DOI: 10.1002/ejoc.200500690

Gram-Scale Preparation of a *p*-(C-Glucopyranosyl)-L-phenylalanine Derivative by a Negishi Cross-Coupling Reaction

Malika Ousmer,[a] Valérie Boucard,[a] Nadège Lubin-Germain,[a] Jacques Uziel,*[a] and Jacques Augé*[a]

Keywords: Amino acids / C-Glycosides / Cross-coupling / Palladium / Zinc

A *p*-(C-glucopyranosyl)-L-phenylalanine derivative protected to be directly incorporated into a peptidic chain is prepared in 37% yield from glucose on a gram scale, with a Negishi cross-coupling reaction as the key step. Zincated glucal and *p*-iodo-L-phenylalanine are involved in this organometallic coupling, which gives rise to a link between the sugar and amino acid moieties in 90% yield; the β-*gluco* configuration of the C-glycopyranosyl amino acid is ascertained by a stereoselective hydroboration of the double bond of the glucal.

Introduction

The importance of glycoproteins in a number of biological processes such as cell adhesion, cell differentiation, or regulation of cell growth is now well established.[1] Changes in glycosylation are often a hallmark of disease states, and have given rise to new strategies in therapeutics and diagnostics.[2] Generally, glycopeptides present two different types of junction between the peptidic and the oligosaccharidic parts, depending on whether a serine/threonine or an asparagine is glycosylated. However, O-glycosylation of phenolic units is also encountered,[3] and a tyrosine residue is glycosylated in glycopeptide antibiotics such as vancomycin or mannopeptimycin for example.[4] To probe and understand biological mechanisms it is always attractive to prepare C-glycosylated amino acids due to their chemical and metabolic stability.[5] C-Glycosyl analogues of tyrosine[6] or phenylalanine[7] have been described previously and they can be used as tools incorporated into more relevant frameworks through supported peptide synthesis. Since such syntheses often need an excess of reagents, it is important to be able to prepare a large amount (up to one gram) of the target C-glycosylated amino acid. We describe herein an efficient gram-scale preparation of a suitably functionalized *p*-(C-glucopyranosyl)phenylalanine derivative with a Negishi coupling reaction as the key step, followed by a regio- and stereoselective hydroboration.

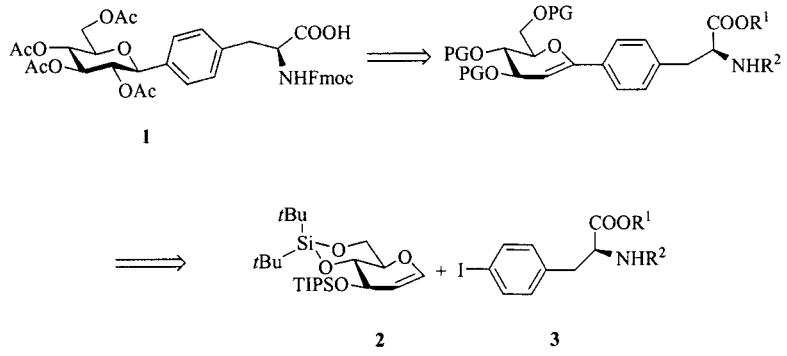
[a] UMR CNRS-UCP-ESCOM, Synthèse Organique Sélective et Chimie Organométallique,
5 mail Gay-Lussac, Neuville-sur Oise, 95031 Cergy-Pontoise Cedex, France
E-mail: jacques.uziel @chim.u-cergy.fr
jacques.auge@chim.u-cergy.fr

Results and Discussion

Our target was *p*-(C-glucopyranosyl)phenylalanine (**1**) (see Scheme 1), which bears protective groups compatible with solid-phase peptide synthesis, such as acetyl groups on the carbohydrate and 9-fluorenylmethoxycarbonyl (Fmoc) for the amino group, with the free carboxylic acid allowing further peptide linkage. As the key step of our strategy is a palladium-catalyzed cross-coupling reaction using a metalated glucal derivative, precursor **2** was chosen as the 4,6-di-*tert*-butylsilylidene liberates the anomeric position for steric and conformational reasons,[8] thereby facilitating subsequent glucal C-1 deprotonation and metalation.[9]

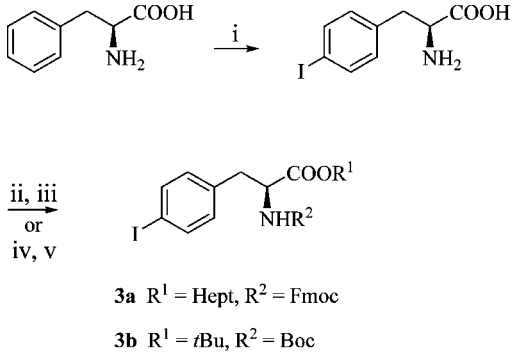
Thus, the silylated glucal **2** was prepared from the easily accessible tri-*O*-acetyl-D-glucal[10] according to the following sequence: methanolysis of the acetyl groups, silylation of the hydroxy groups at positions 4 and 6 at low temperature, and then silylation of the 3-hydroxy group.[11] An overall yield of 88% from glucose was achieved.

