Protective group strategies in carbohydrate and peptide chemistry

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Protecting group strategies in carbohydrate and peptide chemistry

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Contents

List of abbreviations

A	alanine
AA	amino Acid
Abu	2-aminobutyric acid
Ac	acetyl
ACN	acetonitril
Ada	adamantanyl
Ala	alanine
All	allyl
aq	aqueous
Arg	arginine
arom	aromatic
Asn	asparigine
Asp	aspartic acid
Az	azulen-1-yl-dicarbonyl
B	2-aminobutyric acid
BDA	butanediacetal
Bn	benzyl
Boc	*tert*-butoxycarbonyl
bs	broad singlet
BSP	1-Benzenesulfinyl Piperidine
Bu	butyl
Bz	benzoyl
CA	chloroaceyl
CDI	1,1'-carbonyldiimidazole
CEM	cyanoethoxymethyl
C_q	quartnary carbon
Cys	cysteine
D	aspartic acid
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dd	doublet of doublets
DDQ	2,3-Dichloro-5,6-dicyanobenzoquinone
DG	diglycolyl
Dipea	diisopropylethylamine
DMAP	dimethylaminopyridine
DMDO	dimethyl dioxirane
DMF	dimethylformamide
DMG	dimethylglutaryl
DMM	dimethylmaloyl
DMP	dimethylphosphoryl
DMSO	dimethylsulfoxide
DMT	dimethoxytrityl
DPM	diphenylmaloyl
dt	doublet of triplets
DTBMP	di-*tert*-butylmethylpyridine
DTBS	4,6-di-*tert*-butylsilylene
E	glutamic acid
E^+	electrophlie
EDC	1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide
eq.	equivalent
Et	ethyl
FHPLC	fluorous high performance liquid chromatography
FLLE	fluorous liquid liquid extraction
Fmoc	9-fluorenylmethyl carbonyl
FMsc	[1H,1H,2H,2H]-perfluorodecylsulfonyl ethoxy carbonyl
Fmsem	[1H,1H,2H,2H]-perfluorodecylsulfonyl ethoxymethyl
FPsc	[1H,1H,2H,2H,3H,3H]-perfluoroundecyl sulfonylethoxy carbonyl
Fpsem	[1H,1H,2H,2H,3H,3H]-perfluoroundecyl sulfonylethoxymethyl
FSPE	fluorous solid phase extraction
FTIPS	fluorous di-*iso*-propylsilanyl
G	glycine
G	guajazulene
Gln	glutamine
Glu	glutamic acid
Gly	glycine

H	histidine
h	hours
HCTU	2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
His	histidine
HRMS	high resolution mass spectrometery
I	isoleucine
IAD	intramolecular aglycon delivery
IDCP	iodonium-di-*sym*-collidine perchlorate
Ile	isoleucine
IR	infrared
J	coupling constant
K	lysine
L	leucine
LCMS	liquid chromatography mass spectroscopy
Leu	leucine
Lev	levulinoyl
Lys	lysine
M	methionine
M	molar
MBHA	methylbenzylhydraylamine
m-CPBA	3-chloroperoxybenzoic acid
Me	methyl
Met	methionine
min.	minutes
MS	molecular sieves
Msc	methylsulfonylethoxymethylcarbonyl
Msem	methylsulfonylethoxymethyl
MTM	methylthiomethyl
N	asparigine
Nap	2-naphthylmethyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMNO	*N*-methylmorpholine *N*-oxide
NMP	*N*-methylpyrolidone
NMR	nuclear magnetic resonance spectroscopy
NPB	Nitrophthalimidobutyric
Nphth	naphthaloyl
NSEC	2-[dimethyl(2-naphthylmethyl)silyl]ethoxy carbonyl
p	para
P	proline
P	protective group
PFD-OH	[1H,1H,2H,2H]-perfluorodecanoic acid
Ph	phenyl
Phth	phthaloyl
Piv	pivaloyl
PMB	*p*-methoxybenzyl
pmc	2,24,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
Pro	proline
PST	phenylsulfenyltriflate
pyr	pyridine
Q	glutamine
R	arginine
Rf	fluorinated alkyl
R_f	retension factor
RNA	ribosnucleic acid
RT	room temperature
S	serine
s	singlet
SE	2-(trimethylsilyl)ethyl
Ser	serine
SPPS	solid phase peptide synthesis
t	tertiary
T	threonine
t	triplet
TBABr	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBAS	tetrabutylammonium sulfonate
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TBDS	di-*tert*-butylsilyl
TCA	trichloroacetyl

TDG	thiodiglyocolyl
TEA	triethylamine
tert	Tertiary
Tf	Trifyl
TFA	trifluoroacetic acid
TFT	trifluorotoluene
THF	tetrahydrofuran
Thr	threonine
TIS	triisopropylsilane
TLC	thin layer chromatography
TMS	trimethylsilyl
TNBS	trinitrobenzenesulfonic acid
tol	toluene
TOM	tri-*iso*-propylsilyloxymethyl
Troc	trichloroethylcarbonyl\
Trp	tryptophan
Trt	trityl
Ts	tosyl
TTBP	tri-*tert*-butylpyrimidine
Tyr	tyrosine
UV	ultravoilet
V	valine
Val	valine
W	tryptophan
Y	tyrosine

CHAPTER

1

General Introduction: Novel protecting groups in carbohydrate chemistry

Protecting groups play a key role in the synthesis of complex natural products.[1] This holds especially true for the synthesis of oligosaccharides,[2] of which the monomeric carbohydrate building blocks usually contain up to five different hydroxyl functions. The discrimination of these hydroxyl functions requires a careful protecting group strategy and typically involves multistep protocols. Although protecting groups primarily function to mask a given functionality on the carbohydrate core, they also have a profound effect on the overall reactivity of a carbohydrate building block[3] and can control the stereochemical outcome of a glycosylation reaction.[4] Furthermore protecting groups can be used to introduce extra functionality on the carbohydrate core, such as visualization and/or

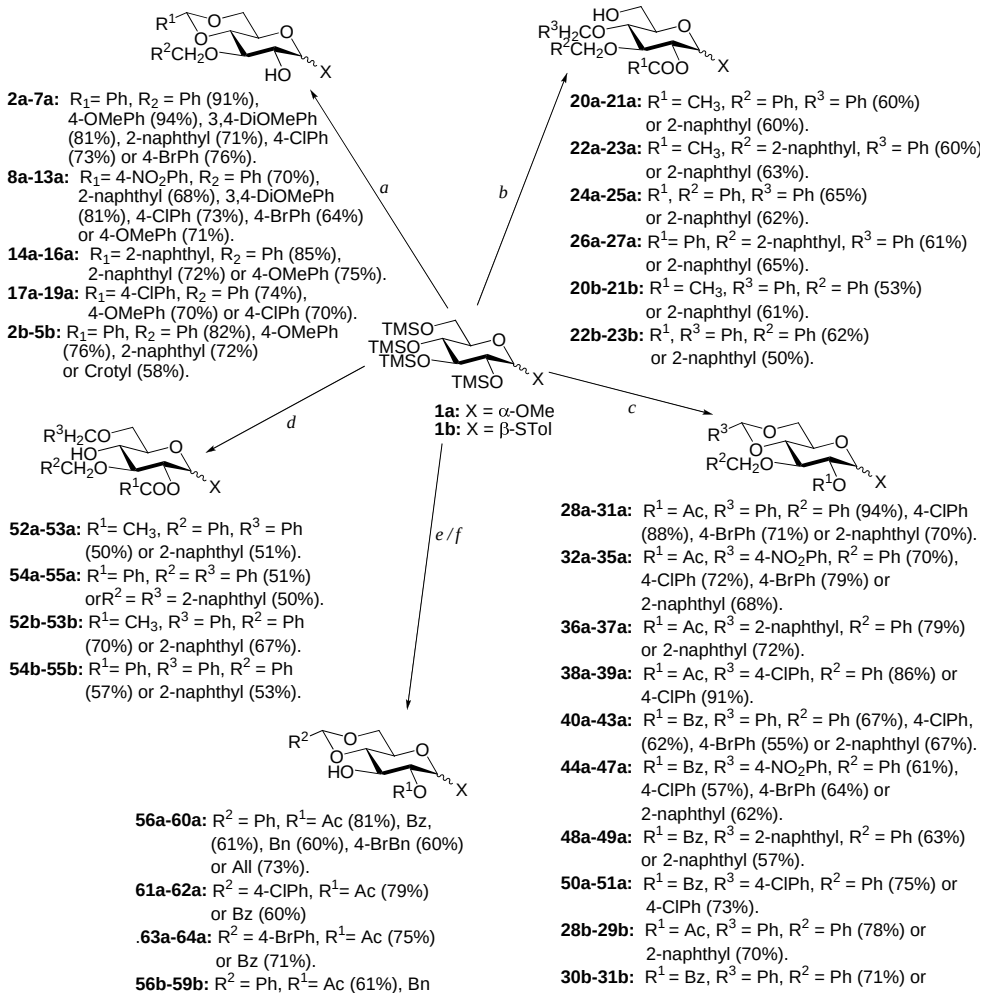
purification handles.[5] This chapter describes selected examples of novel protecting groups and protection strategies in carbohydrate chemistry from the beginning of the 21st century and highlights how protecting group chemistry has evolved from a necessary time consuming burden to a sophisticated synthetic tool for the efficient and stereoselective assembly of oligosaccharides.[6]

Advances in the regioselective protection of carbohydrates:

The regioselective manipulation of the different hydroxyl groups on a carbohydrate monomer is key to any protecting group strategy. Although all hydroxyl groups are of comparable reactivity they can be discriminated by exploiting their subtle reactivity differences and their relative orientation. The nucleophilicity of the different hydroxyls under neutral or acidic conditions increases from the anomeric to the secondary to the primary alcohol function. The anomeric hydroxyl group is most acidic and therefore selective protection of the hemiacetal -OH can be achieved in the presence of other secondary hydroxyls using basic reaction conditions. The general reactivity difference between an axially and an equatorially oriented hydroxyl group can often be exploited to attain a regioselective protection step. Commonly, the use of cyclic protecting groups presents a more robust way to discriminate between the different functionalities on a carbohydrate ring. For example, benzylidene type acetals are widely used to selectively mask the C-4 and C-6 alcohols,[7] whereas isopropylidene ketals are used to block two neighboring *cis* hydroxyls,[8] and butane 2,3-bisacetals to protect vicinal diequatorial diols.[9]

Recently several sequential procedures have been developed to streamline the regioselective protection of carbohydrates. Hung and co-workers disclosed that anomerically protected per-silylated carbohydrate monomers can be transformed into a wide array of differentially protected building blocks using a one-pot protocol, which combines up to five reaction steps (Scheme 1).[10] Because all steps are consecutively executed in the same reaction vessel the intermediate work-up and purification steps are omitted making this process highly efficient. The strategy builds on the trimethylsilyltriflate (TMSOTf) catalyzed installment of a O-4, O-6 arylidene function, which is followed by the

Scheme 1: One-pot regioselective protection of glucosides.

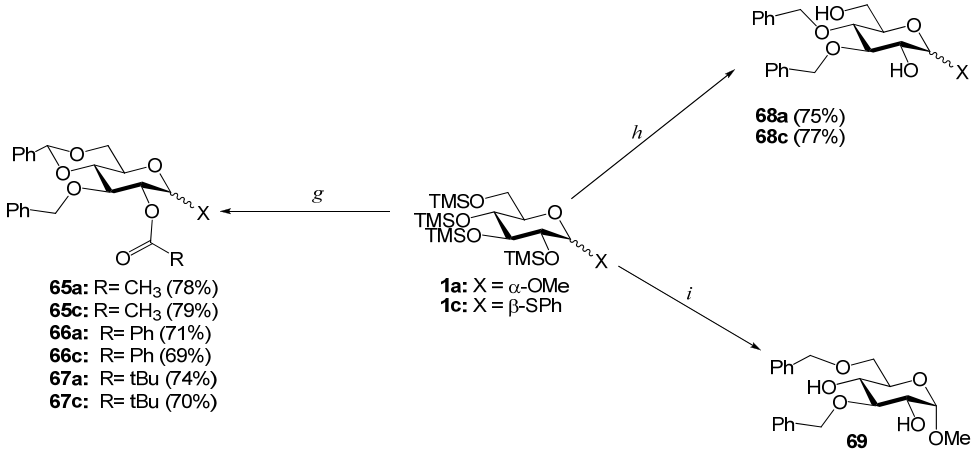


Reagents and conditions; *a*) i- TMSOTf (*cat*), R^1CHO, DCM, 3Å MS, -86 ºC; ii- R^2CHO, Et_3SiH, -86 ºC; iii- TBAF (1 M); *b*) i- TMSOTf (*cat*), R^3CHO, DCM, 3Å MS, -86 ºC; ii- R^2CHO, Et_3SiH, -86 ºC; iii- $(R^1CO)_2O$; iv- BH_3/THF *c*) i- TMSOTf (*cat*), R^3CHO, DCM, 3Å MS, -86 ºC; ii- R^2CHO, Et_3SiH, -86 ºC; iii- TBAF (1 M); iv- base, electrophile; *d*) i- TMSOTf (*cat*), R^3CHO, DCM, 3Å MS, -86 ºC; ii- R^2CHO, Et_3SiH, -86 ºC; iii- $(R^1CO)_2O$; iv- $HCl_{(g)}$, $NaCNBH_3$; *e*) i- TMSOTf (*cat*), R^2CHO, DCM, 3Å MS, -86 ºC; ii- 4-OMePhCHO, Et_3SiH, -86 ºC; iii- TBAF; iv- electrophlie; v- DDQ; *f*) i- TMSOTf (*cat*), R^2CHO, DCM, 3Å MS, -86 ºC; ii- 2-$C_{10}H_7CHO$, Et_3SiH, -86 ºC; iii- Acid anhydride; iv- DDQ.

regioselective formation of a benzyl type ether at O-3. Next, the C-2-OH can be acylated and the arylidene opened to liberate either the C-4-OH or C-6-OH. Alternatively the C-3-benyl ether is removed to expose the C-3-alcohol. Instead of the introduction of a O-2 acyl functionality also the incorporation of various ethers was described. Using the one-pot protocol, Hung and co-workers reported the synthesis of a large panel of differentially protected glucosides, two galactosides, a mannoside and one glucosamine building block.

Simultaneously, Beau and co-workers reported a closely related procedure in which per-silylated glucosides were functionalized with a O-4, O-6 benzylidene acetal and a O-3 benzyl ether using benzaldehyde using $Cu(OTf)_2$ catalysis (Scheme 2).[11] They also demonstrated the possibility to extend the one-pot reaction sequence with an acylation step or a reductive opening of the benzylidene acetal.

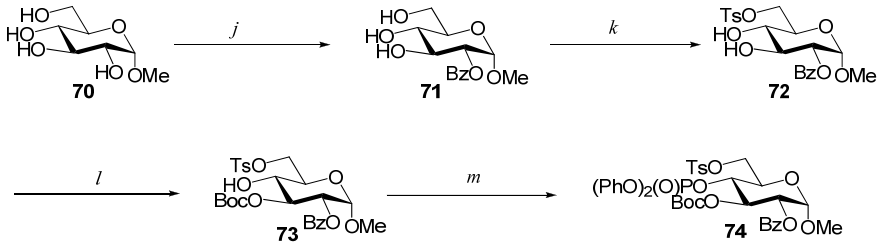
Scheme 2: $Cu(OTf)_2$ catalyzed one-pot regioselective protection of glucosides.



Reagents and conditions; *g*) i- PhCHO, Et_3SiH, $Cu(OTf)_2$, DCM/ACN (4:1), RT (X = α-OMe) or 0 ºC (X = β-SPh), 10 min; ii- Ac_2O, DCM, RT, 1 h or Bz_2O, DCM, reflux, 24 h or Piv_2O, DCM, reflux, 24 h; *h*) i- PhCHO, Et_3SiH, $Cu(OTf)_2$, DCM/ACN (4:1), RT (X = α-OMe) or 0 ºC (X = β-SPh), 10 min; ii- BH_3, THF, $Cu(OTf)_2$, RT, 3 h; *i*) i- PhCHO, Et_3SiH, $Cu(OTf)_2$ (10 mol%), DCM/ACN (4:1), RT (X = α-OMe) or 0 ºC (X = β-SPh), 10 min; ii- Et_3SiH, $Cu(OTf)_2$ (5 mol%), RT, 2 h, 58%.

Stannyl ethers and dialkylstannylene acetals have found wide application in the regioselective protection of carbohydrates ever since their introduction in 1974. Onomura and co-workers recently described the use of a catalytic amount of dimethyltin dichloride (Me_2SnCl_2) for the regioselective protection of various monosaccharides.[12] The regioselectivity in the Me_2SnCl_2 benzoylation was shown to depend on the relative stereochemistry of the hydroxyl functions present. A fully protected α-*O*-methyl glucopyranose was obtained as depicted in Scheme 3. Thus, benzoylation of glucoside **70** provided the C-2 acylated compound **71** in 82% yield. The subsequent tosylation occurred selectively at the C-6 hydroxyl to give diol **72** in 88%. Next a *tert*-butyl carbonate was introduced at the C3-OH and phosphorylation of the remaining alcohol provided the fully functionalized glucoside **74**.

Scheme 3: Dimethyltin dichloride catalyzed regioselective protection of glucose.



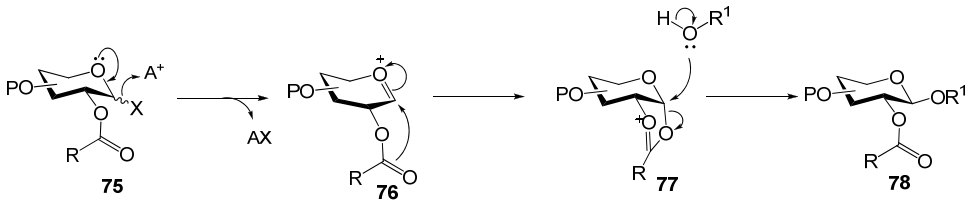
Reagents and conditions; *j*) Me_2SnCl_2, BzCl, DIPEA, THF, RT, 82%; *k)* Me_2SnCl_2, TsCl, DIPEA, THF, RT, 88%; *l)* Me_2SnCl_2, Boc_2O, DIPEA, DMAP, THF, RT, 93%; *m)* $ClP(O)(OPh)_2$, pyr, DMAP, DCM, RT, 95%.

Protecting groups in the stereoselective construction of glycosidic bonds:

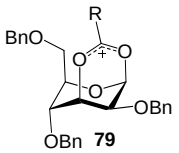
Although the primary purpose of a protecting group is to prevent a given hydroxyl from reacting, it is now well established that the nature of the protecting group has a major effect on the reactivity of glycosyl building blocks and the stereoselectivity and yield of a glycosylation reaction. This is of course best demonstrated considering a C-2 acyl protecting group in a donor glycoside, which not only deactivates this donor species as compared to its C-2 ether counterpart, but also reliably provides anchimeric assistance in the glycosylation process to provide 1,2-*trans* glycosidic bonds (see Scheme 4a). It is now

Scheme 4: Protecting groups providing anchimeric assistance during glycosylation.

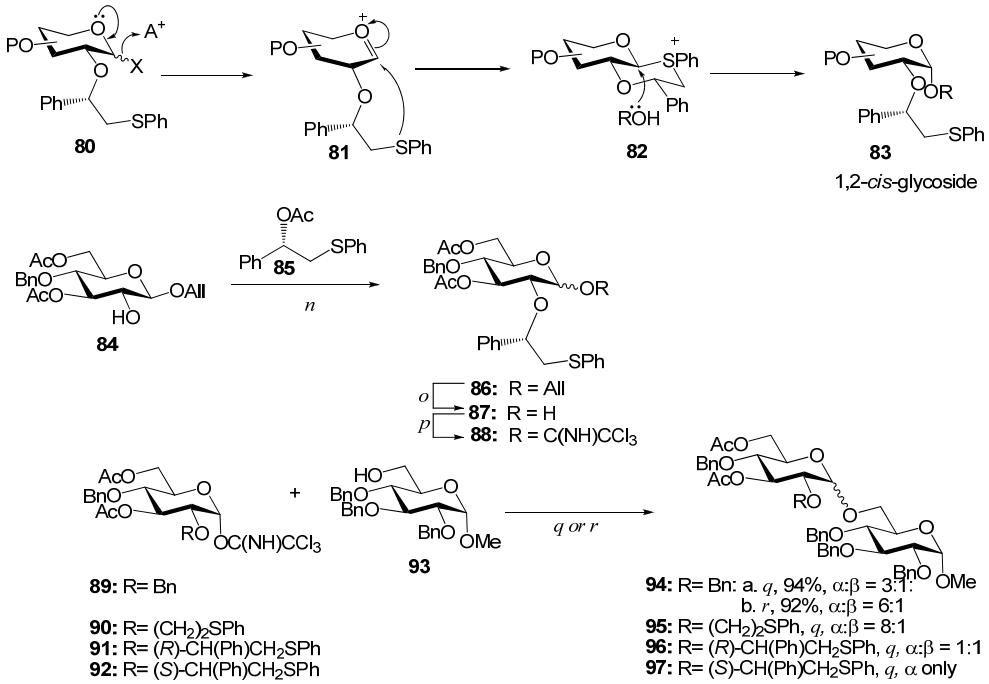
a- Acyl at C-2: Classical neighboring group participation by C-2 ester leading to 1,2-trans glycosides.



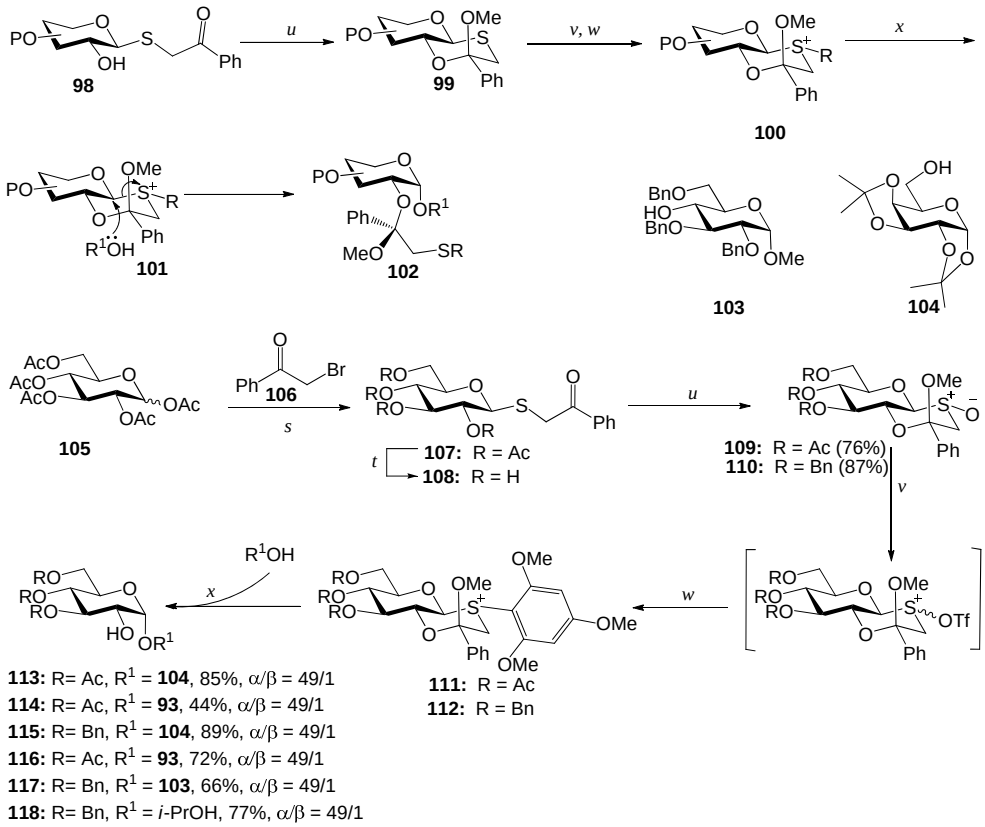
b- Acyl at C-3 in mannosides: participation from (C-3).



c- Boons auxiliary

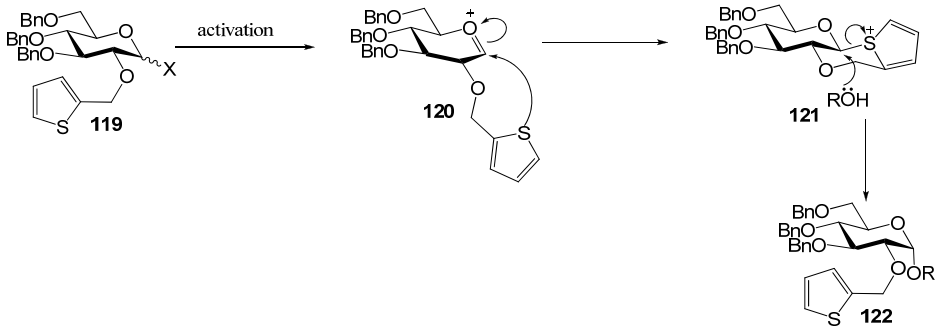


d- Turnbull's strategy.

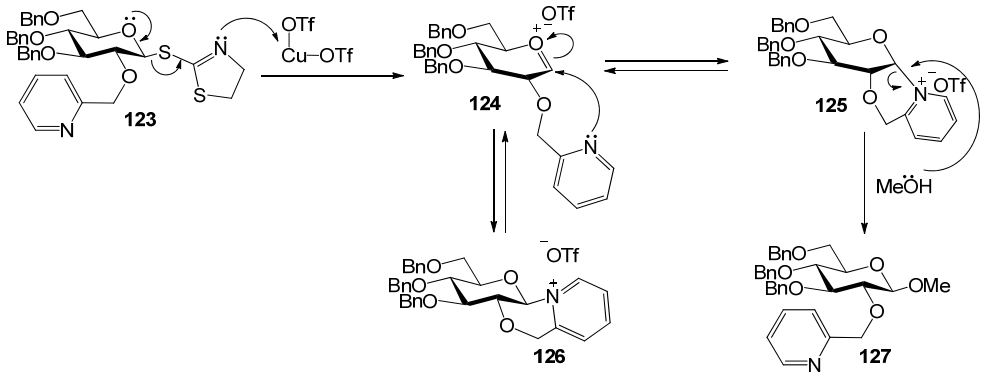


113: R= Ac, R^1 = **104**, 85%, α/β = 49/1
114: R= Ac, R^1 = **93**, 44%, α/β = 49/1
115: R= Bn, R^1 = **104**, 89%, α/β = 49/1
116: R= Ac, R^1 = **93**, 72%, α/β = 49/1
117: R= Bn, R^1 = **103**, 66%, α/β = 49/1
118: R= Bn, R^1 = *i*-PrOH, 77%, α/β = 49/1

e- Fairbanks' strategy.



f- Demchenko's picolyl ether protecting group



Reagents and conditions; *n*) $BF_3{\cdot}OEt_2$, AcOH, DCM, 0 ºC, 10 min, 74%; *o*) $Pd(Ph_3P)_4$, AcOH, RT, 24 h, 90%; *p*) DBU, trichloroacetonitril, DCM, 0 ºC, 1 h, 93%; *q*) TMSOTf, DCM; ; *r*) TMSOTf, PhSEt, DCM; *s*) i- thiourea, $BF_3{\cdot}OEt_2$, ACN; ii- **92**, Et_3N, 64%; *t*) NaOMe, MeOH, 99%; *u*) i- TsOH, MeOH; ii- Ac_2O, pyr or BnBr, NaH, DMF iii- *m*-CPBA; *v*) Tf_2O; *w*) 1,3,5-trimethoxybenzene, DIPEA, -30 ºC; *x*) DIPEA, R^1OH, -10 ºC-50 ºC, 18h.

becoming more and more clear that protecting groups at other positions than the C2-OH can have a powerful stereodirecting effect. For example, Kim and co-workers recently demonstrated that the installment of an acyl function on the C-3 hydroxyl of a mannosyl donor leads to the stereoselective formation of α-mannosides (Scheme 4b) through neighboring group participation.[13]

The stereoselective formation of 1,2-*cis* glycosidic bonds has been a long standing problem in carbohydrate synthesis. In 2005 the group of Boons developed two C-2 OH protecting groups that were capable of promoting the formation of 1,2-*cis* glycosidic bonds by neigbouring group participation.[14] As depicted in Scheme 4C, the (1*S*)-phenyl-2-(phenylsulfanyl)ethyl ether can be introduced at the C2 hydroxyl of glucoside **84** with (1*S*)-phenyl-2-(phenylsulfanyl)ethyl acetate **85** in the presence of $BF_3{\cdot}OEt_2$. The configuration of the chiral center of the newly introduced ether is retained in this reaction because of the intermediate formation of an episulfonium ion, which is displaced at the benzylic position. Using standard protecting group manipulations, glucoside **86** was transformed into trichloroacetimidate **88**. This donor could be (pre)-activated with TMSOTf to provide a meta-stable sulfonium ion **82**, having a *trans*-decalin structure. In this constellation the

phenyl substituent of the C-2 chiral auxiliary occupies an equatorial position. The alternative *cis*-decalin system is not formed because this would place the phenyl group in an unfavorable axial orientation. Nucleophilic displacement of the intermediate sulfonium ion then provides the 1,2-*cis* products. The influence of the C2-chiral auxiliary was compared to the effects of an external sulfide, a non-chiral internal sulfide and the effect of the same auxiliary of opposite chirality. As can be seen in Scheme 4c, the best stereoselectivity was obtained with the (1*S*)-phenyl-2-(phenylsulfanyl)ethyl donor. Along the same line, the Boons laboratory developed the ethoxycarbonylbenzyl ether for the stereoselective construction of α-glucosyl and α-galactosyl linkages. It should be noted that the best selectivities were obtained with electron withdrawing protecting groups on the C-3 OH.[15]

To circumvent the extra synthetic effort that is required to introduce the Boons auxiliary in a suitable donor, Turnbull introduced oxathiane type donors as depicted in Scheme 4d.[16] This type of donor can be activated by transformation into the corresponding sulfoxide which can then be treated with triflic anhydride to provide a glycosylating species. Because the activated leaving group remains attached to the molecule upon glycosylation and thus ends up in the final product, the resulting sulfenyl triflate was transformed into arylsulfonium ion **111** and **112** by treatment with trimethoxybenzene. This species can be displaced with the acceptor alcohol (Scheme 4d). Because of the stability of the intermediate trimethylphenyl sulfonium ion relatively high temperatures are required for this substitution and best results were obtained with primary alcohols. Notably the stereoselectivity of the glycosylations did not depend on the nature of the other protecting groups on the donor glycoside.

The 2-*O*-(thiophen-2-yl)methyl group was introduced by Fairbanks to provide a similar kind of anchimeric assistance as Boons' phenyl-2-(phenylsulfanyl)ethyl ether.[17] Good to excellent α-selectivities were reported for otherwise benzylated donors. No conditions were reported for the removal of the 2-*O*-(thiophen-2-yl)methyl group (Scheme 4e).

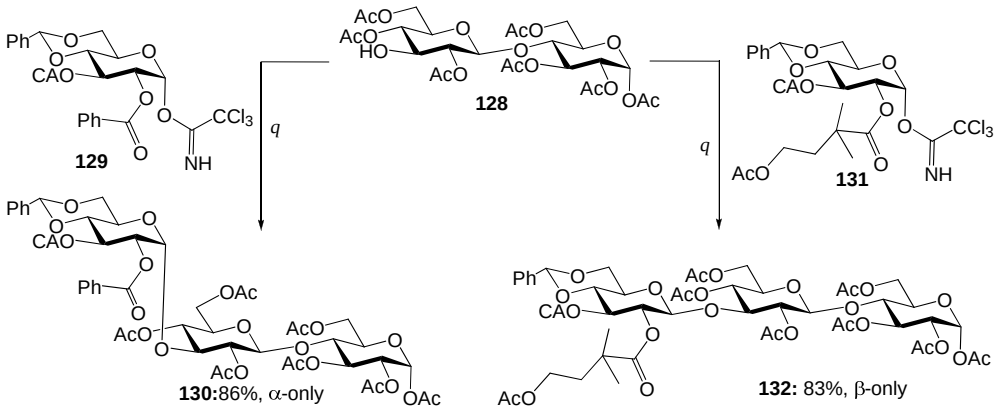
The *trans*-directing effect of the 2-picolyl ether described by Demchenko and co-workers stands in contrast to the α-directing effect of the sulfur-based participating groups (scheme 4f).[18,19] The C-2 picolyl ether was introduced as a "non-disarming" alternative for the participating C-2 acyl function. It was demonstrated that C-2 picolyl *S*-thiazolinyl donor **123** was transformed into a mixture of two bicyclic products, in which the α-oriented pyridinium ion **125** prevailed (20 : 1 at room temperature, 5 : 1 at 50 ^{o}C). The predominant formation of the 1,2-cis bicycle obviously differs from the generation of the β-sulfonium ions described above. Possibly, active participation of the picolyl ether in the expulsion of the *S*-thiazolyl under the mild activation conditions ($Cu(OTf)_2$) is at the basis of this contrasting behavior. The α-pyridinium intermediate could be displaced by a glycosyl nucleophile at elevated temperature (50 ^{o}C) to provide the 1,2-*trans* products. The β-pyridinium ion proved to be inert under these conditions and was isolated after the reaction.

Besides the conceptually novel participating groups described above, several new acyl type protecting functions have recently been reported. For example, the 4-acetoxy-2,2-dimethylbutanoate was introduced as a pivaloyl analogue, which can be removed under relatively mild conditions (Scheme 5a).[20] The 3-(2'-benzyloxyphenyl)-3,3-dimethylpropanoate ester has been developed as a participating group that can be removed by catalytic hydrogenolysis in concert with regularly used benzyl ethers (Scheme 5b).[21] Iadonisi and co-workers introduced alkoxycarbonates as participating functionalities to circumvent orthoester formation, which is a common side reaction when C-2-*O*-acyl protected donors are used in combination with mild activating conditions (Scheme 5c).[22]

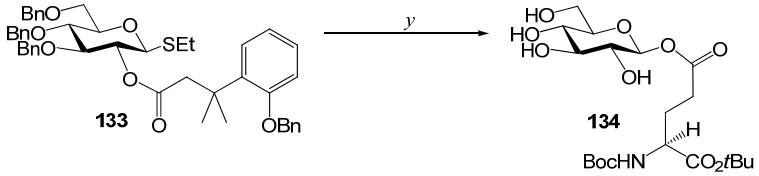
Chapter 2 of this thesis describes that the methylsulfonylethoxycarbonyl group is an orthogonal protecting group for hydroxyl functions in oligosaccharide synthesis that provides anchimeric assistance and excludes orthoester formation, when placed on the C2-OH of a glycosyl donors.[23] The group of Yamago showed that dialkylphosphate esters at the C-2-OH position are stereodirecting protecting groups for the synthesis of 1,2-*trans*-glycosides.

Scheme 5: Novel participating acyl groups.

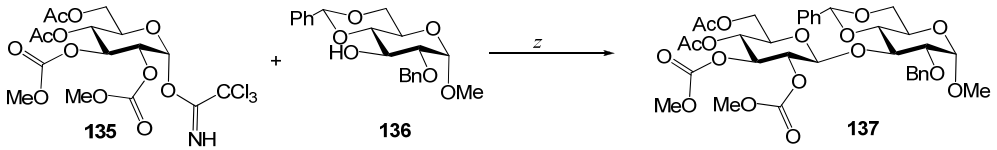
a- 4-acetoxy-2,2-dimethylbutanonyl as 1,2-*trans* directing group.



b- Crich's 3-(2'-benzyloxyphenyl)-3,3-dimethylpropanoate ester.



c- Iadonisi's alkoxycarbonate.



Reagents and conditions; *q*) TMSOTf, DCM; y) i- BocNH-L-Glu-O-*t*-Bu, NIS, TfOH, DCM, -40 ºC, 90%; ii- Pd/C, 3 atm H_2, RT, 86%; *z*) $Yb(OTf)_2$, tol., 50 ºC, 2h, 82%.

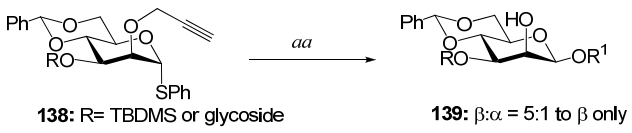
Protecting group size can have a major effect on the stereochemical outcome of a glycosylation reaction. For example, the large C-6 trityl ether has been shown to enhance the α-selectivity of glucosylations, presumably by steric shielding of the β-face. Crich and co-workers have reported on steric buttressing of large protecting groups at the C-3 hydroxyl in 2-*O*-benzyl-4,6-benzylidene mannosyl donors, which typically react in a highly β-selective fashion.[24] Placement of a large *tert*-butyldimethylsilyl (TBDMS) group at the C-3 hydroxyl caused erosion of this selectivity because the large silyl group pushes the C-2 substituent towards the anomeric center of the mannosyl donor thereby obstructing the

nucleophilic attack on the β-face of the molecule. To overcome the poor selectivities of mannosyl donors with bulky C-3 substituents, Crich and co-workers introduced various propargyl ethers as minimally intrusive hydroxyl protecting groups. Firstly, the use of an unsubstituted propargyl ether was reported, which efficiently restored the β-selectivity of mannosyl donor **138** as depicted in Scheme 6a.[25] Because the removal of the propargyl ether required a two step sequence, namely base induced isomerisation followed by oxidative cleavage of the intermediate allene ether by catalytic OsO_4, substituted propargyl ethers were developed next (Scheme 6b). The 1-naphthylpropargyl can be cleaved in a single step using DDQ in wet DCM, but proved to be incompatible with the commonly used sulfonium activator systems BSP/Tf_2O and Ph_2SO/Tf_2O.[26] Furthermore, when placed at the C-2 hydroxyl, it engages in nucleophilic attack of the activated anomeric center. Therefore the 4-trifluoromethylbenzyl propargyl ether group was introduced.[27] This ether was shown to be sterically minimally demanding and compatible with electrophilic activators, while it could be cleaved using lithium naphtalenide (Scheme 6c).

The C-2 propargyl ether was exploited by Fairbanks and co-workers in an intramolecular aglycon delivery (IAD) strategy towards β-mannosides.[28] As depicted in Scheme 6d, the propargyl ether in **144** was isomerised to the allenyl ether, which provided the mixed acetal **147** upon treatment with an glycosyl alcohol and iodonium ions. Dimethyldisulfide-Tf_2O mediated intramolecular glycosylation led to the completely stereoselective formation of the disaccharide **148**.

Scheme 6: Propargyl ethers in carbohydrate chemistry.

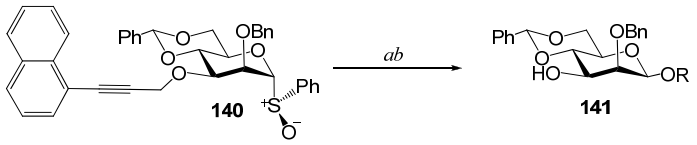
a- unsubstituted propargyl in the synthesis of β-mannosides.



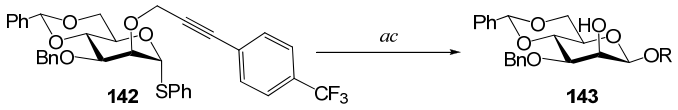
138: R= TBDMS or glycoside

139: β:α = 5:1 to β only

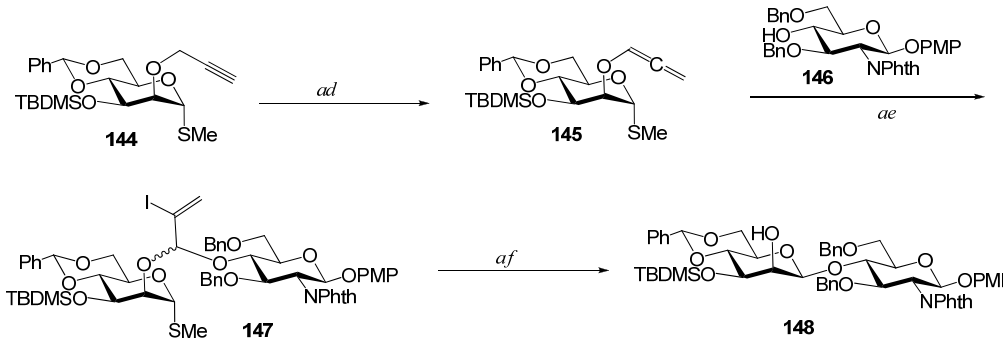
b- 1-Naphthylpropargyl in the synthesis of β-mannosides.



c- 4-Trifluoromethylbenzylpropargyl protective group the synthesis of β-mannosides.



d- Intramolecular aglycon delivery (IAD).



Reagents and conditions; *aa*) i- BSP, TTBP, Tf_2O, DCM; ii- R^1OH; iii- *t*-BuOK, OsO_4, NMNO; *ab*) i- 1-octene, TTBP, Tf_2O, DCM; ii- ROH; iii- DDQ, DCM; *ac*) i- BSP, TTBP, Tf_2O, DCM; ii- ROH; iii- lithium naphthalenide; *ad*) *t*-BuOK, Et_2O, 66%; *ae*) I_2, AgOTf, DTBMP, DMC, -78 °C-RT, 88%; *af*) Me_2S, Tf_2O, DTBMP, DCM, 0 °C-RT, 81%.

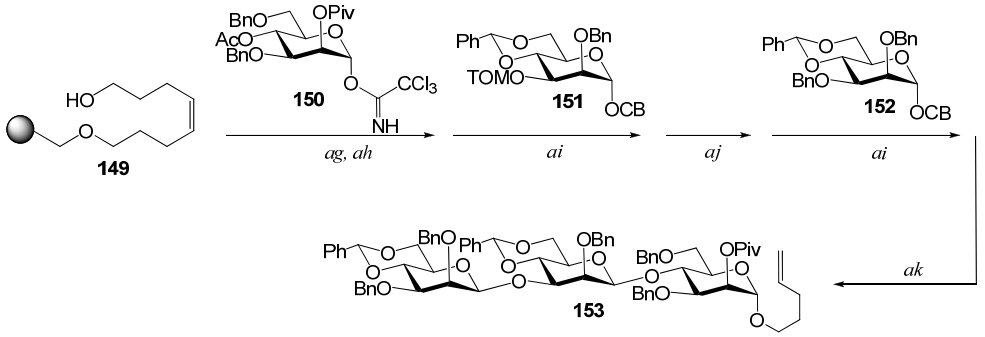
Seeberger and co-workers described another solution to overcome the steric buttressing of the large TBDMS ether described above. They showed that the tri-*iso*-propylsilyloxymethyl (TOM) group, in which the oxymethylene moiety moves the bulk to the silyl group away from the mannosyl core, could be used to install the β-mannosidic linkage.[29] The C-3-*O*-TOM mannosyl donors were used in the automated solid phase synthesis of β-mannosides (Scheme 7a).

Chapter 4 of this thesis describes the methylsulfonylethoxymethyl (Msem) group as a new hydroxyl protecting group in oligosaccharide synthesis.[30] The Msem group is sterically unbiased and could be used for the synthesis of an all *cis*-linked 1,3-*O*-mannotrioside.

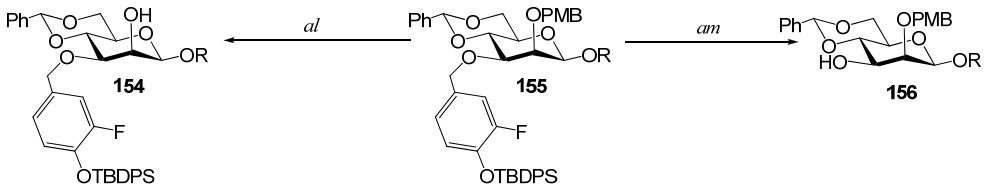
The 4-(*tert*-butyldipehenylsiloxy)-3-fluorobenzyl group was developed as a fluorine labile benzyl ether, attuned to the synthesis of β-mannosides.[31] The fluorine in this *p*-siloxybenzyl type ether was introduced to enhance its stability under acidic and oxidative conditions. The 4-(*tert*-butyldiphenylsiloxy)-3-fluorobenzyl group was introduced using the corresponding benzyl bromide, which was synthesized in three steps from commercially available 3-fluoro-4-hydroxybenzoic acid, and cleaved with tetrabutyl ammonium fluoride (TBAF) at elevated temperatures under microwave irradiation (Scheme 7b). Lower temperatures only led to removal of the silyl group.

Scheme 7: Sterically minimally intrusive silyl based protecting groups in the construction of β-mannosides.

a- TOM protective group.



b- Silyl substituted benzyl ether.

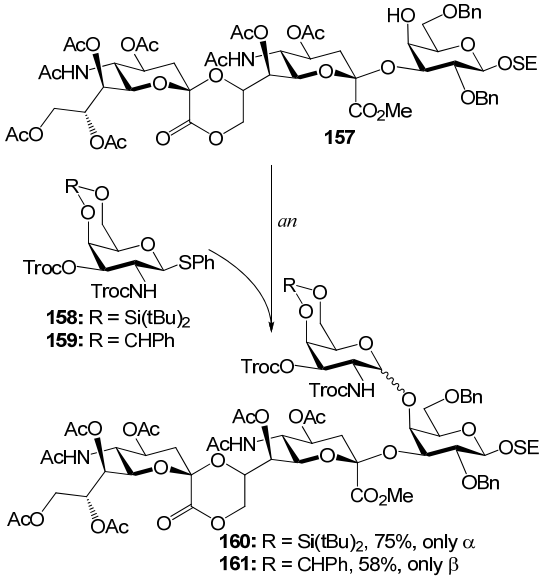


Reagents and conditions; *ag*) TMSOTf, tol., DCM, 15 min, repeat; *ah*) NaOMe, MeOH, DCM, repeat; *ai*) Tf_2O, DTBMP, DCM, -30 °C, 2 h, repeat; *aj*) TBAF, THF, 20 min, repeat; *ak*) the Grubbs catalyst 1st generation, DCM, ethylene atmosphere, overnight; *al*) DDQ, DCM/H_2O, RT, 80-84%; *am*) TBAF, THF, 90 °C, microwave, 74-78%.

The 4,6-di-*tert*-butylsilylene (DTBS) group was introduced in carbohydrate chemistry by Nishimura as a more acid stable alternative to the commonly used cyclic ketal

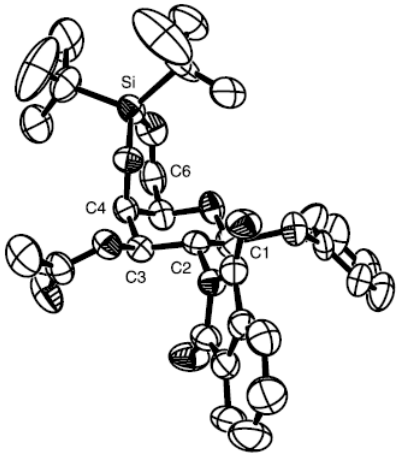
and acetal functions, such as the isopropylidene and benzylidene groups.[32] Dinkelaar *et al.* employed this group for the protection of glucosamine synthons in the assembly of a set of hyaluronan oligosaccharides, where the benzylidene group proved to be insufficiently stable towards the slightly acidic coupling conditions used.[33] Besides its acid stability the 4,6-DTBS has attracted considerable attention because of the α-directing effect it has when mounted on a galactosyl donor.[34] Although the reasons for this stereodirecting effect are not completely clear yet, it has been hypothesized based on a crystal structure of a DTBS-protected thiogalactoside (Figure 1) that the near half chair conformation of the silylene group places one of the *tert*-butyl groups over the β-face of the galactosyl donor during glycosylation (Scheme 8). Notably the α-directing effect is so strong that it can override neighboring group participation by C-2 acyl functions, such as the C-2-*O*-benzoyl, C-2-*N*-trichloroethylcarbonate (Troc), and C-2-N-Phthaloyl (Phth).

Scheme 8: Stereoselective α-galactosylation using 4,6-silylidene galactosides.



Reagents and conditions; *an*) NIS, TfOH, DCM, MS 4Å, 0 °C.

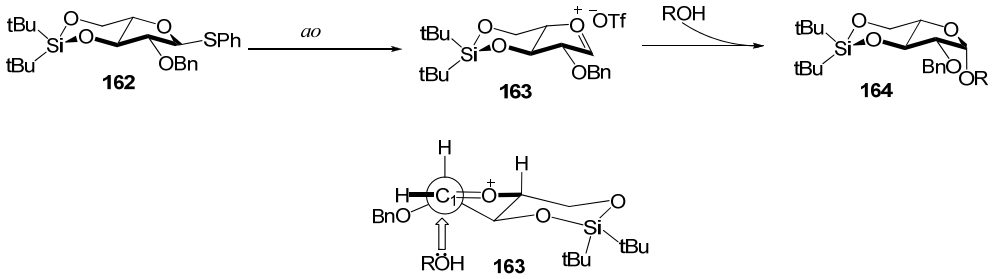
Figure 1: Crystal structure of DTBS protected thioglycoside **158**.



The DTBS has also been applied in the stereoselective synthesis of β-arabinofuranosides.[35] As depicted in Scheme 9, it was proposed that the 3,5-DTBS group

locks the arabinosyl oxacarbenium ion in the E_3 conformation, which is attacked from the β-face in order to avoid eclipsing interaction on the α-face, to provide the 1,2-*cis* arabinosides.

Scheme 9: Stereoselective arabinofuranosylation using a 3,5-DTBS group.

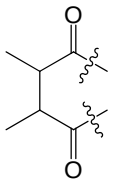


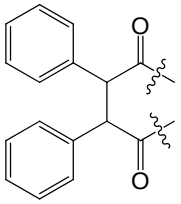
Reagents and conditions; *ao*) NIS, AgOTf, DCM, -30 °C.

