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Carbohydrate Research 332 (2001) 23–31

CARBOHYDRATE RESEARCH

Synthesis of a carbocyclic sialic acid analogue for the inhibition of influenza virus neuraminidase

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Received 5 June 2000; received in revised form 22 February 2001; accepted 8 March 2001

Abstract

The influenza virus neuraminidase (NA) is essential for viral infection and offers a potential target for antiviral drug development. We prepared a carbocyclic sialic acid analogue, potentially able to inhibit NA. Its structure is an analogue of the transition-state of the reaction catalysed by NA. As starting material, quinic acid was selected owing to its ready availability and its stereochemical feature suitable for the target structure. The quinic acid was first converted in the shikimic acid; then two of the three hydroxyl functions of this product were selectively functionalised to obtain the target molecule (3*R*,4*S*,5*R*)-4-acetamido-3-guanidino-5-hydroxycyclohex-1-ene-1-carboxylic acid.

Keywords: Antiviral agents; Sialic acid; Shikimic acid

1. Introduction

The first step of infection by influenza viruses subtypes A and B is mediated by the interaction of sialic acid with two major surface viral glycoproteins, haemagglutinin (HA) and neuraminidase (NA). These proteins are both essential for infection and offer potential targets for antiviral drug development. The HA of influenza viruses A and B is responsible for viral attachment to host cells by binding to terminal sialic acid residues of surface glycoconjugates.[1,2] The NA (EC 3.2.1.18) catalyses the hydrolysis of the α-(2→3) and α-(2→6) glycosidic linkage between a terminal sialic acid and its adjacent carbohydrate moiety on a variety of glycoconjugates. The inhibition of NA activity is lethal to the virus which cannot be released by the cell membrane; this limits the mobility of the virus itself and so it prevents the progress of the viral infection. It has been proposed a mechanism of influenza virus NA reaction in which the driving force comes solely from the induction and stabilisation of the oxocarbonium intermediate **A**[3,4] (see Scheme 1).

On the basis of this mechanism, it is possible to inhibit the NA enzyme by structurally analogue molecules to the oxonium cation transition state. Several NA inhibitor analogues have been synthesised;[5–7] in particular, Kim et al. reported the synthesis of compound GS4071 (Scheme 2) which is a potent NA inhibitor with IC_{50} value of 1 nM.[8] Subsequently, GS4104 (the ethyl ester prodrug of

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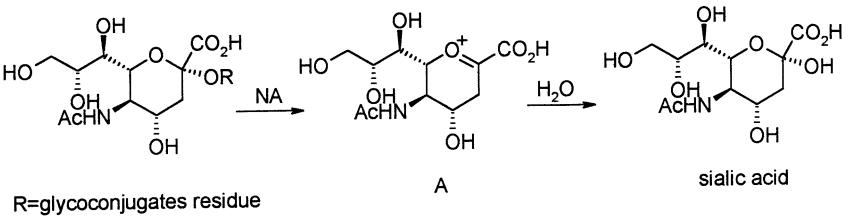
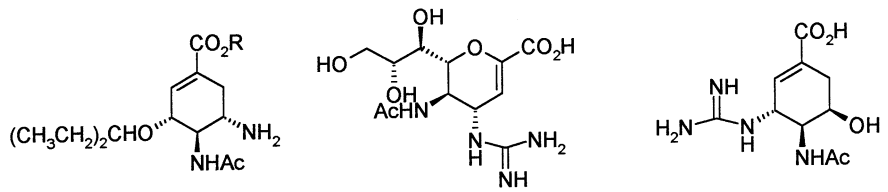
0008-6215/01/$ - see front matter
PII: S0008-6215(01)00079-9



Scheme 1.



Scheme 2.

GS4071) was found to be highly orally bioavailable in several animal species and efficacious in both the mouse and ferret models of influenza infection by oral administration.[9,10] Zanamivir (GG167, see Scheme 2)[11–13] is another 'transition state analogue' of **A** which exhibits the potent NA inhibitory activity. Based on the results previously obtained, we aimed our research at the synthesis of new compounds by using a carbocyclic template instead of the dihydropyran ring of the DANA system (DANA is the 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid).

