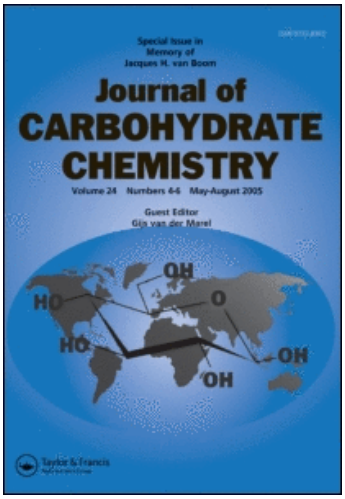


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Application of Phenyl 1-Selenoglycosides in the Synthesis of a Cell-Wall Tetrameric Fragment of *Proteus Vulgaris* Strain 5/43

H. M. Zuurmond[a]; P. A. M. van der Klein[a]; J. de Wildt[a]; G. A. van der Marel[a]; J. H. van Boom[a]
[a] Gorlaeus Laboratoria, Leiden, The Netherlands

To cite this Article Zuurmond, H. M. , van der Klein, P. A. M. , de Wildt, J. , van der Marel, G. A. and van Boom, J. H.(1994) 'Application of Phenyl 1-Selenoglycosides in the Synthesis of a Cell-Wall Tetrameric Fragment of *Proteus Vulgaris* Strain 5/43', Journal of Carbohydrate Chemistry, 13: 2, 323 — 339
To link to this Article: DOI: 10.1080/07328309408009196
URL: http://dx.doi.org/10.1080/07328309408009196

J. CARBOHYDRATE CHEMISTRY, 13(2), 323-339 (1994)

APPLICATION OF PHENYL 1-SELENOGLYCOSIDES IN THE SYNTHESIS OF A CELL-WALL TETRAMERIC FRAGMENT OF *PROTEUS VULGARIS* STRAIN 5/43

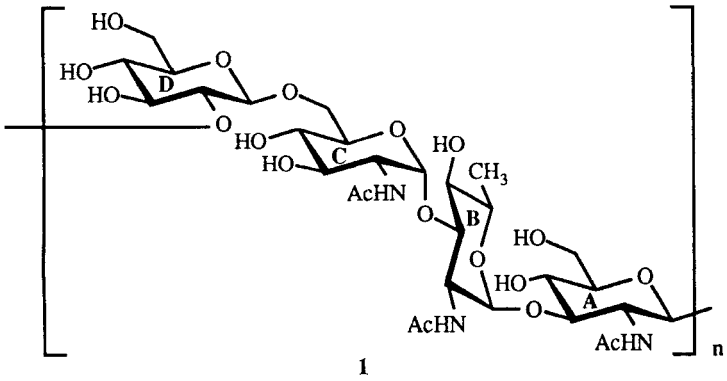
H.M. Zuurmond, P.A.M. van der Klein, J. de Wildt, G.A. van der Marel, J.H. van Boom*

Gorlaeus Laboratoria, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Received June 10, 1993 - Final Form November 2, 1993

ABSTRACT

DAST-assisted rearrangement of 3-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranosyl azide followed by treatment of the generated fluorides with ethanethiol and $BF_3{\cdot}OEt_2$ gave glycosyl donor ethyl 3-*O*-allyl-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio-α/β-L-glucopyranoside. Stereoselective glycosylation of methyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside with ethyl 3-*O*-allyl-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio-α/β-L-glucopyranoside, under the agency of NIS/TfOH afforded methyl 3-*O*-(3-*O*-allyl-2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside. Removal of the allyl function of the latter dimer, followed by condensation with properly protected 2-azido-2-deoxy-glucosyl donors, in the presence of suitable promoters, yielded selectively methyl 3-*O*-(3-*O*-[6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-



1

Figure 1

D-glucopyranoside. Deacetylation and subsequent glycosylation of the free HO-6 with phenyl 2,3,4,6-tetra-*O*-benzoyl-1-seleno-β-D-glucopyranoside in the presence of NIS/TfOH furnished a fully protected tetrasaccharide. Deprotection then gave methyl 3-*O*-(3-*O*-[6-*O*-{β-D-glucopyranosyl}-2-acetamido-2-deoxy-α-D-glucopyranosyl]-2-acetamido-2,6-dideoxy-α-L-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside.

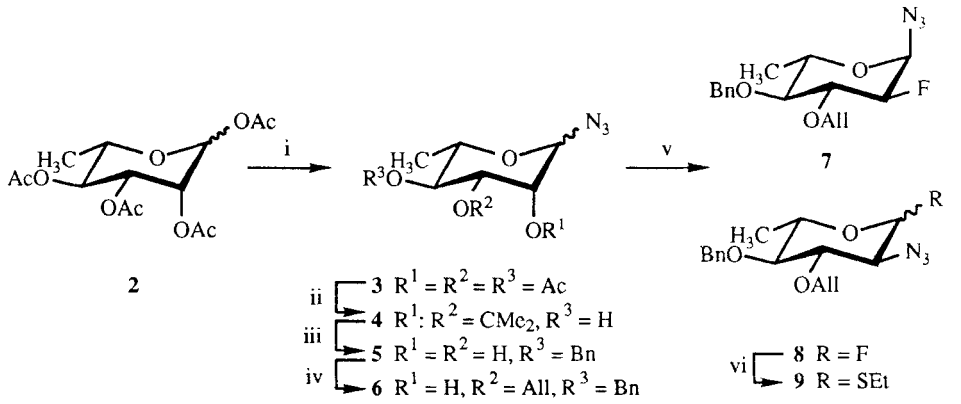
INTRODUCTION

It is well-recognized now that bacteria of the genus *Proteus* are a causative agents of urinary-tract infections.[1] It has also been established that certain strains of the *Proteus* genus may react with antibodies raised against *Rickettsia*[2] and that the *O*-specific polysaccharide chains of the surface lipopolysaccharide are responsible for the cross-reaction between these unrelated Gram-negative bacteria.

Recently, Kochetkov *et al.*[3] showed that the *O*-specific polysaccharide (OPS) of *P. vulgaris* 5/43 is a linear polymer of repeating units (see Figure 1). A striking feature of the OPS repeating unit is the presence of the sugar 2-acetamido-2,6-dideoxy-L-glucose (*N*-acetyl-L-quinovosamine), which is a rather uncommon sugar for bacterial polysaccharides.

In order to gain more insight into the immunological properties of the *P. vulgaris* cell-wall polysaccharides, we report here the synthesis of the methyl β-glycoside of the repeating unit (*i.e.*, the tetrameric fragment **29**) of the *O*-specific polysaccharide.

Scheme 1[a]

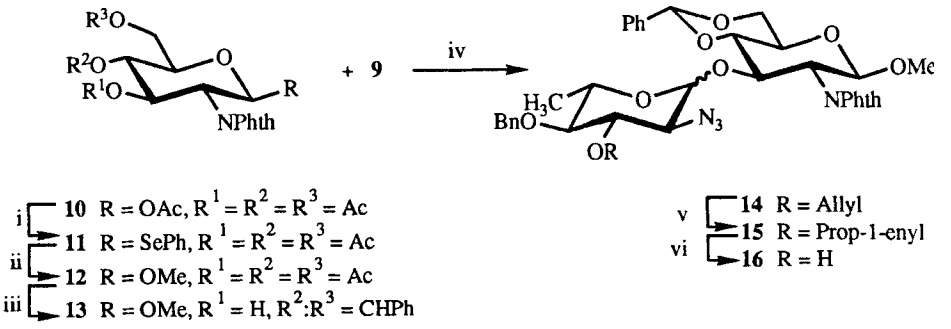


[a]**Key:** (i) $TMSN_3$, $SnCl_4$, CH_2Cl_2 (95%); (ii) KO*t*Bu, MeOH, then $(CH_3O)_2C(CH_3)_2$, acetone, *p*-TsOH; (iii) BnBr, NaH, DMF, then $HOAc/H_2O$ [4:1], 50 °C (81% based on **3**); (iv) Bu_2SnO, MeOH, Δ, then AllBr, CsF, DMF (82%); (v) DAST, dioxane, 95 °C (70%, combined yield of **7** and **8**); (vi) EtSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2 (46% based on **6**).

