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A New Chemoenzymatic Synthesis of 6'-*O*-Acylsucroses.

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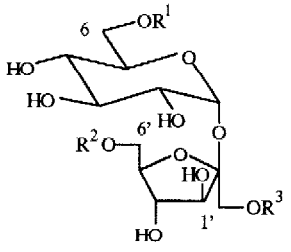
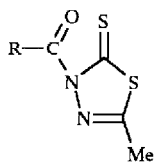
Abstract : *6'-O-acylsucroses were synthesized for the first time in two steps, including a new chemical selective acylation of free sucrose followed by an enzymatic hydrolysis of the 6-O-acylated by-products.*

Partially acylated sucroses are important compounds i) as derivatives of commercial significance and ii) as intermediates in sucrochemistry.[1] As an example of the former, commercially available sucroesters are currently used as emulsifying agents in foods, cosmetics and pharmaceuticals. [2] Although these applications need only mixtures of acylated compounds, pure and clearly defined derivatives of sucrose become actually of primary interest Selective monoacylation of sucrose remains at present difficult to achieve due to i) near relative reactivities of the hydroxyl groups, [1,3] and to ii) facile intramolecular acyl migrations in the unprotected derivatives. [3] Nevertheless some chemical modifications of the free substrate have been already proposed. Acylations of the least hindered primary hydroxyls lie with the use of exceptionally mild conditions, [1] sterically hindered reagents [4] or bulky intermediates, thus applying the Mitsunobu reaction in the carbohydrate field. [5] Under suitable conditions, these reactions can lead to 6,1',6'-triesters or 6,6'-diesters or 6-monoesters according to the well established [6-OH ≥ 6'-OH > 1'-OH > secondary -OH] reactivity order of hydroxyls towards electrophiles commonly used in esterifications. On the other hand, Klibanov [6] succeeded recently in a selective acylation of the neopentylic - like 1'-OH by a protease-catalysed transesterification of sucrose in anhydrous dimethylformamide. All these reports show that selective acylation of sucrose remains difficult to foresee and 6'-*O*-acylsucroses have not yet been isolated to our knowledge.



1 , $R^1 = R^2 = R^3 = H$
3 , $R^1 = R$ - CO, $R^2 = R^3 = H$
4 , $R^2 = R$ - CO, $R^1 = R^3 = H$
5 ; $R^3 = R$ - CO, $R^1 = R^2 = H$



2

a ; $R = CH_3 - (CH_2)_6$ -
b ; $R = CH_3 - (CH_2)_{10}$
c ; $R = CH_3 - (CH_2)_{16}$
d ; $R = C_6H_5 - (CH_2)_2$ -
e , $R = C_6H_5 - (CH_2)_3$ -

We wish now to report on an easy route to these compounds, that is based on a novel chemoenzymatic methodology. Owing to this work these compounds could be obtained in a pure state for the first time. 3-Acyl-5-methyl-1,3,4-thiadiazole-2(3H)-thiones **2** were previously shown by us to be very effective in selective acylations of hexopyranoses, methyl-D-glycosides as well as glycosylamines. [7] Indeed they could acylate the primary hydroxyl of α-D-glucose in pyridine at room temperature without additional initiator or catalyst. When the reaction was performed under similar conditions with sucrose **1**, reagent **2c** disappeared slowly. As shown in table (entry 1), selectivity was low and the reaction gave a mixture of monoesters containing compounds **3c-5c** along with 2-O-stearoylsucrose (7 %), 3-O-stearoylsucrose (4 %) and two undefined monoesters (2 and 6 %).

Table. Acylations of unprotected sucrose with 3-acyl-5-methyl-1,3-4-thiadiazole-2(3H)-thiones.

