Convergent Functional Groups. 13. High-Affinity Complexation of Adenosine Derivatives within Induced Binding Pockets†

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Abstract: Receptors based upon 3,6-diaminocarbazole have been synthesized that bind to adenosine derivatives with an interaction energy of approximately 8 kcal/mol in $CDCl_3$ and over 3 kcal/mol in CD_3OD. The purine nucleus is bound within a cavity by simultaneous Watson–Crick and Hoogsteen base-pairing, hydrogen bonding to N^3, and stacking on both of its aromatic faces. Hydrogen bond interactions can be estimated at approximately 0.5–0.75 kcal/mol in methanol. The structure of the complex in solution has been deduced through binding assays of incremental derivatives, one- and two-dimensional NMR studies, and molecular modeling.

Introduction

In this work, we introduce a family of receptors designed to bind adenosine derivatives within a pocket formed through induced fit. The architecture of these receptors consists of a 3,6-diaminocarbazole-based receptor functionalized with a pendant tail which can stack on top of an adenosine within the hydrogen-bonding cleft. This 'scorpion-like'[1] binding geometry is shown schematically in Figure 1 and provides a mechanism of sequestering the bound nucleoside from the bulk solvent. This work discusses the behavior of these receptors in polar and nonpolar organic solvents. In aqueous solution, such an architecture would both protect the hydrogen-bonding surface from competition with water and introduce a favorable entropic contribution to binding through the release of bulk water.

Molecular recognition of nucleic acid components has attracted considerable attention in recent years, and all of the naturally-occurring nucleosides have been the focus of such studies. Successful strategies typically employ receptor surfaces that simultaneously interact with the target through hydrogen bonding and aromatic stacking, while such additional features as salt bridges assist in the recognition of phosphate derivatives.[2-11] In aqueous solution, interactions among the nucleosides are weak and primarily involve aggregation through base-stacking forces[12] whereas hydrogen-bonding dominates in nonpolar organic solvents.[13,14] Correspondingly, molecular recognition studies have predominantly been carried out in organic solvents in order to exploit hydrogen bonding as the major contributor to binding.

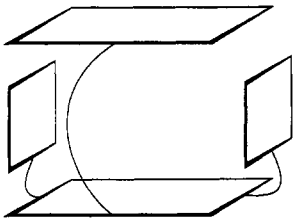

Figure 1. Schematic of binding mode for scorpion receptors.

Because of the vital role it plays as a component of a number of important biological cofactors, adenosine has been the focus of especial attention in molecular recognition studies. In addition to the aromatic system, adenosine presents two separate hydrogen-bonding surfaces for recognition. Base pairing with these two faces results in two modes of binding—the Watson–Crick mode typically seen in DNA and the Hoogsteen mode. A number of widely different receptor designs are possible with these multiple binding modes, including macrocycles,[3] tweezers,[5] and clefts.[15] Refinement of these designs has resulted in receptors with very high affinity for adenosine derivatives. This laboratory has previously reported[16] the synthesis of a cleft based on 2,7-diaminonaphthalene able to complex in both the Hoogsteen and Watson–Crick modes at once while simultaneously providing additional stabilization through aromatic stacking. This receptor binds to 9-ethyladenine with an affinity of 10^5 M^{-1} (–6.7 kcal) in $CDCl_3$.[16] Preliminary reports with extraction[17] suggest that this is the most efficient receptor to date.

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(1) To the best of our knowledge, this term was first used to describe bifunctional crown ethers: Olsher, U.; Frolow, F.; Dalley, N. K.; Weiming, J.; Yu, Z.-Y.; Knobeloch, J. M.; Bartsch, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 6570–6574.

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(12) Ts'o, P. O. P. In *Basic Principles in Nucleic Acid Chemistry*; Ts'o, P. O. P., Ed.; Academic Press: New York, 1974; Vol, I, pp 454–584.

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(17) Benzing, T.; Tjivikua, T.; Wolfe, J.; Rebek, J., Jr. *Science* **1988**, *242*, 266–268.

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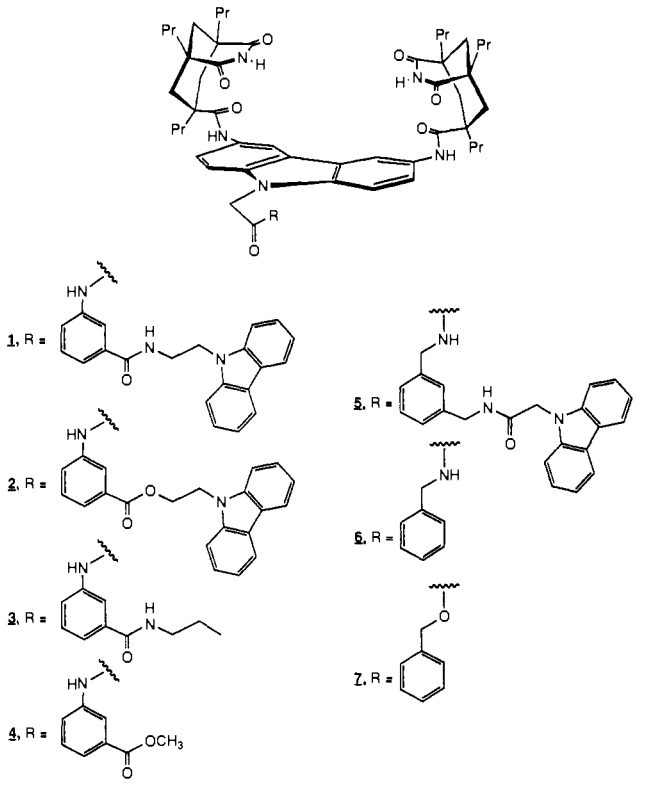
Chart I



The significant advantage of biological receptors over synthetic ones is their ability to strongly bind substrates in aqueous solution. This is due to hydrophobic binding pockets within the structure of the protein. The entropy gained from the release of water from these pockets is an important driving force for complexation.[18] A practical strategy for the construction of binding-pocket models would involve modification of the skeleton of the most efficient pre-existing receptor. Ideally, this would be synthetically simple and general to allow rapid assembly of clefts for structure–function studies. While 2,7-diaminonaphthalene does not lend itself to this easily, a receptor based on 3,6-diaminocarbazole allows facile functionalization of the aromatic moiety while maintaining optimum orientation of the hydrogen-bonding surfaces. We have recently reported the use of this binding motif, coupled with a guanidinium unit, for the complexation of the adenine dinucleotide[19] and cyclic adenosine monophosphates.[20]

Chart I shows the receptors synthesized in this study. Receptors **1** and **5** represent the two different backbone geometries explored in this study, while **2–4** and **6–7** are intended to evaluate the binding contribution of individual design elements.

The receptors in this study were readily assembled using standard synthetic methods. Scheme I summarizes the assembly of the core adenine-binding unit. Dinitrocarbazole (**8**) was alkylated and reduced to give diaminocarbazole derivative **10**. Acylation of this with the tripropyl derivative of the Kemp's triacid imide acyl chloride, followed by hydrolysis, gave the adenine-binding unit ready for condensation with the tail subunits. Schemes II–IV summarize the synthesis of the tail fragments and final receptors.

Results and Discussion

Extraction Studies. We have previously shown the application of adenosine-binding clefts to transport across liquid membranes.[17]

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(19) Galan, A.; de Mendoza, J.; Toiron, C.; Bruix, M.; Deslongchamps, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 9424–9425.
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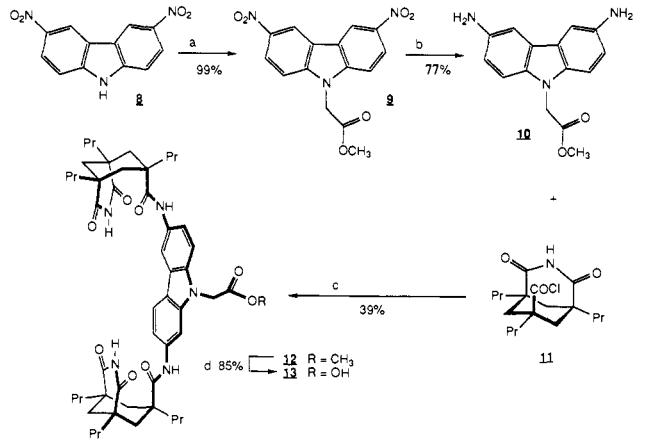
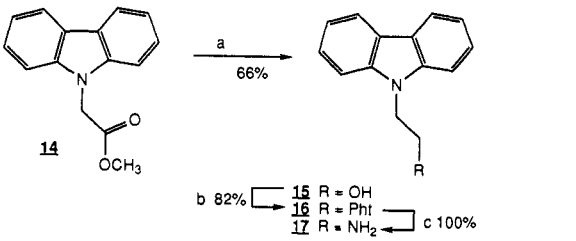
Scheme I[a]



[a] (a) K_2CO_3, DMF, 40–60 °C, 1.5 h; methyl bromoacetate, 50 °C, 2 h; (b) H_2 (1 atm), 10% Pd/C, MeOH, glacial HOAc, room temperature, 5.5 h; (c) pyridine reflux, 6.5 h; (d) THF, 95% EtOH, 1 N NaOH, room temperature, 30 min.

Scheme II[a]



[a] (a) $LiAlH_4$, THF, 0–22 °C, 1.75 h; (b) DEAD, PPh_3, phthalimide, THF, room temperature, 22 h; reflux, 4 h, room temperature, 44 h; (c) hydrazine hydrate, THF, 95% ethanol, reflux, 18 h.

The receptors in this study were examined to evaluate the affinity of these receptors for adenosine derivatives at the chloroform–water interface. As in our initial study, we have found that receptors **1** and **5** are able to extract up to a *full equivalent* of adenosine derivatives from aqueous solution into chloroform (Table I).

