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First Selective Synthesis of Thio-β-Cyclodextrin Derivatives by a Direct Mitsunobu Reaction on Free β-Cyclodextrin.

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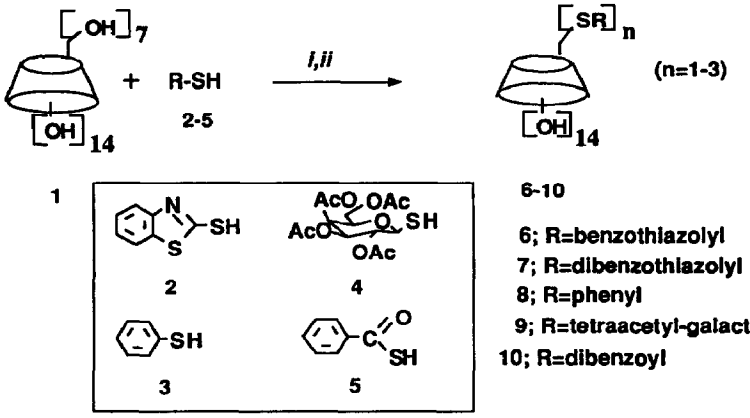
Abstract : The synthesis of mono and disubstituted β-CD thio derivatives has been successfully achieved by a thio-Mitsunobu reaction with a thio-saccharide and aromatic thiols directly on unprotected β-CD.

Some papers recently reported selective primary 6-hydroxyl group substitutions of protected hexapyranosides through the Mitsunobu reaction involving heteroaryl mercaptans[1] or the thio-functionalization of carbohydrates under various Mitsunobu conditions, both on 3 and 6 positions on unprotected mono and disaccharides.[2,3] Moreover, a previous work in the field describes a multistep approach to thioglycosyl cyclodextrins (cycloamyloses, CDs)[4] from a peracetylated 6-deoxy-6-iodo-CD followed by a deprotection step. The reactivity of primary hydroxyls towards nucleophiles under Mitsunobu conditions[5] led us to attempt the direct reaction of thiol nucleophiles on unprotected CDs. We wish to report here, the first successful results of this synthesis.

The reactions were carried out under modified Mitsunobu conditions, using anhydrous β-CD, triphenylphosphane (PPh_3), diisopropyl azodicarboxylate (*i*PrAD) and the desired thiol **2-5** (Scheme 1) in dry pyridine under a dry argon atmosphere. Diisopropyl azodicarboxylate must be added dropwise to the stirred mixture of β-CD, PPh_3 and the thiol. The reaction was followed by thin layer chromatography. The work-up of the reaction mixture containing the thio-β-CD derivative is fairly simple and involves the evaporation of the solvent, the addition of acetone to precipitate the products and filtration over a sintered glass. The crude precipitate of the thio-β-CD and unreacted β-CD was then chromatographed. Pure monosubstituted major and (or) minor disubstituted 6-thio-β–CD derivatives **6-10** (Scheme 1) were obtained after preparative reversed-phase HPLC purifications.[6] A large excess of thiol (12 eq.) and a long reaction time (22 hours), (*i*) were necessary to give 6-monothio compounds as major products. Working in the dark with 4 Å molecular sieves (*ii*) requires only 6 eq. of thiol and shorter reaction times (7.5 to 8 h). These conditions (*ii*) were found to be the most efficient but fairly poor in selectivity (see Table 1).



Scheme 1. Reagents and conditions : *i*, PPh3 / iPrAD / pyridine / room temp.; *ii*, PPh3 / iPrAD / DMF /dark / Mol. sieves 4 Å / room temp. *Yields (after HPLC sep.)* : **6**, n =1; *i*; yield=28%; **7**, n=2; *i*; yield=14%; **8**, n=1; *i*; yield=15%; **9**, n=1; *i*; yield= 8%; *ii*; yield=15%; **10**, n=2, *i*; yield=29%.

