Unusual regioselective acylation of the primary hydroxy groups of β-cyclodextrin

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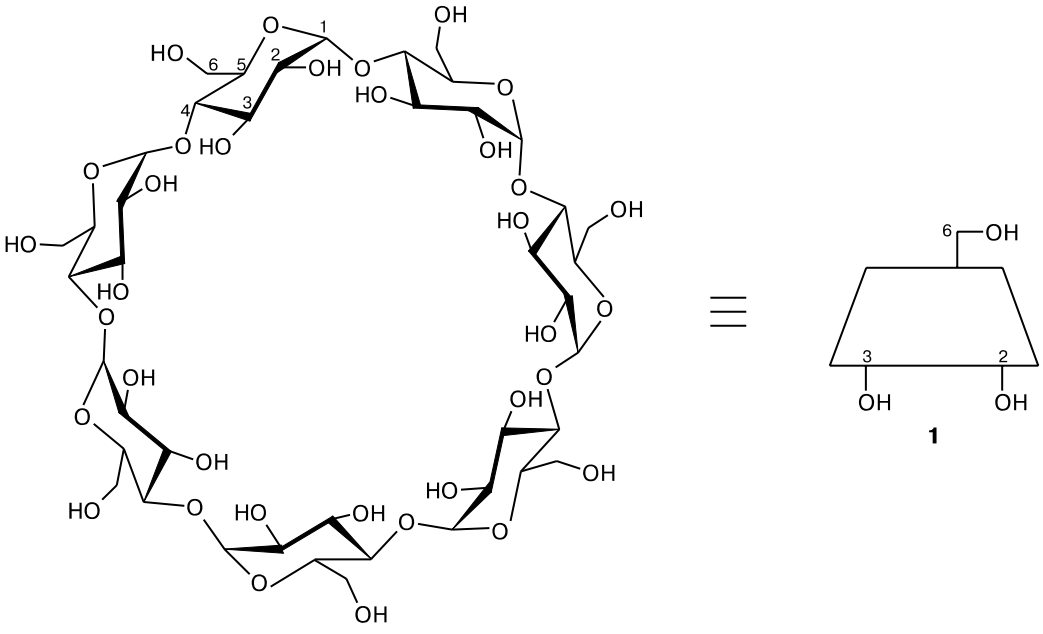
The reaction of β-cyclodextrin (**1**) with palmitoyl (**2**) and valeryl (**4**) chlorides in DMF or Py, unlike previously studied acetylation of **1**, involves only the primary hydroxy groups of **1**. The outcome of the reaction depends on the reaction conditions and the nature of the acid scavenger used (Et_3N, Pr^i_2NEt, $PhNMe_2$, Py). ^{13}C NMR spectroscopy was shown to be an effective tool in determining the number and position of aliphatic carboxylic acid residues introduced into **1**. A hypothesis stating that preliminary formation of a reactive inclusion complex (acid chloride⊂**1**) is required for the acylation of **1** to occur is proposed and substantiated. This hypothesis provides a unified explanation for a variety of unusual facts observed in the acylation of **1** and its derivatives.

Key words: cyclodextrins, inclusion complexes, acylation, regioselectivity, aliphatic carboxylic acids, carboxylic acid chlorides, amines, NMR spectroscopy.

Cyclodextrins (CDs) are cyclic oligosaccharides in which D-glucopyranose residues are connected by α-1-4-glycosidic bonds. Owing to the presence of a chiral inner cavity, CDs possess a number of unique properties, the most important one being the ability to form host—guest type inclusion compounds. This ability ac-

Scheme 1



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counts for the interest in fundamental and applied studies of CDs and their derivatives (first of all, the most accessible β-CD (**1**)) over several decades.[1]

Many properties of CDs such as the solubility in water and organic solvents and the ability to form inclusion compounds can be deliberately changed by selective modification[2] of CDs. Of special interest are amphiphilic derivatives[3—5] that contain, for example, fatty acid residues and find application as substrate transfer agents in phase transfer catalysis,[6—8] in the transmembrane transport of biologically active molecules,[9] in preparing Langmuir—Blodgett films,[10] in investigations of micelles and vesicles based on them,[11] and as sensing elements in the sensors meant for the determination of other molecules.[12]

Targeted functionalization of CDs is an experimental challenge because their molecules contain hydroxy groups of different nature susceptible to the formation of intra- and intermolecular hydrogen bonds.[13,14] Commonly, the reaction products result from nonselective replacement of both primary and secondary hydroxy groups. Meanwhile, regioselectively substituted CDs are most important for practical purposes. Currently, these compounds are regarded as building blocks in the design of supramolecular structures.[15] Syntheses of such CD derivatives often require laborious multistep procedures involving the use of protective groups.[2]

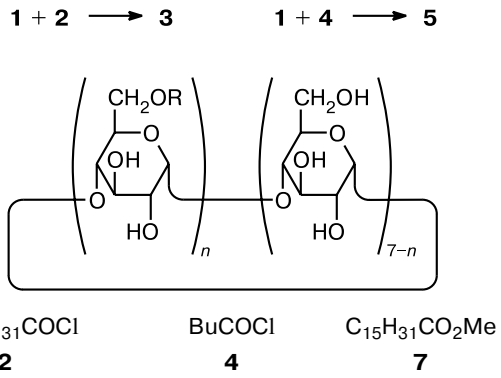
Approaches to selective functionalization of CDs have been considered in a review.[2] One-step methods based on direct acylation of unsubstituted CDs appear to be the most attractive for the introduction of the fatty acid residues into CDs. Although selective acylation of hydroxy groups (first of all, primary) with various acylating reagents is well-known in the chemistry of monosaccharides and acyclic oligosaccharides,[16—21] the reaction conditions often cannot be directly extended to CDs.[2,4,22—28] The acylation of CDs is often nonselective, the selectivity and the degree of substitution of the hydroxy groups substantially depending on the reaction conditions. Despite the large number of publications dealing with this topic, targeted modification of CDs is still a complicated experimental task and study of the methods of synthesis of CD derivatives is a topical line of research in modern organic chemistry.