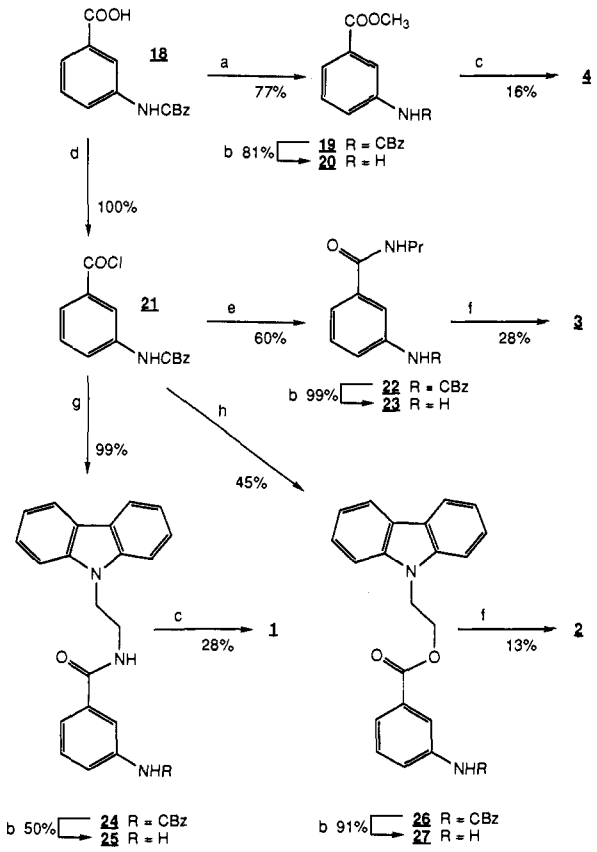
It is known that extraction efficiency depends strongly on the water–chloroform partition coefficient of the target.[21] This dependence manifests itself as a decrease in extraction with increasing hydrophilicity (Table I)—a pattern which mirrors that from our earlier study, despite the higher binding affinity of the receptors in this work. In this case, it seems that formation of a binding pocket around the purine base is not sufficient to overcome the unfavorable effect of partition coefficients on extraction.

Induced Binding Geometry. In chloroform, NMR spectra of the larger scorpions (**1–5**) are extremely broadened and show concentration dependence. Low-temperature NMR (–53 °C) in $CDCl_3$ failed to resolve any identifiable species. In contrast, spectra in methanol are well-resolved, indicating that the broadening results from the slow tumbling of intermolecular aggregates formed through hydrogen bonding.

Addition of 9-ethyladenine to a solution of scorpion receptor **1** in $CDCl_3$ causes a dramatic reorganization, giving a single species by NMR (Figure 2). The substrate–receptor complex exists as a pair of exchanging enantiomers, based upon the position of the N^9-substituent of adenine relative to the pendant tail of the receptor (i.e. to the right versus the left of the tail—Figure 3). Because of this, the chemically equivalent protons become diastereotopic (magnetically nonequivalent) in the complex. The racemization process requires at least partial opening of the

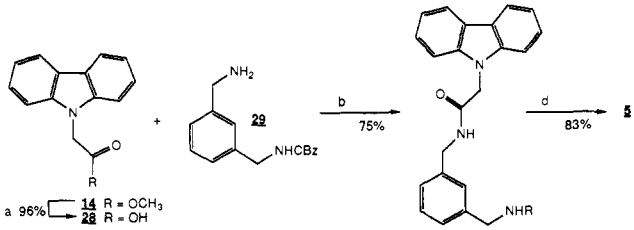
(21) Connors, K. A. *Binding Constants*, 1st ed.; John Wiley & Sons: New York, 1987; p 411.

Scheme III[a]



[a] (a) $SOCl_2$, MeOH, room temperature, 5 h; (b) H_2 (1 atm), 10% Pd/C, THF, 95% EtOH, room temperature; (c) **13**, CDI, THF, room temperature; (d) $SOCl_2$, reflux, 3 h; (e) propylamine, pyridine, CH_2Cl_2, room temperature, 8 h; (*f*) **13**, BOP-Cl, triethylamine, CH_2Cl_2, room temperature; (g) **17**, pyridine, CH_2Cl_2, room temperature, 10 h; (h) **15**, pyridine, CH_2Cl_2, room temperature, 50 h.

Scheme IV[a]



[a] (a) MeOH, 1 N KOH, room temperature, 50 min; (b) CDI, THF, room temperature, 1.5 h; (c) H_2 (1 atm), 10% Pd/C, glacial HOAc, THF, 95% EtOH, room temperature, 4 h; (d) **13**, CDI, THF, room temperature, 15 h.

Table I. Equivalents of Nucleoside Derivatives Extracted from Aqueous Solution by Receptors **1** and **5** (2 mM in Chloroform)

nucleoside	1	5	none
adenine	1.12	1.00	0
2-deoxyadenosine	0.64	0.80	~0.1
adenosine	0.3	0.30	0
guanosine		0	
3′,5′-cAMP		0	

complex, so the bound adenine can leave and re-enter in the opposite orientation. However, the protons on the carbazole lid can exchange by a rotation without adenine leaving the cleft. NMR signals due to these protons are noticeably sharp.

Racemization can be easily slowed at subambient temperature (Figure 4). The rate of exchange at the coalescence temperature[22] (approximately 10 °C) is 170 s^{-1} ($\Delta G^{\ddagger} \sim 13$ kcal/mol). No unbound 9-ethyladenine is observed at this temperature.

In addition to the splitting of the diastereotopic proton signals, the two imide protons display different chemical shifts at low temperatures, reflecting the different chemical environments of the Watson–Crick and Hoogsteen modes. Another significant feature evident is the downfield movement of the amide proton NH-2, suggesting its participation in hydrogen-bonding with N^3 of the 9-ethyladenine.

Binding Studies. A. Chloroform. The choice of 3,6-diaminocarbazole as the scaffolding from which to build receptors involves some sacrifice in binding ability. Despite the fact that both receptors can participate in simultaneous Watson–Crick and Hoogsteen binding, the simplest carbazole-based receptors (**7**, **12**) bind 9-ethyladenine with an interaction energy up to 0.9 kcal/mol (Table II) less than the 2,7-diaminonaphthalene-based receptor.[16] The cause of this is an intramolecular hydrogen bond between the two imide groups which must be broken for binding to occur. Molecular modeling indicates that the curvature of the carbazole groups allows the two imides to interact with each other by rotating into the plane of the aromatic surface. Indeed, the imide protons of **7** in $CDCl_3$ are shifted downfield (10.7 ppm) relative to unbound imide signals (7.6 ppm[15]) in the proton NMR spectrum. The geometry of the naphthalene-based receptor does not allow close approach of the imide groups.

There is negligible steric effect from the groups on the N^9 of carbazole. This can be seen from the near identical binding affinities of **7** and **12** for 9-ethyladenine (Table II). We also found[23] that when conformational flexibility is removed from this position by placing a methyl group at the carbazole N^9 position, the binding affinity for 9-ethyladenine remains the same. If anything, the marginally greater binding affinity of **12** over **7** suggests, possibly, involvement of edge-to-face aromatic interactions between the benzyl ester and bound 9-ethyladenine.

Mutation of ester **7** to amide **6** leads to a 0.6 kcal/mol increase in binding energy in chloroform. As borne out by our low-temperature studies (Figure 4), this increase can be ascribed to a hydrogen bond between the amide and N^3 of the adenine. Molecular modeling shows the hydrogen bond in a nonoptimum geometry, which is also suggested by the magnitude of the interaction (the strength of a base-pair hydrogen bond in chloroform has been estimated at between 1 and 1.5 kcal/mol[13,15,24]).

The fully elaborated receptors (**1–5**) show extremely large binding affinities (Table II) for 9-ethyladenine in $CDCl_3$—up to an *order of magnitude* greater than anything previously observed.[5,16,25] The apparent binding constants were derived from curve fitting of 9-ethyladenine peak shifts upon addition of receptor and lie far outside of the range for which NMR titration is expected to provide accurate association constants.[4] Remarkably, these numbers likely represent *lower* limits on the binding constants, since no attempt was made during the curve-fitting procedure to correct for aggregation of the receptors in solution.

B. Methanol. The inaccuracy of the binding constants determined in $CDCl_3$ is too great to allow any comparison between them. For that reason, we determined the association constants of the 'scorpion' receptors with 9-ethyladenine in CD_3OD (Table III). Despite competition from the increased dipole moment and hydrogen-bonding capability of the solvent, these receptors demonstrate significant affinity for adenosine derivatives. Due to the flexibility inherent in these receptors, all titrations were performed by observing the change in the purine chemical shifts upon addition of receptor to a solution of the nucleoside guest (a

