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Solid-Phase Synthesis of Thio-oligosaccharides**

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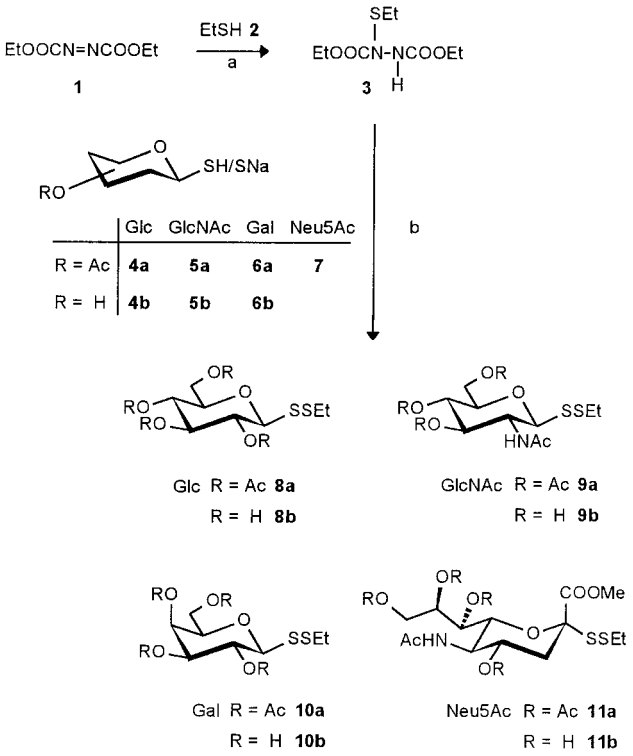
Thioglycosides are of special interest in enzyme-inhibition studies because they are much more stable to the action of glycosidases than O-glycosides and can thus inhibit enzymatic hydrolysis.[1] Thio-oligosaccharides, which contain sulfur in the glycosidic linkage, have previously been synthesized by a variety of methods including S_N2 displacement of a leaving group by a 1-thioglycopyranose derivative,[2] substitution of a glycosyl halide by a sugar thiolate,[3] Lewis acid catalyzed glycosylation between a glycosyl acceptor with an SH group and a glycosyl donor,[4] or Michael addition of 1-thiosugars to α,β-conjugated systems.[5] These methods generally require several steps and result in low overall yield. Furthermore, the formation of unsaturated side products, loss of stereochemistry at the anomeric center, and thiol oxidation products complicate the isolation of the desired product.[6] We present here a new and efficient method for the solid-phase synthesis of thio-oligosaccharides. The key feature of this method is that a highly reactive nucleophilic sugar-1-thiolate *without protecting groups* is used as the nucleophile for coupling to trifluoromethanesulfonate (triflate)-activated glycosides. The required free thiolate is generated on the solid phase from its protected unsymmetrical ethyl disulfide.

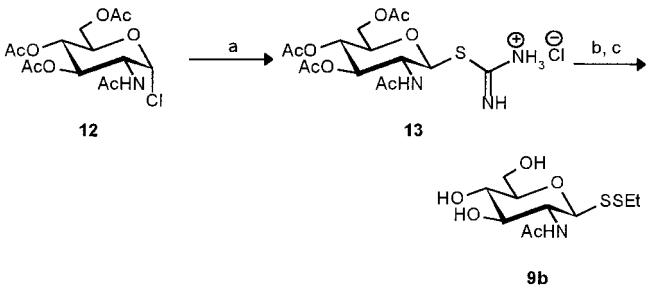
Diethyl-*N*-(ethylsulfanyl)hydrazodicarboxylate (**3**) was prepared by the reaction of diethylazodicarboxylate (**1**) and ethanethiol (**2**) in dichloromethane (Scheme 1).[7] Compound **3** was isolated as a colorless syrup in 76% yield after column chromatography. The thiol groups of readily available pro-



Scheme 1. a) CH_2Cl_2, 20 °C, 2 h, 75%; b) for R = Ac: CH_2Cl_2, THF or CH_3CN; for R = H: MeOH or MeOH/H_2O, 20 °C, 5 min, 100%. Ac = acetyl.

