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Synthesis of (3*R**,4*R**)-4-acetylamino-3-(pent-3-oxy)cyclohex-1-ene-1-carboxylic acid and its (3*S**,4*R**)-isomer and their inhibitory action against influenza virus sialidases

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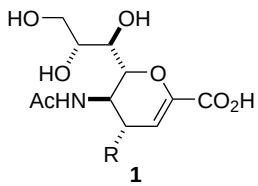
Abstract—(3*R**,4*R**)-4-Acetylamino-3-(pent-3-oxy)cyclohex-1-ene-1-carboxylic acid and its (3*S**,4*R**)-isomer are synthesised in five steps from the Diels–Alder cycloadduct of (*E*)-1-acetoxy-3-methylbuta-1,3-diene and nitroethene; the former acid is a moderately potent inhibitor of influenza A sialidase. © 2001 Elsevier Science Ltd. All rights reserved.

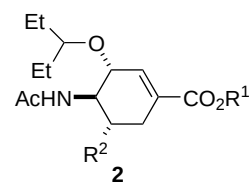
Zanamavir **1a**[1] and GS 4071 **2a**[2] (administered in prodrug form as oseltamivir **2b**[3]) have recently emerged as clinically effective anti-influenza agents. They interfere with viral replication by acting as potent inhibitors of influenza A and B sialidases. The enzymes, located on the viral surfaces, hydrolytically cleave the α-glycosidic bond of terminal sialic acid residues associated with glycoproteins and glycolipids present on host cell surfaces.[4] This hydrolytic action facilitates passage of the virus through the respiratory tract mucus to the target epithelial cells, promotes the release of viral offspring from infected cells and prevents 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 zanamivir **1a**, each of the four substituents is 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 sidechain by a dialkylcarbamoyl entity leads to compounds, e.g. **5a**, that retain potency against influenza A sialidase but lose effectiveness against influenza B sialidase.[9]



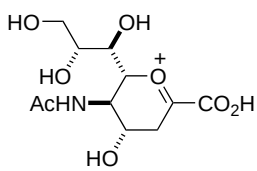
1

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



2

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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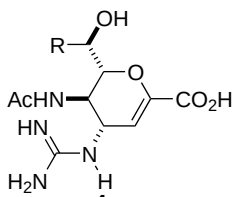
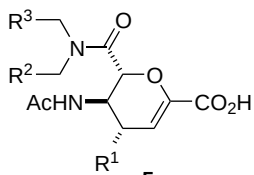


4

a R = CH$_2$OH
b R = H



5

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

Keywords: antivirals; Diels–Alder reactions; oxidation (allylic); nitro compounds.

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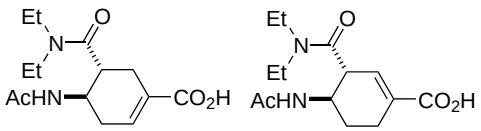
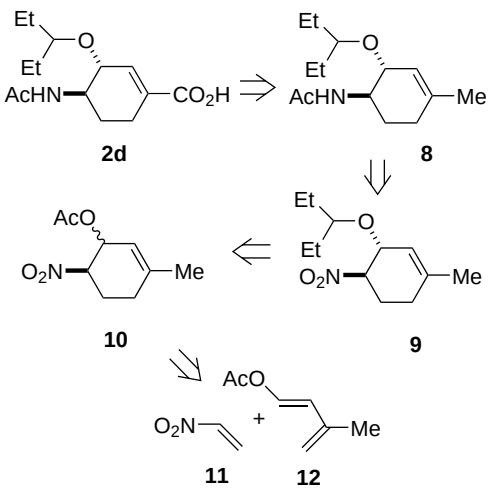
Surprisingly, in this series, the guanidino function appears to play no role in binding to influenza A sialidase since compounds **5b–e** are of comparable activity.[10] The location of the olefinic bond in GS 4071 **2a** seems to be critical for effectiveness (in accord with the postulated mimicry of **3**).[2] Replacement of the amino group by a guanidino unit, i.e. compound **2c**, provides little advantage.[2] Although relatives of GS 4071 **2a** with other alkoxy side chains are active, the pent-3-oxy moiety is considered to be the optimal substituent.[3]



