N-Cholyl Amino Acid Alkyl Esters − A Novel Class of Organogelators

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Several *N*-cholyl amino acid alkyl esters were found to act as novel, potent organogelators for aromatic solvents and cyclohexene. These novel organogelators afford stable, transparent, and thermoreversible gels. Modification of the molecular structure and IR measurements show that gelation takes place by means of a hydrogen-bonded network and involves at least the amide bond and several hydroxy groups of the cholic acid component. The chiral center of the amino acid component seems to play an important role in gelation. Although a wide variety of derivatives display gelation behavior, a small change in molecular structure can have a dramatic effect on the gelling capability. Electron microscopy reveals a fibrous structure in the gels. Differential scanning calorimetry shows several transitions in the melting behavior of the gels.

Introduction

Research in the field of organogels has received more and more attention over the last several years, and the number of low molecular mass organogelators is rapidly growing.[1−8] The gelling capability of these compounds is not yet understood in detail, but one important requirement is the ability of the gelator molecules to form spontaneous aggregates, which encapsulate the solvent in a three-dimensional network. For many organogelators, the gel network consists of fibers, which in turn assemble into larger aggregates.[9] Aggregation of low molecular mass organogelators is usually driven by specific noncovalent intermolecular forces, such as hydrogen bond formation, metal coordination bond formation, hydrophobic interactions, dipole−dipole interactions, or van der Waals interactions. Cholesterol-based organogelators usually aggregate by means of hydrophobic or van der Waals interactions.[5,10] Many other low molecular mass organogelators contain strongly hydrogen-bonding groups. The literature reports several organogelators containing amide and urea groups[11−14] and gelators based on amino acids.[15−19]

Generally, the gelled state can be seen as a metastable state between solution and crystallization. If a compound is moderately soluble, it will have the tendency to crystallize or precipitate upon cooling. However, if crystal packing is hampered, the occurrence of a metastable state, like the gelled state, will be more likely. Cholic acid, one of the main bile acids, has frequently been used in crystal inclusion chemistry. With cholic acid or its derivatives as a host, inclusion crystals have been formed with small ketones, alcohols, and esters. The cholic acid molecules are arranged in stacked bilayers, providing hydrophobic, channel-like voids in which the guest molecules can be accommodated.[20,21] It was found by chance that one of these compounds, *N*-isopropylcholamide, produced not only inclusion crystals and guest-free crystals but also organogels, depending on the mixture of solvents used.[22] This example illustrates the influence of the solvent on the type of aggregation that is observed. The literature reports one other example of bile acid derived organogelators: a two-component gel system mediated by electron donor−acceptor interaction and based on the bile acid backbone, with two derivatized hydroxy groups.[23]

We found that several compounds in which cholic acid is coupled to an amino acid ester act as gelators for aromatic solvents, producing transparent and stable gels. Thus, a novel class of organogelators, composed of two natural building blocks, has been found. Structural modifications were performed in order to assess the structural elements necessary for gel formation, and infrared spectroscopy was used to study the hydrogen-bonding interactions in the gel. Scanning electron microscopy gave information on the fibrous structure, while the melting points and thermal behavior of all gels were measured using differential scanning calorimetry (DSC). As occasionally reported in the literature,[24−26] DSC is an easy and precise method for investigation of the thermal behavior of organogels.

Results and Discussion

A series of novel compounds was prepared by coupling cholic acid to amino acid esters, with diethylphosphoryl cyanide (DEPC) as a coupling reagent, with formation of an amide bond.[27] The compounds synthesized are shown in Scheme 1. Thin layer chromatography, ^{1}H NMR, and elemental analyses confirmed the structures and purities of the compounds. Most compounds were obtained as glasses ($T_g \approx 100$ °C). Upon prolonged heating or solvation, some of the compounds slowly crystallized.

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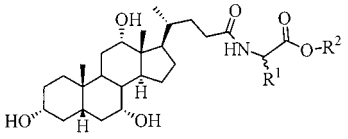


comp	R^1	R^2
1	H	Me
2a	$-CH_3$	Me
2b	$-CH_3$	Me
3	$-CH(CH_3)_2$	Me
4a	$-CH_2-CH(CH_3)_2$	Me
4b	$-CH_2-CH(CH_3)_2$	Me
4c	$-CH_2-CH(CH_3)_2$	n-Bu
5a	$-CH_2-C_6H_5$	Me
5b	$-CH_2-C_6H_5$	Et
5c	$-CH_2-C_6H_5$	n-Bu
6a	$-CH_2-C_6H_4-OH$	Me
6b	$-CH_2-C_6H_4-OMe$	Me
7a	$-C_6H_5$	Me
7b	$-C_6H_5$	Me

Scheme 1

All compounds were tested for their gelling abilities in several solvents. In a typical gelation experiment, a capped test tube was filled with solvent and about 1.5 wt-% of compound, and was then heated until the solid had dissolved. After cooling, gelation was checked by monitoring the disappearance of flow. In several cases, lower concentrations of gelator were sufficient. It was found that the absence of water is crucial for gelation. Addition of a small drop of water or methanol to a gel caused immediate restoration of the solvent's flow and sometimes precipitation of the gelator. To obtain reproducible and stable gels, a small quantity of 4-Å molecular sieves was added to the gelation mixture. This observation indicates that hydrogen bonds play an important role in assembling the gel network. Given the structure of the gelators, which possess an amide bond, an ester bond, and three hydroxy groups, this seems a reasonable assumption.