RESULTS AND DISCUSSION

A crucial element in a successful assembly of the target tetramer **29** entails *inter alia* the stereoselective introduction of two 1,2-*cis* linkages connecting the L-quinovosamine residue **B** with its neighbouring D-glucosamine units **C** and **A**. Retrosynthetic analysis indicated that a stepwise elongation-deprotection of an appropriately protected L-quinovosamine building block would be desirable. In order to meet the above formulated requirements, we adopted Nicolaou's DAST-mediated rearrangement[4] of the properly protected α-L-rhamnopyranosyl azide **6** for the preparation of the versatile L-quinovosamine unit **9**. The route of synthesis commences, as delineated in Scheme 1, by transforming 1,2,3,4-tetra-*O*-acetyl-α/β-L-rhamnopyranose (**2**) into the azide derivative **3** with trimethylsilyl azide in the presence of $SnCl_4$.[5] Zemplén deacetylation of **3** followed by acetalisation gave the acetonide derivative **4**. Benzylation of **4** followed by acid hydrolysis of the isopropylidene group provided diol **5**. Regioselective allylation of **5** to give **6** was effected by the reaction of the intermediate 2,3-dibutylstannylidene complex of **5** with allyl bromide in the presence of cesium fluoride.[6] DAST-mediated rearrangement of **6** under less mild conditions than earlier reported[4] (*cf.* dioxane at 95 °C versus CH_2Cl_2 at 45 °C), resulted in the isolation of an

Scheme 2[a]



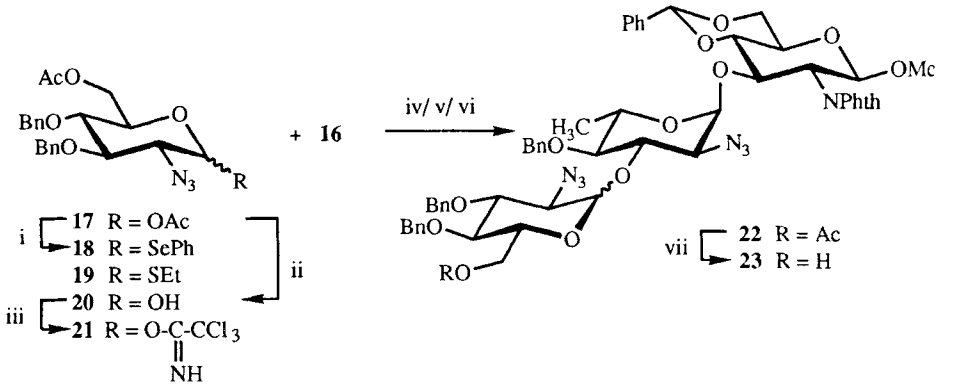
[a]**Key:** (i) PhSeH, $BF_3{\cdot}OEt_2$, $(ClCH_2)_2$ (96%); (ii) MeOH, NIS/TfOH, $(ClCH_2)_2/Et_2O$ [1:1] (87%); (iii) KO*t*Bu, MeOH, then $PhCH(OCH_3)_2$, *p*-TsOH, CH_3CN (77%); (iv) NIS/TfOH, $(ClCH_2)_2/Et_2O$ [1:5] (75%); (v)$\{Ir(COD)[PCH_3(C_6H_5)_2]_2PF_6\}$, $(ClCH_2)_2$ (87%); (vi) HgO, $HgCl_2$, acetone (75%).

inseparable mixture of the major fluoride **8** and the minor unexpected[4] fluoride **7** in a ratio of 5:1. Treatment of the mixture with ethanethiol and $BF_3{\cdot}OEt_2$[7] and subsequent separation of the resulting ethyl thioglycoside from unreacted fluoride **7** by column chromatography, provided target compound **9** in 46% yield based on **6**.

At this stage, we turned our attention to the introduction of the 1,2-*cis* linkage between the 2,6-dideoxy-L-glucosyl donor **9** and the D-glucosamine acceptor **13**.

Earlier studies[8] revealed that phenyl 1-selenoglycosides showed great promise in the future synthesis of oligosaccharides. In order to substantiate this expectation, we purposely prepared the three phenyl selenoglycosyl donors **11**, **18** and **25**, the former of which will be used for the synthesis of the terminal unit **13** and the others for the introduction (see later) of two glycosidic bonds in the target molecule **29**. First of all, building block **13** was prepared (see Scheme 2) in 64% overall yield by the following four step process. Reaction of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose[9] (**10**) with phenylselenol and $BF_3{\cdot}OEt_2$ gave phenyl selenoglycoside **11**. Glycosidation of **11** with methanol using the promoter *N*-iodosuccimide and catalytic trifluoromethanesulfonic acid (NIS/TfOH) afforded the known[10] derivative **12**. Saponification of the latter and subsequent acid catalysed acetalisation of the generated triol according to Alais *et al.*[11] yielded the required acceptor **13**. Glycosylation of **13** with the ethyl thioglycosyl donor **9** under the agency of the promoter NIS/TfOH yielded disaccharide **14** as an inseparable mixture of anomers (α/β ratio of 5:1, determined by ^1H NMR spectroscopy). Two-step deallylation[12] of **14** and purification of the crude

Scheme 3[a]

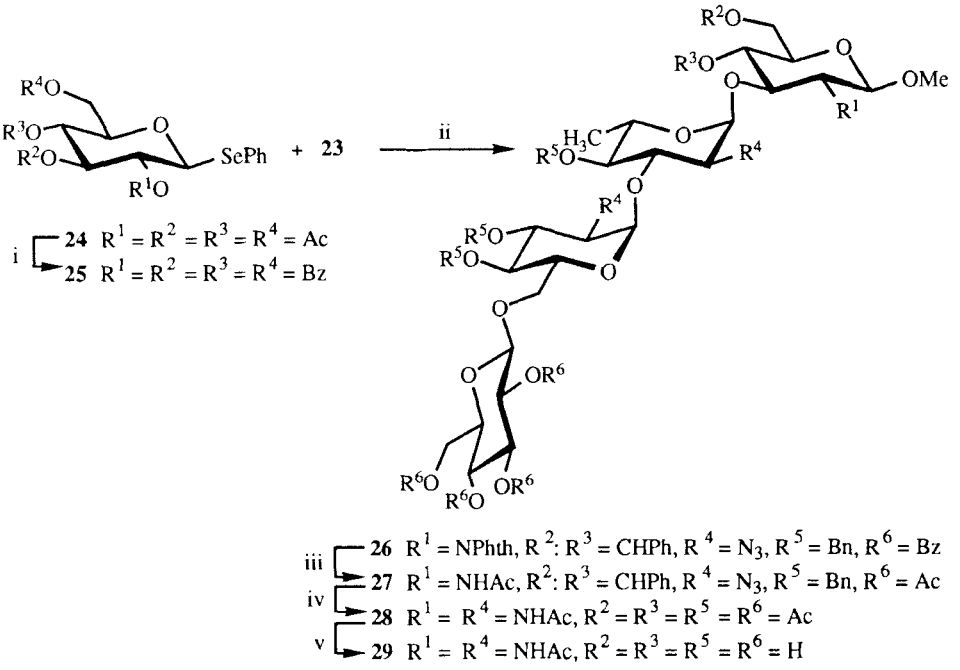


[a]**Key:** (i) PhSeH, $BF_3{\cdot}OEt_2$, $(ClCH_2)_2$ (36%); (ii) $(CH_3)_2NH$, CH_3CN (81%); (iii) Cl_3CCN, K_2CO_3 (76%); (iv) **18**, NIS/TfOH, $(ClCH_2)_2/Et_2O$ [1:5] (21%); (v) **19**, NIS/TfOH, $(ClCH_2)_2/Et_2O$ [1:5] (26%); (vi) **21**, TMSOTf, $(ClCH_2)_2/Et_2O$ [1:5] (40%); (vii) KO*t*Bu, MeOH (57%).

mixture by silica gel chromatography gave the homogeneous 1,2-*cis* linked disaccharide **16**. The latter dimer was then elongated (see Scheme 3) with the earlier mentioned phenyl selenoglycoside **18**, prepared in a reasonable yield from diacetate **17**[13] under the same conditions as applied for the transformation of **10** to **11**, using the promoter NIS/TfOH. Work-up of the glycosylation gave trimer **22** in a disappointing yield and with a low degree of stereoselectivity (*i.e.*, α/β ratio of 4:1), as evidenced by ^{13}C NMR spectroscopy. Similarly, NIS/TfOH-mediated glycosylation of **16** with the known ethyl thioglycosyl donor **19**[13] to give **22** proceeded with the same degree of stereoselectivity but resulted in a slightly higher recovery of the required product. On the other hand, condensation of the trichloroacetimidate derivative **21**, obtained by regioselective deacetylation[14] of **17** and subsequent treatment of **20** with trichloroacetonitrile in the presence of potassium carbonate,[15] with **16** using trimethylsilyl triflate[16] as the promoter gave trimer **22** in 40% yield. However, also in this case, no increase in stereoselectivity was observed. Zemplén deacetylation of **22**, followed by silica gel purification, resulted in the isolation of the anomerically pure acceptor **23**.

