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Complex Formation of Crown Ethers with α-Amino Acids: Estimation by NMR Spectroscopy

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Abstract—Complex formation of 18-crown-6 and dibenzo crowns with glycine, leucine, and norleucine was studied by NMR spectroscopy. The efficiency of non-valence interactions with participation of different active centers of the host and guest molecules is determined by solvation effects, mutual arrangement of benzene rings in dibenzo crowns, and the presence of bulky aliphatic substituents in the α-amino acid. The complexation of dibenzo crowns with α-amino acids in acid medium involves a system of different non-valence interactions, the most efficient of which are $NH_3^+\cdots O$ hydrogen bond between the ammonium group in the guest molecule and ether oxygen atoms in the host molecule and dipole–dipole interaction between the guest ammonium group and host benzene ring ($NH_3^+\cdots Ar$). The efficiency of $NH_3^+\cdots O$ hydrogen bonding decreases in going from 18-crown-6 to dibenzo crowns due to distortion of symmetry of the macroring cavity and violation of geometric complementarity of some ether oxygen atoms. The integral efficiency of non-valence interactions in the system dibenzo crown–α-amino acid was estimated on a quantitative level by ^1H NMR relaxation technique.

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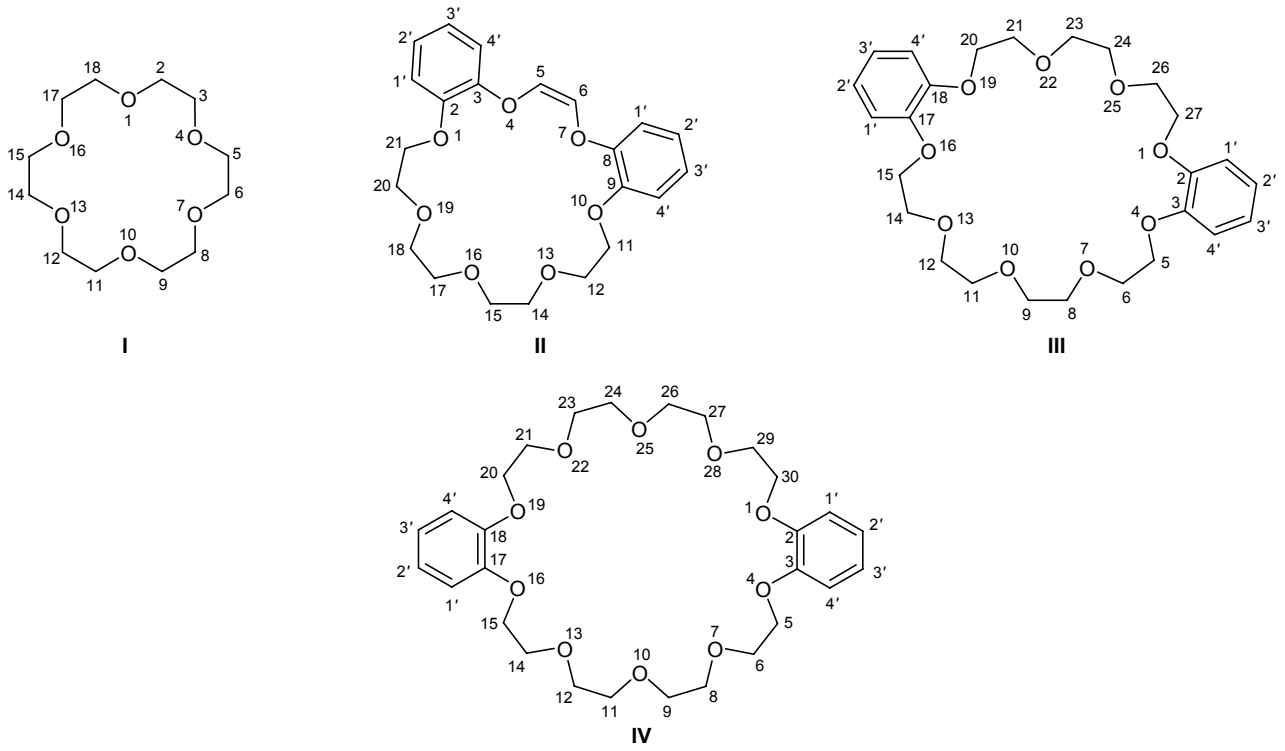
All known fields of application of crown ethers are based on their unique ability to selectively bind cations and neutral molecules [1]. Quite promising is the use of crown ethers and their derivatives for the separation of biomolecules, including α-amino acids, and for the design of new complex compounds possessing unique properties [1–3].

It is known that α-amino acids bind to macrocyclic crown ethers through the amino group. The interaction involves hydrogen bonding between amino hydrogen atoms of the guest molecule and electron-donor oxygen atoms of the host molecule, which is supplemented by ion–dipole interactions provided that the amino group is protonated. Important factors determining the complexing power of a crown ether toward α-amino acids are effects of the medium and solvation of the host and guest molecules, conformity of the geometric parameters of the host and guest molecules, and asymmetry of the crown ether cavity. From the viewpoint of studying complex formation processes, of particular interest are crown ethers containing benzene fragments. On the one hand, benzene ring (in addition to ether oxygen atoms) provides an additional coordination center capable of participating in various interactions with α-amino acids [dipole–dipole interaction between the amino group in α-amino acid and benzene ring in crown ether, which is supplemented by hydrogen bonding; dipole–dipole interaction between the carboxy group in α-amino acid and benzene ring, which is also supplemented by hydrogen bonding; charge-transfer interaction (π–π or *p*–π stacking) between the carboxy group and benzene ring]. Furthermore, side-chain groups in α-amino acids may be involved in additional interactions with both ether oxygen atoms and benzene rings of crown ether. On the other hand, benzene ring constituting a part of the crown ether macroring restricts conformational mobility of the latter and distorts its symmetry, which should impair the complexing power of crown ether. If two benzene rings are fused to a crown ether macroring (dibenzo crowns), the size and shape of the cavity are determined by mutual arrangement of the benzene fragments. The more distant are the latter from each other, the more probable is "sandwich" conformation



of the macroring with intramolecular π–π stacking of the benzene rings. The conformational mobility of such sandwich structure is essentially reduced as compared to non-benzo analogs, and the size of the cavity therein is smaller.

