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**Original article**

# Synthesis and influenza virus sialidase inhibitory activity of analogues of 4-Guanidino-Neu5Ac2en (Zanamivir) modified in the glycerol side-chain

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**Abstract** – Analogues of 4-Guanidino-Neu5Ac2en (Zanamivir) have been prepared containing carbamate substituents at the 7-hydroxy position. (4S,5R,6R)-5-Acetylamino-6-{1R-[(6-aminohexyl)carbamoyloxy]-2R,3-dihydroxypropyl}-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid and (4S,5R,6R)-5-Acetylamino-6-{1R-[heptylcarbamoyloxy]-2R,3-dihydroxypropyl}-4-guanidino-5,6-dihydro4H-pyran-2-carboxylic acid were the two analogues possessing activity comparable to Zanamivir, showing potent inhibition of influenza virus sialidases and good antiviral activity in vitro. © 1999 Éditions scientifiques et médicales Elsevier SAS

**influenza virus sialidase neuraminidase Zanamivir / antiviral activity**

## 1. Introduction

Several classes of potent and selective influenza virus sialidase inhibitors have now been reported based upon either dihydropyrans **1** and **2** (Zanamivir, Glaxo-Wellcome) [1–4]; cyclohexene **3** (GS4104, GS4071, Gilead) [5–7]; or cyclopentane **4** (Biocryst) [8] core templates *(figure 1)*. Several recent reports from our co-workers describe structural modifications to Zanamivir which demonstrate the important contributions to overall binding made by each of the dihydropyran substituents *(figure 2)* [9–13]. In particular, studies have been carried out in which the glycerol side chain has been truncated in a stepwise fashion, confirming that the interaction which the 8,9-diol makes with the conserved $Glu^{276}$ residue makes an important contribution to overall binding of sialic acid derivatives to influenza sialidase. A previous communication describes the complete oxidative cleavage of the glycerol side-chain and replacement with a series of carboxamide derivatives, producing a series of compounds with exceptionally potent activity against influenza A, but which demonstrate lower activity against influenza B [14]. X-ray crystallography indicates that

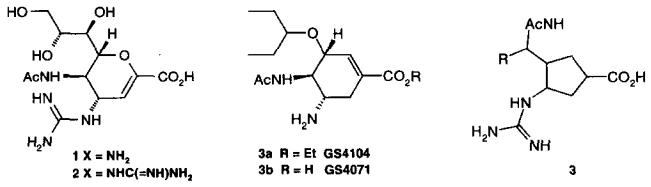


**Figure 1.** Structural comparison of lead classes of influenza sialidase inhibitors.

these more lipophilic compounds bind to the enzyme, making interactions which are quite distinct from those characterised for the glycerol side-chain. Small variations in the position of amino acid sidechains can be used to account for the separation in activity which this series shows when tested against influenza A and B sialidases.

The X-ray crystal structures of sialic acid and Zanamivir bound to influenza sialidase show that the 7-hydroxyl group makes no direct interactions with the protein and is exposed to bulk solvent, indicating that it may be possible to modify the glycerol sidechain at this position, whilst retaining the important 8,9-diol moiety [15, 16]. 7-modification is of particular interest since it allows the synthesis of a wide range of Zanamivir

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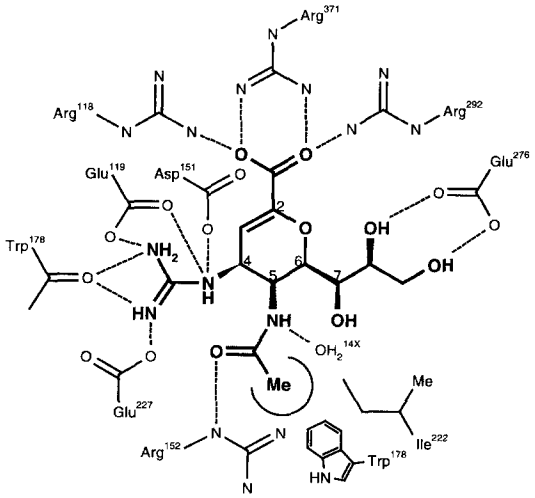



**Figure 2.** Schematic representation of interactions between Zanamivir and influenza A sialidase.

derivatives, which, unlike 6-carboxamides, are potentially A/B non-selective and could display modified physicochemical properties. The analogue set was chosen to probe a wide range of physicochemical properties to see how these influence compound pharmacokinetics. Acylation of the 7-hydroxyl group offered a potentially quick route into testing this hypothesis, but given the known tendency for acyl groups to migrate within sugars onto less hindered positions [17], in this case the 9-hydroxyl group, this approach was not pursued. We chose instead to exploit the resistance to migration which is displayed by the carbamoyl linker, and this paper describes the synthesis and influenza virus sialidase inhibition of a series of 7-carbamate compounds **9**, **11**, **16** and **18** which are derivatives of **1** and **2** *(figure 3)*.

## 2. Chemistry

The syntheses of compounds **9**, **11**, **16** and **18** are outlined in *figures 4–8*. Initially, formation of the required carbamate linker was by reaction of isocyanates with the cyclic carbonate **6**, which had, itself, been synthesised by the method of Zbiral [18] from the known methyl ester **5** [11] using carbonyl diimidazole or phosgene in toluene *(figure 4*, method A). In our preferred route (method B), **5** was treated with 1.2 equivalents of 4-nitrophenyl chloroformate in dry pyridine *(figure 5)*. TLC monitoring first revealed formation of the intermediate cyclic carbonate **6** and addition of a further portion of 4-nitrophenyl chloroformate and DMAP afforded a good yield of the

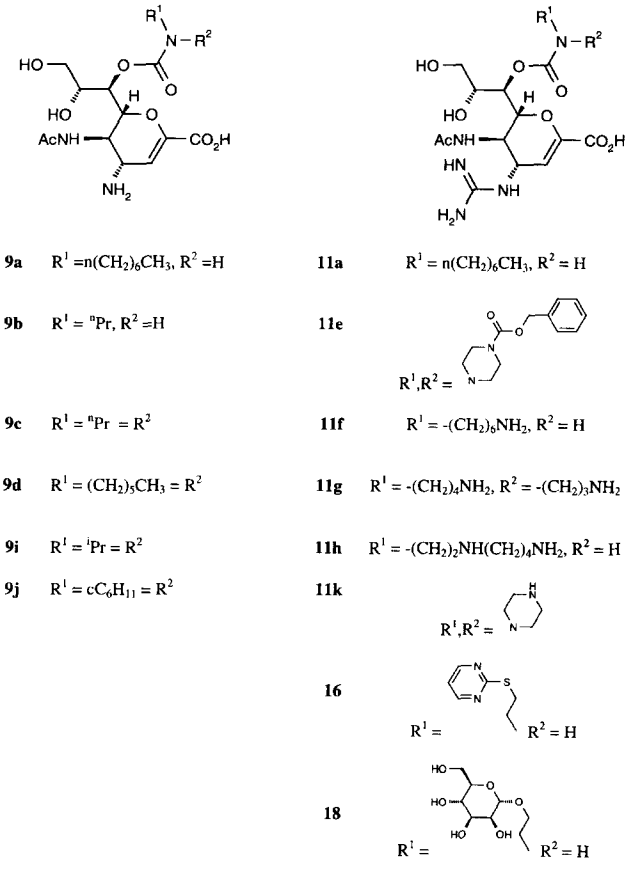



**Figure 3.** Structure of compounds **9**, **11**, **16** and **18**.

active ester **7**. It should be noted that portionwise addition of 4-nitrophenyl chloroformate and later addition of DMAP are essential for efficient conversion. Treatment of **7** with suitable primary and secondary amines furnished a range of 7-carbamates **8**. Method B was adopted as the preferred route since reaction of amines with **7** permits synthesis of a more diverse range of analogues. The use of commercial amines such as mono-Boc-diaminohexane, along with in-house synthesised amines gave access to potentially dendrimeric compounds such as **11f** and **11g**. In the case of **9i** and **9j**, the desired disubstituted carbamates were more conveniently obtained by reaction of the commercially available carbamoyl chloride with **6**.