Results and Discussion

Palmitoylation

As a continuation of our studies on the selective acylation of CDs by aliphatic carboxylic acid chlorides[29] aimed at the preparation of new CD-based surfactants, we studied direct acylation of β-CD (**1**) with palmitoyl chloride (**2**) in DMF and in Py (Scheme 2). The choice of these particular solvents was dictated, first of all, by solubility issues. Since it was shown previously[29] that the outcome of the reaction of **1** with AcCl in DMF depended substantially on the acceptor of hydrogen chloride evolved during the reaction, we studied the effect of various bases on the reaction of **1** with **2**. For this purpose, 3 equiv. of **2** were added over a period of 1 h to a solution of **1** in DMF containing 3.3 equiv. of a base (Et_3N, $PhNMe_2$, or Pr^i_2NEt) and the reaction mixture was stirred for 2 h at 20 °C. Similar experiments were also carried out in DMF without a base and in Py. The reaction was quenched by adding excess MeOH, and product **3** was precipitated with pentane (the yields are given in Table 1). The regioselectivity of substitution in products **3** was determined by ^1H and ^{13}C NMR spectroscopy. The signal assignment in the ^1H NMR spectra was carried out using $^1H—^1H$ COSY 2D spectroscopy, and the ^{13}C NMR signals were assigned using selective $\{^1H\}—^{13}C$ heteronuclear resonance and the JMODXH procedure. The latter made it possible to identify the signals of the methylene groups (C(6) and C(6A)*). The degree of substitution of the hydroxy groups in **3** was estimated from the ratio of the integral intensities of the proton and carbon signals in unsubstituted and acylated* glucopyranose residues (by comparing the integral intensities of the H(5) + H(5A) + H(6), C(6) and H(6A), C(6A) signals, respectively) and from the ratio of integral intensities of the signals for anomeric protons in unsubstituted and substituted glucopyranose residues (δ_H 5.46—5.56 (for H(1)) and δ_H 5.35—5.42 (for H(1A)), respectively). The number of introduced palmitoyl residues was determined by an independent method from the integral intensity ratio of the signals for all protons in the pyranose rings (δ_H 3.70—5.60)

Scheme 2



R = H (*n* = 0) (**1**), $C_{15}H_{31}CO$ (**3**), BuCO (**5**), $SiMe_2Bu^t$ (*n* = 7) (**6**)

Note. The reaction conditions and the degrees of substitution (*n*) for compounds **3** and **5** are presented in Table 1.

* The letter A is added to the designation for the corresponding atom in the acylated residue.

Table 1. Conditions of acylation of β-cyclodextrin (**1**) and product characteristics

Entry	Acyl chloride	N [a]	HCl acceptor[b]	Solvent	Product	n[c]	R_f[d]	Yield (%)	Chemical shifts of acylation products (δ, J/Hz)[e]					
									C(1) + C(1A) H(1)[f]/H(1A)[f]	C(2) + C(2A) H(2) + H(2A)	C(3) + C(3A) H(3) + H(3A)	C(4) + C(4A) H(4) + H(4A)	C(5)/C(5A) H(5) + H(5A)	C(6)/C(6A) H(6)/H(6A)
1	**2**	3	NEt_3	DMF	**3**[g]	1.6	0.75 (A)	90	104.0 5.45 d[h]/5.34 d[i]	74.3 3.86—4.03	73.9 4.48—4.65	83.4 4.03—4.16	74.7/70.7 4.21—4.41	61.5/64.3 4.21—4.41/4.66—4.95
2	**2**	3	Pr^i_2NEt	DMF	[j]		—	90	104.0 5.54 (d, J = 2.6)	74.3 4.01 dd[k]	73.9 4.68 t[l]	83.4 4.15 t[m]	74.7 4.28—4.46	61.6 4.28—4.46
3	**2**	3	Py	Py	**3**[g]	1.8	0.82 (A)	91	104.0 5.55/5.42	74.3 3.87—4.10	73.9 4.54—4.74	83.4 4.11—4.26	74.7/70.6 4.29—4.50	61.5/64.2 4.29—4.50/4.76—5.08
4	**2**	3	$PhNMe_2$	DMF	**3**[g]	2.3	0.79 (A)	94	104.0 5.54/5.42	74.2 3.85—4.09	73.8 4.50—4.79	83.4 4.12—4.23	74.6/70.6 4.27—4.48	61.5/63.8,64.3 4.27—4.48/4.85—5.02
5	**2**	7	Py	Py	**3**[g]	4.9	0.88 (A)	73	103.5—104.4[f] 5.50/5.39	74.3 3.82—4.10	73.9 4.57—4.80	82.8—84.1[f] 4.13—4.23	74.7/70.6 4.24—4.40	61.5/63.8,64.3 4.41—4.57/4.81—5.10
6	**2**	9	Py	Py		[n]	—	[o]	—	—	—	—	—	—
7	**2**	3	[p]	DMF	**3**[g]	1.1	0.79 (A)	93	103.9,104.3 5.46 d[q]/5.35 d[i]	74.3 3.89—4.04	73.9 4.49—4.66	83.5,84.1 4.04—4.16	74.7/70.7 4.20—4.41	61.7/64.2 4.20—4.41/4.68—4.96
8	**4**	7	NEt_3	DMF	**5**[r]	1.5	0.85 (B)	91	104.0 5.56/5.42	74.3 3.92—4.09	73.9 4.54—4.78	83.4,84.1 4.11—4.27	74.7/70.7 4.27—4.52	61.6/64.3 4.27—4.52/4.87—5.01
9	**4**	2	Pr^i_2NEt	DMF	**5**[r]	1.5	0.84 (B)	83	104.0 5.56/5.41	74.3 3.81—4.09	73.9 4.53—4.75	83.4 4.09—4.23	74.7/70.7 4.26—4.51	61.5/64.2 4.26—4.51/4.83—5.03
10	**4**	7	Pr^i_2NEt	DMF	**5**[r]	1.9	0.85 (B)	81	104.0 5.54/5.40	74.3 3.94—4.07	73.9 4.56—4.74	83.4 4.09—4.24	74.7/70.8 4.26—4.48	61.5/64.3 4.26—4.48/4.85—4.97
11	**4**	2	$PhNMe_2$	DMF	**5**[r]	1.9	0.86 (B)	78	104.0 5.50/5.36	74.3 3.92—4.07	73.9 4.47—4.72	83.5,84.2 4.07—4.21	74.7/70.8 4.23—4.45	61.6/63.9,64.3 4.23—4.45/4.83—4.99
12	**4**	7	$PhNMe_2$	DMF	**5**[r]	3.3	0.83 (B)	84	104.0 5.51/5.38	74.3 3.80—3.94	74.1 4.45—4.70	83.5 3.94—4.09	74.7/70.8 4.25—4.44	61.5/64.4 4.25—4.44/4.84—5.01
13	**4**	7	Py	Py	**5**[r]	4.5	0.84 (B)	88	104.0,104.3 5.52/5.38	74.6 3.74—4.01	74.0 4.43—4.78	83.6,84.0 4.01—4.43	74.7/70.7 4.12—4.43	61.2/63.9 4.12—4.43/4.74—5.05
14	**4**	7	[p]	DMF	**5**[r]	3.2	0.84 (B)	91	103.9 5.55/5.36	74.2 3.79—4.09	74.0 4.43—4.72	83.4 4.10—4.21	74.6/70.5 4.22—4.46	61.3/63.8,64.2 4.22—4.46/4.81—5.03
15	—	—	—	—	**1**			—	103.9 5.55 d[h]	74.3 3.91 dd[s]	73.9 4.69 t[m]	83.4 4.16 t[l]	74.6 4.30—4.47	61.5 4.30—4.47

