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Synthetic Tripodal Squaramido-Based Receptors for the Complexation of Antineoplastic Folates in Water

David Quiñonero,[a] Kenia A. López,[a] Pere M. Deyà,[a] M. Nieves Piña,[a] and Jeroni Morey*[a]

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Three suitably oriented squaramido ammonium groups form the basis of the first abiotic receptor that employs the noncovalent ammonium–carboxylate electrostatic interaction and a hydrogen bond to achieve high-affinity molecular recognition in water. These interacctions have been studied experimentally by isothermal calorimetry (ITC), NMR spectroscopy, and MS. Also, we have proposed a model for coordination from DFT calculations and NMR spectroscopy studies. Finally, a fluorimetric ensemble of the receptor with 5-carboxyfluorescein was useful for the quantitative fluorimetric determination of folate and methotrexate in commercial samples using competition assays.

Introduction

The recognition and sensing of anionic substrates by positively charged or neutral synthetic receptors has attracted considerable attention during the last decade because of the fundamental role that anions play in many chemical and biological processes.[1] For instance, anions are present in biochemical processes such as the activity of enzymes, DNA regulation, protein biosynthesis, and the transport of hormones.[2] Furthermore, in recent publications, large hydrocarbon anions, namely, C_6H^- and C_8H^-, have been detected in interstellar media.[3] Generally, the study of real analytical problems – complex mixtures of bioanalytes[4] (urine, blood, saliva) environmental analytes (industrial wastes),[5] cellular imaging, and quality control – concerning anionic species are carried out in water media and at a specific pH value, for example, phosphate[6] only exists in a particular pH range. One important part of anion coordination chemistry is the selective complexation of carboxylate anions by synthetic hosts.[7] For example, sensing and selective detection of carboxylate anions, such as citrate, malate, and tartrate, have been the subject of several studies in food science.[8]

Folic acid is a hematopoietic water-soluble B vitamin that has received a lot of attention because of its important role in the pathogenesis of cardiovascular diseases, neural tube defects, and certain kinds of cancer.[9] Carboxylate anion recognition is usually based on charged binding units (guanidinium and amidinium groups), neutral binding units (urea, thiourea, amide, and pyrrole moieties), and natural amino acids (biomimetic receptors). In fact, in nature recombinant human dihydrofolate reductase (DHFR) is known to bind the glutamate moiety of folate through an arginine residue[10] (i.e., a guanidinium group) in the solid state. However, in the bacterial proteins R67 DHFR[11] and

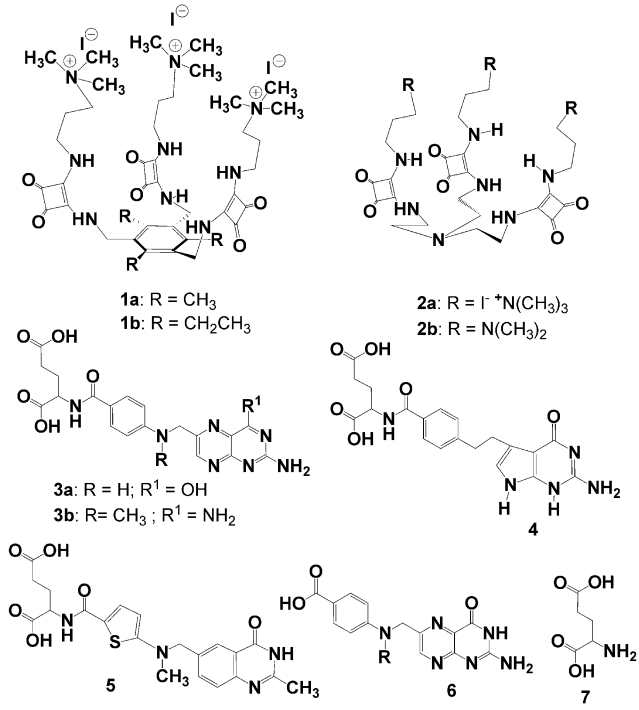

Figure 1. Structure of receptors **1a**–**2b** and guests **3a**–**7**.

[a] Department of Chemistry, Universitat de les Illes Balears, Cra. Valldemossa, Km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain
Fax: +34-971-173-426
E-mail: jeroni.morey@uib.es

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thymidylate synthase[12] the glutamate tail of folate and methotrexate, respectively, is bound to a lysine residue (ammonium group).

Herein, we describe simple abiotic, tripodal, squaramido-based receptors capable of tight binding of folate anions through electrostatic interactions between tetraalkylammonium groups and the cooperative action of squaramide units through hydrogen bonding (Figure 1).

Molecule **1b** represents the first folate-binding receptor composed of an abiotic squaramide that is capable of achieving high enough affinity to sense folate-like guests, namely, folic acid (**3a**), methotrexate (**3b**), alimta (**4**, pemetrexed), and raltitrexed (**5**) in water.

Nowadays, compounds **3b**, **4** and **5** are used as antifolates drugs,[13] and represent one of the most extensively investigated classes of antineoplastic agents, along with aminopterin, which initially demonstrating clinical activity more than 50 years ago.[14]

Results and Discussions

In a previous paper,[15] we reported a tripodal receptor capable of recognizing several tricarboxylate salts and quantifying citrate concentrations. Since this receptor was partially soluble in water, it was impossible to use it for sensing slightly soluble guests in this solvent. All receptors presented herein, **1a**–**b** and **2a**–**b** (Figure 1), have one extra carbon atom in the side chains and all show excellent solubility in water, making it possible to sense slightly soluble guests in this solvent. Alkyl ammonium salts are strongly solvated in water,[16] showing a solvation shell that extends to the carbon in the alpha position to the ammonium group. Accordingly, we propose that in these new receptors, **1a**–**b** and **2a**–**b**, the solvation shell is not sterically hindered (the alpha carbon is accessible by the solvent) and can be formed freely due to the length of the side chain, suggesting that this structural feature is key for to the correct solubility in water. It should be mentioned that **3a** is sparingly soluble in cold (0.0016 mg mL^{-1} at 25 °C) and boiling water (1%), but is soluble in very acidic, pH 1–3, and alkaline, pH > 8, aqueous solutions and insoluble in usual apolar organic solvents. These facts make **3a** a very demanding guest for sensing.[17] The design of the trisubstituted receptors **1a** and **1b** is based on 1,3,5-tris(aminomethyl)-2,4,6-trimethylbenzene[18] and 1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene,[15,19] respectively.

