Synthesis, Metal-binding Properties and Polypeptide Solubilization of 'Crowned' Arborols

Takeshi Nagasaki, Onari Kimura, Masakatsu Ukon, Susumu Arimori, Itaru Hamachi and Seiji Shinkai *

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan

The first dendritic crown ether polymers ('crowned' arborols: G1, G2 and G3) have been synthesized and characterized. The convergent synthetic method was found to be more convenient than the divergent synthetic method. The building block in the branch was prepared from *N*-benzyloxycarbonyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane and 3,5-bis(ethoxycarbonylmethoxy)benzoyl chloride. Since the basic repeating unit is the amino dicarboxylic acid, one can utilize the methods developed for peptide synthesis. Final products were obtained in moderate yields by coupling of each generational fragment with a core (benzene-1,3,5-tricarbonyl trichloride) followed by reduction of all amide linkages to tertiary amine linkages by borane–dimethyl sulfide. The complexation ability of these 'crowned' arborols was estimated by two-phase solvent extraction of alkali picrate salts. We found that some of these arborols can solubilize proteins in organic solvents.

The novel class of cascade molecules, arborols, have recently been of much synthetic interest.[1–9] Tomalia *et al.*[4,5,9] have also reported a similar class of cascade molecules, 'starburst-dendritic' polymers. They have several unique characteristics which conventional polymers do not have: for example, they feature monodispersed relative molecular mass, a globular conformation, and generational increase in relative molecular mass, *etc.* To the best of our knowledge, however, functions of arborols which may stem from these unique characteristics have been little exploited so far.

In order to increase the variety of functional groups that can be used in dendritic structures we have attempted to introduce crown ethers into arborols in the hope of producing novel functional properties. We expected that crown ethers would act as 'nests' in arborols and that metals ions would perch on them like 'birds'. As a result, the metal-binding event would induce some changes in the physical properties, for which one can expect some novel chemical features. For the synthesis of such 'crowned' arborols we chose 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane as a crown ether because the two secondary amines would be useful for the chain elongation. To increase the generation number we adopted the reaction of the secondary amine and a carboxylic acid, which reaction has been well studied in peptide synthesis. Finally, we reduced the amide groups in the arborols to the tertiary amines, which increased the ion-binding ability of the crown ether moiety. We here address the first successful approach to the synthesis of 'crowned' arborols and some aspects of the chemical functionality arising from the crown-metal interactions.

Results and Discussion

Syntheses.—There are two different strategies for the synthesis of arborols, the divergent method going from a nucleus to branches [1–5] and the convergent method going from branches to a nucleus.[6–8] First we synthesized compound Z-B1-CO_2Et (B1 is the abbreviation of the branch for the first generation) which serves as an arborol 'trunk' (Scheme 1). This compound was synthesized from *N*-benzyloxycarbonyl-1,4,-10,13-tetraoxa-7,16-diazacyclooctadecane in moderate yield. According to the divergent method we synthesized the first generation (G1-CO_2Et) from benzene-1,3,5-tricarbonyl trichloride (as a core) and B1-CO_2Et in tetrahydrofuran (THF) in the presence of triethylamine.[10] G1-CO_2Et was identified on the basis of IR, NMR and mass spectral evidence and elemental analysis.[10] After the hydrolysis of the ester groups, the product was treated with B1-CO_2Et, pivaloyl chloride and triethylamine.[10] We here met with a few synthetic difficulties. The reaction of phenoxyacetic acid (reference compound) and B1-CO_2Et proceeded quantitatively to give an amide linkage, whereas the reaction of this hexacarboxylic acid (G1 with X = CO and R = CO_2H) and B1-CO_2Et gave Bu^{t}CO-B1-CO_2Et but not the second-generation product (G2-CO_2Et).[10] The amide synthesis from carboxylic acids (RCO_2H) with the aid of pivaloyl chloride proceeds *via* a mixed acid anhydride, Bu^t-CO_2COR, and the two carbonyl groups can react competitively with nucleophiles. Without exception (to the best of our knowledge), however, the carbonyl group near R reacts preferentially

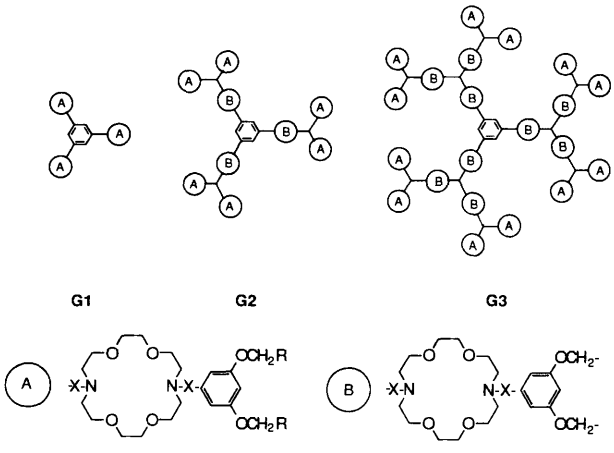
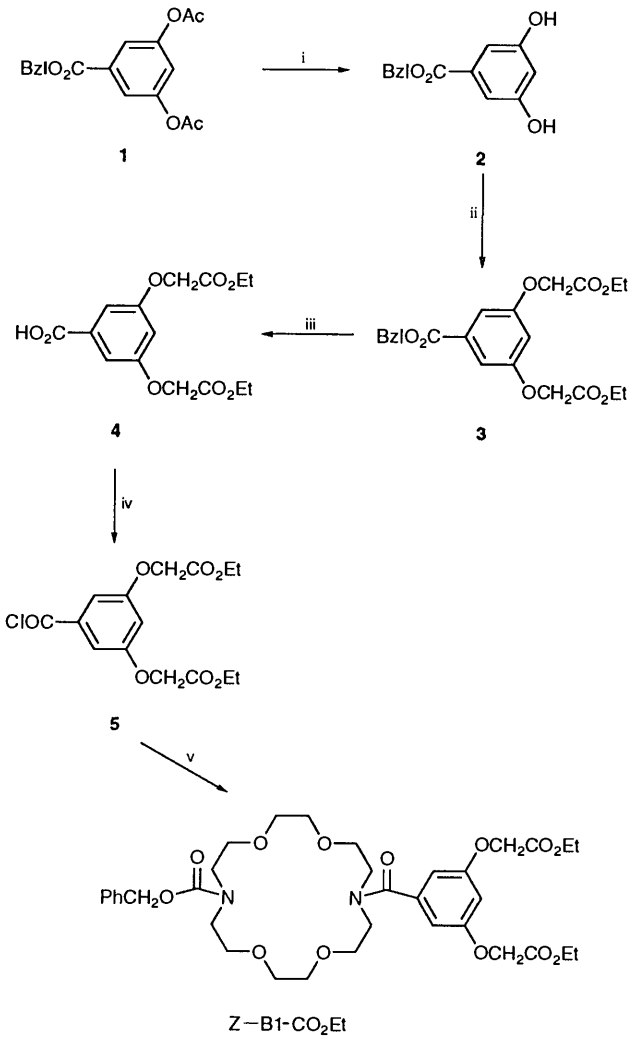