Entry	Reagent	Solvent [a]	Conditions		Monoesters of sucrose					Yield of isolated ester 4 (%) [d]	
			Time/h	T/°c	Overall yield (%)	Product composition (GLC, %) [b]					
						3	4	5		A	B
1	**2c**	C_5H_5N	70	RT	67 [c]	42	27	11		-	
2	**2c**	DMF	15	RT	75	35	45	19	**4c**	29	-
3	**2a**	DMF	2	- 20 to - 10	74	29	63	8	**4a**	40	40
4	**2b**	DMF	4	- 30 to - 15	63	28	63	7	**4b**	35	45
5	**2c**	DMF	7 5	- 30 to - 20	62	26	63	8	**4c**	35	44
6	**2d**	DMF	2 5	-30 to -15	75	20	61	12	**4d**	40	-
7	**2e**	DMF	2 5	- 20	75	30	60	6	**4e**	43	-

a) Reagent, DABCO and sucrose were used in the respective molar ratio of . 1/2/2.
b) The mixtures compositions were determined by GLC analysis after conventional silylation [12], on a Altech OV1 column (0,33 mm x 15 m) , initial temperature . 270°C ; final temperature : 305°C , rate, 2 deg/min.
c) In addition, 2-O-stearoylsucrose (7 %), 3-O-stearoylsucrose (4 %) and other undefined isomers (2 and 6 %) were contained in this mixture
d) Method A : compounds **4a-e** were purified after acylation by column chromatography for separating them from 6-*O*-acyl and 1'-*O*-acylsucroses (Merck 60H silica gel , solvent . ethyl acetate/methanol . 12/1 then 9/1, v/v). Method B . the mixtures obtained in acylations were purified by a previous enzymatic hydrolysis (*vide infra*), and then by a simple filtration on silica gel (ethyl acetate/methanol 9·1, v/v)

On the other hand, when the acylation was achieved in anhydrous DMF, which is a more convenient solvent of sucrose, no reaction was observed without adding a base or an initiatior. In the presence of 1,4-diazabicyclo [2.2.2] octane (DABCO) as an initiator or/and a catalyst, acylation occured and selectivity was enhanced towards 6'-O-acylsucroses **4** (see table, entry 2). At last, the best results were obtained when the addition of DABCO and the acylation were performed at lower temperatures (see table, entries 3-7). In a typical protocol 3,4 g of **1** and then 1,4 g of **2e** were dissolved in anhydrous DMF (30 ml). The mixture was then cooled at - 20°C and 1,1 g of DABCO was added dropwise The mixture was shaken at the same temperature for 2.5 hours after which time all **2e** reacted. The base was then neutralized with AcOH and the solvent was removed under reduced pressure. The mixture was next subjected to our usual procedure. [7]

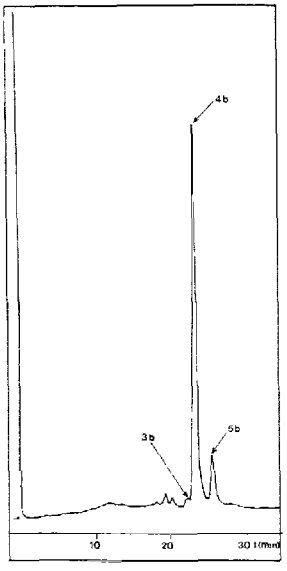


Fig. 1. GLC chromatogram of a monolaurates mixture after selective hydrolysis of ester 3b using CCL. conditions : see table b).

Surprisingly, the major product, isolated as a single pure compound (43 % yield) by column chromatography, revealed its structure as the 6'-O-acylsucrose **4c** , resulting from acylation of the fructose moiety at the C6' position [8] . Similar experiments conducted with reagents **2a-d** gave the results which are presented in the table. In order to explain these data, one can assume that DABCO probably acts as a base, as well as a nucleophilic catalyst. In the former part, selective activation of the more acidic hydroxyl (s) to the more nucleophilic alkoxide (s) may occur at the C-2 and the C-6' hydroxyls, that are intramolecularly hydrogen bonded. [9] On the other hand, reagents **2** whose carbonyl infrared absorptions are located at a high frequency (ν C=O $\equiv$1760 cm^{-1}), meaning that the 3-nitrogen atom conjugates with the thiocarbonyl group of the heterocycle rather than with the carbonyl one, could easily lead with DABCO to bulky loose ion pairs which preferably acylate the least hindered alkoxide, that is to say the C-6' one.

