Synthetic Routes to Thiooligosaccharides and Thioglycopeptides

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1. Introduction

The ongoing ability of bacteria to resist current antibiotic treatments highlights the need for alternative strategies for inhibiting their pathogenicity. For many pathogens, including bacteria, complex carbohydrate structures of glycoproteins on host cell surfaces provide the binding point.[1] The attachment of carbohydrate chains to proteins is a ubiquitous post- and cotranslational modification known as protein glycosylation. This glycosylation is linked to several biological processes such as protein transport, cell adhesion, and signal transduction.[2] Because of these biologically relevant issues, there is a considerable amount of interest in glycoproteins. This in turn creates a need for glycoproteins with a defined composition to correlate structure and function of specific glycoprotein motifs. However, glycoproteins are biosynthesized as complex mixtures of proteins with varying degrees of glycosylation at specific sites, each different component of which is called a glycoform. The presence of mixtures of glycoforms complicates isolation of homogeneous glycoproteins from natural sources. As a result, the development of efficient methods for synthesizing homogeneous glycoproteins is a goal for organic chemists around the world.

Oligosaccharides are attached to proteins mainly through the hydroxy group of serine and threonine (*O*-glycans) and the amido group of asparagine (*N*-glycans). Although solid-phase peptide synthesis (SPPS) can be adapted to allow incorporation of glycosylated amino acids,[3] the synthesis of glycopeptides using SPPS is not entirely routine due to size limitations and problems with deglycosylation during peptide

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Kandasamy Pachamuthu was born at Salem, India, in 1973. He obtained his B.Sc. degree (Chemistry) in 1994 from Kandaswamy Kandar's College, Velur (Salem), affiliated with University of Madras and M.Sc. degree (physical chemistry) from University of Madras, Chennai, India, in 1996. He received his Ph.D. degree (2001) in organic chemistry (synthetic carbohydrate) from the Indian Institute of Technology, Kanpur, India, under the supervision of Professor Yashwant D. Vankar. He worked with professor Richard R. Schmidt as an Alexander von Humboldt postdoctoral fellow, on development of new methodologies for peptide and glycopeptide synthesis at the University of Konstanz, Germany (2001–2003). He then moved to university of Montreal, Canada, where he is currently working with professor Stephen Hanessian as a postdoctoral fellow in medicinal chemistry since 2004. His research interests are carbohydrates and medicinal chemistry with special emphasis on target-oriented glycopeptide synthesis, oligosaccharide synthesis, carbohydrate-based natural product synthesis, and aminoglycoside antibiotics.

Richard R. Schmidt received his Ph.D. degree at the University of Stuttgart in 1962, where he worked under the guidance of Professor Gommper on push–pull-stabilized quinone methides. From 1965 to 1966, he had a postdoctoral fellowship with Professor Frank M. Huennekens at the Scripps Research Foundation in La Jolla, CA, on coenzyme B_{12} metabolism. He returned to Stuttgart and became Dozent and Associate Professor. Since 1975, he has been a Full Professor at the University of Konstanz; he has stayed there since 1975 and denied calls to other universities. His main research interests are devoted to carbanion chemistry, stereochemistry, cycloaddition reactions, chemistry of heterocycles, and natural products. In recent years, his work has been mainly dedicated to carbohydrate and, in particular, to glycoconjugate chemistry and their biological relevance.

synthesis. Direct site-specific N- or O-glycosylation of proteins is not possible due to the presence of many competing functional groups. Also the enzymes are only partly available for carrying out this task. However, a method that has potential to become routine is the chemoselective ligation of a carbohydrate chain to a full-length protein, thus generating glycoprotein mimetics. This method requires two functional groups that selectively react with each other under mild conditions. This could be achieved, for instance, by the high nucleophilicity of the sulfhydryl group in comparison with any other functional group.

Replacement of the anomeric oxygen of *O*-glycopeptides by sulfur (Scheme 1) or nitrogen and carbon, thus furnishing S-, N-, and C-linked glycopeptides, leads to modifications that are tolerated by most biological systems and are less susceptible to acid/base or enzyme-mediated hydrolysis.[4]

This review will cover synthetic routes available for the construction of thio-linked oligosaccharides (1999 to mid-2005) and thio-linked glycopeptide/proteins (up to mid 2005). The reader should also refer to other excellent reviews that have previously covered aspects of thiooligosaccharide synthesis[5−9] and others reporting partially on thio-linked glycopeptides/proteins.[10−12]

Contrary to *O*-glycoside bond formation, thioglycoside bond formation can be readily based on S_N2 displacement of halogenoses because of the high nucleophilicity of thiolate groups. This reaction is particularly convenient for equatorial thioglycoside bond formation because the halogen atom generally adopts the axial position on halogenoses. Yet, the question remains whether introduction of a mercapto group into the glycosyl acceptor prior to reaction with the halogenose (Scheme 1, "base-promoted S-glycosylation") is superior to the most commonly applied procedure, consisting first of halogenose transformation into an anomeric thiol group, which is generally configurationally stable, followed by "anomeric thiol group S-alkylation" with an O-activated sugar or any other alkylating agent. Because ready access to axial thioglycosides is also required, alternatively, thioglycoside bond formation with typical glycosyl donors, such as *O*-glycosyl trichloroacetimidates, has been successfully performed ("acid-catalyzed S-glycosylation" following an S_N1-type reaction course).

Most of the work presented in this review employs these convenient and generally high-yielding reactions, where disulfide formation (via air oxidation) is often the main competing reaction. Closely related alternatives to these basic reactions have successfully been performed for thioglycoside formation, for instance, use of S_N2' reactions, Michael-type additions of thiolates to sugar enones, and use of thiirane intermediates as alkylating agents.

2. Thiooligosaccharide Syntheses

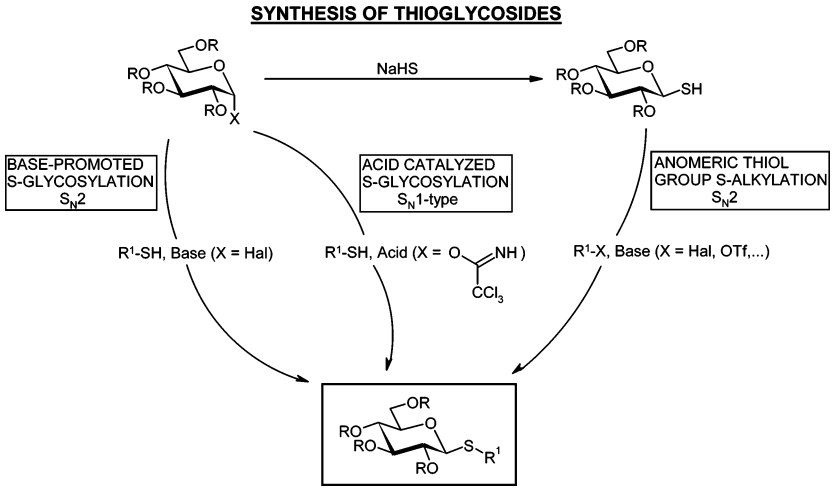
2.1. Synthesis of (1,1)-Thiodisaccharides

A new synthetic method has been reported for the synthesis of 1,1-thiodisaccharides via glycosylthioiminium salts in a two-phase system using a phase transfer catalyst (PTC).[13] The reaction of glyosylthioiminium salts **2**, which were prepared in situ from glycosyl bromides **1** and thioacetamide, with different glycosyl bromides **3** in a two-phase system in the presence of tetrabutylphosphonium bromide (TBPB) and aqueous NaOH gave the corresponding thiodisaccharides **4** via a combination of anomeric thiol group S-alkylation and base-promoted S-glycosylation as shown in Scheme 2. Tetrabutylammonium bromide (TBAB) and tetrabutylphosphonium bromide (TBPB) were highly effective phase transfer catalysts among those studied.

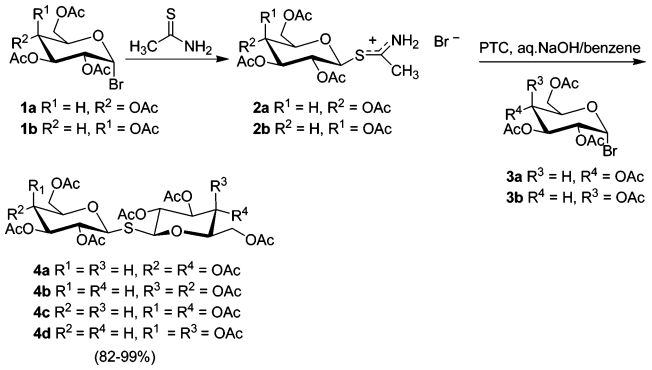
Taking advantage of disulfide formation of mercaptans, Knapp and co-workers[14] obtained product **6** in 80% yield during oxidative coupling of 1-thiosugar **5** in the presence of *meta*-chloroperoxybenzoic acid (*m*-CPBA) (Scheme 3).

Another important approach for S−S-linked disaccharide synthesis has been reported. This method involves the reaction of readily available tetra-*O*-acetyl-*β*-D-glucopyra-

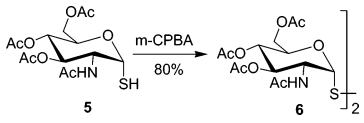
Scheme 1



Scheme 2

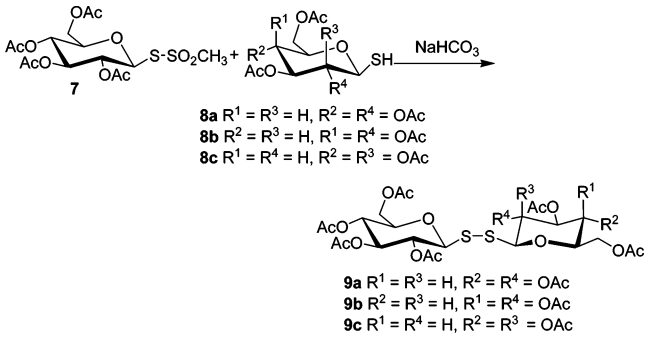


Scheme 3



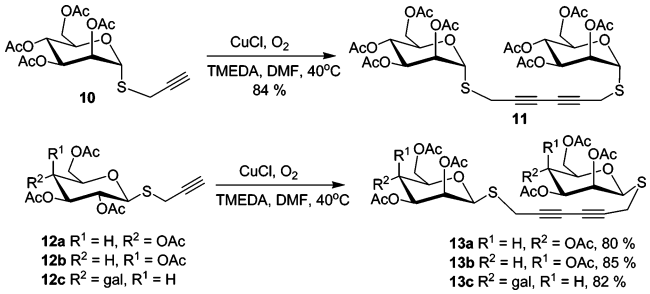
nosyl methanethiolsulfonate (**7**) with O-acetylated 1-thio-sugars **8a**−**c** in the presence of aqueous $NaHCO_3$ in methanol, thus affording the corresponding products **9a**−**c** in good yields (Scheme 4).[15] This method permits the synthesis of mixed disulfides.

Scheme 4



Roy et al.[16] have employed a copper(I)-catalyzed Glaser reaction and a palladium-catalyzed Sonogashira reaction to synthesize divalent "rodlike" thioglycoside clusters via acetylene tethers (Scheme 5). Dimerized products **11** and **13a**−**c** were obtained by bubbling oxygen into a mixture of thioglycosides **10** and **12a**−**c** in the presence of CuCl and

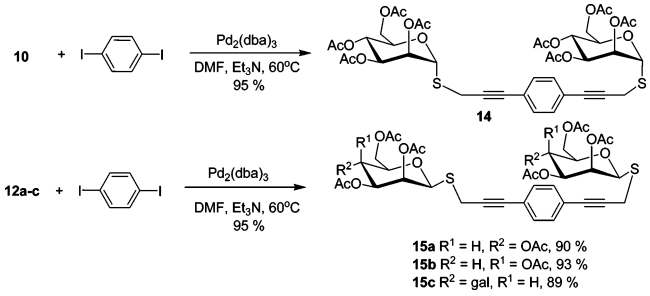
Scheme 5



tetramethylethylenediamine (TMEDA) in DMF. The authors found that TMEDA dramatically increased the yield of the reaction.

The Sonogashira coupling reaction between thioglycosides **10**, **12a**−**c**, and 1,4-diiodobenzene was performed in the presence of $Pd_2(dba)_3$, CuI, PPh_3, and DMF and Et_3N solution (1:1, v/v) under nitrogen at room temperature (Scheme 6). This method gives easy access to a variety of rigid carbohydrate ligands, such as **14** and **15a**−**c**, and the reaction conditions are compatible with acetate protecting groups.

Scheme 6

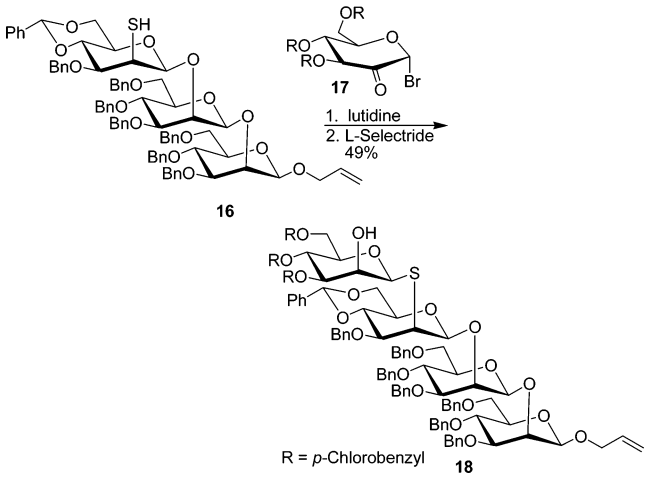


2.2. Synthesis of (1→2) S-Linked Thiodisaccharides

2.2.1. Anomeric Thiol Group S-Alkylation and Base-Promoted S-Glycosylation

Bundle and co-workers[17,18] synthesized β-(1→2)-thio-linked mannopyranosides to study the unique immunological properties of the cell wall mannan of *Candida albicans*. Their synthesis involves the displacement of the anomeric bromide of uloside **17** with 2-thio sugar **16**, in the presence of lutidine

Scheme 7



(Scheme 7); after reduction of the 2-keto functionality employing L-selectride, **18** was obtained in 49% yield. The corresponding 1-thio-α-gluco epimer was also isolated from the reaction (∼12%). This is presumably formed from *β*-ulosyl bromide present in the reaction mixture, although base-catalyzed isomerization of the ulosyl thio-linked glycoside or an S_N1-type reaction with the ulosyl bromide may also be possible.

Knapp et al.[19] have observed, unexpectedly, a mixture of oligomeric thioglycosides **23**−**25** bearing the 2-mercapto substituent and the expected product **22** during deacetylation of **19** under standard Zemplén deacetylation conditions (Scheme 8). Thiirane **21** formation is a very slow process because of the poor leaving group character of the phenylthio moiety. Once the thiirane is formed, it is trapped by the more reactive thiolate present in the medium, thus leading to oligomers. The authors attributed the ring closure reaction of **20** to a favorable S_N2 trajectory and softness match between the participating thiolate at C-2 and the trans−anti anomeric leaving group (PhS^-), as well as to the stability of phenythiolate as a leaving group relative to alkylthioate.

2.2.2. Michael-Type Addition

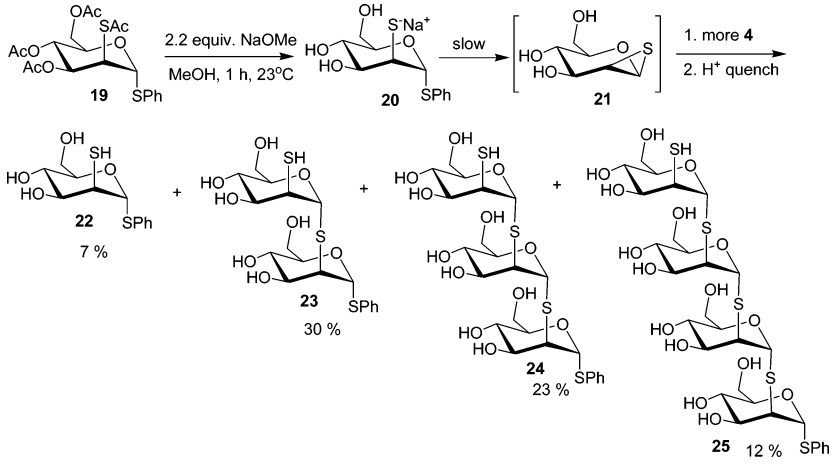
As a variation of anomeric S-alkylation, Witczak and coworkers[20] have reported a new method for the synthesis of 3-deoxy-(1→2)-2-*S*-thiodisaccharides via base-catalyzed Michael-type addition of 1-thiosugars to isolevoglucosenone followed by the reduction of the C-4 keto function (Scheme 9). Michael-type addition of 1-thiosugars **8a**, **8b**, and **27** to the enone **26** proceeded smoothly with the formation of *β*-(1→2)-linked 2,3-dideoxy-2-thio-disaccharides **28a**−**c** in 63−70% yields. The 2-axial adducts were always obtained as the only addition product. The conjugate addition proceeded by the attack of the incoming nucleophile at the alkene face opposite to the 1,6-anhydro ring. The sterically demanding 1,6-anhydro bridge in isolevoglucosenone is assumed to effectively prevent an attack from the upper side of the molecule, which would give access to the 2-equatorial addition products.

The reduction of the C-4 keto function of ketones with L-selectride, followed by in situ acetylation, proceeded stereoselectively with the formation of the D-ribo isomers **28a**−**c** in 79% yield. Only trace amounts of the corresponding D-xylo isomers have been detected by ^1H NMR spectroscopy. The cleavage of the 1,6-anyhydro ring followed by in situ acetylation in **28a**−**c** was examined using different Lewis acids such as $BF_3{\cdot}OEt_2/Ac_2O$, TFA/Ac_2O, and $Et_3SiOSO_2CF_3/Ac_2O$. Each was compatible with compounds **28a** and **28b**, but compound **28c** decomposed under the reaction conditions. Among the Lewis acids, the $CF_3SO_3SiEt_3/Ac_2O$ system gave the best yields.

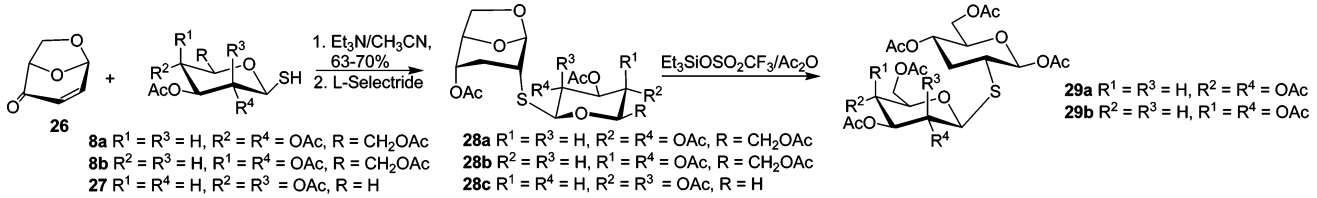
2.2.3. (1→2)-Thioglycosyl Migration

To investigate the binding of substrate analogues and inhibitors to glycosidase enzymes, a novel method has been reported for the conversion of (1-1)-thio-linked glucopyra-

Scheme 8

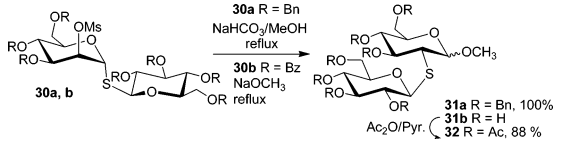


Scheme 9



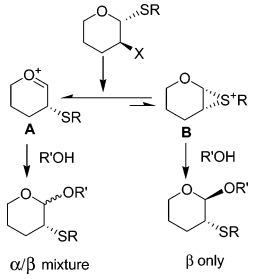
nosyl α-D-mannopyranosides to (1→2)-thio-linked methyl sophorosides or methyl kojibiosides.[21] The method involves the (1→2)-migration of the thioglucopyranosyl moiety of the nonreducing disaccharide with inversion of configuration at C-2 of the mannopyranose ring and concomitant formation of the methyl glucopyranoside (Scheme 10).

