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Recognition of α-amino acid derivatives by *N*,*N*′-dibenzylated *S*,*S*-(+)-tetrandrine †

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Complexation of free and *N*-acetylated α-amino acid anions (Gly, Ala, Phe) and some structurally related guests by a dicationic cyclophane-type *N*,*N*′-dibenzylated chiral derivative of a bisisoquinoline macrocyclic alkaloid *S*,*S*-(+)-tetrandrine (DBT) has been studied by ^{1}H-NMR titrations in D_2O. In contrast to other macrocyclic hosts like cyclodextrins and calixarenes, DBT shows highest affinity and large enantioselectivity ($K(S)/K(R) \geq 10$) toward smaller *N*-acetylalanine and binds larger phenylalanine derivatives more weakly and non-selectively. With 1,2,3,4-tetrahydroisoquinoline-3-carboxylate, a rigid analog of phenylalanine, binding again becomes enantioselective with $K(S)/K(R) = 3.8$. The binding specificity of DBT is rationalized on the basis of molecular mechanics calculations.

Introduction

Amino acids and their derivatives are important components of chemical and biological systems, and their recognition by synthetic macrocyclic receptors, in particular, chiral recognition attracts considerable interest.[1] Various approaches involve the use of metal complexes,[2] imprinted polymers,[3] natural[4] and modified[5] cyclodextrins, synthetic macrocycles (mainly calixarenes)[6] and different types of acyclic compounds[7] as host molecules. Often a chiral host is prepared by using a natural chiral compound as a precursor or modifier.[8] When such a natural precursor already is a macrocycle this may substantially facilitate the preparation of the host molecule. Moreover, natural macrocyclic compounds themselves, in particular macrocyclic antibiotics, already find applications as chiral hosts for analytical separations of enantiomers by HPLC and/or capillary electrophoresis.[9]

Previously we reported the enantioselective recognition of *N*-acylated and free aromatic amino acids by a bisisoquinoline alkaloid (+)-*d*-tubocurarine in water.[10] The ratio of binding constants for *S* and *R* enantiomers of phenylalanine with the zwitterionic form of the alkaloid was *ca.* 3 and the major "driving force" for the binding was the hydrophobic interaction with the guest phenyl group. Other chiral hosts, such as cyclodextrins, cyclophanes, and calixarenes employed for recognition of α-amino acids and their simple *N*- or *O*-protected derivatives in aqueous solutions also use principally hydrophobic interactions in combination with electrostatic attraction of an ionic guest and typically show very low affinities to small amino acids like glycine and alanine in water. Receptors operating in non-aqueous media *via* hydrogen bonding often bind even small amino acids tightly and selectively (see *e.g.*[6e–g]), but in practically more interesting aqueous solutions the size of a guest is very important. Table 1 shows results for some typical systems proposed for recognition of free and *N*-acylated amino acids in water. Only with aromatic amino acids the binding constants reach the order of *ca.* 50 M^{-1} and the enantioselectivity is always rather low.[11] Amino acids derivatized by voluminous dansyl[12a] or 2,4-dinitrophenyl[12b] groups form more stable complexes, but of course with lower selectivity. At the same time, biological receptors may be highly specific to small guests. For example, vancomycin binds *N*-acetylglycine and *N*-acetyl-*R*-alanine with *K* equaling 80 and 300 M^{-1} respectively and does not bind at all *N*-acetyl-*S*-alanine.[13]

Recently we found that a semisynthetic cyclophane type receptor obtained by quaternization of nitrogen atoms of another bisisoquinoline alkaloid *S*,*S*-(+)-tetrandrine (DBT, Scheme 1) showed a significant affinity and selectivity for dicarboxylate anions in aqueous solution.[14] The macrocycle DBT possesses four chiral atoms and exists as a single diastereomer that makes it an attractive object for possible use as a chiral receptor. Simple aliphatic monoanions like acetate or propionate did not form detectable complexes with DBT, but benzoate showed a measurable affinity.[14] This paper describes the binding of a series of anions of free and *N*-acetylated α-amino acids to DBT which demonstrates remarkably high binding enantioselectivity and specificity of this host toward *N*-acetylalanine.

Results and discussion

The crystal structure of *S*,*S*-(+)-tetrandrine shows that it has a conformation of a triangle with a small cavity formed by the inner surface of the phenyl ring D and bounded by the edges of rings C and B.[15] Quaternization of both nitrogen atoms of the alkaloid with benzylic groups transforms it into a dicationic receptor DBT (Scheme 1), which may in principle have a different conformation.