At the same time the *p*-iodo-L-phenylalanine derivatives **3a** and **3b** were prepared from L-phenylalanine as shown in Scheme 2. Iodination of phenylalanine was performed as described by Schwabacher et al.[12] and *p*-iodo-L-phenylalanine, which was obtained as a precipitate in 87% yield, was used without purification. Two kinds of protection of the amino acid were investigated. The first protection that we tested dealt with the Fmoc carbamate/heptyl ester protective groups; this ester is known as an orthogonal ester of carbohydrate acetyl groups cleaved by enzymatic hydrolysis.[13] Fmoc-Phe(4-I)-OHept (**3a**) was obtained in 45% yield from 4-iodo-L-phenylalanine by esterification followed by carbamoylation.[14] In the second protection of the amino acid that we tested, we found it more convenient to first protect the amino group as the *tert*-butyl carbamate, then the carboxylic acid as the *tert*-butyl ester, both protect-



Scheme 1.

ing groups being prone to cleavage under acidic conditions. In the esterification step the carboxylic acid was first transformed into its potassium salt, which was allowed to react with an excess of *tert*-butyl bromide in the presence of a phase-transfer catalyst, leading to Boc-Phe(4-I)-O*t*Bu (**3b**) in 73% yield over the three steps from phenylalanine.



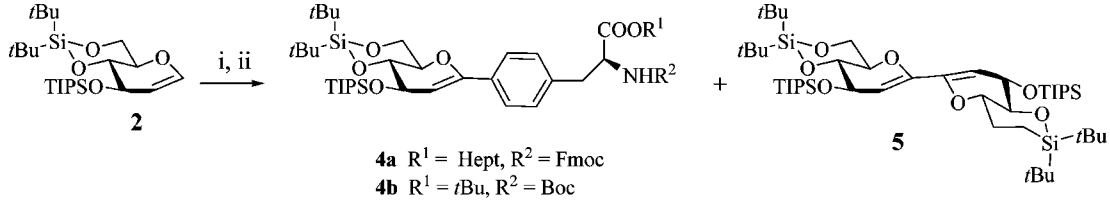
Scheme 2. Reagents and conditions: i) I_2, $NaIO_3$, H_2SO_4/AcOH, 70 °C, $NaIO_4$, 87%; ii) heptanol, TsOH, 80 °C, 53%; iii) $NaHCO_3$ then FmocOSu, dioxane, 86%; iv) Boc_2O, NaOH, dioxane, 90%; v) *t*BuBr, K_2CO_3, nBu_4NBr, DMAC, 93%.

As the coupling reaction of a stannylated glucal with simple aryl bromides has been described previously,[8] we first tested several conditions of the Stille reaction, although homocoupling made the methodology inefficient for a large-scale synthesis. The preliminary results obtained in the coupling of **3a** with the zincated glucal gave encouraging results.[14] The transient organozinc derivative formed in situ by deprotonation of the glucal **2** with *t*BuLi and reaction with zinc dichloride was also applied in the reaction with Boc-Phe(4-I)-O*t*Bu (**3b**) in the presence of a catalytic amount of $[Pd_2(dba)_3]\cdot CHCl_3$ and the bulky tri-*o*-tolylphosphane (Scheme 3). The coupling product **4b** was isolated in 90% yield along with the homocoupling by-product **5** (5%).

The functionalization of the double bond of the coupling products in order to gain access to the pyranosyl ring might be achieved either by hydroboration followed by oxidation[15] or by dihydroxylation followed by reduction.[16] Compound **4b** was chosen as it was obtained in a better yield and it bears the most-stable protective groups.

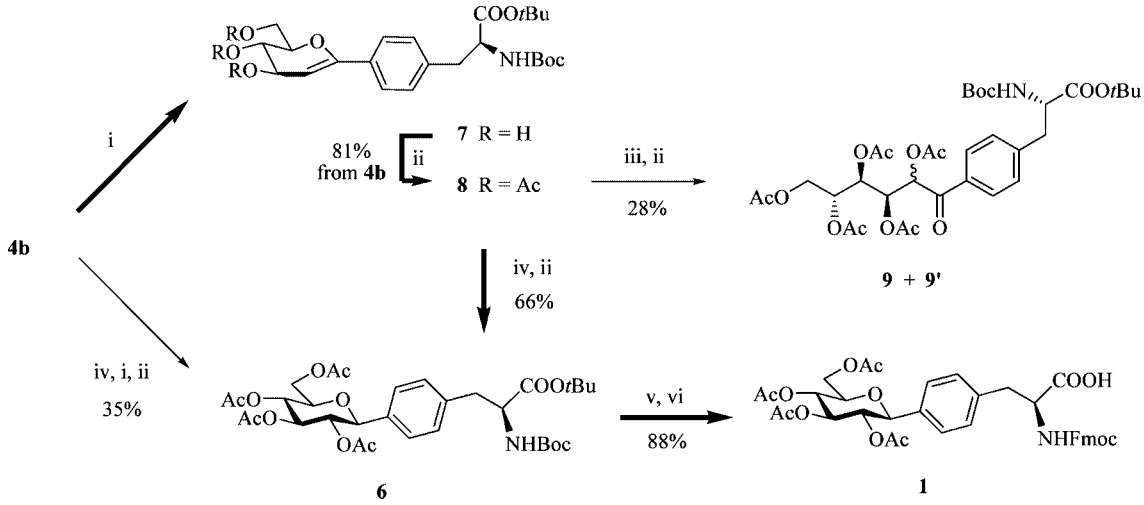
Hydroboration of **4b** followed by oxidation, desilylation, and acetylation led to compound **6** in only 35% yield. This compound, which arises from attack from the α face of the glucal, was accompanied by the compound arising from the attack from the β face (Scheme 4).

As the silylidene group liberates the β face, we thought it more judicious to remove the silyl groups immediately and replace them by acetyl groups, which are especially



Compound	R^1	R^2	Product **4**	Yield	Product **5**
3a	Hept	Fmoc	**4a**	69%	0%
3b	*t*Bu	Boc	**4b**	90%	5%

Scheme 3. Reagents and conditions: i) *t*BuLi, THF, –78 °C to 0 °C then $ZnCl_2$; ii) **3a** or **3b**, $[Pd_2(dba)_3]\cdot CHCl_3$, P(*o*-tolyl)$_3$, THF, 69% from **3a**, 90% from **3b**.



