JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Pseudopeptidic Cages as Receptors for *N*-Protected Dipeptides

Enrico Faggi,[†] Alejandra Moure,[†] Michael Bolte,[‡] Cristian Vicent,[§] Santiago V. Luis,*[,∥] and Ignacio Alfonso*[,†]

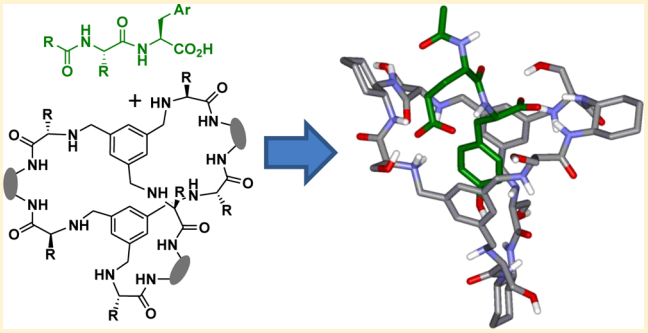
[†]Departamento de Química Biológica y Modelización Molecular, Instituto de Química Avanzada de Cataluña (IQAC-CSIC), Jordi Girona 18-26, E-08034 Barcelona, Spain

[‡]Institut für Anorganische Chemie, J.-W.-Goethe-Universität, Max-von-Laue-Strasse 7, D-60438 Frankfurt/Main, Germany

[§]Serveis Centrals d'Instrumentació Científica and [∥]Departamento de Química Inorgánica y Orgánica, Universitat Jaume I, Avda. Sos Baynat, s/n, E-12071 Castellón, Spain

Supporting Information

ABSTRACT: The molecular recognition of short peptides is a challenge in supramolecular chemistry, and the use of peptide-like cage receptors represents a promising approach. Here we report the synthesis and characterization of a diverse family of pseudopeptidic macrobicycles, as well as their binding abilities toward *N*-protected dipeptides using a combination of different techniques (NMR, ESI-MS, and fluorescence spectroscopy). The cage hosts were assayed for dipeptide binding using competition ESI-MS experiments as high-throughput screening to obtain general trends for the recognition phenomena. Selected hosts were additionally studied by NMR spectroscopy (^{1}H NMR titration and diffusion-ordered spectroscopy experiments) in different solvents. The results unambiguously demonstrated the formation of the [cage·dipeptide] supramolecular complexes and rendered quantitative information about the strength of the interaction (K_{ass}). The structural variables within the pseudopeptidic cage framework that produced a stronger and more selective recognition were thus identified. The cages showed a remarkable selectivity for *N*-protected dipeptides with an aromatic amino acid at the carboxylic terminus, which prompted us to propose a mode of binding based on polar and nonpolar noncovalent interactions. Accordingly, we faced the molecular recognition of a target dipeptide (Ac-EY-OH) mimicking a biologically relevant sequence by NMR and fluorescence spectroscopy in highly competitive media.



INTRODUCTION

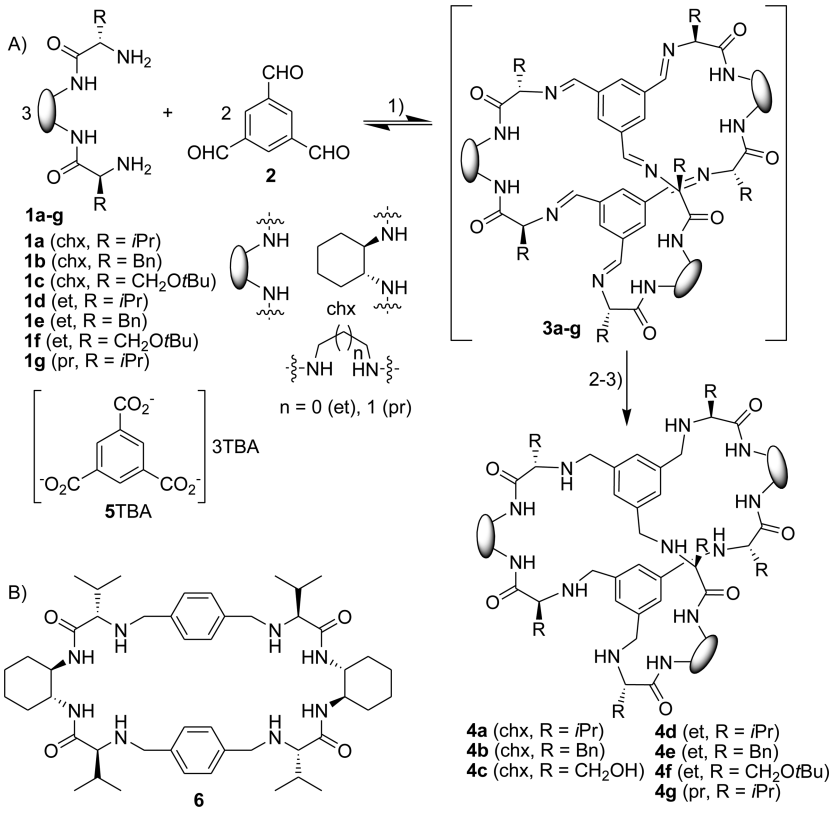
Macrocyclic structures derived from amino acids are attractive molecules with a formidable potential in the molecular recognition field.[1] The different functionalities of the side chains could implement a large molecular diversity and, concomitantly, many potential noncovalent binding sites and different chemical properties (such as charge or polarity). Moreover, the peptide bond also represents a structural and interactional functional group.[2] This combination has allowed the use of pseudopeptidic macrocycles for the recognition of different species.[3] Regarding that, the addition of another dimension to the binding motif could improve the recognition abilities, both by increasing the strength of the interaction and by improving the selectivity of the process.[4] In this context, molecular cages have recently emerged as interesting entities for the binding of specific guests.[5] Accordingly, the macrobicyclic compounds containing amino acid moieties can be considered as pseudopeptidic cages with an inner cavity surrounded by functional groups defining a binding pocket for potential substrates.[6] On the other hand, although some important biological processes are closely related to the noncovalent interaction with specific peptide sequences,[7] the molecular recognition of short peptides is still a challenge in supramolecular chemistry.[8] The external intervention on these interactions could serve for the design of therapeutic or diagnostic tools.[9] The use of synthetic receptors for dipeptides is a fundamental approach to the problem, since the relative simplicity of the systems could allow the full understanding of the rules controlling the process.[10]

During the past decade, we have designed and prepared different pseudopeptidic macrocycles with interesting supramolecular properties.[11] They were shown to be efficient receptors for different species with positive or negative charge.[12] More interestingly, they are hosts for *N*-protected amino acids and dipeptides.[13] However, the strength and selectivity of the binding are moderate, and in most cases, the process has been studied in noncompetitive solvents. More recently, we have increased the complexity of the pseudopeptidic receptors by adding a third arm defining a cage structure.[14] This second generation of pseudopeptidic receptors is able to partially or completely include the guest even in more

Received: March 18, 2014
Published: April 21, 2014

   dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590−4601

Scheme 1. (A) Synthesis of Pseudopeptidic Cages[a] and (B) Chemical Structure of the Monomacrocycle Analogue 6



[a]Conditions: (1) equilibrium at rt either in the absence or in the presence of **5**·TBA; (2) BH_3·py; (3) hydrolysis.

competitive media, leading to interesting functional molecules with potential biological applications.[15] Following our work in this field, here we aimed to prepare larger pseudopeptidic cages able to recognize more elaborate substrates such as dipeptides. We envisioned that the three-dimensional structure of the cages should display more efficient recognition of these challenging substrates through a well-defined network of noncovalent bonds. Besides, the understanding of the intimate interaction would allow the better design of improved binders with potential applications in different fields such as sensing, catalysis, and biomedicine.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Pseudopeptidic Cages. Our proposal for the preparation of the intended cages was based on a multicomponent [3 + 2] reductive amination condensation between a bipodal pseudopeptidic bis(amidoamine) (**1a**–**g**) and a simple aromatic trialdehyde (**2**) (Scheme 1).[16] This approach allows a high degree of modularity in the pseudopeptidic moiety, which will control the size, flexibility, polarity, and binding sites of the final cage. Thus, we faced the preparation of a representative family of receptors (**4a**–**g**) bearing aliphatic, aromatic, or polar side chains and displaying different conformational properties through the use of different aliphatic spacers in the pseudopeptidic bis(amidoamine). The overall process comprises the initial formation of the corresponding hexaimine intermediate (**3**) by a dynamic covalent chemistry methodology[17] followed by the in situ reduction of the imine bonds. Therefore, the success of the reaction highly depends on the ability of the system to self-correct, leading to the formation of the precise [3 + 2] hexaimine cage. Previous studies showed that the process is mainly controlled by the preorganization of the bis(amidoamine) precursor in a U-shaped conformation. Thus, for pseudopeptidic diamines containing a rigid scaffold (such as *trans*-cyclohexane-1,2-diamine, chx) with a *match* combination of the stereocenters ((*R*,*R*)-diamine and (*S*)-amino acids), the formation of the correct imine intermediate is highly favored.[18] For the more flexible aliphatic linear spacers, the formation of the macrocyclic oligoimine is not so favorable, and the use of the suitable anionic template improves the outcome of the reaction.[19] Taking into account these data, we envisioned the preparation of different pseudopeptidic cages with a representative molecular diversity. As expected, the use of the chx spacer induced the formation of the cages with good yields considering that six simultaneous C–N bonds in two steps were formed (Scheme 1, Table 1). We obtained cages **4a**–**c** bearing aliphatic (**4a**, entry 1), aromatic (**4b**, entry 2), or polar (**4c**, entry 3) residues, which allowed us to map a wide range of chemical properties (such as solubility or potential noncovalent interactions). The formation of the hexaimine intermediate in the case of **3a** was additionally studied by NMR and molecular modeling.[16] Most remarkably, the corresponding pseudopeptidic cage derived from serine was obtained in an excellent overall yield (entry 3), calculated from **1c** and including the reductive amination reaction, hydrolysis with the *t*Bu deprotection, and a final reversed-phase chromatographic purification necessary to obtain the pure compound.