Treatment of **8** with triphenylphosphine generated an iminophosphorane which was hydrolysed in situ at 40 °C with aqueous triethylamine; concomitant hydrolysis of the cyclic carbonate and methyl ester protecting groups afforded **9** in good yield *(figure 6)*. The azide **8** can also be reduced by hydrogenation over Lindlar catalyst, but

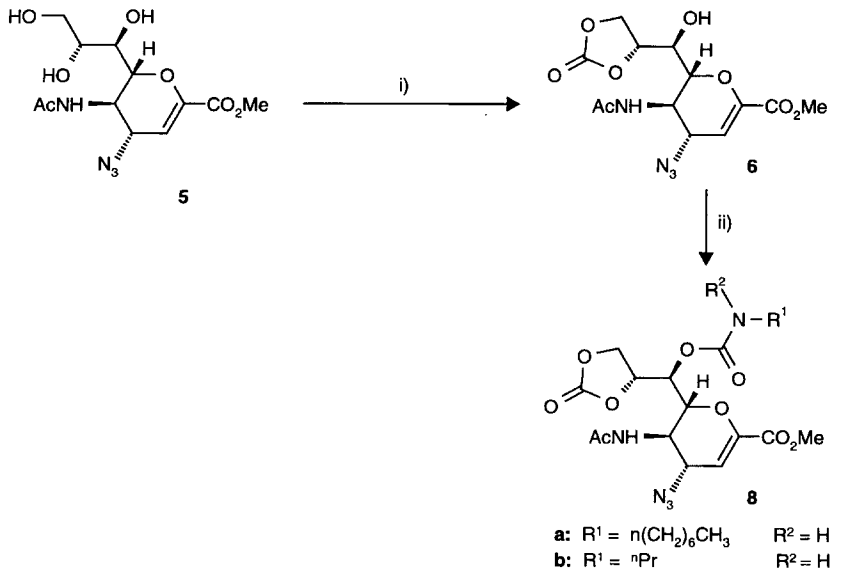



**Figure 4.** Synthetic method A. i) $COCl_2$, DMAP, MeCN, DCM; ii) $R^1NCO$, DMAP, DCM.

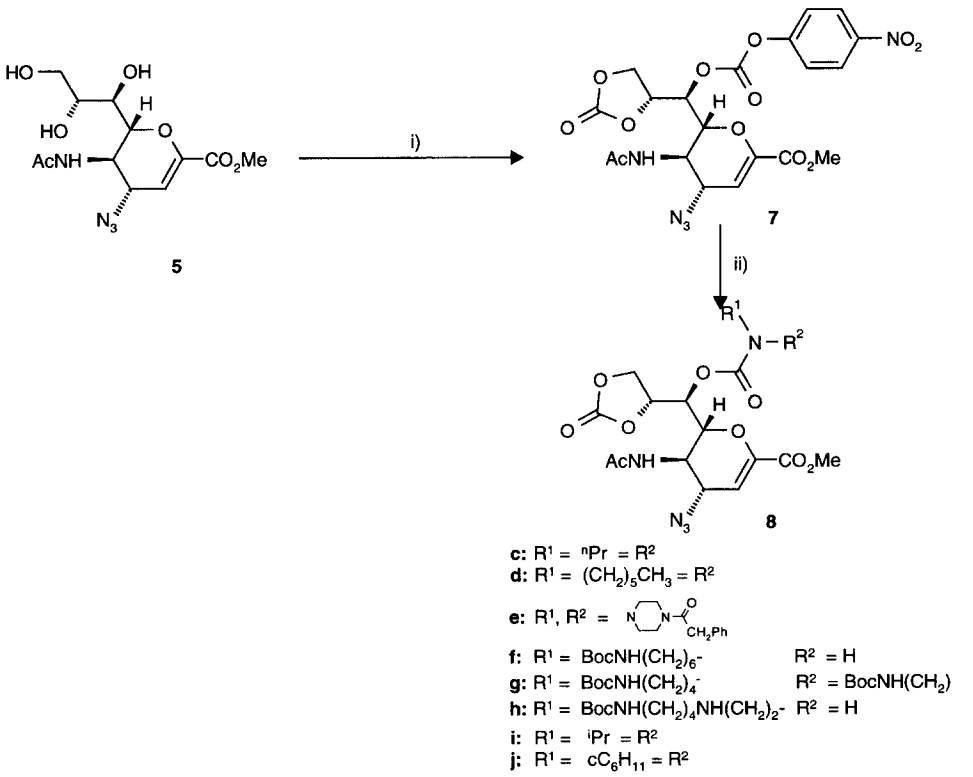



**Figure 5.** Synthetic method B. i) 3 eq. p-$NO_2C_6H_4OCOCl$, DMAP, pyr; ii) $R^1R^2NH$, DMAP, pyr.

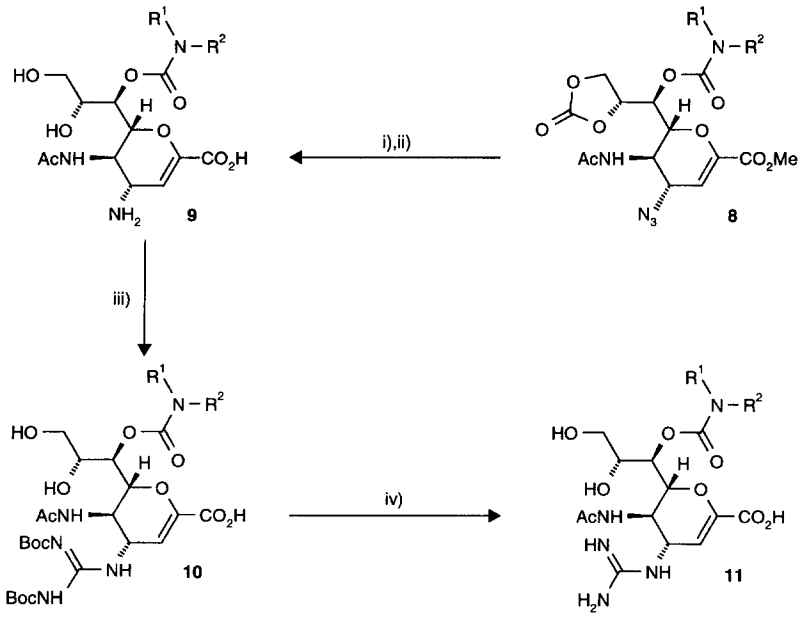



**Figure 6.** Transformation of azido ester to guanidino acid. i) $Ph_3P$, THF; ii) $Et_3N$, $H_2O$, $\Delta$; iii) Bis(Boc)PCH, MeOH, $Et_3N$; iv) TFA, DCM.

