Modular incorporation of 1-benzyltryptophan into dipeptide hosts that bind acetylcholine in pure water

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Abstract: Proteins that recognize and bind quaternary ammonium ions depend on "aromatic-cage" structural motifs that use multiple aromatic residues to engage the side chain's ammonium cation. We introduce herein the use of 1-benzyltryptophan (Trp(Bn)) residues as synthetic, unnatural partial analogues of natural aromatic cages. We demonstrate the modular incorporation of these building blocks into simple dipeptide hosts and show that they are capable of binding quaternary ammonium ions in buffered water and in chloroform.

Key words: molecular recognition, tryptophan, peptides, cation–π interaction, hydrophobic effect.

Résumé : Les protéines qui reconnaissent et qui se lient à des ions ammonium quaternaires dépendent sur des motifs structuraux « cage aromatique » qui font appel à de multiples résidus aromatiques pour s'engager avec le cation ammonium de la chaîne latérale. Dans ce travail, on introduit l'utilisation des résidus 1-benzyltryptophane (Trp(Bn)) comme analogues partiels de synthèse, non naturels, des cages aromatiques naturelles. On démontre que l'incorporation modulaire des ces blocs dans des hôtes dipeptidiques et on montre qu'ils sont capables de se lier à des ions ammonium quaternaires dans l'eau tamponnée et dans le chloroforme.

Mots-clés : reconnaissance moléculaire, tryptophane, peptides, interaction cation–π, effet hydrophobe.

Introduction

The cation–π interaction is implicated in many recognition events in biology.[1–5] Its importance for the recognition of cholinergic agents by their receptors[3,6,7] has long been understood. Its role in the recognition of post-translationally methylated lysine residues has more recently come to light,[4,8–10] and the protein data bank (PDB) now contains many examples of trimethyllysine-containing quaternary ammonium complexes.[11] In these structures, the binding of the quaternary ammonium ion is accomplished by motifs referred to as "aromatic cages" in which clusters of three to four aromatic side chains make close cation–π contacts with the $RNMe_3^+$ portion of the side chain (Fig. 1). The most electron-rich aromatic side chain, tryptophan (Trp), is present as at least one of the binding partners in all trimethyllysine-binding aromatic cage motifs currently reported in the PDB, where it is accompanied by additional phenylalanine (Phe) and (or) tyrosine (Tyr) residues.

Inspired by the use of multiple aromatic rings in the protein domains that bind quaternary ammonium ions, we considered the unnatural modified amino acid 1-benzyltryptophan (Trp(Bn), **1**, Fig. 1) as a building block that could be easily incorporated into peptide-based hosts. A prior report of the dipeptide Boc-Trp-Trp-OMe (**2**) as a simple receptor for ammonium ions in organic solvents[12] led us to wonder if it could be adapted to achieve the far more difficult task of binding cations in pure water by the incorporation of Trp(Bn) residues. We report here, the synthesis of 1-benzyltryptophan based reagents and their rapid and modular incorporation into quaternary ammonium ion binding dipeptide hosts related to the parent Trp-Trp dipeptide (**2**).

Results and discussion

We were surprised to find very few reports of the apparently simple 1-benzyltryptophan (**1**) in the literature. Only six literature references were found in a Scifinder structure-based search,[13–18] and none of these references report the appended 1-benzyl group being used as a functional element. The obvious routes for the synthesis of **1** start with simple tryptophan analogs and involve deprotonation and alkylation of the side chain nitrogen atom. We first targeted the treatment of tryptophan methyl ester with 1 equiv. of NaH followed by treatment with a benzyl halide. Reactions were attempted in THF and DMF using a variety of temperatures and reaction times. While selective benzylation of the side chain nitrogen could be achieved, we found significant racemization of the α-carbon under all conditions that we tested (racemization is most easily detected by the observation of diastereomeric α-CH ^{1}H NMR resonances once dipeptides are made from a Trp(Bn) precursor). We next attempted to

Received 9 April 2010. Accepted 27 June 2010. Published on the NRC Research Press Web site at canjchem.nrc.ca on 23 September 2010.

In memory of Michael Pollard.

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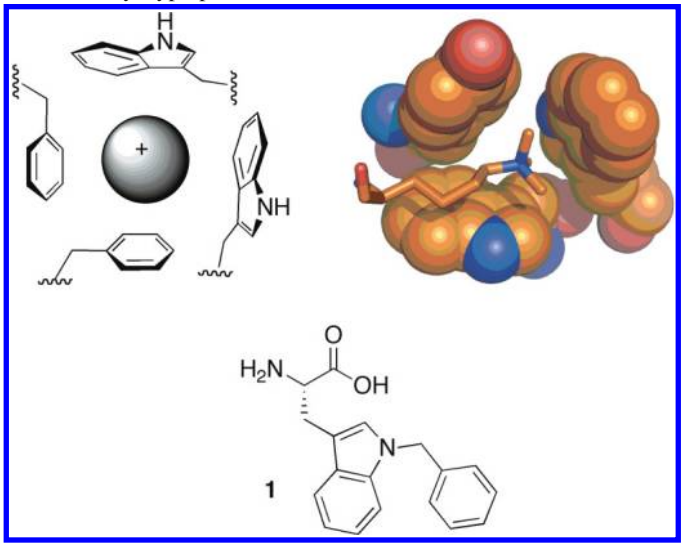
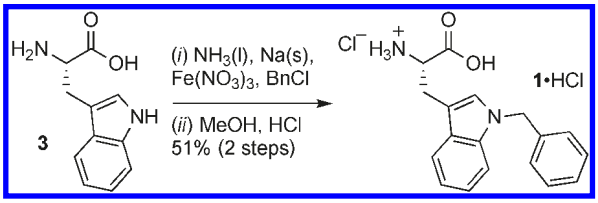
Fig. 1. Top: aromatic cage binding motifs that are used by proteins that recognize biologically important ammonium ions of the form $RNMe_3^+$. The structure on the right is an example of an aromatic cage taken from the PDB entry 1PDQ. Bottom: the unnatural amino acid 1-benzyltryptophan.



treat the free-acid Trp (**3**) with two or more equivalents of NaH to generate the doubly deprotonated carboxylate–indole dianion, with the idea that the more reactive indole anion could be reacted selectively with 1 equiv. of benzyl halide. These efforts effectively prevented racemization, but generated intractable mixtures and low yields arising from mixtures of O- and N-benzylation products. We subsequently adapted a reported deprotonation of unprotected Trp under dissolving metal conditions (Na–NH_3) and subsequent treatment with BnCl in liquid NH_3 (Scheme 1).[14] We found that this method was successful only when performed on ≥5 g of **3**, and crude yields of **1**, uncontaminated by *O*-benzyl or racemized by-products, reached a high of 70% when the reaction was carried out on a 40 g scale. We found an efficient purification for **1** by first dissolving the crude in a mixture of MeOH and concentrated aqueous HCl; precipitation by the addition of Et_2O gave the pure product as the HCl salt (**1**·HCl) in a 51% overall yield.

