Convergent and Sequential Synthesis of Dendritic, Multivalent Complexing Agents

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Abstract: Two series of macrocyclic polyamine derivatives with various length of linkers were synthesized as dendritic ligands containing two, three, four, five or six terminal nitrilotriacetic acid (NTA) groups through convergent and sequential pathways. Tetrafluorophenyl esters were employed as activating reagents in the coupling step of assembling NTA groups to the cyclic polyamine head. A key step of the sequential synthesis is the use of the coupling reagent, 2-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) for the formation of amide bonds between the cyclic polyamine and pendant groups. Finally, two representative compounds were used to demonstrate the formation of stoichiometric protein assemblies.

Key words: complexing agents, polyamines, coupling, nitrilotriacetic acid

Immobilized metal affinity chromatography (IMAC) has become a widely used technique in molecular biology and protein engineering since it was first reported by Porath in 1975. In particular, it has been used for the isolation of proteins, peptides, and nucleic acids. During IMAC, a metal ion, such as Ni$^{2+}$, Zn$^{2+}$, or Cu$^{2+}$, is complexed and immobilized by a chelator, which is covalently linked to a solid matrix. Our interest is based on the use of nitrilotriacetic acid (NTA) which has been employed as a chelator to purify proteins since 1987. NTA chelates a Ni(II) ion through four of its potential six coordination sites, the remaining sites bind two histidine residues forming an octahedral complex. The formation of this type of complex relies on incorporation of 2–6 histidines at one terminus of a protein. The histidine-tagged protein is bound by the metal immobilized on the NTA-derivatized stationary phase, while other proteins are washed off the column. The binding of Ni(II) by NTA is reversible, and the reversibility can be achieved by the use of ethylenediamine tetraacetic acid (EDTA) which has a higher binding constant for Ni(II) ions or imidazole which competes with the histidine binding.

In the course of our study on biomembrane dynamics, we reported a series of macrocyclic polyamine amphiphiles both with and without fluorescent nitrobenzoxadiazole (NBD) labels. These compounds have varied, but defined sizes and shapes and have been useful for investigation of diffusion in biological membranes. The diffusion coefficients of these molecules are size dependent. It is well known that aggregation of membrane proteins plays a key role in signal transduction, so it is of interest to prepare membrane protein aggregates with defined sizes and shapes. To generate aggregates of proteins, two series of complexing agents containing multiple NTA functional groups were designed as shown in Figure 1. These compounds have dendritic arms, which can chelate to histidine-tagged proteins through nickel ions. Changing the size of the central ring will give a corresponding change in the number of dendritic arms. Each dendritic chelating arm can bind to a protein so that different sizes of protein clusters can be created with the various reagents. It may also be possible to control the tightness of the clusters formed by varying the length of the linker chain. In this paper, we report two synthetic strategies for making den...
dritic ligands with a macrocyclic polyamine head group and multiple nitrilotriacetic acid pendant groups. The use of these ligands to assemble dimers, trimers, tetrakers, pentamers, or hexamers of histidine-tagged proteins will be reported separately, but we illustrate the principle here with making dimers and trimers.

The macrocyclic polyamine derivatives with multiple NTA functional groups shown in Figure 1 were synthesized as outlined in Scheme 1 and Scheme 2. Their structures were confirmed by mass spectrometry (MS), NMR spectroscopy and FTIR spectroscopy. The synthesis was achieved by both a convergent and a sequential pathway to provide versatility. All of the syntheses started from cyclic polyamines, some of which are not commercially available as free bases. For example, 1,4,7,10,13-pentaazacyclopentadecane (3d) and 1,4,7,10,13-hexaazacyclooctadecane (3e) can be isolated as such easily from 1,4,7,10,13-pentaazacyclopentadecane pentahydrochloride and hexacyclen trisulfate, respectively. To avoid the formation of byproducts with incomplete carboxymethylation during the preparation of 1a,b, we used lysine-NTA23b (8), to couple with tetrafluorophenyl activated esters of 4a,b as shown in Scheme 1. However, carboxymethylation of the polyamino acids (10a–e) as shown in the sequential pathway (Scheme 2) yielded, under controlled pH the fully substituted species, 2a–e, as the major products. The synthesis of polyamides rather than polyamines is chosen to control the shape of the central ring. The rotation about the amide bond is restricted at room temperature decreasing the conformational mobility of the ring. This has been observed previously and will be evident from the NMR data reported here as well. Also, a polyamine ring may act as a chelator as was demonstrated previously in the use as a radionuclide carrier attached to monoclonal antibodies (mAbs) for cancer chemotherapy. In the case of the macrocyclic system, introduction of

