Approaches to carbocyclic analogues of the potent neuraminidase inhibitor 4-guanidino-Neu5Ac2en. X-Ray molecular structure of *N*-[(1*S*,2*S*,6*R*)-2-azido-6-benzyloxymethyl-4-formylcyclohex-3-enyl]acetamide

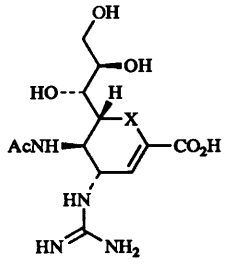
Malcolm Chandler,[a] Richard Conroy,[a] Anthony W. J. Cooper,[a] R. Brian Lamont,[b] Jan J. Scicinski,*[,a] James E. Smart,[a] Richard Storer,[a] Niall G. Weir,[a] Richard D. Wilson[a] and Paul G. Wyatt[a]

Departments of [a] Medicinal and [b] Structural Chemistry, Glaxo Research and Development Limited, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK

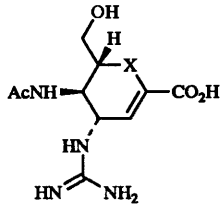
Various approaches using Diels–Alder chemistry have been established for the synthesis of truncated carbocyclic analogues **4** and **6** of 4-guanidino-Neu5Ac2en. In the case of compound **4**, elaboration of an initial adduct from Danishefsky's diene and the dienophile **7** allowed access to the key enone **26**. Methylenation of the carbonyl group and azide-induced opening of an intermediate oxazoline established the required framework regio- and stereo-specifically. Compounds **4** and **6** were found to retain interesting levels of antiviral activity comparable to those shown by their oxygen-containing counterparts.

In the preceding papers[1,2] we describe the synthesis of the nonenonic acid compound **1**, a potent neuraminidase inhibitor, currently under development as a potential drug for the prophylaxis and treatment of disease caused by influenza A and B viruses,[3] and a series of analogues in order to examine the contribution of the glycerol side-chain. In our ongoing studies to define the structure–activity relationships in this class we were interested in preparing the carbocyclic analogue **2** of the lead compound **1** in order to help us understand the contribution made by the ring oxygen to the overall profile of this series. Ogawa *et al.*[4] have previously reported the preparation of the carbocyclic analogue of Neu5Ac, which they found to be a weak sialidase inhibitor.

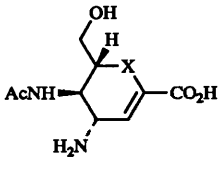
Our earlier work[2] had shown that truncation of the glycerol side-chain of compound **1** to give heptenonic acid derivative **3** resulted in retention of significant activity in the influenza plaque-reduction assays. To enable a rapid entry into the carbocyclic series we chose the simplified target **4** having a hydroxymethyl substituent rather than the full glycerol side-chain. The 4-amino compound **5** also showed activity though at a lower level.[2] Compounds **4** and **6** should provide an adequate indication of the potential of the carbocyclic series.



1 X = O
2 X = CH_2



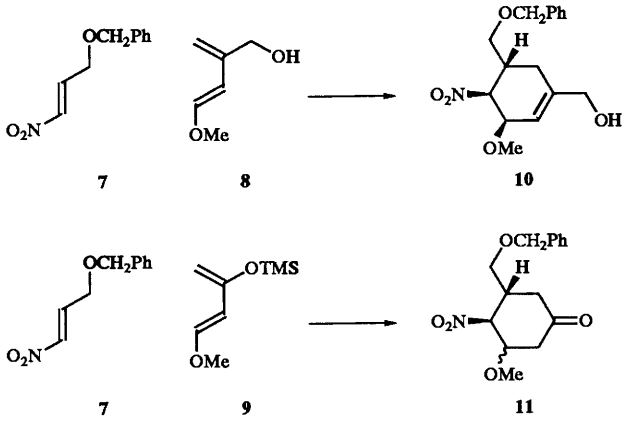
3 X = O
4 X = CH_2



5 X = O
6 X = CH_2

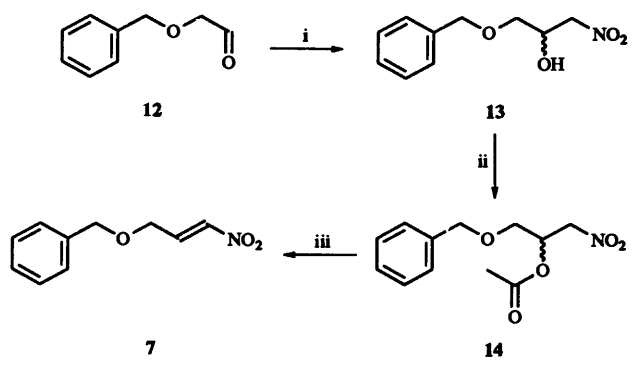
Discussion

A Diels–Alder reaction between the readily available nitro olefin **7** and either the known diene **8** or Danishefsky's diene **9** was chosen as the method of ring construction (Scheme 1).

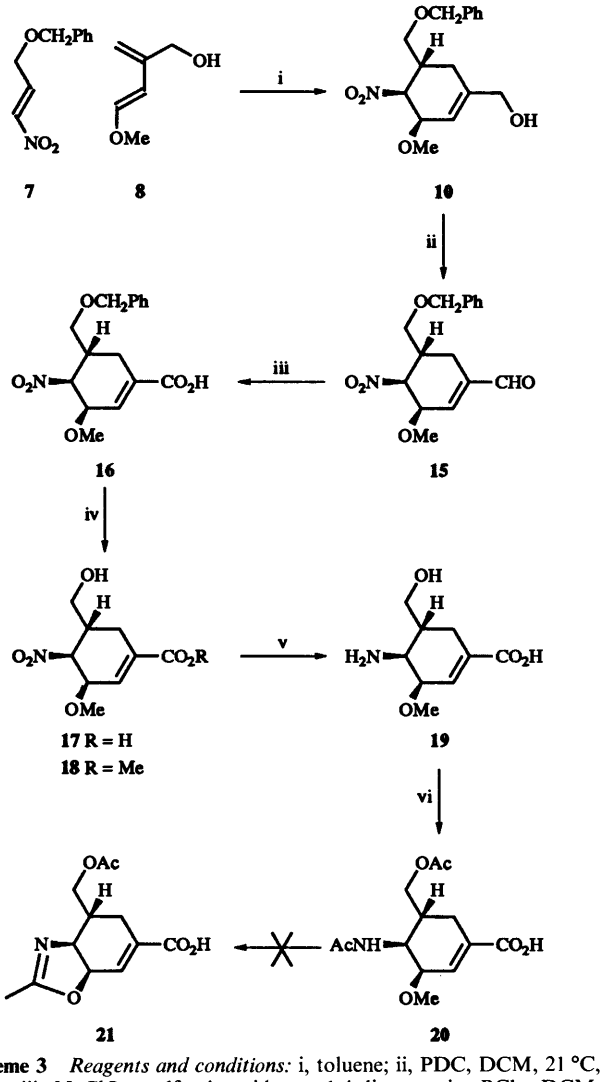


Scheme 1

Treatment of benzyloxyacetaldehyde diethyl acetal with acetic acid gave the corresponding aldehyde **12** (Scheme 2). This was subjected to a Henry reaction with nitromethane in the presence of neutral alumina[5] to give adduct **13** in high yield over the two stages. This and the subsequent dienophile precursors could be purified by Kugelruhr distillation; however, this incurred unacceptable losses. Therefore unpurified adduct **13** was acetylated in acetic anhydride with sulfuric acid catalysis to give acetate **14** in 69% yield. Elimination of acetic acid by treatment with sodium hydrogen carbonate in toluene at 80 °C then gave the required dienophile **7** in 45% yield after purification by flash chromatography. Diels–Alder reaction of dienophile **7** with (*E*)-4-methoxy-2-methylenebut-3-en-1-ol[6] **8** in toluene at ambient temperature afforded a mixture of *exo*/*endo* adducts, from which the major isomer **10** was isolated by flash chromatography in 89% yield (Scheme 3).



