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Synthesis of *N*-Thioacylated Amino Sugars

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Abstract: *Unsubstituted amino sugars readily react with dithiocarboxylic esters or* O*-ethyl thioformate to yield the hitherto unknown free* N*-thioacylated sugars which are of biological interest.*

N-acetylated amino sugars and sialic acids are common constituents of glycoproteins, glycolipids, and glycosaminoglycans and play an important role in the biological function of cell receptors, hormones, blood group substances etc., that is to say in the interaction between macromolecules. Since it is known that even a small chemical modification frequently influences the biological activity of a compound, e.g. towards the enzymes of its metabolism or towards recognition proteins, the synthesis of slightly modified amino sugars is of considerable interest.

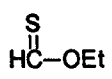
A close analogue of a carbohydrate is the thio derivative. The change from oxygen to its congener sulfur causes a larger size, higher polarizability, and decreased ability to form hydrogen bonds. The most studied compound of this class is 5-thio-D-glucopyranose, whose biological properties differ widely from those of the natural sugar.[1]

We have previously shown that the substitution of the pyranose ring oxygen atom of *N*-acetylneuraminic acid by sulfur produces remarkable effects on its chemical and biological properties.[2] In this context we were now interested in obtaining amino sugars, and in addition sialic acids, with the acetamido oxygen replaced by sulfur. For use in enzymatic tests such compounds should be present in unsubstituted form. In this communication we describe a pathway to such *N*-thioacylated amino sugars.

Unprotected *N*-thioacylated amino sugars have not been reported so far. Only substituted derivatives were obtained from the corresponding *N*-acetyl compounds by replacing oxygen by sulfur on treatment with phosphorus pentasulfide[3, 4, 5, 6] or by coupling a free amino group with dithioacetic acid in the presence of dicyclohexylcarbodiimide.[7] *N*-Thioformamido sugars were prepared from isothiocyanate precursors by reduction with tributyltin hydride.[8] Nevertheless, in none of the cases complete deprotection was accomplished.

At the outset we started from peracetylated *N*-acetyl hexosamines, thiation of which with Lawesson's reagent[9] followed by hydrolysis of *O*-acetyl groups gave the corresponding free *N*-thioacetyl amino sugars. Although we gained access to the desired class of compounds in this manner, some disadvantages remained. First, the use of protective groups leads to an increased number of steps involved, thereby diminishing the yield; second, only thioacetyl amino sugars are directly accessible from commercial educts but not thioformylated and thiopropionylated derivatives; and third, application of Lawesson's method to complex carbohydrates, especially to sialic acids, failed. Seeking for an approach of a more comprehensive scope, the most promising one seemed to be the introduction of the entire amino substituent, hence thioacylation of easily available amino sugars. *O*-ethyl thioformate (1)[10] is known to be the reagent for introducing the thioformyl

group[11], while methyl dithioacetate (**2**)[12] and methyl dithiopropionate (**3**)[12] are suitable reagents for thioacetylation and thiopropionylation, respectively.[11, 13]



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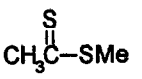

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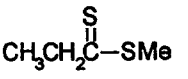


3

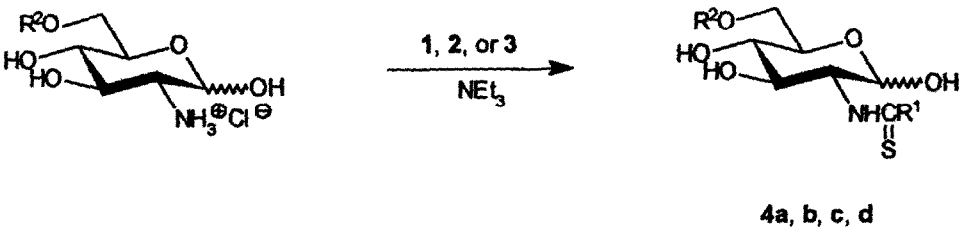
Applying reagents **1**, **2**, or **3** to amino sugars we found that they react smoothly to yield the corresponding *N*-thioacylated derivatives **4**, **5** and **6** (for Scheme, see next page). Reactions were performed in aqueous methanol in the presence of triethylamine to ensure at least a minimum of solubility of hexosamine hydrochlorides as well as to prevent separation of the hydrophobic thioacylation reagents. Employing reagents **1**, **2**, or **3** in fivefold excess TLC showed the conversion to be complete after three to sixteen hours. Removal of volatile material followed by one single column chromatography afforded the title compounds as colourless, amorphous solids which were pure according to TLC, NMR spectroscopy, and elemental analysis. Only *N*-thioacetyl-D-galactosamine crystallized spontaneously.

It should be noted that thioacylation proceeds equally well with an unprotected phosphorylated amino sugar to give, for example, *N*-thioacetyl-D-glucosamine 6-phosphate (**4d**).

