JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2007; **20:** 506–513
Published online 29 April 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/poc.1189

Experimental evidence of chiral crown ether complexation with aromatic amino acids

Małgorzata Koźbiał and Jarosław Poznański*

Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

Received 29 September 2006; revised 9 March 2007; accepted 14 March 2007

ABSTRACT: The complexation of L- and D-enantiomers of phenylglycine, phenylalanine, and tryptophan with D-mannonaphto-crown-6-ether in methanol solution was studied by NMR and isothermal titration calorimetry (ITC) at 298.15 K. The total heat effects attributed to the binding phenomena were measured in the range of 1.8 to 7.7 mJ, and the complexation was found stereo-specific. The binding topologies were estimated basing on ^{1}H 2D-ROESY experiments. The analysis of Job plots obtained from ^{1}H NMR-monitored titrations proved the coexistence of 1:1 and 1:2 (crown ether:amino acids) complexes, which thermodynamic parameters, K_s, ΔG, ΔH°, and $T\Delta S$ were determined with the aid of ITC. The 1:1 complexes were found enthalpically stabilized, generally by electrostatic interactions between the charged NH_3^+ group of amino acid and crown ether macrocyclic moiety, while the binding of the second amino acid molecule was driven entropically due to solvatophobic effect. Strong enthalpy–entropy compensation points towards the uniform binding mode of all complexes studied. The mode of complex formation was found solvent dependent. For phenylalanine guest studied in various solvent systems, in contrast to the aqueous media, the noticeable chiral recognition is observed in methanol solution, and the complex stoichiometry (1:2 ether:Phe) differs from the 2:1 one, determined previously for the same host-guest system in water (J. Thermal. Anal. Cal. 2006; 83: 575–578). Copyright © 2007 John Wiley & Sons, Ltd.
Supplementary electronic material for this paper is available in Wiley InterScience at http://www.interscience.wiley.com/jpages/0894-3230/suppmat

KEYWORDS: chiral crown ether; amino acids; complexation; ITC; NMR

INTRODUCTION

Amino acids and peptides are biologically active substances of great importance. Their interaction patterns with enzymes and antibodies as well as DNA are nowadays the subject of intensive studies. In the last decades, numerous macromolecular systems were engineered to achieve a stereo-specific recognition of peptide systems. Some of such systems exhibits so large specificity towards given amino acid residues that they act as an artificial receptors.[1,2] Although cyclodextrin,[3] calixarene,[4] or porfirin[5,6]-based hosts molecules were successfully applied in sequence-specific amino acid recognition, crown macrocycles were commonly used as binders of *N*-terminal amino groups.[5,7,8] Crown ethers are known to form complexes with amines, amino acids, and peptides by forming hydrogen bonds between oxygen atoms of macrocyclic rings and protonated NH_3^+ group.[9–14] The ability of crown ethers to bind amines is used in separation processes and analytical chemistry for the modification of either the stationary or mobile phase in chromatography and capillary electrophoresis.

Our previous investigations demonstrated the chiral differentiation of the analyzed amino acids upon water–chloroform extraction, by HPLC, and upon transport through either liquid or supported membranes by means of chiral crown ethers coupled with sugar units.[15–18] Sugar-attached crown ethers were also used to achieve enantiomeric recognition of amino acids in Langmuir monolayers.[19]

The example of such type of compound is D-mannonapho-crown-6-ether **1** (Fig. 1), which was originally designed and synthesized by Pietraszkiewicz[15] to obtain the chiral recognition of amino acids. The crown ether **1** consists of sugar and naphthalene moieties attached to the 18-crown-6 macrocyclic skeleton. The macrocyclic ring of chiral crown ether **1** is a major binding site for primary chiral ammonium cations. Sugar unit serve a source of chirality providing hydroxyl groups capable to form a pattern of hydrogen bonds with side chains of amino acid molecules. Naphthalene moiety creates an additional binding site enabling π–π stacking interactions, enabling specific recognition of amino acid aromatic side chains.[5,7] Thus, interaction with chiral

**Correspondence to:* J. Poznański, Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland.
E-mail: jarek@ichf.edu.pl

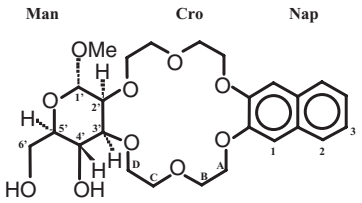

Figure 1. Structure of D-mannonaphto-crown-6-ether **1**. The sugar and naphthalene moieties are attached to the 18-crown-6 macrocycle

macrocyclic molecules could be applied in separation of amino acids' enantiomers.

