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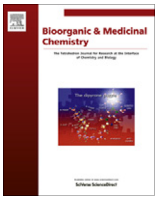

Synthesis of 5-aryl-1,4-benzodiazepine derivatives attached in resorcinaren-PAMAM dendrimers and their anti-cancer activity

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ABSTRACT

A series of resorcinaren-PAMAM dendrimers with benzodiazepines in the periphery were synthesized and their anticancer properties studied. The synthesized dendrimers showed potential anticancer activities, which were enhanced in the presence of a chloro-substituent in the second ring of the 5-aryl-1,4-benzodiazepine. The dendrimers were characterized by IR, ^{1}H and ^{13}C NMR, UV–vis absorption, electrospray (ES) and/or MALDI-TOF mass spectrometries.

1. Introduction

Dendrimers[1] are synthetic, nanometer-sized macromolecules that can be modified to suit a specific application with unique architecture, given that they have well-defined, compartmentalized structure and narrow polydispersity. They have been extensively investigated as drug delivery carriers[2–7] due to the presence of nanocompartments that can encapsulate guest molecules.[8–10] Dendrimers are commercially available, being PAMAM dendrimers the most extensively studied for biological applications.[11–15] They have a unique architecture based on β-alanine subunits with primary amine groups on the surface that are available for the attachment of several types of biological materials.[16] Their aqueous solubility and biocompatibility are well suited to carry ligands, fluorochromes, and drugs for targeting, imaging,[17] and drug delivery.[18,1,19,20] Benzodiazepines represent a large and still expanding group of synthetic heterocyclic derivatives, which have attracted much attention in the field of drugs and pharmaceuticals in the last 30 years. The biological relevance of the benzodiazepine family explains our interest in exploring the anticancer activities of the dendrimers with benzodiazepines. In the present paper, we describe the synthesis of 5-aryl-1,4-benzodiazepines derivatives attached to resorcinaren-PAMAM dendrimers and their anticancer activity.

2. Synthesis

It has been established that the introduction of activated ester groups on the upper rim of resorcinarenes provides an excellent possibility for further chemical modification of resorcinarenes. The ester group of resorcinarenes can be ammonolysized by diamine to obtain amide derivatives with terminal amino groups. Resorcinarene-core amine dendrimers reacting with esters will introduce a benzodiazepine at the periphery of resorcinarenes. The synthetic routes for the preparation of the first and second generations of dendritic resorcinaren-polyamidoamine and Schiff bases are shown in Scheme 1.

The dendritic core resorcinarenes **1** and **2** were prepared by the reaction of resorcinol with dodecanaldehyde or hydrocinnamaldehyde,[21] they have eight functionalized phenolic hydroxyl groups, which were primarily full alkylated with methyl bromoacetate in a K_2CO_3/acetone system to afford active ester derivatives **3**, **4**. Compounds **3** and **4** were used as the starting building blocks for constructing high-generation dendrimers. Refluxing compounds **3** and **4** with a large excess of 1,2-diaminoethane in a mixture of methanol and benzene 1:1 for 24 h gave the corresponding first generation amide dendrimers **5** and **6** containing free terminal amino groups in excellent yields (90% and 88%) (Scheme 1).

Generation 1.5 was obtained from a mixture of methyl acrylate in MeOH cooled to 0 °C, this was added to compounds **5** or **6** in MeOH cooled to 0 °C. The MeOH and excess of methyl acrylate, as volatiles, were evaporated on a rotary evaporator at 34 °C, and

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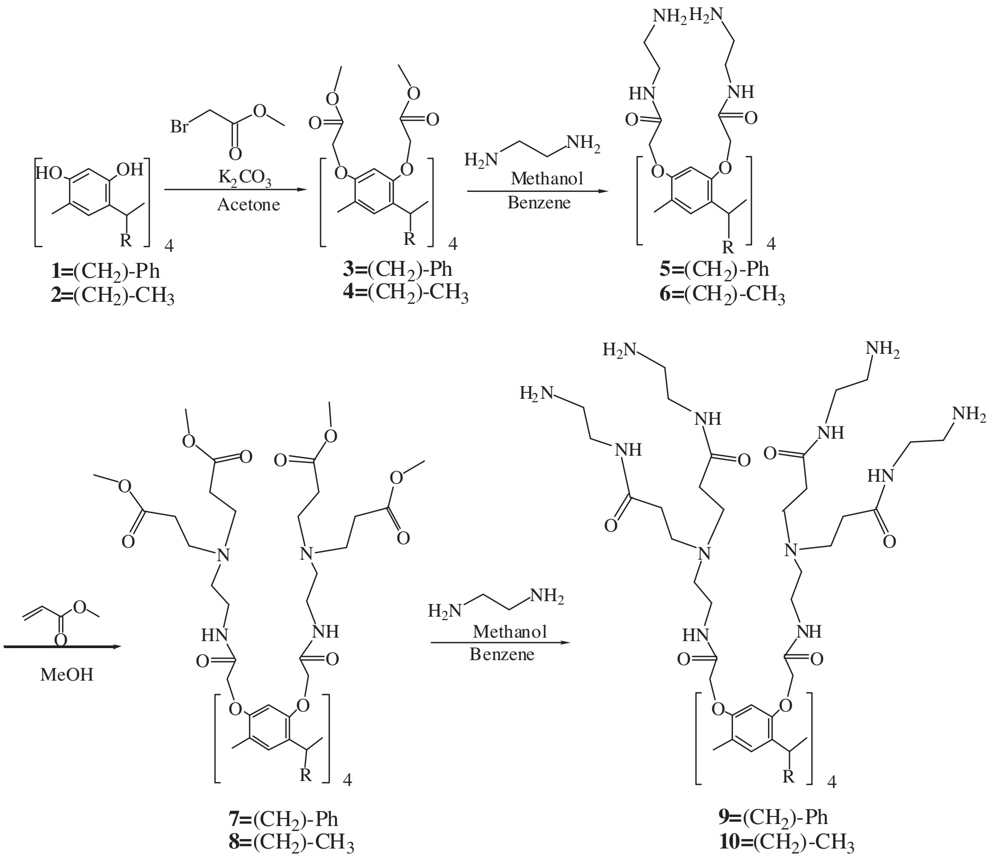
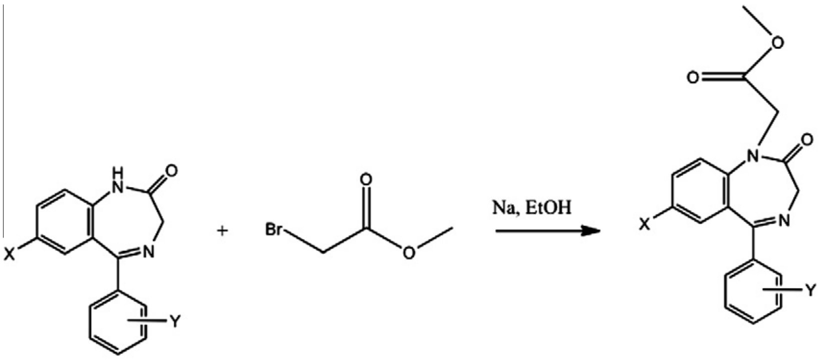


Scheme 1. Synthesis of the resorcinaren-PAMAM dendrimers first and second generation.