the efficiency of this reaction is very variable, depending on the size and bulk of the 7-carbamate substituent. Guanidinylation of the 4-amino group in **9** with N, N′-bis-t-butoxycarbonyl-1H-pyrazole-1-carboxamidine (BisBocPCH) [19] produced the protected guanidine **10**. The Boc protecting groups were removed from **10** by treatment first with TFA, followed by water; lyophilisation of the resulting solution afforded pure compounds **11** in good yield. It is noteworthy that the product of the TFA deprotection was significantly contaminated with its 9-trifluoroacetate ester; this proved to be very labile to hydrolysis and allowed the generation of final products of sufficient purity that no further purification was required.

*Figure 7* describes a modification to the synthesis which permitted access to the ethanolamine derivative **12**. Under standard acylation conditions using an excess of the amine component, very little of the desired **12** was formed, ethanolamine being sufficiently reactive to ring-open the initially formed carbonate **12** to generate the dicarbamate **13**. This unwanted side-reaction consistently presented problems when more nucleophilic amines were used. Reducing the stoichiometry of ethanolamine permitted clean conversion to **12**. This was a very versatile intermediate which was converted to the bromide **15** in three steps and could be displaced by a range of amines and thiols to yield nitrogen- or sulfur-linked heterocyclic compounds such as **16**. In addition, ethanolamine **12** could be glycosylated under standard Koenigs-Knorr conditions to furnish saccharide derivatives of the type **14** *(figure 8)*. In nearly all cases, the intermediate coupled products bearing 8,9 cyclic carbonate protection were highly lipophilic and proved very difficult to purify. Partial deprotection of the intermediates using sodium methoxide in anhydrous methanol, in all cases yielded the BisBoc guanidino esters which were more polar and considerably easier to purify to homogeneity, although partial loss of Boc protection complicated the recovery of product in some cases.

A route utilising the 8,9 isopropylidene protected intermediate **19** was considered but discounted on the basis that a flatter, less sterically demanding protecting group would have wider applicability *(figure 9)*. The carbonate protection exemplified in *figures 4–8*, is very labile to the conditions used for the final basic hydrolysis of the azide-derived iminophosphorane, permitting elegant final deprotection. The protection strategy can be modified, however, to permit a final acidolytic deprotection by employing the 8,9 ethylidene acetal **20** in conjunction with diphenyl methyl ester protection of the carboxylic acid [20]. A further advantage which accrues from the use of ethylidene protection is that when used in conjunction with the more acid-stable methyl ester, it can be isomerised under acid catalysis to a readily separable mixture of the 8,9 (**21**) and 7,9 (**22**) acetals, allowing

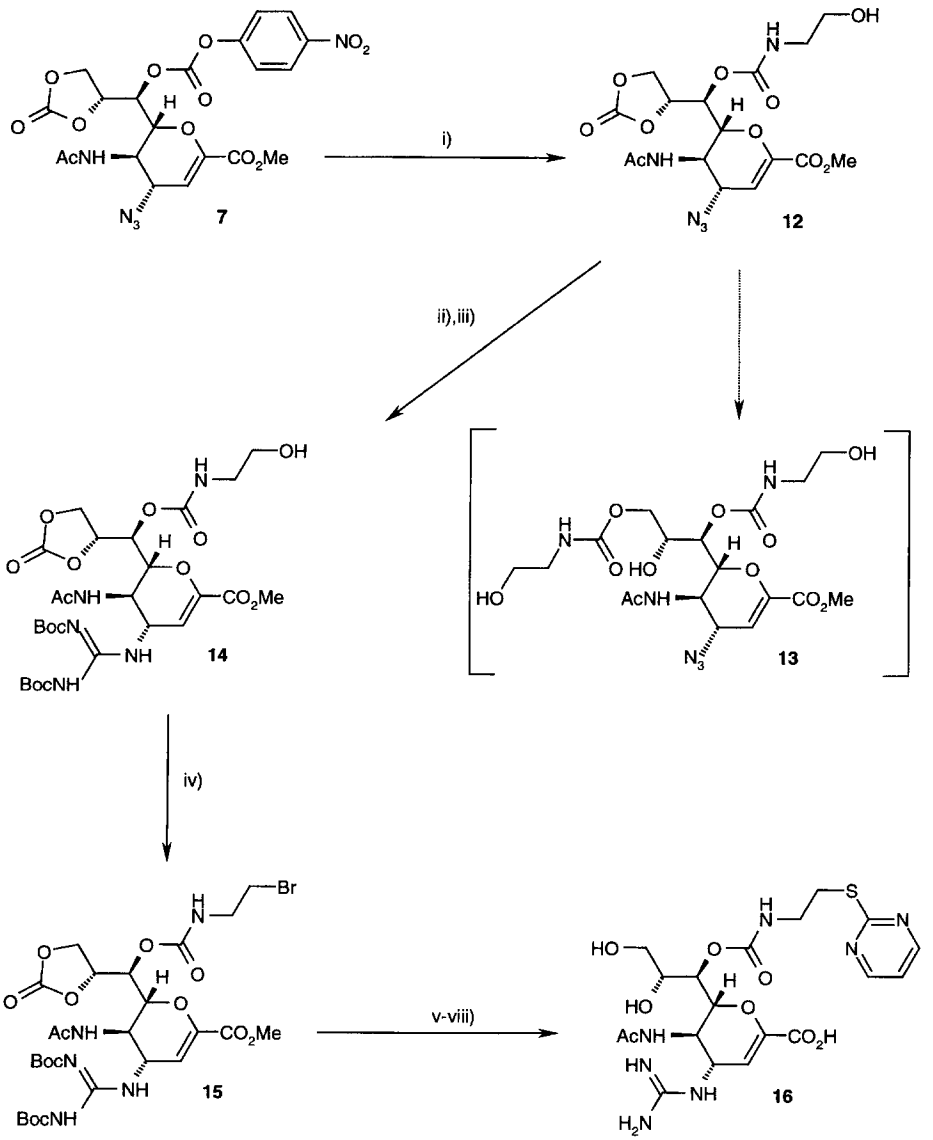



**Figure 7.** Synthetic method C. i) $H_2NCH_2CH_2OH$, MeCN, $Et_3N$; ii) $H_2$, Lindlar catalyst, MeOH; iii) Bis(Boc)PCH, MeOH; iv) $CBr_4$, $Ph_3P$, DCM; v) 2-mercaptopyrimidine, DIPEA, MeCN, 50 °C vi) $Et_3N$, $H_2O$, THF, 50 °C; vii) TFA; viii) $H_2O$.

access to a range of 8-functionalised Zanamivir derivatives. The detail and applicability of this methodology is the subject of a separate communication (unpublished data).

