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Synthesis of (4*R**,5*R**)-4-acetylamino-5-diethylcarbamoyl-cyclohex-1-ene-1-carboxylic acid and (3*R**,4*R**)-4-acetylamino-3-diethylcarbamoylcyclohex-1-ene-1-carboxylic acid: new inhibitors of influenza virus sialidases

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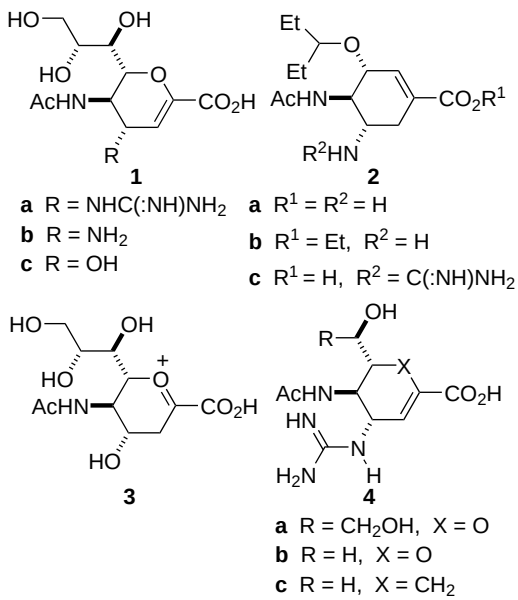
Abstract—(4*R**,5*R**)-4-Acetylamino-5-diethylcarbamoylcyclohex-1-ene-1-carboxylic acid and (3*R**,4*R**)-4-acetylamino-3-diethylcarbamoylcyclohex-1-ene-1-carboxylic acid have been synthesised using a Diels–Alder tactic; both compounds are selective inhibitors of influenza A sialidase, the latter compound being particularly potent. © 2001 Elsevier Science Ltd. All rights reserved.

Zanamivir **1a**[1] and GS 4071 **2a**[2] (administered in prodrug form as oseltamivir **2b**[3]) have recently emerged as effective anti-influenza agents. They interfere with viral replication by acting as potent inhibitors of influenza A and B sialidases. These enzymes, located on the viral surfaces, cleave the α-glycosidic bond of terminal sialic acid residues associated with glycoproteins and glycolipids present on host-cell surfaces.[4] The hydrolytic action is vital to the viral-life cycle, allowing access of the virus to the target epithelial cells by facilitating passage through the respiratory tract mucus, promoting the release of viral offspring from infected cells, and preventing self-aggregation of the offspring.[5,6]

Compounds **1a** and **2a**, which function as transition state analogues of the sialosyl cation **3**, bind to the active site of a sialidase in an analogous manner.[3,7] In the case of the $NHC(NH_2)$:NH_2^+/NH_3^+ moieties, common charge–charge type hydrogen bonding interactions are involved. Whereas the trihydroxypropyl side chain binds to a hydrophilic pocket via hydrogen bonding interactions, the pentyloxy function is accommodated in a hydrophobic pocket (arising from a reorganisation of the hydrophilic pocket).

Each of the four substituents of zanamivir **1a** are important for inhibitory action. For example, the contribution of the guanidino unit is demonstrated by the declining potency of compounds **1b** and **1c**.[1] Similarly, the influence of the trihydroxypropyl moiety is revealed by the diminishing effectiveness of compounds **4a** and **4b**.[8] Replacement of the trihydroxypropyl side chain by a dialkylcarbamoyl entity leads to compounds, e.g. **5a**,



Keywords: antivirals; cyclohexenes; dehydration; Diels–Alder reactions; nitro compounds; regioselection.

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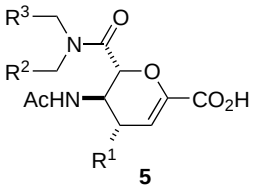

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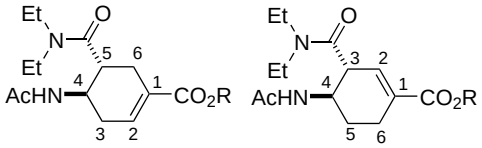
a R^1 = NHC(:NH)NH_2, R^2 = R^3 = Me

