The Anatomy of the Host-Guest Binding

Energetics of Bicyclic Guanidinium-Oxoanion Ion-Pairs.

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<th>Description</th>
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<tbody>
<tr>
<td>Ac</td>
<td>acetate</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>EDIPA</td>
<td>ethyldiisopropylamine</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray-ionization</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FAB</td>
<td>fast-atom-bombardment</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
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<td>isothermal titration calorimetry</td>
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<td>literature</td>
</tr>
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<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>O.N.</td>
<td>over night</td>
</tr>
<tr>
<td>RP</td>
<td>reversed phase</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>R,</td>
<td>retention volume</td>
</tr>
<tr>
<td>TBA</td>
<td>tetrabutylammonium</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TEAI</td>
<td>tetraethylammonium iodide</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>tetramethylsilan</td>
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</tbody>
</table>
1. Introduction

1.1 Supramolecular Chemistry

Supramolecular chemistry is one of the most popular and fastest growing areas of experimental chemistry. It is highly interdisciplinary in nature and, as a result, attracts not just chemists but biochemists, biologists, environmental scientists, and others [1].

Chemistry at the microscopic level is dominated by the chemistry of the covalent bond. Supramolecular chemistry extends beyond the realm of individual molecules to focus on intermolecular non-covalent interactions between two or more entities to create an organized association or structure. Jean-Marie-Lehn, who won the Nobel prize for his work in the area in 1987, has defined supramolecular chemistry as the “the chemistry of molecular assemblies and of the intermolecular bond” or “the chemistry beyond the molecule” [2].

Non-covalent interactions between molecules are the basis of the processes that occur in biology, such as substrate binding to an enzyme or a receptor, the assembling of protein complexes, intermolecular reading of the genetic code, signal induction by neurotransmitters, cellular recognition, and so on [1-3].

The weak noncovalent interactions ubiquitous in the living systems have provoked scientists to mimic these bonds by the design of artificial receptor (host) molecules capable of binding substrate (guest) species strongly and selectively, forming supramolecular entities or host-guest complexes of well defined structures and functions [2].

Commonly, the host is a large molecule or aggregate such as an enzyme or synthetic cyclic compound. The guest may be a monoatomic cation, a simple inorganic anion, or a more sophisticated molecule such as a hormone, pheromone or neurotransmitter. The relationship with the resulting host-guest complex has been defined by Donald Cram (1986) as “complexes that are composed of two or more molecules or ions held together in unique structural relationships by intermolecular forces”. Forces such as, ion-pairing (electrostatic interactions), hydrogen bonds, hydrophobic interactions, π-acid to π-base interaction, metal-to-ligand binding, van der Waals attractive forces and solvent structure [1].

A host-guest relationship involves a complementary stereoelectronic arrangement of binding sites in host and guest. In addition to these sites, the host (receptor) may
bear reactive sites that can transform the bound guest (substrate), which would make the host a molecular reagent or catalyst. Furthermore, the host may act as a molecular carrier if it is fitted with lipophilic groups that allow it to dissolve in a membrane. Thus, the functional properties of a supermolecule cover molecular recognition, catalysis, and transport etc.

Host-guest chemistry goes back more than a century and its roots are based upon three historical concepts [1,2]:

1. The fact that selective binding must involve attraction or mutual affinity between host and guest. This is, in effect, a generalization of Alfred Werner’s 1893 theory of coordination chemistry, in which metal ions coordinate ligands into a sphere.

2. The recognition by Emil Fisher [9] (1894) that binding must be selective, as part of the study of receptor-substrate binding by enzymes. He described this by a ‘lock and key’ image of steric fit in which the guest has a geometric size or shape complementarity to the receptor or host. This concept laid the basis for molecular recognition.

3. It was Paul Ehrlich in 1906 who recognized that molecules don’t act if they don’t bind, in this way he introduced the concept of a biological receptor.

In the course of the development of the field of supramolecular chemistry, enormous progress has been made on quantifying the details of receptors with affinity to guests that fit inside them. The lock-and-key notion went through successive waves of revision provoked by the introduction of the concepts chelation, preorganization and complementarity, solvation and the alteration in the very definition of ‘molecular shape’.

1.2 Nature of supramolecular interaction (noncovalent interactions)

One of the noncovalent interactions, van der Waals interactions, were first recognized by J. D. van der Waals in the nineteenth century [4]. The role of the noncovalent interaction in nature was fully recognized only in the past two decades. Noncovalent interactions lead to the formation of molecular clusters while covalent interactions lead to the formation of classical molecules. The formation of a noncovalent cluster does affect the properties of the subsystems, thereby inducing changes that are important for the detection of cluster formation. The stronger the interaction is, the larger are the changes in the properties of the subsystem. For
example, the marked changes that occur in H-bonded systems, result in large variations of the stretch frequencies upon complex formation.

A covalent bond is formed when partially occupied orbitals of interacting atoms overlap and form a new molecular orbital of lower energy, which is occupied by a pair of electrons shared by these atoms. This bond is generally shorter than 2 Å and highly directional. Noncovalent interactions are induced by the electrical properties of the subsystem and are normally effective over short distances, shorter than few angstroms, but can occasionally form bonds at distances as large as tens of angstroms. The stabilizing energy of noncovalent complexes generally consists of various energy terms such as electrostatic (or coulombic), induction, dispersion, repulsion and charge-transfer [1,3,4]. The repulsive contribution, which is called exchange-repulsion, prevents the subsystems from drawing too close together. The term induction, refers to the general ability of charged molecules to polarize neighboring species. The dispersion interaction term, results from the interactions between fluctuating multipoles. In charge-transfer (CT) interactions the electron translocates from the donor to the acceptor. The term “van der Waals forces” is frequently used to describe dispersion and exchange-repulsion contributions. All of these interactions involve host and guest as well as their surroundings (e.g. solvation).

1.2.1 Coulomb interactions

Coulomb interaction is one type of the most important noncovalent interactions (ion-pairing, ion-dipole, dipole-dipole etc.) in synthetic host-guest complexes as well as in many biological systems [1,3]. The driving force for these interactions is naturally electrostatic (coulombic). In general, the coulomb interaction between two point charges is described by the coulomb potential energy [5],

$$ V = \frac{q_1 q_2}{4 \pi \varepsilon r} \quad (1) $$

where \( q_1 \) and \( q_2 \) are the electric charge of two point masses at a distance \( r \) in a medium of permittivity \( \varepsilon \). It is common to express the permittivity as a multiple of the vacuum permittivity \( \varepsilon_0 \), and to write \( \varepsilon = \varepsilon_r \varepsilon_0 \), where \( \varepsilon_r \) is the relative permittivity (or dielectric constant) of the medium. Note that in general \( \varepsilon_r \geq 1 \), where it assumes the value of unity in vacuum. The coulomb potential energy is equal to the work that must be done to bring up a charge \( q_1 \) from infinity to a distance \( r \) from a charge \( q_2 \). The
coulomb energy is inversely proportional to the interionic distance. The electrical force, $F$, exerted by a charge $q_1$ on a second charge $q_2$ has magnitude

$$F = \frac{q_1 q_2}{4\pi\varepsilon r^2} \quad (2)$$

the force itself is a vector directed along the line joining the two charges.

In organic ions the charge is heavily delocalized therefore the point charges formulae 1 and 2 do not hold anymore, a fact that complicates the theoretical analysis of ion-pairing. Fortunately, it is possible to describe interactions approximately by referring to the whole group where the ions are considered as spherical point like ions that follow the Debye-Hückel theory [3,5].

According to this theory, oppositely charged ions attract one another. As a result, anions are more likely to be found near cations in solution, and vice versa. Overall the solution is electrically neutral, but near any given ion there is an excess of counter ions. Averaged over time, counter ions are more likely to be found near any given ion. This time-averaged, spherical haze around the central ion, in which counter ions outnumber ions of the same charge as the central ion, has a net charge equal in magnitude but opposite in sign to that on the central ion, and is called its ionic atmosphere. The energy, and therefore the chemical potential, of any given central ion is lowered as a result of its electrostatic interaction with its ionic atmosphere. This lowering of energy appears as a difference between the molar Gibbs energy $G_m$ and the ideal value $G_m^{\text{ideal}}$ of the solute, and hence can be identified by $RT \ln \gamma_i$, where $\gamma$ is the activity coefficient.

The well-known Debye-Hückel equation for the activity coefficient of an ion $i$ with a charge number $z_i$ in water has the form,

$$\log \gamma_i = -Az_i^2 I^{1/2} \sqrt{1 + 0.33 a_i I^{1/2}} \quad (3)$$

where $a_i$ (Å) is the distance of the closest approach between ions and $A = 0.509$ is a constant for an aqueous solution at 25°C. $I$, is the dimensionless ionic strength of the solution, related to the charge number of the ion and its molality. In very diluted electrolyte solutions ($I < 0.01$ M) the term $0.33a_i I^{1/2}$ in the denominator may be neglected and equation (3) can be simplified to an equation linear with respect to $I^{1/2}$,

$$\log \gamma_i = -Az_i^2 I^{1/2} \quad (4)$$

known as the limiting Debye-Hückel law. The Debye-Hückel theory applies to the case of hard-sphere charges in a uniform medium and small electric potentials.
For spherical ions A and B with point charges Bjerrum has, on the basis of the Debye-Hückel theory, described the association constant, \( K \), as a function of the charges \( z_A, z_B \), the dielectric constant \( \varepsilon \), and a factor \( Q(b) \) which depends on \( \varepsilon, z \) and on the distance of closest approach \( a \) between A and B [3]:

\[
K = (4\pi N/1000)\left(z_A z_B e^2/\varepsilon kT\right)^3 Q(b) \quad (5)
\]

where \( b = z_A z_B e^2/\varepsilon kTa \), \( z_A z_B \) is taken by its absolute value. The distance \( a \) equals the sum of ionic radii of A and B and the parameter \( b \) is related to the so-called critical distance \( q = ab/2 = z_A z_B e^2/2\varepsilon kT \), defined as the distance at which the mutual electric energy of ions A and B equals 2kT. Bjerrum theory considers that the ion-pairing occurs if the centers of ions A and B approach each other at a distance shorter than or equal to \( q \), which becomes very large in solvents of lower dielectric constants. Large ions with \( a > q \) according to this theory don’t associate at all.

For contact ion pairs the Fuoss equation [87] gives

\[
K = \left(4\pi Na^3/3000\right)\exp\left(z_A z_B e^2/\varepsilon kT a\right) \quad (6)
\]
or (at 298 K)

\[
K = 0.00252 a^3 \exp\left(560 z_A z_B e^2/\varepsilon a\right) \quad (7)
\]

This equation is applicable also for solvent-separated pairs and for loose solvated complexes provided the solvent molecule size is included in evaluation of \( a \). The experimental results do not allow a clear distinction between Bjerrum and Fuoss equations, however, the latter is often preferred because of its simpler mathematical form and conceptual basis.

For strongly interacting ions, when association free energies are >>kT, even a simple equation,

\[
K = \exp\left(560 z_A z_B e^2/\varepsilon kT a\right) \quad (8)
\]
derived from consideration of ion-pairing in terms of the Born cycle gives satisfactory results. The theoretical expressions for enthalpic and entropic contributions to association free energy can be obtained by differentiation of ln\( K \) by T (van’t Hoff equation, see later). Thus, from the Fuoss equation it follows that

\[
\Delta H = \left(z_A z_B e^2/\varepsilon kT a\right)\left(\frac{d\ln \varepsilon}{dT + 1/T}\right) \quad (9)
\]
Considerable improvement has been achieved by application of new numerical methods of solution of the Poisson equation [6,7], which allows one to take into account also polarization effects and charge delocalizations. Salt effects, particularly for DNA, are well described by Manning’s counter-ion condensation theory [8].

An example of ion-ion (ion-pairing) interaction is the electrostatic binding of tricarboxylates with host 1. An example for ion-dipole interaction is an alkali metal cation e.g. K⁺ with crown ether 2 in which the ether oxygen lone pairs are attracted to the cation positive charge. Between neutral polar molecules like organic carbonyl compounds the electrostatic contribution mostly derives from dipole-dipole interactions.

![Images of 1 and 2](image)

### 1.2.2 Hydrogen bonding

A hydrogen bond is a particular kind of dipole-dipole interactions [10] in which a hydrogen atom, attached to an electronegative atom (or electron withdrawing group), is also attracted to a neighboring dipole on an adjacent molecule or functional group. The hydrogen bond is a complex interaction composed of several constituents that are different in their nature [11]. The total energy of a hydrogen bond is split into contributions from electrostatics, polarization, charge transfer, dispersion, and exchange repulsion [12]. The distance and angular characteristics of these constituents are very different. In particular, it is important that of all the constituents, the electrostatic contribution reduces slowest with increasing distance. The hydrogen bond potential for any particular donor-acceptor combination is, therefore, dominated by electrostatics at long distances, even if charge transfer plays an important role at

\[ \Delta S = R \ln \left( \frac{4\pi N a^3}{3000} \right) - \left( z_A z_B e^2 / e k T a \right) R T \left( \frac{d \ln \varepsilon}{dT} \right) \] (10)
optimal geometry [10]. Chemical variations of donor, acceptor, and the environment, can gradually change a hydrogen bond to another interaction type. The transition to pure van der Waals interaction is very common. The polarity of A–H or B (or both) in the array A–H⋯B can be reduced by suitable variation of A or B. In consequence, the electrostatic part of the interaction is reduced while the van der Waals component gains relative weight, and the angular characteristics gradually change from directional to isotropic with interaction energies independent of the contact angle $\theta$.

A hydrogen bond has relatively strong and directional nature, an angular lower cutoff can be set at >90° or, somewhat more conservatively, at >110°. A necessary geometric criterion for hydrogen bonding is a positive directionality preference, that is, linear A–H⋯B angles must be statistically favored over bent ones.

![Figure 1](image)

**Figure 1.** Carboxylic acid dimer bound by hydrogen bonding and base pairing in DNA associated by hydrogen bonding.

Examples of hydrogen bonding are [1]: the formation of carboxylic acid dimers (figure 1), the hydrogen bonding network feature of water, the creation of the elementary secondary structures in proteins, the binding of substrates to numerous enzymes, and the formation of the double helix structure of DNA. A base pairing in DNA by hydrogen bonding is shown in figure 1. The hydrogen bonds exist with a continuum of strengths, nevertheless it is useful to introduce a classification, such as “weak”, “strong”, and “moderate”. Table 1 follows the system described by Jeffrey [13], who called hydrogen bonds moderate if they resemble those between water molecules or in carbohydrates, and are associated with energies in the range 4-15 kcal mol$^{-1}$. Hydrogen bonds with energies above and below this range are termed strong and weak, respectively. It must be stressed that there are no “natural” borderlines between these categories. Unlike moderate and weak hydrogen bonds, strong hydrogen bonds are quasi-covalent in nature [14]. If the hydrogen bond is understood as an incipient proton-transfer reaction, a moderate hydrogen bond represents an early
stage of such a reaction, while a strong one represents an advanced stage. It is called as well the symmetric hydrogen bonds A–H–A, where an H atom is equally shared between two chemically identical atoms A, no distinction can be made between a donor and an acceptor, or a “covalent” A–H and “noncovalent” H···A bond. A key finding of spectroscopy is that very strong hydrogen bonds are formed only if the pKₐ values of the partners suitably match. If the pKₐ values are very different, either a moderate A–H···B or an ionic A⁻···H···B⁺ hydrogen bond is formed, both of which are not very covalent. The concept of hydrogen bonding has also been extended to the weaker C-H···O type [15,16]. Although, this type of interaction is at the weaker end of the energy scale, the presence of electronegative atoms near the carbon can enhance significantly the acidity of the C-H proton, resulting in a significant dipole.

Table 1. Strong, moderate, and weak hydrogen bonds in the gas phase following the classification of Jeffrey. The numerical data are guiding values only.

<table>
<thead>
<tr>
<th>A–H···B interaction</th>
<th>strong</th>
<th>moderate</th>
<th>weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>bond enthalpy (kcal mol⁻¹)</td>
<td>15–40</td>
<td>4–15</td>
<td>&lt;4</td>
</tr>
<tr>
<td>bond lengths (Å): H···B</td>
<td>1.2–1.5</td>
<td>1.5–2.2</td>
<td>&gt;2.2</td>
</tr>
<tr>
<td>A···E</td>
<td>2.2–2.5</td>
<td>2.5–3.2</td>
<td>3.2–4.0</td>
</tr>
<tr>
<td>bond angles (°)</td>
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<td>directionality</td>
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<td>examples</td>
<td>gas phase dimers with strong acids/bases</td>
<td>acids</td>
<td>C–H hydrogen bonds</td>
</tr>
</tbody>
</table>

An example of the C-H···O bond, is the interaction of the methyl group of nitromethane with pyridyl crown ether [17].

1.2.3 Cation-π interaction

Cation-π interaction is normally an interaction between a cation and a π-face of an aromatic structure [1,3], such as K⁺ ion interacting with negatively charged π-electron cloud of benzene. The main force behind cation-π interaction is electrostatic, though modern theories also involve other forces like induced dipole, dispersion and CT [20].
Recently it was shown by Mandolini [18] that the cavity of calix[5]-arene 3, fixed in the cone conformation by the presence of a polyoxyethylene bridge between the phenolic units, is suitable to host a large variety of quats (tetramethylammonium, acetylcholine, N-methylpyridinium salts) with medium to high affinities by cation-π interactions.

1.2.4 π-π interaction

This weak electrostatic interaction occurs between aromatic moieties, where one is relatively electron rich and one is electron poor. Two general types of π-interaction are face-to-face and edge-to-face [1,3,19] (figure 2). Dispersion energy plays an important role in stabilizing π-π interactions. Edge-to-face interactions are weak and are the result of the interaction between positively charged hydrogen atoms and negatively charged π-face of aromatic system. For example, these interactions are responsible for the characteristic herringbone packing in the crystal structures of a range of small aromatic hydrocarbons including benzene.

In the face-to-face interaction there is an orientation offset since direct overlap is repulsive. For example, this kind of π-stacking between the aryl rings of nucleobase pairs helps to stabilize the DNA double helix.

**Figure 2.** (a) face-to-face (interplanar distance about 3.3-3.8 Å).
(b) edge-to-face orientation
1.2.5 Hydrophobic effects

Hydrophobic interactions dominate many important processes, such as folding of proteins, protein-ligand (e.g. enzyme substrate) and protein-protein association, solubilization of non-polar substances by surfactant aggregates, and supramolecular complexation of guests with non-polar parts. The origin of hydrophobic interactions lies in the fact that non-polar molecules tend to avoid aqueous surrounding, as is evident from very low solubility of non-polar substances, in particular hydrocarbons, in water and in the positive transfer free energies of such substances from organic solvents to water. Water possesses a large internal cohesion energy density which is manifested in large vaporization enthalpy and high surface tension. Therefore, the unfavorable hydration free energy of the non-polar molecules can be partially compensated if such molecules associate in the aqueous solution, thus reducing the surface area accessible for water and causing it to form stronger intermolecular hydrogen bonding [1-4]. Such association represents hydrophobic interactions which is considered as a partial reverse of the transfer process ‘solute in organic solvent → solute in water’ in the sense that the nearest surrounding of the solute inside the associate is partially organic like.

These interactions to some extent compensate the inefficiency of polar interactions in water, which results from the high dielectric constant and strong proton-acceptor capacity of this solvent.

Hydrophobic effects are of crucial importance in the binding of organic guests by cyclodextrins and cyclophane hosts in water and are divided into an enthalpic and entropic energetic components. The association is accompanied by little change in enthalpy and is governed by entropy effects upon release of solvent molecules into the bulk.

1.3 Design principles of supramolecular host

In general all compounds capable of binding another molecular species with a somewhat higher affinity than what must be expected from their fundamental molecular properties are termed molecular hosts. Association between host and guest molecules are usually based on simultaneous non-covalent interactions between single binding sites, acceptor and donor, which can be combinations like cation-anion, hydrogen-bond-acceptor-donor, etc.
In order to plan a suitable host for a target guest one should consider several parameters [1-3]. First, since non-covalent interactions are rather weak compared to covalent bonds, it is desirable to obtain multiple interaction sites to enhance complex formation and stability. The principle of multi-site complexation is very general in living systems, where it ensures the efficiency of replication, of enzyme-substrate and of antigen-antibody interactions etc. One can view multi-site complexation as a generalized chelate effect, well known in coordination chemistry, which refers to the increased stability of complexes with polydentate ligands, e.g., as 1,2-diaminoethane with metal ion Ni\(^{2+}\), as compared to those with chemically equivalent monodentate ligands, e.g., ammonia, the chelate complex is more than \(10^8\) times more stable. An important requirement for multi-site binding is complementarity between binding sites in the correct disposition of host and guest molecules.

In designing a host that would bind a specific guest with a strong affinity and selectivity, one should bear in mind the necessary host-guest binding equilibrium. Equilibration is anticipated to be reached during the physical measurement time scale. Thus a prime target for host design is to enable kinetically labile complex formation, which allows rapid guest exchange. An upper limit for the association constant and the free energy of binding should be taken into account.

In host-guest complexation the equilibrium constant \(K_{\text{ass}}\) is determined from the ratio of the rate constants of complex formation (\(k_{\text{form}}\)) and dissociation (\(k_{\text{diss}}\)) reactions,

\[
\text{Host} + \text{Guest} \quad \overset{k_{\text{form}}}{\underset{k_{\text{diss}}}{\rightleftharpoons}} \text{Complex} \quad (1)
\]

\[
K_{\text{ass}} = \frac{k_{\text{form}}}{k_{\text{diss}}} \quad (12)
\]

Where the dissociation rate,

\[
\nu = k_{\text{diss}}[\text{complex}] \quad (13)
\]

and dissociation half time,

\[
\tau_{1/2} = \frac{\ln 2}{k_{\text{diss}}} \quad (14)
\]

A crude estimate for a 1:1 stoichiometric association arrives at \(K_{\text{ass}} \sim 10^{13} \text{ M}^{-1}\) (\(\Delta G^\circ = -18 \text{ kcalmol}^{-1}\)). The rate of bimolecular association is taken at the diffusion
limit of $10^9 \text{ M}^{-1} \text{s}^{-1}$ and the half-time for dissociation of the complex at $T_{1/2} = 3 \text{ h}$. If a supramolecular complex formed with higher stability, one can predict that the bond formed must have a dissociation enthalpy $\Delta H_{\text{dis}}$ of less than 25-30 kcal/mol and $T\Delta S$ maximum at $-(13-14)$ kcal/mol. Therefore a formation of kinetically labile complex is a fundamental criterion in the definition of molecular hosts.

In addition to the above requirements, solvent effects play a fundamental role in host-guest design as their presence is obvious in all associations in condensed phases. Therefore, the net free energy of the complexation process depends strongly on solvent features that favor or impede host-guest association and stability. Hence, a qualified host that complexes the peculiar guest with activation of all the mutual interactions (according to the parameters discussed above) in one solvent may completely fail in another.