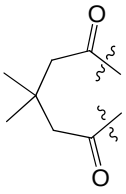
Protecting groups for the amino group in glycosamines:

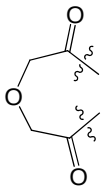
Several new protecting groups for the glucosamine nitrogen function have also been reported recently. Schmidt and co-workers investigated several C_2-symmetric *N,N*-diacyl groups, such as the dimethylmaloyl (DMM), diphenylmaloyl (DPM), dimethylglutaryl (DMG), diglycolyl (DG) and thiodiglycolyl (TDG) group (Figure 2).[36] Of these the DG group proved to perform best in terms of ease of introduction, removal and compatibility in glycosylation reactions. Yang and Yu introduced the *N*-dimethylphosphoryl (DMP) group for the protection of the glucosamine nitrogen. The DMP-group was used in the synthesis of several β-glucosamines, and shown to be stable to certain basic and acidic reaction conditions and could be readily removed using NaOH or hydrazine. Alternatively the *N*-DMP could be transformed into *N*-acyl derivatives using an acyl chloride in refluxing pyridine.[37]

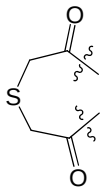
Figure 2: The dimethylmaloyl (DMM), diphenylmaloyl (DPM), dimethylglutaryl (DMG), diglycolyl (DG), thiodiglycolyl (TDG) and dimethylphosphoryl (DMP) groups.

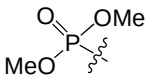

165, DMM


166, DPM


167, DMG


168, DG


169, TDG


170, DMP

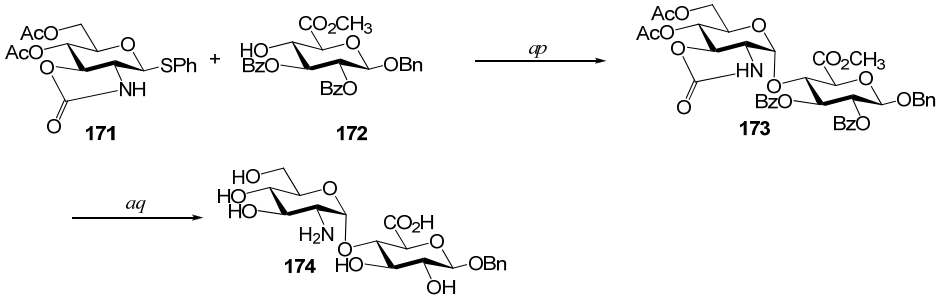
While there is a plethora of *trans*-directing nitrogen protecting groups, very few groups are available to mask the glycosyl amino function with a non-participating group. In fact the azide group has almost exclusively been used for the installation of 1,2-*cis* glycosylamine linkages. In 2001 Kerns and co-workers introduced the oxazolidinone group for the protection of 2-aminoglycosides and it was shown that oxazolidinone protected glucosamine donors stereoselectively provided 1,2-*cis* linked products.[38] Mechanistic studies revealed that the stereochemical outcome of condensations of these donors depends strongly on the nature of the activator and acceptor nucleophile used (Scheme 10c and 10e).[39,40] Kerns and co-workers described that 2,3-oxazolidinone-*N*-acetyl protected glucosamine donor **171** can be activated with benzenesulfinylpiperidine (BSP) and triflic anhydride (Tf_2O) in the presence of tri-*tert*-butylpyrimidine (TTBP), to mainly provide an α-anomeric triflate intermediate. Relatively reactive nucleophiles stereoselectively provided β-linked products, whereas the use of stererically hindered, less reactive nucleophiles led to the predominant formation of the α-products (Scheme 10a). These results were interpreted by assuming that the reactive nucleophiles can displace the α-triflate, but that less reactive nucleophiles can only substitute the more reactive β-triflate, or intermediate oxacarbenium ion. In subsequent studies the groups of Oscarson, Ye and Ito showed that the stereochemical outcome of 2,3-oxazolidinone-*N*-acetyl and 2,3-oxazolidinone-*N*-benzyl protected glucosamine donors could be controlled by tuning the

(Lewis)-acidity of the employed activator systems.[40] Less acidic conditions mainly led to the isolation of β-linked products, where a more acidic milieu favored the formation of α-isomeric products. Convincing evidence has been forwarded that the glucosamine donors initially provide the β-linked products, which rapidly anomerise to the more stable α-isomers under acidic conditions through an *endo*-cyclic ring opening pathway (Scheme 10b and 10d).

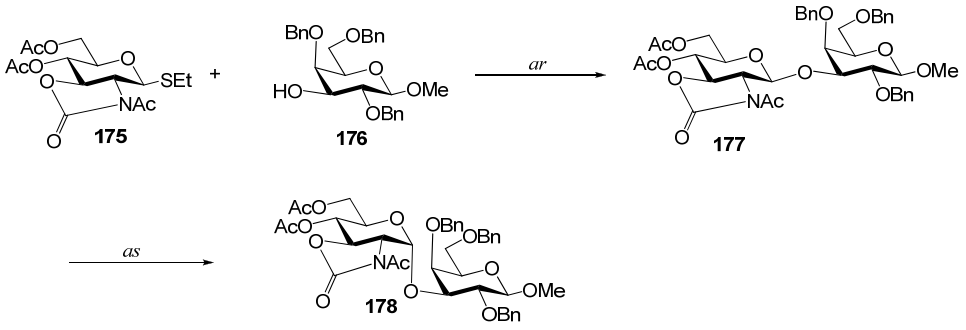
It is also of interest to note the beneficial effect of the 2,3-oxazolidinone-*N* group on the nucleophilicity of the C-4 hydroxyl in *N*-acetyl glucosamines. The reason for this enhanced reactivity probably originates from the tied back nature of the oxazolidinone group.[41]

Scheme 10: Stereoselective condensations of 2,3-oxazolidinone protected glucosamine donors.

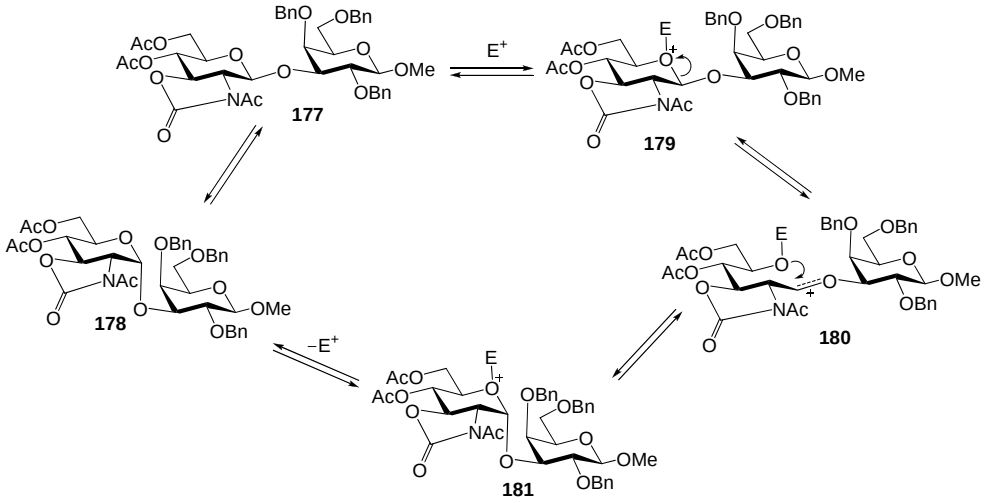
a- Synthesis of a deprotected disaccharide using a 2,3-oxazolidinone protected glucosamine.



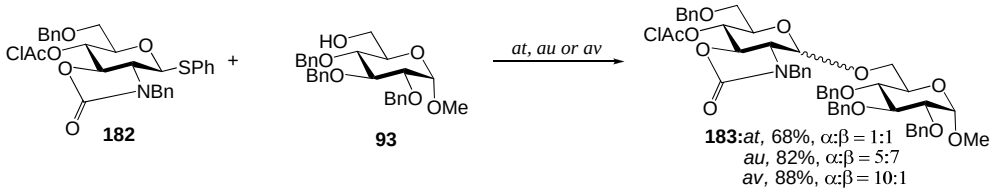
b- Conversion of the stereochemistry under Lewis acid conditions.



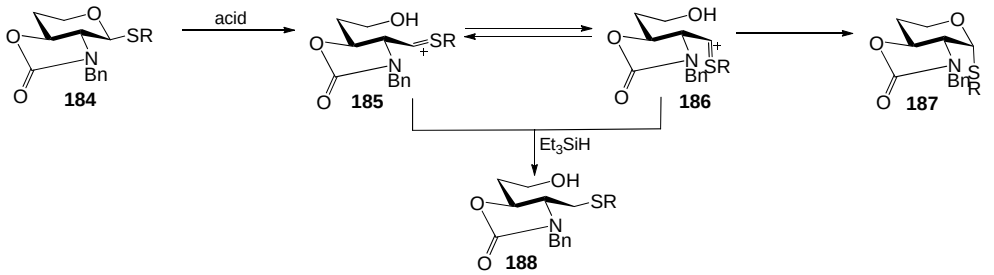
c- Mechanism of the change is stereochemistry.



d- Difference in stereoselectivities with different activator system



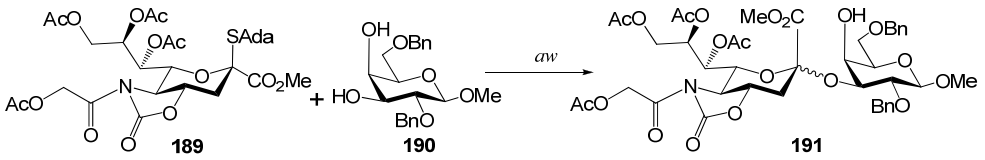
e- Mechanistic explanation of difference is stereoselectivities.



Reagents and conditions; *ap*) PST, DCM, -78 °C, 75%; *aq*) NaOH, H_2O/THF, 80%; *ar*) NIS, AgOTf (0.1 eq) , DCM, RT; *as*) AgOTf (0.4 eq), 82%; *at*) PhSCl, AgOTf, DTBMP, DCM, RT; *au*) *N*-(phenylthio)-e-caprolactam, Tf_2O, DCM, RT; *av*) PhSCl, AgOTf, DTBMP, tol/1,4-dioxane (3:1), 0 °C- RT.

Finally, the application of the 4,5-oxazolidinone group in the synthesis of α-sialosides deserves mentioning. Various groups have reported on the excellent stereoselectivity achieved with sialic acid donors bearing a 4,5-oxazolidinone group, culminating in various one-pot multi-step glycosylation procedures (Scheme 11).[41a,42]

Scheme 11: Stereoselective sialylations of 4,5-oxazolidinone protected *N*-acetyl sialic acids.



Reagents and conditions; *aw*) NIS, TfOH, DCM/ACN (2:1), -78 °C, 64%, α:β = 15:1.

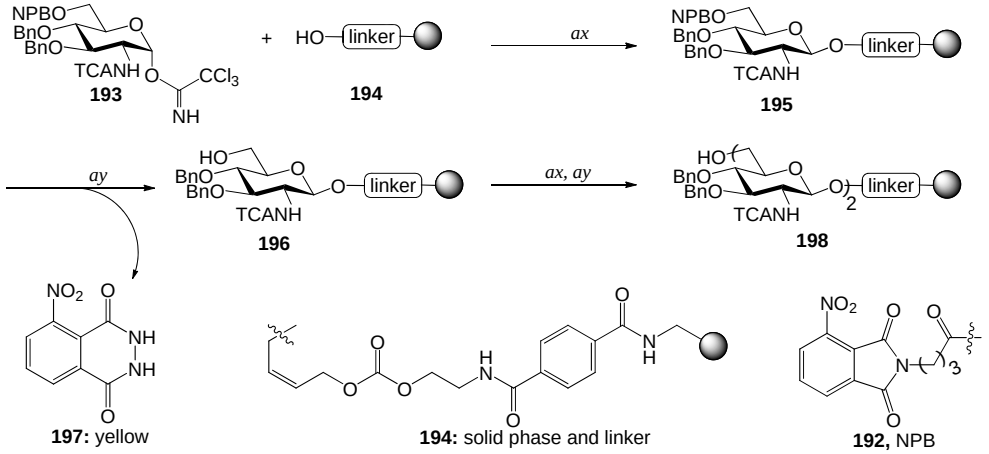
Removal of the oxazolidinone moiety can be accomplished using a variety of nucleophiles (NaOMe in MeOH, LiOH/LiCl in THF/H_2O) but the selective removal of the oxazolidinone in *N*-acyl oxazolidinones has been shown to be difficult in many cases.[39,41a,42b,43]

Protecting groups as visualization tags:

Besides the primary role of masking a functional group on a carbohydrate, protecting groups can be used to introduce extra functionality in a carbohydrate building block. The UV-active 9-fluorenylmethoxycarbonyl (Fmoc) group has been extensively used as an amine protecting group in automated solid phase peptide chemistry to monitor the efficiency of the coupling steps.[44] This group has also found application as a hydroxyl protecting group in the automated synthesis of oligosaccharides.[45] Because the Fmoc is rather base labile when mounted on an alcohol, Pohl and co-workers set out to develop an alternative UV-active hydroxyl protecting group. They introduced the nitrophthalimidobutyric (NPB) ester, which can be introduced on a given hydroxyl function using the corresponding acid.[46] Cleavage of the NPB ester can be accomplished with hydrazine acetate in DMF at elevated temperature (50 ^{o}C) to provide the orange 3-nitrophthalhydrazide **197**, which can be used in the colorimetric monitoring of reaction cycles. To illustrate its applicability Pohl and co-workers described the solid phase synthesis of a resin bound glucosamine dimer **198** as displayed in Scheme 12. Aminomethylated polystyrene, functionalized with an allyl carbonate linker **194**, was glycosylated with trichloroacetimidate donor **193** using a double glycosylation cycle. Cleavage of the NPB ester liberated the primary alcohol for further chain elongation and

simultaneously allowed the colorimetric monitoring of the coupling efficiency, which was determined to be 98%. The second coupling/deprotection cycle proceeded in 96% yield. The dimer was not cleaved form the resin.

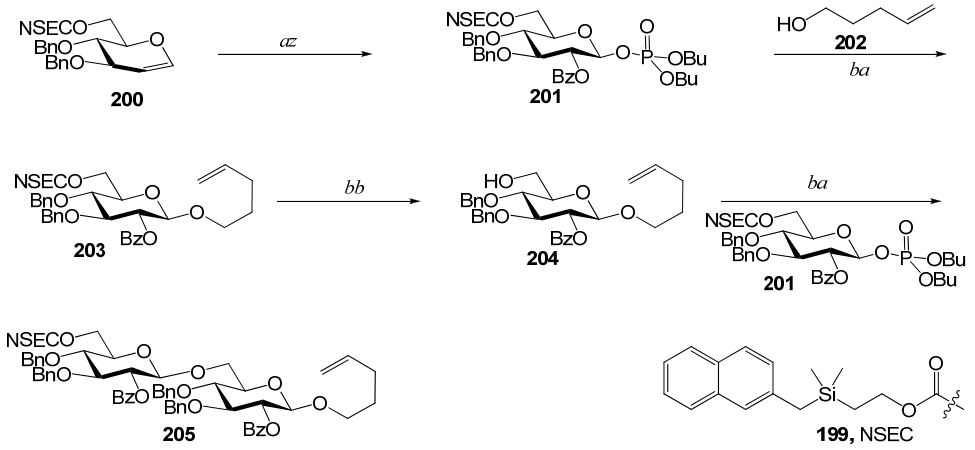
Scheme 12: The NPB ester in the synthesis of resin-bound dimer **198**.



Reagents and conditions; *ax*) i- TMSOTf, DCM, 15 min; ii- rinse; iii- repeat i and ii; *ay*) i- $H_2NNH_2 \cdot AcOH$, DMF, 15 min; ii- rinse.

The Seeberger laboratory introduced the UV-active 2-[dimethyl(2-naphtylmethyl)silyl]ethoxycarbonyl (NSEC) as a novel group to mask carbohydrate hydroxyl functions.[47] The NSEC group was shown to be compatible with various reactions commonly employed in carbohydrate synthesis and could be selectively cleaved with tetrabutylammonium fluoride (TBAF) (Scheme 13). The NSEC group was developed to allow UV-monitoring of glycosylation efficiency during automated synthesis but no such application has been reported yet.

Scheme 13: The NSEC group.

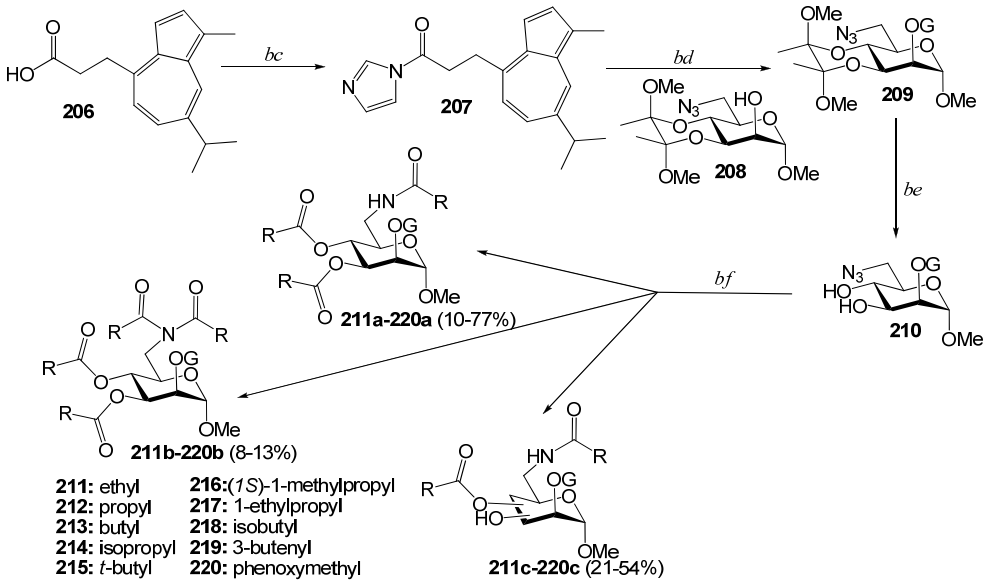


Reagents and conditions; *az*) i- DMDO, DCM, 30 min; ii- $HOP(O)(OBu)_2$, DCM, -78 °C, 30 min; iii- BzCl, DMAP, 0 °C, 3h, 47%; *ba*) TMSOTf, DCM, -78 °C to -30 °C, 3h ; *bb*) TBAF, THF, 0 °C, 50 min, 96%.

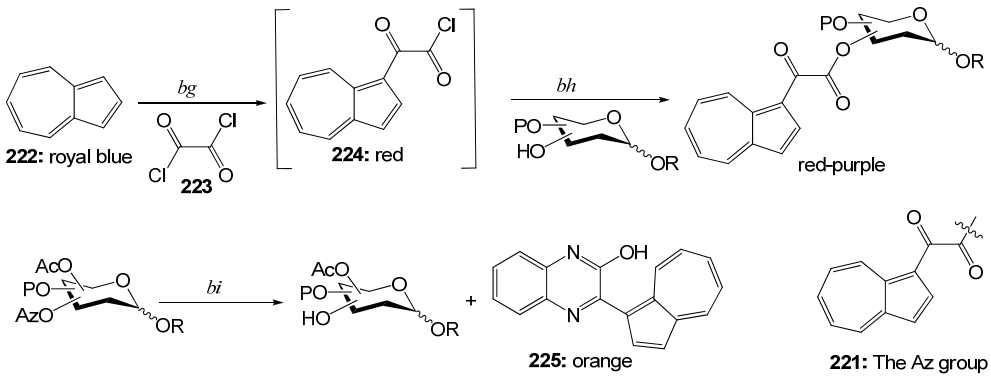
Several recent reports have described the use of azulene derived protecting groups. Azulene is a bicyclic hydrocarbon having a fused 7- and 5-membered ring and a 10-electron π-system, and an intense blue color. Lindhorst and Aumüller used guajazulene derived acid **206** for the protection of mannosyl alcohol **208**.[48] After deprotection of the BDA-acetal, the mannoside was used in the synthesis of mixtures of acylated products. The blue color of the products allowed the visualization of the products during silica gel column chromatography (Scheme 14a). Timmer *et al.* introduced the azulen-1-yl-dicarbonyl (Az) group **221**, which was used to protect a variety of carbohydrate alcohols.[49] It was introduced from the corresponding acid chloride, and could be removed using catalytic NaOMe in methanol or diaminobenzene and acetic acid in refluxing ethanol. The latter deprotection conditions allowed the selective removal of the Az-ester in the presence of acetyl groups, providing the colored benzopyrazine **225** as a side product (Scheme 14b). The Az-group was shown to be compatible with glycosylation conditions involving trichloroacetimidate donors (catalytic TMSOTf), but NIS-TfOH mediated activation of an Az-protected thiophenyl galactoside led to thiophenylation of the Az-group. The color of the Az-group aided in the monitoring of reactions by TLC analysis and purification via column chromatography.

Scheme 14: Azulene based protecting groups in carbohydrate chemistry.

a- The gaujazulene (G) protective group.



b- The Az protective group.



Reagents and conditions; *bc*) CDI, DMF, RT, 1h; *bd*) DBU, DMF, 0 °C, overnight, 46% over two steps; *be*) DMP, $H[BF_4]$, DCM, RT, 2 h, 78%; *bf*) PMe_3, $(RCO)_2O$, THF, RT, overnight; *bg*) toluene; *bh*) DCM, pyr., 89-99%; *bi*) diaminobenzene, AcOH, EtOH, reflux, 94-98%.

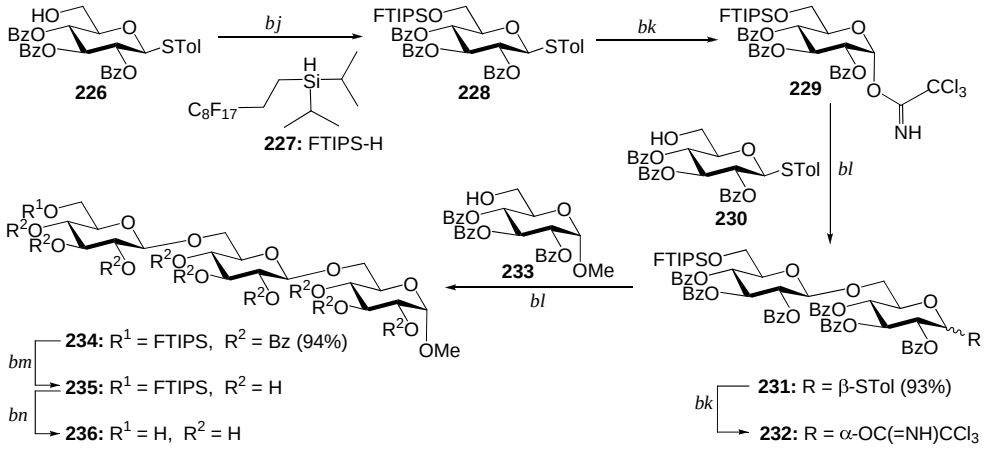
Protecting groups as purification handles:

Fluorous chemistry has been applied in various chemistry areas,[50] including catalysis,[51] combinatorial and parallel synthesis,[52] and selective tagging of biomolecules.[53] The properties of fluorous tags, being both hydrophobic and lipophobic, have been widely used in protecting group chemistry. Both "heavy" and "light" fluorous tags have been introduced as purification handles and their use has been extensively reviewed. "Heavy" fluorous groups are characterized by the presence of many fluorine atoms (39 or more) on multiple alkyl chains, often described as ponytails.[54] The high fluorine content of these groups renders the molecules to which it is attached soluble in a fluorous solvent but insoluble in both organic and aqueous solvents. "Heavy" fluorous compounds can therefore be purified from non-fluorous compounds by simple liquid-liquid extractions. "Light" fluorous groups contain significantly less fluorine atoms, typically between 9 and 17. Because of the lower fluorine content these molecules are often cheaper, easier available and much more soluble in organic solvents.[55] Purification of "light" fluorous compounds can be effected by fluorous solid phase extraction (FSPE) techniques.[56] Light fluorous versions of the most commonly used carbohydrate protecting groups have been described, including fluorous benzyl,[57] trityl,[58] allyl,[59] and pentenyl ethers,[60] the fluorous benzylidene acetal,[61] and various fluorous acyl[62,59d] and silyl based[63] groups.

In analogy to oligosaccharide synthesis on solid or soluble polymeric supports, two strategies can be followed in the fluorous supported assembly of oligosaccharides. In a "donor-bound" strategy, the growing oligosaccharide chain is build up from the non-reducing end, with a donor glycoside bearing a fluorous protecting group/tag. The "acceptor-bound" strategy on the other hand starts with a fluorous acceptor building block. Both strategies have been employed, but the latter has found most applications, because most side reactions in a glycosylation reaction take place on the donor glycosides. Liu and co-workers described the synthesis and application of fluorous glycosyl donor, in which the light fluorous tag was attached to the C-6 hydroxyl *via* a di-*iso*-propylsilyl ether.[63c] As depicted in Scheme 15, *S*-tolyl glucoside **226** was silylated with fluorous di-*iso*-

propylsilane under the agency of triflic acid (TfOH) and subsequently converted into trichloroacetimidate **229**. This donor was glycosidated with an excess of primary acceptor **230** to provide the disaccharide **231** in 93% yield. The *S*-tolyl disaccharide was transformed into a trichloroacetimidate to be coupled to reducing end glucoside **233** in the next step. After both glycosylation steps, FSPE was used to purify the products and recover the excess acceptor. Purification by silicagel chromatography was required in the transformation of the thioglycosides into the corresponding trichloroacetimidates. The fluorous di-*iso*-propylsilyl ether was cleaved at the end of the synthesis using 0.02 M HCl in MeOH/H_2O.

Scheme 15: Donor-bound fluorous synthesis of a trisaccharide using a fluorous di-*iso*-propylsilyl ether (FTIPS).

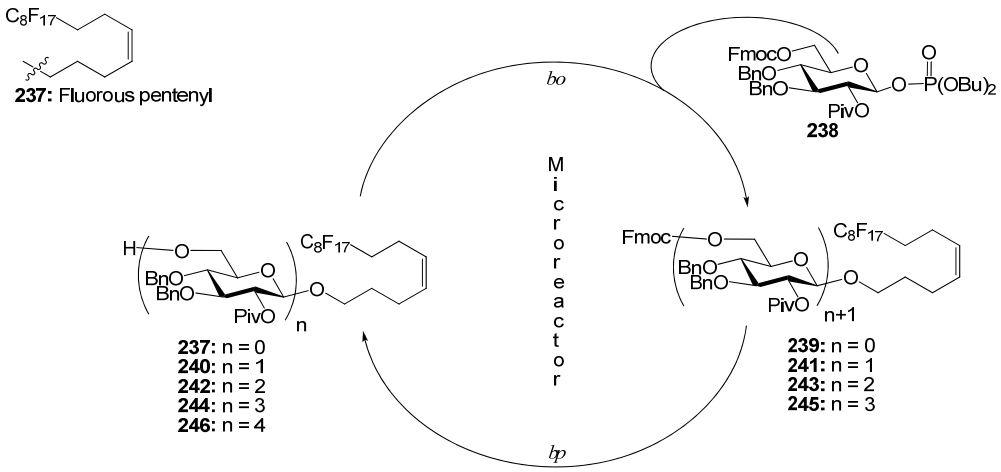


Reagents and conditions; *bj*) i- TfOH, 0 °C; ii- 2,6-lutidine, DCM, RT, 99%; *bk*) i- NBS, TMSOTf, DCM/H_2O, 0 °C-RT; ii- Cl_3CCN, DBU, DCM, RT, 76%; *bl*) i- TMSOTf, DCM, MS 4Å, -40 °C; ii- FSPE; *bm*) i- NaOMe, MeOH/DCM, RT; ii- FSPE, 94%; *bn*) i- $HCl_{(aq)}$, MeOH, RT; ii- FSPE, 84%.

An example of an "acceptor-bound" oligosaccharide assembly strategy is depicted in Scheme 16. In 2007, Seeberger and co-workers introduced the fluorous version of the *n*-pentenyl group **237**, which was employed in the assembly of a tetrasaccharide.[60] A silicon-glass microreactor was used for the optimization of the glycosylation reactions. It was described that β-glycosyl phosphate donor **238** could be employed at room temperature using reaction times ranging from 20 to 60 seconds (Scheme 16). The first glycosylation had to be executed in a mixture of dichloromethane and trifluorotoluene (TFT) to keep the

fluorous pentenyl alcohol in solution. The lipophilic O-6 Fmoc protecting group was removed in the quenching step to facilitate purification by FSPE (Scheme 16). After oligosaccharide assembly, the fluorous *n*-pentenyl group could be cleaved using *N*-bromosuccinimide or transformed into different functional groups.

Scheme 16: Acceptor-bound fluorous synthesis of a oligosaccharide using the fluorous pentenyl group.

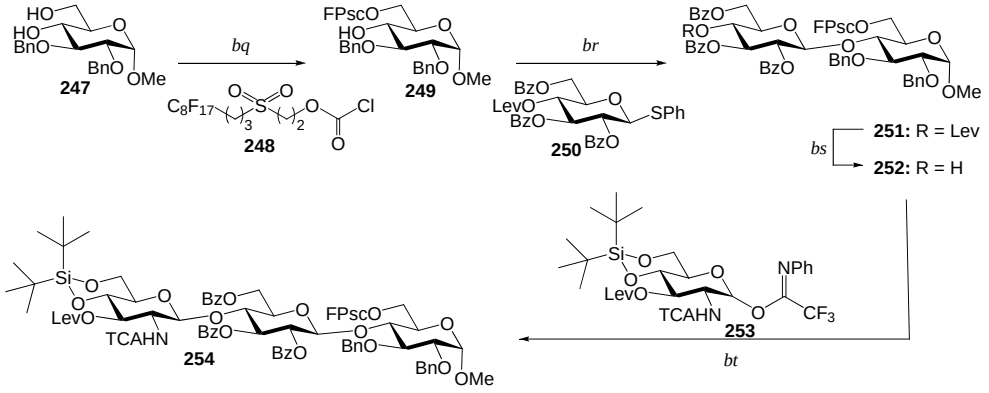


Reagents and conditions; *bo*) TMSOTf, DCM or TFT, 0 °C or 20 °C; *bp*) i- piperdine/DMF (1:4), TBAF; ii- FSPE .

In chapter 3, the fluorous version of the Msc-group is introduced.[50e] It was found that an ethylene insulator, which is commonly used to spacer a fluorous tail from a functional group in a fluorous protecting group, made the FMsc very base labile. Therefore the C_8F_{17}-moiety was removed from the sulfonyl group by a C3 spacer to provide the fluorous propylsulfonylethoxycarbonyl (FPsc) group.[23] This sulfonyl carbonate was successfully employed in the synthesis of a trisaccharide as depicted in Scheme 17. First the FPsc was regioselectively introduced at the primary alcohol of glucoside **247**. The resulting acceptor was coupled with levulinoyl protected thioglucoside **250** to provide the fluorous dimer **251** in 93% yield after FSPE. Deprotection of the Lev-ester then gave disaccharide

252 (81% after FSPE), which was elongated with glucosamine **253** to yield the trisaccharide **254** in 78% after FSPE.

Scheme 17: Oligosaccharide synthesis using the FPsc group.



Reagents and conditions; *bq*) pyr., DCM, -40 ºC-RT, 4 h, 94%; *br*) i- NIS, TMSOTf, DCM, 0 ºC-RT, 1h; ii- FSPE, 93%; *bs*) i- $H_2NNH_2.H_2O$, pyr./HOAc, 5 min; ii- FSPE, 94%; *bt*) i- TfOH, DCM, -20 ºC-RT, 15 min; ii- FSPE, 78%.

Besides fluorous supports, large lipophilic moieties and ionic liquids have also been recently introduced for the construction of oligosaccharides.

Conclusion:

Protecting groups take up a central role in carbohydrate chemistry and hold a key position in controlling the stereoselectivity of glycosylation reactions. Over the years several new and ingenious protecting groups have been added to the broad palette of carbohydrate protecting groups to allow the stereoselective construction of both 1,2-*cis* and 1,2-*trans* linkages. New protecting groups, with tailor-made properties in terms of chemical stability and lability, allow ever more sophisticated glycosylation schemes to be developed, while colored or UV-active groups and purification tags continue to increase the efficiency of oligosaccharide assembly. Given the growing demand for ever more and complex, pure and well-defined oligosaccharides in all fields of glycoscience, it is anticipated that the

development of new protecting groups and protection/deprotection schemes will continue to be a major theme in carbohydrate chemistry.

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CHAPTER

2

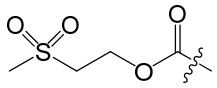
The methylsulfonylethoxycarbonyl (Msc) as hydroxyl protecting group in carbohydrate chemistry[1]

Introduction:

The synthesis of (complex) oligosaccharides is a multi-step process, in which protective group manipulations play a central role.[2] Protecting groups in the donor and acceptor molecules, the reaction partners in a glycosylation event, not only control the regioselectivity but also determine the productivity and stereochemistry of glycosylation reactions.[3] Additionally, elongation of oligosaccharides generally requires the selective removal of one of the protecting groups in the growing chain. Therefore, progress in the assembly of oligosaccharides can be realized by the development of new protecting groups with improved properties, such as ease of introduction and removal and orthogonality toward other protective groups.[4] This chapter describes the use of the

methylsulfonylethoxycarbonyl group (Msc, **1**, Figure 1) as hydroxyl protecting group in carbohydrate chemistry.

Figure 1: The Msc protecting group.



1, Msc

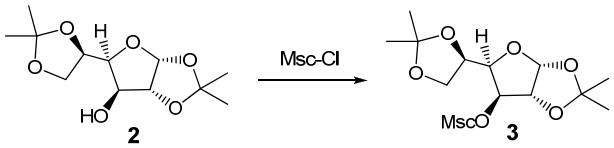
The methylsulfonylethoxycarbonyl (Msc) group, developed by Tesser and coworkers,[5] is well known in peptide chemistry for the protection of amino functions. The Msc-group is removed by base-mediated β-elimination while it resists catalytic hydrogenation and is highly stable in acidic media. The Msc group has also proven to be suitable for the protection of the guanidino function in the side chain of arginine during solid phase peptide synthesis.[6] In addition, methylsulfonylethyl esters have been employed to mask carboxylic acids[7] and used as protective groups en route to phosphate mono- and diesters.[8,9] Conversely, the sulfonylethoxycarbonyl groups, related to the Msc group, have only scarcely been applied to protect alcohol functions.[10,11] In the meantime, the 9-fluorenylmethyl carbonate (Fmoc) is becoming increasingly popular in carbohydrate chemistry for the protection of alcohol functions.[12,13] It was envisaged that the Msc group could be a promising hydroxyl protecting group as it would be equally stable but sterically less demanding and less lipophilic than the Fmoc carbonate.

Results and discussion:

As a first research objective, the optimal conditions for the introduction of the Msc group were investigated using glucofuranose **2** as a model compound (Table 1). In the first attempt a DCM solution of compound **2** was treated with methylsulfonylethoxycarbonyl chloride (Msc-Cl) and 3 equivalents of triethylamine (TEA) (Table 1, Entry 1). The reaction did not proceed and the starting material could be recovered. Employment of the same solvent and 2,6-lutidine (3 eq.) as a base led to the isolation of 1,2:5,6-di-*O*-isopropylidene-3-*O*-methylsulfonylethoxycarbonyl-α-D-glucofuranose **3** in moderate yield (41%, Table 1, Entry 2). Using pyridine (3 eq.) as base in dioxane (Table 1, Entry 3) the

reaction proceeded equally sluggishly, but the yield of **3** was improved to 55%. Returning to the use of DCM as a solvent not only reduced the reaction time to 4 hours, but also increased the yield of **3** to 99% (Table 1, Entry 4).

Table 1: Installation of the Msc group on carbohydrate hydroxyls.

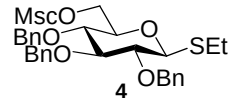
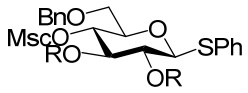
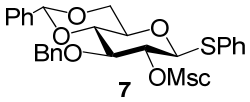
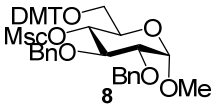
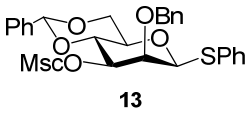
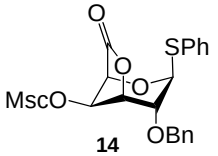
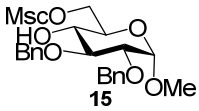


2 **3**

Entry	Conditions	Time	Yield
(1)	DCM, Et_3N (3 eq.), 0 ^oC-rt	90h	No Conversion
(2)	DCM, Lutidine (3 eq.), 0 ^oC-rt	90h	41%
(3)	Dioxane, Pyridine (3 eq.), 0 ^oC-rt	90h	55%
(4)	DCM, Pyridine (3 eq.), 0 ^oC-rt	4h	99%

The applicability of the DCM/pyridine conditions was further evaluated by the introduction of the Msc group onto a range of partially protected pyranose building blocks. The Msc group was readily introduced on the primary hydroxyl function of ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside to give **4** in 98% yield (Table 2, Entry 1). The protection of a variety of secondary hydroxyl functions with the Msc group proceeded uneventfully, leading to high yields of the expected products (Table 2, Entry 2-6).[14] It is of interest to note that migration of the benzoyl group in the starting compound (Table 2, entry 2) was not observed and that the labile galacturonic acid lactone endured the mild conditions (Table 2, Entry 6). Moreover, subjection of ethyl 2,3-di-*O*-benzyl-α-D-glucopyranoside to these conditions, albeit at a lower temperature (-20 ^oC), led to the regioselective introduction of the Msc group at the primary position of the diol starting compound (Table 2, Entry 7).

Table 2: Installation of the Msc on carbohydrate hydroxyls.[a]

Entry	Product	Temperature	Time	Yield
1	 **4**	0 ºC-RT	3 h	98%
2	 **5** R = Bn **6** R = Bz	0 ºC-RT	5 h	**5** (R = Bn): 78% **6** (R =Bz): 76%
3	 **7**	0 ºC-RT	4 h	79%
4	 **8**	0 ºC-RT	4 h	79%
5	 **13**	0 ºC-RT	4 h	93%
6	 **14**	0 ºC-RT	3 h	88%
7	 **15**	-20 ºC-RT	5 h	90%

[a] Msc-Cl (2 eq.), pyridine (3 eq.), DCM (0.2 M).

Next, the most favorable conditions for cleavage of the Msc group were examined using 1,2:5,6-di-*O*-isopropylidene-3-*O*-methylsulfonylethoxycarbonyl-α-D-glucofuranose **3**. As summarized in Table 3, the use of a catalytic amount of sodium methoxide (NaOMe, 0.1 eq.) in methanol required 18 hours to completely remove the Msc group (Table 3, Entry

1). The deblocking of the Msc on **3** *via* a β-elimination with the aid of 30 equivalents of triethylamine reached completion after 20 hours (Table 3, Entry 2). On the other hand, tetrabutylammonium fluoride (TBAF, 0.1 eq.) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.1 eq.) eliminated the Msc group within 30 minutes (Table 3, Entries 3 and 4). The removal of the Msc group from **6** went smoothly and left all the benzoyl groups intact, thereby illustrating the mildness of the cleavage conditions (Table 3, Entry 5). Analogously, cleavage of the Msc group from galacturonic acid lactone **14**[15] was accomplished without compromising the integrity of the labile lactone ring to afford the expected alcohol in 97% yield (Table 3, Entry 6).

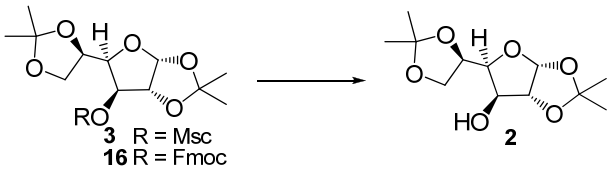
Table 3: Cleavage of the Msc group.

Entry	Substrate	Conditions	Quantity	Time	Yield
1	**3**	NaOMe, MeOH	0.1 eq	18 h	100%
2	**3**	Et_3N, DCM	30 eq	20 h	100%
3	**3**	TBAF, THF	0.1 eq	30 min.	100%
4	**3**	DBU, DMF	0.1 eq	25 min.	100%
5	**6**	DBU, DMF	0.1 eq	30 min	98%
6	**14**	DBU, DMF	0.1 eq	1 min.	97%

Having established the conditions for both installation and cleavage of the Msc group, the stabilities of the Msc group and the 9-fluorenylmethoxycarbonyl (Fmoc) group were compared. With 0.1 equivalents DBU the Fmoc group was cleaved from glucofuranose **16** within 5 minutes while the removal of the Msc group in the corresponding glucofuranose **3** needed 25 minutes (Table 4, Entry 1 and 2). The removal of the Fmoc group in **16** required 2 hours when triethylamine (TEA, 30 eq.) was used in DCM (Table 4, Entry 3) while cleavage of the Msc group in **3** under identical conditions took 20

hours (Table 4, Entry 4). The outcome of these experiments indicates that the Msc group is slightly more stable than the Fmoc group.

Table 4: Comparison of the stability of the Msc group and the Fmoc group.

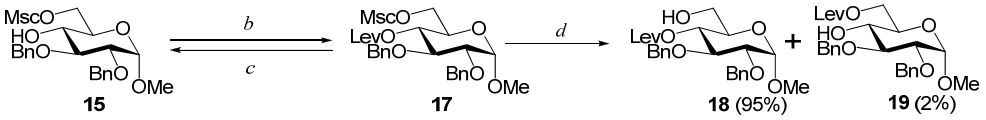


Entry	Conditions	R	Quantity	Time	Yield
1	DBU, DMF	Fmoc	0.1 eq	5 min	87%
2		Msc	0.1 eq	25 min.	92%
3	Et_3N, DCM	Fmoc	30 eq	2 h	89%
4		Msc	30 eq	20 h	93%

Protecting groups that can be selectively cleaved en route to a target oligosaccharide are of prime importance in synthetic carbohydrate chemistry. As the Msc group could be selectively cleaved in the presence of benzoyl esters (*vide supra*), the orthogonality of the Msc group and the levulinoyl (Lev) ester was explored. To this end alcohol **15** was levulinoylated to provide fully protected glucopyranoside **17** (Scheme 1).

The levulinoyl group of **17** could be cleaved without affecting the Msc carbonate at the primary C6-OH position by standard treatment with hydrazine hydrate in a mixture of

Scheme 1: Orthogonality of the Msc and the Lev.

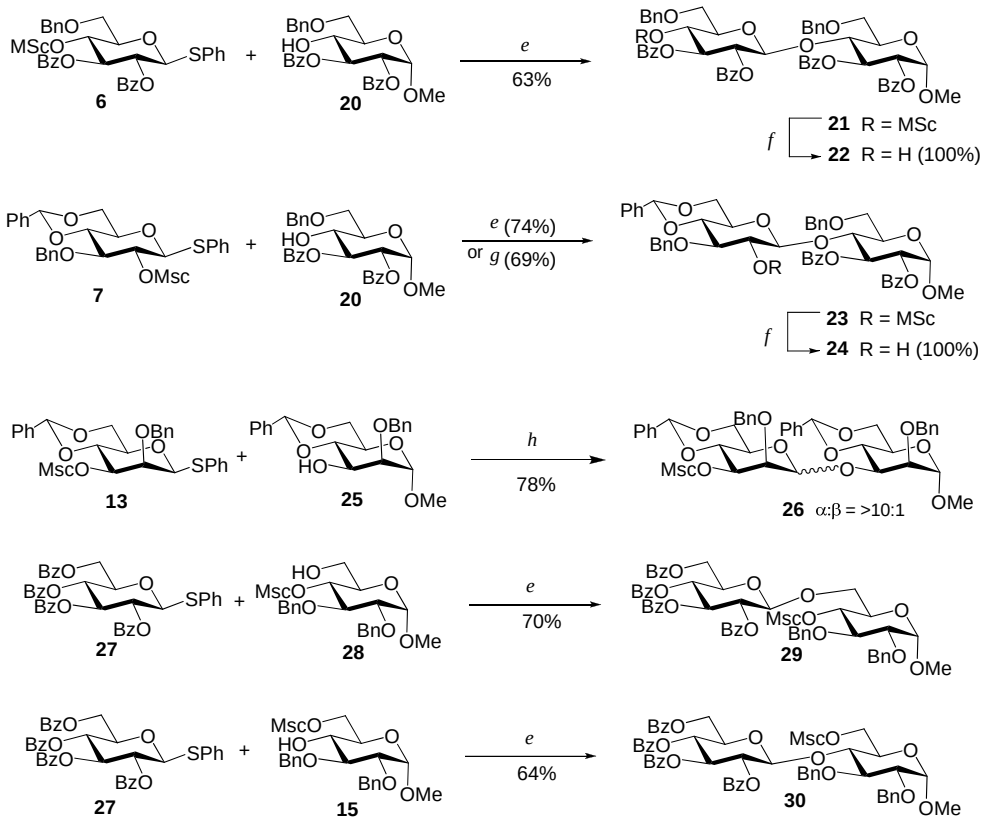


Reagents and conditions; *b*) LevOH, DMAP, EDC.HCl, DCM, 1 h, 89%; *c*) $H_2NNH_2.H_2O$, pyridine/HOAc, 5 min, 96%; *d*) DBU, DMF, 25 min.

pyridine and acetic acid. Alternatively, cleavage of the Msc group at C4-OH in **17** was accomplished with catalytic amount of DBU to provide primary alcohol **18** in 95% yield. Apart from this, 2% of 6-*O*-levulinoylated side product was isolated, originating from migration of the levulinoyl group from the secondary C4-OH to the primary C6-OH. These experiments indicate that the Msc and the Lev protective groups are orthogonal.[16]

Next the feasibility of Msc-protected carbohydrates in a set of glycosylation reactions was investigated (Scheme 2). In the first example the Msc-protected thioglucoside **6** was condensed with methyl glucoside **20** under the influence of *N*-iodosuccinimide (NIS)

Scheme 2: Glycosylation reactions using donors or acceptors containing the Msc group.



Reagents and conditions; *e*) NIS, TMSOTf, DCM, -40 ºC-RT, 1h; *f*) DBU, dioxane, 30 min; *g*) Ph_2SO, Tf_2O TTBP, DCM, -60 ºC-RT; *h*) Ph_2SO, Tf_2O TTBP, DCM, -78 ºC.

and a catalytic amount of trimethysilyltriflate (TMSOTf) to provide disaccharide **21** in 63% yield. The Msc group could be selectively removed from this disaccharide leaving all of the benzoyl functionalities untouched to give **22** in excellent yield. The second glycosylation employed thioglucose donor **7**, having the Msc group located on the C2-OH, acceptor **20** and the same activator system. The β-linked dimer **23** was obtained in 71% yield, showing that the methylsulfonylethyl carbonate provided efficient anchimeric assistance in the glycosylation reaction. When the same donor (**7**) and acceptor (**20**) were condensed, using diphenylsulfoxide (Ph_2SO) in combination with trifluoromethanesulfonic anhydride (Tf_2O)[17] and an excess tri-*tert*-butylpyrimidine (TTBP)[18] disaccharide **23** was isolated in similar yield (67%). This result indicates that the presence of the Msc carbonate at C2 excludes the unwanted formation of orthoester, even under non-acidic conditions. Treatment of dimer **23** with a catalytic amount of DBU quantitatively liberated the C2'-OH to afford **24**. Coupling of Msc-protected thiomannoside **13** with methyl mannoside **25** using the Ph_2SO/Tf_2O[19] activator system and an excess of TTBP afforded disaccharide **26** in 78% yield as an anomeric mixture (α:β >10:1), indicating that the Msc group also provides anchimeric assistance from the 3-position (for a more detailed discussion, see Chapter 4).[20] The Msc group was also tolerated when present in acceptor building blocks as shown in the next glycosylations in which the perbenzoylated *S*-phenyl glucoside **27** was coupled to both primary alcohol **28** and secondary alcohol **15** to furnish dimers **29** and **30** in 70% and 64% yield respectively.

Conclusion:

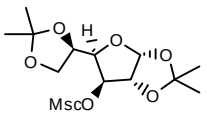
This chapter described the successful application of the methylsulfonylethoxycarbonyl (Msc) group as a non-lipophilic protecting group for hydroxyl functions in oligosaccharide synthesis. The Msc group can be introduced using standard conditions for the formation of carbonates and can be cleaved *via* β-elimination using mildly basic conditions to which commonly used ester protecting groups are stable. The Msc group is slightly more stable than the Fmoc group and is orthogonal with the levulinoyl group. The Msc group is completely stable to acid mediated glycosylation conditions, provides anchimeric assistance and excludes orthoester formation, when placed on the C2-OH of a glycosyl donor.

Experimental:

General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water in donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. Molecular sieves 3Å were flame dried before use. All other chemicals (Acros, Fluka, Merck) were used as received. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). Size exclusion chromatography was performed on Sephadex LH20 (eluent MeOH/DCM = 1/1). TLC analysis was conducted on DC-alufolien (Merck, kiesel gel 60, F_{245}). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10g/L) in 10% $H_2SO_{4\,(aq)}$ followed by charring at ~150 ºC. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 MHz and 100 MHz respectively), AV 500 (500 MHz and 125 MHz respectively). NMR spectra were recorded in $CDCl_3$ unless stated otherwise. Chemical shift are relative to tetramethylsilane and are given in ppm. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High resolution mass spectra were recorded on a LTQ-Orbitrap (thermo electron).

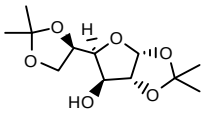
General method for the introduction of the Msc group: A solution of alcohol in DCM (0.2 M) was cooled to 0 ºC before pyridine (3 eq) was added. Methylsulfonylethoxycarbonyl chloride (Msc-Cal, 10% in DCM, 2 eq) was added drop-wise at 0 ºC over the span of 30 minutes. The reaction mixture was allowed to warm to room temperature. The reaction mixture was quenched with methanol, diluted with DCM, washed with $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography.

General method for glycosylations using NIS/TMSOTf: A solution of 1-thio-β-D-glucopyranoside (donor) and acceptor in DCM (0.05 M) was stirred over activated MS3Å for half an hour before *N*-iodosuccinimide (1.3 eq with respect to the donor) was added. The mixture was cooled to -40 ºC followed by the addition of trimethylsilyl trifluoromethanesulfonate (0.1 eq). The mixture was allowed to warm to room temperature. The reaction mixture was quenched with triethylamine (5 eq), filtered, diluted with EtOAc and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with EtOAc thrice, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography.



