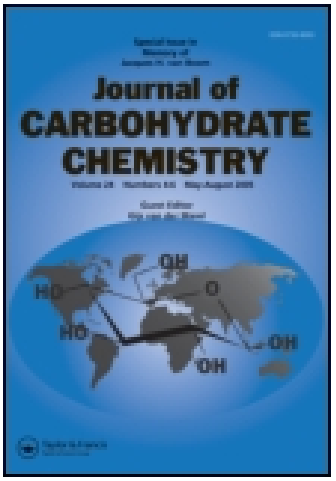

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Convenient Synthesis and Purification of N-Trifluoroacetylated Neuraminic Acid Tetraol: A Key Precursor for the Synthesis of New Sialyl Donors Containing Labile O-Acyl Protecting Groups

Nikita M. Podvalnyy[a], Alexander I. Zinin[a], Nelly N. Malysheva[a], Roman A. Kunetskiy[a] & Leonid O. Kononov[a]
[a] N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Moscow 119991, Russian Federation
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Convenient Synthesis and Purification of *N*-Trifluoroacetylated Neuraminic Acid Tetraol: A Key Precursor for the Synthesis of New Sialyl Donors Containing Labile *O*-Acyl Protecting Groups

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N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Moscow 119991, Russian Federation

A convenient synthesis and separation of α- and β-anomers of methyl (phenyl 3, 5-dideoxy-2-thio-5-trifluoroacetamido-D-*glycero*-D-*galacto*-nonulopyranosid)onate (**6a** and **6b**) on a multigram scale was developed. Both α- and β-isomers of **6** were obtained as crystals suitable for safe storage. The β-isomer forms a crystalline solvate with methanol. Fully *O*-trichloroacetylated and *O*-trifluoroacetylated *N*-trifluoroacetyl thiosialosides were synthesized in an efficient manner from the β-tetraol **6b**.

Keywords Sialic acid; Trifluoroacetamide; Crystal solvate; *O*-Trifluoroacetylation

INTRODUCTION

Biological significance of α-glycosides of sialic acids[1–3] stimulates continuous search of new approaches for their synthesis (for the reviews see refs. [4–6]).

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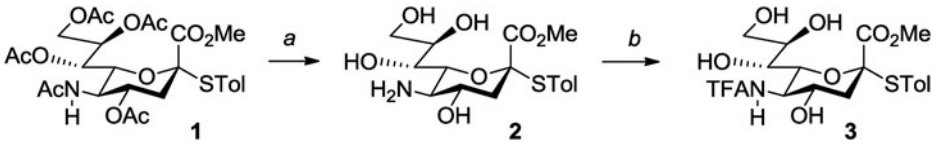
Address correspondence to Leonid O. Kononov, N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Leninsky prosp., 47, 119991, Moscow, Russian Federation. E-mail: kononov@ioc.ac.ru; leonid.kononov@gmail.com

Since both naturally occurring *N*-acetyl- and *N*-glycolyl-substituted sialooligosaccharides are required for biological studies, the use of sialyl donors with a suitable temporary protection at N(5)[7,8] (e.g., trichloroethoxycarbonyl [Troc][9–13] and trifluoroacetyl [TFA] group[14–21]) is considered reasonable.

Moreover, *N*-TFA is not only a convenient protecting group.[7,8] The task of the construction of a naturally occurring equatorial sialic bond is not a simple one. Although various approaches to stereoselective α-sialylation have been suggested,[4–6] the efficient introduction of sialic acid residues into oligosaccharides remains problematic. Considerable influence of *N*[7–16]- and *O*[17,22]-protecting groups in the glycosyl donor on yields of products and stereoselectivity of glycosylation has been demonstrated.