The goal of the present work was to examine the complexing power toward α-amino acids of 18-crown-6 and dibenzo crowns with different sizes of the macroring cavity and different mutual arrangements of the benzene rings. As host molecules we selected 18-crown-6 (**I**), [2,8]dibenzo-21-crown-7 (**II**), [2,14]dibenzo-27-crown-9 (**III**), and [2,17]dibenzo-30-crown-10 (**IV**). 18-Crown-6 was assumed to be a reference compound with symmetric cavity, which binds ammonium salts via three hydrogen bonds to form perch-like complex [1]. The ammonium group in such complexes is located above the mean plane of the macroring rather than inside the cavity {the size of the cavity in molecule **I** is 2.60–3.20 Å [1] which is comparable with the size of a hydrogen atom (van der Waals radius 1.19 Å [4]) rather than with the size of ammonium group}. Glycine, leucine, and norleucine were selected as model α-amino acids. The complex formation process was studied using several NMR techniques (^{1}H, ^{13}C, ^{1}H relaxation). The efficiency of complex formation was assessed by comparing variations of the chemical shifts of the host molecule (**I**–**IV**) and α-amino acid and spin–lattice relaxation times of protons in α-amino acid upon mixing of equimolar amounts of the reactants.

Solvent nature largely affects the complexation of crown ethers with α-amino acids. Protic solvents enhance the complexing ability of the amino group in α-amino acids. As concerns crown ether, the effect of protic solvent is not so definite. Depending on the solvent structure, crown ethers in solution either retain hydrophobic outer surface of their molecules where lone electron pairs on the oxygen atoms are oriented inward or become completely hydrophilic thus masking their lipophilic ethylene backbone. Therefore, in the first step of our study we examined solvation of 18-crown-6 and dibenzo crowns with protic organic solvents possessing different acid–base properties (trifluoroacetic acid and methanol). The type and efficiency of interactions between crown ether and protic solvent were judged by the change of chemical shifts of protons and carbon nuclei in molecules **I**–**IV** in going from aprotic solvent (DMSO) to protic. The chemical shifts of crown ethers **I**–**IV** in different solvents are given in Table 1.

The formation of hydrogen bond between a protic solvent and crown ether where ether oxygen atoms act as proton acceptors is reflected in upfield shift of the ^{1}H and ^{13}C signals from the ethylene fragments of the macroring. As follows from the data in Table 1, dissolution of **I**–**IV** in MeOH or TFA is not accompanied

Table 1. Chemical shifts of ^{1}H and ^{13}C ($\Delta\delta$, $\Delta\delta_C$, ppm) in the NMR spectra of crown ethers **I**–**IV** in different solvents[a]

18-Crown-6 (**I**)

Solvent	Nucleus	CH_2
DMSO	^{1}H	3.51 s
	^{13}C	69.82
MeOH	^{1}H	3.64
	^{13}C	71.59
TFA	^{1}H	3.48
	^{13}C	73.34

[2,8]Dibenzo-21-crown-7 (**II**)

		C^2, C^3, C^8, C^9	C^5H, C^6H	$C^{11}H_2$, $C^{21}H_2$	$C^{12}H_2$, $C^{20}H_2$	$C^{14}H_2$, $C^{18}H_2$	$C^{15}H_2$, $C^{17}H_2$	$C^{1'}H$, $C^{4'}H$	$C^{2'}H$, $C^{3'}H$
DMSO	^{1}H		4.31	4.09	3.73	3.58	3.53	6.97, 7.01	6.89, 6.91
	^{13}C	147.76, 148.38	67.24	67.82	68.77	70.09	70.20	113.27, 114.33	120.77, 121.34
MeOH	^{1}H		4.34	4.16	3.83	3.69	3.64	6.97, 7.02	6.90, 6.93
	^{13}C	150.02, 150.82	69.92	69.97	70.73	71.78	72.00	115.55, 117.34	122.57, 123.31
TFA	^{1}H		4.08	3.90	3.66	3.56	3.48	6.65	6.60
	^{13}C	151.23, 151.71	71.80	72.55	73.32	73.98	73.44	118.40	126.50, 127.04

[2,14]Dibenzo-27-crown-9 (**III**)

		C^2, C^3, C^{14}, C^{15}	C^5H_2, $C^{12}H_2$, $C^{17}H_2$, $C^{27}H_2$	C^6H_2, $C^{11}H_2$	C^8H_2, C^9H_2	$C^{18}H_2$, $C^{26}H_2$	$C^{20}H_2$, $C^{24}H_2$	$C^{21}H_2$, $C^{23}H_2$	$C^{1'}H$, $C^{2'}H$, $C^{3'}H$, $C^{4'}H$
DMSO	^{1}H		4.06	3.75	3.75	3.66	3.63	3.55	6.95, 6.88
	^{13}C	148.51, 148.45	68.54, 68.60	69.04	69.09	69.92	70.12	70.11	114.04, 114.27, 121.12, 121.20
MeOH	^{1}H		4.12	3.86	3.84	3.78	3.73	3.67	6.88, 6.94
	^{13}C	149.02, 149.08	68.92, 69.03	69.52	69.57	70.30	70.49	70.54	114.37, 114.62, 121.29, 121.36
TFA	^{1}H		3.87	3.68	3.64	3.64	3.57	3.51	6.57
	^{13}C	151.57	71.95	73.37	73.82	72.08	73.80	73.43	118.32, 118.42, 126.22, 126.25

[2,17]Dibenzo-30-crown-10 (**IV**)