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(23) Deslongchamps, G. Unpublished results.
(24) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417–1436.
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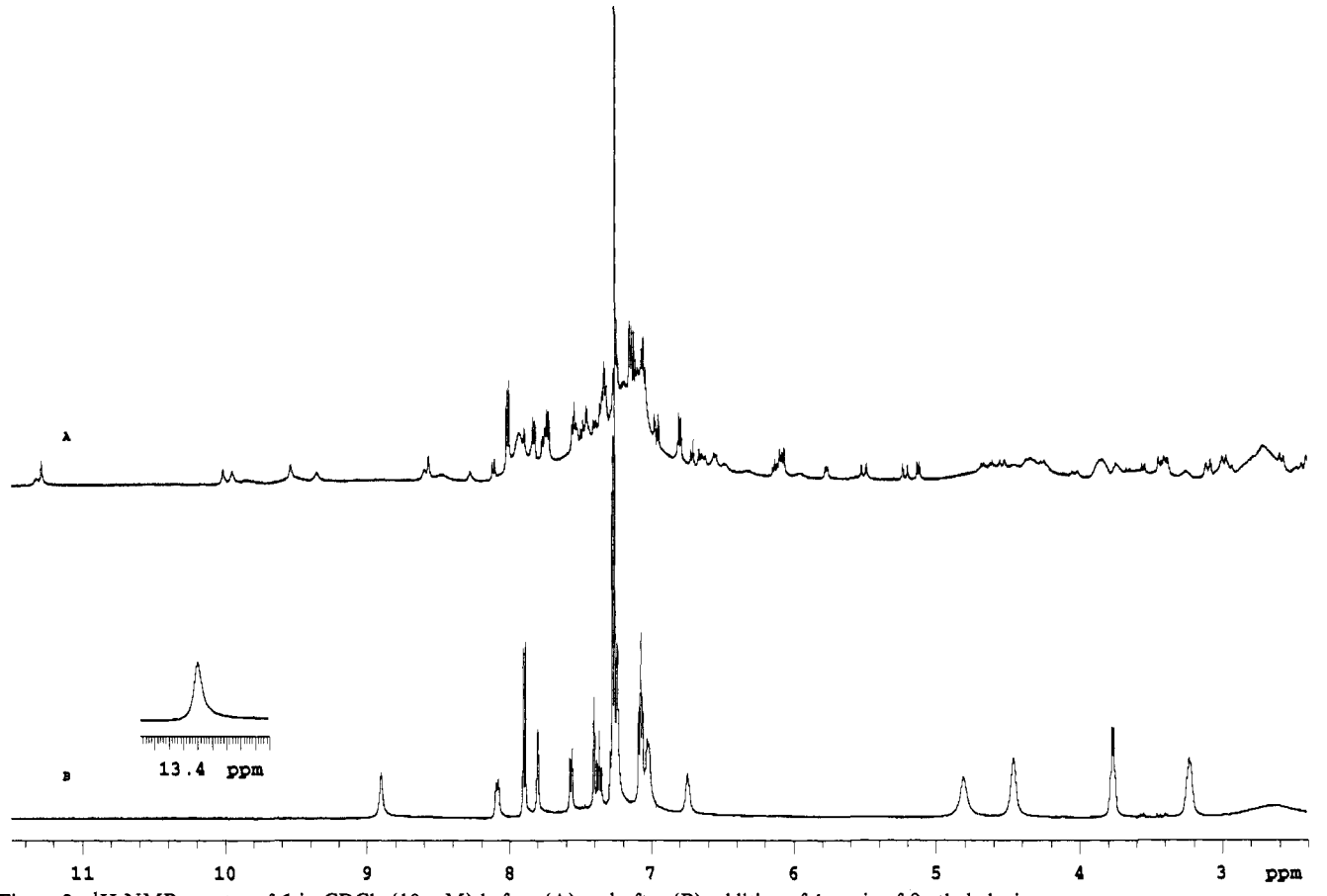

Figure 2. ^1H NMR spectra of **1** in $CDCl_3$ (10 mM) before (A) and after (B) addition of 1 equiv of 9-ethyladenine.

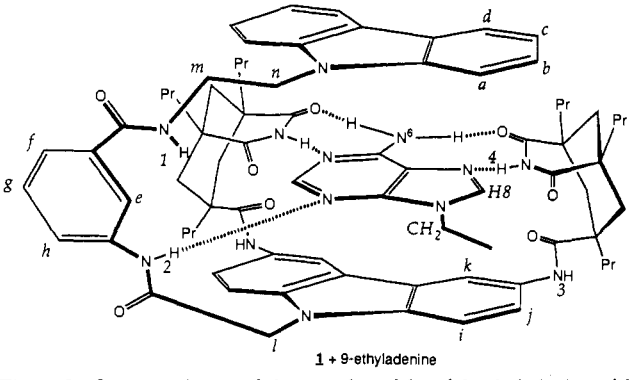

Figure 3. One enantiomer of the complex of **1** and 9-ethyladenine with labeling of protons.

'reverse' titration). By monitoring purine signals, binding constants are derived from protons that are in identical environments in each of the different receptor–nucleoside complexes, thus reducing the effects of difference in local movement. Nevertheless, on the basis of previous experience, these numbers are expected to be accurate only within 10%.[15]

Despite the hydroxylic nature of the solvent, there is only modest propensity for aromatic-stacking interactions in methanol. Titration of both 9-ethylguanine and 9-ethyladenine (1.5 mM) with 20 equiv of **32** (Figure 5) in CD_3OD resulted in less than 0.004 ppm upfield shifts of the purine aromatic signals.

The involvement of hydrogen bonding as a driving force for complexation was confirmed by a binding study performed with **1** and 9-ethyladenine in CD_3OH. Binomial solvent suppression[26] was used to reduce the signal from the solvent hydroxyl protons. Upon addition of 2 equiv of 9-ethyladenine at −20 °C, the imide proton signals of **1** broadened and shifted 1.5 ppm downfield (10.7 ppm to 12.2 ppm). While broadening may be due to base-catalyzed exchange by adenine,[27] the sizable downfield imide shifts provide convincing evidence for hydrogen bond interaction.

Comparison of the binding affinities of receptors **1** and **5** and their incremental derivatives (**2–4**, **6**, **7**) allows an estimate of the contributions to binding by various structural elements (Table III). Energy changes of only 0.1 kcal/mol are at the limit of experimental error, but changes greater than this are expected to be significant. A large proportion of the binding energy is derived from the simplest carbazole diimide motif (**7**). The simultaneous Watson–Crick and Hoogsteen hydrogen-bonding 'chelation' of adenine provides the greatest stabilization while there is also a contribution from aromatic stacking to the carbazole. The involvement of a hydrogen bond between the adenine N^3 and amide NH (**6**) stabilizes complex formation by an additional 0.3 kcal/mol.

Elucidating the exact individual contribution by the upper amide (NH-1) and the aromatic cap is not possible. While the aromatic lid would be expected to provide stabilization through aromatic-stacking interactions, the amide could be expected to participate in a hydrogen bond to N^3, in a fashion similar to NH-2. Singly, though, each element appears to have a minor role in stabilizing the complex (0.1 kcal/mol, **2** → **1** and **3** → **1**). When the two are added together (**4** → **1** or **6** → **5**), significant stabilization (0.4–0.5 kcal/mol) results. The nonadditivity of these 'mutations' complicates the analysis but suggests that both binding elements contribute to the binding energy of complexation, though not simultaneously. As a result, removal of one group allows the other to participate more fully, partially compensating for the loss.

There is a 0.2 kcal/mol increase in binding energy associated with the movement of the pendant phenyl ring one carbon away

(26) Hore, P. J. *J. Magn. Reson.* **1983**, *54*, 539–542.

(27) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; p 556.

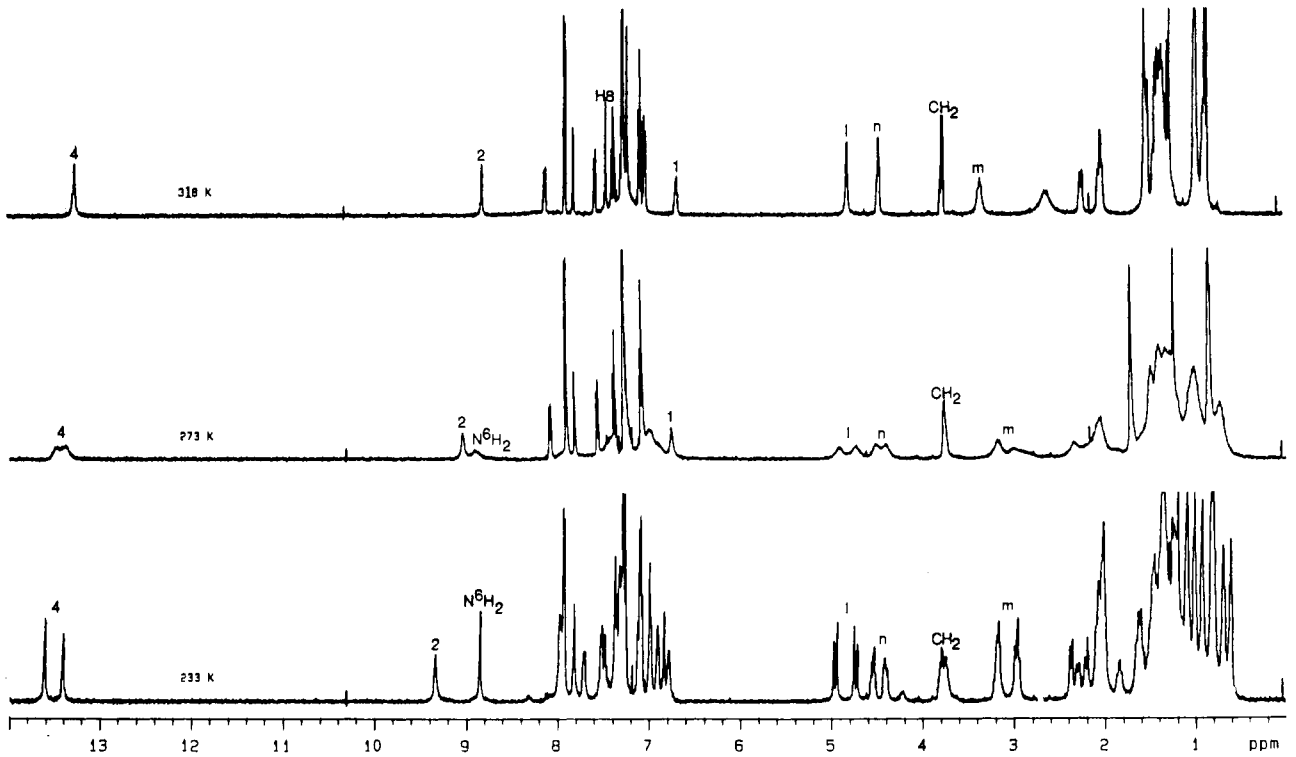

Figure 4. Variable-temperature ^1H NMR spectra of the complex of **1** and 9-ethyladenine (5.5 mM) at 500 MHz in $CDCl_3$.

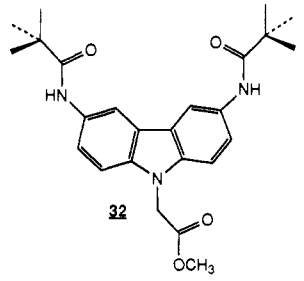

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Figure 5.