Unfortunately, however, due to the fundamental lock-and-key metaphor of Emil Fisher [9], which assumes that the mutual geometric fit of a host and guest dominates their thermodynamic affinity, the importance of the solvent in complexation was ignored for long time. Only recently, all the components of the structure-energy relationship, which depends on the host, guest and the solvent, have started being taken into account. Systematic exploration of the interplay between the various components of the structure-energy relationship is one of the main purposes of this dissertation.

### 1.4 A correlation between structure and energetics

For many years, the ultimate judge of successful complexation in studies launched to optimize the direct interactions between a host and a given guest by modifying the covalent structure of the host was the Gibbs free energy of complex formation $\Delta G^\circ$ and the equilibrium constant for association ($K_{\text{ass}}$), not paying attention to the entropic components and solvent contributions. In many cases this approach didn’t live up to the expectation and met with only limited success thereby exposing the shortcomings of the lock-and-key model.

The Gibbs free energy, $\Delta G^\circ$, which is a combination of enthalpic and entropic components that satisfy the Gibbs-Helmholtz equation, $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$, may change only marginally with host structure because of enthalpy-entropy compensation [21,22]
that accompanies all weak interactions in solution and therefore does not reflect the structural achievements. The detailed knowledge of the energetic parameters allows a better determination of the nature of the complexation, \textit{i.e.}, whether it is $\Delta H^\circ$ or $\Delta S^\circ$ driven, and therefore imposes more stringent constraints on explanatory attempts and on host design.

To demonstrate the last point we present three specific cases of complexation with ion-pairing and H-bonding. In these examples, it is shown that the Gibbs free energy alone fails to convey the accurate picture of the nature of the interaction and solvent contribution. However, when dissecting the energy components and following their behavior during complexation, separately, a better understanding of the true nature of the binding process in each case is achieved.

Case 1: The stability of the host-guest binding between tetraethylammonium acetate and the guanidinium 4 in acetonitrile at 30°C [23], is not only due to a strong exothermic enthalpic attraction, reflected in a strongly negative $\Delta H^\circ$ (-3.7 kcal mol$^{-1}$) and a high affinity constant ($K_{\text{ass}} = 2.0 \times 10^5$ M$^{-1}$), but also to a favorable positive entropic component $\Delta S^\circ$ (+12.0 cal mol$^{-1}$ K$^{-1}$). This association process combines two molecular species to form one complex, yet the overall entropy of the system increases owing to the release of bound solvent molecules. Here, a non hydrogen-bonding solvent was chosen that at the same time would minimize unspecific ion-pairing by its high dielectric permittivity $\varepsilon$ ($\varepsilon = 36$).

Similar entropic effects are widespread in aqueous systems due to hydrophobic effects that have crucial importance in the binding of organic guests by hydrophobic host cavities in water. Here the binding process is driven by both the entropy and enthalpy that give rise to the high affinity of the complex.

Case 2: The binding of sulfate ions by host 5 in a highly polar and competitive solvent like methanol at 30°C [24], is strongly endothermic and gives $\Delta H^\circ = +7.7$ kcal mol$^{-1}$ and $K_{\text{ass}} = 6.8 \times 10^6$ M$^{-1}$. Thus, at room temperature association is strongly entropy-driven $\Delta S^\circ = +56.7$ cal K$^{-1}$ mol$^{-1}$ by far outweighing the unfavorable $\Delta H^\circ$ value. Since both the guanidinium group and the sulfate anions are solvated very effectively in protic solvents, the positive $\Delta H^\circ$ value observed reflects the endothermic reorganization of the solvent shell upon complexation. The complex is less solvated
than the sum of its free components, and the release of solvent molecules thus leads to the entropic overcompensation of the unfavorable positive $\Delta H^\circ$ of desolvation.

Case 3: Host-guest complexation of $\{\text{Na}^+\text{cryptand}[2.2.2]\}_2$ p-Nitrophenyl phosphate by compound 6 in acetonitrile [23] shows a strong negative enthalpy ($\Delta H^\circ = -9$ kcal mol$^{-1}$) and a strong binding affinity ($K_{\text{ass}} = 1.6\times10^5$ M$^{-1}$) which readily discloses a negative entropy contribution ($\Delta S^\circ = -5.9$ cal K$^{-1}$ mol$^{-1}$). Owing to the very negative value of the enthalpy and the negative value of the entropy this complexation is enthalpy driven. The negative entropy implies that this interaction has reduced the degrees of freedom in the system and therefore, the complexation here is selective.

$$
\begin{array}{c}
\text{H}_2\text{C} - \text{O} - \text{N} + \text{N} - \text{H} - \text{H} - \text{CH}_2 \\
\text{R}' \\
\text{X} = \text{OSiMe}_\text{t-C}_6\text{H}_3 \\
\text{R} = \text{Si-BuPh}_2 \\
\end{array}
$$

$$
\begin{array}{c}
\text{H}_2\text{C} - \text{O} - \text{N} + \text{N} - \text{H} - \text{H} - \text{O} \\
\text{R} \\
\text{Cl} \\
\text{X} = \text{OSiMe}_\text{t-C}_6\text{H}_3 \\
\text{R} = \text{Si-BuPh}_2 \\
\end{array}
$$

$$
\begin{array}{c|c}
\text{X} & \text{R} \\
\hline
\text{OCH}_2\text{C}_6\text{H}_6 & \text{Si-BuPh}_2 \\
\text{OH} & \text{Si-BuPh}_2 \\
\end{array}
$$

Case 4: Here we discuss a case in which the association between the host and the guest causes positive enthalpy ($+\Delta H^\circ$) and negative entropy ($-\Delta S^\circ$). This process in energetically unfavorable and hence unstable, and its time scale is normally very short rendering it practically undetectable. When the enthalpy response in an association process is exothermal then the binding of the two partners has occurred successfully. The entropy value depends on the amount of solvent release in the newly organized system and can, therefore, be positive or negative. Whereas, an endothermal enthalpy response necessitates a consumption of energy by the new noncovalent association, which is compensated in turn with entropy increase inducing the stabilization of the system. However, if the number of degrees of freedom in the system is significantly
decreased the interaction becomes energetically very expensive leading to a fast
dissociation and to the break down of the noncovalent bond.

As a conclusion, the examination of the energetic parameters is very critical to
understand the influence of the host character and the environment (\textit{i.e.}, solvent
molecules), on complexation. To measure these parameters, van’t Hoff analysis
derived from different instrumental methods like NMR and UV, and Isothermal
Titration Calorimetry (ITC) [25] methods can be used. The NMR titration is an
indispensable tool in the wide-range collection of information on supramolecular
associations and can provide clues on the structural mode of host-guest relationships.
However, the determination of the thermodynamic parameters $\Delta G^\circ$, $\Delta H^\circ$ and $\Delta S^\circ$ by
this instrumental method requires the use of the laborious, insensitive, and error-prone,
van’t Hoff analysis of the binding data (see explanation below). A more direct access
to those important energetic parameters is offered by isothermal titration calorimetry
(ITC). The calorimetry method doesn’t need any change in spectral characteristics to
be induced by complexation. It requires, however, the reaction heat effect to be large
enough to produce a measurable temperature change. This is at variance to
spectroscopic methods for which the measured signal itself depends on the strength,
specifically the $\Delta H^\circ$ of the complex. Since calorimetric measurements faithfully report
on the cumulative heat response of the entire system it is of utmost importance to
design a host-guest system simple enough to allow deconvolution of all the processes
happening simultaneously in solution and ascribe the heat effect to just one association
reaction.

The main advantage of the ITC is that it allows the immediate determination of
$\Delta H^\circ$ as a primary parameter of measurement; $\Delta G^\circ$ and the host-guest stoichiometry $n$
are estimated from titration curve fitting. The reaction entropy $\Delta S^\circ$ may then be easily
calculated from the Gibbs-Helmholtz equation. The ITC can also provide a precise
determination of heat capacity changes ($\Delta C_p$) from measurements at different
temperatures. There are clear advantages of the use of the ITC measured $\Delta H^\circ$ and $\Delta C_p$
over the determination of the temperature dependence of an equilibrium constant by
the van’t Hoff equation,

$$\frac{d \ln K}{dT} = \frac{\Delta H}{RT^2} \quad (15)$$
The usual assumption is that in a small temperature interval, $\Delta H^\circ$ can be considered as temperature-independent and, accordingly, the integral form of the equation can be presented as

$$R \ln K = -\frac{\Delta H}{T} + \Delta S \quad (16)$$

The temperature dependence of $\Delta H^\circ$ is given by

$$\left( \frac{d\Delta H}{dT} \right)_p = \Delta C_p \quad (17)$$

and the aforementioned assumption of a constant $\Delta H^\circ$ value implies that $\Delta C_p = 0$. The available data on $\Delta C_p$ values for various host-guest equilibria show, however, that this parameter can be even higher than 96 cal mol$^{-1}$ K$^{-1}$. With such values of $\Delta C_p$ the variation of $\Delta H^\circ$ in a temperature interval of, for example, 50°C is more than 4.9 kcal mol$^{-1}$. Unfortunately the temperature dependence of $\Delta H^\circ$ is often simply ignored.

### 1.5 Isothermal Titration Calorimetry (ITC)

In this work the ITC-MCS method introduced by MicroCal [26] was used for host-guest complexation study. This method directly measures the heat evolved or absorbed in liquid samples as a result of injecting precise amounts of reactants [27]. A spinning syringe is used for injecting and subsequent mixing. For other calorimetry methods see for instance [28].

In the ITC a pair of cells is enclosed in an adiabatic jacket (see figure 3), one cell is a sample cell filled with host or guest in a certain solvent, and the other cell is a reference cell, filled with a plane solvent. During an experiment the temperature of the reference cell is controlled and maintained constant by a steady small power supply, the reference offset. The temperature difference between the two cells is constantly measured and a proportional power is increased or reduced to the sample cell by the cell feedback system to keep the temperature difference very small. A signal proportional to the cell feedback (CFB), and with the instrument temperature and time, constitutes the relevant raw data. The CFB is calibrated in units of $\mu$cal/sec.
A negative CFB signal occurs when an injection of a titrant into a sample cell causes a chemical evolution of heat (exothermic reaction). In this case, owing to the exothermic nature of the reaction, the cell feedback is no longer required to be active. The opposite is true for endothermic reactions that result in positive CFB signals. Here the temperature is reduced and the cell feedback is required to provide power for equilibrating temperature again. Since the cell feedback has units of power, the time integral of the peak, yields a measurement of thermal energy, $\Delta H^\circ$.

For a ligand X binding to a single set of $n$ identical sites on a host molecule M, i.e,

\begin{align}
M + X &= MX \\
MX + X &= MX_2 \\
&\vdots \\
MX_{n-1} + X &= MX_n
\end{align}

where the single-site binding constant is

$$K_{ass} = \frac{[\text{filled sites}]}{[\text{empty sites}][X]} \quad (19)$$

and

$$\Delta G^\circ = RT \ln K_{ass} = \Delta H^\circ - T\Delta S^\circ \quad (20)$$

Where $\Delta G^\circ$, $\Delta H^\circ$, and $\Delta S^\circ$ are the free energy, enthalpy, and entropy change for single site binding.

As mentioned before the parameters $K_{ass}$, $\Delta H^\circ$, and $n$ are determined directly in a single experiment, by a non-linear least squares fit, and $\Delta G^\circ$ and $\Delta S^\circ$ are then calculated. Measuring the binding isotherm at a second temperature allows additional determination of the change in heat capacity of binding through the relation,
\[ \Delta C_p = \frac{\Delta H_0(T_2) - \Delta H_0(T_1)}{T_2 - T_1} \]  

(21)

It is worth mentioning here that \( \Delta C_p \) is a good indicator of changes in hydrophobic interactions with binding, being negative if hydrophobic bonds are formed and positive if they are broken.

The critical parameter which determines the shape of the binding isotherm is the dimensionless constant \( c \), defined as,

\[ c = K_{\text{ass}} M_{\text{tot}} n \]  

(22)

where \( M_{\text{tot}} \) is the total host concentration in the cell at the start of the experiment, and \( n \) is the stoichiometry parameter.

![Figure 4](image)

**Figure 4.** A plot showing the ITC binding isotherms with varying \( c \) values.

Very large \( c \) values lead to a very tight binding and the isotherm is rectangular in shape with the height corresponding exactly to \( \Delta H^\circ \) with the sharp drop occurring precisely at the stoichiometric equivalence point \( n \) in the molar ratio \( X_{\text{tot}}/M_{\text{tot}} \) (see figure 4). The shape of this curve is invariant to changes in \( K_{\text{ass}} \) so long as the \( c \) value remains above 5000. As \( c \) is reduced by decreasing \( M_{\text{tot}} \), the drop near the equivalence point becomes broadened and the intercept at Y axis becomes lower than the true \( \Delta H^\circ \). In the limit of very low initial \( M_{\text{tot}} \) concentration (cf., \( c = 0.1 \)), the isotherm becomes featureless and traces a nearly horizontal line indicative of very weak binding. The shape of these isotherms is sensitive to binding constant only for \( c \) values in the range \( 1 \leq c \leq 1000 \). For ideal measurements the range is even more restricted \( 5 \leq c \leq 500 \).
1.6 Hosts for cationic guest molecules

Many molecular receptors of varied structural types for binding cations, anions and neutral species have been investigated. The goal of their design was to achieve structural control through preorganization and led to the development of macrocyclic ligands from acyclic ones.

Crown ethers are macrocyclic receptors that are known for their selectivity and binding strengths towards alkali and alkaline earth metal cations [1-3]. The important characteristic of crown ethers are the number and type of donor atoms, the dimension of the macrocyclic cavity and the preorganization of the host molecule for most effective coordination. The so-called “macrocyclic effect” of crown ethers is related to the last two characteristics. Dibenzo-18-crown-6 2, which complexes Rb⁺ ion, was first discovered by Pedersen in 1967. Similar inclusion in natural macrocycles takes place in ionophores such as valinomycin which gives a strong and selective complex with K⁺. The stability and selectivity of the complexes depend on the size of the polyoxyethylene crown ether ring, the best bound cation is the one that fits into the cavity. Crown ether complexes with other cations such as ammonium, guanidinium, arenediazonium and pyridinium have also been studied.

Shortly after Pedersen’s work, Jean-Marie Lehn has designed three-dimensional analogues of the crown ethers called cryptates like host 7. In this way it was anticipated that metal ions could be encapsulated entirely within a crown-like host with consequent gains in cation selectivity and enhancement in ionophore-like transport properties. The bicyclic cryptate ligands showed stability with alkali cations much higher than those of natural or synthetic macrocyclic ligands. The key to dramatically enhanced metal cation binding ability of cryptands over crown ethers is the three-dimensional nature of their cavity. They show selectivity as a function of relative sizes of the cation (complementarity) and the intramolecular cavity, which enables spherical recognition of the metal ion to take place [29].
1.7 Hosts for anions in particular oxoanions like phosphate and carboxylates.

In comparison to cations, the area of supramolecular anion complexation has developed more slowly, but has attracted increasing attention in recent years [30]. Between 70 and 75% of enzyme substrates and cofactors are anions, very often phosphate residues (as in ATP and ADP) or inorganic phosphate. Anions such as sulphate and carboxylates also occur frequently in biochemical systems. In the gas phase almost all elements can form stable single-charged anions according to their electron affinities [31]. In condensed phases, especially in the presence of water and oxygen, many elements are more stable at higher oxidation states, which combine with water to form oxoanions in which the net charge is distributed over few atoms. Correspondingly, the charge density is lowered with notable changes in properties. Generally, anions possess larger ionic radii and higher solvation energy in protic solvents relative to cations. Electrostatic stabilization of anions is particularly efficient in polar protic solvents due to hydrogen-bonding interactions [32]. Although anions are strongly hydrated, the binding of oppositely charged species is not suppressed. Essential to hydrogen bonding stabilization of anions is their Lewis-base character [33]. The presence of lone electron pairs serving as H-bond acceptor sites (apart from exceptions like AlH$_4^-$, BPh$_4^-$, etc.) their Lewis basicity, however, varies within broad limits. Nevertheless, it is a common feature of anions and may be used as a basic interaction type in the construction of anion hosts. In combination with the covalently insured topology, it adds directionality to the system and renders it sensitive to the spatial arrangement and orientation of binding groups. These form the bases for the distinction between, for example, sulphate and hydrogen phosphate [34,35], which is vital to all biological processes involving these oxoanions. Anions are also of more diverse shapes than cations: spherical, linear, angular etc.

The mutual recognition pattern of host and guest must be defined with the aim of maximizing discrimination of similar guest species. But it is not the only matter, since the solvent shells around the binding partners will unavoidably be changed on complexation, and this can either be a costly process of the net free energy or restructuring the solvent may rather favor complex formation. The solvent will affect complex stability in a way that the association constant may change dramatically
depending on the sheer size of solvent molecules [36]. Therefore one should consider these points in anion host design.

Anion receptors can be neutral Lewis acids, neutral proton donors, metal cations or positively charged organic groups, such as ammonium or guanidine centers, which can also serve as proton donors.

![Chemical structures](image)

Cationic hosts capable of forming ion-pairs with anions in solution are most easily prepared by protonation of suitable basic compounds. Basic open-chain polyamine compounds like spermine or spermidine, are known [37] to bind to phosphate anions or polyanions in water at neutral pH, they most likely adopt a flexible extended conformation that presumably will not incorporate all the ammonium sites because of the far proximity to each other. In contrast, at low pH, polyprotonated monocycle azacrown ethers like 8 possess a greater charge density and thus a greater predisposition to anion binding. The hexaprotonated azacrown 8 shows strong complexes with a variety of anions in water (log $K_{ass} = 4.7$ with AMP$^{2-}$, 7.7 with ADP$^{3-}$ and 9.1 with ATP$^{4-}$). From the general trend that complex stability increases with guest charge, one can infer the dominance of coulombic interactions [38]. Complexation with fumarate$^{2-}$ (log $K_{ass} = 2.2$) and oxalate$^{3-}$ (log $K_{ass} = 4.7$) shows a good discrimination between anions of the same charge. A much greater improvement in stability and selectivity of anion complexation was achieved by rigidification of the binding sites, several bicyclic cryptands were prepared by Lehn such as cryptant 9, which in its penta- or hexaprotonated forms, complexes a variety of well-solvated anions in aqueous solution [39–42]. Host 9 complexes anions like oxalate and malonate strongly by an inclusion process (log $K_{ass} = 4.95$, 3.10 respectively), in which the guest anion penetrates the molecular cavity and is held there by an oriented set of hydrogen bonds. Furthermore, X-ray structures show the ellipsoidal shape of the host cavity and
the topology of nitrogen H-bonding sites provide an optimal complementarity with azide anion translating into an extraordinary high complex stability in water \((\log K_{\text{ass}} = 4.3)\). Halides fit less well, and the decrease in binding free enthalpy from fluoride \((\log K_{\text{ass}} = 4.1)\) to iodide \((\log K_{\text{ass}} = 2.15)\) testifies to the importance of H bonding as the main attractive binding force.

Calix[4]pyrrole 10 has been known for over a century but has been characterized only recently as an electroneutral host for halide anions \([43]\) with a strong preference for fluoride \((K_{\text{ass}} = 1.7 \times 10^4 \text{ M}^{-1})\) over chloride \((K_{\text{ass}} = 350 \text{ M}^{-1})\) and dihydrogen phosphate \((K_{\text{assn}} = 97 \text{ M}^{-1})\) in dichloromethane. Recently it has been shown by Schmidtchen that complexation of 10 in dry acetonitrile resulted in a higher association constant by at least a power of 10 \((K_{\text{ass}} = 1.5 \times 10^5 \text{ M}^{-1} \text{ for fluoride, } 1.8 \times 10^5 \text{ M}^{-1} \text{ for chloride and } 1.6 \times 10^4 \text{ M}^{-1} \text{ for dihydrogenphosphate} [44])\). These results clearly repeat the message that in condensed phases selectivity, with competing guests is not a function of the host structure alone but is heavily dependent on the actual solvent used. Thus, the designation of calixpyrroles as a fluoride receptor at large appears not justified, because the fluoride specificity in dichloromethane or in the gas phase, too, is compromised and eventually vanishes totally in more polar solvents such as acetonitrile or DMSO.

The advantage behind the concept of crown ethers, the complexation of even very weakly coordinating cations, can be utilized for binding anionic species too. The placement of multiple Lewis-acid moieties with their electron-deficient sites in a preorganized molecular framework can result in a host-guest complexation with the lone electron pairs of anions. This is the mutual arrangement used in crown ethers and thus the term “anticrown chemistry” has been made up to clarify this relationship \([45]\). Electroneutral hosts do not face the problem of competitive counterion binding, which is unavoidable with cationic hosts. On the other hand Lewis-acid hosts have to encounter the natural competition of solvents with their guests. Most solvents except for hydrocarbons are quite Lewis basic and in general exceed the molar concentration of a guest anion by several orders of magnitude. (Therefore, solvation design is of great importance). But the examples of natural metalloproteines processing small inorganic anions clearly show that binding is to be possible even in Lewis basic...
solvents, hence proposed that it might well be the preferable concept, if small anions are used.

Examples of hosts that involve electron-deficient atoms such as uranium and silicon, are hosts 11 and 12, respectively. Anion complexations of 11 in organic solvents (MeCN, DMSO) [46,47], reveal a general selectivity for H$_2$PO$_4^-$ ($K_{ass}$=10$^5$ M$^{-1}$), over Cl$^-$ ($K_{ass}$ = 10$^3$ M$^{-1}$) or NO$_2^-$ ($K_{ass}$ = 10$^2$ M$^{-1}$) in MeCN. The analysis of the crystal structure reveals that dihydrogen phosphate is coordinated with the uranyl Lewis acidic center and builds supplementary hydrogen bonds to the methoxy and amido functions of the ligand.

Anion binding (Br$^->$ Cl$^->$ F$^-$, I$^-$) by macrocycle 12 [48] was tested due to its capacity to accelerate anion transport through water-organic solvent interface.

![Diagram](image)

The majority of organic host compounds interacting by charge attraction with anionic species are based on cationic nitrogen compounds. However, the introduction of positive charge into the organic skeleton as an alternative to protonation can be very efficiently attained by metal cation ligation and as a result it requires the careful design of suitable coordination sites. Receptors which use transition metal cations as anion binding sites frequently have the ability to give readily detectable spectral or electrochemical signals upon anion binding, therefore they can act as anion sensors. The cationic acyclic receptors such as 13 [49,50] and 14 [50] are examples of designed receptors for this purpose and the uptake of anions was characterized by cathodic shifts of their reduction potentials. Structures 15 and 16 are more sophisticated macrocyclic derivatives of ruthenium(II) bipyridyl [51]. Anion binding was clearly
visible by optical or cyclovoltammetric methods. Host 15 shows, as expected, pronounced specificity to the more basic dihydrogen phosphate anion ($K_{	ext{ass}} = 2.8 \times 10^4$ M$^{-1}$ in DMSO), even in the presence of ten-fold excess of sulfate and chloride. Host 16, which is a cyclic analog of host 14, is specific for chloride ($K_{	ext{ass}} = 4 \times 10^4$ M$^{-1}$ in DMSO), but practically doesn’t bind to dihydrogenphosphate. At the same time the acyclic compound 14 binds phosphate more strongly than chloride, the selectivity inversion is attributed to the rigid structure of the macrocycle.