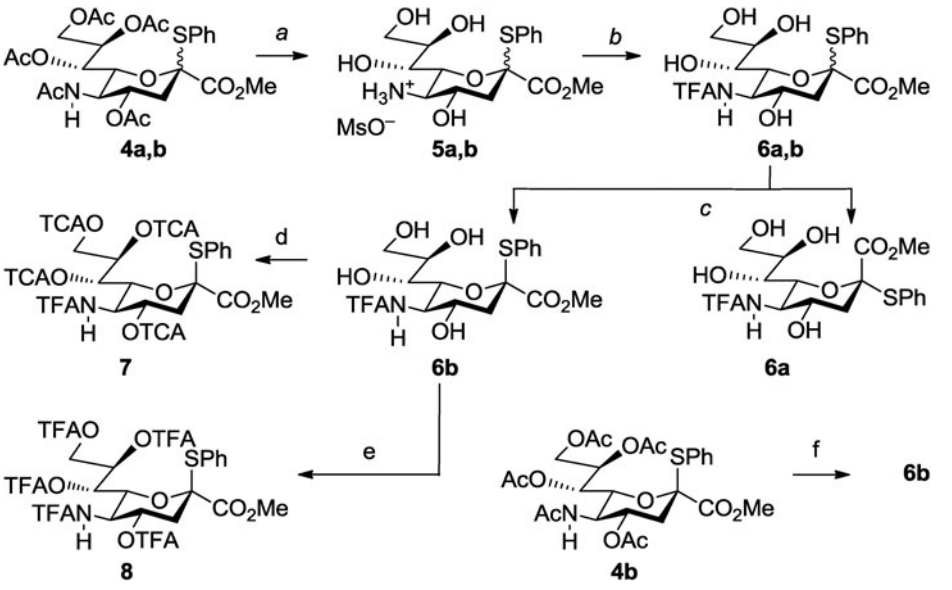
The reason for this high sensitivity of the sialylation outcome to a protecting group pattern of sialyl donor is currently unknown, although various hypotheses have been advanced.[22–29] According to the supramer approach,[25–29] various protecting groups are the sites capable of intermolecular interactions, which may determine the structure of supramer formed in each particular case and hence its reactivity.[29] For this reason, rational introduction of different protecting groups at specific positions of the sialyl donor molecule may change the structure of supramers formed and hence might be a novel way of modulating its reactivity and stereoselectivity.

Such an approach requires reliable access to the *O*-unprotected sialic acid derivatives with various *N*-protecting groups. One of the possible key building blocks for the preparation of these derivatives could be a sialic acid tetrahydroxy amine like **2** (Sch. 1) or **5** (Sch. 2). In fact, both α- and β-anomers of amines **2**[18,30] and **5**[13,31] and related compounds[18] with other aglycons are known and have been successfully used for the preparation of selectively protected sialyl donors with various protecting groups. Such compounds are commonly generated by acidic methanolysis of per-*N*,*O*-acetylated derivatives such as **1**[32] (Sch. 1) and **4**[33] (Sch. 2) and used in situ for the introduction of the desired *N*- and *O*-protecting groups. The target products have been isolated by a combination of standard extractive workup and silica gel chromatography.



Scheme 1: The known[19,34] synthesis of neuraminic acid *p*-tolyl thioglycoside **3**. *Reagents and conditions*: *a*. (1) MsOH, MeOH, Δ; (2) treatment with anion-exchange resin; (3) chromatography on silica gel (55%). *b*. CF_3CO_2Et, Et_3N, MeOH (94%). TFA = CF_3CO, Tol = 4-methylphenyl.

For the synthesis of derivatives, which can be purified by chromatography and survive aqueous workup, such an approach is quite reasonable. However,



Scheme 2: Synthesis of neuraminic acid phenyl thioglycoside **6a**, **6b** and *O*-acyl derivatives **7** and **8**. *Reagents and conditions*: *a*. (1) MsOH, MeOH, Δ; (2) extraction with Et_2O. *b*. CF_3CO_2Et, Et_3N, CH_2Cl_2. *c*. Chromatography and crystallization (combined isolated yield of **6a** and **6b** is 71% from anomeric mixture **4a,b**). *d*. CCl_3COCl, Py, CH_2Cl_2, 0→20°C, 14 h (95%). *e*. $(CF_3CO)_2O$, CF_3CO_2Na (cat.), 0°C, 30 min (99%). *f*. (1) MsOH, MeOH, Δ; (2) extraction with Et_2O; (3) CF_3CO_2Et, *N*,*N*-diisopropylethylamine, MeOH; (4) chromatography and crystallization (89% from β-anomer **4b**). TCA = CCl_3CO, TFA = CF_3CO.

for introduction of highly labile (acyl and probably silyl) groups, exclusion of the possibility of hydrolysis and minimization of the number of operations during isolation of the product are required. Therefore, it seems reasonable to have at hand an *N*-protected sialic acid derivative with free hydroxyl groups that can be purified by chromatography and/or crystallization prior to introduction of *O*-protecting groups.