Scheme 2 depicts the synthesis of Boc-Trp(Bn)-OH (**4**) and H_2N-Trp(Bn)-OMe (**5**) that were used in the subsequent synthesis of a family of organic- and water-soluble host dipeptides. Treatment of **1**·HCl with thionyl chloride in methanol gives methyl ester **5** in a 87% yield, whereas treatment of **1**·HCl with NaOH in the presence of $(Boc)_2O$ under mixed aqueous-organic conditions gives the Boc-protected compound **4** in a 78% yield. Compound **5** was coupled with commercially available Boc-Trp-OH (**6**) to form the monobenzylated dipeptide host (Boc-Trp-Trp(Bn)-OMe, **7**) in a 49% yield. Compound **4** was combined with **5** under standard solution-phase HBTU coupling conditions to yield the dibenzylated dipeptide host (Boc-Trp(Bn)-Trp(Bn)-OMe, **8**) in a 61% yield. To complete the series, **4** was coupled with commercially available H_2N-Trp-OMe (**9**) to afford Boc-Trp-Trp-OMe (**2**) with free indole side chains in a 76% yield. Unlike the prior synthesis of host **2**,[12] all transformations reported herein were optimized to allow purification without resorting to flash chromatography.

Scheme 1. Selective 1-benzylation of Trp and isolation as the HCl salt.



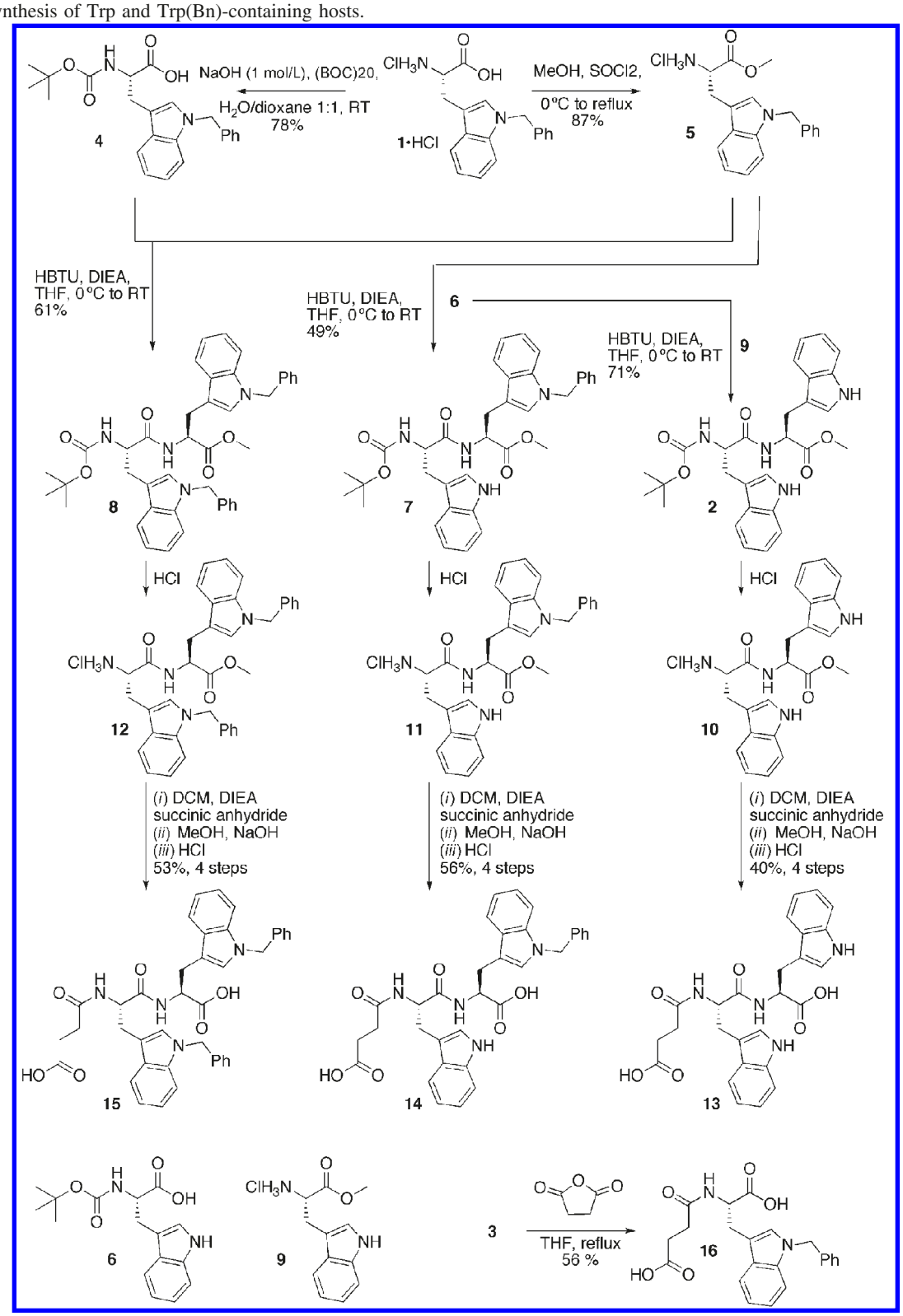
To create water-soluble analogs that retained an overall anionic charge, we doubly deprotected compounds **2**, **7**, and **8** and created simple N-terminal acetylated derivatives of the form Ac-Trp-Trp-OH (not shown). Unfortunately, dipeptides of this form were insoluble in phosphate-buffered D_2O (pD = 7.4). In an effort to increase water solubility while leaving the Trp(Bn) binding motifs unchanged, we targeted structures with N-terminal succinyl groups. Thus, compounds **2**, **7**, and **8** were Boc-deprotected in HCl–AcOH to give the dipeptide hydrochloride salts **10**, **11**, and **12**, respectively, as pure white solids after filtration and washing with Et_2O. The suspension of **10**–**12** in CH_2Cl_2, neutralization with *i*-Pr_2EtN, and treatment with succinic anhydride gave the succinyl (Suc) derived Suc-Trp-Trp-OMe dipeptides, which were directly saponified in methanol–water to yield, after acidification of the aqueous work-up layer, dipeptides **13**–**15** (40%–56% overall yield over four synthetic steps; Scheme 2). Compounds **13**–**15** are all soluble in phosphate-buffered D_2O (pD = 7.4) at concentrations up to 10 mmol/L.

Acetylcholine chloride ($AcCh^+$ Cl^-) was used as a prototypical $RNMe_3^+$ guest because of its high solubility in both water and chloroform, which is a critical feature that we did not find in halide or BArF salts of other guest candidates trimethyllysine, tetramethylammonium, or benzyltrimethylammonium. Titration of $AcCh^+$ Cl^- into the dipeptide hosts **13**–**15** in phosphate-buffered D_2O (10 mmol/L Na_2HPO_4–NaH_2PO_4, pD = 7.4) revealed guest-induced chemical shifts that fit to 1:1 binding isotherms to provide K_{assoc} values (Fig. 2 and Table 1). Even at the highest host and guest concentrations tested (10 and 930 mmol/L, respectively), the unbenzylated host **13**, which is most closely related to the literature host **2** that can bind $AcCh^+$ in $CDCl_3$,[12] showed no detectable binding of $AcCh^+$ under these competitive solvent conditions.