When using a simple linear aliphatic spacer in the pseudopeptidic moiety, the reaction also led to the formation of the cage, though in a lower yield and selectivity. Actually, the HPLC trace of the crude reaction showed the presence of other

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590–4601

Table 1. Synthesis of Pseudopeptidic Molecular Cages

entry	1a–g	spacer	R	template	cage (yield, %)[a]
1	**1a**	chx	*i*Pr		**4a** (47)
2	**1b**	chx	Bn		**4b** (30)
3	**1c**	chx	CH_2OtBu[b]		**4c** (59)[b]
4	**1d**	et	*i*Pr		**4d** (15)
5	**1d**	et	*i*Pr	**5a**·TBA	**4d** (24)
6	**1e**	et	Bn	**5a**·TBA	**4e** (24)
7	**1f**	et	CH_2OtBu	**5a**·TBA	**4f** (11)[c]
8	**1g**	pr	*i*Pr	**5a**·TBA	**4g** (13)

[a]Values in parentheses correspond to the overall isolated yields. [b]During the reaction procedure, the *t*Bu protecting group was cleaved. [c]The final hydrolysis was performed at less acidic pH to avoid *t*Bu elimination, and thus, the isolated cage retained the CH_2OtBu side chain.

undesired side products. Interestingly, the addition of the suitable anionic template (**5**·TBA) improved the yield of the reaction (compare entries 4 and 5 in Table 1) and, more importantly, rendered a more selective process toward cage **4d**. The template **5**·TBA promoted the formation of the correct hexaimine intermediate (**3d**) from the dynamic covalent mixture of pseudopeptidic imines. A detailed study of this intermediate using NMR (including diffusion-ordered spectroscopy (DOSY) and rotating frame Overhauser effect spectroscopy (ROESY)), ESI-MS, CD, and molecular modeling allowed us to propose the formation of a supramolecular complex where the tricarboxylate **5** is included within the cavity of the hexaimine cage **3d**, stabilized by six amide–carboxylate H-bonds.[16] The formation of this complex increased the rate of formation and the thermodynamic stability of the cage, finally leading to an improvement of the overall process. The template methodology was also applied to the synthesis of cages bearing different side chains (**4e–f**) or a longer aliphatic spacer (**4g**) with reasonably good overall yields (Table 1).

The final structures of all the cages were confirmed by the full spectroscopic characterization of the compounds (see the Supporting Information). The ^1H and ^{13}C NMR data showed the expected D_3 symmetry of the systems, while the high-resolution MS spectra confirmed the identity of the macrobicyclic architecture. Moreover, we were able to grow crystals of **4a** (chx; R = *i*Pr) as the corresponding perchlorate salt, suitable for X-ray diffraction studies. The macrobicyclic molecule appeared fully protonated, and thus, six perchlorate anions were found per cage unit, accompanied by several water molecules. The cagelike architecture was fully confirmed in the solid state (Figure 1A), although the average D_3 symmetry found in solution by NMR was broken in the crystal structure. The three cyclohexane rings showed a perfect chair conformation with the amide substitutions in a *trans*-diequatorial conformation, setting the amide NH *anti* to the CH of the chiral centers of the cyclohexane. The isopropyl side chains are set in a pseudoequatorial conformation, pointing out of the macrobicyclic cavity. Besides, each hydrogen atom attached to the chiral center of the amino acid moiety is *syn* to the closest amide NH. Overall, all these conformational preferences are in good agreement with the characteristic behavior found in related macrocycles and also with the results obtained by molecular modeling of the imine precursor.

Among the six perchlorate anions present in the asymmetric unit, four of them are in closer contact with the macrobicyclic structure (Figure 1B). These perchlorate anions are partially

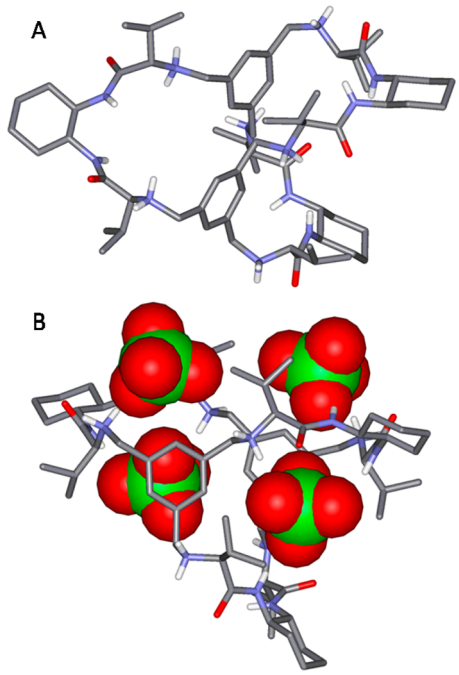


Figure 1. X-ray diffraction analysis of the crystal structure of **4a**·$6HClO_4$: (A) molecular cage structure without perchlorate anions and crystallization water molecules, (B) a different view of the cage with the four perchlorate anions (in a CPK representation) in close contact. The nonpolar H atoms have been omitted for clarity.

inserted into the macrocyclic cavity, connected by a complex network of hydrogen bonds, implicating both the ammonium and amide NH protons. This observation suggested that these cages would be suitable hosts for the binding of anionic entities, where the amino amide moieties would serve as H-bonding donor sites.

Molecular Recognition of *N*-Protected Dipeptides. Although most of the pseudopeptidic cages were purified and isolated as salts of trifluoracetic acid, they were transformed into their corresponding free amino bases by an ion-exchange resin prior to their use for the binding experiments (see the Supporting Information for details).

ESI-MS Experiments. Motivated primarily by the potential binding abilities of the pseudopeptidic cages **4a–4g**, we faced first the use of a convenient experimental approach for high-throughput screening of different guests. Soft ionization mass spectrometric techniques are among the most versatile and widespread for this purpose as exemplified in high-throughput investigations on drug discovery.[20] This is mainly due to the ability of soft ionization MS methods to gently transfer noncovalent complexes from the solution to the gas phase, and excellent reviews in the context of molecular recognition can be found elsewhere.[21] In particular, competition ESI-MS methods have been extensively used to estimate binding affinities (both qualitatively and quantitatively) in protein–ligand complexes that proved to correlate well with the binding constants determined by solution methods.[22] The molecular recognition properties of smaller synthetic receptors can also be addressed by ESI-MS-based methods,[23] for example, in the study of alkylammonium,[24] anion,[25] or amino acid[26] complexation.

In competition ESI-MS experiments, the ESI mass spectrum of a selected host is investigated in the presence of a set of potential guests (or a selected guest in the presence of a group of hosts), so that identification and quantification of different

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590–4601

host−guest complexes are simultaneously measured. In the present work, we used competition ESI-MS for the initial screening of the binding abilities of the synthetic pseudopeptidic cages toward *N*-protected dipeptides. In the first round of experiments, we selected four representative cages to gauge how the structure of each host modulates the binding process. These cages bear (1) the rigid cyclohexane moiety (chx) with aliphatic (**4a**; R = *i*Pr) and polar (**4c**; R = CH_2OH) side chains or (2) a more flexible linear spacer of different lengths (Et/Pr) and from different amino acids (**4e**, ethylene spacer with R = Bn; **4g**, propylene spacer with R = *i*Pr). Besides, to determine the effect of the macrobicyclic structure, an open-chain tripodal derivative was included in the study as a control and revealed a lesser extent of adduct formation in agreement with lower binding affinities (data not shown). As the substrates, we considered three *N*-(benzyloxy)carbonyl (Z) dipeptides derived from alanine and phenylalanine in different sequences, namely, Z-AA-OH, Z-AF-OH, and Z-FF-OH. The competition ESI-MS experiments were carried out as follows: To a stock solution containing an equimolar (5×10^{-5} M) mixture of the **4a**, **4c**, **4e**, and **4g** receptors in CH_3CN/MeOH (2:1) in the presence of 0.5% trifluoroacetic acid were added equimolar amounts of each dipeptide in separated experiments, and the resulting solutions were analyzed by positive ESI-MS. We observed the formation of the supramolecular complexes between every cage and every substrate, all of them showing a 1:1 cage:dipeptide stoichiometry. These noncovalent species were observed in their respective ESI mass spectra mainly as doubly ($[M + 2H]^{2+}$) charged species accompanied by singly charged ($[M + H]^{+}$) species, though to a lesser extent. We took the ratio between the peak intensities of every 1:1 complex (in all charge states) with respect to the peak intensities of the free host (in all charge states) as an indication of the relative binding affinities between the **4a**, **4c**, **4e**, and **4g** hosts and each *N*-protected dipeptide. The obtained results are plotted in Figure 2.

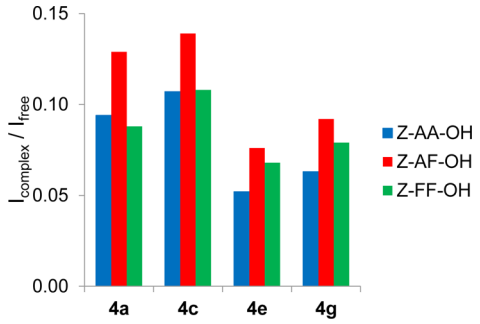


Figure 2. Relative intensities of the complexed/free receptor observed by ESI-MS competition experiments.

We can extract very important information from the comparison of the obtained data (Figure 2). First, the results suggested that the best receptor should be **4c**, since it showed invariably the highest proportion of bound species. We hypothesized that this cage could establish a higher number of H-bond interactions, implicating the OH groups of the Ser side chains. On the other hand, cage **4e** was shown to be the less efficient host, probably due to a more flexible structure (from the ethylene spacer) and to the presence of benzyl side chains, which disfavor the participation of the amino amide moieties in noncovalent interactions.[27] From the results for **4a**/**4g** cages, we concluded that the rigidity imposed by the cyclohexane moiety is beneficial for the molecular recognition of the dipeptides. Overall, the full analysis of the ESI-MS data rendered several general trends: (1) the rigidity of the cyclohexane moiety within the cage architecture improves the binding and (2) the side chains of the receptors are not innocent in the recognition phenomena: Ser and Val improve the interaction, while Phe reduces it. Interestingly, the combination of the cyclohexane spacer with the Val side chain had proved to be the best receptor in the case of the macrocyclic family (compound **6** in Scheme 1B).[13b]

Focusing on the most efficient receptor **4c**, we faced a second round of experiments directed to find out the binding abilities of **4c** toward a wider scope of dipeptide sequences. Accordingly, additional systematic variations in the sequence of the dipeptide substrates were studied (entries 8 and 10−15 in Table 3). The complex formation ability of **4c** with the guest ions was investigated by competition ESI-MS on equimolecular solutions of **4c** and a mixture of all guests. Incidentally, two pairs of guests were isobaric, namely, Z-AF-OH/Z-FA-OH and Z-AE-OH/Ac-EF-OH, so two separate experiments containing the nonoverlapped guests were conducted. Figure 3 illustrates the competitive ESI mass spectrum of an equimolecular solution of host **4c** and a mixture of Z-AA-OH, Ac-EY-OH, Ac-EF-OH, Z-FA-OH, Z-AY-OH, Z-AW-OH, and Z-FF-OH.