The final step in the assembly of the fully protected tetrameric unit **26** could be concluded (Scheme 4) successfully using the phenyl 1-selenoglycoside **25** as a donor for the introduction of the requisite 1,2-*trans* linkage. Thus, NIS/TfOH-promoted

Scheme 4[a]



[a]**Key:** (i) KO*t*Bu, MeOH, then BzCl, C_5H_5N (75%); (ii) NIS/TfOH, $(ClCH_2)_2/Et_2O$ [1:1] (90%); (iii) KO*t*Bu, MeOH, then H_2NNH_2, EtOH, Δ, then Ac_2O, C_5H_5N (86% over three steps); (iv) $Pd(OH)_2$, H_2, 2-propanol, Ac_2O [5:1], then Ac_2O, C_5H_5N (63% over two steps); (v) KO*t*Bu, MeOH (95%).

glycosylation of trimer **23** with **25**, prepared by saponification of phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-β-D-glucopyranoside (**24**)[8] and reacylation with benzoyl chloride, afforded the expected tetramer **26** in an excellent yield. Deblocking of **26** was accomplished by executing the following sequential six step procedure. Debenzoylation was followed by hydrazinolysis to give, after acetylation of the free amino- and hydroxyl groups, compound **27**. Hydrogenolysis of the benzylidene, azido- and benzyl groups in the presence of acetic anhydride and subsequent acetylation of the resulting hydroxyl groups, afforded the fully acetylated derivative **28**. Finally, purification of the crude product, obtained after saponification of **28**, gave the homogeneous target tetramer **29**, the ^1H and ^{13}C NMR data of which were in excellent accord[3] with those of the naturally occurring polymer **1**.

In conclusion, the results thus far obtained indicate that the not completely satisfactory glycosylating properties of phenyl 1-selenoglycosides may discourage their future use as donors in a planned synthesis of complex oligosaccharides.

EXPERIMENTAL

General methods and materials. Dioxane, pyridine and acetonitrile were dried by boiling with CaH_2 (5 g/L) and then distilled. Dichloromethane, 1,2 dichloroethane and toluene were distilled from P_2O_5. DMF was stirred with CaH_2 at room temperature and distilled under reduced pressure. Ether was distilled from $LiAlH_4$. Methanol was dried by refluxing with magnesium methoxide and then distilled. Dioxane, pyridine, acetonitrile and DMF were stored over molecular sieves 4Å (Aldrich) and methanol over molecular sieves 3Å (Aldrich). Toluene and ether were stored over sodium wire and dichloromethane and 1,2-dichloroethane over alumina. Schleicher and Schüll DC Fertigfolien F 1500 LS 254 were used for TLC analysis. Compounds were detected by charring with 20% sulfuric acid in methanol. Optical rotations were recorded at 20 °C with a Perkin-Elmer 241 polarimeter for solutions in $CHCl_3$, unless stated otherwise. Column chromatography was performed on silica gel 60, 230-400 mesh (Merck). Gel filtration was performed on Sephadex LH20 (Pharmacia). ^1H NMR spectra (300 MHz) were recorded at 25 °C with a Brucker WM 300 spectrometer. ^{13}C NMR spectra (50 MHz) were recorded with a Jeol JNM-FX 200 spectrometer. ^1H and ^{13}C chemical shifts (δ) are given in ppm relative to that of Me_4Si ($CDCl_3$ or MeOD) or sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D_2O). Mass experiments were performed with a TSQ-70 (Finnigan MAT, San Jose, USA) equipped with a Finnigan Thermospray (TSP 1) interface. Samples are introduced in column bypass mode at a concentration level of 1μg/inj. The eluent was 50/50 methanol/water E-4 M sodium acetate at a flow rate of 1.2 mL/min. Vaporizer, repeller and source temperature were set at 70 °C, 50V and 250 °C, respectively.

2,3,4-Tri-*O*-Acetyl-α/β-L-rhamnopyranosyl azide (3). To a solution of 1,2,3,4-tetra-*O*-acetyl-α/β-L-rhamnopyranose (**2**) (23 mmol, 7.6 g) in CH_2Cl_2 (150 mL) was added trimethylsilyl azide (27.6 mmol, 3.2 g). Then, a solution of $SnCl_4$ (3.5 mmol, 0.9 g) in CH_2Cl_2 (100 mL) was added and the reaction mixture stirred for 1 h at room temperature. The mixture was diluted with CH_2Cl_2 (100 mL), washed with M KF (50 mL), 0.9M $NaHCO_3$ (50 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel chromatography (95:5 CH_2Cl_2-acetone) gave compound **3** (6.9 g, 95%); IR (neat): 2100 cm^{-1}. $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ (α-anomer) 16.8 (C-6), 19.9, 20.0 (CH_3COO), 67.8, 68.1, 68.8 (C-2, C-3, C-4, C-5), 87.0 (C-1, $J_{C\text{-}1,H\text{-}1}$ 170 Hz), 169.1 (CH_3COO); (β-anomer) 84.2 (C-1, $J_{C\text{-}1,H\text{-}1}$ 158 Hz).

2,3-*O*-Isopropylidene-α/β-L-rhamnopyranosyl azide (4). Compound **3** (21.8 mmol, 6.9 g) was dissolved in MeOH (50 mL). Potassium *tert*-butoxide (100 mg) was added. After 1 h at room temperature, the reaction was neutralised with Dowex (H^+ form), filtered and concentrated. A mixture of the residue, 2,2-dimethoxypropane (218

mmol, 22.7 g) and acetone (100 mL) was treated with *p*-TsOH (30 mg) and stirred for 2 h at room temperature. CH_2Cl_2 (100 mL) was added to the mixture, washed with 0.9 M $NaHCO_3$ (50 mL), water (50 mL), dried ($MgSO_4$), and concentrated to give **4** (4.8 g, 96%), which was used in the next step without further purification. $^{13}C\{^1H\}$ NMR ($CDCl_3$) (α-anomer) δ 17.2 (C-6), 26.2, 28.0 ($C(CH_3)_2$), 68.1, 74.2, 75.5, 78.0 (C-2, C-3, C-4, C-5), 87.1 (C-1), 109.9 ($C(CH_3)_2$).

4-*O*-Benzyl-α/β-L-rhamnopyranosyl azide (5). Compound **4** (20.9 mmol, 4.8 g) was dissolved in DMF (100 mL). Sodium hydride (27.2 mmol, 1.1 g 60% suspension) and benzyl bromide (25.1 mmol, 4.3 g) were added and the reaction mixture was stirred for 2 h at 20 °C. Excess sodium hydride and benzyl bromide were destroyed by addition of MeOH (20 mL) and the mixture concentrated. The mixture was redissolved in CH_2Cl_2 (100 mL), washed with water (2 x 50 mL), dried ($MgSO_4$), and concentrated. The residue was redissolved in 4:1 acetic acid-water (v/v, 100 mL) and heated at 50 °C for 4 h, then concentrated and toluene (2 x 100 mL) was evaporated from the residue. Column chromatography (1:0 to 97:3 CH_2Cl_2-MeOH) of the residue furnished diol **5** (5.1 g, 87%). $^{13}C\{^1H\}$ NMR ($CDCl_3$) (α-anomer) δ 17.5 (C-6), 69.3, 70.3, 70.5, 80.3 (C-2, C-3, C-4, C-5), 74.4 (OCH_2Ph), 88.1 (C-1), 127.3, 127.9 ($CH_{arom.}$), 137.8 ($C_{arom.}$).

3-*O*-Allyl-4-*O*-benzyl-α-L-rhamnopyranosyl azide (6). To a solution of **5** (18.2 mmol, 5.1 g) in dry MeOH (100 mL) was added dibutyltin oxide (23.7 mmol, 5.9 g). The mixture was heated under reflux, then concentrated under reduced pressure, and toluene (2 x 100 mL) was evaporated from the residue. A solution of the residue in DMF (100 mL) was stirred with cesium fluoride (23.7 mmol, 3.6 g) and allyl bromide (27.3 mmol, 3.3 g) for 17 h at 20 °C. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with M KF (50 mL), water (50 mL), dried ($MgSO_4$), and concentrated. Purification by column chromatography on silica gel [1:0 to 1:2 light petroleum (bp 40-60 °C)-ether] afforded **6** (4.8 g, 82%), $[\alpha]_D$ -216° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.35, (d, 3H, H-6, $J_{5,6}$ 6.2 Hz), 3.42 (t, 1H, H-4, $J_{4,5}$ 9.3 Hz), 3.64 (dd, 1H, H-3, $J_{3,4}$ 9.4 Hz, $J_{2,3}$ 3.3 Hz), 3.84 (m, 2H, H-2, H-5), 4.15 (m, 2H, $OCH_2CH{=}CH_2$), 4.81-4.88 (AB, 2H, OCH_2Ph), 5.19-5.27 (m, $OCH_2CH{=}CH_2$), 5.36 (d, 1H, H-1, $J_{1,2}$ 1.5 Hz), 5.83-6.00 (m, 1H, $OCH_2CH{=}CH_2$), 7.72-7.81 (m, 5H, $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 17.3 (C-6), 67.8, 69.3, 78.1, 78.7 (C-2, C-3, C-4, C-5), 70.4 ($OCH_2CH{=}CH_2$), 74.5 (OCH_2Ph), 88.9 (C-1), 116.7 ($OCH_2CH{=}CH_2$), 127.0-127.7 ($CH_{arom.}$), 133.9 ($OCH_2CH{=}CH_2$), 137.8 ($C_{arom.}$).