b R^1 = NHC(:NH)NH_2, R^2 = Bn, R^3 = Et

c R^1 = NH_2, R^2 = Bn, R^3 = Et

d R^1 = OH, R^2 = Bn, R^3 = Et

e R^1 = H, R^2 = Bn, R^3 = Et



6 **a** R = H **b** R = Et

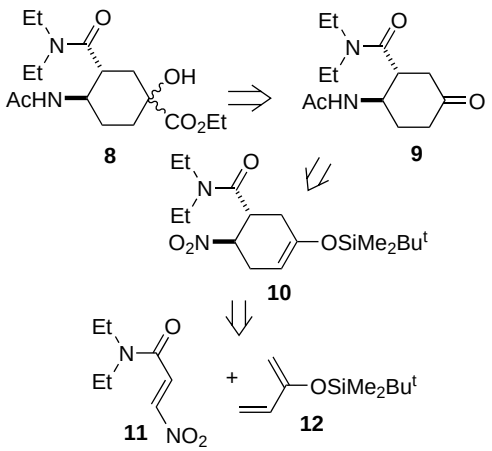
7 **a** R = H **b** R = Et

that retain potency against influenza A sialidase but lose effectiveness against influenza B sialidase.[9] Surprisingly, in this series, the guanidino function appears to play no role in binding to influenza A sialidase since compounds **5b–e** are of comparable activity.[10]

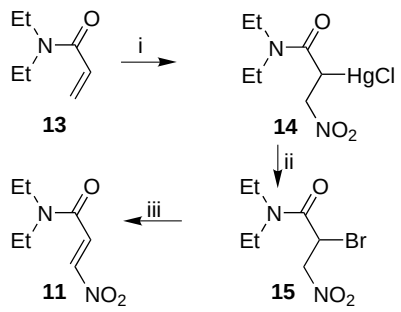
The location of the olefinic bond in GS 4071 **2a** seems to be critical for effectiveness (in accord with the postulated mimicry of **3**).[2] Replacement of the amino group by a guanidino entity, i.e. compound **2c**, provides little advantage.[2] Although relatives of GS 4071 **2a** with other alkoxy side chains are active, the pentyloxy moiety is considered to be the optimal substituent.[3]

Based on the above considerations, we decided to prepare and evaluate compounds **6a** and **7a**. It was envisaged that the targets would be accessible from a common precursor, i.e. **8**, by dehydration and ester-hydrolysis steps. The retrosynthesis of compound **8** is outlined in Scheme 1. The key steps would involve: the addition of an ethoxycarbonyl group equivalent to the ketone function of compound **9**; the reductive acetylation of the nitro function of compound **10**; and the regioselective Diels–Alder reaction of the nitro acrylamide **11** and the siloxydiene **12**.

The synthesis of the nitro acrylamide **11**, shown in Scheme 2, was effected using Corey's method.[11,†] Thus, nitromercuration of the acrylamide **13**[12] gave compound **14** which reacted with bromine to afford mainly the nitro bromide **15**; without purification, the last-cited compound was subjected to the action of sodium acetate in diethyl ether to furnish the nitro acrylamide **11**. The route provided multigram quantities of the dienophile **11** in a moderately efficient manner (~20% overall yield based on CH_2:CHCOCl). A literature procedure, involving enolsilylation of methyl vinyl ketone ($LiNPr^i_2$, Bu^tMe_2SiCl, THF–HMPA),[13] was used to prepare the diene **12**.

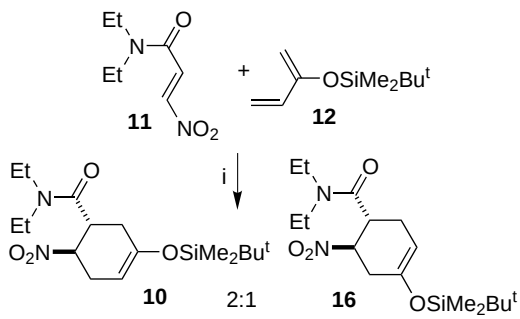


Scheme 1.



Scheme 2. *Reagents and conditions*: (i) $HgCl_2$ (100 mol%), $NaNO_2$ (200 mol%), H_2O, 20 h; (ii) Br_2 (180 mol%), H_2O–Et_2O (1:2), 24 h; (iii) NaOAc (320 mol%), Et_2O, 24 h.

As indicated in Scheme 3, the Diels–Alder reaction of the nitro acrylamide **11** and the diene **12** (used in 2.5 fold excess) proceeded in boiling toluene to give a 2:1 mixture of the cycloadducts **10** and **16**, separable by column chromatography. The fact that the major cycloadduct, isolated as a syrup in 40% yield, possessed the regiostructure **10** was established by a COSY 90° experiment.[‡] The modest regioselectivity displayed in the cycloaddition reaction was surprising in view of Danishefsky's finding that a single cycloadduct (that possessing a 1,4-relationship of the nitro and siloxy



Scheme 3. *Reagents and conditions*: (i) PhMe, reflux.

