Orthogonally Protected Sugar Diamino Acids as Building Blocks for Linear and Branched Oligosaccharide Mimetics**

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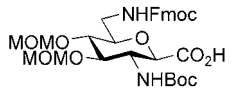
Sugar amino acids (SAAs),[1] which are carbohydrate derivatives with both an amino group and a carboxyl group connected to the carbohydrate frame, have found wide application as building blocks for oligosaccharide[2,3] and peptide mimetics,[4,5] as secondary-structure inducing elements, and as pharmacophore-presenting scaffolds[6] for the generation of combinatorial libraries.[7] Used as monomers with a rigid pyran ring, functional pharmacophoric groups attached to the hydroxy, amino, and carboxyl groups can be presented in a distinct spatial arrangement as was demonstrated in seminal studies by Hirschmann et al.[8] Linear and cyclic oligomers of SAAs have been synthesized, taking advantage of well-established peptide chemistry, and in certain cases they adopt defined secondary structures.[3,5] Sugar amino acids with an additional amino group, that is, sugar diamino acids, would be an attractive extension of this concept, giving access to novel branched oligomeric structures.[9] However, their synthesis has not been reported until now.

Here we introduce the protected derivatives **1** and **2** of 2,6-diamino-2,6-dideoxy-β-D-glucopyranosyl carboxylic acid,



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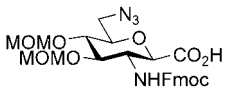

2

the first examples of sugar diamino acids (SDAs) that are amenable to peptide synthesis following standard Fmoc strategy (Fmoc = 9-fluorenylmethoxycarbonyl) in solution and on solid phase. The additional amino group in SDAs can be used to form branched amide-linked oligosaccharide mimetics. Besides that, oligomeric SDAs with unprotected amino groups represent a new class of potential aminoglycoside mimetics.[10] Such structures are of great significance as potential ligands for the new RNA targets emerging in the post-genome era.[11]

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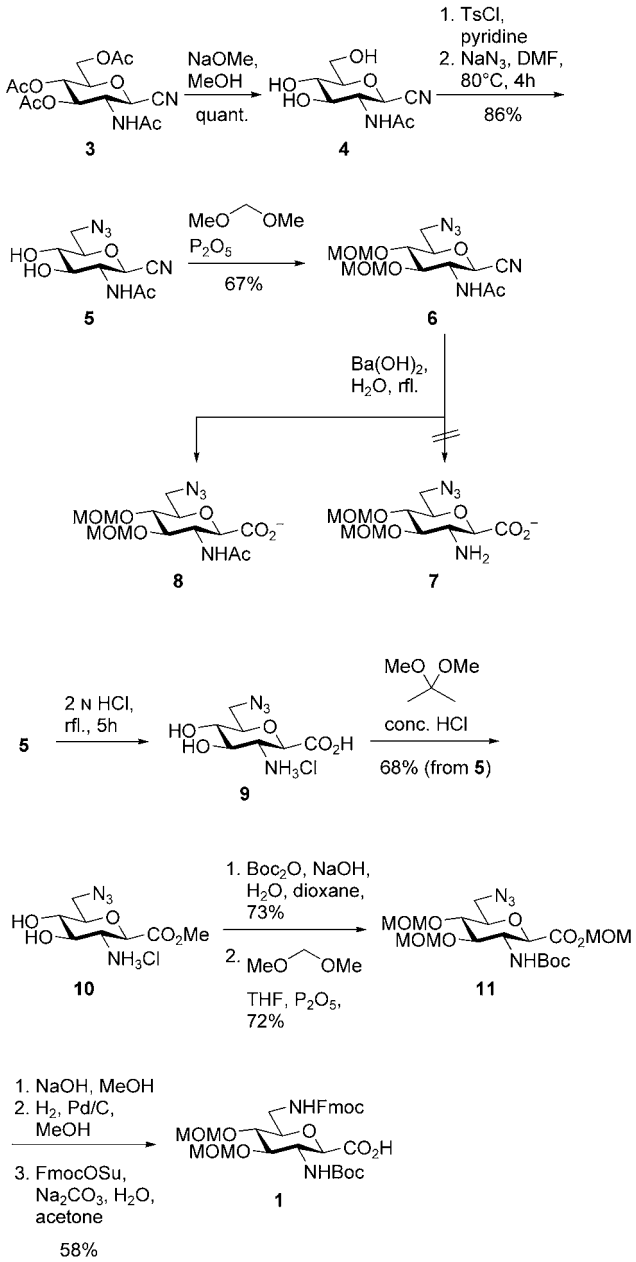
Whereas the protecting-group pattern of SDA **1** was conceived for the construction of linear oligomers through the amino group in the 6-position[12] following Fmoc strategy, SDA **2** can be employed for the synthesis of branched structures since the two amino groups are orthogonally protected with the Fmoc group and as an azide, respectively. We decided to use methoxymethyl (MOM) groups for hydroxy protection, because they are small and easily removable together with Boc (*tert*-butyloxycarbonyl) groups under acidic conditions, and they do not deactivate adjacent nucleophiles as it is known from electron-withdrawing acyl or sterically demanding benzyl protecting groups.[3] Finally, since **2** has protected hydroxy groups, a large excess of the activated amino acid can be used during peptide-bond formation typically employed during solid-phase peptide synthesis.