		C^2, C^3, C^{17}, C^{18}	C^5H_2, $C^{15}H_2$, $C^{20}H_2$, $C^{30}H_2$	C^6H_2, $C^{14}H_2$, $C^{21}H_2$, $C^{29}H_2$	C^8H_2, $C^{12}H_2$, $C^{23}H_2$, $C^{27}H_2$	C^9H_2, $C^{11}H_2$, $C^{24}H_2$, $C^{26}H_2$	$C^{1'}H$, $C^{4'}H$	$C^{2'}H$, $C^{3'}H$
DMSO	^{1}H		4.06	3.74	3.63	3.55	6.95, 6.94	6.88, 6.86
	^{13}C	148.42	68.45	69.00	70.04	69.87	114.13	121.16
MeOH	^{1}H		4.12	3.84	3.74	3.67	6.94, 6.93	6.89, 6.88
	^{13}C	150.49	70.39	70.99	71.89	71.74	116.04	122.81
TFA	^{1}H		3.86	3.65	3.57	3.51	6.56	6.56
	^{13}C	151.60	72.01	73.35	73.73	73.44	118.54	126.26

[a] For atom numbering, see formulas **I**–**IV** on p. 1387.

Table 2. Variations ($\Delta\delta$, $\Delta\delta_C$,[a] ppm) of the ^1H and ^{13}C chemical shifts of crown ethers **I**–**IV** in going from DMSO to methanol and trifluoroacetic acid

Solvent	Nucleus	CH_2CH_2
18-Crown-6 (**I**)		
DMSO→MeOH	^1H	–0.13
	^{13}C	–1.77
DMSO→TFA	^1H	0.03
	^{13}C	–3.52

[2,8]Dibenzo-21-crown-7 (**II**)									
		C^2, C^3, C^8, C^9	C^5H, C^6H	$C^{11}H_2$, $C^{21}H_2$	$C^{12}H_2$, $C^{20}H_2$	$C^{14}H_2$, $C^{18}H_2$	$C^{15}H_2$, $C^{17}H_2$	$C^{1'}H$, $C^{4'}H$	$C^{2'}H$, $C^{3'}H$
DMSO→MeOH	^1H		–0.03	–0.07	–0.10	–0.11	–0.11	0, –0.01	–0.01, –0.02
	^{13}C	–2.26, –2.44	–2.68	–2.15	–1.96	–1.69	–1.80	–2.28, –3.01	–1.80, –1.97
DMSO→TFA	^1H		0.23	0.19	0.07	0.02	0.05	0.32, 0.36	0.29, 0.31
	^{13}C	–3.47, –3.33	–4.26	–4.73	–4.55	–3.89	–3.24	–5.13, –4.07	–5.73, –5.70

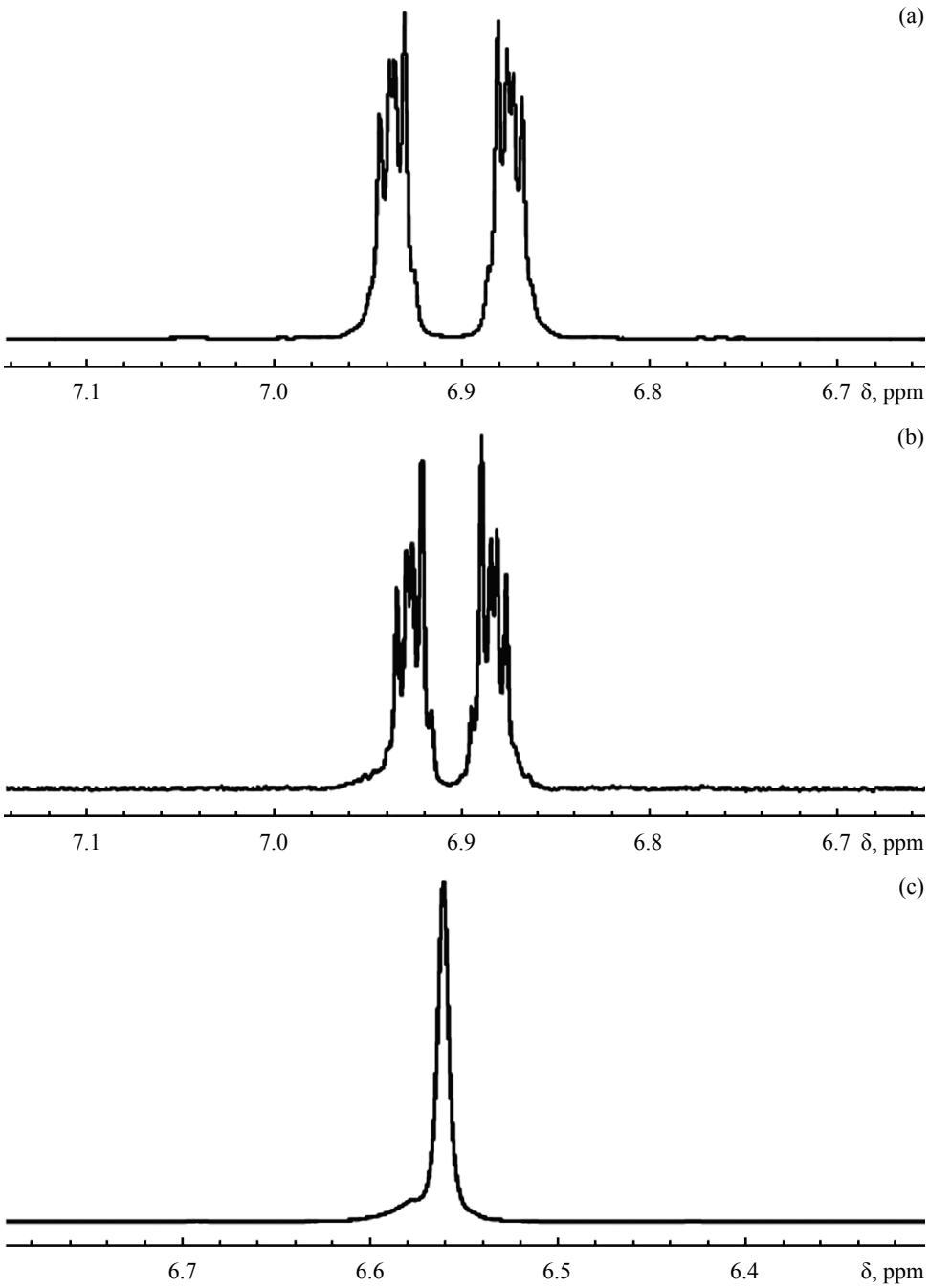
[2,14]Dibenzo-27-crown-9 (**III**)									
		C^2, C^3, C^{14}, C^{15}	C^5H_2, $C^{12}H_2$, $C^{17}H_2$, $C^{27}H_2$	C^6H_2, $C^{11}H_2$	C^8H_2, C^9H_2	$C^{18}H_2$, $C^{26}H_2$	$C^{20}H_2$, $C^{24}H_2$	$C^{21}H_2$, $C^{23}H_2$	$C^{1'}H$, $C^{2'}H$, $C^{3'}H$, $C^{4'}H$
DMSO→MeOH	^1H		–0.06	–0.11	–0.09	–0.12	–0.10	–0.12	–0.07, –0.06
	^{13}C	–0.51, –0.63	–0.38, –0.43	–0.48	–0.48	–0.38	–0.37	–0.43	–0.33, –0.35, –0.17, –0.16
DMSO→TFA	^1H		0.19	0.08	0.11	0.02	0.06	0.04	0.38, 0.31
	^{13}C	–3.06, –3.12	–3.41, –3.35	–4.33	–4.73	–2.16	–3.68	–3.32	–4.28

