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The acidic hydrolysis of *N*-acetylneuraminic 4,5-oxazoline allows a direct functionalization of the C5 position of Neu5Ac2en (DANA)†

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The acidic hydrolysis of *N*-acetylneuraminic 4,5-oxazoline affords the corresponding 2,3-unsaturated amino ester, which was not previously detected (nor isolated) due to the unexpected rearrangement into its corresponding alcohol. In this work, we unveiled the mechanism of these reactions and optimized the conditions to obtain either synthetic intermediate.

Several 2,3-unsaturated derivatives of the *N*-acetylneuraminic acid (Neu5Ac, **1**) have been developed as potent inhibitors of sialidases, the glycolytic enzymes critical for bacteria and virus spread.[1–6] This class of inhibitors includes the 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid **2a** (Neu5Ac2en, DANA), the *N*-trifluoroacetylated congener **2b** (FANA), the *N*-isobutyrylated one **2c** (BCX 2798), and the 4-guanidino derivative **2d** (Zanamivir), which has been widely used in the clinic against the influenza virus (Fig. 1).[1–6]

Interestingly, the 4,5-oxazoline of Neu5Ac **3a** has been shown[1–12] to be a key intermediate for the synthesis of various C4- or C4- and C5-modified DANA derivatives (*e.g.* BCX 2798 **2c** and Zanamivir **2d**),[1,5,6] of the 4-*epi* DANA **4a**[3] (or its protected 4-*epi* hydroxyl derivative **4b**[6,9–13]), as well as of several 3,4-unsaturated DANA-homologs, which were obtained through a Ferrier reaction.[8,14] In this context, our group has revealed the conditions to perform a critical intermolecular nucleophilic attack on **3a** at its C4 position, while avoiding its intramolecular formation, and reported the synthesis of several C5-perfluorinated 4-*epi* DANA derivatives.[2,3,7,15,16] Overall, the synthetic strategies involving the synthon **3a** could be summarized with three distinctive reactions (Scheme 1): (a) a nucleophilic attack – *via* S_N2 – on the carbon at C4, which eventually opens up the oxazoline ring, thus restoring the acetyl group at C5 (analogously to the reaction used for the insertion of the azido group at C4),[17] (b) a hydrolytic ring-opening caused by the direct attack of a water molecule on the sp^2-carbon of the oxazoline ring, which affords the 4β-hydroxy derivative **4b**,[6,9–13] and (c) a nucleophilic attack on the anomeric carbon (C2), followed by a shift of the double bond and the restoration of the *N*-acetyl group through a Ferrier reaction.[8,14]

To date, the formation of the amino ester **5**, an isomer of **4b** and derived from the C=N bond cleavage of the oxazoline ring, has never been described. Indeed, differently to the well-established oxazoline chemistry,[18] it was reported that the acidic treatment of the oxazoline **3a** afforded the 4-*epi* hydroxyl derivative **4b**, as a single product, in variable yields (42–88%), calling for further studies.[6,9–13]

As a result of our continuing interest in the sialic acid chemistry/biochemistry,[2,3,7,14–16,19–23] in this work we solved this mechanistic puzzle, as we unveil that, the acidic hydrolysis of the 4,5-oxazoline **3a** affords the amino ester **5**, which could be isolated in good yields and fully characterized under appropriate work-up conditions. Furthermore, we detailed the shortened synthesis of the precursor of BCX 2798 (**2c**) and other sialidase inhibitors (such as BCX 2755 and various C4 triazole derivatives) further highlighting the synthetic utility of **5**.[1,24,25] Finally, we report an improved procedure

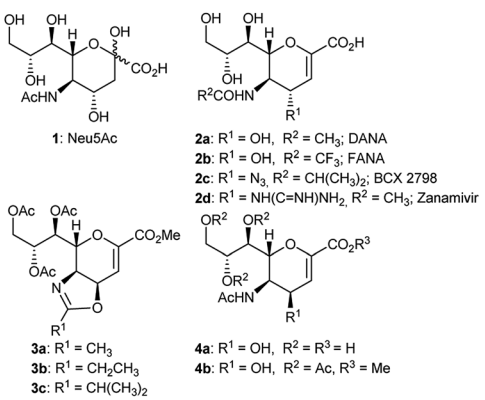

Fig. 1 Sialic acid (Neu5Ac), some sialidase inhibitors (DANA, FANA, BCX 2798, Zanamivir) and some 4,5-oxazolines of Neu5Ac.

