Sialoside clusters as potential ligands for siglecs (sialoadhesins)

Zhonghong Gan and René Roy

Abstract: Clusters of *O*- and *S*-linked α-sialosides with valencies of two to four were constructed to serve as potential multivalent inhibitors towards sialoadhesins (siglecs). Thus, *O*- and *S*-prop-2-ynyl α-sialosides (**3**, **7**), together with 4-iodophenyl sialoside **5** were prepared from acetochloroneuraminic acid derivative **1** using silver salicylate and propargyl alcohol for **3** and phase-transfer catalysis for **5** and **7**, respectively. Oxidative acetylenic homocoupling of **3** and **7** under Glaser conditions (CuCl, O_2) provided 1,3-diynes **8** and **9** in 83–86% yields. Palladium catalyzed cross-coupling of *O*-prop-2-ynyl sialoside **3** with **5** using $Pd_2(dba)_3$ and PPh_3 gave nonsymmetrical dimer **10** (82%). Alternatively, symmetrical clusters were then prepared as above under Sonogashira cross-coupling conditions with 1,4-diiodobenzene (**11**), 1,3,5-triodobenzene (**14**), and finally 1,2,4,6-tetraiodobenzene (**17**) to provide both *O*- and *S*-linked dimers **12** (93%) and **13** (88%), trimers **15** (81%) and **16** (76%), while only *O*-linked tetramer **18** was prepared in 87% yield. Finally, treatment of the *O*-linked prop-2-ynyl sialoside **3** with Grubbs' metathesis catalyst $Cl_2Ru(PCy_3)_2$=CHPh (**19**) gave, as expected, benzeneannulation regioisomeric trimers **20a**, **20b** in 68% yield.

Key words: siglec, sialoadhesins, sialic acid, Sonogashira, palladium cross-coupling.

Résumé : On a préparé une série d'agrégats d'α-sialosides liés par l'oxygène ou le soufre et de valence allant de deux à quatre qui pourraient éventuellement servir d'inhibiteurs multivalents vis-à-vis des sialoadhésines (siglecs). On a ainsi préparé les α-sialosides de *O*- et de *S*-prop-2-ynyle (**3**, **7**) ainsi que le sialoside de 4-iodophényle (**5**) à partir du dérivé **1** de l'acide acétochloroneuraminique, en utilisant le salicylate d'argent et l'alcool propargylique pour le composé **3** et une catalyse de transfert de phase pour les composés **5** et **7** respectivement. L'homocouplage acétylénique oxydant des composés **3** et **7** dans des conditions de Glazer (CuCl, O_2) a permis d'obtenir les composés **8** et **9** avec des rendements allant de 83 à 86%. Un couplage croisé catalysé par le palladium du sialoside de *O*-prop-2-ynyle **3** avec le composé **5**, à l'aide de $Pd_2(dba)_3$ et de PPh_3, fournit le dimère non symétrique **10** (82%). De façon alternative, des agrégats symétriques ont alors été préparés de la façon mentionnée ci-dessus, en utilisant les conditions de couplage croisé de Sonagashira et du 1,4-diiodobenzène (**11**), du 1,3,5-triiodobenzène (**14**) et finalement du 1,2,4,6-tétraiodobenzène (**17**); on a ainsi obtenu des produits liés par l'oxygène et le soufre dont les dimères **12** (93%) et **13** (88%) et les trimères **15** (81%) et **16** (76%) ainsi que le produit **18** (87%) un tétramère lié uniquement à l'oxygène. Enfin, le traitement du sialoside de prop-2-ynyle lié à l'oxygène, **3**, avec le catalyseur de métathèse de Grubbs, $Cl_2Ru(PCy_3)_2$=CHPh (**19**), conduit, tel que prévu, aux trimères de benzèneannellation régioisomérique **20a**, **20b** avec un rendement de 68%.

Mots clés : siglec, sialoadhésines, acide sialique, Sonogashira, couplage croisé au palladium.

Introduction

Sialic acid is a generic term representing over 40 different structures derived from neuraminic acid, a nine-carbon amino acid sugar (1). The most prevalent member, *N*-acetylneuraminic acid, is usually present as a capping residue on natural glycoproteins and glycolipids. As such, it plays the role of a "terminal elaboration factor" (2) and is thus well-suited in mediating molecular recognition at the cell surface, a property well exploited by several pathogens (1). Conversely, siglecs, a subgroup of immunoglobulin-type animal lectins that mediate cell adhesion and signaling, recognize sialoconjugates. Several siglecs are expressed on cells of the innate immune system and sialoadhesin (or siglec-1) is found on macrophages and has been implicated in lymphocyte adhesion and myeloid cell development (3).

Although siglecs are known to possess only one carbohydrate recognition domain (CRD) (4), multivalent sialosides have been shown to dramatically increase their inhibitory potency towards sialoadhesin (5). Given the importance of multivalent glycoforms in the study of carbohydrate–protein interactions at the molecular level (6) and their potential

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Dedicated to the late Raymond U. Lemieux, an outstanding pioneer in carbohydrate chemistry.

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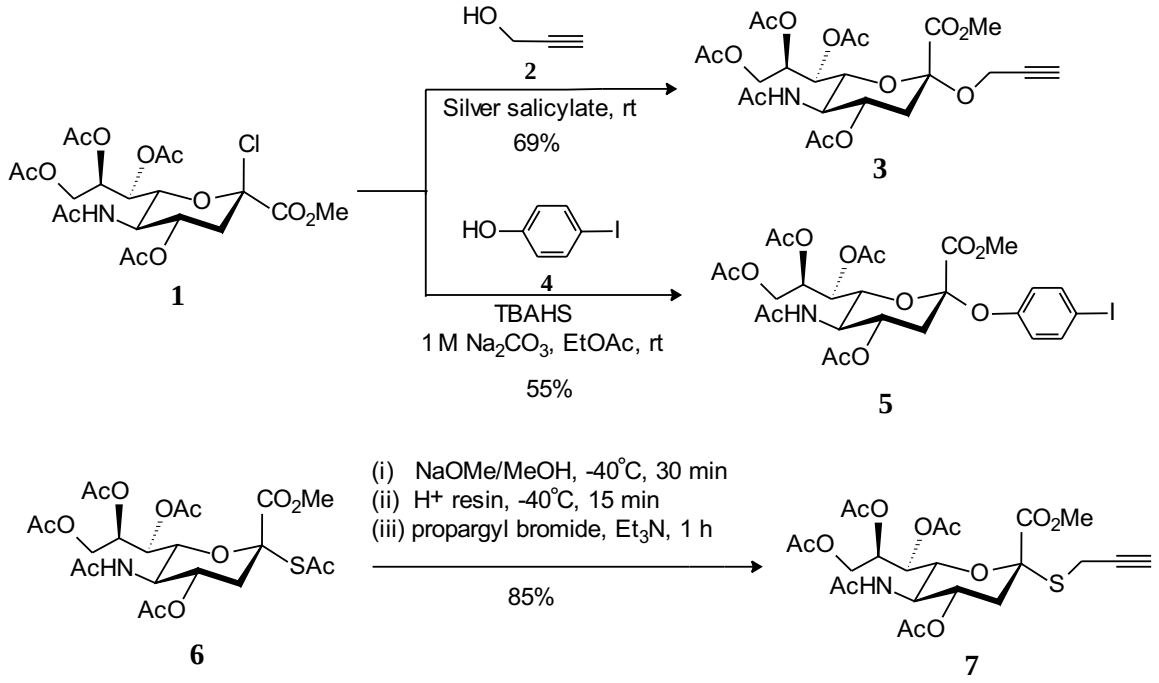
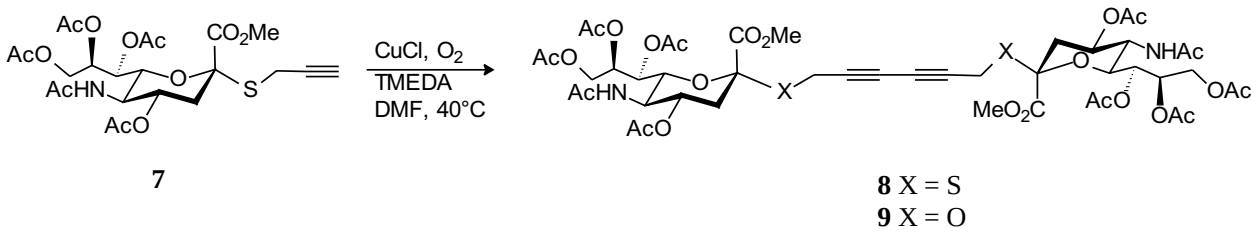
Scheme 1.