The monobenzylated host **14** and dibenzylated host **15** both showed evidence of binding that, while weak, was reproducibly observed over many determinations with the highest affinity arising from dibenzylated **15**. Direct evidence for the interaction of benzyl groups with the bound cation was given by the large chemical shift changes among the benzylic methylenes of dipeptide **15** (Fig. 2B), while the shifts of both benzyl and indolyl resonances in the aromatic regions (Fig. 2A) further support the participation of both types of aromatic rings in binding.

To examine the role of solvent effects in these hosts, we carried out binding studies of the chloroform-soluble analogs **2**, **7**, and **8** using the same titration methodology. In this solvent, the unbenzylated host **2** bound $AcCh^+$ Cl^- with a K_{assoc} of 85 $(mol/L)^{-1}$, which is in agreement with the value previously reported.[12] Suprisingly, our new benzylated hosts **7** and **8** bound $AcCh^+$ more weakly than did the unbenzylated

Scheme 2. Synthesis of Trp and Trp(Bn)-containing hosts.



parent (Table 1), despite the presence of additional aromatic binding surfaces. We also found that host **2** is also the most potent host for the binding of the the noncoordinating $BnNMe_3^+$ $BArF^-$, suggesting that this result is not an artifact arising from counterion effects in chloroform.[19] The cation–π interaction is an electrostatic attraction that should be stronger in nonpolar media; the fact that we see the opposite trend in $CDCl_3$ suggests that it is not a major contributor to interactions between the benzyl groups and cationic guests. Instead, it is likely the increase in hydrophobic surface area provided by the benzyl groups that leads to the higher cation affinities observed for benzylated hosts in D_2O.

Fig. 2. Concentration-dependent chemical shift changes displayed by the host **15** (10 mmol/L) in its (*a*) aromatic and (*b*) methylene regions upon addition of $AcCh^+$ Cl^- in D_2O (phosphate buffer, pD 7.4, 10 mmol/L). Concentrations of $AcCh^+$ Cl^- are indicated. (*c*) A plot of titration data and the 1:1 binding isotherm arising from nonlinear least-squares regression (see Experimental).

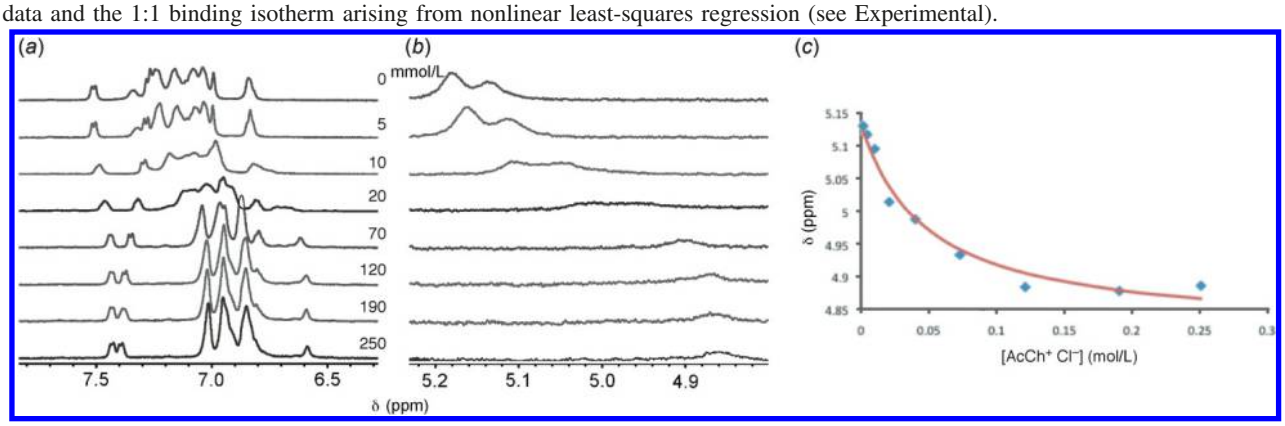


Table 1. K_{assoc} values for cations with hosts **13**–**16** in D_2O and **2**, **7**, and **8** in $CDCl_3$.

Host	Guest	Solvent	K_{assoc} ($(mol/L)^{-1}$)
13 (Trp-Trp)	$AcCh^+$ Cl^{-a}	D_2O	≤1[b]
14 (Trp-Trp(Bn))	$AcCh^+$ Cl^{-a}	D_2O	3±1
15 (Trp(Bn)-Trp(Bn))	$AcCh^+$ Cl^{-a}	D_2O	14±6
16 (Trp(Bn))	$AcCh^+$ Cl^{-a}	D_2O	≤1[b]
2 (Trp-Trp)	$AcCh^+$ Cl^{-a}	$CDCl_3$	85±17
7 (Trp-Trp(Bn))	$AcCh^+$ Cl^{-a}	$CDCl_3$	22±4
8 (Trp(Bn)-Trp(Bn))	$AcCh^+$ Cl^{-a}	$CDCl_3$	1±0.5
2 (Trp-Trp)	$BnNMe_3^+$ $BArF^{-c}$	$CDCl_3$	63±12
7 (Trp-Trp(Bn))	$BnNMe_3^+$ $BArF^{-c}$	$CDCl_3$	27±5
8 (Trp(Bn)-Trp(Bn))	$BnNMe_3^+$ $BArF^{-c}$	$CDCl_3$	47±3

Note: K_{assoc} was determined using at least three replicate titrations, tracking two to three proton resonances during each titration. Errors represent the upper and lower limits of replicate titrations. "D_2O" also contained 10 mmol/L NaH_2PO_4–Na_2HPO_4 adjusted to pD 7.4.

[a]Guest solution (ranging from 80 to 930 mmol/L) was titrated into a solution of the host (10 mmol/L).

[b]Titration resulted in insignificant Δδ values.

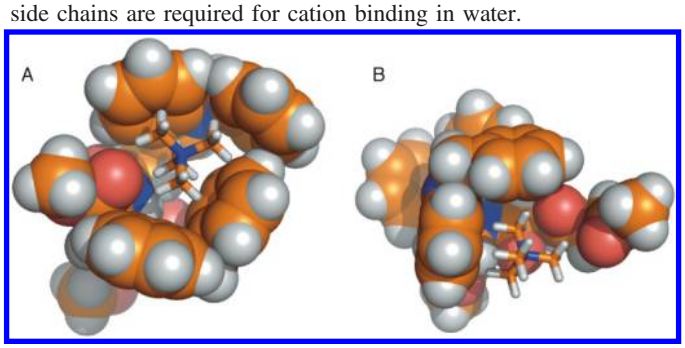
[c]A concentrated solution of the host (80–250 mmol/L) was titrated into the guest (2 mmol/L) due to low solubility of the $BnNMe_3^+$ $BArF^-$ salt in $CDCl_3$.

