

Preparation of ^{14}C -labelled 1,4-Dideoxy-1,4-imino-D-arabinitol: Cyanosilylation of Cyclic Imines using KCN in a One-Pot Synthesis

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SUMMARY

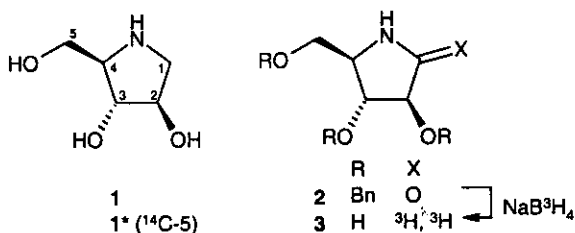
A new method for C-C bond formation was developed based on *in situ* cyanosilylation of cyclic Schiff bases using KCN, TMSCl, KI and ZnI₂. This method was used to prepare the potent α -glucosidase inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol ^{14}C labelled at C-5.

Keywords: *in situ cyanosilylation; ^{14}C labelled hydroxylated pyrrolidines; glycosidase inhibitors.*

INTRODUCTION

Since 5- and 6-membered iminosugars were discovered to be glycosidase inhibitors their synthesis has attracted major attention [1,2,3,4]. Specific inhibitors of glycosidases are of importance in a number of quite different applications which include mechanistic studies of the enzymatic reactions caused by the glycosidases. Studies of the mechanism of the enzymatic hydrolysis of glycosidic linkages and the exact nature of the transition state for such reactions have been the subject of much discussion, and approaches to identify the active site of the glycosidases have been targeted by studying the behaviour of specific inhibitors [2,3,4]. Strong and specific glycosidase inhibitors have potential medical interest and some have been used in the study or treatment of diabetes, cancer and viral diseases [4].

1,4-Dideoxy-1,4-imino-D-arabinitol (DAB1, **1**, Scheme 1) is a potent α -glucosidase inhibitor which has been isolated from the Fern *Arachniodes standishii* and *Angylocalyx boutiqueanus* (Leguminosae) [5,6,7]. The biological importance of α -glucosidase inhibitors has initiated a number of different synthetic approaches for preparation of **1**. The first synthesis was reported by Fleet and co-workers who used a protected derivative of D-xylofuranose as the starting material [5,8]. Later, a chemoenzymatic synthesis of **1** has been described where an enzyme catalysed aldol condensation of azidoacetaldehyde and dihydroxyacetone phosphate was the key step [9,10,11]. Recently, two additional syntheses of the iminoalditol **1** have appeared, one starting from D-(-)-diethyl tartrate in a multistep procedure via a 2-deoxy pentose derivative [12], and the other using D-gluconolactone as the synthon [13].



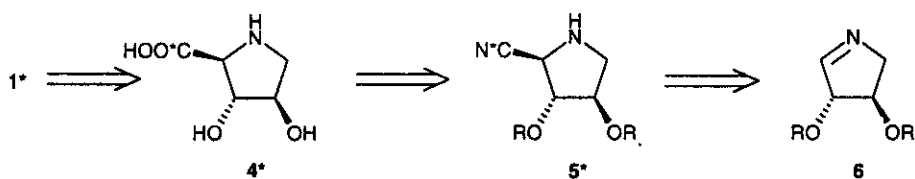
Scheme 1

Due to the potential medicinal use of DAB1 a study of the metabolic and distributional fate of the molecule was undertaken. In order to follow the metabolism of the inhibitor, access to radiolabelled analogues was crucial. Two isotopes that may be considered are ³H and ¹⁴C, either of which may be preferred, depending on the method used for the measurements. Previously, we have prepared the C-1 tritiated version of DAB1 (**3**) in two steps from 4-amino-4-deoxy-2,3,5-tri-*O*-benzyl-D-arabinono-1,4-lactam (**2**) by reduction of the lactam moiety with NaB³H₄ followed by deprotection [14] (Scheme 1). This procedure gave tritiated DAB1 **3** with a specific activity of 4 Ci/mmol and a radiochemical purity > 95 %. The lactam **2** was prepared from 2,3,5-tri-*O*-benzyl-D-arabinono-1,4-lactone in four steps following literature procedures [15].

For some metabolic studies, however, the preferred isotope would be ¹⁴C, and the aim of the present work was to synthesise ¹⁴C-labelled DAB1 in an efficient and practical way involving only a few synthetic steps using radioactive material.

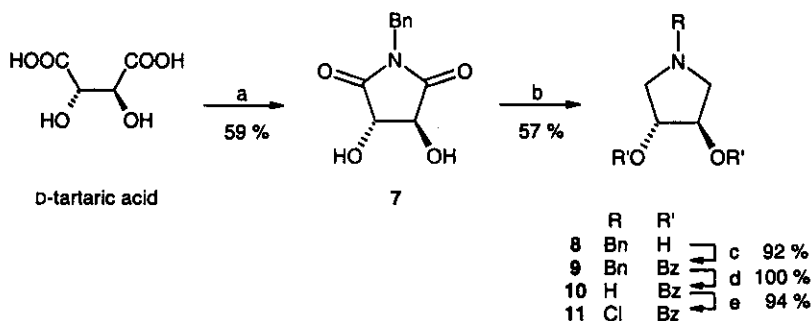
RESULTS AND DISCUSSION

A strategy for establishing C-C bonds and thereby introducing ^{14}C was needed, and this step should be performed late in the synthesis. Retrosynthetic analysis led to a strategy based on cyanosilylation of the imine **6** (Scheme 2). With the aim of using ^{14}C -labelled material we here present a one-pot procedure for the cyanosilylation *in situ* of an imine based on the inexpensive potassium cyanide (KCN). Hydrolysis of the thus-formed nitrile **5*** to the carboxylic acid **4*** followed by reduction required only two steps with radioactive material to obtain ^{14}C -labelled DAB1 (**1***).



