ELSEVIER

Carbohydrate Research 309 (1998) 367–374

CARBOHYDRATE
RESEARCH

Note

Synthesis of regio- and stereospecifically *C*-deuterated derivatives of glycosidase inhibitors 1-deoxymannonojirimycin and 2,5-dideoxy-2,5-imino-D-mannitol by intramolecular reductive amination employing deuterium gas[1]

Martin H. Fechter, Günther Gradnig, Andreas Berger, Christian Mirtl,
Werner Schmid, Arnold E. Stütz *

Institut für Organische Chemie der Technischen Universität Graz, Stremayrgasse 16, A-8010 Graz, Austria

Received 7 May 1998; accepted 16 June 1998

Abstract

6-Azido-1,3,4-tri-*O*-benzyl-6-deoxy-D-fructofuranose can be easily obtained in two steps from the known 6,6′-diazido-6,6′-dideoxysucrose, which is available in two steps from sucrose. Cyclisation of this material by controlled hydrogenation with $^2H_2$ over Raney nickel and concomitant intramolecular reductive amination gave 3,4,6-tri-*O*-benzyl-1,5-dideoxy-1,5-imino-D-(5-^{2}H)mannitol, a partially protected derivative of 1-deoxymannonojirimycin. Conventional deprotection furnished 1-deoxy-(5-^{2}H)mannonojirimycin. Reduction and intramolecular reductive amination of free 5-azido-5-deoxy-D-fructopyranose gave 2,5-dideoxy-2,5-imino-D-(5-^{2}H)mannitol in one step. Likewise, 2,5-dideoxy-2,5-imino-D-(5-^{2}H)glucitol was obtained from 5-azido-5-deoxy-L-sorbopyranose.

Keywords: C-Deuteration; Deuterium gas; Glycosidase inhibitor

Over the past decade, sugars with nitrogen instead of oxygen in the ring (sometimes referred to as aza-sugars) have emerged as the most important class of reversible glycosidase inhibitors [1]. Spurred by biochemical and medicinal research, glycosidases involved in the modification of glycoproteins and glycolipids have become of eminent interest due to their crucial roles in a wide variety of biological processes. In context with a programme aiming for the structure elucidation of glycosidase active sites, as well as approaches to non-invasive in vivo inhibition studies, we have resorted to NMR spectroscopic investigations of interactions

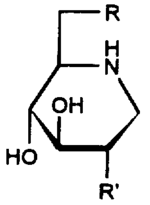
---

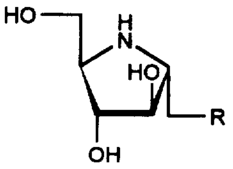
[1] Dedicated to Professor Herfried Griengl on the occasion of his 60th birthday.

* Corresponding author. Fax: +43-316-873-8740; e-mail: stuetz@orgc.tu-graz.ac.at

0008-6215/98/$—see front matter
PII S0008-6215(98)00159-1

of selected glycosidases with suitable iminoalditols as model systems. For the purpose of these experiments, we have recently synthesised several potent glycosidase inhibitors substituted with stable isotopes such as ^{19}F and ^{13}C. This improves detection under the assay conditions employed. A number of ^{19}F-substituted derivatives of the powerful glycosidase inhibitors 1-deoxynojirimycin (**1**), 2-acetamido-1,2-dideoxynojirimycin (**2**) and 2,5-dideoxy-2,5-imino-D-mannitol (**3**) such as, for example, 1,2- [2] and 1,6- [3] dideoxyfluoronojirimycins (**4** and **5**), 1,2, 5-trideoxy-2-fluoroacetamido-1,5-imino-D-glucitol (**6**) [4], as well as 1,2,5-trideoxy-1-fluoro-2,5-imino-D-mannitol (**7**) [2], have been prepared. For ^{13}C NMR applications, ^{13}C-substituted compounds such as 1-deoxy-(1-^{13}C)nojirimycin [5] have been synthesised. In a related line of research aiming for compounds exhibiting simplified coupling patterns as well as derivatives substituted with two or more different stable isotopes (^{13}C, ^{15}N) in the same molecule, selected regio- and stereospecifically *C*-deuterated inhibitors have now become available.





