

Synthesis of nojirimycin C-glycosides

Laura Cipolla,* Amalia Palma, Barbara La Ferla and Francesco Nicotra*

Department of Biotechnology and Biosciences, University of Milano-Bicocca,
P.za della Scienza 2, 20126 Milano, Italy. E-mail: francesco.nicotra@unimib.it;
Fax: + (39) 02 64483565; Tel: + (39) 02 64483457

Received (in Cambridge, UK) 8th July 2002, Accepted 30th July 2002

First published as an Advance Article on the web 29th August 2002

Nojirimycin α -C-glycosides, which have 1- α -allyl-1-deoxy-*N*-benzyl-2,3,4,6-tetra-*O*-benzylnojirimycin (**9**) as a key intermediate for further derivatisation, have been synthesized from commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**3**) through a highly stereoselective procedure which involves treatment of **3** with benzylamine, reaction of the obtained glucosylamine **4** with allylmagnesium bromide and cyclization of the elongated open-chain aminosugar **5** by Fmoc-protection, oxidation of the free hydroxy group, and intramolecular reductive-amination, affording **9** in 59% overall yield. The efficient manipulation of the allylic appendage of **9** has required *N*-debenzylation and Fmoc-protection of the ring-nitrogen.

Introduction

The relevant role played by the glycosidic part of cell-wall glycoconjugates in cell-cell and cell-pathogen recognition phenomena¹ has stimulated interest in compounds which are able to inhibit carbohydrates processing enzymes, such as glycosidases and glycosyltransferases. With reference to glycosidase inhibitors, attention has been focused mainly on analogues of the oxonium ion intermediate which participates in the hydrolysis of the glycosidic bond. In order to mimic the oxonium ion intermediate, two main strategies have been adopted: the introduction of a stable positive charge at the ring-oxygen site, and/or constraint to flatness of the sugar-like structure in order to reproduce the conformation of the reactive intermediate. The best and probably easiest way to generate a positive charge in a sugar-like structure at the ring-oxygen position is to replace the oxygen with a basic nitrogen which will assume the stable protonated form in the active site of the enzyme, generating compounds mimicking the structure of monosaccharides, usually referred to as aza-sugars or iminosugars.²

Nojirimycin (**1**) (Fig. 1) was discovered as the first natural

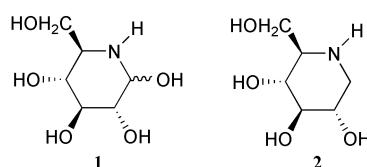


Fig. 1 Structures of nojirimycin (**1**) and 1-deoxynojirimycin (**2**).

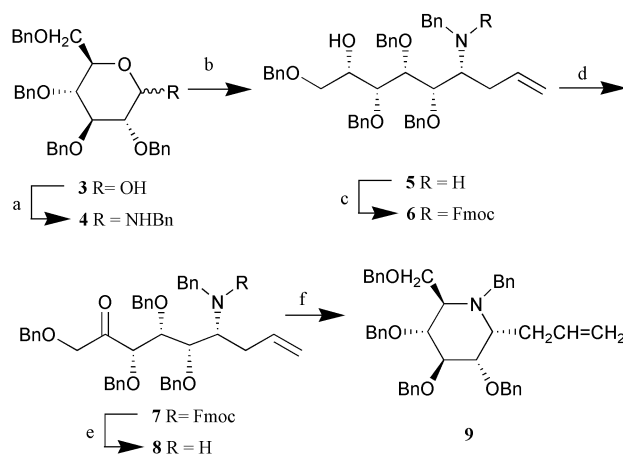
glucose mimic in 1966,³ and since then iminosugars have been found to be widespread in plants and microorganisms. The lability of the hemiaminal function of iminosugars has shifted the interest of chemists to the stable 1-deoxy derivatives, such as 1-deoxynojirimycin (**2**). Deoxynojirimycin derivatives are powerful inhibitors of the *N*-linked glycosylation pathway, where they prevent the endoplasmic reticulum processing of immature glycoproteins.⁴ The observation that iminosugars have promising therapeutic potential in many diseases,⁵ such as cancer, diabetes, and viral infections, has led to increased interest and demand. Homologues,⁶ deoxygenated,⁷ *N*-alkylated,⁸ C-glycosylated,⁹ amino acids conjugates,¹⁰ and a number of other deoxynojirimycin derivatives² have been synthesized during the last few years, both by chemical and enzymatic methods. Some C-glycosides of nojirimycin have already been

synthesized in both the α ¹¹ and β ¹² anomeric configurations, but the development of new efficient and flexible procedures for the synthesis of these compounds is still an area of great interest.

Results and discussion

We describe herein an efficient approach for the synthesis of nojirimycin C-glycosides from commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**3**) through an efficient and stereoselective procedure exploiting, as a key intermediate for further derivatisation, 1- α -allyl-1-deoxy-*N*-benzyl-2,3,4,6-tetra-*O*-benzylnojirimycin (**9**).

