Dendritic receptors designed to bind polyanions in both organic and aqueous media

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This paper reports the synthesis of dendrons containing a spermine unit at their focal point. The dendritic branching is based on L-lysine building blocks, and has terminal oligo(ethyleneglycol) units on the surface. As a consequence of the solubilising surface groups, these dendrons have high solubility in solvents with widely different polarities (*e.g.*, dichloromethane and water). The protonated spermine unit at the focal point is an effective anion binding fragment and, as such, these dendrons are able to bind to polyanions. This paper demonstrates that polyanions can be bound in both dichloromethane (using a dye solubilisation assay) and in water (competitive ATP binding assay). In organic media the dendritic branching appears to have a pro-active effect on the solubilisation of the dye, with more dye being solubilised by higher generations of dendron. On the other hand, in water the degree of branching has no impact on the anion binding process. We propose that in this case, the spermine unit is effectively solvated by the bulk solvent and the dendritic branching does not need to play an active role in assisting solubility. Dendritic effects on anion binding have therefore been elucidated in different solvents. The dendritic branching plays a pro-active role in providing the anion binding unit with good solubility in apolar solvent media.

Introduction

Dendrimers and dendrons make a vital contribution to the field of nanochemistry as a consequence of their unique structural features.[1] In particular, the inherent branched structures of dendritic molecules can be exploited in the field of molecular recognition to achieve intriguing binding phenomena.[2] The multiple surface groups of dendritic molecules are able to amplify binding strengths by multivalency phenomena.[3] On the other hand, binding at the encapsulated core (or focal point) of a dendritic structure takes place in a unique micro-environment, which can have a direct impact on the binding event.[4] This mimics the way in which the three-dimensional peptidic architecture places the active site of an enzyme in a well-defined local environment.[5]

Over recent years, anion binding has developed into a key area of supramolecular chemistry,[6] as a consequence of the wide-ranging importance of anions in environmental and biological processes. Whilst binding anions in organic solvents is relatively widespread, anion binding in water is less common and requires specific solutions. The most successful strategies involve the use of transition metal based receptors,[7] which bind anions through dative bond formation, or protonated polyamines,[8] which bind anions using a combination of electrostatics and hydrogen bonding.

Perhaps surprisingly, dendritic receptors for anions remain relatively unexplored. Dendrimers with metallocene-functionalised surfaces have been investigated by the groups of Astruc and Kaifer.[9] These multivalent anion receptors were demonstrated to be capable of binding multiple anions in organic media, and exhibited dendritic amplification of their ability to electrochemically sense the anionic target. We used a similar anion binding strategy within a dendrimer, only located the metallocene unit at the core, rather than on the surface. We demonstrated that in this case, the branching inhibited the magnitude of electrochemical response to halide anions.[10] Vögtle and co-workers prepared polyvalent dendritic ureas, which were able to extract multiple oxo-anions from water into an organic phase.[11] The groups of van Koten and Stoddart prepared dendrimers with internal quaternised amines, which were able to bind anions in organic media.[12] They used anionic dyes to demonstrate that binding had taken place. There have also been a number of studies of dendrimers containing multiple protonated amines which have been shown to effectively internalise anionic dyes.[13]

As part of a wider program investigating supramolecular dendrimer chemistry,[14] we recently employed spermine groups on the surface of dendrons, in order to achieve ultra high-affinity DNA binding in water.[15] Spermine is a simple polyamine used extensively by biological systems as a nucleic acid binder.[16] Protonated polyamines such as spermine, although good anion receptors in aqueous media, are usually ineffective in apolar media due to their poor solubility. We became interested in employing spermine at the focal point of a dendron. We reasoned that with an appropriate choice of dendritic framework, we could generate highly soluble spermine derivatives, which would operate in solvent media with widely different polarities—for example, both water and organic media. This paper reports the results of our initial investigations into this anion binding strategy.

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Results and discussion

Synthesis

We targeted first and second generation L-lysine based dendritic structures **G1** and **G2**, and model system **G0**, each of which has an anion binding spermine unit at the focal point and oligo ethylene oxide surface group(s) (Scheme 1). We chose these surface groups because they provide solubility across a broad range of solvents and furthermore, do not have any innate affinity for anions. It is worth noting that the dendrons are wholly constructed from biocompatible building blocks.

