

Synthesis of S-Linked *N*-Acetylneuraminic Acid Derivatives via Photoinduced Thiol–ene and Thiol–yne Couplings

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Abstract: Thio-linked mono- and bivalent mimetics of $\alpha(2\rightarrow3)$ and $\alpha(2\rightarrow6)$ -linked sialosides were prepared by photoinduced hydrothiolation of alkenes and alkynes with the 2-mercapto sialic acid. Thiosialylation of 6-*O*-allyl- or 3-*O*-allyl-substituted galactose derivatives has been carried out by the thiol–ene click reaction. Double thiosialylation has also been achieved via thiol–yne chemistry using propargylated galactose derivatives as the alkyne components and the peracetylated 2-mercapto sialic acid as the thiol.

Key words: carbohydrates, *N*-acetylneuraminic acid, radical additions, thiol–ene/thiol–yne coupling, thiosialosides

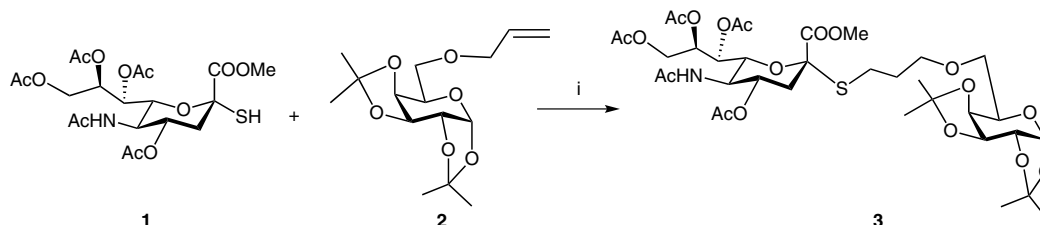
N-Acetylneuraminic acid (Neu5Ac), the most abundant sialic acid, is an important constituent of various cell-surface glycolipids and O- and N-linked glycoproteins. It is typically found at the nonreducing end of glycan chains anchored to galactosides through the $\alpha(2\rightarrow3)$ or $\alpha(2\rightarrow6)$ linkage, or to *N*-acetyl galactosamine through the $\alpha(2\rightarrow6)$ linkage. Being at the terminus of glycoconjugates, Neu5Ac is ideally positioned to mediate a wide variety of biological processes, such as cell–cell interactions, cell differentiation, extravasation of leukocytes, tumor metastasis, and bacterial or viral infections.¹

Thiosialosides are hydrolytically stable analogues of the native O-glycosides of Neu5Ac that have attracted particular interest from synthetic and medicinal chemists, as biological probes and potential inhibitors of sialic acid recognizing proteins.² Sialic acid thioglycosides are generally synthesized by S-alkylation of a 2-mercapto sialic acid derivative or glycosylation of a thiol with a conventional sialyl donor.³

While anionic addition of 1-thiolates to sugar enones (termed thiol Michael addition)⁴ or free-radical addition of thiols to alkenes (termed thiol–ene coupling)⁵ have already been successfully applied to generate thioglycosidic linkages, the neuraminic acid thiol has not been incorporated within these strategies until now. Recently, the radical-mediated hydrothiolation of terminal alkyne, which serves to introduce two thiol fragments across a carbon–carbon triple bond, has been exploited as an approach to the synthesis of dendrimers,⁶ highly cross-linked polymer networks,⁷ or polyfunctional materials.⁸ However, the thiol–yne reaction has not been widely used in the field of carbohydrate chemistry, with only few articles published recently,⁹ none of which report the synthesis of thiosialoside derivatives. Thus, the potential of free-radical hydrothiolation as a powerful, metal-free ligation process for the synthesis of S-linked *N*-acetylneuraminic acid derivatives remains to be established.

Herein, we report on the synthesis of thiosialosides by photoinduced hydrothiolation of allyl- and propargyl-functionalized galactose derivatives with the peracetylated 2-mercapto sialic acid **1**.

