Carbohydrate Research, 216 (1991) 323–335
Elsevier Science Publishers B.V., Amsterdam

Application of *n*-pentenyl glycosides in the regio- and stereo-controlled synthesis of α-linked *N*-glycopeptides*

Andrew J. Ratcliffe, Peter Konradsson, and Bert Fraser Reid†
Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706 (U.S.A.)

(Received November 27th, 1990; accepted for publication March 6th, 1991)

ABSTRACT

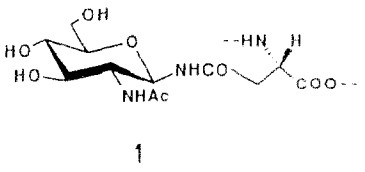
The *N*-glycopeptides α-Glc-(1→Asn and α-Glc-(1→6)-β-Glc-(1→6)-α-Glc-(1→Asn have been synthesized efficiently from pent-4-enyl D-glucopyranoside derivatives. The methodology illustrates (*a*) a novel route for formation of the *N*-α-D-glucosyl-asparagine link, and (*b*) stereo-controlled construction of the glycan backbone α-Glc-(1→6)-β-Glc-(1→6)-Glc in which the reactivity of each pentenyl glycosyl donor is controlled by an appropriate promoter. This strategy minimizes the number of changes of protecting groups that is required.

INTRODUCTION

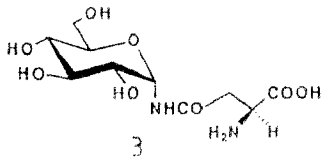
It is well established that naturally occuring *N*-glycopeptides contain characteristic structural features, the most notable being the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine unit (**1**) that links the oligosaccharide and polypeptide components[1]. Consequently, the isolation[2] and identification[3] of an *N*-glycopeptide, named nephritogenoside, from the glomerular basement membrane of rats that contained a glycan backbone which corresponded to α-Glc-(1→6)-β-Glc-(1→6)-Glc and a glycan–protein α-linkage through the amido nitrogen of an *N*-terminal asparagine residue belonging to a 21-amino acid peptide, was a noteworthy exception. The proposed structure has been supported by synthesis of the model structure, α-Glc-(1→6)-β-Glc-(1→6)-α-Glc-(1→Asn[4] (**2**), and the smaller sub-unit α-Glc-(1→Asn[5] (**3**). From degradation studies of nephritogenoside, α-Glc-(1→6)-β-Glc-(1→6)-α-Glc-(1→Asn-Pro has been isolated[3d,e], and details of the synthesis of this *N*-glycopeptide have appeared recently[6].

* Dedicated to Professor Grant Buchanan on the occasion of his 65th birthday.
† To whom correspondence should be addressed.

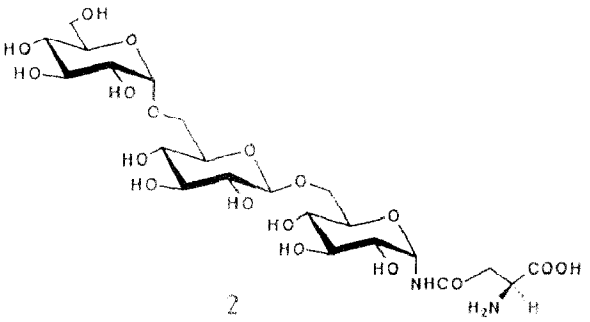
0008-6215/91/$ 03.50



1



3



2

The usual strategy for obtaining the *N*-α-D-glucosyl-asparagine linkage involves reduction of the corresponding α-D-glucopyranosyl azide and condensation of the resulting amine with a protected L-aspartate. However, in practice, the reduction of α-D-glucopyranosyl azides leads to α,β-mixtures of amines and thence to α,β-glycopeptides, which require chromatographic separation[4–6].

We now report details of a synthesis of **3** in which α,β-mixtures are not encountered. Extension to the synthesis of **2**[7], which incorporates new applications of pent-4-enyl glycosides for the construction of oligosaccharides, is also discussed.

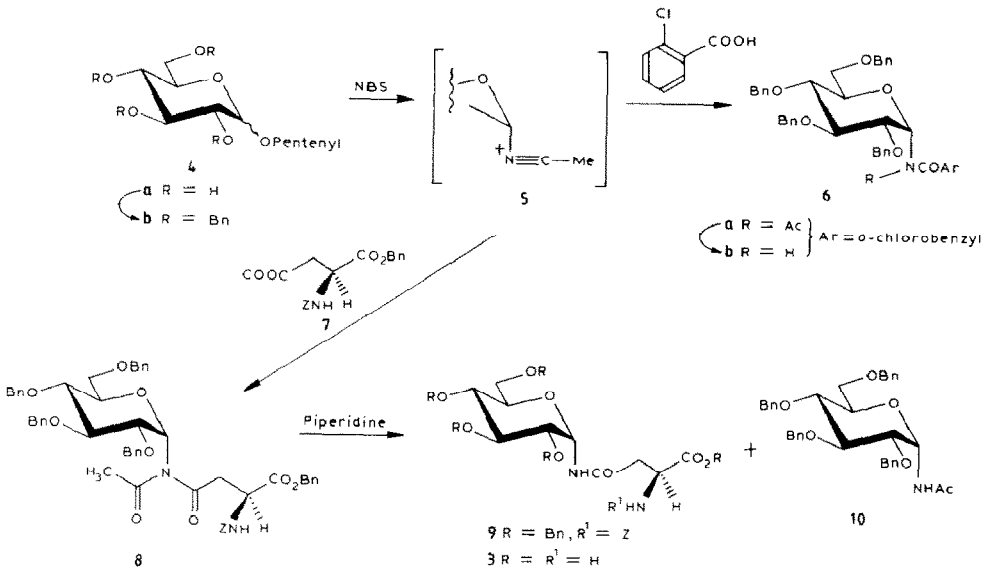
RESULTS AND DISCUSSION

Synthesis of α-Glc-(1→Asn (**3**). – We have reported that treatment of pent-4-enyl 2,3,4,6-tetra-*O*-benzyl-α- or -β-D-glucopyranoside (**4b**) with *N*-bromosuccinimide and 2-chlorobenzoic acid in dry acetonitrile led stereospecifically to the α-*N*-acetyl-2-chlorobenzamide **6a**[8], the nitrilium ion[9] **5** being the logical intermediate. This result contrasts with expectations based on the reverse anomeric effect[10] and differs from results on the trapping of acetonitrilium ions with carboxylic acids[11].

It was shown further[8] that the *N*-acetyl moiety of **6a** was cleaved specifically by sodium methoxide[11] to give the α-2-chlorobenzamide **6b**.