Scheme 2

The target molecule for introduction of ^{14}C in DAB1 (**1***) was thus the dihydroxylated pyrroline **6**. This can be prepared by elimination of HCl from (3*R*,4*R*)-3,4-dibenzoyloxy-1-chloropyrrolidine (**11**) which in turn could be obtained from D-tartaric acid in five steps by a slightly modified literature procedure [16] (Scheme 3).

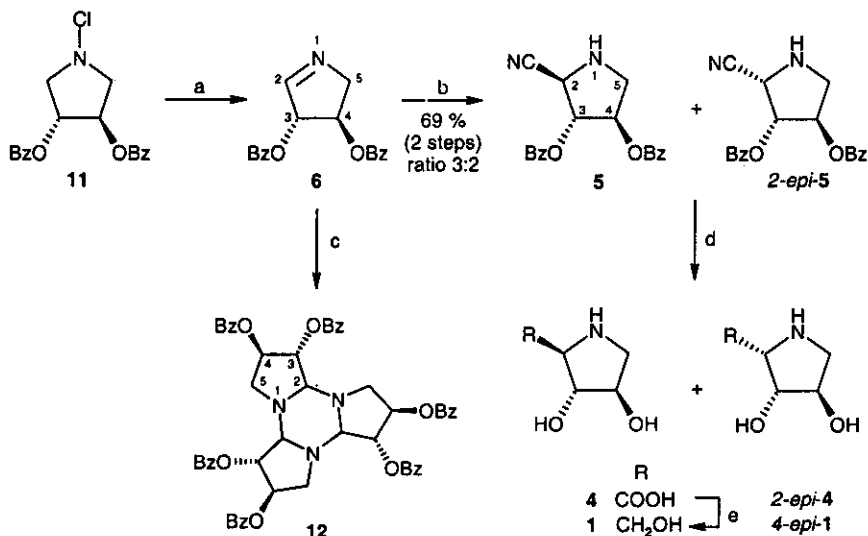


a) BnNH₂, xylene, reflux; b) NaBH₄, BF₃·Et₂O, diglyme; c) BzCl, Na₂CO₃, CH₂Cl₂, H₂O; d) H₂, Pd/C, AcOH, e) NCS, Et₂O.

Scheme 3

Thus, reaction of D-tartaric acid with benzylamine in refluxing xylene gave the *N*-benzylated imide **7** which by reduction using NaBH₄ and boron trifluoride etherate in diethyleneglycol dimethylether (diglyme) afforded the pyrrolidine **8** [17]. In this step

we have successfully substituted LiAlH_4 with NaBH_4 [16,18]. A modified benzoylation of **8** followed by catalytic reduction afforded the pyrrolidine dibenzoate **10** which by treatment with *N*-chlorosuccinimide gave the *N*-chloropyrrolidine **11** in 94 % yield [16]. This compound was stable only at -20°C or below and was kept at this temperature until use. Elimination of HCl to give the cyclic Schiff base **6** in a quantitative yield was accomplished by using 2 eq. of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene (Scheme 4). This product has been prepared and used *in situ* [16], but we have been able to isolate and characterise **6** by NMR spectroscopy and HPLC. During work-up we have, however, also observed a spontaneous polymerisation to give a product to which we have assigned the trimeric structure **12** based on ^{13}C -NMR spectroscopy. These results are in accordance with previously reported polymerisation of cyclic imines [19,20,21]. In order to avoid this trimerisation the imine **6** was used directly without previous concentration of the purified toluene solution.



a) DBU, toluene, rt; b) i) KCN, TMSCl, KI, MeCN, rt, overnight, ii) **6**, cat. ZnI_2 , toluene, rt; c) aq. workup and evaporation at rt; d) 6 N HCl, AcOH, 100°C , overnight; e) i) HMDS, TMSCl, pyridine, 80°C , 1h, ii) $\text{H}_3\text{B}\cdot\text{SMe}_2$, dioxane, 100°C , 5h.

Scheme 4

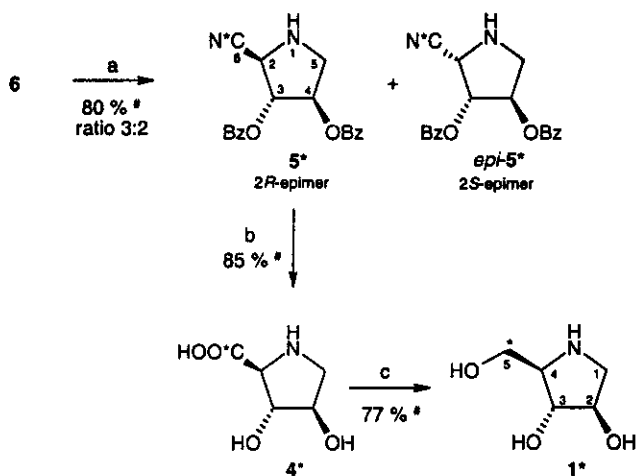
Synthesis of α -aminonitriles can be performed by cyanosilylation of Schiff bases (imines) using trimethylsilylcyanide (TMSCN) [22,23] and our synthetic strategy for introduction of ^{14}C was based on this reaction using ^{14}C -labelled TMSCN. Thus, various methods for generation and reaction of TMSCN *in situ* from commercially available potassium ^{14}C -cyanide were investigated. The method was developed using non-radioactive material and is of general interest because of the easily available reagents potassium cyanide and trimethylsilyl chloride (TMSCl).