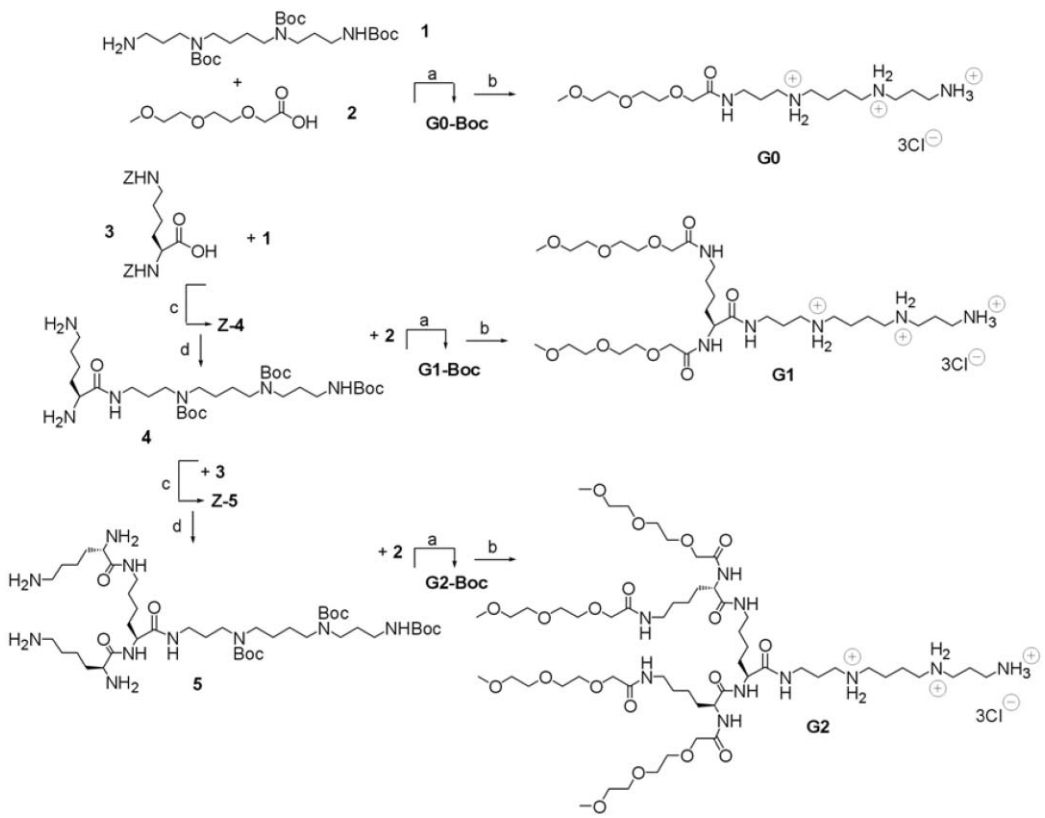
The synthesis of these dendrons was achieved using a divergent strategy (Scheme 1). To synthesise non-dendritic control receptor **G0**, tri-Boc protected spermine **1** was made according to a methodology published by the Blagbrough group,[17] and coupled to 2-(2-(2-methoxyethoxy)ethoxy)acetic acid (**2**) using DCC and HOBt to yield **G0-Boc**. The Boc protecting groups were subsequently removed from the spermine unit using HCl gas in methanol to provide **G0**. To synthesise **G1**, Z-protected lysine **3** was coupled with protected spermine **1** using DCC methodology to yield compound **Z-4**. Removal of the Z protecting groups from the product with palladium hydroxide on carbon and ammonium formate gave key intermediate **4**. Coupling of **4** with acid **2** gave compound **G1-Boc**, and subsequent removal of the Boc groups with HCl gas in methanol provided receptor **G1** in a good overall yield of 41% (four steps). To synthesise **G2**, intermediate **4** was coupled with Z-protected lysine **3** to give compound **Z-5**. The product had the Z protecting groups removed using $Pd(OH)_2$ and ammonium formate to give compound **5**. Coupling compound **5** with acid **2** gave compound **G2-Boc**, which subsequently had the Boc protecting groups removed using HCl in methanol. This sequence gave receptor **G2** in a good overall yield of 27% (six steps) from commercially available starting materials. The compounds were characterised using all the usual spectroscopic methods, and full data can be found in the Experimental section.

Anion binding in organic media

We developed assays to probe the potential of these receptors in both organic and aqueous media. In common with other studies of anion binding in organic media which use dendritic hosts,[12] we decided to use a dye solubilisation assay to probe the affinity of these hosts for anions.

We chose this strategy in preference to NMR titration methods as the N^+–H protons were not readily observed and the other protons in the receptor did not give rise to significant shifts. This is partly a consequence of the fact that the protonated spermine unit in these receptors already has chloride counteranions associated with it, and consequently all anion binding experiments are effectively competition experiments. We therefore decided to use a highly charged anionic guest in order to maximise the binding interaction. However, as highly charged anions are not soluble in organic media, it was necessary to employ a solubilisation assay approach.

Aurin tricarboxylic acid is a powerful inhibitor of cellular processes that are dependent on the formation of protein nucleic acid



Scheme 1 Synthesis of dendritic anion binders **G0**, **G1** and **G2**. (a) DCC, HOBt, Et_3N, DCM; (b) $HCl_{(g)}$, MeOH; (c) DCC, HOBt, Et_3N, DCM; (d) $Pd(OH)_2/C$, $HCOONH_4$, EtOH.

complexes,[18] and is commercially available as the tri-carboxylate anion (Fig. 1). This polyanion is soluble in water, but not in organic media such as dichloromethane (DCM). Our receptors have good solubility in DCM as a consequence of the oligo-ether surface groups. We therefore decided to probe the ability of our receptors to solubilise aurin tricarboxylate into this apolar solvent.