[2,17]Dibenzo-30-crown-10 (**IV**)								
		C^2, C^3, C^{17}, C^{18}	C^5H_2, $C^{15}H_2$, $C^{20}H_2$, $C^{30}H_2$	C^6H_2, $C^{14}H_2$, $C^{21}H_2$, $C^{29}H_2$	C^8H_2, $C^{12}H_2$, $C^{23}H_2$, $C^{27}H_2$	C^9H_2, $C^{11}H_2$, $C^{24}H_2$, $C^{26}H_2$	$C^{1'}H$, $C^{4'}H$	$C^{2'}H$, $C^{3'}H$
DMSO→MeOH	^1H		–0.06	–0.10	–0.11	–0.12	0.01	–0.01, –0.02
	^{13}C	–2.07	–1.94	–1.99	–1.85	–1.87	–1.91	–1.65
DMSO→TFA	^1H		0.20	0.09	0.06	0.04	0.39, 0.38	0.32, 0.30
	^{13}C	–3.18	–3.56	–4.32	–3.69	–3.57	–4.51	–5.10

[a] Paramagnetic shifts are assumed to be negative, and diamagnetic, positive.

by variation of the above chemical shifts, which unambiguously indicates the lack of hydrogen bonding between the acidic proton of the solvent molecule and ether oxygen atoms of **I**–**IV**. A probable reason is that the hydroxy group in methanol and carboxy group in trifluoroacetic acid are efficiently involved in a complex H-bond system where these groups act as proton donors and acceptors simultaneously. The sensitivity to steric effects suggests that crown ethers and their dibenzo derivatives in MeOH and TFA retain hydrophobic outer surface of their molecules, i.e., they remain preorganized for complex formation.

The solvation effects were identified by analysis of the variations in the chemical shifts of structural fragments in crown ethers **I**–**IV** in going from DMSO to MeOH and from DMSO to TFA (Table 2). The data in Table 2 show that dissolution of **I**–**IV** in methanol is accompanied by downfield shifts of the ^1H and ^{13}C signals from both ethylene fragments of the macroring and from the benzene rings. The downfield shift of the ethylene proton signals is considerably larger than that observed for the aromatic protons, and differentiation of signals from protons in different positions of the benzene ring is retained. Figure shows signals from



Signals from aromatic protons in the 1H NMR spectra of [2,17]dibenzo-30-crown-10 (**IV**) in (a) DMSO, (b) MeOH, and (c) TFA.

aromatic protons in the ^{1}H NMR spectra of **IV**, recorded from solutions in DMSO, MeOH, and TFA. The downfield shift of the aromatic ^{13}C nuclei included into the macroring or neighboring thereto is larger than that observed for the distant ^{13}C nuclei. The downfield shift of ^{13}C nuclei increases as the macroring becomes conformationally more rigid. These findings allowed us to presume that the main solvation effect of MeOH is related to van der Waals interactions of atoms constituting the macroring cavity with the methyl group of solvent molecules. Van der Waals interactions induce deformation of the electron shell surrounding nuclei, and reduction of the symmetry of electron distribution provides a paramagnetic contribution to the shielding constant [5].

Dissolution of crown ethers **I**–**IV** in TFA is accompanied by upfield shift of protons and downfield shift of carbon nuclei, the maximum shifts being observed

for the benzene ring, and the minimum, for the ethylene fragments of the macroring containing no fused benzene ring or remote from the latter. This means that the interaction with TFA involves the benzene rings in dibenzo crown ethers rather than ether oxygen atoms. The upfield shift of the aromatic proton signals increases in the series **II** < **III** < **IV**. Following the same series, signals from protons in different positions of the benzene ring become more similar in their multiplicities and δ values. For instance, in the ^{1}H NMR spectrum of a solution of **II** in DMSO, signals from the aromatic protons appear as d.d (1′-H), t.d (2′-H), t.d (3′-H), and d.d (4′-H). In going from DMSO to TFA, the four aromatic multiplets are transformed into one broadened doublet and one broadened triplet. In the ^{1}H NMR spectra of **III** and **IV** in TFA, the 1′-H–4′-H protons give rise to one broadened singlet (see figure). These data indicate that the benzene rings in dibenzo crown ethers act as integrated electron systems in the interaction with TFA. The negative field of the carboxy group dipole deshields carbon nuclei (downfield or paramagnetic shift) and induces displacement of the C–H bond electrons toward hydrogen atoms (upfield or diamagnetic shift).

