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Solution and solid-phase chemoselective synthesis of (1-6)-amino(methoxy) di- and trisaccharide analogues

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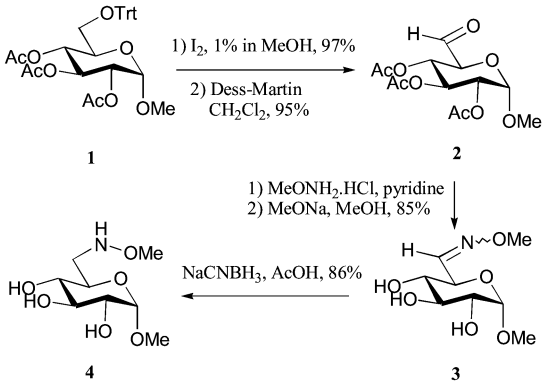
Received (in Cambridge, UK) 15th April 2002, Accepted 15th May 2002
First published as an Advance Article on the web 12th June 2002

Disaccharide and trisaccharide mimics containing the amino(methoxy) interglycosidic linkage were obtained by chemoselective condensation of unprotected aldoses in an aqueous environment both in solution and in solid phase.

The observation that a large number of biological processes are based on oligosaccharide recognition by protein receptors, inspired the synthesis of oligosaccharide analogues in which the *O*-glycosidic linkage, generally not directly involved in the interaction with the receptor, is replaced by other bonds. C–C linked oligosaccharides are an interesting class of mimetics presenting resistance to chemical degradation and glycosidase digestion, while having biological activity and conformational properties suitable to substitute natural oligosaccharides in protein–sugar interactions.[1] Analogues containing a sulfur[2] atom at the interglycosidic linkage as well as carbopeptoids[3] or carbonucleotoids[4] in which the interglycosidic bond has been replaced by a more rigid carboxyamide or phosphoramide bond, have been prepared. From a methodological point of view, the synthesis of oligosaccharides and their analogues is still far from routine, both in solution and in solid phase,[5] requiring extensive use of orthogonal protecting groups and strictly anhydrous conditions. Synthetic strategies based on peptide-like bond formation (carbopeptoids)[3] or on the principle of chemoselective ligation, provide a powerful tool for the convergent preparation of oligosaccharide mimics. In particular, sugars bearing an aminoxy group at the anomeric position have been reacted with keto functions present in a second sugar.[6,7] Preliminary studies also indicate the possibility to link chemoselectively a molecule with an aminoxy function to the anomeric centre of a free aldose. Exploiting an R-ONH$_2$, an open chain sugar-oxime is formed, whereas when a R-ONHR′ is used, the cycle of the sugar is restored.[8] Following this last approach, if R′ is a sugar unit, oligosaccharide mimics could be obtained in aqueous media, without protections and in a iterative way.

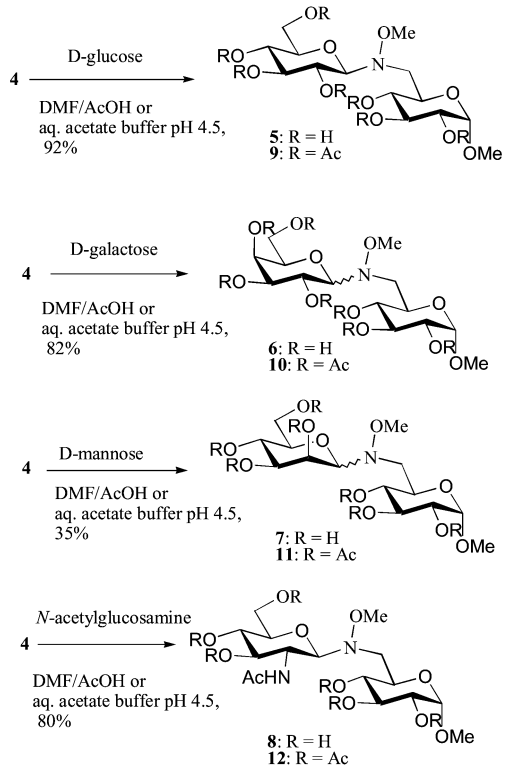
In this communication, we describe our preliminary results on the development of this promising approach. Compounds **5**–**8**, a new class of disaccharide mimics, isosteric to natural disaccharides, have been synthesised from unprotected aldoses such as D-glucose, D-mannose, D-galactose and D-*N*-acetylglucosamine, and **4**, an unprotected sugar bearing a methoxyamino group at C-6.†

