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A Terphenyl Scaffold for π-Stacked Guanidinium Recognition Elements

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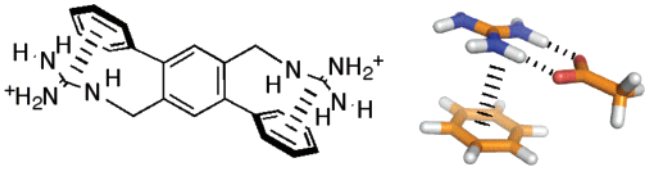
ABSTRACT



A new synthetic model of arginine–carboxylate–aromatic triads—common motifs at sites of protein–protein interactions—is reported. Binding studies in mixed methanol/water solvent systems suggest that the carboxylate-binding ability of π-stacked guanidinium ions is improved relative to a non-stacked control.

Arginine side chains are key mediators of a variety of protein binding events, frequently forming charged hydrogen bonds with phosphate, sulfate, and carboxylate binding partners. Arginine's guanidinium group also frequently interacts with nearby aromatic side chains via π-stacking—surveys of protein structures show that up to 74% of all arginine side chains are in close contact with aromatic residues,[1] and that among these contact pairs the parallel π-stacked and offset-stacked orientations are predominant.[2−4] Arginines also commonly participate in guanidinium−carboxylate−aromatic triads in which both of the above modes of interaction operate simultaneously. These motifs exist within monomeric proteins and also as "hot-spots" of several protein−protein interactions (Figure 1).[5−8] Despite the importance of these π-stacked salt bridges, the energetic influence of these two interactions on each other is not well understood. Does π-stacking increase or decrease the hydrogen bonding potential of a guanidinium ion? Studies of guanidinium hydrogen bonding and π-stacking individually are numerous, but synthetic models for the study of guanidinium ions participating in *both* hydrogen bonding and π-stacking are few.[9,10] We report herein the creation of a new synthetic model in which guanidinium ions and aromatic rings are pre-arranged in a π-stacked geometry and studies on its complexation of carboxylate partners.

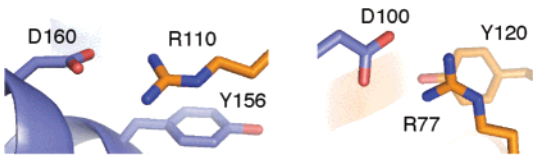


Figure 1. Examples of guanidinium−aromatic−carboxylate triads that mediate protein−protein interactions. (a) Residues at the interface of a complex between Importin-β (blue) and Ran (orange) (PDB code 1IBR).[7] (b) Residues at the interface of the complex of the Fyn SH3 domain (blue) and HIV-1 Nef (orange) (PDB code 1AVZ).[8]

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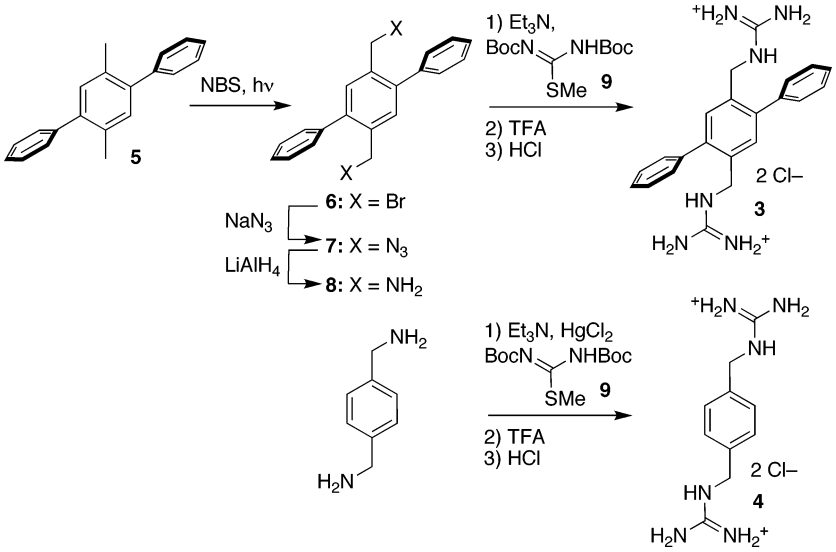
Scheme 1. Synthesis of Hosts **3** and **4**



