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Synthesis and Optimization of ‘Sugar tongs’ Lock Neutraligands of the Chemokine CXCL12

Junjun Ma

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Synthesis and Optimization of 'Sugar tongs' Lock Neutraligands of the Chemokine CXCL12

Thèse de doctorat de l'Université Paris-Saclay
préparée à l'Université Paris-Sud

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Spécialité de doctorat : Chimie

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Abstract

Abstract

Heparan sulfate (HS) is a class of linear and highly sulfated polysaccharides widely present in animal tissues, onto the cell surface or into the extracellular matrix. They are composed of β -(1 \rightarrow 4)-linked disaccharide repetitive units, made of a D-glucosamine (GlcN) α -(1 \rightarrow 4)-linked to a hexuronic acid. HS is one of the most heterogeneous polymers, since various *O*- and/or *N*-sulfation patterns and C5-epimerization of the uronic acid may occur along the chain, which represents the first level of molecular diversity in HS. Besides, HS chains present an alternation of negatively charged regions: highly sulfated domains rich in IdoA and *N*-sulfated Glucosamine (S domains) and weakly sulfated domains rich in GlcA and *N*-acetylated Glucosamine (A domains). Moreover, the length of S and A domains varies from an HS chain to another, therefore, HS presents a second level of diversity attributed to multiple SAS charge topologies. Exposition of those different SAS domains at the cell surface permits the establishment of specific interactions with proteins displaying complementary charge topologies, leading to the regulation of their biological activity.

CXCL12, a HS-binding protein, is a member of chemokine family of pro-inflammatory mediators and the unique natural ligand of CXCR4 receptor, also known as the co-receptor of the T-tropic form of HIV-1. CXCL12/CXCR4 signaling is involved in several biological processes, including cardiovascular organogenesis, hematopoiesis, immune response and cancer metastasis. Therefore, the design of HS type ligands that could bind specifically to CXCL12 to block or modulate its interaction with its receptor should permit us to develop a therapeutic substance being able to modulate the activity of CXCL12 and the treatment of several cancer types. To this aim, we planned to construct a symmetric tridentate ligand of CXCL12 in which the reducing end (RE) and non-reducing end (NRE) of a short synthetic HS fragment (dp4) would be connected *via* a spacer to ligands able to occupy part of the CXCR4 binding site.

Thanks to previous investigation onto the interaction between HS fragments and IFN- γ , a cytokine that binds tightly to HS chains, our already demonstrated that the preparation of SAS mimics should be reached by connecting two synthetic HS fragments (S domains), ranging from tetra- to octa-saccharides, by their reducing end through a PEG type spacer differing in length to mimic internal A domain. To extent this strategy of construction of SAS mimics to the preparation of CXCL12

Abstract

neutraligands and new type of SAS mimics, this PhD program aimed (i) to establish a general strategy of modification of the RE and NRE of HS fragments by using a set of efficient orthogonal reactions and use it to (ii) to prepare specific HS type neutraligands of CXCL12 to modulate its interaction with CXCR4 and (iii) to generate a second generation of SAS mimics to evaluate the influence of the topology of presentation of those synthetic compounds (C2 “head to head” presentation *i.e.* two S domains linked together by their RE, C2 “tail to tail” presentation *i.e.* two S domains linked together by their NRE and classical “head to tail” presentation *i.e.* one S domain linked to the NRE of the second one by its RE).

We selected as orthogonal modifications two Click Chemistry reactions: “the CuAAC” triazole formation and the “oxime ligation”. In order to setup the practicability of this strategy of modification of the two extremities of HS fragments, we optimized reaction conditions onto a model disaccharide derived from cellobiose. On one hand, by using thiol-ene coupling procedure reported by our lab, we introduced an amino group to the reducing end of this disaccharide and optimized reactions conditions of a one-pot diazotransfer reaction/CuAAC sequence, allowing the selective conversion of the amino group into azide and its reaction with alkyne derivatives. This optimized sequence was also applicable to the modification of free amino acids and amino sugars and allowed use the preparation of organofluorine derivatives. On the other hand, the installation of an aldehyde motif onto the non-reducing end of was obtained *via* a three steps sequence involving a Tsuji-Trost decarboxylative allylation of the position O-4 of the NRE unit, dihydroxylation of the obtained allyl group and finally oxidative cleavage of the formed diol. Besides exploring the possibility of oxidative cleavage of vicinal acyclic diols without affecting cyclic diols situated on the backbone of the disaccharide, we also optimized the reaction conditions of oxime ligation to obtain a procedure allowing the one-pot oxidative cleavage/oxime formation. This strategy of functionalization to introduce orthogonal groups to reducing-end and non-reducing end of oligosaccharides can be implemented into the current synthetic strategy of preparation of HS fragments of the lab and was representatively applied to a tetrasaccharide, the HS fragment needed to synthesize CXCL12 neutraligands.

Résumé

Résumé

L'Héparane Sulfate (HS) est un polysaccharide linéaire hautement sulfaté largement présent à la surface des cellules ou dans la matrice extracellulaire des tissus animaux. L'HS est l'un des polymères les plus hétérogènes et présente une alternance de domaines fortement sulfatés (domaines S) et faiblement sulfatés (domaines A). L'exposition à la surface cellulaire de ces domaines SAS permet d'établir des interactions spécifiques avec des protéines basiques présentant des topologies de charges complémentaires et permet la régulation de leurs activités biologiques.

CXCL12, une protéine de la famille des chimiokines se liant aux chaînes d'HS, est l'unique ligand naturel du récepteur CXCR4. La signalisation CXCL12/CXCR4 est impliquée dans divers processus biologiques dont l'hématopoïèse, la réponse immunitaire et la migration des cellules cancéreuses et leur prolifération. La conception de glycoligands pouvant se lier spécifiquement à CXCL12 pourrait permettre de moduler son interaction avec son récepteur naturel et donc donner accès à une substance thérapeutique innovante pour le traitement de divers pathologies dont différents types de cancer. Nous avons ainsi imaginé la construction d'un ligand tridentate symétrique comportant une partie centrale de type fragment d'HS synthétique (dp4) dont les extrémités réductrice (ER) et non réductrice (ENR) seraient reliées à des ligands capables d'occuper une partie du site de liaison à CXCR4.

Grâce à de précédents travaux sur l'IFN- γ , une cytokine se liant également aux chaînes HS, notre laboratoire a déjà démontré que la préparation de mimes de SAS pouvait être obtenue en reliant deux fragments d'HS synthétiques (domaines S) par leur ER *via* des bras espaceur de type PEG et de longueur différente pour mimer les domaines A. Afin d'adapter cette stratégie à la préparation de neutraligands de CXCL12 et d'une nouvelle génération de mimes de SAS, ce programme doctoral visait (i) à établir une stratégie générale de modification de l'ER et l'ENR de fragments d'HS, (ii) à établir des conditions efficaces de réactions de couplage pour (iii) synthétiser des ligands de CXCL12 ainsi que de nouveaux mimes de SAS.

Nous avons sélectionné deux réactions orthogonales de couplage dans le panel de Chimie Click, à savoir la formation de triazole «CuAAC» et la «ligation oxime». Afin de déterminer la faisabilité de transformation des deux extrémités de fragments d'HS pour réaliser ces réactions de couplage, nous avons optimisé les conditions de ces

Résumé

modifications sur un disaccharide modèle dérivé du cellobiose. Tout d'abord, en utilisant la procédure de couplage thiol-ène décrite par notre équipe, nous avons introduit une amine sur l'ER de ce disaccharide et optimisé les conditions d'une séquence monotope transfert de diazo/CuAAC permettant la conversion sélective de cette amine en azoture puis son couplage avec des alcynes vifs. Cette séquence a également pu être appliquée à des acides aminés libres pour la préparation de dérivés organofluorés. Ensuite, l'installation d'un motif aldéhyde sur l'ENR du composé modèle a été obtenue par une séquence en trois étapes comportant une allylation d'acétylante de type Tsuji-Trost de la position O-4 de l'unité NR, une dihydroxylation de l'éther d'allyle obtenu et enfin une coupure oxydante du diol formé. En plus d'explorer la sélectivité de la coupure oxydante en faveur de diols vicinaux acycliques en présence de diols cycliques portés par le squelette saccharidique, nous avons également optimisé les conditions de la réaction de formation d'oximes pour obtenir une seconde procédure monotope de coupure oxydante/formation d'oxime pour la modification rapide de l'ENR de fragments d'HS. Cette stratégie de fonctionnalisation sélective de l'ER et l'ENR d'oligosaccharides a été implémentée dans notre voie actuelle de synthèse de fragments d'HS : elle a été appliquée de manière représentative à un fragment tétrasaccharidique d'HS qui permettra la préparation de neutraligands de CXCL12 et de mimes de SAS.

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List of Abbreviations and Acronyms

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Ac	Acetyl
Ac₂O	Acetic anhydride
AgOTf	Silver trifluoromethanesulfonate
All	Allyl
AT III	Antithrombin III
BAIB	Bis(acetoxy)iodobenzene
BARAC	Biarylazacyclooctynone
BCN	Bicyclononyne
Bn	Benzyl
BnBr	Benzyl bromide
Boc	<i>tert</i> -butoxycarbonyl
CAN	Ceric ammonium nitrate
Cbz	Carboxybenzyl
COMBO	Carboxymethylmonobenzocyclooctyne
CS	Chondroitin sulfate
CuAAC	Copper(I)-catalyzed azide-alkyne cycloaddition
DCC	<i>N,N'</i> -Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIAD	Diisopropyl azodicarboxylate
DIBAC	Dibenzoazacyclooctyne
DIBO	Dibenzocyclooctyne
DIFBO	Difluorobenzocyclooctyne
DIFO	Difluorocyclooctyne
DIMAC	Dimethoxyazacyclooctyne
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide

List of Abbreviations and Acronyms

DNA	Deoxyribonucleic acid
DS	Dermatan sulfate
DTT	Dithiothreitol
EDCI.HCl	<i>N</i> -Ethyl- <i>N</i> '-(3-dimethylaminopropyl)carbodiimide hydrochloride
eq.	equivalent
ESI	Electrospray ionization mass
FDA	Food and Drug Administration
[¹⁸F]FDG	2-deoxy-2-[¹⁸ F]fluoro-D-glucose
GAGs	Glycosaminoglycans
GalN	D-Galactosamine
GlcA	D-Glucuronic acid
GlcN	D-Glucosamine
GPCRs	G-protein-coupled seven-helix transmembrane receptors
HA	Hyaluronic acid
HIV	Human immunodeficiency virus
HP	Heparin
HPLC	High performance liquid chromatography
HS	Heparan sulfate
HSBPs	HS-bonding proteins
hν	Irradiation with light
IC₅₀	Half maximal inhibitory concentration
IdoA	L-Iduronic acid
IFN-γ	Interferon-Gamma
ISA.HCl	Imidazol-1-sulfonyl azide hydrochloride
kD	Kilodalton
K_d	Dissociation constant
KS	Keratan sulfate
LMWH	Low-molecule-weight heparin
LTA	Lead tetraacetate
M	Mol/L
Min	Minute
NBS	<i>N</i> -Bromosuccinimide
Nd(OTf)₃	Neodymium(III) trifluoromethanesulfonate

List of Abbreviations and Acronyms

NEt₃	Trimethylamine
NHC	<i>N</i> -heterocyclic carbene
NHS	<i>N</i> -Hydroxysuccinimide
NIS	<i>N</i> -Iodosuccinimide
NMO	<i>N</i> -Methylmorpholine <i>N</i> -oxide
NMR	Nuclear magnetic resonance
OCT	Cyclooctyne
PCC	Pridinium chlorochromate
PEG	Polyethylene glycol
PG	Proteoglycan
PIDA	Phenyliodine diacetate
PMB	4-Methoxybenzyl
PPh₃	Triphenylphosphine
Pyr	Pyridine
RT	Room temperature
SDF-1	The stromal cell-derived factor-1
SET	Single-electron transfer
S_N2	Substitution nucleophilic bi-molecular
SPAAC	Strain-promoted azide-alkyne [3+2] cycloaddition
SPPS	Solid phase peptides synthesis
TBTA	Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine
TCEP	Tris(2-carboxyethyl)phosphine
TEC	Thiol-ene coupling
TEMPO	2,2,6,6-Tetramethylpiperidine 1-oxyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMSN₃	Trimethylsilyl azide
TfN₃	Trifluoromethanesulfonyl azide
Tf₂O	Trifluoromethanesulfonic anhydride
TLC	Thin layer chromatography
TMTH	3,3,6,6-Tetramethylthiaheptyne
TOF-MS	Time-of-flight mass spectrometry
TsCl	4-Toluenesulfonyl chloride

List of Abbreviations and Acronyms

TsN₃	Tosyl azide
Tf₂O	Trifluoromethanesulfonic anhydride
Tyr	Tyrosine
UV	Ultra violet
V	Volume

Chapter 1. General Introduction

I. Glycosaminoglycans (GAGs)

I.1. Generality and Classification

Glycosaminoglycans (GAGs) are a class of linear, highly sulfated and polydisperse polysaccharides that are often covalently linked to a protein core, called a proteoglycan (PG). GAGs are widely present on mammalian cell surfaces and within the extracellular matrix and play critical roles in a wide range of physiological processes, including viral invasion, blood coagulation, cell proliferation and migration, inflammatory responses, as well as spinal cord injury¹⁻⁸ Depending on the nature of both the repeating unit and glycosidic linkage between them, GAGs can be classified into five main families: (I) hyaluronic acid (HA), (II) chondroitin sulfate (CS), (III) dermatan sulfate (DS), (IV) keratan sulfate (KS) and (V) heparin (HP) and heparan sulfate (HS). With the exception of KS, GAGs are composed of a repeating disaccharide unit containing a hexuronic acid (L-iduronic acid (IdoA) or D-glucuronic acid (GlcA)) and a hexosamine (D-glucosamine (GlcN) or D-galactosamine (GalN)) and are differentially sulfated at the hydroxyl and/or amino positions.

I.1.1. Hyaluronic Acid (HA)

In comparison with other GAGs, HA is a non-sulfated polymer, composed of GlcA β -(1 \rightarrow 3) linked to GlcNAc.⁹ These disaccharide units are connected together by β -

-
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Chapter 1. General Introduction

(1→4) linkages (Figure 1).^{10,11} First isolated from the bovine vitreous body of eyes,^{12,13} HA is a major component of soft connective, epithelial and neural tissues¹⁴ and extracellular matrix,^{15,16} plays a crucial role in embryogenesis,^{17,18} inflammation,^{19,20} cell migration and proliferation.^{21,22}

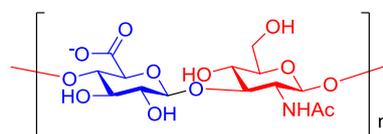


Figure 1. Structure of hyaluronic acid (HA).

I.1.2. Chondroitin Sulfate (CS)

Chondroitin sulfate (CS) occurs in many tissues, and can be found on the cell surface or in extracellular matrix.²³ CS saccharides are consisted of GlcA β -(1→3) linked to GalNAc. Each disaccharide units are connected together by β -(1→4) glycosidic linkages (Figure 2).³ Commonly, CS can be sulfated at positions *O*-4 and *O*-6 of GalNAc as well as positions *O*-2 and *O*-3 of the GlcA. Diverse sulfation patterns can be observed and result in various biological properties.^{24–29} Depending on their modifications and the specific sulfation patterns of the disaccharide units, CSs are

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classified into 5 different types, CS-A, CS-B (also called Dermatan Sulfate (DS)), CS-D, CS-E and CS-K (Figure 2).

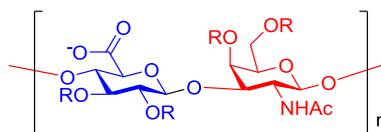


Figure 2. Structure of chondroitin sulfate (CS) (R = H or SO₃⁻).

I.1.3. Dermatan Sulfate (DS)

Dermatan sulfate (DS) was ever classified as a subform of CS, both share the GalNAc unit in its skeleton. Nonetheless, DS distinguishes from CS by the presence of at least one IdoA motif, the C5 epimer of GlcA^{30,31}, into the oligosaccharide chain. Moreover, this IdoA motif can be sulfated at position O-2 (Figure 3). IdoA is a rare sugar, not commercially available, which complicates the syntheses of DS.

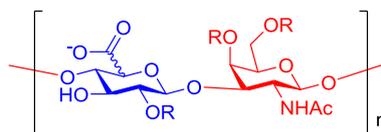


Figure 3. Structure of dermatan sulfate (DS) (R = H or SO₃⁻).

I.1.4. Keratan Sulfate (KS)

KS structurally differs from the other members of GAG due to the replacement of hexuronic acid moiety by a neutral D-galactose (Gal) into the repeating disaccharide. Each of the disaccharide units is composed of Gal β-(1→3) linked to GlcNAc.³² In common, KS can be sulfated at position O-6 of Gal or GlcNAc (Figure 4).³³ Depending of the core proteins that it attached to, KS can be divided into two main classes: KS I and KS II. KS I is linked via an N-glycosidic linkage to L-asparagine residues.³⁴ On the other hand, KS II is glycosidically attached to L-serine or L-threonine.³⁵

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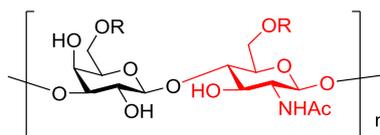


Figure 4. Structure of Keratan sulfate (KS) (R = H or SO₃⁻).

I.1.5. Heparin (HP)/Heparan Sulfate (HS)

The more complex members of GAG family are heparin (HP) and heparan sulfate (HS), which share identical backbone disaccharide units that are composed of an hexuronic acid β -(1 \rightarrow 4)-linked to D-glucosamine (GlcN).^{36–41} The structural diversity of HP/HS is remarkable and due to: (i) large number of sulfation patterns of hydroxyl groups, (ii) presence of a free, sulfated or acetylated amino group, (iii) the C5-epimerization of hexuronic acid that can be either GlcA or IdoA (Figure 5).^{41–43}

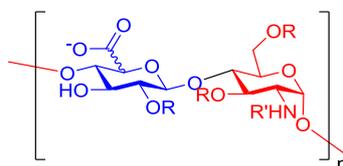


Figure 5. Structure of heparin (HP)/heparan sulfate (HS) (R = H or SO₃⁻, R' = H, Ac or SO₃⁻).

I.1.5.1 Conformation of HP/HS

HP/HS exist primarily as a helical structure.^{44,45} NMR studies and X-ray structures indicate that glucuronic acid and glucosamine residues prefer the chair (⁴C₁) conformation (Figure 6a),^{46–48} whereas the conformation of IdoA is more flexible, depending on its substitution patterns and its relative position in the chains (Figure

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6b).^{49–53} Generally, unsubstituted IdoA and IdoA residues at non-reducing end of HS predominantly resides in ¹C₄ form.⁵⁴ However, for *O*-2-sulfated and internal IdoA units of the oligosaccharide chain, both ¹C₄ and skew-boat (²S₀) conformers are formed.^{55,56}

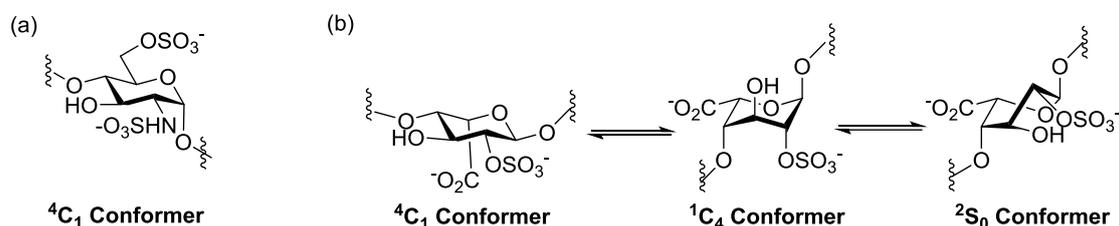


Figure 6. Conformational flexibility of residues in heparin/HS. **(a)** The primary ⁴C₁ form of GlcN; **(b)** the ⁴C₁, ¹C₄ and ²S₀ conformers of IdoA2S present in HP/HS.

I.1.5.2 Differences between HP/HS

Even though HP and HS are structurally related, they are differentiated by their locations and content of sulfation groups and IdoA units (as shown in Table 1). Heparins are exclusively expressed in granules of mast cells, whereas HS are ubiquitously located on all cell surfaces and in extracellular matrix.⁵⁷ In general, heparin have a higher sulfation level than HS, containing an average of 2 sulfate groups per disaccharide subunit compared to an average of 1 sulfate group for HS.^{58,59} In addition, the uronic acid moiety of heparin is made of up to 90% IdoA while HS are consisted of about 40% IdoA.^{60,61} Moreover, HS chains typically have an average molecular weight of about 60 kDa ranging from 20 to 100 kDa. In contrast, heparin is much shorter, generally consists of chains ranging from 7-20 kDa.

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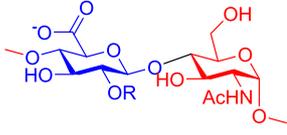
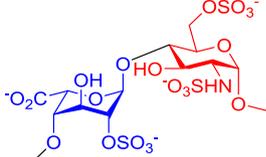
	Heparan sulfate	Heparin
Average sulfate/disaccharide	0.6-1.8	1.8-2.6
N-Sulfate	30-60%	≥ 80%
Site of synthesis	Virtually all cells	Mast cells
IdoA	20-50%	≥ 80%
Major sequence		
Size	20-100 kDa	7-20 kDa
Commercial availability	Milligrams	Kilograms

Table 1. Differences between heparin and heparan sulfate.

I.1.5.3 Regulation of physiological and pathological activities by HP/HS

Owing to the numerous structural variations, HP and HS display a sheer structural diversity and remarkable heterogeneity, which enable HP/HS to interact with a wide range of proteins, generally called HS-binding proteins (HSBPs), regulating their biological activities,⁶²⁻⁷¹ playing a key role, for example, in anticoagulation,^{72,73} cell adhesion, migration, proliferation and differentiation,^{74,75} embryogenesis,⁷⁶ homeostasis, assisting against viral and bacterial invasion,^{77,78} tumor metastasis⁷⁹⁻⁸¹. More than 500 HSBPs have been identified and demonstrated the important and large

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implication of HS into physiological processes and by the way into many human diseases (Table 2).^{62–71}

Class	Proteins	Physiological/Pathological role
Proteases/esterases	AT-III, PCI, HCII, SLPI, C1 INH, VCP...	Blood coagulation, apoptosis, metabolic pathway
Grow factors	FGFs, VEGF, HGF, PDGF, neuropilin-1	Cell proliferation, differentiation, morphogenesis, and angiogenesis
Cytokines	IL-5, IL-10, IFN γ , TNF α ...	Inflammation and wound healing
Chemokines	SDF-1/CXCL12, RANTES, PF4, IL-8, TNF- α ...	Cheotaxis, cell migration, immune response, angiogenesis
Lipid-binding proteins	APoA-5, ApoB, APoE, Annexin II, Annexin V,...	Lipid metabolism, cell membrane functions, AD risk factor
Adhesion proteins	Selectins, vitronectin, fibronectin, HB-GAM, AP,...	Cell adhesion and migration, traction, inflammation
Pathogen proteins	HIV-1 gp120, Dengue virus envelope, HSV, CypA, Streptococcus pneumonia...	Virus infection, entry, fusion and localization
Morphogens	Retinoic acid, Wnt, BMP, sonic hedgehog...	Development, regeneration, bone formation, embryogenesis

Table 2. Selected examples of biological processes regulated by heparin/HS-proteins interactions.

In general, many proteins interact with HP/HS *via* non-specific electrostatic interactions, as the negatively charged sulfate and carboxylate groups form ion pairs with positively charged amino acid residues located on the protein.⁸² However, it has been shown that ionic contacts are not sufficient to explain the functional selectivity of HP/HS. A growing number of biochemical and biophysical studies have demonstrated that the specific interactions of HP/HS with proteins are manifested at different levels.^{83–87}

-
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The structure of HP/HS chains can be characterized by the sequence variations of HS arising from the sulfation and epimerization patterns, which only represent a first level of molecular diversity in HS.

I.2.SAS Domains

I.2.1 Definition of SAS domains

Alternatively, HP/HS is also characterized by an alternation of different domains along the oligosaccharide chains:^{83–87}

(a) S domains bearing a high rate of sulfation, generally rich in IdoA and *N*-sulfated Glucosamine (so-called S).

(b) A domains that are none or weakly sulfated, generally rich in GlcA and *N*-acetylated Glucosamine (so-called A).

(c) Transition domains, including A/S units, located in the transition zone between S and A domains.

It has been demonstrated that the length of A and S domains varies from an HS chain to another, generally S domains contain three to eight disaccharides while the A domains around 15 disaccharides. Moreover, A domains are more regular and encompass a larger area.⁸⁸ Hence, HS presents a second level of diversity attributed to multiple SAS charge topologies along the chain, which are generated from various arrangements of S, A and transition domains.

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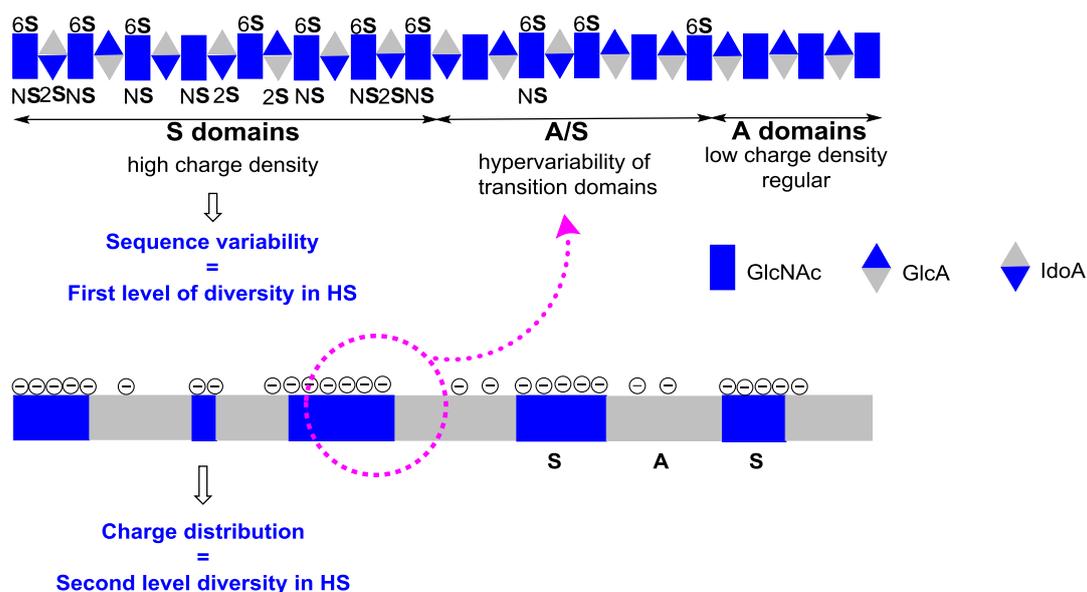


Figure 7. Two levels of molecular diversity in HS chains.⁸⁹

I.2.2 Proteins/SAS interactions

If HP/HS displays SAS domains whose charge topology is complementary to that of basic clusters of the protein, a high affinity may occur (Figure 8a). On the contrary, lower affinity is obtained (Figure 8b). It has been proposed that the HS chain may adapt its conformation in order to meet the needs of recognition of a protein. However, this will have an enthalpic and entropic cost and will thus favor the binding of domains with the correct performed geometry. In this regard, HS glycoconjugates, in which a linker of an appropriate length separates two S-domains, should be a selective functional mimetic of the binding site of a given protein on HS (Figure 8c). Such considerations drove our laboratory when designing a general strategy for the preparation of libraries of HS/SAS long fragments mimetics (see Figure 8d).

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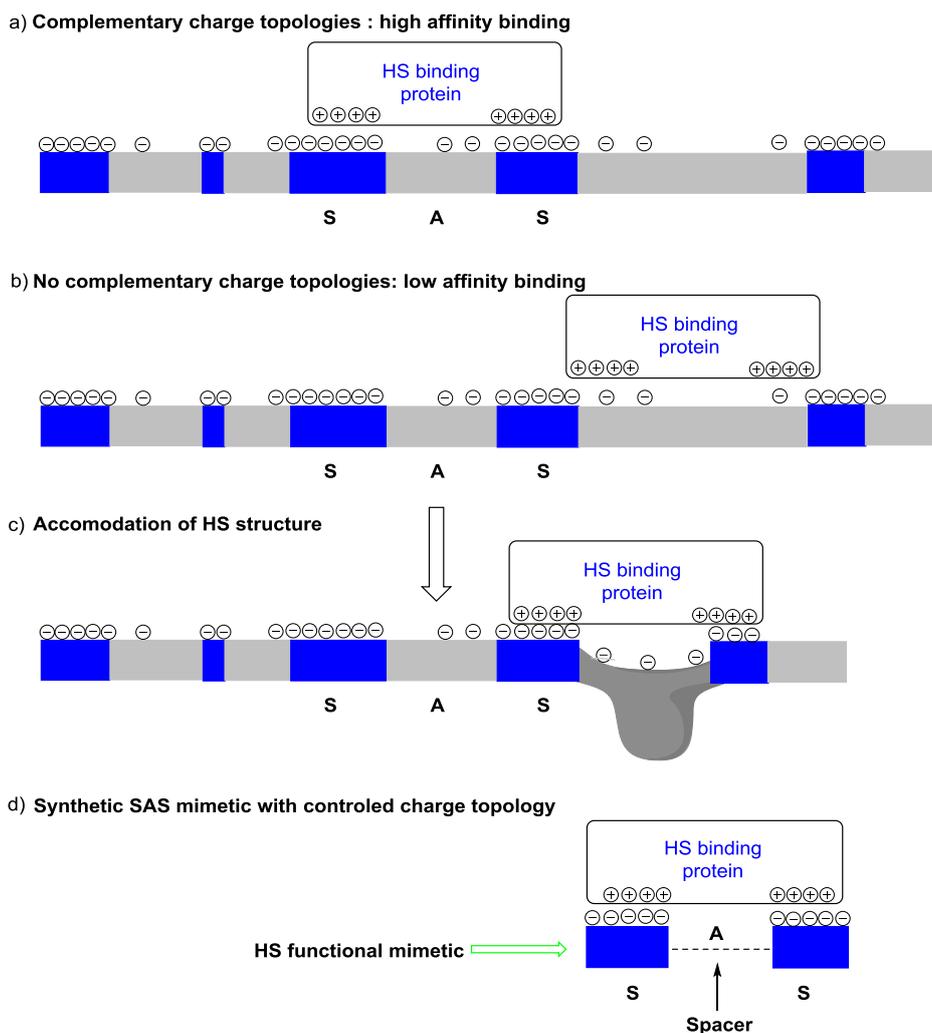


Figure 8. A model for the binding of HS oligosaccharides with a protein that more than a single NS domain is needed.⁸⁹

II. Examples of Modification of SAS/Proteins Interactions for Their Pharmacomodulation

II.1. Interaction of HP/HS with Antithrombin III

So far, the most thoroughly studied heparin-binding protein is antithrombin III (AT-III), a serine protease inhibitor, *i.e.* a serpin that interacts with thrombin and factor Xa in the blood coagulation cascade.^{90,91} The heparin/AT-III interaction is responsible for the anticoagulant activity of heparin. AT-III binds to heparin with an extreme

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specificity for a pentasaccharide sequence (Figure 9a) found in about 30% of heparin chains.^{91–95} It has been demonstrated that AT-III binds heparin chains containing this pentasaccharidic motif with 1,000-fold higher affinity than to chains free of it.⁹² It has also been demonstrated that heparin activates AT-III through an allosteric activation,^{96,97} inducing conformational changes upon binding to heparin that specifically enhances factor Xa binding (Figure 10).⁹²

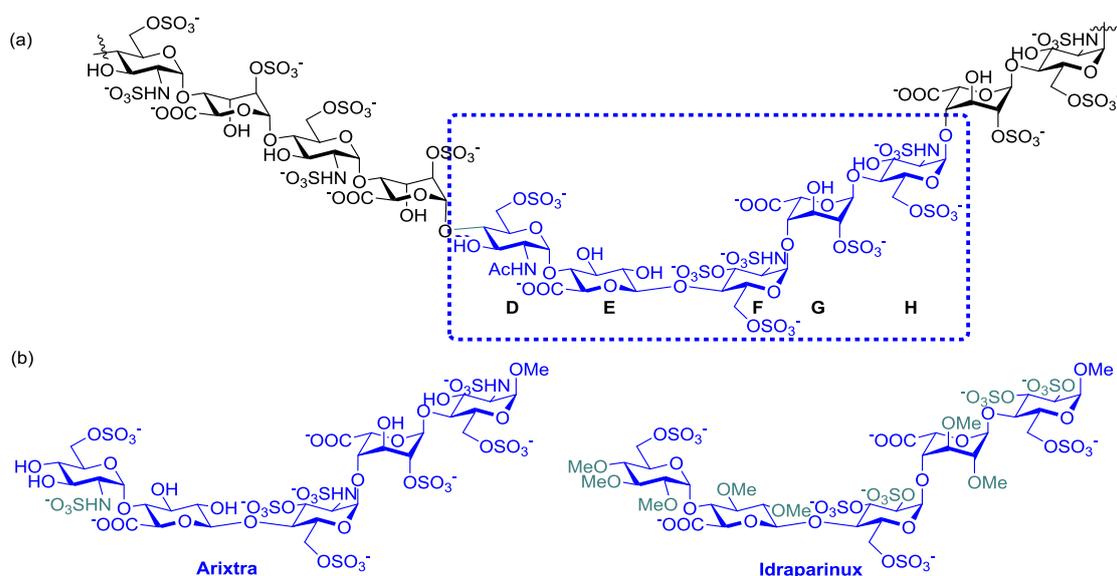


Figure 9. (a) Structure of the AT-III binding pentasaccharide; (b) synthetic pentasaccharide anticoagulant Fondraparinux (left) and Idraparinux (right).

The structure-function relationships of the pentasaccharide sequence have been elucidated by crystallographic studies of AT-III/pentasaccharide complexes (with various combination of sulfo and carboxyl groups onto the pentasaccharide).⁹² Besides the 3-*O* and *N*-sulfate groups of F residue,^{98,99} the 6-*O*-sulfate group of GlcN D^{100,101} as well as the carboxylate group of the IdoA G are required for binding to AT-III and affect

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anticoagulant activity. The 2-*O*-sulfate group of G unit and the 6-*O*-sulfate group of H residue are not essential for binding but contribute to the overall binding affinity.¹⁰² Based on those considerations, the synthetic anticoagulant pentasaccharides Fondraparinix (trade name Arixtra)^{103–105} and Idraparinix have been prepared and the former has been approved by the US Food and Drug Administration. In Idraparinix, *N*-sulfates of units F and H have been replaced by *O*-methylation and by OMe in unit D, while free hydroxyl groups are methylated (Figure 9b). This mimetic exhibits a higher lipophilicity and therefore a longer elimination half-life than Fondraparinix due to the replacement of *N*-sulfate by *O*-sulfate groups and methylation of free hydroxyl groups (Figure 9b).¹⁰⁶

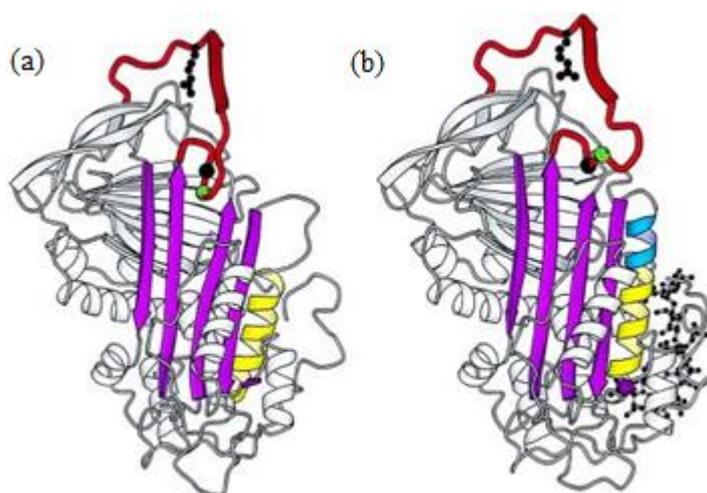


Figure 10. Ribbon representations of conformational changes of antithrombin upon binding to heparin. **(a)** Crystal structure of AT-III and **(b)** AT-III binds to pentasaccharide leading to the change of conformation at the pentasaccharide binding site loop (red) and expulsion of residues P14 (green sphere) and P15 (black sphere).⁹²

However, this pentasaccharide/AT-III complex is not sufficient to inhibit thrombin. In contrast to the extreme specificity of antithrombin binding to heparin, thrombin binds nonspecifically to heparin and the inhibition entirely relies on a template effect (entropic activation): it is electrostatically attracted by heparin and the trimeric complex has to be stabilized by extra interaction.¹⁰⁷ Therefore, a much longer heparin chain (14-

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20 saccharides) than the short pentasaccharide is required for effective thrombin inhibition, allowing the presentation of thrombin to activate AT-III and stabilization of this complex.^{108–111}

Thus, a variety of synthetic SAS mimics have been synthesized to test their ability to inhibit both factor Xa and thrombin. They are composed of a highly sulfated thrombin-binding site (S₁) bound to the non-reducing end of antithrombin-binding pentasaccharide (S₂) through a nonsulfated and neutral linker (A).^{112–115} Initially, a compound containing a flexible polyethylene glycol (PEG) type linker have been synthesized by van Boeckel and co-workers (Figure 11a), displaying both AT-III mediated anti-Factor Xa and anti-thrombin activity.^{112,113} The use of PEGs is not only because of their water-solubility and commercial availability in different lengths, but also because of their flexibility that permits the adoption of specific conformation to realize desired biological interactions. Additionally, PEGs are essentially non-immunogenic and their introduction into a drug is not a problem from the pharmacological point of view. Soon after, Petitou and coworkers prepared a compound bearing a sulfated hexasaccharide (S₁ domain) and a pentasaccharide (S₂ domain) binding to antithrombin with high affinity, linked by a neutral methylated hexasaccharide (Figure 11b), displaying excellent anticoagulant activity (5- to 10-fold more potent than standard heparin and low-molecular-weight heparin (LMWH)) without exhibiting side effects.¹¹⁴ Then, in 2001, a synthetic hexadecasaccharide (SR123781) that displays excellent anticoagulant activity with regard to factor Xa (IC₅₀ = 77 ± 5 ng/ml) and thrombin (IC₅₀ = 4.0 ± 0.5 ng/ml) was developed by Petitou and coworkers (Figure 11c).¹¹⁵

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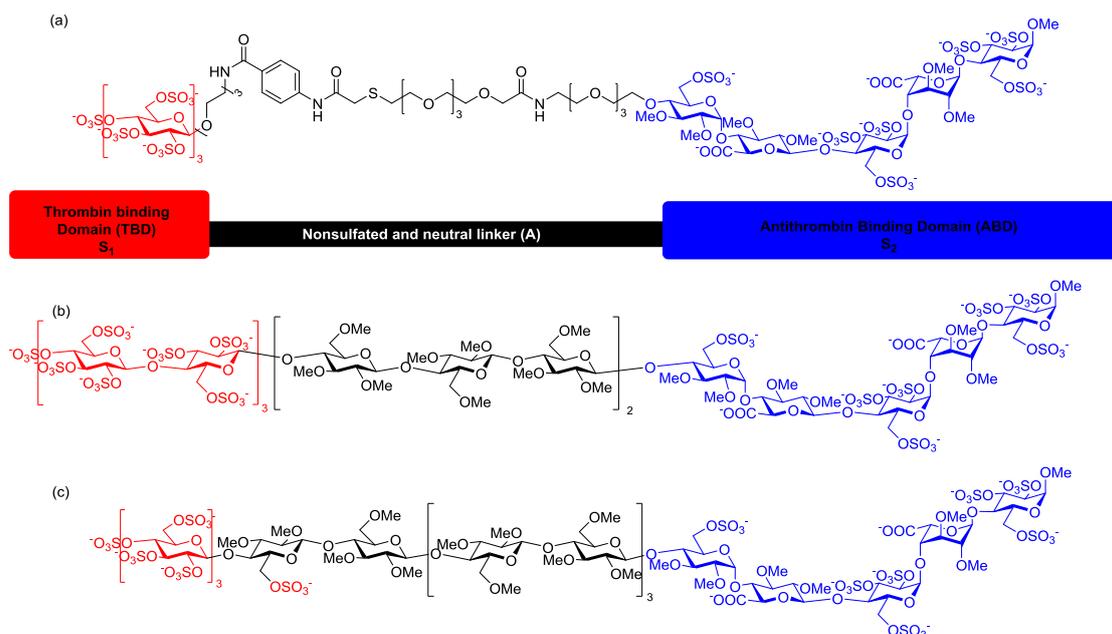


Figure 11. Synthetic heparin mimics, composed of SAS domain, having excellent anticoagulant activity. **(a)** The mimic of S₂ domain binding to antithrombin comprises an Idraparinux motif while the one binding to thrombin is composed of a persulfated cellotriase. The mimic of A domain contains a flexible PEG-type linker. **(b)** Second generation of anticoagulant SAS mimics exhibiting a sulfated hexasaccharide as S₁ domain and a rigid and neutral *O*-methyl-β-D-cellohexaoside as A domain; **(c)** Third generation of SAS mimics composed of a rigid *O*-methyl-β-D-celloheptoside as A domain and 2,3-di-*O*-methyl-6-*O*-sulfate-β-D-glucoside as transition unit between A domain and S₁ domain.

The preparation of heparin mimics enabled chemists to explore the molecular antithrombotic mechanism of heparin by the crystallographic study of the ternary complex formed by HS fragments with AT-III and thrombin (Figure 12a).¹¹⁶ It has been confirmed that heparin fragments and mimics allows for the presentation of thrombin to AT-III, the stabilization of this ternary complex and that the A domain does not interact with the two proteins (Figure 12b).¹¹⁷

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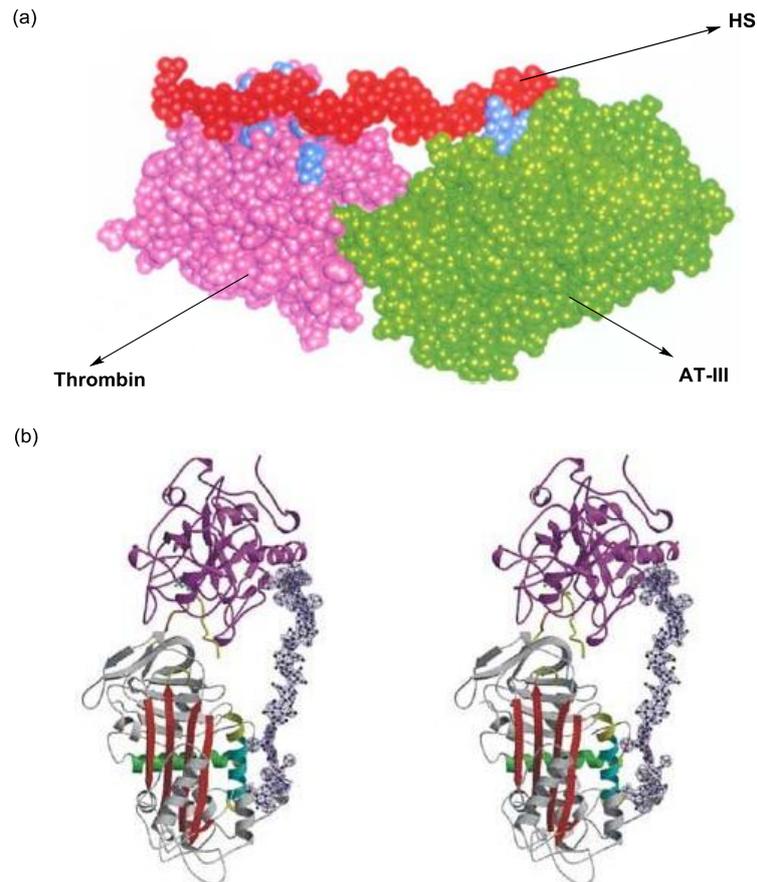


Figure 12. Stabilization of AT III/thrombin complex by a HS fragment or a mimic of SAS domain. **(a)** A space-filling molecular model of the ternary AT III/heparin/thrombin complex;¹¹⁶ **(b)** Stereorepresentation of the crystal structure of the ternary complex between antithrombin (colored as above), thrombin (magenta) and a heparin mimetic (ball-and-stick). Thrombin is docked toward the heparin-binding site of antithrombin, and makes several exosite interactions.¹¹⁷

II.2. Interaction of HP/HS with IFN- γ

Interferon-Gamma (IFN- γ), a cytokine primarily secreted by natural killer cells and activated T cells, not only regulates antiviral/antibacterial immunity and activates functions of macrophage but also can control cellular proliferation and apoptosis.^{118,119} IFN- γ mediates its activities by binding to a specific transmembrane receptor (IFN- γ R) expressed on the surface of nearly all cells.^{120,121}

In solution, this cytokine is a C2-symmetric homodimer and its C-terminal part is

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believed to be highly flexible, lacking a rigid formation.^{122,123} Even though the conditions and role of IFN- γ processing remains not totally elucidated, it has been shown that the C-terminal residues, especially the highly positively charged D1 domain and its downstream D2 domain, clustered in the C-terminus, are critical for its biological activities (Figure 13).^{124,125} The D1 domain appears to be the most important, as the removal of D1 domain will inactivate IFN- γ , nonetheless, the proteolysis of D2 will increase the biological activity of the cytokine.^{126–128}

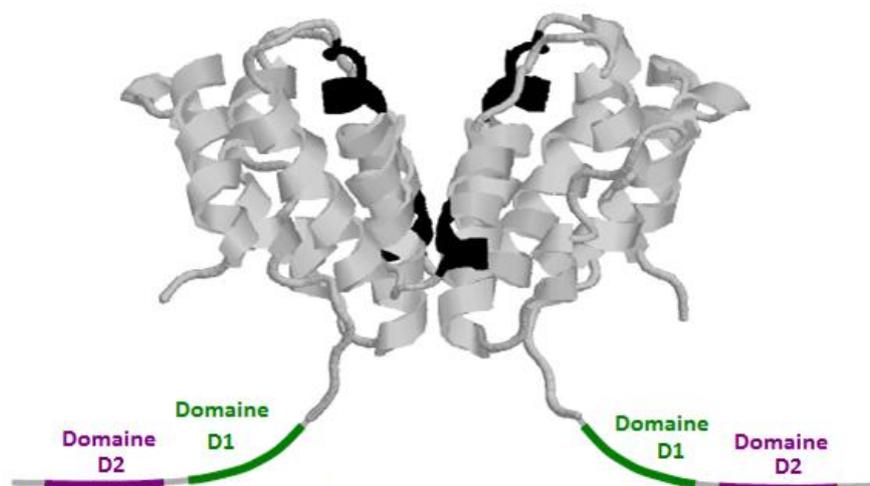


Figure 13. Structure of IFN- γ . Domain D1 and D2 are regions rich in basic amino acids, situated at C-terminus.

IFN- γ also binds to HS with a high affinity interaction with the basic residues at the C terminus of the two subunits, controlling the blood clearance, the subsequent tissue targeting and the local accumulation of the cytokine.^{84,129,130} The modulation of the cellular response to IFN- γ by HS is also a result of the competitive inhibition of receptor binding because the heparin-binding regions of IFN- γ are also involved in receptor recognition.¹²⁷

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To identify the HS oligosaccharide sequences that interact with IFN- γ with high affinity, HS polymer has been enzymatically digested in presence of IFN- γ to retain the binding portion of the full length polysaccharide.⁸⁴ It has been revealed that the HS oligosaccharides were composed of two small S domains, ranging from hexa- to octa-saccharides, bridged by a more extended A domain, ranging from 15 to 16 disaccharides (130-140 Å). Therefore, the high affinity of IFN- γ with HS oligosaccharides was proposed to be achieved by an electrostatic interaction between the D1 domains¹²⁴ located at the C-terminus of each subunit of the IFN- γ dimer and the highly negatively charged S-domains.^{84,130} However, the distance between the two Ala123 residues of IFN- γ subunits is much shorter than the length of A domain (about 23 Å, as revealed by the X-ray diffraction crystal structures of C-termini-truncated IFN- γ dimer^{122,131}), indicating that the chain of HS oligosaccharide will adapt its conformation to achieve a high affinity with IFN- γ .

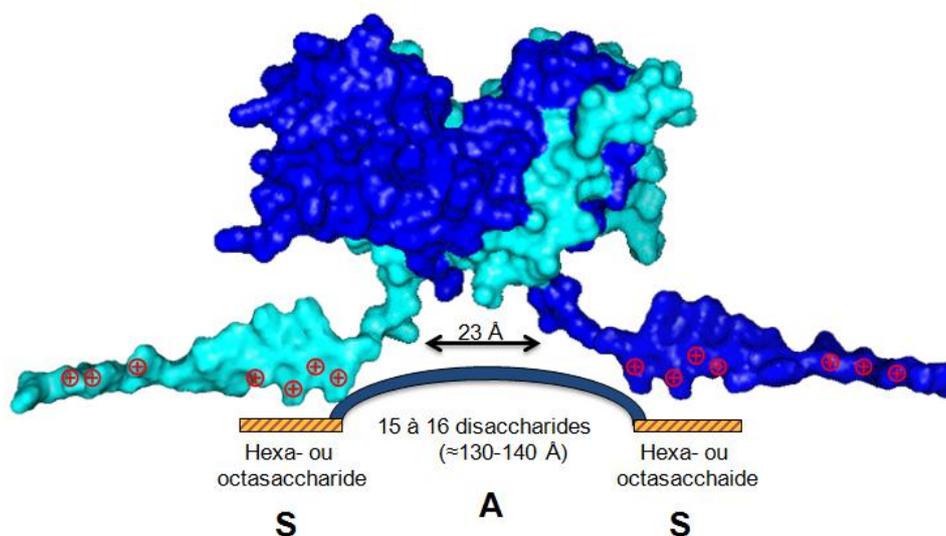


Figure 14. Structure proposed for IFN- γ /SAS complex.

To confirm this hypothesis, our lab prepared a set of C2-symmetric SAS mimics in which two S domains, ranging from tetra- to octa-saccharides, have been linked by their reducing end to PEG-type spacer to mimic internal A domain, differing in length having 5, 10, or 32 ethylene glycol units (Figure 15).¹³² PEG linkers have been chosen because of their flexibility that may be significant to mimic A domain whose

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conformational plasticity is thought to be important for high affinity. In addition, the C2-symmetric glycoconjugates have been furnished by the addition of α,ω -bis(thio)PEGs to the allyl anomeric derivatives through photo-activated thiol-ene coupling reaction in water.

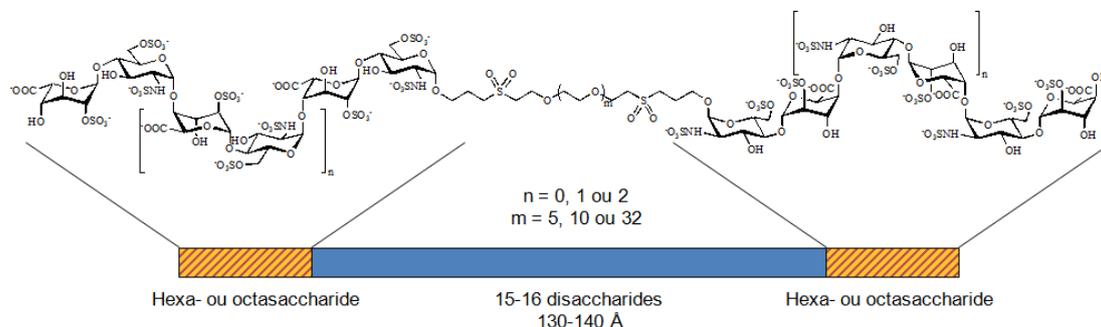


Figure 15. Structure of synthetic SAS mimetics prepared in our lab.

Then these glycoconjugates have been tested at various concentration for their ability to inhibit IFN- γ /Heparin interactions using surface plasmon resonance. The obtained results demonstrated that at 40 nM the inhibition by the sole oligosaccharide is minor, as the tetra- and hexasaccharides were completely inactive and the octasaccharide displayed a slight inhibition activity. Moreover, the inhibition activity of HS glycoconjugates appeared to be well correlated with the linker length. While the octasaccharide conjugated to spacer containing 10 ethylene glycol units, *i.e.* 50 Å linker length, is the most active molecule (IC_{50} is about 35 nM), the glycoconjugate containing 32 ethylene glycol units, 114 Å linker length, is the less active inhibitor and the glycoconjugate containing 5 ethylene glycol units, 33 Å linker length, has a medium activity (Figure 16). These results confirmed the hypothesis that the HS oligosaccharide has to adapt its conformation in order to acquire a high affinity with IFN- γ , as the optimal linker length is two to three times smaller than the A domain found in the natural IFN- γ /HS binding site (50 Å vs 130-140 Å).

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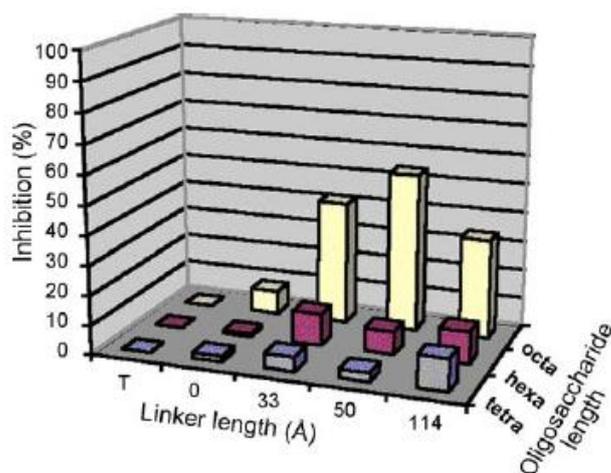


Figure 16. IFN- γ /HP binding inhibition by synthetic glycoconjugates at 40 nM (T: buffer, 0: non-conjugated tetra, hexa- and octasaccharides).

Additionally, we demonstrated that these glycoconjugates are able to inhibit IFN- γ /IFN- γ -receptor interaction, and in a cellular model, the glycoconjugates could downregulate the pro-inflammatory activity of IFN- γ (IC_{50} close to 1.5 mg/mL, 250 nM).¹³³ All of these results permit us to identify a ‘hit compound’ for the potential development of a therapeutic substance being able to modulate the activity of IFN- γ .

II.3. Interactions of HP/HS with Chemokine CXCL12

Chemokines, are a large family comprising approximately 50 structurally related chemotactic cytokines (8-12 kDa in molecular mass). They signal through a family of G-protein-coupled seven-helix transmembrane receptors (GPCRs) on cell surfaces to mediate the immune and inflammatory responses, primarily through controlling the activation and migration of leukocytes.^{134–137} As such, they also control diverse critical processes, including immune cell adhesion, development and differentiation, metastasis

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and angiogenesis, neuronal patterning, hematopoiesis, viral infection and wound healing.^{138–145} The GPCRs are predominantly expressed by leukocytes and 20 of which have been identified to date.^{146–148}

The peptidic sequences and crystal structures of chemokines revealed that they share a remarkably conserved tertiary structures, consisting of: (i) a triple-stranded antiparallel β -sheet ($\beta_{(1)}$, $\beta_{(2)}$ and $\beta_{(3)}$), (ii) a C-terminal α -helix ($\alpha_{(C)}$), (iii) a more or less disordered and extended N-terminal domain of 6-10 residues, (iv) a long loop (N-loop), (v) a short 3_{10} helix, and (vi) a triple helix. The overall structure is stabilized by disulfide bridges that anchored the N-terminal to the rest of the molecule (Figure 17).^{146,149}

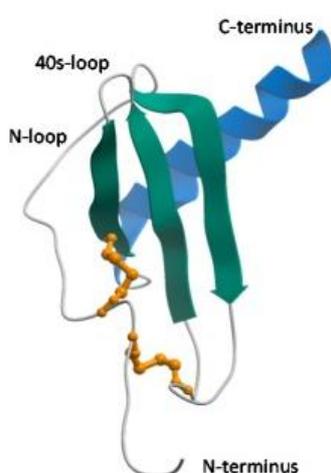


Figure 17. The tertiary structure of a typical chemokine, illustrated by a monomeric subunit of CXCL8.^{150,151}

Hence, according to the structural and functional criteria, chemokines are divided

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into subgroups. Structurally, based on the number and location of cysteine residues in the N-terminal domain of the molecule, chemokines are traditionally divided into four major subfamilies: CC (β chemokines), CXC (α chemokines), CX₃C (γ chemokines) or C chemokines (δ chemokines) (Figure 18).^{137,146} According to a new nomenclature established by Zlotnik and Yoshie,¹⁵² chemokines are named using this motif followed by a L for ligand and an identification number n, like CCL_n or CXCL_n. From the functionally point of view, chemokines are broadly categorized as homeostatic chemokines, which are involved in homeostatic relocation and cellular homing or as inflammatory chemokines, which are active in the recruitment of leukocytes toward inflamed areas.^{135,153} Some of these chemokines fall into both categories.^{154,155}

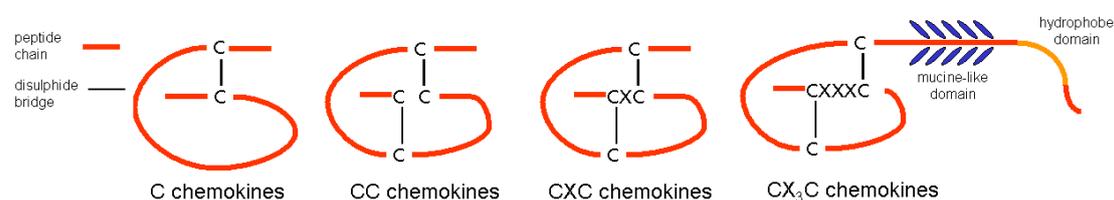


Figure 18. The structural differences between the structural subgroups of chemokines.

Due to the high prevalence of basic residues in their peptidic sequences, chemokines can also bind with heparin/HS (Figure 19)^{156,157} to stabilize concentration gradients along the surface of endothelial cells,^{158,159} providing directional cues for migrating leukocytes,¹⁶⁰ and also to protect chemokines from proteolytic degradation, thus enhancing the local concentration of chemokines in the vicinity of G-protein-coupled receptors.¹⁶¹

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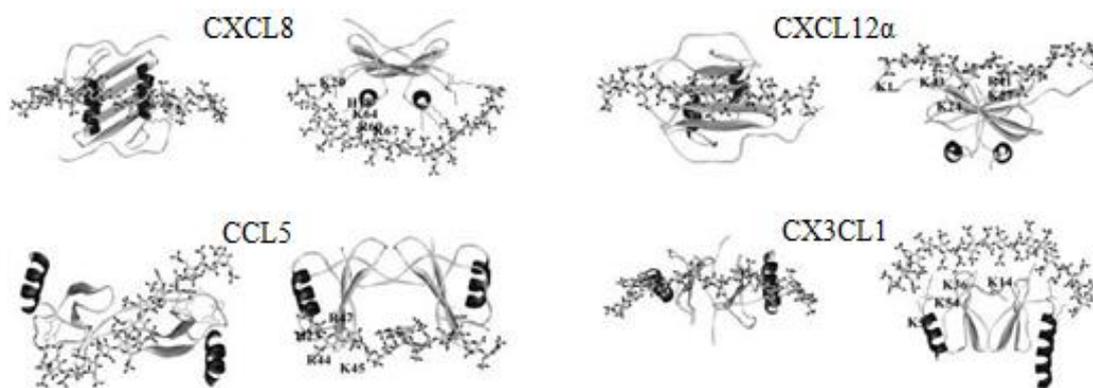


Figure 19. Representation of the lowest energy models of several chemokine-heparin fragment interactions. The proteins are represented by ribbons except for the side chains of the basic amino acids directly involved in polysaccharide binding. The heparin molecule is represented by sticks. Hydrogen atoms are not displayed.¹⁵⁷

In addition, HS or HS derived oligosaccharides could modulate the monomer/dimer equilibrium of chemokines, induce larger oligomerization states of chemokine, or even promote chemokine heterodimerization, to modulate and enhance their biological activities.

For example, CXCL12, also known as SDF-1 (Stromal cell-Derived Factor-1), is a member of the CXC chemokine family of pro-inflammatory mediators and a potent chemoattractant for mono and lymphocytes.¹⁶² It could also compete with the T-tropic form of human immunodeficiency virus (HIV) for binding to its natural co-receptor CXCR4.^{163–167} To date, three different isoforms (α , β and γ) and up to six isoforms (α , β , γ , δ , ϵ and ϕ) of CXCL12 have been found respectively in human¹⁶⁸ and rodents.¹⁶⁹ All these isoforms share the same first 68 amino acid residues, corresponding to the α isoform, comprising the CXCR4-binding domain and a well-defined HS binding site,

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but all differ in their C-terminal domain that contains additional HS-binding motifs. In particular, the γ isoform of CXCL12 is characterized by an unusual and distinctive 30 amino acids long C-terminal peptide (Figure 20).¹⁷⁰

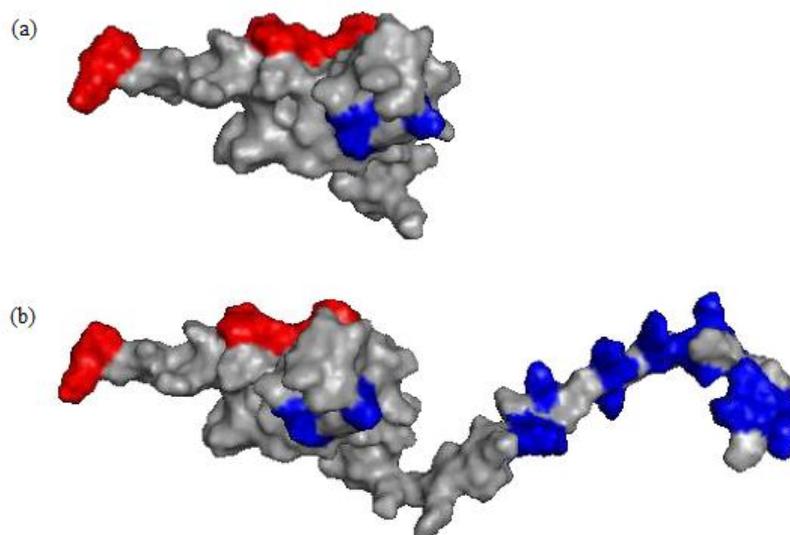


Figure 20. Surface representation of structures of (a) CXCL12 α and (b) CXCL12 γ . The amino acid residues known to contribute to binding to HS and CXCR4 are shown in blue and red, respectively. HS-binding sites include the residues K24, H25, K27 and R41, which are common to CXCL12 α and CXCL12 γ , and several basic clusters within the C-terminal region that characterizes the CXCL12 γ isoform.¹⁷¹

The binding of CXCL12 α and CXCL12 γ with the receptor CXCR4 and HS were thoroughly investigated by Lortat-Jacob and coworkers.^{170–173} They demonstrated that, even though CXCL12 α and CXCL12 γ present identical CXCR4-binding domains within their first 68 amino acid residues, CXCL12 γ has an enhanced affinity for CXCR4, which could be attributed to the basic residues within the C-terminal extension of CXCL12 γ , which distinguishes these two chemokine isoforms from each other.¹⁷¹ However, the binding of CXCL12 γ to CXCR4 was substantially decreased in presence of HS, while HS has no effect on the binding of CXCL12 α to CXCR4.^{170,171} They proposed that the HS-binding site of the C-terminal region of CXCL12 γ interacts with the three sulfotyrosines (at position 7, 12 and 21) contained within the N terminus of CXCR4 (mimicking to some extent the anionic characteristics of HS), thus leading to

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170. Laguri, C.; Sadir, R.; Rueda, P.; Baleux, F.; Gans, P.; Arenzana-Seisdedos, F.; Lortat-Jacob, H. *PLoS One* **2007**, *2*, e1110.
171. Connell, B. J.; Sadir, R.; Baleux, F.; Laguri, C.; Kleman, J. P.; Luo, L.; Arenzana-Seisdedos, F.; Lortat-Jacob, H. *Sci. Signal.* **2016**, *9*, 1–12.
172. Lortat-Jacob, H.; Grosdidier, A.; Imberty, A.; Sadir, R.; Baleux, F. *J. Biol. Chem.* **2001**, *276*, 8288–8296.
173. Sadir, R.; Imberty, A.; Baleux, F.; Lortat-Jacob, H. *J. Biol. Chem.* **2004**, *279*, 43854–43860.

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these differences in activity. Consistent with the proposal, they found that mutations of basic residues in the C-terminal region of CXCL12 γ restored binding to a level comparable to that of CXCL12 α .¹⁷⁰

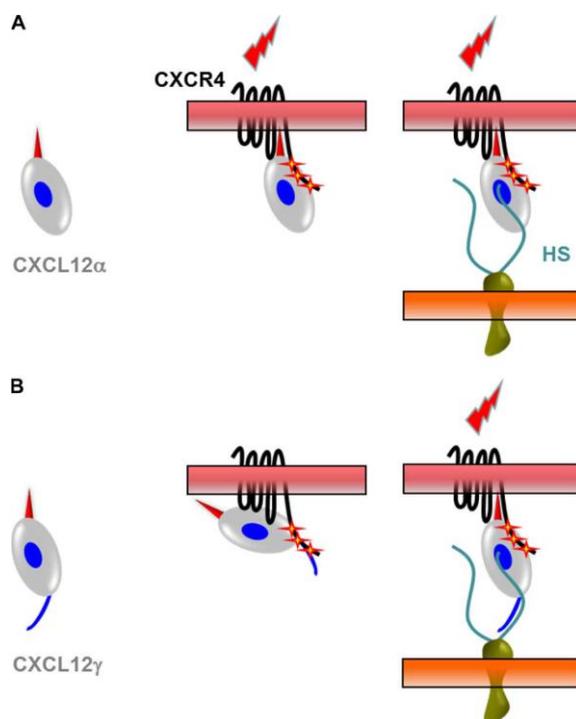


Figure 21. Schematic model of the HS-mediated CXCL12 α and CXCL12 γ presentation to CXCR4. **(A)** CXCL12 α interacts with HS (turquoise) through a small cluster (blue spot) of basic residues (K24/K27) that does not interfere with CXCR4 binding and activation (top). Therefore, both free and HS-bound CXCL12 α similarly stimulate CXCR4 signaling (jagged arrows). **(B)** For CXCL12 γ , in the absence of HS, the C-terminal extension (blue) that characterizes this isoform interacts with the sulfotyrosine-containing N-terminal region of CXCR4, which prevents the formation of a functional association between the chemokine and its receptor. HS (green), which interacts with both the K24/K27 cluster and the C terminus of the chemokine, repositions CXCL12 γ to promote a functional chemokine-receptor interaction and restore the signaling potential of this isoform.¹⁷⁴

Hence, HS binding sites being located at the distant *N*- and *C*-terminal residues on the protein surface, CXCL12 γ can bind to SAS motif within HS chains (Figure 22).

174. Connell, B. J.; Sadir, R.; Baleux, F.; Laguri, C.; Kleman, J.-P.; Luo, L.; Arenzana-Seisdedos, F.; Lortat-Jacob, H. *Sci. Signal.* **2016**, 9, ra107.

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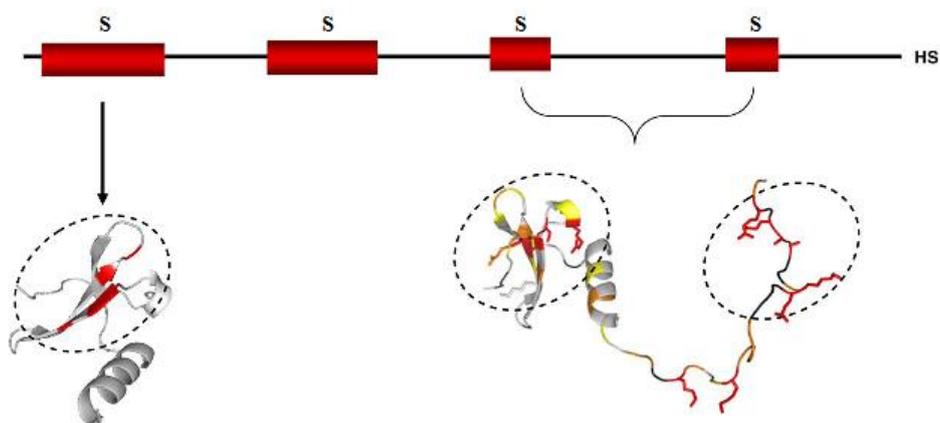


Figure 22. The monomeric structures of CXCL12 α (left) and γ (right) are shown. CXCL12 α (left) displays a single HS binding site and interacts with an S-domain, while CXCL12 γ (right) which features additional regions of interaction.¹⁷⁵

CXCL12 α also interact with HS chain that promote chemokine oligomerization. However, CXCL12 α does not need a SAS topology for efficient binding since, in its dimeric form, the HS binding site located at the protein-protein interface, on one face of the chemokine dimer (Figure 23).^{172,173,176}

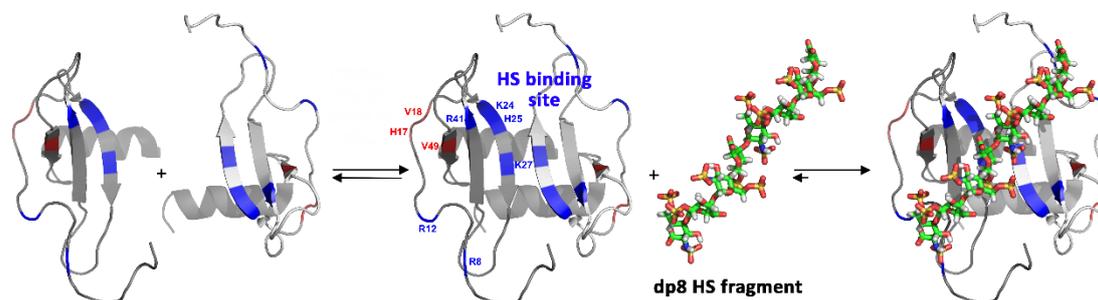


Figure 23. Dimerization of CXCL12 and HS binding site. Basic domains of the monomeric and dimeric form of CXCL12 constituting the binding domain of HS are represented in blue.

The structural models of the chemokine CXCL12 α in complex with heparin derived oligosaccharides indicated that short dp4 HS oligosaccharides should induce dimerization of the chemokine by interacting with the central HS binding domain (K24, H25, K27) while a longer HS oligosaccharide, dp12, extends away from the central binding site and permit the terminal sugar residues to interact with the N-terminal residues R8 and R12. However, in the maximum extended model, only oligosaccharides with dp16 or more would interact with the N-terminal residues (Figure 24).¹⁷³

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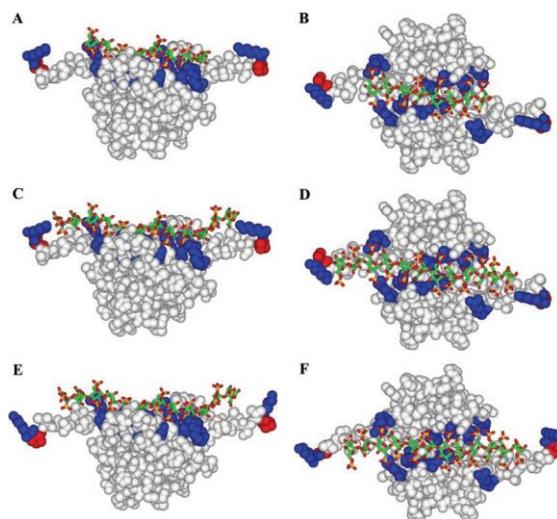


Figure 24. Orthogonal representations of the model for the complex of CXCL12 α with heparin derived oligosaccharides. Space-fill representation of the CXCL12 α dimer in complex with heparin-derived octasaccharide (A and B) or dodecasaccharide (C and D). A fully extended model of the chemokine in complex with a dodecasaccharide is also shown (E and F). The basic amino acids involved in the complex are shown in blue, the proline residue at position 2 is shown in red, and the heparin oligosaccharides are represented as sticks.¹⁷³

To date, only few synthetic heparin/HS oligosaccharides have been prepared to investigate their ability to interact with CXCL12 α .^{177–179} In 2014, three HS-like hexasaccharides were chemically synthesized by Kaundinya and coworkers, their following heparin-protein binding studies implied the significance of the 6-*O*-sulfation of the GlcN in high-affinity binding to CXCL12 α .¹⁷⁸ Consistently, Gardiner and coworkers demonstrated that the 6-*O*-sulfation of GlcN situated at the terminus of the synthetic heparin-like dodecasaccharide induced a switch between inhibition of CXCL8 and CXCL12 α .¹⁷⁹

Nevertheless, even if the design of HS fragments should allow the preparation of a high affinity ligand for CXCL12 and the preparation of SAS mimetics the possible selection of the γ -isoform, such compounds would not modulate the CXCR4/CXCL12 interaction but only CXCL12 interaction with HS of the extracellular matrix and cell surface. However, modulation of the CXCL12/CXCR4 should be of relevant importance for the treatment of aggressive cancers and metastatic invasive tumors: gradients of CXCL12 allow migration of cancer cells, their survival to chemotherapy

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and their proliferation, permit to certain metastases expressing CXCR4 to invade new tissues. For example, CXCL12 produced by ovarian epithelial tumors controls the antitumor response of the host, participates into the neovascularization of these tumors and is therefore involved in their invasive ability.^{180,181}

As previously mentioned, CXCR4 and HS have different binding sites with CXCL12.¹⁷¹ The CXCR4-binding determinant was thoroughly investigated by Volkman and coworkers. They demonstrated that the most functionally relevant mode of CXCL12-CXCR4 interaction involves specific recognition of sulfotyrosines (sTyr) at position 7, 12 and 21, and additional interactions with the receptor N-terminus that are independent of tyrosine sulfation. They also demonstrated the relative contribution to CXCL12 recognition of each tyrosine and revealed the highly important contribution of sTyr21.¹⁸² Using this observation, they identified a CXCL12 neutraligand family able to bind to the sTyr21 pocket on the chemokine through a structure-based drug design approach. Those compounds inhibit the CXCL12/CXCR4 interaction as well as activation of the receptor and the identified “hit” compound from the ZINC library, 3-(naphthalene-2-carbonylthiocarbamoylamino)-benzoic acid (ID 310454), exhibits a high affinity for the chemokine ($K_d = 64 \pm 15 \mu\text{M}$).¹⁸³

More interestingly, this sTyr21 pocket stays accessible in the CXCL12 dimer and, as for HS interaction, it appears that the binding of the sulfopeptide at the Tyr21 recognition site should be coupled to the chemokine dimerization, leading us to envisage the preparation of neoglycoconjugates able to specifically recognize CXCL12 monomer and dimer.

III. Presentation of The Project

The subject of this these is a part of a long-term project of our lab, synthesizing a library of HS fragments/SAS mimics to study the specificity of interactions between those glycoderivatives and cytokines. Based on previous obtained results of preparing

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SAS mimics (with various oligosaccharides and PEG-linker lengths) to modulate the activity of IFN- γ , we plan to design HS type ligands that could bind specifically to CXCL12 to block or modulate its interaction with its protein receptor (CXCR4) and therefore its implications in numerous pathological processes. We also would like to construct a second generation of SAS mimics to bind to IFN- γ .

III.1. Conception of Three Points Lock Neutraligands of CXCL12

Taking into account that HS and CXCR4 have different binding sites and the importance of the specific recognition of sTyr21 in CXCR4-CXCL12 interaction, the analysis of CXCL12 dimer/CXCR4 N-terminus complex containing the sTyr21 residue (pdb code 2K05) revealed that two large grooves connect the central HS-binding domain to the two Tyr21 pocket of each monomer (Figure 25).

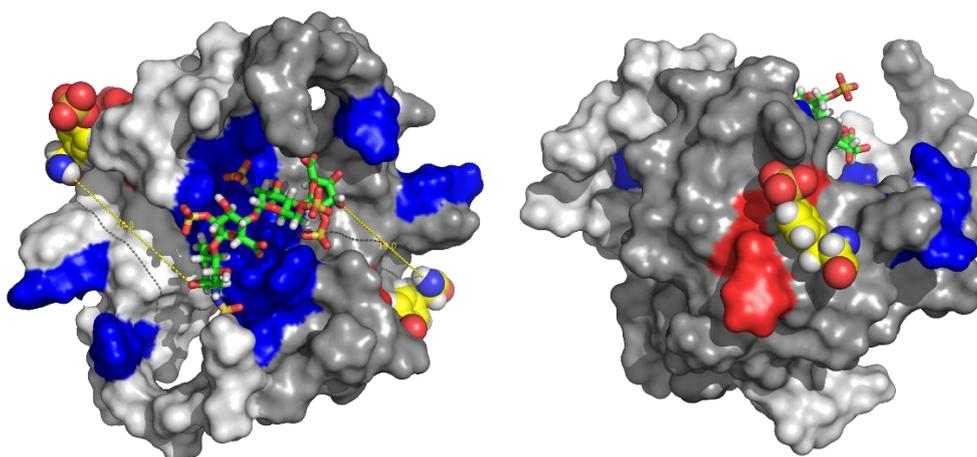


Figure 25. Superposition of a dp4 HS fragment onto the CXCL12 dimer/CXCR4 N-terminus complex. For a better visualization of the two grooves, only the sTyr21 residue of CXCR4 N-terminus are represented.

This observation allows us the design a symmetric tridentate ligand in which the reducing end and non-reducing end of a synthetic HS fragment (dp4) would be connected to a sTyr. In addition, a PEG-type spacer of about 20 Å should be suitable to interconnect the HS fragment to sTyr and allow the interaction of the three parts of the ligand with its specific binding site (Figure 26).

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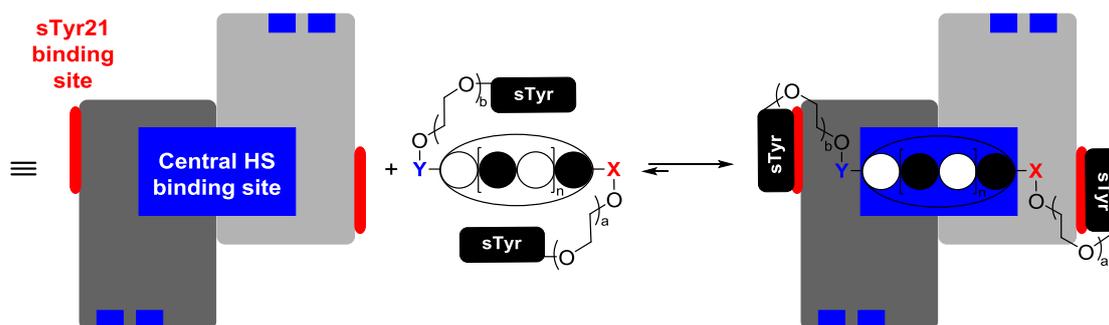


Figure 26. Schematic representation of the possible selective interaction between a tridentate neutraligand and CXCL12 dimer.

In order to synthesize such glycoconjugates, it is necessary to set up different and selective modifications of the reducing end and non-reducing end of HS fragments with two different functional groups allowing orthogonal reaction with two different partners and leading to the formation of two covalent linkages, X and Y. This strategy of orthogonal ligation is a very challenging objective that could be also used to prepare a second generation of SAS mimics.

III.2. Conception of Second Generation of SAS Mimics

Even if excellent results have been obtained concerning the interaction between SAS mimics and IFN- γ through optimizing the length of S and A domains, an intriguing question emerged: Is the influence of the C2-symmetric "head to head" presentation on the biological affinity of these SAS mimics?

To answer this question, it is necessary to design a general and efficient strategy that could promptly conduce to a library of SAS mimics presenting all three possible geometrics (Figure 27):

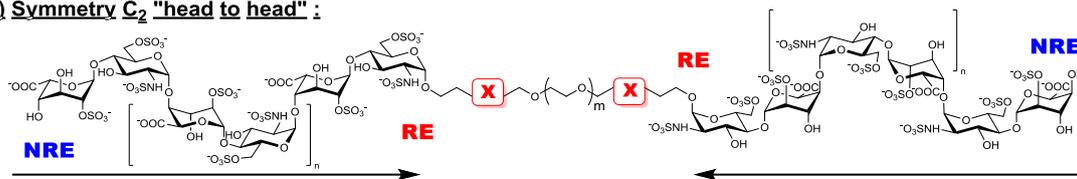
(a) The symmetric C2 'head to head' presentation, in which the two S domains are linked together by their reducing end (RE);

(b) The symmetric C2 'tail to tail' presentation, in which the two S domains are linked together by their non-reducing-end (NRE);

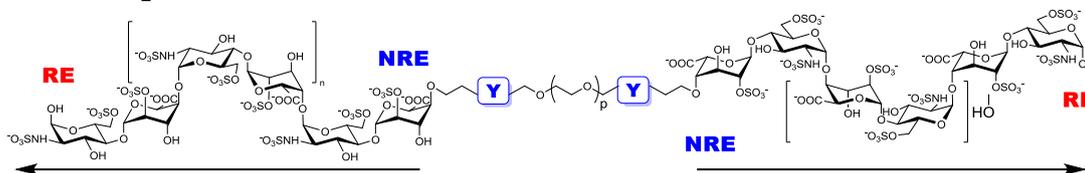
(c) The classical oligosaccharidic topology 'head to tail' presentation, in which the reducing end (RE) of one S domain is linked to the non-reducing end (NRE) of the second S domain.

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(a) Symmetry C_2 "head to head" :



(b) Symmetry C_2 "tail to tail" :



(c) Classic Topology "head to tail" :

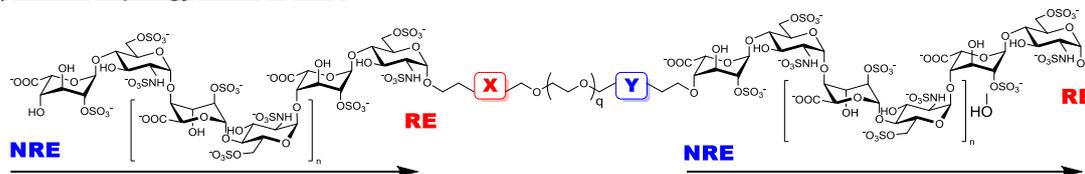


Figure 27. Representation of SAS mimics in three different geometries to study the interactions between HS and proteins. RE = reducing end, NRE = non-reducing end, X and Y are chemical motifs obtained by conjugation of HS fragments with PEG-type linker.

Thus, the preparation of these new synthetic SAS mimics would enlarge the scope of our strategy of functionalization of the two ends of HS fragments, demonstrate its robustness and could provide additional information for a better understanding of HS/IFN- γ interactions. Moreover, its extension to a general preparation of SAS mimics (bearing different S domains in length and sequence as well as different length of PEG-type linker miming A domain) should give access to a large library of SAS mimics to decipher HS/proteins interactions.