† Originally, this procedure was used to convert alkenes into nitro alkenes. We have shown that it can be adapted for the transformation of acrylic acid esters into (*E*)-nitro acrylates: Adams, D. R.; Stoodley, R. J., unpublished work.

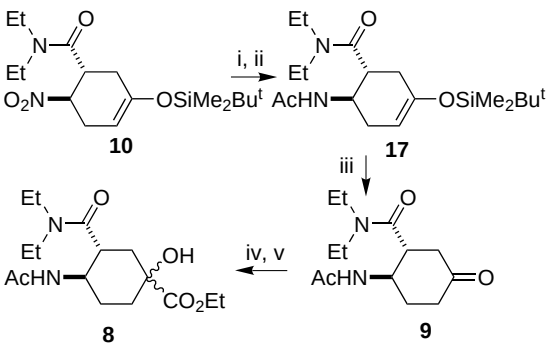
groups) arose from the reaction of methyl (*E*)-3-nitroacrylate and 2-trimethylsiloxybuta-1,3-diene.[14]

Scheme 4 depicts the route employed for the synthesis of the hydroxy ester **8**. Thus, reduction of the nitro group of compound **10**, using Corey's method,[15] followed by acetylation of the product provided the acetamido compound **17** which underwent hydrolysis to afford the ketone **9** (32% overall yield after chromatography). Installation of the ethoxycarbonyl group was achieved by a two-step sequence, involving a Baldwin ethoxyvinylation[16] followed by an immediate ozonolytic cleavage of the vinyl group;[17] after chromatography, the hydroxy ester **8** was isolated as a 3:1 mixture of diastereomers in 53% yield.

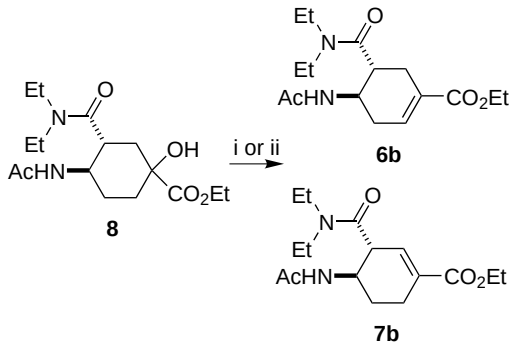
The two methods shown in Scheme 5 were successful in effecting the dehydration of the hydroxy ester **8**. Surprisingly, the use of phosphorus oxychloride[18] gave only the conjugated ester **6b** (61% yield after chromatography). However, Martin's sulfurane[19,20] provided a 1:1.3 mixture of compounds **6b** and **7b** (68% yield after chromatography), separable by HPLC.

Hydrolysis of the ester **6b** [LiOH, MeOH–H_2O (9:1), 2 h] was uneventful, leading to the desired acid **6a**[§] in 70% yield. However, under corresponding conditions, the ester **7b** afforded a 4:2:2:1 mixture of products; the most prevalent component [isolated in a near-pure state as a syrup (24% yield) by addition of EtOAc to the mixture and decantation of the liquid] was identified as the required acid **7a**.[¶,‖]

The inhibitory activities of the acids **6a** and **7a**, compared with zanamivir **1a**, against influenza A and B sialidases are shown in Table 1.** Both compounds are highly selective for influenza A sialidase (by a factor of



Scheme 4. *Reagents and conditions*: (i) Al(Hg), MeOH–H_2O (99:1), 1.5 h; (ii) Ac_2O, pyridine, 15 h; (iii) HOAc–H_2O (1:1), 36 h; (iv) CH_2:CH(OEt), Bu^{t}Li, THF, −78°C, 30 min; (v) O_3, MeOH, −78°C, 10 min.



Scheme 5. *Reagents and conditions*: (i) $POCl_3$, pyridine, 0°C, 20 h [**6b**:**7b** (1:0)]; (ii) $Ph_2S[OC(CF_3)_2Ph]_2$, $CDCl_3$, −78 to 20°C, 40 min [**6b**:**7b** (1:1.3)].

Table 1. Sialidase inhibitory activities (IC_{50}, μM)

Compound	Influenza A	Influenza B
6a	0.210	150
7a	0.017	23
1a	0.005	0.004

‡ The double triplet (*J* 6 and 11 Hz) at δ 5.04, attributed to the nitromethine proton, showed cross-peaks connected with the multiplets at δ 2.42–2.58 and 2.77–2.89, ascribed to the adjacent methylene protons; in turn, each multiplet displayed a cross-peak with the multiplet at δ 4.77–4.81, assigned to the olefinic proton.

