Application of thioglycoside chemistry to the synthesis of trisaccharides and deoxy-trisaccharides related to the *Shigella flexneri* Y polysaccharide[1]

FRANCE-ISABELLE AUZANNEAU AND DAVID R. BUNDLE[2]
Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ont., Canada K1A OR6

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The *Shigella flexneri* lipopolysaccharide has a biological repeating unit, →2)α-L-Rha*p*(1→2)α-L-Rha*p*(1→3)α-L-Rha*p*(1→3)β-D-GlcNAc*p*(1→ ABCD, but the residue segment BCD suffices to fill the binding site of an O-antigen specific monoclonal antibody, SYA/J6. Synthetic modifications to this trisaccharide have been designed to investigate the involvement in binding of the acetamido moiety, the 4- and 6-OH groups of the GlcNAc residue D, the 4′-OH group of the Rha residue C, and the 3″- and 4″-OH groups of the Rha residue B. Sequential chain extension provided the protected trisaccharides **18**, **24**, **26**, **28**, **30**, **32**, and **35** using thioglycoside glycosyl donors activated by iodonium ions generated *in situ* from *N*-iodosuccinimide and triflic acid. Trisaccharides each monodeoxygenated in either ring B **25** and **27** or ring C **29** were accessed by the use of 3,6-dideoxy or 4,6-dideoxy glycosyl donors **14** and **17** and when these were used in sequential steps trisaccharides **31** and **33**, each deoxygenated at double sites in adjacent residues, were obtained. Selective protection of the glucosamine residue as its *N*-benzyloxycarbonyl derivative provided a facile route to the trisaccharide amino compound **20**, from which *N*-acetyl **21**, *N*-propionyl **22**, and *N*-trifluoroacetyl **23** derivatives were directly prepared. Orthoester intermediates were detected in several glycosylation reactions and culminated in an orthoacetate **34** as a major product rather than the target trisaccharide **35**. When triflic acid concentration was increased these products were avoided but acid-catalyzed migration of a 2-*O*-acetyl group led to both 1→2 and 1→3 linked trisaccharides **35** and **37**. To avoid similar undesirable 1,2-linked products, a block synthetic strategy using the 2-*O*-benzoylated disaccharide glycosyl donor **40** was chosen so that the propensity for orthoester formation was minimized in reactions leading to the trisaccharide analogue deoxygenated at C-6 of the glucosamine unit D.

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Le tétrasaccharide →2)-α-L-Rha*p*(1→2) α-L-Rha*p*(1→3) α-L-Rha*p*(1→3)β-D-GlcNAc*p*, ABCD constitue l'unité biologique répétitrice du lipopolysaccharide de *Shigella flexneri*, mais le segment BCD suffit à remplir le site de reconnaissance de l'anticorps monoclonal SYA/J6 spécifique de cet antigène-O. La préparation de trisaccharides modifiés a été effectuée afin d'évaluer l'importance pour la reconnaissance des groupes acétamido, 4- et 6-OH du résidu GlcNAc ainsi que des groupes 4′-, 3″- et 4″-OH des résidus rhamnosyles C et B. Les trisaccharides protégés **18**, **24**, **26**, **28**, **30**, **32** et **35** ont été obtenus en suivant une stratégie d'élongation de chaîne séquentielle utilisant comme donneurs de glycosyles des thioglycosides activés par des ions iodonium générés *in situ* depuis la *N*-iodosuccinimide et l'acide triflique. Les trisaccharides monodésoxygénés sur les résidus B **25**, **27** ou C **29** furent obtenus en utilisant les thioglycosides 3,6- ou 4,6-didésoxy **14** et **17**, dont l'emploi séquentiel combiné permit également l'accès aux trisaccharides **31** et **33** chacun didésoxygénés sur les résidus adjacents B et C. La protection sélective de la glucosamine par un groupe *N*-benzyloxycarbonyle permit d'obtenir facilement le trisaccharide amino **20**, précurseur des composés *N*-acétyl **21**, *N*-propionyl **22** et *N*-trifluoroacétyl **23**. La formation intermédiaire d'orthoesters, détectée au cours de plusieurs glycosylations, fut culminante durant la synthèse du trisaccharide **35** à l'issue de laquelle l'orthoacétate **34** fut isolé majoritairement. Sa formation fut évitée en augmentant la concentration d'acide triflique, mais la migration acido-catalysée du groupe 2-*O*-acétyl conduisit, dans ces conditions, à la formation des trisaccharides **37** et **35** respectivement 1→2 et 1→3 liés. Pour éviter la formation indésirable de composés similaires 1,2 liés, l'analogue **46**, désoxygéné en C-6 de l'unité de glucosamine D, fut préparé en suivant une stratégie de synthèse par bloc, utilisant un disaccharide 2-*O*-benzoylé comme donneur de glycosyle afin de minimiser sa tendance à former un orthoester.

Introduction

In our studies of the antibody combining sites that are generated by immunization of mice with killed vaccines of variant Y *Shigella flexneri*, we have concentrated on two antibodies that are typical of the majority that bind to the Y polysaccharide antigen, an IgM GC-4 and an IgG SYA/J6 (1–3). In their binding patterns with native antigen and glycoconjugates both exhibit a specificity that is directed toward an epitope occurring at the junction of the biological repeating unit (1). Preliminary investigations were based on enzyme immunoassay (EIA) screening with synthetic ligands coupled to BSA and a limited set of modified oligosaccharides (1). These results identified the probable extent of the oligosaccharide–protein contacts and, of the four residues that make up the biological repeating unit of the bacterial *O*-antigen:

[→2)-α-L-Rha*p*(1→2)-α-L-Rha*p*(1→3)-α-L-Rha*p*(1→3)-β-D-GlcNAc*p*(1−]
A B C D

it seemed most probable that the residue sequence BCDA represented the extent of the oligosaccharide epitope. Although the contribution to the binding by the A residue was estimated to be very small (1), we did not properly consider the possibility that the trisaccharide BCD rather than the tetrasaccharide BCDA filled the binding site. Conclusions that the acetamido group of residue D was exposed to

[1]Issued as NRCC No. 34287.
[2]Author to whom correspondence may be addressed.

solvent were based on the use as inhibitor of partially *N*-deacetylated trisaccharide BCD. The residual activity of the trisaccharide α-L-Rha*p*(1→3)-α-L-Rha*p*(1→3)-β-D-GlcNAc*p*-1→*O*Me, which was a 40% contaminant of the intended α-L-Rha*p*(1→3)-α-L-Rha*p*(1→3)-β-D-GlcNH$_2$*p*-1→*O*Me inhibitor, led to an erroneous conclusion that the acetamido group was not a contact residue in the site. To complete the mapping of the SYA/J6 binding site a reliable synthesis of these two trisaccharides as well as various *N*-acylated derivatives was required, together with a complete investigation via monodeoxy derivatives of the hydroxyl groups that form hydrogen bonds to the antibody. The results of this study will provide an important comparison with the detailed oligosaccharide–antibody interactions that should be available now that the crystal structures have been solved for antibody SYA/J6 Fab cocrystallized with penta- and trisaccharide ligands (4).

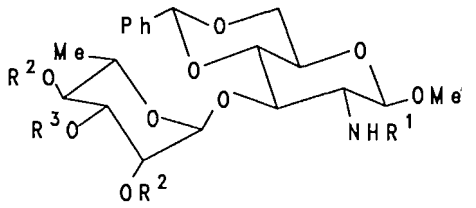
In this report, we describe the use of the *N*-benzyloxycarbonyl protecting group to provide the amino congener of the naturally occurring trisaccharide Rha→Rha→GlcNAc, as well as other acylamido analogues. Early work (1) had suggested that the B residue contributed some 1.5–2.0 kcal/mol of binding energy but its attribution to hydrogen bonding involving the 3- or 4-hydroxyl groups was not investigated. It was also suggested that a hydrogen bond involving the 4-hydroxyl group of the glucosamine unit was crucial to the binding of the antigen to SYA/J6 but that neither the 6-OH group of unit D, or the 4-OH group of residue C were involved in important polar contacts ($\Delta(\Delta G) < 0.5$ kcal/mol). However, the latter results were measured using monodeoxydisaccharide analogues (1) of CD and therefore had to be confirmed using analogues of the trisaccharide BCD that represents the smallest antigen epitope that could fill the SYA/J6 IgG binding site.

To identify the trisaccharide hydroxyl groups that form the most important polar contacts with the protein, five monodeoxy analogues involving various positions within units B, C, or D were synthesized. Two dideoxygenated trisaccharides were also prepared to evaluate the additivity effects to binding energy when changes were made in both the units B and C.

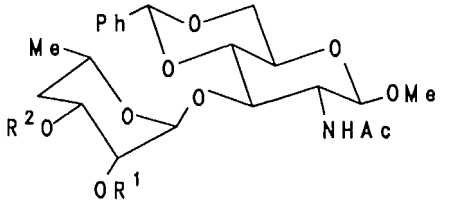
The 11 trisaccharide target compounds were synthesized from thioglycosides. These are flexible and stable intermediates that are well suited for oligosaccharide chemistry since the thioalkyl group can be easily converted to glycosyl halide under mild conditions or activated, *in situ*, by thiophilic reagents (5). Sequential chain extension strategies involving activation of thioglycosides with iodonium ions generated, *in situ*, from *N*-iodosuccinimide and triflic acid (6, 7) were employed for most of these oligosaccharide syntheses.

Results

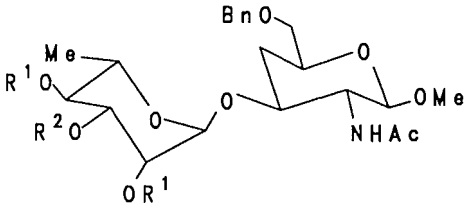
The known (8) disaccharides **1**, **4**, **7**, and **9** were employed as precursors of the glycosyl acceptors needed to prepare the protected trisaccharides **18**, **24**, **26**, **28**, **30**, **32**, and **35**. The triacetates **1** and **4** were transesterified to the triol derivatives **2** and **5**. Regioselective acetylations of **2**, **4**, and **9** to give respectively the acceptors **3**, **6**, and **10** were then accomplished in three steps. Reaction of the triols with trimethylorthoacetate gave 2′,3′-orthoesters that were acetylated at OH-4 prior to acid-catalyzed regioselective opening



1 $R^1 = CO_2CH_2Ph$, $R^2 = R^3 = Ac$
2 $R^1 = CO_2CH_2Ph$, $R^2 = R^3 = H$
3 $R^1 = CO_2CH_2Ph$, $R^2 = Ac$, $R^3 = H$
4 $R^1 = R^2 = R^3 = Ac$
5 $R^1 = Ac$, $R^2 = R^3 = H$
6 $R^1 = R^2 = Ac$, $R^3 = H$



7 $R^1 = R^2 = H$
8 $R^1 = Ac$, $R^2 = H$

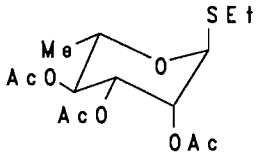


9 $R^1 = R^2 = H$
10 $R^1 = OAc$, $R^2 = H$

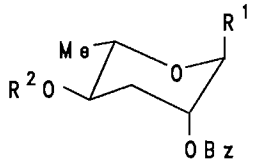
to O-2 by treatment in aqueous acetic acid. The diol **7** was converted into the acceptor **8** analogously by formation of a 2′,3′-orthoacetate and regioselective opening to the axial position with mild acidic treatment.

The thioethylrhamnopyranoside **11**, and the thioethyldideoxyhexopyranosides **14** and **17** were used as glycosyl donors. Preparation of a rhamnosyl donor deoxygenated at C-3 was accomplished from methyl 2-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (9) **12**. The glycoside was converted by acetolysis to a mixture of anomeric acetates **13** (α) and **15** (β) from which the α anomer crystallized from hexanes. Reaction of **13** with ethanethiol in the presence of BF_3–etherate gave the α-L and β-L thioglycosides **14** (75%) and **16** (11%), which were separated by chromatography.