Scheme 1  Convergent pathway to target molecules 1a,b
the side chain was achieved efficiently and in good yields by direct amidation with succinic anhydride or L-glutamic acid N-benzyloxycarbonyl-1-benzyl ester (Z-Glu-OBzl) using coupling agents. Compounds 4a and 4b could be obtained quantitatively by reacting 3a or 3b with succinic anhydride using the method of Kung and Izatt, 8 and the products were easily purified (Scheme 1) in 45–65% yields. However, only two polysuccinoyl amines were successfully obtained. This route failed for the larger cyclic polyamines presumably due to steric factors and/or decreased reactivity. The requisite activated macrocyclic polysuccinoyl amine derivatives 5a and 5b were prepared as the tetrafluorophenyl (TFP) esters in 76–86% yields by the procedure described by Wilbur et al. 9 They are preferred in our work since they are stable to hydrolysis and give good aminolysis yields for products 1a, b while other activated esters, such as N-hydroxysuccinimide (NHS) esters, are more sensitive to ambient moisture. The benzyloxycarbonyl protecting group of compound 7 was removed by hydrogenolysis (10 bar H2, room temperature, Pd/C) afforded polyamino acids. The crude polyamino acids 10a–e were collected in 80–90% yields by recrystallization from ethanol. The last step of the sequential pathway was the carboxymethylation of the polyamino acids 10a–e to provide dendritic ligands 2a–e by a standard procedure in good yields.

The key step in Scheme 2 is the coupling of the polyamine with the commercially available derivative of glutamic acid, which was protected by a benzyloxy carbonyl (Z) group at the N-terminus and benzyl at the C-terminus [L-glutamic acid N-(benzyloxy carbonyl)-1-benzyl ester]. The coupling agent, 2-(1H-benzotriazole-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), was first used in peptide synthesis by Knorr in 1989, 10 and was reported to be very effective for couplings that involve a proline nitrogen, which is similar to the secondary, cyclic polyamines used in this study. The subsequent cleavage of protecting groups (Z and benzyl) in compounds 9a–e by hydrogenolysis (10 bar H2, room temperature, Pd/C) afforded polyamino acids. The crude polyamino acids 10a–e were collected in 80–90% yields by recrystallization from ethanol. The last step of the sequential pathway was the carboxymethylation of the polyamino acids 10a–e to provide dendritic ligands 2a–e by a standard procedure in good yields.

The final compounds 1a, b and 2a–e are extremely polar and isolation by standard chromatographic techniques such as silica gel column chromatography was difficult. Anion exchange chromatography was used for the purification of 2a, b and 1a, b. It was noted that as the number of NTA groups increases, the solubility decreases. So compounds 2c–e precipitate out from the mixture of etha-
nol and water, and their purification can be performed by simple filtration. The crude products $2_{c-e}$ were further purified by recrystallization from methanol and collected in 32–56% yields. Being concerned about the polarity of the second set of dendritic compounds $2_{a-e}$, we initially tried another route to obtain $2_e$ as shown in Scheme 3. We aimed to make a representative methyl ester, i.e. $10_e$, followed by carboxymethylation with methyl bromoacetate to give its complete methyl ester $11_e$. Subsequent hydrolysis of all the methyl groups under basic conditions would give the desired compound $2_e$. However, it was difficult to limit the reaction to tertiary alkylation of the amine $11_e$ since the quaternary ammonium product formed easily in organic solvents. In contrast, direct alkylation of the amino acid was successfully carried out using bromoacetic acid in aqueous solution under basic conditions (pH 11–12) for a few days and followed by acidification with 6 M hydrochloric acid to pH 1.