The preorganization of two aryl groups into offset-stacked geometries was previously accomplished using structures such as **1** (Figure 2a).[11−13] We used molecular modeling of compound **2** to examine the effect of replacing one of the aryl residues with a guanidinium ion (Figure 2b). The covalent tether preserves the offset-stacked geometry (i) in gas-phase energy minimizations (HF/6-31+G*).[14] In a second set of calculations, structure (i) was soaked in a 30 Å diameter droplet of explicit water molecules and the whole structure was minimized at the MMFFaq level. The offset-stacked geometry is preserved.

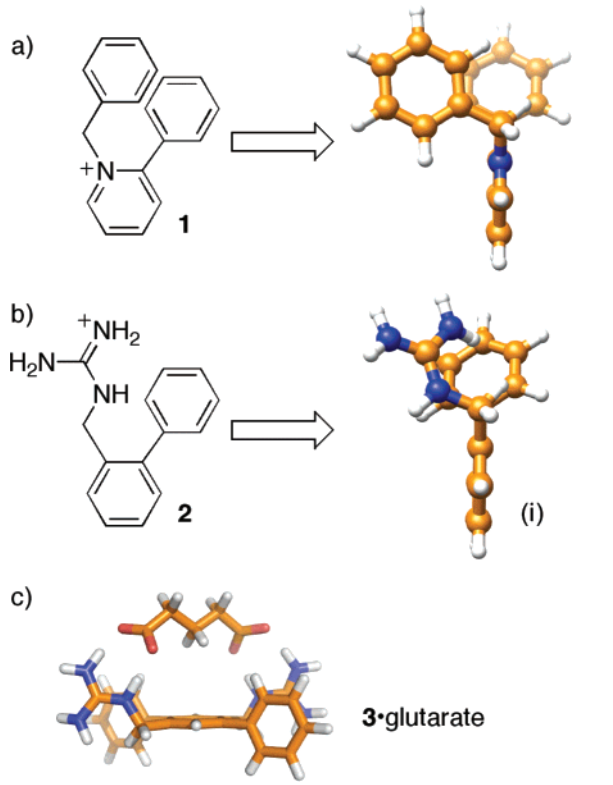


Figure 2. (a) The appended benzene rings of compound **1** are known to adopt an offset-stacked geometry. (b) Calculations (see text) predict that compounds such as **2** can also preorganize an offset-stacked geometry (i) for a guanidinium−benzene pair. (c) A model of receptor **3** (HF/6-31+G*) showing that each guanidinium binding element can simultaneously participate in π-stacking and salt bridge formation when binding to glutarate.

To allow experimental studies of this system, we designed terphenyl receptor **3**, which consists of two copies of this stacked guanidinium−benzene motif on either side of a central benzene spacer. Modeling of **3** in complex with glutarate shows that both guanidinium ions can adopt the stacked geometry of interest while each engaging one of the guest's carboxylate ions (Figure 2c). Comparing the behavior of **3** with the binding of similar guests by control receptor **4**,[15] which lacks π-stacking elements but is predicted to bind in a similar geometry, will provide insight into the beneficial or deleterious effects of π-stacking on guanidinium−carboxylate interactions.