1,2:5,6-di-*O*-isopropylidene-3-*O*-methylsulfonylethoxycarbonyl-α-D-glucofuranose (3): Compound **3** was prepared according to the general procedure for the introduction of the Msc group from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **2** (1.30 g, 5.0 mmol) yielding the compound **9** (1.950 g, 4.8 mmol, 95%). TLC (50% n-hexane in EtOAc): R_f = 0.45; $[\alpha]_D^{22}$: -28.6º (c = 1, DCM); IR (neat, cm^{-1}): 731, 1215, 1757; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.32 (s, 6H, 2xCH_3 isopropylidene), 1.41 (s, 3H, CH_3 isopropylidene), 1.52 (s, 3H, CH_3 isopropylidene), 3.00 (s, 3H, CH_3 Msc), 3.40 (m, 2H, $MeSO_2C\underline{H}_2CH_2$-), 4.00 (m, 1H, H-6), 4.07 (m, 1H, H-6), 4.19 (m, 2H, H-4 and H-5),

4.61 (m, 3H, H-2 and $MeSO_2CH_2C\underline{H}_2$-), 5.13 (d, 1H, *J* = 2.0 Hz, H-3), 5.90 (d, 1H, *J* = 3.6 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 25.0 (CH_3 isopropylidene), 25.9 (CH_3 isopropylidene), 26.4 (CH_3 isopropylidene), 26.6 (CH_3 isopropylidene), 42.1 (CH_3 Msc), 53.3 ($MeSO_2\underline{C}H_2CH_2$-), 61.4 ($MeSO_2CH_2\underline{C}H_2$-), 66.9 (C-6), 72.0, 79.4 (C-4 and C-5), 79.9 (C-3), 82.8 (C-2), 104.8 (C-1), 109.2 (C_q isopropylidene), 112.1 (C_q isopropylidene), 153.1 (C=O Msc); HRMS $[M+H]^+$ calcd for $C_{16}H_{27}O_{10}S$ 411.13194 was found 411.13201, $[M+NH_4]^+$ calcd for $C_{16}H_{30}O_{10}SN$ 428.15849 was found 428.15854, $[M+Na]^+$ calcd for $C_{16}H_{26}O_{10}SNa$ 433.11389 was found 433.11364.

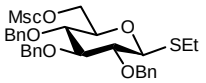


1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (2) (Cleavage of Msc from 3): Method I: To a solution of **3** (80 mg, 200 μmol) in methanol (5 ml, 0.04 M) was added sodium methoxide (1% in MeOH, 370 μl, 20 μmol, 0.1 eq) and the reaction mixture was stirred for 18 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **2** (52 mg, 199 μmol, 100%).

Method II: To a solution of **3** (50 mg, 122 μmol) in DCM (2 ml, 0.06 M) was added triethylamine (500 μl, 360 μmol, 30 eq) and the reaction mixture was stirred for 20 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **2** (32 mg, 122 μmol, 100%).

Method III: To a solution of **3** (50 mg, 122 μmol) in THF (3 ml, 0.04 M) was added TBAF (1 M in THF, 12.5 μl, 12 μmol, 0.1 eq) and the reaction mixture was stirred for 30 minutes. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **2** (32 mg, 121 μmol, 100%).

Method IV: To a solution of **3** (80 mg, 200 μmol) in DMF (5 ml, 0.04 M) was added DBU (0.1 M in DMF, 370 μl, 20 μmol, 0.1 eq) and the reaction mixture was stirred for 25 minutes. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **2** (52 mg, 199 μmol, 100%).

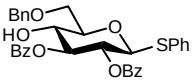


Ethyl 2,3,4-tri-*O*-benzyl-6-*O*-methylsulfonylethoxycarbonyl-1-thio-β-D-glucopyranoside (4): Compound **4** was prepared according to the general procedure for the introduction of the Msc group from ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (0.120 g, 0.242 mmol) yielding the compound **4** (0.153 g, 0.237 mmol, 98%). TLC (50% n-hexane in EtOAc): R_f = 0.5; $[\alpha]_D^{22}$: 10.0º (c = 0.8, DCM); IR (neat, cm^{-1}): 698, 1733; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.31 (t, 3H, *J* = 8.5 Hz, CH_3 Et), 2.68-2.78 (m, 2H, CH_2 Et), 2.97 (s, 3H, CH_3 Msc), 3.29-3.35 (m, 2H, $MeSO_2C\underline{H}_2CH_2$-), 3.41 (t, 1H, *J* = 9.5 Hz, H-2), 3.50 (m, 2H, H-4 and H-5), 3.70 (t, 1H, *J* = 8.5 Hz, H-3), 4.23 (dd, 1H, *J* = 5.0 Hz, *J* = 12.5 Hz, H-6), 4.42 (dd, 1H, *J* = 1.5 Hz, *J* = 12.0 Hz, H-6), 4.46 (d, 1H, *J* = 10.0 Hz, H-1),

4.53 (m, 2H, $MeSO_2CH_2C\underline{H}_2$-), 4.58 (d, 1H, *J* = 11.0 Hz, CH<u>H</u> Bn), 4.73 (d, 1H, *J* = 10.5 Hz, CH<u>H</u> Bn), 4.84 (d, 1H, *J* = 11.0 Hz, CH<u>H</u> Bn), 4.87 (d, 1H, *J* = 11.0 Hz, CH<u>H</u> Bn), 4.91 (d, 1H, *J* = 10.4 Hz, CH<u>H</u> Bn), 4.94 (d, 1H, *J* = 11.0 Hz, CH<u>H</u> Bn), 7.25-7.37 (m, 15H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 15.1 (CH_3 Et), 25.1 (CH_2 Et), 42.6 (CH_3 Msc), 53.8 ($MeSO_2\underline{C}H_2CH_2$-), 61.3 ($MeSO_2CH_2\underline{C}H_2$-), 67.2 (C-6), 75.0 ($CH_2$ Bn), 75.5 (CH_2 Bn), 75.8 (CH_2 Bn), 76.5 (C-5), 77.3 (C-4), 81.6 (C-2), 85.2 (C-1), 86.5 (C-3), 127.7-129.0 (CH arom), 137.5 (C_q Bn), 137.7 (C_q Bn), 138.2 (C_q Bn), 154.1 (C=O Msc); HRMS $[M+NH_4]^+$ calcd for $C_{33}H_{44}O_9S_2N$ 662.24520 was found 662.24536, $[M+Na]^+$ calcd for $C_{33}H_{40}O_9S_2Na$ 667.20060 was found 667.20038.

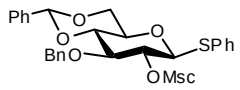
Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-methylsulfonylethoxycarbonyl-1-thio-β-D-glucopyranoside (5): Compound **5** was prepared according to the general procedure for the introduction of the Msc group from phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (0.154 g, 0.28 mmol) yielding the compound **5** (0.155 g, 0.22 mmol, 78%). TLC (50% n-hexane in EtOAc): R_f = 0.6; $[\alpha]_D^{22}$: -8.0º (c = 0.25, DCM); IR (neat, cm^{-1}): 694, 732, 1026, 1247, 1755; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.74 (s, 3H, CH_3 Msc), 3.06 (m, 2H, $MeSO_2C\underline{H}_2CH_2$-), 3.56 (t, 1H, *J* = 9.0 Hz, H-2), 3.62-3.71 (m, 4H, H-3, H-5 and 2xH-6), 4.35 (m, 2H, $MeSO_2CH_2C\underline{H}_2$-), 4.52 (m, 2H, 2xCH<u>H</u> Bn), 4.61-4.71 (m, 3H, H-1 and 2xCH<u>H</u> Bn), 4.88 (m, 3H, H-4 and 2xCH<u>H</u> Bn), 7.23-7.56 (m, 20H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 42.1 (CH_3 Msc), 53.4 ($MeSO_2\underline{C}H_2CH_2$-), 61.3 ($MeSO_2CH_2\underline{C}H_2$-), 69.6 (C-6), 73.5 ($CH_2$ Bn), 75.5 (2xCH_2 Bn), 75.7 (C-4), 76.7 (C-5), 80.5 (C-2), 84.0 (C-3), 87.6 (C-1), 127.3-132.1 (CH arom), 133.2 (C_q SPh), 137.6 (C_q Bn), 137.9 (C_q Bn), 138.0 (C_q Bn), 153.6 (C=O Msc); HRMS $[M+NH_4]^+$ calcd for $C_{37}H_{44}O_9S_2$ N 710.24520 was found 710.24548, $[M+Na]^+$ calcd for $C_{37}H_{40}O_9S_2Na$ 715.20060 was found 715.20074.

Phenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-methylsulfonylethoxycarbonyl-1-thio-β-D-glucopyranoside (6): Compound **6** was prepared according to the general procedure for the introduction of the Msc group from phenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside (0.160 g, 0.28 mmol) yielding the compound **6** (0.155 g, 0.21 mmol, 76%). TLC (50% n-hexane in EtOAc): R_f = 0.5; $[\alpha]_D^{22}$: +39.4º (c = 1, DCM); IR (neat, cm^{-1}): 1242, 1728; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.75 (s, 3H, CH_3 Msc), 2.97 (t, 2H, *J* = 6.0 Hz, $MeSO_2C\underline{H}_2CH_2$-), 3.72-3.79 (m, 2H, 2xH-6), 3.92 (m, 1H, H-5), 4.27 (m, 1H, $MeSO_2CH_2C\underline{H}$H-), 4.36 (m, 1H, $MeSO_2CH_2CH\underline{H}$-), 4.54 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 4.62 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.93 (d, 1H, *J* = 8.4 Hz, H-1), 5.17 (t, 1H, *J* = 9.6 Hz, H-4), 5.46 (t, 1H, *J* = 9.6 Hz, H-2), 5.70 (t, 1H, *J* = 9.6 Hz, H-3), 7.12-7.96 (m, 20H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.0 (CH_3 Msc), 53.4 ($MeSO_2\underline{C}H_2CH_2$-), 61.8 ($MeSO_2CH_2\underline{C}H_2$-), 68.7 (C-6), 70.1 (C-2), 73.5 (CH_2 Bn), 73.7 (C-4), 74.5 (C-3), 76.8 (C-5), 86.1 (C-1), 127.5-133.6 (CH arom), 128.9 (C_q Bz), 129.7 (C_q Bz), 131.8 (C_q SPh), 137.7 (C_q Bn), 153.2 (C=O Msc), 164.9 (C=O Bz), 165.7 (C=O Bz); HRMS $[M+NH_4]^+$ calcd for $C_{37}H_{40}O_{11}S_2$ N 738.20373 was found 738.20386, $[M+Na]^+$ calcd for $C_{37}H_{36}O_{11}S_2Na$ 743.15912 was found 743.15897.

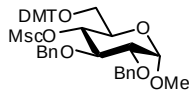


Phenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-glucopyranoside (Cleavage of the Msc from 6): To a solution of **6** (82 mg, 161 µmol) in DMF (8 ml, 0.02 M) was added DBU (1% in DMF, 241 µl, 16 µmol, 0.1 eq) and the reaction mixture was stirred for 1

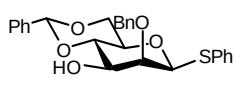
minute. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\ (aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford phenyl 2-*O*-benzyl-1-thio-β-D-galactopyranosidurono-3,6-lactone **(**56 mg, 156 μmol, 98%).



Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methylsulfonylethoxycarbonyl-1-thio-β-D-glucopyranoside (7): Compound **7** was prepared according to the general procedure for the introduction of the Msc group from phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (0.460 g, 1.0 mmol) yielding the compound **7** (0.485 g, 0.81 mmol, 79%). TLC (50% n-hexane in EtOAc): R_f = 0.6; $[\alpha]_D^{22}$: -8.0º (c = 1, DCM); IR (neat, cm^{-1}): 743, 1265, 1747; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.86 (s, 3H, CH_3 Msc), 3.19-3.30 (m, 2H, $MeSO_2C\underline{H}_2CH_2$-), 3.49 (m, 1H, H-5), 3.71-3.83 (m, 3H, H-4, H-6 and H-3), 4.38 (dd, 1H, *J* = 4.8 Hz, *J* = 10.4 Hz, H-6), 4.54 (m, 1H, $MeSO_2CH_2C\underline{H}H$-), 4.59 (m, 1H, $MeSO_2CH_2CH\underline{H}$-), 4.65 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.73 (d, 1H, *J* = 10.0 Hz, H-1), 4.80 (t, 1H, *J* = 8.4 Hz, H-2), 4.90 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 5.56 (s, 1H, CH benzylidene), 7.24-7.49 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.3 (CH_3 Msc), 53.7 ($MeSO_2\underline{C}H_2CH_2$-), 61.7 ($CH_2$ $MeSO_2CH_2\underline{C}H_2$-), 68.3 (C-6), 70.5 (C-5), 74.5 (CH_2 Bn), 76.0 (C-2), 79.9 (C-3), 81.0 (C-4), 86.2 (C-1), 101.2 (CH benzylidene), 125.9-132.8 (CH arom), 131.5 (C_q SPh), 136.9 (C_q CHPh), 137.9 (C_q Bn), 153.4 (C=O Msc); HRMS $[M+H]^+$ calcd for $C_{30}H_{33}O_9S_2$ 601.15605 was found 601.15636, $[M+NH_4]^+$ calcd for $C_{30}H_{36}O_9S_2$ N 618.18260 was found 618.18264, $[M+Na]^+$ calcd for $C_{30}H_{32}O_9S_2Na$ 623.13800 was found 623.13795.

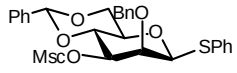


Methyl 2,3-di-*O*-benzyl-6-*O*-dimethoxytrityl-4-*O*-methylsulfonylethoxycarbonyl-α-D-glucopyranoside (8): Compound **8** was prepared according to the general procedure for the introduction of the Msc group from methyl 2,3-di-*O*-benzyl-6-*O*-dimethoxytrityl-α-D-glucopyranoside (0.750 g, 1.11 mmol) yielding the compound **8** (0.770 g, 0.87 mmol, 79%). TLC (33% EtOAc in PE): R_f = 0.4; $[\alpha]_D^{22}$: +26.4º (c = 0.5, DCM); IR (neat, cm^{-1}): 726, 1247, 1508, 1759; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.61 (s, 3H, CH_3 Msc), 2.98 (t, 2H, *J* = 6.0 Hz, $MeSO_2C\underline{H}_2CH_2$-), 3.14-3.23 (m, 2H, 2xH-6), 3.43 (s, 3H, CH_3 OMe), 3.63 (dd, *J* = 3.6 Hz, *J* = 9.6 Hz, 1H, H-2), 3.70 (s, 6H, 2xCH_3 DMT), 3.87 (m, 1H, H-5), 3.98 (t, 1H, *J* = 9.2 Hz, H-3), 4.24 (m, 2H, $MeSO_2CH_2C\underline{H}_2$-), 4.62 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.65 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.72 (d, 1H, *J* = 3.2 Hz, H-1), 4.76 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 4.86 (t, 1H, *J* = 10.0 Hz, H-4), 4.93 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 6.79-7.50 (m, 23 H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 41.6 (CH_3 Msc), 53.1 ($MeSO_2\underline{C}H_2CH_2$-), 54.8 (2x$CH_3$ DMT), 54.9 (CH_3 OMe), 60.9 ($MeSO_2CH_2\underline{C}H_2$-), 62.2 (C-6), 68.1 (C-5), 73.0 (CH_2 Bn), 75.0 (C-4), 75.1 (CH_2 Bn), 79.2 (C-3), 79.5 (C-2), 85.7 (C_q DMT), 97.5 (C-1), 112.8 (CH DMT) 126.5-129.8 (CH arom), 135.5 (C_q DMT), 137.6 (C_q Bn), 138.2 (C_q Bn), 144.3 (C_q DMT), 153.1 (C=O Msc) 158.1 (C_q DMT); HRMS $[M+Na]^+$ calcd for $C_{46}H_{50}O_{12}SNa$ 849.29152 was found 849.29230.

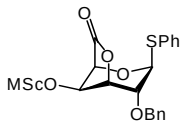


Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-mannopyranoside (11): To a solution of phenyl 4,6-*O*-benzylidene-1-thio-β-D-mannopyranoside **(9)** (0.355 g, 1.0 mmol) in DCM (13 ml, 0.08 M) was added benzyl bromide (0.14 ml, 1.2 mmol, 1.2

eq), tetrabutylammonium sulfonate (0.067 g, 0.20 mmol, 0.2 eq) and $NaOH_{(aq)}$ (1M, 5 ml, 5.0 mmol, 5 eq). The reaction mixture was refluxed at 40 ºC for 18 hours, after which the reaction was quenched with $NH_4Cl_{(aq)}$. The mixture was diluted with EtOAc and extracted thrice with EtOAc. The combined organic layers were washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\,(aq)}$, brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel chromatography to get **10** (0.071 g, 0.16 mmol, 16%), **11** (0.196 g, 0.44 mmol, 44%) and **12** (0.058 g, 0.11 mmol, 11%); TLC (33% EtAcO in PE): R_f = 0.8 (**12**), R_f = 0.6 (**10,11**); TLC (33% Et_2O in PE): R_f = 0.3 (**11**), R_f = 0.2 (**10**); (compound **10** and **12**) analytical data for the compound **10** and **12** was found in accordance to the earlier reports. (Compound **11**) $[\alpha]_D^{22}$: 21.2º (c = 1, DCM); IR (neat, cm^{-1}): 695, 1047; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.56 (s, 1H, OH-3), 3.36 (m, 1H, H-5), 3.82-3.90 (m, 2H, H-3 and H-6), 3.97 (t, 1H, *J* = 9.6 Hz, H-4), 4.08 (d, 1H, *J* = 2.4 Hz, H-2), 4.29 (dd, 1H, *J* = 5.2 Hz, *J* = 10.8 Hz, H-6), 4.85 (d, 1H, *J* = 1.2 Hz, H-1), 4.85-4.97 (m, 2H, 2xCH<u>H</u> Bn), 5.53 (s, 1H, CH benzylidene), 7.24-7.37 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 68.3 (C-6), 71.2 (C-5), 72.8 (C-3), 76.6 (CH_2 Bn), 78.6 (C-4), 80.5 (C-2), 88.8 (C-1), 102.0 (CH benzylidene), 126.1-131.1 (CH arom), 134.7 (C_q SPh), 137.1, 137.8 (C_q CHPh and C_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 88.8 (*J* = 153 Hz, C-1); HRMS $[M+Na]^+$ calcd for $C_{26}H_{26}O_5S_1Na$ 473.13932 was found 473.13904.

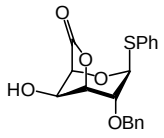


Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxycarbonyl-1-thio-β-D-mannopyranoside (13): Compound **13** was prepared according to the general procedure for the introduction of the Msc group from phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-mannopyranoside (0.140 g, 0.31 mmol) yielding compound **13** (0.182 g, 0.30 mmol, 97%); TLC (50% EtOAc in PE): R_f = 0.2; $[\alpha]_D^{22}$: -42.2º (c = 1, DCM); IR (neat, cm^{-1}): 523, 1267, 1752; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.75 (s, 3H, CH_3 Msc), 3.15-3.20 (m, 1H, $MeSO_2CH\underline{H}CH_2$-), 3.24-3.31 (m, 1H, $MeSO_2CH\underline{H}CH_2$-), 3.48 (m, 1H, H-5), 3.90 (t, 1H, *J* = 10.4 Hz, H-6), 4.24 (t, 1H, *J* = 9.6 Hz, H-4), 4.30 (dd, 1H, *J* = 4.8 Hz, *J* = 10.4 Hz, H-6), 4.36 (d, 1H, *J* = 2.8 Hz, H-2), 4.48 (t, 2H, *J* = 6.4 Hz, $MeSO_2CH_2C\underline{H}_2$-), 4.79 (d, 1H, *J* = 11.2 Hz, CH<u>H</u> Bn), 4.85 (d, 1H, *J* = 11.2 Hz, CH<u>H</u> Bn), 4.93-4.97 (m, 2H, H-1 and H-3), 5.53 (s, 1H, CH benzylidene), 7.24-7.42 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.3 (CH_3 Msc), 53.4 ($MeSO_2\underline{C}H_2CH_2$-), 61.5 ($MeSO_2CH_2\underline{C}H_2$-), 68.2 (C-6), 71.3 (C-5), 75.2 (C-4), 76.4 (CH_2 Bn), 77.7 (C-3), 78.1 (C-2), 88.6 (C-1), 101.7 (CH benzylidene), 126.0-134.0 (CH arom), 134.0 (C_q SPh), 136.9, 137.1 (C_q CHPh and C_q Bn), 153.5 (C=O); CH Gated NMR (100 MHz, $CDCl_3$) δ = 88.6 (*J* = 154 Hz, C-1); HRMS $[M+Na]^+$ calcd for $C_{30}H_{32}O_9S_2Na$ 623.13800 was found 623.13767.

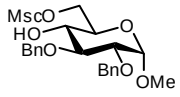


Phenyl 2-*O*-benzyl-4-*O*-methylsulfonylethoxycarbonyl-1-thio-β-D-galactopyranosidurono-3,6-lactone (14): Compound **14** was prepared according to the general procedure for the introduction of the Msc group from phenyl 2-*O*-benzyl-1-thio-β-D-galactopyranosidurono-3,6-lactone (0.414 g, 1.16 mmol) yielding the compound **14** (0.514 g, 1.01 mmol, 88%); TLC (50% EtOAc in PE): R_f = 0.3; $[\alpha]_D^{22}$: -232.4º (c = 1.0, DCM); IR (neat, cm^{-1}): 734, 1264; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.94 (s, 3H, CH_3 Msc), 3.33 (t, 2H, *J* = 5.6 Hz, $MeSO_2C\underline{H}_2CH_2$-), 4.20 (s, 1H, H-5), 4.34 (d, 1H, *J* = 4.8 Hz, H-2), 4.58 (t, 2H, *J* = 5.2 Hz, $MeSO_2CH_2C\underline{H}_2$-), 4.65 (m, 2H, 2xCH<u>H</u>

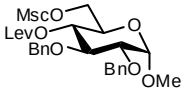
Bn), 4.99 (d, 1H, *J* = 4.8 Hz, H-3), 5.41 (s, 1H, H-4), 5.46 (s, 1H, H-1), 7.25-7.43 (m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.3 (CH_3 Msc), 53.2 ($MeSO_2\underline{C}H_2CH_2$-), 61.9 ($MeSO_2CH_2\underline{C}H_2$-), 69.8 (C-5), 72.9 ($CH_2$ Bn), 75.1 (C-4), 78.2 (C-2 and C-3), 85.9 (C-1), 128.0-132.4 (CH arom), 133.0 (C_q SPh), 136.1 (C_q Bn), 152.6 (C=O Msc), 171.2 (C-6); HRMS $[M+Na]^+$ calcd for $C_{23}H_{24}O_9S_2Na$ 531.07539 was found 531.07525.



Phenyl 2-*O*-benzyl-1-thio-β-D-galactopyranosidurono-3,6-lactone (Cleavage of Msc from 14): To a solution of **14** (82 mg, 161 μmol) in DMF (8 ml, 0.02 M) was added DBU (1% in DMF, 241 μl, 16 μmol, 0.1 eq) and the reaction mixture was stirred for 1 minute. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\ (aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford phenyl 2-*O*-benzyl-1-thio-β-D-galactopyranosidurono-3,6-lactone (56 mg, 156 μmol, 97%).

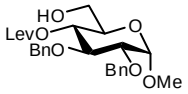


Methyl 2,3-di-*O*-benzyl-6-*O*-methylsulfonylethoxycarbonyl-α-D-glucopyranoside (15): Compound **15** was prepared according to the general procedure for the introduction of the Msc group from methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (0.214 g, 0.57 mmol) at -20 °C yielding the compound **15** (0.270 g, 0.52 mmol, 90%). TLC (50% EtOAc in PE): R_f = 0.6; $[\alpha]_D^{22}$: +49.6° (c = 1, DCM); IR (neat, cm^{-1}): 741, 1055, 1265, 1751, 2927; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.08 (bs, 1H, C4-OH), 2.99 (s, 3H, CH_3 Msc), 3.36 (t, 2H, *J* = 5.6 Hz, $MeSO_2C\underline{H}_2CH_2$-), 3.41 (s, 3H, CH_3 OMe), 3.49 (t, 1H, *J* = 9.6 Hz, H-4), 3.54 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.77-3.84 (m, 2H, H-3 and H-5), 4.43 (m, 2H, 2xH-6), 4.59 (t, 2H, *J* = 6.0 Hz, $MeSO_2CH_2C\underline{H}_2$-), 4.65 (d, 1H, *J* = 3.2 Hz, H-1), 4.70 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.75 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 4.81 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 5.05 (d, 1H, *J* = 11.2 Hz, CH<u>H</u> Bn), 7.30-7.42 (m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.5 (CH_3 Msc), 53.7 ($MeSO_2\underline{C}H_2CH_2$-), 55.3 ($CH_3$ OMe), 61.4 ($MeSO_2CH_2\underline{C}H_2$-), 67.2 (C-6), 68.9 (C-5), 69.6 (C-4), 73.1 (CH_2 Bn), 75.4 (CH_2 Bn), 79.5 (C-2), 81.0 (C-3), 98.1 (C-1), 128.0-128.6 (CH arom), 137.8 (C_q Bn), 138.5 (C_q Bn), 154.4 (C=O Msc); HRMS $[M+NH_4]^+$ calcd for $C_{25}H_{36}O_{10}S$ N 542.20544 was found 542.20528, $[M+Na]^+$ calcd for $C_{25}H_{32}O_{10}SNa$ 547.16084 was found 547.16053.

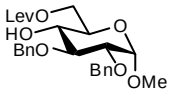


Methyl 2,3-di-*O*-benzyl-4-*O*-levulinoyl-6-*O*-methylsulfonylethoxycarbonyl-α-D-glucopyranoside (17): To a solution of the compound **15** (0.196 g, 0.37 mmol) in DCM (1.8 ml, 0.2 M) was added LevOH (0.434 g, 3.74 mmol, 10 eq) and the reaction mixture was stirred for 30 minutes. A solution of EDC.HCl (0.358 g, 1.87 mmol, 5 eq) and DMAP (2 mg) in DCM (0.5 ml) was added and stirring was continued for 1 hour. The reaction mixture was diluted with DCM, washed with water, $NaHCO_{3\ (aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford compound **17** (0.208 g, 3.34 mmol, 89%). TLC (10% Methanol in DCM): R_f = 0.5; $[\alpha]_D^{22}$: +34.6° (c = 1, DCM); IR (neat, cm^{-1}): 735, 1130, 1251, 1716, 1749; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.15 (s, 3H, CH_3 Lev), 2.24-2.30 (m, 1H, $MeCOCH_2CH\underline{H}COO$-), 2.44-2.50 (m, 1H, $MeCOCH_2CH\underline{H}COO$-), 2.58 (m, 1H, $MeCOCH\underline{H}CH_2COO$-), 2.70 (m, 1H, $MeCOCH\underline{H}CH_2COO$-), 2.99 (s, 3H, CH_3 Msc), 3.29 (m, 1H,

$MeSO_2CH\underline{H}CH_2$-), 3.38 (m, 4H, CH_3 OMe and $MeSO_2CH\underline{H}CH_2$-), 3.55 (dd, 1H, *J* = 3.5 Hz, *J* = 9.5 Hz, H-2), 3.86 (m, 1H, H-5), 3.93 (t, 1H, *J* = 9.5 Hz, H-3), 4.15 (dd, 1H, *J* = 2.0 Hz, *J* = 12.0 Hz, H-6), 4.32 (dd, 1H, *J* = 4.5 Hz, *J* = 12.0 Hz, H-6), 4.49 (m, 1H, $MeSO_2CH_2CH\underline{H}$-), 4.58-4.67 (m, 4H, H-1, $MeSO_2CH_2CH\underline{H}$- and 2x$CH\underline{H}$ Bn), 4.79 (d, 1H, *J* = 12.0 Hz, $CH\underline{H}$ Bn), 4.87 (d, 1H, *J* = 11.5 Hz, $CH\underline{H}$ Bn), 4.96 (t, 1H, *J* = 10.0 Hz, H-4), 7.27-7.35 (m, 10H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 27.7 ($MeCOCH_2\underline{C}H_2COO$-), 29.7 ($CH_3$ Lev), 37.8 ($MeCO\underline{C}H_2CH_2COO$-), 42.5 ($CH_3$ Msc), 53.8 ($MeSO_2\underline{C}H_2CH_2$-), 55.5 ($CH_3$ OMe), 61.5 ($MeSO_2CH_2\underline{C}H_2$-), 66.1 (C-6), 67.3 (C-3), 69.6 (C-4), 73.5 (CH_2 Bn), 75.4 (CH_2 Bn), 78.9 (C-2), 79.4 (C-5), 98.2 (C-1), 127.6-128.5 (CH arom), 137.8 (C_q Bn), 138.4 (C_q Bn), 154.0 (C=O Msc), 171.7 (C=O ($MeCOCH_2CH_2\underline{CO}O$-), 206.3 ($Me\underline{CO}CH_2CH_2COO$-); HRMS $[M+NH_4]^+$ calcd for $C_{30}H_{42}O_{12}S$ N 640.24222 was found 640.24206, $[M+Na]^+$ calcd for $C_{33}H_{38}O_{12}SNa$ 645.19762 was found 645.19721.

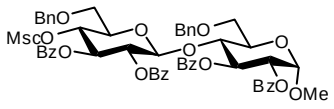


Methyl 2,3,-di-*O*-benzyl-4-*O*-levulinoyl-1-thio-α-D-glucopyranoside (18): To a solution of compound **17** (34 mg, 54 μmol) in DMF (1.1 ml, 0.04 M) was added DBU (10% in DMF, 81μl, 5.4 μmol, 0.1 eq) and the reaction mixture was stirred for 25 minutes. The reaction mixture was quenched with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\ (aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford the compound **17** (23.7mg, 52 μmol, 95%). TLC (66% EtOAc in toluene): R_f = 0.65; $[\alpha]_D^{22}$: +24.8° (c = 1, DCM); IR (neat, cm^{-1}): 738, 1028, 1716, 1739, 2918; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.15 (s, 3H, CH_3 Lev), 2.32 (m, 1H, $MeCOCH_2CH\underline{H}COO$-), 2.48-2.54 (m, 1H, $MeCOCH_2CH\underline{H}COO$-), 2.56-2.62 (m, 1H, $MeCOCH\underline{H}CH_2COO$-), 2.74-2.80 (m, 1H, $MeCOCH\underline{H}CH_2COO$-), 3.39 (s, 3H, CH_3 OMe), 3.56 (dd, 1H, *J* = 3.5 Hz, *J* = 9.5 Hz, H-2), 3.60-3.66 (m, 3H, H-5 and 2xH-6), 3.99 (t, 1H, *J* = 9.0 Hz, H-3), 4.61 (d, 1H, *J* = 4.0 Hz, H-1), 4.64 (d, 1H, *J* = 12.5 Hz, $CH\underline{H}$ Bn), 4.69 (d, 1H, *J* = 11.5 Hz, $CH\underline{H}$ Bn), 4.79 (d, 1H, *J* = 12.0 Hz, $CH\underline{H}$ Bn), 4.89 (m, 2H, H-4 and $CH\underline{H}$ Bn), 7.26-7.36 (m, 10H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 27.8 ($MeCOCH_2\underline{C}H_2COO$-), 29.7 ($CH_3$ Lev), 37.8 ($MeCO\underline{C}H_2CH_2COO$-), 55.4 ($CH_3$ OMe), 60.9 (C-6), 69.5 (C-3), 70.9 (C-4), 73.5 (CH_2 Bn), 75.4 (CH_2 Bn), 78.9 (C-2), 79.4 (C-5), 98.2 (C-1), 127.6-128.5 (CH arom), 137.9 (C_q Bn), 138.7 (C_q Bn), 173.2 (C=O ($MeCOCH_2CH_2\underline{CO}O$-), 206.4 ($Me\underline{CO}CH_2CH_2COO$-); HRMS $[M+NH_4]^+$ calcd for $C_{26}H_{36}O_8N$ 490.24354 was found 490.24324, $[M+Na]^+$ calcd for $C_{26}H_{32}O_8Na$ 495.19894 was found 495.19847.

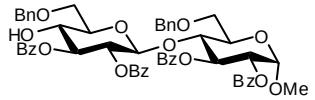


Methyl 2,3,-di-*O*-benzyl-6-*O*-levulinoyl-1-thio-α-D-glucopyranoside (19): Collected as a by-product during the synthesis of **18**; TLC (66% EtOAc in toluene): R_f = 0.8; $[\alpha]_D^{22}$: +22.6° (c = 0.3, DCM); IR (neat, cm^{-1}): 715, 1026, 1150, 1705; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.17 (s, 3H, CH_3 Lev), 2.58 (m, 2H, $MeCOCH_2C\underline{H}_2COO$-), 2.74 (t, 2H, *J* = 6.5 Hz, $MeCOC\underline{H}_2CH_2COO$-), 3.38 (s, 3H, CH_3 OMe), 3.44 (t, 1H, *J* = 9.5 Hz, H-4), 3.50 (dd, 1H, *J* = 3.5 Hz, *J* = 9.5 Hz, H-2), 3.72-3.75 (m, 1H, H-5), 3.79 (t, 1H, *J* = 9.0 Hz, H-3), 4.22 (dd, 1H, *J* = 2.0 Hz, *J* = 12.0 Hz, H-6), 4.42 (dd, 1H, *J* = 4.5 Hz, *J* = 12.0 Hz, H-6), 4.61 (d, 1H, *J* = 3.5 Hz, H-1), 4.66 (d, 1H, *J* = 12.0 Hz, $CH\underline{H}$ Bn), 4.77 (t, 2H, *J* = 11.5 Hz, 2x$CH\underline{H}$ Bn), 4.99 (d, 1H, *J* = 11.5 Hz, $CH\underline{H}$ Bn), 7.26-7.37 (m, 10H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 27.8 ($MeCOCH_2\underline{C}H_2COO$-), 29.8 ($CH_3$ Lev), 37.9 ($MeCO\underline{C}H_2CH_2COO$-), 55.2 ($CH_3$ OMe), 63.4 (C-6), 69.3 (C-5), 69.9 (C-4), 73.2 (CH_2 Bn), 75.5 (CH_2 Bn), 79.5 (C-2), 81. (C-3), 98.2 (C-1), 127.9-129.5 (CH

arom), 137.9 (C_q Bn), 138.6 (C_q Bn), 173.0 (C=O ($MeCOCH_2CH_2\underline{CO}O$-), 206.5 ($Me\underline{CO}CH_2CH_2COO$-); HRMS $[M+Na]^+$ calcd for $C_{26}H_{32}O_8Na$ 495.19894 was found 495.19849.

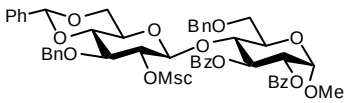


Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-methylsulfonylethoxycarbonyl-β-D-glucopyranosyl)-α-D-glucopyranoside (21): Disaccharide **21** was prepared form donor **6** (0.113 g, 0.16 mmol, 1 eq) and acceptor **20** (0.115 g, 0.24 mmol, 1.5 eq) according to the general procedure for glycosylations as described above yielding compound **21** (0.109 g, 0.10 mmol, 63%); TLC (33% EtOAc in Toluene): R_f = 0.45; $[\alpha]_D^{22}$: +17.6º (c = 0.25, DCM); IR (neat, cm^{-1}): 707, 1247, 1724; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.70 (s, 3H, CH_3 Msc), 2.86 (t, 2H, *J* = 5.0 Hz, $MeSO_2C\underline{H_2}CH_2$-), 3.07 (dd, 1H, *J* = 5.0 Hz, *J* = 10.0 Hz, H-6'), 3.19 (dd, 1H, *J* = 4.0 Hz, *J* = 10.0 Hz, H-6'), 3.30 (s, 3H, CH_3 OMe), 3.47 (m, 1H, H-6), 3.55 (m, 1H, H-5'), 3.71 (dd, 1H, *J* = 3.0 Hz, *J* = 10.5 Hz, H-6), 3.77 (m, 1H, H-5), 4.05-4.10 (m, 2H, $MeSO_2CH_2C\underline{H}H$- and $CH\underline{H}$ Bn), 4.12 (d, 1H, *J* = 12.0, $CH\underline{H}$ Bn), 4.20-4.26 (m, 2H, H-4 and $MeSO_2CH_2CH\underline{H}$-), 4.37 (d, 1H, *J* = 12.0 Hz, $CH\underline{H}$ Bn), 4.71 (m, 2H, H-1' and $CH\underline{H}$ Bn), 4.91 (t, 1H, *J* = 9.5 Hz, H-4'), 5.10 (d, 1H, *J* = 3.5 Hz, H-1), 5.16 (dd, 1H, *J* = 4.0 Hz, *J* = 10.5 Hz, H-2), 5.35 (dd, 1H, *J* = 8.0 Hz, *J* = 10.0 Hz, H-2'), 5.46 (t, 1H, *J* = 9.5 Hz, H-3'), 5.92 (t, 1H, *J* = 9.5 Hz, H-3), 7.20-8.03 (m, 30H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 42.0 (CH_3 Msc), 53.5 ($MeSO_2C\underline{H_2}CH_2$-), 55.4 ($CH_3$ OMe), 61.4 ($MeSO_2CH_2C\underline{H_2}$-), 67.2 (C-6), 69.4 (C-6'), 69.4 (C-5), 70.7 (C-3), 71.5 (C-2'), 71.5 (C-5'), 72.0 (C-2), 73.1 (CH_2 Bn), 73.1 (C-3'), 73.6 (CH_2 Bn), 74.9 (C-4'), 75.5 (C-4), 96.9 (C-1), 100.2 (C-1'), 128.2-133.5 (CH arom), 128.9 (C_q Bz), 129.1 (C_q Bz), 130.2 (C_q Bz), 130.4 (C_q Bz), 137.7 (C_q Bn), 137.7 (C_q Bn), 153.1 (C=O Msc), 164.5 (C=O Bz), 165.2 (C=O Bz), 165.7 (C=O Bz), 165.9 (C=O Bz); HRMS $[M+NH_4]^+$ calcd for $C_{59}H_{62}O_{19}SN$ 1120.36313 was found 1120.36426, $[M+Na]^+$ calcd for $C_{59}H_{58}O_{19}SNa$ 1125.31852 was found 1125.31946.



Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside 22 (Cleavage of Msc from 21): To a solution of **21** (90 mg, 82 µmol) in dioxane (1.5 ml, 0.05 M) was added DBU (5% in DMF, 23 µl, 8 µmol, 0.1 eq) and the reaction mixture was stirred for 30 minutes. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside **22** (78 mg, 82 µmol, 100%). TLC (33% EtOAc in Toluene): R_f = 0.66; $[\alpha]_D^{22}$: +31.8º (c = 1.0, DCM); IR (neat, cm^{-1}): 706, 1025, 1068, 1093, 1261, 1451, 1723; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 3.00 (dd, 1H, *J* = 5.0 Hz, *J* = 9.5 Hz, H-6'), 3.28 (m, 1H, H-6'), 3.30 (s, 3H, CH_3 OMe), 3.36 (m, 1H, H-5'), 3.46 (dd, 1H, *J* = 1.5 Hz, *J* = 10.5 Hz, H-6), 3.70 (m, 2H, H-4' and H-6), 3.75 (m, 1H, H-5), 4.18 (t, 1H, *J* = 9.5 Hz, H-4), 4.21 (m, 2H, 2x$CH\underline{H}$ Bn), 4.36 (d, 1H, *J* = 12.0 Hz, $CH\underline{H}$ Bn), 4.66 (m, 2H, H-1' and $CH\underline{H}$ Bn), 5.09 (d, 1H, *J* = 3.5 Hz, H-1), 5.16 (dd, 1H, *J* = 4.0 Hz, *J* = 10.5 Hz, H-2), 5.29 (m, 2H, H-2' and H-3'), 5.90 (t, 1H, *J* = 9.5 Hz, H-3'), 7.19-8.01 (m, 30H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 55.4 (CH_3 OMe), 67.3 (C-6), 69.5 (C-5), 70.8 (C-3), 71.0 (C-6'), 71.6 (C-2'), 72.1 (C-2), 72.3 (C-4'), 72.6 (C-5'), 73.5 (CH_2

Bn), 73.6 (CH_2 Bn), 75.5 (C-4), 75.7 (C-3'), 96.9 (C-1), 100.4 (C-1'), 127.5-133.2 (CH arom), 129.2 (C_q Bz), 129.2 (2xC_q Bz), 130.4 (C_q Bz), 137.2 (C_q Bn), 137.8 (C_q Bn), 164.8 (C=O Bz), 165.1 (C=O Bz), 165.9 (C=O Bz), 166.5 (C=O Bz); HRMS $[M+NH_4]^+$ calcd for $C_{55}H_{56}O_{15}N$ 970.36445 was found 970.36603, $[M+Na]^+$ calcd for $C_{55}H_{52}O_{15}Na$ 975.31984 was found 975.32080.

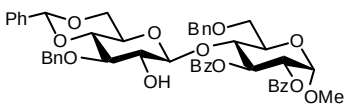


Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methylsulfonylethoxycarbonyl-β-D-glucopyranosyl)-α-D-glucopyranoside (23):

Method I: Disaccharide **23** was prepared form donor **7** (0.091g, 0.15 mmol, 1eq) and acceptor **20** (0.109 g, 0.22 mmol, 1.5 eq) according to the general procedure for glycosylations as described above yielding the compound **23** (0.103 g, 0.10 mmol, 71%).

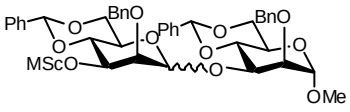
Method II: To a solution of compound **7** (0.127 g, 0.21 mmol, 1q) in DCM (4.2 ml, 0.05 M) was added diphenyl sulfoxide (0.556 g, 0.28 mmol, 1.3 eq) and tri-*tert*-butylpyrimidine (0.157 g, 0.63 mmol, 3 eq) and mixture was stirred over molecular sieve 3Å for 30 minutes. After that the mixture was brought to -60ºC and triflic acid anhydride (0.046 ml, 0.28 mmol, 1.3 eq) was added and the mixture was stirred for 15 minutes. Next a solution of compound **20** (0.156 g, 0.32 mmol, 1.5 eq) in DCM (2.1 ml, 0.15 M) was added and stirring was continued for 10 minutes. The reaction mixture was quenched with triethylamine (5 eq), diluted with DCM, washed with water and extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to yield **23** (0.13 g, 0.14 mmol, 67%).

TLC (33% Toluene in EtOAc): R_f = 0.45; $[\alpha]_D^{22}$: +54.4º (c = 0.5, DCM); IR (neat, cm^{-1}): 696, 1093, 1722; ^1H NMR (500 MHz, $CDCl_3$) δ = 2.72 (t, 1H, *J* = 10.5 Hz, H-6'), 2.79 (s, 3H, CH_3 Msc), 2.98-3.03 (m, 1H, H-5'), 3.20 (t, 2H, *J* = 5.5 Hz, $MeSO_2C\underline{H}_2CH_2$-), 3.41 (m, 4H, CH_3 OMe and H-4'), 3.49 (t, 1H, *J* = 9.5 Hz, H-3'), 3.61 (dd, 1H, *J* = 5.0 Hz, *J* = 11.0 Hz, H-6'), 3.73 (d, 1H, *J* = 10.0 Hz, H-6), 3.87 (dd, 1H, *J* = 3.0 Hz, *J* = 11.0 Hz, H-6), 3.90 (m, 1H, H-5), 4.13 (t, 1H, *J* = 9.5 Hz, H-4), 4.39 (d, 1H, *J* = 8.0 Hz, H-1'), 4.47-4.57 (m, 4H, 2xCH<u>H</u> Bn and $MeSO_2CH_2C\underline{H}_2$-), 4.64 (t, 1H, *J* = 8.5 Hz, H-2'), 4.75 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.85 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 5.14 (d, 1H, *J* = 4.0 Hz, H-1), 5.18 (dd, 1H, *J* = 3.5 Hz, *J* = 10.0 Hz, H-2), 5.24 (s, 1H, CH Benzylidene), 5.88 (t, 1H, *J* = 10.0 Hz, H-3), 7.22-8.00 (m, 25H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 42.0 (CH_3 Msc), 53.4 ($MeSO_2\underline{C}H_2CH_2$-), 55.4 ($CH_3$ OMe), 61.3 ($MeSO_2CH_2\underline{C}H_2$-), 65.8 (C-5'), 67.5 (C-6), 67.7 (C-6'), 69.6 (C-5), 70.6 (C-3), 71.8 (C-2), 73.6 (CH_2 Bn), 73.9 (CH_2 Bn), 76.0 (C-4), 77.5 (C-2'), 78.5 (C-3'), 80.8 (C-4'), 96.9 (C-1), 100.6 (C-1'), 100.9 (CH Benzylidene), 125.9-133.2 (CH arom), 129.0 (C_q Bz), 130.2 (C_q Bz), 136.8 (C_q CHPh), 137.8 (C_q Bn), 138.1 (C_q Bn), 153.3 (C=O Msc), 165.2 (C=O Bz), 165.8 (C=O Bz); HRMS $[M+H]^+$ calcd for $C_{52}H_{55}O_{17}S$ 983.31545 was found 983.31689, $[M+NH_4]^+$ calcd for $C_{52}H_{58}O_{17}SN$ 1000.34200 was found 1000.34326, $[M+Na]^+$ calcd for $C_{52}H_{54}O_{17}SNa$ 1005.29739 was found 1005.29822.

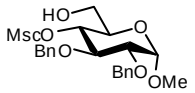


Methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-α-D-glucopyranoside (24) (Cleavage of Msc from 23): To a solution of **23** (94 mg, 96 μmol) in dioxane (1.9 ml, 0.05 M) was added DBU (1% in DMF, 71 μl, 10 μmol,

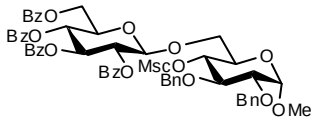
0.1 eq) and the reaction mixture was stirred for 30 minutes. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-α-D-glucopyranoside **23** (78 mg, 96 μmol, 100%); TLC (33% Toluene in EtOAc): R_f = 0.6; $[\alpha]_D^{22}$: +55.2º (c = 1.0, DCM); IR (neat, cm^{-1}): 696, 709, 1026, 1067, 1277, 1722; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.72 (t, 1H, *J* = 10.4 Hz, H-6'), 2.94-2.98 (m, 1H, H-5'), 3.41 (m, 7H, CH_3 OMe, H-2', H-3', H-4' and H-6'), 3.79 (dd, 1H, *J* = 1.60 Hz, *J* = 10.8 Hz, H-6), 3.9 (m, 1H, H-5), 3.06 (dd, 1H, *J* = 2.8 Hz, *J* = 10.8 Hz, H-6), 4.14 (t, 1H, *J* = 9.2 Hz, H-4), 4.32 (d, 1H, *J* = 7.2 Hz, H-1'), 4.56 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.71 (m, 2H, 2xCHH Bn), 4.88 (d, 1H, *J* = 12.0 Hz, CHH Bn), 5.16 (m, 2H, H-1 and H-2), 5.25 (s, 1H, CH Benzylidene), 5.94 (t, 1H, *J* = 9.2 Hz, H-3), 7.25-8.00 (m, 25H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 55.4 (CH_3 OMe), 66.1 (C-5'), 67.9 (C-6'), 68.0 (C-6), 69.6 (C-5), 71.1 (C-3), 72.0 (C-2), 73.6 (CH_2 Bn), 74.3 (CH_2 Bn), 74.4 (C-2'), 80.2 (C-4'), 80.9 (C-3'), 97.0 (C-1), 100.9 (CH Benzylidene), 103.8 (C-1'), 125.9-133.2 (CH arom), 129.2 (C_q Bz), 130.3 (C_q Bz), 137.2 (C_q Benzylidene), 137.7 (C_q Bn), 138.4 (C_q Bn), 165.3 (C=O Bz), 166.0 (C=O Bz); HRMS $[M+Na]^+$ calcd for $C_{48}H_{48}O_{13}Na$ 855.29871 was found 855.29927.



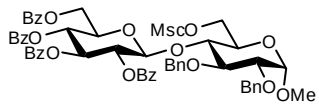
Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-α-D-mannopyranoside (26): To a solution of compound **13** (0.160 g, 0.27 mmol, 1 eq) in DCM (5.3 ml, 0.05 M) was added diphenyl sulfoxide (0.070 g, 0.35 mmol, 1.3 eq) and tri-*tert*-butylpyrimidine (0.199 g, 0.80 mmol, 3 eq) and the mixture was stirred over molecular sieve 3Å for 30 minutes. After that the reaction mixture was brought to -78 ºC and triflic acid anhydride (58 μl, 0.35 mmol, 1.3 eq) was added and the mixture was stirred for 15 minutes. Next a solution of compound **25** (0.148 g, 0.40 mmol, 1.5 eq) in DCM (2.7 ml, 0.15 M) was added and stirring was continued for 18 hours at -78 ºC. The reaction mixture was quenched with triethylamine (5 eq), diluted with DCM, washed with water and extracted with DCM thrice. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to yield **26** (0.178 g, 0.21 mmol, 78%); TLC (33% Toluene in EtOAc): R_f = 0.6; IR (neat, cm^{-1}): 697, 734, 1020, 1066, 1108, 1829; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.79 (s, 3H, CH_3 Msc), 3.18-3.22 (m, 1H, $MeSO_2CHHCH_2$-), 3.27-3.33 (m, 1H, $MeSO_2CHHCH_2$-), 3.38 (s, 3H, CH_3 OMe), 3.80-3.90 (m, 5H, H-2, H-5, H-5', H-6 and H-6'), 4.05 (m, 1H, H-2'), 4.08-4.16 (m, 2H, H-4', CHH Bn), 4.20-4.29 (m, 5H, H-3, H-4, H-6, H-6' and CHH Bn), 4.37 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.48 (m, 2H, $MeSO_2CH_2$ CH_2O-), 4.76 (d, 1H, *J* = 1.5 Hz, H-1), 4.79 (s, 2H, H-1' and CHH Bn), 5.17 (dd, 1H, *J* = 3.5 Hz, *J* = 10.5 Hz, H-3'), 5.53 (s, 1H, CH Benzylidene), 5.61 (s, 1H, CH Benzylidene), 7.00-7.50 (m, 20H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 42.4 (CH_3 Msc), 53.7 ($MeSO_2CH_2CH_2$-), 55.0 (CH_3 OMe), 61.4 ($MeSO_2CH_2CH_2$-), 63.8, 64.4, 77.3 (C-2, C-5 and C-5'), 68.7, 68.9 (C-6 and C-6'), 72.6 (CH_2 Bn), 73.5 (CH_2 Bn), 73.8, 79.3 (C-3 and C-4), 75.4 (C-3'), 75.7 (C-2'), 76.0 (C-4'), 99.2, (C-1'), 100.1, (C-1), 101.9 (CH Benzylidene), 102.1 (CH Benzylidene), 126.2-129.7 (CH arom), 137.2, 137.3, 137.5 (2xC_q Benzylidene and 2xC_q Bn), 153.5 (C=O Msc); CH Gated NMR (125 MHz, $CDCl_3$) δ = 99.2 (*J* = 170 Hz, C-1'), 100.1 (*J* = 182 Hz, C-1). HRMS $[M+Na]^+$ calcd for $C_{45}H_{50}O_{15}SNa$ 885.27626 was found 885.27625.