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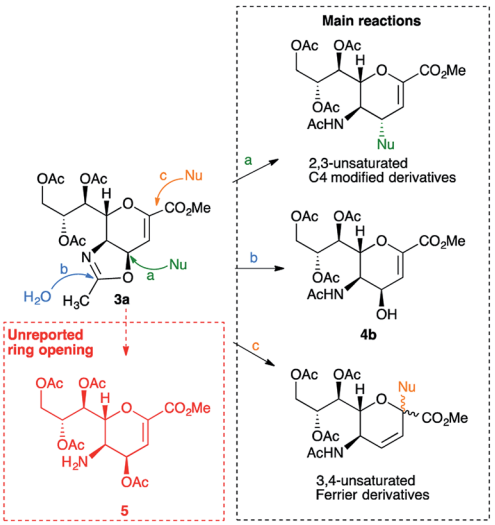
for the quantitative preparation of the isomeric alcohol **4b** and clarified the chemical structure of several by-products observed during the acid hydrolysis[12,13] of the oxazoline **3a**.

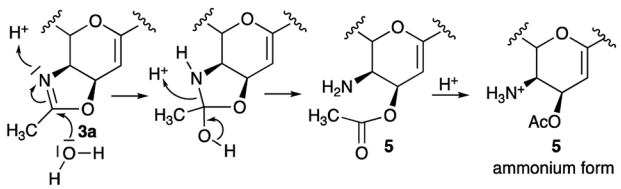
To this purpose, we dissolved 1 mmol of the oxazoline **3a** in a solution of THF–H_2O (15 mL, 2 : 1 v/v) containing TFA (0.2 mL), at 23 °C for 5–15 minutes (alternatively CH_3CN–H_2O, 15 mL, 1 : 1 v/v as a solvent system), under the same experimental conditions previously described for the preparation of **4b**.[9] The reaction was monitored by TLC on silica (AcOEt eluent). After a few minutes, the starting oxazoline **3a** ($R_f = 0.34$) disappeared and was completely converted into a single more polar compound ($R_f = 0.22$). After a careful isolation using weak basic resin (IRA-67), we could assign to this molecule the structure of the 2,3-unsaturated amino ester **5**, on the bases of its physicochemical properties (correct mass, ^1H and ^{13}C NMR spectra), and reactivity (such as a brownish-yellow spot when sprayed with an alcoholic solution of ninhydrin).‡ The obtained compound **5** was sufficiently stable under anhydrous conditions, but, as expected, in the presence of water, it could undergo a slow rearrangement into the alcohol **4b**, possibly explaining previous inconsistent results reported in the literature.

Moreover, we observed that the amino ester **5** was partially transformed into the isomeric amido alcohol **4b** ($R_f = 0.11$) when the reaction mixture was neutralized[12,13] with a saturated sodium hydrogen carbonate solution or when the crude residue of the reaction was chromatographed[9–11] on silica gel column (ethyl acetate–methanol 100 : 1 v/v).§

Time-laps NMR experiments further clarified the reaction mechanism by revealing the initial formation of compound **5** as an ammonium salt, its successive transformation into the free amine form and its slow rearrangement into the alcohol **4b**. Remarkably, by performing the reaction directly in an NMR tube, by dissolving oxazoline **3a** in CD_3CN–D_2O (1 : 1 v/v) and successively adding TFA, we could observe the fast and complete transformation of the oxazoline **3a** into the ammonium form of **5** (see ESI, S6†). The successive elution of the NMR solution through a small column containing a weak basic resin (IRA-67) resulted in the formation of the free amine **5**, as



Scheme 1 Main reported reactions on the 4,5-oxazoline **3a**: (a) nucleophilic attack *via* S_N2 at the C4; (b) hydrolytic ring-opening with the formation of **4b**; (c) nucleophilic attack at the C2 and formation of 3,4-unsaturated Ferrier products.