Each *N*-protected dipeptide formed noncovalent complexes with the **4c** host with a 1:1 stoichiometry as judged by ESI-MS. We made the assumption that the ion efficiencies for the 1:1 adducts and the free hosts are similar. This essentially means that the relative intensities in the ESI mass spectrum directly reflect the relative concentrations in the injected solution. We are aware that this only holds when the presence of the substrate does not affect the ionization efficiency in the 1:1 complex, which is dominated by the contribution of the receptor. Closely related competition ESI-MS experiments were described by Jorgensen and Heck[22a] for a series of vancomycin group antibiotics and bacterial cell wall peptide analogues, for which association constants could be quantitatively extracted directly from equimolar solutions of a given host and different guests. Using this approach (see details in Table S3 in the Supporting Information), we have estimated the association binding constants of the **4c** host and the series of *N*-protected dipeptides. Estimated K_{ass} values derived from ESI-MS competition experiments are collected in Table 2 for receptor **4c** in CH_3CN/CH_3OH (2:1) mixtures. In general, we observed a good correlation between the K_{ass} values derived from ESI-MS and those values determined by strictly solution-based methods such as NMR (see below). Notice that both techniques (i) gave comparable absolute K_{ass} values, (ii) suggest moderate selectivity for *N*-Ac dipeptides, and (iii) clearly identify the Ac-EF-OH and Ac-EY-OH sequences as the most specific for the **4c** host.[28]

^{1}H NMR and DOSY Experiments. We also aimed to tackle the interaction between the pseudopeptidic cages and the dipeptides by NMR, which provides more precise information about the binding process. Initially, we decided to study the complex between **4a** and Z-AF-OH, since the structurally related system was deeply characterized in the case of the macrocyclic hosts (such as compound **6** depicted in Scheme 1B, which interacts with Z-AF-OH dipeptide). Thus, we acquired the ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of a 1:1 host−guest mixture, and we compared it with those of the partners alone (Figure 4). Several signals changed their

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590−4601

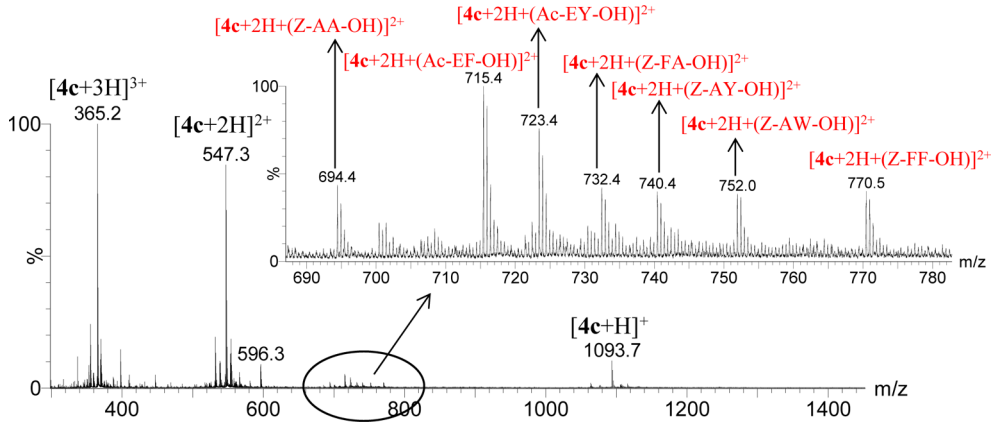


Figure 3. Positive ESI mass spectrum of equimolar (5×10^{-5} M) solutions of the host **4c** and selected *N*-protected dipeptides using 2:1 CH_3CN/CH_3OH mixtures.

Table 2. Binding Constants (K_{ass}, M^{-1}) Obtained by ^1H NMR Titration (500 MHz, 298 K) and ESI-MS Competition Experiments

entry	host	substrate	solvent	$K_{ass}{}^a$ (M^{-1})	$K_{ass}{}^b$ (M^{-1})	ΔG^c (kJ·mol^{-1})
1	**6**	Z-AF-OH	$CDCl_3/CD_3OH$ (100:1)	161[d]	*e*	−12.6
2	**4a**	Z-AF-OH	$CDCl_3/CD_3OH$ (100:1)	955	*e*	−17.0
3	**4a**	Z-AF-OH	CD_3CN/CD_3OH (100:15)	190	*e*	−13.0
4	**4a**	Z-FF-OH	CD_3CN/CD_3OH (100:15)	166	*e*	−12.7
5	**4a**	Z-AA-OH	CD_3CN/CD_3OH (100:15)	112	*e*	−11.7
6	**4a**	Z-FA-OH	CD_3CN/CD_3OH (100:15)	44	*e*	−9.4
7	**4a**	Z-AF-OH	CD_3CN/CD_3OH (2:1)	123	*e*	−11.9
8	**4c**	Z-AF-OH	CD_3CN/CD_3OH (2:1)	209	220	−13.2
9	**4e**	Z-AF-OH	CD_3CN/CD_3OH (2:1)	66	*e*	−10.4
10	**4c**	Z-FF-OH	CD_3CN/CD_3OH (2:1)	224	240	−13.4
11	**4c**	Z-AA-OH	CD_3CN/CD_3OH (2:1)	162	206	−12.6
12	**4c**	Z-FA-OH	CD_3CN/CD_3OH (2:1)	141	214	−12.3
13	**4c**	Z-AY-OH	CD_3CN/CD_3OH (2:1)	178	215	−12.8
14	**4c**	Z-AW-OH	CD_3CN/CD_3OH (2:1)	166	212	−12.7
15	**4c**	Z-AE-OH	CD_3CN/CD_3OH (2:1)	229	234	−13.5
16	**4a**	Ac-EF-OH	CD_3CN/CD_3OH (2:1)	55	*e*	−9.9
17	**4c**	Ac-EF-OH	CD_3CN/CD_3OH (2:1)	324	501	−14.3
18	**4a**	Ac-EY-OH	CD_3CN/CD_3OH (2:1)	69	*e*	−10.5
19	**4c**	Ac-EY-OH	CD_3CN/CD_3OH (2:1)	417	414	−15.0
20	**4e**	Ac-EY-OH	CD_3CN/CD_3OH (2:1)	36	*e*	−8.9

[a]Obtained by ^1H NMR titration, estimated error ≤15% (see the Supporting Information for details). [b]Obtained by ESI-MS, estimated error ≤7% (see the Supporting Information for details). [c]Calculated from the NMR-based binding constant. [d]Taken from ref 13b. [e]Not measured by ESI-MS.

chemical shifts, supporting the interaction between them. All the amide and carbamate NH signals moved downfield, supporting the establishment of strong H-bonding interactions. Besides, the proton attached to the α carbon of the Phe residue of the dipeptide (Fα in Figure 4) moved upfield, while the benzylic and CαH signals of the cage (d and c protons, respectively) moved downfield. These observations are consistent with a proton transfer from the carboxylic acid of the substrate to the secondary amine of the receptor, leading to the formation of an ion pair. An interesting behavior was also observed for the aromatic signals of the Phe side chain of the dipeptide. The protons in the *meta* and *para* positions (F3 and F4 in Figure 4) moved upfield, while the *ortho* hydrogens (F2) practically did not alter. Besides, these signals appeared as a broad band within the complex, which suggests a restricted motion of the Phe side chain. On the contrary, the aromatic signals sourcing from the Z protecting group were less affected by the interaction. These observations highlight the importance of the aromatic residue in the carboxylic terminus and suggest a close interaction with the aromatic rings of the receptor.[29]

The self-diffusion rate obtained by DOSY is also a useful parameter for the characterization of supramolecular species in solution.[30] The formation of stable intermolecular complexes is accompanied by an increase of the apparent size of the molecules with the concomitant reduction of the self-diffusion rate (D). In the case of macrocyclic receptor **6** with Z-AF-OH, we also observed a change of the diffusion properties of both the host and guest upon binding, which could be used to confirm the formation of the corresponding complex (Table 3, entries 1−3).[13b] The DOSY spectra of the macrobicyclic receptor **4a** showed a self-diffusion rate very similar to that found for the [**6** + Z-AF-OH] complex, in good agreement with their similar molecular sizes (entry 4 in Table 3). Moreover, in the DOSY spectrum of a sample with an equimolecular mixture of **4a** and Z-AF-OH, all the signals diffused with the same rate, demonstrating the presence of a unique supramolecular species

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590−4601

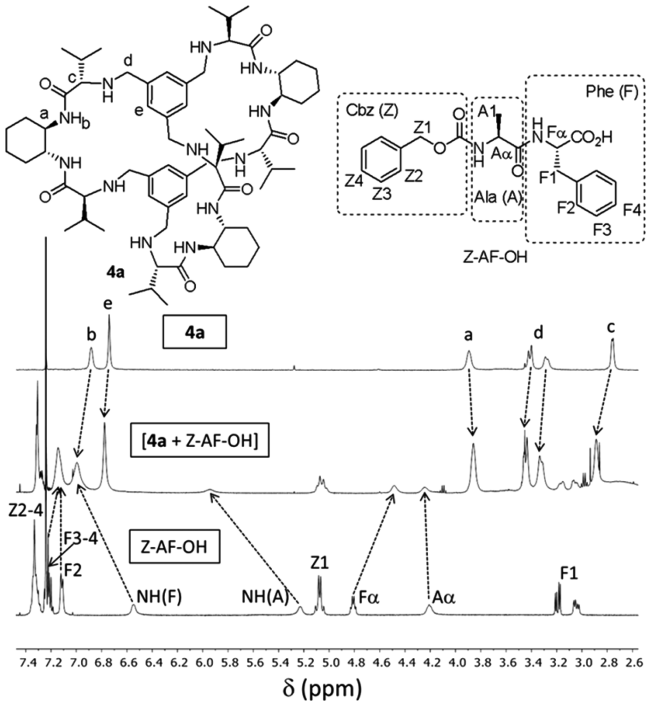


Figure 4. ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectra of **4a**, Z-AF-OH, and an equimolecular mixture of both (with arbitrary numbering/lettering of the signals).

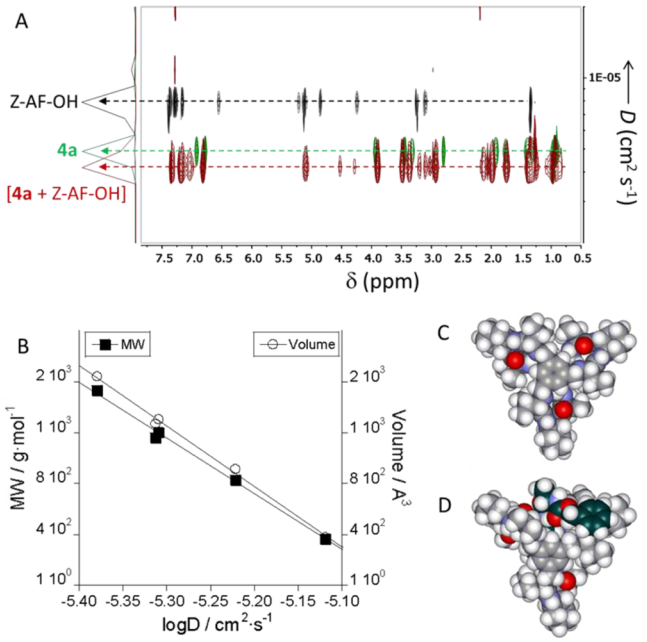


Figure 5. (A) Superposed DOSY (500 MHz, $CDCl_3$, 298 K) spectra for **4a**, Z-AF-OH, and an equimolecular mixture of both. (B) Molecular size (molar mass or molecular volume) versus the self-diffusion rate. CPK models for (C) cage **4a** and (D) the complex of **4a** with Z-AF-OH. For clarity, the carbon atoms of the dipeptide substrate are represented in dark green.

in solution (entry 5 in Table 3, Figure 5A). Thus, also in this case, both the cage and the substrate increased their apparent molecular size in solution upon binding (Table 3 and Figure 5A). Very remarkably, the data displayed in Table 3 indicate the internal consistency of the DOSY experiments performed with these systems, where a good correlation ($R^2 > 0.99$) between the molecular sizes and the DOSY-measured self-diffusion rates was obtained (Figure 5B).

