*Polish J. Chem.*, **77**, 333–340 (2003)

# 1:3 Complex between $\beta$-Cyclodextrin and Dendrimer with Three Branches Involving Four Glycine and One Adamantyl Group

**by H. Dodziuk[1*], O. Demchuk[2], W. Koźmiński[3] and G. Dolgonos[1]**

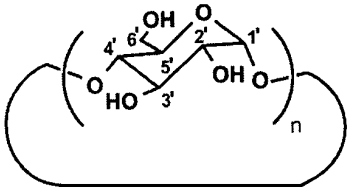
[1]*Institute of Physical Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Kasprzaka 44, Poland*
[2]*Institute of Organic Chemistry, Polish Academy of Sciences, 01-224 Warsaw, Kasprzaka 44, Poland*
[3]*Department of Chemistry, Warsaw University, 02-093 Warsaw, Pasteura 1, Poland*

*(Received October 29th, 2002; revised manuscript December 19th, 2002)*

The first-generation dendrimer, benzene-*sym*-tris-*N,N,N*-carbonyltriglycylglycine *N*′-1-adamantylamide, was synthesized by a modification of a described procedure. Its complexation with $\alpha$-, $\beta$- and $\gamma$-cyclodextrins was studied by NMR. The complexation induced fit and NOESY studies indicate that, in agreement with molecular mechanics calculations, the complex with $\beta$-cyclodextrin is considerably stronger than those with $\alpha$- and $\gamma$-cyclodextrins.

**Key words**: dendrimers, synthesis, cyclodextrin complexes, NMR

Dendrimers [1] with their compact structure and high end-group functionality have found application in medical diagnostics as contrast agents in magnetic resonance imaging, MRI, [2]. Several other biomedical applications of dendrimers such as DNA biosensors, drug delivery systems, antimicrobial agents and in boron neutron-capture terapy (used in the treatment of cancer) are intensively studied [2]. Therefore, studies of dendrimers containing amino acid moieties seem of value. Few dendrimers involving amino acids as end-groups [3] or in their branches [4] are known. The latter produced by Schering AG, Berlin, Germany are used in MRI diagnostics. To our best knowledge, only one dendrimer having amino acids in branches and adamantyl end-groups was reported [5].