Scheme 2 Oxazoline ring opening with the formation of the ammonium salt of **5**.

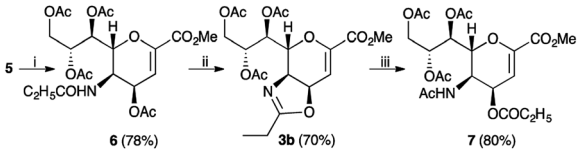
revealed by the shift of the H-4, H-5 and H-6 proton signals (δ_{H4} 5.50, δ_{H5} 3.82 and δ_{H6} 4.59 ppm for ammonium salt of **5** and δ_{H4} 5.25, δ_{H5} 3.04 and δ_{H6} 4.12 ppm for **5**), together with the formation of some traces of the isomeric alcohol **4b**. The reaction was further monitored by ^1H-NMR, revealing the complete transformation of amine **5** into the 4-hydroxyl derivative **4b** as the only product after six days.

Overall, our results support a reaction mechanism which is in line with other oxazoline chemistry and in disagreement with the state of art of the oxazoline.[18] In particular, the opening of the oxazolinic ring and its transformation into the ammonium salt of **5** could involve both the nucleophilic attack of a water molecule on the sp^2 carbon atom of the oxazoline ring and, at the same time, the protonation of the nitrogen group (Scheme 2). Then, the subsequent formation of the alcohol **4b** could be explained by the relative instability of **5** due to the presence of a free amino group in a *cis* configuration, supporting the migration of the acetyl group between the C-4 and C-5 position.

Finally, to further confirm the structure of compound **5**, a classical reactivity-based approach was performed. In particular, the amino ester **5**, reacted with propionyl chloride in the presence of a basic resin (IRA-67) affording the desired propionylamido derivative **6**, as expected (Scheme 3). Moreover, we

‡ Preparation of compound **5**: to a solution of oxazoline **3a**[1] (413 mg, 1.0 mmol) in THF–H_2O, 2 : 1 v/v (15 mL) or in CH_3CN–H_2O, 1 : 1 v/v (15 mL), TFA (0.2 mL) was added and the solution was stirred at 23 °C until the complete disappearance of the starting material (5–15 minutes), by monitoring with TLC (AcOEt). At this time, the reaction was quenched by the addition of a weak basic resin (IRA-67) until neutral pH, filtered and evaporated to give the desired amine **5** (401 mg, 93%). Compound **5** appeared as white foam: $[\alpha]_D$ −130.3 (*c* 1.0 in CH_2Cl_2); δ_H (500 MHz, $CDCl_3$) 6.15 (1H, d, $J_{3,4} = 5.7$ Hz, 3-H), 5.63 (1H, dd, $J_{7,6} = 1.4$, $J_{7,8} = 6.1$ Hz, 7-H), 5.44 (1H, ddd, $J_{8,9a} = 2.3$, $J_{8,7} = J_{8,9b} = 6.1$ Hz, 8-H), 5.28 (1H, dd, $J_{4,5} = 4.2$, $J_{4,3} = 5.7$ Hz, 4-H), 4.69 (1H, dd, $J_{9a,8} = 2.3$, $J_{9a,9b} = 12.6$ Hz, 9a-H), 4.27 (1H, dd, $J_{9b,8} = 6.1$, $J_{9b,9a} = 12.6$ Hz, 9b-H), 4.01 (1H, dd, $J_{6,7} = 1.4$, $J_{6,5} = 10.8$ Hz, 6-H), 3.79 (3H, s, $COOCH_3$), 2.93 (1H, dd, $J_{5,4} = 4.2$, $J_{5,6} = 10.8$ Hz, 5-H), 2.15 (3H, s, $OCOCH_3$), 2.10 (6H, overlapping, 2 × $OCOCH_3$), 2.06 (3H, s, $OCOCH_3$) and 1.65–1.47 (2H, overlapping, NH_2); δ_C (125 MHz, $CDCl_3$) 170.6 (2C, $OCOCH_3$ at C-7 and $OCOCH_3$ at C-9), 170.2 ($OCOCH_3$ at C-4), 169.9 (1C, $OCOCH_3$ at C-8), 162.0 (C-1), 146.2 (C-2), 105.9 (C-3), 75.5 (C-6), 70.8 (C-8), 68.6 (C-7), 65.7 (C-4), 62.0 (C-9), 52.5 ($COOCH_3$), 47.3 (C-5), 20.9 (2C, 2 × $OCOCH_3$), 20.7 ($OCOCH_3$) and 20.6 ($OCOCH_3$); MS (ESI positive): *m*/*z* 432.1 $[M + H]^+$, 454.1 $[M + Na]^+$; elemental analysis (found: C, 50.24; H, 5.79; N, 3.10. $C_{18}H_{25}NO_{11}$ requires C, 50.12; H, 5.84; N, 3.25%).

