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Synthesis of (4*R**,5*S**)-5-acetylamino-4-diethylcarbamoyl-5,6-dihydro-4*H*-pyran-2-carboxylic acid and its inhibitory action against influenza virus sialidases

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Abstract—(4*R**,5*S**)-5-Acetylamino-4-diethylcarbamoyl-5,6-dihydro-4*H*-pyran-2-carboxylic acid has been synthesised from methyl (*E*)-4-diethylcarbamoyl-2-oxobut-3-enoate and 2-nitroethanol; the acid is a moderately active inhibitor of influenza A sialidase.

Sialidases catalyse the hydrolysis of the glycosidic bonds of terminal α-linked sialic acid residues that are associated with glycoproteins and glycolipids. Such reactions play a crucial role in the life cycle of the influenza virus, being implicated in passage of the virus through the respiratory tract mucus to the target epithelial cells, release of the viral offspring from infected cells, and prevention of self-aggregation of the offspring. Accordingly, inhibitors of the enzymes are of interest as potential anti-influenza agents.[1]

Several compounds, which are believed to function as transition state analogues of the sialosyl cation **1**, have been discovered. The leading example that features the 5,6-dihydro-4*H*-pyran ring is GG 167 **2a** (now referred to as zanamavir and administered intranasally); it displays high potency against both influenza A and influenza B sialidases (IC_{50} ~0.005 μM).[2] In the case of compounds of type **2**, the guanidino unit is considered to be the optimal 4-substituent; thus, compound **2a** is more active than the amino relative **2b** (IC_{50} ~0.35 μM) and much more active than the hydroxy relative **2c** (IC_{50} ~12 μM).[3] Replacement of the trihydroxypropyl side-chain by a dialkylcarbamoyl entity leads to compounds, e.g. **3a**, that retain potency against influenza A sialidase (IC_{50} ~0.025 μM) but lose effectiveness against influenza B sialidase (IC_{50} ~110 μM).[4] Surprisingly, in this series, the basic substituent at position 4 appears to play no role in binding to influenza A sialidase, since compounds **3b–d** show only marginal differences in activity.[5]

The outstanding example of an inhibitor that features the cyclohexene ring is GS 4071 **4a** (administered orally in prodrug form as oseltamivir **4b**); it is very active against A and B sialidases (IC_{50} ~0.002 μM).[6] With compounds of type **4**, the guanidino function appears to offer little advantage since compound **4c** shows only a two-fold improvement in activity over compound **4a**.[7] Interestingly, compound **4d** (as a racemate) retains significant activity against A sialidase (IC_{50} ~0.070 μM) and moderate activity against B sialidase (IC_{50} ~0.83 μM).[8]

Recently, we have prepared and evaluated the cyclohexene carboxylic acids **5** and **6** (as racemates).[9] Both compounds display high selectivity for A sialidase. However, whereas the cyclohexene carboxylic acid **5** is less active (IC_{50} ~0.21 μM) than its heterocyclic relative **3d** (IC_{50} ~0.007 μM), the acid **6** is of comparable potency (IC_{50} ~0.017 μM). On the basis of the aforecited findings, it was of interest to prepare and evaluate the dihydropyran carboxylic acid **7a**. We now report our results.

The retrosynthesis of compound **7a** is outlined in Scheme 1. The key steps would involve the reductive acetylation of the nitro group and the hydrolysis of the ester function of the precursor **8**; the dehydration of compound **9**; and the regio- and stereoselective Michael

Keywords: antivirals; cyclisation; enones; Michael reactions; nitro compounds; pyrans.

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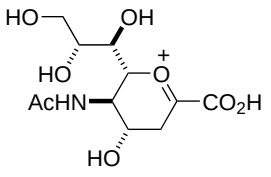
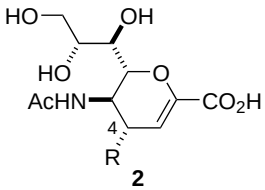
addition of the enone **10** and 2-nitroethanol **11** with concomitant cyclic hemiacetal formation.

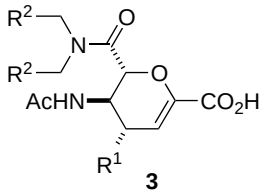


1



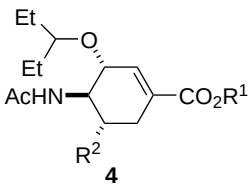
2

a R = NHC(:NH)NH$_2$
b R = NH$_2$
c R = OH



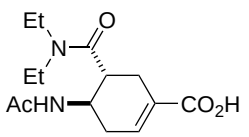
3

a R^1 = NHC(:NH)NH$_2$, R^2 = H
b R^1 = NH$_2$, R^2 = Bn
c R^1 = OH, R^2 = Bn
d R^1 = H, R^2 = Bn