To make the purification of esters **4** easier, we then tried to hydrolyse selectively **3** and **5** by-products. Indeed, Sweers [10] and Klibanov [11] reported that selected lipases could hydrolyse di-or pentaesters of glucose virtually in the C-6 position. We predicted that *Candida cylindracea* lipase (CCL) can also deacylate 6-O-esters of sucrose. This phenomenon was thus used to eliminate compounds **3** from the crude mixtures obtained in the previous acylations.

These predictions were experimentally verified when 0.5 g of a complex mixture of sucrose monoesters was dissolved in a mixture of phosphate buffer (pH 7.7) and DMF (10:1, v/v) [14] followed by addition of 0.25 to 0.38 g of *Candida cylindracea* lipase, and shaking of the mixture at 35°C. A nearly quantitative hydrolysis of esters **3a-c** was detected by gas chromatography after 4.5 to 24 hours without hydrolysis of other esters, whereas any hydrolysis was detected when the same experiment was achieved without lipase.We could thus obtain highly purified 6'-*O*-acylsucroses **4a-c** and yields of the isolated esters could be enhanced by using this procedure (table, method B). Nevertheless, the previous enzymatic hydrolysis was not successful, as expected, for the purification of aromatic esters **4d-e**

Concludingly, the method outlined above provides a new and simple synthesis of 6'-*O*-acylsucroses, which should be widely recognized as an efficient route to new sucrose esters with potential useful properties.

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8 The positions of acylation were established by ^{13}C NMR following the general method of Yoshimoto.[13] In the case of compounds **4a-e**, acylation of the fructose moiety at the C-6' position results in a downfield shift (3.4-3.6 ppm) of the peak at C-6' and a similar upfield shift of the peak at C-5'. Physical and spectroscopic data for **4a** : amorphous product ; $[\alpha]^{20}_D$: + 32,0 (c = 1, MeOH) , IR (HCB) ν (cm^{-1}) 3400 (broad band, OH), 1730 (C=O), 1100-1000 (C-O) ; ^{13}C {^1H} NMR δ (ppm) (DMSO-d_6) : 13.88 (CH_3), 22.00, 24.41, 28.34, 31.08 [$(CH_2)_5$], 33.38 ($\underline{C}H_2$-CO), 60.82 (C_6), 61.77 ($C_{1'}$), 65 53 ($C_{6'}$), 70.14 (C_4), 71.68 (C_2), 72.82 and 72 93 (C_3 and C_5), 74.93 ($C_{4'}$), 76.61 ($C_{3'}$), 79.18 ($C_{5'}$), 91.78 (C_1), 104 29 ($C_{2'}$), 172.85 (C=O) ; MS (EI, 70 eV) m/z (after silylation of the remainder free hydroxyls) 972 $M^{+\cdot}$ (not observed) ; 505 (19.76 %) M_1^+ (M-467)$^+$ (calculated mass for $C_{23}H_{49}O_6Si_3$: 505.28367, found : 505.2827) ; 451 (1,92 %) M_2^+ (M-521)$^+$ (calculated mass for $C_{18}H_{43}O_5Si_4$: 451.21874, found : 451 2187) ; 594 (1.61 %) (M_1 + TMSO)$^{+\cdot}$; 491 (20.14 %) (M_1 + TMSO-TMSOCH$_2$)$^+$, 271 (100.00 %) (M_1 - CH_3 -$(CH_2)_6$-CO_2H-TMSOH)$^{+\cdot}$ and (M_2 - 2 TMSOH)$^+$; 103 (21.57 %) $(TMSOCH_2)^+$; 73 (99 71 %) $(TMS)^+$.

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14 DMF or THF was added to the phosphate buffer in order to dissolve the monostearates and monolaurates, and our experiments revealed a more significant activity of CCL with DMF rather than with THF.

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