Scheme 10



The mesylate **30a** was refluxed in methanol in the presence of $NaHCO_3$ resulting in smooth 1,2-thioglycosyl migration with concomitant capture of the cationic reactive intermediate by methanol to produce 2-thio-linked methyl sophorosides **31a** in quantitative yield (3:1, β/α mixture, Scheme 10). It is interesting to note that the (1→2)-thio migration proceeded with complete retention of configuration at the anomeric center of the migrating 1-thioglucopyranose residue. However, the methyl glucoside was formed with limited stereoselectivity suggesting that the reactive intermediate for 1→2-thio migration in pyranosides exists as an oxocarbenium ion **A** rather than the alternative episulfonium ion **B** (Scheme 11).

Scheme 11



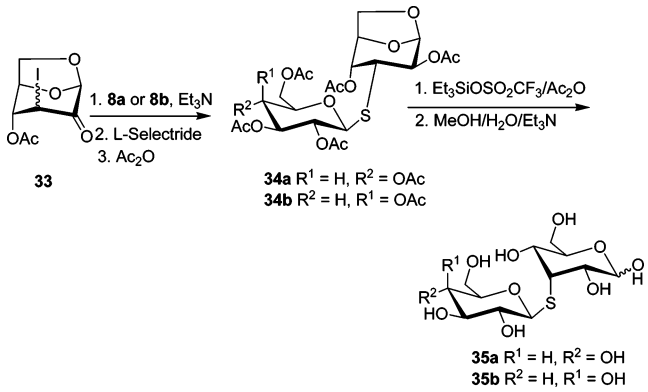
By employment of similar reaction conditions, the synthesis of methyl 2-thio-kojibioside was also achieved. The thioglucosyl migration does not occur under these reaction conditions when electron-withdrawing benzoate protecting groups are present. However, refluxing benzoyl derivative **30b** under Zemplén deacetylation conditions resulted in methanolysis of the benzoate esters followed by formation of (1→2)-thio migrated methyl sophoroside **31b**. This crude mixture was acetylated using Ac_2O and pyridine. The β-linked disaccharide **32** was obtained in 88% yield as an anomeric mixture in a ratio of α/β = 3:2. According to the authors, it is not known precisely at which stage this (1→2)-thio migration occurs. However, the good yields of (1→2)-thio migration products obtained from **30b** indicate that this migration reaction occurs before any extensive methanolysis or displacement of the mesylate group takes place (Scheme 10).

2.3. Synthesis of β-(1→3)-S-Linked Oligosaccharides

2.3.1. Anomeric Thiol Group S-Alkylation

Witczak and co-workers[22] have reported a stereoselective synthesis of (1→3)-3-*S*-thio-disaccharides in two steps by (i) nucleophilic substitution reaction of 1-thio sugars with levoglucosenone-derived iodoketone, (ii) followed by reduction of the C-2 keto functionality (Scheme 12).

Scheme 12



The reaction of a diastereomeric mixture (1:1) of the 3-iodoketone **33** with 1-thiosugars **8a** and **8b** proceeded smoothly with the formation of β-(1→3)-3-*S*-thiodisaccharides in 60−70% yield. It is interesting to note that no epimerization or β-elimination was observed during the coupling reaction. Furthermore, the 4-*O*-acetyl protecting group of **33** was sufficiently stable under the above reaction conditions, and no deacetylation or epoxide formation has been reported. The reduction of the C-2 keto function with L-selectride, followed by in situ acetylation, proceeded stereoselectively to afford the D-altro isomers **34a** and **34b** in 79% yield. The cleavage of the 1,6-anhydro ring followed previously reported procedures (→ **35a**,**b**).

2.3.2. Enzymatic Synthesis of α-(1→3)-S-Linked Oligosaccharides

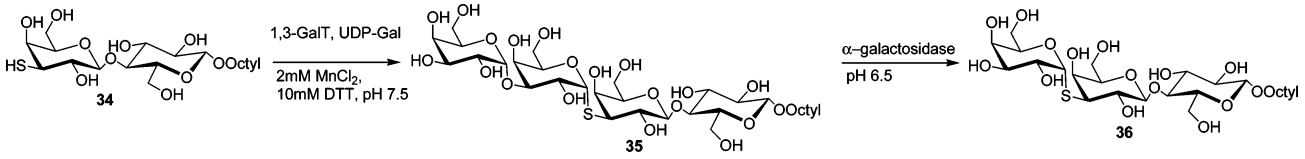
Bundle and co-workers[23] have reported a novel enzyme-catalyzed technique for thiooligosaccharide synthesis. Incubation of a mixture of the thiol **34** and its disulfide (∼3:1) with UDP-Gal and unit quantities of α-1,3-GalT in the presence of dithiothreitol (DTT) afforded, unexpectedly, the thio-linked tetrasaccharide **35** in 92% yield instead of trisaccharide **36** (Scheme 13). A second glycosyl transfer occurred following initial transfer of a galactosyl residue to the thio acceptor. Selective hydrolysis of the terminal *O*-glycosidic linkage in **35** by α-galactosidase (from green coffee beans) gave the expected thio-trisaccharide **36** in quantitative yield. The anomeric octyl group at the glycosyl acceptor was introduced to ease purification of the product by solid-phase extraction on C-18 silica gel after the enzymatic glycosylation reaction. According to the authors, this enzymatic method is claimed to possess several advantages. For instance, (i) it is highly stereo- and regioselective, (ii) it gives high yields, and (iii) there is no need for the final deprotection. Further, this method may be more suitable for the formation of thioglycosidic linkages in molecules containing sensitive functionalities.

2.4. Synthesis of β-(1→4)- and α-(1→4)-S-Linked Oligosaccharides

2.4.1. Acid-Catalyzed S-Glycosylation

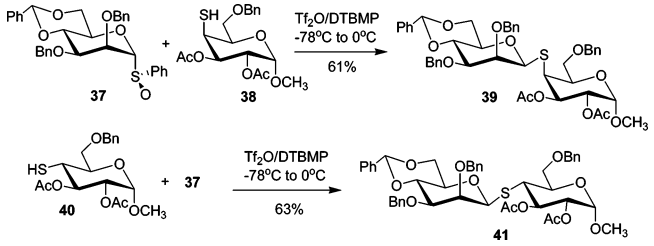
Crich and co-workers[24] have reported a direct stereoselective synthesis of β-thiomannosides in which anomeric *S*-phenyl sulfoxide **37** acts as glycosyl donor (Scheme 14). Coupling of sugar thiol **38** with sulfoxide **37** activated by

Scheme 13



Tf_2O and 2,6-di-(*tert*-butyl)-4-methylpyridine (DTBMP) at −78 °C (→ 0 °C) afforded the disaccharide **39** in 61% yield. Likewise, thiodisaccharide **41** was prepared in 63% yield by a reaction of **40** with **37**. Deprotection of these products was performed by Birch reduction (Bn groups) and by Zemplén deacetylation.

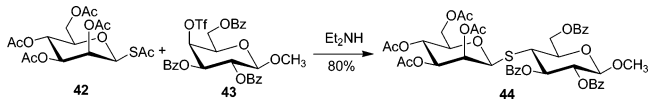
Scheme 14



2.4.2. Anomeric Thiol Group S-Alkylation and Base-Promoted S-Glycosylation

Bundle and co-workers[25] have synthesized β-(1→4) thiomannopyranoside **44** in 80% yield by S_N2 displacement of triflate **43** with thiosugar **42** in the presence of diethylamine (Scheme 15). The reaction was carried out at −5 °C, and the observed anomeric ratio was 95:5 (β/α).

Scheme 15

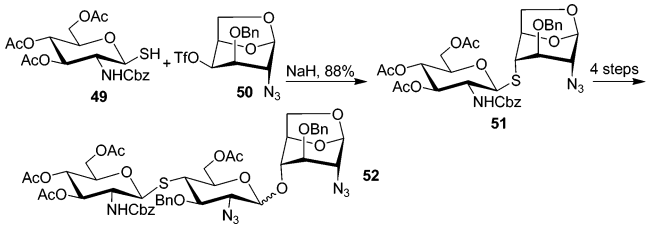


The reaction of 1,2-*trans*-glycosyl acetate **45** with thiourea in the presence of $BF_3{\cdot}OEt_2$ afforded the corresponding isothiourea derivative **46**, and this derivative was transformed into 1-thiosugar by treatment with Et_3N (Scheme 16). After in situ generation, the above 1-thio sugar was alkylated with sugar triflate **47** to give the desired thiodisaccharide **48** in 85% yield. Likewise, the (1→6) S-linked disaccharide has also been synthesized in 83% yield.[26]

In another approach, 1-thiosugar **49** was treated with triflate **50** in the presence of NaH furnishing the corresponding thiodisaccharide **51** in 88% yield (Scheme 17). Compound **51** was further transformed into a trichloroacetimidate donor (anomeric mixture in favor of the α-anomer α/β, 8:2). Glycosylation of an acceptor with this donor (2 molar equiv) in the presence of $BF_3{\cdot}OEt_2$ in dichloromethane as solvent afforded trisaccharides, for instance, **52** (α/β, 1:4.7) in 57% yield.[27]

Ibatullin and co-workers[28] have reported a stereoselective method for the synthesis of thioxylo-di-, tri-, tetra- and pentasaccharides from *S*-glycosyl isothiourea precursors (Scheme 18). The reaction of 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl isothiouronium bromide **53** with 1,2,3-tri-*O*-benzoyl-4-*O*-trifluoromethanesulfonyl-β-D−L-arabinopyranose **57** in the presence of Et_3N afforded the corresponding product **58**. The resulting 4-thio-xylobiose was then converted into the corresponding isothiouronium bromide and used for the synthesis of 4,4′-dithioxylotriose. Higher homologues (**59**−**61**) of the series have also been prepared. The yields of these reactions were between 53% and 82%.

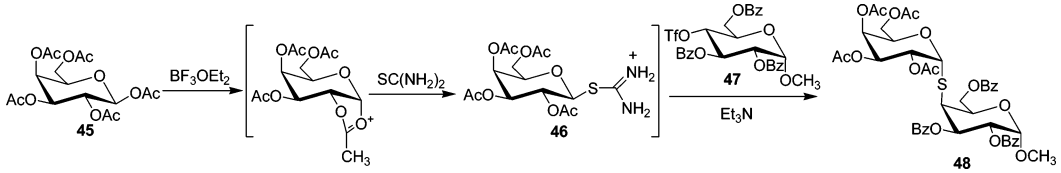
Scheme 17



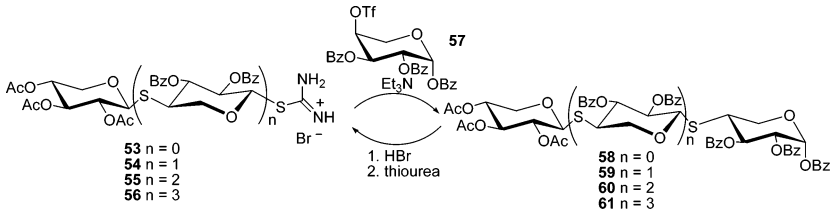
Withers and co-workers[29] have synthesized a thio-linked disaccharide based on the structure of the glycosaminoglycan chondroitin as a potential inhibitor of chondroitin AC lyase from *Flavobacterium heparinum* to provide a structural analysis of the active site (Scheme 19). The coupling of the two monosaccharides was accomplished via anomeric S-alkylation by reaction of 1-thiosugar **62** with triflate **63** in the presence of NaH. The selective oxidation of the primary hydroxy group, which was derived from product **64** by deprotection using TFA, over that of the sulfur moiety was accomplished in 73% yield using sonicative Jones oxidation with CrO_3 and H_2SO_4 at 35 °C. Deprotection gave the disaccharide **65** in 46% yield. It was observed that compound **65** was slowly cleaved by chondroitin AC lyase from *F. heparinum*. The enzyme binds disaccharide **65** with a lower affinity than the monosaccharide substrate suggesting that disaccharide **65** is not a good inhibitor of the polysaccharide lyase enzyme.

Hindsgaul et al.[30] have reported an efficient solid-phase technique for the synthesis of thio-linked oligosaccharides (Schemes 20 and 21). Trityl chloride derivatized polystyrene bound resin thiols were treated with NaOMe in THF to enhance the nucleophilicity of the mercapto group. The resulting thiolate **66a** was reacted with triflate **67** in the presence of 15-crown-5 or kryptofix 221. The S-linked product **68a** was obtained in 64% yield after deprotection followed by cleavage from the resin. Likewise, thiolates **66b**, **66c**, and **69** were reacted with **67**, thus providing the corresponding products **68b**, **68c**, and **70** in similar yields.

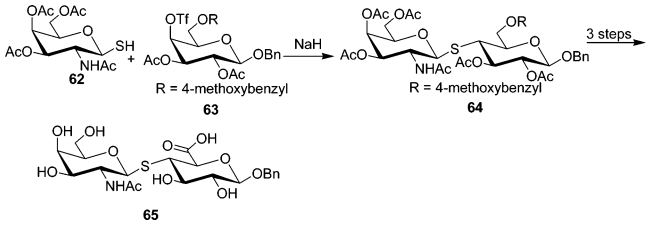
Scheme 16



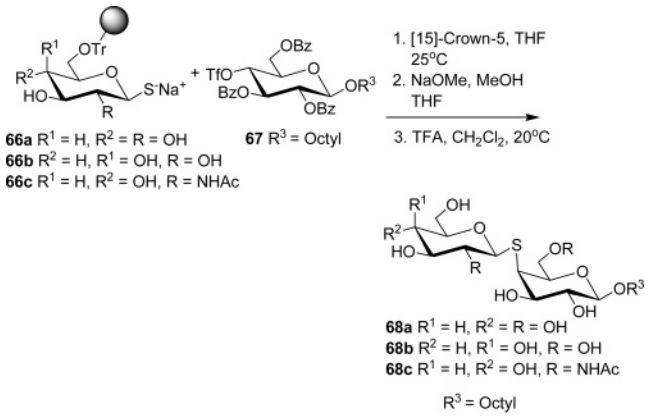
Scheme 18



Scheme 19

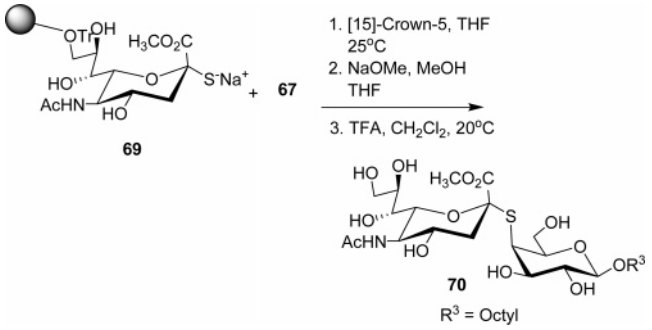


Scheme 20



This method was also extended to the construction of larger thiooligosaccharides.

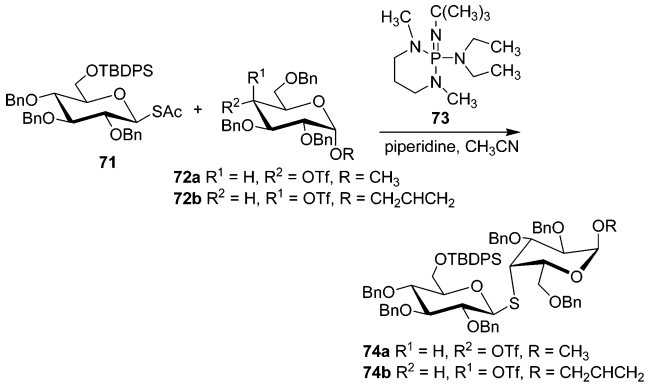
Scheme 21



An improvement of the direct anomeric S-alkylation yield was accomplished via S_N2 displacement of *O*-triflate with 1-thiosugars in the presence of iminophosphorane bases (Scheme 22). The reaction of the thiolate, generated from in situ deprotection of **71** using piperidine, with **72a** or **72b** in the presence of iminophosphorane base **73** provided excellent yields of thio-disaccharides without substantial elimination products.[31]

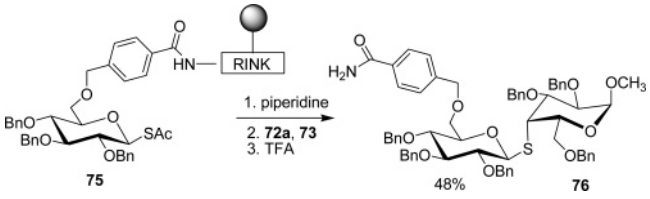
The excellent yields obtained ranged between 85% and 91%. The iminophosphorane base **73** also proved to be useful in solid support-bound coupling of 1-thiolate with 4-*O*-triflate **75** (Scheme 23). The resulting product was cleaved from the solid support using TFA to afford thiodisaccharide **76** in an overall yield of 48%. The iminophosphorane **73** used

Scheme 22



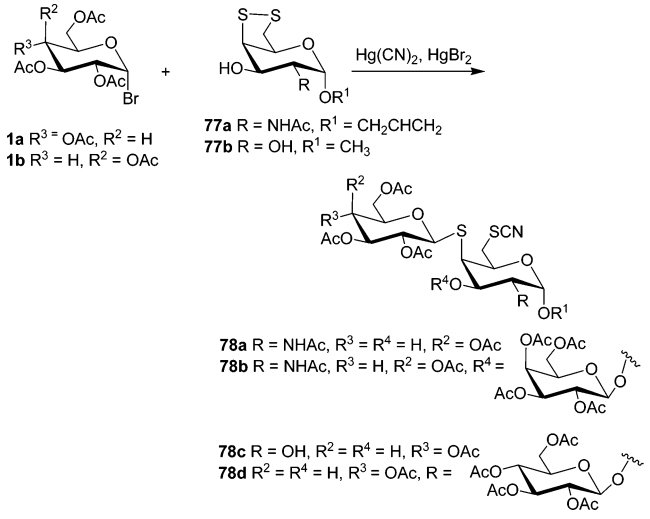
in this approach is expensive. However, these reaction conditions have been shown to avoid the competing elimination reactions and the transesterification of *O*-acyl or *O*-benzoyl protecting groups to the anomeric thiolate.

Scheme 23



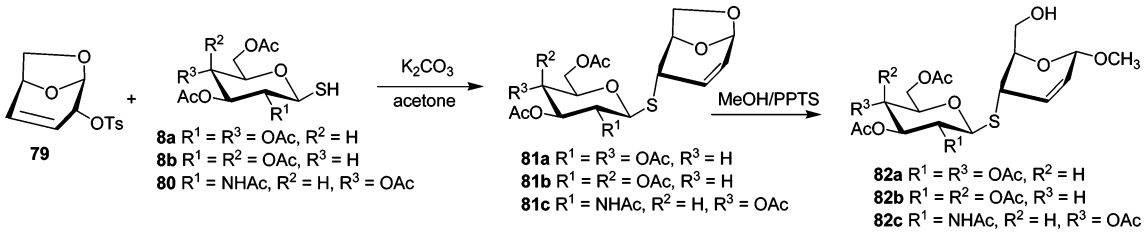
Reaction of disulfide **77a** with a mixture of $Hg(CN)_2$ and $HgBr_2$ followed by addition of an excess of acetobromogalactose **1b** yielded thiodisaccharide **78a** in 50% yield (Scheme 24). The formation of trisaccharide **78b** was also observed, albeit, in low yields.[32]

Scheme 24



Likewise, unprotected acceptor analogue **77b** reacted with acetobromoglucose derivative **1a** as donor providing the

Scheme 25



expected product **78c** in 19%. In addition to the disaccharide, trisaccharide **78d** was also isolated.