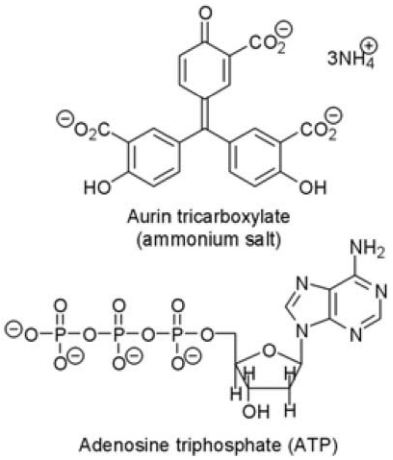


Fig. 1 Structures of anions investigated in this paper.

Solid aurin tricarboxylate (as its tri-ammonium salt) was suspended in a 1 mM solution of the anion receptor (dissolved in DCM) and stirred for 24 h.[19] The mixture was then filtered to remove excess dye, and the solution analysed by UV-Vis spectrometry. Fig. 2 provides a visual assessment of the degree of dye solubilisation in each case and a quantitative measure is given in Table 1 (normalised with respect to the solubilisation caused by **G0**). In the absence of receptor, effectively no dye was solubilised into DCM; however, in the presence of the receptors solubilisation occurred. The extent of solubilisation increased in the order **G0** < **G1** < **G2**. There is, therefore, a clear dendritic effect on the uptake of the anionic dye into organic solvent.

Fig. 2 Solutions resulting from the solubilisation study performed with **G0**, **G1** and **G2** (each 1 mM) in dichloromethane solution with aurin tricarboxylate (ammonium salt).

Table 1 Solubilisation of aurin tricarboxylate into dichloromethane as assessed by UV-Vis spectrometry using absorption at λ_{max} (522 nm) and normalised relative to the uptake exhibited by compound **G0**

Host	Degree of solubilisation
None (DCM alone)	0.02
G0	1.00
G1	1.81
G2	3.64

We have previously demonstrated that interactions between protonated amines and carboxylate anions play an essential role in the solubilisation of aurin-based dyes by dendritic systems.[19b] We propose that in this case, the dendritic effect is a consequence of the dendritic branching, which is compatible with organic solvents such as DCM. Indeed, without the presence of this dendritic branching, the highly charged spermine unit would not be soluble in the solvent (DCM) in the first place, as protonated spermine is not compatible with low-polarity media. Consequently, the ability of the dendritic branching to interact favourably with the solvent enhances the solubility of the overall complex and thus higher generation systems are better able to solubilise the dye.

There is also the possibility of secondary interactions between the anionic dye and hydrogen bonding amide groups in the dendritic branching, which will enhance the degree of uptake—such interactions are well-known in the literature.[20]

Furthermore, it should be noted that the ether units in the dendritic branches may interact with the NH_4^+ cation and further enhance the solubilisation process. However, these ether units also have the potential to interact with the protonated spermine unit at the focal point of the dendron itself, and so we do not believe that ether–NH_4^+ interactions provide the main driving force behind the solubilisation event.

Anion binding in water

We then decided to determine whether our receptors would operate in aqueous solution. We chose to probe the interaction between our polyanionic receptors and adenosine triphosphate (ATP), a biologically-relevant phosphate polyanion. In 1977, Nakai and Glinsmann developed a simple and innovative competition assay to determine the binding between protonated amines and ATP and we applied their methodology in this study.[21] Their method involves placing a constant amount of ATP and cationic resin (Dowex AG1-X2) in tris-chloride buffered water (pH 7.5). Various amounts of soluble receptor are then added. The receptor competes with the solid cationic resin for binding the ATP, and the residual amount of ATP in the solution, which can be determined by UV-Vis spectrometry, reflects the affinity of the receptor for ATP. The Igarashi group have since published minor modifications to handling the data from this procedure.[22] We applied both data-handling approaches to investigate ATP binding in water at pH 7, and for the purposes of comparison also determined the binding of spermine and spermidine (Fig. 3) to the target anion.

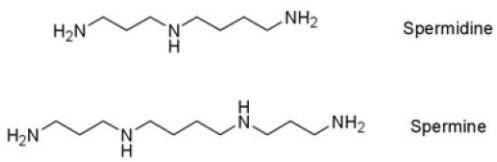


Fig. 3 Structures of spermidine and spermine.

Importantly, the binding constants for spermine and spermidine, generated using the assumption of 1 : 1 binding with the polyanion, were in good agreement with the data in the literature, validating our use of the assay (Table 2). We then investigated the performance of receptors **G0**–**G2** in this assay. It is clear from the data that receptors **G0**, **G1** and **G2** show very similar affinities for

Table 2 Log *K* values determined for ATP binding with the receptors at pH 7 in tris-buffered water using competitive binding assay and assuming a 1 : 1 binding model using methods from ref. 21 and 22

Host	Log K^{21}	Log K^{22}
Spermine	4.06	3.76
Spermidine	3.08	3.22
G0	2.80	3.28
G1	2.74	3.28
G2	2.82	3.37

ATP as spermidine. Although our receptors contain four nitrogen atoms, they are actually more similar to spermidine than spermine, as one of the four nitrogen atoms has been converted to an amide and thus cannot be protonated. Therefore the similarity in behaviour to spermidine is to be expected.

