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Synthesis of Isofagomine, a Novel Glycosidase Inhibitor

Tina M. Jespersen, Mikael Bols*

Department of Organic Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark

Michael R. Sierks

Department of Chemical and Biochemical engineering, University of Maryland, Baltimore County, Baltimore MD 21228-5998, USA

Troels Skrydstrup

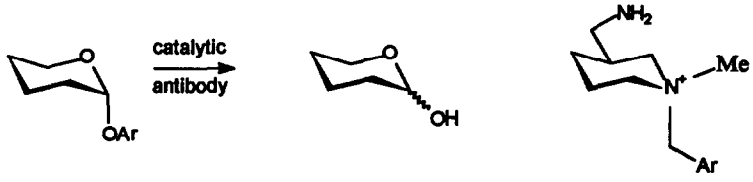
Laboratoire de Biochimie Structurale, Universite d'Orleans, URA 499, BP 6759, F-45067, Orleans Cedex 2, France

Abstract: (3R, 4R, 5R)-3,4-Dihydroxy-5-hydroxymethyl-piperidine (Isofagomine, **1**) a potent β-glucosidase inhibitor was synthesised in a 6 step synthesis in an overall yield of 34% starting from 1,6:2,3-dianhydro-4-*O*-benzyl-β-D-mannopyranose (**7**). A hydroxymethyl group was introduced at C-2 in **7** by epoxide opening with vinylmagnesium bromide followed by ozonolysis with reductive workup to give 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-*C*-hydroxymethyl-β-D-glucopyranose, **9**. Hydrolysis of the anhydro bond and subsequent oxidative carbon chain cleavage gave a pentodialdose, which was cyclised by reductive amination with ammonia affording the 4-*O*-benzyl derivative of **1**. After removing the protecting group by hydrogenation under acidic conditions **1** was isolated as its hydrochloride. Synthesis of **1** was also attempted from its lactam precursor. For this purpose 5-amino-2,3-anhydro-5-deoxy-4-*O*-*tert*-butyldimethylsilyl-D-lyxono-1,5-lactam **6** was synthesised in 5 steps from D-galactose. However, epoxide opening of **6** or **7** with the α-alkoxy cuprate dilithium benzoxymethyl-2-thienylcyanocuprate was unsuccessful.

INTRODUCTION

Many pyranoses with the ring oxygen replaced by an iminogroup are natural products and useful as potent glycosidase inhibitors.[1c] In the last decade it has been of interest to develop effective syntheses of such compounds and their analogues[1] for the investigation of glycosidase action and for the development of specific glycosidase inhibitors for treating metabolic disorders such as diabetes[2] or as antiviral, antibacterial and anticancer agents.[1b] A subject of debate is how these inhibitors interact with the enzyme. It is commonly thought that the glycosidase inhibitor should mimic the transition state in the hydrolysis of a glycosidic bond,[1d] and

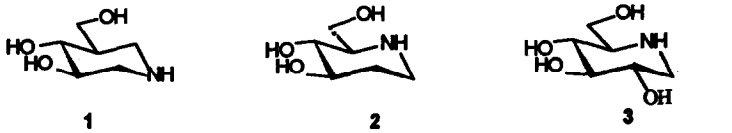
that in the design of new inhibitors both charge, configuration and conformation must be taken into account.[1e] It is also normally supposed that an important feature of a good glycosidase inhibitor is the replacement of the ring oxygen of the parent sugar by nitrogen.[1] Recently a catalytic antibody was developed that catalysed the tetrahydropyranyl ether hydrolysis reaction shown in Scheme 1.[3] It can be argued that this antibody acts as a glycosidase though on an extremely simple level. In the field of catalytic antibodies a transition state analog (TA) for the desired reaction is used as antigen to generate the antibodies,[4] and in this case the antigen employed was the piperidinium-ion shown in Scheme 1. With the knowledge of the composition of the usual inhibitors of glycosidases, it seemed surprising that this piperidine-derivative apparently was a TA for this simplified glycosidase model, because the nitrogen has replaced the anomeric carbon and not the ring oxygen.



TA for
catalytic antibody

Scheme 1

With this example in mind, it was of interest to synthesise a monosaccharide derivative with a nitrogen in place of the anomeric carbon to investigate its properties as a glycosidase inhibitor, particularly because no such compounds have been prepared previously. We decided to prepare a glucose analog (3R, 4R, 5R)-3,4-dihydroxy-5-hydroxymethyl-piperidine **1**, and named this isofagomine since the difference between **1** and fagomine **2** is the position of the nitrogen. We here report the preparation of **1** from levoglucosan in a 10 step synthesis giving an overall yield of 6%.