Scheme 1. Synthesis of SDA **1**; rfl. = reflux.

SDA **1** was synthesized from the known glycosyl cyanide **3**[13] (Scheme 1). After *O*-deacetylation, triol **4** was tosylated regioselectively at the 6-position. Nucleophilic substitution with sodium azide gave **5** in 86% yield over three steps. MOM groups were introduced by treatment with dimethoxymethane and P_2O_5[14] (in order to circumvent toxic MOM-Cl) leading to **6**. Initial attempts to hydrolyze the nitrile and acetamide simultaneously under basic conditions with aqueous $Ba(OH)_2$, however, failed. Under these conditions the reaction stopped at the acetamidocarboxylate stage (**8**). Other bases such as methanolic KOH and aqueous NaOH either led to the same result or to complete decomposition. Finally, heating **5** in 2 N aqueous HCl at reflux successfully effected amide and nitrile hydrolysis to give the free amino acid **9**. To facilitate its purification, methyl ester **10** was formed by treatment with dimethoxypropane and HCl. The amino group of **10** was protected by the Boc group with concomitant cleavage of the methyl ester. Compound **11** was obtained by MOM protection using the procedure mentioned earlier. The MOM ester in **11** was cleaved with NaOH. Finally, hydrogenation of the azide and subsequent Fmoc protection of the amine gave SDA building block **1** (Table 1).

SDA **2** was synthesized commencing with methyl ester **10** (Scheme 2). The 2-amino function was masked temporarily,

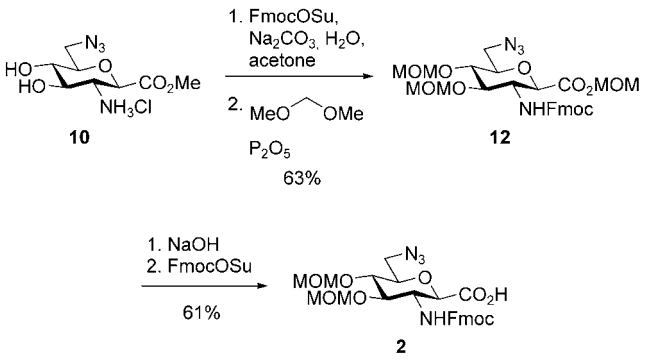
Table 1: Selected physical properties of compounds **1**, **2**, **19**, and **20**.

1: White amorphous solid; $R_f = 0.24$ (silica, MeOH/CH_2Cl_2 9/1); ^{1}H NMR (600 MHz, DMSO, 300 K, TMS) (major conformation): $\delta = 7.87$ (d, $J = 7.4$ Hz, 2H, arenes), 7.68 (m, 2H, arenes), 7.38–7.40 (m, 3H, arenes, NH-Fmoc), 7.29–7.32 (m, 2H, arenes), 6.86 (br s, 1H, NH-Boc), 4.75 (d, $J = 6.4$ Hz, 1H, O-CH_2-O), 4.62–4.65 (m, 3H, O-CH_2-O), 4.11–4.24 (m, 3H, H-9^{Fmoc}, CH_2^{Fmoc}), 3.64 (m, 1H, H-1), 3.55 (m, 1H, H-2), 3.55 (m, 1H, H-3), 3.51 (m, 1H, H-6), 3.30 (s, 3H, O-CH_3), 3.26 (s, 3H, O-CH_3), 3.24 (m, 1H, H-5), 3.23 (m, 1H, H-4), 3.00 (m, 1H, H-6′), 1.33 ppm (s, 9H, C$(CH_3)_3$); ^{13}C NMR (150 MHz, DMSO, 300 K, TMS): $\delta = 171.1$ (COOH), 156.4 (C=O), 155.0 (C=O), 143.9, 140.8, 127.7, 127.2, 125.3, 120.2 (arenes), 97.9 (O-CH_2-O), 97.3 (O-CH_2-O), 81.3 (C-3), 79.2 (CMe_3), 78.3 (C-1), 77.8 (C-5), 77.1 (C-4), 65.6 (CH_2^{Fmoc}), 56.0 (O-CH_3), 55.5 (O-CH_3), 53.8 (C-2), 46.7 (CH^{Fmoc}), 42.0 (C-6), 28.3 ppm (C$(CH_3)_3$); MS (MALDI-TOF), calcd for $C_{31}H_{40}N_2NaO_{11}$ [M+Na$^+$]: 639.25, found: 639.5.