As a preliminary demonstration of the utility of these dendritic reagents for formation of stoichiometric protein assemblies, we used compounds $1_a, b$ to aggregate His-tagged thioredoxin in solution. Thioredoxin was chosen as a model monomeric protein since it is water-soluble, heat-stable, of low molecular weight and locally available (Dr. Eric Ball, Department of Biochemistry, The University of Western Ontario). Figure 2 shows the native polyacrylamide gel electrophoresis analysis of solutions containing mixtures of $1_a$ and $1_b$ with His-tagged thioredoxin (lanes 5 and 2, respectively) compared to a solution of protein alone (lanes 1 and 4). It is evident that the majority of the protein in the mixtures move more slowly and that the complex with $1_b$ moves most slowly. The relative rate of migration is consistent with the majority of the protein moving as monomers in lanes 1 and 4, as dimers in lane 5 and as trimers in lane 2. Lanes 3 and 6 confirm that formation of the stoichiometric assemblies is mediated by the NTA-Ni-His complex since addition of excess EDTA causes the proteins to move as monomers. It should be noted that the mixtures in lanes 3 and 6 were prepared by adding EDTA to solutions after the formation of the protein assemblies but prior to running the gels showing that
complex of E. coli His-tagged thioredoxin is observed predominantly as a single oligomeric structure as long as appropriate ratios of ligand:Ni²⁺:protein were used in solutions. This is consistent with the expectation that the dendritic ligands bind to the His-tagged protein with a high affinity. Although the molecular weight (MW) of the complexes were not determined directly, the gels provide direct evidence that the size of the His-tagged thioredoxin oligomers can be controlled in a reversible fashion by changing the number of NTA groups on the complexing reagents.

In this work, only intermediates 4a,7, and 8 have previously been reported in the literature. We have detailed straightforward methods for the synthesis of two sets of polyfunctional chelators 1a,b and 2a–e. We have tested these compounds to show their ability to form stoichiometric complexes. These and other applications will be reported separately. Access to appropriate ligands with multiple binding features may be a useful step in many aspects of molecular biology or cell biology and protein engineering. The formation constants for 1:1 trivalent metal complexes of NTA (typically 10¹⁰–10¹⁲) may be inadequate for in vivo applications, but the ability to form protein-NTA-metal-NTA-protein linkages may provide a non-denaturing and readily reversible means for cross-linking two proteins, such as antibodies and enzymes, for in vitro use. The bifunctional NTA ligands may also prove useful for tethering ternary metal complexes to proteins, as was done by Kline.¹¹ The ability to introduce a protein-reactive substituent into polyamino polycarboxylate chelator by the two pathways described here, should facilitate the identification of additional areas where an interface between coordination chemistry and protein biochemistry may bear fruit.

Piperazine, 1,4,7-triazacyclononane, 1,4,7,10-tetraazacyclodecane, hexacylen trisulfate, 2,3,5,6-tetrafluorophenol (TFP-OH), di-cyclohexylcarbodiimide (DCC), bromoacetic acid, and succinic anhydride were bought from BDH Chemicals. Bio-Rad NaCl, sodium dodecyl sulfate (SDS), KCl and ethylenediamine tetra-acetic acid (EDTA) were bought from Sigma Chemical Co. CaCl₂, cyclohexylurea was filtered out, and the filtrate was evaporated under vacuum. The residue of reaction was triturated with MeCN–EtOAc (1:1, 15 mL). A white solid (0.49 g) was collected by vacuum.

1,4,7-Tris-(3-carboxypropyl)triazacyclononane (4b)

Compounds 4b (0.21 g, 0.49 mmol) was prepared from 1,4,7-triazacyclononane (3b, 0.14 g, 1.08 mmol) and succinic anhydride (0.33 g, 3.24 mmol) according to the procedure used for 4a in 45% yield as a white solid; mp 126–130 °C.

1H NMR (D₂O): δ = 2.56 (deformed t, J = 8 Hz, 4 H, CH₃CH₂CO₂H), 2.66 (deformed t, J = 8 Hz, 4 H, NOCOCCH₂CH₃), 3.62–3.48 (at r.t.; br s, at 55 °C, due to the existence of rotamers, 8 H, OCNCH₂CH₃NO).

IR (KBr): 3030 (OH stretch), 1720 (C=O stretch for carboxylic acid), 1600 (C=O stretch for tertiary amide), 1486 cm⁻¹ (CH₂ deformation).

1H NMR (DMSO-d₆): δ = 27.7, 29.3, 44.5, 52.4, 170.3, 174.3.