Thus, the solvation of dibenzo crown ethers by MeOH and TFA differently affects the complexing ability of the ether oxygen atoms. The ether oxygen atoms in dibenzo crowns **II**–**IV** are not involved in interaction with TFA, and their complexing ability does not change. Dissolution of **II**–**IV** in methanol is accompanied by shielding of the macrocycle cavity by methyl group of solvent molecules via van der Waals interactions between the solvent and atoms that form the cavity, which creates some steric hindrances to the complexation of dibenzo crown with α-amino acid. For comparison, Table 3 contains the chemical shifts of the methylene fragments in crown ether **I** in solution in TFA and MeOH in the absence and in the presence of an equimolar amount of glycine. It is seen that there is no diamagnetic shift of protons in crown ether upon dissolution of equimolar amounts of **I** and glycine in MeOH; this means that there is no hydrogen bonding between the ether oxygen atoms of **I** and amino group of the amino acid. An insignificant paramagnetic shift (which does not exceed 3σ) may be attributed to weak van der Waals interactions. An equimolar mixture of **I** and glycine in TFA displayed a diamagnetic shift of protons in the crown ether (Δδ = 0.12 ppm), indicating hydrogen bonding between host and guest molecules. This example clearly demonstrates advantage of TFA as solvent which enhances complexing ability of the amino group of α-amino acid but does not impair complexing ability of the ether oxygen atoms in crown ethers and their dibenzo derivatives. Therefore, further study on the complexation of dibenzo crowns with α-amino acids was performed using TFA as solvent.

Table 3. Chemical shifts of the ethylene protons (δ, ppm) in 18-crown-6 (**I**) and its mixture with glycine in trifluoroacetic acid and methanol

Solvent	**I**	**I**–glycine
Trifluoroacetic acid	3.48	3.36
Methanol	3.64	3.66

In the next step we examined the efficiency of hydrogen bonding between the ammonium group of α-amino acid and ether oxygen atoms of crown ethers **I**–**IV**. Variations of the proton and carbon chemical shifts of the ethylene fragments in **I**–**IV** and of the ammonium groups in α-amino acids in equimolar mixtures of crown ethers and amino acids relative to the corresponding parameters of the initial components are given in Tables 4–7.

It is seen (Tables 4, 5) that the set of signals of mixtures of **I**–**IV** with amino acids differs from the overall set of signals of the initial components, which indicates interaction between the components. Protons in the ammonium group of α-amino acids displayed a paramagnetic shift, while protons and carbon nuclei in the ethylene fragments of **I**–**IV** showed a diamagnetic shift. This means that the interaction is hydrogen bonding. The efficiency of hydrogen bonding is not high, for the corresponding Δδ values insignificantly exceed those induced by van der Waals interaction. It should be noted that the paramagnetic shift of protons in the ammonium group of α-amino acids in mixtures with crown ether **I** increases in the series leucine ≈ norleucine < glycine and that the diamagnetic shift of ^{1}H and ^{13}C in the ethylene fragments of **I** decreases in the same series. This apparent contradiction is rationalized by the fact that the total Δδ value is contributed

Table 4. Variations (Δδ, $\Delta\delta_C$, ppm) of the ^{1}H and ^{13}C chemical shifts of the ethylene fragments in 18-crown-6 (**I**) and of the ^{1}H chemical shift of the ammonium group in α-amino acids upon mixing their equimolar amounts

Fragment	Nucleus	**I**–glycine	**I**–leucine	**I**–norleucine
CH_2	^{1}H	0.12	0.13	0.13
	^{13}C	0.14	0.36	0.27
NH_3^+	^{1}H	–0.32	–0.27	–0.26

Table 5. Variations ($\Delta\delta$, $\Delta\delta_C$, ppm) of the ^1H and ^{13}C chemical shifts of the ethylene fragments in crown ethers **II**–**IV** and of ^1H chemical shifts of the ammonium group in α-amino acids upon mixing their equimolar amounts

System	Nucleus	C^5H, C^6H	$C^{11}H_2$, $C^{21}H_2$	$C^{12}H_2$, $C^{20}H_2$	$C^{14}H_2$, $C^{18}H_2$	$C^{15}H_2$, $C^{17}H_2$	NH_3^+
II–glycine	^1H	0.01	0.05	0.10	0.06	009	–0.16
	^{13}C	0.96	1.28	0.34	0.45	0.35	
II–leucine	^1H	–0.02, 0.09	0.06, 0.11	0.24	0.18, 0.19	0.14, 0.15	–0.19
	^{13}C	0.02	0.73	0.24	0.55	0.06	
II–norleucine	^1H	0.02	0.08	0.22	0.19	0.11	–0.21
	^{13}C	0.02	0.55	0.23	0.56	0.02	

		C^5H_2, $C^{12}H_2$, $C^{17}H_2$, $C^{27}H_2$	C^6H_2, $C^{11}H_2$	C^8H_2, C^9H_2	$C^{18}H_2$, $C^{26}H_2$	$C^{20}H_2$, $C^{24}H_2$	$C^{21}H_2$, $C^{23}H_2$	
III–glycine	^1H	0.05	0.07	0.12	0.09	0.09	0.05	–0.10
	^{13}C	0.23	0.17	0.49	–0.29	0.55	1.07	
III–leucine	^1H	0.01	0.03	0.07	0.03	0.04	0.01	–0.15
	^{13}C	0.17	0.17	0.23	0.06	0.47	0.13	
III–norleucine	^1H	0.01	0.03	0.05	0.03	0.04	0.40	–0.18
	^{13}C	0.04	0.14	0.21	0.03	0.01	0.13	