Scheme 2 *Reagents and conditions:* i, $MeNO_2$, neutral alumina, 21 °C, 24 h; ii, Ac_2O, H_2SO_4, 21 °C, 3 h; iii, $NaHCO_3$, toluene, 80 °C, 24 h



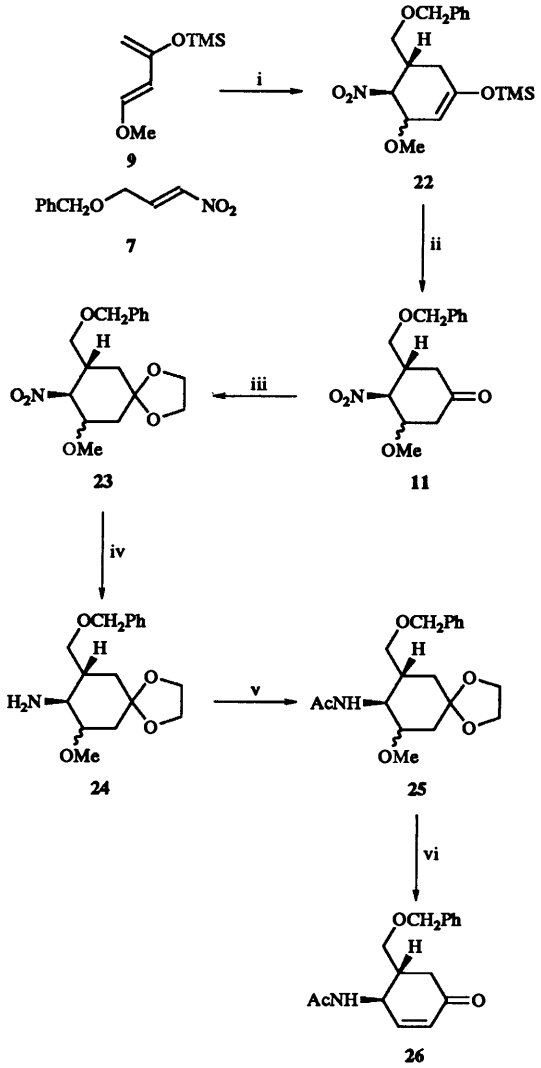
Scheme 3 *Reagents and conditions:* i, toluene; ii, PDC, DCM, 21 °C, 24 h; iii, $NaClO_2$, sulfamic acid, aq. 1,4-dioxane; iv, BCl_3, DCM, −75 °C, 0.5 h; v, Zn, AcOH, ultrasound, 0.5 h; vi, Ac_2O, DCM, Et_3N, pyridine, DMAP, 21 °C, 24 h

Concerned that the benzyl protecting group might prove difficult to remove, we elected to deprotect early in the synthesis. As this liberated a second hydroxymethyl substituent, at C-5, this required the C-1 substituent of compound **10** to be carried through to the required carboxylic acid oxidation state. Best results were obtained by a two-stage process: oxidation to the aldehyde **15** with pyridinium dichromate (PDC) in dichloromethane (DCM), followed by treatment with sodium chlorite in aq. 1,4-dioxane, gave acid **16** in 62% overall yield.

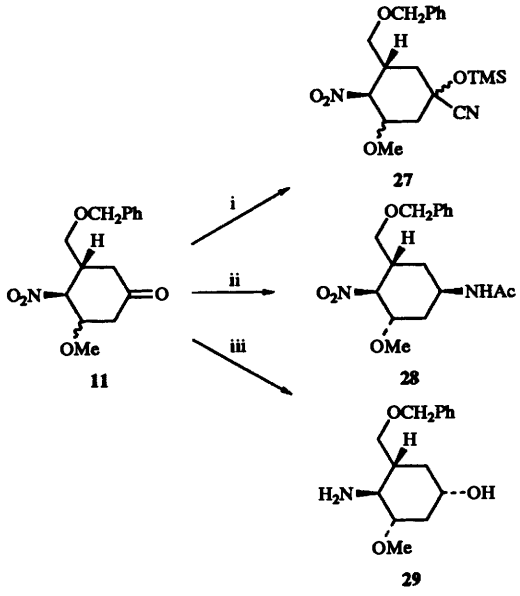
Treatment of acid **16** with boron trichloride in DCM[7] at −75 °C followed by quenching with methanol gave a mixture of the carboxylic acid **17** and the corresponding methyl ester **18**. The acid was separated from the ester by trituration with chloroform to give pure crystalline acid **17** in 55% yield. Reduction of the nitro group of acid **17** was achieved using zinc in acetic acid with ultrasound to give amine **19** in 70% yield. Acetylation of amine **19** gave the acetamide **20** in 77% yield. Attempts to prepare[1,8] the key oxazoline **21** from either methoxy amide **20** or the corresponding 3-hydroxy compound proved unsuccessful, thereby necessitating an alternative approach to introduction of an azide function at C-3.

This work demonstrated the validity of a Diels–Alder approach for the construction of the carbocyclic framework and established conditions for removal of the protecting groups. As the diene proved very difficult to prepare in sufficient quantities, we decided to adopt an alternative approach, using a simplified diene lacking the C-1 substituent, and to explore methods for C-1 homologation. Appropriate activation of the carbonyl functionality would give us the opportunity of using the oxazoline methodology for introduction of nitrogen at C-3, trapping the double bond in the correct position in the ring.

Reaction of the crude dienophile **7** with Danishefsky's diene **9**[9] (Scheme 4) gave the trimethylsilyl enol ether **22**, which on



Scheme 4 *Reagents and conditions:* i, toluene, 80 °C, N_2; ii, HCl, aq. THF; iii, $HOCH_2CH_2OH$, $(CO_2H)_2$, Pr^iOAc, reflux; iv, $NaBH_4$, $CoCl_2$, EtOH, 80 °C; v, Ac_2O, pyridine, DMAP, DCM; vi, 2 mol dm^{-3} HCl, acetone

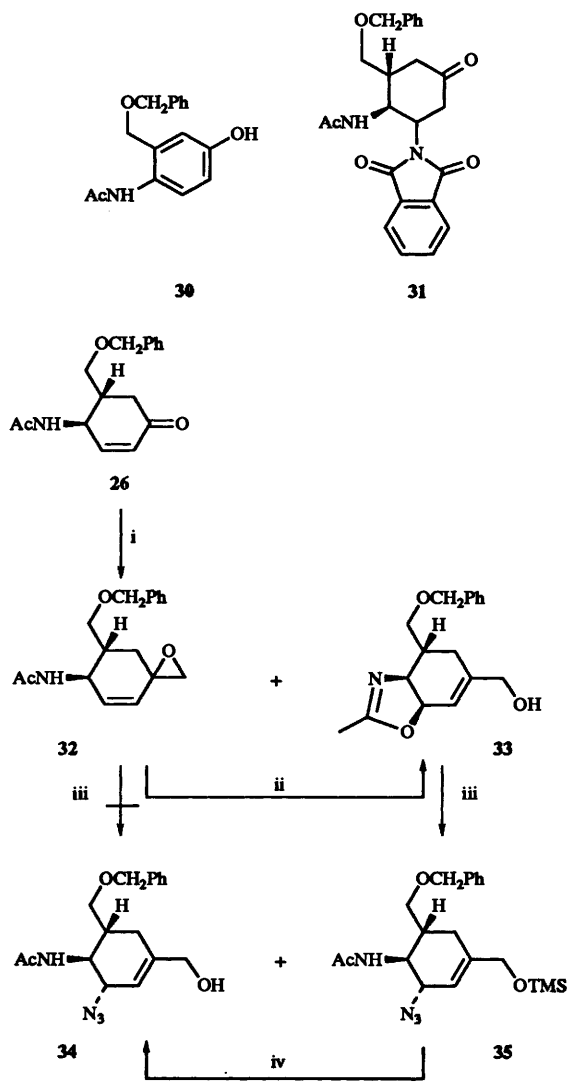


Scheme 5 *Reagents and conditions:* i, TMSCN, TMSOTf (cat.), DCM, −78 °C; ii, 10% Pd/C, NH_4OAc, MeOH; iii, $NaBH_4$, $CoCl_2$, EtOH, 80 °C

hydrolysis with 2 mol dm^{-3} aq. HCl[10] in tetrahydrofuran (THF) gave the methoxy nitro ketone **11** in 58% yield from the dienophile as a 65:35 mixture of isomers, which were conveniently processed without purification. Although C-homologation could be achieved at this stage (Scheme 5) by reaction of the nitro ketone **11** with trimethylsilyl cyanide (TMSCN) in the presence of catalytic trimethylsilyl triflate (TMSOTf)[11] to give the cyanohydrin **27**, attempts at hydrolysis proved unsuccessful. Similar problems were encountered with a Wittig reaction of methoxymethyl(triphenyl)phosphorane.[12] The cause of these problems was presumed to be the acidity of the proton α to the nitro group at this stage.

Reduction of the nitro group proved troublesome and a variety of conditions, including zinc in acetic acid, failed. Interestingly, attempted phase-transfer hydrogenolysis under anhydrous conditions,[13] followed by acylation gave the reductive amination product **28**, substantiated by ^1H NMR spectroscopy (Scheme 5). However, treatment with sodium boranuide in the presence of cobalt(II) chloride[14] in ethanol at elevated temperature resulted in the smooth formation of the amino alcohol **29** in 86% yield. As selective acylation on nitrogen[15] gave mixtures and lower yields, the ketone was protected as the ketal **23** before reduction. Reduction under the same conditions gave the amino ketal **24** in varying yields (55–72%) due to acidic hydrolysis of the ketal during work-up. The amine, without purification, was acylated to give the amide **25** in high yield, which was then hydrolysed to the enone **26** in 59% overall yield. Treatment of the enone **26** with azidotrimethylsilane in *tert*-butyl alcohol[1] at 80 °C gave the unstable 1,2-addition product rather than the required 1,4-product. The structure of the 1,2-adduct was deduced by ^1H NMR spectroscopy. Use of sodium azide[16] in dimethylformamide (DMF) gave mixtures of starting material and the phenol **30**. Addition of phthalimide[17] to the enone **26** on a small scale gave the product **31**; however, this reaction was not amenable to scale-up.