tected or unprotected 1-thioaldoses (Glc, GlcNAc, Gal, Neu5Ac)[8] were selectively protected by using **3** in dichloromethane (**4a**, **5a**, **6a**, **7**) or in methanol (**4b**, **5b**, **6b**). The corresponding sugar disulfides were obtained in quantitative yields. The unprotected disulfides, obtained either directly from **4b, 5b**, and **6b** or by deacetylation of **8a**, **9a**, **10a**, and **11a** with sodium methoxide in methanol, were used directly for immobilization on the solid phase.

For the preparation of larger quantities of 1-dithioethyl-glycosides an alternative reaction sequence was developed (Scheme 2, shown for GlcNAc). 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**12**)[9] was heated



Scheme 2. a) $SC(NH_2)_2$, acetone, reflux, 15 min, 82%; b) EtSSEt, MeOH, NEt_3, 20 °C, 2 h, 89%; c) NaOMe, MeOH, 20 °C, 2 h, then H^+, IR-120, 96%.

with thiourea in acetone for 15 min under reflux to give **13** in 89% yield. Subsequent treatment of **13** with diethyl disulfide in methanol and triethylamine followed by deacetylation with sodium methoxide in methanol afforded the unprotected disulfide **9b** in 85% yield.

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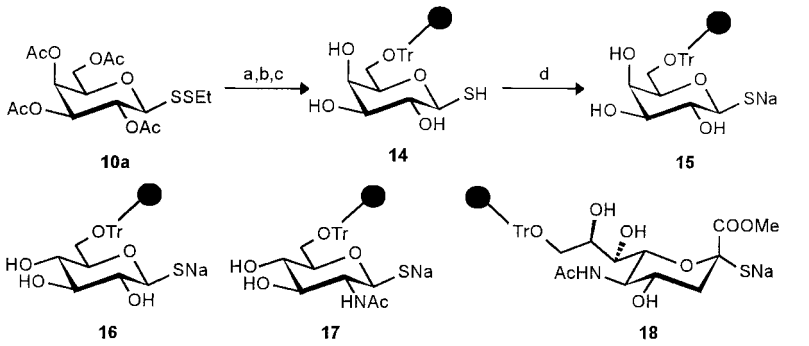
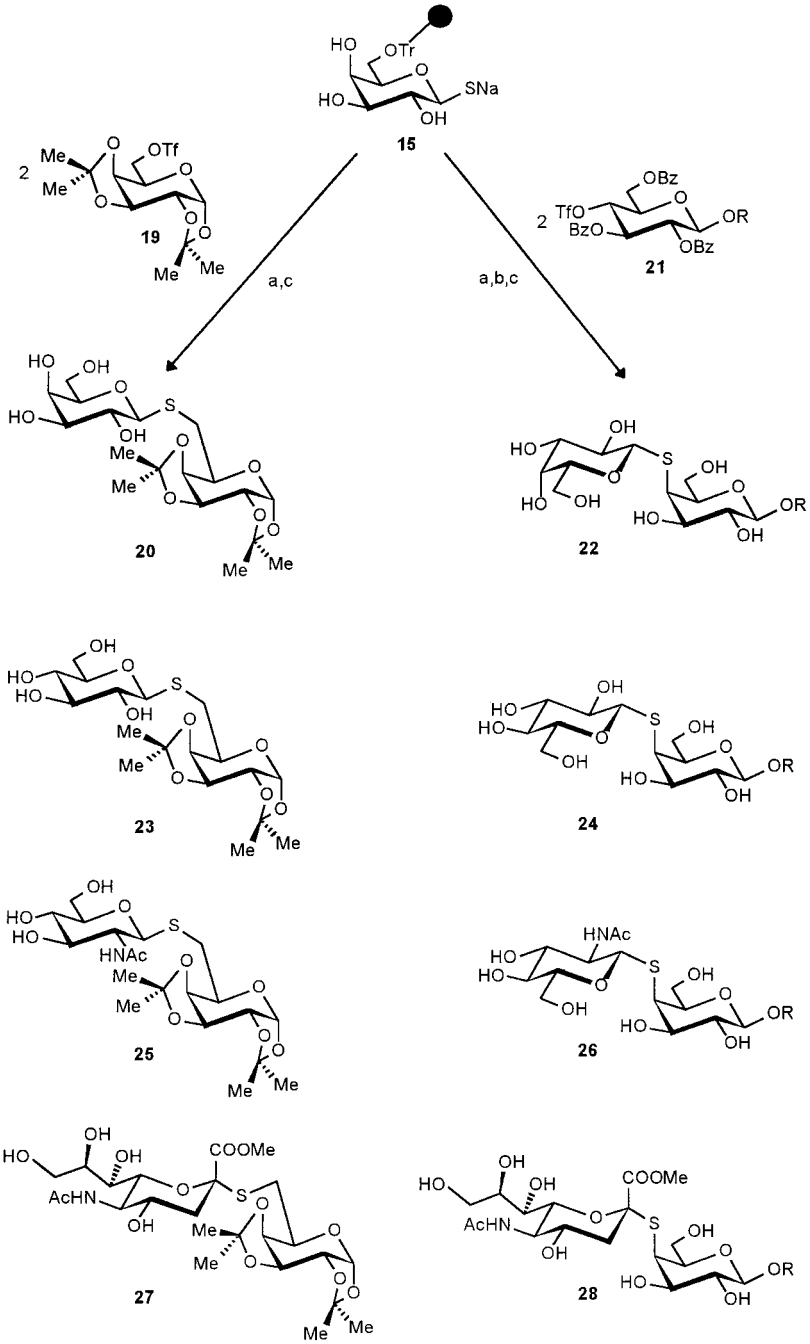
Each of the unprotected sugar disulfides (**8b**, **9b**, **10b**, and **11b**) was immobilized on a trityl chloride derivatized polystyrene resin (0.95 mmol g^{-1}) (shown for Gal, Scheme 3).[10] The loading of the disulfides on the solid support was determined by elemental analysis (sulfur content) and ranged from 0.56 to 0.70 mmol g^{-1} depending on the glycoside. The free thiol function was generated on the solid support by reduction of the disulfide with dithiothreitol (DTT) in a mixture of THF, MeOH, and Et_3N (10:2:1). The progress of the reduction could be easily monitored by IR spectroscopy directly on crushed beads ($\tilde{\nu}$(S–H) ≈ 2555 cm^{-1}). Attempted reaction of the immobilized thiol **14** with 6-*O*-triflate **19** (see Scheme 4) did not produce the desired thioglycoside, therefore, the nucleophilicity of **14** was enhanced by treatment with NaOMe/THF (Scheme 3).

