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Synthesis of potential inhibitors of carbohydrate processing enzymes

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Abstract

The review article describes the synthesis of different C-glycoside mimetics of glycosylphosphates, glycolipids, glycosyl amino acids and oligosaccharides. In particular, the phosphono analogues of α-L-rhamnose 1-phosphate, *N*-acetyl-α-D-glucosamine and *N*-acetyl-α-D-mannosamine-1-phosphate were synthesised; the last two from a C-2-deprotected α-C-glucopyranoside, the free hydroxyl group of which was finally converted into an amino group. In this context a method for the deprotection of allyl C-glycosides at C-2 is also reported. Analogues of glycosyl glycerols were obtained by reaction of a malonyl radical with glycoexoenitols and reduction of the obtained β-C-glycosyl malonate. Acylation gave the analogues of glyceroglycolipids. A C-glucosyl amino acid was obtained exploiting a Wittig rearrangement of compound 38. Naphthoquinone was attached, through C–C bonds, to the anomeric centre of a sugar and the non-reducing end of a different sugar, so affording a mimic of a trisaccharide. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Glycomimetics; C-glycosides; Enzyme inhibitors

1. Introduction

The role of membrane glycoconjugates in a variety of pharmacologically relevant recognition phenomena has stimulated interest in the synthesis and biological evaluation of analogues of carbohydrates, generally defined as glycomimetics. These molecules could, in fact, act as inhibitors of carbohydrate processing enzymes, provided that the functional groups involved in the enzyme-catalysed process are modified. Carbohydrate metabolism mainly involves the anomeric centre. This centre commonly links a phosphate, in glycosyl phosphates and NDP-sugars, a lipid in glycolipids, an amino acid in glycoproteins, or another sugar in di- and oligosaccharides. The substitution of the glycosidic oxygen with a methylene group gives rise to a class of compounds, generally defined as C-glycosides, in which the ‘pseudoglycosidic’ linkage is stable. As a consequence, these compounds can act as inhibitors of carbohydrate processing enzymes.

We describe herein our recent efforts in the synthesis of C-glycosidic analogues of glycosyl phosphates, glycolipids, glycosyl amino acids and disaccharides of biological interest.

2. Synthesis of phosphono analogues of glycosyl phosphates

The interest in glycosyl phosphates lies in the fact that these molecules and NDP-sugars, which directly derive from them, are the main glycosylating agents in nature, respectively, in non-Leloir and Leloir processes. Different C-glycosidic analogues of glycosyl phosphates have already been synthesised by us and other research groups, usually employing multistep procedures.

In a project devoted to synthetic vaccines, we are interested in the synthesis of the phosphono analogue of the repeating unit of the antigenic determinant of *Streptococcus pneumoniae* 19F (Fig. 1). To reach this goal we studied the possibility of converting easily, stereoselectively and in high yield, protected L-rhamnose into the desired α-phosphonate. After some attempts we found that the Horner–Emmons reaction of 2,3,4-tri-O-benzyl-L-rhamnose **1** with tetraethyl methylenediphosphonate and NH in diglyme, directly affords in 73% yield the desired compound **3** through a spontaneous and stereoselective Michael-type cyclisation of the α,β-unsaturated phosphonate intermediate **2** (Scheme 1). The cyclisation reaction, which is under thermodynamic control, afforded preferentially (60% d.e.) the more stable α-‘anomer’ **3**. The configuration at C-1 (for simplicity we maintain the numbering of the parent sugar) of **3** was determined by n.O.e. experiments, which indicate the 1,3-diaxial correlation among CH_2P, H-3 and H-5.