Scheme 4. Reagents and conditions: i) nBu$_4$NF, THF, 0 °C to room temp.; ii) Ac$_2$O, pyridine, DMAP, room temp.; iii) OsO$_4$, NMO, THF, tBuOH, pyridine, H$_2$O; iv) BH$_3$·THF, then NaOH, H$_2$O$_2$; v) TFA, CH$_2$Cl$_2$; vi) FmocOSu, NEt$_3$, CH$_3$CN/H$_2$O.

suited to peptidic solid-phase synthesis. The silyl groups were therefore removed by treatment with tetrabutylammonium fluoride to afford the triol **7**, which was used without purification in the acetylation step to give **8** with 81% yield from **4b** (Scheme 4). Dihydroxylation of **8** was first attempted, but it led to a complex mixture of compounds. In order to get more insight into the outcome of the reaction, the mixture was acetylated to afford a mixture of epimers **9** and **9′** in the ratio 77:23, in only 28% yield (Scheme 4). The structure of these inseparable isomers indicates the opening of the pyranosyl ring during the acetylation step and a low stereoselectivity during the approach of osmium tetroxide.

Since dihydroxylation gave such unsatisfactory results, we decided to return to the hydroboration pathway, which is known to be regioselective.[15] Thus, hydroboration of **8** with an excess of BH$_3$·THF, followed by oxidative quenching (H$_2$O$_2$, NaOH) of the organoborane and then acetylation of the free hydroxy group, afforded the *p*-(C-glucopyranosyl)phenylalanine derivative **6** in 66% yield over two steps (Scheme 4). The β configuration of this new C-glycoside was assigned by the coupling constant of 9.6 Hz between the H^1 and H^2 protons of the carbohydrate ring. Simultaneous deprotection of the Boc and *t*Bu groups with trifluoroacetic acid followed by protection of the amino group by an Fmoc group led to the target *p*-(β-C-glucopyranosyl)phenylalanine **1** in 88% yield over two steps (Scheme 4).

Conclusion

We have shown that a very efficient Negishi-type catalyzed coupling reaction can be applied to functionalized carbohydrate and amino acids to create a carbon link between these two moieties. Such an organometallic reaction between the appropriate glucal and 4-iodo-L-phenylalanine, followed by a stereoselective hydroboration, leads from glucose to a *p*-(β-C-glucopyranosyl)-L-phenylalanine derivative, with an overall yield of 37%, which will be directly incorporated into a peptide chain using automated solid-phase synthesis.

Experimental Section

General Remarks: ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance 250 DPX (^{1}H: 250 MHz; ^{13}C: 62.9 MHz) or a JEOL ECX-400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) spectrometer. IR spectra were measured with a Bruker Tensor 27 spectrophotometer. Optical rotations were determined at 25 °C in chloroform on a JASCO DIP 370 instrument. Melting points (uncorrected) were determined on a Büchi B-545. Elemental analyses were done at the Central Service of Analysis (CNRS, Vernaison). High resolution mass spectra were obtained with an MS JEOL 700 at the Ecole Normale Supérieure (Paris). All solvents were dried and distilled by standard techniques. Thin layer chromatography was carried out on silica gel plates (Macherey–Nagel); spots were detected with UV light and revealed with a 10% H$_2$SO$_4$ solution in EtOH. Flash chromatography was performed with silica gel 60 (40–63 μm).

***N*-[(1,1-Dimethylethoxy)carbonyl]-4-iodo-L-phenylalanine:** Iodine (5.24 g, 20.64 mmol) and sodium iodate (2.04 g, 10.32 mmol) were added to a solution of L-phenylalanine (8.52 g, 51.6 mmol) in acetic acid (47 mL) and concentrated sulfuric acid (6.2 mL). The mixture was heated at 70 °C and stirred vigorously during 20 h. Sodium periodate (2 × 0.2 g) was then added. The reaction was complete when the solution became orange. Acetic acid was removed under reduced pressure and the crude mixture was diluted with H$_2$O (80 mL) and washed with Et$_2$O (2 × 25 mL) and CH$_2$Cl$_2$ (2 × 25 mL). The aqueous layer was decolored with Norit (1.1 g), then filtered and basified to pH 5 with aqueous concentrated NaOH. The precipitate was filtered under vacuum and washed with H$_2$O (170 mL) and ethanol (65 mL) to afford 13.09 g (87%) of 4-iodophenylalanine as a white solid (m.p. 255–265 °C; ref.[12] m.p. 261–262 °C). Di-*tert*-butyl dicarbonate (11.8 g, 54 mmol) was added to a solution of this crude compound (13.09 g, 45 mmol) in dioxane (45 mL) and 1 M NaOH (90 mL), at 0 °C. The mixture was warmed to room temperature and stirred overnight. The pH was controlled and adjusted to pH 9. After stirring for a further 3 h, the solvent was evaporated and the residue was diluted with H$_2$O and washed with Et$_2$O. The aqueous layer was acidified to pH 2–3 with 2 M HCl and extracted with EtOAc. The combined organic

phases were washed with brine, dried with $MgSO_4$, and then concentrated to afford 15.94 g (90%) of the carbamate derivative as a white solid. This compound was used in the next step without purification.