2. Results and discussion

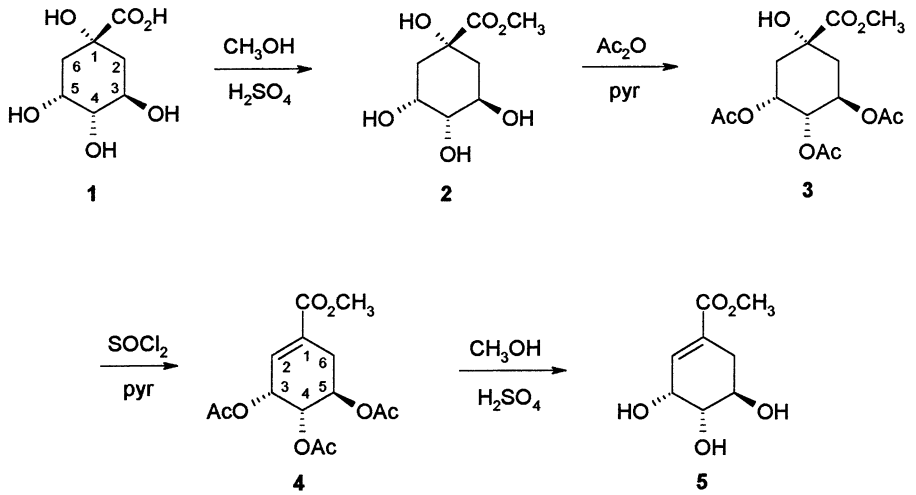
Considering the oxocarbonium ion (Scheme 1) as the transition-state, we prepared a cyclohexene analogue **17** (Scheme 2) which has the necessary conditions to imitate the transition state. These consist on acidic, acetamido functions and the planar carbon corresponding to C-2 of the sialic acid. Also the guanidino group has been introduced on the allylic position; that should be right to occupy the region of the NA active site that is normally filled to glyceric chain of sialic acid.

The carbocyclic analogue **17** was prepared according to the synthetic strategy depicted in Schemes 3 and 5 using as chiral starting material quinic acid **1**. Quinic acid **1** was converted into methyl shikimate **5** as shown in Scheme 3.[14] Quinic acid **1** was transformed into methyl ester **2** which was then acetylated, by controlling the reaction conditions, to obtain the triacetyl derivative **3** in which the tertiary hydroxyl remains free.

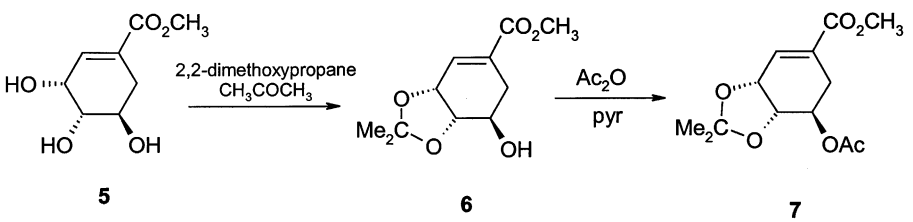
The two hydroxyls at C-3 and at C-4, in cis position to each other, were protected by formation of the *O*-isopropylidene derivative **6** (Scheme 4) and then the free hydroxyl was protected by acetylation to afford compound **7**.

To explain this regioselectivity, there is not an obvious reason; probably the hydroxyl at C-1 in quinic acid could take an axial orientation to undergo the elimination with the anti proton and the substituents at C-3 and C-5 may have an important role in the dehydration process. In fact, on the basis of the ^{1}H NMR coupling constants observed for H-4 in **3** ($J_{4,5}$ 4, $J_{4,3}$ 12 Hz), the acetoxy group at C-3 adopts an equatorial position while that at C-5 adopts an axial orientation. Obviously the transition state, which corresponds to the dehydration that produces **4**, is more energetically favoured than that which would produce the other isomer.

The synthetic strategy for the preparation of **17** continues, as described in Scheme 5,



Scheme 3.



Scheme 4.

starting from methyl shikimate **5**. While this work was in progress, as a part of a PhD[15] thesis project on inhibitors of NA and HA, Kim and co-workers[8] described the synthesis of compound GS4071 (see Scheme 2) with a strategy that is similar to that described in Scheme 5.

Methyl shikimate **5** was esterified with thionyl chloride to the cyclic sulfite **8** as a stable intermediate in 97% yield. In the ^{1}H NMR spectrum of **8**, there were two multiplets at δ 4.87 and at δ 5.58, ascribable to the protons at C-4 and at C-3, deshielded owing to esterification of the attached hydroxyls. Then the hydroxyl at C-5 of **8** was protected by means of acetylation, affording product **9**. The sulfite ring opening of **9** has been carried out with sodium azide in dimethylformamide; this reaction allows the introduction of the azido group in the allylic position and releases the hydroxylic function at C-4, providing derivative **10**. This complete regiospecific ring opening is a consequence of a favoured azide ion attack at the allylic C-3 position of **9**. The structure **10** was verified by the presence of the characteristic absorption band of the azido group at 2090 cm^{-1} in the IR spectrum. Compound **10** was treated with methanesulfonyl chloride to provide the mesyl derivative **11**, in which the three hydroxyl functions at C-3, -4 and -5 are protected selectively with three different groups. The ^{1}H NMR spectrum of **11** showed the presence of a multiplet at δ 4.77 due to the proton at C-4, now deshielded by the esterification of OH-4 with methanesulfonic acid, and an additional singlet at δ 3.12 due to the three protons of the mesyl group.