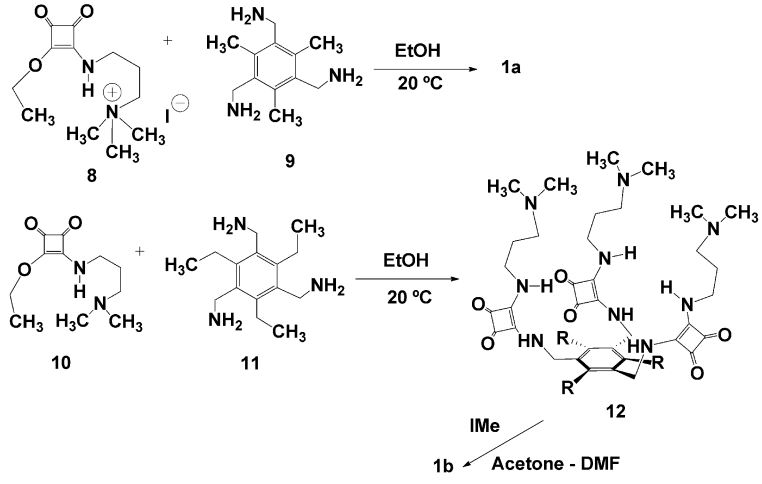
Synthesis of the Receptors

The synthetic route to obtain the receptors **1a**–**b** and **2a**–**b** was adapted from similar syntheses carried out in our group.[15] Receptor **1a** (Scheme 1) in an acceptable yield is obtained by coupling an ammonium squaramide iodide salt (**8**)[20] with a spacer 1,3,5-tris(aminomethyl)-2,4,6-trimethylbenzene (**9**) in ethanol for 8 h at room temperature. Likewise, the preparation of receptor **1b** is carried out by condensing the key intermediate **8** with the spacer 1,3,5-tris-(aminomethyl)-2,4,6-triethylbenzene (**11**), but the receptor is obtained in a very low yield. Better results are obtained (see Scheme 1) in a two-step process, first by obtaining the intermediate **12**, by coupling the squaramide **10**[20] with spacer **11**, followed by complete methylation with methyl iodide.

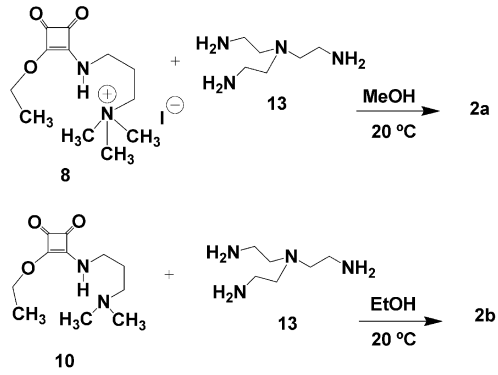
Receptors **2a** and **2b**, based on the more flexible tris(2-aminoethyl)amine (**13**),[21] (see Scheme 2), are obtained by coupling **13** with the intermediate squaramide **8** or **14**, respectively. At this point, it should be noted that receptor **2a** can be prepared on a multigram scale.

The purification of the receptors is simple (chromatography is not required), since they all precipitate in ethanol or methanol and then are exhaustively washed with diethyl ether or pentane.

Receptors **1a**–**b** are more rigid and with a higher degree of preorganization than the trisubstituted receptors **2a**–**b**. The three alkylamino groups present in the hosts are the responsible for their excellent solubility in water. In fact, in



Scheme 1. Synthetic route to prepare receptors **1a**–**b**.



Scheme 2. Synthetic route to prepare receptors **2a**–**b**.

their ammonium form, the coulombic force becomes the dominant interaction between the receptor and the glutamate moiety of folates.

In Table 1 we show the binding constants and thermodynamic experimental results obtained by isothermal calorimetry (ITC; for details see the Supporting Information) for complexation between receptors **1a**–**b** and **2a**–**b** and dicarboxylate folate-related guests **3a**, **3b**, **4**, **5**, pteroic acid (**6**), and the glutamate anion (**7**). All host–guest complexes were studied in water at 293 K.

Table 1. Binding constants (K_{ass}, M^{-1}), association Gibbs free energies (ΔG_{ass} kJ/mol), association enthalpies (ΔH_{ass}, kJ/mol), and association entropies ($T\Delta S_{ass}$, kJ/mol) for guests **3a**–**7** and receptors **1a**–**2b** in H_2O at pH 9.0 in a 0.25 M Tris-HCl buffer system, at 293 K.

Host·guest	K_{ass}	ΔG_{ass}	ΔH_{ass}	$T\Delta S_{ass}$
[1a·3a]	$(3.9 \pm 0.1) \times 10^3$	–20.3	–46.0	–25.7
[1b·3a]	$(1.8 \pm 0.1) \times 10^4$	–23.9	–31.3	–7.4
[2a·3a]	$(1.20 \pm 0.01) \times 10^3$	–17.4	–22.3	–4.9
[2b·3a]	$(5.8 \pm 0.4) \times 10^2$	–15.5	–2.2	13.3
[1b·7]	$(6.0 \pm 0.9) \times 10^2$	–15.6	–2.0	13.6
[1b·3b]	$(4.5 \pm 0.2) \times 10^4$	–26.1	–27.6	–1.5
[1b·4]	$(7.20 \pm 0.05) \times 10^3$	–21.7	–6.4	15.3
[1b·5]	$(2.9 \pm 0.4) \times 10^4$	–25.0	–18.3	6.7
[1b·6]	$(6.2 \pm 0.4) \times 10^3$	–21.3	–25.4	–4.1

To regulate the pH, a 0.25 M Tris-HCl buffer system was used, which provided the pH of 9.0 required to ensure the existence of the anionic form of the assayed guests. In all examples, the stoichiometry *n* factor for the complexes was between 0.7 and 1. The ammonium squarate–carboxylate association, in all cases, was an enthalpically driven process and was clearly exothermic.[22] The significant effect of "preorganization" in folate binding is evident from the data shown in Table 1: the folate affinities of tridentate receptors **1a** (with a central trimethyl-substituted benzene), **2a** (with **13** as a spacer) and **2b** (receptor similar to **2a**, without ammonium salts) are lower than that of **1b**, which has a central triethyl-substituted benzene ring.[23] The alternating arrangement of groups around the benzene ring provides a rigid template for a convergent cavity binding site. The binding association constants obtained for receptor **1b** with **3a**, **3b**, and **5** are in the order of 10^4 M^{-1}.[24] These high values confirm well-established, strong complexation in water. To the best of our knowledge, this is the first example in the literature to address a complexation study between an abiotic receptor and folate-related guests. The low binding association constant obtained for receptor **1b** and the glutamate anion **7** (6.0×10^2 M^{-1}) suggests that the a priori main binding force, that is, the ammonium–carboxylate electrostatic interaction, is not the only one responsible for the high association constants obtained with bigger guests. This fact is in accordance with the results found for complex formation between a dianionic guest and a tricationic host: the association constant obtained for receptor **1b** (tricationic host) interacting with folate derivative **3b** (dianionic guest) is surprisingly high (4.5×10^4 M^{-1}).

In other words, another positive cooperative binding force is needed to explain these high association constants. Nevertheless, in water this cooperativity effect has been rarely observed for synthetic hosts.[24b] In our case, this positive cooperative binding force is most likely to be due to the existence of a well-established hydrogen bond between the OH group in the 4-position of the pterin ring of the **3a**, or the NH_2 group of the **3b**, and one carbonyl group.

ESI-MS supports the formation of the **1b·3a** complex with 1:1 stoichiometry (*m*/*z* 1274; see the Supporting Information). Adducts **1b·3b** (*m*/*z* 1539) and **1b·4** (*m*/*z* 1260) were also observed by ESI-MS, which confirmed the formation of the proposed 1:1 complexes. Complexes of higher stoichiometry were not found in the ESI mass spectra.