1 Isofagomine

2 Fagomine

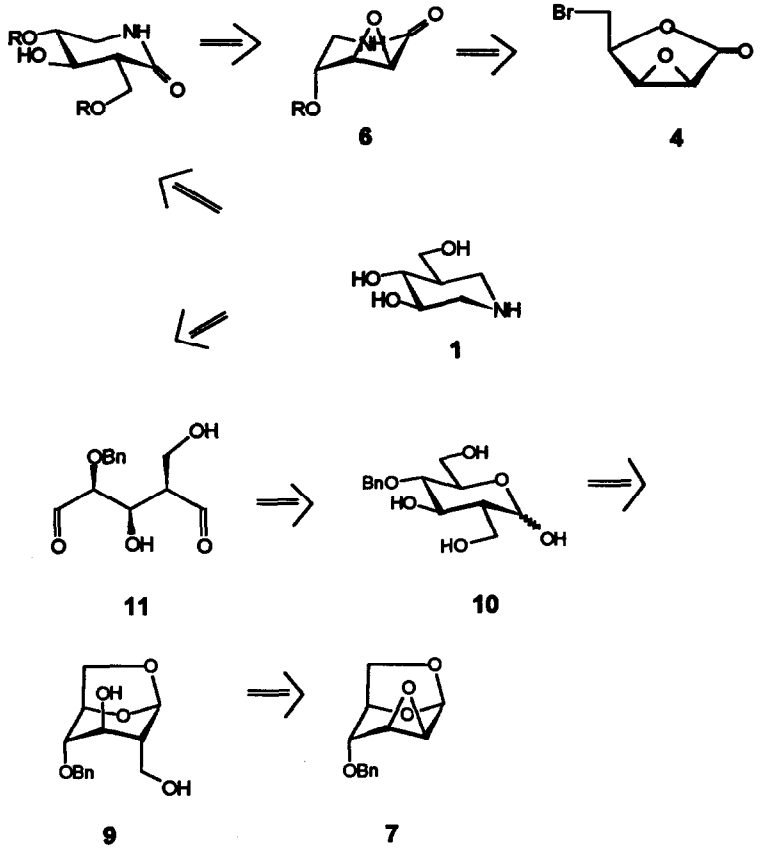
3 1-Deoxynojirimycin

Scheme 2

RESULTS AND DISCUSSION

Two routes for the synthesis of **1** were proposed as shown in the retrosynthesis Scheme 3. In strategy 1, **1** could be obtained from the corresponding lactam by reduction and removal of protecting groups. This lactam could be obtained by opening of the epoxylactam **6** with a nucleophile (for example an α-alkoxy-

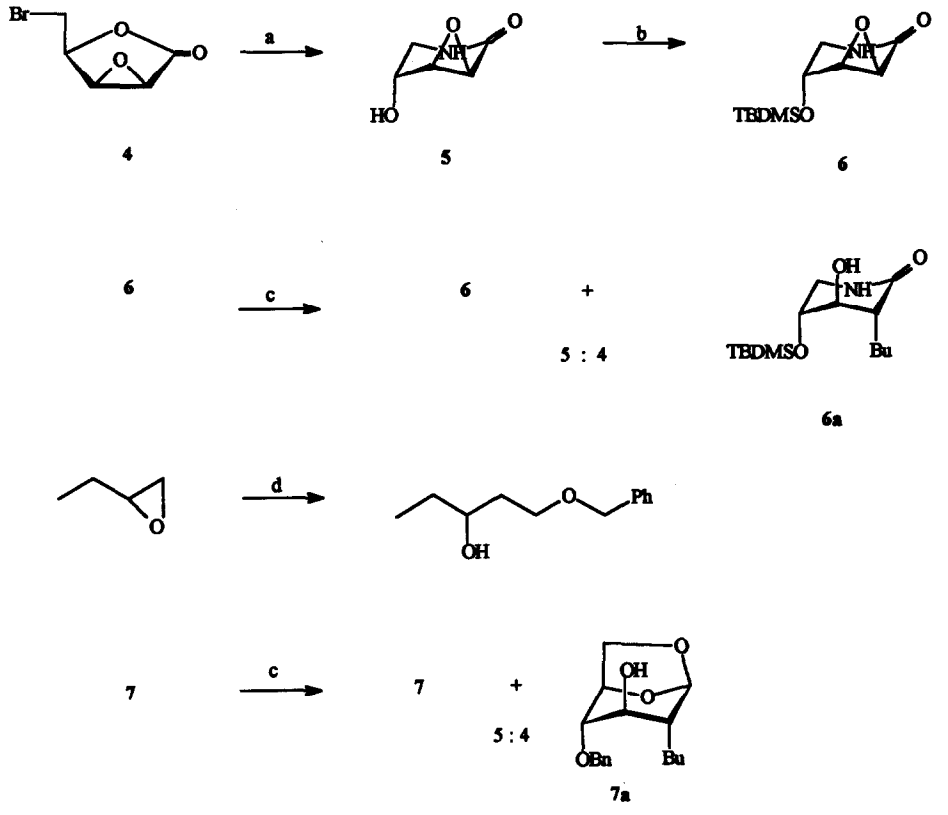
cuprate) to introduce the hydroxymethyl group. 6 Would be obtainable from the bromolactone 4 by treatment with aqueous ammonia[6] and protection of the hydroxy group. In strategy 2 the starting material was the chiral Cerny epoxide 7,[7] which could be opened with a suitable reagent to introduce a hydroxymethyl group at C-2 in a similar manner as proposed in strategy 1. After hydrolysis of the anhydro bond, the pentodialdose could be obtained by oxidative cleavage of the carbon chain. The piperidine 1 would be the expected product after reductive amination. Strategy 1 was first followed because it seemed a short promising route to the synthesis of 1.



Scheme 3: Retro synthesis of 1

The starting material, 2,3-anhydro-5-bromo-5-deoxy-D-lyxonolactone, easily prepared from D-galactose in 3 steps,[5] was treated with aqueous ammonia under controlled conditions to give the 5-amino-2,3-anhydro-5-deoxy-D-lyxono-1,5-lactam 5 in 79% yield as a crystalline compound. It was found by monitoring the reaction by ^{13}C-NMR that a reaction time of 45 minutes was optimal. 5 Was known as an intermediate in the synthesis of 2,5-diamino-2,5-dideoxy-D-lyxonolactam,[6] but had not been isolated before. The conformation of 5 was found to be $^{5}H_{4}$. This conclusion was based on assignment of the chemical shifts and the coupling constants. A long range coupling was observed between H-3 and H-5 eq (1.5 Hz), and no coupling constants