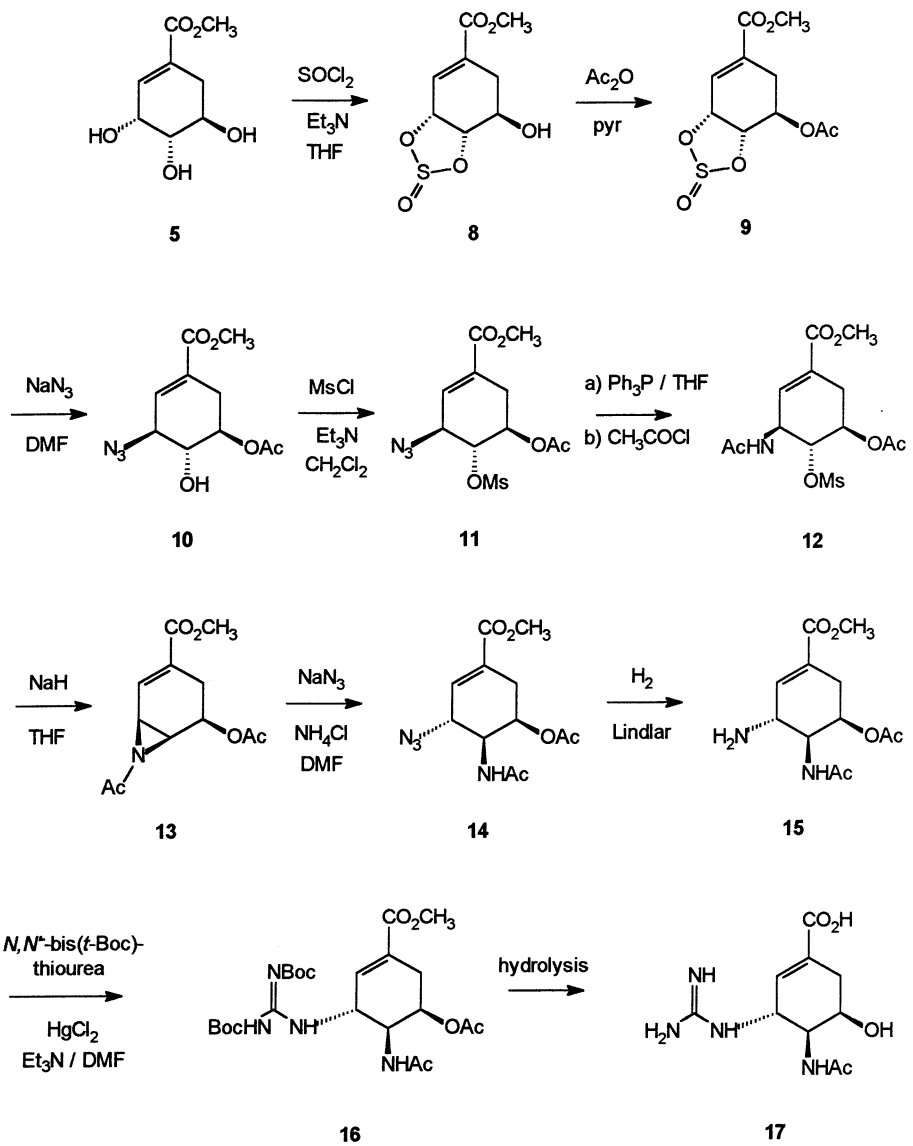
Conversion of the azido group in **11** to the acetamido derivative **12** was efficiently accomplished in 50% yield via a two-step sequence: reduction of the azide functionality with triphenylphosphine in tetrahydrofuran and acetylation of the amine with acetyl chloride. In the ^{1}H NMR spectrum of **12**, it is noted the presence of a singlet at δ 2.09 due to the acetyl group on nitrogen. Product **12** was treated with sodium hydride in tetrahydrofuran to provide *N*-acetylaziridine **13** by means of a S_N2 type intramolecular reaction.