Table II. Estimates of Association Constants of Receptors with 9-Ethyladenine in $CDCl_3$ As Determined by NMR Titration

receptor	K_a (M^{-1})	ΔG^{295} (kcal/mol)
1	10^6	−8
2	10^5	−7
3	10^6	−8
4	10^6	−8
5	10^6	−8
6	53000	−6.4
7	21000	−5.8
12	15000	−5.6

Table III. Estimates of Association Constants of Receptors with 9-Ethyladenine in CD_3OD

receptor	K_a (M^{-1})	ΔG^{295} (kcal/mol)
1	351	−3.4
2	278	−3.3
3	296	−3.3
4	150	−2.9
5	425	−3.5
6	209	−3.1
7	115	−2.8

from the 3,6-diaminocarbazole (**4**→**6**). It is tempting to speculate that this is due to stabilization from edge-to-face aromatic interaction of the benzyl ring with the bound adenine. Theoretical studies suggest that this is more favorable for aromatic interactions than the planar-stacked geometry.[28] Molecular modeling indicates that, in the complex of 9-ethyladenine and **4**, the geometry

Table IV. Selectivity of **1** for Various Nucleoside Derivatives in CD_3OD

guest	K_a (M^{-1})	ΔG^{295} (kcal/mol)
9-ethyladenine	351	−3.4
9-ethylguanine	105	−2.7
2′-deoxyadenosine	577	−3.7
2′-deoxyguanosine	72	−2.5

of the phenyl amide derivative precludes any interaromatic interaction when the amide NH-2 to N^3 hydrogen bond is formed.

Examination of the magnitude of the binding energies in methanol (as compared to chloroform) allows an estimate of the strength of a hydrogen bond in this solvent. Both the overall affinity of **7** for 9-ethyladenine and the increase in binding energy associated with the amide NH-2 to N^3 hydrogen bond (**6**) are reduced to half in methanol solution. From this, we can infer that the strength a single base-pair hydrogen bond contributes approximately 0.5–0.75 kcal/mol to binding in this solvent—half of the contribution in chloroform solution.

C. Selectivity. The selectivity of these receptors was probed by titration of different nucleoside derivatives with **1** in CD_3OD (Table IV). The receptor is more efficient at binding 2′-deoxyadenosine than 9-ethyladenine. The 0.3 kcal/mol increase in binding energy is likely due to additional hydrogen-bonding interactions between amide groups in the tail and polar functionalities in the ribose group, but the complex possible hydrogen-bonding patterns and conformational flexibility make this impossible to determine without more study.

While there is a distinct preference for adenine over guanine (3- to 8-fold), receptor **1** shows surprisingly high affinity for guanosine derivatives. Molecular modeling studies indicate that the geometry of the imides allows a three-hydrogen-bond interaction between carbazole bis-imide receptors and guanosine derivatives (Figure 6). The reorientation of the imides and the subsequently different positioning of guanine within the cleft is likely responsible for the greater preference of the receptor for adenine in the nucleoside series versus the alkyl series (via altered

(28) Linse, P. *J. Am. Chem. Soc.* **1992**, *114*, 4366–4373 and references therein.

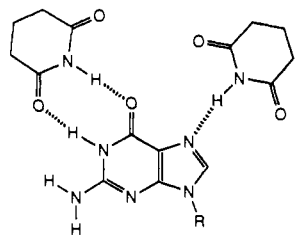

Figure 6. Predicted hydrogen-bonding mode of guanosine derivatives in the scorpion receptor.

Table V. Intermolecular NOESY Correlations in CD_2Cl_2 Solution (5 mM)

1 and 9-ethyladenine	**5** and 9-ethyladenine
NH-4 and H8	NH-4 and H8
NH-4 and H2	NH-4 and H2
H_e and CH_2	H_e and CH_2
NH-2 and CH_2	NH-2 and CH_2
H_h and CH_2	

hydrogen bonding to ribose). These studies suggest that selectivity can only be achieved with receptors having extreme rigidity or specifically designed to select *against* the amino group on the guanine ring.

Solution Structural Studies. Two-dimensional ^1H NMR studies in CD_2Cl_2 were used to obtain information on the geometry of the one-to-one complexes of **1** and **5** with 9-ethyladenine. Peak assignments were made with the assistance of TOCSY[29] experiments. The *inter*molecular contacts observed in NOESY[30] studies are summarized in Table V—all NOE's expected from intramolecular cross-relaxation were seen (see Figure 3 for identification of protons). Observation of the NOE between imide protons and adenine protons (H8 and H2) is direct evidence of the presence of adenine within the cleft. Cross-relaxation between protons in the pendant tail and the purine (CH_2 and H2) show the folding of the tail over the purine, driven by amide hydrogen bonding to N^3.

No NOE's were observed between the stacked aromatic rings. Observation of the interatomic cross-relaxation is complicated by three factors: the lower sensitivity of NOESY relative to steady-state NOE, competitive relaxation from coupling, and the inherent small magnitude of the NOE associated with this tumbling rate.[31] All of the crosspeaks observed were small and positive in CD_2Cl_2 but only intramolecular correlations could be observed in $CDCl_3$, indicating that the tumbling rate of the complex is in the range where the NOE crosses from positive to negative.[31] Rotating-frame NOE experiments (ROESY[32]) are known to give rise to stronger crosspeaks in this motional range but were not used in this study because of the difficulty in suppressing crosspeaks arising from homonuclear coupling (TOCSY).[33-35] It was impossible to separate ROE interactions from the interfering TOCSY-like correlations in our highly aromatic systems.

Despite the absence of interaromatic NOE's, evidence for stacking of the cap over bound adenine was obtained from a titration of scorpion **1** with 9-ethyladenine (a 'forward' titration) in CD_3OD. The proton NMR signals due to the carbazole cap showed significant (0.05–0.1 ppm) upfield shifts during the titration. Since the binding control studies show that medium effects cause *much* smaller shifts, this is consistent with complexation-induced stacking of the carbazole on bound adenine. This same study, by way of downfield shifts of carbazole proton H_k, also indicates inward orientation of the carbonyls of the amides that link the carbazole and Kemp's moieties.

These structural features were modeled through a stochastic dynamics simulation at 300 K of the complex of **1** and 9-ethyladenine using chloroform solvation (see Experimental Section for details). Throughout the simulation, the adenine remains bound between both imides and shows hydrogen bonding to one or both of the amides in the tail. Interestingly, as the simulation proceeded, the carbazole cap moved away from the adenine (about 31 ps into the simulation), simulating, rather accurately, the small driving force for aromatic stacking in organic solvents. The lowest energy conformer, generated by minimization of structures periodically sampled from the simulation, is shown in Figure 7. This structure is consistent with all experimental data—chelated hydrogen bonding of adenine, hydrogen bonding to N^3, and stacking of the carbazole lid. The flexibility of these receptors is such that it is unlikely that there is only one solution conformation of the complex. There are, in fact, a number of low-energy conformations from the dynamics simulation. Rotation of a Kemp's imide moiety so that the adenine is bound in a bifurcated sense[15] on one edge and tilting of the adenine so that hydrogen bonding can occur to NH-1 constitute the major forms of conformational variation. Conformations that show the carbazole lid *not* stacking onto adenine lie more that 20 kJ/mol above the lowest energy conformer.

Conclusions

In conclusion, the use of 3,6-diaminocarbazole as a scaffold allows ready assembly of receptors with very high affinity for adenosine derivatives. The high binding affinity derives from chelation of the adenine by simultaneous Watson–Crick and Hoogsteen base pairing, aromatic stacking, and hydrogen bonding to the N^3 of adenine.

We are currently pursuing the synthesis of receptors that are alternatively functionalized at the carbazole nitrogen in efforts to tailor the specificity and function of adenosine receptors. We will report on our progress in this area in due course.

Experimental Section

Apparatus. ^1H NMR measurements were performed on Bruker AC-250 and AMX-300 and Varian XL-300, GE-300, UN-300, and VXR-500 spectrometers in solvents as indicated. Chemical shifts are reported as parts per million (δ) relative either to tetramethylsilane or to residual solvent peak. Melting points were obtained on an Electrothermal IA9100 digital melting point apparatus and are calibrated. IR spectra were recorded on a Mattson Cygnus 100 FT-IR spectrometer. High-resolution mass spectra were obtained on a Finnegan MAT 8200 mass spectrometer. HPLC was performed on a Waters 600 Multisolvent Delivery System equipped with a Lambda Max Model 481 LC spectrophotometer using a Waters μ-Porasil column.

Materials and Methods. All commercially available compounds (Aldrich) were used without further purification unless otherwise indicated. CD_3OD (99.8% D, Cambridge, Isotope Labs), CD_3OH (99% D, Cambridge Isotope Labs), CD_2Cl_2 (99.6% D, Aldrich Chemical), and DMSO-d_6 (99.9% D, MSD Isotopes) were used from freshly opened vials. $CDCl_3$ (99.8% D, MSD Isotopes) was stored over molecular sieves and passed through dry basic alumina just prior to use. Tetrahydrofuran and ether used in anhydrous conditions were distilled from sodium–benzophenone ketyl; dichloromethane and pyridine were distilled from calcium hydride; and *N,N*-dimethylformamide was dried over molecular sieves for several days. Thin-layer chromatography was performed on Merck Silica 60 F-254 precoated TLC plates. Column chromatography was performed on Merck Silica Gel 60 (230–400 mesh) according to Still et al.[36] Glassware used for anhydrous conditions either was baked overnight at 150 °C, assembled hot, cooled under vacuum, and filled with argon or was flamed dry under vacuum, cooled, and filled with argon

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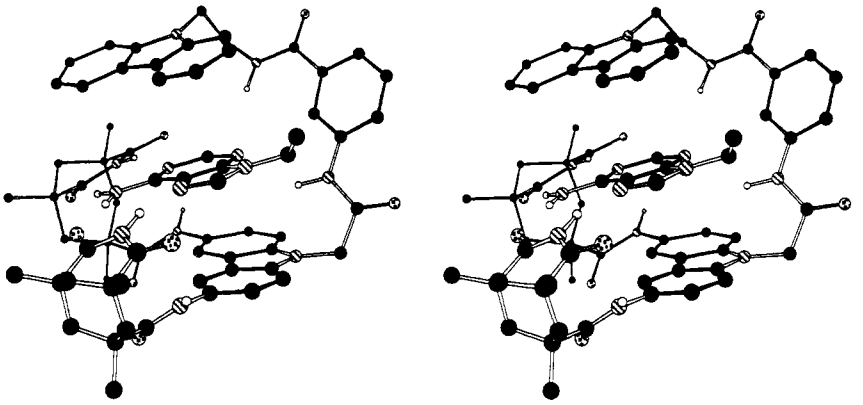

Figure 7. Stereoview of the predicted structure of the complex between **1** and 9-ethyladenine. Hydrogens connected to carbon atoms have been omitted for clarity.

before use. Standard inert-atmosphere techniques were used for syringe and cannula transfers of liquids and solutions.