The observation of thermal effects of complex formation between macrocyclic receptors and biological compounds expand our knowledge about real biological systems. The majority of biological processes undergo in aqueous solutions, and therefore in our previous investigations, we have studied the complexation phenomena between chiral crown ether **1** and enantiomers of phenylalanine (in three ionic forms: zwitterion, potassium, and hydrochloride salts) in aqueous solutions with the aid of both conduction and titration calorimetry.[20,21] The heat effects of the complexation were found differentiating, both in the sign and the magnitude, for the three analyzed forms of phenylalanine, but for each couple of D,L enantiomers, the thermal responses were found very close to each other. The main reasons for the poor chiral recognition upon the complexation in aqueous solution were attributed to the strong interaction of amino acids and crown ether with the solvating water molecules. This was confirmed by X-ray-derived molecular structure, where in crystal unit cell, the two conformers of chiral crown ether **1** were solvated by seven well-resolved water molecules (Lipkowski and Koźbiał, unpublished data).

Since the strong entropic partition rise from solvation changes of host and guest molecules upon binging,[22] majority of available data concerning the binding of amino acids by crown ethers were obtained from measurements in methanol solution.[11–13] In the presented study, we have also followed the complexation phenomena between chiral crown ether **1** and amino acids enantiomers in methanol solutions. The presented study was performed to help in rational functionalization of ether **1** improving its stereo-specific discrimination of *N*-terminal aromatic amino acids. In order to recognize the mechanism of the complexation, and to determine the thermodynamic parameters and stability constants (K_s) for complexes formed by chiral crown ether **1** and enantiomers of phenylglycine, phenylalanine, and tryptophan, the isothermal calorimetric titrations were carried out. Additionally, the ^{1}H NMR-monitored titration experiments were performed, and ^{1}H 2D-ROESY spectra were recorded to investigate the intermolecular interaction patterns characterizing the complexes formed.

EXPERIMENTAL

Materials

Amino acids, Phenylglycine (PhGly), Phenylalanine (Phe), and Tryptophan (Trp) were purchased from Fluka, and used as received. Crown ether **1** was synthesized individually by Pietraszkiewicz[15]. Methanol (Chempur, Piekary Śląskie, Poland) was of analytical grade. Methanol-d4 (99.5% ^{2}H enriched) was purchased from ARMAR (Switzerland).

Titration calorimetry

Titration Calorimetry experiments were carried out using isothermal titration calorimeter Omega-ITC (Microcal, Inc., Northampton, MA). The microcalorimetric titrations were performed at 298.15 K in methanol solutions. In each run, a constant volumes (8 μl/injection) of 10 mM crown ether **1** methanol solution initially placed in the 250 μL syringe were injected into stirred (400 rpm) sample cell containing 1.3186 cm^3 of 1 mM amino acids methanol solution. Each titration experiment consisted of 30 successive injections.

The ORIGIN software (Microcal, Inc.) was used to estimate the binding constants and enthalpy changes (ΔH°) of reaction in a single titration experiment. Prior to the analysis, the resulted heat effects were corrected for the dilution of reactants, both determined in the separate experiments.

Initially, for all the analyzed systems, the 1:1 binding model was applied. Its thermodynamics parameters were then used as the starting points for 1:2 complexation model. The final model, either 1:1 or 1:2 one, was selected with the aid of F-Snedecor test, assuming the 0.01 significance level.

NMR spectroscopy

^{1}H NMR spectra were recorded on 500 MHz spectrometer (Unity Plus Varian) at 298 K in d4-methanol solution. Methanol resonances (^{1}H 3.31 ppm, quintet, ^{13}C 49.15 ppm, septuplet) were used as the internal references. The stoichiometry of the complexes was determined using Job's method.[23] Solutions (2 mM) of crown ether and amino acids enantiomers were mixed at different volumetric ratios. The spectra were processed with the aid of MestRe-C 2.3a.[24] Zero-filling up 64k data points and $\pi/3$ shifted square sine-bell filter were applied prior to Fourier transformation. All 2-D spectra were acquired in d4-methanol solution using 384 increments of 1k data points in direct proton dimension, and processed with the aid of NmrPipe[25] software using $\pi/3$ shifted sine-bell filter and zero-filling up to 1k data points in indirect dimension prior to Fourier transformation. All 2D spectra were analyzed with the help of Sparky 3.111

DOI: 10.1002/poc

Table 1. ^{1}H and ^{13}C chemical shifts of free crown ether **1** assigned in deuterated methanol solution at 298.15 K

Crown ether moiety/resonance	$\Delta\sigma$ (ppm)	
	^{13}C	^{1}H
Crown		
A	69.16	4.32
B	69.95	4.02/3.93
C	72.48; 71.59	3.90/3.75; 3.89/3.72
D	72.48; 69.16	3.82/4.06; 3.69/3.90
Mannopyranoside		
1′	98.39	4.88
2′	73.99	3.92
3′	81.30	3.64
4′	68.88	3.79
5′	74.56	3.47
6′	62.57	3.80/3.68
Naphtyl		
1	108.19	7.34/7.29
2	127.21	7.79/7.64
3	125.33	7.01
Methanol-d_4	49.14	3.31

Crown, Mannopyranoside, and Naphthalene moieties – Fig. 1

program.[26] In order to identify crown ether spin systems, 10 and 80 ms mixing time TOCSY spectra were recorded for both free and 1:1 amino acid–ether mixture. ^{1}H and ^{13}C resonance assignments were done basing on the heteronuclear ^{1}H-^{13}C HSQC spectrum combined with the 10 ms and 80 ms mixing time TOCSY and 300 ms mixing time ROESY spectra. Additional set of TOCSY and ROESY spectra recorded in the presence of amino acid was used to identify the intermolecular interaction pattern. The full assignment of free crown ether is reported in Table 1.