The crucial steps for the synthesis of **9** (Scheme 1), previously



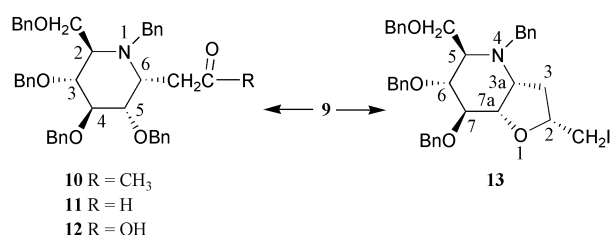
Scheme 1 Reagents and conditions: a) BnNH_2 , $p\text{TsOH}$, CH_2Cl_2 ; b) allylmagnesium bromide, Et_2O ; c) FmocCl, *N,N*-diisopropylethylamine (DIPEA), CH_3CN ; d) PCC, CH_2Cl_2 ; e) Et_2NH , CH_3CN ; f) $\text{NaBH}(\text{OAc})_3$, Na_2SO_4 , DCE, AcOH.

reported in a note,¹³ involve the introduction of the amino function and the allylic appendage, and then cyclisation to the piperidine ring, with particular attention to the stereochemical outcome of the allylation and cyclisation reactions. In more detail, the reaction of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**3**) with benzylamine, in the presence of toluene-*p*-sulfonic acid and 4 Å molecular sieves, afforded the corresponding glucosylamine **4** in quantitative yield. Stereoselective allylation of **4** by allylmagnesium bromide in dry diethyl ether gave the open

chain amino alcohol **5** in 90% de and 92% yield. The high stereo-selection of this reaction can be ascribed to the formation of a Cram-chelate intermediate, in which the magnesium coordinates to the nitrogen of the imine, in equilibrium with the glycosyl amine, and the oxygen belonging to the benzyloxy group at C-2, allowing the attack of the nucleophilic carbon preferentially from the less hindered *Si* face. Cyclisation of compound **5** to the desired piperidine derivative was accomplished by reductive amination,¹⁴ after oxidation of the unprotected hydroxy group. This oxidation was somewhat troublesome, as any attempt to oxidise the hydroxy group of **5** failed under a wide variety of experimental conditions. In conclusion, the oxidation was possible only after protection of the amino function as a carbamate. Two different carbamates were prepared from **5**: first, a *tert*-butoxycarbonyl derivative was synthesized by performing a one-pot deprotection and reductive amination under acidic conditions. However, the acidic conditions required for the reductive amination by means of Na(OAc)₃BH were not effective in the *tert*-butoxycarbonyl hydrolysis, while stronger acidity decomposed the product.¹⁵ Finally, fluorenylmethoxycarbonyl derivative **6** (Fmoc-Cl, 91% yield) turned out to be the carbamate of choice. Oxidation of compound **6** with pyridinium chlorochromate afforded ketone **7** (90% yield); subsequent cleavage of the Fmoc protecting group with diethylamine, then reductive amination of crude amino ketone **8** with Na(OAc)₃BH, afforded the iminosugar **9** in 78% yield over two steps, and 90% de. It is worthy of note that, following this four-step procedure, the nojirimycin C-glycoside **9** was obtained in 59% overall yield from a commercially available starting material. The ¹H NMR data at room temperature showed broad signals in deuterobenzene, due to the conformational equilibrium between the ³C₆ and ⁶C₃ conformations. When the temperature was raised to 30 °C, the signals became sharper and the coupling constants showed that the ³C₆ was the only conformation present [as supported by the H(4)–H(5) coupling constant value of 9.5 Hz].

Compound **9** is a multifunctional key intermediate for further derivatisation, since it possesses an allylic substituent which can be properly derivatised. However, it became immediately clear that oxidative conditions were not fully compatible with the presence of the tertiary nitrogen atom of the ring.

The formation of methyl ketone **10** (Scheme 2), by treatment



Scheme 2 Functionalisation of the allylic appendage.

of **9** with Na₂PdCl₄ and water, proceeded in low yield (50%); ¹H NMR of methyl ketone **10** showed a preference for the ³C₆ conformation in deuterioacetone as solvent at room temperature (see Experimental section). The stereochemical integrity of the C-6 stereocentre was not altered by possible epimerisation due to elimination/addition reactions, as confirmed by the value of 5.4 Hz for the H(1)–H(2) coupling constant.