Table 1 Abbreviations of 'crowned' arborols

Generation	R = CO_2Et X = C=O	R = H X = C=O	R = H X = CH_2
G1	G1-CO_2Et	G1-H	G1-Reduced
G2	G2-CO_2Et	G2-H	G2-Reduced
G3	G3-CO_2Et	G3-H	G3-Reduced

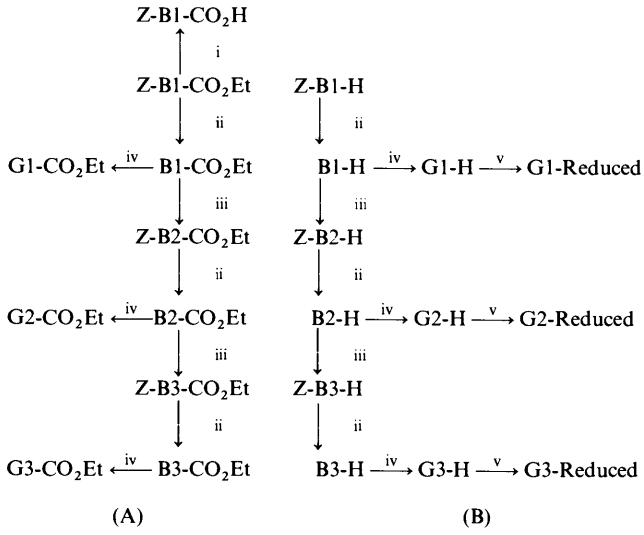


Scheme 1 *Reagents:* i, OH^-; ii, K_2CO_3, $BrCH_2CO_2Et$; iii, H_2/Pd-C; iv, $SOCl_2$; v, *N*-benzyloxycarbonyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane, Et_3N

because of the steric hindrance of the Bu^t moiety. Furthermore, this hexacarboxylic acid was poorly soluble in most solvents. Finally, we abandoned the divergent method.

In order to use the convergent method we first synthesized branches. Compound Z-B1-CO_2Et was hydrolysed to Z-B1-CO_2H, which was allowed to react with B1-CO_2Et in the presence of pivaloyl chloride and triethylamine. As expected, the nucleophilic reaction with the carbonyl group near Z-B1-CO_2H occurred preferentially and Bu^{t}CO-B1-CO_2Et was not detected in the product mixture. The product was deprotected, and treated with benzene-1,3,5-tricarbonyl trichloride (as a core) to give a second-generational 'crowned' arborol G2-CO_2Et. G2-CO_2Et (oil) was identified on the basis of IR and NMR spectral evidence. The reactions illustrated in Scheme 2(A) occurred smoothly in moderate yield, with no solubility problems. The results indicate the superiority of the convergent method over the divergent method for the present purpose. We could synthesize 'crowned' arborols of higher generation numbers as well [Scheme 2(A)].

In a similar manner we synthesized G1-H, G2-H and G3-H with methoxy groups as terminal groups (Scheme 2(B)]. These arborols were synthesized for metal-binding experiments under alkaline conditions. They were obtained in moderate yields comparable with those for a G*n*-CO_2Et series. Finally, the amide linkages were reduced quantitatively to give tertiary



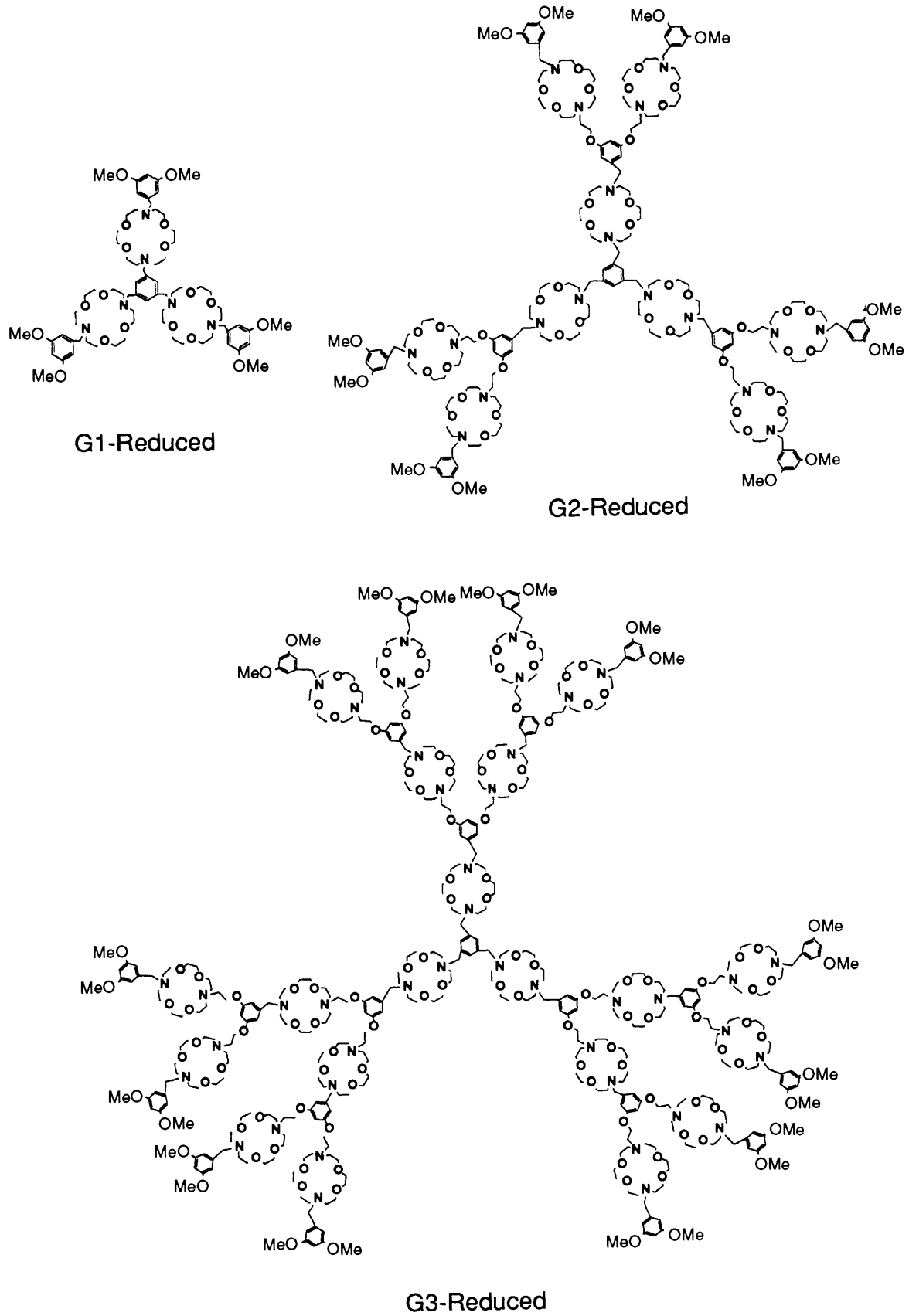
Scheme 2 *Reagents:* i, OH^-; ii, H_2/Pd-C; iii, Z-B1-CO_2H, Me_3CCOCl, Et_3N; iv, benzene-1,3,5-tricarbonyl trichloride, Et_3N; v, borane–dimethyl sulfide