Extractions. Extractions were performed by vigorously shaking 2 mL of a saturated aqueous solution of the nucleoside derivative with 2 mL of a 1 mg/mL solution of **1** of **5** in chloroform for 1 min, drying the organic layer over Na_2SO_4, removing solvent under reduced pressure, and dissolving the residue in DMSO-d_6. Equivalents were calculated by integration of host and guest signals in the ^{1}H NMR spectrum. Extractions were run in duplicate. The absolute quantity of nucleoside extracted by the host was calculated by assuming 100% recovery of the receptor from the original extraction experiments. Blank extractions were performed similarly, but with no substrate in the chloroform. The absolute amount of nucleoside derivatives extracted was quantified using 3,6-dinitrocarbazole (1.14 mM in DMSO-d_6) as an internal standard.

Titrations. 'Reverse' NMR titrations were performed at 250 MHz on a Bruker AC-250 instrument in a 5 mm o.d. NMR tube at ambient temperature (approximately 22 °C) by treating 400 or 500 μL of purine (1–2 mM) with aliquots of scorpion (4–6 mM) and monitoring the changes in the chemical shift of the substrate protons. For 'forward' titrations, scorpions (4 mM) were titrated with 9-ethyladenine (40 mM) in CD_3OD.

Nonlinear least squares regression curve fitting was used to determine binding constants, along with the final chemical shift of each of the monitored protons. In all cases, at least 50% of saturation was experimentally observed. The association constants given are the average of the binding constants obtained for each of the protons on the purine ring system. On the basis of our previous results,[15] the calculated values are expected to be no more accurate than within 10% of the actual value. For chloroform titrations, where association constants are extremely large, a concentration correction was introduced into the regression analysis. The variations in concentration so determined were less than 1% but resulted in a significantly better fit of the data.

NMR Spectroscopy. Processing and analysis of spectra were done on instrument. Experiments performed on Bruker instruments used shifted sinebell FID weighting while those done on Varian instruments employed a Gaussian multiplication of FID's. Except for the Bruker AC-250, which used time proportional phase increments (TPPI),[37] phase cycling was accomplished through the use of hypercomplex data.[38]

Longitudinal relaxation times (T_1) were measured using the inversion–recovery method as implemented in the Varian software (VNMR version 3.1). Proton resonance assignments of **1** were made in DMSO-d_6 from DQF-COSY[39,40] (AC-250, 256 × 512 data points, 292-Hz spectral width, zero-filled to 512 × 512) and NOESY[30,41,42] (UN-300, mixing time of 1 s, 512 × 4K data points, 3919-Hz spectral width, zero-filled to 2K × 4K).

Assignment of resonances in the proton NMR spectrum of the one-to-one complex of **1** and 9-ethyladenine in $CDCl_3$ were based on a DQF-COSY experiment on UN-300 (150 × 512 data points, 1220 Hz, zero-filled to 512 × 512). Low-temperature studies were performed with **1** in $CDCl_3$ on a Varian GE-300 (2.1 and 12.2 mM in chloroform) and with the one-to-one complex of **1** and 9-ethyladenine on a Varian VXR-500 in $CDCl_3$(5.5 mM) and in CD_3OH (2 mM). Hydroxyl protons of CD_3OH were suppressed using 1:3:3:1 binomial solvent suppression.[26,43]

Peak assignments and through-space correlations of the one-to-one complex of **1** and 9-ethyladenine in CD_2Cl_2 were made on the basis of TOCSY[29,44] (mixing time of 25 ms, 512 × 4K data points, 4200 Hz in ω_1 and 5434 Hz in ω_2, zero-filled to 2K × 4K) and NOESY (mixing times of 250 and 500 ms, 256 × 4K data points, 4200 Hz in ω_1 and 5434 Hz in ω_2, zero-filled to 2K × 4K) experiments on AMX-300. These experiments were performed at the Universitat de les Illes Balears by Prof. Pablo Ballester of U.I.B. and Dr. Alain Pagelot of Sadis Bruker Spectrospin SA.

Peak assignments and through-space correlation of the one-to-one complex of **5** and 9-ethyladenine in CD_2Cl_2 were made on the basis of TOCSY (mixing time of 30 ms, 512 × 4K data points, 4500 Hz, zero-filled to 2K × 4K) and NOESY (mixing time of 500 ms, 384 × 4K data points, 4425 Hz, zero-filled to 2K × 4K) on UN-300.

Molecular Modeling. All molecular modeling was performed on Silicon Graphics Personal Iris workstations (4D25 or 4D30) using MacroModel 3.5X.[45] Stochastic dynamics[46] simulation of **1** and 9-ethyladenine in chloroform was run in the MacroModel version of the AMBER[47] force field at 300 K with GB/SA solvation,[48] extended nonbonded distance cutoffs, constrained bond lengths (SHAKE), and a 1.5-fs timestep using a minimized conformation of the complex as the starting point. After a 5.0-ps initialization period, structures were sampled every 0.2 ps for 60 ps. The 300 structures generated during the stochastic dynamics simulation were minimized using the MULT routine in MacroModel to yield 31 unique conformations, using 0.4 Å as the maximum allowed deviation for comparison of all heavy atoms.

Synthesis: 3,6-Dinitrocarbazole (8) was synthesized according to Grotta et al.[49] and was purified by dissolving the crude mixture in hot 5:1 DMF/MEK (8 mL/g), filtering, cooling overnight at 3 °C, filtering, and washing with cold 5:1 DMF/MEK, 5:3 DMF/MEK, and ether. The recovered solid was dried under vacuum (hot water bath) to yield the pure 3,6-dinitrocarbazole.

***N*-((Methoxycarbonyl)methyl)-3,6-dinitrocarbazole (9).** 3,6-Dinitrocarbazole **8** (10.12 g, 39 mmol) was stirred with dry K_2CO_3 (11 g, 2 equiv) in dry DMF (130 mL) at 40–60 °C for 1.5 hours under argon. Methyl bromoacetate (8 mL, 2.2 equiv) was added to the dark-red solution. The resulting cloudy orange mixture was stirred for 2 h at room

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temperature and for 1 h at 45–55 °C. The reaction was quenched with water (130 mL) and cooled on ice. The yellow product was filtered, washed with water, sucked dry overnight, and dried under vacuum (hot water bath) to yield **9** (12.73 g, 99%) as a yellow solid: mp 314–315 °C; IR (KBr) 1750, 1340, 1310 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.538 (d, 2 H, J = 2.4 Hz), 8.445 (dd, 2 H, J = 9, 2.4 Hz), 7.924 (d, 2 H, J = 9 Hz), 5.639 (s, 2 H), 3.708 (s, 3 H); HRMS (EI) calcd for $C_{15}H_{11}N_3O_6$, 329.064 78; found, 329.0645.

***N*-((Methoxycarbonyl)methyl)-3,6-diaminocarbazole (10).** A mixture of dinitro compound **9** (2.41 g, 7.3 mmol), 10% palladium on carbon (520 mg, 22 wt %), methanol (500 mL), and glacial acetic acid (50 mL) was hydrogenated at atmospheric pressure under a balloon for 5.5 h. The product mixture was filtered through a cake of Celite and the methanol removed by rotary evaporation under reduced pressure. Saturated aqueous $NaHCO_3$ was added to neutralize the acetic acid, and the crude suspension was extracted with chloroform. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure yielded 1.52 g (77%) of white solid **10**, which darkens rapidly on exposure to light or air. The crude product is extremely sensitive to air and light and was used without further purification. ^1H NMR (product only) (300 MHz, $CDCl_3$) δ 7.314 (dd, 2 H, J = 2.1, 1.2 Hz), 7.079 (dd, 2 H, J = 8.7, 1.5 Hz), 6.865 (dd, 2 H, J = 8.4, 1.8 Hz), 4.879 (s, 2 H), 3.695 (s, 3 H), 3.596 (br s, 2 H).

***cis,cis*-1,3,5-Tripropylcyclohexane-1,3,5-tricarboxylic acid imide chloride (11)** was made according to Jeong et al.[50]

***N*-((Methoxycarbonyl)methyl)-3,6-bis[((*cis,cis*-2,4-dioxo-1,5,7-tripropyl-3-azabicyclo[3.3.1]non-7-yl)carbonyl)amino]carbazole (12).** Diamine **10** (0.4 g, 1.5 mmol) and acid chloride **11** (1.055 g, 2.06 equiv) were refluxed in dry pyridine under argon for 6.5 h. Solvent was removed by rotary evaporation, and the crude was suspended in chloroform (100 mL). The organic layer was washed with 10% aqueous HCl (100 mL) and brine (60 mL), and the aqueous layers were back extracted with chloroform (100 mL). The combined organic layers were concentrated by rotary evaporation to give a brown oil, which was triturated with cold methanol (6 mL) to give a beige powder, which was filtered and sucked dry. A second crop was recovered from the filtrate. Both crops of crystals and the final filtrate were purified by column chromatography to yield **12** as a pale-beige powder (520 mg, 39%): mp 227–229 °C; IR 3450, 3381, 3227, 3111, 2960, 2935, 2873, 1700, 1492, 1467, 1198 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 10.40 (s, 2 H), 9.16 (s, 2 H), 8.11 (s, 2 H), 7.43 (dd, 4 H), 5.30 (s, 2 H), 3.63 (s, 3 H), 2.66 (d, 4 H, J = 13.7 Hz), 2.03 (d, 2 H, J = 12.3 Hz), 1.8–0.77 (m, 48 H); HRMS (EI) calcd for $C_{51}H_{69}N_5O_8$, 879.5146; found, 879.5146.

