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TETRAHEDRON: *ASYMMETRY*

Preparation of glycosyl amino acids as building blocks for the combinatorial synthesis of neoglycoconjugates

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Abstract—Several neoglycosyl amino acids possessing a sugar residue, a spacer and a trifunctional amino acid moiety were synthesized both in solution and solid phase by activating the carboxylic group as its pentafluorophenyl ester for condensation. The methodology is useful for application in combinatorial syntheses of neoglycoconjugates as potential mimics for oligosaccharides.

Glycosyl amino acids are versatile building blocks for the synthesis of structurally diverse glycopeptides and glycoconjugates due to the fact that they combine the structural features of amino acids and carbohydrates.[1] Glycosyl amino acids that bear an amino and a carboxylate group attached to the pyranose ring of a glycose were previously used by Lehmann[1d] for the preparation of glycopeptide oligomers (carbopeptoids[2]). Similarly, carbopeptoids have been shown to exhibit inhibition of infection of cells by HIV[3] and binding properties to DNA and RNA.[4] Recently, Wong,[5] Meldal,[6] Hummel,[7] and Arya[8] used *O*-, *N*- and *C*-glycosylated amino acids for the construction of highly diverse glycopeptide libraries, which can—to some extent—mimic carbohydrate–protein interactions and thus, demonstrate the potential of glycosyl amino acids as building blocks for the preparation of structurally diverse neoglycoconjugates.

As part of a project toward the combinatorial synthesis of structurally highly diverse glycopeptide libraries as oligosaccharide mimics, we constructed a series of suitably blocked *O*- and *N*-linked L-asparaginyl aminopentyl-D-glycopyranosides of type **A** as well as 3,7-anhydro-2-deoxy-D-glycero-D-gulo-octanoyl L-lysines of type **B** (Fig. 1). The latter types of glycosyl amino acids were furthermore shown to be useful building blocks for either homogeneous solution synthesis or solid-phase synthesis of corresponding glycopeptides.

The *O*-glycosyl amino acids of type **A** (Fig. 1) were prepared starting from readily available[9] 5-benzyloxycarbamidopentyl 2,3,4,6-tetra-*O*-acetyl-D-glycopyranosides **1a–c** which were hydrogenated[10] to give 5-aminopentyl 2,3,4,6-tetra-*O*-acetyl-D-glycopyranosides **2a–c** in 92–96% yield. Similarly, hepta-*O*-acetyl-β-D-cellobiosyl azide[11] was first hydrogenated to give the corresponding cellobiosyl amine which in turn was reacted with freshly prepared 6-benzyloxycarbamidocapronoyl chloride[12] to afford **1d**.[13] Next, the latter gave disaccharide **2d** upon hydrogenation (Scheme 1).

Since the projected glycosyl amino acids were thought to be used as versatile building blocks for the solution-

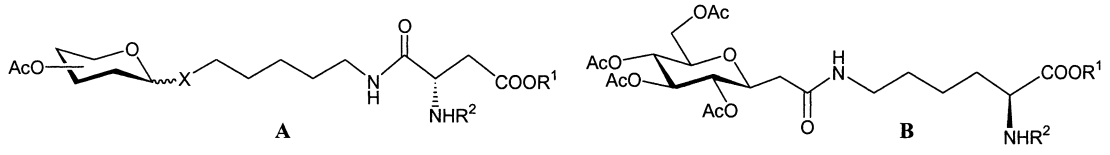

Figure 1. (A) Sugar = glucose, galactose, mannose, cellobiose, *N*-acetyl-glucosamine; X = α- or β-O or β-NHCO; R^1 = *t*-Bu, H, pentafluorophenyl; R^2 = Z, H, Boc, Fmoc. (B) R^1 = *t*-Bu, H, pentafluorophenyl; R^2 = Z, H, Boc, Fmoc