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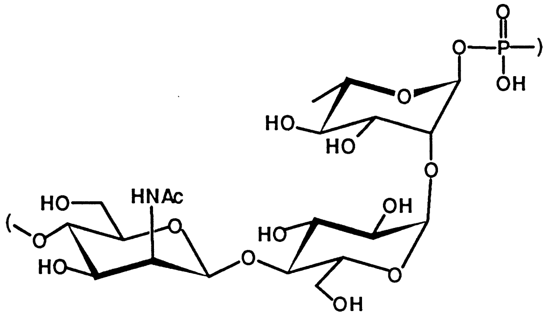
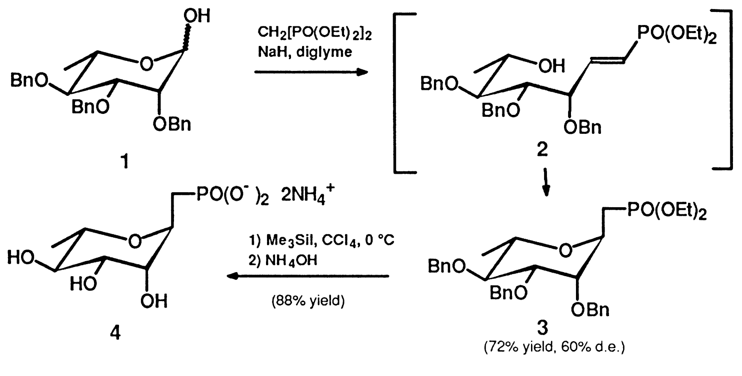


Fig. 1. Repeating unit of the antigenic determinant of *Streptococcus pneumoniae*.

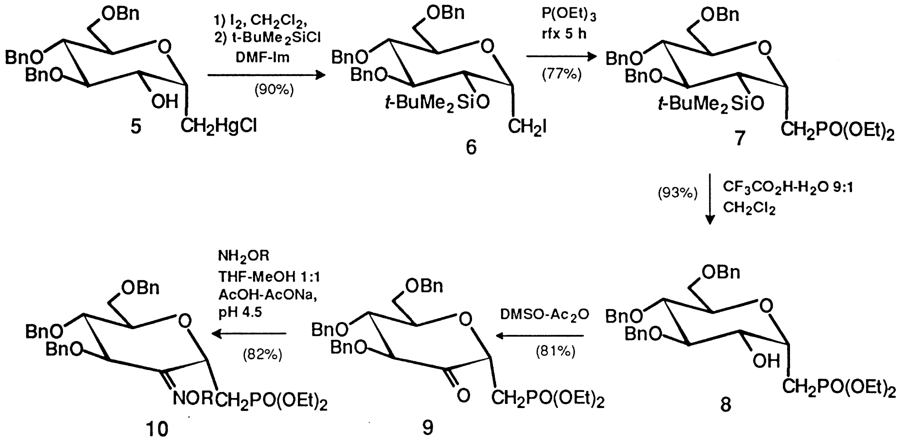
Moreover, the chemical shift value of the 'anomeric' hydrogen of **3** (δ 4.43) compared with that of its β-epimer (δ 3.75) clearly indicates the equatorial orientation of the first, which resonates at lower fields (Cipolla et al., 1997a). Treatment of **3** with Me_3SiI directly afforded **4**, the deprotected phosphono analogue of α-L-rhamnose-1-phosphate. This approach is so efficient that it can be applied to the trisaccharidic structure already synthesised (Panza et al., 1987) to afford the phosphono analogue of the repeating unit of the antigenic determinant of *Streptococcus pneumoniae* 19F.

Much more difficult was the synthesis of the phosphono analogue of *N*-acetyl-α-D-glucosamine 1-phosphate, the interest in which lies inter alia in the fact that *N*-acetyl-D-glucosamine is the main component of bacterial cell wall oligosaccharides such as mureine and teichoic acid. In this case all the attempts we made to convert directly properly protected D-glucosamine into the corresponding phosphonate failed. In addition, attempts to convert the C-glycosidic carbon atom of glucosaminoderivatives into an electrophile, in order to introduce the phosphonic group, were unsuccessful. It seems that this carbon atom, which is close to the nucleophilic amino group, cannot be converted into an electrophile, even when the nucleophilicity of the amino group is lowered by acetylation. Finally we reached the conclusion that a successful strategy to synthesise the phosphono analogue of *N*-acetyl-α-D-glucosamine 1-phosphate requires the introduction of the amino group later, at the final stages of the synthesis, following the introduction of the phosphonate. This approach requires the availability of an α-C-glucopyranoside selectively deprotected at the C-2 hydroxyl group, which will be converted into an amino group at the end of the synthesis. To obtain this compound we exploited our vinylation–mercuriocyclisation procedure for the synthesis of C-glycosides (Boschetti et al., 1988) which afforded stereoselectively (>95% d.e.) and in high yields (75%) the α-C-glucopyranoside **5**.

