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SIALIDASE INHIBITORS RELATED TO ZANAMIVIR: Synthesis and biological evaluation of 4H-Pyran 6-ether and ketone

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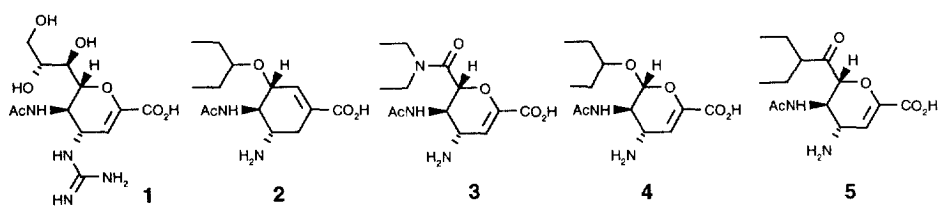
Abstract: Synthesis of 5R-Acetamido-4S-amino-4H-pyran-6R-O-(1-ethyl)propyl and 6R-(1-oxo-2-ethyl)butyl 2-carboxylic acids (**4** and **5**) and their evaluation as inhibitors of influenza virus sialidase is described. Both compounds showed good inhibitory activity with marked selectivity for influenza A sialidase.

Inhibitors of influenza virus sialidases are showing promise in clinical trials for the treatment of influenza.[1] Zanamivir **1** and GS 4071 **2** are both highly potent and selective inhibitors of sialidases from both influenzas A and B. These cyclic inhibitors both adopt similar orientations within the active site of the enzyme, mimic the geometry of the transition state for sialoside hydrolysis, and achieve excellent binding through appropriate presentation of four pendant substituents. Three of these (acid, acetamide and basic group) are similar in both **1** and **2**. However, whilst zanamivir contains a hydrogen bonding glycerol sidechain, GS 4071 possesses a branched pentyl ether substituent. The latter achieves alternative favourable hydrophobic interactions with the viral sialidases.[2,3] In the search for further clinical candidates, a number of reports have also documented other potent sialidase inhibitors[4-7]. In particular we have described 4-H-pyran-6-carboxamides such as **3**.[4,5] These compounds resemble GS 4071 in that they contain a substituent capable of forming hydrophobic interactions with sialidases. However, unlike GS 4071 these compounds show marked (> 100 fold) selectivity for influenza A sialidase. We speculated that this difference might be attributed to the greater conformational flexibility of the pentyl ether sidechain enabling GS 4071 to adopt a favourable binding conformation within the influenza B enzyme which was inaccessible to the relatively rigid amide. Herein we report the synthesis and biological activity of the dihydropyran analogues **4** and **5** containing 6-substituents with greater conformational flexibility.



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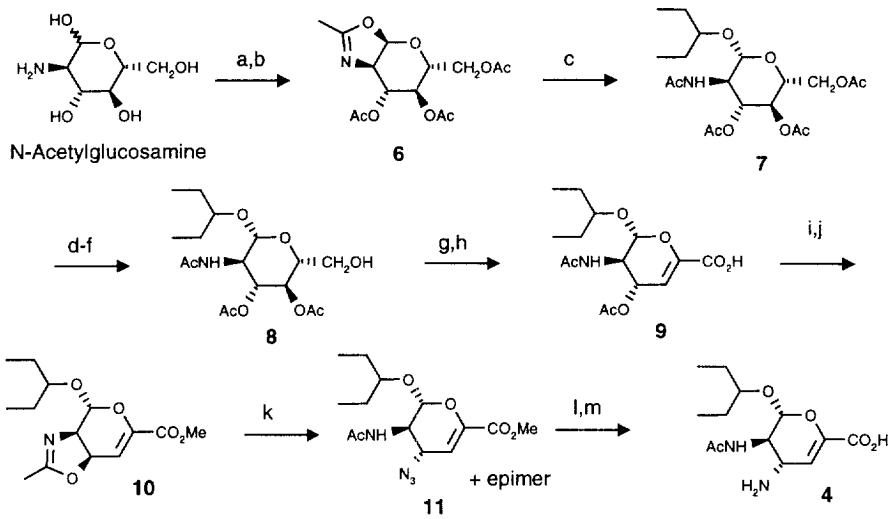
1. Synthesis of compound 4



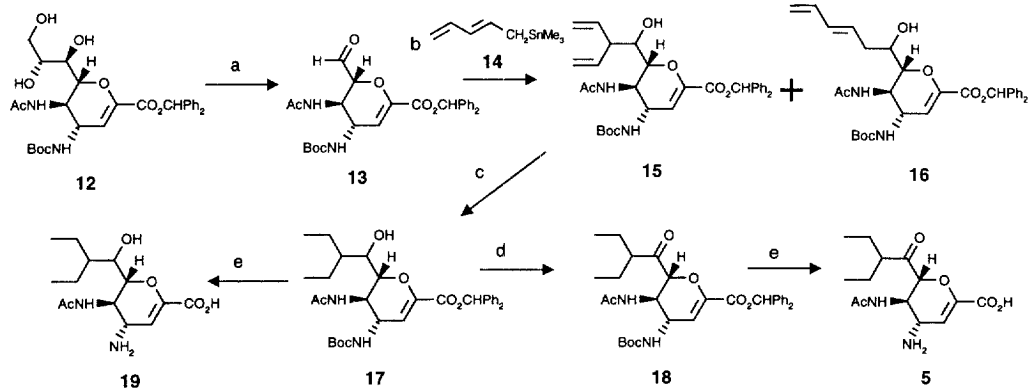
Reagents and conditions: a) CH_3COCl (79%) b) Et_4NCl, $NaHCO_3$, MeCN (73%) c) 3-Pentanol, $CHCl_3$ pTSA d) NaOMe, MeOH (64% over 2 stages) e) $(pMeOPh)_3CCl$ then Ac_2O (84%) f) AcOH aq (83%) g) SO_3-pyridine DMSO/CF_3CO_2H h) $NH_2SO_3H/NaClO_2$ (86% over 2 stages) i) MeOH, TBTU (91%) j) TMS-triflate (93%) k) TMS-azide, tBuOH (12% + 35% of epimer) l) $SnCl_2$, MeOH (100%) m) Et_3N aq (100%)