1: R = OH, R' = OH
2: R = OH, R' = NHAc
4: R = OH, R' = F
5: R = F, R' = OH
6: R = OH, R' = $NHCOCH_2F$

3: R = OH,
7: R = F

Compounds substituted with deuterium atoms have conventionally been prepared by reduction of suitable functional groups with complex metal hydrides [6–9]. In the case of aldehydes and ketones, mono-*C*-deuterio derivatives of the corresponding alcohols can be prepared. On the other hand, *gem*-dideuterated compounds are available from carboxylic esters as demonstrated in the preparation of 1,1,6-trideuterated 1-deoxynojirimycin by Leontein and co-workers [10]. Other approaches in the carbohydrate field have taken advantage of a deuterioboration protocol [11] or hot D_2O in the presence of Raney Ni [12] as well as the nucleophilic displacement of halides [13,14] or sulfonates [14,15] employing $LiAlD_4$. Another example in this class of compounds was given in context with the elucidation of the biosynthesis of compound **1** [16]. A common disadvantage of these approaches is the comparably expensive deuterating step that is required at an early or intermediate step of the synthesis. Additional synthetic transformations and subsequent deprotection reactions lower the yields of the desired final products. Furthermore, depending on the chemical environment of the deuterium substituent and the reaction conditions, isotopic scrambling, as well as partial exchange of the introduced isotope, may occur [7,17,18]. Consequently, we decided to combine the advantage of the comparably moderate price of deuterium gas [18,19] with the possibility to introduce the desired labeling as late as possible in the synthesis. Indeed this can be achieved in the last step of the preparation, which is conventionally [20] the formation of the iminoalditol ring by intramolecular reductive amination of an azidodeoxyaldose or -ketose.

In order to avoid large losses of deuterium gas through manipulations of the reaction vessel, the glass apparatus depicted in Fig. 1 was employed. This allowed purging of the reaction vessel with nitrogen, replacing N_2 with deuterium gas and, upon work-up, the storage of excess D_2 (albeit diluted with nitrogen) in a storage flask for convenient recycling of up to 70% of the deuterium. Preliminary experiments had shown that with short

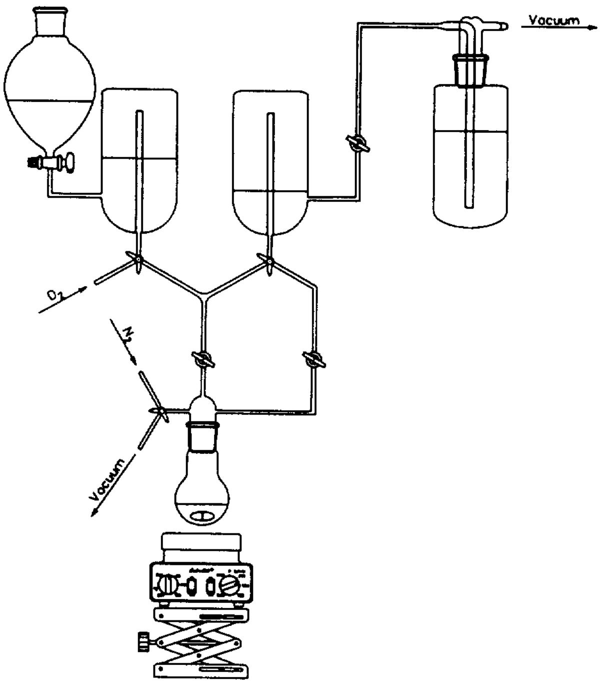
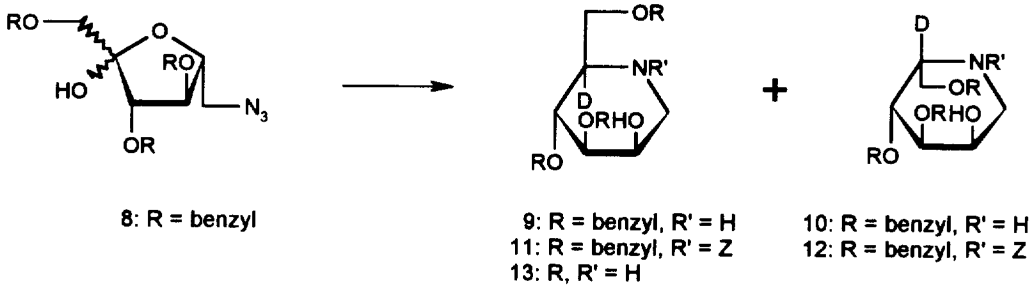


Fig. 1. Deuteration apparatus with D_2 recycling option.

reaction times water was sufficient to pump the gases between the reaction vessel and the storage flasks despite some hydrogen/deuterium exchange with the gas phase.