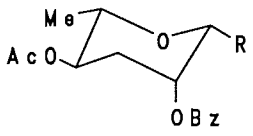
Ethyl tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (10–12) **11** activated by iodonium ions (6) generated *in situ* from *N*-iodosuccinimide and triflic acid (0.13 equivalent) reacted



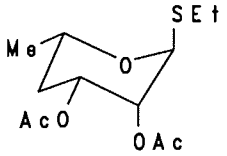
11



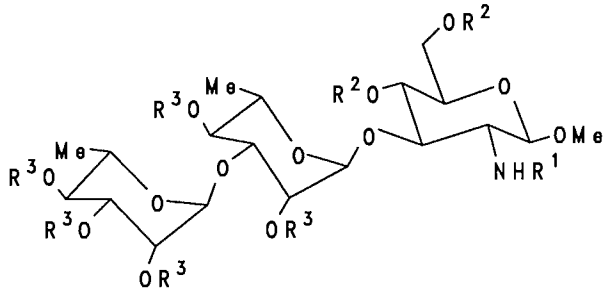
12 $R^1 = OMe$, $R^2 = H$
13 $R^1 = OAc$, $R^2 = Ac$
14 $R^1 = SEt$, $R^2 = Ac$



15 R = OAc
16 R = SEt

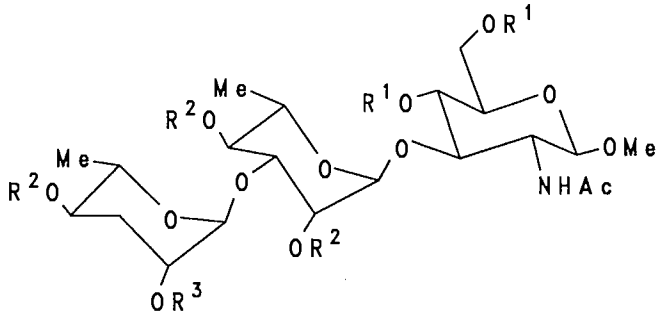


17

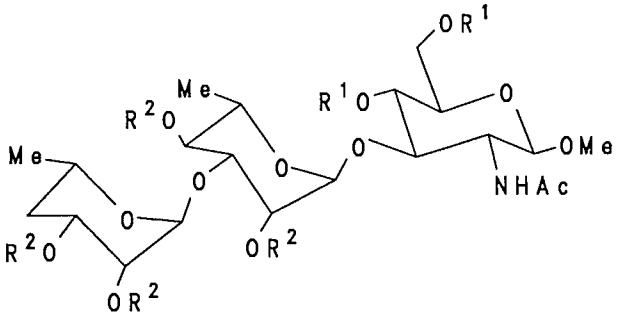


18 $R^1 = CO_2CH_2Ph$, $R^2 = >CHPh$, $R^3 = Ac$
19 $R^1 = CO_2CH_2Ph$, $R^2 = R^3 = H$
20 $R^1 = R^2 = R^3 = H$
21 $R^1 = COCH_3$, $R^2 = R^3 = H$
22 $R^1 = COCH_2CH_3$, $R^2 = R^3 = H$
23 $R^1 = COCF_3$, $R^2 = R^3 = H$

with the disaccharide acceptor **3** to give the protected trisaccharide **18** (84%). Using identical reaction conditions glycosylation of the *N*-acetylated disaccharide **6** with the 3,6-dideoxythiohexoside **14** required further addition of both glycosyl donor and triflic acid to afford the trisaccharide **24** in only 57% yield. Consequently, glycosylation of disaccharide **6** with the thioglycoside **17** was started using more triflic acid (0.26 equivalent) than for the preparation of **18** and gave trisaccharide **26** (86%) without further addition of acid. In view of this result, the same glycosylation conditions were employed to condense the acceptor deoxydisaccharide **8** with the thioglycosides **11**, **14**, and **17**. When **14** and **17** were reacted with **8**, trisaccharides **30** and **32** were isolated in good yields (respectively 82% and 89%). Surprisingly, glycosylation of **8** with the triacetate **11** gave the trisaccharide **28** in only 55% yield. In this case TLC monitoring of the reaction showed complete disappearance of the glycosyl acceptor with simultaneous consumption of the

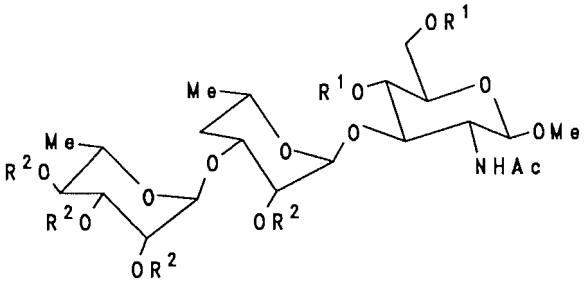


24 $R^1 = >CHPh$, $R^2 = Ac$, $R^3 = Bz$
25 $R^1 = R^2 = R^3 = H$

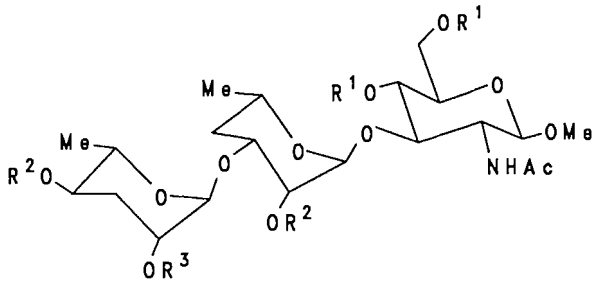


26 $R^1 = >CHPh$, $R^2 = Ac$
27 $R^1 = R^2 = H$

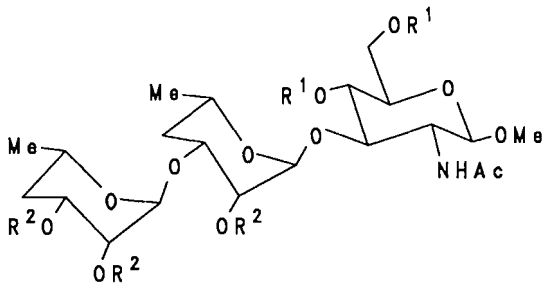
rhamnosyl donor but formation of two UV-absorbent compounds. Upon further addition of triflic acid the less polar product was converted into the more polar compound, which was isolated and identified as the desired trisaccharide **28**. These observations suggested the formation of an orthoester intermediate that rearranged to give **28**. Similarly, when disaccharide **10** was glycosylated with the triacetate **11**, the formation of two products was observed. Work-up and chromatography of the reaction mixture afforded the orthoester **34** (54%) and the target trisaccharide **35** (11%). Their structures were assigned on the basis of ^1H and ^{13}C NMR spectra (Tables 6 and 7). The orthoester **34** showed signals at 26.4 ppm (^{13}C) and 1.63 ppm (^1H) characteristic of an orthoacetate C-CH_3 group (13). As well, the typical signal of a quaternary carbon at 124.7 ppm was evident, while only five signals corresponding to quaternary carbons of carbonyl groups were observed. Since only one stereoisomer was observed, and as the nucleophilic attack of an alcohol on an intermediate 1,2-cyclic acyloxonium ion is sterically favored from the side *trans* (*exo*) to the pyranose ring (14), it is most likely that compound **34** has the *exo* configuration. As expected, the formation of this orthoester could be avoided by adding twice as much triflic acid (0.56 equivalent) when starting the glycosylation. Under these conditions a TLC homogenous mixture (75:25) of the isomeric trisaccharides **35** (53%) and **37** (18%) was obtained. The formation of trisaccharide **37** can be explained by the acid-catalyzed (2′→3′) migration of the 2′-*O*-acetyl group in disaccharide **10** leading to a glycosyl acceptor with a 2-hydroxy group on the rhamnose unit, which can be easily glycosylated by the



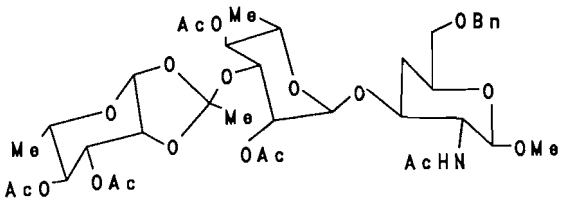
28 $R^1 = >CHPh$, $R^2 = Ac$
29 $R^1 = R^2 = H$



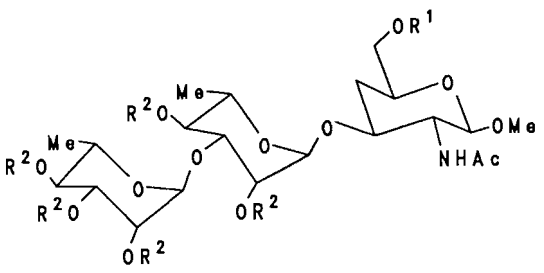
30 $R^1 = >CHPh$, $R^2 = Ac$, $R^3 = Bz$
31 $R^1 = R^2 = R^3 = H$



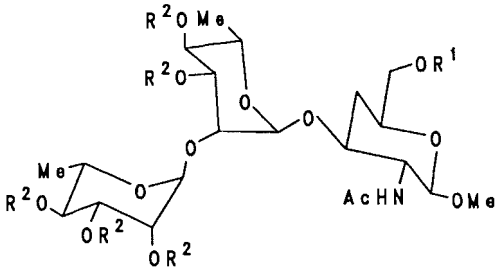
32 $R^1 = >CHPh$, $R^2 = Ac$
33 $R^1 = R^2 = H$



34



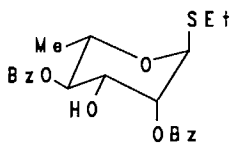
35 $R^1 = Bn$, $R^2 = Ac$
36 $R^1 = R^2 = H$



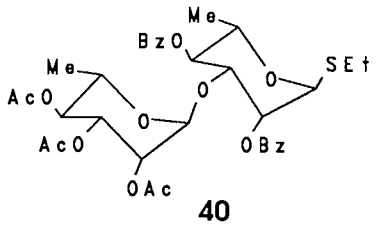
37 $R^1 = Bn$, $R^2 = Ac$
38 $R^1 = R^2 = H$

activated donor **11**. The results described above showed that the success of the iodonium-catalyzed glycosylation of analogous disaccharides such as **3**, **6**, **8**, or **10** was dependent on the structure of both acceptors and donors. Ethyl thiorhamnopyranosides with a 2-*O*-acetyl group react with the acceptor alcohols to form the glycosidic linkage via an orthoacetate, which may be stable in the weakly acidic reaction medium. This observation led us to investigate a block synthetic strategy to prepare trisaccharide **42**, the precursor of the 6-deoxy trisaccharide **46**. The alcohol **39** (12) was glycosylated with acetobromorhamnose (15) (AgOTf) to give disaccharide **40**, which was condensed with monosaccharide **41** (16) under iodonium ion catalysis. As no acetyl group migration was likely to occur when using **41** as glycosyl acceptor, the glycosylation was performed with 0.5 equivalent of triflic acid to avoid orthoester formation and trisaccharide **42** was isolated in 71% yield. The disaccharide donor **40** avoids troublesome orthoester products (17) and thus provides a more convenient route to trisaccharides that are modified in the Glc*N*Ac residue D.