Indeed, a procedure for the synthesis of *N*-TFA tetraol **3** (Sch. 1) with *p*-tolyl substituent at the sulfur atom and α-configuration of the glycoside bond was described recently.[19] The essential feature of this procedure was the use of pure amine precursor **2**.[34] After the deacetylation step, the reaction mixture was treated with a large amount of an anion-exchange resin (in order to remove 10-fold excess of methanesulfonic acid), followed by column chromatography on silica gel to give purified amine **2** in 55% yield. *N*-Trifluoroacetylation of amine **2** gave tetraol **3** in 94% yield. The overall yield of tetraol **3** obtained by this procedure was only 52%.

Here we describe an optimized practical synthesis of both α- and β-anomers of related thioglycosides **6a** and **6b** (Sch. 2) on a multigram scale.

RESULTS AND DISCUSSION

We started from the anomeric mixture of the known[33] peracetylated thioglycosides **4a** and **4b**, which is difficult (although possible) to separate either by crystallization or chromatography. We followed the commonly used procedure for acidic methanolysis of **4a,b** and obtained (as others did[13,18,30,31]) the salt of amine **5a,b** in the mixture with excess MsOH.

The key feature of our approach is the modified procedure for removal of excess MsOH, which is required for methanolysis of *O*- and *N*-acetyl groups. As was mentioned above, at the step of acidic methanolysis of *O*- and *N*-acetyl groups of **4a,b**, treatment of the reaction mixture containing 10-fold excess of MsOH with an anion-exchange resin and a column chromatography would lead to significant losses.[19,34] This problem was obviated by removal of the excess MsOH by extraction with diethyl ether. Then the crude *O*- and *N*-deprotected thiosialoside amine **5a,b** in the form of the salt with MsOH was subjected to *N*-trifluoroacetylation with ethyl trifluoroacetate in the presence of Et_3N. At this step it was found convenient to separate the anomers of *N*-trifluoroacetyl derivative **6a** and **6b** by silica gel column chromatography and crystallization. It is interesting to note that compound **6b** crystallizes only after addition of methanol to the solution, forming a solvate with methanol. This important feature of **6b** allows efficient crystallization of **6a** from the anomeric mixtures. The combined yield of **6a** and **6b** obtained from the anomeric mixture **4a,b** was 71% on a 16-g scale. When the individual β-isomer **4b** was involved in our procedure, separation of anomers was not required, which led to the increased yield (89%) of the target tetraol **6b**.

Elaboration of **6a** and **6b** in pure form allows access to a number of compounds containing labile acyl groups. Synthesis of sialyl donors with labile *O*-protecting groups like **7** and **8** from **5** or **6**, prepared in situ using the previously developed methods, would be impossible due to their instability under chromatographic purification on silica gel.

O-Trichloroacetate **7** was obtained by treatment of **6b** with CCl_3COCl in dichloromethane in the presence of pyridine. Chromatographic purification of **7** on silica gel was possible but associated with losses due to hydrolysis. An alternative workup procedure involving liquid–solid extraction with toluene (most of the salts remain in the solid residue), liquid–liquid extraction with cold aqueous $NaHCO_3$ and $NaHSO_4$ solutions, and quick filtration through a very short (3-mm) layer of silica gel enabled us to obtain the pure product **7** in 95% yield.

O-Trifluoroacetylation of **6b** with $(CF_3CO)_2O$ in the presence of soluble bases (Py or Et_3N)[35] did not allow isolation of the product **8** in pure form apparently due to hydrolysis of *O*-trifluoroacetyl groups during aqueous extractions. Chromatography on silica gel was also absolutely unacceptable because of the known lability of *O*-trifluoroacetyl groups.[36] We managed to obtain pure

8 by conducting the acylation of **6b** in the presence of CF_3CO_2Na as a base[37] with subsequent removal of volatile components and extraction of the product with warm anhydrous benzene. Five distinct signals of trifluoroacetyl groups are present in the ^{19}F NMR spectrum of compound **8**. It was earlier noted[38,39] that ^{19}F NMR spectra of trifluoroacetates of methyl glycosides can be used for identification purposes.

CONCLUSIONS

In summary, a convenient and efficient method for the synthesis and separation of α- and β-anomers of methyl (phenyl 3,5-dideoxy-2-thio-5-trifluoroacetamido-D-*glycero*-D-*galacto*-nonulopyranosid)onate **6a** and **6b** on a multigram scale was developed. It was shown that the obtained thiosialoside tetraol can be utilized as a direct precursor of sialyl derivatives containing labile *O*-acyl groups.