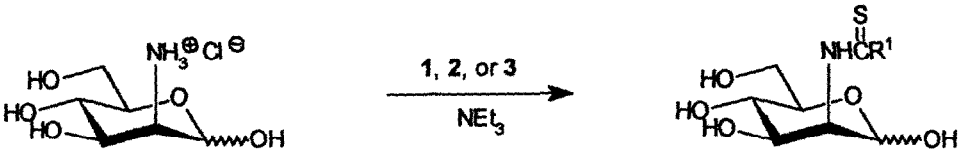
As common features, compounds **4**, **5**, and **6** exhibit strong UV absorption, a well-known characteristic of thioamides; IR spectrum of **4b** shows strong absorption at 1627 cm^{-1} ("thioamide B" band).[14] In the ^{13}C NMR spectra all compounds show low field signals ($\delta \sim 200$ ppm) of thiocarbonyl carbons, and in the proton spectra H-2 protons appear in the anomeric region because of the adjacent thioacylamino group. In the case of thioformamido derivatives **4a**, **5a**, and **6a**, NMR spectra are somewhat intricate owing to *E/Z* isomerism about the NH-CHS bond.[6, 15] Of each compound, not only α, β anomers are present but also *E/Z* rotamers, resulting in four spectroscopically distinguishable species. Primarily, *E/Z* configurations can be assigned by $J_{NH,CHS}$ measurements. For *N*-thioformyl-D-glucosamine (**4a**), ^1H NMR spectrum in DMSO-d_6 shows thioformyl proton signals as doublets with significantly larger coupling constants for *E* ($J_{NH,CHS}$ 14.4 and 14.6 Hz, resp.) than for *Z* ($J_{NH,CHS}$ 6.5 and 6.6 Hz, resp.) isomers. Furthermore, in D_2O as well as in DMSO-d_6 the CHS proton resonates at slightly higher field for *E* than for *Z* rotamers and the same is true for the H-2 proton, but with even greater difference between the rotamers ($\Delta\delta \sim 1.2$ ppm). The deshielding of the *Z*-H-2 proton is a consequence of the anticipated anti-periplanar conformation about the sugar-NHCHS bond. Syn-periplanar disposition seems not to be significant, since no long-range coupling between CHS proton and H-2 proton is observed, which is possible due to W arrangement in *Z*-configuration and was reported in a related case.[15c]

Additional evidence for discrimination between *E* and *Z* configuration is provided by ^{13}C NMR data:[6, 16] (a) the thiocarbonyl carbon is more deshielded for *E* than for *Z* rotamers and (b) C-2 carbon resonates at higher field for *Z* than for *E* isomers. *Z*-configuration is predominant for all of the thioformamido sugars synthesized; α, β ratio is similar to that of thioacetamido analogues.

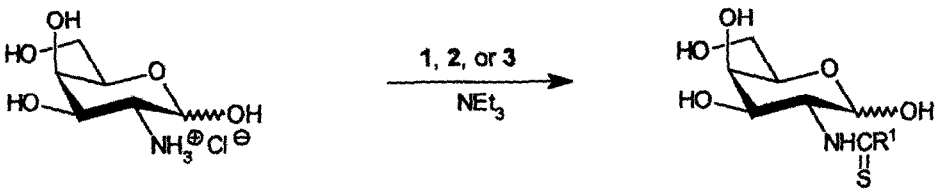
Peracetylation of **4a** afforded the known[8] tetra-*O*-acetyl derivative **7** obtained as a mixture of *Z*α, *E*α, *Z*β, and *E*β isomers, and in addition the crystalline penta acetyl compound, *N*-acetyl-1,3,4,6-tetra-*O*-acetyl-*N*-thioformyl-α-D-glucosamine (**8**) in 23% yield after separation via column chromatography.



4a, b, c, d



5a, b, c



6a, b, c

4a: $R^1 = H$, $R^2 =$ } H
4b: $R^1 = CH_3$, $R^2 =$ } H
4c: $R^1 = CH_2CH_3$, $R^2 =$ } H
4d: $R^1 = CH_3$, $R^2 = HPO_3^{\ominus}NH_4^{\oplus}$

5a, 6a: $R^1 = H$
5b, 6b: $R^1 = CH_3$
5c, 6c: $R^1 = CH_2CH_3$

In summary, the method described here for the preparation of the title compounds proved to be convenient and effective. Both, completely unsubstituted *N*-thioacyl hexosamines and phosphate **4d** are under investigation in biological tests. Among the former, mannosamine derivatives might be used in enzymatic condensation with pyruvate to yield sialic acid analogues. In preliminary experiments, *N*-thioacetyl-D-glucosamine 6-phosphate (**4d**) turned out to be inhibitory towards the enzymes of the sialic acid metabolism. Work is in progress on the extension of *N*-thioacylation to more complex carbohydrates. Results will be reported soon.