6 **7**

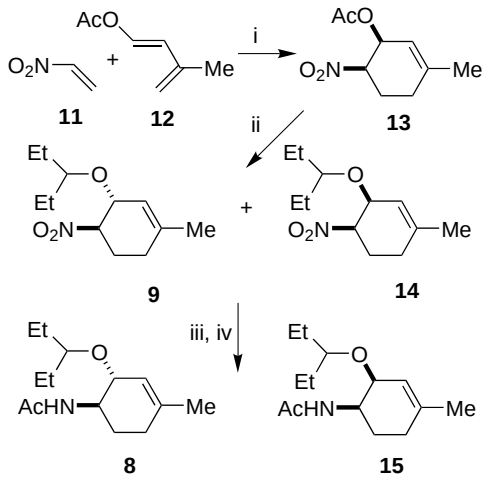
Recently, we prepared and evaluated the acids **6** and **7** in racemic form.[11] Both compounds were found to be highly selective for influenza A sialidase (by a factor of over 700 for **6** and over 1300 for **7**). Against influenza A sialidase, the acid **6** was ~40 times less active than zanamavir **1a**, whereas the acid **7** was ~three times less effective. Clearly, in this series (as in the case of compounds of type **5**) a basic function is not required for high potency. Moreover, the position of the olefinic bond contributes to but is not critical for high activity.

Based upon the above considerations, we decided to prepare and evaluate compound **2d** (in racemic form)—the deamino analogue of GS 4071 **2a**. The retrosynthesis of compound **2d** is outlined in Scheme 1. The key steps would involve: the oxidation of the allylic methyl group of compound **8**; the reductive acetylation of the nitro group of compound **9**; the replacement of the acetoxy group of compound **10** by a pent-3-oxy function with *anti*-stereoselectivity; and the regioselective Diels–Alder reaction of nitroethene **11** and the diene **12**.



Scheme 1.

The synthesis of compound **8** is shown in Scheme 2. Thus, nitroethene **11** (prepared in 30% yield after distillation by the phthalic anhydride induced dehydration of nitroethanol)[12] reacted with the diene **12** (prepared in 72% yield after distillation from the reaction of 3-methylbut-2-enal with Ac_2O and NaOAc under reflux)[13] to give a single cycloadduct (56% yield after chromatography) assigned as structure **13**. The high *endo*-stereoselectivity observed in the Diels–Alder reaction is of note; the result is consistent with the finding that the formation of *endo*-nitro cycloadducts in reactions involving 1-oxybuta-1,3-dienes is promoted by *O*-acetyl substitution (compared with *O*-methyl/silyl substitution).[14] When heated in pentan-3-ol with boron trifluoride diethyl etherate, the cycloadduct **13** was transformed into a 63:37 mixture of compounds **9** and **14** (60% yield after chromatography). Nitro group reduction using Kende's method[15] and subsequent acetylation gave, after chromatography, the minor pentyl ether **15**, mp 114–116°C, in 19% yield and the major pentyl ether **8** in 37% yield. The *syn*-relationship of the acetylamino and pent-3-oxy substituents in compound **15** was established by an X-ray crystallographic investigation (Fig. 1).† The analysis also revealed that the cyclohexene ring adopted a half-chair conformation with a pseudoaxial arrangement of the pentoxy function and an equatorial disposition of the acetylamino group.



Scheme 2. *Reagents and conditions*: (i) PhMe, 20°C, 18 h; (ii) Et_2CHOH, $BF_3{\cdot}OEt_2$, 85°C, 1 h; (iii) SmI_2, THF–MeOH, 1 h; (iv) Ac_2O, pyridine, 2 h.

† Crystal data for compound **15**: $C_{14}H_{25}NO_2$, $M=239.4$, triclinic, space group $P\bar{1}$, $a=4.8989(5)$, $b=10.6663(12)$, $c=13.9566(16)$ Å, $\alpha=97.863(10)$, $\beta=94.198(10)$, $\gamma=92.513(10)°$, $V=719.41(14)$ Å^3, $Z=2$, $D_{calcd}=1.105$ g cm^{-3}, $\mu=0.073$ mm^{-1}, Mo Kα ($\lambda=0.71073$ Å) radiation, $F(000)=264$, $T=203(2)$ K. Nonius MACH3 diffractometer, crystal size 0.35×0.35×0.25 mm, θ_{max} 24.97°, 5050 reflections measured, 2525 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 constrained in calculated positions. The final cycle converged to $R=0.0360$ [for reflections $I>2\sigma(I)$] and $wR^2=0.1021$ (for all reflections). Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 167304.