2: White amorphous solid; $R_f = 0.63$ (silica, H_2O/MeCN 1/4); ^{1}H NMR (600 MHz, DMSO, 300 K, TMS): $\delta = 7.86$ (m, 2H, arenes), 7.69 (d, $J = 7.4$ Hz, 1H, arenes), 7.66 (d, $J = 7.4$ Hz, 1H, arenes), 7.46 (d, 1H, NH), 7.39 (m, 2H, arenes), 7.30 (m, 2H, arenes), 4.72 (d, $J = 6.5$ Hz, 1H, O-CH_2-O), 4.59 (d, $J = 6.5$ Hz, 1H, O-CH_2-O), 4.57 (d, $J = 6.5$ Hz, 1H, O-CH_2-O), 4.53 (d, $J = 6.5$ Hz, 1H, O-CH_2-O), 4.29 a. 4.20 (2 m, 2H, CH_2^{Fmoc}), 4.15 (m, 1H, H-9^{Fmoc}), 3.73 (d, $J = 10.0$ Hz, 1H, H-1), 3.61 (m, 1H, H-2), 3.55 (m, 1H, H-3), 3.54 a. 3.45 (2 m, 2H, 2 H-6), 3.38 (m, 1H, H-4), 3.33 (m, 1H, H-5), 3.27 (s, 3H, O-CH_3), 3.09 ppm (s, 3H, O-CH_3); ^{13}C NMR (150 MHz, DMSO, 300 K, TMS): $\delta = 170.9$ (COOH), 155.8 (C=O^{Fmoc}), 144.0, 143.8, 140.8, 127.6, 127.1, 125.3, 120.1 (arenes), 97.9 (O-CH_2-O), 97.3 (O-CH_2-O), 81.3 (C-3), 79.2 (C-1), 77.5 (C-5), 76.9 (C-4), 65.4 (CH_2^{Fmoc}), 55.9 (O-CH_3), 55.4 (O-CH_3), 54.2 (C-2), 51.0 (C-6), 46.7 ppm (CH^{Fmoc}); MS (MALDI-TOF), calcd for $C_{26}H_{30}N_4NaO_9$ [M+Na$^+$]: 565.19, found: 565.4.

19: HRMS (MALDI-FTICR), calcd for $C_{83}H_{110}N_8O_{27}$: 1673.73730 [M+Na$^+$], found: 1673.73587, $\Delta m = 0.8$ ppm.

20: HRMS (ESI-FTICR, MeCN/H_2O), calcd for $C_{31}H_{50}N_8O_{13}$: 743.35696 [M+H$^+$], found: 743.35563, $\Delta m = 1.8$ ppm.