The mercurioderivative **5** was converted into the iodide **6** at 90% yield, the hydroxyl group of **6** was protected as *t*-butyldimethylsilyl ether and the iodide was transformed into the phosphonate by treatment with triethylphosphite (Scheme 2). Hydrolysis of the silyl ether with trifluoracetic acid–water (9:1), oxidation with DMSO–Ac_2O and treatment of the obtained ketone **9** with an hydroxylamino derivative, allowed the introduction of nitrogen at C-2. The reduction of the oxime **10**, effected with diborane, occurred as expected stereoselectively (64% d.e.) from the less hindered β-face, so affording the desired glucosamino derivative **11** after acetylation of the nitrogen (Scheme 3). Treatment of **11** with iodotrimethylsilane finally afforded the deprotected phosphono analogue **12** of *N*-acetyl-α-D-glucosamine 1-phosphate. Interestingly, when we reduced the oxime **10** by catalytic hydrogenation, either with $Pd(OH)_2$ or with Ni–Raney, the manno isomers **13** and **14**, respectively, were obtained; a result that indicates a co-ordination of the catalyst with the phosphonate. This approach, in conclusion, allows the synthesis of the phosphono analogues of both *N*-acetyl-α-D-glucosamine-1-phosphate (**12**) and *N*-acetyl-α-D-mannosamine-1-phosphate (**15**) depending on the type of reduction of the oxime (Casero et al., 1996). This example clearly indicates that, to synthesise specific C-glycosides of 2-amino sugars, the late introduction of the amino group can solve many synthetic problems. This approach, however, requires the availability of C-glycosides selectively deprotected at C-2, and moreover the vinylation–mercuriocyclisation procedure described is limited and has the disadvantage of using toxic mercuric salts. In the light of these considerations we studied the possibility of deprotecting regioselectively easily available C-glycosides.



Scheme 1. Stereoselective synthesis of α-L-rhamnose 1-phosphonate.



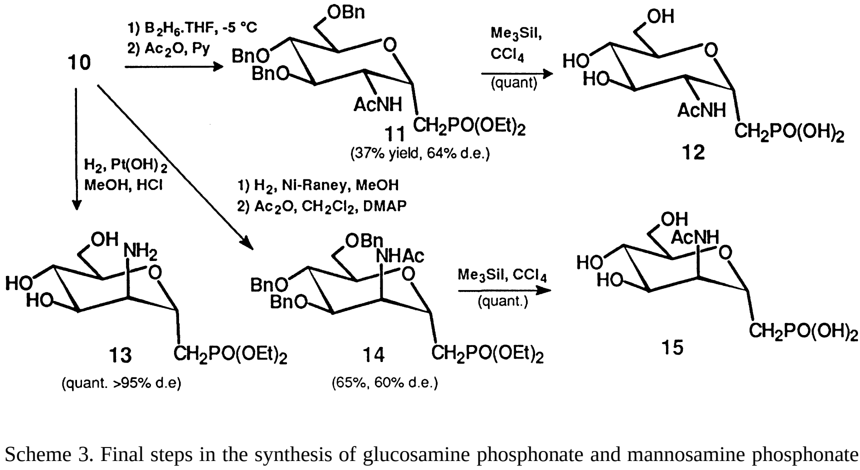
Scheme 2. Conversion of mercurioderivative **5** into the corresponding phosphonate.