Anion binding by these hosts involves electrostatic and hydrogen bonding contributions.

Other receptors were prepared by utilizing peptide bonds in natural proteins for anion complexation. Cyclo-hexapeptide 17, composed of dipeptide building blocks
containing $m$-aminobenzoic acid, possessed a structure with organized H-bonds donating groups converging to the center of the macrocycle [52]. Host 17 showed, through UV spectroscopic analysis, an exceptional binding with $p$-nitrophenyl phosphate in DMSO $K_{\text{ass}} = 1.2 \times 10^6 \text{M}^{-1}$.

Of particular importance in anion binding of proteins and enzymes is the arginine residue 18, which contains the guanidinium moiety. Guanidinium, the protonated and therefore positively charged, form of guanidine is an excellent anion binder. It stays protonated over an extremely wide range of pH ($pK_a = 13.5$ in water for the parent $\text{CN}_3\text{H}_6$), and therefore, in addition to the electrostatic attraction (ion-pairing), it can participate in double hydrogen bonding with o xo anions such as carboxylates, phosphates, etc. (figure 5), a structural motif that can be found in many crystal structures of enzyme complexes with o xo anions as well as in simple guanidinium salts [53,54]. Guanidinium is also involved in the stabilization of protein tertiary structures via internal salt bridges with carboxylate functions.

![Figure 5. Schematic binding patterns of the guanidinium group with oxoanions as observed in many X-ray crystal structures.](image)

Some low molecular weight natural products contain a guanidino functionality as well. Alkaloids such as ptilomycalin A 19 was first isolated in 1989 [55] from the Caribbean sponge *ptilocalulis spiculifer* and the Red Sea sponge *Hemimycale*.. A related series of alkaloids such as crambescidines 20 were obtained from the mediterranean sponge *Crambe crambe*. These antitumor, antiviral and antifungal compounds possess a unique pentacyclic guanidinium core that has a hydroxyspermidine residue attached by a long chain of an $\omega$-hydroxycarboxylic acid
spacrer. Some toxins as well are characterized by their guanidinium moiety, e.g., the puffer fish poison tetrodotoxin 21 [56], the paralytic shellfish poison saxitoxin 22 [57], the peptide antibiotics capreomycin, viomycin, tuberactinomycin, and the anti-fungal agent stendomycin.

![Chemical structures](image)

19: \( \text{R} = \text{H} \) Ptilomycalin A  
20: \( \text{R} = \text{OH} \) Crambescidin 800

The hydrophilic guanidinium moiety enhances the receptor’s solvation in water very efficiently, therefore ion-pairing and H-bonding with oxoanions in aqueous solution is negligible (\( K_{\text{ass}} < 5 \text{ M}^{-1} \)). In spite of these solvation properties that hinder the attempts to mimic the guanidines in artificial receptors, the aforementioned attractive features of this moiety and its participation in natural host-guest binding has encouraged researchers to design abiotic guanidinium host compounds.

The macrocyclic guanidinium based receptors 23 and 24 were prepared by Lehn et al [58], to compare anion binding abilities relative to azacrown ethers. Binding of these receptors with \( \text{PO}_4^{3-} \) in methanol/water showed weak complexation with \( \log K_{\text{ass}} \approx 1.7 \) and 2.4, respectively. This result alongside many other similar examples [59] led to the conclusion that anion binding was governed by electrostatic interactions. Host 25 was designed by Hamilton [60] to mimic the enzymatic cleavage of phosphodiesters [61]. compound 25 indeed complexes phosphodiesters monoanions with \( K_{\text{ass}} = 5 \times 10^4 \text{ M}^{-1} \) in
acetonitrile, and gave rate enhancements for transesterifications by a factor of 300. More preorganized hosts like 26 [62] showed that phosphate binding could stand up to more competitive aqueous solvation conditions and also showed an enhancement of imidazole-catalyzed mRNA hydrolysis by 20 folds in water [63]. Despite the catalytic effects in phosphate ester hydrolysis shown by these simple bis-guanidinium salts, they can’t reach the degree of efficiency seen, for example, in the metalloenzyme mimics.

In order to maintain the structure of the guanidinium group and to enhance its binding abilities, one may incorporate it into a rigid bicyclic framework, which should reduce hydration of the charged moiety. The addition of hydrophobic hydrocarbon residues will lead to a well defined structure, therefore, binding to oxyanions can happen in only one mode with precise positioning of the guest relative to the host structure. The guanidinium moiety in the rigid, strain-free bicycle will make the host more chemically stable and more basic than the parent guanidine. Some natural products contain the guanidino functionality as part of a cyclic or bicyclic system.

The desirable attributes of bicyclic guanidinium groups were recognized by Schmidtchen more than 20 years ago when symmetrically tetrasubstituted derivatives like 27 and 28 became available [64]. Host 27 forms a very stable ion-pair with p-nitrobenzoate in chloroform with $K_{as}=1.4 \times 10^5$ M$^{-1}$ [54]. Host 28 with four hydroxy propel substituents showed that the host-guest binding pattern with acetate was a part of a greater hydrogen bonding network. Later, chiral analogs of the bicyclic guanidines were obtainable [66,67]. Host 29 with its aromatic moieties allows two different recognition sites with aromatic carboxylate anions, ion-pairing and aromatic π-stacking [68,69]. Binding of 29 to chiral carboxylates, formed diastereomeric complexes. These

![Image of diagrams](image-url)
complexes were able to extract N-acetyl- and N-BOC-tryptophan from a racemic aqueous solution into chloroform with moderate selectivity.

Further addition of anchor groups to the bicyclic guanidinium framework can introduce other binding sites with certain guests and increase the specificity of guest binding. Many receptors were designed, with different anchor groups, to recognize amino acids in their zwitterionic form. Anchor groups like crown ethers [70], azacrown ethers [71] and calixarenes [72] will recognize the ammonium moiety of the amino acid.

Several guanidinium receptors were designed with complementary anchor groups, e.g., tweezer-like Kemp acid derivatives, in order to selectively bind nucleotides [73]. Hosts like 30 could complex cyclo-adenosine monophosphate with some preference over guanosine analogs in two phase extractions. The guanidinium-phosphates ion-pairing adhered to in 1:1 stoichiometry. The binding pattern in these complexes as evident from NMR studies, is a combination of ion-pairing, π-stacking and a network of hydrogen bonds. Receptor 31, prepared by Rebek, complexed 2’-3’-c-AMP with ion-pairing contributing about 0.6 kcal/mol on average to the total binding affinity of 3.65 kcal/mol [74].
The development of these receptors to enhance binding of di- and oligonucleotides, led to the preparation of receptors 32 [75] and 33. Receptor 32 showed high affinity for dinucleoside phosphate dApA while 33 brought about phase transfer of nucleotides with a molecular weight of up to 25 kDa [76]. Hence these hosts show a high extraction affinity for oligonucleotides into organic solvents such as dichloroethane.

The continuous design improvements of guanidinium receptors to selectively bind tetrahedral oxoanions in more competitive solvents led to the development of ditopic and polytopic host molecules. In hosts 34-37 [77-81], two bicyclic guanidinium groups were linked by a linear and flexible spacer, a tetrahedral anionic guest binding would begin a folding of the receptor in order to place the main planes of the bicyclic moieties perpendicular to each other. Host 34 formed a 1:1 complex with nucleotides in methanol and 35 formed the same complexes even in water [77]. Compound 36 formed complexes with biologically important phosphates in methanol with binding of constants of (1.8-3.8)×10⁴ M⁻¹. It also showed an impressive preference for binding malonate (dicarboxylate) over its shorter or longer chain analogs [80]. Removal of the silyl ether groups in 37 resulted in the formation of complexes with phosphates with higher stoichiometries in methanol, but in water 1:1 complexation was observed with \( K_{\text{ass}} = 10^3 \) M⁻¹. The influence of spacer flexibility upon complexation was examined on rigidification by using mannitol-derived spacer units [81]. The varying \( K_{\text{ass}} \) led to the conclusion that spacer flexibility in these hosts does not play a major role in guest binding.

The synthesis, spectral properties, and anion-controlled assembly of porphyrin-bicyclic guanidine conjugates such as 38 in aqueous solutions has been recently
reported [82]. The design of the receptors was based on the concept of cooperative interactions of both porphyrin and the chiral bicyclic guanidine moieties with an anionic compound of interest. The coulombic and H-bonding attractive forces are predominantly governed by the peripheral bicyclic guanidines, which, in combination with π-π stacking of porphyrin units, impose additional geometrical restrictions with respect to the mutual distance and orientation of the guanidines. These porphyrin assemblies upon addition of small anions such as acetate, dihydrogenphosphate, terephthalate etc. form chiral structures controlled by the anion. Binding of these anions was indicated by UV/vis, fluorescence, and CD spectroscopy.

The reliable usefulness of the bicyclic guanidinium core in oxoanion complexation triggered the development of more rigid guanidinium systems, such as 39 [83], 40 [84] and 41 [85]. The conjugation of the nitrogen sites into the aromatic moieties make the compounds much less basic than ordinary guanidines and therefore complexation experiments is restricted to a smaller pH range. When host 41 was set up into a liquid membrane in slightly acidic solution it acted as an electrochemical sensor for hydrosulfite with great selectivity [86]. Receptor 40 also showed strong interactions with carboxylates [84]. Nevertheless, the utility of these systems needs further study and improvements.
2. Aim of this work

The aim of this work is to study the structure-energy relationship of a simple host-guest system in a non-hydrogen-bonding solvent in order to develop more reliable guidelines for molecular recognition. Evaluating the energetics means the dissection of $\Delta G^\circ$ into its $\Delta H^\circ$ and $\Delta S^\circ$ components depending on host structure variation in order to understand the bimolecular association process and the role that solvent plays in binding. The latter point can donate a guideline for host-guest design into more competitive solvents, such as water. The importance of solvent participation was explicitly neglected in the earliest studies, therefore ignoring the entropic components of association as well as all solvent contributions.

![Figure 5. Hydrogen bonding and electrostatic interaction between the guanidinium moiety and oxoanions](image)

In this study, the structure-energy relationship in guanidinium-oxoanion systems is explored. The interaction mode between the host and guest follows 1:1 stoichiometry and the prime structural motif features cation-anion arrangement, assisted by two parallel hydrogen bonds as shown in figure 5. Formation of hydrogen-bonded ion pair complexes is expected to depend on the competition with solvent. Thus, a non-hydrogen-bonding solvent was chosen that at the same time would minimize nonspecific ion-pairing by virtue of its high dielectric permittivity $\varepsilon$. Dry acetonitrile ($\varepsilon = 36$) appeared to be an optimal choice for this kind of complexions.

In order to unravel the structure-affinity correlation of host-guest binding modules based on guanidinium-oxoanion interaction, we have attempted to meet the following points:

1) The preparation of a series of bicyclic guanidines like compounds 48 and 70.

The concept behind the synthesis of the designed bicyclic guanidinium cations was to introduce substituents directly adjacent to the binding site with different steric
properties, such as phenyl groups or fluorene moieties in hosts 48 and 70 respectively, without harming the preferential binding mode. The importance of these residues lies in their ability to minimize the solvation shell near the binding site and thus reduce the enthalpy penalty paid to disrupt the solvation shell. Comparison with other hosts in the series like compounds 71 and 132 that were prepared with more flexible substituents will clarify if decreasing flexibility will affect binding properties. These guanidinium compounds constitute a suitable series for trend analysis.

2) The preparation of optimal oxoanions building block, such as phosphate 78 and phosphinate 92 in an attempt to increase the binding directionality towards guanidines (the above hosts) assisted by the two side arms that will project above and beneath the main plain of the guanidinium moiety upon complexation. As a result the number of solvent molecules around the binding site will be reduced and consequently cut down on solvation effects. Although the intrinsic properties of the anionic moiety may not be touched, host-guest complexation is sensible to the overall structure of the guest and the nonionic part may well dominate the binding interaction.

To evaluate the real effect of the newly designed guest anions, the parent anions 84 and 128, which are missing the side substituents, will be used in a trend analysis as well.
3. Synthesis

3.1 Synthesis of bicyclic guanidine hosts 48 and 70

3.1.1 The synthetic strategies for host 48

We have chosen to start off by preparing the fluorene bicyclic guanidine 48 through the reaction scheme strategy shown in scheme 1. The first stage in the step-wise reaction was successfully carried out, and fluorene dicarboxamide 44 was obtained in 80% yield by using the starting materials 9-fluorene carboxamide 42 and N,N-bis(2-iodoethyl)-4-cyanoamide 43. Compound 42 was obtained from the amidation of 9-fluorene carboxylic acid methyl ester 145 starting from 9-fluorene carboxylic acid 144. Compound 45 was then prepared in 85% yield by applying the Hofmann rearrangement to 44 using pifa ([Bis-(trifluoracetoxy)-iodo]benzene) instead of sodium hypobromide as a modern oxidation reagent. Hydrolysis of the intermediate 45 offered the putative compound 46. Deprotonation of 46 with 4N NaOH yielded 47. In order to carry out the final step in the reaction, guanidylation of compound 47 was performed and compound 48 was produced. Although this last step has proven difficult (see below), fortunately, we managed to realize it.

The high basicity of the amino groups in compound 47 enables prompt protonation. In such a case the protonated 47 looses the amine’s nucleophilicity and might therefore prevent the reaction with the reagent thiocarboxyldiimidazole 49, which in turn contributes the additional carbon in compound 48. Scheme 3 describes the mechanism of the guanidylation with thiocarboxyldiimidazole 49. In order to insure complete deprotonation of 46 to 47 during the reaction, different bases were added (scheme 2, table 2, Experiments 1-3). However, despite base addition, the desired product was not observed. The reaction was repeated without the addition of base (Exp. 4 in Table 2) in the same solvent, CH$_3$CN, but did not generate any different result.
Scheme 1. A schematic representation for the preparation of fluorene-guanidinium 48

Scheme 2
These negative results have provoked us to inspect whether the poor solubility of the compound 47 in CH₃CN acted as a hurdle in the guanidylation step. Therefore, the solvent as well as the temperature was varied (exp. 5-7). Unfortunately, however, the guanidylation of 47 still could not be achieved even with the new modifications.

The outcome of experiments 1-7 (Table 2) suggested to further restrict the potential solvents to those that satisfy the following characteristics: 1) Provide an efficient solvation environment for the starting materials. 2) Maintain the integrity of the reagent throughout the interaction. 3) Possess a high boiling point as to enable higher reaction temp. It was found that such conditions are met by ethers with high boiling point, such as 1,4 dioxan (bp.102°C) or diethyleneglycoldimethyl ether (bp.162°C).

The experiments 8-9 (Table 2) using the same stoichiometries as in previous cases were conducted over night to the temperatures shown in Table 2. At the end of the heating period an oily precipitate was observed in Exp. 8, while in Exp. 9 a more solid precipitate was detected. In addition, monitoring the reaction by HPLC, a signal of the monocycle 51 was still observed, hence the alkylation reagent CH₃I was added to the reaction mixture to convert the remaining intermediate monocyclo compound 51 to the bicycle product 48 and subsequently to provide the I⁻ counter ion to the produced

<table>
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<th>base</th>
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<tr>
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<td>—</td>
<td>100</td>
<td>no</td>
</tr>
<tr>
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<td>ethyldiisopropylamine</td>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>chloroform</td>
<td>—</td>
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</tr>
<tr>
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<td>—</td>
<td>105</td>
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</tr>
<tr>
<td>9</td>
<td>diethyleneglycoldimethyl ether</td>
<td>—</td>
<td>140</td>
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</tr>
</tbody>
</table>

Table 2. Different conditions used for the guanidylation step of 47.
charged bicyclic guanidine 48. Analysis of the precipitated material by HPLC, NMR and MS confirmed it to be the desired compound 48.

Scheme 3. The mechanism of guanidylation of 47 with thiocarboxyldiimidazole

The main goal of the use of the reagent thiocarboxyldiimidazole in the previously discussed guanidylation stage, is to provide the guanidinium carbon. Therefore, if the extra carbon atom required in cyclization of 47 were already present in the starting material, there will be no need for the reagent 49 in the guanidylation reaction. Actually, in the presence of a suitable catalyst, one can exploit the cyano (nitrile) group in compound 45 (scheme 1) as a donor of the guanidine carbon. This idea had been previously applied in the Lanthanide(III) ion-catalyzed reactions of amines with nitriles [88]. Here we have employed the same principle for compound 45 in the presence of the catalyst La(OTF)₃, conducting the reaction in different alcoholic solvents at different heating temperatures (scheme 4). In all of our attempts, we obtained only the monocyclic product 53 and not the desired bicyclic one 48.
In an attempt to complete the cyclization of the monocyclic product 53 to 48 we added different catalysts (scheme 5 and table 3) to a solution of the monocycle 53 in 3-pentanol and heated the mixtures in pressure tubes (scheme 5). Monitoring the reaction by HPLC analysis for several days, we didn't observe the desired product in any of the experiments.
Due to reasons that will be discussed later, the route we followed for the preparation of fluoreneguanidinium iodide 48 (scheme 1), didn't give a sufficient yield (5%). Therefore, in order to synthesize compound 48 we tried the novel strategy shown in scheme 6. This route, however, proved to be even less productive than the previous one.

The strategy of the new route is to add the "guanidinium carbon" in compound 56 in the first step. Then, in order to facilitate the substitution of the secondary amine ligand 58, thiourea 56 was activated by alkylation and produced 57. A successful substitution should give compound 59. Biscyclization of 59 by intramolecular interaction after deprotonation might occur under the reaction conditions to obtain the final product 48.

![Scheme 6](image)

**Scheme 6.** A schematic representation of the new strategy for the fluorene-guanidinium preparation.

The preparation of fluorenethiourea 56 was carried out successfully (50-70% yield) by addition of one half-equivalent of thiophosgene 55 to a dry solution of 9-aminofluorene hydrochloride 54 with a base like triethylamine in dichloromethane.
Activating the thiourea carbon in 56 with methyliodide resulted in 57 in a yield range of 90-95%.

Different secondary amine ligands, presented in table 4, were used to carry out the reaction shown in scheme 8. To ensure the nucleophilicity of the secondary amine ligand and to facilitate cyclization (see scheme 7), a variety of bases were added. Unfortunately, none of these attempts (Exps. 1-7 in table 4) were successful. Possible explanations hinge on the fact that fluorene units are too bulky and therefore prevent the reaction by covering the interacting site. Another possibility is that the bases used are either too strong or too weak to promote such a reaction.

Scheme 7. A proposed mechanism for the reaction route shown in scheme 6

Scheme 8
Since the desired product couldn't be obtained even after testing many different conditions (exp.1-7, table 4), we decided to return to the first route and try to improve the yield of 48 produced in the guanidylation stage (scheme 2). This stage was probed
several additional times all resulting in a varying, yet unsatisfactorily, low yield (≤ 5%).
To understand the variation of the yield in the repeated reactions, the starting material of the guanidation step, the triamine compound 47 in scheme 2 was re-characterized.

In revisiting the characterization issue it was realized that the existence of a very small peak in the $^{13}$C-NMR results was initially ignored, which together with the $^1$H-NMR data that pointed to the existence of symmetry in the compound, led to believe that the starting material was correctly obtained. On additional inspection of the compound with ESI-MS it was realized that the compound fed into the cyclization reaction did not have the correct mass for the triamine. Furthermore, the IR spectrum showed a vibration band of an amide unit that should not exist in the triamine. To add to the confusion, the results of the HPLC run that followed the hydrolysis of 45 by 6 N HCl showed a complete conversion of 45 to a new compound, which had a shorter retention time, consistent with what was expected from triamine 46 production.

After careful re-examination of the ESI-MS, IR- and $^{13}$C-NMR results it was concluded that the compound used was the fluorene urea 65 (scheme 9), an intermediate compound produced during the triamine preparation, rather than the desired starting material 46. Therefore the aim became to complete the last step of the hydrolysis procedure shown in scheme 9.

To obtain the triamine salt 46, the urea product 65, which was produced in 80-85% yield, was heated with concentrated HCl (35%-37%) at 145°C for 48h. The requirement of continuous heating under such extreme conditions might be explained by the existence of the two bulky fluorene units that could hide the reacting site and therefore hamper the hydrolysis with HCl. When the fluorene units were replaced by phenyl or methyl groups, the hydrolysis was completed in a much shorter time and under less harsh overnight conditions (6 N HCl at 110° C). After the unambiguous preparation of the triamine compound 46 in a yield of 45%, the guanidylation step (scheme 2) was carried out more successfully in a yield of 35%.

3.1.2 The synthesis of hosts 70,71

The bicyclic tetraphenylguanidine 70 was prepared in a manner similar to compound 48 (see scheme 9). The triammonium salt 66 was converted to the free base 68 by filtration through a basic anion exchange column. The starting material 68 had a good
solubility in dry acetonitrile for the guanidylation step. The reaction with thiocarbonyldiimidazole 49 and subsequently with methyl iodide resulted in product 70 in 80% yield.

The guanidinium host 71, first prepared in our lab. [64], was produced in large quantities so as to have relatively sizable amount of product for the ITC measurements. These different bicyclic guanidines 48, 70 and 71 might probe the effect of host rigidity (hydrophobicity) on host-guest interactions.

![Scheme 9. The preparation of guanidinium compounds 70 and 71.](image)

3.2 Synthesis of the phosphate and phosphinate guests

3.2.1 Synthetic strategy for phosphates 78 and 84

To obtain the desired phosphate guest 78 we followed the synthetic strategy shown in scheme 10. The 2,2'-dihydroxy-3,3'-diiodo-1,1'-biphenyl 62 and 2,2'-dimethoxy-3,3'-diiodo-1,1'-biphenyl 73 were prepared according to the procedures given in [89]. A Sonogashira coupling of 73 with phenylacetylene 74 and Palladium catalyst gave 75 in 85% yield. Demethylation of 75 with BBr₃ [90] in dry dichloromethane failed to provide the dihydroxy compound 76. Therefore the hydroxy groups in 72 were protected with methylnmethoxy (MOM) groups [91] to obtain 79 (scheme 11), which in turn could be hydrolyzed more easily than the methyl groups in 75.
Compound 79 was obtained in 92% yield and then underwent a cross coupling reaction with phenylacetylene to afford 80 in 90% yield. For MOM-ether deprotection it was sufficient to use very mild acidic conditions in order to avoid the cyclization of the free OH groups with the adjacent ethynyl moieties [92]. Thus, the dihydroxy product 76 was available in 75% yield. When compound 76 was treated with POCI₃ (scheme 10) followed by H₂O/THF hydrolysis the successful formation of the cyclic phosphordiester 77 was observed (60% yield). Compound 77 was converted to the final TBA-salt 78 by ion-exchange in an organic solvent mixture. This conversion was necessary for obtaining the phosphate guest anion in a soluble form which will be used later in the complexation with the guanidinium cations.