To gain more insight into the interaction pattern of our complexes, we carried out geometry optimizations of complexes **1b·3a** and **1b·3b** by means of DFT calculations at the BP86/TZVP level of theory. We optimized the geometry of all compounds using restricted Kohn–Sham DFT.[25] In these calculations we used the exchange-correlation BP86 functional, which combined the Becke88 (B) exchange functional,[26] in conjunction with a combination of the Slater–Dirac exchange and Vosko, Wilk, and Nusair 1980,[27] and Perdew's 1986[28] correlation functionals. In these calculations, the Ahlrichs triple-ζ basis for all atoms (def-TZVP basis set, TZ hereafter)[29] was used.

Our DFT calculations were carried out using the resolution of the identity (RI) BP86. Because of the time-consuming nature of the calculations, we used the parallel RI-DFT[30,31] methodology, which uses an auxiliary fitting basis set to avoid treating the complete set of two-electron repulsion integrals, thus speeding up calculations by a factor of 10. Fast evaluation of the Coulomb potential for electron densities by using a multipole-accelerated RI approximation linear scaling [O(*N*)] for large molecules was also used.[32] It significantly reduced calculation times for molecules with more than 1000–2000 basis functions.

Moreover, for all compounds, we carried out optimization calculations by simulating water as the solvent within the conductor-like screening model (COSMO)[33] at the RI-BP86/TZ level of theory. COSMO is a continuum solvation model in which the solute molecule forms a cavity within the dielectric continuum of permitivity, ε (78.39 for water), that represents the solvent.

Geometries of all structures were optimized with the analytical gradient method without symmetry constraints.

All of the theoretical calculations described herein were carried out by using TURBOMOLE version 5.10.[34]

The resulting complex structure for **1b·3a** is shown in Figure 2. The substrate lies atop in an asymmetric composition. One carboxylate group of the **3a** is bound to one of the ammonium arms by ion-pair formation, (average $N^+R_4\cdots O^-$ distance of 3.24 Å) and the other carboxylate moiety interacts with two ammonium cation arms. In addition, hydrogen-bonding interactions are observed between the N–H groups of squaramide and the oxygen atoms of carboxylate groups. In fact, the carboxylate group that is interacting with two ammonium units is hydrogen bonded to one squaramide and the carboxylate bound to one ammonium unit is hydrogen bonded to two squaramide moieties. Furthermore, there is an additional hydrogen bond between the O–H group of the pterin ring of **3a** and the carbonyl group of the squaramide unit (with an HO···O=C distance of 2.78 Å).

Therefore, the asymmetric binding mode observed, which explains the high stability of these folate complexes, is a consequence of three factors: first, the existence of electrostatic forces due to the binding of ammonium residues to carboxylate anions; second, the hydrogen-bonding donor capability of the squaramide unit, which is superior to urea and thiourea,[35] is one important reason for this increase of the aromatic character of the squaramide ring upon complexation of the anion;[36] and third, the formation of one additional hydrogen bond between the guest and a carbonyl group of the squaramide ring.

Positive cooperativity among the above-mentioned nonbonded interactions enlarges the binding phenomena in water and, consequently, our hosts efficiently compete with water molecules that are initially tightly bonded in the solvation shell of the anion. The chemistry of life mainly takes place in water; therefore, the design and synthesis of receptors that are competitive in aqueous media are of special importance, particularly for folate-type guests. For this reason, the low association constant of **7** can be explained by the fact that the guest cannot establish the additional hydrogen-bonding interaction with receptor **1b**.

However, despite being a monocarboxylate anion, pteroic acid (**6**) is structurally adequate to establish this sort of hydrogen bond, giving rise to an acceptable association constant of 6.2×10^3 M^{-1}. This unexpectedly large association constant can also be due to the existence of a cation–π interaction between an aminomethyl ammonium moiety of the host and the phenyl ring of **6**. In fact, this interaction is observed in the theoretical calculations for complex [**1b·3b**] with a calculated distance of 3.7 Å between the ammonium moiety and the phenyl ring of **3b**.[37]

The overall binding scheme proposed herein is in good agreement with the experimental signals and shift changes observed in the ^{1}H NMR spectra. When one equivalent of **3a** is added to an NMR sample of receptor **1b** in D_2O,[37] at basic pH, the signals for the e and f methylene protons of the squaramide receptor arms and the c protons of the methylene group of the central benzene ring of **1b** are split into three new narrow groups of signals (see the Supporting Information). Moreover, the F and G proton signals of the glutamate moiety of **3a** are also split in the same way.[37] The new appearance of proton signals in the ^{1}H NMR spectra confirms the formation of a strong complex in an asymmetric binding mode in D_2O. In addition, the ^{1}H NMR spectra show clear differentiation of proton signals. Our DFT calculations showed that two ammonium units were hydrogen bonded to carboxylates, but in the ^{1}H NMR spectrum there was no significant chemical shift or splitting of the signal of the d protons. These two results are not con-

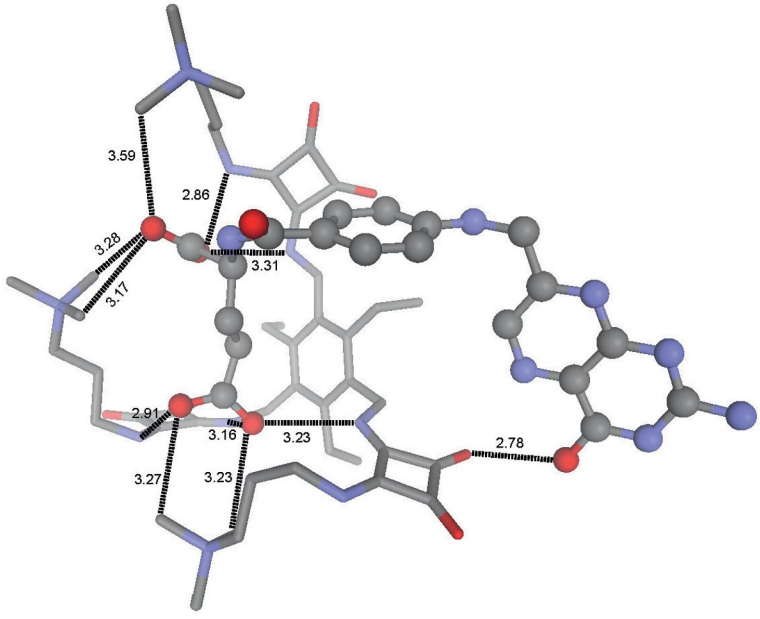


Figure 2. Calculated structure of the **1b·3a** complex. (C gray; O red; N blue). Distances marked in angstroms.

tradictory because the same experimental behavior is often observed in the recognition of anions with squaramide ammonium salts.[38]

Encouraged by the results obtained with receptor **1b**, we planned to obtain a sensing system to be employed for practical uses. Our aim was to check the potential applicability of our approach with a commercial sample of folic acid and extend this approach to related folate samples, such as **3b**. We chose to employ the competitive indicator method [Indicator-Displacement Assay (IDA)][39] that has been widely used in the literature for determining association constants, for instance, in the determination of inorganic anions and carboxylates. This methodology is relatively simple and precise and allows synthetic receptors to work as sensors without introducing additional changes in covalent architecture. In our approach, we used a fluorescence indicator, such as 5-carboxyfluorescein.