## 3. Results and discussion

New compounds **9**, **11**, **16** and **18** were evaluated for their ability to inhibit the hydrolysis of 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid by sialidases of representative strains of influenza A and B viruses by the previously reported method [21, 22]. The more active inhibitors which emerged from this assay were further evaluated for their in vitro antiviral activity in a plaque reduction assay using MDCK cells [22, 23]. The results obtained are shown in *table I* together with values for the known inhibitors **1** and **2**.

The most active compounds in this series are highly potent and are around an order of magnitude less active than their glycerol counterparts **1** and **2**; the approxi-

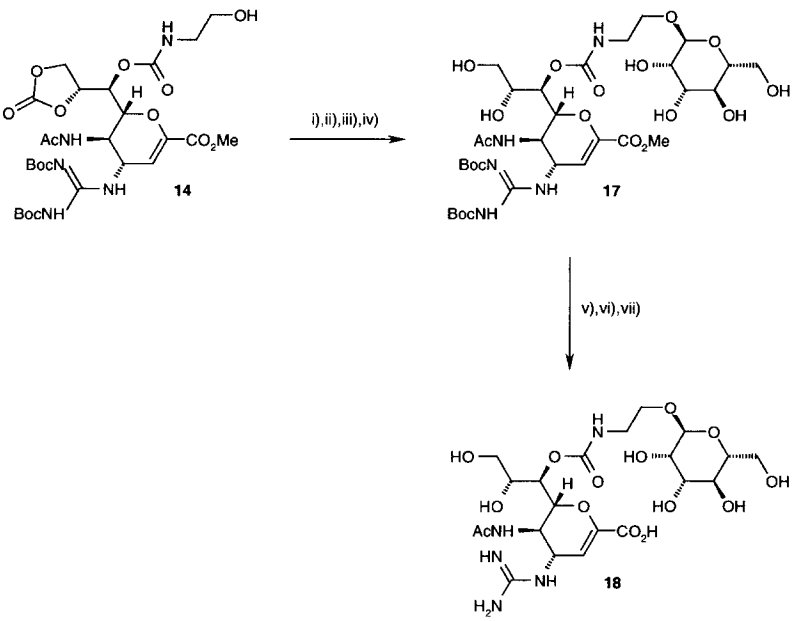



**Figure 8.** Synthetic method D. i)acetobromomannose, AgOTf, DCM –30 °C; ii) 2,4,6-collidine; iii) NaOMe, MeOH; iv) Dowex 50W X 8; v) NaOH, MeOH, $H_2O$; vi) 95% TFA; $H_2O$.

mately 100 to 1 000-fold gain in activity previously seen when the 4-amine is converted to the 4-guanidine is maintained (**9a** cf. **11a**). Generally, disubstituted carbamates are marginally less active than monosubstituted (**9b** cf. **9c**), but a more important trend is that compounds bearing bulky substituents (**9i**) and those which are highly lipophilic (**9d**) show poorer activity than those bearing polar or ionisable substituents (e.g. **11e–11h** and **18**). These results are quite consistent with the X-ray analysis of Zanamivir bound to influenza A sialidase [15]; the poorer activity of the lipophilic compounds may be a result of the fact that when bound to the enzyme, the 7-position is solvent exposed. A large lipophilic carbamate substituent could experience adverse interactions with the polar amino acid residues at the protein surface, or it may simply exist in a highly collapsed form, minimising the surface area which it presents to solvent, making the 7-position much more sterically crowded than is the case for Zanamivir. Of particular importance is that unlike the 6-carboxamides previously described, none of the 7-carbamate compounds investigated shows any A/B selectivity.

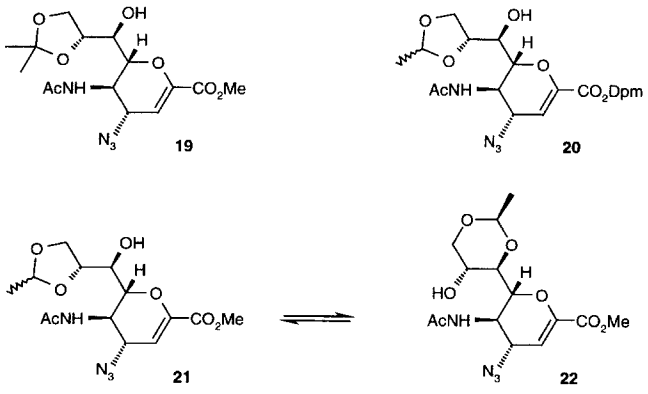
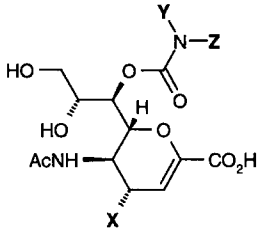


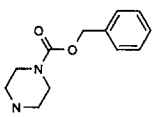
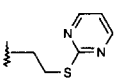
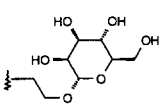

**Figure 9.** Acetal protection strategies for glycerol sidechain.

## 4. Conclusions

We have described the synthesis and properties of a series of carbamate derivatives of Zanamivir. Several compounds were shown to possess potent influenza sialidase inhibitory activity, although in all cases, measured activity was slightly lower than that of Zanamivir. The most potent compounds of the series are **11a** and the closely analogous **11f**, which in addition to good enzyme activity, displays potent anti-viral activity in vitro. Although none of the compounds described herein are as

**Table I.** Influenza sialidase inhiition and plaque reduction by compounds **1**, **2**, **9**, **11**, **16** and **18a**.




| Compound | X | Y | Z | Method of synthesis | Flu A Aichi | | Flu B Victoria | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Enzyme $IC_{50}$ (μM) | Plaque $IC_{50}$ (μg/mL) | Enzyme $IC_{50}$ (μM) | Plaque $IC_{50}$ (μg/mL) |
| **1** | $NH_2$ | – | – | | 0.32 | 0.47 | 0.41 | 0.02 |
| **2** | $NHC(=NH)NH_2$ | – | – | | 0.005 | 0.005 | 0.004 | 0.002 |
| **9a** | $NH_2$ | $-(CH_2)_6CH_3$ | H | A | 6.0 | 1.8 | 1.95 | 0.27 |
| **11a** | $NHC(=NH)NH_2$ | $-(CH_2)_6CH_3$ | H | A | 0.0037 | 0.05 | 0.0033 | 0.023 |
| **9b** | $NH_2$ | nPr | H | A | 4.2 | 1.25 | 1.3 | 1.1 |
| **9c** | $NH_2$ | nPr | nPr | B | 9.8 | 2.95 | 2.0 | 0.52 |
| **9d** | $NH_2$ | $nC_6H_{13}$ | $nC_6H_{13}$ | B | 76 | – | 2.2 | – |
| **9i** | $NH_2$ | iPr | iPr | B | 48 | – | 24 | – |
| **9j** | $NH_2$ | $cC_6H_{11}$ | $cC_6H_{11}$ | B | 100 | – | 40 | – |
| **11e** | $NHC(=NH)NH_2$ |  | | B | 0.014 | | 0.34 | |
| **11f** | $NHC(=NH)NH_2$ | $-(CH_2)_6NH_2$ | H | B | 0.004 | 0.005 | 0.12 | 0.009 |
| **11g** | $NHC(=NH)NH_2$ | $-(CH_2)_4NH_2$ | $-(CH_2)_3NH_2$ | B | 0.72 | 0.018 | 0.62 | 0.034 |
| **11h** | $NHC(=NH)NH_2$ | $-(CH_2)_4NH(CH_2)_2NH_2$ | H | B | 0.077 | 0.019 | 0.04 | 0.27 |
| **11k** | $NHC(=NH)NH_2$ |  | | B | 0.038 | 0.031 | 0.033 | > 0.1 |
| **16** | $NHC(=NH)NH_2$ |  | H | C | 0.0062 | 0.0001 | 0.084 | 0.027 |
| **18** | $NHC(=NH)NH_2$ |  | | D | 0.013 | < 0.1 | 0.16 | 0.88 |