Scheme 10. The preparation of phosphate 78
When the desired product 78 became available, the parent phosphate anion 84 was prepared, which lacks the two side arms. The phosphate 83 (scheme 12) was prepared according to the hints provided by reference [93]. 2,2’-dihydroxy-1,1’-biphenyl, 81, reacted with POCl₃ in the presence of a catalytic amount of DMF, then the intermediate acid chloride 82 was hydrolyzed with acetic acid to give 83 (70% yield). The acid 83 in MeOH was transformed into the TBA-salt using a TBA-charged ion-exchange column to produce the phosphate salt 84, ready for ITC measurements.
3.2.2 Synthetic strategy for the phosphinate 92

The preparation of phosphinate 92 starting from 2,2'-dihydroxy-3,3'-dinitrobiphenyl 85, (scheme 13). The rational behind this strategy included the mesylation of the hydroxy groups in 85 to facilitate in the next step the reaction of 86 with phenylphosphine and butyllithium producing compound 87. Then, a cleavage of

Scheme 13. A schematic representation for the preparation of phosphinate 92
the phosphole 87 by an alkali metal in THF, followed by oxidation with H₂O₂ would result in the phosphinic acid product 88. Hydrogenation of 88 would give 89. Bisdiazoitization of compound 89 was presumed to give 90. The coupling reaction of phenylacetylene and 90 in the presence of a Pd-catalyst should give the desired product 91. Finally, conversion to 92 would be achieved by transfer of 91 through cation exchange column, loaded with tetrabutylammonium cation.

2,2'-dihydroxy-3,3'-dinitro biphenyl 85 was prepared in 30% yield by reacting 2,2'-dihydroxy biphenyl 81 with nitric acid in AcOH as described in the literature procedure [94]. In this reaction several isomers, related to the substitution partition of the nitro groups on the phenyl rings, were obtained. The desired isomer 85 was acquired after purification. For characterization in this case it was insufficient to rely solely on the NMR and ESI-MS spectra as both 85 and the isomer 2,2'-dihydroxy 6,6'-dinitrobiphenyl lead to exactly the same results. Consequently, verification the offered isomer was sought from acid-base titration for pKa determination and comparison with literature data. Fortunately, the obtained pKa matched that of the desired isomer 85 [95] thereby confirming the isolation of the correct isomer.

Scheme 14. The mechanism for obtaining the phosphole 87
The hydroxy groups in 85 were converted into better leaving groups by reaction with mesylchloride; compound 86 was then readily obtained in 73% yield. The leaving groups in compound 86 were reacted with phenylphosphine 93 and butyllithium (see mechanism in scheme 14) in order to obtain the phosphole 87. Unfortunately, despite several attempts using freshly dried solvents and authentic reagents, product 87 was never observed. In order to obviate the possible deprotonation of the mesyl groups in 86 their replacement by less acidic and more reactive leaving groups was necessary. Therefore, compound 97 possessing the triflate groups was produced as shown in scheme 15 in 85% yield. However the reaction with phenylphosphide 94 did not yield product 87 either.

Scheme 15.

It is worth noting that phenylphosphine 93 can be rapidly oxidized to a higher oxidation state, requiring storage under argon. Despite the extra precautions taken to avoid contact with air or humidity, there is no absolute guarantee that the reaction was not hampered by oxidation. The failure to produce the expected compound might be attributable to adventitious oxidation resulting from the small scale conduction of this reaction.
To salvage this approach a modification was attempted so that the two hydroxy
groups in 85 were to be replaced by halides, e.g., the bromides in compound 99 (see
scheme 16). The advantage of this change lies in the easy metalation of compound 99
by butyllithium, which, upon addition of phosphortrichloride (PCl₃), would lead
directly to product 100. In the same reaction mixture this product can be hydrolyzed
by water and oxidized with H₂O₂ to yield product 88. Attempts to replace the hydroxy
groups in 85 by bromide using triphenylphosphine-dibromide 98, as described in
reference [96], were unsuccessful. More attempts to convert the triflate groups in 97
to iodides by reacting 97 with NaI in refluxing acetone or in dry DMF heated to
140°C, did not yield any positive results as well.

Owing to these disappointing results, a completely new strategy which utilizes the
commercially available compound 2,6-dibromoaniline, 101, was adopted (see scheme
17). A protection of the amino group in aniline 101 is favorable due to the acidic
amino protons that could be reactive and functional in the various stages of the
reactions. For this purpose, compound 101 was reacted with the Grignard reagent
methylmagnesium chloride (MeMgCl) 102 and subsequently with the protecting group
bis(dimethylchlorosilyl)ethane (stabase) 103 to produce product 104 in 51% yield. A
complete conversion of the aniline 101 by adding a large excess of the stabase reagent

Scheme 16. A strategy for preparing compound 88
led to the formation of side product, which was very difficult to separate from 104, while a stepwise conversion by which only 2.3 equivalents of stabase 103 were added to the reaction mixture allowed a simple separation from the starting material 101 in a standard chromatographic procedure. This explains the relatively low reaction yield.

Scheme 17. A schematic representation for the preparation of compound 91

Surprisingly, an attempt to carry out the coupling reaction of 104 with butyllithium to yield the biphenyl 105 was not successful, probably due to the complexity of the structure involved in the reaction.

To overcome the last hurdle and decrease the complexity of the reaction, we tried Sonogashira coupling of 104 with phenylacetylene to arrive at compound 109, (scheme 18). In this case one bromide is left and the coupling reaction of 109 with itself might
hopefully yield 110 in one step. However, this approach, even with the reaction repetition with different bases and temperatures, did not yield product 109. The reaction was repeated with aniline 101 to produce 111 and no product formation was observed as well. The unsuccessful coupling might be due to the electron donating amino group in 104 and 101 that causes the low reactivity of the bromides.

Scheme 18. A different strategy for the preparation of 107

Within the framework of the adopted strategy, we attempted to synthesize the main building block 106, from the starting material 101, and then pass it through several modifications to reach the final product. A search in the literature revealed that a Suzuki coupling between compound 101 and any boronic acid in the presence of a catalyst was successful without protecting the amino group in the aniline 101 [97]. Therefore, applying a similar strategy as shown in scheme 19 was the next step to try.
The addition of 3 equivalents of BuLi to compound 101 led to metalation in three positions, the two protons of the amino group and one bromide. A subsequent addition of trimethyl borate 112 to the metalted compound followed by hydrolysis produced the desired boronic acid 113 in 60% yield. Suzuki coupling between the intermediate compound 113 and 2,6-dibromoaniline 101 in the presence of a catalyst finally gave the building block 106 in 66% yield. A bis-diazotization of 106 by sodium-nitrite in a concentrated acid gave the salt 114 (scheme 20). After careful drying of the salt the reaction with phosphortrichloride (PCl₃) and Cu¹⁺-catalyst to form the dibenzophosphole derivative was applied. In this reaction the desired product 115 was not observed instead mainly product 116 was formed (scheme 20), perhaps due to
incomplete tetra-azotization of 106. A similar observation was found in the literature [98] as well. To counter this problem the complete conversion of all the amino groups to the diazonium moiety must be insured. After extensive drying, this salt can be introduced into the next steps. Unfortunately, the reaction with \( \text{PCl}_3 \) and \([\text{Cu}^{1+}(\text{CH}_3\text{CN})_4]\text{PF}_6\) in THF at 0°C then at 40°C did not produce the required product.

Scheme 21. A schematic representation for preparing the phosphinate 121
On the basis of this experience the conventional route using halides instead of diazonium groups in the reaction with PCl₃ appeared more reliable. The metalation of halides, like bromides, by BuLi followed by an addition of PCl₃ has already been shown to easily proceed in the desired direction [99]. Therefore, to follow this route, a Suzuki coupling between 106 and trans-2-phenylvinylboronic acid 117 in benzene (a commercially available boronic acid) taking Pd-catalyst was performed to obtain compound 118 (scheme 21). The reaction proceeded successfully in 70% yield. The next step required bis-diazotization of 118 to yield the salt 119. The subsequent conversion to the bromide 120 would be carried out by heating with KBr. Only then, a reaction between a metalated 120 by BuLi and PCl₃ might furnish the product 121.

![Scheme 21](image)

Scheme 22. A schematic representation for the preparation of phosphinic acid 126

Disappointingly, the diamine 118 underwent bis-diazotization at -20°C by sodium-nitrite to a compound different than 119. A careful analysis of the ESI-MS spectrum
strongly suggested that a cyclization to compound 122 had probably happened instead of 119. This could occur following addition of diazotate to the double bond (or its prior hydration followed by ring closure) on the “arm” followed by cyclization with the diazonium moiety.

In order to obviate the complications seen with the styryl substituent the target was switched to phosphinic acid 126. Presumably the preparation would not suffer from problems with diazotization step. p-Tolueneboronic acid 123 was chosen to couple with compound 106 as shown in scheme 22. Following the standard procedure for this coupling we obtained the tetraphenyl product 124. Further inspection of the literature in order to find some extra insight regarding the diazotization step, revealed good chances for success. For instance, the Cornforth group had applied many bis-diazotization reactions on starting materials similar to 124. In addition, they had converted the bis-diazonium salt in the tetraphenyl framework to the iodide or bromide and prepared, thereafter, phosphinic acids [99]. These are exactly the steps we planned to carry out to obtain our product. At this stage the bis-diazotization step of 124 is still under experimentation.

In a separate attempt, the preparation of the phosphinic acid 128, as a parent for compound 126, was carried out as shown in chart 23. 1,2-dibromo benzene was reacted with 1 equivalent of BuLi in THF to yield compound 126 [100]. The dibromo-biphenyl 126 was metalated with 2 equivalents of BuLi in THF followed by the addition of PCl₃. Hydrolysis and oxidation were continued to obtain the phosphinic
acid 127 in 70% yield. The phosphinic acid was converted to the required TBA-salt 128 by using a TBA-charged cation exchange column.
4. Results and discussion

4.1 Complexation of different guanidinium cation hosts with carboxylate 129.

Here we describe an experimental attempt to evaluate the energetics of a simple host-guest system based on guanidiniums and o xoanions to develop more reliable guidelines for molecular recognition. Namely, one does not only focus on the free energy of association $\Delta G^\circ$, that may not reflect the real contributions of host-guest association and solvent influence, but rather on the dissection of this parameter ($\Delta G^\circ$) into its enthalpy ($\Delta H^\circ$) and entropy ($\Delta S^\circ$) components. In condensed phases all weak interactions are accompanied by enthalpy–entropy compensation [21,22], therefore, the factorization into the energetic components is necessary to measure the enthalpic gain on binding of host and guest and the magnitude of its entropy counteraction. As the enthalpy and entropy components can compensate, the free energy of association $\Delta G^\circ$ may change only marginally and will not reflect the structural achievements. Connection of the structural modification in a tailored host–guest relationship to the experimental energetics, can be determined by using the ITC diagnostic method [27]. Since calorimetric measurements faithfully report on the cumulative heat response of the entire system it is required to design a host–guest system simple enough to allow the disentanglement of all the processes happening simultaneously in solution and ascribe the heat effect to just one association reaction.

Studying a series of structurally related compounds should unfold the structure-affinity correlation, and in addition, the risk of misinterpretation is lowered by using trend analysis.

After completing the preparation of the guanidinium compounds they were kept in non-basic solution in order to prevent hydrolysis of the guanidinium moiety by a strong nucleophile to the cyclic urea. Based on various experimental data the hydrolysis of cyclic guanidinium salts seems to be affected by the antiperiplanar or syn lone pairs of the amino groups in the guanidine [65]. The stereoelectronic effects of the lone pairs in the cleavage of guanidinium salts is still not completely determined. If stereoelectronic control by antiperiplanar lone pairs is operative, then they are expected to hydrolyze
with endocyclic C-N cleavage to acyclic ureas. However, hydrolysis in basic media produces mixtures of cyclic and acyclic products, as determined by $^1$H-NMR analysis. The results reported in [65] show that in the six-membered ring antiperiplanar lone pairs provide a weak acceleration of the breakdown of the tetrahedral intermediate, but in five- and seven-membered rings there is no evidence for such acceleration, which instead can be provided by syn lone pairs.

![Scheme 24](image)

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<td>134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-C₆H₅</td>
<td>135 136 70</td>
<td>----</td>
<td>137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₁ R₂ = [structure]</td>
<td>138 139 48</td>
<td>----</td>
<td>140</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Scheme 24.** Overview of the associations between different bicyclic guanidinium–X⁻ compounds and 129.

The association of bicyclic guanidinium cations (e.g. 130) with carboxylate 129 (scheme 24) may provide a suitable host–guest system. The interaction mode follows strict 1:1 stoichiometry and was characterized in solution and in the solid state in numerous examples [23, 53, 101]. The prime structural motif features an almost planar
cation–anion arrangement, assisted by two parallel hydrogen bonds as shown by the X-ray crystal structures of the tetraphenyldianidinium-benzoate (figure 6a), tetraallyldianidinium-trifluoroacetate (figure 6b), and fluoreneganidinium-trifluoroacetate complexes (figure 6c).

**Figure 6.** X-ray crystal structures of tetraphenyldianidinium benzoate (a), tetraallyldianidinium trifluoroacetate (b) and fluoreneganidinium trifluoroacetate (c).
injectant result in a sizable heat of dilution which is measured in a separate titration and subtracted from the raw data to produce the final binding curve, shown in the bottom panel. Also shown is the non-linear least squares fit of the subtracted curve using a one-site-binding model. The integration of the heat pulses obtained in each titration step gives a titration curve rendering the molar enthalpy $\Delta H^\circ$ as the step height and the free energy $\Delta G^\circ$ from the slope in the inflection point. The stoichiometry $n$ is derived as an independent parameter from the curve fit, and the molar entropy $\Delta S^\circ$ is calculated from the Gibbs–Helmholtz equation. The binding isotherm of the tetraphenyl guanidinium bromide 136 with TEA-benzoate 129 is characteristic of a strong, exothermic 1:1 complex ($\Delta H^\circ = -4.17$ kcal/mol and $K_{\text{ass}} = 2.18 \times 10^4 \text{ M}^{-1}$) displayed in figure 7. The exothermic nature of the association suggests a complex held together by strong hydrogen bonding such as the bidentate interaction shown in scheme 24.

![Figure 7](image)

**Figure 7.** The raw data (CFB = cell feedback current, upper panel) and the integration isotherm (lower panel) is a typical ITC plot of the titration of tetraphenylguanidinium iodide 70 (1.09 mM) with TEA-benzoate 129 (22.48 mM) in acetonitrile at 30°C.
Competition of the desired guest species with the counter ions is inevitable when using charged abiotic hosts. Though straightforward deliberations can reduce this interference its influence has occasionally been noted [102, 103] but has seldom been quantified. The calorimetric determination of host–guest binding in acetonitrile of the tetraallyl-substituted guanidinium compounds 130-134 [64] with 129 revealed a surprisingly strong dependence on the counter ion (Table 5) [104]. Switching the counter anion from chloride to hexafluorophosphate shifts the experimental host–guest affinity by more than a power of ten, with the larger, less hydrogen-bonding anions yielding the higher $K_{ass}$ values. The anion influence is easily detectable even at submillimolar concentrations in high dielectric solvent acetonitrile. The clear-cut dependence on charge density points to an unspecific ion-pairing process which is also indicated by inspection of the entropy of host–guest binding. In all cases the association is accompanied by a positive entropy contribution testifying to the inadequacy of the lock-and-key principle [105] to explain the experimental outcome. While the combination of just two binding partners to form a tight complex must superficially result in a more negative $\Delta S^\circ$ because of the loss of degrees of freedom for translation and rotation [106] the observation of the opposite result can be explained by the release of solvent molecules and counter ions engaged in the solvation of the binding sites. The contribution of the entropic component $T\Delta S^\circ$ to the free energy $\Delta G^\circ$ is not a marginal factor but may even constitute the major part of $\Delta G^\circ$ (e.g. in the case involving chloride; Table 5). Again a subtle enthalpy–entropy compensation ensures the more drastic change in the association enthalpy $\Delta H^\circ$ is not expressed in the resulting Gibbs enthalpy $\Delta G^\circ$.

The results listed in Table 5 suggest that there is a grading in the formation of ion-pairs between the guanidinium host and the series of counter anions. Chloride forms the most stable complex with the host, thus showing the lowest exothermicity ($-\Delta H$) in the exchange of chloride for benzoate, while the PF$_6^-$ ion, at the other extreme, obviously enables the strongest enthalpic response because of its weak binding to the guanidinium group (see figure 8). There is no direct evidence that the limit of complete dissociation of the host-counterion ion-pair has been reached even with the least hydrogen-bonding ion PF$_6^-$ nor that the second process to be considered in the trend
Table 5. Experimental stoichiometries \( n \), binding affinities \( K_{\text{ass}} \), and thermodynamic parameters of the reactions of tetraallyl-guanidinium\(^+\)-X with TEA benzoate \( 129 \) in acetonitrile at 30°C (303°K), from ITC titration (the error limits refer to the fit of the data).

<table>
<thead>
<tr>
<th>Tit. #</th>
<th>Host</th>
<th>TEA Benzoate [mM]</th>
<th>( n )</th>
<th>( K_{\text{ass}} ) [M(^{-1})]</th>
<th>( \Delta H^\circ ) [kcal M(^{-1})]</th>
<th>( \Delta S^\circ ) [cal K(^{-1})M(^{-1})]</th>
<th>T( \Delta S^\circ ) [kcal M(^{-1})]</th>
<th>( \Delta G^\circ ) [kcal M(^{-1})]</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.02 mM</td>
<td>130</td>
<td>23.70</td>
<td>1.17</td>
<td>38 000 ± 3 000</td>
<td>−2.93 ± 0.05</td>
<td>+11.3 ± 0.3</td>
<td>+3.42 − 6.35</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>1.07 mM</td>
<td>131</td>
<td>23.17</td>
<td>1.07</td>
<td>118 000 ± 7 000</td>
<td>−4.13 ± 0.02</td>
<td>+9.5 ± 0.2</td>
<td>+2.89 − 7.03</td>
<td>121</td>
</tr>
<tr>
<td>3</td>
<td>1.03 mM</td>
<td>132</td>
<td>23.17</td>
<td>1.05</td>
<td>280 000 ± 22 000</td>
<td>−4.52 ± 0.02</td>
<td>+10.0 ± 0.2</td>
<td>+3.03 − 7.55</td>
<td>289</td>
</tr>
<tr>
<td>4</td>
<td>1.04 mM</td>
<td>133</td>
<td>23.17</td>
<td>1.09</td>
<td>414 000 ± 8 000</td>
<td>−4.58 ± 0.01</td>
<td>+10.6 ± 0.1</td>
<td>+3.20 − 7.78</td>
<td>180</td>
</tr>
<tr>
<td>5</td>
<td>1.14 mM</td>
<td>134</td>
<td>21.40</td>
<td>1.00</td>
<td>380 000 ± 17 000</td>
<td>−5.21 ± 0.01</td>
<td>+8.3 ± 0.1</td>
<td>+2.52 − 7.73</td>
<td>211</td>
</tr>
</tbody>
</table>
In a similar study Hamilton and Linton [108] had reported that association characteristics between the simple guanidinium host 144 and TBA-acetate 145 in DMSO are dependent on counterion and solvent as well, while they found that the association strength of the guanidinium chloride counterion with 145 is smaller (2.9×10³ M⁻¹) in comparison to that of the iodide (5.2×10³ M⁻¹) or tetraphenylborate (TPB) (5.6×10³ M⁻¹) counterions. This could be attributed to the chloride-guanidinium attraction which reduces the net energy gain from acetate complexation. This finding is consistent with the trend analysis result shown in table 5. They also reported a similar, albeit weaker, trend for the enthalpy values.

![Diagram](image)

**Figure 8.** A plot showing ΔG°, ΔH° and TΔS° in (kcal M⁻¹) of table 5 as they vary with the host counterion (titrations 1-5).

The calorimetric determination of the fluorenyl-substituted guanidinium compounds with 129 (table 6) in acetonitrile revealed results almost similar to those shown in table 5. Switching the counter anion from chloride to iodide shifts the experimental host-guest affinity by a ratio of about 28, with the large iodide ion yielding the highest $K_{as}$
values. In all cases the association is accompanied by a positive entropy contribution that again is explained by the release of solvent molecules and counter anions to the bulk.

Table 6. Energetics of the complexation between fluorenyl-guanidinium\(^+\) X\(^-\) with benzoate 129 in acetonitrile at 30\(^\circ\)C (303K).

<table>
<thead>
<tr>
<th>Tit. #</th>
<th>Host</th>
<th>TEA Benzoate [mM]</th>
<th>(n)</th>
<th>(K_{\text{ass}}) [M(^{-1})]</th>
<th>(\Delta H^\circ) [kcal M(^{-1})]</th>
<th>(\Delta S^\circ) [cal K(^{-1})M(^{-1})]</th>
<th>(T\Delta S^\circ) [kcal M(^{-1})]</th>
<th>(\Delta G^\circ) [kcal M(^{-1})]</th>
<th>(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1.05 mM 138</td>
<td>22.47</td>
<td>0.99</td>
<td>21 300 ± 1 170</td>
<td>−3.56 ± 0.03</td>
<td>+ 8.0 ± 0.2</td>
<td>+ 2.44 ± 0.03</td>
<td>−6.00 ± 0.03</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>0.96 mM 139</td>
<td>21.86</td>
<td>1.08</td>
<td>51 600 ± 5 500</td>
<td>−4.07 ± 0.05</td>
<td>+ 8.1 ± 0.4</td>
<td>+ 2.46 ± 0.06</td>
<td>−6.53 ± 0.06</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>0.2 mM 48</td>
<td>5.30</td>
<td>1.06</td>
<td>592 000 ± 34 000</td>
<td>−6.03 ± 0.03</td>
<td>+ 6.5 ± 0.2</td>
<td>+ 1.97 ± 0.04</td>
<td>−8.00 ± 0.04</td>
<td>115</td>
</tr>
<tr>
<td>9</td>
<td>0.91 mM 140</td>
<td>22.57</td>
<td>1.00</td>
<td>67 400 ± 3 000</td>
<td>−5.07 ± 0.02</td>
<td>+ 5.4 ± 0.2</td>
<td>+ 1.63 ± 0.03</td>
<td>−6.70 ± 0.03</td>
<td>59</td>
</tr>
</tbody>
</table>

The results in table 6 show the lowest exothermicity (−\(\Delta H^\circ\)) in the exchange of chloride for benzoate, while the \(\Gamma\) ion enables the strongest enthalpic response (see figure 9). Surprisingly the affinity of the larger, less hydrogen-bonding PF\(_6\)\(^-\) counter anion is smaller than that of the iodide by a factor of about 9, and its enthalpy is less negative by 1 kcal, contradicting the expected behavior of these anions, namely, that it should display the strongest enthalpic response upon complexation with carboxylate because of its weak, mainly electrostatic binding to the guanidinium group. An
explanation of this amazing result can emerge from the assumption that part of the PF$_6^-$ counterion had been hydrolyzed to the difluorophosphate stage. An inspection of the X-ray crystal structure of the tetraphenyl-guanidinium salt (figure 12c), which nominally was expected to be the PF$_6^-$-salt, shows a hydrogen bonding between a tetraphenyl-guanidinium cation and one of the oxygen atoms in the tetrahedral difluorophosphate (PF$_2$O$_2^-$) anion indicating that a hydrolysis of the PF$_6^-$ counter anion of the tetraphenylguanidinium host had occurred resulting in an X-ray structure different from what was expected with the PF$_6^-$ anion. This structure proves the adventitious presence of hydrolyzed anion species and thus might explain the unusual energetic data obtained for the PF$_6^-$ anion (table 6). During the titration experiment the counter anion PF$_6^-$ had probably been hydrolyzed and simultaneously associated by hydrogen bonding with the guanidinium moiety which will definitely lead to a stronger complex because of the additional hydrogen bonding. The enthalpy of the guanidinium-PF$_2$O$_2$ will result in a lower exothermicity in the exchange for benzoate than with guanidinium-iodide.