We chose methylenation of the ketone as the C-homologation method. As azides have been shown to add to epoxides,[16] we wanted to extend this rationale to a 1,4-addition of azide to the α,β-unsaturated epoxide **32**. Reaction of the enone **26** with trimethylsulfonium iodide in the presence of butyllithium[18] at 0 °C gave the unstable epoxide **32** (Scheme 6). The reaction was



Scheme 6 *Reagents and conditions:* i, Me_3SI, BuLi, THF, 0 °C; ii, SiO_2, EtOAc; iii, $TMSN_3$, Bu^{t}OH, 80 °C; iv, 2 mol dm^{-3} HCl, MeOH

found to be very sensitive to air, owing to small quantities of highly coloured impurities in the enone. In the neuraminic acid series[8] we employed a Lewis acid (optimally trimethylsilyl triflate) to effect cyclisation of the 5-acetamido group. The epoxide **32** formed the corresponding oxazoline even without Lewis acid catalysis; however, adsorption onto silica gel from ethyl acetate was found to be the most convenient method for effecting this rearrangement. Treatment of the oxazoline **33** with azidotrimethylsilane in *tert*-butyl alcohol at 80 °C resulted in attack of azide from the least hindered side to give the alcohol **34** and the trimethylsilyl ether **35**. Acidic hydrolysis of the mixture gave the required azido alcohol **34** in 27% overall yield from the enone as a single isomer. The X-ray crystal structure (Fig. 1) of the aldehyde **36**, determined by single-crystal analysis of a sample recrystallised from isopropyl alcohol, confirmed the relative stereochemistry around the ring. Reaction of the epoxide **32** with azidotrimethylsilane in *tert*-butyl alcohol at 80 °C did not give the required alcohol.

Two-step oxidation (*vide infra*) of the alcohol gave the required carboxylic acid **37** in 22% yield from the alcohol **34** (Scheme 7). Treatment of the acid **37** with boron trichloride gave, after methanolic work-up, a mixture of the carboxylic acid **38** and the methyl ester **39**. Hydrolysis of this mixture with triethylamine in water gave the required carboxylic acid **38** in 36% yield. Carefully controlled hydrogenation of the acid in water over 5% Pd on carbon for 1 h gave the required amine **6**.

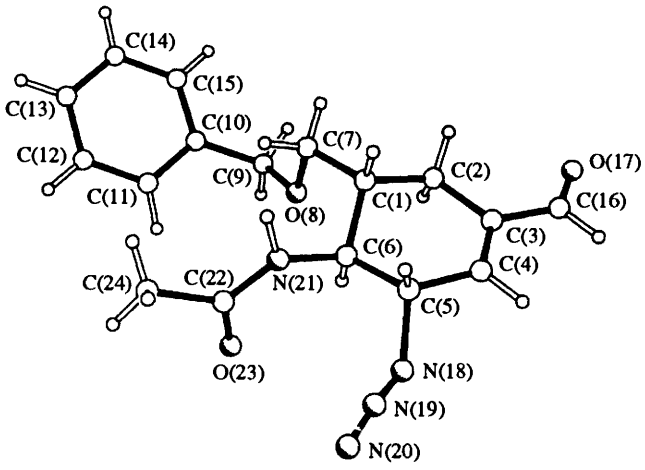


Fig. 1 X-Ray molecular structure of compound **36**, with crystallographic numbering scheme

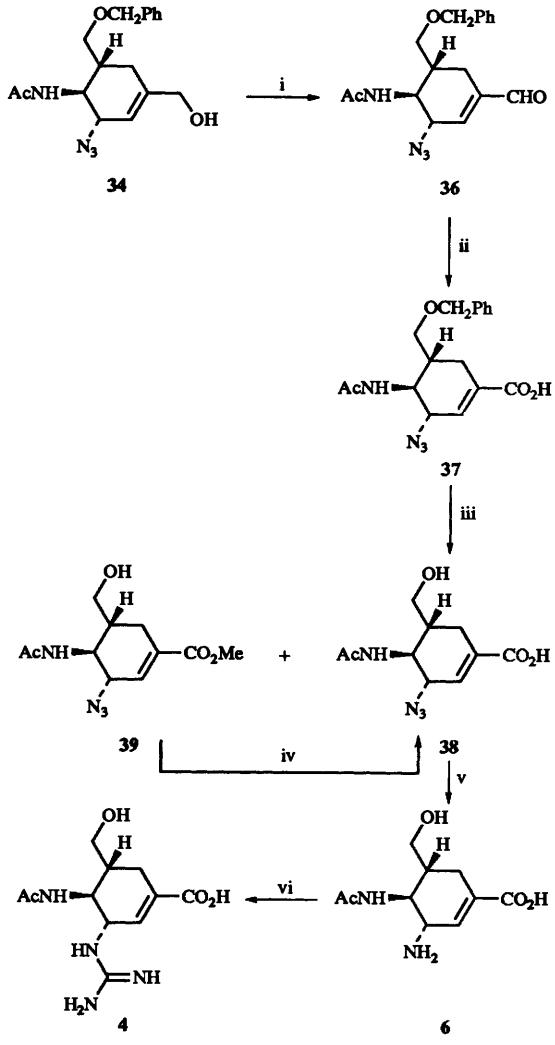
Although transformation of the amine **6** into the guanidine **4** could be achieved by using aminoiminomethanesulfonic acid (AIMSA),[19] better results were obtained by treatment with buffered cyanogen bromide[2,20] in methanol to give the cyanamide. Reaction of the crude cyanamide with concentrated ammonia followed by preparative HPLC gave the required guanidine **4**.

Biological results

The carbocyclic products **4** and **6** were tested as inhibitors of viral neuraminidase and in the influenza A and B plaque-reduction assays. Data for these compounds were compared with data for the neuraminic acid derivatives (Table 1). Although compound **4** was equipotent against viral neuraminidase with the neuraminic acid derivative **3**, it had enhanced activity in the influenza A plaque-reduction assay though not against influenza B. Interestingly, the anti-influenza A activity of the carbocycle **6** was much better than the parent **5**. Although in the neuraminic acid series the guanidine **3** was more active than the amine **5** in the plaque assays, in the carbocyclic series the amine **6** was more active than the guanidine **4**. In view of these results, the full side-chain carbocyclic analogue of compound **1** as well as the corresponding 4-amino derivative may prove interesting.

Experimental

Mps were determined in an open capillary using a Mettler FP51 automatic melting point apparatus and are expressed as *M-y* where *y* is the insertion temperature, the heating rate being 2 °C min^{-1}. ^1H NMR spectra were run on a Bruker 250 or 400 MHz spectrometer with Me_4Si as internal standard. ^{13}C NMR spectra were run on a Varian VXR 500 MHz spectrometer. *J*-Values are given in Hz. IR spectra were obtained using a Nicolet 5SxC FT-IR spectrometer. Preparative silica chromatography was run on Merck 9385 silica under flash conditions or on Merck 7734 silica under gravity conditions. HPLC analysis: conditions 1: S5-ODS2 column; eluent: acetonitrile in water; flow: 1 cm^3 min^{-1}; detection: UV, 220 nm; temperature: ambient. Conditions 2: Dynamax C_{18} column; eluent: 15% acetonitrile in pH 3 phosphate buffer; flow: 1 cm^3 min^{-1}; detection: UV, 230 nm; temperature: ambient. Conditions 3: Dynamax C_{18} column; eluent: 20% acetonitrile + 0.1% trifluoroacetic acid (TFA) in water + 0.1% TFA; flow: 1 cm^3 min^{-1}; detection: UV, 230 nm; temperature: ambient. Conditions 4: S5-ODS2 column; eluent: 10% methanol in pH 9.3 borate buffer; detection: UV, 210 nm; temperature: ambient. Preparative HPLC: conditions: Dynamax C_{18} 2″ i.d. column; eluent: A = 0.1% TFA in water; B = 0.05% TFA in water: 0% B for 15 min, then gradient to 95% B over a period of 5 min, then 95% B for 5 min; detection: UV, 220 nm; flow: 45 cm^3 min^{-1}. Enzyme and virus-inhibitory activities were conducted as previously reported.[3] Light petroleum refers to the fraction with distillation range 40–60 °C.