above 6 Hz were observed, indicating that H-4 was equatorial, and ruling out the $^4H_5$ conformation. This somewhat unexpected conformation explains why **5** was selectively opened at C-2 by ammonia[6] (in contrast to acyclic 2-epoxy amides which were opened at C-3) since opening at C-2 was a favorable *trans* diaxial opening. The hydroxy group of **5** was protected as its *tert*-butyl-dimethylsilyl (TBDMS) ether,[8] to give **6** in 84% yield as a crystalline compound. Next step was the introduction of a hydroxymethyl group. This was expected to be possible with the dilithium benzoxymethyl-2-thienyl-cyanocuprate. A higher order cyanocuprate ($R_2Cu(CN)Li_2$) was chosen because these are known to be more reactive than lower order cuprates (R_2CuLi).[9] The reagent was used together with borontrifluoroetherate, known to improve yields.[10] The cuprate was prepared by mixing benzyloxymethyl lithium, pregenerated from benzyloxymethyltributylstannane and butyllithium,[11] and lithium 2-thienyl cyanocuprate at -78 °C. It is important to realise that since α-alkoxyalkyllithiums are thermally unstable the reagent had to be prepared at -78 °C, which can be done by making it from a stable lower order cuprate.[12] The 2-thienyl moiety is a dummy group and will not be transfe-



a: NH_4OH (25%), 20 °C; b: TBDMSCl, imidazol, DMF, 20 °C; c: 1) $Bu_2Cu(CN)Li_2$, THF, -78 °C; 2) $BF_3.OEt_2$, -78 °C; d: 1) $(BnOCH_2)(2\text{-Th})Cu(CN)Li_2$, THF, - 78 °C; 2) $BF_3.OEt_2$, -78 °C.

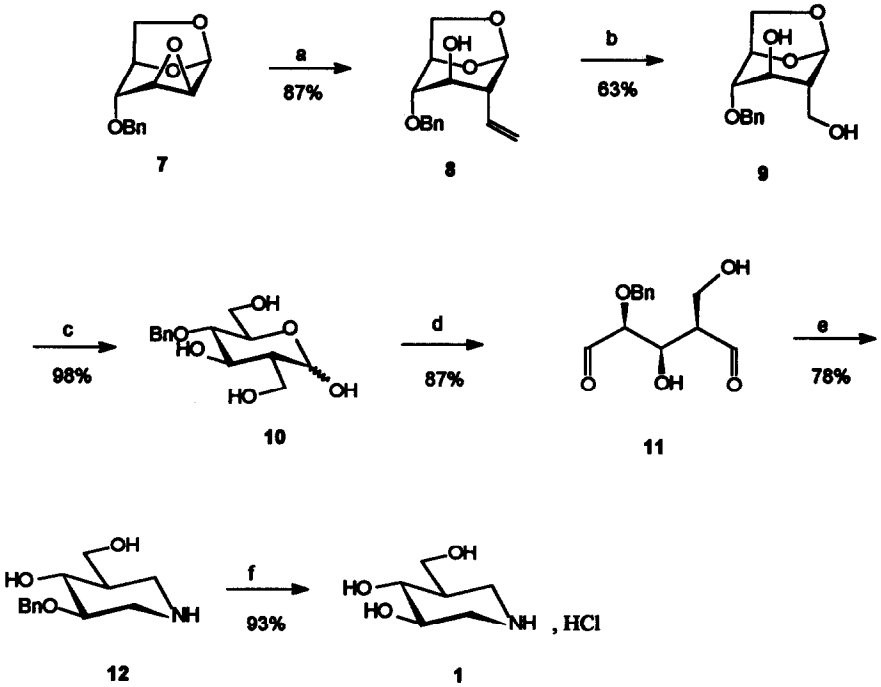
Scheme 4: Synthesis of epoxylactam and epoxide-openings

red.[9b,12] Before epoxide opening with the benzyloxymethyl cuprate was attempted, the simpler dilithium dibutyl-cyanocuprate[9a,9c] was used in a model experiment. Use of 2.8 equivalent of the butyl cuprate resulted in a 45% conversion of **6** into the 2-butyl lactam as verified by ^{13}C NMR (Scheme 4). The product was not isolated and the reaction was not optimised. With this positive result in mind the benzoxymethyl cyanocuprate was prepared and attempted to react with the epoxide **6**, but no reaction was observed and the epoxylactam was reisolated. With the primary epoxide 1,2-epoxybutane the cuprate reacted, however, to give 3-hydroxypentylbenzyl ether (Scheme 4) as verified by ^{13}C NMR. This indicated that the epoxylactam **6** was too stable or the cuprate too unreactive. Strategy 1 was abandoned at this stage and Strategy 2 was pursued.