Prolonged deuteration procedures resulted in reduced deuterium content of the products. Moisture, as well as chemically bound water on the catalyst, were found to have negative effects on the yields of deuterium substitution. Under optimised conditions, palladium-on-charcoal (10%) was pre-deuterated in methanol-d_l for 24 h. Dry azidodeoxyhexose was added in a minimal amount of the same solvent, and hydrogenation was performed under ambient pressure. Reaction times of 20 min were found to be sufficient for the complete conversion of the starting materials. Starting materials were prepared according to previously established procedures. Taking advantage of the known regioselectivity of the reaction [21], in initial model experiments applying the established [4] five-step approach from sucrose but employing Raney nickel and $^2H_2$ instead of hydrogen gas in the hydrogenation step of 6-azido-1,3,4-tri-*O*-benzyl-6-deoxy-D-fructofuranose (**8**), a 10:1 mixture of 3,4,6-tri-*O*-benzyl-1,5-dideoxy-1,5-imino-D-(5-^{2}H)-mannitol (**9**) and the corresponding L-*gulo* epimer (**10**) were obtained in nearly 80% isolated yield from compound **8**. Separation could be achieved after protection of the ring nitrogen as the *N*-benzyloxycarbonyl derivatives **11** and **12**. Intermediate **11** was smoothly deprotected under standard conditions to yield 1-deoxy-(5-^{2}H)mannonojirimycin (**13**) in 60% overall yield (Scheme 1). From the integral over the ^{1}H NMR spectrum of this product incorporation of deuterium at C-5 was found to be in the range of around 80%. In accordance with the aim to introduce deuterium as late in the sequence as possible, *C*-deuteration by catalytic reduction of selected unprotected sugar derivatives was probed next. As could be expected from published data [22,23], catalytic intramolecular reductive amination of 5-azido-5-deoxy-D-fructopyranose (**14**) in methanol-d_l employing palladium-on-activated-charcoal (10%) gave mono-*C*-deuterated 2,5-dideoxy-2,5-imino-D-mannitol (**15**) in approximately 80% isolated yield (Scheme 2). As previously observed with the non-deuterated counterpart, this compound was formed practically stereospecifically over its C-2 epimer, which could not be detected to have been formed under the conditions employed. Applying a reaction time of 20 min, the yield of deuterium introduction was found to range between 90 and 95%, as determined by integration over the ^{1}H NMR spectrum of this product (Fig. 2, spectrum **A**). After a reaction time of 120 min, this value dropped to approximately 80%. Likewise, employing 5-azido-5-deoxy-L-sorbose (**16**) [23], C-5-deuterated 2,5-dideoxy-2,5-imino-D-glucitol (**17**) was identified as the sole product of the reaction. In this case, yields of deuterium incorporation were found to be around 95% (Fig. 3, spectrum **A**) with isolated yields of compound **17** of 75 to 80%. As expected, *N*/*O*-perdeuterated product **17** showed a molecule ion of 169 mass units (Fig. 4). All fragments were found to contain one mass unit more than the corresponding *N*/*O*-perdeuterated, unsubstituted parent compound (Fig. 5). Symmetrical compound **15** exhibited a very similar fragmentation pattern.

In conclusion, catalytic reduction of cyclic aldimines with deuterium gas was, for the first time, conveniently applied to the synthesis of selected iminoalditols. By optimisation of the reaction conditions as well as the selection of suitable starting materials, satisfying deuterium substitution could be achieved. Deuterium incorporation was found to proceed with perfect regio- and practically complete stereoselectivity. However, the need for



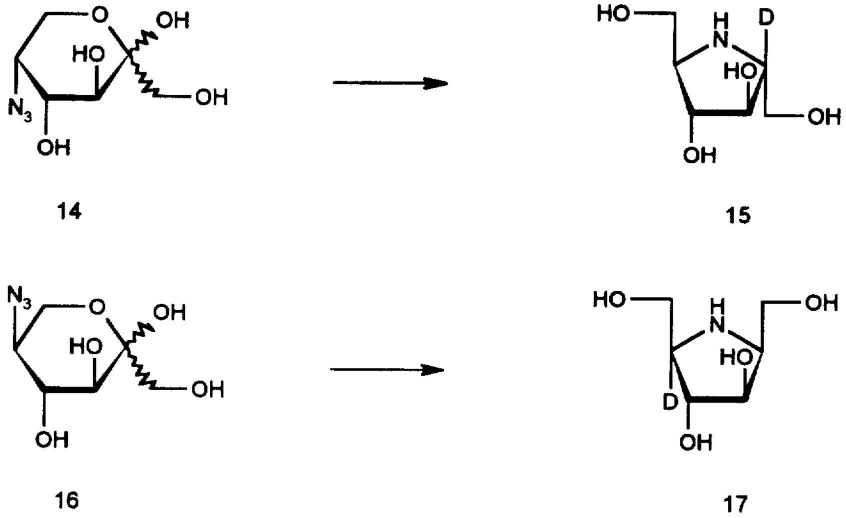
8: R = benzyl

9: R = benzyl, R' = H
11: R = benzyl, R' = Z
13: R, R' = H

10: R = benzyl, R' = H
12: R = benzyl, R' = Z

Z, benzyloxycarbonyl

Scheme 1.



Scheme 2.