***N*-(Carboxymethyl)-3,6-bis[((*cis,cis*-2,4-dioxo-1,5,7-tripropyl-3-azabicyclo[3.3.1]non-7-yl)carbonyl)amino]carbazole (13).** To a solution of ester **12** (499 mg, 0.57 mmol) in THF (20 mL) was added 95% ethanol (60 mL), followed by 1 N aqueous NaOH (20 mL). The reaction solution was stirred under argon for 30 min and acidified to pH 1 with concentrated aqueous HCl. Solvent was removed by rotary evaporation, and the crude material was triturated in water (20 mL) on ice, filtered, washed with cold water, sucked dry, and dried under vacuum to yield a pale-beige solid (421.7 mg, 85%), which was used without further purification: ^1H NMR (300 MHz, DMSO-d_6) δ 10.39 (s, 2 H, imide), 9.16 (s, 2 H, amide), 8.11 (s, 2 H), 7.43 (dd, 4 H, J = 14.4, 9 Hz), 5.16 (s, 2 H), 2.67 (d, 4 H, J = 13.2 Hz), 2.03 (d, 2 H, J = 12.6 Hz), 1.6–0.7 (m, 48 H).

***N*-((Methoxycarbonyl)methyl)carbazole (14).** Carbazole (10.11 g, 60.5 mmol) was stirred with dry K_2CO_3 (22.24 g, 2.7 equiv) in dry DMF (100 mL) under argon for 45 min at 50 °C before methyl bromoacetate (17 mL, 3 equiv) was added portionwise over 3.5 h. The reaction mixture was stirred at this temperature for 14 h and cooled. The reaction was quenched with cold water (200 mL) and triturated for 1 h on ice. The precipitate was filtered, washed with cold water (200 mL), and sucked dry. The product was dried under vacuum to yield white, crystalline **14** (13.24 g, 91%): mp 97–98 °C; IR (KBr) 3054, 2936, 1731, 1457, 1328, 1267 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.103 (d, 2 H, J = 8.1 Hz), 7.493 (t, 2 H, J = 6.9 Hz), 7.293 (m, 4 H), 5.02 (s, 2 H), 3.718 (s, 3 H); HRMS (EI) calcd for $C_{15}H_{13}NO_2$, 239.0946; found, 239.0944.

***N*-(2-Hydroxyethyl)carbazole (15).** An anhydrous THF (30 mL) solution of the carbazole ester **14** (5.3 g, 22 mmol) was cannulated over 15 min into an ice-cooled suspension of LAH (0.94 g, 1.14 equiv) in THF (35 mL). The reaction mixture was stirred at room temperature for 1.75 h and was quenched with water (1 mL), 10% NaOH (1 mL), and water (3 mL).[51] After the reaction mixture was stirred on ice for 10 min, the inorganic salts were removed by vacuum filtration and washed with ether (100 mL). The combined organic layers were washed with water to neutrality and with brine and were dried over anhydrous $MgSO_4$. Solvent was removed by rotary evaporation under reduced pressure, and the residue was pumped free of solvent under vacuum. The crude brown oil recovered (4.48 g, 96%) was purified by column chromatography (25% ethyl acetate/hexanes) to yield white crystalline **15** (3.06 g, 66%): mp 83–84 °C; IR (KBr) 3199, 3047, 1592, 1452, 1324 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.108 (dd, 2 H, J = 6.3, 1.2 Hz), 7.474 (d, 2 H, J = 3.9 Hz), 7.254 (m, 4 H), 4.486 (t, 2 H, J = 5.4 Hz), 4.062 (apparent q, 2 H, J = 4.5 Hz), 1.530 (s, OH); HRMS (EI) calcd for $C_{14}H_{13}NO$, 211.0997; found, 211.0995.

***N*-(2-Phthalimidoethyl)carbazole (16).** DEAD (2 mL, 1.02 equiv) was added to a stirred solution of alcohol **15** (2.62 g, 12.4 mmol), triphenylphosphine (3.29 g, 1.01 equiv), and phthalimide (1.88 g, 1.03 equiv) in anhydrous THF (150 mL) under argon. The reaction solution was stirred at room temperature for 22 h, refluxed for 4 h, and stirred at room temperature for an additional 44 h. Solvent was removed by rotary evaporation and the crude mixture pumped free of solvent under vacuum. The crude solid was triturated with ice-cold methanol (100 mL), and the off-white precipitate was filtered, washed with cold methanol, and dried to give crystalline **16**: mp 143.1–143.6 °C; IR (KBr) 1772, 1710, 1598, 1384 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.082 (d, 2 H, J = 7.5 Hz), 7.816 (apparent dd, 2 H, J = 5.4, 3 Hz), 7.712 (apparent dd, 2 H, J = 5.7, 2.7 Hz), 7.534 (d, 2 H, J = 8.4 Hz), 7.437 (td, 2 H, J = 6.9, 1.5 Hz), 7.222 (td, 2 H, J = 7.5, 1.2 Hz), 4.595 (t, 2 H, J = 7.5 Hz), 4.124 (t, 2 H, J = 7.8 Hz); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{22}H_{16}N_2O_2$, 340.1212; found, 340.1213.

***N*-(2-Aminoethyl)carbazole (17).** The phthalimido compound **16** (1.81 g, 5.3 mmol) was dissolved in THF (50 mL) and 95% ethanol (50 mL) with hydrazine monohydrate (530 μL, 2 equiv), and the resulting solution was refluxed for 18 h. Water (25 mL) was added, and the mixture was concentrated by rotary evaporation. Additional water (50 mL) and ether were added, and the mixture was brought to pH 14. The aqueous layer was extracted with ether, and the combined organic layers were washed with water and brine and dried over anhydrous $MgSO_4$. The solvent was removed by rotary evaporation, and product was dried under vacuum to give off-white crystalline **17** (1.16 g, 100%): mp 68–70 °C; IR (KBr) 3350, 1886, 1594, 1455, 1229 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.120 (dd, 2 H, J = 7.2, 1 Hz), 7.448 (d, 4 H, J = 3.9 Hz), 7.253 (m, 2 H), 4.413 (t, 2 H, J = 6.3 Hz), 3.229 (t, 2 H, J = 6 Hz), 1.152 (br s, 2 H); HRMS (EI) calcd for $C_{14}H_{14}N_2$, 210.1157; found, 210.1156.

3-(((Benzyloxy)carbonyl)amino)benzoic acid (18). Benzyl chloroformate (1.15 mL, 1 equiv) in anhydrous THF (30 mL) was added to 3-aminobenzoic acid (1 g, 7.3 mmol) and dry pyridine (0.7 mL, 1.2 equiv) in anhydrous THF (200 mL) under argon. The reaction was stirred at room temperature for 2.5 h and solvent removed by rotary evaporation. The crude was triturated in water, acidified, filtered, sucked dry, and dried under vacuum to yield white crystalline product (1.945 g, 98%): mp 217.6–218.7 °C; IR (KBr) 3292, 1696, 1594, 1542, 1443, 1242 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 8.97 (s, 1 H), 8.307 (s, 1 H), 7.82 (d, 1 H, J = 9 Hz), 7.70 (d, 1 H, J = 8 Hz), 7.415 (m, 5 H), 5.189 (s, 2 H); HRMS (EI) calcd for $C_{15}H_{13}NO_4$, 271.0844; found, 271.0843.

Methyl 3-(((Benzyloxy)carbonyl)amino)benzoate (19). Thionyl chloride (4 mL) was added to a suspension of acid **18** (431.3 mg, 1.59 mmol) in dry methanol (40 mL). The mixture was stirred under a drying tube at room temperature for 5 h. Solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (chloroform) to give white solid ester **19** (349 mg, 77%): mp 124–124.6 °C; IR (KBr) 3344, 1736, 1697, 1547, 1211 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.97 (s, 1 H), 7.71–7.76 (m, 2 H), 7.36–7.42 (m, 6 H), 6.81 (br s, 1 H), 5.22 (s, 2 H), 3.90 (s, 3 H); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{16}H_{16}NO_4$ (M + H), 286.1079; found, 286.1077.

Methyl 3-Aminobenzoate (20). CBz-protected ester **19** (230 mg, 0.81 mmol) was hydrogenated at balloon pressure for 10.5 h in THF (10 mL) and 95% ethanol (15 mL) with 10% palladium on carbon (40.5 mg, 18 wt %). Catalyst was removed by filtration through Celite and solvent removed by rotary evaporation under reduced pressure. The residue was dissolved in chloroform and dried over $MgSO_4$. Removal of solvent under reduced pressure followed by evacuation yields 98.8 mg (81%) of a pale-brown oil, which was used without further purification: ^1H NMR (250 MHz, $CDCl_3$) δ 7.43 (d, 1 H, J = 7.6 Hz), 7.35 (t, 1 H, J = 1.8

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Hz), 7.22 (t, 1 H, J = 7.8 Hz), 6.86 (dd, 1 H, J = 7.8, 2.3 Hz), 3.89 (s, 3 H), 3.77 (br s, 2 H).

3-(((Benzyloxy)carbonyl)amino)benzoyl Chloride (21). Acid **18** (510.2 mg, 1.88 mmol) was refluxed in $SOCl_2$ (15 mL) for 3 h. Thionyl chloride was removed by rotary evaporation with addition of benzene and dried under vacuum to give white crystalline **21** (545 mg, 100%), which was used without further purification: IR ($CHCl_3$) 3430, 2253, 1793, 1745, 1741, 1606, 1593, 1530, 1489, 1439, 1431 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.083 (s, 1 H), 7.775 (d, 2 H, J = 7.2 Hz), 7.352 (m, 5 H), 7.113 (s, 1 H), 5.195 (s, 2 H).