The starting material 1,6:2,3-dianhydro-4-*O*-benzyl-β-D-mannopyranose **7** was synthesised as described by Cerny *et al*[7a,13] in a four step synthesis from the easily available 1,6-dianhydro-β-D-glucopyranose.[14] To introduce a hydroxymethyl group use of the cuprate was again attempted. As in the first case 45% of epoxide **7** was opened at C-2 with $Bu_2Cu(CN)Li_2$ (2 eq) and borontrifluoroetherate as was verified by ^{13}C-NMR. Reaction of $(BnOCH_2)(2\text{-Thienyl})Cu(CN)Li_2$ with **7**, however, gave only the starting material and tetrabutylstannane. The use of the α-alkoxycuprate was therefore abandoned. Ganem has described a procedure using alkyllithium and borontrifluoroetherate in substitution reactions.[15] **7** Was reacted with benzyloxymethyl lithium following this procedure, but without success. A third possibility to introduce a hydroxymethyl group was to open the epoxide with vinylmagnesium bromide followed by ozonolysis of the double bond and reduction. Treatment of **7** with 10 equivalents vinylmagnesium bromide[16] gave the known compound 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-*C*-vinyl-β-D-glucopyranose[17] **8** in 87% yield. Vinylmagnesium bromide was used in large excess to avoid/minimize bromohydrin byproduct. Ozonolysis of **8** followed by reductive work-up gave, as expected, 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-*C*-hydroxymethyl-β-D-glucopyranose **9** in 63% yield. Hydrolysis of the anhydro bond was easily achieved by gentle reflux in dilute sulfuric acid giving in quantitative yield an anomeric mixture of 4-*O*-benzyl-2-deoxy-2-*C*-hydroxymethyl-D-glucopyranose **10** as an oil. The β-anomer could be crystallised from ethyl acetate in 27% yield. Dissolution of the crystalline compound resulted in mutarotation. The optical rotation rose from +32.7° to +84.4° indicating that the crystalline material was the β-anomer, which was verified by proton NMR of the freshly dissolved crystals. H-1 gave a doublet at 4.48 ppm with a coupling constant of 10 Hz.

The next reaction was periodate cleavage of **10**. It is known that periodate cleavage between C-5 and C-6 in 4-O-alkylated glucose derivatives is slow.[18] To speed up the reaction $NaIO_4$ was used in excess, and a 1:1 mixture of methanol and water was used as solvent. Use of 3 equivalents for 192 h at 20 °C resulted in 57% yield of the product, but when 5 eq. $NaIO_4$ was used at 45 °C the yield of the pentadialdose was 72 to 87% after 3 h. ^{13}C-NMR of the product was very complex, due to several conformers and hydrates of the pentadialdose **11**. To verify that the product was the expected pentodialdose, a sample was reduced with sodium borohydride, which gave the corresponding polyol. Reductive amination using 12 M ammonia in ethanol under hydrogen pressure (3500 kPa) and Palladium on charcoal as catalyst was expected to yield the piperidine **1**. However, ^{13}C-NMR showed two products and surprisingly both had the 4-*O*-benzylgroup intact. The minor compound was tentatively identified as the 1,5-diamine resulting from reductive amination of both carbonyls of **11** with ammonia. This was confirmed by the fact that the amount of byproduct could be reduced by lowering the ammonia concentration. The concentration was therefore reduced from 12 M to 0.23 M which decreased the amount of byproduct from 22% to 5%. After chromatography the piperidine **12** was

obtained in 78% yield. The benzyl group was therefore reduced in acidic media by hydrogenation at 101 kPa with Palladium as catalyst to yield the piperidine **1** as its hydrochloride, a colourless syrup, in 93%. Confirmation and the stereochemistry of **1** was based on ^1H, decoupled ^1H, ^{13}C and 2D NMR spectra. Isofagomine clearly had the $^4C_1$ conformation as seen from the large ^1H-NMR proton-couplings (11-13 Hz) of the axial protons next to nitrogen, H-2ax and H-6ax, with H-3 and H-5 respectively.



a: $MgBrCHCH_2$, reflux, THF; b: 1) O_3, EtOH, 0 °C; 2) $NaBH_4$, EtOH/H_2O; c: 1M H_2SO_4, reflux; d: $NaIO_4$, H_2O/MeOH, 45 °C; e: NH_3, EtOH, H_2, Pd/C, 35 atm., 20 °C; f: HCl (aq), H_2, Pd/C, 1 atm.

Scheme 5

In this paper we have reported an efficient synthesis of **1** from the Cerny epoxide **7**. In addition we have found that a shorter route from a 2,3-epoxylactam, **6** not was possible due to difficulties in opening the epoxide. While the epoxide of **7** was equally unreactive it was a more stable compound that could be subjected to more vigerous reaction conditions.

Isofagomine **1** was found to be a potent glucosidase inhibitor. It is the strongest inhibitor of sweet almond β-glucosidase known, displaying a remarkable K_i value (K_i = 0.11 μM) which is 440 times lower than the K_i of 1-deoxy nojirimycin (K_i = 47.5 μM). Further details of its biological activity will be reported elsewhere.[19]

EXPERIMENTAL

^{13}C-NMR and ^{1}H-NMR spectra were recorded on Bruker instruments AC 200, AC 250 and AM 500. D_2O was used as solvent with DHO (^{1}H-NMR: 4.7 ppm) and acetone (^{1}H-NMR: 2.05 ppm; ^{13}C-NMR: 29.8 ppm) as reference. With $CHCl_3$ as solvent TMS and $CHCl_3$ (^{13}C-NMR: 76.93 ppm) were used as references. Mass spectra were obtained on a VG TRIO-2 instrument. Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter. Microanalyses were carried out by Leo Microanalytical Laboratory. Concentrations were performed on a rotary evaporator at a temperature below 40 °C. Dry tetrahydrofuran and diethyl ether were prepared by distillation from sodium and benzophenone. Borontrifluoroetherate was distilled (bp 124-125 °C) and stored under argon at 5 °C and used within a week. Cuprous cyanide was dried under vacuum at 125 °C for 16 h. Lithium 2-thienylcyanocuprate from Aldrich was used.