3-*O*-Allyl-2-azido-4-*O*-benzyl-2,6-dideoxy-α/β-L-glucopyranosyl fluoride (8). To a solution of **6** (5 mmol, 1.6 g) in dioxane (20 mL) was added DAST (10 mmol, 1.6 g). After stirring for 5 min at 90 °C, TLC analysis [4:1 light petroleum (40-60 °C)-ether]

showed complete conversion of **6**. The mixture was allowed to reach room temperature and neutralised with triethylamine (3 mL). CH_2Cl_2 (50 mL) was added to the reaction mixture, washed with 0.9 M $NaHCO_3$ (25 mL), water (25 mL), dried ($MgSO_4$), and concentrated. Column chromatography [1:0 to 5:1 light petroleum (bp 40-60 °C)-ether] of the residue gave **8** (1.1 g, 70% together with 10% of compound **7**). $^{13}C\{^1H\}$ NMR ($CDCl_3$) (α-anomer) δ 17.4 (C-6), 66.0 (d, C-2, $J_{2,F}$ 22 Hz), 71.4, 81.6, 81.8, 82.3 (C-2, C-3, C-4, C-5), 74.1 ($OCH_2CH=CH_2$), 75.1 (OCH_2Ph), 107.4 (d, C-1, $J_{1,F}$ 212 Hz), 117.4 ($OCH_2CH=CH_2$), 127.6-128.3 ($CH_{arom.}$), 134.1 ($OCH_2CH=CH_2$), 137.5 ($C_{arom.}$)

Ethyl 3-*O*-Allyl-2-azido-4-*O*-benzyl-2,6-dideoxy-1-thio-α/β-L-glucopyranoside (9). Compound **8** (3.2 mmol, 1.0 g) was dissolved in CH_2Cl_2 (20 mL). This solution was treated with EtSH (6.4 mmol, 0.4 g) and $BF_3{\cdot}OEt_2$ (1.6 mmol, 230 mg). After 2 h at room temperature, the reaction mixture was neutralised with triethylamine (2 mL) and CH_2Cl_2 (20 mL) was added. The organic layer was washed with 0.9 M $NaHCO_3$ (10 mL), water (10 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel chromatography [1:0 to 5:1 light petroleum (bp 40-60 °C)-ether] afforded ethyl thioglycoside **9** (0.8 g, 46% based on **6**). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.3 (SCH_2CH_3), 17.4, 17.5 (C-6α, C-6β), 24.4 (SCH_2CH_3), 63.8, 63.9, 67.4, 75.5, 81.1, 82.7, 83.5 (C-1α-C-5α, C-1β-C-5β), 73.9 ($OCH_2CH=CH_2$), 75.0 (OCH_2Ph), 117.1 ($OCH_2CH=CH_2$), 127.7-128.2 ($CH_{arom.}$), 134.2 ($OCH_2CH=CH_2$).

Anal. Calc. for $C_{18}H_{25}N_3O_3S$: C, 59.50; H, 6.88. Found: C, 59.62; H, 6.81.

Phenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-1-seleno-β-D-glucopyranoside (11). To a solution of **10**[9] (2 mmol, 954 mg) in 1,2-dichloroethane (10 mL) was added PhSeH (2.4 mmol, 377 mg) and $BF_3{\cdot}OEt_2$ (8 mmol, 1.1 g). After 2 h at room temperature, the reaction mixture was neutralised with triethylamine (6 mL) and concentrated. The residue was purified by silica gel chromatography [1:0 to 3:7 light petroleum (bp 40-60 °C)-ether] to give phenyl selenoglycoside **11** (1.1 g, 96%), $[\alpha]_D$ +11° (*c* 1). ^1H NMR ($CDCl_3$) δ 1.83, 2.02, 2.10 (3 x s, 9H, CH_3COO), 3.90 (m, 1H, H-5), 4.25 (m, 2H, H-6, H-6'), 4.39 (t, 1H, H-2, $J_{2,3}$ 10.4 Hz), 5.13 (t, 1H, H-4, $J_{4,5}$ 9.6 Hz), 5.79 (t, 1H, H-3, $J_{3,4}$ 10.0 Hz), 5.90 (d, 1H, H-1, $J_{1,2}$ 9.2 Hz), 7.18-7.84 (m, 9H, $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 20.1, 20.4, 20.5 (CH_3COO), 54.5 (C-2), 61.9 (C-6), 68.4, 71.2, 76.7 (C-3, C-4, C-5), 78.1 (C-1), 123.4-135.1 ($CH_{arom.}$), 131.3 ($C_{arom.}$), 169.2, 169.7, 170.2 (CH_3COO, NCOC).

Methyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12). To a stirred solution of **11** (0.5 mmol, 287 mg) and dry MeOH (63 μL) in 1:1 1,2-dichloroethane-ether (v/v, 6 mL) was added dropwise a solution of NIS (0.5 mmol, 113 mg) and TfOH (0.05 mmol, 4 μL) in 1:1 1,2-dichloroethane-ether (v/v, 5 mL). After stirring for 5 min at 20 °C, TLC analysis (97:3 CH_2Cl_2-acetone) revealed the reaction

to be complete. The mixture was diluted with CH_2Cl_2 (20 mL), washed with M $Na_2S_2O_3$ (10 mL), 0.9 M $NaHCO_3$ (10 mL), dried ($MgSO_4$), and concentrated. Purification of the residue on silica gel [1:0 to 3:7 light petroleum (bp 40-60 °C)-ether] afforded pure **12**[16] (203 mg, 87%), $[\alpha]_D$ +42° (*c* 1); {Lit.[10] $[\alpha]_D$ +38°, Lit.[17] $[\alpha]_D$ +46°, Lit.[18] $[\alpha]_D$ +44°}. ^1H NMR ($CDCl_3$) δ 1.85, 2.02, 2.11 (3 x s, 9H, CH_3COO), 3.44 (OCH_3), 3.87 (dq, 1H, H-5, $J_{5,6}$ 4.5 Hz, $J_{5,6'}$ 2.4 Hz), 4.18 (dd, 1H, H-6', $J_{6,6'}$ 12.3 Hz), 4.30 (dd, 1H, H-2, $J_{2,3}$ 10.7 Hz), 4.34 (dd, 1H, H-6), 5.17 (t, 1H, H-4, $J_{4,5}$ 10.1 Hz), 5.29 (d, 1H, H-1, $J_{1,2}$ 8.5 Hz), 5.77 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz), 7.70-7.86 (m, 4H, $H_{arom.}$).