[a] The number of equivalents of acyl chloride **2** or **4** relative to CD **1**. [b] A 10% molar excess of amine relative to acyl chloride **2** or **4**. [c] The degree of substitution (n) according to NMR data (see p. 238). [d] The solvent system used for TLC is given in parentheses (see Experimental). [e] All signals in the NMR spectra are broadened multiplets (m) unless otherwise indicated. [f] The signals are broadened. [g] Other signals for **3**: ^{1}H NMR (pyridine-d_5), δ: 0.64—0.82 (m, 3 H, $(CH_2)_{12}C\underline{H}_3$); 1.03—1.30 (m, 24 H, $(C\underline{H}_2)_{14}Me$); 1.44—1.63 (m, 2 H, $COCH_2C\underline{H}_2$); 2.17—2.47 (m, 2 H, $CO\underline{C}H_2CH_2$); ^{13}C NMR (pyridine-d_5), δ: 14.3 ($(CH_2)_{14}\underline{C}H_3$); 22.9, 25.2, 29.0—31.0, 32.1, 34.2 ($(\underline{C}H_2)_{14}Me$); 173.7 (C=O). [h] J = 3.2 Hz. [i] J = 3.0 Hz. [j] The reaction does not proceed. Only the complex Pr^i_2NEt—**1** (1 : 1) was isolated, yield 90%. Other signals (for Pr^i_2NEt—**1** (1 : 1)): ^{1}H NMR (pyridine-d_5), δ: 1.07 (d, 12 H, $CHC\underline{H}_3$, J = 6.6 Hz); 1.15 (t, 3 H, $CH_2C\underline{H}_3$ J = 7.6 Hz); 2.57—2.76 (m, 2 H, $C\underline{H}_2Me$); 3.13—3.31 (m, 2 H, $C\underline{H}Me$); ^{13}C NMR (pyridine-d_5), δ: 18.7, 18.8 (Me); 41.0 (CH_2); 51.9 (CH). [k] J = 9.6 Hz, J = 3.6 Hz. [l] J = 9.2 Hz. [m] J = 9.4 Hz. [n] We were unable to characterize the product (see p. 244). [o] The weight of the product was ~95% of the weight of CD **1** taken in the reaction. [p] Without addition of a base. [q] J = 3.4 Hz. [r] Other signals for **5**: ^{1}H NMR (pyridine-d_5), δ: 0.44—0.68 (m, 3 H, $(CH_2)_3C\underline{H}_3$); 0.89—1.16 (m, 2 H, $CH_2C\underline{H}_2Me$); 1.23—1.45 (m, 2 H, $CH_2C\underline{H}_2Et$); 2.09—2.30 (m, 2 H, $COC\underline{H}_2CH_2$); ^{13}C NMR (pyridine-d_5), δ: 13.7 ($(CH_2)_3\underline{C}H_3$); 22.3 ($CH_2\underline{C}H_2Me$); 27.1 ($CH_2\underline{C}H_2Et$); 33.8 ($CO\underline{C}H_2CH_2$); 173.6 (C=O). [s] J = 9.4 Hz, J = 2.6 Hz.

Table 2. Dependence of the regioselectivity and the degree of acylation of β-cyclodextrin (**1**) on the nature of acyl chloride and the base

HCl acceptor[a]	Solvent	Acyl chloride								
		$C_{15}H_{31}COCl$ (**2**)			BuCOCl (**4**)			AcCl [b]		
		N^c	Regio-selectivity	n^d	N^c	Regio-selectivity	n^d	N^c	Regio-selectivity	n^d
NEt_3	DMF	3	O(6)	1.6	7	O(6)	1.5	7	O(2), O(3)	2.0
Pr^i_2NEt	DMF	3	[e,f]		2	O(6)	1.5			
					7	O(6)	1.9	7	O(6)	2.2
$PhNMe_2$	DMF	3	O(6)	2.3	2	O(6)	1.9			
					7	O(6)	3.3	7	[e]	
[g]	DMF	3	O(6)	1.1	7	O(6)	3.2	7	O(2), O(3), O(6)	7.0
Py	Py	3	O(6)	1.8						
		7	O(6)	4.9	7	O(6)	4.5	7	O(2), O(3)	3.8
		9	[h]							

[a] A 10% molar excess of amine relative to acyl chloride **2** or **4** was used.
[b] Data of Ref. 29.
[c] The number of equivalents of acyl chloride **2** or **4** relative to the CD **1** taken.
[d] Degree of substitution (n) according to NMR (see the text).
[e] The reaction does not proceed.
[f] Only the Pr^i_2NEt—**1** complex (1 : 1) was isolated, yield 90%.
[g] Without addition of a base.
[h] We were unable to characterize the product (see p. 244). The weight of the product was ~95% of the weight of CD **1** taken in the reaction.

to the alkyl-group protons in the palmitic acid residues (δ_H 0.69—2.44). The degrees of substitution determined by different methods coincided to within the error of measurements. It is noteworthy that the ^{13}C NMR spectra proved to be most convenient for the location of the acyl substituents and determination of the degree of substitution. Detailed interpretation (and integration of signals) of the ^1H NMR spectra of the acylation products is often hampered due to substantial signal broadening, especially in the case of high degrees of substitution. The results are summarized in Tables 1 and 2; the spectral data for the starting cyclodextrin **1** are given for comparison.

In all cases (including the reaction carried out in DMF in the absence of an HCl acceptor), the direct acylation of β-CD (**1**) with palmitoyl chloride (**2**) involved exclusively primary hydroxy groups and gave products **3** containing different numbers of the palmitic acid residues at O(6) (the degrees of substitution (n) are indicated in Table 1; n = 1.1—4.9; the maximum theoretically possible value of n is 7). This regioselectivity of acylation followed from the fact that the characteristic (*cf.* Ref. 30) signals for H(6A) and C(6A) in the ^1H and ^{13}C NMR spectra of products **3** are shifted downfield compared to the corresponding signals in the starting **1**: from δ_H 4.30—4.47 (for H(6) in **1**) to δ_H 4.66—5.10 (for H(6A) in **3**) and from δ_C 61.5 (for C(6) in **1**) to δ_C ~64 (for C(6A) in **3**). It should be noted that at higher degrees of substitution (starting from $n \approx 2$), the C(6A) atoms of compound **3** are responsible for two (δ_C 63.8 and 64.3) rather than one (δ_C 64.2—64.3) signals in the ^{13}C NMR spectrum, which is indicative of different environments of the C(6A) atoms in highly substituted compounds **3**. The fact that it is the O(6) atom that is acylated is confirmed additionally by the characteristic upfield shift[30] of the C(5A) signal (from δ_C 74.6 for C(5) in **1** to δ_C 70.6—70.8 for C(5A) in **3**).* It is significant that acylation of **1** with palmitoyl chloride (**2**) does not entail any significant shifts of signals of secondary carbon atoms in the spectra of the reaction products (the signals for C(2), C(2A), C(3), and C(3A) are located in a very narrow region, δ_C 73.8—74.7, Fig. 1), which points indirectly to the absence of substitution of secondary hydroxy groups in **3**. The ^{13}C NMR spectra of the products of acylation[26] and sulfonylation[31—33] of the secondary hydroxy groups in CD exhibit low-field signals at δ_C 74—80. However, it should be admitted that the real situation is much more intricate: indeed, the signals for C(2A) and C(3A) in the spectra of peracetylated and perbenzoylated CDs do not display substantial shifts compared to the C(2) and C(3) signals in unsubstituted CDs and, besides, their chemical shifts depend substantially on the solvent used to record the NMR spectra (the most pronounced dependence is found for C(2)).[22]

In order to find out whether the palmitoylation of secondary hydroxy groups of CD **1** with chloride **2** is, in principle, possible, we attempted acylation of a derivative

* The integral intensity of the signal for C(5A) coincided with the intensity of the signal for C(6A).