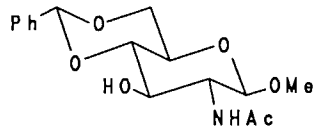
The *N*-benzyloxycarbonyl derivative **19** was obtained as an analytically pure white powder after acetal hydrolysis and transesterification of the protected trisaccharide **18**. Removal of the benzyloxycarbonyl group was accomplished by hydrogenolysis of a methanolic solution of **19** and the amine **20** was precipitated as its hydrochloride salt. Each of the trisaccharides **21**–**23** was prepared from **19** by hydrogenolysis of the *N*-protecting group and *N*-acylation of the crude amine **20**. A methanolic solution of **20** was treated with acetic or propionic anhydride to give **21** (85%) and **22** (73%). The acetylation proceeded much faster than the propionylation, which required the addition of triethylamine to reach completion. Trifluoroacetylation was achieved by treating a solution of **20** in anhydrous pyridine with an excess of trifluoroacetic anhydride, followed by the *in situ* hydrolysis of the *O*-trifluoroacetyl groups, by adding water to the crude reaction mixture. The *N*-trifluoroacetate **23** (50%) was pu-



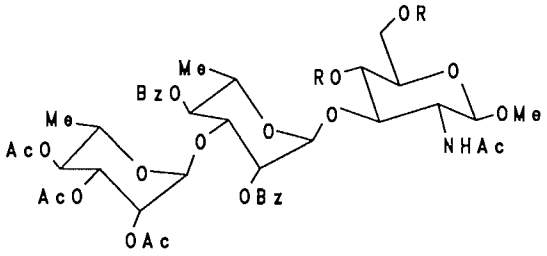
39



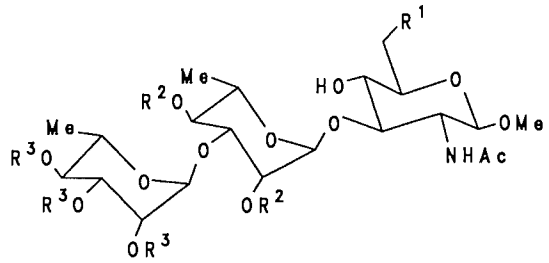
40



41



42 R = > CHPh
43 R = H



44 R^1 = Br, R^2 = Bz, R^3 = Ac
45 R^1 = H, R^2 = Bz, R^3 = Ac
46 $R^1 = R^2 = R^3$ = H

rified by chromatography and obtained free of salt by gel permeation chromatography on a Biogel P2 column eluted with water.

The 3″-, 4″-, and 4′-monodeoxygenated trisaccharides **25**, **27**, and **29** as well as the dideoxy analogues **31** and **33** were obtained by a two-step procedure from the protected trisaccharides **24**, **26**, **28**, **30**, and **32**. The transesterification of the *O*-acyl groups in methanol was followed by the hydrogenolysis of the benzylidene acetal. The latter reaction was preferred to an acid hydrolysis in order to preserve the acid-labile dideoxyhexopyranoside linkages. An analogous sequence of reactions was applied to the mixture of **35** and **37** to obtain the deprotected trisaccharides **36** and **38** that were separated by reversed-phase HPLC.

Deoxygenation at C-6 of the glucosamine unit was achieved in four steps from the protected trisaccharide **42**. Hydrolysis of the benzylidene acetal gave the diol **43**, which was selectively brominated at C-6 using conditions analogous to those described by Garegg *et al.* for the selective chlorination of sugar (18). The brominated trisaccharide **44** (55%) was then reduced to give the 6-deoxy derivative **45**, which, upon transesterification of the acyl groups, led to the deprotected analogue **46** (84%, from **44**).

New compounds and intermediates were fully characterized by ^{1}H NMR spectroscopy (Tables 1 and 3–6). Glycosylation and bromination products as well as final compounds were also characterized by ^{13}C NMR spectroscopy (Tables 2 and 7) and the configurations of the anomeric linkages were assigned according to the heteronuclear coupling constants measured between anomeric carbons and protons (19). The spectroscopic data were in full accord with the proposed structures.

Discussion

The synthesis of 11 trisaccharides reported here underscores the convenience and efficacy of thioglycosides in both block and sequential oligosaccharide synthesis, especially when the glycosyl donors are activated by *N*-iodosuccimide/triflic acid (6). The acidic conditions of activation are sufficiently mild that intermediate orthoesters are observed under typical glycosylation conditions, e.g., synthesis of **34** from reaction of the donor **11** with the acceptor disaccharide **10**. In several reactions TLC monitoring suggested the formation of intermediate orthoesters that rearranged during the normal reaction period, 4–7 h. The formation of orthoester was minimized by increasing the acidity of the reaction mixture; however, in general this is potentially hazardous because of the likelihood of acyl group migration, especially when acetate esters are employed for selective protection. The simplest explanation for the formation of both the 1,2- and 1,3-linked trisaccharide products **35** and **37** was the acid-catalyzed migration of an acetate from *O*-2 to *O*-3 of the terminal rhamnose residue of disaccharide **10**.

Orthoester formation in the *manno* series is well documented (13) and it could be anticipated that the formation of acetoxonium ions following activation of the acylated thioglycoside would lead to orthoester formation under appropriate circumstances of donor and acceptor reactivity (17, 20).

The use of functional group replacement, particularly monodeoxy analogues to map protein binding sites and to draw conclusions about three-dimensional features of the carbohydrate–protein binding sites (21), assumes that only minor perturbations of the conformational equilibria about glycosidic linkages result from these synthetic changes. An initial indication of the validity of this assumption may be drawn from an examination of the ^{1}H NMR and ^{13}C NMR chemical shift changes that accompany the modifications. Carbon-13 chemical shifts of anomeric and aglyconic carbon atoms have been shown to be sensitive to torsional and valence angle changes (22–24). Proton NMR shifts are also diagnostic of predominant conformations (24, 25).

Examination of the ^{1}H NMR chemical shifts for D_2O solutions of the modified and deprotected trisaccharides relative to the native trisaccharide **21** shows a consistent pattern of chemical shifts. Both α-L-rhamnopyranose residues B and C adopt the typical $^1C_4$(L) chair conformation as judged by the ^3J values, and in similar fashion the β-D-GlcNAc residue D has ^1J coupling constants consistent with the $^4C_1$(D) chair conformation (Table 1). The ^{13}C NMR chemical shifts also show closely related values and a consistent pattern for the native trisaccharide and its cogeners. Together, the chemical shift similarities suggest that the conformational equilibria of each trisaccharide are closely related. This is in agreement with calculations that predict closely related potential energy surfaces for each glycosidic linkage of the series of monodeoxy derivatives and, furthermore, shows no indication of substantially shifted conformational equilibria. This conclusion then provides a sound basis for direct comparison of the inhibitory power of each structure and its correlation with a three-dimensional model for the epitope bound to its antibody.

Experimental

General methods

The methods employed were described in an earlier publication (8). NMR data are presented in tables, ^{1}H NMR in Tables 1 and 3–6, ^{13}C NMR in Tables 2 and 7.

*Methyl 2-amino-4,6-*O*-benzylidene-2-*N*-benzyloxycarbonyl-2-deoxy-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**2**)*

A solution of the protected disaccharide **1** (8) (2.02 g, 2.94 mmol) in methanolic NaOMe (0.1 M, 200 mL) was stirred for 1 h at room temperature and deionized with Amberlite IR-120 (H^+) resin. The solution was filtered and concentrated to give the triol **2** (1.61 g, 98%) as a white powder that was isolated by filtration from Et_2O: mp 222–224°C (dec.); $[\alpha]_D^{25}$ −99.2 (*c* 0.6, MeOH). Anal. calcd. for $C_{28}H_{35}NO_{11}$: C 59.9, H 6.3, N 2.5; found: C 59.6, H 6.3, N 2.3.

*Methyl 2-amino-3-*O*-(2′,4′-di-*O*-acetyl-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-*N*-benzyloxycarbonyl-2-deoxy-β-D-glucopyranoside (**3**)*

Trimethylorthoacetate (520 μL, 1.5 mol-equiv.) was added to a suspension of the triol **2** (1.53 g, 2.72 mmol) in anhydrous CH_3CN (60 mL) containing *p*-toluenesulfonic acid (87 mg), and the mixture was stirred for 1 h under N_2 at room temperature. NEt_3 (5 mL) was added, the solution was concentrated to dryness, and residual traces of MeOH were coevaporated with a mixture of anhydrous pyridine–NEt_3 (4:1, 25 mL). *N*,*N*-Dimethylaminopyridine (40 mg) and acetic anhydride (520 μL, 2 mol-equiv.) were then added to a stirred solution of the residue in anhydrous pyridine (60 mL). After 1 h at room temperature more anhydride (100 μL) was added and stirring was continued for another hour. MeOH (200 μL) was added to destroy excess reagent, solvents were evaporated, and the residue, dissolved in EtOAc (200 mL), was washed successively with 1 M HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), saturated aqueous NaCl (50 mL), and dried. The solid obtained after concentration was dissolved in aqueous 80% acetic acid (100 mL), stirred 30 min at room temperature, and solvents were evaporated at 25°C under high vacuum. Residual traces of acid were coevaporated with toluene (3 × 20 mL), and flash chromatography ($CHCl_3$–acetone, 10:1.5) of the residue gave the alcohol **3** (1.3 g, 73%), which crystallized on standing: mp 221.6°C; $[\alpha]_D^{25}$ −45.3 (*c* 0.7, $CHCl_3$). Anal. calcd. for $C_{32}H_{39}NO_{13}$: C 59.5, H 6.1, N 2.2; found: C 59.1, H 6.2, N 2.2.

*Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**5**)*

Deacetylation of disaccharide **4** (8) (1.25 g, 2.1 mmol), as well as work-up of the reaction, was performed in the same manner as the preparation of disaccharide **2**. Triol **5** was obtained as a pure white powder (951 mg, 96%) by crystallization from Et_2O: mp 232.5–234.2°C; $[\alpha]_D^{25}$ −112.5 (*c* 1.0, MeOH). Anal. calcd. for $C_{22}H_{31}NO_{10}$: C 56.3, H 6.7, N 3.0; found: C 55.8, H 6.7, N 3.3.

*Methyl 2-acetamido-3-*O*-(2′,4′-di-*O*-acetyl-α-L-rhamnopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**6**)*

Selective acetylation of triol **5** (860 mg, 1.83 mmol) was performed in three steps, using conditions similar to those used to prepare the alcohol **3**. The alcohol **6** was crystallized from MeOH–Et_2O and obtained as a white powder (904 mg, 89%): mp 255–257°C (dec.); $[\alpha]_D^{25}$ −46.2 (*c* 0.55, $CHCl_3$). Anal. calcd. for $C_{26}H_{35}NO_{12}$: C 56.4; H 6.4, N 2.5; found: C 56.2, H 6.4, N 2.3.

*Methyl 2-acetamido-3-*O*-(2′-*O*-acetyl-4′-deoxy-α-L-*lyxo*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**8**)*

Treatment of the diol **7** (8) (763 mg, 1.68 mmol) with trimethylorthoacetate and acid opening of the intermediate 2′,3′-orthoacetate using identical conditions to those used to prepare the alcohol **3** gave the alcohol **8** as an amorphous white solid isolated by filtration from EtOAc (687 mg, 82%); $[\alpha]_D^{25}$ −76.0 (*c* 0.7, DMSO). Anal. calcd. for $C_{24}H_{33}NO_{10}$: C 58.2, H 6.7, N 2.8; found: C 58.3, H 6.7, N 2.8.

*Methyl 2-acetamido-3-*O*-(2′,4′-di-*O*-acetyl-α-L-rhamnopyranosyl)-6-*O*-benzyl-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**10**)*

Triol **9** (8) (444 mg, 0.97 mmol) was selectively acetylated in the conditions used to prepare alcohol **3**. The alcohol **10** was isolated as a white solid (290 mg, 55%) from MeOH–Et_2O. Chromatography of the mother liquors ($CHCl_3$–MeOH; 40:1, 150 mL; 30:1, 90 mL; 20:1, 80 mL) gave more alcohol (101 mg, 19%), which was also filtered off in MeOH–Et_2O: mp 212.3–212.9°C (dec.); $[\alpha]_D^{25}$ −23.2 (*c* 0.6, $CHCl_3$). Anal. calcd. for $C_{26}H_{37}NO_{11}$: C 57.9, H 6.9, N 2.6; found: C 57.5, H 6.8, N 2.6.