Methyl 2,3-di-*O*-benzyl-4-*O*-methylsulfonylethoxycarbonyl-α-D-glucopyranoside (28): To a solution of **8** (0.240 mg, 0.29 mmol) in DCM (2.9 ml, 0.1 M) was added EtOH (1 ml) and acetic acid (7.6 ml) and the mixture was stirred for 18 hours. The reaction mixture was neutralized with $NaHCO_{3\ (aq)}$, diluted with EtOAc, washed with $NaHCO_{3\ (aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford **28** (0.123 g, 0.23 mmol, 81%). TLC (50% EtOAc in PE): R_f = 0.2; $[\alpha]_D^{22}$: +37.4º (c = 1.0, DCM); IR (neat, cm^{-1}): 630, 1262, 1757; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.35 (bs, 1H, C6-OH), 2.83 (s, 3H, CH_3 Msc), 3.20-3.27 (m, 2H, $MeSO_2C\underline{H}_2CH_2$-), 3.38 (s, 3H, CH_3 OMe), 3.57 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.64 (m, 3H, H-5 and 2xH-6), 3.98 (t, 1H, *J* = 9.6 Hz, H-3), 4.42-4.48 (m, 1H, $MeSO_2CH_2CH\underline{H}$-), 4.51-4.56 (m, 1H, $MeSO_2CH_2CH\underline{H}$-), 4.61 (d, 1H, *J* = 3.6 Hz, H-1), 4.62-4.66 (m, 2H, 2xCH<u>H</u> Bn), 4.76-4.83 (m, 2H, H-4 and CH<u>H</u> Bn), 4.94 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 7.26-7.35 (m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.0 (CH_3 Msc), 53.4 ($MeSO_2\underline{C}H_2CH_2$-), 55.4 ($CH_3$ OMe), 60.9 (C-6), 61.3 ($MeSO_2CH_2\underline{C}H_2$-), 69.0 (C-5), 73.4 ($CH_2$ Bn), 74.8 (C-4), 75.3 (CH_2 Bn), 78.9 (C-3), 79.3 (C-2), 98.0 (C-1), 127.4-128.4 (CH arom), 137.6 (C_q Bn), 138.4 (C_q Bn), 154.2 (C=O Msc); HRMS $[M+Na]^+$ calcd for $C_{25}H_{32}O_{10}SNa$ 547.16084 was found 547.16056.



Methyl 2,3-di-*O*-benzyl-4-*O*-methylsulfonylethoxycarbonyl-6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranoside (29): Disaccharide **29** was prepared form acceptor **28** (0.088 g, 0.17 mmol, 1 eq) and donor **27** (0.168 g, 0.25 mmol, 1.5 eq) according to the general procedure for glycosylations as described above yielding compound **29** (0.130 g, 0.12 mmol, 70%). TLC (50% EtOAc in PE): R_f = 0.65; $[\alpha]_D^{22}$: +39.2º (c = 1, DCM); IR (neat, cm^{-1}): 1249, 1725; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.78 (s, 3H, CH_3 Msc), 3.11-3.20 (m, 5H, CH_3 OMe and $MeSO_2C\underline{H}_2CH_2$-), 3.37-3.39 (m, 1H, H-2), 3.63 (dd, 1H, *J* = 6.4 Hz, *J* = 11.2 Hz, H-6), 3.81-3.89 (m, 2H, H-3 and H-5), 4.02 (d, 1H, *J* = 10.8 Hz, H-6), 4.17 (m, 1H, H-5'), 4.34 (m, 1H, $MeSO_2CH_2C\underline{H}H$-), 4.40-4.56 (m, 5H, H-1, H-6', $MeSO_2CH_2C\underline{H}H$- and 2xCH<u>H</u> Bn), 4.67 (m, 3H, H-4, H-6' and CH<u>H</u> Bn), 4.87 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 4.92 (d, 1H, *J* = 8.0 Hz, H-1'), 5.54 (t, 1H, *J* = 9.2 Hz, H-2'), 5.69 (t, 1H, *J* = 9.6 Hz, H-4'), 5.90 (t, 1H, *J* = 9.6 Hz, H-3'), 7.22-7.96 (m, 30H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.1 (CH_3 Msc), 53.4 ($MeSO_2\underline{C}H_2CH_2$-), 55.0 ($CH_3$ OMe), 61.2 ($MeSO_2CH_2C\underline{H}_2$-), 63.0 (C-6'), 68.0 (C-5), 68.4 (C-6), 69.6 (C-3'), 71.8 (C-2'), 72.1 (C-5'), 72.7 (C-4'), 73.2 (CH_2 Bn), 75.0 (C-4), 75.2 (CH_2 Bn), 79.0 (C-3), 79.2 (C-2), 97.5 (C-1), 101.4 (C-1'), 127.2-138.5 (CH arom), 128.7 (2xC_q Bz), 129.4 (C_q Bz), 129.7 (C_q Bz), 137.7 (C_q Bn), 138.5 (C_q Bn), 153.7 (C=O Msc), 165.0 (C=O Bz), 165.1 (C=O Bz), 165.7 (C=O Bz), 166.0 (C=O Bz); HRMS $[M+H]^+$ calcd for $C_{59}H_{59}O_{19}S$ 1103.33658 was found 1103.33850.



Methyl 2,3-di-*O*-benzyl-6-*O*-methylsulfonylethoxycarbonyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranoside (30): Disaccharide **30** was prepared form acceptor **15** (0.068g, 0.13 mmol,

1 eq) and donor **27** (0.130 g, 0.20 mmol, 1.5 eq) according to the general procedure for glycosylations as described above yielding compound **30** (0.92 g, 0.08 mmol, 64%). TLC (50% EtOAc in PE): R_f = 0.7; $[\alpha]_D^{22}$: +46.6º (c = 1, DCM); IR (neat, cm^{-1}): 1250, 1728; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.98 (s, 3H, CH_3 Msc), 3.28 (s, 3H, CH_3 OMe), 3.30-3.38 (m, 2H, $MeSO_2C\underline{H}_2CH_2$), 3.44 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.71 (m, 1H, H-5), 3.81 (t, 1H, *J* = 9.2 Hz, H-4), 3.98 (t, 1H, *J* = 9.2 Hz, H-3), 4.03 (m, 1H, H-5'), 4.23-4.32 (m, 3H, 2xH-6 and H-6'), 4.39 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H-6'), 4.45 (m, 1H, $MeSO_2CH_2C\underline{H}H$), 4.50 (d, 1H, *J* = 3.2, H-1), 4.56-4.62 (m, 2H, $MeSO_2CH_2CH\underline{H}$ and $CH\underline{H}$ Bn), 4.71 (d, 1H, *J* = 12.0 Hz, $CH\underline{H}$ Bn), 4.91 (d, 1H, *J* = 11.2 Hz, $CH\underline{H}$ Bn), 5.06 (d, 1H, *J* = 11.6 Hz, $CH\underline{H}$ Bn), 5.10 (d, 1H, *J* = 8.0 Hz, H-1'), 5.54 (t, 1H, *J* = 9.6 Hz, H-2'), 5.66 (t, 1H, *J* = 9.6 Hz, H-4'), 5.91 (t, 1H, *J* = 9.6 Hz, H-3'), 7.16-8.00 (m, 30H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.0 (CH_3 Msc), 53.5 ($MeSO_2\underline{C}H_2CH_2$), 55.4 ($CH_3$ OMe), 60.9 ($MeSO_2CH_2\underline{C}H_2$), 62.8 (C-6'), 66.1 (C-6), 67.7 (C-5), 69.4 (C-4'), 71.8 (C-5'), 72.4 (C-2'), 73.0 (C-3'), 73.4 (CH_2 Bn), 75.0 (CH_2 Bn), 78.0 (C-4), 79.1 (C-2), 79.5 (C-3), 98.0 (C-1), 100.9 (C-1'), 126.9-133.4 (CH arom), 128.7 (C_q Bz), 128.8 (2xC_q Bz), 129.5 (C_q Bz), 137.9 (C_q Bn), 138.9 (C_q Bn), 154.0 (C=O Msc), 164.9 (C=O Bz), 165.0 (C=O Bz), 165.7 (C=O Bz), 165.9 (C=O Bz); HRMS $[M+H]^+$ calcd for $C_{59}H_{59}O_{19}S$ 1103.33658 was found 1103.33871, $[M+Na]^+$ calcd for $C_{59}H_{58}O_{19}SNa$ 1125.31852 was found 1125.31962.

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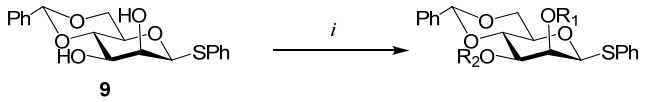
13. During the introduction and cleavage of the Fmoc, dibenzofulvene polymers can be formed which can complicate purification.

14. Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-mannopyranoside (**11**) was obtained from phenyl-4,6-*O*-benzylidene-1-thio-β-D-mannopyranoside (**9**) as described in the experimental section.[21]



9

10 R_1 = H, R_2 = Bn (16%)
11 R_1 = Bn, R_2 = H (44%)
12 R_1 = Bn, R_2 = Bn (11%)

Reagents and conditions; *i*) BnBr, TBAS, $NaOH_{(aq)}$, DCM, 40 ºC, 18 h.

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CHAPTER

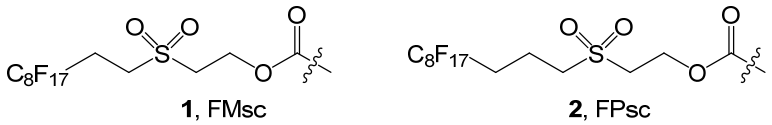
3

([1*H*,1*H*,2*H*,2*H*,3*H*,3*H*]-perfluoroundecyl)sulfonylethoxycarbonyl (FPsc): a fluorous hydroxyl protecting group in carbohydrate chemistry[1]

Introduction:

In 1997, Curran and coworkers reported fluorous solid phase extraction (FSPE) as a new purification method in synthetic organic chemistry.[2] FSPE involves the chromatographic separation of fluorous and non-fluorous components from a mixture by the use of a fluorous solid phase in combination with fluorophilic solvents.[3] Silica gel functionalized with a perfluoroalkyl chain is typically used as a fluorous solid phase in combination with methanol, acetonitrile or tetrahedrofuran as fluorophilic solvents and water as a fluorophobic solvent.[4] FSPE can be executed with the aid of both low- and high-pressure techniques.[5]

Figure 1: The FMsc and the FPsc protecting group.



1, FMsc **2**, FPsc

In recent times the application of FSPE in synthetic organic chemistry is greatly stimulated by the development of numerous fluorous reagents such as catalysts,[6] chiral auxiliaries[7] and scavengers.[8] A variety of fluorous protective groups, serving the dual purpose of protective group and purification handle in FSPE, have also been reported. Fluorous protecting groups for many relevant functional groups, such as amino[9] and hydroxyl functions[10] have been developed. Generally a fluorous protective group is obtained by the appendage of a perfluoroalkyl moiety to the core of a known protective group such as the benzyl,[11] benzyloxycarbonyl,[12] *tert*-butyl,[13] *tert*-butyloxycarbonyl,[14] trityl[15] and benzylidene[16] group. Most commonly, an ethylene spacer is incorporated to isolate the electron withdrawing effect of the fluorous tail, which can undesirably alter the properties of the protecting group.[12]

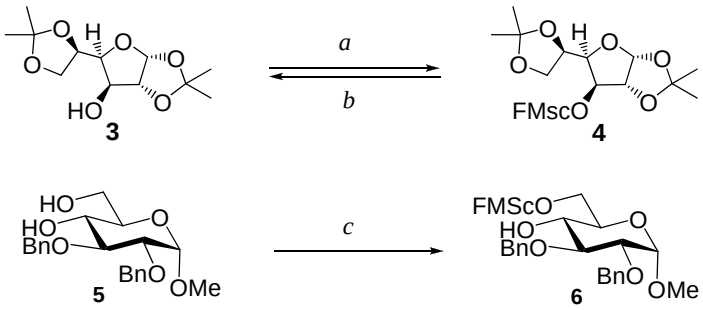
A few years ago, the [1*H*,1*H*,2*H*,2*H*]-perfluorodecylsulfonylethoxycarbonyl (FMsc, **1**, Figure 1) group was introduced as a fluorous version of the methylsulfonylethoxycarbonyl (Msc) protective group for amines.[17] The FMsc group was used as a purification handle for oligopeptides, assembled by Fmoc-based solid phase peptide synthesis (SPPS). The FMsc group could be introduced at the amino terminus of a target oligopeptide at the final stage of the SPPS. Cleavage from the solid support and purification of the resulting mixture by fluorous HPLC (FHPLC) effected separation of the fluorous target oligopeptide and non-fluorous deletion sequences. Removal of the FMsc group using mild basic conditions afforded the pure oligopeptide. This result together with the successful application of the (Msc) group for the protection of hydroxyl functions, as described in chapter 2, was an incentive to explore the use of fluorous Msc-based protective groups in carbohydrate chemistry. The outcome of this study, showing the favorable properties of the [1*H*,1*H*,2*H*,2*H*,3*H*,3*H*]-perfluoroundecylsulfonylethoxycarbonyl (FPsc) group **2** is outlined in the present chapter.

Results and discussion:

The assessment of the optimal conditions for the introduction of the methylsulfonylethoxycarbonyl (Msc) group is described in chapter 2. It turned out that pyridine as base and DCM as solvent were most efficient. Whether these conditions are also suitable for the introduction of the FMsc group (**1**) was checked by the treatment of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **3** with 2 equivalents of [1*H*,1*H*,2*H*,2*H*]-perfluorodecylsulfonylethoxycarbonyl chloride (FMsc-Cl) and 3 equivalents of pyridine in DCM (Scheme 1). After 4 hours the reaction was complete and glucofuranose **4** was isolated in 95% yield. Next, the FMsc group could be introduced regioselectively at the primary C6-OH of diol **5** using the same conditions, albeit at lower temperature (-20 ^{o}C), to give alcohol **6** in 95% yield.

The optimal condition for the removal of the Msc group, *i.e.* 0.1 eq. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), (see Chapter 2), also proved to be appropriate for the cleavage of the FMsc group, although removal of the latter carbonate proved to be significantly faster. Subjection of compound **4** to these conditions for 1 minute gave the expected alcohol **3** in 98% yield.

Scheme 1: Installation and cleavage of the FMsc group.

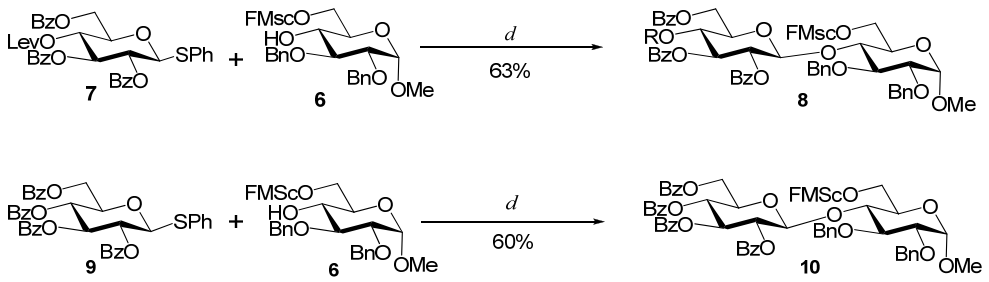


Reagents and conditions; *a*) FMS-Cl, pyr, DCM, 0 ºC-RT, 4h, 95%; *b*) DBU, dioxane, 1 min, 98%; *c*) FMS-Cl, pyr, DCM, -20 ºC-RT, 4h, 95%.

Next, the feasibility of the FMsc-protective group in *N*-iodosuccinimide (NIS) and trimethylsilyltriflate (TMSOTf) mediated glycosylation reactions was investigated. In the

first example, the FMsc-protected methyl glucoside **6** was condensed with thioglucoside **7** to provide disaccharide **8** (Scheme 2). TLC analysis of the crude reaction mixture showed the presence of a main product together with side products, probably derived from the donor. Purification by FSPE using a gradient of acetonitrile in water (50% to 100%) provided disaccharide **8**. Although TLC analysis of the combined fractions after FSPE showed the presence of one product, subsequent evaporation of the solvents caused the formation of an unwanted non-fluorous side product. A second FSPE purification gave the same result and ensuing purification by silica gel column chromatography afforded disaccharide **8** in 63% overall yield. In the second glycosylation event, FMsc-protected methyl glucoside **6** was coupled via the same procedure with perbenzoylated *S*-phenyl glucoside **9** to provide disaccharide **10** (Scheme 2). Disaccharide **10** was purified by FSPE to afford the fluorous product **8** in 80% yield. Unfortunately, again a non-fluorous side product was generated during the evaporation of the solvents. Homogeneous disaccharide **10** was obtained by silica gel column chromatography in 60% yield.

Scheme 2: Glycosylation reactions using acceptors containing the FMsc group.



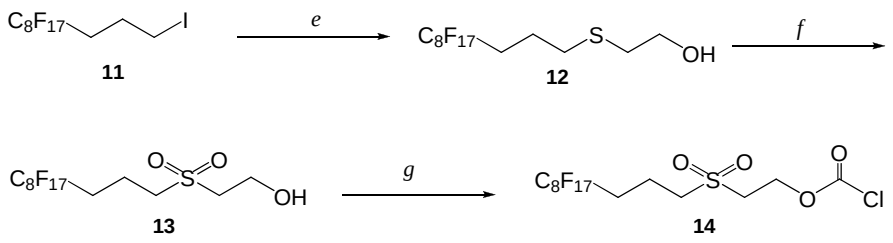
Reagents and conditions; *d*) i- NIS, TMSOTf, DCM, 0 ºC-RT, 1h; ii- FSPE.

The formation of the non-fluorous products, as described above, indicates that simple evaporation of aqueous acetonitrile can lead to partial removal of the FMsc from primary OH functions. This undesirable instability of the FMsc can be explained by the increased susceptibility of the FMsc for β-elimination by the inductive electron withdrawing effect of the perfluoroalkyl chain. It was envisaged that the

[1*H*,1*H*,2*H*,2*H*,3*H*,3*H*]-perfluoroundecylsulfonylethoxycarbonyl (FPsc, **2**, Figure 1) group, in which the distance between the sulfonyl functionality and the perfluoro moiety is increased by an additional methylene group, should be more stable.

The synthesis of [1*H*,1*H*,2*H*,2*H*,3*H*,3*H*]-perfluoroundecylsulfonylethoxycarbonyl chloride (FPsc-Cl, **14**) started from the commercially available [1*H*,1*H*,2*H*,2*H*,3*H*,3*H*]-perfluoroundecyl iodide **11** as depicted in Scheme 3.[18] The iodide was substituted with mercaptoethanol in refluxing *tert*-butylalcohol to give thioether **12** in 95% yield (Scheme 3). In the next step, compound **12** was oxidized using peracetic acid in AcOH/H_2O. The resulting sulfone **13** was isolated in 96% yield. Treatment of primary alcohol **13** with phosgene in THF gave chlorocarbonate **14**.

Scheme 3: Synthesis of FPsc-Cl.

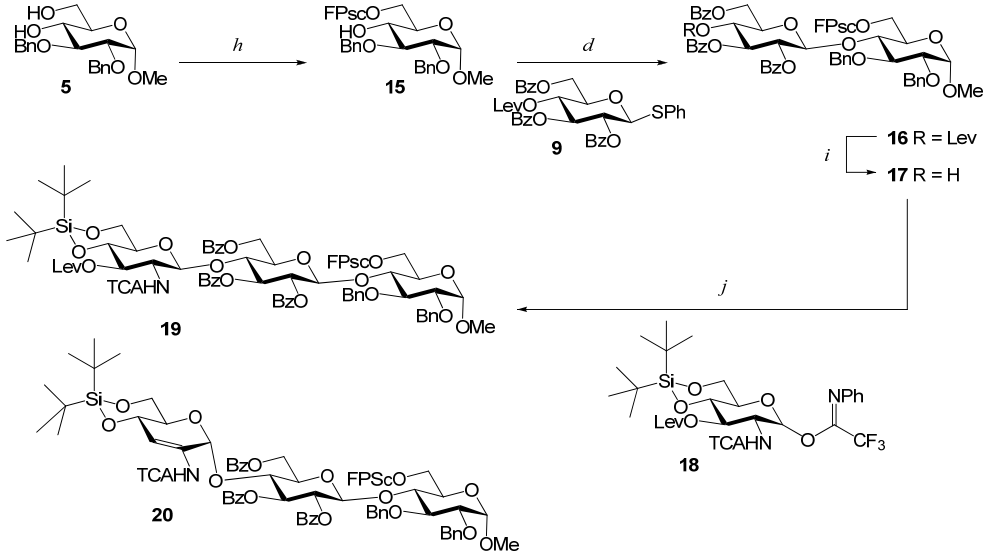


Reagents and conditions; *e*) mercaptoethanol, t-BuOH, NaOH, Reflux, 4h, 95%; *f*) AcOOH, AcOH, H_2O, EtOAc, 2h, 96%; *g*) phosgene, THF, 0 ºC, 16h, 100%.

Next, the usefulness of the FPsc group for oligosaccharide synthesis was assessed with the assembly of trisaccharide **19**.[19] First, the FPsc group was introduced selectively at the primary C6-OH of diol **5** by treatment with 1 equivalent FPsc chloride **14** at low temperature to give the acceptor glycoside **15** in 94% yield (Scheme 4). NIS/TMSOTf mediated condensation of fluorous acceptor **15** with excess of thiodonor **9** (3 equivalents), bearing a levulinoyl group at its C4-OH proceeded uneventfully. TLC analysis of the crude reaction mixture showed the presence of several products. Subsequent purification by FSPE, using a gradient of acetonitrile in water (50% to 100%) provided a single fluorous product. FPsc containing dimer **16** was isolated in excellent yield demonstrating the improved stability of the FPsc group with respect to its ethylene counterpart. The stability

of the FPsc was further substantiated by the selective removal of the levulinoyl group in **16** and ensuing purification by FSPE to afford alcohol **17** in 81% yield. Disaccharide **17** was elongated using an excess of (*N*-phenyl)trifluoro acetimidate **18**[20] (3 equivalents) and a catalytic amount of TfOH at -20 ^{o}C. After purification by FSPE, fluorous trimer **19** was isolated in 78%. It is of interest that executing the coupling of dimer **17** with **18** at 0 ^{o}C instead of -20 ^{o}C and purification by FSPE led to the isolation of a mixture of trimer **19** and a fluorous side product. After separation by silica gel chromatography, this side product was identified as FPsc protected trimer **20**, containing dehydroglucosamine. Formation of this product can be explained by β-elimination of the imidate group of donor **18**, followed by a Ferrier glycosylation on the resulting glycal.[21]

Scheme 4: Oligosaccharide synthesis using the FPsc group.



Reagents and conditions; *d*) i- NIS, TMSOTf, DCM, 0 ºC-RT, 1h; ii- FSPE, 93%; *h*) FPsc-Cl (**14**), pyr., DCM, -40 ºC-RT, 4, 94%; *i*) i- $H_2NNH_2.H_2O$, pyr./HOAc, 5 min; ii- FSPE, 94%; *j*) i- TfOH, DCM, -20 ºC-RT, 15 min; ii- FSPE, 78%

Conclusion:

In conclusion the FPsc group is a new fluorous hydroxyl-protecting group, suitable for implementation in oligosaccharide synthesis. The FPsc group can be introduced under

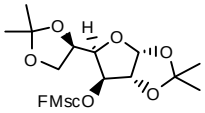
mild conditions and where necessary in a regioselective manner. It is cleaved under mild basic conditions, under which commonly used ester protecting groups stay intact. The FPsc group survives both acidic glycosylation conditions and the removal of the levulinoyl group.

Experimental:

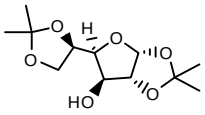
General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Traces of water in donor and acceptor glycosides were removed by co-evaporation with toluene. Molecular sieves 3Å were flame dried before use. All other chemicals (Acros, Fluka, Merck, Fluorous Technologies Inc.) were used as received. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on DC-alufolien (Merck, kiesel gel 60, F_{245}). Compounds were visualized by UV absorption (245 nm), by spraying with an aqueous solution of $KMnO_4$ (20%) and K_2CO_3 (10%), by spraying with 20% H_2SO_4 in ethanol or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10g/L) in 10% $H_2SO_{4\,(aq)}$ followed by charring at ~150 ºC. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 MHz and 100 MHz respectively), AV 500 (500 MHz and 125 MHz respectively) or DMX 600 (600 MHz and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ unless stated otherwise. Chemical shift are relative to tetramethylsilane and are given in ppm. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High resolution mass spectra were recorded on a LTQ-Orbitrap (thermo electron).

General method for glycosylations using NIS/TMSOTf: A solution of 1-thio-β-D-glucopyranoside (donor) and acceptor in DCM (0.05 M) was stirred over activated MS3Å for 30 minutes before *N*-iodosuccinimide (1.3 eq with respect to the donor) was added. The mixture was cooled to -40 °C followed by the addition of trimethylsilyl trifluoromethanesulfonate (0.1 eq). The mixture was allowed to warm to room temperature. The reaction mixture was quenched with triethylamine (5 eq), filtered, diluted with ETOAc and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with EtOAc thrice, after which the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by fluorous solid phase extraction (FSPE).

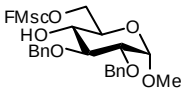
General procedure for fluorous solid phase extraction (FSPE): A FSPE Cartridge preloaded with 10 g of fluorous silica gel was eluted with DMF (20 ml), acetonitrile (30 ml) and 50% acetonitrile in H_2O (50 ml) before loading the crude product in acetonitrile (1.5 ml). The cartridge was eluted with 50% acetonitrile in H_2O (50 ml) and 70% acetonitrile in H_2O (50 ml) to wash the fluorophobic fraction. Next, fluorophilic fraction was eluted with acetonitrile (50 ml) to afford the target compound.



1,2:5,6-di-*O*-isopropylidene-3-*O*-([1H,1H,2H,2H]-perfluorodecyl)sulfonylethoxycarbonyl-α-D-glucofuranoside (4): A solution of 1,2:5,6-*O*-isopropylidene-α-D-glucofuranose **3** (0.039 g, 0.14 mmol) in DCM (1.5 ml, 0.1 M) was cooled to 0 °C before pyridine (36 µl, 0.45 mmol, 3 eq) was added. Next, ([1H,1H,2H,2H]-perfluorodecyl)sulfonylethoxycarbonyl chloride (FMsc-Cl, 10% in DCM, 0.185 g, 0.30 mmol, 2 eq) was added drop-wise at 0 °C over the span of 30 minutes. The mixture was allowed to warm to room temperature. The reaction mixture was quenched with methanol, diluted with DCM, washed with $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford compound **9** (0.120 g, 142 µmol, 95%). TLC (50% n-hexane in EtOAc): R_f = 0.8; $[\alpha]_D^{22}$: +10.8º (c = 0.5, DCM); IR (neat, cm^{-1}): 494, 1023, 1091, 1145, 1199, 1373, 1748; ^1H NMR (600 MHz, $CDCl_3$) δ = 1.28 (s, 3H, CH_3 isopropylidene), 1.29 (s, 3H, CH_3 isopropylidene), 1.40 (s, 3H, CH_3 isopropylidene), 1.50 (s, 3H, CH_3 isopropylidene), 2.67 (m, 2H, $RfC\underline{H}_2CH_2SO_2(CH_2)_2$-), 3.32 (m, 2H, $RfCH_2C\underline{H}_2SO_2(CH_2)_2$-), 3.40 (m, 2H, $Rf(CH_2)_2SO_2C\underline{H}_2CH_2$-), 4.02 (m, 2H, 2xH-6), 4.15 (m, 2H, H-4 and H-5), 4.55 (d, 1H, *J* = 3.6 Hz, H-3), 4.61 (m, 2H, $Rf(CH_2)_2SO_2CH_2C\underline{H}_2$-), 5.14 (d, 1H, *J* = 2.4 Hz, H-2), 5.85 (d, 1H, *J* = 3.6 Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 23.5 (t, *J* = 22.0 Hz, $RfC\underline{H}_2CH_2SO_2(CH_2)_2$-), 24.5 ($CH_3$ isopropylidene), 25.6 (CH_3 isopropylidene), 26.2 (CH_3 isopropylidene), 26.5 (CH_3 isopropylidene), 45.8 ($RfCH_2\underline{C}H_2SO_2(CH_2)_2$-), 52.3 ($Rf(CH_2)_2SO_2\underline{C}H_2CH_2$-), 60.8 ($Rf(CH_2)_2SO_2CH_2\underline{C}H_2$-), 66.7 (C-6), 71.8, 79.1 (C-4 and C-5), 79.8 (C-3), 82.6 (C-2), 104.5 (C-1), 109.1 (C_q isopropylidene), 112.0 (C_q isopropylidene), 152.6 (C=O FMsc); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -126.4, -123.7, -123.0, -122.2, -122.0, -114.8 (CF_2), -81.1 (CF_3); HRMS $[M+Na]^+$ calcd for $C_{25}H_{27}O_{10}SNa$ 865.09457 was found 865.09521.

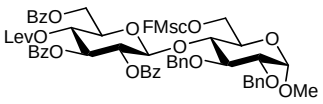


1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranoside (Cleavage of FMsc from 4): To a solution of **4** (24 mg, 28 µmol) in dioxane (0.6 ml, 0.05 M) was added DBU (1% in dioxane, 42 µl, 2.9 µmol, 0.1 eq) and the reaction mixture was stirred for 1 minute. The reaction mixture was neutralized with $NH_4Cl_{\,(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford 1,2:5,6-di-*O*-isopropylidene- -α-D-glucofuranose (7.3 mg, 28 µmol, 98%)

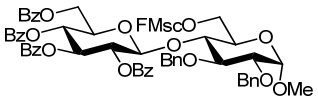


Methyl 2,3-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H]-perfluorodecyl)sulfonylethoxycarbonyl -α-D-glucopyranoside (6): A solution of methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside **5** (0.127 g, 0.34 mmol) in DCM (1 ml, 0.3 M) was cooled to -20 °C before pyridine (82 ml, 1.0 mmol, 3 eq) was added. Next, ([1H,1H,2H,2H]-perfluorodecylsulfonylethoxycarbonyl chloride (FMsc-Cl in 0.1 ml DCM, 0.419 g, 0.68 mmol, 2 eq) was added drop-wise over the span of 45 minutes. The mixture was allowed to warm to room temperature and stirring was continued for 4 hours. The reaction mixture was quenched with methanol, diluted with DCM, washed with $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford **6** (0.310 g, 0.32 mmol , 95%). TLC (50% EtOAc in n-hexane): R_f = 0.9; $[\alpha]_D^{22}$: +11.6º (c = 1, DCM); IR (neat, cm^{-1}): 1056, 1134,

1145, 1199, 1749; ^{1}H NMR (600 MHz, $CDCl_3$) δ = 2.61-2.69 (m, 2H, RfC<u>H</u>$_2$CH$_2$SO$_2$(CH$_2$)$_2$-), 3.30 (m, 2H, RfCH$_2$C<u>H</u>$_2$SO$_2$(CH$_2$)$_2$-), 3.33 (s, 3H, CH_3 OMe), 3.37 (m, 3H, H-4 and Rf(CH$_2$)$_2$SO$_2$C<u>H</u>$_2$CH$_2$-), 3.47 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.74 (m, 2H, H-3 and H-5), 4.33 (dd, 1H, *J* = 5.4 Hz, *J* = 11.4 Hz, H-6), 4.37 (dd, 1H, *J* = 1.8 Hz, *J* = 11.4 Hz, H-6), 4.55 (t, 2H, *J* = 4.8 Hz, Rf(CH$_2$)$_2$SO$_2$CH$_2$C<u>H</u>$_2$-), 4.57 (d, 1H, *J* = 3.6 Hz, H-1), 4.62 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.67 (d, 1H, *J* = 11.4 Hz, CH<u>H</u> Bn), 4.74 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.99 (d, 1H, *J* = 11.4 Hz, CH<u>H</u> Bn), 7.22-7.41 (m, 10H, H arom); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 24.0 (t, *J* = 22.5 Hz, Rf<u>C</u>H$_2$CH$_2$SO$_2$(CH$_2$-)$_2$), 46.3 (RfCH$_2$<u>C</u>H$_2$SO$_2$(CH$_2$-)$_2$), 52.8 (Rf(CH$_2$)$_2$SO$_2$<u>C</u>H$_2$CH$_2$-), 55.2 (CH_3 OMe), 61.0 (Rf(CH$_2$)$_2$SO$_2$CH$_2$<u>C</u>H$_2$-), 67.6 (C-6), 68.8 (C-3 or C-5), 69.7 (C-4), 73.2 (CH_2 Bn), 75.4 (CH_2 Bn), 79.6 (C-2), 81.0 (C-3 or C-5), 98.1 (C-1), 128.0-129.0 (CH arom), 137.8 (C_q Bn), 138.5 (C_q Bn), 154.3 (C=O FMsc); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -129.6, -126.7, -126.2, -125.4, -125.2, -117.3 (CF_2), -84.3 (CF_3); HRMS $[M+Na]^+$ calcd for $C_{34}H_{33}O_{10}F_{17}SNa$ 979.14152 was found 979.14211.

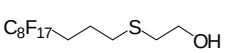


Methyl 2,3-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H]-perfluorodecyl)sulfonylethoxycarbonyl-4-*O*-(2,3,6-tri-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl)-α-D-glucopyranoside (8): Disaccharide **8** was prepared form acceptor **6** (0.055 g, 57.5 μmol, 1 eq) and donor **7** (0.117 g, 172.5 μmol, 3 eq) according to the general procedure for glycosylations as described above. The crude product was purified by general procedure of FSPE as described above. The side product generated during the evaporation of solvent was removed by silica gel chromatography to afford compound **8** (0.055 g, 36 μmol, 63%). TLC (33% EtOAc in toluene): R_f = 0.3; $[\alpha]_D^{22}$: +34.4º (c = 1, DCM); IR (neat, cm^{-1}): 713, 1147, 1207, 1269, 1732; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.92 (s, 3H, CH_3 Lev), 2.28-2.55 (m, 4H, 2xCH_2 Lev), 2.69 (m, 2H, RfC<u>H</u>$_2$CH$_2$SO$_2$(CH$_2$)$_2$-), 3.25 (s, 3H, CH_3 OMe), 3.34 (m, 4H, RfCH$_2$C<u>H</u>$_2$SO$_2$(CH$_2$)$_2$- and Rf(CH$_2$)$_2$SO$_2$C<u>H</u>$_2$CH$_2$-), 3.42 (dd, 1H, *J* = 3.5 Hz, *J* = 9.5 Hz, H-2), 3.73 (m, 2H, H-4 and H-5), 3.79 (m, 1H, H-5'), 3.96 (t, 1H, *J* = 9.0 Hz, H-3), 4.19 (dd, 1H, *J* = 3.0 Hz, *J* = 11.0 Hz, H-6), 4.25 (m, 2H, H-6 and H-6'), 4.34 (dd, 1H, *J* = 2.0 Hz, *J* = 12.0 Hz, H-6'), 4.41-4.45 (m, 1H, Rf(CH$_2$)$_2$SO$_2$CH$_2$CH<u>H</u>-), 4.48 (d, 1H, *J* = 3.5 Hz, H-1), 4.56 (m, 2H, Rf(CH$_2$)$_2$SO$_2$CH$_2$CH<u>H</u>- and CH<u>H</u> Bn), 4.67 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.91 (d, 1H, *J* = 11.5 Hz, CH<u>H</u> Bn), 5.02 (m, 2H, H-1' and CH<u>H</u> Bn), 5.38-5.46 (m, 2H, H-4' and H-2'), 5.68 (t, 1H, *J* = 9.5 Hz, H-3'), 7.20-8.02 (m, 25H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 27.7 (MeCOCH$_2$<u>C</u>H$_2$COO), 29.3 (CH_3 Lev), 29.7 (Rf<u>C</u>H$_2$CH$_2$SO$_2$(CH$_2$)$_2$), 37.7 (MeCO<u>C</u>H$_2$CH$_2$COO), 45.9 (RfCH$_2$<u>C</u>H$_2$SO$_2$(CH$_2$)$_2$), 52.5 (Rf(CH$_2$)$_2$SO$_2$<u>C</u>H$_2$CH$_2$), 55.2 (CH_3 OMe), 60.5 (Rf(CH$_2$)$_2$SO$_2$CH$_2$<u>C</u>H$_2$), 62.2 (C-6'), 66.5 (C-6), 67.6 (C-4 or C-5), 68.5 (C-4'), 71.3 (C-5'), 71.9 (C-2'), 72.4 (C-3'), 73.4 (CH_2 Bn), 74.9 (CH_2 Bn), 78.2 (C-4 or C-5), 79.4 (C-2), 79.6 (C-3), 97.9 (C-1), 100.9 (C-1'), 126.7-133.3 (CH arom), 128.8 (C_q Bz), 137.9 (C_q Bn), 139.0 (C_q Bn), 153.8 (C=O FMsc), 165.0 (C=O Bz), 165.7 (C=O Bz), 165.9 (C=O Bz), 171.3 (C=O MeCOCH$_2$CH$_2$<u>CO</u>O), 205.5 (C=O Me<u>CO</u>CH$_2$CH$_2$COO-); ^{19}F NMR (376 MHz, ($CDCl_3$) δ = -126.4, -123.7, -123.0, -122.2, -122.0, -114.7 (CF_2), -81.1 (CF_3); HRMS $[M+Na]^+$ calcd for $C_{66}H_{62}O_{20}F_{17}S$ 1551.30977 was found 1551.30844.

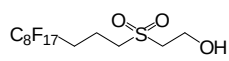


Methyl 2,3-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H]-perfluorodecyl)sulfonylethoxycarbonyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-

β-D-glucopyranosyl)-α-D-glucopyranoside (10): Disaccharide **10** was prepared from acceptor **6** (0.045 g, 47 μmol, 1 eq) and donor **9** (0.094 g, 0.141 mmol, 3 eq) according to the general procedure for glycosylations as described above. The crude product was purified by general procedure of FSPE as described above. The side product generated during the evaporation of solvent was removed by silica gel chromatography to afford compound **10** (0.043 g, 28 μmol, 60%). TLC (50% EtOAc in PE): R_f = 0.6; $[\alpha]_D^{22}$: +40.4º (c = 0.5, DCM); IR (neat, cm^{-1}): 708, 1026, 1091, 1205, 1244, 1733; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.67-2.73 (m, 2H, RfC$\underline{H}_2$CH$_2$SO$_2$(CH$_2$)$_2$-), 3.27 (s, 3H, CH$_3$ OMe), 3.38 (m, 4H, RfCH$_2$C$\underline{H}_2$SO$_2$(CH$_2$-)$_2$ and Rf(CH$_2$)$_2$SO$_2$C$\underline{H}_2$CH$_2$-), 3.44 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.75 (m, 2H, H-5 and H-4), 3.94 (m, 2H, H-3 and H-5'), 4.24 (m, 3H, H-6, H-6 and H-6'), 4.37 (dd, 1H, *J* = 3.2 Hz, *J* = 12.0 Hz, H-6'), 4.41-4.47 (m, 1H, Rf(CH$_2$)$_2$SO$_2$CH$_2$CH$\underline{H}$-), 4.49 (d, 1H, *J* = 3.6 Hz, H-1), 4.55 (m, 2H, Rf(CH$_2$)$_2$SO$_2$CH$_2$CH$\underline{H}$- and CH$\underline{H}$ Bn), 4.69 (d, 1H, *J* = 12.0 Hz, CH$\underline{H}$ Bn), 4.92 (d, 1H, *J* = 11.6 Hz, CH$\underline{H}$ Bn), 5.04 (d, 1H, *J* = 11.6 Hz, CH$\underline{H}$ Bn), 5.08 (d, 1H, *J* = 8.0 Hz, H-1'), 5.52 (dd, 1H, *J* = 8.0 Hz, *J* = 9.6 Hz, H-2'), 5.64 (t, 1H, *J* = 9.6 Hz, H-4'), 5.87 (t, 1H, *J* = 9.6 Hz, H-3'), 7.19-7.98 (m, 30H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 24.1 (t, *J* = 22.0 Hz, Rf$\underline{C}$H$_2$CH$_2$SO$_2$(CH$_2$)$_2$-), 54.9 (RfCH$_2$$\underline{C}H_2SO_2$(CH$_2$)$_2$-), 52.6 (Rf(CH$_2$)$_2SO_2$$\underline{C}H_2$CH-$_2$), 55.3 (CH$_3$ OMe), 60.5 (Rf(CH$_2$)$_2$SO$_2$CH$_2$$\underline{C}H_2$-), 62.8 (C-6'), 66.6 (C-6), 69.4 (C-4 or C-5), 72.0 (C-4'), 72.5 (C-5'), 72.6 (C-2'), 73.0 (C-3'), 73.5 (CH$_2$ Bn), 74.9 (CH$_2$ Bn), 78.1 (C-4 or C-5), 79.4 (C-2), 79.6 (C-3), 98.0 (C-1), 101.0 (C-1'), 126.9-133.3 (CH arom), 128.7 (C$_q$ Bz), 128.8 (C$_q$ Bz), 128.9 (C$_q$ Bz), 129.6 (C$_q$ Bz), 138.0 (C$_q$ Bn), 139.0 (C$_q$ Bn), 153.9 (C=O FMsc), 165.0 (C=O Bz), 165.0 (C=O Bz), 165.7 (C=O Bz), 166.0 (C=O Bz); ^{19}F NMR (376 MHz, ($CDCl_3$) δ = -126.4, -123.4, -123.0, -122.2, -121.9, -113.9 (CF_2), -81.1 (CF_3); HRMS $[M+H]^+$ calcd for $C_{68}H_{60}O_{19}F_{17}S$ 1535.31726 was found 1535.31891, $[M+Na]^+$ calcd for $C_{68}H_{58}O_{19}F_{17}SNa$ 1557.29920 was found 1557.30047.

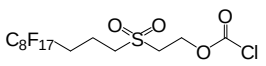


([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfidylethanol (12): NaOH (0.714 g, 17.9 mmol, 1.5 eq) and 2-mercaptoethanol (2.1 ml, 29.8 mmol, 2.5 eq) were refluxed in *t*-BuOH (40 ml) for 30 minutes. ([1H,1H,2H,2H,3H,3H]-perfluoroundecyl iodide (7.0 g, 11.9 mmol, 1 eq) was added and the mixture was refluxed for 2h. After evaporation of all volatiles, the crude product was subjected to silica gel column chromatography to give the compound **12** (6.11 g, 11.4 mmol, 95%). TLC (33% EtOAC in Toluene): R_f = 0.7; IR (neat, cm^{-1}): 528, 1197, 3341; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.94 (m, 2H, RfCH$_2$C$\underline{H}_2$CH$_2$S(CH$_2$)$_2$OH), 2.24 (m, 2H, RfC$\underline{H}_2$(CH)$_2$S(CH)$_2$OH), 2.67 (t, 2H, *J* = 7.2 Hz, Rf(CH$_2$)$_2$C$\underline{H}_2$S(CH$_2$)$_2$OH), 2.76 (t, 2H, *J* = 6.0 Hz, Rf(CH$_2$)$_3$SC$\underline{H}_2$CH$_2$OH), 3.79 (t, 2H, *J* = 6.0 Hz, Rf(CH$_2$)$_3$SCH$_2$C$\underline{H}_2$OH), 3.87 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 20.2 (RfCH$_2$$\underline{C}H_2CH_2$S(CH$_2$)$_2$OH), 29.6 (t, *J* = 22.0 Hz, Rf$\underline{C}$H$_2$(CH$_2$)$_2$S(CH$_2$)$_2$OH), 30.9 (Rf(CH$_2$)$_2$$\underline{C}H_2$S(CH$_2$)$_2$OH), 34.4 (Rf(CH$_2$)$_3S\underline{C}H_2CH_2$OH), 60.8 (Rf(CH$_2$)$_3SCH_2$$\underline{C}H_2$OH), 107.8-120.8 (CF); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -127.4, -124.4, -123.8, -123.0, -122.7, -115.1 (CF_2), -81.3 (CF_3); HRMS $[M+H]^+$ calcd for $C_{13}H_{12}O_1F_{17}S_1$ 539.03319 was found 539.03327.

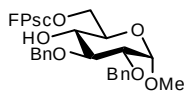


([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethanol (13): 39% AcOOH (4.9 ml, 28.4 mmol, 2.5 eq) and H_2O (2 ml) were added to a solution of **12** (6.11 g, 11.4 mmol) in ice cooled AcOH (3.8 ml). If gel formation occurred, EtOAc (5 ml) was added. The mixture was stirred for 90 minutes. The mixture was neutralized by careful addition of $NaHCO_{3\ (s)}$, extracted using large excess of

EtOAc, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford white crystalline **13** (6.21 g, 10.9 mmol, 96%). TLC (75% EtOAc in PE): R_f = 0.6; IR (neat, cm^{-1}): 506, 1115, 3475; ^1H NMR (400 MHz, $(CD_3)_2CO$) δ = 2.18 (m, 2H, $RfCH_2C\underline{H}_2CH_2SO_2(CH_2)_2OH$), 2.42-2.56 (m, 2H, $RfC\underline{H}_2(CH_2)_2SO_2(CH_2)_2OH$), 3.29 (t, 2H, *J* = 5.6 Hz, $Rf(CH_2)_2C\underline{H}_2SO_2(CH_2)_2OH$), 3.35 (t, 2H, *J* = 7.6 Hz, $Rf(CH_2)_3SO_2C\underline{H}_2CH_2OH$), 4.03 (dt, 2H, *J* = 5.2 Hz, *J* = 5.6 Hz, $Rf(CH_2)_3SO_2CH_2C\underline{H}_2OH$), 4.26 (t, 1H, *J* = 5.2 Hz, OH); ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ = 13.6 ($RfCH_2\underline{C}H_2CH_2SO_2(CH_2)_2OH$), 29.0 (m, $Rf\underline{C}H_2(CH_2)_2SO_2(CH_2)_2OH$), 52.9 ($Rf(CH_2)_2\underline{C}H_2SO_2(CH_2)_2OH$), 55.3 ($Rf(CH_2)_3SO_2\underline{C}H_2CH_2OH$), 55.9 ($Rf(CH_2)_3SO_2CH_2\underline{C}H_2OH$), 111.1-121.0 (CF); ^{19}F NMR (376 MHz, $(CD_3)_2CO$) δ = -127.2, -124.5, -123.7, -122.8, -122.7, -114.7 (CF_2), -81.2 (CF_3); HRMS $[M+H]^+$ calcd for $C_{13}H_{12}O_3F_{17}S_1$ 571.02302 was found 571.02302.

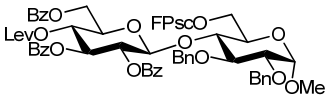


([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethoxycarbonyl chloride (14): To the solution of **13** (5.90 g, 10.4 mmol,) in freshly distilled THF (65 ml, 0.16 M) was added phosgene (20% in toluene, 9.4 ml, 18.6 mmol, 1.8 eq) at 0 ºC and the reaction mixture was stirred for 16 hours. Next the solvents and phosgene were removed *in vacuo* to give **14** (6.54 g, 10.4 mmol, 100%); IR (neat, cm^{-1}): 1139, 1769; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.26 (m, 2H, $RfCH_2C\underline{H}_2CH_2SO_2(CH_2)_2O$-), 2.36 (m, 2H, $RfC\underline{H}_2(CH_2)_2SO_2(CH_2)_2O$-), 3.18 (t, 2H, *J* = 7.2 Hz, $Rf(CH_2)_2C\underline{H}_2SO_2(CH_2)_2O$-), 3.40 (t, 2H, *J* = 5.6 Hz, $Rf(CH_2)_3SO_2C\underline{H}_2CH_2O$-), 4.76 (t, 2H, *J* = 5.6 Hz, $Rf(CH_2)_3SO_2CH_2C\underline{H}_2O$-); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.7 ($RfCH_2\underline{C}H_2CH_2SO_2(CH_2)_2O$-), 29.2 (m, $Rf\underline{C}H_2(CH_2)_2SO_2(CH_2)_2O$-), 51.8 ($Rf(CH_2)_2\underline{C}H_2SO_2(CH_2)_2O$-), 53.5 ($Rf(CH_2)_3SO_2\underline{C}H_2CH_2O$-), 64.4 ($Rf(CH_2)_3SO_2CH_2\underline{C}H_2O$-); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -126.5, -123.8, -123.1, -122.3, -122.0, -114.7 (CF_2) , -81.2 (CF_3); HRMS $[M+H]^+$ calcd for $C_{14}H_{10}O_4F_{17}S_1Na$ 654.96091 was found 654.95760.