The resulting thiolate **15** was coupled with triflate **19** after addition of a complexing agent ([15]crown-5 or Kryptofix 221) in THF (Scheme 4, shown for galactose). After 16 h the resin was washed and the thioglycoside **20** was cleaved from the resin by treatment with 2% TFA in CH_2Cl_2 (76% yield; for NMR and MS data see Table 1).[11] The same reaction with the 4-*O*-triflate **21** gave the corresponding thioglycoside **22** in 64% yield;[11] reactions of the triflates **19** and **21** with the immobilized sugar thiolates **16**–**18** led to the corresponding Glc, GlcNAc, and Neu5Ac thioglycosides **23**–**28** in similar yields. The structures of all thioglycosides were confirmed by ^{1}H NMR spectroscopy and mass spectrometry after adsorption on reverse-phase silica gel, washing with water, and elution with methanol.[11]

To demonstrate the suitability of this method for the construction of larger thiooligosaccharides, the 1-dithioethyl galactose derivative **29** was synthesized in five steps from **10b**. Glycosylation was performed in the same manner as described in Scheme 4 with triflate **29** and the immobilized thiolate **16** (Scheme 5) to give the immobilized disaccharide **30**. After removal of the benzoyl groups on the resin by using sodium methoxide in THF, the anomeric disulfide was reduced with DTT, deprotonated (NaOMe/THF), and again treated with triflate **29** in the presence of [15]crown-5. After removal of the benzoyl protecting groups with sodium methoxide in THF and cleavage from the resin, trisaccharide **32** was obtained in 92% yield based on **16** (NMR and MS data are given in Table 1). This high yield con-



