Pergamon

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Tetrahedron Letters 44 (2003) 6413–6416

TETRAHEDRON LETTERS

Synthesis of a new conformationally constrained glycoamino acid building block

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Received 2 June 2003; revised 24 June 2003; accepted 26 June 2003

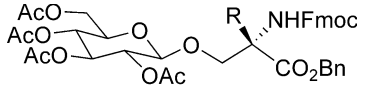
Abstract—The synthesis of a suitably protected β-D-glucopyranosyl-(*S*)-α-methylserine derivative—a new conformationally constrained glycosylated quaternary amino acid analogue of β-D-glucopyranosyl-L-serine—is described. This compound can be used as an attractive building block for the synthesis of new, constrained glycopeptides.

The synthesis of α,α-disubstituted α-amino acids has received enormous attention because it represents a great advance in the design of modified peptides with altered biological activity.[1,2] For example, α-methylserine can be regarded as a potential *C*-terminal α-helix-stabilising building block.[3]

On the other hand, a vast number of natural proteins contain oligosaccharide side chains—glycoproteins—and the saccharide residue is covalently linked to the peptide backbone. In the majority of cases this linkage is *O*-glycosidic through serine. It is well known that the saccharide moiety plays an important role in biological processes by contributing to the membrane permeability and stabilising certain secondary conformations of the peptide backbone.[4–7] Nevertheless, while significant research has been undertaken in the design of restricted peptides, the field of conformationally constrained glycopeptides remains relatively unexplored, and most of the restrictions studied are centered in the saccharide moiety.[8–11]

We have recently combined the aspects mentioned above and focused our attention on the synthesis of conformationally constrained glycosylated amino acids. Such compounds are building blocks for the construction of constrained glycopeptides, which can be regarded as potential tools in the elucidation of the conformational requirements for binding to receptors and enzymes. In this sense, a number of glycoamino acids that contain conformational constraints have been synthesised and the majority of unnatural glycosylated amino acids reported are *C*-glycoside derivatives,[12–15] including *C*-glycosylated α-amino acids that possess a quaternary center (Cα).[16–19] Examples of exclusively *O*-glycosylated quaternary α-amino acids have not been reported to date.

Taking into account this observation and the fact that various biologically active *O*-glycopeptides or *O*-glycoproteins incorporate the unusual β-D-glucopyranosyl-L-serine (β-D-Glc-L-Ser) substructure, we decided to synthesise a suitably protected β-D-glucopyranosyl-α-methylserine derivative β-D-Glc-(*S*)-α-MeSer (**1**) as a conformationally restricted analogue of β-D-Glc-L-Ser (**2**) (Fig. 1). The novel unnatural glycoamino acid **1** is an attractive building block for incorporation into peptides, since only a limited number of glycosylated amino acid building blocks suitable for glycopeptide synthesis are commercially available.



1: R = Me
2: R = H

Figure 1. Protected β-D-glucopyranosyl-(*S*)-α-methylserine [β-D-Glc-(*S*)-MeSer] (**1**) and β-D-glucopyranosyl-L-serine (β-D-Glc-L-Ser) (**2**).

Keywords: amino acids and derivatives; azides; glycosidation; peptide analogues/mimetics.
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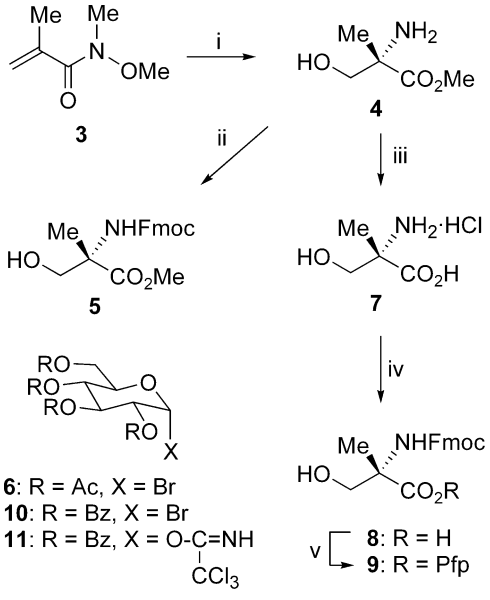
doi:10.1016/S0040-4039(03)01586-7

Several examples of *O*-glycopeptides or *O*-glycoproteins that incorporate the β-D-Glc-L-Ser linkage are present in the first epidermal growth factor-like domains,[20] certain synthetic enkephalin analogues[21] and the blood-clotting factors VII and IX (two plasma glycoproteins that are involved in the blood coagulation cascade).[22–24] In these proteins the serine residue bears either a disaccharide α-D-Xyl-1,3-β-D-Glc chain in factor VII or a trisaccharide α-D-Xyl-1,3-α-D-Xyl-1,3-β-D-Glc chain in factor IX. More specifically, factor IX consists of a single chain of 416 amino acids and the glycopeptide sequence containing the residues 51–56, including the trisaccharide, has been synthesised.[25]