The reaction of the easily available 6-*O*-allyl substituted galactose derivative **2**¹⁰ with thiol **1**¹¹ was investigated initially, in the presence of the cleavable photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone (DPAP)¹² at room temperature by irradiation with UV lamp ($\lambda = 365$ nm).¹³ Using a thiol/ene ratio of 2:1 the reaction went to completion after 30 minutes affording the thio-linked pseudo-disaccharide **3**¹⁴ in 85% isolated yield (Scheme 1).



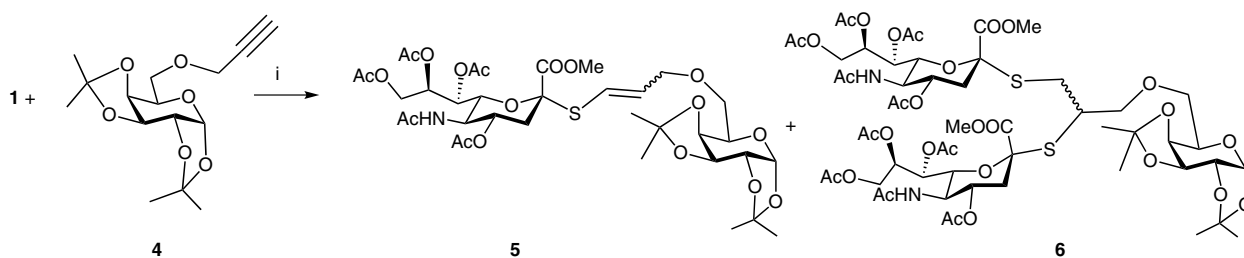
Scheme 1 Reagents and conditions: **1** (2 equiv), DPAP (2 × 0.1 equiv), toluene, r.t., $h\nu$ (365 nm), 2 × 15 min (85%).

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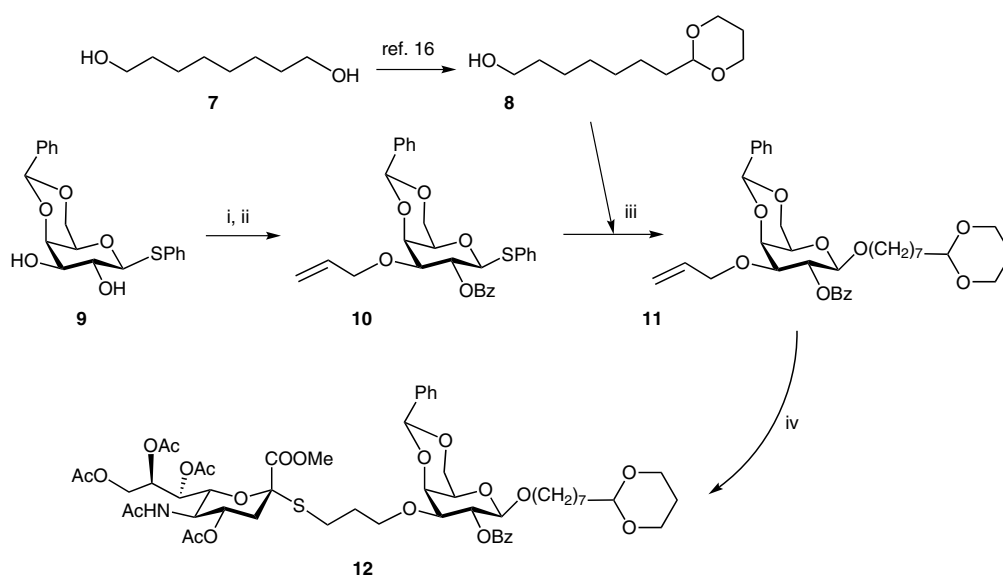


Scheme 2 Reagents and conditions: **1** (4 equiv), DPAP (2×0.1 equiv), $h\nu$ (365 nm), toluene, r.t., 90 min (82% for **5**, 11% for **6**).