[a]Sialidase assay: the $IC_{50}$ values are calculated from the percent inhibition of enzyme activity in the presence of inhibitor, relative to a positive (no inhibitor) control. All reactions were carried out in triplicate, and the mean values of these replicates used in the analysis of data. Plaque assay: the percent inhibition of plaque formation relative to controls was calculated for each inhibitor concentration used. At each concentration, data from three experiments were pooled in order to accurately determine the 50% inhibitory concentration ($IC_{50}$) for each compound. In most cases corresponding $IC_{50}$s from different experiments differed by a factor of ≤ 5.

potent as Zanamivir, they demonstrate that the 7-position is capable of tolerating groups of greatly differing size and chemical functionality, with retention of activity. We have synthesised a wide diversity of analogues at this position, exemplifying the adaptability of this compound class.

## 5. Experimental protocols

### *5.1. General*

FTIR spectra were recorded using a Nicolet 20SXB or a Bio-Rad FTS-7. $^1$H-NMR spectra were recorded either at 250 MHz using a Bruker AC or AM 250 or at 400 MHz with a Varian VXR 400. Mass spectra were measured on an HP Engine (Thermospray positive) or VG Autospec Q (LSIMS). Routine microanalyses were performed on a Leco CHNS-932 or Carlo-Erba instrument. Flash chromatography was performed with Merck Kieselgel 9385. Flash reverse-phase chromatography was performed with Merck C18 13900. Analytical HPLC was performed using a Rainin C18 8 μM column, eluting on a gradient of 0.1% aqueous trifluoroacetic acid in water to 90:10:0.1 acetonitrile/water/trifluoroacetic acid at a flow rate of 1 mL per min.

### 5.2. Syntheses

#### *5.2.1. (4S,5R,6R)-5-Acetylamino-4-azido-6-[(S)-hydroxy-(2-oxo-[1,3]dioxolan-4R-yl)-methyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **6**

To a suspension of the triol **5** [11] (8.2 g, 25 mmol) in dry acetonitrile (100 mL) and dry dichloromethane (200 mL) was added 4-dimethylaminopyridine (8.8 g, 72 mmol). A 20% solution of phosgene in toluene (18 mL, 36 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 2 h. The solution was then added to ice cold 1 M potassium dihydrogen orthophosphate solution (400 mL) and extracted with ethyl acetate (350 mL × 3). The combined organic extracts were washed with saturated aqueous NaCl (30 mL), dried ($Na_2SO_4$), and the solvent removed under vacuum to yield an orange foam which was purified by flash chromatography (eluant, chloroform/methanol 15:1) to give **6** (6.01 g, 67%) as an off-white foam. IR (KBr): $\nu_{max}$ 2 100, 1 785, 1 735, 1 662 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 8.30 (1 H, d, AcN*H*), 5.89 (1 H, d, H-3), 5.67 (1 H, d, OH-7), 4.60 (1 H, m, H-9), 4.57 (1 H, m, H-9), 4.99 (1 H, m, H-8), 4.44 (1 H, dd, H-4), 4.15 (1 H, m, H-6), 4.03 (1 H, m, H-7), 3.98 (1 H, m, H-5), 3.78 (3 H, s, $CO_2CH_3$), 1.94 (3 H, s, NHCOC$H_3$); MS 374 $(M + NH_4)^+$.

#### *5.2.2. (4S,5R,6R)-5-Acetylamino-4-azido-6-[(S)-heptylcarbamoyloxy-(2-oxo-[1,3]dioxolan-4R-yl)-methyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **8a**

To an ice-cold solution of **6** (1 g, 2.81 mmol) in dry dichloromethane (10 mL) was added 4-dimethylaminopyridine (349 mg, 2.86 mmol) and n-heptyl isocyanate (1 g, 7.1 mmol). The solution was warmed to room temperature and stirred for 21 h under nitrogen. The reaction was then quenched by the addition of methanol (1 mL) and the solution stirred for 15 min then added to ice cold 1 M potassium dihydrogen orthophosphate solution (100 mL) and extracted with ethyl acetate (175 mL × 3). The combined organic extracts were washed with concentrated aqueous NaCl (30 mL), dried ($Na_2SO_4$) and the solvent removed under vacuum to yield an orange solid which was purified by flash chromatography (eluant, chloroform/methanol 20:1) to give **8a** (560 mg, 40%) as a white foam. IR (KBr): $\nu_{max}$ 2 905 (br), 2 100, 1 805, 1 732, 1 664 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 7.99 (1 H, d, AcN*H*), 7.33 (1 H, m, OCONH), 5.87 (1 H, d, H-3), 5.43 (1 H, m, H-8) 5.16 (1 H, m, H-7), 4.61 (1 H, t, H-6), 4.51 (1 H, t, H-9), 4.37 (1 H, dd, H-9), 4.26 (1 H, dd, H-4), 4.02 (1 H, q, H-5), 3.74 (3H, s, $CO_2CH_3$), 2.90 (2 H, q, OCONHC$H_2$), 1.79 (3H, s, NHCOC$H_3$), 1.23 (10 H, brs, $(CH_2)_5$), 0.85 (3 H, t, heptyl $CH_3$); MS 498 $(M + H)^+$.

#### *5.2.3. (4S,5R,6R)-5-Acetylamino-4-azido-6-[(S)-(4-nitrophenoxycarbonyloxy)-(2-oxo-[1,3]dioxolan-4R-yl)-methyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **7**

To a solution of the triol **5** (3.3 g, 10 mmol) in dry pyridine (30 mL), under nitrogen, was added 4-nitrophenylchloroformate (2.42 g, 12 mmol). After stirring at room temperature for 2.5 h a further portion of 4-nitrophenylchloroformate (2 g, 10 mmol) was added followed by 4-dimethylaminopyridine (3.05 g, 25 mmol). Stirring was continued at room temperature for 3.5 h. The solution was concentrated under vacuum and the residue was partitioned between 2 M HCl (200 mL) and ethyl acetate (100 mL). The layers were separated and the aqueous phase was further extracted with ethyl acetate (100 mL × 2). The combined organic extracts were washed with saturated aqueous NaCl (60 mL), dried ($Na_2SO_4$) and the solvent removed under vacuum to yield a beige foam which was purified by flash chromatography (eluant, ethyl acetate/cyclohexane 2:1) to give **7** (3.21 g, 62%) as a white foam. IR (KBr): $\nu_{max}$ 4 218 (br) 2 101, 1 792, 1 784, 1 664 cm$^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 8.36 (2H, d, Ar), 8.22 (1H, d, AcN*H*), 7.61 (2H, d, Ar), 5.94 (1H, d, H-3), 5.50 (1H, t, H-7), 5.35 (1H, m, H-8), 4.72 (1H, t, H-9), 4.60 (1H, t, H-9), 4.40–4.50 (2H, m, H-6, H-4), 4.23 (1H, t, H-5), 3.78 (3H, s, $CO_2CH_3$), 1.86 (3H, s, NHCOC$H_3$); MS 522 $(M + H)^+$; anal $C_{20}H_{19}N_5O_{12}$ (C, H, N).