Methyl 3-*O*-(3-*O*-Allyl-2-azido-4-*O*-benzyl-2,6-dideoxy-α/β-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (14). A solution of NIS (1.4 mmol, 315 mg) and TfOH (0.14 mmol, 20 μL) in 1:1 1,2-dichloroethane-ether (v/v, 7 mL) was added dropwise to a cooled (0 °C) mixture of donor **9** (1.4 mmol, 520 mg), **13** (0.9 mmol, 380 mg) and molecular sieves (4Å, 2g) in 1:5 1,2-dichloroethane-ether (v/v, 12 mL). After stirring for 10 min, the reaction mixture was filtered, diluted with CH_2Cl_2 (30 mL), washed with M $Na_2S_2O_3$ (15 mL), 0.9 M $NaHCO_3$ (15 mL), dried ($MgSO_4$), and concentrated. Column chromatography on silica gel [1:0 to 1:1 light petroleum (bp 40-60 °C)-ether] of the residue gave a 5:1 α/β mixture of **14** (0.5 g, 75%). ^1H NMR ($CDCl_3$) (α-anomer) δ 0.70 (d, 3H, H-6^B, $J_{5,6}$ 6.2 Hz), 2.91 (dd, 1H, H-4^B, $J_{4,5}$ 9.7 Hz), 3.11 (dd, 1H, H-2^B, $J_{2,3}$ 10.2 Hz), 3.44 (s, 3H, OCH_3), 3.60 (dd, 1H, H-3^B, $J_{3,4}$ 9.0 Hz), 3.62-3.70 (m, 2H, H-4^A, H-6^A), 3.80-3.91 (m, 2H, H-5^A, H-5^B), 4.03-4.22 (m, 2H, $OCH_2CH{=}CH_2$), 4.27 (dd, 1H, H-2^A, $J_{2,3}$ 10.5 Hz), 4.55 (dd, 1H, H-$6'^A$, $J_{5,6'}$ 4.2 Hz, $J_{6,6'}$ 10.4 Hz), 4.48-4.74 (m, 3H, H-3^A, OCH_2Ph), 4.56 (d, 1H, H-1^B, $J_{1,2}$ 3.5 Hz), 5.11 (d, 1H, H-1^A, $J_{1,2}$ 8.5 Hz), 5.10-5.18 (m, 2H, $OCH_2CH{=}CH_2$), 5.52 (s, 1H, CHPh), 5.79-5.92 (m, 1H, $OCH_2CH{=}CH_2$), 7.20-7.95 (m, 14H, $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) (α-anomer) δ 17.1 (C-6^B), 55.5 (OCH_3), 57.0 (C-2^A), 64.0, 66.3, 67.7, 76.4, 80.0, 81.1, 84.1 (C-3^A-C-5^A, C-2^B-C-5^B), 68.6 (C-6^A), 74.0 ($OCH_2CH{=}CH_2$), 74.9 (OCH_2Ph), 98.8 (C-1^B, $J_{C\text{-}1,H\text{-}1}$ 169 Hz), 99.8 (C-1^A), 101.8 (CHPh), 117.3 ($OCH_2CH{=}CH_2$), 123.2-129.0 ($CH_{arom.}$), 134.0 ($OCH_2CH{=}CH_2$).

Anal. Calc. for $C_{38}H_{40}N_4O_{10}$: C, 64.04; H, 5.62. Found: C, 63.96; H, 5.69.

Methyl 3-*O*-(2-Azido-4-*O*-benzyl-2,6-dideoxy-3-*O*-*trans*-prop-1-enyl-α/β-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (15). To a solution of compound **14** (0.82 mmol, 582 mg) in 1,2-dichloroethane (10 mL) under an inert argon atmosphere was added 1,5-cyclooctadiene-bis [methyldiphenylphosphine]iridium hexafluorophosphate[12] (20 mg). The catalyst was activated by passing over a stream of hydrogen for 2 min. The solution was degassed and left under a stream of argon for 16 h at room temperature. The reaction mixture was

concentrated *in vacuo* and the residue was purified by silica gel chromatography [1:0 to 1:1 light petroleum (bp 40-60 °C)-ether] to give **15** (490 mg, 87%). ^{1}H NMR ($CDCl_3$) (α-anomer) δ 0.70 (d, 3H, H-6^B, $J_{5,6}$ 6.2 Hz), 1.52 (dd, 3H, OCH=CHCH_3), 2.89 (dd, 1H, H-4^B, $J_{4,5}$ 9.5 Hz), 3.11 (dd, 1H, H-2^B, $J_{2,3}$ 10.2 Hz), 3.44 (s, 3H, OCH_3), 3.57-3.68 (m, 2H, H-4^A, H-6^A), 3.79-3.88 (m, 3H, H-3^B, H-5^A, H-5^B), 4.26 (dd, 1H, H-2^A, $J_{2,3}$ 10.4 Hz), 4.30-4.71 (m, 4H, H-3^A, H-6'^{A}, OCH_2Ph), 4.57 (d, 1H, H-1^B, $J_{1,2}$ 3.5 Hz), 4.91 (dq, 1H, OCH=C*H*CH$_3$, $J_{2,3}$ 6.8 Hz), 5.12 (d, 1H, H-1^A, $J_{1,2}$ 8.5 Hz), 5.51 (s, 1H, CHPh), 6.09 (dq, 1H, OC*H*=CHCH$_3$, $J_{2,3}$ 12.2 Hz, $J_{1,3}$ 1.6 Hz), 7.21-7.89 (m, 14H, $H_{arom.}$). ^{13}C{^{1}H} NMR ($CDCl_3$) (α-anomer) δ 11.8 (OCH=CH*C*H$_3$), 16.7 (C-6^B), 55.2 (OCH_3), 56.6 (C-2^A), 63.1, 66.0, 67.1, 76.1, 80.7 (C-3^A-C-5^A, C-2^B-C-5^B), 68.6 (C-6^A), 74.4 (OCH_2Ph), 98.3 (C-1^B), 99.5 (C-1^A), 100.5 (OCH=*C*HCH$_3$), 101.7 (CHPh), 122.8-133.6 ($CH_{arom.}$), 136.9, 137.6 ($C_{arom.}$), 146.5 (O*C*H=CHCH$_3$).

Methyl 3-*O*-(2-Azido-4-*O*-benzyl-2,6-dideoxy-α-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (16). A solution of compound **15** (0.67 mmol, 490 mg) in 15:1 acetone-water (v/v, 8 mL) was treated with HgO (1 mmol, 217 mg) and $HgCl_2$ (0.67 mmol, 274 mg) for 16h at room temperature and then filtered through Celite. The filtrate was diluted with CH_2Cl_2 (20 mL) and successively washed with M KI (10 mL), water (10 mL), dried ($MgSO_4$), and concentrated. Silica gel chromatography [2:1 to 3:7 light petroleum (bp 40-60 °C)-ether] afforded **16** (340 mg, 75%), $[\alpha]_D$ -39° (*c* 1). ^{1}H NMR ($CDCl_3$) δ 0.68 (d, 3H, H-6^B, $J_{5,6}$ 6.2 Hz), 2.84 (t, 1H, H-4^B, $J_{4,5}$ 9.0 Hz), 3.07 (dd, 1H, H-2^B, $J_{2,3}$ 10.2 Hz), 3.43 (s, 3H, OCH_3), 3.53 (m, 2H, H-4^A, H-6^A), 3.66-3.88 (m, 3H, H-3^B, H-5^A, H-5^B), 4.37 (dd, 1H, H-2^A, $J_{2,3}$ 9.5 Hz), 4.41 (dd, 1H, H-6'^{A}, $J_{5,6'}$ 4.2 Hz, $J_{6,6'}$ 10.6 Hz). 4.54 (dd, 1H, H-1^B, $J_{1,2}$ 3.6 Hz), 4.55-4.59 (m, 3H, H-3^A, OCH_2Ph), 5.09 (d, 1H, H-1^A, $J_{1,2}$ 8.5 Hz), 5.52 (s, 1H, CHPh), 7.26-7.90 (m, 14H, $H_{arom.}$). ^{13}C{^{1}H} NMR ($CDCl_3$) δ 17.7 (C-6^B), 55.6 (OCH_3), 56.9 (C-2^A), 63.8, 66.3, 67.0, 72.0, 74.7, 75.9, 80.8, 84.3 (C-3^A-C-5^A, C-2^B-C-5^B), 68.7 (C-6^A), 98.6, (C-1^B), 99.7 (C-1^A), 101.9 (CHPh), 123.2-134.0 ($CH_{arom.}$), 138.0 ($C_{arom.}$).

Anal. Calc. for $C_{35}H_{36}N_4O_{10}$: C, 62.50; H, 5.36. Found: C, 62.58; H, 5.30.

Phenyl 6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-1-seleno-α/β-D-glucopyranoside (18). To a solution of compound **17**[13] (1.4 mmol, 672 mg) in 1,2-dichloroethane (15 mL) and phenylselenol (1.7 mmol, 270 mg) was added $BF_3{\cdot}OEt_2$ (2.2 mmol, 312 mg). After stirring for 4 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with 0.9 M $NaHCO_3$ (25 mL) and water (15 mL). The organic layer was dried ($MgSO_4$) and concentrated to give, after column chromatography on silica gel [1:0 to 7:3 light petroleum (bp 40-60 °C)-ether] glycosyl donor **18** [289 mg, 50% based on recovered **17** (172 mg)]. ^{13}C{^{1}H} NMR ($CDCl_3$) δ

20.8 (CH_3COO), 62.7 (C-6α, C-6β), 64.6 (C-2α), 66.0 (C-2β), 71.6, 77.7, 82.7 (C-3α-C-5α), 75.1, 75.8 (OCH_2Ph), 77.1, 78.1, 81.1 (C-3β-C-5β), 84.5 (C-1α), 85.1 (C-1β), 128.0-135.9 ($CH_{arom.}$), 137.4 ($C_{arom.}$), 170.4 (CH_3COO).