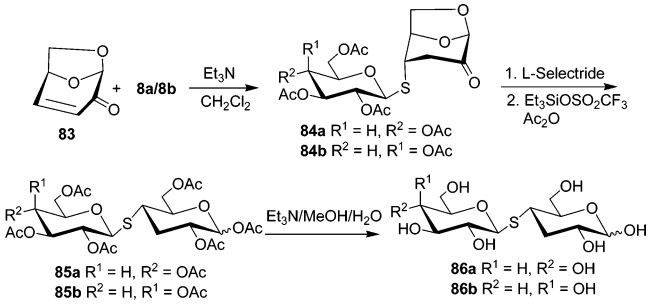
2.4.3. S_N2' Type Reaction

The coupling of 1-thiosugar **80** with the unsaturated tosylhexenose **79** in the presence of K_2CO_3 in acetone proceeded smoothly via S_N2' type reaction with retention of the configuration to give disaccharide **81** and subsequently derivative **82** (Scheme 25). K_2CO_3 in acetone proved to be the best base, whereas lithium carbonate in acetone or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in oxolane were not effective. It is interesting to note that the addition of a small amount of water to the reaction mixture accelerated the reaction dramatically when **80** was used as a nucleophile. On the other hand, dry K_2CO_3 in dry acetone gave almost no conversion.[33,34] This method gives access to a variety of α,β-unsaturated disaccharides.

2.4.4. Michael-Type Addition

Witczak et al.[35,36] have reported a stereoselective synthesis of 3-deoxy-4-thiocellobiose from levoglucosenone by Michael-type addition of 1-thiosugars (Scheme 26). The reaction of

Scheme 26

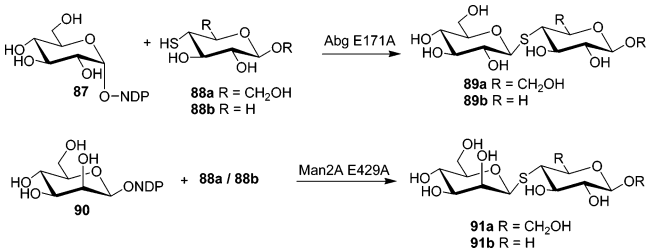


1-thiosugars **8a**/**8b** with **83** in the presence of Et_3N afforded the corresponding S-linked products **84a**/**84b**. The reduction of the C-2 keto function of ketones **84a**/**84b** with L-selectride, followed by acetylation, proceeded stereoselectively with the formation of D-ribo isomers **85a**/**85b** in 91% yield. Only a trace amount of D-arabino isomers was detected by ^1H NMR. The cleavage of the 1,6-anhydro ring afforded the corresponding thiodisaccharides **86a**/**86b**.

2.4.5. Enzymatic Synthesis

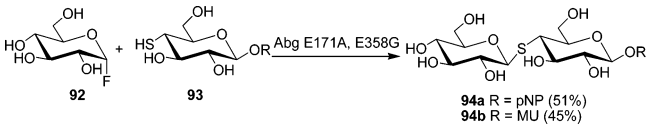
Withers and co-workers[37] reported a novel enzymatic method for the synthesis of β-(1→4) thiooligosaccharides. Incubation of acceptors **88a** and **88b** with the glycosyl donors of nature **87** and **90** and mutant enzymes in phosphate-buffered solution at pH 6.8 provided the corresponding products **89a**, **89b**, **91a**, and **91b** in good yields as depicted in Scheme 27. Two different enzymes from two different glycosidase families such as (i) the β-glucosidase from *Agrobacterium sp.* Abg E171A and (ii) the mannosidase from *Cellulomones fimi* Man 2A E429A were employed to effect the thiodisaccharide formation. It is important to mention that the reaction of the wild-type enzymes with the appropriate nucleoside diphosphate sugar donors and thiosugar acceptors resulted in no detectable formation of disaccharide. These results proved the stereo- and regiospecificity of the reaction.

Scheme 27



Another approach deals with a double mutant[38] retaining glycosidase that lacks both the catalytic nucleophile and the catalytic acid/base residues that efficiently catalyzes the glycoside bond formation from a glycosyl fluoride and thio sugar acceptors. The readily available donor glucosyl fluoride **92** and acceptor *p*-nitrophenyl (pNP) 4-deoxy-4-thio-β-D-glucopyranoside **93** were incubated with the double mutant Abg E171A, E358G overnight at neutral pH (Scheme 28).

Scheme 28



The expected thiodisaccharide **94a** was observed on TLC. Further, the same thioglycosylation event was observed when the acceptor was changed to methylumbelliferyl (MU) 4-deoxy-4-thio-β-D-glucopyranoside (→ **94b** as product). The yield of the products isolated in each case was between ~45% and 51%. The products, obtained from both of the above techniques, were stable toward hydrolysis by both the wild-type and mutant enzymes.

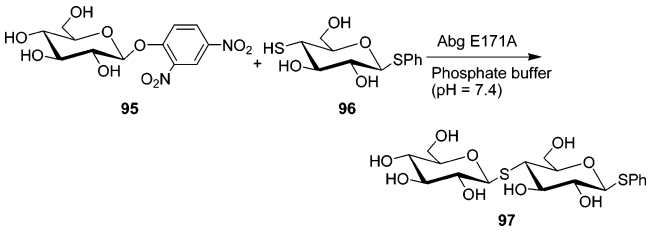
Stick et al.[39] have employed the original method of Withers to synthesize different types of S-linked disaccharides (Scheme 29). Reaction of the glucose derivative **95** with the thiol **96** in the presence of Abg 171A in phosphate buffer at pH 7.4 gave the expected (1→4)-S-linked disaccharide **97**, which was isolated after acetylation as acetate derivative.

In addition to this, the authors have synthesized (1→3)- and (1→6)-S-linked disaccharides employing this methodology and found that it does not work for the synthesis of (1→2)-S-linked disaccharide.

2.4.6. α-(1→4)-S-Linked Maltooligosaccharides

Driguez and co-workers[40] have synthesized an α-thio-linked pentasaccharide by a convergent approach (Scheme

Scheme 29



30). Coupling of triflate **99** with *S*-acetyl protected thiol **98** in the presence of diethylamine afforded the corresponding triphenylmethyl 1,4-dithio-α-maltoside, which was then transformed to the nucleophilic coupling compound **100** in 59% overall yield. The same two-step procedure using compounds **99** and **100** gave the expected trisaccharide **101**, which was then converted into *S*-acetyl protected thiol **102**. Coupling of **102** with triflate **103** in the presence of diethylamine afforded the corresponding pentasaccharide **104** in 68% yield.

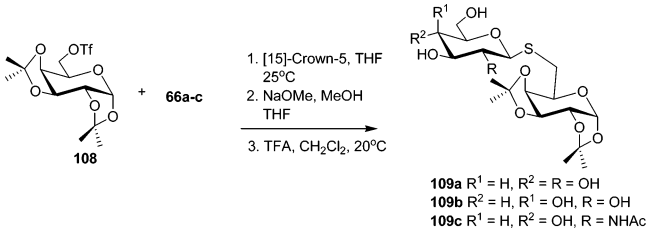
2.5. Synthesis of (1→6)-S-Linked Thiooligosaccharides

2.5.1. Linear (1→6)-S-Linked Thiooligosaccharides

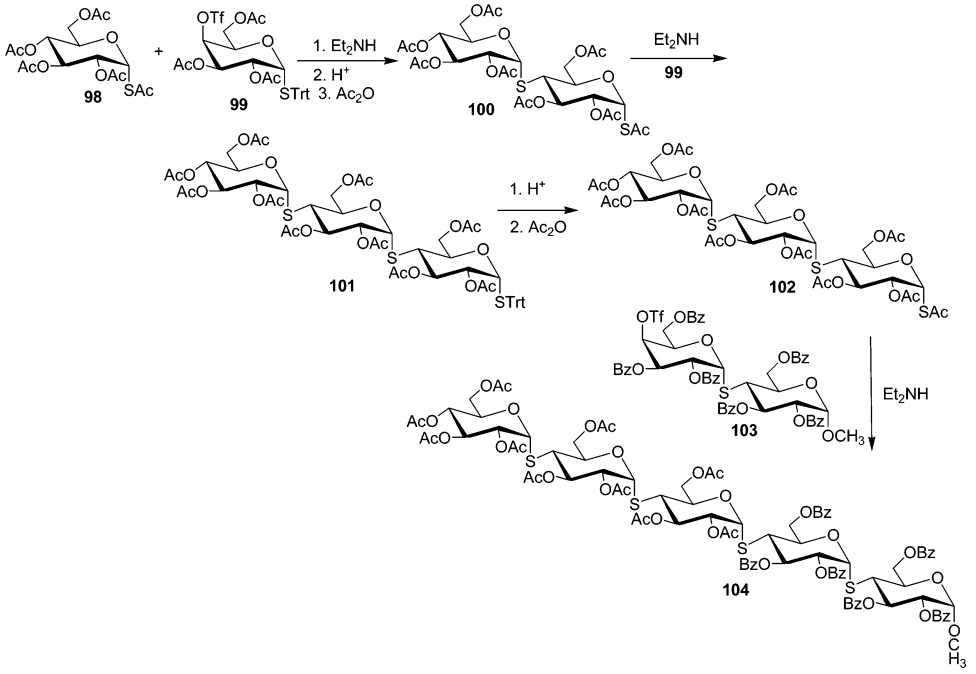
Driguez and co-workers[41] have reported on a convergent approach for the synthesis of β-(1→6)-thio-linked linear as well as branched oligosaccharides by anomeric S-alkylation via S_N2 displacement of 6-halides from *S*-acetyl protected 1-thiosugars. For instance, condensation of sugar iodide **105** with 1-acetylthio sugars **106a** and **106b** in the presence of diethylamine afforded the corresponding products **107a** and **107b** in 76% yield (Scheme 31). In addition to linear oligosaccharide syntheses, branched thio-linked oligosaccharides were also obtained in a similar manner.

Hindsgaul and co-workers[30] described the synthesis of β-(1→6)-S-linked thiooligosaccharides employing solid support. Trityl chloride derived polymer bound thiolates **66a−c** were coupled with triflate **108** in the presence of 15-crown-5 or Kryptofix 221 in THF (Scheme 32). After 16 h, the thioglycosides were cleaved from the resin by treatment with TFA in dichloromethane. The yields obtained of products **109a−c** were approximately 76%.

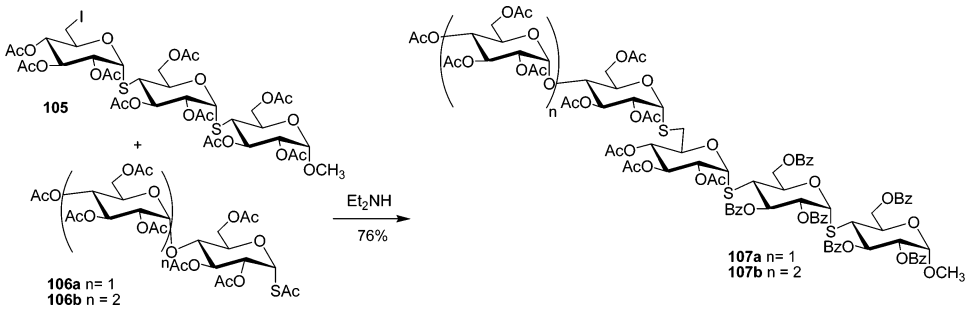
Scheme 32



Scheme 30

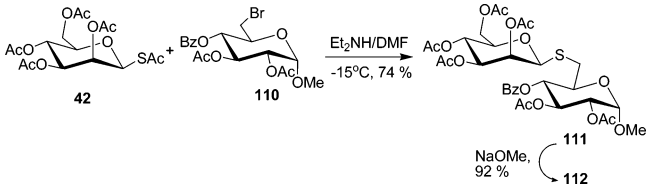


Scheme 31



The condensation of the 6-bromo glucoside **110** with **42** was carried out at −15 °C using von Itzstein conditions (Et_2-NH/DMF), thus providing the desired product **111** in 74% yield with excellent β-selectivity (α/β = 1:15, Scheme 33).

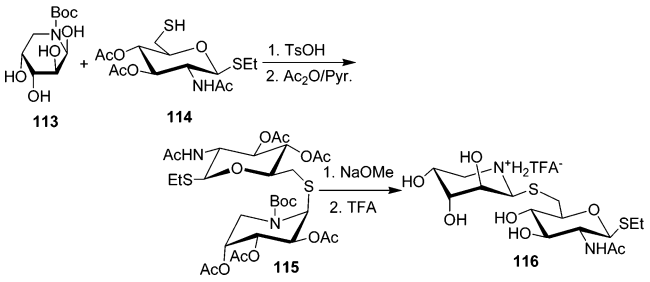
Scheme 33



The authors[25] observed poor selectivity when the reaction was carried out at higher temperature. To avoid poor anomeric selectivity, it is important to run the reaction at lower temperatures. Deprotection of **111** was conducted using NaOMe in methanol, and the product **112** was obtained in 92% yield.

Hashimoto et al.[42] have synthesized a disaccharide bearing an imino sugar residue at the nonreducing end by linking the glycone and aglycone moieties through a thioglycosidic linkage because the corresponding *O*-glycosidic linkage is highly unstable (Scheme 34). Reaction of the 6-thiosugar

Scheme 34



114 with *O*,*N*-acetal **113** in the presence of TsOH gave the corresponding thioglycoside as a single anomer in 80% yield, which was quantitatively converted to the pentaacetate **115**. The conformation of the 5-amino-5-deoxyglycopyranose ring was confirmed to be $^1C_4$ by proton NMR. O-Deacetylation of the thioglycoside **115**, followed by deprotection of the *N*-Boc group with TFA gave **116** quantitatively with a change in conformation from $^1C_4$ to $^4C_1$. This pseudo-disaccharide **116** was found to be stable at low pH but was hydroloyzed rapidly at pH > 5.

All these examples demonstrate that the synthesis of (1→6)-S-linked oligosaccharides can be quite readily performed.

2.5.2. β-(1→3), β-(1→6)-S-Linked Thiooligosaccharides

The sulfur-linked pentathiohexasaccharide $3^{I},3^{IV}$-di-β-D-glucopyranosylthiogentiotetraose **119** has been synthesized in 83% yield by a convergent approach involving the reaction of iodo compound **118** with 1-thiosugar **117** in the presence of NaH at room temperature (Scheme 35).[43,44]

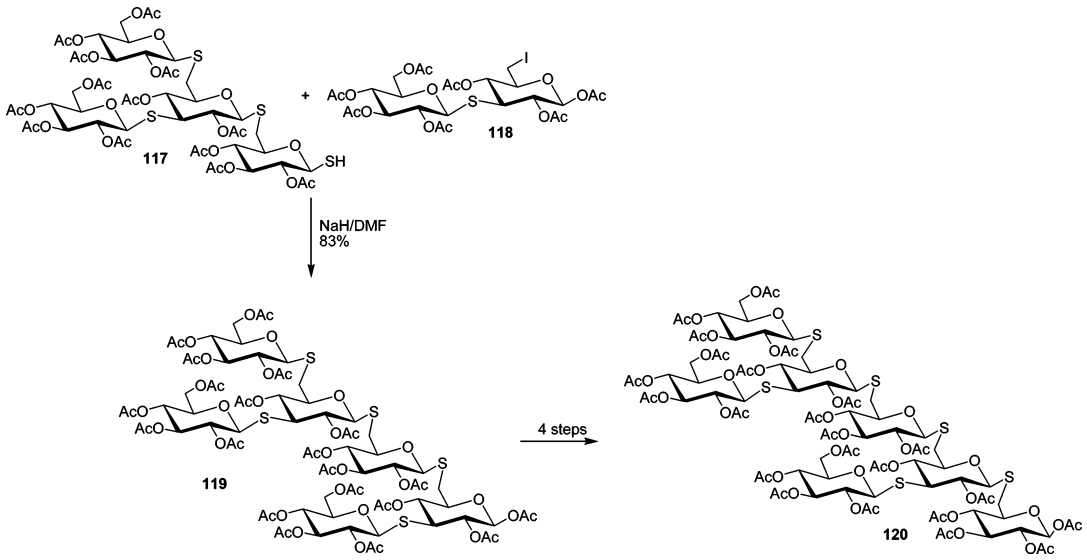
The product **119** was further converted to compound **120** in four steps. Some other thio-linked oligosaccharides were also synthesized in high yields by employing this convergent approach.

The ability of the thioglucosides to induce phytoalexin accumulation in soybean cotyledon tissue was determined. The concentrations resulting in phytoalexin production at the 50% level (EC_{50} value) were much higher than those of the *O*-glycosides giving a similar response in the bioassay. Thiooligosaccharides having a higher elicitor activity were also more efficient competitors of binding of the radiolabeled HG-APEA to the membrane-localized β-glucan binding sites of soybean.[44]

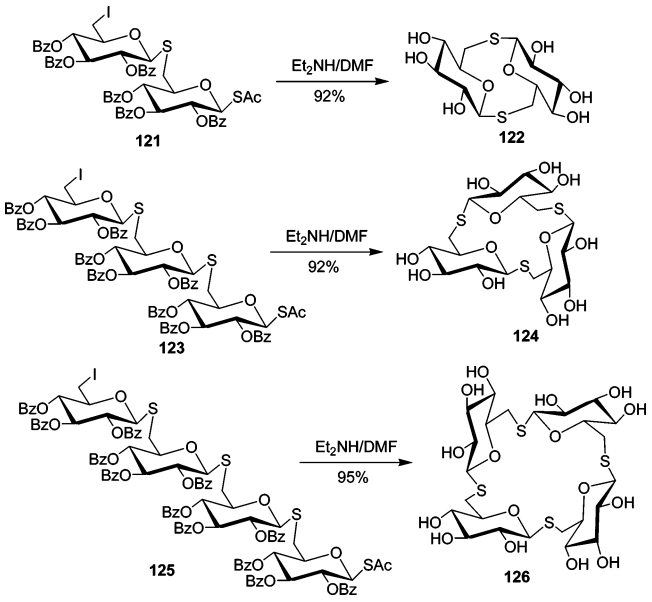
2.5.3. β-(1→6)-S-Linked Cyclic Thiooligosaccharides

A novel protocol for the synthesis of cyclic β-(1→6)-S-linked thioglucopyranosides was developed by Hindsgaul and co-workers.[45] The macrocyclization was accomplished through base-promoted intramolecular S_N2 glycosylation of an intermediate, which carries an iodo group at C-6 of the nonreducing sugar and a thioacetyl group at the anomeric center of the reducing end sugar. Compounds **122**, **124**, and **126** were prepared in 92−95% yield by intramolecular condensation of **121**, **123**, and **125** in the presence of diethylamine (Scheme 36).

Scheme 35



Scheme 36

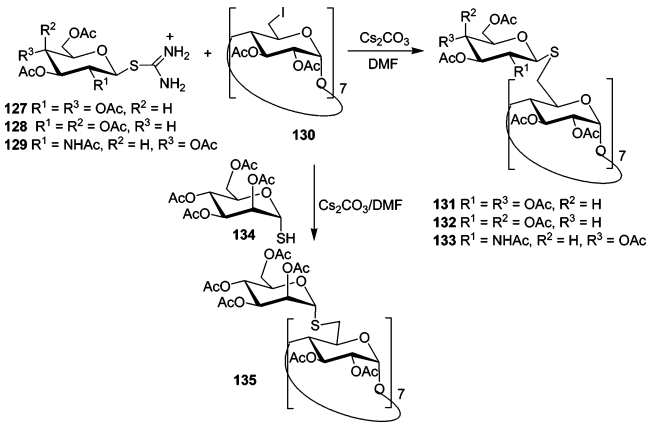


2.5.4. (1→6)-S-Branched β-Cyclodextrin

Several groups[46−49] have described the synthesis of (1→6)-branched β-cyclodextrins (β-CDs) employing two different strategies, (i) direct linkage of the 6-position of the CDs and (ii) linkage via a spacer arm.