It was planned to use the suitably protected α-methylserine **5** (*N*-Fmoc-MeSer-OMe) as the starting material for the glycosidation reaction with the donor 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[26] **6** using the Koenigs–Knorr methodology with silver triflate (AgOTf). Compound **5** was obtained from methyl α-methylserinate **4**, which was synthesised using our methodology involving the Sharpless asymmetric dihydroxylation of the Weinreb amide of 2-methyl-2-propenoic acid **3**, followed by several transformations (Scheme 1).[27] However, this approach proved unsuccessful and further attempts were made using more conveniently protected α-methylserine **9** (*N*-Fmoc-MeSer-OPfp). Compound **9** was synthesised from α-methylserine hydrochloride **7** by protection of the amino group with FmocCl, followed by formation of the Pfp-ester. However, glycosidation of *N*-Fmoc-Ser-OPfp **9** with the donors 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[28] **10** with AgOTf or 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[29] **11** with $BF_3{\cdot}Et_2O$ failed (Scheme 1).

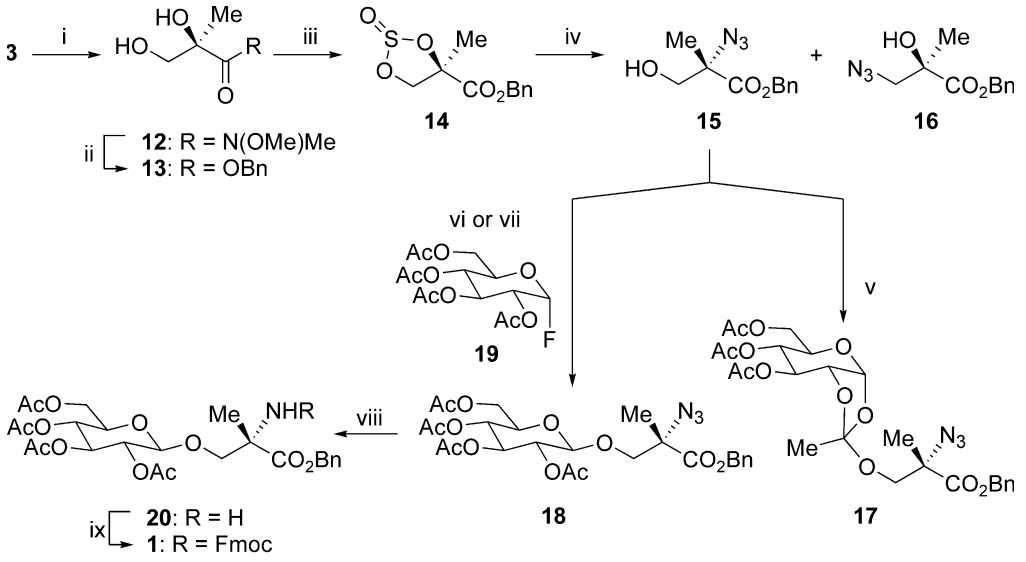


Scheme 1. *Reagents and conditions:* (i) Ref. 27: (a) AD-mix α, $MeSO_2NH_2$, $^tBuOH/H_2O$ (1:1), 0°C, 12 h, 81%; (b) $LiOH{\cdot}H_2O$, H_2O/MeOH (1:3), rt, 2 h; (c) AcCl, MeOH, reflux, 12 h, 85%; (d) $SOCl_2$, CCl_4, reflux, 4 h, 85%; (e) NaN_3, DMF, 50°C, 2 days, 75%; (f) H_2/Pd–C, MeOH, rt, 24 h, 83%; (ii) Fmoc-OSu, TEA, MeCN, rt, 12 h, 55%; (iii) 6N HCl, reflux, 12 h, 100%; (iv) FmocCl, K_2CO_3, H_2O/THF (1:1), 0°C to rt, 12 h, 70%; (v) PfpOH, DCC, AcOEt, 0°C, 2 h, 70%.

The glycosidation reaction with these classical approaches has given good results in the case of serine.[25] However, given the disappointing results obtained here, we decided to change the methodology and start from compound **15**—a precursor of the α-methylserine **7**. Compound **15** was obtained by a modified version of the procedure cited above[27] and required formation of the benzyl ester instead of the methyl ester. Diol **12** was prepared by Sharpless asymmetric dihydroxylation of **3** with AD-mix α and the product was hydrolysed with HCl under reflux, treated with Cs_2CO_3 in MeOH and esterified with BnBr to give compound **13**. Diol **13** was transformed into its 2,3-cyclic sulfite **14** with thionyl chloride and the ring-opening reaction of this sulfite with azide ions in dimethylformamide gave a mixture of the azido esters **15** and **16**. A regioselectiviy of 4.5:1 in favour of compound **15** was obtained and this corresponds to attack at the quaternary centre with clean inversion of configuration[30] (Scheme 2).