First, we determined the binding constants of **1b** with 5-carboxyfluorescein by evaluating the decrease in the fluorescence intensity of 5-carboxyfluorescein in the presence of increasing amounts of receptor **1b** in water with 0.25 M Tris-HCl buffer (pH 9), at room temperature (23 °C). The binding constant determined by this method was found to be $K_{ass} = 4.2 \times 10^3$ M^{-1} with a 1:1 binding algorithm. This binding constant is 4.2-fold lower than that obtained for **3a** by ITC experiments under the same conditions, which allow the use of competition assays for the determination of **3a** and **3b** in water. We have determined a calibration plot at a maximum fluorescence emission band, $\lambda = 525$ nm, for the 5-carboxyfluorescein ensemble upon addition of **3a** (see Figure 3) and **3b**.[37] The increase in the intensity of the emission band upon incremental additions of **3a** is shown in Figure 4. This progressive increase is due to displacement of 5-carboxyfluorescein from the receptor by the **3a** guest, restoring the original fluorescence and, therefore, signaling the presence of **3a**.

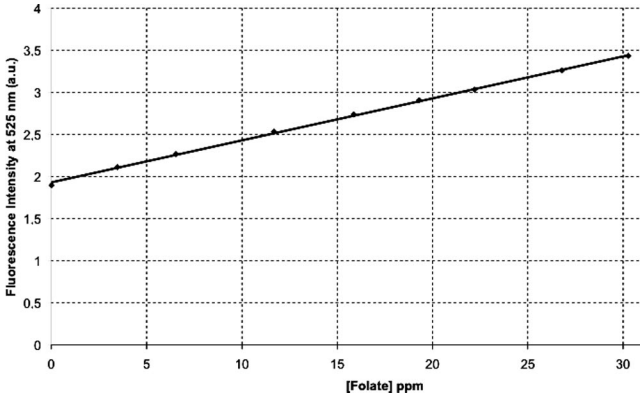


Figure 3. Calibration curve at $\lambda = 525$ nm for the ensemble of **1b** and 5-carboxyfluorescein upon addition of **3a** in H_2O, at pH 9.0 in 0.25 M Tris-HCl ($R = 0.9995$).

The viability and potential applicability[39] of our fluorescence ensemble was checked for the fluorimetric quantitative determination of **3a** and **3b**[40] in commercially available pills of folic acid (acfol®)[41] and **3b** (Metoject®),[42] respectively. The relative error of these measurements was lower than 7% and the interval of determination was between 1 and 50 ppm of **3a** or **3b**.

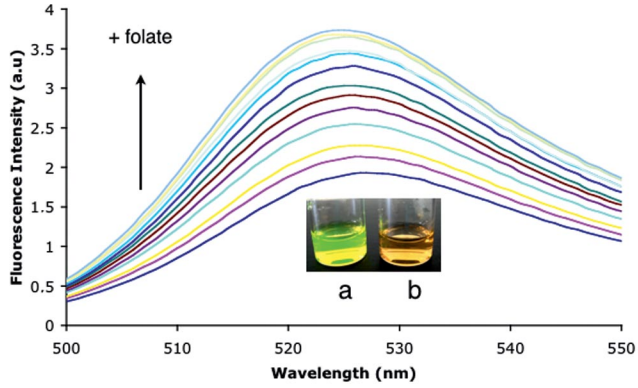


Figure 4. Fluorescence titration of **1b** (2×10^{-5} M; 0.25 M Tris-HCl buffer) with **3a**. $\lambda_{exc} = 525$ nm. Upper curve corresponds to a final concentration of **3a** of 2.1×10^5 M. Inset: (a) final and (b) initial solutions ($\lambda = 365$ nm excitation).

This method for the determination of **3a** and **3b** is cheaper than existing ones,[43] the results are obtained quickly (within hours), and does not require extensive knowledge of specific techniques for carrying it out, for instance, enzymatic methods. In certain circumstances, it could be an excellent method for the routine process of determining quantities of **3a** and **3b** in solids or liquids.

Conclusions

The effective sensing of antineoplastic folate anions has been successfully achieved by using simple tripodal sensors in water. We have demonstrated the potential utility of the squaramide ammonium binding unit for the molecular recognition of dicarboxylate anions, such as **3a** and antineoplastic folate-like guests, in water. In addition, we have shown that a sensing ensemble of **1b** and 5-carboxyfluorescein is capable of quantitatively determining the amount of **3a** and **3b** anions of a commercial preparation. Finally, this study proposes a mechanism for how these new tritopic receptors are coordinated with the folate guests, emphasizing the important role of the hydrogen bond between the squarate and pteridine rings.

Experimental Section

General: Reactions were carried out in oven-dried glassware under an atmosphere of argon, unless otherwise indicated. All commercially available reagents: glutaric acid, **3a**, Pteroic acid, **13**, 1,3,5-triethylbenzene, 2,4,6-tris-(bromomethyl)mesitylene, 3,4-diethoxy-3-cyclobutene-1,2-dione, and *N,N*-dimethyl-1,3-propanediamine were supplied by Sigma Aldrich. Compound **3b** was supplied by Fluka. Compound **4** was supplied by Sequoia Research Products. Compound **5** was purchased from AK Scientific and used without further purification, unless otherwise stated. Diethyl ether and ethanol used for synthesis were supplied by Scharlau Chemie and were distilled from calcium hydride immediately before use. All other reagents and solvents were purchased from Scharlau Chemie or

Sigma Aldrich and used without further purification. NMR solvents were purchased from Euriso-top. For the ITC solvent, doubly distilled deionized water was obtained from a Millipore-Q system with 18 MΩcm resistivity.

Instrumentation: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 300 and 75 MHz at 23 °C. Chemical shifts are reported as part per million (δ, ppm) referenced to the residual protium signal of deuterated solvents. Spectral features are tabulated in the following order: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = complex multiplet), number of protons, coupling constants (J, Hz), assignment. Electrospray mass spectra (ESMS) were recorded with a Micromass, Autospec3000 spectrometer equipped with an electrospray module. The ITC experiments were carried out on a Microcal, ultramicrocalorimeter MCS-ITC instrument. The ITC instrument was periodically calibrated by using an internal electric heater, following the procedures recommended by the manufacturer. The fluorescence titrations were registered with an Aminco-Browman series2 spectrophotomers. IR spectra were obtained on a Nicolet Impact 400 instrument in the solid state. Spectral features are tabulated as follows: wavenumber (cm^{-1}), intensity (s = strong, m = medium, w = weak). Melting points were measured on a Tottoli apparatus.

Determination of Thermodynamic Parameters by ITC

Titration Conditions: 40–45 injections of an 8–10 mM solution of the folates (6 μL each) in H_2O with a 0.25 M Tris-HCl buffer system (pH 9.0) were introduced into a sample cell at 293 K containing a solution of guest (1.5 mL) in H_2O with a 0.25 M Tris-HCl buffer system. The heat of dilution was subtracted prior to data analysis by Origin MicroCal software. In all cases the c parameter, defined as $c = K_{ass}[G]_t n$, was kept between 10 and 1000. Errors were calculated at a confidence level of 95%. K_{ass}, ΔH, and ΔS were obtained at 293 K by curve fitting by using Origin 5.0 software, as implemented by MicroCal™.