Scheme 2.



roles in receptor clustering, we propose herein the synthesis of sialic acid clusters using a variety of metal-catalyzed cross-coupling strategies (7).

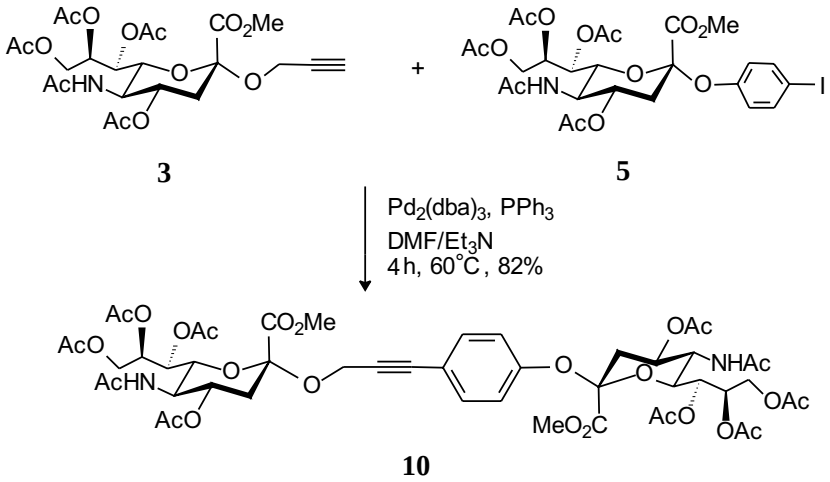
Results and discussion

The syntheses of the requisite prop-2-ynyl α-sialosides **3**, **5**, and **7** necessary for the palladium-catalyzed homo- and cross-coupling reactions are depicted in Scheme 1. Prop-2-ynyl α-*O*-sialoside **3** was readily accessible from β-acetochloroneuraminic acid **1** using propargyl alcohol in the presence of silver salicylate. The Koenigs–Knorr glycosylation occurred with complete stereocontrol giving rise to the α form as previously observed for analogous cases (8). The pure product can be obtained by recrystalizing the crude sample from ethanol (69%). 4-Iodophenyl α-sialoside **5** was also prepared from **1** using improved phase-transfer catalysis (PTC) conditions developed in our laboratory (9). The synthesis of aryl α-sialosyl derivatives was achieved at room temperature in a two-phase system using ethyl acetate, 1 M sodium carbonate, and tetrabutylammonium hydrogensulfate as phase-transfer catalyst. The reaction was entirely stereoselective and provided **5** in 55% yield. Prop-2-ynyl α-thiosialoside **7** was synthesized from the corresponding thioacetate derivative **6**, itself obtained from **1** under PTC conditions (10). Chemoselective de-*S*-acetylation of **6** under low-temperature Zemplén conditions followed by low-temperature quenching by acidic (H^+) resin generated a sialic acid thiol intermediate (10, 11). The thiol intermediate was then coupled to propargyl bromide to give **7** in 85% yield as previously observed from other glycosyl thioacetates (12) (Scheme 1).

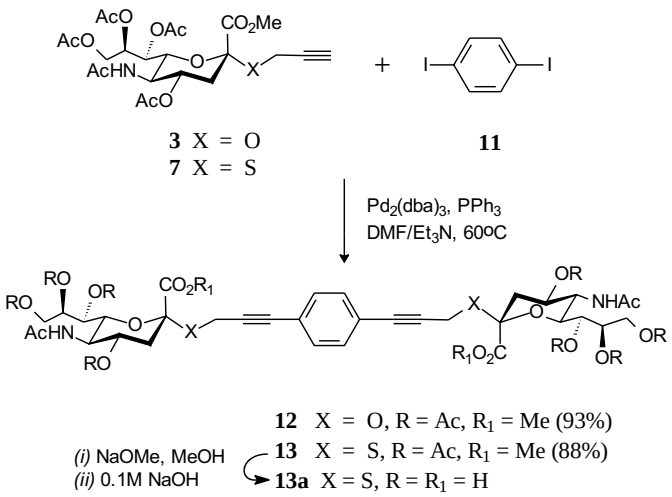
The syntheses of homo-coupling symmetrical diynes can be accomplished by using the Glaser or alternative methods (13). Thus, oxygen was bubbled through a solution of prop-2-ynyl α-sialoside **3**, copper(I) chloride, and TMEDA in DMF at 40°C for 2 h. The desired dimer (**9**) was obtained in 86% yield. The structure of (**9**) was confirmed from its ^{1}H NMR spectrum which did not contain a signal with a chemical shift close to δ 2.24 ppm, the position of the acetylenic proton in the spectrum of the monomer. The mass spectrum of compound (**9**) gave a $M^+ + 1$ peak at 1057.2 Da. This procedure was also successfully applied to prop-2-ynyl α-thiosialoside **7** to afford the corresponding dimer **8** in 83% yield (Scheme 2).

According to our previous studies (14), the use of Sonogashira cross-coupling conditions modified by removal of the copper(I) iodide cocatalyst, can eliminate the formation

Scheme 3.



Scheme 4.



of symmetrical diyne. This new protocol was thus used for the treatment of prop-2-ynyl α-sialoside **3** (1.1 equiv) and 4-iodophenyl *O*-α-sialoside **5** (1 equiv) using tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) as catalyst at 60°C to afford cross-coupling dimer **10** in 82% yield. As expected, no symmetrical diyne (**9**) was found (Scheme 3).

Similarly, treatment of **3** with 1,4-diiodobenzene (**11**, 1 equiv) under the above reaction conditions provided dimer **12** in 93% yield (Scheme 4). Again, no symmetrical diyne (**9**) was observed. The distinction between the homodimer (**9**) and the crossdimer **12** can be clearly distinguished from two marked differences between their respective ^{13}C NMR spectra. The acetylene peaks of the crossdimer **12** were shifted downfield at δ 86.2 and at 85.6 ppm compared with those of the homodimer (**9**) at δ 74.7 and at 70.1 ppm and the spectra of **12** contained peaks in the aromatic region while those of **9** did not. Previous results showed that thioethers could also undergo cross-coupling with aryl halides under Sonogashira conditions (15). Thus, prop-2-ynyl α-thiosialosides **7** reacted with 1,4-diiodobenzene in the presence of $Pd_2(dba)_3$ at 60°C to afford dimer **13** in 88% yield. Fully protected dimer **13** was sequentially hydrolyzed with NaOMe in methanol followed by 0.1 M NaOH to provide water-soluble **13a**.

Encouraged by the successful synthesis of divalent sialoside derivatives by the Sonogashira reaction, we further expanded this reaction toward the synthesis of larger clusters. As already mentioned, the formation of symmetrical diynes as minor by-products that cause purification problems can be avoided using CuI. However, CuI is a particularly effective co-catalyst which allows the reaction to occur at room temperature. Without CuI, the reaction has to be accomplished at higher temperatures and longer reaction times are required. Since sialic acid trimers and tetramers are easily separated from their corresponding symmetrical diynes, the addition of CuI was employed to help the reactions to occur smoothly and rapidly with more hindered tri- or tetraiodobenzenes.

