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Carboxybetaine and Carboxybetaine Ester Derivatives of Tetra(dodecyloxyphenyl)-calix[4]resorcinarene: Synthesis, Self-Assembly and In Vitro Toxicity

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Abstract: Amphiphilic calix[4]resorcinarenes are a class of macrocyclic compounds with broad potential utility including nanomedicine. Here the synthesis of new carboxybetaine and carboxybetaine ester calix[4]resorcinarene bearing 4-(dodecyloxy)phenyl groups on the lower rim is presented. The compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, 2D NMR, IR, ESI and elemental analysis. The critical association concentration values are 1.00×10^{-5} and 1.18×10^{-5} mol·L^{-1} for carboxybetain and ester, respectively. The hemolytic activity of the macrocycles and their cytotoxicity against normal (WI-38, Chang liver) and tumor cells (M-HeLa) are also estimated.

Keywords: calix[4]resorcinarene; carboxybetaine; hemolytic activity; cytotoxicity

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1. Introduction

Amphiphilic calix[4]resorcinarenes as well as calix[n]arenes are one of the unique classes of synthetic macrocycles, which have the potential to be used in different areas including nanomedicine [1–3]. Calix[4]resorcinarenes have the aromatic cavity, constructing from four resorcinol units linked together by methylene bridges bearing substituents [4]. The upper and lower rims of the cavity are distinguished; they consist of substituents of hydroxyl groups or of o-position of resorcinol rings and substituents at methylene bridges, respectively. Calixresorcinarenes' molecules can exist in the form of five conformation isomers [4]; usually amphiphilc calixresorcinarenes exist in boat conformation, in which the upper rim hydrophilic substituents and the lower rim hydrophobic substituents are located on opposite sides of the aromatic cavity. At the present time, the row of calix[4]resorcinarene derivatives was synthesized for the purpose of the complexation and delivery of biologically active compounds [5–13]. It was shown that amphiphilic calix[4]resorcinarenes spontaneously self-organized in aqueous solutions at low concentrations with the formation of nanoassociates of different diameter [14–17]. The functionalization of the upper rim of calix[4]resorcinarene (by modification of hydroxyl groups or into *o*-position of resorcinol rings) promotes the solubility of macrocycles' molecules in aqueous solutions and provides the low toxicity of the macrocycles.

Hydrophilic nanosystems containing PEG or zwitter ionic particles are known to be biocompatible because they reduce bioadhesion [18]. The stability of the zwitterionic materials and the fact that they do not lead to the immune response of the living organism [19] encourage the synthesis of novel zwitterionic compounds. Carboxybetaine compounds have high hydration as well as an absence of intra- and intermolecular interactions between the zwitterionic groups, which leads to the high stability of the carboxybetaine materials to changes in temperature and ionic strength of the solution. Additionally, the

carboxylic anionic groups of the carboxybetain fragments can be functionalized for additional conjugation with biomolecules, antibodies, target ligands, and fluorescent dyes to create polyfunctional materials [20].

The traditional method of synthesis of a carboxybetaine compound includes the quaternization of dimethylamino derivatives to obtain carboxybetaine esters and their further saponification [21]. Carboxybetaine esters are intriguing substances for application in medicine themselves. The polycationic nature of carboxybetaine esters can contribute to the antibacterial properties of surfaces modified by these compounds, and their saponification as a result of interaction with bacteria and proteins will promote the transformation of the material surface into zwitterionic, and, therefore, the absence of immune response of the organism on the medical material [22].