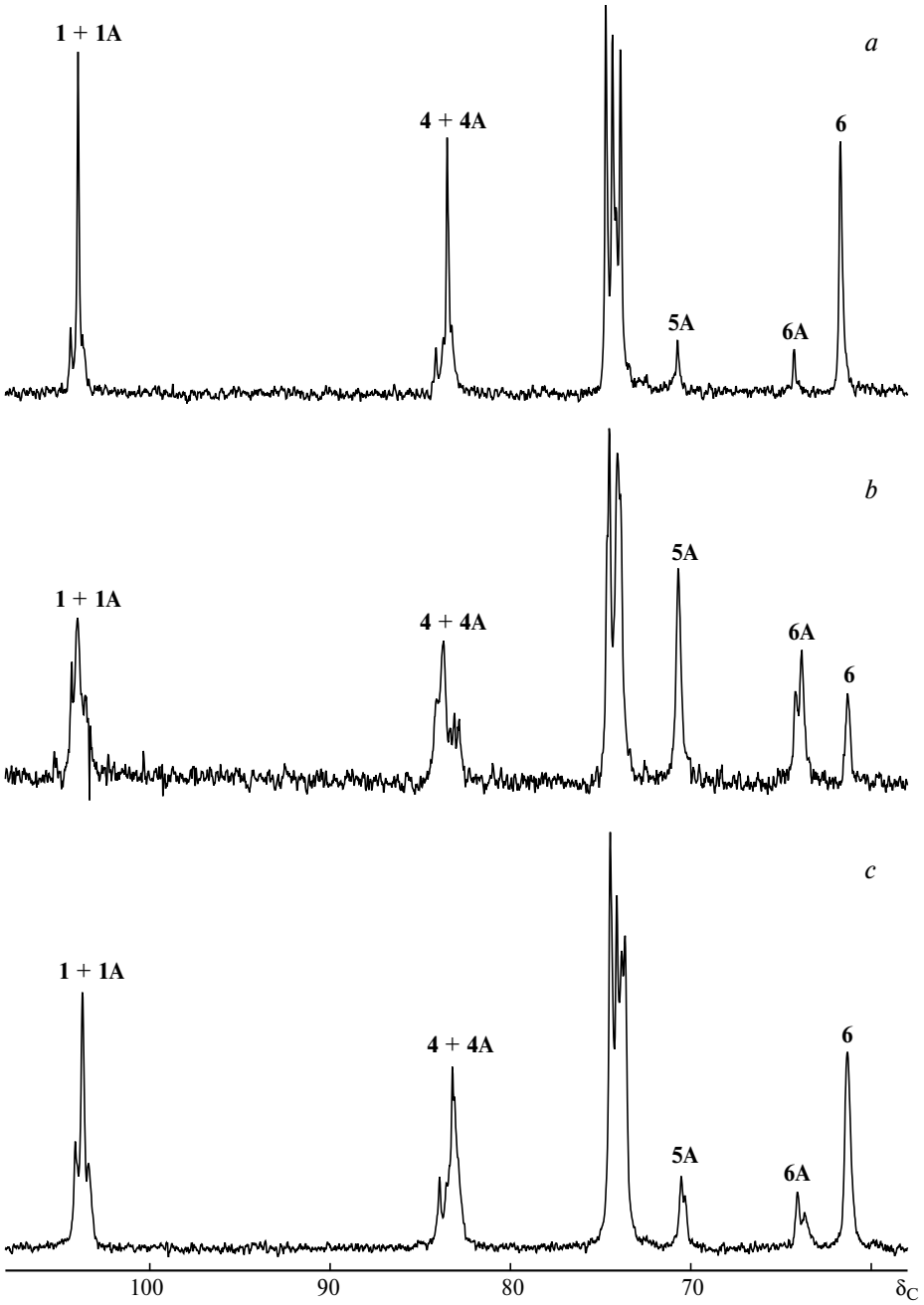


Fig. 1. ^{13}C NMR spectra of acylated cyclodextrins **3** (*a*, *b*) and **5** (*c*) with different degrees of substitution: (*a*) run 7, compound **3**, $n = 1.1$; (*b*) run 5, compound **3**, $n = 4.9$; (*c*) run 11, compound **5**, $n = 1.9$.

with selectively protected primary hydroxy groups (see Scheme 2). To this end, per-6-*O*-[*tert*-butyl(dimethyl)silyl]-β-cyclodextrin (**6**)[34] was made to react with 7 equiv. of chloride **2** in the presence of 7.7 equiv. of Et_3N. The reaction was carried out under more drastic conditions (DMF, 60 °C, 8 h) than palmitoylation of unsubstituted CD **1** (for acylation of the secondary hydroxy groups in **6**, see Ref. 4). The isolation procedure was modified in such a way as to *rule out completely* the possible loss of CD hydrophobic derivatives soluble in organic solvents. Therefore, following the work-up, the reaction mixture mainly contained products formed from palmitoyl chloride (**2**), *i.e.*, palmitic acid and methyl palmitate. The ^1H NMR spectrum of this mixture provided little information, as it contained signals for the long aliphatic chain (δ_H 0.7—2.4) together with weak highly broadened signals at δ_H 4.0 and 4.7 corresponding apparently to cyclodextrin. The ^{13}C NMR spectrum contained no signals for the carbon atoms of the cyclodextrin cage (only a slight elevation of the base line in the region of δ_C 70—75 could be de-

tected), and the signal for the $\underline{C}Me_3$ carbon atom of the *tert*-butyldimethylsilyl substituent (δ_C ~18.5) could be distinguished with difficulty from the noise. The following signals were the most intense: a series of signals for the long aliphatic chain (δ_C 14.5—35.0), the signal for the MeO group of the methyl ester (δ_C 54.2), an unidentified signal (δ_C 101.4), and the signals for two carbonyl groups (δ_C 170.1 and 175.9) corresponding to the acid and the ester, respectively.