In benzene and toluene, a very clear, transparent, and thermoreversible gel was found for most compounds. Some compounds provide transparent gels at concentrations as high as 20 wt-%. The organogels have a remarkably high degree of stability towards mechanical agitation. Furthermore, they can be stored for several months at room temperature without disruption or precipitation. Several other aromatic solvents were found to be less suitable for gelation. Butylbenzene, chlorobenzene, and anisole gave isotropic solutions for most compounds; only a few compounds were able to gel these solvents. The only nonaromatic solvents that were found to be capable of gel formation with these compounds were cyclohexene and cyclooctene; these gave similar results and so only those for cyclohexene are reported here. Results of the gelation experiments are summarized in Table 1. The melting behavior of 25 mM gels (about 1.5 wt-%) in benzene, as determined using DSC, is given in Table 2. Higher concentrations of gelator resulted in higher melting points, which is analogous to increasing solubility of a compound at higher temperatures.

Compound **1** showed no gelling behavior, but was moderately soluble in benzene. This result is intriguing, because compounds **2a**, **3** and **4a**, with small, apolar tails in the amino acid, gave stable gels in benzene, with similar melting points. Comparison of compound **1** with compound **2a** shows that the former lacks only a methyl group. One explanation for this phenomenon is that the presence of an alkyl group at this position results in lower solubility and therefore gelation. Another explanation could be that an asymmetric group next to the amide bond is essential for gelation of these types of compounds.

To study the influence of the chiral center at the amino acid α-carbon atom, compounds **2b** and **4b** (D isomers) were synthesized and their gelling ability compared to that of compounds **2a** and **4a** (L isomers). It was found that compound **2b** was insoluble in benzene and in the other solvents and gave no gelation. This is probably connected with the fact that this compound was directly obtained in a crystalline state, whereas other compounds were obtained as glasses. Compound **4b** was soluble in warm benzene and gave gelation upon cooling. Interestingly, the melting point of the gel with compound **4b** was 13 °C higher than that with compound **4a**. However, when heated in benzene for a longer period, precipitation of compound **4b** in the crystalline state occurred, after which it was insoluble. Furthermore, compound **4b** produced a gel in anisole, whereas compound **4a** did not. These findings indicate a crucial role for the chiral center at the amino acid α-carbon atom. Similar results were recently reported for another class of chiral organogelators.[28]

Introduction of a phenyl group into the amino acid component of the molecule seems to have little effect on the gelling ability. Compound **5a** in benzene afforded a stable, transparent gel similar to those produced by compounds **2a**, **3**, and **4**. The tyrosine derivative, compound **6a**, showed no gelling behavior: this compound was insoluble in benzene. However, compound **6b**, which was prepared from compound **6a** by methylation of the hydroxy group, proved to be a potent gelator for benzene. Apparently, compound **6a** is too polar to dissolve in benzene, even at high temperature. Compounds **7a** and **7b** were not soluble in the tested solvents and did not form any gels. This lack of gelation is comparable to the case of compound **2b**, since compound **7a** was also obtained in a crystalline state and compound **7b** easily crystallized in solution.

For the behavior of compounds **4c**, **5b**, and **5c**, with alkyl ester groups longer than methyl moieties, no clear trend was observed. The ethyl ester derivative, compound **5b,** was a poor gelator for many solvents. In most cases a gel was formed initially, but was not stable. Within 1 d the gelator had precipitated, indicating that for this compound the energetically more favorable crystalline state is easily reached. On the other hand, the butyl ester derivatives **4c** and **5c**

Table 1. Gelation results for several solvents with different *N*-cholyl amino acid alkyl esters (the observation was made immediately after cooling; changes after 24 h are indicated in parentheses; g_t = transparent gel; g_c = cloudy gel; s = soluble; n = not soluble; p = precipitation)

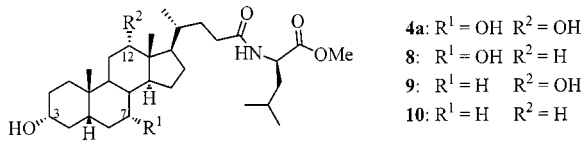
Compound	Benzene	Toluene	Butylbenzene	Chlorobenzene	Anisole	Cyclohexene
1	s	n	n	p	s (p)	n
2a	g_t	g_t	p	s	s	p
2b	n	n	n	n	n	n
3	g_t	g_t	g_c (p)	s (p)	s	g_c (p)
4a	g_t	g_t	g_t	g_t	s	g_t
4b	g_t	g_t	g_t	g_t	g_t	g_t
4c	g_t	g_t	g_t	s	s	g_t
5a	g_t	g_t	g_t	s	s	g_t (g_c)
5b	g_t (g_c)	g_t (g_c)	g_t (g_c)	s (p)	s	g_t (g_c)
5c	g_t	g_t	g_t	s	s	g_t
6a	n	n	n	n	n	n
6b	g_t	g_t	g_c (p)	s	s	p
7a	n	n	n	n	n	n
7b	n	n	n	n	n	n
8	s	s	s	s	s	p
9	s (p)	s (p)	s (p)	s	s	p
10	p	s (p)	n	s	s	n

Table 2. Melting behavior of 25 mM gels in benzene, determined using DSC (heating rate 0.5 °C/min; b = broad peak, bs = broad shoulder)

Compound	Pre-melting transitions [°C]	M.p. [°C]
2a	49 (b)	53
3	40 (b)	42
4a	40 (b)	43
4b	54 (b)	56
4c	32 (b)	33
5a	30 (b)	42 (b)	46
5b	26 (b)	27
5c	25 (bs)	32 (b)	33
6b	35 (b)	43 (b)	48

gave stable gels in the same solvents as had the corresponding methyl ester derivatives, although the melting points of gels of the butyl esters were about 10 °C lower than those of the methyl esters.