Furthermore, oxidation (NaClO₂–NaH₂PO₄¹⁶ or CrO₃–H₂SO₄) of the crude aldehyde **11**, obtained by cleavage of the allylic double bond (OsO₄–NaIO₄), did not afford the expected carboxylic acid **12**. In addition, attempts to convert nojirimycin derivative **9** to the bicyclic structure **13** gave poor yields. The desired product **13**, in fact, was obtained in 42% yield by the competitive formation of the quaternary ammonium salt **14** (Fig. 2) (identified by mass spectrometry of the reaction mixture), which decomposed to the starting material

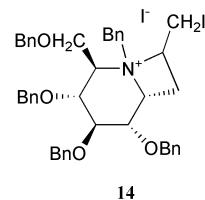


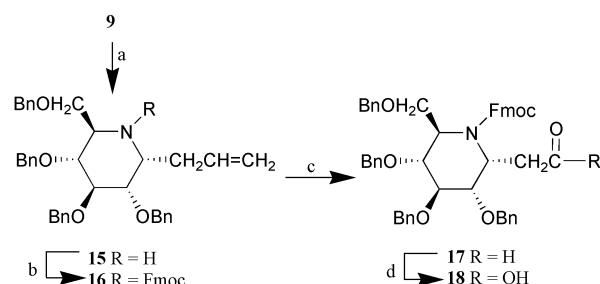
Fig. 2 Structure of the by-product obtained in the iodocyclisation reaction.

9 during work-up with sodium thiosulfate. This result can be ascribed, once more, to the presence of the nucleophilic nitrogen, which competes in the iodocyclisation/debenzylation reaction,¹⁷ toward derivative **13**. It is noteworthy that bicyclic compound **13** was obtained stereospecifically (only one stereoisomer was detected by NMR analysis), the new stereocentre having an *R* configuration, as determined by NOESY experiments (NOE effect between H-2 and H-3, correlated in turn with H-3a).

In order to functionalise more efficiently the double bond of the allylic appendage, it was clear that the nitrogen lone pair reactivity had to be quenched by the introduction of a suitable electron-withdrawing protecting group.¹⁸

We performed the synthetic sequence using *p*-methoxybenzylamine, instead of benzylamine, in order to achieve the orthogonal deprotection of the nitrogen. Unfortunately, the selective deprotection under both oxidative¹⁹ and acidic conditions²⁰ was unsuccessful.

We were forced to go back to the fully benzylated nojirimycin derivative **9**, basing our strategy on the possibility of selectively hydrolysing an *N*-benzyl group, without affecting the other benzyl groups present in the molecule. A number of methods have been reported for the cleavage of tertiary to secondary amines (the cleaved group being that with the best leaving group tendency in an S_N2-type mechanism) and for one-pot conversion of tertiary amines into a carbamate²¹ or amide.²² Unfortunately, compound **9** did not react under any of these experimental conditions (benzoyl chloride, acetyl chloride, or benzyl, fluorenylmethyl, *p*-nitrophenyl, trichloroethyl and 1-chloroethyl chloroformate). We were able to effect efficiently selective *N*-debenzylation by modifying a recently reported procedure²³ which exploits oxidative cleavage with ammonium ceric nitrate (Scheme 3).



Scheme 3 Reagents and conditions: a) CAN, THF–H₂O, 5 : 1; b) FmocCl, DIPEA, CH₃CN; c) OsO₄, NaIO₄; d) NaClO₂, NaH₂PO₄.

In detail, the reported reaction conditions were found to be unsuitable, when applied to our substrate **9**; but just by changing the solvent system from the reported acetonitrile–water to THF–water, † we were able to obtain efficiently the debenzylated compound **15**. The secondary amino group of compound **15** was protected as the fluorenylmethoxycarbonyl derivative, affording compound **16** (Fmoc-Cl, MeCN, in 85% yield over two steps), which was then subjected to oxidative cleavage in a

† Many different solvent mixtures were tested in order to optimise the reaction yield: toluene–H₂O, 1,4-dioxane–H₂O, benzene–H₂O, dichloromethane–H₂O, pyridine–H₂O, DMSO–H₂O, diethyl ether–H₂O.

two-step procedure (OsO_4 – NaIO_4 , then NaClO_2 – NaH_2PO_4), affording the amino acid derivative **18** in 94% yield.

In conclusion, starting from commercially available tetra-benzylglucose, by treatment with benzylamine, allylation with allylmagnesium bromide, oxidation with pyridinium chlorochromate and subsequent reductive amination, it is possible to obtain stereoselectively and in very good yield a nojirimycin C-glycoside, which can undergo further functionalisation both at the C-1 substituent and at the nitrogen atom,²⁴ thus opening the way to the synthesis of a large variety of new nojirimycin derivatives.

Experimental

All solvents were dried over molecular sieves, for at least 24 h prior to use. Thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ plates (Merck) with detection with UV light when possible, or charring with a solution containing conc. H_2SO_4 – EtOH – H_2O in a ratio of 5 : 45 : 45 or a solution containing $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ (21 g), $\text{Ce}(\text{SO}_4)_2$ (1 g), conc. H_2SO_4 (31 mL) in 500 mL of water. Flash column chromatography was performed on silica gel 230–400 mesh (Merck). ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz instrument using CDCl_3 as solvent unless otherwise stated. Chemical shift assignments, reported in ppm, are referenced to the corresponding solvent peaks. Due to conformational equilibria,^{11f} ^1H NMR for compounds **6**, **7**, and **18** showed unclear resolution, and are not reported, whereas ^{13}C NMR refers to the major conformer at equilibrium. Mass spectra were recorded on a MALDI2 Kompakt Kratos instrument, using gentisic acid (DHB) as the matrix. IR spectra were recorded on a Biorad FT/IR Spectrometer FTS-40A coupled to an IR microscope Biorad UMA-40A. Optical rotations were measured at room temperature with a Perkin-Elmer 241 polarimeter and are reported in units of 10^{-1} deg $\text{cm}^2 \text{g}^{-1}$.

