Stereoselective synthesis of the *C*-analogue of β-D-glucopyranosyl serine

Luigi Lay,[a] Morten Meldal,[b] Francesco Nicotra,[a] Luigi Panza*[a] and Giovanni Russo[a]

[a] *Dipartimento di Chimica Organica e Industriale, Centro per lo Studio delle Sostanze Organiche Naturali del CNR, Università di Milano, via Venezian 21, I-20133 Milano, Italy*
[b] *Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*

The [2,3]-Wittig sigmatropic rearrangement of an appropriate glucose derivative followed by further chemical modification afforded stereoselectively the *C*-analogue of β-D-glucopyranosyl serine.

Protein glycosylation plays fundamental roles in inter- and intra-cellular recognition.[1] Thus, *N*-linked protein glycosylation is involved in protein sorting and the ability of the immune system to recognize and discriminate *O*-linked tumour associated glycoprotein antigens of malignant tissue as foreign structures has been well documented.[1] Furthermore, *O*-glycosylation of a peptide or a protein can increase their solubility and their resistance towards enzymatic degradation, can modify the conformation of the peptide backbone and modulate the biological activity. These properties have recently stimulated great interest in glycopeptides as therapeutic agents.[2]

One limitation of the use of these compounds as potential drugs is the sensitivity of the glycosidic linkage between the sugar and the peptide towards both chemical and enzymatic deglycosylation.[1] For these reasons it is of great interest to have access to stable analogues of *O*-glycosylated amino acids, such as *C*-glycosyl amino acids, in which the glycosidic oxygen atom can be substituted with a methylene group or omitted to give nor analogues. Few examples of the synthesis of *C*-glycosyl amino acids as well as their incorporation in biologically active peptides appear in the literature.[3] However, such compounds are not easily obtainable, particularly by direct carbon–carbon bond forming reactions between a sugar and an amino acid,[4] and have usually been prepared as diastereoisomeric mixtures at the α-carbon of the amino acid moiety.[3]

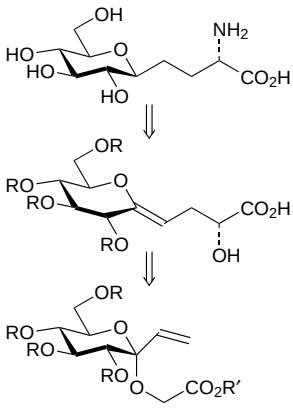
Here we describe the synthesis of the *C*-analogue of glucopyranosyl serine using a [2,3]-Wittig sigmatropic rearrangement[5] in which the chirality of the sugar induces the stereoselective formation of the amino acid moiety, according to the retrosynthetic pathway in Scheme 1.

The appropriate precursor **2**† for Wittig rearrangement was prepared in 70% yield (Scheme 2) from known compound **1**[5] by treatment with methyl glycolate (5 equiv.) and $BF_3{\cdot}Et_2O$ (1.5 equiv.) in the presence of 4 Å molecular sieves.

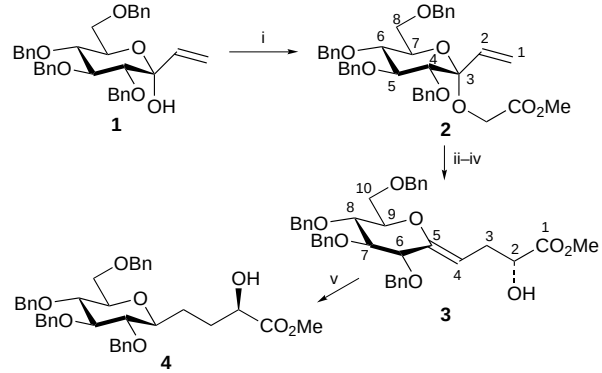
Since preliminary attempts to effect silyl trifluoromethanesulfonate-mediated Wittig rearrangement[6] on **2** were unsuccessful, the dianionic [2,3]-Wittig rearrangement[7] was considered. Compound **2** was then hydrolysed with 2% NaOH in MeOH. The carboxylic acid obtained was treated with excess LDA (3 equiv.) in THF at −78 °C and the reaction mixture was allowed to warm to 0 °C. The crude product was then treated wih CH_2N_2 in Et_2O–MeOH and the α-hydroxy ester **3** was isolated in 70% yield from **2** (de > 95%). The geometry of the newly formed double bond was shown to be *Z* by an NOE experiment.‡ Compound **3** was hydrogenated with freshly prepared Raney-Ni in EtOH to give **4** in 80% yield. The absolute configuration of the stereocentre at C-2 was determined converting **4** into the (*R*)- and (*S*)-MTPA esters by treatment with (*R*)- and (*S*)-MTPA chloride, respectively, in CH_2Cl_2–pyridine. The ^{1}H NMR spectrum of the MTPA esters showed a $\Delta\delta$ value ($\delta S - \delta R$) of +0.1 ppm for the signals of the hydrogen atoms at C-3 and C-4, allowing the assigment of the absolute configuration *R* at C-2.[8]