***N*-[(1,1-Dimethylethoxy)carbonyl]-4-iodo-L-phenylalanine 1,1-Dimethylethyl Ester (3b):** *tert*-Butyl bromide (65.77 g, 480 mmol) was added dropwise, at room temperature, to a solution of *N*-[(1,1-dimethylethoxy)carbonyl]-4-iodo-L-phenylalanine (3.91 g, 10 mmol) in *N*,*N*-dimethylacetamide (75 mL) in the presence of benzyltriethylammonium chloride (2.28 g, 10 mmol) and anhydrous potassium carbonate (35.93 g, 260 mmol). The mixture was stirred at 55 °C for 2 h (TLC monitoring). After cooling, cold water (1000 mL) was added to the reaction mixture, which was extracted with EtOAc. The combined extracts were washed (H_2O, brine), dried (Na_2SO_4), and concentrated. The crude product was purified through silica gel (0%→20% EtOAc/cyclohexane) to afford 4.16 g (93%) of **3b** as a white solid. M.p. 59–61 °C. R_f = 0.4 (EtOAc/cyclohexane, 1:9). $[\alpha]_D^{25}$ = +36.5 (*c* = 1, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 2978 cm^{-1}, 2930, 1745 (C=O, ester), 1697 (NHCOO, carbamate). ^1H NMR (250 MHz, $CDCl_3$): δ = 7.60 (d, *J* = 8.3 Hz, 2 H, $H_{arom.}$), 6.91 (d, *J* = 8.3 Hz, 2 H, $H_{arom.}$), 5.00 (br. d, *J* = 8.0 Hz,1 H, N*H*), 4.44–4.38 (m, 1 H, C*H*), 3.10–2.94 (m, 2 H, CH_2), 1.43–1.35 (m, 18 H, 6 CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.52 (*C*O), 154.88 (*C*O), 137.28 ($C_{arom.}$), 136.04 ($C_{quat.}$ arom.), 131.47 ($C_{arom.}$), 92.11 ($C_{quat.}$ arom.), 82.13, 79.63 ($C_{quat.}$ *t*Bu), 54.50 (*C*H), 37.90 (CH_2) 28.20, 27.86 (CH_3) ppm. $C_{18}H_{26}INO_4$ (447.31): calcd. C 48.33, H 5.86, N 3.13; found C 47.93, H 5.83, N 3.01.

Cross-Coupling Reaction: *t*BuLi (1.5 M in pentane solution, 8 mL) was slowly added to a solution of protected glucal **2** (2.66 g, 3 mmol) in freshly distilled THF (30 mL), in a Schlenk flask, and stirred at –78 °C. The solution was then stirred for 30 min at 0 °C. In another Schlenk tube, $ZnCl_2$ (2.25 g, 16.5 mmol) was heated under vacuum for 15 min, then THF (41 mL) was added. This second solution was transferred into the first one via a cannula at 0 °C and stirred for 1 h at room temperature. In another Schlenk tube, a solution of the catalyst was prepared by dissolving $[Pd_2(dba)_3]\cdot CHCl_3$ (154 mg, 0.15 mmol) and tri-(*o*-tolyl)phosphane (228 mg, 0.75 mmol) in THF (15 mL). A solution of the 4-iodophenylanine derivative **3b** (1.34 g, 3 mmol) in THF (15 mL) was slowly added at room temperature. Finally, the solution of organozinc compound was transferred via cannula into the solution containing the catalyst and the aryl iodide. The mixture was stirred overnight at room temperature. The reaction mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. Purification by flash chromatography (0%→10% EtOAc/petroleum ether) provided 2.06 g (90%) of **4b** as a colorless foam. R_f = 0.42 (EtOAc/cyclohexane, 1:9). $[\alpha]_D^{25}$ = +2.1 (*c* = 1, $CHCl_3$). IR (ATR): $\tilde{\nu}$ = 2934, 2863, 1715, 1652, 1496, 1472, 1391, 1365, 1281, 1248, 1154, 1109, 1059, 1020, 890 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ = 7.42 (d, *J* = 8.2 Hz, 2 H, $H_{arom.}$), 7.12 (d, *J* = 8.2 Hz, 2 H, $H_{arom.}$), 5.19 (d, $J_{2,3}$ = 2.3 Hz, 1 H, 2-H), 4.96 (br. d, *J* = 8.0 Hz, 1 H, N*H*), 4.57 (dd, $J_{3,4}$ = 6.5 Hz, 1 H, 3-H), 4.48–4.40 (m, 1 H, C*H*NH), 4.31 (dd, $J_{6eq,6ax}$ = 10.1, $J_{5,6eq}$ = 4.9 Hz, 1 H, 6-H_{eq}), 4.17–4.04 (m, 2 H, 4-H and 6-H_{ax}), 3.96 (ddd, $J_{4,5}$, $J_{5,6ax}$ = 10.2 Hz, 1 H, 5-H), 3.12–2.90 (m, 2 H, CH_2), 1.53–1.30 [m, 18 H, CH_3 (Boc)], 1.25–0.90 [m, 39 H, CH_3 (C–Si)] ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 170.82 (CO), 155.05 (CO), 150.81 (C-1), 136.95, 132.77 ($C_{quat.}$ arom.), 129.36, 124.96 ($CH_{arom.}$), 101.00 (C-2), 82.08, 79.64 ($C_{quat.}$ *t*Bu), 77.41 (C-4), 72.93 (C-5), 70.72 (C-3), 66.08 (C-6), 54.66 (*C*HNH), 38.12 (CH_2) 28.28, 27.95, 27.43, 26.92 (CH_3 *t*Bu), 22.73, 19.85 ($C_{quat.}$ *t*BuSi), 18.16, 17.68 (CH_3 *i*Pr), 12.47 (CH *i*Pr) ppm. $C_{41}H_{71}NO_8Si_2$ (762.18): calcd. C 64.61, H 9.39, N 1.84; found C 64.13, H 9.59, N 1.45.