NMR Titration Experiments. The quantitative measurement of the interaction between **4a** and Z-AF-OH was also performed by ^{1}H NMR titration experiments in the same solvent as that used for the structurally related monomacrocycle **6**. The simultaneous nonlinear fitting of the variations of different NMR signals (both from the host and from the guest) rendered an association constant of 955 M^{-1} (Table 2, entry 2), which is nearly 1 order of magnitude higher than that found for the monomacrocyclic derivative (entry 1 in Table 2), thus supporting the beneficial effect of the cage architecture for the binding of dipeptides.

Several additional cage−*N*-protected dipeptide host−guest interactions were also analyzed by ^{1}H NMR titration procedures. The somehow stronger binding allowed us to study the interaction in more polar and competitive solvent mixtures that would avoid the complication of the analysis of the data due to dipeptide self-aggregation.[13b] The value of the binding constant between **4a** and Z-AF-OH was markedly reduced in the CD_3CN/CD_3OH (100:15) mixture (entry 3, Table 2), reflecting the importance of the intermolecular H-bonding interactions for the stability of the supramolecular complex. We studied the substrate sequence selectivity of **4a** by measuring the corresponding *N*-protected dipeptides with Ala and Phe residues (entries 3−5), and we obtained the same stability order as that observed by ESI-MS (Z-AF-OH > Z-FF-OH > Z-AA-OH). Even more strikingly, the scrambled sequence Z-FA-OH (entry 6) showed a much weaker binding to **4a** ($\Delta\Delta G$ = 3.6 kJ mol^{-1}), meaning that the presence of an aromatic residue at the C-terminus modulates the selectivity toward dipeptides. To explain these results, we hypothesized the inclusion of this aromatic residue within the inner cavity of the host. Thus, a tentative proposal for the main interactions between the pseudopeptidic cage and the dipeptide is shown in Figure 6, where the combined action of electrostatic, H-bonding, and hydrophobic contacts would explain the observed selectivity.

Table 3. Self-Diffusion Rates Measured by DOSY Experiments (500 MHz, $CDCl_3$, 298 K) with Different Pseudopeptide−Dipeptide Host−Guest Systems

entry	sample	molar mass (g·mol^{-1})	volume[a] (Å^3)	D (10^{-6} cm^2·s^{-1})
1	Z-AF-OH	370.4	379.6	7.6 ± 0.2[b]
2	**6**	829.2	914.1	6.0 ± 0.2[b]
3	[**6** + Z-AF-OH]	1202.6	1305.3	4.9 ± 0.3[b]
4	**4a**	1165.7	1273.8	4.86 ± 0.15
5	[**4a** + Z-AF-OH]	1536.1	1645.8	4.17 ± 0.20

[a]CPK volume calculated with Spartan '06. [b]Taken from ref 13b.

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590−4601

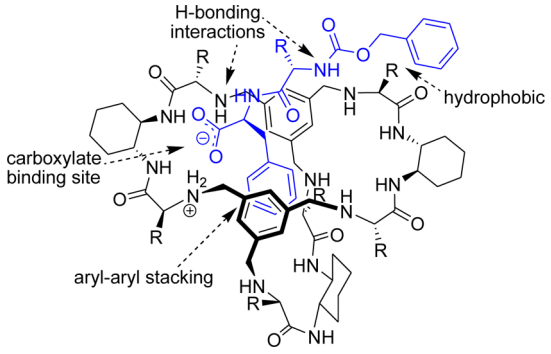


Figure 6. Schematic representation of the tentative proposed model for the interaction between a pseudopeptidic cage and an *N*-Z-protected dipeptide bearing an aromatic residue at the carboxylic terminus.

For the comparison between different receptors, we had to use a slightly more polar mixture of solvents (2:1 CD_3CN/CD_3OH) due to solubility issues of **4c**. In this case, the comparison of the binding constants between three different cages and the Z-AF-OH dipeptide (entries 7–9 in Table 2) rendered the stability order **4c** > **4a** > **4e**. These results are fully consistent with the data obtained by ESI-MS competition experiments and underscore the importance of the overall flexibility and of the nature of the side chains in the molecular recognition properties of the pseudopeptidic cages. The combination of a rigid cage structure with polar side chains produced a more efficient host.

Focusing on the best receptor **4c**, several systematic variations in the sequence of the dipeptide substrates were studied (entries 8 and 10–15). Also for this host, the presence of an aromatic residue at the C-terminus favored the interaction (for instance, compare entries 8 and 12 for scrambled sequences), although the selectivity was lower than that with **4a** (Val derivative). This effect could be due to the coexistence of structurally different supramolecular complexes in solution, where the aromatic ring of the Z protecting group would displace the aromatic side chain of the amino acid from the host cavity. This possibility could be present for all the hosts, but for **4a**, an additional hydrophobic interaction between the Z group and the *i*Pr side chain (Figure 6) would stabilize the outer disposition of the *N*-protecting group. In the case of **4c**, the Ser side chains form a highly polar surrounding periphery, favoring the competition between the two aromatic rings (side chain and Z) for the host cavity. Since the Z group is present in all the dipeptides, the observed overall effect is a decreased selectivity in **4c**. Little difference was observed by comparing the nature of the aromatic amino acid residue at the C-terminus (entries 13 and 14), which also points to the possible competition of the aromatic group of the carbamate. Interestingly, the introduction of an additional carboxylic acid at the C-terminus (entry 15) had a stabilizing effect comparable to that of an aromatic ring, suggesting the possibility of implementing both residues in a cooperative fashion. Following this rationale, we envisioned that a dipeptide bearing an aromatic residue at the C-terminus and a polar carboxylic residue at the N-terminus should display much stronger binding with **4c** than with **4a**. Besides, to improve the selectivity, the *N*-protecting group was replaced by acetyl, which lacks the aromatic ring and is also a more realistic model of a peptide bond. Very gratifyingly, the binding constant of Ac-EF-OH was much higher with **4c** than with **4a** (entries 16 and 17 in Table 2; $\Delta\Delta G$ = 4.4 kJ mol^{-1}), strongly supporting our hypothesis for the explanation of the observed trends.

Taking into account the binding behavior with the studied cages, we decided to investigate the replacement of phenylalanine by tyrosine in the C-terminus of the dipeptide. The obtained sequence (Ac-EY-OH) bears an electron-rich aromatic residue with additional H-bonding properties and would allow the binding phenomena to be monitored with a different technique, taking advantage of the fluorescence emission of the phenol group (see the next section). The ^{1}H NMR titration experiments with the three cages and the Ac-EY-OH dipeptide showed a much stronger complexation with **4c** (see entries 18–20 in Table 2; $\Delta\Delta G$ = 4.5–6.1 kJ mol^{-1}), which again reinforces our general model for the interaction. Besides, by comparing the binding of **4c** with both the Ac-EF-OH and Ac-EY-OH dipeptides (entries 17 and 19, respectively), we concluded that the phenolic OH group must be implicated in an additional stabilizing ($\Delta\Delta G$ = 0.7 kJ mol^{-1}) interaction with the host. Interestingly, in spite of the much weaker interaction of these dipeptides with **4a**, a very similar difference was obtained due to the presence of the phenolic OH (entries 16 and 18; $\Delta\Delta G$ = 0.6 kJ mol^{-1}).

Fluorescence Spectroscopy Experiments. As anticipated and due to the presence of the Tyr residue, the Ac-EY-OH dipeptide shows a strong fluorescence emission band with a maximum at 304 nm upon excitation at 275 nm (1.5×10^{-4} M in a 2:1 CH_3CN/CH_3OH mixture). The addition of increasing amounts of the host **4c** produced a quenching of the emission (Figure 7). The decrease of the emission intensity at 304 nm

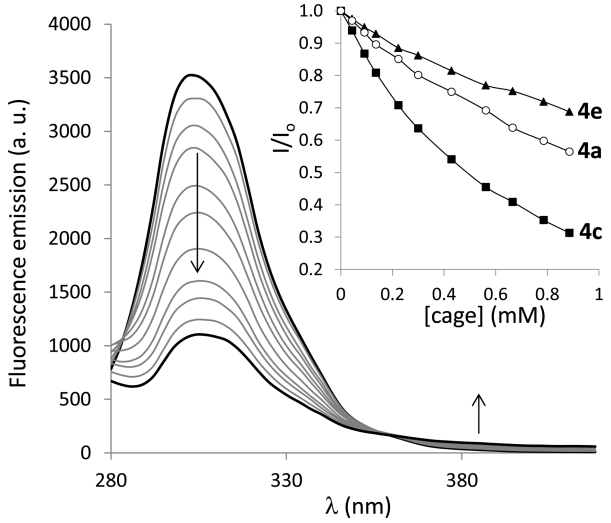


Figure 7. Emission fluorescence spectra of Ac-EY-OH (1.5×10^{-4} M in 2:1 CH_3CN/CH_3OH) at increasing concentrations ((0–9) $\times 10^{-4}$ M) of the receptor **4c**. Inset: relative fluorescence quenching (I/I_o) at 304 nm versus the receptor concentration (mM) for **4a**, **4c**, and **4e**.

was accompanied by a slight increase of the emission at ~400 nm, with an isosbestic point at 360 nm that can be taken as proof of the formation of a new species in solution. The analysis of the quenching process with the Stern–Volmer equation (see the Supporting Information) indicated that both dynamic and static quenching mechanisms are present in the system. This fact additionally supports the formation of a supramolecular complex between **4c** and Ac-EY-OH in the ground state (static quenching). Moreover, this static quenching suggests the participation of the phenol residue of

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590–4601

the Tyr moiety in the binding with **4c**. The binding abilities of the different cages were also studied by fluorescence spectroscopy. The plot of the decrease of the fluorescence intensity at 304 nm versus the cage concentration for the three hosts **4a**, **4c**, and **4e** (Figure 7, inset) confirmed the data obtained by NMR titrations, showing the stability order **4c** > **4a** > **4e**.