X= Cl	Y = H	**11** (61%)
X= Cl	Y = ortho Cl	**12** (62%)
X= Cl	Y = ortho F	**13** (75%)
X= NO_2	Y = H	**14** (85%)

Scheme 2. Synthesis of the benzodiazepine derivatives.

G-1.5 was dried in vacuum obtaining compounds **7** and **8**. Finally the compounds **7** and **8** were reacted with an excess of 1,2-diaminoethane in a mixture of methanol and benzene 1:1 for 24 h resulting in the corresponding second generation amide dendrimers **9** and **10** (Scheme 1).

It should be pointed out that compounds **5**, **6**, **9** and **10** have good solubility in most organic solvents. In the IR spectra of amides **5** and **6** the absorption band of C=O appears at 1675 cm^{-1}, while the absorption band of C=O in the ester derivatives **3** and **4** appears at 1750 cm^{-1}, which indicates that all the ethyl ester groups were transformed into amide groups.

The 5-aryl-1,4-benzodiazepines (**11–14**) were synthesized in agreement with the literature procedure[22] and modified in N1 with MeO^{-} Na^{+} and methyl bromoacetate (Scheme 2).

The structure of compound (**11**) was confirmed by X-ray diffraction analysis of a single crystal prepared by crystallization from chloroform. The general view of compound (**11**) is shown in Figure 1.

Finally the benzodiazepine derivatives were attached to the resorcinaren-PAMAM dendrimers in methanol/benzene 1:1 at reflux for 3 days, the compounds **19–34** were washed with acetone and methanol to remove the excess of benzodiazepine (Scheme 3).

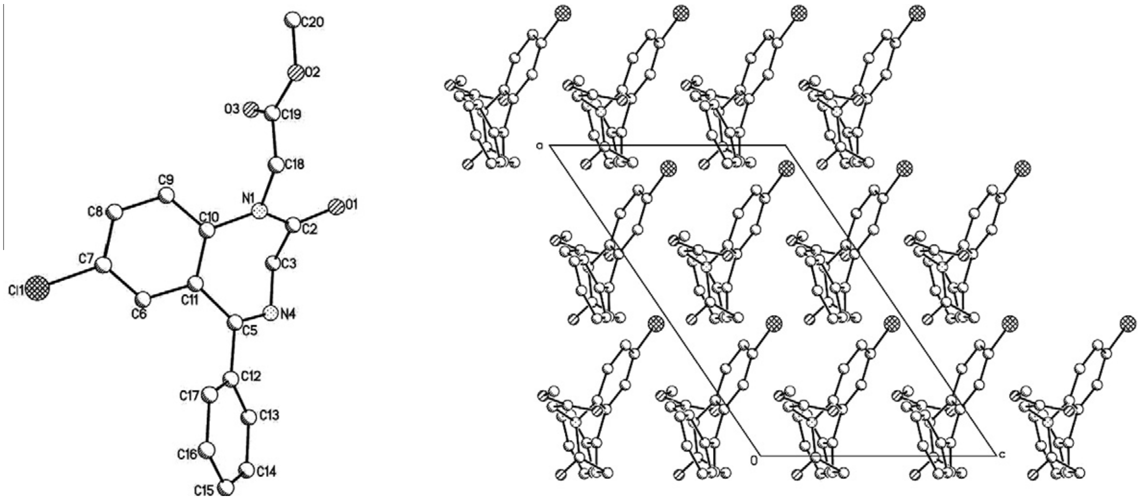
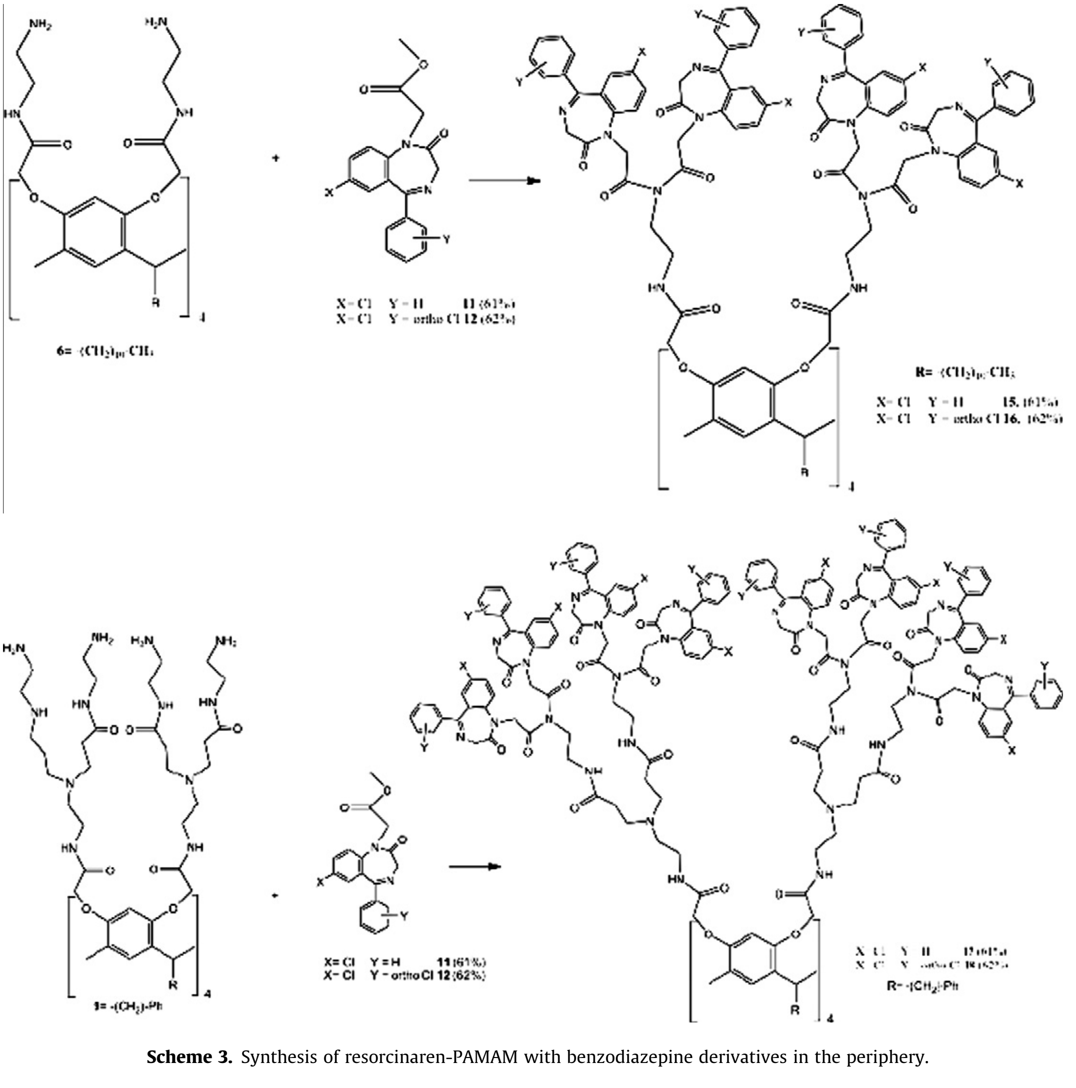