III.3. Retrosynthetic Analysis

As previously mentioned, in order to efficiently prepare CXCL12 tridentate neutral ligands and the three different geometries of SAS mimics, it is necessary to set up different and selective modifications of the reducing end and non-reducing end of HS fragments with two different functional groups Xa and Ya. In the meantime, the ends of PEG-type linkers might be modified with functional groups Xb and/or Yb. Significantly, the functional groups Xb and Yb could orthogonally react with Xa and Ya to form two covalent linkages, X and Y respectively. Thus, based on the selection of reactions and the functional groups contained in the PEG-type linkers, we could

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I. Preparation of HS Fragments

As mentioned in chapter 1 (Figure 28), the strategy applied to develop tridentate ligands and the three different geometries of HS/SAS mimics should be compatible with the synthetic approach previously developed in our lab for the preparation of HS fragments. That is to say that the selected modification reactions should be compatible with previously validated, in our group, set of orthogonal protecting groups (Ac, N₃, Bn, PMB, All, CO₂Me) that allowed, the synthesis of HS fragments exhibiting a well-defined sulfation pattern (Figure 29).¹³²

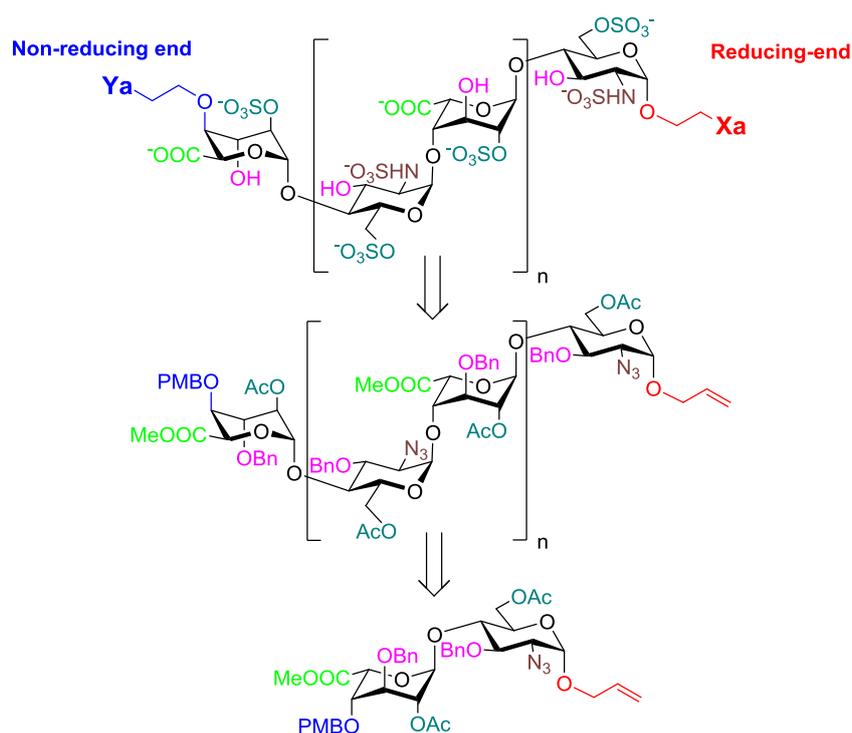


Figure 29. The strategy of protecting groups employed by our lab to prepare HS fragments.

Therefore, the selective removal of acetate groups under Zemplen conditions permit to liberate the positions where sulfate groups need to be installed, while benzyl groups (Bn) are used as permanent protection and will be removed by hydrogenolysis in the final step of the synthesis to give free hydroxyl groups. The anomeric center of

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the reducing unit and the C-4 hydroxyl group of the non-reducing unit are protected as an allyl ether (**All**) and a para-methoxybenzyl group (**PMB**), respectively. In addition to the fact that selective removal of these two protecting groups can give an easy entry into glycosyl donors and acceptors for the assembly of HS oligosaccharides, they should also permit or give access to selective conjugations with linker ends.

In general, there are two strategies to conjugate HS fragments to linkers, realizing the conjugation (i) onto the fully or partly protected HS fragments or (ii) onto the fully deprotected HS fragments. The latter strategy appears to be more efficient, not only because it avoids the validation of the stability of linkage with reaction conditions of deprotection steps, but also because it will permit the preparation of a variety of glycoconjugate/SAS mimics in much shorter time. Therefore, the selected functional groups X_a and Y_a must: (i) be introduced using reaction conditions compatible with orthogonal protecting groups, (ii) be stable under deprotection conditions and (iii) permit the realization of conjugation reactions on fully deprotected HS fragments, *i.e.* must be operated in aqueous media.

II. Click reactions In Aqueous Media

II.1. General Concept of Click Chemistry

The concept of ‘Click Chemistry’ has been introduced by Sharpless and coworkers in 2001 and is undeniably one of the most noticeable synthetic trends in contemporary chemistry.^{184,185} Click chemistry was defined as a set of reactions allowing the reliable and efficient formation of complex substances starting from simple precursors in high yield and with high purity. Connection between molecular blocks is made by the formation of energetically favorable carbon-heteroatom bond. To be considered as part of the Click Chemistry panel, a reaction must fulfill several requirements:

- (a) give very high yields
- (b) be modular and wide in scope of starting compounds
- (c) be regiospecific and/or stereospecific (but not necessarily enantioselective)
- (d) give by-products (if any) that are inoffensive and could be separated by non-

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chromatographic processes (such as crystallization or distillation)

(e) have simple and mild reaction conditions (ideally, the process should be insensitive to oxygen and water)

(f) be highly atom-economical

(g) be solventless or use easily removed solvent (preferably water)

(h) give stable products under physiological conditions

All those criteria cannot be really measured and unfortunately it is difficult to find a chemical reaction that will fit all of them. Nonetheless, some type of chemical transformations meet parts of the desired standards and constitute the current panel of Click Chemistry reactions. Concurrently, click reactions can be classified into four major categories: a) cycloadditions of unsaturated species, such as copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC)¹⁸⁶ and several Diels-Alder cycloaddition (Scheme 1a),^{187,188} b) nucleophilic ring-opening of strained heterocyclics, such as epoxides, aziridines, aziridiniumions and episulfoniumions (Scheme 1b),¹⁸⁴ c) carbonyl chemistry of non-adol type transformation, such as formation of oxime ethers,¹⁸⁹ hydrazones,¹⁹⁰ amides, ureas, thioureas (Scheme 1c), d) addition reactions to carbon-carbon multiple bonds, such as epoxidation,¹⁹¹ aziridination,¹⁹¹ thiol-ene reaction and certain Michael-type conjugate additions (Scheme 1d).¹⁹³

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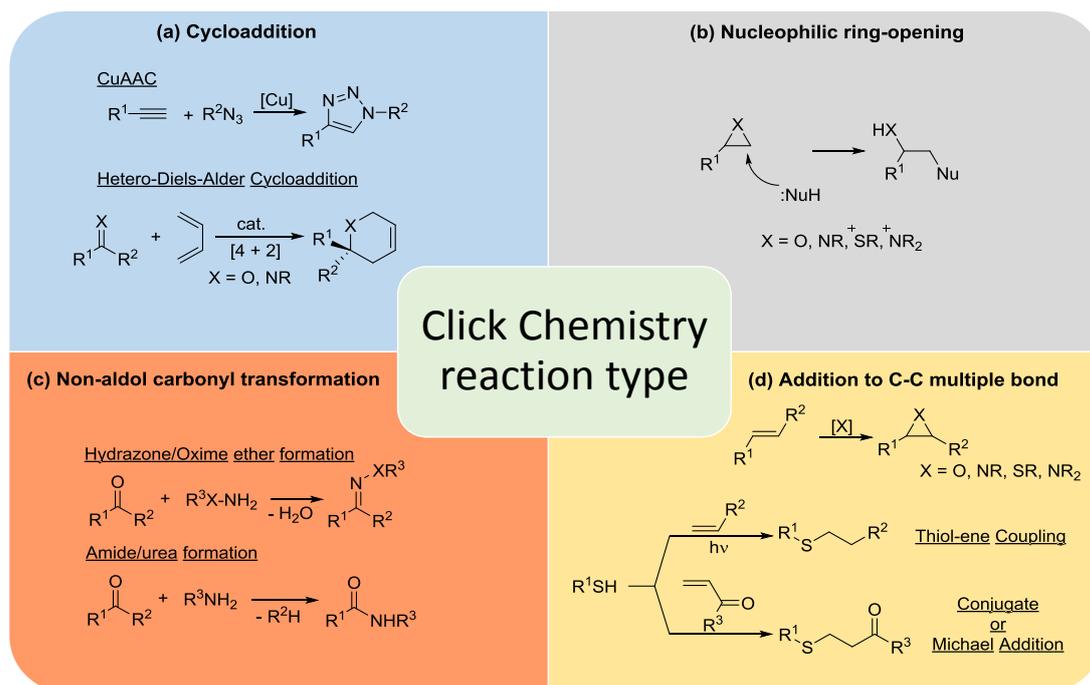
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Scheme 1. Current classification of the click chemistry reactions panel.

In view of properties of click reactions and requirements of conjugation reactions needed for our project, we decided to select our ligation reactions into this panel. Firstly, we excluded hetero-Diels Alder reactions not only due to difficulty in introduction of the two insaturated partners to complex molecules but also because of the potential reduction of the needed insaturation during the hydrogenolysis step. Moreover, the use of epoxide is also incompatible with our synthetic approach: Zemplen conditions for acetate removal as well as saponification of methyl uronic esters should lead to epoxide opening. Thus, we selected CuAAC, non-aldol type carbonyl transformations and thiol-ene coupling as potential click reaction for the selective modification of the reducing and non-reducing ends of HS fragments.

II.2. Copper Catalyzed Azide-Alkyne Cycloadditions (CuAAC)

The formation of 1,2,3-triazoles by 1,3-dipolar cycloaddition of azides and alkynes was initially discovered by Arthur Michael at the end of 19th century¹⁹⁴ and significantly developed by Rolf Huisgen in the 1960s.^{195,196} However, the traditional

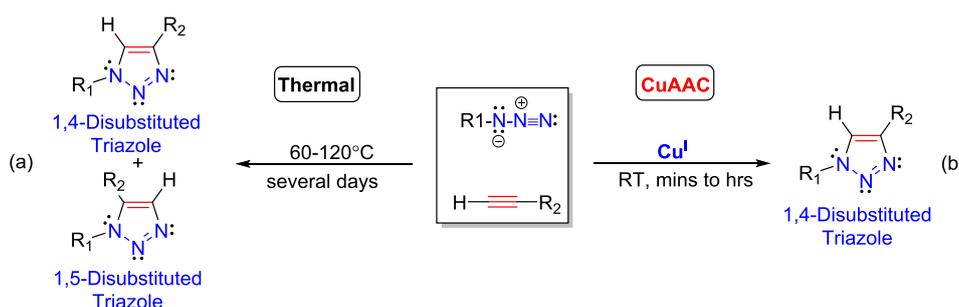
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thermally promoted [3+2]-cycloaddition suffers from several major disadvantages: (i) high reaction temperatures, (ii) slow rate of reaction and (iii) poor regioselectivity (Scheme 2a). In 2002, the groups of Sharpless¹⁸⁶ and Meldal¹⁹⁷ independently reported their pioneering studies on copper-catalyzed azide-alkyne cycloaddition, providing a mild, fast and highly efficient synthesis of 1,4-disubstituted 1,2,3-triazoles at 10⁷ times faster rate than thermal reaction (Scheme 2b). Owing to its compatibility with aqueous conditions and biological media, wide scope of reactants, good tolerance to steric hindrance, excellent orthogonality to usual functional groups and complete regioselectivity, CuAAC has evolved into an indispensable tool with applications extending to organic synthesis,^{198,199} biotechnology,^{200,201} polymer and material science,^{202,203} for example. The constant increasing number of publications on the use and application of CuAAC demonstrate that it is a true example of efficient and versatile ‘Click Chemistry’ reaction.^{204–206}



Scheme 2. Thermal (a) & Copper(I)-catalyzed (b) conditions of azide-alkyne cycloaddition reaction.

To date, the azide and alkyne groups have secured a prominent role as unique chemical tools for bioconjugation. Indeed, it is a result of (i) their small size, and absence from generic organisms, (ii) their inertness to most biological and common organic reaction conditions, including highly functionalized biological molecules,

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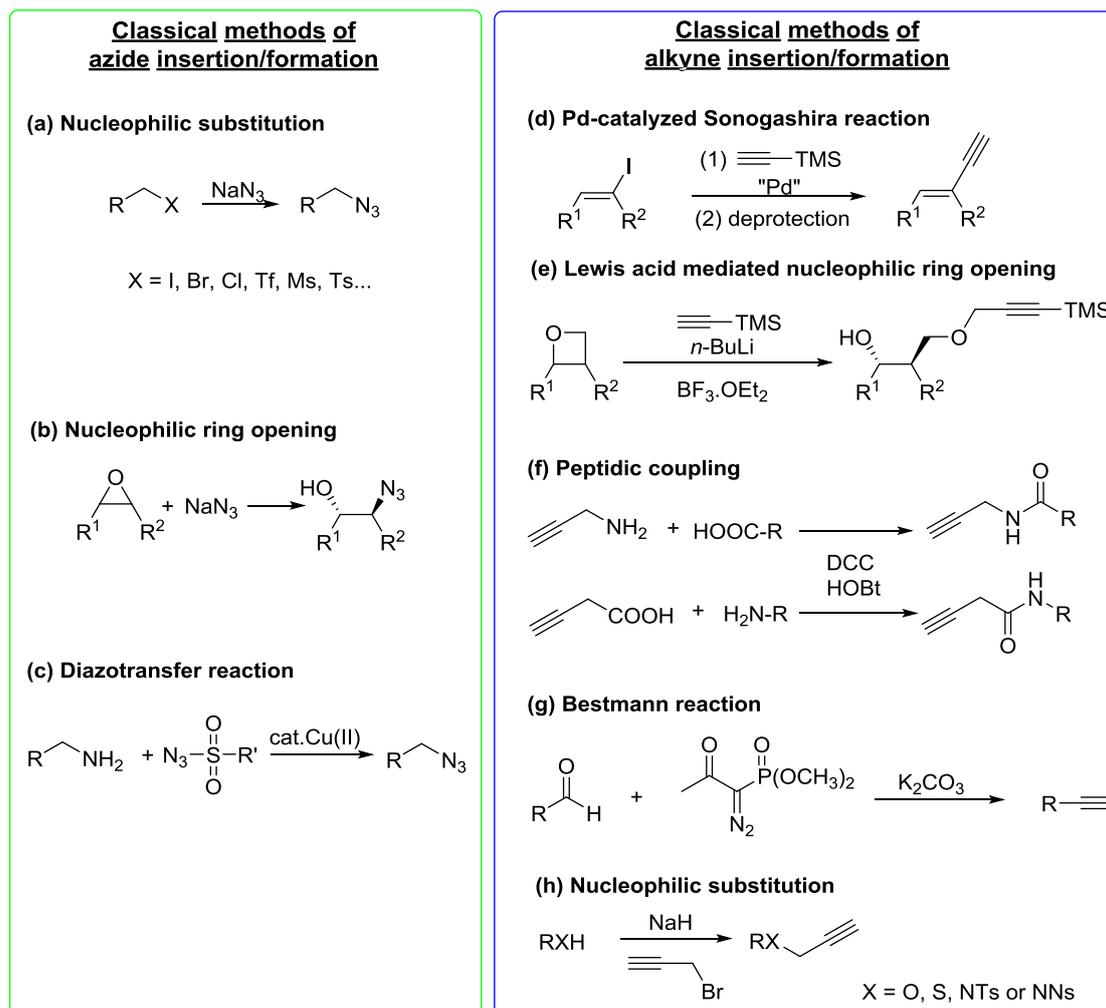
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molecular oxygen and water.

In most cases, the two functionalities can be installed conveniently and remain unaffected through a number of subsequent transformations. In general, an azide group can be easily introduced by nucleophilic substitution of a leaving group (Scheme 3a) or strained ring opening (Scheme 3b) with an azide anion or by Cu(II)-catalyzed conversion of an amine group into an azide one using a diazotransfer reagent (Scheme 3c). A variety of methods can be applied to incorporate a terminal alkyne functionality into organic scaffolds, including Pd-catalyzed Sonogashira coupling between a halogenated arene or alkene and a (trimethylsilyl)acetylene (Scheme 3d), Lewis acid-mediated nucleophilic opening of a strained ring by (trimethylsilyl)acetylene (Scheme 3e), amide bond formation between propargylamine and carboxylic acids (Scheme 3f), Bestmann reaction (Scheme 3g) as well as nucleophilic substitution (Scheme 3h).²⁰⁷ The latter strategy is probably the most exceedingly investigated one and allows propargylation of alcohol, sulfonamidic amine or thiol under basic Williamson conditions. Therefore, this modification can be easily applied to modify PEG-type spacers. In other words, it means that an azide group has to be introduced onto HS fragments.

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Scheme 3. General methods for the introduction of an azide functional group (a, b, c) and a terminal alkyne (d, e, f, g, h).

II.3. Strain-promoted Azide-Alkyne Cycloaddition (SPAAC)

While CuAAC is often seen as the quintessential click reaction, the cytotoxicity of the required Cu-catalyst precludes its *in vivo* applications.^{208–210} To address this issue, in 2004, Bertozzi and coworkers developed a copper-free and strain-promoted azide-alkyne [3+2] cycloaddition (SPAAC) with enhanced biocompatibility but with reduced reaction kinetics and yielding isomeric mixtures (Scheme 4).²¹¹ The development of this reaction was inspired by the pioneering work of Wittig and Krebs in the 60s, who described the exothermic cycloaddition of cyclooctyne with phenyl azide leading to the

208. Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13–21.

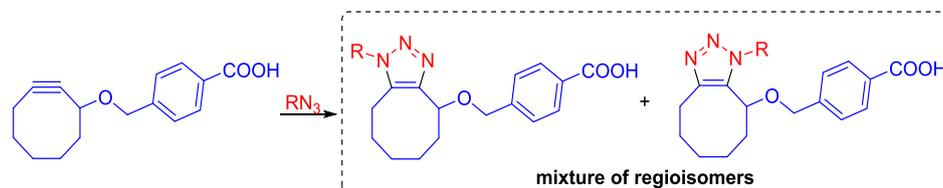
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corresponding triazole in good yields.²¹² The heightened reactivity of cyclooctynes, compared to linear alkynes, was attributed to ring strain released and close geometry to formed triazole, leading to the reaction to spontaneously occur at ambient temperature and pressure without the need for any catalyst.



Scheme 4. Strain-promoted azide-alkyne cycloaddition (SPAAC).

Since the first example of SPAAC, significant efforts have been focused on design and syntheses of various cycloalkyne analogues, aiming to enhance the relatively slow reaction rate constant of plain cyclooctyne (Figure 30).^{213,214,223–229,215–222} The activation of reactivity can be achieved in three ways: (i) through electronic activation, for example, by placing geminal fluorine atoms at the propargylic position (DIFO),^{213,216} (ii) *via* increase of the strain energy through fused aryl ring (DIBO,²¹⁵ COMBO),²²⁷

213. Lo, A.; Baskin, J. M.; Miller, I. A.; Agard, N. J.; Prescher, J. A.; Bertozzi, C. R.; Laughlin, S. T.; Chang, P. V.; Codelli, J. A. *Proc. Natl. Acad. Sci.* **2007**, *104*, 16793–16797.
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cyclopropyl ring (BCN)²²³ or through reducing ring size (TMTH);²²⁵ (iii) by combination of the two previous methods (DIFBO).²²² TMTH is the fastest so far reported cycloalkyne with a rate constant of $4.0 \text{ M}^{-1}\text{s}^{-1}$. However, the introduction of anchor points into TMTH turned the resulting molecules either unreactive or unstable. Moreover, those cyclooctynes exhibit low water solubility. Therefore, Bertozzi and coworkers developed a more hydrophilic cyclooctyne DIMAC, in which a nitrogen atom was introduced into the ring to break its hydrophobic character and two additional methoxy groups were introduced to enhance polarity and water solubility of the molecule.²¹⁴ It has a rate constant of $0.003 \text{ M}^{-1}\text{s}^{-1}$, slightly faster than OCT, but significantly lower than DIFO. Adopting a similar approach, DIBAC^{218,219} and BABAC^{217,224} were synthesized and have a high rate constant of $0.310 \text{ M}^{-1}\text{s}^{-1}$ and $0.960 \text{ M}^{-1}\text{s}^{-1}$, respectively.

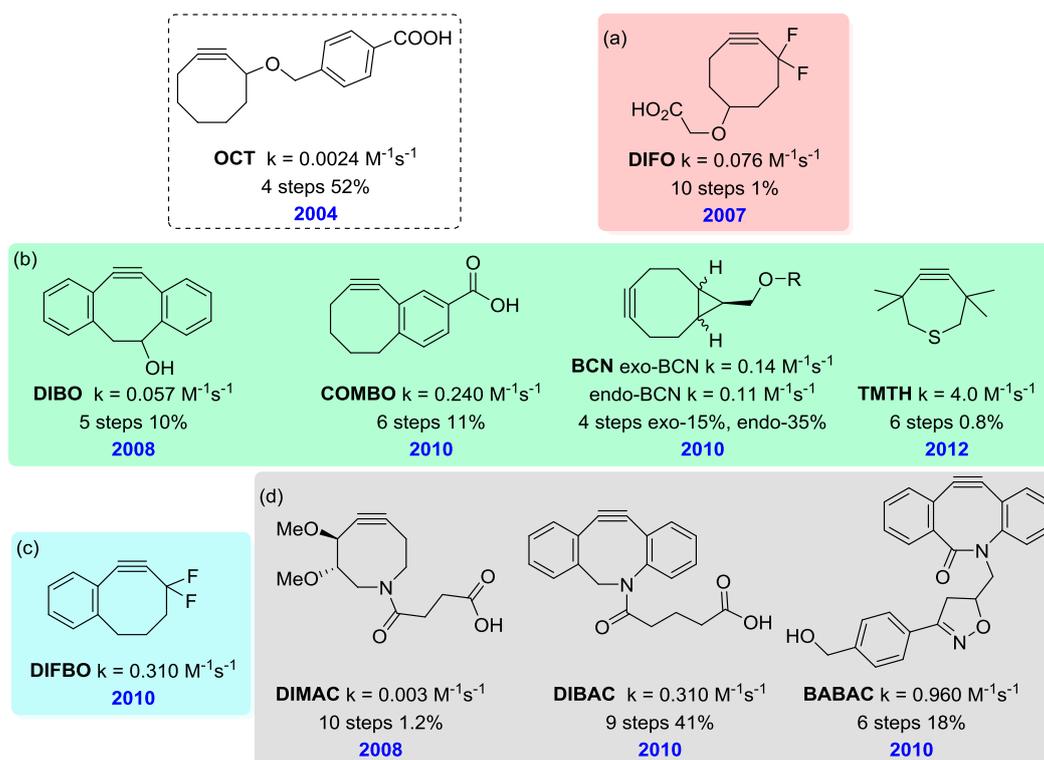


Figure 30. Cycloalkynes developed for SPAAC and associated second-order rate constants determined in AcCN or MeOH. Cyclooctynes activated by (a) electronic factors; (b) structural modifications; (c) a combination of the two methods; (d) Cyclooctynes with enhanced water solubility.

Although SPAAC has been initially designed for biological applications, it has found a wide variety of applications in material sciences, surface functionalization, bioconjugation, and chemical biology. Nevertheless, great challenges remain in SPAAC

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approach, especially in the design of appropriate cyclooctyne derivatives to improve their current:

- (a) instability (air-sensitivity,²¹⁷ possible rearrangement²²⁸ and polymerization²²²);
- (b) large size and hydrophobicity, which could affect their distribution and modify the biological properties of the species to which they are attached;
- (c) limited commercial availability and typically long synthetic preparation;
- (d) reactivity with naturally occurring thiols, which might be the source of complication during *in vivo* experiments;^{221,226,229}
- (f) poor regioselectivity of the reaction, producing a mixture of regioisomers;
- (g) slow rate of reaction (the fastest one still reacts more than tenfold slower than terminal alkynes in CuAAC ($k_{\text{obs}} \approx 4 \text{ M}^{-1}\text{s}^{-1}$ for TMTH compared to $k_{\text{obs}} \approx 10\text{-}100 \text{ M}^{-1}\text{s}^{-1}$ per $10\text{-}100 \text{ }\mu\text{M}$ $\text{Cu}^{\text{I}}/\text{Cu}^{\text{II}}$ for CuAAC).

All these factors hampered the scope and application of this variant of azide-alkyne click reaction, in comparison to the copper-catalyzed version.

II.4. Thiol-ene Coupling

The century-old radical-mediated addition of thiols to alkenes, termed as thio1-ene coupling (TEC), emerged as a useful alternative click reaction to CuAAC (Scheme 5).¹⁹³ This reaction is highly efficient, orthogonal to a wide range of functional groups and compatible with water. In addition, the thioether linkage is stable in a wide range of chemical environments such as acid and basic media as well as reducing conditions. Over the years, TEC has been extensively exploited in polymer chemistry, materials synthesis and as a synthetic tool in chemical biology studies.^{230–234}



Scheme 5. Thiol-ene coupling with anti-Markovnikov regioselectivity.

The reaction of thiols with enes can proceed through two different mechanisms (Scheme 6)²³⁴:

230. Hoyle, C. E.; Lee, T. Y.; Roper, T. *J. Polym. Sci. Part A Polym. Chem.* **2004**, *42*, 5301–5338.

231. Killops, K. L.; Campos, L. M.; Hawker, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 5062–5064.

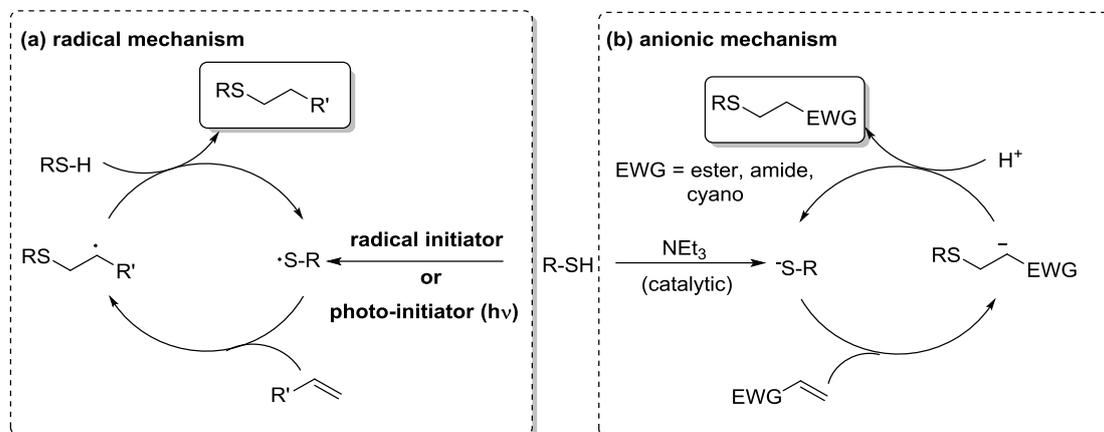
232. Hoyle, C. E.; Lowe, A. B.; Bowman, C. N. *Chem. Soc. Rev.* **2010**, *39*, 1355–1387.

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- (a) a photochemically/thermally induced free-radical addition
- (b) an anionic catalyzed Michael-type addition of thiols to electron-deficient conjugated enes

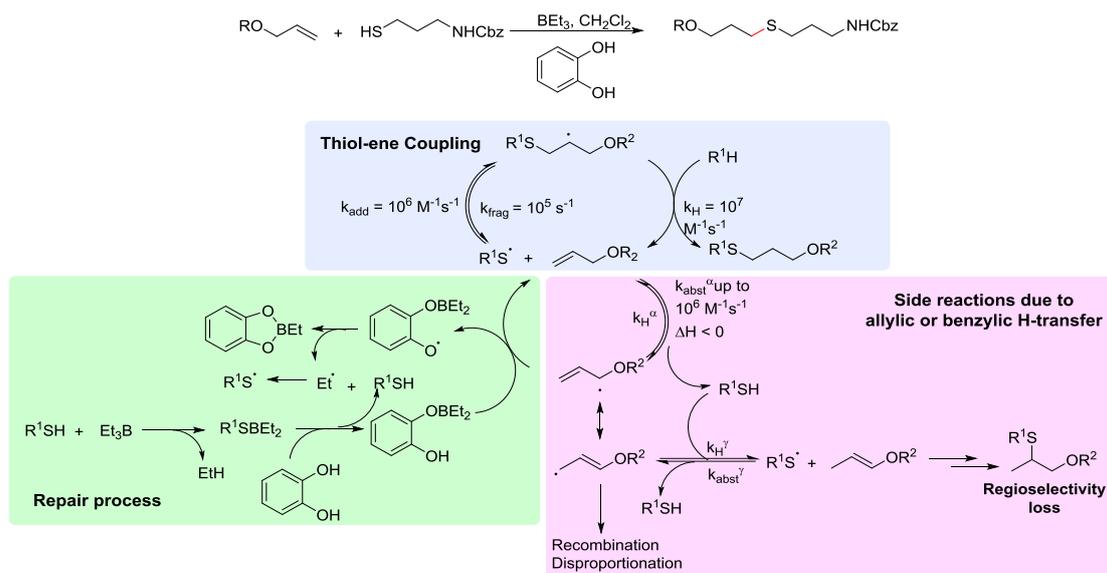


Scheme 6. The two thiol-ene coupling methods to form thioethers: (a) free-radical and (b) Michael addition reactions.

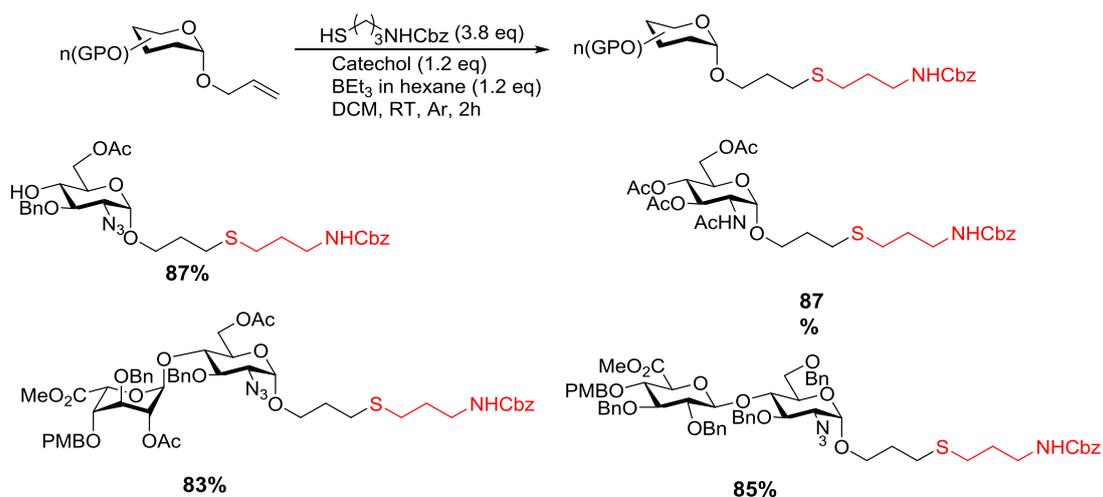
However, it is worth noting that TEC suffers from a major drawback, the incompatibility with several functional groups. For example, allylic, benzylic and propargylic position could react with the radical formed, leading to formation of side-products if the TEC is not well controlled.²³⁵ Therefore, our group, in collaboration with the one of Phillippe Renaud, recently reported a highly efficient TEC utilizing Et₃B as initiator and catechol as a co-reagent, avoiding radical chain to be terminated by allylic or benzylic radical formation and recombination or disproportionation, thanks to a repair process mediated by catechol borane formation (Scheme 7).

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As demonstrated by our group, this efficient version of radical TEC reaction allows an easy incorporation of benzyl 3-mercaptopropylcarbamate chain onto the reducing end of protected HS fragments: Indeed, the reaction of the anomeric allyl moiety of diverse saccharides allows us to introduce a protected amine onto the reducing end (Scheme 8). Interestingly, the Cbz group can be selectively removed in presence of benzyl groups if needed.²³⁶ In our case, this strategy followed by diazotransfer reaction will permit us to attain an azide group onto the reducing end of HS fragments (In chapter 3).

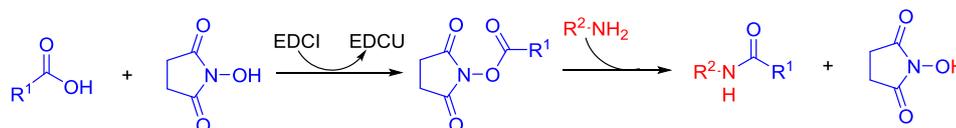


236. Liu, W. Université Paris-Sud, 2015.

II.5. Carbonyl Ligations

II.5.1. Formation of amide bond

Amide bonds are not only limited to peptides and biological systems, but also present in a huge array of molecules, including several marketed drugs.^{237,238} As carboxylic acids and amines cannot react spontaneously,²³⁹ several strategies for amide bond formation have been developed in recent years.^{240,241} By far, the most widely used method is the formation of activated carboxylic acid. The carboxylic acid is dehydrated by a carbodiimide reagent, EDCI²⁴² or DCC²⁴³ for example, in presence of an additive such as N-hydroxysuccinimide (NHS)^{244,245} or pentafluorophenol,²⁴⁶ followed by the addition of the amine partner in a one-pot or stepwise process (Scheme 9).^{247,248} This method is now popular in peptide chemistry, lactam formation, bioconjugate chemistry as well as fluorophore dyes tagging using N-hydroxysuccinimidyl activated esters (Figure 31).^{249–252}



Scheme 9. Formation of amide with NHS-activated esters.

237. Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Comb. Chem.* **1999**, *1*, 55–68.
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The activated ester could not only selectively react with primary amines in presence of alcohols, but also permit the reaction to proceed in aqueous solutions if the pH is well controlled (avoiding the hydrolysis of the corresponding activated ester). In our case, considering the carboxylic acid groups in HS fragments, only the PEG-type linkers could be converted into an activated ester. In the meantime, a free primary amine would be present onto the HS fragments after introduction of the benzyl 3-mercaptopropylcarbamate chain at the reducing end of HS fragments through thiol-ene coupling. Thus, amide bond formation should represent an alternative ligation strategy to CuAAC.

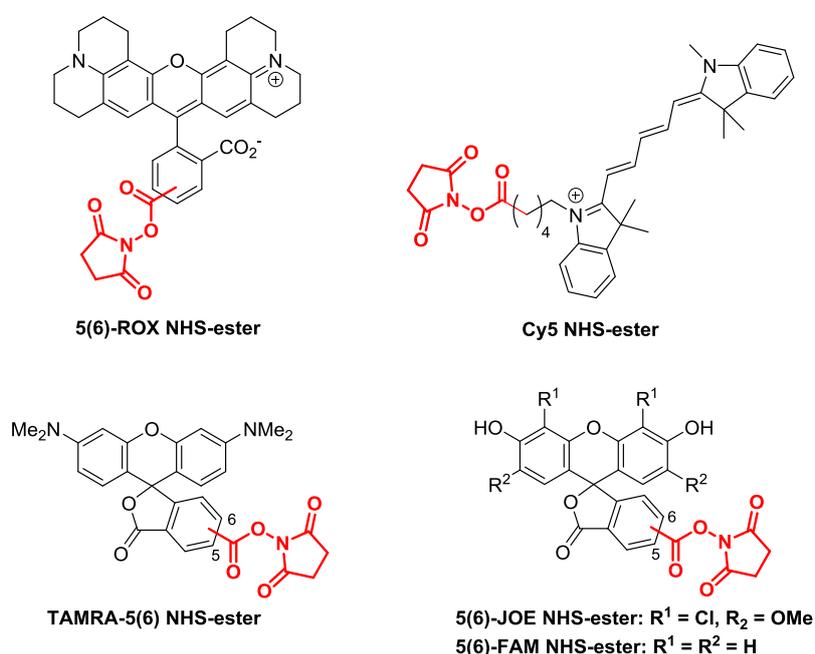


Figure 31. Examples of commercially available NHS-ester fluorophore dyes.

II.5.2. Hydrazone and oxime formation

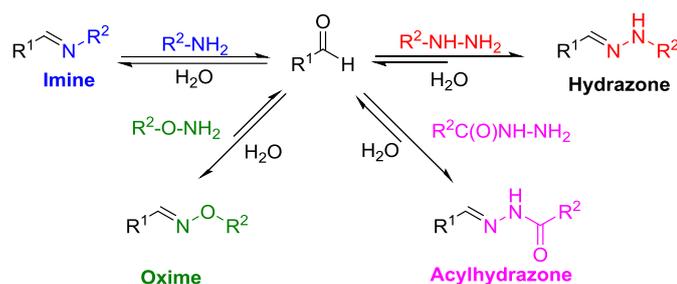
The condensation of a primary amine, aminoxy group or hydrazine with a carbonyl group leads to the formation of a Schiff base, oxime ether or hydrazone respectively and provides a powerful and simple ligation method (Scheme 10).^{253–255}

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Scheme 10. Imine, hydrazone and oxime formation. E and Z isomers may be present depending on the nature of substituents and reaction conditions.

However, their hydrolytic instability strongly limits their utilization for biological application. On one hand, the imine is highly sensible to acidic conditions and the equilibrium favors the free carbonyl compound. On the other hand, hydrazones and oximes are more stable in aqueous media.²⁵⁶ Their improved stability stems from electron delocalization of the lone pairs of the nitrogen or oxygen heteroatom adjacent to the sp^2 nitrogen one, termed as α -effect (contribution of resonance forms **II** and **IV**, Figure 32).^{257–259} Additionally, attractive inductive effects also reduce the basicity of the sp^2 nitrogen atom, slowing down the rate of acid-catalyzed hydrolysis. However, oximes display substantially higher hydrolytic stability than hydrazones: the equilibrium constant K_{eq} , the ratio between the rate constant of formation k^1 and the rate constant of hydrolysis k^{-1} , is typically in the range of 10^4 - 10^6 M^{-1} for hydrazone linkages and greater than 10^8 M^{-1} for oxime bonds.²⁶⁰ Hence, oxime ligation has emerged as a powerful tool for the chemoselective conjugation of polysaccharides, which can be performed in an aqueous medium, under mild acidic conditions.

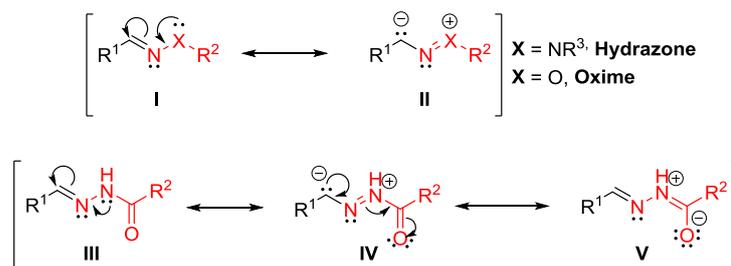


Figure 32. Major resonance forms of conjugates and the α -effect.²⁶⁰

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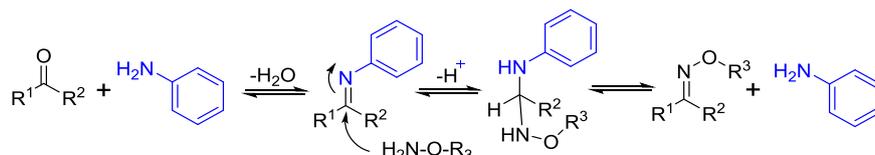
258. Buncel, E.; Um, I.-H. *Tetrahedron* **2004**, *60*, 7801–7825.

259. Ren, Y.; Yamataka, H. *Chem. - An Asian J.* **2007**, *13*, 677–682.

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The oxime/hydrazone ligation become even more popular due to the discovery by Dawson and coworkers of its nucleophilic aniline catalysis.^{261–266} This organocatalytic process proceeds through a highly reactive protonated aniline Schiff base, followed by transamination, leading to the desired oxime bond (Scheme 11). To improve biocompatibility and increase reaction rate at neutral pH, substituted anilines such as 5-methoxyanthranilic, 3,5-diaminobenzoic²⁶⁷ and 2-aminobenzenephosphonic acid²⁶⁸ have been used to accelerate the oxime formation.



Scheme 11. Mechanism of nucleophilic catalysis by aniline in oxime ligation.²⁶⁹

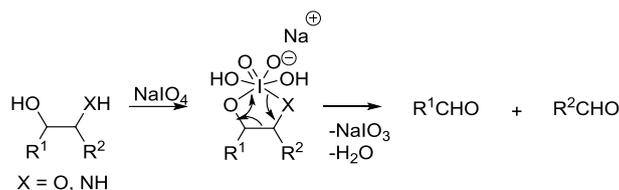
Although aniline is known to ‘catalyze’ the oxime formation reaction, this strategy suffers from several drawbacks: (i) the high concentrations of catalyst needed to complete the reaction over reasonable time periods (for a concentration of 10 μ M in aldehyde or/and hydrazine, up to 100 mM of aniline, *i.e.* 10000 equivalents, is required for the hydrazone formation);^{263–266} (ii) the toxicity of aniline to cells.²⁷⁰

II.5.3. Introduction of carbonyl group

As mentioned above, aldehydes, ketones and carboxylic acids are very attractive chemical patterns for modifying biomolecules due to their synthetic accessibility and small size, despite the fact that they are present in numerous secondary metabolites. Several strategies have been developed for the functionalization of biomolecules with carbonyl groups.²⁶⁹ Among them, the most well-known and widely used is the oxidative cleavage of vicinal diols with sodium periodate (Scheme 12).²⁷¹

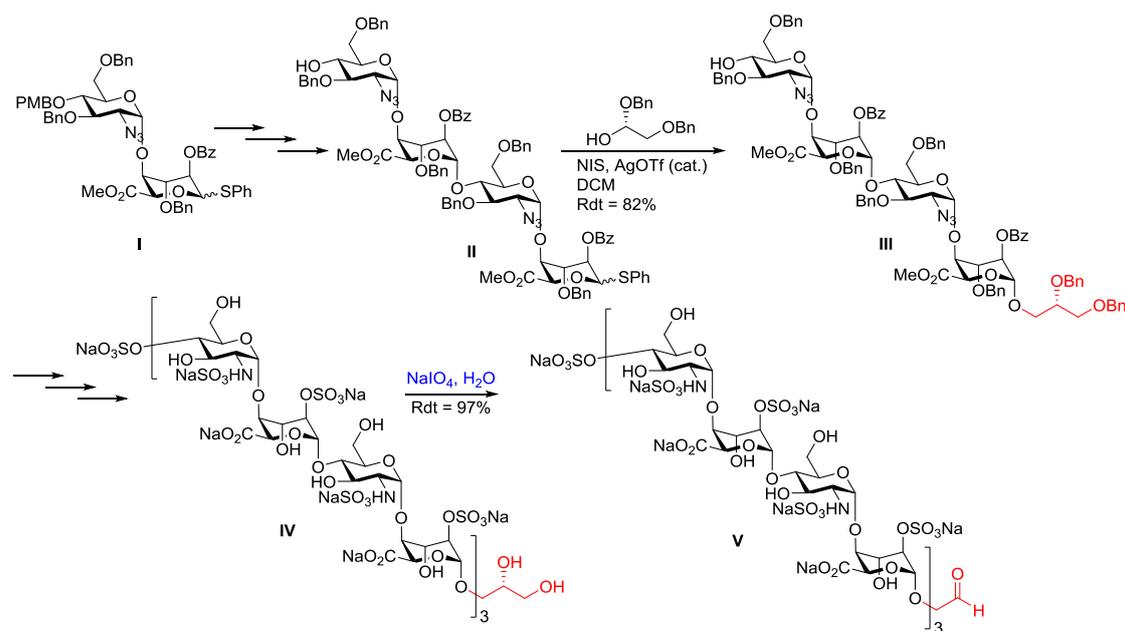
261. Cordes, E. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1962**, *84*, 826–831.
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Scheme 12. Sodium periodate-mediated oxidative cleavage of vicinal diols or 1,2-amino alcohols.

It is important to note that, in 2013, Gardiner and coworkers reported a first example of application of vicinal diol oxidative cleavage for labeling the reducing-end of HS fragments.²⁷² Glycosylation of (S)-2,3-bis(benzyloxy)propanol with the tetrasaccharidic thioglycoside II, using the NIS/AgOTf system as promoter, allowed the introduction of a vicinal diol as a latent group. After hydrogenolysis of benzyl ether, the 1,2-diol was liberated to furnish the unique reactive motif for the final sodium periodate-mediated oxidative cleavage (Scheme 13).



Scheme 13. Insertion and utilization of 1,2-diol as precursor of aldehyde at HS fragment's reducing-end.

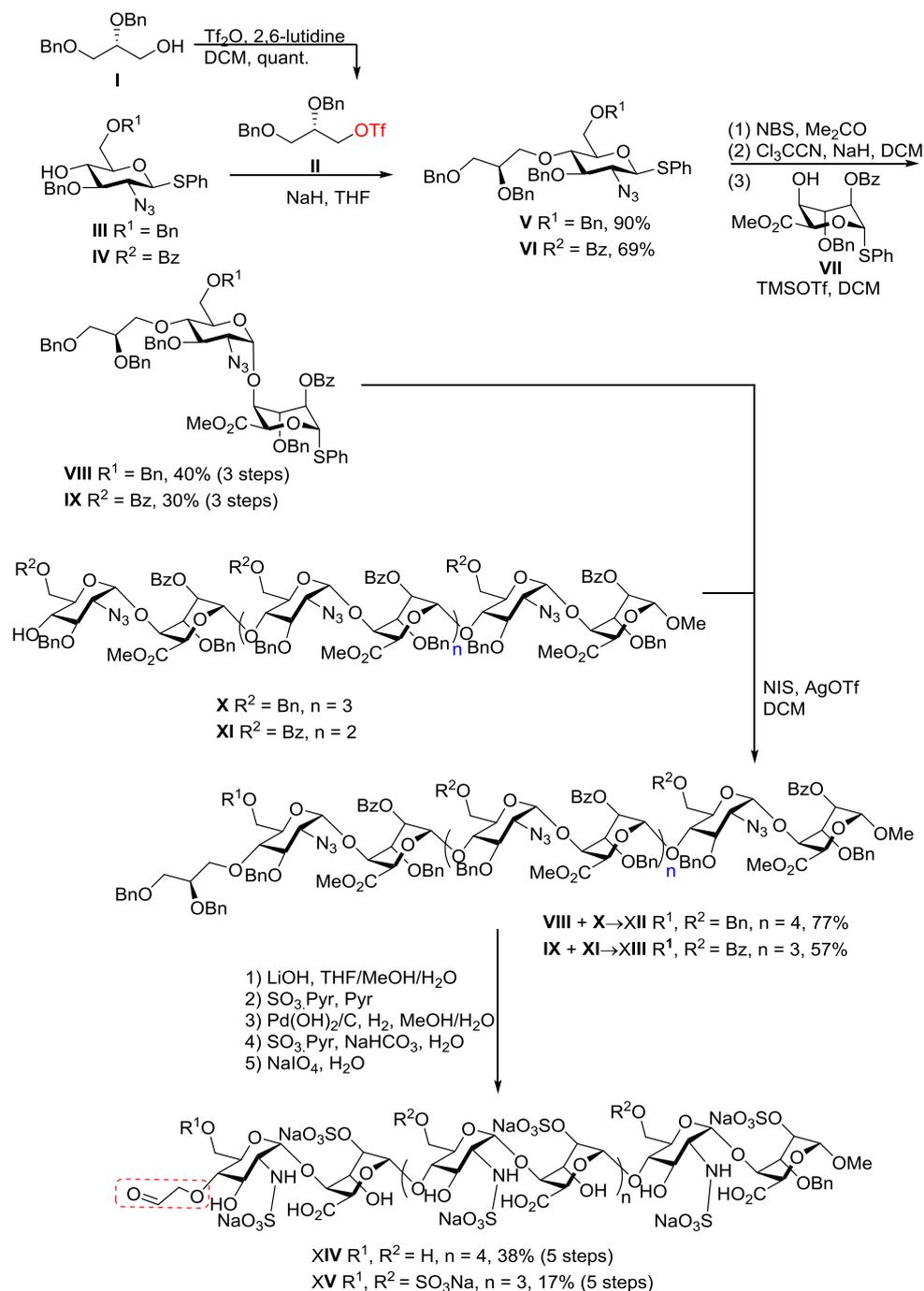
Moreover, in 2015, they extended this tagging strategy to the modification of the non-reducing end of HS fragments.²⁷³ This time, the vicinal diol was introduced as a latent motif by modifying a disaccharidic building block used for the chain elongation at the non-reducing end (Scheme 14). The main drawback of such strategy is either the

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preparation of specific building blocks or the recruitment of protective groups for the latent motif that have to be compatible with all reaction conditions (glycosylation, deprotection & sulfation). Nevertheless, those results demonstrate the compatibility of the oxidative cleavage reaction conditions with synthesis of HS fragments, convincing us to introduce an aldehyde group into HS fragments for conjugation reaction.



Scheme 14. Insertion and utilization of 1,2-diol as precursor of aldehyde at HS fragment's non-reducing end.

III. Orthogonal Reactions Applied to Modifications of HS/PEG Spacer

In aforementioned click reactions, Sharpless described that ‘although click components are necessarily highly reactive, the reactions are orthogonal to an unusually broad range of reagent, solvents and other functional groups’. For this reason, CuAAC and oxime ligation were chosen as orthogonal conjugation reactions for our project: an azide and an alkyne cannot react with either an aldehyde or an aminoxy in physiological or aqueous media. By bifunctionalizing the HS fragment with an azide and an aldehyde and other partners with a propargyl and/or an aminoxy group, the two ligation reactions could be sequentially and selectively performed (Figure 33).

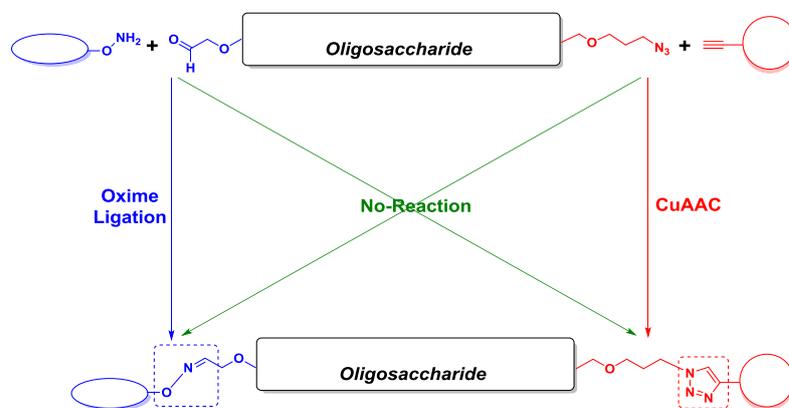


Figure 33. Orthogonality of CuAAC and oxime ligation reactions for the controlled HS fragments modification/conjugation.

III.1. Modifications of HS Oligosaccharides

Taking into account required modifications, we decided to introduce an azide group at the reducing-end of HS oligosaccharides for the application of CuAAC as the first ligation reaction. The azide group will be obtained *via* a diazotransfer reaction onto an amine group, the latter being introduced through thiol-ene coupling between the allyl moiety of the reducing-end unit of HS fragments and benzyl 3-mercaptopropylcarbamate chain.

In parallel, we have to introduce an aldehyde to the non-reducing end of HS fragments, that motif can be implemented through the oxidative cleavage of 1,2-diol at *O*-4 position of the terminal uronic acid unit. This vicinal diol will come from the dihydroxylation of an allyl ether, which will be introduced by a Tsuji-Trost

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decarboxylative allylation^{274–276} on the free hydroxyl group at *O*-4 position (Figure 34).

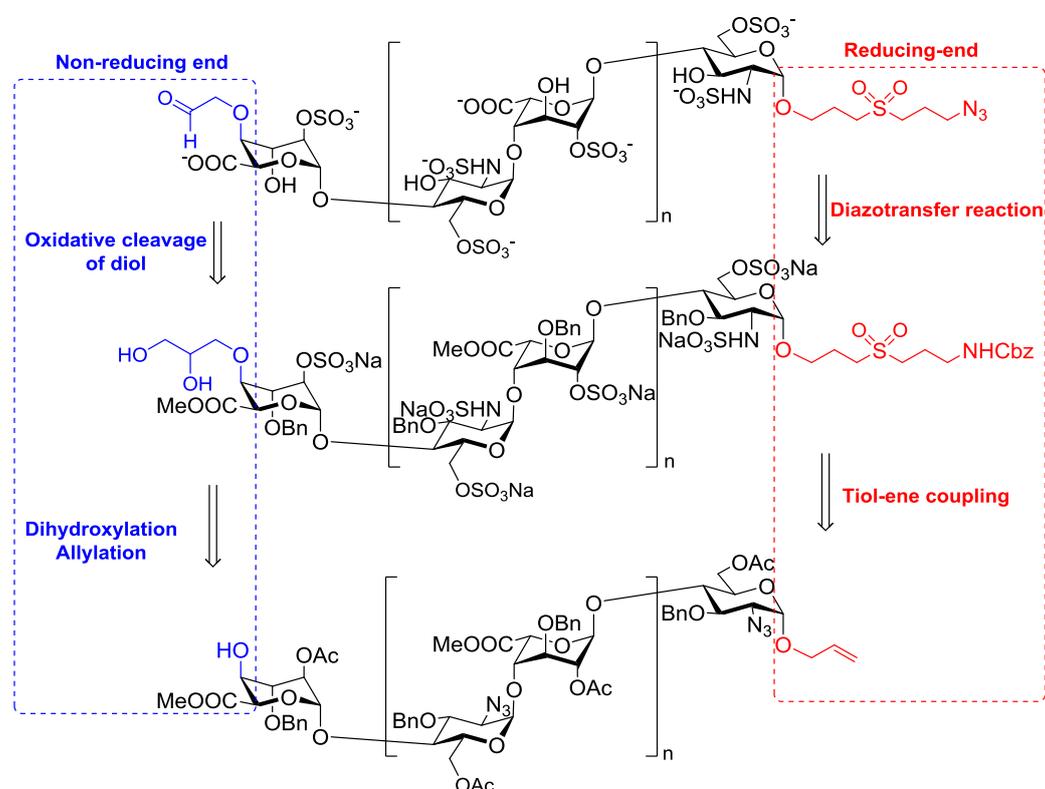


Figure 34. Proposed retrosynthetic scheme allowing the modifications of the reducing and non-reducing end of HS fragments.

III.2. Modifications of PEG Spacers

With regard to modifications of PEG-type spacers, they have to be functionalized with a propargyl and/or an aminoxy group at one or two of their extremities for ligation reaction but also coupled to a sulfotyrosine for the preparation of CXCL12 neutral ligands. Moreover, those modifications have to be applicable to all type of spacers, whatever their length. From a retrosynthetic point of view, those different kinds of modification of PEG-type spacers could be achieved through (Figure 35):

(i) Williamson alkylation reaction with propargyl bromide to introduce the propargyl group;

(ii) Mitsunobu reaction to introduce a hydroxyphthalimide group, followed by its deprotection to obtain the aminoxy motif;

274. Shimizu, I.; Yamada, T.; Tsuji, J. *Tetrahedron Lett.* **1980**, *21*, 3199–3202.

275. Lakhmiri, R.; Lhoste, P.; Sinou, D. *Tetrahedron Lett.* **1989**, *30*, 4669–4672.

276. Lakhmiri, R.; Lhoste, P.; Kryczka, B.; Sinou, D. *J. Carbohydr. Chem.* **1993**, *12*, 223–235.

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(iii) A one-pot amide formation with NHS-activated *N*-acetyl-tyrosine to introduce Tyr motif;

(iv) Application of a combination of these three methods of modification to synthesize asymmetric PEG-type spacers or doubled the reagents amounts to prepare symmetric PEG-type spacers.

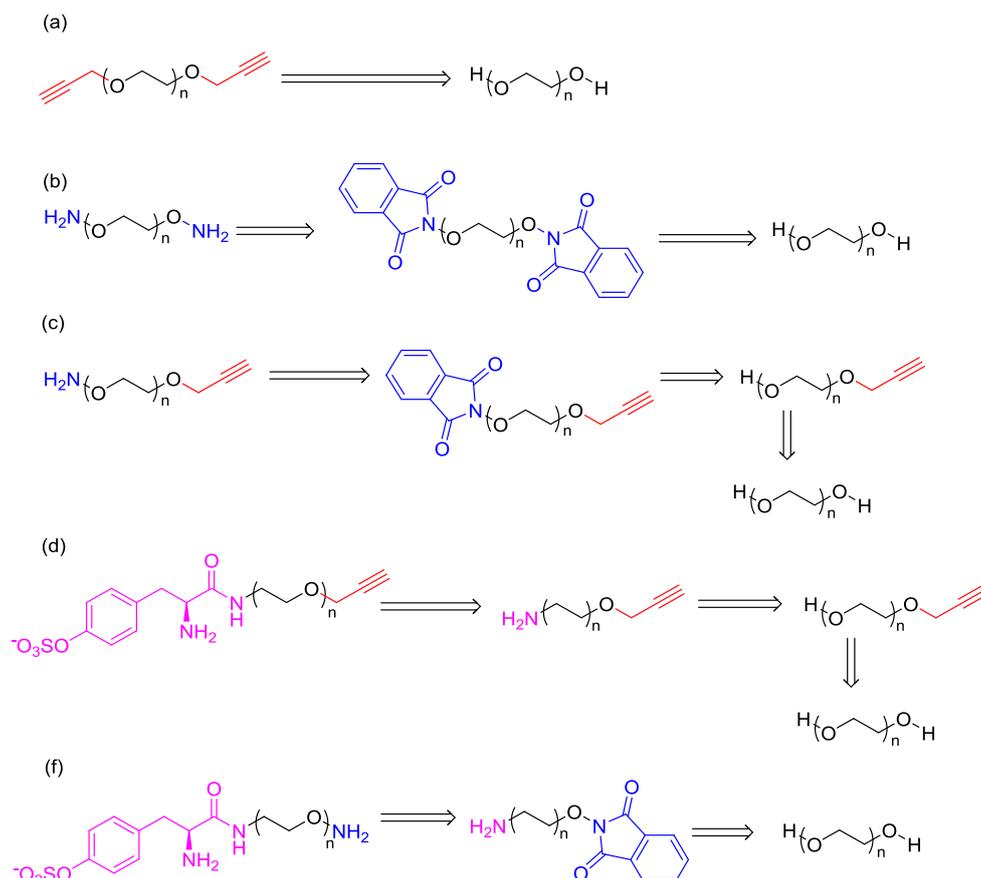


Figure 35. Retrosynthetic analysis for introduction of the three desired motifs and for the preparation of all PEG-type spacers.

IV. Preparation of A Model Disaccharide To Evaluate The Set of Modifications/Ligation Reactions

To confirm the practicability of the selected modifications/ligation reaction at the two ends of HS fragments, we decided to apply them onto a model disaccharide, displaying the principal functions found our synthesized HS fragments: (i) an All group at reducing end; (ii) an uronic ester at its non-reducing end bearing a poorly nucleophilic *O*-4 position; (iii) benzyl groups to evaluate the compatibility of deprotection step conditions with introduced functions.

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IV.1. Retrosynthetic Analysis

As aforementioned for HS fragments, modifications of the two ends of the model disaccharide will be done by:

(a) introducing an azide group to the reducing end of the disaccharide starting from an amine precursor, which will be incorporated onto the disaccharide using the thiol-ene coupling procedure reported by our lab.

(b) installing an aldehyde into the non-reducing end *via* a three steps process involving a Tsuji-Trost decarboxylative allylation of position *O*-4', dihydroxylation of the obtained All group and, finally, oxidative cleavage of the formed diol. (Figure 36).

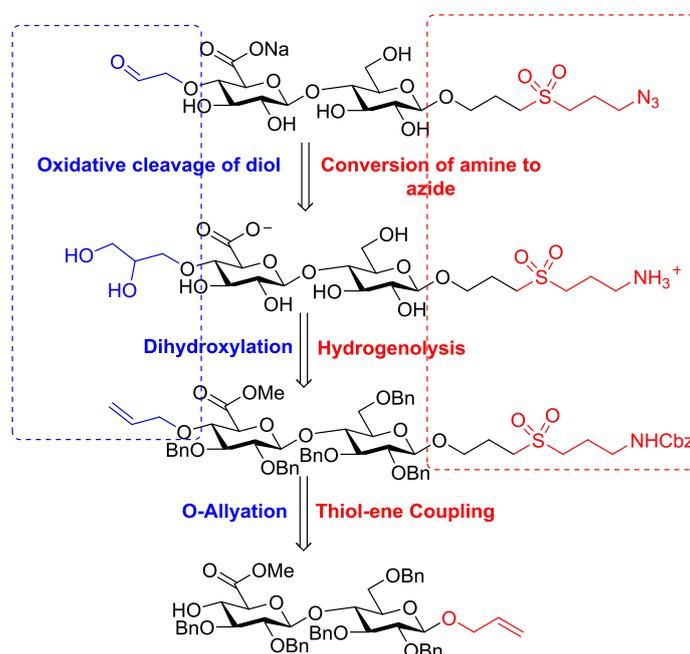


Figure 36. Envisaged retrosynthetic scheme for the modifications of the two ends of the model disaccharide.

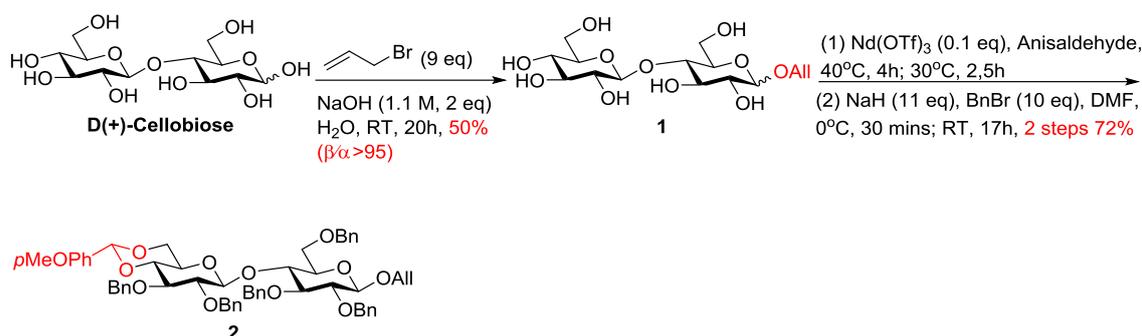
IV.2. Modifications of Model Disaccharide

The synthesis of the model disaccharide, following procedures optimized by Dr. Wenqing Liu during her PhD in our group, were carried out starting from D-cellobiose, which is not only cheap in price but also a renewable resource available in large quantities. A selective anomeric allylation of cellobiose using 9 equivalents of allyl bromide in water in presence of 2 equivalents of sodium hydroxide allowed the selective formation of allyl-D-cellobioside **1** in 61% yield after stirring at room temperature for 20 h ($\alpha/\beta = 17/83$). Two recrystallizations of the obtained anomeric mixture in isopropanol permit to isolate allyl- β -D-cellobioside in 50% yield with a

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anomeric purity higher than 95 % as judged by ^1H NMR.

Selective acetalization of the 4,6-diol was achieved by treatment of **1** with anisaldehyde in presence of a catalytic amount of $\text{Nd}(\text{OTf})_3$ under reduced pressure. After the quench of the reaction with triethylamine, dilution of the resulting solution with a dichloromethane/diethyl ether mixture (v/v = 3/1) allowed the extraction of the desired product with water in a satisfactory purity for the next step. Subsequently, remaining hydroxyl groups were benzylated with benzyl bromide in presence of NaH in DMF to yield fully protected disaccharide **2** (Scheme 15).



Scheme 15. Synthetic route for the preparation of the key building block **2**.

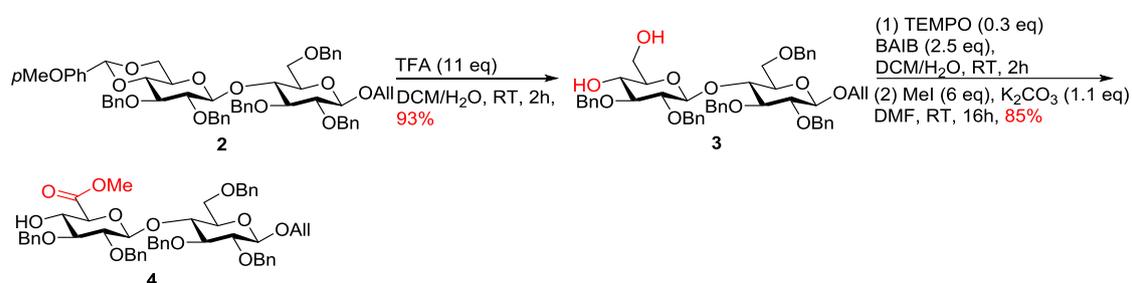
Numerous methods have been reported to convert glycopyranoside into uronic acid. However, these methods primarily relied on the use of toxic chromium reagents or a two-step protocols, such as Swern or Dess-Martin oxidation followed by the treatment with PDC or Pinnick oxidation, frequently giving low yields of the desired product.^{277–281} Subsequently, we overcame these problems by using the mild 2,2,6,6-tetramethyl-1-piperidinyloxy-mediated oxidation (TEMPO-mediated oxidation), which are typically effected with a co-oxidant such as NaOCl ^{282–284} or iodobenzene

277. van Boeckel, C. A. A.; Beetz, T.; Vos, J. N.; de Jong, A. J. M.; Van Aelst, S. F.; van den Bosch, R. H.; Mertens, J. M. R.; van der Vlugt, F. A. *Synthesis of a Pentasaccharide Corresponding to the Antithrombin III Binding Fragment of Heparin*; 1985; Vol. 4.
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280. Petitou, M.; Bono, F.; Tabeur, C.; Herbert, J.-M.; Mallet, J.-M.; SinaÿP. *Bioorg. Med. Chem.* **1999**, *7*, 2003–2012.
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diacetate (BAIB),^{285,286} in presence of water for the direct formation of the carboxylic acid derivative.^{287–290} Moreover, a wide range of sensitive functional groups, such as PMB and allyl ethers, glycosidic linkages, electron-rich aromatic rings are little affected by this oxidation method.

Hence, the benzylidene acetal of compound **2** was hydrolyzed by treatment with TFA in DCM and water at room temperature to afford the corresponding diol **3** in 93% yield, which was regioselectively oxidized with TEMPO in a dichloromethane-water biphasic system containing BAIB as cooxidant. After the selective oxidation of the primary alcohol of **3**, the resulting carboxylic acid was protected as methyl esters by treatment with iodomethane, in presence of potassium carbonate, to give compound **4** in an overall yield of 85% (Scheme 16).



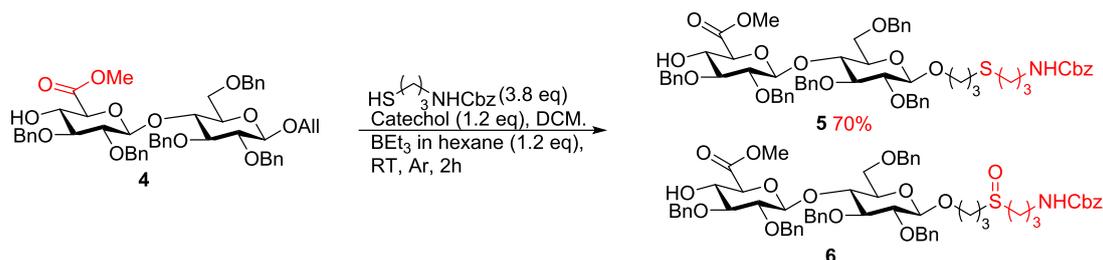
Scheme 16. Selective conversion of the glycopyranoside unit at the non-reducing end of **3** into methyl urinate **4**.

Since the free hydroxyl group at position C-4 is compatible with thiol-ene coupling, benzyl 3-mercaptopropylcarbamate chain was directly incorporated onto the reducing end of disaccharide **4** through our repairing conditions: compound **4** was treated with 3.8 equivalents of benzyl 3-mercaptopropylcarbamate in presence of 1.2 equivalents of triethylborane, catechol under argon atmosphere at room temperature for 2 hours, to give the corresponding thioether **5** in 70% yield (Scheme 17). It has to be noted that this low yield should be attributed to the easy oxidation of thioether **5** into its

285. Van den Bos, L. J.; Codée, J. D. C.; Van der Toorn, J. C.; Boltje, T. J.; Van Boom, J. H.; Overkleeft, H. S.; Van der Marel, G. A. *Org. Lett.* **2004**, *6*, 2165–2168.
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289. Wang, Z.; Xu, Y.; Yang, B.; Tiruchinapally, G.; Sun, B.; Liu, R.; Dulaney, S.; Liu, J.; Huang, X. *Chem. - A Eur. J.* **2010**, *16*, 8365–8375.
290. Tiruchinapally, G.; Yin, Z.; El-Dakdouki, M.; Wang, Z.; Huang, X. *Chem. - A Eur. J.* **2011**, *17*, 10106–10112.

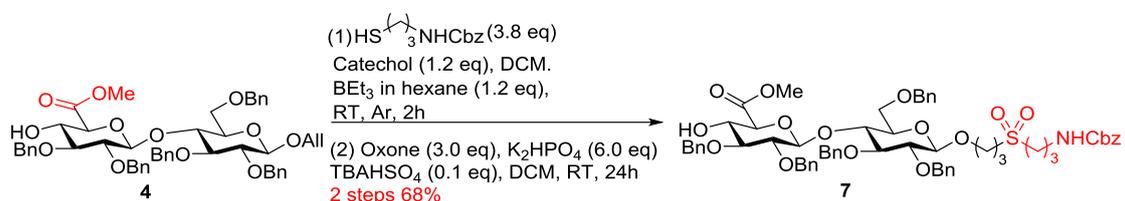
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corresponding sulfoxide **6** during the purification step.



Scheme 17. Thiol-ene coupling to introduce benzyl 3-mercaptopropylcarbamate at reducing-end of compound **4**.

To solve this problem, we decided to directly oxidize the thioether **5** into its stable sulfone, a more easily handled compound. The oxidation was carried out using Oxone® as oxidative reagent instead of mCPBA or DMDO to make sure the full conversion of thioether into sulfone instead of sulfoxide, under phase-transfer conditions using TBAHSO₄ as transfer reagent.^{291,292} Moreover, K₂HPO₄ was used to adjust the pH of the solution to nearly neutral and avoid possible secondary reactions occurring in acidic conditions. In this way, sulfone **7** was obtained with an overall yield of 68% in two steps starting from **4**, which in one way confirmed the efficiency of the oxidation step as the moderate mainly arose from the first TEC step (Scheme 18).



Scheme 18. Application of Thiol-ene/oxidation sequence to access to a stable and easy isolated compound **7**.

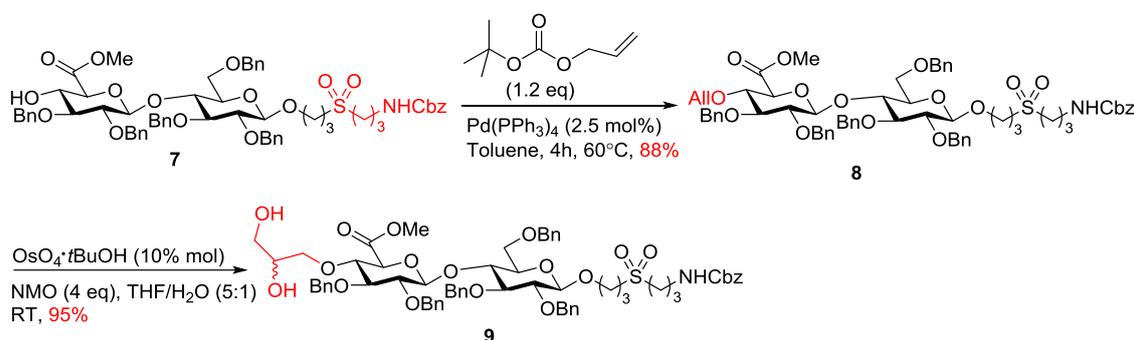
The free hydroxyl group at position C4 of **7** was selectively allylated through a Tsuji-Trost decarboxylative allylation reaction using allyl *tert*-butylcarbonate as allylation reagent in presence of 2.5 mol% of Pd(PPh₃)₄ in toluene at 60 °C for 4 hours. Using these conditions, the free alcohol was selectively allylated even in the presence of the reactive benzyloxycarbonyl moiety. The perfect control of the temperature and time of reaction limited the formation of the diallylated compound, *O*- and *N*-allylated, gave the desired compound **8** with 88% and allowed the recovery of the unreacted

291. Trost, B. M.; Curran, D. P. *Tetrahedron Lett.* **1981**, 22, 1287–1290.

292. Hussain, H.; Green, I. R.; Ahmed, I. *Chem. Rev.* **2013**, 113, 3329–3371.

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starting material. The obtained allyl ether was then converted into 1,2-diols. A variety of methods have been reported for dihydroxylation of alkene, among them,²⁹³ the commonly used one is Upjohn dihydroxylation reaction,²⁹⁴ using catalytic amount of osmium tetroxide in presence of stoichiometric amount of co-oxidant, potassium chlorate (KClO₃) or *N*-methylmorpholine *N*-oxide (NMO) for example. Due to its mild reaction conditions and the good solubility of NMO in aqueous media, disaccharide **8** was treated with a catalytic amount of osmium tetroxide in presence of stoichiometric amount of NMO in a mixture of tetrahydrofuran and water (ratio is 5/1) at room temperature to afford vicinal diol **9** in an overall yield of 95% (Scheme 19).



Scheme 19. Insertion of an acyclic vicinal diol at the non-reducing end of the model disaccharide using a sequence of decarboxylative allylation/dihydroxylation.

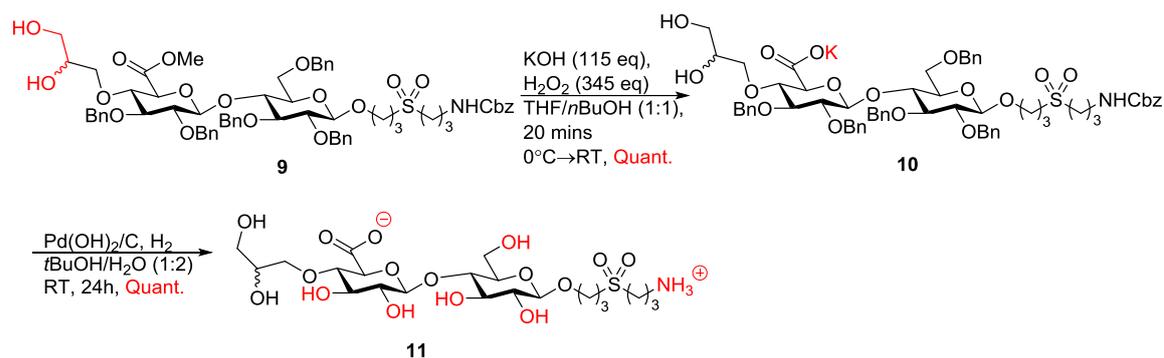
To avoid possible β -elimination of uronate moieties, the saponification of ester of HS fragments is generally performed using a strong base (KOH, NaOH or LiOH) in presence of hydrogen peroxide (H₂O₂). This conditions permits an *in situ* generation of HOO⁻, a specie which is sufficiently nucleophilic for saponification to occur but not sufficiently basic for the occurrence of β -elimination (pK_a = 11.6). Moreover, under those conditions, the benzyloxycarbonyl group can also be cleaved to liberate the amine groups. Thus, saponification of disaccharide **9** was performed using KOH and H₂O₂ in a mixture of THF and *n*BuOH to remove methyl and Cbz group at the same time. However, we failed to remove the Cbz group even through to a prolongation of the reaction time to 2 days at 37 °C. On the contrary, the saponification proceeded rapidly, and was completed in 20 minutes at room temperature to afford the corresponding disaccharide **10** in a quantitative yield. Finally, the water-soluble disaccharide **11** was obtained *via* hydrogenolysis of disaccharide **10**, in presence of palladium dihydroxide,

293. Schroeder, M. *Chem. Rev.* **1980**, *80*, 187–213.

294. Cha, J. K.; Kim, N.-S. *Chem. Rev.* **1995**, *95*, 1761–1795.

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with concomitant removal of the benzyl and Cbz groups (Scheme 20).



Scheme 20. Saponification and hydrogenolysis.

V. Summary

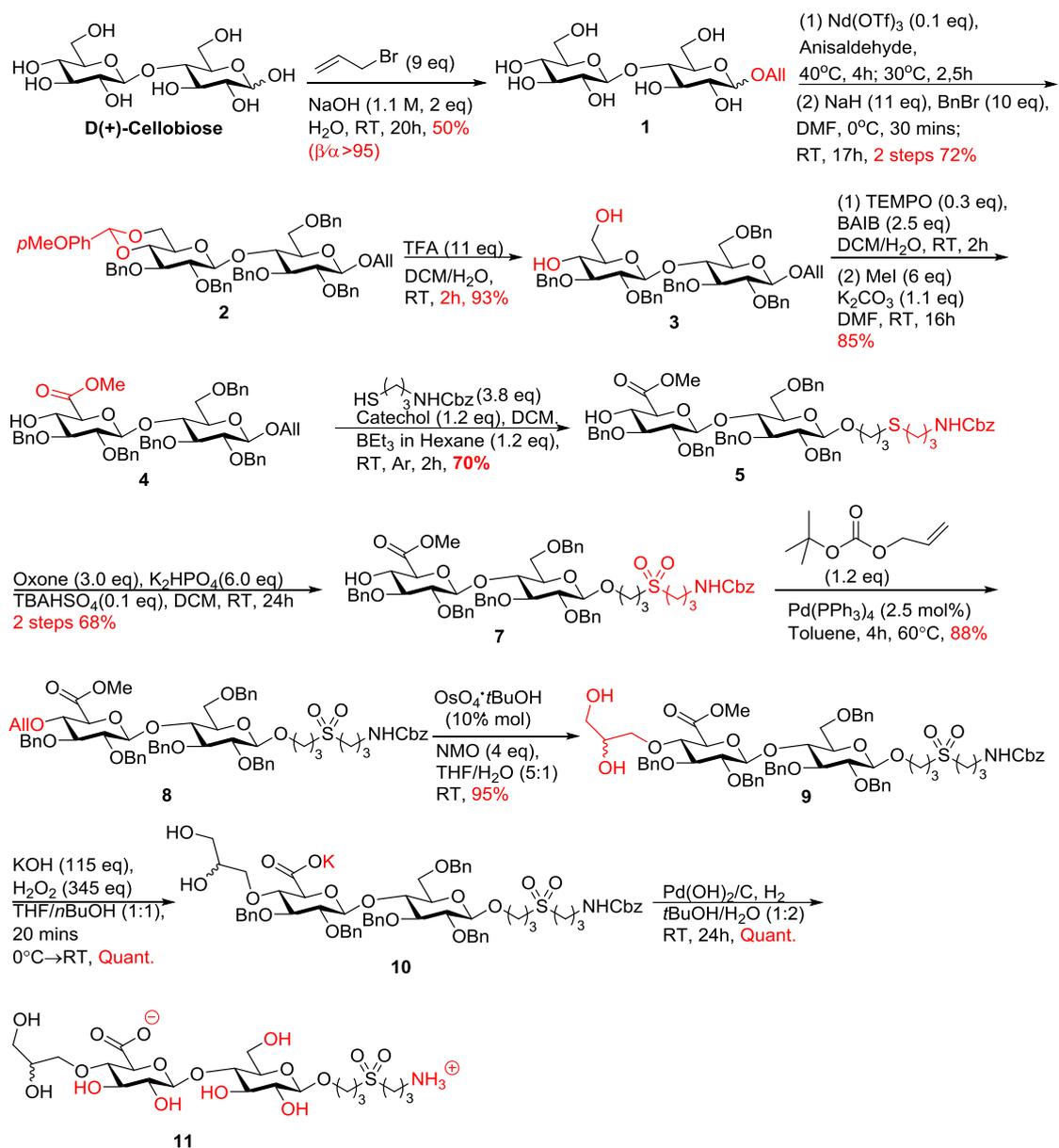
In this chapter, we not only describe the protecting group strategy used to synthesize HS fragments but also propose modification method to prepare SAS mimetics with variable geometries, in which S domains were composed of HS fragments and A domains were mimed by PEG-type linkers in different lengths.

Among Click Chemistry panel, we finally selected two orthogonal reactions: CuAAC and oxime ligation to make modifications of two ends of HS fragments (S domains) and PEG-type linkers (A domains). Therefore, on one hand, a propargyl group, an aminooxy group and/or a Tyr motif can be introduced to the two ends of PEG-type linkers. On the other hand, an azide group can be introduced to the reducing end of HS fragments. The azide group will be obtained *via* a diazotransfer reaction from an amine group, which is introduced through thiol-ene coupling between the allyl moiety at the reducing-end of HS fragments and a benzyl 3-mercaptopropylcarbamate chain. In the meantime, an aldehyde group was installed onto the non-reducing end of HS fragments through the oxidative cleavage of 1,2-diol at *O*-4 position of the terminal uronic acid unit. This vicinal-diol will be formed through the dihydroxylation of an allyl ether, introduced by a Tsuji-Trost decarboxylative allylation of the free hydroxyl group at *O*-4 position.

In order to confirm the practicability of two end modifications of HS fragments, we decided to apply them onto a model disaccharide that displays the characteristic ends of synthesized HS fragments. Thus, using D-cellobiose as starting material, we synthesized disaccharide **11** in 12 steps with an overall yield of 11% (Scheme 21). The rest modifications of the reducing-end and non-reducing end as well as the conjugation

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reactions will be discussed in Chapter 3 and Chapter 4 in detail.



Scheme 21. Synthetic routes to prepare model disaccharide and modifications of the two ends of model disaccharide.

Chapter 3. One-pot CuAAC Reaction from *In situ* Generated Azides

I. Copper Catalyzed Azide-Alkyne Cycloaddition (CuAAC)

The CuAAC is probably the model example of the click chemistry concept and is frequently and improperly named ‘click reaction’. If this transformation perfectly satisfies all criteria of Click Chemistry, is reliable and easy to use, it still suffers from a major drawback: the instability to air of its Cu(I) catalyst as well as the low solubility in organic and aqueous solvents of Cu(I) salts.

I.1. Copper Catalyst

Since the development of CuAAC, three major approaches have been commonly used to generate catalytic amount of Cu(I) species in the reaction:¹⁹⁸ (i) the reduction of copper (II) into Cu(I), (ii) the direct utilization of Cu(I) salts; and finally (iii) oxidation of copper metal into Cu(I) (Figure 37).

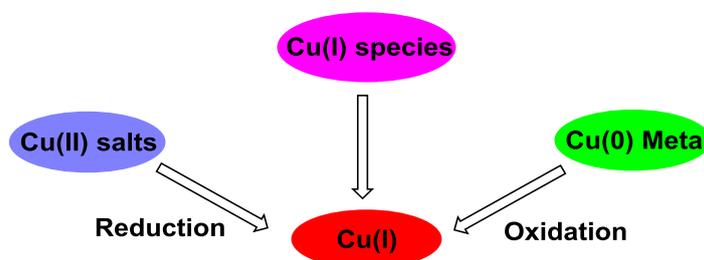


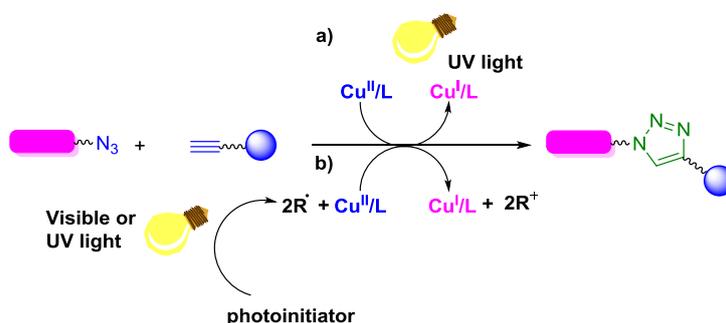
Figure 37. Sources of Cu (I) species.

The most widely used strategy is the *in situ* generation of Cu(I) from Cu(II) salts, such as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{Cu}(\text{OAc})_2$, using a sacrificial reducing agent, since it does not require an inert atmosphere,²⁹⁵ it can be carried out in aqueous medium, whilst maintaining a high Cu(I) concentration during the course of reaction and, more importantly, it is accessible to non-experts in Chemistry. Till now, sodium ascorbate¹⁸⁶ is the most frequently used sacrificial reducing agents, offering excellent results when

295. Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2005**, 2006, 51–68.