Compound 1a: A solution of **9** (207 mg, 1 mmol) in ethanol (20 mL) was added dropwise to a stirred solution (1.10 g, 3.0 mmol) of **8** in ethanol (10 mL). The reaction mixture was stirred overnight at room temperature (20 °C) in an atmosphere of argon. After this time, the white precipitate was collected from the reaction mixture by centrifugation, washed with cold ethanol (3 × 10 mL) and Et_2O (2 × 10 mL), and dried under high vacuum to give host **1a** (0.65 g, 56%); m.p. 254 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.92 (s, 1 H, NH), 7.35 (s, 5 H, NH), 4.89 (s, 6 H, NCH_2 ArH), 3.56 [q, J(H,H) = 5.6 Hz, 6 H, NCH_2 chain], 3.31 [t, J(H,H) = 5.6 Hz, 6 H, $(CH_3)_3N^+CH_2$ chain], 3.07 [s, 27 H, $^+N(CH_3)_3$], 2.50 (9 H, CH_3 methyl ArH), 1.97 (m, 6 H, CH_2 chain), ppm. ^{13}C NMR (75.4 MHz [D$_6$]DMSO): δ = 183.1, 182.7, 168.2, 167.4, 144.1, 132.3, 63.3, 54.6, 53.0, 42.0, 41.7, 35.1, 27.2, 25.0, 16.1 ppm. FTIR (KBr): $\tilde{\nu}$ = 3445 (s), 3222 (w), 1799 (w), 1656 (m), 1597 (s), 1544 (s), 1477 (m), 1342 (w) cm^{-1}. HRMS (ESI, H_2O/EtOH): calcd. for $C_{42}H_{66}N_9O_6I_2$ ([M – I]$^+$ 1046.3220; found 1046.3226.

Compound 12: A solution of **11** (0.21 g, 0.84 mmol) in ethanol (20 mL) was added dropwise to a stirred solution (0.67 g, 2.9 mmol) of **10** in ethanol (20 mL). The reaction mixture was stirred overnight at room temperature (20 °C) in an atmosphere of argon. After this time, the white precipitate was collected from the reaction mixture by centrifugation, washed with cold ethanol (3 × 10 mL), and dried under high vacuum to give **12** (0.43 g, 66%); m.p. > 300 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.25 (s, 3 H, NH), 7.18 (s, 3 H, NH), 4.84 [d, J(H,H) = 4.5 Hz, 6 H, NCH_2 ArH], 3.84 [q, J(H,H) = 5.6 Hz, 6 H, NCH_2 chain], 2.75 [q, J(H,H) = 7.1 Hz, 6 H, CH_2 ethyl ArH], 2.20 [t, J(H,H) = 5.6 Hz, 6 H, $(CH_3)_2NCH_2$ chain], 2.11 [s, 18 H, $N(CH_3)_2$], 1.63 [quintuplet, J(H,H) = 5.6, 7.1 Hz, 6 H, CH_2 chain], 1.10 [t, J(H,H) = 7.3 Hz, 9 H, CH_3 ethyl ArH] ppm. ^{13}C NMR (75.4 MHz [D$_6$]DMSO): δ = 182.8, 182.7, 168.2, 167.2, 144.1, 132.8, 56.4, 45.5, 42.0, 41.7, 29.2, 23.1, 16.8 ppm. FTIR (KBr): $\tilde{\nu}$ = 3169 (s), 2934 (s), 2779 (m), 1797 (m), 1653 (s), 1582 (s), 1341 (s), 1096 (m), 747 (w) cm^{-1}. HRMS (ESI, H_2O/EtOH): calcd. for $C_{42}H_{64}N_9O_6$ [M + H]$^+$ 790.4901, $C_{42}H_{63}N_9O_6Na$ [M + Na]$^+$ 812,4799; found 790.5446, 812.5301.

Compound 1b: Methyl iodide (79 μL, 1.27 mmol) was added, using a syringe, to a solution of **12** (0.22 g, 0.28 mmol) in acetone (50 mL) and DMF (25 mL). The mixture was heated under reflux in an atmosphere of argon for 12 h, after which time it was cooled to room temperature. The resulting white solid was isolated by centrifugation, after decanting the supernatant, and purified by washing with cold acetone (3 × 10 mL) then it was dried to give **1b** (0.46 g, 39%); m.p. 209 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.35 (s, 3 H, NH), 7.28 (s, 3 H, NH), 4.84 (s, 6 H, NCH_2 ArH), 3.54 [q, J(H,H) = 5.6 Hz, 6 H, NCH_2 chain], 3.31 [t, J(H,H) = 5.6 Hz, 6 H, $(CH_3)_3N^+CH_2$ chain], 3.05 [s, 27 H, $^+N(CH_3)_3$], 2.72 [q, J(H,H) = 7.1 Hz, 6 H, CH_2 ethyl ArH], 1.94 (m, 6 H, CH_2 chain), 1.10 [t, J(H,H) = 7.3 Hz, 9 H, CH_3 ethyl ArH] ppm. ^{13}C NMR (75.4 MHz [D$_6$]DMSO): δ = 183.1, 182.7, 168.2, 167.4, 144.1, 132.6, 63.3, 54.6, 53.0, 42.0, 41.7, 26.4, 25.0, 23.1, 16.8 ppm. FTIR (KBr): $\tilde{\nu}$ = 3451 (s), 3222 (s), 2962 (m), 1798 (m), 1662 (m), 1595 (s), 1536 (s), 1447 (m), 1340 (w), 1256 (w) cm^{-1}. HRMS (ESI, H_2O/EtOH): calcd. for $C_{45}H_{72}N_9O_6I_2$ [M – I]$^+$ 1088.3684, $C_{45}H_{72}N_9O_6I_3Na$ [M + Na]$^+$ 1238.2632; found 1088.3682, 1238.3047.

Compound 2a: A solution of **13** (6.82 g, 0.047 mol) in methanol (50 mL) was added dropwise to a stirred solution (51.5 g, 0.140 mol) of **8** in methanol (100 mL). The reaction mixture was stirred overnight at room temperature (20 °C) in an atmosphere of argon. After this time, the white precipitate was collected from the reaction mixture by filtration, washed with cold diethyl ether (70 mL) and methanol (120 mL), and dried under high vacuum to give **2a** (34 g, 65%); m.p. 152 °C ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.61 (s, 3 H, NH), 7.38 (s, 3 H, NH), 3.58 (m, 12 H), 3.32 (m, 6 H), 3.10 [s, 27 H, $^+N(CH_3)_3$], 2.72 [q, J(H,H) = 7.1 Hz, 6 H], 1.98 (m, 6 H, CH_2 chain), ppm. ^{13}C NMR (75.4 MHz [D$_6$]DMSO): δ = 183.1, 182.6, 168.2, 63.4, 55.0, 52.8, 42.1, 41.0, 24.9 ppm. FTIR (KBr): $\tilde{\nu}$ = 3445 (s), 3220 (m), 2953 (w), 1799 (w), 1653 (m), 1592 (s), 1544 (s), 1447 (m), 1345 (w) cm^{-1}. HRMS (ESI, H_2O/EtOH): calcd. for $C_{36}H_{63}N_{10}O_6I_2^+$ [M – I]$^+$ 985.3022; found m/z 985.2855.