amine linkages by borane–dimethyl sulfide to give a G*n*-Reduced series.

Characterizations.—The theoretical relative molecular masses of G1-Reduced, G2-Reduced and G3-Reduced were computed to be 1351.9, 3899.5 and 8994.7, respectively. Since these compound contain protonizable tertiary amines, one can estimate the relative molecular masses by electrospray mass spectrometry with positive secondary ionization mass spectrometry (SIMS) in acidic media. *m*-Nitrobenzyl alcohol, which easily dissolves these arborols, was used as a matrix. For G1-Reduced, m/z = 1352, which corresponds to G1-Reduced·H^+, was detected. On the other hand, the relative molecular masses of G2-Reduced and G3-Reduced were too high to allow us to detect their parent peaks. Instead, we could detect m/z 780.6 (G2-Reduced·5H^+), 650.8 (G2-Reduced·6H^+) and 558.3 (G2-Reduced·7H^+) for G2-Reduced and 1124.3 (G3-Reduced·8H^+) and 999.5 (G3-Reduced·9H^+) for G3-Reduced. These values are in good accord with the theoretical relative molecular masses.

The stepwise synthesis of arborols should afford the monodispersed molecular mass. We estimated the molecular mass by gel permeation chromatography (GPC). The relation between the molecular mass and the retention volume was calibrated by using monodispersed poly(styrene)s (filled circles in Fig. 1). Here, we tested three samples, G1-CO_2Et, G2-CO_2Et and G3-CO_2Et. The M_w/m_n values, used as a measure of the monodispersity, were estimated to be 1.01, 1.05 and 1.10, respectively. The result allowed us to conclude that these crowned arborols have a monodispersed nature (within experimental error). It is also interesting that the relative molecular masses estimated by GPC were always lower than the theoretical molecular masses: 1528 for G1-CO_2Et (theoretical 1868), 3429 for G2-CO_2Et (theoretical 4945), and 8246 for G3-CO_2Et (theoretical 11 310). We consider that this discrepancy reflects the polymeric conformation of the arborols: poly(styrene)s used for standardization are linear polymers and should be linearly extended in good solvents (THF) whereas crowned arborols have a globular conformation which tends to be more strongly trapped in the gel phase.

Metal-binding Properties.—It is known that polymeric crown ethers behave differently from monomeric crown ethers.[11–14] The most outstanding difference is the formation of a 1:2



Structures of 'crowned' arborols, G1-Reduced, G2-Reduced and G3-Reduced

metal:crown sandwich complex with the crown ethers appended along the polymer chain.[11-14] Hence, polymeric 15-crown-5 and 18-crown-6 show selectivity towards Cs^+ rather than towards Na^+ or K^+. To test if such a cooperative metal-binding is operating in crowned arborols, we estimated their metal-binding ability by two-phase solvent extraction of alkali picrates. As shown in Fig. 2, G1-CO_2Et, G2-CO_2Et, and G3-CO_2Et, in which diaza-18-crown-6 units are connected by

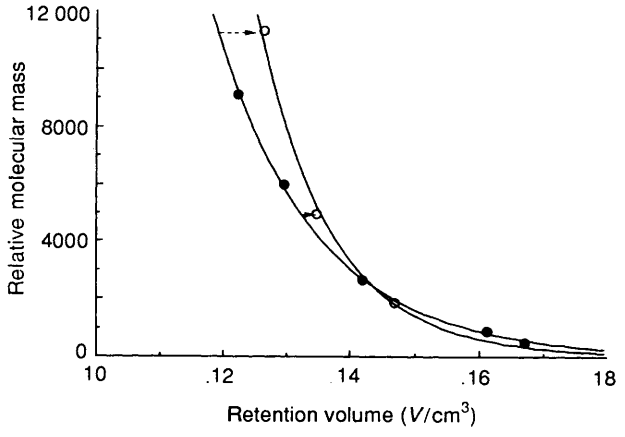


Fig. 1 Plots of relative molecular mass *vs.* retention volume: two serial columns of TSK gel GMHXL-L (7.8 mm × 30 cm), eluent THF, flow rate 1.0 cm^3 min^{-1}, 40 °C: ●, poly(styrene); ○, crowned arborols

