Recognition of solvent exposed protein surfaces using anthracene derived receptors†

Andrew J. Wilson,‡ Jason Hong, Steven Fletcher and Andrew D. Hamilton*

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A new class of receptor is described that can selectively bind to the solvent exposed surface of proteins such as cytochrome *c* and lysozyme with low micromolar affinity over cytochrome c551, α-lactalbumin, myoglobin and RNase A, under physiologically relevant conditions (5 mM phosphate, pH 7.4). The use of anthracene as a hydrophobic scaffold allows the receptor to act as a selective chemosensor *via* fluorescence quenching or FRET. The study reveals that co-operative electrostatic interactions over a large surface area dominate binding. Further investigations reveal that the receptor binds to the solvent exposed heme edge of cytochrome *c* inhibiting its reaction with small reducing agents and validating the strategy for the disruption of protein function.

Introduction

The design of molecular receptors capable of binding protein surfaces and disrupting their function is an active area of research due to the importance of protein–protein interactions in many cellular processes.[1,2] Traditionally, this disruption of function is achieved through recognition of well-defined cavities within the protein,[1] while recognition of the solvent-exposed exterior is less explored.[2] Every protein presents a unique distribution of hydrophobic and charged residues at its surface (Fig. 1)[3] that influences the selectivity and affinity with which it interacts with other proteins.[4] Recognition of small peptide fragments has been achieved in aqueous solution using combinations of hydrophobic, electrostatic and metal–ligand interactions, an approach that has been extended further to the recognition of proteins.[5,6] Similarly, numerous reports describing model peptides or miniature proteins focus upon stabilizing and presenting the features of secondary structure critical to a protein–protein interface[7] and in turn this has inspired the development of protein surface mimetics (*proteomimetics*).[8]

In recent years, we have reported synthetic receptors that are capable of binding to the exterior surfaces of cytochrome *c*, α-chymotrypsin, platelet-derived growth factor and other proteins through complementary electrostatic and hydrophobic interactions.[9] Latterly, we have validated this approach for the modulation of protein function by (a) disrupting protein–protein interactions[10] and (b) inducing denaturation of the target protein.[11] In further developing these studies we sought to construct a broader range of receptor designs with the added potential for function in the recognition and sensing of a wider variety of proteins.

In this paper we describe the synthesis and binding properties of a series of anthracene-derived receptors. These compounds have the advantages, relative to our previously-reported porphyrins, of being of lower molecular weight, virtually colourless and exhibiting an efficient fluorescence resonance energy transfer (FRET) based quenching of fluorescence on protein binding. The design strategy leads to selectivity in the recognition and sensing of cytochrome *c* and lysozyme when compared to cytochrome c551, α-lactalbumin, RNase A and myoglobin.[3]

Results and discussion

We chose to focus upon two primary targets in this work with the added goal of incorporating a ready readout of recognition into the receptor design. Cytochrome *c* was an attractive target as we had already shown that the unparied electrons in the ferro form could quench fluorescence upon binding to a fluorogenic receptor.[9a,c] Lysozyme was chosen as a complementary non-metalloprotein that might promote a different fluorescent response. Cytochrome *c* is a highly basic protein (12 kDa, pI 9.79) and plays key roles in electron transfer and apoptosis, that are mediated by complex formation with proteins such as cytochrome *c* peroxidase and Apaf1.[12] A critical recognition region on the surface of cytochrome *c* involves a hydrophobic patch centered on the solvent exposed Fe(III) heme unit that is surrounded by lysine and arginine residues. This domain has been characterised in terms of its interaction with a complementary domain of cytochrome *c* peroxidase.[12b,13] The interaction is dominated by large entropic and small enthalpic contributions and a large negative heat capacity.[13] Likewise the highly basic and helical hen egg white lysozyme is a monomeric protein (14 kDa, pI 9.80) that destroys the cell wall in certain bacteria *via* cleavage of the β-glycosidic bond between the C1 of *N*-acetylmuramic acid and the C4 of *N*-acetylglucosamine of the bacterial peptidoglycan.[14] While there is no specific protein partner associated with lysozyme, there are several crystal structures of lysozyme-antibody complexes with affinities ranging from 10^{-5}–10^{-11} M. Additional investigations have demonstrated that the entire surface of the protein is antigenic.[14,15] In each case, the

Department of Chemistry, PO Box 208107, 225 Prospect Street, Yale University, New Haven, CT 06520-8107, USA. E-mail: Andrew.Hamilton@yale.edu

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‡ *Current address:* School of Chemistry, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK.

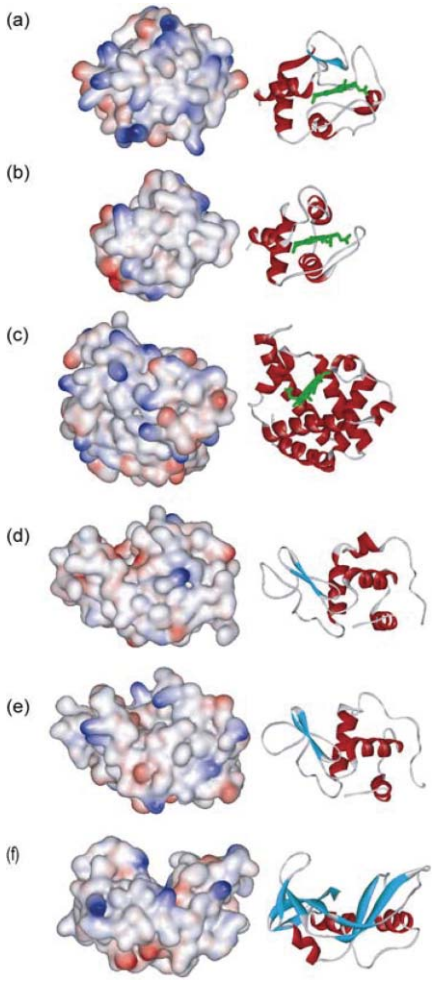


Fig. 1 Electrostatic and ribbon representations of (a) horse heart cytochrome *c*, (b) *Pseudomonas aeruginosa* cytochrome c551, (c) horse skeletal myoglobin, (d) hen egg white lysozyme, (e) bovine milk α-lactalbumin, and (f) bovine RNase A (hydrophobic patches are represented in white and grey, acidic patches in red and basic patches in blue. α-Helical regions are shown in red, β-sheet blue and random coil light grey. Prosthetic heme groups are shown as green cylinders).[3]

interaction between antigen and antibody is characterised by a large number of hydrophobic contacts coupled with critical ion pair interactions.[15]

We reasoned that a fluorescent, hydrophobic group such as anthracene could act as a useful core around which to attach different charged groups. The synthesis of receptor **1a** was achieved in 6 steps and was tailored to match the basic nature of the targeted proteins. Starting with dimethyl-5-hydroxyisophthalate, alkylation and then hydrolysis yielded the benzyl protected di-acid as described previously.[16] EDCI mediated coupling with aspartic acid dimethyl ester followed by hydrogenation over 10% Pd(C) gave 5-hydroxy(bisdimethylaspartate)isophthalamide in 70% yield. Di-alkylation using 9,10-bis(bromomethylanthracene) was achieved with K_2CO_3 as base (32%) followed by ester hydrolysis to yield receptor **1a** (87%) (Scheme 1). Manual docking of receptor **1a** to the known structure of cytochrome *c*[3,12b] indicates that the anthracene scaffold could bind to the solvent exposed hydrophobic patch of the protein with additional interactions resulting from electrostatic contact between the carboxylates of the receptor and basic residues of the protein (Fig. 2a, b).