For the isolation the cyclodextrin derivatives whose molecules are much larger than the molecules of palmitic acid derivatives, we used gel chromatography (with BioBeads SX-3 as the stationary phase and toluene as the eluent). Even a single chromatographic separation gave a product whose ^{13}C NMR spectrum had sufficiently narrow signals (the ^1H NMR was still of little use because the signals were too broad). Apart from signals for the initial silyl ether **6**, the ^{13}C NMR spectrum exhibited a series of signals for the aliphatic chain (δ_C 14.5, 23.1, 25.8, 26.1, 26.9, 28.7, 29.8, 30.1, 31.2, 32.3, 33.5, and 35.0) with comparable intensities. Since the spectrum of the carbohydrate moiety remained unchanged, we assumed that these signals are not associated with the acylation of **6** but are due to the free (and/or incorporated in the CD cavity) palmitic acid impurity (but not its methyl ester because no signals for the MeO group were observed in the ^1H or ^{13}C NMR spectra), which could arise during isolation. Indeed, repeated gel chromatography resulted in a substantially low intensity of the signals for the aliphatic chain in the NMR spectra. However, the signals for this impurity did not disappear completely even after chromatography has been repeated several times. As a result, a product containing no more than two fatty acid residues per five CD molecules ($n \approx 0.4$, ^1H NMR data) was obtained in 36% yield. The positions of carbon signals of the glucose residue and the *tert*-butyldimethylsilyl fragment in the ^{13}C NMR spectrum of the purified product did not differ from those in the spectrum of the starting silyl ether **6**, which points to the absence of any chemical transformation of the starting compounds. These data also confirm indirectly the conclusion that only primary hydroxy groups of CD **1** were affected in the palmitoylation described above.

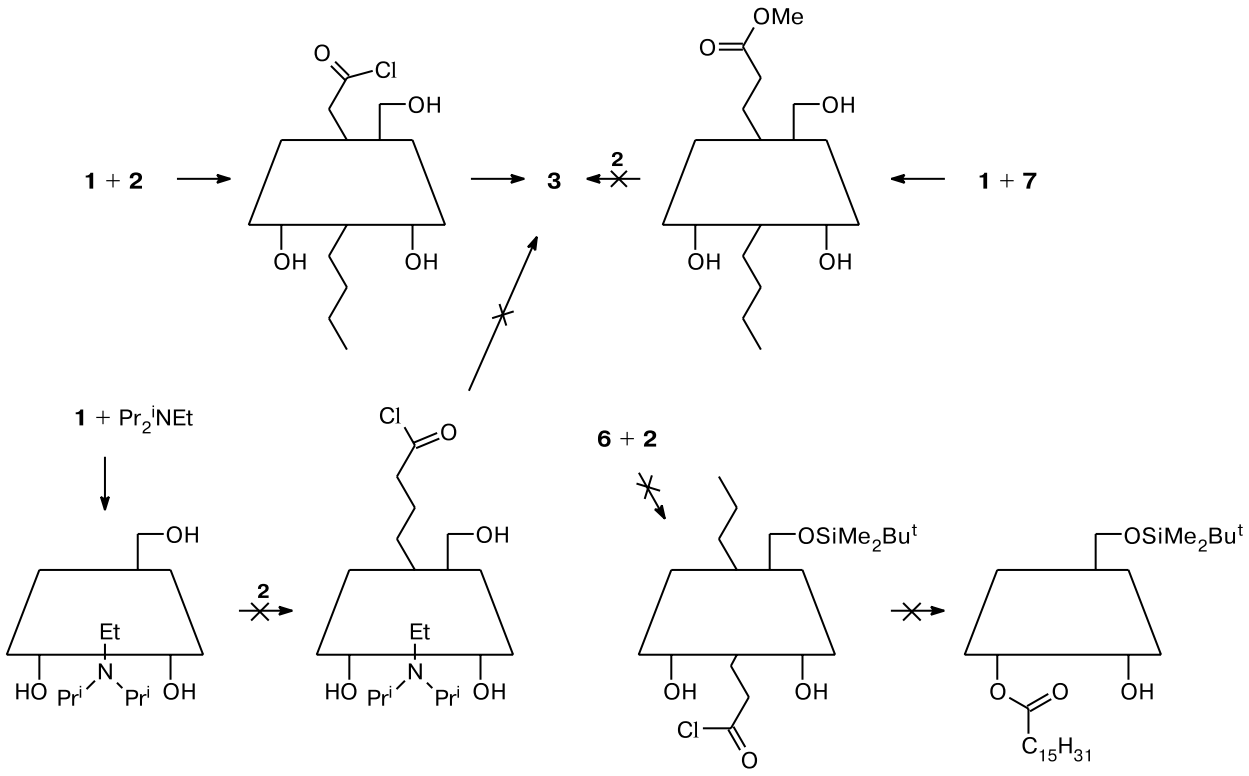
Study of the possibility of clathrate-induced regioselectivity of acylation

This high regioselectivity acylation of primary hydroxy groups in CD **1** by palmitoyl chloride *irrespective* of the presence or the nature of the base is unusual and has no analogy with the acetylation of **1**.[23,29] In principle, this could be attributed to the greater bulk of the long-chain carboxylic acid chlorides compared to the short-chain ones. However, to perform *selective* 6-*O*-acylation of β-CD (**1**) with really sterically crowded pivaloyl chloride or diphenylacetyl chloride in pyridine, the reaction has to be carried out at a reduced temperature,[25] which is entirely in line with the regularities of acylation of "common" sugars. Therefore, we attribute the obtained results to the influence of more complex supramolecular interactions between CD and the reagents. For example, derivatives of higher fatty acids are known[35] to form inclusion compounds with CDs. A similar incorporation of acylating agent **2** into the cavity of CD **1** could change the reactivity of acyl chloride **2** and influence the acylation selectivity. The selectivity of palmitoylation we observe suggests the formation of such a host—guest inclusion complex **2**⊂**1** where it is the primary hydroxy groups of CD that the carbonyl group of chloride **2** is closely adjacent to (for yet unknown reasons). In our opinion, this *spatial proximity* is responsible for the observed reaction pathway. Uncomplexed molecules **2** or inclusion complexes **2**⊂**1** with the opposite (with respect to the CD cavity) orientation of the palmitoyl chloride **2** molecules (if they are formed) appear to be much less reactive and unable to acylate CD under the reaction conditions (Scheme 3). This is quite natural because the reactivity of long-chain carboxylic acid chlorides is lower than that of the short-chain chlorides.