Furthermore, it is clear that receptors **G0**–**G2** all give similar degrees of binding, irrespective of the extent of functionalisation. This indicates that the dendritic branching does not inhibit the ability of the polyamine unit at the focal point to bind ATP anions in water. Furthermore, it indicates that in water, unlike in organic media, the dendritic branching does not assist the anion binding process. In aqueous solution, the polar dendron structure and the spermine binding unit will both be heavily solvated, and we propose it is therefore unable to generate a specific microenvironment at the core. In addition, the affinity of neutral amides for anions is limited in polar media, and there will be no additional anion binding within the dendritic framework. For this reason, we argue there is no dendritic effect in water and the binding afforded by the protonated (solvated) polyamine unit is unaffected by dendritic functionalisation.

Conclusions

In summary, we have demonstrated that these new dendritic structures enable anion binding to be achieved by the same receptor in solvent media with large difference in polarities (DCM, $\varepsilon = 9.1$, H_2O, $\varepsilon = 80$). The dendritic branching plays a pro-active role in enhancing the solubility of the system in apolar media. The dendritic structure has a direct impact on the binding in organic media where it can help ensure the charged spermine unit and its charged guest remain compatible with the surrounding apolar solvent. The more extensive the dendritic branching, the more able it is to solubilise the charged complex, and hence higher generation systems cause enhanced uptake of the dye—a 'dendritic effect'.[23] However, there is no dendritic effect in water, where the whole structure will be largely solvated and the branching will have less impact on the charged binding unit. We propose that these systems, which are compatible with a broad range of solvent systems may have future potential as anion transport agents. Furthermore, the incorporation of additional functionality into the dendritic structures may enable the development of medically-relevant anion complexation agents.

Experimental

Materials and methods

Silica column chromatography was carried out using silica gel provided by Fluorochem Ltd. (35–70 μ). Thin layer chromatography was performed on commercially available Merck aluminium backed silica plates. Preparative gel permeation chromatography was carried out using a 2 m glass column packed with Biobeads SX-1 supplied by Biorad, or a shorter length column (0.5 m) packed with Sephadex LH-20. Proton and carbon NMR spectra were recorded on a Jeol 400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz). Samples were recorded as solutions in $CDCl_3$ and chemical shifts (δ) are quoted in parts per million, referenced to residual solvent. Coupling constant values (J) are given in Hz. DEPT experiments were used to assist in the assignment of ^{13}C NMR spectra. Melting points were measured on an Electrothermal IA 9100 digital melting point apparatus and are uncorrected. Positive ion electrospray mass spectra were recorded on a Finnigan LCQ mass spectrometer. Positive ion fast atom bombardment mass spectra were recorded on a Fisons Instruments Autospec mass spectrometer, with 3-nitrobenzyl alcohol as matrix. Polyethylene glycols and/or polyethylene glycol monomethyl ethers were used as calibrants for HRMS determinations. The isotope distribution observed for mass spectral ions of the larger molecules is consistent with data calculated from isotopic abundances. Infra-red spectra were recorded using an ATI Mattson Genesis Series FTIR spectrometer.

Compound **1** was synthesised according to the methodology previously published by Blagbrough and co-workers.[17] Compounds **2** and **3** were commercially available. The full synthetic methodology and characterisaton data for **G0** can be found in the supplementary information of our previous publication.[15a]

Synthesis and characterisation

Compound Z-4. Compound **1** (4.0 g, 8.75 mmol, 1.1 eq.) and compound **3** (3.63 g, 8 mmol, 1 eq.) were dissolved in EtOAc (100 mL). DCC (2.48 g, 12 mmol, 1.5 eq.), HOBt (1.62 g, 12 mmol, 1.5 eq.) and Et_3N (1.21 g, 12 mmol, 1.5 eq.) were added and the mixture was placed in an ice bath for 1 h and then left to stir at rt for 2 d. Dicyclohexylurea (DCU) was removed by filtration, and the solvents removed by rotary evaporation. The crude product was purified by silica column chromatography (70 : 30 cyclohexane–EtOAc), a white solid was recovered (5.56 g, 62%). ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.28–7.13 (10H, m, C*H* aromatic), 6.98–6.87 (1H, *br* m, N*H* amide), 6.54–6.39 (3H, *br* m, N*H*BOC, N*HZ*), 4.97 (2H, s, OCH_2Ph), 4.94 (2H, s, OCH_2Ph), 3.90 (1H, dd, $J = 5.2$, 8.9 Hz, COC*H*(R)NH), 3.28–3.14 (14H, *br* m, CH_2N), 3.11 (2H, t, $J = 6.6$ Hz, CH_2N), 3.01 (2H, t, $J = 7.3$ Hz, CH_2N), 1.71–1.53 (4H, *br* m, CH_2CH$_2$NH), 1.50–1.21 (37H, *br* m, (CH_3)$_3$C, CH_2CH$_2$NBOC, CH$_2$); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 175.12 (*C*ONH, amide), 158.92, 158.41, 157.78 (*C*ONBoc × 2, *C*ONHBoc, *C*ONHZ × 2, overlapping), 138.43, 138.15, 129.47, 129.44, 128.75 (Ar–C, overlapping), 80.92, 79.93 (*C*(CH$_3$)$_3$ × 3, overlapping), 67.67, 67.31 (*C*H$_2$ benzylic), 56.01 (CO*C*H(R)NH), 46.56, 46.37, 41.81, 40.66, 38.23, 37.61, 37.30, 30.46 (*C*H$_2$, overlapping), 28.78 ((*C*H$_3$)$_3$C × 9, overlapping), 27.64, 25.41, 24.09 (*C*H$_2$CH$_2$N, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3444, 3336 (NH), 1600 (C=O), 1639 (C=O), 1600 (C=O), 1580, 1552 (CONH, amide 2), 1509, 1251 (OCONH carbamate); ESI-MS (m/z) calculated value for $C_{47}H_{74}N_6O_{11}$ 898.5: (ES$^+$) found 921.4 ([M + Na]$^+$, 100%); HR-FAB calculated value for $C_{47}H_{74}N_6O_{11}Na$ 921.5313: found 921.5316; R_f 0.13