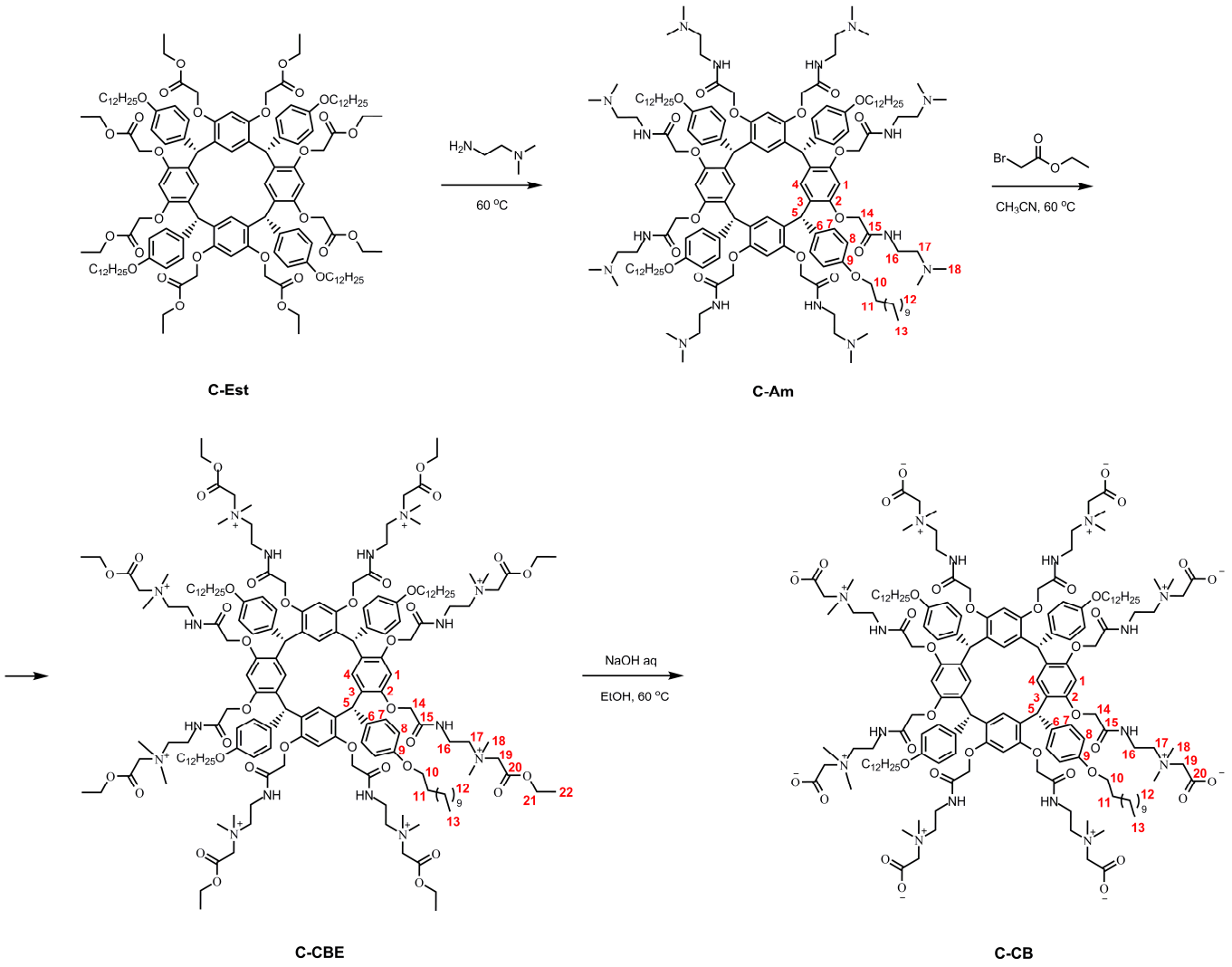
These advantages of carboxybetaine and their esters have inspired us to implement the synthesis of carboxybetaine ester (C-CBE) and carboxybetaine (C-CB) derivatives of calix[4]resorcinarene, bearing 4-(dodecyloxy)phenyl substituents on the lower rim. For both new compounds, self-association data, in vitro hemolytic activity, and cytotoxicity on normal and tumor cells are acquired.