The glycosidation of **15**, once separated from **16** by column chromatography, with donor **6** using the Koenigs–Knorr methodology with a mixture of silver carbonate (Ag_2CO_3) and silver perchlorate ($AgClO_4$) gave 70% of the undesired orthoformiate derivative **17** with 25% of unreacted **6** recovered. Fortunately, the glycosidation of **15** using trichloloracetimidate **11** gave 50% yield of the desired derivative **18**. This yield was later improved by using the donor 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride[31,32] **19** in the presence of $BF_3{\cdot}Et_2O$ and triethylamine (TEA),[33] a process that gave **18** in 60% yield. The azido group of this compound was readily transformed into the amino group by the action of Ph_3P and water in tetrahydrofuran. This reaction gave β-D-Ac_4Glc-(*S*)-MeSer-OBn (**20**), which was easily converted into the required building block **1** by protection of the amino group as the Fmoc carbamate (Scheme 2).[34]

In conclusion, we have synthesised the suitably protected glycosylated amino acid β-D-Glc-(*S*)-α-MeSer (**1**). This compound is an important building block in the synthesis of constrained glycopeptides in which the conformational restriction is only present in the amino acid moiety (quaternary α-amino acid). To the best of our knowledge this is the first time that a glycosylated amino acid with these features has been synthesised. The nearest previous example is the glycosylated amino acid recently obtained by Lane and Halcomb,[19] although in this case the restriction affects not only the amino acid moiety but also the carbohydrate. We intend to explore the incorporation of other carbohydrates into different hydroxylated quaternary α-amino acids, using the methodology described here, in order to assess the applicability of this approach in the synthesis of conformationally restricted glycopeptides.



Scheme 2. *Reagents and conditions:* (i) Ref. 27: AD-mix α, $MeSO_2NH_2$, $^tBuOH/H_2O$ (1:1), 0°C, 12 h, 81%; (ii) (a) 6N HCl, reflux, 1 h, (b) Cs_2CO_3, MeOH, rt, 20 min. (c) BnBr, DMF, rt, 48 h, 84%; (iii) $SOCl_2$, CCl_4, reflux, 4 h, 81%; (iv) NaN_3, DMF, 50°C, 12 h, column chromatography (hexane/AcOEt = 4/1), 77% of **15** and 17% of **16**; (v) **6**, Ag_2CO_3, $AgClO_4$, CH_2Cl_2, MS 4 Å, −10°C to rt, 12 h; 70% (vi) **11**, $BF_3{\cdot}EtO_2$, CH_2Cl_2, rt, 12 h; 50% (vii) **19**, $BF_3{\cdot}Et_2O$, TEA, CH_2Cl_2, rt, 12 h; 60%; (viii) Ph_3P (2 equiv.), H_2O (2 equiv.), THF, reflux, 4 h, 71%; (ix) FmocCl, DIEA, CH_2Cl_2, rt, 12 h, 58%.

Acknowledgements

We thank the Ministerio de Ciencia y Tecnología (project PPQ2001-1305), the Gobierno de La Rioja (ANGI2001/30) and the Universidad de La Rioja (project API-02/03).

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34. Compound **20**: Mp: 65–67°C. $[\alpha]_D^{25}=-14.3$ (*c* 1.01, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 3.50 (d, 1H, $J=9.6$ Hz), 3.64 (ddd, 1H, $J=10.0$ Hz, $J=4.6$ Hz, $J=2.5$ Hz), 4.11 (dd, 1H, $J=12.3$ Hz, $J=2.5$ Hz), 4.17 (d, 1H, $J=9.6$ Hz), 4.25 (dd, 1H, $J=12.3$ Hz, $J=4.6$ Hz), 4.57 (d, 1H, $J=8.0$ Hz), 4.96 (dd, 1H, $J=9.6$ Hz, $J=8.0$ Hz), 5.07 ('t', 1H, $J=9.6$ Hz), 5.15 (s, 2H), 5.18 ('t', 1H, $J=9.6$ Hz), 7.33–7.37 (m, 5H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 20.5, 20.6, 22.7, 58.5, 61.9, 67.0, 68.4, 71.3, 71.8, 72.7, 76.1, 101.1, 128.0, 128.3, 128.5, 132.0, 132.1, 135.7, 167.1, 169.3, 170.1, 170.5, 175.3. Anal. calcd for $C_{25}H_{33}NO_{12}$: C, 55.65; H, 6.17; N, 2.60. Found; C, 55.71; H, 6.22; N, 2.84.