Thiol **3** (eq.)	*i* PrAD (eq.)	Reaction Solvent	Time (h)	Isomer ratios of **8** (%)[d]		
				n=1[c]	n=2	n=3
12	12	pyridine	8	57	30	13
6	12	DMA	7.5	25	47	28
12	12	pyridine[a]	8	81	14	5
12	12	DMA[a]	8	82	13	5
6	12	DMF[a,b]	7.5	39	44	17
3	6	DMF[a,b]	7.5	35	48	16

Table 1. Isomer ratios of **8** *versus* the conditions of reaction. [a] Presence of 4Å molecular sieves in the medium. [b] In the dark. [c] n = number of subtituents. [d] Isomers ratios of **8** separated by HPLC with UV detection wavelength (λ_{max} = 254 nm).

Calculated on the pure compounds, the yields were usually between 14-29%. In the case of **9** (8 to 15%), the unreacted starting products (the thiol and β-CD) may be easily recycled for other runs. We can therefore confirm that the direct proposed method clearly remains competitive compared to a multistep approach.[4] As expected, the best thiol reactivity was found with aromatic mercaptans. Because of it highest reactivity in the same reaction conditions the thioacid **5** readily gave the disubstituted adduct **10**. Monosubstitution probably works in low temperature conditions with a less amount of the thiol reagent. New attempts in this sense are under investigation and will be published elsewere.

Compounds **6-10** were analyzed by ^{13}C NMR and FABMS and the collected data matched with the proposed structures.[7] Chromatographic study by analytical reversed-phase HPLC (gradient elution : Methanol : Water)[8] of compound **8** (as example) permits a quantitative analysis of the different isomers and an evaluation

of the relative percentage *vs.* the reaction conditions (Table 1). Theoretically, each polysubstituted compound consists of several isomers (3 disubstituted and 4 trisubstituted for β-CD) for the same substitution degree. The sum of the peak areas (corresponding to the different isomers of a compound with the same substitution level) have been taken in account for the percentage determination of mono, di and trisubstituted thio-β-CDs respectively. The retention times increased as the substitution degree of CDs increased. Using reference compounds, we have verified that the molecular absorbance was proportional to the substitution degree of CDs by the chromophoric thiol unit 3. The following empirical formula (1) was used for the calculations:

$$100.\left[An.\sum_{1}^{3} n.Mr_n / An\right] / Mr_n = \%\ \text{of substituted } CD.^{9} \qquad (1)$$

A similar approach has been reported recently in the case of β-CD sulfoalkylethers using capillary electrophoresis as separative method, coupled to indirect UV detection.[10] The authors established that the response factor was dependent on the substitution degree of CD. Our results are in full agreement with this conclusion. We observed that the chromatographic properties of compounds **6-10**, with a same substitution degree, were unaffected by the nature of the thiol radical. The relative simplicity of the above-described method suggests a possible extension in analysis of other chromophoric CD mixtures.[11]

In conclusion, we demonstrate that 6-thio-β-CD derivatives could be synthesized in a one step reaction, starting from native β-CD and thiols using the direct thio-Mitsunobu approach. Satisfactory reaction conditions have been found to obtain a good selectivity in substitution compared to *e.g.* tosylation[12], and new attempts will be made to reach better yields particularly in the case of thio-carbohydrates. Further modifications and applications of this method with other important classes of nucleophiles (*e.g.* amines) are underway.

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References and Notes

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6. Preparative scale purifications were performed on a LiChroprep RP 18 ® Merck column, (particle size 5-20 µm, 230 x 36 mm I.D.); the products were eluted with different MeOH : H_2O ratios (30 : 70 to 70 : 30, v/v).
7. Structures of all compounds were assigned by ^{13}C NMR on a Bruker AM 400 spectrometer (double dashed numbers refer to the carbon of the unsubstituted unit of β-CD, dashed numbers to the carbon of

the 1-thioglycosyl unit, numbers of the substituted glycosyl unit of β-CD are not dashed). Mass spectra were recorded on a R-1010 Nermag spectrometer, in FAB^+ mode with glycerol as matrix. All new compounds gave satisfactory spectroscopic data.