Compound 2b: A solution of **13** (50 mg, 0.34 mmol) in ethanol (15 mL) was added dropwise to a stirred solution (0.27 g, 1.2 mmol) of **10** in ethanol (20 mL). The reaction mixture was stirred overnight at room temperature (20 °C) in an atmosphere of argon. After this time, the white precipitate was collected from the reaction mixture by centrifugation, washed with cold ethanol (3 × 10 mL), and dried under high vacuum to give **2b** (0.182 g, 78%); m.p. 241 °C, ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.45 (s, 3 H, NH), 7.32 (s, 3 H, NH), 3.58 (m, 12 H), 2.65 (t, 6 H), 2.21 (t, 6 H), 3.10 [s, 18 H, $N(CH_3)_2$], 1.63 (m, 6 H), ppm. ^{13}C NMR (75.4 MHz [D$_6$]DMSO): δ = 182.9, 182.7, 168.6, 168.0, 56.5, 55.2, 45.6, 42.1, 29.1 ppm. FTIR (KBr): $\tilde{\nu}$ = 3445 (s), 3220 (m), 2952 (w), 1799 (w), 1648 (m), 1594 (s), 1546 (s), 1435 (m), 1345 (w) cm^{-1}. HRMS (ESI, H_2O/EtOH): calcd. for $C_{33}H_{55}N_{10}O_6$ [M + H]$^+$ 687.4306; found 687.4304.

Supporting Information (see footnote on the first page of this article): ITC experiments. ^{1}H and ^{13}C NMR spectra, MS (ESI) characterization, and fluorescence titration data including fitting curves; details of the quantum chemical calculations.

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[1] For comprehensive reviews of anion recognition, see: a) P. A. Gale, *Coord. Chem. Rev.* **2001**, *213*, 79–128; b) P. D. Beer, P. A. Gale, *Angew. Chem.* **2001**, *113*, 502; *Angew. Chem. Int. Ed.* **2001**, *40*, 486–516; c) P. A. Gale, *Coord. Chem. Rev.* **2000**, *199*, 181–233; d) C. Caltagirone, P. A. Gale, *Chem. Soc. Rev.* **2009**, *38*, 520–563; e) C. R. Bondy, S. J. Loeb, *Coord. Chem. Rev.* **2003**, *240*, 77–99; f) *Supramolecular Chemistry of Anions* (Eds.: A. Bianchi, K. Bowman-James, E. García-España), Wiley-VCH, Weinheim, Germany, **1997**; g) *Anion Receptor Chemistry* (Eds.: J. L. Sessler, P. A. Gale, W.-S. Cho), Royal Society of Chemistry, Cambridge, U.K., **2006**; h) *Encyclopedia of Supramolecular Chemistry* (Eds.: J. L. Atwood, J. W. Steed), Marcel Dekker, New York, **2004**; i) E. A. Katayev, Y. A. Ustynyuk, J. L. Sessler, *Coord. Chem. Rev.* **2006**, *250*, 3004–3037; j) M. D. Best, S. L. Tobey, E. V. Anslyn, *Coord. Chem. Rev.* **2003**, *240*, 191–221; k) Z. Xu, S. K. Kim, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 1457–1466; l) Z. Xu, N. J. Singh, S. K. Kim, D. R. Spring, K. S. Kim, J. Yoon, *Chem. Eur. J.* **2011**, *17*, 1163–1170.

[2] For reviews, see: a) R. Martínez-Máñez, F. Sancenón, *Chem. Rev.* **2003**, *103*, 4419–4476; b) L. Pu, *Chem. Rev.* **2004**, *104*, 1687–1716; c) C. Schmuck, *Coord. Chem. Rev.* **2006**, *250*, 3053–3067; d) B. Kuswandi, Nuriman, W. Verboom, D. N. Reinhoudt, *Sensors* **2006**, *6*, 978–1017, and references cited therein.

[3] a) M. C. McCarthy, C. A. Gottlieb, H. Gupta, P. Thaddeus, *ApJ.* **2006**, *652*, L141–L143; b) S. Brünken, H. Gupta, C. A. Gottlieb, M. C. McCarthy, P. Thaddeus, *ApJ.* **2007**, *664*, L43–L46.

[4] a) S. L. Tobey, E. V. Anslyn, *Org. Lett.* **2003**, *5*, 2029–2031; b) S. Van Arman, A. W. Czarnik, *J. Am. Chem. Soc.* **1990**, *112*, 5376–5377; c) S. K. V. Yathavakilla, M. Fricke, P. A. Creed, D. T. Heitkemper, N. V. Shockey, C. Schwegel, J. Caruso, J. T. Creed, *Anal. Chem.* **2008**, *80*, 775–782; d) A. W. Martínez, S. T. Phillips, M. J. Butte, G. M. Whitesides, *Angew. Chem.* **2007**, *119*, 1340; *Angew. Chem. Int. Ed.* **2007**, *46*, 1318–1320; e) Z. Lin, M. Wu, M. Schäferling, O. S. Wolfbeis, *Angew. Chem.* **2004**, *116*, 1767; *Angew. Chem. Int. Ed.* **2004**, *43*, 1735–1738.

[5] a) B. A. Moyer, J. F. Birdwell, P. V. Bonnesen, L. H. Delmau, in: *Macrocyclic Chemistry: Current Trends and Future Perspectives* (Ed.: K. Gloe), Springer, Dordrecht, **2005**, pp. 383–405; b) *Analytical Methods in Supramolecular Chemistry* (Ed.: C. A. Schalley), Wiley-VCH, Weinheim, Germany, **2007**; c) *Core Concepts in Supramolecular Chemistry and Nanochemistry* (Eds.: J. W. Steed, D. R. Turner, K. J. Wallace), Wiley, Chichester, U.K., **2007**; d) M. A. Palacios, R. Nishiyabu, M. Márquez, P. Anzenbacher Jr., *J. Am. Chem. Soc.* **2007**, *129*, 7538–7544, and references cited therein.

[6] a) T. Zhang, E. V. Anslyn, *Tetrahedron* **2004**, *60*, 11117–11124; b) S. L. Tobey, B. D. Jones, E. V. Anslyn, *J. Am. Chem. Soc.* **2003**, *125*, 4026–4027; c) R. Prohens, G. Martorell, P. Ballester, A. Costa, *Chem. Commun.* **2001**, 1456–1457; d) H. N. Lee, Z. Xu, S. K. Kim, K. M. K. Swamy, Y. Kim, S.-J. Kim, J. Yoon, *J. Am. Chem. Soc.* **2007**, *129*, 3828–3829; e) G. V. Zyryanov, M. A. Palacios, P. Anzenbacher Jr., *Angew. Chem.* **2007**, *119*, 7995; *Angew. Chem. Int. Ed.* **2007**, *46*, 7849–7852; f) G. V. Oshovsky, D. N. Reinhoudt, W. Verboom, *Angew. Chem.* **2007**, *119*, 2418; *Angew. Chem. Int. Ed.* **2007**, *46*, 2366–2393; g) M. Boiocchi, M. Bonizzoni, L. Fabbrizzi, G. Piovani, A. Taglietti, *Angew. Chem. Int. Ed.* **2004**, *43*, 3847–3852.