EXPERIMENTAL

General methods

Melting points were determined with a Tottoli-Büchi apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter after 24 hours keeping at ambient temperature (c 0.5, H_2O). UV spectra were recorded in aqueous solution with a Hitachi U-2000 spectrophotometer. TLC was performed on aluminium sheets coated with Silica Gel 60 F_{254} (Merck) using 5:1 ethyl acetate-methanol (solvent A) or 5:1 n-propanol-water (solvent B). Compounds were detected by UV light and by spraying TLC plates with 2 M H_2SO_4 and charring at 200°C for a few minutes. Column chromatography was performed on Silica Gel Merck 60 (70-230 mesh) by elution with 9:1 ethyl acetate-methanol (solvent C). Proton and carbon NMR spectra were recorded in D_2O at 25°C with a Bruker AC-300 spectrometer at 300 MHz (^1H) and at 75 MHz (^{13}C), respectively, unless otherwise stated. Chemical shifts are reported in ppm relative to the solvent; HOD in D_2O at 4.76 ppm, $D_2HCSOCD_3$ in DMSO-d_6 at 2.50 ppm, CD_3OH in methanol-d_4 at 4.82 ppm, and $CHCl_3$ in $CDCl_3$ at 7.25 ppm for the proton spectra. For carbon spectra, the reference is CD_3OD = 49.0 ppm in D_2O. FAB MS (matrix: glycerol; ion energy 15 kV) were recorded on a MAT 95 mass spectrometer (Finnigan MAT, Bremen, Germany).

General procedure for the synthesis of compounds 4, 5, and 6

A suspension of D-hexosamine hydrochloride (216 mg, 1.0 mmol) in water (0.25 ml) was diluted with methanol (2.5 ml). After cooling to 0°C, triethylamine (0.56 ml, 4.0 mmol) was added followed by **1**, **2**, or **3** (~0.5 ml, 4-5 mmol). The reaction mixture was allowed to warm up to room temperature. Complete conversion to **4**, **5**, or **6** required 3-16 h (TLC, solvent A). Volatile material was removed under reduced pressure and the residue was purified by column chromatography on silica gel (for compounds **4a-c**, **5a-c**, and **6a-c**; solvent C) or by ion exchange chromatography on DEAE-Sephadex A-25 (HCO_3^-) (for phosphate **4d**; elution with 0.04 M NH_4HCO_3 solution). After evaporation of the solvent, re-solution in distilled water and freeze-drying, **4a-c**, **5a-c**, and **6a-c** were obtained as colourless amorphous solids. **4d** was obtained as monoammonium salt after removal of NH_4HCO_3 by three times repeated lyophilization.

N-*Thioformyl-D-glucosamine* (*GlcNThFo*, **4a**). Yield 174 mg (78%); R_F 0.44 (A); $[\alpha]_D^{20}$ +12.4°; λ_{max} 265.0 nm (ε_M 12960); ^1H NMR δ 9.52 (s, 0.2 H, *Z*-HC(S)-β), 9.43 (s, 0.5 H, *Z*-HC(S)-α), 9.23 (s, 0.15 H, *E*-HC(S)-α), 9.20 (s, 0.15 H, *E*-HC(S)-β), 5.40 (d, $J_{1,2}$ 3.4 Hz, 0.5 H, *Z*-H-1α), 5.32 (d, $J_{1,2}$ 3.3 Hz, 0.15 H, *E*-H-1α), 4.92-4.67 (m, ~0.55 H, presumably *Z*-H-1β, *E*-H-1β, *Z*-H-2β; unequivocal identification and assignment are not possible due to coincidence with HOD peak), 4.74 (dd, $J_{2,3}$ 10.5 Hz, 0.5 H, *Z*-H-2α), 4.00-3.62 (m, ~3.9 H), 3.58-3.35 (m, ~1.4 H); data in CD_3OD (300 MHz) δ 9.47 (s, 0.03 H, *Z*-HC(S)-β), 9.36 (s, 0.71 H, *Z*-HC(S)-α), 9.19 (s, 0.17 H, *E*-HC(S)-α), 9.14 (s, 0.09 H, *E*-HC(S)-β), 5.35 (d, $J_{1,2}$ 3.5 Hz, 0.71 H, *Z*-H-1α), 5.18 (d, $J_{1,2}$ 3.5 Hz, 0.17 H, *E*-H-1α), 4.72 (d, $J_{1,2}$ 8.2 Hz, 0.03 H, *Z*-H-1β), 4.64 (dd, $J_{2,3}$ 10.6 Hz, 0.71 H, *Z*-H-2α), 4.62-4.54 (m, ~0.12 H, presumably *E*-H-1β, *Z*-H-2β), 3.95-3.65 (m, ~4.0 H), 3.51 (dd, $J_{2,3}$ 10.3 Hz, 0.17 H, *E*-H-2α), 3.55-3.18 (m, ~1.1 H); data in DMSO-d_6 (300 MHz) δ 10.25 (apparent t, $J_{NH,CHS}$ = $J_{NH,2}$ 7.0 Hz, 0.50 H, *Z*-NH-α), 10.11-9.98 (m, 0.31 H, 2 NH), 9.62 (dd, $J_{NH,CHS}$ 14.5, $J_{NH,2}$ 8.6 Hz, 0.19 H, *E*-NH), 9.30 (d, $J_{NH,CHS}$ 6.5 Hz, 0.12 H, *Z*-HC(S)-β), 9.19 (d, $J_{NH,CHS}$ 6.6 Hz, 0.50 H, *Z*-HC(S)-α), 9.07 (d, $J_{NH,CHS}$ 14.6 Hz, 0.19 H, *E*-HC(S)-α), 8.92 (d, $J_{NH,CHS}$ 14.4 Hz, 0.19 H, *E*-HC(S)-β), 6.91 (d, $J_{1\text{-}OH,1}$ 6.9