		C^5H_2, $C^{15}H_2$, $C^{20}H_2$, $C^{30}H_2$	C^6H_2, $C^{14}H_2$, $C^{21}H_2$, $C^{29}H_2$	C^8H_2, $C^{12}H_2$, $C^{23}H_2$, $C^{27}H_2$	C^9H_2, $C^{11}H_2$, $C^{24}H_2$, $C^{26}H_2$	
IV–glycine	^1H	0.06	0.07	0.07	0.04	–0.10
	^{13}C	0.0	0.22	0.52	0.12	
IV–leucine	^1H	0.01	0.03	0.04	0.0	–0.14
	^{13}C	0.24	0.22	0.21	0.14	
IV–norleucine	^1H	0.01	0.03	0.04	–0.01	–0.13
	^{13}C	0.16	0.12	0.15	0.06	

not only by hydrogen bonding but also by short-range van der Waals interactions. Both these factors induce paramagnetic shift of the NH_3^+ protons, whereas the effects of hydrogen bonding and van der Waals interactions on the ethylene fragment are opposite: H-bonding induces diamagnetic shift, and van der Waals interactions, paramagnetic. The closer the H-bonded atoms to each other, the stronger the deformation of their electronic shells, the larger the contribution of the paramagnetic component to $\Delta\delta$, and the weaker the overall diamagnetic shift of the ethylene fragments. On the basis of the above reasonings we can conclude that hydrogen bonding of crown ether **I** with glycine is more efficient than with leucine and norleucine. This conclusion is very consistent with the available published data on steric effects of alkyl substituents in alkylammonium salts upon complex formation with crown ethers [1].

The efficiency of hydrogen bonding with the ammonium group of α-amino acids decreases in going from 18-crown-6 (**I**) to dibenzo crowns **II**–**IV**. This follows from the ranges of paramagnetic shifts of the NH_3^+ protons: $\Delta\delta$ –0.26 to –0.32 (**I**) –0.16 to –0.21 (**II**), –0.10 to –0.18 (**III**), and –0.10 to –0.14 ppm (**IV**). The most probable reason is violation of complementarity of the binding centers in the host and guest molecules, resulting from steric effects of the benzene rings (increase of conformational rigidity and asymmetry of the macrocycle) and alkyl substituents in α-amino acids. Steric hindrances created by alkyl groups in α-amino acids hamper most appropriate orientation of the ammonium group with respect to the ether oxygen atoms in dibenzo crown ether. As a result, the contribution of paramagnetic shift (due to van der Waals interactions) to the $\Delta\delta$ value of NH_3^+ increases. Therefore, the paramagnetic shift of the ammonium protons in mixtures of crown ethers **II**–**IV** with glycine is smaller than in mixtures of **II**–**IV** with leucine and norleucine.

Comparison of the ranges of diamagnetic shifts for the ethylene fragments of **II**–**IV** in mixtures with α-amino acids [$\Delta\delta$, $\Delta\delta_C$, ppm: 0.24–0.01, 1.28–0.02 (**II**); 0.12–0.01, 1.07–0.01 (**III**); 0.07 to –0.01, 0.52–

0.0 (**IV**)] also indicates weakening of H-bonding in the series **II** > **III** > **IV**. The hydrogen bonds formed by different ether oxygen atoms in **II**–**IV** are nonequivalent due to their unsymmetrical orientation with respect to the NH_3^+ group. As a result, the chemical shifts of the ethylene fragments change differently. These differences increase when the ethylene fragments are additionally involved in van der Waals or dipole–dipole interactions with structural fragments of α-amino acids. Not only different ethylene fragments but also geminal protons in each methylene group in **II** become nonequivalent in an equimolar mixture of **II** with leucine whose molecule contains an isobutyl group.

Thus analysis of the variation of chemical shifts of crown ethers **I**–**IV** in mixtures with equimolar amounts of α-amino acids led us to conclude that the efficiency of hydrogen bonding between the ammonium group of α-amino acid and ether oxygen atoms decreases in going from 18-crown-6 (**I**) to dibenzo crowns **II**–**IV**. Increase in the macrocycle size does not favor enhancement of hydrogen bonds, for extension of the aliphatic chain between the benzene rings in molecules **II**–**IV** creates conditions for intramolecular π–π stacking. This interaction essentially distorts the symmetry of the macrocycle cavity, reduces its real size, and restricts conformational mobility of the macrocycle. The presence of alkyl substituents in α-amino acid molecule also weakens hydrogen bonds as a result of increased steric hindrances to approach of the ammonium group to the ether oxygen atoms.

We then proceeded to analysis of the efficiency of interaction between α-amino acids and the second binding site of dibenzo crowns **II**–**IV**, benzene rings. For this purpose, we examined variations in the chemical shifts of the aromatic protons and carbon nuclei in **II**–**IV** and carbon nuclei in the carboxy group of amino acids (Tables 6, 7). The data in Table 6 show that mixtures of **I** with α-amino acids are characterized by a small paramagnetic shift of the carboxy carbon nucleus; its magnitude does not exceed 3σ for leucine and norleucine. The downfield shifts of the C=O signals correlate with those of the NH_3^+ protons. Therefore, we presumed that the observed paramagnetic shift of the C=O carbon nucleus results from deshielding of the neighboring α-hydrocarbon fragment due to participation of the ammonium group in hydrogen bonding with the ether oxygen atoms in **I**.

The downfield shift of the C=O signal increases in going to dibenzo crown **II**, while the efficiency of $NH_3^+\cdots O$ hydrogen bonding decreases. This means that the ammonium group in α-amino acids bound to crown **II** is involved in additional dipole–dipole interaction (probably, hydrogen bonding) with the benzene rings.