Anal. Calc. for $C_{28}H_{29}N_3O_5Se$: C, 59.36; H, 5.12. Found: C, 59.42; H, 5.24.

6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranose (20). To a solution of compound **17**[13] (1 mmol, 467 mg) in acetonitrile (10 mL) was added dimethylamine (4 mmol, 180 mg). The solution was left at 20 °C for 16 h, when TLC analysis (97:3 CH_2Cl_2-acetone) indicated complete disappearance of **17**. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with water (10 mL), dried ($MgSO_4$), and concentrated. The residual oil was purified by column chromatography (97:3 CH_2Cl_2-acetone) to furnish **20** (346 mg, 81%). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 20.4 (CH_3COO), 62.7 (C-6α, C-6β), 63.7 (C-2α), 66.0 (C-2β), 68.3, 77.7, 79.9 (C-3α-C-5α), 74.6, 75.2 (OCH_2Ph), 72.6, 76.9, 82.8 (C-3β-C-5β), 91.3 (C-1α), 95.7 (C-1β), 127.6-128.2 ($CH_{arom.}$), 137.4 ($C_{arom.}$), 171.0 (CH_3COO).

6-*O*-Acetyl-2-Azido-3,4-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranosyl trichloroacetimidate (21). To a cooled (0 °C) mixture of compound **20** (0.81 mmol, 346 mg) and trichloroacetonitrile (4.1 mmol, 592 mg) in 1,2-dichloroethane (5 mL) was added K_2CO_3 (3.0 mmol, 416 mg). After stirring for 4 h at 0 °C, TLC analysis (97:3 CH_2Cl_2-acetone) showed the conversion of **20** into **21**. The mixture was diluted with CH_2Cl_2 (20 mL), washed with water (10 mL), dried ($MgSO_4$), and concentrated. The residual oil was applied onto a column of silica gel [1:0 to 2:1 light petroleum (bp 40-60 °C)-ether] to yield **21** (315 mg, 76%). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 20.6 (CH_3COO), 62.3 (C-6α, C-6β), 62.8 (C-2α), 65.6 (C-2β), 71.6, 77.2, 78.0 (C-3α-C-5α), 74.3, 75.2 (OCH_2Ph), 73.6, 76.4, 82.1 (C-3β-C-5β), 93.2 (C-1α), 96.5 (C-1β), 127.9-128.4 ($CH_{arom.}$), 137.1, 137.4 ($C_{arom.}$), 160.8 ($OC(=NH)CCl_3$), 171.0 (CH_3COO).

Anal. Calc. for $C_{24}H_{25}Cl_3N_4O_5$: C, 51.86; H, 4.50. Found: C, 51.93; H, 4.57.

Methyl 3-*O*-(3-*O*-[6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α/β-D-glucopyranosyl]-2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (22).

Method A. To a cooled (0 °C) mixture of phenyl selenoglycoside **18** (0.3 mmol, 170 mg), acceptor **16** (0.22 mmol, 150 mg) and molecular sieves (4Å, 0.5 g) in 1:5 1,2-dichloroethane-ether (v/v, 6 mL) was added a solution of NIS (0.36 mmol, 81 mg) and TfOH (36 μmol, 5 μL) in 1:1 1,2-dichloroethane-ether (v/v, 3.6 mL). After stirring for 5 min at 0 °C, the mixture was diluted with CH_2Cl_2 (20 mL), washed with M $Na_2S_2O_3$ (10 mL), 0.9M $NaHCO_3$ (10 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel column chromatography [1:0 to 1:1 light petroleum (bp 40-60 °C)-ether] gave a 4:1 α/β mixture of trimer **22** (51 mg, 21%).

Method B. To a cooled (0 °C) mixture of ethyl thioglycoside **19**[13] (0.44 mmol, 207 mg), acceptor **16** (0.27 mmol, 181 mg) and molecular sieves (4Å, 0.5 g) in 1:5 1,2-dichloroethane-ether (v/v, 6 mL) was added a solution of NIS (0.44 mmol, 99 mg) and TfOH (40 μmol, 6 μL) in 1:1 1,2-dichloroethane-ether (v/v, 4.4 mL). After stirring for 5 min at 0 °C, the mixture was diluted with CH_2Cl_2 (20 mL), washed with M $Na_2S_2O_3$ (10 mL), 0.9 M $NaHCO_3$ (10 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel column chromatography [1:0 to 1:1 light petroleum (bp 40-60 °C)-ether] gave a 4:1 α/β mixture of trimer **22** (76 mg, 26%).

Method C. To a cooled (0 °C) mixture of trichloroacetimidate **21** (0.58 mmol, 330 mg), acceptor **16** (0.34 mmol, 229 mg) and molecular sieves (4Å, 1 g) in 1:5 1,2-dichloroetane-ether (v/v, 12 mL) was added a solution of TMSOTf (0.02 mmol, 4 μL) in 1,2-dichloroethane (1 mL). After 5 min at 0 °C, the mixture was diluted with CH_2Cl_2 (20 mL), washed with 0.9 M $NaHCO_3$ (10 mL) and water (10 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel column chromatography [1:0 to 1:1 light petroleum (bp 40-60 °C)-ether] gave a 4:1 α/β mixture of trimer **22** (158 mg, 40%). $^{13}C\{^1H\}$ NMR ($CDCl_3$) (α-anomer) δ 17.2 (C-6^B), 20.7 (CH_3COO), 55.7 (OCH_3), 56.9 (C-2^A), 62.0 (C-6^C), 63.4, 65.7, 66.4, 67.7, 69.0, 75.0, 77.6, 80.3, 80.8, 82.5 (C-3^A-C-5^A, C-2^B-C-5^B, C-2^C-C-5^C), 68.5 (C-6^A), 97.2 (C-1^C), 98.6 (C-1^B), 99.8 (C-1^A), 100.9 (CHPh), 123.5-134.1 ($CH_{arom.}$), 137.0, 137.3, 137.6 ($C_{arom.}$), 170.4 (CH_3COO).

Anal. Calc. for $C_{57}H_{60}N_7O_{15}$: C, 63.21; H, 5.55. Found: C, 63.12; H, 5.62.

Methyl 3-*O*-(3-*O*-[2-Azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (23). To a solution of compound **22** (0.21 mmol, 222 mg) in MeOH (10 mL) was added a catalytic amount of potassium *tert*-butoxide (10 mg). After 2 h at room temperature, the reaction mixture was neutralised with Dowex (H^+ form), filtered and concentrated. Purification of the residue on silica gel (1:0 to 97:3 CH_2Cl_2-acetone) afforded **23** (122 mg, 57%), $[\alpha]_D$ +11° (*c* 1) . ^1H NMR ($CDCl_3$) δ 0.75 (d, 3H, H-6^B, $J_{5,6}$ 6.2 Hz), 2.92 (t, 1H, H-4^B, $J_{4,5}$ 9.4 Hz), 3.19 (t, 1H, H-2^B, $J_{2,3}$ 9.1 Hz), 3.20 (t, 1H, H-2^C, $J_{2,3}$ 9.2 Hz), 3.24-3.31 (m, 1H, H-6^C), 3.41 (dd, 1H, H-$6'^C$, $J_{5,6'}$ 2.3 Hz, $J_{6,6'}$ 9.4 Hz), 3.44 (s, 1H, OCH_3), 3.49 (m, 1H, H-5^C), 3.51 (dd, 1H, H-4^C, $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 10.0 Hz), 3.64 (m, 1H, H-6^A), 3.69 (t, 1H, H-4^A, $J_{4,5}$ 9.0 Hz), 3.79-3.94 (m, 4H, H-3^B, H-3^C, H-5^A, H-5^B), 4.30 (dd, 1H, H-2^A, $J_{2,3}$ 10.5 Hz), 4.43 (dd, 1H, H-$6'^A$, $J_{5,6'}$ 4.3 Hz, $J_{6,6'}$ 10.6 Hz), 4.48-4.83 (AB, 6H, OCH_2Ph), 4.61 (t, 1H, H-3^A, $J_{3,4}$ 8.4 Hz), 4.69 (d, 1H, H-1^B, $J_{1,2}$ 3.5 Hz), 5.01 (d, 1H, H-1^C, $J_{1,2}$ 3.8 Hz), 5.12 (d, 1H, H-1^A, $J_{1,2}$ 8.5 Hz), 5.56 (s, 1H, CHPh), 7.10-7.92 (m, 24H, $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 17.3 (C-6^B), 55.6 (OCH_3), 57.0 (C-2^A), 61.0 (C-6^C), 63.4, 65.8, 66.3,67.8,

71.2, 74.5, 75.9, 77.9, 80.0, 81.0, 82.4 (C-3^A-C-5^A, C-2^B-C-5^B, C-2^C-C-5^C), 68.6 (C-6^A), 74.9, 75.2 (OCH_2Ph), 97.0 (C-1^C), 98.8 (C-1^B), 99.7 (C-1^A), 101.9 (CHPh), 126.3-129.1 ($CH_{arom.}$), 131.6 ($C_{arom.}$).