The ^{1}H NMR spectrum of **13** shows the shielding of two protons at C-3 and at C-4 (by about 1 ppm) that give a multiplet at about δ

3.17. Treatment of **13** with sodium azide in dimethylformamide in the presence of ammonium chloride allowed ring opening of the acetylated aziridine and formation of **14**. The ring-opening reaction was completely regiospecific, giving azide **14** as the only product. In the ^{1}H NMR spectrum of **14**, one could notice the presence of two multiplets at δ 4.08 and at δ 4.35 due to protons at C-3 and at C-4, respectively, which were deshielded.

The azido group in allylic position was then subject to catalytic reduction with hydrogen in the presence of the Lindlar catalyst resulting in the selective reduction of the azide in the amino group without interference with the C–C double bond. This reaction afforded derivative **15**.

Treatment of **15** with *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea,[16] provided bis-(BOC) protected guanidine **16**. Finally, the hydrolysis of the methyl ester and the acetyl group at C-5 followed by the removal of the BOC groups, furnished the final product **17**. Biological tests on the sialic acid analog **17** are in progress.



Scheme 5.

3. Experimental

General methods.—^{1}H NMR spectra were measured with a Varian Gemini 200 MHz spectrometer and chemical shifts are expressed in ppm from TMS. Infrared spectra were registered with a Shimadzu IR-470 spectrometer. The starting material, quinic acid **1**, was obtained from Aldrich Chemical Co. Product purification was obtained by solid–liquid column chromatography on E. Merck 0.063–0.20 nm Silica Gel; the elution mixtures were determined case by case. E. Merck TLC plates coated with Kiesel-Gel 60 F_{254} were employed to monitor the reactions using 2 N H_2SO_4 and heating at 120 °C.

Methyl quinate (**2**).—Quinic acid **1** (1 g) was dissolved in MeOH; concd H_2SO_4 was added in catalytic quantity. The reaction mixture was allowed to stand at rt overnight. When the reaction was complete as evidenced by TLC (3:2 $CHCl_3$–EtOAc), the solution was neutralised with Dowex-21K (Cl^-) previously regenerated with NaOH. The mixture was filtered on gooch and the filtrate was evaporated under pressure to give methyl ester **2** (1.070 g, quantitative yield) as a white solid which crystallised from MeOH, mp 118 °C (Lit. 120 °C[17]). Compound **2** did not give a correct carbon analysis but was pure enough to be utilized in the next step. Quinic acid **1**, ^{1}H NMR (300 MHz, D_2O): δ 1.78 (m, 1 H, H-2ax), 1.90 (m, 1 H, H-6ax), 1.96 (m, 1 H, H-2eq), 2.07 (m, 1 H, H-6eq), 3.41 (dd, 1 H, *J* 3.3, 9.3 Hz, H-4ax), 3.90 (m, 1 H, H-3ax), 4.02 (m, 1 H, H-5eq). Methyl ester **2**, ^{1}H NMR (300 MHz, D_2O): δ 2.01 (m, 4 H, H-2ax, H-2eq, H-6ax, H-6eq), 3.49 (dd, 1 H, H-4ax), 3.69 (s, 3 H, CO_2CH_3), 4.05 (m, 2 H, H-3ax, H-5eq). Anal. Calcd for $C_8H_{14}O_6$: C, 46.60; H, 6.84. Found: C, 50.48; H, 6.19.

Methyl 3,4,5-tri-O-*acetylquinate* (**3**).—Methyl ester **2** (500 mg) was dissolved in pyridine (0.5 mL) and Ac_2O (1 mL) was added. The reaction mixture was allowed to stand at rt. After 2 h, compound **3** was partially formed as evidenced by TLC (9:1 $CHCl_3$–EtOAc); after this time there is a part of substrate that has not yet reacted; nevertheless the reaction was interrupted because longer reaction times afforded principally the tetraacetylated product. To the reaction mixture was added MeOH and the volatile materials were removed under diminished pressure. The residue was purified by chromatography (9:1 $CHCl_3$–EtOAc) to give **3** (380 mg, 47%) as a colourless amorphous solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.84 (s, 3 H, AcO), 1.90 (s, 3 H, AcO), 1.96 (s, 3 H, AcO), 2.05 (m, 4 H, H-2ax, H-2eq, H-6ax, H-6eq), 3.71 (s, 3 H, CO_2CH_3), 4.98 (dd, *J* 4, 12 Hz, 1 H, H-4), 5.35 (m, 2 H, H-3, H-5). Anal. Calcd for $C_{14}H_{20}O_9$: C, 50.60; H, 6.07. Found: C, 50.48; H, 6.19.