Methyl 6-deoxy-6-methoxyamino-D-glucopyranoside **4** was prepared according to Scheme 1. Compound **1** was detritylated (97% yield) and oxidized at C-6 (Dess–Martin periodinane, 95% yield) to afford aldehyde **2** which was converted into the *O*-methyloxime by treatment with *O*-methylhydroxylamine hydrochloride in pyridine. Zemplén de-*O*-acetylation gave compound **3** (85% yield over the two steps) the reduction of which with NaCNBH$_3$ in glacial acetic acid afforded compound **4** in 86% yield. Compound **4** was reacted with D-glucose, D-galactose, D-mannose and D-*N*-acetylglucosamine affording, respectively, disaccharide analogues **5**, **6**, **7** and **8** (Scheme 2). The coupling reactions proceeded in good yields (except for **7**) at room temperature in 4–6 hours, and were carried out either in aqueous acetic acid–sodium acetate buffer (pH = 4.5) or in 1:1 (v/v) water–acetic acid or in 1:2 acetic acid–DMF as solvent



Scheme 1

mixtures. After complete removal of the solvents, crude reaction products were treated with Ac$_2$O, pyridine and DMAP, and fully acetylated disaccharides **9**, **10**, **11** and **12** were isolated and characterised by MALDI-TOF mass spectrometry and NMR spectroscopy.‡ Disaccharides **9** and **12** were obtained in the β form with total stereoselectivity, whereas the ^{1}H-NMR analysis of **10** revealed the presence of a small amount of the α form (β:α = 7:1). In the case of **11** a mixture of α and β anomers was formed with α being the most abundant (β:α = 1:5).§ These results are coherent with the stereochemical

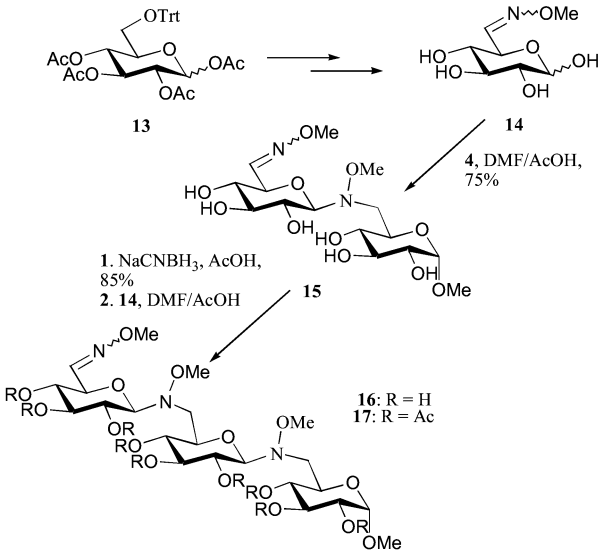


Scheme 2

DOI: 10.1039/b203605c

behaviour of the same reducing sugars in the reaction with *N*,*O*-dialkyl hydroxylamines and *N*-methylhydroxylamino peptides described in a precedent paper.[8]

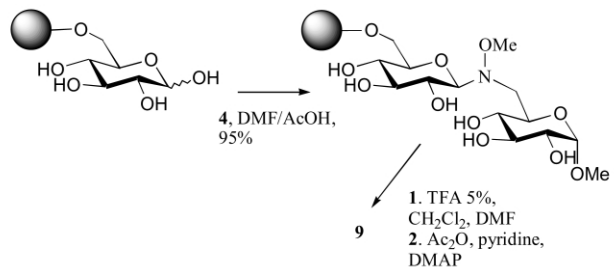
In order to investigate the possibility of using this approach iteratively for the preparation of oligosaccharide mimetics, sugar **14** (Scheme 3), obtained from **13** following the procedure of Scheme 1, was reacted with **4** to afford disaccharide **15** (75% yield). Upon reduction of the oxime group of **15** with $NaCNBH_3$, and subsequent reaction of the obtained amino(methoxy) disaccharide with **14**, trisaccharide mimic **16** was obtained (65% yield), which can be reduced for further chemoselective elongation. Compound **16** was fully *O*-acetylated and converted into derivative **17** which was characterised. Monomer **14** is an excellent scaffold for the synthesis of oligosaccharide analogues, presenting the two complementary functionalities required for chemoselective ligation, one of which, the amino(methoxy) group, masked as *O*-methyloxime.



Scheme 3

Finally, we aimed to extend the chemoselective ligation to polymer-bound sugars in order to evaluate the possibility of automation of the process on solid phase. In sharp contrast with the majority of solid phase glycosylation methods, this condensation can be effected in the presence of water, does not require any glycosylation promoter and, in the case of D-glucose or *N*-acetylglucosamine, is stereoselective affording only the β-disaccharide mimic.