1,4,7-Trimethyl-1,4,7-triazacyclononane (5a): Typical Procedure

To a stirred solution of compound 4a (0.28 g, 0.98 mmol) in DMF (10 mL) under argon was added 2,3,5,6-tetrafluorophenol (TFP-OH) (0.41 g, 2.50 mmol) at 50 °C. A solution of dicyclohexylcarbodiimide (DCC, 0.45 g, 2.04 mmol) in DMF (10 mL) was added dropwise at this temperature. The reaction mixture was stirred overnight, and the solution was cooled to r.t. The white precipitate of dicyclohexylurea was filtered out, and the filtrate was evaporated under vacuum. The residue of reaction was triturated with MeCN–EtOAc (1:1, 15 mL). A white solid (0.49 g) was collected by vacuum filtration; yield: 86%; mp140–142 °C.

IR (KBr): 3422, 3086 (CH stretch for aromatic compound), 2970, 2930. (CH stretch), 1778 (C=O stretch for ester), 1696 (C=O stretch), 1615 (C=O stretch for tertiary amide), 1485, 1451 (CH₂ deformation), 1216 (C–F stretch), 851 cm⁻¹ (CH out of plane deformation).

1H NMR (CDCl₃): δ = 2.80 (t, J = 6 Hz, 4 H, NOCOCCH₂CH₃COO), 3.08 (t, J = 6 Hz, 4 H, NOCOCCH₂CH₃), 3.76–3.52 (2 m, J = 6 Hz, 8 H, OCNCH₂CH₃NO, conformational isomer), 6.98 (m, J = 5 Hz, 2 H, 2 C₆H₅).
HRMS (EI, 70 V): m/z calcd for C_{23}H_{40}F_{2}N_{3}O_{5}; 582.1037 [M]^+; found: 582.1040 [M]^+.

1,4,7-Tris[3-(2,3,5,6-tetrafluorophenoxycarbonyl)propanoyl]-triazacyclononane (5b)
Compound 5b (0.15 g, 0.17 mmol) was obtained from 4b (0.11 g, 0.25 mmol), TFP-OH (0.15 g, 0.88 mmol) and DCC (0.18 g, 0.82 mmol) according to the procedure used for 5a as a white solid in 67% yield; mp 120–122 °C.

IR (KBr): 3412, 3085 (CH stretch for aromatic compound), 2950, 1720 (C=O stretch for carboxylic acid), 1685 (C=O stretch for tertiary amide), 1594 (C=C stretch), 1464 (CH_2 deformation), 1431, 1381 cm⁻¹ (C–N stretch).

IR (KBr): 3412, 3085 (CH stretch for aromatic compound), 2950, 1720 (C=O stretch for carboxylic acid), 1685 (C=O stretch for tertiary amide), 1594 (C=C stretch), 1464 (CH_2 deformation), 1431, 1381 cm⁻¹ (C–N stretch).

HRMS (EI, 70 V): m/z calcd for C_{40}H_{62}N_{3}O_{18}; 747.4551 [M]^+; found: 747.4556 [M]^+.

**N-(5-Benzoylcarbonylamo-1-carboxypentyl)imidodiacetic Acid [Nγ-Z-Lys-NTA, 7]**
Bromoacetic acid (4.17 g, 30 mmol) was dissolved in NaOH solution (15 mL, 2 M) and cooled to 0 °C. Nγ-Benzoylcarbonyl-1-lysine (6, 4.20 g, 15 mmol) in aq 2 M NaOH solution (22.5 mL) was added dropwise at 0 °C with stirring. After 2 h, the ice bath was removed and the mixture was stirred overnight at r.t. The reaction was then raised to 50 °C for 2 h and the mixture was stirred further. After 12 h, the reaction mixture was cooled and was stirred at r.t. The reaction was monitored by TLC, the developed component was visualized by ninhydrin spray. When the reaction was complete, it was quenched by the addition of aq 1 M HCl to pH 1. After extraction with CHCl₃, the aqueous layer was evaporated to dryness under vacuum and the resulting product redissolved in H₂O. The resulting residue was purified twice by anion exchange column chromatography and collected to give 1a (0.13 g) in 35% yield; mp 170 °C (dec.).