Scheme 7 *Reagents and conditions:* i, PDC, DCM, room temp.; ii, $NaCl_2O$, sulfamic acid, aq. 1,4-dioxane; iii (*a*) BCl_3, DCM, −78 °C, (*b*) MeOH; iv, aq. Et_3N; v, H_2, 5% Pd/C, water; vi (*a*) BrCN, NaOAc, MeOH, (*b*) NH_4OH, HCO_2NH_4

Table 1 Viral neuraminidase (IC_{50} NA), and influenza A and B (IC_{50} FluAPL and FluBPL) plaque-reduction activities of the carbocyclic analogues **4** and **6** compared with the parents **3** and **5** (assays were conducted as previously described)[3]

Compound	IC_{50} NA (μmol dm^{-3})	IC_{50} FluAPL (μg cm^{-3})	IC_{50} FluBPL (μg cm^{-3})
3	9.2	17	1.9
4	7.8	4.0	43
5	270	> 100	19
6	> 880	1.2	11

1-Benzyloxy-3-nitropropan-2-ol 13

Benzyloxyacetaldehyde diethyl acetal (50.6 g, 257.8 mmol) was dissolved in THF (160 cm^3) and the solution was treated with water (160 cm^3) and glacial acetic acid (230 cm^3). The reaction mixture was then heated at reflux. After TLC [ethyl acetate–cyclohexane (1:2 v/v)] indicated reaction was complete, the reaction mixture was cooled, then evaporated. Residual acetic

acid was removed by co-evaporation with toluene (2 × 300 cm^3). Evaporation to dryness gave the aldehyde **10** as an oil (36.5 g). The crude material was dissolved in cooled nitromethane (70 cm^3, 1293 mmol), then neutral alumina (120 g) was added to the stirred mixture. The resultant slurry was stored at 21 °C for 24 h. DCM (1700 cm^3) was added and the mixture was filtered, then evaporated to give the *title compound* **13** as an oil (50.74 g, 98%). Purification of this could be achieved by Kugelruhr distillation (Found: C, 56.85; H, 6.0; N, 6.5. $C_{10}H_{13}NO_4$ requires C, 56.87; H, 6.20; N, 6.63%); $\nu_{max}(CHBr_3)/cm^{-1}$ 3578, 2865 and 1553; $\delta_H(CDCl_3)$ 7.33 (5 H, m, Ph), 4.60–4.40 (5 H, m, CH_2OCH_2CH), 3.52 (2 H, m, CH_2NO_2) and 3.11 (1 H, d, *J* 5, OH).

1-Benzyloxy-3-nitropropan-2-yl acetate 14

Compound **13** (50.74 g, 240.2 mmol) was cooled in an ice-bath, then was treated with acetic anhydride (105 cm^3). The mixture was stirred in an ice-bath for 5 min and then conc. sulfuric acid (3 drops) was added cautiously. Once the temperature had stabilised, the cooling bath was removed and the mixture was stirred at ambient temperature. After 1.5 h TLC [ethyl acetate–cyclohexane (1 : 2 v/v)] showed the reaction to be complete. The mixture was poured onto a mixture of diethyl ether (500 cm^3) and saturated aq. sodium hydrogen carbonate (250 cm^3). Solid sodium hydrogen carbonate was added to the stirred mixture. When effervescence had ceased, the layers were separated, and the organic phase was dried ($MgSO_4$) and evaporated to give the crude product as an oil (53.5 g). This could be purified by Kugelruhr distillation [oven temperature 185 °C at 1 mbar (10^2 Pa) pressure] to give the *title compound* **14** as an oil (84.6 g, 69%), $\nu_{max}(CHBr_3)/cm^{-1}$ 1748 and 1557; $\delta_H(CDCl_3)$ 7.35 (5 H, m, Ph), 5.60 (1 H, m, CH), 4.70–4.50 (4 H, m, $PhCH_2$ and CH_2NO_2), 3.65 (2 H, m, OCH_2CH) and 2.12 (3 H, s, AcN) (Found: $[M + H]^+$, 254.103 546. $C_{12}H_{15}NO_5$ requires *m/z*, 254.102 848).

3-Benzyloxy-1-nitroprop-1-ene 7

A solution of acetate **14** (53.5 g, 211.25 mmol) in dry toluene (300 cm^3) was treated with sodium hydrogen carbonate (60 g). The resulting suspension was stirred with an efficient mechanical stirrer and heated at 80 °C under nitrogen. After 24 h the cold mixture was diluted with diethyl ether (500 cm^3). The organic layer was decanted and the residual solids were washed with diethyl ether (2 × 500 cm^3). The combined organic phases were washed with water (500 cm^3) and the aqueous phase was re-extracted with diethyl ether (300 cm^3). The combined organic phases were dried ($MgSO_4$) and evaporated to give the crude title compound **7** as an oil (38.3 g). In earlier runs the material was purified by flash chromatography. In later preparations it was more convenient to purify the material at the methoxy ketone **11** stage. An overall yield of 45% from the diethyl acetal was obtained. Flash chromatography using gradient elution [ethyl acetate–light petroleum (0 : 1–1 : 4 v/v)] gave the title compound **7** as an oil, $\nu_{max}(CHBr_3)/cm^{-1}$ 1658, 1529 and 1355; $\delta_H(CDCl_3)$ (5 H, m, Ph), 7.28 (2 H, s, 3- and 2-H), 4.60 (2 H, s, $PhCH_2O$) and 4.28 (2 H, s, 1-H_2); *m/z* 211 (MNH_4^+); TLC [R_f 0.47 in ethyl acetate–light petroleum (1 : 4 v/v)].

(3*R*,4*S*,5*R*)-5-Benzyloxymethyl-3-methoxy-4-nitrocyclohex-1-enyl)methanol 10

A solution of (*E*)-4-methoxy-2-methylenebut-3-en-1-ol **8** (1.19 g, 10.4 mmol) in dry toluene (20 cm^3) was treated with hydroquinone (2 mg) followed by nitro olefin **7** (4.76 g, 24.6 mmol). The resulting solution was stirred at 21 °C under nitrogen. After 48 h no further change was indicated by TLC [EtOAc–cyclohexane (1 : 1 v/v)] and the solvent was evaporated off to give an oil. This was purified by gravity column chromatography [DCM, then DCM–MeOH (9 : 1 v/v)] to give recovered dienophile **7** (3.2 g) as the first eluted component [R_f 0.8, ethyl acetate–cyclohexane (1 : 1 v/v)] followed by the title compound **10** (2.87 g, 89.6%); $\delta_H(CDCl_3)$ 7.40–7.22 (5 H, m, Ph), 5.98 (1 H, s, 2-H), 4.82 (1 H, dd, *J* 4 and 12, 4-H), 4.44 (2 H, m, $PhCH_2O$), 4.27 (1 H, dd, *J* 4, 3-H), 4.08 (2 H, m, CH_2OH), 3.75 (1 H, dd, *J* 4.5 and 10, CH_2O), 3.48 (1 H, dd, *J* 2 and 10, CH_2O), 3.36 (3 H, s, OMe), 2.88–2.72 (1 H, m, 5-H) and 2.35–2.24 (2 H, m, 6-H_2). Assigned signals from minor α-isomer: 5.78 (2-H), 4.70 (4-H), 2.63–2.49 (5-H) (Found: $[M + NH_4]^+$, 325.175 568. $C_{16}H_{25}N_2O_5$ requires *m/z* 325.176 347) (R_f 0.4, ethyl acetate–cyclohexane (1 : 1 v/v); 91.6% pure by HPLC (conditions 1; 40% MeCN in water).

(3*R*,4*S*,5*R*)-5-Benzyloxymethyl-3-methoxy-4-nitrocyclohex-1-enecarbaldehyde 15

To a solution of the alcohol **10** (3.12 g, 10.15 mmol) in dry DCM (10 cm^3) was added PDC (1.435 g, 3.81 mmol) and the resulting suspension was stirred at 21 °C for 24 h. The mixture was then diluted with DCM (50 cm^3) and filtered through Celite. The filtrate was evaporated to give an oil (630 mg). Purification by column chromatography [cyclohexane–ethyl acetate–acetic acid (30 : 30 : 1 v/v)] gave the *title compound* **15** as an oil (2.42 g, 78.4%); $\nu_{max}(CHBr_3)/cm^{-1}$ 1719, 1690 and 1553; $\delta_H(CDCl_3)$ 9.55 (1 H, s, CHO), 7.40–7.21 (5 H, m, Ph), 6.80 (1 H, s, 2-H), 4.97 (1 H, dd, *J* 5 and 10, 4-H), 4.53–4.40 (3 H, m, 3-H and $PhCH_2$), 3.77 (1 H, dd, *J* 3.75 and 10, CH_2O), 3.54–3.40 (1 H, m, CH_2O), 3.49 (3 H, s, OMe), 2.82 (2 H, m, 5-H and 6-H^a) and 2.34 (1 H, dd, *J* 10 and 17.5, 6-H^b) (Found: $[M + NH_4]^+$, 323.160 950. $C_{16}H_{23}N_2O_5$ requires *m/z* 323.160 697); ratio of α-methoxy and β-methoxy isomers: 66% and 29% by HPLC (conditions 1; 40% MeCN in water).