TMSCN can be prepared from TMSCl and KCN catalysed by potassium iodide (KI), whereby the highly reactive trimethylsilyl iodide is formed as an intermediate [24]. The cyanosilylation of an imine with TMSCN is catalysed by ZnI_2 since the Lewis acid activates the reaction by complex formation with the imine [16]. By combination of these requirements we investigated the *in situ* cyanosilylation by addition of the imine **6** and ZnI_2 to a solution of KCN, TMSCl and a catalytic amount of KI. The solvents investigated were acetonitrile and *N*-methylpyrrolidone at room temperature and at 90 °C. The choice of a polar aprotic solvent should favour the solvation of K^+ , thereby increasing the nucleophilicity of CN^- [25,26]. The best results were obtained using acetonitrile at room temperature, since the imine **6** trimerised at higher temperatures (Scheme 4). Reaction of **6** under the optimised conditions gave the diastereomeric products **5** and 2-*epi*-**5**, which were formed in a total yield of 69 % based on **11**. The procedure can be extended to other imines and is advantageous since inexpensive chemicals are used which are also easy to handle.

The configurations of **5** and 2-*epi*-**5** were determined by $^1\text{H-NMR}$ spectroscopy to be 2,3-*trans* (2*R*) and 2,3-*cis* (2*S*), respectively (Scheme 5). The doublet at 4.26 ppm ($J = 1.5$ Hz) was assigned to the H-2 proton of the product having the 2*R* configuration (**5**), and the doublet at 4.71 ppm ($J = 6$ Hz) to the H-2 proton of the 2*S* epimer (2-*epi*-**5**). The pattern of the signals and the coupling constants for *cis* vs *trans* substituents in cyclic compounds have been reported for the cyclopropane system [27]. It was found that protons *cis* to a given substituent in general resonate upfield compared to protons *trans* to the same substituent. Normally the vicinal coupling constant between *cis* protons is larger than the coupling constant between *trans* protons in 5-membered rings.

The epimeric mixture of the cyanopyrrolidines **5** and 2-*epi*-**5** was hydrolysed in acetic acid/ 6 N HCl to give the corresponding amino acids **4** and 2-*epi*-**4** in quantitative yield. Finally, **1** and 4-*epi*-**1** were obtained in 75 % yield by reduction of the amino acids using $\text{BH}_3\text{-SMe}_2$ in dioxane (Scheme 4). NMR data of the epimeric mixture of **1** and 4-*epi*-**1** were in accordance with similar literature data of DAB1 (**1**) [13,28] and 1,4-dideoxy-1,4-imino-L-xylitol (4-*epi*-**1**) [13]. This also confirmed the assigned stereochemistry to **5** and 2-*epi*-**5**.

In synthesis using radiolabelled compounds it is important to secure complete conversion of the radioactive material. Thus the imine **6** was added in slight excess to the preformed solution of TMS^{14}CN as described above. In this case 1.0 molar eq. K^{14}CN , 2.0 molar eq. TMSCl and a catalytic amount of KI were stirred in acetonitrile overnight. Then 1.2 molar eq. of 3,4-dibenzoyloxy-1-pyrroline **6** in toluene and a catalytic amount of ZnI_2 were added. After 4 hours at room temperature work up gave a diastereomeric mixture of labelled cyanopyrrolidines **5*** and 2-*epi*-**5*** in the ratio 3:2 according to HPLC analysis (Scheme 5).



^a radiochemical yield

a) i) 1 eq. K¹⁴CN, 2 eq. TMSCl, cat. KI, MeCN, rt, overnight, ii) 1.2 eq. 6, cat. ZnI₂, toluene, rt, 4h; b) 6 N HCl, AcOH, 100 °C, overnight; c) i) HMDS, TMSCl, pyridine, 80 °C, 1h, ii) H₃B·SMe₂, dioxane, 100 °C, 5h.

Scheme 5

Separation of the two nitriles 5* and 2-*epi*-5* by preparative HPLC led to the pure labelled compounds (> 98%). ¹H-NMR spectra of the individual compounds were in agreement with the assignment of the epimeric mixture 5 and 2-*epi*-5.

The cyanopyrrolidine 5* was hydrolysed as described above to give the ¹⁴C-carboxylic acid 4*, which was separated from benzoic acid by eluting an aqueous solution of the product through a RP-SepPak. The product 4* was obtained in a 85 % radiochemical yield.

The amino acid 4* was silylated *in situ* prior to reduction using H₃B·SMe₂ in dioxane at 100 °C for 4.5 hours [29]. After work up [¹⁴C]-1,4-dideoxy-1,4-imino-D-arabinitol (1*) was isolated as its hydrochloride in 77 % radiochemical yield and 95 % radiochemical purity, with a specific radioactivity of 55 mCi/mmol as determined by mass spectrometry.

CONCLUSION

In summary, we have developed a convenient one-pot method for ¹⁴C-cyanosilylation of 1-pyrrolines, a method which might be useful for generating C-C bonds in similar systems. In the synthesis inexpensive and readily available potassium cyanide and trimethylsilyl chloride have been used as an alternative to trimethylsilyl cyanide. The cyanopyrrolidines were hydrolysed to the amino acids which in turn were reduced to the primary alcohols to give the target molecules 1,4-dideoxy-1,4-imino-pentitols.

This work has resulted in development of a one-pot procedure for the synthesis of ^{14}C -labelled 3,4-dibenzoyloxy-2-cyanopyrrolidine (**5*** and 2-*epi*-**5***) from the precursor 3,4-dibenzoyloxy-1-pyrroline (**6**) and K^{14}CN . The labelled TMSCN was generated *in situ* by reaction of TMSCl and K^{14}CN .