In view of the supposed importance of hydrogen bond formation in production of the gel network, the question arises of whether all the hydroxy groups of the cholic acid component are required for gelation to take place. In order to investigate this, compounds **8**, **9**, and **10** were prepared (see Scheme 2). These compounds are also based on natural bile acids: chenodeoxycholic acid, deoxycholic acid, and lithocholic acid, respectively. All of them lack one or two hydroxy groups at the 7 and 12 positions, compared to cholic acid. Compounds **8, 9**, and **10** did not form gels in any of the tested solvents; these results are also summarized in Table 1. Apparently, the hydroxy groups at the 7 and 12 positions are both needed to build the hydrogen-bonded gel network. This is an important difference from cholesterol-based organogelators, since the cholesterol skeleton lacks both these hydroxy groups.



4a: R^1 = OH R^2 = OH
8: R^1 = OH R^2 = H
9: R^1 = H R^2 = OH
10: R^1 = H R^2 = H

Scheme 2

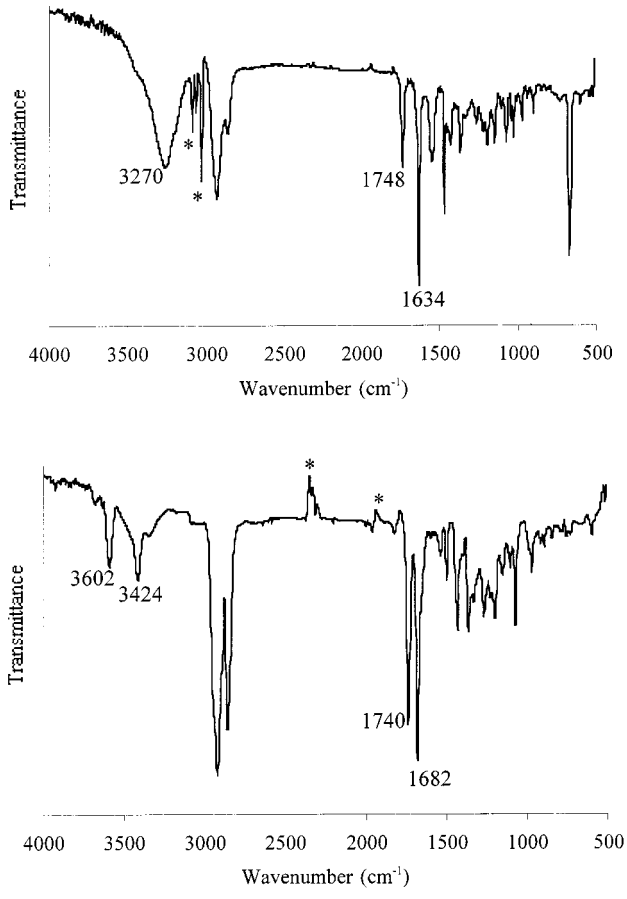


Figure 1. FT-IR spectra of compounds **4b** (top) and **8** (bottom) in benzene (* = residual solvent peak)

Table 3. FT-IR data for several gelating and nongelating compounds in benzene (b = broad peak, s = sharp peak)

Compound	State	ν(OH/NH) [cm^{-1}]	ν(C=O) ester [cm^{-1}]	ν(C=O) amide [cm^{-1}]
3	gel	3265 (b)	1746	1636
4b	gel	3270 (b)	1748	1634
6b	gel	3270 (b)	1746	1636
1	solution	3580 (s)/3400 (s)	1750	1686
8	solution	3602 (s)/3424 (s)	1740	1682

The presence of hydrogen bonds in the gels was further investigated with FT-IR measurements. IR spectra of gels in benzene were recorded for compounds **3**, **4b**, and **6b**. Compounds **1** and **8** were measured as solutions in benzene. Typical spectra are shown in Figure 1 and the relevant data are listed in Table 3.

All compounds showed two strong and sharp carbonyl signals. The signal from the ester carbonyl group was found at about 1745 cm^{-1} in all cases. The signal from the amide carbonyl group gave different values: about 1634 cm^{-1} for gelating compounds and about 1682 cm^{-1} for nongelating compounds. These values agree with literature values for amides in the solid and dissolved states, respectively. Intermolecular hydrogen bonding of amides causes a marked decrease in the amide carbonyl stretching frequency.[29] Another difference between the spectra of the gelating and nongelating compounds was seen in the O−H and N−H stretch signals. For the gels, one broad signal around 3270 cm^{-1} was found, which points to hydrogen-bonded N−H. This peak had a shoulder between 3450 and 3500 cm^{-1}, which is in the range for hydrogen-bonded hydroxy groups. The nongelating compounds gave two sharp peaks at about 3602 and 3424 cm^{-1}, indicative of dissolved O−H and N−H groups, not involved in hydrogen bonding.

Scanning electron microscopy (SEM) of a 25 mM gel of compound **4b** in benzene showed fibers with a diameter of about 50 nm and a length of several μm, as can be seen in Figure 2. No helical shape is visible within the fibers.

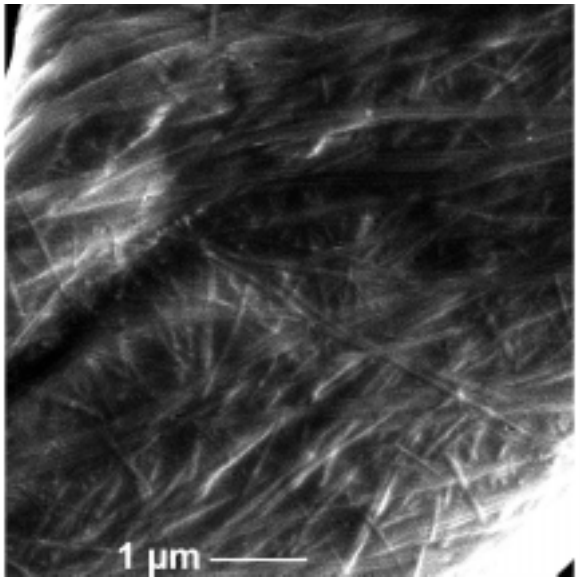


Figure 2. SEM image of a 25 mM gel of compound **4b** in benzene

Occasionally, several single fibers seem to aggregate in a bundle, with a parallel arrangement.