The direct linkage of D-glucose, D-galactose, D-*N*-acetylglucosamine, and D-mannose to β-CD was achieved by a reaction of the pseudo-thioureas **127**−**129** and the thiol **134** with peracetylated 6-iodo-β-CD **130** (Scheme 37). Peracetylated glycosylated CDs **131**−**133** and **135** were obtained in high yields (85−94%). The reactions were performed with Cs_2CO_3 in DMF at room temperature, and the acetate groups were deprotected using standard deacetylation conditions.

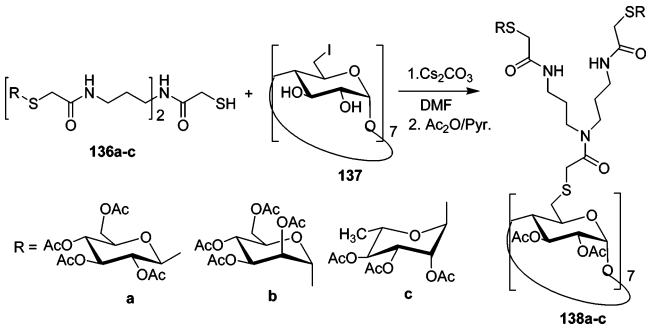
Scheme 37



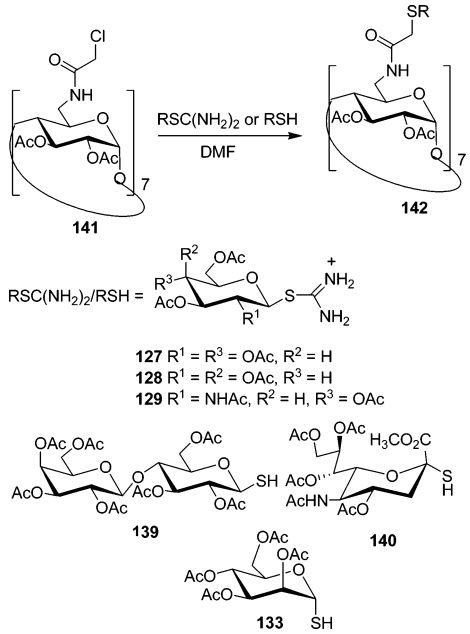
The attachment of dendrons to the CD core was accomplished by the reaction of thiols **136a**−**c** with per-6-deoxy-6-iodo-β-CD **137** in the presence of Cs_2CO_3 in dry DMF at 60 °C (Scheme 38). The S-linked products were isolated as their acetate derivatives **138a**−**c**, obtained by conventional acetylation (Ac_2O, Pyr, DMAP), in 70%, 88%, and 83% yield, respectively.

Alternatively, a few methods involve an attachment of 1-thiosugars to the 6-position of the cyclodextrin through a linker. For instance, 1-thiosugars such as **127**−**129** and **133**, **139**, and **140** were reacted with **141** in the presence of diethylamine or Cs_2CO_3 in DMF to afford the corresponding thioethers **142** in excellent yields (Scheme 39).

Scheme 38



Scheme 39



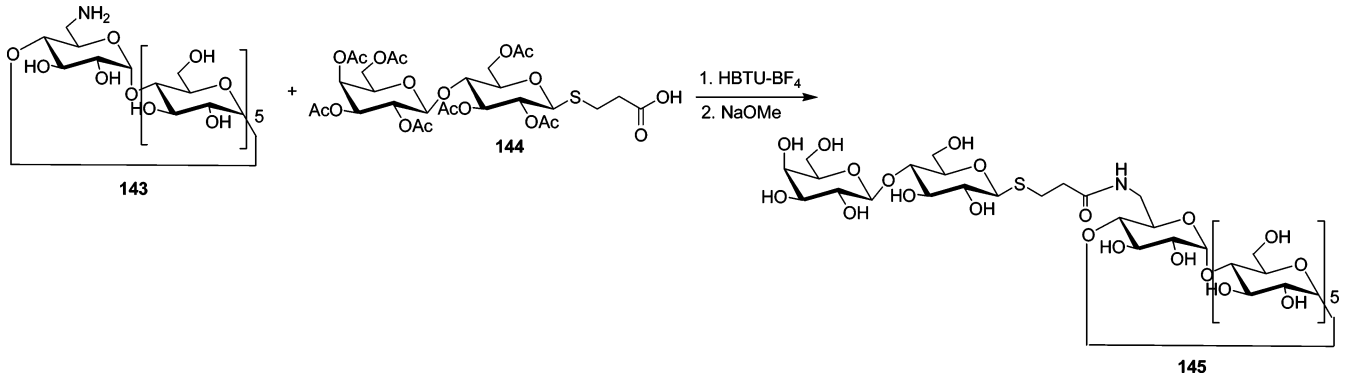
Stoddart and co-workers[50] have synthesized a variety of cyclodextrin derivatives branched at the 6-OH. For instance, the lactose bearing α-cyclodextrin **145** was synthesized by coupling the lactosyl propionic acid derivative **144** with the monoamino cyclodextrin **143** using HBTU-BF_4, followed by deacetylation with sodium methoxide in methanol (Scheme 40).

Another efficient approach involves the photoaddition of 1-thiosugars **139** and **147** to the *O*-allyl ether functions of per-6-*O*-allyl **146**, per-2-*O*-allyl **149,** and per-2,6-*O*-diallyl-β-cyclodextrin **151**, as depicted in Scheme 41, thus affording the corresponding products **148**, **150**, and **152** in yields above 70% in all cases.[51−53]

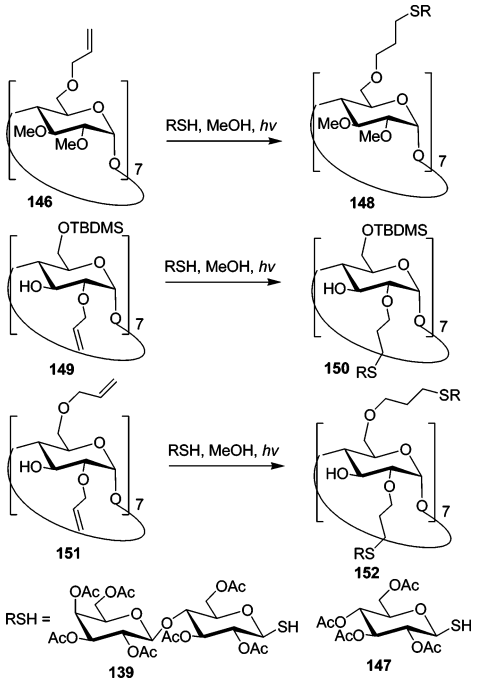
2.5.5. Sulfur-Bridged β-Cyclodextrin Dimers

Also some closely related S-connected cyclodextrins are presented here, although they do not contain a thioglycosidic linkage. Epoxide opening of β-cyclodextrin **153** with Na_2S in DMF afforded **154** and **155** in 30% and 13% yields, respectively (Scheme 42).[54]

Scheme 40

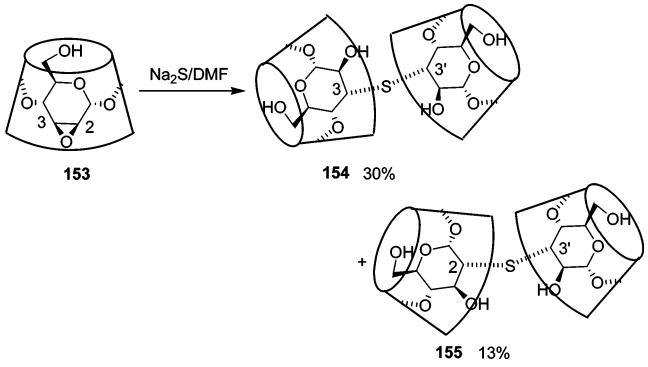


Scheme 41

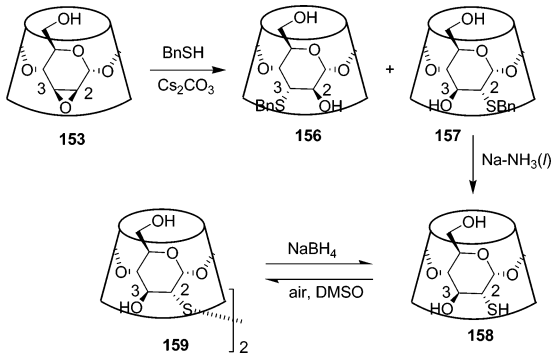


In another approach, epoxide **153** was opened with BnSH (→**156** and **157**), followed by debenzylation of product **157** to furnish β-cyclodextrin thiol **158**, which was dimerized oxidatively to **159** (Scheme 43).[55]

Scheme 42

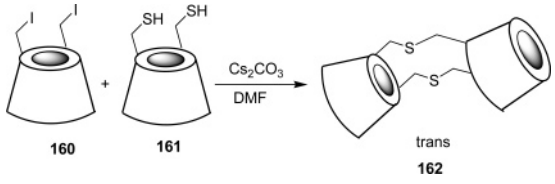


Scheme 43



Cross-coupling of **160** and **161** in the presence of Cs_2CO_3 in DMF afforded a single head-to-head coupled β-cyclodextrin dimer **162** with two sulfur linkages at adjacent 6-methylene carbons. NMR and X-ray analysis revealed the trans-type linkage of both β-cyclodextrin units (Scheme 44).[56]

Scheme 44

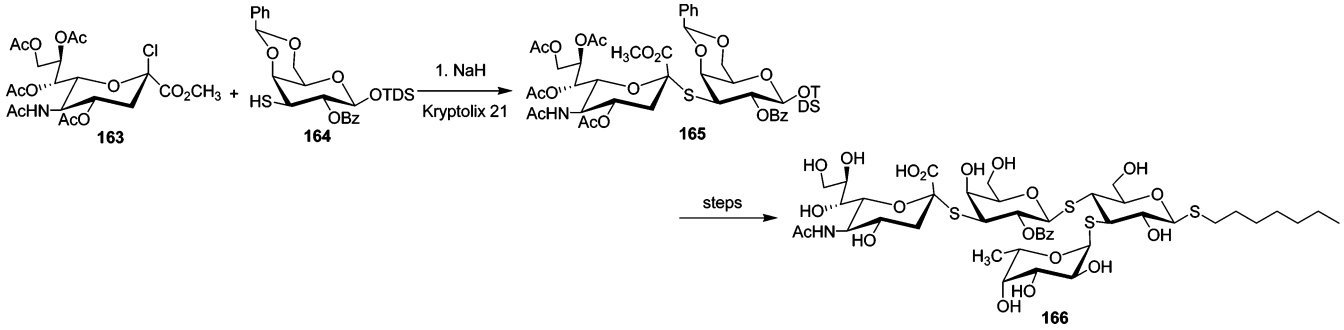


2.6. Synthesis of S-Linked Thiosialosides

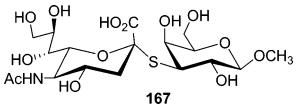
2.6.1. Synthesis of (2→3)-S-Linked Thiosialosides

Fully thio-linked sialyl Lewis^X analogue **166** is one of the most complex oligosaccharides synthesized to date to compare the conformation and the relative binding to selectins with the natural epitope (Scheme 45). Base-promoted (NaH) S-alkylation of thiosugar **164** with anomeric chloride of *N*-acetylneuraminic acid **163** in the presence of Kyrptofix 21 afforded the corresponding thio-linked product **165** in 75% yield. This was further converted to target compound **166**.[57,58]

Scheme 45



Von Itzstein et al.[59] applied this methodology in their synthesis of thiodisaccharide **167**.



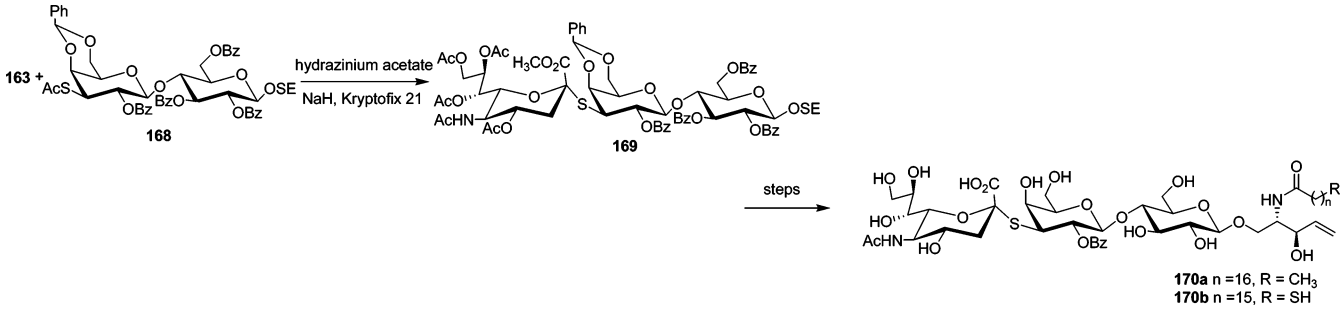
Based on this approach, Bundle et al.[60] have synthesized several S-linked ganglioside GM_3 analogues to allow for their incorporation into carbohydrate−protein congjugates for use as conjugate vaccines. The reaction involves a coupling of sugar thiol **168** with the anomeric chloride of *N*-acetyl neuraminic acid **163** in the presence of NaH and Kryptofix 21 affording product **169** in 65% yield (Scheme 46). This was further converted to S-linked ganglioside analogues **170a** and **170b**. In addition, the authors mentioned that the intermediates **168** and **169** would serve as advanced synthetic precursors to other ganglioside analogues such as GM_2, GM_1, and GD1a.

Field and co-workers[61] have reported an alternative method for the synthesis of such type of compounds. Reaction of *N*-acetylneuraminic acid thioacetate **171** and 1.3 equiv of triflate **172** in the presence of diethylamine in DMF gave the desired thiodisaccharide **173** in 68% yield, along with 14% unchanged triflate **172** and the galactofuranose disulfide **174** in 4% yield (Scheme 47). Disaccharide **173** was treated with TFA, followed by acetylation; this way compounds **175** and **176** were obtained in a ratio of 4:1.

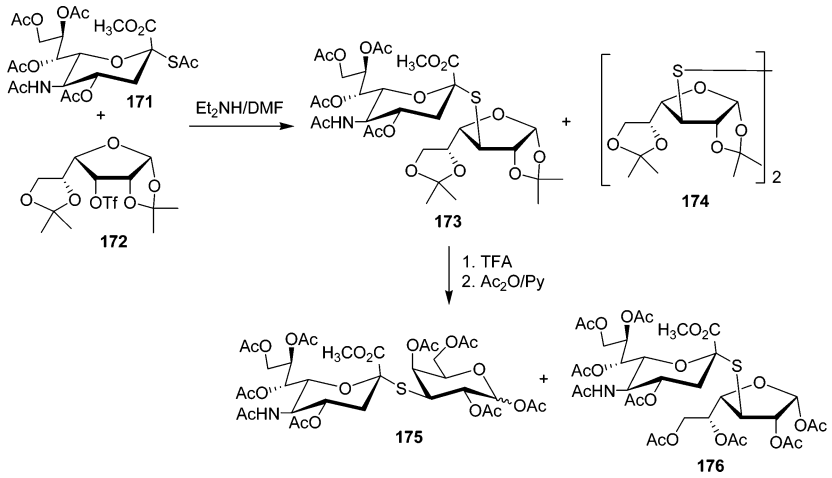
2.6.2. Synthesis of (2→6)-S-Linked Thiosialosides

Von Itzstein et al.[62] have developed a new synthesis and purification method for the α-(2→6)-S-linked thiosialosides that involves the selective in situ thiodeacetylation of the thioacetyl-Neu5Ac derivative **171** using diethylamine and coupling of the resultant thiolate with activated acceptors such as **177** or **178** (Scheme 48). Coupling proceeds efficiently to produce the corresponding S-linked products **179a** and **179b**. However, these products were contaminated with the eliminated product **180**, which arises during the preparation of the starting material **171**. Generally, this impurity is not easily separable by chromatographic methods at this stage. Even after coupling steps, the products and the

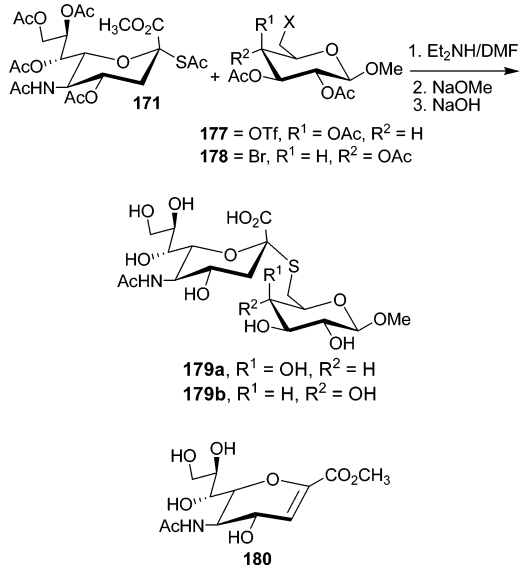
Scheme 46



Scheme 47



Scheme 48



eliminated products have similar chromatographic behavior. To find a solution to this problem, the authors introduced a two-step deprotection strategy, (i) NaOMe treatment and (ii) methyl ester hydrolysis using NaOH. Accordingly, the thiosialosides were treated with NaOMe in methanol to give the desired methyl esters along with the impurity **180**. At this stage, the products and **180** were separable by HPLC. Then the methyl esters were hydrolyzed using NaOH to yield the corresponding carboxylic acid **179a** and **179b**.

2.7. Thioglycoside-Derived Nucleosides

2.7.1. CMP–Sialic Acid Analogues

Halcomb and co-workers[63] have synthesized an anomeric sulfur analogue of CMP-sialic acid to improve the synthetic utility of sialyltransferases to sialylate oligosaccharides and glycoconjugates (Scheme 49). The reaction involves tetrazole-promoted activation of phosphoramidite **182** in the presence of glycosylthiol **181** at −40 °C affording the corresponding thiophosphite product, which was oxidized in situ at −40 °C with dimethyldioxirane to give the corresponding product **183** in 80% yield as a 2:1 mixture of diastereomers. The final product **184** was obtained after cleavage of the protecting groups.