4

a R^1 = H, R^2 = NH$_2$
b R^1 = Et, R^2 = NH$_2$
c R^1 = H, R^2 = NHC(:NH)NH$_2$
d R^1 = R^2 = H



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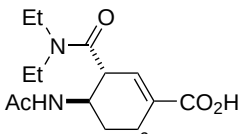

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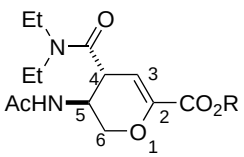
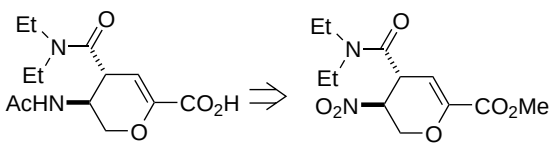
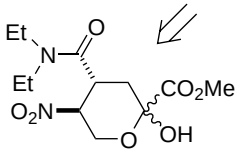


7

a R = H
b R = Me



7a **8**



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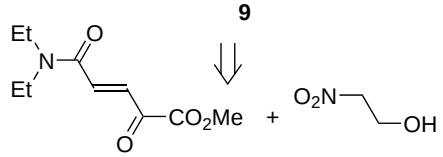


10 **11**

Scheme 1.

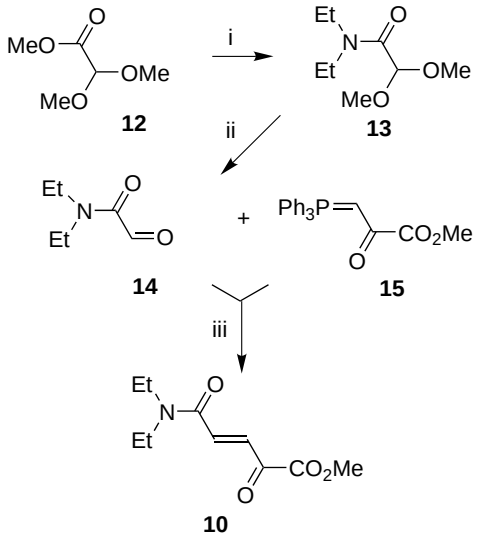
The synthesis of the enone **10** is shown in Scheme 2. Thus, using Weinreb's procedure,[10] the commercially available ester **12** was converted into the amide **13** (71% yield), which afforded the aldehyde **14**† (~84% yield) under acidic conditions. A Wittig condensation of the last-cited compound with the phosphorane **15**[11] provided the enone **10** (64% yield after chromatography), mp 37°C.

In the presence of methanolic sodium methoxide, the enone **10** and 2-nitroethanol **11** reacted to give a mixture of products from which the adduct **16** was isolated in 8% yield. After screening a range of basic conditions, those of Ballini (but using MeCN as solvent)[12] were found to be the most effective, providing the tetrahydropyranol **16**‡ in 56% yield after chromatography (Scheme 3). The stereochemistry of compound **16** was deduced from conformational considerations, which pointed to the adoption of the chair conformer **17**. Thus, a diequatorial disposition of the diethylcarbamoyl and nitro groups was supported by the large coupling constant (J 11 Hz) between the 4- and 5-protons; an axial orientation of the hydroxy function was anticipated on the basis of the anomeric effect.[13,14] As Fig. 1 reveals, X-ray crystallography§ fully corroborated the stereochemical and conformational assignments.

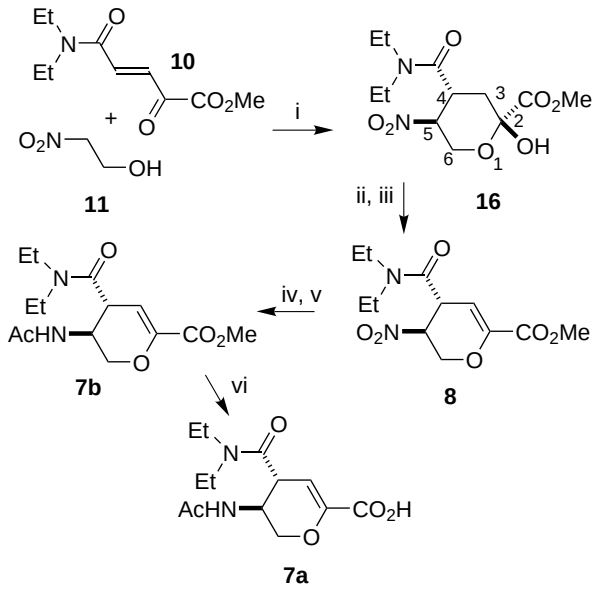
† Compound **14** was obtained partially as a hydrate (~60%) by ^{1}H NMR spectroscopy.