**2** n = 6

**3** n = 7

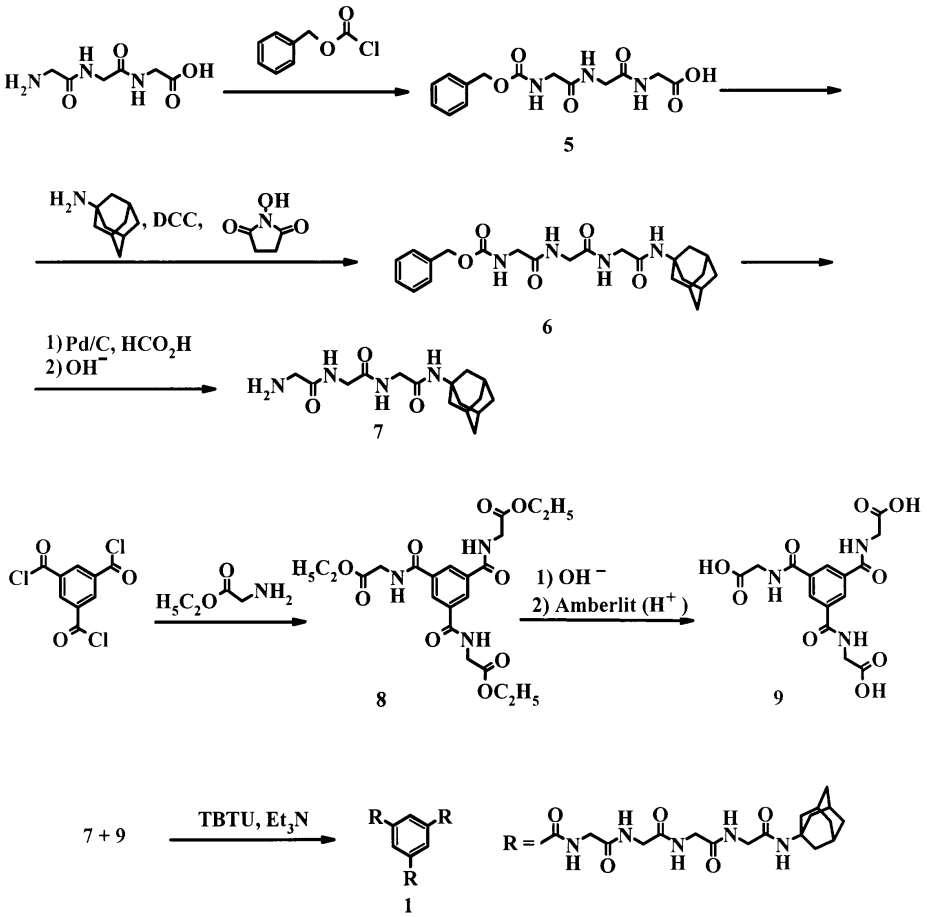
**4** n = 8

*Author for correspondence. E-mail:dodziuk@ichf.edu.pl

In this paper, the synthesis of first generation dendrimer **1** and manifestations of its complexation with $\alpha$-, $\beta$- and $\gamma$-cyclodextrins, CDs, **2–4**, respectively, in NMR spectra are reported.

## RESULTS AND DISCUSSION

**Synthesis.** The scheme of reactions leading to **1** is shown in Fig. 1. Compounds **1, 5-9** were synthesized using a modified peptide-coupling methodology. Details may be found in the Experimental Section. All but one intermediate products were utilized as pure materials. In the case of Z-Gly-Gly-Gly-NH-1-adamantane **6**, the 1:1 mixture of **6** with 1,3-dicyclohexylurea was used in view of a low yield of neat product **6** after recrystallization, and the simplicity of resolution of the reaction mixture after the next step. The atom numbering for these molecules is shown in Fig. 2.




TBTU = *o*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate

**Figure 1.** The scheme of the synthesis of dendrimer **1**.

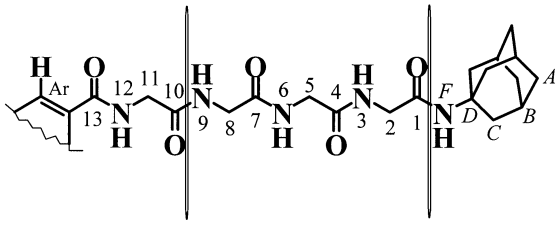



**Figure 2.** The scheme of atom numbering. The vertical lines divide fragments stemming from different molecules. Left side corresponds to **9**, the central part describes the numbering in triglycine residue and the right fragment shows 1-amino-adamantane end group.

**NMR complexation studies.** As expected, the complexation increased the solubility of **1** in water. A comparison of the signals of adamantyl protons of free guest **1** (Fig. 3a) with the corresponding signals of the complexes with **2–4** (Fig. 3b-d) clearly reveals small but definite complexation induced shifts, CIS. As shown in Fig. 4, small CIS were also detected in the carbon spectrum of the complex of **1** with **3** measured by the HSQC method [6]. The inclusion character of the complex with $\beta$-CD is unequivocally revealed by NOESY spectrum (Fig. 5), clearly exhibiting equally strong