Methyl 3,4,5-tri-O-*acetylshikimate* (**4**).—Triacetyl derivative **3** (500 mg) was dissolved in pyridine; dropwise a solution of thionyl chloride (0.2 mL) in pyridine (0.3 mL) was added. The reaction mixture was allowed to stand at rt. After 2 h a brown syrup was formed; it was diluted with water and the reaction product **4** was extracted with EtOAc; the organic phase was washed first with 2 N HCl and then with water. The organic extract was dried on anhyd Na_2SO_4 and filtered; the solvent was evaporated and the residue was purified by chromatography (9:1 benzene–EtOAc) to give **4** (285 mg, 61%) as a colourless solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.00 (s, 3 H, AcO), 2.02 (s, 3 H, AcO), 2.03 (s, 3 H, AcO), 2.41 (m, 1 H, H-6ax), 2.91 (m, 1 H, H-6eq), 3.72 (s, 3 H, CO_2CH_3), 5.25 (m, 2 H, H-4, H-5), 5.71 (m, 1 H, H-3), 6.85 (m, 1 H, H-2). $[\alpha]_D$ −129° (EtOH, *c* 1.88); Lit. −168° (MeOH)[14d]. Anal. Calcd for $C_{14}H_{18}O_8$: C, 53.50; H, 5.77. Found: C, 53.37; H, 5.86.

Methyl shikimate (**5**).—Triacetyl derivative **4** (500 mg) was dissolved in MeOH (10 mL); an alcoholic solution of concd H_2SO_4 (0.5 mL in 5 mL of MeOH) was added. The reaction mixture was allowed to stand at rt overnight. The reaction mixture was neutralised with a methanolic suspension of barium carbonate; the solution was filtered through Celite and the solvent was evaporated under diminished pressure. The residue was purified by chromatography (9:1 EtOAc–$CHCl_3$) to give **5** (275 mg, 92%) as a colourless solid which crystallised from AcOH–pet. ether, mp 112–113 °C (Lit. 113–114 °C[14a]). ^{1}H NMR (200 MHz, D_2O): δ 2.19 (dd, 1 H, H-6ax), 2.67 (dd, 1 H, H-6eq), 3.68 (m, 1 H, H-5), 3.73 (s, 3 H,

CO_2CH_3), 3.97 (m, 1 H, H-4), 4.36 (m, 1 H, H-3), 6.77 (m, 1 H, H-2). Anal. Calcd for $C_8H_{12}O_5$: C, 51.06; H, 6.43. Found: C, 50.84; H, 6.56.

Methyl 3,4-O-isopropylideneshikimate (**6**).—Methyl shikimate **5** (500 mg) was dissolved in anhyd acetone (5 mL); 2,2-dimethoxypropane (5 mL) and Dowex-50 (H^+) (catalytic quantity) were added. The reaction mixture was stirred at rt for 3 h. The mixture was filtered on gooch and the filtrate was evaporated under pressure to give **6** (596 mg, quantitative yield). For its characterisation, a crude sample (100 mg) was purified by chromatography (3:2 EtOAc–$CHCl_3$) to give **6** (92 mg) as a colourless solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (s, 3 H, CCH_3), 1.41 (s, 3 H, CCH_3), 1.69 (bs, 1 H, OH), 2.23 (m, 1 H, H-6ax), 2.76 (dd, 1 H, H-6eq), 3.73 (s, 3 H, CO_2CH_3), 3.85 (m, 1 H, H-5), 4.05 (t, 1 H, H-4), 4.71 (m, 1 H, H-3), 6.89 (m, 1 H, H-2). Anal. Calcd for $C_{11}H_{16}O_5$: C, 57.89; H, 7.07. Found: C, 57.73; H, 7.18.

Methyl 5-O-acetyl-3,4-O-isopropylideneshikimate (**7**).—Isopropylidene derivative **6** (100 mg) was dissolved in pyridine (0.5 mL); Ac_2O (1 mL) was added. The reaction mixture was allowed to stand at rt for 1 h. After this time, MeOH was added to the reaction mixture and the solvents were concentrated under diminished pressure. The residue was diluted with EtOAc; to the organic phase 2 N HCl acid was added; then the solution was washed with water until neutralisation. The organic extract was dried on anhyd Na_2SO_4, filtered and evaporated under diminished pressure to afford derivative **7** (118 mg, quantitative yield). For its characterisation, a crude sample (20 mg) was purified by chromatography (7:3 $CHCl_3$–EtOAc) to give **7** (17 mg) as a colourless solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (s, 3 H, CCH_3), 1.40 (s, 3 H, CCH_3), 2.08 (s, 3 H, AcO), 2.25–2.38 (dd, 1 H, H-6ax), 2.72–2.83 (dd, 1 H, H-6eq), 3.78 (s, 3 H, CO_2CH_3), 4.20 (t, 1 H, H-4), 4.71 (m, 1 H, H-3), 5.15 (m, 1 H, H-5), 6.89 (m, 1 H, H-2). Anal. Calcd for $C_{13}H_{18}O_6$: C, 57.77; H, 6.71. Found: C, 57.60; H, 6.84.