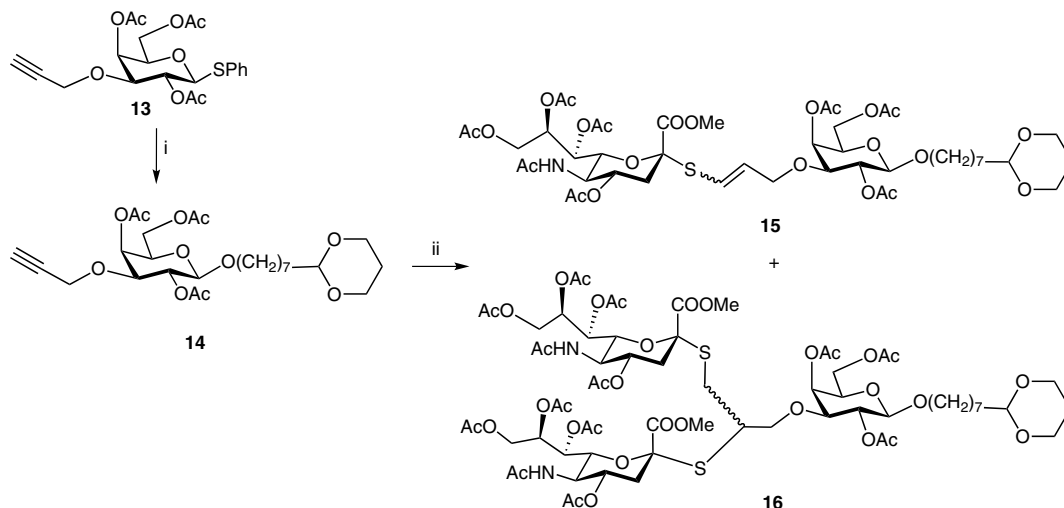
Then, photoinduced reaction¹³ between thiol **1** and the 6-O-propargylated galactose derivative **4**¹⁵ was examined. It is important to note that the radical thiol–yne reaction is a two-step process. The first step involves the addition of a thiol to the C≡C bond to yield an intermediate vinyl sulfide that subsequently undergoes a second, formally thiol–ene, reaction with additional thiol yielding the dithioether with exclusive 1,2-addition mode.^{9a} In our case, with a thiol/yne ratio of 4:1, the formation of two products was observed after 30 minutes, the ratio of which remained unchanged even in prolonged exposure to UV light. The main product turned out to be an inseparable 7:3 mixture of the *E*- and *Z*-isomers of the vinyl sulfide **5**. The ratio of the isomers was determined by ¹H NMR spectroscopy of the mixture based on the coupling constants of the vinyl hydrogens.¹⁶ Despite the high excess of the thiol reagent, the doubly sialylated pseudotrisaccharide **6** was formed as the minor product of the thiol–yne coupling reaction (Scheme 2). Reduced rates and conversions for 1,2-substituted internal enes have been observed previously, which was due to steric considerations and a reversible addition of the thyl radical to the internal ene.¹² The low efficiency of the second addition step between thiol **1** and the disubstituted alkene **5** could also be explained by the reversibility of the reaction and steric hindrance of the major,

thermodynamically more stable, *E*-isomer of the intermediate vinyl sulfide **5**. ¹³C NMR analysis revealed that compound **6** had been obtained as a ca. 1:1 mixture of diastereomers as evidenced by four signals for C-2 of the sialyl residues at $\delta = 82.8$ –83.5 ppm.¹⁷ Unfortunately, our efforts to separate the isomers were unsuccessful due to their very similar chromatographic behavior.

Next, compound **11** bearing a 3-O-allyl group and attached to an acetal-protected octylaldehyde aglycon was prepared to access a mimetic of the $\alpha(2 \rightarrow 3)$ -linked sialosides (Scheme 3). We envisaged that introduction of the aldehyde spacer at the anomeric position would make the pseudodisaccharide able to be conjugated for biological testing via reductive amination, a methodology that is compatible with the carboxylic acid group of the *N*-acetylneuraminic acid. The appropriately protected hydroxylaldehyde **8** was obtained from octane-1,8-diol (**7**) by a literature procedure.¹⁸ The synthesis of galactoside **11** was accomplished from diol **9**¹⁹ in three steps. Dibutylstannylene-mediated regioselective alkylation²⁰ and subsequent benzoylation afforded thiogalactoside **10**, reaction of which with the masked hydroxylaldehyde **8** upon NIS–TfOH activation provided **11**. Photoinduced free-radical addition of thiol **1** to the 3-O-allylated galactoside