‡ A mixture of the enone **10** (1.57 g, 7 mmol), 2-nitroethanol (0.900 g, 9 mmol) and Amberlyst A-21 ion-exchange resin (4.5 g) in acetonitrile (150 cm^3) was stirred for 18 h. The mixture was then filtered and the filtered material washed with methanol (300 cm^3). Evaporation of the combined filtrate and washings and subjection of the resultant oil to silica gel column chromatography [EtOAc–hexanes (1:1) as eluent] gave methyl (2*R**,4*R**,5*S**)-4-diethylcarbamoyl-2-hydroxy-5-nitrotetrahydropyran-2-carboxylate **16** (1.20 g, 56%); mp 76°C (from Me$_2$CHOH); ν_{max} (KBr)/cm^{-1} inter alia 3250br (OH), 1750 (ester CO), 1625 (amide CO), 1550 and 1360 (NO$_2$); δ_H (400 MHz; CDCl$_3$) 1.10 and 1.30 (each 3H, t, J 7 Hz, 2×*M*eCH$_2$), 1.99 and 2.29 [each 1H, dd (J 4 and 14 Hz) and dt (J 1 and 14 Hz), 3-H$_2$], 3.26–3.35 and 3.38–3.49 (1 and 3H, each m, 2×C*H*$_2$Me), 3.77 (1H, ddd, J 4, 11 and 14 Hz, 4-H), 3.85 (3H, s, MeO), 4.07 (1H, br s, OH), 4.18 and 4.32 [each 1H, t (J 11 Hz) and dd (J 6 and 11 Hz), 6-H$_2$] and 5.22 (1H, dt, J 6 and 11 Hz, 5-H); δ_C (100 MHz; CDCl$_3$) 13.3 and 14.9 (2×CH$_3$), 34.0 (3-CH$_2$), 36.7 (4-CH), 41.3 and 42.5 (2×CH$_2$N), 54.1 (CH$_3$O), 61.6 (6-CH$_2$), 80.8 (5-CH), 93.4 (2-C), (2-C), 170.0 and 170.1 (ester and amide CO); m/z (FAB) 305 (MH$^+$, 50%), 287 [(M–OH)$^+$, 60], 100 (70), 81 (70), 72 (95), 55 (100) and 43 (100); found: C, 47.6; H, 6.7; N, 9.5. $C_{12}H_{20}N_2O_7$ requires C, 47.4; H, 6.6; N, 9.2%.

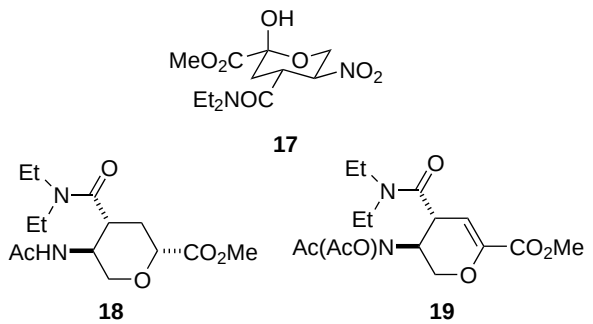
§ Crystal data for compound **16**: $C_{12}H_{20}N_2O_7$, M=304.3, monoclinic, space group $P2_1/c$, a=6.8968(10), b=15.668(2), c=13.542(2) Å, β=102.43(2), V=1429.0(3) Å^3, Z=4, D_{calcd}=1.414 g cm^{-3}, μ=0.117 mm^{-1}, Mo Kα (λ=0.71073 Å) radiation, $F(000)$=648, T=203(2) K. Nonius MACH3 diffractometer, crystal size 0.30×0.15×0.15 mm, θ_{max} 25.0°, 2512 reflections measured, 2290 independent. Structure solution by direct methods, full-matrix least-squares refinement on F^2 using SHELX97-2 with all non-hydrogen atoms anisotropic and hydrogen atoms isotropic. The final cycle converged to R=0.0747 [for reflections $I>2\sigma(I)$] and wR^2=0.1989 (for all reflections). Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 169424.



Scheme 2. *Reagents and conditions*: (i) $AlMe_3$, Et_2NH, PhMe, Δ, 3 h; (ii) 2 M HCl–1,4-dioxane (1:1), Δ, 0.5 h; (iii) PhMe, Δ, 24 h.



Scheme 3. *Reagents and conditions*: (i) Amberlyst A-21, MeCN, 18 h; (ii) Ac_2O (160 mol%), pyridine, 16 h; (iii) Me_3SiOTf (200 mol%), MeCN, argon, ~2°C, 6 h, then pyridine; (iv) Al(Hg), MeOH–H_2O (99:1), 1 h; (v) Ac_2O (200 mol%), pyridine, 16 h; (vi) LiOH, MeOH–H_2O (9:1), 1 h.