DSC measurements on the thermoreversible benzene gels showed several transitions in the heating curves. For gels produced with compounds **2a, 3,** and **4**, two endothermic transition peaks were found: a broad one at lower temperature and a sharp one at higher temperature. For gels with the aromatic amino acid compounds **5a, 5c**, and **6b**, three endothermic transition peaks were found: a second broad peak appeared at low temperature. Typical thermograms of both kinds of compounds are shown in Figure 3. The melting points of the gels (defined as the temperature above which macroscopic flow appears) were also determined by visually monitoring the flow of the gels in a thermally controlled bath. If these values are compared to the DSC thermograms, it can be concluded that the sharp transition peak in the thermogram corresponds with the actual melting of the gel. At the temperatures of the broad transitions, there was no visual change in the gels at macroscopic level. An explanation for the appearance of these pre-melting transitions is partial melting of the gelator network. It can be imagined that an assembly of fibers is broken down upon heating, prior to complete disintegration of the single fibers.

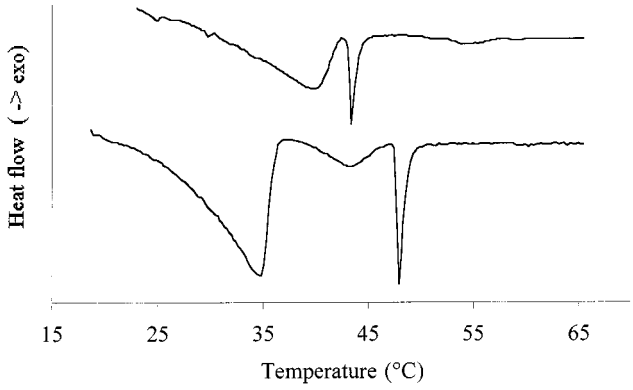


Figure 3. Heating curves of 25 mM gels of compounds **4a** (top) and **6b** (bottom) in benzene (heating rate 0.5 °C/min)

Conclusion

A variety of *N*-cholyl amino acid alkyl esters were found to behave as novel organogelators, forming stable, transparent, and thermoreversible gels in aromatic solvents. Gelation was only observed for compounds that did not crystallize easily and takes place through a network of fibers of about 50 nm thickness. Molecular requirements for gelation

are the amide bond and several hydroxy groups of the cholic acid component, since these groups are responsible for the hydrogen-bonded aggregation within the fibers. There are indications that chirality also plays a role in the aggregation of these novel organogelators. It was found that a small difference in structure can have a large influence on the gelling capability; it is assumed that in these cases a change in structure leads to a change in packing of the molecules. The gels go through one or two pre-melting transitions before actual melting takes place, indicating a partial melting of the network upon heating.

Experimental Section

General Remarks: ^{1}H NMR spectra (200 MHz) were recorded with a Bruker AC200 spectrometer at ambient temperature. − Melting points were measured using an Olympus BH-2 microscope with a Mettler FP82HT hot stage and a FP80HT temperature controller. − Elemental analysis was performed with an Elemental Analyzer EMASyst1106. − FT-IR spectra were recorded with a BIO-RAD FTS-7 spectrophotometer with a resolution of 4 cm^{-1}. Compounds were measured as solids in KBr or as solutions or gels in benzene. − For SEM, drops of gel were air-dried on carbon adhesive taps (Electron Microscopy Sciences, Washington), that were mounted on specimen stubs. Specimens were placed in a dedicated preparation chamber (CT 1500 HF, Oxford Instruments), sputtercoated with Pt, 10 nm. Samples were analyzed on the SEM (JSM 6300 F, Jeol Japan) at ambient temperature and images were digitally recorded. − DSC thermograms were recorded with a Setaram micro-DSC III. Approximately 500 mg of a 25 mM solution of gelator in benzene above the gel melting temperature was introduced into a metal, screw-capped cup and was allowed to cool to room temperature, thus forming a gel. A reference cup contained the same amount of benzene. Two heating and cooling cycles were recorded, with rates of 1 °C/min and 0.5 °C/min, respectively, and the second heating curves were compared. This procedure ensured that gels were formed under reproducible conditions. − The different bile acids were purchased, as were glycine methyl ester hydrochloride, L-leucine methyl ester hydrochloride, L-phenylalanine ethyl ester hydrochloride, L-tyrosine methyl ester hydrochloride, and diethylphosphoryl cyanide (DEPC). Solvents used were of p.a. quality. − L-Valine methyl ester hydrochloride, D-leucine methyl ester hydrochloride, and L-phenylalanine methyl ester hydrochloride were prepared by adding the amino acids to a cold solution of thionyl chloride in methanol.[30] Acid-catalyzed esterification of L-leucine to 1-butanol has been described previously.[31] The purity of the products was verified with NMR, TLC, and elemental analysis.