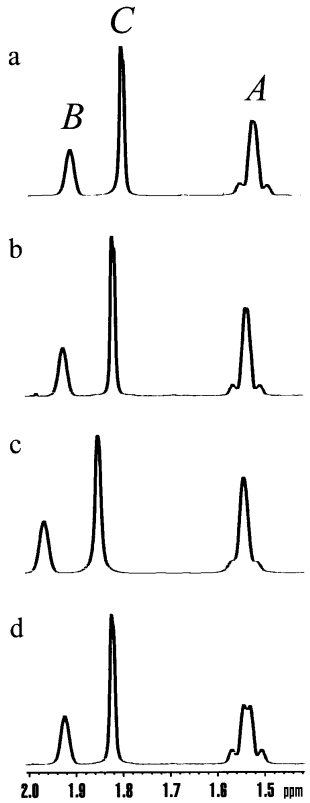



**Figure 3.** The adamantyl protons region in $^1$H NMR spectra of a) free dendrimer **1**, b–d) its complexes with $\alpha$- **2**, $\beta$- **3** and $\gamma$- **4** cyclodextrins, respectively.

cross-peaks between the adamantyl protons of the guest and H3 and H5 CD protons pointing inside the cavity. On the other hand, the cross peaks of the complex of **1** with $\gamma$-CD involving H3 protons are considerably weaker than those involving H5 ones. No cross-peaks were obtained for the complex with $\alpha$-CD **2**. Taking into account the results of molecular mechanics [7] modeling of the complexes, the above observations may be interpreted in the following way: the $\alpha$-CD cavity is too small to host the adamantyl group, thus, this group is located on the top of the macrocycle. Its size fits best the $\beta$-CD cavity and in this case the cross-peaks involving both H3 and H5 CD protons and all protons of the adamantyl group are of approximately equal intensity. On the other hand, the $\gamma$-CD seems to be slightly too big for this guest resulting in a deeper guest entrance into the CD cavity and a weaker complex with cross-peaks involving cyclodextrin H5 protons considerably stronger than those involving H3 ones.

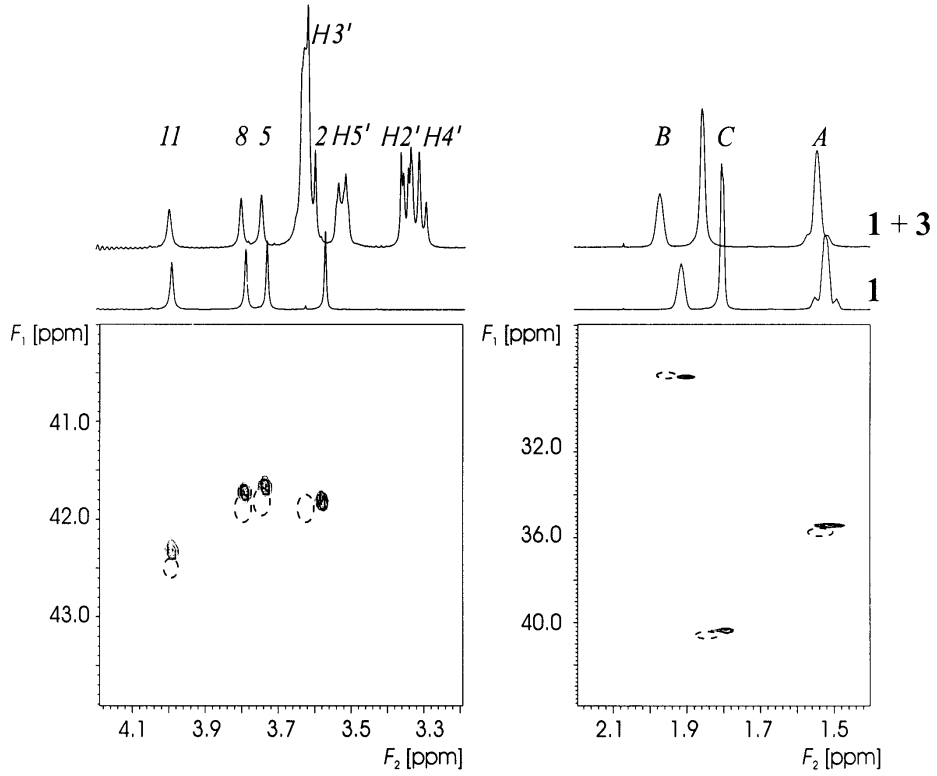



**Figure 4.** Expansion of two regions of HSQC spectra of free dendrimer **1** (solid shapes) and its complex with $\beta$-CD **3** (dash line).