*5.2.4. (4S,5R,6R)-5-Acetylamino-4-azido-6-{(S)-[(2-oxo-[1,3]dioxolan-4R-yl)-(6-tert-butoxycarbonylamino-hexyl)-carbamoyloxy]-methyl}-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **8f**

To a solution of **7** (1.15 g, 2.2 mmol) in dry pyridine (10 mL) was added 4-dimethylaminopyridine (648 mg, 5.3 mmol) and N-Boc-1,6-diaminohexane hydrochloride (670 mg, 2.65 mmol). The solution was stirred at room temperature for 18 h. 2 M aqueous hydrochloric acid (150 mL) was added to the solution which was then extracted with ethyl acetate (50 mL × 2). The combined organic extracts were washed with saturated aqueous NaCl (10 mL), dried ($Na_2SO_4$) and the solvent removed under vacuum to yield a yellow foam which was purified by flash chromatography (eluant, ethyl acetate/cyclohexane 4:1) to give **8f** (1.155 g, 90%) as a pale yellow foam. IR (KBr): $\nu_{max}$ 3 326 (br), 2 099, 1 799, 1 737, 1 693 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) δ 6.31 (1 H, br.d, N*H*-Boc), 5.95 (3 H, d, H-3), 5.42 (1 H, dd, AcN*H*), 5.10 (2 H, m, H-7, H-4), 4.95 (1 H, m, H-8) 4.5–4.8 (3 H, m, H-5, H-9), 3.80 (3H, s, $CO_2CH_3$), 3.0–3.4 (4 H, m $NHCH_2(CH_2)_4CH_2NH$), 2.06 (3H, s, $NHCOCH_3$), 1.50 (4H, m, $NHCH_2(CH_2)_4CH_2NH$), 1.44 (9H, s, NH*Boc*), 1.34 (4H, m, $NHCH_2(CH_2)_4CH_2NH$); anal $C_{25}H_{38}N_6O_{11}$.0.35 EtOAc (C, H, N).

*5.2.5. (4S,5R,6R)-5-Acetylamino-4-amino-6-{2R,3-dihydroxy-1R-[(6-tert-butoxy-carbonylaminohexyl)-carbamoyloxy]-propyl}-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate* **9f**

To a solution of **8f** (1.05 g, 1.75 mmol) in dry tetrahydrofuran (40 mL) was added triphenylphosphine (610 mg, 2.32 mmol) and the solution was stirred at room temperature for 18 h. To this was added triethylamine (12 mL, 86 mmol) and water (31 mL) and the solution was heated at 40 °C for a further 28 h. The reaction mixture was evaporated to yield a yellow gum which was purified by flash reverse-phase chromatography (eluant, water (containing trifluoroacetic acid 0.1%)/acetonitrile 8:2). The gum obtained by freeze-drying was triturated vigorously with diethyl ether for 1 h at room temperature to give **9f** (0.79 g, 70%) as a buff powder. IR (KBr): $\nu_{max}$ 3 310 (br), 1 685 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 7.92 (1 H, d, AcN*H*), 7.13, 6.75 (2 H, t, OCON*H*, N*H*Boc), 5.70 (1 H, s, H-3) 4.85 (1 H, d, H-7), 4.34 (1 H, d, H-6), 4.02 (1 H, q, H-5), 3.85 (1 H, t, H-8), 3.77 (1 H, d, H-4), 3.25, 3.44 (2 H, m, H-9), 2.90 (4 H, m, $NHCH_2(CH_2)_4CH_2NH$), 1.82 (3H, s, $NHCOCH_3$), 1.18–1.42 (8H, m, $NHCH_2(CH_2)_4CH_2NH$), 1.38 (9H, s, Boc); MS 533 (M + H)$^+$; anal $C_{23}H_{40}N_4O_{10}$.TFA.0.25$Et_3N$ (C, H, N).

*5.2.6. (4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxy-carbonyl]guanidino-6-{1R-[(6-tert-butoxycarbonylamino-hexyl)-carbamoyloxy]-2,3-dihydroxypropyl}-5,6-dihydro-4H-pyran-2-carboxylic acid* **10f**

A solution of **9f** (0.75 g, 1.16 mmol), triethylamine (0.5 mL, 3.6 mmol) and N,N′-bis-t-butoxycarbonyl-1H-pyrazole-1-carboxamidine Bis(Boc)PCH (0.54 g, 1.74 mmol) [15] in a mixture of dry tetrahydrofuran (10 mL) and dry methanol (3 mL) was stirred under nitrogen for 20 h and then evaporated under vacuum. The residue was dissolved in ethyl acetate (100 mL), stirred vigorously with water (20 mL) and the pH of the mixture was adjusted to pH 2 with 2 M hydrochloric acid. The organic layer was washed with saturated aqueous NaCl (20 mL), dried ($Na_2SO_4$), and the solvent removed under vacuum to yield a yellow foam which was purified by flash chromatography (eluant, chloroform/methanol 7:1) to give **10f** (0.37 g, 42%) as a white solid. IR (KBr): $\nu_{max}$ 3 291 (br), 1 686, 1 610 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 11.42 (1 H, s, guanidine N*H*Boc), 8.25 (1 H, d, NH-4), 7.95 (1 H, d, AcN*H*), 7.16 (1 H, t, OCONH), 6.75 (1 H, t, heptyl N*H*Boc) 5.80 (1 H, s, H-3), 4.82 (1 H, d, H-7), 4.74 (1 H, t, H-4), 4.43 (1 H, d, H-6), 4.0 (4 H, m, H-5,-8,-9), 3.25 (1 H, m, OH), 2.90 (4 H, m, $NHCH_2(CH_2)_4CH_2NH$) 1.76 (3 H, s, $NHCOCH_3$), 1.49 (9 H, s, Boc), 1.38 (18 H, s, Boc), 1.2–1.5 (8 H, m, $NHCH_2(CH_2)_4CH_2NH$); MS 775 (M + H)$^+$; anal $C_{34}H_{58}N_6O_{14}$ (C, H, N).