EXPERIMENTAL

General Methods

Melting points are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on Bruker AC-200 and AC-250 instruments. Chemical shifts (δ) were measured in ppm and coupling constants (J) in Hz. For NMR spectra in CDCl_3 the solvent ($\delta = 76.93$ ppm) was used as the internal reference for ^{13}C NMR-spectra while TMS ($\delta = 0$) was used for ^1H NMR-spectra. For NMR spectra in D_2O dioxane ($\delta = 67.4$ ppm) was used as internal reference for ^{13}C NMR-spectra and HDO ($\delta = 4.63$ ppm) for ^1H NMR-spectra. All evaporations were carried out below 40°C in vacuo. Column chromatography was performed using Merck 60 Å (35-70 μ) silica gel.

Potassium [^{14}C]cyanide was obtained from Amersham (58 mCi/mmol) in vials containing 10 mCi. KCN and KI were dried in vacuo at 120°C for 24 h before use. All solvents used were of analytical grade. Determination of total radioactivity was carried out on a Packard 1000 CA tri-carb liquid scintillation analyzer, using 20 mL counting vials and Pico-aqua™ Packard liquid scintillator. High performance liquid chromatography was performed using a Merck HPLC pump L-6200 with a rheodyne injector (20 μL loop) and a Merck UV-detector L-4000 (operating at 214 nm). Separations were performed at room temperature with a C-18 column (250 \times 4.6 mm, 5 μm) using a mixture of water and acetonitrile containing 0.1 % trifluoroacetic acid (TFA). The flow rate was 1.0 mL/min. Radioactivity in the column effluent was monitored with a Radiomatic/Canberra Flo-One beta detector A-200 using a 500 μL liquid flow cell. The ratio of column effluent to liquid scintillator (Picoaqua™, Packard) was 1:2. Two eluent systems were used: A-eluent (90/10 water/acetonitrile, 0.1 % TFA) and B-eluent (10/90 water/acetonitrile, 0.1 % TFA). Two different HPLC solvent systems were used: System I: Gradient from 100/0 to 0/100 A-eluent/B-eluent was used for analytical purpose. System II: Isocratic, 75/25 A-eluent/B-eluent was used for semipreparative separations. Thin layer chromatography (TLC) was performed on alumina plates coated with 0.25 mm silica gel 60 F₂₅₄ (Merck). For detecting **1*** TLC analysis was performed using a Bioscan Imaging

Scanner System 200-IBM with Autochanger 1000. Mass spectroscopy was performed on a triple quadrupole LC/MS mass spectrometer.

(3S,4S)-N-Benzyl-3,4-dihydroxy-2,5-dioxopyrrolidine (7)

A solution of D-tartaric acid (20.1 g, 134 mmol) in xylene (500 mL) was heated to reflux in a 3-necked flask fitted with a water separator and condenser. To the vigorously stirred and refluxing xylene solution was added benzylamine (17.8 mL, 153 mmol) over a period of 30 min and reflux was continued for 3 h. The solution was cooled in an ice bath and the crystalline product was filtered. The product was washed twice with cold toluene. Recrystallization successively from H₂O and EtOH gave **7** as colourless crystals (17.4 g, 59%); mp 194 °C, $[\alpha]_D^{21} - 139.1$ (c 1, MeOH), (lit. [16] L-form, mp 197-198 °C, $[\alpha]_D^{22} + 138.0$). ¹³C NMR (50 MHz, DMSO-d₆): δ 41.24 (PhCH₂O), 74.56 (C-3, C-4), 127.56, 128.61, 135.69 (ArC-H), 174.62 (C-2, C-5).

(3R,4R)-N-Benzyl-3,4-dihydroxypyrrolidine (8)

To a solution of compound **7** (15.7 g, 71 mmol) and BF₃·Et₂O (35 mL, 0.27 mol) in diethylene glycol dimethyl ether (142 mL) was added NaBH₄ (7.0 g, 185 mmol) in small portions during 1 h at 0 °C. When the evolution of hydrogen had stopped the mixture was allowed to come to room temperature followed by heating to 70 °C for 2 h. After cooling to room temperature 6N HCl (94 mL, 0.56 mol) was added slowly, then the reaction was heated to 70 °C for 15 min and NaF (43.5 g, 1.04 mol) was added in one portion with stirring. The mixture was immediately acidified by adding 6 N HCl (60 mL) to pH 1 followed by heating to 100 °C for 30 min. After cooling to room temperature 20 % NaOH (90 mL, 0.55 mol) was added (pH ~ 9-10). The salts were removed by filtration and the organic and aqueous phases were separated. The organic phase was concentrated affording a colourless solid. This residue was dissolved in H₂O (56 mL) and extracted 10 times with Et₂O. The combined organic phases were dried (Na₂SO₄) and concentrated to a pale yellow oil, which solidified upon standing. Recrystallization from EtOAc (50 mL) gave crystalline **8** (4.2 g). The water phase was then extracted continuously for 8 h with Et₂O, which was dried and concentrated to give a residue which was recrystallized from EtOAc (40 mL) to give more crystalline **8** (3.6 g) raising the total yield of **8** to 7.8 g (57 %); mp 98-99 °C, $[\alpha]_D^{21} - 7.3$ (c 1.1, CHCl₃), (lit. [16] L-form, mp 100 °C, $[\alpha]_D^{22} + 8.3$). ¹H NMR (200 MHz, D₂O): δ 2.35 (2H, dd, *J* = 10.5, 4.0, *trans* H-2, *trans* H-5), 2.80 (2H, dd, *J* = 10.5, 6.0, *cis* H-2, *cis* H-5), 3.45 (2H, two d, *J* = 13.0, PhCH₂N), 7.20 (5H, m, Ar-H).

^{13}C NMR (50 MHz, D_2O): δ 58.9 (C-2, C-5), 59.7 (PhCH_2N), 76.9 (C-3, C-4), 127.6, 128.5, 129.6, 137.2 (ArC-H).