Methyl shikimate 3,4-cyclic sulfite (**8**).—To a solution of **5** (400 mg) in THF (8 mL) at 0 °C was added triethylamine (0.8 mL), followed by dropwise addition of thionyl chloride (0.4 mL). The reaction mixture was warmed to rt and stirred for 2 h. After this time, the reaction was complete (TLC 7:3 $CHCl_3$–EtOAc). The mixture was diluted with $CHCl_3$ and was washed with water and brine and dried on anhyd Na_2SO_4. The solvents were evaporated under diminished pressure, and the residue was purified by chromatography (9:1 $CHCl_3$–EtOAc) to give **8** (480 mg, 97%) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.26–2.42 (m, 1 H, H-6a), 2.85 (m, 1 H, H-6b), 3.81 (s, 3 H, CO_2CH_3), 3.95 (m, 1 H, H-5), 4.87 (m, 1 H, H-4), 5.58 (m, 1 H, H-3), 6.91 (m, 1 H, H-2). Anal. Calcd for $C_8H_{10}O_6S$: C, 41.02; H, 4.30; S, 13.69. Found: C, 40.83; H, 4.42; S, 13.74.

Methyl 5-O-acetylshikimate 3,4-cyclic sulfite (**9**).—Compound **8** (300 mg) was dissolved in pyridine (0.5 mL); Ac_2O (1 mL) was added. The reaction mixture was allowed to stand at rt for 1 h. After this time MeOH was added to the reaction mixture and the solvents were concentrated under diminished pressure. The residue was diluted with EtOAc. To the organic phase 2 N HCl was added; then the solution was washed with water until neutralisation. The organic extract was dried on anhyd Na_2SO_4, filtered and evaporated under pressure to give **9** (320 mg, 91%). Compound **9** is pure enough to be used in the following synthetic step. For its characterisation, a crude sample (20 mg) was purified by chromatography (9:1 $CHCl_3$–EtOAc) to give **9** (17 mg) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.10 (s, 3 H, AcO), 2.38 (m, 1 H, H-6ax), 2.89 (dd, 1 H, H-6eq), 3.79 (s, 3 H, CO_2CH_3), 4.91 (m, 1 H, H-4), 5.11 (m, 1 H, H-5), 5.54 (m, 1 H, H-3), 6.91 (m, 1 H, H-2). Anal. Calcd for $C_{10}H_{12}O_7S$: C, 43.48; H, 4.38; S, 11.60. Found: C, 40.33; H, 4.49; S, 11.52.

Methyl (3S,4R,5R)-5-acetoxy-3-azido-4-hydroxycyclohex-1-ene-1-carboxylate (**10**).—Compound **9** (200 mg) was dissolved in anhyd DMF (4 mL); sodium azide (200 mg) was added. The mixture was stirred at rt for 20 h. The reaction mixture was then diluted with EtOAc, washed with saturated ammonium chloride, water, and brine, and dried on anhyd Na_2SO_4. The solvents were evaporated and the residue was purified by chromatography

(4:1 $CHCl_3$–EtOAc) to give **10** (168 mg, 92%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.08 (s, 3 H, AcO), 2.13–2.32 (m, 1 H, H-6ax), 2.79–2.98 (m, 1 H, H-6eq), 3.70 (s, 3 H, CO_2CH_3), 3.79 (m, 1 H, H-4), 4.11 (m, 1 H, H-3), 4.98 (m, 1 H, H-5), 6.62 (m, 1 H, H-2). IR: 2090 cm^{-1} (N_3). Anal. Calcd for $C_{10}H_{13}N_3O_5$: C, 47.06; H, 5.13; N, 16.46. Found: C, 46.82; H, 5.21; N, 16.33.