To synthesize this novel terphenyl-derived receptor, 2′,5′-dimethyl-*p*-terphenyl **5**[16] is first brominated using NBS, giving bis(bromomethyl) terphenyl **6** in 80% yield (Scheme

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(15) Hamilton has conducted a thorough study of dicarboxylate binding by a non-stacked receptor analogous to control host **4** but constructed using closely related 2-amino-1,3-imidazoline binding elements. Linton, B. R.; Goodman, M. S.; Fan, E.; van Arman, S. A.; Hamilton, A. D. *J. Org. Chem.* **2001**, *66*, 7313−7319.
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1). Conversion to the bis(azidomethyl) terphenyl **7** by treatment with NaN_3 is followed by $LiAlH_4$ reduction to give the diamine **8** (65% for two steps). Treatment with 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (**9**) followed by deprotection with TFA gives the bis(guanidinium) terphenyl compound **3** as the TFA salt. Finally, the trifluoroacetate counterions are exchanged with Cl^- by repeated cycles of treatment with excess HCl (3 M in dioxane) and evaporation (36% yield, three steps). The non-stacked control compound **4** is similarly synthesized from *p*-xylylenediamine in three steps with an overall yield of 29%.

Compound **3** binds various dicarboxylate guests (tetrabutylammonium glutarate (**10**), tetrabutylammonium Cbz-glutamate (**11**), and tetrabutylammonium glutamate (**12**) were investigated) in mixtures of CD_3OD and D_2O, as evidenced by NMR titrations (Figure 3). In all cases, we obtain good

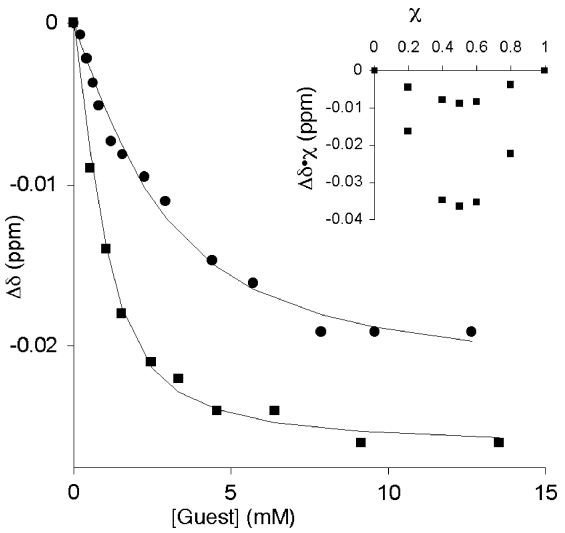
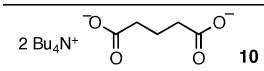
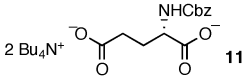
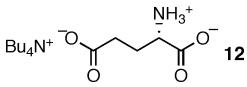


Figure 3. ^1H NMR titration data for the complexation of $(n\text{-}Bu_4N^+)_2$ glutarate by stacked host **3** (■, host concn = 1.2 mM) and non-stacked control host **4** (●, host concn = 2.4 mM) in 90:10 (v/v) CD_3OD/D_2O at 298 K. The lines represent fitted 1:1 binding isotherms. See the Supporting Information for details. Inset: a Job plot (90:10 (v/v) CD_3OD/D_2O; [**3**] + [**10**] = 5 mM; T = 298 K) demonstrates the formation of a 1:1 complex. The two curves in the Job plot track the movement of two different signals on host **3**.

fits of titration data to a 1:1 binding isotherm, and analysis by the method of continuous variation (Job plot) shows that binding between host **3** and glutarate occurs in the proposed 1:1 stoichiometry (Figure 3 inset).[17] The resulting equilibrium constants are shown in Table 1 alongside values for the non-stacked control receptor **4**.[15]

In 90:10 CD_3OD/D_2O, both hosts have dramatically lower affinity for glutamate than for glutarate and Cbz-glutamate,

Table 1. Binding Constants of Dicarboxylate Guests for Hosts **3** and **4** in Mixtures of CD_3OD and D_2O as Determined by ^1H NMR Titration