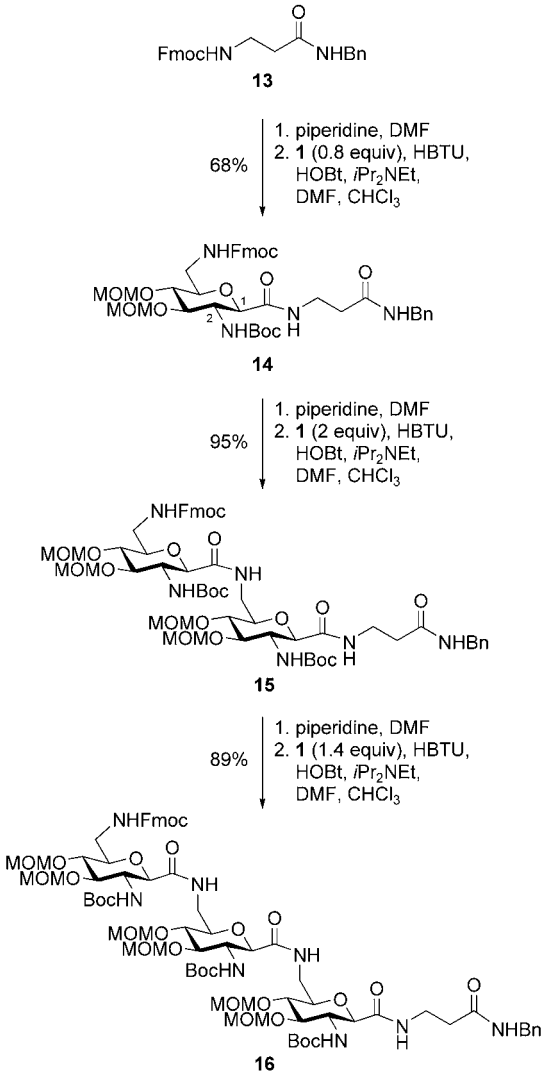


Scheme 2. Synthesis of SDA **2**.

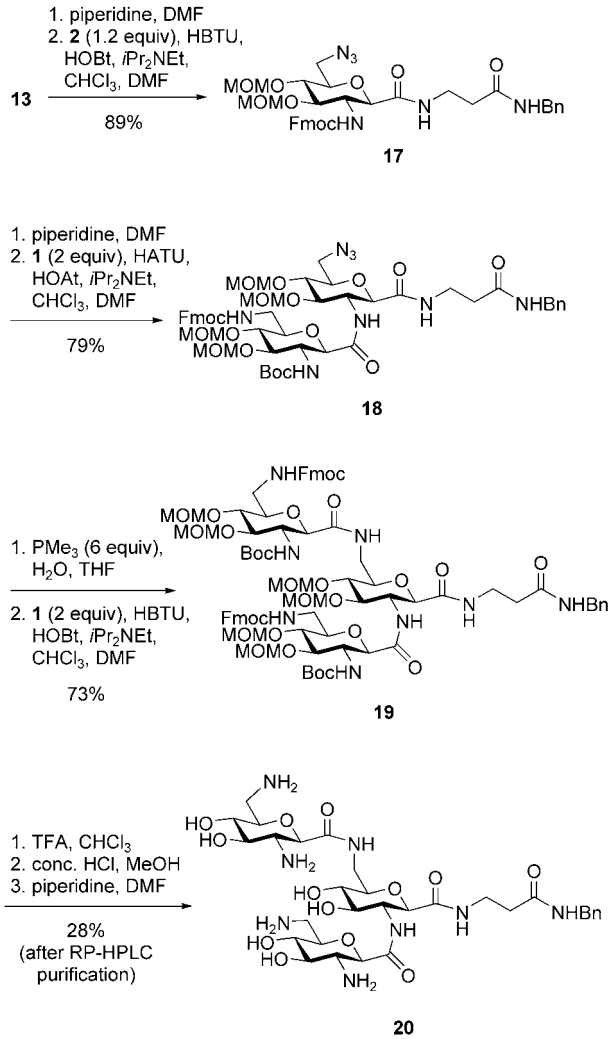
and introduction of MOM groups gave **12**, which was treated with NaOH. Reprotection of the amino group at the 2-position gave building block **2**.

Scheme 3 illustrates the application of SDA building block **1** in the peptide synthesis of aminoglycoside mimetic **16** following the standard Fmoc protocol. We carried out the synthesis in solution to be able to follow the outcome of each step. Starting with β-alanine amide **13**, stepwise coupling of **1** using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxy-1*H*-benzotriazole (HOBt) as coupling reagents led to linear pseudotrisaccharide **16** in six steps. When SDA building block **1** was applied in excess, peptide couplings proceeded smoothly with no side products detectable by TLC and yields up to 95% after column chromatography. The integrity of the chirality at the α-carbon of the sugar diamino acids was verified by ^{1}H NMR spectroscopy (e.g. **14**: $\delta_{H\text{-}1} = 3.56$ ppm, $^3J_{H\text{-}1,H\text{-}2} = 9.8$ Hz).