Figure 1. Crystal structure and crystal packing of compound **11**. Selected bond lengths (Å): C(1)–C(7) 1.736(2); N(1)–C(18) 1.458(3); N(1)–C(18) 1.458(3); N(1)–C(2) 1.367(3); C(5)–C(11) 1.486(3); C(5)–C(12) 1.499(3); Cl(1)–C(7) 1.736(2). Selected bond angles (°): C(11)–C(5)–C(12) 118.83(17); N(4)–C(5)–C(11) 124.53(17); C(2)–N(1)–C(10) 123.91(16); C(2)–N(1)–C(18) 115.44(17).



Scheme 3. Synthesis of resorcinaren-PAMAM with benzodiazepine derivatives in the periphery.

Table 1
Inhibition on the growth (%) of human tumor cell lines for (**1**, **2**, **5**, **6**, **9**, **10–18**) at 50 μM in DMSO

Cell line	U251	PC-3	K256	HCT-15	MCF-7	SKLU-1
Compound						
1	47.57	5.4	60.78	14.73	21.46	14.85
2	11.80	1.78	17.67	—	8.54	6.01
5	37.00	28.96	35.33	34.91	33.21	30.92
6	18.05	—	11.19	31.54	—	16.24
9	—	—	—	—	—	—
10	56.52	38.54	100	91.27	91.98	100
11	11.72	12.30	19.71	12.0	19.29	13.23
12	18.6	12.83	15.92	12.9	11.8	12.50
13	20.72	22.41	27.23	26.0	24.5	19.8
14	33.72	22.33	30.62	25.2	22.2	27.5

Table 2
Inhibitory concentration (IC_{50}) (μM) values obtained in PC-3, HCT-15 and SKLU-1 cell lines for compounds (**15–18**) in DMSO

Dendrimers	PC-3	HCT-15	SKLU-1
15	38.54 ± 4.60	35.43 ± 2.50	39.71 ± 2.30
16	15.40 ± 2.30	18.85 ± 0.75	15.69 ± 0.24
17	20.86 ± 1.60	23.62 ± 1.90	22.64 ± 1.00
18	40.84 ± 1.60	33.52 ± 1.90	32.44 ± 1.00
cis-Platin	15.20 ± 1.40	13.83 ± 0.70	7.13 ± 0.20

2.1. Cytotoxic activity

From the series of synthesized compounds, resorcinarenes **1** and **2**, as well as the resorcinaren-dendrimers of first and second generation **5**, **6**, **9**, **10**, were tested for anticancer activity and were screened in vitro against five human cancer cell lines. U251 (human glyoblastoma), PC-3 (human prostatic adenocarcinoma), K562 (human chronic myelogenous leukemia cells), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma) and SKLU-1 (human lung adenocarcinoma). Cell lines were supplied by the National Cancer Institute (USA). The human tumor cytotoxicity was determined using the protein-binding dye sulforhodamine B (SRB) in microculture assay to measure cell growth, as described in the protocols established by the NCI (Monks et al., 1991).[23] The cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 10,000 units/ml penicillin G sodium, 10,000 μg/ml streptomycin sulfate and 25 μg/ml amphotericin B (Gibco) and 1% non-essential amino acids (Gibco). They were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The viability of the cells used in the experiments exceeds 95% as determined with trypan blue. The initially obtained cytotoxic screening data (Table 1) shows that they have significant anticancer activities, especially compounds **6**, **9**, **11** and **12**, and that their activity depends on the nature of the human cancer cell line.

The inhibitory concentration (IC_{50}) values were only determined for those lines (PC-3, HCT-15 and SKLU-1), for four resorcinaren-PAMAM with benzodizepine derivatives samples, and were compared to the results obtained with the *cis*-platin cytotoxic agent (Table 2). For the three selected cell lines, the first generation dendrimer **6** and the second generation dendrimer **9** showed good activity and in the case of the benzodiazepine derivatives **11**, **12** showed promising activity as well. The first generation dendrimer with the benzodiazepine attached (**16**) was found to be the most potent among the benzodiazepines studied, exhibiting the lowest inhibitory concentrations (15.40 ± 2.3, 18.85 ± 0.75 and 15.69 ± 0.24 μM, Table 2). The comparison of the activity of chloro-derivative (dendrimer **16**) with that of the corresponding hydrogen-derivative (dendrimer **15**) indicates that the presence of chloro in the second ring of the benzodiazepines leads to better results in inhibition of cancer cell proliferation. In the case of the second generation dendrimers **17** and **18**, the dendrimer **18** was the most active.

The results of the present experiments clearly indicate that compounds **15–18** inhibit, in all cell lines, the proliferation of cancer cells. The rank order of potency was: **16** > **17** > **18** > **15**, at the concentration assayed. Dendrimers **16** and **18** emerged as the most promising PC-3, HCT-15 and SKLU-1 inhibitors. In summary, these results reveal that this class of resorcinaren-PAMAM with benzodiazepine derivatives in the periphery gives rise to an inhibitory growth of cancerous cells, although further investigation is warranted to establish the mechanism of inhibitory activity.