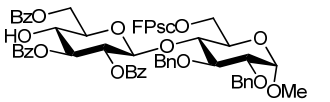


Methyl 2,3,-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethoxycarbonyl-α-D-glucopyranoside (15): A solution of **5** (0.153 g, 0.41 mmol) in DCM (1.4 ml, 0.3 M) was cooled to -40 ºC before pyridine (0.1 ml, 1.22 mmol, 3 eq) was added. Next, ([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethoxycarbonyl chloride (FPsc-Cl in 0.1 ml DCM, 0.387 g, 0.61 mmol, 1.5 eq) was added drop-wise over the span of 45 minutes. The reaction mixture was allowed to warm to room temperature and the stirring was continued for 4 hours. The reaction mixture was quenched with methanol, diluted with DCM, washed with $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and the crude product was purified by silica gel column chromatography to afford **15** (0.372 g, 0.38 mmol, 94%). TLC (5% Et_2O in DCM): R_f = 0.8; $[\alpha]_D^{22}$: +20.2º (c = 1, DCM); IR (neat, cm^{-1}): 734, 1200, 1748, 2927; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.14-2.21 (m, 2H, $RfCH_2C\underline{H}_2CH_2SO_2(CH_2)_2$-), 2.23-2.34 (m, 2H, $RfC\underline{H}_2(CH_2)_2SO_2(CH_2)_2$-), 2.72 (s, 1H, C4-OH), 3.09 (t, 2H, *J* = 7.6 Hz, $Rf(CH_2)_2C\underline{H}_2SO_2(CH_2$-$)_2$), 3.29 (t, 2H, *J* = 5.6 Hz, $Rf(CH_2)_3SO_2C\underline{H}_2CH_2$-), 3.36 (s, 3H, CH_3 OMe), 3.44 (t, 1H, *J* = 10.0 Hz, H-4), 3.49 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.73-3.80 (m, 2H, H-3 and H-5), 4.37 (m, 2H, 2xH-6), 4.51 (t, 2H, *J* = 6.0 Hz, $Rf(CH_2)_3SO_2CH_2C\underline{H}_2$-), 4.61 (d, 1H, *J* = 3.2 Hz, H-1), 4.64 (d, 1H, *J* = 12.4 Hz, CH<u>H</u> Bn), 4.74 (m, 2H, 2xCH<u>H</u> Bn), 4.99 (d, 1H, *J* = 11.2 Hz, CH<u>H</u> Bn), 7.26-7.35 (m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.4 ($RfCH_2\underline{C}H_2CH_2SO_2(CH_2)_2$-), 29.3 (t, *J* = 22.0 Hz, $Rf\underline{C}H_2(CH_2)_2SO_2(CH_2)_{2}$-), 52.0 ($Rf(CH_2)_2\underline{C}H_2SO_2(CH_2)_2$-),

52.9 (Rf$(CH_2)_3SO_2\underline{C}H_2CH_2$-), 55.1 ($CH_3$ OMe), 61.0 (Rf$(CH_2)_3SO_2CH_2\underline{C}H_2$-), 67.3 (C-6), 68.9 (C-5), 69.6 (C-4), 73.0 (CH_2 Bn), 75.3 (CH_2 Bn), 79.5 (C-2), 81.0 (C-3), 98.0 (C-1), 108.3-118.4 (CF), 127.4-128.4 (CH arom), 137.8 (C_q Bn), 138.6 (C_q Bn), 154.3 (C=O FPsc); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -126.4, -123.7, -123.0, -122.2, -122.0, -114.8 (CF_2), -81.1 (CF_3); HRMS $[M+Na]^+$ calcd for $C_{35}H_{35}O_{10}F_{17}SNa$ 993.15717 was found 993.15814.

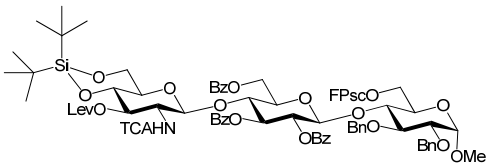


Methyl 2,3-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethoxycarbonyl-4-*O*-(2,3,6-tri-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl)-α-D-glucopyranoside (16): Disaccharide **16** was prepared from acceptor **15** (0.31 g, 0.39 mmol, 1 eq) and donor **9** (0.57 g, 0.84 mmol, 2.6 eq) according to the general procedure for glycosylations as described above. The crude product was purified by general procedure of FSPE as described above to afford compound **16** (0.46 g, 0.30 mmol, 93%). TLC (5% Methanol in DCM): R_f = 0.85; $[\alpha]_D^{22}$: +24.4° (c = 0.5, DCM); IR (neat, cm^{-1}): 710, 1203, 1722; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.90 (s, 3H, CH_3 Lev), 2.15-2.21 (m, 2H, Rf$CH_2\underline{C}H_2CH_2SO_2(CH_2)_2$-), 2.25-2.43 (m, 4H, 2x$CH_2$ Lev), 2.49 (m, 2H, Rf$\underline{C}H_2(CH_2)_2SO_2(CH_2)_2$-), 3.18 (t, 2H, *J* = 7.6 Hz, Rf$(CH_2)_2\underline{C}H_2SO_2(CH_2)_2$-), 3.25 (s, 3H, CH_3 OMe), 3.26-3.32 (m, 1H, Rf$(CH_2)_3SO_2CH\underline{H}CH_2$-), 3.38 (m, 1H, Rf$(CH_2)_3SO_2CH\underline{H}CH_2$-), 3.42 (dd, 1H, *J* = 4.8 Hz, *J* = 11.6 Hz, H-2), 3.72 (m, 1H, H-5), 3.79 (t, 1H, *J* = 8.8 Hz, H-4), 3.91 (m, 1H, H-5'), 3.97 (t, 1H, *J* = 9.2 Hz, H-3), 4.22 (m, 1H, H-6), 4.26-4.32 (m, 2H, H-6 and H-6'), 4.38 (dd, 1H, *J* = 2.0 Hz, *J* = 12.0 Hz, H-6'), 4.41-4.47 (m, 1H, Rf$(CH_2)_3SO_2CH_2CH\underline{H}$-), 4.50 (d, 1H, *J* = 3.6 Hz, H-1), 4.52-4.58 (m, 2H, Rf$(CH_2)_3SO_2CH_2CH\underline{H}$- and C$\underline{H}$ Bn), 4.69 (d, 1H, *J* = 12.0 Hz, C$\underline{H}$ Bn), 4.90 (d, 1H, *J* = 11.6 Hz, C$\underline{H}$ Bn), 5.06 (m, 2H, H-1' and C$\underline{H}$ Bn), 5.41-5.49 (m, 2H, H-4' and H-2'), 5.72 (t, 1H, *J* = 9.6 Hz, H-3'), 7.17-8.04 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.5 (Rf$CH_2\underline{C}H_2CH_2SO_2(CH_2)_2$-), 27.6 (MeCOC$H_2\underline{C}H_2$COO-), 29.1 ($CH_3$ Lev), 29.3 (t, *J* = 22.0 Hz, Rf$\underline{C}H_2(CH_2)_2SO_2(CH_2)_2$-), 37.6 (MeCO$\underline{C}H_2CH_2$COO-), 51.7 (Rf$(CH_2)_2\underline{C}H_2SO_2(CH_2)_2$-), 52.7 (Rf$(CH_2)_3SO_2\underline{C}H_2CH_2$-), 55.2 ($CH_3$ OMe), 60.5 (Rf$(CH_2)_3SO_2CH_2\underline{C}H_2$-), 62.2 (C-6'), 66.3 (C-6), 67.6 (C-5), 68.5 (C-4'), 71.6 (C-5'), 72.1 (C-2'), 72.9 (C-3'), 79.3 (CH_2 Bn), 74.9 (CH_2 Bn), 78.0 (C-4), 79.4 (C-2), 79.4 (C-3), 97.9 (C-1), 100.7 (C-1'), 108.0-120.4 (CF), 127.9-133.3 (CH arom), 128.8 (C_q Bz), 137.8 (C_q Bn), 138.9 (C_q Bn), 153.9 (C=O FPsc), 164.9 (C=O Bz), 165.7 (C=O Bz), 165.9 (C=O Bz), 171.3 (C=O MeCOC$H_2CH_2\underline{CO}$O-), 205.5 (C=O Me$\underline{CO}CH_2CH_2$COO-); ^{19}F NMR (376 MHz, ($CDCl_3$) δ = -126.4, -123.7, -123.0, -122.2, -122.0, -114.7 (CF_2), -81.1 (CF_3); HRMS $[M+H]^+$ calcd for $C_{67}H_{64}O_{20}F_{17}S$ 1543.34347 was found 1543.34541.



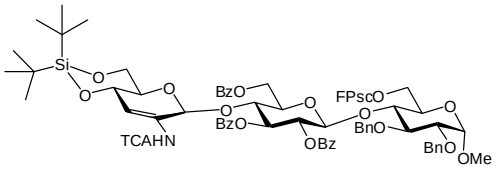
Methyl 2,3-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethoxycarbonyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranoside (17): To a solution of **16** (0.44 g, 0.29 mmol) in pyridine (2.4 ml, 0.1 M) and acetic acid (0.6 ml) was added hydrazine hydrate (72 µl, 1.49 mmol, 5 eq) and the mixture was stirred for 5 minutes, quenched with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\ (aq)}$ and brine, dried over $MgSO_4$, filtered and concentrated. . The crude product was purified by general procedure of FSPE as described above to afford compound **16** (0.332 g, 0.23 mmol, 81%). TLC (40% Toluene in EtOAc): R_f = 0.6; $[\alpha]_D^{22}$: +44.0° (c = 1.0, DCM); IR (neat, cm^{-1}): 1044, 1246, 1719, 3395; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.20 (m, 2H, Rf$CH_2\underline{C}H_2CH_2SO_2(CH_2)_2$-), 2.26-2.33 (m, 2H, Rf$\underline{C}H_2(CH_2)_2SO_2(CH_2)_2$-),

3.14-3.21 (m, 2H, Rf(CH_2)$_2$C<u>H</u>$_2$SO$_2$(CH_2)$_2$-), 3.28 (m, 4H, CH_3 OMe and Rf(CH_2)$_3$SO$_2$CH<u>H</u>CH_2-), 3.40 (m, 1H, Rf(CH_2)$_3$SO$_2$CH<u>H</u>CH_2-), 3.45 (dd, 1H, *J* = 3.2 Hz, *J* = 9.6 Hz, H-2) , 3.52 (d, 1H, *J* = 4.8 Hz, C4'-OH), 3.69-3.84 (m, 4H, H-5, H-5', H-4 and H-4'), 3.96 (t, 1H, *J* = 9.2 Hz, H-3), 4.20 (d, 1H, *J* = 11.2 Hz, H-6'), 4.30 (dd, 1H, *J* = 2.8 Hz, *J* = 9.6 Hz, H-6'), 4.39-4.50 (m, 3H, H-6, H-1 and Rf(CH_2)$_3$SO$_2$CH$_2$CH<u>H</u>-), 4.54-4.65 (m, 3H, H-6, Rf(CH_2)$_3$SO$_2$CH$_2$CH<u>H</u>-, and CH<u>H</u> Bn), 4.69 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.88 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 4.99 (d, 1H, *J* = 8.0 Hz, H-1'), 5.13 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 5.42 (t, 1H, *J* = 9.6 Hz, H-3'), 5.55 (t, 1H, *J* = 9.2 Hz, H-2'), 7.19-8.00 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.4 (RfCH$_2$<u>C</u>H$_2$CH$_2$SO$_2$(CH_2)$_2$-), 29.4 (t, *J* = 22.0 Hz, Rf<u>C</u>H$_2$(CH_2)$_2$SO$_2$(CH_2)$_2$-), 51.7 (Rf(CH_2)$_2$<u>C</u>H$_2$SO$_2$(CH_2)$_2$-), 52.8 (Rf(CH_2)$_3$SO$_2$<u>C</u>H$_2$CH$_2$-), 55.2 (CH_3 OMe), 60.4 (Rf(CH_2)$_3$SO$_2$CH$_2$<u>C</u>H$_2$-), 63.0 (C-6'), 66.4 (C-6), 67.7 (C-5), 69.0 (C-4'), 72.1 (C-2'), 73.4 (CH_2 Bn), 74.4 (C-5'), 75.0 (CH_2 Bn), 75.8 (C-3'), 78.2 (C-4), 79.1 (C-2), 79.6 (C-3), 98.0 (C-1), 100.9 (C-1'), 108.1-120.4 (CF), 126.9-133.3 (CH arom), 128.8 (C_q Bz), 128.9 (C_q Bz), 129.4 (C_q Bz), 137.9 (C_q Bn), 139.1 (C_q Bn), 154.0 (C=O FPsc), 165.1 (C=O Bz), 166.8 (C=O Bz), 167.0 (C=O Bz); ^{19}F NMR (376 MHz, ($CDCl_3$) δ = -126.5, -123.7, -123.1, -122.3, -122.0, -114.7 (CF_2) , -81.1 (CF_3); HRMS $[M+H]^+$ calcd for $C_{67}H_{58}O_{18}F_{17}S$ 1445.30724 was found 1445.30859, $[M+Na]^+$ calcd for $C_{62}H_{57}O_{18}F_{17}SNa$ 1467.28864 was found 1467.28959.



Methyl 2,3-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethoxycarbonyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-{2-deoxy-4,6-*O*-di-*tert*-butylsilyl-3-*O*-levulinoyl-2-*N*-trichloroacetamido-β-D-glucopyranosyl}-β-D-glucopyranosyl]-α-D-glucopyranoside (19): To the solution of **17** (0.288 g, 0.20 mmol) and **18** (0.394 mg, 0.60 mmol, 3 eq) in DCM (2 ml) was added triflic acid (5% in DCM, 67 µl, 0.02 mmol, 0.1 eq) at -20 ºC and the mixture was stirred at same temperature for 15 minutes before TLC showed complete disappearance of the acceptor. The mixture was diluted with EtOAc and washed with $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by FPSE to give **19** (0.312 mg, 0.16 mmol, 78%). TLC (50% Toluene in EtOAc): R_f = 0.65; $[\alpha]_D^{22}$: +2.6º (c = 1.0, DCM); IR (neat, cm^{-1}): 710, 1069, 1728; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.76 (s, 9H, 3xCH_3 TBDS), 0.84 (s, 9H, 3xCH_3 TBDS), 2.11 (s, 3H, CH_3 Lev), 2.13-2.25 (m, 3H, RfCH$_2$C<u>H</u>$_2$CH$_2$SO$_2$(CH_2)$_2$-) and MeCOC<u>H</u>HCH$_2$COO-), 2.49 (m, 1H, H-6''), 2.51-2.58 (m, 3H, MeCOCH$_2$C<u>H</u>$_2$COO- and MeCOC<u>H</u>HCH$_2$COO-), 2.67 (m, 2H, RfC<u>H</u>$_2$(CH_2)$_2$SO$_2$(CH_2)$_2$-), 2.75-2.81(m, 1H, H-5''), 3.24 (m, 5H, CH_3 OMe, Rf(CH_2)$_2$C<u>H</u>HSO$_2$(CH_2)$_2$- and Rf(CH_2)$_3$SO$_2$CH<u>H</u>CH_2-), 3.35-3.46 (m, 4H, H-2, H-4'', H-6'' and Rf(CH_2)$_3$SO$_2$CH<u>H</u>CH_2-), 3.57 (m, 1H, Rf(CH_2)$_3$SO$_2$CH<u>H</u>CH_2-), 3.63 (m, 1H, H-5), 3.80 (m, 1H, H-2''), 3.85-3.92 (m, 3H, H-3, H-4 and H-4'), 4.00 (m, 1H, H-5'), 4.06 (d, 1H, *J* = 10.4 Hz, H-6), 4.12 (d, 1H, *J* = 12.4 Hz, H-6'), 4.23 (d, 1H, *J* = 8.4 Hz, H-1''), 4.38 (dd, 1H, *J* = 2.8 Hz, *J* = 11.6 Hz, H-6), 4.46 (d, 1H, *J* = 3.6 Hz, H-1), 4.51-4.57 (m, 2H, CH<u>H</u> Bn and Rf(CH_2)$_3$SO$_2$CH$_2$CH<u>H</u>-), 4.68-4.76 (m, 2H, Rf(CH_2)$_3$SO$_2$CH$_2$CH<u>H</u>- and CH<u>H</u> Bn), 4.84 (m, 3H, H-6', H-3'' and CH<u>H</u> Bn), 4.97 (d, 1H, *J* = 8.0 Hz, H-1'), 5.17 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 5.44 (dd, 1H, *J* = 8.4 Hz, *J* = 10.0 Hz, H-2'), 5.73 (t, 1H, *J* = 9.6 Hz, H-3'), 7.12-8.04 (m, 26H, H arom and NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.5 (RfCH$_2$<u>C</u>H$_2$CH$_2$SO$_2$(CH_2)$_2$-), 19.5 (C_q TBDS), 22.2 (C_q TBDS), 26.6 (3xCH_3 TBDS), 27.0 (3xCH_3 TBDS), 27.8 (MeCOCH$_2$<u>C</u>H$_2$COO-), 29.1 (t, *J* = 22.0 Hz, Rf<u>C</u>H$_2$(CH_2)$_2$SO$_2$(CH_2)$_2$-),

29.6 (CH_3 Lev), 37.9 (MeCO<u>C</u>H_2CH_2COO-), 50.5 (RfC$(CH_2)_2$<u>C</u>$H_2SO_2(CH_2)_2$-), 53.3 (Rf$(CH_2)_3SO_2$<u>C</u>H_2CH_2-), 55.3 (CH_3 OMe), 55.4 (C-4''), 60.2 (Rf$(CH_2)_3SO_2CH_2$<u>C</u>H_2-), 62.1 (C-6'), 64.7 (C-6''), 66.0 (C-6), 67.6 (C-5), 70.6 (C-5''), 71.9 (C-2'), 72.6 (C-3'), 72.6 (C-4), 73.6 (CH_2 Bn), 73.9 (C-3''), 74.4 (C-5'), 75.0 (CH_2 Bn), 75.7 (C-3), 78.7 (C-4' and C-2), 79.6 (C-2''), 92.2 (CCl_3), 98.3 (C-1), 100.8 (C-1''), 101.1 (C-1'), 108.3-118.5 (CF), 127.0-133.7 (CH arom), 128.4 (C_q Bz), 128.6 (C_q Bz), 128.7 (C_q Bz), 137.9 (C_q Bn), 139.2 (C_q Bn), 154.0 (C=O FPsc), 162.3 (C=O TCA), 165.2 (C=O Bz), 165.4 (C=O Bz), 166.8 (C=O Bz), 172.2 (C=O MeCOCH_2CH_2<u>CO</u>O-), 205.5 (C=O Me<u>CO</u>CH_2CH_2COO-); ^{19}F NMR (376 MHz, ($CDCl_3$) δ = -126.4, -123.7, -123.0, -122.2, -122.0, -114.6 (CF_2) , -81.1 (CF_3); HRMS $[M+H]^+$ calcd for $C_{83}H_{89}NO_{25}Cl_3F_{17}SSi$ 1988.40805 was found 1988.40959.



Methyl 2,3-di-*O*-benzyl-6-*O*-([1H,1H,2H,2H,3H,3H]-perfluoroundecyl)sulfonylethoxycarbonyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-{2,3-dideoxy-4,6-*O*-di-*tert*-butylsilyl-2-*N*-trichloroacetamido-*erythro*-hex-2-eno-pyranosyl}-β-D-glucopyranosyl]-α-D-glucopyranoside (20): When the synthesis of **19** was carried at 0 ºC, two fluorous products were obtained after FSPE. **19** was separated from **20** (14%) by silica gel column chromatography. TLC (50% Toluene in EtOAc): R_f = 0.75; $[\alpha]_D^{22}$: +52.8º (c = 0.8, DCM); IR (neat, cm^{-1}): 731, 1264; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.94 (s, 9H, 3xCH_3 TBDS), 0.99 (s, 9H, 3xCH_3 TBDS), 2.11-2.35 (m, 4H, RfCH_2<u>C</u>$H_2CH_2SO_2(CH_2)_2$- and RfC<u>H</u>$_2(CH_2)_2SO_2(CH_2)_2$-), 3.07 (t, 2H, *J* = 7.6 Hz, Rf$(CH_2)_2$<u>C</u>$H_2SO_2(CH_2)_2$-), 3.19-3.26 (m, 5H, CH_3 OMe, Rf$(CH_2)_3SO_2$<u>C</u>H_2CH_2-), 3.41 (dd, 1H, *J* = 3.6 Hz, *J* = 9.2 Hz, H-2), 3.68 (m, 5H, H-4, H-5, H-6'', H-5', H-5''), 3.97 (t, 1H, *J* = 9.2 Hz, H-3), 4.03-4.11 (m, 2H, H-6 and H-6'), 4.16 (dd, 1H, *J* = 3.6 Hz, *J* = 11.6 Hz, H-6''), 4.23 (dd, 1H, *J* = 3.2 Hz, *J* = 11.6 Hz, H-6), 4.30 (t, 1H, *J* = 9.2 Hz, H-4'), 4.34-4.47 (m, 3H, H-4'' and Rf$(CH_2)_3SO_2CH_2$<u>C</u>H_2-), 4.49 (d, 1H, *J* = 3.6 Hz, H-1), 4.52 (d, 1H, *J* = 12.4 Hz, CH<u>H</u> Bn), 4.65 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.70 (dd, 1H, *J* = 3.0 Hz, *J* = 12.0 Hz, H-6'), 4.90 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.97-5.08 (m, 3H, ‚H-1', H-1'' and CH<u>H</u> Bn), 5.38 (dd, 1H, *J* = 8.0 Hz, *J* = 9.6 Hz, H-2'), 5.61 (t, 1H, *J* = 9.6 Hz, H-3'), 6.86 (s, 1H, H-3''), 7.15-8.06 (m, 26H, H arom and NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.6 (RfCH_2<u>C</u>$H_2CH_2SO_2(CH_2)_2$-), 20.0 (C_q TBDS), 22.3 (C_q TBDS), 26.8 (3xCH_3 TBDS), 27.3 (3xCH_3 TBDS), 29.5 (t, *J* = 22.0 Hz, Rf<u>C</u>$H_2(CH_2)_2SO_2(CH_2)_2$-), 52.0 (RfC$(CH_2)_2$<u>C</u>$H_2SO_2(CH_2)_2$-), 52.8 (Rf$(CH_2)_3SO_2$<u>C</u>H_2CH_2-), 55.3 (CH_3 OMe), 60.2 (Rf$(CH_2)_3SO_2CH_2$<u>C</u>H_2-), 62.5 (C-6), 66.1 (C-6''), 66.6 (C-6'), 67.7, 68.5, 72.9, 77.4 (C-4, C-5, C-5' and C-5''), 69.5 (C-4''), 71.2 (C-4'), 72.6 (C-2'), 73.3 (CH_2 Bn), 74.6 (CH_2 Bn), 76.4 (C-3'), 78.9 (C-3), 79.6 (C-2), 92.2 (CCl_3), 92.6 (C-1''), 98.0 (C-1), 99.6 (C-1'), 118.2 (C-3''), 120.9 (C-2''), 126.5-134.0 (CH arom), 128.4 (C_q Bz), 128.6 (C_q Bz), 128.7 (C_q Bz), 137.9 (C_q Bn), 138.9 (C_q Bn), 153.9 (C=O FPsc), 159.9 (C=O TCA), 165.1 (C=O Bz), 165.8 (C=O Bz), 166.6 (C=O Bz); HRMS $[M+Na]^+$ calcd for $C_{78}H_{81}NO_{22}Cl_3F_{17}SSi$ 1894.34265 was found 1894.34094.

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18. Fluorous Fmoc-Cl is commercially available from Fluorous Techonologies Inc. for $95/mmol. 3-([1H,1H,2H,2H,3H,3H]-Perfluoroundecyl iodide (**11**, Scheme 3) used for the synthesis of fluorous propylsulfonylethoxycarbonyl chloride (FPsc-Cl, **14**) is available from Fluorous Techonologies Inc. for $15/mmol. See http://www.fluorous.com.
19. For F-tag oligosaccharide synthesis, see for example: (a) T. Miura, A. Satoh, K. Goto, Y. Murakami, N. Imai, T. Inazu, *Tetrahedron*: *Asymm.* **2005**, *16*, 3. (b) L. Manzoni, R. Castelli, *Org. Lett.* **2006**, *8*, 955. (c) F. A. Jaipuri, N. L. Pohl, *Org. Biomol. Chem.* **2008**, *6*, 2686. (d) M. Tojino, M. Mizuno, *Tetrahedron Lett.* **2008**, *49*, 5920. (e) M. Mizuno, K. Goto, T. Miura, T. Inazu, *QSAR Comb. Sci.* **2006**, *25*, 742.

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CHAPTER 4

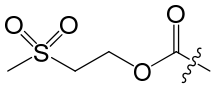
The methylsulfonylethoxymethyl (Msem) as a hydroxyl protecting group in oligosaccharide synthesis

Introduction:

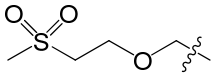
The development of suitable protecting groups is an important objective in synthetic organic chemistry. Although numerous protecting groups for hydroxyl functions are available,[1] the palette of protecting groups that is commonly applied *en route* to an oligosaccharide is quite limited. Benzyl ethers (Bn)[2] and benzoyl (Bz)[3] or pivaloyl (Piv)[4] esters are usually selected as permanent protecting groups, to be removed only at the end of the synthesis of the target oligosaccharide. Among the temporary protecting groups that allow chain elongation by selective deprotection, the levulinoyl (Lev),[5] the 9-fluorenylmethoxycarbonyl (Fmoc),[6] the *p*-methoxybenzyl (PMB) ether,[7] and silyl ethers such as *tert*-butyldimethylsilyl (TBDMS)[8] and *tert*-butyldiphenylsilyl (TBDPS)[9] are most often used. In addition, diol protecting groups, such as the benzylidene acetal,[10] and the

isopropylidene[11] and di-*tert*-butylsilyl ketal[12] are often employed. With the current state of the art in oligosaccharide synthesis it is becoming increasingly clear that the nature of the protecting group at each position on the core of the reacting donor and acceptor glycosides.

Figure 1: The Msc protecting group.



1, Msc



2, Msem

may exert influence on the stereochemical outcome and yield of a glycosylation reaction. Consequently, not only the armed-disarmed concept on the reactivity of glycosyl donors is continuously adjusted and expanded,[13] but also the knowledge of the stereodirecting power of various substituents on the core of the glycosyl donors is progressing.[14] A striking example of the influence of a remote protecting group is presented by the 4,6-*O*-benzylidene protection in mannose donors that allow the easy introduction of the challenging 1,2-cis mannose linkage.[15] On the other hand, the *cis*-directing power of the 4,6-*O*-benzylidene acetal in mannopyranose donors can be overshadowed by the presence of bulky ether or participating acyl groups at the C-3 OH.[16] In this framework alkoxymethyl protecting groups have recently attracted attention.[16,17] A range of alkoxymethyl groups, such as the cyanoethoxymethyl group have been developed in the field of RNA synthesis.[18] Protecting groups at the C-2 hydroxyl of an RNA building block must meet strict requirements to prevent both unwanted removal *en route* to the fully protected oligoribonucleotide and phosphate diester migration at the end of the synthesis. The endeavors on the methylsulfonylethoxycarbonyl (Msc) group **1**, as described in Chapter 2,[19] together with the favorable properties of the cyanoethoxymethyl group in RNA chemistry, in terms of intermediate stability and ease of removal at the end of the oligo nucleotide assembly were an incentive to explore the methylene analogue of the Msc group in oligosaccharide synthesis. In this chapter the methylsulfonylethoxymethyl (Msem, **2**) is introduced for the protection of carbohydrates and its applicability in the synthesis of β−1,3-*O*-mannotriose is demonstrated.

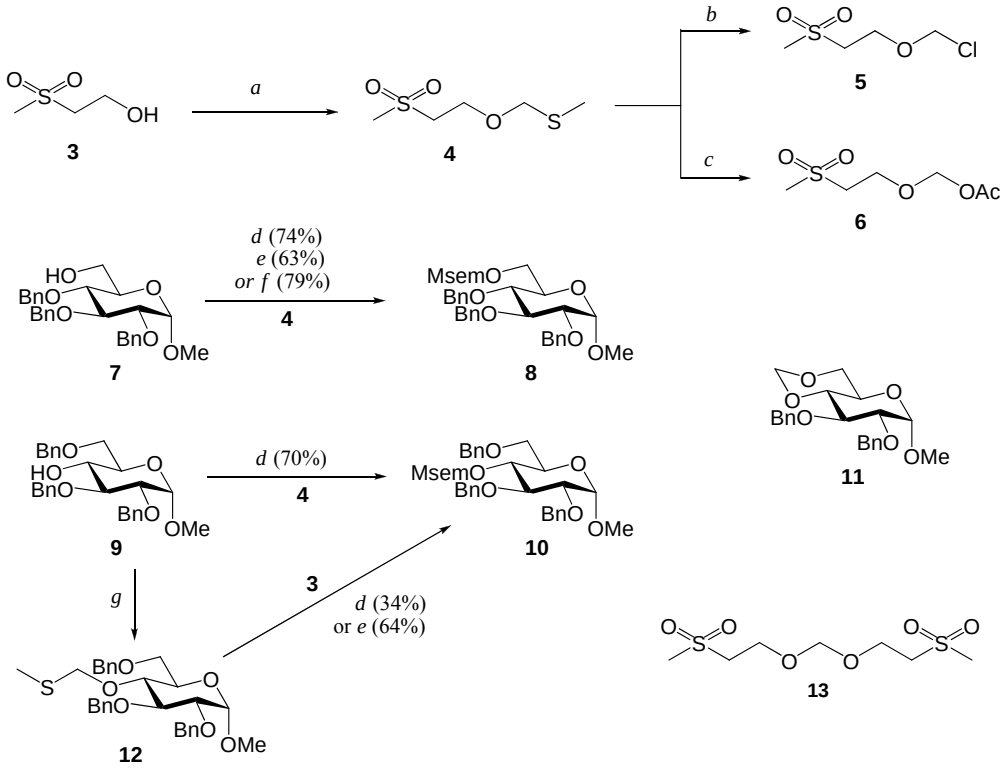
Results and discussion:

The most efficient way to introduce various alkoxymethyl protecting groups relies on the use of thiomethyl intermediates.[5b,20] Therefore it was decided to explore two complementary strategies to introduce the methylsulfonylethoxymethyl (Msem) group on a hydroxyl function. In the first approach, an alkoxymethyl thiomethyl ether reagent is prepared while in the second procedure, the hydroxyl function to be protected is converted into the corresponding methylthiomethyl ether. First attention was focused on the former approach and to this end commercially available methylsulfonylethanol **3** was converted to thiomethyl ether **4** in 57% yield by treatment with dimethylsulfoxide (DMSO) and acetic anhydride (Ac_2O) in acetic acid (Scheme 1). Thiomethyl ether reagent **4** can be used for the introduction of the Msem group at hydroxyl functions using chemistry developed for glycosylations of thioglycosides. Condensation of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **7** with reagent **4** under the influence of *N*-iodosuccinimide (NIS) and trimethylsilyltriflate (TMSOTf) produced Msem protected **8** in 70% yield (Scheme 1). The preparation of Msem protected **10** from methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **9** using reagent **4** and the same activator system indicate that this procedure is also suitable to protect secondary hydroxyl functions with the Msem group. Using the milder iodonium di-*sym*-collidine perchlorate (IDCP) as iodonium source, the condensation of methyl glycoside **7** and thiomethyl ether **4** led to the isolation of Msem protected **8** in 63% yield. The yield of this reaction could be increased to 79% by activation of **4** with diphenylsulfoxide (Ph_2SO) in combination with trifluoromethanesulfonic anhydride (Tf_2O) and an excess of tri-*tert*-butylpyrimidine (TTBP) as a proton scavenger. This reaction was accompanied by the formation of side-product **13**.

Since reagent **4** and thioglycosides can both be activated with iodonium or sulfonium ions, orthogonal conditions were sought that are suitable for introduction of the Msem group at hydroxyl functions of thioglycosides. To this end, the thiomethyl ether **4** was transformed into methylsulfonylethoxymethyl chloride **5** by treatment with sulfuryl chloride in DCM. Unfortunately, attempts to introduce the Msem group to the primary hydroxyl in compound **7** with methylsulfonylethoxymethyl chloride **5**, employing either sodium hydride, diisopropylethylamine (Dipea), 2,6-lutidine or 2,4,6-*syn*-collidine as a base

failed and resulted only in the recovery of starting compound **7**. Apparently, the chloride **5** is not stable under the applied conditions. Since thioglycosides can withstand acidic conditions, attention was shifted to acetyl acetal **6**, which was produced by reaction of thioether **4** with AcOH under the influence of NIS in 95% yield. Unfortunately the reaction of (2-(methylsulfonyl)ethoxy)methyl acetate **6** and methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **9** under influence of TfOH or $SnCl_4$ mainly led to the formation of the methylene acetal **11** instead of the desired Msem protected **10**, indicating that the Msem can be introduced using acidic conditions, but that the resulting ketal also reacts under these conditions.

Scheme 1: Introduction of the Msem group.



Reagents and conditions; *a*) AcOH, Ac_2O, DMSO, RT, 48 h, 57%; *b*) SO_2Cl_2, DCM, RT, 2 h, 100%; *c*) NIS, AcOH, DCM, -20 ºC to RT, 2 h, 95%; *d*) NIS, TMSOTf, DCM, -20 ºC to RT, 24h; *e*) IDCP, DCM, RT, 2h; *f*) DPS, TTBP, Tf_2O, DCM, -60 ºC, 2h. *g*) NaH, MTM-Cl, DMF, 1h, 73%.

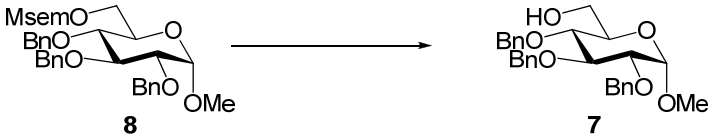
The second approach, in which a hydroxyl function in a monosaccharide is firstly transformed into the methylthiomethyl ether and subsequently into the Msem ether was next pursued. 2,3,4-Tri-*O*-benzyl-α-D-glucopyranoside **9** was converted into fully protected **12** by treatment with sodium hydride and methylthiomethyl chloride (MTM-Cl) in DMF (Scheme 1). Condensation of thiomethyl ether **12** with 2-(methylsulfonyl)ethanol **3** using the NIS/TfOH combination gave methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylsulfonylethoxymethyl-α-D-glucopyranoside **10** in only 34% yield. The low yield can be explained by the unwanted formation of methylene acetal **11**. Employing IDCP (4 equivalents) as a more mildly activating system improved the yield of **10** to 64% but did not completely circumvent the formation of side product **11**. The fluorous analogue of the Msem group could also be constructed under these conditions in combination with ([1H,1H,2H,2H]-perfluorodecyl)sulfonylethanol as a nucleophile. Because of the low reactivity of this alcohol, the side product **11** prevailed in the reaction mixture and the fluorous Msem protected glucose **14** was obtained in unproductive yield.

With two methods at hand for the introduction of the Msem group, the most favorable conditions for cleavage of the Msem group were sought. Therefore, 2,3,4-tri-*O*-benzyl-6-*O*-methylsulfonylethoxymethyl-α-D-glucopyranoside **8** was subjected to conditions that normally effectuate β-elimination. As summarized in Table 1, the Msem group is reasonably stable under basic conditions, and significantly more robust than its carbonate counterpart. The use of 2 equivalents 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) required 3h at elevated temperature (100 ºC) to completely remove the Msem group (Table 1, entry 1). Addition of thiophenol as the scavenger retarded the time for cleavage considerably (Table 1, entry 2). The deblocking of the Msem group with the aid of 5 equivalents of potassium *tert*-butoxide (KOtBu) reached completion after 24 hours at 40 ºC (Table 1, entry 3). Gratifyingly, treatment of **8** with a catalytic amount of tetrabutyl ammonium fluoride (TBAF, 0.1 equivalents) led to the cleavage of the Msem group after 24 hours at room temperature (Table 1, Entry 4).

The feasibility of the Msem group as hydroxyl protecting group in oligosaccharide synthesis was investigated in the context of the construction of 1,2-*cis*-mannosidic bonds. In a seminal study of the group of Crich, it was discovered that glycosylations using 4,6-*O*-benzylidene mannosyl sulfoxides or thiomannosides as glycosyl donors led to the formation

of β-mannosides with high stereoselectivity.[15] Although the presence of 4,6-*O*-benzylidene acetal in several types of mannose donors proved to be effective to obtain β-selective mannosylations, the nature of protective groups at the 3-OH position has also been shown to have a major effect on the α/β-ratio. For instance, it has become clear that the bulky 3-*O*-*tert*-butyldimethylsilyl ether reduces the β-selectivity by a steric interaction with the C-2 hydroxyl protecting group,[21] while 3-*O*-carboxylate esters essentially give pure α–mannosides, presumably *via* neighboring group participation.[16] In this respect, the comparison of the here presented Msem group and the methylsulfonylethoxycarbonyl (Msc) group, both relatively small protecting groups and having the methylsulfonylethoxy moiety in common, is relevant.

Table 1: Conditions for cleavage of the Msem group.

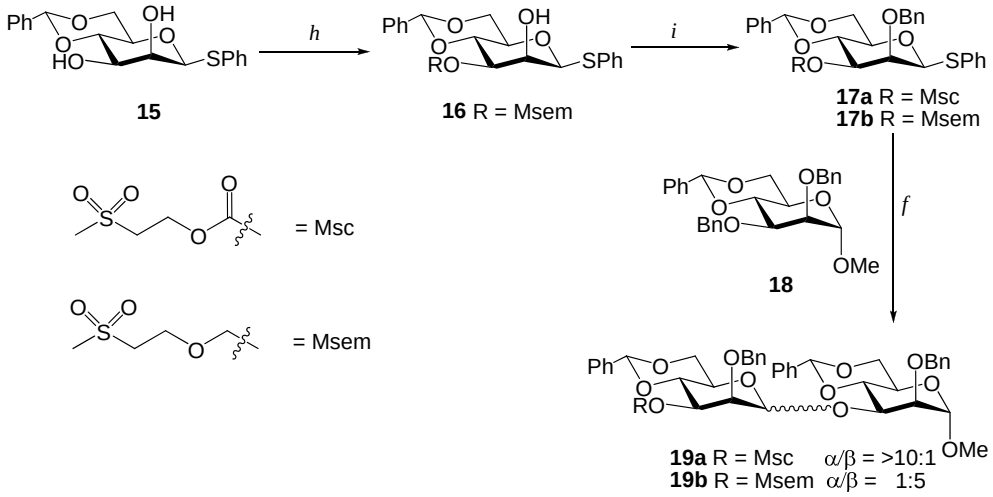


8 **7**

Entry	Conditions	Conc.	Temperature	Time	Yield
1	DBU, DMF	2 eq	100º C	3h	91
2	DBU, DMF, PhSH	2 eq	100º C	20h	93
3	KOtBu, MeOH	5 eq	40º C	24h	89
4	TBAF, THF	0.1 eq	RT	24h	94

In Chapter 2, it was described that the Msc carbonate is an orthogonally removable hydroxyl protecting group that efficiently provides anchimeric assistance during glycosylation reactions. It was shown that the Ph_2SO /Tf_2O mediated condensation of 3-*O*-Msc donor **17a** with acceptor **18** led to the predominant formation of the α-mannopyranoside linkage (Scheme 2). This result underlines that not only carboxylate esters but also carbonates such as the Msc-group at the C-3 hydroxyl of benzylidene mannosides direct mannosylation reactions towards the α products. To investigate the effect of the Msem ether instead of the Msc carbonate in a similar condensation, the synthesis of donor **17b** was required (Scheme 2). Guided by ample literature precedent describing the use of tin ketals to introduce alkoxymethyl ethers, the regioselective alkylation of the 2,3-*O*-dibutylstannylidene of diol **15** with methylsulfonylethoxymethyl chloride **5** was undertaken. A mixture of **15** and dibutyltin oxide in toluene was heated for 2 hours and after evaporation of the solvents, the crude product was treated with Msem-Cl **5**

Scheme 2: Coupling of both Msc protected **17a** and Msem protected **17b** with acceptor **18**.



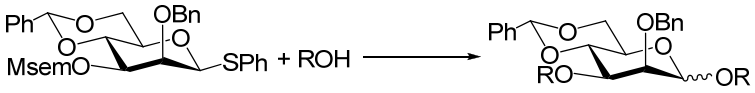
Reagents and conditions; *f*) TTBP, Ph_2SO, Tf_2O, DCM, -78 º C-RT, 2h; *h*) i- Bu_2SnO, tol, Reflux, 2h: ii- Msem-Cl, CsF, TBABr, tol, 18 h, 81% ; *i*) NaH, DMF, 0 ºC, 15 min, 75%.

in the presence of cesium fluoride and tetrabutylammonium bromide (TBABr). 3-*O*-Msem protected mannopyranoside **16** was obtained in high yield as the sole regio isomer. The key

Ph_2SO /Tf_2O mediated condensation of 3-*O*-Msem donor **17b** with acceptor **18** led to the predominant formation of a *cis*-mannopyranoside linkage (α:β = 1:5) (Scheme 2). The outcome of this glycosylation indicates that the Msem group does not act as a remote neighboring group and is sterically minimally intrusive, allowing the selective formation of the β-mannoside bond in line with a comparable study of Codée et al. on the use of [triisopropyl)silyloxy]methyl group.[16a]

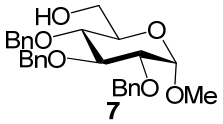
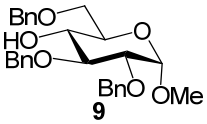
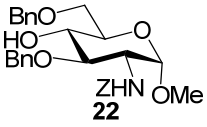
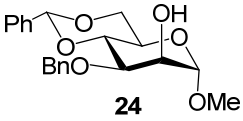
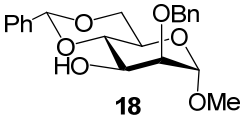
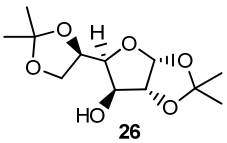
The glycosylating properties of 3-*O*-Msem protected mannopyranose **17b** were further examined in a set of Ph_2SO /Tf_2O-mediated condensation with a range of different nucleophiles (Table 2). Surprisingly, the coupling with primary acceptor **7** furnished the α- and β-isomers of disaccharide **20** in almost equal amounts (Table 2, Entry 1, **20**). Secondary alcohol **9**, which has previously been shown to be a relatively challenging substrate to β-mannosylate, reacted with donor **17b** to provide the α/β-disaccharide in a 1/3 ratio (Table 2, Entry 2, **21**). When glucosamine acceptor **22**, also a notoriously difficult substrate for the β-mannosylation reaction, was employed, equal amounts of α and β products were obtained (Table 2, Entry 3, **23**). Condensation of donor **17b** with methyl 4,6-*O*-benzylidine-3-*O*-benzyl-α-D-mannopyranoside **24**, on the other hand gave disaccharide **25** with good β-selectivity again (α/β = 1:5, Table 1, Entry 4, **25**). The same result, in terms of stereoselectivity and yield was obtained earlier (see Scheme 2) with the corresponding 2-*O*-benzyl acceptor **18**. Executing this reaction for a longer period at -78 ºC led to the same selectivity and a slight increase in yield (Table 2, Entry 5,**19b**). Finally, the use of 1,2:5,6-di-*O*-isopropylidene-3-*O*-α-D-glucofuranose **26** led to the formation disaccharide **27** in 1:10 α/β ratio (Table 2, Entry 6, **27**). These experiments clearly show that the glycosylations of **17b** can proceed with good to moderate 1,2-*cis* selectivity. However, the reactivity of the hydroxyl function in the acceptor glycoside also plays an important role. Although poor selectivities for acceptors **9** and **22** have been reported before,[22,23] the outcome of the mannosylation of primary alcohol **7** stands in sharp contrast to the β-slective mannosylations commonly reported for this acceptor.[14,15a] This result highlights how minor changes in a glycosylation system can result in major changes in the outcome of the reaction, and for this unexpected result there is currently no adequate explanation.

Table 2: Glycosylation of donor **17b** with various acceptors.[f]



17b R = Msem

20 R = 7
21 R = 9
23 R = 22
25 R = 24
19b R = 18
27 R = 26

Entry	Acceptor	Time	Temp.	Yield	α/β
1	 **7**	2h	-78 °C to -72 °C	74	4:5
2	 **9**	4h	-78 °C to 0 °C	72	1:3
3	 **22**	4h	-78 °C to 0 °C	70	1:1
4	 **24**	4h	-78 °C to 0 °C	72	1:5
5	 **18**	4h 18 h	-78 °C to 0 °C -78 °C	75 84	1:5 1:5
6	 **26**	2h	-78 °C to -60 °C	75	1:10

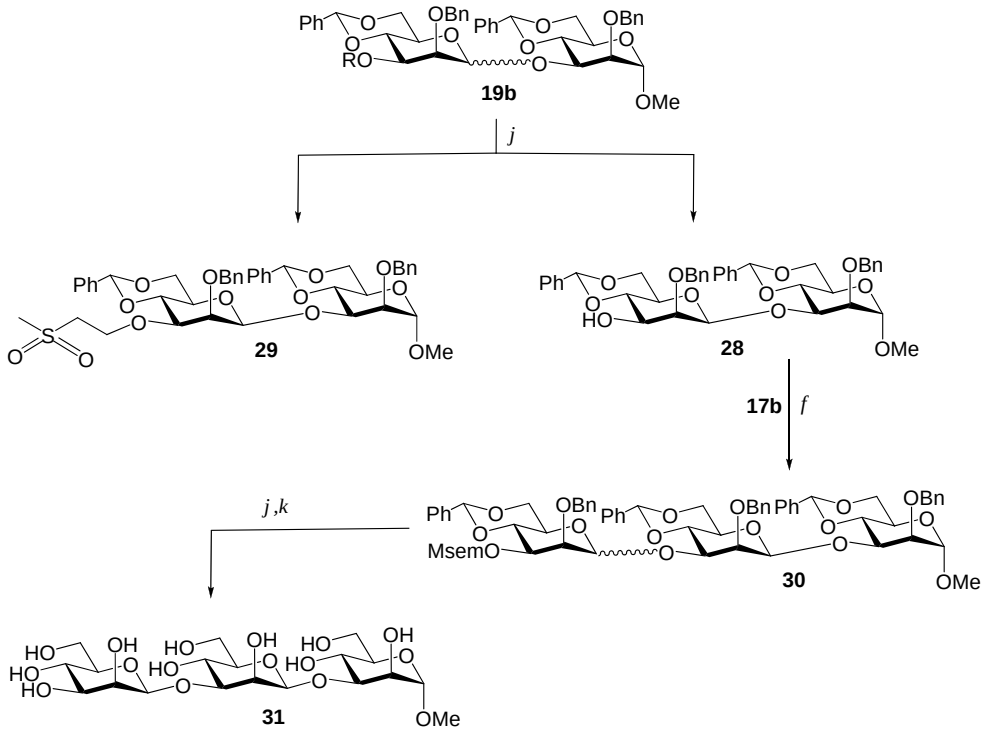
[f] TTBP, Ph_2SO, Tf_2O, DCM.

Finally the assembly of β-1,3-mannotriose **31** was undertaken as depicted in Scheme 3. To this end, the α- and β-anomers of compound **19b** were separated by silicagel column chromatography and the Msem group in β-dimer **19b** was cleaved by treatment with the TBAF to give disaccharide **28** in 60% yield (Scheme 3). Apart from target **28**, a substantial amount of side product **29** was isolated, the formation of which can be explained by Michael addition of the released (methylsulfonyl)ethene to the free C-3 hydroxyl in **28**. Notably this side reaction has not been observed for any other Msem substrate investigated so far. To circumvent the formation of side product **29**, piperidine was added to the reaction mixture to scavenge the released vinylsulfone. In this case disaccharide **28** was obtained in 88% yield. Elongation of **28** by preactivation of 2 equivalents of thioglycoside **17b** with Ph_2SO/Tf_2O in the presence of an excess of TTBP furnished trisaccharide **30** in 83% yield, as an anomeric mixture (α:β = 1:5). Also in this case, the α- and β-anomers could be separated by silica gel chromatography. Anomerically pure **30** was then deprotected in two steps. First, the Msem group in **30** was removed by treatment with TBAF in the presence of piperidine and subsequent hydrogenolysis of the remaining benzylidene and benzyl groups using palladium hydroxide on charcoal and hydrogen gas led to the isolation of trisaccharide **31** in 60% yield over two steps.

Conclusion:

The methylsulfonylethoxymethyl (Msem) group has been introduced as a new hydroxyl protecting group that meets the requirements for productive oligosaccharide synthesis. It can be introduced at primary and secondary hydroxyl functions of *O*-glycosides with thiomethyl ether reagent **4** and a thiophilic activator. For installation of the Msem-group at the hydroxyl functions of thioglycosides, the conversion of the hydroxyl functions into dibutylstannylidene acetals followed by reaction with Msem-Cl **5** is the method of choice. The methylsulfonylethoxymethyl ether is sterically unbiased, does not provide remote neighboring group participation and is easily removed by a catalytic amount of TBAF in the presence of piperidine as scavenger. The usefulness of the Msem group is illustrated by the synthesis of an all *cis*-linked 1,3-*O*-mannotrioside.

Scheme 3: The synthesis of β-1,3-mannan **30**.



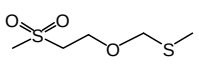
Reagents and conditions; *f*) TTBP, Ph_2SO, Tf_2O, DCM, -78 º C-RT, 2h; *j*) TBAF, piperdine, THF, 24 h; *k*) $Pd(OH)_2/C$, H_2, 24 h.

Experimental:

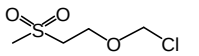
General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water in donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. Molecular sieves 3Å were flame dried before use. All other chemicals (Acros, Fluka, Merck, Fluorous Technologies Inc.) were used as received. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). Size exclusion chromatography was performed on Sephadex LH20 (eluent MeOH/DCM = 1/1). Gel filtration was performed on Sephadex HW40 (0.15 M Et_3NHOAc in H_2O). TLC analysis was conducted on DC-alufolien (Merck, kiesel gel 60, F_{245}). Compounds were visualized by UV absorption (245 nm), by spraying with an aqueous solution of $KMnO_4$ (20%) and K_2CO_3 (10%), by spraying with 20% H_2SO_4 in ethanol or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10g/L) in 10% $H_2SO_{4\,(aq)}$ followed by charring at ~150 ºC. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic

polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 MHz and 100 MHz respectively), AV 500 (500 MHz and 125 MHz respectively) or DMX 600 (600 MHz and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ unless stated otherwise. Chemical shift are relative to tetramethylsilane and are given in ppm. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High resolution mass spectra were recorded on a LTQ-Orbitrap (thermo electron).

