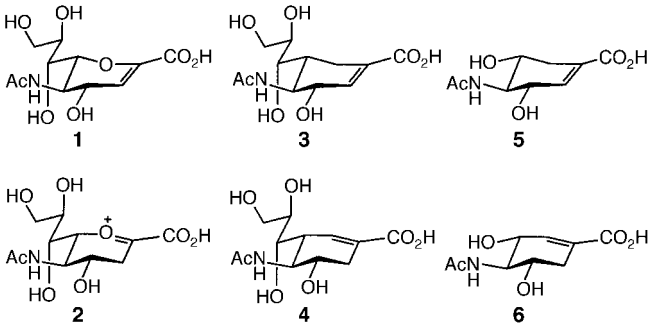
Carbocyclic Analogues of *N*-Acetyl-2,3-didehydro-2-deoxy-D-neuraminic Acid (Neu5Ac2en, DANA): Synthesis and Inhibition of Viral and Bacterial Neuraminidases**

Steffen Vorwerk and Andrea Vasella*

The influenza virus neuraminidase is essential for viral replication and infectivity;[1] inhibition of this enzyme forms the basis of a chemotherapy for influenza.[2] In contrast, little is known about the relevance of bacterial neuraminidases to the pathogenicity of many bacteria.[3] *N*-Acetyl-2,3-didehydro-2-deoxy-D-neuraminic acid (Neu5Ac2en, DANA, **1**)[4] is the prototypical inhibitor of neuraminidases. It inhibits viral and a number of bacterial neuraminidases.[5a] However, it hardly binds to *Salmonella typhimurium* LT2 neuraminidase.[5b] This is surprising, considering that X-ray analyses have shown a large degree of similarity between the active sites of the neuraminidases from *Vibrio cholerae*, *Salmonella typhimurium*, and several strains of *Influenza* virus.[6] DANA (**1**) is considered a transition state analogous inhibitor.[7]

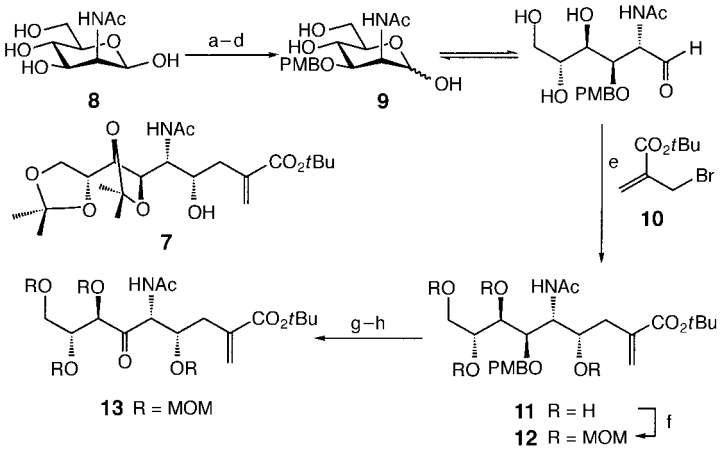
One expects the carbocyclic compound **4** to be a stronger inhibitor than DANA as it more closely resembles the proposed reactive intermediate **2**[8] of the enzymatic glycoside cleavage. For the same reasons, **4** should be a more potent inhibitor than its isomer **3**. This is in keeping with the finding that the cyclohexene **6** is a stronger inhibitor of *Influenza* A neuraminidase (N2) than its isomer **5**.[9] The significance of



this result is not quite clear since both compounds lack the glycerol side chain. We have now synthesized the carbocyclic analogues **3** and **4** of DANA (**1**) to investigate more closely the influence of the position of the double bond on the inhibition of viral and *Salmonella typhimurium* neuraminidases.

6a-Carba-*N*-acetyl-D-neuraminic acid[10] is the only known carbaneuraminic acid possessing an intact side chain. Carbocyclic analogues lacking the side chain,[9] or possessing a truncated[11] or modified[9] side chain, have been prepared either from quinic acid or by total synthesis based on a Diels–Alder cycloaddition. We planned to synthesize **3** and **4** by a ketyl–olefin cyclization.