(3*R*,4*S*,5*R*)-5-Benzyloxymethyl-3-methoxy-4-nitrocyclohex-1-enecarboxylic acid 16

A cold (ice-bath), stirred solution of the aldehyde **15** (2.0 g, 6.55 mmol) in 1,4-dioxane (25 cm^3) was treated with a solution of sulfamic acid (356 mg, 3.66 mmol) in water (8.8 cm^3) followed by the dropwise addition of aq. sodium chlorite (80%; 814 mg, 7.21 mmol in 5.5 cm^3) to give rise to a slight exotherm (temperature rise of ~ 7–12 °C). The reaction mixture was then allowed to warm to 20 °C and was stirred at this temperature for 1 h. Although TLC [cyclohexane–ethyl acetate (1 : 1 v/v)] at this stage showed a slight trace of aldehyde further reaction time was ineffective. The mixture was diluted with water (50 cm^3), saturated with sodium chloride, and extracted with ethyl acetate (3 × 25 cm^3). The extracts were extracted with 50% saturated aq. sodium hydrogen carbonate (3 × 25 cm^3). The combined alkaline extracts were then acidified with conc. phosphoric acid (to pH 1) and saturated with sodium chloride. This mixture was then extracted with ethyl acetate (3 × 25 cm^3), dried ($MgSO_4$), and evaporated to give the *title compound* **16** as a solid (1.66 g, 79%), mp (*M* − 100) 142.9 °C (Found: C, 59.6; H, 5.95; N, 4.4. $C_{16}H_{19}NO_6$ requires C, 59.81; H, 5.96; N, 4.36%); ν_{max}(Nujol)/cm^{-1} 2958, 2903, 2858, 1694, 1552 and 1463; $\delta_H(CDCl_3)$ 7.20–7.40 (5 H, m, Ph), 7.13 (1 H, s, 2-H), 4.88 (1 H, dd, *J* 5 and 10, 4-H), 4.47 (2 H, m, $PhCH_2$), 4.35 (1 H, m, 3-H), 3.78 (1 H, dd, *J* 3.5 and 10, CH_2O), 3.51–3.41 (1 H, m, CH_2O), 3.45 (3 H, s, OMe), 2.62–2.86 (2 H, m, 5-H, and 6-H^a) and 2.46 (1 H, dd, *J* 10 and 18.75, 6-H^b) (Found $[M - H]^-$, 320.115 112. $C_{16}H_{18}NO_6$ requires *m/z*, 320.113 413).

(3*R*,4*S*,5*R*)-5-Hydroxymethyl-3-methoxy-4-nitrocyclohex-1-enecarboxylic acid 17

A stirred solution of the benzyl ether **16** (500 mg, 1.55 mmol) in dry DCM (16 cm^3) under nitrogen was cooled to −75 °C. To this was added dropwise a solution of boron trichloride in DCM (7.8 cm^3, 7.8 mmol; 1 mol dm^{-3}) at such a rate so as to maintain the temperature in the range −72 to −74 °C. After

stirring of the mixture for 30 min at −74 °C, TLC [$CHCl_3$–MeOH (5:1 v/v)] indicated complete reaction. The reaction mixture was allowed to warm to 21 °C, quenched by being poured into methanol (80 cm^3), then was evaporated to give a dark oil. Further portions of methanol (2 × 50 cm^3) were added and removed under reduced pressure to remove boron residues. The residue was then trituated with chloroform (5 cm^3), and filtered to give the *title compound* **17** as a solid (199 mg, 55%), mp (*M*-100) 159.2 °C (Found: C, 45.6; H, 5.3; N, 6.0. $C_9H_{13}NO_6$·0.05$CHCl_3$ requires C, 45.83; H, 5.55; N, 5.91%); ν_{max}(Nujol)/cm^{-1} 3402, 1706, 1554 and 1242; δ_H(400 MHz; D_2O) 6.95 (1 H, d, *J* 2, 2-H), 5.05 (1 H, dd, *J* 5 and 10, 4-H), 4.48 (1 H, m, 3-H), 3.63 and 3.60 (2 H, m, 7-H_2), 2.61 (1 H, m, 6-H^a), 2.55 (1 H, m, 5-H) and 2.20 (1 H, m, 6-H^b); *m/z* 230 $(M - H)^-$; 87.9% pure by HPLC (conditions 2) [evaporation of the mother liquors gave an oil (150 mg, 39.5%), found to be methyl (3*R*,4*S*,5*R*)-5-hydroxymethyl-3-methoxy-4-nitrocyclohex-1-enecarboxylic ester, **18**].

(3*R*,4*S*,5*R*)-4-Amino-5-hydroxymethyl-3-methoxycyclohex-1-enecarboxylic acid 19

A solution of nitro acid **17** (190 mg, 0.82 mmol) in acetic acid (5 cm^3) was treated with zinc dust (1 g). The resulting mixture was sonicated in an ultrasonic bath for 30 min. The mixture was then filtered and the filtrate was evaporated to give the *title compound* **19** as an oil (116 mg, 70%); ν_{max}(Me_2SO)/cm^{-1} 2530, 1716, 1603 and 1379; δ_H(D_2O) 6.75 (1 H, d, *J* 5, 2-H), 4.14 (1 H, dd, *J* 5, 3-H), 3.80–3.58 (3 H, m, 4-H and CH_2O), 3.54 (3 H, s, OMe), 2.52 (1 H, dd, *J* 5 and 17.5, 6-H^a) and 2.35–2.00 (2 H, m, 5-H and 6-H^b) (Found: $[M + H]^+$, 202.108 459. $C_9H_{16}NO_4$ requires *m/z*, 202.107 933); 91.8% pure by HPLC (conditions 3).

(3*R*,4*S*,5*R*)-4-Acetamido-5-acetoxymethyl-3-methoxycyclohex-1-enecarboxylic acid 20

A solution of the amino acid **19** (116 mg, 0.58 mmol) in dry DCM (4 cm^3) containing dry pyridine (1 cm^3) was treated with acetic anhydride (238 mm^3, 3 mmol) followed by triethylamine (84 mm^3, 0.6 mmol). 4-(Dimethylamino)pyridine (DMAP) (3 mg) was then added and the resulting solution was stirred under nitrogen at 21 °C. After 24 h, toluene (100 cm^3) was added and the mixture was evaporated under reduced pressure to give an oil. This was then dissolved in ethyl acetate (10 cm^3) and the solution was washed with 2 mol dm^{-3} hydrochloric acid (5 cm^3). The aqueous layer was re-extracted with ethyl acetate (5 cm^3) and the combined extracts were dried ($MgSO_4$) and evaporated to give the *title compound* **20** as an oil (180 mg, 77%); δ_H($CDCl_3$) 7.15 (1 H, d, *J* 4, 2-H), 6.00 (1 H, d, *J* 10, NH), 4.17 (1 H, m, 4-H), 4.10 (2 H, m, CH_2O), 3.81 (1 H, dd, *J* 4, 3-H), 3.48 (3 H, s, OMe), 2.70 (1 H, m, 6-H^a), 2.25 (2 H, m, 5-H and 6-H^b), 2.05 (3 H, s, Ac) and 2.04 (3 H, s, Ac) (Found: $[M + H]^+$, 286.128 204. $C_{13}H_{20}NO_6$ requires *m/z*, 286.129 063); 81.3% pure by HPLC (conditions 2).

(3*R*,4*S*,5*RS*)-3-Benzyloxymethyl-5-methoxy-4-nitrocyclohexanone 11

To a solution of the dienophile **7** (10.12 g, 58.78 mmol) in dry toluene (150 cm^3) was added 1-methoxy-3-trimethylsiloxybuta-1,3-diene **9** (9.89 g, 57.39 mmol). After 4.5 h heating at 80 °C under nitrogen, TLC [ethyl acetate–cyclohexane (1:4 v/v)] showed the reaction to be complete. The reaction mixture was cooled, then was evaporated under reduced pressure to give the crude TMS enol ether **22** as a brown oil (29.77 g). This was dissolved in dry THF (555 cm^3) and was then treated with 2 mol dm^{-3} hydrochloric acid (210 cm^3) and the mixture was stirred for 10 min at 21 °C before being poured into water (2000 cm^3) and extracted with DCM (2 × 500 cm^3, then 250 cm^3). The combined extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown gum (23.91 g). Flash chromatography [light petroleum–ethyl acetate (5:1 v/v)] gave the *title compound* **11** as a semi-crystalline yellow oil (10.05 g, 58.3%) (Found: C, 61.2; H, 6.2; N, 4.6. $C_{15}H_{19}NO_5$ requires C, 61.63; H, 6.21; N, 4.79%) as a 65:35 mixture of isomers. Major isomer: δ_H($CDCl_3$) 7.21–7.50 (5 H, m, Ph), 4.96 (1 H, t, *J* 9, 4-H), 4.48 (2 H, ABq, CH_2Ph), 4.01 (1 H, ddd, *J* 11, 9 and 5, 5-H), 3.42 (2 H, d, *J* 3, CH_2O), 3.37 (1 H, m, 3-H), 3.35 (3 H, s, OMe), 2.95 (1 H, dd, *J* 14 and 5, 6-H^a), 2.35–2.65 (3 H, m, 2-H and 6-H^b).