*5.2.7. (4S,5R,6R)-5-Acetylamino-6-{1R-[(6-aminohexyl)-carbamoyloxy]-2R,3-dihydroxypropyl}-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid* **11f**

A solution of **10f** (325 mg, 0.42 mmol) in trifluoroacetic acid (10 mL) was stirred under nitrogen for 1 h and evaporated under vacuum. The residue was dissolved in water (10 mL), stirred for 1 h and the water removed by lyophilisation to give **11f** (255 mg, 86%) as a white solid. IR (KBr): $\nu_{max}$ 3 307 (br), 1 735 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 7.97 (1 H, d, AcN*H*), 7.2–7.8 (6 H, m, guanidine, hexylamine $NH_2$s), 7.6 (1 H, d, NH-4), 5.72 (1 H, s, H-3), 5.07 (1 H, d, OH), 4.85 (1 H, d, H-7), 4.4 (2 H, m, H-6, OH), 4.33 (1 H, t, H-4), 4.02 (1 H, q, H-5), 3.83 (1 H, m, H-8), 3.3 (1 H, m, H-9), 3.4 (1 H, m, H-9), 2.91 (2 H, m, $OCONHCH_2$), 2.78 (2 H, m, $CH_2NH_2$), 1.81 (3 H, s, $NHCOCH_3$), 1.2–1.6 (8 H, m, $NHCH_2(CH_2)_4CH_2NH$); MS 475 (M + H)$^+$; anal: found C 38.2; H 5.1; N 11.5. $C_{19}H_{34}N_6O_8$.2TFA.$H_2O$ requires C 38.3; H 5.3; N 11.6.

*5.2.8. (4S,5R,6R)-5-Acetylamino-4-azido-6-[(S)-(2-hydroxyethylcarbamoyloxy)-(2-oxo-[1,3]dioxolan-4R-yl)-methyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **12**

To a solution of **7** (7.60 g, 14.58 mmol) in dry acetonitrile (60 mL) was added triethylamine (0.86 mL, 14.42 mmol), followed by ethanolamine (0.86 mL, 14.3 mmol). The solution was stirred at room temperature for 1 h and the solvent removed under vacuum to yield an orange foam which was purified by flash chromatography (eluant, ethyl acetate) to yield **12** as a yellow foam (5.78 g, 90%). IR (KBr): $\nu_{max}$ 3 300 (br), 2 100, 1 792, 1 733, 1 653 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 8.09 (1 H, d, AcN*H*), 7.35 (1 H, t, OCONH), 5.88 (1 H, d, H-3), 5.41 (1 H, s, H-7), 5.16 (1 H, m, H-8), 4.6 (1 H, m, OH), 4.6 (1 H, m, H-9), 4.5 (1 H, m, H-9), 4.37 (1 H, m, H-6), 4.29 (1 H, m, H-4), 4.07 (1 H, m, H-5), 3.76 (3 H, s, $COOCH_3$), 3.39 (2 H, m, $CH_2CH_2OH$), 3.05, 2.92 (2 H, m, $CH_2CH_2OH$), 1.82 (3 H, s, $NHCOCH_3$); MS 461 (M + $NH_4$)$^+$; anal $C_{16}H_{21}N_5O_{10}$ (C, H, N).

*5.2.9. (4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-6-[(S)[(2-hydroxyethyl)-carbamoyloxy]-(2-oxo-[1,3]dioxolan-4R-yl)-methyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **14**

A solution of **12** (5.7 g 12.9 mmol) in methanol (250 mL) was added to Degussa CE407 Pb/Pd on $CaCO_3$ R/D 5 + 5% (i.e. 5% Pd on $CaCO_3$, poisoned with Pb 5%) dry catalyst and stirred under hydrogen for 2 h. The resulting mixture was filtered through celite, washed with methanol (500 mL) and the combined filtrate and washings evaporated. The yellow foam obtained was dissolved, with heating, in ethanol and evaporated to a slurry which was triturated with ethyl acetate (20 mL) to yield the intermediate amine as an off-white solid. A mixture of the intermediate amine (3.64 g, 8.72 mmol) and BisBocPCH (3.39 g, 10.9 mmol) in methanol (150 mL) was warmed briefly to give a solution which was allowed to cool to room temperature and stirred for 5 h. A further portion of BisBocPCH (0.68 g, 2.19 mmol) was added and the solution stirred for 18 h; solvent was removed under vacuum to give a white amorphous solid which was purified by flash chromatography (eluant, ethyl acetate/cyclohexane 4:1 to 9:1) to yield **14** as a white foam (4.32 g, 57%). IR (KBr): $\nu_{max}$ 3 290 (br) 1 800, 1 738, 1 646 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 11.4 (1 H, s BocN*H*), 8.18 (1 H, d, AcN*H*), 8.05 (1 H, d, NH-4), 7.35 (1 H, t, OCONH), 5.84 (1 H, d, H-3), 5.43 (1 H, m, H-7), 5.16 (1 H, m, H-8), 4.8 (1 H, m, H-4), 4.62 (2 H, m, H-9), 4.62 (1 H, m, H-6), 4.52 (1 H, t, OH), 4.03 (1 H, q, H-5), 3.64 (3 H, s, $COOCH_3$), 3.41 (2 H, m, $CH_2CH_2OH$), 2.98 (2 H, m, $CH_2CH_2OH$), 1.75 (3 H, s, $NHCOCH_3$), 1.46, 1.40 (9 H, 9 H, s, s, Boc, Boc); HRMS: found MH$^+$ 660.273025, calc for $C_{27}H_{42}N_5O_{14}$ 660.272827.

*5.2.10. (4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-6-[(S)[(2-bromoethyl)-carbamoyloxy]-(2-oxo-[1,3]dioxolan-4R-yl)-methyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **15**

To a solution of **14** (533 mg, 0.81 mmol) in dry dichloromethane (6 mL) was added tetrabromomethane (407 mg, 1.23 mmol) and triphenylphosphine (318 mg, 1.2 mmol) and the solution was stirred at room temperature for 30 min. The reaction mixture was evaporated to yield a colourless foam which was purified by flash chromatography (eluant, ethyl acetate/cyclohexane 1:1) to yield **15** as a colourless foam (395 mg, 68%). IR (KBr): $\nu_{max}$ 3 310 (br), 1 807, 1 732, 1 642, $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 11.4 (1 H, s BocN*H*), 8.18 (1 H, d, AcN*H*), 7.97 (1 H, d, NH-4), 7.76 (1 H, t, OCONH), 5.85 (1 H, d, H-3), 5.46 (1 H, m, H-7), 5.18 (1 H, m, H-8), 4.8 (1 H, m, H-4), 4.64 (1 H, m, H-9), 4.53 (1 H, m, H-6), 4.46 (1 H, m, H-9), 4.02 (1 H, q, H-5), 3.75 (3 H, s, $COOCH_3$), 3.45 (2 H, m, $CH_2CH_2Br$), 3.3 (2 H, m, $CH_2CH_2Br$), 1.75 (3 H, s, $NHCOCH_3$), 1.46, 1.40 (9 H, 9 H, s, s, Boc, Boc); HRMS: found MH$^+$ 722.188633 calc for $C_{27}H_{41}N_5O_{13}Br$ 722.188423 (for $^{79}$Br).