3. Conclusions

In the present work it was observed that resorcinaren-PAMAM with benzodiazepine derivatives in the periphery can be obtained in good yields. Biological activity tests showed that the synthesized resorcinaren-PAMAM with benzodiazepine derivatives compounds have potential activity against cancer. Inhibition of cancer cell proliferation was enhanced in the presence of a chloro-substituent in the second ring of the 5-aryl 1,4-benzodiazepines.

4. Experimental section

4.1. Materials and equipments

Solvents and reagents were purchased as reagent grade and used without further purification. Ether and toluene were distilled from sodium and benzophenone, acetone was distilled over calcium chloride, and methanol was distilled over calcium oxide powder. Column chromatography was performed on Merck Silica Gel 60 Å (70–230 mesh). ^1H and ^{13}C NMR spectra were recorded on a Varian Unity-300 MHz with tetramethylsilane (TMS) as an internal reference. Infrared (IR) spectra were measured on a Nicolet FT-SSX spectrophotometer. Elemental analysis was determined by Galbraith Laboratories, INC Knoxville.

4.2. General procedure for preparation of dendrimers, generation 0.5

A mixture of the methyl bromoacetate (1 mmol), potassium carbonate (3 mmol) in dry acetone (50 mL) was heated to reflux and stirred vigorously under an N_2 atmosphere for 20 min. The compounds **1** or **2** (0.2 mmol) dissolved in dry acetone (40 mL) were added drop wise and the reaction was continued for 7 days. The mixture was cooled and the precipitate filtered. The filtrate was evaporated to dryness under pressure. The residue dissolved in diethyl ether was washed with an aqueous solution of 5% Na_2CO_3 (three times). The organic layer was dried and evaporated to obtain the esters **3** and **4**.

4.2.1. Compound 3

Yield: 93%. Brown solid. mp: >300 °C. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 2.20 (br, 8H, CH_2), 2.66 (br, 8H, CH_2), 3.75 (s, 24H, O–CH_3), 4.28 (s, 16H, CH_2–O), 4.72 (t, 4H, CH, $J = 7.0$ Hz), 6.23 (s, 4H, Ar), 6,76 (s, 4H, Ar), 7.06–7.15 (m, 20H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 34.3 (CH_2), 35.58 (CH_2), 36.7 (CH), 51.9 (O–CH_3), 66.9 (CH_2-O), 100.5 (Ar), 125.4 (Ar), 126.2 (Ar_{ipso}), 128.0 (Ar), 128.1 (Ar), 128.3 (Ar), 142.5 (Ar_{ipso}), 154.2 (Ar-O), 169.7 (C=O). MS (MALDI-TOF) m/z: 1480 (M^+). Anal. Calcd for $C_{84}H_{88}O_{24}$: C, 68.10; H, 5.99. Found: C, 68.10; H, 5.97.

4.2.2. Compound 4

Yield: 95%. Brown solid. mp: >300 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 0.86 (t,12H, CH_3, $J = 6.8$ Hz), 1.23 (s, 56H, CH_2), 1.29 (s, 16H, CH$_2$), 1.83 (m, 8H, CH$_2$), 3.75 (s, 24H, O–CH$_3$), 4.27 (s, 16H, CH$_2$–O), 4.25 (t, 4H, CH, *J* = 7.5 Hz), 6.21 (s, 4H, Ar), 6.61 (s, 4H, Ar); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C: 14.0 (CH$_3$), 22.6 (CH$_2$), 28.0 (CH$_2$), 29.3 (CH$_2$), 29.6 (CH$_2$), 29.7 (CH$_2$), 29.8 (CH$_2$), 29.9 (CH$_2$), 31.9 (CH$_2$), 34.5 (CH), 51.8 (CH$_3$-O), 67.1 (CH$_2$-O), 100.8 (Ar), 126.5 (Ar), 128.5 (Ar$_{ipso}$), 154.4 (Ar-O), 169.7 (C=O). MS (MALDI-TOF) *m/z*: 1681 (M$^+$). Anal. Calcd for $C_{96}H_{144}O_{24}$: C, 68.54; H, 8.63. Found: C, 68.52; H, 8.60.

4.3. General procedure for preparation of dendrimers, Generation 1.0 and 2.0

A mixture of ester-containing derivatives **3**, 4, **7** and 8 (1.0 mmol) and 1,2-diaminoethane (15.0 mL) in ethanol (15 mL) and toluene (15 mL) were refluxed for 24 h in an atmosphere of nitrogen. The organic solvent and the excess 1,2-diaminoethane were removed in vacuo. The residue was washed several times with alcohol to obtain the amide products **5**, **6**, **9** and **10**.

4.3.1. Compound 5

Yield: 90%. Brown solid. mp: >300 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ_H: 1.96 (br, 16H, CH$_2$), 2.89 (s,16H,CH$_2$–NH$_2$), 3.39 br, 16H, CH$_2$–NH), 4.37 (br, 16H, CH$_2$–O), 4.47 (br, 16H, NH$_2$), 4.68 (t, 4H, CH, *J* = 7.0 Hz), 6.44 (s, 4H, Ar), 6,51 (s, 4H, Ar), 7.06–7.22 (m, 20H, Ar); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C: 30.9 (CH$_2$), 34.5 (CH$_2$), 37.2 (CH), 40.5 (CH$_2$–NH$_2$), 41.2 (CH$_2$–NH), 68.5 (CH$_2$–O), 101.2 (Ar), 125.7 (Ar), 127.2 (Ar$_{ipso}$), 128.4 (Ar), 128.6 (Ar), 141.6 (Ar$_{ipso}$), 147.9 (Ar-O), 162.3 (C=O). MS (MALDI-TOF) *m/z*: 1704 (M$^+$). Anal. Calcd for $C_{92}H_{120}N_{16}O_{16}$: C 64.77, H 7.09; N, 13.14. Found: C, 64.74; H, 7.11; N, 13.12.