2. Results and Discussion

The synthesis of the macrocycles was performed by Scheme 1 on the base of calix[4]reso rcinarene with peripheral dimethylamino groups C-Am (boat conformation), bearing 4-(dodecyloxy)phenyl substituents on the lower rim. C-Am was obtained by aminolysis of ester derivative C-Est [8]. The synthesis was carried out similar to ref [13] in an excess of N,N-dimethylethylenediamine at 60 °C, and the yield was 96%. The structure of C-Am is confirmed by ^{1}H and ^{13}C NMR spectra (Figures S1 and S2). In the ^{1}H-NMR spectrum, the proton peak of the dimethylamino groups is fixed as two singlets at 2.44 and 2.26 ppm (Figure S1). On the next stage, the alkylation of the dimethylamino groups of macrocycle C-Am was performed by reaction with ethyl bromoacetate in acetonitrile at 60 °C. The yield of C-CBE was 86% (total yield is 82%), and ^{1}H and ^{13}C NMR spectra validate the macrocycle's structure (Figures S3–S5). The signals were assigned using 2D-NMR spectroscopy (COSY, HSQC, HMBC, Figures S6–S8).

It should be highlighted that both the bulky substituents on the upper rim of calix[4] resorcinarenes and self-aggregation are responsible for the broadening of some signals in the NMR spectra of C-CBE. The intramolecular mobility of the substituents is typically slowed down when the bulky fragments connect to calix[4]resorcinarenes. Additionally, the influence of the magnetically anisotropic groups and the various substituent orientations with respect to them in various spatial forms has an impact on the spectral view [23]. The structure of C-CBE is further supported by the ESI spectrum, in which positive molecular ions without bromide ions are registered (Figure S9). In the ^{1}H NMR spectrum of C-CBE, the downfield shift of the dimethylamino groups' signals (about 3.39 ppm) as a result of quartenization is observed. Additionally, signals from the C-CBE peripheral ester groups are seen at 1.22–1.12 and 4.29–4.14 (Figures S3 and S4).

The zwitterionic macrocycle C-CB was produced via the saponification of the ester groups of C-CBE by an aqueous base solution in ethanol. By using the dialysis method, the reaction's product was separated from inorganic salt, and elemental analysis data revealed that no inorganic elements were present. C-CB has the yield of 67% (the overall yield is 55%). ^{1}H and ^{13}C NMR spectra were used to confirm the structure of C-CB (Figures S10 and S11). The signals were assigned using 2D-NMR spectroscopy (COSY, HSQC, HMBC, Figures S12–S14). The absence of the ester groups is seen in the ^{1}H NMR spectrum (Figure S10). It should be noted that while macrocycle C-CBE is soluble in both methanol and water, macrocycle C-CB is only soluble in water; as a result, the NMR spectra of C-CB were recorded only in D_2O solution and contain the broadening signals. Furthermore, it is well-known that the zwitterionic groups have a strong ability to bind water molecules [24], and that strong intra- and intermolecular hydrogen interactions cause multiplets to overlap and broaden in the NMR spectra.



Scheme 1. The synthesis of C-CBE and C-CB.

The IR spectra of C-Am, C-CBE, and C-CE in Figures S15–S17 provide an illustration of the structural modifications in the macrocycles. The Amide I band can be seen at 1686 cm^{-1} in the spectrum of C-Am. New C=O and C-O-C bands can be seen at 1747 and 1243 cm^{-1} in the spectrum of C-CBE, but they are absent in the spectrum of C-CB. It should be noted that the presence of a broad and intense band at a wavelength over 3400 cm^{-1} in the spectra of C-CBE and C-CE indicates the presence of water molecules in the compounds, which corresponds with the results of the elemental analysis.

Due to the presence of the hydrophobic substituents on the lower rim of the aromatic cavity, macrocycles C-CBE and C-CB are amphiphilic and can form self-associates in an aqueous media. Thus, the study of their self-association can be performed. The self-association of the macrocycles C-CBE and C-CB was studied by the fluorimetry method with pyrene [25]. Pyrene is a fluorescent probe that is used to measure the critical association concentration (cac) and critical micelle concentration (cmc) of amphiphilic compounds and surfactants due to the sensitivity of its emission to the surrounding polarity. It is evident that the approach is based on changes in the ratio between the dye's first (I, 373 nm) and third (III, 385 nm) emission bands at the various amphiphil's concentrations. The I/III(logC) plot typically has a sigmoid shape, and cmc (cac) is calculated by locating the middle of the middle segment on the sigmoid curve. For the macrocycles C-CBE and C-CB, the cac values are 1.18×10^{-5} and 1.00×10^{-5} mol L^{-1}, respectively (Figure S18). The amplification of hydrophobicity of C-CB, apparently, can be explained by stronger interaction of the

zwitterionic groups of C-CB with water molecules, which leads to the enhancement of the hydrophobic interaction.