Scheme 3 Reagents and conditions: i) 1. Bu₂SnO, toluene, reflux, 3 h; 2. allyl bromide, CsF, DMF, 12 h (76%); ii) BzCl, pyridine, 3 h, 0 °C (82%); iii) NIS–TfOH, CH₂Cl₂, 4 Å MS, 0 °C, 30 min (54%); iv) **1** (2 equiv), DPAP (2×0.1 equiv), toluene, r.t., $h\nu$ (365 nm), 2×15 min (85%).

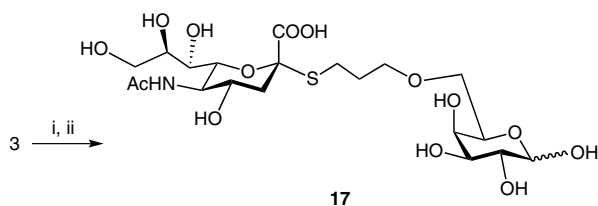


Scheme 4 Reagents and conditions: i) **8** (1.5 equiv), NIS–TfOH, CH₂Cl₂, 4 Å MS, 0 °C, 30 min (46%); ii) **1** (4 equiv), DPAP (2 × 0.1 equiv), *hν* (365 nm), toluene, r.t., 2 × 15 min (82% for **15**, 12% for **16**).

11 proceeded efficiently to produce the thiosialomimetic **12**²¹ in 85% isolated yield.

Finally, the 3-O-propargylated galactoside **14** was prepared via NIS–TfOH promoted glycosylation of **8** with thioglycoside **13**²² and subjected to photoinduced coupling with thiol **1** using a thiol/yne ratio of 4:1. The outcome of the reaction was similar to that observed for hydrothiolation of **4**, showing the vinyl sulfide derivative **15** as the major product. The ¹H NMR analysis of **15** revealed, again, the presence of two diastereomers with the prevalence of the *E*-isomer.²³ Compound **16** as a doubly sialylated mimetic of the α(2→3)-linked sialoside was obtained in 12% yield (Scheme 4).

Deprotection of the sialylmimetics was demonstrated on pseudodisaccharide **3**. Zemplén deacetylation followed by removal of the isopropylidene groups with HCl in methanol and subsequent hydrolysis of the carboxylic ester provided compound **17** in 27% overall yield (Scheme 5).



Scheme 5 Reagents and conditions: i) NaOMe, MeOH, overnight (82%); ii) 1 M HCl, MeOH, 2 d, 50 °C (52%); iii) 0.2 M NaOH, 1,4-dioxane (64%).

In conclusion, thiol–ene and thiol–yne couplings have been used for the first time to achieve thio-linked mono- and bivalent mimetics of the α(2→3)- and α(2→6)-linked sialosides. The thiol–ene reactions took place by click-type chemistry affording the pseudodisaccharides under mild conditions and with excellent yields. During hydrothiolation of the propargyl moiety with the 2-mercapto si-

alic acid, incomplete reaction in the second addition step and lack of diastereoselectivity were observed. Nevertheless, the thiol–yne approach provides an easy access to novel vinyl sulfide type or dually glycosylated sialyl mimetics of biological interest. Further studies of these types of compounds are in progress in our laboratory.

Acknowledgment

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Supporting Information for this article is available online at <http://www.thieme-connect.com/ejournals/toc/synlett>.