To gain some insight into the possible structures of these host–guest complexes, we carried out Monte Carlo conformational searches using a representative dibenzylated dipeptide host (the methyl ester analog of **15**) and the truncated guest Me_4N^+. These gas-phase calculations are limited in scope and capabilities; they are subject to unrealistic overemphasis of electrostatic attractions (hence our use of the methyl ester host analog) and they are incapable of representing the hydrophobic driving forces that are critical in aqueous solution. However, they are very good at determining energetically reasonable bond angles and intra- and intermolecular contacts, and are therefore useful for gaining a qualitative sense of what types of structures might be allowable for the host–guest complex. The two most common types of structures among low-energy conformations obtained from our Monte Carlo calculation involve (*i*) the interaction of the cation with both benzyl indole moieties in a pocket-like geometry ("pocket", Fig. 3A) or (*ii*) the interaction of the cation with only a single Trp(Bn) side chain ("cleft", Fig. 3B). The cleft structure in Fig. 3B suggests that only one benzyl indole group and a neighboring carbonyl might be needed to engage the cationic guest. To test this hypothesis, we designed compound **16** (Suc-Trp(Bn)-OH) to see if this monomeric host fragment was a competent cation binder in water. Compound **16** was synthesized in a 56% yield by the reaction of succinic anhydride with precursor **3** (Scheme 2). NMR titrations of **16** with $AcCh^+$ Cl^- in buffered D_2O, carried out as for the other hosts, gave no observable sign of binding, proving that two Trp residues are required for cation binding in water by these dipeptides.

Conclusions

These studies with simple dipeptide hosts introduce the readily synthesized Trp(Bn) building block as a new quaternary ammonium ion binding motif that can function in pure, buffered water. The weakness of the observed binding by these dipeptides in either medium almost certainly arises from their lack of preorganization relative to more rigid

Fig. 3. Representative structures, (A) "pocket" and (B) "cleft", arising from Monte Carlo conformation searches at the PM3 level of theory. Structures like that shown in Fig. 3A are consistent with both of our key experimental observations in water: the *N*-benzyl subsitituents play a significant role in binding, and both amino acid side chains are required for cation binding in water.



macrocyclic systems.[9,10] We are curious to see if rigidification of these building blocks by incorporation into small, folded peptide scaffolds increases the potency of Trp(Bn) building blocks as cation binders.

Experimental

General procedure

All solvents and reagents were used as purchased from Sigma-Aldrich and used without further purification, except solvents that were dried using an MBraun solvent purification system. NMR spectra and NMR titration data were obtained on a Brüker Avance 500 MHz NMR spectrometer with the exception of ^{19}F NMR, which was obtained on a Brüker Avance 300 MHz spectrometer. Coupling constants are reported in Hertz. ^{1}H and ^{13}C spectra were referenced to residual solvent signals, and the ^{19}F spectrum was externally referenced to $FCCl_3$. HR-MS were acquired on a Waters/Micromass LCT TOF instrument. Melting points were determined with the use of a GallenKamp melting point apparatus. Specific rotations (c = g/100 mL) were aquired with a Rodolf Research Analytical Autopol I instrument and are given in units of 10^{-1} deg cm^2 g^{-1}.

The typical titration procedure is as follows: The molecule of interest was dissolved in the appropriate solvent (dispensed with a micropipette) to make a solution (4 mL) of a predetermined concentration (2–10 mmol/L). The stock (600 μL) was then introduced into an NMR tube and to the remaining stock solution was added an excess of host or guest. The NMR spectrum of the stock solution was used as a reference for unbound host or guest and the solution titrated with predetermined volumes (micropipette) of titrant, followed by the collection of new NMR spectra. This method ensures that the concentration of the host remains constant throughout the titration. The Δδs were recorded and the data worked-up and fitted to the 1:1 binding isotherm using the spreadsheet made freely available by Sanderson.[20] Initial guesses for host or guest concentrations during the course of the experiments were obtained by the method discussed by Hirose.[21]

Calculations were carried out at the PM3 level of theory using the conformational search routine in Spartan'04.[22] Structures within 5 kcal/mol (1 cal = 4.186 8 J) of the global minimum were retained, and examination revealed that all such low-energy structures could be sorted into two groups by considering the number of aryl side chains making contact with the quaternary ammonium ion (pocket = two Trp(Bn) side chains in contact, Fig. 3A; cleft = one Trp(Bn) residue in contact, Fig. 3B).

1-Benzyltryptophan hydrochloride (1·HCl)

Liquid ammonia (1000 mL) was condensed into a 2 L flask containing Na(s) (10.4 g, 452 mmol) and $Fe(NO_3)_3$ (0.6 g, 0.01 mmol) under argon. To facilitate the addition of ammonia, the flask was cooled in a dry ice – acetone bath and equipped with a cold finger (dry ice – acetone) via a Claisen adapter. The resultant mixture turns blue immediately and slowly fades to a grey color. Tryptophan (40 g, 196 mmol) was introduced to the grey solution as a slurry in ether, and benzyl chloride (22.5 mL, 196 mmol) was added immediately thereafter via syringe through the septum. The reaction refluxed on an ice bath for 1 h, and evaporated overnight. The crude product dissolved in hot water and a small amount of solid residue was removed by filtration. Acetic acid was added until the product precipitated, and filtration yielded the 1-benzyltryptophan as an off-white solid (41 g, 71%). A portion of this product (24 g, 81 mmol) was suspended in methanol (200 mL) at room temperature and concentrated aqueous hydrochloric acid was added dropwise until the starting material was completely dissolved. The reaction was stirred for 5 min, and was then concentrated to half volume. The solution was then poured into 1.6 L of ether and the resultant slurry was filtered to yield **1**·HCl as a white solid that was left to air-dry overnight (19 g, 72%); mp 204–206 °C. $[\alpha]_D^{25}$ +5.0° (c 0.08 in MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.32 (2H, J = 6.3, d), 4.12 (1H, bs), 5.38 (2H, s), 7.03 (1H J = 7 and 1, td), 7.10 (1H, J = 8 and 1, td), 7.18–7.33 (5H, m), 7.37 (1H, J = 8, d), 7.39 (1H, s), 7.63 (1H, J_{HH} = 7, d), 8.31 (3H, bs), 13.77 (1H, bs). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 26.0, 49.1, 52.6, 106.8, 110.2, 118.7, 118.9, 121.4, 127.0, 127.3, 127.7, 128.5, 128.7, 136.1, 138.1, 170.7. ESI (accurate mass) m/z: 295.1452 (M^+ $C_{18}H_{19}N_2O_2$ requires 295.1447).

N-(tert-*Butoxycarbonyl)-1-benzyltryptophan (4)*

3 (1.0 g, 3.0 mmol) was suspended in 1:1 dioxane–water (12 mL) at room temperature. NaOH (aq, 1 mol/L, 6 mL, 6 mmol) was added, followed by the addition of $(Boc)_2O$ (0.84 mL, 3.6 mmol), and this mixture was stirred for 18 h. The reaction was acidified to pH 2.5 with HCl (2 mol/L), and diluted further with water (50 mL). Ethyl acetate was then used to extract the mostly aqueous mixture. The organic layers were combined, dried, and concentrated to yield a white solid (0.928 g, 78%); mp 181–182 °C (EtOAc). $[\alpha]_D^{25}$ +9.1° (c 0.08 in MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.13 (9H, s), 2.99 (1H, J = 15 and 9, dd), 3.14 (1H, J = 14 and 5, dd), 4.16 (1H, J = 9 and 5, td), 5.36 (2H, s), 6.97–7.1 (3H, m), 7.13–7.16 (2H, m), 7.19–7.31 (4H, m), 7.37 (1H, J = 8, d), 7.55 (1H, J = 7, d), 12.55 (1H, bs). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 26.7, 28.1, 48.9, 54.4, 77.9, 110.0, 110.3, 118.6, 121.2, 126.9, 127.2, 127.4, 127.8, 128.4, 135.9, 138.3, 155.3, 173.8. ESI (accurate mass) m/z calcd.: 417.1790; found: 417.1789.