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used in 3- to 10-fold excess.²⁹⁵ In addition to sodium ascorbate, other sacrificial reducing agents, such as hydrazine²⁹⁶ and tris(2-carboxyethyl)phosphine (TCEP)^{297–299} have also been successfully implemented. However, the application of sodium ascorbate can be complicated by the over reduction of Cu(II) to Cu(0) and the formation of radical oxygen. Moreover, strongly electrophilic byproducts of dehydroascorbate have been reported to react with protein chains³⁰⁰ and alter the chemical structure of DNA³⁰¹. Recently, a UV light-mediated *in situ* generation of Cu(I) by means of photoinduced reduction of Cu(II) salts has emerged as an alternative strategy for bioconjugation studies, showing interesting results (Scheme 22).^{302–304}



Scheme 22. Photoinduced CuAAC click reactions mediated by a) direct and b) indirect reduction of Cu^{II} to Cu^I.³⁰³

An alternative protocol is to directly add Cu(I) salts without using any additional reducing agents. Over the past years, Cu(I) salts (iodide, bromide, chloride, acetate)^{305,306} and coordination complexes such as [Cu(CH₃CN)₄]PF₆¹⁸⁴ and [Cu(CH₃CN)₄]OTf³⁰⁷ have been commonly employed.³⁰⁸ However, Cu(I) is unstable since it can be relatively easily oxidized to catalytically inactive Cu(II) or undergo

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disproportion into Cu(0) and Cu(II).³⁰⁹ Therefore, deoxygenated organic solvent (THF, toluene, dichloromethane, acetonitrile) is required. Additionally, the assistance of a base, such as trimethylamine (NEt₃), diisopropylethylamine (DIPEA), 2,6-lutidine, and pyridine, will be probably demanded to both stabilize the Cu (I) specie and facilitate the Cu (I)-acetylide formation.³¹⁰

Catalytic amounts of Cu(I) can also be introduced into the reaction through oxidation of elemental Cu(0) in various form,^{311,312} such as copper wire, copper nanoparticles^{312,313} or copper nanoparticles absorbed on charcoal.³¹⁴ Cu(II) sulfate may be added to accelerate the reaction, but this is usually not mandatory as the patina on the copper surface is sufficient to initiate the catalytic cycle.³¹⁵ Due to the poor solubility of copper metal, the preferable used solvent is a polar organic one, such as THF, DMF as well aqueous alcohol. Even though this route is less efficient and takes longer reaction time till completion (12-48 hours) than using standard catalyst systems mentioned above³¹⁵, it usually yields the product with high purity, simplifying the experimental procedure. Additionally, this strategy can be dramatically sped up by applying microwave (MW) irradiation,^{316,317} ultrasound or combination of the two.^{318,319}

I.2.Mechanism of The CuAAC Reaction

Based on DFT calculations and kinetic evidence, Sharpless and co-workers initially proposed a stepwise mechanism involving a mononuclear Cu-acetylide

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 319. Cravotto, G.; Fokin, V. V.; Garella, D.; Binello, A.; Boffa, L.; Barge, A. *J. Comb. Chem.* **2010**, *12*, 13–15.

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intermediate (Figure 38, Path 1).^{186,311} After the formation of Cu(I)-acetylides **Ia**³²⁰, complexation of the azide to the Cu(I)-acetylide allows the addition of the C-4 carbon atom of the acetylide onto the terminal azote atom (N-3) of the azide moiety³²¹ to generate the Cu(III) metallocycle **IIIa** (supported by Micouin and coworkers).³²² Regioselectivity of the reaction therefore can be explained by the binding of both azide and alkyne to copper catalyst prior to the formation of the C-N bonds.³²² A transannular ring contraction followed by a proton-demetalation would finally give the 1,4-triazole and also leads to easy dissociation of the copper complex which could further reacts with another terminal alkyne and gives Cu(I)-acetylide **Ia**.

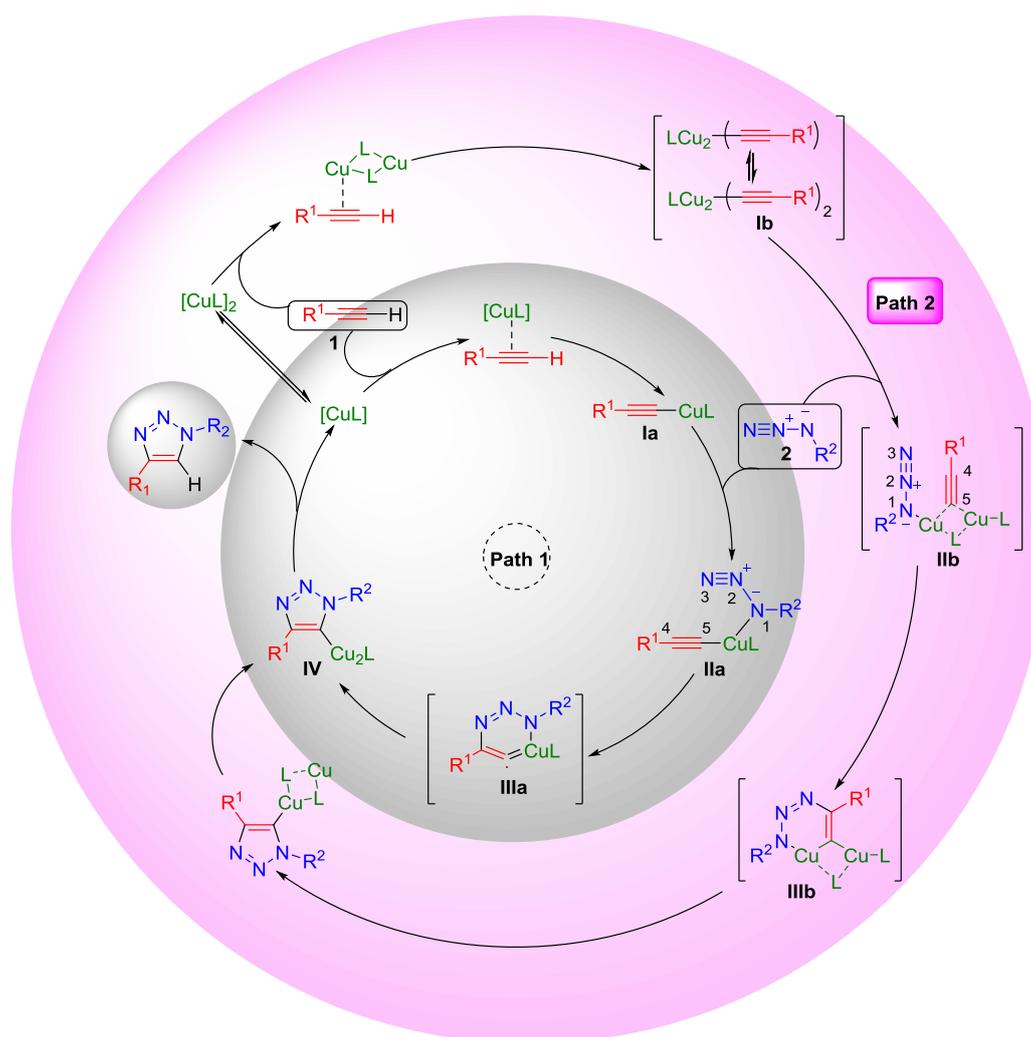


Figure 38. Two proposed mechanisms for the Cu (I)-catalyzed azide-alkyne cycloaddition (CuAAC).³²³

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However, in 2013, Fokin and coworkers have questioned this mechanism by demonstrating that the preformed mononuclear copper acetylide complexes **Ia** were unreactive toward organic azides and presenting their evidence for a dinuclear copper intermediate **Ib**.³²⁴ With an isotopically enriched exogenous copper catalyst and by using time-of-flight mass spectrometry (TOF-MS) experiments, they demonstrated the stepwise nature of the C-N bonds formation and that the two copper centers were equivalent within the cycloaddition steps (Figure 38, Path 2).

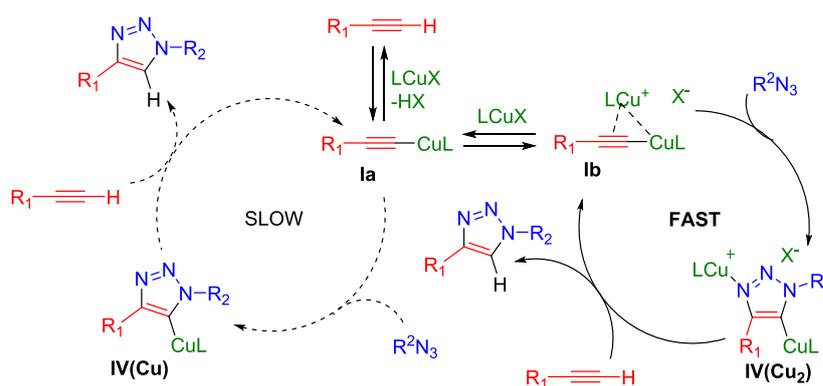


Figure 39. Bertrand's conclusive mechanism of CuAAC: both the mono- and bis-copper pathways are active in the CuAAC but the latter is kinetically favored.³²⁵

Very recently, Bertrand's group confirmed Fokin's hypothesis by reporting the successful isolation of previously proposed π,σ -biscopper acetylide **Ib**.^{325,326} They also isolated a 3,5-bismetallated triazole complex, **IV(Cu₂)**, and revealed that the biscopper pathway is kinetically more favored than the monocopper pathway. Kinetic studies of the Cu-catalyzed reaction of phenylacetylene with benzyl azide revealed that dinuclear complexes **Ib** and **IV(Cu₂)** are catalytically more reactive than their mononuclear counterparts **Ia** and **IV(Cu)** (Figure 39).

I.3.Ligands

The addition of extra ligands are by no means required to carry out the CuAAC reaction, however, they are generally employed to prevent the degradation of Cu(I) by oxidation or disproportionation and therefore significantly enhance the reaction rate.^{186,197}

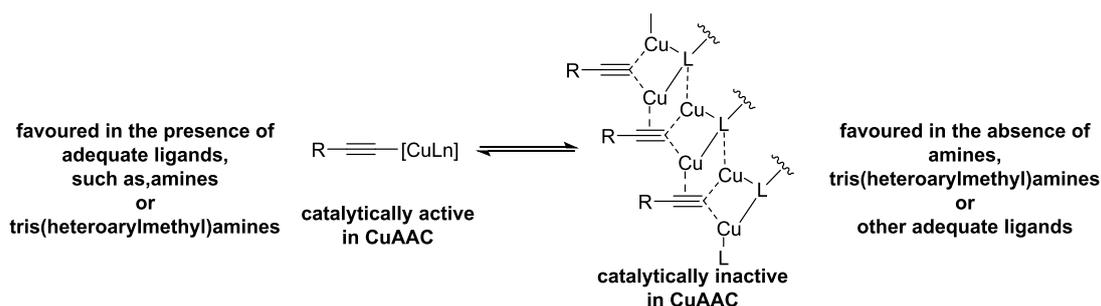
324. Worrell, B. T.; Malik, J. A.; Fokin, V. V. *Science*. **2013**, *340*, 457 LP – 460.

325. Jin, L.; Tolentino, D. R.; Melaimi, M.; Bertrand, G. *Sci. Adv.* **2015**, *1*, e1500304.

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To date, a variety of ligands have been found to be effective under different conditions. Several phosphine-based complexes, such as $\text{Cu}(\text{PPh}_3)_2\text{OAc}$,³²⁷ $\text{Cu}(\text{P}(\text{OMe})_3)_3\text{Br}$ ³²⁸ and $\text{Cu}(\text{PPh}_3)_3\text{Br}$ ^{329,330}, have been shown excellent catalytic activity under optimized reaction conditions. Nevertheless, their applications have been limited by the sole solubility of this cuprous complexes in organic solvents. Additionally, it has been demonstrated that several *N*-heterocyclic carbene (NHC) ligands can efficiently catalyze the CuAAC reaction in aqueous-alcohol media at slightly elevated temperature.^{331,332} It has been demonstrated that these catalysts are very active under the solvent-free conditions, achieving turnover numbers as high as 20000.^{331,332} However, they are less reactive than other catalytic systems when used in solution.³³³ Furthermore, simple amine-based ligands,^{334,335} such as trimethylamine,^{336,337} DIPEA,¹⁹⁷ 2,6-lutidine³³⁸ and phenylendiamine³³⁹ facilitate the CuAAC reactions. Indeed, the use of amines allows the prevention of the formation of unreactive polynuclear copper(I) aggregates as well as increases the solubility of the copper complex (Scheme 23).^{340,341}



Scheme 23. Off-cycle equilibrium between unreactive polymeric copper(I) acetylide species (right) and reactive monomeric copper(I) acetylides (left) as proposed by Fokin.^{340,341} For reason of simplicity, we represented a monomeric acetylide complex instead of the actual catalytic dimeric one.

To date, the most widely used accelerating ligands for CuAAC reaction were based

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331. Déz-González, S.; Correa, A.; Cavallo, L.; Nolan, S. P. *Chem. - A Eur. J.* **2006**, 12, 7558–7564.
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on tris(triazolymethyl)amine, including TBTA (tris-triazole tris[(1-benzyl-1H-1,2,3-triazol-4-yl)-methyl]amine) and its derivatives (Figure 40). These ligands themselves were prepared using the CuAAC reaction. Indeed, during the synthesis of polytriazole compounds, Fokin and coworkers found that the reaction rate was higher than usual and they assumed to observe an autocatalytic process.³¹⁷ Thus, they hypothesized that the polytriazole products themselves could potentially serve as accelerating ligands for CuAAC reaction. Their subsequent study indicated that those oligotriazole ligands were effective in protecting Cu(I) ions from aerobic aqueous conditions and thus increase the CuAAC reaction rate.^{315,317} Further investigation by Finn and coworkers revealed a possible mechanism for the acceleration effect of these triazole ligands in CuAAC reaction: the central nitrogen donor provides electron density at the copper atom, the three-armed motif, carrying relatively weak coordinating heterocyclic ligands, bind to the copper(I) atom with sufficient strength while providing access to necessary coordination sites.³⁴²

As the first generation of triazole ligands, TBTA could significantly accelerate the reaction and stabilize the Cu(I) in aqueous media.^{307,343,344} However, its application in CuAAC reaction was hindered not only by the poor solubility of TBTA in aqueous media but also by the severe toxicity of TBTA-Cu(I) complex in living cells.^{345,346} To circumvent these problems, Finn and coworkers developed a more water-soluble and less toxic ligand THPTA (tris(3-hydroxypropyltriazolyl methyl)-amine).^{307,347} Later on, Wu and coworkers developed two novel TBTA analogues, BTES and BTAA.^{348,349} A detailed and side-by-side comparison for ligand-assisted CuAAC in the presence of TBTA, THPTA, BTAA or BTES demonstrated that these two newly developed TBTA analogues (BTAA and BTES) accelerated the reaction more efficiently than the other ligands.³⁴⁹ Moreover, other tris(triazole)amine-based ligands, such as

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333. Nolte, C.; Mayer, P.; Straub, B. F. *Angew. Chemie. Int. Ed.* **2007**, *46*, 2101–2103.
 334. Diez-Gonzalez, S. *Current Organic Chemistry*. 2011, pp 2830–2845.
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TEOTA,^{351,352} BTTP and BTTPS³⁵² as well as tris(benzimidazole) (BimC₄A)₃^{353,354} also exhibit excellent rate accelerating properties.

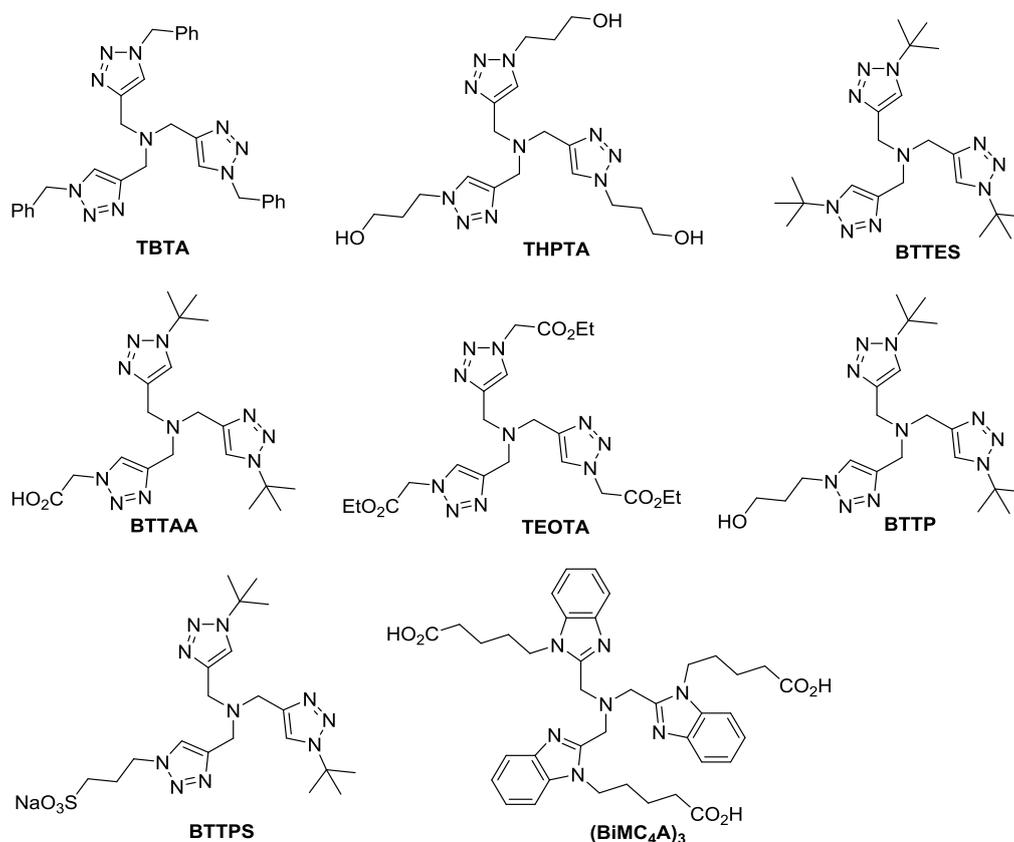


Figure 40. Structure of effective ligands used in CuAAC reaction.

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II. One-Pot CuAAC Procedure Starting from Amino Groups

II.1. Synthesis of Organo-Azides

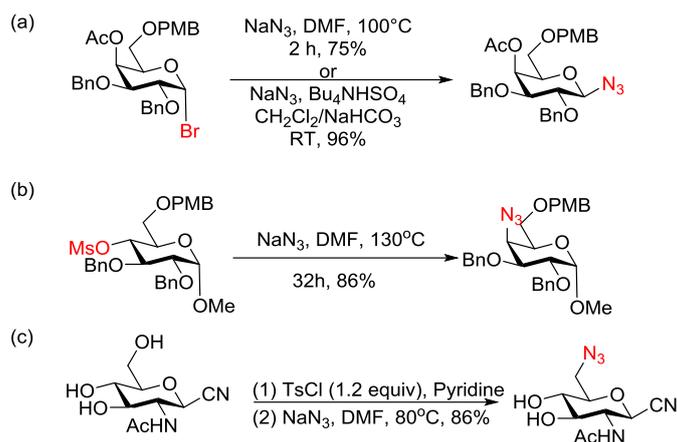
In spite of their potential explosive hazards, organic azides are highly versatile intermediates for organic synthesis³⁵⁵ due to their capabilities to participate in 1,3-dipolar cycloadditions,³⁵⁶ aza-Wittig reaction,³⁵⁷ Staudinger ligation,³⁵⁸ and C-H bond amination.³⁵⁹ In carbohydrates chemistry, organic azides are frequently used either as masked amine moieties for the preparation of 1,2-cis glycosidic linkages,^{361,362} or to construct glycoclusters, glycopeptides and glycodendrimers, through CuAAC reaction.³⁶² Furthermore, glycosyl azides are generally stable and inert under diverse reaction conditions, less sterically hindered than carbamates and amides as well as highly soluble in organic solvents. In general, organic azides can be mostly prepared through two different methods: (i) nucleophilic substitution (S_N2 type) of a leaving group with nucleophilic azide anions or (ii) direct conversion of amino groups by using a diazotransfer reaction.^{355,363}

II.1.1. Classic nucleophilic substitution

Traditionally, the functionalization of carbohydrates compounds with an azide substituent can be proceeded through the nucleophilic displacement of a suitable nucleofuge by an azide ion via a S_N2 process, with inversion of stereochemical configuration. The frequently used glycosyl nucleofuges are glycosyl halides and sulfonates (Scheme 24).

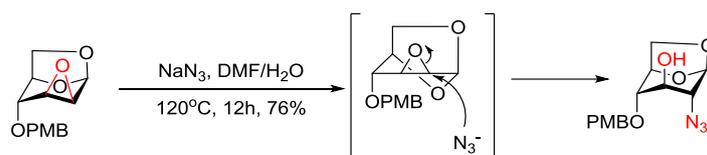
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Scheme 24. Synthesis of glycosyl azides via $\text{S}_{\text{N}}2$ reaction with inversion of configuration.

An alternative strategy widely employed is the selective ring opening of epoxides with azide ions, providing another interesting methodology for generation of an azido group into carbohydrate series. For example, 2-azido-2-deoxyglucopyranose was prepared regioselectively from epoxide by reaction with NaN_3 , using DMF/ H_2O as the solvent systems under heating conditions (Scheme 25)³⁶⁴



Scheme 25. Regio- and stereoselective epoxide ring opening in compound.

II.1.2. Diazo transfer: a simple synthesis of azides from amino groups

In contrast to the former approach, the diazo-transfer reaction could be conducted under mild reaction conditions to convert an amine into azide by using a diazo-transfer reagent, circumventing the formation of sensitive diazonium ions, with complete retention of configuration. The commonly used diazo-transfer reagents are shown in Figure 41.^{365,366,375,367–374}

365. Alper, P. B.; Hung, S. C.; Wong, C. H. *Tetrahedron Lett.* **1996**, 37, 6029–6032.
366. Jiang, J.-W.; Sheng, S.-R.; Zhang, X.-L.; Fang, Z.; Cai, M.-Z. *Tetrahedron* **1996**, 52, 10823–10826.
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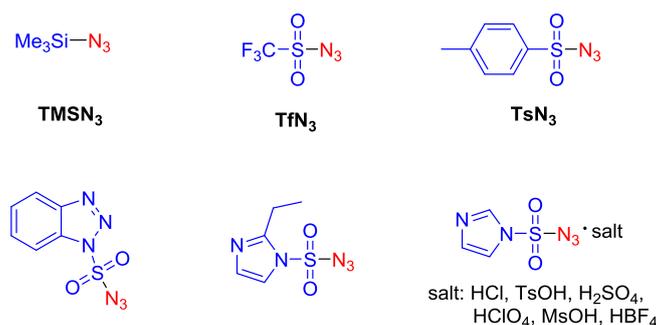
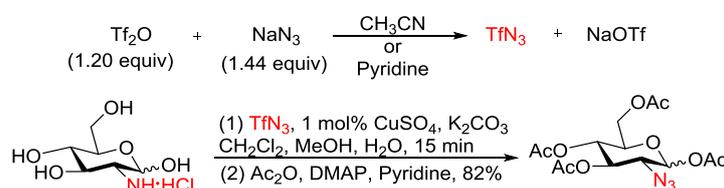


Figure 41. Diazotransfer reagents.

Trifluoromethanesulfonyl azide (TfN₃) must be freshly prepared prior to its use and can be synthesized by reacting trifluoromethanesulfonic anhydride with sodium azide.^{365,368,376} Tf₂O is easily hydrolyzed under aqueous reaction conditions, therefore, the use of an excess of Tf₂O and NaN₃ is required. Then, an alternative procedure was reported by Ye and coworkers, using anhydrous acetonitrile or pyridine as solvent to reduce the addition of excess reagents, which are highly toxic (Scheme 26).³⁶⁸ However, the utilization of TfN₃ is still restricted, because of the high price of Tf₂O and poor shelf life of TfN₃. Most importantly, the procedure of preparation of TfN₃ can be troublesome and not reproducible.



Scheme 26. Modified procedure for preparation of TfN₃ and Diazo transfer reaction utilizing TfN₃ reported by Ye et coworkers.

Wong and coworkers demonstrated that the diazotransfer reaction using TfN₃ can be catalyzed by Cu(II), Ni(II), Zn(II), tetrabutylammonium hydrogen sulfate, or 18-crown-6.³⁶⁵ However, to date, the exact mechanism of diazo transfer reaction is still controversial. In 2002, Wong and coworkers proposed the mechanism to incorporate a divalent metal ion, as depicted in the following scheme (Figure 42).³⁶⁷

-
- 2010, 75, 6532–6539.
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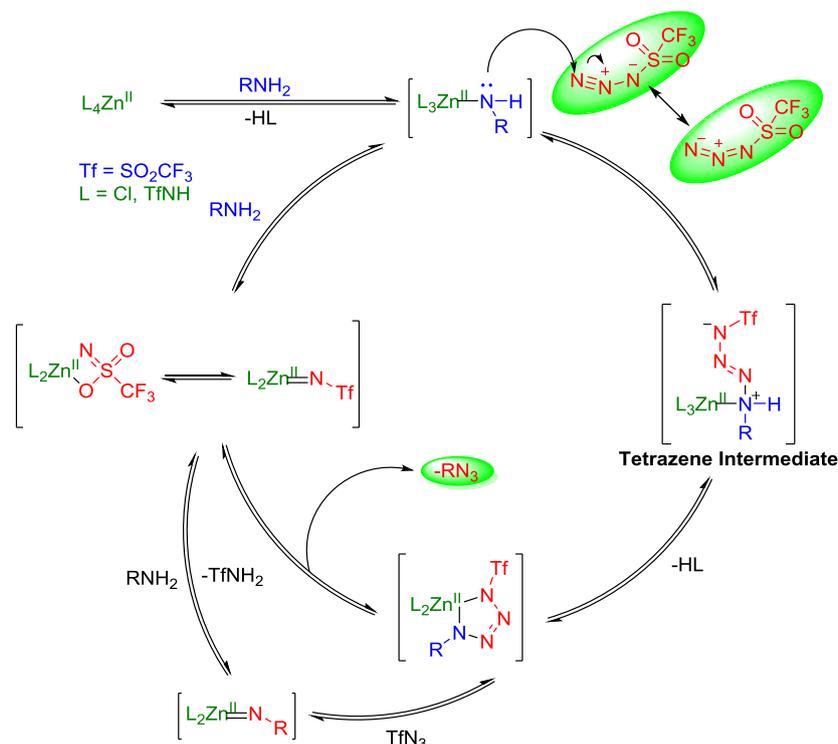
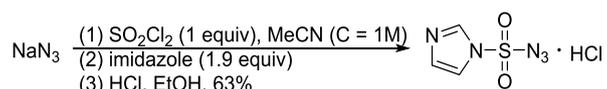


Figure 42. Possible mechanism for the metal-catalyzed diazo-transfer reaction with TfN_3 proposed by Wong and coworkers.³⁶⁷

Then, in 2007, Goddard-Borger and coworkers developed the use and preparation of another diazo-transfer reagent, imidazol-1-sulfonyl azide hydrochloride ($\text{ISA}\cdot\text{HCl}$) (Scheme 27).³⁷⁰ In comparison with TfN_3 , this reagent is more stable, can be prepared on large scale, in good yields and high purity, without the need of chromatography.



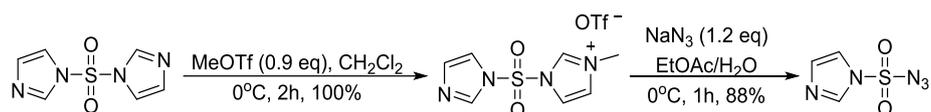
Scheme 27. Preparation of imidazolyl-1-sulfonyl hydrochloride ($\text{ISA}\cdot\text{HCl}$).³⁷⁰

However, there are some safety concerns regarding the potential risk of explosion

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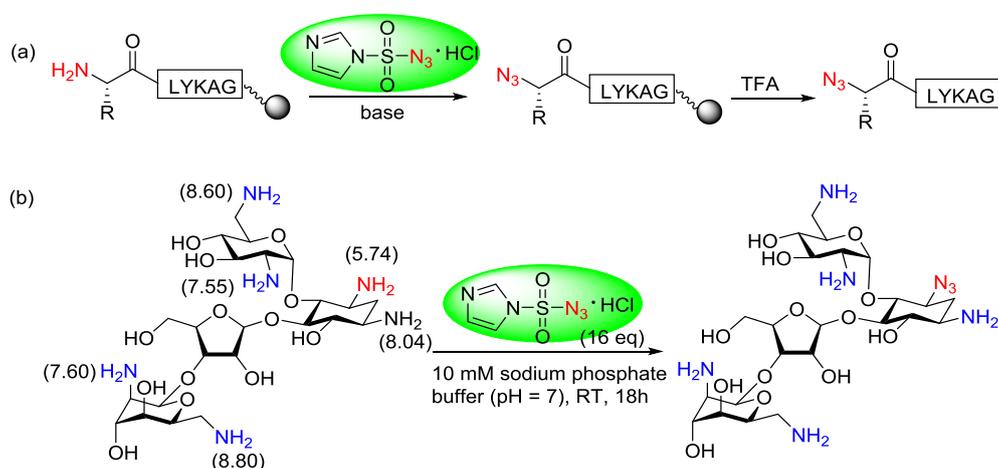
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of this reagent. To identify safe-to handle forms of this compound, several types of imidazol-1-sulfonyl azide salts were prepared and their sensitivity to heat, impact, friction, and electrostatic discharge were quantitatively determined.³⁷⁴ It has been revealed that several salts exhibited improved properties and could be considered safer than ISA·HCl. In addition, in 2013, Wang and coworkers reported a second protocol to synthesize imidazole-1-sulfonyl azide (Scheme 28) that could be easily used in polar solvents or aqueous media.³⁷⁷



Scheme 28. Facile protocol to prepare imidazole-1-sulfonyl azide by Wang et coworkers.³⁷⁷

The use of crystalline ISA·HCl found a wide range of application in organic synthesis, generally offering the corresponding organic azides with excellent yields.^{378–384} In 2012, Hansen and coworkers used ISA·HCl as a diazotransfer reagent for the efficient preparation of azidopeptides on solid phase (Scheme 29a).³⁸¹ In 2013, Herrmann and coworkers demonstrated their application of ISA·HCl to selectively introduce azide to neomycin B, based on the pKa differences of amino groups (Scheme 29b).³⁸²



Scheme 29. Applications of imidazolyl-1-sulfonyl azide.

II.2. Development of One-pot Azide Formation/CuAAC Reaction Sequence

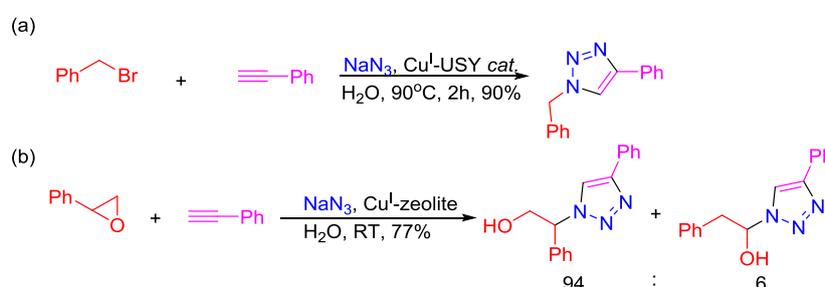
Although organic azides are generally stable at room temperature, compounds of low weight (it is believed that six carbon atoms per one azide group makes the molecule safe, “rule of six”), or those containing several azide functionalities such as precursors

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of multivalent structures, tend to be explosive and their isolations/purifications should be problematic. Therefore, it is desirable to devise a safer one-pot procedure for CuAAC reaction in which the *in situ* generated azides can be captured by the afterwards added alkynes to directly lead to the corresponding 1,4-triazole products, avoiding isolation and handling of potentially unstable azide intermediates.^{371,385–391}

Several approaches have been already investigated to realize this cascade of reactions, involving the *in situ* generation of azides through: (i) nucleophilic substitution or the selective ring opening of epoxides with azide ions or (ii) diazotransfer reaction.

In 2010, Pale and coworkers reported a three components procedure for the preparation of triazoles: organic azides were *in situ* generated by the substitution of halides with sodium azide followed by a CuAAC reaction using a heterogeneous copper catalyst (Scheme 30a).³⁹¹ However, this approach cannot be general because nucleophilic replacement of halides only fastly proceeds into the case of activated halides, such as allylic, propargylic, benzylic or anomeric ones. They also reported a second multicomponent process in which organic azides were *in situ* generated by a highly regioselective ring-opening of epoxides with azide ions in presence of Cu^I (Scheme 30b).³⁹⁰

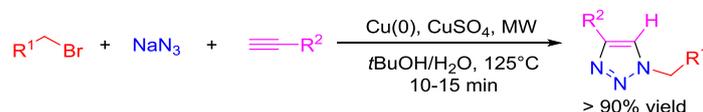


Scheme 30. One-pot three-component reaction involving the *in situ* generation of azides through nucleophilic substitution of halides or selective ring-opening of epoxides.

385. Blass, B. E.; Coburn, K. R.; Faulkner, A. L.; Seibel, W. L.; Srivastava, A. *Tetrahedron Lett.* **2003**, *44*, 2153–2155.
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391. Chassaing, S.; Alix, A.; Boningari, T.; Sani Souna Sido, K.; Keller, M.; Kuhn, P.; Louis, B.; Sommer, J.; Pale, P. *Synthesis.* **2010**, *2010*, 1557–1567.

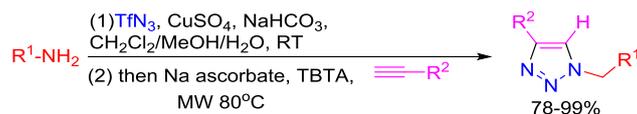
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In 2004, Fokin and coworkers demonstrated that microwave irradiation allowed for the acceleration of the rate of this reaction mainly starting from benzyl bromide and few other activated alkyl halides (Scheme 31).³⁸⁷ However, the harsh reaction conditions needed (125°C) may not be compatible with sensitive substrates such as sulfated oligosaccharides.



Scheme 31. A microwave-assisted one-pot Cu(I)-catalyzed three-component click reaction.

A more efficient and convenient one-pot procedure has been reported by Wittmann and coworkers in 2007, involving the *in situ* generation of azides from an amino group through Cu(II)-catalyzed diazo transfer using TfN₃ (Scheme 32).³⁷¹ The essential requirement of this procedure is to add a reducing agent after accomplishment of the Cu(II)-catalyzed diazo transfer.



Scheme 32. One-pot CuAAC reaction from *in situ* generated azides by diazotransfer reaction.

However, in spite of the advantages of the use of ISA·HCl for preparation of organoazides, its use in a one-pot azide formation/CuAAC cascade reactions could be problematic. Indeed, sulfonyl azides can react with alkynes to yield other products than the desired 1,4-disubstituted 1,2,3-triazole depending on the conditions and reagents employed.^{315,392,401,393–400} Presumably, the reaction of terminal alkynes with sulfonyl azides initially appears to follow the same pathway than a classical CuAAC reactions, yielding to the cuprated triazole intermediate **IV** (Figure 43). Apart from being

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protonated to furnish the sulfonyltriazole product **V** (path a), the intermediate **IV** which is destabilized by the strong electron withdrawing *N*-sulfonyl group, can also undergo a ring-chain isomerization to form the cuprated triazole intermediate **VII**, providing the *N*-sulfonylketenimine **VIII** upon subsequent loss of dinitrogen (path c). Alternatively, copper(I) alkynamide **VI** can be generated with a concomitant elimination of dinitrogen and, after protonation, would also generate the same reactive *N*-sulfonylketenimine species **VIII** (Scheme 46).^{315,395,398}

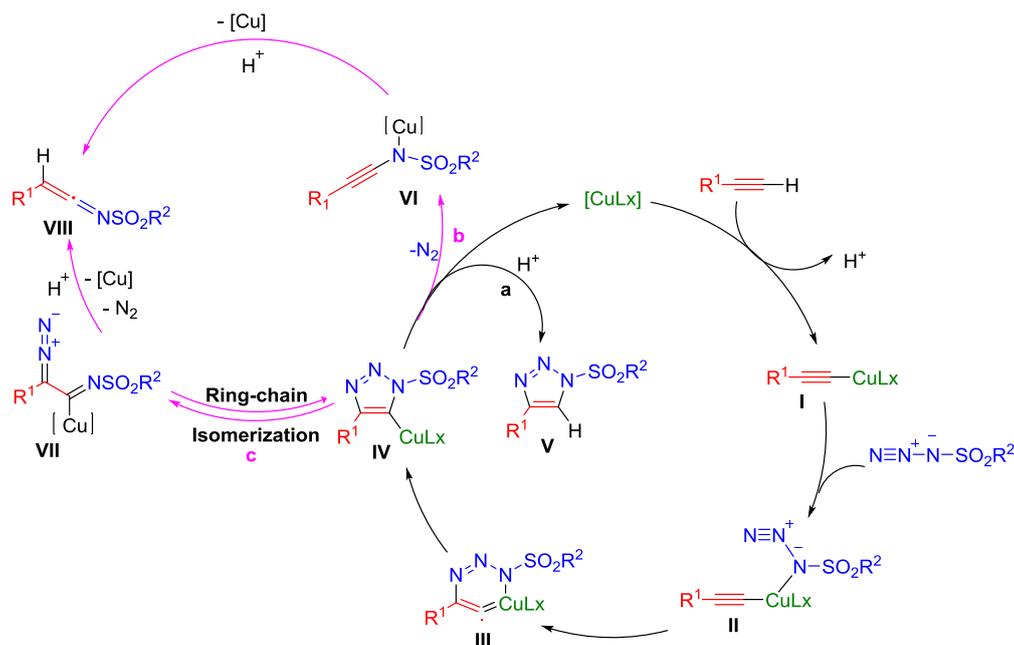
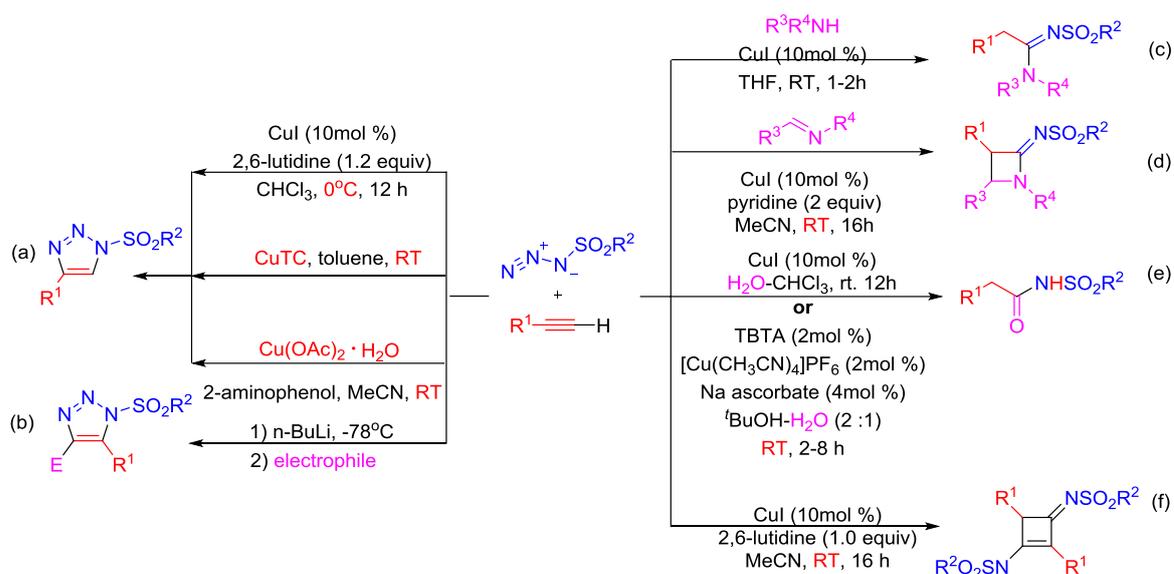


Figure 43. Possible mechanisms leading to secondary products in presence of diazotransfer reagent (a) by a classical CuAAC or (b,c) secondary pathways to form the reactive *N*-sulfonylketenimine intermediate **VIII**.

Therefore, when the reaction is conducted in organic solvents and in presence of amines and imines, *N*-sulfonyl amidines (Scheme 33c)³⁹³ and *N*-sulfonyl azetidinimines (Scheme 33d)³⁹³ can be furnished respectively. However, ketenimine dimers can be isolated in absence of a nucleophilic reagent (Scheme 33f).³⁹³ When conducted under aqueous media, *N*-sulfonyl amides are formed as major products (Scheme 33e), providing an amenable way to the solid-phase amide synthesis, as the *N*-sulfonyl group can be easily removed with sodium in naphthalene.^{392,394} Nevertheless, the 4-substituted 1-(*N*-sulfonyl)-1,2,3-triazoles can also be furnished by using copper iodine, copper (I) thiophen-2-carboxylate (CuTC) and combination of $Cu(OAc)_2 \cdot H_2O$ with 2-aminophenol as catalyst (Scheme 33a).^{396,399,400} Recently, the Croatt group reported a selective synthesis of 4,5-disubstituted *N*-sulfonyl triazoles under strongly basic conditions, in which the 5-substituted triazole anion can be trapped with various

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electrophiles (Scheme 33b).⁴⁰¹



Scheme 33. Products of CuAAC reaction with sulfonyl azides.

As we planned to carry out the one-pot azide formation/CuAAC reaction in aqueous media, the possible side-reactions involving $ISA \cdot HCl$ is the formation of *N*-sulfonyl amides or 4-substituted 1-(*N*-sulfonyl)-1,2,3-triazoles. Therefore, the quantity of $ISA \cdot HCl$ should be well controlled.

Therefore, based on the different aspects discussed above, we decided to carry out an optimization of a one-pot diazotransfer/CuAAC reaction using $ISA \cdot HCl$ as diazotransfer reagent. Wishing to extend this strategy to the preparation of modified HS fragments, establishment of optimal reaction conditions for this procedure represents an important challenge due to: (i) the liability of *N*-sulfate groups at high temperature in presence of Lewis acid, such as copper salts, (ii) the chelating properties of sulfated oligosaccharides that should scavenge the catalyst and (iii) the exclusive solubility of HS fragments in water. To validate the robustness of the one-pot conversion of amino derivatives into azides then into triazoles ones, evaluate the limitation of this process and for pharmacomodulation concerns, we decided to use low water-soluble or water-insoluble fluorinated alkynes as partners for the CuAAC reaction.

III. Development of a One-Pot Diazotransfer/CuAAC Sequence for the Conjugation of Sensitive Compounds and Preparation of Organofluorine Saccharides and Amino Acids

III.1. Importance of Organofluorine Compounds

Owing to the unique physical/chemical properties of fluorine, organofluorine compounds have been widely used in pharmaceuticals,^{402,403} agrochemicals^{404,405} and materials.^{406,407} Fluorine is a small atom, between the size of hydrogen and oxygen atoms, closer to an hydroxyl group (Van der Waals radii: H = 1.2 Å, F = 1.47 Å, OH = 1.4 Å, O = 1.52 Å).⁴⁰⁸ Therefore, the replacement of C-H or C-OH with C-F would not dramatically affect the overall molecular size, and fluorine is often considered as an isostere of hydrogen atom or hydroxyl groups. However, this substitution can profoundly change the electronic properties of the molecule. Fluorine is the most electronegative element of the periodic table (value of 3.98 in Pauling scale).⁴⁰⁸ When covalently bound to carbon, the difference of electronegativity of the two elements conduces to a highly polarized C-F bond and introduces a dipole into the molecule, leading to electronic and conformational modifications.^{402,409} Hence, despite suggestions that H/OH and F is interchangeable, this substitution can dramatically alter the physical, chemical, and biological properties of target molecule.

In medicinal chemistry, the use of isostere generally induces modifications of structural, physical, chemical and biological properties of compounds. This strategy of modification is generally adopted to modify the lipophilicity of compound and improve its ADMET cycle (absorption, distribution, metabolism, exertion and toxicity).⁴¹⁰ In this way, the metabolic stability of drugs can be enhanced by metabolically blocking a

402. O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37*, 308–319.

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410. Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 4359–4369.

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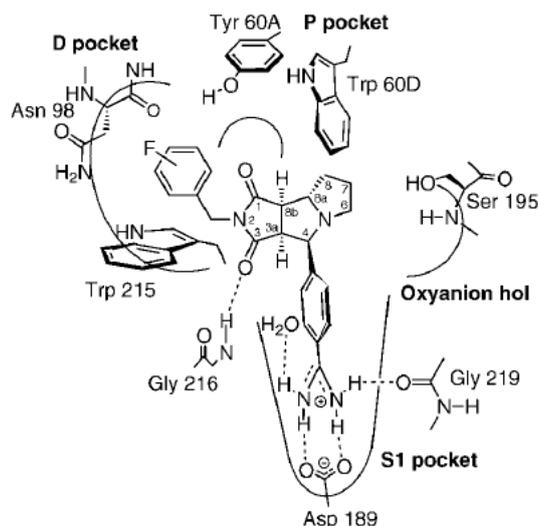
fluorine substituent to slow down its elimination.

Furthermore, fluorine substitution could modulate the pKa of proximal functional groups, due to its high electronegativity. It was found that the introduction of fluorine could lower the basicity of proximal basic groups, thereby resulting in enhanced membrane permeability and increased bioavailability of compound.^{411–414}

Fluorine substitution is also known to significantly enhances the binding affinity of a compound, as the dipole of C-F bond could help to optimize the orientation of drugs at the binding site with their target protein.^{415–417} After a survey of a set of fluorine-substituted thrombin inhibitors, Olsen and coworkers demonstrated that C-F...C=O interactions can significantly increase binding affinities of compounds/proteins, discovered that a monofluorinated compound could bind five times strongly to thrombin than the nonfluorinated parent compound (Table 3).⁴¹⁸

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411. Böhm, H.-J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M. *ChemBioChem* **2004**, *5*, 637–643.
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Inhibitor	Substitution position of F	K _i [uM] ^[a]	Selectivity ^[b]
(±)-1	---	0.31	15
(±)-2	2-F	0.50	9.8
(±)-3	3-F	0.36	26
(±)-4	4-F	0.057	67
(±)-5	2,6-F ₂	0.61	9.0
(±)-6	3,5-F ₂	0.59	25
(±)-7	1,2,3,4,5-F ₅	0.27	44

[a]. The uncertainty of the measured K_i values is ±20%. [b]. K_i(trypsin)/K_i(thrombin).

Table 3. Representation of the fluorinated thrombin inhibitors in the active site of thrombin and their activities & selectivities with respect to trypsin.

Additionally, the isotope ¹⁸F is widely used and preferred in positron emission tomography (PET), as it has longer half-lives compared to other commonly used radionuclides (¹⁵O t_{1/2} = 2 min, ¹³N t_{1/2} = 10 min, ¹²C t_{1/2} = 20 min, ¹⁸F t_{1/2} = 110 min).⁴¹⁹ 2-deoxy-2-[¹⁸F]fluoro-D-glucose ([¹⁸F]FDG) is the most frequently used radiopharmaceutical in early disease detections. Therefore, ¹⁸F usually used in molecular imaging of biological and biochemical processes and the synthesis of fluorinated organic molecules has received considerable attentions in last decades.⁴¹⁹

In drug discovery, there are two efficient approaches to introduce fluorine and fluorinated functional groups into organic compounds: (i) the ‘fluorinated synthon’ approach, involving the use of fluorinated building blocks to assembly the target

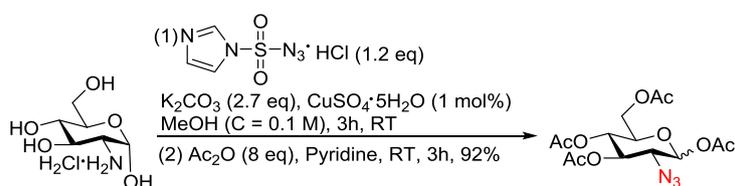
419. Van Der Born, D.; Pees, A.; Poot, A. J.; Orru, R. V. A.; Windhorst, A. D.; Vugts, D. J. *Chem. Soc. Rev.* **2017**, *46*, 4709–4773.

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molecule (there are a variety of fluorine-containing blocks currently available) ; (ii) the use of various electronic or nucleophilic fluorine reagents to selectively introduce fluorine atoms.⁴²⁰ In this context, we assumed that the development of a rapid and efficient diazotransfer/CuAAC sequence of transformations of an amino group at room temperature (no microwaves heating or activation), in water using fluorinated alkynes as cycloaddition partner should represent a convenient method for the preparation of fluorinated thermosensitive bioactive compounds such as fluorinated oligosaccharides, peptides or proteins.

III.2. Preliminary Optimization of Reaction Conditions of the Diazotransfer/CuAAC Sequence using D-Glucosamine Hydrochloride as Substrate

According to the Goddard-Borger and coworkers study on the easy use of ISA·HCl as diazotransfer reagent, D-glucosamine hydrochloride can be conveniently converted into the corresponding azide in methanol and subsequently peracetylated for an easier isolation of the diastereoisomer mixture (Scheme 34).³⁷⁰ To evaluate the feasibility of development of a one-pot diazotransfer/CuAAC procedure and its use for the conjugation of HS fragments amino derivatives or preparation of fluorinated compounds, we decided to use D-glucosamine hydrochloride as a model substrate.



Scheme 34. Model CuAAC reaction using D-glucosamine hydrochloride reported by Goddard-Borger.³⁷⁰

As partner of the cycloaddition reaction and for optimization of reaction conditions, we decided to use 1.5 equivalents of two different and easy to prepared fluorine-contained alkynes (**12** and **13**). Moreover, since the operation of CuAAC reaction should be applied to water-soluble amino derivatives (*i.e.* deprotected HS fragments for our project), we decided to use water and a mixture of *t*BuOH/H₂O (*v/v* = 1:1, preferable for water-insoluble alkynes such as **13**) as solvent and to add 1 equivalent of

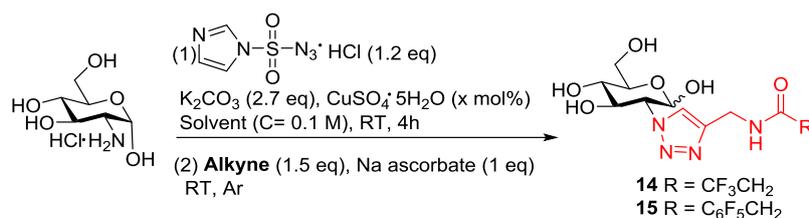
420. Richardson, P. *Expert Opin. Drug Discov.* **2016**, *11*, 983–999.

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sodium ascorbate after the diazotransfer reaction to *in situ* generate active Cu(I) species. We also decided to only evaluate by TLC the time of reaction needed for completion of the sequence depending on the copper(II) sulfate loading and the solvent of the reaction (Table 4).

First, as demonstrated by Goddard-Borger and coworkers, only 1 mol% of copper(II) sulfate is required for the full conversion of D-glucosamine hydrochloride into the corresponding azide in less than 4 h whatever the solvent or the Cu(II) loading used and we applied this time of reaction for all reaction. However, regardless of the alkyne and solvent used, after addition of sodium ascorbate and alkyne, days are required for a full completion of the cycloaddition reaction probably due to a faster rate of oxidation of copper catalyst by dissolved oxygen than rate of cycloaddition reaction (Table 4, entries 1, 5, 9 & 13). To avoid solvent degassing and develop the simplest reaction conditions, we increased the catalyst loading. As expected, the more the catalyst loading and the faster the cycloaddition reaction: the use of 5 mol% of copper(II) sulfate allowed to reduce the reaction time of the CuAAC to hours (Table 4, entries 2, 6, 10 & 14) when 10 to 20 mol% of catalyst were used, the reaction time dropped to a half hour (Table 4, entries 3-4, 7-8, 11-12 & 15-16).

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Entry	Alkyne	Solvent	CuSO ₄ loading (mol%)	Reaction time (TLC evaluation)
1	 12	H ₂ O	1	> 17 days
2			5	3 h 20 min
3			10	30 min
4			20	20 min
5		<i>t</i> BuOH/H ₂ O (v/v = 1:1)	1	> 11 days
6			5	2 h
7			10	40 min
8			20	20 min
9	 13	H ₂ O	1	> 11 days
10			5	> 6 h
11			10	2 h 10 min
12			20	40 min
13		<i>t</i> BuOH/H ₂ O (v/v = 1:1)	1	> 6 days
14			5	1 h
15			10	30 min
16			20	20 min

Table 4. Optimization of reaction conditions of the diazotransfer/CuAAC sequence using D-glucosamine hydrochloride as amino substrate. All reactions were performed using 0.12 mmol of D-glucosamine hydrochloride as starting material.

Moreover, concerning the reaction media, even if the one-pot sequence should be performed in water, under 10 mol% of catalyst loading, the reaction rate was sped up by using the *t*BuOH/H₂O mixture (v/v = 1:1) as solvent due to a good solubility of fluorinated alkynes in this media (Table 4, entries 1-2 vs. 5-6 & 9-10 vs. 13-14). Unlike reactions in water, for low catalyst loadings, fluorinated alkyne **12** reacted slower than alkyne **13** in *t*BuOH/H₂O (Table 4, entries 5-6 vs. 13-14). Finally, in *t*BuOH/H₂O, all CuAAC reactions should be completed in a maximum of 2 h for a copper(II) sulfate loading of 5 mol% or greater. However, no important decrease in reaction time had been observed by increasing the catalyst loading from 10 to 20 mol% (Table 4, entries 7 & 15 vs. 8 & 16 respectively), indicating that an optimal catalyst loading of 10 mol% in *t*BuOH/H₂O (v/v = 1:1) should be sufficient for an efficient one-pot sequence of

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conversion of amino groups into 1,4-disubstituted triazoles. Nevertheless, when we tried to isolate those triazole derivatives **14** and **15**, even if a complete conversion of D-glucosamine was observed, we were confronted to difficulties of separation of the desired products from copper salts and oxidized ascorbate derivatives. Fortunately, in parallel to the development of this transformations sequence into D-glucosamine hydrochloride, we started to apply it to several free amino acids to extend the scope of our sequence and to demonstrate the possibility of preparation of organofluorine amino acids synthons exhibiting the well-recognized amide bond surrogate 1,2,3-triazole without using protective groups.^{421,422}

III.3. Complete Optimization of The Diazotransfer/CuAAC Sequence *via* Organofluorine Amino Acids Preparation

Replacement of amide bond with bioisosteres to improve the stability of potential drug candidates is a continual goal of many laboratories.^{423,424} The 1,4-disubstituted triazole displays structural similarity with a *Z*-amide bond: the lone pair of the 3-nitrogen atom mimics the carbonyl oxygen atom one of the amide bond, the polarized C(5)-H bond can act as a hydrogen bonding donor, just like the amide N-H bond. Moreover, the distances between substituents are 3.9 Å in amide and 5.0 Å in 1,4-disubstituted triazole, and the electrophilic and polarized 4-carbon is electronically similar to the carbonyl carbon (Figure 44). Since the overall dipolar moment of the triazole system is larger than that of the amide bond, its hydrogen bonding donor and acceptor properties are more marked than those of an amide bond, with an enhanced peptide mimicry.^{425,426}

421. Pedersen, D. S.; Abell, A. *Eur. J. Org. Chem.* **2011**, 2011, 2399–2411.

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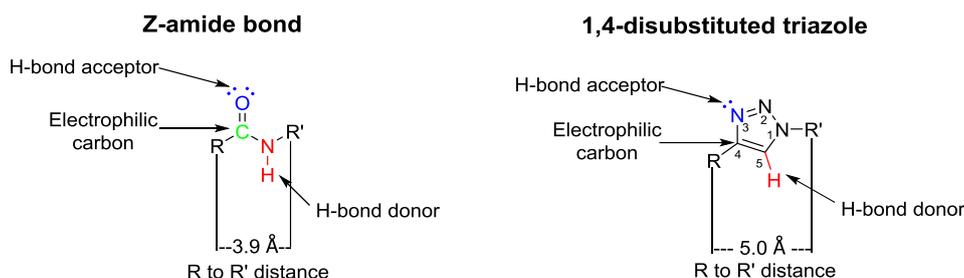
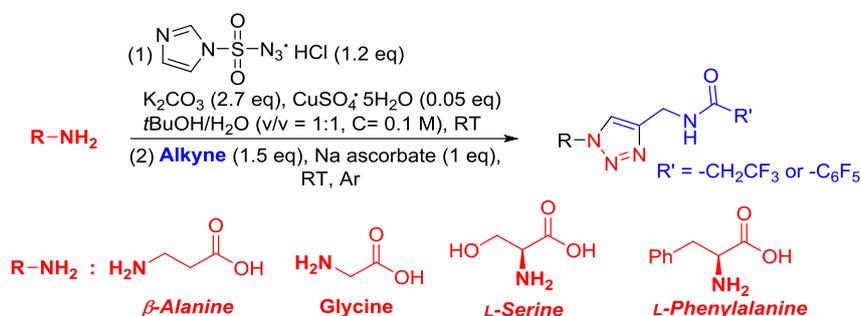


Figure 44. Structural similarities between Z-amide bond and the 1,4-disubstituted 1,2,3-triazole moiety.

In addition to the properties of fluorine atoms, these latter considerations convinced us to apply our one-pot diazotransfer/CuAAC sequence to free amino acids using fluorinated alkynes as partners of the cycloaddition reaction (Scheme 35).



Scheme 35. Application of the one-pot preparation of organofluorine amino acids.

III.3.1. One-pot diazotransfer-CuAAC reaction cascade applied to amino acids

By applying the aforementioned one-pot diazotransfer/CuAAC sequence to a series of unprotected amino acids and fluorinated alkynes, due to a high polarity of amino acids and corresponding azido or triazolyl derivatives, the standard monitoring of reactions by TLC was replaced by a ^1H NMR determination of the time of completion for each reaction. For this purpose, at specific reaction time, the magnetic stirring was stopped and, after decantation of the reaction media, an aliquot of the solution was taken up and diluted into D_2O . For example, using β -alanine as starting amino acid, the characteristic chemical shifts of $-\text{CH}_2-$ groups at α and β positions of the carboxyl moiety, respectively noted H-2 and H-3 on ^1H NMR spectra, allowed a good monitoring of the reaction completion (Figure 45b). By using 5 mol% of copper(II) sulfate as catalyst, 1.2 equivalents of ISA.HCl in a mixture of $t\text{BuOH}/\text{H}_2\text{O}$ (v/v = 1:1), the diazotransfer was completed in less than one hour as confirmed by the displacement of the H-3 signal from 3.11 ppm to 3.47 ppm (Figure 45c). After addition of 1.5 equivalent

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of alkyne and 1 equivalent of sodium ascorbate to the solution of formed azide, completion of the CuAAC was monitored by the appearance of the triazole proton H-4 signal at 7.84 ppm and displacement of the H-3 signal from 3.47 ppm to 4.58 ppm. Finally, even we observed an overlapping of the H-9 signals of the desired compound and excess of alkyne (Figure 45a vs. 45d), thanks to the characteristic signal H-4 of terminal alkynes, we observed that after 2 hours of reaction, only 0.5 equivalent of alkyne remained, corresponding to the excess used in the reaction (Figure 45d).

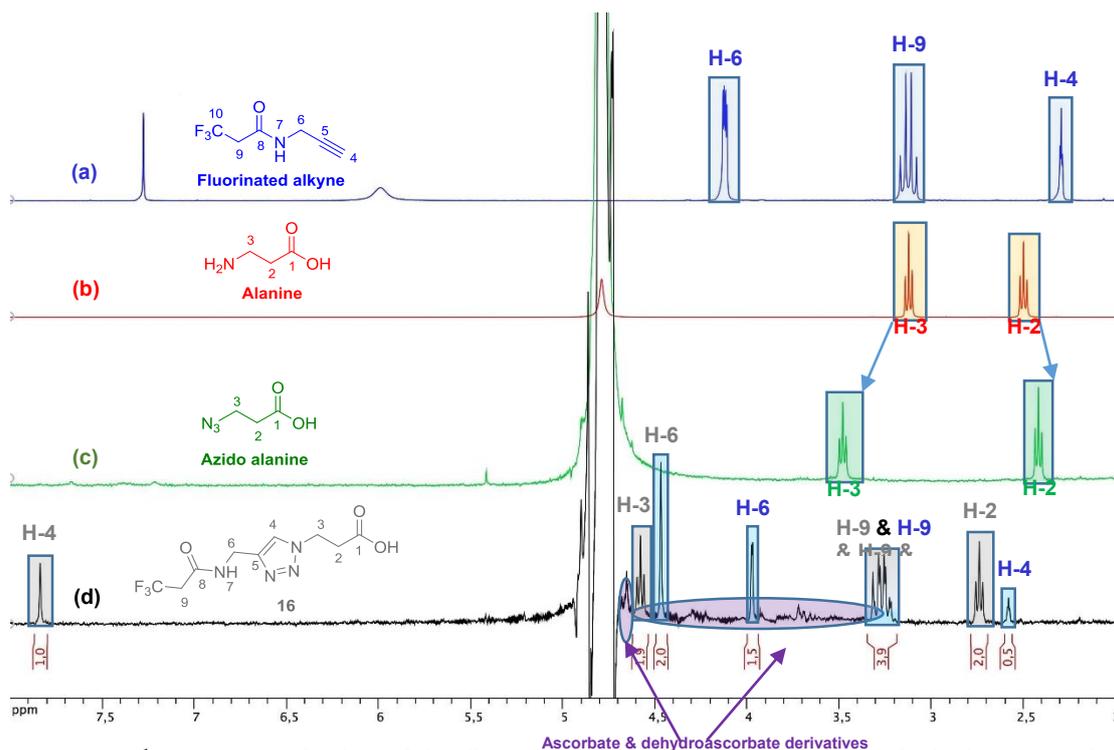
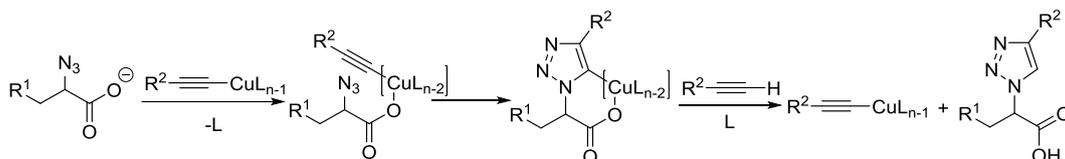


Figure 45. ^1H NMR monitoring of the diazotransfer/CuAAC sequence using β -alanine as starting amino acid. (a) In blue, the ^1H NMR spectrum of fluorinated alkyne; (b) In red, the ^1H NMR spectrum of β -alanine; (c) In green, ^1H NMR spectrum of the reaction media after 1h of diazotransfer reaction; (d) In black, ^1H NMR spectrum after 2h of CuAAC reaction.

Applying this ^1H NMR monitoring to the four amino acids engaged into the one-pot reactions sequence, we observed that the amino group was fully converted into azido group in less than 1 hour. Concerning the formation of the triazolyl derivatives, whatever the alkyne used, the CuAAC reaction was also completed in less than one hour, except for β -alanine that required to extend the time of reaction with alkyne **12** to 2 hours as in the case of D-glucosamine. We postulated that the acceleration of reaction rate in the case of glycine, L-serine and L-phenylalanine was due to the carboxylate group of the *in situ* formed α -azido acids that can act as a chelation site for copper to accelerate the CuAAC reaction (Scheme 36).

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Scheme 36. Putative chelation-assisted catalysis of CuAAC reaction using L-amino acids. For reason of simplicity, we represented a monomeric acetylide complex instead of the actual catalytic dimeric one.

Indeed, as demonstrated by Brotherton and coworkers in 2009, the concept of chelation-assisted catalysis is a complementary approach to ligand-based acceleration of reaction rate in CuAAC reactions.⁴²⁷ Further mechanistic study demonstrated that organic azide bearing a copper-chelating moiety greatly enhanced the association of azide with the catalytic copper center, lowering the kinetic barrier of nucleophilic addition of the acetylide to form the key metallacycle intermediate (*vide infra*).^{205,428}

III.3.2. Optimization of the final purification

Even if preparation of 1,4-disubstituted triazoles derived from amino acids are generally performed using fully protected azides and alkynes,⁴²¹ such strategy does not fulfill with the concept of Click Chemistry due to the requirement of additional protection-deprotection operations and cumbersome chromatographic separations. To consolidate the pertinence and the value of our one-pot process, we looked for the implementation of a simple purification protocol to circumvent those problems. Inspired by the work of Delmas and coworkers onto the preparation of N₃Asp(OtBu)-OH and N₃Lys(Boc)-OH,⁴²⁹ we decided to use the acid-base properties of the carboxylic moiety to establish an extractive protocol. Nonetheless, in comparison with Delmas, we not only had to remove copper salts, ISA salts and imidazole-1-sulfonamide but also water-soluble ascorbate derivatives and excess of alkyne reactant. For this purpose, after completion of the reactions sequence, the resulting mixture was diluted with water and washed with DCM to firstly remove the excess of alkyne. Due to the basic pH of the aqueous phase, the slight excess of ISA and the corresponding sulfonamide were also mainly removed during this step as shown by the ¹H NMR spectrum of the dried

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and concentrated organic phase (Figure 46a vs. 46b). Then, the aqueous phase was acidified to pH = 1~2 with a 1N HCl solution and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated to mostly recover the desired triazolyl acid derivative (Figure 46c).

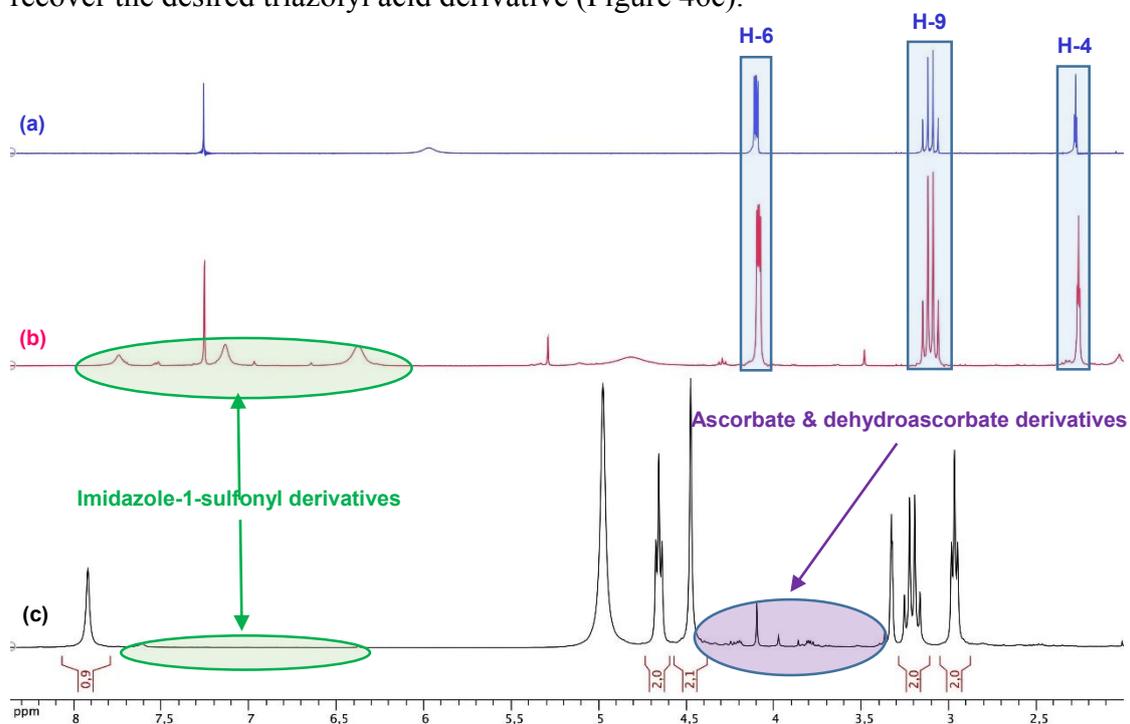


Figure 46. ¹H NMR monitoring of the extractive process of purification of **16**. (a) In blue, the ¹H NMR spectrum of fluorinated alkyne in CDCl₃; (b) In magenta, the ¹H NMR spectrum of the washing organic phase in CDCl₃; (c) In black, the ¹H NMR spectrum of the extractive organic phase in MeOD for a better visualization of ascorbate and dehydroascorbate derivatives as well as traces of imidazole-1-sulfonyl derivatives.

As the final step of the purification process, we attempted to recrystallize triazolyl acids with different pure or mixture of solvents. Disappointingly, even if the organofluorine amino acids can be recrystallized in water, this final purification can only be applicable for gram scale synthesis due to the low volume of water required for a good recrystallization.

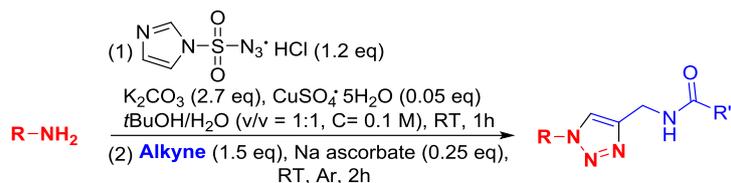
Considering that the major side products to separate from desired products are ascorbate derivatives such as dehydroascorbates, a first solution should be to decrease the amount of used sodium ascorbate to perform the CuAAC reaction. From few control experiments, we observed that at least a loading of 0.25 equivalent of sodium ascorbate is sufficient for the completion of the cycloaddition of α -azido acids with fluorinated alkynes. However, we assumed that this amount of reducing agent is probably applicable only to chelation-assisted accelerated cycloaddition and we still observed traces of ascorbate derivatives after extraction.

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So, after the work-up procedure, instead of a recrystallization, we added a filtration step on Florisil[®]. Florisil[®] is a synthetic magnesium silicate adsorbent (MgO:SiO₂ ratio 15:85) used for the purification of polyols, such as carbohydrates, and nitrogen compounds. After drying and concentration of the organic phase, the obtained crude product was solubilized into a minimal volume of DCM/MeOH (v/v = 4:1) and deposited onto the pad of Florisil[®] (4 mL/mmol of product to extract). After washing the pad of Florisil[®] with 20 volumes of DCM/MeOH (v/v = 4:1) to remove ascorbate derivatives, the desired pure triazolyl acid was eluted with 20 volumes of DCM/MeOH (v/v = 1:1). With this complete one-pot sequence of transformation of amino acids into triazolyl acid derivatives as well as the adapted purification procedure, we used it for the preparation of 8 different triazolyl acids (Table 5).

Whatever the amino acid and alkyne used, good to excellent isolated yields were obtained. In the case of glycine and β -alanine, the use of *N*-propargyl trifluoropropyl- or pentafluorobenzylamide gave similar yields (Table 5, entries 1 vs. 2 & 3 vs. 4). In the case of L-phenylalanine, due to the increased lipophilicity of **22** compared to **21**, we assumed that the drop in isolated yield should be explained by a loss of product during the DCM washing of the reaction media (Table 5, entry 6 vs. 5). The only way to prove this assumption is to show that the product is indeed present in the CH₂Cl₂ phase by the use of L-serine. In this case, if the higher hydrophilicity limited the loss of product during the first washing, the recovery of it from the Florisil[®] pad was difficult due to more important interactions with the adsorbent and probably a loss of **19** on it (Table 5, entry 7 vs. 8).

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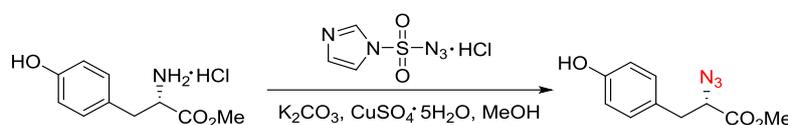
Entry	Amino Acid RNH ₂	Alkyne	Product	Yield (%)
1				86%
2				84%
3				78%
4				84%
5				100%
6				88%
7				78%
8				87%

Table 5. Prepared triazolyl acids using our optimized reactions conditions and purification process. All reactions were performed using 0.5 mmol of amino acids as starting material.

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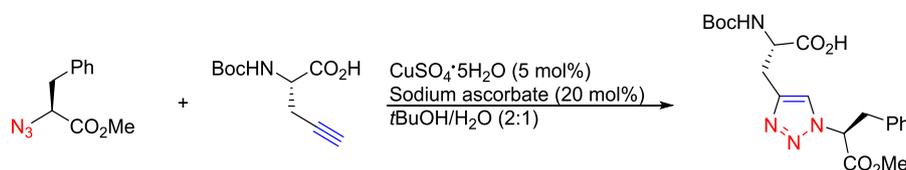
III.3.3. Verification of the Retention of Configuration of Amino Acids

For a successful implementation of our one-pot sequence in peptidic synthesis or preparation of chiral α -triazolyl acids, optical pure compounds must be obtained after our one-pot procedure with retention of configuration of chiral center. In 2007, Goddard-Borger and coworkers demonstrated that, under conditions of their diazotransfer reaction using ISA.HCl, no racemization was observed when the methyl ester of L-tyrosine hydrochloride was used as starting material (Scheme 37).³⁷⁰ A similar observation was done by Pelletier and coworkers when they reported the preparation of α -azido acids using the Wong's copper(II)-catalyzed diazotransfer method⁴³⁰ and by Delmas and coworkers using the Goddard-Borger's one.⁴²⁹



Scheme 37. Retention of the chiral center of tyrosine methyl ester using ISA.HCl under diazotransfer conditions developed by Goddard-Borger and coworkers.

In 2010, with the preparation of AMPA analogues, Abell and coworkers demonstrated that no epimerization of chiral center was detected when α -azido acids were used as partner in CuAAC reactions (Scheme 38).⁴³¹



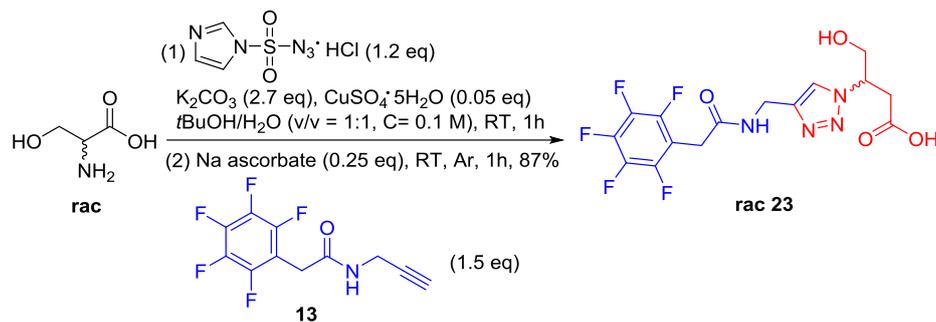
Scheme 38. Synthesis of 1,4-disubstituted triazolyl amino acids by Cu(II)-catalyzed cycloaddition described by Abell and coworkers.

In light of these results, we assumed that no epimerization should happen during our one-pot sequence. To confirm this hypothesis, D,L-serine was engaged into the reactions sequence using alkyne **13** as partner of the CuAAC reaction. As for pure L-serine, the racemic mixture of **23** was obtained in 87% overall yield (Scheme 39).

430. Lundquist IV, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781–783.

431. Stanley, N. J.; Pedersen, D. S.; Nielsen, B.; Kvist, T.; Mathiesen, J. M.; Bräuner-Osborne, H.; Taylor, D. K.; Abell, A. D. *Bioorganic Med. Chem. Lett.* **2010**, *20*, 7512–7515.

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Scheme 39. Application of the one-pot azide formation/CuAAC reaction sequence to the racemic mixture of serine.

HPLC analysis confirmed that the previously synthesized compound **23** is enantiomerically pure (Figure 47).

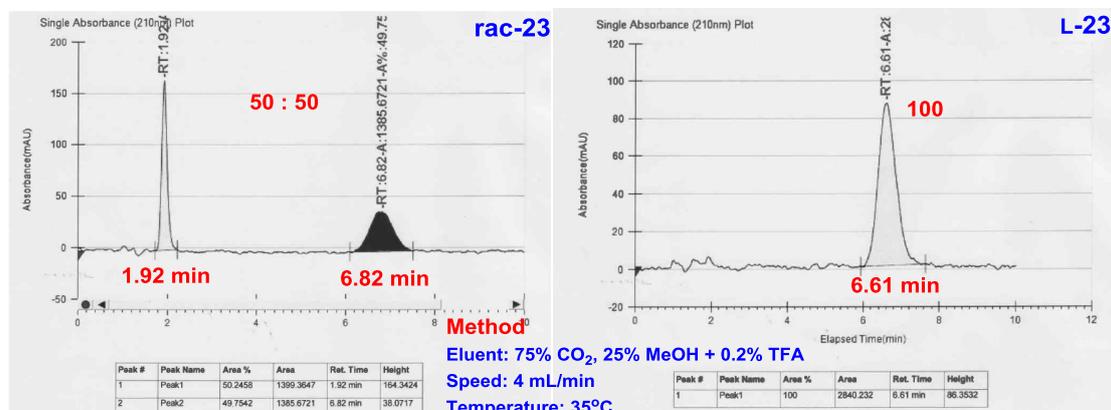


Figure 47. HPLC analysis of compound **23** & racemic **23**.

III.3.4. Transposition of the completely optimized protocol to amino carbohydrate derivatives

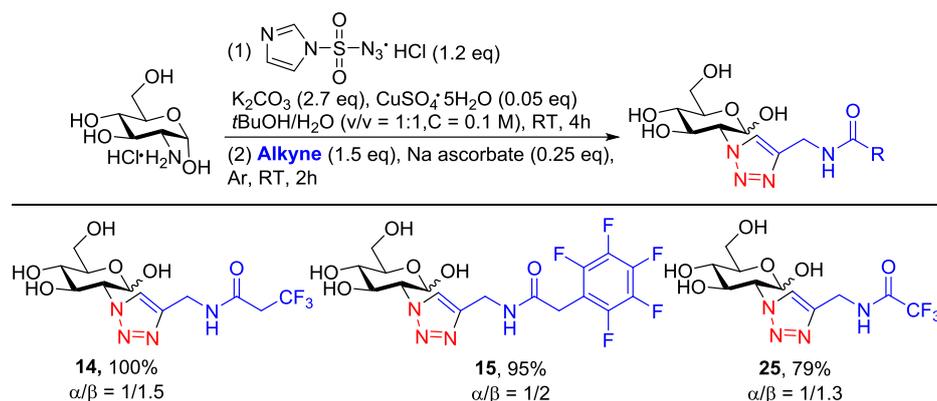
III.3.4.1. Application to D-glucosamine hydrochloride

After the optimization of the purification protocol on amino acids, we evaluated its efficiency to purify carbohydrate triazolyl derivatives. Compared with amino acids, carbohydrate derivatives cannot be extracted from aqueous phase by using acid-base properties and most of it stayed in water along with copper ions and ascorbate derivatives. After monitoring of the completion of the one-pot sequence by TLC, the resulting solution was stirred with QuadraPure™ metal scavenger resin (QuadraPure TU, 1 mg/mL) to remove copper ions. After filtration of the solution and washing of the resin with water, the combined aqueous solution can be extracted with DCM to remove the excess of alkyne as well as imidazole-1-sulfonyl derivatives but this step is

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not required due to the great difference of polarity of products to separate: the aqueous phase was concentrated under reduced pressure, took up in a minimum of DCM/MeOH mixture (v/v = 8:1) and deposited on a plug of Florisil[®], washed with 20 volumes of DCM/MeOH (v/v = 8:1) to remove excess of alkyne, imidazole-1-sulfonyl and ascorbate derivatives. The desired pure triazolyl acid was eluted with 20 volumes of DCM/MeOH (v/v = 4:1). to get the pure D-glucosamine triazolyl derivative.

With this purification protocol in hand, we carried out the one-pot diazotransfer/CuAAC sequence using three different fluorinated alkynes as partner of the cycloaddition reaction. (Scheme 40). With alkynes **12** and **13**, the one-pot sequence gave almost quantitative yields (> 95%). Using the base-sensitive 2,2,2-trifluoro-*N*-(prop-2-yn-1-yl)acetamide **24** as alkyne partner for the CuAAC reaction, the corresponding click product **25** was obtained with a moderate yield of 79%, probably due to the base sensitivity of the trifluoroacetamide moiety that conducted to a loss of a part of the desired product.



Scheme 40. One-pot model reaction with different alkynes.

As expected for reducing sugars, the 1H NMR data showed the presence of α/β two anomers. For example, as depicted in Figure 48, the formation of the triazolyl compound **15** was confirmed by the chemical shifts of the H-7 proton of the two formed diastereoisomers, 8.01 ppm and 8.06 ppm, in a 1:2 integration ratio. This ratio is also measured by integration of the two H-1 signals, 5.44 ppm for the α isomer and 5.25 ppm for the β one, or the two H-2 signals, 4.39 ppm for the α isomer and 4.34 ppm for the β isomer.

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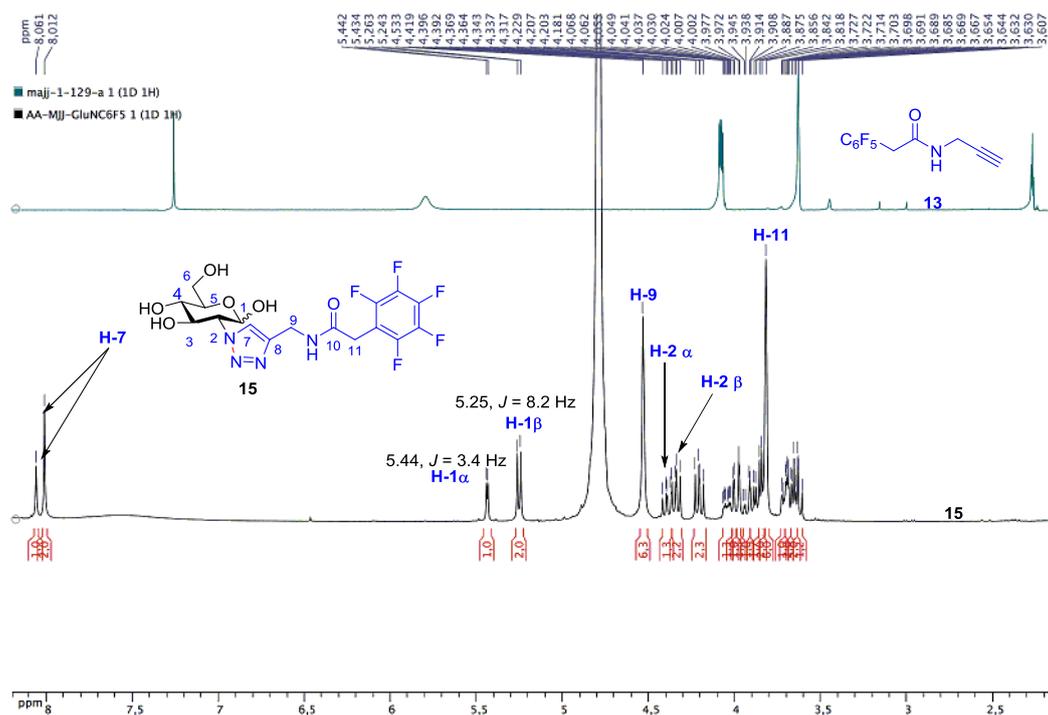
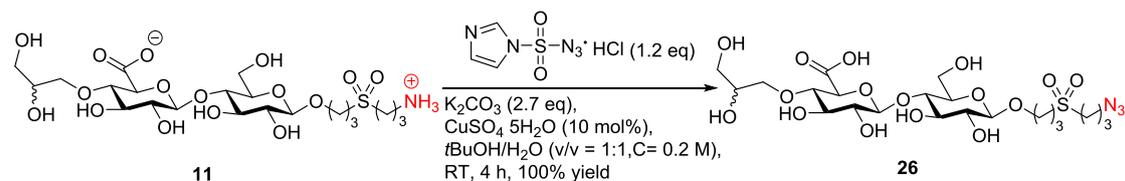


Figure 48. ^1H NMR of the α/β mixture of the obtained compound **15**.

With these results in hand, we decided to apply our one-pot sequence to our model disaccharide to prepare its fluorinated triazolyl derivatives.

III.3.4.2. Application to the model disaccharide **11**

First, we verify that our optimized conditions of diazotransfer reaction allow the full conversion of **11** into azide **26**. However, with a 0.1 M solution of disaccharide, whatever the combination of solvent, loading of copper catalyst (5 or 10 mol%) and reaction temperature, only traces of product were afforded after days. Therefore, we increased the concentration to 0.2 M and, satisfyingly, in presence of 10 mol% of copper(II) sulfate, the primary amine **11** was fully converted into azide **26** in 4 hours and could be quantitatively isolated (Scheme 41).

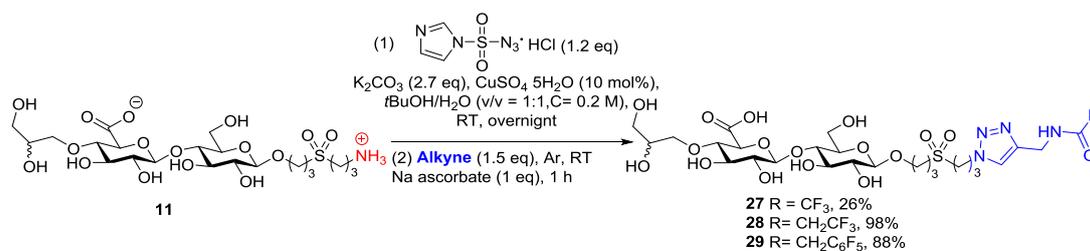


Scheme 41. Preparation of compound **26** via copper-catalyzed diazotransfer reaction.