4.3.2. Compound 6

Yield: 88%. Brown solid. mp: >300 °C. ^{1}H NMR (200 MHz, CDCl$_3$) δ_H: 0.86 (t, 12H, CH$_3$, *J* = 6.2 Hz), 1.21 (br, 72H, CH$_2$), 1.95 (br, 8H, CH$_2$), 2.9 (br, 16H, CH$_2$–NH$_2$), 3.4 (s, 16H, CH$_2$–NH), 4.58 (s, 16H, CH$_2$–O), 4.71 (t, 4H, CH, *J* = 7.0 Hz), 5.89 (br, 4H, NH$_2$), 6.49 (s, 4H, Ar), 7.25 (s, 4H, Ar); ^{13}C NMR: (75 MHz, CDCl$_3$) δ_C: 14.0 (CH$_3$), 22.6 (CH$_2$), 29.3 (CH$_2$), 29.7 (CH$_2$), 29.8 (CH$_2$), 31.9 (CH$_2$), 34.5 (CH), 37.3 (CH$_2$–NH$_2$), 41.2 (CH$_2$–NH), 68.1 (CH$_2$–O), 99.0 (Ar), 126.8 (Ar), 129.5 (Ar$_{ipso}$), 154.4 (Ar-O), 168.6 (C=O). MS (MALDI-TOF) *m/z*: 1905 (M$^+$). Anal. Calcd for $C_{104}H_{176}N_{16}O_{16}$: C, 65.51; H, 9.30; N, 11.75. Found: C, 65.49; H, 9.32; N, 11.72.

4.3.3. Compound 9

Yield: 89%. Brown solid. mp: >300 °C: ^{1}H NMR (300 MHz, CDCl$_3$) δ_H: 2.34 (br, 8H, CH$_2$), 2.59 (br, 16H, CH$_2$–N), 2.74 (br, 8H, CH$_2$), 2.97 (s, 32H, CH$_2$–NH$_2$), 3.01 (s, 32H, CH$_2$–NH), 3.10 (br, 32H, N–CH$_2$), 3.23 (br, 32H, CH$_2$–C=O), 3.46 (br, 16H, CH$_2$–NH), 4.44 (br, 16H, CH$_2$–O), 4.80 (t, 4H, CH, *J* = 7.0 Hz), 6.43 (s, 4H, Ar), 6.50 (s, 4H, Ar), 7.06–7.22 (m, 20H, Ar); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C: 32.2 (N–CH$_2$), 33.9 (CH$_2$), 34.4 (CH$_2$), 37.2 (CH), 40.9 (CH$_2$–NH), 41.7 (CH$_2$–NH$_2$), 43.6 (CH$_2$–NH), 45.6 (CH$_2$–N), 52.2 (N–CH$_2$), 67.2 (CH$_2$–O), 101.2 (Ar), 125.9 (Ar), 126.7(Ar$_{ipso}$), 127.4 (Ar), 128.4 (Ar), 141.8 (Ar$_{ipso}$), 154.9 (Ar-O), 169.3 (C=O), 173.6 (C=O). MS (MALDI-TOF) *m/z*: 3530 (M$^+$). Anal. Calcd for $C_{172}H_{280}N_{48}O_{32}$: C, 58.48; H, 7.99; N, 19.03. Found: C, 58.44; H, 7.95; N, 19.92.

4.3.4. Compound 10

Yield: 89%. Brown solid. mp: >300 °C: ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H: 0.87 (s, 12H, CH$_3$), 1.23 (s, 80H, CH$_2$), 2.36 (br, 16H, CH$_2$–N), 2.77 (br, 32H, CH$_2$–NH$_2$), 3.02 (br, 32H, CH$_2$-NH), 3.27 (br, 32H, CH$_2$–C=O), 3.36 (br, 16H, CH$_2$–NH), 4.57 (br, 16H, CH$_2$–O), 4.71 (t, 4H, CH, *J* = 7.0 Hz), 5.96 (br, 32H, NH$_2$), 6.37 (s, 4H, Ar), 7.25 (s, 4H, Ar); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C: 14.1 (CH$_3$), 22.7 (CH$_2$), 29.5 (CH$_2$), 29.8 (CH$_2$), 29.9 (CH$_2$), 31.2 (CH$_2$), 34.0 (CH), 37.6 (CH$_2$–NH), 41.1(CH$_2$–NH$_2$), 41.9 (CH$_2$–NH), 50.4 (CH$_2$–N), 52.5 (CH$_2$–C=O), 68.9 (CH$_2$–O), 96.4 (Ar), 124.4 (Ar), 127.1 (Ar$_{ipso}$), 154.7 (Ar-O), 169.2 (C=O), 173.7 (C=O). MS (MALDI-TOF) *m/z*: 3730 (M$^+$). Anal. Calcd for $C_{184}H_{336}N_{48}O_{32}$: C, 59.20; H, 9.07; N, 18.01. Found: C, 59.23; H 9.10; N, 18.01.

4.4. General procedure for preparation of dendrimers, generation 1.5

A mixture of each of the amides **5** and **6** (0.5 mmol) and methyl acrylate (15 mL) in methanol (15 mL) was stirred in an atmosphere of nitrogen at 45–50 °C for 5 d. Methanol and excessive methyl acrylate were removed in vacuo. The residue was crystallized from alcohol/ether to obtain the ester compounds **7** and **8**.

4.4.1. Compound 7

Yield: 78%. Brown solid. mp: >300 °C: ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H: 2.04 (s, 8H, CH$_2$), 2.39 (br, 16H, CH$_2$–N), 2.57 (s, 8H, CH$_2$), 2.73 (s,16H,CH$_2$–NH), 3.10 (br, 32H, CH$_2$–C=O), 3.49 (br, 32H, N–CH$_2$), 3.58 (s, 48H, –OCH$_3$), 4.42 (br, 16H, CH$_2$–O), 4.46 (t, 4H, CH, *J* = 7.0 Hz), 5.12 (br, 8H, NH), 6.54 (s, 4H, Ar), 6.67 (s, 4H, Ar), 6.9–7.22 (m, 20H, Ar); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C: 32.1 (CH$_2$), 32.5 (CH$_2$), 32.8 (CH$_2$–C=O), 34.2 (CH), 37.1 (CH$_2$–NH), 49.2 (N–CH$_2$–), 51.5 (O–CH$_3$), 52.7 (CH$_2$–N), 68.4 (CH$_2$–O), 101.2 (Ar), 125.0 (Ar), 125.2 (Ar$_{ipso}$), 128.3 (3-Ar), 141.8 (Ar$_{ipso}$), 154.2 (Ar-O),167.9 (C=O, 1), 172.9 (C=O, 2). MS (MALDI-TOF) *m/z*: 3081 (M$^+$). Anal. Calcd for $C_{156}H_{216}N_{16}O_{48}$: C, 60.77; H, 7.06; N, 7.27. Found: C, 60.78; H, 7.05; N, 7.25.