Hz, 0.19 H, *E*-1-OH-β), 6.72 (broadened d, presumably due to W-coupling with H-2, $J_{1\text{-}OH,1}$ 4.6 Hz, 0.19 H, *E*-1-OH-α), 6.64 (d, $J_{1\text{-}OH,1}$ 6.5 Hz, 0.12 H, *Z*-1-OH-β), 6.62 (d, $J_{1\text{-}OH,1}$ 4.2 Hz, 0.50 H, *Z*-1-OH-α); addition of D_2O caused disappearance of signals due to NH and OH protons; signals due to thioformyl protons collapsed to singlets; H-1 protons were assigned after H/D exchange: δ 5.17 (d, $J_{1,2}$ 3.2 Hz, 0.5 H, *Z*-H-1α), 5.00 (d, $J_{1,2}$ 3.1 Hz, 0.19 H, *E*-H-1α), 4.56 (d, $J_{1,2}$ 9.2 Hz, 0.12 H, *Z*-H-1β), 4.49 (d, $J_{1,2}$ 8.0 Hz, 0.19 H, *E*-H-1β). ^{13}C NMR *Z*-rotamer, α-form δ 191.6 (C=S), 90.2 (C-1), 72.6, 71.7, 71.0 (C-3,4,5), 61.6 (C-6), 57.9 (C-2); the other three isomers, *Z*β, *E*α, *E*β, are present in nearly equal quantities; the important assignments are listed: δ 194.7, 194.0 (2 *E*-C=S), 192.7 (*Z*-C=S-β), 95.6, 94.6, 92.0 (3 C-1), 77.0, 76.9, 75.0, 74.0, 72.5, 71.3, 70.7, 70.6 (C-3,4,5), 68.4, 65.6 (2 *E*-C-2), 61.7, 61.6, 61.5 (3 C-6), 60.0 (*Z*-C-2β).

Negative FAB MS: m/z 222 [4.6%, $(M-H)^-$]; positive FAB MS: m/z 224 [3.8%, $(M+H)^+$]. Anal. Calc. for $C_7H_{13}NO_5S \cdot 0.25\ H_2O$ (223.25+4.50): C 36.92, H 5.97, N 6.15, S 14.08. Found: C 36.84, H 5.77, N 6.00, S 13.84.

N-*Thioacetyl-D-glucosamine* (*GlcNThAc*, **4b**). Yield 198 mg (84%); R_F 0.39 (A); $[\alpha]_D^{20}$ +14.1°; λ_{max} 264.0 nm (ε_M 11680); ν_{max} (KBr) 1627 cm^{-1} (NHCS); ^1H NMR δ 5.29 (d, $J_{1,2}$ 3.6 Hz, 0.65 H, H-1α), 4.71 (d, $J_{1,2}$ 8.5 Hz, 0.35 H, H-1β), 4.53 (dd, $J_{2,3}$ 10.2 Hz, 0.35 H, H-2β), 4.50 (dd, $J_{2,3}$ 10.7 Hz, 0.65 H, H-2α), 3.87 (dd, $J_{3,4}$ 9.1 Hz, 0.65 H, H-3α), 3.85-3.62 (m, 2.65 H, H-5α, 6α, 6'α, 6β, 6'β), 3.60-3.52 (m, 0.35 H, H-5β), 3.42 (dd, $J_{4,5}$ 9.7 Hz, 0.65 H, H-4α), 3.41-3.37 (m, 0.7 H, H-3β, 4β), 2.45 (s, 3H, C(S)CH_3). ^{13}C NMR δ 205.4 (C=S-β), 204.4 (C=S-α), 95.8 (C-1β), 90.2 (C-1α), 77.0 (C-5β), 75.2 (C-3β), 72.6 (C-5α), 71.5 (C-3α), 71.2 (C-4α), 70.8 (C-4β), 63.3 (C-2b), 61.7 (C-6β), 61.6 (C-6α), 61.2 (C-2α), 33.9 (C(S)$\underline{C}H_3$-β), 33.5 (C(S)$\underline{C}H_3$-α).

Negative FAB MS: m/z 473 [4.1%, $(2M-H)^-$], 328 [5.7%, $(M-H+glycerol)^-$], 236 [52.7%, $(M-H)^-$]; positive FAB MS: m/z 352 [4.9%, $(M+Na+glycerol)^+$], 330 [3.3%, $(M+H+glycerol)^+$], 260 [5.3%, $(M+Na)^+$], 238 [31.8%, $(M+H)^+$], 220 [4.2%, $(M-OH)^+$]. Anal. Calc. for $C_8H_{15}NO_5S$ (237.27): C 40.50, H 6.37, N 5.90, S 13.51. Found: C 40.32, H 6.60, N 5.82, S 13.70.