Subsequently, with this diazotransfer conditions, we carried out three one-pot

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azide formation-CuAAC reaction cascade using the three fluorinated alkynes to prepare the corresponding organofluorine disaccharides **27** to **29**. To insure the full conversion of amine into azide (TLC monitoring using EtOH/H₂O = 6:3 as eluent), the first step was run overnight. Then, 1.5 equivalents of the alkyne (**14**, **15** or **24**) and 1 equivalent of sodium ascorbate were sequentially added. After 1 hour of reaction, the azide was fully converted into the corresponding organofluorine disaccharide **27** to **29** (TLC monitoring using EtOH/H₂O = 6:1 as eluent). After removal of copper ions using the QuadraPure TU resin, the obtained solution was filtrated, concentrated, deposited onto a G-15 Sephadex column to isolate the products. As for amino acids and D-glucosamine, the pentafluorobenzylamide and trifluoropropylamide derivatives, **29** and **27** respectively, were isolated in excellent yield whereas the trifluoroacetamide derivative **28** was recovered with a poor yield of 26% probably due to the instability of the trifluoroacetamide moiety in basic media (Scheme 42).

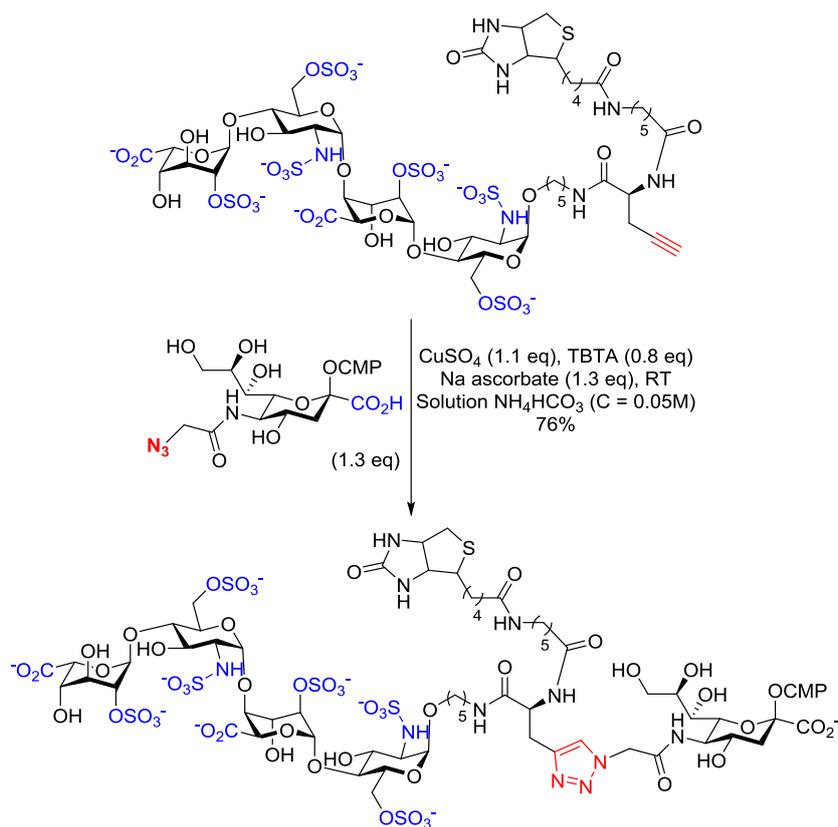


Scheme 42. Preparation of compound **27** to **29** in one-pot CuAAC reaction.

We assumed that the loss of reactivity observed during the diazotransfer should be due to chelation of copper ions to the carboxylate moiety of **11** and that sulfate groups of HS fragments should also represent chelating groups and will reduce the efficiency of our one-pot sequence when apply to them. During the course of our study, in 2017, Boons and coworkers reported the application of CuAAC reaction for the synthesis of a synthetic HS fragment glycoconjugate. In this example, an excess of copper(II) sulfate and sodium ascorbate were used as well as addition of TBTA as copper ligand (Scheme 43) but no explanation of the catalytic loading of copper was given into the publication.⁴³²

432. Capicciotti, C. J.; Zong, C.; Sheikh, M. O.; Sun, T.; Wells, L.; Boons, G.-J. *J. Am. Chem. Soc.* **2017**, *139*, 13342–13348.

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Scheme 43. Application of CuAAC reaction on synthesis of HS oligosaccharides reported by Boons and coworkers.

So we planned to investigate the possibility of using catalytic amount of copper(II) sulfate for the modification of HS fragments and to decipher possible limitation of a one-pot sequence of transformation of a primary amine into a triazolyl moiety.

III.3.4.3. Evaluation of the limitation of the one-pot process due to the presence of chelating groups

Supposing that in the case of Boons' example the requirement of excess of copper(II) sulfate should be due to chelating properties of sulfate and carboxylate groups of the HS fragments, we investigated the effect of these functional groups on the preparation of triazolyl **14**. For this purpose, we added sodium acetate or sulfate derivatives, sodium sulfate or persulfate allyl- β -D-lactose, to the reaction media to mimic the effect of these chelating moieties. We also looked at the effect of addition of triazolyl-type ligand onto the rate of reaction of the one-pot process. All reactions were run using 0.05 equivalent of copper(II) sulfate as catalyst and 1 equivalent of sodium ascorbate (Table 6).

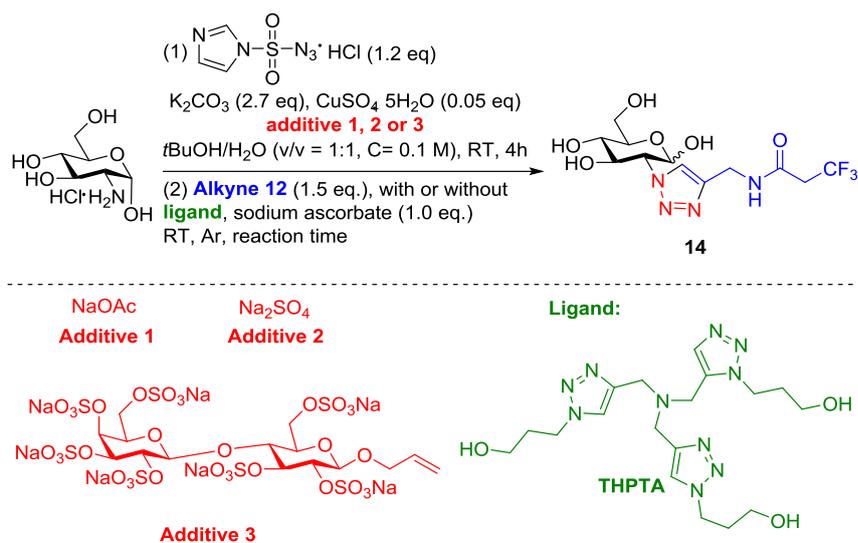
When one equivalent of sodium acetate was added to the starting solution to mimic

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the chelating effect of carboxylate groups, even if the diazotransfer proceeded well, the CuAAC reaction was slowed down (Table 6, entry 2 *vs.* 1). To restore the complete activity of copper catalyst, we added THPTA ligand to the second step of the transformation. Addition of the same amount of ligand than catalyst permitted to accelerate the CuAAC reaction (Table 6, entry 3 *vs.* 2) whereas the use of a greater amount of ligand did not speed up the reaction (Table 6, entry 4 *vs.* 3). To verify this observation, two control experiments were run and demonstrated that the quantity of ligand used was an important parameter of the CuAAC reaction: in absence of additive, when THPTA ligand was added in equal amount than copper catalyst, the reaction rate was increased (Table 6, entry 5 *vs.* 1) while an excess of ligand seems to block the accelerating effect of ligand, probably by sequestering copper ions (Table 6, entry 6 *vs.* 5). These results confirmed that a too important quantity of ligand can affect the reaction of cycloaddition.

To evaluate the effect of HS fragments sulfate groups on the time of reactions of the one-pot sequence, sodium sulfate was firstly used and added into the reaction solution before the diazotransfer reaction. As indicated in entries 7 and 8, no effect on the one-pot procedure have been observed by using sodium sulfate as additive, whatever the added quantity. Nevertheless, the use of 0.5 equivalent of persulfate allyl- β -D-lactose as additive to the reaction solution conducted to the scavenging of copper(II) and blocked the diazotransfer reaction (Table 6, entry 9). Restoration of the catalytic activity of copper(II) for the diazotransfer reaction was obtained by addition of 0.05 equivalent of THPTA to the starting solution and the rate of the CuAAC step was strongly increased (Table 6, entry 10 *vs.* 1 & 9). By adding persulfate allyl- β -D-lactose without ligand prior to the second step, we observed the same increase of CuAAC reaction rate, suggesting that persulfate compounds were responsible of the observed acceleration rate of reaction (Table 6, entry 11 *vs.* 10).

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Entry	Reaction Conditions	Reacting Time (by TLC)
1	Without ligand and additive	2h
2	$NaOAc$ (1 eq)	2 h 40 min
3	$NaOAc$ (1 eq) + Ligand (0.05 eq)	1 h 40 min
4	$NaOAc$ (1 eq) + Ligand (0.1 eq)	2 h
5	Ligand (0.05 eq)	40 min
6	Ligand (0.1 eq)	2 h
7	Na_2SO_4 (1.0 eq)	2h
8	Na_2SO_4 (10 eq)	2 h
9	Additive 3 (0.5 eq)	No full diazotransfer
10	Ligand (0.05 eq) + Additive 3 (0.5 eq) simultaneously added	1 h 10 min
11	Additive 3 (0.5 eq) added for the second step	1 h 10 min

Table 6. Effects of additives and ligand on the CuAAC reaction rate of the one-pot sequence of transformation. Additives were added prior to the first step; Ligand was added concomitantly to sodium ascorbate and alkyne.

Finally, we verified that copper salts, due to their Lewis acid properties, would not conduce to the hydrolysis of *N*- and/or *O*-sulfate groups of HS fragments. For this purpose, we treated the experiment of entry 11 to verify the integrity of persulfate allyl- β -D-lactose during the one-pot procedure. Copper ions were removed from the reaction solution using the metal scavenger resinQuadraPure TU. Then, the solution was concentrated to remove *tert*-butanol, followed by Na^+ ion-exchange. The click product and the persulfated compound were presented in the final mixture as revealed by the 1H NMR of the obtained residue (Figure 49). Moreover, MS analysis confirmed the integrity of persulfate lactose and 1HNMR data revealed that the two compound were present as a 2:1 ratio, in accordance with the reaction loading (Figure 49).

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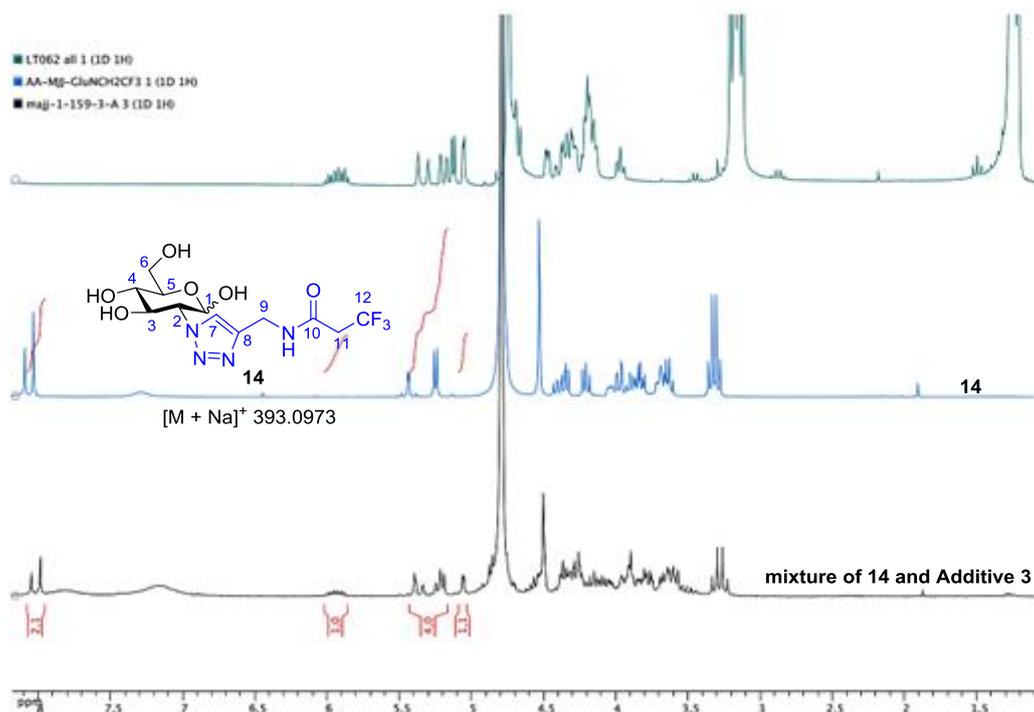
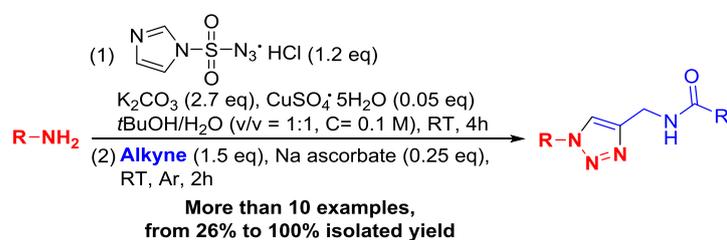


Figure 49. ¹H NMR of the obtained residue of entry 11.

IV. Summary

As discussed above, before applying the diazotransfer and CuAAC reactions to the model disaccharide and to HS fragments, we investigated the possibility of developing a one-pot sequence of azide formation/CuAAC reaction. Firstly, the reaction conditions were optimized using D-glucosamine hydrochloride and amino acids as primary amine and fluorinated alkynes as partners of the cycloaddition reaction. Under optimized reaction conditions, we synthesized several fluorinated triazolyl derivatives (Scheme 44) and set up a simplified protocol of isolation of fluorinated derivatives.



Scheme 44. Preparation of fluorinated triazolyl derivatives of amino acids and D-glucosamine using our optimized reactions conditions and purification process.

Moreover, due to observation of possible limitation of the one-pot process in presence of copper chelating groups, we evaluated the limitation of our one-pot process

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in presence of carboxylate and sulfate groups on the rate of the copper-catalyzed one-pot procedure. We observed that carboxylate groups slowed down the diazotransfer reaction whereas sulfate groups totally blocked this reaction. Addition of a equimolar quantity of triazolyl ligand THPTA permitted to restore the catalytic activity of copper(II) salts for the diazotransfer reaction, sped up the 1,3-dipolar cycloaddition and confirmed to us that a catalytic loading of copper and ligand should be required to apply our one-pot sequence to modified HS fragments.

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

I. Oxidative Cleavage of Diol

I.1. General Introduction On Oxidative Cleavage of Vicinal Diol

The oxidative carbon-carbon bond cleavage of vicinal diols, or glycol cleavage is a very important and frequently used transformation in synthetic organic chemistry with a broad array of applications (Scheme 45). Up to now, not only stoichiometric but also catalytic oxidants as well as metal-free approaches have been developed to carry out such oxidative cleavage.^{433,434}



Scheme 45. Oxidative cleavage of vicinal diol.

I.1.1. Stoichiometric procedures

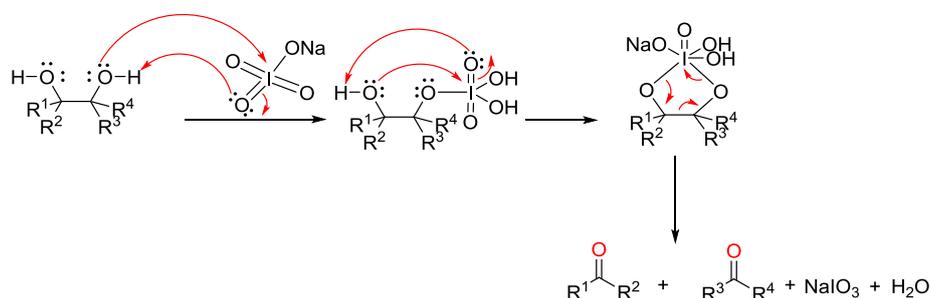
One of the two major reagents used for oxidative cleavage is periodic acid or its periodate salts, and is also known as Malaprade reaction.⁴³⁵ The glycol cleavage reaction using sodium periodate (NaIO_4) is generally rapid under mild conditions and gives aldehydes and ketones in quantitative yields. Significantly, the generated carbonyl compounds are inert towards further oxidation under classical reaction conditions. Sodium periodate is a stable, easy to handle and commercially available solid. It can be used in water or aqueous organic solvents^{436,437} and is compatible with a wide range of chemical groups.⁴³⁸ Even if it can be used in stoichiometric quantities, an excess of reagent, i.e. 1.2-1.5 equiv is frequently employed to reduce the time of reaction.

In general, the oxidative cleavage of vicinal diols using sodium periodate proceeds

-
433. Perlin, A. S. *Adv. Carbohydr. Chem. Biochem.* **2006**, *60*, 183–250.
 434. Schmidt, A. K. C.; Stark, C. B. W. *Synthesis.* **2014**, *46*, 3283–3308.
 435. Malaprade, L. *Bull. Soc. Chim. Fr.* **1928**, *43*, 683–696.
 436. Domingos, J. L. D. O.; Vilela, G. V. M. D. A.; Costa, P. R. R.; Dias, A. G. *Synth. Commun.* **2004**, *34*, 589–598.
 437. Vu, C. C.; Peterson, L. A. *Chem. Res. Toxicol.* **2005**, *18*, 1012–1017.
 438. Fleet, G. W. J.; Shing, T. K. M. *J. Chem. Soc. Chem. Commun.* **1984**, 835, 835–837.

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via a cyclic mechanism, involving the formation of a five-membered ring intermediate between the diol and the oxidant that oxidatively collapses to the corresponding carbonyl compounds (Scheme 46).⁴³⁴



Scheme 46. Accepted mechanism for Malaprade reaction.

The cleavage step is presumably concerted and the reaction is second-ordered. Moreover, the reaction rate is fairly sensitive to the relative stereochemistry of the reacting hydroxyl groups: (i) *cis*-diols are cleaved more rapidly than the corresponding *trans*-diols and *threo*-1,2-diols undergo oxidation faster than the *erythro* isomers, as the *trans*-diols can impede the formation of the cyclic intermediate due to structural constraints; (ii) vicinal diols that cannot form a cyclic intermediate, for example *diaxial trans*-1,2-diols, are inert to the reagent.^{439,440} Therefore, this reaction can be used to differentiate several vicinal diol moieties in the same molecule. Before the advent of modern spectroscopic instrumentation, sodium periodate was extensively used to elucidate the structure of carbohydrates. For example, the periodate mediated oxidative cleavage can be used to determine the prominent chair conformation (⁴C₁ or ¹C₄) of L-iduronate (Figure 50). Compared to ¹C₄ conformation of L-iduronate, the periodate mediated oxidative cleavage proceeds much faster when it is in ⁴C₁ conformation, since the cyclic intermediate can be formed much easily due to a small dihedral angle between the *O*-2 and *O*-3 substituents.⁴⁴¹

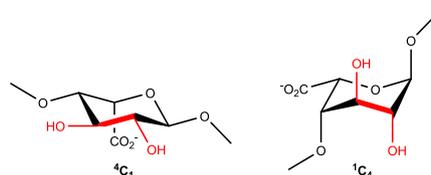


Figure 50. Two different chair conformations of L-iduronate.

439. Criegee, R.; Buchner, E.; Walther, W.; Geschwindigkeit, D.; Iv-acetat, B. *Chem. Ber.* **1940**, *73*, 571–575.

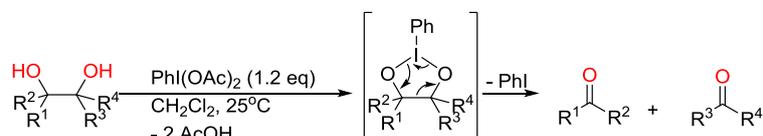
440. Bergel, F.; Stock, J. A. *J. Chem. Soc.* **1957**, 4567.

441. Rees, D. A.; Morris, E. R.; Stoddart, J. F.; Stevens, E. S. *Nature* **1985**, *317*, 480.

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

In order to carry out periodic oxidation in non-aqueous media, heterogeneous reactions with NaIO₄-supported silica, easily prepared by adding silica gel to aqueous sodium periodate⁴⁴² and homogeneous reactions with quaternary ammonium periodates, such as, (n-Bu)₄NIO₄, usually prepared *in situ* from tetrabutylammonium hydrogen sulfate and sodium periodate, have been used.^{443,444} Compared to aqueous periodate, these reagents are more convenient to use, due to a simplest way of isolation of corresponding carbonyl compounds: a simple filtration of the reaction solution and subsequent evaporation of solvent generally leads to compounds of sufficient purity for direct use in further transformation. Alternatively, polymer-supported quaternary ammonium periodate has been recently reported and used for the oxidative cleavage of vicinal diols.⁴⁴⁵

Besides periodate, other hypervalent iodine reagents have been reported to cleave 1,2-diols. One of them is phenyliodine diacetate (PIDA), which is commercially available and can also be used in organic solvent.⁴⁴⁶ Even though its ability to cleave vicinal diols was demonstrated by Criegee and Beucker seven decades ago, it is not commonly used for that ability⁴⁴⁷ until 2010, when Nicolaou and coworkers reported the first systematic study of PIDA as an oxidative cleavage reagent (Scheme 47).⁴⁴⁸

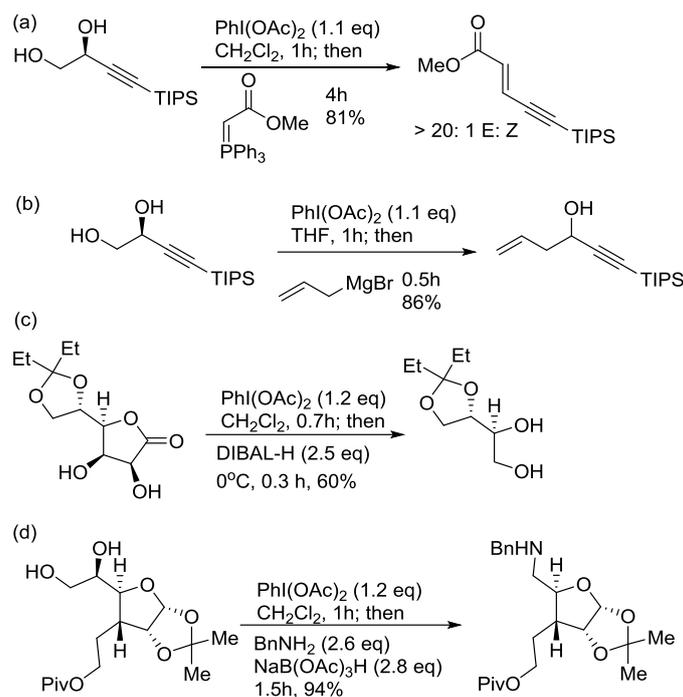


Scheme 47. Cleavage of vicinal diols to carbonyl derivatives by PhI(OAc)₂. This oxidation also proceeds *via* a cyclic intermediate and generated acetic acid as by-product.

The procedure involves slightly excess of PIDA (1.2 equiv) to give the corresponding carbonyl products. Significantly, this method only generates iodobenzene and acetic acid as secondary products and provides an opportunity for one-pot sequential reactions, such as oxidative cleavage followed by Wittig (Scheme 48a)/Grignard reaction (Scheme 48b)/carbonyl reduction (Scheme 48c) or reductive amination (Scheme 48d).

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442. Gupta, D. N.; Hodge, P.; Davies, J. E. *J. Chem. Soc. Perkin Trans. 1* **1981**, No. 2970, 2970–2973.
443. Arata, K.; Tanabe, K. *Bulletin of the Chemical Society of Japan*. 1980, pp 299–303.
444. Santaniello, E.; Manzochi, A.; Farachi, C. *Synth.* **1980**, No. 2, 563.
445. Harrison, C. R.; Hodge, P. *J. Chem. Soc. Perkin Trans. 1* **1982**, No. 0, 509–511.
446. Sharefkin, J. G.; Saltzman, H. *Org. Synth.* **1963**, 43, 62.
447. Criegee, R.; Beucker, H. *Justus Liebigs Ann. Chem.* **1939**, 541, 218–238.
448. Nicolaou, K. C.; Adsool, V. A.; Hale, C. R. H. *Org. Lett.* **2010**, 12, 1552–1555.

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation



Scheme 48. One-Pot sequential reactions consisting in a PhI(OAc)_2 mediated oxidative cleavage of 1,2-Diols prior to (a) Wittig olefination; (b) Grignard addition; (c) DIBAL-H reduction; (d) reductive amination.

Moreover, even if Dess-Martin periodinane (DMP) and IBX (2-Iodoxybenzoic acid) are generally used to oxidize alcohols into aldehydes or ketones, they can also allow oxidative cleavage of 1,2-diols.^{449–451} This process involves the irreversible formation of spirocyclic periodinane intermediates with vicinal diols that undergo oxidative fragmentation. This process spontaneously happens with DMP (Scheme 49a)^{450,451} while it requires assistance of trifluoroacetic acid (TFA) or dimethyl sulfoxide (DMSO) under elevated temperatures for IBX (Scheme 49b).⁴⁵²

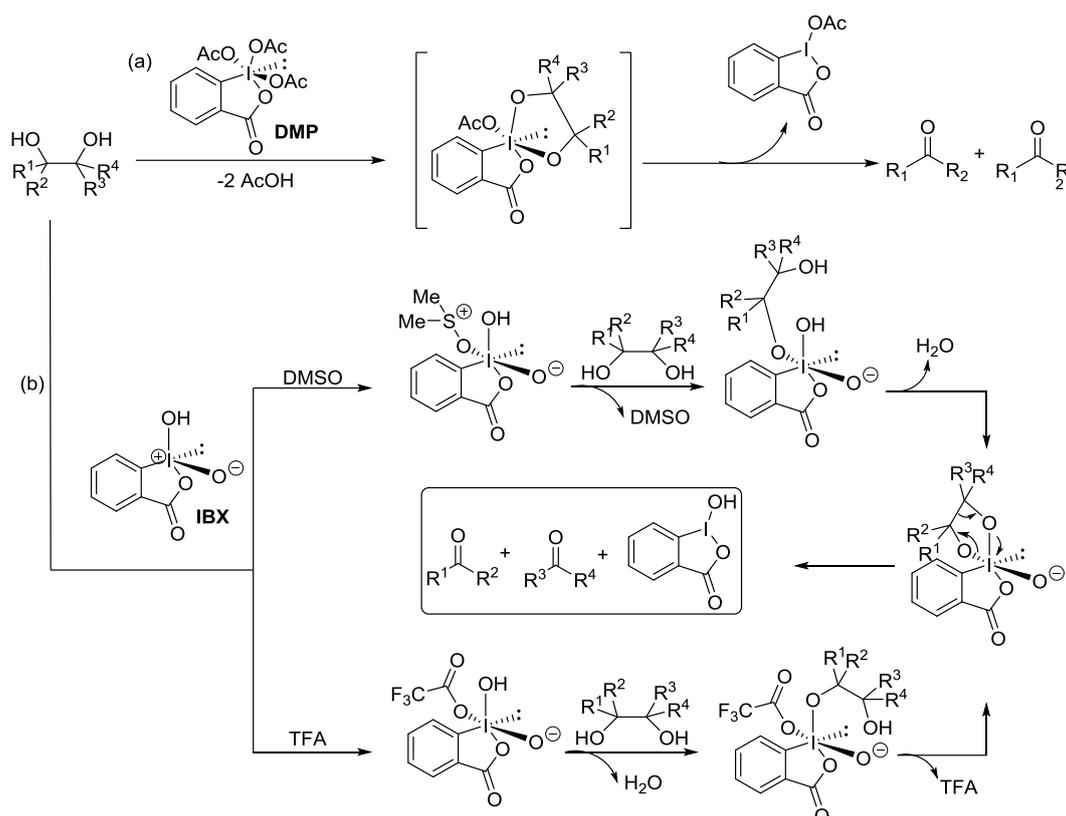
449. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.

450. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.

451. De Munari, S.; Frigerio, M.; Santagostino, M. *J. Org. Chem.* **1996**, *61*, 9272–9279.

452. Moorthy, J. N.; Singhal, N.; Senapati, K. *Org. Biomol. Chem.* **2007**, *5*, 767–771.

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Scheme 49. Mechanism of oxidative cleavage of 1,2-diols with DMP & IBX.

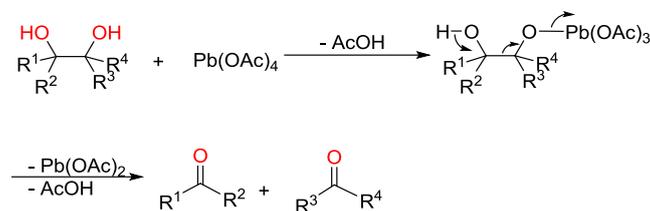
The other representative oxidant used for oxidative cleavage of vicinal diols is lead tetraacetate (LTA), also referred to as Criegee oxidation.⁴⁵³ Compared to aqueous Malaprade reaction, Criegee oxidation usually proceeds under mild conditions in organic solvents (aprotic solvents such as dichloromethane). The Criegee oxidation also affords aldehydes or ketones and the carbonyl products cannot be further oxidized under standard reaction conditions. In addition, LTA is less selective than periodate and can react with reactants that are inert to periodate, but an excess of reagent is frequently required compared to periodate mediated cleavage.

Consistent with Malaprade reaction, the Criegee oxidation also proceeds *via* a five-membered ring intermediate (Scheme 46).⁴⁵³ Support for such mechanism comes from the observations that the reaction is second-order and the addition of acetic acid decreased the reaction rate as well as *cis*-diols reacts substantially faster than the *trans* isomers. However, if the cyclic intermediate is too highly strained, the oxidation is able to proceed, probably *via* an acyclic, monodentate intermediate between diol and oxidant.⁴³⁴ Then, the acyclic intermediate directly converted into aldehydes and/or

453. Criegee, R.; Kraft, L.; Rank, B. *Justus Liebigs Ann. Chem.* **1933**, 507, 159–197.

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ketones in a two-electron oxidative fragmentation (Scheme 50). Therefore, vicinal diols oxidized through this mechanism is not sensitive to stereochemistry and cis- and trans-diols display similar reactivities and reaction rates.



Scheme 50. Acyclic mechanism with LTA involving a two-electron oxidative fragmentation.

Occasionally, other reagents such as chromium trioxide (CrO_3),⁴⁵⁴ pyridinium chlorochromate (PCC),⁴⁵⁵ manganese dioxide (MnO_2),^{456,457} N-iodosuccinimide (NIS),⁴⁵⁸ ceric ammonium nitrate (CAN)⁴⁵⁹ or sodium bismuthate,^{460,461} for example, have also been used to cleave vicinal diols. However, these oxidants led to the formation of toxic secondary products in equimolar quantities.

Up till to now, the two classical oxidants, periodate and LTA are still the reagents of choice for most synthetic transformations due to their exceptional efficiency, functional tolerance and wide range of applications in modern organic chemistry. Even though stoichiometric or excess quantities are required, it can be compensated by the high-value of starting materials and small-scale applications.

However, from the view point of green and atom-economical chemistry, new efficient catalytic methods for the oxidative cleavage of vicinal diols have been recently developed as an advantageous alternative to the classical aforementioned reagents. The catalytic procedure generally involves a metal catalyst and a sacrificial stoichiometric organic or inorganic oxidant.

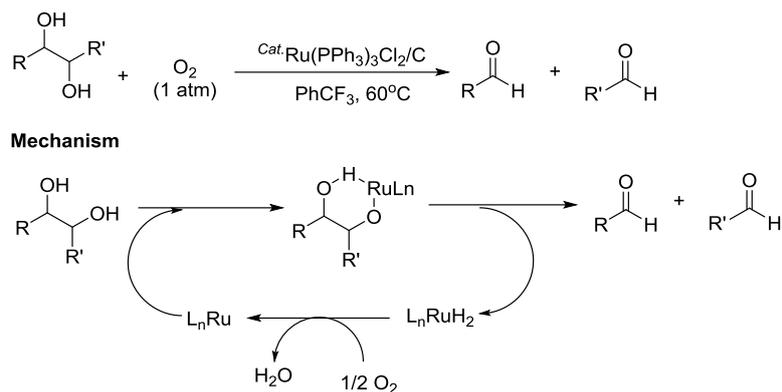
I.1.2. Catalytic procedures

To date, various reagent systems have been reported. One of them, reported by

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454. Rocek, J.; Westheimer, F. H. *J. Am. Chem. Soc.* **1962**, *84*, 2241–2246.
455. Cisneros, A.; Fernández, S.; Hernández, J. E. *Synth. Commun.* **1982**, *12*, 833–838.
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457. Outram, H. S.; Raw, S. A.; Taylor, R. J. K. *Tetrahedron Lett.* **2002**, *43*, 6185–6187.
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459. Trahanovsky, W. S.; Young, L. H.; Bierman, M. H. *J. Org. Chem.* **1969**, *34*, 869–871.
460. Rigby, W. *J. Chem. Soc.* **1950**, No. 0, 1907–1913.
461. Uskoković, M.; Gut, M.; Trachtenberg, E. N.; Klyne, W.; Dorfman, R. I. *J. Am. Chem. Soc.* **1960**, *82*, 4965–4969.

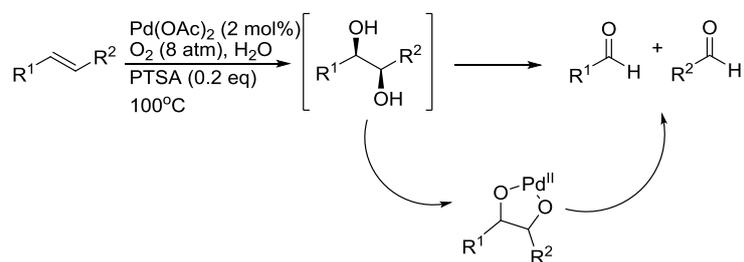
Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

Ishii and co-workers in 1999, is based on a combination of a catalytic amount of $\text{Ru}(\text{PPh}_3)_3\text{Cl}_2$ on active carbon and oxygen as oxidant (1 atm) (Scheme 51).⁴⁶² This system allowed to cleave a variety of vicinal diols to the corresponding aldehydes. It has been proposed that this reaction also proceeds through a cyclic mechanism, involves a six-membered intermediate, forming the corresponding aldehydes and a ruthenium hydride, the latter is oxidized by dioxygen to regenerate the original ruthenium complex and gives water as coproduct.



Scheme 51. Oxidative cleavage of vicinal diols by a combination of catalytic $\text{Ru}(\text{PPh}_3)_3\text{Cl}_2$ and O_2 .

In a similar way, in 2010, Jiang and coworkers developed a catalytic process for the oxidative cleavage of olefins using palladium diacetate as catalyst and oxygen as oxidant to afford the corresponding aldehydes (Scheme 52).⁴⁶³ It is important to note that acid was employed as an additive in this reaction. Even though the effect of this catalytic amount of acid has not been answered, the cleavage reactions of dioxo- Pd^{II} complexes indicate that 1,2-diols is an intermediate product to formation of the desired carbonyl derivatives.



Scheme 52. Oxidative cleavage of alkenes by palladium to give the corresponding aldehydes *via* a putative 1,2-diols intermediate.

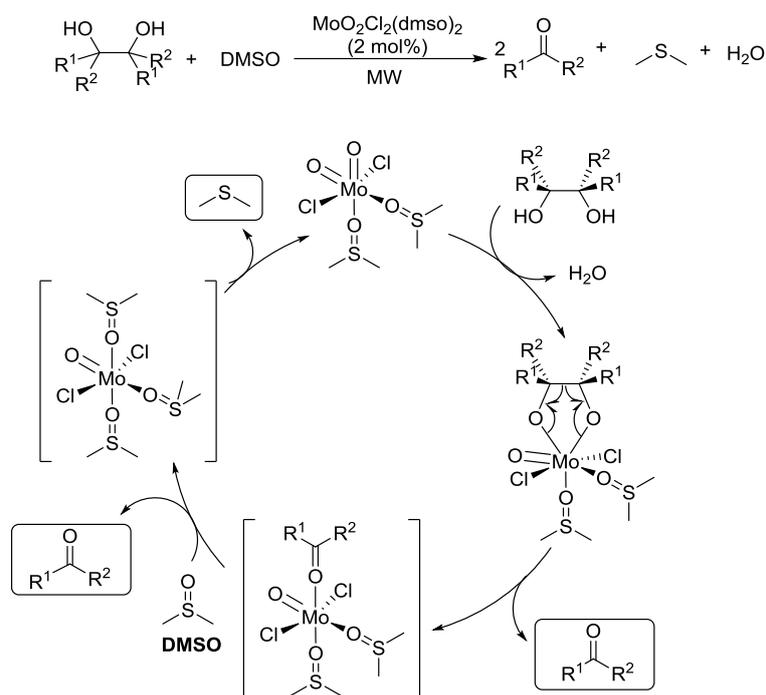
Few years later, in 2016, Sanz and coworkers reported a novel and efficient method

462. Takezawa, E.; Sakaguchi, S.; Ishii, Y. *Org. Lett.* **1999**, *1*, 713–715.

463. Wang, A.; Jiang, H. *J. Org. Chem.* **2010**, *75*, 2321–2326.

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for oxidative cleavage of vicinal diols using environmentally friendly $\text{MoO}_2\text{Cl}_2(\text{dmsO})_2$ as catalyst and non-toxic DMSO as oxidant (Scheme 53).⁴⁶⁴ All reported examples afforded solely the corresponding aldehydes and/or ketones, without observation of the overoxidation into the corresponding carboxylic acids. Additionally, with this method, the products could be isolated by a simple extraction, enabling recycle the catalyst. It has also been proposed that the cleavage proceeds *via* a five-membered intermediate. However, the reaction was performed under microwave irradiation in air and therefore a high temperature is required for the reaction.



Scheme 53. Oxidative cleavage of vicinal diols with a combination of $\text{MoO}_2\text{Cl}_2(\text{dmsO})_2$ as catalyst and DMSO as oxidant & proposed catalytic cycle.

In addition, catalytic procedures using copper,^{465,466} cobalt,^{467,468}

464. Garc ía, N.; Rubio-Presa, R.; Garc ía-Garc ía, P.; Fern ández-Rodr íguez, M. A.; Pedrosa, M. R.; Arn áz, F. J.; Sanz, R. *Green Chem.* **2016**, *18*, 2335–2340.

465. Sedai, B.; D áz-Urrutia, C.; Baker, R. T.; Wu, R.; Silks, L. A. P.; Hanson, S. K. *ACS Catal.* **2011**, *1*, 794–804.

466. Prati, L.; Rossi, M. *J. Mol. Catal. A Chem.* **1996**, *110*, 221–226.

467. Mastrorilli, P.; Suranna, G. P.; Nobile, C. F.; Farinola, G.; Lopez, L. *J. Mol. Catal. A Chem.* **2000**, *156*, 279–281.

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469. Felthouse, T. R. *J. Am. Chem. Soc.* **1987**, *109*, 7566–7568.

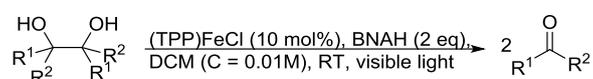
470. Shiota, Y.; Herrera, J. M.; Juh ász, G.; Abe, T.; Ohzu, S.; Ishizuka, T.; Kojima, T.; Yoshizawa, K. *Inorg. Chem.* **2011**, *50*, 6200–6209.

471. Shimizu, M.; Orita, H.; Suzuki, K.; Hayakawa, T.; Hamakawa, S.; Takehira, K. *J. Mol. Catal. A Chem.* **1996**, *114*, 217–220.

472. Alexander M. Khenkin, R. N. *Adv. Synth. Catal.* **2002**, *344*, 1017–1021.

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ruthenium^{462,469,470} or polyoxometalate complexes^{471,472} have also recently been reported. However, all these methods require a high loading of precious noble metals as catalyst and harsh reaction conditions. Driven by the need of mild conditions and the use of sustainable catalysts, catalysts based on more abundant metals, such as Fe,^{473–476} Mn^{477–480} and V^{481–489} complexes have been reported recently. For example, in 1988, Okamoto and coworkers reported an aerobic selective oxidative cleavage of vicinal diols to the corresponding carbonyl compounds using the biomimetic system of iron porphyrin complex, oxygen and l-benzyl-3-carbamoyl-1,4-dihydropyridine (BNAH).⁴⁷⁴ It was proposed that the iron catalyst was reversibly reduced by BNAH and with oxygen to cleave the vicinal diols into two carbonyl compounds (Scheme 54).



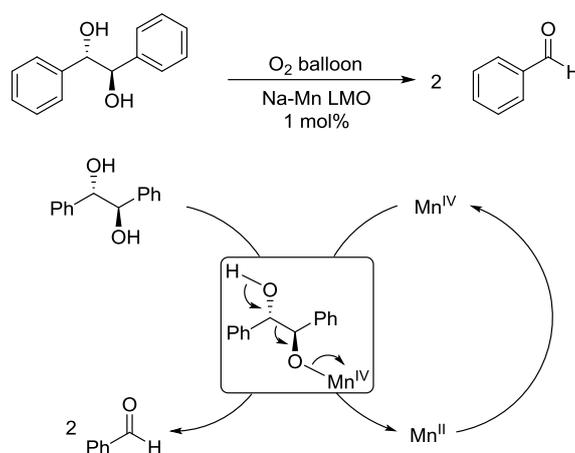
Scheme 54. Iron-complex catalyzed oxidative cleavage of vicinal diols.

In 2017, Escande, Anastas and coworkers reported a highly selective method for aerobic oxidative cleavage of 1,2-diols catalyzed by a sodium manganese layered oxide (Na-Mn LMO) to afford the corresponding carbonyl derivatives (Scheme 55).⁴⁸⁰ The procedure was operated at a high temperature of 100°C and produced water as the

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473. Okamoto, T.; Sasaki, K.; Shimada, M.; Oka, S. *J. Chem. Soc., Chem. Commun.* **1985**, 381–383.
474. Okamoto, T.; Sasaki, K.; Oka, S. *J. Am. Chem. Soc.* **1988**, *110*, 1187–1196.
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486. Amadio, E.; Di Lorenzo, R.; Zonta, C.; Licini, G. *Coord. Chem. Rev.* **2015**, *301–302*, 147–162.
487. Hanson, S. K.; Baker, R. T. *Acc. Chem. Res.* **2015**, *48*, 2037–2048.
488. Tomishige, K.; Tamura, M.; Hirasawa, S.; Obara, N.; Nakagawa, Y. *ChemCatChem* **2016**, *8*, 1732–1738.
489. Amadio, E.; González-Fabra, J.; Carraro, D.; Denis, W.; Gjoka, B.; Zonta, C.; Bartik, K.; Cavani, F.; Solmi, S.; Bo, C.; et al. *Adv. Synth. Catal.* **2018**, *360*, 3286–3296.

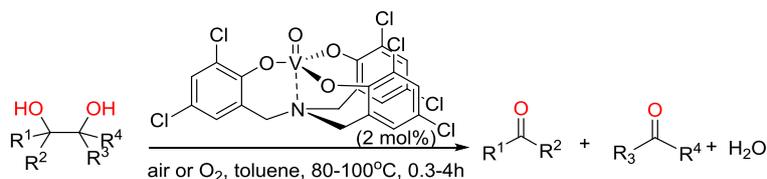
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only byproduct. This catalyst can be easily prepared from inexpensive and abundant manganese and sodium, can be recycled and reused several times without loss of activity. It is compatible with a wide range of functional groups and conditions, representing an efficient alternative to the classical reagents, such as, periodic acid or LTA, for the cleavage of vicinal diols. However, benzylic and allylic diols can be cleaved, aliphatic (linear or cyclic) diols were inactive. Mechanistic investigations indicated that the cleavage proceeds through an acyclic intermediate to the monodentate and a two-electron oxidative fragmentation process involving a monodentate Mn^{IV} complex.



Scheme 55. Na-Mn LMO catalyzed oxidative cleavage of vicinal diols.

Besides manganese, the earth-abundant vanadium based oxidative C-C cleavage of vicinal diols have also been thoroughly investigated.^{481–489} In 2018, Licini and coworkers reported an effective method for aerobic oxidative cleavage of vicinal diols catalyzed by vanadium amino triphenolates (Scheme 56).⁴⁸⁹ The reaction is highly selective with a wide scope of substrate, not only tertiary and benzylic diols but also cyclic and linear aliphatic diols (internal and terminal) were cleaved to afford the corresponding carbonyl compounds with high yields. This reaction can be considered as the most general and effective homogeneous catalytic system reported to date for the aerobic oxidative cleavage of vicinal diols (with catalyst loadings down to 0.001%, TONs of up to 81,000 and TOFs of up to 4150 h^{-1}).



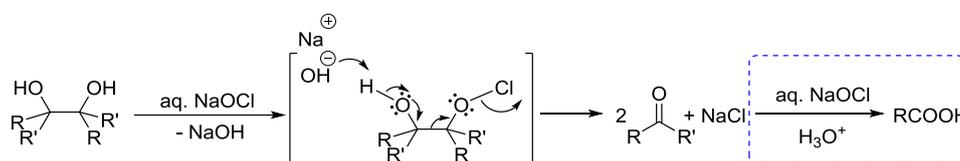
Scheme 56. Vanadium amino triphenolates catalyzed aerobic oxidative cleavage of vicinal diols.

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Compared to the stoichiometric procedures, the catalytic methods of oxidative cleavage of vicinal diols are of equivalent efficiency but more environmentally friendly, especially methods employing earth-abundant metal catalysts. However, most of these catalytic systems are non-recyclable and generally require a high temperature for a good conversion of diols into corresponding carbonyl derivatives. Therefore, oxidative cleavage of vicinal diols involving transition-metal-free reagent systems still remains a challenging issue for organic chemist.

I.1.3. Metal-free procedures

In 1989, a metal-free oxidative cleavage of vicinal diols with stoichiometric amounts of NaBO_3 as oxidant was reported by Banerjee and coworkers, but afforded the corresponding carboxylic acids.⁴⁹⁰ The reaction was operated under acidic conditions and the products could be isolated by a simple work-up. Then in 2007, Khurana and coworkers also reported a metal-free oxidative cleavage of vicinal diols with stoichiometric aqueous sodium hypochlorite (aq. NaOCl) as oxidant in acetonitrile (CH_3CN).⁴⁹¹ Even if the corresponding carboxylic acids could be easily formed (molar ratio of substrate to NaOCl is 1:20), the corresponding aldehydes and/or ketones can be generated as major products, if the molar ratio is 1:10 (Scheme 57).



Scheme 57. Metal-free oxidative cleavage of vicinal diols using excess NaOCl as oxidant.

Instead of using stoichiometric oxidants, Vinod and coworkers developed a catalytic metal-free protocol for oxidative cleavage of alkenes and vicinal diols to generate the corresponding ketone and carboxylic acid (Scheme 58).⁴⁹² The cleavage of alkenes was proposed to proceed *via* a diol intermediate, obtained by action of Oxone[®], that was oxidatively cleaved by the *in situ* generated [hydroxyl(4-carboxyphenyl)iodonium]ion (oxidation of the catalytic amount of 4-IBA in presence

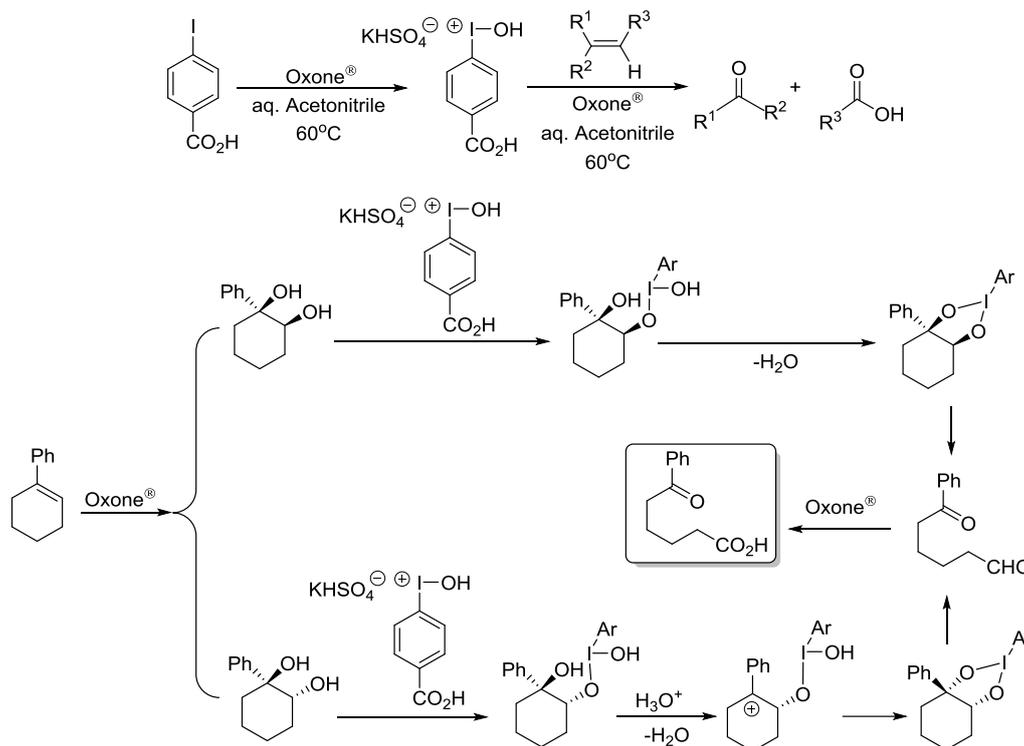
490. Banerjee, A.; Hazra, B.; Bhattacharya, A.; Banerjee, S.; Banerjee, G. C.; Sengupta, S. *Synthesis*. **1989**, No. 2, 765–766.

491. Khurana, J. M.; Sharma, P.; Gogia, A.; Kandpal, B. M. *Org. Prep. Proced. Int.* **2007**, 39, 185–189.

492. Thottumkara, P. P.; Vinod, T. K. *Org. Lett.* **2010**, 12, 5640–5643.

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of Oxone[®]) to give the corresponding carbonyl derivatives, themselves overoxidized into carboxylic acid by the excess of Oxone[®].

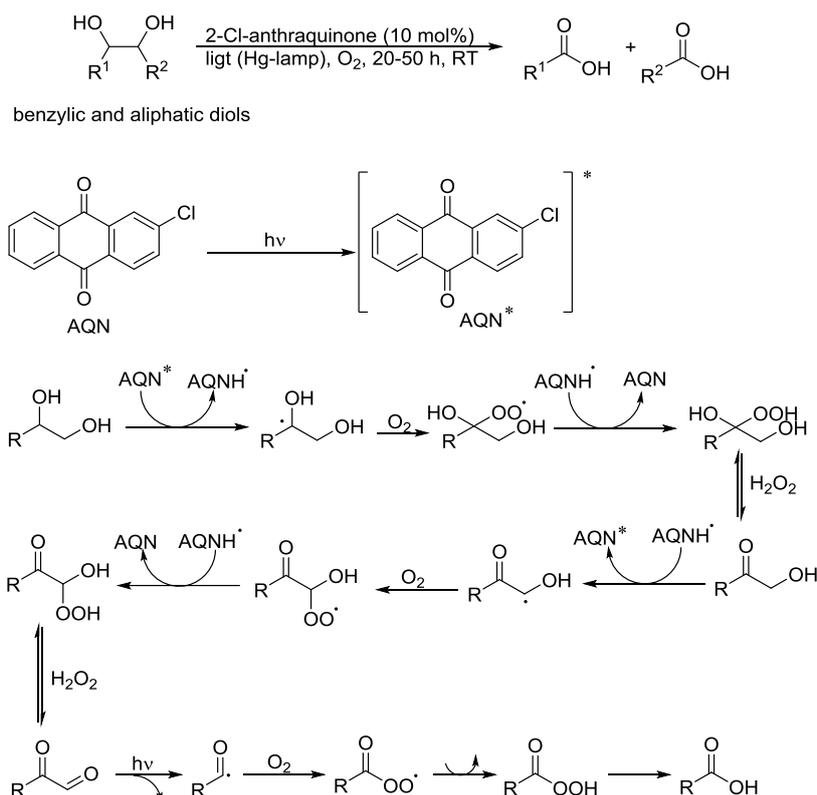


Scheme 58. Metal-free oxidative cleavage of vicinal diols with a catalytic amount produced hypervalent iodine ion and Oxone[®] as oxidant.

While the cleavage could proceed with no requirements of transition-metals, however, non-atom-economical stoichiometric oxidants were involved, thus producing large amounts of waste and leading to the carboxylic acid but not the aldehydes. Therefore, in 2012, Itoh and coworkers reported a catalytic aerobic photoirradiation cleavage of vicinal diols catalyzed by 2-chloroanthraquinone to the corresponding carboxylic acids.⁴⁹³ The reaction was photoirradiated by a high-pressure mercury lamp. Significantly, this is the first metal-free catalytic reaction using oxygen as the terminal oxidant (Scheme 59).

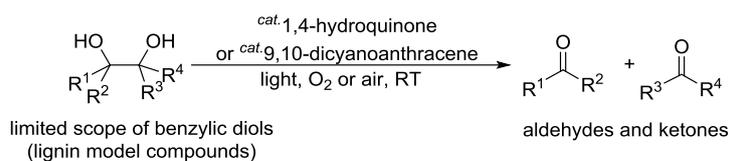
493. Matsusaki, Y.; Yamaguchi, T.; Tada, N.; Miura, T.; Itoh, A. *Synlett* **2012**, 23, 2059–2062.

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Scheme 59. Catalytic aerobic photoirradiation cleavage of vicinal diols & plausible mechanism.

Since this first example, some other light-mediated catalytic metal-free oxidative cleavage of vicinal diols have been reported, *e.g.* with hydroquinone⁴⁹⁴ or 9,10-dicyanoanthracene,^{495,496} to afford aldehydes and/or ketones (Scheme 60). However, these methods are limited to aromatic substituted diols, usually lignin model compounds.



Scheme 60. Light-mediated catalytic metal-free oxidative cleavage of vicinal diols.

In summary, since the initial reports of periodate and LTA-mediated oxidative cleavages of vicinal diols, a variety of novel and powerful reagents and methods have been investigated and reported. While several catalysts are highly efficient and selective,

494. Mitchell, L. J.; Moody, C. J. *J. Org. Chem.* **2014**, *79*, 11091–11100.

495. Lim, S. H.; Lee, W. S.; Kim, Y. Il; Sohn, Y.; Cho, D. W.; Kim, C.; Kim, E.; Latham, J. A.; Dunaway-Mariano, D.; Mariano, P. S. *Tetrahedron* **2015**, *71*, 4236–4247.

496. Cho, D. W.; Latham, J. A.; Park, H. J.; Yoon, U. C.; Langan, P.; Dunaway-Mariano, D.; Mariano, P. S. *J. Org. Chem.* **2011**, *76*, 2840–2852.

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the two classical reagents, periodate and LTA, are still of choice for most synthetic applications. Even though the catalytic procedures and metal-free protocols are still rare, their positive outcomes present a big advance and perhaps will substitute the classical transformations. However, compared to other reported catalysts, the classical reagent NaIO_4 has found a much wider application in organic transformations, especially in carbohydrate chemistry.

I.2. The Selectivity of Oxidative Cleavage of Diol

As discussed in Chapter 2, we planned to use oxidative cleavage of vicinal diols to incorporate aldehyde group to the non-reducing end of disaccharide **26**. However, from a structural point of view, disaccharide **26** not only contains one acyclic vicinal diol motif situated at side chain of the non-reducing end but also contains two cyclic vicinal diols motifs onto the backbone of the disaccharide (Figure 51). Control of the regioselectivity of the oxidative cleavage for the unique transformation of the non-reducing end represented a challenging issue that we would address.

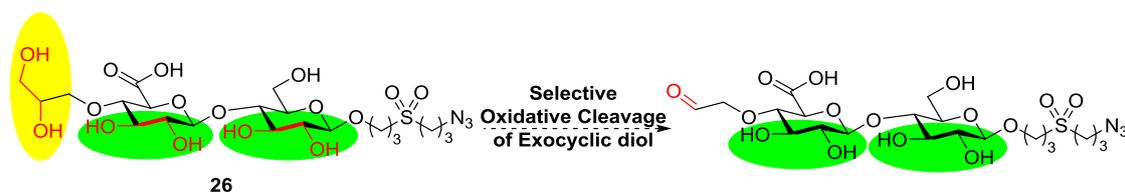


Figure 51. Oxidative cleavage of diol situated at non-reducing end of disaccharide.

In 1959, Miles and coworkers have extensively studied the mechanism of oxidative cleavage of vicinal diols by NaIO_4 .⁴⁹⁷ They demonstrated that cis-cyclohexanediol can be oxidatively cleaved faster than trans-cyclohexanediol. The oxygen atoms of both the cis- and trans-cyclohexanediol are able to coordinate with the octahedral iodine atom to form a strain-free puckered ring with only slight changes in their original interatomic distances: the calculated inter-oxygen distance is ca. 2.86 Å for both the cis- and trans-diols, whereas the distance between adjacent oxygen atoms in the $\text{H}_3\text{IO}_6^{2-}$ ion is close to 2.73 Å (Figure 52).

497. Buist, G. J.; Bunton, C. A.; Miles, J. H. *J. Chem. Soc.* **1959**, 743–748.

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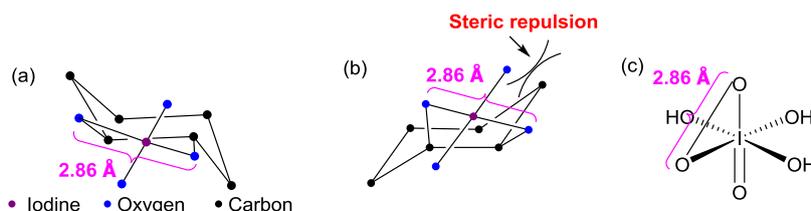
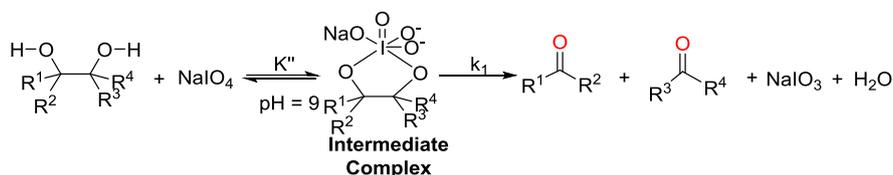


Figure 52. Intermediate-complex of the (a) trans-diol and (b) cis-diol.

However, the formed intermediate-complex of cis-cyclohexanediol collapsed more rapidly than of trans-cyclohexanediol (the rate of breakdown for cis-cyclohexanediol is 20 times faster than that for the trans-complex, $3300 \times 10^{-4} \text{ s}^{-1}$ vs $165 \times 10^{-4} \text{ s}^{-1}$, Table 7), which was caused by an internal steric repulsion within cis-cyclohexanediol between one of the oxygen atoms attached to the iodine atom and one carbon as well as one hydrogen atom of the ring (Figure 51b).



	$10^4 k_1 \text{ (s}^{-1}\text{)}$	K''
Cis-cyclohexanediol	3300	400
Trans-cyclohexanediol	165	1000
(-)-Butane-2,3-diol	308	8000
Meso-butane-2,3-diol	182	373

k_1 : rate constant for breakdown of the single negatively charged intermediate complex; K'' : the equilibrium constant between the periodate dianion, $\text{H}_3\text{IO}_6^{2-}$, the diol, and the dinegatively charged intermediate complex.

Table 7. Rate and equilibrium constants of cis- & trans-cyclohexanediol and butane-2,3-diol.

Moreover, as indicated in Table 7, even though they have a similar structure, the oxidative cleavage of meso-butane-2,3-diol has a smaller velocity than the one of cis-cyclohexanediol ($182 \times 10^{-4} \text{ s}^{-1}$ vs $3300 \times 10^{-4} \text{ s}^{-1}$), as the repulsions of the more rigid intermediate complex of cis-cyclohexanediol is more severe than the open-chain meso-butane-2,3-diol. However, the situation can be different when it comes to the oxidative cleavage of (-)-butane-2,3-diol and trans-cyclohexanediol. Even though their corresponding intermediate complexes could be collapsed at almost same speed, it is more difficult for trans-cyclohexanediol to form the intermediate complex, which may be caused by the disruption of some intramolecular hydrogen-bonding or the distortion of the ring during the formation of intermediate complex. Thus, the oxidative cleavage of trans-cyclohexanediol is slower than (-)-butane-2,3-diol.

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Based on these results, we planned to use NaIO_4 as the cleavage reagent to differentiate these vicinal diols moieties in disaccharide **26**. This study will complete and should rationalize the one performed by the group of Gardiner, since their proof of concept was made on HS fragments, in which the sulfation pattern prevent the presence of 1,2-diols.^{272,273}

I.3. Validation of The Selectivity of The Oxidative Cleavage of Vicinal Diols Mediated by NaIO_4

Before applying it to the non-reducing end of model disaccharide, we verified that the rate of reaction of oxidative cleavage were in favor of the oxidation of the exocyclic diol by applying this reaction to a series of compounds mimicking the different vicinal diols of compound **26**: compound **31**, a glycerol derivative containing an acyclic diol, compound **32**, **33** and **36** containing cyclic diols with different configuration onto their saccharidic backbone (Figure 52). From a structural point of view, compound **32** contains a 1,2-trans-diol onto a ${}^4\text{C}_1$ conformation, whereas compound **33** contains a 1,2-cis-diol onto a ${}^4\text{C}_1$ conformation and compound **36** contains two 1,2-trans-diols onto a ${}^1\text{C}_4$ conformation. In addition to their structural characters, these compounds were chosen not only due to their water solubility, but also because they can be easily prepared.

I.3.1. Preparation and synthesis of substrates for oxidative cleavage reaction

From a retrosynthetic point of view, model compound **31-33** can be synthesized from commercially available glycerol, α -D-methylglucoside and α -D-methylmannoside, respectively, while compound **36** could be easily prepared from compound **34**, which is available in our lab (Figure 53).

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

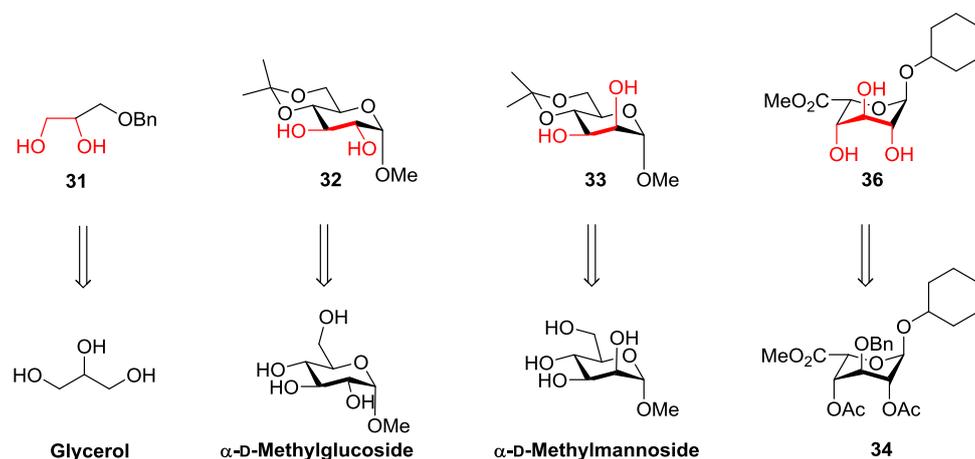
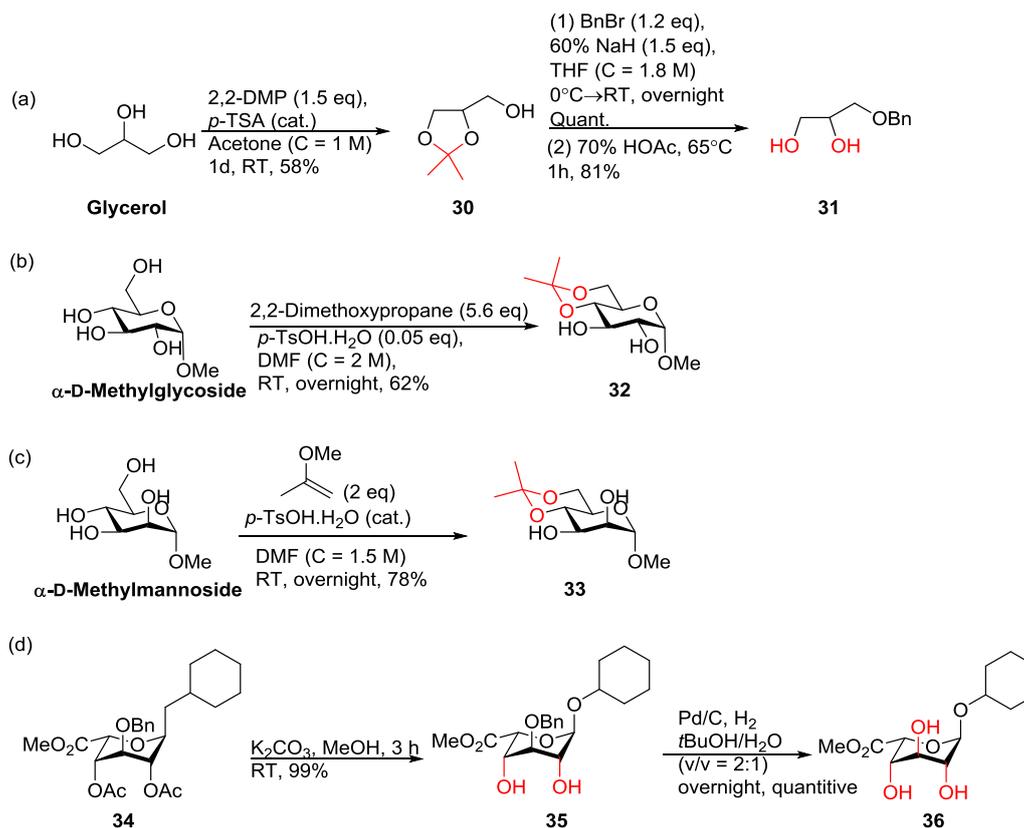


Figure 53. Retrosynthetic scheme for the preparation of compounds **31-33** and **36**.

Therefore, starting from glycerol, isopropylidene compound **30** can be obtained in 58% yield by using 1.5 equivalents of 2,2-dimethoxypropane in presence of catalytic amount of *p*-toluenesulfonic acid in acetone, then its free hydroxyl group was quantitatively protected as benzyl ether using 1.2 equivalents of benzyl bromide and 1.5 equivalents of NaH in THF. The subsequent acidic hydrolysis of the acetal led to the diol **31** in 81% yield, an overall yield of 47% in three steps.⁴⁹⁸⁻⁵⁰² Similarly, the 4,6-diol of α -D-methylglucoside was protected using a large excess of 2,2-dimethoxypropane in presence of *p*-toluenesulfonic acid as a catalyst to yield isopropylidene compound **32** in 62% yield.⁵⁰³ This protocol did not offer the desired product in acceptable yield when applied to α -D-methylmannoside, thus 4,6-isopropylidene- α -D-methylmannoside **33** was synthesized using 2-methoxypropene instead of 2,2-dimethoxypropane leading to 78% yield.⁵⁰⁴ Finally, compound **36** was prepared in quantitative yield from monosaccharide **34** using a sequence of deacetylation followed by hydrogenolysis of the benzyl ether to give **36** in 99% yield over two steps (Scheme 61).

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Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

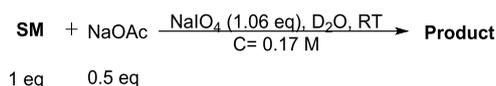


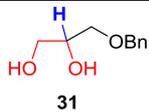
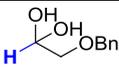
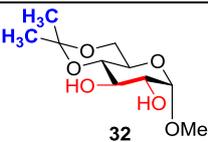
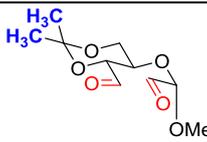
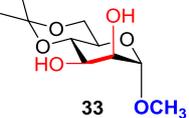
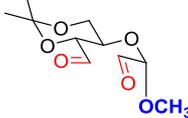
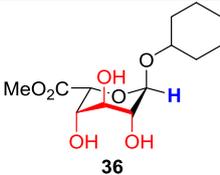
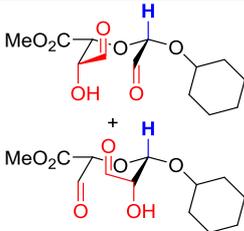
Scheme 61. Preparation of compound 31-33 and 36.

I.3.2. Results and discussions of oxidative cleavage reactions

With those compounds in hands, we carried out the kinetic studies of the oxidative cleavages to determine the reaction time required for the completion of the reaction. All reactions were followed by 1H NMR, using sodium acetate (0.5 equiv) as an internal standard. At a specific time, the reaction was stopped by addition of 2 equivalents sodium thiosulfate before acquiring the 1H NMR spectrum. In those conditions, the average pH of reactions was neutral and sodium acetate was totally compatible with all substrates and sodium periodate (Table 8).

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation



Entry	Substrate	Signal for quantification (δ , ppm)	Product	Signal for quantification (δ , ppm)
1	 31	3.76		5.04
2	 32	1.39, 1.30		1.44, 1.33
3	 33	3.51		3.43
4	 36	5.07		N.D.

a. General conditions: SM (dissolved in D₂O, C = 0.85 M), NaIO₄ (dissolved in D₂O, C = 0.9 M), internal standard NaOAc (dissolved in D₂O, C = 0.425 M). Every NMR experiment, SM was taken 100 μ L, NaIO₄ was taken 100 μ L, internal standard NaOAc was taken 100 μ L and D₂O was taken 200 μ L. b. The shift of the bond in blue was chosen for monitor the consumption of SM.

Table 8. ¹HNMR parameters of oxidative cleavage of vicinal diols contained in compound **31-33** and **36**.

All results are represented in Figure 54. When sodium periodate was added to the solution of diol **31**, we observed a total consumption of the diol in 3 minutes while it took hours for diols **32** and **33** to complete the reaction. Interestingly and in accordance to the published data reported above for cis and trans cyclohexane-1,2-diols, the trans 1,2-diol **32** reacted slower than the cis 1,2-diol **33**. Indeed, diol **33** was totally consumed in 8 hours while diol **32** didn't. In addition, when sodium periodate was added, triol **36** hardly reacted and no changes ever occurred even though we prolonged the reacting time to days. Therefore, based on these results, we proposed that at neutral pH it should be possible to selectively cleave the acyclic diol of the side chain of non-reducing end of disaccharide **26** using a slight excess of sodium periodate without oxidizing trans 1,2-diols of the backbone of disaccharide **26**.

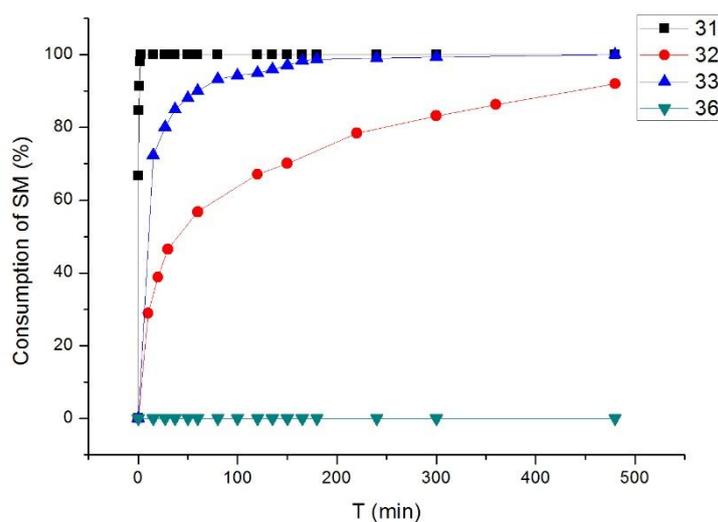


Figure 54. Kinetics of the consumption of 1,2-diols **31-33** and **36** using ^1H NMR.

II. Oxime Ligation

II.1. Optimization of The Reaction Conditions for Oxime Ligation

After the investigation of oxidative cleavage of vicinal diols, we started to optimize the reaction conditions for oxime ligation using commercially available benzaldehyde and *O*-benzylhydroxylamine hydrochloride (Table 9).⁵⁰⁵⁻⁵⁰⁷ In this reaction, sodium bicarbonate was used as base to liberate the active species, *O*-benzylhydroxylamine. As shown in Table 9, when 2.0 equivalents of *O*-benzylhydroxylamine hydrochloride was used, the concentration of the reaction has little influence on the reaction: no change in reaction time between 0.5 and 0.1 M apart from a slight decrease in isolated yields of oxime (entry 1-2). However, even though excellent yield could be obtained when the concentration is 0.5 M, it could be problematic when we applied the condition to oxime ligation of HS fragments, which have a high molecule weight. Unfortunately, reduction of the amount of *O*-benzylhydroxylamine hydrochloride to 1.2 equivalent, led to an extensive slow down of the reaction. Restoration of a short reaction

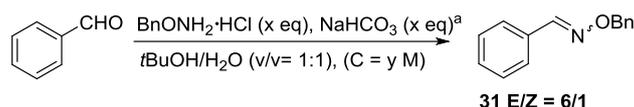
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time can be obtained by increasing the concentration of the reaction but it conducted to a slight decrease in isolated yield (entry 4-5 vs 6-8). In addition, the quantity of *O*-benzylhydroxylamine hydrochloride should be well controlled (entry 2-4), the less the better, since for synthesis of SAS mimetics, the aminoxy has to be in default amount with regard to the aldehyde. Therefore, based on the discussions above, the optimized condition is to use 1.2 equivalents of *O*-benzylhydroxylamine hydrochloride when the concentration is 0.1 M.



Entry	BnONH ₂ .HCl (x equiv)	Concentration (y M)	Reaction Time Needed for full conversion	Isolated Yield (%)
1	2.0	0.5	2 h	97%
2	2.0	0.1	2 h	84%
3	1.5	0.1	2 h	80%
4	1.2	0.1	20 h	80%
5	1.2	0.2	20 h	83%
6	1.2	0.3	2 h	77%
7	1.2	0.4	10 min	84%
8	1.2	0.5	10 min	70%

a. Reaction scale: benzaldehyde (0.2 mmol, 1 eq), *t*BuOH/H₂O (v/v = 1/1), equimolar quantities of NaHCO₃ and BnONH₂.HCl were used.

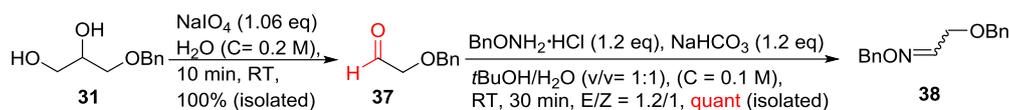
Table 9. Model oxime ligation using benzaldehyde and *O*-benzylhydroxylamine hydrochloride.

Then, as realized by Nicolaou and coworkers, we investigated the possibility of performing oxidative cleavage of vicinal diols/oxime ligation in a one-pot reaction sequence. As shown in Table 10, the stepwise or one-pot procedure conducted to the isolation of **38** in different yields. When the reaction was conducted in stepwise manner, 1.2 equivalents of BnONH₂.HCl is enough for the full conversion of aldehyde **37** and final compound **38** was isolated quantitatively. However, the use of 1.2 equivalents of BnONH₂.HCl into the one-pot process conducted to a decrease of the reaction rate, conversion of intermediate aldehyde into ether oxime as well as isolated yield (entry 1). An increase of the quantity of BnONH₂.HCl and base to 2 equivalents permitted to restore the full conversion of aldehyde into oxime **38** (entries 2-3). These results can be rationalized when considering the formaldehyde formed during the oxidative cleavage also reacted with 1 equivalent of BnONH₂.HCl, thus leading to incomplete reaction. Nevertheless, the one-pot procedure has the further advantage to limit purification steps

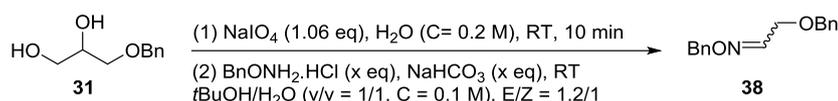
Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

and manipulation of compounds.

(a) *Stepwise*



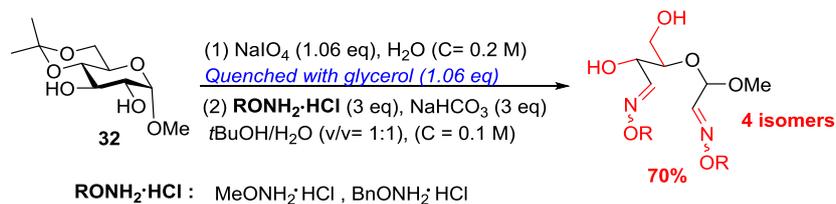
(b) *One-pot*



Entry	BnONH ₂ .HCl (eq)	NaHCO ₃ (eq)	Reaction Time	Conversion (%)	Yield (%)
1	1.2	1.2	4 h	50%	44%
2	1.5	1.5	4 h	70%	70%
3	2.0	2.0	40 min	100%	Quant.

Table 10. A stepwise (a) or one-pot (b) oxidative cleavage of vicinal diols/oxime ligation reaction using diol **31** as starting material.

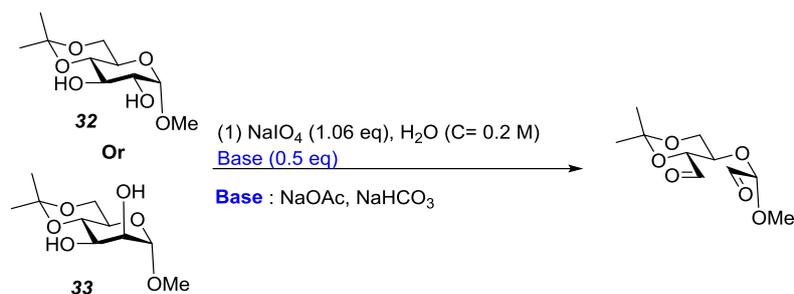
With this result in hand, we applied the one-pot oxidative cleavage /oxime ligation reaction sequence was to diol **32** (Scheme 62). During our previous kinetic study, no side reactions were ever observed. Surprisingly, even if the sequence was successfully conducted, the intermediate product was deprotected during the course of the oxidative cleavage. The only difference between kinetic study of oxidative cleavage of diol **32** and the one-pot oxime ligation of diol **32** is the use of sodium acetate as internal base that should limit the drop of pH during the oxidation step due to the formation of the acidic cyclic intermediate.



Scheme 62. Application of the one-pot sequence for the formation of ether oxime using diol **32**.

Therefore, we investigated the effect of addition of a base, sodium acetate or sodium bicarbonate, on the oxidative cleavage of diol **32** and **33**. As depicted in Table 11, when sodium bicarbonate was used, the oxidative cleavage almost didn't proceed, even if the reaction time was prolonged to several days while the reaction was completed in one night using sodium acetate whatever the diol configuration.

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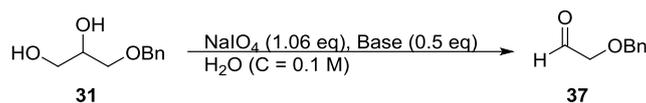


	32 + NaOAc	32 + NaHCO ₃	33 + NaOAc	33 + NaHCO ₃
Conversion	Overnight 100%	13 days, 30%	Overnight 100%	13 days, trace

Table 11. The effect of base on oxidative cleavage of diol **32** and **33**.

To have a better understanding of the effect of the base, we screened different basic conditions (organic and inorganic bases) of the oxidative cleavage of diol **31** to facilitate analysis of reactions. As shown in Table 12, when sodium acetate (entry 1), bicarbonate (entry 2) or methoxide (entry 4) was used, the oxidative cleavage could be all completed in 10 minutes, while the use of sodium carbonate (entry 3), trimethylamine (entry 5) or DBU (entry 6) dramatically slowdown the reaction, it took days for the full conversion of the diol into corresponding aldehydes. These results are in agreement with previously reported studies, demonstrating that the oxidative cleavage efficiently proceeds at an optimal pH of 4-6⁴⁹⁷ and can explain the observed deceleration of reaction rate using sodium carbonate, triethylamine and DBU. With respect to NaOMe, its addition to aqueous solution will lead to the direct formation of hydroxide and methanol. We assumed the excellent rate of oxidative cleavage in presence of NaOMe was due to the formation of methanol, which can participate in the reaction and may speed up the formation of the intermediate complex by deprotonation of the starting diol and favor the collapse of the intermediate complex.

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

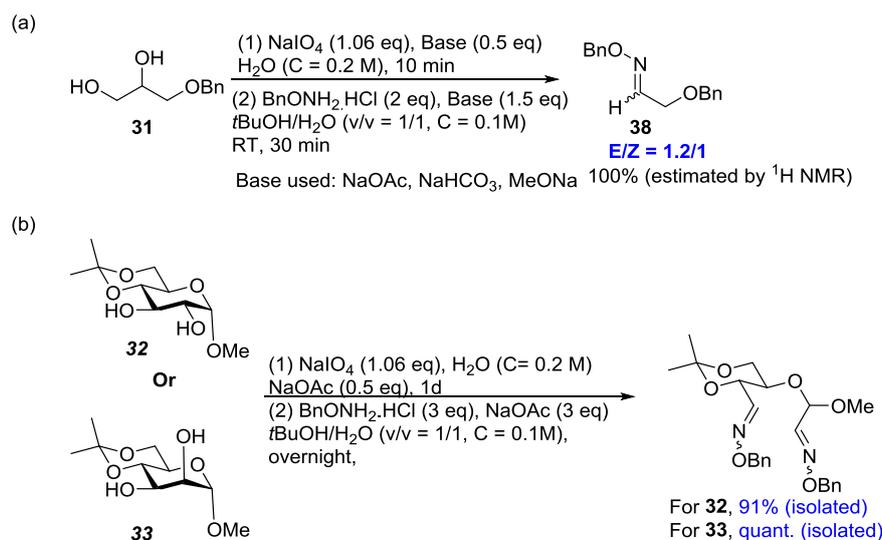


Entry	Base	pK _a values of corresponding conjugate acid	Reaction Time
1	NaOAc	4.73	10 min
2	NaHCO ₃	6.3	10 min
3	Na ₂ CO ₃	10.3	> 2 d
4	NaOMe	15.5	10 min
5	Et ₃ N	10.75	> 2 d
6	DBU	12	> 2 d

a. Reaction conditions: **30** (0.16 mmol, 1 equiv), NaIO₄ (0.17 mmol, 1.06 equiv), Base (0.5 equiv), and H₂O (C = 0.1 M).

Table 12. The effect of base on oxidative cleavage of diols.

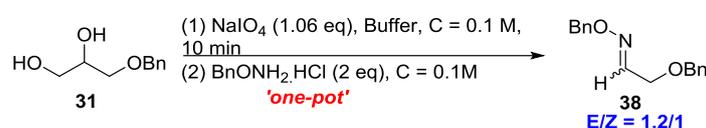
So we decided to revise the conditions of the one-pot sequence of oxidative cleavage/ether oxime formation. After cleavage of the diol in presence of 0.5 equivalent of base, we decided to add a new loading of this base prior to the oxyamine hydrochloride. Moreover, as the side product of oxidative cleavage, formaldehyde, would consume part of aminoxy, we used 2 equivalents of BnONH₂.HCl for the one-pot oxime ligation of diol **31**. In these conditions, as judged by ¹H NMR, whatever the base used, the complete oxime ligation could be completed in 30 minutes (Scheme 63a). As for diol **32-33**, 3 equivalents of BnONH₂.HCl were used and corresponding oxime could be isolated in an overall of 91% and quantitative yield, respectively (Scheme 63b).



Scheme 63. New one-pot conditions of oxidative cleavage /oxime ligation sequence to convert diol **31-33** into corresponding oxime.

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

We then decided to evaluate the use of a Na₂HPO₄-NaH₂PO₄ buffer instead of a base to control the one-pot procedure (Table 13). Irrespective of its concentration, the oxime ligations conducted in buffer with pH value of 7 reacted faster than oxime ligations conducted in buffer with pH value of 6 (entries 1, 3, 5 vs. 2, 4, 6 respectively). Moreover, higher the concentration, faster the oxime formation: with a buffer concentration of 1M, intermediate **37** can be fully converted to the target compound **38** in quantitative yield in less than 40 minutes (entry 1). A decrease of the buffer concentration to 0.5 M led to a longer reaction time (entry 3) while a decrease to 0.1M conducted to a uncomplete conversion of the intermediate aldehyde into the final product **37** (entry 5).