Scheme 3. a) NaOMe, MeOH, 20 °C, 2 h; b) trityl chloride resin, pyridine, 4-dimethylaminopyridine (DMAP), 60 °C, 48 h; c) DTT, THF, MeOH, Et_3N, 20 °C, 18 h; d) NaOMe, THF, 20 °C, 2 h. Tr = trityl = triphenylmethyl.



Scheme 4. a) [15]Crown-5, THF, 20 °C, 16 h; b) NaOMe, MeOH, THF; c) trifluoroacetic acid (TFA), CH_2Cl_2, 20 °C. **21**, **22**, **24**, **26**, **28**: R = octyl, Bz = benzoyl, Tf = trifluormethylsulfonyl.

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Table 1. Selected ^{1}H NMR signals (360 MHz, CD_3OD) and MS data of **20**, **22**–**28**, and **32**.

20: $\delta = 4.35$ (d, 1H, $J_{1,2} = 9.7$ Hz; 1b-H, 1c-H), 5.45 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z: 461.2 [$M^+ - Na$]

22: $\delta = 4.18$ (d, 1H, $J_{1,2} = 7.6$ Hz; 1b-H), 4.42 (d, 1H, $J_{1,2} = 9.6$ Hz; 1a-H); MS: m/z: 493.2 [$M^+ - Na$]

23: $\delta = 4.40$ (d, 1H, $J_{1,2} = 9.6$ Hz; 1b-H), 5.45 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z: 461 [$M^+ - Na$]

24: $\delta = 4.18$ (d, 1H, $J_{1,2} = 7.6$ Hz; 1a-H), 4.48 (d, 1H, $J_{1,2} = 9.7$ Hz; 1b-H); MS: m/z: 493.2 [$M^+ - Na$]

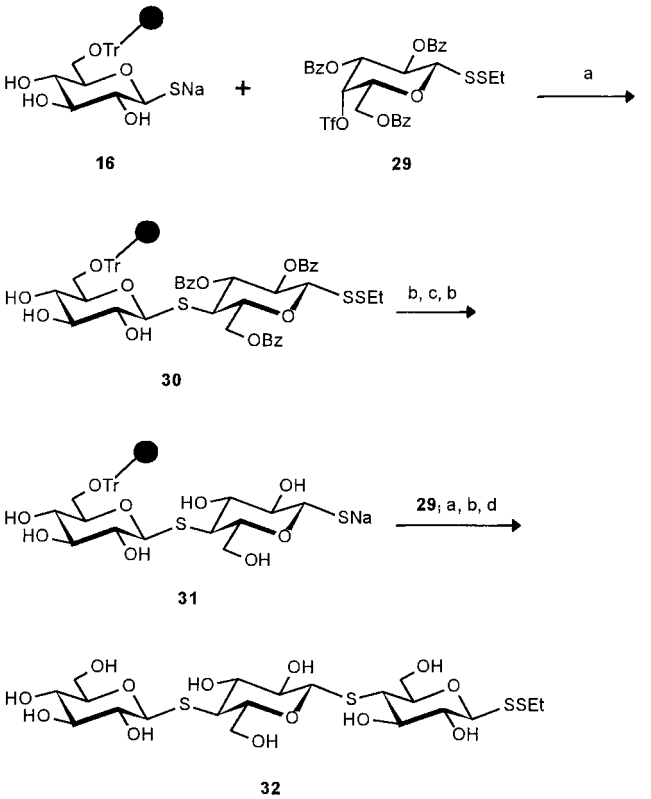
25: $\delta = 4.55$ (d, 1H, $J_{1,2} = 10.3$ Hz; 1b-H), 5.45 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z: 502.2 [$M^+ - Na$]