N-*Thiopropionyl-D-glucosamine* (*GlcNThPr*, **4c**). Yield 191 mg (76%); R_F 0.48 (A); $[\alpha]_D^{20}$ +17.0°; λ_{max} 264.0 nm (ε_M 11490); ^1H NMR δ 5.28 (d, $J_{1,2}$ 3.5 Hz, 0.6 H, H-1α), 4.71 (d, $J_{1,2}$ 8.4 Hz, 0.4 H, H-1β), 4.53 (dd, $J_{2,3}$ 9.9 Hz, 0.4 H, H-2β), 4.49 (dd, $J_{2,3}$ 10.8 Hz, 0.6 H, H-2α), 3.85 (dd, $J_{3,4}$ 9.0 Hz, 0.6 H, H-3α), 3.85-3.50 (m, 3 H, H-5, 6, 6'), 3.41 (dd, $J_{4,5}$ 9.7 Hz, 0.6 H, H-4α), 3.40-3.36 (m, 0.8 H, H-3β, 4β), 2.60 (q, J 7.6 Hz, 2H, $C\underline{H}_2CH_3$), 1.13 (t, 3H, $CH_2C\underline{H}_3$).

Negative FAB MS: m/z 501 [7.2%, $(2M-H)^-$], 250 [100%, $(M-H)^-$]; positive FAB MS: m/z 525 [7.0%, $(2M+Na)^+$], 274 [24.6%, $(M+Na)^+$], 252 [100%, $(M+H)^+$]. Anal. Calc. for $C_9H_{17}NO_5S$ (251.30): C 43.02, H 6.82, N 5.57, S 12.76. Found: C 42.59, H 6.78, N 5.54, S 12.49.

N-*Thioacetyl-D-glucosamine 6-phosphate monoammonium salt* (*GlcNThAc 6-P*, **4d**). Yield 224 mg (67%); R_F 0.27 (B); $[\alpha]_D^{20}$ +12.8°; λ_{max} 264.5 nm (ε_M 11870); ^1H NMR (500 MHz, D_2O) δ 5.44 (d, $J_{1,2}$ 3.6 Hz, 0.67 H, H-1α), 4.87 (d, $J_{1,2}$ 8.4 Hz, 0.33 H, H-1β), 4.69 (dd, $J_{2,3}$ 10.0 Hz, 0.33 H, H-2β), 4.67 (dd, $J_{2,3}$ 10.7 Hz, 0.67 H, H-2α), 4.18 (ddd, $J_{5,6}$ 1.2, $J_{6,6'}$ 11.8, $J_{P,6}$ 5.6 Hz, 0.33 H, H-6β), 4.15-4.06 (m, 1.67 H, H-6α, 6'α, 6'β), 4.03 (m, 0.67 H, H-5α), 4.00 (dd, $J_{3,4}$ 9.1 Hz, 0.67 H, H-3α), 3.70 (m, 0.33 H, H-5β), 3.66-3.62 (m, 0.67 H, H-3β, 4β), 3.64 (dd, $J_{4,5}$ 10.0 Hz, 0.67 H, H-4α), 2.589, 2.587 (2 s, 3 H, 2 C(S)CH_3). ^{13}C NMR δ 205.3 (C=S-β), 204.3 (C=S-α), 95.8 (C-1β), 90.1 (C-1α), 76.1 (C-5β), 74.8 (C-3β), 71.5 (C-5α), 71.0 (C-3α), 70.4 (C-4α), 70.0 (C-4β), 64.8 (C-6α,β), 62.9 (C-2β), 60.9 (C-2α), 33.8 (C(S)CH_3-β), 33.4 (C(S)CH_3-α).

Anal. Calc. for $C_8H_{19}N_2O_8PS \cdot H_2O$ (334.28+18.02): C 27.27, H 6.01, N 7.95, S 9.10. Found: C 26.97, H 6.06, N 8.33, S 9.28.