In subsequent experiments directed at the synthesis of **3**, replacement of 2-chlorobenzoic acid with α-benzyl-*N*-benzyloxycarbonyl-L-aspartic acid (**7**) afforded[12] the corresponding imide **8** (53%), and none of the β anomer was detected (^{1}H-n.m.r. analysis).

Attempts to selectively *N*-deacetylate **8** by treatment with sodium methoxide led to complex mixtures, in contrast to the results obtained[8] with **6a**. However, *N*-deacetyla-



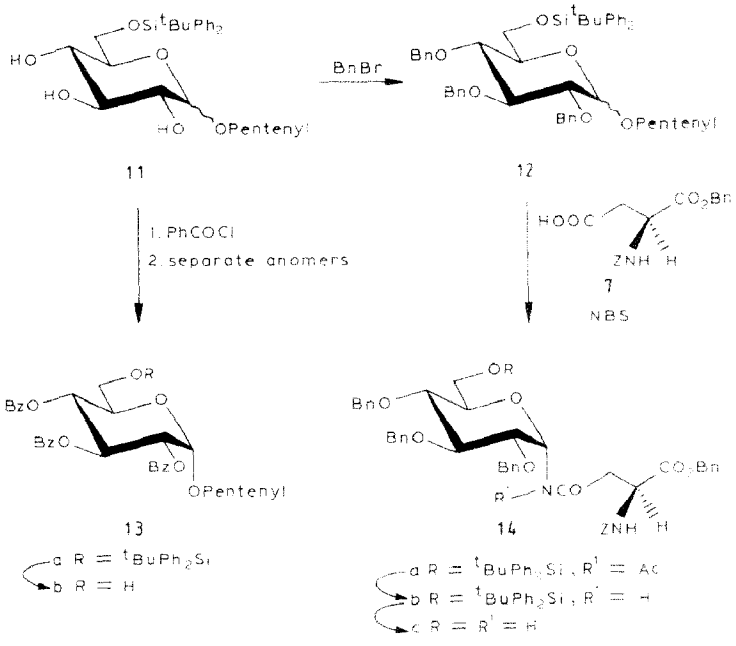
tion could be achieved with secondary amines in *N*,*N*-dimethylformamide. Thus, reaction of **8** with diethylamine for 39 h afforded the known[5a] α-linked asparagine derivative **9** (71%) and the acetamide **10** (12.5%). The use of piperidine led to higher selectivity and shorter reaction times, and gave **9** (89%) and **10** (3.5%) after only 5.75 h. The use of diisopropylamine resulted in no reaction.

Catalytic hydrogenolysis of **9** in the presence of 10% Pd/C, or transfer hydrogenolysis using ammonium formate[13], removed the protecting groups to furnish **3**, which exhibited spectroscopic data [notably, H-1 δ 5.48 (d, J 5.4 Hz), C-1 δ 79.04] in accord with those reported by Ogawa and co-workers[5a]. Compounds **2** and **3** were purified by chromatography on ion-exchange resins, and the chemical shifts of the ^{13}C resonances for the aspartoyl residue accorded with those reported by Ogawa and co-workers[4a,4b,5a] but differed considerably from those reported by Takeda's group[4c,5b]. This situation may be a result of different distribution of charges within the amino acid chain, resulting from the different modes of preparation and purification of **2** and **3**.

Synthesis of α-*Glc*-(1→6)-β-*Glc*-(1→6)-α-*Glc*-(1→*Asn* (**2**). — The desired *N*-aspartoyl component **14c** was prepared as follows. Pent-4-enyl α,β-D-glucopyranoside[8] (**4a**) was *tert*-butyldiphenylsilylated at the primary position[14] to give **11** (82%) then benzylated to afford **12** (83%). Reaction of **12** with α-benzyl *N*-benzyloxycarbonyl-L-aspartate[12] (**7**) and *N*-bromosuccinimide in acetonitrile gave only the α-imide **14a** (61%) which, on treatment with piperidine in *N*,*N*-dimethylformamide, led exclusively to the *N*-deacetylated glycopeptide **14b** (89%).

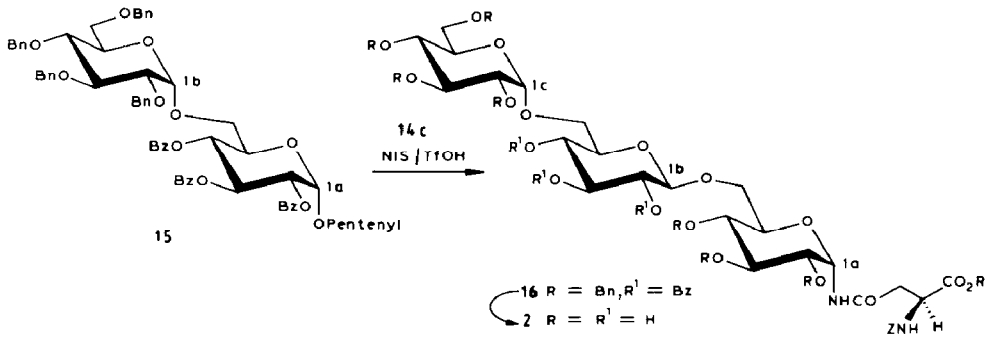
Initial attempts to desilylate either the α-imide **14a** or the glycopeptide **14b** with tetrabutylammonium fluoride gave complex mixtures. However, desilylation of **14b** with hydrogen fluoride in pyridine[15] afforded **14c** (95%), the protecting groups in the peptide chain being unaffected[16].

The "middle" component of **2** was conceived as the 2,3,4-tribenzoate **13b**. Benzoylation of **11** enabled convenient separation of the α and β anomers and facilitated interpretation of the ^{1}H-n.m.r. spectra of the products from the subsequent coupling reactions. Desilylation of the α anomer **13a**, using hydrogen fluoride in pyridine[15], afforded **13b** (41% from **4a**).



We recently adumbrated the concept of "armed" and "disarmed" glycosyl donors in oligosaccharide coupling[17], whereby the presence of an ether or an ester, respectively, at position 2 controls the reactivity of the pent-4-enyl glycosides and hence the coupling process(es). Since the disclosure of these armed/disarmed effects for saccharide coupling, the phenomenon has been identified in other glycosyl donors in other laboratories[18], and we have rationalized these phenomena[19].

Thus, the disarmed benzoylated alcohol donor **13b** was coupled with the armed benzylated glycosyl donor **4b** in the presence of iodonium dicollidine perchlorate in ether–dichloromethane to give the α-linked disaccharide derivative **15** (63%) together with minor products that were not investigated (ether has been used previously[20] to induce preferential α-coupling). The α configurations at C-1b and C-1a of **15** were assigned[21] on the basis of the ^{13}C resonances at δ 97.13 and 95.89, respectively.