![Figure 9](image-url)  
**Figure 9.** A plot showing $\Delta G^\circ$, $\Delta H^\circ$ and $T\Delta S^\circ$ in (kcal M$^{-1}$) of table 6 as they vary with the host counteranion titrations 6-9.

The titration results of the tetraphenylguanidinium hosts with benzoate 129 shown in table 7, mark an increase in the association constant when replacing the chloride counter anion with the largest PF$_6^-$ counter anion. The enthalpic values show higher exothermicity when shifting the counter anion to the iodide and not the PF$_6^-$ anion. The positive entropy component is the highest for the PF$_6^-$ counter anion indicating that
more solvent molecules were released to the bulk (see figure 10). Again, this unexpected result can arise from the assumption that part of the PF₆⁻ counter anion had been hydrolyzed to the difluorophosphate. It seems that the percentage of the PF₆⁻ counter anion involved in the hydrolysis process varies from one experiment (see differences between tables 6 and 7) to the other therefore, the trend behavior of this anion upon complexation is ill determined.

Table 7. Energetics of host–guest binding of the tetraphenylguanidinium cations with benzoate 129 in acetonitrile at 30°C (303K).

<table>
<thead>
<tr>
<th>Tit. #</th>
<th>Host</th>
<th>TEA Benzoate [mM]</th>
<th>n</th>
<th>Kₘₙ [M⁻¹]</th>
<th>ΔH° [kcal M⁻¹]</th>
<th>ΔS° [cal K⁻¹M⁻¹]</th>
<th>TΔS° [kcal M⁻¹]</th>
<th>ΔG° [kcal M⁻¹]</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.02 mM</td>
<td>51.22</td>
<td>1.03</td>
<td>7600</td>
<td>-3.59</td>
<td>+5.9</td>
<td>+1.80</td>
<td>-5.38</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±172.0</td>
<td></td>
<td>±0.02</td>
<td>±0.2</td>
<td>±0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.01 mM</td>
<td>21.40</td>
<td>1.06</td>
<td>21800</td>
<td>-4.17</td>
<td>+6.0</td>
<td>+1.81</td>
<td>-5.99</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1700</td>
<td></td>
<td>±0.06</td>
<td>±0.2</td>
<td>±0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.09 mM</td>
<td>22.48</td>
<td>1.01</td>
<td>111000</td>
<td>-5.18</td>
<td>+6.0</td>
<td>+1.81</td>
<td>-6.99</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±7000</td>
<td></td>
<td>±0.02</td>
<td>±0.2</td>
<td>±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.91 mM</td>
<td>22.36</td>
<td>1.01</td>
<td>125000</td>
<td>-4.61</td>
<td>+8.1</td>
<td>+2.50</td>
<td>-7.07</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±8500</td>
<td></td>
<td>±0.03</td>
<td>±0.2</td>
<td>±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

66
examined in the experiments performed hereafter.

Table 8. The enthalpy values in kcal/mol for the different counter anions of the guanidinium hosts with benzoate 129.

<table>
<thead>
<tr>
<th>X^-</th>
<th>[\Delta G^\circ]</th>
<th>[\Delta H^\circ]</th>
<th>[\Delta S^\circ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl^-</td>
<td>-2.93</td>
<td>-3.56</td>
<td>-3.59</td>
</tr>
<tr>
<td>Br^-</td>
<td>-4.13</td>
<td>-4.07</td>
<td>-4.17</td>
</tr>
<tr>
<td>I^-</td>
<td>-4.52</td>
<td>-6.03</td>
<td>-5.18</td>
</tr>
<tr>
<td>PF_6^-</td>
<td>-5.21</td>
<td>-5.07</td>
<td>-4.61</td>
</tr>
</tbody>
</table>

Figure 10. A plot showing \[\Delta G^\circ\], \[\Delta H^\circ\] and \[\Delta S^\circ\] in (kcal M\(^{-1}\)) of table 7 as they vary with the host counteranion (titrations 10-13).

The enthalpy results presented in tables 5, 6, and 7 – concisely collected in table 8 – clearly indicate that the complexation of guanidinium chloride counter anion with benzoate produce the lowest enthalpy values in the series, ranging from -3.0 to -3.6 kcal/mole. Switching the counter anion to bromide shifts the experimental enthalpy values to about -4.1 kcal/mole. The highest enthalpic response range (-4.5 to -6 kcal/mole) is obtained with iodide, the largest halide counter anion. Note that, as discussed earlier, the enthalpy values obtained with the largest counter anion in the series, PF_6^- , has values unexpectedly lower than those with iodide. Therefore, it is sufficient to use the guanidinium iodide ion-pair in the exchange for the o xoanions examined in the experiments performed hereafter.
The impact of host design on binding affinity represents the ultimate goal of molecular-recognition studies. Here this was evaluated using an ensemble of bicyclic guanidinium iodides 48, 70, 132, 141, 142 (table 9). All of these compounds contain the same primary binding motif and are probed by complex formation with the same guest species 129. However, the lining of the binding site was modified synthetically to introduce substituents with different steric properties, but without harming the preferential binding mode. Some of these compounds were newly prepared and the others were previously obtained in our lab [64, 109]. This specific set of differently substituted guanidinium compounds was chosen due to their varying hydrophobicity properties, from the more flexible allyl groups in 132 to the more rigid fluorenyl units in 48. The logic behind complexing these substituted guanidinium cations with the same guest anion is to investigate the effect of the guanidinium hosts rigidity on the complex desolvation. Solvation effects may be supplemented to some degree by a field effect that lowers the dielectric constant in the vicinity of the charged guanidinium function which results in desolvation enhancement near the binding site and thereby enhances the coulombic attraction and hydrogen bonding for the anionic guest [104].

The approach is analogous to the site-directed mutagenesis of biological receptors in which single amino acid residues in the substrate binding pocket may be deliberately exchanged to probe or alter the binding characteristics. Unlike the biological example, in the present artificial host–guest system the substituents available are not restricted to the natural amino acids side chains.

![Figure 11](image.png)

**Figure 11.** A plot showing $\Delta G^\circ$, $\Delta H^\circ$ and $T\Delta S^\circ$ in (kcal M$^{-1}$) of guanidinium iodide hosts, table 9 as they vary with benzoate 129 (titrations 14-18).
Table 9. Energetics of host – guest binding of different guanidinium iodide host compounds with benzoate 129 in acetonitrile at 30°C (303K).

<table>
<thead>
<tr>
<th>Tit. #</th>
<th>Host</th>
<th>TEA Benzoate [mM]</th>
<th>$K_{ass}$ [M$^{-1}$]</th>
<th>$\Delta H^\circ$ [kcal M$^{-1}$]</th>
<th>$\Delta S^\circ$ [cal K$^{-1}$M$^{-1}$]</th>
<th>$T\Delta S^\circ$ [kcal M$^{-1}$]</th>
<th>$\Delta G^\circ$ [kcal M$^{-1}$]</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td><img src="image1" alt="141" /></td>
<td>24.51 0.90</td>
<td>203 000 ± 12 000</td>
<td>−4.40 ± 0.02</td>
<td>+11.8 ± 0.3</td>
<td>+2.95 ± 0.3</td>
<td>−7.35 ± 0.04</td>
<td>211</td>
</tr>
<tr>
<td>15</td>
<td><img src="image2" alt="142" /></td>
<td>24.51 0.90</td>
<td>203 000 ± 12 000</td>
<td>−4.40 ± 0.02</td>
<td>+11.8 ± 0.3</td>
<td>+2.95 ± 0.3</td>
<td>−7.35 ± 0.04</td>
<td>211</td>
</tr>
<tr>
<td>16</td>
<td><img src="image3" alt="132" /></td>
<td>23.17 1.05</td>
<td>280 000 ± 22 000</td>
<td>−4.52 ± 0.02</td>
<td>+10.0 ± 0.2</td>
<td>+3.03 ± 0.3</td>
<td>−7.55 ± 0.05</td>
<td>289</td>
</tr>
<tr>
<td>17</td>
<td><img src="image4" alt="70" /></td>
<td>22.48 1.01</td>
<td>111 000 ± 7 000</td>
<td>−5.18 ± 0.02</td>
<td>+6.0 ± 0.2</td>
<td>+1.81 ± 0.3</td>
<td>−6.99 ± 0.04</td>
<td>121</td>
</tr>
<tr>
<td>18</td>
<td><img src="image5" alt="48" /></td>
<td>5.30 1.06</td>
<td>592 000 ± 34 000</td>
<td>−6.03 ± 0.03</td>
<td>+6.5 ± 0.2</td>
<td>+1.96 ± 0.3</td>
<td>−8.00 ± 0.04</td>
<td>115</td>
</tr>
</tbody>
</table>

The calorimetric results (Table 9) report a clear trend that assigns a decisive role to solvation even in a typical organic solvent, such as acetonitrile. We observe a distinct variation of the binding free energy $\Delta G^\circ$ with the substitution pattern of the host (see figure 11). The differences are manifest in the enthalpies as well as in the entropies of association. The less positive $\Delta S^\circ$ values seen in 70 and 48 with the aromatic substituents indicate the release of fewer solvent molecules on benzoate binding than in
In view of the considerable distance of the guest from the residues in the \( \alpha \)-positions of the host (see Figure 6), a direct influence of the guest on the internal motility of the host is unlikely. Thus, it is most plausible to assume that the receptor site in 70 and 48, lined by aromatic residues, is less solvated than the others and thus the enthalpic penalty paid to disrupt the solvation shell is smaller (see figure 11). This smaller unfavorable contribution results in an enhancement of the total exothermicity. The same arguments serve to explain the energetics of binding to the hosts 132, 141 and 142. The tetrasubstitution with “lean” residues directly adjacent to the binding site in 132 or by disubstitution with much more bulky silylether groups in 141 and 142 gives states of solvation that seem to be very much alike.

The fluorenyl compound 48 shows solvation changes almost identical to those of the tetraphenyl analogue 70 (cf. \( \Delta S^o \) values), but exhibits a significantly enhanced enthalpic contribution. The result relates to the presumed higher rigidity of 48 which opposes the reorientation of the solvent dipoles to cope with the change of the electrostatic field on complex formation. Comparison of 132 and 70 also provides a good example for the benefit of dissecting \( \Delta G^o \) into the enthalpy and entropy components. Considering affinity only (\( K_{as} \)) the allyl compound 132 would be a better host than 70. Most molecular recognition studies, however, would rather select for better structural definition of the noncovalent complex since this constitutes the basis of goals from selectivity to self-assembly. With respect to precise structuring of the host–guest complex, 70 holds an edge over 132 because of the stronger enthalpic interaction and thus provides a strong argument to take enthalpy instead of the almost exclusively used free energy as a ranking criterion.

4.2 Different conformations of the tetraphenylguanidinium cation with different counter anions.

The tetraphenylguanidinium compound is considered to be less flexible than the tetraallyl- 132 and the tetramethyl- 71 hosts. Several X-ray structures for the tetraphenyl compound with different counter anions (figure 12) show that this host in the solid phase can adopt different conformations due to a degree of flexibility that allows the conversion from one conformation to another depending on the nature of the counter anion. Tetraphenylguanidinium iodide and bromide shown in figures 8a and
8b respectively, adopt a trans conformation in which one phenyl group is in the axial position perpendicular to the plane of the bicyclic guanidine while the other axial phenyl group in the neighboring ring is below the plane. The other two phenyls groups are in the equatorial positions. On replacing the counter anion to benzoate the structure adopts the cis conformation (figure 12d) where the two axial phenyls point in the same direction, however, when the counter anion is replaced with PF$_2$O$_2$ the structure adopts a conformation in between cis and trans (figure 12c). These observation reveals that the tetraphenylguanidinium compound cannot be considered rigid.

Figure 12. X-ray crystal structure of tetraphenyl guanidinium–iodide (a), –bromide (b), –difluorophosphate (c), –benzoate (d).

An examination of the association properties with ITC of the different guanidinium hosts with carboxylate 129 in methanol, did not show any marked binding characteristics. The complexation is hampered due to the strong polar and competitive hydrogen bonding solvent that decreased the association constant to a limit that was
not detectable from the ITC experiment. An increase in the concentration of both host and guest did not result in a significant association as well.

About a decade ago, a theoretical Monte Carlo study [110] of the free energy profile for the guanidinium-acetate ion-pair in water was carried out in order to model putative interactions between solvated arginine and glutamic or aspartic acid residues. These simulations showed that best geometric configuration for the ion-pair is to be maintained in a $C_{2v}$ symmetry. The determined potential showed three minima, corresponding to an ion-pair, solvent separated and “infinitely” separated ion-pairs. There was no clear preference within the ion-pairs. Whereas, the intermediate state displayed cooperative binding between the ions via two water molecules forming a double hydrogen-bond bridge. The two extreme states of ion-pair and infinitely separated ions have a large energy barrier. Therefore, the weak association in methanol between the guanidinium compounds and the benzoate found in the current study could be caused by an intermediate state in which binding between the two ions has taken place through methanol molecules.

In this work, the guanidinium host molecules were complexed with carboxylate or phosphate oxoanions in acetonitrile. It is wise to note that despite the similar preferential binding mode of these oxoanions with the guanidinium moiety, there are some differences in their stereochemistry when each interacts with Lewis acid [111].

The stereochemistry of carboxylate–Lewis acid interaction is defined as syn or anti, the carboxylate group typically prefers a syn-oriented interaction with metal ion, a hydrogen bond donor, or a covalently bound proton. Additionally, the interaction preferably occurs in the plane of the carboxylate, presumably in order to complex the oxygen sp$^2$ lone electron pair. The anionic phosphinyl portion ($\text{--PO}_2^-$) of the phosphate group, as it comprises the backbone of nucleic acids, may interact with Lewis acids such as metal ions or hydrogen bond donors as well. However, the geometry of phosphate–Lewis acid interactions has been examined by using the Cambridge Structural Database [111]. Interactions involving metal ions display preferentially syn, unidentate, and out-of-plane coordination stereochemistry with regard to the phosphinyl ($\text{--PO}_2^-$) group. In contrast, hydrogen bond donors display a very slight preference for anti stereochemistry with the phosphinyl group, and these interactions also do not tend toward in-plane interaction with the phosphinyl group.
Therefore, in general, the preferential phosphate–Lewis acid geometry contrasts markedly with carboxylate–Lewis acid geometry. These results were useful in the interpretation of nucleic acid structures and protein–nucleic acid interactions observed by macromolecular X-ray crystallography.

4.3 Complexation of different guanidinium cation hosts with phosphate 78.

The impact of guest design on binding affinity with different guanidinium iodide hosts was evaluated using a newly designed phosphate anion 78 (figure 13 shows the X-ray crystal structure of the phosphoric acid form of 78) with comparison to the parent 84. The new phosphate anion contains the same primary guanidinium binding motif as in all oxoanions, however, the lining of the guest binding site was modified synthetically to introduce substituents (arms) at positions adjacent to it but without harming the preferential binding mode. The motive behind the additional substituted arms in the guest anion, is to strengthen the directionality towards the guanidinium binding site and restrict it into one possible direction only. The new phosphate might wrap around the host in order to reach an optimal orientation upon complexation that will lead to selectivity enhancement within this specific guest anion.

![Diagram showing complexation](image)

The calorimetric results (table 10) of the complexation between the phosphate guest anion 78 and the different guanidinium iodide hosts, starting from the more flexible tetramethyl-substituted guanidinium compound 71 down to the most rigid fluoreneguanidinium compound 48, report again a clear trend of increasing host-guest affinity with host rigidity, yielding the highest $K_{\text{ass}}$ for the fluorene-guanidinium compound 48. A variation of the binding enthalpy $\Delta H^\circ$, where the tetraphenylguanidinium compound 70 shows the strongest enthalpic response despite having the lowest affinity ($K_{\text{ass}}$) in the series (see slope of ITC plot, figure 15).
variation in the $\Delta S^\circ$ values was noticeable as well, for example, the complexation between the phosphate 78 and compound 70 yields the first negative entropy value that was observed in this work (see $T\Delta S^\circ$ in figure 14 titration # 21).

**Figure 13.** X-ray crystal structure of the Phosphoric acid 9, the acid form of the 78.

![X-ray crystal structure of the Phosphoric acid 9](image)

**Figure 14.** A plot showing $\Delta G^\circ$, $\Delta H^\circ$ and $T\Delta S^\circ$ in (kcal M$^{-1}$) of guanidinium iodide hosts, table 10 as they vary with phosphate 78 (titrations 19-22).
Table 10. Energetics of host–guest binding of different guanidinium iodide hosts with phosphate 78 in acetonitrile at 30°C (303K).

<table>
<thead>
<tr>
<th>Tit. #</th>
<th>Host [mM]</th>
<th>phosphate 78 [mM]</th>
<th>$K_{ass}$ [M⁻¹]</th>
<th>$ΔH^°$ [kcal M⁻¹]</th>
<th>$ΔS^°$ [cal K⁻¹M⁻¹]</th>
<th>$TΔS^°$ [kcal M⁻¹]</th>
<th>$ΔG^°$ [kcal M⁻¹]</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>0.52 mM</td>
<td>16.00</td>
<td>1.04</td>
<td>12 500 ± 290</td>
<td>−4.14 ± 0.02</td>
<td>+ 5.0 ± 0.1</td>
<td>−5.68 ± 0.01</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><img src="image1.png" alt="Image" /> 71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.50 mM</td>
<td>16.00</td>
<td>0.94</td>
<td>19 800 ± 680</td>
<td>−4.97 ± 0.03</td>
<td>+ 3.3 ± 0.2</td>
<td>−5.96 ± 0.02</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><img src="image2.png" alt="Image" /> 132</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.75 mM</td>
<td>16.00</td>
<td>1.09</td>
<td>9 900 ± 466</td>
<td>−5.77 ± 0.05</td>
<td>−0.8 ± 0.3</td>
<td>−5.54 ± 0.03</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><img src="image3.png" alt="Image" /> 70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0.20 mM</td>
<td>4.60</td>
<td>0.92</td>
<td>23 000 ± 1 000</td>
<td>−5.10 ± 0.07</td>
<td>+ 3.15 ± 0.3</td>
<td>−6.04 ± 0.03</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><img src="image4.png" alt="Image" /> 48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The negative entropy value might reflect a release of few solvent molecules near the binding site upon complexation and formation of a tight complex that results in the loss of degrees of freedom. The other guanidinium hosts have more positive entropy values compared to 70, however, lower than the entropy values obtained from complexation of the same hosts to carboxylate (tables 5-7 and 9). It is clear that the tetraphenyl-compound 70 shows, unexpectedly, the highest $ΔH^°$ and $ΔS^°$ values within this series. The energetic values of fluorene-guanidinium host 48 is the second in order in this family in terms of binding strength. Comparison between the host-guest complexes in table 10 is another good example of the insight provided by dissecting $ΔG^°$ into its enthalpy and entropy components. Considering the affinity ($K_{ass}$) and the Gibbs energy
of complex formation ($\Delta G^\circ$) only, the tetraphenyl compound 70 would be the weakest host in this group. However, by studying the detailed energetic parameters of complex formation, host 70 holds a benefit over the others due its strongest exothermicity ($-\Delta H^\circ$) and its negative entropy value, pronouncing the higher selectivity toward the guest anion 78 with respect to the rest of the series.

Table 11 shows the association properties of the same hosts as in table 10 with the parent phosphate anion 84 which resembles phosphate 78 but lacks the two side arms. The trend behavior of these hosts with the guest anion 84 is very much like the results shown in table 10 (see figure 16). A comparison between the two tables 10 and 11 highlights the differences in binding properties of the same guanidinium hosts with anion 78 and the parent anion 84. The comparison unveils the influence of the two side substituents in phosphate 78 on the binding properties.
Table 11. Energetics of host–guest binding of different guanidinium hosts with phosphate 84 in acetonitrile at 30°C (303K).

<table>
<thead>
<tr>
<th>Tit. #</th>
<th>Host</th>
<th>Phosphate 84 [mM]</th>
<th>$n$</th>
<th>$K_{\text{ass}}$ [M$^{-1}$]</th>
<th>$\Delta H^\circ$ [kcal M$^{-1}$]</th>
<th>$\Delta S^\circ$ [cal K$^{-1}$M$^{-1}$]</th>
<th>$T\Delta S^\circ$ [kcal M$^{-1}$]</th>
<th>$\Delta G^\circ$ [kcal M$^{-1}$]</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>1.04 mM</td>
<td>22.56</td>
<td>1.10</td>
<td>10 000 ± 150</td>
<td>$-3.21 \pm 0.02$</td>
<td>$+7.7 \pm 0.06$</td>
<td>$+2.33 \pm 0.01$</td>
<td>$-5.54 \pm 0.01$</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>0.54 mM</td>
<td>11.56</td>
<td>0.98</td>
<td>18 000 ± 1 129</td>
<td>$-3.51 \pm 0.04$</td>
<td>$+7.8 \pm 0.3$</td>
<td>$+2.39 \pm 0.04$</td>
<td>$-5.90 \pm 0.04$</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>0.60 mM</td>
<td>23.12</td>
<td>1.05</td>
<td>10 400 ± 517</td>
<td>$-5.19 \pm 0.06$</td>
<td>$+1.3 \pm 0.3$</td>
<td>$+0.38 \pm 0.03$</td>
<td>$-5.57 \pm 0.03$</td>
<td>6</td>
</tr>
<tr>
<td>26</td>
<td>0.20 mM</td>
<td>4.62</td>
<td>1.09</td>
<td>22 000 ± 873</td>
<td>$-4.54 \pm 0.05$</td>
<td>$+4.9 \pm 0.3$</td>
<td>$+1.48 \pm 0.02$</td>
<td>$-6.02 \pm 0.02$</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 16. A plot showing $\Delta G^\circ$, $\Delta H^\circ$ and $T\Delta S^\circ$ in (kcal M$^{-1}$) of guanidinium iodide hosts, table 11 as they vary with phosphate 84 (titrations 23-26).
Inspection of the binding affinity between pairs of titration experiments (19-23, 20-24, 21-25, 22-26) of the same guanidinium host with phosphate 78 and phosphate 84, indicates that there is no significant difference in the association affinity ($K_{ass}$) toward either guest anions. However a clear enhancement in the binding enthalpy $\Delta H^o$ is evident when the phosphate anion 78 is used. The value of the binding enthalpy of tetramethyl compound 71 with anion 78 is 1 kcal greater than its value with anion 84. The enthalpic response of the tetraallyl compound 132 with 78 shows an exothermicity of about 1.5 kcal higher than with the parent anion, 84. When using anion 78, the tetraphenyl-70 and the fluorenyl compound 48 have an increased exothermicity of about 0.5 kcal more than those obtained with 84. Therefore, it is clear that the binding strength of all the guanidinium hosts was higher on binding with phosphate 78. The less positive $\Delta S^o$ values seen in all the titration experiments with anion 78 indicate the release of fewer solvent molecules than on phosphate 84 binding.