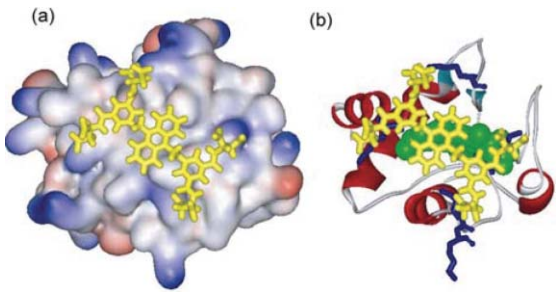


Fig. 2 Receptor **1a** manually docked to cytochrome *c* (a) electrostatic representation and (b) ribbon representation (colours are as for Fig. 1 except the prosthetic heme group is shown in CPK format. The receptor is shown as yellow cylinders. The basic residues proposed as important to the binding interaction are shown as blue cylinders).

Titration of horse heart cytochrome *c* into a solution of receptor **1a** (5 mM phosphate, pH 7.4) resulted in efficient fluorescence quenching (ex 330 nm, similar results are obtained upon excitation at 285 nm) (Fig. 3a) as a result of complex formation with the heme edge region of the protein; a well known recognition site for binding various substrates.[9a,c,e,10b,11,12b,13] Curve fitting of this data to a 1 : 1 binding model gave a dissociation constant of 0.66 μM, reflecting good affinity for a first generation receptor. Job analysis was used to confirm the predominant formation of a 1 : 1 complex (Fig. 3b).

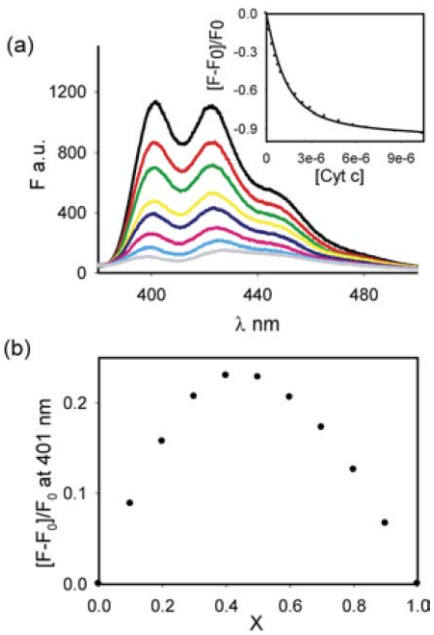
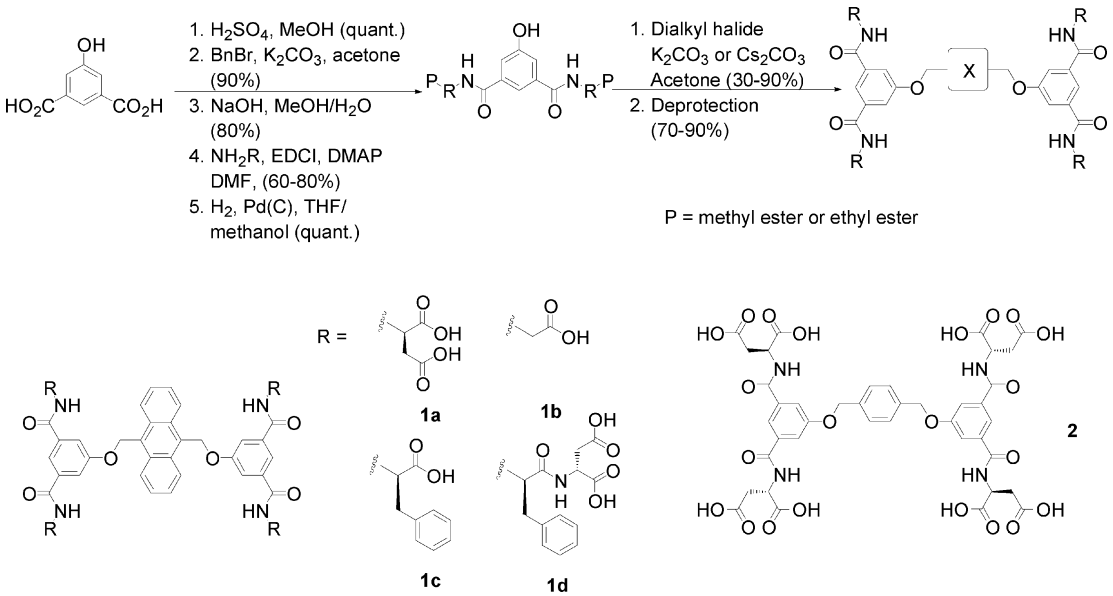


Fig. 3 Fluorescence spectra (ex 330 nm, 5 mM phosphate, pH 7.4) (a) 1 μM receptor **1a** on addition of cytochrome *c*, inset shows the change in signal at 401 nm over the course of the titration as a function of the concentration of cytochrome *c* and the curve derived by regression analysis. (b) Job's plot: emission of receptor **1a** was monitored at 401 nm as a function of the mole fraction of cytochrome *c*. The total concentration of the two species was held constant at 2 μM.



Scheme 1 Synthesis of receptors **1–2**.

This result prompted us to probe the structural properties of the receptor that are key to protein affinity. Receptors **1b–d** and **2** were synthesised in a similar manner to **1a** (Scheme 1). Due to weak fluorescence emission from receptor **2**, the fluorescence recovery of **1a** upon titration of **2** into a 1 : 1 mixture of **1a** and cytochrome *c* was used to assess the binding with cytochrome *c*. In a control experiment, no changes in fluorescence of **1a** were observed upon titration with **2**. There is a deviation from simple 1 : 1 stoichiometry observed for binding of receptor **1c** to cytochrome *c* and this is further reflected in a non-ideal 1 : 1 Job's plot. It has been proposed on several occasions that a second weak binding site on cyctochrome *c* exists[11b] and this may account for the observed deviation from a simple 1 : 1 stoichiometry with the more hydrophobic receptor **1c**. A summary of the results obtained from binding assays is shown in Table 1. Receptors **1a**, **1d** and **2** all have affinity for cytochrome *c* below 1 μM whereas receptors **1b** and **c** bind an order of magnitude less efficiently. This result indicates that the dominant contribution to the binding interaction is derived from the presence of eight appropriately spaced carboxylate residues that act in a cooperative manner across a large surface area. Curiously no significant increase in affinity results from additional hydrophobic residues (**1a** v **1d** and **1b** v **1c**) as was observed for porphyrin derived receptors.[9a,c]

Table 1 Dissociation constants of receptors **1a–d** and **2** with cytochrome *c*[a]

Receptor	Stoichiometry	Dissociation constant
1a	1 : 1	0.66 (±0.048) μM
1b	1 : 1	3.32 (±0.097) μM
1c	1 : 1[b]	1.4 (±0.192) μM
1d	1 : 1	0.30 (±0.013) μM
2	n/d	0.57 (±0.036) μM[c]

[a] 5 mM phosphate, pH 7.4. [b] Non-ideal 1 : 1 Job's plot (see ESI†). [c] Competition assay.

Since the readout of binding relied upon fluorescence quenching, we sought to assess the versatility of the receptor design in the recognition of other proteins. Titration of lysozyme into a solution of receptor **1a** (5 mM phosphate buffer, pH 7.4, ex 285 nm) results in fluorescence resonance energy transfer (FRET). This is due to the overlap in emission spectra of the protein tryptophan residues (em_{max} 340 nm) and absorption spectra of the anthracene moitey (ex 354 nm) suggesting a mutual proximity (<25 Å) from complex formation (data not shown). However, this approach proved unsuitable for studying the binding because of signal overlap at higher concentrations of protein. Consistent with this observation, titration of receptor **1a** into a solution of lysozyme causes a decrease in intensity of the protein emission due to FRET (Fig. 4a). These data were used to derive a dissociation constant of 0.52 μM by curve fitting to a 1 : 1 binding isotherm and the stoichiometry of the interaction was once again confirmed using Job analysis (Fig. 4b).