EXPERIMENTAL

General Methods

All reactions were carried out under argon atmosphere with anhydrous solvents. The reactions were performed with the use of commercial reagents (Aldrich, Fluka, Acros Organics) and distilled solvents purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (40–63 μm, Merck). Thin-layer chromatography was carried out on plates with silica gel 60 F_{254} on glass or on aluminum foil (Merck). Spots of compounds were visualized under UV light and by heating the plates after immersion in a 1:10 (v/v) mixture of 85% aqueous H_3PO_4 and 95% EtOH. ^1H, ^{13}C, and ^{19}F NMR spectra were recorded for solutions in $CDCl_3$ or CD_3OD on a Bruker AC-200 instrument (200.13, 50.32, and 188.31 MHz, respectively), a Bruker AM-300 instrument (300.13, 75.48, and 282.38 MHz, respectively), or a Bruker AVANCE 600 spectrometer (600.13, 150.9, and 564.69 MHz, respectively). The ^1H NMR chemical shifts are referred to the residual signal of $CHCl_3$ (δ_H 7.27) or CHD_2OD (δ_H 3.31) and the ^{13}C NMR shifts to the $CDCl_3$ signal (δ_C 77.0) or CD_3OD signal (δ_C 49.0). Assignments of the signals in the NMR spectra were performed using 2D-spectroscopy (COSY, HSQC, HMBC) and DEPT-135 experiments. High-resolution mass spectra (electrospray ionization, HR-ESI-MS) were measured in a positive mode on a Bruker micrOTOF II mass spectrometer for 2×10^{-5} M solutions in MeCN or MeOH. Optical rotations were measured using a PU-07 automatic digital polarimeter (Russia).

Methyl (phenyl 3,5-dideoxy-2-thio-5-trifluoroacetamido-α- and β-D-*glycero*-D-*galacto*-nonulopyranosid)onate (6a and 6b)

Method A. To a stirred solution of the anomeric mixture of **4a** and **4b** (16.0 g, 27.4 mmol, α:$\beta \sim$ 1:4.5)[33] in anhydrous MeOH (274 mL), neat MsOH (17.8 mL, 274 mmol) was added. The reaction mixture was heated under argon at 67°C (bath temperature) for 24 h with a reflux condenser, then cooled to rt and concentrated under reduced pressure (bath temperature 40°C). The syrupy residue was triturated with Et_2O (100 mL) for 15 min followed by decantation of the solvent, and the procedure was repeated three more times with Et_2O (3 $\times$ 100 mL) until most of the MsOH was extracted. The solid residue (crude tetraol **2** containing free amino group in the form of the salt with MsOH) was dried in vacuo and dissolved in MeOH (55 mL). Triethylamine (30.5 mL, 220 mmol) and CF_3COOEt (17.1 mL, 144 mmol) were added in that order with stirring. After 24 h at rt ($\sim$20°C), the reaction mixture was concentrated under reduced pressure, and the residue was co-concentrated with MeCN (3 $\times$ 20 mL) and dried in vacuo to give a crude anomeric mixture **6a,b** as a white solid (33.7 g).

At the first step of isolation, the anomeric mixture **6a,b** was purified by chromatography on silica gel with gradient elution (acetone–toluene 3:7 v/v $\rightarrow$ 100% acetone). Fractions containing β-anomer **6b** as the major component (R_f = 0.45, $CHCl_3$–MeOH, 4:1 v/v) were combined and concentrated under reduced pressure; the residue was dissolved in acetone (25 mL) and diluted with Et_2O (50 mL); and then petroleum ether–Et_2O mixture (1:1 v/v) was added dropwise until a slight turbidity appeared. After addition of MeOH (5 mL), colorless crystals (prisms) started to precipitate. After precipitation of a major part of the product ($\sim$1 h), an additional amount of petroleum ether–Et_2O (1:1 v/v, total 100 mL) was added dropwise to the mother liquor containing crystals, and the mixture was kept at +4°C overnight. The product was filtered off and air-dried to give β-anomer **6b** as a solvate with MeOH containing a minor admixture of α-anomer. The crystalline product was dissolved in a minimal volume of acetone, the solution was concentrated to dryness, and the residue was recrystallized one more time as mentioned above, leading to pure β-anomer **6b** as a 1:1 solvate with MeOH (**6b**·MeOH) (6.70 g, 49%).