3. Selective deprotection at C-2 of allyl C-glycosides

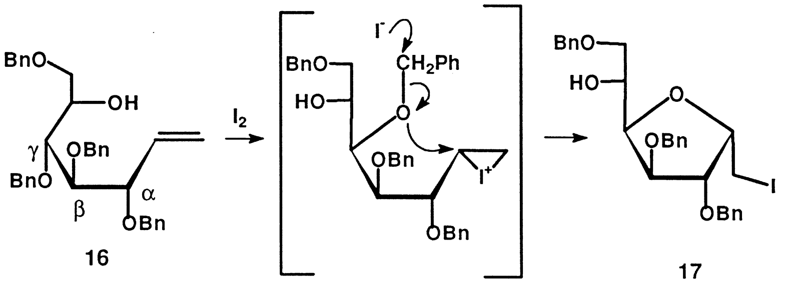
Polybenzylated allyl C-glycosides are probably among the most easily available C-glycosides. The α-isomer can be stereoselectively obtained treating a polybenzylated D-glycoside with allyltrimethylsilane in the presence of a Lewis acid (Lewis et al., 1982), whereas the β-isomer is easily and stereoselectively obtainable by reaction of the corresponding glyconolactone with allylmagnesium bromide and subsequent reduction of the obtained lactol with triethylsilane (Lewis et al., 1982). We next studied the possibility to deprotect selectively at C-2 these easily available polybenzylated allyl C-glycosides in order to obtain 2-amino-2-deoxy C-glycosides through the elaboration of the free hydroxyl group. The idea came from our previous work (Nicotra et al., 1987), in which we observed that glucoenitol **16** obtained from 2,3,4,6-tetra-O-benzyl-D-glucopyranose according to Sinaÿ's procedure (Pougny et al., 1981) treated with iodine afforded the furanosidic structure **17** instead of the expected C-glucopyranoside. This result indicates that the benzyloxy group in γ-position with respect to the double bond preferentially acts as a nucleophile on the iodonium ion, with loss of the benzyl protecting group and formation of a cyclic iodoether (Scheme 4).

The previous result has suggested that treatment of an allyl C-glycoside with iodine should result in a selective debenzylation in the γ-position with respect to the double bond, that is at C-2 of the sugar ring. Reductive elimination of the obtained iodoether, by treatment for example with zinc and acids, should then regenerate the original double bond so resulting in a deprotection at C-2 (Scheme 5). The free hydroxyl group can then be converted into an amino group with retention or with inversion of configuration. The idea has been first applied on the allyl α-C-glucopyranoside **18** (Scheme 6). Reaction with iodine in THF at 0°C and subsequent treatment of the obtained iodoether with zinc and acetic acid in Et_2O-MeOH (1:1) resulted in the deprotection at C-2 in 81% overall yield. The same procedure applied on the corresponding β-anomer **20** (Scheme 6) indicates that debenzylation also occurs in this case, in 72% overall yield (Cipolla et al., 1997b). Furthermore, preliminary results indicate that the procedure is applicable in the same way to galactose and lactose.

The conversion of the free hydroxyl group into an amino group has been effected according to known procedures, but



Scheme 3. Final steps in the synthesis of glucosamine phosphonate and mannosamine phosphonate.



Scheme 4. Unexpected result in the cyclisation of derivative **16**.

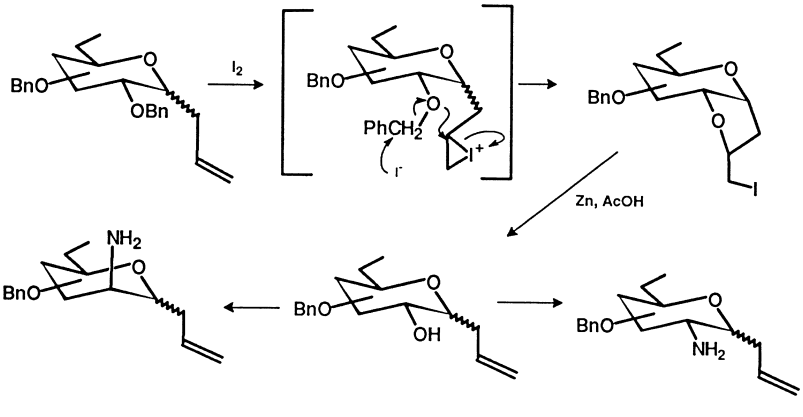
the stereochemistry of the processes requires some comments. In case of the *α*-gluco compound **19**, oxidation with PCC and treatment of the ketone obtained with methylhydroxylamine allowed the introduction of the nitrogen at C-2 (Scheme 7). The reduction of the obtained oxime **22**, either by catalytic hydrogenation or with LiA1H$_4$, stereoselectively occurred from the less hindered *β*-face affording the glucosamino derivatives **23** and **24** in a diastereomeric excess higher than 95% and 67%, respectively. To obtain the mannosamino derivative **26**, the free hydroxyl group of **19** was converted into an azido group with inversion of configuration (treatment with triflic anhydride and then with tetrabutylammonium azide), and the azido group of **25** was finally reduced with LiA1H$_4$ (Cipolla et al., 1997b).