The macrocycles C-CBE and C-CB were tested for their hemolytic activity and cytotoxicity as a preliminary test for their prospective use in nanomedicine (Table 1, Figure 1, Tables S1 and S2). According to the research, C-CB does not exhibit hemolytic activity at the examined concentrations, while C-CBE results in the death of red blood cells, which is most likely accounted for by the macrocycle's cationic charge. It is evident that cationic chemicals have been shown to have antibacterial effects that are frequently associated by the cytotoxicity [26]. Two normal cell lines (human lung cell lines WI38 and human hepatocytes cells Chang liver) and one tumor cell line (human epithelioid cervical carcinoma M-HeLa) are used to investigate the cytotoxicity of macrocycles. According to Table 1, both macrocycles have lower toxicity on tumor cells than normal, but the cytotoxicity of C-CBE is larger than of C-CB. Therefore, during the creation of nanosystems based on the studied macrocycles, it will be crucial to choose the concentrations of the macrocycles and/or the size of the nanosystems (to promote the Enhanced Permeability and Retention Effect [27]) in order to minimize adverse effects on healthy cells.

Table 1. Hemolytic activity and cytotoxicity of compounds C-CBE and C-CB.

	HC_{50} (μM)	IC_{50} (μM)		
		WI-38	**Chang Liver**	**M-HeLa**
C-CBE	500 ± 40	1800 ± 100	1200 ± 90	3900 ± 300
C-CB	>5000	2900 ± 200	>5000	>5000