Crystals of DBT were obtained by precipitation from acetonitrile, but unfortunately appeared to be too unstable to allow one to obtain a complete structure. Therefore the likely structure of DBT was determined by MM simulation starting from the known macrocycle crystal structure. Due to the rigidity of the cyclophane macrocycle one may expect that benzylic

† Electronic supplementary information (ESI) available: simulated structures of DBT complexes with *S*- and *R*-**7**. See http://www.rsc.org/suppdata/ob/b4/b402698e/

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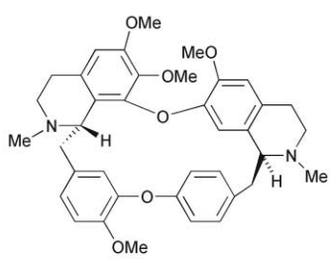
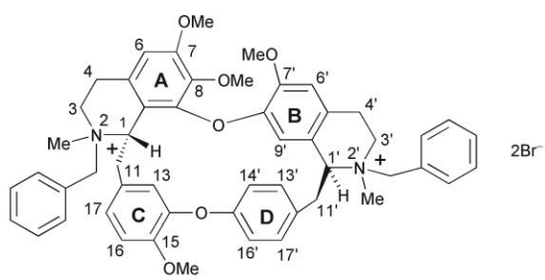
Table 1 Selected formation constants for complexes of free and *N*-acetylated α-amino acids with macrocyclic hosts in water

Guest[a]	Host	K, M^{-1}	K_S/K_R	Ref.
Gly (±1)	β-cyclodextrin	–[b]		4*a*
S-Ile (±1)		4.9		
S-Leu (±1)		3.3		
S-Phe (±1)	α-cyclodextrin	8.0	1.0	4*b*
S-Phe (−1)		15.5	0.86	4*c*
N-Ac-*S*-Phe (−1)	β-cyclodextrin	67.5	1.11	4*d*
N-Ac-*S*-Trp (−1)		17.1	1.35	
N-Ac-*S*-Phe (−1)	β-cyclodextrin-NH_3^+	67	1.2	5*a*
N-Ac-*S*-Leu (−1)		58	1.2	
S-Ala (±1)	tetrasulfonatocalix[4]arene	–[b]		6*a*
S-Val (±1)		16		
S-Leu (±1)		50		
S-Phe (±1)		63		

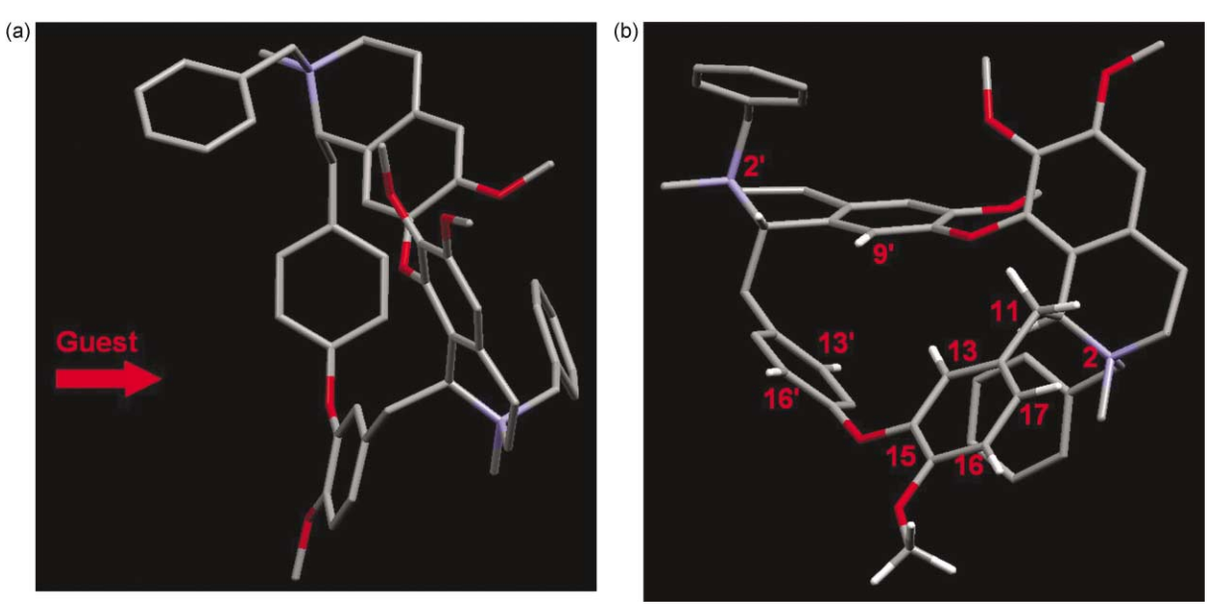
[a] The ionic state of the guest is indicated in parentheses. [b] No interaction.