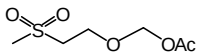
General method for glycosylations using Ph_2SO/Tf_2O: A solution of 1-thio-β-D-mannopyranoside (donor), diphenylsulfoxide (1.3 eq), and tri-*tert*-butylpyrimidine (3 eq) in DCM (0.05 M) was stirred over activated MS3Å for 30 minutes. The mixture was brought to -78 °C before triflic acid anhydride (1.3 eq) was added. The mixture was allowed to warm to -60 °C in 15 minutes followed by the addition of the acceptor (1.5 eq). The reaction mixture was stirred at the temperature described in table 2. The reaction mixture was quenched with triethylamine (5 eq), filtered, diluted with DCM and washed with water. The aqueous layer was extracted with DCM thrice, the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by size exclusion and silica gel column chromatography.



((Methylsulfonylethoxy)methyl)methylsulfane (4): To a solution of methylsulfonylethanol **3** (6.55 g, 52.8 mmol) in DMSO (15 ml, 211 mmol, 4 eq) was added acetic acid (6 ml, 106 mmol, 2 eq) and acetic anhydride (9.9 ml, 106 mmol, 2 eq). The reaction mixture was stirred for 48 hours. The mixture was neutralized by careful addition of $NaHCO_{3\,(s)}$, extracted using a large excess of EtOAc, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford **4** (5.54 g, 30.0 mmol, 57%) as yellow a oil. TLC (75% EtOAc in toluene): R_f = 0.75; IR (neat, cm^{-1}): 730, 1129, 1286; ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 2.15 (s, 3H, -CH_2SCH_3), 2.99 (s, 3H, CH_3SO_2-), 3.31 (t, 2H, *J* = 5.2 Hz, $MeSO_2CH_2CH_2OCH_2SCH_3$), 3.95 (t, 2H, *J* = 5.6 Hz, $MeSO_2CH_2CH_2OCH_2SCH_3$), 4.68 (s, 2H, $MeSO_2(CH_2)_2OCH_2SCH_3$); ^{13}C NMR (100 MHz, ($CDCl_3$) δ = 13.3 (-CH_2SCH_3), 42.0 (CH_3SO_2-), 53.9 ($MeSO_2CH_2CH_2OCH_2SCH_3$), 60.9 ($MeSO_2CH_2CH_2OCH_2SCH_3$), 74.7 ($MeSO_2(CH_2)_2OCH_2SCH_3$); HRMS $[M+NH_4]^+$ calculated for $C_5H_{16}O_3S_2N$ 202.05661, found 202.05662.

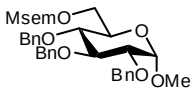


Methylsulfonylethoxymethyl chloride (5): To a solution of ((methylsulofnylethoxy)methyl)methylsulfane **4** (1.39 g, 7.55 mmol) in DCM (25 ml, 0.3 M) was added sulfuryl chloride (0.6 ml, 7.6 mmol, 1 eq) and the mixture was stirred for 2 hours. Next the solvents were removed *in vacuo* to give **5**; IR (neat, cm^{-1}): 643, 944, 1112, 1288; ^{1}H NMR (400 MHz, ($CDCl_3$) δ = 2.92 (s, 3H, CH_3SO_2-), 3.27 (t, 2H, *J* = 5.2 Hz, $MeSO_2CH_2CH_2OCH_2Cl$), 4.07 (t, 2H, *J* = 5.6 Hz, $MeSO_2CH_2CH_2OCH_2Cl$), 5.46 (s, 2H, $MeSO_2(CH_2)_2OCH_2Cl$); ^{13}C NMR (100 MHz, ($CDCl_3$) δ = 42.5 (CH_3SO_2-), 53.9 ($MeSO_2CH_2CH_2OCH_2Cl$), 63.6 ($MeSO_2CH_2CH_2OCH_2Cl$), 81.9 ($MeSO_2(CH_2)_2OCH_2Cl$); HRMS $[M+NH_4]^+$ calculated for $C_4H_{13}ClO_3S_2N$ 190.02992, found 190.02882.



Methylsulfonylethoxymethylacetate (6): To a solution of ((methylsulfonylethoxy)methyl)methylsulfane **4** (1.05 g, 5.7 mmol) in DCM (29 ml, 0.2

M) was added *N*-iodosuccinimide (1.52 g, 6.83 mmol, 1.2 eq). The mixture was cooled to -20 °C followed by the addition of acetic acid (0.65 ml, 11.4 mmol, 2 eq). The mixture was allowed to warm to rt and was stirred for 2 hours. The reaction mixture was quenched with triethylamine (5eq), filtered, diluted with DCM and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with DCM thrice and the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford **6** (1.06 g, 5.41 mmol, 95%). TLC (66% EtOAc in PE): R_f = 0.6; IR (neat, cm^{-1}): 489, 961, 1124, 1285, 1740; ^1H NMR (400 MHz, ($CDCl_3$) δ = 2.12 (s, 3H, CH_3 -OAc), 2.98 (s, 3H, $C\underline{H}_3SO_2$-), 3.26 (t, 2H, *J* = 5.2 Hz, $MeSO_2C\underline{H}_2CH_2OCH_2OAc$), 4.09 (t, 2H, *J* = 5.6 Hz, $MeSO_2CH_2C\underline{H}_2OCH_2OAc$), 5.27 (s, 2H, $MeSO_2(CH_2)_2OC\underline{H}_2OAc$); ^{13}C NMR (100 MHz, ($CDCl_3$) δ = 20.7 (CH_3 OAc), 42.8 (CH_3 $\underline{C}H_3SO_2$-), 54.7 (CH_2 $MeSO_2\underline{C}H_2CH_2OCH_2OAc$), 63.5 ($CH_2$ $MeSO_2CH_2\underline{C}H_2OCH_2OAc$), 88.0 ($CH_2$ $MeSO_2(CH_2)_2O\underline{C}H_2OAc$); HRMS $[M+Na]^+$ calculated for $C_6H_{12}O_5S_1Na$ 219.02977, found 219.02982.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-methylsulfonylethoxymethyl-α-D-glucopyranoside (8):

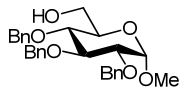
Method I: A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **7** (0.525 g, 1.14 mmol) and ((methylsulfonylethoxy)methyl)methylsulfane **4** (0.314 g, 1.70 mmol, 1.5 eq) in DCM (23 ml, 0.05 M) was stirred over activated MS3Å for 30 minutes before *N*-iodosuccinimide (0.304 g, 1.36 mmol, 1.2 eq) was added. The mixture was cooled to -20 °C followed by the addition of trimethylsilyltrifluoromethanesulfonate (10% in DCM, 0.41 ml, 0.23 mmol, 0.2 eq). The reaction mixture was stirred for 1.5 hours. The reaction mixture was quenched with triethylamine (5eq), filtered, diluted with DCM and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with DCM thrice, the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to get **8** (0.570 g, 0.949 mmol, 84%).

Method II: A solution of ((methylsulfonylethoxy)methyl)methylsulfane **4** (0.058 g, 0.31 mmol, 1.5 eq), diphenyl sulfoxide (0.083 g, 0.41 mmol, 1.3 eq), and tri-*tert*-butylpyrimidine (0.234 g, 0.942 mmol, 3 eq) in DCM (6.3 ml, 0.05 M) was stirred over activated MS3Å for 30 minutes. The mixture was brought to -60 °C before triflic acid anhydride (69 µl, 0.41 mmol, 1.3 eq) was added. The mixture was allowed to warm to -40 °C in 15 minutes followed by the addition of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **7** (0.097 g, 0.21 mmol, 1 eq). The reaction mixture was stirred for 1 hour. The reaction mixture was quenched with triethylamine (5 eq), filtered, diluted with DCM and washed with water. The aqueous layer was extracted with DCM thrice, the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford **8** (0.099 g, 0.165 mmol, 79%).

Method III: A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **7** (0.102 g, 0.22 mmol) and ((methylsulfonylethoxy)methyl)methylsulfane **4** (0.061 g, 0.33 mmol, 1.5 eq) in DCM (4.5 ml, 0.05 M) was stirred over activated MS3Å for 30 minutes before iodonium di-*sym*-collidine perchlorate (IDCP, 0.412 g, 0.88 mmol, 8 eq) was added in the dark. The reaction mixture was stirred in the dark for 24 hours. The reaction mixture was quenched with $NH_4Cl_{\,(aq)}$, filtered, diluted with DCM and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with DCM thrice, the combined organic layers were washed with $NH_4Cl_{\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried

over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to get **8** (0.070 g, 0.12 mmol, 63%).

TLC (50% EtOAc in PE): R_f = 0.4; $[\alpha]_D^{22}$: +43.0º (c = 1.0, DCM); IR (neat, cm^{-1}): 696, 1026, 1717; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.91 (s, 3H, CH_3 Msem), 3.15 (t, 2H, *J* = 5.2 Hz, $MeSO_2C\underline{H}_2CH_2OCH_2$-), 3.38 (s, 3H, OMe), 3.50-3.55 (m, 2H, H-2 and H-4), 3.73-3.77 (m, 3H, H-5 and 2xH-6), 3.88-4.03 (m, 3H, H-3 and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 4.57-4.63 (m, 3H, H-1, $MeSO_2(CH_2)_2OCH\underline{H}$- and CH<u>H</u> Bn), 4.65-4.70 (m, 2H, $MeSO_2(CH_2)_2OCH\underline{H}$- and CH<u>H</u> Bn), 4.78-4.82 (m, 2H, 2xCH<u>H</u> Bn), 4.92 (d, 1H, *J* = 11.2 Hz, CH<u>H</u> Bn), 4.99 (d, 1H, *J* = 10.8 Hz, CH<u>H</u> Bn), 7.26-7.37 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.8 (CH_3 Msem), 55.0 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.2 ($CH_3$ OMe), 61.8 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 66.8 (C-6), 69.7 (C-5), 73.3 (CH_2 Bn), 74.9 (CH_2 Bn), 75.7 (CH_2 Bn), 77.5, 79.8 (C-2 and C-4), 82.0 (C-3), 95.8 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 98.1 (C-1), 127.6-128.4 (CH arom), 138.0 (C_q Bn), 138.2 (C_q Bn), 138.6 (C_q Bn); HRMS $[M+Na]^+$ calculated for $C_{32}H_{40}O_9S_1Na$ 623.22852, found 623.22834.



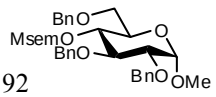
Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (7) (Cleavage of Msem from 8):

Method I: To a solution of **8** (24 mg, 40 µmol) in DMF (0.8 ml, 0.05 M) was added DBU (1 M in DMF, 80 µl, 80 µmol, 2 eq) and the reaction mixture was heated at 100 ºC for 3 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3,6-*tri*-*O*-benzyl-α-D-glucopyranoside **7** (17 mg, 36 µmol, 91%).

Method II: To a solution of **8** (35 mg, 58 µmol) in DMF (1.2 ml, 0.05 M) was added thiophenol (0.2 M in DMF, 0.3 ml, 64 µmol, 1.1 eq) and DBU (1 M in DMF, 116 µl, 116 µmol, 2 eq) and the reaction mixture was heated at 100 ºC for 20 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3,6-*tri*-*O*-benzyl-α-D-glucopyranoside **7** (25 mg, 54 µmol, 93%).

Method III: To a solution of **8** (24 mg, 40 µmol) in MeOH (0.8 ml, 0.05 M) was added KOtBu (23 mg, 200 µmol, 5 eq) and the reaction mixture was heated at 40 ºC for 24 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3,6-*tri*-*O*-benzyl-α-D-glucopyranoside **7** (16 mg, 35 µmol, 89%).

Method IV: To a solution of **8** (34 mg, 57 µmol) in THF (1.1 ml, 0.05 M) was added TBAF (0.1 M in DMF, 57 µl, 5.7 µmol, 0.1 eq) and the reaction mixture was stirred for 24 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3,6-*tri*-*O*-benzyl-α-D-glucopyranoside **7** (25 mg, 53 µmol, 94%).



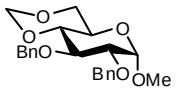
Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylsulfonylethoxymethyl-α-D-glucopyranoside (10):

Method I: A solution of methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylthiomethyl-α-D-glucopyranoside **9** (0.160 g, 0.31 mmol) and methylsulfonylethanol (0.095 g, 0.77 mmol, 2.5 eq) in DCM (3 ml, 0.1 M) was stirred over activated MS3Å for 30 minutes before *N*-iodosuccinimide (0.102 g, 0.48 mmol, 1.5 eq) was added. The mixture was cooled to -20° C followed by the addition of triflic acid (1% in DCM, 0.4 ml, 0.045 mmol, 0.14 eq). The mixture was allowed to warm to room temperature. The reaction mixture was quenched with triethylamine (5 eq), filtered, diluted with DCM and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with DCM thrice, the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to provide **10** (0.062 g, 0.10 mmol, 34%) and side product **11** (0.029 g, .08 mmol, 25%).

Method II: A solution of methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylthiomethyl-α-D-glucopyranoside **9** (0.200 g, 0.381 mmol) and methylsulfonylethanol (0.118 g, 0.95 mmol, 2.5 eq) in DCM (7.6 ml, 0.1 M) was stirred over activated MS3Å for 30 minutes before iodonium di-*sym*-collidine perchlorate (IDCP, 0.712 g, 1.524 mmol, 4eq) was added in dark. The mixture was stirred in the dark for 24 hours. The reaction mixture was quenched with $NH_4Cl_{\,(aq)}$, filtered, diluted with DCM and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with DCM thrice, the combined organic layers were washed with $NH_4Cl_{\,(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to provide **10** (0.146 g, 0.24 mmol, 64%). and side product **11** (0.024 g, .06 mmol, 16%).

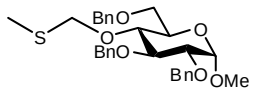
Method III: A solution of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **9** (0.553 g, 1.2 mmol) and ((methylsulfonylethoxy)methyl)methylsulfane **4** (0.330 g, 1.8 mmol, 1.5 eq) in DCM (24 ml, 0.05 M) was stirred over activated MS3Å for 30 minutes before *N*-iodosuccinimide (0.320 g, 1.435 mmol, 1.2 eq) was added. The mixture was cooled to -20 °C followed by the addition of trimethylsilyltrifluoromethanesulfonate (10% in DCM, 0.43 ml, 0.239 mmol, 0.2 eq). The mixture was stirred for 2 hours. The reaction mixture was quenched with triethylamine (5 eq), filtered, diluted with DCM and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with DCM thrice, the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to get **10** (0.530 g, 0.74 mmol, 70%).

TLC (50% EtOAc in PE): R_f = 0.4; $[\alpha]_D^{22}$: +70.4° (c = 1.0, DCM); IR (neat, cm^{-1}): 524, 1027, 1311; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.75 (s, 3H, CH_3 Msem), 2.78-2.90 (m, 2H, $MeSO_2C\underline{H}_2CH_2OCH_2$-), 3.39 (s, 3H, OMe), 3.54 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.60-3.67 (m, 3H, H-4 and 2xH-6), 3.71 (m, 1H, H-5), 3.75 (m, 2H, $MeSO_2CH_2C\underline{H}_2OCH_2$-), 3.88 (t, 1H, *J* = 9.6 Hz, H-3), 4.50 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.59-4.68 (m, 5H, H-1, $MeSO_2(CH_2)_2OCH\underline{H}$- and 3xCH<u>H</u> Bn), 4.73-4.78 (m, 2H, $MeSO_2(CH_2)_2OCH\underline{H}$- and CH<u>H</u> Bn), 5.02 (d, 1H, *J* = 10.8 Hz, CH<u>H</u> Bn), 7.23-7.35 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.6 (CH_3 Msem), 54.6 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.1 ($CH_3$ OMe), 62.3 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 68.4 (C-6), 69.6 (C-5), 72.9 (CH_2 Bn), 73.2 (CH_2 Bn), 75.1 (C-4), 75.3 (CH_2 Bn), 79.8 (C-2), 81.0 (C-3), 96.2 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 97.6 (C-1), 127.5-128.3 (CH arom), 137.7 (C_q Bn), 138.3 (C_q Bn); HRMS $[M+Na]^+$ calculated for $C_{32}H_{40}O_9S_1Na$ 623.22852, found 623.22826.

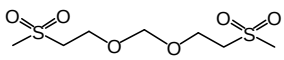


Methyl 2,3-di-*O*-benzyl-4,6-*O*-methylidine-α-D-glucopyranoside (11): A solution of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **9** (0.117 g, 25 mmol) in DCM (2.5 ml, 0.1

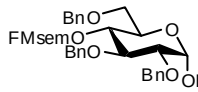
M) was brought to -30 °C before the addition of methylsulfonylethoxymethylacetate **6** (0.099 g, 51 mmol, 2 eq) followed by the addition of tin tetrachloride (45 µl, 380 mmol, 1.5 eq). The TLC analysis showed that compound **10** started to appear after 15 minutes while starting material was still present in addition to a side product. On continuing stirring, the amount of side product increased with the consumption of starting material and compound **10.** After 20 hours all the starting material is gone and the compound **11** (0.062 g, 16 mmol, 63%) is the only product; TLC (50% toluene in EtOAc): R_f = 0.7; $[\alpha]_D^{22}$: +57.8° (c = 1.0, DCM); IR (neat, cm^{-1}): 696, 1049; ^1H NMR (400 MHz, $CDCl_3$) δ = 3.31 (t, 1H, *J* = 9.6 Hz, H-4), 3.38-3.44 (m, 4H, H-6 and CH_3 OMe), 3.50 (dd, 1H, *J* = 3.6 Hz, *J* = 9.2 Hz, H-2), 3.72 (m, 1H, H-5), 3.96 (t,1H, *J* = 9.2 Hz, H-3), 4.11 (dd, 1H, *J* = 4.8 Hz, *J* = 10.0 Hz, H-6), 4.55 (d, 1H, *J* = 4.0 Hz, H-1), 4.60 (d, 1H, *J* = 6.0 Hz, CHH methylene), 4.65 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.80-4.89 (m, 2H, 2xCHH Bn), 4.87 (d, 1H, *J* = 11.2 Hz, CHH Bn), 5.07 (d, 1H, *J* = 6.4 Hz, CHH methylene), 7.24-7.35 (m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 55.3 (CH_3 OMe), 62.4 (C-5), 68.8 (C-6), 73.6 (CH_2 Bn), 75.2 (CH_2 Bn), 78.5 (C-3), 79.3 (C-2), 82.0 (C-4), 93.7 (CH_2 methylene), 99.1 (C-1), 125.8-130.2 (CH arom), 138.0 (C_q Bn), 138.7 (C_q Bn); HRMS $[M+NH_4]^+$ calculated for $C_{22}H_{30}O_6N$ 404.20676, found 404.20671.



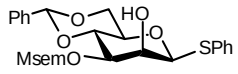
Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylthiomethyl-α-D-glucopyranoside (12): To a solution of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **9** (0.907 g, 2.1 mmol) in DMF (4.2 ml, 0.05 M) was added methylthiomethyl chloride (0.43 ml, 5.2 mmol, 2.5 eq). The reaction mixture was brought to 0° C before sodium hydride (60% in oil, 0.150 g, 3.75 mmol, 1.8 eq) was added in small portions and the stirring was continued for 1 hour. The reaction mixture was diluted with diethyl ether and washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\ (aq)}$ and brine, dried over MgSO4, filtered, concentrated and purified by silica gel chromatography to get compound **12** (0.802 g, 1.5 mmol, 73%). TLC (50% toluene in EtOAc): R_f = 0.8; $[\alpha]_D^{22}$: +178.0° (c = 0.3, DCM); IR (neat, cm^{-1}): 530, 1049; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.99 (s, 3H, CH_3 MTM), 3.38 (s, 3H, CH_3 OMe), 3.52 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.57 (t, 1H, *J* = 10.0 Hz, H-4), 3.64-3.74 (m, 3H, H-5 and 2xH-6), 3.94 (t, 1H, *J* = 9.2 Hz, H-3), 4.56 (m, 2H, 2xCHH Bn), 4.60-4.62 (m, 2H, H-1 and CHH Bn), 4.68 (d, 1H, *J* = 10.8 Hz, CHH MeSCHH-), 4.74-4.78 (m, 3H, CHH MeSCHH- and 2xCHH Bn), 4.97 (d, 1H, *J* = 10.8 Hz, CHH Bn), 7.24-7.37 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.7 (CH_3 MTM), 55.2 (CH_3 OMe), 68.8 (C-6), 69.7 (C-5), 73.3 (CH_2 Bn), 73.4 (CH_2 Bn), 75.6 (CH_2 Bn), 76.1 (C-4), 76.7 (CH_2 MeSCH$_2$-), 79.9 (C-2), 81.8 (C-3), 97.9 (C-1), 127.6-128.4 (CH arom), 138.0 (C_q Bn), 138.0 (C_q Bn), 138.5 (C_q Bn); HRMS $[M+Na]^+$ calculated for $C_{30}H_{36}O_6S_1Na$ 574.21248, found 574.21196.



Di-(2-(methylsulfonyl)ethoxy)methane (13): Collected as by-product during the preparation of the compound **8** (Method II) (14 mg, 54 µmol, 17% w.r.t to the compound **4** used in the reaction). TLC (50% EtOAc in PE): R_f = 0.75; IR (neat, cm^{-1}): 1029, 1277; ^1H NMR (400 MHz, ($CDCl_3$) δ = 3.01 (s, 6H, 2xCH_3 CH_3SO$_2$-), 3.30 (t, 4H, *J* = 5.6 Hz, 2x CH_2 (($MeSO_2CH_2CH_2O)_2CH_2$), 4.02 (t, 4H, *J* = 5.6 Hz, 2x CH_2 (($MeSO_2CH_2CH_2O)_2CH_2$), 4.75 (s, 2H, (($MeSO_2(CH_2)_2O)_2CH_2$); ^{13}C NMR (100 MHz, ($CDCl_3$) δ = 43.1 (2xCH_3 CH_3SO_2-), 54.8 (2xCH_2 (($MeSO_2CH_2CH_2O)_2CH_2$), 61.8 (2xCH_2 (($MeSO_2CH_2CH_2O)_2CH_2$), 95.4 (CH_2 (($MeSO_2(CH_2)_2O)_2CH_2$); HRMS $[M+H]^+$ calculated for $C_7H_{17}O_6S_2$ 261.04611, found 261.04626, $[M+NH_4]^+$ calculated for $C_7H_{20}O_6S_2N$ 278.07266, found 278.07269.

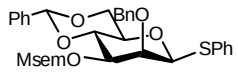


Methyl 2,3,6-tri-*O*-benzyl-4-*O*-([1H,1H,2H,2H]-perfluorodecyl)sulfonylethoxymethyl-α-D-glucopyranoside (14): A solution of methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylthiomethyl-α-D-glucopyranoside **12** (0.145 g, 0.28 mmol) and ([1H,1H,2H,2H]-perfluorodecyl)sulfonylethanol (0.384 g, 0.70 mmol, 2.5 eq) in DCM (5.6 ml, 0.05 M) was stirred over activated MS3Å for 30 minutes before iodonium di-*sym*-collidine perchlorate (IDCP, 0.712 g, 1.52 mmol, 4 eq) was added in the dark. The mixture was stirred in the dark for 24 hours. The reaction mixture was quenched with $NH_4Cl_{(aq)}$, filtered, diluted with DCM and washed with $Na_2S_2O_{3\,(aq)}$. The aqueous layer was extracted with DCM thrice, the combined organic layers were washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\,(aq)}$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to get **14** (0.036 g, 0.03 mmol, 11%) and the side product **11** (0.033 g, 0.9 mmol, 31%)**;** TLC (50% EtOAc in PE): R_f = 0.9; $[\alpha]_D^{22}$: +23.2º (c = 0.6, DCM); IR (neat, cm^{-1}): 696, 1042; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.54-2.69 (m, 2H, CH_2 RfC<u>H</u>$_2$CH$_2$SO$_2$(CH$_2$)$_2$OCH$_2$O-), 2.75-2.88 (m, 2H, CH_2 Rf(CH$_2$)$_2$SO$_2$C<u>H</u>$_2$CH$_2$OCH$_2$O-), 3.18 (m, 2H, CH_2 RfCH$_2$C<u>H</u>$_2$SO$_2$(CH$_2$)$_2$OCH$_2$O-), 3.39 (s, 3H, OMe), 3.54-3.59 (m, 2H, H-2 and H-4), 3.62-3.66 (m, 2H, 2xH-6), 3.69 (m, 1H, H-5), 3.74 (m, 2H, CH_2 Rf(CH$_2$)$_2$SO$_2$CH$_2$C<u>H</u>$_2$OCH$_2$O-), 3.87 (t, 1H, *J* = 9.6 Hz, H-3), 4.50 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.60-4.67 (m, 5H, H-1, CH_2 Rf(CH$_2$)$_2$SO$_2$(CH$_2$)$_2$OC<u>H</u>HO- and 3xCH<u>H</u> Bn), 4.73 (d, 1H, *J* = 6.4 Hz, Rf(CH$_2$)$_2$SO$_2$(CH$_2$)$_2$OC<u>H</u>HO-), 4.75 (d, 1H, *J* = 12.4 Hz, CH<u>H</u> Bn), 5.05 (d, 1H, *J* = 10.4 Hz, CH<u>H</u> Bn), 7.26-7.37 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 24.1 (Rf<u>C</u>H$_2$CH$_2$SO$_2$(CH$_2$)$_2$OCH$_2$O-), 46.5 (RfCH$_2$<u>C</u>H$_2$SO$_2$(CH$_2$)$_2$OCH$_2$O-), 53.7 (Rf(CH$_2$)$_2$SO$_2$<u>C</u>H$_2$CH$_2$OCH$_2$O-), 55.3 (CH_3 OMe), 62.1 (Rf(CH$_2$)$_2$SO$_2$CH$_2$<u>C</u>H$_2$OCH$_2$O-), 68.5 (C-6), 69.8 (C-5), 73.3 (CH_2 Bn), 73.6 (CH_2 , Bn), 75.4 (C-2 or C-4), 75.5 (CH_2 Bn), 80.1 (C-2 or C-4), 81.1 (C-3), 96.4 (Rf(CH$_2$)$_2$SO$_2$(CH$_2$)$_2$O<u>C</u>H$_2$O-), 97.9 (C-1), 127.6-128.5 (CH arom), 137.8 (C_q Bn), 137.9 (C_q Bn), 138.6 (C_q Bn); HRMS $[M+Na]^+$ calculated for $C_{41}H_{41}F_{17}O_9S_1Na$ 1055.20920, found 1055.20965.

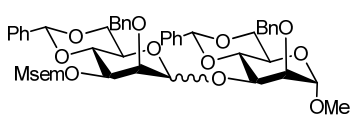


Phenyl 4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-1-thio-β-D-mannopyranoside (16): To a solution of phenyl 4,6-*O*-benzylidene-1-thio-β-D-mannopyranoside **(15)** (3.0 g, 8.3 mmol) in toluene (55 ml, 0.15 M) was added dibutyltin oxide (2.18 g, 8.77 mmol, 1.05 eq) and the reaction mixture was refluxed for 2 hours. The solvents were evaporated and the residue was co-evaporated with toluene. The mixture was re-dissolved in toluene (55ml) followed by the addition of tetrabutylammonium bromide (3.23 g, 10 mmol, 1.2 eq), cesium fluoride (1.51 g, 10 mmol, 1.2 eq) and methylsulfonylethoxymethyl chloride (1.86 g, 10.8 mmol, 1.3 eq) and stirring was continued for 18 hours. The reaction mixture was diluted with EtOAc, washed with $NaHCO_{3\,(aq}$ and extracted thrice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography to get **16** (3.48 g, 6.85 mmol, 82%); TLC (66% EtOAc in PE): R_f = 0.4; $[\alpha]_D^{22}$: -225.0º (c = 1, DCM); IR (neat, cm^{-1}): 696, 732, 1020, 1310; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.83 (s, 3H, CH_3 Msem), 2.95-3.01 (m, 1H, CH<u>H</u> MeSO$_2$CH<u>H</u>CH$_2$OCH$_2$-), 3.10-3.17 (m, 1H, CH<u>H</u> MeSO$_2$CH<u>H</u>CH$_2$OCH$_2$-), 3.32 (d, 1H, *J* = 2.8 Hz, 2-OH), 3.45 (m, 1H, H-5), 3.85-3.93 (m, 3H, H-3, H-6 and CH<u>H</u> MeSO$_2$CH$_2$CH<u>H</u>OCH$_2$-), 3.98-4.04 (m, 1H, CH<u>H</u> MeSO$_2$CH$_2$CH<u>H</u>OCH$_2$-), 4.10 (t, 1H, *J* = 9.6 Hz, H-4), 4.29 (dd, 1H, *J* = 4.8 Hz, *J* = 10.4 Hz, H-6), 4.34 (bs, 1H, H-2), 4.80 (d, 1H, *J* = 7.2 Hz, CH<u>H</u> MeSO$_2$(CH$_2$)$_2$CH<u>H</u>O-), 4.86 (d, 1H, *J* = 7.2 Hz, CH<u>H</u>

$MeSO_2(CH_2)_2CH\underline{H}O$-), 4.95 (s, 1H, H-1), 5.53 (s, 1H, CH benzylidene), 7.22-7.42 (m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.6 (CH_3 Msem), 54.5 (CH_2 $MeSO_2\underline{C}H_2CH_2OCH_2$-), 61.5 ($CH_2$ $MeSO_2CH_2\underline{C}H_2OCH_2$-), 68.2 (C-6), 71.1 (C-5), 71.3 (C-2), 76.0 (C-3), 77.0 (C-4), 87.8 (C-1), 94.5 (CH_2 $MeSO_2(CH_2)_2O\underline{C}H_2$-), 101.6 (CH benzylidene), 125.9-130.9 (CH arom), 134.2 (C_q SPh), 137.1 (C_q CHPh); CH Gated NMR (100 MHz, $CDCl_3$) δ = 87.8 (J = 152 Hz, C-1); HRMS $[M+Na]^+$ calculated for $C_{23}H_{28}O_8S_2Na$ 519.11178, found 519.11140.



Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-1-thio-β-D-mannopyranoside (17b): To a solution of phenyl 4,6-*O*-benzylidene-3-methylsulfonylethoxymethyl-1-thio-β-D-mannopyranoside **(16)** (3.3 g, 6.65 mmol) in DMF (33 ml, 0.2 M) was added benzyl bromide (2 ml, 17.0 mmol, 2.5 eq) and tetrabutylammonium iodide (2.46 g, 6.65 mmol, 1 eq). The reaction mixture was brought to 0 ºC and sodium hydride (60%, 0.266 g, 6.65 mmol, 1 eq) was added subsequently in small portions. The reaction mixture was allowed to warm to rt and stirring was continued for 2 hours. The reaction mixture was quenched with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\ (aq)}$,brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography to get **17b** (2.91g, 4.97 mmol, 75%); TLC (50% EtOAc in PE): R_f = 0.6; $[\alpha]_D^{22}$: -30.2º (c = 1, DCM); IR (neat, cm^{-1}): 738, 1089, 1282; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.76 (s, 3H, CH_3 Msem), 2.83-2.89 (m, 1H, $CH\underline{H}$ $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.02-3.09 (m, 1H, $CH\underline{H}$ $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.42 (m, 1H, H-5), 3.80-3.96 (m, 4H, H-3, H-6 and CH_2 $MeSO_2CH_2C\underline{H}_2OCH_2$-), 4.16-4.20 (m, 2H, H-2 and H-4), 4.27 (dd, 1H, J = 4.8 Hz, J = 10.4 Hz, H-6), 4.71 (d, 1H, J = 6.8 Hz, $CH\underline{H}$ $MeSO_2(CH_2)_2OC\underline{H}H$-), 4.79 (d, 1H, J = 6.8 Hz, $CH\underline{H}$ $MeSO_2(CH_2)_2OC\underline{H}H$-), 4.82 (d, 1H, J = 11.2 Hz, $CH\underline{H}$ Bn), 4.91 (s, 1H, H-1), 4.99 (d, 1H, J = 10.8 Hz, $CH\underline{H}$ Bn), 5.53 (s, 1H, CH benzylidene), 7.22-7.50 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.5 (CH_3 Msem), 54.4 (CH_2 $MeSO_2\underline{C}H_2CH_2OCH_2$-), 61.4 ($CH_2$ $MeSO_2CH_2\underline{C}H_2OCH_2$-), 68.1 (C-6), 71.4 (C-5), 75.8 (CH_2 Bn), 76.4 (C-3), 77.5, 78.7 (C-2 and C-4), 88.7 (C-1), 94.0 (CH_2 $MeSO_2(CH_2)_2O\underline{C}H_2$-), 101.4 (CH benzylidene), 125.9-131.1 (CH arom), 134.5 (C_q SPh), 137.2, 137.5 (C_q CHPh and C_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 88.7 (J = 153 Hz, C-1); HRMS $[M+Na]^+$ calculated for $C_{30}H_{34}O_8S_2Na$ 609.15873, found 609.15848.



Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-α-D-mannopyranoside (19b): Disaccharide **19b** was prepared from donor **17b** (0.26 g, 0.44 mmol, 1 eq) and acceptor **18** (0.248 g, 0.67 mmol, 1.5 eq) according to the general procedure for glycosylations as described above at -78 °C to afford compound **19b** (0.317 g, 0.37 mmol, 84%, α/β = 1:5).

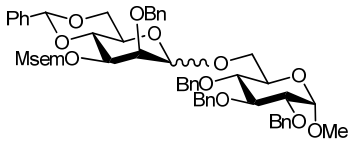
α-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.66; $[\alpha]_D^{22}$: -2.5º (c = 0.4, DCM); IR (neat, cm^{-1}): 698, 1067; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.64 (s, 3H, CH_3 Msem), 2.70-2.77 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 2.95 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.38 (s, 3H, CH_3 OMe), 3.78-3.89 (m, 8H, H-2, H-2', H-6, H-6', $MeSO_2CH_2C\underline{H}_2OCH_2$- and two of the H-3, H-4, H-5, H-3', H-4' and H-5'), 4.05-4.16 (m, 3H, H-6 or H-6' and two of the H-3, H-4, H-5,

H-3', H-4' and H-5'), 4.18-4.29 (m, 4H, H-6 or H-6', CHH Bn and two of the H-3, H-4, H-5, H-3', H-4' and H-5'), 4.42 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.59 (d, 1H, *J* = 7.2 Hz, $MeSO_2(CH_2)_2OCHH$-), 4.70-4.77 (m, 4H, H-1 or H-1', 2xCHH Bn and $MeSO_2(CH_2)_2OCHH$-), 5.34 (s, 1H, H-1 or H-1'), 5.57 (s, 1H, CH benzylidene), 5.64 (s, 1H, CH benzylidene), 7.02-7.53 (m, 20H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.7 (CH_3 Msem), 54.8 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.0 ($CH_3$ OMe), 61.6 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 63.9, 64.8, 72.9, 73.9, 76.3, 77.7, 78.1, 79.2 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', and C-5'), 68.8, 68.9 (C-6 and C-6'), 72.5 (CH_2 Bn), 73.2 (CH_2 Bn), 94.6 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 99.69, 99.7(C-1 and C-1'), 101.8 (CH benzylidene), 102.2 (CH benzylidene), 125.3-129.3 (CH arom), 137.5, 137.6, 137.6 (2xC_q benzylidene and 2xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 99.69 (*J* = 173 Hz, C-1), 99.71 (*J* = 177 Hz, C-1'); HRMS $[M+Na]^+$ calculated for $C_{45}H_{52}O_{14}SNa$ 871.29700 found 871.29542.

β-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.4; $[\alpha]_D^{22}$: -68.4º (c = 1.0, DCM); IR (neat, cm^{-1}): 750, 1088; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.74 (s, 3H, CH_3 Msem), 2.82 (dt, 1H, *J* = 4.8 Hz, *J* = 15.2 Hz, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.01-3.08 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.14 (m, 1H, H-5'), 3.38 (CH_3 OMe), 3.61 (dd, 1H, *J* = 3.2 Hz, *J* = 9.6 Hz, H-3'), 3.69-3.92 (m, 7H, H-2, H-2', H-5, H-6, H-6' and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 4.05 (t, 1H, *J* = 9.6 Hz, H-4'), 4.17-4.22 (m, 2H, H-4 and H-6'), 4.27 (dd, 1H, *J* = 4.4 Hz, *J* = 9.6 Hz, H-6), 4.33 (dd, 1H, *J* = 3.2 Hz, *J* = 10.4 Hz, H-3), 4.47 (s, 1H, H-1'), 4.56 (d, 1H, *J* = 7.2 Hz, $MeSO_2(CH_2)_2OCHH$-), 4.66-4.76 (m, 4H, 3xCHH Bn and $MeSO_2(CH_2)_2OCHH$-), 4.80 (s, 1H, H-1), 4.96 (d, 1H, *J* = 12.0 Hz, CHH Bn), 5.46 (s, 1H, CH benzylidene), 5.63 (s, 1H, CH benzylidene), 7.19-7.51 (m, 20H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.6 (CH_3 Msem), 54.7 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 54.9 ($CH_3$ OMe), 61.2 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 64.0 (C-2, C-2' or C-5), 67.6 (C-5'), 68.5 (C-6'), 68.8 (C-6), 73.1 (CH_2 Bn), 73.6 (C-3), 74.6 (CH_2 Bn), 74.8 (C-3'), 75.2 (C-2, C-2' or C-5) 76.0 (C-2, C-2' or C-5), 77.4 (C-4'), 77.6(C-4), 93.9 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 99.1 (C-1'), 99.5 (C-1), 101.6 (CH benzylidene), 101.6 (CH bBenzylidene), 126.0-129.1 (CH arom), 137.4, 137.5, 137.8, 138.4 (2xC_q benzylidene and 2xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 99.1 (*J* = 155 Hz, C-1'), 99.5 (*J* = 172 Hz, C-1); HRMS $[M+Na]^+$ calculated for $C_{45}H_{52}O_{14}SNa$ 871.29700, found 871.29669.



Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-α-D-glucopyranoside (20): Disaccharide **20** was prepared from donor **17b** (0.147 g, 0.25 mmol, 1 eq) and acceptor **7** (0.174 g, 0.38 mmol, 1.5 eq) according to the general procedure for glycosylations as described above to afford compound **20** (0.171 g, 0.18 mmol, 74%, α/β = 4:5).

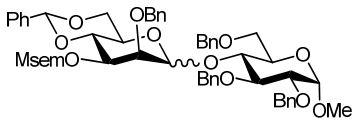
α-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.65; $[\alpha]_D^{22}$: +52.2º (c = 0.5, DCM); IR (neat, cm^{-1}): 697, 1027; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.74 (s, 3H, CH_3 Msem), 2.77-2.83 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 2.98-3.05 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.36 (s, 3H, CH_3 OMe), 3.48 (t, 1H, *J* = 9.2 Hz, H-4), 3.51 (dd, 1H, *J* = 3.6 Hz, *J* = 10.0 Hz, H-2), 3.65 (dd, 1H, *J* = 1.6 Hz, *J* = 11.2 Hz, H-6), 3.71 (m, 1H, H-5), 3.80-3.90 (m, 6H, H-6, H-2', H-5', H-6' and $MeSO_2CH_2C\underline{H}_2OCH_2O$-), 3.98-4.04 (m, 2H, H-3 and H-4'), 4.10-4.16 (m, 2H, H-6 and H-3'), 4.57 (d, 1H, *J* =

3.6 Hz, H-1), 4.60 (d, 1H, *J* = 11.2 Hz, CHH Bn), 4.68-4.72 (m, 4H, 3xCHH Bn and $MeSO_2(CH_2)_2OCHH$-), 4.75-4.82 (m, 3H, 2xCHH Bn and $MeSO_2(CH_2)_2OCHH$-), 4.90 (d, 1H, *J* = 1.2 Hz, H-1'), 4.93 (d, 1H, *J* = 11.2 Hz, CHH Bn), 5.00 (d, 1H, *J* = 10.4 Hz, CHH Bn), 5.55 (s, 1H, CH benzylidene), 7.25-7.42 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.8 (CH_3 Msem), 54.8 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.2 ($CH_3$ OMe), 61.6 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 64.3 (C-5), 66.2 (C-6'), 68.7 (C-6), 69.7 (C-2'or C-5'), 73.3 (CH_2 Bn), 73.4 (CH_2 Bn), 73.6 (C-3 or C-4'), 74.9 (CH_2 Bn), 75.9 (CH_2 Bn), 76.6 (C-2' or C-5'), 77.4 (C-4), 78.1 (C-3'), 80.0 (C-2), 82.0 (C-3 or C-4'), 94.7 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 98.0 (C-1), 99.2 (C-1'), 100.8 (CH benzylidene), 126.1-129.1 (CH arom), 137.5, 137.8, 138.0, 138.1, 138.4 (C_q benzylidene and 4xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 97.9 (*J* = 166 Hz, C-1), 99.2 (*J* = 170 Hz, C-1'); HRMS $[M+Na]^+$ calculated for $C_{52}H_{60}O_{14}SNa$ 963.35960, found 963.35948.

β-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.4; $[\alpha]_D^{22}$: +1.5º (c = 0.5, DCM); IR (neat, cm^{-1}): 696, 1026; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.73 (s, 3H, CH_3 Msem), 2.79-2.85 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.01-3.08 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.29 (m, 1H, H-5'), 3.36 (s, 3H, CH_3 OMe), 3.45 (t, 1H, *J* = 9.6 Hz, H-4), 3.50 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-2), 3.55 (dd, 1H, *J* = 5.2 Hz, *J* = 10.4 Hz, H-6), 3.70 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H-3'), 3.74-3.87 (m, 4H, H-5, H-2', and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 3.90 (t, 1H, *J* = 10.0 Hz, H-6'), 4.01-4.10 (m, 2H, H-3 and H-4'), 4.14 (dd, 1H, *J* = 1.6 Hz, *J* = 10.4 Hz, H-6), 4.28 (dd, 1H, *J* = 4.8 Hz, *J* = 10.4 Hz, H-6), 4.31 (s, 1H, H-1'), 4.51 (d, 1H, *J* = 7.2 Hz, $MeSO_2(CH_2)_2OCH\underline{H}O$-) 4.54-4.60 (m, 2H, H-1, CHH Bn), 4.64-4.69 (m, 2H, CHH Bn and $MeSO_2(CH_2)_2OCH\underline{H}O$-), 4.73 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.79 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.83 (d, 1H, *J* = 11.2 Hz, CHH Bn), 4.87 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.92 (d, 1H, *J* = 12.0 Hz, CHH Bn), 5.01 (d, 1H, *J* = 10.8 Hz, CHH Bn), 5.53 (s, 1H, CH benzylidene), 7.23-7.44 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.6 (CH_3 Msem), 54.7 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.1 ($CH_3$ OMe), 61.2 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 67.6 (C-5'), 68.5 (C-6'), 68.6 (C-6), 69.6 (C-2'), 73.3 (CH_2 Bn), 74.7 (CH_2 Bn), 74.7 (C-3'), 74.7 (CH_2 Bn), 75.1 (C-5), 75.7 (CH_2 Bn), 77.6 (C-4), 77.6 (C-4'), 79.8 (C-2), 82.0 (C-3), 93.8 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 97.8 (C-1), 101.7 (CH benzylidene), 102.2 (C-1'), 126.0-129.2 (CH arom), 137.4, 138.0, 138.2, 138.3, 138.7 (C_q benzylidene and 4xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 97.8 (*J* = 168 Hz, C-1), 102.2 (*J* = 156 Hz, C-1'); HRMS $[M+Na]^+$ calculated for $C_{52}H_{60}O_{14}SNa$ 963.35960, found 963.36030.



Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-α-D-glucopyranoside (21): Disaccharide **21** was prepared from donor **17b** (0.117 g, 0.2 mmol, 1 eq) and acceptor **9** (0.138 g, 0.3 mmol, 1.5 eq) according to the general procedure for glycosylations as described above to afford compound **21** (0.135 g, 0.14 mmol, 72%, α/β = 1:3).

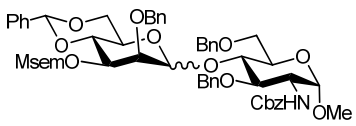
α-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.80 (m, 4H, CH_3 Msem and $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.00-3.13 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.41 (s, 3H, CH_3 OMe), 3.55 (m, 1H, one of the H-2, H-2', H-3, H-3', H-4, H-4'), 3.76 (m, 6H, (H-5 or H-5'), 2xH-6 and 2xH-6' and one of the H-2, H-2', H-

3, H-3', H-4, H-4'), 3.84 (m, 4H, (H-5 or H-5') and $MeSO_2CH_2C\underline{H}_2OCH_2$- and one of the H-2, H-2', H-3, H-3', H-4, H-4'), 3.98 (m, 2H, two of the H-2, H-2', H-3, H-3', H-4, H-4'), 4.09 (m, 1H, one of the H-2, H-2', H-3, H-3', H-4, H-4'), 4.20 (d, 1H, *J* = 12.0, CH<u>H</u> Bn), 4.25 (d, 1H, *J* = 12.0, CH<u>H</u> Bn), 4.68 (m, 8H, H-1, 5xCH<u>H</u> Bn and $MeSO_2(CH_2)_2OC\underline{H}_2$-), 5.17 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 5.41 (s, 1H, H-1'), 5.54 (s, 1H, CH benzylidene), 7.23-7.43 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.7 (CH_3 Msem), 54.8 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.4 ($CH_3$ OMe), 61.5 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 65.1 (C-5 or C-4), 68.6, 69.1 (C-6 and C-6'), 69.6 (C-2' or C5'), 73.0 (CH_2 Bn), 73.1 (CH_2 Bn), 73.4 (C-3 or C-4'), 73.6 (CH_2 Bn), 74.8 (CH_2 Bn), 76.2 (C-4 or C-5), 77.5 (C-2' or C-5'), 77.8 (C-3'), 79.9 (C-2), 81.6 (C-3 or C-4'), 94.4 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 97.7 (C-1), 100.5 (C-1'), 101.8 (CH benzylidene), 126.0-129.1 (CH arom), 137.5, 138.0, 138.3, 138.3, 139.4 (C_q benzylidene and 4xC_q Bn); HRMS $[M+Na]^+$ calculated for $C_{52}H_{60}O_{14}SNa$ 963.35960, found 963.36029.

β-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.35; $[\alpha]_D^{22}$: +7.2º (c = 0.5, DCM); IR (neat, cm^{-1}): 696, 1026; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.76 (s, 3H, CH_3 Msem), 2.80-2.83 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.05-3.13 (m, 2H, H-5' and $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.41 (s, 3H, CH_3 OMe), 3.48-3.58 (m, 3H, H-2, H-3 and H-6'), 3.61-3.68 (m, 3H, H-5, H-6 and H-6), 3.75-3.83 (m, 3H, H-2' and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 3.87 (t, 1H, *J* = 9.2 Hz, H-3'), 3.94-4.02 (m, 2H, H-4 and H-4'), 4.09 (dd, 1H, *J* = 5.2 Hz, *J* = 10.8 Hz, H-6'), 4.46 (d, 1H, *J* = 12.0, CH<u>H</u> Bn), 4.53-4.57 (m, 2H, H-1' and $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.60-4.85 (m, 8H, H-1 and 6xCH<u>H</u> Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 5.05 (d, 1H, *J* = 10.8 Hz, CH<u>H</u> Bn), 5.46 (s, 1H, CH benzylidene), 7.23-7.43 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.8 (CH_3 Msem), 54.6 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.4 ($CH_3$ OMe), 61.1 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 67.3 (C-5'), 68.5, 68.6 (C-6 and C-6'), 69.7 (C-5), 73.4 (CH_2 Bn), 73.6 (CH_2 Bn), 75.0 (C-3), 75.1 (CH_2 Bn), 75.3 (CH_2 Bn), 76.7 (C-2'), 77.4 (C-4 or C-4'), 77.9 (C-4 or C-4'), 79.0 (C-2), 80.3 (C-3'), 94.0 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 98.4 (C-1), 101.4 (C-1'), 101.6 (CH Benzylidene), 126.1-129.2 (CH arom), 137.5, 138.0, 138.3, 138.3, 139.4 (C_q Benzylidene and 4xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 98.4 (*J* = 170 Hz, C-1), 101.4 (*J* = 156 Hz, C-1'); HRMS $[M+Na]^+$ calculated for $C_{52}H_{60}O_{14}SNa$ 963.35960, found 963.36034.



Methyl 2-deoxy-3,6-di-*O*-benzyl-2-(*N*-carboxybenzyl)-amino-4-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-α-D-glucopyranoside (23): was prepared from donor **17b** (0.142 g, 0.24 mmol, 1 eq) and acceptor **22** (0.168 g, 0.36 mmol, 1.5 eq) according to the general procedure for glycosylations as described above to afford compound **23** (0.177 g, 0.19 mmol, 78%, α/β = 1:1).