RESULTS AND DISCUSSION

The study of complexation process in methanol solution was carried out with the aid of titration calorimetry at 298.15 K. The total heat responses corresponding to the studied complexation phenomena were negative, failing in the range of −16.1 to −48.0 kJ per mol of injectant. The largest effects were found for the PhGly: −48.0 kJ mol^{-1} and −37.2 kJ mol^{-1} for D- and L-enantiomers, respectively. The differences between heat effects of the complexation enantiomers of the same amino acid, $\Delta\Delta H_{D,L}$, varied in the order of −1.1 kJ mol^{-1}, −10.8 kJ mol^{-1}, and −13.8 kJ mol^{-1} for Phe, PhGly, and Trp, respectively. The overall enthalpy changes upon titration of amino acids enantiomers by crown ether **1** per mol of injectant are summarized in Table 2. The heat effects of the complexation between crown ether **1** and enantiomers of phenylglycine, phenylalanine, and tryptophan were found higher than those previously observed in aqueous solution.[20,21] In contrast to the aqueous environment, all the complexes are enthalpically stabilized, and the chiral

Table 2. Total enthalpy effects of complex formation measured by isothermal titration microcalorimetry in methanol solution at 298.15 K

Amino acid	Total heat effect of complexation per mol of crown ether **1** (kJ mol^{-1})	$\Delta\Delta H_{D,L}$(kJ mol^{-1})
D-PhGly	−48.0	−10.8
L-PhGly	−37.2	
D-Phe	−17.2	−1.1
L-Phe	−16.1	
D-Trp	−22.9	−13.8
L-Trp	−36.7	

differentiation is observed – the largest for Trp, and the lowest for Phe. Experimental calorimetric titration curves for all the three amino acids studied are presented in Fig. 2. The observed dependence of ΔH° on crown ether concentration displays the same tendencies for both Trp

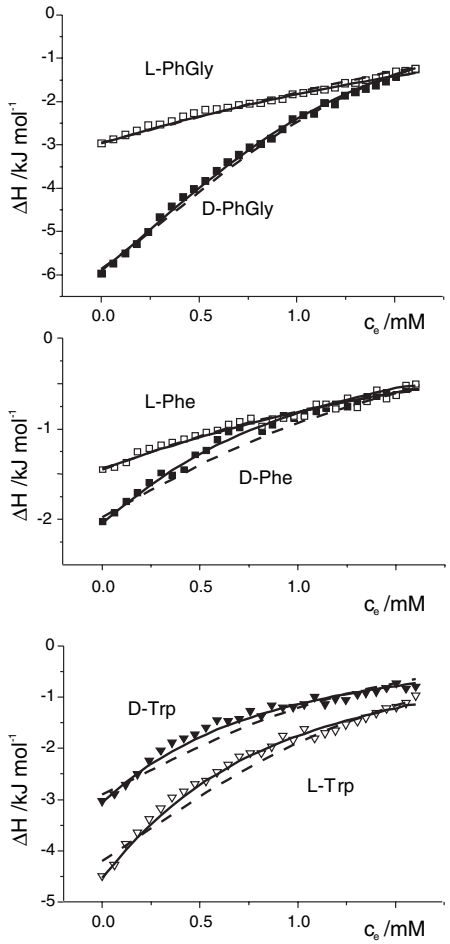

Figure 2. Calorimetric titration curves obtained for the complexation of PhGly, Phe, and Trp by crown ether **1**. The changes in enthalpy of complexation are presented as a function of ether **1** concentration. Dashed lines represents 1:1 model, and solid ones correspond to 1:2 complexation model. In the case of PhGly and L-Phe 1:1 model was found, at the significance level of 0.01, sufficiently describing the titration experiment

DOI: 10.1002/poc

and Phe enantiomers, but differs markedly for D and L-enantiomers of PhGly. In all the cases, the largest heat flow were observed at the beginning of titrations process. Our earlier study on D- and L-phenylalanine complexation by chiral crown ether **1** in aqueous media (in a form of zwitterions, hydrochloride, and potassium salt) indicated that two ether molecules may be involved in binding of single amino acid residue, and the model of two binding sites was found to properly describe the complexation phenomena. In aqueous solutions, the relative ratio of 1:1 and 2:1 complexes depends on the reagent concentrations – the excess of ether drives the system to the 2:1 complexation mode, while 1:1 complex dominates at high amino acid concentrations.