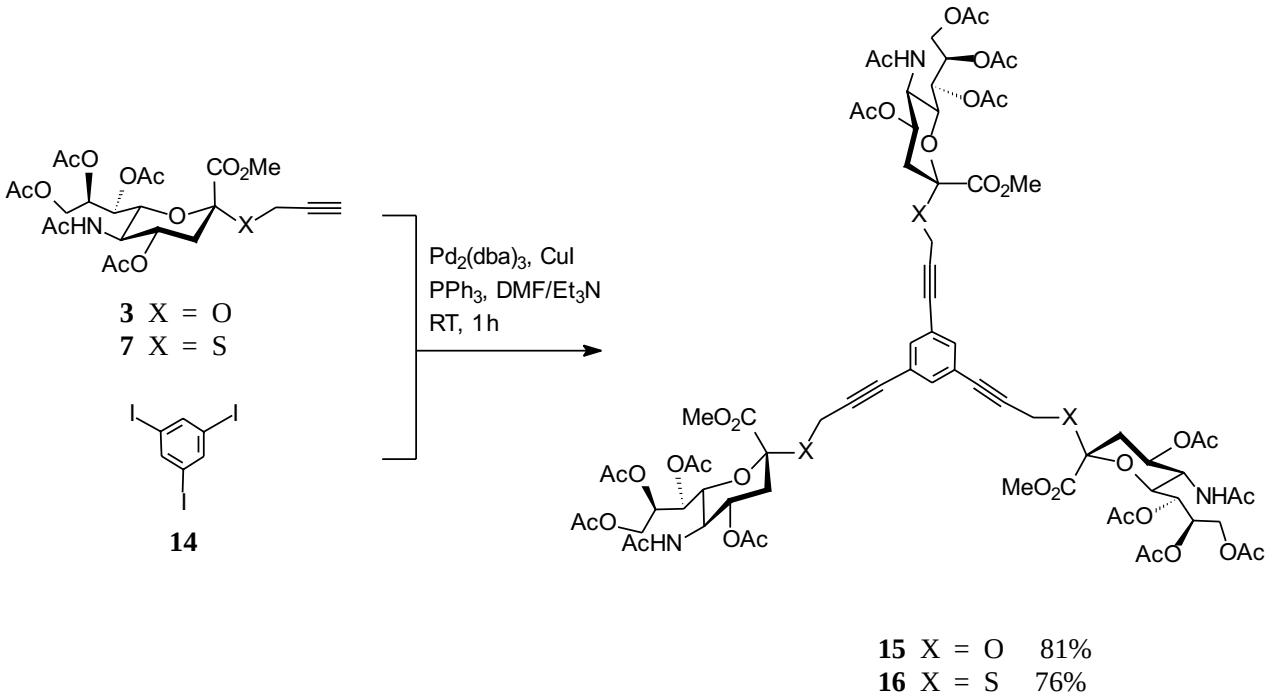
1,3,5-Triiodobenzene (**14**) was prepared from 1,3,5-tribromobenzene following a known procedure (16). Treatment of 1,3,5-tribromobenzene with KI, I_2, and Ni powder in refluxing DMF afforded 1,3,5-triiodobenzene in 52% yield. Direct polyiodination of benzene with periodic acid and iodine in sulfuric acid at room temperature (rt) provided 1,2,4,6-tetraiodobenzene (**17**) in good yield (73%) (17).

Prop-2-ynyl α-sialoside **3** was treated with 1,3,5-triiodobenzene (**14**) ($Pd_2(dba)_3$, CuI, PPh_3, DMF–Et_3N, rt, 1 h) to give trimer **15** (81%). Trimer **15** was readily separated from a small amount of symmetrical diyne (**9**) as a by-product by silica gel chromatography. Trimer **16** was analogously prepared from prop-2-ynyl α-thiosialoside **7** in 76% yield (Scheme 5).

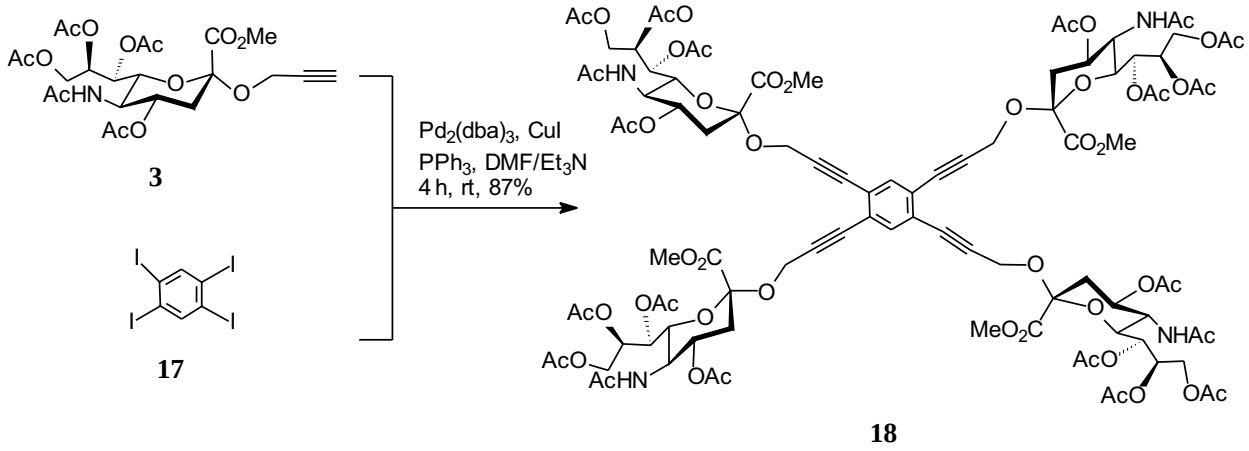
Treatment of prop-2-ynyl α-sialoside **3** with 1,2,4,6-tetraiodobenzene (**17**) using the above conditions gave *O*-linked tetramer **18** in 87% yield (Scheme 6). Because of the steric hindrance, the reaction with tetraiodobenzene needed longer reaction time for completion (4 h). The structure for **18** was confirmed by its ^1H NMR and ^{13}C NMR spectra and from the molar mass determined by FAB-MS.

Grubbs' catalyst **19** can also be used to catalyze intermolecular cyclotrimerization of three terminal alkynes according to our previous findings (18). Prop-2-ynyl α-sialoside **3** was treated with Grubbs' catalyst **19** in refluxing 1,2-dichloroethane for 24 h. The desired regioisomeric trisubstituted

Scheme 5.



Scheme 6.



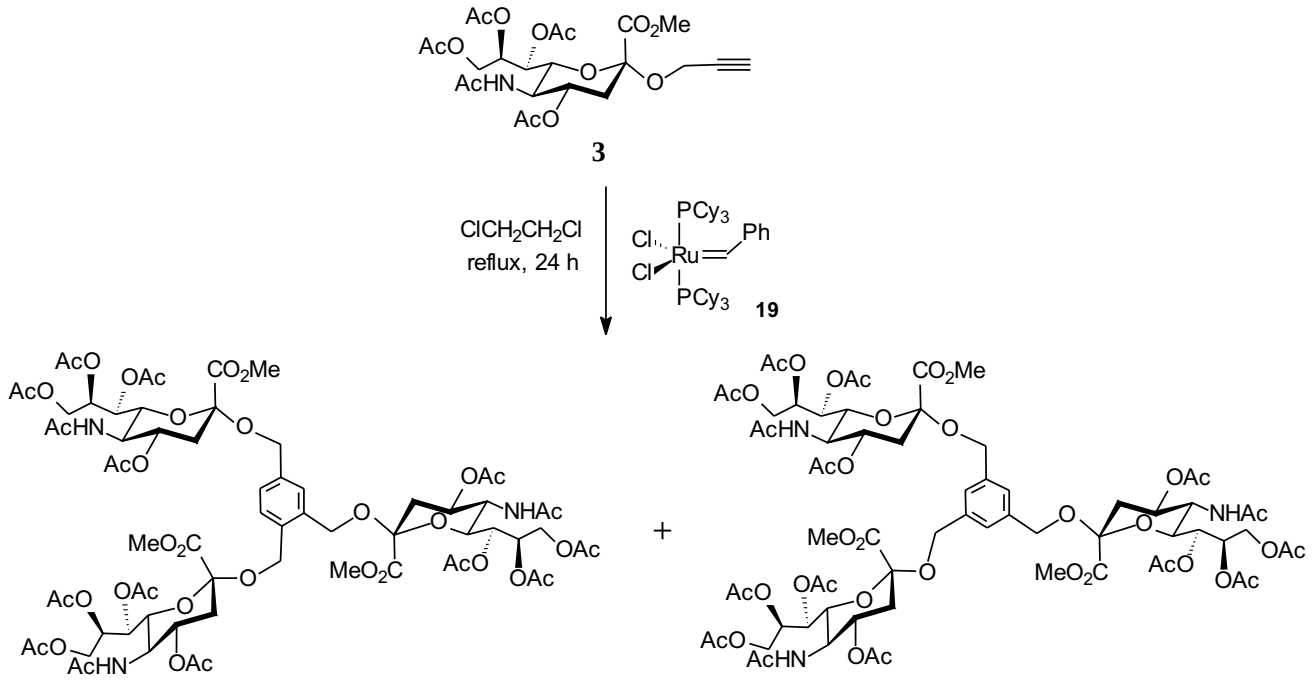
benzene derivatives **20a**, **20b** were isolated as a mixture of 1,2,4-(**20a**) and 1,3,5-(**20b**) regioisomers in 68% yield (Scheme 7). The reaction was found to be highly regioselective in favor of the 1,2,4-regioisomer **20a** over that of **20b** (4.5:1). The regioisomeric structures and the ratio were determined from the ^{1}H NMR spectrum by comparing the data of analogous products reported in the literature (18, 19). The aromatic protons for the 1,2,4-isomer **20a** appeared at δ 7.27 ppm (d, J = 7.8 Hz), 7.23 (s), and 7.19 (d, J = 7.8 Hz) and for the symmetrical 1,3,5-isomer **20b** as a singlet at δ 7.14 ppm. The ^{13}C NMR showed aromatic carbon signals at 136.4, 135.4, 135.0 ppm (quaternary carbons) and 128.7, 127.9, 127.1 ppm (tertiary carbons) for the 1,2,4-isomers and those for 1,3,5-isomer appeared at 137.2 ppm (quaternary carbon) and at 126.4 ppm (tertiary carbon). This novel procedure is therefore complementary to that using dicobalt octacarbonyl [$Co_2(CO)_8$] (19).

