Mimicking biological systems through modular control of solvation: a synthetic receptor that binds in both water or chloroform

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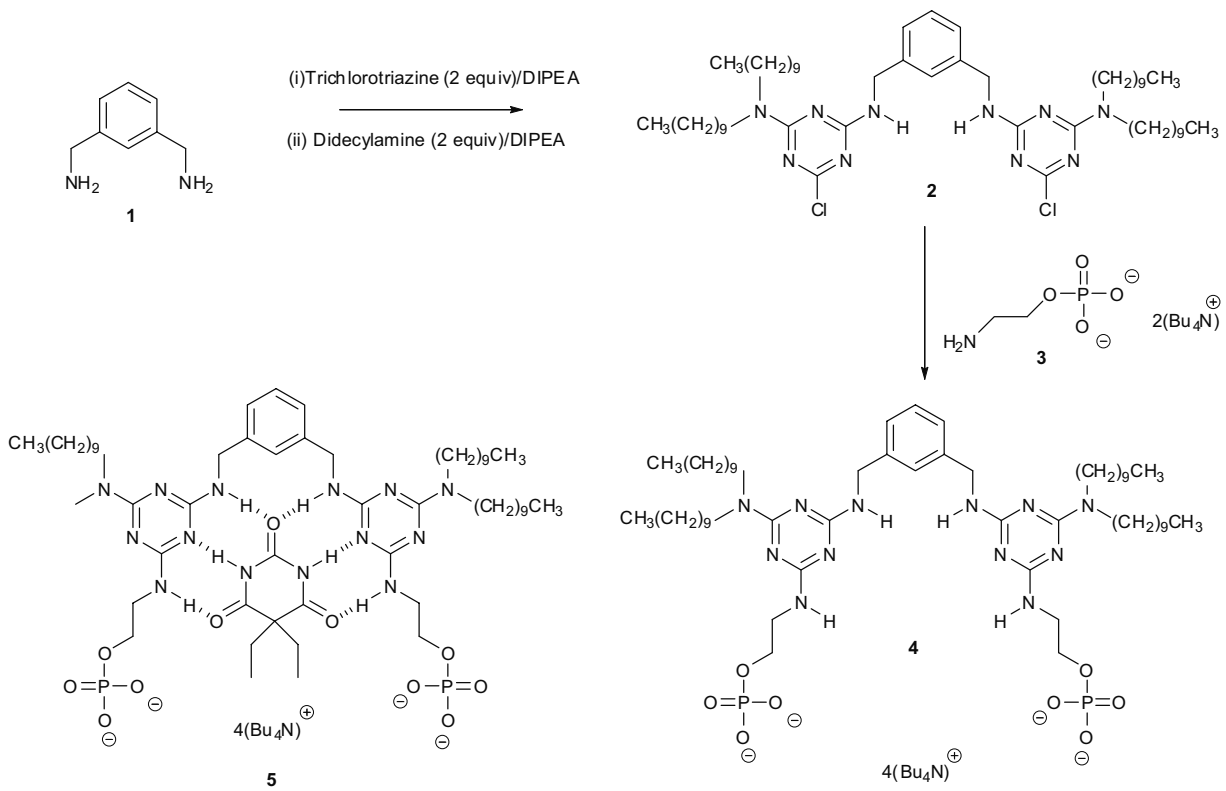
Two receptors are described which are designed to complex barbiturates and cyanuric acids. The functional groups at the binding pocket are polar and are designed to enable guest exchange from water to occur by a dispersion of the host. One receptor has a charged binding pocket lined with two phosphate groups resembling a surfactant. Evidence for the partial extraction of diethylbarbituric acid from water is presented. The more polar guest sodium alloxan 5-f-sulfonatophenylhydrazone was not complexed. The receptors were both used to solubilise two insoluble bis-isocyanuric acids by forming 2:1 complexes in chloroform.