At the second step of isolation, the remaining chromatographic fractions were combined with all mother liquors from the previous crystallizations, concentrated under reduced pressure, and dried in vacuo. The residue was purified by column chromatography on silica gel with gradient elution with $CHCl_3$–acetone (3:1$\rightarrow$1:1 v/v). Fractions containing α-anomer **6a** (R_f = 0.38, $CHCl_3$–acetone 1:1 v/v) as the major component were combined, concentrated under reduced pressure, and dried in vacuo. The residue was dissolved in acetone (10 mL) and diluted with Et_2O (20 mL), and then the petroleum

ether–Et_2O mixture (1:1 v/v) was added dropwise until a slight turbidity appeared. Colorless crystals formed were collected and dried in vacuo to give pure α-anomer **6a** (1.72 g, 13%).

The remaining fractions of the second chromatography and the mother liquor from the previous crystallization were combined, concentrated under reduced pressure, and dried in vacuo. The residue was dissolved in acetone (10 mL) and diluted with Et_2O (20 mL), and then the petroleum ether–Et_2O mixture (1:1 v/v) was added until a slight turbidity appeared. After addition of MeOH (2 mL), the product (**6b**·MeOH) precipitated. Colorless crystals were collected and air-dried, leading to an additional amount of pure β-anomer **6b** as solvate with MeOH (**6b**·MeOH) (1.26 g, 58% [overall yield of β-anomer **6b**]).

Data for 6a: R_f 0.38 ($CHCl_3$–acetone, 1:1). m.p. 191–195°C (from acetone–Et_2O–petroleum ether). $[\alpha]_D^{22}$ +55.0 (*c* 1.0, MeOH). HR-ESI-MS: found *m*/*z* 492.0913 $[M + Na]^+$. Calcd for $C_{18}H_{22}F_3NO_8SNa$: 492.0910. Elemental analysis: calcd (%) for $C_{18}H_{22}F_3NO_8S$ (469.43): C 46.05, H 4.72, N 2.98; found: C 46.08, H 4.86, N 2.99. ^{1}H NMR (600 MHz, CD_3OD): δ ppm 7.56–7.59 (m, 2 H, Ph), 7.41–7.45 (m, 1 H, Ph), 7.35–7.39 (m, 2 H, Ph), 4.02 (dd∼t, $J_{4,5}$ = 10.3 Hz, $J_{5,6}$ = 10.3 Hz, 1 H, H-5), 3.76–3.81 (m, 2 H, H-8, H-9b), 3.69 (ddd, $J_{3ax,4}$ = 11.6 Hz, $J_{4,5}$ = 10.3 Hz, $J_{3eq,4}$ = 4.8 Hz, 1 H, H-4), 3.67 (dd, $J_{5,6}$ = 10.3 Hz, $J_{6,7}$ = 1.6 Hz, 1 H, H-6), 3.62 (s, 3 H, OCH_3), 3.59–3.62 (m, 1 H, H-9a), 3.44 (dd, $J_{7,8}$ = 9.0 Hz, $J_{6,7}$ = 1.6 Hz, 1 H, H-7), 2.85 (dd, $J_{3ax,3eq}$ = 12.8 Hz, $J_{3eq,4}$ = 4.8 Hz, 1 H, H-3_{eq}), 1.90 (dd, $J_{3ax,3eq}$ = 12.8 Hz, $J_{3ax,4}$ = 11.6 Hz, 1 H, H-3_{ax}). ^{19}F NMR (282 MHz, CD_3OD) δ ppm −77.07. ^{13}C NMR (151 MHz, CD_3OD): δ 171.0 ($COOCH_3$), 159.8 (q, $^3J_{C,F}$ = 36.8 Hz, $COCF_3$), 138.0, 131.3, 130.3, 130.1 (Ph), 117.6 (q, $^2J_{C,F}$ = 287.2 Hz, CF_3), 88.2 (C-2), 76.4 (C-6), 73.4 (C-8), 70.2 (C-7), 69.1 (C-4), 64.6 (C-9), 53.8 (C-5), 53.4 (OCH_3), 41.9 (C-3).