(3R,4R)-N-Benzyl-3,4-dibenzoyloxypyrrolidine (9)

To a solution of **8** (4.24 g, 21.9 mmol) in CH_2Cl_2 (35 mL) was added a solution of Na_2CO_3 (5.7 g, 53 mmol in 35 mL H_2O). The mixture was stirred vigorously at 0 °C. Benzoylchloride (6.3 mL, 54.3 mmol) was added dropwise at 0 °C and stirring was continued at room temperature for 6 h. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 \times 35 mL). The combined organic phases were washed with H_2O (35 mL), dried (Na_2SO_4) and concentrated to a crystalline residue. Recrystallization from EtOH yielded **9** as colourless crystals (8.14 g, 92 %); mp 107–108 °C, $[\alpha]_{\text{D}}^{22}$ - 92.2 (c 0.51, CHCl_3), (lit. [16] L-form, mp 110–112 °C, $[\alpha]_{\text{D}}^{22}$ + 86.6). ^1H NMR (200 MHz, CDCl_3): δ 2.70 (2H, dd, J = 10.5, 4.0, *trans* H-2, *trans* H-5), 3.20 (2H, dd, J = 10.5, 6.0, *cis* H-2, *cis* H-5), 3.62 (2H, m, PhCH_2N), 5.50 (2H, m, H-3, H-4), 7.1–7.5 (11H, m, Ar-H), 7.9–8.0 (4H, m, Ar-H). ^{13}C NMR (50 MHz, CDCl_3): δ 58.1 (C-2, C-5), 59.7 (PhCH_2N), 78.1 (C-3, C-4), 127.2, 128.3, 128.7, 129.7, 133.1 (ArC-H), 165.9 ($\text{PhC}=\text{O}$).

(3R,4R)-3,4-Dibenzoyloxypyrrolidine (10)

Compound **9** (5.15 g, 12.90 mmol) was hydrogenolysed in AcOH (70 mL) in the presence of 500 mg Pd/C (5 %) at 35 atm. for 24 h. The catalyst was filtered off and washed with AcOH and the filtrate was concentrated to give the acetic acid salt of **10**. This was dissolved in MeOH (35 mL) and 1N KOH was added slowly with stirring to pH 8 followed by addition of H_2O (35 mL). The solution was extracted with toluene (3 \times 30 mL) and the combined organic phases were dried (Na_2SO_4). Concentration gave **10** as a pale yellow oil (3.63 g) which solidified upon standing. Repeating the workup of the aqueous phase by treatment with 1 N KOH in MeOH and extraction gave an additional amount (0.54 g) of **10** raising the total yield to 4.17 g (100 %). ^1H NMR (200 MHz, CDCl_3): δ 2.45 (1H, br. s, NH), 3.18 (2H, dd, J = 13.0, 2.0, *trans* H-2, *trans* H-5), 3.55 (2H, dd, J = 13.0, 5.0, *cis* 2-H, *cis* 5-H), 5.52 (2H, m, H-3, H-4), 7.4–7.6 (6H, m, Ar-H), 8.0–8.1 (4H, m, Ar-H). ^{13}C NMR (50 MHz, CDCl_3): δ 52.3 (C-2, C-5), 79.1 (C-3, C-4), 128.3, 129.6, 133.2 (ArC-H), 165.5 ($\text{PhC}=\text{O}$).

(3R,4R)-3,4-Dibenzoyloxy-N-chloropyrrolidine (11)

3,4-Dibenzoyloxypyrrolidine (**10**) (4.05 g, 13.10 mmol) was dissolved in Et_2O (50 mL) by gentle heating. *N*-Chlorosuccinimide (NCS) (1.92 g, 14.41 mmol) was added

and the reaction was stirred under nitrogen at room temperature for 3 h. Then H₂O (80 mL) and Et₂O (80 mL) was added. The aqueous phase was extracted with Et₂O (2 × 40 mL). The combined organic phases were washed with 10 % Na₂S₂O₃ (80 mL), H₂O (40 mL) and dried (Na₂SO₄). Concentration afforded **11** as a colourless oil (4.21 g, 94 %), which was kept at -20 °C due to its instability. ¹H NMR (200 MHz, CDCl₃): δ 3.41 (2H, dd, *J* = 12.0, 4.0, *trans* H-2, *trans* H-5), 3.90 (2H, dd, *J* = 12.0, 6.0, *cis* H-2, *cis* H-5), 5.64 (2H, m, H-3, H-4), 7.40-7.60 (6H, m, Ar-H), 8.00-8.10 (4H, m, Ar-H). ¹³C NMR (50 MHz, CDCl₃): δ 66.4 (C-2, C-5), 77.5 (C-3, C-4), 128.4, 129.1, 129.7, 133.4 (ArC-H), 165.6 (PhC=O).

(3R,4R)-3,4-Dibenzoyloxy-1-pyrroline (**6**)

The *N*-chloropyrrolidine **11** (216 mg, 0.6 mmol) in toluene (4.5 mL) was added to a solution of 1,8-diazabicyclo[5.4.0.]undec-7-ene (DBU) (185 mg, 1.2 mmol) in toluene (2.8 mL), and the mixture was stirred overnight at room temperature. Then H₂O (5 mL) and toluene (4 mL) were added and the phases separated. The organic phase was washed with H₂O (2 × 5 mL), and the combined aqueous phases were re-extracted with toluene (4 mL). The combined organic phases were dried (Na₂SO₄), filtered and concentrated to a pale yellow oil (190 mg, 100 %), which was kept at -20 °C due to its instability. ¹H NMR (200 MHz, CDCl₃): δ 4.08 (1H, ddd, *J* = 17.5, 3.0, 3.0, *trans* H-5), 4.60 (1H, dddd, *J* = 17.5, 6.5, 3.0, 1.5, *cis* H-5), 5.61 (1H, ddd, *J* = 6.5, 3.0, 3.0, H-4), 6.10 (1H, m, H-3), 7.40-7.65 (6H, m, Ar-H), 7.82 (1H, ddd, *J* = 3.0, 3.0, 1.0, H-2), 8.0-8.1 (4H, m, Ar-H). ¹³C NMR (50 MHz, CDCl₃): δ 66.5 (C-5), 76.8 (C-4), 84.1 (C-3), 128.6-134.1 (ArC-H), 163.4 (C-2), 166.0, 166.4 (PhC=O).