N-*Thioformyl-D-mannosamine* (*ManNThFo*, **5a**). Yield 176 mg (79%); R_F 0.42 (A); $[\alpha]_D^{20}$ +15.1°; λ_{max} 264.0 nm (ε_M 11430); ^{1}H NMR (500 MHz, D_2O) δ 9.64 (s, 0.33 H, *Z*-HC(S)-β), 9.53 (s, 0.39 H, *Z*-HC(S)-α), 9.30 (s, 0.15 H, *E*-HC(S)-β), 9.29 (s, 0.13 H, *E*-HC(S)-α), 5.45 (dd, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 4.3 Hz, 0.33 H, *Z*-H-2β), 5.38 (d, $J_{1,2}$ 1.0 Hz, 0.13 H, *E*-H-1α), 5.32 (d, $J_{1,2}$ 1.3 Hz, 0.39 H, *Z*-H-1α), 5.21 (d, 0.33 H, *Z*-H-1β), 5.17 (dd, $J_{2,3}$ 4.7 Hz, 0.39 H, *Z*-H-2α), 5.15 (d, $J_{1,2}$ 1.4 Hz, 0.15 H, *E*-H-1β), 4.27 (dd, $J_{3,4}$ 9.8 Hz, 0.39 H, *Z*-H-3α), 4.20 (dd, $J_{2,3}$ 4.4 Hz, 0.13 H, *E*-H-2α), 4.15 (dd, $J_{2,3}$ 4.4 Hz, 0.15 H, *E*-H-2β), 4.13 (dd, $J_{3,4}$ 9.5 Hz, 0.15 H, *E*-H-3β), 4.06 (dd, $J_{3,4}$ 9.6 Hz, 0.33 H, *Z*-H-3β), 4.00-3.82 (m, c.2.8 H), 3.70 (dd, $J_{4,5}$ 9.9 Hz, 0.39 H, *Z*-H-4α), 3.67 (dd, $J_{4,5}$ 9.6 Hz, 0.15 H, *E*-H-4β), 3.62 (dd, $J_{4,5}$ 9.9 Hz, 0.13 H, *E*-H-4α), 3.59-3.50 (m, c.0.66 H); ^{13}C NMR *Z*-rotamer δ 193.8 (C=S-β), 192.3 (C=S-α), 93.9 (C-1β), 92.6 (C-1α), 77.6 (C-5β), 73.5 (C-3β), 73.1 (C-5α), 69.8 (C-3α), 68.2 (C-4α), 67.9 (C-4β), 61.7 (C-6β), 61.6 (C-6α), 57.9 (C-2β), 57.3 (C-2α); *E*-rotamer δ 195.9, 195.1 (2 C=S), 93.9, 93.2 (2 C-1), 66.5, 65.2 (2 C-2), 61.5, 61.4 (2 C-6).
Negative FAB MS : m/z 445 [7.1%, $(2M-H)^-$], 314 [7.0%, $(M-H+glycerol)^-$], 222 [64.6%, $(M-H)^-$]; positive FAB MS: m/z 447 [2.5%, $(2M+H)^+$], 338 [5.3%, $(M+Na+glycerol)^+$], 316 [6.6%, $(M+H+glycerol)^+$], 246 [8.2%, $(M+Na)^+$], 224 [32.4%, $(M+H)^+$]. Anal. Calc. for $C_7H_{13}NO_5S \cdot 0.5\ H_2O$ (223.25+9.01): C 36.20, H 6.07, N 6.03, S 13.81. Found: C 36.60, H 6.22, N 5.99, S 13.71.

N-*Thioacetyl-D-mannosamine* (*ManNThAc*, **5b**). Yield 199 mg (84%); R_F 0.42 (A); $[\alpha]_D^{20}$ +18.2°; λ_{max} 264.5 nm (ε_M 11340); ^{1}H NMR δ 5.28 (dd, $J_{1,2}$ 1.1 Hz, $J_{2,3}$ 4.6 Hz, 0.4 H, H-2β), 5.14 (d, $J_{1,2}$ 0.5 Hz, 0.6 H, H-1α), 5.03 (d, 0.4 H, H-1β), 5.00 (dd, $J_{2,3}$ 4.9 Hz, 0.6 H, H-2α), 4.08 (dd, $J_{3,4}$ 9.9 Hz, 0.6 H, H-3α), 3.88 (dd, $J_{3,4}$ 9.9 Hz, 0.4 H, H-3β), 3.84-3.72 (m, 2.6 H, H-5α, 6α, 6'α, 6β, 6'β), 3.63 (dd, $J_{4,5}$ 9.9 Hz, 0.6 H, H-4α), 3.50 (dd, $J_{4,5}$ 9.9 Hz, 0.4 H, H-4β), 3.37 (ddd, $J_{5,6}$ 2.8, $J_{5,6'}$ 3.9 Hz, 0.4 H, H-5β), 2.52 (s, 1.2 H, C(S)CH_3-β), 2.48 (s, 1.8 H, C(S)CH_3-α); ^{13}C NMR α-anomer δ 205.9 (C=S), 92.9 (C-1), 72.9 (C-5), 70.0 (C-3), 67.8 (C-4), 61.3 (C-6), 60.5 (C-2), 33.6 (C(S)$\underline{C}H_3$); β-anomer δ 207.4 (C=S), 94.1 (C-1), 77.4 (C-5), 73.6 (C-3), 67.4 (C-4), 61.3, 61.2 (C-2,6), 33.7 (C(S)$\underline{C}H_3$).
Negative FAB MS: m/z 473 [4.1%, $(2M-H)^-$], 236 [66.4%, $(M-H)^-$]; positive FAB MS: m/z 260 [12.3%, $(M+Na)^+$], 238 [4.9%,$(M+H)^+$]. Anal. Calc. for $C_8H_{15}NO_5S$ (237.27): C 40.50, H 6.37, N 5.90, S 13.51. Found: C 40.39, H 6.22, N 5.97, S 13.76.