Moreover, the chemical shift of the hydrogen atom at C-5 of **4**, which resonates at δ 3.27, suggests the presence of an equatorial substituent which was confirmed subsequently on compound **8**.

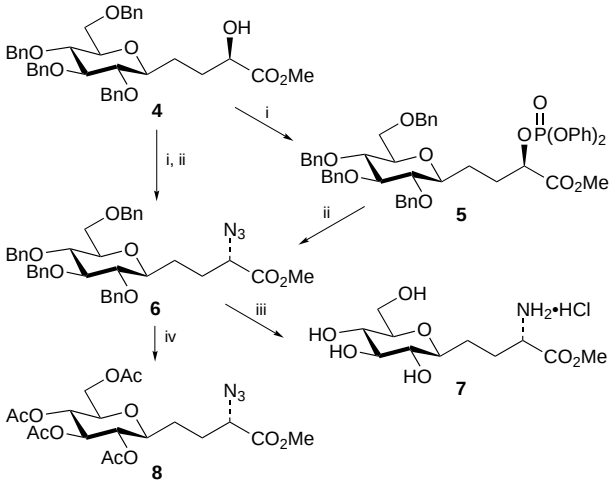
The substitution of the hydroxy group with a nitrogen was attempted using diphenylphosphoryl azide (DPPA),[9] which should have allowed the direct conversion of an α-hydroxy ester to the corresponding azide with inversion of configuration. When compound **4** was treated with DPPA (2.5 equiv.) and DBU (0.98 equiv.) in toluene at 20 °C, only the intermediate phosphate **5** was isolated in good yield (Scheme 3). Moreover, when the reaction mixture was heated, the substitution reaction with the azide was incomplete even at reflux (110 °C) and gave many byproducts. However, treatment of the phosphate with



Scheme 1



Scheme 2 *Reagents and conditions*: i, $HOCH_2CO_2Me$, $BF_3{\cdot}Et_2O$, 20 °C, 70%; ii, NaOH, MeOH, room temp.; iii, LDA, THF, −78 → 0 °C; iv, CH_2N_2, Et_2O–MeOH, 70% from **2**; v, H_2, Raney-Ni, MeOH, 80%



Scheme 3 *Reagents and conditions*: i, DPPA, DBU, toluene; ii, Bu_4NN_3, toluene; iii, H_2, $Pd(OH)_2$, EtOH, quant.; iv, Ac_2O, $Me_3SiOSO_2CF_3$, 67%

Bu_4NN_3 in toluene gave the desired substitution product **6** in 88% yield. The reaction conditions were then modified as follows to obtain a one-pot transformation: the phosphate ester was formed as described above, but with heating to 55 °C. Then a solution of Bu_4NN_3[10] (3 equiv., 0.2 M solution in toluene) was added and the mixture was stirred at the same temperature until the phosphate ester disappeared on TLC: in this way the desired azido ester **6** was obtained in 92% yield.§

Reduction of the azido group and contemporary hydrogenolysis of the benzyl ethers was carried out with $Pd(OH)_2$ in EtOH containing few drops of 5% aq. HCl to finally afford the deprotected compound **7** in an almost quantitative yield.