(70 : 30 EtOAc–hexane, ninhydrin stain); m.p. 52–54 °C; $[\alpha]_D^{293}$ −5.1 ($c = 1.0$, CH_3OH).

Compound 4. Compound **Z-4** (5.0 g, 5.5 mmol, 1 eq.), ammonium formate (1.0 g, 16.5 mmol, 3 eq.) and palladium hydroxide on carbon (20 wt%, wet) (0.35 g, 2.75 mmol, 0.5 eq.) were refluxed in ethanol (100 mL) for 48 h. The mixture was allowed to cool to room temperature and filtered over celite to remove the solids, and the solvents were removed on a rotary evaporator. The mixture was dissolved in DCM (50 mL), washed with conc. NH_4OH and brine, dried over $MgSO_4$, and the solvents removed on a rotary evaporator. A light yellow viscous oil was recovered (3.05 g, 88%). ^{1}H NMR (CD_3OD, 400 MHz) δ_H 6.71–6.69 (1H, *br* m, N*H* amide), 5.62–5.60 (1H, *br* m, N*H*BOC), 3.60 (1H, dd, $J = 5.2$, 8.9 Hz, COC*H*(R)NH), 3.29–3.15 (10H, *br* m, CH_2N), 3.16 (2H, t, $J = 6.6$ Hz, CH_2NH), 2.73 (2H, t, $J = 7.3$ Hz, CH_2NH$_2$), 1.71–1.53 (4H, *br* m, CH_2CH$_2$N), 1.50–1.21 (37H, *br* m, (CH_3)$_3$C, CH_2CH$_2$NBoc, CH$_2$); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 177.03 (*C*ONH, amide), 157.60, 155.63 (*C*ONBoc × 2, *C*ONHBoc, overlapping), 79.77, 79.72, 78.70 (*C*(CH$_3$)$_3$), 54.96 (CO*C*H(R)NH), 47.76, 47.55, 44.313, 41.04, 37.56, 35.32, 34.92, 32.30, (*C*H$_2$, overlapping), 27.45 ((*C*H$_3$)$_3$C × 9, overlapping), 25.65, 22.72 (*C*H$_2$CH$_2$N, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3443, 3336 (NH), 1638 (C=O), 1553 (CONH, amide 2), 1509, 1251 (OCONH carbamate); ESI-MS (*m*/*z*) calculated value for $C_{31}H_{62}N_6O_7$ 630: (ES$^+$) found 653 ([M + Na]$^+$, 100%). HR-FAB calculated value for $C_{31}H_{62}N_6O_7Na$ 653.4578: found 653.4568.