The ester **7** (Scheme 1) is an intermediate in one of our syntheses of *N*-acetyl-D-neuraminic acid.[12] It contains the carbon atoms required for the preparation of a carbaneuraminic acid and is readily available. However, for the regioselective generation of a radical, the OH groups must



Scheme 1. a) Allylic alcohol, $BF_3 \cdot OEt$, 95 °C, 80 %; b) (4-methoxyphenyl)acetaldehyde dimethyl acetal, cat. *p*TsOH, MeCN, 0 °C, 70 %; c) 4-methoxybenzyl 2,2,2-trichloroacetimidate, cat. trifluoromethanesulfonic acid, THF/Et_2O, 0 °C, 88 %; d) 1. cat. $[Pd(PPh_3)_4]$, HCO_2H, Et_3N, dioxane, 65–95 °C, 2. acetic acid (AcOH), room temperature (RT), 92 %; e) In, MeCN/0.1M HCl 20/1, TBAI (0.1 equiv), 40 °C, 60–70 % (d.e. > 90 %); f) MOMCl, iPr_2NEt, 1,2-dichloroethane, cat. TBAI, cat. 4-PP, 0–25 °C, 91 %; g) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH_2Cl_2/H_2O, RT, 92 %; h) Dess–Martin periodinane, CH_2Cl_2, RT, 98 %. PMB = 4-methoxybenzyl; MOM = methoxymethyl.

be differentiated at an earlier stage of the synthesis. Therefore, *N*-acetyl-β-D-mannosamine (**8**) was transformed into the partially protected aldose **9** (five steps, 45 % overall yield). To perform the chain elongation[12] with *tert*-butyl bromomethacrylate (**10**) in aqueous solution, we replaced zinc by indium[13] and obtained the homoallylic alcohol **11**.[14] The diastereoselectivity of this reaction strongly depends upon the MeCN/H_2O ratio and on the amount of tetra-*n*-butylammonium iodide (TBAI).

Attempts to protect the OH groups of **11** by methoxymethylation under standard conditions[15] gave inseparable mixtures of the desired compound **12**, incompletely O-alkylated, and O- and N-alkylated products. The methoxymethylation was, however, successful with methoxymethyl chloride (MOMCl), ethyl(diisopropyl)amine and a catalytic amount of 4-pyrrolidinylpyridine (4-PP)[16] in the presence of TBAI. Selective removal of the 4-methoxybenzyl (PMB) group[17] and oxidation of the intermediate alcohol[18] with periodinane[19] gave the ketone **13** in 70 % overall yield without epimerization of adjacent chiral centers.

Treatment of **13** with samarium(II) iodide in THF/hexamethyl phosphoric acid triamide (HMPT) and *tert*-butyl alcohol as the proton source[20] led to a mixture of the

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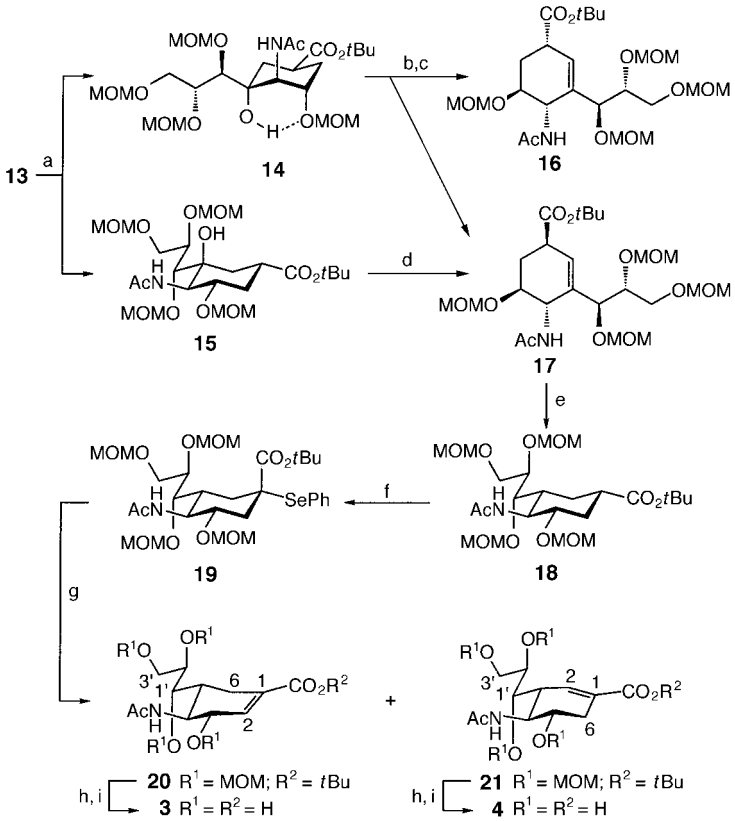
carbocyclic esters **14**[14] and **15**, which were separated by chromatography (Scheme 2). Regioselective dehydration to form an alkene was only successful with Martin's sulfurane.[21] This reagent transformed the tertiary alcohol **15** at 0 °C[22] into the desired β,γ-unsaturated ester **17**. The analogous treatment