(2R,3R,4R,5S,6R)-6-[N-Benzyl-N-(fluoren-9-ylmethoxycarbonyl)amino]-1,3,4,5-tetrakis(benzyloxy)non-8-en-2-ol (6)

Amino alcohol **5**²⁵ (1.16 g, 1.73 mmol) was dissolved in dry CH_3CN (30 mL), and *N*-ethyl-diisopropylamine (355 μL , 2.07 mmol) and Fmoc-Cl (0.895 g, 3.46 mmol) were added. After 5 h, the reaction mixture was neutralized with AcOH, then the solvent was evaporated under reduced pressure. The crude residue was purified by flash chromatography (toluene–EtOAc 9 : 1), affording compound **6** (1.54 g) as colourless oil in 97% yield. $[\alpha]_{\text{D}}^{25} +15.6$ (c 1, CHCl_3); ^{13}C NMR (100.57 MHz) δ 157.49 (s), 144.27, 144.18, 141.72, 141.60 (4s), 140.03, 138.74, 138.74, 138.36, 137.97 (5s), 135.26 (d), 130.24–128.89 (m), 125.51 (d), 120.31 (d), 118.06 (t), 81.14, 79.97, 78.40 (3d), 76.29, 75.42, 74.06, 73.81, 71.75 (5t), 70.25 (d), 66.78, 65.18 (2t), 57.10 (d), 47.72 (d), 35.62 (t); MALDI-MS: calcd for $\text{C}_{59}\text{H}_{59}\text{NO}_7$, 894.10; found $[\text{M} + \text{H}]^+$ 895. Anal. calcd. for $\text{C}_{59}\text{H}_{59}\text{NO}_7$: C, 79.26; H, 6.65; N, 1.57; found: C, 79.24; H, 6.68; N, 1.59%.

(3R,4R,5S,6R)-6-[N-Benzyl-N-(fluoren-9-ylmethoxycarbonyl)amino]-1,3,4,5-tetrakis(benzyloxy)non-8-en-2-one (7)

To a solution of alcohol **6** (0.160 g, 0.179 mmol) in dry dichloromethane (39 mL) were added 4 Å powdered molecular sieves (0.180 g), and pyridinium chlorochromate (0.116 g, 0.537 mmol). After 24 h stirring the suspension was filtered and the solvent evaporated under reduced pressure. The crude residue was purified by flash chromatography (petroleum ether–EtOAc 9 : 1) giving ketone **7** (0.145 g) as yellowish oil (90% yield). $[\alpha]_{\text{D}}^{25} +11.3$ (c 1, CHCl_3); ^{13}C NMR (C_6D_6 , 100.57 MHz) δ 206.20 (s), 169.80 (s), 144.26, 144.25, 141.61, 141.56 (4s), 138.73, 138.49, 138.43, 138.29, 137.82 (5s), 135.12 (d), 129.03–127.91 (m), 125.14, 120.07 (2d), 117.57 (t, C-3'), 84.28, 82.88, 81.84 (3d), 75.88, 75.39, 75.05, 74.45, 73.21 (5t), 65.89,

60.11 (2t), 47.74 (d), 35.40 (t); MALDI-MS: calcd for $\text{C}_{59}\text{H}_{57}\text{NO}_7$, 892.09; found $[\text{M} + \text{Na}]^+$ 915, $[\text{M} + \text{K}]^+$ 931. Anal. calcd. for $\text{C}_{59}\text{H}_{57}\text{NO}_7$: C, 79.44; H, 6.44; N, 1.57; found: C, 79.48; H, 6.41; N, 1.55%.

(2R,3R,4R,5S,6R)-N-Benzyl-3,4,5-tris(benzyloxy)-2-benzyl-oxymethyl-6-(prop-2-enyl)piperidine (9)