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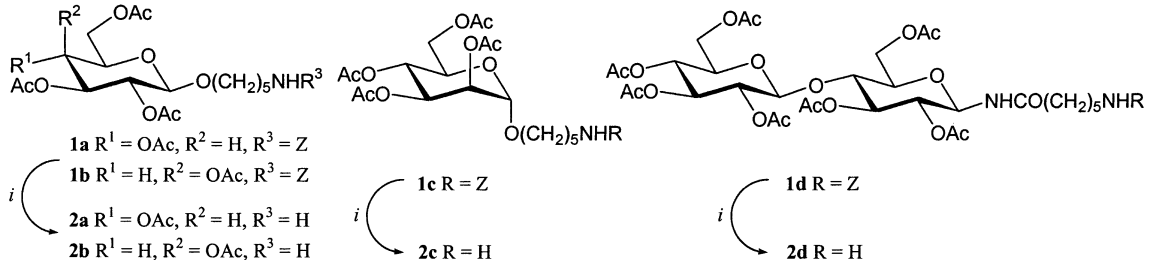
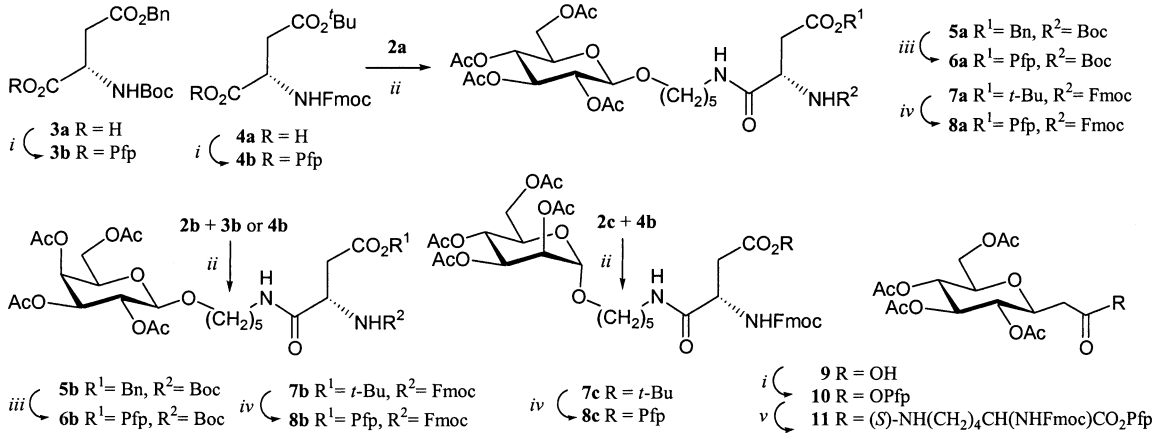


Scheme 1. *Reagents and conditions*: (i) **1a–d**, H_2 (100 kPa), cat. Pd/C, EtOH/AcOH (1:1), 1 h, 25°C; 96% **2a**, 94% **2b**, 92% **2c**, 100% **2d** (crude).

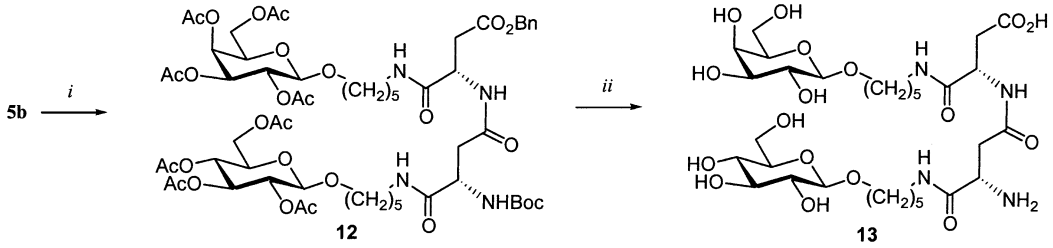
based or solid-phase preparation of glycopeptides by the Bn/BOC and *t*-Bu/Fmoc methodology we first tested several literature procedures for condensing Boc-Asp(OBn)-OH **3a** with the amino group of compound **2a** (Scheme 2). However, neither of the 'standard' procedures (DCC/1-hydroxybenzotriazole,[14a] EEDQ,[14b] TBTU,[14c] WSC[14d]) did result in a satisfactory yield of the product. Therefore, the pentafluorophenyl ester (Pfp) **3b** was prepared from **3a** and condensed with **2a** next. The reaction proceeded smoothly at room temperature and gave the corresponding glucosyl amino acid **5a** in 95% isolated yield. Similarly, derivatives **2a–c** were subsequently converted with **3b** and **4a–b**, respectively into the corresponding glycosyl amino acids **5b**, **7c**, and **7c** which in turn were debenzylated or hydrolyzed and esterified with pentafluorophenol (Pfp-OH) to give derivatives **6a–b** and **8a–c**, respectively. Compound **9** was converted via **10** into **11** in 72% overall yield (Scheme 2).