Compound G1-Boc. Compound **4** (0.30 g, 0.6 mmol, 1 eq.) and carboxylic acid **2** (0.24 g, 0.2 mL, 1.32 mmol, 2.2 eq.) were dissolved in DCM (75 mL). DCC (0.30 g, 1.5 mmol, 2.5 eq.), HOBt (0.202 g, 1.5 mmol, 2.5 eq.) and Et_3N (0.15 g, 1.5 mmol, 2.5 eq.) were added and the mixture was placed in an ice bath for 1 h and then left to stir at rt for 2 d. Dicyclohexylurea (DCU) was removed by filtration, and the solvents removed by rotary evaporation. The crude product was purified by preparative gel permeation chromatography (MeOH, Sephadex), a clear oil was recovered (0.42 g, 0.45 mmol, 75%). ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.99–7.83 (2H, *br* m, N*H* amide), 7.06–7.02 (1H, *br* m, N*H* amide), 6.46–6.36 (1H, *br* m, N*H*BOC), 4.23 (1H, dd, $J = 5.2$, 8.9 Hz, COC*H*(R)NH), 4.01–3.89 (4H, *br* s, OCH_2CONH), 3.80–3.40 (16H, *br* m, OCH_2), 3.35–3.30 (6H, *br* s, OCH_3), 3.28–3.14 (10H, *br* m, CH_2N), 3.13–3.09 (2H, t, $J = 6.6$ Hz, CH_2NH), 3.06–2.95 (2H, t, $J = 7.3$ Hz, CH_2NH), 1.71–1.53 (4H, *br* m, CH_2CH$_2$N), 1.52–1.22 (37H, *br* m, (CH_3)$_3$C, CH_2CH$_2$N, CH$_2$); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 173.78, 172.60 (*C*ONH × 3, amide, overlapping), 158.92, 157.78 (*C*ONBoc × 2, *C*ONHBoc, overlapping), 80.87, 79.93 (*C*(CH$_3$)$_3$ × 3, overlapping), 71.56, 71.37, 71.36, 71.34, 71.28 (*C*H$_2$O, overlapping), 59.11 (OCH$_3$), 55.25 (CO*C*H(R)NH), 46.56, 46.37, 41.81, 40.66, 38.23, 37.61, 37.30, 30.08 (*C*H$_2$, overlapping), 28.78 ((*C*H$_3$)$_3$C × 9, overlapping), 27.64, 25.41, 24.32 (*C*H$_2$CH$_2$N, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3444, 3336 (NH), 1641 (C=O), 1553 (CONH, amide 2), 1509 (CONH, carbamate), 1366 (ether), 1251 (C–O, carbamate). ESI-MS (*m*/*z*) calculated value for $C_{45}H_{86}N_6O_{15}Na$ 973.6 (100.0%), 974.6 (50.2%), 975.6 (16.5%): (ES$^+$) found 973.5 (100%), 974.5 (48.2%), 975.5 (12.4%); R_f 0.61 (70 : 30 EtOAc–hexane, ninhydrin stain); $[\alpha]_D^{293}$ −5.2 ($c = 1.0$, CH_3OH).

Anion receptor G1. Compound **G1-Boc** (0.060 g, 0.79 mmol) was dissolved in MeOH (150 mL) and gaseous hydrogen chloride was bubbled through for 30 seconds. The reaction was allowed to stir for 2 h, after which time the solvent was removed under reduced pressure, and a white solid was recovered (0.39 g, 81%). Yield calculated for HCl salt, FW: 760. ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.99–7.83 (2H, *br* m, N*H* amide), 7.06–7.02 (1H, *br* m, N*H* amide), 4.23 (1H, dd, $J = 5.3$, 8.9 Hz, COC*H*(R)NH), 4.01–3.89 (4H, *br* s, OCH_2CONH), 3.70–3.40 (16H, *br* m, OCH_2), 3.35–3.30 (6H, *br* s, OCH_3), 3.28–3.14 (10H, *br* m, CH_2N), 3.15–2.93 (4H, *br* m, CH_2NH), 2.05–1.27 (14H, *br* m, CH_2CH$_2$N (amide), CH$_2$); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 174.25, 174.19, 173.96 (*C*ONH × 3), 73.07, 71.56, 71.03, (*C*H$_2$O, overlapping), 59.38 (OCH$_3$), 53.25 (CO*C*H(R)NH), 39.34, 37.61, 37.30, 32.03, 30.32, 28.38, 24.50, 24.45 (*C*H$_2$, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3444, 3336 (NH), 1641 (C=O), 1604, 1553 (CONH, amide 2), 1366 (O–CH$_3$, ether); ESI-MS (*m*/*z*) calculated value for $C_{30}H_{62}N_6O_9Na$ 673.5 (100.0%), 674.5 (33.5%): (ES$^+$) found 673.5 (100.0%), 674.5 (30.5%); $[\alpha]_D^{293}$ −5.3 ($c = 1.0$, CH_3OH).