In order to incorporate the obtained analogue into glycopeptides using either solution or solid phase synthesis, it would be useful to maintain the azido group[11] and to change the protecting groups on the sugar moiety. The benzylated analogue **6** was thus submitted to acetolysis[12] by treatment with trimethylsilyl trifluoromethanesulfonate in Ac_2O at room temp. for 40 h to give the acetylated derivative **8** in 67% yield.

The ^{1}H NMR spectrum of compound **8** showed a signal at δ 3.42 due to the hydrogen atom at C-5 in which the coupling constant with the adjacent ring hydrogen at C-6 (*J* 9.5 Hz) clearly confirmed that the equatorial configuration had been obtained at position 5, corresponding to the β-anomer of the *O*-linked analogue.

The reported procedure allows a simple and stereoselective access to the *C*-linked analogue of glucopyranosyl serine which can be incorporated in synthetic peptides. As the sugar moiety is not directly involved into chemical modifications, the procedure could be, in principle, applicable to other monosaccharides. Work is in progress to extend the scope of the procedure.

Part of the present work was supported by the EU-Carenet 2 program (ERB4061PL95-0372).

Footnotes

* E-mail: panza@imiucca.csi.unimi.it

† All new compounds gave satisfactory elemental analysis and specroscopic data. *Selected data*: $[\alpha]_D$: **2**, 25.3 (*c* 0.8, $CHCl_3$); **3**: 45.6 (*c* 1.0, $CHCl_3$); **4**, 4.5 (*c* 0.9, $CHCl_3$); **5**, 5.4 (*c* 1.0, $CHCl_3$); **6**, 8.9 (*c* 1.0, $CHCl_3$); **7**, −8.1 (*c* 1.0, MeOH); **8**, 16.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): **2**, 5.96 (dd, 1 H, *J* 17.5, 10.8 Hz, H-2), 5.60 (dd, 1 H, *J* 17.5, 1.7 Hz, H-1a), 5.30 (dd, 1 H, *J* 10.8, 1.7 Hz, H-1b), 4.22 (t, 1 H, *J* 9.5 Hz, H-5), 4.10 and 4.02 (2 d, 2 H, *J* 16.1 Hz, C*H*$_2$CO$_2$Me), 3.72 (s, 3 H, Me), 3.42 (d, 1 H, *J* 9.5 Hz, H-4); **3**, 4.94 (t, 1 H, *J* 7.4 Hz, H-4), 4.26 (br t, 1 H, *J* 5.1 Hz, H-2), 3.91 (d, 1 H, *J* 5.7 Hz, H-6), 3.71 (s, 3 H, Me), 2.94 (br s, 1 H, OH), 2.67–2.58 (m, 2 H, H-3); **4**, 4.18 (dd, 1 H, *J* 7.5, 4.4 Hz, H-2), 3.72 (s, 3 H, Me), 3.27 (m, 1 H, H-5), 3.04 (br s, 1 H, OH), 2.03 (m, 1 H, H-4a), 1.88 (m, 2 H, H-3), 1.57 (m, 1 H, H-4b); **5**, 5.04 (dt, 1 H, *J* 7.7, 4 Hz, H-2), 3.65 (s, 3 H, Me), 3.18 (m, 1 H, H-5), 2.15 (m, 1 H, H-3a), 1.93 (m, 2 H, H-3b and H-4a), 1.57 (m, 1 H, H-4b); **6**, 3.94 (dd, 1 H, *J* 8.8, 4.6 Hz, H-2), 3.74 (s, 3 H, Me), 3.26 (m, 1 H, H-5), 2.03 (m, 1 H, H-3a), 1.81 (m, 2 H, H-3b and H-4a), 1.56 (m, 1 H, H-4b); **7** (D_2O, 50 °C), 4.39 (t, 1 H, *J* 6.3 Hz, H-2), 4.02 (s, 3 H, Me), 3.48 (m, 1 H, H-5), 2.38 (m, 1 H, H-3a), 2.21 (m, 2 H, H-3b and H-4a), 1.73 (m, 1 H, H-4b); **8**, 5.14 (t, 1 H, *J* 9.5 Hz, H-7), 5.01 (t, 1 H, *J* 9.5 Hz, H-8), 4.85 (t, 1 H, *J* 9.5 Hz, H-6), 4.18 (dd, 1 H, *J* 12.4, 5.2 Hz, H-10a), 4.08 (dd, 1 H, *J* 12.4, 2.3 Hz, H-10b), 3.89 (dd, 1 H, *J* 8.8, 4.5 Hz, H-2), 3.77 (s, 3 H, Me), 3.60 (ddd, 1 H, *J* 9.5, 5.2, 2.3 Hz, H-9), 3.42 (dt, 1 H, *J* 9.5, 2.4 Hz, H-5), 2.1–1.9 (m, 13 H, H-3a and 4 Ac), 1.8–1.6 (m, 2 H, H-3b and H-4a), 1.57 (m, 1 H, H-4b).