*1,4-Di-*O*-acetyl-2-*O*-benzoyl-3-deoxy-α-L-*arabino*-hexopyranoside (**13**) and 1,4-di-*O*-acetyl-2-*O*-benzoyl-3-deoxy-β-L-*arabino*-hexopyranoside (**15**)*

A solution of sulfuric acid in glacial acetic acid (0.47 M, 5 mL) was added dropwise to a stirred solution of the glycoside **12** (9) (2.21 g, 8.7 mmol) in a mixture of glacial acetic acid and acetic anhydride (2:5, 185 mL). After 1 h at 20°C (water bath) NaOAc (5 g) was added and the reaction mixture was poured slowly into an ice-cold saturated solution of $NaHCO_3$ (250 mL). The product was extracted in EtOAc (2 × 250 mL), washed with saturated aqueous NaCl (200 mL), dried, and concentrated. Chromatography (hexanes–EtOAc, 7:3) of the oily residue gave a mixture (2.5 g) of the α-(**13**) and β-(**15**) anomers (94:6). Monosaccharide **13** crystallized from hexanes (2.0 g, 71%): mp 90.2–90.8°C; $[\alpha]_D^{25}$ −31.7 (*c* 0.95, $CHCl_3$). Anal. calcd. for $C_{17}H_{20}O_7$: C 60.7, H 6.0; found: C 60.7, H 6.0.

*Ethyl 4-*O*-acetyl-2-*O*-benzoyl-3-deoxy-1-thio-α-L-*arabino*-hexopyranoside (**14**) and ethyl 4-*O*-acetyl-2-*O*-benzoyl-3-deoxy-1-thio-β-L-*arabino*-hexopyranoside (**16**)*

The di-*O*-acetate **13** (1.80 g, 5.35 mmol) dissolved in dry CH_2Cl_2 (100 mL) was reacted for 30 min at room temperature with EtSH

TABLE 1. ^{1}H NMR chemical shifts for D_2O solutions of the trisaccharides

Protons (J, Hz)	**21**	**20**	**22**	**23**	**25**	**27**	**29**	**31**	**33**	**36**	**46**	**38**
α-L-Rha unit B												
H-1	5.01	5.06	5.02	5.01	4.85	5.05	4.94	4.78	4.99	4.99	5.01	4.87
($J_{1,2}$)	(1.5)		(1.5)	(1.5)						(1.5)	(1.0)	(1.0)
H-2	4.05	4.08	4.05	4.05	4.05	3.89	3.93	3.93	3.76	4.05	4.05	4.05
($J_{2,3}$)	(3.5)		(3.5)	(3.0)	(3.0)	(~3.5)	(3.0)		(3.5)	(3.0)	(3.5)	(3.0)
H-3	3.82	3.85	3.82	3.81	1.87	4.08	3.79	1.84	4.05	3.82	3.82	3.77
($J_{3,4}$)	(9.0)	(10.0)	(10.0)	(9.5)	(~11.5)	(12.0)	(9.5)		(12.0)	(9.5)	(9.5)	(9.5)
($J_{3,3'}$)					(~12.0)			(13.5)				
H-3′					2.04			2.03				
($J_{2,3'}$)								(~3.5)				
($J_{3',4}$)					(4.5)			(~3.5)				
H-4	3.45	3.47	3.47	3.45	3.61	1.60	3.44	3.61	1.59	3.45	3.46	3.45
($J_{4,5}$)	(10.0)	(10.0)	(10.0)	(9.5)	(~11.5)	(12.0)	(9.5)		(12.0)	(9.5)	(9.5)	(9.5)
H-4′						1.76			1.75			
($J_{3,4'}$)						(~3.5)			(4.5)			
($J_{4,4'}$)						(12.0)			(12.0)			
H-5	3.78	3.85	3.77	3.73	3.76	4.04	3.74	3.73	3.99	3.77	3.78	3.72
($J_{5,6}$)	(6.5)	(6.5)	(6.5)	(6.0)	(6.0)	(6.5)	(6.0)	(6.0)	(6.5)	(6.5)	(6.0)	(6.0)
H-6	1.30	1.30	1.31	1.29	1.27	1.24	1.30	1.27	1.23	1.29	1.30	1.28
α-L-Rha unit C												
H-1	4.81	4.99	4.82	4.78	4.82	4.82	4.87	4.88	4.87	4.83	4.80	4.99
($J_{1,2}$)	(~1.5)	(~1.0)	(~1.5)						(1.0)	(~1.5)		
H-2	3.86	4.20	3.86	3.82	3.89	3.85	3.71	3.74	3.70	3.86	3.85	3.72
($J_{2,3}$)	(~2.5)		(~2.0)	(3.5)	(3.0)		(~4.0)	(~4.0)		(~2.5)	(3.0)	(3.5)
H-3	3.78	3.84	3.78	3.76	3.80	3.76	4.01	4.04	4.00	3.76	3.77	3.82
($J_{3,4}$)		(10.0)	(10.0)	(9.5)	(9.5)	(~9.0)	(12.0)	(12.0)		(9.5)	(10.0)	(10.0)
H-4	3.52	3.58	3.52	3.53	3.52	3.49	1.57	1.58	1.56	3.50	3.51	3.45
($J_{4,5}$)		(10.0)	(10.0)				(12.0)	(12.0)	(12.0)	(9.5)	(10.0)	(9.5)
H-4′							1.85	1.85	1.85			
($J_{3,4'}$)							(~4.0)	(~3.5)				
($J_{4,4'}$)							(12.0)	(12.0)	(12.0)			
H-5	4.03	3.99	4.04	4.02	4.04	4.03	4.23	4.24	4.23	3.80	4.05	3.72
($J_{5,6}$)	(6.0)	(6.0)	(6.5)	(6.5)	(6.5)	(6.5)	(6.0)	(6.5)	(6.0)	(6.5)	(6.0)	(6.0)
H-6	1.24	1.29	1.23	1.24	1.24	1.24	1.18	1.18	1.18	1.27	1.23	1.27
β-D-GlcNAc unit D												
H-1	4.47	4.68	4.48	4.58	4.47	4.47	4.47	4.48	4.47	4.41	4.45	4.45
($J_{1,2}$)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)	(8.5)
H-2	3.82	3.20	3.83	3.92	3.82	3.82	3.80	3.81	3.81	3.65	3.81	3.62
($J_{2,3}$)	(9.5)	(9.0)	(10.0)	(~9.0)	(~9.5)	(9.0)	(10.0)	(10.0)	(10.0)		(9.5)	(9.5)
H-3	3.58	3.86	3.60	3.69	3.59	3.58	3.57	3.58	3.58	3.77	3.52	3.74
($J_{3,4}$)	(9.0)	(~9.5)	(10.0)	(10.0)	(9.5)	(9.0)				(11.5)	(9.5)	(10.5)
($J_{3,4'}$)												(4.0)
H-4	3.52	3.66	3.52	3.58	3.52	3.52	3.50	3.50	3.49	1.59	3.28	1.58
($J_{4,5}$)		(~9.5)	(10.0)	(10.0)						(11.5)	(9.5)	(12.5)
H-4′										2.07		2.04
($J_{4,4'}$)										(11.5)		(12.5)
H-5	3.47	3.54	3.48	3.50	3.47	3.46	3.47	3.48	3.48	3.71	3.49	3.71
H-6	3.76	3.78	3.76	3.77	3.76	3.75	3.75	3.75	3.74	3.64	1.33	3.64
($J_{6,5}$)	(6.0)	(5.5)	(6.0)	(6.0)		(~6.0)					(6.0)	
H-6′	3.94	3.95	3.95	3.96	3.94	3.94	3.94	3.94	3.94	3.71		3.71
($J_{6',5}$)	(2.0)	(2.0)	(1.5)	(1.5)	(~1.0)	(~2.0)						
($J_{6,6'}$)	(12.5)	(12.0)	(12.0)	(12.5)	(12.0)	(11.5)			(13.0)			
OCH_3	3.51	3.60	3.51	3.53	3.52	3.51	3.51	3.52	3.51	3.51	3.49	3.52
NCOC*H*R	2.03		2.30		2.05	2.04	2.05	2.06	2.05	2.04	2.05	2.06
NCOCCH_3			1.13									

(700 μL, 1.8 mol-equiv.) and BF_3–etherate (800 μL, 1.2 mol-equiv.). NEt_3 (1.1 mL) was added slowly and the solution was concentrated to dryness. The residue dissolved in EtOAc (200 mL) was washed successively with saturated aqueous $NaHCO_3$ (2 × 100 mL), saturated aqueous NaCl (100 mL), and dried. Concentration and flash chromatography (hexanes–EtOAc; 9:1, 400 mL; 8:2, 400 mL) gave the α-L-thioglycoside **14** (1.36 g, 75%), which crystallized on standing: mp 84.3–84.8°C; $[\alpha]_D^{25}$ −103.9 (*c* 0.9, $CHCl_3$). Anal. calcd. for $C_{17}H_{22}O_5S$: C 60.3, H 6.6; found: C 60.1, H 6.7.

TABLE 2. ^{13}C NMR chemical shifts for D_2O solutions of the trisaccharides[a]

Carbon	21	20	22	23	25	27	29	31	33	36	46	38
α-L-Rha unit B												
C-1	103.2	103.5	103.1	103.1	102.0	103.9	99.2	98.0	99.8	103.2	103.2	103.5
($J_{C,H}$)	(172)	(172)	(172)	(172)	(171)	(171)	(171)	(169)	(170)	(172)	(172)	(168)
C-2	71.0	71.0	70.9	70.8	68.2	68.6	71.3	68.5	68.9	71.0	71.0	70.9
C-3	71.0	70.8	70.9	70.8	34.2	65.9	71.0	34.2	65.9	71.0	71.0	70.9
C-4	72.8	72.8	72.7	72.8	67.8	35.4	72.9	67.8	35.3	72.8	72.8	72.9
C-5	69.9	70.0	69.8	69.8	70.9	66.4	69.8	70.9	66.5	69.8	69.9	70.0
C-6	17.5	17.5	17.5	17.1	17.6	20.9	17.5	17.6	20.9	17.5	17.5	17.4
α-L-Rha unit C												
C-1	102.2	102.0	102.1	102.3	102.2	102.2	103.0	103.0	103.0	102.6	102.2	101.2
($J_{C,H}$)	(170)	(171)	(170)	(170)	(170)	(171)	(170)	(170)	(170)	(170)	(170)	(171)
C-2	71.3	70.9	71.2	71.2	71.4	71.4	68.9	68.9	68.9	71.1	71.3	80.5
C-3	79.0	79.4	78.9	78.7	78.8	78.8	72.0	71.9	71.8	78.8	79.1	70.5
C-4	72.1	71.9	72.1	72.0	72.1	72.0	32.4	32.6	32.5	72.2	72.1	72.9
C-5	69.9	70.6	69.8	69.8	69.8	69.8	66.5	66.5	66.4	69.9	69.8	69.8
C-6	17.3	17.5	17.1	17.1	17.3	17.3	20.7	20.7	20.7	17.4	17.2	17.7
β-D-GlcNAc unit D												
C-1	102.2	100.6	102.2	101.4	102.2	102.1	102.2	102.2	102.2	102.7	102.2	102.4
($J_{C,H}$)	(162)	(161)	(162)	(163)	(162)	(162)	(163)	(162)	(162)	(162)	(162)	(162)
C-2	55.9	55.8	55.8	56.4	55.9	55.9	55.9	55.9	55.9	56.4	56.1	56.6
C-3	82.6	82.2	82.4	82.5	82.5	82.5	82.6	82.6	82.5	78.6	82.4	78.7
C-4	69.3	69.5	69.3	69.2	69.3	69.3	69.4	69.4	69.4	34.6	74.5	34.6
C-5	76.9	76.9	76.8	76.9	76.8	76.8	76.8	76.8	76.8	73.3	72.9	73.3
C-6	61.6	61.3	61.6	61.4	61.6	61.6	61.6	61.6	61.6	64.4	17.5	64.4

[a]The numbers in parentheses denote the one-bond $^{13}C-^{1}H$ coupling constants for the anomeric carbon atoms (Hz).