Guest	Host	K (M^{-1})[a] 10% D_2O	K (M^{-1})[a] 50% D_2O
2 Bu_4N^+  **10**	**3**	2680	390
	4	570	< 20[b]
2 Bu_4N^+  **11**	**3**	2780	210
	4	1730	140
Bu_4N^+  **12**	**3**	150	< 20[b]
	4	170	< 20[b]

[a] Receiving solutions and titrants contained host at 1−3 mM. Titrant solutions additionally contained guest at 30−50 mM. Shifts of host CH_2 protons were tracked and fit to a 1:1 binding isotherm in order to determine values for K. All K values are the result of 2−3 repetitions with an estimated error of ±10%. See Supporting Information for details. [b] Small or nonexistent chemical shifts indicate binding is weaker than the lower limit for determination by NMR (20 M^{-1}).[18]

explained by the repulsive electrostatic influence and increased desolvation potential of glutamate's ammonium functionality. The stacked guanidinium host **3** binds glutarate (**10**) and Cbz-protected glutamate (**11**) 1.6-fold to 4.7-fold more strongly than does non-stacked host **4**. The weaker association constants observed for glutamate (**12**) (150 M^{-1} for host **3** and 170 M^{-1} for host **4**) agree within experimental error. In the more competitive 50:50 CD_3OD/D_2O solvent system, the stacked host again wins out, this time binding Cbz-glutamate 1.5-fold more strongly and glutarate 20-fold more strongly than non-stacked host **4**.

What can account for the increased affinity of **3** for carboxylate guests relative to **4**? The preorganizing influence of the newly introduced phenyl ring may contribute, but in our hands, other diguanidinium hosts preorganized by adjacent methyl groups do not show a significant effect (unpublished data). Stereoelectronic considerations would have the acidity of the guanidinium ions decreased by the nearby electron density of the aromatic π-cloud, thereby decreasing their hydrogen bonding ability;[19,20] this effect, though possibly a minor contributor in this system, runs opposite to the trends observed here. The most likely explanation is rooted in a solvation effect, in which the nearby aromatic surface[21] shields the salt bridge from disruption by competitive solvent. In two prior experimental

(17) This does not rule out the formation of oligomeric *n*:*n* complexes, but the binding interactions of a single carboxylate group to receptor **3** on which oligomer formation would rely is extremely weak (K < 10 M^{-1}) in the methanol/water mixtures employed here. We assume that the contribution of non-1:1 structures to the observed binding isotherms is negligible.

(18) The exchangeable guanidinium protons that participate directly in hydrogen bonding are not observed in the deuterated aqueous mixtures used here. In these systems, the chemical shifts of the observable neighboring methylene protons are inherently small even for strong binding events, leading us to set a lower limit of 20 M^{-1} for binding constant determination in this setting.

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(21) The proximity of guanidinium and aromatic elements in **3** is supported by modeling and by upfield chemical shifts (~0.3 ppm in DMSO; see Supporting Information) of guanidinium NH protons in **3** relative to those in **4**. Determining the exact structure of the guanidinium−aromatic interactions of **3** and related compounds in aqueous media will be the focus of future work.

studies of guanidinium−carboxylate[9] and ammonium−carboxylate[19] pairs near aromatic surfaces in mixed organic/aqueous solvents, the presence of an aromatic surface has also driven the formation of a stronger salt bridge. However, hydration effects are greatly perturbed by organic cosolvents such as those used here and in other studies.[9,15,22,23] Compound **3** is unable to bind dicarboxylate guests in pure water, so our study leaves the role of hydration in the formation of guanidinium−carboxylate−aromatic triads open to discussion. The current results provide a new model of these important motifs, and we are now pursuing variations on this synthetic model system that will allow us to carry out studies in the medium of life—warm, salty water.

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Supporting Information Available: Synthetic procedures, characterization of new compounds, and a detailed description of NMR titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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