S,S-(+)-tetrandrine



DBT



Scheme 1 Chemical structure of *N*,*N*′-dibenzylated *S*,*S*-(+)-tetrandrine (DBT) and simulated three-dimensional structure of the macrocycle: (a) side view (hydrogens omitted), (b) top view (shown hydrogens at chiral carbons and those whose NMR signals undergo largest complexation-induced shifts).

groups will appear at positions occupied by the lone electron pairs of nitrogen atoms of the alkaloid. Scheme 1 shows the simulated conformation of the macrocycle according to which the benzyl group attached to the N(2′) atom is directed out of the macrocycle cavity and the benzyl group attached to the N(2) atom closes the cavity from the right side. This makes more probable entrance of guests from the left side (Scheme 1a). This prediction is supported by observation of larger complexation-induced shifts of the ^{1}H NMR signals of protons at carbon atoms more accessible from this side with all guests studied (see below).

Chemical structures of all guests used in this study are shown in Scheme 2. Additions of aromatic guests induced upfield shifts of proton signals of DBT, but purely aliphatic guests induced small downfield shifts. The plots of observed chemical shifts *vs.* guest concentration followed the equation (1) derived for a 1 : 1 complexation scheme:

$$\delta_{obs} = \delta_H + 0.5\Delta\delta([H]_T + [G]_T + 1/K - \sqrt{([H]_T + [G]_T + 1/K)^2 - 4[H]_T[G]_T}\,)/[H]_T \quad (1)$$

where δ_H is the chemical shift of a given proton in free DBT, $\Delta\delta$ is the difference in chemical shifts of the proton in complexed and free DBT (complexation-induced shift at saturation), $[H]_T$ and $[G]_T$ are the total concentrations of the host and the guest, K is the binding constant. Also a simplified equation (2) which assumes the free guest

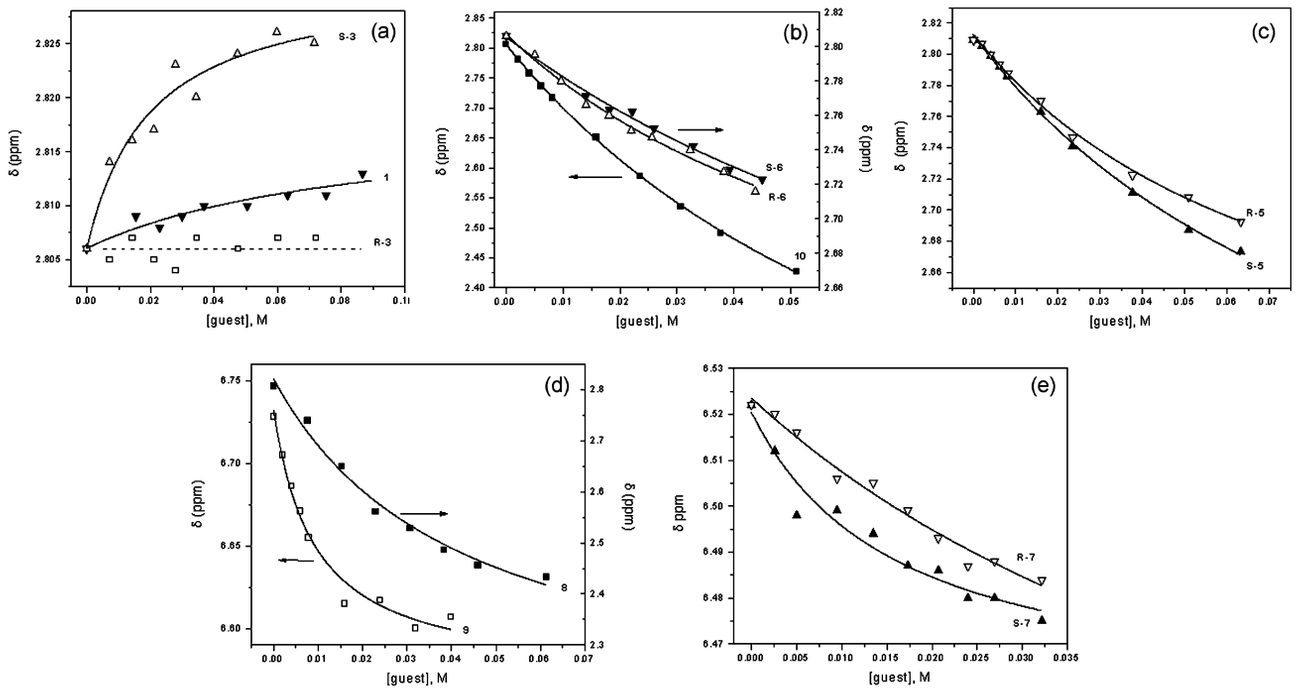
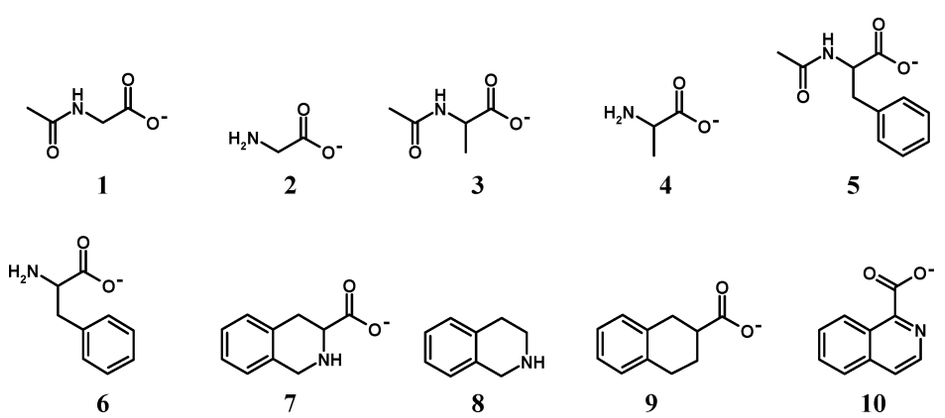