IR (KBr): 3330 (OH stretch), 2948, 2854 (CH stretch), 1730 (C=O stretch for carboxylic acid), 1680 (C=O stretch for tertiary amide), 1436 (CH_3 deformation), 1380 cm⁻¹ (C–N stretch).

IR (KBr): 3330 (OH stretch), 2948, 2854 (CH stretch), 1730 (C=O stretch for carboxylic acid), 1680 (C=O stretch for tertiary amide), 1436 (CH_3 deformation), 1380 cm⁻¹ (C–N stretch).

HRMS (FAB, oxalic acid/glycerol): m/z calcd for C_{63}H_{70}N_{6}O_{2}_{10}; 775.3362 [M + H]^+; found 775.3368 [M + H]^+.

1,4,7-Tris[5,N,N-di(carboxymethyl)amino-5-carboxypentylamino-3-oxopropanoyl]triazacyclononane (1b)
Compound 1b was obtained from 5b (0.25 g, 0.28 mmol) and lysine-NTA (8, 0.28 g, 1.17 mmol) according to the method given for 1a as a white solid (0.1 g, 32%); mp 200 °C (dec.).

IR (KBr): 3331 (OH stretch), 2945, 2857 (CH stretch), 1732 (C=O stretch for carboxylic acid), 1685 (C=O stretch for tertiary amide), 1434 (CH_3 deformation), 1381 cm⁻¹ (C–N stretch).

IR (KBr): 3331 (OH stretch), 2945, 2857 (CH stretch), 1732 (C=O stretch for carboxylic acid), 1685 (C=O stretch for tertiary amide), 1434 (CH_3 deformation), 1381 cm⁻¹ (C–N stretch).

HRMS (FAB, oxalic acid/glycerol): m/z calcd for C_{32}H_{50}N_{6}O_{18}; 1162.5003 [M + 1]^+; found 1162.4982 [M + 1]^+.

1,4-Bis[(N-benzyloxy carbonyl)amino-4-benzyloxy carbonylbutanoyl]diazacyclohexane (9a); Typical Procedure
Z-Glu-OBzl (2.5 g, 6.0 mmol) and TBTU (1.926 g, 6.0 mmol) were dissolved in DMF (60 mL) followed by the addition of Et₃N (2 mmol). The reaction mixture was stirred at r.t. for 12 h. After extraction with CHCl₃, the aqueous layer was evaporated to dryness under vacuum and the resulting product redissolved in H₂O. The resulting residue was purified twice by anion exchange column chromatography and collected to give 9a (1.36 g) as a white solid in 89% yield; mp 172–174 °C.
HRMS (EI, 70 V): 128.5, 128.7, 135.3, 136.3, 156.8, 170.4, 171.7 (CH out of plane deformation).

1H NMR (CDCl3): δ = 2.05 (br, 4 H, CH2CH2CH2), 2.26 (t, J = 7 Hz, 4 H, COCH2CH2), 3.20 (br, s, 4 H, OCNCH2CH2NCO, isomer), 3.48 (br, s, 4 H, OCNCH2CH2NCO), 4.45 (t, J = 7 Hz, 2 H, CH2CH(OOC)), 5.06 (s, 4 H, C6H5CH2OOC), 5.16 (q, J = 3 Hz, 4 H, NCOCH2CH2), 5.70 (d, J = 8 Hz, 2 H, CHNHCNO), 7.34–7.25 (br s, 20 H, 4 C6H5).

13C NMR (CDCl3): δ = 27.6, 29.1, 41.4, 45.0, 53.7, 67.1, 128.2, 128.5, 135.3, 136.4, 156.8, 170.4, 171.7.


1,4,7-Tris-[4-(N-benzyloxycarbonylamino-4'-benzylxocarbonylbutanoyl]triazacyclononane (9b)

Compound 9b (0.95 g, 0.52 mmol) was obtained as a white solid from 1,4,7-triazacyclononane (3b, 0.10 g, 0.80 mmol), Z-Glu-OBzl (1.35 g, 3.60 mmol) and TBTU (1.15 g, 3.60 mmol) according to the procedure used for 9a in 65% yield; mp 40–43°C.

IR (KBr): 3305 (NH stretch), 3030 (CH stretch for aromatic compound), 2947 (CH stretch for alkane), 1720 (C=O stretch for ester), 1628 (C=O stretch for tertiary amide), 1535 (C=O stretch, 1458 (CH3 deformation), 1215 (C–O–C stretch), 697 cm–1 (CH out of plane deformation).