N-*Thiopropionyl-D-mannosamine* (*ManNThPr*, **5c**). Yield 203 mg (81%); R_F 0.51 (A); $[\alpha]_D^{20}$ +5.2°; λ_{max} 265.0 nm (ε_M 11400); ^{1}H NMR δ 5.30 (dd, $J_{1,2}$ 1.0 Hz, $J_{2,3}$ 4.5 Hz, 0.4 H, H-2β), 5.14 (d, $J_{1,2}$ 0.5 Hz, 0.6 H, H-1α), 5.05 (d, 0.4 H, H-1β), 5.01 (dd, $J_{2,3}$ 4.9 Hz, 0.6 H, H-2α), 4.10 (dd, $J_{3,4}$ 9.8 Hz, 0.6 H, H-3α), 3.89 (dd, $J_{3,4}$ 9.8 Hz, 0.4 H, H-3β), 3.85-3.72 (m, 2.6 H, H-5α, 6α, 6'α, 6β, 6'β), 3.65 (dd, $J_{4,5}$ 9.8 Hz, 0.6 H, H-4α), 3.51 (dd, $J_{4,5}$ 9.8 Hz, 0.4 H, H-4β), 3.44-3.34 (m, 0.4 H, H-5β), 2.76-2.63 (m, 2 H, C$\underline{H}_2$CH$_3$), 1.19 (t, J 7.5 Hz, 1.2 H, CH$_2$C$\underline{H}_3$-β), 1.16 (t, J 7.5 Hz, 1.8 H, CH$_2$C$\underline{H}_3$-α).
Negative FAB MS: m/z 250 [45.9%, $(M-H)^-$]; positive FAB MS: m/z 525 [2.3%, $(2M+Na)^+$], 274 [36.1%, $(M+Na)^+$], 252 [43.0%, $(M+H)^+$]. Anal. Calc. for $C_9H_{17}NO_5S \cdot 0.25\ H_2O$ (251.30+4.50): C 42.27, H 6.85, N 5.48, S 12.52. Found: C 42.13, H 6.98, N 5.47, S 13.07.

N-*Thioformyl-D-galactosamine* (*GalNThFo*, **6a**). Yield 158 mg (71%); R_F 0.37 (A); $[\alpha]_D^{20}$ +145.2°; λ_{max} 265.0 nm (ε_M 12690); ^{1}H NMR δ 9.49 (s, 0.2 H, *Z*-HC(S)-β), 9.40 (s, 0.43 H, *Z*-HC(S)-α), 9.19 (s, 0.15 H, *E*-HC(S)-α), 9.17 (s, 0.22 H, *E*-HC(S)-β), 5.39 (d, $J_{1,2}$ 3.7 Hz, 0.43 H, *Z*-H-1α), 5.31 (d, $J_{1,2}$ 3.6 Hz, 0.15 H, *E*-H-1α), 4.94 (dd, $J_{2,3}$ 11.0 Hz, 0.43 H, *Z*-H-2α), 4.86 (dd, $J_{1,2}$ 8.4, $J_{2,3}$ 10.5 Hz, 0.2 H, *Z*-H-2β), 4.75 (d,

0.2 H, *Z*-H-1β), 4.73 (d, $J_{1,2}$ 7.8 Hz, 0.22 H, *E*-H-1β), 4.13-3.60 (m, ~5.15 H), 3.54 (dd, $J_{2,3}$ 10.5 Hz, 0.22 H, *E*-H-2β); signals due to furanose forms (in all ~10% of **6a**): δ 9.38 (s), 9.25 (s), 5.54 (d, $J_{1,2}$ 5.0 Hz), 5.36 (d, $J_{1,2}$ 2.7 Hz).

Negative FAB MS: m/z 222 [6.9%, $(M-H)^-$]; positive FAB MS: m/z 224 [3.3%, $(M+H)^+$]. Anal. Calc. for $C_7H_{13}NO_5S$ (223.25): C 37.66, H 5.87, N 6.27, S 14.36. Found: C 37.49, H 5.82, N 6.11, S 14.22.

N-*Thioacetyl-D-galactosamine* (*GalNThAc*, **6b**). Yield 208 mg (88%), **6b** crystallized from solvent C after column chromatography, m.p. 167°C; R_F 0.40 (A); $[\alpha]_D^{20}$ +31.7°; λ_{max} 264.5 nm (ε_M 11800); ^1H NMR pyranose form (~92%) δ 5.31 (d, $J_{1,2}$ 3.7 Hz, 0.7 H, H-1α), 4.73 (dd, $J_{2,3}$ 11.2 Hz, 0.7 H, H-2α), 4.71 (d, $J_{1,2}$ 8.8 Hz, 0.3 H, H-1β), 4.64 (apparent t, $J_{2,3}$ 8.8 Hz, 0.3 H, H-2β), 4.02 (apparent t, $J_{5,6} = J_{5,6'}$ 5.7 Hz, 0.7 H, H-5α), 4.01 (dd, $J_{3,4}$ 2.8 Hz, 0.7 H, H-3α), 3.89 (d, 0.7 H, H-4α), 3.83 (d, $J_{3,4}$ 3.0 Hz, 0.3 H, H-4β), 3.75-3.58 (m,~2.6 H, H-3β, 5β, 6α, 6'α, 6β, 6'β), 2.43 (s, 3H, $C(S)CH_3$); furanose form (~8%) δ 5.46 (d, $J_{1,2}$ 4.8 Hz, 0.6 H, H-1α), 5.26 (d, $J_{1,2}$ 2.8 Hz, 0.4 H, H-1β).