Keywords: barbiturate, Hamilton receptor

Applications of hydrogen bonded based artificial receptors have been restricted because of their incompatibility with aqueous or polar organic solvents.[1] For strong hydrogen bonding interactions to occur a non-competing solvent medium must be used.[2-3] As a consequence of this many artificial receptor systems are not applicable to aqueous biological fluids or for analysis in bioanalytical problems. Additional selective binding forces that are more independent of the solvent polarity have been investigated. These are hydrophobic bonding,[4-5] π–π stacking,[6] π-cation interactions,[1,7] ion-pairing[8-9] and metal–ion coordination.[10-11] Receptors that bind biologically active barbiturates have gained attention since the publication of the 'Hamilton Receptor'.[12] For example the binding characteristics of bis(triazine) receptors with barbiturates have been studied in organic solvents.[13] The interactions between melamines and cyanuric acids have also been extensively studied with binding modes well delineated in organic solvents.[14-15] However these systems have rarely been studied on an aqueous interface which is much more difficult. These types of receptors are of interest for investigation in aqueous systems because six hydrogen bonds can form between host and guest that leads to strong binding. Optimisation of this system might therefore stand a greater chance of success and indicate modulation design for applications in aqueous systems. Barbiturate binding in aqueous media was recently achieved with an amphiphilic copolymer which provided a microenvironment (a micelle) for the aqueous solvation of a hydrogen bonding barbiturate receptor.[16] This paper reports our studies on a barbiturate receptor initially designed to work as a surfactant. Reduced solvation of the polar-head group (the receptor), occurring because it is only partially dissolved, was expected to still allow a dynamic equilibrium to occur and favour hydrogen bonding to a barbiturate. In thermodynamic terms the reduced solvation of the surfactant or receptor head-group has enhanced the entropic disorder of the system, an explanation normally reserved for hydrophobic bonding which shows a marked temperature dependence.[17] Our interest in such systems arose because many biological systems, in which binding occurs from water, are not entirely dissolved in water such as cell membranes, mitochondria,



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DNA, RNA, ribosomes, the rough and smooth endoplasmic reticulum, enzymes, the golgi apparatus and the cytoskeleton, *etc.*[18] Much of a cells machinery is in fact immobilised and hence partially insoluble. Aqueous molecular recognition on this interface for a simple molecular receptor has rarely been investigated and is of topical interest alongside studies using sodium dodecyl sulfate (SDS) micellar microenvironment for facilitating hydrogen bonding molecular recognition in water.[19] The receptor reported in this paper was also used to bind to insoluble bis-isocyanuric acids[20] in an organic solvent giving a soluble complex.

Discussion

Receptor **4** was readily constructed by a stepwise displacement of chlorine atoms from trichlorotriazine firstly with 1,3-bis(aminomethyl)benzene **1** followed by didecylamine to give compound **2** in a one pot procedure. The first two chlorines of trichlorotriazine are readily displaced in a stepwise manner under mild conditions but the third chlorine is more stable and typically requires heating at reflux for displacement to occur.[21] 2-Aminoethyl-di-(tetrabutylammonium)phosphonate **3** was prepared as an ionic liquid by mixing a solution of aminoethyldihydrogen phosphonate with tetrabutylammonium hydroxide in a 1:2 ratio followed by drying *in vacuo*. The counterions were chosen to ensure solubility of the charged receptor in organic solvents as well as allowing it to have surfactant properties. Building block **2** was refluxed in THF for 3 days with 3 equivalents of aminoethyl-di-(tetrabutylammonium)phosphonate **3**. The product was purified by repeated extraction with a water/chloroform mixture then centrifugation to allow the liquids to be decanted. This left receptor **4** as a pure light brown gum. Whereas this synthesis worked a similar attempt to displace both chlorines on 1,3-*N,N'*-bis[4-chloro-6-(diethylamino)-1,3,5-triazin-2-yl]xylylenediamine with dibenzylamine by reflux for 6 days was unsuccessful.[13] Prior to this study the synthesis and crystal structure of 1,3-*N,N'*-bis[4-chloro-6-(dibutylamino)-1,3,5-triazin-2-yl]xylylenediamine was reported by the author.[22] Both guests chosen for this study diethylbarbituric acid **6** and sodium alloxan 5-f-sulfonatophenylhydrazone **7** [23] have some water solubility.