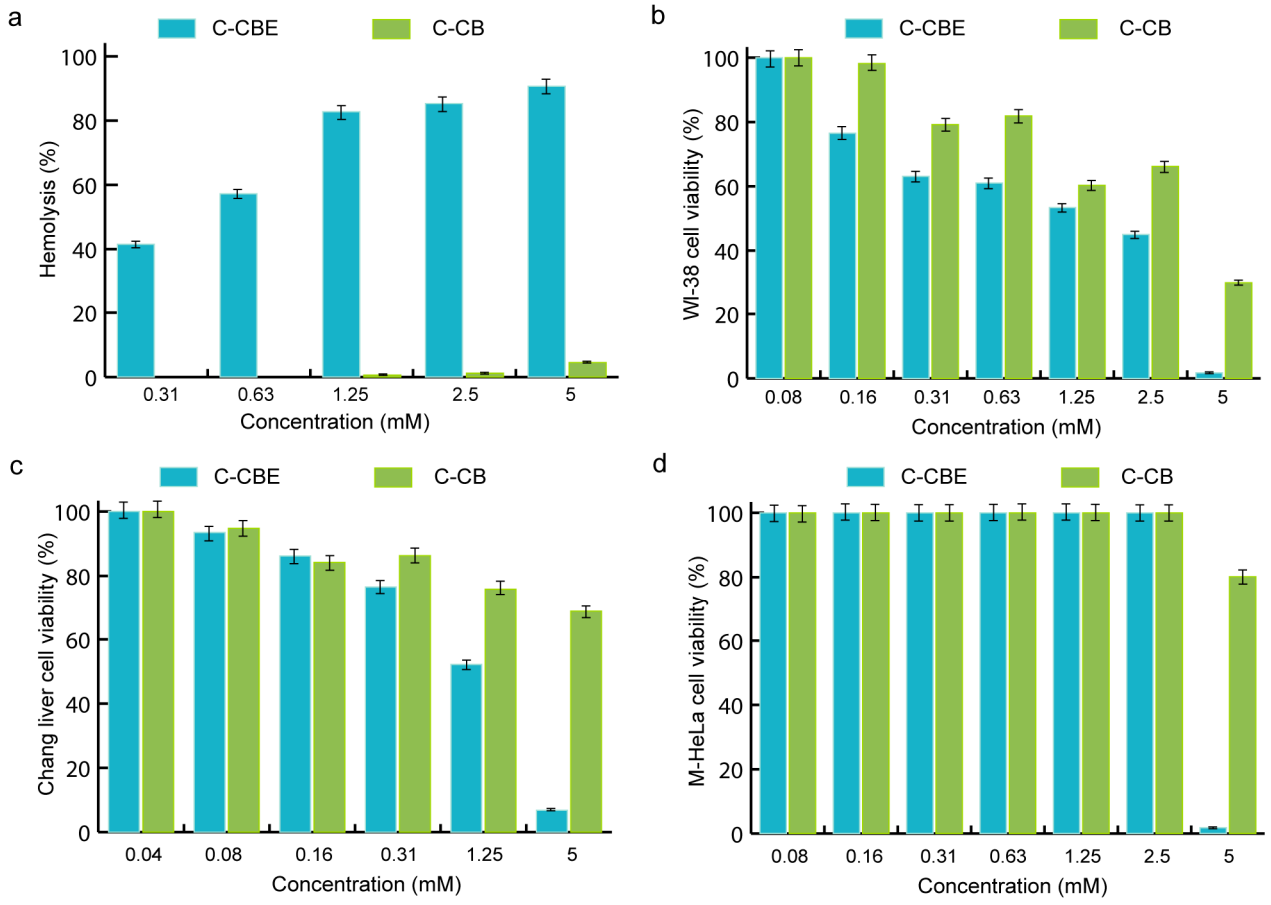


Figure 1. The hemolysis (%) of human red blood cells (**a**), and cell viability (%) of WI-38 cells (**b**), Chang liver cells (**c**), and M-HeLa cells (**d**) in the presence of C-CBE and C-CB.

3. Materials and Methods

NMR spectra were recorded on a Bruker Avance III 500 spectrometer. IR spectra were recorded using a Vector-27 IR spectrometer (Bruker, Germany) in KBr pellets. Electrospray ionization mass spectra (ESI) were obtained on 428 an AmazonX mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The elemental analysis was carried out on a CHNS analyzer Vario Macro cube (Elementar Analysensysteme GmbH, Germany). Fluorescence spectra were registered on Hitachi F-7100 fluorimeter. Macrocycle C-Est was synthesized as described in [9], and macrocycle C-Am was obtained similarly to method described in [13].

Synthesis of 2,8,14,20-Tetra(4-dodecyloxy-phenyl)pentacyclo[19.3.1.13,7.19,13.115,19]-octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaen-4,6,10,12,16,18,22,24-octakis(N-(2-(dimethylamino)ethyl)-2-methoxyacetamide) C-Am.

A total of 3.635 g (1.64 mmol) of macrocycle C-Est was dissolved in 15 mL of N,N-dimethylethylenediamine and heated at 60 °C for 100 h. Then the amine was removed at reduced pressure and acetonitrile (50 mL) was added to residue. The product was filtered, washed with acetonitrile (100 mL) and dried at reduced pressure to yield C-Am as a light yellow solid, m.p. 175–178 °C. Yield was 4.00 g (96%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.87 (12H, t, *J* 8.0 Hz, H-13), 1.46–1.26 (72H, m, H-12), 1.78 (8H, q, *J* 8.0 Hz, H-11), 2.44–2.26 (48H, 2s, H-18), 3.41–3.13 (32H, m, H-16 and H-17), 3.87 (8H, t, *J* 6.0 Hz, H-10), 4.45 and 4.19 (16H, m, H-14), 5.71 (4H, s, C-5), 6.04 and 5.77 (8H, 2s, H-1), 6.24 (4H, s, NH), 6.37 (2H, s, H-4), 6.64–6.58 (18H, m, H-4, H-7 and H-8), 7.01 (4H, s, NH). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 167.9 (C-15), 167.1 (C-15), 157.4 (C-9), 154.2 (C-2), 153.5 (C-2), 133.9 (C-6), 128.9 (C-7), 125.6 (C-4), 124.9 (C-3), 113.9 (C-8), 100.0 (C-1), 68.7 (C-10, C-14), 67.7 (C-10, C-14), 67.2 (C-10, C-14), 57.9 (C-17), 45.1 (C-18), 42.7 (C-5), 36.5 (C-16), 31.6 (C-11), 29.4 (C-12), 29.1 (C-12), 25.9 (C-12), 22.4 (C-12), 13.8 (C-13). IR (KBr, cm^{-1}): ν˜ = 3399 m (N-H), 2924–2854 s (C-H), 2820–2770 w (CH), 1686 s (Amid I), 1537 m (Amid II), 1509 s (C=C_{Ar}). Anal. Calcd for $C_{148}H_{232}N_{16}O_{20}$: C, 69.56; H, 9.15; N, 8.77. Found: C, 69.50; H, 9.11; N, 8.79.