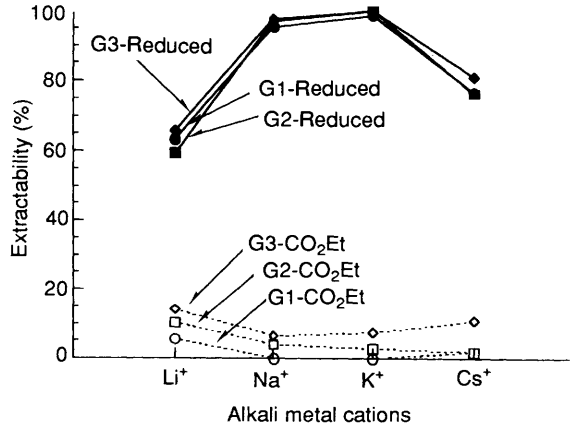


Fig. 2 Extraction of alkali picrates (M^+ Pic^-) from water to CH_2Cl_2. Aq. phase (5 cm^3) contains M^+ Pic^- (2.5×10^{-4} mol dm^{-3}), MOH (0.10 mol dm^{-3}) and MCl (0.50 mol dm^{-3}). Organic phase (5 cm^3) contains arborol ([crown ether unit] = 2.5×10^{-3} mol dm^{-3}).

amide linkages, showed low affinity towards alkali metal cations. The extractabilities (Ex%) were generally low (0–14%). The Ex%-values for Li^+ are somewhat higher than those for Na^+ or K^+. This may be due to the interaction between the amide carbonyls and Li^+. On the other hand, G1-Reduced, G2-Reduced and G3-Reduced, in which the amide groups connecting the diaza-18-crown-6 moiety are reduced to tertiary amines, showed high metal affinity. In particular, the Ex%-values for Na^+ and K^+ are very high (92–100%). The highest affinity was always observed for K^+. This indicates that the crown ethers in a G*n*-Reduced series behave rather independently and do not form a sandwich complex. Examination of CPK molecular models suggested that two crown ethers branching from the same benzene ring can form a sandwich complex with Cs^+ if it is allowed to turn the bonds in the spacer in the same direction and to increase the steric crowding somewhat. Our experimental results indicate, however, that they do not form such a sandwich complex which would be energetically disfavoured, but rather form a 1:1 complex.

Dissolution of Myoglobin into Organic Solvents.—From two-phase solvent extraction of alkali picrates we learned that crown ethers in a G*n*-Reduced series can bind Na^+ or K^+ very strongly and tend to behave independently. This finding suggests that a G*n*-Reduced series would be useful for the dissolution of polyanionic species with Na^+ and/or K^+ countercations or polycationic species with RNH_3^+ groups into organic media. It is known that myoglobin has many NH_3^+ groups and CO_2^- M^+ groups on its surface.[15] If these NH_3^+ and CO_2^- M^+ groups could be bound to the crown ethers in a G*n*-Reduced series, myoglobin would be solubilized into organic media.* As myoglobin has a strong absorption band at λ ~400 nm, one could readily estimate the amount of dissoluted myoglobin.

Here, we tested G1-CO_2Et, G1-Reduced, G2-Reduced, G3-Reduced and a reference compound (7,16-dibenzyl-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane: G0-Reduced). Myoglobin is slightly soluble in dimethyl formamide (DMF) (8.4×10^{-7} mol dm^{-3}). Addition of G1-CO_2Et, G2-Reduced, G3-Reduced and G0-Reduced could not increase the myoglobin concentration significantly. We found, in contrast, that when G1-Reduced was added, the concentration of myoglobin *was* markedly increased. When a DMF solution containing G1-Reduced and myoglobin powder was stirred at 37 °C, the concentration of myoglobin increased with time and reached an equilibrium after 2 h. Fig. 3 shows a plot of myoglobin concentration *vs.* G1-Reduced concentration. Very interestingly, dissolution occurs in an allosteric manner: at [crown ether unit] < 3.9×10^{-5} mol dm^{-3} myoglobin is scarcely solubilized whereas at [crown ether unit] > 3.9×10^{-5} mol dm^{-3} myoglobin is acceleratively solubilized. The result can be rationalized by assuming that myoglobin is not solubilized by complexation with a few G1-Reduced molecules but becomes soluble only when the surface is totally covered by G1-Reduced.

Why is only G1-Reduced able to solubilize myoglobin? We can offer two plausible answers. The first is related to the steric crowding around the crown ether. The crown ethers in G1-Reduced are exposed to the surface of the globular arborol, so that they can easily interact with the NH_3^+ and CO_2^- $-M^+$ groups in myoglobin. In contrast, the crown ethers in G2-Reduced and G3-Reduced are more or less buried in the globular arborols and find it more difficult to approach the surface of myoglobin. The second answer is related to the 'bulkiness' of G2-Reduced and G3-Reduced. The molecular size of G1-Reduced is small enough to cover the myoglobin surface through interaction with the NH_3^+ and CO_2^- M^+ groups. On the other hand, the molecular size of G2-Reduced and G3-Reduced is so large that the interaction may interfere with complexation of the second G2- (or G3)-Reduced with the neighbouring NH_3^+ or CO_2^- M^+ groups. At present, it is not clear which factor is more important. It *is* clear, however, that sterically less crowded 'crowned' arborols are more powerful for dissolution of myoglobin.

Conclusions.—The present paper demonstrates the syntheses and metal- and polypeptide-binding properties of 'crowned' arborols. In the synthesis it was shown that the convergent method is much superior to the divergent method, particularly for the synthesis of high-generation arborols. Through the metal-binding studies it was shown that the crown ether units behave rather independently. Finally, we found that G1-Reduced is a powerful reagent for the solubilization of myoglobin into organic media. We are now synthesizing more lipophilic G1-Reduced analogues, which are expected to solubilize myoglobin and other polypeptides into non-polar solvents.

* Reinhoudt *et al.* recently found that crown ethers can solubilize certain enzymes in organic solvents.[16]

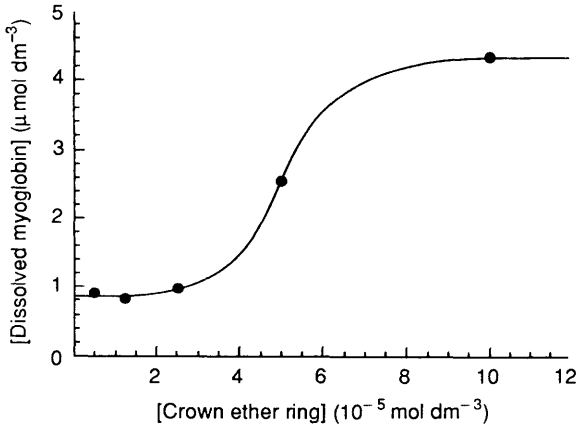


Fig. 3 Plots of dissolved myoglobin *vs* [crown ether] in G1-Reduced: DMF (1.5 cm^3), myoglobin 0.10 mg; mixture was stirred for 3 h at 37 °C

Experimental

General Directions.—All chemicals were of commercial reagent quality and were used without further purification with the following exceptions: benzene and THF were distilled over sodium; DMF was distilled over sodium hydride; triethylamine was distilled over sodium hydroxide; thionyl dichloride was freshly distilled prior to use; chloroform, methanol and ethanol were purified by distillation. Analytical TLC was performed on commercial Merck plates coated with silica gel KF254. Silica gel used for chromatography was Wakogel C-300. Gel filtration was performed with Sephadex LH-20 with methanol as eluent.