Mixtures of dibenzo crown **III** with α-amino acids showed a more complex pattern of the variation of the C=O chemical shift. The paramagnetic shift of the C=O carbon nucleus of glycine in a mixture with **III** was larger than in its mixtures with **I** and **II**. This may be due to either enhanced alternative non-valence interaction with the ammonium group or steric factors. Mixtures of **III** with leucine and norleucine displayed a diamagnetic shift of the C=O carbon nucleus as a result of dipole–dipole interaction where the carboxy group acts as negative pole. Likewise, paramagnetic shift of the C=O carbon nucleus was observed for a mixture of **IV** with glycine, whereas the $\Delta\delta_C$ values for its mixtures with leucine and norleucine were positive. Therefore, the system of non-valence interactions between α-amino acids and dibenzo crown **IV** is the same as with **III**, the only difference is the efficiency of these interactions.

An insignificant diamagnetic shift for aromatic protons is observed in mixtures of **II**–**IV** with α-amino acids (Table 7); this shift exceeds 3σ for mixtures of **II** with all the examined amino acids and for a mixture of **III** with glycine. These data suggest that protons in the benzene rings are involved in neither dipole–dipole interaction nor hydrogen bonding with the carboxy group of α-amino acid; otherwise, a paramagnetic shift of the aromatic protons would be observed. The diamagnetic shift of aromatic carbon nuclei exceeds 3σ for mixtures of **II** with the three amino acids, of **III** with glycine, and of **IV** with leucine. Presumably, the observed diamagnetic shift of aromatic ^{13}C and ^{1}H nuclei is induced by dipole–dipole interaction (probably, H-bond) between the benzene rings and ammonium group, which is supplemented by shielding of the neighboring ether oxygen atoms which are involved in hydrogen bonding with the ammonium group. These data are very consistent with the assumption made on

Table 6. Variations of the chemical shifts of the C=O carbon nucleus ($\Delta\delta_C$, ppm) in α-amino acids on mixing with an equimolar amount of crown ethers **I**–**IV**

Amino acid	**I**	**II**	**III**	**IV**
Glycine	–0.52	–0.72	–1.22	–0.86
Leucine	–0.05	–0.82	0.45	0.72
Norleucine	–0.21	–1.00	0.44	0.42

Table 7. Variations of the ^{1}H and ^{13}C chemical shifts ($\Delta\delta$, $\Delta\delta_C$) of the benzene rings of crown ethers **II**–**IV** on mixing with an equimolar amount of α-amino acid

Mixture	Nucleus	$\Delta\delta$, $\Delta\delta_C$, ppm	
		1′, 4′	2′, 3′
II–glycine	^{1}H	0.05	0.0
	^{13}C	1.14, 1.48	0.43, 0.97
II–leucine	^{1}H	0.03	0.02
	^{13}C	0.37	0.02, 0.37
II–norleucine	^{1}H	0.04	0.01
	^{13}C	0.17	0.01, 0.46
III–glycine	^{1}H	0.09	0.04
	^{13}C	0.20, –0.51	–0.03, –0.32
III–leucine	^{1}H	0.01	0.01
	^{13}C	–0.01, 0.28	–0.10, –0.23
III–norleucine	^{1}H	0.01	0.01
	^{13}C	–0.03, 0.13	–0.09, –0.15
IV–glycine	^{1}H	0.02	0.02
	^{13}C	0.11	–0.06
IV–leucine	^{1}H	0.01	0.01
	^{13}C	0.47	0.34
IV–norleucine	^{1}H	0.01	0.01
	^{13}C	0.0	–0.10

the basis of variation of the chemical shift of the C=O carbon nuclei in α-amino acids: unlike 18-crown-6 (**I**), the ammonium group of α-amino acid interacts with not only ether oxygen atoms but also with the benzene rings of dibenzo crowns **II**–**IV**. The largest diamagnetic shifts of ^{1}H and ^{13}C in the benzene rings were observed for a mixture of **II** with glycine; therefore, the $NH_3^+ \cdots$ Ar dipole–dipole interaction in that mixture is the most efficient.

A mixture of **III** with glycine is characterized by the opposite variations of the ^{13}C chemical shifts of the two benzene rings. This may be due to different orientations of the benzene rings with respect to the ammonium group. Frontal orientation of the benzene ring with respect to the ammonium group should lead to diamagnetic shifts of the aromatic protons and paramagnetic shifts of the aromatic carbon nuclei. If the ammonium group is located above (or below) the benzene ring plane, dipole–dipole interaction between them should result in paramagnetic shifts of both ^{13}C and ^{1}H nuclei.

Mixtures of dibenzo crown **III** with leucine and norleucine and of **IV** with glycine and norleucine displayed no significant (>3σ) variations of the chemical shifts of aromatic ^{13}C and ^{1}H nuclei. This means that the benzene rings in **III** and **IV** are not involved in appreciable non-valence interactions with the corresponding amino acids. Then, the larger paramagnetic shift of the C=O carbon nucleus of glycine in its mixture with **IV**, unlike a mixture of glycine with 18-crown-6 (**I**), is produced not by additional dipole–dipole interaction between the benzene ring and NH_3^+ group but exclusively by enhanced van der Waals interaction between the carboxy group and the macrocycle. Correspondingly, significant diamagnetic shifts of the C=O carbon nuclei in mixtures of **III** with leucine and norleucine and of **IV** with norleucine should be attributed to dipole–dipole interaction between the carboxy group and ethylene fragments rather than between the former and the benzene ring.