Experimental section

General methods

^{1}H and ^{13}C NMR were recorded on a Bruker AMX500 spectrometer at 500 MHz for protons and 125.7 MHz for carbons, respectively. Proton chemical shifts (δ) are given relative to internal chloroform (7.24 ppm) for $CDCl_3$. For carbon spectra, chemical shifts were given relative to $CDCl_3$ (77.0 ppm). Spectral analyses were performed as first-order approximations and were based on correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and one- and two-dimensional distortionless

Scheme 7.



enhancement by polarization transfer (DEPT) experiments. Optical rotations were measured on a PerkinElmer 241 polarimeter. FAB-MS spectra were recorded on a Kratos Concepts IIH with Cs^+ beam and are not high resolution unless stated otherwise. Thin layer chromatography (TLC) was performed using silica gel 60-F_{254} glass plates. Reagents used for developing plates include ceric sulfate (1% w/v) and ammonium sulfate (2.5% w/v) in 10% (v/v) aqueous sulfuric acid, iodine, dilute aqueous potassium permanganate, and UV light. TLC plates were heated to approximately 150°C when necessary. Purifications were performed by gravity or flash chromatography on silica gel 60 (230–400 mesh, E. Merck no. 9385). Solvents were evaporated under reduced pressure using a Büchi rotary evaporator connected to a water aspirator. All chemicals used in the experiments were of reagent grade. Solvents were purified by the published procedures.

*Methyl (prop-2-ynyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-*α*-*D*-galacto-2-nonulopyranosid) onate (3)*

To a solution of freshly prepared β-acetochloroneuraminic acid **1** (1.18 g, 2.31 mmol) in dry propargyl alcohol (20 mL) containing 4 Å molecular sieves (2 g) was added freshly prepared silver salicylate (0.85 g, 3.47 mmol). The reaction was stirred for 2 h under nitrogen at room temperature in the dark. The slurry was filtered over Celite, washed with dichloromethane, and evaporated to dryness. The residue was dissolved in dichloromethane (30 mL). The organic solution was successively washed with sat. $NaHCO_3$ solution, 5% $Na_2S_2O_3$ solution, and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude syrup was crystallized from ethanol to afford pure **3** (840 mg, 69%) as a colorless solid: mp 162–164°C; $[\alpha]_D$ +69.4° (*c* 0.5, CH_3CN). HR-MS calcd. for $C_{23}H_{32}N_1O_{13}(MH^+)$ (*m*/*z*): 530.1874; found: 530.1895. ^{1}H NMR ($CDCl_3$) δ: 5.44 (d, 1H, $J_{5,NH}$ = 9.0 Hz, NH), 5.34 (m, 1H, H-8), 5.25 (dd, 1H, $J_{6,7}$ = 1.3 Hz, $J_{7,8}$ = 8.3 Hz, H-7), 4.80 (m, 1H, H-4), 4.35 (dd, 1H, *J* = 15.7 Hz, -OC*H*$_2$C≡), 4.22 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, $J_{8,9a}$ = 2.7 Hz, H-9a), 4.10 (dd, 1H, -OC*H*$_2$C≡), 4.04–3.97 (m, 3H, H-5, H-6, H-9b), 3.75 (s, 3H, OCH_3), 2.57 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.39 (dd, 1H, *J* = 2.4 Hz, -C≡C*H*), 2.09, 2.07, 1.98, 1.96, 1.81 (s, 15H, OAcs, NHAc), 1.91 (dd, 1H, $J_{3a,4}$ = 12.5 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.8–170.0 (O=*C*s), 167.8 (C-1), 98.2 (C-2), 78.9 (-*C*≡CH), 74.4 (-C≡*C*H), 72.6 (C-6), 68.8 (C-4), 68.4 (C-8), 67.2 (C-7), 62.3 (C-9), 52.8 (OCH_3), 52.7 (-O*C*H$_2$C≡), 49.2 (C-5), 37.8 (C-3), 23.0, 21.0, 20.9, 20.7, 20.6 (NHAc, OAcs).

*Methyl (4-iodophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-*α*-*D*-galacto-2-nonulopyranosid) onate (5)*

To a solution of freshly prepared acetochloroneuraminic acid **1** (680 mg, 1.33 mmol) in EtOAc (20 mL) was added a solution of *p*-iodophenol (587 mg, 2.67 mmol) and TBAHS (453 mg, 1.33 mmol) in 1 M Na_2CO_3 (20 mL). The mixture was stirred vigorously for 1 h at room temperature and next diluted with EtOAc (20 mL). The organic phase was separated and washed with brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2–EtOH (30:1, v/v) as eluent to give compound **5** (509 mg, 55%) as a white solid, mp 88–90°C; $[\alpha]_D$ +10.8° (*c* 1.0, $CHCl_3$). FAB-MS calcd. for $C_{26}H_{32}IO_{13}N_1$: 693.45; found: 694.1 ($[M + 1]^+$, 33.9%). ^{1}H NMR ($CDCl_3$) δ: 7.49

(d, 2H, J = 8.8 Hz, aromatic H), 6.76 (d, 2H, aromatic H), 5.58 (bs, 1H, NH), 5.29 (m, 2H, H-7, H-8), 4.87 (m, 1H, H-4), 4.38 (d, 1H, $J_{5,6}$ = 10.8 Hz, H-6), 4.21 (d, 1H, $J_{9a,9b}$ = 12.6 Hz, H-9a), 4.09–4.01 (m, 2H, H-9b, H-5), 3.59 (s, 3H, OCH_3), 2.62 (dd, 1H, $J_{3a,3e}$ = 12.9 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.14 (dd, 1H, $J_{3a,4}$ = 12.5 Hz, H-3a), 2.08, 2.06, 1.99, 1.97, 1.84 (s, 15 H, OAcs, NHAc). ^{13}C NMR ($CDCl_3$) δ: 170.8–170.0 (O=Cs), 168.0 (C-1), 153.7, 138.2, 121.8, 87.1 (aromatic C), 99.8 (C-2), 73.4 (C-6), 69.1 (C-8), 68.6 (C-4), 67.3 (C-7), 62.0 (C-9), 52.9 (OCH_3), 49.2 (C-5), 38.1 (C-3), 23.0, 20.9, 20.7, 20.6 (NHAc, OAcs).