2.7.2. UDP–Sugar Analogues

Recently, we reported the synthesis of thioglycoside derived UDP−sugar derivatives **187a**−**c** as potential substrate-based inhibitors (Scheme 50). The glycosylthiomethyl (GTM) phosphonates **186a**−**c** should mimic the naturally occurring glycosyl phosphates, which play a vital role in carbohydrate metabolism. Arbuzov reaction of *O*-acetyl-protected glycosylthiomethyl chlorides **185a**−**c** with triethyl phosphite and then phosphonate ethyl ester cleavage with trimethylsilyl bromide afforded glycosylthiomethyl phosphonates **186a**−**c**. These intermediates were coupled with UMP-morpholidate in the presence of tetrazole, followed by deacetylation to afford the corresponding UDP−sugar analogues **187a**−**c** in good yields.[64]

2.8. Thiooligosaccharides as Acceptors for Enzymes

Southwick and co-workers[65] have tested the activity of Nod proteins to create Nod factor analogues built on thio-linked *N*-acetylglucosamine backbones (thiochitooligosaccharides) rather than chitooligosaccharide backbones (Figure 1A,B). Authentic Nod factors are susceptible to degradation

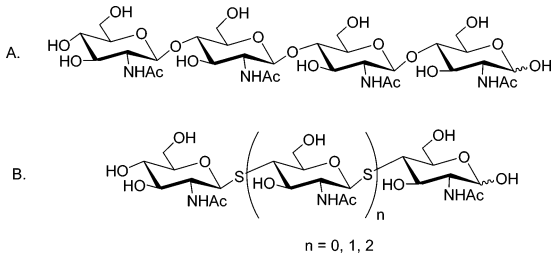
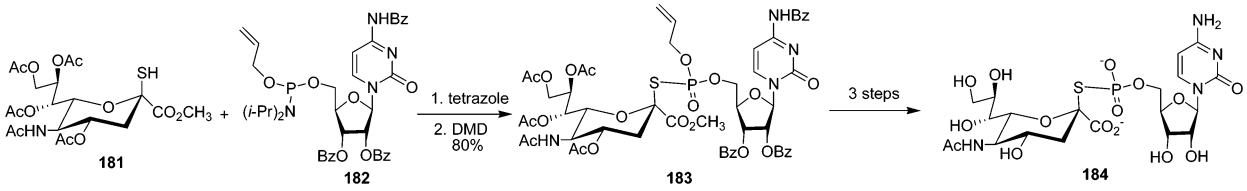


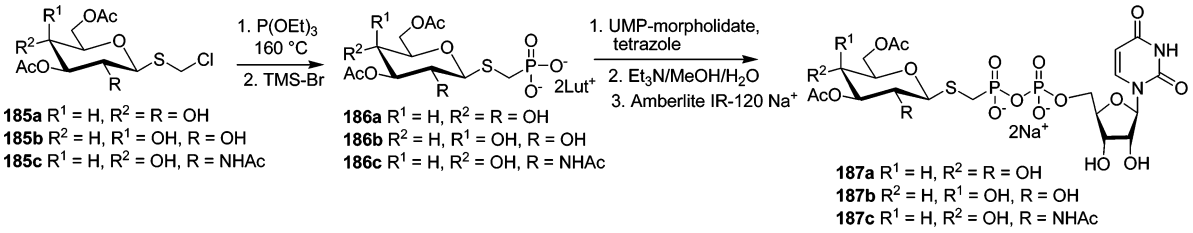
Figure 1. Chitotetrasaccharide and thiochitooligosaccharides employed in Nod factor studies.

in vitro by chitinases and lysozymes. The thio linkage is resistant to hydrolysis by chitinases; thus, the successful synthesis of thiooligosaccharide Nod factor analogues will prove that the host plant uses chitinase degradation in the process of specific Nod factor recognition and response. The authors demonstrated that NodA, NodB, and NodH are active in modifying thiochitooligosaccharide backbones, thus allowing the synthesis of chitooligosaccharide Nod factor analogues. The activity of NodA and NodB on the reducing thiochitooligosaccharides showed a preference for tetrameric substrates rather than the shorter oligomers observed with

Scheme 49



Scheme 50



natural substrates. These enzymes were also able to modify the thiochitooligosaccharide substrates used in this study. The reaction end product with the reducing thiochitotetramer is a novel Nod factor analogue with an oligosaccharide backbone predicted to be resistant to chitinase enzymatic hydrolysis.

Snaar-Jagalska and co-workers[66] reported in vivo stimulation of embryonic zebra fish cells ZF13 and ZF29 with chitin tetrasaccharides at 10^{-9} M concentration, which transiently induced activation/phosphorylation of extracellular regulated kinases (EFKs) with a maximum after 15 min. Furthermore, the biological specificity of chitin tetrasaccharides and various derivatives was examined. The replacement of one or two GlcNAc residues of the chitin backbone by glucose and fucosylation of chitin tetrasaccharides at the reducing end caused a complete loss of their activity. On the other hand, thiochitotetrasaccharide was as potent an inducer as chitin tetrasaccharide, excluding a role of possible degradation products.

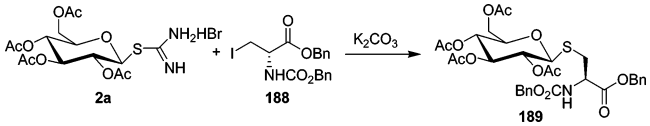
3. Synthesis of Thio-Linked Glycopeptides and Glycoprotein Mimetics

3.1. Synthesis of Thio-Linked Glycosyl Amino Acids and Glycopeptides

3.1.1. Anomeric Thiol Group S-Alkylation

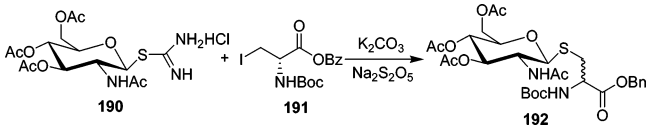
1-Thio sugar **2a** was coupled with β-iodoalanine **188** under basic conditions (K_2CO_3, DMSO/H_2O) to afford the corresponding S-linked sugar amino acid **189** in 72% yield (Scheme 51).[67]

Scheme 51



Similar reaction conditions (K_2CO_3, $Na_2S_2O_5$/acetone/H_2O) were employed to link 1-thio GlcNAc **190** with β-iodoalanine **191** (Scheme 52).[68] Under these reaction conditions some

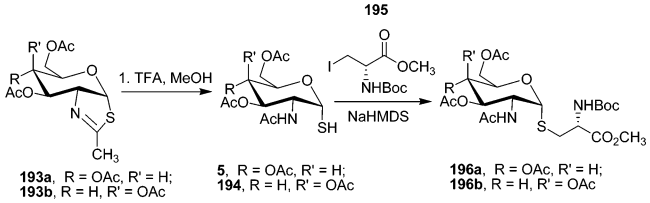
Scheme 52



of the iodo derivative **191** underwent β-elimination to give the α,β-unsaturated ester. This served as Michael acceptor, which produced a mixture of two diastereomers of **192** (L and D, 5:1). However, coupling of 1-thio sugar **190** with iodoalanine **191** in the presence of a phase transfer catalyst (Bu_4NI in CH_2Cl_2/H_2O) gave the corresponding S-linked glycosyl amino acid **192** in 34% yield without epimerization.

Knapp and co-workers,[69,70] reported a novel method for the synthesis of α-GlcNAc **5** and α-GalNAc **194** thiols from the respective thiazolines **193a** and **193b**, which were derived from their acetate derivatives using Lawesson's reagent (Scheme 53). Alkylation of α-GlcNAc **5** and α-GalNAc **194** thiols with *N*-Boc β-iodoalanine methyl ester **195** in the presence of NaHMDS provided the corresponding α-thio linked glycosyl amino acids **196a** and **196b** (Scheme 53).

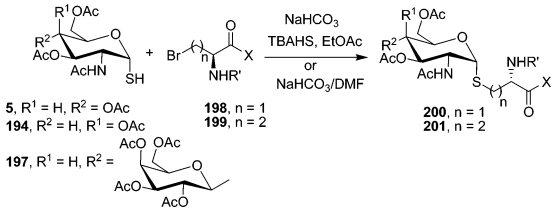
Scheme 53



Also, the synthesis of various thio-linked α-GlcNAc and α-GalNAc conjugates is described.

A similar glycosylthiol S-alkylation approach was employed by Schmidt et al. to effect the attachment of the sugar unit to the peptide backbone under phase transfer conditions.[71] Coupling of α-GlcNAc thiol **5**, β-GlcNAc thiol, α-GalNAc thiol **194**, and β-lactosyl thiol **197** with various β-bromoalanine **198** and γ-bromohomoalanine **199** containing peptides in the presence of tetra-*n*-butylammonium hydrogen sulfate (TBAHS) in ethyl acetate and an aqueous solution of $NaHCO_3$ at pH 8.5 produced the corresponding thio-linked glycopeptides **200** and **201** in good yields (Scheme 54). The above reaction could also be performed

Scheme 54

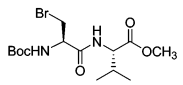
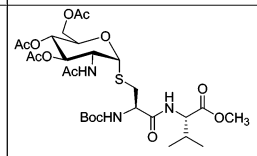
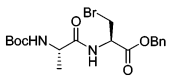
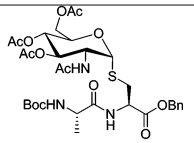
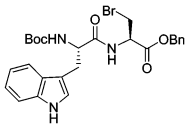
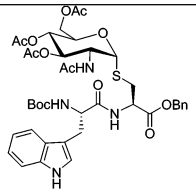
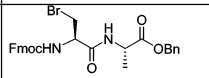
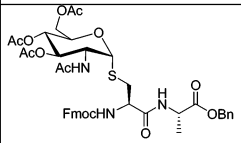
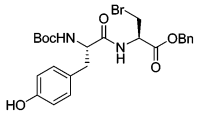
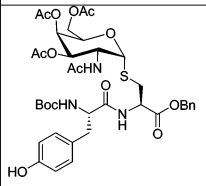
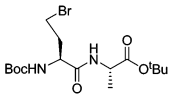
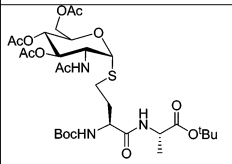
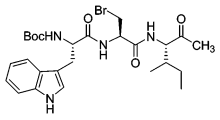
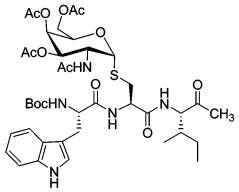
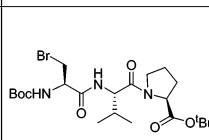
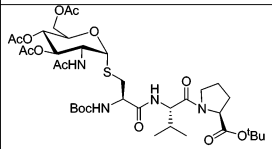
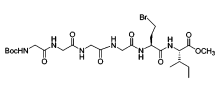
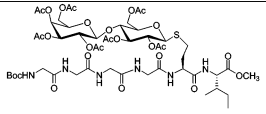


in a mixture of DMF/H_2O in the presence of $NaHCO_3$ at pH 8.5. These conditions worked efficiently for more complex peptides. Due to the pH of the medium, racemization was not observed under the above reaction conditions. These conditions also worked well for Fmoc-protected peptides. Some representative examples are listed in Table 1. It is noteworthy to mention that reaction of O-unprotected 1-thio sugar **202** with β-bromoalanine containing tripeptide **203** afforded the corresponding product **204** in 70% yield, as shown in Scheme 55.[72] Furthermore, the syntheses of several S-farnesylated and S-palmitoylated peptide conjugates including lipidated N-Ras hexapeptide have been achieved by employing β-bromoalanine containing peptides as electrophiles.[73]

Wong et al.[74] have slightly modified this methodology to a two-step one-pot reaction of BrAla derivative with in situ generated sugar thiolate to generate the thio-linked glycosyl amino acid products (Scheme 56).

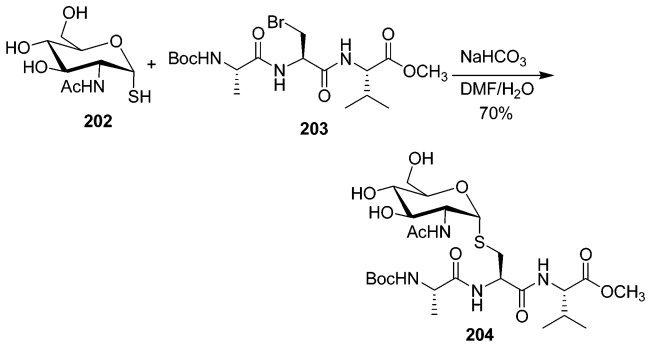
The condensation of thiolate salt **206**, which was prepared in situ by selective deacetylation of **205** with NaOH in MeOH (pH $\approx$ 7.5), with β-bromoalanine **207**/**208** in dry DMF gave the desired S-linked glycosyl amino acid derivatives **209**/**210** (Table 2). These reaction conditions worked well at room temperature for *N*-Boc-protected bromoalanine and at −78 °C for *N*-Fmoc-protected bromoalanine without any β-elimination of the bromoalanine. The authors have employed one of the S-linked glycosyl amino acids **209a** as Fmoc building block to synthesize the more complicated glycopeptide **213**, which is a linear analogue of tyrocidin A (Scheme 57). Tyrocidine A is a cyclic cationic decapeptide anitibiotic produced in *Bacillus brevis*, which possesses an antiparallel amphipathic β sheet conformation in solution. To this end, compound **209a** was treated with $Pd(PPh_3)_4$ and

Table 1. Reaction of Bromoalanine-Containing Peptides with Glycosylthiols

Sugar nucleophile	Peptide electrophile	*S*-linked glycopeptide	Yield (%)
5			78
5			83
5			79
5			89
194			63
5			89
194			82
5			72
197			76

morpholine in dichloromethane to give the Fmoc-modified building block **211**, which was used for the solid-phase synthesis without purification to afford the protected S-linked glycodecapeptide **213** (Scheme 57). Also the synthesis of a cyclic glycopeptide analogue of tyrocidine A was achieved. This method works for both *N*-Boc- and *N*-Fmoc-protected bromoalanine. However, the authors have not really tested this methodology for the chemoselective coupling of sugar

Scheme 55



Scheme 56

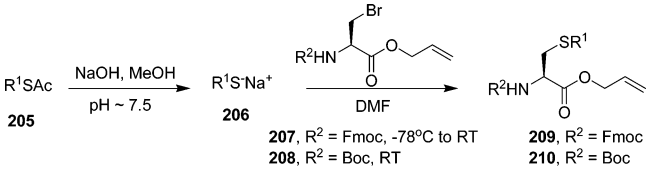
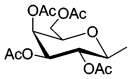
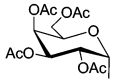
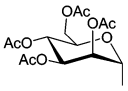
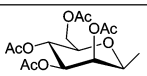
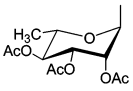
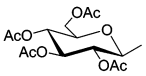
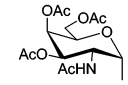
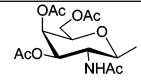
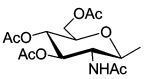
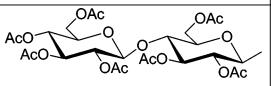


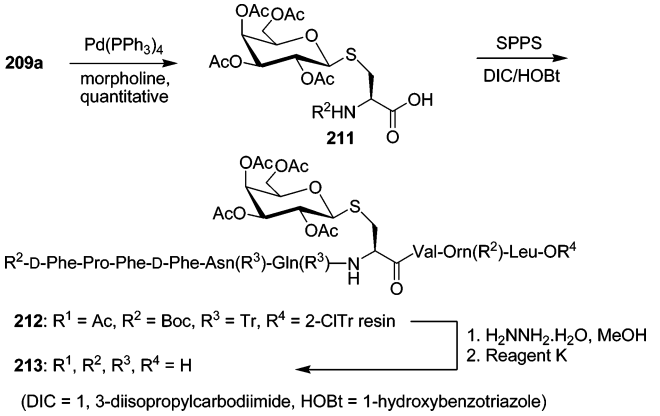
Table 2. Reaction of Glycosyl Thioacetates with Allyl Bromoalanine

R^1SAc	R^1	R^2 = Fmoc (Yield, %)	R^2 = Boc (Yield, %)
205a		**209a** (79)	
205b		**209b** (74)	
205c		**209c** (77)	
205d		**209d** (81)	
205e		**209e** (75)	
205f		**209f** (71)	
205g		**209g** (66)	**210g** (75)
205h		**209h** (68)	**210h** (70)
205i		**209i** (71)	**210i** (78)
205j		**209j** (78)	

thiolate with bromoalanine containing polypeptides.

Jobron and Hummel[75] described the use of unprotected sugar 1-thiolates as the nucleophile on solid phase for couplings with iodine-activated Fmoc-protected amino acids (Scheme 58). Trityl chloride derivatized resin bound thiol

Scheme 57



Scheme 58

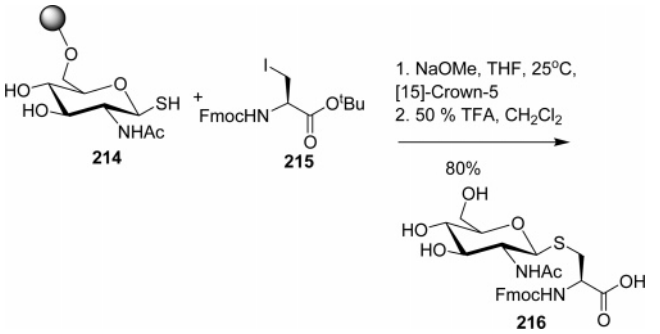
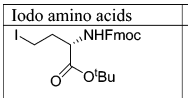
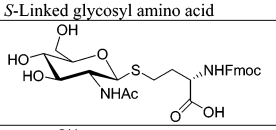
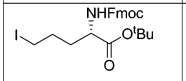
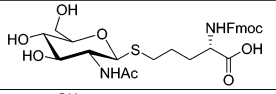
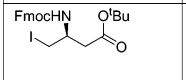
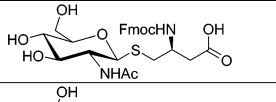
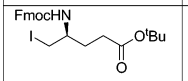
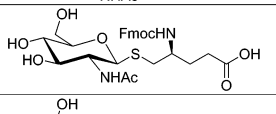
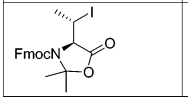
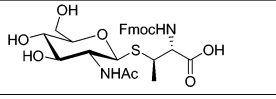


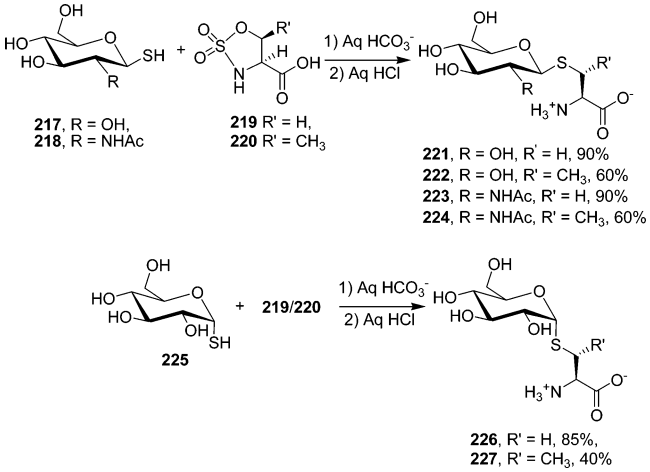
Table 3. Reaction of Iodo-Substituted Amino Acids with Glycosylthiols

Iodo amino acids	*S*-Linked glycosyl amino acid	Yield (%)
		76
		82
		75
		76
		50

214 was coupled in the presence of NaOMe and 15-crown-5 with iodine-activated amino acid **215**, thus affording the S-linked glycosyl amino acid **216**, after resin and *tert*-butylester cleavage using 50% trifluoroacetic acid in dichloromethane, in 80% yield. Various S-linked glycosyl amino acids were synthesized using this approach in good yields (50−79%); the examples are listed in Table 3. This method led to the synthesis of both glycosyl serine and threonine analogues on solid support.

Halcomb et al.[76,77] reported an efficient method for the synthesis of S-linked glycosyl amino acids using 1-thio sugars in aqueous buffer solution. In this way, both S-linked glycosyl serine and S-linked glycosyl threonine conjugates were obtained (Scheme 59). Reaction of 1-thio sugars **217**, **218**, and **225** with pre-prepared cyclic sulfamidates **219/220** under basic buffer conditions afforded the corresponding

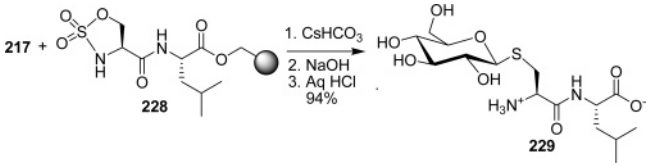
Scheme 59



glycosyl amino acids **221**−**224** and **226** and **227** in excellent yields (40−90%) following acid hydrolysis.

The cyclic sulfamidate moiety was then incorporated into peptides in solution and on solid support to generate reactive peptide substrates for chemoselective ligation with an unprotected 1-thio sugar. 1-Thio sugar **217** was added to cyclic sulfamidate **228** bound to a modified polystyrene resin under basic conditions followed by acid treatment to afford the S-linked glycopeptide **229** in almost quantitative yield (Scheme 60). Although, this method requires two additional

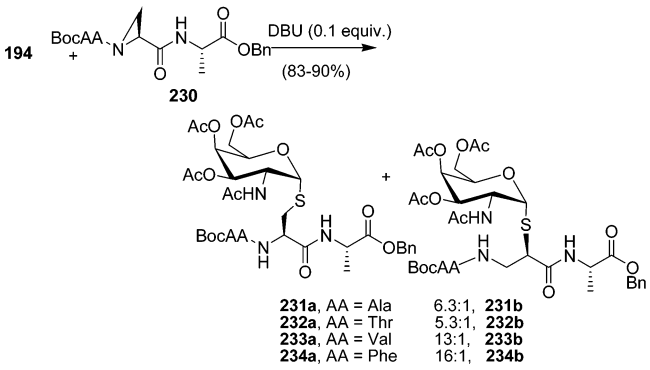
Scheme 60



steps (attachment to the solid support and hydrolysis) to obtain the expected S-linked products, it has the potential to yield analogues of both S-linked glycosyl serine conjugates and also S-linked glycosyl threonine conjugates, which are less readily obtained. This method is limited to monosaccharides because the use of strong acid for the cleavage of the sulfate group can also cleave the glycosidic bond.