1H NMR (CDCl3): δ = 2.05 (br, 10 H, CH2CH2CH2), 2.45 (br, 10 H, COCH2CH2), 3.55–3.23 (2 br s, 20 H, OCNCH2CH2NCO), 4.43 (br, 5 H, CH2CH(OOC)), 5.04 (s, 10 H, CH2CH2OOC), 5.15 (br, 10 H, C6H5CH2O), 5.96 (d, J = 8 Hz, 5 H, CHNHCNO), 7.35–7.25 (br s, 50 H, 10 C6H5).

13C NMR (CDCl3): δ = 27.0, 29.8, 38.7, 49.9, 53.5, 66.8, 67.3, 128.2, 128.9, 136.1, 157.5, 171.8, 172.0.


1,4,7,10-Tetraakis-[4-(N-benzyloxycarbonylamino-4'-benzylxocarbonylbutanoyl]tetraazacyclooctadecane (9c)

Compound 9c (0.17 g, 0.11 mmol) was obtained from cyclen or 1,4,7,10-tetraazacyclododecane (3e, 0.043 g, 0.24 mmol), Z-Glu-OBzl (0.54 g, 1.44 mmol) and TBTU (0.46 g, 1.44 mmol) according to the procedure used for 9a as a white solid in 44% yield; mp 60–62°C.

IR (KBr): 3305 (NH stretch), 3030 (CH stretch for aromatic compound), 2946 (CH stretch for alkane), 1723 (C=O stretch for ester), 1630 (C=O stretch for tertiary amide), 1527 (C=O stretch), 1455 (CH3 deformation), 1215 (C–O–C stretch), 697 cm–1 (CH out of plane deformation).

1H NMR (CDCl3): δ = 1.95 (br, 6 H, COCH2CH2CH2), 2.25 (br s, 6 H, COCH2CH2), 3.30 (br s, 9 H, OCNCH2CH2NCO, due to isomer), 3.64 (br s, 3 H, OCNCH2CH2NCO), 4.38 (t, J = 8 Hz, 3 H, CH2CH(OOC)), 5.05 (s, 6 H, C6H5CH2OOC), 5.15 (m, J = 2 Hz, 6 H, C6H5CH2OCON), 6.01 (d, J = 8 Hz, 3 H, CHNHCNO), 7.33–7.25 (br s, 30 H, 6 C6H5).

13C NMR (CDCl3): δ = 26.5, 30.0, 46.0, 50.9, 54.0, 67.1, 67.3, 128.3, 128.6, 128.8, 136.3, 158.1, 172.2, 173.4.


1,4,7,10,13-Hexaazacyclooctadecane or Hexacyclen (3e)

Hexacyclen trisulfate (0.46 g, 0.83 mmol) was dissolved in 4 mL NaOH (40% NaOH). The solution was extracted with CHCl3 (5 mL) and dried to yield (Na2SO4). Removal of the solvent gave 3e (0.14 g) in 65% yield.

1,4,7,10,13,16-Hexakis-[4-(N-benzyloxycarbonylamino-4'-benzylxocarbonylbutanoyl]hexaazacyclooctadecane (9e)

Compound 9e (0.07 g, 0.03 mmol) was obtained as a white solid from hexacyclen (3e) (0.024 g, 0.093 mmol), Z-Glu-OBzl (0.35 g, 0.93 mmol) and TBTU (0.30 g, 0.93 mmol) according to the procedure used for 9a as a white solid in 33% yield; mp 56–58°C.

IR (KBr): 3304 (NH stretch), 3030 (CH stretch for aromatic compound), 2947 (CH stretch for alkane), 1728 (C=O stretch for ester), 1628 (C=O stretch for tertiary amide), 1521 (C=O stretch), 1455 (CH3 deformation), 1210 (C–O–C stretch), 697 cm–1 (CH out of plane deformation).

1H NMR (CDCl3): δ = 2.05 (br, 12 H, CH2CH2CH2), 2.45 (br, 12 H, COCH2CH2), 3.54–3.27 (2 br s, 24 H, OCNCH2CH2NCO), 4.40 (br, 6 H, CH2CH(OOC)), 5.02 (s, 12 H, CH2CH2OOC), 5.12 (br, 12 H, C6H5CH2OCON), 6.05 (br, 6 H, CHNHCNO), 7.35–7.18 (br s, 60 H, 18 C6H5).