Fig. 1 Typical ^{1}H NMR titration plots of DBT with guests studied. For **1**, **3**, **5**, **6**, **8**, **10** chemical shifts of protons at C(11) are shown, for **7** – at C(13′) and for **9** – at C(16′).



Scheme 2 Chemical structures of guests employed.

concentration to be approximately equal to its total concentration was used when appropriate.

$$\delta_{obs} = \delta_H + \Delta\delta K[G]_T/(1 + K[G]_T) \quad (2)$$

Typical titration plots for all guests are shown in Fig. 1a–e and the binding constants K are collected in Table 2. The $\Delta\delta$ values for selected protons, which undergo the largest changes of chemical shifts, are given in Table 3.

With guests **1** and *S*-**3** signals of only a few DBT protons were shifted sufficiently for a titration experiment. Other guests induced larger shifts, but often it was impossible to determine K and $\Delta\delta$ values from a given signal because of overlapping of signals of protons of added guests with DBT signals. For this reason, Table 3 does not show complexation-induced shifts at saturation for the same protons with each guest. Negative (upfield) complexation-induced shifts observed with guests **5**–**10** are typical for interactions with aromatic groups producing the shielding effect due to the π-system ring current.[16] The largest $\Delta\delta$ values are observed with purely aromatic guest **10**. Guests **5**–**9** which have both aromatic and aliphatic fragments not only produce smaller upfield shifts, but also induce downfield shifts of the signals of some protons (C(6)H, C(7)OMe and C(7′)OMe, Table 3). Positive (downfield) shifts observed with aliphatic guests **1** and *S*-**3** most probably result from changes in DBT hydration due to the contacts with guest hydrophobic groups. Such deshielding microscopic solvation effects were reported for other host–guest complexes.[16] Also speaking in favor of this explanation is the downfield shift of the signals of all DBT protons on going from water to DMSO solvent.[14]

Table 2 Formation constants for complexes of anions of α-amino acids, their derivatives and some related compounds with DBT at 25 °C in D_2O and ionic strength 0.05 M

Guest	K (M^{-1})	Guest	K (M^{-1})
1	6.3 ± 1.9	*S*-**6**	8.8 ± 0.9
2	—[a]	*R*-**6**	10.6 ± 1.5
S-**3**	72 ± 9	*S*-**7**	59.5 ± 8.3
R-**3**	—[a]	*R*-**7**	15.8 ± 1.7
S-**4**	—[a]	**8**	24.0 ± 2.8
R-**4**	—[a]	**9**	92.4 ± 10.0
S-**5**	17.4 ± 1.6	**10**	11.2 ± 1.8
R-**5**	16.4 ± 1.2	$PhCOO^-$	11.6[b]

[a] No interaction. [b] From Ref. 14.

Inspection of Table 3 shows that with all guests the largest $\Delta\delta$ values, both negative and positive, are observed for protons at C(11). Other significantly affected signals belong to protons at C(16′), C(13), C(13′), C(9′), and the methyl group at N(2). As

Table 3 Complexation-induced shifts in different signals at saturation ($\Delta\delta$)