Negative FAB MS: m/z 473 [3.9%, $(2M-H)^-$], 328 [4.7%, $(M-H+glycerol)^-$], 236 [62.2%, $(M-H)^-$]; positive FAB MS: m/z 352 [3.1%, $(M+Na+glycerol)^+$], 330 [2.4%, $(M+H+glycerol)^+$], 260 [6.3%, $(M+Na)^+$], 238 [27.6%, $(M+H)^+$], 220 [2.5%, $(M-OH)^+$]. Anal. Calc. for $C_8H_{15}NO_5S$ (237.27): C 40.50, H 6.37, N 5.90, S 13.51. Found: C 40.68, H 6.49, N 5.72, S 13.04.

N-*Thiopropionyl-D-galactosamine* (*GalNThPr*, **6c**). Yield 213 mg (85%); R_F 0.47 (A); $[\alpha]_D^{20}$ +75.3°; λ_{max} 264.5 nm (ε_M 11750); ^1H NMR pyranose form (~90%) δ 5.32 (d, $J_{1,2}$ 3.7 Hz, 0.75 H, H-1α), 4.75 (dd, $J_{2,3}$ 11.0 Hz, 0.75 H, H-2α), 4.74 (d, $J_{1,2}$ 10.2 Hz, 0.25 H, H-1β), 4.66 (apparent t, $J_{2,3}$ 10.2 Hz, 0.25 H, H-2β), 4.03 (apparent t, $J_{5,6} = J_{5,6'}$ 5.7 Hz, 0.75 H, H-5α), 4.02 (dd, $J_{3,4}$ 3.0 Hz, 0.75 H, H-3α), 3.91 (d, 0.75 H, H-4α), 3.85 (d, $J_{3,4}$ 3.0 Hz, 0.25 H, H-4β), 3.77-3.61 (m, ~2.6 H, H-3β, 5β, 6α, 6'α, 6β, 6'β), 2.63 (~q, J 7.5 Hz, 0.5 H, $C\underline{H}_2CH_3$-β), 2.61 (~q, J 7.5 Hz, 1.5 H, $C\underline{H}_2CH_3$-α), 1.14 (~t, J 7.5 Hz, 3H, $CH_2C\underline{H}_3$-α,β); furanose form (~10%) δ 5.48 (d, $J_{1,2}$ 4.8 Hz, ~0.55 H, H-1α), 5.27 (d, $J_{1,2}$ 2.3 Hz, ~0.45 H, H-1β), 4.82 (dd, $J_{2,3}$ 9.0 Hz, ~0.55 H, H-2α).

Negative FAB MS: m/z 501 [11.5%, $(2M-H)^-$], 250 [100%, $(M-H)^-$]; positive FAB MS: m/z 525 [6.1%, $(2M+Na)^+$], 366 [6.1%, $(M+Na+glycerol)^+$], 274 [28.7%, $(M+Na)^+$], 252 [100%, $(M+H)^+$]. Anal. Calc. for $C_9H_{17}NO_5S$ (251.30): C 43.02, H 6.82, N 5.57, S 12.76. Found: C 43.30, H 6.94, N 5.68, S 12.74.

1,3,4,6-Tetra-O-acetyl-N-thioformyl-D-glucosamine (**7**) and *N-acetyl-1,3,4,6-tetra-O-acetyl-N-thioformyl-α-D-glucosamine* (**8**). To remove traces of water, **4a** (230 mg, ~1 mmol) was coevaporated twice with abs. pyridine. To the colourless oil in abs. pyridine (5 ml) freshly distilled acetic anhydride (2.5 ml) was added under ice-cooling, and the mixture was kept at 4°C overnight. TLC (2:1 toluene-ethyl acetate) showed two spots (R_F 0.45 and 0.36, resp.) in a ratio of ~ 1:3. After evaporation and coevaporation with toluene, ^1H NMR spectrum in $CHCl_3$ revealed the formation of **7** and **8** in a ratio of 2.5:1. Compound **7** appeared in four isomeric forms: *Z***7**α, *E***7**α, *Z***7**β, and *E***7**β (ratio ~22:5:1:1). Of derivative **8**, only the α anomer could be identified. Column chromatography (2:1 toluene-ethyl acetate) afforded 101 mg (23%) of **8** and 220 mg (56%) of **7**. ^1H NMR data of **7** were in agreement with those reported in literature.[8] Data of compound **8**: yellow needles, m.p. 99°C; $[\alpha]_D^{20}$ -22.1° (c 0.5 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 10.31 (s, 1 H, HC(S)), 6.53 (dd, $J_{2,3}$ 11.5, $J_{3,4}$ 9.0 Hz, 1 H, H-3), 6.28 (d, $J_{1,2}$ 3.2 Hz, 1 H, H-1), 5.84 (m, 1 H, H-2), 5.11 (dd, $J_{4,5}$ 10.1 Hz, 1 H, H-4), 4.36-4.27 (m, 2 H, H-5, 6), 4.12 (dd, $J_{5,6'}$ 3.4, $J_{6,6'}$ 12.6 Hz, 1 H, H-6'), 2.42 (s, 3 H, $NC(O)CH_3$), 2.10, 2.07, 2.04, 1.94 (4 s, 12 H, $OC(O)CH_3$). Anal. Calc. for $C_{17}H_{23}NO_{10}S$ (433.43): C 47.11, H 5.36, N 3.23, S 7.40. Found: C 46.98, H 5.71, N 3.06, S 6.91.

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