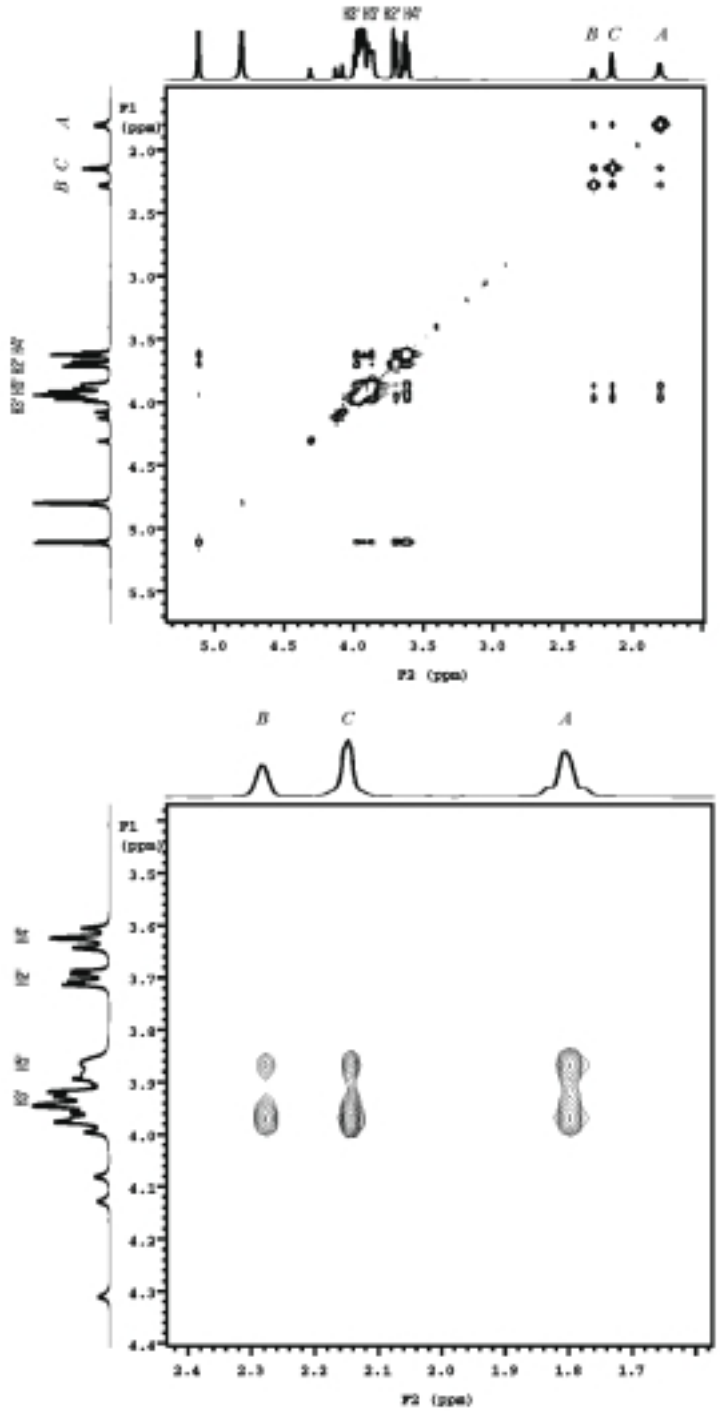



**Figure 5.** Two fragments of the NOESY spectrum of the complex between **1** and **3**.

## EXPERIMENTAL

**General:** Solvents and reagents were commercial materials (Sigma-Aldrich AG) purified according to standard procedures. All reactions were carried out with dry, freshly distilled solvents. Thin-layer chromatography was done on Merck pre-coated aluminium sheets of Silica gel 60 $F_{254}$ with the following solvent systems: $R_f^1$ $CHCl_3$-MeOH (3:1, v/v); $R_f^2$ $CHCl_3$-MeOH-$Et_3N$ (3:1:0.125, v/v). The compounds with a free amino group were detected on a thin-layer plate by spraying with ninhydrin. Products with a blocked amino group were detected with 25% hydrogen bromide in acetic acid and then ninhydrin or on the basis of UV fluorescence. Column chromatography was performed on Silica Gel (Merck 60, 0.04–0.063 mm). Solvents were evaporated under reduced pressure. NMR spectra of intermediate compounds were recorded with a Varian Gemini BB-2000 Fourier-transform (200 MHz, internal $Me_4Si$ or the residual solvent standards), while the complexation studies were carried out on 500 Varian Unity Plus spectrometer, using the ID_PFG probehead with actively shielded z-gradient coil. Mass spectra were recorded on: high resolution ESI MS-Mariner (PerSeptive Biosystem) and LSI MS-AMD 604 (AMD Intectra GmbH, Germany). All melting points are uncorrected.