Trimeric structure of (3R,4R)-3,4-dibenzoyloxy-1-pyrroline (**12**)

The reaction was performed as for the described synthesis of **6**. In some experiments concentration of the toluene gave a colourless oil which was assigned the trimeric structure **12**, based on the ¹³C-NMR spectrum. ¹³C NMR (50 MHz, CDCl₃): δ 51.7 (C-5), 76.5, 79.2 (C-4, C-3), 83.7 (C-2), 128.1-133.3 (ArC-H), 165.5 (PhC=O).

The chemical shift for C-2 in this structure is in accordance with literature values [30].

(2R,3R,4R)-3,4-dibenzoyloxy-2-cyanopyrrolidine (**5**) and the *(2S,3R,4R)*-epimer (*2-epi-5*)

To a suspension of dry KCN (0.72 g, 11.08 mmol) and dry KI (0.19 g, 1.14 mmol) in dry CH_3CN (13 mL) was added TMSCl (1.4 mL, 11.08 mmol) and the mixture was stirred at room temperature overnight protected from moisture. The toluene solution containing **6** prepared from **11** (1.09 g, 3.18 mmol) was added together with ZnI_2 (80 mg, 0.25 mmol) and the reaction was stirred at room temperature overnight. Then H_2O (30 mL) and dioxane (30 mL) were added and stirring was continued for 1 h, whereupon more H_2O (30 mL) was added. The mixture was extracted with toluene (2×30 mL), which was washed with H_2O (60 mL), dried (Na_2SO_4), treated with active charcoal and filtered through a plug of Celite. Concentration gave a crude product (1.07 g) which was purified by flash chromatography on silica gel (EtOAc/hexane 2:3, $R_f = 0.23$) to give a mixture of **5** and *2-epi-5* in the ratio 3:2 (0.74 g, 69 %). ^1H NMR (250 MHz, CDCl_3): δ 2.6-2.7 (1H, br. s, NH), 3.28 (0.4 H, dd, $J = 12.5, 2.0$, *trans* H-5), 3.38 (0.6 H, dd, $J = 12.5, 3.0$, *trans* H-5), 3.71 (0.6 H, dd, $J = 12.5, 6.0$, *cis* H-5), 3.72 (0.4 H, dd, $J = 12.5, 6.0$, *cis* H-5), 4.25 (0.6 H, d, $J = 1.5$, H-2, **5**), 4.70 (0.4 H, d, $J = 6.0$, H-2, *2-epi-5*), 5.60 (1H, m, H-4), 5.68 (1 H, m, H-3), 7.4-7.7 (6H, m, Ar-H), 8.0-8.15 (4H, m, Ar-H). ^{13}C NMR (62.5 MHz, CDCl_3): δ 50.8; 51.4 (C-5), 52.2; 53.2 (C-2), 76.9 (C-3), 80.4 (C-4), 116.3; 117.8 (CN), 128.4-133.8 (ArC-H), 165.0; 165.4 (PhC=O).

(2S,3R,4R)-2-Carboxy-3,4-dihydroxypyrrolidine (**4**) and the *(2R,3R,4R)* epimer (*2-epi-4*)

The 2-cyanopyrrolidine (**5** and *epi-5*) (0.70 g, 2.08 mmol) was suspended in AcOH/6 N HCl (20 mL/20 mL) and heated at reflux for 24 h. The solution was cooled to room temperature and H_2O (30 mL) and toluene (30 mL) were added. The aqueous phase was washed with toluene (2×30 mL) and the combined organic phases were reextracted with H_2O (15 mL). The combined aqueous phases were treated with active charcoal, filtered and concentrated to give **4** and *2-epi-4* as a yellow salt (0.38 g, 100 %). C_a and C_b indicate the two diastereomers **4** and *2-epi-4*, respectively. ^{13}C NMR (50 MHz, D_2O): δ (**4**): 51.3 (C_a -5), 65.9 (C_a -2), 73.6 (C_a -4), 77.8 (C_a -3); (*2-epi-4*): 51.3 (C_b -5), 63.8 (C_b -2), 74.3 (C_b -4), 75.2 (C_b -3); 168.6, 169.0 (COOH).

1,4-Dideoxy-1,4-imino-D-arabinitol (1) and -L-xylitol (4-epi-1)

2-Carboxy-3,4-dihydroxypyrrolidine hydrochloride (**4** and *2-epi-4*) (0.383 g, 2.09 mol) (dried in vacuo overnight) was suspended in dry pyridine (20 mL) and hexamethyldisilazane (HMDS) (1.86 mL, 8.92 mmol) and TMSCl (56 μ L, 0.45 mmol) were added. After stirring for 1 h at 80 °C a precipitate had formed which was filtered off, and washed with CHCl₃. The combined filtrates were concentrated. The residue was dissolved in dioxane (18 mL) and H₃B·SMe₂ (10 M, 1.1 mL, 10.6 mmol) was added under an argon atmosphere. The mixture was stirred for 5 h at 100 °C and cooled to room temperature. Then HCl (1 N, 18 mL) was slowly added and the reaction mixture was heated at 100 °C for 1 h. The reaction mixture was concentrated and co-concentrated with 1 % conc. HCl in MeOH (2 \times 10 mL) to give **1** and *4-epi-1* as the hydrochlorides (264 mg, 75 %). The ratio of **1**: *4-epi-1* was 3:2. ¹³C NMR (50 MHz, D₂O): δ 49.9 (t, C_a-1), 58.9 (t, C_a-5), 66.5 (d, C_a-4), 50.5 (t, C_b-1), 57.2 (t, C_b-5), 63.0 (d, C_b-4), 74.3, 75.6 (4 \times d, C-2, C-3 for **1** and *4-epi-1*).