In the original armed/disarmed concept[17], changing the 2-substituent from *O*-acyl to *O*-alkyl would be required in order to activate the disaccharide derivative for further coupling. However, this transformation is no longer necessary because efficient coupling of disarmed pent-4-enyl glycosides occurs in the presence of iodonium ion, generated from *N*-iodosuccinimide and trifluoromethanesulfonates[22]. The lower potency of the iodonium dicollidine perchlorate may be due to the collidine ligands that are necessary in order to stabilize iodonium perchlorate, which is otherwise explosive[23]. The advantages of this development are not only that two steps are saved, but that neighboring group participation of the ester at C-2 will control the stereochemistry of the glycosidation. Consequently, the desired β linkage was created by use of the 2-benzoylated glycosyl donor **15**.

Thus, coupling of the disaccharide derivative **15** with **14c** proceeded in the presence of *N*-iodosuccinimide–trifluoromethanesulfonic acid[22] to give the trisaccharide derivative **16** (42%). The modest yield in this step reflects the instability of the benzyloxycarbonylamino moiety to trifluoromethanesulfonic acid[24]. The β configuration of the newly formed glycosidic bond in **16** was evident from the n.m.r. spectra which contained a resonance for H-1b at δ 5.14 (d, *J* 7.9 Hz), and for C-1c and C-1b at δ 95.99 and 99.79, respectively.

O-Debenzoylation of **16**, using methanolic sodium hydroxide, proceeded smoothly but, unlike in the conversion **9**→**3**, transfer hydrogenolysis with ammonium formate/Pd/C caused decomposition. However, catalytic hydrogenolysis of the crude debenzoylated product gave the target compound **2** (71%), which had resonances for H-1a, H-1b, and H-1c at δ 5.46 (d, *J* 4.9 Hz), 4.37 (d, *J* 7.1 Hz), and 4.86 (d, *J* 2.3 Hz), respectively, and for C-1a, C-1b, and C-1c at δ 79.09, 105.21, and 100.38, respectively, similar to those reported[4].

The above efficient regio- and stereo-controlled route to the glycopeptide **2** illustrates the potential of pent-4-enyl glycosides in coupling reactions. Other notable features are the ability to trap the nitrilium ion intermediate with an aspartic acid derivative, and the selective *N*-deacetylation. The ability to change a disarmed (2-acylated) substrate to a reactive substrate by altering the *inorganic* promoter[22] is a development which promises to simplify saccharide coupling reactions.

EXPERIMENTAL

General methods. — Column chromatography was carried out on Kieselgel (230–400 mesh). All reactions that required anhydrous conditions were conducted in an oven-dried apparatus under argon. Organic extracts were dried over $MgSO_4$ and concentrated at aspirator pressure using a rotary evaporator, unless otherwise stated. Light petroleum refers to the fraction b.p. 35–60°. Before use, pyridine, piperidine, dichloromethane, acetonitrile, triethylamine, tetrahydrofuran, and *N,N*-dimethylformamide were dried and distilled, using standard methods[25]. *N*-Bromosuccinimide was recrystallized from hot water and dried *in vacuo* over phosphorus pentaoxide. *N*-Iodosuccinimide was recrystallized from dioxane–carbon tetrachloride. ^{1}H-N.m.r. spectra were recorded at 300 MHz with a Varian XL-300 spectrometer on solutions in $CDCl_3$ (internal Me_4Si). When $(CD_3)_2SO$ was the solvent, the reference was the Me_2SO signal at 2.49 p.p.m. When D_2O was the solvent, the reference was the water signal at 4.63 p.p.m. ^{13}C-N.m.r. spectra were recorded at 75 MHz with a Varian XL-300 spectrometer. The solvent was used as internal standard when spectra were recorded in $CDCl_3$ (77.0 p.p.m.) or $(CD_3)_2SO$ (39.5 p.p.m.); *tert*-butyl alcohol (32.2 p.p.m.) was used as the internal standard for solutions in D_2O. Optical rotations were measured on a Perkin–Elmer 297 instrument. C.i.-mass spectra were recorded with a VG-705 instrument (70eV and ~10 000 resolution), and f.a.b.-mass spectra with dithioerythritol–dithiothreitol as the matrix. T.l.c. was conducted on Kieselgel $60F_{254}$ (Merck 5554) and detection with 2.5% ammonium molybdate(VI) and 1.0% cerium(IV) sulphate tetrahydrate in aqueous 10% sulphuric acid. M.p.s were recorded with a Büchi 510 apparatus and are uncorrected. Elemental combustion analyses were performed by Atlantic Microlab, Inc. (P.O. Box 2288, Norcross, GA).

N-Acetyl-2,3,4,6-tetra-O-benzyl-N-(benzyl-N-benzyloxycarbonyl-L-β-aspartyl-α-D-glucopyranosylamine (**8**). — To a solution of **4b**[7] (337 mg, 0.55 mmol) in dry acetonitrile (11 mL) was added **7** (215 mg, 0.60 mmol) and *N*-bromosuccinimide (156 mg, 0.88 mmol). The mixture was stirred in the dark at room temperature under argon. After 2.75 h, aqueous 10% $Na_2S_2O_3$ (2 mL) was added to the light green solution, the bulk of organic solvent was evaporated under reduced pressure, and the residue was partitioned between water (50 mL) and chloroform (50 mL). The aqueous layer was extracted with chloroform (4 × 50 mL), and the combined organic solutions were dried and then concentrated under reduced pressure. Flash-column chromatography (chloroform–acetone, 80:1 then 40:1) of the residue gave **8** (271 mg, 53%), isolated as an oil, $[\alpha]_D^{25}$ −17° (*c* 0.6). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.28–7.18 (m, 30 H, 6 Ph), 5.90 (d, 1 H, *J* 8.4 Hz, NHCOBn), 5.59 (d, 1 H, *J* 5.7 Hz, H-1), 5.20–5.02 (m, 4 H, 2 CO_2CH_2Ph), 4.71–4.39 (m, 9 H, 4 OCH_2Ph and $COCH_2CH$), 4.17–4.14 (m, 1 H, H-5), 4.04 (t, 1 H, *J* 6.8 Hz, H-3), 3.82 (t, 1 H, *J* 6.1 Hz, H-2), 3.72–3.64 (m, 2 H, H-4,6a), 3.56 (dd, 1 H, *J* 10.5 and 1.9 Hz, H-6b), 3.34 (dd, 1 H, *J* 17.8 and 4.2 Hz, $COCH_AH_BCH$), 3.26 (dd, 1 H, *J* 17.8 and 5.5 Hz, $COCH_AH_BCH$), and 2.12 (s, 3 H, NAc). F.a.b.-mass spectrum: *m/z* 921 (3.5%, M + 1) and 92 (100).