***N*-Benzyloxycarbonyl-diglicylglycine (5)**: Z-Gly-Gly-Gly-OH (**5**) was synthesized from the free peptide, which was treated with carbobenzyloxy chloride under modified Schotten-Baumann conditions [8,9] usual for benzyloxycarbonylation of amino acids (0°C, pH controlled at 10–11 in aqueous-dioxane solution). Free peptide (0.005 mol) was dissolved in NaOH (1 M, 15 ml); then 10 ml of dioxane were added to this solution. The mixture was stirred in ice bath, and solutions of Z-Cl (0.01 mol) in dioxane (15 ml) and NaOH (1 M, 10 ml) were added dropwise while pH was maintained at above 10. Then the mixture was left stirred at 0°C overnight, dioxane was evaporated and the water solution was washed with ether (3×20 ml), cooled, and acidified with saturated $KHSO_4$ solution to pH 3. The crystalline product was collected by filtration, washed with cold water and the product was recrystallized from hot water; yield 1.15 g (71%), m.p. 200°C, reported [10] 195°C. $^1$H NMR (DMSO-$d_6$) $\delta_H$: 3.68 (2H, d, $^3J$ = 6.03 Hz, *2*-C***H***$_2$), 3.76 (2H, d, $^3J$ = 5.55 Hz, *5*-C***H***$_2$), 3.77 (2H, d, $^3J$ = 5.87 Hz, *8*-C***H***$_2$), 5.04 (2H, s, Ph-C***H***$_2$), 7.37 (5H, br s, Ph), 7.51 (1H, t, $^3J$ = 6.03 Hz, *3*-N***H***), 8.18 (2H, m, *6,9*-N***H***).