Methyl 1-benzyltryptophanate hydrochloride (5)

3 (5.0 g, 15.1 mmol) was dissolved in methanol (300 mL) and cooled to 0 °C. Thionyl chloride (1.1 mL, 15.1 mmol) was added dropwise and the mixture was then allowed to warm to room temperature before being brought to reflux. After overnight stirring at reflux, the reaction was cooled and concentrated. Trituration with diethyl ether provided a white powder with no further purification needed (4.55 g, 87%); mp 115–117 °C (ether). $[\alpha]_D^{25}$ +11.3° (*c* 0.15 in MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.26 (1H, *J* = 15 and 7, dd), 3.38 (1H, *J* = 15 and 5, dd), 3.60 (3H, s), 4.19 (1H, *J* = 7 and 5, dd), 5.38 (2H, s), 7.04 (1H, *J* = 7 and 1, td), 7.11 (1H, *J* = 8 and 1, td), 7.18–7.33 (5H, m), 7.37 (1H, s), 7.41 (1H, *J* = 8, d), 7.59 (1H, *J* = 7, d), 8.71 (3H, bs). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 26.1, 49.0, 52.5, 52.7, 106.6, 110.2, 118.5, 119.0, 121.5, 127.0, 127.3, 127.5, 128.4, 128.5, 136.0, 138.1, 169.7. ESI (accurate mass) *m*/*z* calcd.: 309.1603; found: 309.1603.

Dipeptide coupling general procedure

N-terminally protected (Boc) amino acid (2.0 mmol) and HBTU (0.78 g, 2 mmol) were added to a flask containing THF (70 mL). The mixture was cooled to 0 °C and 1 equiv. of DIEA (0.35 mL, 2.03 mmol) was added. The mixture was stirred for 10 min. C-terminally protected (methyl ester) amino acid (2 mmol) and 1.2 more equiv. of DIEA (0.42 mL, 2.4 mmol) were added to the reaction, which was then warmed to room temperature and stirred for 2 h, regardless of completeness. Once complete, the reaction was concentrated, taken up in ethyl acetate, and washed with 1 mol/L HCl (×2), satd. $NaHCO_3$ (×2), water, and brine. The organic layer was dried with Na_2SO_4 and concentrated to produce a white powder (or foam) after exposure of the residue to high vacuum overnight.

Methyl* N-*[*N-*(*tert-*butoxycarbonyltryptophyl)]-tryptophanate (2)

After initial concentration, the product was precipitated from a minimum volume of CH_2Cl_2 at –20 °C as a white solid (1.51 g, 71%); mp 162–164 °C (CH_2Cl_2). $[\alpha]_D^{25}$ –7.0° (*c* 0.08 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.26 (9H, s), 2.84 (1H, *J* = 15 and 9, dd), 3.00 (1H, *J* = 15 and 4, dd), 3.06 (1H, *J* = 15 and 7, dd), 3.13 (1H, *J* = 15 and 6, dd), 3.52 (3H, s), 4.21 (1H, *J* = 9 and 4, td), 4.53 (1H, *J* = 7.0 and 6, td), 6.69 (1H, *J* = 8, d), 6.92–6.97 (2H, m), 7.00–7.06 (3H, m), 7.14 (1H, *J* = 2, d), 7.28–7.33 (2H, m), 7.44 (1H, J_{HH} = 8, d), 7.55 (1H, *J* = 8, d), 8.23 (1H, *J* = 8, d), 10.77 (1H, s), 10.86 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 27.1, 27.7, 28.1, 51.8, 53.1, 55.0, 78.1, 109.2, 110.1, 111.3, 111.5, 118.0, 118.2, 118.46, 118.51, 118.9, 120.8, 121.0, 123.7, 127.1, 127.4, 136.06, 136.10, 155.1, 172.1, 172.2. ESI (accurate mass) *m*/*z* calcd.: 527.2270; found: 527.2271.

Methyl* N-*[*N-*(*tert-*butoxycarbonyl)tryptophyl]-1-benzyltryptophanate (7)

A white powder was isolated (1.16 g, 49%); mp 76–78 °C then 99–100 °C (EtOAc). $[\alpha]_D^{25}$ –15.6° (*c* 0.09 in MeOH). ^{1}H NMR (500 MHz) δ: 1.26 (9H, s), 2.89 (1H, *J* = 15 and 9, dd), 3.06 (1H, *J* = 15 and 4, dd), 3.10 (1H, *J* = 15 and 7, dd), 3.18 (1H, *J* = 15 and 6, dd), 3.54 (3H, s), 4.26 (1H, *J* = 9 and 4, td), 4.60 (1H, *J* = 7 and 6, td), 5.35 (2H, s), 6.72 (1H, *J* = 8, d), 6.97(1H, *J* = 7, t), 7.00–7.10 (3H, m), 7.12–7.21 (3H, m), 7.25–7.27 (2H, m), 7.31–7.33 (2H, m), 7.38 (1H, *J* = 8, d), 7.51 (1H, *J* = 8, d), 7.60 (1H, *J* = 8, d), 8.38 (1H, *J* = 7, d), 10.81 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 27.0, 27.7, 28.1, 49.0, 51.8, 53.0, 55.0, 78.0, 109.3, 110.07, 110.12, 111.2, 118.1, 118.4, 118.5, 118.8, 120.8, 121.3, 123.7, 126.9, 127.2, 127.4, 127.5, 127.7, 128.5, 135.8, 136.0, 138.2, 155.1, 172.1, 172.2. ESI (accurate mass) *m*/*z* calcd.: 617.2740; found: 617.2754.

Methyl* N-*[*N-*(*tert-*butoxycarbonyl)-1-benzyltryptophyl)]-1-benzyltryptophanate (8)

A white powder was isolated (0.83 g, 61%); mp 149–151 °C (EtOAc). $[\alpha]_D^{25}$ +4.0° (*c* 0.08 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.28 (9H, s), 2.91 (1H, *J* = 15 and 9, dd), 3.05 (1H, *J* = 15 and 4, dd), 3.09 (1H, *J* = 15 and 7, dd), 3.18 (1H, *J* = 15 and 6, dd), 3.52 (3H, s), 4.27 (1H, *J* = 9 and 4, td), 4.58 (1H, *J* = 7 and 6, td), 5.32 (2H, s), 5.35 (2H, s), 6.74 (1H, *J* = 8, d), 6.98–7.03 (1H, m), 7.05–7.09 (2H, m), 7.12–7.28 (11H, m), 7.32–7.38 (2H, m), 7.51 (1H, *J* = 8, d), 7.61 (1H, *J* = 8, d), 8.31 (1H, *J* = 7, d). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 27.0, 27.6, 28.1, 48.90, 48.94, 51.8, 53.0, 55.0, 78.0, 109.3, 109.9, 110.1, 110.2, 118.4, 118.5, 118.8, 118.9, 121.1, 121.3, 126.8, 126.9, 127.2, 127.5, 127.7, 128.0, 128.4, 135.8, 135.9, 138.2, 138.3, 155.1, 171.9, 172.2. ESI (accurate mass) *m*/*z* calcd.: 707.3209; found: 707.3201.