In methanol solutions, the stoichiometry of studied complexes was determined by Job's method using ^{1}H NMR measurements. The variations in chemical shifts of proton resonances derived from both sugar and aromatic parts of crown ether could be detected upon amino acid addition. The changes in chemical shift of aromatic and mannose (HM3′, HM4′ HM6′b) protons of ether **1** are summarized in Table 3. These changes are stereo-specific and they are larger for D enantiomers of PhGly and Phe and L-enantiomer of Trp. The significant differentiation of enantiomers were also observed for all amino acids, but in the case of PhGly, overlapped signals of sugar ring protons did not split, what should be attributed to the restricted flexibility of PhGly side chain shortened by the lack of methylene C(β)H$_2$ group separating amino acid's main chain and aromatic ring, and in consequence different modes in binding to crown ether **1**. The changes in resonance line positions upon complexation of D- and L-enantiomers of Phe and Trp are close, but in this case, the analyzed signals were well separated. These amino acid molecules carry more flexible side-chains, which can adopt a conformation enabling effective magnetic shielding of mannose protons upon complex formation. The changes in pattern of

Table 3. The bound shifts, $\Delta\delta_{CSI}$, of chiral crown ether **1** resonances determined for complexation of aromatic amino acids enantiomers in methanol solution at 298.15 K

ether **1** resonance	$\Delta\delta_{CSI}$, ppb estimated for a given guest molecule					
	D-PhGly	L-PhGly	D-Phe	L-Phe	D-Trp	L-Trp
H 1a	6	6	1	−1	2	4
H 1b	6	6	15	12	3	4
H 2a	0.3	−0.3	6	5	26	0
H 2b	2	10	7	6	6	0
H 3a	−13	5	16	12	−36	−37
H 3b	4	−0.3	15	13	−38	−37
H 3′	60	54	80	35	30	42
H 4′	60	54	85	35	34	36
H 6′b	60	54	89	36	39	31

The data, calculated from NMR monitored titration experiments and ITC-determined stability constants (K), are biased at ∼25% level due to K uncertainty propagation.

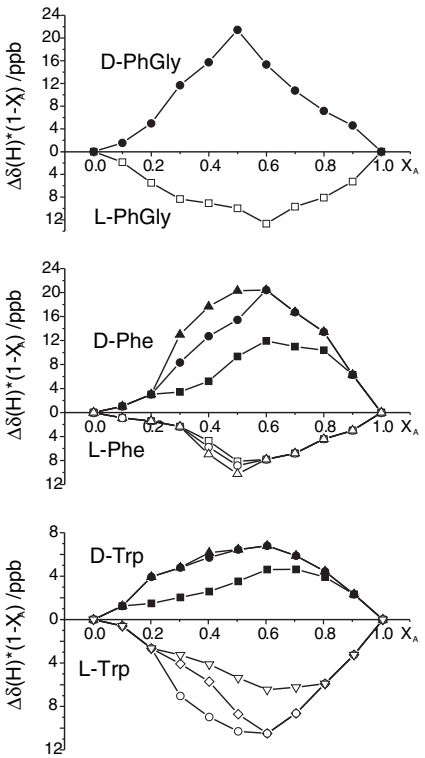

Figure 3. Job plots obtained for phenylglycine, phenylalanine, and tryptophan-crown ether **1** complexes by 1H NMR titrations. The traces corresponding to L-enantiomers (empty marks) are mirrored

resonance signals of macrocyclic ring and naphthalene moiety proton resonances are smaller, but their existence indicates that these parts of chiral host are also involved in complexation of aromatic amino acids. The largest deviations in aromatic part of crown ether NMR spectra were observed upon binding Trp enantiomers (protons H2b and H3). Small changes in the position of amino acid's side chain aromatic proton resonances were also observed upon binding.

The variations in chemical shifts of crown ether caused by changes in amino acid concentration were used for the determination of complex stoichiometry. The variation of sugar protons D- and L-PhGly upon complexation by crown ether **1** were also analyzed. The Job plots obtained for pairs of enantiomers of amino acids studied are collected in Fig. 3. The asymmetry of the shape of the curves clearly indicates the dominance of either 1:1 or 1:2 (crown ether: amino acid) complexes, which proportions depends on both the amino acid residue type and the reagents concentration.

Amino acid residues also display changes in chemical shift upon complexation, but due to the strong signal overlapping with ether resonances those of aliphatic Hα and Hβ could be hardly assigned, while strongly coupled spin systems of aromatic protons practically precludes the quantitative analysis. Generally, the tendencies in chemical shift changes observed for the amino acid stereoisomers reflect the stability of their complexes.