^1H NMR spectra were recorded on a JEOL GSX-400 spectrometer using tetramethylsilane as reference. *J* Values are given in Hz. IR spectra were recorded on a JASCO A-100 or an FT/IR-5000 spectrometer. GPC was carried out on a HPLC instrument (Tohso Co. Ltd., Type HLC-8020) with THF as solvent. Mass spectra were obtained on a Hitachi M-2500 spectrometer.

Benzyl 3,5-*Diacetoxybenzoate* **1**.—To a benzene solution (75 cm^3) containing benzyl alcohol (1.27 cm^3, 12.2 mmol) and triethylamine (5.10 cm^3, 36.6 mmol) was added, 3,5-diacetoxybenzoyl chloride (12.2 mmol). The solution was stirred at room temperature for 12 h. The precipitate was removed by filtration. The filtrate was evaporated to dryness and the oily product was used for the next step without further purification: yield ~ 100%; ν_{max}(neat)/cm^{-1} 1780 and 1730 (C=O); δ($CDCl_3$, 30 °C) 2.27 (6 H, s, Me), 5.34 (2 H, s, PhCH_2), 7.31 and 7.79 (3 H, s × 2, ArH of benzoate) and 7.37 (5 H, s, ArH of benzyl).

Benzyl 3,5-*Dihydroxybenzoate* **2**.—To a methanol solution (20 cm^3) containing crude benzyl 3,5-diacetoxybenzoate **1** (1.0 g, 2.54 mmol) was added aq. 5% sodium hydrogencarbonate (8.1 cm^3, 2 mol equiv.) and the solution was stirred at room temperature for 1 h. After acidification of the solution to pH 3 (detected with a pH test paper) by aq. 1 mol dm^{-3} HCl the product was taken up into chloroform and the chloroform solution was dried over $MgSO_4$, and then concentrated to dryness. The solid residue was recrystallized from dichloromethane–hexane (77%), m.p. 131.5–134.7 °C; ν_{max}(KBr)/cm^{-1} 3400 (OH) and 1710 (C=O); δ($CDCl_3$, 30 °C) 5.30 (2 H, s, $PhCH_2$), 6.46 and 6.94 (3 H, s × 2, ArH of benzoate) and 7.31–7.41 (5 H, m, ArH of benzyl).

Benzyl 3,5-*Bis(ethoxycarbonylmethoxy)benzoate* **3**.—To an acetone solution (30 cm^3) containing benzyl 3,5-dihydroxybenzoate **2** (1.0 g, 4.1 mmol) and potassium carbonate (4.5 g, 32.7 mmol) was added an acetone solution (5 cm^3) containing ethyl bromoacetate (2.25 cm^3, 20.4 mmol). After reflux for 4.5 h the product was taken up into ethyl acetate. The organic layer was washed successively with aq. 1 mol dm^{-3} HCl solution and brine. After being dried ($MgSO_4$) the mixture was evaporated to dryness to give a yellow oil (100%), ν_{max}(neat)/cm^{-1} 1770 and 1730 (C=O); δ($CDCl_3$; 30 °C) 1.28 (6 H, t, Me), 4.25 (4 H, q, CH_2Me), 4.62 (4 H, s, CH_2CO), 5.33 (2 H, s, PhCH_2), 6.37 and 7.26 (3 H, s × 2, ArH of benzoate) and 7.38 (5 H, s, ArH of benzyl).

3,5-*Bis(ethoxycarbonylmethoxy)benzoic Acid* **4**.—The benzyl group of triester **3** was deprotected by catalytic hydrogenation with Pd on charcoal in ethanol at room temperature. The product was recrystallized from dichloromethane–hexane (77%), m.p. 111.9–113.3 °C; ν_{max}(neat)/cm^{-1} 3200 (OH), 1750 and 1730 (C=O); δ($CDCl_3$; 30 °C) 1.31 (6 H, t, *J* 7.5, Me), 4.30 (4 H, q, CH_2Me), 4.66 (4 H, s, CH_2CO) and 6.79 and 7.27 (3 H, s × 2, ArH).

3,5-*Bis(ethoxycarbonylmethoxy)benzoyl Chloride* **5**.—A solution of 3,5-bis(ethoxycarbonylmethoxy)benzoic acid **4** (1.0 g, 3.07 mmol) in thionyl dichloride (10 cm^3) was refluxed for 1 h. After evaporation of excess of thionyl dichloride with suction the residual solid was dried *in vacuo*. The product was identified as the acid chloride by IR spectroscopy. The crude product was used for the next step without further purification: ν_{max}(KBr)/cm^{-1} 1750 (C=O).

Z-Bl-CO_2Et.—To a benzene solution (30 cm^3) containing *N*-benzoyloxycarbonyl-1,4,7,13-tetraoxa-7,16-diazacyclooctadecane (1.21 g, 3.07 mmol) was added another benzene solution (55 cm^3) containing 3,5-bis(ethoxycarbonylmethoxy)benzoyl chloride **5** and the solution was stirred at room temperature for 10 h. The mixture was then taken up into ethyl acetate and the solution was washed successively with water, 1 mol dm^{-3} HCl, 5% aq. sodium hydrogencarbonate and brine. After being dried ($MgSO_4$) the mixture was evaporated to dryness to give a new compound Z-Bl-CO_2Et as a yellow oil (95%), ν_{max}(neat)/cm^{-1} 1740, 1700 and 1640 (C=O); δ($CDCl_3$; 30 °C) 1.28 (6 H, m, Me), 3.57–3.62 (20 H, m, OCH_2 and O_2CNCH_2 of crown ether), 3.76 (4 H, s, CH_2HCOAr), 4.25 (4 H, q, CH_2Me), 4.59 (4 H, s, CH_2CO), 5.11 (2 H, s, PhCH_2), 5.56–6.51 (3 H, m, ArH of benzoate) and 7.35–7.36 (5 H, m, ArH of benzyl) (Found: C, 59.45; H, 6.9; N, 3.95. Calc. for $C_{35}H_{48}N_2O_{13}$: C, 59.65; H, 6.87; N, 3.97%).

