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A New Method for the Solid Phase Synthesis of Oligosaccharides[1]

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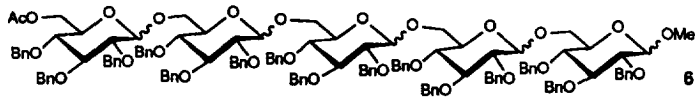
Abstract: Trichloroacetimidates are employed as sugar donors in solid phase oligosaccharide synthesis as demonstrated for the glucose (1→6)-linkage. The use of a new thiol linker allows the direct attachment of the first sugar residue to the resin and a fast and convenient quantitative monitoring of all solid phase reactions. The synthesis of pentasaccharides **6** (n = 5) exhibits the efficiency of the method and the potential in combinatorial syntheses.



6 (n = 5)

The progress in solid phase chemistry[2] has not yet been extended considerably on oligosaccharides[3,4]. Hitherto advances in this field have been essentially restrained by the lack of powerful analytical tools for reactions on solid phases. Our rationale was to enable the analysis of each reaction step by employing a linker which enables the attachment of any sugar by a standard glycosylation procedure but which is cleaved from a small resin sample fast enough for immediate analysis of the products. We expected these demands to be met by an oxidatively cleavable linker, as for instance by thioglycosides, which can be cleaved with thiophilic reagents like dimethylmethylthiosulfonium triflate (DMTST) or the corresponding tetrafluoroborate (DMTSB).

As polymer, chloromethylated polystyrene crosslinked with 1% divinylbenzene (**1**) was employed (Figure 1). The linker can be attached to the resin in two ways: S-dimethoxytrityl-protected mercaptopropanol is treated with the resin preferably with NaHMDS as base and then deprotected with TFA/dichloromethane to yield a 0.2 mmol/g loading. Alternatively, propane-1,3-dithiol can be attached to the resin with DBU as base[5] yielding resin **2**. In this case a thiol concentration of 0.6 mmol/g is achieved; no deprotection step is necessary and convenient attachment of the first sugar residue was possible. With three equivalents of a sugar trichloroacetimidate as donor quantitative glycosylation of the thiol groups was obtained according to a corresponding gain in weight and the complete disappearance of the thiol band at 2565 cm^{-1} in the IR spectrum.

For the investigation of the chain elongation reaction we chose the glucose (1→6)-linkage as example. Several protecting groups were tested for permanent and temporary protection. Finally donor **3** was selected, because it should be suitable for the synthesis of anomeric mixtures of sugar oligomers[6] which are also of interest in combinatorial syntheses. Several analytic protocols for the investigation of the glycosylation reactions yielding **4** were developed. The glycosylation of shorter sugars (→ **4**) and the ensuing deprotection reactions (→ **5**) can be monitored by TLC ten minutes after the cleavage solution has been applied[7]. For quantitative analysis RP-18 HPLC (dioxane/water gradient) was employed. To ease detection of the oligosaccharide products the thiolinker was cleaved with the thiophilic reagents in the presence of

chromophores such as nitroaniline as nucleophiles. The HPLC chromatogram gives then a quantitative picture of product distribution. Variation of the reaction conditions exhibited that the best glycosylation results are obtained in CH_2Cl_2 as solvent, at room temperature, with three equivalents of donor **3**, and 0.2 equivalents of TMSOTf as catalyst. Excellent yields (> 95%)[7] were finally achieved by an improved washing procedure (15-crown-5,CH_2Cl_2/MeOH 20:1) and mechanical shaking of the reaction vessel. Most efficiently MALDI-TOF-MS can be employed for the monitoring of solid phase glycosylations: a 2 mg resin sample is treated with the cleavage solution containing DMTSB in CH_2Cl_2/MeOH for 15 min; an aliquot of this solution is then mixed with dihydroxybenzoic acid (DHB) and directly applied on the laser target for measurement.

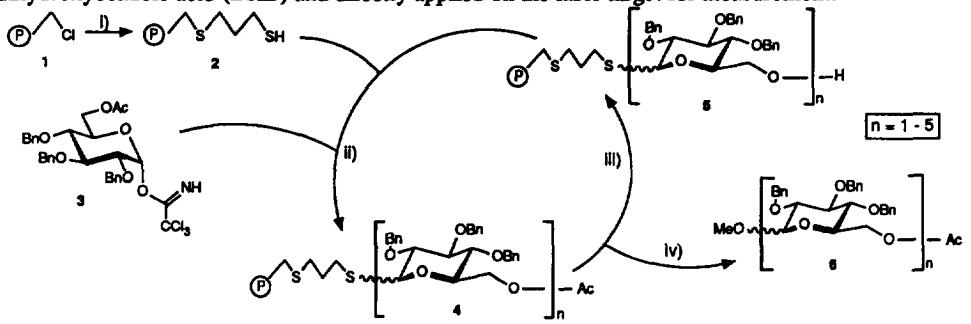


Figure 1. Synthesis cycle of solid phase oligosaccharide synthesis. i) 10 eq. propanedithiol, DBU (2eq), toluene, r.t., 16 h. ii) **3** (3 eq), TMSOTf, (0.2 eq), CH_2Cl_2, r.t., 1 h. iii) CH_2Cl_2/0.5 M sodium methoxide in MeOH (9:1), r.t., 2 h; 15-crown-5 (2 eq), CH_2Cl_2/MeOH 20:1. iv) DMTSB (2 eq), Hünig's base (2 eq), CH_2Cl_2/MeOH 9:1, 1 h.

Finally, preparative isolation of the glycosylation products **6** was carried out: The cleavage products were chromatographed over silica gel with toluene/ethyl acetate 5:1. The fully protected sugars were separated with a gradient of acetone/water over RP-18 flash silica gel. It was possible to isolate the di-, tri-, tetra- and pentamer as methyl glucosides[8]. For all oligosaccharides correct mass spectra were obtained. The α:β ratio of anomeric protons (as expected close to one) could be obtained from the integrated H1-C1-crosspeaks in the HMQC spectra[9]. Therefore, the isolated n-oligomer fractions contain 2^n (= 32 for the pentamer **6**) diastereoisomers.

In summary, the method presented is efficient for the synthesis of smaller oligosaccharides. The linker and the analytical tools should be of value for further applications especially in combinatorial syntheses.

References and Notes

1. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. J.R. acknowledges a grant from the Landesgraduiertenförderung Baden-Württemberg.
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7. Completion is indicated by the disappearance of any educt signals in the MALDI-MS.
8. In toluene/ethyl acetate 4:1 the 6-O-acetyl-protected oligosaccharides **6** have an R_f-value between 0.45 and 0.55, the 6-O-deprotected sugars around 0.2.
9. NMR data of compounds **6**: ^{1}H-NMR (600 MHz) δ in ppm: 4.95-5.02 H-1α, 4.25-4.30 H-1β. ^{13}C-NMR (150.9 MHz) δ: 97-99 C-1α, 104-106 C-1β.

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