*Methyl (3*S*,4*R*,5*R*)-5-acetoxy-3-azido-4-(methylsulfonyloxy)cyclohex-1-ene-1-carboxylate* (**11**).—Compound **10** (200 mg) was dissolved in CH_2Cl_2 (10 mL) and to this solution, at 0 °C, was added triethylamine (0.4 mL) followed by dropwise addition of methanesulfonyl chloride (0.2 mL). The reaction mixture was stirred at 0 °C for 1 h. Then it was diluted with CH_2Cl_2. The organic phase was washed with water and brine, dried on anhyd Na_2SO_4, and the volatile materials were removed under diminished pressure. The residue was purified by chromatography (9:1 $CHCl_3$–EtOAc) to give **11** (186 mg, 72%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.12 (s, 3 H, AcO), 2.32–2.51 (m, 1 H, H-6ax), 2.91–3.09 (m, 1 H, H-6eq), 3.12 (s, 3 H, SO_2CH_3), 3.79 (s, 3 H, CO_2CH_3), 4.30 (m, 1 H, H-3), 4.77 (m, 1 H, H-4), 5.19 (m, 1 H, H-5), 6.72 (m, 1 H, H-2). Anal. Calcd for $C_{11}H_{15}N_3O_7S$: C, 39.64; H, 4.54; N, 12.61; S, 9.62. Found: C, 39.48; H, 4.61; N, 12.53; S, 9.48.

*Methyl (3*S*,4*R*,5*R*)-3-acetamido-5-acetoxy-4-(methylsulfonyloxy)cyclohex-1-ene-1-carboxylate* (**12**).—To a solution of **11** (200 mg) in THF (15 mL) was added triphenylphosphine (140 mg) and the solution was stirred at rt for 3 h. The solvent was evaporated, the crude residue was dissolved in CH_2Cl_2 (15 mL) and cooled at 0 °C, and pyridine (0.5 mL) was added followed by dropwise addition of acetyl chloride (0.25 mL). The reaction mixture was stirred at 0 °C for 1 h. Then it was diluted with EtOAc. The organic phase was washed with water and brine, dried on anhyd Na_2SO_4, and the volatile materials were removed under diminished pressure. The residue was purified by chromatography (3:2 EtOAc–$CHCl_3$) to give **12** (106 mg, 50%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.08 (s, 3 H, NHAc), 2.15 (s, 3 H, AcO), 2.34 (m, 1 H, H-6ax), 2.90 (m, 1 H, H-6eq), 3.12 (s, 3 H, SO_2CH_3), 3.77 (s, 3 H, CO_2CH_3), 4.31 (m, 1 H, H-3), 4.77 (m, 1 H, H-4), 5.20 (m, 1 H, H-5), 5.61 (d, 1 H, NH), 7.19 (m, 1 H, H-2). Anal. Calcd for $C_{13}H_{19}NO_8S$: C, 44.69; H, 5.48; N, 4.01; S, 9.18. Found: C, 44.31; H, 5.62; N, 4.15; S, 9.32.

*Methyl (3*S*,4*S*,5*R*)-5-acetoxy-3,4-(acetylepimino)cyclohex-1-ene-1-carboxylate* (**13**).—Compound **12** (100 mg) was dissolved in THF (10 mL); NaH (25 mg) was added. The reaction mixture was allowed to stand at rt for 4 h. The excess NaH was neutralised with CO_2; then the volatile material was evaporated, the crude residue was purified by chromatography (1:1 $CHCl_3$–EtOAc) to give **13** (48 mg, 65%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.10 (s, 3 H, NAc), 2.16 (s, 3 H, AcO), 2.35 (m, 1 H, H-6ax), 2.85 (m, 1 H, H-6eq), 3.14 (m, 2 H, H-3, H-4), 3.77 (s, 3 H, CO_2CH_3), 5.58 (m, 1 H, H-5), 7.19 (m, 1 H, H-2). Anal. Calcd for $C_{11}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.70; H, 6.07; N, 5.47.

*Methyl (3*R*,4*S*,5*R*)-4-acetamido-5-acetoxy-3-azidocyclohex-1-ene-1-carboxylate* (**14**).—A mixture of **13** (70 mg), sodium azide (70 mg), and ammonium chloride (35 mg) in DMF (4 mL) was stirred at rt for 20 h. The reaction mixture was diluted with EtOAc, washed with water and brine, and dried on anhyd Na_2SO_4. The solvent was evaporated under diminished pressure, and the residue was purified by chromatography (1:1 $CHCl_3$–EtOAc) to give a mixture of products which was dissolved in Ac_2O (1 mL) and stirred for 2 h. Excess Ac_2O was evaporated to afford **14** (52 mg, 61%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.08 (s, 3 H, NHAc), 2.12 (s, 3 H, AcO), 2.51 (m, 1 H, H-6ax), 2.91 (m, 1 H, H-6eq), 3.78 (s, 3 H, CO_2CH_3), 4.08 (m, 1 H, H-4), 4.31 (m, 1 H, H-3), 5.05 (m, 1 H, H-5), 5.53 (d, 1 H, NH), 6.76 (m, 1 H, H-2). IR: 2090 cm^{-1} (N_3). Anal. Calcd for $C_{12}H_{16}N_4O_5$: C, 48.65; H, 5.44; N, 18.91. Found: C, 48.33; H, 5.62; N, 18.70.