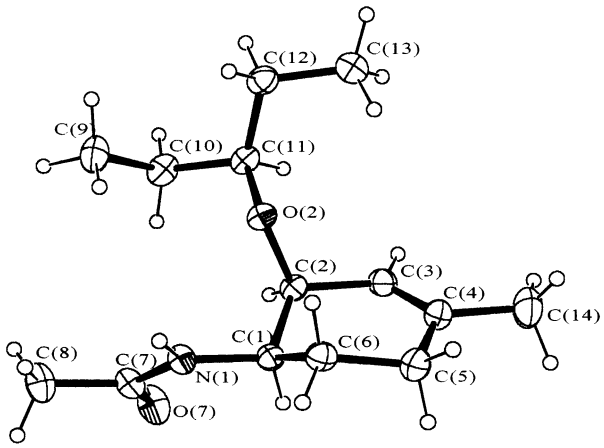
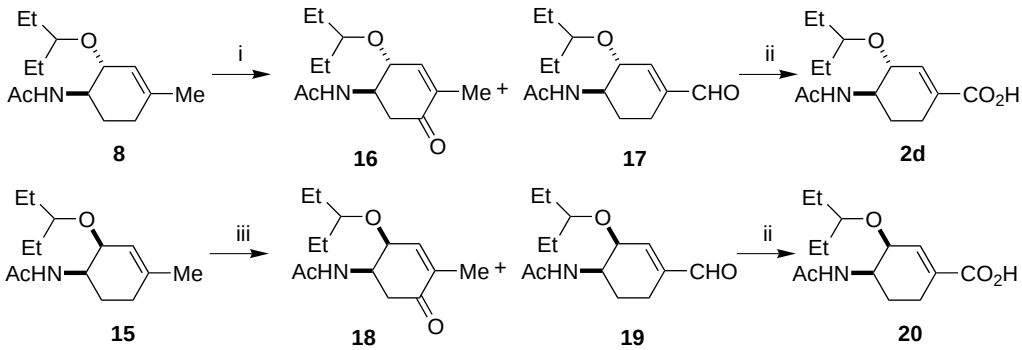


Figure 1. X-Ray crystal structure of compound **15**.

With compound **8** in hand, efforts were directed at effecting an oxidation of its allylic methyl group. It is well established that selenium dioxide is capable of effecting such oxidations.[16] However, in the case of 1-methylcyclohexenes lacking 6-substituents, the allylic methylene group is usually oxidised preferentially (with selectivities of >3:1).[17] It was a surprise, therefore, to find that the oxidant converted compound **8** into a 1:1 mixture of products (75% yield after chromatography) identified as the enone **16** and the enal **17**; after HPLC fractionation, the enal **17** was isolated in 33% yield and the enone **16** in 38% yield. Lindgren oxidation[18] of the enal **17** provided the target acid **2d**‡ in 72% yield. Oxidation of compound **15** with selenium dioxide provided a 1:2 mixture of the enone **18** and the enal **19**; after chromatography, the enal **19** was isolated in 16% yield and the enone **18** in 9% yield. Finally, Lindgren oxidation[18] of the enal **19** afforded the acid **20**§ in 71% yield. The aforecited reactions are illustrated in Scheme 3.

The inhibitory activities of the acids **2d** and **20**, in comparison with GS 4071, against influenza A and B sialidases are shown in Table 1.¶ Only compound **2d** showed any appreciable activity. Compared with GS 4071, it was ~70 times less active against influenza A sialidase and ~280 times less active against influenza B sialidase. The findings indicate that the amino group of GS 4071 contributes to but is not obligatory for high potency although, seemingly, it plays a more important role in binding to B sialidase than to A sialidase. The poor activity of the acid **20** demonstrates the inadequacy of the *syn*-arrangement of the pentoxy and acetylamino groups.