*Methyl (prop-2-ypnyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-*D*-glycero-α-*D*-galacto-2-nonulopyranosid) onate (7)*

To a solution of thioacetate sialoside **6** (220 mg, 0.40 mmol) in dry methanol (10 mL), cooled to –40°C, was added a 1 M solution of sodium methoxide (380 μL, 0.38 mmol). The mixture was stirred at –40°C under nitrogen for 30 min. The solution was then neutralized with Amberlite IR-120 H^+ resin at –40°C for 15 min. The solution was filtered. To the filtrate were added prapargyl bromide (80% in toluene, 67 μL, 0.60 mmol) and triethyl amine (112 μL, 0.8 mmol). The solution was stirred at room temperature for 1 h. The solution was poured then into water and exacted by dichloromethane (3 × 10 mL). The combined organic layers were washed with 5% HCl and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The compound **7** (185 mg, 85%) was purified by recrystallization from CH_2Cl_2–hexanes as white solids, mp 186 to 187°C; $[\alpha]_D$ +51° (*c* 0.2, CH_3CN). HR-MS calcd. for $C_{23}H_{32}NO_{12}S$ (MH^+) (*m*/*z*): 546.1645; found: 546.1653. ^{1}H NMR ($CDCl_3$) δ: 5.37 (m, 1H, H-8), 5.31–5.27 (m, 2H, NH, H-7), 4.84 (m, 1H, H-4), 4.25 (dd, 1H, $J_{9a,9b}$ = 12.5 Hz, $J_{8,9a}$ = 2.7 Hz, H-9a), 4.08–3.99 (m, 2H, H-9b, H-5), 3.84 (dd, 1H, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 2.2 Hz, H-6), 3.79 (s, 3H, OCH_3), 3.41, 3.40 (d, 2H, J = 2.6 Hz, -SCH_2C=), 2.69 (dd, 1H, $J_{3a,3e}$ = 12.7 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.14 (t, 1H, -C≡C*H*), 2.13, 2.09, 2.00, 1.99, 1.83 (s, 15H, OAcs), 1.95 (dd, 1H, $J_{3a,4}$ = 12.3 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.8–170.0 (O=Cs), 168.0 (C-1), 82.7 (C-2), 79.5 (-*C*≡CH), 74.2 (C-6), 70.6 (-C≡*C*H), 69.4 (C-4), 68.4 (C-8), 67.2 (C-7), 62.2 (C-9), 53.0 (OCH_3), 49.2 (C-5), 37.4 (C-3), 23.1, 21.1, 20.7, 20.7 (NHAc, OAcs), 16.8 (-S*C*H_2C≡).

General procedure for the homo-coupling of acetylenic thiosugar by Glaser reaction

In a mixture of thiosugar (1 mmol), CuCl (0.3 mmol), and TMEDA (0.6 mmol) in *N*,*N*-dimethylformamide (10 mL) was bubbled O_2 at 40°C for 2 h. Then the brownish green solution was poured into water (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography.

General procedure for the cross-coupling of acetylenic thiosugar by Sonogashira reaction

To acetylenic thiosugar (1 mmol) and 1,4-diiodobenzene (0.45 mmol) in a degassed solution of DMF and Et_3N (5 mL, 1:1, v/v) were added $Pd_2(dba)_3$ (0.05 mmol) and PPh_3 (0.1 mmol). The solution was stirred at 60°C under nitrogen atmosphere for 2 h. The solution was diluted with CH_2Cl_2 (20 mL), and then washed successively with 5% HCl and brine. The organic phase was dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography.

1,3,5-Triiodobenzene (14)

1,3,5-Tribromobenzene (1.10 g, 3.5 mmol), KI (3.50 g, 21 mmol), Ni powder (2.00 g), I_2 (5.10 g), and DMF (12.5 mL) were added into a 50 mL round-bottomed flask. The flask was evacuated on the vacuum line at 0°C for 15 min. The mixture was refluxed under N_2 for 3 h. After cooling, the solution was poured into a separation funnel. The flask was washed with 3% aq HCl (50 mL) and CH_2Cl_2 (50 mL) until all material, except for Ni powder, was transferred into the separation funnel. The CH_2Cl_2 layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic phase was washed with distilled water and dried over Na_2SO_4. The solvent was evaporated, leaving a light-brown crude product. This was further purified by sublimation at 60°C overnight. The residue was sublimed at 120–140°C to afford compound **15** (0.83 g, 52%).^{13}C NMR ($CDCl_3$) δ: 144.4, 95.2 (lit. (16) value).

1,2,4,5-Tetraiodobenzene (17)

H_5IO_6 (1.59 g, 6.97 mmol) was dissolved with stirring in conc. H_2SO_4 (25 mL), iodine (5.20 g, 20.5 mmol) was crushed and added to the clear solution. After 30 min of stirring, the dark mixture was placed in an ice bath. Benzene (0.95 g, 12.20 mmol) was then added slowly. The reaction was allowed to stir at room temperature for 2 days. The mixture was poured onto crushed ice. The resulting solid was collected by suction filtration and washed well with methanol to remove iodine. The crude lavender powder was crystallized from 2-methoxyethanol, giving white needles **17** (5.16 g, 73%), mp 253 to 254°C (lit. (17) value mp 253°C). ^{1}H NMR ($CDCl_3$) δ: 7.98 (s, 2H, aromatic H).

General procedure for the homo-coupling of acetylenic sialic acid derivatives (3, 7)

In a mixture of propargyl glycoside (0.2 mmol), CuCl (0.06 mmol), and TMEDA (0.12 mmol) in DMF (5 mL) was bubbled O_2 at 40°C. Then the brownish green solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with 5% HCl and brine, dried overNa_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography CH_2Cl_2–MeOH (20:1, v/v) as eluent.

*1,6-Di-*S*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-hex-2,4-di-yne (8)*

Reaction time: 3 h; yield: 83%; mp 131 to 132°C, $[\alpha]_D$ +131.7° (*c* 0.6, $CHCl_3$). FAB-MS calcd.: 1089.12; found: 1089.2 (M^+, 12.2%). ^{1}H NMR ($CDCl_3$) δ: 5.40 (d, 2H, $J_{5,NH}$ = 10.1 Hz, NH), 5.33 (m, 2H, H-8), 5.25 (dd, 2H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 8.6 Hz, H-7), 4.80 (m, 2H, H-4), 4.20 (dd, 2H, $J_{9a,9b}$ = 12.5 Hz, $J_{8,9a}$ = 2.7 Hz, H-9a), 4.02 (dd, 2H, $J_{8,9b}$ = 5.5 Hz, H-9b), 3.99 (ddd, 2H, $J_{4,5}$ = 10.6 Hz, H-5), 3.83 (dd, 2H, $J_{5,6}$ = 10.8 Hz, H-6), 3.78 (s, 6H, OCH_3), 3.51 (ABq,

2H, J = 16.9 Hz, -SCH_2C≡), 3.38 (ABq, 2H, -SCH_2C≡), 2.65 (dd, 2H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.11, 2.07, 1.98, 1.96, 1.81 (s, 30H, OAcs, NHAc), 1.89 (dd, 2H, $J_{3a,4}$ = 12.2 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.7–170.0 (O=Cs), 167.9 (C-1), 82.5 (C-2), 74.2 (-SCH_2*C*≡C-), 74.1 (C-6), 69.3 (C-4), 68.2 (C-8), 67.1 (C-7, -SCH_2C≡*C*-), 62.2 (C-9), 53.3 (OCH_3), 49.1 (C-5), 37.4 (C-3), 23.0, 21.1, 20.7, 20.6, 20.6 (NHAc, OAcs), 17.6 (-SCH_2C≡). Anal. calcd. for $C_{46}H_{60}N_2O_{24}S_2$: C 50.73, H 5.55, N 2.57; found: C 50.83, H 5.69, N 2.56.

*1,6-Di-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylonate)-hex-2,4-di-yne (9)*

Reaction time: 2 h; yield: 86%; mp 118–120°C, $[\alpha]_D$ +23.0° (*c* 1.0, $CHCl_3$). FAB-MS calcd.: 1057.0; found: 1057.2 (M^+, 2.3%). ^{1}H NMR ($CDCl_3$) δ: 5.39–5.31 (m, 4H, NH, H-8), 5.24 (dd, 2H, $J_{6,7}$ = 1.7 Hz, $J_{7,8}$ = 8.6 Hz, H-7), 4.80 (m, 2H, H-4), 4.36 (ABq, 2H, J = 16.2 Hz, -OCH_2C≡), 4.22 (ABq, 2H, -OCH_2C≡), 4.21 (dd, 2H, $J_{9a,9b}$ = 12.4 Hz, $J_{8,9a}$ = 2.7 Hz, H-9a), 4.03–3.98 (m, 6H, H-5, H-6, H-9b), 3.76 (s, 6H, OCH_3), 2.55 (dd, 2H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.09, 2.08, 1.98, 1.97, 1.81 (s, 30 H, OAcs, NHAc), 1.88 (dd, 2H, $J_{3a,4}$ = 12.6 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.8–169.9 (O=Cs), 167.7 (C-1), 97.9 (C-2), 74.7 (-OCH_2*C*≡C-), 72.6 (C-6), 70.1 (-OCH_2C≡*C*-), 68.8 (C-4), 68.2 (C-8), 67.1 (C-7), 62.3 (C-9), 53.1 (-O*C*H_2C≡), 52.9 (OCH_3), 49.2 (C-5), 37.7 (C-3), 23.0, 21.0, 20.7, 20.7, 20.6 (NHAc, OAcs). Anal. calcd. for $C_{46}H_{60}N_2O_{26}$: C 52.26, H 5.72, N 2.65; found: C 51.92, H 5.75, N 2.65.