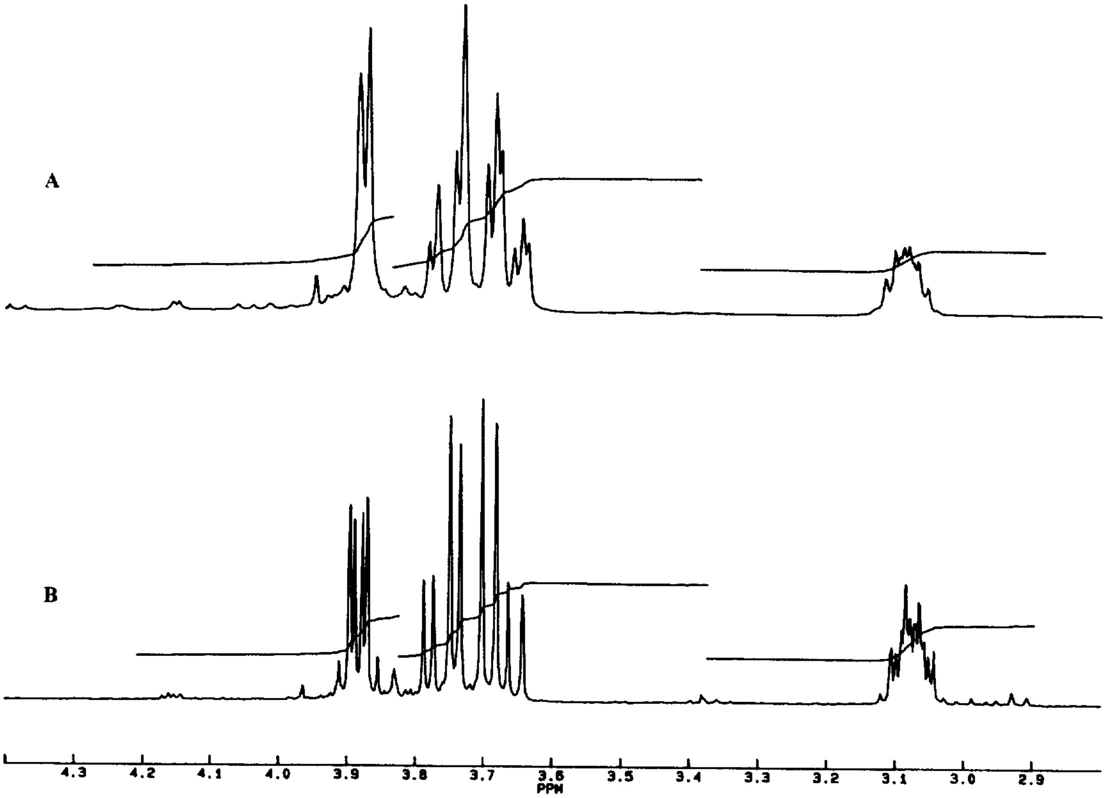


Fig. 2. ^{1}H NMR Spectrum of compound **15** (**A**) and non-deuterated 2,5-dideoxy-2,5-imino-D-mannitol (**B**) recorded at 300 MHz.

methanol-d_l as the reaction medium as well as the pre-deuteration of the catalyst remain a disadvantage of this method. Nonetheless, definite advantages of this approach are the introduction of deuterium in the last step with compounds such as **15** and **17**, together with the high deuterium content found in the afforded products.

1. Experimental

General methods.—Melting points of crystalline intermediates were recorded on a Tottoli apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 341 Digital Polarimeter with a path length of 10 cm. NMR spectra were