***N*-Benzyloxycarbonyl-diglicylglycine *N′*-1-adamantylamide (6)**: The solution of DCC (0.0055 mol) in DMF (25 ml) was slowly added to a solution of *N*-hydroxysuccinimide (0.006 mol) and **5** (0.003 mol) in DMF (25 ml) at 0°C. The mixture was stirred for 3 h at 0°C and at room temperature for another half an hour. A precipitate quickly formed and the mixture was cooled to 0°C. Next, 0.005 mol of adamantyl amine was added and the mixture was stirred for 48 h at 0°C. After that 0.004 mol of acetic acid was added and stirring was continued for 2 h at room temperature. The solvent was evaporated under a reduced pressure and the crude material was purified by $SiO_2$ chromatography ($R_f^1$ = 0.33) to afford the 1:1 mixture of **6** and 1,3-dicyclohexylurea (70% yield calculated for pure **6**). Pure Z-Gly-Gly-Gly-NH-1-adamantane can be obtained after recrystallization from MeOH, as a colorless crystalline solid with low yield. M.p. 229°C; $^1$H NMR (DMSO-$d_6$) $\delta_H$: 1.61 (6H, br s, *A*-C***H***$_2$), 1.92 (6H, br s, *C*-C***H***$_2$), 1.99 (3H, br s, *B*-C***H***), 3.61–3.74 (6H, m, *2,5,8*-C***H***$_2$), 5.04 (2H, s, Ph-C***H***$_2$), 7.20 (1H, br s, *F*-N***H***), 7.35–7.39 (5H, m, Ph), 7.52 (1H, t, $^3J$ = 5.87 Hz, *3*-N***H***), 7.99 (1H, t, $^3J$ = 5.39 Hz, *6*-N***H***), 8.23 (1H, t, $^3J$ = 5.56 Hz, *9*-N***H***); ESI MS (high resolution), *m/z* obsd.: 479.2297, calcd.: 479.2265 for $C_{24}H_{32}N_4O_5Na$ $[M + Na]^+$.

**Diglicylglycine *N′*-1-adamantylamide (7)**: Suspension of 1g of 1:1 mixture of **6** and dicyclohexylurea and 1g of 10% Pd/C in 60 ml 70% formic acid in water and 20 ml of methanol was vigorously stirred under $H_2$ atmosphere for 96 h at room temperature. Then the unsolved material was removed by filtration *via* Celite and the solvent was evaporated under a reduced pressure. Next, the residue was dissolved in 50 ml of $CHCl_3$ and the crude product **7** was extracted back from water solution with 20 ml of 1 M HCl, the organic phase was removed and **7** was extracted with 100 ml of $CH_2Cl_2$ after basification up to pH 12. The solution was washed with 30 ml of brine, dried ($Na_2SO_4$) and concentrated. The product was purified by column chromatography ($R_f^2$ = 0.25) to afford 240 mg (~70% yield) of **7** as white foam**.** $^1$H NMR (methanol-$d_4$) $\delta_H$: 1.72 (6H, br s, *A*-C***H***$_2$), 2.04 (9H, br s, *B,(C)*-C***H***,(C***H***$_2$)), 3.37 (2H, s, *2*-C***H***$_2$), 3.78 (2H, s, *5*-C***H***$_2$), 3.92 (2H, s, *8*-C***H***$_2$); ESI MS (high resolution) *m/z* obsd.: 323.2081, calcd.: 323.2078 for $C_{16}H_{27}N_4O_3$ $[M + H]^+$.

**Benzene-*sym*-tri-*N,N,N*-carbonylglycine ethyl ester (8)**: Compound **8** was synthesized *via* a modified described procedure [11]. Solution of 3 mmol of benzene-1,3,5-tricarbonyl chloride in $CH_2Cl_2$ (10 ml) and DMF (5 ml) was slowly added over a period of 2 h, to a stirred mixture of Gly-OEt× HCl (12

mmol) and $Et_3N$ (30 mmol) in $CH_2Cl_2$/DMF (2:1) (15 ml) at 0°C. The stirring was continued at the same temperature for 4 h and at room temperature for 16 h. The reaction mixture was then filtered and the solvents were evaporated. The resulting residue was dissolved in $CHCl_3$ (120 ml) and washed successively with 5% aqueous HCl solution (2×25 ml), saturated aqueous $NaHCO_3$ solution (2×25 ml) and $H_2O$ (15 ml), dried ($MgSO_4$), evaporated and crystallized (toluene/hexane). The product **8** was obtained as white crystalline powder with yield 0.67 g (50%), m.p. 110.5–111.5°C; $^1$H NMR ($CDCl_3$) $\delta_H$: 1.36 (9H, t, $^3J$ = 7.00 Hz, C***H***$_3$), 4.21 (6H, d, $^3J$ = 6.03 Hz, NC***H***$_2$), 4.29 (6H, q, $^3J$ = 7.00 Hz, OC***H***$_2$), 8.51 (3H, t, $^3J$ = 6.03 Hz, N***H***), 8.27 (3H, s, Ar***H***); LSI-MS *m/z* obsd.: 466, calcd.: 466 for $C_{21}H_{28}N_3O_9$ $[M + H]^+$.