D-glucose was reacted with 4-methoxytrityl chloride resin (Novabiochem) in the presence of TBAI and *sym*-collidine in DMF–pyridine (4 : 1) for 12 h, affording sugar linked to polymer through C-6 position (loading: 0.8 mmol g^{-1}). The polymer-bound sugar was reacted with a 4-fold excess of **4** in DMF–AcOH (5:1) for 48 h at rt (Scheme 4). The disaccharide **5** was then cleaved from the polymer (5% TFA in CH_2Cl_2–DMF 1 : 1). After solvent evaporation, the crude product was acetylated giving pure **9** (95% yield without purification). Interestingly,



Scheme 4

the interglycosidic amino(methoxy) bond of **9** proved to be resistant to prolonged TFA treatment.

In conclusion, this chemoselective and iterative approach for the synthesis of oligosaccharide mimics is promising either in solution and on solid phase. Work is in progress to apply the method to the synthesis of other oligosaccharide mimics and study their conformational properties.

Notes and references

† The synthesis of (1 → 4)amino(methoxy) disaccharides containing glucose and galactose was recently achieved in a non-chemoselective way by reacting protected 4-deoxy-4-methoxyaminoglycosides acceptors with glycosyl bromide donors in Koenigs–Knorr conditions.[7]

‡ Selected spectral data (^{1}H-NMR, 400 MHz, $CDCl_3$); protons of the monosaccharide unit derived from **4** are numbered 1-6, 1′-6′ and 1″-6″numbers refer to the other units, methyl signals of acetates have been omitted. Compounds **10-β** and **11-α** are described as selected peaks from spectra of anomeric mixtures. Compound **9**: δ (ppm) 5.43 (t, 1H, J = 9.3 Hz, H-3), 5.20 (m, 2H, H-2′ and H-3′), 5.04 (t, 1H, J = 9.6 Hz, H-4′), 4.87 (d, 1H, J = 3.7 Hz, H-1), 4.81 (t, 1H, J = 9.4 Hz, H-4), 4.80 (dd, 1H, J = 3.7, 9.3 Hz, H-2), 4.28 (d, 1H, J = 9.0 Hz, H-1′), 4.24 (dd, 1H, J = 5.3, 12.2 Hz, H-6′a), 4.08 (dd, 1H, J = 2.0, 12.2 Hz, H-6′b), 3.95 (m, 1H, H-5), 3.62 (ddd, 1H, J = 2.0, 5.3, 9.5 Hz, H-5′), 3.45 (s, 3H, OCH_3), 3.40 (s, 3H, OCH_3), 3.16 (dd, 1H, J = 8.1, 14.6 Hz, H-6a), 3.03 (dd, 1H, J = 2.2, 14.6 Hz, H-6b). Compound **10-β** (major isomer): δ (ppm) 5.45 (t, 1H, J = 9.3 Hz, H-3), 5.36 (m, 2H, H-2′ and H-3′), 5.01 (dd, 1H, J = 3.5, 9.9 Hz, H-4′), 4.88 (d, 1H, J = 3.7 Hz, H-1), 4.83 (t, 1H, J = 9.3 Hz, H-4), 4.81 (dd, 1H, J = 3.7, 9.3 Hz, H-2), 4.26 (d, 1H, J = 9.3 Hz, H-1′), 4.14 (m, 2H, H-6′a, H-6′b), 3.98 (m, 1H, H-5), 3.86 (m, 1H, H-5′), 3.49 (s, 3H, OCH_3), 3.41 (s, 3H, OCH_3), 3.16 (dd, 1H, J = 8.2, 14.6 Hz, H-6a), 3.03 (dd, 1H, J = 2.0, 14.6 Hz, H-6b). Compound **11-α** (major isomer): δ (ppm) 5.57 (dd, 1H, J = 1.8, 3.4 Hz, H-2′), 5.41 (t, 1H, J = 9.4 Hz, H-3), 5.32 (dd, 1H, J = 3.4, 9.7 Hz, H-3′), 5.19 (t, 1H, J = 9.7 Hz, H-4′), 4.85 (d, 1H, J = 3.6 Hz, H-1), 4.79 (t, 1H, J = 9.4 Hz, H-4), 4.75 (dd, 1H, J = 3.6, 9.4 Hz, H-2), 4.2–4.0 (m, 5H, H-1′, H-6′a, H-6′b, H-5, H-5′), 3.45 (s, 3H, OCH_3), 3.40 (s, 3H, OCH_3), 3.11 (dd, 1H, J = 7.7, 14.6 Hz, H-6a), 2.80 (br d, 1H, J = 14.6 Hz, H-6b). Compound **12** δ (ppm) 5.85 (d, 1H, J = 8.8 Hz, H-1′), 5.47 (t, 1H, J = 10.2 Hz, H-3), 5.10 (t, 1H, J = 9.4 Hz, H-3'), 5.05 (m, 2H, H-4′, H-4), 4.91 (d, 1H, J = 3.6 Hz, H-1), 4.81 (dd, 1H, J = 3.6, 10.2 Hz, H-2), 4.31 (t, 1H, J = 9.3 Hz, H-2′), 4.25 (dd, 1H, J = 5.3, 12.2 Hz, H-6'a), 4.11 (dd, 1H, J = 2.3, 12.2 Hz, H-6′b), 3.94 (m, 1H, H-5), 3.58 (m, 1H, H-5′), 3.50 (s, 3H, OCH_3), 3.40 (s, 3H, OCH_3), 3.26 (dd, 1H, J = 2.1, 15.3 Hz, H-6a), 2.93 (dd, 1H, J = 5.9, 15.3 Hz, H-6b). Compound **17** δ (ppm) 7.16 (d, 1H, J = 7.1 Hz, H-6″), 5.09 (m, 1H, H-5), 5.01 (t, 1H, J = 9.3 Hz, H-2′), 5.40–4.80 (m, 9H, H-2″, H-3″, H-4″, H-3′, H-4′, H-1, H-2, H-3, H-4), 4.22 (d, 1H, J = 9.3 Hz, H-1″), 4.20 (d, 1H, J = 9.3 Hz, H-1′), 3.90 (m, 2H, H-5′, H-5″), 3.76 (s, 3H, OCH_3), 3.41 (s, 3H, OCH_3), 3.38 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 3.20 (dd, 1H, J = 2.5, 14.6 Hz, H-6a), 2.92 (dd, 1H, J = 8.6, 14.6 Hz, H-6b), 2.82 (m, 1H, H-6′a), 2.72 (dd, 1H, J = 7.3, 14.1 Hz, H-6′b).