Along with compound **4b**, flash chromatography also provided 0.133 g (5%) of **5** as a white solid. M.p. 276–278 °C. $[\alpha]_D^{25}$ = –37.9 (*c* = 1, $CHCl_3$). R_f = 0.63 (EtOAc/cyclohexane, 5:95). IR (ATR): $\tilde{\nu}$ = 2935, 2863, 1632, 1467, 1388, 1270, 1165, 1107, 1061, 1013, 878 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ = 5.11 (dd, $J_{2,3}$ = 2.0 Hz, 2 H, 2-H), 4.45 (dd, $J_{3,4}$ = 6.9, $J_{3,1}$ = 2.0 Hz, 2 H, 3-H), 4.20 (dd, $J_{6eq,6ax}$ = 10.2, $J_{5,6eq}$ = 4.9 Hz, 2 H, 6-H_{eq}), 4.00 (dd, $J_{4,5}$ = 10.2, $J_{4,3}$ = 6.9 Hz, 2 H, 4-H), 3.97 (t, $J_{5,6ax}$ = 10.2 Hz, 2 H, 6-H_{ax}), 3.82 (ddd, $J_{4,5}$ = 10.2, $J_{5,6ax}$ = 10.2, $J_{5,6eq}$ = 4.9 Hz, 2 H, 5-H), 1.18–0.80 (m, 78 H, CH_3) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 142.19 (C-1), 102.72 (C-2), 77.32 (C-4), 72.70 (C-5), 71.14 (C-3), 65.93 (C-6), 27.42, 26.91 (CH_3 *t*Bu), 22.73, 19.84 ($C_{quat.}$ *t*Bu), 18.09, 18.05 (CH_3 *i*Pr), 12.41 (CH *i*Pr) ppm. HRMS (CI) calcd. for $C_{46}H_{90}O_8Si_4$ $[M]^+$: 883.5791; found 883.5786.

***N*-[(1,1-Dimethylethoxy)carbonyl]-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-phenylalanine 1,1-Dimethylethyl Ester (6):** $BH_3\cdot$THF (1 M in THF solution, 6 mL, 12 equiv.) was added to a solution of C-glycoside intermediate **8** (0.296 g, 0.5 mmol) in freshly distilled THF (5 mL) at 0 °C. The mixture was stirred for 4 h at 0 °C (TLC monitoring). NaOH (10 mL, 1 M, 10 mmol) and then hydrogen peroxide (2.1 mL, 35% in water, 25 mmol) were added dropwise, and the resulting solution was stirred for 30 min at 0 °C, then warmed to room temperature and stirred again for 2 h. The mixture was then extracted with EtOAc. The combined organic layers were washed with brine and dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The crude product was acetylated with pyridine (2.37 g, 30 mmol), acetic anhydride (3.06 g, 30 mmol), and DMAP (73.3 mg, 0.6 mmol) at room temperature and the mixture was stirred overnight. The solution was diluted with EtOAc and washed with HCl (0.1 M) and brine. The organic layer was dried (Na_2SO_4) and concentrated. Purification of the residue over silica gel (10%→40% EtOAc/cyclohexane) provided 0.215 g (66%) of **6** as a colorless foam. $[\alpha]_D^{25}$ = +5.1 (*c* = 1, $CHCl_3$), R_f = 0.36 (EtOAc/cyclohexane, 2:3). IR (ATR): $\tilde{\nu}$ = 2978, 1746, 1714, 1502, 1451, 1368, 1220, 1154, 1103, 1036, 919 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.26 (d, *J* = 8.2 Hz, 2 H, $CH_{arom.}$), 7.14 (d, *J* = 8.2 Hz, 2 H, $CH_{arom.}$), 5.32 (t, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 5.22 (t, $J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 5.12 (t, $J_{1,2}$ = 9.6 Hz, 1 H, 2-H), 4.94 (br. d, *J* = 8.3 Hz, 1 H, NH), 4.46–4.39 (m, 1 H, C*H*NH), 4.37 (d, $J_{1,2}$ = 9.6 Hz, 1 H, 1-H), 4.28 (dd, $J_{6,6'}$ = 12.3, $J_{5,6}$ = 4.5 Hz, 1 H, 6-H), 4.14 (dd, $J_{5,6'}$ = 2.3 Hz, 1 H, 6′-H), 3.82 (ddd, $J_{4,5}$ = 9.6, $J_{5,6}$ = 4.5, $J_{5,6'}$ = 2.3 Hz, 1 H, 5-H), 3.07–2.98 (m, 2 H, CH_2), 2.08 (s, 3 H, $CH_{3\ Ac}$), 2.05 (s, 3 H, $CH_{3\ Ac}$), 1.98 (s, 3 H, $CH_{3\ Ac}$), 1.78 (s, 3 H, $CH_{3\ Ac}$), 1.42 (s, 9 H, $CH_{3\ Boc}$), 1.40 (s, 9 H, $CH_{3\ Boc}$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.77, 170.76, 170.42, 169.52, 168.81 (CO), 155.04 (CO), 137.19, 134.72 ($C_{quat.}$ arom.), 129.64, 127.19 ($CH_{arom.}$), 82.12 ($C_{quat.}$ *t*Bu), 80.02 (C-1), 79.69 ($C_{quat.}$ *t*Bu), 76.08 (C-3), 74.23 (C-5), 72.48 (C-2), 68.48 (C-4), 62.30 (C-6), 54.53 (*C*HNH), 37.91 (CH_2), 28.28, 27.91 (CH_3 *t*Bu), 20.79, 20.67, 20.38 (CH_3 Ac) ppm. $C_{32}H_{45}NO_{13}$ (651.70): calcd. C 58.98, H 6.96; found C 58.50, H 7.15.