**Benzene-*sym*-tri-*N,N,N*-carbonylglycine (9)**: **9** was synthesized *via* a modified procedure [11]. To a stirred solution of the triester **8** (1 mmol) in MeOH (15 ml) at 0°C, 2 M aqueous NaOH solution (5 ml) was added. The reaction mixture was left to stir for 3 h. The precipitating solid was dissolved by addition of $H_2O$ (5 ml), neutralized with Amberlite IR-120 ($H^+$ form) ion-exchange resin, which was later filtered off. Next, the solvents were evaporated and the resulting solid was crystallized from hot water, dried thoroughly under *vacuum* to obtain **9** as a white powder (90%). M.p. 226°C; $^1$H NMR (DMSO-d$_6$) $\delta_H$: 3.98 (6H, d, $^3J$ = 5.85 Hz, C***H***$_2$), 8.51 (3H, s, Ar***H***), 9.12 (3H, t, $^3J$ = 5.87 Hz, N***H***), ~12 (~2H, br s, COO***H***); LSI-MS: *m/z* obsd.: 380, calcd.: 380 for $C_{15}H_{14}N_3O_9$ $[M-H]^-$.

**Benzene-*sym*-tris-*N,N,N*-carbonyltriglycylglycine *N*′-1-adamantylamide (1)**: **1** was synthesized *via* a modification of a described procedure [12]. To a stirred solution of **7** (0.68 mmol), **9** (0.155 mmol) and $Et_3N$ (5 mmol) in DMF (20 ml) was added TBTU (0.78 mmol) at 0°C. The resulting mixture was stirred at 0°C for 96 h and at room temperature for another 24 h. The solvent was evaporated and the residue was washed with 0.5 M HCl (2×5 ml), saturated $NaHCO_3$ (5 ml), $H_2O$ (5 ml) and recrystallized from methanol to obtain **1** as a light yellow powder (yield 0.11g, 55%). M.p. 192°C; $^1$H NMR (DMSO-d$_6$) $\delta_H$: 1.61 (18H, br s, *A*-C***H***$_2$), 1.92 (18H, br s, *C*-C***H***$_2$), 2.00 (9H, br s, *B*-C***H***), 3.62 (6H, d, $^3J$ = 5.75 Hz, *2*-C***H***$_2$), 3.73 (6H, d, $^3J$ = 5.59 Hz, *5*-C***H***$_2$), 3.79 (6H, d, $^3J$ = 5.59 Hz, *8*-C***H***$_2$), 3.99 (6H, d, $^3J$ = 5.40 Hz, *11*-C***H***$_2$), 7.19 (3H, s, *F*-N***H***), 7.97 (3H, t, $^3J$ = 5.76 Hz, *3*-N***H***), 8.21 (3H, t, $^3J$ = 5.60 Hz, *6*-N***H***), 8.31 (3H, t, $^3J$ = 5.59 Hz, *9*-N***H***), 8.54 (3H, s, Ar***H***), 9.00 (3H, t, $^3J$ = 5.39 Hz, *12*-N***H***); $^1$H NMR (DMSO-d$_6$ – D$_2$O) $\delta_H$: 0.89 (18H, br s, *A*-C***H***$_2$), 1.18 (2H, br s, *B,(C)*-C***H***,(C***H***$_2$)), 2.93 (6H, s, *2*-C***H***$_2$), 3.13 (6H, s, *5*-C***H***$_2$), 3.22 (6H, s, *8*-C***H***$_2$), 3.34 (6H, s, *11*-C***H***$_2$), 7.63 (3H, s, Ar***H***); ESI MS *m/z* obsd.: 1316.7, calcd.: 1316.6 for $C_{63}H_{87}N_{15}O_{15}Na$ $[M + Na]^+$ (the observed isotopic profile corresponds to the calculated one).