***N'*-Propyl-3-(((benzyloxy)carbonyl)amino)benzamide (22).** Acid chloride **21** (985 mg, 3.4 mmol) was dissolved in anhydrous CH_2Cl_2 (150 mL) under argon. Pyridine (280 μL, 1.02 equiv) and propylamine (560 μL, 2 equiv) were added. The solution was stirred at room temperature for 8 h and then washed with 5% (w/v) citric acid (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was recrystallized from ether/CH_2Cl_2 to give 636 mg (60%) of fluffy white crystals: mp 145.4–146 °C; IR (KBr) 3334, 3299, 2961, 1736, 1624, 1553 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.84 (s, 1 H), 7.61 (d, 1 H, J = 7.8 Hz), 7.45–7.33 (m, 7 H), 7.07 (br s, 1 H), 6.18 (br s, 1 H), 5.21 (s, 2 H), 3.39 (q, 2 H, J = 7.5 Hz), 1.609 (q, 2 H, J = 7.3 Hz), 0.964 (t, 3 H, J = 7.3 Hz); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{18}H_{21}N_2O_3$ (M + H), 313.1522; found, 313.1550.

***N'*-Propyl-3-aminobenzamide (23).** The CBz-protected amine **22** (361 mg, 1.16 mmol) was hydrogenolyzed using the same procedure as for **20** to give colorless oil **23** (203 mg, 99%). The oil darkened rapidly on exposure to light and air and was used without further purification: ^1H NMR (250 MHz, $CDCl_3$) δ 7.27 (s, 1 H), 7.16 (s, 1 H), 7.04 (br t, 1 H), 6.90 (m, 1 H), 4.15 (br s, 2 H), 3.49 (q, 2 H, J = 7.3 Hz), 1.08 (t, 3 H, J = 7.4 Hz).

***N'*-(2-(9-*N*-Carbazolyl)ethyl)-3-(((benzyloxy)carbonyl)amino)benzamide (24).** Amine **17** (316.6 mg, 1 equiv) and acid chloride **21** (435.8 mg, 1.5 mmol) were condensed using the same procedure as for **22** to give off-white solid **24** (686.3 mg, 99%), which was used without further purification: mp 207–211 °C dec; ^1H NMR (250 MHz, $CDCl_3$) δ 8.1 (d, 2 H, J = 6 Hz), 7.1–7.6 (m, 15 H), 5.200 (s, 2 H), 4.619 (t, 2 H, J = 6 Hz), 3.89 (apparent q, 2 H, J = 6 Hz).

***N'*-(2-(9-*N*-Carbazolyl)ethyl)-3-aminobenzamide (25).** The CBz-protected amide **24** (586 mg, 1.26 mmol) was hydrogenolyzed using the procedure for **20**. Following column chromatography (3.3% MeOH/$CHCl_3$), aromatic amine **25** was recovered as an off-white foam (~50%), which resisted recrystallization attempts and was used without further purification: ^1H NMR (250 MHz, acetone-d_6) δ 8.129 (d, 2 H, J = 8 Hz), 7.82 (br m, 1 H), 7.613 (d, 2 H, J = 8 Hz), 7.419 (t, 2 H, J = 8 Hz), 7.189 (m, 2 H), 7.043 (t, 1 H, J = 7 Hz), 6.92 (d, 1 H, J = 8 Hz), 6.75 (dd, 1 H, J = 8, 2 Hz), 4.645 (t, 2 H, J = 6 Hz), 3.811 (apparent q, 2 H, J = 6 Hz).

***N'*-(2-(9-*N*-Carbazolyl)ethyl)-3-(((benzyloxy)carbonyl)amino)-benzoate (26).** Acid chloride **21** (515 mg, 1.78 mmol) and alcohol **15** (375.8 mg, 1 equiv) were condensed using the procedure of **22**. Recrystallization from chloroform gave 375 mg (45%) of **26** as a white solid: mp 200–200.3 °C; IR (KBr) 3295, 3126, 1700, 1545, 1214 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 8.12 (d, 2 H, J = 7.9 Hz), 7.8 (d, 1 H, J = 8 Hz), 7.54–7.24 (m, 9 H), 6.5 (s, 1 H), 5.24 (s, 2 H), 4.73–4.67 (m, 4 H); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{29}H_{24}N_2O_4$, 464.1736; found, 464.1733.

***N'*-(2-(9-*N*-Carbazolyl)ethyl)-3-aminobenzoate (27).** The CBz-protected ester **26** (199 mg, 0.428 mmol) was hydrogenolyzed using the procedure for **20** to give 129.3 mg (91%) of **27** as a colorless oil, which was used without further purification: ^1H NMR (250 MHz, $CDCl_3$) δ 8.12 (d, 2 H, J = 7.8 Hz), 7.48–7.55 (m, 2 H), 7.22–7.37 (m, 5 H), 7.12 (t, 1 H, J = 7.8 Hz), 6.92 (t, 1 H, J = 1.9 Hz), 6.80 (dd, 1 H, J = 7, 2 Hz), 4.68 (m, 4 H), 3.62 (br s, 2 H).

***N*-(Carboxylmethyl)carbazole (28).** To a stirred solution of ester **14** (5.0 g, 20.9 mmol) in MeOH (430 mL) was added 1 N KOH (70 mL) dropwise under argon. The mixture was stirred for 50 min and neutralized with 1 N HCl (7 mL). Organic solvents were evaporated in vacuo. Cold water was added to the residue, and the precipitate was filtered off, washed with cold water, and dried in vacuo overnight to afford 4.53 g (96%) of white solid **28**: mp 182–184 °C; IR (KBr) 3422, 3052, 2928, 1706 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 8.11 (d, 2 H, J = 8.1 Hz), 7.48 (t, 2 H, J = 7.4 Hz), 7.34–7.25 (m, 4 H), 5.05 (s, 2 H); HRMS (EI) calcd for $C_{14}H_{11}NO_2$, 225.0790; found, 225.0789.

3-((((Benzyloxy)carbonyl)amino)methyl)benzylamine (29). To a vigorously stirred solution of *m*-xylylenediamine (34.05 g, 0.25 mol) in dry CH_2Cl_2 (300 mL) was added a CH_2Cl_2 solution (300 mL) of benzyl chloroformate (42.65 g, 1 equiv) dropwise over 1 h. The resulting solution was stirred for 12 h. Solid K_2CO_3 (34.6 g, 1 equiv) was added, and stirring was continued for another 30 min. Solids were removed by vacuum filtration, and the filtrate was washed with 0.5 N aqueous KOH and brine, dried over Na_2SO_4, and evaporated under reduced pressure. Purification by column chromatography (5% MeOH/CH_2Cl_2 and 10% MeOH/CH_2Cl_2) afforded 6.15 g (9%) of the monoprotected diamine **29** as a waxy solid: IR 3450, 3371, 3314, 3013, 2954, 1719 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36–7.22 (m, 9 H), 5.14 (s, 2 H), 4.38 (d, 2 H, J = 6.1 Hz), 3.84 (s, 2 H), 1.51 (s, 2 H); HRMS (EI) calcd for $C_{16}H_{18}N_2O_2$, 270.1368; found, 270.1368.

9-[(((3-((((Benzyloxy)carbonyl)amino)methyl)benzyl)amino)carbonyl)-methyl]carbazole (30). To a stirred solution of acid **28** (225 mg, 1.0 mmol) in dry THF (10 mL) was added carbonyldiimidazole (170 mg, 1.05 equiv) under argon. After the reaction mixture was stirred for 1 h, amine **29** (270 mg, 1.0 equiv) was added and stirring was continued for 1.5 h. Solvents were evaporated in vacuo, and the residue was taken up in 2% MeOH/$CHCl_3$, from which 270.1 mg of pure product **30** was filtered. The mother liquor was concentrated under reduced pressure and purified by column chromatography (2% MeOH/$CHCl_3$) to afford another 88.7 mg of product. The combined yield of solid **30** was 358.8 mg (75%): mp 211–212 °C; IR (KBr) 3297, 1687, 1652 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, 2 H, J = 8.0 Hz), 7.56–7.10 (m, 10 H), 5.83 (br s, 1 H, NH), 5.12 (s, 2 H), 4.99 (s, 2 H), 4.35 (d, 2 H, J = 6.1 Hz), 4.21 (d, 2 H, J = 5.8 Hz); HRMS (EI) calcd for $C_{30}H_{27}N_3O_3$, 477.2052; found, 477.2051.

9-[(((3-(Aminomethyl)benzyl)amino)carbonyl)methyl]carbazole (31). A solution of CBz derivative **30** (330.2 mg, 0.69 mmol) in 95% EtOH (6 mL) and THF (24 mL) containing glacial AcOH (15 drops) was hydrogenated over 10% Pd/C (165 mg, 50 wt %) for 4 h at atmospheric pressure under a hydrogen balloon. The catalyst was removed by filtration through Celite, and solvent was evaporated in vacuo. The residue was dissolved in CH_2Cl_2, washed with basic (NH_4OH) brine, dried over Na_2SO_4, and concentrated by rotary evaporation under reduced pressure. Column chromatography (10% MeOH/$CHCl_3$ containing a few drops of Et_3N) afforded 180.3 mg (76%) of pure amine **31**: mp 191–194 °C; IR (KBr) 3280, 3053, 2926, 1654 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.12–6.84 (m, 12 H), 5.82 (br s, 1 H, amide), 4.99 (s, 2 H), 4.37 (d, 2 H, J = 6.1 Hz), 3.67 (s, 2 H), 1.43 (s, 2 H, amine); HRMS (EI) calcd for $C_{22}H_{21}N_3O$, 343.1685; found, 343.1682.

***N*-((Methoxycarbonyl)methyl)-3,6-bis(pivaloylamino)carbazole (32).** Diamine **10** (770 mg, 2.86 mmol) in pyridine (50 mL) was treated with pivaloyl chloride (370 μL, 1.05 equiv) and stirred under argon at room temperature for 70 h. The solvent was removed by rotary evaporation under reduced pressure, and the residue was dissolved in chloroform, washed with 4% HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. After concentration under reduced pressure, the crude product was purified by flash chromatography (3% MeOH/$CHCl_3$), trituration in cold methanol, and flash chromatography (10% EtOAc/CH_2Cl_2) to give 211 mg (17%) of an off-white powder: mp 242 °C dec; IR (KBr) 3425, 3297, 2963, 1732, 1657, 1529, 1479, 1198 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 9.22 (s, 2 H), 8.34 (d, 2 H, J = 1.4 Hz), 7.55 (dd, 2 H, J = 8.7, 1.6 Hz), 7.44 (d, 2 H, J = 8.8 Hz), 5.29 (s, 2 H), 3.65 (s, 3 H), 1.26 (s, 18 H); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{25}H_{32}N_3O_4$ (M + H), 438.3293; found, 438.2391.