***N*-[(1,1-Dimethylethoxy)carbonyl]-4-(1,2-dideoxy-D-*arabino*-hexenopyranosyl)-L-phenylalanine 1,1-Dimethylethyl Ester (7):** A solution of TBAF·$3H_2O$ (4.58 g, 14.51 mmol) in THF (10 mL) was added dropwise, with a cannula, to a solution of compound **4b** (1.58 g, 2.073 mmol) in THF (11 mL) at 0 °C. The mixture was stirred for 2 h at 0 °C and then it was warmed to room temperature and stirred for 20 h. The mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed (brine) and dried (Na_2SO_4), and the solvent was evaporated under

reduced pressure. Purification of the residue over silica gel (30:70→100% EtOAc/cyclohexane) provided 0.83 g (86%) of **7** as a white solid. M.p. 138–140 °C. $[\alpha]_D^{25}$ = +28.9 (*c* = 1, EtOH), R_f = 0.24 (pure EtOAc). IR (ATR): $\tilde{\nu}$ = 3422, 3367, 2971, 2925, 1731, 1707, 1664, 1517, 1252, 1172, 1070, 1049, 1022, 1011, 986 cm^{-1}. ^1H NMR (250 MHz, MeOD): δ = 7.62 (d, *J* = 8.2 Hz, 2 H, $H_{arom.}$), 7.23 (d, *J* = 8.2 Hz, 2 H, $H_{arom.}$), 5.32 (d, $J_{2,3}$ = 2.7 Hz, 1 H, 2-H), 4.32 (dd, $J_{3,4}$ = 5.3 Hz, 1 H, 3-H), 4.26 (br. dd, *J* = 8.4, *J* = 6.3 Hz, 1 H, C*H*NH), 4.08–3.85 (m, 3 H, 5-H, 6-H), 3.70 (br. t, $J_{4,5}$ = 8.4 Hz, 1 H, 4-H), 3.07 (dd, *J* = 13.8 Hz, 1 H, CH_2), 2.93 (dd, *J* = 13.8 Hz, 1 H, CH_2), 1.45–1.33 (m, 18 H, CH_3 Boc) ppm. ^{13}C NMR (62.5 MHz, MeOD): δ = 173.73 (CO), 158.59 (CO), 154.02 (C-1), 139.84, 135.31 ($C_{quat.}$ arom.), 131.03, 127.04 ($CH_{arom.}$), 100.97 (C-2), 83.62 ($C_{quat.}$ *t*Bu), 81.70 (C-5), 81.37 ($C_{quat.}$ *t*Bu), 72.41 (C-3), 71.85 (C-4), 63.24 (C-6), 57.89 (*C*HNH), 39.35 (CH_2), 29.54, 29.07 (CH_3 *t*Bu) ppm. $C_{24}H_{35}NO_8$ (465.54): calcd. C 61.92, H 7.58, N 3.01; found C 61.81, H 7.67, N 3.03.

***N*-[(1,1-Dimethylethoxy)carbonyl]-4-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hexenopyranosyl)-L-phenylalanine 1,1-Dimethylethyl Ester (8):** A solution of TBAF·3H_2O (1.10 g, 3.5 mmol) in THF (3 mL) was added dropwise, with a cannula, to a solution of compound **4b** (0.381 g, 0.5 mmol) in THF (4 mL) at 0 °C. The mixture was stirred for 2 h at 0 °C and then it was warmed to room temperature and stirred for 20 h. The mixture was quenched with water and extracted with EtOAc, then the solvent was dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was acetylated under standard conditions [pyridine (2.37 g, 30 mmol), acetic anhydride (3.06 g, 30 mmol), DMAP (73.3 mg, 0.6 mmol), room temperature, overnight stirring]. The solution was diluted with EtOAc and washed with 0.1 M HCl and brine. The organic layer was dried (Na_2SO_4) and concentrated. Chromatography of the residue (0%→60% EtOAc/cyclohexane) afforded 0.24 g (81% over two steps) of **8** as a colorless foam. $[\alpha]_D^{25}$ = –17.5 (*c* = 1, $CHCl_3$), R_f = 0.55 (EtOAc/cyclohexane, 1:1). IR (ATR): $\tilde{\nu}$ = 3398, 2977, 2933, 1734, 1693, 1657, 1524, 1448, 1367, 1220, 1157, 1096, 1035, 963 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ = 7.49 (d, *J* = 8.2 Hz, 2 H, $CH_{arom.}$), 7.14 (d, *J* = 8.2 Hz, 2 H, $CH_{arom.}$), 5.49 (dd, $J_{3,4}$ = 5.3, $J_{2,3}$ = 3.7 Hz, 1 H, 3-H), 5.35 (d, *J* = 3.7 Hz, 1 H, 2-H), 5.28 (dd, $J_{4,5}$ = 7.2 Hz, 1 H, 4-H), 4.96 (br. d, *J* = 8.0 Hz, 1 H, N*H*), 4.48–4.26 (m, 4 H, C*H*NH, 5-H, 6-H), 3.07–3.01 (m, 2 H, CH_2), 2.14–2.00 (m, 9 H, CH_3 Ac), 1.42–1.31 (m, 18 H, CH_3 Boc) ppm. ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 170.63, 170.53, 170.45, 169.59 (CO), 154.94 (CO), 153.34 (C-1), 137.80, 132.04 ($C_{quat.}$ arom.), 129.43, 125.15 ($CH_{arom.}$), 94.07 (C-2), 82.08, 79.60 ($C_{quat.}$ *t*Bu), 74.32 (C-5), 68.60 (C-3), 67.31 (C-4), 61.32 (C-6), 54.60 (*C*HNH), 38.02 (CH_2), 28.21, 27.90 (CH_3 *t*Bu), 21.00, 20.76, 20.67 (CH_3 Ac) ppm. $C_{30}H_{41}NO_{11}$ (591.65): calcd. C 60.90, H 6.98, N 2.37; found C 61.12, H 7.05, N 2.34.