	1	*S*-**3**	*S*-**5**	*R*-**5**	*S*-**6**	*R*-**6**	*S*-**7**	*R*-**7**	**8**	**9**	**10**
C(1)H					−0.075			−0.122	−0.72		
C(1′)H			−0.167	−0.175	−0.079	−0.089					
N(2)CH_3		+0.008	−0.126	−0.120	−0.151	−0.135	−0.078	−0.13			−0.482
N(2)CH_2		+0.014			−0.015	−0.041					
N(2′)CH_2		+0.013			−0.007	−0.037					
C(3)H			−0.054	−0.041	−0.039						
C(6)H	+0.011	+0.014	0	0	+0.052	0	+0.027	+0.046	+0.087	+0.036	−0.168
C(6′)H	+0.010	+0.010				−0.037	−0.050	−0.094			
C(7′)OMe									+0.06	+0.049	0
C(7)OMe		+0.013	+0.017		+0.076	+0.03			−0.100	−0.039	−0.24
C(9′)H			−0.166	−0.192	−0.129	−0.133	−0.07	−0.137	−0.301	−0.084	−0.658
C(11)H	+0.018	+0.019	−0.268	−0.235	−0.332	−0.277	−0.324	−0.493	−0.680	−0.307	−1.025
C(11′)H					−0.077						
C(13)H			−0.141	−0.143	−0.184	−0.168	−0.115	−0.094	−0.207	−0.100	−0.576
C(13′)H			−0.129	−0.127	−0.102	−0.104	−0.064	−0.136	−0.229	−0.100	−0.655
C(15)OMe			−0.065	−0.067	−0.028	−0.04		−0.040	−0.098	−0.029	−0.254
C(16)H			−0.045	−0.041	+0.018	0	−0.018	−0.058	−0.063	−0.029	−0.226
C(16′)H	+0.010	+0.014	−0.192	−0.250	−0.15	−0.133	−0.097	−0.207	−0.463	−0.165	−1.010
C(17)H	+0.010	+0.012	−0.068	−0.060	−0.032	−0.050	−0.051	−0.073	−0.143	−0.070	−0.387
C(17′)H		+0.012	−0.031	−0.052	−0.017					−0.086	−0.564

one can see from Scheme 1a,b all these atoms are more accessible from one side of the macrocycle opposite to the benzyl group at N(2) and are closer to N(2) than to the N(2′) atom. This suggests that the binding occurs predominantly as is illustrated in Scheme 1a, probably with the carboxylate group of the guest directed towards the N(2) ammonium center. Smaller but still detectable shifts are observed also for the signals of protons situated at the periphery and even at the opposite side of the macrocycle, such as those at C(15)OMe, C(6), C(6′) and C(7)OMe. Apparently, there are several different possible modes of binding and complexes of different structures are in a fast equilibrium with each other.

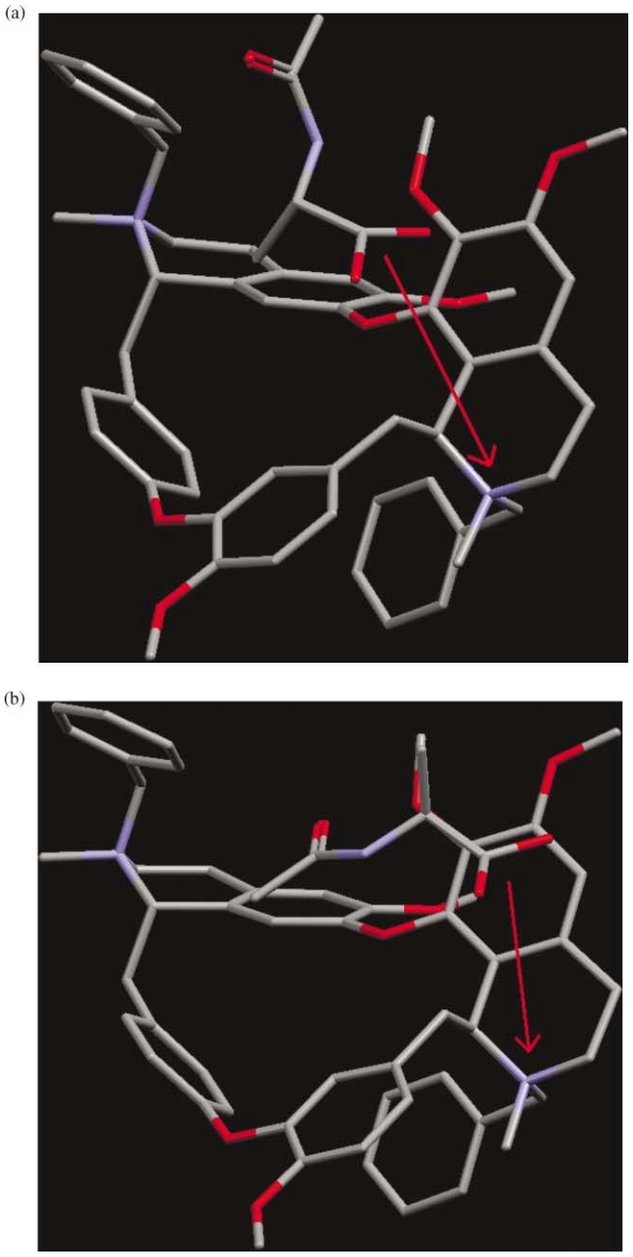
Aliphatic monoanions such as acetate or propionate do not form detectable complexes with DBT.[14] Nevertheless, monoanions of *N*-acetylated aliphatic amino acids **1** and *S*-**3** interact with DBT (Table 2). The presence of the *N*-acetyl group is important: free glycine and both enantiomers of alanine (guests **2** and **4**) in their anionic forms do not interact with DBT. Binding of *N*-acetylalanine is highly enantioselective. Fig. 1a illustrates the observed difference in titration curves for both enantiomers for protons at C(11). Since *R*-**3** did not affect signals of not only the usually more sensitive C(11), but of any of the DBT protons, we conclude that the absence of complexation-induced changes in the signals of protons at C(11) is not due to a different binding mode of the *R*-enantiomer, but the binding constant with this anion is indeed very small, at least below that with **1**. Therefore, the binding enantioselectivity factor can be estimated as $K(S\text{-}\mathbf{3})/K(R\text{-}\mathbf{3}) \geq 10$.