DOI: 10.1002/poc

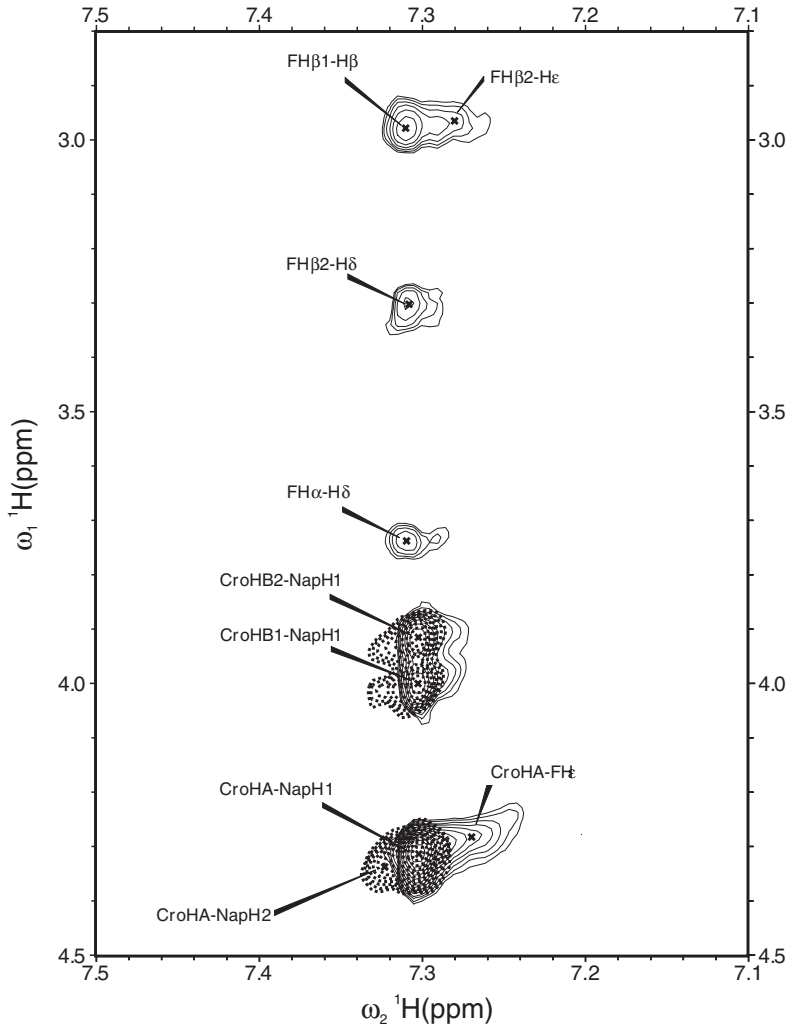

Figure 4. Fragment of 2-D ROESY spectrum of L-Phe-ether **1** complex in methanol-d_4. Chopped lines correspond to the spectrum of ether 1, while the solid ones represent the spectrum recorded after Phenylalanine addition. One of the cross-peaks involving Phe(H^{ε}) is unequivocally assigned to the intermolecular interactions

Thus, aromatic signals of L- and D- stereoisomers of PhGly experience the CIS up to 30 ppb and 13 ppb, respectively, while for Trp, the tendency is reversed −3 ppb and 4 ppb. In the case of Phe guests, the effect for L-isomer is negligible, while for D-one is in the order of 1–2 ppb.

The intermolecular magnetization transfer evidenced in ROESY experiments, confirmed that the crown ether macrocyclic ring is directly involved in the complexation of phenylalanine. The efficient intermolecular magnetization transfer was undoubtfully assigned between Hε resonance of Phenylalanine and naphtyl H2 and HA/HB of crown, respectively. The rest of expected intermolecular contacts are observed, but could not been unequivocally assigned due to the strong signal overlapping (Fig. 4). Moreover, the two HA (crown) resonances degenerated in the free ether **1** are well resolved upon amino acid binding. All these changes point to the topology of the complex, indicating that the phenylalanine benzene ring is protected from the solvent being placed just between the naphthyl and crown moieties of ether **1**. The mannose part putatively interacts both with backbone carboxyl group and side chain of amino acid, supporting weak source of stereospecificity.

Basing on the stoichiometry obtained from ^{1}H NMR titration, the heat effects measured upon isothermal calorimetry titrations were analyzed. The stability constant values and thermodynamic parameters of complexation processes for all amino acids studied were determined according to the complexation equilibria:

$$E + A \overset{K1}{\longleftrightarrow} EA \quad (1)$$

$$EA + A \overset{K2}{\longleftrightarrow} EA_2 \quad (2)$$

where E is crown ether **1** and A is amino acid. The estimated values of K_s, ΔG, ΔH° and $T\Delta S$ are collected in Table 4.