4.4.2. Compound 8

Yield: 75%. Brown solid. mp: >300 °C: ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H: 0.83 (t, 12 H, CH$_3$, *J* = 7.2 Hz), 1.20 (s, 16H, CH$_2$), 1.25 (s, 40H, CH$_2$), 1.34 (s, 24H, CH$_2$), 2.40 (br, 32H, CH$_2$–C=O), 2.60 (br, 16H, CH$_2$–N), 2.75 (br, 32H, N–CH$_2$), 3.50 (br, 16H, CH$_2$–NH), 3.62 (s, 48H, –OCH$_3$), 4.5 (br, 16H, CH$_2$–O), 4.69 (br, 4H, CH), 6.97 (s, 4H, Ar), 7.18 (s, 4H, Ar); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C: 14.1 (CH$_3$), 22.6 (CH$_2$), 28.6 (CH$_2$), 29.4 (CH$_2$), 29.7 (CH$_2$), 29.9 (CH$_2$), 30.3 (CH$_2$), 31.9 (CH$_2$–C=O), 32.6 (CH$_2$), 36.7 (CH$_2$-NH), 37.2 (CH), 49.3 (N–CH$_2$), 51.54 (–OCH$_3$), 52.7 (CH$_2$–N), 67.9 (CH$_2$–O), 98.1 (Ar), 125.8 (Ar), 128.3 (Ar$_{ipso}$), 155.4 (Ar-O), 169.7 (C=O, 1), 173.2 (C=O, 2). MS (MALDI-TOF) *m/z*: 3281 (M$^+$). Anal. Calcd for $C_{168}\ H_{272}N_{16}\ O_{48}$: C, 61.44; H, 8.35; N, 6.82. Found: C, 61.41; H, 8.37; N, 6.84.

4.5. General procedure for preparation of benzodiazepine derivatives

To a solution of 1,4-benzodiazepines **11**–**14** (7.9 mmol) in 50 mL of anhydrous methanol, NaMeOH (9.8 mmol) was added, the mixture was stirred for 30 min. Afterwards, was cooled at 10 °C and methyl bromoacetate (9.8 mmol) was added, the reaction was stirred for 4 h. The solvent was evaporated under vacuum and the product was chromatographed in a mixture of hexanes/ethyl acetate 6:4.

4.5.1. Methyl-2-(7-chloro-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-1-yl) acetate 11

Yield: 58%. White solid. mp: 134–135 °C. ^{1}H NMR: (300 MHz, CDCl$_3$) δ_H: 3.71 (s, 3H, O–CH$_3$), 3.87 (d, 1H, CH$_2$, *J* = 10.8 Hz), 4.55 (s, 2H, CH$_2$), 4.86 (d, 1H, CH$_2$, *J* = 10.8 Hz), 7.29 (d, 1H, Ar-9, *J* = 2.4 Hz), 7.43 (s, 1H, Ar-6), 7.44–7.46 (m, 2H, Ar-6′, 2′), 7.48–7.50 (m, 1H, Ar-4′), 7.51–7.53 (m, 2H, Ar-5′, 3′), 7.64 (d, 1H, Ar-8, *J* = 7.9 Hz); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C: 49.3 (CH$_2$), 52.6 (O–CH$_3$), 56.0 (CH$_2$), 123.0 (Ar-9), 128.5 (Ar-5′, 3′), 129.1 (Ar-7), 129.7 (Ar-6′, 2′), 130.1 (Ar-6), 130.5 (Ar-5a), 131.0 (Ar-4′), 131.9 (Ar-8), 137.7 (Ar-1′), 141.3 (Ar-9a), 168.9 (C=N), 169.0 (C=O), 169.7 (C═O). MS (IE) *m/z*: 342 (M^+). Anal. Calcd for $C_{18}H_{15}ClN_2O_3$: C, 63.07; H, 4.41; N, 8.17. Found: C, 63.06; H, 4.40; N, 8.15.

4.5.2. Methyl-2-(7-chloro-5-(2-chlorophenyl)-2-oxo-2, 3-dihydro-1*H*-benzo[*e*][1,4] diazepin-1-yl)acetate 12

Yield: 64%. White solid. mp: 113–114 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 3.72 (s, 3H, O–CH_3), 4.22 (d, 2H, CH_2, *J* = 11.5 Hz), 4.6 (s, 2H, CH_2), 7.12 (d,1H, Ar-9, *J* = 8.7 Hz), 7.37 (d, 1H, Ar-6, *J* = 2.4 Hz), 7.42–7.45 (m, 1H, Ar-3′), 7.47–7.49 (m, 1H, Ar-4′), 7.52–7.53 (m, 1H, Ar-5′), 7.55–7.58 (m, 1H, Ar-8), 7.63–7.65 (m, 1H, Ar-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 50.1 (CH_2), 52.5 (–OCH_3), 56.8 (CH_2), 122.8 (Ar-9), 127.0 (Ar-5′), 129.0 (Ar-5a), 129.2 (Ar-7), 129.3 (Ar-6), 130.2 (Ar-3′), 131.0 (Ar-4′, Ar-6′), 131.9 (Ar-8), 133.2 (Ar-2′), 136.6 (Ar-1′), 138.3 (Ar-9a), 169.4 (C═N), 171.3 (C═O), 173.2 (C═O). MS (IE) *m/z*: 376 (M^+). Anal. Calcd for $C_{18}H_{14}Cl_2N_2O_3$: C, 57.31; H, 3.74; N, 7.43. Found: C, 57.30; H, 3.73; N, 7.44.

4.5.3. Methyl-2-(7-chloro-5-(2-fluorophenyl)-2-oxo-2, 3-dihydro-1*H*-benzo[*e*][1,4] diazepin-1-yl)acetate 13

Yield: 70%. Brown solid. mp: 114–115 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 3.76 (s, 3H, O-CH_3), 3.89 (dd, 1H, CH_2, *J* = 11.8 Hz), 4.53 (s, 2H, CH_2), 4.92 (d, 1H, CH_2, *J* = 11.8 Hz), 7.05 (d, 1H, Ar-3′, *J* = 0.9 Hz), 7.18 (d, 1H, Ar-9, *J* = 2.4 Hz), 7.25 (d, 1H, Ar-5′, *J* = 4.6 Hz), 7.43 (s, 1H, Ar-6), 7.45–7.48 (m, 1H, Ar-4′), 7.67 (d, 1H, Ar-8, *J* = 1.8 Hz), 7.70 (d, 1H, Ar-6′, *J* = 1.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 49.7 (CH_2), 52.6 (O-CH_3), 56.5 (CH_2), 116.4 (Ar-3′), 122.8 (Ar-9), 124.5 (Ar-5′), 126.6 (Ar-1′), 126.7 (Ar-5a), 130.4 (Ar-6), 131.3 (Ar-6′), 131.4 (Ar-7), 131.6 (Ar-8),132.4 (Ar-4′), 140.2 (Ar-9a), 162.15 (Ar-2′), 166.0 (C═N), 168.7 (C═O), 169.1 (C═O). MS (IE) *m/z*: 360 (M^+). Anal. Calcd for $C_{18}H_{14}ClFN_2O_3$: C, 59.93; H, 3.91; N, 7.76. Found: C, 59.91; H, 3.92; N, 7.75.