General Procedure for the Synthesis of *N*-Cholyl Amino Acid Alkyl Esters: Cholic acid (2.05 g, 5.0 mmol) and the appropriate amino acid alkyl ester (5.0 mmol) were dissolved in dry *N*,*N*-dimethylformamide (35 mL). After cooling to 0 °C, DEPC (0.825 mL, 5.5 mmol) was added. An excess of triethylamine (3.5 mL) was gradually added over a period of 10 min. The mixture was stirred at 0 °C for 45 min and subsequently at room temperature for 24 h. Afterwards the triethylammonium salt was filtered off and DMF and triethylamine were evaporated under vacuum. The residue was purified by flash column chromatography on silica gel, using dichloromethane/methanol (95:5) as eluent. Concentration of the product-containing fractions under vacuum gave a white solid, generally in 70% yield.

***N*-Cholyl Glycine Methyl Ester (1):** ^{1}H NMR ($CDCl_3$): δ = 6.27 (br. t, 1 H, NH), 4.03 (d, 2 H, CH_2), 3.97 (br. s, 1 H, 12α-CH), 3.83 (br. s, 1 H, 7α-CH), 3.75 (s, 3 H, OCH_3), 3.48 (m, 1 H, 3α-CH), 2.32−1.02 (m, 24 H, aliphatic H), 0.99 (d, 3 H, 21-CH_3), 0.88 (s, 3 H, 19-CH_3), 0.67 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3421, 2929, 2861, 1744, 1654, 1540. − $C_{27}H_{45}NO_6 \cdot 0.9H_2O$: C 65.40, H 9.51, N 2.83, found C 65.37, H 9.76, N 2.85.

***N*-Cholyl L-Alanine Methyl Ester (2a):** ^{1}H NMR ($CDCl_3$): δ = 6.41 (br. d, 1 H, NH), 4.57 (m, 1 H, C*H), 3.97 (br. s, 1 H, 12α-CH), 3.84 (br. s, 1 H, 7α-CH), 3.73 (s, 3 H, OCH_3), 3.46 (m, 1 H, 3α-CH), 2.28−1.05 (m, 27 H, aliphatic H), 0.98 (d, 3 H, 21-CH_3), 0.87 (s, 3 H, 19-CH_3), 0.67 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3388, 2935, 2867, 1743, 1650, 1540. − $C_{28}H_{47}NO_6 \cdot 0.7H_2O$: C 66.42, H 9.64, N 2.77; found C 66.35, H 9.97, N 2.76.

***N*-Cholyl D-Alanine Methyl Ester (2b):** ^{1}H NMR ($CDCl_3$): δ = 6.60 (br. s, 1 H, NH), 4.58 (m, 1 H, C*H), 3.98 (br. s, 1 H, 12α-CH), 3.84 (br. s, 1 H, 7α-CH), 3.73 (s, 3 H, OCH_3), 3.45 (m, 1 H, 3α-CH), 2.26−1.09 (m, 27 H, aliphatic H), 0.98 (d, 3 H, 21-CH_3), 0.87 (s, 3 H, 19-CH_3), 0.67 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3448, 3364, 2937, 2868, 1758, 1641, 1544. − HMRS calcd. for $C_{28}H_{47}NO_6$ [M^+]: 493.3403; found 493.3403.

***N*-Cholyl L-Valine Methyl Ester (3):** ^{1}H NMR ($CDCl_3$): δ = 6.06 (br. d, 1 H, NH), 4.55 (dd, 1 H, C*H), 3.98 (br. s, 1 H, 12α-CH), 3.84 (br. s, 1 H, 7α-CH), 3.73 (s, 3 H, OCH_3), 3.48 (m, 1 H, 3α-CH), 2.30−1.03 (m, 25 H, aliphatic H), 0.99 (d, 3 H, 21-CH_3), 0.93 (d, 6 H, 2 Val-CH_3), 0.88 (s, 3 H, 19-CH_3), 0.68 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3428, 2934, 2861, 1739, 1656, 1536. − $C_{30}H_{51}NO_6 \cdot 0.7H_2O$: C 67.43, H 9.89, N 2.62; found C 67.35, H 10.10, N 2.72.

***N*-Cholyl L-Leucine Methyl Ester (4a):** ^{1}H NMR ($CDCl_3$): δ = 6.06 (br. d, 1 H, NH), 4.55 (dd, 1 H, C*H), 3.98 (br. s, 1 H, 12α-CH), 3.84 (br. s, 1 H, 7α-CH), 3.73 (s, 3 H, OCH_3), 3.48 (m, 1 H, 3α-CH), 2.30−1.04 (m, 27 H, aliphatic H), 0.99 (d, 3 H, 21-CH_3), 0.93 (d, 6 H, 2 Leu-CH_3), 0.88 (s, 3 H, 19-CH_3), 0.68 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3418, 2948, 2869, 1740, 1655, 1545. − $C_{31}H_{53}NO_6 \cdot H_2O$: C 67.23, H 10.01, N 2.53; found C 67.33, H 10.35, N 2.86.

***N*-Cholyl D-Leucine Methyl Ester (4b):** ^{1}H NMR ($CDCl_3$): δ = 6.42 (br. s, 1 H, NH), 4.63 (m, 1 H, C*H), 3.98 (br. s, 1 H, 12α-CH), 3.85 (br. s, 1 H, 7α-CH), 3.71 (s, 3 H, OCH_3), 3.48 (m, 1 H, 3α-CH), 2.27−1.02 (m, 27 H, aliphatic H), 0.98 (d, 3 H, 21-CH_3), 0.92 (d, 6 H, 2 Leu-CH_3), 0.88 (s, 3 H, 19-CH_3), 0.67 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3397, 2948, 2869, 1744, 1655, 1540. − $C_{31}H_{53}NO_6 \cdot 0.7H_2O$: C 67.90, H 10.00, N 2.55, found C 67.90, H 10.29, N 2.67.