Anal. Calc. for $C_{55}H_{56}N_2O_{11}$: C, 71.72; H, 6.13; N, 3.04. Found: C, 71.79; H, 6.17; N, 2.98.

Reactions of **8** *with piperidine.* — A solution of **8** (282 mg, 0.31 mmol) in dry *N,N*-dimethylformamide (9 mL) and dry piperidine (0.1 mL, 1.01 mmol) was stirred under argon for 5.75 h, then concentrated at 0.1 mmHg. The residue was partitioned between water (50 mL) and chloroform (50 mL), the aqueous layer was extracted with chloroform (4 × 50 mL), and the combined organic solutions were dried and concentrated under reduced pressure. Flash-column chromatography (toluene–ethyl acetate, 5:1) of the residue afforded 2,3,4,6-tetra-*O*-benzyl-*N*-(benzyl *N*-benzyloxycarbonyl-L-β-aspartyl)-α-D-glucopyranosylamine (**9**; 239 mg, 89%), isolated as an oil, $[\alpha]_D^{21}$ +44.5° (*c* 0.75); lit.[5a] $[\alpha]_D^{25}$ +40.8° (*c* 0.6).

Further elution provided *N*-acetyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosylamine (**10**; 6 mg, 3.5%), isolated as an oil, $[\alpha]_D^{21}$ +55° (*c* 1.08). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.34–7.11 (m, 20 H, 4 Ph), 6.14 (d, 1 H, *J* 6.6 Hz, N*H*Ac), 5.78 (t, 1 H, *J* 5.9 Hz, H-1), 4.95–4.45 (m, 8 H, 4 CH_2Ph), 3.86–3.61 (m, 6 H, H-2,3,4,5,6a,6b), and 2.04 (s, 3 H, NAc), E.i.-mass spectrum: *m/z* 581.277 (calc. for $C_{36}H_{39}NO_6$: *m/z* 581.2768).

N-(L-β-*Aspartyl*)-α-D-*glucopyranosylamine* (**3**). — A mixture of **9** (135 mg, 0.15 mmol) and 10% Pd/C (183 mg) in tetrahydrofuran (8 mL), ethanol (6 mL), and water (9 mL) was hydrogenated at 25 p.s.i. at room temperature. After 30 h, the mixture was filtered, the 10% Pd/C residues were washed with ethanol–water (2 × 30 mL, 1:1), and the combined filtrate and washings were concentrated under reduced pressure. A solution of the residue in methanol–water (2 mL, 1:1) was treated with sodium methoxide to pH 8 (pH paper). The mixture was sitrred for 1 h, concentrated to ~0.5 mL, and passed through a column (0.5 × 2.5 cm) of Dowex 50-X8-100 (H^+) resin (methanol–water, 1:1). The appropriate fractions were concentrated to give **3** (29 mg, 64%) as a colorless glass, $[\alpha]_D^{22}$ +88° (*c* 0.43, water); lit.[5a] $[\alpha]_D^{25}$ +106.9° (water); lit.[5b] $[\alpha]_D^{26}$ +34.1° (water). N.m.r. data (D_2O): ^{1}H, δ 5.48 (d, 1 H, *J* 5.4 Hz, H-1), 3.92 (dd, 1 H, *J* 7.2 and 4.2 Hz, $COCH_2CH$), 3.71–3.55 (m, 4 H, H-2,3,6a,6b), 3.43–3.38 (m, 1 H, H-5), 3.30 (t, 1 H, *J* 9.4 Hz, H-4), 2.95 (dd, 1 H, *J* 17.1 and 4.2 Hz, $COCH_AH_BCH$) and 2.84 (dd, 1 H, *J* 17.3 and 7.3 Hz, $COCH_AH_BCH$); ^{13}C, δ 175.86, 175.62 (C=O), 79.04 (C-1), 75.54, 75.23, 71.89, 63.03, 53.58 ($COCH_2CH$), and 37.51 ($COCH_2CH$). F.a.b.-mass spectrum: *m/z* 295 (37%, $M^+ + 1$) and 115 (100).

Anal. Calc. for $C_{10}H_{18}N_2O_8 \cdot 2H_2O$: C, 36.36; H, 6.71; N, 8.48. Found: C, 36.72; H, 6.34; N, 8.51.

Pent-4-enyl 6-O-tert-*butyldiphenylsilyl*-D-*glucopyranoside* (**11**). — To a solution of **4a**[7] (1.06 g, 4.27 mmol) in dry triethylamine (1.5 mL) and dry dichloromethane (10 mL) and 0° under argon was added *tert*-butyldiphenylsilyl chloride (1.2 mL, 4.62 mmol) and 4-dimethylaminopyridine (53 mg). The solution was stirred and allowed to warm to room temperature. After 9 h, saturated aqueous $NaHCO_3$ was added (50 mL), followed by dichloromethane (100 mL). The aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic solutions were dried (Na_2SO_4), and concentrated under reduced pressure. Flash-column chromatography (ethyl acetate–dichloromethane, 1:1) of the residue gave **11** (1.71 g, 82%), isolated as an oil.

Anal. Calc. for $C_{27}H_{38}O_6Si$: C, 66.30; H, 7.87. Found: C, 66.53; H, 7.88.

*Pent-4-enyl 2,3,4-tri-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-*D*-glucopyranoside* (**12**). — To a solution of **11** (1.07 g, 2.20 mmol) in dry *N*,*N*-dimethylformamide (15 mL) under argon were added tetrabutylammonium iodide (105 mg) and sodium hydride (60% dispersion in oil, 453 mg, 11.33 mmol). After cooling the stirred suspension to 0°, benzyl bromide was added (1.5 mL, 12.61 mmol), and the mixture was stirred for another 1.5 h in the cold, then at room temperature for 16.5 h. The solvent was then evaporated at 0.1 mmHg, and the residue was partitioned between water (100 mL) and chloroform (100 mL). The aqueous layer was extracted with chloroform (3 × 100 mL), and the combined organic solutions were dried and concentrated under reduced pressure. Flash-column chromatography (light petroleum–ethyl acetate, 40:1, 20:1, and 10:1) of the residue gave **12** (1.38 g, 83%), isolated as an oil.

Anal. Calc. for $C_{48}H_{56}O_6Si$: C, 76.15; H, 7.45. Found: C, 76.01; H, 7.47.