Compound **7** (0.050 g, 0.056 mmol) was dissolved in dry CH_3CN (400 μL) and diethylamine (100 μL) was added. After 2 h the solvent was evaporated under reduced pressure, avoiding heating, and the unstable deprotected derivative **8** was submitted to subsequent reaction without any further purification. Hence, amine **8** was dissolved in dry 1,2-dichloroethane (2 mL), the reaction mixture cooled to -35°C , and anhydrous sodium sulfate (0.199 g, 1.4 mmol), glacial acetic acid (19 μL , 0.336 mmol) and sodium triacetoxyborohydride (0.047 g, 0.224 mmol) were added. After 24 h, the suspension was filtered, the solvent evaporated and the residue purified by flash chromatography (toluene, 0.2% Et_3N), affording pure **9** and its epimer at C-1 in 78% overall yield, and 90% de. Compound **9**: $[\alpha]_{\text{D}}^{25} +21.4$ (c 1, CHCl_3); ^1H NMR (500 MHz, C_6D_6) δ 7.41–7.00 (m, 25 H), 5.92–5.78 (m, 1 H, H-2''), 5.13–4.98 (m, 2 H, H-3''), 5.03, 4.84 (ABq, 2 H, $J = 11.2$ Hz, PhCH_2O), 5.04, 4.65 (ABq, 2 H, $J = 11.4$ Hz, PhCH_2O), 4.34 (s, 2 H, PhCH_2O), 4.20, 4.14 (ABq, 2 H, $J = 11.9$ Hz, PhCH_2O), 4.04 (d, 1 H, $J = 14.0$ Hz, PhCHN), 3.90–3.84 (m, 3 H, H-3, H-4, PhCHN), 3.83–3.75 (m, 2 H, H-1'a, H-5), 3.63 (dd, 1 H, $J = 10.3$, 2.1 Hz, H-1'b), 3.16 (dt, 1 H, $J = 8.6$, 5.4 Hz, H-6), 3.11 (ddd, 1 H, $J = 9.5$, 4.9, 2.1 Hz, H-2), 2.53 (dt, 1 H, $J = 14.0$, 5.4 Hz, H-1'a), 2.39 (dt, 1 H, $J = 14.0$, 8.6 Hz, H-1'b); ^{13}C NMR (100.57 MHz, C_6D_6) δ 141.19, 139.74, 139.58, 138.79, 138.51 (5s), 138.08 (d), 115.30 (t), 84.12, 79.44, 78.80 (3d), 75.27, 74.98, 73.02, 72.14 (4t), 68.70 (t), 57.97, 57.50 (2d), 53.22 (t), 30.24 (t). MALDI-MS: calcd for $\text{C}_{44}\text{H}_{47}\text{NO}_4$, 653.85; found $[\text{M} + \text{H}]^+$ 655. Anal. calcd. for $\text{C}_{44}\text{H}_{47}\text{NO}_4$: C, 80.82; H, 7.25; N, 2.14; found: C, 80.77; H, 7.28; N, 2.11%.

(2R,3R,4R,5S,6R)-N-Benzyl-3,4,5-tris(benzyloxy)-2-benzyl-oxymethyl-6-(2-oxopropyl)piperidine (10)

To a solution of compound **9** (0.027 g, 0.041 mmol) in DMF–THF– H_2O (0.8 mL : 0.5 mL : 0.1 mL) was added Na_2PdCl_4 (0.016 g, 0.054 mmol). The solution was concentrated *in vacuo* and, after purification by flash chromatography (petroleum ether–EtOAc 85 : 15), methyl ketone **10** was obtained in 50% yield. $[\alpha]_{\text{D}}^{25} +9.6$ (c 1, CHCl_3); ^1H NMR (400 MHz, acetone- d_6) δ 7.24–7.05 (m, 25 H), 4.81, 4.64 (ABq, 2 H, $J = 11.2$ Hz, PhCH_2O), 4.75, 4.45 (ABq, 2 H, $J = 11.0$ Hz, PhCH_2O), 4.30, 4.24 (ABq, 2 H, $J = 11.4$ Hz, PhCH_2O), 4.23 (br s, 2 H, PhCH_2O), 3.88 (d, 1 H, $J = 14.3$ Hz, PhCHN), 3.66–3.62 (m, 3 H, H-6, H-1'), 3.61 (t, 1 H, $J = 8.8$ Hz, H-4), 3.58 (d, 1 H, $J = 14.3$ Hz, PhCHN), 3.54 (dd, 1 H, $J = 8.8$, 5.4 Hz, H-5), 3.48 (t, 1 H, $J = 8.8$ Hz, H-3), 2.80–2.74 (m, 2 H, H-2, H-1'a), 2.47 (dd, 1 H, $J = 15.1$, 5.4 Hz, H-1'b), 1.90 (s, 3 H, H-3''); ^{13}C NMR (100.57 MHz, C_6D_6) δ 207.35 (s), 128.60–126.63 (m), 83.88, 79.77, 79.13 (3d), 74.94, 74.92, 72.33 (4t), 68.22 (t), 59.39, 54.85 (2d), 53.04 (t), 37.61 (t), 30.21 (q). MALDI-MS: calcd for $\text{C}_{44}\text{H}_{47}\text{NO}_5$, 669.85; found $[\text{M} + \text{H}]^+$ 671. Anal. calcd. for $\text{C}_{44}\text{H}_{47}\text{NO}_5$: C, 78.89; H, 7.07; N, 2.09; found: C, 78.92; H, 7.08; N, 2.06%.