§ One or more of these occurrences, could explain the transformation of the compound **5** into the isomer **4b** and the discrepancy with literature data.



Scheme 3 Exchange of an acyl group between the C5 and C4 positions. Reaction conditions: (i) propionyl chloride, weak basic resin IRA 67, CH_2Cl_2, 0 °C to 23 °C, 0.5 h; (ii) BF_3 Et_2O, CH_2Cl_2, 23 °C to 80 °C, 20 min; (iii) TFA, $CH_3CN–H_2O$, 1 : 1 v/v, 23 °C, 5–15 min, then acetyl chloride, weak basic resin IRA 67, CH_2Cl_2, 0 °C to 25 °C, 0.5 h.

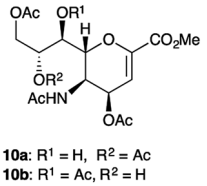


Fig. 2 Unreported byproducts of acetic acid hydrolysis of oxazoline **3a**.

tested whether the exchange reaction of the acyl group between the C5 and C4 was general in scope. To this purpose, we transformed compound **6** into its oxazoline derivative **3b**, using the standard reaction conditions.[7] Compound **3b** was achieved in good yield (70%) and high purity (>98% by ^{1}H NMR). Then, the propionyl-oxazoline **3b** was treated with moist TFA and, after its disappearance, directly reacted with an excess of acetyl chloride in the presence of a basic resin (IRA-67), affording compound **7** in high yield (80%).

The discovery that **3a** could be easily converted into the amino ester **5** is of high synthetic value, as the direct acylation of its C5 amino group opens up a novel and easy way to replace the acetamido function present in DANA derivatives with different suitable acyl amido substituents. Indeed, as a proof of concept, we were able to directly functionalize the C5 position, ultimately achieving the desired isobutyl amide derivative **8** with this new one-pot reaction from the starting oxazoline **3a** in good overall yield (78%, Scheme 4), which could be easily converted into **9**, the key precursor of several sialidase inhibitors such as BCX 2798 **(2c)** and BCX 2755.[1,24,25]