Accordingly, we sought to assess the selectivity of receptor **1a** for the surface of cytochrome *c* and lysozyme against other proteins. Cytochrome c551 from *Pseudomonas aeruginosa* has a similar tertiary structure and performs a similar biological function to cytochrome *c* yet lacks lysine residues surrounding the prosthetic heme. Likewise α-lactalbumin, which forms a complex with galactosyl transferase, has a similar tertiary structure to that of lysozyme.[3,14a,17] The protein fold and electrostatic representations of both lysozyme and α-lactalbumin (bovine milk) highlight the similar evolutionary origins of the two proteins (Fig. 1).[3,14a] Furthermore, α-lactalbumin contains several tryptophans that are close to the surface suggesting its compatibility with the FRET assay. In addition, RNase A (bovine) containing no tryptophans and myoglobin (horse skeletal),[3] were selected to provide a further measure of selectivity.

The results of the binding assays are summarized in Table 2 and highlight a clear electrostatic dependence upon the binding of receptor **1a** to different proteins. Both cytochrome c551 and myoglobin behaved well in the quenching assay and showed an affinity

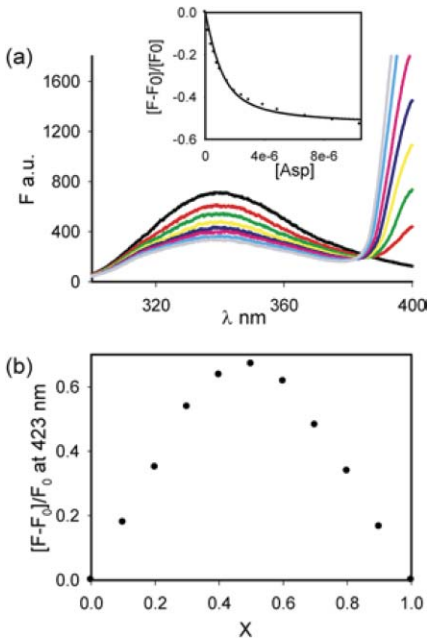


Fig. 4 Fluorescence spectra (ex 285 nm, 5 mM phosphate, pH 7.4) (a) 1 μM lysozyme on addition of receptor **1a**, inset shows the change in signal at 340 nm over the course of the titration as a function of the concentration of receptor **1a** and the curve derived by regression analysis. (c) Job's plot: emission of receptor **1a** was monitored at 423 nm as a function of the mole fraction of lysozyme. The total concentration of the two species was held constant at 2 μM.

Table 2 Dissociation constants for binding of proteins to receptor **1a**[a]

Protein	p*I*	MW	Dissociation constant
Cytochrome *c*	9.8	12 kDa	0.66 (±0.048) μM[b]
Cytochrome c551	5.7	11 kDa	7.36 (±0.960) μM[b]
Myoglobin	7.4	16 kDa	3.56 (±0.160) μM[b]
Lysozyme	9.8	14 kDa	0.52 (±0.062) μM[c]
α − *Lactalbumin*	4.7	14 kDa	>5 μM
RNase A	8.6	14 kDa	n/d

[a] pH 7.4, 5 mM sodium phosphate buffer. [b] Determined by following quenching of anthracene emission (em_{max} = 401 nm, ex 330 nm) upon addition of protein. [c] Determined by following the decrease in protein emission (em_{max} = 340 nm, ex 285 nm) due to FRET from tryptophan of protein (ex = 285 nm) to anthracene upon addition of receptor.

for **1a** an order of magnitude lower than lysozyme and cytochrome *c* reflecting their less basic nature (Table 2). At concentrations up to 5 μM of protein, no FRET was observed between **1a** and α-lactalbumin. At higher concentrations, deviations from a linear dependence upon fluorescence occurred for both receptor and protein suggesting aggregation. However, Beers law was obeyed in both cases up to at least 2×10^{-4} M. Furthermore, no exciplex emission was observed for the anthracene group indicating that inner filter effects are probably responsible for the non-linear behaviour. This suggests that **1a** binds to α-lactalbumin with $K_d >$ 5 μM. Only small changes in the fluorescence of 5 μM **1a** were observed upon titration with RNase A indicating that the binding is likely quite weak. Although RNase A contains no tryptophan residues, the high tyrosine content should still permit some FRET to occur.

The electrophoretic mobility of a protein should in principle be affected upon complex formation with an anionic receptor such as **1a**. A gel shift assay of lysozyme with and without **1a** shows that a discernable change in electrophoretic mobility occurs in the presence of receptor **1a**. No effect is seen with α-lactalbumin further supporting the selectivity of **1a** for lysozyme over α-lactalbumin (Fig. 5).

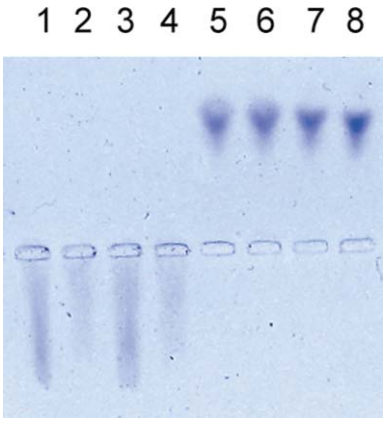


Fig. 5 Agarose gel shift assay: lanes 1 and 3 lysozyme, lanes 2 and 4 lysozyme and **1a**, lanes 5 and 7 α-lactalbumin, lanes 6 and 8 α-lactalbumin and **1a**.

The reaction of cyctochrome *c* with reducing agents represents a useful model of the reaction that occurs between cytochrome *c* and its natural protein partner—cytochrome *c* peroxidase. Previously, it has been shown that the pseudo first order reduction of Fe(III) cytochrome *c* to Fe(II) cytochrome *c* with ascorbate can be inhibited through binding of proteins or surface receptors.[8d,18] Reduction of cytochrome *c* by ascorbate was clearly inhibited in the presence of the receptor **1a** K_{rel} = 2 and **1d** K_{rel} = 4 (Fig. 6) indicating the receptor sterically blocks approach of the reducing agent (Fig. 2). Furthermore, the receptors both have affinity greater than that of the natural protein partner (2.4 μM, 5 mM phosphate, pH 7.0).[12d] Therefore, these receptors could in principle disrupt protein function by binding to the solvent exposed surface of cytochrome *c* displacing the natural protein partner and preventing the redox reaction. The enhanced effect of receptor **1d** is presumably a result of the greater surface area (*i.e.* greater steric impedence to the reducing agent) that it can provide compared to **1a**.

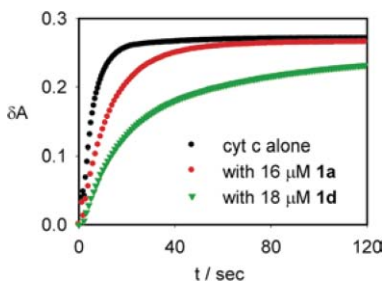


Fig. 6 Reduction kinetics of 10 μM cytochrome *c* by 2 mM ascorbate (5 mM phosphate, pH 7.4) in the presence and absence of receptors **1a** and **1d**.

Conclusions

In summary, the synthesis and characterization of a new class of receptors for exterior protein surfaces represents a strategy that allows for the detection of high affinity binding to a broad range of proteins. We observe marked selectivity among these proteins suggesting this strategy can form the basis for more selective receptors with higher affinity.

Experimental

All solvents were purchased from Malinkroft and all reagents were purchased from Aldrich unless otherwise stated and used without further purification. Yields are not optimized. Purification by column chromatography was carried out using silica gel (230–400 mesh size). Analytical thin layer chromatography (TLC) was conducted using Baker 25 mm silica gel pre-coated glass plates with fluorescent indicator active at UV_{245}. Preparative TLC was conducted using Analtech 1000 mm silica gel pre-coated plates with fluorescent indicator active at UV_{245}. Melting points were obtained using an Electrothermal capillary melting point apparatus and are uncorrected. Both ^{1}H and ^{13}C NMR spectra were acquired on either Bruker DPX 400 or DPX 500 series spectrometers at 400 and 100 MHz, or 500 and 125 MHz, respectively. Chemical shifts are expressed as parts per million using solvent as internal standard and coupling constants are expressed in Hz. The following abbreviations are used: s for singlet, d for doublet, t for triplet, q for quartet, p for pentet, m for multiplet and br for broad. Preparative HPLC was performed on a Waters 600E controller in conjunction with a Waters 490E multiwavelength UV detector. Analytical HPLC was performed on a Rainin HP controller with a Rainin UV detector, both attached to a Dell Optiplex PC running Varian StarWorkstation software. Mass spectrometry data were obtained by the University of Illionois at Urbana-Champaign Mass Spectrometry Laboratory, under the guidance of Dr Steve Mullen. CHN analyses were performed at Atlantic Microlabs New Jersey. UV spectra were measured using an Agilent A453 spectrophotometer or a molecular devices spectramax250 plate reader spectrophotometer. Fluorescence spectra were acquired on a Hitachi F-4500 instrument.