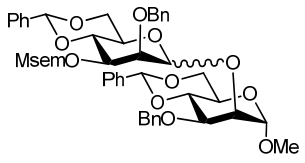
α-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.5; $[\alpha]_D^{22}$: +58.4º (c = 0.5, DCM); IR (neat, cm^{-1}): 733, 1311, 1717; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.78-2.85 (m, 4H, CH_3 Msem and $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.03-3.10 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.37 (s, 3H, CH_3 OMe), 3.70-3.83 (m, 6H, H-2, H-4, H-5, H-6, H-6' and (H-6 or H-6')), 3.84-3.89 (m, 3H, H-5' and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 3.95 (t, 1H, *J* = 9.2 Hz, H-4'), 4.01 (dd, 1H, *J* = 2.8 Hz, *J* = 10.0 Hz, H-3'), 4.08-4.15 (m, 3H, H-3, H-2' and (H-6 or H-6')), 4.21-4.32 (m, 2H, 2xCH<u>H</u> Bn), 4.55-4.78 (m, 7H, H-1', 4xCH<u>H</u> Bn and $MeSO_2(CH_2)_2OC\underline{H}_2$-), 4.92 (d, 1H, *J* = 10.0 Hz, NH), 4.98-5.05 (m, 2H, 2xCH<u>H</u> Cbz), 5.36

(s, 1H, H-1), 5.55 (s, 1H, CH benzylidene), 7.12-7.54 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.8 (CH_3 Msem), 54.4 (C-2' or C-3), 54.8 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.3 ($CH_3$ OMe), 61.6 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 65.3 (C-5'), 67.0 ($CH_2$ Cbz), 68.6, 69.2 (C-6 and C-6'), 70.5 (C-2, C-5 or C-4'), 73.0 (CH_2 Bn), 73.3 (C-3'), 73.6 (CH_2 Bn), 73.8 (CH_2 Bn), 76.0 (C-4), 77.4 (C-2, C-5 or C-4'), 78.0 (C-2' or C-3), 81.1 (C-2, C-5 or C-4'), 94.5 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 99.0 (C-1), 100.4 (C-1'), 101.8 (CH benzylidene), 126.2-129.2 (CH arom), 137.6, 137.9, 137.9 (C_q benzylidene and 2xC_q Bn), 155.8 (C=O Cbz); CH Gated NMR (100 MHz, $CDCl_3$) δ = 99.0 (*J* = 169 Hz, C-1), 100.4 (*J* = 174 Hz, C-1'); HRMS $[M+H]^+$ calculated for $C_{53}H_{62}NO_{15}S$ 984.38347, found 984.38438, $[M+Na]^+$ calculated for $C_{53}H_{61}NO_{15}SNa$ 1006.36541, found 1006.36591.

β-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.35 $[\alpha]_D^{22}$: +16.4º (c = 0.5, DCM); IR (neat, cm^{-1}): 522, 1028, 1717; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.76 (s, 3H, CH_3 Msem), 2.78-2.85 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.06-3.15 (m, 2H, $MeSO_2CH\underline{H}CH_2OCH_2$- and H-5'), 3.36 (s, 3H, CH_3 OMe), 3.46 (t, 1H, *J* = 10.0 Hz, H-6'), 3.53 (t, 1H, *J* = 9.6 Hz, H-4), 3.59 (dd, 1H, *J* = 2.8 Hz, *J* = 10.0 Hz, H-3'), 3.65-3.68 (m, 2H, 2xH-6), 3.83 (m, 4H, H-2', H-3 and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 3.95-4.03 (m, 2H, H-2 and H-4'), 4.04-4.12 (m, 2H, H-5, H-6'), 4.50-4.59 (m, 4H, H-1, 2xCH<u>H</u> Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.71-4.74 (m, 3H, H-1' and CH<u>H</u> Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.80 (m, 2H, NH and CH<u>H</u> Bn), 4.87 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 5.00-5.12 (m, 3H, CH<u>H</u> Bn and 2xCH<u>H</u> Cbz), 5.45 (s, 1H, CH benzylidene), 7.23-7.43 (m, 25H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.9 (CH_3 Msem), 54.6 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.3 ($CH_3$ OMe), 61.1 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 66.8 ($CH_2$ Cbz), 67.2 (C-5'), 68.5 (C-6 and C-6'), 70.5 (C-3), 73.5 (CH_2 Bn), 74.3 (CH_2 Bn), 75.0 (C-3), 75.1 (CH_2 Bn), 75.3 (CH_2 Bn), 76.7 (C-2'), 77.8 (C-4'), 77.9 (C-2 and C-5), 78.5 (C-4), 94.0 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 98.9 (C-1), 101.6 (CH benzylidene), 101.8 (C-1'), 126.0-129.2 (CH arom), 137.5, 138.0, 138.3, 138.3, 139.4 (C_q benzylidene and 4xC_q Bn), 155.9 (C=O Cbz); CH Gated NMR (100 MHz, $CDCl_3$) δ = 98.9 (*J* = 173 Hz, C-1), 101.6 (*J* = 157 Hz, C-1'); HRMS $[M+H]^+$ calculated for $C_{53}H_{62}NO_{15}S$ 984.38347, found 984.38458; $[M+Na]^+$ calculated for $C_{53}H_{61}NO_{15}SNa$ 1006.36541, found 1006.36608.



Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-α-D-mannopyranoside (25): Disaccharide **25** was prepared from donor **17b** (0.112 g, 0.19 mmol, 1 eq) and acceptor **24** (0.107 g, 0.29 mmol, 1.5 eq) according to the general procedure for glycosylations as described above to afford compound **20** (0.117 g, 0.14 mmol, %, α/β = 1:5).

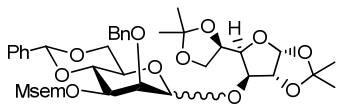
α-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.6; $[\alpha]_D^{22}$: -8.5º (c = 0.3, DCM); IR (neat, cm^{-1}): 696, 1040, 1312; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.81.2.87 (m, 4H, CH_3 Msem and $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.04-3.11 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.37 (s, 3H, CH_3 OMe), 3.77-3.94 (m, 7H, H-5, H-5', H-6, H-6', $MeSO_2CH_2C\underline{H}_2OCH_2$- and one of the H-2, H-2', H-3, H-3', H-4, H-4'), 3.98 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, one of the H-2, H-2', H-3, H-3', H-4, H-4'), 4.00-4.17 (m, 4H, four of the H-2, H-2', H-3, H-3', H-4, H-4'), 4.27 (m, 2H, H-6 and H-6'), 4.42 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.51 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.67-4.69 (m, 3H, H-1, CH<u>H</u> Bn and

$MeSO_2(CH_2)_2OCH\underline{H}$-), 4.81-4.85 (m, 2H, $CH\underline{H}$ Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 5.33 (d, 1H, *J* = 0.8 Hz, H-1'), 5.58 (s, 1H, CH benzylidene), 5.69 (s, 1H, CH benzylidene), 7.23-7.54 (m, 20H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.9 (CH_3 Msem), 54.8 (CH_3 OMe), 54.9 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 61.5 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 63.9, 64.6 (C-5 and C-5'), 68.6, 68.7 (C-6 and C-6'), 72.6, 75.4, 76.3, 76.5, 78.3, 79.0 (C-2, C-2', C-3, C-3', C-4 and C-4'), 73.0 (CH_2 Bn), 73.7 (CH_2 Bn), 94.5 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 100.3 (C-1'), 101.1 (C-1), 101.4 (CH benzylidene), 101.8 (CH benzylidene), 126.0-129.2 (CH arom), 137.4, 137.5, 137.8, 138.3 (2xC_q benzylidene and 2xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 100.3 (*J* = 170 Hz, C-1'), 101.1 (*J* = 171 Hz, C-1); HRMS $[M+Na]^+$ calculated for $C_{45}H_{52}O_{14}SNa$ 871.29700, found 871.29667.

β-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.35; $[\alpha]_D^{22}$: -61.8º (c = 1.0, DCM); IR (neat, cm^{-1}): 696, 1084, 1312; ^1H NMR (400 MHz, $CDCl_3$) δ = 2.77 (s, 3H, CH_3 Msem), 2.84 (dt, 1H, *J* = 3.6 Hz, *J* = 16.4 Hz, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.04-3.11 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.30-3.38 (m, 4H, CH_3 OMe and H-5'), 3.71-3.82 (m, 5H, H-3, H-5, H-6 and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 3.88 (t, 1H, *J* = 10.4 Hz, H-6'), 3.94-3.98 (m, 2H, H-3 and H-2'), 4.09-4.18 (m, 2H, H-4 and H-4'), 4.23 (m, 1H, H-2), 4.27-4.30 (m, 2H, H-6 and H-6'), 4.53 (d, 1H, *J* = 6.8 Hz, $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.69 (s, 1H, H-1'), 4.72-4.79 (m, 4H, H-1, 2x$CH\underline{H}$ Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.93 (d, 1H, *J* = 12.0 Hz, $CH\underline{H}$ Bn), 5.06 (d, 1H, *J* = 12.4 Hz, $CH\underline{H}$ Bn), 5.51 (s, 1H, CH benzylidene), 5.55 (s, 1H, CH benzylidene), 7.23-7.39 (m, 20H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.7 (CH_3 Msem), 54.7 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 54.9 ($CH_3$ OMe), 61.1 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 64.0 (C-3' or C-5), 67.8 (C-5'), 68.5 (C-6'), 68.9 (C-6), 71.4 (CH_2 Bn), 74.0 (C-3 or C-2'), 74.3 (C-3' or C-5), 74.5 (CH_2 Bn), 75.4 (C-3 or C-2'), 75.8 (C-2), 77.5 (C-4'), 78.7 (C-4), 93.8 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 99.5 (C-1), 101.2 (C-1'), 101.6 (CH benzylidene), 101.6 (CH benzylidene), 126.0-129.1 (CH arom), 137.3, 137.5, 138.2, 138.8 (2xC_q benzylidene and 2xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 99.5 (*J* = 167 Hz, C-1), 101.2 (*J* = 153 Hz, C-1'); HRMS $[M+Na]^+$ calculated for $C_{45}H_{52}O_{14}SNa$ 871.29700, found 871.29692.



1,2:5,6-Di-*O*-isopropylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-α-D-glucofuranoside (27): Disaccharide **27** was prepared from donor **17b** (0.147 g, 0.25 mmol, 1 eq) and acceptor **26** (0.098 g, 0.38 mmol, 1.5 eq) according to the general procedure for glycosylations as described above to afford compound **27** (0.139 g, 0.187 mmol, 75%, α/β = 1:10).

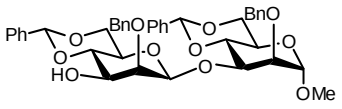
α-anomer:

TLC (50% Toluene in EtOAc): R_f = 0.6; $[\alpha]_D^{22}$: +50º (c = 0.5, DCM); IR (neat, cm^{-1}): 697, 1026; ^1H NMR (400 MHz, $CDCl_3$) δ = 1.33 (s, 3H, CH_3 isopropylidene), 1.36 (s, 3H, CH_3 isopropylidene), 1.43 (s, 3H, CH_3 isopropylidene), 1.51 (s, 3H, CH_3 isopropylidene), 2.84-2.88 (m, 4H, CH_3 Msem and $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.07-3.10 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.79-3.92 (m, 5H, H-2', H-5', H-6' and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 4.00 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H-3'), 4.05-4.08 (m, 2H, H-4 and H-6), 4.15-4.20 (m, 2H, H-4' and H-6), 4.23 (m, 1H, H-5), 4.31-4.35 (m, 2H, H-3' and H-6), 4.57 (d, 1H, *J* = 3.6 Hz, H-2), 4.62 (d, 1H, *J* = 7.2 Hz, $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.66 (d, 1H, *J* = 12.4 Hz, $CH\underline{H}$ Bn), 4.76-4.79 (m, 2H, $CH\underline{H}$ Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 5.30 (s, 1H, H-1'), 5.61 (s, 1H, CH benzylidene), 5.84 (d, 1H, *J* = 3.6 Hz, H-1), 7.17-7.47

(m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 25.4 (CH_3 isopropylidene), 26.2 (CH_3 isopropylidene), 26.8 (CH_3 isopropylidene), 26.9 (CH_3 isopropylidene), 42.8 (CH_3 Msem), 54.8 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 61.6 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 65.0 (C-5'), 67.8 (C-6), 68.7 (C-6'), 72.4 (C-5), 73.0 (C-3'), 73.0 (CH_2 Bn), 75.9 (C-2'), 78.0 (C-4'), 80.1 (C-3), 81.4 (C-4), 84.0 (C-2), 94.7 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 99.4 (C-1'), 101.7 (CH benzylidene), 105.2 (C-1), 109.5 (C_q isopropylidene), 112.2 (C_q isopropylidene), 125.9-129.2 (CH arom), 137.3, 137.6 (C_q benzylidene and C_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 99.4 (*J* = 172 Hz, C-1), 105.2 (*J* = 181 Hz, C-1'); HRMS $[M+Na]^+$ calculated for $C_{36}H_{48}O_{14}SNa$ 759.26570, found 759.26596.

β-anomer:

TLC (50% Toluene in EtOAc): R_f = 0.4; $[\alpha]_D^{22}$: -43.0º (c = 0.5, DCM); IR (neat, cm^{-1}): 697, 733, 1025; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.33 (s, 3H, CH_3 isopropylidene), 1.34 (s, 3H, CH_3 isopropylidene), 1.44 (s, 3H, CH_3 isopropylidene), 1.51 (s, 3H, CH_3 isopropylidene), 2.80 (s, 3H, CH_3 Msem), 2.82-2.88 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.08-3.15 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.35 (m, 1H, H-5'), 3.73-3.85 (m, 3H, H-3' and $MeSO_2CH_2C\underline{H}_2OCH_2$-), 3.90-3.96 (m, 2H, H-2' and C-6'), 4.05-415 (m, 3H, 2xH-6 and H-4'), 4.30-4.32 (m, 3H, H-3, H-4 and H-6'), 4.42 (m, 1H, H-5), 4.51 (d, 1H, *J* = 4.0 Hz, H-2), 4.55 (d, 1H, *J* = 6.8 Hz, $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.64 (s, 1H, H-1'), 4.70-4.74 (m, 2H, CH<u>H</u> Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.88 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 5.56 (s, 1H, CH benzylidene), 5.93 (d, 1H, *J* = 3.6 Hz, H-1), 7.15-7.45 (m, 10H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 25.4 (CH_3 isopropylidene), 26.2 (CH_3 isopropylidene), 26.5 (CH_3 isopropylidene), 26.6 (CH_3 isopropylidene), 42.8 (CH_3 Msem), 54.5 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 61.0 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 66.0 (C-6), 67.7 (C-5'), 68.3 (C-6'), 72.9 (C-5), 74.4 (C-3'), 74.8 (CH_2 Bn), 75.9 (C-2'), 77.6 (C-4'), 80.3, 80.9 (C-3 and C-4), 82.6 (C-2), 93.9 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 100.2 (C-1'), 101.5 (CH benzylidene), 104.8 (C-1), 108.6 (C_q isopropylidene), 111.9 (C_q isopropylidene), 125.2-129.1 (CH arom), 137.2, 137.8 (C_q benzylidene and C_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 100.2 (*J* = 154 Hz, C-1), 104.8 (*J* = 181 Hz, C-1'); HRMS $[M+Na]^+$ calculated for $C_{36}H_{48}O_{14}SNa$ 759.26570, found 759.26588.

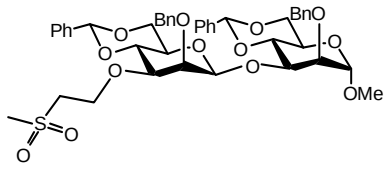


Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-α-D-mannopyranoside (28):

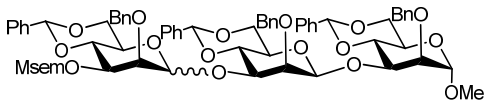
Method I (Without scavenger): To a solution of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene3-*O*-methysulfonylethoxymethyl-β-D-mannopyranosyl)-α-D-mannopyranoside **19b(β)** (0.340 g, 0.40 mmol) in THF (4 ml, 0.1 M) was added tetrabutylammonium fluoride (0.05 M/THF, 0.8 ml, 0.04 mmol, 0.1 eq). The reaction mixture was stirred for 24 hours. The reaction mixture was quenched with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3\,(aq)}$, brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography to give **28** (0.175 g, 0.248 mmol, 62%) and **29** (0.091 g, 0.111 mmol, 27%).

Method II (With scavenger): To a solution of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene3-*O*-methysulfonylethoxymethyl-β-D-mannopyranosyl)-α-D-mannopyranoside **19b(β)** (0.148g, 0.17 mmol) in THF (3.5 ml, 0.05 M) was added pipperdine (35 µl, 0.35 mmol, 2 eq) followed by the addition of tetrabutylammonium fluoride (0.01M, 1.74 ml, 0.1 eq). The reaction mixture was stirred for 24 hours. The reaction

mixture was quenched with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3(aq)}$, brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography to give **28** (0.114 g, 0.160 mmol, 92%). TLC (50% EtOAc in PE): R_f = 0.8; $[\alpha]_D^{22}$: -48.0º (c = 0.6, DCM); IR (neat, cm^{-1}): 535, 698, 1093; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.59 (bs, 1H, OH-3'), 3.12 (m, 1H, H-5'), 3.35 (CH_3 OMe), 3.63.-3.71 (m, 2H, H-5' and H-6'), 3.71 (d, 1H, *J* = 4.0 Hz, H-2'), 3.79-3.88 (m, 4H, H-2, H-5, H-6, H-4'), 4.11-4.15 (m, 2H, H-4 and H-6'), 4.25 (dd, 1H, *J* = 4.0 Hz, *J* = 9.5 Hz, H-6), 4.30 (dd, 1H, *J* = 3.5 Hz, *J* = 10.0 Hz, H-3), 4.44 (s, 1H, H-1'), 4.58-4.62 (m, 2H, 2xCH<u>H</u> Bn), 4.70 (d, 1H, *J* = 12.0 Hz, CH<u>H</u> Bn), 4.79 (s, 1H, H-1), 4.97 (d, 1H, *J* = 11.0 Hz, CH<u>H</u> Bn), 5.20 (bs, 1H, CH benzylidene), 5.57 (s, 1H, CH benzylidene), 7.16-7.49 (m, 20H, H arom); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 54.8 (CH_3 OMe), 64.0 (C-2 or C-5), 66.6 (C-5'), 68.5 (C-6'), 68.7 (C-6), 70.0 (C-3'), 72.6 (C-3), 72.9 (CH_2 Bn), 74.3 (C-2 or C-5), 74.5 (CH_2 Bn), 77.1 (C-4 and C-2'), 79.7 (C-4'), 98.0 (C-1'), 99.4 (C-1), 101.4 (CH benzylidene), 101.8 (CH benzylidene), 126.1-128.9 (CH arom), 137.2, 137.4, 137.5, 138.0 (2xC_q benzylidene and 2xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 98.0 (*J* = 158 Hz, C-1'), 99.4 (*J* = 168 Hz, C-1); HRMS $[M+H]^+$ calculated for $C_{41}H_{45}O_{11}$ 713.29564, found 713.29657; $[M+Na]^+$ calculated for $C_{41}H_{44}O_{11}Na$ 735.27758, found 735.27777.



Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethyl-D-mannopyranosyl)-α-D-mannopyranoside (29): Collected as a side product during the preparation of **28** by the cleavage of Msem without scavenger (0.91 g, 0.111 mmol, 27 %). TLC (50% EtOAc in PE): R_f = 0.6; $[\alpha]_D^{22}$: -33.4º (c = 1.0, DCM); IR (neat, cm^{-1}): 730, 1061; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.79 (s, 3H, CH_3 Mse), 3.01-3.16 (m, 2H, $MeSO_2C\underline{H}_2CH_2$-), 3.36 (m, 1H, H-5'), 3.39 (CH_3 OMe), 3.70-3.80 (m, 3H, H-2', H-6' and $MeSO_2CH_2CH\underline{H}_2$-), 3.80-3.93 (m, 4H, H-2, H-5, H-6 and $MeSO_2CH_2CH\underline{H}_2$-), 4.04 (t, 1H, *J* = 8.8 Hz, H-4'), 4.08-4.20 (m, 2H, H-4 and H-6'), 4.29 (dd, 1H, *J* = 4.4 Hz, *J* = 10.0 Hz, H-6), 4.32 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H-3), 4.39 (s, 1H, H-1'), 4.58 (d, 1H, *J* = 12.4 Hz, CH<u>H</u> Bn), 4.63 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 4.74 (d, 1H, *J* = 12.4 Hz, CH<u>H</u> Bn), 4.82 (s, 1H, H-1), 4.96 (d, 1H, *J* = 11.6 Hz, CH<u>H</u> Bn), 5.26 (bs, 1H, CH benzylidene), 5.63 (s, 1H, CH benzylidene), 7.19-7.50 (m, 20H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 43.1 (CH_3 Mse), 54.9 (CH_3 OMe), 55.3 ($MeSO_2\underline{C}H_2CH_2$-), 63.8 ($MeSO_2CH_2\underline{C}H_2$-), 64.0 (C-2 or C-5), 67.0 (C-5'), 68.6 (C-6'), 68.8 (C-6), 72.6 (C-3), 72.9 (CH_2 Bn), 74.2 (CH_2 Bn), 74.4 (C-2 or C-5), 74.9 (C-2'), 77.2 (C-4), 77.3 (C-4'), 77.7 (C-3'), 99.1 (C-1'), 99.4 (C-1), 101.2 (CH benzylidene), 101.8 (CH benzylidene), 126.0-129.0 (CH arom), 137.2, 137.5, 137.6, 138.2 (2xC_q benzylidene and 2xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 99.1 (*J* = 160 Hz, C-1'), 99.5 (*J* = 167 Hz, C-1); HRMS $[M+Na]^+$ calculated for $C_{44}H_{50}O_{13}SNa$ 841.28643, found 841.28680.



Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-methylsulfonylethoxymethyl-D-mannopyranosyl)-β-D-mannopyranosyl)-α-D-mannopyranoside (30): Trisaccharide **30** was prepared from donor **17b** (0.172 g, 0.30 mmol, 1.5 eq) and acceptor

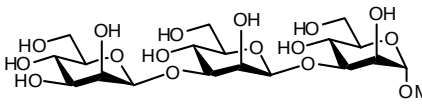
28 (0.144 g, 0.20 mmol, 1 eq) according to the general procedure for glycosylations as described above at -78 ºC yielding compound **35** (0.199 g, 0.17 mmol, 83%, α/β = 1:5).

α-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.66; $[\alpha]_D^{22}$: -2.5º (c = 0.4, DCM); IR (neat, cm^{-1}): 698, 1067; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.59 (s, 3H, CH_3 Msem), 2.72-2.76 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 2.91-2.98 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.05-3.10 (m, 1H, H-5') 3.36 (s, 3H, CH_3 OMe), 3.68 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H-3'), 3.75-3.92 (m, 10H, H-2, H-2', H-2'', H-6, H-6', H-6'', $MeSO_2CH_2C\underline{H}_2OCH_2$- and two of the H-3, H-4, H-5, H-3'', H-4'' and H-5''), 4.01-4.36 (m, 10H, H-1', H-4', H-6, H-6', H-6'', C$\underline{H}$ Bn and four of the H-3, H-4, H-5, H-3'', H-4'' and H-5''), 4.39 (d, 1H, *J* = 12.0 Hz, C$\underline{H}$ Bn), 4.56-4.60 (m, 2H, C$\underline{H}$ Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.73-4.79 (m, 3H, 2xC$\underline{H}$ Bn and $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.85 (s, 1H, H-1), 4.97 (d, 1H, *J* = 12.0 Hz, C$\underline{H}$ Bn), 5.27 (s, 1H, H-1''), 5.55 (s, 1H, CH benzylidene), 5.59 (s, 1H, CH bnzylidene), 5.63 (s, 1H, CH benzylidene), 7.03-7.51 (m, 30H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.8 (CH_3 Msem), 54.8 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.0 ($CH_3$ OMe), 61.5 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 64.0, 64.8, 67.4, 72.9, 72.9, 74.2, 76.2, 76.5, 78.0, 78.2, 78.2, 78.6 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2'', C-3'', C-4'', and C-5''), 68.6, 68.8 (C-6, C-6' and C-6''), 72.6 (CH_2 Bn), 72.7 (CH_2 Bn), 75.0 (CH_2 Bn), 94.8 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 98.7, 99.1, 99.8 (C-1, C-1' and C-1''), 101.7 (CH benzylidene), 101.8 (CH benzylidene), 102.0 (CH benzylidene), 126.1-129.3 (CH arom), 137.5, 137.6, 137.6 (3xC_q benzylidene and 3xC_q Bn); HRMS $[M+Na]^+$ calculated for $C_{65}H_{72}O_{19}SNa$ 1211.42807, found 1211.42847.

β-anomer:

TLC (33% Toluene in EtOAc): R_f = 0.45; $[\alpha]_D^{22}$: -136.4º (c = 1.0, DCM); IR (neat, cm^{-1}): 698, 1092; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.72 (s, 3H, CH_3 Msem), 2.72-2.82 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 2.99-3.00 (m, 1H, $MeSO_2CH\underline{H}CH_2OCH_2$-), 3.10-3.15 (m, 2H, H-5' and H-5"), 3.40 (CH_3 OMe), 3.52 (dd, 1H, *J* = 3.2 Hz, *J* = 10.0 Hz, H-3' or H-3''), 3.71 (m, 1H, H-2' or H-2"), 3.75 (m, 1H, $MeSO_2CH_2CH\underline{H}OCH_2$-), 3.82-3.95 (m, 8H, H-2, H-5, H-6, (H-2' or H-2''), (H-3' or H-3''), H-6', H-6" and $MeSO_2CH_2CH\underline{H}OCH_2$-), 4.01 (m, 1H, H-4' or H-4"), 4.08 (m, 1H, H-4'or H-4''), 4.15-4.22 (m, 3H, H-4, H-6' and H-6"), 4.28 (dd, 1H, *J* = 3.6 Hz, *J* = 9.2 Hz, H-6), 4.34-4.38 (m, 2H, H-3 and (H-1' or H-1''), 4.43 (s, 1H, H-1'or H-1''), 4.49 (d, 1H, *J* = 6.8 Hz, $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.64 (d, 1H, *J* = 7.2 Hz, $MeSO_2(CH_2)_2OCH\underline{H}$-), 4.66-4.75 (m, 3H, 3xCH$\underline{H}$ Bn), 4.78 (d, 1H, *J* = 12.0 Hz, C$\underline{H}$ Bn), 4.85 (s, 1H, H-1), 4.96 (d, 1H, *J* = 12.0 Hz, C$\underline{H}$ Bn), 5.04 (d, 1H, *J* = 12.0 Hz, C$\underline{H}$ Bn), 5.46 (s, 1H, CH Benzylidene), 5.48 (s, 1H, CH Benzylidene), 5.58 (s, 1H, CH Benzylidene), 7.15-7.48 (m, 30H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 42.5 (CH_3 Msem), 54.7 ($MeSO_2\underline{C}H_2CH_2OCH_2$-), 55.0 ($CH_3$ OMe), 61.3 ($MeSO_2CH_2\underline{C}H_2OCH_2$-), 64.0, 74.2, 74.2, 74.4 (C-2, C-3 C-2'and C-5), 67.5, 67.8 (C-5' and C-5"), 68.5, 68.6 (C-6' and C-6"), 68.8 (C-6), 72.7 (CH_2 Bn), 72.8 (C-3), 74.3 (CH_2 Bn), 74.5 (CH_2 Bn), 74.6 (C-2"), 75.2 (C-3"), 77.0, (C-4"), 77.3 (C-4), 77.4 (C-4'), 93.7 ($MeSO_2(CH_2)_2O\underline{C}H_2$-), 98.1, 98.4 (C-1' and C-1"), 99.2 (C-1), 101.5 (CH Benzylidene), 101.7 (CH Benzylidene), 101.7 (CH Benzylidene), 125.3-129.7 (CH arom), 137.3, 137.4, 137.5, 137.5, 138.3, 138.6 (3xC_q Benzylidene and 3xC_q Bn); CH Gated NMR (100 MHz, $CDCl_3$) δ = 98.1 (*J* = 153 Hz, C-1' or C-1"), 98.1 (*J* = 155 Hz, C-1' or C-1"), 99.2 (*J* = 167 Hz, C-1); HRMS $[M+Na]^+$ calculated for $C_{65}H_{72}O_{19}SNa$ 1211.42807, found 1211.42842.



Methyl 3-*O*-[3-*O*-(β-D-mannopyranosyl)-β-D-mannopyranosyl)-α-D-mannopyranoside (31): To a solution of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene3-*O*-methysulfonylethoxymethyl-β-D-mannopyranosyl)-β-D-mannopyranosyl] α-D-mannopyranoside **30β** (40 mg, 35 μmol) in THF (0.7 ml, 0.05 M) was added pipperdine (7 μl, 70 μmol, 2 eq) followed by the addition of tetrabutylammonium fluoride (0.01M, 0.35 ml, 3.5 μmol, 0.1 eq). The reaction mixture was stirred for 24 hours. The reaction mixture was quenched with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_{3(aq)}$, brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel chromatography to get methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-β-D-mannopyranosyl)-α-D-mannopyranoside. The resulting compound was dissolved in MeOH (1 ml) and H_2O (0.7 ml) before the addition of catalytic amount of $Pd(OH)_2$ on charcoals. The reaction was stirred for 24 hours under an H_2-atmosphere and filtered and purified by Gel filteration to afford the desired trisaccharide **31** (11 mg, 21 μmol, 60%); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 3.30-3.37 (m, 5H, H-5, (H-5' or H-5'') and CH_3 OMe), 3.49 (t, 1H, *J* = 9.6 Hz, H-4' or H-4''), 3.58-3.61 (m, 2H, H-5 and (H-3' or H-3'')), 3.63-3.67 (m, 5H, H-4, (H-4' or H-4"), H-6, H-6' and H-6''), 3.83-3.88 (m, 3H, H-6, H-6', H-6"), 3.91 (dd, 1H, *J* = 2.4 Hz, *J* = 9.6 Hz, H-3' or H-3''), 3.95 (dd, 1H, *J* = 3.0 Hz, *J* = 9.6 Hz, H-3), 3.98 (d, 1H, *J* = 2.4 Hz, H-2' or H-2''), 4.06 (s, 1H, H-2), 4.19 (s, 1H, H-2' or H-2''), 4.73 (s, 1H, H-1), 4.74 (s, 1H, H-1' or H-1''), 4.79 (s, 1H, H-1' or H-1''); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 53.7 (CH_3 OMe), 61.8, 61.9 (C-6, C-6'and C-6''), 66.1, 66.2 (C-4 and C-4'), 67.8, 67.9 (C-2 and C-4' or C-4''), 68.7, 71.7 (C-2 and C-2''), 73.3, 73.8 ((C-5' or C-5'') and (C-3' or C-3")), 77.0, 77.3 (C-5'and C-5''), 78.2, 79.8 (C-3' and C-3'') 97.6, 97.7 (C-1' and C-1''), 101.6 (C-1); HRMS $[M+Na]^+$ calculated for $C_{19}H_{34}O_{16}Na$ 541.17391, found 541.17358.

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A two-step fluorous capping procedure in solid phase peptide synthesis

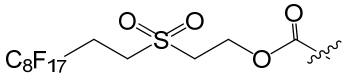
CHAPTER 5

Introduction:

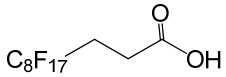
The synthesis of peptides by solid phase procedures has reached a high level of efficiency and oligopeptides up to a length of 50 amino acids are routinely prepared[1]. Nonetheless, present state of the art in solid phase peptide synthesis (SPPS) dictates that the outcome of a specific peptide synthesis cannot always be predicted. Although in a standard SPPS protocol the reagents and protected amino acid building blocks are used in five or more fold excess, the required near quantitative yields in the corresponding reactions are not always attained. In the elongation cycle of SPPS, comprising the removal of the terminal amino-protecting group and coupling of a suitably protected amino acid to the

growing peptide chain, the condensation step is most often incomplete. Consequently the solid phase synthesis of an oligopeptide is accompanied by the formation of truncated and deletion sequences. The accumulation of these unwanted sequences, in particular the (n-1) peptides frequently complicates the purification of the target full-length product[2].

Figure 1: The fluorous capping reagent PFD-OH and the FMsc protecting group.



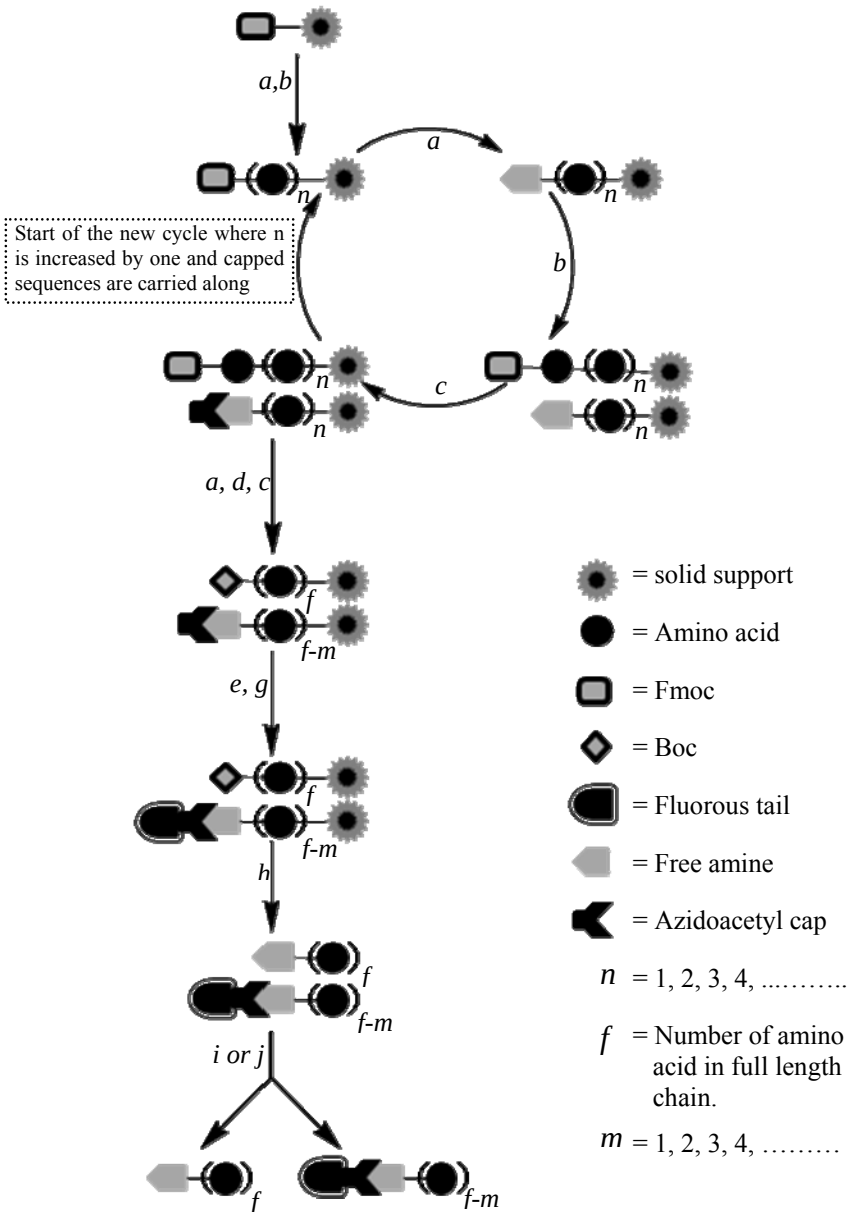
1, FMsc



2, PFD-OH

The advent of light-fluorous techniques, in which compounds differing in fluorine content are separated by chromatography using a fluorous stationary phase, offers an opportunity to improve the purification of oligopeptides obtained by SPPS[3]. Two approaches can be distinguished. In the first approach a suitable fluorous protecting group is developed and installed at the N-terminal end of the target oligopeptide in the final stage of the solid phase synthesis. The fluorous protecting group survives the subsequent cleavage of the peptide from the solid support and the fluorous target peptide is separated from the non-fluorous impurities by fluorous HPLC or fluorous solid phase extraction (F-SPE) [4]. Final steps of this approach entail the removal of the fluorous protecting group and the isolation of the target oligopeptide. Several examples of fluorous protecting groups are reported[5]. An example of such a protecting group is the [1*H*,1*H*,2*H*,2*H*]-perfluorodecylsulfonylethoxycarbonyl (FMsc, Figure 1) group, the application of which in carbohydrate chemistry is described in chapter 3[6]. In the second approach the intermediate and immobilized oligopeptides that resist elongation en route to the target peptide are capped after every condensation step in the SPPS cycle with a fluorous reagent, making the truncated and deletion sequences fluorous and leaving the target compound untouched[7]. The final step of the second approach entails the separation of the non-fluorous target peptide from the fluorous impurities by fluorous HPLC or fluorous solid phase extraction (F-SPE).

Figure 2: General elongation cycle of the SPPS approach, using a two step fluorous capping procedure and ensuing isolation of the target peptide using fluorous techniques.



Reagents and conditions; *a*) Piperdine (20% in NMP), 15 min.; *b*) Fmoc-amino acid, HCTU, Dipea, NMP; *c*) Azidoacetic acid, HCTU, NMP, 20 min; *d*) Boc_2O, Dipea, NMP, 30 min; *e*) PMe_3, H_2O, dioxane, 2h; *g*) PFD-OH, HCTU, Dipea, NMP, 1h; *h*) TFA, TIS, H_2O, 45 min; *i*) F-HPLC; *j*) FSPE.

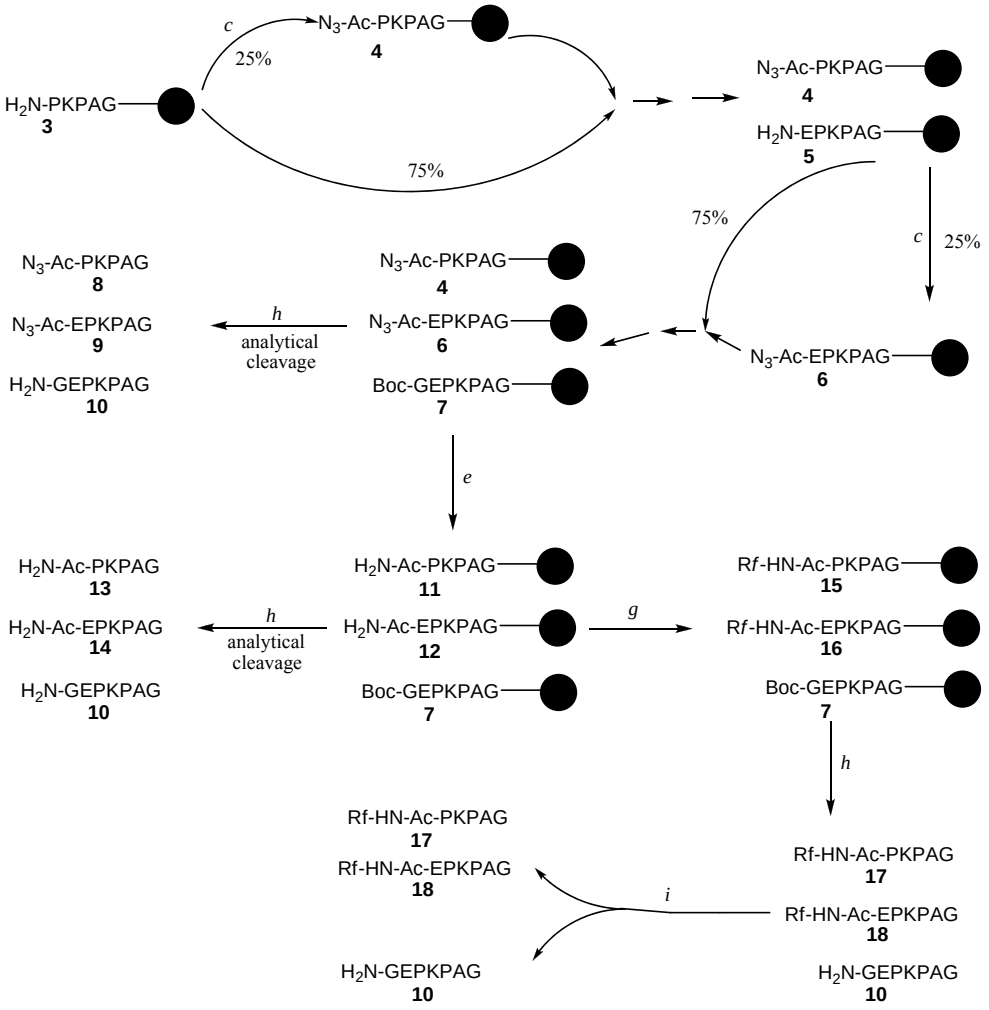
The present chapter describes the exploration of a fluorous capping approach in SPPS. Capping of incomplete sequences in standard SPPS is commonly done with acetic anhydride and it seems obvious to replace acetic anhydride with a suitable fluorous anhydride. However anhydrides with sufficient fluorine content are not commercially available and the corresponding acids are rather expensive. Therefore a new SPPS protocol endowed with a two-step fluorous capping procedure was designed (Figure 2). Each elongation cycle of this SPPS protocol entails (*a*) Fmoc removal, (*b*) coupling of the next suitably protected amino acid and (*c*) capping with azidoacetic acid under influence of HCTU. In this way the azidoacetyl group is introduced at the amino functions of intermediate sequences that failed to react in the condensation steps en route to the oligopeptide target. After completion of the SPPS the Fmoc protecting group in the target peptide is replaced with the Boc group (*d*). In the next event the azides in the immobilized incomplete sequences are reduced to amino functions (*e*) and made fluorous by condensation with (1H,1H,2H,2H)-perfluoroundecanoic acid (PFD-OH, **2**, Figure 1, *g*). Finally the immobilized oligopeptides are deprotected, cleaved from the solid support (*h*) and purified by fluorous HPLC (*i*) or fluorous solid phase extraction (F-SPE, *j*).

Results and discussion:

The feasibility of the two-step capping strategy was examined with the aid of the synthesis of model peptide GEPKPAG (**10**). The manual SPPS of this heptamer was executed in such a manner, that deletion sequences were deliberately obtained (Figure 3). In the SPPS protocol the Fmoc group was removed with piperidine and 5 equivalents of the required amino acids building blocks were used in presence of the activator HCTU and Dipea as base. When the SPPS to **10** had reached the stage of the pentamer **3** with a free amino function, 25% of the resin **3** was taken out and the immobilized pentamer PKPAG **3** was condensed with azidoacetic acid under influence of HCTU and Dipea for 20 minutes (Figure 3). The reaction went to completion, as determined by the chloranil test and the resulting azidoacetyl capped immobilized pentamer **4** was remixed with the untreated resin **3** (Figure 3). The next amino acid was coupled and the terminal amino function was unmasked to give resin **5** (Figure 3). Next, 25% of the resin containing immobilized hexamer **5** and azidoacetyl capped **4** was taken aside as described above, capped with an

azidoacetyl group to give **6** and remixed with the rest of the resin still containing **4** and **5** (Figure 3). The last Boc-protected amino acid Gly was coupled to give full length protected peptide **7** (Figure 3). The Boc group was used instead of Fmoc protection because the Fmoc group is not stable during the forthcoming reduction with trimethylphosphine[8].

Figure 3: Synthesis and purification of heptamer GEPKPAG.



Reagents and conditions; *c*) Azidoacetic acid, HCTU, NMP, 20 min; *e*) PMe_3, H_2O, dioxane, 2h; *g*) PFD-OH, HCTU, Dipea, NMP, 1h; *h*) TFA, TIS, H_2O, 45 min; *i*) FSPE.

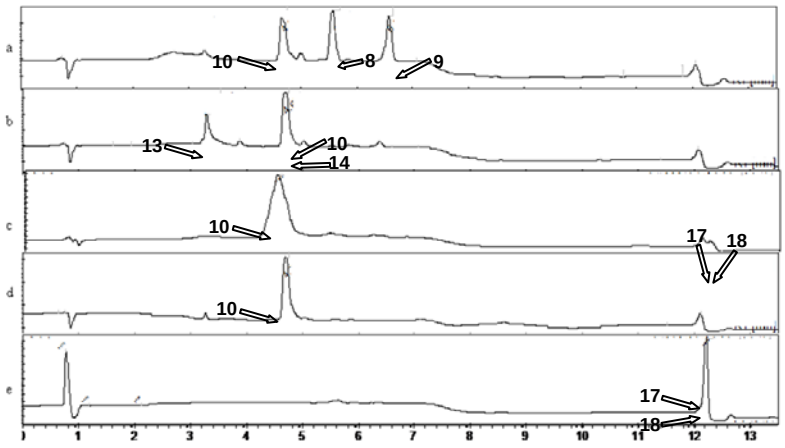
To analyze the product distribution a small part of the thus obtained resin mixture was subjected to standard deblocking and cleavage conditions. The resin mixture containing Boc protected heptamer together with the deliberately introduced azidoacetyl capped deletion sequences pentamer and hexamer was treated with TFA/TIS/H_2O (95:2.5:2.5) to remove the Boc group, the amino acid side chain protecting groups and to release the peptides from the resin[9]. Analysis by LC-MS showed the presence of three major peaks which correspond to the target heptamer (**10**) and the azidoacetyl capped pentamer (**8**) and hexamer (**9**) (Figure 4a).

Next, conditions were explored that allow complete reduction of the azides to amino functions. The resin was treated with trimethylphosphine in a mixture of H_2O and 1,2-dioxane for 2 hours. The reduction of azide to amine was ensured by repetition of the reductive treatment[10]. The result was substantiated by LC-MS analysis of the crude mixture, obtained by subjecting a small amount of resin to the standard deprotection/cleavage conditions (Figure 4b). The remaining resin **7**, **11** and **12**, having deletion sequences with free amine functions was treated with (1H,1H,2H,2H)-perfluoroundecanoic acid (PFD-OH, **1**), the condensing agent HCTU and Dipea in NMP (Figure 3). After completion of the reaction (**11** to **15** and **12** to **16**), as monitored by the TNBS test[11], the peptides were released from the resin and the crude mixture containing **10**, **17** and **18** was analyzed by LC-MS which showed two peaks, representing target heptamer and the fluorous peptides, respectively Figure 4c). The crude mixture was conveniently purified by fluorous solid phase extraction (FSPE) using a stepwise gradient of acetonitrile in H_2O with 0.1%TFA. Mixtures of acetonitrile/H_2O/TFA (20/79.9/0.1) and acetonitrile/H_2O/TFA (50/49.9/0.1) were used to elute the target peptide **10** (Figure 4d). The fluorous penta- and hexamer were eluted with acetonitrile/H_2O/TFA (90/9.9/0.1) and acetonitrile/TFA (99.9/0.1), respectively (Figure 4e). The overall yield of **10** obtained after FSPE was 17% (Table 1, Entry 1).

To further ascertain the feasibility of the two-step capping procedure in oligopeptide synthesis, the decapeptide VEAAIDYIDA (**19**), a peptide derived from acyl carrier protein, was selected as a suitable model[5b,12]. Following the manual SPPS procedure described above tetrameric and nonameric deletion sequences were deliberately produced

by taking out 20% of the resin at the corresponding stage, capping it with the azidoacetyl group and remixing it with the rest of the resin. After completion of the sequence, the Fmoc-group on the last amino acid residue was removed and the Boc group was installed by treatment with di-*tert*-butyl-dicarbonate (Boc_2O) and Dipea[13]. A small portion of the resin was subjected to standard deblocking and cleavage conditions and the obtained mixture was analyzed (Figure 5a)[14]. The resin mixture containing the crude mixture of target peptide and two azido capped tetrameric and nonameric deletion sequences was treated with trimethylphosphine (PMe_3) and the resulting free amines in the deletion sequences were coupled with fluorous PFD-OH (**1**). The peptide was cleaved from the resin and analyzed (Figure 5b). Target peptide **19** could be conveniently purified by FSPE (Figure 5c) and was isolated in 7% overall yield (Table 1, Entry 2).

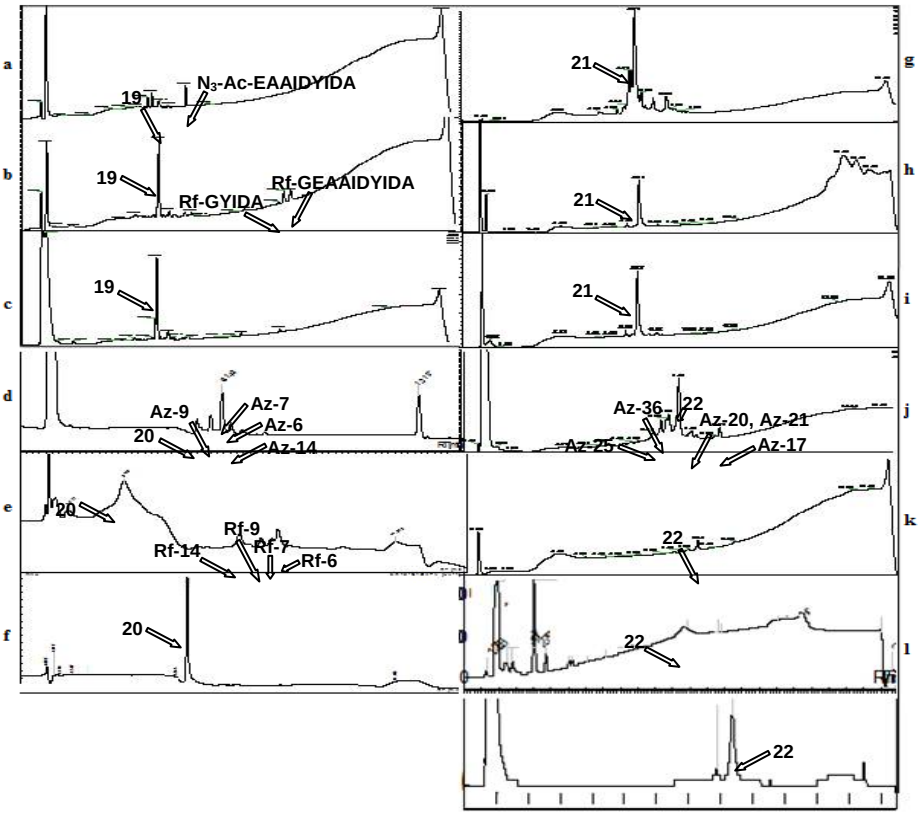
Figure 4: LC Chromatograms of GEPKPAG. a) after synthesis; b) after reduction; c) after fluorous capping; d) after FSPE (target peptide); e) after FSPE (fluorous capped fragments).