Dihydroxylation: Pyridine (0.5 mL), H_2O (1 mL), NMO (94 mg, 0.8 mmol), and OsO_4 (0.05 M solution in *t*BuOH, 0.8 mL, 0.2 equiv.) were added sequentially to a solution of glucal intermediate **8** (0.118 g, 0.2 mmol) in THF (2 mL) and *t*BuOH (2 mL). The mixture was heated to reflux for 3 h, cooled to room temperature, and quenched by addition of 10% $NaHSO_3$ (4 mL). The resulting mixture was stirred for 5 h and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), and the solvent was evaporated. The crude product was acetylated under standard conditions [pyridine (1.02 g, 10 mmol), acetic anhydride (0.79 g, 10 mmol), DMAP (12.2 mg, 0.1 mmol), room temperature, overnight stirring]. The solution was diluted with EtOAc and washed with HCl (0.1 M) and brine. The organic layer was dried (Na_2SO_4) and concentrated. Purification of the residue over silica gel (10%→50% EtOAc/cyclohexane) provided 0.04 g (28%) of compounds **9** and **9′** as a 77:23 mixture of diastereomers as a colorless oil. R_f = 0.28 (EtOAc/cyclohexane: 2:3). IR (ATR): $\tilde{\nu}$ = 2979, 1747, 1706, 1608, 1500, 1451, 1369, 1212, 1153, 1047, 960 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 8.2 Hz, 2 H, $CH_{arom.}$), 7.22 (d, *J* = 8.2 Hz, 2 H, $CH_{arom.}$), 6.05 (d, $J_{2,3}$ = 5.2 Hz, 0.77 H, 2-H for **9**), 5.98 (d, $J_{2,3}$ = 2.3 Hz, 0.23 H, 2-H for **9′**), 5.68 (dd, $J_{3,4}$ = 4.1 Hz, 0.77 H, 3-H for **9**), 5.61 (dd, $J_{3,4}$ = 8.5 Hz, 0.23 H, 3-H for **9′**), 5.46 (dd, $J_{4,5}$ = 7.1 Hz, 0.77 H, 4-H for **9**), 5.44 (dd, $J_{4,5}$ = 3.3 Hz, 0.23 H, 4-H for **9′**), 5.32–5.25 (m, 0.23 H, 5-H for **9′**), 5.14–5.08 (m, 0.77 H, 5-H for **9**), 5.15 (br. d, *J* = 7.6 Hz, 1 H, NH), 4.50–4.40 (m, 1 H, C*H*NH), 4.30 (d, $J_{6,6'}$ = 12.4, $J_{5,6'}$ = 3.2 Hz, 0.77 H, 6′-H for **9**), 4.29 (dd, $J_{6,6'}$ = 11.9, $J_{5,6'}$ = 3.9 Hz, 0.23 H, 6′-H for **9′**), 4.14 (dd, $J_{5,6}$ = 7.3 Hz, 0.23 H, 6-H for **9′**), 4.09 (dd, $J_{5,6}$ = 5.7 Hz, 0.77 H, 6-H for **9**), 3.18–2.98 (m, 2 H, CH_2), 2.15–1.90 (m, 15 H, CH_3 acet), 1.45–1.25 (m, 18 H, CH_3 Boc) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 192.92 (CO-1), 170.57, 170.41, 169.79, 169.65, 169.56, 169.48 (CO), 154.98 (CO), 143.18, 133.67 ($C_{quat.}$ arom.), 129.99, 128.47 ($CH_{arom.}$), 82.45 ($C_{quat.}$ *t*Bu), 79.88 ($C_{quat.}$ *t*Bu), 72.27 (C-2), 69.13 (C-4), 68.58 (C-5), 68.50 (C-3), 61.68 (C-6), 54.47 (*C*HNH), 38.46 (CH_2), 28.26, 27.91 (CH_3 *t*Bu), 20.74, 20.66, 20.52, 20.40, 20.32 (CH_3 Ac) ppm. HRMS (CI) calcd. for $C_{34}H_{53}N_2O_{15}$ $[M + NH_4]^+$: 727.3289; found 727.3285.