Anal. Calc. for $C_{55}H_{58}N_7O_{14}$: C, 63.46; H, 5.58. Found: C, 63.37; H, 5.63.

Phenyl 2,3,4,6-Tetra-*O*-benzoyl-1-seleno-β-D-glucopyranoside (25). To a solution of compound **24**[8] (10.0 mmol, 4.9 g) in MeOH (50 mL) was added a catalytic amount of potassium *tert*-butoxide (50 mg). After 1 h at room temperature, the reaction mixture was neutralised with Dowex (H^+ form), filtered and concentrated. The residue was redissolved in pyridine (50 mL) and benzoyl chloride (52.3 mmol, 7.4 g) was added. After stirring for 4 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with 0.9M $NaHCO_3$ (25 mL), water (25 mL), dried ($MgSO_4$), and concentrated. Purification of the residue on silica gel [1:1 light petroleum (bp 40-60 °C)-ethyl acetate] gave compound **25** (5.6 g, 76%), $[\alpha]_D$ +22° (*c* 1). ^1H NMR ($CDCl_3$) δ 4.10 (m, 1H, H-5), 4.50 (dd, 1H, H-6, $J_{5,6}$ 6.1 Hz, $J_{6,6'}$ 12.1 Hz), 4.70 (dd, 1H, H-6', $J_{5,6'}$ 2.5 Hz), 5.24 (d, 1H, H-1, $J_{1,2}$ 10.0 Hz), 5.52 (t, 1H, H-2, $J_{2,3}$ 9.5 Hz), 5.61 (t, 1H, H-4, $J_{4,5}$ 9.5 Hz), 5.90 (t, H-3, $J_{3,4}$ 8.3 Hz), 7.10-8.05 (m, 25H, $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 63.1 (C-6), 69.2, 74.0, 77.3 (C-2, C-3, C-4, C-5), 81.3 (C-1), 127.0 ($C_{arom.}$), 128.3-135.1 ($CH_{arom.}$).

Anal. Calc. for $C_{40}H_{32}O_9$: C, 65.31; H, 4.35. Found: C, 65.45; H, 4.43.

Methyl 3-*O*-(3-*O*-[6-*O*-{2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl}-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (26). To a stirred and cooled (0 °C) solution of **25** (0.14 mmol, 104 mg), **23** (0.094 mmol, 98 mg) and molecular sieves (4Å, 0.3 g) in 1:1 1,2-dichloroetane-ether (v/v, 3 mL) was added a solution of NIS (0.17 mmol, 38 mg) and TfOH (17 μmol, 1.4 μL) in 1:1 1,2-dichloroethane-ether (v/v, 1.7 mL). After 5 min at 0 °C, TLC analysis (97:3 CH_2Cl_2-acetone) revealed the reaction to be complete. The reaction mixture was filtered and the filtrate diluted with CH_2Cl_2 (10 mL), washed with M $Na_2S_2O_3$ (5 mL), 0.9M $NaHCO_3$ (5 mL), dried ($MgSO_4$), and concentrated. Purification of the residue on silica gel [1:0 to 2:3 light petroleum (bp 40-60 °C)-ether] yielded fully protected tetrasaccharide **26** (136 mg, 90%), $[\alpha]_D$ +8° (*c* 1). ^1H NMR ($CDCl_3$) δ 0.78 (d, 3H, H-6^B, $J_{5,6}$ 6.2 Hz), 2.88 (t, 1H, H-4^B, $J_{4,5}$ 9.4 Hz), 3.05 (dd, 1H, H-2^C, $J_{2,3}$ 10.3 Hz), 3.10 (dd, 1H, H-6'^{C}, $J_{5,6'}$ 2.1 Hz, $J_{6,6'}$ 11.1 Hz), 3.44 (t, 1H, H-4^C, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 10.2 Hz), 3.44 (s, 3H, OCH_3), 3.65-3.86 (m, 7H, H-4^A, H-5^A, H-6^A, H-3^B, H-3^C, H-5^C, H-6^C), 3.92-4.02 (m, 2H, H-5^B, H-5^D), 4.12-4.24 (AB, 2H, OCH_2Ph), 4.29 (dd, 1H, H-2^A, $J_{2,3}$ 10.5 Hz), 4.40-4.68 (m, 8H, H-3^A, OCH_2Ph, H-6'^{A}, H-6^D, H-6'^{D}), 4.53 (d, 1H, H-1^D, $J_{1,2}$ 7.9 Hz), 4.71 (d, 1H, H-1^B, $J_{1,2}$ 3.3 Hz), 4.80 (d, 1H, H-1^C, $J_{1,2}$ 3.7 Hz), 5.14 (d, 1H,

H-1^A, $J_{1,2}$ 8.5 Hz), 5.55 (dd, 1H, H-2^D, $J_{2,3}$ 9.7 Hz), 5.56 (s, 1H, CHPh), 5.68 (t, 1H, H-4^D, $J_{4,5}$ 9.7 Hz), 5.84 (t, 1H, H-3^D, $J_{3,4}$ 9.6 Hz), 7.10-8.15 (m, 44H, $H_{arom.}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 17.3 (C-6^B), 55.4 (OCH_3), 56.9 (C-2^A), 63.0 (C-6^D), 63.1, 66.0, 66.3, 67.7, 69.5, 69.7, 71.7, 72.0, 72.7, 74.6, 75.5, 77.3, 79.9, 81.2, 82.4 (C-3^A-C-5^A, C-2^B-C-5^B, C-2^C-C-5^C, C-2^D-C-5^D), 68.6, 68.8 (C-6^A, C-6^C), 97.0 (C-1^C), 98.5 (C-1^B), 99.7 (C-1^A), 101.1 (C-1^D), 101.8 (CHPh), 126.3-134.0 ($CH_{arom.}$), 128.7, 129.4, 131.5, 137.1, 137.5, 137.8, 138.1 ($C_{arom.}$), 164.8, 165.0, 165.7, 166.0 (PhCOO).

Anal. Calc. for $C_{89}H_{84}N_7O_{23}$: C, 66.00; H, 5.19. Found: C, 66.15; H, 5.25.

Methyl 3-*O*-(3-*O*-[6-*O*-{2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl}-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl]-2-azido-4-*O*-benzyl-2,6-dideoxy-α-L-glucopyranosyl)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (27). A mixture of compound **26** (0.04 mmol, 65 mg), potassium *tert*-butoxide (5 mg) in MeOH (3 mL) was kept for 2 h at room temperature. The reaction mixture was neutralised with Dowex (H^+ form), filtered, and concentrated. The residue was redissolved in 95:5 ethanol-water (v/v, 3 mL). Then hydrazine hydrate (0.1 mL, 80%) was added and the mixture was heated under reflux for 16 h. The mixture was concentrated *in vacuo* and the last traces of hydrazine and water were removed by evaporation of toluene (2 x 5 mL) from the mixture. The residue was dissolved in pyridine (2 mL) and acetic anhydride (1 mL). After 10 h at room temperature, the mixture was concentrated, and toluene (2 x 5 mL) was evaporated from the mixture. Purification on silica gel (1:0 to 97:3 CH_2Cl_2-MeOH) gave **27** (44 mg, 86%), $[\alpha]_D$ -8° (*c* 1) . ^1H NMR ($CDCl_3$) δ 0.67 (d, 3H, H-6^B, $J_{5,6}$ 6.2 Hz), 1.86, 1.95, 1.98, 2.00. 2.01 (5 x s, 15H, CH_3CO), 2.98 (t, 1H, H-4^B, $J_{3,4} \approx J_{4,5}$ 9.4 Hz), 3.09 (ddd, 1H, H-2^A, $J_{2,3}$ 10.2 Hz, $J_{2,NH}$ 7.0 Hz), 3.17 (dd, 1H, H-6^C, $J_{5,6}$ 2.5 Hz, $J_{6,6'}$ 11.1 Hz), 3.27 (dd, 1H, H-2^B, $J_{2,3}$ 10.2 Hz), 3.33 (dd, 1H, H-2^C, $J_{2,3}$ 10.3 Hz), 3.42-3.62 (m, 7H, H-4^A, H-6^A, H-4^C, H-5^D), 3.63 (dd, 1H, H-6'^{C}, $J_{5,6}$ 1.6 Hz), 3.74 (m, 1H, H-5^A), 3.89-3.96 (m, 2H, H-3^C, H-5^C), 3.98-4.07 (m, 3H, H-3^B, H-5^B, H-6'^{D}), 4.18 (dd, 1H, H-6^D, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 12.3 Hz), 4.26 (d, 1H, H-1^D, $J_{1,2}$ 7.9Hz), 4.34 (dd, 1H, H-6'^{A}, $J_{5,6}$ 4.1 Hz, $J_{6,6'}$ 10.7 Hz), 4.59, 4.61, 4.62, 4.64, 4.73, 4.76, 4.79, 4.82, 4.84, 4.87, 4.90, 4.93 (AB, 6H, OCH_2Ph), 4.65 (t, 1H, H-3^A, $J_{3,4}$ 9.5 Hz), 4.93 (d, 1H, H-1^B, $J_{1,2}$ 3.4 Hz), 4.95 (dd, 1H, H-2^D, $J_{2,3}$ 8.0 Hz), 5.01 (t, 1H, H-4^D, $J_{4,5}$ 10.0 Hz), 5.02 (d, 1H, H-1^A, $J_{1,2}$ 7.7 Hz), 5.09 (t, 1H, H-3^D, $J_{3,4}$ 9.2 Hz), 5.41 (d, 1H, H-1^C, $J_{1,2}$ 3.6 Hz), 5.51 (s, 1H, CHPh), 6.15 (d, 1H, NH), 7.00-7.50 (m, 20H, $H_{arom.}$).