A dimeric neoglycopeptide **12** was synthesized in 78% yield by coupling glucosyl amino acid derivative **6a** with a galactosyl moiety carrying a free amine group (prepared in situ from **5b**), and following the pentafluorophenyl ester methodology. Final deblocking of protected intermediate **12** gave glycopeptide **13** in 95% yield (Scheme 3). A spatial closeness of both the sugar molecules in solution could be shown by NMR spectroscopy (NOESY/ROESY, in D_2O/H_2O, 1:9)[15] for **13**.

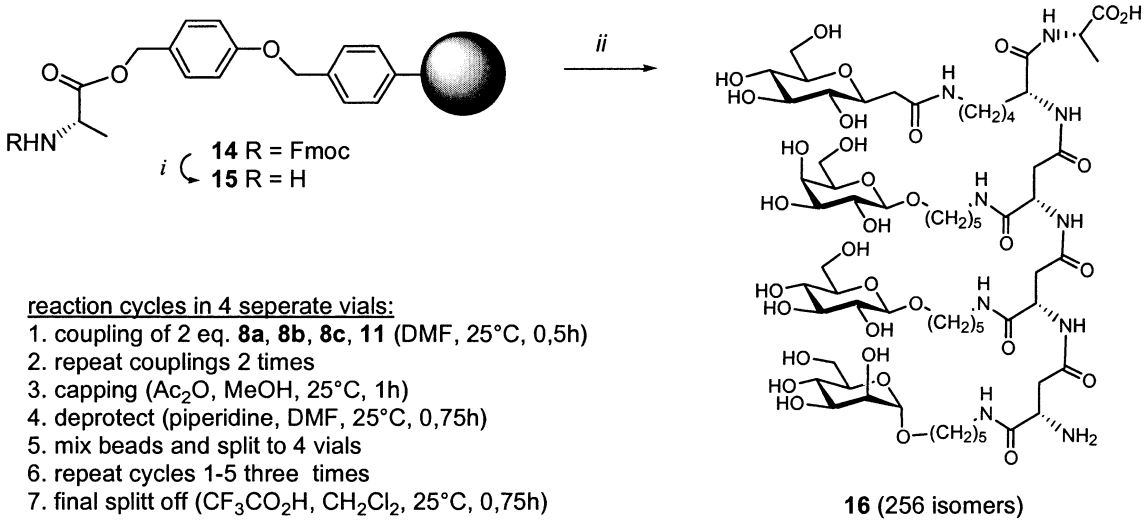
Beside the solution synthesis of glycopeptides, their formation using the polymer supported Rink-amid-



Scheme 2. *Reagents and conditions*: (i) **3a**, Pfp-OH, DCC, EtOAc, 1 h, 0→20°C, 93% **3b**, 92% **4b**, 86% **10**. (ii) **2a–c**, **3a–b** or **4b**, EtOAc, 1.5–3 h, 20°C, 95% **5a**, 87% **5b**, 98% **7a**, 96% **7b**, 94% **7c**. (iii) (1) **5a**, **5b**, H_2 (100 kPa), cat. Pd/C, EtOH/AcOH 1:1, 1 h, 25°C; (2) Pfp-OH, DCC, EtOAc, 1 h, 0→20°C, 85% **6a**, 95% **6b**. (iv) (1) **7a–c**, CF_3CO_2H, CH_2Cl_2, 2 h, 25°C; (2) Pfp-OH, DCC, EtOAc, 1 h, 0→20°C, 93% **8a**, 95% **8b**, 92% **8c**. (v) (1) **10**, L-Fmoc-Lys, DMF, 2 h, 25°C; (2) Pfp-OH, DCC, EtOAc, 1 h, 0→20°C, 84% **11**.