*Methyl (3*R*,4*S*,5*R*)-4-acetamido-5-acetoxy-3-aminocyclohex-1-ene-1-carboxylate* (**15**).—Compound **14** (50 mg) was dissolved in EtOH (2 mL) and treated with the Lindlar catalyst (10 mg) and stirred at rt under 101 kPa of hydrogen for 12 h. The reaction mixture was filtered through Celite, and the catalyst was washed with 1:1 hot MeOH–water. The

filtrate was evaporated on a rotatory evaporator. The residue was purified by chromatography (1:1 MeOH–$CHCl_3$) to give **15** (32 mg, 70%) as a colourless amorphous solid. The compound did not give a correct nitrogen analysis, but was pure enough for further use. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.06 (s, 3 H, NHAc), 2.10 (s, 3 H, AcO), 2.35 (m, 1 H, H-6ax), 2.83 (dd, 1 H, H-6eq), 3.75 (s, 3 H, CO_2CH_3), 3.92–4.08 (m, 2 H, H-3, H-4), 5.01 (m, 1 H, H-5), 5.56 (d, 1 H, NH), 6.26 (m, 1 H, H-2). Anal. Calcd for $C_{12}H_{18}N_2O_5$: C, 53.33; H, 6.71; N, 10.36. Found: C, 53.28; H, 6.82; N, 9.51.

*Methyl (3*R,*4*S,*5*R*)-4-acetamido-5-acetoxy-3-[2,3-bis(*tert-*butoxycarbonyl)guanidino]cyclohex-1-ene-1-carboxylate* (**16**).—To a solution of amine **15** (100 mg), *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (110 mg) and triethylamine (15 mL) in dry DMF (5 mL) cooled to 0 °C was added $HgCl_2$ (100 mg). The heterogeneous reaction mixture was stirred for 45 min at 0 °C and then at rt for 15 min, after which the reaction was diluted with EtOAc and filtered through a pad of Celite. The volatile materials were removed under diminished pressure. The residue was chromatographed (9:1 EtOAc–hexane) to give **16** (156 mg, 84%) as a pale oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.45 (s, 18 H, *t*-Boc), 1.95 (s, 3 H, NHAc), 2.01 (s, 3 H, OAc), 2.33–2.45 (m, 1 H, H-6a), 2.83 (m, 1 H, H-6b), 3.77 (s, 3 H, CO_2CH_3), 4.06–4.13 (m, 1 H, H-4), 4.32–4.43 (m, 1 H, H-3), 4.98 (m, 1 H, H-5), 6.46 (d, 1 H, NH), 6.89 (m, 1 H, H-2). Anal. Calcd for $C_{23}H_{36}N_4O_9$: C, 53.91; H, 7.03; N, 10.93. Found: C, 53.77; H, 7.18; N, 10.85.

*(3*R,*4*S,*5*R*)-4-Acetamido-3-guanidino-5-hydroxycyclohex-1-ene-1-carboxylic acid* (**17**).—To a solution of **16** (100 mg) in THF (5 mL) was added aq KOH (1.5 mL of a 1 N solution). The reaction mixture was stirred at rt for 17 h, cooled at 0 °C, neutralised by bubbling CO_2, and acidified with Dowex-50 (H^+). The mixture was stirred at rt for 4 h. The solution was filtered on gooch and the volatile materials were removed under diminished pressure. The residue was purified by reverse-phase chromatography on Lichroprep RP-8 E. Merck eluting with water to give **17** (40 mg, 84%), as a colourless amorphous solid. ^{1}H NMR (200 MHz, D_2O): δ 1.98 (s, 3 H, NHAc), 2.19 (m, 1 H, H-6a), 2.67 (m, 1 H, H-6b), 3.68 (m, 1 H, H-5), 3.99 (m, 1 H, H-4), 4.24 (m, 1 H, H-3), 6.66 (m, 1 H, H-2). Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.86. Found: C, 46.68; H, 6.13; N, 21.62.

Acknowledgements

This work was supported by Istituto Pasteur-Fondazione Cenci Bolognetti, Università di Roma 'La Sapienza'.

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