The unchanged affinity constant ($K_{ass}$) together with the increase in the affinity strength ($\Delta H^o$) and decrease in the entropy ($\Delta S^o$) values within every pair of experiments indicates very clearly the enhancement of the binding directionality and selectivity upon complexation with phosphate 78. In the complexation process there might be an additional cation-$\pi$ or $\pi$-$\pi$ interaction between the tetrphenylguanidinium host or the fluorenyl host 48 and the phosphate anion 78. This additional interaction could be caused by the aromatic moieties attached to the hosts and to the guest anion, which explains the enhanced enthalpic response upon complexation in comparison with anion 84. The additional cation-$\pi$ and $\pi$-$\pi$ interactions between guanidinium moieties and benzylic o xoanions were noticed and studied before. Certain benzylic biphosphonates are known to bind strongly to guanidinium cations, however, these guests showed enhanced selectivity for aromatic guanidines. The specific recognition pattern of these molecules includes additional $\pi$-$\pi$ interactions, that are absent in the arginine-selective molecular tweezer [112].

Hamilton and de Mendoza [113] have designed a new protein surface that utilizes cation-$\pi$ interactions to enhance binding between an $\alpha$-helical peptide that contains the aspartic acid and the tryptophan carboxylate residues and a cationic bicyclic guanidinium molecule in even aqueous-methanol solution. Therefore, in principle these interactions could occur with the compounds used in the current study, however more
robust data should be obtained, *e.g.*, an X-ray crystal structure, in order to validate the existence of such interactions.

If the determination of the strongest host-guest complex in both tables 10 and 11 would be according to the $\Delta G^\circ$ values alone, that do not vary with significant values for all complexes, it would be not possible to reach the above conclusions nor the structural achievement in phosphate 78 would be observed.

![Chemical structures](image)

In an attempt to increase the selectivity of the guanidinium hosts towards a specific oxoanion, the synthesis of the new phosphinate anion, 92, was envisaged. The guest anion 92 has the same organic framework as the phosphate anion 78 but with the phosphate diester unit in the latter replaced with phosphinic acid while maintaining the same binding motif to the guanidinium compound hosts. In complexation, the whole anion molecule is expected to be much closer to the guanidinium host molecule. The phosphor atom in the phosphinate guest anion is part of a 5-membered ring and directly bound to the biphenyl framework, whereas in the phosphate diester 78 the phosphor atom is a part of a 7-membered ring within which the oxygen atoms are the ones directly attached to the biphenyl framework. Owing to the geometrical difference between the two structures, the opening angle between the two side arms in 92 is tighter which increases the directionality upon complexation. Since the opening angle between the arm substituents in 92 is smaller than in 78, the abundance of solvent molecules in the vicinity of the binding site is diminished. It seems likely that anion 92 will deliberately form tighter complexes with guanidinium hosts like 48 and 70 than those achieved with anion 78. This tightening is surely assisted by the two side arms
that will project above and beneath the main plain of the host to reach optimal orientation at the time of complex formation. Anion 92 is still in preparation.

Table 12. Energetics of host–guest binding of the different guanidinium iodide compounds with phosphinate 128 in acetonitrile at 30°C (303K).

<table>
<thead>
<tr>
<th>Tit. #</th>
<th>Host</th>
<th>phosphinate 128 [mM]</th>
<th>K_{ass} [M^{-1}]</th>
<th>ΔH° [kcal M^{-1}]</th>
<th>ΔS° [cal K^{-1} M^{-1}]</th>
<th>TΔS° [kcal M^{-1}]</th>
<th>ΔG° [kcal M^{-1}]</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>71</td>
<td>0.52 mM</td>
<td>23.08</td>
<td>24700 ± 1393</td>
<td>3.62 ± 0.03</td>
<td>+8.2</td>
<td>+2.47</td>
<td>13</td>
</tr>
<tr>
<td>28</td>
<td>132</td>
<td>0.54 mM</td>
<td>23.08</td>
<td>49500 ± 3057</td>
<td>-5.13 ± 0.04</td>
<td>+4.5</td>
<td>+0.93</td>
<td>27</td>
</tr>
<tr>
<td>29</td>
<td>70</td>
<td>0.20 mM</td>
<td>11.54</td>
<td>33000 ± 758</td>
<td>-10.09 ± 0.06</td>
<td>-15.3</td>
<td>-4.62</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>48</td>
<td>0.50 mM</td>
<td>11.54</td>
<td>78000 ± 2288</td>
<td>-5.14 ± 0.02</td>
<td>+5.4</td>
<td>+1.65</td>
<td>39</td>
</tr>
</tbody>
</table>

A calorimetric study of the complexation between the parent phosphinate 128, prepared for a planned comparison with 92, and different guanidinium cations was obtained (table 12). In this series we observe yet again that affinity to the phosphinate becomes stronger with host rigidity as in the fluorenyl compound 48. The unexpected result reported in table 12 is the strong exothermicity (ΔH°) of the tetraphenyl compound 70 (see also table 11) which differs from the other enthalpy values by about 5 kcal. The unusual negative entropy in the same host indicates a pronounced selectivity to the guest (see figure 17). The high exothermicity and negative entropy
achieved during binding to tetraphenyl guanidinium provide a clear testimony to the strong structural definition toward this phosphinate guest. It is expected that complexation of the same host series with the desired guest anion 92 would reveal even higher energetic values than what is shown in table 12.

Most of the guanidinium host samples for the titration experiments were prepared in dry acetonitrile of about 1mM concentration. Owing to the association between the tetraallylguanidinium iodide (1.03 mM) and TEA-benzoate (23.17 mM) the first several injections in the titration experiment, as seen in figure 18a, deviate from ideal binding behavior. This deviation probably reflects the effect of multiple guanidinium molecules association with the limited benzoate present, or other unspecific interactions. To ensure that this behavior is due to unspecific interactions and not part of the association between the guanidinium and the anion guest, the guanidinium sample was diluted to reach a concentration of 0.27 mM and then complexed with the same guest anion (5.3 mM). Figure 18b shows that the anomaly of the initial injections has disappeared testifying to the specificity of the host-guest interaction in the diluted samples. The energetic values in both cases (figure 18) were identical. In the rest of the titration experiments, when a similar behavior occurs, the first several injections were not included in the curve fitting analysis.

Figure 17. A plot showing ΔG°, ΔH° and TΔS° in (kcal M⁻¹) of the guanidinium iodide hosts, table 12 as they vary with phosphinate 128 (titrations 27-30).
The dilution option can be only used when the association constant of the two partners is relatively high, otherwise the dilution effect might weaken the association.

Figure 18. ITC plots for the association of tetraallyl guanidinium iodide 132 and TEA-benzoate 129 in acetonitrile at 30°C with (a) concentration of 1.03 mM and 23.17 mM respectively and (b) concentration of 0.27 mM and 5.3 mM respectively.
5. Experimental part

5.1 General methods and materials.

Proton and $^{13}$C–NMR spectra were recorded on a Bruker AM 250 or 360 (MHz) and were calibrated to tetramethylsilane as internal standard. $^{31}$P and $^{19}$F NMR were recorded on a Bruker 250 MHz and were calibrated to H$_3$PO$_4$ as external standard and to C$_6$F$_6$ internal standard, respectively. Mass spectra were obtained on:

- Varian MAT CH5 : Fast-Atom-Bombardment (FAB)
- Varian MAT 112S : Electron–Ionization (EI)
- Finnigan LQC : Electrospray–Ionization (ESI, HPLC-MS)

IR spectra were measured on a Perkin-Elmer FTIR 1600 instrument. HPLC–analysis were performed on Merck-Hitachi instrument L6200 or 655A-11 pump connected to Knauer UV–detector or to a Eurosep DDL–31 light-scattering detector.

Solvents were distilled before use except for DMF which was purchased in anhydrous quality from Aldrich and acetonitrile which was purchased in HPLC-quality from Baker. All other chemicals were purchased in reagent quality and used as received. Aqueous solutions were prepared from deionized, glass-distilled water. Most reactions were carried out in an atmosphere of nitrogen and solvents (CH$_2$Cl$_2$, CH$_3$CN) were passed for drying through a small column of activated alumina directly into the reaction vessel.

HPLC analyses were carried out under six sets of conditions which will be referred to as HPLC1, HPLC2, HPLC3, HPLC4, HPLC5 and HPLC6 analysis.

**HPLC1 analysis**: Nucleosil C18 (250×4.6 mm) column, UV detection at 254 nm, flow = 1 ml/min, gradient from 50% MeOH to 90% MeOH in 15 min, 30 mM NaClO$_4$/H$_3$PO$_4$ as buffer.

**HPLC2 analysis**: Nucleosil C18 (250×4 mm) column, UV detection at 254 nm, flow = 1ml/min, gradient from 50% MeOH to 90% MeOH in 15 min, 30 mM NaClO$_4$/H$_3$PO$_4$ as buffer.

**HPLC3 analysis**: Nucleosil C18 (250×4 mm) column, LS detector, UV detection at 254 nm, flow = 1 ml/min, gradient from 50% MeOH to 90% MeOH in 15 min 0.1% TFA as buffer.
HPLC4 analysis: Nucleosil C18 (250×4 mm) column, UV detection at 254 nm, flow = 1 ml/min, gradient from 50% MeOH to 90% MeOH in 10 min and holding 90% MeOH for 5 min, 30 mM NaClO₃/H₃PO₄ as buffer.

HPLC5 analysis: Nucleosil C18 (250×4 mm) column, LS detector, UV detection at 254 nm, flow = 1ml/min, gradient from 50% MeOH to 90% MeOH in 5 min and continuing at 90% MeOH for 25 min 0.1% TFA as buffer.

HPLC6 analysis: Nucleosil C18 (250×4 mm) column, LS detector, UV detection at 254 nm, flow = 1ml/min, gradient from 50% MeOH to 90% MeOH in 10 min and 90% MeOH for 5 min, 0.1% TFA as buffer.

5.2 Synthetic procedures

9-Fluorencarboxylic acid methyl ester 145

![Chemical structure](144)

10 g (47.5mmol) of 9-fluorencarboxylic acid 144 were dissolved in 200 ml of MeOH in 1 liter flask, and 7 ml of thionyl chloride (SOCl₂) were added. The reaction mixture was stirred in a 1 liter flask at RT. At the beginning observed a non homogeneous solution, but after 1.5 h the mixture had a clear yellow colour. After 2 hours all of the starting material was completely converted to 9-fluorencarboxylic acid methyl ester (HPLC analysis). The solvent and SOCl₂ were removed by a stream of N₂ or evaporation in vacuum. 9 g (40mmol, 84%) of product 145 were obtained after recrystallization from MeOH.

145: C₁₃H₁₂O₂ (MW 224.2)
mp: 65°C (lit): 63-63.5°C
HPLC1 analysis: Rᵥ(144) = 10.4 ml, Rᵥ(145) = 11.8 ml
9-Fluorene carboxylic acid amide 42

\[
\begin{align*}
\text{O} & \quad \text{C} & \quad \text{NH}_2 \\
\end{align*}
\]

100 g (1.3 mmol) of ammonium acetate (NH$_4$OAc) were dissolved in 200 ml of MeOH in a 1 liter flask. The mixture was stirred at 0°C, then a stream of ammonia gas was passed through the solution. When the solution was saturated with ammonia, 9 g (40 mmol) crystals of 9-fluorencarboxylic acid methyl ester 145 were added. It took 5 min until the reaction mixture reached homogeneity again. The reaction mixture was kept at 5°C for 3-5 days. A precipitate of 9-fluorencarboxylic acid amide 42 was obtained and collected by filtration, washed with H$_2$O and dried by N$_2$ gas. The white residue was crystallized from ethanol to give 5 g (23.8 mmol) of product (60% yield).

42: C$_{14}$H$_{11}$NO (MW 209.2)
mp: 258°C (lit): 250°C
HPLC analysis: $R_V = 5.8$ ml.

N,N-Bis[2-(9'-carboxamidefluoren-9'-yl)ethyl]cyanoamide 44.

\[
\begin{align*}
\begin{array}{c}
\text{O} & \quad \text{C} & \quad \text{NH}_2 \\
\text{CN} & \quad \text{NH}_2 \\
\end{array}
\end{align*}
\]

2 g (9.57 mmol) of dry 9-fluorenecarboxylic acid amide 42 were suspended in 9 ml of dry DMF (nonhomogeneous solution) and 4.2 ml (33.5 mmol) of 1,1,3,3 tetramethyl guanidine was added. Within 2 min of stirring at RT the reaction mixture became a clear yellow solution. Then 1.34 g (3.83 mmol) of bis-(2-iodo-ethyl)-cyanamide 43 were added slowly. The reaction mixture was stirred at RT over night, then 10 ml diluted acetic acid (50%) was added and the mixture was extracted repeatedly with
chloroform (3-4 times). The combined organic phases were dried over MgSO₄ and then filtered and evaporated. A white product 44 was obtained and crystallized from THF/EtOH or THF/Ether to give 2.4-3.9 g (4.78-7.65 mmol, 50%-80% yield) of 44 (enforced dryness of the starting materials and the solvent resulted in higher yield of product).

44: C₃₃H₂₈N₄O₂ (MW 512.6) mp: 195°C

HPLC2 analysis: Rᵥ(42)= 10 ml, Rᵥ(43)= 8 ml, Rᵥ(44)= 14 ml.

¹³C-NMR of (44) (62.90 MHz; MeOD): δ= 172.0 (carboxamide), 145.9, 142.3, 129.8, 129.2, 125.0, 121.7 (aromatic carbons), 117.5 (cyanoamide), 61.2 (C-9 of fluorene units), 48.3, 34.4 (aliphatic carbons).

¹³C-NMR of (44) (62.90 MHz; DMSO-d₆): δ= 172.8 (carboxamide), 145.0, 140.4, 128.1, 127.7, 123.8, 120.4 (aromatic carbons), 116.4 (cyanoamide), 59.7 (C-9 of fluorene units), 46.8, 33.2 (aliphatic carbons).

¹H-NMR of (44) (250 MHz; MeOD): δ= 7.6, 7.3-7.1 (m, 16H aromatic), 2.28 (t, J= 7.2, 4H aliphatic), 1.7 (t, J= 7.3, 4H aliphatic).

¹H-NMR of (44) (250 MHz; DMSO-d₆): δ= 7.82-7.29 (m, 16H aromatic), 6.88, 6.06 (2×br s, 4H carboxamide), 2.33, 1.86 (8H aliphatic).

¹H-NMR of (44) (250 MHz; CDCl₃): δ= 7.72-7.30 (m, 16H aromatic), 5.14, 4.93 (2×br s, 4H carboxamide), 2.50 (t, J= 7.7, 4H aliphatic), 2.0 (t, J= 7.7, 4H aliphatic).

IR (KBr): 2208 cm⁻¹ for cyanoamide, 1672 cm⁻¹ for carboxamide

Bis-[2-(9-fluorene amine)ethyl]-cyanamide hydrochloride 45

1 g (1.95 mmol) of 44 was added to 20 ml of THF, 3 ml of H₂O and 166 μl of TFA. After 5 min of stirring of the resulting solution at RT 2.6 g (6 mmol) of [bis-(trifluoracetoxy)-iodo]benzene (CF₃CO₂)₂IC₆H₅ (pifa) were added. The reaction mixture was protected from light and was kept at RT over night. After 15 hours the
THF was evaporated, and 10 ml of 6% HCl were added to the reaction mixture. After 15 min at RT the reaction mixture was shaken with ethyl acetate which extracted most of the undesired byproducts (repeated 3-4 times). The desired product 45 stayed in the water phase as a salt. The water phase was evaporated by rotavapor or lyophilization to get 700 mg (1.32 mmol) 85% yield of an oily product.

45: C$_{31}$H$_{30}$N$_4$Cl$_2$ (MW 529.6)

HPLC analysis: $R_v = 4.0$ ml

$^{13}$C-NMR (62.9 MHz; MeOD): $\delta = 142.2$ (CN), 142.0, 141.7, 131.9, 129.8, 124.6, 122.2 (aromatic carbons), 64.7 (C-9 of fluorene units), 44.9, 35.0 (aliphatic carbons).

$^1$H-NMR (250 MHz; MeOD): $\delta = 7.59$-7.12 (m, aromatic-H), 2.27 (t, $J = 7.3$ Hz, aliphatic-H), 1.72 (t, $J = 7.4$ Hz, aliphatic-H).

N,N-Bis-[2-(9-aminofluoren-9-y1)ethyl]-urea 65

500 mg (0.94 mmol) of 45 were dissolved in 5 ml of 6N HCl and heated to 110$^\circ$C for 2 hours, the conversion to 65 was followed by HPLC analysis, the reaction mixture contained two phases (brown and light yellow, respectively). Product 65 was distributed between both phases, mostly however, the light yellow phase contained 65 in much higher purity. The two phases were separated and both were washed with ethyl acetate. The light yellow (water) phase was evaporated and a yellow brown salt of 65 was obtained. The product was treated with 20 ml of 4N NaOH and extracted with 20 ml of CHCl$_3$, the organic phases were combined and dried over MgSO$_4$. After filtration and evaporating the solvent, product 65 (367 mg, 0.77 mmol) was obtained in 80%-85% yield, and crystallized from EtOH/CH$_3$CN.

65: C$_{31}$H$_{30}$N$_4$O (MW 474.6).

mp: 246-249$^\circ$C

HPLC analysis: $R_v = 4$ ml
**MS-ESI:** $m/z = 476$ (M$^+$ 100%)

$^1$H-NMR (360 MHz, DMSO-d$_6$): $\delta = 7.70, 7.42, 7.28$ (d, d, m, $J = 6.5, 6.5$ Hz respectively for aromatic-H), 2.40, 1.92 (8H aliphatic).

$^{13}$C-NMR (90.56 MHz, DMSO-d$_6$): $\delta = 157.6$ (the amide carbon), 151.0, 138.7, 127.7, 127.5, 123.2, 119.8 (aromatic carbons), 63.8 (C-9 of fluorene units), 42.5, 38.9 (aliphatic carbons).

**Bis-[2-(9-aminofluoren-9-yl)-ethyl]-amine 47**

5 ml of concentrated hydrochloric acid were added to 367 mg (0.77 mmol) of 46 and the mixture was heated in a glass pressure tube at 145°C for 2-3 days. A precipitate appeared which proved to be the desired product 46 and was collected. The ammonium tris-hydrochloride salt 46 was dissolved in H$_2$O and DMSO, then 4N NaOH were added until the precipitate of the triamine free base 47 appeared. The product was extracted with 2×10 ml CHCl$_3$. The organic phases were combined, dried over MgSO$_4$ and evaporated to yield a brown solid was partly soluble in methanol. The yield of 47 after crystallization from EtOH/ CH$_3$CN amounted to 40%-45% 151.2 mg (0.35 mmol).

**47:** C$_{30}$H$_{29}$N$_3$ (MW 431.6)

mp: 61-64°C

HPLC4 analysis: $R_v = 8$ ml

**MS-ESI:** $m/z = 432$ (M$^+$ 100%)

$^1$H-NMR of (46) (360 MHz, MeOD): $\delta = 7.71$-7.64 (m, aromatic-H), 7.42-7.33 (m, aromatic-H), 2.7, 1.9 (8H aliphatic).

$^{13}$C-NMR of (46) (90.56 MHz, MeOD): $\delta = 141.8, 141.4, 132.2, 130.1, 125.1, 122.2$ (aromatic carbons), 64.3 (C-9 of fluorene units), 43.7, 33.3 (aliphatic carbons).
H-NMR of (47) (250 MHz, CDCl₃): δ= 8.0-7.65 (m, 16H aromatic), 2.50-2.40 (m, 8H aliphatic).

C-NMR of (47) (62.90 MHz, CDCl₃): δ= 150.4, 139.2, 128.1, 127.8, 123.0, 120.0 (aromatic carbons ), 64.5 (C-9 of fluorene units), 45.5, 40.1 (aliphatic carbons).

2-Amino-3-(2’-[9”-aminofluorene-9”-yl]ethyl)-6-(9”-fluorenylidene)-3,4,5,6-tetrahydro pyrimidine hydrogen carbonate 53.

Product 45 was basified with 4N NaOH and extracted with CHCl₃. After drying and evaporating the solvent the free base of 45 was obtained as an oily product.

50 mg (0.1mmol) of the oily product were dissolved in 2ml of isopropanol, MeOH or 3-pentanol. The reaction mixture stirred at RT over night. During this period 45 cyclized quantitatively to monocycle 53. When the same starting reaction mixture was heated at 60°C, complete conversion to 53 occurred within half an hour.

The solvent was evaporated and the product was dried over night by a stream of N₂.

53: C₃₂H₂₉N₄O₃ (MW 517.6)

HPLC2 analysis: Rᵥ = 10 ml.

MS- ESI: m/z = 457 (M⁺ 100%)

C-NMR (90.50 MHz; CD₃CN): δ= 155.5, 151.9, 148.4, 140.7, 140.4 (aromatic carbons, C-2 of the monocycle), 131.1, 130.0, 129.80, 129.6, 125.3, 124.8, 122.0, 121.8 (aromatic carbons), 65.5, 63.5 (C-9 of fluorene units), 48.0, 45.0, 39.1, 33.1 (aliphatic-carbons)

H-NMR (360 MHz; CDCl₃): δ= 7.67, 7.61, 7.59, 7.42-7.28 (d, d, d, m, J= 7.5, 7.4, 7.6 respectively, aromatic-H), 3.47, 3.40, 2.45, 1.96, (m, aliphatic-H).

600 mg (1.37 mmol) of 47 were dissolved in 15 ml of diethylene glycol dimethyl ether, then a solution of 316 mg (1.78 mmol) of thiocarbonyl diimidazole 49 in 15 ml diethylene glycol dimethyl ether was added slowly. The reaction mixture was stirred at 130°C over night. A precipitate was observed and the reaction mixture was cooled to RT. The precipitated product was isolated by centrifugation and was purified by preparative HPLC using a prontosil nitrile column, 30 μm (UV detection at 245nm, 50% MeOH in 50 mM NH₄OAc, isocratic eluent, flow = 3 ml/min). The product was eluted after 180 ml or after 120 min. Recrystallization from MeOH/ toluene yielded 205 mg (0.41 mmol) 25%-30% yield.

48: C₃₃H₃₀N₃O₂ (MW 500.6)
mp: 250°C
HPLC2 analysis: Rᵥ = 12.8 ml.
ESI MS: m/z = 440.3 (M⁺ 100%)

¹³C-NMR (360 MHz; MeOD): δ= 153.3 (guanidinium carbon), 148.4, 140.5, 130.8, 129.6, 124.9, 121.6 (aromatic carbons), 63.5 (C-9 of fluorene units), 45.9, 33.0 (aliphatic carbons).