The utilization of the orthogonally protected SDA **2** for the preparation of the branched oligomer **20** is demonstrated in Scheme 4. After coupling of **2** to β-alanine amide **13**, the amine at the 2-position was deprotected by treatment with piperidine. Coupling of SDA **1** to this sterically hindered amine proceeded in a yield of 79% after column chromatography on silica gel to give **18**. In this case *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt) were used as coupling reagents. Reduction of the



Scheme 3. Synthesis of linear oligomer **16**; Bn = benzyl.



Scheme 4. Synthesis of branched oligomer **20**.

azide in presence of the hydrogenolytically labile Fmoc group was accomplished under Staudinger conditions.[15] Subsequent coupling with **1** provided the branched oligomer **19** in a yield of 73 % over two steps. Complete deprotection of **19** was carried out by a three-step procedure. Treatment with TFA/$CHCl_3$ (1:1) led to complete Boc removal and partial cleavage of the MOM groups. The latter could be completed by addition of a small amount of water. A cleaner product, however, was obtained removing the MOM groups with conc. HCl in methanol. Finally, Fmoc groups were cleaved by treatment with piperidine in DMF to yield **20**, which was purified by RP-HPLC with added ion-pairing reagent pentafluoropropionic acid.[16] Compound **20** is the first sugar amino acid oligomer in which branching is achieved through two amide linkages.

In conclusion, we have presented a divergent synthesis of the SDA building blocks **1** and **2** and their application in the efficient assembly of oligosaccharide mimetics **16** and **20**, which are the first examples of a new class of aminoglycoside mimetics. The protecting-group scheme of **1** and **2** is compatible with conventional Fmoc solid-phase peptide synthesis and includes the option of generating branched structures. Future applications include the utilization of SDA building blocks in the preparation of combinatorial libraries of aminoglycoside mimetics. Due to the various possibilities with which SDAs can be connected to each other, a high degree of diversity can be achieved by employing only a small set of different sugar diamino acids.

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[1] Reviews: a) T. K. Chakraborty, P. Srinivasu, S. Tapadar, B. K. Mohan, *J. Chem. Sci.* **2004**, *116*, 187 – 207; b) E. Gräfin von Roedern, M. A. Born, M. Stöckle, H. Kessler in *Methods of Organic Chemistry (Houben-Weyl)*, *Vol. E22c* (Eds.: M. Goodman, A. Felix, L. Moroder, C. Toniolo), Thieme, Stuttgart, **2003**, p. 807 – 821; c) S. A. W. Gruner, E. Locardi, E. Lohof, H. Kessler, *Chem. Rev.* **2002**, *102*, 491 – 514; d) F. Schweizer, *Angew. Chem.* **2002**, *114*, 240 – 264; *Angew. Chem. Int. Ed.* **2002**, *41*, 230 – 253.

[2] a) T. Q. Gregar, J. Gervay-Hague, *J. Org. Chem.* **2004**, *69*, 1001 – 1009; b) S. Sabesan, *Tetrahedron Lett.* **1997**, *38*, 3127 – 3130; c) H. P. Wessel, C. Mitchell, C. M. Lobato, G. Schmid, *Angew. Chem.* **1995**, *107*, 2920 – 2921; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2712 – 2713.

[3] Y. Suhara, Y. Yamaguchi, B. Collins, R. L. Schnaar, M. Yanagishita, J. E. K. Hildreth, I. Shimada, Y. Ichikawa, *Bioorg. Med. Chem.* **2002**, *10*, 1999 – 2013.

[4] a) M. Raunkjær, F. El Oualid, G. A. van der Marel, H. S. Overkleeft, M. Overhand, *Org. Lett.* **2004**, *6*, 3167 – 3170; b) E. Lohof, E. Planker, C. Mang, F. Burkhart, M. A. Dechantsreiter, R. Haubner, H.-J. Wester, M. Schwaiger, G. Hölzemann, S. L. Goodman, H. Kessler, *Angew. Chem.* **2000**, *112*, 2868 – 2871; *Angew. Chem. Int. Ed.* **2000**, *39*, 2761 – 2764; c) A. B. Smith III, S. Sasho, B. A. Barwis, P. Sprengeler, J. Barbosa, R. Hirschmann, B. S. Cooperman, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3133 – 3136.