[7] a) A. B. Descalzo, K. Rurack, H. Weisshoff, R. Martínez-Mañez, M. D. Marcos, P. Amoros, K. Hoffmann, J. Soto, *J. Am. Chem. Soc.* **2005**, *127*, 184–200; b) S. K. Kim, B.-G. Kang, H. S. Koh, Y. J. Yoon, S. J. Jung, B. Jeong, K.-D. Lee, J. Yoon, *Org. Lett.* **2004**, *6*, 4655–4658 (dicarboxylates); c) C. Schmuck, *Chem. Eur. J.* **2000**, *6*, 709–718; d) C. Schmuck, *J. Am. Chem. Soc.* **2005**, *127*, 3373–3379; e) A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 682; *Angew. Chem. Int. Ed.* **1998**, *37*, 649–652; f) A. Metzger, V. M. Lynch, E. V. Anslyn, *Angew. Chem.* **1997**, *109*, 911; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 862–865; g) C. Bazzicalupi, A. Bencini, A. Bianchi, L. Borsari, C. Giorgi, B. Valtancoli, *J. Org. Chem.* **2005**, *70*, 4257–4266; h) C. Bazzicalupu, A. Bencini, A. Bianchi, C. Borri, A. Danesi, E. García-España, C. Giorgi, B. Valtancoli, *J. Org. Chem.* **2008**, *73*, 8286–8295; i) C. Gomy, A. R. Schmitzer, *J. Org. Chem.* **2006**, *71*, 3121–3125.

[8] a) A. Metzger, E. V. Anslyn, *Angew. Chem.* **1998**, *110*, 682; *Angew. Chem. Int. Ed.* **1998**, *37*, 649–652; b) M. I. Burguete, F. Galindo, S. V. Luis, L. Vigara, *Dalton Trans.* **2007**, 4027–4033; c) C. Schmuck, M. Schwegmann, *Org. Biomol. Chem.* **2006**, *4*, 836–838; d) L. Fabbrizzi, F. Foti, A. Taglietti, *Org. Lett.* **2005**, *7*, 2603–2606; e) J. L. Lavigne, E. V. Anslyn, *Angew. Chem.* **1999**, *111*, 3903; *Angew. Chem. Int. Ed.* **1999**, *38*, 3666–3669.

[9] a) H. I. Morrinson, D. Schaubel, M. Desmeules, D. T. Wigle, *JAMA J. Am. Med. Assoc.* **1996**, *275*, 1893–1896; b) O. Nygard, S. M. Vollset, H. Refsum, I. Stensvold, A. Tverdal, J. E. Nordrehaug, P. M. Ueland, G. Kvale, *JAMA J. Am. Med. Assoc.* **1995**, *274*, 1526–1533; c) E. B. Rimm, W. C. Willet, F. B. Hu, L. Sampson, G. A. Colditz, J. E. Manson, C. Hennekens, M. J. Stampfer, *JAMA J. Am. Med. Assoc.* **1998**, *279*, 359–364.

[10] J. F. Davies, T. J. Delcamp, N. J. Prendergast, V. A. Ashford, J. H. Freisheim, J. Kraut, *Biochemistry* **1990**, *29*, 9467–9479.

[11] E. E. Howell, *Biochemistry* **2005**, *44*, 12420–12433.

[12] W. Welch, C. R. Sage, T. J. Stout, T. Klein, J. Ruppert, R. M. Stroud, A. Jain, *J. Mol. Biol.* **2001**, *313*, 813–829.

[13] a) G.-M. Liand, S. R. Presnell, in: *Encyclopedia of Biological Chemistry* (Eds.: W. J. Lennarz, M. D. Lane), Royal Society of Chemistry, Cambridge, **2004**, vol. 1, p. 671; b) R. Messmann, C. Allegra, *Antifolates in Cancer Chemotherapy & Biotherapy* (Eds.: B. Chabner, D. Longo), Lippincott Williams & Wilkins, Philadelphia, **2001**, p. 241; c) N. Kohler, C. Sun, J. Wang, M. Zhang, *Langmuir* **2005**, *21*, 8858–8864; d) Pemetrexed: A. Hanauske, V. Chen, P. Paoletti, C. Niyikiza, *Oncologist* **2001**, *6*, 363–373; e) N. J. Vogelzang, J. J. Rusthoven, J. Symanowski, *J. Clin. Oncol.* **2003**, *21*, 2636–2644; f) L. Paz-Ares, S. Bezares, J. M. Tabernero, D. Castellanos, H. Cortes-Funes, *Cancer* **2003**, *97*, 2056–2063; g) C. Shih, V. J. Chen, L. S. Gossett, *Cancer Res.* **1997**, *57*, 1116–1123.

[14] S. Farber, L. K. Diamond, R. D. Mercer, R. F. Sylvester, J. A. Wolff, *N. Engl. J. Med.* **1948**, *238*, 787–793.

[15] A. Frontera, J. Morey, A. Oliver, M. N. Piña, D. Quiñonero, A. Costa, P. Ballester, P. M. Deyà, E. V. Anslyn, *J. Org. Chem.* **2006**, *71*, 7185–7195.

[16] M. Rekharsky, Y. Inoue, *J. Am. Chem. Soc.* **2000**, *122*, 4418–4435.

[17] *The Merck Index*, 11th ed., Merck, New Jersey, **1989**, p. 660.

[18] 1,3,5-Tris(bromomethyl)-2,4,6-trimethylbenzene: a) A. W. van der Made, R. H. van der Made, *J. Org. Chem.* **1993**, *58*, 1262–1263; b) Z. Hou, T. D. P. Stack, C. J. Sunderland, K. N. Raymond, *Inorg. Chim. Acta* **1997**, *263*, 341–355; c) C. E. Willans, K. M. Anderson, P. C. Junk, L. J. Barbour, J. W. Steed, *Chem. Commun.* **2007**, 3634–3636; d) M. G. Sarwar, B. Dragisic, S. Sagoo, M. S. Taylor, *Angew. Chem. Int. Ed.* **2010**, *49*, 1–5.

[19] 1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene: a) K. V. Kilway, J. S. Siegel, *Tetrahedron* **2001**, *57*, 3615–3627; b) K. J. Wallace, R. Hanes, E. V. Anslyn, J. Morey, K. V. Kilway, J. Siegel, *Synthesis* **2005**, 2080–2083; c) A. Vacca, C. Nativi, M. Cacciarini, R. Pergoli, S. Roelens, *J. Am. Chem. Soc.* **2004**, *126*, 16456–16465; d) C. Guarise, G. Zaupa, L. J. Prins, P. Scrimin, *Eur. J. Org. Chem.* **2008**, 3559–3568.