*5.2.11. (4S,5R,6R)-5-Acetylamino-6-{2R,3-dihydroxy-1R-[2-(pyrimidin-2-ylsulfanyl)-ethylcarbamoyloxy]-propyl}-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid bis (trifluoroacetate)* **16**

To a solution of **15** (373 mg, 0.52 mmol) in dry acetonitrile (5 mL) was added 2-mercaptopyrimidine (984 mg, 0.75 mmol) and diisopropylethylamine (130 μL, 0.75 mmol) and the solution was stirred at 50 °C for 2 h. The reaction mixture was evaporated to yield a yellow foam which was purified by flash chromatography (eluant, ethyl acetate/cyclohexane 3:1) to yield the coupled product as a colourless foam (354 mg, 0.47 mmol, 91%). The foam (340 mg, 0.45 mmol) was dissolved in dry THF (1 mL), triethylamine (1 mL) and water (0.5 mL) and heated at 50 °C for 30 h; the solvent was evaporated to yield a cream foam (334 mg) which was dissolved in TFA (2 mL) and stirred for 4 h. Solvent was evaporated and the residue stirred in water (2 mL) for 2 h. This aqueous solution was purified directly by flash reverse-phase chromatography (eluant, water (containing trifluoroacetic acid 0.1%)/acetonitrile 9:1). Fractions of interest were pooled and lyophilised to yield **16** as an amorphous white powder (155 mg, 0.3 mmol, 52%). IR (KBr): $\nu_{max}$ 3 388 (br), 1 615 $cm^{-1}$. $^1$H-NMR (DMSO-$d_6$): δ 8.62 (2 H, d, Ar 3,5-H), 7.98 (1 H, d, AcN*H*), 7.57

(1 H, d, NH-4), 7.48 (1 H, t, OCONH), 7.21 (1 H, m, Ar 4-H), 7.2 (H, br s, HNC(=N*H*)N$H_2$), 5.71 (1 H, d, H-3), 4.9 (1 H, d, H-7), 4.39 (1 H, m, H-6), 4.31 (1 H, m, H-4), 4.02 (1 H, m, H-5), 3.81 (1 H, m, H-8), 3.43 (1 H, m, H-9), 3.23 (1 H, m, H-9), 3.34–3.06 (4 H, m, NHC$H_2$C$H_2$S), 1.8 (3 H, s, NHCOC$H_3$); HRMS: found $MH^+$ 514.171687 calc for $C_{19}H_{28}N_7O_8S$ 514.172008; anal $C_{19}H_{27}N_7O_8S$. 2.5TFA. $H_2O$ (C, H, N, S).

*5.2.12. (4S,5R,6R)-5-Acetylamino-4-[2,3-bis(tert-butoxycarbonyl)-guanidino]-6-{1R-[2-(3S,4S,5S-trihydroxy-6R-hydroxymethyl-tetrahydropyran-2S-yloxy)-ethylcarbamoyloxy]-(2-oxo-[1,3]dioxolan-4R-yl)-methyl}-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester* **17**

To a solution of **14** (1.32 g, 2 mmol) in dry dichloromethane (15 mL) was added silver triflate (0.74 g, 2.88 mmol) and the solution cooled to –30 °C. To this solution was added dropwise over 30 s, a solution of acetobromomannose (1.18 g, 2.9 mmol) in dry dichloromethane (10 mL). The mixture was stirred in the dark at –30 °C for 6 h and quenched by the addition of 2,4,6-collidine (400 µL, 3 mmol) and diethyl ether (150 mL). This mixture was filtered through a celite pad, washed with diethyl ether (100 mL) and the combined filtrate and washings were evaporated to yield a colourless foam which was redissolved in ethyl acetate (100 mL) and washed with 0.5 M HCl (20 mL). The aqueous layer was separated and washed with ethyl acetate (50 mL). The combined organic extracts were washed with brine, dried ($Na_2SO_4$) and evaporated to yield a colourless foam which was purified by flash chromatography (eluant, chloroform/methanol 25:1) to yield the coupled product as an impure colourless foam (1.323 g, 68%). This foam was dissolved in anhydrous methanol (100 mL) and the solution adjusted to pH 10.8 by dropwise addition of sodium methoxide (25% solution in methanol). After 2 h, the solution was adjusted to pH 7 with Dowex 50W X 8 ($H^+$) resin, filtered and evaporated to yield a white solid which was purified by flash chromatography (eluant chloroform/methanol 10:1) to yield **17** as a white amorphous solid (0.27 g, 28%, loss of Boc group). $^1$H-NMR (DMSO-$d_6$): δ 11.4 (1 H, s, N*H*Boc), 8.18 (1 H, d, NH-4), 8.0 (1 H, d, AcN*H*), 7.31 (1 H, t, OCONH), 5.83 (1 H, d, H-3), 5.07 (1 H, d, 2′OH), 4.82 (1 H, d, H -1′), 4.76 (1 H, t, H-4), 4.65 (1 H, m, H-7), 4.49 (1 H, d, H-6), 4.40–4.80 (5 H, m, OH-8,9,3′,4′,6′), 4.05 (1 H, q, H-5), 3.75 (3 H, s, $COOCH_3$), 3.10–3.80 (13 H, m, H-8,9,2′-6′, NHC$H_2$C$H_2$O), 1.86 (3 H, s, NHCOC$H_3$), 1.46, 1.41 (9 H, 9 H, s, s, Boc, Boc); MS: 813 $(M + NH_4)^+$.

*5.2.13. (4S,5R,6R)-5-Acetylamino-6-{2R,3-dihydroxy-1R-[2-(3S,4S,5S-trihydroxy-6R-hydroxymethyl-tetrahydro-pyran-2S-yloxy)-ethylcarbamoyloxy]-propyl}-4-guanidino-5,6-dihydro-4H-pyran-2-carboxylic acid bis(trifluoroacetate)* **18**

A solution of **17** in methanol (20 mL) was treated with water (20 mL) and 0.1 M sodium hydroxide solution (3.25 mL, 0.325 mmol). After 1 h, the solution was evaporated and the residue triturated with diethyl ether (10 mL) to yield a white amorphous powder which was stirred in 95% trifluoroacetic acid (20 mL) at room temperature for 1 h and evaporated to dryness. The residue was stirred in water (5 mL) for 1 h, concentrated and purified by flash reverse-phase chromatography (eluant, water (containing trifluoroacetic acid 0.1%)). Fractions of interest were pooled and lyophilised to a gum which was dissolved in ethanol, concentrated and triturated with diethyl ether (10 mL) and filtered to yield **18** as a white amorphous ethanol-solvated trifluoroacetate salt (198 mg, 84%, corrected). $^1$H-NMR (DMSO-$d_6$ + $D_2O$): δ 5.72 (1 H, d, H-3), 4.84 (1 H, d, H-1′), 4.63 (1 H, m, H-7), 4.39 (1 H, m, H-6), 4.31 (1 H, m, H-4), 4.0 (1 H, q, H-5), 3.10–3.70 (13 H, m, H-8,9,2′-6′, NHC$H_2$C$H_2$O), 1.81 (3 H, s, NHCOC$H_3$); HRMS: found $MH^+$ 582.224769 calc for $C_{21}H_{36}N_5O_{14}$ 582.225876; anal $C_{21}H_{35}N_5O_{14}$. EtOH. TFA (C, H, N).

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