To investigate whether the two-step capping procedure could be transferred to automatic SPPS, the assembly of nonadecamer SSKKSGSYSGSKGSKRRIL **20** using an automated peptide synthesizer and Fmoc chemistry was undertaken. To ensure the acquirement of deletion sequences, 2 eq. instead of the usual 5 eq. of protected amino acid building blocks were employed in the coupling step and the coupling time was reduced

from the usual 45 minutes to 30 minutes. After each coupling, the unreacted resin bound amino functions were capped by reaction with azidoacetic acid in combination with HCTU and Dipea. After the completion of the target sequence, the Fmoc at the N-terminal end was quantitatively replaced by the Boc group, as shown by the TNBS test. A small portion of the resin was taken out, subjected to standard deblocking and cleavage conditions. The

Figure 5: LC Chromatograms. a) crude peptide **19** after synthesis; b) crude peptide **19** after fluorous capping; c) peptide **19** after FSPE; d) crude peptide **20** after synthesis; e) crude peptide **20** after fluorous capping; f) peptide **20** after FSPE; g) crude peptide **21** after synthesis; h) crude peptide **21** after fluorous capping; i) peptide **21** after FSPE; j) crude peptide **22** after synthesis; k) peptide **22** after fluorous capping; l) peptide **22**, F-HPLC-trace; m) peptide **22** HPLC (C_{18}) trace).



mixture comprising target peptide and deletion sequences was analyzed (Figure 5d). The remaining resin was subjected to reducing conditions and the obtained amino functions on the unwanted sequences were provided with a fluorous tail by condensation with PFD-OH (**1**). The peptide was released from the resin and analyzed (Figure 5e). The crude mixture was easily purified by FSPE to give target peptide **20** (Figure 5f) in 9% overall yield (Table 1, Entry 3).

Similarly peptide EPLTSLTPR-Abu-NTAWNRLKL **21**, part of an envelope protein of the Moloney virus, containing a 2-aminobutyric acid residure (Abu) as an isosteric replacement for Cysteine (Cys) was prepared on an automated peptide synthesizer using Fmoc chemistry combined with azidoacetic acid capping (Fmoc/AzidoAc protocol)[15]. This time the usual excess of amino acid building blocks and the coupling time were employed, minimizing the formation of deletion sequences. At the final stage of the SPPS the Fmoc group was replaced with the Boc group. A small portion of the resin was subjected to standard deblocking and cleavage conditions and the obtained mixture was analyzed (Figure 5g). The crude target oligopeptide **21** (Figure 5h) was obtained by the same sequence of events as described for the preparation of oligopeptide **20**. Finally, FSPE purification gave target peptide **21** (Figure 5i) in 21% overall yield (Table 1, Entry 4).

As a last example BDSTLRLBVQSTHVDIRTLEDLLMGTLGIVPBIBSQKP **22**, a peptide derived from E7 protein of HPV16 was assembled using the Fmoc/AzidoAc protocol on automated peptide synthesizer.[16] Again 2-aminobutyric acid residue was introduced as the isostere of Cys. The LC chromatograms of the non-fluorous reaction mixture and the crude reaction mixture containing the fluorous deletion peptides are shown in figures 5j and 5k respectively. Pure target peptide **22** (Figure 5l) was obtained by F-HPLC purification in 7% overall yield (Table 1, Entry 5).

Table 1: Yields of different peptides.

Entry	Peptide	Yield
1	GEPKPAG (**10**)	17%
2	VEAAIDYIDA (**19**)	7%
3	SSKKSGSYSGSKGSKRRIL (**20**)	9%
4	EPLTSLTPRBNTAWNRLKL (**21**)	21%
5	BDSTLRLBVQSTHVSIRTLEDLLMGTLGIVPSIBSQKP (**22**)	7%

Conclusion:

This chapter described the successful implementation of a two-step fluorous capping procedure in an otherwise standard SPPS protocol allowing the improved isolation of five natural and artificially designed oligopeptides. In the elongation cycle of the SPPS protocol amino functions of unwanted sequences were provided with the azidoacetyl group and in the final stage of the oligopeptide synthesis the thus appended azides were reduced to amine functions and subsequently capped with a fluorous tail. The fluorous deletion sequences were conveniently separated from target peptides using FSPE or F-HPLC.

Experimental:

General: All chemicals used in the solid phase peptide synthesis, with the exception of the HCTU were from Biosolve (The Netherlands) and used as received. HCTU was purchased IRIS Biotech GmbH (Germany). Resins were bought at Rapp Polymere GmbH (Germany). (1H,1H,2H,2H)-perfluoroundecanoic acid was bought at Fluorous Technologies Inc. Fmoc amino acids were from SENN Chemicals. Fmoc amino acids used in the synthesis were: Fmoc-Abu-OH, Fmoc-Ala-OH, Fmoc-Arg-(Pmc)-OH, Fmoc-Asn-(Trt)-OH, Fmoc-Asp-(tBu)-OH, Fmoc-Gln-(Trt)-OH, Fmoc-Glu-(tBu)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-His-(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys-(Boc)-OH, Fmoc-Met-OH, Fmoc-Pro-OH, Fmoc-Ser-(tBu)-OH, Fmoc-Thr-(tBu)-OH, Fmoc-Trp-

(Boc)-OH, Fmoc-Tyr-(tBu)-OH, Fmoc-Val-OH. Azidoacetic acid was prepared according to the literature procedure[17]. Analytical LC/MS was conducted on a JASCO system using an Alltima C_{18} analytical column (5 μm particle size, flow 1.0 ml/min). Absorbance was measured at 214 nm and 254 nm. Solvent system: A: 100% H_2O, B: 100% acetonitrile, C: 1% $TFA_{(aq)}$. Gradients of B in 10% C were applied over 13 minutes.

General cycle of peptide syntheis: The solid phase peptide synthesis was performed on ABI 433A (Applied Biosystems) applying Fmoc based protocol starting either from Rink amide MBHA resin or Tentagel preloaded with Fmoc-Leu-OH (Wang linker). Alternatively a mechanical shaker was used and the reagents were introduced manually (manual peptide synthesis). The synthesis was performed on 50 μmol according to established methods. The consecutive steps performed in each cycle were:
1) Deprotection of the Fmoc group with 20% piperdine in NMP for 15 minutes; 2) DMF wash; 3) Coupling of the appropriate amino acid applying a five-fold excess. Generally the Fmoc amino acid (0.25 mmol) were dissolved in 0.25 M HCTU in NMP (1 ml), the resulting solution was transferred to the reaction vessel followed by 0.5 ml of 1 M DIPEA in NMP to initiate the coupling; 4) DMF wash; 5) Capping with azidoacetic acid as described in the general procedure for azide capping; 6) DMF wash

General procedure for azide capping: To the resin bound peptide (1 eq) was added azidoacetic acid (0.2 M/NMP, 5 eq), HCTU (0.25 M/NMP, 5 eq) and DIPEA (1 M/NMP, 10 eq). The mixture was shaken for 20 minutes. Next, solvents were drained off and the resin was subsequently washed with DMF.

General procedure for reduction of azide: To the suspension of resin bound peptide (1 eq) in dioxane was added trimethylphosphine (1 M /toluene, 8 eq) and H_2O (222 eq). The mixture was shaken for 2 hours. Next, the solvents were drained off and the resin was washed with NMP. The procedure was repeated once more and the solvents were drained off and the resin was subsequently washed with NMP and DCM.
General procedure for fluorous capping: To the suspension of resin bound peptide (1 eq) in NMP was added (1H,1H,2H,2H)-perfluoroundecanoic acid (5 eq), HCTU (5 eq) and DIPEA (10 eq). The mixture was shaken for 1 hour. Next, solvents were drained off and the resin was subsequently washed with NMP and DCM.

General procedure for introduction of Boc: To the suspension of resin bound peptide (1 eq) in NMP was added di-*tert*-butyl-dicarbonate (Boc_2O, 5 eq) and DIPEA (5 eq), the mixture was shaken for 30 minutes. Next, solvents were drained off and the resin was subsequently washed with NMP and DCM.

General procedure for cleaving the peptide from the resin: To the resin bound peptide (10 μmol, 1 eq) in a cartridge with a filter was added a cocktail of TFA/TIS/H_2O (95/2.5/2.5). The mixture was kept for 1 hour with occasion swirling. Next, the solvents were filtered into cold diethyl ether. The mixture was centrifuged, the diethyl ether was removed, and precipitates were washed with diethyl ether and air dried.

General procedure for fluorous solid phase extraction (FSPE): A FSPE cartridge preloaded with 2 g of fluorous silica gel was eluted with acetonitrile (10 ml) and acetonitrile/H_2O/TFA (20/79.9/0.1, 15 ml). The crude peptide was dissolved in the minimum amount of water and acetonitrile was added to help in dissolution if necessary, followed by loading to the cartridge. The cartridge was eluted with acetonitrile/H_2O/TFA (20/79.9/0.1, 10 ml) and acetonitrile/H_2O/TFA (50/49.9/0.1, 15 ml) to elute the target peptide. Next, fluorous capped truncated sequences were eluted with acetonitrile/H_2O/TFA (90/9.9/0.1, 15 ml) and acetonitrile/TFA (99.9/0.1, 10 ml).

Gly-Glu-Pro-Lys-Pro-Ala-Gly-NH_2 (10): Resin bound peptide **10** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. At pentamer stage after deprotection, 25% of the resin was taken out and capped with azidoacetyl according to the general procedure as described above. The two resins were remixed, and next amino acid was coupled and Fmoc was deprotected. Again 25% of the resin was taken out and capped with azidoacetyl. The two resins were remixed, and synthesis was continued according to the general procedure. Boc-Gly-OH was coupled as the last amino acid residue. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (40 mg, Initial loading = 0.34 mmol/g, final loading 0.30 mmol/g, 11.9 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **10** (1300 μg, 1.99 μmol, 17%). LCMS: 00-20% B, RT = 4.51 min; ESI-MS: $[M+H]^+$ 654.6 (calc. 654.7).

Val-Glu-Ala-Ala-Ile-Asp-Tyr-Ile-Asp-Ala-NH_2 (19): Resin bound peptide **19** was prepared manually using peptide synthetic cycle as described above. At tetramer stage after deprotection, 20% of the resin was taken out and capped with azidoacetyl according to the general procedure as described above. The two resins were remixed, and standard cycle was followed. Next amino acid was coupled and Fmoc was deprotected. At nonamer stage after deprotection, again 20% of the resin was taken out and capped with azidoacetyl. The two resins were remixed, and synthesis was continued according to the general procedure. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (30 mg, Initial loading = 0.58 mmol/g, final loading 0.37 mmol/g, 11.2 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **19** (900 μg, 0.84 μmol, 7%). LCMS: 00-50% B, RT = 4.59 min; ESI-MS: $[M+H]^+$ 1078.5 (calc. 1078.2).

Ser-Ser-Lys-Lys-Ser-Gly-Ser-Tyr-Ser-Gly-Ser-Lys-Gly-Ser-Lys-Arg-Arg-Ile-Leu-OH (20): Resin bound peptide **20** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (25 mg, Initial loading = 0.26 mmol/g, final loading 0.18 mmol/g, 4.54 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **20** (800 μg, 0.4 μmol, 9%). LCMS: 00-90% B, RT = 3.88 min; ESI-MS: $[M+H]^{2+}$ 1007.7 (calc. 1007.7), $[M+H]^{3+}$ 672.1 (calc. 672.1).

Glu-Pro-Leu-Thr-Ser-Leu-Thr-Pro-Agr-Abu-Asn-Thr-Ala-Trp-Asn-Agr-Leu-Lys-Leu-OH (21): Resin bound peptide **21** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (17 mg, Initial loading = 0.26 mmol/g, final loading 0.18 mmol/g, 3.0 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **21** (1400 μg, 0.64 μmol, 21%). LCMS: 00-90% B, RT = 4.55 min; ESI-MS: $[M+H]^{2+}$ 1099.0 (calc. 1098.8), $[M+H]^{3+}$ 733.2 (calc. 732.9).

Abu-Asp-Ser-thr-Leu-Arg-Leu-Abu-Val-Gln-Ser-Thr-His-Val-Asp-Ile-Arg-Thr-Leu-Glu-Asp-Leu-Leu-Met-Gly-Thr-Leu-Gly-Ile-Val-Pro-Abu-Ile-Abu-Ser-Gln-Lys-Pro-OH (22): Resin bound peptide **22** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **22** in 7% yield. LCMS: 10-90% B, RT = 8.36 min; ESI-MS: $[M+H]^{3+}$ 1363.3 (calc1363.4).

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CHAPTER

6

Summary and future prospects

An efficient organic synthesis of fragments of biopolymers and analogues thereof cannot be reached without a proper use of protective groups. In oligonucleotide and oligopeptide synthesis the applied protective groups generally ensure the regioselective introduction of the phosphodiester and peptide linkages between the monomeric nucleosides and amino acids respectively, as well as minimization of unwanted side reactions en route to the target compounds. Thanks to the developed protective groups and improved coupling methods to introduce phosphate and peptide linkages, DNA and peptide fragments consisting of dozens of monomeric residues can be prepared with the aid of fully automated solid phase procedures. Despite these advances the search for new protective groups in the field of nucleic acid and peptide chemistry still goes on. For instance, improvement of the overall yield in the synthesis of RNA fragments and the synthesis of

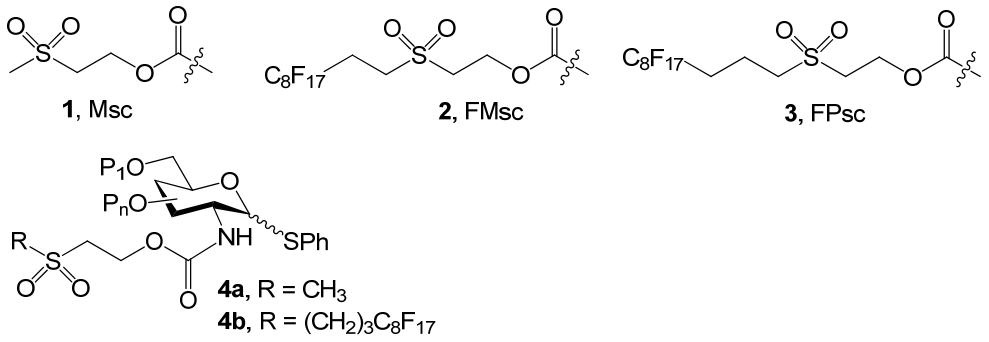
nucleic acid peptide conjugates may benefit from the development of new tailor made protective groups. Compared with the present state of the art in nucleic acid and peptide chemistry the synthesis of oligosaccharides has been relatively slow to mature, [1] which can be explained by the increased structural complexity of carbohydrates. Carbohydrates may occur either as straight or branched chains and may contain various monosaccharides differing in the number, nature and configuration of the substituents. Moreover, the glycosidic linkage between the constituting monosaccharides can occur in two diastereomeric forms. In the synthesis of naturally occurring and artificially designed oligosaccharides the protective group strategy[2] and the glycosylation procedure[3] play a decisive role to attain a productive and stereoselective glycosylation reaction. In this respect it is of interest to note that the protective groups installed on the glycosylating partners (donor and acceptor), not only dictate the regioselectivity[4] and the yield of the glycosylation reactions but also influence the stereoselective outcome.[5]

This thesis reports on the development of new protective groups that can be applied for the synthesis of biopolymer fragments with a focus on oligosaccharides. **Chapter 1** reviews recent advances on protective groups and protective group manipulations in the field of carbohydrate chemistry. With the objective to diminish the efforts to prepare sufficient amounts of suitably protected monosaccharides, several groups have reported on the development of one-pot protocols, entailing up to five reaction steps. One procedure starts from anomerically protected per-silylated carbohydrate monomers and gave access to a wide array of differentially protected monosaccharide donors and acceptors. A lot of attention has been paid to the design of protective groups for the C-2 hydroxyl, that are able to induce the stereoselective formation of 1,2-*cis* glycosidic bonds. An impressive example is presented by the development of two C-2 OH protecting groups capable of promoting the formation of 1,2-*cis* glycosidic bonds by neigbouring group participation, reported by the group of Boons. The discovery of Crich and co-workers that 4,6-benzylidene mannosyl donors react in a highly β-selective fashion was followed by studies on the influence of protecting groups at the C-2 and C-3 positions of these donors and led to the development of various propargyl ethers as minimally intrusive hydroxyl protecting groups. Silyl protecting groups are becoming increasingly popular in carbohydrate chemistry as exemplified by the introduction of the 4,6-di-*tert*-butylsilylene

(DTBS) as a more acid stabile alternative for the usual cylic ketals and acetals. A row of new *trans*-directing protecting groups for the glucosamine nitrogen function has also been reported. Contrary, Kerns and co-workers introduced oxazolidinone protected glucosamine donors that stereoselectively provided 1,2-*cis* linked products. Subsequent studies revealed that the stereochemical outcome of 2,3-oxazolidinone-*N*-acetyl and 2,3-oxazolidinone-*N*-benzyl protected glucosamine donors could be controlled by the (Lewis)-acidity of the employed activator systems.

Several types of protecting groups were published that not only mask a specific functional group on the carbohydrate core, but also add an extra functionality to the carbohydrate building block. For instance, protective groups have been developed which impart colour to the substrate, allowing an easy detection during purification procedures or monitoring of the glycosylation efficiency during automated synthesis. A number of protective groups provided with purification handles became available through the development of fluorous chemistry and ionic liquids.

Figure 1: The Msc protecting group and fluorous derivatives thereof.



Chapter 2 describes the first application of the methylsulfonylethoxycarbonyl (Msc) group (**1**, Figure 1) as protecting group for hydroxyl functions in oligosaccharide synthesis. The Msc group can be introduced using conditions, commonly used to install the 9-fluorenylmethyl carbonate (Fmoc). Contrary, the Msc group is less lipophilic, less bulky and slightly more stable than the Fmoc group. The Msc group can be cleaved *via* β-

elimination using mildly basic conditions and is orthogonal with the levulinoyl (Lev) group. The Msc group was used in donor and acceptor glycosides and was stable during glycosylation reactions. When the Msc group was placed at the C-2 hydroxyl, it provided anchimeric assistance while unwanted orthoester formation was prevented.

It would be interesting to evaluate the Msc group as an amino protecting group in oligosaccharide synthesis. Protection of the glucosamine nitrogen function with the Msc group would afford a glycosyl donor (e.g. **4a**, Figure 1), having a sterically unbiased, participating group.

In bioorganic chemistry fluorous solid phase extraction (FSPE) has emerged a new purification method. In this context a number fluorous protecting groups to facilitate the purification of oligopeptides, (oligo)nucleotides and oligosaccharides is reported. **Chapter 3** deals with the evaluation of fluorous counterparts of the Msc group as purification handles in carbohydrate chemistry. The [1H, 1H, 2H, 2H]-perfluorodecylsulfonylethoxycarbonyl (FMsc, **2**, Figure 1) group, known as a fluorous protective group for amines was found to be too labile for the protection of hydroxyl functions of carbohydrates. Increasing the distance between the sulfonyl functionality and the perfluoro moiety by an additional methylene group led to the development of the [1*H*,1*H*,2*H*,2*H*,3*H*,3*H*]-perfluoroundecylsulfonylethoxycarbonyl (FPsc, **3**, Figure 1) group as a sufficiently stable hydroxyl protecting group. The FPsc group was applied in the assembly of a trisaccharide and found to be orthogonal with levulinyl (Lev) group.

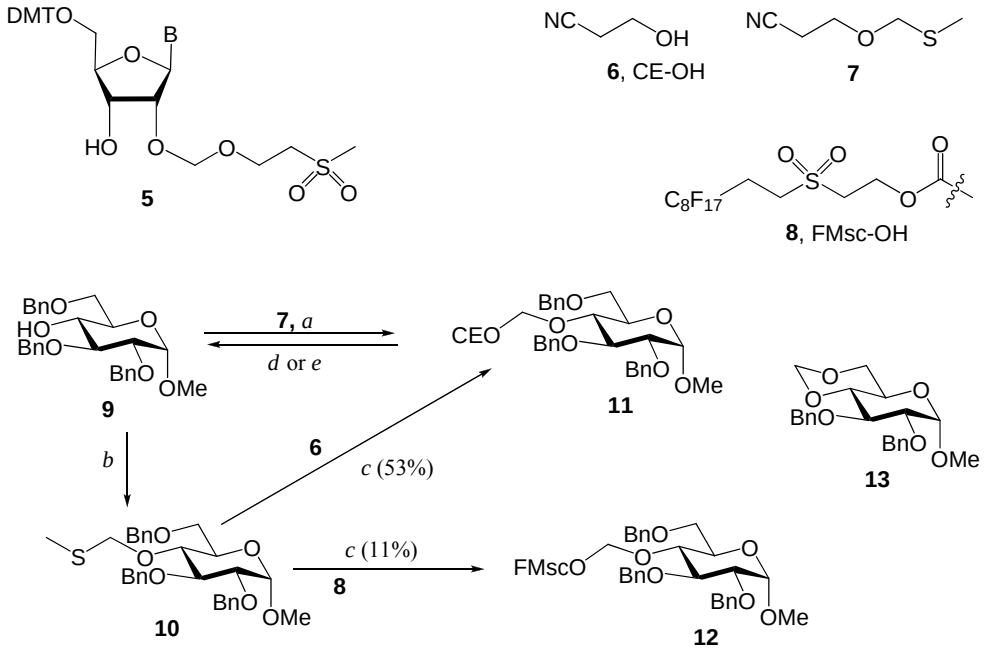
These results indicate that it is worthwhile to investigate whether the FPsc group can be applied for the purification of oligosaccharides and glycopeptides that are assembled by solid phase procedures. In this context the FPsc group can also be used as purification handle at amino functions such as in glucosamine derivative **4b** (Figure 1). In addition it would be interesting to investigate whether the FPsc can function as a fluorous handle to facilitate the purification of different classes of biomolecules, such as oligonucleotides and conjugates thereof.

Chapter 4 describes the methylsulfonylethoxymethyl (Msem) group as a new hydroxyl protecting group, that increases the palette of ether based protecting groups in carbohydrate chemistry. Several methods for the introduction of the Msem group were investigated. The Msem group can be introduced at primary and secondary hydroxyl

functions of *O*-glycosides using the thiomethylether of 2-(methylsulfonyl)ethanol and a thiophilic activator. Diol thioglycosides can be selectively protected by the conversion of the hydroxyl functions into dibutylstannylidene acetals followed by reaction with (methylsulfonylethoxy)methyl chloride (Msem-Cl). The Msem group proved to be relatively base stabile and could be easily removed by a catalytic amount of TBAF in the presence of piperidine as scavenger. Application of the Msem group in the synthesis of an all *cis*-linked 1,3-*O*-mannotrioside showed that this group is sterically unbiased and does not provide remote participation.

The properties of the Msem group indicate that is valuable to investigate whether the Msem group can function as a protecting group for the C-2-OH function in RNA synthesis. Accordingly, protected nucleoside **5** (Figure 2) may be accessible by the synthetic route devised for the introduction of the cyanoethoxymethyl group at the same position of RNA building blocks.[6]

Figure 2: The Msem protecting group, its fluorous derivatives and the cyanoethoxymethyl group.



Reagents and conditions; *a*) NIS, TMSOTf, DCM, -20 ºC to RT, 24h, 72%; *b*) NaH, Methylthiomethylchloride, DMF, 1h, 73%; *c*) IDCP, DCM, RT, 2h; *d*) DBU, DMF, 10h, 93%; *e*) THF, TBAF, 1h, 96%.

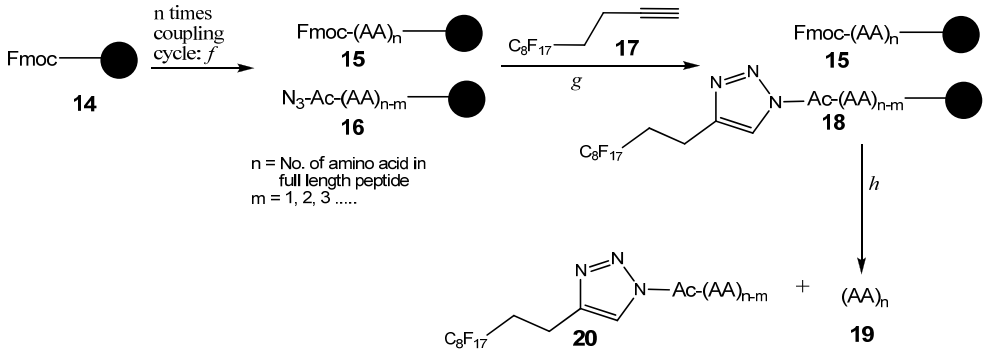
Conversely, it would be interesting to explore protecting groups that are specifically developed to improve RNA synthesis for their application in carbohydrate chemistry. Preliminary results on the use of the cyanoethoxymethyl group in carbohydrate chemistry are depicted in figure 2. The required 3-(methylthiomethoxy)propanenitrile **7** was prepared in 60% yield by treating cyanoethanol with dimethylsulfoxide, acetic acid and acetic anhydride. Condensation of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **9** with thiomethyl ether **7** under the agency of *N*-iodosuccinimide (NIS) and trimethylsilyltriflate (TMSOTf) produced cyanoethoxymethyl protected glucoside **11** in 72% yield (Figure 2). Alternatively, 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside **9** was converted into methylthiomethyl ether **8** by treatment with sodium hydride and methylthiomethyl chloride (MTM-Cl) in DMF. Reaction of **10** and cyanoethanol under influence of IDCP (4 equivalents) as activating agent led to the isolation of the desired monosaccharide **11** (53% yield) together with side product **13**. The cyanoethoxymethyl group could be removed from 2,3,6-tri-*O*-benzyl-4-*O*-cyanoethoxymethyl-α-D-glucopyranoside **11** by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 6h at room temperature to give **9** in 93% yield (Figure 2). Alternatively, treatment of **11** with tetrabutylammonium fluoride (TBAF, 0.1 equivalents) for 30 minutes led to the removal of the cyanoethoxymethyl group in 96% yield. The development of a fluorous version of the methylsulfonylethoxymethyl (Msem) group may be also be advantageous, as the Msem group is a non-participating and more base stabile in comparison with the perfluoroundecylsulfonylethoxycarbonyl (FPsc, see Chapter 3) group. In a preliminary experiment fluorous glucoside **12** could be prepared in 11% yield.

Two approaches can be distinguished for the application of fluorous techniques to purify fragments of biopolymers prepared with the aid of solid phase procedures In the first approach a suitable fluorous protecting group is used and installed in the final stage of the solid phase synthesis to give the fluorous target oligomer. Examples of such fluorous protecting groups are discussed in Chapter 1 and 3. In the second approach intermediate oligomers that resist elongation en route to the target compound are capped after every condensation step in the solid phase cycle with a fluorous reagent, making the truncated and deletion sequences fluorous and leaving the target compound untouched. **Chapter 5**

narrates the idea of a new two-step fluorous capping procedure. Five oligopeptides were prepared by regular solid phase peptide synthesis, in which the standard capping procedure was replaced by treatment with azidoacetic acid, HCTU and Dipea. After the completion of the synthesis of the target oligopeptide, the azides in the truncated and deletion sequences were reduced to amines and subsequently capped with (1H,1H,2H,2H)-perfluoroundecanoic acid. The fluorous sequences were separated from the target peptide by fluorous solid phase extraction (FSPE) or by Fluorous HPLC (F-HPLC).

It is envisaged that the number of reaction steps in this fluorous capping strategy can be reduced by replacing the azide reduction and ensuing capping with a fluorous carboxylic acid by a Huisgen 1,3-dipolar cycloaddition (click reaction) with a suitable alkyne.[7]

Figure 3: fluorous capping strategy using click chemistry.

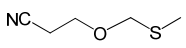


Reagents and conditions; *f*) i- Piperdine (20% in NMP)15 min.; ii- Fmoc-amino acid, HCTU, Dipea, NMP; iii- Azidoacetic acid, HCTU, NMP, 20 min; *g*) $CuSO_4$, Sodium ascorbat; *h*) i- TFA, TIS, H_2O, 45 min; ii- FSPE or F-HPLC.

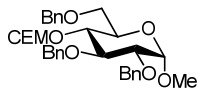
Following the same coupling cycle as described in Chapter 5, immobilized target oligopeptide **15** and a number of azido functionalized deletion sequences **16** will be obtained at the end of the synthesis (Figure 3). With the aid of alkyne **17** the deletion sequences **16** will be made fluorous by a copper catalyzed click reaction. Removal of the protecting groups and cleavage from the solid support will provide target oligopeptide **19** and fluorous sequences **20**. Target peptide **19** can be separated from the fluorous impurities using Fluorous HPLC.

Experimental:

General: Dichloromethane was refluxed with P_2O_5 and distilled before use. All other chemicals (Acros, Fluka, Merck, Fluorous Technologies Inc.) were used as received. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). Compounds were visualized by UV absorption (245 nm), by spraying with an aqueous solution of $KMnO_4$ (20%) and K_2CO_3 (10%), by spraying with 20% H_2SO_4 in ethanol or by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10g/L) in 10% $H_2SO_{4\ (aq)}$ followed by charring at ~150 ºC. IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}. Optical rotations were measured on a Propol automatic polarimeter. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 MHz and 100 MHz respectively), AV 500 (500 MHz and 125 MHz respectively) or DMX 600 (600 MHz and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ unless stated otherwise. Chemical shift are relative to tetramethylsilane and are given in ppm. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High resolution mass spectra were recorded on a LTQ-Orbitrap (thermo electron).



((Cyanoethoxy)methyl)methylsulfane (7) : To a solution of cyanoethanol **6** (4.16 ml, 58.4 mmol) in DMSO (18 ml, 234 mmol, 4 eq) was added acetic acid (7 ml, 117 mmol, 2 eq) and acetic anhydride (12 ml, 117 mmol, 2 eq). The reaction mixture was stirred for 48 hours. The mixture was neutralized by careful addition of $NaHCO_{3\ (s)}$, extracted using a large excess of EtOAc, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to afford **7** (5.54 g, 30.0 mmol, 60%) as a yellow ~~a~~ oil. TLC (75% EtOAc in PE): R_f = 0.75; IR (neat, cm^{-1}): 730, 1129, 1286; ^1H NMR (400 MHz, ($CDCl_3$) δ = 2.16 (s, 3H, -$CH_2SC\underline{H}_3$), 2.66 (t, 2H, *J* = 6.0 Hz, $NCC\underline{H}_2CH_2OCH_2SCH_3$), 3.73 (t, 2H, *J* = 6.0 Hz, $NCCH_2C\underline{H}_2OCH_2SCH_3$), 4.68 (s, 2H, $NC(CH_2)_2OC\underline{H}_2SCH_3$); ^{13}C NMR (100 MHz, ($CDCl_3$) δ = 13.7 (-$CH_2S\underline{C}H_3$), 18.6 ($NC\underline{C}H_2CH_2OCH_2SCH_3$), 62.2 ($NCCH_2\underline{C}H_2OCH_2SCH_3$), 75.1 ($NC(CH_2)_2O\underline{C}H_2SCH_3$); HRMS $[M+Na]^+$ calculated for C_5H_9OSN 154.02971, found 154.02962.

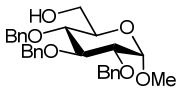


Methyl 2,3,6-tri-*O*-benzyl-4-*O*-cyanoethoxymethyl-α-D-glucopyranoside (11):

Method I: A solution of Methyl 2,3,6-tri-*O*-benzyl-4-*O*-methylthiomethyl-α-D-glucopyranoside **10** (0.203 g, 0.387 mmol) and cyanoethanol (66 µl, 0.968 mmol, 2.5 eq) in DCM (7.7 ml, 0.05M) was stirred over activated MS3Å for half an hour before Iodo dicollidine perchlorate (0.724 g, 1.548 mmol, 4eq) was added in dark. The mixture was stirred in dark for 24 hours. The reaction mixture was quenched with NH_4Cl, filtered, diluted with DCM and washed with $Na_2S_2O_3$. The aqueous layer was extracted with DCM thrice, the combined organic layer was washed with NH_4Cl, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to get compound **11** (0.113 g, 0.206 mmol, 53%);

Method II: A solution of methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside **9** (70 mg, 0.15 mmol) and reagent **7** (30 mg, 0.23 mmol, 1.5 eq) in DCM (10.4 ml, 0.05 M) was stirred over activated MS3Å for 30 minutes before *N*-iodosuccinimide (40 mg, 1.85 mmol, 1.2 eq) was added. The mixture was cooled to -20 ºC followed by the addition of trimethylsilyltrifluoromethanesulfonate (10% in DCM, 55 µl, 30 µmol, 0.2 eq). The mixture was stirred for 2 hours. The reaction mixture was quenched with triethylamine (5 eq), filtered, diluted with DCM and washed with $Na_2S_2O_{3\ (aq)}$. The aqueous layer was extracted with DCM thrice, the combined organic layers were dried over $MgSO_4$, filtered, concentrated and purified by silica gel column chromatography to get **11** (59 mg, 0.11 mmol, 72%).

TLC (50% EtOAc in PE): R_f = 0.6; $[\alpha]_D^{22}$: +59.0º (c = 0.4`, DCM); IR (neat, cm^{-1}): 689, 699, 738, 954, 1016, 1089, 1118, 1281, 1378; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.15-2.27 (m, 2H, NCC<u>H</u>$_2$CH$_2$OCH$_2$O-), 3.39 (s, 3H, OMe), 3.50 (mH-2 and NCCH$_2$C<u>H</u>$_2$OCH$_2$O-), 3.60 (m, 3H, H-4 and 2xH-6), 3.71 (m, 1H, H-5), 3.87 (t, 1H, *J* = 9.2 Hz, H-3), 4.62 (m, 6H, H-1, NC(CH$_2$)OCH<u>H</u>O- and 4xCH<u>H</u> Bn), 4.77 (m, 2H, NC(CH$_2$)OCH<u>H</u>O- and CH<u>H</u> Bn), 5.01 (d, 1H, *J* = 11.2 Hz, CH<u>H</u> Bn), 7.23-7.33 (m, 15H, H arom); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.4 (NC<u>C</u>H$_2$CH$_2$OCH$_2$O-), 55.2 (CH$_3$ OMe), 63.1 (NCCH$_2$<u>C</u>H$_2$OCH$_2$O-), 68.7 (C-6), 69.7 (C-5), 73.1 (CH$_2$ Bn), 73.3 (CH$_2$ Bn), 75.4 (CH$_2$ Bn), 75.5 (C-4), 79.9 (C-2), 81.1 (C-3), 96.5 (NC(CH$_2$)O<u>C</u>H$_2$O-), 97.8 (C-1), 117.6 (<u>C</u>N), 127.5-129.8 (CH arom), 137.8 (C_q Bn), 138.3 (C_q Bn); HRMS $[M+Na]^+$ calcd for $C_{32}H_{37}NO_7Na$ 570.24622 was found 570.24579.



Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (9) (Cleavage of Msem from 11):

Method I: To a solution of **11** (42 mg, 77 µmol) in DMF (0.8 ml, 0.1 M) was added DBU (0.067 M in DMF, 120 µl, 7.7 µmol, 0.1 eq) and the reaction mixture was stirred for 6 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3,6-*tri*-*O*-benzyl-α-D-glucopyranoside **9** (33 mg, 71 µmol, 93%).

Method II: To a solution of **11** (52 mg, 95 µmol) in THF (1.0 ml, 0.1 M) was added TBAF (0.1 M in DMF, 100 µl, 9.5 µmol, 0.1 eq) and the reaction mixture was stirred for 1 hours. The reaction mixture was neutralized with $NH_4Cl_{(aq)}$, diluted with EtOAc, washed with $NH_4Cl_{(aq)}$, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography to afford methyl 2,3,6-*tri*-*O*-benzyl-α-D-glucopyranoside **9** (42 mg, 91 µmol, 96%).

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خلاصہ (Summary in Urdu)

کاربو ھائدیٹ کیمسٹری(کیمیائے نشاستہ)میں نئے پروٹیکٹو گروپس (حفاظتی بند) اوردو مراحل پر مشتمل فلورس کیپنگ (غیر فعالیت)

آرگینک کیمسٹری (نامیاتی کیمیا) میں تمام تر ترقی اور پیش قدمی کے باوجود کاربوہائیڈریٹ کیمسٹری یا شگر کیمسٹری میں ترقی اور بہتری کی بہت زیادہ گنجائش موجود ہے۔کاربوہائیڈریٹ کیمسٹری (کیمیائے نشاستہ)، پروٹین اور نیوکلیوٹائد کیمسٹری کے برعکس زیادہ پیچیدہ ہے۔قدرتی طور پر پائے جانے والے اور مصنوعی طور پر ترتیب دیے گئے اولیگوسیکرائڈ بنانے کے لیے دو عوامل بہت اہم کردار ادا کرتے ہیں۔

1۔پروٹیکٹو گروپس (حفاظتی بند) کا مناسب استعمال

2۔گلائی کوسیلیشن کی حکمت عملی

عام طور پر ہر مونوسیکرائڈ میں پانچ تک ہائڈروآکسل ہوتے ہیں۔اولیگوسیکرائڈ کی تیاری کیلیے ان الکوحلز کی موثر ،امتیازی اور قابل مسابقت پروٹیکشن (حفاظتی بند باندھنا) ضروری ہے۔گلائی کوسیلیشن میں حصہ لینے والے دونوں یونٹ یعنی ڈونر اور ایکسیپٹر پر پروٹیکٹو گروپس کا انتخاب بہت اہم ہے۔کیونکہ یہ نہ صرف مرکب کی رجعت پذیری پر اثر انداز ہوتے ہیں بلکہ ریجیو سیلیکٹیویٹی (گلائی کوسیلیشن کہاں ہوگی) اور سٹیریو سیلیکٹیویٹی (بننے والے بانڈ کی سمت) کا تعین بھی کرتے ہیں اور ہونے والے کیمیائی عمل کی پیداوار اور کامیابی کا دارومدار بھی ان پروٹیکٹو گروپس کے انتخاب اور قسم پر ہوتا ہے۔لہذا اولیگوسیکرائڈ کی تیاری کے لیے پروٹیکٹو گروپس مرکزی حیثیت رکھتے ہیں۔اس لیے بہتر خصوصیات کے حامل نئے پروٹیکٹو گروپس کی تیاری کاربوہائیڈریٹ کیمسٹری کے اہم ترین موضوعات میں سے ایک ہے۔یہ مقالہ اولیگوسیکرائڈ کی تالیف کے لیے نئے پروٹیکٹو گروپس کی تیاری کے بارے میں ہے۔

باب نمبر 1 میں شگر کیمسٹری کے میدان میں حالیہ پیش قدمیوں کے بارے میں بات کی گئی ہے۔ مونوسیکرائڈ کے ہر ہائڈروآکسل کی سلسلہ وار، یک ظروفی، ریجیوسیلیکو (انتخاب جائے حفاظت) پروٹیکشن کے مختلف طریقہ ہائے کار ہر مرحلے کے بعد کی جانے والی تخلیص کے سدبات کے سبب بنے ہیں جو عام طور پر وقت کے ضیاں کا سبب بنتے ہیں۔ ڈونر اور ایکسیپٹر کے باہمی عمل سے بننے والے گلائیکوسیڈک بانڈ 1,2-ٹرانس ہوتے ہیں یا 1,2- سس۔ اگر ہم کسی بھی مونوسیکرائڈ ڈونرز کے کاربن نمبر 2 پر موجود ہائڈروآکسل پر شراکت دار گروپ کا استعمال کرتے ہیں تو 1,2-ٹرانس گلائیکوسیڈک بانڈ نہایت آسانی سے بنائے جاسکتے ہیں. 1,2- سس گلائیکوسیڈک بانڈ بنانے کے لیے کاربن نمبر 2 پر کائرل معاونین یا مسلح کرنے والے شراکت دار گروپس استعمال کیے گئے۔ جو بننے والے عشری آئن کو ٹرانس ہیئت دیتے ہیں۔ جو بعد ازاں سس بانڈ کا موجب بنتا ہے۔ بیٹا مینو سائیڈ تالیف کرنا شگر کیمسٹری کے مشکل ترین کاموں میں سے ایک ہے۔ اس مقصد کے حالیہ برسوں میں 4,6-بینزائلیڈین سے ڈھکے ہوئے مینوز ڈونز استعمال کرنے کا رواج رہا۔ ان کے کاربن نمبر 2 اور 3 پر مختلف اقسام کے ایتھر استعمال کیے گئے۔ اسی طریقہ کار کو ٹھوس حالت (سالڈ فیز) میں بیٹا مینو سائیڈز کی تیاری کے لیے بھی استعمال کیا گیا۔ عام طور پر کاربن نمبر 4,6 کی پروٹیکشن کے لیے استعمال ہونے والے کیٹل اور ایسیٹل کے تیزاب کے زیر اثر غیر مستحکم ہونے کے پیش نظر 4,6-ڈائی-ٹرشری-بیوٹائل سیلین متعارف کروائے گئے۔ جو وقت کے ساتھ مقبولیت حاصل کر رہے ہیں۔ عام طور پر شگر امین کے امین کو پروٹیکٹ کرنے والے گروپس نایاب ہیں۔ اس مقصد کے لیے آکسازولیڈینون کو بطور محافظ امین استعمال کیا گیا۔ نسبتاً زیادہ تیزابی حالات میں آکسازولیڈینون والے ڈونز کی مدد سے 1,2- سس گلائیکوسیڈک بانڈ بنایا گیا۔ ہائڈروآکسل گروپ کو غیر فعال بنانے کے علاوہ، پروٹیکو گروپ چند دیگر اہم کردار بھی ادا کرتے ہیں۔ ایسے پروٹیکو گروپس بنائے جاچکے ہیں۔ جو متعلقہ مادے میں ایک رنگ پیدا کرنے کا موجب بنتے ہیں۔ اس رنگ کی مدد سے عمل کے دوران اس کے بارے میں معلومات حاصل کرنا نسبتاً آسان ہوتا ہے۔ دوسرے یہ سلیکا جیل کروماٹوگرافی کو بھی نہایت سہل بنا دیتے ہیں۔ پروٹیکو گروپس بعض اوقات عمل تخلیص میں بھی مدد کرتے ہیں۔ مثلاً بذریعہ فلورس جامد حالت والا عمل کشید یا بذریعہ فلورس مائع مائع والا عمل کشید۔ عمل تخلیص کو بذریعہ آئنی مائع لنگر اندازی یا ٹھم پسند پروٹیکو گروپس کی مدد سے بھی سہل بنایا جاسکتا ہے۔

میتھائل سلفونائل ایتھوکسی کاربونائل (ایم ایس سی) گروپ کو پہلی بار اولیگوسیکرائڈ کی تالیف کے لیے، ہائڈروآکسل گروپ کو ڈھانپنے کے لیے استعمال کا تذکرہ **باب نمبر 2** میں کیا گیا ہے۔ ایم ایس سی گروپ کا ایم ایس سی کلورائڈ اور پیریڈین کی موجودگی میں کسی بھی ہائڈروآکسل گروپ پر متعارف کروایا جاسکتا ہے۔ جو کہ کاربونیٹ کی قسم کے پروٹیکو گروپ کو لگانے کی معیاری شرائط اطلاق ہیں۔ ایم ایس سی کا جب بہت زیادہ مقبول ایف موک کے ساتھ تقابلہ جائزہ کیا گیا۔ تو یہ عقدہ کھلا کہ ایم ایس سی، ایف موک کی نسبت کم ٹھم پسند اور کم ضخیم ہے۔ یہ

ایف موک کے مقابلے میں زیادہ قیام پذیر ہے۔ایم ایس سی، لیوگروپ کے ساتھ مکمل طور پر آرتھوگونل ہے۔ایم ایس سی اور لیوودونوں کو ایک دوسرے کی موجودگی میں اتارا جاسکتا ہے۔ایم ایس سی کو دونوں ڈونز اور ایکسیپٹر میں استعمال کیا گیا۔اور ان کی مدد سے کی گئی گلائیکوسیلیشن سے حوصلہ افزا نتائج برآمد ہوئے۔جب ایم سی کو کاربن نمبر 2 پر لگایا گیا تو اس نے شراکت کا فرض بھی بخوبی نبھایا۔اس کی شراکت داری کا ایک اور مفید پہلو یہ ہے کہ اس کی مدد سے آرتھوایسٹر نہیں بن سکتا، جو عام طور پر شراکت دار گروپس کا ایک منفی پہلو ہے۔

باب نمبر 3 میں فلورس کیمسٹری کے فوائد سے مستفید ہونے کے لیے ایم ایس سی کا فلورس مماثل (1ایچ، 1ایچ، 2ایچ، 2ایچ، 2ایچ) پر فلوروڈیسائل سلفونائل ایتھوکسی کاربونائل (ایف ایم ایس سی) کو شگر کیمسٹری میں استعمال کیا گیا۔جو نسبتاً غیر قیام پذیر ثابت ہوا۔اس کی قیام پذیری کو مزید مستحکم کرنے کے لیے ایک نیا پروٹیکٹو گروپ (1ایچ، 1ایچ، 2ایچ، 2ایچ، 3ایچ، 3ایچ) پر فلوروڈیسائل سلفونائل ایتھوکسی کاربونائل (ایف پی ایس سی) تیار کیا گیا۔دونوں ایف ایم ایس سی اور ایف پی ایس سی کو عام کاربونیٹ متعارف کروانے والے طریقوں کے مطابق نہایت آسانی سے کسی بھی ہائڈروآکسل پر لگایا جاسکتا ہے۔ان گروپس کو ہلکے اساسی حالات میں اتارا جاسکتا ہے۔یہ گروپ تیزاب کے زیر اثر ہونے والی گلائیکوسیلیشن میں قیام پذیر تھے۔ان دونوں گروپس کا استعمال کا دوسرا فائدہ یہ تھا کہ ان کے حامل مرکبات کو فلورس جامد حالت والے عمل کشید کے ذریعے خالص بنایا جاسکتا ہے۔مزید برآں ایف پی ایس سی کی مدد سے ایک ٹرائی سیکرائڈ بنایا گیا۔جس میں فلورس جامد حالت والے عمل کشید سے استفادہ کیا گیا۔اس ٹرائی سیکرائڈ کی تیاری کے دوران ایف پی ایس کو لیوگروپ کے ساتھ آرتھوگونل پایا گیا۔

1,3-میانومینوسائڈز کی تیاری کے ایک نرالے پروٹیکٹو گروپ میتھائل سلفونائل ایتھوکسی میتھائل (ایم سیم) کے اطلاق کا ذکر **باب نمبر 4** میں کیا گیا ہے۔(میتھائل سلفونائل ایتھوکسی میتھائل) میتھائل تھائیو ایتھر استعمال کر کے ایم سیم کو ان حالات کے تحت لگایا جاسکتا ہے۔جو عام طور پر گلائیکوسیلیشن کے لیے متعارف کروائی گئی ہیں۔دوالکوحل کے حامل تھائیو گلائیکوسائڈ پر ایک پرمنتخب طریقے سے ایم سیم لگانے کے لیے کیمیائی ٹن استعمال کیا گیا۔اس مقصد کے لیے میتھائل سلفونائل ایتھوکسی میتھائل کلورائڈ (ایم سیم کلورائڈ) استعمال کیا گیا۔جب ایم سیم کی مدد سے مینوسائڈ کے کاربن نمبر 3 پر موجود ہائڈروآکسل کو ڈھانپا گیا، تو ان ڈونز کی مدد سے میانومینوسائڈز کی پیداوار حوصلہ افزا تھی۔ایم ایس سی (کاربونیٹ) اور ایم سیم (ایتھر) کو مینوسائڈ کے کاربن نمبر 3 پر متعارف کروا کے سٹیریوسلیکٹیویٹی کا تقابلہ کیا گیا۔ایم ایس سی والا ڈونز ٹرانس ڈائی سیکرائڈ کا سبب بنتا ہے۔جبکہ اس کے برعکس ایم سیم والا ڈونز سس ماحصل بناتا ہے۔جس سے نتیجہ نکلتا ہے کہ ایم ایس سی دور سے شراکت داری سے شراکت داری کرتا ہے۔جبکہ ایم سیم اس خوبی سے عاری ہے۔ایم سیم کی مدد سے 1,3-میانومینو ٹرائی سیکرائڈ بھی بنایا گیا۔

ایسے پیپٹائڈ جن میں امینو تیزابوں کی زنجیر زیادہ لمبی ہوجاتی ہے۔ان میں منقطوع زنجیروں کے پائے جانے کا امکانات زیادہ

ہوتے ہیں۔اور اصل پیپٹائڈ کی ان ٹوٹے سلاسل سے تخلیص بہت مشکل، وقت کش اور تکلیف دہ امر ہے۔اس عمل کو سہل بنانے کے لیے **باب نمبر 5** میں دو مراحل پر مشتمل فلورس غیر فعالیت (کیپنگ) کا تصور پیش کیا گیا ہے۔عام طور پر استعمال ہونے والے ٹھوس حالت میں تالیف پیپٹائڈ کے طریقہ کار کو استعمال کرتے ہوئے قدرتی طور پر پائے جانے والے یا مصنوعی طور پر ترتیب دیے گئے پانچ پیپٹائڈ کو تیار کیا گیا۔عام طریقہ کار میں واحد دو بدل عمومی عامل غیر فعالیت کی ازیڈوایسیٹائل کے ساتھ تبدیلی تھی۔زنجیر کی مکمل تیاری کے بعد ٹوٹے سلاسل پر موجود زائد کو بذریعہ عمل تخفیف امین میں ڈھالا گیا۔ان امیور پر پھر فلورس ذنب لگائی گئی۔نتیجتاً اصل ہدف غیر فلورس پیپٹائڈ کو ان فلورس کثافتوں سے فلورس جامد حالت والے عمل کشید یا فلورس ایچ پی ایل سی کی مدد سے حوصلہ فزا مقدار میں با آسانی خالص بنایا گیا۔

Curriculum Vitae

Asghar Ali was born in Kasur, Pakistan on October 8th, 1978. After completing his high school in 1994, he started his scientific studies at the Govt. Islamia College Civil Lines, Lahore. In 1999, he graduated as a bachelor of science with Chemistry, Zoology and Botany as the major subjects. In 2001, he got his Masters degree in Chemistry from the University of Punjab. During his master, he worked on various research projects especially analyzing the drinking water in the Narowal region.

He started his Ph.D at Leiden University, the Netherlands, in February 2006 with financial support from the Higher Education Commission (HEC) of Pakistan. The research which is described in this thesis was conducted in the Bio-Organic synthesis group (Biosyn) of the Leiden Institute of Chemisty (LIC) under the supervision of Prof. Dr. Gijsbert A. van der Marel, Jeroen D. C. Codée and Dmitri V. Filippov. Part of the work described in this thesis was presented at the 14th European Carbohydrate Conference in Lübeck, Germany (2007) and HRSMC meeting of the Design and synthesis Divison in Luntren, the Netherlands (2007, 2008).

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یہ کامیابیاں، یہ عزت یہ نام تم سے ہے
خدا نے جو بھی دیا ہے مقام تم سے ہے