Z-Bl-H.—This compound was prepared from 3,5-bis(ethoxycarbonylmethoxy)benzoyl chloride in a manner similar to that described for Z-Bl-CO_2Et: yellow oil (97%), ν_{max}(neat)/cm^{-1} 1700 and 1640 (C=O); δ(CD_3OD; 30 °C) 3.47–3.62 (20 H, m, OCH_2 and O_2CNCH_2 of crown ether), 3.78 (10 H, m, CH_2NCOAr and OMe), 5.11 (2 H, s, PhCH_2), 6.44–6.54 (3 H, m, ArH of benzoate) and 7.33 (5 H, m, ArH of benzyl).

Z-Bl-CO_2H.—To a methanol solution (300 cm^3) containing Z-Bl-CO_2Et (13.58 g, 19.28 mmol) was added aq. 1 mol dm^{-3} sodium hydroxide (200 cm^3) and the resultant solution was stirred at room temperature for 4 h. After neutralization (detected with a pH test paper) with 1 mol dm^{-3} HCl the solvent was removed under reduced pressure. Ethyl acetate was added to the residue and then the organic layer was washed with 1 mol dm^{-3} HCl. After being dried ($MgSO_4$) the mixture was evaporated to dryness. The residue was purified by recrystallization from dichloromethane–hexane to give a solid (70%), m.p. 119.1–120.0 °C; ν_{max}(neat)/cm^{-1} 3200 (OH), 1760 and 1700 (C=O); δ(CD_3OD; 30 °C) 3.55–3.63 (20 H, m, OCH_2 and O_2CNCH_2 of crown ether), 3.75 (4 H, s, CH_2NCOAr), 4.67

(4 H, s, CH_2CO), 5.09 (2 H, s, PhCH_2), 6.61 (3 H, s, ArH of benzoate) and 7.30–7.33 (5 H, m, ArH of benzyl).

B1-CO_2Et.—The benzyloxycarbonyl group in Z-B1-CO_2Et was deprotected by catalytic hydrogenation with Pd on charcoal in ethanol in the presence of HCl–1,4-dioxane to give B1-CO_2Et as a yellow oil (96%), ν_{max}(neat)/cm^{-1} 1750 and 1640 (C=O), 1590 (C=C) and 1210 (C–O–C); δ($CDCl_3$; 30 °C) 1.30 (6 H, t, Me), 1.98 (2 H, br s, N^+H), 3.18 (4 H, t, CH_2N^+), 3.64 (16 H, m, OCH_2 of crown ether), 3.92 (4 H, t, CH_2NCOAr), 4.26 (4 H, q, CH_2Me), 4.60 (4 H, s, CH_2CO) and 5.56–6.50 (3 H, m, ArH of benzoate).

General Procedure for the Preparation of Branches.—To a THF solution containing Z-B1-CO_2H and triethylamine (10 mol equiv.) at −5 °C was added slowly a THF solution of pivaloyl chloride (2.0 mol equiv.). The mixture was stirred at −5 °C for 3 h and at room temperature for 1 h. After the solution had been re-cooled to −5 °C a mixture of the amine component (2.1 mol equiv.) and triethylamine (3.0 mol equiv.) was added slowly. The mixture was stirred at −5 °C for 3 h and at room temperature for 10 h. After filtration to remove the precipitate the filtrate was evaporated to dryness. The residue was taken up into ethyl acetate and was washed with water, 1 mol dm^{-3} HCl, 5% aq. sodium hydrogen carbonate and brine. After being dried ($MgSO_4$) the mixture was evaporated. The crude product was purified as described below for each species.

Z-B2-CO_2Et.—This new compound was prepared from B1-CO_2Et and was purified by silica gel chromatography followed by gel filtration: light yellow oil (61%), ν_{max}(neat)/cm^{-1} 1760, 1700 and 1630 (C=O), 1590 (C=C) and 1160 (C–O–C); δ($CDCl_3$; 25 °C) 1.32 (12 H, t, Me), 3.55 (48 H, m, OCH_2 of crown ether), 3.74 (24 H, m, NCH_2), 4.26 (8 H, q, CH_2Me), 4.59 and 4.70 (12 H, s × 2, OCH_2CO), 5.11 (2 H, s, CH_2Ph), 6.53 (9 H, m, ArH of branches) and 7.55 (5 H, m, ArH of benzyl).

Z-B2-H.—This new compound was prepared from B1-H and was purified by gel filtration to give an oil (61%), ν_{max}(neat)/cm^{-1} 1710 and 1630 (C=O) and 1590 (C=C); δ($CDCl_3$; 25 °C) 3.56–3.78 (84 H, m, OCH_2 of crown ether and Me), 4.84 (4 H, s, OCH_2CO), 5.09 (2 H, s, CH_2Ph), 6.54–6.63 (9 H, m, ArH of branches) and 7.33 (5 H, m, ArH of benzyl).

B2-CO_2Et.—This new compound was prepared from Z-B2-CO_2Et (2.14 g, 1.14 mmol) in a manner similar to that described for B1-CO_2Et: light yellow oil (92%), δ(CD_3OD; 25 °C) 1.27 (12 H, t, Me), 3.65 (72 H, m, OCH_2 of crown ether), 4.22 (8 H, q, CH_2Me), 4.71 and 4.88 (12 H, s × 2, OCH_2CO) and 6.60 (9 H, m, ArH).

B2-H.—This new compound was prepared from Z-B2-H (7.10 g, 4.85 mmol) in a manner similar to that described for B1-CO_2Et: oil (95%), ν_{max}(neat)/cm^{-1} 1680–1600 (C=O); δ($CDCl_3$; 25 °C) 3.65–3.78 (84 H, m, OCH_2 of crown ether and Me), 4.86 (4 H, s, OCH_2CO) and 6.54–6.64 (9 H, m, ArH).