**NMR studies:** Because of the low dendrimer **1** solubility in water, $^1$H and $^{13}$C NMR spectra were measured in DMSO-d$_6$:D$_2$O = 2:1 solution on Varian Unity + 500 MHz spectrometer at 300 K. NOESY of the complexes of **1** with **2–4** in $D_2O$ spectra were acquired with 500 ms mixing time.

Acknowledgments

A partial support by the Polish State Committee for Scientific Research, grant nr 1599/T09/2000/19, and by the computational grant from Interdisciplinary Center for Modelling of Warsaw University are gratefully acknowledged. The authors are also grateful to Dr. B. Korybut-Daszkiewicz for his critical remarks to the manuscript.

## REFERENCES

1. *Comprehensive Supramolecular Chemistry*, Pergamon, Oxford, (1996); *Dendrimers I, Topics in Curr. Chem*., Vol. 197, Vögtle, F. (Ed.), (2000); *Dendrimers II. Architecture, Nanostructure and Supramolecular Chemistry, Topics in Curr. Chem*., Vol. 210, Vögtle, F. (Ed), (2000).
2. Stiriba S.E., Frey H. and Haag R., *Angew. Chem. Int. Ed. Engl*., **41**, 1329 (2002).
3. Zhang X.Y., Wilhelm M., Klein J., Pfaadt M. and Meijer E.W., *Langmuir*, **16**, 3884 (2000); Cavallo L. and Fraternali F., *Chem. Eur. J*., **4**, 927 (1998).
4. Brouwer A.J., Mulders S.J.E. and Liskamp R.M.J., *Eur. J. Org. Chem*., 1903 (2001); Gossage R.A., Jastrzebski J.T.B.H., van Ameijde J., Mulders S.J.E., Brouwer A.J., Liskamp R.M.J. and van Koten, G., *Tetrahedron Lett.*, **40**, 1413 (1999).
5. Boas U., Sontjens S.H.M., Jensen K.J., Christensen J.B. and Meijer E.W., *ChemBioChem*, **3**, 433 (2002).

6. Davis A.L., Keeler J., Laue E.D. and Moskau D., *J. Magn. Reson*., **98**, 207 (1992).
7. Osawa E. and Musso H., *Angew. Chem. Int. Ed. Engl.*, **22**, 1 (1983).
8. Greenstein J.P. and Winitz M., *Chemistry of the Amino Acids*, John Wiley & Sons, Inc., NY, (1961), p. 891.
9. Byk G. and Gilon C.J., *J. Org. Chem.*, **57,** 5687 (1992).
10. Langenbeck W., von Rothe M., Brünig H. and Eppert G., *J. Prakt. Chem*., **4**, 323 (1959).
11. Ashton P.R., Boyd S.E., Brown C.L., Jayaraman N., Nepogodiev S.A. and Stoddart J.F., *Chem. Eur. J.*, **2**, 115 (1996).
12. Xue C.B., Wityak J., Sielecki T.M., Pinto D.J., Batt D.G., Cain G.A., Sworin M., Rockwell A.L., Roderick J.J., Wang S.G., Orwat M.J., Frietze W.E., Bostrom L.L., Liu J., Higley C.A., Rankin F.W., Tobin A.E., Emmett G., Lalka G.K., Sze J.Y., DiMeo S.V., Mousa S.A., Thoolen M.J., Racanelli A.L., Hausner E.A., Reilly T.M., DeGrado W.F., Wexler R.R. and Olson R.E.,*J. Med. Chem.,* **40**, 2064 (1997).