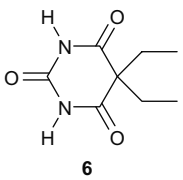
Binding in water

Some simple binding experiments were performed in water. Mixing of the receptor **4** with water gave a fine dispersion reminescent of soapy water like a dispersed surfactant. On standing overnight the dispersion would settle or aggregate forming a clear transparent layer at the top and a layer at the bottom of the vial. The receptor behaviour was advantageous for host-guest studies because it allowed the receptor to be separated from the water layer so that the water could be analysed for guest. A binding experiment was done using the receptor **4** and sodium alloxan 5-f-sulfonatophenylhydrazone **7**. Two standard solutions of the coloured guest were made up and one was stirred with the receptor **4** for 5 hours. The receptor was allowed to settle overnight. The UV-Vis spectra of the standard solution was compared with that of the extracted solution and found to be identical. Sodium 4-[2,4,6-trioxohexahydropyrimidin-5-ylidenehydrazino] benzenesulfonate **7** is a water soluble ionic guest molecule. For comparison a less water soluble and neutral guest was studied, diethylbarbituric acid **6**. Two standard solutions of diethylbarbituric acid **6** were made up in D_2O with a small quantity of a spectator molecule sodium benzoate. One solution was treated for 5 hours with stirring with receptor **4** then allowed to settle overnight. However, the dispersed receptor **4** did not aggregate as in previous experiments and settle even after two weeks and centrifugation. It could not be filtered. It was not possible to assay the recovered solution by NMR to determine if any binding had occurred. The different physical behaviour of receptor **4** in the presence of diethylbarbituric acid **6** however suggests that binding to diethylbarbituric acid **6** may be occurring to cause this change in behaviour. The observation is not unreasonable. When diethylbarbituric acid **6** is complexed in receptor **4** intramolecular hydrogen bonds are formed. In the absence of the guest **6** there is more scope for intermolecular hydrogen bonds to form between the triazine units of **4** leading to aggregation and settling.

Receptor **8** was prepared which possessed a less polar binding pocket. Hydrogen bonding was expected to be stronger in the pocket owing to weaker solvation by water. The two polar alcohols were expected to assist dissolution and partial solvation of the 'head-group' in water but not as much as the charged phosphate groups. Precursor **2** was refluxed with an excess of ethanolamine in THF for 3 days to give receptor **8**. A standard solution of diethylbarbituric acid **6** and sodium benzoate were dissolved in D_2O and split into two. One half of this solution was treated with receptor **8** for 24 h then filtered. The two solutions were then analysed by ^{1}H NMR. The integrations for the two *ortho* hydrogens of the sodium benzoate were both set at 2 and then a comparison of the integrations for the ethyl groups was made. For the standard these were 6.0 (CH_2) and 8.3 (CH_3) and for the extracted solution 5.0 (CH_2) and 7.4 (CH_3). Comparing each pair of integals gives an average percentage extraction of 14%. Repeating the experiment gave similar results. This experiment was easier to perform than the previous experiment using charged surfactant receptor **4** because the suspension of receptor:guest complex or remaining receptor could be filtered from solution. The amount of guest binding is quite low, compared to at least 99% bound in chloroform,[21] but the extraction is an equilibration from water which is a polar hydrogen bonding solvent. To extract a guest at all from water using hydrogen bonding alone is noteworthy.

Binding in chloroform

The receptor **4** is fully soluble in chloroform as well as displaying surfactant like properties. The tetrabutylammonium groups are charged but sufficiently hydrophobic to allow dissolution in organic solvents. As part of a study of dynamic combinatorial libraries of hosts assembled by hydrogen bonding the bis-isocyanuric acids **9–12** had been prepared in the authors group.[20] Despite the hexadecyloxy and decyloxy side chains in compounds **11** and **12** respectively these compounds are still insoluble in chloroform. Binding



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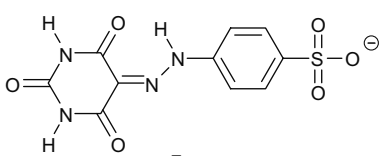