*Model for the [**4c** + Ac-EY-OH] Supramolecular Complex.* Taking into account all the data obtained in our study, we also aimed to propose a reasonable binding mode for the interaction between the receptor **4c** and the dipeptide Ac-EY-OH. We selected this supramolecular complex for several reasons. This was the most stable species analyzed by NMR titration (and ESI-MS) in the most competitive solvents considered in our study. Moreover, this motif can represent a model for one of the target sequences for kinases, with important biological implications.[31] Several general considerations were taken into account for building such a model. First, the main interactions are due to the electrostatic H-bonds between the carboxylates of the dipeptide and the amino amide moieties (as generally observed by the NMR induced chemical shifts in the titration experiments). These anion binding motives resemble the contacts observed between the cage and the perchlorate anions in the crystal structure of the **4a**·6$HClO_4$ salt (Figure 1B). In the case of **4c**, also the OH from the side chains could form H-bonds with the corresponding anions, as suggested from the stronger interaction observed with this Ser derivative. Moreover, our binding studies clearly showed a remarkable preference for aromatic residues at the C-terminus, implying the participation of the aromatic ring in the binding phenomena. This is also supported by the quenching of the tyrosine side chain fluorescence upon the addition of the cages (Figure 7). A proposal for the [**4c** + Ac-EY-OH] supramolecular complex, obtained by molecular mechanics calculations, is shown in Figure 8. The carboxylate at the C-terminus and that of the glutamic side chain set several H-bonds with two of the amino amide moieties of the receptor (with the amino groups partially protonated in the model). Additional H-bonds with the corresponding OH of the side chains are possible. The tyrosine side chain of the dipeptide is included within the cage cavity, showing aryl stacking interactions with the aromatic rings of the receptor. Moreover, the phenolic OH is implicated in additional H-bonding interactions (both as a donor and as an acceptor, Figure 8) with the third amino amide moiety of the cage. This specific interaction could explain the observed quenching of the fluorescence emission of the substrate, since this H-bond pattern would increase the electronic density on the aryl ring (by incipient abstraction of the OH proton). Experimental support for this binding mode was also obtained by ROESY experiments (500 MHz, D_2O, 288 K) of a 6 mM sample of **4c** in the presence of a 5-fold excess of Ac-EY-OH. A relatively weak but measurable NOE was observed between the aromatic proton of the cage and the proton *ortho* to the phenol hydroxyl of the tyrosine moiety, supporting the existence of the proposed complex (Figure 8B). Although we cannot discard the participation of other binding modes in solution, the proposed complex fully agrees with all the experimental data and draws a plausible picture of the recognition process.

CONCLUSIONS

The efficient synthesis of large pseudopeptidic cages has been accomplished by a [3 + 2] multicomponent reductive amination reaction. The synthetic strategy is very robust and

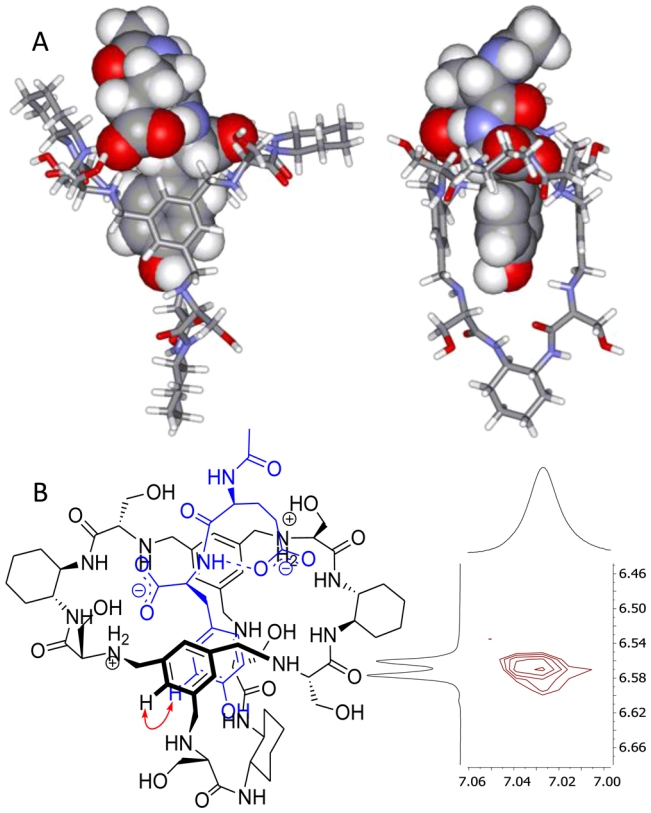


Figure 8. (A) Two views of the proposed model for the structure of the supramolecular complex formed by the receptor **4c** (stick) and the dipeptide Ac-EY-OH (CPK). (B) Schematic representation of the complex with the corresponding substrate in blue and section of the 2D ROESY spectrum showing the intermolecular contacts as a red double-headed arrow in the structure.

allows the implementation of large molecular diversity regarding the size, flexibility, polarity, and potential interaction sites on the molecular cages. The molecular recognition of the obtained cages toward *N*-protected dipeptides has been deeply studied using a combination of different techniques. The competition ESI-MS experiments allowed us to screen the binding abilities for the determination of the most efficient receptors. Moreover, for the best host, we were able to obtain the quantitative assessment of the binding interactions with a family of dipeptides by mass spectrometry. The ESI-MS-obtained binding constants are in very good agreement with those obtained by NMR. In general, ESI mass spectrometric techniques are very restrictive regarding the systems for which absolute binding constants can be estimated directly on the basis of the relative intensities of the complex and the free receptor. In fact, it is important to emphasize that noncovalent binding based on electrostatic interactions is reinforced in the absence of solvent whereas hydrophobic interactions become less important. In this context, it is crucial to validate whether the observed complexes truly reflect the specific interactions that are present in solution. If intensity ratios truly reflect the ratio of the concentrations in solution, as demonstrated in the present work, ESI-MS can be regarded as a powerful alternative method for high-throughput screening of recognition processes.

For selected host–guests pairs, a detailed study of the supramolecular complexes in solution has been performed by NMR, including ^1H NMR titrations and DOSY and ROESY studies. Thus, the quantitative binding constants were

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590–4601

measured, and a reliable picture of the mode of binding can be proposed. More interestingly, the wide structural diversity accessible for the host molecules permitted the study of the molecular recognition process in media with different polarities, ranging from nonpolar organic solvents to very competitive media. We extracted several general trends in the structural parameters of the receptors: the rigid cagelike structure leads to more efficient hosts, and the side chains can participate in the binding. Overall, the data rendered the cage containing the cyclohexane spacer and the serine amino acid (**4c**) as the most efficient receptor. With respect to the dipeptide substrate structure, we observed some selectivity for those bearing an aromatic residue at the C-terminus and an acetyl group at the N-terminus. This trend allowed us to study a biologically interesting sequence (EY) as a model substrate by NMR and fluorescence spectroscopy. Both techniques showed excellent agreement regarding the host efficiencies. The strongest supramolecular complex was qualitatively characterized in water by ROESY experiments. These results seemed promising for the future applications of related systems in biological processes since the EY dipeptide is a target sequence for kinases, which are phosphorylating enzymes with key implications for cell regulation and metabolism. However, in this regard, more efficient receptors able to operate in water at neutral pH must be designed and developed. Studies in these directions are under way and will hopefully render optimized artificial receptors with true biological applications. Moreover, our findings have a fundamental relevance since we were able to build an efficient and selective binding motif from simple and readily accessible pseudopeptidic cages, where further structural modifications can be carried out.

EXPERIMENTAL SECTION

General Experimental Methods. Reagents and solvents were purchased from commercial suppliers and were used without further purification. Compounds **1a**−**g** were prepared as previously described.[16,18,19] Preparative reversed-phase purifications were performed on an MPLC instrument with a C18 column as the stationary phase and CH_3CN/water with 0.1% TFA as the mobile phase. The cages obtained as TFA salts were transformed into the free-base amines using an ion-exchange resin prior to the binding studies. Analytical RP-HPLC was performed using a C8 (15 × 0.46 cm, 5 μm) column. CH_3CN/H_2O mixtures containing 0.1% TFA at 1 mL/min were used as the mobile phase, and the monitoring wavelength was set at 220 and 254 nm. The NMR spectra were performed on spectrometers operating at 500 or 400 MHz for ^{1}H NMR and 125 or 100 MHz for ^{13}C NMR. The chemical shifts are reported in parts per million using tetramethylsilane (TMS) as a reference. High-resolution mass spectrometry (HRMS) was performed on a UPLC system coupled with an orthogonal acceleration time-of-flight (oa-TOF) analyzer equipped with an electrospray ionization source.

General Procedure for the Macrocyclization Reaction with the Preorganized Precursors (Synthesis of 4a−c). A solution of 33.5 mg of benzene-1,3,5-tricarbaldehyde (0.21 mmol) in 15 mL of CH_3OH was added to a solution of **1a** (96.8 mg, 0.31 mmol) in 15 mL of CH_3OH. The mixture was stirred overnight. Then 390 μL of an 8 M solution of BH_3·py complex (3.1 mmol) was carefully added, and the mixture was allowed to react for 16 h before being hydrolyzed (concd HCl, to acidity) and evaporated to dryness. The residue obtained was dissolved in water and basified with 1 N NaOH, and the product was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and the solvents were evaporated in vacuum. The product was purified using reversed-phase flash chromatography, yielding **4a** as a trifluoroacetate salt. Yield: 89 mg, 47%; RP-HPLC (gradient from 10% to 50% CH_3CN). ^{1}H NMR (400 MHz, MeOD-d_4): δ = 6.75 (s, 6H, CH_{Ar}), 3.81 (m, 6H, CH), 3.47 (d, J = 12.6 Hz, 6H, CH_2NH), 3.16 (d, J = 12.6 Hz, 6H, CH_2NH), 2.86 (d, J = 6.8 Hz, 6H, CH), 2.06 (d, J = 9.5 Hz, 6H, CH_2), 1.85 (m, 6H, CH), 1.78 (d, J = 6.9 Hz, 6H, CH_2), 1.45−1.25 (m, 12H, CH_2), 0.99 (t, J = 7.3 Hz, 36H, CH_3). ^{13}C NMR (100 MHz, MeOD-d_4): δ = 176.6 (CO), 140.8 (C_{Ar}), 127.8 (CH_{Ar}), 96.4 (CH), 69.8 (CH), 53.7 (CH_2NH), 34.1 (CH), 33.0 (CH_2), 25.8 (CH_2), 19.9 (CH_3), 19.7 (CH_3). HRMS (m/z) (M + 1): calcd for $C_{66}H_{108}N_{12}O_6$, 1165.8594; found, 1165.8665.