Synthesis of 2,8,14,20-Tetra(4-dodecyloxy-phenyl)pentacyclo[19.3.1.13,7.19,13.115,19]-octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaen-4,6,10,12,16,18,22,24-octakis{N-[3-(dimethyl{ethoxycarbonylmethyl}ammonio)ethyl]aminocarbonylmethoxy}octabromide C-CBE.

A total of 3 g (1.17 mmol) of macrocycle C-Am was dissolved in 100 mL of dry acetonitrile at 70 °C and then reaction mixture was cooled to room temperature. Then 1.04 mL (9.39 mmol) of ethyl bromoacetate was added, and the reaction mixture was heated at 60 °C for 65 h. After cooling, the precipitate was filtered, washed with acetonitrile (50 mL) and dried at reduced pressure to yield C-CBE as a white solid, m.p. 230–231 °C. Yield was 3.92 g (86%). ^{1}H NMR (500 MHz, D_2O): δ = 0.85 (12H, s, H-13), 1.63–1.12 (104H, m, H-11, H-12, H-22), 3.36 (48H, s, H-18), 3.85–3.71 (48H, br s, H-10, H-14, H-17, H-16), 4.29 and 4.14 (16H, 2 br s, H-21), 4.58 and 4.47 (20H, br s,H-14, H-19), 5.88 (4H, s, H-4), 6.17–6.10 (8H, 2s, H-1, H-5), 6.55 (8H, s, H-8), 6.86 (8H, s, H-7). ^{1}H NMR (500 MHz, CD_3OD): δ = 0.89 (12H, t, *J* 8.0 Hz, H-13), 1.52–1.27 (96H, m, H-12, H-22), 1.18 (8H, q, *J* 8.0 Hz, H-11), 3.39–3.35 (48H, m, H-18), 3.95-3.68 (44H, m,H-10, H-14, H-16, H-17), 4.32 (16H, m, H-21), 4.45 (4H, br s,H-14), 4.58 (16H, m, H-19), 5.91 (4H, s, H-4), 6.11–6.10 (8H, 2s, H-1, H-5), 6.68 (8H, s, H-8), 6.82 (8H, s, H-7). ^{13}C NMR (D_2O, 126 MHz) δ = 170.5 (C-15), 169.5 (C-15), 164.5 (C-20), 156.9 (C-9), 153.6 (C-2), 134.2 (C-6), 129.2 (C-7), 126.3 (C-4), 125.9 (C-3), 113.6 (C-8), 100.3 (C-1), 67.8 (C-14), 67.2 (C-10), 63.2 (C-21), 62.6 (C-17), 61.7 C-17), 56.4 (C-19), 52.3 (C-18), 41.8 (C-5), 33.1 (C-16), 31.6–22.3 (C-11, C-12), 13.6 (C-13), 13.1 (C-22). IR (KBr, cm^{-1}): ν˜ = 2925–2854 s (C-H), 1747 s (C=O), 1675 s (Amid I), 1530 m (Amid II), 1510 s (C=C_{Ar}), 1201 s (C-O). MS (ESI): *m*/*z* calcd for $C_{180}H_{288}Br_8N_{16}O_{36}$: 406.3 [M – 8Br$^-$]$^{8+}$; found 406.3; 476.0 [M – 7Br$^-$]$^{7+}$; found 475.8; 568.0 [M – 6Br$^-$]$^{6+}$; found 568.3; 698.4 [M – 5Br$^-$]$^{5+}$; found 698.2. Anal. Calcd for $C_{180}H_{288}Br_8N_{16}O_{36}\cdot 4H_2O$: C, 54.54; H, 7.53; N, 5.65; Br 16.13. Found: C, 54.35; H, 7.54; N, 5.63; Br, 16.19.