Thus, on the basis of the ^{1}H and ^{13}C NMR data we can presume that, unlike 18-crown-6 (**I**), the complexation of α-amino acids with dibenzo crowns **II**–**IV** is characterized by not only weakened hydrogen bonding of the ammonium group in the guest molecule with the ether oxygen atoms in the host but also more complicated system of non-valence interactions between the host and guest molecules. Crown ether **I** binds α-amino acids only via hydrogen bonding between the ether oxygen atoms of **I** and ammonium group of amino acid. Complex formation of **IV** with glycine, apart from the above hydrogen bonding, involves van der Waals interactions between the carboxy group of glycine and the macrocycle. In the complexes of **II** with α-amino acids and of **III** with glycine the ammonium group interacts not only with the ether oxygen atoms but also with the benzene rings. In the complexes of **III** with leucine and norleucine and of **IV** with norleucine hydrogen bonding with participation of the guest ammonium group and host ether oxygen atoms is supplemented by dipole–dipole interaction between the guest carboxy group and host ethylene fragments. The complexation of dibenzo crown **IV** with leucine involves hydrogen bonding between the ammonium group of the amino acid and ether oxygen atoms of the macrocycle, dipole–dipole interaction between the carboxy group and ethylene fragments, and dipole–dipole interaction between the ammonium group and benzene rings.

With a view to quantitatively assess the efficiency of different non-valence interactions in mixtures of

Table 8. Spin–lattice relaxation times T_1(^{1}H-α) (s) of the α-proton in α-amino acids and their mixtures with crown ethers **I–IV**

α-Amino acid	Pure acid	α-Amino acid–**I**	α-Amino acid–**II**	α-Amino acid–**III**	α-Amino acid–**IV**
Glycine	0.665±0.002	0.59±0.02	0.484±0.008	0.516±0.002	0.555±0.005
Leucine	0.876±0.007	0.62±0.05	0.67±0.01	0.660±0.008	0.585±0.009
Norleucine	0.841±0.007	0.64±0.05	0.683±0.003	0.678±0.003	0.682±0.002

crown ethers **I–IV** with α-amino acids, we measured the ^{1}H spin–lattice relaxation times (T_1) of α-amino acids in solution in the absence and in the presence of an equimolar amount of crown ether **I–IV** (Table 8). It is known that T_1 is a quantitative parameter characterizing the molecular dynamics (rotational and translational) which is determined by a number of factors, including the molecular size and shape, temperature, viscosity of solution, and intra- and intermolecular interactions [6–9]. Enhancement of intermolecular interactions, other conditions being equal, leads to increase of the relaxation rate and reduction of T_1. The relaxation rate is an additive quantity which is contributed mainly by the dipole–dipole and spin rotation components [9]. Rapid rotation of the terminal fragments of small molecules may considerably distort T_1 of nuclei in these fragments [8, 9]. Therefore, we confined ourselves to analysis of variation of ^{1}H spin–lattice relaxation times for the central α-hydrocarbon fragment of α-amino acid [T_1(^{1}H-α)] and did not consider T_1 values for the ammonium group and terminal aliphatic substituent.

As follows from the data in Table 8, the T_1(^{1}H-α) values for mixtures of **I–IV** with α-amino acids are lower than those found for individual α-amino acids, which indicates the existence of host–guest non-valence interactions. In going from 18-crown-6 (**I**) to dibenzo crowns **II–IV** T_1(^{1}H-α) values for leucine and norleucine increase (except for leucine–**IV**). This confirms the conclusion drawn previously, according to which hydrogen bonds formed by the ammonium group of the guest molecule and ether oxygen atoms of the host molecule weaken in going from crown **I** to dibenzo crowns **II–IV**. Alternative non-valence interactions (dipole–dipole interaction between the ammonium group and benzene rings or between the carboxy group and ethylene fragments) do not compensate for weakening of $NH_3^+\cdots O$ hydrogen bonds. Only if both these alternative interactions exist (leucine–**IV**) in addition to $NH_3^+\cdots O$ hydrogen bonding, the complexation becomes more efficient [T_1(^{1}H-α) 0.62 and 0.59 s for leucine–**I** and leucine–**IV**, respectively].

The T_1(^{1}H-α) values for mixtures with glycine decrease in going from crown ether **I** to **II–IV**, i.e., the total efficiency of non-valence interactions between glycine and dibenzo crowns **II–IV** is higher than between glycine and 18-crown-6 (**I**). This is consistent with one more previously drawn conclusion that glycine forms more efficient $NH_3^+\cdots O$ hydrogen bonds than those formed by leucine and norleucine. The shortest relaxation time T_1(^{1}H-α) corresponds to the system glycine–dibenzo crown **II** where the dipole–dipole interaction between the ammonium group of amino acid and benzene rings of **II** is the strongest (Table 7).

Thus the results of studying complex formation of crown ethers **I–IV** with α-amino acids by different NMR techniques are very consistent with each other.

EXPERIMENTAL

The NMR spectra were recorded at 25°C on an Agilent DD2 NMR System 600 spectrometer at 599.996 MHz for ^{1}H and 150.882 MHz for ^{13}C using standard 1D and 2D techniques [5]. The T_1 values were measured by the inversion recovery sequence method (IRSM) [6]. Methanol-d_4, DMSO-d_6, and trifluoroacetic acid were used as solvents. Taking into account different solubilities of crown ethers and α-amino acids in these solvents and the necessity of using equimolar amounts of the reactants, their concentration was selected depending on the solvent: 0.1 mmol/mg in TFA and 0.004 mmol/mg in methanol-d_4 and DMSO-d_6. The chemical shifts were measured relative to tetramethylsilane (in methanol-d_4 and DMSO-d_6) or DMSO (δ 2.5, δ_C 39.52 ppm; in TFA) as internal reference. The ^{1}H and ^{13}C signals were assigned on the basis of their position and multiplicity, as well as using two-dimensional homo- (^{1}H–^{1}H COSY) and heteronuclear (^{1}H–^{13}C HSQC, ^{1}H–^{13}C HMBC) correlation techniques. Because of low sensitivity of ^{13}C NMR, only ^{1}H NMR and ^{1}H–^{1}H COSY experiments were carried out for dilute solutions. Variation of a chemical shift was assumed to be significant if the following condition was met: $\Delta\delta > 3\sigma$, where σ is the error in the

determination of chemical shifts (σ = 0.01 ppm for ^{1}H and 0.1 ppm for ^{13}C).

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