4b was isolated as a trifluoroacetate salt. Yield: 48 mg, 30%; RP-HPLC (gradient from 10% to 60% CH_3CN). ^{1}H NMR (500 MHz, $CDCl_3$: MeOD-d_4): δ = 7.23−7.00 (30H, CH_{Ar}), 6.45 (6H, CH_{Ar}), 3.50 (m, 6H, CH), 3.28 (d, J = 12.2 Hz, 6H, CH_2NH), 3.12 (d, J = 12.2 Hz, 6H, CH_2NH), 3.25 (m, 6H, CH), 2.86 (dd, J = 13.1 and 5.5 Hz, 6H, CH_2), 2.75 (dd, J = 13.1 and 9.4 Hz, 6H, CH_2), 1.51 (d, J = 7.9 Hz, 6H, CH_2), 1.45 (d, J = 12.6 Hz, 6H, CH_2), 1.07 (t, J = 9.6 Hz, 6H, CH_2), 0.70 (m, 6H, CH_2). ^{13}C NMR (100 MHz, MeOD-d_4): δ = 174.1 (CO), 138.2 ($6C_{Ar}$), 137.2 ($6C_{Ar}$), 129.3 ($12CH_{Ar}$), 128.5 ($12CH_{Ar}$), 127.9 ($6CH_{Ar}$), 126.8 ($6CH_{Ar}$), 63.1 (CH), 52.2 (CH), 51.5 (CH_2NH), 39.5 (CH_2Ar), 32.0 (CH_2), 24.5 (CH_2). HRMS (m/z) (M + 1): calcd for $C_{90}H_{108}N_{12}O_6$, 1453.8594; found, 1453.8539.

4c was isolated as a trifluoroacetate salt. Yield: 86.7 mg, 59%; RP-HPLC (gradient from 10% to 50% CH_3CN). ^{1}H NMR (500 MHz, MeOD-d_4): δ = 7.04 (6H, CH_{Ar}), 3.88 (dd, J = 10.8 and 5.2 Hz, 6H, CH_2OH), 3.82 (dd, J = 10.8 and 5.2 Hz, 6H, CH_2OH), 3.79 (m, 6H, CH), 3.73 (d, J = 13.1 Hz, 6H, CH_2NH), 3.53 (d, J = 13.1 Hz, 6H, CH_2NH), 3.23 (t, J = 5.2 Hz, 6H, CH), 2.16 (d, J = 12.2 Hz, 6H, CH_2), 1.80 (d, J = 7.3 Hz, 6H, CH_2), 1.48−1.27 (m, 12H, CH_2). ^{13}C NMR (100 MHz, MeOD-d_4): δ = 174.9 (CO), 140.2 (C_{Ar}), 125.4 (CH_{Ar}), 65.0 (CH), 63.1 (CH_2OH), 54.2 (CH), 53.1 (CH), 49.8 (CH_2NH), 33.3 (CH_2), 25.9 (CH_2). HRMS (m/z) (M + 1): calcd for $C_{54}H_{84}N_{12}O_{12}$, 1093.6406; found, 1093.6425.

General Procedure for the Anion-Templated Macrocyclization Reaction (Synthesis of 4d−h). Pseudopeptidic bis(amidoamine) **1d** (68 mg, 0.26 mmol) was dissolved in 8 mL of $CHCl_3/CH_3OH$ (9:1) inside a flask under nitrogen. Benzene-1,3,5-tricarboxylate **5**·TBA (82 mg, 0.09 mmol) was dissolved in 8 mL of $CHCl_3/CH_3OH$ (9:1) and added to the solution of **1d**. Benzene-1,3,5-tricarbaldehyde (**2**) (29 mg, 0.17 mol) was dissolved in 8 mL of $CHCl_3/CH_3OH$ (9:1) and added to the solution mixture of **1d** + **5**·TBA. The mixture was stirred overnight, then a large excess of BH_3·py complex (330 μL of a 8 M solution of BH_3·py, 2.6 mmol) was carefully added, and the mixture was allowed to react for 24 h. before being hydrolyzed (concd HCl, to acidity) and evaporated to dryness. The residue obtained was dissolved in water and basified with 1 N NaOH. The product was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), and the solvents were evaporated in vacuum. The product was purified using reversed-phase flash chromatography, yielding **4d** as a trifluoroacetate salt. Yield: 12 mg, 24%; RP-HPLC (gradient from 5% to 30% CH_3CN). ^{1}H NMR (400 MHz, MeOD-d_4): δ = 7.64 (s, 6H, CH_{Ar}), 4.29 (d, J = 12.8 Hz, 6H, CH_2NH), 4.14 (d, J = 12.8 Hz, 6H, CH_2NH), 3.64 (d, J = 5.9 Hz, 6H, CH), 2.98 (m, 6H, CH_2), 2.84 (m, 6H, CH_2), 2.15 (m, 6H, CH), 0.97 (d, J = 6.8 Hz, 18H, CH_3), 0.89 (d, J = 6.8 Hz, 18H, CH_3). ^{13}C NMR (100 MHz, MeOD-d_4): δ = 169.2 (CO), 163.0 (c, J = 34.9 Hz, CO_{TFA}), 136.4 (CH_{Ar}), 133.9 (C_{Ar}), 67.7 (CH), 51.9 (CH_2NH), 40.0 (CH_2), 31.5 (CH), 19.3 (CH_3), 18.4 (CH_3). HRMS (m/z) (M + 1): calcd for $C_{54}H_{90}N_{12}O_6$,1003.7188; found, 1003.7195.

4e was isolated as a trifluoroacetate salt. Yield: 24 mg, 24%. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 7.69 (s, 6H, CH_{Ar}), 7.37−7.22 (18H, CH_{Ar}), 7.15 (12H, CH_{Ar}), 4.34 (d, J = 12.9 Hz, 6H, CH_2NH), 4.20 (d, J = 12.9 Hz, 6H, CH_2NH), 4.08 (dd, J = 10.2 and 5.1 Hz, 6H, CH), 3.24 (dd, J = 12.9 and 5.1 Hz, 6H, CH_2), 3.02 (dd, J = 12.9 and 10.2 Hz,, 6H, CH_2), 2.61 (m, 6H, CH_2), 2.34 (m, 6H, CH_2). ^{13}C NMR (100 MHz, MeOD-d_4): δ = 169.2 (CO), 162.9 (c, J = 34.9 Hz, CO_{TFA}), 135.6 (C_{Ar}), 135.2 (CH_{Ar}), 134.3 (C_{Ar}), 130.4 (12C, CH_{Ar}), 129.9 (12C, CH_{Ar}), 128.8 (6C, CH_{Ar}), 63.6 (CH), 51.3 (CH_2NH), 39.5 (CH_2), 38.2 (CH_2). HRMS (m/z) (M + 1): calcd for $C_{78}H_{90}N_{12}O_6$, 1291. 7188; found, 1291.7241.

In the case of **4f**, the reaction mixture was hydrolyzed with satd aq NH_4Cl. The product was isolated as a trifluoroacetate salt. Yield: 8.1 mg, 11%. ^{1}H NMR (400 MHz, MeOD-d_4): δ = 7.10 (6H, CH_{Ar}),

dx.doi.org/10.1021/jo500629d | J. Org. Chem. 2014, 79, 4590−4601

3.64−3.48 (24H, 12 × CH_2O*t*Bu + 12 × CH_2NH), 3.44−3.30 (m, 12H, CH_2), 3.26 (dd, *J* = 6.4 and 4.8 Hz, 6H, CH), 1.19 (s, 54H, CH_3). ^{13}C NMR (500 MHz, MeOD-d_4): δ = 175.5 (CO), 140.9 (C_{Ar}), 127.7 (CH_{Ar}), 74.6 (C), 64.1 (CH), 63.5 (CH_2O*t*Bu), 53.1 (CH_2NH), 40.1 (CH_2), 27.9 (CH_3). HRMS (*m*/*z*) (M + 1): calcd for $C_{66}H_{114}N_{12}O_{12}$, 1267.8750; found, 1267.8799.

In the case of **4g**, the product was purified by preparative reversed-phase chromatography and then by flash chromatography on silica gel using $CHCl_3$ as the eluent, slowly increasing the polarity with MeOH and several drops of aqueous ammonia. The product was isolated as the free amine. Yield: 13 mg, 13%. ^{1}H NMR (400 MHz, $CDCl_3$:MeOD-d_4): δ = 7.22 (s, 6H, CH_{Ar}), 3.69 (d, *J* = 13.3 Hz, 6H, CH_2NH), 3.66 (d, *J* = 13.3 Hz, 6H, CH_2NH), 3.13 (m, 12H, CH_2), 2.86 (d, *J* = 5.9 Hz, 6H, CH), 1.92 (m, 6H, CH), 1.57 (m, 6H, CH_2), 0.95 (d, *J* = 3.1 Hz, 18H, CH_3), 0.93 (d, *J* = 3.9 Hz, 18H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$/MeOD-d_4): δ = 175.8 (6 × CO), 140.7 (6 × C_{Ar}), 128.0 (6 × CH_{Ar}), 68.8 (6 × CH), 53.3 (6 × CH_2NH), 36.8 (6 × CH_2), 32.2 (6 × CH), 30.2 (3 × CH_2), 19.7 (6 × CH_3), 19.0 (6 × CH_3). HRMS (*m*/*z*) (M + 1): calcd for $C_{57}H_{96}N_{12}O_6$, 1045.7656; found, 1045.7727.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Further experimental details, characterization data, X-ray diffraction data, ESI-MS determination of K_{ass}, NMR titration procedures and data, fluorescence spectra, additional NMR spectra, and crystal structure data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Fax: +34-964728214. E-mail: luiss@uji.es.

*Fax: +34-932045904. E-mail: ignacio.alfonso@iqac.csic.es.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO; CTQ2012-38543-C03 project). E.F. thanks CSIC and the European Social Fund for personal financial support (JAE-doc program). We thank SCIC of the Universitat Jaume I for providing us with mass spectrometric facilities and Dr. Yolanda Pérez for her helpful assistance with the acquisition of specific NMR experiments.

■ REFERENCES

(1) (a) Gloe, K., Ed. *Macrocyclic Chemistry: Current Trends and Future Perspectives*; Springer: Dordrecht, The Netherlands, 2005. (b) Choi, K.; Hamilton, A. D. *Coord. Chem. Rev.* **2003**, *240*, 101−110. (c) Chmielewski, M. J.; Zielinsky, T.; Jurczak, J. *Pure Appl. Chem.* **2007**, *79*, 1087−1096. (d) Diederich, F., Stang, P. J., Tikwinsky, R. R., Eds. *Modern Supramolecular Chemistry: Strategies for Macrocycle Synthesis*; Wiley-VCH Verlag GmbH & Co.: Weinheim, Germany, 2008.

(2) (a) White, C. J.; Yudin, A. K. *Nat. Chem.* **2011**, *3*, 509−524. (b) Gibson, S. E.; Lecci, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 1364−1377.