¹H-NMR (360 MHz; MeOD): δ= 7.72, 7.63, 7.35 (d, d, m, J= 7.0, 6.8 Hz respectively, 16H aromatic), 3.91, 2.28 (8H aliphatic).
500 mg (2.3 mmol) of dry 9-aminofluorene hydrochloride 54 were suspended in 10 ml dry CH2Cl2, (the solubility improved after the addition of 4-6 eq of Et3N). The reaction mixture was stirred for 15 minutes at RT, then 88 µl (1.15 mmol) of thiophosgene (CSCl2) 55 were slowly added and the color of the mixture turned brown. The mixture was heated to 60°C for 3-4 hours, until all the starting material was converted to the thiourea product (HPLC analysis). The dichloromethane solution was washed with diluted acetic acid pH 4-5, in order to extract the base and the salts that were produced during the reaction. The organic phase was evaporated and the product was washed with the minimum amount of CH3CN, a white solid product appeared and crystallized from hot acetonitrile to yield 325.6 mg (0.8 mmol, 69%). The yield ranged between 50%-70% and dependent on the dryness of the starting material. When 54 was kept under N2 over night or in a desiccator the yield improved.

56: C27H20N2S (MW 404.5)

HPLC1 analysis: Rv (54)= 6 ml, Rv (56)= 20 ml

1H-NMR for (54) (360 MHz, MeOD): δ= 7.72 (m, 4H aromatic), 7.39 (m, 4H aromatic), 5.27 (s, aliphatic-H).

13C-NMR for (54) (90.56 MHz, MeOD): δ= 142.2, 140.4, 131.2, 129.1, 126.0, 121.6 (aromatic carbons), 55.2 (aliphatic carbon)

1H-NMR for (56) (250 MHz, MeOD): δ= 7.65, 7.27 (m, 16H aromatic), 5.12 (s, 2H aliphatic).

13C-NMR for (56) (62.90 MHz, MeOD): δ= 139.8, 128.6, 127.6, 124.8, 120.2 (aromatic-carbon), 57.3, 56.4 (aliphatic carbons).
1,3-Bis-(9H-fluoren-9-yl)-2-methyl-isothioure- iodide  57

\[
\begin{align*}
&\text{C}_{28}\text{H}_{23}\text{N}_2\text{I} \quad (\text{MW} 546.46) \\
\text{HPLC}1 \text{ analysis: } R_v = 14.4 \text{ ml}
\end{align*}
\]

100 mg (0.25 mmol) of 56 were suspended in 5 ml dry CH$_3$CN and heated to 80°C, when 8 eq (2 mmol, 125 µl) of CH$_3$I were added. After 1-2 hours at 80°C the reaction was completed and the product was crystallized from the reaction mixture after cooling. 123 mg (0.225 mmol, 90%) of 57 were obtained.

57: C$_{28}$H$_{23}$N$_2$I$_S$ (MW 546.46)

HPLC1 analysis: $R_v = 14.4$ ml

$^{13}$C-NMR for (57) (90.56 MHz, MeOD): $\delta$= 142.4, 142.1, 130.9, 129.3, 126.0, 121.7 (aromatic carbons), 60.51, 60.3 (aliphatic carbons), 15.75 (CH$_3$).

2H-Pyrimido[1,2-a]pyrimidine,1,3,4,6,7,8-hexahydro-2,2,8,8-tetraphenyl- iodide  70

\[
\begin{align*}
&\text{C}_{38}\text{H}_{36}\text{N}_2\text{I} \quad (\text{MW} 618.39) \\
\text{HPLC}1 \text{ analysis: } R_v = 10.0 \text{ ml}
\end{align*}
\]

673 mg (1.55 mmol) of 68 [114] were dissolved in 10 ml of dry CH$_3$CN (dried over Al$_2$O$_3$ activity-I) and heated to 85°C. A solution of 364 mg (2 mmol) thiocarbonyldiimidazole in 4 ml of dry CH$_3$CN was then added. The reaction was kept at 85°C for 4 h. When the reaction was cooled to RT, 170 µl (2.68 mmol) of CH$_3$I were added. A precipitate appeared, the mixture was stirred for another 16 hours at
The precipitate, was identified as product 70, and was filtered and crystallized from nitromethane (CH₃NO₂) yielding 700 mg (1.24 mmol), 80% yield.

70: C₃₁H₆₀N₃I (MW 571.5)

mp.(70): 340°C

MS ESI: 444.4 (100% M⁺).

HPLC2 analysis: Rᵥ (70)= 13.9 ml.

¹³C-NMR for (70) (62.9 MHz; DMSO-d₆): δ= 162.1 (the guanidinium carbon), 143.6, 129.0, 127.9, 126.0 (phenyl carbons), 60.6, 44.0, 31.2 (aliphatic carbons).

¹³C-NMR for (70) (62.9 MHz; MeOD): δ= 162.0 (the guanidinium carbon), 144.7, 130.1, 129.2, 127.3 (phenyl carbons), 62.6, 45.6, 33.2 (aliphatic carbons).

¹H-NMR for (70) (250 MHz; MeOD): δ= 7.36-7.26 (m, 20H aromatic), 3.08, 2.70 (8H aliphatic).

2H-Pyrimido[1,2-a]pyrimidine,1,3,4,6,7,8-hexahydro-2,2,8,8-tetramethyl-iodide  71

30 g of Dowex resin (2×8 50-100 mesh, OH⁻–form) in H₂O were poured into a column and washed with more distilled water to pH=7, then washing was continued with MeOH in order to replace all the water.

1 g (3.5 mmol) of the white triammonium salt 67 were dissolved in 3-5 ml of MeOH, poured into the column and kept for 15min to allow counter ion exchange, then washed again with MeOH to collect the compound. The solution was evaporated and the oily compound 69 of the free base (600 mg) was kept under N₂ gas to avoid uptake of atmospheric CO₂.

600 mg (3.4 mmol) of 69 were dissolved in 12 ml of dry CH₃CN, the mixture was heated to 85°C, then a solution of 917 mg (5.1 mmol) of thiocarbonyldimidazole in 5 ml of dry CH₃CN was added. The reaction mixture was kept stirring at 85°C over
night, the reaction was cooled to RT and 300 µl of CH₃I were added. After another night at RT, the solvent was removed and 6 ml of CHCl₃ were added to the crude product. Extraction with water was necessary, in order to remove most of the undesired products to the water phase (the extraction was repeated 3-4 times). The organic phase was dried over MgSO₄, filtered and evaporated to obtain 70% 768 mg (2.38 mmol, 70%) of product 71. Which could be recrystallized from MeOH/H₂O.

**71**: C₁₁H₂₂N₃I (MW 323)

mp: 185°C (lit) 183°C

HPLC3 analysis: Rᵥ (69) = 3 ml; Rᵥ (71) = 11.8 ml.

¹³C-NMR of (71) (90.5 MHz; MeOD): δ= 151.0 (guanidinium carbon) 50.1 (2xC(CH₃)₂), 44.9, 33.9 (aliphatic-carbons), 28.5 (4xC₃H₉).

¹H-NMR of (71) (360 MHz; MeOD): δ= 3.37 (t, J= 6.6 Hz, 4H aliphatic), 1.81 (t, J= 6.6 Hz, aliphatic-H), 1.23 (s, 4×CH₃).

**A general method for counter anion exchange for different guanidinium compounds.**

To exchange the counter anions of the different guanidinium compounds to the halogen counter anions (scheme 25), we used an anion exchange resin, acryl matrix AG 4X4, 100-200 mesh, free base form.

**Scheme 25**

3 g of the anion exchange resin in a column were washed with distilled water, then 5-10 ml of an acid (6N HCl, 6N HBr, TFA, acetic acid or hexafluorophosphoric acid which was dissolved in 1:1 water/acetonitrile), were poured slowly into the column till
the resin was saturated with the required acid, and was converted to the tertiary ammonium salt. This was confirmed by pH test, of the column efficient. The column then was washed with distilled water to remove all the excess of the acid till pH = 7. The resin was washed with MeOH in order to replace all the water solvent, due to the poor solubility of the guanidinium compounds in water.

100 mg of the guanidinium compound were dissolved in 3-4 ml of MeOH. The solution was poured into through the column till it hardly covered the top of the resin. After 5-10 min the resin was washed with more MeOH in order to collect the guanidinium compound with its new counter anion. The solvent was evaporated and the compound was recrystallized as it is shown in table 10

### A preparation of Fluoreneguanidinium iodide from Fluorenylguanidinium acetate.

A column of 1.0 g of anion exchange resin AG 4×4, acetate form, was converted into the iodide form by passing 30 ml of a 0.5 M solution of KI in water slowly through it. The column was washed with distilled water until a test with AgNO₃/HNO₃ for soluble iodide in the effluent was negative. Then 30 mg of fluorenylidenguanidinium acetate 48 were dissolved in 1-2 ml of MeOH, the mixture was poured into the column, and kept for 10-20 min to allow counter anion exchange, then washed with more

<table>
<thead>
<tr>
<th>compound’s number</th>
<th>crystallized from</th>
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<tbody>
<tr>
<td>135</td>
<td>CH₃NO₂ or CH₃CN</td>
</tr>
<tr>
<td>136</td>
<td>CH₃NO₂ or CH₃CN</td>
</tr>
<tr>
<td>137</td>
<td>CH₃CN/toluene</td>
</tr>
<tr>
<td>134</td>
<td>MeOH/H₂O</td>
</tr>
<tr>
<td>138</td>
<td>MeOH/toluene or CH₃CN</td>
</tr>
<tr>
<td>139</td>
<td>MeOH/toluene or CH₃CN</td>
</tr>
<tr>
<td>140</td>
<td>MeOH/toluene or CH₃CN</td>
</tr>
</tbody>
</table>
MeOH to collect the compound. The solvent was evaporated and the fluorenlylidenguanidinium iodide was crystallized from MeOH/Toluene.

**2,2'-dihydroxy 3,3'-dinitro 1,1'-biphenyl 85**

reference: [94]

**85:** C$_{12}$H$_8$N$_2$O$_6$ (MW 276.2)  
mp: 180°C  
HPLC6 analysis: Rv = 8.5 ml  

MS- ESI: m/z = 275.5 [100%, (M-H)$-$]  
$^1$H-NMR (360 MHz, CDCl$_3$): δ= 10.98 (s, OH), 8.21 (d, J= 8.2 Hz, 2H), 7.63 (d, J= 7.0 Hz, 2H), 7.10 (t, J= 7.6 Hz, 2H).

$^{13}$C-NMR (90.56 MHz, CDCl$_3$): δ= 153.0 (C-OH), 139.1 (CH), 134.1 (C-NO$_2$), 127.3 (quaternary C), 125.4, 119.6 (aromatic carbons).

**2,2'-methanesulfonyloxy 3,3'-dinitro 1,1'-biphenyl 86**

500 mg (1.81 mmol) of **2,2'-dihydroxy-3,3'-dinitrobiphenyl 85** were dissolved in 40 ml of dry CH$_2$Cl$_2$. The yellow solution was stirred at 0°C-5°C under nitrogen, when 630 µl (4.52mmol) of triethyl amine were added and the resulting orange color of the mixture became again yellow after the dropwise addition of 350 µl (4.52 mmol) of mesyl chloride. A precipitate of Et$_3$NHCl appeared immediately. After 1.5 h stirring at 5°C followed by 15 min at room temperature, the reaction mixture was extracted with aqueous acetic acid followed by another extraction with 5% NaHCO$_3$. The organic
phase was dried with MgSO₄ and evaporated to obtain a yellow product 86 which was recrystallized from acetonitrile to yield 570 mg (1.31 mmol), 73%.

86: C₁₄H₁₂N₂O₁₀S₂ (MW 432)
mp: 218°C
HPLC6 analysis: Rv = 5ml
MS-ESI: m/z = 433.5 [100% (M+H)⁺]
¹H-NMR (250.13, acetone d₆): δ = 8.22 (d, J = 7.9 Hz, 2H), 7.97 (d, J = 7.3 Hz, 2H), 7.75 (t, J = 7.9 Hz, 2H), 3.07 (s, 2×CH₃).
¹H-NMR (250.13, CD₂CN): δ = 8.13 (d, J = 7.7 Hz, 2H), 7.86 (d, J = 7.3 Hz, 2H), 7.66 (t, J = 8.1 Hz, 2H), 2.91 (s, 2×CH₃).
¹³C-NMR (62.90, CD₂CN): δ = 145.2 (C-OMs), 139.5 (C-NO₂), 138.4 (CH), 133.5 (quaternary C), 129.3 (CH), 127.9 (CH), 39.8 (2×CH₃).

2,2'-Trifluoromethanesulfonyloxy 3,3'-dinitro 1,1'-biphenyl 97

400 mg (1.44 mmol) of 2,2'-dihydroxy 3,3'-dinitro biphenyl 85 were added to 10 ml dry pyridine at 0°C, the mixture was stirred under nitrogen when 855 µl (5.18 mmol) of (Tf₂O) trifluoromethansulfonic anhydride were added dropwise. After 3 h at 0°C, the ice bath and nitrogen were removed and the pyridine solvent was evaporated in vacuo, the residue was extracted with CH₂Cl₂/H₂O for 3-4 times. The organic phase was dried with MgSO₄, filtered and evaporated to obtain a yellow-brown product. Recrystallization from acetonitrile yielded 659 mg (1.22 mmol, 85%).

97: C₁₄H₆F₆N₂O₁₀S₂ (MW 540)
mp: 130-132°C
HPLC6 analysis: Rv = 11.0 ml
MS-ESI: m/z = 541.3 [100% (M+H)⁺]

¹H-NMR (250.13 MHz, CDCl₃): δ= 8.25 (d, J= 7.3 Hz, 2H), 7.76 (d, J= 13.1 Hz, 2H), 7.77 (t, J= 11.6 Hz, 2H).

¹³C-NMR (62.90 MHz, CDCl₃): δ= 143.0 (C-OTf), 138.3 (C-NO₂), 137.3 (CH), 131.3 (quaternary C), 129.4 (CH), 127.8 (CH), 117.9 (d, J= 320.9 Hz, 2xCF₃).

¹⁹F-NMR (235.34, CDCl₃) δ= 88.4

2,2’-Dimethoxy-3,3’-bis-phenylethynyl-1,1’-biphenyl 75

A degassed solution of 73.7 mg (0.1 mMol) of [PdCl₂(PPh₃)₂] and 20 mg CuI in 5 ml of dry THF was added to a degassed solution of 1.0 g (2.1 mMol) of 2,2’-Dimethoxy-3,3’-diiodo-1,1’-biphenyl [89] in 18 ml dry THF, 30 ml of dry (i-Pr)₂NH and 1 ml (11.1 mMol) of phenylacetylene at 40°C. The mixture was stirred under N₂ at 40°C for 4 h. 20 ml of sat. aqueous NaCl solution and 40 ml of CH₂Cl₂ were added, the phases were separated, and the aqueous phase was extracted with CH₂Cl₂ (30 ml). The combined organic phases were dried (Na₂SO₄) and concentrated. Column chromatography (CC) (SiO₂ 230-400 mesh; hexane/AcOEt 6:1 containing 0.5% Et₃N), followed by recrystallization from hexane yielded 738 mg (1.78 mmol, 85%) of product 75.

75: C₃₀H₂₂O₂ (MW 414)
mp: 77°C
HPLC5 analysis: Rv = 17.0 ml
MS (GC-MS, EI): m/z = 413.9 (100%)
¹H-NMR (250.13, CDCl₃): δ= 7.55-7.16 (m, 16H), 3.79 (s, -OCH₃)
$^{13}$C-NMR (62.90, CDCl$_3$): $\delta$ = 158.8, 133.2, 131.9, 131.7, 131.5, 128.3, 123.4, 123.4, 123.3, 117.2 (aromatic carbons), 93.7, 86.0 (-C≡C-), 61.1 (-OCH$_3$).

3,3'-Diodo-2,2'-bis-methoxymethoxy-1,1'-biphenyl 79

1.0 g (25 mMol) of NaH (60% dispersion) were washed with 10 ml of dry THF. To the NaH stirred under nitrogen in 60 ml dry THF was added in portions 1.0 g (2.28 mMol) of 3,3’-diodo-2,2’-dihydroxy-1,1’-biphenyl 72 [90]. After H$_2$ evolution stopped, 2.4 ml (30mmol) of chlordimethylether (ClCH$_2$OCH$_3$) was added to the stirred precipitate, and the resulting mixture was stirred for 3 h. 20 ml of H$_2$O were added slowly and the mixture was shaken with 30 ml of CH$_2$Cl$_2$. The aqueous layer was extracted with CH$_2$Cl$_2$ (2x15 ml), and the combined organic layers were washed with saturated KHCO$_3$ solution. The organic layer was dried (MgSO$_4$) and evaporated. Crystallization from petroleum ether yielded 1.17 g (2.22 mmol, 92%) crystals of product 79.

79: C$_{16}$H$_{16}$O$_4$I$_2$ (MW 526)

mp:102°C

HPLC5 analysis: Rv = 12.0 ml

MS (GC-MS, EI): $m/z$ = 525.8 (4%, M$^+$), 449.8 (100%, M-C$_3$H$_8$O$_2$$^+$)

$^1$H-NMR (250.13, CDCl$_3$): $\delta$ = 7.82 (d, $J$= 7.6 Hz, 2H), 7.34 (d, $J$= 7.3 Hz, 2H), 6.91 (t, $J$= 7.6 Hz, 2H), 4.79 (s, 2x-OCH$_2$), 3.02 (s, 2x-OCH$_3$).

$^{13}$C-NMR (62.90, CDCl$_3$): $\delta$ = 154.6, 139.2, 133.3, 132.1, 125.7 (aromatic carbons), 99.5 (OCH$_2$), 92.8, 57.1 (OCH$_3$).
2,2’-Bis-methoxymethoxy-3,3’-bis(phenylethynyl)-1,1’-biphenyl 80

A degassed solution of 38 mg (0.05mMol) of [PdCl₂(PPh₃)₂] and 10 mg (0.05 mMol) of CuI in 3 ml of dry THF was added to a degassed solution of 600 mg (1.07 mMol) of 2,2’-bis-methoxymethoxy-3,3’-diiodo-1,1-biphenyl 79 in 15 ml dry THF, 20 ml of dry (i-Pr)₂NH and 520 µl (4.7 mMol) of phenylacetylene 74 at 40°C. The mixture was stirred under N₂ at 40°C for 6 h. 15 ml of sat. aqueous NaCl solution and 20 ml of CH₂Cl₂ were added, the phases were separated, and the aqueous phase was extracted with CH₂Cl₂ (15 ml). The combined organic phases were dried (Na₂SO₄) and concentrated. Column chromatography (CC) (SiO₂ 230-400 mesh; hexane/AcOEt 6:1 containing 0.5% Et₃N) to the crude product 80, followed by recrystallization from hexane yielded 487 mg (90%) of pure product 80.

80: C₃₂H₂₆O₄ (MW 474)

mp: 123°C
HPLC5 analysis: Rv = 18 ml

MS (GC-MS, EI): m/z = 473.9 (38%, M⁺), 398 (100%, M⁺-C₃H₈O₂).

¹H-NMR (250.13, CDCl₃): δ= 7.53 (m, 6H), 7.37 (d, J= 7.5 Hz, 2H), 7.35 (m, 8H), 7.18 (t, J= 7.5 Hz, 2H), 5.08 (s, -OCH₂-), 3.03 (s, -OCH₃)

¹³C-NMR (62.90, CDCl₃): δ= 156.1, 133.1, 132.8, 132.0, 131.5, 128.4, 123.8, 123.2, 117.7 (aromatic carbons), 99.2 (-OCH₂-), 93.6, 86.0 (-C≡C-), 56.7 (-OCH₃).
To 200 mg (0.4 mmol) of 80 in 140 ml THF, 328 µl of conc. HCl (37%) in 120 ml MeOH were added, and the solution was stirred for 12 h under N2 at room temp. 200 ml of H2O and 200 ml of CH2Cl2 were added, the aq. phase was extracted with 100 ml of CH2Cl2, the combined organic phases were dried (Na2SO4), and evaporated to give 80% 125 mg (0.32 mmol) yield of oily product 76.

76: C28H18O2 (MW 386)

HPLC5 analysis: Rv = 13.0 ml

MS (GC-MS, EI): m/z = 386 (100%, M).

1H-NMR (250.13, CDCl3): δ= 7.51-7.45 (m, 6H), 7.31-7.26 (m, 8H), 6.97 (t, J = 7.32 Hz, 2H), 6.28 (br s, 2H).

13C-NMR (62.9 MHz, CDCl3): δ= 153.5 (-COH, biphenyl), 132.1 (-CH, biphenyl), 131.7 (-CH, biphenyl), 131.5 (aromatic carbon), 128.6 (-CH, biphenyl), 128.3 (Ar), 124.1 (quat. C, biphenyl), 122.4 (-C=C, biphenyl), 120.6 (Ar), 110.7 (-C=C-C, Ar), 95.8 (-C≡C-), 83.6 (-C≡C-).

3,3’-Bis-phenylethynyl-1,1’-biphenyl-2,2’-diylphosphonic acid tetrabutyl ammonium salt 78
To 100 mg (0.24 mMol) of 76 in 35 ml dry CH₂Cl₂, 50 µl (0.54 mMol) of POCl₃ and 105 µl (0.74 mMol) of Et₃N were added at RT under N₂, and the solution was stirred for 5 h. After evaporation in vacuo, 30 ml THF/H₂O 1:1 were added, and the mixture was stirred for 12 h at RT. 50 ml of CH₂Cl₂ and 30 ml of H₂O were added, the separated organic phase was washed with H₂O (2x30 ml), dried (Na₂SO₄), and concentrated to give the free acid 77. Column chromatography (SiO₂; CH₂Cl₂/Et₃N 98:3) to 77, followed by ion-exchange chromatography (Dowex 50WX8, Bu₄N⁺; CH₂Cl₂/CH₃CN 1:1), provided an oily product 78, 99 mg (60%). The oily product was dried in high vacuum, then crystallized from dry CH₂Cl₂/dry ether.

78: C₄₄H₅₂NO₄P (MW 689)
77: C₂₈H₁₇O₄P (MW 448)
mp: (78) 88-90°C
HPLC5 analysis: Rv = 9.0 ml
MS (GC-MS, EI): m/z = 447.8 (100%)
¹H-NMR of 78 (360 MHz, CDCl₃): δ = 7.60 (m, 4H), 7.51 (d, J = 7.3 Hz, 2H), 7.36 (d, J = 7.6 Hz, 2H), 7.26 (m, 6H), 7.12 (t, J = 7.6 Hz, 2H), 2.9 (m, 8H), 1.3 (m, 8H), 1.13 (m, 8H), 0.7 (t, J = 6.7, 12H).

¹³C-NMR of 78 (90.56 MHz, CDCl₃): δ = 151.2 (JPC = 9.0 Hz), 132.6, 131.9, 130.3, 129.2, 128.1, 128.0, 123.7, 123.6, 117.7 (JPC = 3.8 Hz), 93.3, 86.2, 58.2, 23.7, 19.4, 13.5.