[5] a) T. K. Chakraborty, V. Ramakrishna Reddy, G. Sudhakar, S. Uday Kumar, T. Jagadeshwar Reddy, S. Kiran Kumar, A. C. Kunwar, A. Mathur, R. Sharma, N. Gupta, S. Prasad, *Tetrahedron* **2004**, *60*, 8329 – 8339; b) S. Chandrasekhar, M. S. Reddy, B. Jagadeesh, A. Prabhakar, M. H. V. R. Rao, B. Jagannadh, *J. Am. Chem. Soc.* **2004**, *126*, 13586 – 13587; c) R. M. van Well, L. Marinelli, C. Altona, K. Erkelens, G. Siegal, M. van Raaij, A. L. Llamas-Saiz, H. Kessler, E. Novellino, A. Lavecchia, J. H. van Boom, M. Overhand, *J. Am. Chem. Soc.* **2003**, *125*, 10822 – 10829; d) S. A. W. Gruner, V. Truffault, G. Voll, E. Locardi, M. Stöckle, H. Kessler, *Chem. Eur. J.* **2002**, *8*, 4365 – 4376; e) N. L. Hungerford, T. D. W. Claridge, M. P. Watterson, R. T. Aplin, A. Moreno, G. W. J. Fleet, *Perkin 1* **2000**, 3666 – 3679.

[6] M. Ghosh, R. G. Dulina, R. Kakarla, M. J. Sofia, *J. Org. Chem.* **2000**, *65*, 8387 – 8390.

[7] a) A. A. Edwards, O. Ichihara, S. Murfin, R. Wilkes, M. Whittaker, D. J. Watkin, G. W. J. Fleet, *J. Comb. Chem.* **2004**, *6*, 230 – 238; b) M. J. Sofia, N. Allanson, N. T. Hatzenbuhler, R. Jain, R. Kakarla, N. Kogan, R. Liang, D. Liu, D. J. Silva, H. Wang, D. Gange, J. Anderson, A. Chen, F. Chi, R. Dulina, B. Huang, M. Kamau, C. Wang, E. Baizman, A. Branstrom, N. Bristol, R. Goldman, K. Han, C. Longley, S. Midha, H. R. Axelrod, *J. Med. Chem.* **1999**, *42*, 3193 – 3198.

[8] a) R. Hirschmann, K. C. Nicolaou, S. Pietranico, J. Salvino, E. M. Leahy, P. A. Sprengeler, G. Furst, A. B. Smith III, C. D. Strader, M. A. Cascieri, M. R. Candelore, C. Donaldson, W. Vale, L. Maechler, *J. Am. Chem. Soc.* **1992**, *114*, 9217 – 9218. b) For recent reports on the use of carbohydrates as multifunctional scaffolds, see: U. Hünger, J. Ohnsmann, H. Kunz, *Angew. Chem.* **2004**, *116*, 1125 – 1128; *Angew. Chem. Int. Ed.* **2004**, *43*, 1104 – 1107; c) C.-H. Wong, X.-S. Ye, Z. Zhang, *J. Am. Chem. Soc.* **1998**, *120*, 7137 – 7138.

[9] For the synthesis of a branched oligomer employing a glycosylated sugar amino acid, see: C. M. Timmers, J. J. Turner, C. M. Ward, G. A. van der Marel, M. L. C. E. Kouwijzer, P. D. J. Grootenhuis, J. H. van Boom, *Chem. Eur. J.* **1997**, *3*, 920 – 929.

[10] 2,6-Diamino-2,6-dideoxyglucose is a substructure of the aminoglycoside antibiotic neomycin B. For the use of disaccharide-based diamino acid derivatives as inhibitors of bacterial cell-wall biosynthesis, see ref. [7b].

[11] a) Q. Vicens, E. Westhof, *ChemBioChem* **2003**, *4*, 1018 – 1023; b) Y. Tor, *ChemBioChem* **2003**, *4*, 998 – 1007.

[12] Numbering according to the parent compound glucosamine is used throughout this communication.

[13] D. Carrière, S. J. Meunier, F. D. Tropper, S. Cao, R. Roy, *J. Mol. Catal. A* **2000**, *154*, 9 – 22.

[14] K. Fuji, S. Nakano, E. Fujita, *Synthesis* **1975**, 276 – 277.

[15] J. T. Lundquist IV, J. C. Pelletier, *Org. Lett.* **2002**, *4*, 3219 – 3221.

[16] M. McCroskey, V. E. Groppi, J. D. Pearson, *Anal. Biochem.* **1987**, *163*, 427 – 432.