***N*-Cholyl L-Leucine *n*-Butyl Ester (4c):** ^{1}H NMR ($CDCl_3$): δ = 6.18 (d, 1 H, NH), 4.62 (m, 1 H, C*H), 4.10 (t, 2 H, OCH_2), 3.97 (br. s, 1 H, 12α-CH), 3.84 (br. s, 1 H, 7α-CH), 3.45 (m, 1 H, 3α-CH), 2.30−1.03 (m, 31 H, aliphatic H), 0.99−0.87 (m, 15 H, 5 CH_3), 0.66 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3388, 2958, 2870, 1739, 1656, 1544. − $C_{34}H_{59}NO_6 \cdot 0.5H_2O$: C 69.58, H 10.31, N 2.39; found C 69.64, H 10.63, N 2.53.

***N*-Cholyl L-Phenylalanine Methyl Ester (5a):** ^{1}H NMR ($CDCl_3$): δ = 7.25 (m, 3 H, aromatic H), 7.08 (m, 2 H, aromatic H), 6.07 (br. d, 1 H, NH), 4.87 (m, 1 H, C*H), 3.95 (br. s, 1 H, 12α-CH), 3.83 (br. s, 1 H, 7α-CH), 3.71 (s, 3 H, OCH_3), 3.43 (m, 1 H, 3α-CH), 3.10 (m, 2 H, CH_2-Ph), 2.36−1.05 (m, 24 H, aliphatic H), 0.95 (d, 3 H, 21-CH_3), 0.87 (s, 3 H, 19-CH_3), 0.65 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3407, 3033, 2933, 2867, 1741, 1650, 1536. −

$C_{34}H_{51}NO_6 \cdot 1.1H_2O$: C 69.26, H 9.10, N 2.38; found C 69.18, H 9.08, N 2.50.

***N*-Cholyl L-Phenylalanine Ethyl Ester (5b):** ^{1}H NMR ($CDCl_3$): δ = 7.25 (m, 3 H, aromatic H), 7.09 (m, 2 H, aromatic H), 6.07 (br. d, 1 H, NH), 4.85 (m, 1 H, C*H), 4.15 (q, 2 H, OCH_2), 3.94 (br. s, 1 H, 12α-CH), 3.82 (br. s, 1 H, 7α-CH), 3.43 (m, 1 H, 3α-CH), 3.09 (m, 2 H, CH_2-Ph), 2.33−1.02 (m, 27 H, aliphatic H), 0.95 (d, 3 H, 21-CH_3), 0.87 (s, 3 H, 19-CH_3), 0.65 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3407, 3027, 2932, 2861, 1737, 1655, 1530. − $C_{35}H_{53}NO_6 \cdot 0.6H_2O$: C 70.70, H 9.19, N 2.36; found C 70.74, H 9.35, N 2.57.

L-Phenylalanine *n*-Butyl Ester Hydrochloride: L-Phenylalanine (3.30 g, 20 mmol) was dissolved in 1-butanol (15 mL) and heated to 80 °C. Hydrochloric acid (37%, 1.5 mL) was added and the mixture was stirred overnight. After evaporation of the solvent under vacuum, the residue was dissolved in a small amount of methanol and precipitated with ether to give 2.96 g (11.5 mmol, 57%) of a white powder. − ^{1}H NMR ($CDCl_3$): δ = 8.75 (br. s, 2 H, NH_2), 7.29 (m, 5 H, aromatic H), 4.36 (m, 1 H, C*H): 4.08 (t, 2 H, OCH_2), 3.41 (m, 2 H, CH_2-C*), 1.49 (q, 2 H, CH_2), 1.22 (q, 2 H, CH_2), 0.85 (t, 3 H, CH_3). − HRMS calcd. for $C_{13}H_{20}NO_2Cl$: [M^+ − 36] 221.1416; found 221.1419.

***N*-Cholyl L-Phenylalanine *n*-Butyl Ester (5c):** ^{1}H NMR ($CDCl_3$): δ = 7.26 (m, 3 H, aromatic H), 7.09 (m, 2 H, aromatic H), 6.03 (d, 1 H, NH), 4.86 (m, 1 H, C*H), 4.19 (t, 2 H, OCH_2), 3.96 (br. s, 1 H, 12α-CH), 3.83 (br. s, 1 H, 7α-CH), 3.43 (m, 1 H, 3α-CH), 3.09 (m, 2 H, CH_2-Ph), 2.31−1.02 (m, 28 H, aliphatic H), 0.96−0.87 (m, 9 H, 3 CH_3), 0.66 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3403, 3027, 2933, 2869, 1737, 1650, 1536. − $C_{37}H_{57}NO_6 \cdot 0.5H_2O$: C 71.58, H 9.42, N 2.26; found C 71.61, H 9.61, N 2.48.

***N*-Cholyl L-Tyrosine Methyl Ester (6a):** ^{1}H NMR ($CDCl_3$/CD_3OD): δ = 6.88 (m, 2 H, aromatic H), 6.69 (m, 2 H, aromatic H), 6.34 (br. d, 1 H, NH), 4.74 (m, 1 H, C*H), 3.89 (br. s, 1 H, 12α-CH), 3.79 (br. s, 1 H, 7α-CH), 3.69 (s, 3 H, OCH_3), 3.35 (m, 1 H, 3α-CH), 3.06 (m, 2 H, CH_2-Ph), 2.60−1.01 (m, 24 H, aliphatic H), 0.88 (d, 3 H, 21-CH_3), 0.84 (s, 3 H, 19-CH_3), 0.61 (s, 3 H, 18-CH_3). − ^{13}C NMR ($CDCl_3$/CD_3OD): δ = 174.1 (s), 172.6 (s), 155.8 (s), 130.3(d), 126.7(s), 115.6(d), 73.3(d), 71.9(d), 68.5(d), 52.9(d), 52.4(q), 46.5(d), 46.2(s), 45.8(t), 41.6(d), 41.4(d), 39.3(d), 36.9(t), 36.7(s), 35.4(d), 34.7(t), 32.9(t), 31.8(t), 30.2(t), 28.0(t), 27.5(t), 26.3(d), 23.2(t), 22.4(q), 17.2(q), 12.4(q). − IR (KBr): ṽ = 3397, 3030, 2931, 2861, 1741, 1646, 1517.