The spectral data were in accordance with the literature values [31,32].

The hydrochloride was dissolved in water and stirred with ion-exchange resin (Amberlite IRA 420, OH⁻). The resin was washed with H₂O and the eluate was concentrated to give the free amine **1** and *4-epi-1*. ¹H NMR (200 MHz, D₂O): δ 2.51-3.18 (3H, m, H-1, H-4), 3.40-3.69 (3H, m, H-5, H-2), 3.90-4.10 (1H, m, H-3). ¹³C NMR (50 MHz, D₂O): δ (**1**): 50.4 (t, C_a-1), 61.9 (t, C_a-5), 65.0 (d, C_a-4), 77.3 (d, C_a-2), 78.9 (d, C_a-3); (*epi-1*): δ 50.6 (t, C_b-1), 59.9 (t, C_b-5), 60.8 (d, C_b-4), 76.6 (d, C_b-2), 77.0 (d, C_b-3).

[2-¹⁴C]-(2R,3R,4R)-3,4-Dibenzoyloxy-2-cyanopyrrolidine (5) and the [2-¹⁴C]-(2S,3R,4R)-epimer (2-epi-5*)*

To a reaction vial containing potassium [¹⁴C]cyanide (10 mCi; 0.180 mmol, specific radioactivity given by Amersham 56 mCi/mmol) was added TMSCl (45 μ L, 0.357 mmol), KI (6 mg, 0.035 mmol) and CH₃CN (300 μ L). The reaction vial was stirred at room temperature for 20 h.

The precursor **6** was synthesised by reaction of 3,4-dibenzoyloxy-1-chloropyrrolidine (**11**) (139 mg, 0.405 mmol) and DBU (105 mg, 0.690 mmol) in toluene (4.9 mL) at room temperature overnight. To the reaction mixture was added toluene (2.5 mL) and H₂O (3 mL). The toluene layer was washed with H₂O (2 \times 3 mL) and the aqueous layer was re-extracted with toluene (2.5 mL). The combined toluene phase was dried

(Na_2SO_4), filtered and concentrated to 300 μL under reduced pressure at rt. This solution was diluted to 1000 μL by addition of CH_3CN .

To the reaction vial was added ZnI_2 (5 mg) and 1.2 eq. of **6** (600 μL). The reaction was stirred at room temperature. The reaction was followed by HPLC analysis. The reaction was stopped after 4.25h and the supernatant was removed and stored at -20°C until separation. The total radioactivity transferred from the vial was found to be 8.93 mCi. The crude reaction mixture was purified in batches of 100 μL (~ 6 mg) diluted in a mixture of DMSO and A-eluent (40/60, 1 mL). The two diastereomers (**5***, *2-epi-5**) were separated on a semi-preparative C-18 HPLC column using a mixture of A- and B-eluent 75/25 (system II) as eluent. The collected fractions were concentrated by evaporation at room temperature to give colourless solids. Radiochemical yield: *2-epi-5** 2.3 mCi and **5*** 3.3 mCi. Total of 56 %. Radiochemical purity > 98 %, determined by radio-HPLC analysis (system I) with a retention time identical to a standard reference sample. (**5***): ^1H NMR (400 MHz, CDCl_3): δ 3.60 (1H, dd, $J = 12.7, 2.0$, *trans* H-5), 3.84 (1H, dd, $J = 12.7, 5.0$, *cis* H-5), 4.51 (1H, s, H-2), 5.69 (1H, m, H-4), 5.78 (1H, m, H-3), 7.45-8.15 (10H, m, Ar-H). Electrospray MS ($M + 1 = 339.0$). (*2-epi-5**): ^1H NMR (400 MHz, CDCl_3) δ 3.68 (1H, d, $J = 13.2$, *trans* H-5), 4.02 (1H, dd, $J = 13.2, 4.8$, *cis* H-5), 5.07 (1H, d, $J = 4.6$, H-2), 5.71 (1H, d, $J = 4.5$, H-4), 5.90 (1H, d, $J = 4.5$, H-3), 7.46-8.13 (10 H, m, Ar-H). Electrospray MS ($M + 1 = 339.1$).

[$6\text{-}^{14}\text{C}$]-(*2S,3R,4R*)-2-Carboxy-3,4-dihydroxyproline (**4***)

Compound **5*** (1.3 mCi) was suspended in AcOH (1 mL) and 6N HCl (1 mL) in a reaction vial fitted with a condenser. The reaction mixture was heated to reflux at 100°C overnight. The reaction was concentrated on vacuum and dissolved in H_2O (1 mL). Purification was done on Sep-Pak (RP C-18 Plus, WatersTM) by elution with 4 \times 1 mL of H_2O and then with fractions of decreasing polarity (mixtures of H_2O and CH_3CN). Combining the aqueous fractions containing radioactivity gave a solution containing 1.1 mCi of the product (**4***). The benzoic acid was retained on the Sep-Pak. The product solution was concentrated in vacuo and dried in a desiccator. Radiochemical yield: 1.1 mCi (85%).