The synthesis of several S-linked glycopeptides was described by opening of electrophilic aziridine-2-carboxylic acids with nucleophilic 1-thio sugars promoted by DBU (Scheme 61).[78,79] 1-Thio sugar **194** was added to aziridine-

Scheme 61

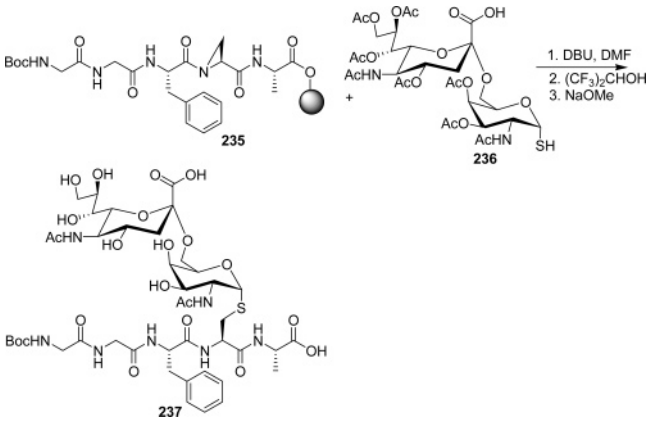


2-carboxylic acid containing peptides **230** in the presence of catalytic amounts of DBU (0.1 equiv), which provided a

regioisomeric mixture of S-linked glycopeptides in good yields strongly favoring the α-amino acid products **231a**−**234a** over the β-isomer **231b**−**234b**.

To demonstrate the versatility of this reaction, the aziridine-2-carboxylic acid was also incorporated into peptides using solid-phase synthesis. For example, ligation of more complex 1-thio-ST_N-antigen **236** with polymer-supported pentapeptide **235** proceeded with high efficiency providing S-linked glycopeptide **237** after release from solid support and removal of the protecting groups (Scheme 62). One

Scheme 62



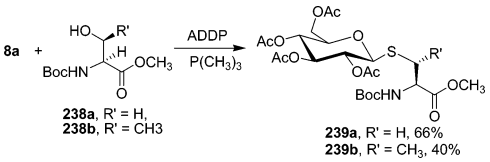
limitation of this method is the formation of two isomeric products. The advantage of this method is that it works well in solution as well as on solid support.

3.1.2. Mitsunobu Condensation

As a variation of anomeric S-alkylation, standard Mitsunobu conditions were utilized both in solution and on solid support to transform alcohols under mild conditions into thioglycosides in good yields.

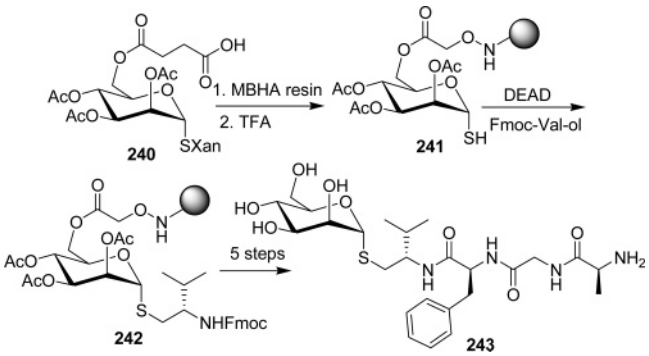
Glycosylthioamino acids **239a** and **239b** were synthesized by displacing the hydroxy groups of *N*-Boc-protected serine methyl ester (**238a**) and *N*-Boc-threonine methyl ester (**238b**) by 1-thiosugar **8a** in the presence of 1,1′-azo-dicarbonyl-dipiperidine (ADDP) (Scheme 63). Yields of these reactions were moderate (40−66%).[80]

Scheme 63



The same group later performed this reaction on a solid-phase system and synthesized C-terminal thio-linked glycopeptides by a linear approach (Scheme 64).[81] After several unsuccessful attempts with different linkers, the mannosyl building block was successfully attached with a succinate linker at the 6-hydroxy group, obtained by reaction with succinic anhydride. This linker proved to be stable to the conditions of *S*-xanthosyl deprotection and peptide chain extension; it could be cleaved using NaOMe along with the removal of *O*-acetyl protecting groups. Xanthosyl (Xan) thioglycoside **240** was loaded onto 4-methylbenzhydroxylamine (MBHA) derivatized polystyrene resin. After removal of the *S*-Xan protecting group (→ **241**), it was reacted smoothly with 2-fold excess of Fmoc-Val-ol under Mitsunobu conditions affording the corresponding S-linked product **242**. After *N*-Fmoc deprotection, the peptide chain was extended using standard *N*-Fmoc-based solid-phase peptide synthesis (SPPS) to yield glycopeptide mimetic **243**.

Scheme 64



According to the authors, this method was very efficiently applied to simple glycopeptides containing no side-chain functionality. A slight modification of the previous reaction conditions was employed to connect the thio GlcNAc **128** with *N*-Boc-protected serine benzyl ester without epimerization at the α-carbon of the amino acid.[68]

3.1.3. Michael-Type Addition as a Variation of Anomeric S-Alkylation and Base-Promoted S-Glycosylation

A convergent approach to the synthesis of several S-linked glycopeptides by Michael-type addition of 1-thio sugars to dehydroalanine-containing peptides under basic conditions was recently described in solution and on solid support (Scheme 65).

Mild oxidation of the selenide **244** and elimination of the selenoxide provided dehydropeptide **245**.[82] Subsequent addition of *O*-acetyl protected 1-thio sugars and O-unprotected 1-thio sugars afforded the corresponding S-linked glycopeptides **246** as mixtures of diastereomers in good yields (62−74%). Wang resin-bound dehydroalanine-containing peptides **247** were reacted with unprotected 1-thio sugar (**217**) in the presence of Et_3N followed by removal of the resin to afford the corresponding S-linked product **248** in 45% yield (Scheme 66).

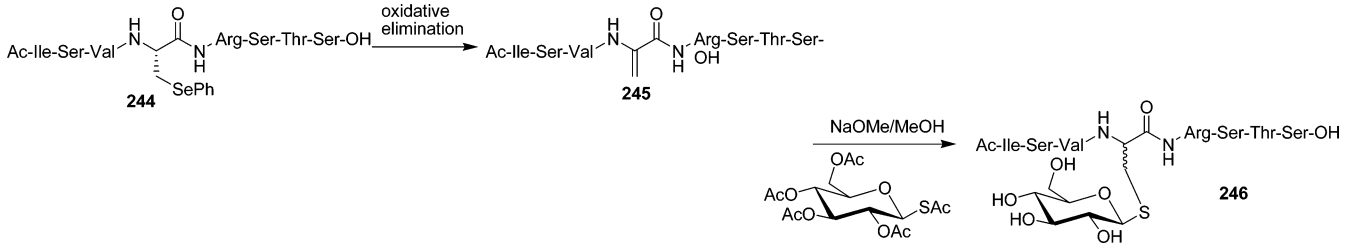
Hindsgaul and co-workers[83] have synthesized libraries of S-linked glycosyl amino acids via Michael-type addition to evaluate their biological activities. The connection of the sugar unit and the amino acids was performed with the help of a hydrophobic tether, for instance, with cyclohexenone, in two steps. Addition of 1-thio sugar **249** to cyclohexenone followed by reductive amination with amino acids such as tryptophan methyl ester afforded a diastereomeric mixture of four products (**250**) after deprotection (Scheme 67). The use of *O*-laurate protecting groups for the 1-thio sugar allowed for purification of the reaction products by means of the solid-phase extraction method.

Kessler and co-workers[84] synthesized S-linked 2-deoxy glycosylamino acids by employing Michael-type reaction (Scheme 68). Reaction of protected cysteine **252** with hex-1-en-3-ulose **251** in the presence of ZnI_2 gave stereoselectively α-*S*-uloside **253** in 41% yield.

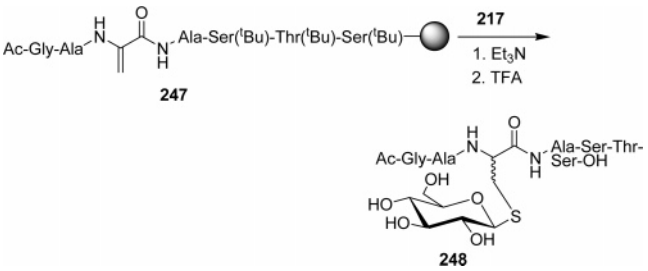
3.1.4. Acid-Catalyzed S-Glycosylation

Lewis acid promoted glycosylation is a traditional method to synthesize a variety of glycosylated products. This method has been employed to glycosylate the reactive mercapto

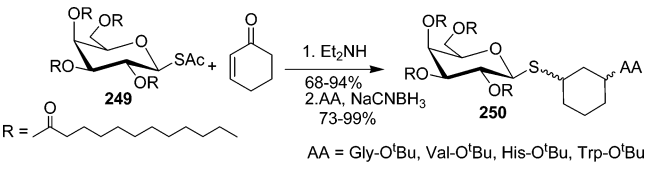
Scheme 65



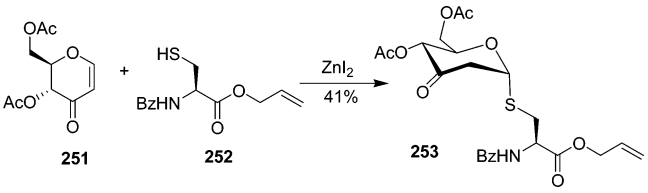
Scheme 66



Scheme 67

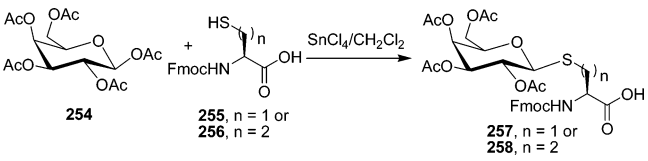


Scheme 68



groups in Fmoc-protected amino acids (cysteine or homocysteine) with 1,2-*trans*-1-*O*-acetyl sugars as glycosyl donors (Scheme 69).[85,86]

Scheme 69



The glycosylation of *N*-Fmoc-cysteine **255** and -homocysteine (Hcy) **256** with β-D-galactose pentaacetate **254** was investigated with $BF_3{\cdot}Et_2O$ and $SnCl_4$ as promoters in dichloromethane or acetonitrile. The use of $SnCl_4$ in dichloromethane was found to give significantly better yields of compounds **257** and **258** than the use of $SnCl_4$ in acetonitrile or $BF_3{\cdot}Et_2O$ in dichloromethane.

Galactosyl ceramide (GalCer) glycosphingolipid recognizes the V3 loop of HIV gp120, which plays a key role in the fusion of the HIV envelope and cellular membrane. Analogues of GalCer were synthesized to inhibit HIV uptake and infection. S-Linked galactosyl derivatives have a higher affinity for the HIV-1 gp120 relative to β-*O*-galactosyl derivatives, and they possess higher stability of the glycosidic bond toward enzymatic hydrolysis.[87]

L-Cysteine derivatives **259** and **260** were prepared in two steps from Fmoc- and Cbz-protected cysteine, respectively, and glycosylated with pentaacetyl galactopyranose **254** in the presence of $BF_3{\cdot}Et_2O$, thus affording the products **261** and **262** in 93% and 78% yields, respectively (Scheme 70). Further, S-linked glycopeptides **261** and **262** were transformed via chain extension and deprotection into derivatives **264** and **265**.

Lewis acid promoted S-glycosylation of **259** with glycosyl donor **266** afforded the corresponding product **267** in 56% yield, which was further transformed into **268** (Scheme 71). However, none of the Gal−Cys derivatives were found to be biologically active.

Recently, glycosylation of *N*-acetyl-L-cysteine ethyl ester with penta-*O*-acetyl galactopyranose **254** in the presence of $BF_3{\cdot}Et_2O$ was reported to give the corresponding protected product in 45% yield. Deprotection with 10% Et_3N in methanol gave 68% of **269**, with partial racemization at the α-carbon of the cysteine moiety (Scheme 72).[88] Glycosyl fluoride has been employed as a glycosyl donor for thio-linked glycosyl amino acid synthesis under similar reaction conditions.[89]

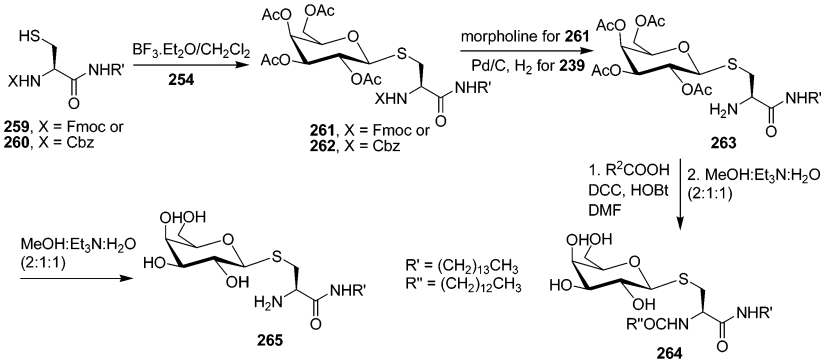
The synthesis of S-linked glycosyl amino acid by S_N2 displacement of anomeric halides by the sulfhydryl group of cysteine has also been reported. Kessler et al.[90,91] employed Koenigs−Knorr conditions to glycosylate the thiol group of cysteine **271** with O-acetylated cellobiosyl bromide **270**, thus affording S-linked glycosyl amino acid **272** in 85% yield (Scheme 73).

3.1.5. Base-Promoted S-Glycosylation in Combination with Native Chemical Ligation (NCL)

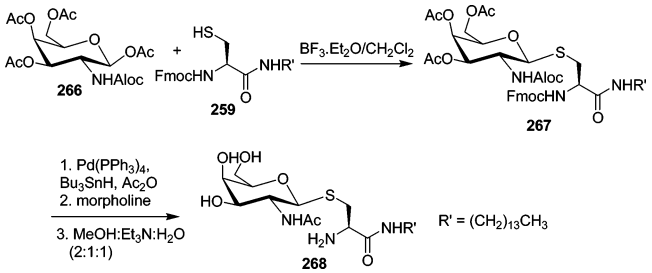
The S_N2 displacement approach has several advantages due to the convenient preparation of starting materials: (i) Glycosyl halides are readily prepared from the peracetylated sugars and (ii) cysteine-containing peptides can be easily synthesized and have gained special attention after the emergence of native chemical ligation (NCL) (Figure 2). Native chemical ligation using peptide segments, often obtained by solid-phase peptide synthesis (SPPS), has greatly facilitated the synthesis of proteins and protein conjugates. The technique involves a mixture of two peptide segments under aqueous conditions in which the C-terminal peptide contains a pre-prepared thioester unit and the N-terminal peptide contains a cysteine residue.[92,93] The first step comprises the formation of a reversible thioester-linked intermediate and the second step irreversible rearrangement through a five- or six-membered transition state to form a native Xaa−Cys peptide bond (Figure 2, $n = 1$ or 2).[94,95]

Recently, we have synthesized several cysteine-containing peptides in pure form via reversed native chemical ligation[96,97] (Figure 2). The designed concept having an *N*-Boc-protected β-bromo-alanyl residue at the N-terminus should avoid any byproduct formation. In addition, this method

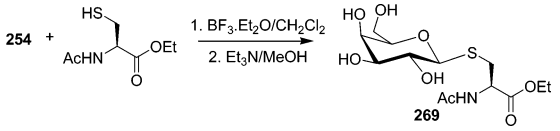
Scheme 70



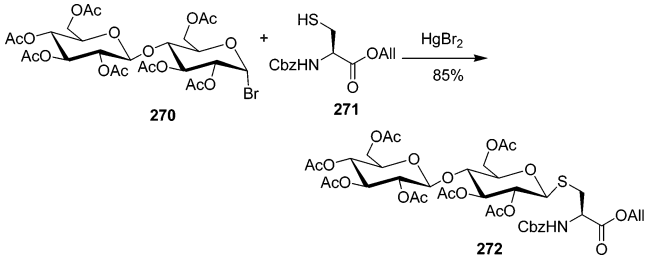
Scheme 71



Scheme 72



Scheme 73



should be applicable to the synthesis of homocysteine (Hcy)-containing peptides and to S-alkylation.[97]

The principle of this reversed chemical ligation is shown in Figure 2: the first step is the chemoselective reaction of a peptide α-thio acid with another peptide segment containing an *N*-Boc-protected terminal *β*-bromoalanine [Ala(Br)] or a *γ*-bromohomoalanine [Hal(Br)] residue to give a stable thioester intermediate under neutral conditions in an irreversible reaction. The second step involves removal of the *N*-Boc group, thus furnishing an intermediate that is identical with the one in native chemical ligation. The ensuing third step is the rearrangement of the thioester intermediate in Tris buffer (pH ≈ 7) to form cysteine- or homocysteine-containing peptides. The formation of any of byproducts was eliminated by first isolating and purifying the stable thioester intermediate and then transforming it into the corresponding peptide.

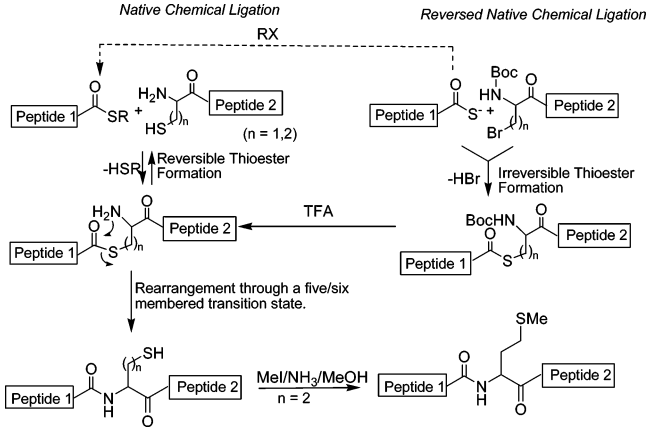


Figure 2. Native chemical ligation and reversed native chemical ligation.