Stronger binding of *S*-**3** as compared to **1** indicates a significant contribution of the α-methyl group of **3** to the binding free energy equaling $\Delta\Delta G^{\circ} = -RT\ln(K(S\text{-}\mathbf{3})/K(\mathbf{1})) = -6.04$ kJ mol^{-1}. Also a large binding contribution can be assigned to the *N*-acetyl group. As one can see from Table 2 neither **2** nor *S*-**4**, which are deacetylated forms of **1** and *S*-**3** respectively, form detectable complexes with DBT. Under the experimental conditions employed the *K* values below *ca.* 3 M^{-1} may be already immeasurably small. Therefore we estimate the contribution of the *N*-acetyl group to the binding free energy to be in the range from −2.4 to −8.1 kJ mol^{-1}. The nature of these binding contributions is most probably the hydrophobic interaction of the guest methyl group with apolar moieties of DBT.

The binding of *S*-**3** was strong enough to allow us to measure the complexation-induced shifts in the NMR signals of all guest protons at saturation which appeared to be equal to −0.091, −0.089 and −0.090 ppm for methyl groups of the amino acid, *N*-acetyl fragment and the α-proton respectively. These limiting upfield shifts indicate that the guest protons are placed in the shielding region of DBT benzene rings. By their magnitude they are close to that observed for the α-methyl group of *R*-alanine on association with ristocetin A (−0.11 ppm).[17]

We attempted to rationalize the binding mode of *S*-**3** and the origin of enantioselectivity by using molecular mechanics simulations. The guest anion in its minimized conformation was positioned at the side shown by the arrow in Scheme 1a with the carboxylate group directed toward the N(2) ammonium center and the subsequent energy minimization of the complex produced two structures of similar energy shown in Scheme 3 in which either the alanine α-methyl group or the methyl group of the *N*-acetyl substituent fits the macrocycle cavity.

In both structures oxygen atoms of the guest carboxylate group are separated from the N(2) ammonium center by distances between 5.5 and 6.1 Å, sufficiently short for significant attractive interaction. The carbonyl oxygen of the amide group is directed outside the macrocycle and the NH proton is directed toward an oxygen atom of the host. Such orientation of the guest anion allows the amide group to minimize its possible unfavorable contact with the hydrophobic surface of the host and at the same time makes possible the hydrophobic interaction with methyl groups. However, it follows from the



Scheme 3 Simulated structures of the DBT complex with *S*-**3**.

observed equal limiting upfield shifts of the NMR signals of guest protons (see above) that both methyl groups of the guest make equally close contacts with aromatic rings of the host, and the molecular modeling clearly shows that the size of DBT is not sufficient to allow this. To find an explanation for this controversy we first performed an estimate of upfield shifts expected for both simulated structures. A proton NMR shift δ_{rc} caused by the ring current of a phenyl group is given by the equation (3)[18]

$$\delta_{rc} = 27.6\,(1 - 3\cos^2\theta)/r^3 \qquad (3)$$

where r is the distance (in Å) of the resonant proton from the centre of the phenyl ring, and θ is the angle between the normal and the vector from the ring centre to the proton. On the basis of the structures shown in Scheme 3 and assuming that the shifts of all three protons of each methyl group are averaged due to the free rotation, the δ_{rc} values for methyl groups of the amino acid and *N*-acetyl fragment and the α-proton respectively were calculated to be −0.2540, −0.1009 and −0.0637 ppm (structure "a") and −0.0829, −0.2676 and −0.3499 ppm (structure "b"). It is highly probable, however, that both structures co-exist in a rapid equilibrium and observed shifts are the average values. Assuming that contributions of structures "a" and "b" are 60 and 40% respectively we obtain the averaged complexation-induced upfield shifts of −0.186, −0.168 and −0.178 ppm for methyl groups of the amino acid and *N*-acetyl fragment and the α-proton. Although the absolute values of calculated shifts do not coincide with experimentally measured values (one hardly could expect such a coincidence taking into account the approximate character of simulated structures and strong distance dependence of δ_{rc}), they predict correctly the relative trend.