5-Amino-1,2-anhydro-5-deoxy-D-lyxono-1,4-lactame, **5**. Aqueous ammonia (25%, 10 ml) was added to 2,3-anhydro-5-bromo-5-deoxy-D-1,4-lyxono-lactone (**4**, 2.02 g, 10.5 mmol) and stirred for 45 min. The reaction mixture was concentrated and co-concentrated with toluene (2 x 20 ml). The residue (a brown syrup) was filtered through a column of silica gel using ethyl acetate followed by ethyl acetate/methanol 9:1 as eluent. One fraction was collected and concentrated to give **5** as a crystalline compound in 79% (1.07 g) yield: mp 144-145 °C. Recrystallisation from ethyl acetate/methanol 10:1 gave mp 147 °C, $[\alpha]_D^{20}$ = -28.7° (*c* 1.0, MeOH). ^{13}C-NMR (50 MHz, D_2O): δ 169.0 (C-1); 62.2 (C-4); 54.1 and 49.3 (C-2 and C-3); 42.3 ppm (C-5). ^{1}H-NMR (200 MHz, D_2O): δ 4.53 (ddd, 1H, H-4, $J_{3,4}$ = 6 Hz, $J_{4,5}$ = 3 Hz, $J_{4,5'}$ = 1.5 Hz); 3.79 (ddd, 1H, H-3, $J_{2,3}$ = 4 Hz, $J_{3,5'}$ = 1.5 Hz); 3.51 (d, 1H, H-2); 3.37 (dd, 1H, H-5, $J_{5,5'}$ = 14 Hz); 3.19 (dt, 1H, H-5'). Anal. Calc. for $C_5H_7NO_3$: C, 46.51; H, 5.46; N 10.85. Found: C, 46.05; H, 5.43; N, 10.82.

5-Amino-2,3-anhydro-5-deoxy-4-O-tert-butyldimethylsilyl-D-lyxono-1,5-lactame, **6**. To a solution of t-butyldimethylchlorosilane (0.42 g, 4.79 mmol) and imidazole (0.397 g, 5.81 mmol) in DMF (1 ml) in a dry flask, **5** (0.30 g, 2.3 mmol) dissolved in DMF (7 ml) was added dropwise. The mixture was stirred for 2 h under a nitrogen atmosphere. Dichloromethane (8 ml) was added, and the mixture was washed with 1N HCl (2 x 15 ml) and saturated $NaHCO_3$ (1 x 15 ml). The organic layer was dried with magnesium sulfate, filtered and concentrated to give **6** as a white crystalline compound in 84% (0.47 g) yield. Recrystallisation from hexane gave: mp 106.5-107 °C, $[\alpha]_D^{20}$ = -34.4° (*c*; 1.0, $CHCl_3$). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 168.0 (C-1); 64.3 (C-4); 54.7 and 50.3 (C-2 and C-3); 43.9 (C-5); 25.4 (t-Bu, 3 x CH_3); 17.8 (t-Bu, C); -5.0 ppm $(CH_3)_2Si$). ^{1}H-NMR (200 MHz, D_2O): δ 6.75 (broad, 1H, N-H); 4.3 (broad s, 1H, H-4); 3.45 (d, 1H, H-5'); 3.0 (dd, 1H, H-5); 2.9 and 2.8 (2 d, 2H, H-2 and H-3); 0.85 (9H, t-Bu); 0.5 (6H, $(CH_3)_2Si$). Anal. Calc. for $C_{11}H_{24}NO_3Si$: C, 54.29; H, 8.70; N, 5.82. Found: C, 54.09; H, 8.64; N, 5.82.

Dilithium dibutylcyanocuprate. To cuprous cyanide (0.115 g, 1.28 mmol) in a dry flask equipped with a septum, dry diethyl ether or THF (1.3 ml) was added under an argon atmosphere and cooled to -78 °C. Butyllithium 1.6 M in hexane (1.6 ml, 2.56 mmol) was added with a syringe. The flask was removed from the cooling bath until a darkening of the solution was observed. The solution was recooled to -78 °C and used *in situ*.

Dilithium benzyloxymethyl-2-thienyl-cyanocuprate. To benzoxymethyltributylstannane (0.822 g, 2.0 mmol), dry THF (2 ml) was added and cooled to -78 °C under an argon atmosphere in a dry flask equiped with a septum. Buthyllithium 1.6 M in hexane (1.25 ml, 2.0 mmol) was added. After stirring for 10 min lithium 2-thienyl-cyanocuprate 0.25 M in THF (8.0 ml, 2.0 mmol) was added slowly to the yellow solution at -78 °C. The colour changed to orange/brown. The cuprate was used *in situ* at -78 °C after stirring for another 20 min.

Reaction between dilithium dibutylcyanocuprate and 6. To a solution of dilithium dibutylcyanocuprate (2.0 mmol) in ether, prepared as described above, a solution of **6** (0.17 g, 0.7 mmol) dissolved in dry diethyl ether (2 ml) was added followed by $BF_3.OEt_2$ (275 µl, 2.2 mmol). The mixture was allowed to warm slowly to 20 °C. After 2 h aq ammonia/saturated ammonium chloride 1:1 (4 ml) was added. The mixture was stirred until 2 homogeneous phases appeared. The aqueous layer was extracted with ethyl acetate (5 x 5 ml). The collected organic layers were dried with magnesium sulfate, filtered and concentrated. The residue was a yellow oil containing 45% of the desired product **6a** and 55% **6** as seen from ^{13}C-NMR. ^{13}C-NMR of **6a** (50 MHz, $CDCl_3$): δ 72.4, 70.7 (C-3, C-4); 50.2 (C-5); 44.8 (C-2); 28.2, 27.8, 22.9, 14.0 (Bu); 25.5, 17.8, -4.9 ppm (TBDMS).