§ It was impossible to isolate anomers from mixtures by chromatography for both compounds **10** and **11**.

1 A. Paveda, J. L. Asensio, T. Palat, R. J. Linhardt and J. Jimenez-Barbero, *Eur. J. Org. Chem.*, 2000, 1805; E. Montero, A. Garcia-Herrero, J. L. Asensio, K. Hirai, S. Ogawa, F. Santoyo-Gonzàles, F. J. Canada and J. Jimenez-Barbero, *Eur. J. Org. Chem.*, 2000, 1805.

2 See, for example, B. D. Johnston and B. M. Pinto, *J. Org. Chem.*, 2000, **65**, 4607 and ref. cited therein.

3 C. M. Timmers, J. J. Turner, C. M. Ward, G. A. van der Marel, M. L. C. E. Kouwijzer, P. D. J. Grootenhuis and J. H. van Boom, *Chem. Eur. J.*, 1997, **3**, 920; T. D. W. Claridge, D. D. Long, N. L. Hungerford, R. T. Aplin, M. D. Smith, D. G. Marquess and G. W. J. Fleet, *Tetrahedron Lett.*, 1999, **40**, 2199; D. Long, N. L. Hungerford, M. D. Smith, D. E. A. Brittain, D. G. Marquess, T. D. W Claridge and G. W. J. Fleet, *Tetrahedron Lett.*, 1999, **40**, 2195; M. D. Smith, D. Long, A. Martin, T. D. W Claridge and G. W. J. Fleet, *Tetrahedron Lett.*, 1999, **40**, 2191; M. D. Smith, D. Long, G. Marquess, T. D. W Claridge and G. W. J. Fleet, *Chem. Commun.*, 1998, 2039.

4 K. C. Nicolaou, H. Florke, M. G. Egan and V. A. Estevez, *Tetrahedron Lett.*, 1995, **36**, 1775.

5 P. H. Seeberger and W.-C. Haase, *Chem. Rev.*, 2000, **100**, 4349.

6 E. C. Rodriguez, K. A. Winans, D. K. King and C. R. Bertozzi, *J. Am Chem. Soc.*, 1997, **119**, 9905; L. A. Marcaurelle and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2001, **123**, 1587.

7 O. Renaudet and P. Dumy, *Tetrahedron*, 2002, **58**, 2127.

8 F. Peri, P. Dumy and M. Mutter, *Tetrahedron*, 1998, **54**, 12269.