The simulated structure of the DBT complex with guest **1** resembles that for *S*-**3** in Scheme 3b with removed α-methyl group. In this structure the methyl group of the *N*-acetyl fragment fits to the host cavity and the carboxylate oxygens are at the same distance from N(2), but an essential difference is that the carbonyl oxygen of the amide group of **1** is directed toward the hydrocarbon moiety of DBT outside the cavity. Therefore it seems that the large binding increment of the α-methyl group in *S*-**3** results from two factors: first, formation of an additional structure "a" in which this group fits the host cavity providing an additional hydrophobic contribution and, second, improvement of the guest conformation in the structure "b" in which the amide carbonyl avoids unfavorable contact with the host apolar moiety.

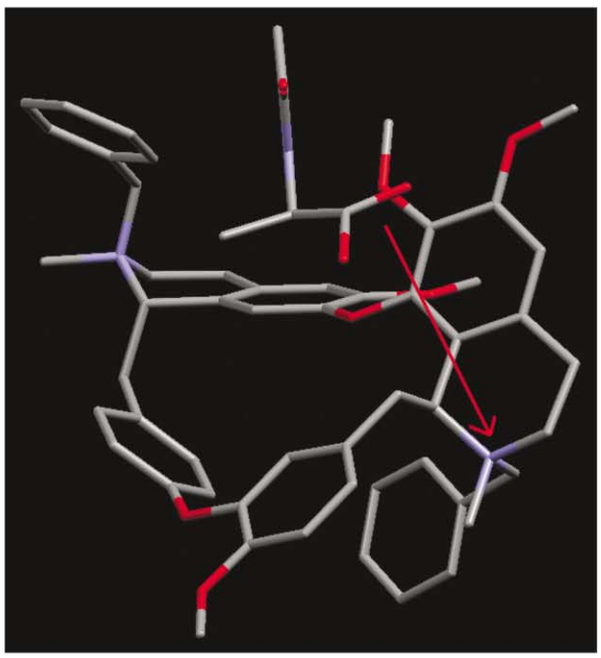
Too weak binding of *R*-**3** did not allow us to use the complexation-induced shifts in NMR spectra for preliminary positioning of the guest anion. Therefore molecular mechanics simulations of complexes with *R*-**3** were performed by interchanging positions of H and CH_3 at the α-carbon in structures "a" and "b" obtained for the *S*-enantiomer and subsequent minimization, Scheme 4. As a result, in structure "a" the α-methyl group becomes directed outside the host cavity and the respective hydrophobic contribution is lost, and in structure "b" this group appears directed toward oxygen atoms of methoxy groups of the ring A of DBT providing unfavorable contacts with the polar moiety of the host. These two factors explain the observed enantioselectivity.

There is a certain analogy in recognition properties of DBT and the vancomycin family of antibiotics. This involves the binding specificity to alanine, although with inverted enantioselectivity and smaller affinity, contribution of ion pairing between guest terminal carboxylate and the host cationic site, similar complexation-induced shifts of proton NMR signals and similar hydrophobic contributions of aliphatic guest moieties. Of course, DBT does not provide hydrogen bonding which is considered to be the major contribution to the binding to vancomycin.[17]

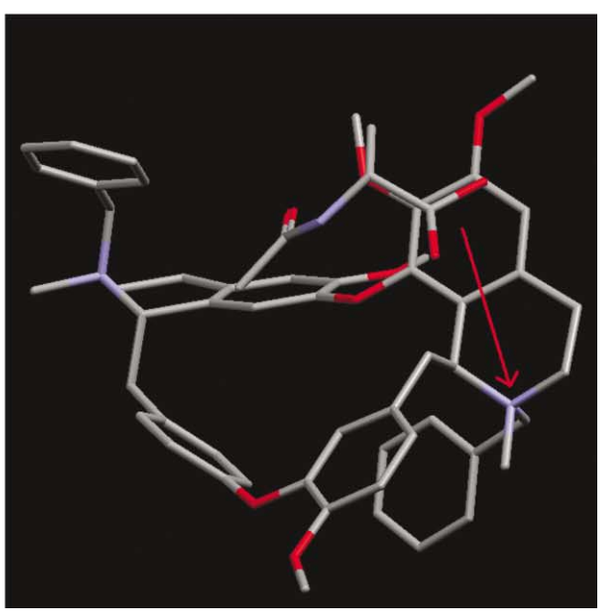
Substitution of the terminal alanine with more voluminous amino acid residues, *e.g.* tryptophan or tyrosine, strongly decreases the binding constant to vancomycin.[19] We observe a similar effect with DBT too: binding of *S*-**5** is weaker than that of *S*-**3** and in addition is not enantioselective, Table 2. Removal of the *N*-acetyl group from **5** leads to only a two-fold decrease in the binding constant (see results for *S*- and *R*-**6** in Table 2). Apparently these guests are too large to fit a small cavity in the DBT macrocycle and their binding is determined primarily by interaction with the phenylalanine benzene rings and the ion pairing with carboxylate. In accordance with this, the binding constants for enantiomers of **6** appear to be close to the *K* value for benzoate anion, 11.6 M^{-1} [14] and somewhat higher values of *K* for **5** reflect a typical *ca.* two-fold hydrophobic contribution of an additional methyl group.