***N*-[(Fluoren-9-ylmethoxy)carbonyl]-4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-phenylalanine (1):** Trifluoroacetic acid (13.68 g, 120 mmol) was added dropwise, at 0 °C, under argon, to a solution of compound **6** (0.782 g, 1.2 mmol) in distilled dichloromethane (12 mL). The mixture was warmed to room temperature and stirred for 5 h. Toluene (3×40 mL) was then added, and the solvent was removed in vacuo. The resultant foam was immediately dissolved in water (10 mL) and acetonitrile (10 mL) and stirred at room temperature. FmocOSu (0.486 g, 1.44 mmol) was then added, followed by the dropwise addition of triethylamine (0.36 g, 3.6 mmol) in order to maintain the pH equal to 9. The solution was stirred for 3 h at room temperature. The mixture was then acidified to pH 2–3 with 1 M HCl. The aqueous layer was extracted with EtOAc (3×20 mL). The combined extracts were washed with brine and dried (Na_2SO_4) and the solvent was evaporated. Chromatography of the residue (0%→10% MeOH/$CHCl_3$) afforded **1** (0.760 g, 88% over two steps) as a white solid. M.p. 126–128 °C. R_f = 0.2 (MeOH/$CHCl_3$, 1:9). $[\alpha]_D^{25}$ = +4.1 (*c* = 1, $CHCl_3$). IR (ATR): $\tilde{\nu}$ = 2927, 1744, 1517, 1370, 1220, 1038 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 7.3 Hz, 2 H, $CH_{arom.}$ Fmoc), 7.55 (d, *J* = 7.3 Hz, 2 H, $CH_{arom.}$ Fmoc), 7.38 (t, *J* = 7.3 Hz, 2 H, $CH_{arom.}$ Fmoc), 7.29 (d, *J* = 7.8 Hz, 2 H, $CH_{arom.}$ Fmoc), 7.23 (d, *J* = 7.8 Hz, 2 H, $CH_{arom.}$), 7.10 (d, *J* = 7.8 Hz, 2 H, $CH_{arom.}$), 5.4 (d, *J* = 8.2 Hz, 1 H, NH), 5.32 (t, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1 H, 3-H), 5.22 (t, $J_{4,5}$ = 9.6 Hz, 1 H, 4-H), 5.10 (t, $J_{2,1}$ = 9.6 Hz, 1 H, 2-H), 4.70–4.63 (m, 1 H, C*H*NH), 4.42 (dd, *J* = 10.1, *J′* = 6.9 Hz, 2 H, CH_2 Fmoc), 4.29 (d, $J_{1,2}$ = 9.6 Hz, 1 H, 1-H), 4.27 (dd, $J_{6,6'}$ = 12.8, $J_{5,6}$ = 4.6 Hz, 1 H, 6-H), 4.18 (d, *J* = 6.9 Hz, 1 H, CH Fmoc), 4.12 (t, $J_{6',6}$ = $J_{6',5}$ = 12.8 Hz, 1 H, 6′-H), 3.82–3.72 (m, 1 H, 5-H), 3.16 (dd, *J* = 13.8, *J* = 5.1 Hz, 1 H, CH_2), 3.05 (dd, *J* = 13.8, *J* = 6.5 Hz, H, CH_2), 2.07–1.97 (m, 9 H, CH_3 Ac), 1.75 (s, 3 H, CH_3 Ac) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.64 (CO acid), 171.13, 170.65, 169.84, 169.36 (CO Ac), 155.89 (CO Fmoc), 143.87, 143.76, 141.38 ($C_{quat.}$ arom. Fmoc), 136.75, 135.08 ($C_{quat.}$ arom.), 129.66 ($CH_{arom.}$), 127.86, 127.56, 127.19($CH_{arom.}$ Fmoc), 127.19 ($CH_{arom.}$), 125.25, 125.15, 120.12 ($CH_{arom.}$ Fmoc), 80.04 (C-1), 76.17 (C-5), 74.24 (C-3), 72.71 (C-2), 68.61 (C-4), 67.08 (CH_2 Fmoc), 62.44 (C-6), 54.57 (*C*HNH), 47.19 (CH Fmoc), 37.62 (CH_2), 20.91, 20.80, 20.46 (CH_3 Ac) ppm. $C_{38}H_{39}NO_{13}$ (717.72): HRMS (CI) calcd. for $C_{38}H_{43}N_2O_{13}$ $[M + NH_4]^+$: 735.2765; found 735.2759.

[1] a) A. Varki, *Glycobiology* **1993**, *3*, 97–130; b) R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683–720; c) C. M. Taylor, *Tetrahedron* **1998**, *54*, 11317–11362.

[2] D. H. Dube, C. R. Bertozzi, *Nat. Rev. Drug Disc.* **2005**, *4*, 477–488.

[3] a) S. A. Ahmad, T. L. Hopkins, K. J. Kramer, *Insect Biochem. Mol. Biol.* **1996**, *26*, 49–57; b) A. M. Jansson, K. Jensen, M. Meldal, J. Lomako, W. M. Lomako, C. E. Olsen, K. Bock, *J. Chem. Soc., Perkin Trans. 1* **1996**, 1001–1006.

[4] a) H. He, R. T. Williamson, B. Shen, E. I. Graziani, H. Y. Yang, S. M. Sakya, P. J. Petersen, G. T. Carter, *J. Am. Chem. Soc.* **2002**, *124*, 9729–9736; b) M. H. D. Postema, *Tetrahedron* **1992**, *48*, 8545–8599.

[5] A. Dondoni, A. Marra, *Chem. Rev.* **2000**, *100*, 4395–4421.

[6] a) A. J. Pearce, S. Ramaya, S. N. Thorn, G. B. Bloomberg, D. S. Walter, T. Gallagher, *J. Org. Chem.* **1999**, *64*, 5453–5462; b) E. Brenna, C. Fuganti, P. Grasselli, S. Serra, S. Zambotti, *Chem. Eur. J.* **2002**, *8*, 1872–1878.

[7] X. Xu, G. Fakha, D. Sinou, *Tetrahedron* **2002**, *58*, 7539–7544.

[8] a) V. Jeanneret, L. Meerpoel, P. Vogel, *Tetrahedron Lett.* **1997**, *38*, 543–546; b) J. O. Hoberg, *Carbohydr. Res.* **1997**, *300*, 365–367.

[9] a) P. Lesimple, J.-M. Beau, G. Jaurand, P. Sinaÿ, *Tetrahedron Lett.* **1986**, *27*, 6201–6204; b) R. Friesen, R. W. Loo, *J. Org. Chem.* **1991**, *56*, 4821–4823.

[10] B. K. Shull, Z. Wu, M. Koreeda, *J. Carbohydr. Chem.* **1996**, *15*, 955–964.

[11] K. A. Parker, A. T. Georges, *Org. Lett.* **2000**, *2*, 497–499.

[12] H. Lei, M. S. Stoakes, K. P. B. Herath, J. Lee, A. W. Schwabacher, *J. Org. Chem.* **1994**, *59*, 4206–4210.

[13] P. Braun, H. Waldmann, W. Vogt, H. Kunz, *Liebigs Ann. Chem.* **1991**, 165–170.

[14] V. Boucard, K. Larrieu, N. Lubin-Germain, J. Uziel, J. Augé, *Synlett* **2003**, *12*, 1834–1837.

[15] M. H. D. Postema, J. L. Piper, R. L. Betta, *J. Org. Chem.* **2005**, *70*, 829–836.

[16] M. H. D. Postema, D. Calimente, L. Liu, T. L. Behrmann, *J. Org. Chem.* **2000**, *65*, 6061–6068.

Received: September 9, 2005
Published Online: December 20, 2005