Further elution of the column gave the β-anomer **16** (200 mg, 11%) as a colourless oil: $[\alpha]_D^{25}$ +36.6 (*c* 1.0, $CHCl_3$). Anal. found: C 60.1, H 6.6.

Methyl 2-amino-3-O-[2',4'-di-O-acetyl-3'-O-(2",3",4"-tri O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-4,6-O-benzylidene-2-N-benzyloxycarbonyl-2-deoxy-β-D-glucopyranoside (18)

A mixture of the alcohol **3** (585 mg, 0.91 mmol) and the glycosyl donor **11** (350 mg, 1.2 mol-equiv.) in anhydrous CH_2Cl_2 (30 mL) containing powdered activated molecular sieves 4Å (2 g) was stirred under N_2 for 18 h at room temperature. *N*-Iodosuccinimide (262 mg, 1.3 mol-equiv.) and a saturated solution of triflic acid in CH_2Cl_2 (0.15 M, 785 μL, 0.13 mol-equiv.) were added to the mixture, which was protected from light, and stirred 30 min at room temperature. NEt_3 (800 μL) was added to the reaction mixture, and solids were removed by filtration and rinsed with EtOAc (75 mL). The combined organic solutions were washed successively with a 5% solution of sodium thiosulfate in aqueous 0.5 M NaOH (3 × 50 mL), 1 M HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), saturated aqueous NaCl (50 mL), and then dried. Concentration and flash chromatography (toluene–EtOAc, 7:3) gave the trisaccharide **18** (697 mg, 84%) as a white powder: mp 119–123°C (dec.); $[\alpha]_D^{25}$ −40.6 (*c* 0.9, $CHCl_3$). Anal. calcd. for $C_{44}H_{55}NO_{20}$: C 57.6, H 6.0, N 1.5; found: C 57.4, H 6.1, N 1.8.

Methyl 2-amino-2-N-benzyloxycarbonyl-2-deoxy-3-O-[3'-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (19)

A solution of the trisaccharide **18** (647 mg, 0.7 mmol) in 60% acetic acid (35 mL) was stirred for 45 min at 100°C and concentrated to a syrup. Residual acid was removed by evaporating a mixture of toluene–MeOH (1:1, 2 × 20 mL). The dry residue was dissolved in a 0.06 M methanolic solution of NaOMe (30 mL) and was stirred for 18 h at room temperature. The reaction mixture was deionized with Amberlite IR-120 (H^+) resin, filtered, and the filtrate was concentrated. The deprotected trisaccharide **18** was obtained as a white powder from EtOH–acetone (397 mg; 91%); mp 210–213°C (dec.); $[\alpha]_D^{25}$ −55.1 (*c* 0.5, H_2O). Anal. calcd. for $C_{27}H_{41}NO_{15}$: C 52.3, H 6.7, N 2.3; found: C 51.9, H 6.5, N 2.2.

Methyl 2-amino-2-deoxy-3-O-[3'-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (20)

A solution of the trisaccharide **19** (56 mg, 0.09 mmol) in MeOH (5.5 mL) containing Pd–C catalyst (10%, 19 mg) was stirred for 2 h at room temperature under a hydrogen atmosphere. The catalyst was removed by filtration, rinsed with MeOH (10 mL), and the combined filtrates were concentrated to dryness. The residue was dissolved in H_2O (2 mL), the pH lowered to 4 by addition of 0.1 M HCl, and the solution was concentrated. The pure trisaccharide **20** was then precipitated (EtOH–acetone) as the hydrochloride salt, centrifuged, washed (acetone 2 × 1 mL), and dried (38 mg, 82%); $[\alpha]_D^{25}$ −56.4 (*c* 0.4 MeOH). Anal. calcd. for $C_{19}H_{36}ClNO_{13}$: C 43.7, H 6.9, N 2.7; found: C 43.6, H 6.8, N 2.5.

Methyl 2-acetamido-2-deoxy-3-O-[3'-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (21)

Hydrogenolysis of the trisaccharide **19** (34 mg, 0.05 mmol) was performed as described for the preparation of **20**. Acetic anhydride (200 μL) was added to the crude reaction mixture, which was stirred for 1 h at room temperature, then filtered and concentrated. The trisaccharide **21** was purified by elution from a Biogel P-2 column (H_2O) and it was obtained as a white powder by freeze-drying (24.6 mg, 85%); $[\alpha]_D^{25}$ −74.9 (*c* 0.4, MeOH). Anal. calcd. for $C_{21}H_{37}NO_{14}$: C 47.8, H 7.1, N 2.6; found: C 47.6, H 7.0, N 2.8.

Methyl 2-deoxy-2-methylacetamido-3-O-[3'-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (22)

The amine obtained from **19** (53.9 mg, 0.09 mmol) by hydrogenolysis was dissolved in MeOH (4 mL) and treated with propionic anhydride (200 μL). After 1 h at room temperature, more

TABLE 3. ^{1}H NMR data for compounds **2**, **3**, **5**, **6**, **8**, and **10**

Protons (J, Hz)	**2**[a]	**3**[a]	**5**[b]	**6**[c]	**8**[a]	**10**[d]
α-L-Rha unit C						
H-1	4.76	4.82	4.77	4.75	4.76	4.87
($J_{1,2}$)						(~1.0)
H-2	3.59	4.97	3.73	4.78	4.67	4.92
($J_{2,3}$)		(~3.0)	(~2.5)	(3.0)		(3.5)
H-3	3.40	3.79	3.64	3.90	3.78	3.96
($J_{3,4}$)	(9.0)	(10.0)	(10.0)	(10.0)	(9.0)	(9.5)
H-4	3.10	4.59	3.26	4.68	1.32	4.81
($J_{4,5}$)	(~9.5)	(10.0)	(10.0)	(10.0)	(12.0)	(10.0)
H-4′					1.41	
($J_{4',4}$)					(12.0)	
H-5	3.66	3.92	3.74	4.86	3.95	3.84
($J_{5,6}$)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)	(6.5)
H-6	0.70	0.49	0.64	0.52	0.56	1.15
β-D-GlcNAc unit D						
H-1	4.37	4.35	4.58	4.55	4.38	4.70
($J_{1,2}$)	(8.5)	(8.5)	(8.5)	(8.5)	(8.0)	(8.5)
H-2	3.44	3.52	3.90	3.62	3.74	3.12
($J_{2,3}$)	(9.5)	(10.0)	(9.5)	(9.0)		(11.0)
H-3	3.74	3.74	3.81	3.93	3.72	4.20
($J_{3,4}$;$J_{3,4'}$)	(9.0)	(10.0)	(9.5)	(~9.0)	(9.0)	(11.0, 5.2)
H-4	3.57	3.65	3.72	3.50	3.56	1.52
($J_{4,5}$)	(9.0)	(9.5)	(9.0)	(9.5)	(9.0)	(11.5)
H-4′						2.04
($J_{4,4'}$;$J_{4',5}$)						(11.5; 5.0)
H-5	3.37	3.39	3.60	3.44	3.42	3.69
H-6	3.76	3.77	3.86	3.71	3.75	3.50
($J_{5,6}$)	(~10.0)	(10.5)	(9.0)	(10.0)	(10.0)	(5.0)
H-6	4.22	4.25	4.36	4.28	4.23	3.59
($J_{5,6'}$;$J_{6,6'}$)	(5.0, 10.0)	(5.0, 10.1)	(5.0, 10.5)	(5.0, 10.5)	(5.0, 10.5)	(5.5. 10.0)
OCH_3	3.36	3.36	3.50	3.42	3.33	3.47
N*H*R	7.5					5.58
C*H*Ph	5.62	5.67	5.67	5.44	5.63	
CH_2Ph	5.01, 5.07	4.84, 5.06				4.56
OAc		1.96, 2.00		1.98, 1.99	1.83	2.11, 2.12
NAc			2.05	2.04	2.01	2.02

[a]In $(CD_3)_2SO$.
[b]In 1:1 CD_3OD–D_2O at 310 K.
[c]In 9:1 $CDCl_3$–CD_3OD.
[d]In $CDCl_3$.

anhydride (100 μL) was added and the reaction mixture was stirred for an additional hour. As a TLC (EtOAC–MeOH–H_2O, 6:3:1, R_f (product) 0.6) showed that the reaction was not proceeding further, NEt_3 (600 μL) and propionic anhydride (200 μL) were added to the solution stirred at 18°C (water bath). The acylation was completed within 1 h. Solvents were evaporated and the residual NEt_3 was coevaporated with MeOH (2 × 2 mL). The residue dissolved in MeOH (1 mL) was deionized on a mixed bed column (20 × 1.5) (top half Rexyn 201 (OH^-) and bottom half Amberlite IR-120 (H^+)). Elution with MeOH and concentration of the effluent gave the trisaccharide **22** as a white powder (35 mg, 73%) that was centrifuged from an acetone suspension (2 mL) and dried; $[\alpha]_D^{25}$ −76.9 (*c* 0.4, MeOH). Anal. calcd. for $C_{22}H_{39}NO_{14}$: C 48.8, H 7.3, N 2.6; found: C 48.6, H 7.3, N 2.4.

*Methyl 2-deoxy-3-*O*-[3′-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-2-trifluoroacetamido-β-D-glucopyranoside (**23**)*

The amine obtained by hydrogenolysis of the trisaccharide **19** (21 mg, 0.03 mmol) was filtered, concentrated, evaporated with anhydrous pyridine (2 × 2 mL), and a solution of the dry residue in anhydrous pyridine (1.5 mL) containing trifluoroacetic anhydride (60 μL) was stirred 48 h at 40°C. Water (100 μL) was added to the reaction mixture, which was concentrated, and residual pyridine was coevaporated with H_2O (2 × 1 mL). Flash chromatography (EtOAc–MeOH–H_2O, 8:1.2:1) of the oily residue gave the trisaccharide **23**, subsequently obtained free of salts by chromatography on a Biogel P2 column (H_2O). Freeze-drying of the appropriate fractions gave **23** as an amorphous white powder (9.6 mg, 50%); $[\alpha]_D^{25}$ −79.2 (*c* 0.4, MeOH). Anal. calcd. for $C_{21}H_{34}F_3NO_{14}$: C 43.4, H 5.9, N 2.4; found: C 43.3, H 5.8, N 2.3.

*Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[2′,4′-di-*O*-acetyl-3′-*O*-(4″-*O*-acetyl-2″-*O*-benzoyl-3″-deoxy-α-L-arabino-hexopyranosyl)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**24**)*

A mixture of the alcohol **6** (216 mg, 0.4 mmol), the thioglycoside **14** (153 mg, 1.3 mol-equiv.), and activated powdered molecular sieves 4Å (1.1 g) in anhydrous CH_2Cl_2 (15 mL) was stirred for 2 h under N_2 at room temperature. *N*-Iodosuccinimide (120 mg,

TABLE 4. ^1H and ^{13}C NMR data[a] for compounds **13**, **14**, and **16**

Proton (J Hz)	^1H NMR			^{13}C NMR[b]			
	13	**14**	**16**	Carbon	**13**	**14**	**16**
L-Rhamnopyranose derivatives							
H-1	6.06	5.29	4.77	C-1	89.8	82.1	84.0
($J_{1,2}$)				($J_{C,H}$)	(176)	(166)	(152)
H-2	5.15	5.28	5.38	C-2	68.9	72.1	71.4
($J_{2,3}$;$J_{2,3'}$)	(−, ~3.5)	(~3.0, −)	(~3.0, ~4.0)				
H-3	2.05	2.02	1.81	C-3	29.5	30.5	34.6
($J_{3,3'}$;$J_{3,4}$)	(13.5, 10.5)	(14.0, 11.5)	(14.0, 11.0)				
H-3′	2.36	2.31	2.50				
($J_{3',4}$)	(4.5)	(~4.5)	(4.5)				
H-4	4.92	4.96	4.83	C-4	69.5	70.2	69.5
($J_{4,5}$)	(10.5)	(~11.0)	(11.0)				
H-5	3.92	4.22	3.56	C-5	68.9	67.0	76.7
($J_{5,6}$)	(6.0)	(6.0)	(6.0)				
H-6	1.23	1.23	1.29	C-6	17.7	17.6	18.1
OAc	2.07, 2.14	2.06	2.0	OAc	20.7, 21.2	21.1	20.9
SCH_2CH_3		2.67	2.71	SCH_2CH_3		25.3	25.5
SCH_2CH_3		1.31	1.25	SCH_2CH_3		15.1	14.8

[a]In $CDCl_3$.
[b]The numbers in parentheses denote the one-bond ^{13}C–^1H coupling constants for the anomerics (Hz).

1.3 mol-equiv.) and a saturated solution of triflic acid in CH_2Cl_2 (0.15 M, 400 μL, 0.15 mol-equiv.) were added to the reaction mixture, which was stirred for 30 min in the dark at room temperature. More glycosyl donor (10.8 mg, 0.2 mol-equiv.) in CH_2Cl_2 (120 μL), and more acid (3 × 200 μL) were added during the following 2 h. NEt_3 (900 μL) was then added to the reaction mixture and work-up was carried out as described for the preparation of **18**. Flash chromatography ($CHCl_3$–MeOH, 30:1) gave the pure trisaccharide **24** (148 mg, 46%) as a colourless glass. Preparative TLC (Silica Gel 60 F254, Merck, 2-mm plate, $CHCl_3$–MeOH, 20:1) of impure fractions gave another 37 mg (11%) of pure **24**; $[\alpha]_D^{25}$ −40.7 (*c* 0.7, $CHCl_3$). Anal. calcd. for $C_{41}H_{51}NO_{17}$: C 59.3, H 6.2, N 1.7; found: C 59.1, H 6.4, N 1.9.

*Methyl 2-acetamido-2-deoxy-3-*O*-[3′-*O*-(3″-deoxy-α-*L*-arabino-hexopyranosyl)-α-*L*-rhamnopyranosyl]-β-*D*-glucopyranoside (25)*

A solution of the protected trisaccharide **24** (46 mg, 0.055 mmol) in methanolic NaOMe (3.2 mL, 0.07 M) was stirred 18 h at room temperature, deionized with Amberlite IR-120 (H^+) resin, and filtered. The resin was rinsed with MeOH (5 mL) and the combined filtrates were concentrated to dryness. A solution of the residue in aqueous acetic acid (90%, 3 mL) was hydrogenated 18 h at 60 psi (1 psi = 6.89 kPa) in the presence of 10% Pd–C catalyst (54 mg). The reaction mixture was filtered, the catalyst rinsed with MeOH, and the combined filtrates concentrated. Chromatography (EtOAc–MeOH–H_2O, 7:2:1) of the residue gave the trisaccharide **25**, finally purified on a Biogel P2 column (H_2O) and obtained as a white amorphous powder by freeze-drying (19 mg, 68%); $[\alpha]_D^{25}$ −92.8 (*c* 0.4, H_2O). Anal. calcd. for $C_{21}H_{37}NO_{13}$: C 49.3, H 7.3, N 2.7; found: C 49.1, H 7.4, N 2.8.

*Methyl 2-acetamido-3-*O*-[2′,4′-di-*O*-acetyl-3′-*O*-(2″,3″-di-*O*-acetyl-4″-deoxy-α-*L*-lyxo-hexopyranosyl)-α-*L*-rhamnopyranosyl]-4,6-*O*-benzylidene-2-deoxy-β-*D*-glucopyranoside (26)*

A mixture of the alcohol **6** (100.4 mg, 0.18 mmol), the thioglycoside **17** (8) (62.5 mg, 1.3 mol-equiv.), and activated powdered molecular sieve 4Å (550 mg) in anhydrous CH_2Cl_2 (7 mL) was stirred 18 h under N_2 at room temperature. *N*-Iodosuccinimide (56 mg, 1.4 mol-equiv.) and a saturated solution of triflic acid in CH_2Cl_2 (320 μL, 0.26 mol-equiv.) were added to the reaction mixture, which was protected from light and stirred 3.5 h at room temperature. NEt_3 was added and work-up was carried out as described for the preparation of **18**. The trisaccharide **26** was purified by flash chromatography ($CHCl_3$–MeOH, 30:1) and obtained as a colourless glass (119 mg, 86%); $[\alpha]_D^{25}$ −49.5 (*c* 0.8, $CHCl_3$). Anal. calcd. for $C_{36}H_{49}NO_{17}$: C 56.3, H 6.4, N 1.8; found: C 56.1, H 6.4, N 1.5.

*Methyl 2-acetamido-2-deoxy-3-*O*-[3′-*O*-(4″-deoxy-α-*L*-lyxo-hexopyranosyl)-α-*L*-rhamnopyranosyl]-β-*D*-glucopyranoside (27)*

The trisaccharide **26** (96 mg, 0.125 mmol) was deprotected using the conditions employed to prepare trisaccharide **25**. Flash chromatography (EtOAc–MeOH–H_2O; 7:1.5:1, 150 mL; 7:2:1, 50 mL) gave trisaccharide **26**, finally purified on a Biogel P2 column (H_2O) and obtained as a white amorphous powder by freeze-drying (47 mg, 73%); $[\alpha]_D^{25}$ −86.4 (*c* 0.8, H_2O). Anal. calcd. for $C_{21}H_{37}NO_{13}$: C 49.3, H 7.3, N 2.7; found: C 49.0, H 7.3, N 2.6.

*Methyl 2-acetamido-3-*O*-[2′-*O*-acetyl-4′-deoxy-3′-*O*-(2″,3″,4″-tri-*O*-acetyl-α-*L*-rhamnopyranosyl)-α-*L*-lyxo-hexopyranosyl]-4,6-*O*-benzylidene-2-deoxy-β-*D*-glycopyranoside (28)*

Glycosylation of the disaccharide **8** (102 mg, 0.2 mmol) with the glycosyl donor **11** (85 mg, 1.22 mol-equiv.) was started in the same conditions as those used to prepare the trisaccharide **26**. After 4 h at room temperature more acid (150 μL, 0.11 mol-equiv.) was added, and the reaction was allowed to proceed during an additional 3 h at room temperature and quenched by addition of NEt_3 (50 μL). Work-up was carried out as described for the preparation of **18** and the trisaccharide **28** was purified by flash chromatography ($CHCl_3$–MeOH, 50:1) to yield a colourless glass (87 mg, 55%); $[\alpha]_D^{25}$ −62.6 (*c* 0.5, $CHCl_3$). Anal. calcd. for $C_{36}H_{49}NO_{17}$: C 56.3, H 6.4, N 1.8; found: C 56.5, H 6.5, N 2.0.

*Methyl 2-acetamido-2-deoxy-3-*O*-[4′-deoxy-3′-*O*-(α-*L*-rhamnopyranosyl)-α-*L*-lyxo-hexopyranosyl]-β-*D*-glucopyranoside (29)*

The trisaccharide **28** (70 mg, 0.1 mmol) was deprotected as described for **25**. Flash chromatography (EtOAc–MeOH–H_2O, 7:1.5:1) gave trisaccharide **29**, finally purified on a Biogel P2 column (H_2O) and isolated as a white powder by freeze-drying (34 mg, 72%); $[\alpha]_D^{25}$ −92.9 (*c* 0.4, H_2O). Anal. calcd. for $C_{21}H_{37}NO_{13}$: C 49.3, H 7.3, N 2.7; found: C 49.2, H 7.4, N 2.7.

TABLE 5. ^{1}H NMR data for compounds **18**, **19**, **24**, **26**, **28**, **30**, and **32**

Protons (J, Hz)	**18**[a]	**19**[b]	**24**[c]	**26**[c]	**28**[c]	**30**[c]	**32**[c]
α-L-Rha unit B							
H-1	~4.89	5.01	4.89	4.90	4.84	4.91	4.88
H-2	~4.94	4.05	4.96	4.84	5.10	5.04	4.93
($J_{2,3}$)		(3.5)			(3.5)	(3.0)	(3.0)
H-3	~4.94	3.83	1.96	5.06	5.19	1.98	5.13
($J_{3,4}$)		(10.5)			(10.0)		(11.5)
($J_{3,3'}$)						(13.5)	
H-3′			2.20			2.21	
($J_{2,3'}$)						(~3.5)	
($J_{3',4}$)						(~3.5)	
H-4′	~4.90	3.45	4.88	1.66	5.01	4.87	1.68
($J_{3,5}$)	(10.0)	(10.0)		(12.0)	(10.0)		
H-4				1.73			1.69
($J_{3,4'}$)							
($J_{4,4'}$)				(12.0)			
H-5	3.87	3.73	3.79	3.91	3.83	3.84	3.93
($J_{5,6}$)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)
H-6	1.13	1.26	1.15	1.17	1.15	1.17	1.18
α-L-Rha unit C							
H-1	~4.90	4.87	4.81	4.77	4.86	4.87	4.83
H-2	5.10	3.87	5.07	5.04	4.89	4.94	4.90
($J_{2,3}$)	(3.0)	(3.0)	(3.5)	(3.5)		(~4.0)	
H-3	4.01	3.78	4.06	3.99	4.03	4.10	4.01
($J_{3,4}$)	(10.0)	(10.0)	(10.0)	(10.0)			
H-4	4.76	3.52	4.84	4.90	~1.55	~1.57	~1.52
($J_{4,5}$)	(10.0)	(11.0)	(10.0)	(8.5)			
H-4′					~1.55	~1.58	~1.52
H-5	3.98	4.04	3.94	3.93	3.96	3.99	3.97
($J_{5,6}$)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)
H-6	0.49	1.24	0.58	0.57	0.65	0.65	0.64
β-D-GlcNAc unit D							
H-1	4.36	4.45	4.95	4.92	4.83	4.85	4.83
($J_{1,2}$)	(8.5)	(8.0)	(8.5)	(8.0)	(9.0)	(~8.5)	(7.5)
H-2	3.54	~3.50	3.14	3.19	3.30	3.27	3.28
($J_{2,3}$)	(10.0)	(9.0)	(~9.0)	(9.5)	(9.5)	(9.5)	(9.5)
H-3	3.76	3.59	4.46	4.42	4.29	4.32	4.28
($J_{3,4}$)	(9.0)	(9.0)	(~9.0)	(9.5)	(9.5)	(9.5)	(9.5)
H-4	3.68	~3.50	3.47	3.48	3.46	3.46	3.45
($J_{4,5}$)	(9.0)		(~10.0)	(9.5)	(9.0)	(9.5)	(9.0)
H-5	3.41	3.45	3.54	3.54	3.53	3.53	3.53
H-6	3.74	3.74	3.73	3.74	3.73	3.73	3.73
($J_{6,5}$)	(9.5)	(6.5)	(10.5)	(10.0)	(10.0)	(10.0)	(10.0)
H-6′	4.24	3.94	4.34	4.34	4.33	4.33	4.33
($J_{5,6'}$)	(5.0)		(5.0)	(5.0)	(5.0)	(4.5)	(4.5)
($J_{6,6'}$)	(10.0)	(12.0)	(10.5)	(10.5)	(10.5)	(10.0)	(10.0)
OCH_3	3.36	3.49	3.47	3.48	3.44	3.43	3.43
N*H*R			5.71	5.70	5.81	5.89	5.88
C*H*Ph	5.67		5.49	5.49	5.46	5.46	5.46
CH_2Ph	4.96, 5.09	5.13, 5.19					
NAc			2.00	1.98	2.08	1.99	1.98
OAc	1.94–2.08		2.04–2.13	1.96–2.09	1.95–2.10	2.04, 2.08	1.95–2.10

[a]In $(CD_3)_2SO$.
[b]Solution: ~2.5 mg in 0.5 mL D_2O.
[c]In $CDCl_3$.