***N*-Cholyl 4-Methoxyphenylalanine Methyl Ester (6b):** Compound **6a** (0.30 g, 0.48 mmol) was dissolved in butanone (15 mL), and methyl iodide (0.5 g) and potassium carbonate (0.5 g) were added. After refluxing the mixture overnight, butanone was evaporated under vacuum. Dichloromethane was added and the salts were filtered off. After evaporation of the solvent under vacuum, the crude product was purified with flash column chromatography on silica gel, using dichloromethane/methanol (95:5) as an eluent, affording 0.28 g (0.40 mmol, 92%) of a white solid. − ^{1}H NMR ($CDCl_3$): δ = 7.00 (m, 2 H, aromatic H), 6.81 (m, 2 H, aromatic H), 5.96 (br. d, 1 H, NH), 4.83 (q, 1 H, C*H), 3.96 (br. s, 1 H, 12α-CH), 3.84 (m, 1 H, 7α-CH), 3.77 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 3.44 (m, 1 H, 3α-CH), 3.04 (m, 2 H, CH_2-Ph), 2.24−1.25 (m, 24 H, aliphatic H), 0.95 (d, 3 H, 21-CH_3), 0.88 (s, 3 H, 19-CH_3), 0.66 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3423, 3040, 2930, 2857, 1740, 1661, 1513. − $C_{35}H_{53}NO_7 \cdot 0.5H_2O$: C 69.05, H 8.94, N 2.30; found C 69.09, H 9.19, N 2.37.

***N*-Cholyl L-Phenylglycine Methyl Ester (7a):** ^{1}H NMR ($CDCl_3$): δ = 7.35 (m, 5 H, aromatic H), 6.80 (m, 1 H, NH), 5.58 (s, 1 H, C*H), 3.95 (br. s, 1 H, 12α-CH), 3.84 (br. s, 1 H, 7α-CH), 3.73 (s, 3 H, OCH_3), 3.42 (m, 1 H, 3α-CH), 2.39−1.05 (m, 24 H, aliphatic H), 0.98 (d, 3 H, 21-CH_3), 0.88 (s, 3 H, 19-CH_3), 0.66 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3449, 3293, 3033, 2930, 2866, 1758, 1659, 1531. − $C_{33}H_{49}NO_6 \cdot 0.5H_2O$: C 70.18, H 8.92, N 2.48; found C 70.20, H 8.86, N 2.27.

***N*-Cholyl D-Phenylglycine Methyl Ester (7b):** ^{1}H NMR ($CDCl_3$): δ = 7.35 (m, 5 H, aromatic H), 6.78 (d, 1 H, NH), 5.59 (d, 1 H, C*H), 3.95 (br. s, 1 H, 12α-CH), 3.83 (br. s, 1 H, 7α-CH), 3.72 (s, 3 H, OCH_3), 3.43 (m, 1 H, 3α-CH), 2.38−1.03 (m, 24 H, aliphatic H), 0.97 (d, 3 H, 21-CH_3), 0.88 (s, 3 H, 19-CH_3), 0.64 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3417, 3033, 2933, 2861, 1745, 1657, 1529. − $C_{33}H_{49}NO_6 \cdot H_2O$: C 69.08, H 8.96, N 2.44; found C 69.23, H 9.11, N 2.42.

***N*-Chenodeoxycholyl L-Leucine Methyl Ester (8):** ^{1}H NMR ($CDCl_3$): δ = 5.92 (br. d, 1 H, NH), 4.66 (m, 1 H, C*H), 3.86 (br. s, 1 H, 7α-CH), 3.73 (s, 3 H, OCH_3), 3.49 (m, 1 H, 3α-CH), 2.35−1.12 (m, 29 H, aliphatic H), 0.94 (m, 9 H, 21-CH_3 + 2 Leu-CH_3), 0.90 (s, 3 H, 19-CH_3), 0.66 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3385, 2931, 2868, 1745, 1655, 1544. − $C_{31}H_{53}NO_5 \cdot 0.2H_2O$: C 71.14, H 10.29, N 2.68; found C 71.12, H 10.54, N 2.77.

***N*-Deoxycholyl L-Leucine Methyl Ester (9):** ^{1}H NMR ($CDCl_3$): δ = 6.02 (br. d, 1 H, NH), 4.66 (m, 1 H, C*H), 3.99 (br. s, 1 H, 12α-CH), 3.73 (s, 3 H, OCH_3), 3.64 (m, 1 H, 3α-CH), 2.32−1.10 (m, 29 H, aliphatic H), 0.96 (d, 3 H, 21-CH_3), 0.92 (d, 6 H, 2 Leu-CH_3), 0.87 (s, 3 H, 19-CH_3), 0.68 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3394, 2938, 2866, 1744, 1650, 1540. − $C_{31}H_{53}NO_5 \cdot 0.3H_2O$: C 70.90, H 10.29, N 2.67; found C 70.88, H 10.59, N 2.81.