Synthesis of 2,8,14,20-Tetra(4-dodecyloxy-phenyl)pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]-octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaen-4,6,10,12,16,18,22,24-octakis{N-[3-(dimethyl{acetoxido}ammonio)ethyl]aminocarbonylmethoxy} C-CB.

A total of 2.74 g (0.84 mmol) of C-CBE was dissolved in 50 mL of ethanol at 60 °C and then 2 mL of an aqueous solution of NaOH (0.84 g, 21.0 mmol) was added under stirring. The reaction mixture was heated at 60 °C for 14 h. After cooling to rt, the precipitate was collected, washed with ethanol, and dried. The precipitate was dissolved in distilled water (3 mL) and the resulting solution was dialyzed in distilled water (molecular weight cutoff 1000 Da) and followed by evaporation of the aqueous solution and drying of the product under reduced pressure to yield C-CBE as a white solid, m.p. > 260 °C. Yield was 1.69 g (67%). ^{1}H NMR (500 MHz, D_2O): δ = 0.86 (12H, s, H-13), 1.53–1.24 (80H, m, H-11, H-12), 4.70–3.02 (120H, m, H-14, H-19, H-10, H-16, H-18, H-17), 5.91 (8H, br s, H-4, H-5), 6.13 (4H, br s, H-1), 6.77–6.55 (16H, 2 br s, H-7, H-8). ^{13}C NMR (D_2O, 126 MHz) δ = 175.5 (C-15), 168.3 (C-20), 156.9 (C-9), 153.4 (C-2), 133.5 (C-6), 131.9 (C-4), 129.3 (C-7), 125.4 (C-3), 113.7 (C-8), 98.9 (C-1), 67.5 (C-14), 67.3 (C-10), 64.0 (C-17), 61.4 (C-19), 51.2 (C-18), 41.8 (C-5), 32.9 (C-16), 32.8–22.3 (C-16, C-11, C-12), 13.6 (C-13). IR (KBr, cm^{-1}): ν˜ = 2924-2853 s (C-H), 1629 s, br (Amid I, C=O), 1535 w (Amid II), 1509 m (C=C_{Ar}). Anal. Calcd for $C_{164}H_{248}N_{16}O_{36}\cdot 8H_2O$: C, 62.26; H, 8.41; N, 7.08. Found: C, 62.46; H, 8.42; N, 7.09.

3.1. Hemolytic Activity Assay

Fresh hRBC with heparin was rinsed 3 times with 0.15 M NaCl by centrifugation at 800 rpm for 10 min and re-suspended in 0.15 M NaCl. Each of the investigated solutions in 0.15 M NaCl was then added to 0.5 mL of a solution of the stock hRBC in 0.15 M NaCl to reach a final volume of 5 mL (final erythrocyte concentration 10% v/v). The resulting suspension was incubated under agitation for 1 h at 37 °C. The samples were then centrifuged at 2000 rpm for 10 min. The release of hemoglobin was monitored by measuring the absorbance of the supernatant at 540 nm by means of a digital photoelectric colorimeter AP-101 (Apel, Japan). Controls for zero hemolysis (blank) and 100% hemolysis consist of hRBC that is suspended in 0.15 M NaCl and bidistilled water, respectively.