It is worthy of note that no reaction of **1** with **2** occurred in DMF in the presence of Pr^i_2NEt and unexpectedly, a 1 : 1 Pr^i_2NEt—**1** complex was isolated from the reaction mixture in 90% yield. The ^1H and ^{13}C NMR spectra of the carbohydrate part of this product were identical to those of the starting CD **1**. This finding is in sharp contrast with our previous results,[29] indicating that the use of Pr^i_2NEt to trap hydrogen chloride does not hamper the reaction between **1** and AcCl in DMF and, moreover, it ensures the most efficient variant of selective acetylation involving *exclusively* the primary hydroxy groups (see Table 2). Since the difference is only in the nature of the acyl chloride ($C_{15}H_{31}COCl$ or AcCl), any interpretation should necessarily take into account this fact. It is natural to suggest that some supramolecular complexes involving *all three components* of the reaction mixture are formed during the process (the participation of solvent cannot also be ruled out) because in the presence of other amines (or without amines), the reaction of **1** with **2** proceeds without complications. However, our hypothesis of selective formation of complexes **2**⊂**1** suggests a simpler interpretation, namely, the formation, in the presence of Pr^i_2NEt, of a more stable inclusion complex Pr^i_2NEt⊂**1**, which precludes the formation of the true acylating reagent (**2**⊂**1**), so that no palmitoylation takes place (see Scheme 3).

A strong argument supporting our hypothesis is the fact that treatment of a mixture of CD **1** with methyl palmitate (**7**, 17 equiv.) (which, apparently, should resemble acyl chloride **2** in its ability to form complexes with CD) with acyl chloride **2** (3 equiv.) in DMF in the

Scheme 3



presence of Et_3N (3.3 equiv.) resulted in a product containing no more than one fatty acid residue per three CD molecules ($n \approx 0.3$). The signals for the aliphatic chain could be detected only by ^1H NMR spectroscopy. Recall that the palmitoylation product formed in the absence of **7** contained a substantial number of fatty acid residues ($n \approx 1.6$; see Tables 1 and 2). These results can be easily explained within the framework of our hypothesis. Methyl ester **7** competes successfully with palmitoyl chloride (**2**) for the formation of the inclusion complex **2**⊂**1** (the true acylating reagent) and palmitoylation proceeds much less efficiently (see Scheme 3).

The hypothesis we propose provides a new insight into the fact that 6-*O*-silyl ether **6**, containing bulky *tert*-butyl groups, is barely palmitoylated. In our opinion, the reason is not the reduced reactivity of the secondary hydroxy groups in **6** but mainly the fact that the formation of the appropriate inclusion complex **2**⊂**6** (see Scheme 3), which could act as the acylating reagent, is either impossible or difficult (recall that according to our hypothesis, uncomplexed chloride **2** cannot react with CD **1**). A weighty reason supporting this interpretation of the experimental results is the fact that silyl ether **6** *does react* with sterically crowded pivaloyl chloride (5 equiv.) at ~20 °C in Py over a period of 24 h to give the mono-3-*O*-pivaloyl derivative in 36% yield,[28] which clearly points to a marked reactivity of the hydroxy groups in **6** toward usual acylating reagents.

A specific feature of palmitoylation of **1** is the fact that, irrespective of the nature of the amine used (or without any amine), acylation with palmitoyl chloride is inefficient (see Table 1). Using 3 equiv. of acyl chloride **2**, one can obtain only products **3** with a substitution degree of $n < 3$. This is not due to insufficient reaction time because it was shown in a special experiment (in which aliquot portions were taken from the reaction mixture at definite intervals) that acylation of β-CD (**1**) with palmitoyl chloride (**2**) in the presence of Et_3N proceeds rapidly and is virtually over 30 min after the end of reagent addition to the reaction mixture, while further reaction (up to 144 h) does not increase the degree of acylation of the product ($n = 1.65 \pm 0.05$), although only half of the introduced chloride **2** has reacted with CD **1**. This deviation of the course of acylation from the expected one is, apparently, due to the presence of complex competing supramolecular interactions between CD **1** and the reagents similar to those we described in a previous publication[36] devoted to transphosphorylation of CD derivatives.

To study the possibility of introducing a larger number of acyl groups in CD **1**, we carried out the synthesis with 7 equiv. of chloride **2**. The reaction was carried out in Py, because the use of this solvent markedly facilitates the product isolation. This gave product **3** with the degree of substitution $n \approx 4.9$.

An unusual result was obtained in the reaction of CD with 9 equiv. of chloride **2**. Once isolated in the solid state, the product became insoluble in any of the available solvents including those from which it had been just isolated by solvent evaporation followed by drying *in vacuo* at 20 °C. This may imply that starting from a particular number of palmitoyl substituents in the CD, the molecules stick together due to numerous supramolecular interactions between neighboring molecules caused by inclusion of the long aliphatic groups into the CD cavity (*cf.* Ref. 35), and this structure is so stable that it cannot be solvated and, hence, it is insoluble.

When evaluating in general the efficiency of using various amines (see Table 2) as activators of nucleophilic substitution and hydrogen chloride scavengers, one should note that the highest degree of palmitoylation in DMF was attained with $PhNMe_2$, whereas in the case of CD acetylation with acetyl chloride,[29] $PhNMe_2$ was found to be least effective (at room temperature, acetylation did not occur at all).

Valerylation. Correction of the hypothesis

Since the selectivity and the mere possibility of acylation of β-CD (**1**) with acetyl (C_2)[29] and palmitoyl (C_{16}) chlorides (see above and Table 2) differ so appreciably (and unpredictably), we decided to try acylation with an acid chloride having an intermediate chain length and chose valeryl chloride for this purpose (**4**, C_5). The acylation products were isolated and characterized similarly to the palmitoylation products. In all cases (see Table 1 and 2), the reactions gave the products of acylation only at the primary hydroxy groups (**5**). As in the case of palmitoylation, the reaction in Py was the most efficient; the maximum degree of substitution (n) reached in this case was 4.5. The acylation of CD **1** with chloride **4** in DMF *in the absence of amines* proceeded with moderate efficiency: about half of the primary hydroxy groups in CD were replaced ($n \approx 3.2$). It is of interest that all experiments on the acylation of CD **1** with chloride **4** in DMF *in the presence of amines* gave products with a moderate degree of substitution ($n \approx 1.3$—2.3), which depended only slightly on the amount of chloride used in the reaction (see Table 2). It is important that the presence of Pr^i_2NEt did not prevent the acylation of CD **1** with chloride **4**; the degree of substitution was relatively low ($n \approx 1.9$) but comparable with this value ($n \approx 2.2$)[29] for the product of regioselective (at O(6)) acetylation of **1** in the presence of Pr^i_2NEt (see Table 2).