Scorpion 1. To a solution of acid **13** (422 mg, 0.49 mmol) in anhydrous THF (40 mL) under argon was added CDI (88.9 mg, 1.12 equiv). The solution was stirred at room temperature for 1.6 h, and amine **25** (188.9 mg, 86% pure, 1 equiv) in anhydrous THF (10 mL) was added. The reaction solution was stirred at room temperature for 23 h and at 55 °C for 3.5 h. Solvent was removed by rotary evaporation. The residue was dissolved in chloroform, washed with 4% aqueous HCl and brine, and dried over anhydrous Na_2SO_4. Solvent was removed by rotary evaporation, and the crude was purified by column chromatography (3% MeOH/$CHCl_3$ and 40% EtOAc/hexane) to give pale-brown foam **1** (163.3 mg, 28%): mp 280 °C dec; IR (KBr) 3377 (br), 2959, 2932, 2872, 1700, 1485, 1465, 1195 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 10.607 (s, 1 H, amide), 10.377 (s, 2 H, imide), 9.169 (s, 2 H, amide), 8.624 (apparent t, 1 H, amide), 8.131 (s, 2 H), 8.115 (d, 2 H, J = 8.2 Hz), 8.036 (s, 1 H), 7.685 (m, 2 H), 7.578 (d, 2 H, J = 8.2 Hz), 7.454 (s, 4 H), 7.380 (t, 2 H, J = 7.3 Hz), 7.319 (d, 1 H, J = 4.9 Hz), 7.153 (t, 2 H, J = 7.4 Hz), 5.220 (s, 2 H), 4.536 (apparent t, 2 H), 3.606 (apparent q, 2 H), 2.667 (d, 4 H, J = 13.6 Hz), 2.023 (d, 2 H, J = 12.6 Hz), 0.772–1.9 (m, 48 H); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{71}H_{85}N_8O_8$ (M + H), 1177.6490; found, 1177.6482.

Scorpion 2. Acid **13** (193.5 mg, 0.22 mmol), amine **27** (83 mg, 1.13 equiv), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl, 64 mg, 1.13 equiv), and triethylamine (69 μL, 2.21 equiv) were mixed in anhydrous CH_2Cl_2 (20 mL), stirred under argon for 5 h, and refluxed for 16 h. The reaction solution was diluted with CH_2Cl_2 (50 mL), washed with 5% (w/v) citric acid (25 mL), saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. After removal of solvent by rotary evaporation under reduced pressure, the crude product was purified by flash chromatography (2.4% MeOH/$CHCl_3$) and preparative TLC (4% MeOH/$CHCl_3$) to give 33.2 mg (13%) of a beige powder: mp 180 °C dec; IR (KBr) 3378 (br), 2959, 2872, 1700, 1485, 1461, 1300, 1194 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.64 (s, 1 H, amide), 10.36 (s, 2 H, imide), 9.16 (s, 2 H, amide), 8.15 (m, 5 H), 7.7 (m, 1 H), 7.67 (d, 2 H, J = 8.1 Hz), 7.46 (m, 4 H), 7.35 (m, 4 H), 7.15 (t, 2 H, J = 6.7 Hz), 5.22 (s, 2 H), 4.79 (br s, 2 H), 4.60 (br s, 2 H), 2.67 (d, 4 H, J = 13.5 Hz), 2.02 (d, 2 H, J = 12.3 Hz), 1.9–0.75 (m, 48 H); HRMS (FAB in glycerol) calcd for $C_{71}H_{84}N_7O_9$ (M + H), 1178.6330; found, 1178.6328.

Scorpion 3. Under argon, BOP-Cl (66 mg, 1.11 equiv) was added to acid **13** (203 mg, 0.234 mmol) and triethylamine (33 μL, 1 equiv) in CH_2Cl_2 (20 mL) on ice. After 15 min of stirring, the solution was allowed to come to room temperature and stirred for an additional 3 h. Amine **23** (50 mg, 1.2 equiv) in CH_2Cl_2 (10 mL) was added with triethylamine (35 μL, 1.1 equiv), and the reaction mixture was stirred for 19 h at room temperature. The product solution was diluted with CH_2Cl_2 (100 mL), washed with 4% (v/v) HCl (50 mL), saturated $NaHCO_3$ (50 mL), and brine, and dried over $MgSO_4$. After removal of solvent under reduced pressure, the beige powder was purified by flash chromatography (EtOAc/$CHCl_3$ gradient) and preparative TLC (50% EtOAc/$CHCl_3$) to give pure **3** as a beige powder (66 mg, 28%): mp 200 °C dec; IR (KBr) 3379, 2959, 2872, 1696, 1533, 1196 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 10.62 (s, 1 H, amide), 10.37 (s, 2 H, imide), 9.17 (s, 2 H, amide), 8.42 (t, 1 H, J = 7.3 Hz, amide), 8.12 (s, 2 H), 8.01 (s, 1 H), 7.73 (d, 1 H, J = 7.4 Hz), 7.51–7.33 (m, 6 H), 5.22 (s, 2 H), 3.17 (q, 2 H, J = 6.6 Hz), 2.67 (d, 4 H, J = 13.7 Hz), 2.02 (d, 2 H, J = 13.8 Hz), 1.9–0.78 (m, 48 H); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{60}H_{80}N_7O_8$ (M + H), 1026.6068; found, 1026.6055.

Scorpion 4. Acid **13** (253 mg, 0.29 mmol) was condensed with amine **20** (67.2 mg, 1.52 equiv) using the method of scorpion **1**. The product was purified by flash chromatography (2% MeOH/$CHCl_3$ and 2.3% MeOH/$CHCl_3$) followed by preparative HPLC (2% MeOH/$CHCl_3$) to remove residual **12**. Pale beige **4** (47.6 mg, 16%) was isolated: mp 193 °C dec; IR (KBr) 3380 (br), 2934, 2872, 1700, 1494, 1468, 1301, 1197 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.71 (s, 1 H, amide), 10.37 (s, 2 H, imide), 9.16 (s, 2 H, amide), 8.27 (d, 1 H, J = 1.8 Hz), 8.12 (s, 2 H), 7.82 (dd, 1 H, J = 7.8, 1.2 Hz), 7.64 (d, 1 H, J = 8.1 Hz), 7.45 (m, 5 H), 5.22 (s, 2 H), 3.81 (s, 3 H), 2.67 (d, 4 H, J = 13.5 Hz), 2.02 (d, 2 H, J = 12.6 Hz), 1.9–0.8 (m, 48 H); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{58}H_{75}N_6O_9$ (M + H), 999.5595; found, 999.5600.

Scorpion 5. Acid **13** (227 mg, 0.26 mmol) and amine **31** (90 mg, 1.0 equiv) were condensed using the method of scorpion **1**. Column chromatography (2% MeOH/$CHCl_3$) afforded 259 mg (83%) of receptor **5**: mp 180 °C dec; IR (KBr) 3449–3383, 3199–3120, 2959, 2935, 2873, 1702, 1696 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.36 (s, 2 H, imide), 9.15 (s, 2 H, amide), 8.7 (m, 2 H, amide), 8.14 (d, 2 H, J = 7.8 Hz), 8.11 (d, 2 H, J = 1.5 Hz), 7.6–7.1 (m, 14 H), 5.09 (s, 2 H), 5.04 (s, 2 H), 4.29 (d, 2 H, J = 6.4 Hz), 4.27 (d, 2 H, J = 6.4 Hz), 2.66 (d, 4 H, J = 13.3 Hz), 2.02 (d, 2 H, J = 12.9 Hz), 1.9–0.7 (m, 48 H); HRMS (EI) calcd for $C_{72}H_{86}N_8O_8$, 1190.6569; found, 1190.6563.

Scorpion 6. The method for scorpion **1** was used to condense crude acid **13** (300 mg, 0.35 mmol) and benzylamine (37.1 mg, 1.0 equiv). Column chromatography (2.5:22.5:25:50 MeOH/CH_2Cl_2/Et_2O/hexane) afforded 267 mg (81%) of benzylamide **6**: mp 190 °C dec; IR (KBr) 3381, 3192–3117, 2959, 2933, 2872, 1700 cm^{-1}; ^1H NMR (250 MHz, DMSO-d_6) δ 10.38 (s, 2 H, imide), 9.16 (s, 2 H, amide), 8.73 (t, 1 H, J = 5.9 Hz, amide), 8.12 (s, 2 H), 7.43–7.27 (m, 9 H), 5.04 (s, 2 H), 4.29 (d, 2 H, J = 5.6 Hz), 2.67 (d, 4 H, J = 13 Hz), 2.03 (d, 2 H, J = 13 Hz), 1.8–0.7 (m, 48 H); HRMS (EI) calcd for $C_{57}H_{74}N_6O_7$, 954.5619; found, 954.5619.

Scorpion 7. Benzyl alcohol (22.0 mg, 1.0 equiv) was condensed with acid **13** (176 mg, 0.20 mmol) as for scorpion **1**. The crude product was purified by column chromatography (11:10:78:1 hexane/Et_2O/CH_2Cl_2/MeOH and 10:89:1 Et_2O/CH_2Cl_2/MeOH) to afford 126.5 mg (65%) of benzyl ester **7**: mp 252–253 °C; IR (KBr) 3382, 3198–3120, 2959, 2932, 2872, 1704, 1696 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.39 (s, 2 H, imide), 9.16 (s, 2 H, amide), 8.12 (s, 2 H), 7.44 (s, 5 H), 7.32 (multiplet, 4 H), 5.38 (s, 2 H), 5.15 (s, 2 H), 2.67 (d, 4 H, J = 13.8 Hz), 2.03 (d, 2 H, J = 12.3 Hz), 1.9–0.7 (m, 48 H); HRMS (EI) calcd for $C_{57}H_{73}N_5O_8$, 955.5459; found, 955.5457.

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