*Pent-4-enyl 2,3,4-tri-*O*-benzoyl-6-*O*-tert-butyldiphenylsilyl-α-*D*-glucopyranoside* (**13a**). — To a solution of **11** (1.02 g, 2.10 mmol) in dry pyridine were added 4-dimethylaminopyridine (254 mg) and benzoyl chloride (1.5 mL). The solution was stirred under argon at room temperature for 12 h, the solvent was evaporated at 0.1 mmHg, and the residue was partitioned between cold (0°) saturated aqueous $NaHCO_3$ (100 mL) and cold (0°) chloroform (100 mL). The aqueous layer was extracted with cold (0°) chloroform (4 × 100 mL), and the combined organic solutions were dried and concentrated under reduced pressure. Flash-column chromatography (light petroleum–ethyl acetate, 19:1 then 9:1) of the residue gave **13a** (884 mg, 53%), isolated as an oil, $[\alpha]_D^{22}$ +60° (*c* 0.49). ^{1}H-N.m.r. data ($CDCl_3$): δ 8.01 (dd, 2 H, *J* 7.7 and 1.3 Hz, *o*-CO_2Ph), 7.69 (dd, 2 H, *J* 7.6 and 1.7 Hz, *o*-*Ph*Si), 7.61 (dd, 2 H, *J* 7.4 and 1.3 Hz, *o*-*Ph*Si), 7.52–7.20 (m, 15 H, 3 *m*- and *p*-CO_2Ph and 2 *m*- and *p*-*Ph*Si), 6.18 (t, 1 H, *J* 9.8 Hz, H-3), 5.74 (qt, 1 H, *J* 16.6, 10.7, and 6.5 Hz, $CH{=}CH_2$), 5.64 (t, 1 H, *J* 9.9 Hz, H-4), 5.37 (d, 1 H, *J* 3.7 Hz, H-1), 5.30 (dd, 1 H, *J* 10.1 and 3.7 Hz, H-2), 4.94–4.88 (m, 2 H, $CHCH_2$), 4.24–4.18 (m, 1 H, H-5), 3.92–3.80 (m, 3 H, H-6a,6b and $OCH_AH_BCH_2$), 3.52–3.44 (m, 1 H, $OCH_AH_BCH_2$), 2.15–2.07 (m, 2 H, $OCH_2CH_2CH{=}CH_2$), 1.77–1.67 (m, 2 H, $OCH_2CH_2CH_2CH{=}CH_2$), and 1.06 (s, 9 H, CMe_3).

Anal. Calc. for $C_{48}H_{50}O_9Si$: C, 72.16; H, 6.31. Found: C, 72.10; H, 6.36.

*Pent-4-enyl 2,3,4-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**13b**). — To a solution of **13a** (876 mg, 1.10 mmol) in dry tetrahydrofuran (10 mL) and dry pyridine (10 mL) at 0° was added hydrogen fluoride–pyridine (6 mL). The solution was stirred in the cold under argon. After 1.5 h, cold water (0°, 25 mL) was added, followed by solid $NaHCO_3$ to pH ~7 (pH paper). The aqueous solution was diluted with cold water (76 mL) and then extracted with chloroform (4 × 100 mL), and the combined organic solutions were dried and concentrated at 0.1 mmHg. Flash-column chromatography (light petroleum–ethyl acetate, 4:1) of the residue gave **13b** (576 mg, 94%), isolated as an oil, $[\alpha]_D^{22}$ +65° (*c* 0.72). N.m.r. data ($CDCl_3$): ^{1}H, δ 8.00 (dd, 1 H, *J* 6.8 and 2.0 Hz, *o*-CO_2Ph), 7.99 (dd, 1 H, *J* 8.6 and 1.6 Hz, *o*-CO_2Ph), 7.89 (dd, 1 H, *J* 7.9 and 1.4 Hz, *o*-CO_2Ph), 7.54–7.25 (m, 9 H, 3 *m*- and *p*-CO_2Ph), 6.26 (t, 1 H, *J* 9.8 Hz, H-3), 5.74 (qt, 1 H, *J* 17.6, 9.6, and 6.6 Hz, $CH{=}CH_2$), 5.53 (t, 1 H, *J* 9.8 Hz, H-4), 5.38 (d, 1 H, *J* 3.7 Hz, H-1), 5.31 (dd, 1 H, *J* 10.0

and 3.8 Hz, H-2), 4.98–4.87 (m, 2 H, CH = CH_2), 4.12–4.08 (m, 1 H, H-5), 3.86–3.71 (m, 3 H, H-6a,6b and OCH_AH$_B$CH$_2$), 3.53–3.45 (m, 1 H, OCH$_A$$H_BCH_2$), 2.85 (t, 1 H, *J* 6.6 Hz, OH), 2.16–2.03 (m, 2 H, OCH$_2$CH$_2$CH_2CH = CH$_2$), and 1.77–1.67 (m, 2 H, OCH$_2$CH_2CH$_2$CH = CH$_2$); ^{13}C, δ 95.98 (C-1).

Anal. Calc. for $C_{32}H_{32}O_9$: C, 68.56; H, 5.75. Found: C, 68.47; H, 5.76.

N-*Acetyl-2,3,4-tri*-O-*benzyl*-N-*(benzyl* N-*benzyloxycarbonyl*-L-*β-aspartyl)-6*-O-tert-*butyldiphenylsilyl*-α-D-*glucopyranosylamine* (**14a**). — To a solution of **12** (1.25 g, 1.65 mmol) in dry acetonitrile (30 mL) were added **7** (652 mg, 1.83 mmol) and *N*-bromosuccinimide (444 mg, 2.50 mmol). The mixture was stirred in the dark at room temperature under argon. After 1 h, aqueous 10% $Na_2S_2O_3$ (10 mL) was added, the bulk of organic solvent was evaporated under reduced pressure, and the residue was partitioned between water (100 mL) and chloroform (100 mL). The aqueous layer was extracted with chloroform (3 × 100 mL). The combined organic layers were washed with water (2 × 75 mL) and dried, and the solvent was removed under reduced pressure. Flash-column chromatography (light petroleum–ethyl acetate, 4:1) of the residue gave **14a** (1.08 g 61%), isolated as an oil, $[\alpha]_D^{22}$ −11° (*c* 0.54). ^{1}H-N.m.r. data ($CDCl_3$): δ 7.64–7.60 (m, 4 H, 2 *o-Ph*Si), 7.39–7.18 (m, 31 H, 3 CH$_2$*Ph*, 2 CO$_2$CH$_2$*Ph*, and 2 *m*- and *p-Ph*Si), 5.80 (d, 1 H, *J* 8.5 Hz, N*H*CO$_2$CH$_2$Ph), 5.61 (d, 1 H, *J* 5.8 Hz, H-1), 5.21–5.00 (m, 4 H, 2 CO$_2$CH_2Ph), 4.78–4.42 (m, 7 H, 3 CH_2Ph and COCH$_2$C*H*), 4.08–4.02 (m, 2 H, H-3,5), 3.91–3.77 (m, 4 H, H-2,4,6a,6b), 3.30 (dd, 1 H, *J* 17.7 and 4.6 Hz, COCH_AH$_B$CH), 3.22 (dd, 1 H, *J* 17.6 and 4.5 Hz, COCH$_A$$H_B$CH), 2.10 (s, 3 H, NAc), and 1.02 (s, 9 H, CMe$_3$). F.a.b.-mass spectrum: *m/z* 1069 (6%, M$^+$ + 1) and 57 (100).