In the case of allyl *β*-C-glucopyranoside, reduction of the methyloxime **28**, obtained by Swern oxidation of **21** followed by treatment with methylhydroxylamine, occurs stereoselectively from the *α*-face, and requires treatment with LiA1H$_4$ at 40°C. The mannosamino derivative **29** was thus obtained in 83% diastereomeric excess. In this case, to obtain the equatorial amino group, ketone **27** was reduced with LiA1H$_4$ at −40°C, so affording stereoselectively (>95% d.e.) the manno-isomer **30**. The axial hydroxyl group of **30** was then converted into the equatorial amine (**31**) by triflation, treatment of the triflate with tetrabutylamonium azide and finally reduction with LiA1H$_4$ (Scheme 8).

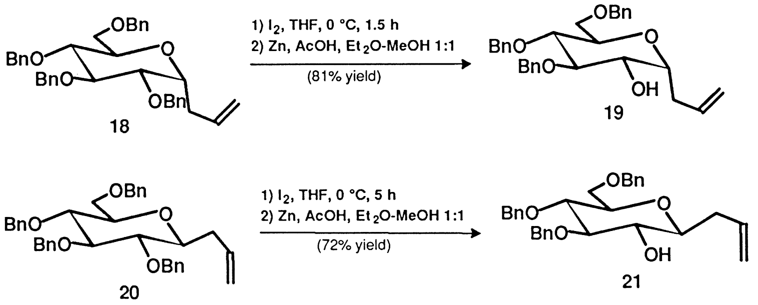
In conclusion, following this simple selective debenzylation procedure, it is possible to prepare stereoselectively *α*- and *β*-C-glycosides of glucosamine and mannosamine, and preliminary results indicate that the approach is also applicable to other C-pyranosides.

4. Synthesis of analogues of glycolipids

Radical chemistry has been recently widely applied to the synthesis of glycomimetics and in particular of C-glycosides. In this field Giese has proposed an interesting approach to obtain C-glycosides by reaction of a glycosyl radical, which has nucleophilic character, with an electron-poor double bond such as that of acrilonitrile (Giese and Dupuis, 1983). The reaction affords stereoselectively *α*-C-glucopyranosides due to the anomeric effect. We decided to study the possibility of synthesising analogues of glyceroglycolipids exploiting a completely different radical approach in which the electron-rich double bond of a glycoxoenitol such as **33** (Scheme 9) is submitted to the intermolecular attack of the electron-poor malonyl radical. Starting from the glycoexoenitols **33**, obtained from 2,3,4,6-tetra-O-benzyl-D-gluconolactone **32a** or 2,3,4,6-tetra-O-benzyl-D-galactonolactone **32b**, the reaction afforded stereoselectively the *β*-C-glucopyranosyl or *β*-C-galactopyranosyl malonates **34a** and **34b** in approx. 50% yield and a diastereomeric excess higher than 95% (only the *β*-isomer was detected by ^{13}C NMR of the mixture) (Cipolla et al., 1997c). From the stereochemical point of view, it is



Scheme 5. General method for the conversion of perbenzylated C-glycosides into 2-amino-2-deoxy derivatives.