Stock solutions of receptor were prepared to a concentration of 0.1–0.9 mM in 5 mM phosphate buffer. Once the receptors had been dissolved, the pH was adjusted to 7.4 *via* addition of 1 N sodium hydroxide or 1 N HCl. Likewise, stock solutions of hen egg white lysozyme, bovine milk α-lactalbumin, horse skeletal myoglobin, bovine RNase A, horse heart cytochrome *c* and *Pseudomonas aeruginosa* cytochrome c551 (all obtained from Sigma and used without further purification except where stated) were prepared to a concentration of 0.3–1.0 mM in 5 mM phosphate and the pH adjusted to 7.4 *via* addition of sodium hydroxide or HCl (extinction coefficients and p*I*'s were calculated using the program "*ProtParam*" available at www.expasy.org). *Pseudomonas aeruginosa* cytochrome c551 was subjected to ultrafiltration (5 kDa molecular cutoff) then dissolved in 5 mM phosphate and subjected to ultrafiltration two more times to remove undesired salts. The concentration of protein in the stock solution was verified from the absorbance at 280 nm in 6 M GdmHCl, 20 mM phosphate, pH 6.5 (ε = 3.84 × 10^4 dm^{-3} mol^{-1} cm^{-1} lysozyme, 2.84 × 10^4 dm^{-3} mol^{-1} cm^{-1} α-lactalbumin, 1.39 × 10^4 dm^{-3} mol^{-1} cm^{-1} myoglobin, 0.82 × 10^4 dm^{-3} mol^{-1} cm^{-1} RNase A) and found to be within 5–10% of that determined by weight. The concentration of horse heart cytochrome *c* and *Pseudomonas aeruginosa* cytochrome c551 was determined using the molar extinction coefficient at 550 nM of 2.95 × 10^4 after reduction using dithionite. No deviations from Beers law were observed for any of the receptors in the concentration ranges studied indicating they were monomeric in solution. During the course of all titrations the guest solution contained a concentration of host equivalent to that in the host solution and the volume added was kept small to minimize large changes in the concentration of guest, which were corrected for in the regression analysis.

Eqn (1) operating in SigmaPlot was used to derive the dissociation constant for a 1 : 1 binding isotherm:

$$f = m[(nc + x + K) - \sqrt{\{(n*c + x + K)^2 - 4ncx\}}]/2nc \qquad (1)$$

f = change in relative fluorescence, m = maximum value of f, n = stoichiometry, c = concentration of receptor, K = dissociation constant, x = concentration of protein added.

Eqn (2) operating in SigmaPlot was used to derive the apparent dissociation constant in the competition assay and then eqn (3) was used to calculate the real dissociation constant

$$f = f_{max}*[(s + x + k_d) - \sqrt{\{(s + x + k_d)^2 - 4xs\}}]/2s \qquad (2)$$

$$K_{dcomp} = K_d/(1 + s/K_{drec}) \qquad (3)$$

f = change in fluorescence, f_{max} = maximum change in fluorescence, s = concentration of protein, x = concentration of competitor added, K_d = apparent dissociation constant, K_{dcomp} = real dissociation constant of competitor, K_{drec} = dissociation constant of receptor as derived from eqn (1).

Electrophoretic mobility shift assay

Non-denaturing gel-shift assay was performed on thin (10 mm), 1% agarose gel in 5 mM sodium phosphate gel buffer. 5 μL of sample (protein = 8 μM, receptor 16 μM) were loaded on the gel, and power (constant at 100 V) was applied for 1 hour. The gel was fixed briefly in acetone, washed with water, and dried. The gel was stained quickly with Coomasie blue R250 and destained in 10% acetic acid solution.

UV–Vis-monitored ascorbate reduction assay

A solution containing cytochrome *c* was incubated with or without synthetic agent in a 2 mL quartz cuvette with a pathlength of 1 cm, and the volume was adjusted to 1.9 mL by addition of 5 mM sodium phosphate buffer (pH 7.4). After 10 min of incubation at rt, 100 μL of a 20 mM stock solution of ascorbate in 5 mM sodium phosphate buffer (pH 7.4) were added to the cuvette. The final concentration of cytochrome *c* was 15 μM and of receptor between 16–18 μM. The cuvette was immediately agitated with a glass rod for <1 second, and the spectrophotometer started. The instrument automatically measured the absorbance at 550 nm, every 0.5 seconds, for at least 1 min. The collected data was analyzed based on the equation derived from a pseudo first order rate expression: $\Delta Abs = max*(1 - e^{-kobs*t})$; where $\Delta_{Abs} = Abs_t - Abs_{t0} = 0$, and t = time in seconds. Both *max* and *k* were floated,

and were determined by regression analysis in the SigmaPlot 2001 software. The parameter *max* corresponds to maximal Δ_{Abs} as *t* approaches infinity or completion of reaction, and the parameter *k*obs corresponds to the observed pseudo-first order rate constant in units of s^{-1}. The relative rates of reaction were then calculated based on the following equation $K_{rel} = K_{(cyt\ c\ +\ receptor)}/K_{cyt\ c}$.

Benzoyloxycarbamoylphenylalanylaspartic acid diethyl ester

To a stirred solution of benzoyloxycarbamoylphenylalanine (3.00 g, 10.0 mmol), aspartic acid hydrochloride diethyl ester (2.26 g, 10.0 mmol), DMAP (1.22 g, 10.0 mmol) in dichloromethane (150 mL) at 0 °C was added EDCI (1.91 g, 10.0 mmol). The reaction mixture was then allowed to warm to room temperature and stirred overnight. The reaction mixture was washed successively with hydrochloric acid (1 N, 25 mL), saturated sodium bicarbonate (25 mL) and saturated sodium chloride then dried over magnesium sulfate, filtered and concentrated to *ca.* 15 mL. The solution was passed through a short pad of silica gel using 10% ethyl acetate in dichloromethane as eluent and concentrated to leave the product (3.91 g, 83%) as a white solid, mp 129.5–132 °C (Found: C, 63.6; H, 6.4; N, 6.0. $C_{26}H_{31}O_7N$ requires C, 63.8; H, 6.4; N, 5.9%); δ_H (400 MHz, $CDCl_3$) 1.27 (6H, dt, *J* 7.0 and 7.0), 2.82 (1H, dd, *J* 17.1 and 4.5), 3.0 (1H, dd, *J* 16.9 and 4.5), 3.15 (2H, m), 4.13 (2H, q, *J* 7.0), 4.22 (2H, q, *J* 7.0), 4.52 (1H, m), 4.81 (1H, dt, *J* 7.8 and 4.5), 5.11 (2H, s), 5.36 (1H, d, *J* 7.3), 6.89 (1H, d, *J* 7.8), 7.31 (10H, m); δ_C (100 MHz, $CDCl_3$) 14.46, 14.49, 36.59, 38.81, 49.08, 56.23, 61.48, 62.33, 67.42, 127.44, 128.44, 128.58, 128.93, 129.05, 129.75, 136.42, 136.55, 156.22, 170.61, 171.07, 171.18; *m/z* (EI) 470 (M^+).