General procedure for BOC deprotection

Starting material (0.6 mmol) was suspended in acetic acid at room temperature. In a separate flask, HCl (concd) was diluted with acetic acid to form a 1.5 mol/L solution of HCl in acetic acid. The resultant HCl in acetic acid solution (9.4 mL, 14 mmol HCl) was added in one portion to the starting material suspension. The suspension was dispersed upon addition of the HCl solution, followed by the appearance of another precipitate 1 h postaddition. The reaction was stirred overnight, filtered, and the cake washed with ether to yield a white solid that needs no further purification.

Methyl* N-*tryptophyl-tryptophanate hydrochloride (10)

The work-up differed as the product was soluble in acetic acid. The acetic acid was concentrated and diluted with ether. A light brown solid resulted, was filtered, and isolated (0.23 g, 90%); mp (foams) 162–170 °C, mp (melts) 178–180 °C (ether). $[\alpha]_D^{25}$ –4.2° (*c* 0.08 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.05–3.30 (4H, m), 3.58 (3H, s), 4.03 (1H, *J* = 8 and 6, dd), 4.62 (1H, *J* = 7, q), 6.98–7.01 (2H, m), 7.08 (2H, *J* = 8, q), 7.21 (1H, s), 7.22 (1H, s), 7.35 (2H, *J* = 8, t), 7.50 (1H, *J* = 8, d), 7.71 (1H, *J* = 8, d), 8.1 (3H, bs), 9.11 (1H, *J* = 7.0, d), 10.96 (1H, s), 11.04 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 27.2, 27.3, 52.0, 52.5, 53.3, 106.8, 108.9, 111.4, 111.5, 117.9, 118.4, 118.47, 118.52, 121.0, 121.1, 124.0, 125.0, 127.0, 127.1, 136.1, 136.3, 168.8, 171.6. ESI (accurate mass) *m*/*z* calcd.: 405.1927; found: 405.1927.

Methyl* N-*tryptophyl-1-benzyltryptophanate hydrochloride (11)

A white solid was isolated (0.112 g, 69%); mp 222–

224 °C (AcOH). $[\alpha]_D^{25}$ +2.3° (*c* 0.08 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.08–3.29 (4H, m), 3.55 (3H, s), 4.04 (1H, *J* = 6, t), 4.62 (1H, *J* = 7, q), 5.37 (2H, s), 5.37 (2H, s), 7.00 (1H, *J* = 7, t), 7.03 (1H, *J* = 7, t), 7.09 (2H, *J* = 7, t), 7.16 (2H, *J* = 7, d), 7.21(2H, *J* = 7, t), 7.27 (2H, *J* = 7, t), 7.35 (1H,s), 7.36 (1H, *J* = 11, d), 7.41(1H, *J* = 8, d), 7.53 (1H, *J* = 8, d), 7.70 (1H, *J* = 8, d), 8.16 (3H, bs), 9.15 (1H, *J* = 6, d) 11.00 (1H, s). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 27.1, 27.3, 48.9, 51.9, 52.4, 53.4, 106.7, 108.9, 110.2, 111.4, 118.4, 118.5, 118.8, 121.1, 121.3, 125.0, 126.9, 127.1, 127.3, 127.6, 127.8, 128.5, 135.9, 136.3, 138.3, 168.6, 171.5. ESI (accurate mass) *m*/*z* calcd.: 495.2396; found: 495.2397.

Methyl N-*(1-benzyltryptophyl)-1-benzyltryptophanate hydrochloride (12)*

A white solid was isolated (0.30 g, 83%); mp 219–220 °C (AcOH). $[\alpha]_D^{25}$ +6.2° (*c* 0.08 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.12–3.28 (4H, m), 3.51 (3H, s), 4.09 (1H, bs), 4.61 (1H, *J* = 7, q), 5.35 (2H, s), 5.37 (2H, s), 7.03 (2H, *J* = 7, t), 7.08–7.12 (2H, m), 7.16–7.31 (10H, m), 7.33 (1H, s), 7.37 (1H, *J* = 8, d), 7.39 (1H, s), 7.41(1H, *J* = 8, d), 7.53 (1H, *J* = 8, d), 7.75 (1H, *J* = 8, d), 8.31 (3H, bs), 9.2 (1H, *J* = 7, d). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 27.7, 27.8, 49.6, 49.8, 52.5, 53.2, 54.1, 107.5, 109.5, 110.7, 110.8, 119.0, 119.4, 119.5, 119.6, 121.97, 122.02, 127.6, 127.7, 127.89, 127.93, 128.2, 128.4, 128.5, 129.1 (×2), 129.4, 136.6, 136.8, 138.7, 138.9, 169.2, 172.2. ESI (accurate mass) *m*/*z* calcd.: 585.2866; found: 585.2875.

General method for the sequential installation of the succinyl group on N-terminal amines and saponification of C-terminal methyl esters

Starting material **10**, **11**, or **12** (0.25 mmol) was suspended in CH_2Cl_2 (10 mL) at room temperature. Succinic anhydride (0.036 g, 0.36 mmol) was added to the reaction, followed by DIEA (0.095 mL, 0.55 mmol), and the mixture stirred overnight. Dilution of the reaction with CH_2Cl_2 (50 mL), followed by extraction with HCl (1 mol/L, 2 × 50 mL), followed by back extraction of the aqueous with CH_2Cl_2 was sufficient to isolate the methyl ester of the desired product. The combined organic layers were washed with brine (1 × 50 mL) and dried over Na_2SO_4 before concentration. The sticky residue was used without further purification to produce the de-esterified product. Dissolution in methanol (3 mL), followed by the addition of NaOH (1 mol/L, 0.75 mL) furnished the final product after 2 h of stirring. The reaction was diluted with water (5 mL) and acidified with HCl (1 mol/L) to pH 2. The suspension was extracted with ethyl acetate and the organic layer was washed with brine and dried over Na_2SO_4 before concentration.