Both enantiomers of the smallest amino acid analyzed, PhGly, were found to bind in the same manner forming

DOI: 10.1002/poc

Table 4. Stability constants and thermodynamic parameters determined for complexation of enantiomeric amino acids with crown ether **1** in methanol solution at 298.15 K

Amino acid	K_1 ($dm^3\ mol^{-1}$) K_2 ($dm^3\ mol^{-1}$)	ΔH° ($kJ\ mol^{-1}$)	$T\Delta S$ ($kJ\ mol^{-1}$)	ΔG ($kJ\ mol^{-1}$)
D-PhGly	1346 (±130)	−9.6 (±0.1)	8.2 (±0.4)	−17.8 (±0.3)
L-PhGLy	441 (±132)	−9.0 (±0.6)	6.1 (±1.9)	−15.1 (±1.0)
D-Phe	616 (±197)	−5.4 (±0.4)	10.5 (±1.3)	−15.9 (±0.9)
	1027 (±127)	3.3 (±0.6)	21.0 (±0.5)	−17.2 (±0.4)
L-Phe	363 (±82)	−5.5 (±0.7)	9.1 (±1.3)	−14.6 (±0.6)
D-Trp	359 (±78)	−9.2 (±1.2)	5.3 (1.8)	−14.5 (±0.6)
	1228 (±292)	3.8 (±0.9)	21.4 (±1.6)	−17.6 (±0.7)
L-Trp	407 (±162)	−14 (±2.0)	0.8 (±1.1)	−14.8 (±1.2)
	1285 (±307)	7.7 (±1.4)	25.4 (±2.0)	−17.7 (±0.7)

1:1 complex, exhibiting the negative enthalpy and positive entropy changes upon binding. The chiral discrimination was achieved due to the difference in the entropic terms. The 1:1 complexes of D- and L-Phe are also stabilized by negative enthalpy and positive entropy, the binding the second molecules of D-Phe is accompanied by positive enthalpy and entropy changes. The thermal effect of complexation process is close for both enantiomers. The complexes of D- and L-enantiomers of Tryptophan have similar origin, the 1:1 complexes for both enantiomers are enthalpically stabilized, and 1:2 ones are entropy driven. The L-enantiomer of Trp is strongly bounded. The values of stability constants and thermodynamic parameters showed that for both enantiomers of PhGly, the complexes are enthalpically stabilized. The complexation processes of both D (1:2 stoichiometry and) and L (1:1 stoichiometry) enantiomers of Phe were connected with small enthalpic and stronger entropic effects. In the case of tryptophan (both 1:2 stoichiometry), the formation of 1:1 complexes was enthalpically stabilized and the binding of the second molecules of tryptophan was entropically stabilized process with positive enthalpy. The values of Gibbs free energy do not differ significantly for amino acids studied (−10.9 to −17.8 kJ mol^{-1}). The similar pairs of values of K_1 and K_2 were obtained for both enantiomers of tryptophan, but higher for L-enantiomer. This suggested similar mechanism of complexation of complexation of D and L-enantiomers of Trp by crown ether. Both 1:2 complexes are stabilized by solvatophobic interactions between naphthyl and indole rings of ether molecule and tryptophan, respectively, while the 1:1 complex is stabilized by electrostatic interactions between the amino groups of amino acids and crown ether macrocyclic ring. Thus, it is strongly suggested that in the 1:1 complex, the molecule of tryptophan is bound to non-chiral part of crown ether, while the other Trp molecule is stabilized by the aromatic π–π stacking. The proposed geometries of 1:2 complexes of D and L-Trp with crown ether **1**, calculated by Molecular Mechanics using HyperChem 6 software, which satisfy all the NMR and calorimetric data, are shown in Fig. 5.

CONCLUSIONS

The results of calorimetric and ^{1}H NMR titration experiments point towards the heterogeneity of the mechanism of binding process in methanol solution. The Job curves obtained from ^{1}H NMR measurements indicated the presence of 1:1 and 1:2 (crown ether:amino acid enantiomers) complexes, and the stoichiometry preferences depend on both the amino acid type and its chirality. The heat effects of complexation were found moderate, but differentiating each couple of enantiomers studied. The results of ^{1}H NMR and calorimetric measurements allowed the interpretation of the details of binding equilibrium, which take place during the titration experiments. The proposed binding model for D and L-Trp suggests that all 1:1 complexes are stabilized enthalpically by direct interaction of charged *N*-terminal amino group with crown macrocycle. The main source of

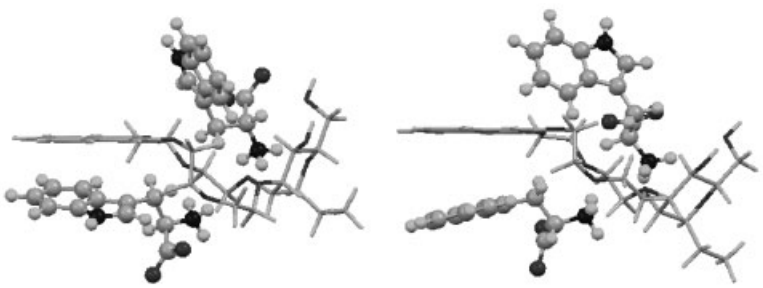

Figure 5. Schema of the organization of 1:2 complexes of crown ether **1** with D (left) and L (right) Tryptophane. The complexes were built with the aid of ChyperChem 6 using Amber forcefield