Phenylalanylaspartic acid diethyl ester

A solution of benzoyloxycarbamoylphenylalanine aspartate diethyl ester (3.00 g, 6.38 mmol) and 10% palladium on carbon (0.16 g) in 1 : 1 tetrahydrofuran–methanol (25 mL) was stirred under an atmosphere of hydrogen for 6 hours. When the reaction was complete, the solution was filtered through celite and concentrated *in vacuo* to yield the product (2.05 g, 96%) as an off yellow glass, mp 207–209 °C; δ_H (400 MHz, $CDCl_3$) 1.25 (6H, m), 2.73 (1H, dd, *J* 13.6 and 9.0), 2.78 (1H, dd, *J* 16.9 and 4.8), 3.00 (1H, dd, *J* 16.7 and 4.8), 3.23 (1H, dd, *J* 13.6 and 4.0), 3.69 (1H, dd, *J* = 9.0 and 4.0), 4.13 (2H, m), 4.21 (2H, m), 4.83 (1H, dt, *J* 8.3 and 4.8), 7.27 (5H, m), 8.12 (1H, d, *J* 8.3); δ_C (100 MHz, $CDCl_3$) 14.50, 14.51, 36.91, 41.16, 48.71, 56.61, 61.40, 62.21, 127.26, 129.10, 129.73, 137.91, 171.09, 171.13, 174.43; *m/z* (EI) 337 (M^+).

General procedure for the synthesis of benzyl protected bis(isophthalamides)

5-Benzyloxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide. To a stirred solution of 5-benzyloxyisophthalic acid (prepared as described previously[16]) (0.40 g, 1.47 mmol), DMAP (0.45 g, 3.67 mmol) and aspartic acid dimethyl ester hydrochloride (0.64 g, 3.23 mmol) in dimethyl formamide (25 mL) was added EDCI (0.70 g, 3.67 mmol). The reaction mixture was stirred overnight then partitioned between water (150 mL) and ethyl acetate (3 × 50 mL). The organic layer was washed successively with HCl (1 N, 50 mL), saturated bicarbonate (50 mL) and saturated sodium chloride (50 mL) then dried over magnesium sulfate, filtered and concentrated. The resultant crude product was passed through a short pad of silica (1% methanol in dichloromethane) to yield the product (0.54 g, 70%) as a white solid, mp 101.5–103.5 °C (Found: C, 57.3; H, 5.3; N, 4.9. $C_{27}H_{30}O_{11}N_2{\cdot}CH_3OH$ requires C, 56.9; H, 5.8; N, 4.7%); δ_H (400 MHz, DMSO-d_6) 2.85 (2H, dd, *J* 16.4 and 8.0), 2.96 (2H, dd, *J* 16.4 and 6.0), 3.62 (6H, s), 3.65 (6H, s), 4.84 (2H, dt, *J* = 8.0, 6.3 Hz), 5.22 (2H, s), 7.36 (1H, d, *J* = 8.3 Hz), 7.42 (2H, t, *J* = 7.0), 7.49 (3H, d, *J* = 7.0), 7.66 (2H, s), 7.95 (1H, s), 9.09 (2H, d, *J* = 7.8 Hz); δ_H (100 MHz, $CDCl_3$) 36.40, 49.51, 52.54, 53.36, 117.45, 118.27, 128.14, 128.71, 129.11, 135.88, 136.36, 159.47, 166.35, 171.48, 171.89; *m/z* (EI) 558.1860 (M^+). $C_{27}H_{30}O_{11}N_2$ requires 558.1850).

5-Benzyloxy-*N*-bis(glycine ethyl ester) isophthalamide. 5-Benzyloxyisophthalic acid[16] (0.40 g, 1.47 mmol), DMAP (0.45 g, 3.67 mmol), glycine ethyl ester hydrochloride and EDCI (3.67 mmol, 0.7 g). Crude product was purified by silica gel chromatography (1% methanol in dichloromethane) to yield the product (0.46 g, 72%) as a clear viscous oil; δ_H (400 MHz, $CDCl_3$) 1.28 (6H, t, *J* 7.0), 4.15 (4H, d, *J* 5.6), 4.21 (4H, q, *J* 7.0), 4.98 (2H, s), 7.32 (5H, m), 7.45 (2H, s), 7.57 (2H, t, *J* 5.6), 7.68 (1H, s); δ_C (100 MHz, $CDCl_3$) 14.55, 42.34, 53.83, 62.09, 117.40, 117.71, 128.07, 128.58, 129.03, 135.72, 136.55, 159.43, 167.13, 170.74; *m/z* (EI) 442.1741 (M^+). $C_{23}H_{26}O_7N_2$ requires 442.1740).

5-Benzyloxy-*N*-bis(phenylalanine methyl ester) isophthalamide. 5-Benzyloxyisophthalic acid[16] (1.00 g, 3.68 mmol), DMAP (1.12 g, 0.919 mmol) phenylalanine methyl ester hydrochloride (1.74 g, 8.09 mmol) and EDCI (1.76 g, 0.919 mmol). The reaction mixture was purified by silica gel chromatography (30% ethyl acetate in dichloromethane) to leave the product (1.76 g, 80%) as an amorphous white solid (Found: C, 70.8; H, 6.0; N, 4.4. $C_{35}H_{34}O_7N_2$ requires C, 70.7; H, 5.8; N, 4.7%); δ_H (400 MHz, $CDCl_3$) 3.20 (2H, dd, *J* 13.9 and 5.8), 3.29 (2H, dd, *J* 13.9 and 5.8 Hz), 3.77 (6H, s), 5.07 (2H, dt, *J* 7.6 and 5.8 Hz), 5.11 (2H, s), 6.62 (2H, d, *J* 7.6), 7.13 (4H, d, *J* 8.0), 7.33 (11H, m), 7.48 (2H, s), 7.61 (1H, s); δ_C (100 MHz, $CDCl_3$) 38.34, 52.90, 54.13, 70.90, 117.32, 117.86, 127.71, 128.06, 128.72, 129.11, 129.68, 136.11, 136.15, 136.34, 159.53, 166.13, 172.23; *m/z* (EI) 594.2358 (M^+). $C_{35}H_{34}O_7N_2$ requires 594.2366.

5-Benzyloxy-*N*-bis(phenylalanylaspartic acid diethyl ester) isophthalamide. 5-Benzyloxyisophthalic acid[16] (0.69 g, 2.55 mmol), DMAP (0.68 g, 5.61 mmol), phenylalanylaspartic acid diethyl ester (1.88 g, 5.61 mmol) and EDCI (1.07 g, 5.61 mmol). Crude product was purified by silica gel chromatography (20% ethyl acetate in dichloromethane) to yield the product (1.45 g, 63%) as a white solid, mp 160–162 °C (Found: C, 64.5; H, 6.2; N, 6.1. $C_{49}H_{57}O_{13}N_4$ requires C, 64.7; H, 6.2; N, 6.2%); δ_H (400 MHz, $CDCl_3$) 1.15 (6H, t, *J* 7.0), 1.20 (6H, t, *J* 7.0), 2.83 (2H, dd, *J* 16.4 and 5.8), 2.92 (2H, dd, *J* 16.2 and 5.8), 3.18 (2H, dd, *J* 14.1 and 8.6), 3.29 (2H, dd, *J* 14.1 and 5.0), 4.70 (1H, dd, *J* 11.3 and 4.3), 4.81 (1H, dd, *J* 11.3 and 3.0), 4.89 (4H, m), 7.26 (19H, m), 7.54 (1H, s), 7.90 (2H, brs); δ_C (100 MHz, $CDCl_3$) 14.45, 14.47, 36.38, 38.27, 49.40, 55.49, 61.43, 62.21, 70.48, 116.69, 118.54, 127.17, 128.27, 128.48, 128.92, 128.96, 129.61, 135.13, 136.71, 137.58, 158.88, 166.23, 171.01, 171.43, 172.37; *m/z* (EI) 909.3925 (M^+). $C_{49}H_{57}O_{13}N_4$ requires 909.3922.