Scheme 6. Selective debenzylation at position 2 of C-ally glucosides.

noteworthy that this approach is complementary to that of Giese, affording β-C-glycopyranosides due to the anomeric effect in the reduction of the intermediate anomeric radical with Bu_3SnH. The C-glycosyl malonates **34** were then reduced with $LiA1H_4$ affording the corresponding diols **35** which are stable C-glycosidic analogues of glycosylglycerols. Esterification with palmitoyl chloride and deprotection of the sugar finally afforded the analogues of glyceroglycolipids **36**. In view of the fact that some glyceroglycolipids and glycosylglycerols, isolated from algae and microorganisms, have shown antitumour activity, their analogues were tested as antitumour agents, but no significant activity was found. The analogue **35a** of glucosylgycerol showed a 56.9% inhibition (IC$_{50}$ = 12.9 μM) of cellular tyrosine phosphorylation at 100 μM concentration, and the analogues of glyceroglucolipid **36a** and glycerogalactolipid **36b** showed, respectively, inhibitions of 26.3% and 37.3% (IC$_{50}$ > 50 μM).

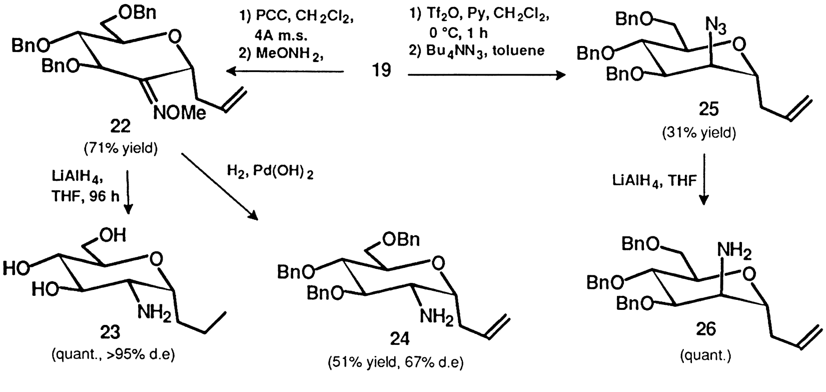
5. Synthesis of a C-glucosyl amino acid

The glycidic part of glycoproteins is linked to serine or threonine through an O-glycosidic bond (O-linked glycoproteins) or to asparagine through a N-glycosidic bond (N-linked glycoproteins). There is a great interest in analogues in which these glycosidic linkages are stable, in order to avoid chemical or enzymatic hydrolysis.

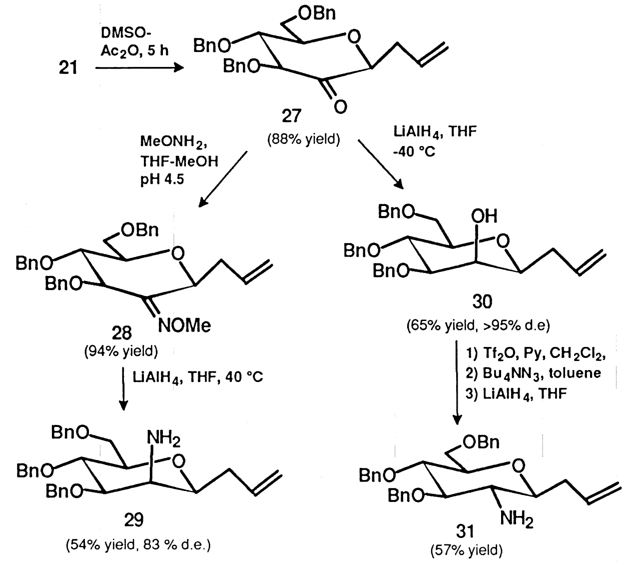
We synthesised the stable analogue of β-glucopyranosyl serine according to Scheme 10. The approach is based on a [2,3]-Wittig sigmatropic rearrangement of compound **38** which stereoselectively (>95% d.e.) afforded compound **39**, the double bond of which was stereoselectively reduced by catalytic hydrogenation with Ni-Raney. The absolute configuration of the stereocentre in α to the carboxylic group was determined converting **38** into the (R)- and (S)-MTPA esters. The ^{1}H NMR spectra of the MTPA esters showed a $\Delta\delta$ value (δS-δR) of $+ 0.1$ ppm for the signals of the hydrogen atoms at C-3 and C-4, allowing the assignment of the absolute configuration R at C-2 (Ohtani et al., 1991). Conversion of the hydroxyl group into an amino group, with inversion of configuration, was then effected by treatment with diphenylphosphoryl azide an DBU followed by treatment with Bu_4NN_3. Catalytic hydrogenation of the obtained azide **41** with $Pd(OH)_2$, finally afforded **42** (Lay et al., 1997).