(3) (a) Kubik, S. Cyclopeptide derived synthetic receptors. In *Artificial Receptors for Chemical Sensors*; Mirsky, V. M., Yatsimirsky, A. K., Eds.; Wiley-VCH: Weinheim, Germany, 2010; pp 135−167. (b) Krause, M. R.; Goddard, R.; Kubik, S. *J. Org. Chem.* **2011**, *76*, 7084−7095. (c) Reyheller, C.; Kubik, S. *Org. Lett.* **2007**, *9* (25), 5271−5274. (d) Kubik, S.; Goddard, R.; Kirchner, R.; Nolting, D.; Seidel, J. *Angew. Chem., Int. Ed.* **2001**, *40* (14), 2648−2651. (e) Kubik, S. Cyclopeptides as macrocyclic host molecules for charged guests. In *Highlights in Bioorganic Chemistry—Methods and Applications*; Wennemers, H., Schmuck, C., Eds.; Wiley-VCH: Weinheim, Germany, 2004; pp 124−137. (f) Kubik, S. *Chem. Soc. Rev.* **2009**, *38*, 585−605. (g) Kubik, S. Synthetic peptide-based receptors. In *Supramolecular Chemistry: from Molecules to Nanomaterials*, Steed, J. W.; Gale, P. A., Eds.; Wiley-VCH: Weinheim, Germany, 2012; pp 1179−1204. (h) Dungan, V.; Ngo, H.; Young, P.; Jolliffe, K. *Chem. Commun.* **2013**, *49*, 264−266. (i) Young, P.; Clegg, J.; Jolliffe, K. *Supramol. Chem.* **2012**, *24*, 77−87. (j) Butler, S.; Jolliffe, K. *Chem.—Asian J.* **2012**, *7*, 2621−2628. (k) Rao, J.; Lahiri, J.; Isaacs, L.; Weis, R. M.; Whitesides, G. M. *Science* **1998**, *280*, 708−711.

(4) (a) Ilioudis, C. A.; Tocher, D. A.; Steed, J. W. *J. Am. Chem. Soc.* **2004**, *126*, 12395−12402. (b) Williams, C. E.; Anderson, K. M.; Junk, P. C.; Barbour, L. J.; Steed, J. W. *Chem. Commun.* **2007**, 3634−3636. (c) Channa, A.; Steed, J. W. *Dalton Trans.* **2005**, 2455−2461. (d) Ok Kang, S.; Day, V. W.; Bowman-James, K. *Inorg. Chem.* **2010**, *49*, 8629−8636. (e) Mason, S.; Clifford, T.; Seib, L.; Kuczera, K.; Bowman-James, K. *J. Am. Chem. Soc.* **1998**, 8899−8900. (f) Edwards, N. Y.; Sager, T. W.; McDewitt, J. T.; Anslyn, E. V. *J. Am. Chem. Soc.* **2007**, *129*, 13575−13583. (g) Bisson, A. P.; Lynch, V. M.; Monahan, M.-K. C.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2340−2342. (h) Klein, E.; Ferrand, Y.; Barwell, N. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2008**, *47*, 2693−2696. (i) Barwell, N. P.; Crump, M. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2009**, *48*, 7673−7676. (j) Sookcharoenpinyo, B.; Klein, E.; Ferrand, Y.; Walker, D. B.; Brotherhood, P. R.; Ke, C.; Crump, M. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2012**, *51*, 4586−4590. (k) Howgego, J. D.; Butts, C. P.; Crump, M. P.; Davis, A. P. *Chem. Commun.* **2013**, *49*, 3110−3112. (l) Acharyya, K.; Mukherjee, S.; Mukherjee, P. S. *J. Am. Chem. Soc.* **2013**, *135*, 554−557. (m) Granzhan, A.; Riis-Johannessen, T.; Scopelliti, R.; Severin, K. *Angew. Chem., Int. Ed.* **2010**, *49*, 5515−5518. (n) Cook, T. R.; Vajpayee, V.; Hyung Lee, M.; Stang, P. J.; Chi, K.-W. *Acc. Chem. Res.* **2013**, *46*, 2464−2474.

(5) (a) Henley, P. D.; Kilburn, J. D. *Chem. Commun.* **1999**, 1335−1336. (b) Fujita, D.; Suzuki, K.; Sato, S.; Yagi-Utsumi, M.; Yamaguchi, Y.; Mizuno, N.; Kumasaka, T.; Tanaka, M.; Noda, M.; Uchiyama, S.; Kato, K.; Fujita, M. *Nat. Commun.* **2012**, *3*, No. 1093. (c) Tashiro, S.; Fujita, M. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 833−837. (d) Hof, F.; Craig, S. L.; Nuckolls, C.; Rebek, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1488−1508. (e) Rebek, J. *Science* **1987**, *235*, 1478−1484.

(6) (a) Soo Yoon, S.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2458−2460. (b) Hong, J.-I.; Namgoong, S. K.; Bernardi, A.; Still, W. C. *J. Am. Chem. Soc.* **1991**, *113*, 5111−5112. (c) Fiehn, T.; Goddard, R.; Seidel, R. W.; Kubik, S. *Chem.—Eur. J.* **2010**, *16*, 7241−7255. (d) Rodriguez-Docampo, Z.; Pascu, S. I.; Kubik, S.; Otto, S. *J. Am. Chem. Soc.* **2006**, *128*, 11206−11210. (e) Wareham, R. S.; Kilburn, J. D.; Turner, D. L.; Rees, N. H.; Holmes, D. S. *Angew. Chem., Int. Ed. Engl.* **1995**, 2660−2662. (f) Young, P.; Jolliffe, K. *Org. Biomol. Chem.* **2012**, *10*, 2664−2672. (g) Tanaka, A.; Fujiyoshi, S.; Motomura, K.; Hayashida, O.; Hisaeda, Y.; Murakami, Y. *Tetrahedron* **1998**, *54*, 5187−5206.

(7) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479−2494 and cited therein.

(8) (a) Chen, C.-T.; Wagner, H.; Still, W. C. *Science* **1998**, *279*, 851−853. (b) Maletic, M.; Wennemers, H.; McDonald, D. Q.; Breslow, R.; Still, W. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1490−1493. (c) Dowden, J.; Edwards, P. D.; Flack, S. S.; Kilburn, J. D. *Chem.—Eur. J.* **1999**, *5*, 79−89. (d) Schmuck, C.; Hernandez-Folgado, L. *Org. Biomol. Chem.* **2007**, *5*, 2390−2394. (e) Shepherd, J.; Gale, T.; Jensen, K. B.; Kilburn, J. D. *Chem.—Eur. J.* **2006**, *12*, 713−720. (f) Schmuck, C.; Wich, P. *Angew. Chem., Int. Ed.* **2006**, *45*, 4277−4281. (g) Yamamura, H.; Rekharsly, M. V.; Ishihara, Y.; Kawai, M.; Inoue, Y. *J. Am. Chem. Soc.* **2004**, *126*, 14224−14233. (h) Heitmann, L. M.; Taylor, A. B. P.; Hart, J.; Urbach, A. R. *J. Am. Chem. Soc.* **2006**, *128*, 12574−12581. (i) Bush, M. E.; Bouley, N. D.; Urbach, A. R. *J. Am. Chem. Soc.* **2005**, *127*, 14511−14517. (j) Rekharsky, M. V.; Yamamura, H.; Ho Ko, Y.; Selvapalam, V.; Kim, K.; Inoue, Y. *Chem. Commun.* **2008**, 2236−2238. (k) Castilla, A. M.; Conn, M. M.; Ballester, P. *Beilstein J. Org. Chem.* **2006**, *6*, 1−15. (l) Botta, B.; D'Acquarica, I.; Delle Monache, G.; Subissati, D.; Uccello-Barretta, G.; Mastrini, M.;

Nazzi, S.; Speranza, M. *J. Org. Chem.* **2007**, *72*, 9283−9290. (m) Schmuck, C.; Geiger, L. *J. Am. Chem. Soc.* **2004**, *126*, 8898−8899. (n) Schmuck, C.; Rupprecht, D.; Wienand, W. *Chem.—Eur. J.* **2006**, *12*, 9186−9195. (o) Schneider, H.-J. *Angew. Chem., Int. Ed.* **2009**, *48*, 3924. (p) Kruppa, M.; Mandl, C.; Miltschitzky, S.; König, B. *J. Am. Chem. Soc.* **2005**, *127*, 3362−3365. (q) Stadlbauer, S.; Riechers, A.; Späth, A.; König, B. *Chem.—Eur. J.* **2008**, *14*, 2536−2541. (r) Mandl, C. P.; König, B. *J. Org. Chem.* **2005**, *70*, 670−674. (s) Schmuck, C. *Coord. Chem. Rev.* **2006**, *250*, 3053−3067. (t) Sinha, S.; Lopes, D. H. J.; Du, Z. M.; Pang, E. S.; Shanmugam, A.; Lomakin, A.; Talbiersky, P.; Tennstaedt, A.; McDaniel, K.; Bakshi, R.; Kuo, P. Y.; Ehrmann, M.; Benedek, G. B.; Loo, J. A.; Klärner, F. G.; Schrader, T.; Wang, C. Y.; Bitan, G. *J. Am. Chem. Soc.* **2011**, *133*, 16958−16969.

(9) (a) Schmuck, C.; Heil, M. *Org. Biomol. Chem.* **2003**, *1*, 633−636. (b) Kuphal, S.; Bauer, R.; Bosserhoff, A. K. *Cancer Metastasis Rev.* **2005**, *24*, 195. (c) Nicolau, K. C.; Boddy, C. N. C.; Bräse, S.; Winssinger, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2096−2152. (d) Logsdon, L. A.; Urbach, A. R. *J. Am. Chem. Soc.* **2013**, *135*, 11414−11416. (e) Ingerman, L. A.; Cuellar, V.; Waters, M. L. *Chem. Commun.* **2010**, *46*, 1839−41. (f) James, L. I.; Beaver, J. E.; Rice, N. W.; Waters, M. L. *J. Am. Chem. Soc.* **2013**, *135*, 6450−6455. (g) Butterfield, S. M.; Goodman, C. M.; Rotello, V. M.; Waters, M. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 724−727. (h) Singh, Y.; Sharpe, P. C.; Hoang, H. N.; Lucke, A. J.; McDowall, A. W.; Bottomley, S. P.; Fairlie, D. P. *Chem.—Eur. J.* **2011**, *17*, 151−160. (i) Hans, D.; Young, P. R.; Fairlie, D. P. *Med. Chem.* **2006**, *2*, 627−646.

(10) (a) Zutshi, R.; Franciskovich, J.; Shultz, M.; Schweitzer, B.; Bishop, P.; Wilson, M.; Chmielewski, J. *J. Am. Chem. Soc.* **1997**, *119*, 4841−4845. (b) Hamuro, Y.; Crego Calama, M.; Park, H. S.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2680−2683. (c) García-Echeverría, C.; Chène, P.; Blommers, M. J. J.; Furet, P. *J. Med. Chem.* **2000**, *43*, 3205−3208. (d) Nguyen, H. D.; Dang, D. T.; van Dongen, J. L. J.; Brunsveld, L. *Angew. Chem., Int. Ed.* **2010**, *49*, 895−898. (e) McGovern, R. E.; Fernandes, H.; Khan, A. R.; Power, N. P.; Crowley, P. B. *Nat. Chem.* **2012**, *4*, 527−533. (f) Dang, D. T.; Nguyen, H. D.; Merkx, M.; Brunsveld, L. *Angew. Chem., Int. Ed.* **2013**, *52*, 2915−2919. (g) Bier, D.; Rose, R.; Bravo-Rodriguez, K.; Bartel, M.; Ramirez-Anguita, J. M.; Dutt, S.; Wilch, C.; Klärner, F.-G.; Sanchez-Garcia, E.; Schrader, T.; Ottmann, C. *Nat. Chem.* **2013**, *5*, 234−239.