Data for 6b·MeOH: R_f = 0.19, $CHCl_3$–acetone (1:1 v/v). R_f 0.45, $CHCl_3$–MeOH (4:1 v/v). m.p. 114–118°C (crystal solvate with MeOH from acetone–Et_2O–petroleum ether–MeOH). $[\alpha]_D^{22}$ −141.2 (*c* 1.0, MeOH). HR-ESI-MS: found *m*/*z* 492.0905 $[M + Na - MeOH]^+$. Calcd for $C_{18}H_{22}F_3NO_8SNa$: 492.0910. Elemental analysis: calcd (%) for $C_{19}H_{26}F_3NO_9S$ (501.47): C 45.51, H 5.23, N 2.79; found: C 45.44, H 5.02, N 2.83. ^{1}H NMR (600 MHz, CD_3OD): δ ppm 7.58–7.61 (m, 2 H, Ph), 7.33–7.40 (m, 3 H, Ph), 4.72 (dd, $J_{5,6}$ = 10.6 Hz, $J_{6,7}$ = 0.8 Hz, 1 H, H-6), 4.20 (ddd, $J_{3ax,4}$ = 11.7 Hz, $J_{4,5}$ = 10.1 Hz, $J_{3eq,4}$ = 4.7 Hz, 1 H, H-4), 4.06 (dd∼t, $J_{5,6}$ = 10.6 Hz, $J_{4,5}$ = 10.1 Hz, 1 H, H-5), 3.82 (dd, $J_{9a,9b}$ = 11.4 Hz, $J_{8,9a}$ = 2.9 Hz, 1 H, H-9a), 3.77 (ddd, $J_{7,8}$ = 9.2 Hz, $J_{8,9b}$ = 5.5 Hz, $J_{8,9a}$ = 2.9 Hz, 1 H, H-8), 3.67 (dd, $J_{9a,9b}$ = 11.4 Hz, $J_{8,9b}$ = 5.5 Hz, 1 H, H-9b), 3.53 (s, 3 H, OCH_3), 3.50 (d, $J_{7,8}$ = 9.2 Hz, 1 H, H-7), 2.70 (dd, $J_{3ax,3eq}$ = 13.6 Hz, $J_{3eq,4}$ = 4.7 Hz, 1 H, H-3eq), 1.99 (dd, $J_{3ax,3eq}$ = 13.6 Hz, $J_{3ax,4}$ = 11.7 Hz, 1 H, H-3ax). ^{19}F NMR (282 MHz, CD_3OD): δ ppm −76.89. ^{13}C NMR (151 MHz, CD_3OD): δ ppm 171.0 ($COOCH_3$), 159.9 (q, $^3J_{C,F}$ = 36.9 Hz,

O*C*OCF$_3$), 137.4, 131.5, 130.7, 130.1 (Ph), 117.7 (q, $^2J_{C,F} = 287.3$ Hz, CF$_3$), 91.6 (C-2), 72.8 (C-6), 71.6 (C-8), 70.9 (C-7), 67.8 (C-4), 65.2 (C-9), 54.6 (C-5), 53.2 (OCH$_3$), 42.4 (C-3).

Method B. To a stirred solution of **4b** (300 mg, 0.514 mmol)[33] in anhydrous MeOH (12 mL), neat MsOH (0.34 mL, 5.2 mmol) was added. The reaction mixture was heated under argon at 67°C (bath temperature) with a reflux condenser for 24 h, cooled to rt, and concentrated under reduced pressure (bath temperature 40°C). The syrupy residue was triturated with Et$_2$O (10 mL) for 15 min followed by decantation of the solvent, and the procedure was repeated three more times with Et$_2$O (3 × 5 mL) until most of the MsOH was extracted. The solid residue was dried in vacuo and dissolved in MeOH (4 mL). *N*,*N*-Diisopropylethylamine (0.72 mL, 4.1 mmol) and CF$_3$COOEt (0.32 mL, 2.7 mmol) were added in that order with stirring. After 24 h at rt (~20°C), the reaction mixture was concentrated under reduced pressure, and the residue was co-concentrated with MeCN (3 × 3 mL) and dried in vacuo to give crude **6b** as a white solid.