Methyl 2-acetamido-3-O-[2′-O-acetyl-4′-deoxy-3′-O-(4″-O-acetyl-2″-O-benzoyl-3″-deoxy-α-L-arabino-hexopyranosyl)-α-L-lyxo-hexopyranosyl]-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (30)

Glycosylation of the alcohol **8** (101 mg, 0.2 mmol) by the glycosyl donor **14** (85 mg, 1.25 mol-equiv.), as well as work-up of the reaction, were performed according to the conditions used to prepare trisaccharide **26**. The trisaccharide **30** was purified by flash chromatography ($CHCl_3$–MeOH, 50:1) and isolated as a colourless glass (127 mg, 82%); $[\alpha]_D^{25}$ −46.9 (*c* 0.7, $CHCl_3$). Anal. calcd. for $C_{39}H_{49}NO_{15}$: C 60.7, H 6.4, N 1.8; found: C 60.2, H 6.4, N 1.9.

TABLE 6. ^{1}H NMR data for $CDCl_3$ solutions of compounds **34**, **35**, **37**, **40**, and **42–45**

Protons (J, Hz)	**34**	**35**	**37**	**40**	**42**	**43**	**44**	**45**
α-L-Rha unit B								
H-1	5.47	4.84	4.74	4.86	4.91	4.92	4.93	4.91
($J_{1,2}$)	(1.0)				(1.5)	(2.0)		
H-2	4.70	5.03	5.24	4.86	4.83	4.89	4.88	4.87
($J_{2,3}$)		(3.0)	(3.5)	(3.0)	(3.5)	(3.0)	(3.5)	(3.5)
H-3	4.72	5.12	5.32	5.03	5.07	5.04	5.04	5.03
($J_{3,4}$)	(9.5)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(9.5)
H-4	5.00	5.00	5.02	4.84	4.84	4.84	4.84	4.83
($J_{4,5}$)	(9.5)	(10.5)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)
H-5	3.45	3.80	3.92	3.81	3.76	3.73	3.73	3.72
H-6	1.21	1.13	1.19	1.01	0.91	0.94	0.94	0.93
($J_{5,6}$)	(6.0)	(~6.0)	(6.5)	(6.0)	(6.0)	(6.0)	(6.5)	(6.0)
α-L-Rha unit C								
H-1	4.67	4.80	4.86	5.40	5.06	5.04	5.03	5.03
($J_{1,2}$)				(1.0)	(1.5)	(~2.0)		
H-2	4.92	5.03	3.91	5.48	5.31	5.35	5.35	5.34
($J_{2,3}$)	(3.0)	(3.5)	(~3.0)	(3.0)	(3.0)	(3.5)	(3.0)	(3.0)
H-3	4.07	4.00	5.11	4.29	4.31	4.34	4.33	4.34
($J_{3,4}$)	(10.0)	(10.0)	(~10.0)	(9.5)	(10.0)	(9.5)	(9.5)	(9.5)
H-4	4.90	5.03	5.01	5.48	5.34	5.48	5.48	5.48
($J_{4,5}$)	(10.0)		(~10.0)	(9.5)	(10.0)	(9.5)	(9.5)	(9.5)
H-5	3.81	3.80	3.85	4.33	4.21	4.24	4.23	4.25
H-6	1.13	1.13	1.18	1.28	0.68	1.30	1.30	1.30
($J_{5,6}$)	(6.5)	(~6.0)	(6.5)	(6.0)	(6.0)	(6.0)	(6.0)	(6.0)
β-D-GlcNAc unit D								
H-1	5.15	4.66	4.76		4.96	4.73	4.74	4.61
($J_{1,2}$)	(8.5)	(8.5)	(8.5)		(8.5)	(8.5)	(8.5)	(8.5)
H-2	2.73	3.12	2.90		3.31	3.46	3.45	3.49
($J_{2,3}$)	(10.5)	(10.0)	(~10.0)		(~8.0)	(8.5)	(8.0)	(8.5)
H-3	4.48	4.18	4.44		4.49	4.01	4.05	3.90
($J_{3,4}$)	(10.5)	(10.5)	(~10.5)			(10.0)		(10.0)
($J_{3,4'}$)	(5.5)	(5.0)	(5.0)					
H-4	1.53	1.50	1.50		3.59	3.56	3.47	3.20
($J_{4,5}$)	(~12.5)	(12.0)	(~12.0)			(10.0)		(9.0)
H-4′	1.97	2.02	2.07					
($J_{4,4'}$)	(~12.5)	(12.0)	(~12.0)					
H-5	3.74	3.67	3.70		3.59	3.40	3.47	3.34
H-6	3.53	3.48	3.48		3.79	3.83	3.56	1.33
($J_{6,5}$)	(4.5)	(5.5)	(5.5)			(4.5)		(6.0)
H-6′	3.58	3.57	3.57		4.38	3.93	3.77	
($J_{6',5}$)	(5.5)	(5.5)	(~5.5)			(3.5)		
($J_{6,6'}$)	(11.0)	(10.0)	(~10.0)		(10.5)	(12.0)	(11.0)	
OCH_3	3.49	3.45	3.45		3.51	3.50	3.50	3.47
N*H*Ac	7.26	5.60	5.64		5.82	5.69	5.85	5.86
C*H*Ph					5.58			
CH_2Ph	4.58	4.54	4.54					
SCH_2CH_3				2.68				
SCH_2CH_3				1.30				
NAc	1.99	1.98	1.97		2.07	2.07	2.07	2.07
OAc	2.03–2.18	1.94–2.14	1.94–2.10	1.80–1.86	1.81–1.90	1.82–1.92	1.81–1.91	1.80–1.90
CCH_3	1.63							

*Methyl 2-acetamido-2-deoxy-3-*O*-[4′-deoxy-3′-*O*-(3″-deoxy-α-L-*arabino*-hexopyranosyl)-α-L-*lyxo*-hexopyranosyl]-β-D-glucopyranoside (**31**)*

The trisaccharide **30** (106 mg, 0.14 mmol) was deprotected using the conditions described for trisaccharide **25**. Flash chromatography (EtOAc–MeOH–H_2O, 7:1.5:1) and gel permeation chromatography (Biogel P2, H_2O) gave pure trisaccharide **31** (56 mg, 82%) isolated as a lyophilized white powder: $[\alpha]_D^{25}$ −110.7 (*c* 0.6, H_2O). Anal. calcd. for $C_{21}H_{37}NO_{12}$: C 50.9, H 7.5, N 2.8; found: C 50.9, H 7.6, N 2.8.

*Methyl 2-acetamido-3-*O*-[2′-*O*-acetyl-4′-deoxy-3′-*O*-(2″,3″-di-*O*-acetyl-4″-deoxy-α-L-*lyxo*-hexopyranosyl)-α-L-*lyxo*-hexopyranosyl]-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**32**)*

Glycosylation of the alcohol **8** (101 mg, 0.2 mmol) by the glycosyl donor **17** (71 mg, 1.3 mol-equiv.), as well as work-up of the

TABLE 7. ^{13}C NMR data[a] for $CDCl_3$ solutions of compounds **18**, **19**, **24**, **26**, **28**, **30**, **32**, **34**, **35**, **37**, **40**, **42**, **44**

Carbon	18	19	24	26	28	30	32	34	35	37	40	42	44
α-L-Rha unit B													
C-1	97.7	103.3	98.2	99.8	96.4	95.2	97.2	97.4	98.8	99.3	99.2	98.9	99.1
($J_{C,H}$)	(175)	(172)	(171)	(171)	(170)	(169)	(170)	(176)	(171)	(171)	(168)	(172)	(172)
C-2	69.3	71.0	70.4	68.1	70.3	70.8	68.2	75.1	70.0	70.0	69.7	69.9	69.5
C-3	68.2	71.0	29.3	66.5	68.8	29.3	66.6	72.7	68.6	68.6	68.4	68.4	68.4
C-4	69.9	72.9	69.6	33.2	70.9	69.8	33.3	69.8	70.8	71.2	70.9	70.9	70.9
C-5	66.8	70.0	67.4	65.1	67.0	67.3	64.9	68.5	—[b]	66.8	67.2	67.2	67.2
C-6	17.0	17.5	17.5	20.9	17.3	17.7	20.9	17.2	17.3	17.4	17.2	17.1	17.1
α-L-Rha unit C													
C-1	98.2	102.2	97.4	97.5	98.5	98.5	98.6	98.5	98.8	99.9	82.1	97.3	99.6
($J_{C,H}$)	(175)	(171)	(173)	(174)	(172)	(172)	(172)	(174)	(171)	(171)	(169)	(175)	(172)
C-2	70.8	71.5	72.1	71.9	69.6	70.0	69.7	71.4	71.4	77.2	74.0	72.4	72.1
C-3	73.9	79.1	75.5	75.3	71.4	70.9	70.9	67.7	75.7	70.4	76.0	76.3	75.7
C-4	72.1	72.2	72.1	72.1	33.3	33.4	33.2	71.4	71.9	71.4	73.5	72.7	72.2
C-5	65.8	70.0	66.4	66.6	64.5	64.6	64.5	66.7	—[b]	67.1	67.5	66.6	68.3
C-6	16.5	17.3	16.4	16.5	20.3	20.3	20.3	17.4	17.3	17.2	17.6	16.7	17.7
β-D-GlcNAc unit D													
C-1	102.3	102.7	100.8	100.9	101.1	101.0	101.1	100.0	100.7	100.4		101.0	100.4
($J_{C,H}$)	(164)	(163)	(165)	(165)	(164)	(164)	(164)	(164)	(162)	(164)		(163)	(163)
C-2	57.3	58.1	58.8	59.0	58.5	58.6	58.5	60.5	58.6	59.5		59.0	56.4
C-3	77.6	82.6	75.1	75.1	75.5	75.3	75.5	74.0	76.1	75.1		74.8	85.0
C-4	79.0	69.5	80.3	80.3	80.3	80.4	80.3	36.0	35.8	35.9		80.3	72.2
C-5	66.0	76.9	66.1	66.1	66.1	66.1	66.1	70.5	70.9	70.9		66.1	74.5
C-6	67.9	61.7	68.8	68.8	68.8	68.8	68.8	72.2	72.2	72.2		68.9	32.5

[a]The numbers in parentheses denote the one-bond ^{13}C–^{1}H coupling constants for the anomerics (Hz).
[b]Unresolved 67.2 or 66.9.

reaction, followed the procedure described for the synthesis of trisaccharide **26**. The trisaccharide **32** was purified by flash chromatography ($CHCl_3$–MeOH, 50:1) and isolated as a colourless glass (129 mg, 89%); $[\alpha]_D^{25}$ −65.2 (*c* 0.65, $CHCl_3$). Anal. calcd. for $C_{34}H_{47}NO_{15}$: C 57.5, H 6.7, N 2.0; found: C 57.2, H 6.5, N 1.8.

*Methyl 2-acetamido-2-deoxy-3-*O*-[4'-deoxy-3'-*O*-(4"-deoxy-α-L-*lyxo*-hexopyranosyl)-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranoside (**33**)*

The trisaccharide **32** (107 mg, 0.15 mmol) was deprotected in similar fashion to trisaccharide **24**. Flash chromatography (EtOAc–MeOH–H_2O, 7:1.5:1) and gel permeation chromatography (Biogel P2, H_2O) gave pure trisaccharide **33** (58 mg, 77%) isolated as a white lyophilized powder; $[\alpha]_D^{25}$ −99.8 (*c* 0.5, H_2O). Anal. calcd. for $C_{21}H_{37}NO_{12}$: C 50.9, H 7.5, N 2.8; found: C 51.0, H 7.5, N 2.7.