[$5\text{-}^{14}\text{C}$]-1,4-Dideoxy-1,4-imino-D-arabinitol (**1***)

The hydrochloride **4*** (1.1 mCi, 0.03 mmol) was dried in a two necked flask in a desiccator under vacuum. Silylation and reduction was performed under the same conditions as described previously: silylation in dry pyridine (500 μL) with HMDS (100 μL , 0.48 mmol) and TMSCl (15 μL , 0.12 mmol); reduction under nitrogen in

dry dioxane (1 mL) with $\text{H}_3\text{B-SMe}_2$ (10 M, 100 μL , 1.0 mmol). The reaction flask was cooled to room temperature and left overnight, followed by addition of 1 N HCl (1 mL) (exothermic). The reaction mixture was heated at 100 °C for 1h. The reaction mixture was concentrated and co-concentrated in MeOH/ conc. HCl (1 mL/ 1 drop) to give a colourless salt. The salt was dissolved in MeOH (2mL) and purified on a normal phase Sep-Pak (NP Si-60 Plus, Waters™). The Sep-Pak was eluted with 5×1 mL MeOH. The resulting fractions contained 960 μCi of the product 1*. Radiochemical yield: 1 mCi (77 %). Radiochemical purity > 92 %, determined by radio-HPLC analysis (Licrosphere Si60 column) and TLC system (isopropylalcohol/ 25 % aq NH_3 , 3:1) with a retention time identical to a standard reference sample. Specific radioactivity was determined using Electrospray MS with DAB1 as reference ($M + 1 = 136$). Further purification on a LiChrospher 100 NH_2 column using a mixture of H_2O and CH_3CN as mobile phase gave the desired product 1* with a radiochemical purity > 95 %.

References

- [1] Ganem, B. *Acc. Chem. Res.* 1996, 29, 340-347.
- [2] Legler, G. *Advances in Carbohydrate Chemistry and Biochemistry* 1990, 48, 319-384.
- [3] Sinnott, M.L. *Chem. Rev.* 1990, 90, 1171-1202.
- [4] Winchester, B.; Fleet, G.W.J. *Glycobiology* 1992, 2, 199-210.
- [5] Fleet, G.W.J.; Nicholas, S.J.; Smith, P.W.; Evans, S.V.; Fellows, L.E.; Nash, R.J. *Tetrahedron Lett.* 1985, 26, 3127-3130.
- [6] Furukawa, J.; Okuda, S.; Saito, K.; Hatanaka, S.I. *Phytochemistry* 1985, 4, 593.
- [7] Nash, R.J.; Bell, E.A.; Williams, J.M. *Phytochemistry* 1985, 24, 1620.
- [8] Fleet, G.W.J.; Smith, P.W. *Tetrahedron* 1986, 42, 5685-5692.
- [9] Osten, G.H.v.; Sinsky, A.J.; Barbas, C.F.; Pederson, R.L.; Wang, Y-F.; Wong, C-H. *J. Am. Chem. Soc.* 1989, 111, 3924-3927.
- [10] Ziegler, T.; Straub, A.; Effenberger, F. *Angew. Chem. Int. Ed. Engl.* 1988, 27, 716-717.
- [11] Hung, R.R.; Straub, J.A.; Whitesides, G.M. *J. Org. Chem.* 1991, 56, 3849-3855.
- [12] Kim, Y.J.; Kido, M.; Bando, M.; Kitahara, T. *Tetrahedron* 1997, 53, 7501-7508.
- [13] Kim, J.H.; Yang, M.S.; Lee, W.S.; Park, K.H. *J. Chem. Soc., Perkin Trans. I.* 1998, 2877-2880.
- [14] Valsborg, J.S.; Folkersen, B.M.; Lundt, I.; Winge-Rasmussen, A.; Lundgren, K.; Foged, C. *Synthesis and Applications of Isotopically Labelled compounds* 1997; John Wiley & Sons. Paper 84.
- [15] Overkleef, H.S.; Wiltenburg, J.v.; Pandit, J.K. *Tetrahedron* 1994, 50, 4215-4224.
- [16] Arakawa, Y.; Yoshifuji, S. *Chem. Pharm. Bull.* 1991, 39, 2219-2224.
- [17] Wong, C.M.; Buccini, J.; Te Raa, J. *Can. J. Chem.* 1968, 46, 3091-3094.
- [18] Nagel, U.; Kinzel, E.; Andrade, J.; Prescher, G. *Chem. Ber.* 1986, 119, 3326-3343.
- [19] Grundon, M.F.; Reynolds, B.E. *J. Chem. Soc.* 1964, II, 2445-2450.
- [20] Behr, J-B.; Defoin, A.; Streith, J. *Heterocycles* 1994, 37, 747-750.
- [21] Bock, H.; Dammel, R. *Chem. Ber.* 1987, 120, 1961-1970.
- [22] Ojima, I.; Inaba, S.; Nakatsugawa, K. *Chem. Lett.* 1975, 331-334.
- [23] Ojima, I.; Inaba, S. *Chem. Lett.* 1975, 737-740.
- [24] Reetz, M.T.; Chatziiosifidis, I. *Synthesis* 1982, 330.
- [25] Hünig, S.; Wehner, G. *Synthesis* 1979, 522-523.
- [26] Rasmussen, J.K.; Heilmann, S.M. *Synthesis* 1979, 523-524.

- [27] Jackman, L.M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*. 2. ed., Pergamon Press, NY, 1969, 227-229, 286-289.
- [28] Fleet, G.W.J.; Smith, P.W. *Tetrahedron* 1986, 42, 5685-5692
- [29] Godskesen, M.; Lundt, I.; Madsen, R.; Winchester, B. *Bioorg. Med. Chem.* 1996, 4, 1857-1865.
- [30] Kalinowski, H.-O.; Berger, S.; Braun, S. *Carbon-13 NMR Spectroscopy*. John Wiley and Sons, 1988, 350.
- [31] Fleet, G.W.J.; Smith, P.W. *Tetrahedron* 1986, 42, 5685-5692.
- [32] Van der Klein, P.A.M.; Filemon, W.; Broxterman, H.J.G.; Van der Marel, G.A.; Van Boom, J.H. *Synth. Commun.* 1992, 22, 1763-1771.