*Methyl [4-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyloxyonate prop-2-ynyl) phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosid] onate (10)*

To a degassed solution of the propargyl sialic acid **3** (58.7 mg, 0.11 mmol) and iodophenyl sialic acid **5** (70 mg, 0.1 mmol) in DMF–Et_3N (4 mL, 1:1) were added $Pd_2(dba)_3$ (5.0 mg, 5.5 μmol) and PPh_3 (5.8 mg, 22 μmol). The mixture was stirred under N_2 at 60°C for 4 h. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with 5% HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (20:1, v/v) as eluent to afford dimer (91 mg, 82%): mp 120 to 121°C, $[\alpha]_D$ +20° (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.29 (d, 2H, J = 8.6 Hz, aromatic H), 6.93 (d, 2H, aromatic H), 5.47–5.25 (m, 6H, NH, NH′, H-7, H-7′, H-8, H-8′), 4.89 (m, 1H, H-4′), 4.82 (m, 1H, H-4), 4.55 (ABq, 2H, J = 15.6 Hz, -OCH_2C≡), 4.41 (dd, 1H, $J_{5',6'}$ = 10.3 Hz, $J_{6',7'}$ = 1.1 Hz, H-6′), 4.29 (ABq, 2H, -OCH_2C≡), 4.25 (dd, 1H, $J_{9a,9b}$ = 12.4 Hz, $J_{8,9a}$ = 2.8 Hz, H-9a), 4.23 (d, 1H, $J_{9a',9b'}$ = 12.6 Hz, H-9a′), 4.10–4.02 (m, 5H, H-5, H-5′, H-6, H-9b, H-9b′), 3.75 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 2.64 (dd, 1H, $J_{3a',3e'}$ = 12.9 Hz, $J_{3e',4'}$ = 4.6 Hz, H-3e′), 2.60 (dd, 1H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.17 (dd, 1 H, $J_{3a',4}$ = 12.6 Hz, H-3a′), 2.12, 2.09, 2.08, 2.07, 2.00, 1.99, 1.99, 1.98, 1.85, 1.83 (s, 30 H, OAcs, NHAcs), 1.96 (dd, 1H, $J_{3a,4}$ = 12.5 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.9–169.9 (O=Cs), 168.0 (C-1′), 167.9 (C-1), 154.0, 133.0, 119.4, 117.8 (aromatic C), 99.9 (C-2′), 98.0 (C-2), 85.6 (-OCH_2*C*≡C-), 83.8 (-OCH_2C≡*C*-), 73.4 (C-6′), 72.6 (C-6), 69.1 (C-8′), 68.9 (C-4), 68.6 (C-4′), 68.4 (C-8), 67.3 (C-7′), 67.2 (C-7), 62.3 (C-9), 62.0 (C-9′), 53.6 (-O*C*H_2C≡), 52.9 (OCH_3, OCH_3′), 49.2 (C-5, C-5′), 38.2 (C-3′), 37.9 (C-3), 23.1, 21.0, 20.9, 20.7, 20.6 (NHAcs, Oacs). Anal. calcd. for $C_{49}H_{62}N_2O_{26}$: C 53.75, H 5.71, N 2.56; found: C 53.63, H 5.76, N 2.59.

General procedure for the cross-coupling of acetylenic sialic acid derivatives with 1,4-diiodobenzene (11)

To a degassed solution of the prop-2-ynyl sialosides (**3**, **7**) (0.2 mmol) and 1,4-diiodobenzene (**11**) (30 mg, 91 μmol) in DMF–Et_3N (4 mL, 1:1) were added $Pd_2(dba)_3$ (9.2 mg, 10 μmol) and PPh_3 (10.5 mg, 40 μmol). The mixture was stirred under N_2 at 60°C. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with 5% HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (20:1, v/v) as eluent.

*1,4-Bis-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyloxyonate prop-2-ynyl)benzene (12)*

Reaction time: 2 h; yield: 93%; mp 119–121°C, $[\alpha]_D$ +18.8° (*c* 0.5, $CHCl_3$). FAB-MS calcd.: 1133.09; found: 1133.3 (M^+, 3.6%). ^{1}H NMR ($CDCl_3$) δ: 7.31 (s, 4H, aromatic H), 5.56–5.36 (m, 4H, NH, H-8), 5.27 (dd, 2H, $J_{6,7}$ = 2.0 Hz, $J_{7,8}$ = 8.5 Hz, H-7), 4.83 (m, 2H, H-4), 4.36 (ABq, 2H, J = 15.8 Hz, -OCH_2C≡), 4.33 (ABq, 2H, -OCH_2C≡), 4.25 (dd, 2H, $J_{9a,9b}$ = 12.4 Hz, $J_{8,9a}$ = 2.8 Hz, H-9a), 4.12–4.00 (m, 6H, H-5, H-6, H-9b), 3.74 (s, 6H, OCH_3), 2.60 (dd, 2H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.11, 2.08, 1.99, 1.98, 1.83 (s, 30H, OAcs, NHAc), 1.96 (dd, 2H, $J_{3a,4}$ = 12.8 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.8–170.0 (O=Cs), 167.9 (C-1), 131.6, 122.6 (aromatic C), 98.1 (C-2), 86.2 (-OCH_2*C*≡C-), 85.6 (-OCH_2C≡*C*-), 72.6 (C-6), 68.8 (C-4), 68.4 (C-8), 67.2 (C-7), 62.3 (C-9), 53.5 (-O*C*H_2C≡), 52.9 (OCH_3), 49.3 (C-5), 37.9 (C-3), 23.1, 21.1, 20.8, 20.7, 20.6 (NHAc, OAcs). Anal. calcd. for $C_{52}H_{64}N_2O_{26}$: C 55.12, H 5.69, N 2.47; found: C 54.82, H 5.75, N 2.49.

*1,4-Bis-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosylthioonate prop-2-ynyl)benzene (13)*

Reaction time 3 h; yield: 88%; mp 134–136°C, $[\alpha]_D$ +95° (*c* 0.7, $CHCl_3$). FAB-MS calcd.: 1165.22; found: 1165.2 (M^+, 3.5%). ^{1}H NMR ($CDCl_3$) δ: 7.23 (s, 4H, aromatic H), 5.50 (d, 2H, $J_{5,NH}$ = 9.9 Hz, NH), 5.36 (m, 2H, H-8), 5.26 (dd, 2H, $J_{6,7}$ = 1.6 Hz, $J_{7,8}$ = 8.3 Hz, H-7), 4.81 (m, 2H, H-4), 4.23 (dd, 2H, $J_{9a,9b}$ = 12.4 Hz, $J_{8,9a}$ = 2.4 Hz, H-9a), 4.04–3.98 (m, 4H, H-9b, H-5), 3.83 (dd, 2H, $J_{5,6}$ = 10.7 Hz, H-6), 3.71 (s, 6H, OCH_3), 3.63 (ABq, 2H, J = 16.9 Hz, -SCH_2C≡), 3.58 (ABq, 2H, -SCH_2C≡), 2.67 (dd, 2H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e), 2.12, 2.05, 1.96, 1.95, 1.81 (s, 30H, OAcs, NHAc), 1.93 (dd, 2H, $J_{3a,4}$ = 12.2 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.7–170.0 (O=Cs), 168.0 (C-1), 131.4, 122.8 (aromatic C), 86.6 (C-2), 82.3 (-SCH_2*C*≡C-), 81.8 (-SCH_2C≡*C*-), 74.2 (C-6), 69.4 (C-4), 68.5 (C-8), 67.2 (C-7), 62.2 (C-9), 53.1 (OCH_3), 49.1 (C-5), 37.5 (C-3), 23.0, 21.1, 20.7, 20.7, 20.6 (NHAc, OAcs), 18.0 (-SCH_2C≡).