[20] M. N. Piña, M. C. Rotger, B. Soberats, P. Ballester, P. M. Deyà, A. Costa, *Chem. Commun.* **2007**, *9*, 963–965.
[21] a) M. Allevi, M. Bonizzoni, L. Fabbrizzi, *Chem. Eur. J.* **2007**, *13*, 3787–3795; b) M. M. Ibrahim, N. Shimomura, K. Ichikawa, M. Shiro, *Inorg. Chim. Acta* **2001**, *313*, 125–136; c) M. Kruppa, B. König, *Chem. Rev.* **2006**, *106*, 3520–3560; d) P. S. Lakshminarayanan, I. Ravlkumar, E. Suresh, P. Ghosh, *Inorg. Chem.* **2007**, *46*, 4769–4771.
[22] In polar media, especially water or aqueous alcoholic solution, the binding phenomena can even be endothermic and entropy driven due to reorganization of the solvent upon complexation: a) M. Haj-Zaroubi, N. W. Mitzel, F. P. Schmidtchen, *Angew. Chem.* **2002**, *114*, 111; *Angew. Chem. Int. Ed.* **2002**, *41*, 104–107; b) R. Prohens, M. C. Rotger, M. N. Piña, P. M. Deyà, J. Morey, P. Ballester, A. Costa, *Tetrahedron Lett.* **2001**, *42*, 4933–4936; c) R. Meissner, X. Garcias, S. Mecozzi, J. Rebek Jr., *J. Am. Chem. Soc.* **1997**, *119*, 77–85.
[23] This fact is due to minor steric effects of methyl groups relative to ethyl groups in the benzene ring between the alternate arrangement of the methyl (ethyl) groups and the aminomethylene substituents in the trisubstituted benzene derivatives; for examples, see: a) G. Hennrich, E. V. Anslyn, *Chem. Eur. J.* **2002**, *8*, 2218–2224; b) K. V. Kilway, J. S. Siegel, *J. Am. Chem. Soc.* **1992**, *114*, 255–261; c) D. J. Iverson, G. Hunter, J. F. Blount, J. R. Damewood, K. Mislow, *J. Am. Chem. Soc.* **1981**, *103*, 6073–6083; d) T. D. P. Stack, Z. Hou, K. N. Raymond, *J. Am. Chem. Soc.* **1993**, *115*, 6466–6467.
[24] Receptors capable of binding dicarboxylate in water have been reported; for example, a receptor that employs tris-cationic units based on the guanidiniumcarbonyl pyrrole group in cooperation with an ammonium group binds *N*-acetylglutamate with a 2:1 association model K_1 = 460 M^{-1} and K_2 = 3.300 M^{-1} (90% water/10% DMSO), see: a) C. Schmuck, L. Geiger, *J. Am. Chem. Soc.* **2005**, *127*, 10486–10487; on the other hand, a receptor employing diguanidinium and Cu^{II} groups binds glutarate and succinate with K_a = 1.400 M^{-1} and K_a = 5.500 M^{-1} in water, respectively, see: b) A. D. Hughes, E. V. Anslyn, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 6538–6543; another receptor with a cholic acid based fluorescent neutral group binds glutarate, succinate, and L-glutamate with K_a = 2.93×10^5 M^{-1}, K_a = 1.73×10^5 M^{-1} and K_a = 5.57×10^6 M^{-1} in CH_3OH/H_2O (1:1), respectively, see c) S.-Y. Liu, L. Fang, Y.-B. He, W.-H. Chan, K.-T. Yeung, Y.-K. Cheng, R.-H. Yuang, *Org. Lett.* **2005**, *7*, 5825–5828.
[25] W. Kohn, L. J. Sham, *Phys. Rev.* **1965**, *140*, A1133–A1138.
[26] A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098–3100.
[27] a) P. A. M. Dirac, *Proc. R. Soc. London Ser. A* **1929**, *123*, 714–733; b) J. C. Slater, *Phys. Rev.* **1951**, *81*, 385–390; c) S. Vosko, L. Wilk, M. Nusair, *Can. J. Phys.* **1980**, *58*, 1200–1211.
[28] J. P. Perdew, *Phys. Rev. B* **1986**, *33*, 8822–8824.
[29] a) A. Schäfer, C. Huber, R. Ahlrichs, *J. Chem. Phys.* **1994**, *100*, 5829–5835; b) K. Eichkorn, F. Weigend, O. Treutler, R. Ahlrichs, *Theor. Chem. Acc.* **1997**, *97*, 119–124.
[30] M. v. Arnim, R. Ahlrichs, *J. Comput. Chem.* **1998**, *19*, 1746–1757.
[31] K. Eichkorn, O. Treutler, H. Öhm, M. Häser, R. Ahlrichs, *Chem. Phys. Lett.* **1995**, *242*, 652–660.
[32] M. Sierka, A. Hogekamp, R. J. Ahlrichs, *J. Chem. Phys.* **2003**, *118*, 9136–9148.
[33] A. Klamt, G. Schüürmann, *J. Chem. Soc. Perkin Trans. 2* **1993**, *5*, 799–805.
[34] R. Ahlrichs, R. M. Bär, M. Hacer, H. Horn, C. Kömel, *Chem. Phys. Lett.* **1989**, *162*, 165–169.
[35] a) D. Quiñonero, A. Frontera, G. A. Suñer, J. Morey, A. Costa, P. Ballester, P. M. Deyà, *Chem. Phys. Lett.* **2000**, *326*, 247–254; b) C. Garau, A. Frontera, P. Ballester, D. Quiñonero, A. Costa, P. M. Deyà, *Eur. J. Org. Chem.* **2005**, 179–183.
[36] D. Quiñonero, R. Prohens, C. Garau, A. Frontera, P. Ballester, A. Costa, P. M. Deyà, *Chem. Phys. Lett.* **2002**, *351*, 115–120.
[37] See the Supporting Information: Figure S6.
[38] M. N. Piña, PhD thesis, Universitat de les Illes Balears (Spain), **2005**, p. 72.
[39] E. V. Anslyn, *J. Org. Chem.* **2007**, *72*, 687–699.
[40] Spanish Patent application P24718ES00 (31 March, 2009).
[41] Acfol, Italfarmaco, Alcobendas, Madrid, Spain.
[42] Metoject, 10 mg mL^{-1}, Gebro Pharma, Medac GmbH, Hamburg, Germany.
[43] For comprehensive reviews of folate determination, see: a) J. Arcot, A. Shrestha, *Trends Food Sci. Technol.* **2005**, *16*, 253–266; b) Microbiological method by AOAC [Method 992.05 (2002) and AACC 7469]; c) C. Wang, S. Li, L. Ting, X. Xu, C. Wang, *Microchim. Acta* **2006**, *152*, 233–238; d) D. Hoegger, P. Morier, C. Vollet, D. Heini, F. Reymond, J. S. Rossier, *Anal. Bioanal. Chem.* **2007**, *387*, 267–275; e) J. D. M. Patring, J. A. Jastrebova, S. B. Hjortmo, T. A. Andlid, I. M. Jägerstad, *J. Agric. Food Chem.* **2005**, *53*, 2406–2411.

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