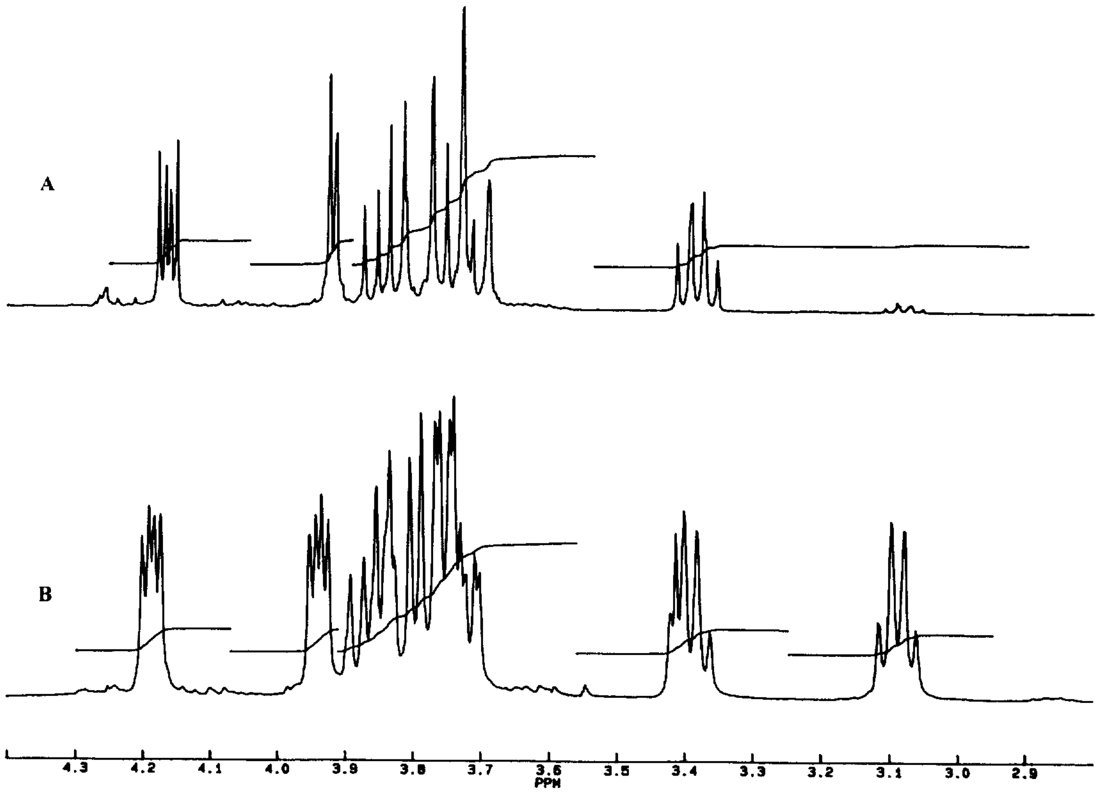


Fig. 3. ^{1}H NMR Spectrum of compound **17** (**A**) and nondeuterated 2,5-dideoxy-2,5-imino-D-glucitol (**B**) recorded at 300 MHz.

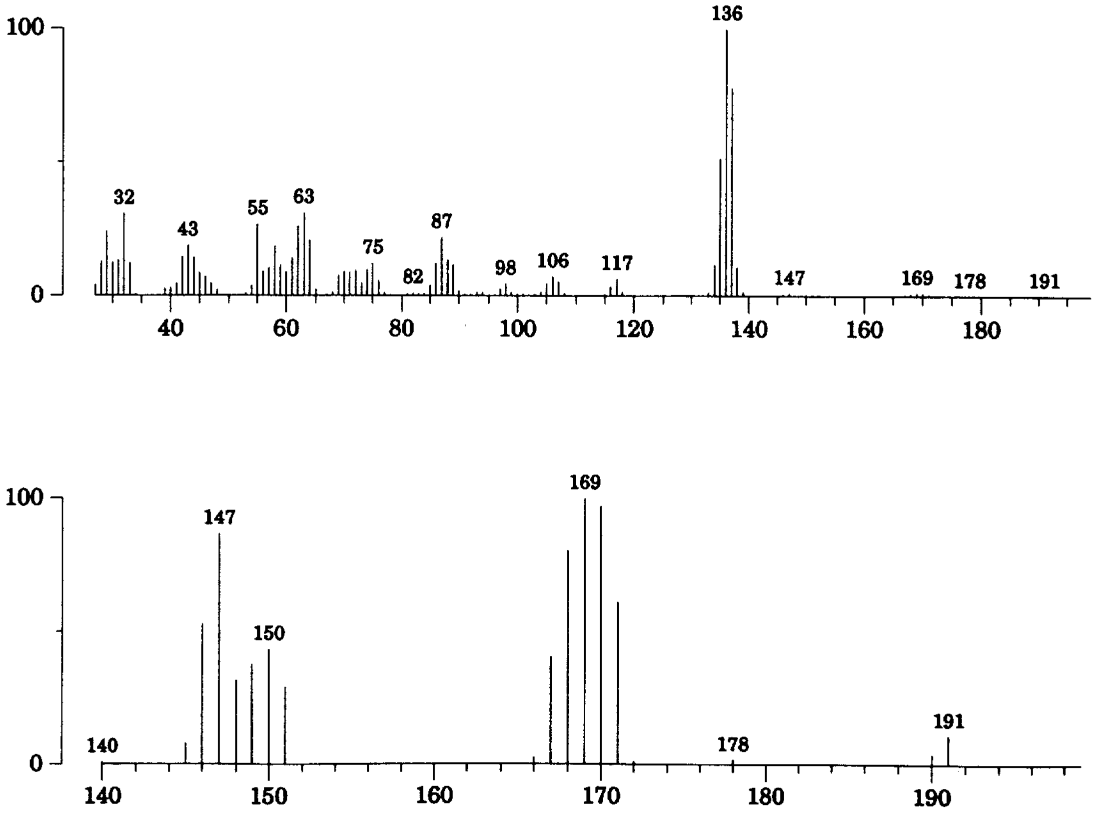


Fig. 4. Mass spectra of *N*/*O*-perdeuterated compound **17**.