Scheme 3. *Reagents and conditions*: (i) (1) CF_3CO_2H, CH_2Cl_2, 1 h, 25°C; (2) **6a**, EtOAc, 12 h, 25°C, 78% **12**. (ii) (1) CF_3CO_2H, CH_2Cl_2, 2 h, 25°C; (2) NH_3/MeOH, 24 h, 25°C, 95% **13**.



Scheme 4. *Reagents and conditions*: (i) Piperidine, DMF, 25°C, 0.75 h, 92% **15**. (ii) Reaction cycles, 54% **16**.

AM,[16] Rink-amid-MBHA,[17] and Wang-Ala-Fmoc[18] (**14**) resins, respectively, was also conducted and used for constructing a neoglycopeptide library containing 256 compounds as follows. The four different glycosyl amino acid moieties **8a**–**c**, and **11** were coupled with polymer supported Wang-Ala resin **15** as shown in Scheme 4 using the split/mix technology. The progress of the couplings was monitored by MAS–NMR spectroscopy. After final removal of the library from the resin, compounds **16** were obtained in pure form as determined by LC in 54% overall yield and characterized by inspecting significant regions of their ^{13}C NMR spectra[19] and by ESI mass spectrometry. All other compounds described here were fully characterized by NMR, specific rotation, and elemental analysis, respectively.

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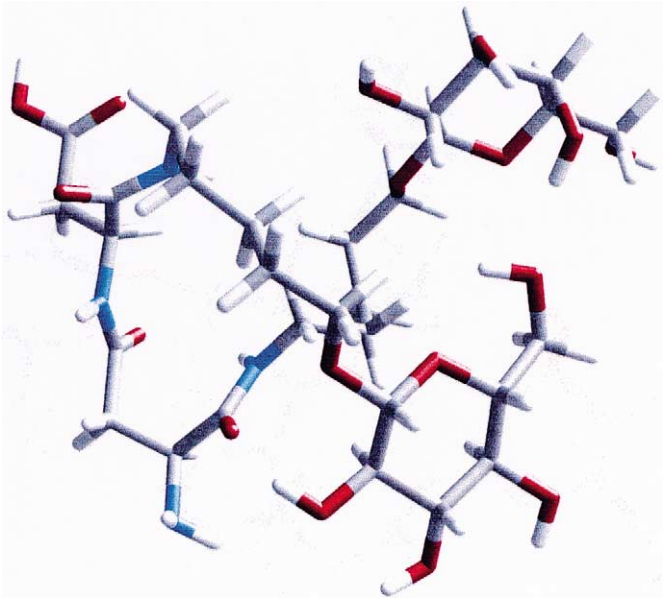
15. The calculation for compound **13** was performed with the program MacroModel 6.5 using a workstation O2 (Sili-



con Graphics Irix 6.3) and forcefield AMBER (Orozco, M.; Jorgensen, W. L.; Tirado-Rives, J. *Biochemistry* **1993**, *32*, 12864–12874). Energy minimization was performed applying a virtual medium of water and using 1000 Monte-Carlo cycles with convergence of ±0.1 kJ/mol. Calculations were repeated until energy minima were found several times and until the calculated structure was in accordance with the measured NOESY and ROESY NMR spectra of compound **13**. The global energy minimum was calculated to be −355.3 kJ/mol.

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19. ^{13}C NMR ($CDCl_3$): $\delta = 101.6$ (C-1 galactose), 100.8 (C-1 glucose), 97.4 (C-1 mannose), 73.9 (C-3 C-glucoside).