(2R,3aR,5R,6R,7R,7aS)-N-Benzyl-6,7-bis(benzyloxy)-5-benzyloxymethyl-2-iodomethyl-2-hydrofuro[3,2-b]pyridine (13)

Nojirimycin derivative **9** (0.100 g, 0.15 mmol) was dissolved in dry THF (10 mL), and *N*-iodosuccinimide (0.338 g, 1.5 mmol) was added; the reaction was quenched with aq. sodium thiosulfate until a colorless solution was obtained, and then the mixture was extracted with dichloromethane. The organic layer was dried over Na_2SO_4 , filtered and concentrated to dryness. The

residue was purified by flash chromatography (petroleum ether–EtOAc 95 : 5), affording a yellowish oil (43 mg, 42% yield) as the only detected isomer. $[a]_D^{25} +11.0$ (c 0.65, CHCl_3); ^1H NMR (400 MHz) δ 7.48–7.13 (m, 20 H), 4.97, 4.78 (ABq, 2 H, $J = 11.4$ Hz, PhCH_2O), 4.61, 4.43 (ABq, 2 H, $J = 11.0$ Hz, PhCH_2O), 4.41 (s, 2 H, PhCH_2O), 4.15 (t, 1 H, $J = 8.3$ Hz, H-7a), 3.97–3.90 (m, 2 H, H-2, PhCHN), 3.86 (t, 1 H, $J = 8.3$ Hz, H-7), 3.72–3.60 (m, 4 H, H-3a, H-1'', PhCHN), 3.55 (dd, 1 H, $J = 8.1$, 5.1 Hz, H-6), 3.30 (dd, 1 H, $J = 9.9$, 4.8 Hz, H-1'a), 3.21 (dd, 1 H, $J = 9.9$, 6.8 Hz, H-1'b), 3.03–2.98 (m, 1 H, H-5), 2.24 (dt, 1 H, $J = 11.0$, 5.7 Hz, H-3), 1.66 (br q, 1 H, $J = 11.0$ Hz, H-3); ^{13}C NMR (100.57 MHz) δ 128.60–126.63 (m), 84.16, 80.75, 80.00, 77.56 (4d) 74.06, 73.26, 72.84 (3t), 67.02 (t), 54.76 (t), 60.83, 59.34, (2d), 36.20 (t), 9.92 (t). MALDI-MS: calcd for $\text{C}_{37}\text{H}_{40}\text{INO}_4$, 689.62; found $[\text{M} + \text{H}]^+$ 691. Anal. calcd. for $\text{C}_{37}\text{H}_{40}\text{INO}_4$: C, 64.44; H, 5.85; I, 18.40; N, 2.03; found: C, 64.60; H, 5.70; I, 18.55; N, 1.99%.

(2R,3R,4R,5S,6R)-3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-N-(fluoren-9-ylmethoxycarbonyl)-6-(prop-2-enyl)piperidine (16)

To compound **9** (1.193 g, 1.825 mmol), dissolved in a 5 : 1 mixture of THF– H_2O (120 mL), ammonium cerium(IV) nitrate (4.0 g, 7.30 mmol) was added in portions. When the reaction was complete, the mixture was treated with an aq. satd. solution of NaHCO_3 , until basic pH was reached, and extracted with Et_2O . The organic phase was dried (Na_2SO_4), filtered and concentrated, then crude **15** was dissolved in dry CH_3CN (15 mL) and directly subjected to the subsequent reaction by treatment with DIPEA (375 μL , 2.19 mmol) and FmocCl (0.944 g, 3.65 mmol, dissolved in 15 mL of dry CH_3CN). After 2 hours the pH was adjusted to neutrality with AcOH and then the solution was concentrated under reduced pressure. Purification by flash chromatography (petroleum ether–EtOAc 9 : 1) afforded compound **16** (1.035 g) in 73% yield (over two steps). $[a]_D^{25} +8.8$ (c 2.3, CHCl_3); ^1H NMR (400 MHz, C_6D_6 , $T = 60^\circ\text{C}$) δ 7.60–6.95 (m, 23 H), 5.98–5.82 (m, 1 H), 5.25 (d, 1 H, $J = 16.9$ Hz), 4.90 (d, 1 H, $J = 10.0$ Hz), 4.80 (dd, 1 H, $J = 10.9$, 5.4 Hz), 4.47–4.20 (m, 11 H), 3.95 (br dd, 1 H, $J = 3.3$, 2.1 Hz), 3.92–3.87 (m, 2 H), 3.66 (dd, 1 H, $J = 7.4$, 5.9 Hz), 3.56 (br s, 1 H), 3.47 (t, 1 H, $J = 8.9$ Hz), 2.74 (dt, 1 H, $J = 14.5$, 7.6 Hz), 2.62 (dt, 1 H, $J = 14.5$, 6.9 Hz); ^{13}C NMR (100.57 MHz) δ 156.07 (s), 144.24, 144.08, 141.61, 141.56 (4s), 138.54, 138.50, 138.36, 138.06 (4s), 136.47 (d), 130.00–120.00 (m), 116.25 (t), 81.75, 80.69, 77.49 (3d), 73.15, 73.01, 72.39, 72.12, 70.20, 66.97 (6t), 54.75, 53.10 (2d), 47.83 (d), 34.05 (t). MALDI-MS: calcd. for $\text{C}_{52}\text{H}_{51}\text{NO}_6$, 785.96; found $[\text{M} + \text{Na}]^+$ 810, $[\text{M} + \text{K}]^+$ 826. Anal. calcd. for $\text{C}_{52}\text{H}_{51}\text{NO}_6$: C, 79.46; H, 6.54; N, 1.78; found: C, 79.41; H, 6.56; N, 1.77%.