§ Data for the acid **6a**: mp 184–186°C; ν_{max} (KBr)/cm^{-1} *inter alia*: 1710 (carboxy CO), 1665 and 1620br (amide CO and C=C); δ_H (400 MHz; CD_3OD): 1.14 and 1.26 [each 3H, t (*J* 7 Hz) and t (*J* 7.5 Hz), 2×*Me*CH_2], 1.92 (3H, s, MeCO), 2.41–2.62 (4H, m, 3- and 6-H_2), 3.20–3.29 (2H, m, 5-H and MeC*H*H), 3.33–3.42, 3.55–3.64 and 3.70–3.79 (each 1H, m, MeC*H*H and MeCH_2), 4.09–4.16 (1H, m, 4-H) and 6.96–6.98 (1H, m, 2-H); δ_c (100 MHz; CD_3OD): 13.7 and 15.4 (2×*C*H_3CH$_2$), 23.2 (*C*H_3CO), 29.4 and 32.0 (3- and 6-CH_2), 41.7 (5-CH), 42.3 and 43.9 (2×CH_3*C*H_2), 48.7 (4-CH), 130.5 (1-C), 138.5 (2-CH) and 170.1, 173.2 and 175.0 (3×CO): *m*/*z* (FAB): 305 [M(Na)$^+$, 35%], 283 (MH$^+$, 70) and 74 (100). Found: *m*/*z* 283.1656. $C_{14}H_{22}N_2O_4$ (MH$^+$) requires 283.1658.

¶ Data for the acid **7a**: ν_{max} (KBr)/cm^{-1} *inter alia*: 1720 (carboxy CO), 1630 (amide CO and C=C); δ_H (400 MHz; CD_3OD): 1.15 and 1.29 (each 3H, t, *J* 7 Hz, 2×*Me*CH_2), 1.76–1.85 and 2.01–2.09 (each 1H, m, 5-H_2), 1.96 (3H, s, MeCO), 2.38–2.56 (2H, m, 6-H_2), 3.28–3.40 (MeC*H*H), 3.44–3.54 (2H, m, MeCH*H* and MeC*H*H), 3.64–3.76 (2H, m, MeCH*H* and 3-H), 4.25–4.30 (1H, ddd, *J* 3.5, 7 and 11, 4-H) and 6.70–6.72 (1H, m, 2-H); δ_c (100 MHz; CD_3OD): 13.5 and 15.4 (2×*C*H_3CH$_2$), 23.1 (*C*H_3CO), 24.2 and 28.0 (5- and 6-CH_2), 42.0 (3-CH), 42.5 and 44.1 (2×CH_3*C*H_2), 46.4 (4-CH), 133.8 (1-C), 136.5 (2-CH) and 170.5, 173.2 and 173.3 (3×CO): *m*/*z* (FAB): 283 (MH$^+$, 50%), 107 (90) and 77 (100). Found: *m*/*z* 283.1663. $C_{14}H_{22}N_2O_4$ (MH$^+$) requires 283.1658.

‖ The other three components were tentatively considered to be the 3-epimer of the acid **7a** and the 1-epimers of the double bond isomer of the acid **7a.**

** Inhibition of influenza sialidase was determined, using a fluorimetric assay, by measuring the ability of compounds to inhibit the hydrolysis of 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid by whole virus (A/Aichi N2 or B Victoria) grown in hen eggs. The IC_{50} value quoted is the concentration of inhibitor required to reduce the enzymic activity by 50%.

over 700 for **6a** and over 1300 for **7a**). Against influenza A sialidase, the acid **7a** is ~3 times less active than zanamivir **1a** whereas the acid **6a** is ~40 times less active. The findings indicate that, in this series, the presence of a basic function is not a requirement for high potency. They also reveal that the position of the double bond contributes to, but is not critical for high activity against influenza A sialidase. In this respect, it is worth noting that the acid **4c** displays activity comparable to the acid **4b**[21] (although the latter compound is ~1800 times less active than **1a**).

The aforecited results are of interest in a number of respects. Thus, the synthesis of the nitro acrylamide **11** shows that the Corey protocol can be extended to electron-deficient alkenes. The **10**→**17** transform illustrates the compatibility of the *tert*-butyldimethylsilyl enol ether function with the nitro group reduction conditions involving aluminium amalgam. The differing regioselectivities displayed in the dehydration reactions of compound **8** by phosphorus oxychloride and Martin's sulfurane are notable. Finally, the sialidase inhibitory properties of the acids **6a** and **7a** provide new insights into structure–activity relationships of anti-influenza agents.

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