This result as well as the possibility of regioselective acetylation of **1** in the presence of Pr^i_2NEt (unlike palmitoylation, which does not occur at all under these conditions) requires some correction of our hypothesis concerning the necessity of formation of a reactive inclusion complex between acyl chloride and CD (**2**⊂**1** in the case of palmitoylation). Apparently, Pr^i_2NEt forms such an inclusion complex (Pr^i_2NEt⊂**1**) in which the approach to the lower rim of the cyclodextrin bearing the secondary hydroxy groups is blocked (recall that the Pr^i_2NEt—**1** complex (1 : 1) was isolated from the **1** + **2** + Pr^i_2NEt reaction mixture). As a result, acyl chloride can form a complex with CD only by approaching it from the opposite side (*i.e.*, from the side of primary hydroxy groups). We believe that selective acylation of primary hydroxy groups of CD with aliphatic carboxylic acid chlorides occurs in those cases where acyl chloride penetrates rather deeply the CD cavity from the side of primary hydroxy groups and forms the reactive acyl chloride—**1** complex in which the hydroxy groups of CD and the carbonyl group of acyl chloride *are proximate in space*. This is possible for acyl chlorides in which the alkyl chain is not too long (AcCl or BuCOCl). If the alkyl chain is long (in our case, this is $C_{15}H_{31}COCl$), the carbonyl group becomes too remote from the primary hydroxy groups and the reaction does not take place (see Scheme 3). It should be emphasized that our hypothesis also provides an interpretation for the unusually high regioselectivity of *acetylation* of CD (**1**) in the presence of Pr^i_2NEt, which proceeds[29] exclusively at primary hydroxy groups at *room* temperature (rather than at a reduced temperature, as it is typical of monosaccharides, for example, at −78 °C).[20] The hypothesis we propose allows one to explain many separate facts from a unified standpoint; however, the data of Table 2 show that the situation is more complicated, and additional experiments are required for elucidating the true reasons for the unusually high regioselectivity of acylation of CD **1** with aliphatic carboxylic acid chlorides.

* * *

Thus, in this work we have proposed procedures for the synthesis of regioselectively (at O(6)) acylated amphiphilic derivatives of β-cyclodextrin (**1**) and studied characteristic features of acylation with palmitoyl (**2**) and valeryl (**4**) chlorides depending on the reaction conditions and the nature of the amines used. ^{13}C NMR spectroscopy is suitable for determining the positions and the number of fatty acid residues introduced in CD **1**. The hypothesis we proposed stating that successful regioselective acylation requires the formation of a reactive host—guest inclusion complex of acyl chloride with CD (acyl chloride⊂**1**) provides a uniform explanation for most of unusual facts observed during acylation of β-cyclodextrin.

Experimental

All experiments with carboxylic acid chlorides were carried out in anhydrous solvents purified by standard procedures and in

an inert atmosphere. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 instrument (200.13 and 50.32 MHz, respectively) in pyridine-d_5. The ^{1}H NMR chemical shifts are referred to the residual signal of Py (δ 8.57 for the low-field signal (s)) and the ^{13}C NMR chemical shifts are referred to the C_5D_5N signal (δ 149.8 for the low-field signal (t)). The degrees of substitution for acylated derivatives **3** and **5** were determined by signal integration in the NMR spectra, as described in the Results and Discussion section*. Thin layer chromatography was carried out on Silufol UV–254 plates using 1 : 2 $CHCl_3$—EtOH (A) and 1 : 1 $CHCl_3$—MeOH (B) systems as eluents.

Dehydration of β-cyclodextrin (1). Toluene (30 mL) was added to a solution of β-cyclodextrin (Novodex, Ufa, Russia) (10 g) in 120 mL of DMF and the mixture was refluxed with a Dean—Stark trap for 4 h. The solution was concentrated *in vacuo* to 30 mL, and acetone (100 mL) was added. The precipitate was filtered off and thoroughly triturated under acetone and the mixture was filtered. The solid was dried in a vacuum desiccator over P_2O_5.

Acylation of β-cyclodextrin (1). ***A.*** *In DMF without hydrogen chloride scavenger.* A solution of the corresponding acid chloride (**2** or **4**, the amount is given in Table 1) in 10 mL of benzene was added dropwise with stirring to a solution of β-cyclodextrin (**1**) (1.5 g, 1.32 mmol) in 50 mL of DMF. Dry nitrogen was bubbled through the solution for 2 h. Methanol (0.5 mL) was added and the mixture was stirred for 2 h and concentrated *in vacuo*. Pentane (50 mL) was added, the precipitate was filtered off, washed with 20 mL of pentane, and triturated with 25 mL of water. The precipitate was filtered off, washed with 10 mL of water and 30 mL of acetone, and dried *in vacuo*. The yields of the products formed as white or yellowish powders (**3** or **5**), the corresponding R_f values, and the ^{1}H and ^{13}C NMR data are presented in Table 1.

B. *In DMF in the presence of bases.* A solution of the corresponding acid chloride (**2** or **4**, the amount is given in Table 1) in 10 mL of benzene was added with stirring at 0 °C over a period of 1 h to a solution of β-cyclodextrin (**1**) (1.5 g, 1.32 mmol) in 50 mL of DMF containing a tertiary amine (1.1 equiv. relative to acyl chloride). The mixture was allowed to stand for 16 h at 20 °C. Methanol (0.5 mL) was added and the mixture was stirred for 2 h and concentrated *in vacuo*. The further isolation was as described in procedure ***A***.

C. *In pyridine.* The reaction was carried out as in procedure ***B*** but Py was used as the solvent instead of DMF (tertiary amines were not added to the reaction mixture).

D. A solution (10.90 g, 39.6 mmol) of chloride **2** in 10 mL of benzene was added dropwise with stirring at 0 °C over a period of 15 min to a solution of β-cyclodextrin (**1**) (15 g, 13.2 mmol) in 500 mL of Py. At regular intervals, 35-mL samples were taken from the reaction mixture. To each sample, MeOH (0.5 mL) was added and the mixture was stirred for 2 h and concentrated *in vacuo*. Pentane (50 mL) was added, the precipitate was filtered off, washed with 20 mL of pentane, and triturated with 25 mL of water. The precipitate was filtered off, washed with 10 mL of water and 30 mL of acetone, and dried *in vacuo*. The degree of substitution (n) in the products was determined by ^{13}C NMR.

Palmitoylation of heptakis[6-*O*-*tert*-butyl(dimethyl)silyl]-β-cyclodextrin (6) in pyridine. A solution of chloride **2** (2.54 g, 9.25 mmol) in 10 mL of benzene was added dropwise with stirring at 20 °C for 1 h to a solution of heptakis[6-*O*-*tert*-butyl(dimethyl)silyl]-β-cyclodextrin (**6**) (2.56 g, 1.034 mmol)[34] in 80 mL of Py. The mixture was kept for 8 h at 80 °C and allowed to stand for 16 h at 20 °C. Methanol (2.0 mL) was added, and the mixture was stirred for 2 h and concentrated *in vacuo*. Water (50 mL) was added, the precipitate was filtered off, dissolved in 40 mL of Et_2O, and washed with water (3×15 mL), and the solvent was evaporated. The product was purified by repeated (5 times) gel chromatography (BioBeads SX-3 as the stationary phase, toluene as the eluent) to give 0.93 g (36%) of a white powder R_f 0.84 (B). ^{1}H NMR (pyridine-d_5), δ: –0.08—0.21 (m, 40.2 H, $Si(C\underline{H}_3)_2$); 0.67—0.99 (m, 66.5 H, $(CH_2)_{12}C\underline{H}_3$), $SiC(C\underline{H}_3)_3$); 1.04—1.17 (m, 9.2 H, $(C\underline{H}_2)_{12}Me$); 1.51—1.66 (m, 0.5 H, $COCH_2C\underline{H}_2(CH_2)_{12}$); 3.93—4.65 (m, 42.0 H, C(2)H—C(5)H, $C(6)H_2$); 5.35—5.57 (m, 6.8 H, C(1)H). ^{13}C NMR (pyridine-d_5), δ: –4.9 (SiMe); 18.6 ($Si\underline{C}Me_3$); 26.2 ($SiC(\underline{C}H_3)_3$); 23.0, 29.9 ($(\underline{C}H_2)_{14}Me$); 62.7 (C(6)), 73.3, 74.2, 74.5 (C(2), C(3), C(5)), 82.3 (C(4)), 103.6 (C(1)). The ^{13}C NMR spectrum coincides with the spectrum of the starting **6** but contains additional low-intensity signals for the carbon atoms of the methylene units of palmitic acid.