Anal. Calc. for $C_{64}H_{68}N_2O_{11}Si$: C, 71.88; H, 6.41; N, 2.62. Found: C, 71.95; H, 6.44; N, 2.63.

2,3,4-Tri-O-*benzyl*-N-*(benzyl* N-*benzyloxycarbonyl*-L-*β-aspartyl)-6*-O-tert-*butyldiphenylsilyl*-α-D-*glucopyranosylamine* (**14b**). — A solution of **14a** (1.06 g, 0.99 mmol) in dry *N,N*-dimethylformamide (15 mL) and dry piperidine (0.35 mL, 3.54 mmol) was stirred under argon for 3.75 h. The solvent was then evaporated at 0.1 mmHg and the residue partitioned between water (100 mL) and chloroform (100 mL). The aqueous layer was extracted with chloroform (4 × 100 mL). The combined organic solvents were dried and concentrated under reduced pressure. Flash-column chromatography (toluene–ethyl acetate, 10:1) of the residue gave **14b** (908 mg, 89%), m.p. 128–129° (from dichloromethane–light petroleum), $[\alpha]_D^{22}$ +29° (*c* 0.64). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 8.68 (d, 1 H, *J* 9.0 Hz, N*H*COCH$_2$), 7.71 (d, 1 H, *J* 7.9 Hz, N*H*CO$_2$CH$_2$Ph), 7.62 (d, 2 H, *J* 7.9 Hz, *o-Ph*Si), 7.59 (d, 2 H, *J* 6.6 Hz, *o-Ph*Si), 7.45–7.12 (m, 31 H, 3 CH$_2$*Ph*, 2 CO$_2$CH$_2$*Ph*, and 2 *m*- and *p-Ph*Si), 5.82 (dd, 1 H, *J* 8.7 and 5.4 Hz, H-1), 5.14–4.52 (m, 11 H, 3 CH_2Ph, 2 CO$_2$CH_2Ph), and COCH$_2$C*H*), 4.09 (t, 1 H, *J* 9.1 Hz, H-3), 3.87 (dd, 1 H, *J* 11.1 and 1.8 Hz, H-6a), 3.75–3.54 (m, 4 H, H-2,4,5,6b), 2.82 (dd, 1 H, *J* 15.4 and 5.9 Hz, COCH_AH$_B$CH), 2.63 (dd, 1 H, *J* 15.7 and 7.3 Hz, COCH$_A$$H_B$CH), and 0.98 (s, 9 H, CMe$_3$). F.a.b.-mass spectrum: *m/z* 1027 (18%, M$^+$ + 1) and 92 (100).

Anal. Calc. for $C_{62}O_{66}N_2O_{10}Si$: C, 72.49; H, 6.47; N, 2.73. Found: C, 72.34; H, 6.52; N, 2.78.

2,3,4-Tri-O-benzyl-N-(benzyl N-benzyloxycarbonyl-L-β-aspartyl)-α-D-glucopyranosylamine (**14c**). — To a solution of **14b** (865 mg, 0.84 mmol) in dry tetrahydrofuran (25 mL) and dry pyridine (14 mL) at 0° was added hydrogen fluoride·pyridine (7 mL). The solution was then allowed to warm to room temperature and stirred under argon. After 2.75 h, the mixture was cooled (0°), saturated aqueous $NaHCO_3$ (300 mL) was added slowly, the aqueous solution was extracted with chloroform (6 × 100 mL), and the combined organic solutions were dried and concentrated under reduced pressure (<45°). Flash-column chromatography (light petroleum–ethyl acetate, 9:1, then 1:1) of the residue provided **14c** (628 mg, 95%), m.p. 139–140° (from toluene), $[\alpha]_D^{22}$ +48° (*c* 0.59). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 8.72 (d, 1 H, *J* 9 Hz, N*H*(CO)CH_2), 7.76 (d, 1 H, *J* 8.1 Hz, N*H*CO_2CH_2Ph), 7.39–7.32 (25 H, 3 CH_2*Ph* and 2 CO_2CH_2*Ph*), 5.78 (dd, 1 H, *J* 8.8 and 5.4 Hz, H-1), 5.21–5.02 (m, 4 H, 2 CO_2CH_2Ph), 4.92–4.53 (m, 8 H, 3 CH_2Ph, $COCH_2CH$, and OH), 4.08 (t, 1 H, *J* 8.8 Hz, H-3), 3.67 (dd, 1 H, *J* 9.6 and 5.3 Hz, H-2), 3.60–3.40 (m, 4 H, H-4,5,6a,6b), 2.85 (dd, 1 H, *J* 15.6 and 5.6 Hz, $COCH_AH_BCH$), and 2.72 (dd, 1 H, *J* 15.6 and 7.8 Hz, $COCH_AH_BCH$). F.a.b.-mass spectrum: *m/z* 787 (17%, $M^+ + 1$) and 92 (100).

Anal. Calc. for $C_{46}H_{48}N_2O_{10}$: C, 70.04; H, 6.13; N, 3.55. Found: C, 70.19; H, 6.16; N, 3.47.