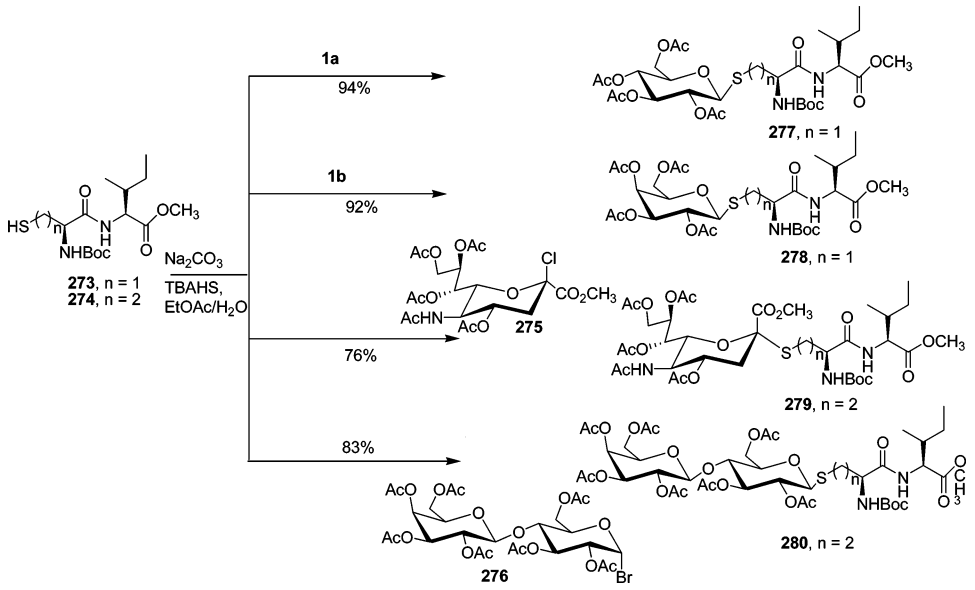
Very recently,[98] cysteine- and homocysteine-containing peptides were S-glycosylated for the first time with a variety of glycosyl donors via S_N2 displacement of their bromides under aqueous basic conditions. Thus, reaction of the mercapto group bearing cysteine- and homocysteine-containing dipeptides **273** and **274** and tripeptide **281** were coupled with sugar anomeric halides **1a**, **1b**, **275**, and **276** in the presence of a $NaHCO_3$/TBAHS/ethyl acetate/H_2O or Na_2CO_3/DMF/H_2O system affording the corresponding S-linked glycopeptides **277**−**280**, **282**, and **283** in yields ranging between 52% and 95% (Schemes 74 and 75). The two-phase system worked well for dipeptide coupling with glycosyl halides, whereas the single-phase system worked well for tripeptides. The different behavior of dipeptides and tripeptides is caused by differences in solubility. Therefore, the procedure was modified by changing the solvent from ethyl acetate to DMF as shown in Scheme 75. When these reaction conditions were employed, several thio-linked glycopeptides were efficiently synthesized.

Native chemical ligation followed by *S*-glycosylation is a versatile method for S-linked glycopeptide synthesis. To this end, homocysteine-containing tripeptides **284** and **289**, which were prepared by employing the chemical ligation approach, were glycosylated in the presence of Na_2CO_3 with sugar halides **1a**, **276**, and **285** to afford the corresponding S-linked glycopeptides **286**−**288** and **290** in yields ranging between 40% and 55% (Schemes 76 and 77).

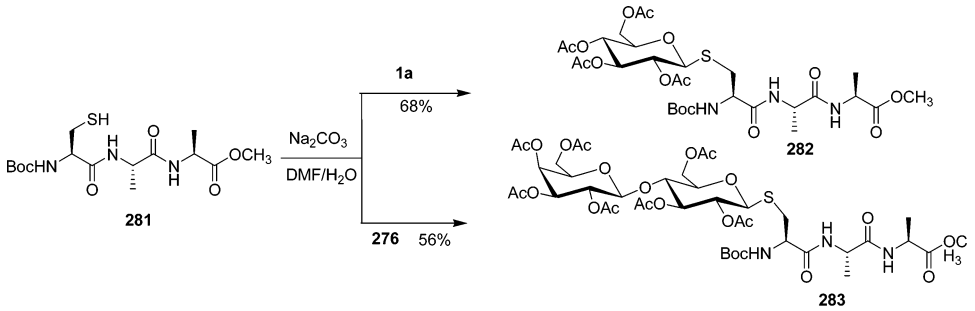
Based on this approach, the synthesis of an S-linked glycopeptide analogue carrying two sugar residues derived from Tamm−Horsfall glycoprotein (Figure 3a), the most abundant glycoprotein present in normal human urine, was achieved.[99] This S-linked glycopeptide analogue (Figure 3b) corresponds to the sequence Ala484−Ala490 in Tamm−Horsfall glycoprotein, containing Thr485 as potential O-glycosylation site and Asn489 as N-glycosylation site.

Chemoselective coupling of the sulfhydryl group of cysteine-containing tripeptide **291** obtained by NCL with

Scheme 74



Scheme 75



lactosyl bromide **276** in the presence of aqueous Na_2CO_3 in DMF provided the product **292** in 65% yield (Scheme 78). Fragment **295** was obtained in 90% yield by coupling of homocysteine-containing dipeptide **294** with *N*-Troc-protected 2-amino glucosyl bromide **285** under phase transfer conditions. The target glycopeptide analogue (Figure 3b) was obtained by connecting glycopeptide fragments **293** and **296** in the presence of PyBOP and DIPEA in DMF followed by cleavage of the protecting groups.

The advantages of this method are the chemoselectivity and its usefulness in couplings of cysteine/homocysteine-containing larger peptides with sugar halides in aqueous DMF, which dissolves even large peptides.

3.1.6. Unusual Base-Promoted S-Glycosylation

An unusual reaction was reported for the synthesis of S-linked glycosyl amino acid **300** (Scheme 79). Reaction of phenyl 2-acetylthio-2-deoxy-1-thiomannopyranoside **297** with protected cysteine **298** in the presence of NaOMe in MeOH led to **300** in 25% yield. The reaction mechanism involves the formation of thiirane intermediate **299**, which acts as a sugar electrophile in a base-promoted S-glycosylation.[19]

Glycosylation of protected cysteine **302** with hydroxyimino sugar chloride **301** via S_N2 displacement has also been reported (Scheme 80). The glycosidation reaction was not stereoselective, affording both anomers **303** and **304** ($\alpha/\beta \approx 2{:}1$) with (*Z*)-configuration of the hydroxyimino group.[100] Oxime **303** had (*Z*)-configuration in chloroform and (*E*)-configuration in dimethyl sulfoxide as solvent.

Glycosyl amino acid isostere **308**, which differs in two atoms from the wild-type structure, was synthesized via chemoselective S_N2 reaction between mesylate **305** and a thiolate derived from cysteine **306**, followed by hydrogenation of intermediate **307** with Wilkinson catalyst, as shown in Scheme 81. It is noteworthy to mention that the reaction of **305** with **306** occurred with complete inversion at the mesylate-bearing stereocenter. Catalytic reduction of **307** provided the corresponding compound **308** as a single isomer in 28% yield.[101]

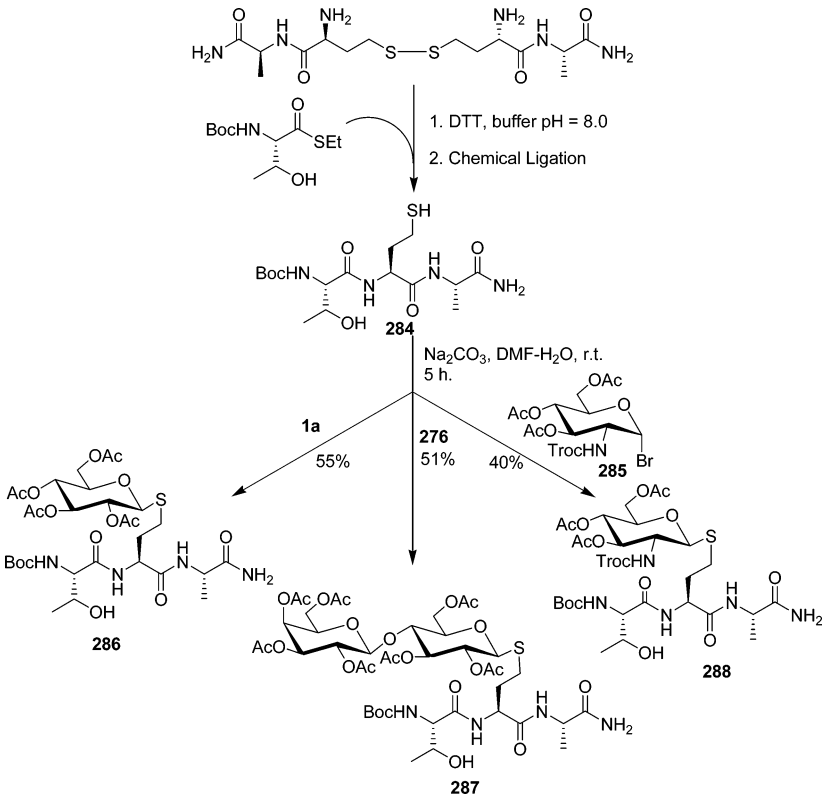
3.2. Synthesis of Thio-Linked Glycoprotein Mimetics

3.2.1. Ligation via Disulfide Linkage

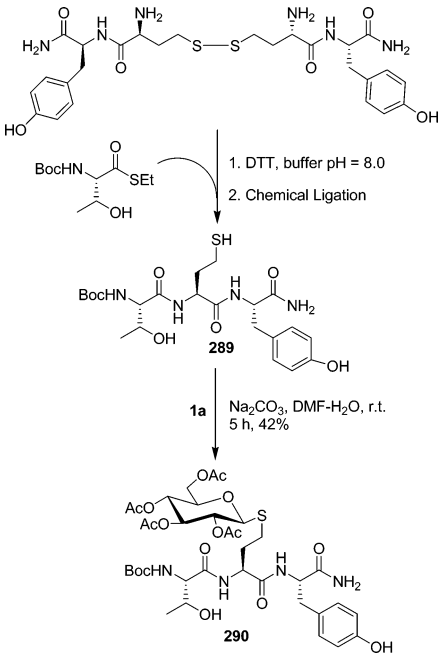
(a) A highly facile method for synthesizing disulfide linked glycopeptides and glycoproteins was reported by Boons et al.[102] Reaction of 1-thiol sugar **218** with 2,2′-dithiobis(5-nitropyridine) (DTNP) led to compound **309** in 58% yield (Scheme 82). This intermediate in which the 1-thio sugar acts as electrophile was reacted with protected cysteine, cysteine-containing peptides, and cysteine-containing protein (BSA, bovine serum albumin) affording the corresponding disulfide-linked glycoconjugates **310** (Table 4).

In a similar approach, Davis and others[103−106] have described the use of glycosyl methanethiosulfonates **7** or **311** (glyco-MTS) as another valuable addition to the cysteine-reactive glycosylthio group transferring reagents that are available for peptide and protein glycosylation (Scheme 83). They described a combined site-directed mutagenesis and

Scheme 76



Scheme 77



chemical modification approach using well-defined and highly specific glycosylating agents. The strategy involves the introduction of cysteine as a chemoselective tag at preselected positions within a given protein and then reaction of its thiol residue **312** with glycosyl methanethiosulfonate reagents.

Glycosyl methanethiosulfonates **7** and **311** were prepared by direct nucleophilic displacement of sugar halides with sodium methanethiosulfonate. Glyco-MTS **7** and **311** were reacted in aqueous buffer with four cysteine mutants of protein serine protease of subtilisin *Bacillus lentus* (SBL) prepared by site-directed mutagenesis (SBL-N62C, -S156C, -S166C, and -L217C). These reactions were rapid and quantitative. Partial deacetylation occurred during the glycosylation conditions, but it is substrate-dependent. For example, modification of L217C with reagent **7** at pH 9.5 was accompanied by complete in situ deacetylation, and the sole product was the fully deprotected glucosylated-SBL, L217C-*S*-β-Glu **313**. Furthermore, deacetylation was controlled by varying the pH of the reaction medium. The reaction of L217C with **7** at pH 7.5 and 5.5 yielded products in which the glucosyl residue introduced retained two and three acetate groups, forming L217C-*S*-β-Glc$(Ac)_2$ and L217C-*S*-β-Glc$(Ac)_3$, respectively. In contrast, treatment of cysteine mutant L217C with **311** under identical conditions resulted in no concomitant deacetylation during glycosylation; the fully protected glucosylated-SBL, L217C-*S*-β-GlcNAc$(Ac)_3$ **314**, was obtained.

(b) Davis and co-workers also reported a selenenyl-mediated glycoprotein synthesis, which allows ligation with mono- and oligosaccharides of up to seven saccharide units

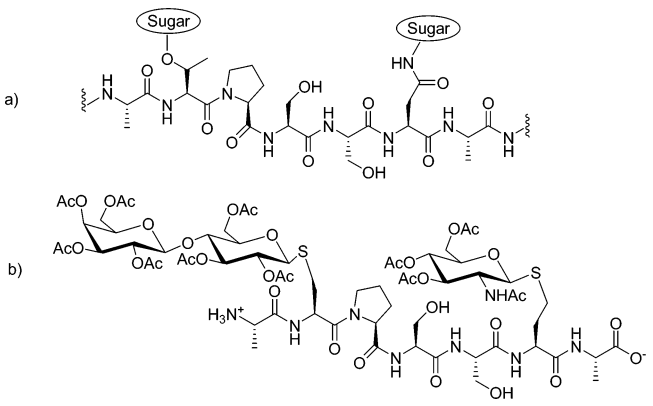
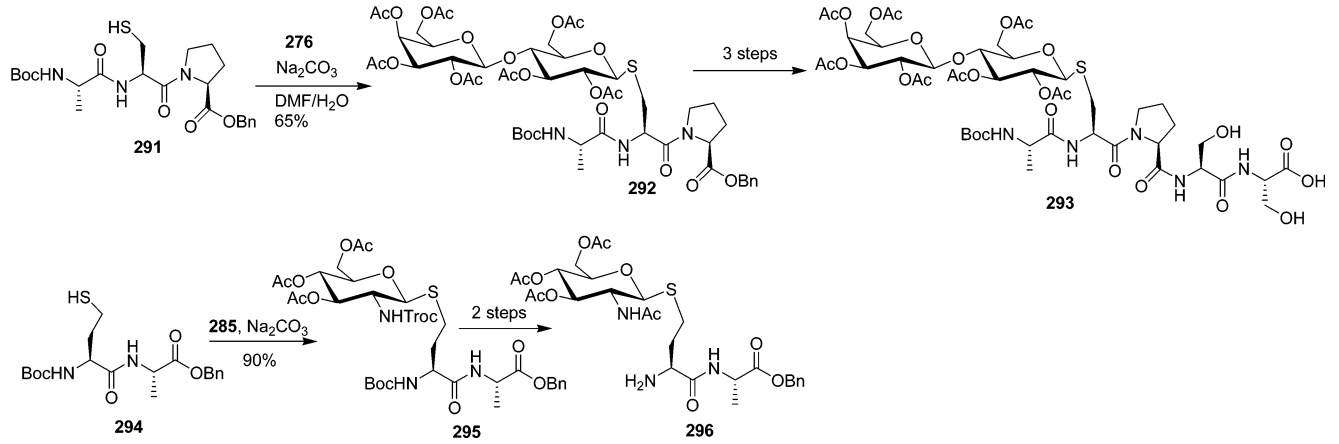
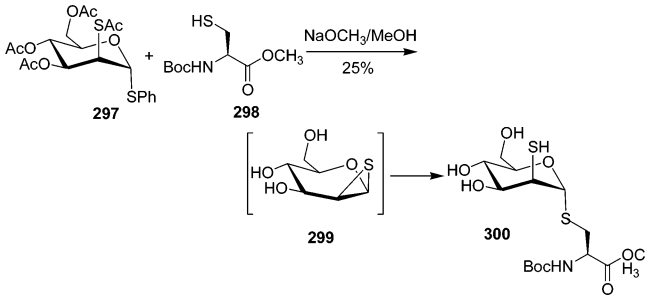


Figure 3. Partial structure of Tamm−Horsfall glycoprotein.

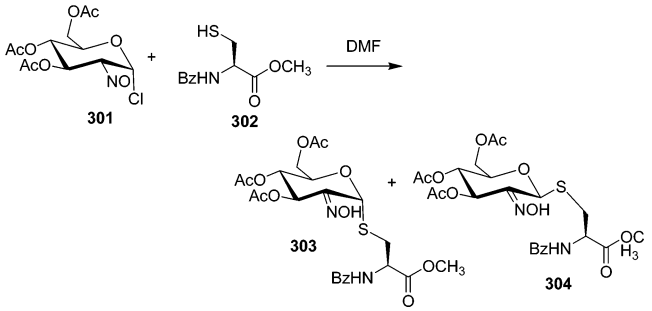
Scheme 78



Scheme 79



Scheme 80



Scheme 82

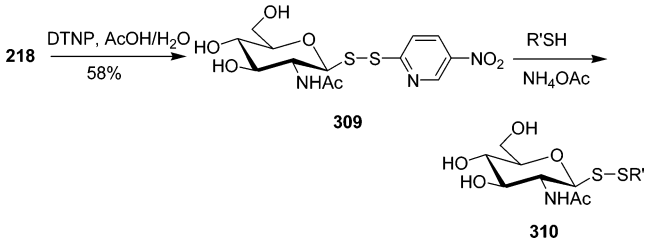


Table 4. Disulfide-Linked Glycopeptides and Glycoproteins

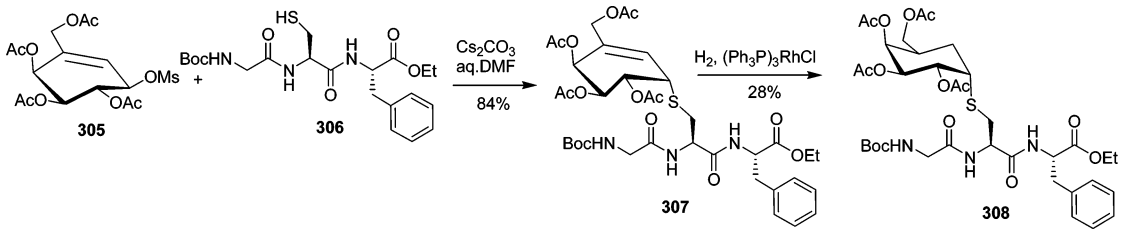
Mole ratio **309**/R'SH	Product	Yield (%)
1/1	AcNHCys-O^{t}Bu	65
5/1	PalmitoylNHCys-O^{t}Bu	53
2/1	AcNHPheCysSerThrPhe-NH_2	77
50/1	BSA-Cys-58	100

in size at single and multiple sites in a variety of proteins.[107] Two parallel strategies were investigated in which the protein cysteine residue plays potentially contrasting electrophilic and nucleophilic roles.

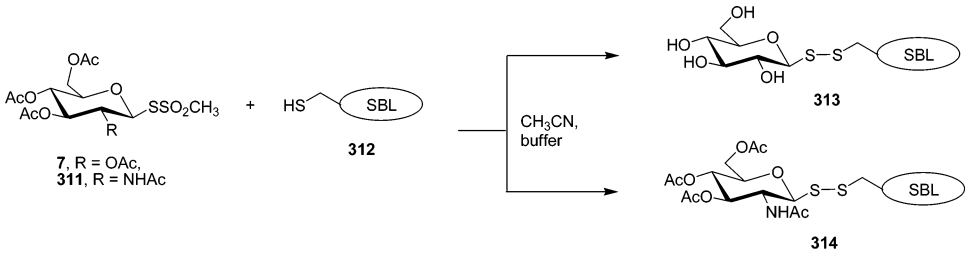
In approach **A**, a cysteine-containing protein is converted into the corresponding phenylselenenyl sulfide in which the electrophilic sulfur atom renders it susceptible to nucleophilic substitution by 1-thio mono- or oligosaccharides (Scheme 84). In the alternative approach **B**, 1-thio mono or oligosaccharides are first converted into their phenylselenenyl sulfide, which can subsequently be coupled to a cysteine-containing protein.