***N*-Lithocholyl L-Leucine Methyl Ester (10):** ^{1}H NMR ($CDCl_3$): δ = 5.76 (d, 1 H, NH), 4.65 (m, 1 H, C*H), 3.72 (s, 3 H, OCH_3), 3.61 (m, 1 H, 3α-CH), 2.32−1.05 (m, 31 H, aliphatic H), 0.94 (d, 3 H, 21-CH_3), 0.91 (d, 6 H, 2 Leu-CH_3), 0.89 (s, 3 H, 19-CH_3), 0.62 (s, 3 H, 18-CH_3). − IR (KBr): ṽ = 3426, 3328, 2942, 2868, 1754, 1656, 1535. − $C_{31}H_{53}NO_4$: C 73.91, H 10.61, N 2.78; found C 74.28, H 11.02, N 2.87.

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[1] P. Terech, R. G. Weiss, *Chem. Rev.* **1997**, *97*, 3133−3159.
[2] K. Yoza, Y. Ono, K. Yoshihara, T. Akao, H. Shinmori, M. Takeuchi, S. Shinkai, D. N. Reinhoudt, *Chem. Commun.* **1998**, 907−908.
[3] R. Oda, I. Huc, S. J. Candau, *Angew. Chem. Int. Ed.* **1998**, *37*, 2689−2691.
[4] G. M. Clavier, J. F. Brugger, H. Bouas-Laurent, J. L. Pozzo, *J. Chem. Soc., Perkin Trans. 2* **1998**, 2527−2534.
[5] C. Geiger, M. Stanescu, L. Chen, D. G. Whitten, *Langmuir* **1999**, *15*, 2241−2245.
[6] N. Amanokura, Y. Kanekiyo, S. Shinkai, D. N. Reinhoudt, *J. Chem. Soc., Perkin Trans. 2* **1999**, 1995−2000.
[7] D. J. Abdallah, R. G. Weiss, *Langmuir* **2000**, *16*, 352−355.
[8] D. J. Abdallah, R. G. Weiss, *Adv. Mater.* **2000**, *12*, 1237−1247.
[9] P. Terech, *Ber. Bunsenges. Phys. Chem.* **1998**, *102*, 1630−1643.
[10] L. Lu, T. M. Cocker, R. E. Bachman, R. G. Weiss, *Langmuir* **2000**, *16*, 20−34.
[11] M. de Loos, J. van Esch, I. Stokroos, R. M. Kellogg, B. L. Feringa, *J. Am. Chem. Soc.* **1997**, *119*, 12675−12676.

[12] K. Hanabusa, C. Koto, M. Kimura, H. Shirai, A. Kakehi, *Chem. Lett.* **1997**, 429−430.
[13] K. Inoue, Y. Ono, Y. Kanekiyo, T. Ishi-i, K. Yoshihara, S. Shinkai, *J. Org. Chem.* **1999**, *64*, 2933−2937.
[14] U. Beginn, S. Keinath, M. Möller, *Macromol. Chem. Phys.* **1998**, *199*, 2379−2384.
[15] K. Hanabusa, H. Kobayashi, M. Suzuki, M. Kimura, H. Shirai, *Colloid Polym. Sci.* **1998**, *276*, 252−259.
[16] V. P. Vassilev, E. E. Simanek, M. R. Wood, C. H. Wong, *Chem. Commun.* **1998**, 1865−1866.
[17] K. Hanabusa, R. Tanaka, M. Suzuki, M. Kimura, H. Shirai, *Adv. Mater.* **1997**, *9*, 1095−1097.
[18] S. Bhattacharya, S. N. Ghanashyam Acharya, *Chem. Mater.* **1999**, *11*, 3121−3132.
[19] K. Hanabusa, M. Matsumoto, M. Kimura, A. Kakehi, H. Shirai, *J. Colloid Interface Sci.* **2000**, *224*, 231−244.
[20] K. Miki, A. Masui, N. Kasai, M. Miyata, M. Shibakami, K. Takemoto, *J. Am. Chem. Soc.* **1988**, *110*, 6594−6596.
[21] K. Sada, T. Kondo, M. Ushioda, Y. Matsuura, K. Nakano, M. Miyata, K. Miki, *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1931−1937.
[22] Y. Hishikawa, K. Sada, R. Watanabe, M. Miyata, K. Hanabusa, *Chem. Lett.* **1998**, 795−796.
[23] U. Maitra, P. V. Kumar, N. Chandra, L. J. D′Souza, M. D. Prasanna, A. R. Raju, *Chem. Commun.* **1999**, 595−596.
[24] K. Hanabusa, K. Shimura, K. Hirose, M. Kimura, H. Shirai, *Chem. Lett.* **1996**, 885−886.
[25] J. van Esch, S. DeFeyter, R. M. Kellogg, F. DeSchryver, B. L. Feringa, *Chem. Eur. J.* **1997**, *3*, 1238−1243.
[26] P. Terech, J. J. Allegraud, C. M. Garner, *Langmuir* **1998**, *14*, 3991−3998.
[27] T. Shioiri, Y. Yokoyama, Y. Kasai, S. Yamada, *Tetrahedron* **1976**, *32*, 2211−2217.
[28] K. Hanabusa, Y. Maesaka, M. Kimura, H. Shirai, *Tetrahedron Lett.* **2000**, *40*, 2385−2388.
[29] D. Dolphin, A. Wick, *Tabulation of infrared spectral data*; John Wiley & Sons, Inc., New York, **1977**.
[30] W. J. Humphlett, C. V. Wilson, *J. Org. Chem.* **1961**, *26*, 2507−2510.
[31] W. T. J. Morgan, *J. Chem. Soc.* **1926**, 79−84.

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