Pent-4-enyl 2,3,4-tri-O-benzoyl-6-O-(2,3,4,5-tetra-O-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (**15**). — To **13b** (576 mg, 1.03 mmol), flame-dried 4Å molecular sieves (2 g), and iodonium dicollidine perchlorate (773 mg, 1.65 mmol) was added a solution of **4b**[7] (762 mg, 1.25 mmol) in dry ether–dichloromethane (37.5 mL, 4:1). The mixture was stirred under argon in the dark at room temperature. After 10 h, aqueous 10% $Na_2S_2O_3$ (10 mL) was added, the mixture was filtered, and the 4Å molecular sieves were washed with chloroform (100 mL). The combined filtrate and washings were washed with cold (0°) aqueous 1% HCl (100 mL) and the aqueous phase was extracted with chloroform (3 × 100 mL). The combined organic extracts were washed with cold (0°) aqueous 10% $NaHCO_3$ (100 mL), then dried, and the solvent was removed under reduced pressure. Flash-column chromatography (light petroleum–ethyl acetate, 9:1 then 4:1) of the residue gave slightly impure **15** (808 mg). Further flash-column chromatography (chloroform–0.75% of ethanol) yielded **15** (701 mg, 63%), isolated as an oil, $[\alpha]_D^{22}$ +69° (*c* 0.83). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.99 (d, 2 H, *J* 8.8 Hz, 2 *o*-CO_2*Ph*), 7.96 (dd, 2 H, *J* 8.7 and 1.1 Hz, 2 *o*-CO_2*Ph*), 7.87 (dd, 1 H, *J* 7.7 and 1.2 Hz, 2 *o*-CO_2*Ph*), 7.53–7.11 (m, 29 H, 3 *m*- and *p*-CO_2*Ph* and 4 CH_2*Ph*), 6.16 (t, 1 H, *J* 9.8 Hz, H-3a), 5.68 (qt, 1 H, *J* 17.9, 9.3, and 6.5 Hz, $CH{=}CH_2$), 5.54 (t, 1 H, *J* 9.9 Hz, H-4a), 5.30 (d, 1 H, *J* 3.7 Hz, H-1a), 5.23 (dd, 1 H, *J* 10.2 and 3.8 Hz, H-2a), 4.94–4.34 (m, 11 H, 4 CH_2Ph, $CH{=}CH_2$ and H-1b), 3.97 (t, 1 H, *J* 9.3 Hz, H-3b), 3.88–3.38 (m, 10 H, H-5a,6a,6′a,2b,4b,5b,6b,6′b and OCH_2CH_2), 2.08–2.01 (m, 2 H, $OCH_2CH_2CH_2$-$CH{=}CH_2$), and 1.69–1.61 (m, 2 H, $OCH_2CH_2CH_2CH{=}CH_2$); ^{13}C, δ 165.75, 165.72, 165.17 (C=O), 114.89 ($CH{=}CH_2$), 97.13 (C-1b), and 95.59 (C-1a).

Anal. Calc. for $C_{66}H_{66}O_{14}$: C, 73.18; H, 6.14. Found: C, 72.81; H, 6.16.

O-*(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)-(1→6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-N-(benzyl N-benzyloxycarbonyl-L-β-*

aspartyl-α-D-glucopyranosylamine (**16**). — To **15** (250 mg, 0.23 mmol), *N*-iodosuccinimide (195 mg, 0.87 mmol), **14c** (204 mg, 0.26 mmol), and flame-dried 4 Å molecular sieves (1 g) was added dry dichloromethane (25 mL). The mixture was stirred at room temperature under argon. After 0.5 h, a saturated solution of trifluoromethanesulphonic acid in dichloromethane was added dropwise until t.l.c. (light petroleum–ethyl acetate, 33:17) indicated total consumption of **15**. The mixture was filtered and the collected 4Å molecular sieves were washed with chloroform (100 mL). To the combined filtrate and washings were added cold (0°) aqueous 10% $Na_2S_2O_3$ (50 mL) and aqueous 10% $NaHCO_3$ (50 mL). The aqueous layer was extracted with chloroform (3 × 100 mL), the combined solutions were washed with water (2 × 100 mL), and dried, and the solvent was removed under reduced pressure. Flash-column chromatography (light petroleum–ethyl acetate, gradient 9:1 to 13:7) of the residue gave **16** (172 mg, 42%), isolated as an oil, $[\alpha]_D^{23}$ +30° (*c* 1.11). N.m.r. data [$(CD_3)_2SO$]: ^1H, δ 8.58 (d, 1 H, *J* 8.6 Hz, N*H*COCH$_2$CH), 5.98 (t, 1 H, *J* 9.6 Hz, H-3b), 5.64–5.57 (m, 2 H, H-1a,4b), 5.37 (t, 1 H, *J* 8.8 Hz, H-2b), 5.14 (d, 1 H, *J* 7.9 Hz, H-1b), 2.76 (dd, 1 H, *J* 15.3 and 5.8 Hz, CO*H*$_A$H$_B$CH), and 2.54 (dd, 1 H, *J* 15.4 and 7.7 Hz, COCH$_A$*H*$_B$CH); ^{13}C, δ 99.79 (C-1b) and 95.99 (C-1c). F.a.b.-mass spectrum: *m/z* 1870 (2%, M^+ + 23) and 475 (100).

Anal. Calc. for $C_{107}H_{104}N_2O_{23}$: C, 71.96; H, 5.87; N, 1.57. Found: C, 71.91; H, 5.95; N, 1.53.

O-*(α-D-Glucopyranosyl)-(1→6)-O-(β-D-glucopyranosyl)-(1→6)*-N-*(β-L-aspartyl)-α-D-glucopyranosylamine* (**2**). — To **16** (105 mg, 0.06 mmol) was added 0.18M NaOH in methanol (3.5 mL). After stirring at room temperature for 0.75 h, the bulk of methanol was removed under reduced pressure and to the residue was added water (25 mL), followed by aqueous 1% HCl to pH 6 (pH paper). The aqueous phase was extracted with chloroform (3 × 50 mL), the combined organic solutions were dried (Na_2SO_4), and the solvent was removed under reduced pressure. A solution of the white solid residue in tetrahydrofuran (8 mL), ethanol (6 mL), and water (9 mL) containing 10% Pd/C (184 mg) was hydrogenated at 25 p.s.i. at room temperature. After 24 h, the mixture was filtered, the 10% Pd/C residue was washed with ethanol–water (2 × 30 mL, 1:1), and the combined filtrate and washings were concentrated under reduced pressure. A solution of the residue in methanol–water (2 mL, 1:1) was treated with sodium methoxide to pH ~8 (pH paper). After stirring for 1 h, the mixture was concentrated to ~0.5 mL and passed through a column (0.5 × 2.5 cm) of Dowex 50-X8-100 (H^+) resin with methanol–water (1:1). The appropriate fractions were concentrated to give **2** (26 mg, 71%) as a colorless glass, $[\alpha]_D^{23}$ +30° (*c* 0.41, water); lit.[4b] $[\alpha]_D^{25}$ +72.8° (water). N.m.r. data (D_2O): ^1H, δ 5.46 (d, 1 H, *J* 4.9 Hz, H-1a), 4.86 (d, 1 H, *J* 2.3 Hz, H-1c), 4.37 (d, 1 H, *J* 7.1 Hz, H-1b), 2.92 (dd, 1 H, *J* 17.2 and 3.5 Hz, COC*H*$_A$H$_B$CH) and 2.82 (dd, 1 H, *J* 17.3 and 7.3 Hz, COCH$_A$*H*$_B$CH); ^{13}C, δ 105.21 (C-1b), 100.38 (C-1c), 79.09 (C-1a), 53.60 (COCH$_2$CH), and 37.60 (COCH$_2$CH).