*3,4-Di-*O*-acetyl-1,2-*O*-[2',4'-di-*O*-acetyl-1'-*O*-(2"-acetamido-6"-*O*-benzyl-2",4"-dideoxy-1"-*O*-methyl-β-D-*xylo*-hexopyranos-3"-yl)-α-L-rhamnopyranos-3'-yl orthoacetyl)-α-L-rhamnopyranose (**34**), methyl 2-acetamido-3-*O*-[2',4'-di-*O*-acetyl-3'-*O*-(2",3",4"-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-6-*O*-benzyl-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**35**), and methyl 2-acetamido-3-*O*-[3',4'-di-*O*-acetyl-2'-*O*-(2",3",4"-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-6-*O*-benzyl-2,4-dideoxy-β-D-*xylo*-hexopyranoside (**37**)*

Method A

Glycosylation of the disaccharide **9** (109 mg, 0.2 mmol) by the glycosyl donor **11** (82 mg, 1.2 mol-equiv.) initially used conditions similar to the synthesis of trisaccharide **26**. After 1.5 h at room temperature more acid (175 μL, 0.13 mol-equiv.) was added and the reaction proceeded for 2 h at room temperature, after which it was quenched by addition of NEt_3 (100 μL). Work-up followed the procedure described for **18**. Flash chromatography (EtOAc–hexanes; 7:3, 100 mL; 8:2, 100 mL) gave the orthoester **34** (87 mg, 54%). Further elution of the column gave the trisaccharide **35** as a colourless glass (18 mg, 11%). Both compounds were characterized by NMR spectroscopy (Tables 6 and 7).

Method B

A mixture of the disaccharide **10** (103 mg, 0.3 mmol), the donor **11** (71 mg, 1.12 mol-equiv.), and activated powdered 4Å molecular sieves (400 mg) in anhydrous CH_2Cl_2 (7 mL) was stirred 18 h under N_2 at room temperature. *N*-Iodosuccinimide (59 mg, 1.4 mol equiv.) and a saturated solution of triflic acid (0.15 M, 740 μL, 0.56 mol-equiv.) were added to the mixture, which was protected from light, and stirred 6 h at room temperature. NEt_3 (100 μL) was added to the mixture and work-up was carried out as described for the preparation of **18**. Flash chromatography ($CHCl_3$–MeOH, 50:1) gave a mixture (75:25, 110 mg, 71%) of trisaccharides **35** and **37** that could not be separated. They were characterized by NMR spectroscopy (Tables 6 and 7).

*Methyl 2-acetamido-2,4-dideoxy-3-*O*-[3'-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-β-D-*xylo*-hexopyranoside (**36**) and methyl 2-acetamido-2,4-dideoxy-3-*O*-[2'-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-β-D-*xylo*-hexopyranoside (**38**)*

The mixture of trisaccharides **35** and **37** (75:25, 110 mg) was dissolved in anhydrous MeOH (9 mL) and a solution of NaOMe (1 M, 200 μL) in MeOH was added. The mixture was stirred 18 h at room temperature, deionized with amberlite IR-120 (H+) resin, and filtered. The resin was rinsed with MeOH (3 mL) and the combined filtrates were concentrated. Pd–C catalyst (10%, 50 mg) was added to a solution of the residue in MeOH (9 mL) and the mixture was hydrogenated for 18 h at 60 psi. The catalyst was fil-

tered off, rinsed with MeOH (3 mL), and the combined filtrates were concentrated. Reversed-phase HPLC (C-18 column, H_2O) gave the pure trisaccharide **36** (43 mg, 84%), isolated as a white powder upon freeze-drying; $[\alpha]_D^{25}$ −73.1 (*c* 0.45, MeOH). Anal. calcd. for $C_{21}H_{37}NO_{13}$: C 49.3, H 7.3, N 2.7; found: C 49.0, H 7.3, N 2.8.

Further elution of the column gave the pure trisaccharide **38** (13 mg, 75%) isolated as a white powder upon freeze-drying; $[\alpha]_D^{25}$ −71.6 (*c* 0.5, MeOH). Anal. found: C 49.0, H 7.4, N 2.9.

*Ethyl 3-O-(2',3',4'-tri-O-acetyl-α-L-rhamnopyranosyl)-2,4-di-O-benzoyl-1-thio-α-L-rhamnopyranoside (**40**)*

A solution of the alcohol **39** (12) (522 mg, 1.25 mmol) in anhydrous CH_2Cl_2 (50 mL) containing powdered activated molecular sieve 4Å (2 g) and silver trifluoromethanesulfonate (651 mg, 2 mol-equiv.) was stirred 2 h in the dark under N_2 and cooled to −75°C. A solution of tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (15) (598 mg, 1.35 mol-equiv.) in anhydrous CH_2Cl_2 (5 mL) was added dropwise and the mixture was allowed to reach −50°C over 2 h, at which temperature it was left for an additional 0.5 h. NEt_3 (270 μL) was added to the reaction mixture and solids were filtered off on Celite. The solids were rinsed with CH_2Cl_2 (150 mL), and the combined filtrates washed with saturated aqueous $NaHCO_3$ (50 mL), H_2O (50 mL), and dried. Concentration and chromatography (hexanes–EtOAc; 6:2, 200 mL; 6:3, 400 mL) gave the disaccharide **40** (620 mg, 72%) as a colourless glass; $[\alpha]_D^{25}$ −1.09 (*c* 1.1, $CHCl_3$). Anal. calcd. for $C_{34}H_{40}O_{13}S$: C 59.3, H 5.8; found: C 59.4, H 6.0.

*Methyl 2-acetamido-3-O-[2',4'-di-O-benzoyl-3'-O-(2",3",4"-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**42**)*

A mixture of the acceptor **41** (274 mg, 0.85 mmol) (16), the disaccharide thioglycoside **40** (698 mg, 1.2 mol-equiv.), and powdered activated molecular sieves 4Å (1.6 g) in anhydrous CH_2Cl_2 (40 mL) was stirred 18 h under N_2 at room temperature. *N*-Iodosuccinimide (259 mg, 1.35 mol-equiv.) and a saturated solution of triflic acid (0.15 M, 2.6 mL, 0.5 mol-equiv.) in CH_2Cl_2 were added to the mixture, which was protected from light and stirred 2 h under N_2 at room temperature. NEt_3 (200 μL) was then added and work-up was carried out as described for the preparation of **18**. Flash chromatography (EtOAc–hexanes, 65:35) gave the pure trisaccharide **42** (544 mg, 67%) as a colourless glass. Combined impure fractions containing mostly **42** were subjected to reversed-phase HPLC (C-18 column, CH_3CN–H_2O 6:4) and gave additional pure trisaccharide **42** (31 mg, 4%); $[\alpha]_D^{25}$ −6.1 (*c* 0.8, $CHCl_3$). Anal. calcd. for $C_{48}H_{55}NO_{19}$: C 60.7, H 5.8, N 1.5; found: C 60.6, H 5.7, N 1.42.

*Methyl 2-acetamido-3-O-[2',4'-di-O-benzoyl-3'-O-(2",3",4"-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-2-deoxy-β-D-glucopyranoside (**43**)*

A solution of the acetal **42** (895 mg, 0.94 mmol) in 60% acetic acid (30 mL) was stirred 1 h at 100°C, cooled to room temperature, and concentrated. Residual acid was coevaporated with a mixture of toluene–MeOH (10:1, 3 × 10 mL) and flash chromatography ($CHCl_3$–MeOH, 30:1) of the dry residue gave the pure diol **43** (637 mg, 78%) as a white powder; $[\alpha]_D^{25}$ +31.0 (*c* 0.3, $CHCl_3$). Anal. calcd. for $C_{41}H_{51}NO_{19}$: C 57.1, H 6.0, N 1.6; found: C 57.3, H 6.0, N 1.8.

*Methyl 2-acetamido-6-bromo-3-O-[2',4'-di-O-benzoyl-3'-O-(2",3",4"-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-2,6-dideoxy-β-D-glucopyranoside (**44**)*

Imidazole (125 mg, 8 mol-equiv.), triphenylphosphine (238 mg, 4 mol-equiv.), and carbon tetrabromide (757 mg, 10 mol-equiv.) were added to a stirred solution of the diol **43** (196 mg, 0.22 mmol) in a mixture of anhydrous CH_3CN and anhydrous pyridine (1:1, 6 mL). After 22 h at room temperature MeOH (400 μL) was added, solvents were evaporated, and the residue dissolved in EtOAc (100 mL) was washed successively with 1 M aqueous HCl (2 × 50 mL), saturated aqueous $NaHCO_3$ (50 mL), and H_2O (50 mL). The washings were reextracted with EtOAc (2 × 50 mL) and the combined organic phases were dried and concentrated. Chromatography ($CHCl_3$–MeOH, 60:1) of the residue gave the trisaccharide **44** contaminated with triphenylphosphine oxide (124 mg) and the pure trisaccharide **44** (58 mg, 27%). Rechromatography (solvent as above) of the impure trisaccharide again yielded a contaminated fraction (80 mg) and the pure trisaccharide **44** (36 mg, 17%). The remaining contaminated trisaccharide was finally isolated pure (21 mg, 11%) by preparative TLC (solvent as above). The trisaccharide **44** was isolated as a colourless glass, $[\alpha]_D^{25}$ +35.0 (*c* 0.83, $CHCl_3$). Anal. calcd. for $C_{41}H_{50}BrNO_{18}$: C 53.2, H 5.4, N 1.5; found: C 53.1, H 5.6, N 1.3.

*Methyl 2-acetamido-2,6-dideoxy-3-O-[3'-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**46**)*

A solution of the brominated trisaccharide **44** (115 mg, 0.14 mmol) in EtOH (9 mL) containing NEt_3 (50 μL, 2.6 mol-equiv.) was hydrogenated 18 h at 60 psi in the presence of Pd–C catalyst (10%, 100 mg). More catalyst (20 mg) was added and hydrogenolysis was continued during another 8 h at 60 psi. The catalyst was filtered off, rinsed with dichloromethane (20 mL), and the combined filtrates were concentrated. A solution of the residue in CH_2Cl_2 (30 mL) was washed successively with 1 M HCl (25 mL), saturated aqueous $NaHCO_3$ (20 mL), and H_2O (20 mL). The washings were reextracted with CH_2Cl_2 (2 × 20 mL) and the combined organic phases were dried and concentrated to give the pure trisaccharide **45**, characterized by ^1H NMR spectroscopy (Table 6). Compound **45** was used directly in the next step. It was concentrated from anhydrous MeOH (2 × 3 mL), dissolved in anhydrous MeOH (6 mL), and a solution of NaOMe, 1 M in MeOH (600 μL), was added. The mixture was stirred 18 h at room temperature, more NaOMe was added portionwise over 3 h (2 × 200 μL), and stirring was continued during 2 h. The solution was deionized with Amberlite IR-120 (H^+) resin, diluted with MeOH (8 mL), filtered and the resin was washed with MeOH (10 mL). The combined filtrates were concentrated to dryness and chromatography (EtOAc–MeOH–H_2O, 7.5:1.5:1) gave **46**, which was finally purified on a Biogel P-2 column (H_2O). The trisaccharide **46** was obtained pure (53 mg, 84%) as a white solid by evaporation from MeOH; $[\alpha]_D^{25}$ −74.9 (*c* 0.6, MeOH). Anal. calcd. for $C_{21}H_{37}NO_{13}$: C 49.3, H 7.3, N, 2.7; found: C 48.9, H 7.5, N 2.8.

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