Compound **4** was synthesised from readily available N-acetylglucosamine. Initial chloroacetylation with acetyl chloride[8] and subsequent cyclisation with tetraethylammonium chloride formed the tri-O-acetyl oxazoline glycoside **6** which was opened by treatment with 3-pentanol in the presence of p-toluenesulfonic acid to form exclusively the β-glucoside **7**. The primary hydroxyl group was next liberated in three simple protecting group manipulation stages to afford alcohol **8** which was then oxidised over two stages (with accompanying elimination of the β-acetyl group) to afford the α,β-unsaturated acid **9**. Acid **9** was next converted into its methyl ester and treatment of this compound with TMS-triflate produced the oxazoline **10** which was opened with TMS-azide in t-BuOH[9] to produce the desired azide **11**, together with its epimer (1:3 ratio). The azide in compound **11** was reduced to the amine with stannous chloride and finally the methyl ester hydrolysed, by the previously published method, to afford the required target **4**.[10,11]

2. Synthesis of compound 5

Compound **5** and the related analogue **19** were prepared from the previously described triol **12** [4] which was initially cleaved to aldehyde **13** with sodium periodate. The key stage in the synthesis

was the homologation of aldehyde **13** with the pentadienyl stannane **14**[12,13]. Thus treatment of **13** with **14** in the presence of zinc chloride afforded a 1.25:1 mixture of regioisomeric diene adducts **15** and **16** (combined yield 70%, stereochemistry at the C-7 position was not determined but appeared to be a single isomer in both products by NMR). The terminal alkenes in the required regioisomer **15** were selectively reduced using Wilkinsons catalyst $(Ph_3P)_3RhH$ producing the saturated intermediate **17**. This was oxidised to the ketone **18** using tetrapropylammonium perruthenate in the presence of N-methylmorpholine-N-oxide.[14] Hydrolysis of compound **18** with trifluoroacetic acid afforded target **5**; similar treatment of alcohol **17** afforded the related analogue **19**.



Reagents and conditions: a) $NaIO_4$, MeOH (100%) b) Stannane **14** + $ZnCl_2$ (70%, 1.25:1 mixture of regioisomers) c) $(Ph_3P)_3RhCl$, H_2, EtOAc (95%) d) nPr_4NRuO_4, N-methylmorpholine-N-oxide, CH_2Cl_2 (89%) e) CF_3CO_2H/CH_2Cl_2 (83% for **5**, 90% for **19**).

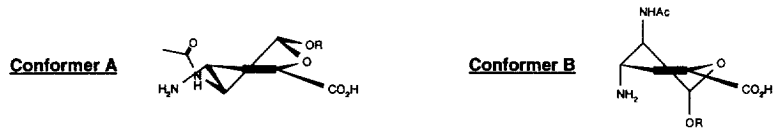
3. Sialidase Inhibitory Activities of **4**,**5** and **19** (Table)

Compound	Influenza A IC50 (μM)	Influenza B IC50 (μM)
Zanamivir **1**	0.005	0.004
GS4071 **2**	0.002	0.032
Carboxamide **3**	0.003	0.360
Ether **4**	0.770	42.000
ketone **5**	0.002	0.470
Alcohol **19**	0.039	7.400

Sialidase inhibitory activities of compounds were determined by previously reported methods[4,15]

The results demonstrate that despite possessing 6-substituents with greater conformational flexibility, dihydropyrans **4**, **5** and **19** all resemble the carboxamide **3** in that they are highly selective inhibitors of influenza A sialidase. The surprisingly low activity of the dihydropyran ether **4** can be explained from its NMR spectrum. The coupling constants between protons around the dihydropyran ring clearly indicate that, in solution, this compound adopts half chair conformation B,

in which the pendant substituents are pseudo-axial, rather than the normally preferred arrangement A (which is similar to the conformation which these compounds must adopt upon sialidase binding).[16] This preference is presumably due to anomeric stabilisation of conformer B for this compound[17] and implies that an energy penalty is incurred upon binding.



In conclusion, it is apparent from this study that a simple argument based on conformational rigidity cannot explain the difference in influenza sialidase selectivity observed between the dihydropyran carboxamides such as **3** and the cyclohexene GS4104 **2**.

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16. Compound **4**: $J_{3,4}$ = 4.5Hz; $J_{4,5}$ = 2Hz; $J_{5,6}$ = 2Hz. Compound **5**: $J_{3,4}$ = 2.5Hz; $J_{4,5}$ = 9Hz; $J_{5,6}$ = 10Hz
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