N-*[*N-*(3-Carboxypropanoyl)-tryptophyl]-tryptophan (13)*

Isolated as a light brown powder (0.055 g, 44%); mp (turns dark brown) 88 °C, mp (melts) 154–156 °C (EtOAc). $[\alpha]_D^{25}$ –11.6° (*c* 0.09 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.25–2.34 (4H, m), 2.88 (1H, *J* = 15 and 9, dd), 3.08 (1H, *J* = 15 and 8, dd), 3.12 (1H, *J* = 15 and 4, dd), 3.19 (1H, *J* = 15 and 5, dd), 4.50 (1H, *J* = 8 and 5, td), 4.58 (1H, *J* = 9.0 and 4, td), 6.97 (1H, *J* = 8, t), 6.98 (1H, *J* = 8, t), 7.05 (1H, *J* = 8, t), 7.06 (1H, *J* = 8, t), 7.11 (1H, *J* = 2.0, d), 7.16 (1H, *J* = 2.0, d), 7.31 (1H, *J* = 8, d), 7.33 (1H, *J* = 8, d), 7.53 (1H, *J* = 8, d), 7.58 (1H, *J* = 8, d), 8.01 (1H, *J* = 8, d), 8.17 (1H, *J* = 8, d), 10.77 (1H, bs), 10.85 (1H, bs), 12.36 (2H, bs), 12.7 (bs, 1H). ^{13}C NMR (126 MHz) δ: 27.0, 27.6, 29.2, 30.0, 53.0, 53.2, 109.7, 110.2, 111.2, 111.4, 118.2, 118.40, 118.44, 120.8, 120.9, 123.5, 123.6, 127.3, 127.4, 136.0, 136.1, 170.9, 171.6, 173.2, 173.9. ESI (accurate mass) *m*/*z* calcd.: 489.1774; found: 489.1773.

N-*[*N-*(3-Carboxypropanoyl)-tryptophyl]-1-benzyltryptophan (14)*

Isolated as a white powder (0.074 g, 88%); mp 180 °C dec (EtOAc). $[\alpha]_D^{25}$ –11.9° (*c* 0.09 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.25–2.32 (4H, m), 2.89 (1H, *J* = 15 and 9, dd), 3.06 (1H, *J* = 15 and 8, dd), 3.11 (1H, *J* = 15 and 4, dd), 3.21 (1H, *J* = 15 and 5, dd), 4.50 (1H, *J* = 8.0 and 5, td), 4.58 (1H, *J* = 9.0 and 4, td), 5.35 (2H, s), 6.96 (1H, *J* = 7, t), 7.01 (1H, *J* = 7, t), 7.04 (1H, *J* = 7, t), 7.07 (1H, *J* = 7, t), 7.12 (1H, *J* = 2, d), 7.16–7.27 (5H, m), 7.31 (1H, *J* = 8, d), 7.32 (1H, s), 7.35 (1H, *J* = 8, d), 7.55 (1H, *J* = 8, d), 7.58 (1H, *J* = 8, d), 8.01 (1H, *J* = 8, d), 8.28 (1H, *J* = 8, d), 10.78 (1H, *J* = 2, d), 12.3 (1H, bs), 12.7 (1H, bs). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 26.8, 27.6, 29.1, 30.0, 49.0, 52.9, 53.3, 109.7, 110.1, 110.2, 111.2, 118.1, 118.4, 118.5, 118.7, 120.8, 121.2, 123.6, 126.9, 127.2, 127.4, 127.6, 127.9, 128.5, 135.8, 136.0, 138.3, 170.9, 171.7, 173.1, 173.8. ESI (accurate mass) *m*/*z* calcd.: 579.2244; found: 579.2231.

N-*[*N-*(3-Carboxypropanoyl)-1-benzyltryptophyl]-1-benzyltryptophan (15)*

A white solid was isolated (0.106 g, 64%); mp (turns brown) 180 °C, mp (melts) 190–192 °C (EtOAc). $[\alpha]_D^{25}$ –1.1° (*c* 0.09 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.24–2.32 (4H, m), 2.88 (1H, *J* = 15 and 9, dd), 3.07 (1H, *J* = 15 and 8, dd), 3.12 (1H, *J* = 15 and 4, dd), 3.21 (1H, *J* = 15 and 5, dd), 4.49 (1H, *J* = 8.0 and 5, td), 4.59 (1H, *J* = 9.0 and 4, td), 5.32 (2H, s), 5.34 (2H, s), 6.99 (1H, *J* = 7, q), 7.05–7.08 (2H, m), 7.14–7.29 (10H, m), 7.34 (2H, *J* = 9, d), 7.35 (2H, *J* = 8, d), 7.56 (1H, *J* = 8, d), 7.60 (1H, *J* = 8, d), 8.05 (1H, *J* = 8, d), 8.31 (1H, *J* = 8, d), 12.7 (2H, bs). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 26.8, 27.5, 29.1, 29.9, 48.9, 49.0, 53.0, 53.2, 109.7, 109.9, 110.0, 110.2, 118.5, 118.6, 118.7, 118.9, 121.1, 121.2, 126.9, 127.2, 127.4, 127.6, 127.9, 128.0, 128.4, 135.8, 138.3, 138.4, 170.8, 171.5, 173.1, 173.8. ESI (accurate mass) *m*/*z* calcd.: 669.2724; found: 669.2713.

Benzyltrimethylammonium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate ($BnNMe_3^+$ $BArF^-$)

Benzyltrimethylammonium chloride (0.107 g, 0.57 mmol) and NaBArF (0.510 g, 0.57 mmol) were stirred overnight in CH_2Cl_2 (40 mL). The resulting suspension was filtered through a syringe filter with a pore size of 0.45 μm and the filtrate was concentrated to produce a white solid. The solid was rinsed with pentane and dried in vacuo for 3 h (0.499 g, 86%); mp 136–137 °C (CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.82 (9H, s), 4.12 (2H, s), 7.22 (2H, *J* = 10, d), 7.47 (2H, *J* = 10, t), 7.51 (4H, bs), 7.57 (1H, *J* = 10, t), 7.67–7.68 (8H, m). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 53.2

(J_{CN} = 4, t), 71.9, 117.8, 124.7 (J_{CF} = 273, q), 124.8, 129.2 (J_{CF} = 29, q), 130.4, 132.3, 132.7, 134.9, 161.9 (J_{CB} = 50, q). ^{19}F NMR (282 MHz) δ: –62.3. ^{11}B NMR (160 MHz) δ: –6.8. ESI (accurate mass) *m/z* calcd. (anion): 862.0685; found: 862.0700; calcd. (cation): 150.1283; found: 150.1281.

N-*(3-Carboxypropanoyl)-1-benzyltryptophan (16)*

5 (0.926 g, 2.8 mmol) was suspended in dry THF and stirred at room temperature. Succinic anhydride (0.28 g, 2.8 mmol) and DIEA (0.49 mL, 2.8 mmol) were then added and the mixture refluxed overnight. The volume was reduced until a thick syrup formed. Ethyl acetate was added (300 mL) and the organic was extracted three times with NaOH (aq, 0.5 mol/L, 3 × 75 mL). The aqueous extracts were combined and acidified to pH 2 with HCl (1 mol/L). The brown oil was extracted with ethyl acetate. Upon drying (Na_2SO_4) and concentrating the residue solidifies on exposure to vacuum overnight. Purification with column chromatography ($CHCl_3$, AcOH, MeOH) with gradient elution from 94:5:1 to 93:5:2 was used to elute pure product. Trituration of the combined and concentrated fractions with ether yielded a white crystalline solid (0.634 g, 56%); mp 148–150 °C (ether). $[\alpha]_D^{25}$ +30° (*c* 0.09 in MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.25–2.34 (4H, m), 3.00 (1H, *J* = 15 and 9, dd), 3.17 (1H, *J* = 15 and 5, dd), 4.88 (1H, m), 7.01 (1H, *J* = 8, t), 5.35 (2H, s), 7.07 (1H, *J* = 8, t), 7.15–7.16 (2H, m), 7.21–7.23 (1H, m), 7.27–7.30 (3H, m), 7.37 (1H, *J* = 8, d), 7.56 (1H, *J* = 8, d), 8.19 (1H, *J* = 8, d), 12.37 (2H, bs). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 27.1, 29.0, 29.8, 48.9, 52.9, 109.9, 110.0, 118.6, 118.7, 121.2, 126.9, 127.2, 127.5, 127.8, 128.4, 135.9, 138.3, 170.9, 173.3, 173.8. ESI (accurate mass) *m/z* calcd.: 393.1456; found: 393.1450.

