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Note

A regio- and stereo-selective introduction of azide at C-4 of 2,3-unsaturated *N*-acetylneuraminic acids

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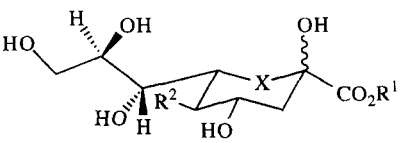
Abstract

The regio- and stereo-selective azidation of the peracetate methyl esters of 4-*epi*-Neu5Ac2en and 6-thio-4-*epi*-Neu5Ac2en has been achieved using Pd(0) chemistry in good yield (> 80%). These 4-*epi*-azido-4-deoxy-Neu5Ac2en derivatives provide interesting biological probes.

Keywords: *N*-acetylneuraminic acid; Sialic acid; Glycal; Ulosonic acid; Thioisostere

Sialic acids, in particular *N*-acetylneuraminic acid (Neu5Ac, **1**), as constituents of glycoproteins, glycolipids, and oligosaccharides, play an important role in many biological processes such as cell adhesion and inflammation [1]. The derivatives, analogues, and glycosides of sialic acids have received much attention in recent years [2]. As part of our research program on sialic acid structure–activity relationships and the synthesis of a range of sialic acid-based biological probes, we have been interested in introducing functional modifications, particularly nitrogen, at C-4. Given the interesting biological properties of some of the C-4 nitrogen-containing Neu5Ac2en derivatives [3] and the corresponding thioisosteres [4], we have directed our attention to the synthesis of the 4-*epi*-azido derivatives of unsaturated sialic acids. C-4 oxygen epimers of Neu5Ac2en, such as methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*talo*-non-2-enonate (**8**), are known [5,6].

We have previously reported [7] a stereoselective and convenient synthesis of the C-4 azido-substituted Neu5Ac2en derivative, methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-*glycero*-D-*galacto*-non-2-enonate (**4**). The preparation of **4** was readily achieved from the peracetate methyl ester of Neu5Ac2en (**5**) via the oxazoline **3**. Thus treatment of **3** with azidotrimethylsilane at 80°C over 4 h afforded the azide **4** in 82% yield.



	X	R^1	R^2
1	O	H	NHAc
2	S	Me	NHAc

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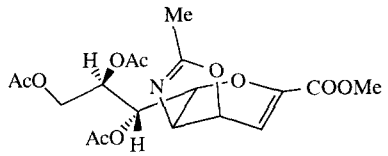

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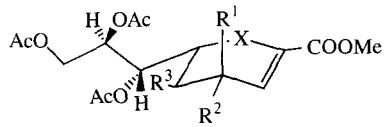


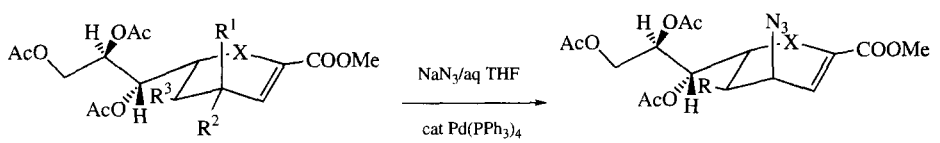
	X	R^1	R^2	R^3
4	O	H	N_3	NHAc
5	O	H	OAc	NHAc
6	O	OH	H	NHAc
7	S	H	OAc	NHAc

However, while a stereoselective synthesis of the azide **4** [7], and the corresponding 6-thioisostere from **7** [4], has been achieved, the same does not apply to the synthesis of the 4-*epi*-azide **10**. Thus, some years ago, Schreiner et al. [8] described the introduction of azide at C-4 of compound **6** via a Mitsunobu reaction (TPP/DEAD/HN_3) resulting in an epimeric mixture of the azides **4** and **10**. The stereochemical outcome of this reaction was reported to be controlled by the choice of solvent employed in the reaction. With toluene as the solvent, compounds **4** and **10** were formed in the ratio of 1:3, whereas with THF, the level of selectivity (3:2) improved in favour of the 4-*epi*-azide **10**. The low selectivities were ascribed [8] to possible competing S_N2 and S_N2' reaction pathways at the activated triphenylphosphonium intermediate. The S_N2' process results in the formation of a 2-β-azido-3,4-didehydrosialic acid intermediate which then undergoes a [3,3]-sigmatropic rearrangement to give azide **10**.

As a part of our research interest in sialic acid chemistry, and because of the importance of C-4-modified sialic acids, we required a highly stereoselective synthesis of 4-*epi*-azido-substituted Neu5Ac2en and its corresponding 6-thio analogue. In this paper, we report on a completely stereoselective synthesis of such compounds.