Entry	Concentration (M)	pH	Reacting Time	Conversion (%)	Isolated Yield (%)
1	1	7	40 min	100	Quant.
2		6	2 h	100	98%
3	0.5	7	3 h 10 min	100	99%
4		6	> 3 days	50	34%
5	0.1	7	> 3 days	80	80%
6		6	> 3 days	40	23%

Table 13. Use of buffer instead of base for the one-pot procedure.

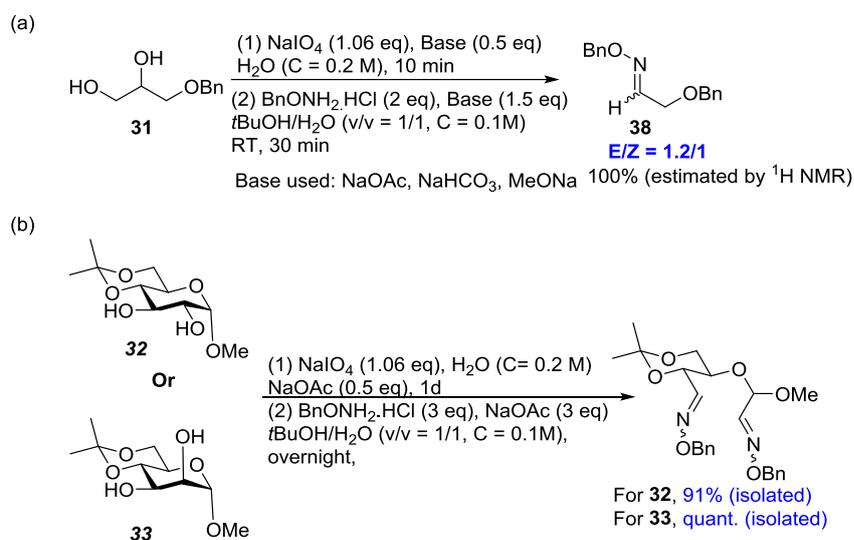
Even if a buffer solution can be used as the reaction media, it appeared that a high concentration is required for a rapid and efficient one-pot sequence, especially for the reaction of oxime formation. Moreover, the final removal of salts of the buffer solution from the crude product should be difficult when synthesizing low molecular weight oxime ether such as oxime ether derivatives of our model disaccharide.

III. Summary

By a simple study of the kinetics of NaIO₄-mediated oxidative cleavage of acyclic and cyclic vicinal diols, we confirmed that acyclic vicinal diols could be cleaved faster than cyclic vicinal diols. Moreover, due to the larger reaction rate constant of oxidative cleavage of cyclic cis-diols than cyclic trans-diols, it appears to us to probably cleave the acyclic diol situated at non-reducing end of disaccharide **26** while cyclic diols sited on the backbone of disaccharide **26** will be unaffected.

Chapter 4. Oxidative Cleavage of Diol & Oxime Ligation

Moreover, we optimized the reaction conditions of oxime ligation by using commercially available benzaldehyde and *O*-benzylhydroxylamine hydrochloride. Using this system of reagents, the use of 1.5 equivalents of *O*-benzylhydroxylamine hydrochloride permitted an easy conversion of benzaldehyde (0.1 M solution in water) into the corresponding oxime in 2 hours. By using diols **31-33** as substrate, we developed a one-pot sequence of transformation of diols into corresponding ether oximes. However, to avoid side reactions, we demonstrated that the use of a base did not affect the oxidative cleavage, prevent the drop of pH of the reaction media and allow a fast oxime formation (Scheme 64).



Scheme 64. Optimal one-pot conditions for the oxidative cleavage /oxime ligation sequence allowing the conversion of diol **31-33** into corresponding ether oxime.

Additionally, to avoid the sequential addition of base, the one-pot oxidative cleavage/oxime ligation should be run in a phosphate buffer (pH = 7, C = 0.5 M). Nevertheless, this later conditions can be applicable only to the preparation of high molecular weight molecules for an efficient desalting of the desired product by size exclusion chromatography.

Chapter 5 Preparation of HS Fragments and Functionalization of a HS Tetrasaccharide & Synthesis of Linkers for The Generation of CXCL12 Tridentate Ligands and SAS Mimics

To perform the detailed structure-activity relationships (SAR) of the interaction between HS and a given protein, as well as to decipher the involvement of HS chains into critical biological processes and key regulatory mechanisms, a straightforward access to HS oligosaccharides is highly desirable. The traditional strategy employed by biologists is to purify HS fragments after enzymatic digestion of GAG chains. However, due to their structural and sulfation pattern heterogeneities, it can be challenging to obtain sufficient quantities of homogeneous materials for SAR studies. Alternatively, an approach involving chemical modification of natural HS fragments should be used. Nonetheless, a complex mixture of partially modified products is generally obtained as a result of incomplete reactions.^{508,509} Therefore, synthetic approaches towards pure and homogenous HS oligosaccharides have been developed and facilitate their structural characterization and study of their biological activities. Moreover, development of innovative and tunable methods of preparation of HS fragments could allow the easy design of new pharmacological agents based on HS structures.

I. Synthetic Approaches Towards HS Fragments

In general, there are two synthetic approaches to access structurally defined HS oligosaccharides:

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Chapter 5. Preparation of HS Fragments and Functionalization of a HS Tetrasaccharide & Synthesis of Linkers for The Generation of CXCL12 Tridentate Ligands and SAS Mimics

(i) Synthetic organic approach, relying on a purely chemical synthesis method to prepare these fragments allowing the full control of the oligosaccharides length, their epimerization and sulfation pattern.^{510–513} An impressive example is the chemical synthesis of the FDA-approved pentasaccharide anticoagulant drug, Arixtra, in more than 50 steps with an overall yield of 0.1%;⁵¹⁴

(ii) chemoenzymatic approach, by integrating enzymatic steps with the chemical synthesis approach to mimic the biosynthetic pathway of HP/HS polysaccharides.^{515–}

522

Even though chemoenzymatic approach is an efficient way to access part of the molecular diversity naturally occurring within HS oligosaccharides, the synthetic organic approach permits to access to a larger diversity of HS fragments, natural and unnatural ones, and represents a powerful tool for the tailor-made synthesis of pure HS oligosaccharides. However, due to their structural complexity and heterogeneity, the chemical synthesis of structurally defined HS oligosaccharides is confronting significant chemical challenges, including (i) difficult access to L-idose and L-iduronic acid, not found in nature in large quantities and available in few quantities from biosources; (ii) formation of challenging glycosidic 1,2-cis linkage using GlcN donors; (iii) selection of appropriate set of orthogonal protecting groups allowing position-specific sulfation and deprotection; (iv) convergent methods for elongation of the

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Chapter 5. Preparation of HS Fragments and Functionalization of a HS Tetrasaccharide & Synthesis of Linkers for The Generation of CXCL12 Tridentate Ligands and SAS Mimics

oligosaccharide backbone.

I.1.Preparation of L-Iduronic Acid and L-Iduronic Derivatives

A key issue in HS fragments synthesis concerns the preparation of L-iduronic acid (L-IdoA) and its corresponding hexopyranose, L-idose, which are not available in large quantities from natural or commercial sources. Therefore, a synthetic strategy to access L-IdoA and its derivatives in multigrams scale is highly needed.

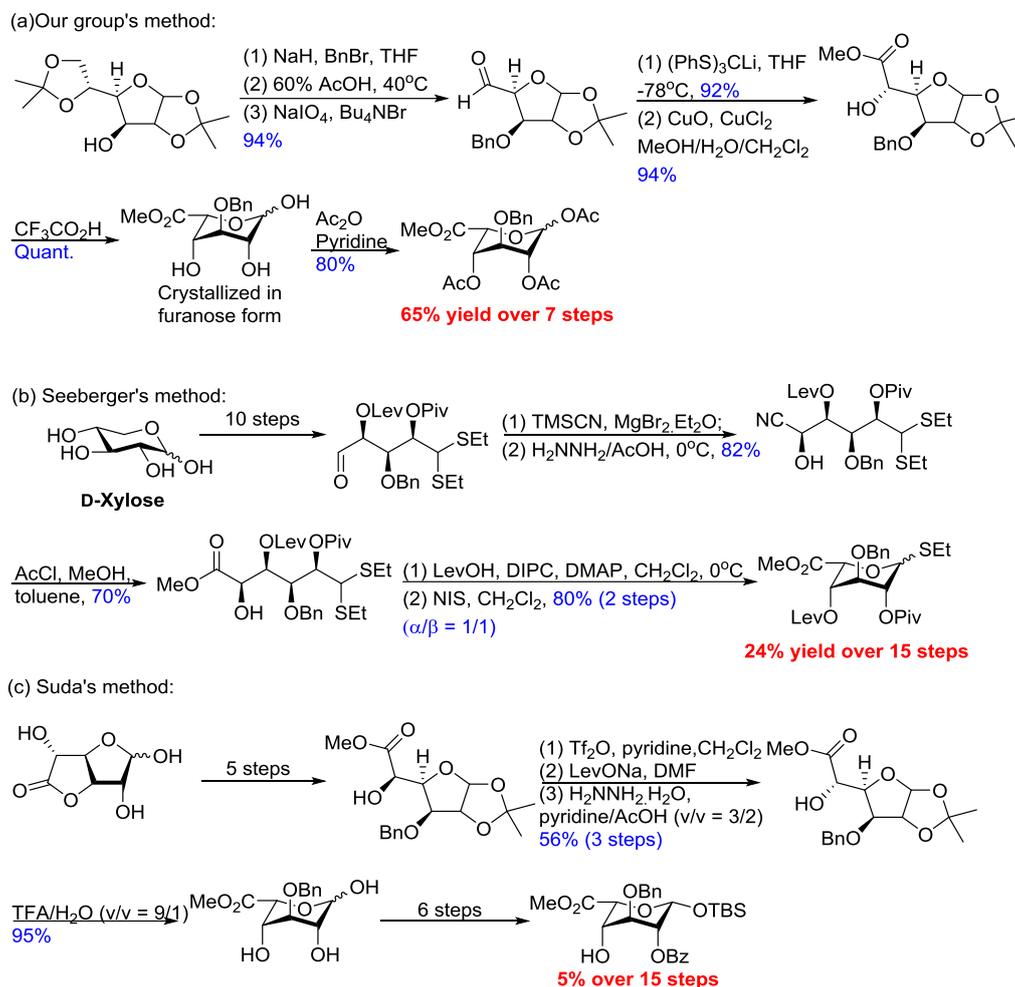
Since the early 1980s, numerous approaches have been developed to prepare L-IdoA derivatives. Many of them use commercially available 1,2,5,6-di-*O*-isopropylidene- α -D-glucofuranose as starting material.^{523–530} For example, our lab reported in 2000 a new and efficient strategy to methyl 3-*O*-benzyl-L-iduronate derivatives, involving an oxidative cleavage of the *O*-5,*O*-6-diol in a three-step process starting from 1,2,5,6-di-*O*-isopropylidene- α -D-glucofuranose, followed by a stereoselective addition of a tris-(phenylthio)methyl group to generate the targeted stereochemistry at C-5 position and conversion of the resulting orthothioester into the corresponding methyl ester using copper salts mixture. Acidic hydrolysis of the 1,2-isopropylidene led to a furanose-pyranose mixture that mainly crystallize in its β -pyranose form that could be further trapped by acetylation (Scheme 65a).^{525,527,528}

More recently, in 2007, Seeberger and coworkers reported a *de novo* synthesis of L-IdoA from D-xylose, a renewable resource commercially available in large quantities. The key step involves a chelation-controlled cyanation for obtaining the L-iduronic acid derivatives (Scheme 65b).^{531,532} Finally, in 2010, Suda and coworkers also realized an

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523. Jacquinet, J.-C.; SinaýP.; Duchaussoy, P.; Choay, J.; Lederman, I.; Petitou, M.; Torri, G. *Carbohydr. Res.* **1984**, *132*, C5–C9.
524. Hung, S.-C.; Puranik, R.; Chi, F.-C. *Tetrahedron Lett.* **2000**, *41*, 77–80.
525. Lubineau, A.; Gavard, O.; Alais, J.; Bonnaffé D. *Tetrahedron Lett.* **2000**, *41*, 307–311.
526. Hung, S.; Thopate, S. R.; Chi, F.; Chang, S.; Lee, J.; Wang, C. *J. Am. Chem. Soc.* **2001**, *123*, 3153–3154.
527. Dilhas, A.; Bonnaffé D. *Carbohydr. Res.* **2003**, *338*, 681–686.
528. Bonnaffé D.; Alais, J.; Dilhas, A.; Hersant, Y.; Gavard, O.; Bascou, A.; Duverger, V. *European J. Org. Chem.* **2003**, *2003*, 3603–3620.
529. Tatai, J.; Osztrovszky, G.; Kajtár-Peredy, M.; Fügedi, P. *Carbohydr. Res.* **2008**, *343*, 596–606.
530. Hansen, S. U.; Baráth, M.; Salameh, B. A. B.; Pritchard, R. G.; Stimpson, W. T.; Gardiner, J. M.; Jayson, G. C. *Org. Lett.* **2009**, *11*, 4528–4531.
531. Timmer, M. S. M.; Adibekian, A.; Seeberger, P. H. *Angew. Chemie. Int. Ed.* **2005**, *44*, 7605–7607.
532. Adibekian, A.; Bindschäler, P.; Timmer, M. S. M.; Noti, C.; Schützenmeister, N.; Seeberger, P. H. *Chem. - A Eur. J.* **2007**, *13*, 4510–4522.

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inversion of the stereochemistry of C-5 position starting from glucurono-6,3-lactone to access to methyl 3-*O*-benzyl-L-idoonate derivatives.⁵³³



Scheme 65. The different efficient approaches towards the synthesis of L-IdoA derivatives based on stereochemistry inversion at position C-5 (a) or stereoselective elongation of a thioacetal protected dialdehyde derived from d-xylose (b).

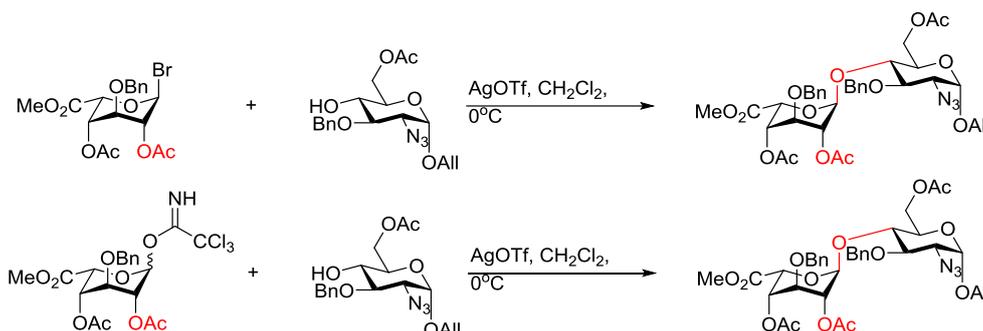
However, despite the great advances that have been achieved onto the elaboration of a simple access to L-IdoA derivatives,^{277,523,532–537,524–531} preparation of L-IdoA derivatives still remains a challenging task.

533. Saito, A.; Wakao, M.; Deguchi, H.; Mawatari, A.; Sobel, M.; Suda, Y. *Tetrahedron* **2010**, *66*, 3951–3962.
534. Schell, P.; Orgueira, H. A.; Roehrig, S.; Seeberger, P. H. *Tetrahedron Lett.* **2001**, *42*, 3811–3814.
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536. Ke, W.; Whitfield, D. M.; Gill, M.; Larocque, S.; Yu, S. H. *Tetrahedron Lett.* **2003**, *44*, 7767–7770.
537. Yu, H. N.; Furukawa, J.; Ikeda, T.; Wong, C. *Org. Lett.* **2004**, *6*, 723–726.

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I.2. Stereochemical Control In Glycosylation Reactions

While the 1,2-trans glycosidic linkage from uronic acid to D-glucosamine units is generally achieved by introducing participating protecting groups at C-2 position of the uronic acid donor (Scheme 66), the stereocontrolled formation of 1,2-cis glycosidic linkage between D-glucosamine donor and uronic acid acceptor still remains challenging.⁵³⁸



Scheme 66. Glycosylation reaction used in our group to prepare the disaccharidic HS repetitive unit.^{528,539,540}

Typically, the non-participating azido group is widely used as masked nitrogen at C-2 position of D-glucosamine building blocks and mainly leads to α -glycoside derivatives (1,2-cis glycosidic linkage).^{360,361} In general, high stereoselectivities can be achieved when L-idosyl acceptors were employed. However, for D-glucuronic acid-based acceptors, anomeric mixtures usually obtained.^{541–543}

In order to achieve high yield and stereoselectivity of such glycosylation reaction, a fine tuning of used protecting groups, leaving group and conditions of glycosylation reaction should be performed. For example, Huang and coworkers demonstrated that replacement of a benzyl ether by *tert*-butyldimethylsilyl ether at *O*-4 position of *p*-tolyl 2-azido-2-deoxy-3,4-di-*O*-benzyl- β -D-1-thioglucopyrannoside donor allowed an

538. Demchenko, A. V. *Synlett* **2003**, 2003, 1225–1240.

539. Dilhas, A.; Bonnaffé D. *Tetrahedron Lett.* **2004**, 45, 3643–3645.

540. Baleux, F.; Loureiro-Morais, L.; Hersant, Y.; Clayette, P.; Arenzana-Seisdedos, F.; Bonnaffé D.; Lortat-Jacob, H. *Nat. Chem. Biol.* **2009**, 5, 743–748.

541. van Boom, J. H.; Stubba, B.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S.; van Boeckel, C. A. A.; Schiattarella, M. *J. Am. Chem. Soc.* **2005**, 127, 3767–3773.

542. Loureiro-Morais, L.; Hersant, Y.; Lucas, R.; Bonnaffé D.; Dilhas, A. *J. Comb. Chem.* **2008**, 10, 166–169.

543. Wang, Z.; Xu, Y.; Yang, B.; Tirachinapally, G.; Sun, B.; Liu, R.; Dulaney, S.; Liu, J.; Huang, X. *Chem. - A Eur. J.* **2010**, 16, 8365–8375.

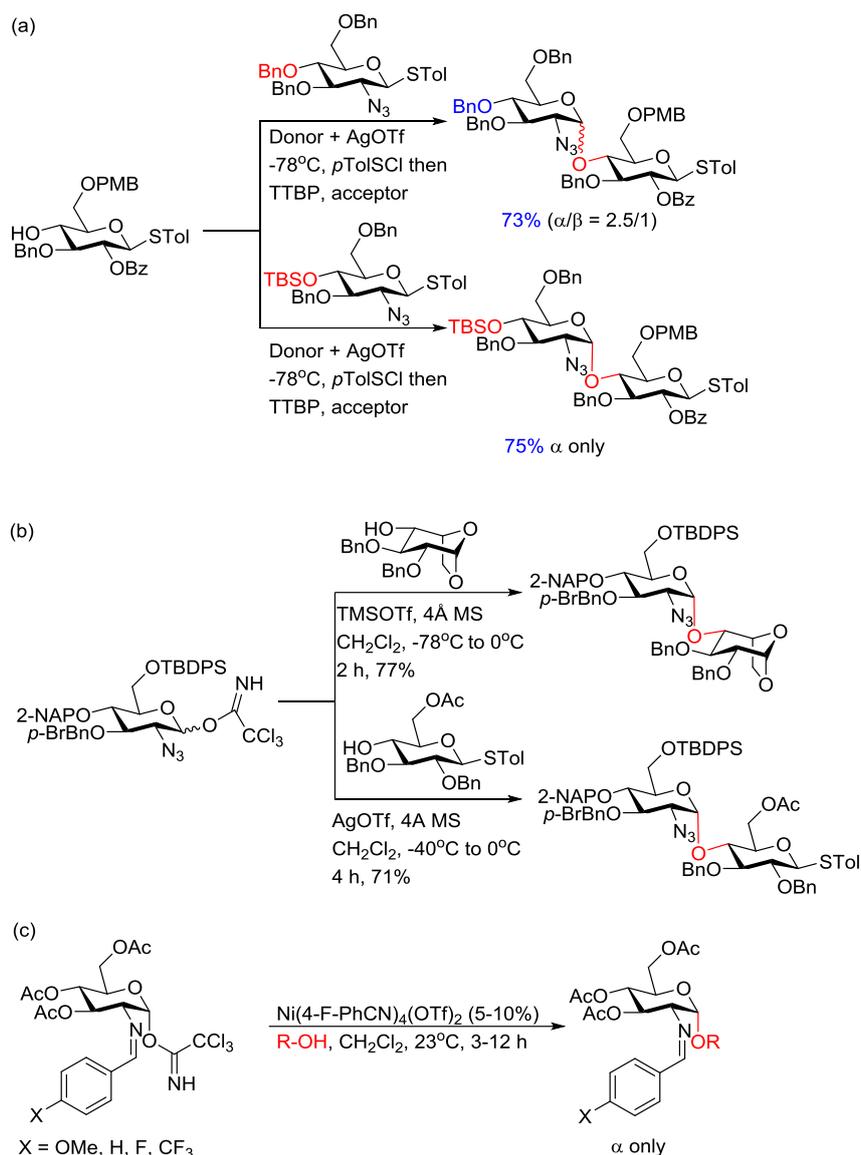
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important improvement of the stereocontrol of the glycosylation reaction (Scheme 67a).⁵⁴³ Hung and coworkers reported glycosylation reaction conditions allowing the formation of pure α -glycosidic bond using a precise set of orthogonal protecting groups on D-glucosamine-derived donors (*tert*-butyldiphenylsilyl, 2-naphthylmethyl and *p*-bromobenzyl groups respectively at *O*-6, *O*-4 and *O*-3 positions), regardless of the leaving group, activator and glycosyl acceptor used (Scheme 67b).⁵⁴⁴ As demonstrated by Nguyen and coworkers, another amino group precursor other than azido can be used to form 1,2-*cis* glycosidic linkage. They developed a novel and stereoselective nickel catalyzed glycosylation reaction using 2-deoxy-2-[(phenyl)methylene]amino- α -D-glucopyranoside trichloroacetimidates as glycosyl donors to form this type of bond in good to excellent yields and high stereoselectivity (Scheme 67c).⁵⁴⁵

544. Zulueta, M. M. L.; Lin, S.; Lin, Y.; Huang, C.; Wang, C.; Ku, C.; Shi, Z.; Chyan, C.; Irene, D.; Lim, L.; et al. *J. Am. Chem. Soc.* **2012**, *134*, 8988–8995.

545. Mensah, E. a; Yu, F.; Nguyen, H. M. *J. Am. Chem. Soc.* **2010**, *132*, 14288–14302.

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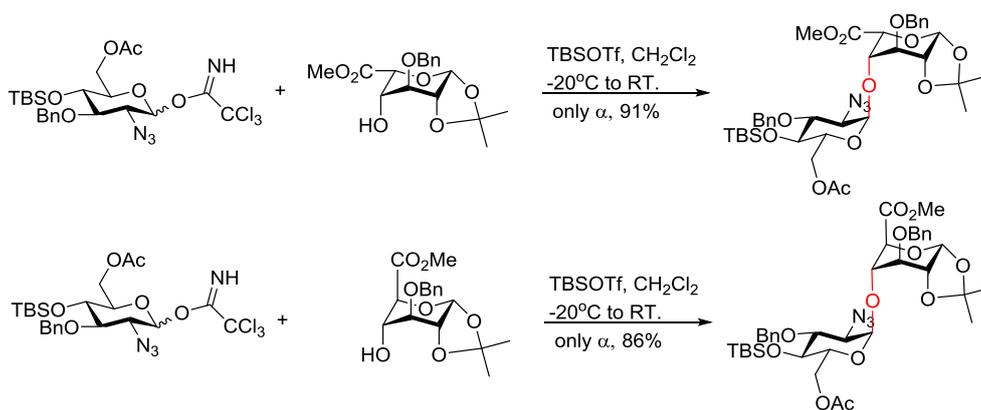
Scheme 67. Examples of developed glycosylation reaction conditions for enhancing α -stereoselectivity in glycosylation reaction using 2-aminoglycoside derivatives as glycosyl donors.

Alternatively, Seeberger and coworkers reported a blocked conformation strategy. By locking the uronate acceptor in ¹C₄ conformation thanks to formation of 1,2-*O*-isopropylidene, they demonstrated that pure α -glycosidic linkages could be formed in excellent yield, using trichloroacetimido-6-*O*-acetyl-2-azido-2-deoxy-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-D-glucopyranoside as donor and TBSOTf as activator in dichloromethane at low temperature (Scheme 68).^{546,547}

546. Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Seeberger, P. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 2128–2131.

547. Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem. - A Eur. J.* **2003**, *9*, 140–169.

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Scheme 68. The influence of conformation of uronate acceptor for stereoselective glycosylation.

However, when the conformation strategy was applied to the preparation of larger HS oligosaccharides, an inseparable anomeric mixture was obtained.⁵⁴⁸ Furthermore, the employment of these conformationally constrained acceptors, makes it difficult to access to several sulfoforms of HS fragments.

I.3. Protecting-Group Strategy to Access to HS Diversity

In addition to participate to the stereocontrol of the glycosylation reaction, the employed set of protecting groups must allow the tailor-made synthesis of HS fragments with a specific sulfation pattern. Therefore, determination of a compatible set of protecting groups with a general glycosylation process represents an important issue.

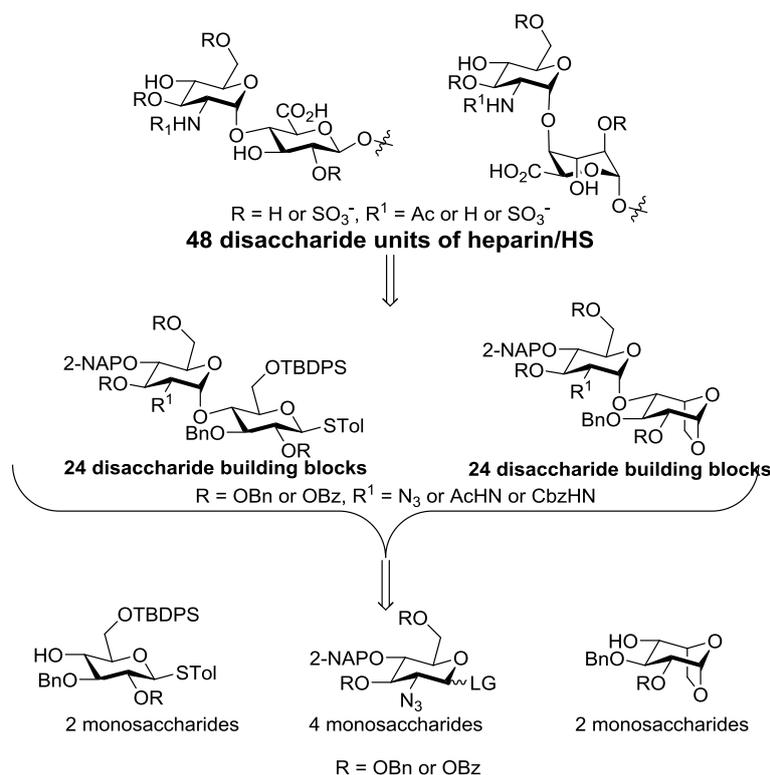
To access to the whole diversity of HS polysaccharides, specific set of orthogonal protecting groups must be used to allow the selective *N*- and *O*-sulfation of specific positions. For this purpose, the most representative works had been done by Hung and coworkers in 2006.⁵⁴⁹ They have prepared the 48 theoretical disaccharides needed to access to the full natural diversity of HS oligosaccharides, starting from eight orthogonally protected monosaccharides (Scheme 69). They combined their previously developed set of protecting groups for stereoselective glycosylation reaction (Scheme 5.3b) with specific protecting groups for site-specific sulfation. This second set is composed of benzoyl groups (Bz) for the protection of hydroxyl groups that would be

548. Lohman, G. J. S.; Seeberger, P. H. *J. Org. Chem.* **2004**, *69*, 4081–4093.

549. Lu, N. D.; Shie, C. R.; Kulkarni, S. S.; Pan, G. R.; Lu, X. A.; Hung, S. C. *Org. Lett.* **2006**, *8*, 5995–5998.

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ultimately sulfated and of benzyl groups (Bn), employed as permanent protecting groups for hydroxyl groups that would remain free in the final products. In addition, The TBDPS group had been used as temporary protecting group to mask the primary alcohol of D-glucopyranoside and L-idopyranoside units that were oxidized to access to corresponding uronic acid motifs. Moreover, the employed azido group for stereoselective formation of α -glycosidic linkage could be selectively reduced to free amine by Staudinger reduction to access to sulfated, acetylated or free amine.



Scheme 69. Synthesis of the 48 theoretical HS disaccharide building blocks from 8 orthogonally protected monosaccharides.

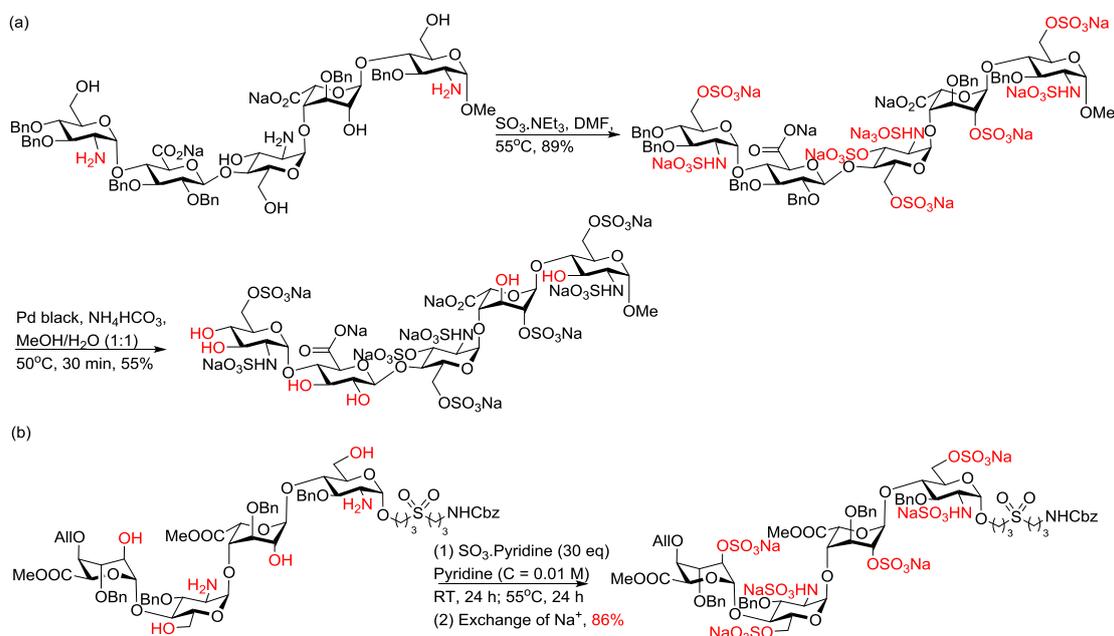
Sulfate groups are generally introduced at the late-stage of HS fragments syntheses that could lead to low yields⁵⁵⁰ and incomplete conversions.^{540,551} To circumvent these problems, several strategies have been reported. For example, de Paz and coworkers demonstrated in 2011 that a microwave-assisted sulfation of HP-type tetra- and hexasaccharides using $\text{SO}_3\cdot\text{NEt}_3$ permitted to concomitantly achieve their *N*- and *O*-

550. Lee, J.-C.; Lu, X.-A.; Kulkarni, S. S.; Wen, Y.-S.; Hung, S.-C. *J. Am. Chem. Soc.* **2004**, *126*, 476–477.

551. Noti, C.; De Paz, J. L.; Polito, L.; Seeberger, P. H. *Chem. - A Eur. J.* **2006**, *12*, 8664–8686.

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sulfation in high yields.^{552,553} Simultaneous *N*- and *O*-sulfation procedures were also developed by Manikowski to synthesize fondaparinux sodium using trimethylamine sulfur trioxide complex (Scheme 70a)⁵⁵⁴ and our group to prepare HS-type glycoconjugates using pyridine sulfur trioxide complex (Scheme 70b),¹³² respectively.



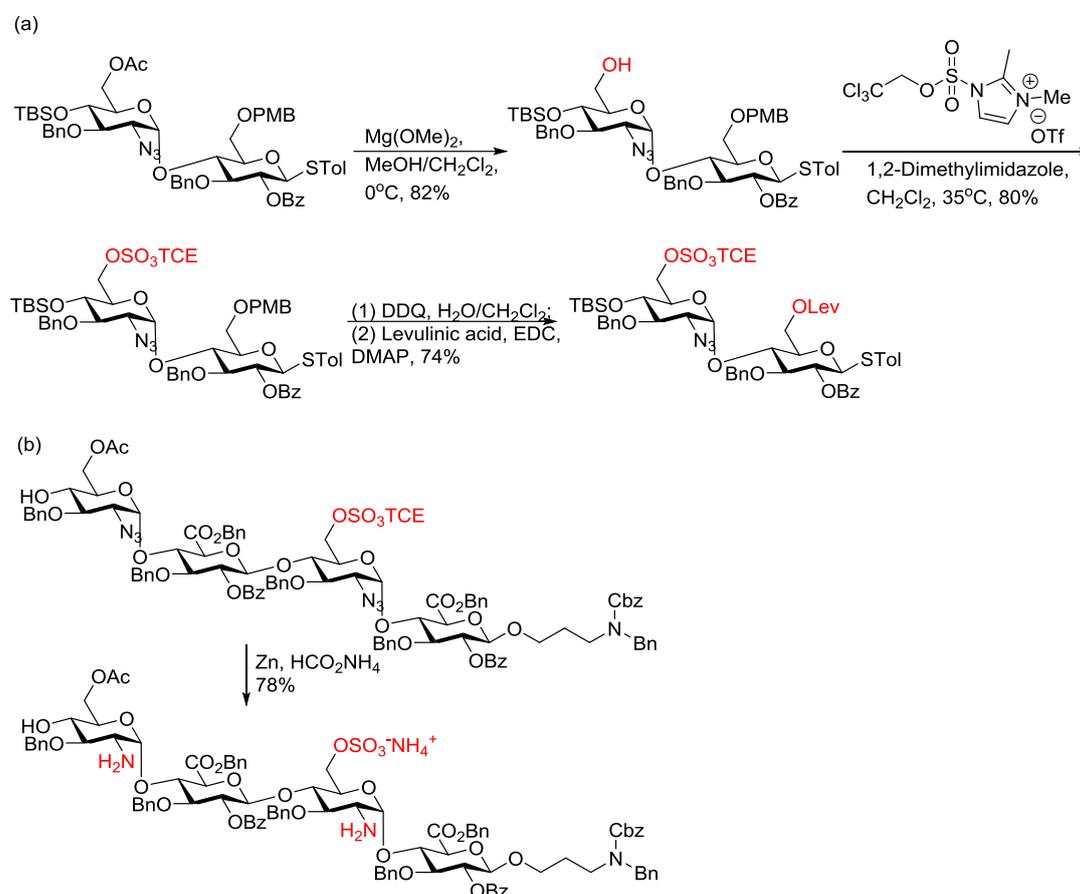
Scheme 70. One-Pot *N*- and *O*-sulfation reported by Manikowski⁵⁵⁴ and our group.¹³²

Alternatively, sulfate groups can be installed onto hydroxyl groups as sulfodiester prior to the assembly of HP/HS fragments and numerous sulfodiester derivatives have been developed for this purpose.^{555–557} In 2012, Huang and coworkers prepared a library of HP/HS oligosaccharides²⁹⁰ by using the 2,2,2-trichloroethyl (TCE) sulfuryl imidazolium salt developed by Taylor and coworkers (Scheme 71a).⁵⁵⁸ In presence of zinc/ammonium formate, not only the TCE sulfate ester can be deprotected but also azide can be cleanly reduced to amine with moderate yield (Scheme 71b). Till now, mono- to tetrasaccharides have been successfully synthesized by using the sulfodiester

552. Maza, S.; De Paz, J. L.; Nieto, P. M. *Tetrahedron Lett.* **2011**, 52, 441–443.
 553. Maza, S.; MacChione, G.; Ojeda, R.; López-Prados, J.; Angulo, J.; De Paz, J. L.; Nieto, P. M. *Org. Biomol. Chem.* **2012**, 10, 2146–2163.
 554. Manikowski, A.; Koziol, A.; Czajkowska-Wojciechowska, E. *Carbohydr. Res.* **2012**, 361, 155–161.
 555. Karst, N. A.; Islam, T. F.; Linhardt, R. J. *Org. Lett.* **2003**, 5, 4839–4842.
 556. Huibers, M.; Manuzi, Á.; Rutjes, F. P. J. T.; Van Delft, F. L. *J. Org. Chem.* **2006**, 71, 7473–7476.
 557. Simpson, L. S.; Widlanski, T. S. *J. Am. Chem. Soc.* **2006**, 128, 1605–1610.
 558. Ingram, L. J.; Taylor, S. D. *Angew. Chemie. Int. Ed.* **2006**, 45, 3503–3506.

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strategy and its suitability to the preparation of longer HP/HS oligosaccharides need to be explored.



However, all these synthetic tools (preparation of uronic moieties, protecting groups strategy for stereocontrol of glycosylation and access to a large HS molecular diversity, method of sulfation) have to be perfectly combined to successfully access the desired length of HS oligosaccharides bearing a well-defined sulfation pattern.

I.4. Elongation of The Oligosaccharidic Backbone

A last but important factor for HP/HS oligosaccharide syntheses lies in the construction of heparin/HS backbones. Several reliable strategies have been developed during the past decades to rapidly access to these oligosaccharides.

I.4.1. Linear strategy

The earliest strategy used in oligosaccharide synthesis is the linear approach

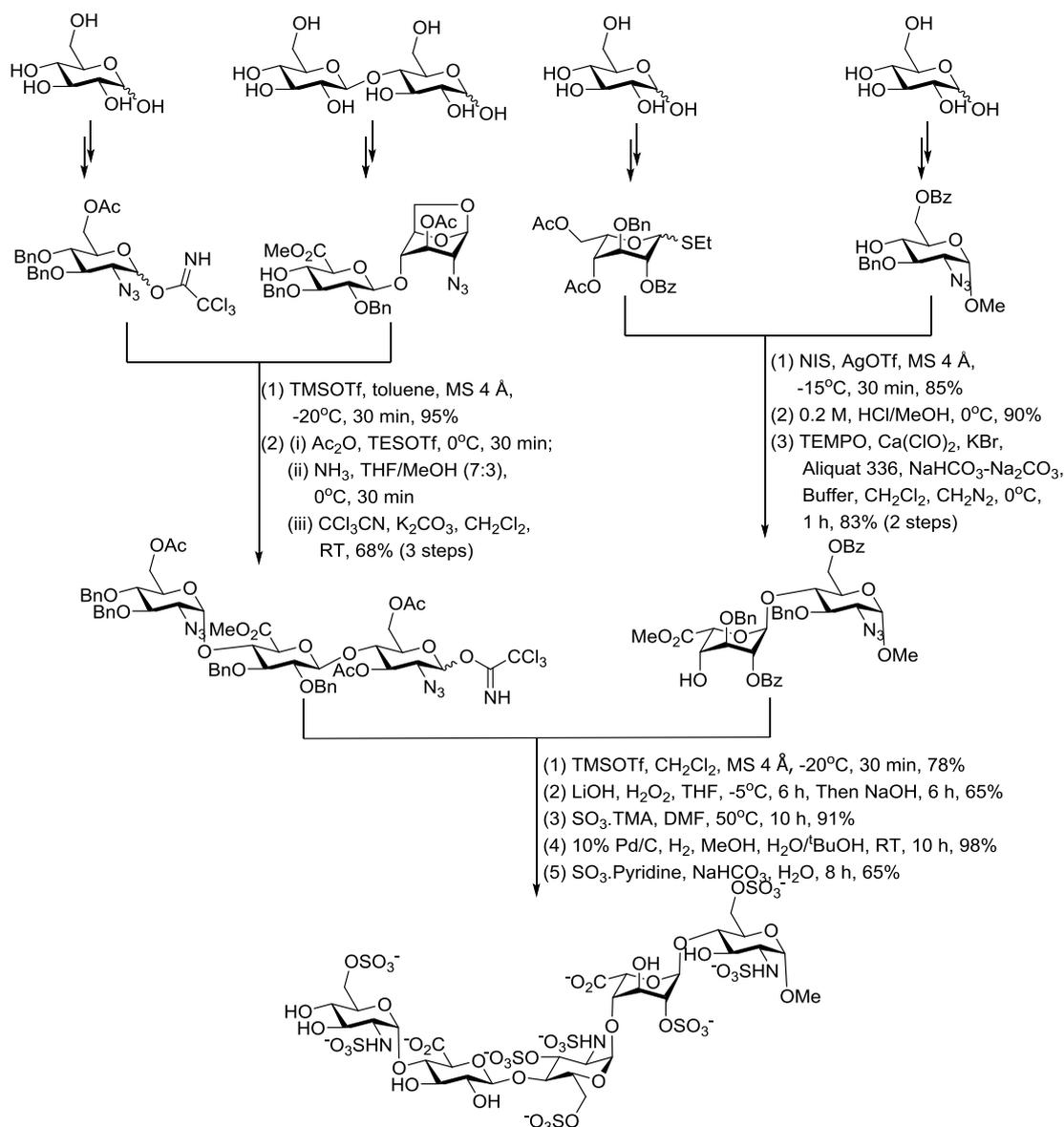
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consisting into the elongation of HP/HS chains from reducing end to non-reducing end. It was achieved *via* the parallel preparation of needed building blocks and their assembly by repetition of a sequence of glycosylation reaction / selective deprotection of 4-hydroxyl group at non-reducing end of newly formed oligosaccharides.

For example, in 2013, a linear approach was employed by Lin and coworkers to synthesize antithromboembolic heparin-related pentasaccharide Fondaparinux (Scheme 72).⁵⁵⁹ Even though only commercially available in large-scale D-glucose and D-cellobiose were used as starting materials, this synthesis was accomplished in 36 linear steps with 0.017% overall yield.

559. Lin, F.; Lian, G.; Zhou, Y. *Carbohydr. Res.* **2013**, *371*, 32–39.

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Scheme 72. Linear approach for total synthesis of Fondaparinux by Lin and coworkers.

The linear approach for the synthesis of the anticoagulant Fondaparinux drug was also investigated by Hung,⁵⁶⁰ Wang⁵⁶¹ and Qin⁵⁶² groups to adapt it to other starting materials and to reduce the number of liner steps. However, if this strategy is applicable to the preparation of short or highly heterogeneous in sequence oligosaccharides, its

560. Chang, C. H.; Lico, L. S.; Huang, T. Y.; Lin, S. Y.; Chang, C. L.; Arco, S. D.; Hung, S. C. *Angew. Chemie. Int. Ed.* **2014**, *53*, 9876–9879.

561. Li, T.; Ye, H.; Cao, X.; Wang, J.; Liu, Y.; Zhou, L.; Liu, Q.; Wang, W.; Shen, J.; Zhao, W.; et al. *ChemMedChem* **2014**, *9*, 1071–1080.

562. Dai, X.; Liu, W.; Zhou, Q.; Cheng, C.; Yang, C.; Wang, S.; Zhang, M.; Tang, P.; Song, H.; Zhang, D.; et al. *J. Org. Chem.* **2016**, *81*, 162–184.

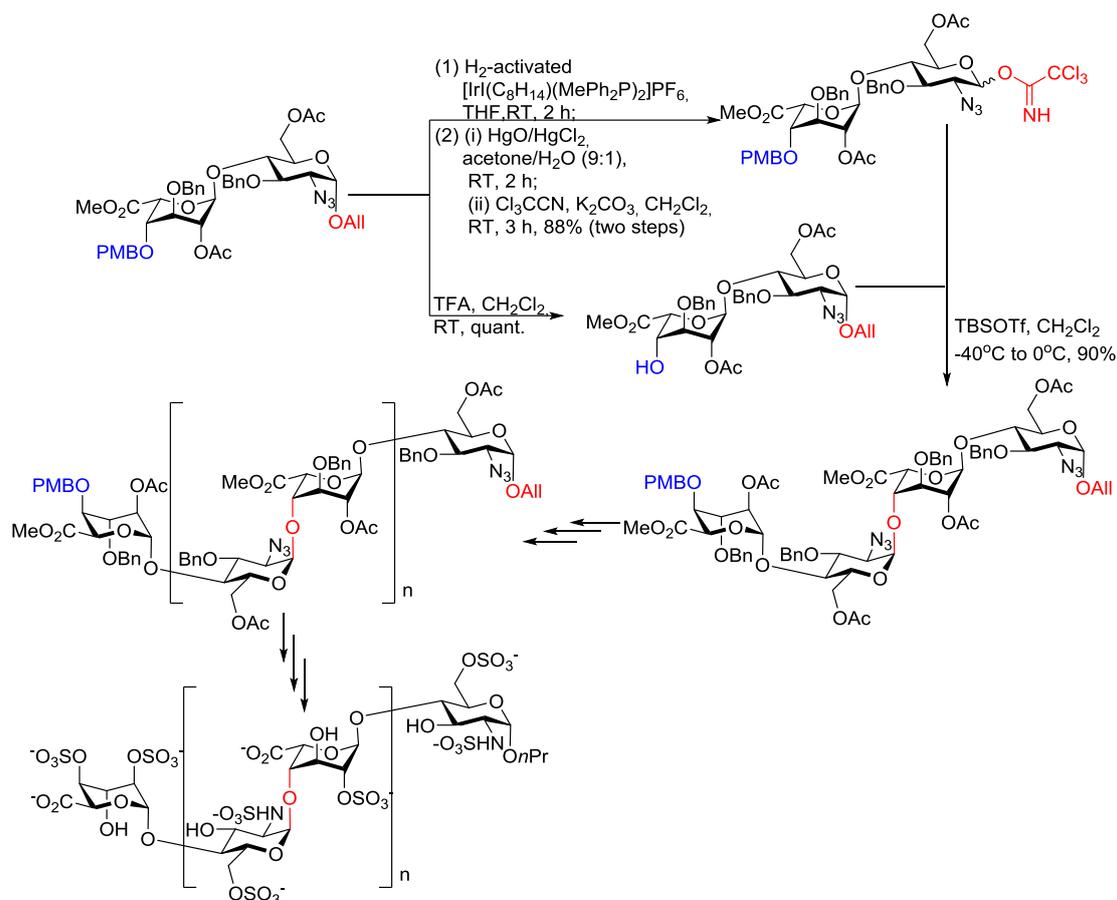
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use is generally limited due to high overall steps and consequent decrease of overall yield. Moreover, due to (i) numerous intermediates generated; (ii) the number of protection/deprotection steps required and (iii) many purification steps needed, this time consuming strategy is commonly abandoned in favor of convergent and iterative approaches that are more adapted to the repetitive chemical structure of HP/HS oligosaccharides.

I.4.2. Convergent approach

In order to prepare longer HP/HS oligosaccharides, a more commonly used strategy is the convergent approach. It allows the convenient and rapid assembly of orthogonally protected building blocks, in a minimum number of synthetic steps, for the preparation of HP/HS oligosaccharides backbones (reduction of the number of generated intermediates and required deprotection steps). In our group, we adopted this strategy to prepare a library of tetra- to octasaccharidic HS-type glycoconjugates targeting the IFN- γ chemokine¹³² as well as the construction of a dodecasaccharidic HS-type glycoconjugate to develop a HIV-1 entry inhibitor.⁵⁴⁰ Our strategy relies onto the use of two orthogonal protecting group, PMB and allyl, to protect the non-reducing end and reducing end of HS fragments, respectively. This dual system allows the preparation of glycoside donor and acceptor from a common building block, to create tetra-, hexa-, octa- and dodecasaccharides by [2+2], [2+4], [4+4] and [4+8]-glycosylation reactions, respectively (Scheme 73). Therefore, this strategy can also give a rapid and efficient access to longer HS fragments.

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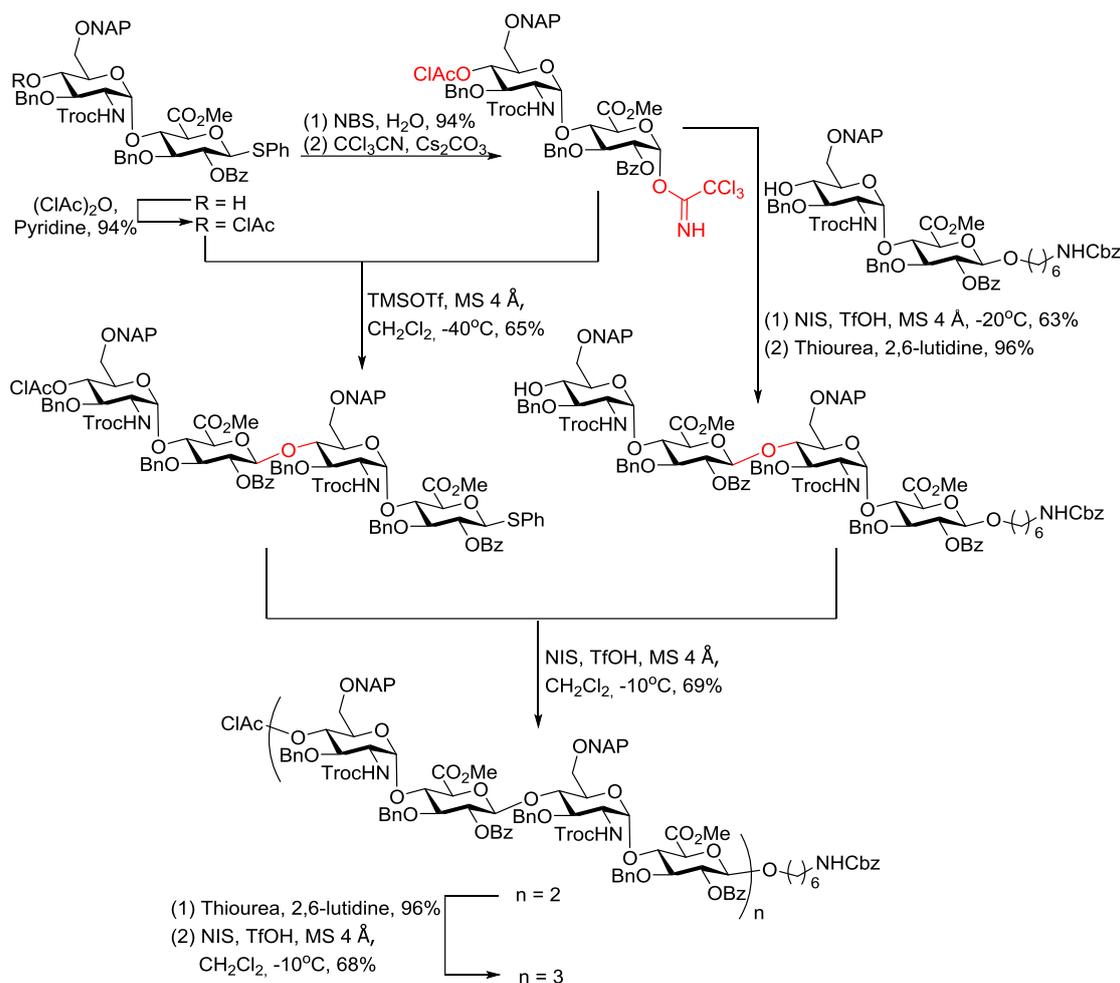


Scheme 73. Convergent approach employed by our group to synthesize HS fragments.

Other groups also used similar convergent strategies such as Tanaka and coworkers. In 2012, they reported a [4 + 4 + 4] glycosylation approach to synthesize HP/HS-type dodecasaccharides (Scheme 74).⁵⁶³ Starting from the same disaccharide building block, trichloroacetimidate donor, thioglycoside acceptor as well as a reducing end amino-modified acceptor were prepared, coupled to afford two different tetrasaccharides, a thioglycoside donor and an acceptor bearing an amino-modified reducing end. Repeated glycosylation reactions using these later compound, including an intermediary deprotection step of the *O*-4 position of the non-reducing unit of the obtained 6-aminohexyl oligosaccharide derivatives, permitted to them to prepare an octa- and dodecasaccharide in good yields.

563. Tanaka, H.; Tateno, Y.; Takahashi, T. *Org. Biomol. Chem.* **2012**, *10*, 9570–9582.

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Scheme 74. Convergent approach towards synthesis of a HP-type dodecasaccharide by Tanaka and coworkers.

More recently, in 2015, Gardiner and coworkers adapted this strategy to propose an iterative synthesis of HP-type oligosaccharides. At each two-step cycle of elongation, consisting into release of the *O*-4 position of the non-reducing end followed by glycosylation reaction, the same thiophenyl tetrasaccharide donor was used to increase the length of the acceptor oligosaccharides with 4 saccharide units (+ [4] elongation). After reporting the first gram-scale synthesis of a structurally defined HP-related dodecasaccharide (12-mer) in 14 steps starting from the first protected disaccharide acceptor with an overall yield of 15% (Scheme 75),⁵⁶⁴ they applied seven more iterative two-step cycles to prepare the 16-mer, 20-mer, 28-mer, 32-mer, 36-mer and 40-mer and validate the viability of the chemical synthesis of long HP/HS-type oligosaccharides

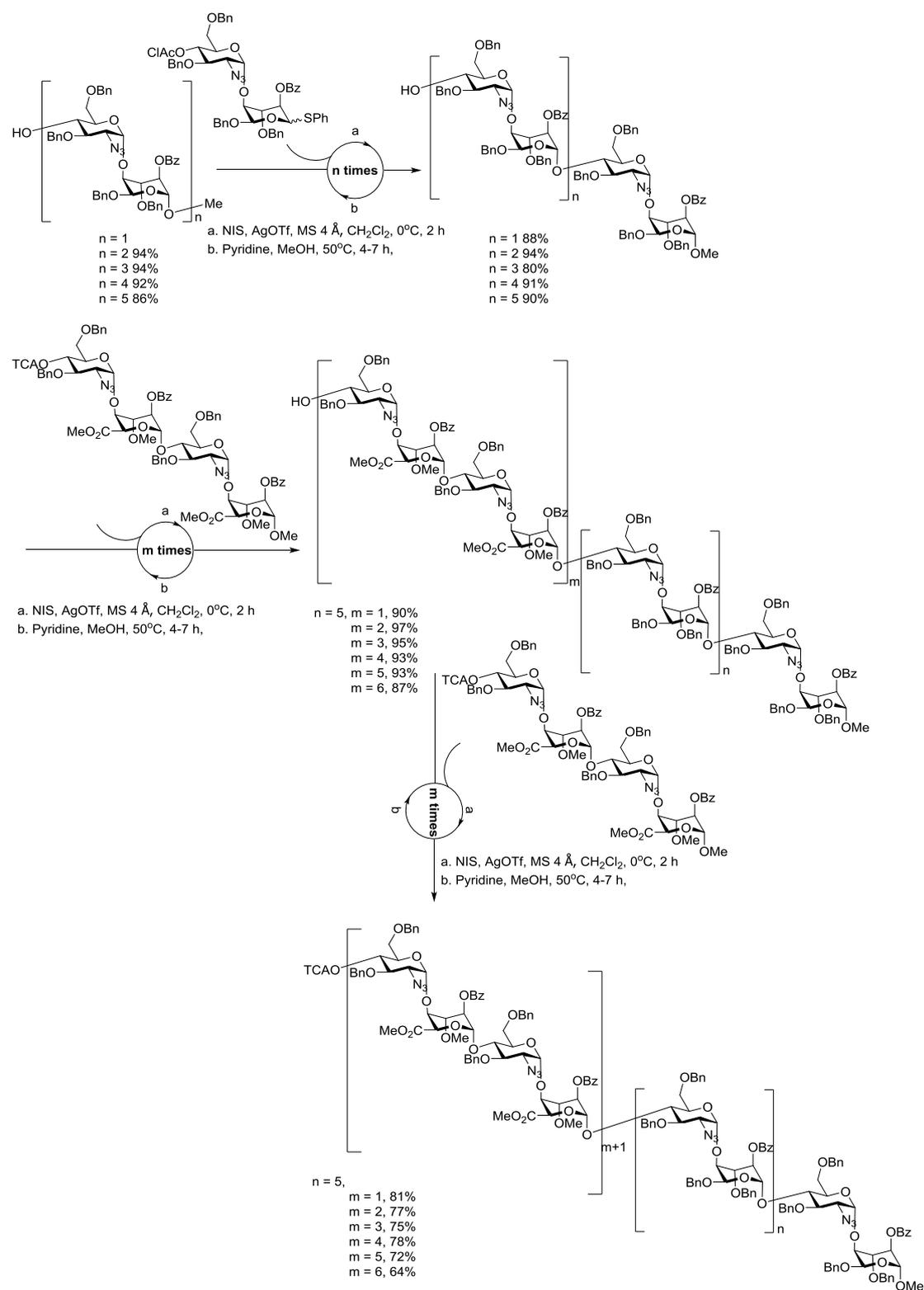
564. Hansen, S. U.; Miller, G. J.; Jayson, G. C.; Gardiner, J. M. *Org. Lett.* **2013**, *15*, 88–91.

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by solution-phase chemical synthesis. However, they only conducted the deprotection and sulfation reaction onto the 20-mer.⁵⁶⁵

565. Hansen, S. U.; Miller, G. J.; Cliff, M. J.; Jayson, G. C.; Gardiner, J. M. *Chem. Sci.* **2015**, *6*, 6158–6164.

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Scheme 75. Two-step cycles developed by Gardiner and coworkers to prepare long HP-type oligosaccharides.

This iterative approach can also be used for the elongation of HP/HS chains from non-reducing end to reducing end into one-pot processes, using the ability of specific

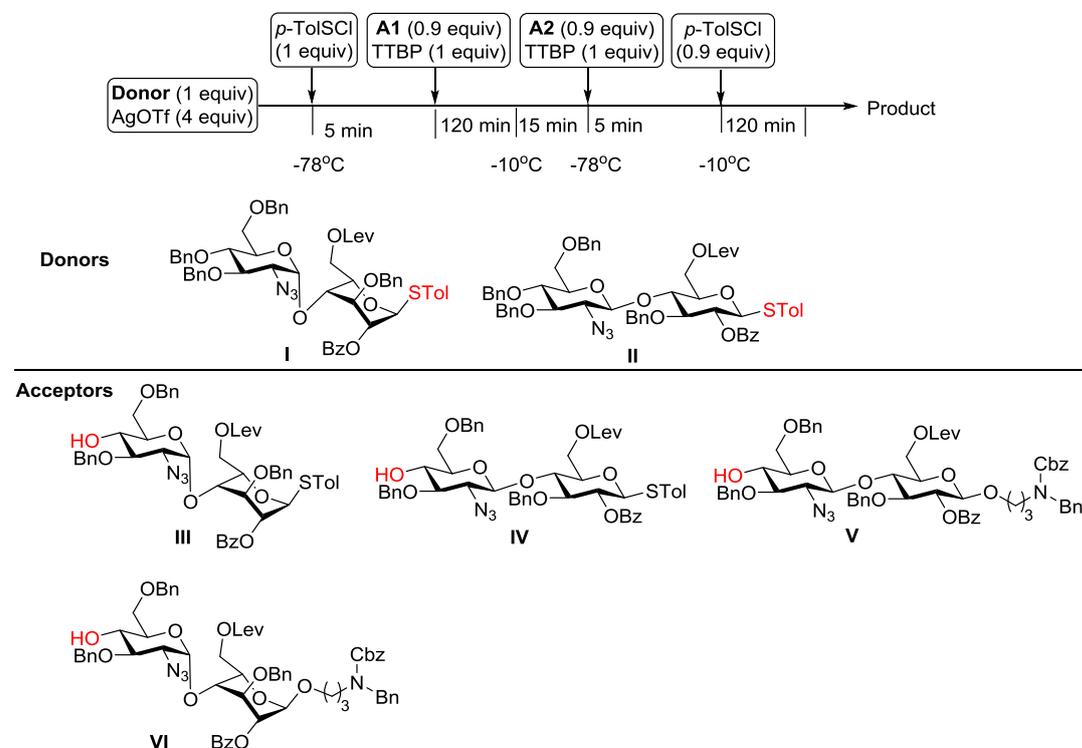
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leaving groups of glycosidic donors to be selectively activated.

I.4.3. Pre-activation and reactivity-based one-pot approach

Thioglycosides exhibited the well-established ability to be pre-activated by a promoter to generate a reactive intermediate in absence of any acceptor. In this way, if a thioglycoside, bearing a free hydroxyl group, is used as an acceptor, after the completion of the glycosylation reaction, the formed thioglycoside derivative can be directly pre-activated into the reaction media prior to the addition of a new acceptor. Therefore, to avoid activation of the acceptor or product during this iterative one-pot process, (i) the promoter must be used in stoichiometric amount, (ii) side products generated by the activation of the donor must not be nucleophilic.

In 2010, an efficient one-pot strategy based on pre-activation was employed by Huang and coworkers to prepare a library of HP/HS hexasaccharides. After the preparation of required six disaccharide building blocks, a library of 6 different hexasaccharides was obtained using the combination of *p*-TolSCI and AgOTf as promoter and specific cycles of reaction time and temperature (Table 14).^{543,566}



566. Huang, X.; Huang, L.; Wang, H.; Ye, X. S. *Angew. Chemie. Int. Ed.* **2004**, *43*, 5221–5224.

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Donor	Acceptor 1 (A1)	Acceptor 2 (A2)	Product	Yield (%)
I	III	V	I-III-V	54%
I	IV	V	I-IV-V	59%
I	IV	VI	I-IV-VI	58%
II	III	V	II-III-V	62%
II	III	VI	II-III-VI	57%
II	IV	VI	II-IV-VI	50%

Table 14. One-pot preparation of heparin/HS hexasaccharides.

Remarkably, using this iterative one-pot pre-activation protocol, they also successfully synthesized two HS proteoglycans, Syndecan-1⁵⁶⁷ and Syndecan-3,^{568,569} laying a groundwork for the preparation of more complex targeted oligosaccharides.

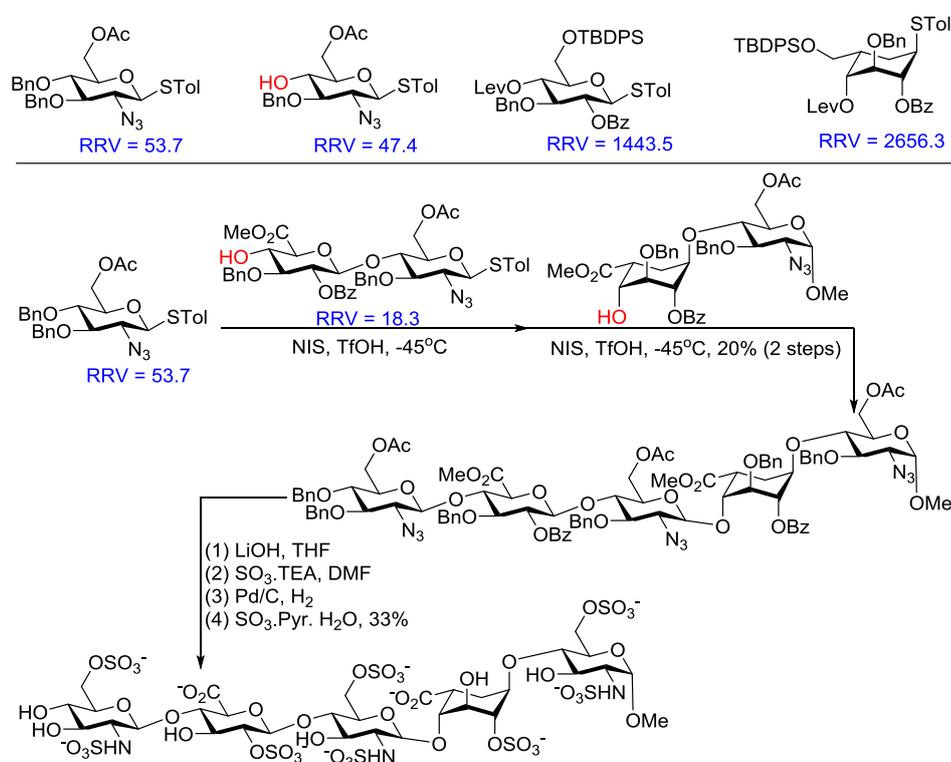
An alternative to iterative one-pot pre-activation protocol is to use the difference of reactivity of glycosyl building blocks to selectively activate one thioglycosides in presence of another one. This reactivity-based approach relies on the armed-disarmed concept⁵⁷⁰ that permit to estimate the differences of reactivity of glycosides regarding the presence of electron-donating (armed = more reactive) and electron-withdrawing (disarmed = less reactive) protecting groups.^{571–576} Differences of reactivity can be quantified as relative reactivity values (RRVs), by setting the reference reactivity of a disarmed glycosyl donor, generally a tetra-*O*-acetylated glycopyranoside, toward methanol as acceptor to 1.0.⁵⁷¹ The general procedure of glycosylation consists to add a limiting amount of promotor to a mixture of a more reactive glycosyl donor (armed glycoside) than glycosyl acceptor (disarmed glycoside). In this case, the armed donor

567. Yang, B.; Yoshida, K.; Yin, Z.; Dai, H.; Kavunja, H.; El-Dakdouki, M. H.; Sungsuwan, S.; Dulaney, S. B.; Huang, X. *Angew. Chemie. Int. Ed.* **2012**, *51*, 10185–10189.
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573. Jensen, H. H.; Nordstrøm, L. U.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205–9213.
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575. Huang, L.; Wang, Z.; Huang, X. *Chem. Commun.* **2004**, No. 17, 1960.
576. Pornsuriyasak, P.; Gangadharmath, U. B.; Rath, N. P.; Demchenko, A. V. *Org. Lett.* **2004**, *6*, 4515–4518.

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is preferentially activated and glycosylation occurs on the disarmed acceptor. The newly formed oligosaccharide directly acts as donor in further glycosylation with a less reactive acceptor.

In 2007, Wong and coworkers reported a one-pot synthesis of a heparin-like pentasaccharide based on the reactivity-based approach (Scheme 76).⁵⁷⁷ They prepared four monosaccharide building blocks with well-defined reactivity. After successful application of the reactivity-based glycosylation for the preparation of two disaccharides with excellent yields and stereoselectivity, they extended this method to the one-pot synthesis of Fondaparinux backbone and its subsequent deprotection/sulfation.



Scheme 76. A one-pot strategy for preparing heparin-like pentasaccharide *via* reactivity-based approach.

However, the application of this strategy for the preparation of longer HP/HS oligosaccharides can be challenging, since different reactivities of building blocks are required. Indeed, the RRV values of building blocks can vary according to reaction conditions, leading to uncertainty of the final outcome of a reaction if predicted results

577. Polat, T.; Wong, C. *J. Am. Chem. Soc.* **2007**, *129*, 12795–12800.

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only rely on RRV values.⁵⁷⁸ Therefore, the pre-activation strategy represents the most reliable one-pot approach for the preparation of oligosaccharides.

In summary, since the accomplishment of the total synthesis of the anticoagulant drug Fondaparinux, significant progress has been achieved into the chemical formation of HP/HS glycosidic linkage using glycosylation methodologies to access to a large variety of pure and well-defined synthetic HP/HS. However, the assembly of highly diversified HP/HS oligosaccharides of significant length remains an important challenge and a daunting task to master. Continued efforts are done to improve the panel of glycosylation techniques, to extend them to automated solid-phase synthesis^{579–586} or to decipher glycosylation processes from a mechanistic point of view.⁵⁸⁷ In our group, preparation of oligosaccharidic fragments constituted of the HP repeating sequence is highly reliable and, for our project, we decided to implement reducing and non-reducing end modifications of HS fragments after their elongation to limit as far as possible modification and/or reoptimization of reaction conditions.

II. Strategy of Synthesis of HS Fragments and Functionalization of a HS-type Tetrasaccharide

During her PhD in our group, Dr. Wenqing Liu implemented the sequence of transformations of the reducing end and non-reducing end to the preparation of a modified dp4 HS fragment.²³⁶ She demonstrated its compatibility with the synthetic pathway and set of protecting groups currently used in our lab to access to well-defined HS fragments. By this way, she obtained 30 mg of the modified tetrasaccharide **53**

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578. Li, X.; Huang, L.; Hu, X.; Huang, X. *Org. Biomol. Chem.* **2009**, *7*, 117–127.
579. Dreef-Tromp, C. M.; Willems, H. A. M.; Westerduin, P.; van Veelen, P.; van Boeckel, C. A. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1175–1180.
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583. Weishaupt, M.; Eller, S.; Seeberger, P. H. *Methods Enzymol.* **2010**, *478*, 463–484.
584. Czechura, P.; Guedes, N.; Kopitzki, S.; Vazquez, N.; Martín-Lomas, M.; Reichardt, N. C. *Chem. Commun.* **2011**, *47*, 2390–2392.
585. Etxebarria, J.; Serna, S.; Beloqui, A.; Martín-Lomas, M.; Reichardt, N. C. *Chem. - A Eur. J.* **2013**, *19*, 4776–4785.
586. Guberman, M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2019**, *141*, 5581–5592.
587. Martín, A.; Arda, A.; D'Esposito, J.; Martín-Mingot, A.; Probst, N.; SinaýP.; Jiménez-Barbero, J.; Thibaudeau, S.; Blériot, Y. *Nat. Chem.* **2015**, *8*, 186.

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(Figure 55). In order to prepare the symmetric tridentate ligand targeting the CXCL12 dimer and to validate one-pot conditions of Click reactions developed onto the model compound, we decided first to reproduce the work of Dr. Wenqing Liu to have a larger amount of the modified dp4 HS fragment **53** in hand. Therefore, we started by the resynthesis of tetrasaccharide **44**.

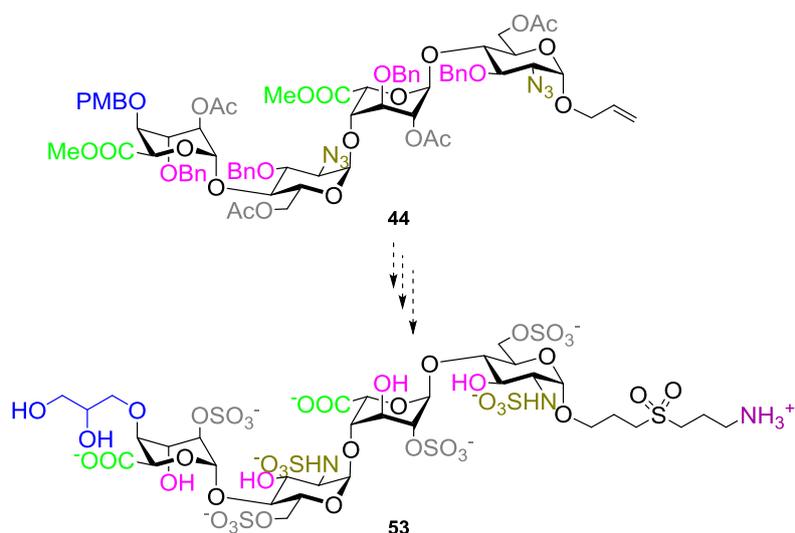


Figure 55. Structure of modified dp4 HS fragment **50** obtained by Dr. Wenqing Liu starting from the tetrasaccharide **44**.

It should be not forgotten that our strategy of access to well-defined HS fragments relies on a carefully selected set of four orthogonal protecting groups (Ac, N₃, CO₂Me, Bn). As depicted in Figure 5.1, these protecting groups are used as following:

(i) Acetyl groups (Ac) are installed as transient protecting groups at positions that will be *O*-sulfated;

(ii) Azido groups (N₃) are used as masked amine, they can be readily reduced into amino groups, sulfated or acetylated in later synthetic steps;

(iii) Methyl ester groups (CO₂Me) allowed the temporary protection of carboxylic moieties of uronic units;

(iv) Benzyl groups (Bn) are utilized as permanent protecting groups to block the hydroxyl groups that would be free in the final compounds.

II.1. Synthesis of HS-type Tetrasaccharide **44**

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II.1.1. Retrosynthetic analysis

From a retrosynthetic point of view, tetrasaccharide **44** can be obtained *via* a [2+2]-glycosylation between a *N*-phenyl-2,2,2-trifluoroacetimidate-type disaccharide donor **42** (NPTFA) and a disaccharide acceptor **43**, both of them being synthesized from the common disaccharide building block **41**, avoiding the preparation of other specific building blocks. Compound **41** can be prepared through a [1+1]-glycosylation between the *N*-phenyl-2,2,2-trifluoroacetimidate-type iduronyl donor **39** and 2-azido-2-deoxy-D-glucose acceptor **40**, while both of them can be easily synthesized from commercial available 1,2,5,6-di-*O*-isopropylidene-D-glucose (Scheme 65) and D-glucosamine hydrochloride, respectively (Figure 56).

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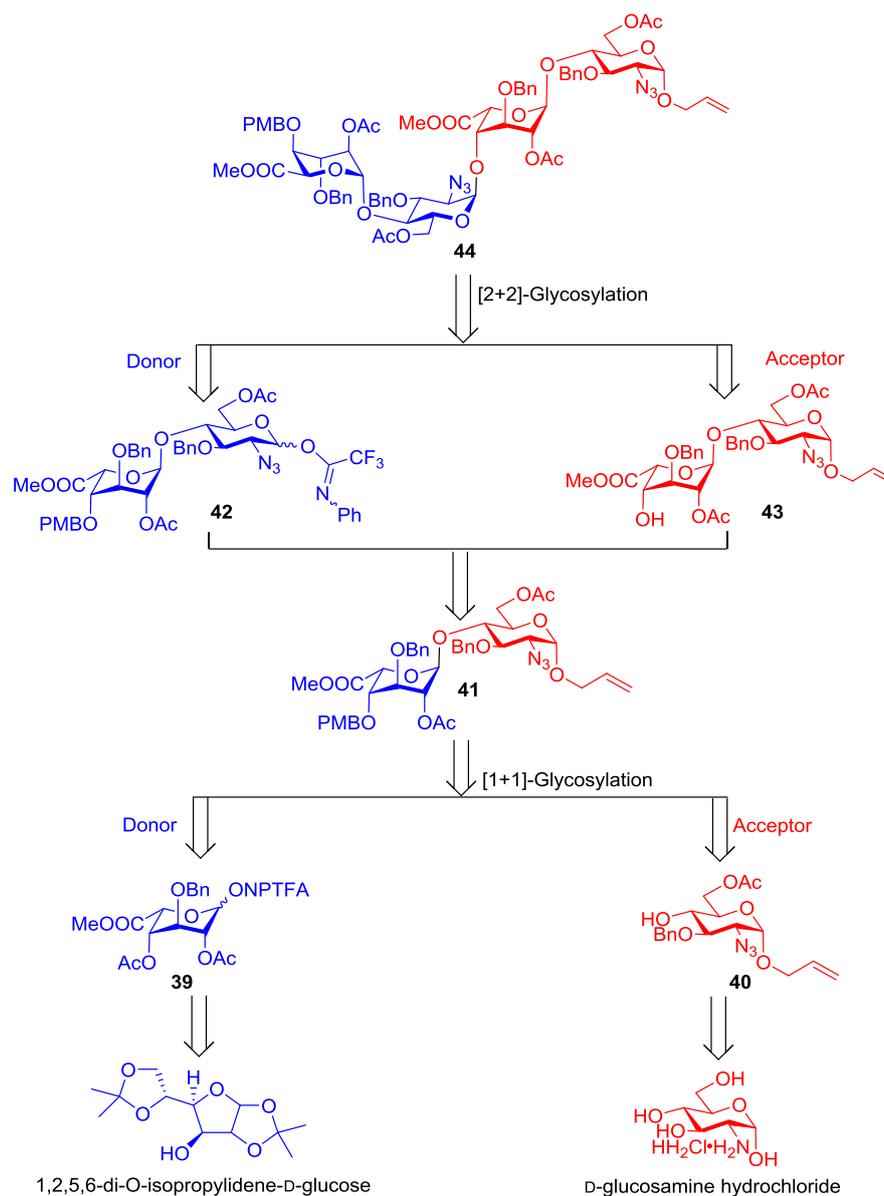


Figure 56. Retrosynthetic analysis to access tetrasaccharide **44**.

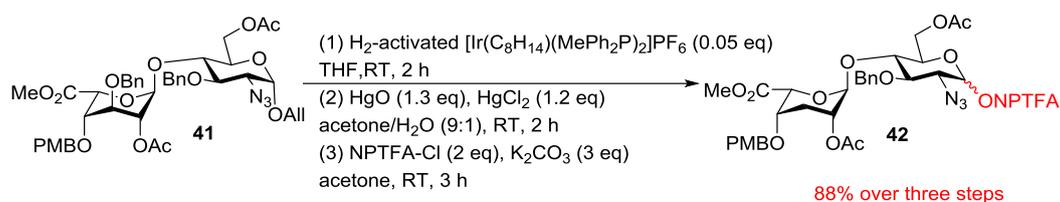
It is worth mentioning that the *O*-2 acetyl group of **39** is not only used as temporary protecting for sulfation of this hydroxyl group but it also confers neighboring group assistance during the formation of the 1,2-*trans* glycosidic bond. Moreover, taking advantage of its nonparticipation in glycosylation, the azido group of **42** permits to favor 1,2-*cis* glycosidic bond formation during glycosylation reaction required for the elongation of HS fragments.

II.1.2. Synthesis of tetrasaccharide **44**

In our lab, the disaccharide **41** is available in large amount and permits to prepare

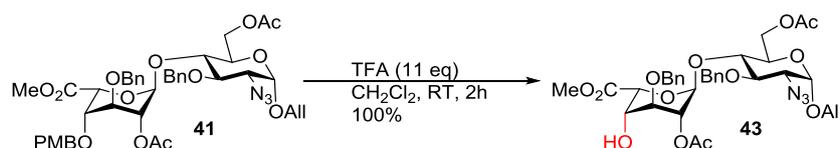
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the glycosyl donor **42** and the glycosyl acceptor **43**. The glycosyl donor was prepared in three sequential steps consisting into the allyl ether isomerization, hydrolysis and conversion of the obtained hemiacetal into *N*-phenyl-2,2,2-trifluoroacetimidate. Starting from disaccharide **41**, isomerization of the allyl ether was performed using 5 mol% of (bis(methyldiphenylphosphine))(1,5-cyclooctadiene)iridium(I) hexafluorophosphate. Addition of dihydrogen to this cationic iridium complex conducted to the *in situ* generation of a highly active catalyst for isomerization of allyl to the corresponding *trans*-propenyl ether.⁵⁸⁸ This latter was hydrolyzed to the hemiacetal of **41** by treatment with a combination of a slight excess of mercuric chloride and mercuric oxide.⁵⁸⁹ After its purification, the hemiacetal was directly transformed into *N*-phenyl-2,2,2-trifluoroacetimidate **42** by treatment with *N*-phenyl-2,2,2-trifluoroacetimidoyl chloride in acetone in presence of potassium carbonate (Scheme 77).



Scheme 77. Preparation of glycosyl donor **42** from disaccharide **41**.

The acceptor **43** was obtained in quantitative yield by acidic cleavage of the PMB ether of disaccharide **41** with 11 equivalents of TFA in DCM after 2 hours of stirring at room temperature (Scheme 78).



Scheme 78. Preparation of glycosyl acceptor **43** from disaccharide **41**.

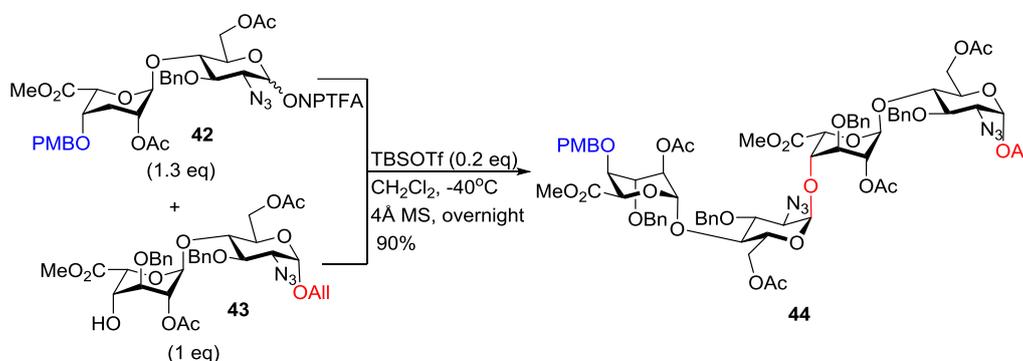
The [2+2]-glycosylation of the acceptor **43** with the donor **42**, in DCM at -40°C in presence of a catalytic amount of TBDMSOTf as promotor, gave the desired tetrasaccharide **44** in 90% yield and full α stereoselectivity (Scheme 79). Even if this result is similar than our previously described glycosylation reaction using the 2,2,2-trichloroacetimidate derivative of **42**,^{132,540} *N*-phenyl-2,2,2-trifluoroacetimidate

588. Oltvoort, J. J.; Van Boeckel, C. A. A.; De Koning, J. H.; Van Boom, J. H. *Synthesis*. **1981**, 1981, 305–308.

589. Gigg, R.; Warren, C. D. *J. Chem. Soc. C Org.* **1968**, No. 0, 1903–1911.

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exhibited an increased stability during its purification step as well as for its storage⁵⁹⁰ and no imidate-amide rearrangement⁵⁹¹ has been observed during other type of glycosylation reactions performed in our group.



Scheme 79. [2+2]-glycosylation reaction for the preparation of the fully protected tetrasaccharide **44** via the *N*-phenyl-2,2,2-acetimide pathway.

In this way, we managed to easily prepared 700 mg of the fully protected tetrasaccharide **44** for reducing end and non-reducing ends functionalization.

II.2. Modification of The Reducing and Non-Reducing Ends of **44**

To access to modified dp4 HS fragment, the previously developed sequence of transformations onto the model disaccharide **11** had been adapted to tetrasaccharide **44** to introduce aldehyde and azido groups onto the non-reducing and reducing end, respectively.

II.2.1. Retrosynthetic analysis

As shown in Figure 57, functionalized tetrasaccharide **53** will be prepared from tetrasaccharide **52** via oxidative cleavage of vicinal diol to afford aldehyde motif at non-reducing end and diazotransfer reaction to afford azide motif at reducing-end, respectively. The tetrasaccharide **52** will be afforded via a simple sequence of saponification of methyl ester of tetrasaccharide **50** followed by hydrogenolysis of benzyl ether. The vicinal diol at reducing-end of this latter compound will be formed

590. Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405–2407.

591. Larsen, K.; Olsen, C. E.; Motawia, M. S. *Carbohydr. Res.* **2008**, *343*, 383–387.

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by dihydroxylation of allyl group at position *O*-4 of non-reducing end unit of tetrasaccharide **49**, itself obtained after *O*- and *N*-sulfation of tetrasaccharide **48**. Compound **48** will be afforded from tetrasaccharide **47** via a sequence of reduction of azide and deacetylation of hydroxyl groups. The allyl group at position *O*-4 of non-reducing end unit of tetrasaccharide **47** will be introduced by a chemoselective Tsuji-Trost decarboxylative allylation after deprotection of the PMB group of tetrasaccharide **45**. Finally, the stable sulfone **45** will be prepared from tetrasaccharide **44** through a sequence of thiol-ene coupling onto the allyl moiety of the reducing-end unit and subsequent oxidation of the formed thioether.