4.5.4. Methyl-2-(7-nitro-2-oxo-5-phenyl-2,3-dihydro-1 *H*-benzo[*e*][1,4]diazepin-1-yl) acetate 14

Yield: 70%. Brown solid. mp: 120–121 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 3.75 (s, 3H. O–CH_3), 4.22 (d, 1H, CH_2, *J* = 9.4 Hz), 5.28 (s, 2H, CH_2), 6.57 (d, 1H, CH_2, *J* = 9.4 Hz), 7.14 (d, 1H, Ar-9, *J* = 2.2 Hz), 7.48–7.50 (m, 2H, Ar-3′, 5′), 7.51–7.53 (m, 1H, Ar-4′), 7.53–7.56 (m, 2H, Ar-2′, 6′), 8.10 (s, 1H, Ar-6), 8.14 (d, 1H, Ar-8, *J* = 2.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 49.7 (CH_2), 52.6 (O-CH_3), 56.5 (CH_2), 116.4 (Ar-3′), 122.8 (Ar-9), 124.5 (Ar-5′), 126.6 (Ar-1′), 126.7 (Ar-5a), 130.4 (Ar-6), 131.3 (Ar-6′), 131.4 (Ar-7), 131.6 (Ar-8), 132.4 (Ar-4′), 140.2 (Ar-9a), 162.15 (Ar-2′), 166.0 (C═N), 168.7 (C═O), 169.1 (C═O). MS (IE) *m/z*: 353 (M^+). Anal. Calcd for $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89. Found: C, 61.17; H, 4.27; N, 11.87.

4.6. Dendrimers with benzodiazepines in the periphery

To a solution of dendrimers **5**, **6**, 9 and **10** (0.5 mmol) in methanol (15 mL), the benzodiazepine ester **11**–**14** (0.5 mmol) in methanol (15 mL) were added, the mixture was stirred in an atmosphere of nitrogen at 45–50 °C for 5 d. The methanol was removed in vacuo. The residue was washed several times with methanol, after with CH_2Cl_2 to obtain desired products **15**–**18**.

4.6.1. First generation

4.6.1.1. Dendrimer 15. Yield: 84%. White solid. mp: >300 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 0.82 (s, 12H, CH_3), 1.03 (s, 40H, CH_2), 1.25 (s, 40H, CH_2), 3.21 (br, 16H, CH_2–NH), 3.38 (br, 16H, CH_2–N), 3.70 (s, 16H, CH_2–O), 3.9 (d, 16H, CH_2, Bz, *J* = 10.8 Hz), 4.6 (d, 32H, CH_2, Bz, *J* = 4.8 Hz), 4.8 (d, 16H, CH_2, Bz, *J* = 10.8 Hz), 7.09 (br, 4H, Ar), 7.14 (br, 4H, Ar), 7.26 (d, 16H, Ar-9 Bz, *J* = 2.4 Hz), 7.32–7.35 (m, Ar-2′,6′ Bz), 7.41–7.45 (m, 16H, Ar-3′,5′ Bz), 7.49–7.51 (m, 16H, Ar-4′ Bz), 7.53–7.56 (m, 16H, Ar-6 Bz), 7.58–7.60 (m, 16H, Ar-8 Bz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 14.1 (CH_3), 22.8 (CH_2), 29.5 (CH_2), 29.8 (CH_2), 29.9 (CH_2), 30.2 (CH_2), 32.1 (CH_2), 34.7 (CH), 37.2 (CH_2–NH), 40.1 (CH_2–N), 52.9 (CH_2 Bz), 56.6 (CH_2 Bz), 67.5 (CH_2-O), 123.8 (Ar, Ar-9 Bz), 126.3 (Ar-*ipso*, Ar-5a Bz), 128.4 (Ar-6 Bz), 128.6 (Ar-3′,5′ Bz), 128.7 (Ar-2′,6′ Bz), 128.9 (Ar-7 Bz), 130.6 (Ar-8 Bz), 131.3 (Ar-4′ Bz), 138.4 (Ar-1′), 141.6 (Ar-O), 142.5 (Ar-9a Bz), 169.6 (C═N, C═0 Bz), 169.8(C═O Bz), 169.9 (C═O). MS (MALDI-TOF) *m/z*: 6903.17 (M+Na). Anal. Calcd for $C_{376}H_{352}Cl_{16}N_{48}O_{48}$: C, 65.66; H, 5.16; N.9.77. Found: C, 60.68; H, 5.17; N, 9.76.

4.6.1.2. Dendrimer 16. Yield: 84%. White solid. mp: >300 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 0.88 (t, 12H, CH_3, *J* = 6.2 Hz), 1.25 (br, 72H, CH_2), 1.95 (br, 8H, CH_2), 3.02 (br, 16H, CH_2–NH), 3.54 (s, 16H, CH_2–N), 3.86 (d, 16H, CH_2 Bz, *J* = 10.9 Hz), 4.35 (s, 16H, CH_2–O), 4.63 (s, 32H, CH_2 Bz), 4.80 (d, 16H, CH_2 Bz, *J* = 10.9 Hz), 6.25 (s, 4H, Ar), 6.84 (s, 4H, Ar), 7.14 (d, 16H, Ar-9 Bz, *J* = 8.7 Hz), 7.37 (d, 16H, Ar-6 Bz, *J* = 2.4 Hz), 7.45–7.49 (m, 16H, Ar-3′ Bz), 7.52–7.56 (m,16H, Ar-4′ Bz), 7.57–7.59 (m, 16H, Ar-5′ Bz), 7.63–7.66 (m, 16H, Ar-8 Bz), 7.70–7.72 (m, 16H, Ar-6′ Bz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 14.1 (CH_3), 22.7 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 34.5 (CH), 36.1 (CH_2–NH), 40.9 (CH_2–N), 52.3 (CH_2 Bz), 57.1 (CH_2 Bz), 67.4 (CH_2–O), 98.2 (Ar), 122.8 (Ar-9), 125.5 (Ar), 126.6 (Ar-5a), 127.0 (Ar-5′), 127.8 (Ar_{ipso}), 129.2 (Ar-7), 129.4 (Ar-6), 130.1 (Ar-3′), 131.0 (Ar-4′, Ar-6′), 131.4 (Ar-8), 133.2 (Ar-2′), 137.4 (Ar-1′), 138.3 (Ar-9a), 156.4 (Ar-O), 167.2 (C═N, C═O Bz), 168.3 (C═O), 169.3 (C═O). MS (MALDI-TOF) *m/z*: 7411.55 (M+). Anal. Calcd for $C_{376}H_{336}Cl_{32}N_{48}O_{48}$: C, 60.79; H, 4.56; N, 9.05. Found: C, 60.78; H, 4.55; N, 9.05.