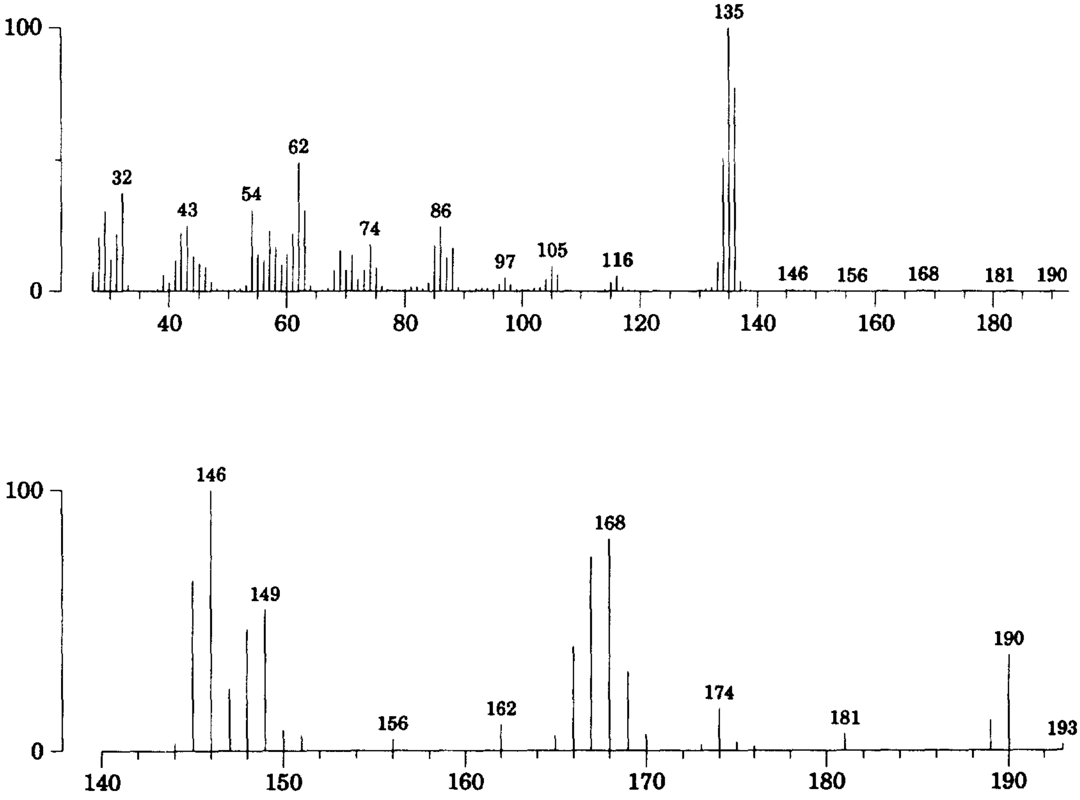


Fig. 5. Mass spectra of N/O-perdeuterated 2,5-dideoxy-2,5-imino-D-glucitol.

recorded at 200 and 300 MHz (^{1}H), and at 50.29 and 75.47 MHz (^{13}C), in D_2O. EI (70 eV) DI/MS data were recorded on a Shimadzu Profile mass spectrometer. TLC was performed on precoated aluminum sheets (E. Merck 5554). For column chromatography Silica Gel 60 (E. Merck) or ion-exchange resin (Amberlite CG 50) was used. Deuterium (99.8%) was purchased from Cambridge Isotope Laboratories (DLM-408). Raney Ni (Fluka) was pre-deuterated by stirring in D_2O for 18 h, after which time the solvent was removed by filtration. The catalyst was then rinsed with a minimal amount of methanol-d_1 and immediately used in the respective hydrogenation experiment.

General procedure for the reaction of free 5-azido-5-deoxyketoses.—Typically, a suspension of Pd/C (10%, 300 mg) in methanol-d_1 (10 mL) was pre-deuterated twice at ambient pressure for 12 h after which time the solvent was decanted. A 2% solution of the respective free 5-azidodeoxyketose (100 mg, 0.49 mmol) in methanol-d_1 was added, and the mixture was stirred under an atmosphere of deuterium gas for 20 min. After removal of the catalyst by filtration, the solvent was removed under reduced pressure (with larger scale reactions, the deuterated solvent can be recycled), and the residue was chromatographed on silica gel employing a mixture of 100:100:1 $CHCl_3$–MeOH–concd aq ammonia as the solvent system.