Z-B3-CO_2Et.—This new compound was prepared from B2-CO_2Et and purified by silica gel chromatography followed by gel filtration: oil (52%), ν_{max}(neat)/cm^{-1} 1760, 1700 and 1630 (C=O), 1590 (C=C) and 1160 (C–O–C); δ($CDCl_3$; 25 °C) 1.30 (24 H, t, Me), 3.65 (168 H, m, OCH_2 of crown ether), 4.26 (16 H, q, CH_2Me), 4.59 and 4.70 (28 H, s × 2, OCH_2CO), 5.11 (2 H, s, CH_2Ph), 6.55 (21 H, m, ArH of branches) and 7.33 (5 H, s, ArH of benzyl).

Z-B3-H.—This new compound was prepared from B2-H and purified by silica gel chromatography followed by gel filtration: oil (47%), ν_{max}(neat)/cm^{-1} 1710–1600 (C=O); δ($CDCl_3$; 25 °C) 3.58–3.78 (192 H, m, OCH_2 of crown ether and Me), 4.73 (12 H, s, OCH_2CO), 5.11 (2 H, s, CH_2Ph), 6.46–6.59 (21 H, m, ArH of branches) and 7.33 (5 H, m, ArH of benzyl).

B3-CO_2Et.—This new compound was prepared from Z-B3-CO_2Et in a manner similar to that described for B1-CO_2Et: light yellow oil (90%), δ(CD_3OD; 25 °C) 1.27 (24 H, t, Me), 3.65 (168 H, m, OCH_2 of crown ether), 4.22 (16 H, q, CH_2Me), 4.71 and 4.88 (28 H, s × 2, OCH_2CO) and 6.60 (21 H, m, ArH).

B3-H.—This new compound was prepared from Z-B3-H (7.10 g, 4.85 mmol) in a manner similar to that described for B1-CO_2Et: oil (95%), ν_{max}(neat)/cm^{-1} 1680–1580 (C=O); δ(CD_3OD; 25 °C) 3.56–3.78 (192 H, m, OCH_2 of crown ether and Me), 4.90 (12 H, s, OCH_2CO) and 6.54–6.64 (21 H, m, ArH).

General Procedure for the Condensation with a Core.—A solution of the amine hydrochloride (3.2 mol equiv.) and triethylamine (6.4 mol equiv.) in dry THF was treated with benzene-1,3,5-tricarbonyl trichloride. The reaction mixture was stirred under a nitrogen stream at room temperature for 12 h. After filtration the filtrate was evaporated to dryness. The crude product was purified as described below for each species.

G1-CO_2Et.—This new compound was prepared from B1-CO_2Et and purified by gel filtration: oil (83%), ν_{max}(neat)/cm^{-1} 1750 (C=O), 1630 (C=O), 1590 (C=C) and 1200 (C–O–C); δ(CD_3OD; 25 °C) 1.27 (18 H, t, Me), 3.58 (48 H, m, OCH_2 of crown ether), 3.76 (24 H, m, NCH_2), 4.22 (12 H, q, CH_2Me), 4.71 (12 H, s, OCH_2CO), 6.59 (9 H, s, ArH of branches) and 7.55 (3 H, s, ArH of core); FAB–MS m/z 1868 (Found: C, 55.1; H, 6.4; N, 4.2. Calc. for $C_{90}H_{126}N_6O_{36}$·1.5NaCl: C, 55.27; H, 6.49; N, 4.30%). In the purification process of G1-CO_2Et the residual solid was dissolved in ethyl acetate and the solution was washed with brine. NaCl may be trapped in this step. This treatment was not appropriate for G2-CO_2Et and G3-CO_2Et because an emulsion resulted with each.

G2-CO_2Et.—This new compound was prepared from B2-CO_2Et and purified by gel filtration: oil (88%), ν_{max}(neat)/cm^{-1} 1750 (C=O), 1630 (C=O), 1590 (C=C) and 1200 (C–O–C); δ(CD_2Cl_2; 25 °C) 1.27 (36 H, t, Me), 3.65 (216 H, m, CH_2 of crown ether), 4.23 (24 H, q, CH_2Me), 4.62 (24 H, s, OCH_2CO_2), 4.74 (12 H, s, OCH_2CON), 6.53 (27 H, s, ArH of branches) and 7.44 (3 H, s, ArH of core).

G3-CO_2Et.—This new compound was prepared from B3-CO_2Et and purified by gel filtration: glass (76%), ν_{max}(KBr)/cm^{-1} 1750 (C=O), 1630 (C=O), 1590 (C=C) and 1200 (C–O–C); δ($CDCl_3$; 25 °C) 1.29 (72 H, t, Me), 3.59 (336 H, m, CH_2 of crown ether), 3.74 (168 H, m, NCH_2), 4.25 (48 H, q, CH_2Me), 4.60 (48 H, s, OCH_2CO_2), 4.89 (36 H, s, OCH_2CON), 6.56 (63 H, s, ArH of branches and 7.40 (3 H, s, ArH of core).

G1-H.—This new compound was prepared from B1-H and purified by gel filtration: oil (89%), ν_{max}(neat)/cm^{-1} 1660–1600 (C=O); δ(CD_3OD; 25 °C) 3.34–3.78 (99 H, m, OCH_2 and OMe), 6.52 (9 H, s, ArH of branches) and 7.56 (3 H, s, ArH of core) (Found: C, 59.6; H, 7.1; N, 5.7. Calc. for $C_{72}H_{102}N_6O_{24}$·H_2O: C, 59.43; H, 7.01; N, 5.78%).

G2-H.—This new compound was prepared from B2-H and purified by gel filtration: viscous oil (100%), ν_{max}(neat)/cm^{-1} 1680–1600 (C=O); δ($CDCl_3$; 25 °C) 3.55–3.77 (252 H, m, OCH_2 of crown ether and OMe), 4.84 (12 H, s, OCH_2CO), 6.53–6.62 (27 H, m, ArH of branches) and 7.56 (3 H, s, ArH of core)

(Found: C, 54.75; H, 6.65; N, 5.5. Calc. for $C_{204}H_{294}N_{18}O_{72}$·3.5$CHCl_3$: C, 54.53; H, 6.57; N, 5.51%).

G3-H.—This new compound was prepared from B3-H and purified by silica gel chromatography followed by gel filtration: viscous glass (98%), ν_{max}(neat)/cm^{-1} 1680–1600 (C=O); δ($CDCl_3$; 25 °C) 3.57–3.78 (612 H, m, OCH_2 and OMe), 6.51–6.62 (63 H, m, ArH of branches) and 7.55 (3 H, s, ArH of core).