Thus, we have found that sodium azide in aqueous THF in the presence of a catalytic amount of palladium(0) provides an efficient method [9] for the azidation (Scheme 1) of the peracetate methyl ester of 4-*epi*-Neu5Ac2en (**8**) [6]. In a typical experiment, equimolar amounts of compound **8** and NaN_3 in aqueous THF were treated with 3 mole-% of $Pd(PPh_3)_4$ until all starting material was consumed, and, following conventional workup, **10** was isolated in 84% yield. No detectable amount of the other C-4 isomer **4** was observed by ^1H NMR spectroscopy.

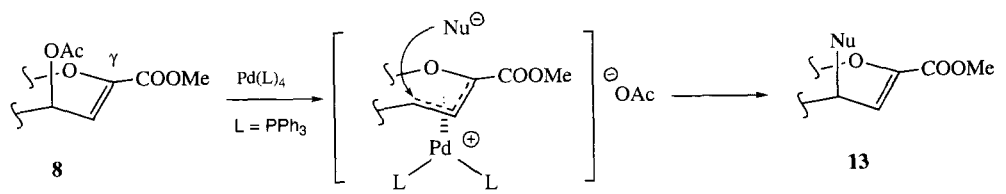
It is thought [9] that the oxidative addition of allyl acetates to Pd(0) occurs at the γ-position of allyl



	X	R^1	R^2	R^3
8	O	OAc	H	NHAc
9	S	OAc	H	NHAc

	X	R
10	O	NHAc
11	S	NHAc

Scheme 1.



8 **12** **13**

Scheme 2.

acetates (C-2 in the present examples) and proceeds with inversion of configuration to yield a (π-allyl)palladium complex such as **12** (Scheme 2). Subsequent reaction of the complex with suitable nucleophiles, such as azide, also proceeds with inversion of configuration [9]. The net result is an overall retention of configuration at C-4 in the product **13** (Scheme 2). Clearly nucleophilic attack may occur directly at C-4 (as shown in Scheme 2) or at the γ-position (C-2) followed by a [3,3]-sigmatropic rearrangement (as proposed for the Mitsunobu reaction [8]). Whilst it is not possible to discern between these mechanisms based on the present data, it is interesting to note that alkoxy allylic acetates [10] react predominantly at the α-position (with respect to the alkoxy group) in Pd(0)-catalysed C–C bond formation reactions. It has been suggested that the alkoxy group exerts a large electronic effect on the regioselectivity of nucleophilic attack [10]. This notion may provide support for an S_N2' mechanism followed by a [3,3]-sigmatropic rearrangement.

Our recent interest [4] in the thioisostere of *N*-acetylneuraminic acid has provided us with 6-thio-Neu5Ac1Me **2**, which was readily converted to the 4-*epi*-6-thio-Neu5Ac2en derivative **9** using previously described acetolysis conditions [6]. The palladium-catalysed azidation of 4-*epi*-6-thio-Neu5Ac2en derivative **9** provided the novel sulfur analogue, methyl 5-acetamido-7,8,9-tri-*O*-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-6-thio-D-*glycero*-D-*talo*-non-2-enonate (**11**). Once again none of the other C-4 isomer was detected by ^{1}H NMR spectroscopy.

Interestingly, the azidation reaction did not proceed when performed on the D-*glycero*-D-*galacto*-compound **5** (data not shown). In this case an excellent recovery of starting material **5** was found. This may well suggest that, under these reaction conditions, a pseudo-axially disposed acetate better facilitates the initial oxidative addition of the allyl acetate **8** to Pd(0). It may also be possible to effect the desired transformation on **5** with a change in the reaction solvent. It is known that glycals may adopt substantially different conformations depending upon solvent polarity [11].

In conclusion, the NaN_3/Pd(0) methodology described above leads to the formation of the 4-*epi*-azides **10** and **11** under mild conditions with exclusive net retention of configuration and in good yields. The stereochemical course is opposite to that of S_N2 type azidation previously observed for the opening of the oxazoline analogue **3** with azide.

1. Experimental

General.—The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra [in δ (ppm), relative to Me_4Si] were recorded on a Bruker AMX 300 spectrometer at 303 K. All NMR spectra were recorded in $CDCl_3$. Both low-resolution (LR) and high-resolution (HR) fast-atom bombardment (FAB) mass spectra were recorded on a Jeol JMS-DX 300 spectrometer. Optical rotations were measured at 24 °C using a JASCO DIP-370 polarimeter. All solvents were distilled and dried before use. Column chromatography was performed on Silica Gel 60 (E. Merck; 0.040–0.063 mm). Reactions were monitored by TLC on Kieselgel $60F_{254}$ plate (E. Merck 5554), and the plates were developed by spraying with a 95% aq EtOH soln containing 5% H_2SO_4 and charring for several minutes.