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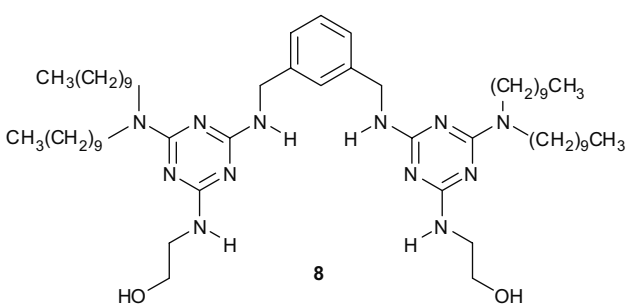

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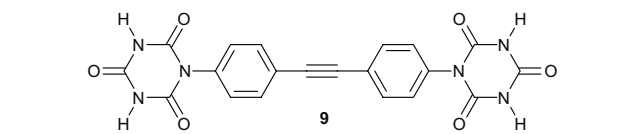
experiments were therefore performed to see if the pincer receptors **4** or **8** could pince each bis-isocyanuric acid at the ends and solubilise it.

A solution of the receptor in chloroform was mixed with a suspension of each guest **9–12** in a 2:1 ratio. The solutions were shaken and sonicated. It was observed that cyanuric acids **9** and **10** remained completely insoluble but cyanuric acids **11** and **12** dissolved completely within one minute. If excess cyanuric acid **11** or **12** was used it did not dissolve. Drawing **13** shows the proposed mode of binding. This mode of hydrogen bonding has been studied previously for receptors of this type which provides preceedent for this.[13] Since two receptors are seen to 'lift-up' the ends of the linear bis-isocyanuric acids **11** and **12** (the bar) by taking them into solution the colloquial term 'weight-lifting' has beeen applied. Although all the guests **9–12** are insoluble (compound **12** shows very slight solubility in chloroform) guests **11** and **12** are more easily solubilised owing to the flexible side chains. Complexation of the receptor to guests **9** and **10** does not overcome the crystal packing forces to allow solubilisation. Solubilisation of insoluble isocyanuric acid components has previously been studied as evidence of binding and stoichiometric complex formation which this system illustrates.[14] This example also illustrates a graded solubility because solubilisation only occurred when side chains were present.

Conclusion

Evidence that the surfactant receptor's **4** and **8** were able to bind to diethylbarbiturate **6** in water was discovered. The physical properties of the receptor **4** changed so that it remained dispersed in water in the presence of **6** but in the absence of **6** aggregation occurred. This was explained by the availability of inter- and intramolecular hydrogen bonding. In the absence of barbiturate **6** intermolecular hydrogen bonding may lead to aggregation, but in the presence of barbiturate **6** intramolecular hydrogen bonds form giving a complex that may be more easily dispersed. Receptor **8** bound weakly to barbiturate **6** from water which was determined by ^{1}H NMR using an internal standard. The receptors **4** and **8** show strong hydrogen bonding to the insoluble bis-isocyanuric acids **10** and **11** leading to their solubilisation in chloroform which is proposed as a 2:1 complex.



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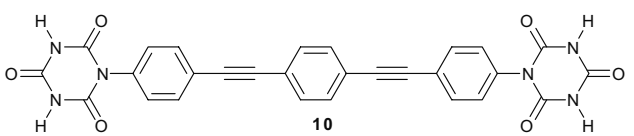

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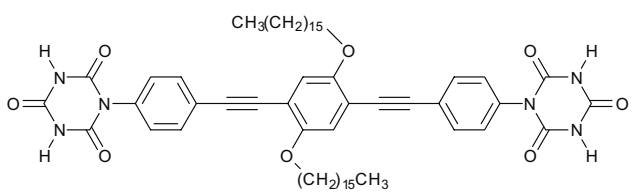

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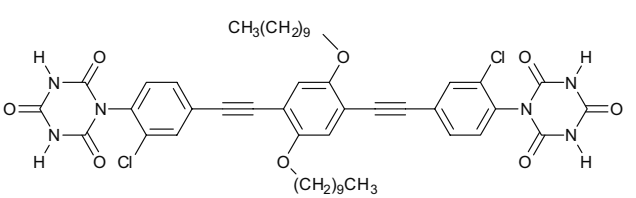