Compound Z-5. Compound **4** (1.25 g, 1.94 mmol, 1 eq.) and compound **3** (1.76 g, 4.26 mmol, 2.2 eq.) were dissolved in DCM (100 mL). DCC (0.88 g, 4.26 mmol, 2.2 eq.), HOBt (0.58 g, 4.26 mmol, 2.2 eq.) and Et_3N (0.43 g, 0.59 mL, 4.26 mmol, 2.2 eq.) were added and the mixture was placed in an ice bath for 1 h and then left to stir at rt for 2 days. Dicyclohexylurea (DCU) was removed by filtration, and the solvents removed by rotary evaporation. The crude product was purified by preparative gel permeation chromatography (Biobeads, 90 : 10 DCM–MeOH), a tacky white solid was recovered (2.26 g, 82%). ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.81–7.70 (2H, *br* m, N*H* amide), 7.28–7.13 (20H, m, C*H* aromatic), 6.71–6.69 (1H, *br* m, N*H* amide G1), 6.63–6.33 (5H, *br* m, N*H*BOC, N*H*Z), 5.05–4.92 (8H, m, OCH_2Ph), 4.25 (1H, dd, $J = 4.5$, 9.1 Hz, COC*H*(R)NH), 4.09 (1H, dd, $J = 5.4$, 8.5 Hz, COC*H*(R)NH), 4.00 (1H, dd, $J = 5.2$, 9.1 Hz, COC*H*(R)NH, 1H), 3.28–3.14 (16H, *br* m, CH_2N), 3.01 (2H, t, $J = 7.3$ Hz, CH_2NH), 1.86–1.04 (53H, *br* m, CH$_2$, CH$_3$); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 175.72, 174.78, 173.69 (*C*ONH, amide), 159.55, 159.04, 158.04 (CONHBoc, CONBoc × 2, CONHZ × 4 overlapping), 138.62, 138.58, 129.93, 129.37 129.23 (Ar–C, overlapping), 81.14, 80.15, (*C*(CH$_3$)$_3$ × 3, overlapping), 67.96, 67.91, 67.47 (Ar–*C*H$_2$, overlapping), 55.85, 55.77 (CO*C*H(R)NH × 3, overlapping), 46.56, 46.37, 41.39, 39.93, 32.86, 32.52, 32.34, 30.42 (*C*H$_2$N, overlapping), 28.74 ((*C*H$_3$)$_3$C × 9, overlapping), 27.64, 25.41, 23.93 (*C*H$_2$CH$_2$N, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3441, 3330 (NH), 1638 (C=O), 1600, 1580 (aromatic), 1553 (CONH, amide 2), 1509, 1251 (OCONH, carbamate); ESI-MS (*m*/*z*) calculated value for $C_{75}H_{110}N_{10}O_{17}Na$ 1445.8 (100.0%), 1446.8 (83.0%), 1447.8 (40.6%): (ES$^+$) found 1445.5 (100%), 1446.6 (70.0%), 1447.8 (30.0%); R_f 0.37 (90 : 10 DCM–MeOH, ninhydrin stain); m.p.: 138–140 °C; $[\alpha]_D^{293}$ −9.4 ($c = 1.0$, CH_3OH).

Compound 5. Compound **Z-5** (2.0 g, 1.40 mmol, 1 eq.), ammonium formate (0.53 g, 8.4 mmol, 6 eq.) and palladium hydroxide on carbon (20 wt%, wet) (0.90 g, 0.71 mmol, 0.5 eq.) were refluxed in ethanol (100 mL) for 48 h. The mixture was allowed to cool to room temperature and filtered over celite to remove the solids, and the solvents were removed on a rotary evaporator. The mixture was dissolved in DCM (50 mL), washed

with conc. NH_4OH and brine, dried over $MgSO_4$, and the solvents removed on a rotary evaporator. A hygroscopic waxy orange solid was recovered (1.02 g, 82%). ^{1}H NMR (CD_3OD, 400 MHz) δ_H 7.80–7.73 (2H, *br* m, N*H* amide G2), 6.71–6.69 (1H, *br* m, N*H* amide G1), 6.31–6.23 (1H, *br* m, N*H*BOC), 4.70–4.50 (8H, br m, NH_2), 4.29–4.04 (3H, br m, COC*H*(R)NH, overlapping), 3.28–3.14 (12H, *br* m, CH_2N), 3.01 (2H, t, $J = 6.5$ Hz, CH_2NH), 2.65 (4H, t, $J = 7.3$ Hz, CH_2NH$_2$), 1.86–1.04 (53H, *br* m, CH_2, CH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 175.73, 174.88, 173.64 (*C*ONH, amide), 159.55, 159.03 (*C*ONBoc × 2, *C*ONHBoc, overlapping), 80.95, 80.91, 79.90, (*C*(CH_3)$_3$), 56.21, 56.18 (CO*C*H(R)NH × 3, overlapping), 42.32, 33.61 (*C*H_2, overlapping), 28.81 ((*C*H_3)$_3$CO × 9, overlapping), 29.53, 27.64, 25.41, 23.93 (*C*H_2CH$_2$N, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3431, 3330 (NH), 1639 (C=O), 1553 (CONH, amide 2), 1509, 1251 (OCONH carbamate); ESI-MS (*m*/*z*) calculated value for $C_{43}H_{86}N_{10}O_9$ 887: (ES$^+$) found 910 ([M + Na]$^+$, 100%); $[\alpha]_D^{293}$ −20.7 ($c = 1.0$, CH_3OH).