DOI: 10.1002/poc

stereo specific differentiation arise from thermodynamic properties of 1:2 complexes, in which one molecule of amino acid is bond to bottom and second to upper part of crown ether, as it could be concluded from the data presented in Table 2. The positive entropy change in 1:2 complexes results from the balance of the desolvation of both host and guest molecules upon complexation and the contribution from host–guest association, since the binding of two partners to forming a complex results in a more negative ΔS° due to the loss of degrees of freedom for translation and rotation,[27] while the opposite effect is observed upon the release of solvent molecules and counter ions engaged in the solvation of the binding sites.[28] This results in the gain of entropy when the contact surfaces are unfavorably solvated in the free state. This effect is also widely observed in the protein crystallization phenomena.[29] Since in the stabilization of 1:2 complexes participate mainly aliphatic and aromatic parts of molecules (c.f. Fig. 5), the desolvation of apolar parts of both molecules overcompensates entropy loss upon complexation of the second amino acid molecule to the 1:1 complex. Contrary, the 1:1 complexes are stabilized by the interaction of ether with the amino acid *N*-amino group, and the entropy gain upon desolvation of the charged NH_3^+ group is significantly lower.

The endothermic binding of the second amino acid residue raise from unfavorable interaction between the two charged *N*-terminal amino groups occupying proximal sites on the both crown macrocycle sides, as it is roughly presented in Fig. 5. Thus preventing binding of the 'bottom' molecule should improve ether **1** affinity and selectivity towards aromatic residues.

This binding scheme is applicable to all amino acid residues studied with the only exception of PhGly and L-Phe, for which the binding of the first amino acid molecule to the crown moiety preclude the succeeding binding of the second one.

The linear enthalpy–entropy dependence estimated independently for both 1:1 and 1:2 complexes formation, as presented in Fig. 6, is frequently observed in

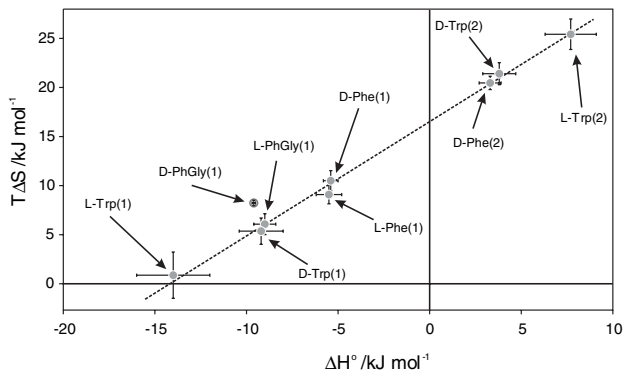

Figure 6. The enthalpy–entropy dependence determined for the complexes of enantiomeric aromatic amino acids with chiral crown ether **1**. The slope of the correlation line, 1.16 ± 0.03, indicates the relatively large reorganization of ether **1** required for efficient binding

various host–guest systems, and validates both the quality of experimental data and data treatment procedure applied. The observed clustering of thermodynamic parameters determined for 1:1 and 1:2 complexes in enthalpy/entropy coordinates confirms the direct observations that both types of complexes differ in the interaction patterns. The common slope of the obtained line characterize the effect of conformational changes of host molecules required for the efficient complexation, while the intercept is a measure of the solvatophobic effect.[30] The ΔH°–$T\Delta S$ linear dependence indicates that the all, but D-PhGly complexes are characterized by a slope of 1.160 ± 0.03, higher than that determined for calixarene- or cyclodextrin-based Host molecules,[31] indicating the larger conformational changes required for efficient binding. The intercept value of 16.5 ± 0.3 kJ mol^{-1} is close to the values found for macrocycles of the similar size. The enthalpy–entropy dependence for D-PhGly complex falls out of the correlation found, indicating the improved spatial compatibility of the crown ether **1** Host molecule towards this PhGly enantiomer.

The ^{1}H NMR data indicated that all three moieties of crown ether:macrocyclic ring, sugar unit, and naphthalene ring are involved in amino acids molecules binding. The additional binding site, enabling 1:2 complexation, defines the overall 'pocket' on the chiral receptor, and thus requires a more defined spatial orientation of chiral guest than the primary one, what should cause higher enantiomer selectivity. In consequence, 1:1 complexes driven by direct electrostatic interactions between *N*-terminal amino group and crown macrocycle exhibits similar thermodynamic parameters, slightly moderated by the properties of amino acid, while 1:2 complexes' stability are much more sensitive for the both amino acid type and its chirality.