*3,4,6-Tri-O-benzyl-1,5-dideoxy-1,5-imino-*D-*(5-^{2}H)-mannitol* (**9**) *and* -L-*(5-^{2}H)gulitol* (**10**).—To a suspension of predeuterated Raney nickel (2 g) (Caution: pyrophoric), a 2% solution of compound **8** (300 mg, 0.63 mmol) in dry methanol-d_1 was added, and the mixture was stirred under an atmosphere of deuterium at ambient temperature for 18 h. After filtration the solvent was removed under reduced pressure to give an inseparable mixture of partially protected iminoalditols **9** and **10**, which was immediately used in the next step.

*3,4,6-Tri-O-benzyl-N-benzyloxycarbonyl-1,5-dideoxy-1,5-imino-*D-*(5-^{2}H)mannitol* (**11**).—To a 5% solution (5:1 dichloromethane–methanol) of the crude mixture of intermediates **9** and **10** obtained from the previous reaction, solid sodium bicarbonate (160 mg, 1.9 mmol, 3 equiv), as well as benzyloxycarbonyl chloride (0.1 mL, 1.2 equiv), were added, and the mixture was stirred at ambient temperature for 30 min. Dichloromethane (100 mL) was added, and the mixture was washed with water, the aqueous layer was extracted once with dichloromethane (100 mL), and the combined organic layers were dried (sodium sulfate). Removal of the solvent under reduced pressure and chromatography of the residue gave compound **11** (250 mg, 70%) as a colourless syrup. This was identical by TLC with the non-deuterated analogue [4] and was directly employed in the subsequent deprotection step. Despite our best efforts, spectra of this compound did not resolve due to the effects of pyramidal inversion at the ring nitrogen as well as the presence of rotamers as was previously reported ([4] and refs. cited therein).

*1,5-Dideoxy-1,5-imino-*D*-(5-^2H) mannitol, (1-deoxy-(5-^2H) mannonojirimycin)* (**13**).—A 2% solution of intermediate **11** (200 mg, 0.35 mmol) in dry MeOH was hydrogenated over Pd/C (10%, 200 mg) at 4×10^5 Pa for 40 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The product was purified by ion-pair chromatography on Amberlite CG 50 (200:1 water–concd aqueous ammonia) to allow the isolation of 38 mg (65%) as an off-white, semi-crystalline material: $[\delta]_D^{20}$ −10.5° (*c* 1.4, water); ^{1}H NMR (D_2O): 3.88 (broad m, 1 H, H-2), 3.68 (d, 1 H, $J_{6,6'}$ 11.8 Hz, H-6), 3.63 (d, 1 H, H-6′), 3.49 (d, 1 H, $J_{3,4}$ 9.6 Hz, H-4), 3.43 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-3), 2.89 (dd, 1 H, $J_{1e,2,}$ 2.5 $J_{1e,1a}$ 14.3 Hz, H-1e), 3.68 (broad d, 1 H, H-1a); ^{13}C NMR (D_2O): 75.9 (C-3), 70.4 (C-4), 69.9 (C-2), 62.2 (C-6), 61.2 (t, $J_{C,D}$ 22 Hz, C-5), 49.4 (C-1); MS of a *N*/*O*-per deuterated sample: found *m*/*z* 169.01, required 169.225.

*2,5-Dideoxy-2,5-imino-*D*-(2-^2H) mannitol* (**15**). Employing the general procedure to 5-azidodeoxyketose **14** [23], compound **15** was isolated (66 mg, 83%) as off-white, semi-crystalline solid, $[\delta]_D^{20}$ +40.0° (*c* 0.61, water); ^{1}H NMR (D_2O): 3.88 (broad m, 2 H, H-3, H-4), 3.75 (dd, 1 H, $J_{5,6}$ 4.0, $J_{6,6'}$ 11.7 Hz, H-6), 3.75 (d, 1 H, $J_{1,1'}$ 11.6 Hz, H-1), 3.67 (dd, 1 H, $J_{5,6'}$ 6.0 Hz, H-6′), 3.66 (d, 1 H, H-1′), 3.09 (broad m, ≥1 H, H-5 plus residual H-2); ^{13}C NMR (D_2O): 78.7, 78.65 (C-3, C-4), 63.0, 62.8 (C-1, C-6), 62.7 (C-5), 62.3 (t, $J_{C,D}$ 21.5 Hz, C-2).