3.2. Cytotoxic Effect Assay

Chang liver cell and M-HeLa clone 11 cell viability was evaluated by means of multifunctional system Cytell Cell Imaging (GE Healthcare Life Sciences, Uppsala, Sweden) using Cell Viability BioApp application, which makes it possible to precisely count the number of cells and estimate their viability from the fluorescence intensity. Cells were cultured in a standard Eagle's nutrient medium manufactured at the Chumakov Institute of Poliomyelitis and Virus Encephalitis (PanEco Company) and supplemented with 10% fetal calf serum and 1% nonessential amino acids. The cells were dispersed on a 24-well «Eppendorf» plate at a concentration of 200 × 10^3 cells/mL, 500 μL of medium per well and cultured in a CO_2 incubator at 37 °C. After 24 h seeding the cells into wells, the examined compounds were added at a preset dilution, 500 μL to each well. The twofold dilutions of the compounds were prepared immediately in nutrient media. The resulting suspensions were incubated for 24 h at 37 °C. The experiments were performed in triplicates. Intact cells cultured in parallel with experimental cells served as a reference. The fraction of the grown-up cells was expressed in % vs. reference cells. The degree of cell growth inhibition under the influence of the testing agent was calculated by the equation: N (%) = (1 – Exp/Control)·100%, where Exp is the quantity of uninhibited cells in the study sample, Control is the quantity of uninhibited cells in the control sample. Then IC_{50} (the concentration which caused 50% cell growth inhibition) was determined from the curve of cell cultural growth versus examined compound concentration.

3.3. The Determination of cac Values of Compounds by Fluorimetry Method with Pyrene Probe

The fluorescence spectra of pyrene (2 × 10^{-6} M) were recorded on a fluorescence spectrophotometer Hitachi F-7100 in a quartz cell of 1 cm path length at 25 °C. The concen-

trations of the macrocycles varied from 1×10^{-7} to 1×10^{-3} M. Fluorescence spectra were obtained using 2.5/2.5 nm (excitation/emission) slit widths. The excitation wavelength was set at 333 nm, and the emission range was from 345 to 500 nm. The ratio of the first (372 nm) and third (381 nm) emission bands I/III for every spectrum was estimated and the cac values were graphically determined from the sigmoidal plots of I/III ratio versus the logarithm of macrocycles' concentration accordingly to ref [25].

4. Conclusions

In summary, the synthesis of new amphiphilic carboxybetaine and carboxybetaine ester calix[4]resorcinarenes bearing 4-(dodecyloxy)phenyl groups on the lower rim starting from macrocycle with peripheral dimethylamino groups was presented. The alkylation of the amino groups by ethyl bromoacetate led to the carboxybetaine ester derivative C-CBE with an 86% yield and the saponification of the ester groups led to the carboxybetaine C-CB with a 67% yield. The macrocycles were characterized by NMR, IR, and ESI-MS (in the case of C-CBE) and elemental analysis data. The study of the macrocycles' self-association in an aqueous solution by fluorimetry provided the cac values of 1.18×10^{-5} and 1.00×10^{-5} mol L^{-1} for C-CBE and C-CB, respectively. The macrocycles' hemolytic activity and in vitro cytotoxicity data were obtained. It was found that the hemo- and cytotoxicity of C-CBE are larger than of C-CB. The obtained data can be used to develop new nanosystems for drug delivery on the base of calix[4]resorcinarene derivatives.

Supplementary Materials: The following supporting information can be downloaded online. Figures S1 and S2: ^{1}H and ^{13}C NMR spectra of C-Am; Figures S3–S8: ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC spectra of C-CBE; Figure S9: ESI spectrum of C-CBE; Figures S10–S14: ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC spectra of C-CB; Figures S15–S17: IR spectra of C-Am, C-CBE, and C-CB. Figure S18: The pyrene I/III values dependence on the logarithmic concentration of macrocycles C-CB and C-CBE in an aqueous solution, 25 °C. Tables S1 and S2: Hemolytic activity and cytotoxicity of compounds C-CBE and C-CB.

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Institutional Review Board Statement: All subjects gave their informed consent for inclusion before they participated in the study. Blood was collected at the Clinic of the FRC "KazSC RAS" from healthy patients with their personal consent.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available in Supplementary Materials.

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Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are available from the authors.

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