Finally, it could be concluded that presented results confirmed that calorimetric and ^{1}H NMR measurements may be successfully used in the studies of chiral recognition of amino acids, and the information obtained from calorimetric and NMR data can help in designing the methods of separation of amino acids enantiomers.

Acknowledgements

The authors thank Prof. Wojciech Zielenkiewicz and Prof. Janusz Lipkowski for their kind interest and constant support, and Oksana and Prof. Marek Pietraszkiewicz for the synthesis of the crown ether **1** and consultancy.

REFERENCES

1. Fokkens M, Schrader T, Klärner F-G. *J. Am. Chem. Soc.* 2005; **127**: 14415–14421.
2. Heitmann LM, Taylor AB, Hart PJ, Urbach AR. *J. Am. Chem. Soc.* 2006; **128**: 12574–12581.

DOI: 10.1002/poc

3. Prokai L, Ramanathan R, Nawrocki J, Eyler J. *J. Inclus. Phenom. Macrocyc. Chem.* 1996; **25**: 117–120.
4. Mutihac L, Buschmann HJ, Mutihac RC, Schollmeyer E. *J. Inclus. Phenom. Macrocyc. Chem.* 2005; **51**: 1–10.
5. Sirish M, Schneider HJ. *Chem. Commun.* 1999; 907–908.
6. Sirish M, Chertkov VA, Schneider HJ. *Chem. Eur. J.* 2002; **8**: 1181–1188.
7. Even P, Boitrel B. *Coord. Chem. Rev.* 2006; **250**: 519–541.
8. Hossain MA, Schneider HJ. *J. Am. Chem. Soc.* 1998; **120**: 11208–11209.
9. Cram DJ, Helgeson RC, Peacock SC, Kaplan LJ, Domeier LA, Moreau P, Koga K, Mayer JM, Chao Y, Siegel MG, Hoffman DH, Sogah GDY. *J. Org. Chem.* 1978; **43**: 1930–1946.
10. Rudiger V, Schneider HJ, Solovev P, Kazachenko P, Raevsky OA. *Eur. J. Org. Chem.* 1999; 1847–1856.
11. Danil de Namor AF. *Pure. Appl. Chem.* 1990; **62**: 2121–2127.
12. Czekalla M, Stephan H, Habermann B, Trepte J, Gloe K, Schmidtchen FP. *Termochimica. Acta.* 1998; **313**: 137–144.
13. Bushmann HJ, Schollmeyer E, Mutihac L. *J. Inclus. Phenom. Macrocycl. Chem.* 2001; **40**: 199–202.
14. Bushmann HJ, Mutihac L. *J. Inclus. Phenom. Macrocycl. Chem.* 2002; **42**: 193–195.
15. Pietraszkiewicz M, Koźbiał M. *J. Inclus. Phenom.* 1992; **14**: 339–348.
16. Pietraszkiewicz M, Koźbiał M, Pietraszkiewicz O. *J. Membrane Sci.* 1998; **138**: 109–113.
17. Pietraszkiewicz M, Koźbiał M, Pietraszkiewicz O. *Enantiomer* 1997; **2**: 319–325.
18. Pietraszkiewicz M, Koźbiał M, Pietraszkiewicz O. *J. Inclus. Phenom.* 1998; **30**: 69–77.
19. Badis M, Tomaszkiewicz I, Joly JP, Rogalska E. *Langmuir* 2004; **20**: 6259–6267.
20. Poznański J, Utzig E. *J. Thermal. Anal. Cal.* 2006; **83**: 565–569.
21. Koźbiał M, Poznański J, Utzig E, Lipkowski J. *J. Thermal. Anal. Cal.* 2006; **83**: 575–578.
22. Biros SM, Ullrich EC, Hof F, Trembleau L, Rebek J. *J. Am. Chem. Soc.* 2004; **126**: 2870–2876.
23. Job P. *Ann. Chim.* 1928; **9**: 113–203.
24. MestRe-C 2.3a Departamento de Quimica Organica, Universidade de Santiago de Compostela www.mestrec.com
25. Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR.* 1995; **6**: 277–293.
26. Goddard TD, Kneller DG. SPARKY 3, University of California, San Francisco www.cgl.uscsf.edu/home/sparky/
27. Grunwald E, Steel C. *J. Am. Chem. Soc.* 1995; **117**: 5687–5692.
28. Böhm H-J, Klebe G. *Angev. Chem.* 1995; **35**: 2588–2614.
29. Derewenda ZS, Vekilov PG. *Acta Cryst.* 2006; **D62**: 116–124. Entropy and surface engineering in protein crystallization.
30. Inoue Y, Liu Y, Tong LH, Shen BJ, Jin DS. *J. Am. Chem. Soc.* 1993; **115**: 475–481.
31. Zielenkiewicz W, Marcinowicz A, Cherenok S, Kalchenko VI, Poznański J. *Supramol. Chem.* 2006; **18**: 167–176.

DOI: 10.1002/poc