Scheme 2. a) SmI_2, THF/HMPT, *t*BuOH, RT, 93% (**14**:**15** = 40:60); b) Martin's sulfurane, CCl_4, RT; c) 5% AcOH, RT, 67% (**16**:**17** = 80:20); d) Martin's sulfurane, CCl_4, 0 °C, 95%; e) H_2, Pd/C, AcOEt, RT, 82%; f) 1. LICA, THF, −78 °C → RT, 2. Ph_2Se_2, THF, −78 °C; g) 1. H_2O_2, CH_2Cl_2, 0 °C, 2. pyridine, RT, 53% (over two steps, **20**:**21** = 64:36); h) 1. HCl/MeOH, 90 °C, 2. CH_2N_2/Et_2O, MeOH, RT, 3. Ac_2O, MeOH, RT, i) 1. Et_3N, H_2O, 0 °C, 2. DOWEX H^+, **3**: 62%; **4**: 57% (over four steps).

of **14** followed by hydrolysis with dilute acetic acid[23] yielded the unsaturated esters **16** and **17**. Attempts to isomerize the olefins **16** and **17** to the conjugated α,β-unsaturated esters failed. We, therefore, hydrogenated **17** to **18**, deprotonated **18** with lithium cyclohexyl(isopropyl)amide (LICA),[24] and treated the ensuing enolate with Ph_2Se_2.[25] The resulting equatorial phenylselenide **19**[14] (64%) proved unstable in solution and was therefore directly treated with H_2O_2 and pyridine at 0–27 °C. The resulting regioisomeric α,β-unsaturated esters **20**[14] and **21** were isolated by preparative HPLC, and transformed into the title compounds **3** and **4** (Table 1).

The cyclohexene **3** ("CarbaDANA") proved to be a weak inhibitor[26] of *Influenza* A neuraminidase (N2; IC_{50} = 0.85 mM). In contrast, isomer **4** ("Iso-CarbaDANA") showed an approximately 40-fold stronger inhibition of *Influenza* A neuraminidase (N2; IC_{50} = 20 μM) and is thus twice as potent as DANA.[27] The differences between **3** and **4** must result from their different half-chair conformations, since the conformations of the side chains of **1**, **3**, and **4** are very

Table 1. Analytical data for **3** and **4**.[a]

3: Colorless lyophilisate; R_f: 0.35 (*n*PrOH/H_2O 7/1); HPLC: R_t: 8.6 min; $[\alpha]_D^{25} = 42.5°$ (c = 0.08, H_2O); ^{1}H NMR: δ = 6.41 (br t, J = 1.9 Hz, 1H; H-2), 4.29 (ddt, J = 9.1, 3.7, 1.8 Hz, 1H; H-3), 3.79 (dd, J = 11.6, 2.0 Hz, 1H; H′3′), 3.76 (dd, J = 11.9, 9.1 Hz, 1H; H-4), 3.60 (AB system with virtual coupling, 2H; H-2′, H-1′), 3.54 (dd with virtual coupling J = 11.6, 6.0 Hz, 1H; H-3′), 2.37 (br dd, J = 17.5, 5.3 Hz, 1H; H_e-6), 2.25 (ddt, J = 17.4, 11.2, 3.2 Hz, 1H; H_a-6), 2.08 (tdd, J = 11.4, 5.4, 1.6 Hz, 1H; H-5), 2.02 (s, 3H, AcN); ^{13}C NMR: δ = 177.87 (s, CO_2H), 177.66 (s, AcN), 138.50 (s, C-1), 135.96 (d, C-2), 73.91 (d, C-2′, 73.27 (d, C-1′), 70.96 (d, C-3), 66.25 (t, C-3′), 55.77 (d, C-4), 40.26 (d, C-5), 26.35 (t, C-6), 24.73 (q, AcN); HR-MS (NESI) calcd for $C_{12}H_{18}NO_7$: 288.1083, found: 288.1083.