General Procedure for Reduction of Gn-H.—To a THF solution containing the amide precursor at 60 °C was added slowly borane–dimethyl sulfide (excess). The solution was heated for 3 h under a nitrogen stream. Disappearance of the $\nu_{C=O}$ band was confirmed by IR spectroscopy. The solution was concentrated under reduced pressure. The residual amine–borane complex was refluxed for 3 h in aq. 6 mol dm^{-3} HCl. After the mixture had cooled, 15% aq. tetramethylammonium hydroxide was added in order to adjust pH to 12 (detected with a pH test paper). The liberated amine was collected, and purified by gel filtration.

G1-*Reduced.*—This new compound was prepared from G1-H: light brown oil (60%), ν_{max}(neat)/cm^{-1} 1600 (C=C); δ(Cl_2DC_2; 130 °C) 2.99 (24 H, s, NCH_2 of crown ether), 3.58–3.71 (48 H, m, OCH_2), 3.76 (18 H, s, OMe), 3.80–3.92 (12 H, m, NCH_3), 6.37 and 6.63 (9 H, s × 2, ArH of branches) and 7.48 (3 H, s, ArH of core); FAB–MS *m*/*z* 1352.

G2-*Reduced.*—This new compound was prepared from G2-H: light brown oil (63%), ν_{max}(neat)/cm^{-1} 1600 (C=C); δ[$(CD_3)_2SO$; 30 °C] 2.70–2.82 (72 H, m, NCH_2 of crown ether), 3.49–3.70 (216 H, m, OCH_2, NCH_2 and OMe), 3.99 (12 H, m, ArOCH_2), 6.35 and 6.50 (27 H, s × 2, ArH of branches) and 7.16 (3 H, s, ArH of core); positive SIMS *m*/*z* 780.6 ([M + 5 H]$^{5+}$), 650.8 ([M + 6 H]$^{6+}$) and 558.3 ([M + 7 H]$^{7+}$).

G3-*Reduced.*—This new compound was prepared from G3-H: light brown oil (55%), ν_{max}(neat)/cm^{-1} 1600 (C=C); δ(CD_2Cl_2; 30 °C) 2.74, 2.90 and 2.95 (168 H, t × 3, NCH_2 of crown ether), 3.61 (420 H, m, OCH_2 of crown ether), 3.75 (72 H, s, OMe), 3.99 (36 H, t, ArOCH_2), 6.30 and 6.50 (63 H, s × 2, ArH of branches) and 7.14 (3 H, s, ArH of core); positive SIMS *m*/*z* 1124.3 ([M + 8 H]$^{8+}$) and 999.5 ([M + 9 H]$^{9+}$).

Two-phase Solvent Extraction.—An organic solution (dichloromethane, 5 cm^3) containing arborol ([crown ether unit] = 2.5 × 10^{-3} mol dm^{-3}) was mixed with an aq. solution (5 cm^3) containing an alkali metal picrate (2.5 × 10^{-4} mol dm^{-3}), an alkali metal hydroxide (0.10 mol dm^{-3}) and an alkali metal chloride (0.50 mol dm^{-3}). The mixture was vigorously shaken for 30 min at 25 °C. The aqueous phase was separated and subjected to spectroscopic analysis to determine the decrease in the picrate concentration (λ_{max} 353 nm).

Dissolution of Myoglobin into Organic Solvents.—Myoglobin (excess) was dispersed in a DMF solution containing arborol (from horse heart, Sigma). This suspension was sealed, and shaken vigorously at 37 °C. After filtration of the mixture to remove the precipitate the concentration of dissolved myoglobin was determined from the absorption band at 400 nm (Soret band of haem porphyrins).

References

1 G. R. Newkome, G. R. Baker, M. J. Saunders, P. S. Russo, V. K. Gupta, Z.-Q. Yao, J. E. Miller and K. Bouillion, *J. Chem. Soc., Chem. Commun.*, 1986, 752.
2 G. R. Newkome, Z.-Q. Yao, G. R. Baker and V. K. Gupta, *J. Org. Chem.*, 1985, **50**, 2003.
3 G. R. Newkome, Z.-Q. Yao, G. R. Baker, V. K. Gupta, P. S. Russo and M. J. Saunders, *J. Am. Chem. Soc.*, 1986, **108**, 849.
4 D. A. Tomalia, V. Berry, M. Hall and D. M. Hedstrand, *Macromolecules*, 1987, **20**, 1164.
5 K. R. Gopidas, A. R. Leheny, G. Caminati, N. J. Turro and D. A. Tomalia, *J. Am. Chem. Soc.*, 1991, **113**, 7335.
6 K. L. Wooley, C. J. Hawker and M. J. Frechet, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1059.
7 K. L. Wooley, C. J. Hawker and M. J. Frechet, *J. Am. Chem. Soc.*, 1991, **113**, 4252.
8 C. J. Hawker, R. Lee and M. J. Frechet, *J. Am. Chem. Soc.*, 1991, **113**, 4583.
9 For a comprehensive review see D. A. Tomalia, A. M. Naylor and W. A. Goddard III, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 138.
10 T. Nagasaki, M. Ukon, S. Arimori and S. Shinkai, *J. Chem. Soc., Chem. Commun.*, 1992, 608.
11 J. Smid, S. Shah, L. Wong and J. Hurley, *J. Am. Chem. Soc.*, 1975, **97**, 5932.
12 K. Kimura, H. Tamura, T. Maeda and T. Shono, *Polym. Bull.*, 1979, **1**, 403.
13 K. Yagi, J. A. Ruiz and M. C. Sanchez, *Makromol. Chem., Rapid Commun.*, 1980, **1**, 263.
14 For a comprehensive review see J. Smid, *Makromol. Chem. Suppl.*, 1981, **5**, 203.
15 E. Antonini and M. Brunori, *Hemoglobin and Myoglobin in their Reactions with Ligands*, North-Holland, Amsterdam and London, 1971, p. 19.
16 D. N. Reinhoudt, A. M. Eendebak, W. F. Nijenhuis, W. Verboom, M. Kloosterman and H. E. Schoemaker, *J. Chem. Soc., Chem. Commun.*, 1989, 399.

Paper 3/04714H
Received 5*th August* 1993
Accepted 7*th September* 1993