1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid (**7**) is a rigidified analog of phenylalanine often employed in peptidomimetics[20] and since the DBT structure involves two 1,2,3,4-tetrahydroisoquinoline fragments we considered it interesting to see a possible effect of such rigidification, in particular, because there are little data on comparative recognition of natural and rigidified amino acids. Results for *S*-**7** and *R*-**7**

(a)



(b)



Scheme 4 Simulated structures of the DBT complex with *R*-**3**.

(Table 2) show that the affinity of the *R* enantiomer is only slightly higher than that for *R*-**6**, but the binding constant for *S*-**7** is significantly increased. The binding enantioselectivity factor equals $K(S\text{-}\mathbf{7})/K(R\text{-}\mathbf{7})$ = 3.8. The binding of neutral 1,2,3,4-tetrahydroisoquinoline **8** is also rather strong: the binding constant for **8** is larger than those for anions of aromatic amino acids (Table 2). Obviously the molecule of **8** fits very well to the surface of DBT and when the carboxylate group appears in the position which allows its contact with the host ammonium group this leads to a significant increase in the binding constant. Molecular modeling of the DBT complexes with enantiomers of **7** (Supplementary Information) confirms this explanation: calculated distances of guest carboxylate oxygens to the N(2) ammonium centre of DBT are 5.6 and 5.7 Å for the *S*-enantiomer, but 7.6 and 8.1 Å for the *R*-enantiomer. We tested also the binding of 1,2,3,4-tetrahydro-2-naphthalenecarboxylate (**9**) as a purely hydrocarbon analog of **7** and observed a significantly increased *K* value (Table 2). On the other hand, the binding constant for 1-isoquinolinecarboxylate (**10**), which is a completely aromatic analog of **7**, is close to those of *R*-**7** and benzoate (Table 2). Apparently the rigid structure of **10** does not allow a good fit of the guest to the DBT surface.

In conclusion, the alkaloid-based host DBT binds anionic forms of *N*-acetylated and free amino acids in water with *K* values similar to other macrocyclic hosts like calixarenes or cyclodextrins (*cf.* results in Tables 1 and 2), but differs from them in pronounced specificity to *N*-acetylalanine, previously observed only with macrocyclic antibiotics. We expect that the use of *S*,*S*-(+)-tetrandrine as a binding block for construction of more sophisticated host molecules with additional binding sites will allow the creation of new receptors for recognition of peptides specific for the presence of alanine residues. Another interesting point of this study is the appearance of a significant binding enantioselectivity on the rigidification of a natural α-amino acid, which by itself interacts with the host non-selectively.

Experimental

Materials

S,*S*-(+)-Tetrandrine, amino acids and their derivatives, inorganic salts and components of buffer solutions were purchased from commercial suppliers and used without further purification. Preparation and characteristics of *N*,*N*′-dibenzyltetrandrine dibromide (DBT) are described elsewhere.[14]

Instrumentation

NMR spectra were recorded on UNITY INOVA 400 and 500 MHz VARIAN spectrometers.

Methodology

The acids used as guest molecules were converted into the respective anions by adjusting pH of their solutions in D_2O 2–3 units above respective pK_a values by adding Na_2CO_3. The ^{1}H-NMR titrations were performed by adding aliquots of the guest stock solutions (typically 0.4 M) to *ca.* 2 mM solution of DBT in D_2O at the ionic strength 0.05 M. The experimental data were fitted using non-linear least-squares regression with the Microcal Origin 5 program. At least 10 signals of different protons of DBT were used for the fitting and obtained binding constants were averaged.

Molecular mechanics simulations were performed with Hypercube's hyperchem package, using the mm+ force field as implemented in the 6.03 version of the program. The structures of DBT–guest complexes were obtained by placing the guest in a position maximally close to the DBT protons which undergo the largest complexation-induced shifts in ^{1}H-NMR spectra, followed by minimization *in vacuo* of the potential energy using a combination of conjugated gradient and Newton–Raphson algorithms. In all cases the DBT macrocycle structure resembled the reported X ray structure [15] very accurately, also in complexes, using a 0.4184 kJ mol^{-1} convergence criterion in the minimization procedure.

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