Reaction between dilithium dibutylcyanocuprate and 7. To a solution of dilithium dibutylcyanocuprate (2.56 mmol) in THF, prepared as described above, a solution of **7** (0.30 g, 1.28 mmol) in dry THF (2 ml) was added followed by BF_3. OEt_2 (356 µl, 2.82 mmol). After stirring for 1.5 h at -78 °C the mixture was allowed to warm slowly to 20 °C. Work-up after 3.5 h as above resulted in a yellow oil containing 45% of the desired product **7a** and 55% **7** as seen from ^{13}C-NMR. ^{13}C-NMR **7a** (50 MHz, $CDCl_3$): δ 138-127 (Ph); 103.4 (C-1), 79.0 (C-4), 74.5 (C-3), 71.1 ($O\underline{CH_2}Ph$), 69.4 (C-5), 65.0 (C-6), 46.1 (C-2), 29.3, 28.5, 22.4, 13.7 (Bu).

Reaction between dilithium benzoxymethyl-2-thienyl-cyanocuprate and 6. To a solution of dilithium benzoxymethyl-2-thienyl-cyanocuprate (2.0 mmol), prepared as described above, epoxylactam **6** (0.25 g, 1 mmol) disolved in THF (2 ml) was added. Finaly $BF_3.OEt_2$ (0.63 ml, 5.0 mmol) was either added or not added. After stirring for 5 h at -78 °C the solution was allowed to warm slowly to 20 °C. After work-up as above starting material and tetrabutylstannane were reisolated independent on the presence or abscence of $BF_3.OEt_2$.

Reaction between dilithium benzoxymethyl-2-thienyl-cyanocuprate and 7. The reaction was carried out as described above but using **7** (1 mmol) in THF instead of **6** in diethyl ether, and using $BF_3.OEt_2$. Tetrabutylstannane and **7** were recovered.

Reaction between benzoxymethyllithium and epoxide 7. To benzoxymethyltributylstannane (1.23 g, 3.0 mmol) dry THF (2 ml) was added under an argon atmosphere, and the solution was cooled to -78 °C. Butyllithium 1.6 M in hexane (1.8 ml, 3.0 mmol) was added through a septum. The solution turned yellow. After stirring for 10 min. epoxide **7** (0.234 g, 1.0 mmol) dissolved in dry THF (2 ml) was added. The yellow colour disappeared, and $BF_3.OEt_2$ (0.63 ml, 5.0 mmol) was added. After stirring for 2 h at -78 °C a saturated solu-

tion of sodium hydrogencarbonate (3 ml) was added, and the mixture was allowed to heat to 20 °C. The aqueous layer was extracted with ethyl acetate (3 x 10 ml). The collected organic layers were dried with magnesium sulfate, filtered and concentrated. The residue contained unreacted **7** and tetrabutylstannane.

3-Hydroxypentyl benzylether. Dilithium benzoxymethyl-2-thienyl-cyanocuprate (2.0 mmol) was prepared as described above. 1,2-epoxybutane (92 µl, 1.0 mmol) was added followed by $BF_3.OEt_2$ (0.40 ml, 3.2 mmol). After stirring for 2 h at -78 °C followed by work-up as above, a crude product was obtained, which was chromatographed using ethyl acetate/pentane 1:2. 2-Hydroxypentyl benzyl ether (0.106 g, 44%) was collected in a fraction which contained also benzylalcohol (50%). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 137.5, 126.1, 127.4 (Ph), 72.7, 72.0 (C-1, O<u>C</u>H_2Ph), 68.6 (C-2), 35.6 (C-3), 29.8 (C-4), 9.6 ppm (C-5). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.25 (s, 5H, Ph), 4.45 (s, 2H, <u>C</u>H_2Ph), 3.65 (m, 3H, 2 H-1, H-3), 3.4-3.1 (broad, 1H, OH), 1.65 (m, 2H, 2 H-2), 1.4 (sext., 2 H, 2 H-4), 0.9 ppm (t, 3H, 3 H-5).

1,6-Anhydro-4-O-benzyl-2-deoxy-2-C-vinyl-β-D-glucopyranose, **8**. To a stirred solution of 1,6:2,3-dianhydro-4-*O*-benzyl-β-D-mannopyranose[7,13] (**7**, 5.0 g, 21.3 mmol) in dry THF (25 ml), was added a solution of 1.77 M vinylmagnesium bromide[16] in THF (120.5 ml, 213 mmol). The mixture was refluxed gently for 3 h at 60 °C. After cooling to room temperature 2 M NH_4Cl (650 ml, pH 8) was slowly added. The aqueous layer was extracted with ethyl acetate (2 x 250 ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The crude product (4.87 g, 87%) was used directly in the next reaction, but could be purified by flash chromatography using ethyl acetate/pentane 1:2 as eluent, to give 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-*C*-vinyl-β-D-glucopyranose (**8**) as a white crystalline compound in 76% (4.23 g) yield. After recrystallization from ethanol: mp. 88-91 °C; $[\alpha]_D^{20}$ = -2.3° (*c* 4.1, $CHCl_3$) (lit[17] mp. 91-93 °C $[\alpha]_D^{20}$ = +1.2° (*c* 1.3, $CHCl_3$)). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 135.5 (CH=), 137.7, 128.3, 127.6, 127.5 (Ph), 117.2 (CH_2=), 103.5 (C-1), 78.8 (C-4), 74.6 (C-3), 71.3, 70.4 (C-5, O<u>C</u>H_2Ph), 65.4 (C-6), 51.8 ppm (C-2).[17] ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.35 (d, 5H, Ph), 5.99 (m, 1H, <u>C</u>H=CH_2), 5.43 (s, 1H, H-1), 5.25 and 5.17 (s, 1H og d, 1H, CH_2=), 4.63 (dd, 3H, O<u>C</u>H_2Ph and H-5), 4.09 and 3.75 (d, 1H and m, 2H, H-3, H-6 and H-6'), 3.43 (s, 1H, H-4), 2.80 (broad s, 1H, OH), 2.45 (d, 1H, H-2).[17] Anal. Calc. for $C_{15}H_{18}O_4$: C, 68.69; H, 6.92. Found: C, 68.58; H, 6.94.