4.6.2. Second generation

4.6.2.1. Dendrimer 17. Yield: 89%. Brown solid. mp: >300 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 2.38 (br, 8H, CH_2), 2.61 (br, 8H, CH_2), 2.8 (br, 16H, CH_2–N), 3.05 (s, 32H, CH_2–NH), 3.12 (s, 32H, CH_2–NH), 3.20 (br, 32H, CH_2–C═O), 3.41 (br, 32H, CH_2–NH), 3.9 (d, 32H, CH_2, *J* = 10.8 Hz), 4.54 (br, 16H, CH_2–O), 4.60 (d, 64H, CH_2-Bz, *J* = 4.8 Hz), 4.77 (d, 32H, CH_2, *J* = 10.8 Hz), 4.80 (t, 4H, CH, *J* = 7.0 Hz), 6.43 (s, 4H, Ar), 6.50 (s, 4H, Ar), 7.10–7.20 (m, 20H, Ar), 7.27 (d, 1H, Ar-9 Bz, *J* = 2.4 Hz), 7.33 (s, 2H, Ar-6′,2′ Bz), 7.36 (s, 1H, Ar-5′,3′ Bz), 7.45–7.50 (m, 2H, Ar-4′ Bz), 7.54 (d, 1H, Ar-6 Bz, *J* = 2.4 Hz), 7.60 (d, 1H, Ar-8 Bz, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 29.8 (CH_2), 29.9 (CH_2), 34.5 (CH), 37.8 (CH_2–NH), 40.1 (CH_2–N), 52.8 (CH_2-Bz, CH_2–N), 56.4 (CH_2–C═O, CH_2-Bz), 67.2 (CH_2–O), 101.2 (Ar), 123.2 (Ar-9 Bz), 123.5 (Ar), 126.1 (Ar-5a, Bz), 128.5 (Ar), 128.8 (Ar), 129.8 (Ar-5′,3′-Bz), 130.4 (Ar-6′,2′,4′-Bz), 130.8 (Ar-7-Bz), 131.2 (Ar_{ipso}), 131.3 (Ar-6 Bz), 132.4 (Ar-8 Bz), 138.3 (Ar-1′ Bz), 141.4 (Ar_{ipso}), 142.3 (Ar-9a, Bz), 157.9 (Ar-O), 169.5 (C═O, Bz, C═N),169.7 (C═O, 2, Bz), 170.1 (C═O, 1). MS (MALDI-TOF) *m/z*: 13,442.95 (M+). Anal. Calcd for $C_{716}H_{632}Cl_{32}N_{112}NaO_{96}$: C, 63.71; H, 4.72; N, 11.62. Found: C, 63.68; H, 4.69; N, 11.59.

4.6.2.2. Dendrimer 18. Yield: 87%. White solid. mp: >300 °C. ^1H NMR: (300 MHz, $CDCl_3$) δ_H: 2.38 (br, 8H, CH_2), 2.61 (br, 8H, CH_2), 2.25 (br, 16H, CH_2–N), 2.77 (br, 32H, CH_2–C═O), 2.92 (br, 32H, N–CH_2), 3.29 (br, 48H, CH_2–NH), 3.27 (br, 32H, CH_2–N), 3.77 (d, 32H, CH_2 Bz, *J* = 10.8 Hz), 4.49 (br, 16H, CH_2–O), 4.44 (d, 32H, CH_2, Bz *J* = 3.3 Hz), 4.72 (d, 64 H, CH_2 Bz, *J* = 10.8 Hz), 6.43 (s, 4H, Ar), 6.50 (s, 4H, Ar), 7.10–7.20 (m, 20H, Ar), 7.29–7.41 (m, 16H, Ar-2′, Ar-6′, Bz), 7.18 (d, 32H, Ar-9, Bz, *J* = 3.3 Hz), 7.48 (d, 32H, Ar-6, Bz, *J* = 1.8 Hz), 7.51 (s, 32H, Ar-8, Bz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 14.3 (CH_3), 22.8 (CH_2), 29.5 (CH_2), 29.8 (CH_2), 29.9 (CH_2), 30.2 (CH_2), 32.1 (CH_2), 34.0 (CH), 49.0 (CH_2–C═O), 49.3 (CH_2–NH), 49.5 (CH), 49.8 (CH_2–NH), 50.2 (CH_2–N), 50.5 (CH_2–N), 52.7 (N–CH_2, CH_2-Bz), 56.4 (CH_2-Bz), 56.7 (CH_2–O), 96.4 (Ar), 122.6 (Ar_{ipso}), 123.0 (Ar, Ar-9 Bz), 128.6 (Ar-3′,5′ Bz), 129.6 (Ar-6 Bz) 130.2(Ar-2′,6′ Bz), 130.4(Ar-7 Bz), 131.0 (Ar-8 Bz), 131.9 (Ar-4′ Bz), 138.1 (Ar-1′ Bz), 142.8 (Ar-9a Bz), 169.2 (C═N, C═0 Bz), 169.4 (C═O, 3, 2), 169.9 (C═O, 1). MS (ES) *m*/*z*: 14561.70 (Na+). Anal. Calcd for $C_{716}H_{600}Cl_{64}N_{112}NaO_{96}$: C, 58.90; H, 4.14; N, 10.74. Found: C, 58.93; H, 4.18; N, 10.76.

4.7. Crystal structure determination

A suitable crystal of compound **11** (obtained by crystallization from $CHCl_3$ at room temperature) was rolled in epoxy resin and mounted on a glass fiber. Bruker Apex AXS CCD area detector X-ray diffractometer was the instrument used for determination. The data were first reduced and corrected for absorption using psi-scans, and then solved using the program SHELL-XS. All non-hydrogen atoms were refined using the anisotropic thermal parameters and the hydrogen atoms were refined at calculated positions applying thermal parameters constrained to the carbon atom on which they were attached. CCDC 848697 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; (e-mail: deposit@ccdc.cam.ac.uk)

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