26: $\delta = 4.16$ (d, 1H, $J_{1,2} = 7.6$ Hz; 1a-H), 4.70 (d, 1H, $J_{1,2} = 10.4$ Hz; 1b-H); MS: m/z: 534.3 [$M^+ - Na$]

27: $\delta = 1.31$, 1.33, 1.39, 1.49 (4 × s, 12H; CH_3), 1.80 (dd, 1H, $J_{3a,4} = 11.3$, $J_{3a,3e} = 12.8$ Hz; 3b-H_a), 1.98 (s, 3H; $NCOCH_3$), 2.75 (dd, 1H, $J_{3e,4} = 4.6$, $J_{3e,3a} = 12.8$ Hz; 3b-H_e), 3.67 (s, 3H; $COOCH_3$), 4.31 (dd, 1H, $J_{1,2} = 5.0$, $J_{2,3} = 2.4$ Hz; 2a-H), 4.61 (dd, 1H, $J_{2,3} = 2.4$, $J_{3,4} = 7.9$ Hz; 3a-H), 5.42 (d, 1H, $J_{1,2} = 5.0$ Hz; 1a-H); MS: m/z: 604.2 [$M^+ - Na$]

28: $\delta = 0.88$ (m, 3H; CH_3), 1.28 (m, 10H; Octyl), 1.56 (m, 2H; OCH_2CH_2), 1.99 (s, 3H; $NCOCH_3$), 1.79 (dd, 1H, $J_{3a,4} = 11.3$, $J_{3a,3e} = 12.8$ Hz; 3b-H_a), 1.97 (s, 3H; $NCOCH_3$), 2.75 (dd, 1H, $J_{3e,4} = 4.6$, $J_{3e,3a} = 12.8$ Hz; 3b-H_e), 3.67 (s, 3H; $COOCH_3$), 4.20 (d, 1H, $J_{1,2} = 7.6$ Hz; 1a-H); MS: m/z: 636.3 [$M^+ -$ Na]

32: $\delta = 1.30$ (t, 3H, $J = 7.4$ Hz; CH_3), 2.83 (q, 2H, $J = 7.4$ Hz; SCH_2), 4.31 (d, 2H, $J_{1,2} = 9.3$ Hz; 1b-H, 1c-H), 4.40 (d, 1H, $J_{1,2} = 9.5$ Hz; 1a-H); MS: m/z: 635.1 [$M^+ - Na$]



Scheme 5. a) [15]Crown-5, THF, 20 °C, 16 h; b) NaOMe, MeOH, THF; c) DTT, THF, MeOH, Et_3N; d) TFA, CH_2Cl_2, 20 °C.

firms that O-glycosidic bond cleavage from the resin was in fact responsible for the lower yields of about 70% found for compounds **20**–**28**.

In conclusion, a highly efficient method for the synthesis of thio-oligosaccharides on the solid-phase has been described. All glycosides were obtained stereoselectively and in high yield. Side products arising from elimination of the triflates could be easily removed by washing the resin after glycosylation. The protection of the anomeric thiol function as an ethyl disulfide proved to be compatible with common carbohydrate reaction conditions and served as an ideal protective group.

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[10] The resin is commercially available from Novabiochem. Immobilization was performed by using pyridine as solvent in the presence of 4-dimethylaminopyridine (DMAP) for 48 h at 60 °C.
[11] Yield of isolated product. The isopropylidene groups of **20**, **23**, **25, 27** and the O-glycosidic bonds of unprotected **22**, **24**, **26**, **28** were partly cleaved under acidic conditions. Cleaving the benzoyl-protected thiodisaccharides from the resin afforded the corresponding disaccharides in quantitative yields since the presence of the electronegative benzoyl esters stabilizes the glycosidic linkages. The use of more acid labile linker systems is currently under investigation.

Characterization of Ligand Binding by Saturation Transfer Difference NMR Spectroscopy**

Moriz Mayer and Bernd Meyer*

The difference between a saturation transfer spectrum and a normal NMR spectrum provides a new and fast method (saturation transfer difference (STD) NMR spectroscopy) to

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