1,6-Anhydro-4-O-benzyl-2-deoxy-2-C-hydroxymethyl-β-D-glucopyranose, **9**. A stream of ozone (0.36 mmol/min) was passed through a solution of 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-*C*-vinyl-β-D-glucopyranose (**8**, 3.36 g, 12.8 mmol) in ethanol (100 ml) for 43.5 min. The solution of the ozonide was transfered to a three-necked round bottomed flask equipped with thermometer and a dropping funnel containing a solution of $NaBH_4$ (3.87 g, 102 mmol) in ethanol/water 1:1 (35 ml). The solution of $NaBH_4$ was added dropwise maintaining the temperature below 20 °C (cooling on ice bath). After stirring for 45 min Amberlite IR 120, H^+ (100 ml) was added. After additional stirring for 30 min. the ion exchange resin was filtered off and rinsed with water. The solution was evaporated and co-concentrated with methanol (3 x 80 ml) leaving a colourless syrup (3.28 g). This syrup was flash chromatographed using ethyl acetate/pentane 2:1 and ethyl acetate as eluent to give **9** (2.14 g, 63%) as a crystalline compound. In some runs the crude syrup crystallised and was recrystallised from chloroform: mp. 111-113°; $[\alpha]_D^{20}$ = -41.4° (*c*; 2.05, CH_3OH). ^{13}C-NMR (50 MHz, D_2O):

δ 139, 130.1, 129.8 (Ph); 102.0 (C-1), 80.5 (C-4), 76.1 (C-3), 72.9 (O<u>CH</u>$_2$Ph), 67.4 (C-5), 66.2 (C-6), 61.7 (C-2'), 49.0 ppm (C-2). Anal. Calc. for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.14; H, 6.79.

1,6-Anhydro-4-O-benzyl-2-deoxy-2-C-hydroxymethyl-D-glucopyranose, 10. 1,6-Anhydro-4-*O*-benzyl-2-deoxy-2-*C*-hydroxymethyl-β-D-glucopyranose (**9**, 1.59 g, 6.0 mmol) was dissolved in 1 M sulfuric acid (30 ml) by heating to reflux and the solution was boiled for 1 h. After cooling to room temperature the solution was poured through a column containing Amberlite IR 67, OH^- (120 ml). The column was rinsed with water followed by methanol (400 ml). Concentration of the eluate gave 1.67 g (98%) of a colourless syrup which was employed in the next reaction. However, purification using flash chromatography with ethyl acetate and ethyl acetate/methanol 10:1 as eluent gave a product in 76% (1.29 g) yield. One of the anomers (β) could be obtained by crystallisation from ethyl acetate in 27% yield as a white crystalline compound. Mp. 102-105 °C, $[\alpha]_D^{20}$ = +32.7° → +84.4° (*c* 1.0, CH_3OH). ^{13}C-NMR (50 MHz, D_2O): δ 136.8, 128.3, 128.0 (Ph), 94.1 (C-1, β), 91.4 (C-1, α), 78.6 (C-4), 74.4 (C-3), 70.3 (O<u>CH</u>$_2$Ph, C-6 β), 69.5 (C-6, α), 60.3 (C-5), 59.1 (C-2', α), 56.6 (C-2', β), 49.9 (C-2, β), 47.7 ppm (C-2, α). ^{1}H-NMR (200 MHz, D_2O): δ 5.13 (d, 1H, H-1 (α), $J_{1,2}$ = 3.5 Hz), 4.48 ppm (d, 1H, H-1 (β), $J_{1,2}$ = 10 Hz). Anal. Calc. for $C_{14}H_{20}O_6$ x 0.3 H_2O: C, 58.04; H, 7.17. Found: C, 58.07; H, 7.19.

4-O-Benzyl-2-deoxy-2-C-hydroxymethyl-D-xylo-pentodialdose, 11. To a solution of 4-*O*-benzyl-2-deoxy-2-*C*-hydroxymethyl-D-glucopyranose **10** (1.41 g, 5.0 mmol) in methanol (15 ml) was added dropwise a solution of sodium periodate (5.35 g, 25.0 mmol) in water (50 ml) over 15 min. Further methanol (25 ml) was added. The mixture was stirred at 45 °C for 3 h. The precipitated iodate was filtered off, and the mixture was concentrated. By solution of the residue in ethyl acetate/ethanol 1:1 (80 ml) more iodate was precipitated and filtered off. The mother liquour was concentrated and the residue (2.07 g) was flash chromatographed using ethyl acetate as eluent giving **11** as a yellow syrup in 87% (1.09 g) yield. A second fraction containing 9% of unreacted starting material (0.13 g) was isolated. The purified product was used immediately in the next reaction. The ^{13}C-NMR spectrum was complex and the product was identified as the reduced polyol: To a solution of **11** (0.16 g, 0.63 mmol) in ethanol/water 1:1 (4 ml) a solution of $NaBH_4$ (0.21 g, 5.6 mmol) in ethanol/water 1:1 (8 ml) was added dropwise keeping the temperature below 10 °C. The reaction mixture was stirred for 45 min at room temperature. Amberlite IR 120, H^+ (6 ml) was added. After stirring for 30 min the Amberlite was filtered off and rinsed with water and ethanol. The filtrate was concentrated and co-concentrated with methanol (3 x 6 ml) to give the polyol. ^{13}C-NMR (50 MHz, D_2O): δ 140.0, 131.1, 130.7 (Ph); 82.0 (C-4); 75.0 (O<u>CH</u>$_2$Ph); 71.8 (C-3); 62.9, 62.2, 61.6 (C-1, C-2', C-5); 46.6 ppm (C-2). ^{1}H NMR (200 MHz, D_2O): δ 7.45 (s, 5H, Ph); 4.70 (2 d, 2H, <u>OCH</u>$_2$Ph); 3.87 (k, 1H); 3.82 (d, 2H); 3.78 (t, 1H); 3.69 (k, 2H); 3.58 (dd, 2H); 1.95 ppm (sext, 1H, H-2). MS (CI, NH_3): m/z 257 ($M + H^+$), 274 ($M + NH_4^+$).