General procedure for the debenzylation of bis(isophthalamides)

5-Hydroxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide. A solution of 5-benzyloxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide (0.32 g, 0.57 mmol) and palladium on carbon (0.05 g) in tetrahydrofuran (15 mL) was stirred under an atmosphere of hydrogen for 18 hours. When the reaction was complete, the solution was filtered through celite and concentrated *in vacuo* to yield the product (0.27 g, 98%) as a clear oil, δ_H (500 MHz, DMSO-d_6) 2.83 (2H, dd, *J* 16.4 and 8.2), 2.94 (2H, dd, *J* 16.4 and 6.4), 3.62 (6H, s), 3.64 (6H, s), 4.82 (2H, dt, *J* 7.6 and 6.0), 7.38 (2H, s), 7.74 (1H, s), 8.98 (2H, d, *J* 7.6), 10.06 (1H, s); δ_C (100 MHz, $CDCl_3$) 36.38, 49.68, 52.63, 53.45, 117.49, 118.40, 135.49, 157.64, 167.00, 171.67, 171.96; *m/z* (EI) 468.1387. $C_{20}H_{24}O_{11}N_2$ requires 468.1380.

5-Hydroxy-*N*-bis(glycine ethyl ester)isophthalamide. 5-Benzyloxy-*N*-bis(glycine ethyl ester)isophthalamide (0.40 g, 0.90 mmol), palladium on carbon (0.05 g). Product isolated (0.31 g, 97%) as a white solid, mp 126.5–129 °C (Found: C, 54.8; H, 5.8; N, 7.9. $C_{16}H_{20}O_7N_2$ requires C, 54.5; H, 5.7; N, 7.9%); δ_H (400 MHz, DMSO-d_6) 1.20 (6H, t, *J* 7.0) 3.98 (4H, d, *J* 5.8), 4.12 (4H, q, *J* 7.0), 7.40 (2H, s), 7.79 (1H, s), 8.94 (2H, t *J* 5.8), 10.04 (1H, s); δ_C (100 MHz, $CDCl_3$) 14.01, 41.93, 61.78, 116.80, 117.77, 134.71, 156.94, 167.59, 170.81; *m/z* (EI) 352.1270 (M^+). $C_{16}H_{20}O_7N_2$ requires 352.1270.

5-Hydroxy-*N*-bis(phenylalanine methyl ester) isophthalamide. 5-Benzyloxy-*N*-bis(phenylalanine methyl ester) isophthalamide (1.74 g, 2.93 mmol) and 10% palladium on carbon (0.17 g). The product was isolated (1.56 g, 95%) as an amorphous white solid (Found: C, 66.1; H, 5.6; N, 5.4. $C_{28}H_{28}O_7N_2$ requires C, 66.7; H, 5.6; N, 5.5%); δ_H (400 MHz, $CDCl_3$) 3.19 (2H, dd, *J* 13.9 and 6.6), 3.27 (2H, dd, *J* 13.9 and 5.8), 3.76 (6H, s), 5.02 (2H, dt, *J* 7.0 and 6.8), 5.30 (2H, s), 6.98 (2H, d, *J* 7.8), 7.16 (4H, m), 7.25 (6H, m), 7.39 (2H, s), 7.46 (1H, s), 7.94 (1H, brs); δ_C (100 MHz, $CDCl_3$) 38.23, 52.99, 54.50, 117.03, 118.35, 127.68, 129.12, 129.63, 135.69, 136.14, 157.60, 166.78, 172.58; *m/z* (CI) 505.1959 (M + H^+). $C_{28}H_{28}N_2O_7$ + H requires 505.1974.

5-Hydroxy-*N*-bis(phenylalanyl aspartic acid diethyl ester) isophthalamide. 5-Benzyloxy-*N*-bis(phenylalanyl aspartatic acid dimethyl ester) isophthalamide (0.505 g, 0.556 mmol), 10% palladium on carbon (0.047 g). Product isolated (0.325 g, 71%) as an amorphous white foam (Found: C, 61.3; H, 6.2; N, 6.6. $C_{42}H_{50}O_{13}N_7$ requires C, 61.6; H, 6.2; N, 6.8%); δ_H (400 MHz, DMSO-d_6) 1.15 (6H, t, *J* 7.0), 1.16 (6H, t, *J* 7.0), 2.72 (2H, dd, *J* 16.4 and 6.8), 2.82 (2H, dd, *J* 16.4 and 6.0), 2.98 (2H, dd, *J* 13.50 and 10.1), 3.10 (2H, dd, *J* 10.1 and 3.3), 4.08 (8H, m), 4.67 (2H, dt, *J* 7.6 and 6.8), 4.74 (2H, m), 7.16 (2H, m), 7.25 (8H, m), 7.33 (2H, m), 7.62 (1H, s), 8.55 (2H, d, *J* 8.6), 8.60 (2H, d, *J* 7.8), 9.91 (1H, s); δ_H (100 MHz, DMSO-d_6) 14.26, 14.32, 36.15, 37.45, 37.40, 49.04, 54.89, 60.73, 61.23, 117.35, 117.66, 126.60, 128.42, 129.46, 137.87, 138.55, 157.33, 166.21, 170.25, 170.84, 171.78; *m/z* (FAB *m*NBA matrix) 819.3454 (M + H^+). $C_{42}H_{51}O_{13}N_4$ requires 819.3452.

9,10-Bis-(methyl-5-oxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide) anthracene. A stirred solution of 5-hydroxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide (0.200 g, 0.427 mmol), potassium carbonate (0.062 g, 0.446 mmol), 9,10-bis(bromomethyl)anthracene (0.071 g, 0.194 mmol) and a few crystals of sodium iodide in acetone (25 mL) was heated at reflux for a period of 16 hours (caution, the product has poor solubility in common organic solvents and can sometimes precipitate from the reaction mixture). The reaction mixture was then filtered and the precipitate washed several times with chloroform. The combined organics were concentrated and dissolved in chloroform (75 mL). The solution was washed successively with hydrochloric acid (1 N 50 mL), saturated sodium bicarbonate (50 mL) and saturated sodium chloride (50 mL) and then dried over magnesium sulfate, filtered and concentrated. The crude material was then purified by silica gel chromatography (2 : 10 : 100 ethanol–acetone–chloroform) followed by size exclusion chromatography (sephadex LH20, 1 : 1 methanol–chloroform) to yield the product (0.0691 g, 32%) as a yellow glassy solid (Found: C, 58.6; H, 5.3; N, 4.5. $C_{56}H_{58}O_{22}N_4 \cdot CH_3OH$ requires C, 58.5; H, 5.3; N, 4.8%); δ_H (400 MHz, $CDCl_3$) 2.98 (4H, dd, *J* 17.2 and 4.8), 3.12 (4H, dd, *J* 17.2 and 4.5), 3.68 (12H, s), 3.79 (12H, s), 5.08 (4H, dt, *J* 7.8 and 4.5), 6.03 (4H, s), 7.39 (4H, d, *J* 7.8), 7.54 (4H, dd, *J* 6.8 and 3.0), 7.73 (4H, s), 7.87 (2H, s), 8.28 (4H, dd, *J* 6.8 and 3.2); δ_C (100 MHz, $CDCl_3$) 36.42, 49.48, 52.63, 53.44, 109.98, 117.30, 124.95, 127.05, 131.22, 136.16, 159.88, 166.38, 171.38, 171.95; *m/z* (FAB *m*NBA matrix) 1139.3624 (M + H^+). $C_{56}H_{58}O_{22}N_4$ + H requires 1139.3621.