Anal. calcd. for $C_{52}H_{64}N_2O_{24}S_2$: C 50.73, H 5.55, N 2.57; found: C 50.83, H 5.69, N 2.56.

Fully deprotected divalent sialoside (13a)

To peracetylated sialoside **13** (75 mg, 0.064 mmol) dissolved in MeOH (2 mL) was added 1 M NaOMe in MeOH (1 mL) and the solution was stirred at 25°C. After 4 h, MeOH was removed and 0.1 N NaOH (5 mL) was added. The mixture was stirred at 25°C overnight. The solution was treated with Amberlite IR-120 H^+ resin for 10 min, and filtrered. The filtrate was then freeze-dried to afford compound **13a** in quantitative yield: $[\alpha]_D$ +32.7° (*c* 2.6, H_2O). MS (ES^+) calcd. for $C_{34}H_{44}N_2O_{16}S_2$: 800.86; found: 801.1 ($[M + 1]^+$). ^1H NMR (D_2O) δ: 7.10 (s, 4H, aromatic H), 2.75 (dd, 2H, $J_{3a,3e}$ = 12.4 Hz, $J_{3e,4}$ = 4.5 Hz, H-3e), 1.91 (s, 6H, NHAc), 1.80 (dd, 2H, $J_{3a,4}$ = 12.2 Hz, H-3a). ^{13}C NMR (D_2O) δ: 175.4 (C=O), 170.8 (C-1), 131.3, 122.7 (aromatic C), 88.2 (-SCH$_2$*C*≡C-), 83.4 (C-2), 82.5 (-CH$_2$C≡*C*-), 75.4 (C-6), 71.7 (C-4), 68.8 (C-8), 69.5 (C-7), 63.2 (C-9), 52.1 (C-5), 40.2 (C-3), 22.5 (NHAc), 18.3 (-S*C*H$_2$C≡).

General procedure for the cross-coupling of acetylenic sialic acid derivatives with 1,3,5-triiodobenzene (14)

To a degassed solution of the propargyl sialic acid (0.2 mmol) and 1,3,5-triiodobenzene (**14**) (27.8 mg, 61 μmol) in DMF–Et_3N (4 mL, 1:1) were added $Pd_2(dba)_3$ (9.2 mg, 10 μmol), CuI (3.8 mg, 20 μmol), and PPh_3 (10.5 mg, 40 μmol). The mixture was stirred under N_2 at room temperature for 1 h. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with 5% HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (15:1, v/v) as eluent.

1,3,5-Tris-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloxyonate prop-2-ynyl)benzene (15)

Yield: (81%); mp 129–132°C, $[\alpha]_D$ +16.2° (*c* 0.5, $CHCl_3$). FAB-MS calcd.: 1660.59; found: 1660.5 (M^+, 1.5%). ^1H NMR ($CDCl_3$) δ: 7.37 (s, 3H, aromatic H), 5.40–5.37 (m, 6H, NH, H-8), 5.28 (dd, 3H, $J_{6,7}$ = 1.9 Hz, $J_{7,8}$ = 8.5 Hz, H-7), 4.82 (m, 3H, H-4), 4.53 (ABq, 3H, *J* = 15.9 Hz, -OC*H*$_2$C≡), 4.28 (ABq, 3H, -OC*H*$_2$C≡), 4.24 (dd, 3H, $J_{9a,9b}$ = 12.6 Hz, $J_{8,9a}$ = 2.7 Hz, H-9a), 4.08–4.00 (m, 9H, H-5, H-6, H-9b), 3.74 (s, 9H, OCH_3), 2.60 (dd, 3H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.12, 2.09, 1.99, 1.98, 1.83 (s, 45H, OAcs, NHAc), 1.95 (dd, 3H, $J_{3a,4}$ = 12.8 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 171.1–170.0 (O=Cs), 167.8 (C-1), 134.6, 123.2 (aromatic C), 98.1 (C-2), 85.8 (-OCH$_2$*C*≡C-), 84.2 (-OCH$_2$C≡*C*-), 72.6 (C-6), 68.8 (C-4), 68.3 (C-8), 67.2 (C-7), 62.4 (C-9), 53.4 (-O*C*H$_2$C≡), 52.9 (OCH_3), 49.3 (C-5), 37.9 (C-3), 23.1, 21.1, 20.8, 20.7, 20.6 (NHAc, Oacs). Anal. calcd. for $C_{75}H_{93}N_3O_{39}$: C 54.25, H 5.64, N 2.53; found: C 54.55, H 5.63, N 2.50.

1,3,5-Tris-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylthioonate prop-2-ynyl)benzene (16)

Yield: (76%); $[\alpha]_D$ +104.7° (*c* 0.3, $CHCl_3$). FAB-MS calcd.: 1708.63; found: 1708.9 (M^+, 2.5%). ^1H NMR ($CDCl_3$) δ: 7.26 (s, 3H, aromatic H), 5.53 (d, 3H, $J_{5,NH}$ = 9.9 Hz, NH), 5.36 (m, 3H, H-8), 5.27 (dd, 3H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 8.3 Hz, H-7), 4.83 (m, 3H, H-4), 4.24 (dd, 3H, $J_{9a,9b}$ = 12.5 Hz, $J_{8,9a}$ = 2.6 Hz, H-9a), 4.05–3.99 (m, 6H, H-9b, H-5), 3.85 (dd, 3H, $J_{5,6}$ = 10.8 Hz, H-6), 3.72 (s, 9H, OCH_3), 3.59 (s, 6H, -SC*H*$_2$C≡), 2.68 (dd, 3H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.13, 2.07, 1.98, 1.97, 1.82 (s, 45H, OAcs, NHAc), 1.93 (dd, 3 H, $J_{3a,4}$ = 12.0 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.8–170.0 (O=Cs), 168.0 (C-1), 134.1, 123.5 (aromatic C), 86.3 (C-2), 82.7 (-SCH$_2$*C*≡C-), 81.0 (-SCH$_2$C≡*C*-), 74.2 (C-6), 69.4 (C-4), 68.6 (C-8), 67.2 (C-7), 62.2 (C-9), 53.1 (OCH_3), 49.1 (C-5), 37.5 (C-3), 23.0, 21.1, 20.7, 20.7, (NHAc, OAcs), 17.8 (-S*C*H$_2$C≡). Anal. calcd. for $C_{75}H_{93}N_3O_{36}S_3$: C 52.72, H 5.49, N 2.46; found: C 52.75, H 5.67, N 2.45.

1,2,4,6-Tetrakis-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloxyonate prop-2-ynyl)benzene (18)

To a degassed solution of the prop-2-ynyl sialoside **3** (100 mg, 0.19 mmol) and 1,2,4,5-tetraiodobenzene (**17**) (25 mg, 43 μmol) in DMF–Et_3N (4 mL, 1:1) were added $Pd_2(dba)_3$ (8.6 mg, 9.4 μmol), CuI (3.6 mg, 18.9 μmol), and PPh_3 (9.9 mg, 37.7 μmol). The mixture was stirred under N_2 at room temperature for 4 h. The solution was poured into water (50 mL). The mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with 5% HCl and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (10:1, v/v) as eluent to afford tetramer **18** (82 mg, 87%): $[\alpha]_D$ +25.6° (*c* 0.5, $CHCl_3$). FAB-MS calcd.: 2188.08; found: 1696.6 [M^+-$C_{20}H_{28}NO_{13}$ -H, 0.7%). ^1H NMR ($CDCl_3$) δ: 7.44 (s, 2H, aromatic H), 5.47 (d, 4H, $J_{5,NH}$ = 8.4 Hz, NH), 5.37 (m, 4H, H-8), 5.28 (dd, 4H, $J_{6,7}$ = 1.8 Hz, $J_{7,8}$ = 8.4 Hz, H-7), 4.82 (m, 4H, H-4), 4.58 (ABq, 4H, *J* = 16.1 Hz, -OC*H*$_2$C≡), 4.40 (ABq, 4H, -OC*H*$_2$C≡), 4.25 (dd, 4H, $J_{9a,9b}$ = 12.5 Hz, $J_{8,9a}$ = 2.6 Hz, H-9a), 4.07–3.98 (m, 12H, H-5, H-6, H-9b), 3.75 (s, 12H, OCH_3), 2.60 (dd, 4H, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 2.12, 2.08, 1.98, 1.97, 1.82 (s, 60 H, OAcs, NHAc), 1.93 (dd, 4H, $J_{3a,4}$ = 12.5 Hz, H-3a). ^{13}C NMR ($CDCl_3$) δ: 170.8–170.0 (O=Cs), 167.8 (C-1), 136.1, 124.8 (aromatic C), 98.1 (C-2), 90.7 (-OCH$_2$*C*≡C-), 83.1 (-OCH$_2$C≡*C*-), 72.5 (C-6), 68.8 (C-4), 68.4 (C-8), 67.1 (C-7), 62.3 (C-9), 53.4 (-O*C*H$_2$C≡), 52.9 (OCH_3), 49.3 (C-5), 37.8 (C-3), 23.0, 21.0, 20.7, 20.7, 20.6 (NHAc, OAcs). Anal. calcd. for $C_{98}H_{122}N_4O_{52}$: C 53.75, H 5.71, N 2.56; found: C 53.46, H 5.67, N 2.50.