6 : ^{13}C NMR ($DMSO_6d$) δ (ppm) = 35.6 (C_6); 59.8-60.2 ($C_{6''}$); 68-69.3 (C_5, $C_{5''}$); 72.3-73 (C_2, $C_{2''}$, C_3, $C_{3''}$); 81.5-82.1 ($C_{4''}$); 85.7 (C_4); 101.5-102.8 (C_1, $C_{1''}$); 121.1, 122.0, 124.6, 126.5 (CH, aromatics); 134.9 (C-S, aromatic); 152.8 (C-N, aromatic); 166.3 (N=C-S). MS (FAB^+): 1306 ($M+H^+ + Na^+$), 1284 ($M+H^+$).

7 : ^{13}C NMR ($DMSO_6d$) δ (ppm) = 35.3 (C_6); 59.7-60.0 ($C_{6''}$); 69.3-69.5 (C_5, $C_{5''}$); 72.1-73.1 (C_2, $C_{2''}$, C_3, $C_{3''}$); 81.2-81.9 ($C_{4''}$); 85.4-85.8 (C_4); 101.5-102.8 (C_1, $C_{1''}$); 120.9, 121.1, 121.7, 124.5, 124.6, 126.3, 126.5 (CH, aromatics); 134.7, 134.9 (C-S, aromatics); 152.6, 152.8 (C-N, aromatics); 166.1, 166.2 (N=C-S). MS (FAB+), 1456 ($M+H^+ + Na^+$), 1433 ($M+H^+$).

8 : ^{13}C NMR ($DMSO_6d$) δ (ppm) = 35.9 (C_6); 60.1 ($C_{6''}$); 72.2-73.2 (C_2, $C_{2''}$, C_3, $C_{3''}$,C_5, $C_{5''}$); 81.6 ($C_{4''}$); 85.2 (C_4); 102.1 (C_1, $C_{1''}$); 126.3, 129.0, 130.3 (CH, aromatics); 136.3 (C, aromatic); MS (FAB^+): 1248 ($M+H^+ + Na^+$), 1226 ($M+H^+$).

9 : ^{13}C NMR ($DMSO_6d$) δ(ppm) = 22.0 (CH_3-CO); 31.3 (C_6); 60.0 ($C_{6''}$); 67.9 (C_5, $C_{5''}$); 68.9 ($C_{6'}$), 72.1-73.1 (C_2, $C_{2''}$, $C_{2'}$, C_3, $C_{3'}$, $C_{3''}$, C_5, $C_{5''}$); 81.6 ($C_{4''}$); 83.8 (C_4); 102.1 (C_1, $C_{1''}$); 169.8 (C= O) MS (FAB^+): 1518 ($M+H^+ + Na^+$), 1496 ($M+H^+$).

10 : ^{13}C NMR ($DMSO_6d$) δ(ppm) = 35.9 (C_6); 59.4 ($C_{6''}$); 69.4-69.8 (C_5, $C_{5''}$); 72.3-73.0 (C_2, $C_{2''}$, C_3, $C_{3''}$); 80.7-80.9 ($C_{4''}$); 85.7-86.1 (C_4); 101.4-102.8 (C_1, $C_{1''}$); 126.7, 128.7, 133.5 (CH, aromatics); 136.3 (C, aromatic); 190.4-190.7 (C=O). MS (FAB^+): 1397 ($M+Na^+$), 1375 ($M+H^+$).

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11. Analytical separations were realized on an end capped column LiChrospher 100 RP18 ® (particle size: 5 µm; 125 x 4 mm I.D.; Merck Clévenot, Nogent-sur-Marne, France) at a temperature of 20 ± 1°C with a gradient elution as follows: isocratic elution with MeOH : H_2O (1 : 99, v/v) for 5 min, then the MeOH content was increased linearly up to 95% (v/v) for 30 min. UV detection was operated either at 254 nm (for β-CD substituted with thiophenol) or at 280 nm (for β-CD substituted with 2-mercapto-benzothiazole). The HPLC system consisted of a two-solvent gradient pump Spectra Physics P2000® (Thermo Separation Products, Les Ulis, France), an injection valve (Rheodyne 7125 ®, Cotati, CA, USA) equipped with a 50 µl sample loop, an UV spectrophotometric detector (Spectra Physics UV2000) and an integrator (Chromjet ®, Spectra Physics), connected to a data station (Winner ®, Spectra Physics).
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