³¹P-NMR (101.25 MHz, CDCl₃): -3.50.
1.1'-Biphenyl -2,2'-diylphosphoric acid tetrabutyl ammonium salt

1.0 g (5.37 mmol) of 2,2'-dihydroxy 1,1'-biphenyl 81 were mixed with 492 µl (5.37 mmol) of POCl₃ and 125 µl of DMF. The reaction mixture was heated at 117°C for 5 h then at 135°C for overnight. The mixture was cooled to room temperature and 5 ml of acetic acid were added. An immediate precipitate product appeared but it was collected only after one hour and was recrystallized over night from MeOH/CH₃CN or immediately from CHCl₃/ether to give the clean free acid 83. The free acid was subjected to cation-exchange chromatography (Dowex 50WX8, Bu₄N⁺ form; MeOH), to provide an oily-solid product 84 which was recrystallized from CHCl₃/ether to give a white precipitate (like needles) in 1.83 g (3.76 mmol, 70%).

83: C₁₂H₉PO₄ (MW 248)

mp: 83 175°C

MS ESI: m/z = 249.1 [100% (M+H)⁺]

HPLC analysis: Rv = 5.0 ml

¹H-NMR of 83 (250.13, MeOD): δ= 7.46 (d, J= 7.0 Hz, 2H), 7.33-7.24 (m, 4H), 7.14 (d, J= 7.3 Hz, 2H).

¹³C-NMR of 83 (62.9 MHz, MeOD): δ= 149.6 (d, J= 9.1 Hz, COP), 131.1 (CH), 130.93 (CH), 130.0, 127.4 (d, J= 1.9 Hz, CH), 122.4 (d, J= 4.3 Hz, CH).

³¹P-NMR of 83 (101.25 MHz, MeOD): 0.32
\textbf{H-NMR of 84 (360.13, CDCl$_3$):} $\delta= 7.43$ (d, $J= 7.3$ Hz, 2H), 7.28-7.20 (m, 4H), 7.15 (t, $J= 7.3$ Hz, 2H), 3.16 (m, 8H), 1.50 (m, 8H), 1.31 (m, 8H), 0.91 (t, $J= 7.0$ Hz, 4CH$_3$).

\textbf{13C-NMR of 84 (62.9 MHz, MeOD):} $\delta= 151.2$ (d, $J= 9.2$ Hz, COP), 130.43 (quaternary carbon), 128.85 (CH), 128.78 (CH), 123.62 (CH), 122.1 (d, $J= 4.3$ Hz, CH), 58.51 (4CH$_2$), 23.91 (4CH$_2$), 19.58(4CH$_2$), 13.60 (4CH$_3$).

\textbf{31P-NMR of 84 (101.25 MHz, MeOD):} -1.04

\textbf{1-(2,6-Dibromophenyl)-2,2,5,5-tetramethyl-2,5-disila pyrroolidine 104}

A solution of 5.0 g (20 mmol) of dibromoaniline 101 in 40 ml dry THF was stirred at 0°C under nitrogen atmosphere. 14 ml (40 mmol) of MeMgCl 102 were added dropwise. The mixture was warmed to room temperature and a solution of 4.3 g (20 mmol) 1,2-bis(chlorodimethylsilyl)ethane (stbase) 103 in 15 ml dry THF was added. The yellow-brown mixture was warmed to 50°C for 2 h, then at 38°C for overnight. The THF was evaporated and the mixture was extracted between water/hexane, the organic phase was concentrated and chromatographed on Silica gel 60 (230-400 mesh) with hexane to yield 51%, 4.0 g (10.2 mmol) of the desired product 104.

104: C$_{12}$H$_{19}$NSi$_2$Br$_2$ (MW 393)

mp: 40°C

MS (GC-MS, EI): m/z =378 (100% M-CH$_3$)

HPLC5 analysis: R$_v$ = 18 ml

\textbf{1H-NMR (250.13, CDCl$_3$):} $\delta= 7.53$ (d, $J= 7.9$ Hz, 2H), 6.74 (t, $J= 7.9$ Hz, 1H), 0.96 (s, 4H, 2SiCH$_2$), 0.19 (s, 12H, 2Si(CH$_3$)$_2$).

\textbf{13C-NMR (62.9 MHz, CDCl$_3$):} $\delta= 144.3$ (C-N-), 132.8 (2×CH), 126.6 (2×CBr), 125.5 (CH), 8.7 (2×CH$_2$), 1.4 (4×CH$_3$).
A solution of 1.0 g (3.98 mmol) of 2,6-dibromoaniline 101 in 15 ml of dry THF was stirring at -78°C under N₂. 7.4 ml of nBuLi (1.6 M in hexane, 11.95 mmol) were added. After stirring at this temperature for one hour, this was followed by addition of 1.3 ml trimethyl borate 112, and stirring was continued at -78°C for another hour, then at room temperature for 2 hours, 5 ml of 3N HCl were poured into the reaction mixture. 1.5 hours later the mixture was extracted with chloroform. Side products accompanied the desired one in the extract. The crude product was dissolved in a minimum amount of MeOH and separated using a preparative reverse phase column (nucleosil C-8, 30µm). The product was eluted with 42% MeOH/0.1%TFA at 254 nm, 4 ml/min to give 515 mg (2.38 mmol, 60%) of 113.

113: C₆H₇BBrNO₂ (MW 215.7)  
mp: 110°C  
MS ESI: 215.1 [24% (M+H)⁺, (C₆H₇¹⁰B⁷⁹BrNO₂⁺H)⁺], 216.1 (96% [(M+H)⁺, (C₆H₇¹¹B⁷⁹BrNO₂⁺H)⁺], 217 [32% (M+H)⁺, (C₆H₇¹⁰B⁸¹BrNO₂⁺H)⁺], 218.0 [92% (M+H)⁺, (C₆H₇¹¹B⁸¹BrNO₂⁺H)⁺].

![Figure 19. ESI-MS of 113](image)

HPLC5 analysis: Rv = 6.0 ml
$^1$H-NMR (360 MHz, MeOD): $\delta = 7.79$ (d, $J = 7.0$ Hz, 1H), 7.70 (d, $J = 7.7$ Hz, 1H), 7.14 (t, $J = 7.5$ Hz, 1H).

$^{13}$C-NMR (90.56 MHz, MeOD): $\delta = 162.2$ (d, $J = 35.7$ Hz, C-B(OH)$_2$), 140.2 (C-NH$_2$), 136.8 (CH), 136.4 (CH), 127.5 (CH), 116.6 (C-Br).

3,3'-Dibromo-2,2'-diamino-1,1'-biphenyl 106

A solution of 800 mg (3.7 mmol) of 6-bromo aniline 2-boronic acid 113 in 5-10 ml EtOH, 14 ml 2M Na$_2$CO$_3$ and 428 mg (10 mol%) (PPh$_3$)$_4$Pd was added to 2.78 g (11.0 mmol) of 2,6-dibromoaniline 101 in 70 ml benzene. After the resulting mixture was purged with nitrogen, it was gently refluxed for 24 h with stirring under nitrogen. After cooling, the organic layer was separated, and the aqueous solution was extracted with benzene. The combined organic layers were dried with MgSO$_4$ and evaporated, and the residue was chromatographed on preparative reverse phase column (nucleosil C-8, 30 $\mu$m) with 66% MeOH / 0.1% TFA eluent, 4ml/min at 254 nm) to obtain the product 106 which was well recrystallized from MeOH in 835 mg (2.44 mmol, 66%).

106: C$_{12}$H$_{10}$N$_2$Br$_2$ (MW 342)

mp: 105°C

MS ESI: 341 [41% (M+H)$^+$ (C$_{12}$H$_{10}$$^{79}$Br$_{79}$BrN$_2$ +H)$^+$], 343 [80% (M+H)$^+$ (C$_{12}$H$_{10}$$^{79}$Br$_{81}$BrN$_2$ +H)$^+$], 345 [41% (M+H)$^+$ (C$_{12}$H$_{10}$$^{81}$Br$_{81}$BrN$_2$ +H)$^+$].

HPLC5 analysis: Rv = 8 ml

$^1$H-NMR (360 MHz, MeOD): $\delta = 7.43$ (d, $J = 7.6$ Hz, 2H), 7.0 (d, $J = 7.0$ Hz, 2H), 6.67 (t, $J = 7.5$ Hz, 2H).

$^{13}$C-NMR (90.56 MHz, MeOD): $\delta = 143.7$ (C-NH$_2$), 133.5 (CH), 131.2 (CH), 126.6 (quaternary), 119.9 (CH), 110.7 (C-Br).
600 mg (1.75 mmol) of 3,3’-dibromo-2,2’-diamino-1,1’-biphenyl 106 and 12 mol% (0.21 mmol, 243 mg) of Pd(PPh₃)₄ were dissolved in 15 ml benzene. A solution of 3 equiv (5.52 mmol, 777 mg) of trans-phenylvinyl boronic acid 117 in 10 ml ethanol and 6 equiv. of 7 ml 2M Na₂CO₃ solution were added to the reaction mixture. The reaction mixture was flushed with nitrogen, then refluxed for 24 h at 75°C. The desired product precipitated from the mixture while refluxing. The product was collected, washed with MeOH several times, then dissolved in CHCl₃ and extracted with water. The organic phase was dried over Na₂SO₄, filtered and evaporated to yield 680 mg (1.75 mmol, 70%) of product.

118: C₂₈H₂₄N₂ (MW 388.5)

mp: 220°C-223°C

MS ESI: m/z = 389 (100% (M+H)⁺).
HPLC5 analysis: \( R_v = 10.0 \text{ ml} \)

\(^1\text{H-NMR} (250.13 \text{ MHz, CDCl}_3): \delta = 7.53-6.85 \text{ (m, 20H of aromatic and vinyl groups), 3.86 (s, 4H, 2×NH}_2).\)

\(^{13}\text{C-NMR} (62.9 \text{ MHz, CDCl}_3): \delta = 142.0, 137.6, 131.0, 130.4, 128.7, 127.7, 126.5, 125.0, 124.5, 124.3, 119.0.\)

2,2’-Dibromo-1,1’-biphenyl \( \text{126} \)

Reference [100]

\( \textbf{126}: \text{C}_{12}\text{H}_8\text{Br}_2 \text{ (MW 312)} \)

mp: 73°C

MS (GC-MS, EI): \( m/z = 312 \) [57% \( \text{C}_{12}\text{H}_8^{79}\text{Br}^{81}\text{Br} \)], 231 [74% \( \text{C}_{12}\text{H}_8^{79}\text{Br} \)], 233 [71% \( \text{C}_{12}\text{H}_8^{81}\text{Br} \)], 152 [100% (M-2Br)]³].

HPLC3 analysis: \( R_v = 14 \text{ ml} \)

\(^1\text{H-NMR} (360 \text{ MHz, CDCl}_3): \delta = 7.65 \text{ (d, } J = 7.7 \text{ Hz, 2H), 7.38-7.34 \text{ (m, 2H), 7.26-7.22 (m, 4H).} \)

\(^{13}\text{C-NMR} (62.9 \text{ MHz, CDCl}_3): \delta = 142.0 \text{ (C-Br), 132.5 (CH), 130.9 (CH), 129.3 (CH), 127.1 (CH), 123.5 (quaternary C).} \)

1,1’-biphenyl- 2,2’-phosphinic acid tetrabutyl ammonium salt \( \text{128} \)

A solution of 1.0 g (3.2 mmol) of the dibromide \( \text{126} \) in 60 ml of dry THF was cooled to -78°C under nitrogen. 4 ml (6.4 mmol) of n-BuLi (1.6 M in hexane) were added with cooling, after 3 h at -78°C, followed by 279 µl (3.2 mmol) of phosphorus
trichloride. After 2.5 h more, still at -78°C, 3 ml of water were added dropwise (which yielded an inhomogenous mixture for 2-3 h only) and the nitrogen cover and cooling were discontinued. Next day the clear pale yellow solution was evaporated and the residue was suspended in 45 ml acetone and warmed to 40°C, the mixture was treated dropwise during 10 min with 8 ml (30%) hydrogen peroxide then boiled gently (reflux) for 4 h, with the addition of more 4 ml H₂O₂ after 1 h. 70 ml of water were added and the reaction mixture was extracted with CHCl₃, most of the undesired products remain in the organic phase, the aqueous phase was evaporated and the free base product 127 was recrystallized by MeOH/CHCl₃ to yield 483.8 mg (2.24 mmol) around 70% product. The phosphinic acid 127 was converted to the TBA-phosphinate salt 128 through ion-exchange chromatography (Dowex 50WX8, Bu₄N⁺; MeOH/CH₃CN 1:1). The oily salt 128 was crystallized by MeOH/water to obtain 872 mg (1.9 mmol, 85%) of a pure product 128.

127: C₁₂H₉P₂O₂ (MW 216)

mp (127): 210°C
MS ESI: m/z = 215.3 (100% (M-H)⁻).
HPLC4 analysis: Rv = 5 ml

¹H-NMR of 127 (250.13 MHz, MeOD): δ = 7.67 (m, 2H), 7.50 (t, J= 8.2 Hz, 2H), 7.39 (t, J= 7.3 Hz, 2H), 7.23 (m, 2H).

¹³C-NMR of 127 (62.9 MHz, MeOD): δ = 141.2 (d, J= 28.1 Hz, quaternary C), 134.4 (d, J= 1.9 Hz, CH), 131.7 (d, J= 137.3 Hz, CP), 130.5 (d, J= 11.9 Hz, CH), 128.7 (d, J= 8.6 Hz, CH), 122.3 (d, J= 11.9 Hz, CH).

³¹P-NMR of 127 (101.25 MHz, MeOD): 37.47

¹H-NMR of 128 (250.13 MHz, MeOD): δ = 7.60 (d, J= 7.5 Hz, 2H), 7.46 (t, J= 7.5, 2H), 7.24-7.21 (m, 2H), 7.14 (d, J= 4.9 Hz, 2H), 2.95 (m, 8H), 1.38 (m, 8H), 1.19-1.11 (m, 8H), 0.77 (t, J= 71 Hz, 2H).

¹³C-NMR of 128 (62.9 MHz, MeOD): δ = 140.3 (d, J= 25.27 Hz, quaternary C), 137.9 (d, J= 132.6 Hz, CP), 131.8 (d, J= 1.9 Hz, CH), 129.4 (d, J= 10.5 Hz, CH), 127.9 (d,
\( J = 8.6, \text{CH}) \), 121.4 (d, \( J = 11.0 \text{ Hz, CH}) \), 59.3 (d, \( J = 2.5 \text{ Hz, 4\times CH}_2 \)), 24.65 (4\times CH2), 20.60 (4\times CH2), 13.95 (4\times CH3).

\(^{31}\text{P-NMR of 128} \) (101.25 MHz, MeOD): 29.9

4,4’’’-Dimethyl-[1,3’,1’’,3’’,1’’’]quaterphenyl-2’,2’’-diamine 124

\[ \text{H}_2\text{C} \]
\[ \text{NH}_2 \]
\[ \text{H}_2\text{N} \]
\[ \text{C}_{26}\text{H}_{24}\text{N}_2 \]
\[ \text{CH}_3 \]

300 mg (0.87 mmol) of 3,3’-dibromo-2,2’-diamino-1,1’-biphenyl 106 and 12 mol% 121 mg of Pd(PPh3)4 were dissolved in 15 ml benzene. A solution of 600 mg (4.41 mmol) of p-tolueneboronic acid 117 in 10 ml ethanol and 2 ml 2N Na2CO3 solution were added to the reaction mixture. The mixture was flushed with nitrogen, then heated gently till reflux at 85°C for 2 h. Extraction between benzene and water phase, drying and evaporating the organic phase provided the crude product 124. The product was collected, washed with MeOH several times, then dissolved in CHCl3 and extracted with water. The organic phase was dried over Na2SO4, filtered and evaporated. The product was recrystallized from CHCl3/ether to yield 205 mg (0.56 mmol, 65%) product 124.

124: C_{26}H_{24}N_2 (MW 364.48)

mp: 122°C

MS ESI: \( m/z = 365.4 \) (100% (M+H)+).

HPLC5 analysis: \( R_v = 12.0 \text{ ml} \)

\(^1\text{H-NMR} \) (250.13 MHz, CDCl3): \( \delta = 7.40-7.37 \) (m, 4H aromatic), 7.26-7.23 (m, 4H aromatic), 7.15-7.13 (m, 4H aromatic), 6.87 (t, \( J = 7.3 \text{ Hz, 2H aromatic} \)), 3.87 (s, 4H 2\times NH2), 2.39 (s, 6H 2\times CH3).

\(^{13}\text{C-NMR} \) (62.9 MHz, CDCl3): \( \delta = 141.5, 136.9, 136.7, 130.2, 130.0, 129.5, 129.1, 128.0, 125.0, 118.3 \) (24C aromatic), 21.1 (2\times CH3).
6. Summary

In the present study, we have investigated the association behavior of a series of guanidinium host derivatives (48, 70, 71, 132, 141, 142), which differ in their steric properties, to tetaethylammonium benzoate (129), tetrabutylammonium phosphates (78, 84) and to tetrabutylammonium phosphinate (128) guests in the non-hydrogen bonding and the highly dielectric solvent acetonitrile at 30°C. The lining of the binding site of the guanidinium hosts was modified synthetically to introduce substituents with different hydrophobicity properties in order to study their effect on complexation with the same guest anion. In comparison to anion 84, the guest anion 78 has two extended substituents adjacent to the binding site, that increase the binding directionality to the guanidinium hosts and may lead to tighter complexes, as the two arms can wrap around the host by projecting above and beneath the main plain of the host and thus to can profit from additional enthalpic interaction securing an optimal orientation.

The guanidinium hosts 48, 70 and the guest anion 78, were newly prepared in this study whereas the other guanidinium hosts were previously prepared in our lab. The phosphate 84 and the phosphinate 128 were prepared with some modifications to literature procedures. The interaction mode between the guanidinium hosts and the different oxoanions follows 1:1 stoichiometry with a planar cation-anion arrangement, assisted by two parallel hydrogen bonds. The modifications in the guanidinium hosts
and the introduced substituents in the phosphate anion guest 78 were carried out without harming the preferential binding mode.

Isothermal titration calorimetry (ITC) was utilized to study the association properties of the host-guest complexation. This technique provides a measure of association strength, stoichiometry of binding, as well as thermodynamic parameters of association from a single experiment. The factorization into the energetic components allowed the deduction of the energetic signature of the complexation, i.e., whether it is an enthalpically or entropically driven process. This outcome is determined by the host and guest character and the environment (i.e., solvent molecules).

The iodide counter anion of the guanidinium hosts 132, 48, and 70 was replaced by different counter anions (Cl\(^-\), Br\(^-\), and PF\(_6\)^-) in order to evaluate its influence on complexation with the benzoate anion 129. The calorimetric determination of the host-guest binding in acetonitrile revealed a surprisingly strong dependence on the counter anion. In general, switching the counter anion from chloride to hexafluorophosphate shifts the experimental host-guest affinity to higher values and to a stronger enthalpic response due to the shift from the more stable hydrogen bonding Cl\(^-\) to the larger and less hydrogen-bonding PF\(_6\)^- in the exchange for benzoate. A positive entropy contribution indicates the release of solvent molecules and counter anions, engaged in the solvation of the binding site, to the bulk.

The trend analysis of the complexation of different guanidinium iodide hosts (141, 142, 132, 70 and 48) with TEA-benzoate 129 unfolded the decisive role of solvation. The less positive \(\Delta S^\circ\) values seen in hosts 70 and 48 with the aromatic substituents indicate the release of fewer solvent molecules on benzoate binding than in the other guanidinium hosts, because these substituents are less solvated than the others. Even though the fluorenyl compound 48 and the tetraphenyl compound 70 showed identical solvation changes (\(\Delta S^\circ = +6.5\) and +6.0 cal K\(^\circ\)M\(^{-1}\), respectively) and thereby enhancement of the coulombic attraction for the anionic guest, however, the exothermicity enhancement was more pronounced for the fluorenyl compound 48 (-6.03 kcal/mol). This result is attributed to the presumed higher rigidity of 48 which opposes the reorientation of the solvent dipoles to cope with the change of the electrostatic field on complex formation.
The complexation of the guanidinium iodide compounds 71, 132, 70, and 48 with the two arms substituted phosphate 78 showed a clear trend of increasing host-guest affinity with host rigidity, yielding the highest $K_{ass}$ for the fluorene-guanidinium host 48 (2.3×10^4 M^{-1}) but the strongest enthalpy response was obtained for the tetraphenyl host 70 (-5.77 kcal/mol). The entropy contribution for host 70 was the lowest in this series (-0.8 cal K^{-1} M^{-1}) indicating the release of fewer solvent molecules and stronger structural definition toward the guest 78.

The conclusion that the anion 78 contributed to the enhancement in binding directionality to the aforementioned hosts was supported by a comparable trend analysis of the same guanidinium hosts with phosphate 84 that lacks the two side arms. This trend actually showed that there was no significant change in the association constants but a clear decrease in the binding enthalpy of about 1-1.5 kcal/mol. (e.g., -5.19 kcal/mol for the tetraphenyl 70) and an increase in the entropy component in the range of 2-4 cal K^{-1} M^{-1} (e.g., +1.3 for 70) due to the release of more solvent molecules than in the previous trend. Therefore, the two arm substituents followed the assumption that this structure would deliberately form tighter complexes and stronger binding enthalpy than the parent phosphate 84. Hence, there was a clear benefit of the structural lay-out in anion 78. This benefit would have never been realized if the analysis were restricted to only the association constant and the free energy of association, because the latter did not significantly change in most of the trend analyses.

An attempt to improve the structural definition by preparing and using phosphinate anion guest 92 was planned. The advantage of 92 over the anion 78 is in the smaller opening angle between the two substituents that would diminish the abundance of solvent molecules in the vicinity of the binding site and form tighter complexes with
the same trend of the guanidinium hosts. Unfortunately, the anion 92 is still in preparation but the complexation with the parent anion 128 that also lacks the two side arms already showed a surprising result with tetraphenyl host 70. Host-guest binding revealed the strongest enthalpy value (\(\Delta H^\circ = -10\) kcal/mole) and the lowest entropy contribution (\(\Delta S^\circ = -15.3\) cal K\(^{-1}\) M\(^{-1}\)) among all titration experiments that were carried out in this study. Therefore, we expect even better complexation energetics if the anion 92 is used.

As a conclusion, the dissection of the free energy \(\Delta G^\circ\) into its enthalpy \(\Delta H^\circ\) and entropy \(\Delta S^\circ\) components was essential for understanding the correlation between structure and thermodynamics of complexation whereas relying exclusively on the association constant and the free energy can easily hamper the interpretation of the binding properties of a given host-guest set.
6. References


[105] The original description refers to a scenario where only two interacting entities perform “rigid-body docking”. Much later the complementarity of functional groups and their preorganization were included aiding substantially in the comprehension of the molecular events especially in solvents of low polarity. Recognition processes in more polar solvents in particular in water so far largely eluded rationalization. To avoid confusion the metaphor is used here in its original sense.
[114] the compound was synthesized in the lab of Prof. F.P. Schmidtchen.
List of compounds

71

132

70

48

142 \( R_1 = R_2 \) OSiBuPh₂

143 \( R_1 = \text{OSiBuPh}_2 \)
\( R_2 = \text{OSiBu(CH}_3)_2 \)

129

78

84

92

128