Methyl 3-*O*-(3-*O*-[6-*O*-{β-D-Glucopyranosyl}-2-acetamido-2-deoxy-α-D-glucopyranosyl]-2-acetamido-2,6-dideoxy-α-L-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside (29). To a solution of compound **27** (0.034 mmol, 44 mg) in 5:1 2-propanol-acetic anhydride (v/v, 6 mL) was added $Pd(OH)_2$ (20%, 100 mg).

The reaction mixture was stirred under an atmosphere of hydrogen for 72 h at 20 °C. The catalyst was removed by filtration and the filtrate concentrated to give a residue, which was redissolved in 1:2 acetic anhydride-pyridine (v/v, 5 mL). After 4 h at room temperature, the reaction mixture was concentrated to give, after purification on a LH20 column (eluens: 1:1 MeOH-CH_2Cl_2), the fully acetylated tetrasaccharide **28**. To a solution of the latter in MeOH (3 mL) was added potassium *tert*-butoxide (10 mg). After 4 h at 20 °C, the reaction mixture was neutralised with Dowex (H^+ form), filtered and concentrated under reduced pressure. The residue was purified by hiload Sephadex S100 HR 26/60 column chromatography to give pure **29** (16 mg, 59% over three steps); $[\alpha]_D$ -4° (*c* 1, H_2O). ^1H NMR (D_2O) δ 1.28 (d, 3H, H-6^B, $J_{5,6}$ 6.2 Hz), 1.98, 1.99, 2.06 (3 x s, 9H, CH_3COO), 3.31 (t, 1H, H-4^B, $J_{4,5}$ 9.9 Hz), 3.35 (dd, 1H, H-2^D, $J_{2,3}$ 8.0 Hz), 3.44 (t, 1H, H-4^D, $J_{4,5}$ 8.5 Hz), 3.46 (t, 1H, H-4^C, $J_{3,4} \approx J_{4,5}$ 8.5 Hz), 3.47 (m, 1H, H-5^D), 3.52 (t, 1H, H-3^D, $J_{3,4}$ 7.2 Hz), 3.69 (t, 1H, H-3^A, $J_{3,4}$ 10.1 Hz), 3.71 (t, 1H, H-4^A, $J_{4,5}$ 9.9 Hz), 3.75-3.85 (m, 4H, H-3^B, H-3^C, H-5^C, H-6^C), 3.88 (dd, 1H, H-2^A, $J_{2,3}$ 10.1 Hz), 3.92-3.99 (m, 3H, H-2^C, H-$6'^A$, H-$6'^C$), 4.10 (dd, 1H, H-2^B, 10.4 Hz), 4.13 (dd, 1H, H-6^A, $J_{5,6}$ 2.3 Hz, $J_{6,6'}$ 11.6 Hz), 4.30 (dq, 1H, H-5^B), 4.32 (m, 1H, H-5^A), 4.39 (d, 1H, H-1^A, $J_{1,2}$ 8.5 Hz), 4.54 (d, 1H, H-1^D, $J_{1,2}$ 7.9 Hz), 4.97 (d, 1H, H-1^B, $J_{1,2}$ 4.0 Hz), 5.07 (d, 1H, H-1^C, $J_{1,2}$ 3.9 Hz). ^{13}C {^1H} NMR (D_2O) δ 17.2 (C-6^B), 22.9 (CH_3CON), 54.3, 54.5 (C-2^B, C-2^C), 56.2 (C-2^A), 58.1 (OCH_3), 61.6 (C-6^A, C-6^D), 68.5 (C-6^C), 68.7, 69.3, 70.2, 70.5, 71.6, 71.8, 73.9, 74.5, 76.2, 76.5, 76.7, 78.7 (C-3^A-C-5^A, C-3^B-C-5^B, C-3^C-C-5^C, C-2^D-C-5^D), 97.9 (C-1^B, C-1^C), 103.0 (C-1^A), 103.5 (C-1^D), 174.2, 174.9 (CH_3CON). LC-MS: *m/z* 810 ($[M+Na]^+$).

Anal. Calc. for $C_{31}H_{53}N_3O_{20}$: C, 47.27; H, 6.73. Found: C, 47.31; H, 6.79.

ACKNOWLEDGEMENTS

We thank R.A.M. van der Hoeven for the LC-MS analysis of **29** and A.W.M. Lefeber and C. Erkelens for recording the ^1H and ^{13}C NMR spectra.

REFERENCES

1. Topley and Wilson, *Principles of Bacteriology, Virology and Immunity*, 7th edition, **3**, 1984, p 300.

2. Burrows, *Textbook of Microbiology*, 21th edition, W.B. Saunders Company, 1979, p 514.

3. E.V. Vinogradov, W. Kaca, A. Rozalski, A.S. Shashkov, M. Cedzynski, Y.A. Knirel and N.K. Kochetkov, *Eur. J. Biochem.*, **200**, 195 (1991).

4. K.C. Nicolaou, T. Ladduwahetty, J.L. Randall and A. Chucholowski, *J. Am. Chem. Soc.*, **108**, 2466 (1986).

5. A. Grouiller, B. Nonga, M.-L. Navarro, P. Molière and H. Pacheco, *J. Carbohydr. Chem.*, **7**, 507 (1988).

6. S. Davis and S. Hanessian, *Tetrahedron*, **41**, 643 (1985). N. Nagashima and M. Ohno, *Chem. Lett.*, 141 (1987).

7. K.C. Nicolaou, A. Chucholowski, R.E. Dolle and J.L. Randall, *J. Chem. Soc. Chem. Commun.*, 1155 (1984).

8. S. Mehta and M. Pinto, *Tetrahedron Lett.*, **32**, 4435 (1991). H.M. Zuurmond, P.A.M van der Klein, P.H. van der Meer, G.A. van der Marel and J.H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **111**, 365 (1992). H.M. Zuurmond, P.A.M. van der Klein, P.H. van der Meer, G.A. van der Marel and J.H. van Boom, *J. Carbohydr. Chem.*, accepted for publication.

9. B.R. Baker, J.P. Joseph, R.E. Schaub and J.H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

10. M.T. Campos-Valdes, J.R. Marino-Albernas and V. Verez-Bencomo, *J. Carbohydr. Chem.*, **6**, 509 (1987).

11. J. Alais and S. David, *Carbohydr. Res.*, **201**, 69 (1990).

12. J.J. Oltvoort, C.A.A. van Boeckel, J.H. de Koning and J.H. van Boom, *Synthesis*, 305 (1981).

13. P.A.M. van der Klein, W. Filemon, G.J.P.H. Boons, G.H. Veeneman, G.A. van der Marel and J.H. van Boom, *Tetrahedron*, **48**, 4649 (1992).

14. J. Fiandor, M.T. Garcia-López, F.G. de las Heras and P.P. Mendéz-Castrillón, *Synthesis*, 1121 (1985).

15. R.R. Schmidt, J. Michel and M. Roos, *Liebigs Ann. Chem.*, 1343 (1984).

16. W. Kinzy and R.R. Schmidt, *Liebigs Ann. Chem.*, 1537 (1985).

17. S. Akija and T. Osawa, *Chem. Pharm. Bull.*, **8**, 593 (1960).

18. G. Grundler and R.R. Schmidt, *Carbohydr. Res.* **97**, 81 (1981).