1,3,4- and 1,3,5-Tris-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloxyonate methylene)benzene (20a, 20b)

To a solution of the prop-2-ynyl sialoside **3** (100 mg, 0.19 mmol) in dry 1,2-dichloroethane (4 mL) was added ruthenium catalyst **19** (8 mg, 9.7 μmol). The mixture was refluxed under N_2 for 24 h. The solution was evaporated under vacuum. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (10:1, v/v) as eluent to afford two isomers **20a**, **20b** (68 mg, 68%) (1,2,4- and 1,3,5-regioisomers, 9:2). ^1H NMR ($CDCl_3$) δ: 7.27 (d, 1H, *J* = 7.8 Hz, H-Ar), 7.23 (s, 1H, H-Ar), 7.19 (d, 1H, H-Ar), 7.14 (s, 1H, H-Ar for the sym. isomer), 3.69, 355, 3.54 (s, OMe), 3.68 (s, OMe for the syn. isomer), 2.64–2.55 (m, 3H,

H-3e). ^{13}C NMR ($CDCl_3$) δ: 170.9–168.1 (C=Os), 137.2, 126.4 (Ar for sym. Isomer), 136.4, 135.4, 135.0, 128.7, 127.9, 127.1 (Ar), 98.6, 98.3, 98.3 (C-2), 72.5, 72.4, 72.3 (C-6), 69.4 (C-4), 68.5, 68.4 (C-8), 67.3, 67.2 (C-7), 66.5, 64.3, 64.1 (CH_2), 62.3, 62.2, 62.1 (C-9′), 52.6, 52.5, 52.5 (OCH_3), 49.4 (C-5), 38.0, 38.0, 37.9 (C-3), 23.1 (NHAc), 21.1, 20.8, 20.7, 20.7 (Oacs).

Conclusions

In summary, the palladium-catalyzed Sonogashira reaction has been successfully applied for the synthesis of sialic acid clusters of restricted conformational mobility. The procedure is general, high yielding, compatible with the easily removable acetate protecting group, and the sialic acid clusters were isolated in excellent yields. The reactions are applicable to thioglycosides. The choice for these "rigidified" scaffolds is based on the assumption that lesser entropic penalty will be paid upon binding with the siglecs, as previously observed by isothermal titration calorimetry (ITC) for analogous mannoside clusters upon binding to the phytohemagglutinin concanavalin A (20).

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References

1. S. Kelm and R. Schauer. Int. Rev. Cytol. **175**, 137 (1997).
2. K. Drickamer and M.E. Taylor. Trends Biochem. Sci. **23**, 321 (1998).
3. (*a*) P.R. Crocker and S. Kelm. The siglec family of I-type lectins. *In* Carbohydrates in chemistry and biology. Vol. 4. *Edited by* B. Ernst, G.W. Hart, and P. Sinaÿ. Wiley–VCH, Weinheim. 2000. Chap. 35. p. 579; (*b*) P.R. Crocker and A. Varki. Trends Immunol. **22**, 337 (2001).
4. J.M. Rini and Y. Lobsanov. Curr. Opin. Struct. Biol. **9**, 578 (1999).
5. Y. Hashimoto, M. Suzuki, P.R. Crocker, and A. Suzuki. J. Biochem. (Tokyo), **123**, 468 (1998).
6. (*a*) R. Roy. Curr. Opin. Struct. Biol. **6**, 692 (1996); (*b*) R. Roy. Top. Curr. Chem. **187**, 241 (1997).
7. R. Roy, S.K. Das, R. Dominique, M.C. Trono, F. Hernández-Mateo, and F. Santoyo-González. Pure Appl. Chem. **71**, 565 (1999).
8. R. Roy and C.A. Lafferière. Can. J. Chem. **68**, 2045 (1990).
9. D. Carrière, S.J. Meunier, F.D. Tropper, and R. Roy. J. Mol. Catal. A: Chem. **154**, 9 (2000).
10. R. Roy, D. Zanini, S.J. Meunier, and A. Romanowska. ACS Symp. Ser. **560**, 104 (1994).
11. A. Hasegawa, J. Nakamura, and M. Kiso. J. Carbohydr. Chem. **5**, 11 (1986).
12. Z. Gan and R. Roy. Tetrahedron Lett. **41**, 1155 (2000).
13. (*a*) C. Glaser. Ann. Chem. Pharm. **154**, 137 (1870). For a review and representative examples see: (*b*) P. Siemsen, R.C. Livingston, and F. Diederich. Angew. Chem. Int. Ed. Engl. **39**, 2632 (2000); (*c*) A.S. Hay. J. Org. Chem. **27**, 3320 (1962); (*d*) G. Eglinton and A.R. Galbraith. Chem. Ind. (London), 737 (1956); (*e*) G. Eglinton. J. Chem. Soc. 889 (1959).
14. (*a*) R. Roy, S.K. Das, F. Hernández-Mateo, F. Santoyo-González, and Z. Gan. Synthesis, 1049 (2001); (*b*) B. Liu and R. Roy. J. Chem. Soc. Perkin Trans. 1, 773 (2001).
15. For examples of Glaser and Sonogashira reactions using organosulfur compounds see: (*a*) T.J.J. Müller Tetrahedron Lett. **40**, 6563 (1999); (*b*) M. Fossatelli, A.C.T.H. van der Kerk, S.F. Vasilevsky, and L. Brandsma. Tetrahedron Lett. **33**, 4229 (1992); (*c*) T.X. Nee and G.M. Whitesides. J. Org. Chem. **53**, 2489 (1988); (*d*) U. Fritzsche and S. Hunig. Tetrahedron Lett. **13**, 4831 (1972). For reviews on general palldium cross-couplings, see: (*e*) K. Sonogashira. *In* Comprehensive organic synthesis. Vol. 3. *Edited by* B.M. Trost and I. Fleming. Pergamon Press, New York. 1991. pp. 521–549; (*f*) L. Brandsma, S.F. Vasilevsky, and H.D. VerKruijsse. *In* Application of transition metal catalysts in organic synthesis. Springer, Berlin. 1998. pp. 67–83; (*g*) K. Sonogashira. *In* Metal-catalyzed cross-coupling reactions. *Edited by* F. Diederich and P.J. Stang. Wiley–VCH, Weinheim, Germany. 1998. pp. 203–229.
16. U. Schöberl, T.F. Magnera, R.M. Harrison, F. Fleischer, J.L. Pflug, P.F.H. Schwab, X. Meng, D. Lipiak, B.C. Noll, V.S. Allured, T. Rudaleige, S. Lee, and J. Michl. J. Am. Chem. Soc. **119**, 3907 (1997).
17. D.L. Mattern. J. Org. Chem. **49**, 3051 (1984).
18. S.K. Das and R. Roy. Tetrahedron Lett. **40**, 4015 (1999).
19. R.J. Kauman and R.S. Sidhu. J. Org. Chem. **47**, 4941 (1982).
20. T.K. Dam, R. Roy, S.K. Das, S. Oscarson, and C.F. Brewer. J. Biol. Chem. **275**, 14 223 (2000).