The aforecited results are of interest in a number of respects. Thus, the excellent *endo*-stereoselectivity realised in the Diels–Alder reaction is deserving of comment; presumably, it is attributable to the presence of the *O*-acetyl substituent in the oxybuta-1,3-diene. The regiochemical outcomes of the reactions of compounds **8** and **15** with selenium dioxide are of note because they are contrary to expectations based on literature examples. Finally, the sialidase inhibitory properties of the acids **2a** and **20** provide new insights into structure–activity relationships of anti-influenza agents.



Scheme 3. *Reagents and conditions*: (i) SeO_2, 1,4-dioxane, reflux, 16 h; (ii) $NaClO_2$, NH_2SO_3H, 1,4-dioxane–H_2O, 1 h; (iii) SeO_2, EtOH, reflux, 15 h.

‡ Data for the acid **2d**: ν_{max} (film)/cm^{-1} inter alia: 1700 (carboxy CO), 1650 (amide CO) and 1625 (C=C); δ_H (400 MHz; $CDCl_3$) 0.90 and 0.93 (each 3H, t, *J* 7.5 Hz, 2×*M*eCH$_2$), 1.45–1.57 (4H, m, 2×C*H*$_2$Me), 1.75–1.83 and 2.03–2.10 (each 1H, m, 5-H$_2$), 1.99 (3H, s, MeCO), 2.20–2.29 and 2.42–2.51 (each 1H, m, 6-H$_2$), 3.45 (1H, quintet, *J* 6 Hz, OC*H*Et$_2$), 3.90–3.93 (1H, m, 3-H), 4.03–4.10 (1H, m, 4-H), 5.56 (1H, d, *J* 8 Hz, NH) and 6.93–6.96 (1H, m, 2-H); δ_C (100 MHz; $CDCl_3$) 9.7 and 10.2 (2×*C*H$_3$CH$_2$), 21.5 and 23.6 (5- and 6-CH$_2$), 23.9 (*C*H$_3$CO), 26.7 and 26.8 (2×*C*H$_2$CH$_3$), 48.4 (4-CH), 73.0 (3-CH), 82.0 (O*C*HEt$_2$), 132.2 (1-C), 138.4 (2-CH) and 170.7 and 171.2 (amide and carboxy CO); *m*/*z* (FAB) 292 [M(Na)$^+$, 10%], 270 (MH$^+$, 30), 182 (55) and 43 (100); found: *m*/*z* 270.1707. $C_{14}H_{24}NO_4$ (MH$^+$) requires 270.1705.

§ Data for the acid **20**: ν_{max} (film)/cm^{-1} inter alia: 1710 (carboxy CO) and 1650 (amide CO and C=C); δ_H (400 MHz; $CDCl_3$) 0.88 and 0.93 (each 3H, t, *J* 7 Hz, 2×*M*eCH$_2$), 1.47–1.58 (4H, m, 2×C*H*$_2$Me), 1.69–1.78 and 1.88–1.96 (each 1H, m, 5-H$_2$), 2.03 (3H, s, MeCO), 2.25–2.34 and 2.41–2.50 (each 1H, m, 6-H$_2$), 3.37 (1H, quintet, *J* 6 Hz, OC*H*Et$_2$), 4.02–4.06 (1H, m, 3-H), 4.10–4.17 (1H, m, 4-H), 6.09 (1H, br d, *J* 8 Hz, NH), 6.3 (1H, br s, CO_2H) and 6.96–7.00 (1H, m, 2-H); δ_C (100 MHz; $CDCl_3$) 9.4 and 9.8 (2×*C*H$_3$CH$_2$), 22.8 and 23.7 (5- and 6-CH$_2$), 23.3 (*C*H$_3$CO), 26.1 and 26.4 (2×*C*H$_2$CH$_3$), 47.4 (4-CH), 69.8 (3-CH), 81.1 (O*C*HEt$_2$), 132.6 (1-C), 137.8 (2-CH) and 170.5 and 170.9 (amide and carboxy CO); *m*/*z* (electrospray) 292 [M(Na)$^+$, 10%], 270 (MH$^+$, 50) and 182 (100); found: *m*/*z* 270.1700. $C_{14}H_{24}NO_4$ (MH$^+$) requires 270.1705.

¶ 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%.

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

Compound	Influenza A	Influenza B
2d	0.071	0.83
20	172	>700
2a[a]	0.001	0.003

[a] Quoted from Ref. 3.

Acknowledgements

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