(7*R*,8*S*,9*RS*)-7-Benzyloxymethyl-9-methoxy-8-nitro-1,4-dioxaspiro[4.5]decane 23

A solution of the methoxy nitro ketone **11** (14.60 g, 49.77 mmol) in isopropyl acetate (360 cm^3) with ethylene glycol (90 cm^3) and oxalic acid (45.2 g) was refluxed through a Dean–Stark trap for 3.5 h, then was cooled, diluted with ethyl acetate (500 cm^3), and washed successively with water (3 × 100 cm^3), saturated aq. sodium hydrogen carbonate (2 × 100 cm^3), and saturated brine (3 × 100 cm^3). The combined aqueous phases were extracted with ethyl acetate (2 × 100 cm^3) and these extracts were washed with saturated brine (2 × 50 cm^3). The total combined organic phases were dried ($MgSO_4$), filtered, and evaporated to give the crude material as a brown liquid (20.23 g). Flash chromatography [Merck 9385 silica (600 g); elution with ethyl acetate–cyclohexane (1:5 v/v)] yielded the *title compounds* **23**, in order of elution: α-isomer (9.35 g, yellow oil) (Found: C, 60.7; H, 6.9; N, 4.15. $C_{17}H_{23}NO_6$ requires C, 60.52; H, 6.03; N, 3.70%), δ_H(400 MHz; $CDCl_3$) 7.40–7.23 (5 H, m, Ph), 4.53 (1 H, dd, *J* 11 and 11, 8-H), 4.48 and 4.43 (2 H, AB, *J* 11, CH_2Ph), 4.03–3.92 (5 H, m, 2- and 3-H_2 and 9-H), 3.40–3.31 (2 H, m, CH_2O), 3.32 (3 H, s, MeO), 2.50 (1 H, m, 7-H), 2.26 (1 H, m, 10-H^{eq}), 1.81 (1 H, ddd, *J* 4.1, 6-H^{eq}), 1.72 (1 H, t, *J* 13, 6-H^{ax}) and 1.53 (1 H, t, *J* 12, 10-H^{ax}); *m/z* 355 (MNH_4^+); mixture of α and β isomers (1.818 g, yellow gum); β-*isomer* (1.823 g, yellow gum) (Found: C, 60.8; H, 6.5; N, 4.5. $C_{17}H_{23}NO_6$ requires C, 60.52; H, 6.03; N, 3.70%), δ_H(400 MHz; $CDCl_3$) 7.40–7.23 (5 H, m, Ph), 4.78 (1 H, dd, *J* 9.9 and 3.9, 8-H), 4.46–4.42 (2 H, AB, *J* 12.6, CH_2Ph), 4.09 (1 H, m, 9-H), 4.03–3.96 and 3.92–3.87 (4 H, 2 m, 2- and 3-H_2), 3.62 and 3.43 (2 H, m, CH_2O), 3.32 (3 H, s, MeO), 2.87 (1 H, m, 7-H), 2.28 (1 H, m, 10-H^{eq}), 1.97 (1 H, d, *J* 4.1, 6-H^{eq}), 1.79 (1 H, t, *J* 13, 6-H^{ax}) and 1.77 (1 H, dd, *J* 3.8, 10-H^{ax}); *m/z* 355 (MNH_4^+).

(7*R*,8*S*,9*RS*)-7-Benzyloxymethyl-9-methoxy-1,4-dioxaspiro-[4.5]decan-8-ylamine 24

Cobalt(II) chloride (3.49 g, 26.8 mmol) was added to a solution of the methoxy nitro ketal **23** (9.05 g, 26.8 mmol) in absolute ethanol (175 cm^3) under nitrogen. Sodium boranuide (10.1 g, 268 mmol) was added very cautiously over a period of 40 min (**exotherm!**). After the addition was complete, the mixture was heated at reflux for 1.5 h, and then TLC [cyclohexane–acetone (3:1 v/v)] showed reaction to be complete. After cooling to room temperature, the mixture was quenched with saturated aq. ammonium chloride and filtered. The residue was washed with saturated aq. ammonium chloride (500 cm^3). The filtrate was concentrated to remove ethanol, then was extracted with ethyl acetate (3 × 200 cm^3). The combined organic phases were washed with saturated brine (200 cm^3), dried ($MgSO_4$), then filtered and evaporated to give the title compound **24** (5.93 g, 72%) as a brown gum; δ_H($CDCl_3$) 7.21–7.40 (5 H, m, Ph), 4.31 (1 H, s, CH_2Ph), 3.95 (4 H, m, 2- and 3-H_2), 3.55 (2 H, d, *J* 5, CH_2O), 3.37 (3 H, s, MeO), 3.12 (1 H, m, 9-H), 2.62 (1 H, dd, *J* 10, 8-H), 2.30 (2 H, br s, NH_2), 2.20 (1 H, m, 10-H^{eq}), 1.78 (2 H, t, *J* 13.75, 7-H and 6-H^{eq}), 1.57 (1 H, t, *J* 12.5, 6-H^{ax}) and 1.45 (1 H, t, *J* 12.5, 10-H^{ax}); *m/z* 308 (MH^+); R_f 0.05 [cyclohexane–acetone (3:1 v/v)].

N-[(7*R*,8*S*,9*RS*)-7-Benzyloxymethyl-9-methoxy-1,4-dioxaspiro[4.5]decan-8-yl]acetamide 25

To a stirred solution of the amine **24** (5.934 g, 1.93 mmol) in

DCM (175 cm^3) was added pyridine (13.8 cm^3, 0.17 mol), acetic anhydride (7.6 cm^3, 8.09 mmol) and a catalytic amount of DMAP. After 2 h, TLC showed absence of starting material. The solution was washed successively with 2 mol dm^{-3} hydrochloric acid (2 × 175 cm^3) and saturated brine (2 × 150 cm^3). The combined aqueous phases were extracted with DCM (50 cm^3) and the total organic phases were dried ($MgSO_4$), filtered, and evaporated to give the *title compound* **25**, as a pale brown solid (6.04 g, 90%), mp (*M*-50) 118.9 °C (Found: C, 64.9; H, 7.8; N, 4.0. $C_{19}H_{27}NO_4$ requires C, 65.31; H, 7.79; N, 4.01%); $\delta_H(CDCl_3)$ 7.21–7.39 (5 H, m, Ph), 5.20 (1 H, d, *J* 10, NH), 4.44 (2 H, d, *J* 5, CH_2Ph), 3.96 (4 H, s, 2- and 3-H_2), 3.70 (1 H, ddd, *J* 10, 8-H), 3.50 (1 H, dd, *J* 10 and 8.75, 9-H), 3.35 (2 H, m, CH_2O), 3.30 (3 H, s, OMe), 2.18 (1 H, m, 10-H^{eq}), 1.95 (3 H, s, *Ac*NH), 1.90 (2 H, m, 6-H^{eq} and 7-H), 1.62 (1 H, dd, *J* 11.9, 6-H^{ax}), 1.53 (1 H, dd, *J* 13.75, 10-H^{ax}); *m/z* 350 (MH^+).

N-[(1*R*,6*R*)-6-Benzyloxymethyl-4-oxocyclohex-2-enyl]acetamide 26

To a solution of the acetamide **25** (6.03 g, 17.26 mmol) in acetone (175 cm^3) was added 2 mol dm^{-3} hydrochloric acid solution (38 cm^3) and the resultant solution was stirred for 90 h. The reaction mixture was neutralised with saturated aq. sodium hydrogen carbonate and concentrated to remove the acetone. The aqueous residue was extracted with ethyl acetate (4 × 100 cm^3) and the combined organic phases were washed with saturated brine (100 cm^3), dried ($MgSO_4$), filtered, and evaporated to give a gum (5.322 g). Flash chromatography [ethyl acetate–cyclohexane (4:1 v/v), then neat ethyl acetate, then ethyl acetate–methanol (19:1 v/v)] gave a yellow–white solid (3.13 g). The solid was dissolved in ethyl acetate (150 cm^3), and the solution was stirred with Norrit Ultra SX+ (33.7 mg) for 40 min. Filtration and evaporation yielded the *title compound* **26** (3.10 g, 66%); mp (*M*-50) 111.2 °C (Found: C, 70.4; H, 7.0; N, 5.1. $C_{16}H_{19}NO_3$ requires C, 70.31; H, 7.01; N, 5.12%); $\nu_{max}(Me_2SO)/cm^{-1}$ 1671 and 1543; $\delta_H(CDCl_3)$ 7.40–7.26 (5 H, m, Ph), 6.80 (1 H, dd, *J* 6 and 2.5, 2-H), 6.02 (1 H, d, *J* 6, 3-H), 5.82 (1 H, d, *J* 7.5, NH), 4.76 (1 H, ddd, *J* 2.5 and 7.5, 1-H), 4.49 (2 H, s, CH_2Ph), 3.50 (2 H, d, *J* 5, CH_2O), 2.61 (1 H, dd, *J* 5 and 17.5, 5-H^a), 2.43 (1 H, d, *J* 17.5, 5-H^b), 2.42–2.25 (1 H, m, 6-H) and 1.97 (3 H, s, Ac); *m/z* 274 (MH^+).