Supplementary data

Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca).

Acknowledgements

This work was funded by the Michael Smith Foundation for Health Research (MSFHR). FH is a MSFHR Career Scholar and Canadian Institutes of Health Research (CIHR) New Investigator.

References

(1) Dougherty, D. A.; Stauffer, D. A. *Science* **1990**, *250* (4987), 1558. doi:10.1126/science.2274786. PMID:2274786.

(2) Dougherty, D. A. *Science* **1996**, *271* (5246), 163. doi:10.1126/science.271.5246.163. PMID:8539615.

(3) Beene, D. L.; Brandt, G. S.; Zhong, W.; Zacharias, N. M.; Lester, H. A.; Dougherty, D. A. *Biochemistry* **2002**, *41* (32), 10262. doi:10.1021/bi020266d. PMID:12162741.

(4) Hughes, R. M.; Benshoff, M. L.; Waters, M. L. *Chem. Eur. J.* **2007**, *13* (20), 5753. doi:10.1002/chem.200601753.

(5) Hughes, R. M.; Wiggins, K. R.; Khorasanizadeh, S.; Waters, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (27), 11184. doi:10.1073/pnas.0610850104. PMID:17581885.

(6) Beene, D. L.; Brandt, G. S.; Zhong, W.; Zacharias, N. M.; Lester, H. A.; Dougherty, D. A. *Biochemistry* **2002**, *41* (32), 10262. doi:10.1021/bi020266d. PMID:12162741.

(7) Schmitt, J. D.; Sharples, C. G. V.; Caldwell, W. S. *J. Med. Chem.* **1999**, *42* (16), 3066. doi:10.1021/jm990093z. PMID:10447950.

(8) Taverna, S. D.; Li, H.; Ruthenburg, A. J.; Allis, C. D.; Patel, D. J. *Nat. Struct. Mol. Biol.* **2007**, *14* (11), 1025. doi:10.1038/nsmb1338. PMID:17984965.

(9) (a) Beshara, C. S.; Jones, C. E.; Daze, K. D.; Lilgert, B. J.; Hof, F. *ChemBioChem* **2010**, *11* (1), 63. doi:10.1002/cbic.200900633. PMID:19937593.; (b) Whiting, A. L.; Neufeld, N. M.; Hof, F. *Tetrahedron Lett.* **2009**, *50* (50), 7035. doi:10.1016/j.tetlet.2009.09.161.

(10) Ingerman, L. A.; Cuellar, M. E.; Waters, M. L. *Chem. Commun. (Camb.)* **2010**, *46* (11), 1839. doi:10.1039/c000255k. PMID:20198226.

(11) (a) Shi, X.; Kachirskaia, I.; Walter, K. L.; Kuo, J.-H. A.; Lake, A.; Davrazou, F.; Chan, S. M.; Martin, D. G. E.; Fingerman, I. M.; Briggs, S. D.; Howe, L.; Utz, P. J.; Kutateladze, T. G.; Lugovskoy, A. A.; Bedford, M. T.; Gozani, O. *J. Biol. Chem.* **2007**, *282* (4), 2450. doi:10.1074/jbc.C600286200. PMID:17142463.; (b) Jacobs, S. A.; Khorasanizadeh, S. *Science* **2002**, *295* (5562), 2080. doi:10.1126/science.1069473. PMID:11859155.; (c) Flanagan, J. F.; Mi, L.-Z.; Chruszcz, M.; Cymborowski, M.; Clines, K. L.; Kim, Y.; Minor, W.; Rastinejad, F.; Khorasanizadeh, S. *Nature* **2005**, *438* (7071), 1181. doi:10.1038/nature04290. PMID:16372014.; (d) Huang, Y.; Fang, J.; Bedford, M. T.; Zhang, Y.; Xu, R.-M. *Science* **2006**, *312* (5774), 748. doi:10.1126/science.1125162. PMID:16601153.

(12) Ito, K.; Nagase, K.; Morohashi, N.; Ohba, Y. *Chem. Pharm. Bull. (Tokyo)* **2005**, *53* (1), 90. doi:10.1248/cpb.53.90. PMID:15635237.

(13) Powers, J. C. *Tetrahedron Lett.* **1965**, *6* (11), 655. doi:10.1016/S0040-4039(00)90013-3.

(14) Yamada, S.; Shioiri, T.; Itaya, T.; Hara, T.; Matsueda, R. *Chem. Pharm. Bull. (Tokyo)* **1965**, *13* (1), 88. PMID:5864290.

(15) Magnus, P.; Mugrage, B.; DeLuca, M. R.; Cain, G. A. *J. Am. Chem. Soc.* **1990**, *112* (13), 5220. doi:10.1021/ja00169a033.

(16) Cornforth, J. W.; Cornforth, R. H.; Dalgliesh, C. E.; Neuberger, A. *Biochem. J.* **1951**, *48* (5), 591. PMID:14838907.

(17) Chen, Y.; Bilban, M.; Foster, C. A.; Boger, D. L. *J. Am. Chem. Soc.* **2002**, *124* (19), 5431. doi:10.1021/ja020166v. PMID:11996584.

(18) Kumaraswamy, G.; Pitchaiah, A.; Ramakrishna, G.; Ramakrishna, D. S.; Sadaiah, K. *Tetrahedron Lett.* **2006**, *47* (12), 2013. doi:10.1016/j.tetlet.2006.01.050.

(19) Ito et al.[12] previously reported that the Trp-Trp dipeptide **2** bound $BnNMe_3^+$ Cl^- with K_{assoc} of 69 $(mol/L)^{-1}$ in chloroform, and they attributed the relative strength to NH–Cl^- hydrogen bonding effects. Our observation that the binding of the $BnNMe_3^+$ salt of the nonhydrogen-bonding $BArF^-$ anion is essentially equal to their reported value indicates that the role of Cl^- may not be as important as previously described.

(20) Sanderson, J. M. *Excel Workbooks for Curve-fitting NMR Titration Data;* The Centre for Bioactive Chemistry, Department of Chemistry University Science Laboratories, Durham University: Durham, UK; Available from http://www.dur.ac.uk/j.m.sanderson/science/downloads.html.

(21) Hirose, K. *J. Incl. Phenom. Macrocycl. Chem.* **2001**, *39* (3–4), 193. doi:10.1023/A:1011117412693.

(22) *Spartan'04;* Wavefunction, Inc.: Irvine, CA, 2004.

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