9,10-Bis-(methyl-5-oxy-*N*-bis(glycine ethyl ester) isophthalamide) anthracene. A stirred solution of 5-hydroxy-*N*-bis(glycine ethyl ester) isophthalamide (0.080 g, 0.227 mmol), potassium carbonate (0.033 g, 0.237 mmol), 9,10-bis(bromomethyl)anthracene (0.038 g, 0.103 mmol) and a few crystals of sodium iodide in acetone (25 mL) was heated at reflux for 48 hours. The reaction mixture was then filtered, to leave a pale yellow solid. The solid was rinsed with water then methanol and dried thoroughly to give the product (0.0654 g, 70%) as a pale yellow amorphous solid, δ_H (400 MHz, DMSO-d_6) 1.21 (12H, t, *J* 7.0), 4.06 (8H, dt, *J* 7.5 and 6.0), 4.14 (8H, q, *J* 7.0), 6.24 (4H, s), 7.67 (4H, dd, *J* 6.8 and 3.0), 7.89 (4H, s), 8.10 (2H, s), 8.50 (4H, dd, *J* 6.8 and 3.2), 9.15 (4H, dt, *J* 9.8 and 5.5); δ_C (100 MHz, DMSO-d_6) 14.47, 41.77, 60.88, 63.15, 116.89, 119.72, 125.27, 126.88, 129.49, 130.67, 135.71, 135.76, 159.10, 166.30, 170.15, 170.65; *m/z* (FAB *m*NBA matrix) 907.3399 (M + H^+). $C_{48}H_{50}O_{14}N_4$ requires 907.3402.

9,10-Bis-(methyl-5-oxy-*N*-bis(phenylalanine methyl ester) isophthalamide)anthracene. A stirred solution of 9,10-bis-(bromomethyl)anthracene (0.164 g, 0.451 mmol), 5-hydroxy-*N*-bis(phenylalanine methyl ester) isophthalamide (0.500 g, 0.992 mmol), potassium carbonate (0.143 g, 1.04 mmol), and a few crystals of sodium iodide in acetone (50 mL) was heated under reflux for 16 hours. The reaction mixture was then filtered, concentrated and dissolved in dichloromethane (75 mL). The solution was washed successively with hydrochloric acid (1 N 50 mL), saturated sodium bicarbonate (50 mL) and saturated sodium chloride (50 mL) and then dried over magnesium sulfate, filtered and concentrated. The solution was then passed down a silica gel column using 10% ethyl acetate in dichloromethane as the eluent to give the product (0.313 g, 57%) as a pale yellow solid, mp 198–200 °C, δ_H (400 MHz, $CDCl_3$) 3.24 (4H, dd, *J* 13.6 and 6.5), 3.36 (4H, dd, *J* 13.8 and 5.6), 3.83 (12H, s), 5.17 (4H, dt, *J* 7.6 and 6.5), 5.97 (4H, q, *J* 10.0), 7.10 (4H, d, *J* 7.6), 7.29 (20H, m), 7.58 (4H, dd, *J* 6.8 and 3.0), 7.67 (4H, s), 7.79

(2H, s), 8.29 (4H, dd, *J* 6.8 and 3.0); δ_C (100 MHz, DMSO-d_6) 38.23, 52.93, 54.40, 63.52, 117.13, 118.14, 124.93, 126.88, 127.62, 128.84, 129.08, 129.60, 131.07, 136.26, 136.34, 159.70, 166.55, 172.64.

9,10-Bis-(methyl-5-oxy-*N*-bis(phenylalanylaspartatic acid dimethyl ester) isophthalamide) anthracene. A stirred solution of 5-hydroxy-*N*-bis(phenylalanylaspartic acid dimethyl ester) isophthalamide (0.1014 g, 0.124 mmol), potassium carbonate (0.0179 g, 0.129 mmol), 9,10-bis(bromomethyl)anthracene (0.0205 g, 0.056 mmol) and a few crystals of sodium iodide in acetone (30 mL) was heated at reflux for a period of 16 hours. The reaction mixture was then filtered, and the resultant precipitate washed with water (2 × 10 mL) then dried thoroughly to yield the product (0.1005 g, 97%) as a slightly yellow glassy solid, δ_H (400 MHz, DMSO-d_6) 1.15 (24H, m), 2.72 (4H, dd, *J* 16.4 and 6.8), 2.82 (4H, dd, *J* 16.4 and 6.0), 2.98 (4H, dd, *J* 12.2 and 11.6), 3.12 (4H, dd, *J* 14.4 and 3.3), 4.06 (16H, m), 4.67 (4H, dt, *J* 6.8 and 6.3), 4.80 (4H, m), 6.15 (2H, d, *J* 12.2), 6.24 (2H, d, *J* 12.2), 7.16 (4H, m), 7.24 (8H, m), 7.35 (8H, m), 7.67 (4H, dd, *J* 7.0 and 3.0), 7.75 (4H, s), 7.89 (2H, s), 8.46 (4H, dd, *J* 6.3 and 3.0 Hz), 8.65 (4H, d, *J* 7.8), 8.74 (4H, d, *J* 8.0); δ_C (100 MHz, DMSO-d_6) 14.26, 14.30, 36.11, 37.46, 49.07, 54.96, 60.66, 61.22, 63.05, 116.69, 120.05, 125.22, 126.62, 126.91, 128.43, 129.46, 130.65, 135.93, 138.45, 138.54, 158.65, 165.81, 170.26, 170.76, 171.77; *m/z* (FAB *m*NBA matrix) 1839 ($M + H^+$).

9,10-Bis-(methyl-5-oxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide) benzene. A stirred solution of 5-hydroxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide (0.200 g, 0.427 mmol), caesium carbonate (0.152 g, 0.460 mmol), *p*-xylylenedibromide (0.0513 g, 0.194 mmol) and a few crystals of sodium iodide in acetone (25 mL) was heated at reflux for a period of 16 hours. The reaction mixture was then filtered, concentrated and dissolved in dichlormethane (75 mL). The solution was washed successively with hydrochloric acid (1 N 50 mL), saturated sodium bicarbonate (50 mL) and saturated sodium chloride (50 mL) and then dried over magnesium sulfate, filtered and concentrated. The crude material was then purified by silica gel chromatography (2 : 10 : 100 ethanol–acetone–chloroform) followed by size exclusion chromatography (sephadex LH20, 1 : 1 methanol–chloroform) to yield the product (0.129 g, 64%) as an amorphous white solid (Found: C, 53.3; H, 5.1; N, 5.1. $C_{48}H_{54}O_{22}N_4 \cdot 2H_2O$ requires C, 53.6; H, 5.4; N, 5.2%); δ_H (500 MHz, $CDCl_3$) 2.97 (4H, dd, *J* 17.0 and 4.4), 3.12 (4H, dd, *J* 17.0 and 4.7), 3.70 (12H, s), 3.79 (12H, s), 5.06 (4H, dt, *J* 7.8 and 4.4), 5.12 (4H, s), 7.33 (4H, d, *J* 7.2), 7.47 (4H, s), 7.56 (4H, s), 7.78 (2H, s); δ_C (100 MHz, $CDCl_3$) 36.41, 49.48, 52.61, 53.42, 117.47, 118.34, 128.48, 135.88, 136.51, 159.41, 166.33, 171.47, 171.98; *m/z* (FAB *m*NBA matrix) 1039.3305 ($M + H^+$). $C_{48}H_{54}O_{22}N_4$ + H requires 1039.3308.

9,10-Bis-(methyl-5-oxy-*N*-bis(aspartic acid) isophthalamide) anthracene 1a. To a stirred solution of 9,10-bis-(methyl-5-oxy-*N*-bis(aspartic acid dimethyl ester) isophthalamide) anthracene (0.0582 g, 0.0514 mmol) in 1 : 1 ethanol in water (10 mL) was added sodium hydroxide (1 N, 0.51 mL, 10 eq.). The reaction mixture was then stirred until deprotection was complete as evidenced by reverse phase HPLC, then concentrated and acidified with 1 N HCl to give a cloudy suspension, which was subjected to centrifugation. The supernatant was decanted and the remaining pellet dissolved in 40% acetonitrile in water and lyophilized to yield the product (0.046 g, 87%) as a yellow fluffy solid, λ_{max} (5 mM phosphate, pH 7.4)/nm 300 (ε/dm^{-3} mol^{-1} cm^{-1} 5773) 354 (7051) 372 (10946) 393 (10617); δ_H (400 MHz, DMSO-d_6) 2.73 (4H, dd, *J* 16.7 and 8.0), 2.87 (4H, dd, *J* 16.7 and 5.8), 4.79 (4H, dt, *J* 7.8 and 6.0), 6.24 (4H, s), 7.66 (4H, dd, *J* 5.5 and 3.0), 7.86 (4H, s), 8.06 (2H, s), 8.49 (4H, dd, *J* 6.5 and 3.0), 8.96 (4H, d, *J* 7.8); δ_C (100 MHz, DMSO-d_6) 36.08, 49.75, 63.16, 118.83, 119.86, 125.28, 126.87, 129.47, 130.65, 135.82, 158.96, 165.76, 166.14, 172.08, 172.78; *m/z* (MALDI-TOF α-hydroxy-4-hydroxycinnamic acid matrix) 1027 ($M + H^+$).