[(3a*S*,4*R*,7a*R*)-4-Benzyloxymethyl-2-methyl-3a,4,5,7a-tetrahydrobenzoxazol-6-yl]methanol 33

To a stirred suspension of powdered trimethylsulfonium iodide (5.86 g, 28.72 mmol) in dry THF (100 cm^3) under nitrogen in an ice-bath was added dropwise a 0.95 mol dm^{-3} solution of butyllithium in hexanes (24.18 cm^3, 22.79 mmol). After stirring of the mixture for 15 min at this temperature, only a slight precipitate remained; a solution of the enone **26** (3.14 g, 11.49 mmol) in dry THF (25 cm^3) was then added dropwise at such a rate that the internal temperature did not rise above 1 °C. The mixture was stirred for 30 min in the ice-bath, and then for 2 h at 21 °C. After this time TLC [acetone–chloroform (1:3 v/v)] indicated almost complete reaction. The reaction mixture was poured onto a chilled (ice/salt), stirred mixture of ethyl acetate (250 cm^3) and water (250 cm^3). The layers were separated and the aqueous phase was saturated with sodium chloride, then was extracted with ethyl acetate (2 × 100 cm^3). The combined extracts were dried ($MgSO_4$), filtered, and evaporated to leave a brown solid (3.93 g). ^1H NMR spectroscopy indicated a mixture of epoxide **32** and the oxazoline **33**. The solid was taken up in ethyl acetate (150 cm^3), treated with silica (Merck 7734, 12 g), and the solvent was removed under reduced pressure. After 1.25 h, TLC [acetone–chloroform (1:3 v/v)] indicated complete rearrangement. The silica was deposited on a fritted column and washed with ethyl acetate (600 cm^3). Evaporation of the filtrate gave the crude *title compound* **33** as a tan oil (2.78 g); $\nu_{max}(CHBr_3)/cm^{-1}$ 1719, 1666 and 1510; $\delta_H(CDCl_3)$ 7.4–7.2 (5 H, m, Ph), 5.90 (1 H, s, 7-H), 4.80 (1 H, d, *J* 9, 7-H^a), 4.55 (2 H, s, CH_2OH), 4.48 (1 H, br, OH), 4.10 (2 H, s, CH_2Ph), 3.97 (1 H, dd, *J* 9, 3-H^a) 3.70 (1 H, dd, *J* 3.75 and 10, 8-H^a), 3.56 (1 H, dd, *J* 10, CH_2O), 2.20 (1 H, m, 5-H^a), 1.90–1.60 (2 H, m, 5-H^b and 4-H) and 1.95 (3 H, s, Me) (Found: $[M + H]^+$, 288.161 194. $C_{17}H_{22}NO_3$ requires *m/z*, 288.159 969); TLC [acetone–chloroform (1:3 v/v)] R_f = 0.35.

N-[(1*S*,2*S*,6*R*)-2-Azido-6-benzyloxymethyl-4-(hydroxymethyl)-cyclohex-3-enyl]acetamide 34

To a solution of the oxazoline **33** (4.02 g, 13.98 mmol) in dry *tert*-butyl alcohol (100 cm^3) was added azidotrimethylsilane (11.15 cm^3, 83.88 mmol) in one portion. The mixture was then heated to 80 °C. After 2 h, TLC (EtOAc) showed disappearance of starting material. The reaction mixture was allowed to cool and was stirred for a further 19 h before being poured into saturated aq. sodium hydrogen carbonate (500 cm^3) and extracted with ethyl acetate (2 × 500 cm^3, 1 × 250 cm^3). The combined extracts were dried ($MgSO_4$), filtered, and evaporated to give a brown oil (4.35 g). ^1H NMR spectroscopy indicated the presence of some silylated product **35**. The oil was taken up in methanol (175 cm^3) and treated with 2 mol dm^{-3} hydrochloric acid (60 cm^3). After stirring of the mixture at 21 °C for 30 min, TLC (EtOAc) showed full desilylation. The mixture was evaporated free of methanol, then was taken up in ethyl acetate (100 cm^3). Saturated aq. sodium hydrogen carbonate (300 cm^3) was added cautiously portionwise and the mixture was extracted with ethyl acetate (3 × 250 cm^3). The combined extracts were dried ($MgSO_4$), filtered, and evaporated to give a tan solid (4.0 g). The material was purified by flash chromatography [ethyl acetate–cyclohexane (4:1 v/v)] to give the *title compound* **34** as a brown oil (1.49 g, 32.3% over 2 steps) (Found: C, 61.95; H, 6.8; N, 16.8. $C_{17}H_{22}N_4O_3$ requires C, 61.80; H, 6.71; N, 16.96%); $\nu_{max}(CHBr_3)/cm^{-1}$ 2095, 1672 and 1512; $\delta_H(CDCl_3)$ 7.41–7.20 (5 H, m, Ph), 5.61 (1 H, s, 3-H), 5.52 (1 H, d, *J* 7.8, 1-H), 4.51 and 4.41 (2 H, AB, *J* 10, CH_2Ph), 4.18–4.00 (3 H, m, 2-H and CH_2OH), 3.54–3.40 (2 H, m, CH_2O), 2.35–2.02 (3 H, m, 6-H and 5-H_2) and 1.43 (3 H, s, Ac); *m/z* 353 $(MNa)^+$ and 331 $(MH)^+$.

N-[(1*S*,2*S*,6*R*)-2-Azido-6-benzyloxymethyl-4-formylcyclohex-3-enyl]acetamide 36

A mixture of the azido alcohol **34** (178 mg, 0.54 mmol) and PDC (304 mg, 0.81 mmol) was treated with DCM (4 cm^3) under nitrogen. The reaction mixture (protected from light with tin foil) was stirred at 21 °C, rapidly becoming dark. After 18 h, TLC (EtOAc) showed that reaction was almost complete. The reaction mixture was diluted with chloroform (15 cm^3) and filtered through Celite, the pad being washed with chloroform (3 × 15 cm^3). The combined filtrates were evaporated and the residue was purified by preparative TLC (EtOAc) to give the title compound **36** as solid (80 mg, 45%), mp (*M*-75) 110.2 °C (Found: C, 61.3; H, 6.1; N, 16.9. $C_{17}H_{20}N_4O_3 \cdot 0.25H_2O$ requires C, 62.18; H, 6.14; N, 17.06%); $\nu_{max}(Me_2SO)/cm^{-1}$ 2095 and 1681; δ_H[400 MHz; $(CD_3)_2SO$] 9.50 (1 H, s, CHO), 8.02 (1 H, d, *J* 9, NH), 7.36–7.23 (5 H, m, Ph), 6.70 (1 H, s, 3-H), 4.42 and 4.39 (2 H, AB, *J* 12, CH_2Ph), 4.40 (1 H, m, 2-H), 3.80 (1 H, dd, *J* 9 and 19.5, 1-H), 3.48 (1 H, dd, *J* 3 and 9, CH_2O), 3.35 (1 H, dd, *J* 6 and 9, CH_2O), 2.50 (4 H, m, Ac, 5-H^a) and 2.1–1.9 (2 H, m, 6-H and 5-H^b); *m/z* 329 $(MH)^+$; 98.4% pure by HPLC (conditions 1; 30% MeCN in water).

(3*S*,4*S*,5*R*)-4-Acetamido-3-azido-5-(benzyloxymethyl)cyclohex-1-enecarboxylic acid 37

A solution of the aldehyde **36** (40 mg, 0.12 mmol) in 1,4-dioxane (0.5 cm^3) was treated with a solution of sulfamic acid (5.7 mg, 0.05 mmol) in water (0.14 cm^3), followed by a solution of

sodium chlorite (12.5 mg, 0.13 mmol) in water (0.1 cm^3). The resulting solution was stirred at 21 °C. After 1 h, TLC indicated no starting material remaining and the reaction mixture was diluted with water (5 cm^3). The resulting solution was saturated with sodium chloride and extracted with ethyl acetate (4 × 10 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to give a foam. Trituration of this with diethyl ether gave the *title compound* **37** as a solid (20 mg, 48%), mp (*M*-75) 176.2 °C [Found: C, 58.75; H, 5.7; N, 15.2. $C_{17}H_{20}N_4O_4 \cdot 0.25(C_4H_8O_2)$ requires C, 59.00; H, 6.05; N, 15.29%]; ν_{max}-[$(CD_3)_2SO$]/cm^{-1} 2977, 2869, 2100, 1700, 1647 and 1142; δ_H[400 MHz; $(CD_3)_2SO$] 12.8 (1 H, br, CO_2H), 7.98 (1 H, d, *J* 10, NH), 7.3 (5 H, m, Ph), 6.50 (1 H, s, 2-H), 4.44 and 4.39 (2 H, AB, *J* 14, PhCH_2), 4.24 (1 H, m, 3-H), 3.78 (1 H, m, 4-H), 3.20–3.60 (2 H, m, CH_2O), 2.50 (1 H, m, 6-H^a), 2.20–1.90 (2 H, m, 5-H and 6-H^b) and 1.80 (3 H, s, Ac); *m/z* 345 $(MH)^+$.