The product was purified by column chromatography on silica gel with gradient elution (acetone–toluene 1:3 v/v → 100% acetone). Fractions containing the product (R_f 0.45, CHCl$_3$–MeOH, 4:1) were combined and concentrated under reduced pressure; the residue was dissolved in acetone (1 mL) and diluted with CH$_2$Cl$_2$ (2 mL) and Et$_2$O (3 mL); then petroleum ether was added dropwise until a slight turbidity appeared. After addition of MeOH (0.2 mL), colorless prisms started to precipitate. After precipitation of a major part of the product, an additional amount of petroleum ether (total 8 mL) was added dropwise and the mixture was kept at +4°C overnight. The crystals were filtered off and air-dried to afford the target product **6b** in the form of solvate with MeOH (**6b**·MeOH) (230 mg, 89%).

Methyl (phenyl 3,5-dideoxy-2-thio-4,7,8,9-tetra-*O*-trichloroacetyl-5-trifluoroacetamido-D-*glycero*-β-D-*galacto*-nonulopyranosid)onate (7)

Tetraol **6b**·MeOH (121.0 mg, 0.241 mmol) was co-concentrated with acetone (2 × 5 mL) and dried in vacuo for ~1 h; then anhydrous CH$_2$Cl$_2$ (5 mL) was added followed by pyridine (240 μL, 2.97 mmol); and the resulting suspension was cooled to 0°C (ice–water bath). Trichloroacetyl chloride (160 μL, 1.425 mmol) was added dropwise under argon (after addition the starting material has dissolved); the reaction mixture was allowed to warm to rt and stirred for 16 h at ~20°C, then concentrated under reduced pressure, co-concentrated with toluene (2 × 10 mL), and dried in vacuo. The residue was triturated with toluene (5 mL), and the solids were filtered off and washed with toluene (2 × 5 mL). The combined filtrate was concentrated to dryness, and the residue was dissolved in toluene (5 mL), washed with ice-cold water

(5 mL), ice-cold saturated aqueous $NaHCO_3$ (5 mL), and ice-cold 1 M aqueous $NaHSO_4$ (5 mL). Back-extraction with ~1.5 mL of toluene at each step was performed. The combined organic layer was washed with ice-cold water (5 mL), dried over Na_2SO_4, and quickly filtered through a very short (~3 mm) layer of silica gel; solids were washed with the 19:1 toluene–EtOAc mixture (10 mL) and concentrated under reduced pressure, and the residue was dried in vacuo to give **7** as a white foam (242.6 mg, 95%).

$[\alpha]_D^{18}$ −66.9 (*c* 1.0, $CHCl_3$). HR-ESI-MS: found *m*/*z* 1062.7108 $[M + NH_4]^+$. Calcd for $C_{26}H_{22}Cl_{12}F_3N_2O_{12}S$: 1062.7102. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.38–7.60 (m, 5 H, Ph), 6.66 (d, $J_{5,NH} = 9.6$ Hz, 1 H, NH), 5.80 (ddd~td, $J_{3ax,4} = 11.6$ Hz, $J_{4,5} = 10.9$ Hz, $J_{3eq,4} = 4.9$ Hz, 1 H, H-4), 5.63 (dd~t, $J_{6,7} = 2.2$ Hz, $J_{7,8} = 2.2$ Hz, 1 H, H-7), 5.13 (ddd~dt, $J_{8,9b} = 9.6$ Hz, $J_{8,9a} = 2.4$ Hz, $J_{7,8} = 2.1$ Hz, 1 H, H-8), 5.06 (dd, $J_{5,6} = 10.5$ Hz, $J_{6,7} = 2.2$ Hz, 1 H, H-6), 4.82 (dd, $J_{9a,9b} = 12.4$ Hz, $J_{8,9a} = 2.4$ Hz, 1 H, H-9a), 4.59 (dd, $J_{9a,9b} = 12.4$ Hz, $J_{8,9b} = 9.6$ Hz, 1 H, H-9b), 4.23 (ddd~q, $J_{4,5} = 10.9$ Hz, $J_{5,6} = 10.5$ Hz, $J_{5,NH} = 9.6$ Hz, 1 H, H-5), 3.77 (s, 3 H, OCH_3), 2.97 (dd, $J_{3ax,3eq} = 13.9$ Hz, $J_{3eq,4} = 4.9$ Hz, 1 H, H-3_{eq}), 2.35 (dd, $J_{3ax,3eq} = 13.9$ Hz, $J_{3ax,4} = 11.6$ Hz, 1 H, H-3_{ax}). ^{19}F NMR (282 MHz, $CDCl_3$): δ ppm −75.71. ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 167.4, 162.1, 161.4, 160.8, 160.8 (CO), 158.5 (q, $^3J_{C,F} = 38.7$ Hz, O*C*OCF_3), 136.0, 131.0, 129.8, 127.2 (Ph), 115.1 (q, $^2J_{C,F} = 285.0$ Hz, *C*F_3), 88.0 (C-2), 76.5 (C-6), 73.1, 73.0 (C-4, C-7), 71.3 (C-8), 65.2 (C-9), 53.2 (OCH_3), 50.2 (C-5), 36.8 (C-3).