6. Synthesis of a spacer-connected C-disaccharide

Analogues of oligosaccharides in which a spacer substitutes one or more sugar units are of great interest as simplified structures recognised by carbohydrate receptors. In this context for example, analogues of Sialyl Lewis X in which the sugars not involved in selectin recognition were substituted with spacers (Huang and Wong, 1995; Banteli and



Scheme 7. Transformation of α-C-glucosides into mannosamine and glucosamino derivatives.

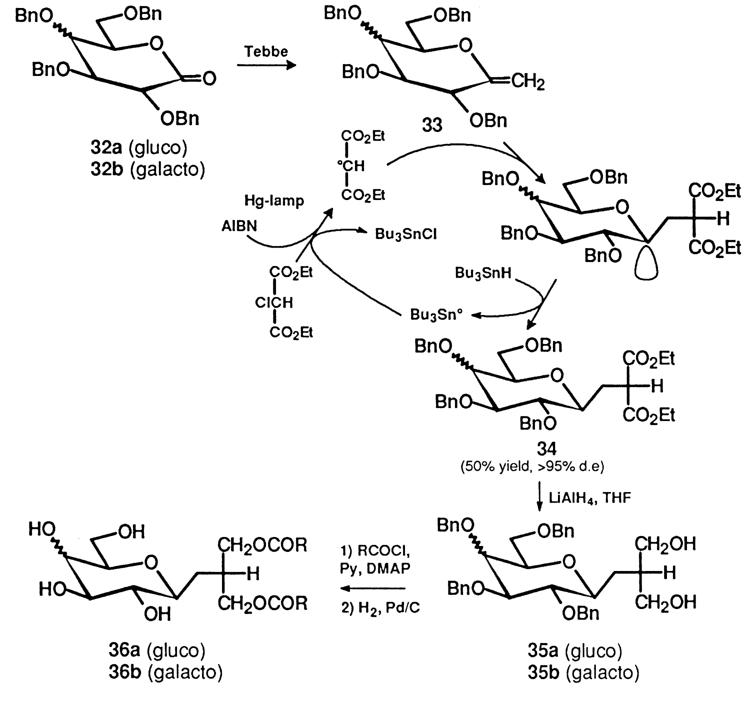


Scheme 8. Transformation of β-C-glucosides into mannosamino and glucosamino derivatives.