(3R, 4R, 5R)-3-Benzyloxy-4-hydroxy-5-hydroxymethyl-piperidine, 12. To a solution of 4-*O*-benzyl-2-deoxy-2-*C*-hydroxymethyl-D-*xylo*-pentodialdose (**11**, 1.77 g) in ethanol (40 ml) was added 0.29 M NH_3 in ethanol (162 ml) and 5% palladium on charcoal (300 mg). The mixture was hydrogenated at 3500 kPa at 20° for 15 h. The reaction mixture was filtered and concentrated. The residue (1.85 g) was flash chromatographed using ethanol/NH_4OH (25% aq)/triethylamine 122:2:1 as eluent giving the product **12** as a colourless syrup (becoming coloured on storage) in 78% yield. $[\alpha]_D^{20}$ = +13.5° (*c*; 1.0, EtOH). ^{13}C-NMR (62.9 MHz,

D_2O): δ 138.1, 128.1, 127.3 (Ph); 80.6 (C-3), 74.9 (C-4); 71.8 ($\underline{OCH_2}Ph$); 62.5 (C-5'); 48.0 (C-2); 46.8 (C-6); 45.0 ppm (C-5). ^{1}H-NMR (500 MHz, D_2O): δ 7.3 (s, 5H, Ph); 4.65, 4.51 (2 d, 2H, $\underline{OCH_2}Ph$, J_{gem} = 12 Hz); 3.66 (dd, 1H, H-5a', $J_{5a',5b'}$ = 10 Hz, $J_{5a',5}$ = 5.5); 3.57 (dd, 1H, H-5'b, $J_{5b',5}$ = 4.5 Hz); 3.41 (dd, 1H, H-4, $J_{3,4}$ = 11 Hz, $J_{4,5}$ = 9 Hz); 3.3 (broad, N-H); 3.27 (dd, 1H, H-3, $J_{3,2ax}$ = 11 Hz, $J_{3,2eq}$ = 4 Hz); 3.22 (dd, 1H, H-2eq, $J_{2eq,2ax}$ = 11 Hz); 2.97 (dd, 1H, H-6 eq, $J_{6eq,\ 6ax}$ = 12, $J_{6eq,5}$ = 4 Hz); 2.38 (t, 1H, H-2ax, $J_{2ax,2eq} = J_{2ax,3}$ = 11 Hz); 2.31 (t, 1H, H-6ax, $J_{6ax,6eq} = J_{6ax,5}$ = 12 Hz); 1.80 ppm (m, 1H, H-5). MS (CI, NH_3): m/z 238 ($M + H^+$).

(3R, 4R, 5R)-3,4-Dihydroxy-5-hydroxymethyl-piperidine hydrochloride, 1. (3R, 4R, 5R)-3-Benzyloxy-4-hydroxy-5-hydroxymethyl-piperidine, (**12**, 0.527 g, 2.2. mmol) was dissolved in ethanol (50 ml). 0.5 M HCl (5.3 ml) and 5% palladium charcoal (300 mg) was added. The mixture was hydrogenated at 101 kPa and 20 °C for 18 h. The reaction mixture was filtrered and concentrated to give **1** in 93% (0.375 g) yield. ^{13}C-NMR (50 MHz, D_2O): δ 70.7 and 68.1 (C-3 and C-4); 58.6 (C-5'); 46.2 and 44.4 (C-2 and C-6); 40.6 ppm (C-5). ^{1}H-NMR (500 MHz, D_2O, pH < 1, ref. HOD 4.63 ppm): δ 3.72 (dd, 1H, H-5b', $J_{5a',5b'}$ = 11.5, $J_{5,5b'}$ = 3.3 Hz); 3.67 (ddd, 1H, H-3, $J_{3,2ax}$ = 11.2, $J_{3,4}$ = 8.9, $J_{3,2eq}$ = 4.9 Hz); 3.64 (dd, 1H, H-5a', $J_{5a',5}$ = 6.2 Hz); 3.43 (ddd, 1H, H-2eq, $J_{2eq,2ax}$ = 12.7, $J_{2eq,3}$ = 4.9, $J_{2eq,6eq}$ = 2.0 Hz); 3.42 (dd, 1H, H-4, $J_{4,5}$ = 10.5 Hz); 3.41 (ddd, 1H, H-6eq, $J_{6eq,6ax}$ = 13.4, $J_{6eq,5}$ = 3.8, $J_{6eq,2eq}$ = 2.0 Hz); 2.87 (dd, 1H, H-6ax, $J_{6ax,5}$ = 12.2 Hz); 2.78 (dd, 1H, H-2ax); 1.86 ppm (ddddd, 1H, H-5). If necessary the product could be chromatographed using ethanol/NH_4OH (25% aq) 10:1 to give the corresponding free base $[\alpha]_D^{20}$ = +19.6° (*c* 0.85, EtOH). MS (CI, NH_3): m/z 148 ($M + H^+$). Anal. Calc. for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52. Found: C, 48.46; H, 9.33; N, 9.17.

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