*2,5-Dideoxy-2,5-imino-*D*-(5-^2H)glucitol* (**17**).—Application of the general procedure to starting material **16** [23] gave compound **17** (63 mg, 79%) as slightly yellow wax, $[\delta]_D^{20}$ +18.1° (*c* 0.61, water); ^{1}H NMR (D_2O): 4.17 (dd, 1 H, $J_{2,3}$ 5.1, $J_{3,4}$ 2.9 Hz, H-3), 3.92 (d, 1 H, H-4), 3.84 (dd, 1 H, $J_{1,2}$ 6.2, $J_{1,1'}$ 11.5 Hz, H-1), 3.79 (d, 1 H, $J_{6,6'}$ 11.5 Hz, H-6), 3.72 (dd, 1 H, $J_{1',2}$ 7.0 Hz, H-1′), 3.71 (d, 1 H, H-6′), 3.38 (dd, 1 H, H-2); ^{13}C NMR (D_2O): 79.9, 78.2 (C-3, C-4), 65.4 (t, $J_{C,D}$ 21.5 Hz, C-5), 63.0 (C-1), 61.8, 61.0 (C-1, C-6).

Acknowledgements

This work was kindly supported by the Austrian Fonds zur Förderung der Wissenschaftlichen Forschung, Vienna, Projects 10067 and 10805 CHE.

References

[1] G. Legler, *Adv. Carbohydr. Chem. Biochem.*, 48 (1990) 319–384.

[2] S.M. Andersen, M. Ebner, C.W. Ekhart, G. Gradnig, G. Legler, I. Lundt, A.E. Stütz, and S.G. Withers, *Carbohydr. Res.*, 301 (1997) 155–166.

[3] K. Dax, V. Grassberger, and A.E. Stütz, *J. Carbohydr. Chem.*, 9 (1990) 903–908; A.Berger, K. Dax, G. Gradnig, V. Grassberger, A.E. Stütz, M. Ungerank, G. Legler, and E. Bause, *Bioorg. Med. Chem. Lett.*, 2 (1992) 27–32.

[4] G. Gradnig, G. Legler, and A.E. Stütz, *Carbohydr. Res.*, 287 (1996) 49–57.

[5] A. Berger, M. Ebner, and A.E. Stütz, *Tetrahedron Lett.*, 36 (1995) 4989–4990.

[6] J.E.G. Barnett and D.L. Corina, *Adv. Carbohydr. Chem. Biochem.*, 27 (1972) 127–190.

[7] R.J. Parry, Y. Li, and E.E. Gomez, *J. Am. Chem. Soc.*, 114 (1992) 5946–5959.

[8] A. (Mei) Ono, T. Shiina, A. Ono, and M. Kainosho, *Tetrahedron Lett.*, 39 (1998) 2793–2796.

[9] Y. Oogo, A. (Mei) Ono, A. Ono, and M. Kainosho, *Tetrahedron Lett.*, 39 (1998) 2873–2876.

[10] K. Leontein, B. Lindberg, and J. Lönngren, *Acta Chem. Scand. B*, 36 (1982) 515–518.

[11] J. Goddat, P. Datta, A.A. Grey, J.P. Carver, and R.N. Shah, *J. Carbohydr. Chem.*, 12 (1993) 793–800.

[12] H.J. Koch and R.S. Stuart, *Carbohydr. Res.*, 67 (1978) 341–348.

[13] E.C. Ashby and C.O. Welder, *J. Org. Chem.*, 62 (1997) 3542–3551.

[14] A.A. Mitrochkine, I. Blain, C. Bit, C. Canlet, S. Pierre, J. Courtieu, and M. Reglier, *J. Org. Chem.*, 62 (1997) 6204–6209.

[15] P. Dietrich and D.W. Young, *Tetrahedron Lett.*, 34 (1993) 5455–5458.

[16] D.J. Hardick, D.W. Hutchinson, S.J. Trew, and E.M.H. Wellington, *Tetrahedron*, 48 (1992) 6285–6296.

[17] A.S. Serianni, T. Vuorinen, and P.B. Bondo, *J. Carbohydr. Chem.*, 9 (1990) 513–541.

[18] M. Oba, T. Terauchi, J. Hashimoto, T. Tanaka, and K. Nishiyama, *Tetrahedron Lett.*, 38 (1997) 5515–5518.

[19] K. Wähälä, P. Kiuru, S. Kaltia, J. Koskimies, and T. Hase, *Synlett*, (1997) 460.

[20] H. Paulsen, *Angew. Chem.*, 78 (1966) 501–556; *Angew. Chem., Int. Ed. Engl.*, 5 (1966) 495–550.

[21] A. de Raadt and A.E. Stütz, *Tetrahedron Lett.*, 33 (1992) 189–192.

[22] R.R. Hung, J.A. Straub, and G.M. Whitesides, *J. Org. Chem.*, 56 (1991) 3849–3855.

[23] G. Legler, A. Korth, A. Berger, C. Ekhart, G. Gradnig, and A.E. Stütz, *Carbohydr. Res.*, 250 (1992) 67–77.