General reaction procedure: To a mixture of the substrate (0.09 mmol) in THF (1.2 mL) and H_2O (0.5 mL) was added NaN_3 (6 mg, 0.09 mmol) and tetrakis(triphenylphosphine)palladium(0) (3 mole-%) under an atmosphere of argon. The resulting mixture was stirred at 50 °C until TLC indicated disappearance of starting material. The reaction mixture was concd to dryness, and the residue was partitioned between diethyl ether (10 mL) and H_2O (5 mL). The organic layer was washed successively with 2 N HCl (2×5 mL), satd $NaHCO_3$ soln (2×5 mL) and H_2O (2×5 mL), and dried (Na_2SO_4). After solvent removal the resulting syrup was chromatographed, eluting with 1:1 EtOAc–hexane to remove triphenylphosphine oxide.

Preparation of methyl 5-acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-glycero-D-talo-*non-2-enonate* (**10**).—Treatment of **8** with NaN_3 under the above conditions provided the known title compound in 84% isolated yield. ^{1}H NMR and IR spectral data were in good agreement with literature values [8].

Methyl 5-acetamido-7,8,9-tri-O-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-6-thio-D-glycero-D-talo-*non-2-enonate* (**11**).—The required substrate, methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-6-thio-D-*glycero*-D-*talo*-non-2-enonate (**9**), was prepared in > 90% yield by acetolysis [6] of the known [4] methyl ester of 6-thio-Neu5Ac (**2**). Treatment of **9** with NaN_3 under the general reaction procedure provided the title compound in 81% isolated yield.

Data for **9**: $[\alpha]_D$ −133° (*c* 1.47, $CHCl_3$); ^{1}H

NMR, δ 2.06, 2.07, 2.08, 2.10 (s, 12 H, OCOCH_3), 3.82 (pseudo t, 1 H, $J_{6,5}$ 6.7, $J_{6,7}$ 6.7 Hz, H-6), 3.82 (s, 3 H, OCH_3), 4.21 (dd, 1 H, $J_{9a,8}$ 6.2, $J_{9a,9b}$ 12.2 Hz, H-9a), 4.38 (dd, 1 H, $J_{9b,8}$ 4.4 Hz, H-9b), 4.85 (ddd, 1 H, $J_{5,4}$ 4.4, $J_{5,NH}$ 9.8 Hz, H-5), 5.35 (ddd, 1 H, $J_{8,7}$ 4.4 Hz, H-8), 5.45 (dd, 1 H, H-7), 5.62 (dd, 1 H, $J_{4,3}$ 3.4 Hz, H-4), 5.85 (d, 1 H, N*H*), 6.82 (d, 1 H, H-3); ^{13}C NMR, δ 20.6, 20.7, 23.1 (OCO*C*H_3), 43.9, 44.3 (C-5, C-6), 52.9 (O*C*H_3), 61.1 (C-9), 66.4, 69.6, 70.1 (C-4, C-7, C-8), 127.8 (C-3), 129.8 (C-2), 163.3, 169.7, 170.0, 170.5 (carbonyls); LRFABMS: 490 ($M^+ + 1$, 35%), 430 (69%); HRFABMS for $C_{20}H_{28}NO_{11}S$ requires 490.1383. Found 490.1364.

Data for **11**: $[\alpha]_D$ −255° (*c* 1.02, $CHCl_3$); ^{1}H NMR, δ 2.01, 2.08, 2.11 (s, 12 H, OCOCH_3), 3.56 (dd, 1 H, $J_{6,5}$ 8.5, $J_{6,7}$ 5.1 Hz, H-6), 3.84 (s, 3 H, OCH_3), 4.13 (dd, 1 H, $J_{9a,9b}$ 12.3 Hz, H-9a), 4.35 (dd, 1 H, $J_{4,3}$ 4.8, $J_{4,5}$ 4.1 Hz, H-4), 4.36 (dd, 1 H, $J_{9b,8}$ 3.9 Hz, H-9b), 4.68 (ddd, 1 H, $J_{5,NH}$ 9.4 Hz, H-5), 5.21 (ddd, 1 H, $J_{8,7}$ 5.5 Hz, H-8), 5.43 (dd, 1 H, H-7), 5.80 (d, 1 H, N*H*), 6.89 (d, 1 H, H-3); ^{13}C NMR, δ 20.6, 20.7, 23.0 (OCO*C*H_3), 42.1 (C-6), 46.2 (C-4), 53.0, 56.9 (C-5, O*C*H_3), 61.4 (C-9), 68.3, 69.9 (C-7, C-8), 125.5 (C-3), 131.1 (C-2), 163.2, 169.7, 170.0, 170.5 (carbonyls); IR (NaCl): ν 2104 cm^{-1} (N_3); LRFABMS: 473 ($M^+ + 1$, 28%), 430 (50%); HRFABMS for $C_{18}H_{25}N_4O_9S$ requires 473.1342. Found 473.1345.

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