(2R,3R,4R,5S,6R)-3,4,5-Tris(benzyloxy)-2-benzyloxymethyl-N-(fluoren-9-ylmethoxycarbonyl)-6-carboxymethylpiperidine (18)

Compound **16** (0.070 g, 0.089 mmol), dissolved in a 1 : 1 : 1 mixture of H_2O –acetone– $t\text{BuOH}$ (600 μL), was treated with OsO_4 (900 μL of a 5 mg mL^{-1} solution in $t\text{BuOH}$) and NaIO_4 (0.057 g, 0.267 mmol). After 3 hours, the mixture was diluted with EtOAc, the precipitate was filtered off, the organic phase washed with water, then concentrated *in vacuo* after drying over Na_2SO_4 and filtration. The crude aldehyde **17** was dissolved in CH_3CN (1 mL), and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (1.25 M solution in H_2O , 700 μL) and NaClO_2 (0.080 g) were added; after 1 hour and 30 min the reaction mixture was concentrated and the residue extracted with CHCl_3 . The organic phase was dried over Na_2SO_4 , filtered and evaporated. Purification by flash chromatography (CHCl_3 and 0.05% AcOH) afforded protected amino acid **21** (0.068 g) in 94% yield over 2 steps. $[a]_D^{25} +6.2$ (c 0.93, CHCl_3); ^{13}C NMR (100.57 MHz) δ 176.38 (s), 155.95 (s), 144.02, 143.74, 141.52, 141.49 (4s), 138.29, 138.07, 137.93,

137.58 (4s), 129.50–120.14 (m), 80.59, 79.56, 75.65 (3d), 73.16, 72.53, 72.19, 71.14, 69.77, 67.58 (6t), 54.41, 49.61 (2d), 47.76 (d), 36.14 (t). MALDI-MS: calcd for $\text{C}_{51}\text{H}_{49}\text{NO}_8$, 803.94; found $[\text{M} + \text{Na}]^+$ 828, $[\text{M} + \text{K}]^+$ 844. Anal. calcd. for $\text{C}_{51}\text{H}_{49}\text{NO}_8$: C, 76.19; H, 6.14; N, 1.74; found: C, 76.23; H, 6.11; N, 1.75%.

Acknowledgements

This work was financially supported by MURST, project COFIN MM03155477.