In a model study, a cysteine-containing protein, serine protease subtilisin *Bacillus lentus* mutant S156C (SBL-Cys156), was treated with PhSeBr to give the corresponding selenenyl sulfide, which was subsequently reacted with 1-thio monosaccharides to afford the corresponding glycosylated proteins (SBL-Glc/SBL-Gal or SBL-GlcNAc) in quantitative yields (strategy **A**). Likewise, bulky trisaccharide and heptasaccharide thiols were also glycosylated (Table 5). A large cysteine-containing protein, bovine serum albumin (BSA-Cys58), was also glycosylated by employing strategy **B**. However, the experimental results suggest that the same glycoconjugation mechanism (i.e., sugar-thiol acts finally as nucleophile) dominates regardless of the strategy used. An enzymatic carbohydrate extension (glycosylation) of a disulfide-linked glycoprotein was successfully carried out, thus demonstrating the stability of the newly formed disulfide

Scheme 81



Scheme 83



Scheme 84

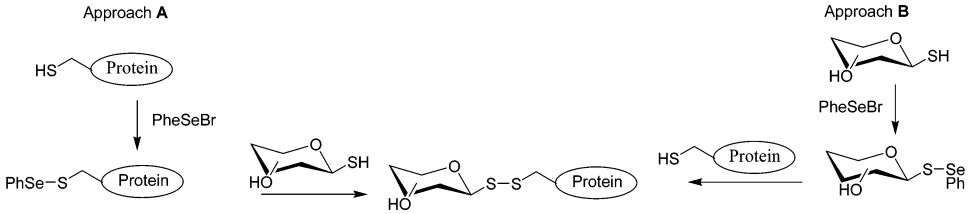
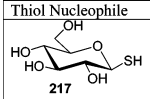
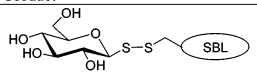
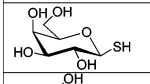
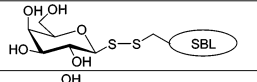
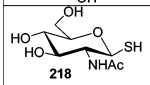
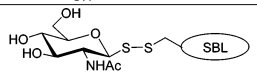
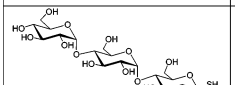
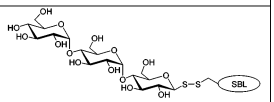
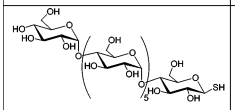
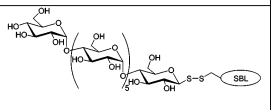
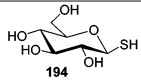
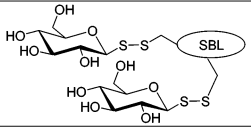
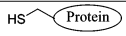
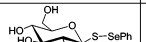
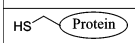
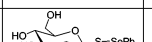


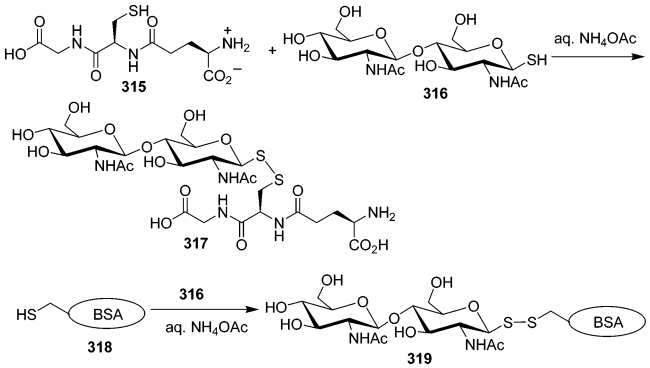
Table 5. Ligation of Sugars to Proteins via Disulfide Linkages

Thiol Nucleophile	Thiol Electrophile	Product
 **217**	SBL-SSePh	
	SBL-SSePh	
 **218**	SBL-SSePh	
	SBL-SSePh	
	SBL-SSePh	
 **194**	SBL-SSePh	
		
		

bond. This approach allows for the preparation of fully deprotected glycoprotein mimetics.

(c) Mixed disulfide formation by air oxidation. Disulfide-linked glycopeptides and glycoproteins were synthesized by oxidative disulfide formation of 1-thio sugars with cysteine-containing peptides and proteins under basic conditions.[108] Equimolar amounts of 1-thio sugar **316** and glutathione **315** in aqueous ammonium acetate at pH = 8.5 afforded the disulfide-linked glycopeptide **317**, the thiosugar homodisulfide, and gluthione disulfide in a ratio of 2:1:1 (Scheme 85). Use of 2 equiv of sugar-thiol afforded these products in a ratio of 1:0.9:0.2, thus increasing the amount of the heterodisulfide compared to glutathione disulfide. However, when a larger excess of the thioaldose (5−20 equiv) was used, only the sugar homodimer and glycopeptide were obtained and the oxidized glutathione disulfide was not

Scheme 85



detected. By this approach, a free cysteine residue at Cys58 of bovine serum albumin (BSA) **318** was linked to 1-thio sugar **316** in aqueous ammonium acetate solution affording glycoprotein mimetic **319**.

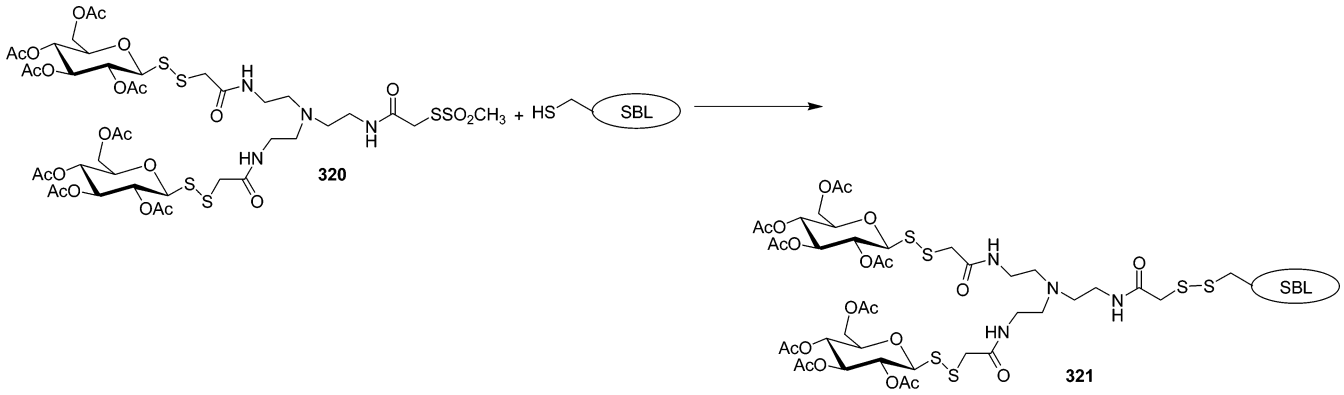
(d) Glycodendriproteins based on disulfide formation. This approach was further extended by Davis[109] to synthesize complex dendriproteins. A pre-prepared bivalent protein glycosylating reagent **320** was treated with mutant protein SBL-S156C in aqueous buffer (Scheme 86) affording the glycoprotein mimetic **321** in quantitative yield. SBL-S156C was obtained through site-directed mutagenesis of SBL as reported in the previous work.

Likewise, a pre-prepared glycoconjugate **322** was ligated with mutant protein SBL-S156C in aqueous buffer leading to glycodendriprotein **323** as shown in Scheme 87.[110]

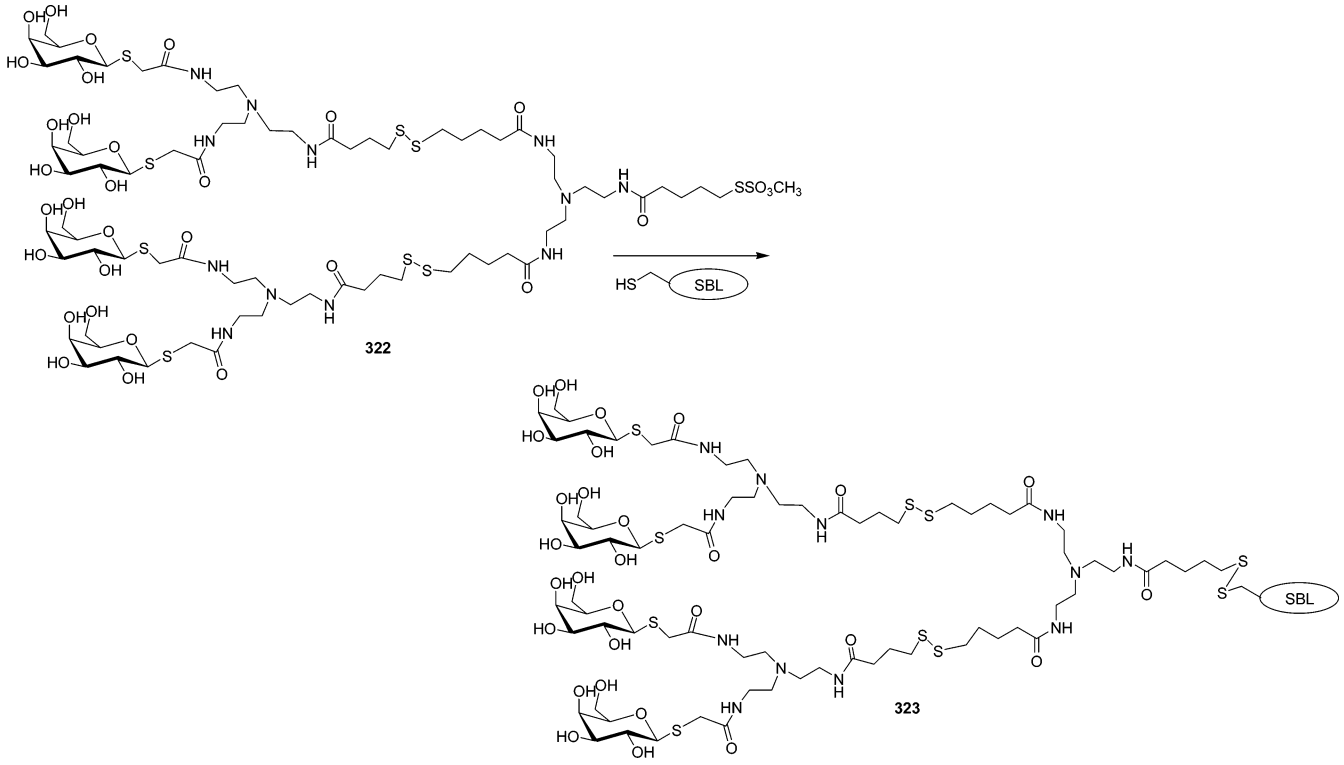
3.2.2. Glycosylthiomethyl Bromide and Azide as Glycosyl Donor[111]

(a) Glycosylthiomethyl bromides. Recently, we described a method to synthesize thioglycosylmethyl halides and their use in successful glycopeptide synthesis.[112] Reaction of glycosylthiomethyl bromide **324** with cysteine derivative **325** under phase transfer conditions afforded the expected product **326** in 92% yield (Scheme 88). Similarly, glycosylthiomethyl bromides **327** and **330** were coupled in a single phase system ($NaHCO_3$/DMF) with cysteine-containing peptide **328** and homocysteine-containing peptide **331** to afford thiolinked glycopeptides **329** and **332**, respectively, as shown in Scheme 89. In addition to these examples, several other cysteine- and homocysteine-linked glycopeptides were synthesized.

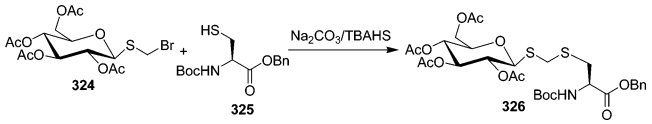
Scheme 86



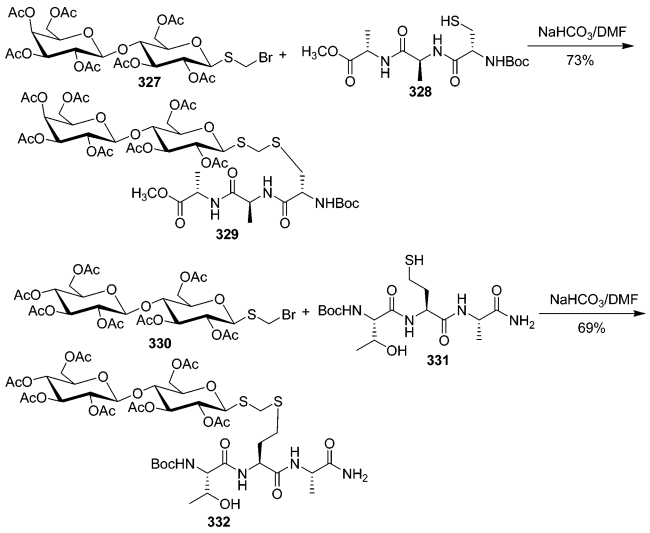
Scheme 87



Scheme 88



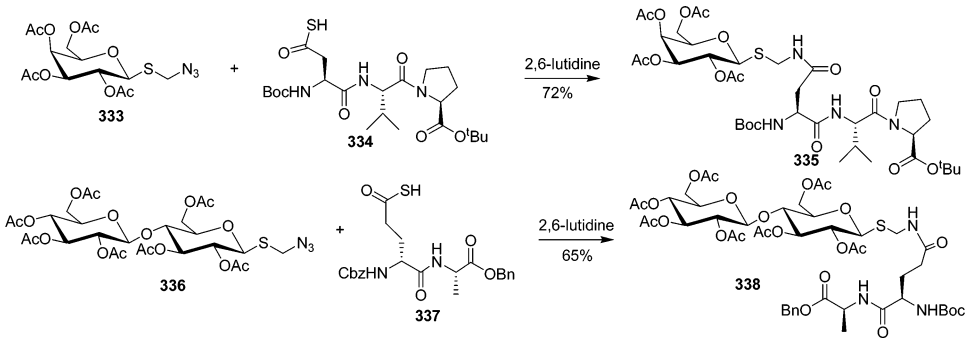
Scheme 89



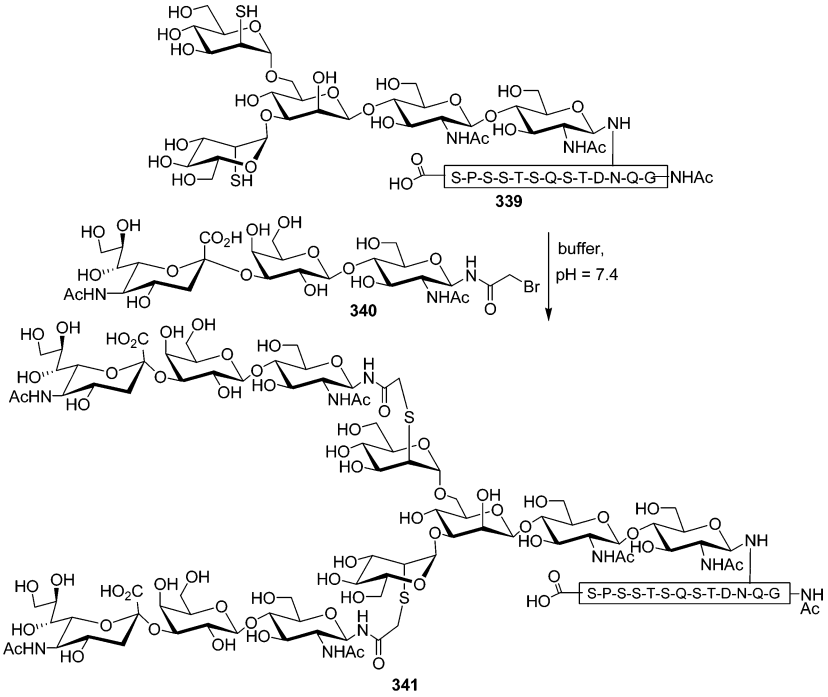
Fmoc-protected amino acids could also successfully be linked to glycosylthiomethyl bromide derivatives.

(b) Reductive coupling with glycosylthiomethyl azides. Another novel strategy to link peptides to sugar moieties employs reductive coupling of sugar-derived azides and peptide thio acids. This method deals with the reaction of glycosylthiomethyl azides with peptide thio acids. For instance, peptide thio acid **334** and galactosylthiomethyl azide **333** in the presence of 2,6-lutidine afforded the corresponding product **335** in 72% yield (Scheme 90). Similarly, glycosylthiomethyl azide **336** was reacted with thio acid **337** under basic conditions furnishing thio-linked glycopeptide **338** in 65% yield. Several other S-linked glycopeptides were obtained very efficiently. This method gives access to a variety of different types of thio-linked glycopeptides and is also amenable to the "click" reaction. The aminomethyl thioglycoside intermediates are also accessible by anomeric

Scheme 90



Scheme 91



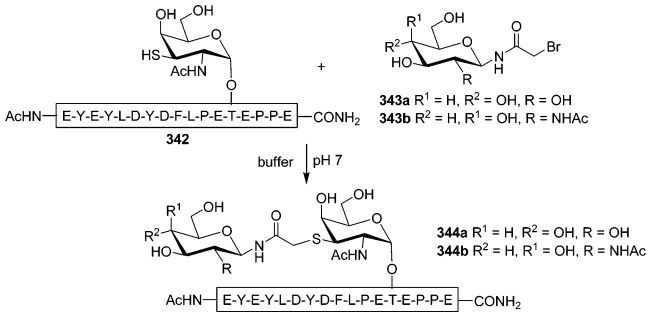
S-alkylation with bromonitromethane followed by nitro group reduction.[113]

3.2.3. Thioacetamido Group as Linker between Sugars

Bertozzi and co-workers[114] employed a powerful chemoselective thioalkylation approach for the synthesis of a complex glycoprotein containing CD52, which bears a biantennary N-linked glycan. CD52 is a glycoprotein expressed on human lymphocytes and sperm cells. Chemoselective ligation of glycopeptide **339** as a thionucleophile with an excess of *N*-glycosyl bromoacetamide **340** at 37 °C for 16 h gave the expected biantennary glycopeptide **341** (Scheme 91). However, this approach is not just a replacement of the anomeric oxygen by a sulfur atom; the connecting oxygen is replaced by a thioacetamido group consisting of four atoms, which imposes quite a structural change on the parent glycopeptide.

Bertozzi et al.[115] also employed chemoselective ligation to attach oligosaccharides to pre-prepared glycopeptides bearing the α-D-GalNAc epitope. This technique is based on the introduction of two mutually and uniquely reactive functional groups onto unprotected fragments and the convergent coupling of these fragments in an aqueous environment. Glycopeptide analogues in which the *β*-(1→3) glycosidic linkage to α-*O*-GalNAc was replaced by a thioacetamido group were synthesized by employing this approach.

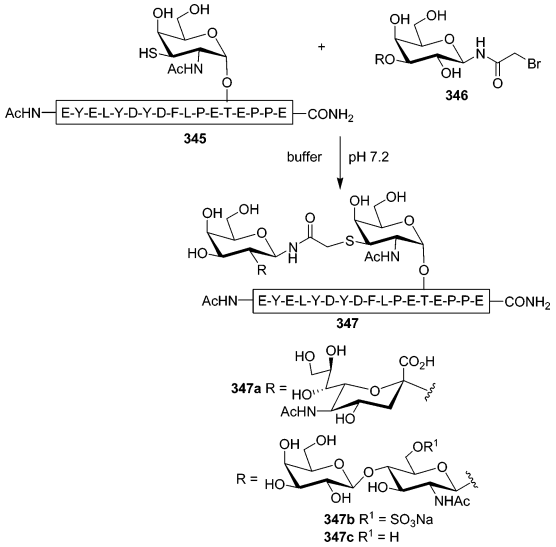
Scheme 92



Thioacetamido-linked glycopeptide mimetics **344a** and **344b** were obtained by treatment of **342** with an excess of the *N*-bromoacetamido sugar (**343a** and **343b**) in sodium phosphate buffer (pH 7) at 37 °C (Scheme 92). In each case, only the desired thioether linkage was generated; no formation of the disulfide-bound homodimer was observed.

Likewise, synthesis of thioacetamido-linked mimetics of the 2,3-sialyl-TF and MECA-79 antigens were achieved. Bromoacetamide derivatives **346a**−**c** were reacted with glycopeptide **345** under buffer (pH 7.2) conditions. The thioether-linked products **347a**−**c** were isolated by RP-HPLC (Scheme 93).[116]

Scheme 93



4. Conclusion

The increasing awareness of the biological importance of oligosaccharide derivatives and the growing interest in their applications to glycobiology have been major driving forces toward the development of strategies for the synthesis of the structurally closely related thiooligosaccharides and thioglycopeptides/proteins. This review summarizes the recent work dedicated to the successful construction of various complex glycoconjugates containing thioglycosidic linkages, including target-oriented molecules. The high nucleophilicity of the thiol and thiolate groups compared with the corresponding hydroxy compounds is the basis of this success. Future challenges are many more applications of these compounds, which are structurally closely related to the less readily available natural products, in biological investigations.

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