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References

1. J.-M. Lehn, *Supramolecular Chemistry. Concepts and Perspectives*, VCH Verlagsgesellschaft mbH, Weinheim, 1995; (b) *Chem. Rev. (special issue)*, 1998, **98**, No. 5.
2. A. R. Khan, P. Forgo, K. J. Stine, and V. T. D´Souza, *Chem. Rev.*, 1998, **98**, 1977 (and references cited therein).
3. H. Schlenk, J. L. Gellerman, and D. M. Sand, *Anal. Chem.*, 1962, **84**, 1529.
4. P. Zhang, C.-C. Ling, A. W. Coleman, H. Parrot-Lopez, and H. Gallons, *Tetrahedron Lett.*, 1991, **32**, 2769.
5. B. Angelova and H.-P. Schmauder, *J. Biotechnol.*, 1999, **67**, 13.
6. T. Lacroix, H. Bricout, S. Tilloy, and E. Monflier, *Eur. J. Org. Chem.*, 1999, 3127.
7. R. Widehem, T. Lacroix, H. Bricout, and E. Monflier, *Synlett*, 2000, 722.
8. H. Bricout, L. Caron, D. Bormann, and E. Monflier, *Catal. Today*, 2001, **50**, 1.
9. K. Uekama, F. Hirayama, and T. Irie, *Chem. Rev.*, 1998, **98**, 2045.

* The ^{13}C relaxation times were not specially determined; however, recording the ^{13}C NMR spectra of the reaction products with different relaxation delays (RD = $1 \cdot 10^{-6}$, 1, and 10 s) showed that the integral intensities of the signals of the C(1)—C(6) atoms of the sugar *do not depend* on the RD value, whereas the integral intensities of the signals of the alkyl residues in the aliphatic carboxylic acid substantially increase with an increase in RD.

10. Y. Kawabata, M. Matsumoto, M. Tanaka, H. Takahashi, Y. Irinatsu, S. Tamura, W. Tagaki, H. Nakahara, and K. Fukuda, *Chem. Lett.*, 1986, 1933.
11. B. J. Ravoo and R. Darcy, *Angew. Chem.*, *Int. Ed. Engl.*, 2000, **39**, 4324.
12. A. Janshoff, C. Steinem, A. Michalke, C. Henke, and H. J. Galla, *Sens. Actuators*, *B*, 2000, **70**, 243.
13. J. Szejtli, *Chem. Rev.*, 1998, **98**, 1743.
14. W. Saenger, J. Jacob, K. Gessler, T. Steiner, D. Hoffmann, H. Sanbe, K. Kozumi, S. M. Smith, and T. Tanaka, *Chem. Rev.*, 1998, **98**, 1787.
15. G. Wenz, *Angew. Chem.*, *Int. Ed. Engl.*, 1994, **33**, 803.
16. A. H. Haines, *Adv. Carbohydr. Chem. Biochem.*, 1976, **33**, 11.
17. Y. Tsuda, M. E. Haque, and K. Yoshimoto, *Chem. Pharm. Bull.*, 1983, **31**, 1612.
18. M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, 1987, **109**, 3977.
19. I. F. Pelyvás, T. K. Lindhorst, H. Streicher, and J. Thiem, *Synthesis*, 1991, 1015.
20. R. Ishihara, H. Kurihara, and H. Yamamoto, *J. Org. Chem.*, 1993, **58**, 3791.
21. T. Ziegler, in *Carbohydrate Chemistry*, Ed. G.-J. Boons, 1998, Blackie Academic and Professional, London, p. 21.
22. J. Boger, R. J. Corcoran, and J.-M. Lehn, *Helv. Chim. Acta*, 1978, **61**, 2190.
23. A. P. Croft and R. A. Bartsch, *Tetrahedron*, 1983, **39**, 1417 (and references cited therein).
24. N. Sakairi and H. Kuzuhara, *Chem. Lett.*, 1993, 2077.
25. F. Santoyo-Gonzalez, J. Isac-Garcia, A. Vargas-Berenguel, R. Robles-Diaz, and F. G. Calvo-Flores, *Carbohydr. Res.*, 1994, **262**, 271.
26. A. Y. Hao, L. H. Tong, F. S. Zhang, and X. M. Gao, *Carbohydr. Res.*, 1995, **277**, 333.
27. X. M. Gao, L. H. Tong, Y. Inoue, and A. Tai, *Synth. Commun.*, 1995, **25**, 703.
28. S.-H. Chiu and D. C. Myles, *J. Org. Chem.*, 1999, **64**, 332.
29. A. A. Sutyagin, A. E. Glazyrin, G. I. Kurochkina, M. K. Grachev, and E. E. Nifant'ev, *Zhurn. Obshch. Khim.*, 2002, **72**, 156 [*Russ. J. Gen. Chem.*, 2002, **72** (Engl. Transl.)].
30. K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 1983, **41**, 27.
31. A. Ueno and R. Breslow, *Tetrahedron Lett.*, 1982, **23**, 3451.
32. K. Fujita, T. Takahara, T. Imoto, and T. Koga, *J. Am. Chem. Soc.*, 1986, **108**, 2030.
33. T. Tahara, K. Fujita, and T. Koga, *Bull. Chem. Soc. Jpn.*, 1990, **63**, 1409.
34. P. Fügedi, *Carbohydr. Res.*, 1989, **192**, 366.
35. S. G. Skoulika, C. A. Georgiou, and M. G. Polissiou, *J. Incl. Phenom. Mol. Recognit. Chem.*, 1999, **34**, 85.
36. E. E. Nifant´ev, A. E. Glazyrin, G. I. Kurochkina, and M. K. Grachev, *Zhurn. Obshch. Khim.*, 2000, **70**, 1752 [*Russ. J. Gen. Chem.*, 2000, **70** (Engl. Transl.)].

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