13C NMR (CDCl3): δ = 27.5, 29.0, 47.5, 53.9, 67.5, 67.7, 127.9, 128.8, 128.9, 136.9, 156.8, 172.5, 173.6.


1,4-Bis-[4-amino-4-carboxybutanoyl]diazacyclohexane (10a)

Typical Procedure

Compound 9a (0.65 g, 0.82 mmol) was dissolved in MeOH (10 mL). A small amount of Pd/C (10%) was added to this solution. After stirring the suspension for 2 min, H2 gas was introduced into the flask at r.t. under normal pressure. The reaction progress was monitored by TLC, visualized by illumination with UV light. The catalyst was filtered off, washed with H2O, and the filtrate was dried in vacuo. The resulting precipitate was dissolved in hot EtOH, and allowed to crystallize at 0°C. The crystals were filtered off and dried in vacuo; yield: 90% (0.25 g); mp 168–170°C.

IR (KBr): 3435 (NH and OH stretch), 1735 (C=O stretch for carboxylic acid), 1634 (C=O stretch for tertiary amide), 1447 (NH₃⁺ deformation), 1426 cm⁻¹ (COO⁻ deformation). compound 10e (0.04 g, 0.04 mmol) was obtained from 9e (0.11 g, 0.05 mmol) according to the procedure used for 10a as a white solid in 80% yield; mp 200 °C (dec.).

IR (KBr): 3436 (NH stretch), 1717 (C=O stretch for carboxylic acid), 1635 (C=O stretch for tertiary amide), 1446 (NH₃⁺ deformation), 1425 cm⁻¹ (CH₂ deformation).

1 H NMR (D₂O): δ = 2.06 (br s, 4 H, CH₂CH₂CH₂), 2.64 (br s, 4 H, COCH₂CH₂), 3.65–3.43 (2 br s, 12 H, OCNCH₂CH₂NCO), 3.89 (br t, J = 8 Hz, 6 H, CH₂CHCOO).

13C NMR (D₂O): δ = 25.5, 28.5, 46.1, 51.0, 173.9, 174.7.


1.4,7-Tris(4-amino-4-carboxybutylyl)diacazoctadecane (10c)

Compound 10c (0.017 g, 0.02 mmol) was obtained from 10b (0.02 g, 0.02 mmol) according to the procedure used for 2a as a white solid in 48% yield; mp 210 °C (dec.).

IR (KBr): 3436 (NH stretch), 1733 (C=O stretch for carboxylic acid), 1631 (C=O stretch for tertiary amide), 1439 cm⁻¹ (COO⁻ deformation).

1 H NMR (D₂O): δ = 2.05 (br s, 4 H, CH₂CH₂CH₂), 2.54 (br s, 4 H, COCH₂CH₂), 3.63–3.43 (2 br, 10 H, CH₂OCNCH₂NCO), 3.67 (br t, 5 H, CH₂CHCOO).

13C NMR (D₂O): δ = 25.2, 29.2, 45.6, 48.0, 50.9, 174.2, 175.1.

1,4,7,10-Tetraakis-[4-N,N-di(carboxymethylamino)-4-carboxybutanoyl]-tetraazaacyclodecane (2c)

Compound 2c (0.04 g, 0.035 mmol) was obtained from 10c (0.056 g, 0.081 mmol) and bromoacetic acid (0.10 g, 0.73 mmol) according to the procedure used for 2a as white solid in 43% yield; mp 220 °C (dec.).

IR (KBrij): 3430 (OH stretch), 1723 (C=O stretch for carboxylic acid), 1634 (C=O stretch for tertiary amide), 1364 cm⁻¹ (N–C stretch).


Compound 2d (0.044 g, 0.03 mmol) was obtained from 10d (0.07 g, 0.08 mmol) and bromoacetic acid (0.12 g, 0.88 mmol) according to the procedure used for 2a as a white solid in 38% yield; mp 230 °C (dec.).

IR (KBrij): 3435 (OH stretch), 1730 (C=O stretch for carboxylic acid), 1637 (C=O stretch for tertiary amide), 1364 cm⁻¹ (N–C stretch).


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