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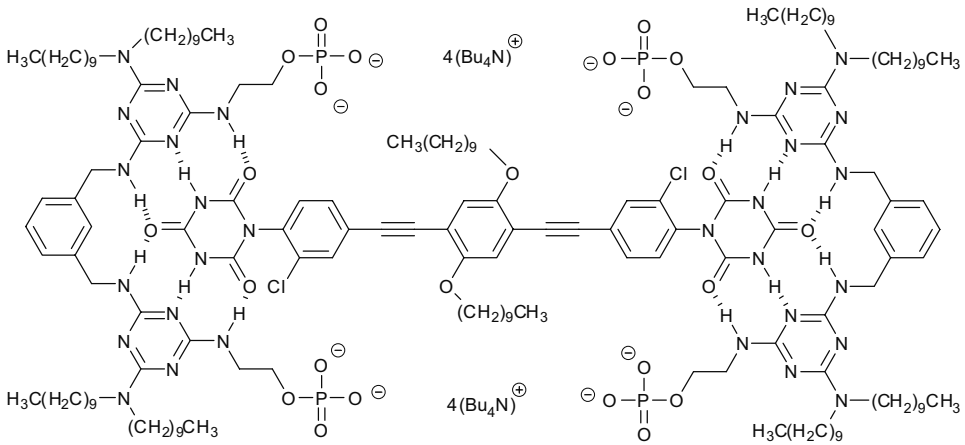
Experimental

UV spectra were recorded on a Perkin-Elmer Lambda 15 UV-VIS spectrometer using $HCCl_3$ as the solvent. IR spectra were recorded on an ATI Mattson FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained at 250 MHz and 62.9 MHz respectively on a Brucker AC 250 spectrometer and at 400 MHz and 100.5 MHz respectively on a Varian 400 spectrometer. Chemical shifts (δ) are given in ppm relative to the residual solvent. Coupling constants (*J* =) are given in Hz. All compounds were prepared to a high standard of purity, greater than 95% as determined by ^{1}H NMR spectra. Low resolution mass spectra were obtained using electrospray ionisation on a Finnigan Navigator Mass Spectrometer and accurate mass at the University of Wales, Swansea using CI and EI modes. Aldrich supplied starting chemicals. THF was dried by distillation from potassium using benzophenone as the indicator.

α,α-Di-N-[(3-didecylamino-5-chloro-1,3,5-triazeno)]amino-m-xylene (**2**)**:** Trichlorotriazine (5.42 g, 30 mmol) and *N,N*-diisopropylethyl amine (7.75 g, 60 mmol) were added to dry THF (100 ml) under nitrogen and cooled to 0 °C. A solution of 1,3-bis(aminomethyl)benzene **1** (2.0 g, 15 mmol) in dry THF (20 ml) was cooled to 0 °C and slowly added dropwise to the trichlorotriazine solution. The solution was stirred for 5 h then allowed to warm to room temperature. A solution of didecylamine (8.74 g, 30 mmol) in THF (10 ml) was added while the temperature was gently increased to 40 °C. The solvent was removed and the residue was dissolved in DCM (50 ml) and washed with dilute HCl (1 M, 100 ml) and water (2 × 100 ml) then dried with sodium sulfate (5 g). The mixture was filtered and the solvent removed *in vacuo* to give the crude product. Baseline contamination was removed by column chromatography (silica gel 50 g; eluent 20% DCM: 80% EtOAc) to yield the title compound (11.2 g, 79%) as a viscous light brown oil; λ_{max} ($CHCl_3$)/nm 293 (log ε 5.0); ν_{max} (NaCl)/cm^{-1} 3259 m, 3112 m, 2965 s, 2905 s, 2865 m, 1750w, 1541vs, 1500w, 1458 m, 1403 m, 1292 m, 1230w, 1073 m, 993 m, 974w, 849w, 801 s, 767w, 656w and 624 m; δ_H (250 MHz; $CDCl_3$) 0.85 (12H, t, *J* = 6.8, CH_3), 1.12–1.61 (64H, m, $CH_3(CH_2)_8R$), 3.39 (8H, m, $N(CH_2R)_2$), 4.57 (4H, d, *J* = 6.0, $PhCH_2N$), and 7.02–7.27 (4H, m, Ph) (H in $PhCH_2NHR$ groups substituted for D); δ_C (62.9 MHz; $CDCl_3$) 14.2, 22.7, 26.7–29.8 (four overlapping resonances) 29.4, 29.6, 31.9, 44.5, 47.5, 125.4, 125.8, 128.4, 139.3, 164.5, 165.5 and 168.4; *m/z* 951.6978 ([M – H]$^+$ ^{35}Cl. $C_{54}H_{93}Cl_2N_{10}$ requires 951.6956), 952.6 (M$^+$ ^{35}Cl, 100%) and 951.6 ([M - H]$^+$ ^{35}Cl, 40%).