‡ By irradiation of the vinylic hydrogen, an NOE of 4.5% was observed on the hydrogen atom at C-6 for compound **3**.

§ ^{1}H and ^{13}C NMR spectra of compounds **6**-**8** each showed a single set of peaks indicating the formation of a single diastereoisomer.

References

1 H. Lis and N. Sharon, *Eur. J. Biochem.*, 1993, **218**, 1; R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683; S.-I., Hakmori, *Adv. Cancer Res.*, 1989, **52,** 257; G. W. Hart, K. D. Greis, D. L.-Y. Dong, M. A. Blomberg, T.-Y. Chou, M.-S. Jiang, E. P. Roquemore, D. M. Snow, L. K. Kreppel, R. N. Cole, F. I. Comer, C. S. Arnold and B. K. Hayes, in *Glycoimmunology*, ed. A. Alavi and J. S. Axford, Plenum Press, New York, 1995, pp. 115–123.

2 J. F. Fisher, A. W. Harrison, G. L. Bundy, K. F. Wilkinson, B. D. Rush and M. J. Ruwart, *J. Med. Chem.*, 1991, **34**, 3140; J. Kihlberg, J. Ahman, B. Walse, T. Drakenberg, A Nilsson, C. Söderberg-Ahlm, B. Bengtsson and H. Olsson, *J. Med. Chem.*, 1995, **38**, 161.

3 K. Michael, V. Wittmann, W. König, J. Sandow and H. Kessler, *Int. J. Pept. Protein Res.*, 1996, **48**, 59 and references cited therein.

4 B. J. Dorgan and R. F. W. Jackson, *Synlett*, 1996, 859.

5 A paper in which glucose is used in a Wittig rearrangement for the stereoselective synthesis of chiral prop-2-ynylic alcohols has recently been published: K. Tomooka, Y. Nakamura and T. Nakai, *Synlett*, 1995, 321.

6 K. Mikami, O. Takahashi, T. Tabei and T. Nakai, *Tetrahedron Lett.*, 1986, **27**, 4511.

7 K. Kakinuma and H.-Y. Li, *Tetrahedron Lett.*, 1989, **31**, 4157.

8 I. Ohtani, T. Kusumi, Y. Kashman and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.

9 A. S. Thompson, G. R. Humphrey, A. M. DeMarco, D. J. Mathre and E. J. J. Grabowski, *J. Org. Chem.*, 1993, **58**, 5886.

10 A. Brändström, B. Lamm and I. Palmertz, *Acta Chem. Scand. Ser. B*, 1974, **28**, 699.

11 M. Meldal, M. A. Juliano and A. M. Jansson, *Tetrahedron Lett.*, 1997, **38**, 2531.

12 J. Alzeer and A. Vasella, *Helv. Chim. Acta*, 1995, **78**, 177.

Received in Cambridge, UK, 6th May 1997; 7/03063K