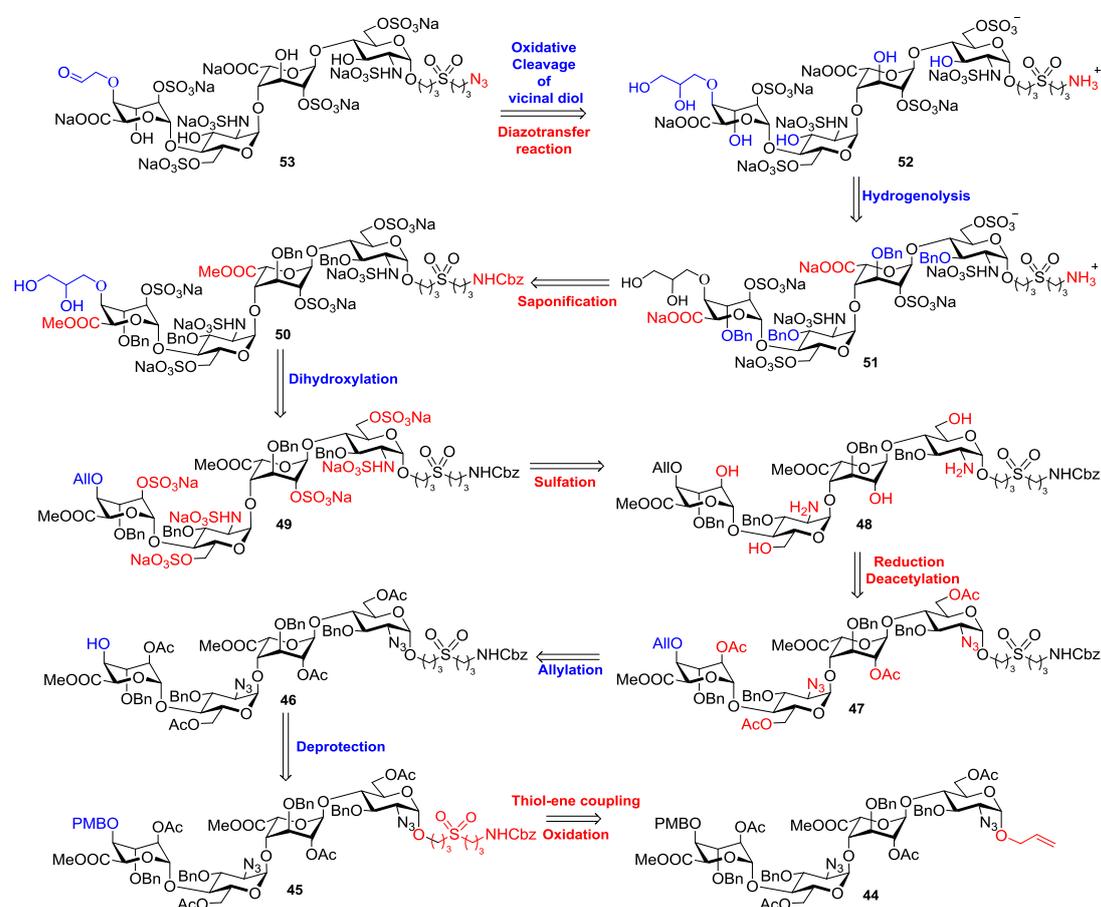


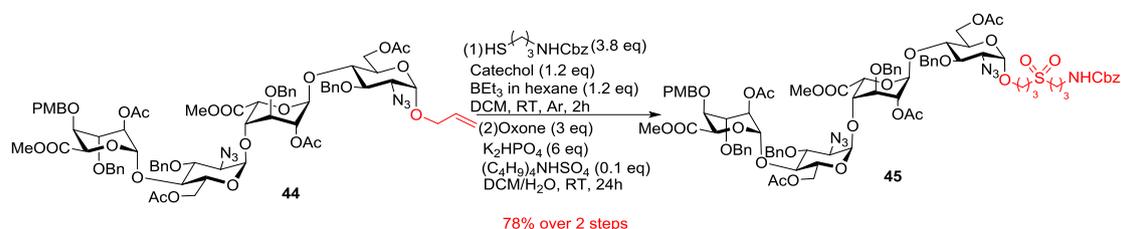
Figure 57. Retrosynthetic scheme for the preparation of tetrasaccharide **53**.

II.2.2. Application of the sequence of chemical modifications

As discussed above, the first steps of preparation of tetrasaccharide **53** consisted into a sequence of radical thiol-ene coupling of the allyl group at the reducing end of **44** followed by oxidation in sulfone of the formed thioether. As for disaccharide **4**, compound **44** was treated with 3.8 equivalents of benzyl 3-mercaptopropylcarbamate

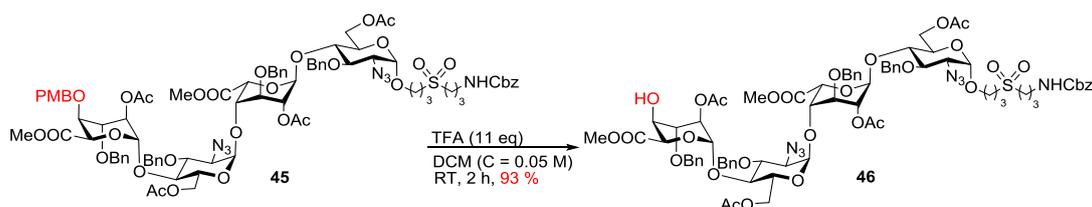
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in presence of 1.2 equivalents of triethylborane and catechol under argon atmosphere at room temperature for 2 hours to give the corresponding thioether that was directly converted into sulfone **45**, at room temperature in DCM under phase transfer conditions, using Oxone® as oxidant, with an overall yield of 78% (Scheme 80).



Scheme 80. Radical TEC on tetrasaccharide **44** and its direct conversion into sulfone **45**.

Then, the PMB ether of tetrasaccharide **45** was cleaved in 93% yield after 2 hours of stirring in DCM at room temperature in presence of 11 equivalents of TFA (Scheme 81).



Scheme 81. Deprotection of PMB group of tetrasaccharide **45**.

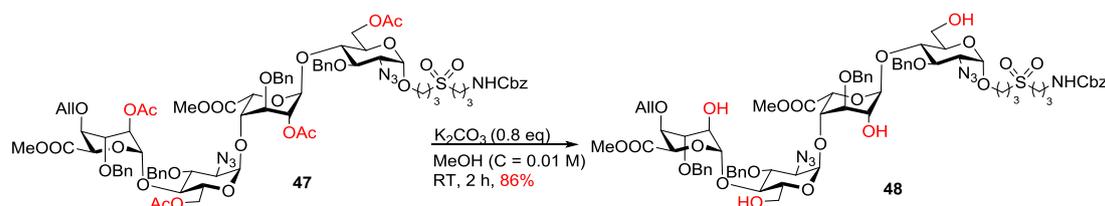
Subsequently, the liberated hydroxyl group at position *O*-4 of the non-reducing end unit of tetrasaccharide **46** was allylated under optimized Tsuji-Trost decarboxylative *O*-allylation conditions (as described in Chapter 2) that allow the limitation of the allylation of the nitrogen atom of the carbamate moiety. After 4 hours of stirring at 60 °C in toluene in presence of 1.2 equivalents of allyl *tert*-butylcarbonate and 2.5 mol% of Pd(PPh₃)₄, 65% of free alcohol was converted in desired allyl ether **47** while 25% of starting material was recovered. Moreover, 5% of *N,O*-diallylated tetrasaccharide **47'** was also isolated (Scheme 82).



Scheme 82. Chemoselective Tsuji-Trost *O*-allylation of tetrasaccharide **46**.

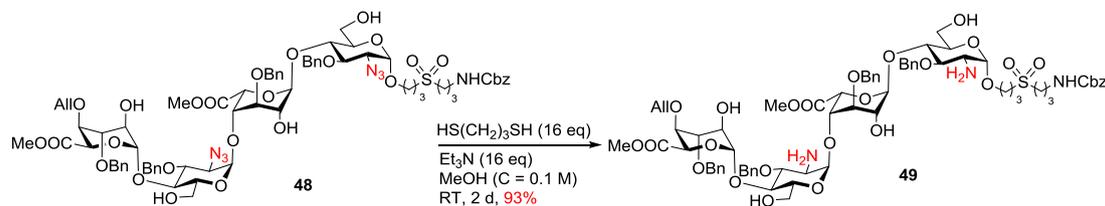
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All acetyl groups were then removed by transesterification using 0.8 equivalent of K_2CO_3 in anhydrous methanol at room temperature for 2 hours. After neutralization of the reaction media with Dowex H^+ resin, filtration and concentration under reduced pressure, tetrasaccharide **48** was isolated in 86% yield. At this stage of the synthesis, about 400 mg of **48** were obtained (Scheme 83).



Scheme 83. Deacetylation of tetrasaccharide **47**.

Deacetylation was followed by reduction of azide into amino groups using propane-1,3-dithiol in presence of triethylamine in methanol for 2 days under argon. The reaction solution must be protected from light and oxygen in order to avoid light promoted TEC side reaction with propane-1,3-dithiol. After size exclusion chromatography using Sephadex LH-20 to remove excess of thiol and its formed polymers, the crude product was concentrated and purified by silica gel chromatography to give tetrasaccharide **49** in 93% yield (Scheme 84).

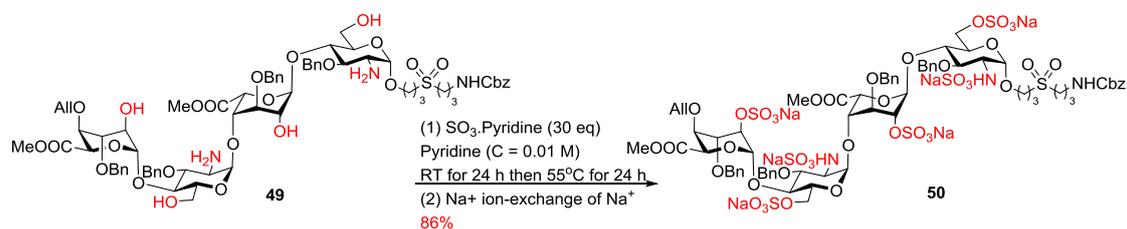


Scheme 84. Reduction of azido groups of **48** to access the amino derivative **49**.

In the lab, for the sulfation step, SO_3 -pyridine was used as sulfating reagent and 5 equivalents of this sulfur trioxide complex was used per free amino and hydroxyl groups to sulfate. The reaction solution was firstly stirred at room temperature for 24 hours to sulfate amino groups, then the temperature was increased to $55^\circ C$ and kept for another 24 hours to sulfate hydroxyl groups. After confirmation of the full conversion of tetrasaccharide **49** into desired persulfated compound **50** by HPLC monitoring, the reaction was quenched by successive addition of trimethylamine and methanol and the resulting solution was stirred at room temperature for an additional hour. Size exclusion chromatography on Sephadex LH-20 gel followed by ion-exchanged using BioRad

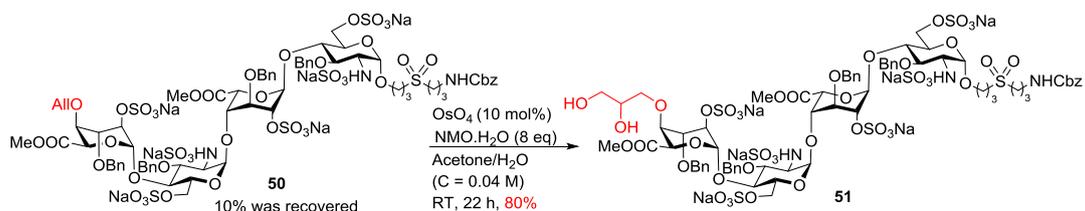
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AG50W-X8 200 (Na⁺) resin and lyophilisation of the resulting aqueous solution permit to isolate the pure hexasulfated product **50** in Na⁺ form in 86% yield (Scheme 85).



Scheme 85. Sulfation of free amine and hydroxyl groups of tetrasaccharide **49**.

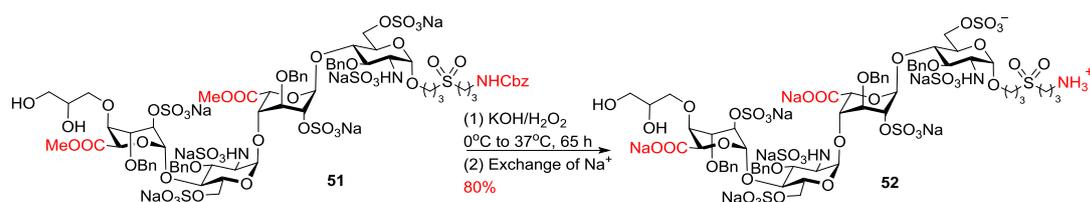
Before saponification of methyl ester and to facilitate the purification of the formed diol, dihydroxylation of the allyl ether of the non-reducing end of **50** was accomplished under previously optimized Upjohn-dihydroxylation conditions on model disaccharide **8**. However, precipitation of black OsO₂ solid was observed in this case and another loading of 4 equivalents of NMO was needed to reoxidize OsO₂ into OsO₄. HPLC monitoring allowed to insure a good conversion of allyl ether **50** into diol **51** and the reaction was quenched with Na₂S₂O₃ after 22 hours of stirring at room temperature. The crude product was purified by RP-18 chromatography and permit to isolate 80% yield of tetrasaccharide **51** (Scheme 86) and to recovery 10% of starting allyl ether.



Scheme 86. Dihydroxylation of allyl ether motif of tetrasaccharide **50**.

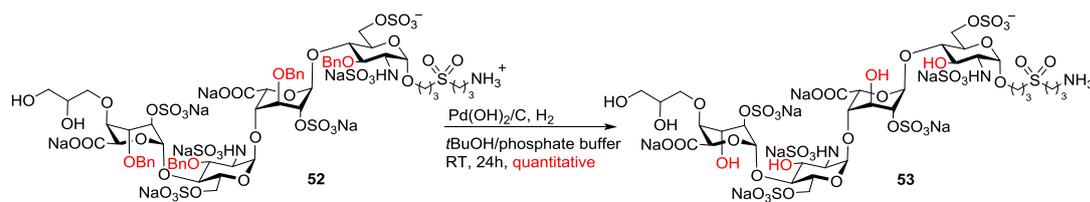
Subsequently, saponification of methyl ester of **51** and liberation of the amino group of its aglycone moiety was performed simultaneously using the combination of KOH/H₂O₂ in water. This time, in contrast to disaccharide **9**, **51** was successfully converted into the desired compound **52** after 65h at 37 °C. After quenching the reaction with AcOH, the mixture was purified by RP-18 chromatography and ion-exchanged with BioRad AG50W-X8 200 (Na⁺) resin to afford **52** in Na⁺ form in 80% yield (Scheme 87).

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Scheme 87. Saponification reaction of methyl ester allowing the concomitant removal of the Cbz group of tetrasaccharide **51**.

Finally, benzyl ether of tetrasaccharide **52** were hydrogenolyzed in presence of palladium hydroxide (20% on charcoal) in a mixture of *t*-BuOH/phosphate buffer (C = 20 mM, pH = 6) under hydrogen atmosphere after 24 hours of stirring at room temperature. Palladium on charcoal was filtered off and rinsed three times by H₂O. Then the obtained residue was desalted on a Sephadex G-15 column to give tetrasaccharide **53** in quantitative yield (Scheme 88).



Scheme 88. Hydrogenation of tetrasaccharide **52** to remove benzyl groups.

With this compound in hand, in order to prepare desired glycoconjugates, we decided to turn our attention to the preparation of PEG-type partners.

III. Preparation of PEG-Type Partners for Click Reactions

As previously described in chapter 1, the synthesis of the tridentate neutral ligand to target CXCL12 dimer necessitates the preparation of 2 different PEG-type spacers, functionalized by a sulfotyrosine at one extremity and by an aminoxy (compound **83**) or propargyl group (compound **77**) at the other one, with a length allowing to cover a distance of about 20 Å (Figure 58a). For the preparation of the three different geometries of SAS mimics targeting INF- γ , 3 different PEG-type spacers have to be synthesized: one bearing two propargyl groups (compound **67**), a second one two aminoxy groups (compound **69**) and a last one exhibiting the two different functional groups (compound **68**) at its extremities and covering a distance of about 50 Å (Figure 58b). The polymerization degree of each starting PEG to use for the preparation of ligation

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partners has been determined by molecular modeling by Aniss Zaoui during his M2 internship in the lab.

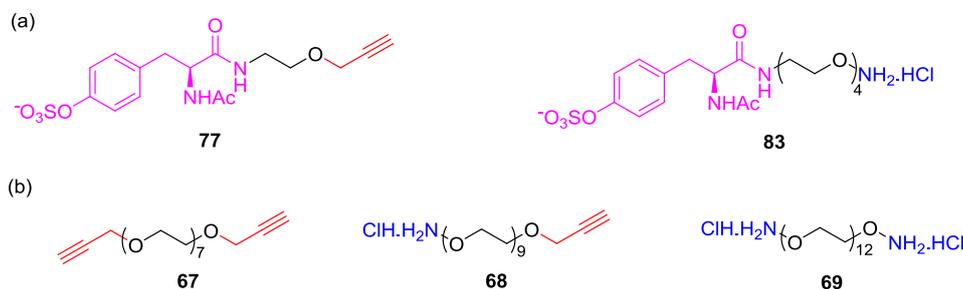


Figure 58. Five different kinds of PEG-type partners of different lengths.

To setup and validate the sequence of chemical modifications of PEG extremities to introduce aminoxy and propargyl groups, we started our optimization of reaction conditions by the preparation of PEG-type spacers required for the synthesis of the three different geometries of SAS mimics.

III.1. Preparation of PEG Type Spacers for SAS Mimics

As previously described in chapter 2, from a retrosynthetic point of view, the three different kinds of PEG type spacers could be synthesized through (Figure 59):

(a) Williamson alkylation reaction with propargyl bromide to synthesize symmetric dipropargyl-PEG;

(b) Introduction of hydroxyphthalimide groups by Mitsunobu reaction onto PEG, followed by their deprotection to synthesize symmetric diaminoxy-PEG;

(c) Apply the combination of the two methods to synthesize asymmetric propargyl-aminoxy-PEG.

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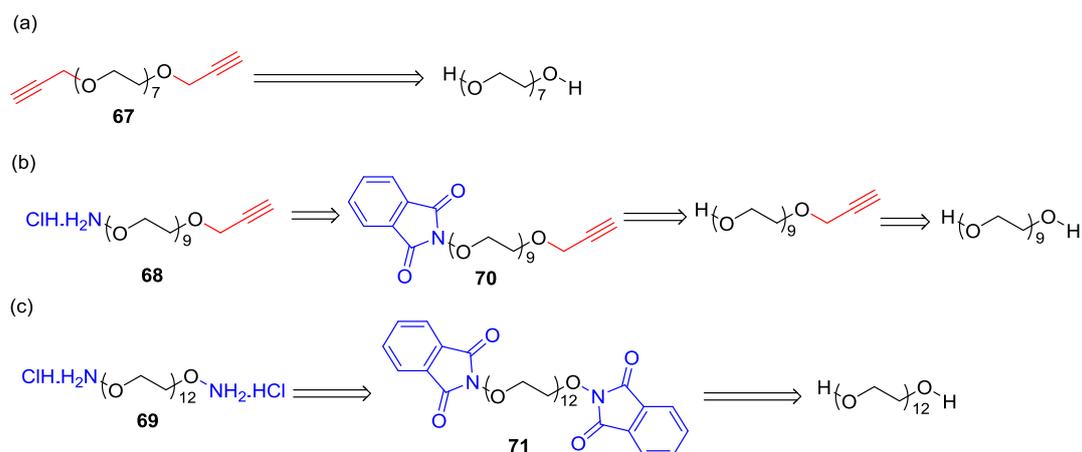


Figure 59. Retrosynthetic scheme for the preparation of the three different PEG-type spacers required for SAS mimics preparation.

Moreover, considering that the hepta-, nona- and dodecaethylene glycol needed to prepare PEG-type linkers **67-69** are not commercially available, we have evaluated two different strategies to prepare these PEG-type linkers:

- (i) Introduction of the desired modification at one extremity of 3 PEG building blocks followed by their coupling
- (ii) elongation of the PEG oligomer followed by introduction of desired modifications at its two extremities

III.1.1. Preparation of PEG-type linkers using strategy (i)

Concerning the first strategy, these three PEG-type linkers can be synthesized from three commercially available and cheap PEGs, ethylene glycol, triethylene glycol and hexaethylene glycol. As depicted in Figure 60 below, the heptaethylene glycol derivative **67** can be prepared *via* a [1+6]-coupling through a S_N2 reaction between *O*-propargyl-ethylene glycol **54** and *O*-propargyl-*O'*-tosyl-hexaethylene glycol **56**, the nonaethylene glycol derivative **68** *via* a [3+6]-coupling between *O*-propargyl-triethylene glycol **57** and *O*-(*N*-phtamimidooxyethyl)-*O'*-tosyl-pentaethylene glycol **59**. This latter compound can be used to prepare the last PEG-type linker **69** *via* a [6+6]-coupling with *O*-(*N*-phtamimidooxyethyl)-pentaethylene glycol **58**).

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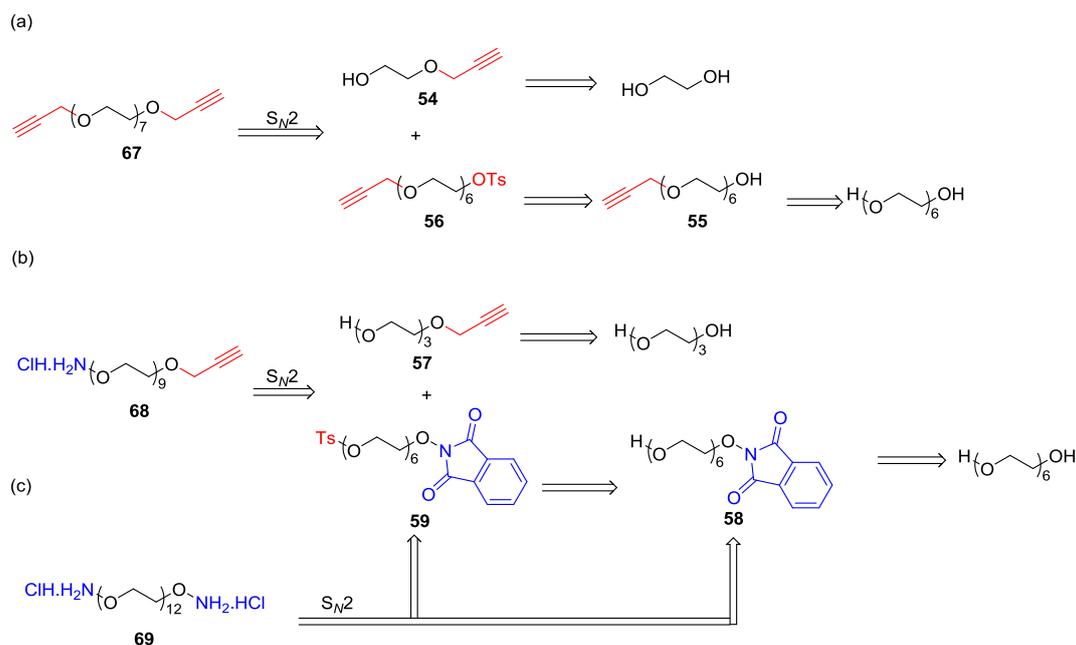


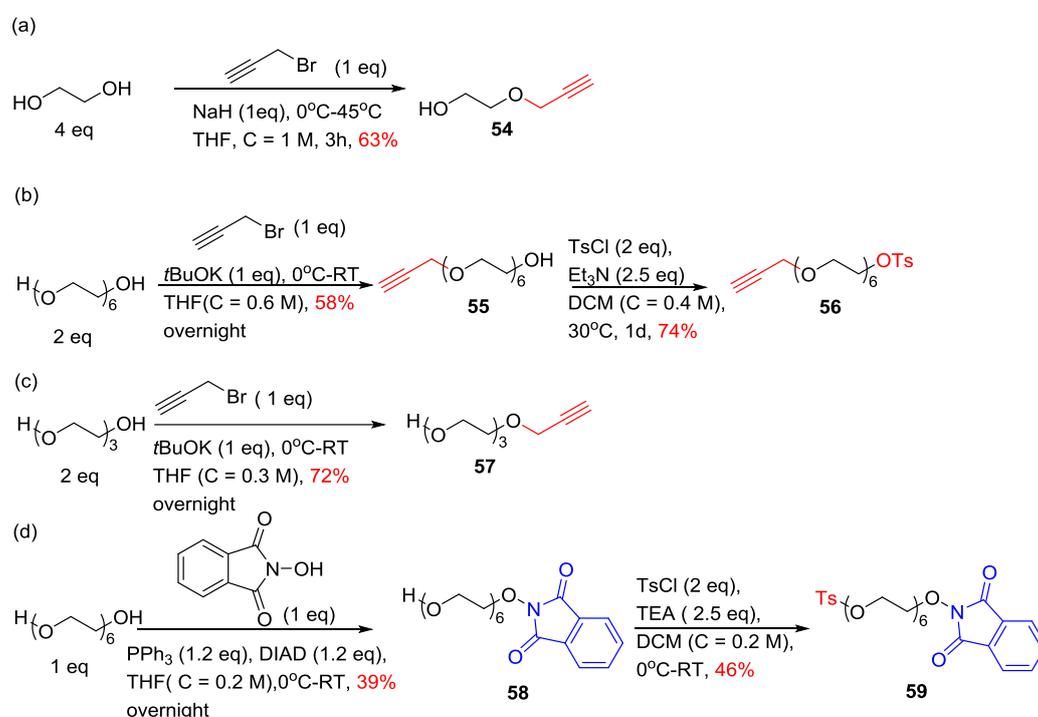
Figure 60. Retrosynthetic scheme for the preparation of PEG-type spacers using strategy i.

Site-selective reactions is a challenging subject for chemists.⁵⁹² In our case, we had to realize a mono-functionalization of one PEG extremity. The simplest method is to use the substrate in a large excess to favor the monofunctionalization over the difunctionalization. Given the relatively low price of the three used PEGs ($n = 2, 3, 6$), we decided to use them in large excess to prepare the monosubstituted PEG-type building blocks **54-59** and to recover as far as possible unreacted starting materials. Hence, to prepare compound **54**, 4 equivalents of ethylene glycol were treated with 1 equivalent of NaH in anhydrous THF at 0 °C. After stirring at 0 °C for 30 minutes, 1 equivalent of propargyl bromide was added and the reaction temperature was raised to 45°C and maintained for 3 h and compound **54** was isolated in 63% yield (Scheme 89a). In a similar way, compound **57** was isolated in 72% yield by using 1 equivalent of *t*-BuOK as base instead of NaH. This modification of protocol allowed us to use only 2 equivalents of triethylene glycol in anhydrous THF and to observe conversion of the starting material at room temperature (Scheme 89c). Compound **55** was isolated in 58% yield by using this modified protocol on hexaethylene glycol. The obtained compound was converted into its tosylate derivative **56** using 2 equivalents of *p*-toluenesulfonyl chloride and 2.5 equivalents of trimethylamine in anhydrous CH₂Cl₂. As for the

592. Dimakos, V.; Taylor, M. S. *Chem. Rev.* **2018**, *118*, 11457–11517.

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preparation of **56**, the reaction media has to be heated at 30°C to observe conversion of **55** and, after 1 day of stirring at this temperature, **56** was isolated in 74% yield (Scheme 89b). Finally, compound **59** was obtained by a two-steps sequence consisting into a Mitsunobu reaction followed by a tosylation reaction. One equivalent of hexaethylene glycol was stirred with 1.2 equivalents of triphenylphosphine (1.2 equivalent) and 1 equivalent of *N*-hydroxyphthalimide in THF before the addition of 1 equivalent of diisopropyl azodicarboxylate. The resulting phthalimidooxy derivative **58** was isolated in 39% yield and almost quantitatively converted into its tosylate derivative **59** in 46% yield by using 2.0 equivalents of *p*-toluenesulfonyl chloride and 2.5 equivalents of triethylamine in anhydrous CH₂Cl₂ (Scheme 89d).



Scheme 89. Preparation of compound **54** to **59**.

With intermediate compounds **54** to **59** in hands, we investigated reaction conditions of the S_N2 reaction allowing the elongation of the PEG oligomers to access to desired PEG-type partners **67** to **69**. Unfortunately, whatever the base and solvent used, the reaction concentration and temperature, we never observed formation of desired compounds and in all case we recovered a mixture of starting materials along with partial hydrolyzed tosylate derivatives. We assumed that the failure of elongations can be attributed to a low reactivity of formed electrophiles. One solution to circumvent

Chapter 5. Preparation of HS Fragments and Functionalization of a HS Tetrasaccharide & Synthesis of Linkers for The Generation of CXCL12 Tridentate Ligands and SAS Mimics

this problem would be to replace the tosylate leaving group by a halide or a triflate one. Another one would be to apply the second strategy of preparation of desired linkers, *i.e.* performing the elongation of the PEG oligomer prior to its functionalization.

III.1.2. Preparation of PEG-type linkers using strategy (ii)

We opted for the second strategy of preparation of PEG-type linkers instead of modifying the leaving group.⁵⁹³ Thus, as depicted in Figure 60, we decided to access to desired PEG-type linkers **67** to **69** *via* intermediary dibenzylated PEG oligomers and to limit the number of PEG-type building blocks to prepare. Indeed, we assumed that di-*O*-benzylated heptaethylene glycol **64** can give access to **67** by a sequence of hydrogenolysis/di-*O*-propargylation. Compound **64** can be synthesized by a [3+4]-coupling between *O*-benzyl-triethylene glycol **60** and *O*-benzyl-*O'*-tosyl-tetraethylene glycol **62**. These two building blocks will come from the mono-*O*-benzylation of triethylene glycol and the application of a mono-*O*-benzylation/tosylation sequence to tetraethylene glycol (Figure 61a). In a similar way, compound **68** can be prepared from di-*O*-benzylated nonaethylene glycol **65** *via* a sequence of hydrogenolysis/mono-*O*-propargylation/Mitsunobu reaction/phtalimido group removal. Compound **65** can be synthesized by a [4+1+4]-coupling between previously prepared tosylate **62** and ethylene glycol (Figure 2.25b). Finally, PEG derivative **69** can be synthesized from di-*O*-benzylated dodecaethylene glycol **66** *via* a sequence of hydrogenolysis/Mitsunobu reaction/phtalimido removal. Compound **66** can be synthesized by a [4+4+4]-coupling between the ditosylated tetraethylene glycol **63** and the previously prepared *O*-benzyl-tetraethylene glycol **61** (Figure 61b).

593. Ghilardi, A.; Pezzoli, D.; Bellucci, M. C.; Malloggi, C.; Negri, A.; Sganappa, A.; Tedeschi, G.; Candiani, G.; Volonterio, A. *Bioconjug. Chem.* **2013**, *24*, 1928–1936.

Chapter 5. Preparation of HS Fragments and Functionalization of a HS Tetrasaccharide & Synthesis of Linkers for The Generation of CXCL12 Tridentate Ligands and SAS Mimics

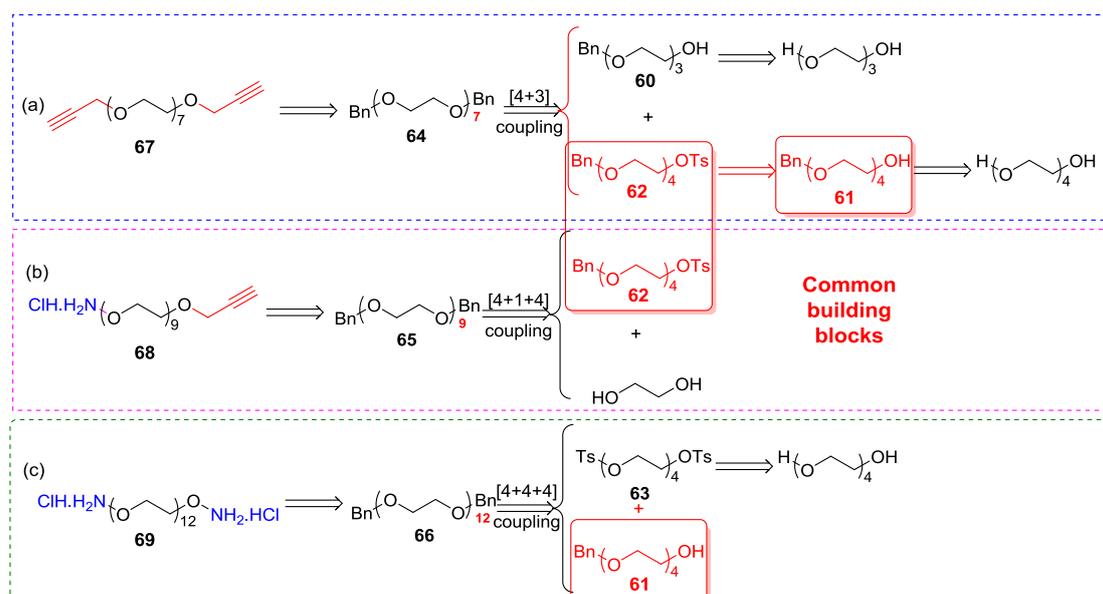
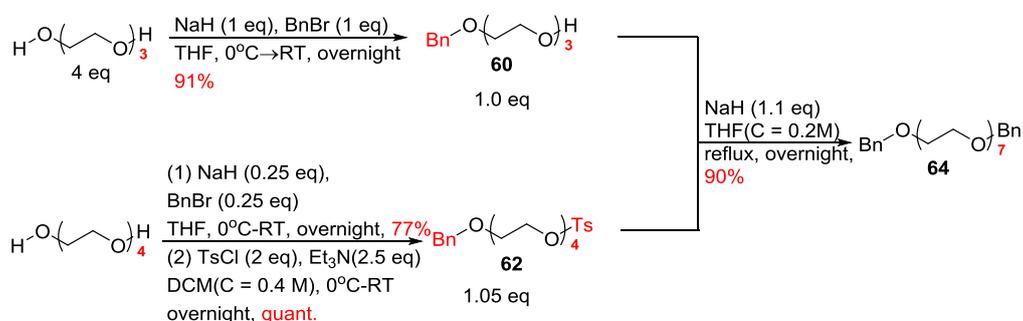


Figure 61. Retrosynthetic scheme for the preparation of PEG-type linkers using strategy (ii).

We started the preparation of the PEG-type linkers by the synthesis of compound **64**. Portionwise addition of NaH over 30 minutes to a stirring cold solution of triethylene glycol in anhydrous THF. Then, to the resulting solution, was dropwise added benzyl bromide to offer the desired mono-*O*-benzylated triethylene glycol **60** in 91%. In the same way, compound **61** was obtained in 77% yield and was then quantitatively tosylated using an excess of tosyl chloride and trimethylamine in DCM to give *O*-benzyl-*O'*-tosyl-tetraethylene glycol **62**. This time, implementation of a similar protocol allowed the [4+3]-coupling to proceed efficiently: after deprotonation of the free alcohol of **60** and addition of a slight excess of **62**. The resulting solution was refluxed overnight to smoothly afford the key dibenzylated intermediate **64** in 90% isolated yield (Scheme 90).



Scheme 90. Synthesis of the key intermediate **64** from tri- and tetraethylene glycol.

Finally, through a stepwise reaction sequence of hydrogenolysis of benzyl groups

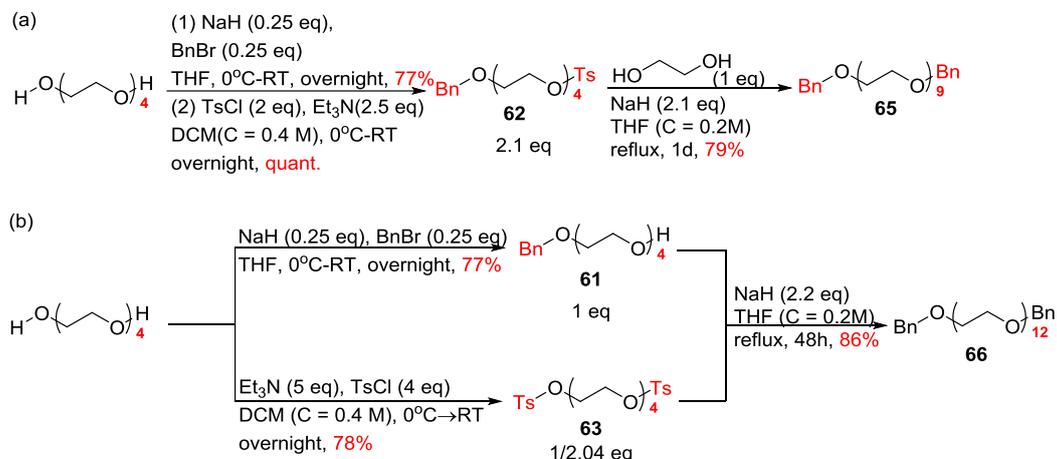
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followed by the di-*O*-propargylation of the intermediary obtained heptaethylene glycol, the targeted PEG-type linker **67** was obtained in 88% yield over two steps (Scheme 91).



Scheme 91. Conversion of di-*O*-benzylated compound **64** into targeted PEG-linker **67**.

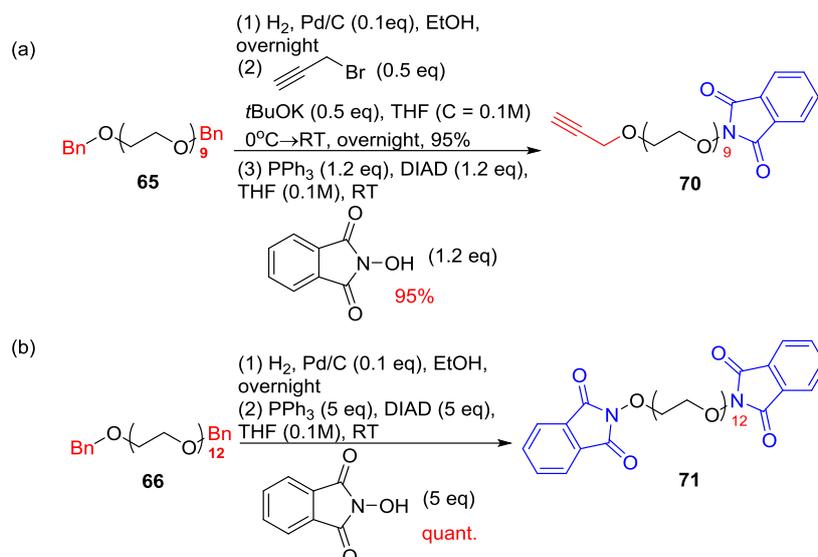
Using similar synthetic pathways, the two others PEG-type linkers **68** and **69** were easily prepared from key intermediates **65** and **66**, respectively. The intermediate **65** was synthesized in 79% isolated yield *via* a [4+1+4]-coupling reaction between an excess of previously prepared compound **62** and ethylene glycol (Scheme 92a). After preparation of di-*O*-tosylated tetraethylene glycol **63** in 78% isolated yield starting from tetraethylene glycol, intermediate **66** was obtained in 86% isolated yield by a [4+4+4]-coupling reaction between the previously prepared tetraethylene glycol derivatives **61** and **63** (Scheme 92b).



Scheme 92. Synthesis of the key intermediate **65** and **66** from tri- and tetraethylene glycol.

Hydrogenolysis of benzyl groups of **65** and **66** gave nonaethylene glycol and dodecaethylene glycol intermediates that have been directly engaged in a sequence of mono-*O*-propargylation/Mitsunobu reaction using *N*-hydroxyphthalimide as nucleophile to give **70** in 95% isolated yield over 3 steps and a double Mitsunobu reaction to give **71** in quantitative isolated yield over two steps, respectively (Scheme 93).

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Scheme 93. Synthesis of protected precursors **70** and **71** for an easy access to PEG-type linkers **68** and **69**, respectively.

We decided to keep PEG-linkers **68** and **69** as their protected derivatives **70** and **71**, respectively, for a better stability during the storage and to deprotect them prior their use. Preparation of all these PEG-type linkers allowed use to develop reliable reaction conditions for the functionalization of extremities of PEG and permitted to us to adapt it for the synthesis of required sulfotyrosine derivatives for the construction of a glycoconjugate targeting CXCL12 dimer.

III.2. Modifications of Sulfotyrosine Derivatives

As depicted in Figure 62, regarding the preparation of the 2 required sulfotyrosine derivatives, we decided to prepare the *N*-acetyl-L-sulfotyrosine derivative **77** for the HS fragment reducing end modification by sulfation of a key *N*-acetyl-L-tyrosine derivative **75**. This amide can be synthesized by coupling *N*-acetyl-L-tyrosine with 2-(propargyloxy)-ethan-1-amine **76** *via* the NHS-activated ester strategy. Compound **76** can be prepared starting from 2-aminoethanol using a sequence of *N*-protection/*O*-propargylation/*N*-deprotection (Figure 61a). The second *N*-acetyl-L-sulfotyrosine derivative **83** needed for the HS fragment non-reducing end modification by sulfation of a key *N*-acetyl-L-tyrosine derivative **82**. A new time, this amide can be obtained by coupling *N*-acetyl-L-tyrosine with the amino PEG derivative **81** *via* the NHS-activated ester strategy. Compound **81** can be prepared starting from tetraethylene glycol using a

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sequence of monotosylation/nucleophilic substitution with sodium azide/reduction to introduce the required amino group for amide bond formation while the other extremity of tetraethylene glycol can be modified by a Mitsunobu reaction to introduce the hydroxyphthalimido moiety (Figure 61b).

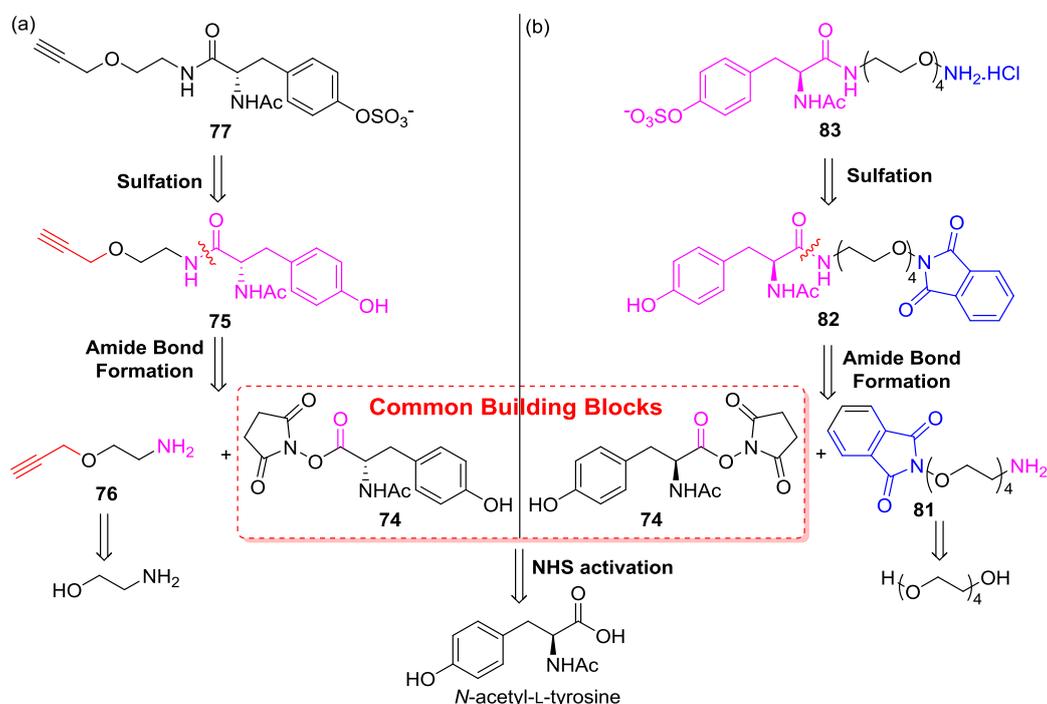


Figure 62. Retrosynthetic scheme for the preparation of sulfotyrosine derivatives.

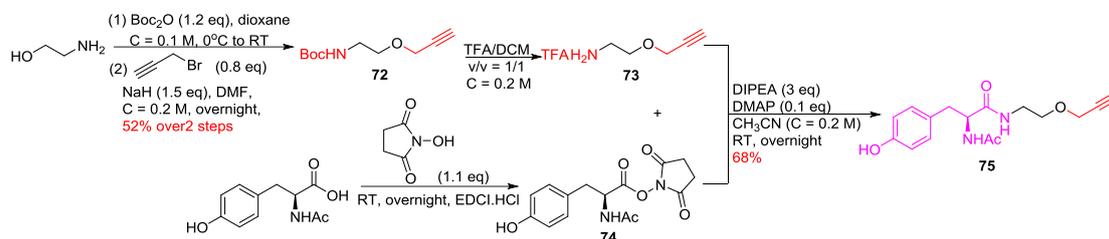
To insure a good stability of click sulfotyrosine partners, we decided to prepare and store their non-sulfated precursors **75** and **82**. The deprotection of the phthalimido group and *O*-sulfation will be performed just prior to their use into conjugation reactions.

III.2.1. Preparation of the sulfotyrosine derivative 77

For the preparation of derivative **77**, a key synthon, 2-(propargyloxy)-ethan-1-amine **76** was obtained starting from commercially available 2-aminoethanol. After the selective protection of the amino group with Boc_2O in dioxane, the propargylation of the hydroxyl group was performed in anhydrous DMF with NaH and propargyl bromide and furnished the desired compound **72** in 52% isolated yield over two steps.⁵⁹⁴ In parallel, we prepared the NHS-activated ester of *N*-acetyl-L-tyrosine **74** by treating commercially available *N*-acetyl-L-tyrosine in CH_3CN with EDCI and *N*-hydroxysuccinimide to give **74**. Then, after the removal of the Boc group of **72** by TFA

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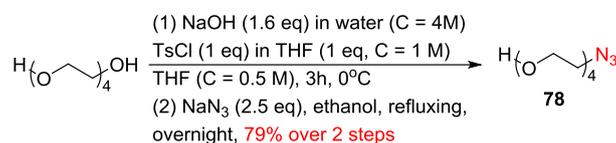
in DCM (v/v = 1/1), the crude product **73** was directly engaged with **74** in reaction of amide bond formation in CH₃CN in presence of DIPEA as base. In this way, **75** was isolated in 68% yield over two steps (Scheme 94).



Scheme 94. Preparation of compound **75** starting from ethanolamine.

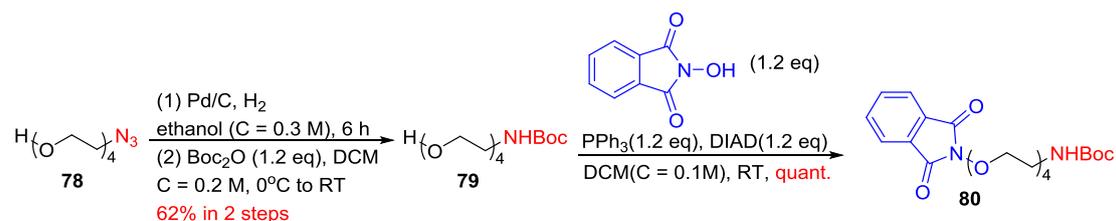
III.2.2. Preparation of the sulfotyrosine derivative **83**

To prepare derivative **83**, the azido intermediate **78** was prepared by the addition of an aqueous solution of NaOH to a large excess of tetraethylene glycol in THF followed by the dropwise addition of TsCl in THF. After 3 hours of reaction at 0 °C, a simple extraction permitted to isolate the formed monotosylate. The crude product was refluxed overnight in ethanol with sodium azide to lead to the desired compound **78** in 79% isolated yield over two steps (Scheme 95).⁵⁹⁴



Scheme 95. Preparation of azide derivative **78** starting from tetraethylene glycol.

After reduction of the azido group with Pd on charcoal under dihydrogen atmosphere, the resulting amine was then protected with Boc group to afford compound **79** in 62% overall yield. Its hydroxyl group was transformed into phthalimidooxy *via* a Mitsunobu reaction in anhydrous DCM, using *N*-hydroxyphthalimide as nucleophile, giving **80** in quantitative yield (Scheme 96).



Scheme 96. Preparation of amine trifluoroacetate salt **80**.

594. DeForest, C. A.; Tirrell, D. A. *Nat. Mater.* **2015**, *14*, 523–531.

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Unfortunately, for the moment, all our attempts of amide bond formation between the NHS-activated ester of *N*-acetyl-L-tyrosine and **81** failed.

IV. Summary

Started from disaccharide **41**, an available compound in our lab, we finally obtained tetrasaccharide **53** with an overall yield of 16% in 12 steps, by employing the synthetic methods developed by our lab to prepare HS fragments and by adapting the previously optimized functionalization strategy of the reducing and nonreducing end of HS fragments (Figure 63).

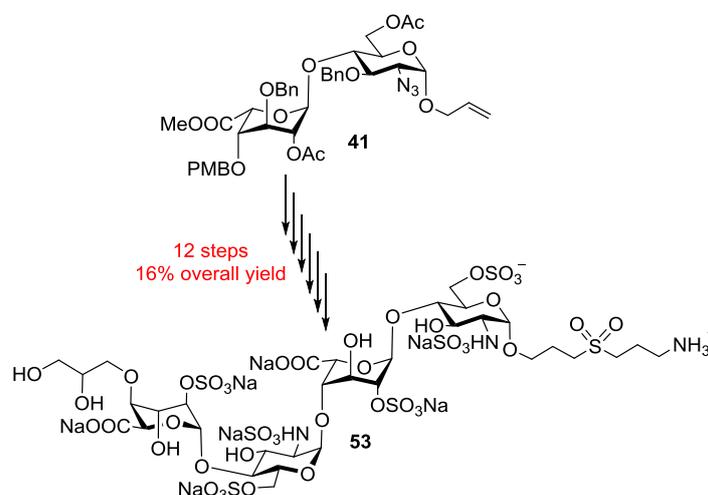


Figure 63. Preparation of a functionalized dp4 HS fragment for one-pot click conjugation.

In parallel, 2 stable precursors of required oxyamine PEG-linkers to prepare SAS mimics as well as the directly usable dipropargyl one have been successfully synthesized using a strategy of PEG oligomers elongation followed by modifications of their two extremities. We also prepared two important intermediates **75** and **80** to access to Click *N*-acetyl-sulfotyrosine partners **77** and **83**, respectively (Figure 64).

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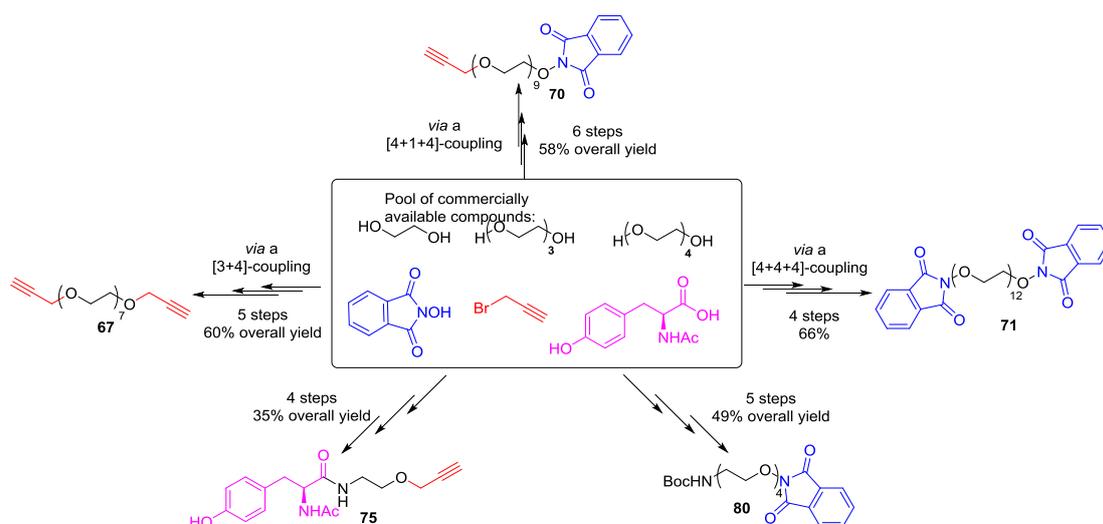


Figure 47. Synthesis of precursors of PEG-type linkers and *N*-acetyl-L-sulfotyrosine partners required for preparation of SAS mimics and glycoconjugates targeting INF- γ and CXCL12 dimer, respectively.

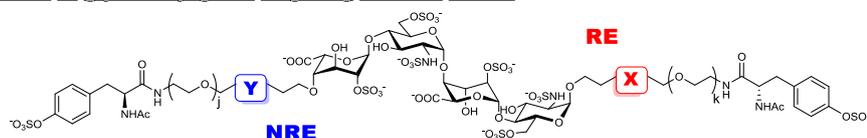
We still have to finalize the synthesis of the *N*-acetyl-L-sulfotyrosine partners **83** and to evaluate the practicability of our two developed one-pot sequence of conjugation onto tetrasaccharide **53** to access to a new generation of SAS mimics to study the interaction between HS fragments and IFN- γ as well as CXCL12.

Chapter 6. Conclusions & Perspectives

I. Conclusions

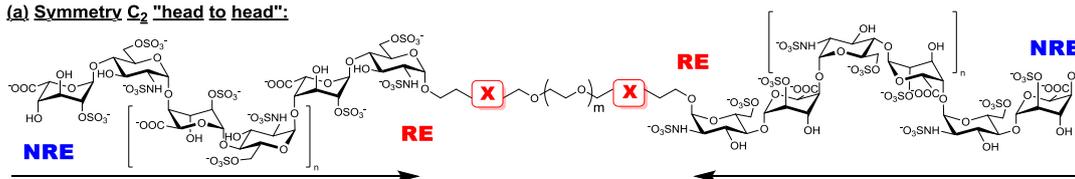
As part of a larger research program aiming at having a better understanding of the interactions between HS and proteins, herein we planned to target two different HSBP, INF- γ and CXCL12, by the chemical synthesis of new glycoconjugates (Figure 65). In both cases, these glycoconjugates have to be prepared by conjugating PEG-type partners to the non-reducing and/or to the reducing end of HS fragments.

(1) Structure of glycoconjugates targeting CXCL12 dimer:

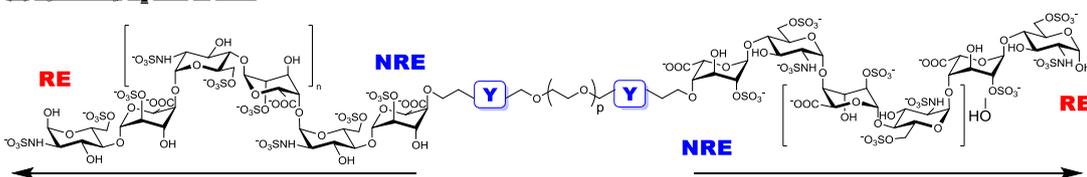


(2) Structure of glycoconjugates mimicking HS long fragments to interact with INF- γ homodimer:

(a) Symmetry C₂ "head to head":



(b) Symmetry C₂ "tail to tail":



(c) Classic Topology "head to tail":

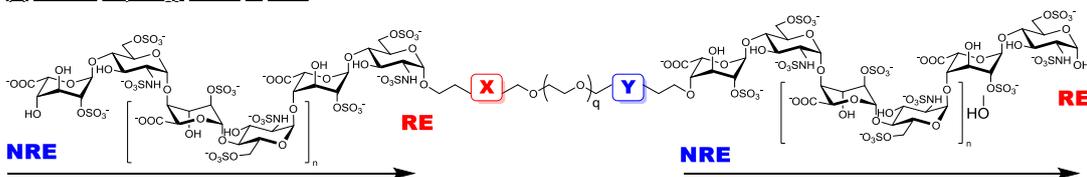


Figure 48. Representation of sulfotyrosine glycoconjugate and SAS mimics in three different geometries to study the interactions between HS and proteins. RE = reducing end, NRE = non-reducing end, X and Y are chemical motifs obtained by conjugation of HS fragments with PEG-type linker.

After the selection of two orthogonal Click reactions as conjugation reactions, CuAAC reaction and oxime ligation, a synthetic pathway allowing the modifications of the two end of HS fragments has been established by Dr. Wenqing Liu during her PhD thesis. She managed to introduce an amino group to the reducing end and a 1,2-diol

Chapter 6. Conclusions & Perspectives

motif onto the non-reducing end of a model disaccharide and onto a dp4 HS fragment (Figure 66). These two moieties are respectively precursors of the azido group required for the CuAAC reaction and aldehyde for the oxime ligation.

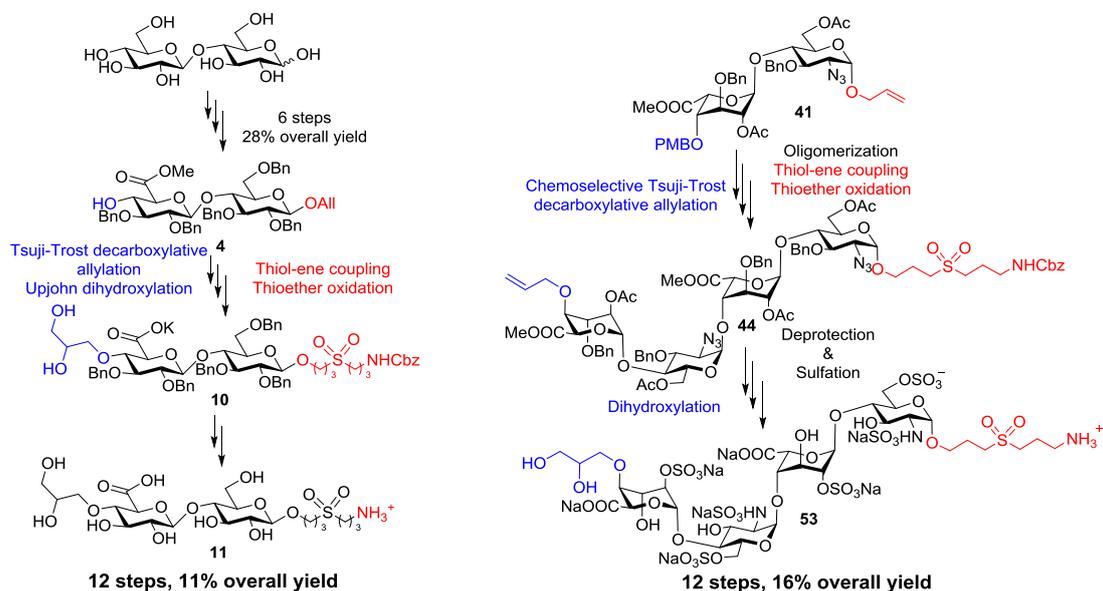
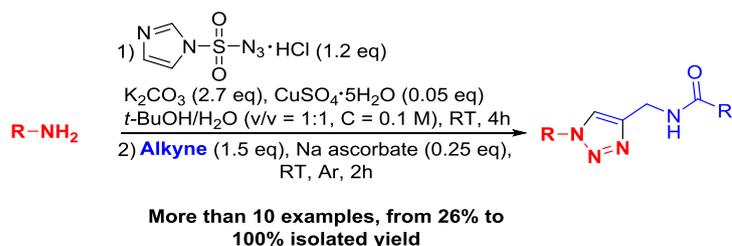


Figure 49. Preparation and modification of the two ends of a model disaccharide **4** & dp4 HS fragment **44**.

After reproduction of her work, two one-pot processes for final transformation and ligation of the two ends of oligosaccharides have been set up: (i) a diazotransfer reaction/CuAAC reaction sequence and (ii) an oxidative cleavage/oxime formation sequence.

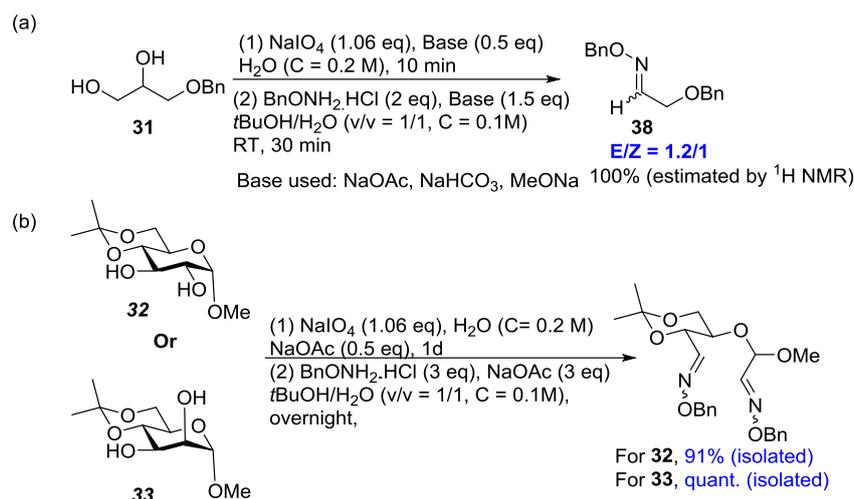
For the first sequence, reaction conditions have been optimized using D-glucosamine hydrochloride and amino acids as primary amine for the diazotransfer reaction and fluorinated alkynes as partners for the cycloaddition reaction (Scheme 97). This allowed us to prepare a series of fluorinated triazolyl derivatives, to set up a simplified protocol of isolation of fluorinated derivatives and to evaluate its practicability to modify the reducing end of oligosaccharides by applying our developed protocol to the model disaccharide **11**.



Scheme 97. Optimized reaction conditions of the one-pot sequence for the transformation of primary amine groups of amino acids and D-glucosamine into fluorinated triazolyl derivatives.

Chapter 6. Conclusions & Perspectives

Concerning the second sequence of one-pot transformation, we not only evaluated the feasibility of a selective oxidative cleavage of the acyclic vicinal diols situated at non-reducing end of oligosaccharides in presence of cyclic 1,2-diols but we also optimized the reaction conditions of the one-pot transformation using 3-(benzyloxy)propane-1,2-diol **31** and **32-33** as starting diols and *O*-benzyloxyamine hydrochloride for oxime formation (Scheme 98).



Scheme 98. Optimal one-pot conditions for the oxidative cleavage /oxime ligation sequence allowing the conversion of diol **31-33** into corresponding ether oxime.

In the meantime, we also developed reliable synthetic routes to access to the desired series of PEG-type linkers and sulfotyrosine derivatives for the preparation of targeted glycoconjugates (Figure 67).

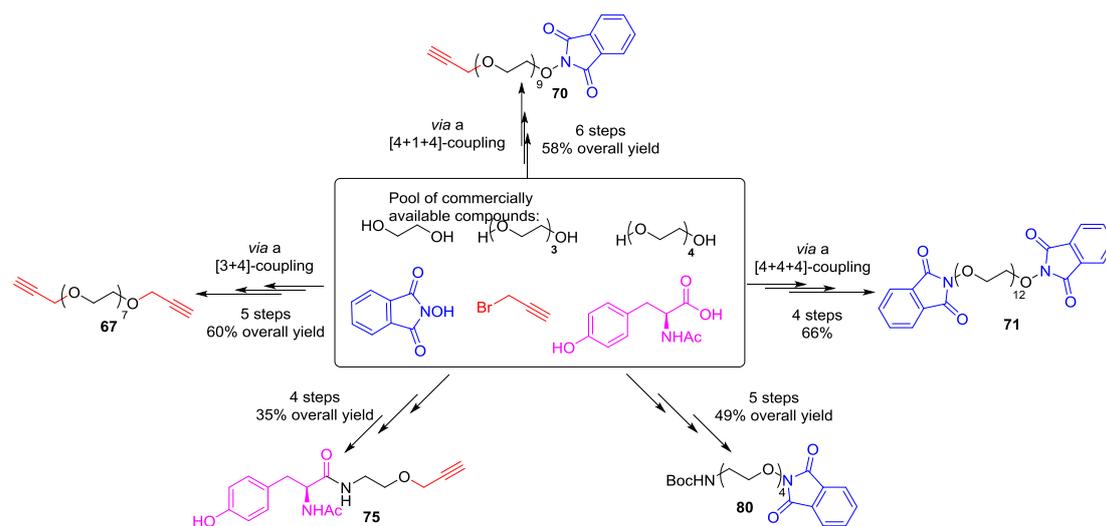


Figure 50. Preparation of PEG-type linkers and sulfotyrosine derivatives using cheap starting materials.

II. Perspectives

In order to prepare targeted glycoconjugates, we still have to:

- (i) complete the preparation of sulfotyrosine derivatives;
- (ii) apply the optimized one-pot diazotransfer/CuAAC reaction conditions onto model disaccharide **11** using the dipropargylated heptaethylene glycol and to transpose it to the dp4 HS fragment **53** in order to validate our strategy of modification of the reducing end of oligosaccharides;
- (iii) validate the regioselectivity of the oxidative cleavage of 1,2-diols onto model disaccharide **11** and oxime ligation using *O*-benzyloxyamine hydrochloride;
- (iv) apply the optimized one-pot oxidative cleavage/oxime ligation reaction conditions onto model disaccharide and HS fragments;
- (v) determine the order of application of the two one-pot sequences of modifications of oligosaccharides reducing and non-reducing end.

Finally, after these complete optimization, preparation of the 4 targeted glycoconjugates will be performed to evaluate the ability of the *N*-acetyl-L-sulfotyrosine derivative to interact with CXCL12 dimer and to validate the strategy of preparation of a second generation of SAS mimics. Application of this synthetic pathway to different HS fragments in length (dp4, dp6 and dp8) and chemical sequence (sulfation patterns and/or uronic configurations) as well as different PEG-type linkers will allow us to prepare a library of new SAS mimics to investigate HS/IFN- γ interactions as well as with other proteins.

Chapter 7. Experimental Part

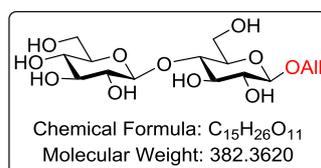
I. General Experimental Techniques

All starting materials were of commercial grade and were used without further purification. Dry dichloromethane, THF, acetonitrile and toluene were obtained using Glass Technology GT S100 device. Reactions were monitored by thin-layer chromatography carried out on silica plates (silica gel 60 F254, Merck) using UV-light, KMnO_4 , ninhydrin, orcinol or 5% sulfuric acid for visualization. Retention factors (R_f) are given for such TLC analysis. Column chromatographies were performed on silica gel 60 (15-40 μm , Merck). Size-exclusion chromatographies were performed on Sephadex LH-20 and G15. Evaporation of solvents was conducted under reduced pressure at temperatures less than 50°C. Optical rotations were measured using a 10 cm quartz cell on a Perkin Elmer 341 Polarimeter (c in g / 100 mL). Values for $[\alpha]_D^T$ were obtained with the D-line of sodium at the indicated temperature T , using solutions of concentration (c) in units of g·100 mL⁻¹. High-resolution mass spectrometry (HRMS) data were recorded using a Bruker Daltonics micro TOF-Q instrument equipped with an electrospray ionization source either in positive or negative mode as appropriate. Nuclear magnetic resonance (NMR) data were acquired on different spectrometers: Bruker DPX250, Bruker AV300, Bruker AV360 and Bruker AV400 operating at 250/300/360/4000 MHz for ¹H, at 62.5/75/90/100 MHz for ¹³C and 235 MHz for ¹⁹F. Chemical shifts (δ) are reported in parts per million, using solvent signals as internal standards (chloroform- d_1 : 7.26 ppm for ¹H and 77.0 ppm for ¹³C; methanol- d_4 : 3.31 ppm for ¹H and 49.0 ppm for ¹³C; DMSO- d_6 : 2.50 ppm for ¹H and 39.5 ppm for ¹³C; D₂O: 4.79 ppm for ¹H). Proton multiplicities for ¹H NMR are designated as: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintuplet), broad singlet (br. s), broad doublet (br. d) or m (multiplet). Coupling constants (J) are reported in Hz. Carbon multiplicities were determined by DEPT135 experiments.

II. Procedures for The Preparation of Compounds and Identification Data

Chapter 7. Experimental Part

Compound 1: Allyl β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranoside



To a stirred solution of D-cellobiose (8.0 g, 23.37 mmol, 1 equiv.) in water (C = 0.13 M) at room temperature, was added allyl bromide (9 equiv.), followed by NaOH solution (C = 1.1 M, 2 equiv.) over 1 h. The resulting mixture was stirred at room temperature for 20 h and then the remaining allyl bromide was carefully removed through an extraction with CH₂Cl₂ (200 mL \times 3). After addition of NaOH (2 equiv.), temperature was increased to 100 °C and the reaction mixture was stirred for 3 h. After cooling to room temperature, activated charcoal (20 g) was added. The suspension was stirred for another 3 h and then filtrated through celite (20 g) and washed by a binary gradient (MeCN/H₂O from 0/100 to 15/85). The filtrate was concentrated under reduced pressure till left around 10 mL which was subsequently poured into a column charged with mixture resins and then rinsed with H₂O for several times. The solution was concentrated under reduced pressure to give the crude product, which was crystallized twice with i-PrOH (volume 1: 180 mL, volume 2: 100 mL) to afford a white crystal (4.4 g, 50 % yield, $\beta / \alpha > 95\%$).

R_f = 0.65 (EtOAc / i-PrOH / H₂O = 3 / 3 / 1); **Revelator**: 5 % H₂SO₄

$[\alpha]_{589}^{20} = -23.0$ (C = 1.0, H₂O).

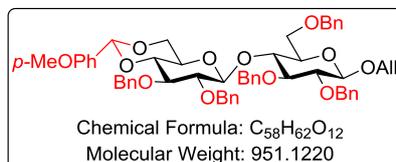
¹H NMR (360 MHz, D₂O) δ 5.99 (dddd, *J* = 17.0 Hz, 10.5 Hz, 6.5 Hz, 5.5 Hz, 1H), 5.39 (dddd, *J* = 17.0 Hz, 1.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 5.30 (dddd, *J* = 10.5 Hz, 1.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 4.55 (d, *J* = 8.0 Hz, 1H), 4.52 (d, *J* = 8.0 Hz, 1H), 4.41 (dddd, *J* = 12.5 Hz, 5.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 4.25 (dddd, *J* = 12.5 Hz, 6.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 4.00 (dd, *J* = 12.5 Hz, *J* = 2.0 Hz, 1H), 3.93 (dd, *J* = 12.5 Hz, *J* = 2.0 Hz, 1H), 3.83 (dd, *J* = 12.5 Hz, *J* = 5.0 Hz, 1H), 3.75 (dd, *J* = 12.5 Hz, *J* = 5.5 Hz, 1H), 3.67-3.58 (m, 2H), 3.64 (dd, *J* = 9.0 Hz, *J* = 8.5 Hz, 1H), 3.53 (dd, *J* = 9.0 Hz, *J* = 9.0 Hz, 1H), 3.51-3.48 (m, 1H), 3.45 (dd, *J* = 9.0 Hz, *J* = 8.5 Hz, 1H), 3.35 (dd, *J* = 9.0 Hz, *J* = 8.0 Hz, 1H), 3.33 (dd, *J* = 9.0 Hz, *J* = 8.0 Hz, 1H).

¹³C NMR (90 MHz, D₂O) δ 133.4, 118.8, 102.6, 101.1, 78.8, 76.0, 75.6, 74.8, 74.4, 73.2, 72.9, 70.7, 69.5, 60.6, 60.1.

HRMS (ESI, negative mode): *m/z*: [M-H]⁻ Calcd. for C₁₅H₂₅O₁₁⁻ 381.1402, found 381.1389.

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Compound 2: Allyl (2,3-di-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)- β -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-2,3,6-tri-*O*-benzyl- β -D-glucopyranoside



Compound 1 (1 g, $\beta/\alpha = 20/1$, 2.62 mmol, 1 equiv.) and 4-methoxybenzaldehyde (27 mL, 219.90 mmol, 84 equiv.) were successively added into a flask under Ar at room temperature, and then treated with ultrasonic for 10 minutes to allow dissolution of compound 1. Nd(OTf)₃ (155 mg, 0.26 mmol, 0.1 equiv.) was subsequently added into the mixture, which was stirred at 40°C for 4 h. The reaction was slowly cooled down to 30°C and stirred under reduced pressure for additional 2.5 h. After adding Et₃N (3 mL) drop by drop into the mixture, the reaction was diluted with Ether/DCM (1 / 3, 400 mL) and later extracted by H₂O (300 mL \times 4). The aqueous layers were concentrated under reduced pressure to give the crude product (1.3 g, white solid), which was directly applied to the next reaction without further purification.

The crude product (1.3 g) was dissolved in anhydrous DMF (26 mL) under Ar at room temperature. After cooling to 0 °C, NaH (1.9 g (60 % mass), 28.80 mmol, 11 equiv.) was added and the mixture was stirred for 10 min, followed by addition of benzyl bromide (3.1 mL, 26.18 mmol, 10 equiv.). The mixture was slowly warmed up to room temperature and stirred overnight. The reaction was quenched by MeOH (10 mL) and kept stirring for 10 min before evaporation. The residue was diluted by Ether (200 mL) and washed by H₂O (150 mL \times 3). The organic layers were dried over MgSO₄ and concentrated in *vacuo*. The residue was purified by flash chromatography on silica (Petroleum Ether / AcOEt, 90 / 10 to 80 / 20 gradient) to give product 2 (1.8 g, 72 %, white solid, β anomer). Crystallization with Petroleum Ether/EtOAc or Ether was tried but without success.

R_f = 0.5 (EtOAc/Petroleum Ether = 3/7), **Revelator**: 5 % H₂SO₄

$[\alpha]_{589}^{20} = -4.2$ (C = 1.0, CHCl₃).

¹H NMR (360 MHz, CDCl₃) δ 7.40 (d, $J = 9.0$ Hz, 2H), 7.37-7.23 (m, 25H), 6.90 (d, 2H, $J = 9.0$ Hz), 5.95 (dddd, 1H, $J = 17.0$ Hz, 10.5 Hz, 6.0 Hz, 5.0 Hz,), 5.45 (s, 1H), 5.33 (dddd, $J = 17.0$ Hz, 2.0 Hz, 1.5 Hz, 1.5 Hz, 1H), 5.20 (dddd, $J = 10.5$ Hz, 2.0 Hz, 1.5 Hz, 1.0 Hz, 1H), 4.92-4.70 (m, 8H), 4.58 (d, $J = 12.0$ Hz, 1H), 4.53 (d, $J = 8.0$ Hz,

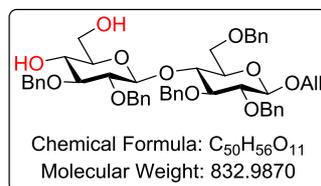
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1H), 4.42 (d, $J = 7.5$ Hz, 1H), 4.41 (dddd, $J = 13.0$ Hz, 5.0 Hz, 1.5 Hz, 1.5 Hz, 1H), 4.38 (d, $J = 12.0$ Hz, 1H), 4.16 (dd, $J = 11.0$ Hz, 5.0 Hz, 1H), 4.13 (dddd, $J = 13.0$ Hz, 6.0 Hz, 1.5 Hz, 1.0 Hz, 1H), 3.98 (dd, $J = 9.5$ Hz, 9.0 Hz, 1H), 3.83 (dd, $J = 11.0$ Hz, 4.0 Hz, 1H), 3.81 (s, 3H), 3.67 (dd, $J = 11.0$ Hz, 1.5 Hz, 1H), 3.60 (dd, $J = 9.0$ Hz, 8.0 Hz, 1H), 3.56 (dd, $J = 9.5$ Hz, 9.0 Hz, 1H), 3.53 (dd, $J = 9.0$ Hz, 9.0 Hz, 1H), 3.46 (dd, $J = 11.0$ Hz, 9.5 Hz, 1H), 3.45 (dd, $J = 9.0$ Hz, 7.5 Hz, 1H), 3.35 (dd, $J = 8.0$ Hz, 8.0 Hz, 1H), 3.31 (ddd, $J = 9.5$ Hz, 4.0 Hz, 1.5 Hz, 1H), 3.13 (ddd, $J = 9.5$ Hz, 9.5 Hz, 5.0 Hz, 1H).

^{13}C NMR (90 MHz, CDCl_3) δ 160.0, 139.0, 138.6, 138.5, 138.3, 138.2, 134.1, 130.0, 128.3, 128.1, 127.9, 127.8, 127.6, 127.4, 127.3, 117.2, 113.6, 102.8, 102.7, 101.1, 82.9, 82.5, 81.7, 81.1, 76.8, 75.4, 75.0, 74.9, 75.1, 73.2, 70.2, 68.7, 68.0, 65.8, 55.3.

HRMS (ESI, positive mode): m/z : $[\text{M}+\text{Na}]^+$ Calcd. for $\text{C}_{58}\text{H}_{62}\text{NaO}_{12}^+$ 973.4133, found 973.4147.

Compound 3: Allyl (2,3-di-*O*-benzyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-2,3,6-tri-*O*-benzyl- β -D-glucopyranoside



To a stirred solution of **2** (2.51 g, 2.64 mmol, 1 equiv.) in wet CH_2Cl_2 (54 mL) was added CF_3COOH (2.2 mL, 29 mmol, 11 equiv.). The mixture was stirred at room temperature for 1 h. Flash chromatography (Petroleum Ether/EtOAc = 80/20 to 60/40 gradient) gave compound **6** (1.8 g, 82 %, white solid).

$R_f = 0.6$ (EtOAc/Petroleum Ether = 6/4); **Revelator**: 5 % H_2SO_4

$[\alpha]_{589}^{20} = +5.4$ ($C = 0.5$, CHCl_3).

^1H NMR (360 MHz, CDCl_3) δ 7.40-7.20 (m, 25H), 5.96 (dddd, $J = 17.0$ Hz, 10.5 Hz, 6.0 Hz, 5.0 Hz, 1H), 5.34 (dddd, $J = 17.0$ Hz, 1.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 5.20 (dddd, $J = 10.5$ Hz, 1.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 4.94-4.70 (m, 6H), 4.92 (d, $J = 11.5$ Hz, 1H), 4.67 (d, $J = 11.5$ Hz, 1H), 4.62 (d, $J = 12.0$ Hz, 1H), 4.44 (d, $J = 8.0$ Hz, 2H), 4.43 (d, $J = 12.0$ Hz, 1H), 4.41 (dddd, $J = 13.0$ Hz, 5.0 Hz, 1.5 Hz, 1.5 Hz, 1H), 4.14 (dddd, $J = 13.0$ Hz, 6.0 Hz, 1.5 Hz, 1.5 Hz, 1H), 3.93 (dd, $J = 9.5$ Hz, 9.0 Hz, 1H), 3.80 (dd, $J = 11.0$ Hz, 4.0 Hz, 1H), 3.70 (dd, $J = 11.0$ Hz, 2.0 Hz, 1H), 3.62 (dd, $J = 12.0$ Hz, 3.5 Hz, 1H), 3.54 (dd, $J = 9.0$ Hz, 9.0 Hz, 1H), 3.45 (dd, $J = 9.0$ Hz, 8.0 Hz, 1H), 3.42 (dd, $J =$

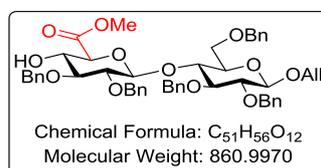
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9.5 Hz, 9.0 Hz, 1H), 3.39-3.32 (m, 1H), 3.36 (dd, $J = 12.0$ Hz, 5.5 Hz, 1H), 3.32-3.25 (m, 2H), 3.07 (ddd, $J = 9.5$ Hz, 3.5 Hz, 5.5 Hz, 1H), 2.31-2.17 (br, 1H), 1.92-1.76 (br, 1H).

^{13}C NMR (90 MHz, CDCl_3) δ 138.9, 138.5, 138.2, 138.1, 134.1, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 117.2, 102.6, 102.4, 84.3, 82.7, 82.4, 81.6, 76.6, 75.3, 75.0, 74.9, 74.8, 73.3, 70.7, 70.2, 68.0, 62.3.

HRMS (ESI, positive mode): m/z : $[\text{M}+\text{Na}]^+$ Calcd. for $\text{C}_{50}\text{H}_{56}\text{NaO}_{11}$, 855.3715, found 855.3715.

Compound 4: Allyl (methyl 2,3-di-*O*-benzyl- β -D-glucopyranosyluronate)-(1 \rightarrow 4)-*O*-2,3,6-tri-*O*-benzyl- β -D-glucopyranoside



To a stirred solution of **3** (1.73 g, 2.08 mmol, 1 equiv.) in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ ($v/v = 2/1$, 19 mL), was added TEMPO (97.5 mg, 0.624 mmol, 0.3 equiv.) and BAIB (1.67 g, 5.2 mmol, 2.5 equiv.). The reaction was stirred at room temperature for 2 h before addition of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ (2 g, 8.06 mmol, 15.5 equiv.), sat. NaHCO_3 (15 mL) and EtOAc (30 mL). After stirring for 1h, the organic layers were separated and the aqueous layers were extracted with EtOAc (50 mL). The combined organic layers were washed by sat. NaHCO_3 (50 mL), dried over MgSO_4 and concentrated to give the intermediate acid product, which was directly applied to the next reaction without further purification.

To a stirred solution of the acid intermediate in anhydrous DMF (15 mL), was added K_2CO_3 (316 mg, 2.28 mmol, 1.1 equiv.). The reaction mixture was stirred at room temperature for 15 min before adding MeI (1.77 mL, 12.48 mmol, 6 equiv.). The suspension was stirred overnight and quenched by MeOH, diluted by EtOAc and washed with H_2O . The organic layers were separated and the combined aqueous layers were extracted with EtOAc. The combined organic layers were dried over MgSO_4 and concentrated in vacuo. Flash chromatography (Petroleum Ether/EtOAc = 80/20) gave product **4** (1.43 g, 85 %, white solid).

$R_f = 0.3$ (EtOAc/Petroleum Ether = 25 / 75); **Revelator**: 5 % H_2SO_4

$[\alpha]_{589}^{20} = +5.1$ ($C = 1.0$, CHCl_3).

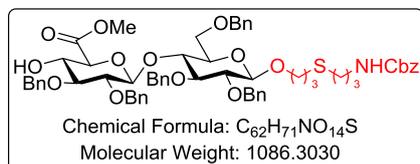
^1H NMR (360 MHz, CDCl_3) δ 7.37-7.23 (m, 25H), 5.95 (dddd, $J = 17.5$ Hz, 11.0 Hz,

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6.0 Hz, 5.5 Hz, 1H), 5.33 (dddd, $J = 17.5$ Hz, 2.0 Hz, 1.5 Hz, 1.5 Hz, 1H), 5.20 (dddd, $J = 11.0$ Hz, 2.0 Hz, 1.5 Hz, 1.0 Hz, 1H), 5.02-4.60 (m, 8H), 4.62 (d, $J = 12.0$ Hz, 1H), 4.54 (d, $J = 7.5$ Hz, 1H), 4.42 (d, $J = 7.5$ Hz, 1H), 4.40 (d, $J = 12.0$ Hz, 1H), 4.40-4.38 (m, 1H), 4.13 (dddd, $J = 13.0$ Hz, 6.0 Hz, 1.5 Hz, 1.0 Hz, 1H), 4.02 (dd, $J = 9.5$ Hz, 9.0 Hz, 1H), 3.83 (dd, $J = 11.0$ Hz, 4.0 Hz, 1H), 3.79 (dd, $J = 3.0$ Hz, 9.0 Hz, 1H), 3.67 (dd, $J = 11.0$ Hz, 1.5 Hz, 1H), 3.59 (s, 3H), 3.58 (dd, $J = 9.0$ Hz, 9.0 Hz, 1H), 3.55 (d, $J = 3.0$ Hz, 1H), 3.48 (dd, $J = 8.5$ Hz, 7.5 Hz, 1H), 3.34 (dd, $J = 9.0$ Hz, 7.5 Hz, 1H), 3.33 (dd, $J = 9.0$ Hz, 8.5 Hz, 1H), 3.32 (ddd, $J = 4.0$ Hz, 1.5 Hz, 9.5 Hz, 1H), 2.81 (br, 1H).
 ^{13}C NMR (90 MHz, CDCl_3) δ 169.9, 138.8, 138.7, 138.5, 138.4, 134.3, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.3, 117.4, 102.9, 102.7, 83.7, 83.1, 81.8, 81.7, 77.2, 75.5, 75.2, 75.1, 74.2, 73.5, 72.3, 70.4, 68.3, 52.8.

HRMS (ESI, positive mode): m/z : $[\text{M}+\text{Na}]^+$ Calcd. for $\text{C}_{51}\text{H}_{56}\text{NaO}_{12}$, 883.3664, found 883.3663.

Compound 5: 3-[(3-Benzyloxycarbonylamino)propylthio]propyl (methyl 2,3-di-O-benzyl- β -D-glucopyranosyluronate)-(1 \rightarrow 4)-O-2,3,6-tri-O-benzyl- β -D-glucopyranoside



To a stirred mixture of **4** (170 mg, 0.20 mmol, 1 equiv), Benzyl *N*-(3-mercapto)propyl carbamate (169 mg, 0.75 mmol, 3.8 equiv.) and catechol (26 mg, 0.24 mmol, 1.2 equiv.) in dry CH_2Cl_2 (748 μL) under Ar, was added BET_3 Hexane (237 μL , 0.24 mmol, C = 1.0 M, 1.2 equiv.). The reaction was stirred at room temperature for 2 h before evaporation in vacuo. Flash chromatography (Toluene/EtOAc, 85/15 to 50/50 gradient) gave product **5** (150 mg, 70 %, colorless liquid).