Aminoethyl-di-(tetrabutylammonium)phosphonate (**3**)**:** Aminoethyldihydrogen phosphonate (1.0 g, 7 mmol) was added to a solution of tetrabutylammonium hydroxide (9.2 g, 14 mmol) in 40 wt% solution of H_2O. This solution was left drying under rotary evaporation for 4 h, which yielded the *title compound* (4.7 g, 99%) as a viscous clear oil; λ_{max} ($CHCl_3$)/nm 203 (log ε 4.4); ν_{max} (NaCl)/cm^{-1} 3331 m, 3249 m, 3194 s, 2971vs, 2944vs. 2876 m, 1647 s, 1476 s, 1377w, 1113 s, 1085 s, 978 m, 884w, 750 m and 683 m; δ_H (250 MHz; $CDCl_3$) 0.92 (24H, t, *J* = 7.3, CH_3), 1.38 (16H, m, CH_3CH_2R), 1.59 (16H, m, $CH_3CH_2CH_2R$), 2.86 (2H, m, NH_2CH_2R), 3.21 (16H, t, *J* = 7.5, $NCH_2CH_2CH_2R$), 3.83 (2H, m, $POCH_2R$) and 4.58 (2H, s, NH_2); δ_C (62.9 MHz; $CDCl_3$) 13.7, 19.6, 24.0, 58.6 and 77.3 (one resonance missing); *m/z* 242.2843 (Bu_4N^+ $C_{16}H_{36}N_1$ requires 242.2823) and 142.0262 ([$H_2NC_2H_4H_2PO_4$ + H]$^+$ $C_2H_9O_4N_1P_1$ requires 142.0264), 242.1 (Bu_4N^+, 100%) and 139.8 ([$H_2NC_2H_4PO_4$ + H]$^-$, 17%).

α,α-Di-N-[3-didecylamino-5-(2-(di-O-tetrabutylaminophosphonato) ethylamino)-1,3,5-triazeno]amino-m-xylene (**4**): α,α-Di-*N*-[(3-didecylamino-5-chloro-1,3,5-triazeno)]amino-*m*-xylene **2** (1.0 g, 1 mmol), aminoethyl-di-(tetrabutylammonium)phosphonate **3** (1.96 g, 3 mmol) and N,N-diisopropylethyl amine (0.14 g, 3 mmol) were added to dry THF (50 ml) under nitrogen. This mixture was refluxed for three days during which a colour change from colourless to blue and eventually to brown was noted. The solvent was removed under vacuum and the residue was brought up in water/chloroform (1/1, v/v, 50 ml) and shaken vigorously. The resultant opaque water/chloroform mixture was centrifuged and the water/chloroform mixture was decanted off the oil layer. This was repeated another three times to remove excess aminoethyldi(tetrabutylammonium)phosphonate 3 and N,N-diisopropylethyl amine hydrochloride that yielded the title compound