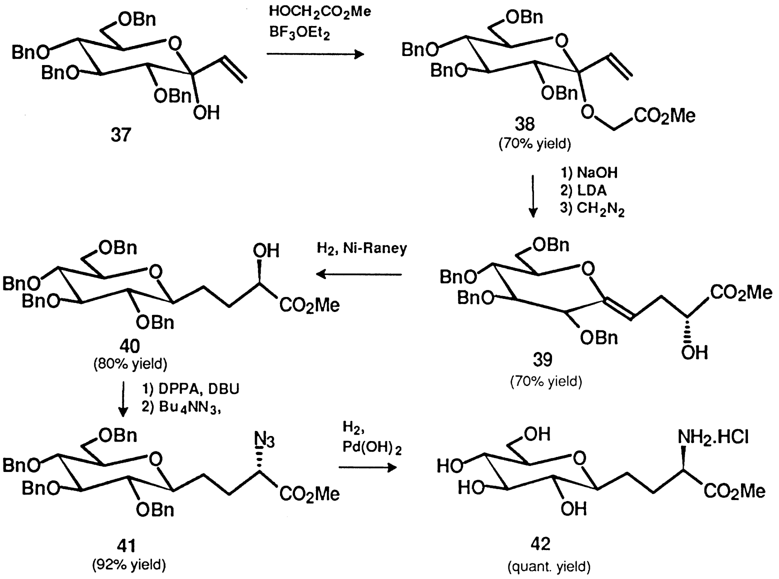
Ernst, 1997) have been synthesised. We were able to link naphthoquinone as a spacer, through a C–C bond, to the anomeric centre of a first sugar and the non-reducing end of a second sugar, according to the radical procedure reported in Scheme 11. Iodide **43** was converted into the corresponding radical by dropwise addition of a solution of *p*-nitrophenyldiazonium tetrafluoroborate in the presence of $FeSO_4$. The radical was trapped by naphthoquinone affording **44** which was, in turn, submitted to the reaction of the C-glycosyl radical obtained in the same way from iodide **45** (Scheme 11). The reaction afforded the disubstituted naphthoquinone **46** in 50% yield. In this compound the ‘anomeric’ carbon atom of the gluco unit links, through a methylene bridge, the quinone which, in turn, links the non-reducing end of the galacto unit through a stable C–C bond (Cipolla et al., 1997d). The product mimics a trisaccharidic structure in which the intermediate sugar is substituted by a napththoquinone spacer. Furthermore, the reducing end of the trisaccharidic structure still exists (in protected form), allowing further elongation by conventional glycosylation procedures. The elongation can, in principle, be effected also at the non-reducing end of the gluco unit, by selective deprotection of the primary hydroxyl group, conversion into an iodide radical and attack to another naphthoquinone unit.

7. Conclusion

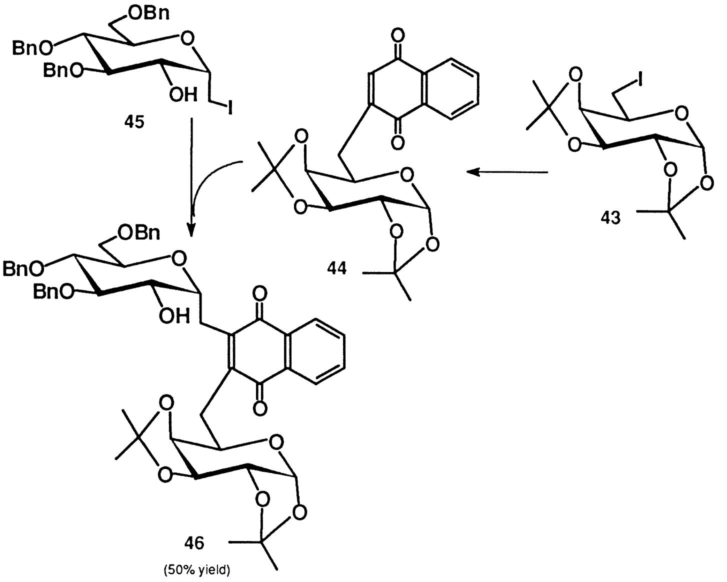
The role of carbohydrates in recognition phenomena, many of which are of great pharmacological relevance, stimulates interest in the synthesis of glycomimetics. These molecules are potential inhibitors of carbohydrate processing enzymes or can act as substitutes of oligosaccharides or glycoconjugates in recognition phenomena. This article is devoted to a specific class of glycomimetics, the C-glycosides, and has described our recent efforts in the synthesis of stable C-glycosidic analogues of glycosyl phosphates, glycolipids, glycosyl aminoacids and di- or oligosaccharides, that is to say all possible natural glycoderivatives. It



Scheme 9. Radical approach to the synthesis of glyceroglycolipid and glycosyl glycerol analogues.



Scheme 10. Synthesis of a C-analogue of *β*-D-glucopyranosyl glycerol analogues.



Scheme 11. Synthesis of spacer-connected C-disaccharides.

is clear from the different examples that chemical and stereochemical problems are encountered in the different synthetic approaches, and that a variety of different synthetic methodologies and reagents are employed in this sort of chemistry. In other words, the synthesis of glycomimetics is interesting, not only for the biological and pharmacological potential of the products, but also as a challange for a synthetic chemist.

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