ACKNOWLEDGMENTS

This work was made possible by grants from the National Science Foundation (CHE 8703916), Glaxo Inc. (Research Triangle Park, NC), and Glycomed, Inc. (Hayward, CA). The S.E.R.C. (United Kingdom) is acknowledged for a NATO Postdoctoral Research Fellowship (to A.J.R.). We thank Dr. G. Dubay for recording the mass spectra.

REFERENCES

1 J. Montreuil, in A. Neuberger and L. L. M. Van Deenen (Eds.), *Comprehensive Biochemistry*, Vol. 19B.II, Elsevier, Amsterdam, 1982, p. 1; P. G. Johansen, R. D. Marshall, and A. Neuberger, *Biochem. J.*, 78 (1961) 518–527.
2 S. Shibata, Y. Miyagawa, T. Naruse, and T. Takuma, *J. Immunol.*, 102 (1969) 593–601.
3 (*a*) S. Shibata and H. Nakanishi, *Carbohydr. Res.*, 81 (1980) 345–348; (*b*) *idem*, *ibid.*, 86 (1980) 316–320; (*c*) S. Shibata, M. Sawaki, T. Takeda, and Y. Ogihara, *Chem. Pharm. Bull.*, 33 (1985) 5134–5136; (*d*) T. Takeda, M. Sawaki, Y. Ogihara, and S. Shibata, *ibid.*, 37 (1989) 54–56; (*e*) S. Shibata, T. Takeda, and Y. Natori, *J. Biol. Chem.*, 263 (1988) 12 483–12 485.
4 (*a*) T. Ogawa, S. Nakabayashi, and S. Shibata, *Carbohydr. Res.*, 86 (1980) C7–C10; (*b*) T. Ogawa, S. Nakabayashi, and S. Shibata, *Agric. Biol. Chem.*, 47 (1983) 1213–1218; (*c*) T. Takeda, Y. Sugiura, C. Hamada, R. Fujii, K. Suzuki, Y. Ogihara, and S. Shibata, *Chem. Pharm. Bull.*, 29 (1981) 3196–3201.
5 (*a*) T. Ogawa, S. Nakabayashi, and S. Shibata, *Agric. Biol. Chem.*, 47 (1983) 281–285; (*b*) T. Takeda, Y. Sugiura, Y. Ogihara, and S. Shibata, *Can. J. Chem.*, 58 (1980) 2600–2603.
6 T. Takeda, A. Utsuno, N. Okamoto, Y. Ogihara, and S. Shibata, *Carbohydr. Res.*, 207 (1990) 71–79.
7 A. J. Ratcliffe, P. Konradsson, and B. Fraser-Reid, *J. Am. Chem. Soc.*, 112 (1990) 5665–5667.
8 A. J. Ratcliffe and B. Fraser-Reid, *J. Chem. Soc., Perkin Trans. 1*, (1990) 747–750.
9 A. J. Ratcliffe and B. Fraser-Reid, *J. Chem. Soc., Perkin Trans. 1*, (1989) 1805–1810; R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, 57 (1979) 1244–1251; A. A. Pavia, S. N. Ung-Chhun, and J.-L. Durand, *J. Org. Chem.*, 46 (1981) 3158–3160; A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 200 (1990) 319–337; C. Amatore, A. Jutand, J.-M. Mallet, G. Meyer, and P. Sinaÿ, *J. Chem. Soc., Chem. Commun.*, (1990) 718–719; A. Marra, L. K. S. Shun, F. Gauffeny, and P. Sinaÿ, *Synlett*, (1990) 445–447; A. Marra, J.-M. Mallet, C. Amatore, and P. Sinaÿ, *ibid.*, (1990) 572–574; R. R. Schmidt, M. Behrendt, and A. Toepfer, *ibid.*, (1990) 694–696.
10 R. U. Lemieux and A. R. Morgan, *Can. J. Chem.*, 43 (1965) 2205–2213.
11 P. Sinaÿ and J. R. Pougny, *Tetrahedron Lett.*, (1976) 4073–4076; R. R. Schmidt and J. Michel, *J. Carbohydr. Chem.*, 4 (1985) 141–169.
12 P. M. Bryant, R. M. Moore, P. J. Pimlott, and G. T. Young, *J. Chem. Soc.*, (1959) 3868–3873.
13 S. Ram and L. D. Spicer, *Tetrahedron Lett.*, 28 (1987) 515–516.
14 S. Hanessian and P. Lavallee, *Can. J. Chem.*, 53 (1975) 2975–2977.
15 K. C. Nicolaou, S. P. Seitz, M. R. Pavia and N. A. Petasis, *J. Org. Chem.*, 44 (1979) 4011–4013.
16 S. Matsuura, C.-H. Niu, and J. S. Cohen, *J. Chem. Soc., Chem. Commun.*, (1976) 451–452.
17 D. R. Mootoo, P. Konradsson, U. E. Udodong, and B. Fraser-Reid, *J. Am. Chem. Soc.*, 110 (1988) 5583–5584.
18 R. W. Friesen and S. J. Danishefsky, *J. Am. Chem. Soc.*, 111 (1989) 6656–6660; H. B. Mereyala and G. V. Reddy, *IUPAC Int. Symp. Chem. Nat. Prod., 17th*, New Delhi, India, February 4–9, 1990; G. H. Venneman, S. H. van Leeuwen, and J. H. van Boom, *Tetrahedron Lett.*, 31 (1990) 1333–1334; G. H. Veeneman and J. H. van Boom, *ibid.*, 31 (1990) 275–278.
19 B. Fraser-Reid, Z. Wu, U. E. Udodong, and H. Ottosson, *J. Org. Chem.*, 55 (1990) 6068–6070.
20 S. Hashimoto, M. Hayashi, and R. Noyori, *Tetrahedron Lett.*, 24 (1984) 1379–1382; Y. Ito and T. Ogawa, *ibid.*, 28 (1987) 4701–4703; B. Fraser-Reid, D. R. Mootoo, P. Konradsson, and U. E. Udodong, *J. Chem. Soc., Chem. Commun.*, (1988) 823–825.
21 A. S. Perlin, *MTP Int. Rev. Sci. Org. Chem., Ser. Two*, 7 (1976) 1–34; P. A. J. Gorin, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 13–104.

22 P. Konradsson, D. R. Mootoo, R. E. McDevitt, and B. Fraser-Reid, *J. Chem. Soc., Chem. Commun.*, (1990) 270–272; P. Konradsson, U. E. Udodong, and B. Fraser-Reid, *Tetrahedron Lett.*, 31 (1990) 4313–4316.
23 H. Carlson, *Ber.*, 68 (1935) 2209–2211.
24 H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, (1974) 107–108.
25 D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, *Purification of Laboratory Chemicals*, Pergamon, Oxford, 1980.