(3*S*,4*S*,5*R*)-4-Acetamido-3-azido-5-(hydroxymethyl)cyclohex-1-enecarboxylic acid 38

To a solution of the benzyl ether **37** (239 mg, 0.69 mmol) in dry DCM (10 cm^3) under nitrogen at −70 °C was added a solution of boron trichloride (1 mol dm^{-3} in DCM; 3.47 cm^3, 3.47 mmol). The reaction mixture was stirred at −70 °C for 45 min, then at ambient temperature for 30 min. The reaction mixture was treated with methanol (5 cm^3), then was evaporated under reduced pressure. The resulting solid residue was co-evaporated with methanol (2 × 20 cm^3) to give a mixture of the carboxylic acid **38** and the methyl ester **39** as a buff foam (219 mg) (ratio of products: 1:1 by HPLC). The mixture was treated with water (24 cm^3) and triethylamine (0.6 cm^3), then was stirred at 21 °C for 4 h before being evaporated. The residue was co-evaporated with methanol (2 × 20 cm^3) to give a tan solid. This was taken up in water (100 cm^3) and loaded onto a 20 × 85 mm (20 cm^3) Dowex 50W-X8 (H^+) column prewashed with water. Elution with water (200 cm^3), at a slow dropwise rate, followed by evaporation of all the eluted solvent gave the *title compound* **38** as tan oil (122 mg, 36%); $\nu_{max}(Me_2SO)$/cm^{-1} 2096, 1788, 1701, 1673, 1548 and 1250; $\delta_H(CD_3OD)$ 6.65 (1 H, s, 2-H), 4.20 (1 H, m, 3-H), 3.82 (1 H, t, 4-H), 3.58 (2 H, m, CH_2OH), 2.60 (1 H, m, 6-H), 2.28 (1 H, m, 6-H), 2.00 (3 H, s, Ac) and 1.10 (1 H, m, 5-H) [Found: $(MH)^+$, 255.109 650. $C_{10}H_{15}N_4O_4$ requires *m/z* 255.109 330]; HPLC: 77.2% (13.5 min) and 11.13% (13.8 min) (conditions 4).

(3*S*,4*S*,5*R*)-4-Acetamido-3-amino-5-(hydroxymethyl)cyclohex-1-enecarboxylic acid 6

A solution of the azido acid **38** (100 mg, 0.393 mmol) in water (5 cm^3) was hydrogenated over 5% Pd on carbon (25 mg, 50% water) for 1 h. The reaction mixture was filtered, then evaporated. Co-evaporation of the residue with MeOH (2 × 10 cm^3) gave the *title compound* **6** as tan oil (115 mg); ν_{max}[$(CD_3)_2SO$]/cm^{-1} 3442, 1698, 1667 and 1552; $\delta_H(D_2O)$ 6.20 (1 H, s, 2-H), 4.00–3.82 (2 H, m, 3- and 4-H), 3.62–3.50 (2 H, m, CH_2OH), 2.50 (1 H, dd, *J* 10 and 17.5, 6-H^a), 2.25 (1 H, m, 6-H^b), 2.00 (3 H, s, Ac) and 1.97 (1 H, m, 5-H) (Found: $[M + H]^+$, 229.118 256. $C_{10}H_{17}N_2O_4$ requires *m/z*, 229.118 832); 89.0% pure by HPLC (conditions 1; 50% MeCN in water).

(3*S*,4*S*,5*R*)-4-Acetamido-3-guanidino-5-(hydroxymethyl)cyclohex-1-enecarboxylic acid 4

A stirred solution of the amine **6** (74 mg, 0.324 mmol) in dry methanol (3 cm^3) was treated with dry sodium acetate (60 mg, 0.729 mmol). To the suspension was added, dropwise, a solution of cyanogen bromide (36 mg, 0.338 mmol) in dry methanol (4.7 cm^3) over a period of 1.5 h. The mixture was stirred at 21 °C for 16 h, then was evaporated to give a tan oil (172 mg). Thermospray (+ve) MS: *m/z* 226, 268, 85.

The oil was treated with ammonium formate (145 mg, 2.3 mmol) and conc. aq. ammonia (2.5 cm^3). After being stirred for 5.25 h at 87 °C the reaction mixture was evaporated under reduced pressure to give a brown solid (302 mg). Co-evaporation with water (3 × 10 cm^3) followed by freeze-drying gave a buff solid (202 mg). Preparative HPLC gave the *title compound* **4** as a solid (25 mg, 28.6%) (preparative retention times: **6**, 13 min; **4**, 18 min); $\delta_H(D_2O)$ 6.60 (1 H, br s, 2-H), 4.28 (1 H, m, 3-H), 3.86 (1 H, dd, *J* 9 and 10, 4-H), 3.63 (1 H, dd, *J* 12 and 3, CH_2OH), 3.56 (1 H, dd, *J* 12 and 6, CH_2OH), 2.57 (1 H, dd, *J* 13 and 4, 6-H^a), 2.26 (1 H, m, 6-H^b) and 2.04 (1 H, m, 5-H); $\delta_C(D_2O)$ 174.7 (CH_3C=O), 170.5 (CO_2H), 157.1 (C=NH), 134.8 (C-2), 133.3 (C-1), 61.9 (CH_2O), 54.5 (C-3), 51.1 (C-4), 38.6 (C-5), 27.3 (C-6) and 22.1 (Me) (Found: $[M + H]^+$, 271.140 813. $C_{11}H_{19}N_4O_4$ requires *m/z*, 271.140 630); >99% pure by HPLC (conditions 1; 50% MeCN in water).

X-Ray experimental data for compound 36

Crystal data. $C_{17}H_{20}N_4O_3$, M = 328.37. Monoclinic, *a* = 16.259(9), *b* = 12.049(3), *c* = 9.558(3) Å, β = 111.76(3)°, *V* = 1739(2) $Å^3$ (by least-squares refinement on diffractometer angles for 12 automatically centred reflections, λ = 1.541 78 Å). Space group *Cc* (No. 9), *Z* = 4, D_c = 1.25 g cm^{-3}, *F*(000) = 696, μ(Cu-Kα) = 6.9 cm^{-1}. The compound crystallised from isopropyl alcohol as prisms.

Data collection and processing. Three-dimensional, room temperature (295 K) X-ray data were collected on a Siemens R3m/V diffractometer with monochromatised Cu-Kα X-radiation. $2\theta/\omega$ mode with scan range (ω) 1.14° plus Kα separation and a variable scan speed (7.32–14.65° min^{-1}). 2389 Reflections were measured ($3 < 2\theta < 115°$, min. *hkl* 0, 0, −11, max. *hkl* 18, 14, 11) of which 1232 were unique [$R(\sigma)$ = 0.011, Friedel opposites merged] and 945 had $I > \sigma(I)$. Two control data monitored every 98 reflections showed no appreciable decay during 15.4 h of exposure of the crystal to the X-rays.

Structure analysis and refinement. Direct methods resulted in the location of all the non-hydrogen atoms. The terminal azide nitrogen proved more difficult to refine than the other atoms in the molecule, showing perhaps a degree of disorder at this atomic position. Full-matrix least-squares refinement was employed with anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms were refined in riding mode. Individual weights were applied according to the scheme $w = [\sigma^2(F_o) + 0.0011|F_o|^2]^{-1}$; refinement converged at *R* 0.067, *Rw* 0.068, goodness-of-fit = 1.28. Maximum and mean shift/error in final cycle of refinement were 0.195 and 0.032, respectively. The final electron-density difference synthesis showed peaks >0.20 or holes < −0.22 e $Å^{-3}$. All computations were carried out using the SHELXTL PLUS (μ-VAX II) system of programs.[21] Full details of crystal data, fractional atomic co-ordinates, bond lengths, bond angles, hydrogen coordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.†

Acknowledgements

We thank Ms Jackie Woods and Dr Richard Bethell of the Virology Department for provision of biological data. Thanks are also due to Dr Paul Smith for the purification of compound **6**.

† For full details of deposition scheme, see Instructions for Authors, 1995, Issue 1.

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Paper 4/06172A
Received 10*th October* 1994
Accepted 22*nd December* 1994