R_f = 0.15 (EtOAc/Toluene = 15/85); **Revelator**: 5 % H_2SO_4

^1H NMR (360 MHz, CDCl_3) δ 7.29-7.12 (m, 30H), 5.00 (s, 2H), 4.93 (d, $J = 11.5$ Hz, 1H), 4.81 (br, 1H), 4.75 (d, $J = 11.0$ Hz, 1H), 4.74 (s, 2H), 4.66 (s, 2H), 4.63 (d, $J = 11.0$ Hz, 1H), 4.60 (d, $J = 11.5$ Hz, 1H), 4.52 (d, $J = 12.0$ Hz, 1H), 4.44 (d, $J = 7.5$ Hz, 1H), 4.31 (d, $J = 12.0$ Hz, 1H), 4.27 (d, $J = 7.5$ Hz, 1H), 3.94 (dd, $J = 9.0$ Hz, 9.5 Hz, 1H), 3.90 (dt, $J = 10.0$ Hz, 6.0 Hz, 1H), 3.75 (dd, $J = 11.0$ Hz, 4.0 Hz, 1H), 3.72 (dd, $J = 8.5$ Hz, 8.0 Hz, 1H), 3.58 (dd, $J = 11.0$ Hz, 2.0 Hz, 1H), 3.52 (dt, $J = 10.0$ Hz, 3.5 Hz,

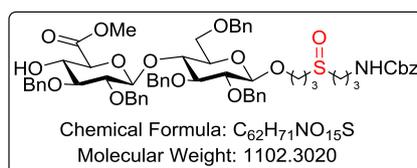
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1H), 3.50 (dd, $J = 9.0$ Hz, 9.5 Hz, 1H), 3.49 (s, 3H), 3.48 (d, $J = 8.0$ Hz, 1H), 3.35 (dd, $J = 9.0$ Hz, 7.5 Hz, 1H), 3.29-3.21 (m, 3H), 3.19-3.08 (m, 2H), 2.83-2.75 (br, 1H), 2.51 (t, $J = 7.0$ Hz, 2H), 2.39 (t, $J = 7.0$ Hz, 2H), 1.86-1.73 (m, 2H), 1.69-1.58 (m, 2H).

^{13}C NMR (90 MHz, CDCl_3) δ 169.6, 156.3, 139.2, 138.5, 138.2, 138.1, 136.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.1, 103.5, 102.5, 83.4, 82.9, 81.6, 81.5, 77.0, 75.2, 75.0, 74.9, 74.8, 74.0, 73.2, 72.1, 68.1, 68.0, 66.6, 52.4, 40.0, 29.7, 29.5, 29.3, 28.6.

HRMS (ESI, positive mode): m/z : $[\text{M}+\text{Na}]^+$ Calcd. for $\text{C}_{62}\text{H}_{71}\text{NNaO}_{14}\text{S}^+$ 1108.4462, found 1108.4487.

Compound 6: 3-[(3-Benzyloxycarbonylamino)propylsulfinyl]propyl (methyl 2,3-di-*O*-benzyl- β -*D*-glucopyranosyluronate)-(1 \rightarrow 4)-*O*-2,3,6-tri-*O*-benzyl- β -*D*-glucopyranoside



R_f = 0.45 (EtOAc); **Revelator:** 5 % H_2SO_4

$[\alpha]_{589}^{20} = +2.3$ (C = 1.0, CHCl_3).

^1H NMR (360 MHz, CDCl_3) δ 7.30-7.10 (m, 30H), 5.11-5.03 (br, 1H), 5.01 (s, 2H), 4.95 (d, $J = 11.0$ Hz, 1H), 4.75 (s, 2H), 4.69 (dd, $J = 11.5$ Hz, 1.5, 1H), 4.66 (s, 2H), 4.64 (dd, $J = 11.5$ Hz, 1.5, 1H), 4.62 (d, $J = 11.0$ Hz, 1H), 4.52 (dd, $J = 12.0$ Hz, 3.5 Hz, 1H), 4.43 (d, $J = 7.5$ Hz, 1H), 4.30 (d, $J = 12.0$ Hz, 1H), 4.27 (dd, $J = 8.0$ Hz, 2.0 Hz, 1H), 3.94 (dd, $J = 9.0$ Hz, 9.0 Hz, 1H), 3.90 (dt, $J = 10.0$ Hz, 5.5 Hz, 1H), 3.76 (dd, $J = 11.0$ Hz, 4.0 Hz, 1H), 3.74-3.70 (m, 1H), 3.62-3.54 (m, 1H), 3.56 (dd, $J = 11.0$ Hz, 2.0 Hz, 1H), 3.51 (s, 3H), 3.50 (dd, $J = 9.0$ Hz, 9.0 Hz, 1H), 3.49 (d, $J = 9.0$ Hz, 1H), 3.34 (dd, $J = 9.0$ Hz, 8.0 Hz, 1H), 3.27-3.24 (m, 2H), 3.23 (ddd, $J = 9.0$ Hz, 4.0 Hz, 2.0 Hz, 1H), 3.17 (m, $J = 6.0$ Hz, 2H), 2.86 (d, $J = 2.5$, 1H), 2.68-2.52 (m, 2H), 2.50-2.30 (m, 2H), 2.03-1.91 (m, 2H), 1.87-1.74 (m, 2H).

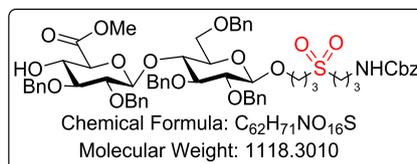
^{13}C NMR (90 MHz, CDCl_3) δ 169.6, 156.4, 139.1, 138.6, 138.5, 138.2, 138.0, 136.5, 128.5, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.2, 103.4+103.3, 102.5, 83.5, 82.90+82.88, 81.6, 81.5, 76.9, 75.3, 75.1, 75.0, 74.8, 74.1, 73.3, 72.1, 68.0+67.9, 67.9, 66.7, 52.4, 49.50, 49.53, 49.3, 49.2, 39.8, 23.6, 23.5, 23.3, 23.1.

HRMS (ESI, positive mode): m/z : $[\text{M}+\text{Na}]^+$ Calcd. for $\text{C}_{62}\text{H}_{71}\text{NNaO}_{15}\text{S}^+$ 1124.4468,

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found 1124.4437.

Compound 7: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 2,3-di-*O*-benzyl- β -D-glucopyranosyluronate)-(1 \rightarrow 4)-*O*-2,3,6-tri-*O*-benzyl- β -D-glucopyranoside



To a stirred solution of **5** (70 mg, 6.45×10^{-2} mmol, 1 equiv.) in CH₂Cl₂ (860 μ L), was added oxone (645 μ L, 0.19 mmol, C = 0.3 M, 3 equiv.), K₂HPO₄ (483 μ L, 0.39 mmol, C = 0.8 M, 6 equiv.) and (C₄H₉)₄NHSO₄ (2 mg, 6.45×10^{-3} mmol, 0.1 equiv.) respectively. The mixture was stirred at room temperature for 24 h. After complete transformation of sulfoxide to sulfone, Na₂S₂O₃ (5.8 mL, 0.58 mmol, C = 0.1 M, 9 equiv.) was added and the suspension was stirred for additional 30 min. The mixture was extracted with CH₂Cl₂ (10 mL \times 3) and the combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. Flash chromatography (Toluene / Acetone, 70 / 30) gave product **7** (65 mg, 90 %, white solid). During the above oxidation step, the intermediate sulfoxide **6** was once isolated after flash chromatography. The rotamers of **6** have been observed and marked by underline in H¹ and C¹³.

R_f = 0.3 (EtOAc/Petroleum Ether = 1/1); **Revelator**: 5 % H₂SO₄

$-\alpha_{589}^{20} = +2.1$ (C = 1.0, CHCl₃).

¹H NMR (360 MHz, CDCl₃) δ 7.36-7.22 (m, 30H), 5.08 (s, 2H), 5.03 (d, *J* = 11.5 Hz, 1H), 5.00-4.95 (br, 1H), 4.82 (s, 2H), 4.74 (s, 4H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.58 (d, *J* = 12.5 Hz, 1H), 4.50 (d, *J* = 7.5 Hz, 1H), 4.36 (d, *J* = 12.5 Hz, 1H), 4.34 (d, *J* = 8.0 Hz, 1H), 4.02 (dd, *J* = 9.5 Hz, 9.0 Hz, 1H), 3.95 (dt, *J* = 10.0 Hz, 5.5 Hz, 1H), 3.83 (dd, *J* = 11.0 Hz, 4.0 Hz, 1H), 3.81-3.76 (m, 1H), 3.68-3.60 (m, 1H), 3.63 (dd, *J* = 11.0 Hz, 2.0 Hz, 1H), 3.58 (dd, *J* = 9.0 Hz, 9.0 Hz, 1H), 3.58 (s, 3H), 3.57 (d, *J* = 8.0 Hz, 1H), 3.41 (dd, *J* = 9.0 Hz, 8.0 Hz, 1H), 3.33-3.27 (m, 3H), 3.27-3.18 (m, 2H), 3.00 (t, *J* = 7.5 Hz, 2H), 2.84 (s, 1H), 2.81 (t, *J* = 7.5 Hz, 2H), 2.13-2.05 (m, 2H, -OCH₂CH₂CH₂SO₂-), 1.92 (m, 2H).

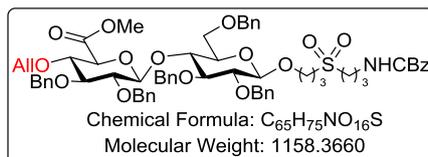
¹³C NMR (90 MHz, CDCl₃) δ 169.7, 156.4, 139.1, 138.5, 138.2, 138.0, 136.4, 128.5, 128.1, 127.9, 127.8, 127.7, 127.6, 127.2, 103.3, 102.6, 83.4, 82.9, 81.6, 81.5, 76.9, 75.3, 75.1, 75.0, 74.9, 74.8, 74.1, 73.3, 72.1, 67.9, 67.4, 66.8, 52.5, 50.1, 50.0, 39.3, 22.5,

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22.4.

HRMS (ESI, positive mode): m/z : $[M+Na]^+$ Calcd. for $C_{62}H_{71}NNaO_{16}S^+$ 1140.4300, found 1140.4386.

Compound 8: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 4-*O*-allyl-2,3-di-*O*-benzyl- β -D-glucopyranosyluronate)-(1 \rightarrow 4)-*O*-2,3,6-tri-*O*-benzyl- β -D-glucopyranoside



To a stirred solution of **7** (100 mg, 8.94×10^{-2} mmol, 1 equiv.) and Allyl *tert*-butylcarbonate (17 mg, 0.11 mmol, 1.2 equiv.) in dry Toluene (447 μ L) under Ar, was added $Pd(PPh_3)_4$ (2.6 mg, 2.24×10^{-3} mmol, 2.5 (mol) %). After stirring at 60°C for 4 h, the mixture was filtered through celite and concentrated in *vacuo*. The residue was purified by flash chromatography (Toluene / AcOEt, 77 / 23 to 50 / 50 gradient) to give product **8** (96 mg, 93 %, white solid), and di-allyl compound (8 mg, 7 %, white solid).

R_f = 0.4 (EtOAc/Petroleum Ether = 1/1); **Revelator:** 5 % H_2SO_4

$[\alpha]_{589}^{20} = +6.0$ (C = 1.0, $CHCl_3$).

1H NMR (360 MHz, $CDCl_3$) δ 7.31-7.14 (m, 30H, Ar), 5.73 (dddd, $J = 17.5$ Hz, 10.5 Hz, 5.5 Hz, 5.5 Hz, 1H), 5.12 (dddd, $J = 17.5$ Hz, 2.0 Hz, 1.5 Hz, 1.0 Hz, 1H), 5.04 (dddd, $J = 10.5$ Hz, 2.0 Hz, 1.5 Hz, 1.0 Hz, 1H), 5.01 (s, 2H), 4.95 (d, $J = 11.0$ Hz, 1H), 4.93-4.86 (br, 1H), 4.72 (d, $J = 3.5$ Hz, 2H), 4.66-4.63 (m, 4H), 4.58 (d, $J = 11.0$ Hz, 1H), 4.50 (d, $J = 12.0$ Hz, 1H), 4.42 (d, $J = 8.0$ Hz, 1H), 4.31 (d, $J = 12.0$ Hz, 1H), 4.25 (d, $J = 8.0$ Hz, 1H), 4.13 (dddd, $J = 12.5$ Hz, 5.5 Hz, 1.5 Hz, 1.0 Hz, 1H), 3.98 (dddd, $J = 12.5$ Hz, 5.5 Hz, 1.5 Hz, 1.0 Hz, 1H), 3.92 (dd, $J = 9.5$ Hz, 9.5 Hz, 1H), 3.87 (dt, $J = 10.0$ Hz, 5.5 Hz, 1H), 3.76 (dd, $J = 11.0$ Hz, 3.5 Hz, 1H), 3.62 (d, $J = 9.5$ Hz, 1H), 3.58-3.54 (m, 1H), 3.58 (dd, $J = 9.5$ Hz, 8.0 Hz, 1H), 3.55 (s, 3H), 3.54 (dd, $J = 11.0$ Hz, 3.0 Hz, 1H), 3.48 (dd, 1H, $J = 9.5$ Hz, 9.0 Hz, 1H), 3.34 (dd, $J = 8.0$ Hz, 9.0 Hz, 1H), 3.30 (dd, $J = 9.0$ Hz, 8.0 Hz, 1H), 3.26 (dd, $J = 9.0$ Hz, 8.0 Hz, 1H), 3.21 (ddd, $J = 9.5$ Hz, 3.5 Hz, 3.0 Hz, 1H), 3.18-3.10 (m, 2H), 2.92 (t, $J = 7.5$ Hz, 2H), 2.74 (t, $J = 7.5$ Hz, 2H), 2.04-1.98 (m, 2H), 1.84 (m, 2H).

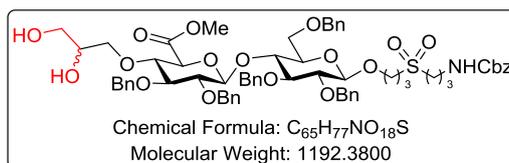
^{13}C NMR (90 MHz, $CDCl_3$) δ 167.8, 155.7, 137.9, 137.6, 137.4, 137.2, 136.9, 135.4, 133.5, 127.5, 127.3, 127.2, 127.1, 126.9, 126.8, 126.7, 126.6, 126.3, 116.0, 102.3, 101.6,

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82.8, 81.9, 81.0, 80.5, 78.4, 76.0, 74.6, 74.4, 74.0, 73.9, 73.4, 72.7, 72.3, 66.8, 66.4, 65.8, 51.2, 49.1, 49.0, 38.2, 21.5, 21.4.

HRMS (ESI, positive mode): m/z : $[M+Na]^+$ Calcd. for $C_{65}H_{75}NNaO_{16}S^+$ 1180.4819, found 1180.4699.

Compound 9: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 2,3-di-O-benzyl-4-O-(2, 3- dihydroxypropyl)- β -D-glucopyranosyluronate)-(1 \rightarrow 4)-O-2,3,6-tri-O-benzyl- β -D-glucopyranoside



To a stirred solution of **8** (100 mg, 8.63×10^{-2} mmol, 1 equiv.) and NMO H_2O (47 mg, 0.35 mmol, 4 equiv.) in the mixture solvents THF/ H_2O (v/v = 5/1, 863 μ L), was added O_5O_4 *tert*-Butanol (108 μ L (2.5 wt %), 8.63×10^{-3} mmol, 10 (mol) %). After stirring at room temperature for 7 h, $Na_2S_2O_3$ (109 mg, 0.86 mmol, 10 equiv.) was added and the mixture was kept stirring for additional 30 min before diluting by H_2O (30 mL) and extracting with CH_2Cl_2 (20 mL \times 3). The combined organic layers were washed with H_2O (20 mL), dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography (Toluene/EtOAc, 50/50 to 10/90 gradient) gave product **9** (98 mg, 95 %, white solid, the ratio of two diastereoisomers is 3/2 according to the spectrum of carbon).

R_f = 0.4 (EtOAc/Toluene = 85/15); **Revelator**: orcinol

$[\alpha]_{589}^{20} = + 8.0$ (C = 1.0, MeOH).

1H NMR (360 MHz, $CDCl_3$) δ 7.32-7.07 (m, 30H), 5.01 (s, 2H), 4.95 (d, J = 11.0 Hz, 1H), 4.91-4.82 (br, 1H), 4.79 (d, J = 11.0 Hz, 1H), 4.71-4.64 (m, 4H), 4.63 (d, J = 11.0 Hz, 1H), 4.60 (d, J = 11.0 Hz, 1H), 4.55 (d, J = 12.5 Hz, 1H), 4.42 (d, J = 7.0 Hz, 1H), 4.32 (d, J = 12.5 Hz, 1H), 4.26 (d, J = 8.0 Hz, 1H), 3.94 (dd, J = 9.5 Hz, 9.0 Hz, 1H), 3.88 (dt, J = 10.5 Hz, J = 5.5 Hz, 1H), 3.77 (dd, J = 11.0 Hz, 3.5 Hz, 1H), 3.73 (dd, J = 10.0 Hz, 3.0 Hz, 1H), 3.65-3.60 (m, 2H), 3.57-3.52 (m, 3H, H_{6b} , H_5 , $H_{4'}$), 3.55 (s, 3H), 3.49 (dd, J = 9.0 Hz, 8.5 Hz, 1H), 3.49-3.36 (m, 2H), 3.46 (dd, J = 10.0 Hz, 6.0 Hz, 1H), 3.32 (dd, J = 8.5 Hz, 8.0 Hz, 1H), 3.30 (dd, 1H, J = 8.0 Hz, 7.0 Hz, 1H), 3.28 (dd, J = 8.5 Hz, 8.0 Hz, 1H), 3.24-3.12 (m, 3H), 2.93 (t, J = 7.5 Hz, 2H), 2.75 (t, J = 7.0 Hz, 2H), 2.28 (s, 1H), 2.06-2.00 (m, 2H), 1.92-1.79 (m, 3H).

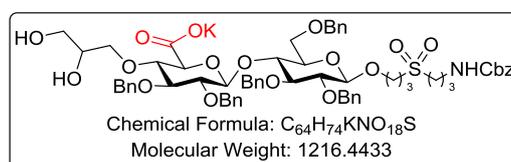
^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 169.0, 156.4, 139.0, 138.6, 138.0, 137.9, 136.4,

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129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.3, 103.3, 102.5, 83.5, 83.3, 82.9, 82.08, 82.12, 81.6, 80.1, 76.9, 75.7, 75.6, 75.4, 75.0, 74.5, 74.5, 74.3, 74.1, 73.9, 73.3, 70.9, 70.8, 67.9, 67.4, 66.9, 63.5, 63.4, 52.6, 50.1, 50.0, 39.4, 22.5, 22.3.

HRMS (ESI, positive mode): m/z : $[M+Na]^+$ Calcd. for $C_{65}H_{77}NNaO_{18}S^+$ 1214.4724, found 1214.4754.

Compound 10: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (2,3-di-O-benzyl-4-O-(2, 3- dihydroxypropyl)- β -D-glucopyranosiduronic acid)-(1 \rightarrow 4)-O-2,3,6-tri-O-benzyl- β -D-glucopyranoside



To a stirred solution of **9** (160 mg, 0.13 mmol, 1 equiv.) and KOH (2.57 mL, 15.43 mmol, $C = 6.0$ M, 115 equiv.) in THF / *n*-BuOH ($v/v = 1/1$, 2.08 mL) at $0^{\circ}C$, was added very carefully H_2O_2 (3.46 mL (35 wt %), 46.29 mmol, 345 equiv.). The temperature was warmed slowly to room temperature and the mixture was stirred for 20 min. After cooling to $0^{\circ}C$, H_3PO_4 (2.57 mL, $C = 2.0$ M) was added to the reaction until $pH = 7$. After evaporation in *vacuo*, the residue was diluted by EtOAc (60 mL), and washed with sat. NaCl (30 mL \times 2). The organic layers were dried over Na_2SO_4 and concentrated. Chromatography (C18, CH_3CN / H_2O , 50 / 50 to 100 / 0 gradient) gave product **10** (138 mg, 87 %, white foam).

$R_f = 0.4$ (EtOAc/MeOH = 8/2); **Revelator:** orcinol

$[\alpha]_{589}^{20} = +4.5$ ($C = 1.0$, MeOH).

1H NMR (360 MHz, CD_3OD) δ 7.30-7.10 (m, 30H), 4.96 (d, $J = 11.5$ Hz, 1H), 4.95 (s, 2H), 4.75-4.69 (m, 2H), 4.69 (d, $J = 11.0$ Hz, 1H), 4.67 (d, $J = 11.5$ Hz, 1H), 4.57 (d, $J = 11.0$ Hz, 1H), 4.54 (d, $J = 11.5$ Hz, 1H), 4.51 (d, $J = 11.5$ Hz, 1H), 4.49 (d, $J = 12.0$ Hz, 1H), 4.44 (d, $J = 8.0$ Hz, 1H), 4.32 (d, $J = 8.0$ Hz, 1H), 4.27 (d, $J = 12.0$ Hz, 1H), 3.83 (dd, $J = 9.5$ Hz, 9.5 Hz, 1H), 3.82 (dt, $J = 10.5$ Hz, 5.5 Hz, 1H), 3.79 (dd, $J = 11.5$ Hz, 3.5 Hz, 1H), 3.76 (dd, $J = 10.0$ Hz, 5.0 Hz, 1H), 3.65-3.53 (m, 5H), 3.51 (d, 1H, $J_{5'-4'} = 9.0$ Hz, 1H), 3.49 (dd, $J = 9.0$ Hz, 9.0 Hz, 1H), 3.46 (dd, $J = 11.0$ Hz, 4.5 Hz, 1H), 3.38 (dd, $J = 11.0$ Hz, 6.0 Hz, 1H), 3.33 (dd, $J = 9.0$ Hz, 8.0 Hz, 1H), 3.32-3.28 (m, 1H), 3.23-3.16 (m, 2H), 3.11-3.05 (m, 2H), 3.01 (t, $J = 7.5$ Hz, 2H), 2.85 (t, J

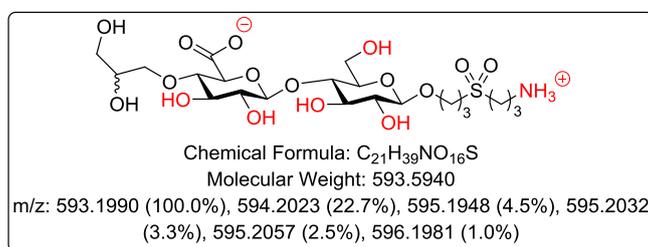
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= 7.5 Hz, 2H), 2.00-1.89 (m, 2H), 1.83-1.72 (m, 2H).

¹³C NMR (90 MHz, CD₃OD) δ 171.2, 158.1, 140.1, 140.0, 139.9, 139.8, 139.4, 129.9, 129.7, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 104.1, 103.6, 85.2, 85.1, 83.3, 82.6, 82.3, 82.2, 78.3, 78.2, 78.0, 76.1, 76.0, 75.9, 75.7, 75.7, 74.9, 74.4, 74.2, 72.4, 72.0, 69.0, 68.4, 67.2, 64.0, 63.8, 50.8, 50.4, 40.0, 23.4, 23.3.

HRMS (ESI, negative mode): m/z: [M-H]⁻ Calcd. for C₆₄H₇₄NO₁₈S⁻ 1176.4627, found 1176.4714.

Compound 11: (2S,3S,4R,5R,6R)-6-(((2R,3S,4R,5R,6R)-6-(3-((3-Ammoniopropyl)sulfonyl)-propoxy)-4,5-dihydroxy-2-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)-oxy)-3-(2,3-dihydroxypropoxy)-4,5-dihydroxytetrahydro-2H-pyran-2 carboxylate



To a solution of compound **10** (278 mg, 0.24 mmol, 1 eq) in *tert*-butanol/water (v/v = 1:2, 53 mL, C = 0.005 M) was added palladium hydroxide on carbon (278 mg, 1 eq) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature until TLC analysis indicated complete consumption of the starting material. Afterwards the mixture was filtered over Celite and the filter cake was washed with water several times. This solution was concentrated *in vacuo* and product **11** was obtained in quantitative yield (142 mg).

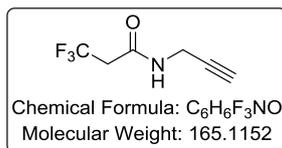
¹H NMR (360 MHz, D₂O) δ 4.37 (d, *J* = 1.9 Hz, 1H), 4.35 (d, *J* = 1.8 Hz, 1H), 3.95 – 3.86 (m, 1H), 3.83 (d, *J* = 11.7 Hz, 1H), 3.76 – 3.66 (m, 3H), 3.66 – 3.59 (m, 2H), 3.59 – 3.38 (m, 8H), 3.33 – 3.21 (m, 6H), 3.21 – 3.13 (m, 1H), 3.08 – 2.97 (m, 2H), 2.12 – 1.96 (m, 4H).

¹³C NMR (75 MHz, D₂O) δ 175.4, 102.2, 102.1, 81.3, 81.1, 78.7, 75.8, 75.0, 74.8, 74.2, 73.4, 73.3, 72.9, 72.8, 70.9, 70.6, 67.9, 62.5, 62.4, 60.0, 49.2, 49.1, 38.0, 21.6, 19.8.

HRMS (ESI, positive mode): m/z: [M + H]⁺ Calcd for C₂₁H₄₀NO₁₆S⁺ 594.2062, found 594.2060; m/z: [M + Na]⁺ Calcd for C₂₁H₃₉NNaO₁₆S⁺ 616.1882, found 616.1879; **(ESI, negative mode):** m/z: [M - H]⁻ Calcd for C₂₁H₃₈NO₁₆S⁻ 592.1917, found 592.1918.

Compound 12: 3,3,3-trifluoro-*N*-(prop-2-yn-1-yl)propanamide

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A solution of propargylamine (679.4 mg, 12.3 mmol, 1.1 eq) in anhydrous dichloromethane (56 mL, C = 0.2 M) was added EDCl.HCl (2.58 g, 13.4 mmol, 1.2 eq) and 3,3,3-trifluoropropionic acid (1.44 g, 11.3 mmol, 1 eq) at 0°C in ice-bath. The mixture was stirred at room temperature for overnight. The solution was then extracted with DCM and washed by water, saturated aqueous NaHCO₃ and brine. The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel with dichloromethane. The product was dried in vacuo until dryness. The product **12** was obtained as white solid (1.81 g, 98%).

R_f = 0.4 (EtOAc/Petroleum Ether = 2/1); **Revelator**: KMnO₄

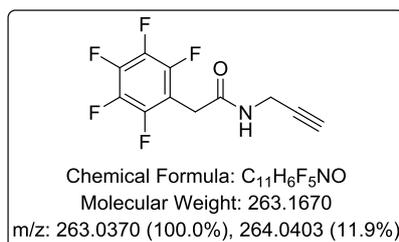
¹H NMR (300 MHz, CDCl₃) δ 4.08 (dd, J = 5.2, 2.5 Hz, 2H), 3.11 (dd, J = 3.2 Hz, 3.1 Hz, 2H), 2.26 (t, J = 2.6 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 162.7, 123.9 (q, J = 276.8 Hz), 78.5, 72.2, 41.3 (q, J = 29.8 Hz), 29.6.

¹⁹F NMR (235 MHz, CDCl₃) δ -62.93 (t, J = 10.5 Hz, 3F).

HRMS (ESI, positive mode): m/z: [M + H]⁺ Calcd for C₆H₇F₃NO⁺ 166.0474, found 166.0465; m/z: [M + Na]⁺ Calcd for C₆H₆F₃NNaO⁺ 188.0294, found 188.0286.

Compound 13: 2-(perfluorophenyl)-N-(prop-2-yn-1-yl)acetamide



A solution of propargylamine (363.5 mg, 6.6 mmol, 1.1 eq) in anhydrous dichloromethane (30 mL, C = 0.2 M) was added EDCl.HCl (1.38 g, 6 mmol, 1 eq) and 2,3,4,5,6-pentafluorophenylacetic acid (363.5 mg, 6.6 mmol, 1.1 eq) at 0°C in ice-bath. The mixture was stirred at room temperature for overnight. The solution was then extracted with DCM and washed by water, saturated aqueous NaHCO₃ and brine. The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel with

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dichloromethane. The product was dried in vacuo until dryness. The product **13** was obtained as white solid (1.43 g, 91%)

R_f = 0.5 (EtOAc/Petroleum Ether = 2/1); **Revelator**: KMnO₄

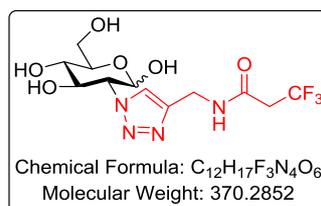
¹H NMR (300 MHz, CDCl₃) δ 4.07 (dd, *J* = 5.2, 2.5 Hz, 2H), 3.63 (s, 2H), 2.26 (t, *J* = 2.5 Hz, 1H).

¹³C NMR (75 MHz, CDCl₃) δ 166.7, 147.2 – 146.7 (m), 143.7 (dd, *J* = 12.8, 9.6 Hz), 142.3 (dd, *J* = 13.6, 6.9 Hz), 139.6 – 138.7 (m), 136.2 – 135.6 (m), 108.2 (d, *J* = 15.1 Hz), 78.8, 72.1, 29.7, 29.4.

¹⁹F NMR (235 MHz, CDCl₃) δ 4.10 (dt, *J* = 5.1, 2.5 Hz, 2F), 3.15 (qd, *J* = 10.5, 2.5 Hz, 2F), 2.28 (q, *J* = 2.5 Hz, 1F).

HRMS (ESI, positive mode): *m/z*: [M + H]⁺ Calcd for C₁₁H₇F₅NO⁺ 264.0442, found 264.0440; *m/z*: [M + Na]⁺ Calcd for C₁₁H₆F₅NNaO⁺ 286.0262, found 286.0258.

Compound 14: 3,3,3-trifluoro-*N*-((1-((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxylmethyl)tetra- hydro-2*H*-pyran-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)propanamide



A mixture of D-glucosamine hydrochloride (25.9 mg, 0.12 mmol, 1 eq), ISA.HCl (30.3 mg, 0.144 mmol, 1.2 eq) and K₂CO₃ (44.8 mg, 0.324 mmol, 2.7 eq) were dissolved in ^tBuOH/H₂O (v/v = 1/1, 1.2 mL, C = 0.1 M), then a catalytic amount of CuSO₄·5H₂O (6 mg, 0.024 mmol 0.2 eq) was added to the solution and the resulting mixture was stirred at room temperature for 4 h. After the complete absence of D-glucosamine hydrochloride (as judged by TLC), 3,3,3-trifluoro-*N*-(prop-2-yn-1-yl)propanamide **12** (24 mg, 0.18 mmol, 1.5 eq) and sodium ascorbate (23.8 mg, 0.12 mmol, 1 eq) were added under argon and the resulting solution was stirred at room temperature for 1 hr. After the complete absence of corresponding azide compound (as judged by TLC), QuadraPureTM metal scavenger (QuadraPure TU) was added to the solution to remove the copper ions, filtered and concentrated. The crude was then filtrated with Florisil 600-100 (DCM/MeOH = 8/1, 40 mL, DCM/MeOH = 4/1, 40 mL) to give **14** as a white solid (42 mg, 95%).

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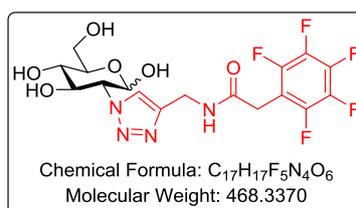
¹H NMR (300 MHz, MeOD) δ 8.03 (s, 1H), 7.92 (s, 1H), 5.29 (d, J = 3.0 Hz, 1H), 5.11 (d, J = 7.1 Hz, 1H), 4.64 (dd, J = 10.9, 3.0 Hz, 1H), 4.50 (s, 4H), 4.31 – 4.20 (m, 1H), 4.19 – 4.07 (m, 2H), 4.03 – 3.89 (m, 2H), 3.79 (ddd, J = 16.2, 15.4, 9.8 Hz, 3H), 3.50 (dt, J = 16.3, 9.0 Hz, 3H), 3.21 (dd, J = 3.3 Hz, 3.2 Hz, 4H).

¹³C NMR (75 MHz, MeOD) δ 165.7, 127.7, 125.7, 124.1, 123.8, 96.2, 92.7, 78.2, 75.6, 73.3, 72.4, 72.2, 71.8, 69.7, 66.9, 62.6, 62.5, 41.2 (q, J = 29.1 Hz), 35.9, 35.8.

¹⁹F NMR (235 MHz, MeOD) δ -64.56 (t, J = 10.7 Hz, 3F).

HRMS (ESI, positive mode): m/z : $[M + H]^+$ Calcd for C₁₂H₁₈F₃N₄O₆⁺ 371.1173, found 371.1159; m/z : $[M + Na]^+$ Calcd for C₁₂H₁₇F₃N₄NaO₆⁺ 393.0992, found 393.0981.

Compound 15: 2-(perfluorophenyl)-*N*-((1-((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)- tetrahydro-2*H*-pyran-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide



A mixture of D-glucosamine hydrochloride (25.9 mg, 0.12 mmol, 1 eq), ISA.HCl (30.3 mg, 0.144 mmol, 1.2 eq) and K₂CO₃ (44.8 mg, 0.324 mmol, 2.7 eq) were dissolved in ^tBuOH/H₂O (v/v = 1/1, 1.2 mL, C = 0.1 M), then a catalytic amount of CuSO₄·5H₂O (6 mg, 0.024 mmol, 0.2 eq) was added to the solution and the resulting mixture was stirred at room temperature for 4 h. After the complete absence of D-glucosamine hydrochloride (as judged by TLC), 2-(perfluorophenyl)-*N*-(prop-2-yn-1-yl)acetamide **13** (47.4 mg, 0.18 mmol, 1.5 eq) and sodium ascorbate (23.8 mg, 0.12 mmol, 1 eq) were added under argon and the resulting solution was stirred at room temperature for 1 hr. After the complete absence of corresponding azide compound (as judged by TLC), QuadraPureTM metal scavenger (QuadraPure TU) was added to the solution to remove the copper ions, filtered and concentrated. The crude was then filtrated with Florisil 600-100 (DCM/MeOH = 8/1, 40 mL, DCM/MeOH = 4/1, 40 mL) to give product **15** as a white solid (56 mg, quantitative).

¹H NMR (300 MHz, MeOD) δ 8.02 (s, 1H), 7.91 (s, 1H), 5.29 (d, J = 2.3 Hz, 1H), 5.12 (d, J = 6.4 Hz, 1H), 4.64 (dd, J = 10.6, 2.2 Hz, 1H), 4.49 (s, 4H), 4.32 – 4.21 (m,

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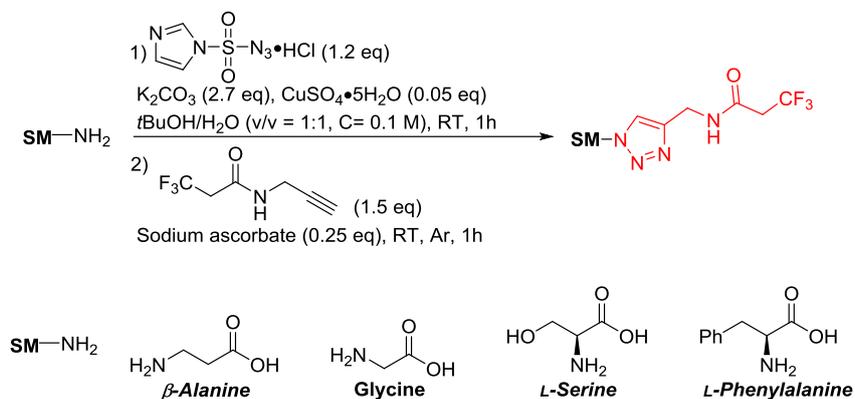
¹H), 4.21 – 4.07 (m, 2H), 3.95 (t, *J* = 9.9 Hz, 2H), 3.84 (dd, *J* = 14.8, 7.7 Hz, 2H), 3.77 (d, *J* = 3.9 Hz, 2H), 3.74 (s, 3H), 3.59 – 3.41 (m, 3H).

¹³C NMR (75 MHz, MeOD) δ 170.2, 148.5 (dd, *J* = 19.4, 8.9 Hz), 145.4 (dd, *J* = 20.0, 12.2 Hz), 143.9 – 142.8 (m), 141.7 – 139.2 (m), 137.2 (dd, *J* = 22.5, 10.2 Hz), 125.7, 123.8, 110.9 (t, *J* = 17.5 Hz), 96.2, 92.7, 78.2, 75.6, 73.3, 72.5, 72.2, 71.8, 69.7, 66.9, 62.6, 62.5, 36.1, 36.0, 29.6.

¹⁹F NMR (235 MHz, MeOD) δ -143.95 – -144.89 (m, 2F), -159.24 – -159.65 (m, 2F), -165.65 – -166.22 (m, 1F).

HRMS (ESI, positive mode): *m/z*: [M + H]⁺ Calcd for C₁₇H₁₈F₅N₄O₆⁺ 469.1141, found 469.1116; *m/z*: [M + Na]⁺ Calcd for C₁₇H₁₇F₅N₄NaO₆⁺ 491.0960, found 491.0940.

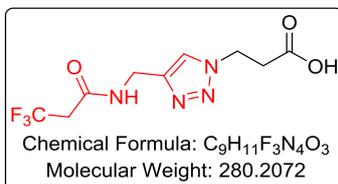
A general procedure of preparation of compound 16-19



A mixture of the amino acid (0.50 mmol, 1 eq), K₂CO₃ (187 mg, 1.35 mmol, 2.7 eq) and CuSO₄·5H₂O (6 mg, 0.025 mmol, 0.05 eq) were dissolved in *tert*-butanol/water (v/v = 1:1, 5 mL, C = 0.1 M), then Imidazole-1-sulfonyl azide hydrochloride (126 mg, 0.60 mmol, 1.2 eq) was added and the mixture was stirred at room temperature for 1 hr. After the complete absence of starting material (as judged by ¹H NMR), 3,3,3-trifluoro-*N*-(prop-2-yn-1-yl)propanamide **12** (124 mg, 0.75 mmol, 1.5 eq) and sodium ascorbate (25 mg, 0.125 mmol, 0.25 eq) were added under argon, and the resulting solution was stirred at room temperature for 1h. After the complete absence of corresponding azide compound (as judged by ¹H NMR), the reaction solution was diluted with water and washed by CH₂Cl₂, then the aqueous phase was acidified to pH = 1~2 with a 1 M HCl solution and extracted three times with ethyl acetate. The combined organic layer was dried over NaSO₄, filtered and concentrated in vacuo. The crude was then purified by flash chromatography on Florisil® 600-100 mesh (eluent: CH₂Cl₂/MeOH = 4/1 40 ml, then CH₂Cl₂/MeOH = 1 : 1 40 ml) to give the desired product.

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Compound 16: 3-(4-((3,3,3-trifluoropropanamido)methyl)-1H-1,2,3-triazol-1-yl)propanoic acid



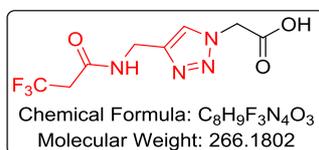
1H NMR (360 MHz, MeOD) δ 7.88 (s, 1H), 4.63 (t, $J = 6.6$ Hz, 2H), 4.45 (s, 2H), 3.19 (dd, $J = 3.2$ Hz, 3.2Hz, 2H), 2.88 (t, $J = 6.6$ Hz, 2H).

^{13}C NMR (75 MHz, MeOD) δ 175.3, 165.6, 145.4, 125.8 (q, $J = 275.8$ Hz), 124.7, 47.6, 39.6 (dd, $J = 29.1$ Hz), 36.3, 35.7.

^{19}F NMR (235 MHz, MeOD) δ -64.68 (t, $J = 10.7$ Hz, 3F).

HRMS (ESI, positive mode): m/z : $[M + H]^+$ Calcd for $C_9H_{12}F_3N_4O_3^+$ 281.0856, found 281.0849; m/z : $[M + Na]^+$ Calcd for $C_9H_{11}F_3N_4NaO_3^+$ 303.0675, found 303.0675; (ESI, negative mode): m/z : $[M - H]^-$ Calcd for $C_9H_{10}F_3N_4O_3^-$ 279.0710, found 279.0721.

Compound 17: 2-(4-((3,3,3-trifluoropropanamido)methyl)-1H-1,2,3-triazol-1-yl)acetic acid



1H NMR (360 MHz, MeOD) δ 7.87 (s, 1H), 5.09 (s, 2H), 4.48 (s, 2H), 3.19 (dd, $J = 3.2$ Hz, 3.2Hz, 2H).

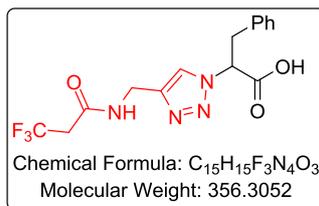
^{13}C NMR (75 MHz, MeOD) δ 171.9, 165.7, 145.4, 125.8, 125.8 (q, $J = 275.8$ Hz), 53.2, 41.1 (q, $J = 29.1$ Hz), 35.9, 35.7.

^{19}F NMR (235 MHz, MeOD) δ -64.66 (t, $J = 10.7$ Hz, 3F).

HRMS (ESI, positive mode): m/z : $[M + H]^+$ Calcd for $C_8H_{10}F_3N_4O_3^+$ 267.0700, found 267.0692; m/z : $[M + Na]^+$ Calcd for $C_8H_9F_3N_4NaO_3^+$ 289.0519, found 289.0509; (ESI, negative mode): m/z : $[M - H]^-$ Calcd for $C_8H_8F_3N_4O_3^-$ 265.0554, found 265.0564.

Compound 18: 3-phenyl-2-(4-((3,3,3-trifluoropropanamido)methyl)-1H-1,2,3-triazol-1-yl)-propanoic acid

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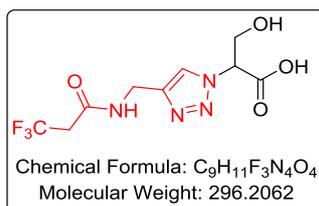
¹H NMR (360 MHz, MeOD) δ 7.87 (s, 1H), 7.19 – 7.05 (m, 5H), 5.36 (dd, *J* = 10.9, 4.5 Hz, 1H), 4.40 (d, *J* = 2.8 Hz, 2H), 3.62 (dd, *J* = 14.4, 4.4 Hz, 1H), 3.39 (dd, *J* = 14.4, 3.2 Hz, 3.2Hz, 1H), 3.17 (q, *J* = 10.8 Hz, 2H).

¹³C NMR (75 MHz, MeOD) δ 174.9, 165.5, 144.8, 138.4, 129.8, 129.4, 127.7, 125.8 (q, *J* = 275.8 Hz), 124.5, 68.7, 41.1 (q, *J* = 29.1 Hz), 39.9, 35.8, 35.7.

¹⁹F NMR (235 MHz, MeOD) δ -64.61 (t, *J* = 10.7 Hz, 3F).

HRMS (ESI, positive mode): *m/z*: [M + H]⁺ Calcd for C₁₅H₁₆F₃N₄O₃⁺ 357.1170, found 357.1161; *m/z*: [M + Na]⁺ Calcd for C₁₅H₁₅F₃N₄NaO₃⁺ 379.0988, found 379.0983.

Compound 19: 3-hydroxy-2-(4-((3,3,3-trifluoropropanamido)methyl)-1H-1,2,3-triazol-1-yl)propanoic acid



¹H NMR (360 MHz, MeOD) δ 8.02 (s, 1H), 5.32 (dd, *J* = 6.9, 3.9 Hz, 1H), 4.50 (s, 2H), 4.26 (dd, *J* = 11.8, 7.0 Hz, 1H), 4.12 (dd, *J* = 11.8, 3.9 Hz, 1H), 3.20 (dd, *J* = 3.3 Hz, 3.2Hz, 2H).

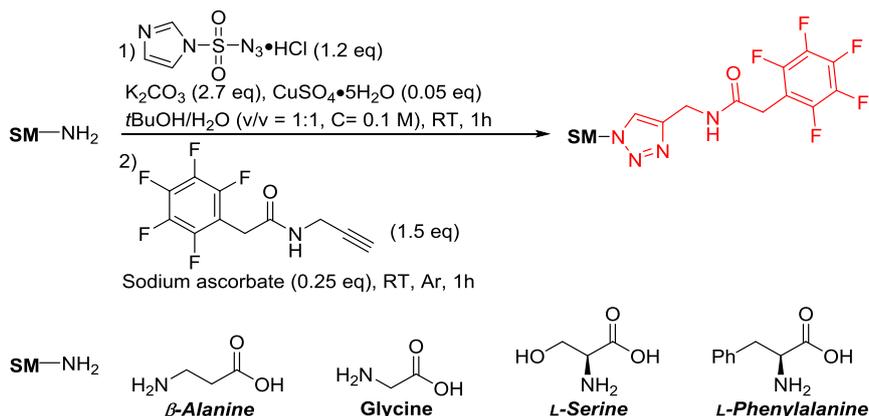
¹³C NMR (75 MHz, MeOD) δ 172.4, 165.7, 144.9, 125.8 (q, *J* = 275.8 Hz), 125.0, 68.1, 63.3, 41.1 (q, *J* = 29.2 Hz), 35.9, 35.8.

¹⁹F NMR (235 MHz, MeOD) δ -64.67 (t, *J* = 10.7 Hz, 3F).

HRMS (ESI, positive mode): *m/z*: [M + H]⁺ Calcd for C₉H₁₂F₃N₄O₄⁺ 297.0805, found 297.0795; *m/z*: [M + Na]⁺ Calcd for C₉H₁₁F₃N₄NaO₄⁺ 319.0625, found 319.0616.

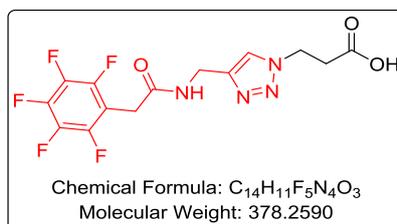
A general procedure of preparation of compound 20-23:

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A mixture of the amino acid (0.50 mmol, 1 eq), K_2CO_3 (187 mg, 1.35 mmol, 2.7 eq) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (6 mg, 0.025 mmol, 0.05 eq) were dissolved in *tert*-butanol/water (v/v = 1:1, 5 mL, C = 0.1 M), then imidazole-1-sulfonyl azide hydrochloride (126 mg, 0.60 mmol, 1.2 eq) was added to the solution and the resulting mixture was stirred at room temperature for 1 hr. After the complete absence of starting material (as judged by ^1H NMR), 2-(perfluorophenyl)-*N*-(prop-2-yn-1-yl)acetamide **13** (197 mg, 0.75 mmol, 1.5 eq) and sodium ascorbate (25 mg, 0.125 mmol, 0.25 eq) were added under argon, and the resulting solution was stirred at room temperature for 1 hr. After the complete absence of corresponding azide compound (as judged by ^1H NMR), the solution was diluted with water and washed by CH_2Cl_2 , then the aqueous phase was acidified to pH = 1~2 with a 1 M HCl solution and extracted three times with ethyl acetate. The collected organic layer was dried over NaSO_4 , filtered and concentrated under in vacuo. The crude was then purified by flash chromatography on Florisil[®] 600-100 mesh (eluent: $\text{CH}_2\text{Cl}_2/\text{MeOH}$ = 4 : 1 40 ml, then $\text{CH}_2\text{Cl}_2/\text{MeOH}$ = 1 : 1 40 ml) to give the desired product.

Compound 20: 3-(4-((2-(perfluorophenyl)acetamido)methyl)-1*H*-1,2,3-triazol-1-yl)propanoic acid



^1H NMR (360 MHz, MeOD) δ 7.88 (s, 1H), 4.63 (t, J = 6.6 Hz, 2H), 4.45 (s, 2H), 3.72 (s, 2H), 2.87 (t, J = 6.6 Hz, 2H).

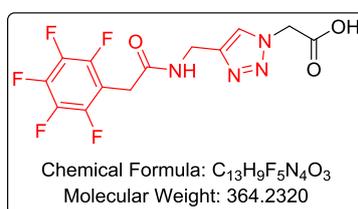
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¹³C NMR (75 MHz, MeOD) δ 175.8, 170.1, 149.0 – 148.1 (m), 145.7, 145.5 – 144.8 (m), 143.6 – 142.7 (m), 140.1 (dt, J = 13.6, 12.0 Hz), 137.1 (t, J = 16.7 Hz), 124.7, 110.9 (t, J = 18.7 Hz), 47.7, 36.6, 35.9, 29.6.

¹⁹F NMR (235 MHz, MeOD) δ -144.66 (dd, J = 21.5, 8.2 Hz, 2F), -159.53 (t, J = 20.0 Hz, 1F), -165.82 – -166.24 (m, 2F).

HRMS (ESI, positive mode): m/z : $[M + H]^+$ Calcd for $C_{14}H_{12}F_5N_4O_3^+$ 379.0824, found 379.0807; m/z : $[M + Na]^+$ Calcd for $C_{14}H_{11}F_5N_4NaO_3^+$ 401.0644, found 401.0630.

Compound 21: 2-(4-((2-(perfluorophenyl)acetamido)methyl)-1H-1,2,3-triazol-1-yl)acetic acid



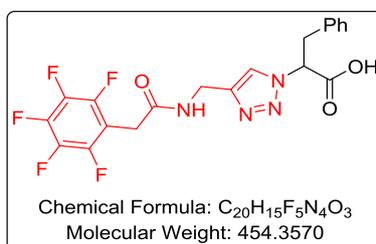
¹H NMR (300 MHz, MeOD) δ 7.86 (s, 1H), 5.10 (s, 2H), 4.48 (s, 2H), 3.73 (s, 2H).

¹³C NMR (75 MHz, MeOD) δ 171.6, 170.1, 149.1 – 148.1 (m), 145.6, 145.2 (d, J = 9.1 Hz), 143.7 – 142.8 (m), 140.0 (ddd, J = 27.0, 21.2, 10.0 Hz), 137.1 (t, J = 15.6 Hz), 125.7, 110.9 (t, J = 18.7 Hz), 53.1, 36.0, 29.6.

¹⁹F NMR (235 MHz, MeOD) δ -144.62 (dd, J = 21.3, 7.8 Hz, 2F), -159.55 (t, J = 20.0 Hz, 1F), -166.06 (dt, J = 21.5, 8.2 Hz, 2F).

HRMS (ESI, positive mode): m/z : $[M + H]^+$ Calcd for $C_{13}H_{10}F_5N_4O_3^+$ 365.0668, found 365.0657; m/z : $[M + Na]^+$ Calcd for $C_{13}H_9F_5N_4NaO_3^+$ 387.0487, found 387.0475.

Compound 22: 2-(4-((2-(perfluorophenyl)acetamido)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylpropanoic acid



¹H NMR (300 MHz, MeOD) δ 7.86 (s, 1H), 7.14 (m, 5H), 5.42 (dd, J = 10.8, 4.5 Hz, 1H), 4.41 (s, 2H), 3.70 (s, 2H), 3.62 (dd, J = 14.4, 4.4 Hz, 1H), 3.41 (dd, J = 14.3, 10.9 Hz, 1H).

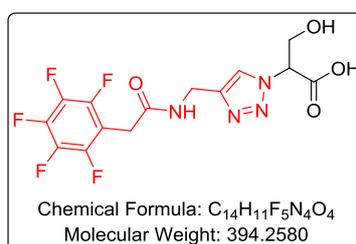
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¹³C NMR (75 MHz, MeOD) δ 173.3, 170.1, 148.7 – 148.0 (m), 145.6 – 144.3 (m), 143.2 (dd, $J = 18.5, 8.3$ Hz), 140.7 – 139.4 (m), 137.8, 137.0 (t, $J = 15.8$ Hz), 129.8, 129.4, 127.8, 124.7, 110.7 (t, $J = 18.6$ Hz), 67.7, 39.4, 35.9, 35.8, 29.5.

¹⁹F NMR (235 MHz, MeOD) δ -144.56 (dd, $J = 21.1, 7.7$ Hz, 2F), -159.50 (t, $J = 20.0$ Hz, 1F), -165.68 – -166.36 (m, 2F).

HRMS (ESI, positive mode): m/z : $[M + H]^+$ Calcd for $C_{20}H_{16}F_5N_4O_3^+$ 455.1137, found 455.1128; m/z : $[M + Na]^+$ Calcd for $C_{20}H_{15}F_5N_4NaO_3^+$ 477.0957, found 477.0949.

Compound 23: 3-hydroxy-2-(4-((2-(perfluorophenyl)acetamido)methyl)-1H-1,2,3-triazol-1-yl)propanoic acid



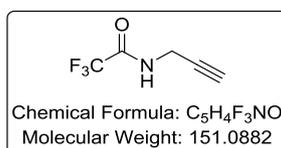
¹H NMR (360 MHz, MeOD) δ 8.03 (s, 1H), 5.41 (dd, $J = 6.6, 3.7$ Hz, 1H), 4.50 (s, 2H), 4.29 (dd, $J = 11.8, 6.7$ Hz, 1H), 4.11 (dd, $J = 11.8, 3.7$ Hz, 1H), 3.73 (s, 2H).

¹³C NMR (75 MHz, MeOD) δ 171.5, 170.2, 148.5 (t, $J = 9.3$ Hz), 145.5 - 144.7 (m), 145.3, 143.7 – 142.7 (m), 141.1 – 139.3 (m), 137.1 (t, $J = 16.3$ Hz), 125.0, 110.9 (t, $J = 18.7$ Hz), 67.4, 63.2, 36.1, 36.0, 29.6.

¹⁹F NMR (235 MHz, MeOD) δ -144.59 (dd, $J = 21.5, 8.2$ Hz, 2F), -159.55 (t, $J = 20.0$ Hz, 1F), -165.79 – -166.35 (m, 2F).

HRMS (ESI, positive mode): m/z : $[M + H]^+$ Calcd for $C_{14}H_{12}F_5N_4O_4^+$ 395.0773, found 395.0765; m/z : $[M + Na]^+$ Calcd for $C_{14}H_{11}F_5N_4NaO_4^+$ 417.0593, found 417.0591.

Compound 24: 2,2,2-trifluoro-*N*-(prop-2-ynyl)acetamide



Propargylamine (440.64 mg, 8 mmol, 1.07 eq) was dissolved in anhydrous dichloromethane (15 mL), the solution was cooled to 0°C. A solution of the trifluoroacetic anhydride (157.2 mg, 7.48 mmol, 1 eq) in dichloromethane (7 mL) was added dropwise. Then the flask was sealed with rubber septa, and the reaction mixture was poured into 20 mL H₂O, the organic phase was separated, and the aqueous phase

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was extracted with 10 mL dichloromethane. The combined organic phases were washed with saturated NaHCO₃ and brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel with dichloromethane. The product was dried in vacuo until dryness. The product **24** was obtained as colorless liquid (973.9, 86%).

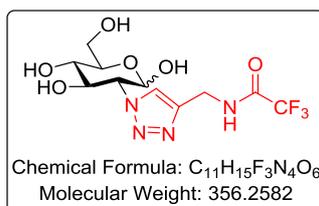
$R_f = 0.5$ (PE/EA = 3/1)

¹H NMR (360 MHz, CDCl₃) δ 4.11 (dd, $J = 5.4, 2.3$ Hz, 2H), 2.30 (t, $J = 2.5$ Hz, 1H).

¹³C NMR (91 MHz, CDCl₃) δ 157.1 (dd, $J = 76.5, 37.0$ Hz), 115.6 (dd, $J = 574.8, 287.7$ Hz), 73.1, 73.0, 29.6.

¹⁹F NMR (235 MHz, CDCl₃) δ -75.99 (s, 3F).

Compound 25: 2,2,2-trifluoro-*N*-((1-((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxylmethyl)tetra- hydro-2*H*-pyran-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide



A mixture of D-glucosamine hydrochloride (25.9 mg, 0.12 mmol, 1 eq), ISA.HCl (30.3 mg, 0.144 mmol, 1.2 eq) and K₂CO₃ (44.8 mg, 0.324 mmol, 2.7 eq) were dissolved in ^tBuOH/H₂O (v/v = 1/1, 1.2 mL, C = 0.1 M), then a catalytic amount of CuSO₄·5H₂O (6 mg, 0.024 mmol 0.2 eq) was added to the solution and the resulting mixture was stirred at room temperature for 4 h. After the complete absence of D-glucosamine hydrochloride (as judged by TLC), 2,2,2-trifluoro-*N*-(prop-2-ynyl)acetamide **24** (27.2 mg, 0.18 mmol, 1.5 eq) and sodium ascorbate (23.8 mg, 0.12 mmol, 1 eq) were added under argon and the resulting solution was stirred at room temperature for 1 hr. After the complete absence of corresponding azide compound (as judged by TLC), QuadraPureTM metal scavenger (QuadraPure TU) was added to the solution to remove the copper ions, filtered and concentrated. The crude was then filtrated with Florisil 600-100 (DCM/MeOH = 8/1, 40 mL, DCM/MeOH = 4/1, 40 mL) to give product **25** a white solid (34 mg, 79%).

¹H NMR (300 MHz, MeOD) δ 8.06 (s, 1H), 7.96 (s, 1H), 5.29 (d, $J = 3.3$ Hz, 1H), 5.12 (d, $J = 7.9$ Hz, 1H), 4.65 (dd, $J = 11.0, 3.3$ Hz, 1H), 4.57 (s, 4H), 4.25 (dd, $J =$

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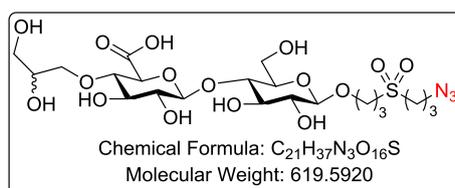
11.0, 8.7 Hz, 1H), 4.18 – 4.09 (m, 2H), 4.01 – 3.86 (m, 2H), 3.78 (ddd, $J = 17.0, 9.6, 3.8$ Hz, 3H), 3.58 – 3.42 (m, 3H).

^{13}C NMR (75 MHz, MeOD) δ 159.7, 159.2, 158.7, 158.3, 144.2, 144.0, 126.0, 124.1, 117.5 (q), 96.2, 92.7, 78.2, 75.5, 73.3, 72.5, 72.2, 71.8, 69.7, 66.9, 62.6, 62.5, 35.8, 35.8, 30.7.

^{19}F NMR (235 MHz, MeOD) δ -76.87 (s, 3F).

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{11}\text{H}_{16}\text{F}_3\text{N}_4\text{O}_6^+$ 357.1016, found 357.1018; m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{11}\text{H}_{15}\text{F}_3\text{N}_4\text{NaO}_6^+$ 379.0836, found 379.0833.

Compound 26: (2*S*,3*S*,4*R*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*R*)-6-(3-((3-Azidopropyl)sulfonyl)propoxy)-4,5-dihydroxy-2-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)oxy)-3-(2,3-dihydroxypropoxy)-4,5-dihydroxytetrahydro-2*H*-pyran-2-carboxylic acid



A mixture of compound **11** (10 mg, 17 μmol , 1 eq), K_2CO_3 (6.7 mg, 45 μmol , 2.7 eq) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.4 mg, 1.7 μmol , 0.1 eq) were dissolved in *tert*-butanol/water ($v/v = 1:1$, 85 μL , $C = 0.2$ M), then Imidazole-1-sulfonyl azide hydrochloride (4.3 mg, 20 μmol , 1.2 eq) was added and the mixture was stirred at room temperature for 4 hours. After the complete absence of corresponding amine compound (as judged by TLC), the reaction solution was added QuadraPureTM metal scavenger (QuadraPure TU) to remove the copper ions. Then the solution was filtrated, concentrated and the crude product were purified by G15 to obtain product **26** (10 mg, quantitative).

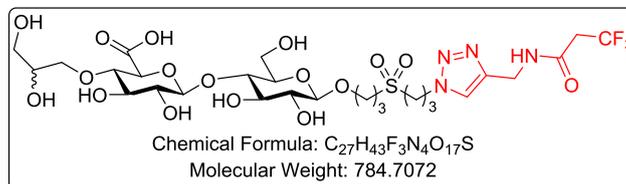
^1H NMR (360 MHz, D_2O) δ 4.40 (d, $J = 0.7$ Hz, 1H), 4.38 (d, $J = 0.7$ Hz, 1H), 3.97 – 3.89 (m, 1H), 3.86 (dd, $J = 11.9, 0.6$ Hz, 1H), 3.78 – 3.70 (m, 3H), 3.69 – 3.63 (m, 2H), 3.62 – 3.57 (m, 1H), 3.57 – 3.51 (m, 3H), 3.51 – 3.47 (m, 3H), 3.47 – 3.40 (m, 3H), 3.29 (dt, $J = 7.5, 3.2$ Hz, 4H), 3.23 (dt, $J = 16.8, 5.4$ Hz, 3H), 2.09 – 1.95 (m, 4H).

^{13}C NMR (91 MHz, D_2O) δ 175.4, 102.1, 102.0, 81.2, 81.0, 78.7, 75.6, 74.9, 74.7, 74.1, 73.4, 73.2, 72.9, 72.7, 70.8, 70.6, 67.9, 62.4, 62.3, 59.9, 49.3, 49.2, 49.0, 21.6, 20.9.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{21}\text{H}_{37}\text{N}_3\text{NaO}_{16}\text{S}^+$ 642.1787, found 642.1788.

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Compound 27: (2*S*,3*S*,4*R*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*R*)-4,5-Dihydroxy-2-(hydroxymethyl)-6-(3-((3-(4-((3,3,3-trifluoropropanamido)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)-sulfonyl)propoxy)tetrahydro-2*H*-pyran-3-yl)oxy)-3-(2,3-dihydroxypropoxy)-4,5-dihydroxytetrahydro-2*H*-pyran-2-carboxylic acid



A mixture of compound **11** (10 mg, 17 μ mol, 1 eq), K₂CO₃ (6.7 mg, 45 μ mol, 2.7 eq) and CuSO₄·5H₂O (0.4 mg, 1.7 μ mol, 0.1 eq) were dissolved in *tert*-butanol/water (v/v = 1:1, 85 μ L, C = 0.2 M), then Imidazole-1-sulfonyl azide hydrochloride (4.3 mg, 20 μ mol, 1.2 eq) was added and the mixture was stirred at room temperature for 4 hours. After the complete absence of corresponding amine compound (as judged by TLC), alkyne **12** (4.2 mg, 26 μ mol, 1.5 eq) and sodium ascorbate (3.4 mg, 17 μ mol, 1 eq) were added under argon, and the resulting solution was stirred at room temperature for 1h. After the complete absence of corresponding azide compound (as judged by TLC), the reaction solution was added QuadraPure™ metal scavenger (QuadraPure TU) to remove the copper ions. Then the solution was filtrated, concentrated and the crude product were purified by G15 to obtain product **27** (13 mg, 98%).

¹H NMR (360 MHz, D₂O) δ 7.84 (s, 1H), 4.52 (t, *J* = 6.4 Hz, 1H), 4.41 (s, 2H), 4.40 – 4.36 (m, 2H), 3.99 (dd, *J* = 5.7, 3.0 Hz, 1H), 3.92 – 3.84 (m, 4H), 3.77 – 3.74 (m, 1H), 3.71 (s, 1H), 3.71 – 3.66 (m, 1H), 3.65 – 3.60 (m, 1H), 3.59 – 3.49 (m, 5H), 3.48 – 3.44 (m, 1H), 3.27 (dd, *J* = 17.3, 8.3 Hz, 2H), 3.23 – 3.15 (m, 2H), 3.14 (d, *J* = 7.7 Hz, 2H), 2.98 (dd, *J* = 13.0, 6.1 Hz, 1H), 2.41 – 2.29 (m, 2H), 2.05 – 1.96 (m, 2H), 1.18 (t, *J* = 7.1 Hz, 2H).

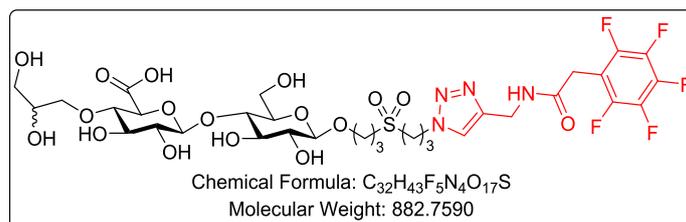
¹³C NMR (91 MHz, D₂O) δ 173.6, 172.4, 123.9, 102.1, 102.0, 88.0, 81.2, 81.1, 78.7, 75.7, 74.9, 74.7, 74.1, 73.3, 73.2, 72.9, 72.8, 72.3, 72.0, 70.6, 67.9, 64.1, 63.2, 62.8, 62.6, 62.4, 62.4, 59.9, 59.8, 49.0, 48.7, 48.4, 42.2 (q, *J* = 29.1 Hz), 34.7, 28.8, 22.1, 21.6.

HRMS (ESI, positive mode): *m/z*: [M - H]⁻ Calcd for C₂₇H₄₂F₃N₄O₁₇S⁻ 783.2210, found 783.2223.

Compound 28: (2*S*,3*S*,4*R*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*R*)-4,5-Dihydroxy-2-(hydroxymethyl)-6-(3-((3-(4-((2-(perfluorophenyl)acetamido)methyl)-1*H*-1,2,3-

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triazol-1-yl)propyl)-sulfonyl)propoxy)tetrahydro-2H-pyran-3-yl)oxy)-3-(2,3-dihydroxypropoxy)-4,5-dihydroxytetrahydro-2H-pyran-2-carboxylic acid



A mixture of the compound **11** (10.7 mg, 18 μ mol, 1 eq), K₂CO₃ (6.7 mg, 49 μ mol, 2.7 eq) and CuSO₄·5H₂O (0.45 mg, 1.8 μ mol, 0.1 eq) were dissolved in *tert*-butanol/water (v/v = 1:1, 90 μ L, C = 0.2 M), then Imidazole-1-sulfonyl azide hydrochloride (4.6 mg, 22 μ mol, 1.2 eq) was added and the mixture was stirred at room temperature for overnight. After the complete absence of starting material (as judged by TLC), Alkyne **13** (7.1 mg, 27 μ mol, 1.5 eq) and sodium ascorbate (3.6 mg, 18 μ mol, 1 eq) were added under argon, and the resulting solution was stirred at room temperature for 1h. After the complete absence of corresponding azide compound (as judged by TLC), the reaction solution was added QuadraPure™ metal scavenger (QuadraPure TU) to remove the copper ions. Then the solution was filtrated, concentrated and the crude product were purified by G15 to obtain product **28** (14 mg, 88%).

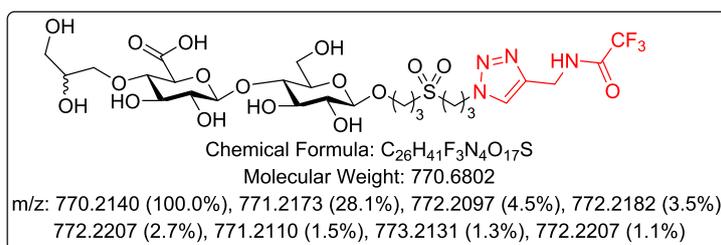
¹H NMR (300 MHz, D₂O) δ 7.90 (s, 1H), 4.56 (d, *J* = 6.6 Hz, 1H), 4.46 (s, 2H), 4.44 (d, *J* = 4.6 Hz, 1H), 4.42 (d, *J* = 4.5 Hz, 1H), 4.10 – 4.00 (m, 2H), 3.92 (ddd, *J* = 9.5, 7.7, 2.8 Hz, 4H), 3.83 – 3.69 (m, 4H), 3.68 – 3.46 (m, 6H), 3.36 – 3.27 (m, 2H), 3.24 (s, 2H), 3.20 (d, *J* = 4.4 Hz, 1H), 3.16 (d, *J* = 8.0 Hz, 1H), 2.45 – 2.33 (m, 2H), 2.09 – 2.00 (m, 2H), 1.30 – 1.20 (m, 2H).

¹³C NMR (75 MHz, D₂O) δ 175.4, 171.2, 123.4, 102.2, 102.1, 81.3, 81.1, 78.7, 75.8, 75.0, 74.8, 74.2, 73.4, 73.3, 72.9, 72.8, 70.8, 70.6, 70.5, 67.9, 65.3, 62.9, 62.5, 62.4, 60.0, 59.8, 49.1, 48.8, 48.4, 40.3, 34.7, 23.2, 22.1, 21.6.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₃₂H₄₃F₅N₄NaO₁₇S⁺ 905.2156, found 905.2168; **(ESI, negative mode):** m/z: [M - H]⁻ Calcd for C₃₂H₄₂F₅N₄O₁₇S⁻ 881.2191, found 881.2196.

Compound 29: (2*S*,3*S*,4*R*,5*R*,6*R*)-6-(((2*R*,3*S*,4*R*,5*R*,6*R*)-4,5-Dihydroxy-2-(hydroxymethyl)-6-(3-((3-(4-((2,2,2-trifluoroacetamido)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)sulfonyl)-propoxy)tetrahydro-2*H*-pyran-3-yl)oxy)-3-(2,3-dihydroxypropoxy)-4,5-di-hydroxytetrahydro-2*H*-pyran-2-carboxylic acid

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A mixture of the amine (29.9 mg, 50 μ mol, 1 eq), K₂CO₃ (18.7 mg, 135 μ mol, 2.7 eq) and CuSO₄·5H₂O (1.2 mg, 5 μ mol, 0.1 eq) were dissolved in *tert*-butanol/water (v/v = 1:1, 2.5 mL, C = 0.2 M), then Imidazole-1-sulfonyl azide hydrochloride (12.6 mg, 60 μ mol, 1.2 eq) was added and the mixture was stirred at room temperature for overnight. After the complete absence of starting material (as judged by TLC), 3,3,3-trifluoro-*N*-(prop-2-yn-1-yl)propanamide **24** (11.4 mg, 76 μ mol, 1.5 eq) and sodium ascorbate (10 mg, 50 μ mol, 1 eq) were added under argon, and the resulting solution was stirred at room temperature for 1h. After the complete absence of corresponding azide compound (as judged by TLC), the reaction solution was added QuadraPure™ metal scavenger (QuadraPure TU) to remove the copper ions. Then the solution was filtrated, concentrated and the crude product were purified by G15 to obtain product **29** (10 mg, 26%).

¹H NMR (360 MHz, D₂O) δ 7.91 (s, 1H), 4.84 – 4.79 (m, 1H), 4.50 (s, 2H), 4.38 (d, *J* = 4.6 Hz, 1H), 4.36 (d, *J* = 4.6 Hz, 1H), 4.31 (d, *J* = 3.9 Hz, 1H), 4.00 (dt, *J* = 9.1, 4.6 Hz, 2H), 3.92 – 3.81 (m, 4H), 3.78 – 3.62 (m, 4H), 3.56 – 3.40 (m, 5H), 3.25 (dt, *J* = 11.8, 7.0 Hz, 4H), 3.14 (dd, *J* = 14.0, 6.8 Hz, 2H), 2.34 (dt, *J* = 13.9, 6.7 Hz, 1H), 2.04 – 1.94 (m, 2H).

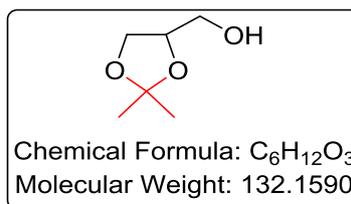
¹³C NMR (75 MHz, D₂O) δ 178.6, 172.4, 124.4, 116.6 (q, *J* = 29.1), 102.1, 102.0, 83.0, 81.2, 81.0, 78.7, 75.6, 74.7, 74.1, 73.4, 73.2, 72.9, 72.7, 72.3, 72.0, 70.6, 68.5, 67.8, 64.0, 63.2, 62.8, 62.4, 62.3, 59.9, 59.8, 49.1, 48.7, 48.4, 34.5, 22.0, 21.6, 20.0.

¹⁹F NMR (235 MHz, D₂O) δ -75.92 (s, 3F).

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₂₆H₄₁F₃N₄NaO₁₇S⁺ 793.2032, found 793.1983; **(ESI, negative mode):** m/z: [M - H]⁻ Calcd for C₂₆H₄₀F₃N₄O₁₇S⁻ 769.2067, found 769.2072.

Compound 30: (2,2-dimethyl-1,3-dioxolan-4-yl)methanol

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A solution of glycerol (4.6 g, 50 mmol, 1 eq) in anhydrous acetone (50 mL, C = 1 M) was added 2,2-dimethoxypropane (7.8 g, 75 mmol, 1.5 eq) and a catalytic amount of *p*-TsOH.H₂O (19 mg, 0.1 mmol, 0.2% eq) sequentially. Then the mixture was stirred at room temperature for 24 h and was concentrated under vacuum, then the mixture was purified by a flash column chromatography (PE/EA = 1/1), isolated product **30** as a colorless oil (8.678 g, 66%)

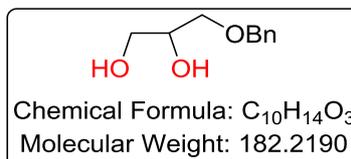
R_f = 0.3 (Petroleum ether/EtOAc = 50/50); **Relevator**: KMnO₄

¹H NMR (300 MHz, CDCl₃) δ 4.23 (tdd, *J* = 6.5, 5.2, 3.8 Hz, 1H), 4.03 (dd, *J* = 8.2, 6.6 Hz, 1H), 3.78 (dd, *J* = 8.2, 6.5 Hz, 1H), 3.72 (dd, *J* = 11.7, 3.7 Hz, 1H), 3.58 (dd, *J* = 11.7, 5.2 Hz, 1H), 1.43 (s, 3H), 1.37 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 109.4, 76.1, 65.7, 63.0, 26.7, 25.3.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₆H₁₂NaO₃ 155.0679, found 155.0672.

Compound 31: 3-(benzyloxy)propane-1,2-diol



To a solution of compound **30** (11.756 g, 88.95 mmol, 1 eq) in anhydrous THF (50 mL, C = 1.8M) 60% NaH (5.34g, 133.43 mmol, 1.5 eq) was portionwise added at 0°C (ice-bath). The reaction mixture was stirred for 30 minutes at 0°C, then Benzyl bromide (18.26 g, 106.74 mmol, 13 mL, 1.2 eq) was then dropwise added and the reaction mixture was warmed slowly to room temperature. After being stirred for overnight, MeOH was added at 0°C to quench the reaction. The solution then was extracted with EtOAc and was washed successively with water, saturated aqueous NaHCO₃ and brine. The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude was purified by a flash column chromatography on silica gel with EA/PE (v/v = 1/10), gave a colorless oil (24.945 g, quantitative) and used directly in the next step.

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The colorless oil was then dissolved in HOAc/H₂O (v/v = 7/3, 40 mL, C = 2 M) and stirred at 65°C for 1 hour. Then the reaction mixture was concentrated and neutralized with saturated aqueous NaHCO₃ solution and extracted with DCM. The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude was purified with column chromatography on silica gel with PE/EA (v/v = 1/1) gave product **31** as a colorless oil (6.147 g, 81%).

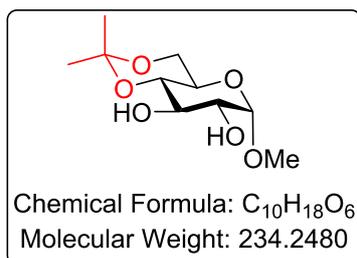
R_f = 0.4 (Petroleum ether/EtOAc = 50/50); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 7.38 – 7.25 (m, 5H), 4.52 (s, 2H), 3.88 (tt, *J* = 6.0, 4.0 Hz, 1H), 3.66 (dd, *J* = 11.5, 3.7 Hz, 1H), 3.58 (dd, *J* = 11.5, 6.0 Hz, 1H), 3.55 – 3.46 (m, 2H).

¹³C NMR (91 MHz, CDCl₃) δ 137.7, 129.7, 128.5, 127.9, 73.6, 71.7, 70.8, 64.0.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₁₀H₁₄NaO₃⁺ 205.0835, found 205.0831.

Compound 32: Methyl 4,6-O-isopropylidene- α -D-glucofuranoside



To a solution of α -D-methylglucoside (260 mg, 1.3 mmol, 1eq) in dry DMF (0.6 mL, C = 2 M) was added 2,2-dimethoxypropane (753 mg, 0.89 mL, 5.6 eq) and *p*-TsOH.H₂O (12.7 mg, 0.07 mmol, 0.05 eq). The mixture was stirred at room temperature for overnight and neutralized with Et₃N, then the solution was concentrated under reduced pressure and the mixture was purified by column chromatography (DCM/MeOH = 9/1), afforded product **32** as a white solid (190 mg, 62%)

R_f = 0.5 (DCM/MeOH = 90/10); **Relevator**: Orcinol

¹H NMR (360 MHz, CDCl₃) δ 4.70 (d, *J* = 3.8 Hz, 1H), 3.82 (dd, *J* = 10.6, 5.2 Hz, 1H), 3.76 (d, *J* = 9.7 Hz, 1H), 3.73 – 3.66 (m, 1H), 3.56 (ddd, *J* = 15.2, 9.8, 5.1 Hz, 2H), 3.51 – 3.43 (m, 1H), 3.37 (s, 3H), 3.24 (d, *J* = 8.5 Hz, 1H), 1.47 (s, 3H), 1.39 (s, 3H).

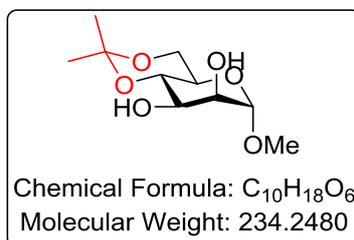
¹³C NMR (91 MHz, CDCl₃) δ 99.9, 99.7, 77.5, 77.1, 76.8, 73.6, 72.9, 71.6, 63.2, 62.33, 55.3, 29.0, 19.1.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₁₀H₁₈NaO₆ 257.0996, found

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257.0990.

Compound 33: Methyl 4,6-*O*-isopropylidene- α -D-mannopyranoside



A solution of α -D-methylmannoside (540 mg, 2.78 mmol, 1 eq) in anhydrous DMF (2 mL, C = 1.5 M) was added 2-methoxypropene (410 mg, 5.56 mmol, 0.52 mL, 2 eq) and *p*-TsOH·H₂O (2 mg, 0.01 mmol, 0.4% eq). The mixture was stirred at room temperature for overnight and quenched with Et₃N, then the solution was concentrated under reduced pressure and purified by column chromatography (PE/EA = 1/2) gave product **33** as a white solid (505 mg, 78%).

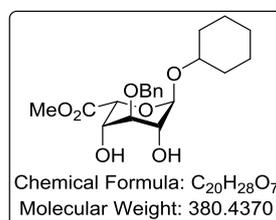
R_f = 0.3 (Petroleum ether/EtOAc = 30/60); **Relevator**: Orcinol

¹H NMR (360 MHz, CDCl₃) δ 4.74 (d, 1H, *J* = 1.2 Hz), 4.02 (dd, 1H, *J* = 3.2, 1.2 Hz), 3.97 – 3.89 (m, 2H), 3.89 – 3.79 (m, 2H), 3.66 – 3.58 (m, 1H), 3.37 (s, 3H), 1.52 (s, 3H), 1.43 (s, 3H).

¹³C NMR (75 MHz, CDCl₃) δ 101.3, 100.1, 71.4, 70.9, 69.1, 63.8, 62.2, 55.0, 29.2, 19.3.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₁₀H₁₈NaO₆ 257.0996, found 257.0991.

Compound 35: methyl (2R,3S,4S,5R,6S)-4-(benzyloxy)-6-(cyclohexyloxy)-3,5-dihydroxytetrahydro-2H-pyran-2-carboxylate



A solution of **34** (304 mg, 0.654 mmol, 1 eq) in anhydrous methanol (26 mL, C = 0.03 M) was added K₂CO₃ (36.2 mg, 0.26 mmol, 0.4 eq). The mixture was stirred at room temperature and monitored by TLC until the starting material was consumed. The reaction was quenched the with resin (H⁺) until pH = 7, the solution was filtered and

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concentrated under reduced pressure. The crude was then purified by a flash column chromatography, afforded product **36** as a white solid (246 mg, 99%).

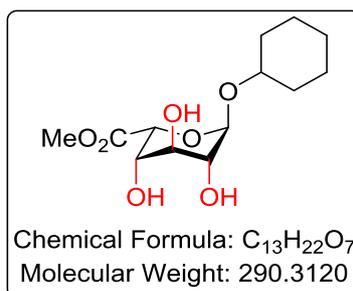
R_f = 0.3 (Petroleum ether/EtOAc = 60/30); **Relevator**: Orcinol

¹H NMR (300 MHz, CDCl₃) δ 7.41 – 7.27 (m, 5H), 5.11 (s, 1H), 4.83 (d, *J* = 1.8 Hz, 1H), 4.76 (d, *J* = 11.5 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.11 (s, 1H), 3.89 – 3.76 (m, 5H), 3.69 (ddd, *J* = 12.6, 8.8, 3.9 Hz, 2H), 1.88 (d, *J* = 11.0 Hz, 2H), 1.72 (d, *J* = 6.2 Hz, 2H), 1.54 – 1.39 (m, 2H), 1.34 – 1.19 (m, 4H).

¹³C NMR (75 MHz, CDCl₃) δ 171.2, 138.1, 128.4, 127.7, 127.5, 99.3, 75.9, 75.7, 71.8, 68.9, 68.2, 67.3, 52.5, 33.4, 31.4, 25.6, 24.0, 23.8.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₂₀H₂₈NaO₇ 403.1727, found 403.1722.

Compound 36: methyl (2R,3S,4S,5R,6S)-6-(cyclohexyloxy)-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-carboxylate



A solution of compound **34** (200 mg, 0.526 mmol, 1 eq) in ^tBuOH/H₂O (v/v = 1/2, 36 mL, C = 0.01 M) was added Pd/C (10%). The solution was bubbled with a H₂ balloon and stirred at room temperature, then was filtered and concentrated under reduced pressure, afforded product **35** as a white solid (153 mg, quantitative).

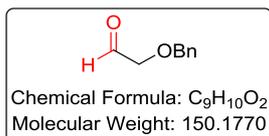
¹H NMR (250 MHz, CDCl₃) δ 5.07 (d, *J* = 4.9 Hz, 1H), 3.95 (dd, *J* = 6.3, 4.4 Hz, 1H), 3.80 – 3.72 (m, 4H), 3.68 (d, *J* = 10.3 Hz, 1H), 3.44 (dd, *J* = 7.2, 5.0 Hz, 1H), 1.98 – 1.80 (m, 2H), 1.75 – 1.61 (m, 2H), 1.50 (dd, *J* = 10.7, 3.5 Hz, 1H), 1.35 – 1.06 (m, 6H).

¹³C NMR (75 MHz, D₂O) δ 171.6, 99.1, 78.2, 71.1, 70.8, 70.7, 70.1, 52.6, 32.9, 31.3, 24.9, 23.9, 23.7.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₁₃H₂₂NaO₇ 313.1258, found 313.1267.

Compound 37: 2-(benzyloxy)acetaldehyde

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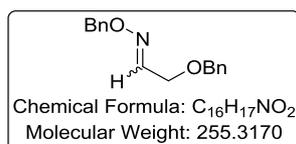
To a solution of compound **31** (354 mg, 1.94 mmol, 1 eq) in water (10 mL, C = 0.2 M) was added NaIO₄ (440 mg, 2.06 mmol, 1.06 eq). The solution was stirred at room temperature for 10 mins and the reaction was quenched with glycerol. Then the solution was diluted with water and extracted with CH₂Cl₂ and The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give product **37** (291 mg, quantitative, colorless oil).

R_f = 0.78 (Petroleum: EA = 30: 60); Relevator: KMnO₄.

¹H NMR (360 MHz, CDCl₃) δ 9.71 (s, 1H), 7.42 – 7.35 (m, 5H), 4.63 (s, 2H), 4.10 (s, 2H).

¹³C NMR (91 MHz, CDCl₃) δ 200.5, 136.8, 128.6, 128.3, 128.1, 75.3, 73.7.