References

- (a) For recent reviews see: R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683–720; (b) R. A. Dwek, *Science*, 2001, **291**, 2370; (c) A. Helenius and M. Aeby, *Science*, 2001, **291**, 2364.
- Iminosugars as Glycosidase Inhibitors—Nojirimycin and Beyond*, ed. A. Stütz, Wiley-VCH, Weinheim, 1999.
- S. Inoue, T. Tsuruoka and T. Niida, *J. Antibiot.*, 1966, **19**, 288.
- J. S. Jacob, P. Scudder, T. D. Butters, I. Jones and D. C. Tiemeier, in *Natural Products as Antiviral Agents*, eds. C. K. Chu and H. G. Cutler, Plenum Press, New York, 1992, p. 137.
- (a) For recent reviews see: N. Asano, R. J. Nash, R. J. Molyneux and G. W. J. Fleet, *Tetrahedron: Asymmetry*, 2000, **11**, 1645; (b) R. A. Dwek, T. D. Butters, F. M. Platt and N. Zitzmann, *Nat. Rev. Drug Discovery*, 2002, **1**, 65.
- (a) Selected references: G. C. Kite, L. E. Fellows, G. W. J. Fleet, P. S. Liu, A. M. Scofield and N. G. Smith, *Tetrahedron Lett.*, 1988, **29**, 6483; (b) I. Bruce, G. W. J. Fleet, I. Cenci di Bello and B. Winchester, *Tetrahedron*, 1992, **48**, 183; (c) K. E. Holt, F. J. Leeper and S. Handa, *J. Chem. Soc., Perkin Trans. 1*, 1994, 231; (d) C.-H. Wong, L. Provencher, J. A. Porco Jr, S.-H. Jung, Y.-F. Wang, L. Chen, R. Wang and D. H. Steensma, *J. Org. Chem.*, 1995, **60**, 1492; (e) N. Asano, M. Nishida, A. Kato, H. Kizu, K. Matsui, Y. Shimada, A. A. Watson, R. J. Nash, P. M. de Q. Lilley, T. Itoh, M. Baba and G. W. J. Fleet, *J. Med. Chem.*, 1998, **41**, 2565.
- (a) Selected references: A. Arnone, P. Bravo, A. Donadelli and G. Resnati, *Tetrahedron*, 1996, **52**, 131; (b) J. Fitremann, A. Duréault and J.-C. Depezay, *Tetrahedron Lett.*, 1994, **35**, 1201; (c) A. Kilonmda, F. Compennolle, K. Peeterers, G. J. Joly, S. Toppet and G. J. Hoornaert, *Tetrahedron*, 2000, **56**, 1005; (d) D. D. Dhavale, N. N. Saha and V. N. Desai, *J. Org. Chem.*, 1997, **62**, 7482.
- (a) Selected references: K. K.-C. Liu, T. Kajimoto, L. Chen, Z. Zhong, Y. Ichikawa and C.-H. Wong, *J. Org. Chem.*, 1991, **56**, 6280; (b) T. Kajimoto, K. K.-C. Liu, R. L. Pederson, Z. Zhong, Y. Ichikawa, J. A. Porco Jr and C.-H. Wong, *J. Am. Chem. Soc.*, 1991, **113**, 6187.
- (a) B. A. Johns, Y. T. Pan, A. D. Elbein and C. R. Johnson, *J. Am. Chem. Soc.*, 1997, **119**, 4856; (b) F. D'Andrea, G. Catelani, M. Mariani and B. Vecchi, *Tetrahedron Lett.*, 2001, **42**, 1139 and references cited therein.
- T. Fuchss and R. R. Schmidt, *Synthesis*, 2000, **2**, 259.
- (a) H. Boshagen, W. Geiger and B. Unge, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 806; (b) P. S. Liu, *J. Org. Chem.*, 1987, **52**, 4717; (c) S. Aoyagi, S. Fujimaki and C. Kibayashi, *J. Chem. Soc., Chem. Commun.*, 1990, 1457; (d) O. R. Martin, L. Liu and F. Yang, *Tetrahedron Lett.*, 1996, **75**, 1991; (e) T. K. Chakraborty and S. Jayaprakash, *Tetrahedron Lett.*, 1997, **38**, 8899; (f) T. Fuchss, H. Streicher and R. R. Schmidt, *Liebigs Ann./Recl.*, 1997, 1315.
- (a) O. M. Saavedra and O. R. Martin, *J. Org. Chem.*, 1996, **61**, 6987; (b) M. A. Leeuwenburgh, S. Picasso, H. O. Overkleeft, G. A. van der Marel, P. Vogel and J. H. van Boom, *Eur. J. Org. Chem.*, 1999, 1185.
- L. Cipolla, B. La Ferla, F. Peri and F. Nicotra, *Chem. Commun.*, 2000, 1289.
- (a) H. S. Overkleeft, J. Van Wiltenburg and U. K. Pandit, *Tetrahedron*, 1994, **50**, 4215; (b) R. Hoos, A. B. Naughton and A. Vasella, *Helv. Chim. Acta*, 1993, **76**, 2666.
- Unpublished results.
- G. W. J. Fleet and J. C. Son, *Tetrahedron*, 1988, **44**, 2637.
- L. Cipolla, L. Lay and F. Nicotra, *J. Org. Chem.*, 1997, **62**, 6678.
- (a) Similar observations have been reported for *N*-benzylpyrrolidine derivatives: C. Herdeis, A. Aschenbrenner, A. Kirfel and F. Schwabenländer, *Tetrahedron: Asymmetry*, 1997, **8**, 2421; (b) C. A. Weir and C. M. Taylor, *J. Org. Chem.*, 1999, **64**, 1554.
- I. T. Forbes, C. N. Johnson and M. Thompson, *J. Chem. Soc., Perkin Trans. 1*, 1992, 275.

- 20 L. Yan and D. Kahne, *Synlett*, 1995, 523.
- 21 (a) R. A. Olofson, J. T. Martz, J.-P. Senet, M. Piteau and T. Malfroot, *J. Org. Chem.*, 1984, **49**, 2081; (b) M. Marzi and D. Misiti, *Tetrahedron Lett.*, 1989, **30**, 6075; (c) V. H. Rawal, R. J. Jones and M. P. Cava, *J. Org. Chem.*, 1987, **52**, 19; (d) B. W. Yang, D. O'Rourke and J. Li, *Synlett*, 1993, 195; (e) E. Magnier, Y. Langlois and C. Mérienne, *Tetrahedron Lett.*, 1995, **36**, 9475.
- 22 J. H. Cooley and E. J. Evain, *Synthesis*, 1989, 2.
- 23 S. D. Bull, S. G. Davies, G. Fenton, A. W. Mulvaney, R. S. Prasad and A. D. Smith, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3765.
- 24 L. A. G. N. M. van der Broeck, D. J. Vermaas, B. M. Heskamp, C. A. A. van Boeckel, M. C. A. A. Tan, J. G. M. Bolscher, H. L. Ploegh, F. J. van Kemenade, R. E. Y. de Goede and F. Miedema, *Recl. Trav. Chim. Pays-Bas*, 1993, **112**, 82 and references cited therein.
- 25 L. Cipolla, L. Lay, F. Nicotra, C. Pangrazio and L. Panza, *Tetrahedron*, 1995, **51**, 4679.