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- (13) **General Method for the Photoinduced Addition of 2-Mercapto Sialic Acid 1 to Alkenes 2 and 8 and Alkynes 4 and 11**
To a solution of the starting alkene or alkyne (1.00 mmol) in dry toluene (5 mL), thiol (2.0–4.0 equiv) and 2,2-dimethoxy-2-phenylacetophenone (DPAP, 2 × 25 mg, 2 × 0.10 mmol) were added. The solution was deoxygenated and irradiated with a UV lamp ($\lambda = 365\text{nm}$) at r.t. for 2 × 15 min. Then the solution was concentrated, and the residue was purified by column chromatography.
- (14) **NMR Data for Compound 3**
 ^1H NMR (500 MHz, CDCl_3): $\delta = 5.52$ (d, 1 H, H-1, $J_{1,2} = 5.0$ Hz), 5.34–5.31 (m, 3 H), 5.29 (d, 1 H, $J = 10.5$ Hz, NH), 4.89–4.83 (m, 1 H, H-4'), 4.59 (dd, 1 H, $J_1 = 8.0$ Hz, $J_2 = 2.5$ Hz, H-5'), 4.32–4.29 (m, 2 H), 4.25 (dd, 1 H, $J_1 = 8.0$ Hz, $J_2 = 2.0$ Hz), 4.11 (dd, 1 H, $J_1 = 12.5$ Hz, $J_2 = 4.5$ Hz), 4.07–4.01 (m, 1 H), 3.95–3.93 (m, 1 H), 3.83–3.80 (m, 1 H), 3.79 (s, 3 H, COOCH_3), 3.64–3.48 (m, 3 H), 2.77–2.64 (m, 4 H), 2.15, 2.13, 2.04, 2.03 (4 × s, 12 H, 4 × CH_3 , Ac), 1.87 (s, 3 H, CH_3 , NHAc), 1.85–1.77 (m, 2 H), 1.54, 1.44, 1.34, 1.33 (4 × s, 12 H, 4 × CH_3) ppm. ^{13}C NMR (125 MHz, CDCl_3): $\delta = 170.9$ – 168.5 (6 × CO), 109.1, 108.4 [2 × (CH_3)₂C], 96.3 (C-1), 83.2 (C-2'), 74.1, 71.1, 70.6, 69.6, 68.7, 67.3, 66.6 (C-2, C-3, C-4, C-5, C-4', C-6', C-7', C-8'), 69.7, 69.4 (C-6, OCH_2), 62.1 (C-9'), 52.9 (OCH_3), 49.3 (C-5'), 38.0 (C-3'), 29.4, 25.9 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{S}$), 26.0, 25.7, 24.9, 24.4, 23.1 (4 × CH_3 , Ac , CH_3 , NHAc), 21.1, 20.8, 20.7 (4 × CH_3) ppm.
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- (16) **NMR Data for Compound 5**
 ^1H NMR (500 MHz, CDCl_3): $\delta = 6.43^*$ (d, 0.7 H, $J = 15.5$ Hz, $\text{SCH}=\text{CHCH}_2$), 6.41^{*} (d, 0.3 H, $J = 8.5$ Hz, $\text{SCH}=\text{CHCH}_2$), 5.93–5.88 (m, 1 H, $\text{SCH}=\text{CHCH}_2$), 5.59–5.52 (m, 2 H), 5.39–5.31 (m, 3 H), 4.89–4.85 (m, 1 H), 4.60–4.57 (m, 1 H), 4.32–4.24 (m, 3 H), 4.16–4.03 (m, 4 H), 3.96–3.88 (m, 2 H), 3.85–3.78 (m, 4 H), 3.63–3.53 (m, 2 H), 2.76–2.71 (m, 1 H), 2.20–1.87 (m, 15 H), 1.54, 1.53, 1.44, 1.43, 1.34, 1.33, 1.32 (7 × s, 12 H, CH_3 , ip) ppm. ^{13}C NMR (125 MHz, CDCl_3): $\delta = 170.7$, 170.5, 170.2, 169.9, 168.0, 167.9 (CO), 132.3, 128.5, 121.2, 120.3 ($\text{SCH}=\text{CHCH}_2$), 109.0, 108.4 [(CH_3)₂C], 96.2 (C-1), 84.2, 83.3 (2 × C-2'), 75.0, 74.3, 74.2, 71.0, 70.5, 70.4, 70.2, 69.5, 69.4, 68.9, 68.5, 67.6, 67.3, 67.2, 66.7, 66.6 (skeleton carbons), 71.1, 68.8, 68.5, 67.7, 62.1, 62.0 (C-6, C-9', CH_2), 53.0, 52.9 (2 × COOCH_3), 49.0 (2 × C-5'), 37.5, 37.3 (2 × C-3'), 25.9, 25.8, 24.8, 24.3, 22.9, 21.0, 20.6 (CH_3 , Ac , CH_3 , NHAc , CH_3) ppm; *overlapping signals.
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- (21) **NMR Data for Compound 12**
 ^1H NMR (360 MHz, CDCl_3): $\delta = 8.06$ – 7.26 (m, 10 H, arom.), 5.60–5.43 (m, 3 H), 5.30–5.27 (m, 2 H), 5.14–5.12 (m, 1 H), 4.85–4.75 (m, 2 H), 4.57 (d, 1 H, $J = 7.9$ Hz), 4.50 (t, 1 H, $J = 10.0$ Hz), 4.45–4.35 (m, 3 H), 4.29–4.24 (m, 1 H), 4.15–3.87 (m, 8 H), 3.80–3.49 (m, 16 H), 2.60–2.54 (m, 2 H), 2.14–1.25 (m, 23 H) ppm. ^{13}C NMR (90 MHz, CDCl_3): $\delta = 170.9$, 170.6, 170.1, 170.0 (6 C, CO), 133.5–126.4 (10 C, arom), 102.3, (2 C, C_{ac}), 101.1 (C-1), 83.3 (C-2'), 78.5, 73.9, 73.2, 69.6, 68.3, 67.1, 66.7, 66.4 (8 C, skeleton carbons), 70.5, 69.3, 69.1, 68.0, 66.8 (5 C, 4 × CH_2 , C-6), 63.0 (C-9'), 52.8 (C-5'), 35.1, 32.7, 29.3, 29.2, 29.1, 25.8, 25.7, 25.5, 23.8, 23.7 (10 C, CH_2), 23.1 (CH_3 , NHAc), 21.2, 20.8 (4 C, CH_3 , Ac) ppm.
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- (23) **NMR Data for Compound 15**
 ^1H NMR (500 MHz, CDCl_3): $\delta = 6.46$ (d, 0.17 H, $J = 9.5$ Hz, $\text{SCH}=\text{CHCH}_2$), 6.35 (d, 0.83 H, $J = 15.5$ Hz, $\text{SCH}=\text{CHCH}_2$), 5.72–5.69 (m, 1 H, $\text{SCH}=\text{CHCH}_2$), 5.48–5.31 (m, 3 H), 5.04–5.01 (m, 1 H), 4.90–4.85 (m, 2 H), 4.51–4.49 (m, 2 H), 4.40–4.17 (m, 3 H), 4.15–4.05 (m, 3 H), 4.01–3.75 (m, 10 H), 3.60–3.42 (m, 3 H), 2.75–2.72 (m, 2 H), 2.22–1.84 (m, 24 H), 1.59–1.28 (m, 14 H) ppm. ^{13}C NMR (125 MHz, CDCl_3): $\delta = 170.7$, 170.5, 170.4, 170.3, 170.2, 170.1, 170.0, 169.8, 169.4, 169.3, 168.1, 167.7 (CO), 130.5, 128.4, 122.2, 121.9 (CH), 102.3 (C_{ac}), 101.2 (C-1), 77.2, 77.0, 76.7, 76.6, 76.1, 74.9, 74.4, 74.1, 70.7, 70.4, 69.8, 69.2, 68.8, 68.0, 67.3, 67.0, 66.7, 66.5, 65.9 (skeleton carbons), 69.5, 69.4, 67.5 (CH_2), 62.2, 62.1, 61.9 (C-6), 53.0 (OCH_3), 49.2, 49.0 (C-5), 37.5, 37.3, 35.1, 29.3, 29.1, 25.74 (CH_2), 25.6, 23.7, 23.0, 21.1, 21.0, 20.9, 20.7, 20.6 (CH_3) ppm.

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