Compound 38: 2-(benzyloxy)acetaldehyde O-benzyl oxime



To a solution of compound **37** (50 mg, 0.33 mmol, 1 eq) in *tert*-butanol/water (v/v = 1:1, 3.4 μL, C = 0.1 M) was added NaHCO₃ (34 mg, 0.4 mmol, 1.2 eq) and BnONH₂.HCl (64 mg, 0.4 mmol, 1.2 eq) sequentially. The solution was stirred at room temperature for 40 mins and the solution was diluted with water and extracted with CH₂Cl₂. The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give product **38** as a colorless oil (85 mg, E/Z = 1.2/1, quantitative).

R_f = 0.83 (Petroleum/EtOAc = 60/30); Relevator: KMnO₄, UV

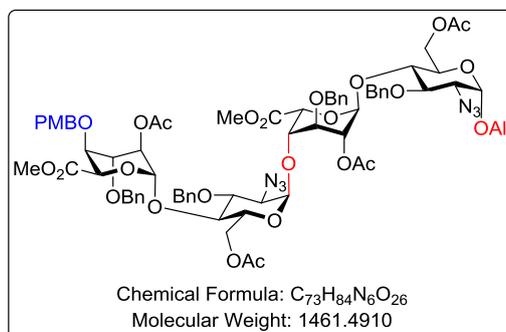
¹H NMR (300 MHz, CDCl₃) δ 7.56 (t, *J* = 5.8 Hz, 1H), 7.40 – 7.33 (m, 20H), 6.95 (t, *J* = 3.6 Hz, 1H), 5.12 (d, *J* = 3.5 Hz, 4H), 4.53 (d, *J* = 6.5 Hz, 4H), 4.38 (d, *J* = 3.6 Hz, 2H), 4.13 (d, *J* = 5.7 Hz, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 150.8, 147.7, 137.7, 137.6, 137.5, 137.5, 129.9, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 76.4, 76.1, 73.3, 72.6, 66.8, 64.8.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₁₆H₁₇NaO₂ 278.1151, found 278.1151; . [M + H]⁺ Calcd for C₁₆H₁₈O₂ 256.1332, found 256.1332.

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Compound 44: Allyl [(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4-methoxybenzyl)- α -l-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -d-glucopyranoside)-(1 \rightarrow 4)-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl- α -l-idopyranosyluronate)]-(1 \rightarrow 4)-*O*-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -d-glucopyranoside



Acceptor **43** (460 mg, 0.66 mmol) and imidate **42** (785 mg, 0.85 mmol, 1.3 equiv) were azeotropically dried with toluene and dissolved in CH₂Cl₂ (2 mL). Powdered 4Å molecular sieves (1.86 g) were added and the mixture was then stirred for 30 min at room temperature. The solution was cooled to -40°C and TBDMSOTf (0.1m in CH₂Cl₂, 1.3 mL, 0.13 mmol, 0.2 equiv) was added. The reaction was stirred at this temperature for 30 min and then the temperature was raised to 0°C over 30 min. The reaction was then quenched with a solution of NEt₃ (0.1m in CH₂Cl₂, 400 mL, 0.4 mmol, 0.6 equiv), directly applied to the top of a flash chromatography column filled with silica gel and eluted (CH₂Cl₂/EtOAc/petroleum ether (85:10:5 to 70:25:5)) to give tetrasaccharide **44** (860 mg, 90%).

$[\alpha]_D^{30} = +1.7$ (C = 1.0, CHCl₃)

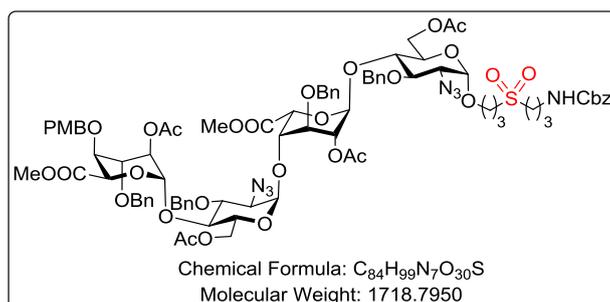
¹H NMR (400 MHz, CDCl₃) δ 7.37±7.24 (m, 20H), 7.13 (d, *J* = 8.5 Hz, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 5.94 (dddd, *J* = 17.0, 10.5, 6.0, 5.0 Hz, 1H), 5.36 (dq, *J* = 17.0, 1.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 5.28 (d, *J* = 5.0 Hz, 1H), 5.26 (dq, *J* = 10.5, 1.5 Hz, 1.5 Hz, 1.5 Hz, 1H), 5.24 (d, *J* = 3.5 Hz, 1H), 4.94 (d, *J* = 3.5 Hz, 1H), 4.93 (dd, *J* = 4.0, *J* = 3.5 Hz, 1H), 4.91 (d, *J* = 3.5 Hz, 1H), 4.90 (d, *J* = 10.5 Hz, 1H), 4.86 (dd, *J* = 5.5, *J* = 5.0 Hz, 1H), 4.82 (d, *J* = 10.5 Hz, 1H), 4.73 (s, 2H), 4.69 (d, *J* = 10.5 Hz, 1H), 4.68 (d, *J* = 4.0 Hz, 1H), 4.66 (s, 2H), 4.63 (d, *J* = 10.5 Hz, 1H), 4.59 (d, *J* = 5.0 Hz, 1H), 4.46 (d, *J* = 11.5 Hz, 1H), 4.42 (d, *J* = 11.5 Hz, 1H), 4.40 (brd, *J* = 12.0 Hz, 1H), 4.34 (dd, *J* = 12.5, 2.0 Hz, 1H), 4.24 (dd, *J* = 12.0, 3.0 Hz, 1H), 4.20 (ddt, *J* = 13.0, 5.0, 1.5 Hz, 1.5 Hz, 1H), 4.16 (dd, *J* = 12.5, 3.0 Hz, 1H), 4.05 (ddt, *J* = 13.0, 6.0, 1.5 Hz, 1.5 Hz, 1H), 3.98 (dd, *J* = 5.0, 4.0 Hz, 1H), 3.95 (dd, *J* = 10.0, 9.0 Hz, 1H), 3.92 (dd, *J* = 5.0, 4.0 Hz, 1H), 3.90±3.85

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(m, 3H), 3.82±3.78 (m with s at δ =3.80, 5H), 3.76 (t, J = 5.5 Hz, 5.5 Hz, 1H), 3.65 (dd, J =10.0, 9.0 Hz, 1H), 3.56 (s, 3H), 3.49 (s, 3H), 3.39 (dd, J = 10.0, 3.5 Hz, 1H), 3.29 (dd, J =10.0, 3.5 Hz, 1H), 2.12 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H).

^{13}C NMR (100.6 MHz, CDCl_3) δ 170.6, 170.5, 169.9, 169.8, 169.7, 169.3, 159.4, 137.8, 137.7, 137.6, 137.3, 133.0, 129.6, 129.2, 128.4, 128.3, 128.0, 127.8, 127.6, 127.5, 127.4, 118.3, 113.7, 97.9, 97.8, 97.4, 96.5, 78.3, 77.8, 75.7, 75.5, 75.4, 75.1, 74.9, 74.7, 73.7, 73.5, 73.3, 72.8, 72.5, 71.1, 70.9, 69.6, 69.3, 69.1, 68.8, 68.7, 63.2, 62.8, 62.1, 61.6, 55.2, 51.9, 51.7, 20.8, 20.7, 20.6.

Compound 45: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(4-methoxybenzyl)- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranoside



The starting compound **44** (210 mg, 0.125 mmol, 1 equiv.) was treated as described for the preparation of **7**. Flash chromatography (Petroleum Ether/AcOEt, 40/60) gave product **44** (180 mg, 84 %, white foam).

R_f = 0.3 (EtOAc/Petroleum Ether = 6/4); **Revelator**: orcinol

$[\alpha]_{589}^{20} = +15.5$ ($C = 1.0$, CHCl_3).

^1H NMR (300 MHz, CDCl_3) δ 7.32-7.17 (m, 25H), 7.05 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 5.21 (d, J = 5.5 Hz, 1H), 5.20 (d, J = 3.5 Hz, 1H), 5.13 (t, J = 6.5 Hz, 1H), 5.00 (s, 2H), 4.85 (dd, J = 4.5 Hz, 3.5 Hz, 1H), 4.84 (d, J = 3.5 Hz, 1H), 4.85-4.78 (br, 2H), 4.81 (d, J = 3.5 Hz, 1H), 4.79 (dd, J = 5.5 Hz, 5.5 Hz, 1H), 4.66 (s, 2H), 4.62 (d, J = 10.5 Hz, 1H), 4.59 (s, 2H), 4.58 (d, J = 4.0 Hz, 1H), 4.55 (d, J = 10.5 Hz, 1H), 4.52 (d, J = 4.5 Hz, 1H), 4.39-4.34 (br, 2H), 4.34 (dd, J = 12.5 Hz, 3.0 Hz, 1H), 4.27 (dd, J = 12.5 Hz, 2.0 Hz, 1H), 4.15 (dd, J = 12.5 Hz, 3.0 Hz, 1H), 4.08 (dd, J = 12.5 Hz, 3.0 Hz, 1H), 3.91 (dd, J = 5.0 Hz, 4.0 Hz, 1H), 3.88 (dd, J = 9.0 Hz, 10.0 Hz, 1H), 3.84 (dd, J = 5.0 Hz, 4.5 Hz, 1H), 3.82-3.74 (m, 4H), 3.74-3.69 (m with s at δ =

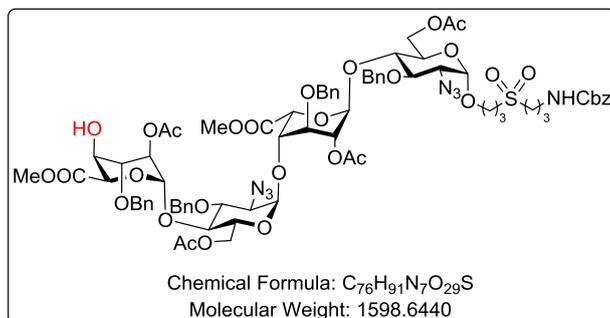
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3.71, 5H), 3.68 (dd, 1H, $J = 5.5$ Hz, 6.0 Hz, 1H), 3.56 (dd, $J = 10.5$ Hz, 9.0 Hz, 1H), 3.51-3.47 (br, 1H), 3.48 (s, 3H), 3.44 (s, 3H), 3.29-3.25 (br, 2H), 3.28 (dd, $J = 10.0$ Hz, 3.5 Hz, 1H), 3.22 (dd, $J = 10.5$ Hz, 3.5 Hz, 1H), 3.10-3.00 (br, 2H), 2.99-2.89 (br, 2H), 2.14-2.05 (br, 2H), 2.04 (s, 3H), 2.01-1.93 (br, 2H), 1.97 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ 170.5, 169.7, 169.8, 169.6, 169.3, 159.4, 156.5, 137.7, 137.6, 137.2, 136.2, 129.5, 129.2, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 113.6, 97.9, 97.7, 97.6, 97.4, 78.0, 77.7, 75.7, 75.4, 75.1, 74.8, 74.7, 74.1, 73.5, 73.4, 72.9, 72.5, 71.1, 70.9, 69.7, 69.6, 69.3, 69.2, 66.7, 65.9, 63.0, 62.7, 61.9, 61.5, 55.2, 51.9, 51.7, 50.3, 49.5, 39.3, 22.6, 21.9, 20.7, 20.6, 20.5.

HRMS (ESI, positive mode): m/z : $[\text{M}+\text{Na}]^+$ Calcd. for $\text{C}_{84}\text{H}_{99}\text{N}_7\text{NaO}_{30}\text{S}^+$ 1740.6044, found 1740.6049; $[\text{M}+2\text{Na}]^{2+}$ Calcd. for $\text{C}_{84}\text{H}_{99}\text{N}_7\text{Na}_2\text{O}_{30}\text{S}^{2+}$ 881.7981, found 881.7971.

Compound 46: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 2-*O*-acetyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranoside



To a stirred solution of compound **45** (170 mg, 0.1 mmol, 1 equiv.) in dry CH_2Cl_2 (2 mL) under Ar, was added CF_3COOH (82 μL , 1.1 mmol, 11 equiv.). After stirring at room temperature for 1 h, the suspension was quenched by Et_3N at 0 $^\circ\text{C}$ and then evaporated. The residue was purified by Flash chromatography (Petroleum Ether/ EtOAc , 45/55 to 30/70 gradient) gave product **46** (147 mg, 93%, white foam).

$R_f = 0.3$ (EtOAc /Petroleum Ether = 7/3); **Revelator**: orcinol

$[\alpha]_{589}^{20} = +22.5$ ($C = 1.0$, CHCl_3).

^1H NMR (360 MHz, CDCl_3) δ 7.33-7.16 (m, 25H), 5.17 (d, $J = 3.5$ Hz, 1H), 5.08-5.02

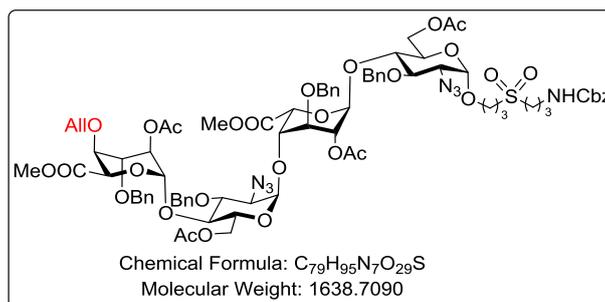
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(br, 1H), 5.01 (s, 2H), 5.00 (d, 1H, $J = 1.5$ Hz, 1H), 4.86 (d, 1H, $J = 3.5$ Hz, 1H), 4.85 (dd, $J = 4.5$ Hz, 3.5 Hz, 1H), 4.82-4.79 (br, 1H), 4.81 (dd, $J = 3.5$ Hz, 1.5 Hz, 1H), 4.80 (d, $J = 3.5$ Hz, 1H), 4.76 (d, $J = 2.5$ Hz, 1H), 4.71 (d, $J = 11.0$ Hz, 1H), 4.69 (d, $J = 11.5$ Hz, 1H), 4.66 (d, $J = 4.0$ Hz, 1H), 4.64 (d, $J = 12.0$ Hz, 2H), 4.63 (d, $J = 11.0$ Hz, 1H), 4.56 (d, $J = 11.5$ Hz, 1H), 4.55 (d, $J = 11.0$ Hz, 1H), 4.36-4.29 (br, 2H), 4.17 (dd, $J = 12.5$ Hz, 3.5 Hz, 1H), 4.11 (dd, 1H, $J = 12.5$ Hz, 3.0 Hz, 1H), 3.93 (dd, $J = 5.0$ Hz, 4.0 Hz, 1H), 3.91-3.86 (br, 1H), 3.84 (dd, $J = 4.5$ Hz, 5.0 Hz, 1H), 3.82-3.70 (m, 6H), 3.64 (dd, $J = 4.0$ Hz, 3.5 Hz, 1H), 3.59 (dd, $J = 10.0$ Hz, 8.5 Hz, 1H), 3.53-3.47 (br d, 1H), 3.42 (s, 3H), 3.39 (s, 3H), 3.32-3.27 (br, 2H), 3.28 (dd, $J = 10.0$ Hz, 3.5 Hz, 1H), 3.21 (dd, $J = 10.0$ Hz, 3.5 Hz, 1H), 3.11-3.01 (br, 2H), 3.00-2.90 (br, 2H), 2.56 (d, $J = 10.5$ Hz, 1H), 2.14-2.07 (br, 2H), 2.04-1.95 (br, 2H), 2.03 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H).

^{13}C NMR (90 MHz, CDCl_3) δ 170.6, 170.4, 169.9, 169.1, 169.4, 169.2, 156.7, 137.7, 137.5, 137.2, 137.1, 136.3, 128.6, 128.5, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 98.1, 97.7, 97.3, 78.1, 78.1, 75.6, 74.9, 74.8, 74.7, 74.3, 73.6, 73.3, 72.7, 72.6, 69.8, 69.4, 69.3, 69.0, 68.9, 67.9, 67.7, 66.8, 66.0, 63.2, 62.1, 61.7, 52.0, 51.8, 50.4, 49.7, 39.3, 22.0, 20.8, 20.7.

HRMS (ESI, positive mode): m/z : $[\text{M}+\text{Na}]^+$ Calcd. for $\text{C}_{76}\text{H}_{91}\text{N}_7\text{NaO}_{29}\text{S}^+$ 1620.5474, found 1620.5463; m/z : $[\text{M}+2\text{Na}]^{2+}$ Calcd. for $\text{C}_{76}\text{H}_{91}\text{N}_7\text{Na}_2\text{O}_{29}\text{S}^{2+}$ 821.7683, found 821.7692.

Compound 47: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 2-*O*-acetyl-4-*O*-allyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy- α -D-glucopyranoside



To a stirred solution of compound **46** (105 mg, 6.6×10^{-2} mmol, 1 equiv.) and Allyl

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tert-butylcarbonate (12.5 mg, 1.2 equiv.) in dry Toluene (330 μ L) under Ar, was added Pd(PPh₃)₄ (1.9 mg, 1.65×10^{-3} mmol, 2.5 (mol) %). After stirring at 60°C for 4 h, the mixture was filtered through celite and concentrated in *vacuo*. The residue was purified by flash chromatography (Petroleum Ether/EtOAc, 40/60 to 0/100 gradient) gave product **47** (70 mg, 65 %, colorless liquid), the recovery starting compound **46** (30 mg, conversion rate 71 %). The diallyl product (2 mg, 2 %, white solid) was obtained and confirmed by mass spectra: **HRMS** (ESI, positive mode): *m/z*: [M+Na]⁺ Calcd. for C₈₂H₉₉N₇NaO₂₉S⁺ 1700.6150, found 1700.6100.

R_f = 0.2 (EtOAc/Petroleum Ether = 6/4); **Revelator**: orcinol

[α]_D²⁰₅₈₉ = + 14.5 (C = 1.0, CHCl₃)

¹H NMR (300 MHz, CDCl₃) δ 7.30-7.18 (m, 25H), 5.71 (dddd, *J* = 17.5 Hz, 10.5 Hz, 5.5 Hz, 5.5 Hz, 1H), 5.21 (brd, 2H), 5.12 (dddd, *J* = 17.5 Hz, 1.5 Hz, 1.5 Hz, 1.0 Hz, 1H), 5.08 (dddd, *J* = 10.5 Hz, 1.5 Hz, 1.0 Hz, 1.0 Hz, 1H), 5.06-5.02 (br, 1H), 5.01 (s, 2H), 4.87 (d, *J* = 3.5 Hz, 1H), 4.85 (d, *J* = 3.5 Hz, 1H), 4.84 (d, *J* = 10.0 Hz, 2H), 4.82 (d, *J* = 5.0 Hz, 1H), 4.80 (d, *J* = 4.0 Hz, 1H), 4.67 (s, 2H), 4.63 (s, 2H), 4.63 (d, *J* = 10.5 Hz, 1H), 4.58 (d, *J* = 4.5 Hz, 1H), 4.57 (d, *J* = 10.5 Hz, 1H), 4.55 (d, *J* = 5.0 Hz, 1H), 4.34 (dd, *J* = 12.5 Hz, 1.5 Hz, 1H), 4.28 (dd, *J* = 12.5 Hz, 1.5 Hz, 1H), 4.16 (dd, *J* = 12.5 Hz, 3.5 Hz, 1H), 4.10 (dd, *J* = 12.5 Hz, 3.5 Hz, 1H), 3.94-3.86 (m, 4H), 3.85 (dd, *J* = 4.5 Hz, 3.5 Hz, 1H), 3.84-3.81 (m, 1H), 3.80-3.67 (m, 6H), 3.58 (dd, *J* = 10.0 Hz, 9.0 Hz, 1H), 3.53 (s, 3H), 3.52-3.48 (br, 1H), 3.46 (s, 3H), 3.34-3.26 (br, 2H), 3.28 (dd, *J* = 10.0 Hz, 4.0 Hz, 1H), 3.23 (dd, *J* = 10.0 Hz, 3.5 Hz, 1H), 3.11-3.02 (br, 2H), 3.01-2.92 (br, 2H), 2.15-2.08 (br, 2H), 2.05 (s, 3H), 2.04-1.97 (br, 2H), 2.01 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H).

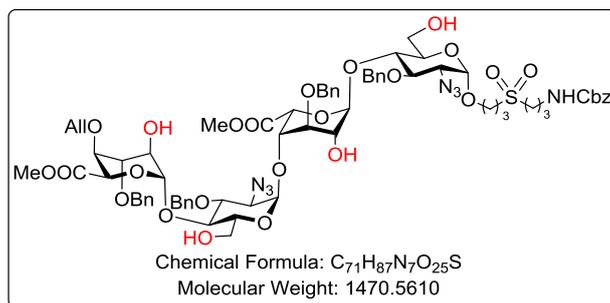
¹³C NMR (75 MHz, CDCl₃) δ 170.6, 169.9, 169.7, 169.9, 169.4, 156.5, 137.8, 137.7, 137.3, 136.3, 134.0, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.8, 127.7, 127.6, 117.6, 98.0, 97.5, 97.8, 97.7, 78.0, 77.8, 75.8, 75.5, 74.9, 74.8, 74.3, 73.6, 73.0, 71.8, 71.1, 70.9, 69.8, 69.6, 69.4, 69.3, 66.8, 66.0, 63.1, 62.8, 62.0, 61.6, 52.0, 51.8, 50.3, 49.6, 39.3, 22.6, 22.0, 20.8, 20.7.

HRMS (ESI, positive mode): *m/z*: [M+Na]⁺ Calcd. for C₇₉H₉₅N₇NaO₂₉S⁺ 1660.5755, found 1660.5787; Calcd. for C₇₉H₉₅N₇Na₂O₂₉S²⁺ 841.7848, found 841.7840.

Compound 48: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 4-*O*-allyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(2-azido-3-*O*-benzyl-2-

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deoxy- α -D-glucopyranosyl)-(1 \rightarrow 4)-O-(methyl 3-O-benzyl- α -L-idopyranosyl-uronate)-(1 \rightarrow 4)-O-2-azido-3-O-benzyl-2-deoxy- α -D-glucopyranoside



To a stirred solution of compound **47** (95 mg, 5.8×10^{-2} mmol, 1 equiv.) in MeOH (5.8 mL), was added K₂CO₃ (6.4 mg, 0.8 equiv.). The mixture was stirred at room temperature for 2 h and then neutralized by Dowex H⁺ resin, filtered and concentrated in vacuo. Flash chromatography (CH₂Cl₂/MeOH, 98 / 2) gave product **48** (73 mg, 86 %, white foam).

R_f = 0.5 (MeOH/CH₂Cl₂ = 1/9); **Revelator**: orcinol

[α]₅₈₉²⁰ = + 16.5 (C = 1.0, CHCl₃).

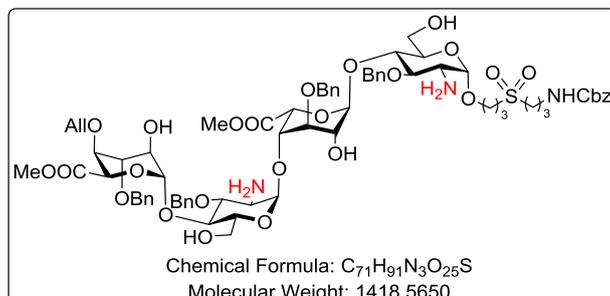
¹H NMR (300 MHz, CDCl₃) δ 7.33-7.11 (m, 25H), 5.66 (dddd, *J* = 17.5 Hz, 10.5 Hz, 6.0 Hz, 5.5 Hz, 1H), 5.20 (d, *J* = 1.5 Hz, 1H), 5.15 (d, *J* = 2.5 Hz, 1H), 5.09 (dddd, *J* = 17.5 Hz, 1.5 Hz, 1.5 Hz, 1.0 Hz, 1H), 5.06 (dddd, *J* = 10.5 Hz, 1.5 Hz, 1.0 Hz, 1.0 Hz, 1H), 5.05-5.02 (brd, 1H), 5.01 (s, 2H), 4.89 (d, *J* = 3.5 Hz, 1H), 4.84-4.80 (br, 2H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.67 (d, *J* = 11.5 Hz, 2H), 4.63 (d, *J* = 4.5 Hz, 1H), 4.60 (d, *J* = 12.5 Hz, 1H), 4.56 (d, *J* = 10.0 Hz, 1H), 4.54 (d, *J* = 11.0 Hz, 1H), 4.53 (d, *J* = 12.5 Hz, 1H), 4.49 (d, *J* = 12.5 Hz, 1H), 4.00-3.96 (m, 1H), 3.93-3.85 (m, 3H), 3.83-3.61 (m, 12H), 3.54-3.47 (m, 1H), 3.61-3.55 (m, 1H), 3.53 (dd, *J* = 10.0 Hz, 9.5 Hz, 1H), 3.44 (dd, *J* = 10.0 Hz, 3.5 Hz, 1H), 3.41-3.36 (m, 1H), 3.38 (s, 3H), 3.33-3.23 (m, 2H), 3.30 (s, 3H), 3.29 (dd, *J* = 10.0 Hz, 3.5 Hz, 1H), 3.13-3.00 (m, 2H), 3.01-2.90 (m, 2H), 2.49-2.24 (br, 2H), 2.17-2.04 (m, 2H), 2.04-1.92 (m, 2H).

¹³C NMR (75 MHz, CDCl₃) δ 169.6, 169.4, 156.6, 137.8, 137.7, 137.4, 137.2, 136.3, 133.5, 128.6, 128.5, 128.2, 128.1, 128.0, 127.3, 127.2, 127.1, 118.2, 100.9, 100.7, 97.9, 95.6, 79.1, 78.2, 75.1, 74.8, 74.4, 74.5, 74.3, 73.5, 72.6, 72.5, 72.2, 72.0, 71.9, 68.4, 67.9, 67.7, 67.4, 66.8, 66.0, 63.9, 63.4, 61.4, 61.0, 52.0, 51.8, 50.2, 49.8, 39.3, 22.5, 22.1.

HRMS (ESI, positive mode): *m/z*: [M+Na]⁺ Calcd. for C₇₁H₈₇N₇NaO₂₅S⁺ 1492.5387, found 1492.5365; *m/z*: [M+2Na]²⁺ Calcd. for C₇₁H₈₇N₇Na₂O₂₅S²⁺ 757.7607, found 757.7628.

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Compound 49: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 4-*O*-allyl-3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(2-amino-3-*O*-benzyl-2-deoxy- α -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-(methyl 3-*O*-benzyl- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-2-amino-3-*O*-benzyl-2-deoxy- α -D-glucopyranoside

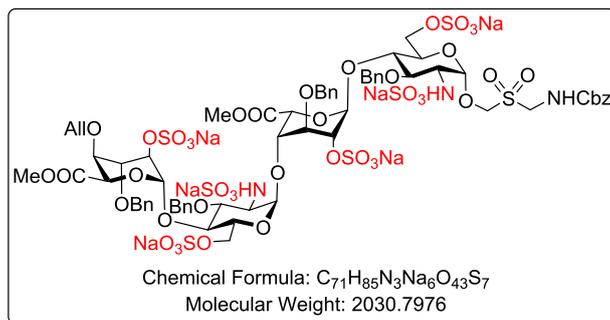


To a stirred solution of **48** (70 mg, 47.6 μ M, 1 equiv.) and dry triethylamine (106 μ L, 16 equiv.) in MeOH (476 μ L), was added propane-1, 3-dithiol (76.5 μ L, 16 equiv.). The mixture was stirred at room temperature for 2 days with protection from light. After filtering through Sephadex LH-20 chromatography (CH₂Cl₂/MeOH, 50/50 with 0.1 % Et₃N) to remove excess thiol and its side products, the fractions were concentrated. Flash chromatography on silica (CH₂Cl₂/MeOH, 90/10) gave product **49** (63 mg, 93 %, white solid).

HRMS (ESI, positive mode): m/z: [M+Na]⁺ Calcd. for C₇₁H₉₁N₃NaO₂₅S⁺ 1440.5655, found 1440.5555; m/z: [M+H]⁺ Calcd. for C₇₁H₉₂N₃O₂₅S⁺ 1418.5820, found 1418.5735; m/z: [M+Na+H]²⁺ Calcd. for C₇₁H₉₂N₃NaO₂₅S²⁺ 720.7818, found 720.7814; m/z: [M+2H]²⁺ Calcd. for C₇₁H₉₃N₃NaO₂₅S²⁺ 709.7903, found 709.7904.

Compound 50: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 4-*O*-allyl-3-*O*-benzyl-2-*O*-sulfonato- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfonamino-6-*O*-sulfonato- α -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-(methyl 3-*O*-benzyl-2-*O*-sulfonato- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-3-*O*-benzyl-2-deoxy-2-sulfonamino-6-*O*-sulfonato- α -D-glucopyranoside hexasodium salt

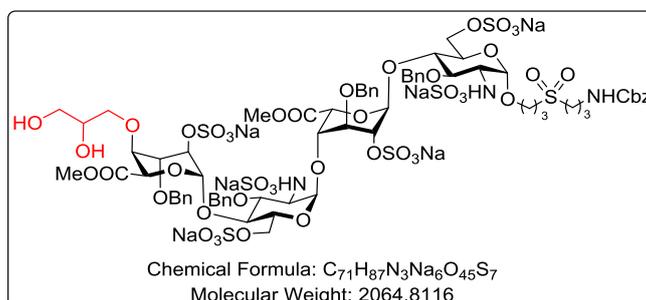
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To a stirred solution of **49** (60 mg, 42.3 μ M, 1 equiv.) in pyridine (4.2 mL), was added sulfur trioxide pyridine complex (202.1 mg, 30 equiv.). The mixture was stirred at room temperature for 24 h and then heated to 55°C for additional 24 h. The reaction was quenched by MeOH (93 μ L, 54 equiv.) and Et₃N (318 μ L, 54 equiv.) and kept stirring for another 1 h. After purification with Sephadex LH-20 (CH₂Cl₂ / MeOH, 50 / 50 with 0.1 % Et₃N), ion exchange with BioRad AG50W-X8 200 (Na⁺) resin and lyophilisation, the product **50** was obtained as white solid (86 %).

HRMS (ESI, positive mode): the sample analysed was in form of Et₃N salt: m/z: [M+2H]²⁺ Calcd. for C₁₀₇H₁₈₃N₉O₄₃S₇²⁺ 1253.0246, found 1253.0222; m/z: [M+Na+H]²⁺ Calcd. for C₁₀₇H₁₈₂N₉NaO₄₃S₇²⁺ 1264.0157, found 1264.0132.

Compound 51: 3-[(3-Benzyloxycarbonylamino)propylsulfonyl]propyl (methyl 3-O-benzyl-4-O-(2,3-dihydroxypropyl)-2-O-sulfonato- α -L-idopyranosyluronate)-(1 \rightarrow 4)-O-(3-O-benzyl-2-deoxy-2-sulfonamino-6-O-sulfonato- α -D-glucopyranosyl)-(1 \rightarrow 4)-O-(methyl 3-O-benzyl-2-O-sulfonato- α -L-idopyranosyluronate)-(1 \rightarrow 4)-O-3-O-benzyl-2-deoxy-2-sulfonamino-6-O-sulfonato- α -D-glucopyranoside hexasodium salt



To a stirred solution of **50** (53 mg, 26.1 μ M, 1 equiv.) in Acetone / H₂O (v/v = 1/1, 653 μ L), was added NMO·H₂O (14.1 mg, 4 equiv.) and OsO₄·*tert*-Butanol (33 μ L (2.5 wt %), 10 (mol) %) respectively. After stirring at room temperature overnight, NMO·H₂O (14.1 mg, 4 equiv.) was added again to the mixture. After stirring totally for 22 h, the

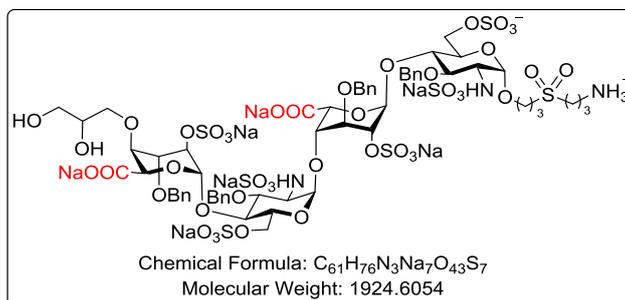
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reaction was quenched by Na₂S₂O₃ (2.6 mL, C = 0.1 M, 10 equiv.) and then concentrated in vacuo. RP-18 flash chromatography (H₂O/CH₃CN, 100/0 to 85/15 gradient) (the fractions were detected by HPLC) gave product **51** (43 mg, 80 %, white solid).

¹H NMR (360 MHz, D₂O) (partial) δ 7.47-7.26 (m, 25H), 5.39 (br, 1H), 5.22 (d, *J* = 3.5 Hz, 1H), 5.12 (br, 1H), 5.09 (d, *J* = 3.5 Hz, 1H), 4.97 (s, 2H), 4.92 (d, *J* = 1.0 Hz, 1H), 4.84 (d, *J* = 1.5 Hz, 1H), 4.56 (d, *J* = 2.0 Hz, 1H), 4.41 (br, 1H), 3.35 (s, 3H), 3.33 (s, 3H), 2.15-2.00 (m, 2H), 1.97-1.85 (m, 2H).

HRMS (ESI, negative mode): *m/z*: [M-2Na]²⁻ Calcd. for C₇₁H₈₇N₃Na₄O₄₅S₇²⁻ 1008.6145, found 1008.6129.

Compound 52: 3-(3-Amino propylsulfonyl)propyl (3-*O*-benzyl-4-*O*-(2,3-dihydroxypropyl)-2-*O*-sulfonato- α -L-idopyranosyluronate)-(1→4)-*O*-(3-*O*-benzyl-2-deoxy-2-sulfonamino-6-*O*-sulfonato- α -D-glucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfonato- α -L-idopyranosyluronate)-(1→4)-3-*O*-benzyl-2-deoxy-2-sulfonamino-6-*O*-sulfonato- α -D-glucopyranoside heptasodium salt



To a stirred solution of **51** (40 mg, 19.4 μ M, 1 equiv.) and KOH (0.74 mL, C = 6 M, 230 equiv.) in water (184 μ L) at 0 °C, was added very carefully H₂O₂ (35% in water, 1.15 mL, 690 equiv.). After warming slowly to 37 °C, the reaction was stirred for 65 h before quenching by AcOH (0.5 mL, C = 6 M) at 0 °C to pH 8. The mixture was purified by RP-18 flash chromatography (50 mM, AcOH-NEt₃/CH₃CH, 100/0 to 85/15 gradient) with detection by HPLC. Product **52** was obtained after ion exchange with BioRad AG50W-X8 200 (Na⁺) resin and lyophilisation (30 mg, 80 %, white solid).

¹H NMR (360 MHz, D₂O) δ 7.53-7.37 (m, 20H), 5.63 (br, 1H), 5.34 (br, 1H), 5.30 (d, *J* = 3.5 Hz, 1H), 5.18 (d, *J* = 3.5 Hz, 1H), 4.90 (d, *J* = 11.0 Hz, 1H), 4.87-4.64 (m, 7H), 4.77 (d, *J* = 4.5 Hz, 1H), 4.70 (d, *J* = 4.5 Hz, 1H), 4.64 (br, 1H), 4.53 (br, 1H), 4.41 (dd, *J* = 11.0 Hz, 1.5 Hz, 1H), 4.38-4.28 (m, 3H), 4.30 (dd, *J* = 4.0 Hz, 3.5 Hz, 1H), 4.25-4.13 (m, 4H), 4.00 (dd, *J* = 10.0 Hz, 9.5 Hz, 1H), 4.00-3.90 (m, 2H), 3.94 (dd, *J* = 10.0

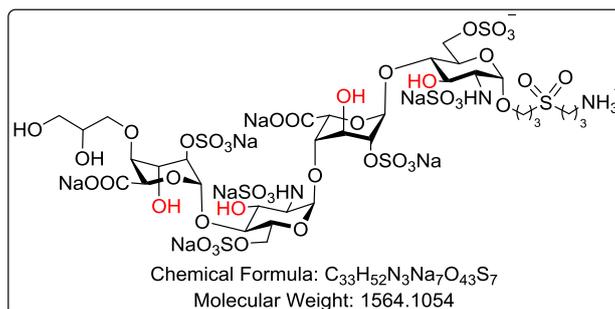
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Hz, 9.5 Hz, 1H), 3.84 (dd, $J = 10.0$ Hz, 9.5 Hz, 1H), 3.81 (br, 1H), 3.79-3.68 (m, 2H), 3.63-3.27 (m, 10H), 3.18 (td, $J = 7.5$ Hz, 1.5 Hz, 2H), 2.29-2.16 (m, 4H).

^{13}C NMR (90 MHz, D_2O) δ 175.5, 174.4, 137.7, 137.4, 137.3, 137.0, 129.1, 129.1, 129.0, 128.8, 128.7, 128.6, 128.3, 128.2, 128.0, 97.9, 97.3, 96.9, 96.4, 76.8, 76.5, 75.1, 74.9, 74.7, 74.6, 74.5, 74.2, 73.1, 72.7, 72.4, 71.9, 71.4, 70.8, 70.5, 70.3, 70.2, 69.2, 68.5, 68.4, 68.3, 67.2, 66.6, 66.3, 62.5, 62.4, 58.0, 57.5, 49.6, 49.3, 38.0, 21.1, 19.5.

HRMS (ESI, negative mode): m/z : $[\text{M-K-H}]^{2-}$ Calcd. for $\text{C}_{61}\text{H}_{75}\text{N}_3\text{K}_6\text{O}_{43}\text{S}_7^{2-}$ 997.4862, found 997.4826.

Compound 53: 3-(3-Amino propylsulfonyl)propyl (4-*O*-(2,3-dihydroxypropyl)-2-*O*-sulfonato- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-(2-deoxy-2-sulfonamino-6-*O*-sulfonato- α -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-(2-*O*-sulfonato- α -L-idopyranosyluronate)-(1 \rightarrow 4)-*O*-2-deoxy-2-sulfonamino-6-*O*-sulfonato- α -D-glucopyranoside heptasodium salt



To a stirred solution of **52** (35 mg, 18.0 μM , 1 equiv.) in phosphate buffer (2.7 mL, $C = 20$ mM, $\text{pH} = 6.0$) and *tert*-BuOH (1.3 mL), was added $\text{Pd}(\text{OH})_2$ (20 % on charcoal, 132 mg). After degassing, the reaction was stirred at room temperature for 24 h under H_2 . The mixture was filtered through celite and rinsed by H_2O . After lyophilisation, the residue was desalted on a Sephadex G-15 column and lyophilised to give product **53** (30 mg, quantitative, white solid).

^1H NMR (300 MHz, D_2O) δ 5.38 (d, $J = 3.5$ Hz, 1H), 5.26 (d, $J = 2.5$ Hz, 1H), 5.20 (br, 1H), 5.13 (d, $J = 3.5$ Hz, 1H), 4.87 (d, $J = 2.0$ Hz, 1H), 4.76 (d, $J = 2.5$ Hz, 1H), 4.36 (dd, $J = 11.5$ Hz, 2.0 Hz, 1H), 4.34-4.23 (m, 6H), 4.18 (dd, $J = 6.0$ Hz, 3.5 Hz, 1H), 4.07 (dd, $J = 6.0$ Hz, 2.5 Hz, 1H), 4.05-3.96 (m, 2H), 3.91-3.48 (m, 12H), 3.46-3.35 (m, 4H), 3.27 (dd, $J = 10.0$ Hz, 3.5 Hz, 1H), 3.25 (dd, $J = 10.0$ Hz, 3.5 Hz, 1H), 3.18 (t, $J = 7.5$ Hz, 2H), 2.27-2.08 (m, 4H).

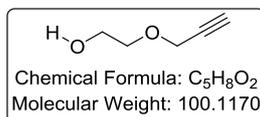
^{13}C NMR (75 MHz, D_2O) δ 176.2, 174.6, 98.9, 97.0, 96.5, 77.0, 76.5, 75.9, 75.7, 73.5, 71.0, 70.7, 70.6, 69.7, 69.5, 69.3, 69.0, 68.8, 68.7, 68.3, 67.0, 66.3, 66.0, 64.9, 62.5,

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62.4, 57.9, 57.7, 49.3, 49.2, 37.9, 21.1, 19.5.

HRMS (ESI, negative mode): m/z : $[M-2Na]^{2-}$ Calcd. for $C_{33}H_{52}N_3Na_5O_{43}S_7^{2-}$ 758.4757, found 758.4759.

Compound 54: 2-(Prop-2-ynoxy)ethanol



To a solution of ethylene glycol (4.37 g, 70.4 mmol, 4 eq) in anhydrous THF (20 mL) at 0°C under argon atmosphere was portionwise added sodium hydride (60% in oil dispersion, 704 mg, 17.6 mmol, 1 eq) and the resulting mixture was stirred at 0°C for 30 mins. Then, propargyl bormide (80% in toluene) (1.9 mL, 17.6 mmol, 1 eq) was injected dropwise to the reaction flask. The mixture was stirred at 0°C for 30 mins and at 45°C for 3 h. The mixture was poured into water, extracted with CH_2Cl_2 and dried over Na_2SO_4 . The crude product was purified by flash chromatography eluting with petroleum ether/ Et_2O (1 : 1) to afford product **54** as a light yellow oil (1.12 g, 63%).

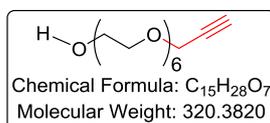
R_f = 0.3 (Petroleum ether / Et_2O = 50/50); **Relevator:** $KMnO_4$

1H NMR (360 MHz, $CDCl_3$) δ 4.19 (dd, J = 2.4, 0.7 Hz, 2H), 3.78 – 3.73 (m, 2H), 3.64 (m, 2H), 2.45 (t, J = 2.4 Hz, 1H).

^{13}C NMR (91 MHz, $CDCl_3$) δ 79.5, 74.7, 71.2, 61.7, 58.4.

HRMS (ESI, positive mode): m/z : $[M + Na]^+$ Calcd for $C_5H_8NaO_2^+$ 123.0417. Found 123.0416.

Compound 55: 3,6,9,12,15,18-hexaoxahenicos-20-yn-1-ol



A solution of hexaethylene glycol (1.2 g, 4.1 mmol, 2 eq) in anhydrous THF (6 mL, C = 0.6 M) was portionwise added $tBuOK$ (229 mg, 2.0 mmol, 1 eq) at 0°C in ice-bath under argon atmosphere. The reaction mixture was allowed to stir at room temperature for 30 minutes then moved back to 0°C and propargyl bromide (242 mg, 0.18 mL, 1 eq) in THF (1 mL) was added dropwise. The resulting mixture was allowed to stir at room temperature for overnight. After completion of the reaction as indicated by TLC, the mixture was diluted with THF and filtered through Celite®. The filtrate was

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concentrated and the residue was purified by column chromatography eluting with petroleum ether/Acetone (6 : 4) to give the product **55** as a yellow oil (757 mg, 58%).

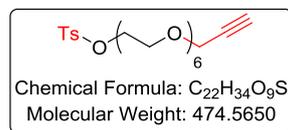
R_f = 0.2 (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 4.20 (d, *J* = 2.4 Hz, 2H), 3.72 (m, 2H), 3.70 – 3.67 (m, 4H), 3.65 (m, 16H), 3.60 (m, 2H), 2.43 (t, *J* = 2.4 Hz, 1H).

¹³C NMR (91 MHz, CDCl₃) δ 79.7, 74.5, 72.5, 70.6, 70.5, 70.4, 70.3, 69.1, 61.7, 58.4.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₁₅H₂₈NaO₇⁺ 343.1727. Found 343.1710.

Compound 56: 3,6,9,12,15,18-hexaoxahenicos-20-yn-1-yl 4-methylbenzenesulfonate



To a solution of compound **55** (200 mg, 0.62 mmol, 1 eq) in anhydrous CH₂Cl₂ (1.5 mL, C = 0.4 M) was dropwise added triethylamine (0.22 mL, 1.56 mmol, 2.5 eq) at 0°C in ice-bath and the reaction mixture was stirred at 0°C for additional 30 minutes. Then *p*-toluenesulfonyl chloride (238 mg, 1.25 mmol, 2 eq) was added slowly at 0°C under argon atmosphere. The resulting mixture was stirred at 0°C for 30 minutes and at 30°C for 1 day under argon. The reaction mixture was poured into water, extracted with EtOAc and dried over Na₂SO₄. The crude product was purified by flash chromatography eluting with petroleum ether/acetone (1 : 1) to afford product **56** as a colorless oil (220 mg, 0.46 mmol) in 74% yield.

R_f = 0.4 (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO₄

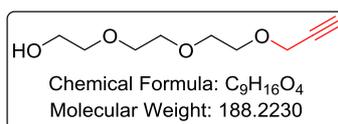
¹H NMR (360 MHz, CDCl₃) δ 7.79 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 4.18 (dd, *J* = 11.6, 2.0 Hz, 2H), 4.15 (td, *J* = 5.0, 1.6 Hz, 2H), 3.77 – 3.51 (m, 22H), 2.46 – 2.39 (m, 4H).

¹³C NMR (91 MHz, CDCl₃) δ 144.8, 129.8, 128.0, 79.7, 74.5, 70.8, 70.6, 70.6, 70.5, 70.4, 69.2, 69.1, 68.7, 58.4, 21.6.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₂₂H₃₄NaO₉S⁺ 497.1816. Found 497.1796.

Compound 57: 2-(2-(2-(Prop-2-ynyloxy)ethoxy)ethoxy)ethanol

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Into a suspension of ^tBuOK (1.98 g, 17.6 mmol, 1 eq) in anhydrous THF (60 mL, C = 0.3 M) was added triethylene glycol (5.3 g, 35.3 mmol, 2 eq) at 0°C under argon atmosphere. The reaction mixture was allowed to stir at room temperature for 30 minutes then moved back to 0°C and propargyl bromide (2.09 g, 1.5 mL, 17.6 mmol, 1eq) was added dropwise. The resulting mixture was allowed to stir at room temperature for overnight. After completion of the reaction as indicated by TLC, the mixture was diluted with THF and filtered through Celite®. The filtrate was concentrated and the residue was purified by column chromatography eluting with petroleum ether/EtOAc (2 : 1) to give the product **57** as a faint yellow oil (2.38 g, 72%).

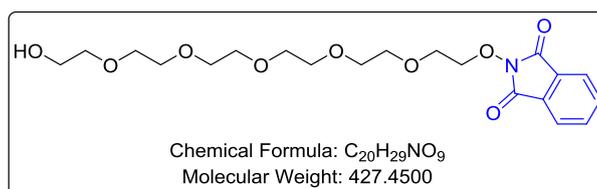
R_f = 0.3 (EtOAc); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 4.20 (d, *J* = 2.4 Hz, 2H), 3.74 – 3.71 (m, 1H), 3.71 – 3.65 (m, 8H), 3.63 – 3.59 (m, 1H), 2.43 (t, *J* = 2.4 Hz, 1H).

¹³C NMR (91 MHz, CDCl₃) δ 79.7, 74.7, 72.6, 70.8, 70.5, 70.5, 69.2, 61.9, 58.6.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₉H₁₆NaO₄⁺ 211.0941. Found 211.0941.

Compound **58**: 2-((17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl)oxy)isoindoline-1,3-dione



To a solution of hexaethylene glycol (1.5 g, 5.3 mmol, 1 eq) in anhydrous tetrahydrofuran (27 mL, C = 0.2 M) was added successively triphenylphosphine (1.67 g, 6.4 mmol, 1.2 eq) and *N*-hydroxyphthalimide (871 mg, 5.3 mmol, 1 eq) under Argon atmosphere at room temperature, then Diisopropyl azodicarboxylate (0.42 mL, 428 mg, 2.11 mmol, 1.2 eq) was dropwise added at 0°C in ice-bath, and the mixture was stirred overnight at room temperature. The crude reaction mixture was concentrated under reduced pressure and purified by flash chromatography to yield product **58** as a colorless oil (880 mg, 2.1 mmol) in 39% yield.

R_f = 0.1 (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO₄

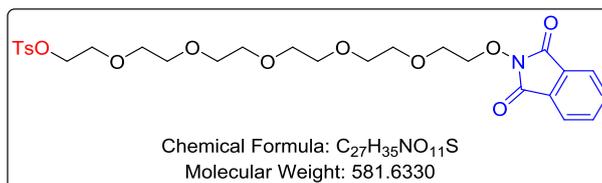
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¹H NMR (360 MHz, CDCl₃) δ 7.85 – 7.79 (m, 2H), 7.76 – 7.71 (m, 2H), 4.39 – 4.33 (m, 2H), 3.88 – 3.82 (m, 2H), 3.73 – 3.68 (m, 2H), 3.67 – 3.55 (m, 18H).

¹³C NMR (91 MHz, CDCl₃) δ 163.4, 134.4, 129.0, 123.5, 77.2, 72.5, 70.8, 70.6, 70.5, 70.3, 69.3, 61.7.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₂₀H₂₉NNaO₉⁺ 450.1735. Found 450.1717.

Compound 59: 17-((1,3-dioxoisindolin-2-yl)oxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate



To a solution of compound **58** (120 mg, 0.28 mmol, 1 eq) in anhydrous CH₂Cl₂ (1.4 mL, C = 0.2 M) was dropwise added triethylamine (0.1 mL, 0.70 mmol, 2.5 eq) at 0°C in ice-bath and the reaction mixture was stirred at 0°C for additional 30 mins. Then *p*-toluenesulfonyl chloride (107 mg, 0.56 mmol, 2 eq) was portionwise added at 0°C under argon. The mixture was stirred at room temperature for overnight under argon. The reaction mixture was poured into water, extracted with EtOAc and dried over Na₂SO₄. After removal of the solvent in vacuo, the crude product was purified by flash chromatography eluting with petroleum ether/EtOAc (3 : 2) to afford product **59** as a colorless oil (75 mg, 0.13 mmol) in 46% yield.

R_f = 0.3 (Petroleum ether/Acetone = 60/40); **Relevator:** KMnO₄

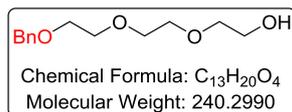
¹H NMR (360 MHz, CDCl₃) δ 7.82 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.74 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 4.39 – 4.34 (m, 2H), 4.18 – 4.12 (m, 2H), 3.88 – 3.83 (m, 2H), 3.69 – 3.63 (m, 4H), 3.58 (m, 14H), 2.43 (s, 3H).

¹³C NMR (91 MHz, CDCl₃) δ 163.4, 144.8, 134.4, 133.1, 129.8, 129.0, 128.0, 123.5, 77.2, 70.8, 70.6, 70.5, 69.3, 69.2, 68.7, 21.6.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₂₇H₃₅NNaO₁₁S⁺ 604.1823. Found 604.1813.

Compound 60: 2-(2-(2-(benzyloxy)ethoxy)ethoxy)ethan-1-ol

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To a stirred solution of triethylene glycol (6.0 g, 40 mmol, 4 eq) in anhydrous tetrahydrofuran (10 mL) at 0°C was portionwise added NaH (60% oil dispersion, 400 mg, 10 mmol, 1 eq) and the resulting mixture was stirred for 30 mins at room temperature. Then benzyl bromide (1.71 g, 10 mmol, 1 eq) was added and the resulting mixture was stirred overnight at room temperature. After quenching the reaction with methanol, the mixture was diluted with EtOAc, washed by water, NaHCO₃ (sat.) and brine, then dried over Na₂SO₄, concentrated and purified by flash chromatography on silica gel to give the alcohol **60** as a clear oil (2.18 g, 9.1 mmol) in 91% yield.

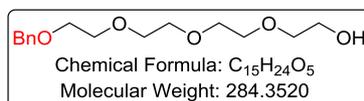
R_f = 0.4 (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 7.34-7.33 (m, 4H), 7.30 – 7.24 (m, 1H), 4.56 (s, 2H), 3.73 – 3.59 (m, 12H).

¹³C NMR (91 MHz, CDCl₃) δ 138.2, 128.4, 127.8, 127.7, 73.3, 72.6, 70.7, 70.6, 70.4, 69.4, 61.8.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₁₃H₂₀NaO₄ 263.1362. Found 263.1246.

Compound 61: 1-phenyl-2,5,8,11-tetraoxatridecan-13-ol



To a stirred solution of tetraethylene glycol (11.66 g, 60 mmol, 4 eq) in anhydrous tetrahydrofuran (15 mL) at 0°C was portionwise added NaH (60% oil dispersion, 600 mg, 15 mmol, 1 eq) and the resulting mixture was stirred for 30 minutes at room temperature. Then benzyl bromide (2.57 g, 15 mmol, 1 eq) was added and the resulting mixture was stirred overnight at room temperature. After quenching the reaction with methanol, the mixture was diluted with EtOAc, washed by water, NaHCO₃ (sat.) and brine, then dried over Na₂SO₄, concentrated and purified by flash chromatography on silica gel to give the alcohol **61** as a clear oil (3.3 g, 11.5 mmol) in 77% yield.

R_f = 0.3 (Petroleum/Acetone = 60/40); **Relevator**: KMnO₄

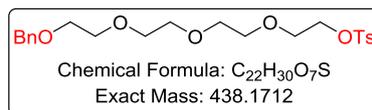
¹H NMR (360 MHz, CDCl₃) δ 7.34 – 7.33 (m, 4H), 7.29 – 7.24 (m, 1H), 4.56 (s, 2H), 3.71 – 3.57 (m, 16H).

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^{13}C NMR (91 MHz, CDCl_3) δ 138.2, 128.4, 127.8, 127.6, 73.3, 72.8, 70.7, 70.6, 70.3, 69.4, 61.7.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{15}\text{H}_{24}\text{NaO}_5^+$ 307.1516. Found 307.1513.

Compound 62: 1-phenyl-2,5,8,11-tetraoxatridecan-13-yl 4-methylbenzenesulfonate



To a solution of alcohol **61** (3.23 g, 11.4 mmol, 1 eq) in anhydrous CH_2Cl_2 (30 mL, $C = 0.4$ M) was dropwise added triethylamine (3.46 g, 34.2 mmol, 2.4 mL, 3 eq) at 0°C in ice-bath and the reaction mixture was stirred at 0°C for additional 30 mins. Then *p*-toluenesulfonyl chloride (4.34 g, 22.8 mmol, 2 eq) was added portion by portion. This solution was stirred at room temperature for overnight, then Et_3N was removed by rotary evaporation. The residue was extracted with EtOAc , washed with brine, and dried over MgSO_4 . After removal of the solvent *in vacuo*, the crude product was purified with flash column chromatography to give product **62** as a colorless oil (4.98 g, 11.4 mmol) in quantitative yield.

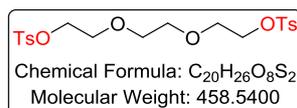
$R_f = 0.4$ (Petroleum ether/ $\text{EtOAc} = 50/50$); **Relevator**: KMnO_4

^1H NMR (360 MHz, CDCl_3) δ 7.79 (d, $J = 8.1$ Hz, 2H), 7.50 – 7.29 (m, 6H), 7.29 – 7.19 (m, 1H), 4.55 (s, 2H), 4.18 – 4.10 (m, 2H), 3.71 – 3.53 (m, 14H), 2.43 (s, 3H).

^{13}C NMR (91 MHz, CDCl_3) δ 144.8, 138.3, 133.0, 129.8, 128.4, 128.0, 127.8, 128.6, 73.3, 70.8, 70.7, 70.6, 69.5, 69.3, 68.7, 21.7

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{22}\text{H}_{30}\text{NaSO}_7$ 461.1604. Found 461.1595.

Compound 63: (ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate)



To a solution of tetraethylene glycol (1.94 g, 10 mmol, 1 eq) in CH_2Cl_2 (25 mL, $C = 0.4$ M) was dropwise added triethylamine (5.06 g, 50 mmol, 6.9 mL, 5 eq) at 0°C in ice-bath for 30 minutes. Then *p*-toluenesulfonyl chloride (7.62 g, 40 mmol, 4 eq) was added

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portion by portion. This solution was stirred at room temperature for overnight, then Et₃N was removed by rotary evaporation. The residue was extracted with EtOAc, washed with brine, and dried over MgSO₄. After removal of the solvent *in vacuo*, the crude product was purified with flash column chromatography to give product **63** as a colorless oil (3.93 g, 7.8 mmol) in 78% yield.

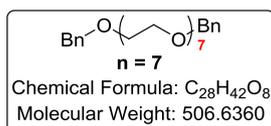
R_f = 0.2 (Petroleum/EtOAc = 50/50); **Relevator**: KMnO₄

¹H NMR (300 MHz, CDCl₃) δ 7.79 (d, J = 8.3 Hz, 4H), 7.33 (d, J = 8.2 Hz, 4H), 4.18 – 4.12 (m, 4H), 3.70 – 3.65 (m, 4H), 3.56 (m, 8H), 2.44 (s, 6H).

¹³C NMR (75 MHz, CDCl₃) δ 144.8, 133, 130, 128, 70.7, 70.5, 69.3, 68.7, 21.6.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₂₂H₃₀NaS₂O₉ 525.1223. Found 525.1204.

Compound 64: 1,24-diphenyl-2,5,8,11,14,17,20,23-octaoxatetracosane



To a solution of the alcohol **60** (1 g, 4.16 mmol, 1 eq) in anhydrous tetrahydrofuran (10 mL) at 0°C was portionwise added sodium hydride (60% in oil dispersion, 184 mg, 4.58 mmol, 1.1 eq) and the reaction mixture was stirred at 0°C for an additional 30 minutes. Then the tosylated **62** (1.91 g, 4.36 mmol, 1.05 eq) in anhydrous tetrahydrofuran (12 mL) was added dropwise over 30 minutes. Then the mixture was heated up to reflux for overnight. The mixture was cooled to 0°C and neutralized with a saturated ammonium chloride solution and the mixture was diluted with EtOAc, washed by water, NaHCO₃ (sat.) and brine, then dried over Na₂SO₄, concentrated *in vacuo* and further purified by column chromatography to obtain product **64** as a colorless oil (1.88g, 3.71 mmol) in 90% yield.

R_f = 0.5 (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO₄

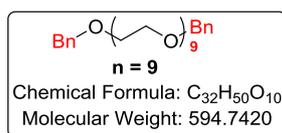
¹H NMR (360 MHz, CDCl₃) δ 7.40 – 7.20 (m, 10H), 4.56 (s, 4H), 3.72 – 3.55 (m, 28H).

¹³C NMR (91 MHz, CDCl₃) δ 138.3, 128.4, 127.8, 127.6, 73.2, 70.7, 70.6, 69.4.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₂₈H₄₂NaO₈⁺ 529.2772. Found 529.2759.

Compound 65: 1,30-diphenyl-2,5,8,11,14,17,20,23,26,29-decaoxatriacontane

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To a solution of ethylene glycol (168 mg, 2.7 mmol, 1 eq) in anhydrous tetrahydrofuran (6 mL) at 0°C was portionwise added sodium hydride (60% in oil dispersion, 228 mg, 5.7 mmol, 2.1 eq) and the reaction mixture was stirred at 0°C for an additional 30 mins. Then the tosylated **62** (2.5 g, 5.7 mmol, 2.1 eq) in anhydrous tetrahydrofuran (8 mL) was dropwise added over 30 minutes. Then the mixture was heated up to reflux for 24 hour. The mixture was cooled to 0°C and neutralized with a saturated ammonium chloride solution and the mixture was diluted with EtOAc, washed by water, NaHCO_3 (sat.) and brine, then dried over Na_2SO_4 , concentrated *in vacuo* and further purified by column chromatography to obtain product **65** as a colorless oil (1.25g, 2.11 mmol) in 79% yield.

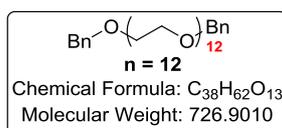
R_f = 0.5 (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO_4

¹H NMR (360 MHz, CDCl_3) δ 7.38 – 7.25 (m, 10H), 4.57 (s, 4H), 3.69 – 3.63 (m, 36H).

¹³C NMR (91 MHz, CDCl_3) δ 138.3, 128.4, 127.7, 127.6, 73.2, 70.7, 70.6, 69.4.

HRMS (ESI, positive mode): m/z: $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{32}\text{H}_{50}\text{NaO}_{10}^+$ 617.3296. Found 617.3277.

Compound 66: 1,39-diphenyl-2,5,8,11,14,17,20,23,26,29,32,35,38-tridecaoxanonatriacontane



To a solution of alcohol **61** (1.94 g, 6.84 mmol, 2.04 eq) in anhydrous tetrahydrofuran (9 mL) at 0°C was portionwise added sodium hydride (60% in oil dispersion, 298 mg, 7.44 mmol, 2.2 eq) and the reaction mixture was stirred at 0°C for an additional 30 minutes. Then the tosylated **63** (1.68 g, 3.35 mmol, 1 eq) in anhydrous tetrahydrofuran (8 mL) was dropwise added over 30 minutes. Then the mixture was heated up to reflux for 48 hours. The mixture was cooled to 0°C and neutralized with a saturated ammonium chloride solution and the mixture was diluted with EtOAc, washed by water, NaHCO_3 (sat.) and brine, then dried over Na_2SO_4 , concentrated *in vacuo* and further purified by column chromatography to obtain product **61** as a colorless oil (2.1 g, 2.87

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mmol) in 86% yield.

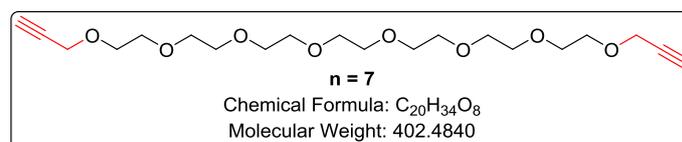
$R_f = 0.4$ (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO_4

$^1\text{H NMR}$ (360 MHz, CDCl_3) δ 7.39 – 7.20 (m, 10H), 4.56 (s, 4H), 3.64 (m, 48H).

$^{13}\text{C NMR}$ (91 MHz, CDCl_3) δ 138.26, 128.36, 127.75, 127.60, 73.24, 70.64, 70.57, 69.42.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{38}\text{H}_{62}\text{NaO}_{13}$ 749.4083. Found 749.4050.

Compound 67: 4,7,10,13,16,19,22,25-octaooxaoctacos-1,27-diyne



To a solution of the dibenzylated **64** (172 mg, 0.34 mmol, 1 eq) in EtOH (3.5 mL, C = 0.1 M) was added palladium on carbon (18 mg, 0.1 eq) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature until TLC analysis indicated complete consumption of the dibenzylate. Afterwards the mixture was filtered over Celite and the filter cake was washed with MeOH three times. This solution was concentrated *in vacuo* and the pure OEG diol was obtained without further purification. To a solution of the OEG diol (111 mg, 0.34 mmol, 1 eq) in anhydrous tetrahydrofuran (3.5 mL, C = 0.1 M) was portionwise added sodium hydride (60% in oil dispersion, 41 mg, 1.02 mmol, 3 eq) and the reaction mixture was stirred at 0°C for an additional 30 mins. Then propargyl bromide (101 mg, 0.85 mmol, 70 μL , 2.5 eq) was added dropwise and the resulting mixture was stirred at room temperature for 1hr. After quenching the reaction with saturated ammonium chloride, the mixture was diluted with EtOAc, washed by water, NaHCO_3 (sat.) and brine, then dried over Na_2SO_4 , concentrated and purified by flash chromatography on silica gel to give the product **67** as a clear oil (121 mg, 0.3 mmol) in 88% yield.

$R_f = 0.3$ (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO_4

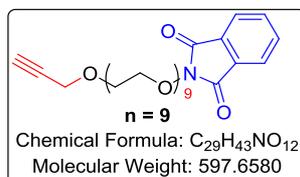
$^1\text{H NMR}$ (360 MHz, CDCl_3) δ 4.18 (d, $J = 2.3$ Hz, 4H), 3.69 – 3.66 (m, 8H), 3.63 (d, $J = 2.5$ Hz, 20H), 2.42 (t, $J = 2.4$ Hz, 2H).

$^{13}\text{C NMR}$ (91 MHz, CDCl_3) δ 79.7, 74.6, 70.6, 70.4, 69.1, 58.4.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{20}\text{H}_{34}\text{NaO}_8^+$ 425.2146. Found 425.2136.

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Compound 70: 2-((3,6,9,12,15,18,21,24,27-nonaoxatriacont-29-yn-1-yl)oxy)isoindoline-1,3-dione



To a solution of the dibenzylated **65** (2.10 g, 3.5 mmol, 1 eq) in EtOH (35 mL, C = 0.1 M) was added palladium on carbon (210 mg, 0.1 eq) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature until TLC analysis indicated complete consumption of the dibenzylate. Afterwards the mixture was filtered over Celite and the filter cake was washed with MeOH three times. This solution was concentrated *in vacuo* and the pure OEG diol was obtained without further purification. To a solution of the OEG diol (1.45 g, 3.5 mmol, 1 eq) in anhydrous tetrahydrofuran (35 mL, C = 0.1 M) was portionwise added sodium hydride (60% in oil dispersion, 70 mg, 1.75 mmol, 0.5 eq) and the reaction mixture was stirred at 0°C for an additional 30 mins. Then propargyl bromide (208 mg, 1.75 mmol, 150 μ L, 0.5 eq) was added dropwise and the resulting mixture was stirred at room temperature for 1hr. After quenching the reaction with saturated ammonium chloride, the mixture was diluted with EtOAc, washed by water, NaHCO₃ (sat.) and brine, then dried over Na₂SO₄, concentrated and purified by flash chromatography on silica gel to give the mono-propargylated OEG as a clear oil (749 mg, 1.66 mmol) in 95% yield.

R_f = 0.1 (Petroleum ether/Acetone 60/40); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 4.09 (d, *J* = 2.3 Hz, 2H), 3.65 – 3.61 (m, 2H), 3.56 (dd, *J* = 8.6, 2.8 Hz, 32H), 3.50 – 3.47 (m, 2H), 2.38 (t, *J* = 2.3 Hz, 1H).

¹³C NMR (91 MHz, CDCl₃) δ 79.5, 74.7, 71.8, 70.2, 70.0, 69.9, 69.8, 69.7, 69.6, 69.4, 69.1, 68.9, 60.4, 58.2.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₂₁H₄₀NaO₁₀ 475.2624. Found 475.2627.

To a solution of the mono-propargylated OEG (749 mg, 1.66 mmol, 1 eq) in anhydrous tetrahydrofuran (17 mL, C = 0.1 M) was added successively triphenylphosphine (522 mg, 1.99 mmol, 1.2 eq) and *N*-hydroxyphthalimide (325 mg, 1.99 mmol, 1.2 eq) under Argon atmosphere at room temperature, then Diisopropyl azodicarboxylate (0.4 mL, 402 mg, 1.99 mmol, 1.2 eq) was dropwise added, and the mixture was stirred overnight at room temperature. The crude reaction mixture was concentrated under reduced

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pressure and purified by flash chromatography to yield product **70** as a colorless oil (992 mg, 1.66 mmol) in quantitative yield.

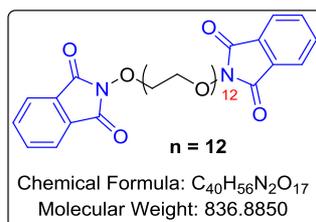
R_f = 0.1 (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 7.85 – 7.80 (m, 2H), 7.76 – 7.71 (m, 2H), 4.09 (d, *J* = 2.3 Hz, 2H), 3.65 – 3.61 (m, 2H), 3.56 (m, 32H), 3.50 – 3.47 (m, 2H), 2.38 (t, *J* = 2.3 Hz, 1H).

¹³C NMR (91 MHz, CDCl₃) δ 163.4, 134.4, 129.0, 123.5, 79.7, 74.8, 71.9, 70.23, 70.1, 70.0, 69.8, 69.7, 69.7, 69.5, 69.2, 68.8, 60.5, 58.4.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₂₉H₄₃NaO₁₂ 620.2783. Found 620.2785.

Compound 71: 2,2'-((3,6,9,12,15,18,21,24,27,30,33-undecaioxapentatriacontane-1,35-diyl)bis(oxy)) bis(isoindoline-1,3-dione)



To a solution of the dibenzylated **66** (2.1 g, 3.87 mmol, 1 eq) in EtOH (29 mL, C = 0.1 M) was added palladium on carbon (209 mg, 0.1 eq) and the reaction mixture was stirred under a hydrogen atmosphere at room temperature until TLC analysis indicated complete consumption of the dibenzylate. Afterwards the mixture was filtered over Celite and the filter cake was washed with MeOH three times. This solution was concentrated in vacuo and the pure OEG diol was obtained without further purification. To a solution of the OEG diol (1.57 g, 2.87 mmol, 1 eq) in anhydrous tetrahydrofuran (30 mL, C = 0.1 M) was added successively triphenylphosphine (3.76 g, 14.35 mmol, 5 eq) and *N*-hydroxyphthalimide (2.34 g, 14.35 mmol, 5 eq) under Argon atmosphere at room temperature, then Diisopropyl azodicarboxylate (2.8 mL, 2.9 g, 14.35 mmol, 5 eq) was dropwise added, and the mixture was stirred overnight at room temperature. The crude reaction mixture was concentrated under reduced pressure and purified by flash chromatography to yield product **71** as a colorless oil (2.4 g, 2.87 mmol) in quantitative yield.

R_f = 0.3 (CH₂Cl₂/Methanol = 20/1); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 7.85 – 7.80 (m, 4H), 7.77 – 7.73 (m, 4H), 4.39 – 4.35

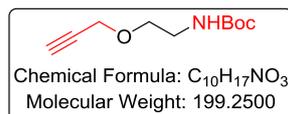
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(m, 4H), 3.88 – 3.83 (m, 4H), 3.64 (m, 30H), 3.59 – 3.55 (m, 10H).

^{13}C NMR (91 MHz, CDCl_3) δ 163.44, 134.46, 128.98, 123.50, 77.20, 70.78, 70.56, 70.50, 69.31.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{40}\text{H}_{56}\text{N}_2\text{NaO}_{17}$ 859.3471. Found 859.3438.

Compound 72: *N*-(*tert*-Butyloxycarbonyl)-2-(propargyloxy)-aminoethane



To a solution of ethanolamine (3.05 g, 50 mmol, 1 eq) in dioxane (50 mL) was added $(\text{Boc})_2\text{O}$ (10.45 g, 60 mmol, 1.2 eq) dropwise at 0°C in ice-bath, then the temperature was slowly raised to room temperature and the resulting mixture was stirred at room temperature overnight. After the complete absence of starting material (as judged by TLC), the volatiles were removed under reduced pressure, and the residue was redissolved in EtOAc. The solution was washed successively with 1M HCl and brine, then dried over Na_2SO_4 , filtered and concentrated to yield *N*-(*tert*-butoxycarbonyl)ethanolamine, which was used directly in the subsequent steps.

R_f = 0.48 (Petroleum ether : AcOEt 50 : 50)

^1H NMR (360 MHz, CDCl_3) δ 3.66 (d, J = 2.9 Hz, 2H), 3.25 (d, J = 3.2 Hz, 2H), 1.42 (s, 9H).

^{13}C NMR (91 MHz, CDCl_3) δ 156.85, 79.74, 62.30, 43.27, 28.39.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_7\text{H}_{15}\text{NNaO}_3$ 184.0944. Found 184.0937.

To a suspension of NaH (60% oil dispersion, 373 mg, 9.3 mmol, 1.5 eq) in dry DMF (3 mL) was dropwise added a solution of *N*-(*tert*-butoxycarbonyl)ethanolamine (1.0 g, 6.2 mmol, 1 eq) at 0°C in ice-bath and under a Argon atmosphere. The temperature was slowly raised to r.t. and the suspension stirred for 30 minutes. The mixture was cooled to 0°C and a solution of propargyl bromide (900 μL , 4.8 mmol, 0.8 eq) in dry DMF (5 mL) was added dropwise. The resulting mixture was stirred overnight at r.t., diluted with water and extracted with EtOAc. The collected organic layers were anhydriified over Na_2SO_4 , filtered and evaporated. The crude was purified by flash chromatography affording the product **72** (640 mg, 3.2 mmol) in 52% yield (2 steps).

R_f = 0.4 (Petroleum ether/EtOAc = 80/20); **Relevator**: KMnO_4

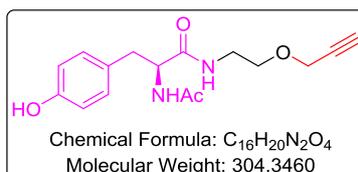
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¹H NMR (360 MHz, CDCl₃) δ 4.14 (d, *J* = 2.4 Hz, 2H), 3.57 (t, *J* = 5.1 Hz, 2H), 3.32 (dd, *J* = 10.3, 5.2 Hz, 2H), 2.43 (t, *J* = 2.3 Hz, 1H), 1.42 (s, 9H).

¹³C NMR (91 MHz, CDCl₃) δ 155.9, 79.4, 79.3, 74.7, 69.1, 58.3, 40.3, 28.4.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₁₀H₁₇NNaO₃⁺ 222.1101. Found 222.1091.

Compound 75: (*S*)-2-acetamido-3-(4-hydroxyphenyl)-*N*-(2-(prop-2-yn-1-yloxy)-ethyl)propanamide



To a solution of *N*-Acetyl-Tyrosine (134 mg, 0.6 mmol, 1 eq) in dry CH₃CN (3 mL, C = 0.2 M) was added *N*-Hydroxysuccinimide (100 mg, 0.7 mmol, 1.1 eq) and EDCl.HCl (127 mg, 0.7 mmol, 1.1 eq) at room temperature and stirred for overnight. After the complete absence of starting material (as judged by ¹H NMR), the solution was diluted by EtOAc, then washed successively with NaHCO₃ (sat.) and brine, dried over Na₂SO₄, filtered and concentrated to yield *N*-Acetyl-Tyrosine NHS ester **74**, which was used directly in the subsequent steps.

After Compound **72** (100 mg, 0.5 mmol, 1 eq) was dissolved in TFA/DCM (v/v = 1/1, 1.2/1.2 mL, C = 0.2 M) at 0°C then stirred at room temperature for 1 hour. The reaction mixture was then concentrated and the residue **73** was redissolved in dry CH₃CN (2.5 mL, C = 0.2 M), then DIPEA (194 mg, 0.25 mL, 1.5 mmol, 3 eq) was added dropwise at 0°C in ice-bath, and a mixture of *N*-Acetyl-Tyrosine NHS ester (304 mg, 1.0 mmol, 1 eq) and DMAP (6 mg, 0.5 mmol, 0.1 eq) were added. The resulting mixture was stirred overnight at room temperature, diluted with EtOAc and washed successively with NaHCO₃ (sat.) and brine, then the collected organic layers were anhydried over Na₂SO₄, filtered and evaporated. The crude was purified by flash chromatography affording the product **75** (104 mg, 0.34 mmol) in 68% yield.

R_f = 0.7 (Petroleum ether/Acetone = 20/80); **Relevator:** KMnO₄

¹H NMR (360 MHz, MeOD) δ 7.07 – 7.01 (m, 2H), 6.72 – 6.67 (m, 2H), 4.49 (dd, *J* = 8.1, 6.8 Hz, 1H), 4.11 (dd, *J* = 2.3, 1.2 Hz, 2H), 3.51 (ddd, *J* = 9.4, 6.1, 5.0 Hz, 1H), 3.43 (ddd, *J* = 9.6, 6.7, 4.6 Hz, 1H), 3.39 – 3.31 (m, 1H), 3.31 – 3.22 (m, 1H), 2.97

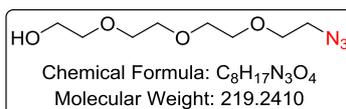
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(dd, $J = 13.7, 6.7$ Hz, 1H), 2.83 (t, $J = 2.3$ Hz, 1H), 2.79 (dd, $J = 13.7, 8.2$ Hz, 1H), 1.91 (s, 3H).

^{13}C NMR (91 MHz, MeOD) δ 172.4, 171.6, 155.9, 129.9, 127.6, 114.8, 79.1, 74.6, 67.7, 57.5, 55.1, 38.7, 37.0, 21.1.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{NaO}_4$ 327.1315. Found 327.1303.

Compound 78: 2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol



To a solution of tetraethylene glycol (25.2 g, 130 mmol, 10 eq) in tetrahydrofuran (5 mL) was added an aqueous solution (5 mL) of sodium hydroxide (832 mg, 20.8 mmol, 1.6 eq). Then a solution of *p*-toluenesulfonyl chloride (2.5 g, 13 mmol, 1 eq) in tetrahydrofuran (15 mL) was dropwise added to the mixture at 0°C and stirred while maintaining this temperature for 3 hours. The reaction was diluted with ice-cold water and extracted into dichloromethane. The combined organic layers were washed with water, dried over MgSO_4 , filtered, and concentrated to yield a clear oil (4.6 g, 13 mmol, quantitative yield).

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{15}\text{H}_{24}\text{SNaO}_7$ 371.1243. Found 371.1122.

Sodium azide (2.1 g, 32.5 mmol, 2.5 eq) was added to the tosylate intermediate, and the mixture was dissolved in absolute ethanol (100 mL). The reaction was stirred under reflux overnight, cooled to room temperature, and then diluted with water (50 mL). The mixture was concentrated by rotary evaporation to approximately 50 mL, and the product was extracted into ethyl acetate, dried with MgSO_4 , filtered, and concentrated to yield product **78** as a faint yellow liquid (2.2 g, 10.2, 79% yield over two steps).

$R_f = 0.4$ (Petroleum ether/Acetone = 60/40); **Relevator**: KMnO_4

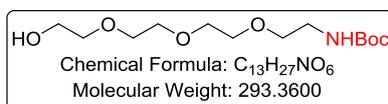
^1H NMR (360 MHz, CDCl_3) δ 3.72 – 3.62 (m, 12H), 3.59 (m, 2H), 3.37 (t, $J = 4.6$ Hz, 2H), 2.70 (s, 1H).

^{13}C NMR (91 MHz, CDCl_3) δ 72.49, 70.67, 70.64, 70.57, 70.31, 70.03, 61.69, 50.64.

HRMS (ESI, positive mode): m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_8\text{H}_{17}\text{N}_3\text{NaO}_4^+$ 242.1111. Found 242.1102.

Chapter 7. Experimental Part

Compound 79: *tert*-butyl (2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-carbamate



To a solution of 2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol (1 g, 4.56 mmol, 1 eq) in methanol (15 mL) was added palladium on carbon (10%, 200 mg) and the mixture was stirred under a hydrogen atmosphere for 6 hour. Then mixture was then filtered through a pad of celite, and the celite cake was washed with methanol for three times. The filtrates were combined and the solvent removed under reduced pressure to give 2-(2-(2-amineethoxy)ethoxy)ethoxy)ethanol (881 mg, 4.56 mmol, quantitative) as a colorless oil which was used without further purification.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₈H₁₉NNaO₄⁺ 216.1206. Found 216.1203.

To a solution of 2-(2-(2-amineethoxy)ethoxy)ethoxy)ethanol (650 mg, 3.36 mmol, 1 eq) in CH₂Cl₂ (17 mL) was dropwise added Boc₂O (703 mg, 4.04 mmol, 1.2 eq) at 0°C in ice-bath, and the mixture was stirred for overnight at room temperature. Then the solution was diluted with CH₂Cl₂ and washed with water, dried over anhydrous Na₂SO₄ and evaporated. The crude was purified by flash chromatography affording product **79** as a colorless oil (610 mg, 2.1 mmol) in 62% yield.

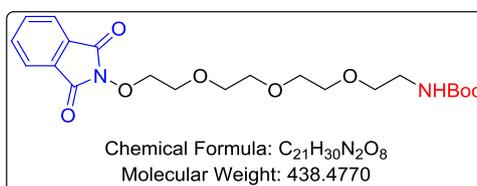
R_f = 0.4 (Petroleum ether/Acetone 50/50); **Relevator:** KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 3.75 – 3.65 (m, 4H), 3.61 (m, 8H), 3.51 (t, *J* = 4.6 Hz, 2H), 3.29 (t, *J* = 4.3 Hz, 2H), 1.42 (s, 9H).

¹³C NMR (91 MHz, CDCl₃) δ 156.2, 79.1, 72.7, 70.6, 70.4, 70.2, 70.1, 61.6, 40.5, 28.4.

HRMS (ESI, positive mode): m/z: [M + Na]⁺ Calcd for C₁₃H₂₇NNaO₆ 316.1838. Found 316.1724.

Compound 80: *tert*-butyl (2-(2-(2-(2-((1,3-dioxoisindolin-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-carbamate



Chapter 7. Experimental Part

To a solution of **79** (517 mg, 1.76 mmol, 1 eq) in anhydrous dichloromethane (18 mL, C = 0.1 M) was added successively triphenylphosphine (555 mg, 2.11 mmol, 1.2 eq) and *N*-hydroxyphthalimide (345 mg, 2.11 mmol, 1.2 eq) under argon atmosphere at room temperature, then Diisopropyl azodicarboxylate (0.42 mL, 428 mg, 2.11 mmol, 1.2 eq) was dropwise added at 0°C in ice-bath, and the mixture was stirred for overnight at room temperature. The crude reaction mixture was concentrated under reduced pressure and purified by flash chromatography to yield product **80** as a colorless oil (773 mg, 1.76 mmol) in quantitative yield.

R_f = 0.4 (Petroleum ether/Acetone = 60/30); **Relevator**: KMnO₄

¹H NMR (360 MHz, CDCl₃) δ 7.86 – 7.80 (m, 2H), 7.77 – 7.71 (m, 2H), 4.40 – 4.34 (m, 2H), 3.88 – 3.83 (m, 2H), 3.67 (dd, *J* = 6.0, 3.5 Hz, 2H), 3.60 – 3.53 (m, 6H), 3.50 (t, *J* = 5.2 Hz, 2H), 3.28 (t, *J* = 5.1 Hz, 2H), 1.42 (s, 9H).

¹³C NMR (91 MHz, CDCl₃) δ 163.4, 156.0, 134.5, 129.0, 123.5, 79.2, 77.3, 70.8, 70.5, 70.2, 69.3, 28.4.

HRMS (ESI, positive mode): *m/z*: [M + Na]⁺ Calcd for C₂₁H₃₀N₂NaO₈ 461.1894.

Found 461.1876.

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Titre : Synthèse et optimisation de neutraligands tridentates de la chimiokine CXCL12

Mots clés : glycochimie, Héparane Sulfate, mimes de sucres, Chimie Click, CuAAC, formation d'oximes, réactions monotopes

Résumé : L'Héparane Sulfate (HS) est un polysaccharide linéaire exprimé à la surface des cellules de mammifères et dans la matrice extracellulaire, constitué d'une alternance de régions hautement (S) et plus faiblement (A) sulfatés. Ces motifs SAS interagissent avec des protéines basiques impliquées dans divers processus biologiques et suscitent un intérêt considérable pour le développement de nouveaux agents thérapeutiques. Nous avons ainsi supposé que l'activité de CXCL12, une

protéine se liant aux HS et impliquée dans des cancers, pourrait être modulée par un ligand comportant une partie centrale de type HS lié à deux sulfotyrosines. Pour assembler ces trois, deux réactions de Chimie Click, la CuAAC et la formation d'oxime, ont été sélectionnées et les extrémités de fragments d'HS modifiées en conséquence. Après optimisation des conditions réactionnelles, nous avons démontré que cette stratégie d'assemblage moléculaire permettrait la synthèse chimique de ligands de CXCL12 et de mimes de SAS.

Title : Synthesis and optimization of "sugar tongs" lock neutraligands of the chemokine CXCL12

Keywords : glycochemistry, Heparan Sulfate, glycomimetics, Click Chemistry, CuAAC, oxime ligation, one-pot reactions

Abstract : Heparan Sulfate (HS) is a linear and sulfated polysaccharide widely expressed on cell surfaces and in the extracellular matrix of animal tissues, displaying alternation of highly negatively charged regions (S) and less charged one (A). Those SAS motifs, interacting with several basic proteins playing essential roles in biological processes, represent attractive scaffold for the development of new innovative therapeutic compounds. For example, we assume that the HS-binding protein CXCL12, involved in

cancer metastasis, should be targeted by a well-designed tridentate ligand bearing a central HS fragment linked to two sulfotyrosines. To prepare it, we selected two Click Chemistry reactions, "the CuAAC" triazole formation and the "oxime ligation" and accordingly modified the reducing end and non-reducing end of HS fragments. After optimization of those reactions on model compounds, we demonstrated that this strategy should be applied to CXCL12 ligands and SAS mimics preparation.