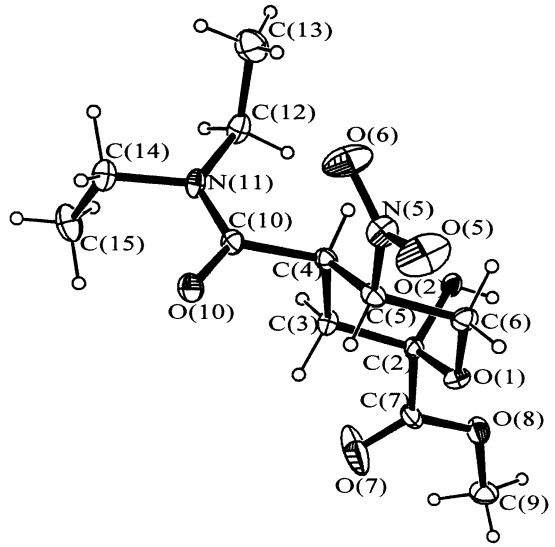


Figure 1. X-Ray crystal structure of compound **16**.

The conversion of the tetrahydropyranol **16** into the dihydropyran **8** proved troublesome. Complex mixtures resulted using the dehydration conditions reported by Rapoport ($POCl_3$, pyridine, 0°C)[15] and Martin {$Ph_2S[OC(CF_3)_2Ph]_2$, $CDCl_3$, −78°C}[16] (employed earlier by ourselves[9] in the synthesis of compounds **5** and **6**). The conditions of Tamoo et al. ($SOCl_2$, HMPA, PhH, 100°C)[17] did provide the desired dehydration product **8** but in only 20% yield. The two-step acetylation–elimination sequence of Claesson and Luthman[18] offered a better solution, affording compound **8** in 47% overall yield (Scheme 3). Subjection of compound **8** to the nitro group reduction conditions of Kende (SmI_2, THF–MeOH)[19] followed by acetic anhydride and pyridine resulted in the isolation of the tetrahydropyran **18** (70% yield). When compound **8** was treated with aluminium amalgam in moist methanol[20] and the product acetylated, the desired acetamide **7b** was isolated (37% yield after chromatography) (Scheme 3); compound **19** (54% yield) was also obtained. Saponification of the ester **7b** provided the target acid **7a**¶ (89% yield).

The inhibitory activity of the acid **7a** against A and B sialidases, compared with its relative **6**, is shown in Table 1.‖ Although displaying high selectivity for A

¶ Data for the acid **7a**: ν_{max} (film)/cm^{-1} inter alia 1725 (carboxy CO) and 1640 (amide CO and C=C); δ_H (400 MHz; CD_3OD) 1.16 and 1.31 (each 3H, t, *J* 7 Hz, 2×*M*eCH_2), 1.99 (3H, s, MeCO), 3.37–3.49, 3.50–3.56 and 3.64–3.71 (2, 1 and 1H, each m, 2×CH_2Me), 3.59–3.62 (1H, m, 4-H), 4.16 and 4.36 [each 1H, dd (*J* 3 and 11 Hz) and dd (*J* 2 and 11 Hz), 6-H_2], 4.21–4.25 (1H, m, 5-H) and 5.98 (1H, dd, *J* 1 and 4 Hz, 3-H); δ_C (100 MHz; CD_3OD) 13.5 and 15.3 (2×CH_3), 22.8 (*C*H_3CO), 41.3 (4-CH), 42.4 and 43.9 (2×CH_2N), 46.3 (5-CH), 68.0 (6-CH_2), 108.0 (3-CH), 146.7 (2-C), 165.8 (carboxy CO), 172.4 and 173.7 (amide CO); *m*/*z* (FAB) 285 (MH^+, 5%), 69 (60), 55 (90) and 43 (100) [(after addition of KI) 323 (MK^+, 10%)]; found: *m*/*z* 285.1443. $C_{13}H_{20}N_2O_5$ (MH^+) requires 285.1450.

‖ Inhibition of influenza sialidase was determined, using a fluorimetric assay, by measuring the ability of the compound 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%.

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

Compound	Influenza A	Influenza B
7a	1.5	>100
6[a]	0.017	23

[a] Quoted from Ref. 9.

sialidase, the acid **7a** is ~90 times less active than compound **6**. Clearly, the replacement of the 6-methylene group of compound **6** by an oxygen atom leads to a significant loss in potency.

The aforecited results are of interest in a number of respects. Thus, the reaction leading to the tetrahydropyranol **16**, which exemplifies a simple but little-used route to such heterocycles,[21–25] is notable for its regio- and stereoselectivity. The olefinic reduction associated with the **8→18** transformation is unusual; in spite of the wide application of samarium iodide in organic synthesis,[26,27] we are aware of only one reference which documents such a reaction.[28] Finally, the sialidase inhibitory properties of the acid **7a** provide new insights into structure–activity relationships of anti-influenza agents.

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