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(1.75 g, 78%) as a light brown gum; λ_{max} ($CHCl_3$)/nm 252 (log ε 5.6); ν_{max} (NaCl)/cm^{-1} 3633w, 3455 m, 3379 m, 3287 m, 3187w, 2956w, 2920 s, 2854 m, 1641 m, 1568 m, 1546 s, 1530 m, 1482 m, 1417w, 1313 m, 1284 m, 1160w 1078w, 1044 m, 768 m and 653 m; δ_H (400 MHz; $CDCl_3$) 0.85 (48H, t, J = 4.8, Bu CH_3), 0.92 (12H, t, J = 7.2, Decyl CH_3), 1.12–1.69 (128H, m, $NCH_2CH_2CH_2CH_3$ and $N[CH_2(CH_2)_8CH_3)_2]$, 3.32–3.48 (44H, m, $N(CH_2R)_2$ and $N(CH_2R)_4$ and NCH_2CH_2OP), 3.94 (4H, bs, $POCH_2R$), 4.50 (4H, bs, $PhCH_2N$) and 7.1 (4H, m) (2 resonance's missing, H on NH substituted for D); δ_C (100 MHz; $CDCl_3$) 13.4, 13.7, 19.8, 22.8, 24.0, 26.9, 27.4, 28.0, 28.3, 29.5, 29.8, 32.1, 47.0 - 47.5 (3 overlapping resonances), 53.9, 58.4, 126.9, 127.7, 129.3, 129.9, 166.6, 167.0 and 170.9; *m/z* 580.3865 ($[M - 4(Bu_4N^+) + 2H^+]^{2-}$ $C_{58}H_{106}N_{12}O_8P_2$ requires 580.3860), 580.6 ($[M - 4(Bu_4N^+) + 2H^+]^{2-}$, 100%).

α,α-*Di-N-[3-didecylamino-5-(2-(hydroxy)ethylamino)-1,3,5-triazeno]amino-m-xylene* **(8):** α,α-Di-*N*-[(3-didecylamino-5-chloro-1,3,5-triazeno)]amino-*m*-xylene **2** (1.0 g, 1 mmol), ethanolamine (0.64 g, 10 mmol) and *N,N*-diisopropylethyl amine (1.35 g, 10 mmol) were added to dry THF (50 ml) under nitrogen. This mixture was refluxed for three days during which a white precipitate could be seen forming. The solvent was removed under vacuum and the residue partially dissolved in chloroform (40 ml) and extracted with water (2 × 60 ml). This solution was dried with $MgSO_4$ (4 g) before being filtered through a cinter (no. 4). The crude product was purified by column chromatography (silica gel, 200 g; eluent 100% EtOAc gradiented to 80% EtOAc: 20% MeOH, R_f 0.66) to yield the title compound (0.73 g, 69%) as a yellow tinted gum; λ_{max} ($CHCl_3$)/nm 251 (log ε 5.2); ν_{max} (NaCl)/cm^{-1} 3407 m, 3336 s, 3292 m, 2931 s, 2854vs, 1572 s, 1506vs, 1458 m, 1434 m, 1374w, 1352 m, 1312w, 1066 m and 810 m; δ_H (250 MHz; $CDCl_3$) 0.85 (12H, t, J = 6.8, RCH_3), 1.12 – 1.62 (32H, m, $NCH_2(CH_2)_8CH_3$), 3.39 (12H, t + m, J = 4.8, NCH_2CH_2OH and NCH_2R), 3.67 (4H, t, J = 4.8, RCH_2OH), 3.89 (2H, bs, ROH), 4.45 (4H, d, J = 6.0, NCH_2Ph), and 7.11–7.25 (4H, m, Ph); δ_C (62.9 MHz; $CDCl_3$) 14.1, 22.7, 27.1, 28.0, 29.1, 29.4, 29.5, 29.6, 29.7, 31.9, 43.8, 44.4, 47.1, 63.6, 126.1, 128.4, 140.1, 164.6, 165.4 and 166.4; *m/z* 1003.8616 ($[M + H]^+$. $C_{58}H_{107}N_{12}O_2$ requires 1003.8616), 1003.9 ($[M + H]^+$, 100%) and 1005.0 (M^+, 58%).

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