Compound G2-Boc. Compound **5** (0.35 g, 0.4 mmol, 1 eq.) and acid **2** (0.36 g, 0.3 mL, 2 mmol, 5 eq.) were dissolved in DCM (50 mL). DCC (0.41 g, 2 mmol, 5 eq.), HOBt (0.27 g, 2 mmol, 5 eq.) and Et_3N (0.2 g, 2 mmol, 5 eq.) were added and the mixture was placed in an ice bath for 1 h and then left to stir at rt for 2 d. Dicyclohexylurea (DCU) was removed by filtration, and the solvents removed by rotary evaporation. The crude product was purified initially by preparative gel permeation chromatography (MeOH, Sephadex), and subsequently by silica column chromatography (90 : 10, DCM–MeOH), a clear oil was recovered (0.44 g, 0.29 mmol, 73%). ^{1}H NMR (CD_3OD, 400 MHz) δ_H 8.12–7.81 (6H, *br* m, N*H* amide), 7.06–7.02 (1H, *br* m, N*H* amide), 6.55–6.36 (1H, *br* m, N*H*BOC), 4.35 (1H, dd, $J = 5.6$, 8.7 Hz, COC*H*(R)NH), 4.27 (1H, dd, $J = 5.2$, 9.1 Hz, COC*H*(R)NH), 4.16 (1H, dd, $J = 5.2$, 9.1 Hz, COC*H*(R)NH), 4.01–3.80 (8H, *br* m, OCH_2CONH), 3.80–3.40 (32H, *br* m, OCH_2), 3.35–3.30 (12H, *br* s, OCH_3), 3.28–3.14 (14H, *br* m, CH_2N), 3.13–3.09 (2H, t, $J = 6.6$ Hz, CH_2NH), 3.01 (2H, t, $J = 7.3$ Hz, CH_2NH), 1.71–1.16 (53H, *br* m, CH_2, CH_3). ^{13}C NMR (CD_3OD, 100 MHz) δ_C 173.62, 172.59, 172.54 (*C*ONH × 7, amides, overlapping), 159.32, 157.87 (*C*ONBoc × 2, *C*ONHBoc, overlapping), 80.91, 79.90 (*C*(CH_3)$_3$ × 3, overlapping), 72.91, 71.39, 71.37, 71.34 (*C*H_2O, overlapping), 59.17 (O*C*H_3), 55.25, 55.02 (CO*C*H(R)NH, overlapping), 46.58, 46.37, 41.83, 40.65, 39.68, 39.65, 38.23, 37.61, 37.30, 30.13 (*C*H_2, overlapping), 28.81 ((*C*H_3)$_3$C × 9, overlapping), 24.31 (*C*H_2CH$_2$N, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3444, 3341 (NH), 1659 (C=O), 1538 (CONH, amide 2), 1509 (CONH, carbamate), 1366 (O–CH_3, ether), 1250 (OCONH, carbamate); ESI-MS (*m*/*z*) calculated value for $C_{71}H_{134}N_{10}O_{25}Na$ 1549.9 (100.0%), 1550.9 (79.3%), 1551.9 (36.2%): (ES$^+$) found 1549.7 (100.0%), 1550.7 (71.8%), 1551.8 (30.6%); also found $[C_{71}H_{134}N_{10}O_{25}Na_2]^{2+}$, with peaks at 786.2 (15.1% of the M$^+$), and others at 786.7 (77% of the M^{2+}), 787.2 (38% of the M^{2+}); R_f 0.25 (90 : 10 DCM–MeOH, ninhydrin stain); $[\alpha]_D^{293}$ −9.2 ($c = 1.0$, CH_3OH).

Anion receptor G2. Compound **G2-Boc** (0.196 g, 0.79 mmol) was dissolved in MeOH (150 mL) and gaseous hydrogen chloride was bubbled through for 30 seconds. The reaction was allowed to stir for 2 h, after which time the solvent was removed under reduced pressure, and a white solid was recovered (0.170 g, 0.79 mmol, 100%). Yield calculated for HCl salt, FW: 1336. ^{1}H NMR (CD_3OD, 400 MHz) δ_H 8.14–7.80 (6H, *br* m, N*H* amide), 7.06–7.02 (1H, *br* m, N*H*), 4.35 (1H, dd, $J = 5.6$, 8.7 Hz, COC*H*(R)NH), 4.27 (1H, dd, $J = 5.2$, 9.1 Hz, COC*H*(R)NH), 4.16 (1H, dd, $J = 5.2$, 9.1 Hz, COC*H*(R)NH), 4.10–3.80 (8H, *br* m, OCH_2CONH), 3.80–3.40 (32H, *br* m, OCH_2), 3.35–3.29 (12H, *br* s, OCH_3), 3.28–3.14 (10H, *br* m, CH_2N), 3.13–2.95 (4H, *br* m, CH_2NH, 8H), 2.11–1.14 (26H, *br* m, CH_2); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 173.63, 172.59, 172.53 (*C*ONH × 7, amides, overlapping), 72.65, 71.72, 71.37, 71.11 (*C*H_2O, overlapping), 58.94 (O*C*H_3), 55.23, 54.99 (CO*C*H(R)NH, overlapping), 39.34, 37.61, 37.35, 32.03, 30.87, 29.78 28.38, 24.50, 24.45 (*C*H_2, overlapping); IR (KBr disc) ν_{max} cm^{-1} 3444, 3336 (NH), 1641 (C=O), 1602 (NH_2^+, NH_3^+), 1553 (CONH, amide 2), 1366 (O–CH_3, ether); ESI-MS (*m*/*z*) calculated value for $C_{56}H_{110}N_{10}O_{19}Na$ 1249.8 (100.0%), 1250.8 (62.6%), 1251.8 (25.4%): (ES$^+$) found 1249.8 (100.0%), 1250.8 (65.5%), 1251.8 (20.5%); also found $[C_{56}H_{110}N_{10}O_{19}Na_2]^{2+}$, with a peak at 637.0 (10% of the intensity of the M$^+$ ion). $[\alpha]_D^{293}$ −2.34 ($c = 1.0$, CH_3OH).

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