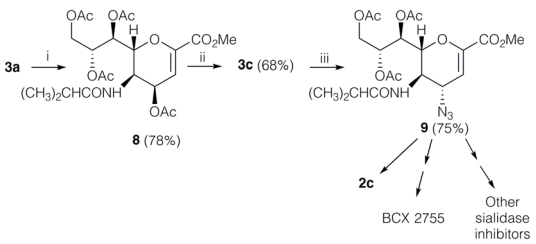
Briefly, by simple treatment of **8** with BF_3 Et_2O in dichloromethane, we obtained the unreported isobutyl-oxazoline **3c** (68% yield). Remarkably, we performed the C4α-insertion of an azido group using azidotrimethylsilane ($TMSN_3$) in *t*-butanol, under the same reaction conditions reported[17] to obtain the azido derivative on the oxazoline **3a**. Thus, we were able to isolate the key intermediate **9** in high yield (75%). The hydrolysis[1] of the azide **9** afforded the final BCX 2798 (**2c**).

Overall, this novel procedure avoids several steps of protection and deprotection at C5 that were needed in previous synthetic routes for the synthesis of BCX 2798 (**2c**), as well as of several sialidase inhibitors.[1,24,25]

Finally, while studying and optimizing the conditions for **3a** ring-opening we were able to develop a more efficient synthetic route to the 4-*epi* hydroxyl alcohol **4b**, which was previously obtained with variable yields (42–88%).[6,9–13] Remarkably, we were able to obtain **4b** in high yields by a "one-pot" reaction from oxazoline **3a**. To this purpose, **3a** (1 mmol) was treated with TFA in THF–H_2O as previously described and, after its disappearance (monitored by TLC), triethylamine (5 mmol) was added. After 40 min at r.t. the intermediate **5** was completely transformed into a single spot (by TLC) corresponding to the desired alcohol **4b**. The ^{1}H NMR analysis of crude product of reaction confirmed the quantitative formation of this compound, which, after chromatographic purification from the salts, was obtained in 89% isolated yield.

Intrigued by these results, we carefully scrutinized the more widely used hydrolytic method (AcOH in of AcOEt–H_2O mixture) to obtain **4b**, in order to assess the possible formation of other byproducts. Interestingly, after 1 h, we observed by TLC the partial reduction of the starting oxazoline **3a**, to afford a spot (R_f = 0.22) corresponding to the expected compound **5**, and two additional spots (R_f = 0.16 and R_f = 0.11), the most polar one corresponded to **4b**. As the reaction proceeded overnight, both **3a** and **5** disappeared and were transformed in the two most polar spots in TLC, which remain unchanged even after the basic ($NaHCO_3$) work-up extraction. After two flash chromatographies (see ESI†), three different products were isolated, corresponding to the expected alcohol **4b** (51%), and to two less polar products **10a** (7%) and **10b** (18%) that previously unreported (Fig. 2).

Indeed, under acidic conditions, the acetyl scrambling between C4 and C5 positions could compete with that among either the C7 or the C8 and the C5, differently from what we observed under basic conditions. However, it cannot be excluded that one of the two products **10a** or **10b** may derive from the simple migration of the acetyl group between the hydroxyl functions present in the glycerol side chain.



Scheme 4 Synthesis of precursor **9** of BCX 2798, BCX2755 and other sialidase inhibitors. Reaction conditions: (i) TFA, $CH_3CN–H_2O$, 1 : 1 v/v, 23 °C, 5–15 min, then isobutyric anhydride, weak basic resin IRA 67, CH_2Cl_2, 0 °C to 25 °C, 1 h; (ii) BF_3 Et_2O, CH_2Cl_2, 23 °C to 80 °C, 20 min; (iii) $TMSN_3$, *t*BuOH, 80 °C, overnight.

Conclusions

In this work, we report the direct conversion of **3a** in the corresponding amine **5**, a useful synthon for the preparation of several sialidase inhibitors with anti-viral activity. The understanding of this reaction mechanism clarified several inconsistent and unexplained results in the literature, and it allowed

the revision and improvement of the synthesis of alcohol **4b**, another key intermediate for the preparation of sialic acid derivatives.

Conflicts of interest

There are no conflicts to declare.

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