(11) Luis, S. V.; Alfonso, I. *Acc. Chem. Res.* **2014**, *47*, 112−124.

(12) (a) Alfonso, I.; Burguete, M. I.; Luis, S. V.; Miravet, J. F.; Seliger, P.; Tomal, E. *Org. Biomol. Chem.* **2006**, *4*, 853−859. (b) Alfonso, I.; Bolte, M.; Bru, M.; Burguete, M. I.; Luis, S. V. *CrystEngComm* **2009**, *11*, 735−738. (c) Blasco, S.; Burguete, M. I.; Clares, M. P.; García-España, E.; Escorihuela, J.; Luis, S. V. *Inorg. Chem.* **2010**, *49*, 7841−7852. (d) Martí-Centelles, V.; Kumar, D. K.; White, A.J. P.; Luis, S. V.; Vilar, R. *CrystEngComm* **2011**, *13*, 6997−7008. (e) Martí, I.; Ferrer, A.; Escorihuela, J.; Burguete, M. I.; Luis, S. V. *Dalton Trans.* **2012**, *41*, 6764−6776. (f) Martí-Centelles, V.; Burguete, M. I.; Galindo, F.; Izquierdo, M. A.; Kumar, D. K.; White, A. J. P.; Luis, S. V.; Vilar, R. *J. Org. Chem.* **2012**, *77*, 490−500.

(13) (a) Alfonso, I.; Burguete, M. I.; Galindo, F.; Luis, S. V.; Vigara, L. *J. Org. Chem.* **2009**, *74*, 6130−6142. (b) Alfonso, I.; Bolte, M.; Bru, M.; Burguete, M. I.; Luis, S. V.; Vicent, C. *Org. Biomol. Chem.* **2010**, *8*, 1329−1339.

(14) (a) Martí, I.; Rubio, J.; Bolte, M.; Burguete, M. I.; Vicent, C.; Quesada, R.; Alfonso, I.; Luis, S. V. *Chem.—Eur. J.* **2012**, *18*, 16728−16741. (b) Martí, I.; Bolte, M.; Burguete, M. I.; Vicent, C.; Alfonso, I.; Luis, S. V. *Chem.—Eur. J.* **2014**, DOI: 10.1002/chem.201303604.

(15) (a) Alfonso, I.; Quesada, R. *Chem. Sci.* **2013**, *4*, 3009−3019. (b) Gokel, G. W.; Negin, S. *Adv. Drug Delivery Rev.* **2012**, *64*, 784−796. (c) Fernandez-Lopez, S.; Kim, H.-S.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature* **2001**, *412*, 452−455. (d) Sanchez-Quesada, J.; Kim, H. S.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 2503−2507. (e) Prins, L. J.; Scrimin, P. Artificial (pseudo)peptides for molecular recognition and catalysis. In *Functional Synthetic Receptors*; Schrader, T., Hamilton, A. D., Eds.; Viley-VCH: Weinheim, Germany, 2005; pp 1−44. (f) Cheng, P.-N.; Liu, C.; Zhao, M.; Eisenberg, D.; Nowick, J. S. *Nat. Chem.* **2012**, *4*, 927−933. (g) Cheng, P.-N.; Pham, J. D.; Nowick, J. S. *J. Am. Chem. Soc.* **2013**, *135*, 5477−5492. (h) Zheng, J.; Liu, C.; Sawaya, M. R.; Vadla, B.; Khan, S.; Woods, R. J.; Eisenberg, D.; Goux, W. J.; Nowick, J. S. *J. Am. Chem. Soc.* **2011**, *133*, 3144−3157. (i) Kang, S.-W.; Gothard, C. M.; Maitra, S.; Atia-tul-Wahab; Nowick, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 1486−1487.

(16) Moure, A.; Luis, S. V.; Alfonso, I. *Chem.—Eur. J.* **2012**, *18*, 5496−5500.

(17) (a) Rowan, S. J.; Cantrill, S. J.; Cousins, G. R. L.; Sanders, J. K. M.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **2002**, *41*, 898−952. (b) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Chem. Commun.* **1999**, 1575−1576.

(18) Alfonso, I.; Bolte, M.; Bru, M.; Burguete, M. I.; Luis, S. V. *Chem.—Eur. J.* **2008**, *14*, 8879−8891.

(19) (a) Bru, M.; Alfonso, I.; Burguete, M. I.; Luis, S. V. *Angew. Chem., Int. Ed.* **2006**, *45*, 6155−6159. (b) Alfonso, I.; Bolte, M.; Bru, M.; Burguete, M. I.; Luis, S. V.; Rubio, J. *J. Am. Chem. Soc.* **2008**, *130*, 6137−6144. (c) Bru, M.; Alfonso, I.; Bolte, M.; Burguete, M. I.; Luis, S. V. *Chem. Commun.* **2011**, *47*, 283−285.

(20) (a) Zhang, J.; McCombie, G.; Guenat, C.; Knochenmuss, R. *Drug Discovery Today* **2005**, *10*, 635−642. (b) Deng, G. J.; Sanyal, G. *J. Pharm. Biomed. Anal.* **2006**, *40*, 528−538. (c) Hofstadler, S. A.; Sannes-Lowery, K. A. *Nat. Rev. Drug Discovery* **2006**, *5*, 585−595. (d) Jecklin, M. C.; Touboul, D.; Bovet, C.; Wortmann, A.; Zenobi, R. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 332−343. (e) Yao, C. X.; Na, N.; Huang, L. Y.; He, D. C.; Ouyang, J. *Anal. Chim. Acta* **2013**, *794*, 60−66.

(21) (a) Loo, J. A. *Mass Spectrom. Rev.* **1997**, *16*, 1−23. (b) Schalley, C. A. *Int. J. Mass Spectrom.* **2000**, *194*, 11. (c) Daniel, J. M.; Friess, S. D.; Rajagopalan, S.; Wendt, S.; Zenobi, R. *Int. J. Mass Spectrom.* **2002**, *216*, 1−27. (d) Di Tullio, A.; Reale, S.; De Angelis, F. *J. Mass Spectrom.* **2005**, *40*, 845−865. (e) Chen, Z.; Weber, S. G. *TrAC, Trends Anal. Chem.* **2008**, *27*, 738−748. (f) Rosu, F.; De Pauw, E.; Gabelica, V. *Biochimie* **2008**, *90*, 1074−1087. (g) Kitova, E. N.; El-Hawiet, A.; Schnier, P. D.; Klassen, J. S. *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 431−441.

(22) (a) Jorgensen, T. J. D.; Roepstorff, P.; Heck, A. J. R. *Anal. Chem.* **1998**, *70*, 4427−4432. (b) Jen, C. H.; Leary, J. A. *Anal. Biochem.* **2010**, *407*, 134−140. (c) Cubrilovic, D.; Biela, A.; Sielaff, F.; Steinmetzer, T.; Klebe, G.; Zenobi, R. *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 1768−1777.

(23) (a) Brodbelt, J. S. *Int. J. Mass Spectrom.* **2000**, *200*, 57−69. (b) Schalley, C. A. *Mass Spectrom. Rev.* **2001**, *20*, 253−309. (c) Schafer, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 1896−1899. (d) Baytekin, B.; Baytekin, H. T.; Schalley, C. A. *Org. Biomol. Chem.* **2006**, *4*, 2825−2841. (e) Weimann, D. P.; Schalley, C. A. *Supramol. Chem.* **2008**, *20*, 117−128.

(24) (a) Schalley, C. A.; Martin, T.; Obst, U.; Rebek, J. *J. Am. Chem. Soc.* **1999**, *121*, 2133−2138. (b) Mansikkama, H.; Schalley, C. A.; Nissinen, M.; Rissanen, K. *New J. Chem.* **2005**, *29*, 116−127. (c) Beyeh, N. K.; Weimann, D. P.; Kaufmann, L.; Schalley, C. A.; Rissanen, K. *Chem.—Eur. J.* **2012**, *18*, 5552−5557. (d) Beyeh, N. K.; Göth, M.; Kaufmann, L.; Schalley, C. A.; Rissanen, K. *Eur. J. Org. Chem.* **2014**, 80−85.

(25) (a) Oshovsky, G. V.; Verboom, W.; Fokkens, R. H.; Reinhoudt, D. N. *Chem.—Eur. J.* **2004**, *10*, 2739−2748. (b) Oshovsky, G. V.; Reinhoudt, D. N.; Verboom, W. *J. Am. Chem. Soc.* **2006**, *128*, 5270−5278.

(26) Torvinen, M.; Neitola, R.; Sansone, F.; Baldini, L.; Ungaro, R.; Casnati, A.; Vainiotalo, P.; Kalenius, E. *Org. Biomol. Chem.* **2010**, *8*, 906−915.

(27) Faggi, E.; Luis, S. V.; Alfonso, I. *RSC Adv.* **2013**, *3*, 11556−11565.

(28) Previous competition ESI-MS studies on molecular recognition of *N*-protected dipeptides and smaller macrocyclic pseudopeptides (for example, receptor **6**) were used to evaluate the relative strength of the host−guest interactions[13b] for which relative binding abilities could only be extracted in a semiquantitative range. We believe that, for the

smaller macrocycles, such as **6**, the criterion of preservation of ionization efficiencies for the 1:1 complex and the free host is not fulfilled. In addition, macrocyclic hosts displayed inherently low K_{ass} values toward *N*-protected dipeptides, which may originate potential disadvantages for their ESI-MS screening due to in-source dissociation effects or nonspecific interactions (due to the high concentrations of guest required to produce detectable levels of the host−guest complex) typically found for low-affinity host−guest complexes.

(29) We tried to get additional information on the supramolecular complex by through-space dipolar interactions, but unfortunately, we did not observe intermolecular cross-peaks in NOESY or ROESY experiments, probably due to unfavorable relaxation rates of this system in this solvent.

(30) (a) Macchioni, A.; Ciancaleoni, G.; Zuccaccia, C.; Zuccaccia, D. Diffusion ordered NMR spectroscopy (DOSY). In *Supramolecular Chemistry: From Molecules to Nanomaterials*; Steed, J. W., Gale, P. A., Eds.; Wiley-VCH: Weinheim, Germany, 2012; pp 319−330. (b) Gafni, A.; Cohen, Y. *J. Org. Chem.* **1997**, *62*, 120−125.

(31) (a) Braun, S.; Raymond, W. E.; Racker, E. *J. Biol. Chem.* **1984**, *4*, 20151−2054. (b) Glickman, J. F. Assay development for protein kinases and phosphatases. In *A Practical Guide to Assay Development and High-Throughput Screening in Drug Discovery*; Chen, T., Ed.; CRC Press: Boca Raton, FL, 2010; pp 1−24.