Methyl (phenyl 3,5-dideoxy-2-thio-4,7,8,9-tetra-*O*-trifluoroacetyl-5-trifluoroacetamido-D-*glycero*-*β*-D-*galacto*-nonulopyranosid)onate (8)

Tetraol **6b**·MeOH (240.0 mg, 0.47 mmol) was co-concentrated with anhydrous MeCN (2 × 10 mL) and dried in vacuo. Sodium trifluoroacetate (80 mg, 0.58 mmol, dried at 80°C in vacuo for 1 h) was added, and the reaction flask was flushed with argon and cooled to 0°C. Trifluoroacetic anhydride (6.4 mL) was added, and the reaction mixture was stirred for 30 min at 0°C (ice–water bath) and then concentrated to dryness; the residue was triturated with warm (+40°C) anhydrous benzene (2 × 10 mL), the extracts were filtered through a silanized glass wool (Serva) plug, solids were washed with warm anhydrous benzene, and the combined filtrate was concentrated and dried in vacuo to give per-trifluoroacetylated compound **8** as a white foam (404.4 mg, 99%). (Caution! Benzene has been identified as a carcinogen. All procedures involving benzene should be carried out in a well-ventilated hood, and glove protection is required.) The product shows limited solubility in $CDCl_3$ (after addition of $CDCl_3$ to the sample of **8** it starts to crystallize).

m.p. 191–192°C (from $CDCl_3$). $[\alpha]_D^{20}$ −102.1 (*c* 2.04, $CHCl_3$). HR-ESI-MS: found *m*/*z* 891.9946 $[M + K]^+$. Calcd for $C_{26}H_{18}F_{15}NO_{12}SK$: 891.9942. ^{1}H NMR

(300 MHz, $CDCl_3$): δ ppm 7.37–7.50 (m, 5 H, Ph), 6.55 (d, $J_{5,NH} = 9.9$ Hz, 1 H, N*H*), 5.69–5.80 (m, 1 H, H-4), 5.55 (dd∼t, $J_{7,8} = 1.8$ Hz, $J_{6,7} = 2.2$ Hz, 1 H, H-7), 4.92 (dd, $J_{5,6} = 10.5$ Hz, $J_{6,7} = 2.2$ Hz, 1 H, H-6), 4.98–5.04 (m, 1 H, H-8), 4.72 (dd, $J_{9a,9b} = 12.5$ Hz, $J_{8,9b} = 2.6$ Hz, 1 H, H-9b), 4.29 (dd, $J_{9a,9b} = 12.5$ Hz, $J_{8,9a} = 9.1$ Hz, 1 H, H-9a), 4.18 (ddd∼q, $J_{5,6} = 10.5$ Hz, $J_{4,5} = 10.2$ Hz, $J_{5,NH} = 9.9$ Hz, 1 H, H-5), 3.80 (s, 3 H, OCH_3), 2.92 (dd, $J_{3ax,3eq} = 14.1$ Hz, $J_{3eq,4} = 5.0$ Hz, 1 H, H-3eq), 2.36 (dd, $J_{3ax,3eq} = 14.1$ Hz, $J_{3ax,4} = 11.6$ Hz, 1 H, H-3ax). ^{19}F NMR (282 MHz, $CDCl_3$): δ ppm −74.13, −74.68, −74.71, −74.84, −75.75. ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.4 (CO), 158.2–155.5 (m, 5 × *C*OCF_3), 136.0, 131.0, 129.7, 127.0 (Ph), 119.7–110.6 (m, 5 × CO*C*F_3), 87.5 (C-2), 75.0 (C-6), 71.9, 71.5, 71.2 (C-4, C-7, C-8), 63.8 (C-9), 53.3 (OCH_3), 49.8 (C-5), 36.5 (C-3).

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