4: Colorless lyophilisate; R_f: 0.27 (*n*PrOH/H_2O 7/1); HPLC: R_t: 10.8 min; ^{1}H NMR: δ = 6.48 (br t, J = 2.4 Hz, 1H; H-2), 3.90 (t, J = 10.1 Hz, 1H; H-4), 3.80 (dd, J = 12.2, 2.8 Hz, 1H; H′-3′), 3.76 (td, J = 10.3, 5.0 Hz, 1H; H-5), 3.73 (ddd, J = 9.5, 6.5, 2.9 Hz, 1H; H-2′), 3.54 (dd, J = 11.9, 6.4 Hz, 1H; H-3′), 3.50 (dd, J = 9.6, 1.7 Hz, 1H; H-1′), 2.73 (ddd, J = 16.7, 5.2, 1.6 Hz, 1H; H_e-6), 2.69 (ddt, J = 9.7, 3.9, 2.0 Hz, 1H; H-3), 2.18 (dddd, J = 16.8, 10.0, 4.0, 2.9 Hz, 1H; H_a-6), 2.00 (s, 3H; AcN); ^{13}C NMR: δ = 177.65 (2s, CO_2H, AcN), 137.61 (s, C-1), 133.52 (d, C-2), 73.68 (d, C-2′), 72.09 (d, C-1′), 72.02 (d, C-5), 66.14 (t, C-3′), 55.09 (d, C-4), 45.89 (d, C-3), 35.88 (t, C-6), 24.91 (q, AcN); HR-MS (NESI) calcd for $C_{12}H_{18}NO_7$: 288.1083, found: 288.1078.

[a] HPLC: Hibar (Merck), RP-18 (7 μm), column: 250 × 25 mm, eluent: H_2O, flow rate: 10 mL min^{-1}. ^{1}H NMR: 500 MHz, D_2O; ^{13}C NMR: 125 MHz, D_2O.

similar. Iso-CarbaDANA (**4**) inhibits *S. typhimurium* neuraminidase (IC_{50} = 39 μM), while **1** and isomer **3** did not show any inhibition at concentrations up to 350 μM.

The reasons for this decisive influence of the position of the double bond for the inhibition of the *S. typhimurium* neuraminidase are largely obscure. Conceivably, the active site of this enzyme is less flexible than that of other neuraminidases and consequently more demanding on the precise structure of an inhibitor.[28] The mechanistic details of action of the *S. typhimurium* neuraminidase are largely unknown and partially controversial.[29]

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Ring-Closing Metathesis of Functionalized Acetylene Derivatives: A New Entry into Cycloalkynes

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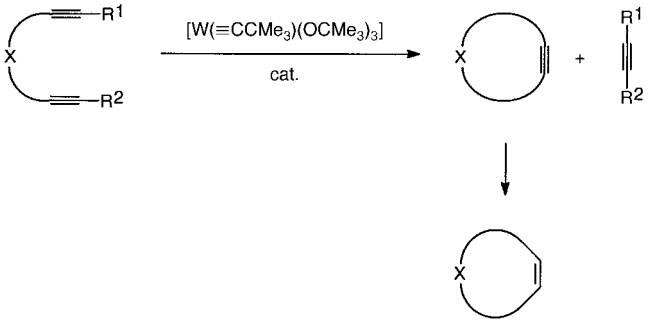
Olefin metathesis is rapidly evolving into a prosperous field of research, and as a result of the development of a new generation of performance catalysts with a high tolerance towards functional groups has recently found many applications in organic synthesis.[1, 2] In particular, ring-closing metathesis (RCM) of dienes to cycloalkenes provides good access to carbo- and heterocycles and has been proved to be effective in numerous syntheses of natural products.[2] Medium-sized and macrocyclic rings can also be forged by RCM.[3, 4] The latter, however, are usually obtained as mixtures of *E* and *Z* isomers, the ratio of which can, at present, be neither predicted nor properly controlled. This is a major drawback in target-oriented syntheses as exemplified, for example, by several approaches to epothilone: Although various research teams succeeded in forming the 16-membered ring of this promising chemotherapeutic agent by RCM, separation of the resulting stereoisomeric mixtures were inevitable because only epoxidation of the *Z*-configured cycloalkene leads to the desired target molecule.[5]

In striking contrast to olefin metathesis, the metathesis of alkynes presently plays only a minor role in organic chemistry.[6] Even though the close mechanistic ties between both types of transformations were noticed early on,[7] and various well-defined alkyne metathesis catalysts are available,[8] the applications of alkyne metathesis have until now been confined to the preparation of some special polymers[9] and to the dimerization or cross-metathesis of simple acetylene derivatives.[10]

We now describe the first efficient syntheses of functionalized macrocycles by ring-closing metathesis of diyne substrates (Scheme 1). Partial reduction of the cycloalkyne molecules thus obtained by one of the conventional methods (e.g. Lindlar hydrogenation or hydroboration/protonation) also consitutes a stereoselective route to *Z*-configured cycloalkenes[11] which cannot yet be directly prepared in pure form by RCM.



Scheme 1. Ring-closing metathesis of diynes with **1**.

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