9,10-Bis-(methyl-5-oxy-*N*-bis(glycine) isophthalamide) anthracene 1b. To a stirred solution of 9,10-bis-(methyl-5-oxy-*N*-bis(glycine ethyl ester) isophthalamide) anthracene (0.020 g, 0.0221 mmol) in 1 : 1 ethanol in water (10 mL) was added sodium hydroxide (1 N, 0.13 mL, 6 eq.). The reaction mixture was then stirred until deprotection was complete as evidenced by reverse phase HPLC, then concentrated and acidified with 1 N HCl to give a cloudy suspension, which was filtered and dried thoroughly to yield the product (0.015 g, 86%) as a yellow solid, λ_{max} (5 mM phosphate, pH 7.4)/nm 300 (ε/dm^{-3} mol^{-1} cm^{-1} 5928) 353 (7173) 372 (10933) 393 (10582); δ_H (400 MHz, DMSO-d_6) 3.97 (8H, d, $J = 5.8$ Hz), 6.24 (4H, s), 7.66 (4H, dd, $J = 7.0$, 3.3 Hz), 7.87 (4H, s), 8.08 (2H, s), 8.49 (4H, dd, $J = 7.0$, 3.3 Hz), 9.01 (4H, t, $J = 5.8$ Hz); δ_C (100 MHz, DMSO-d_6) 49.86, 63.16, 117.33, 119.70, 125.08, 126.89, 129.50, 130.66, 135.86, 159.04, 166.51, 171.61; *m/z* (FAB *m*NBA matrix) 795.2149 ($M + H^+$). $C_{40}H_{34}O_4N_{14}$ + H requires 795.2150.

9,10-Bis-(methyl-5-oxy-*N*-bis(phenylalanyl) isophthalamide) anthracene 1c. To a stirred solution of 9,10-bis-(methyl-5-oxy-*N*-bis(phenylalanyl methyl ester) isophthalamide) anthracene (0.244 g, 0.201 mmol) in 1 : 1 water in ethanol (15 mL) was added sodium hydroxide (1 N, 2.01 mL, 10eq). The reaction mixture was then stirred until deprotection was complete as evidenced by reverse phase HPLC, then concentrated and acidified with 1 N HCl to give a cloudy suspension, which was subjected to centrifugation. The supernatant was decanted and the remaining oily pellet dissolved in 30% acetonitrile in water and lyophilized to yield the product (0.154 g, 66%) as a slightly yellow solid, mp 167–169 °C; λ_{max} (5 mM phosphate, pH 7.4)/nm 298 (ε/dm^{-3} mol^{-1} cm^{-1} 4587) 355 (5277) 374 (8228) 394 (7885); δ_H (400 MHz, DMSO-d_6) 3.12 (4H, dd, *J* 13.4 and 10.6), 3.20 (4H, dd, *J* 13.4 and 4.0), 4.66 (4H, m), 6.21 (4H, m), 7.16 (4H, m), 7.27 (8H, m), 7.34 (8H, m), 7.66 (4H, dd, *J* 6.7 and 3.0), 7.80 (4H, s), 8.00 (2H, s), 8.49 (4H, dd, *J* 7.0 and 3.0), 8.96 (4H, d, *J* 7.8), 12.82 (8H, brs); δ_C (100 MHz, DMSO-d_6) 36.54, 54.74, 63.11, 116.79, 119.98, 125.29, 126.71, 126.90, 128.56, 129.43, 129.50, 130.65, 135.83, 138.55, 158.81, 165.93, 166.01, 173.41; *m/z* (MALDI-TOF α-hydroxy-4-hydroxycinnamic acid matrix) 1178 ($M + Na^+$).

9,10-Bis-(methyl-5-oxy-*N*-bis(phenylalanyl aspartic acid) isophthalamide) anthracene 1d. To a stirred solution of 9,10-bis-(methyl-5-oxy-*N*-bis(phenylalanyldiethylaspartate) isophthalamide) anthracene (0.0725 g, 0.0394 mmol) in 1 : 1 ethanol in water (10 mL) was added sodium hydroxide (1 N, 0.39 mL, 10 eq.). The reaction mixture was then stirred until deprotection was complete as evidenced by reverse phase HPLC, then concentrated

and acidified with 1 N HCl to give a cloudy suspension, which was subjected to centrifugation. The supernatant was decanted and the remaining oily pellet dissolved in 30% acetonitrile in water and lyophilized to yield the product (0.058 g, 90%) as a slightly yellow fluffy solid, λ_{max} (5 mM phosphate, pH 7.4)/nm 300 (ε/dm^{-3} mol^{-1} cm^{-1} 5685) 353 (7175) 372 (11183) 393 (10819); δ_H (400 MHz, DMSO-d_6) 2.62 (4H, m), 2.74 (4H, dd, J = 16.7, 5.8 Hz), 2.96 (4H, m), 3.12 (4H, m), 4.58 (4H, q, J 6.6), 4.82 (4H, m), 6.18 (4H, p, J 11.6), 7.15 (4H, m), 7.22 (8H, m), 7.34 (8H, m), 7.67 (4H, d, J 7.6), 7.74 (4H, s), 7.91 (2H, m), 8.49 (8H, m), 8.71 (4H, m), 12.64 (8H, brs); δ_C (100 MHz, DMSO-d_6) 36.36, 36.53, 37.57, 39.24, 49.09, 54.96, 116.74, 125.25, 126.59, 126.92, 128.41, 129.50, 130.66, 135.85, 138.64, 158.68, 165.82, 171.44. 171.63, 172.03, 172.57; m/z (MALDI-TOF α-hydroxy-4-hydroxycinnamic acid matrix) 1639 (M + Na$^+$).

9,10-Bis-(methyl-5-oxy-*N*-bis(aspartyl) isophthalamide) benzene 2. To a stirred solution of 9,10-bis-(methyl-5-oxy-*N*-bis(dimethylaspartate) isophthalamide) benzene (0.0474 g, 0.0457 mmol) in 1 : 1 tetrahydrofuran in water (10 mL) was added lithium hydroxide (1 N, 0.54 mL, 12 eq.). The reaction mixture was then stirred until deprotection was complete as evidenced by reverse phase HPLC, then concentrated and acidified with 1 N HCl and concentrated. The resulting white solid was dissolved in 1 : 1 chloroform–methanol, filtered and concentrated then redissolved in 40% acetonitrile in water and lyophilized to yield the product (0.038 g, 89%) as a white powder, δ_H (400 MHz, DMSO-d_6) 2.72 (4H, dd, J 16.4 and 7.8 Hz), 2.85 (4H, dd, J 16.4 and 6.0), 4.74 (4H, dt, J 8.0 and 6.0), 5.24 (4H, s), 7.53 (4H, s), 7.68 (4H, s), 8.02 (2H, s), 8.96 (4H, d, J 7.8 Hz); δ_C (100 MHz, DMSO-d_6) 36.10, 39.84, 71.72, 116.87, 120.62, 128.28, 135.71, 136.72, 158.53, 165.65, 172.06, 172.76; m/z (MALDI-TOF α-hydroxy-4-hydroxycinnamic acid matrix) 927 (M + H$^+$).

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