27. Calorimetric Measurements of the Complexation of Cyclosporin A, Ascomycin, Fujimycin, and Rapamycin with Lithium Chloride and with an Immunophilin

by **Dieter Seebach*** and **Hans G. Bossler**[1])

Laboratorium für Organische Chemie der Eidgenössischen Technischen Hochschule, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich

and **Robert Flowers** and **Edward M. Arnett***

Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27708-0354, USA

(12.VII.93)

Solutions (2 ml) of small linear and cyclic peptides (**4–11**), of a peptolide containing nine amino acids and a lactate moiety (**12**), of the cyclic undecapeptide cyclosporin A (CS, **1**), and of the macrolides ascomycin, fujimycin, and rapamycin (**13–15**) in THF were added to excess LiCl, LiBr, or $LiClO_4$ (up to 3000 equiv. in 40 ml THF) in a calorimeter (calorimetric titration). The enthalpies of interaction measured are in the range of $\Delta H = -8$ to -37 kcal/mol. A similar experiment was carried out with one of the binding proteins of cyclosporin, the human cyclophilin A, to give the thermodynamic parameters for the complexation $\Delta H° = -16$, $\Delta G° = -10$ kcal/mol, and $\Delta S° = -20$ cal/mol·deg. at 25° which corresponds to an equilibrium constant $K = 2 \cdot 10^7$ l/mol, in good agreement with the result of independent measurements using different methods. NMR Measurements of the macrolides in (D_8)THF containing LiCl show strong down-field shifts of signals of the H-atoms next to C=O and C–OH groups in these molecules.

1. Introduction: Salt Effects on Peptides in Non-polar Solvents. – The dramatic effects of inorganic salts, notably Li salts, on the solubility in non-polar organic solvents, such as tetrahydrofuran (THF), and on the reactivity of amides and peptides under aprotic conditions have been described and discussed extensively [1] [2]. The effect, which is not to be confused with the so-called chaotropic effect used in peptide chemistry [3], was exploited for synthetic purposes, for instance for the generation and alkylation of peptide enolates [2a] [4] or for improved peptide-coupling procedures [5][2])[3])[4]). The complexation of peptides with Li salts in THF [1] [10–12], but also in trifluoroethanol [13], usually leads to a new conformation of the peptide backbone. In the case of the immunosuppressive cyclic undecapeptide cyclosporin A (CS, **1**) we were able to determine the structure in

[1]) Part of the Ph.D. thesis of *H.G.B.*, Dissertation No. 10254, ETH-Zürich, 1993.

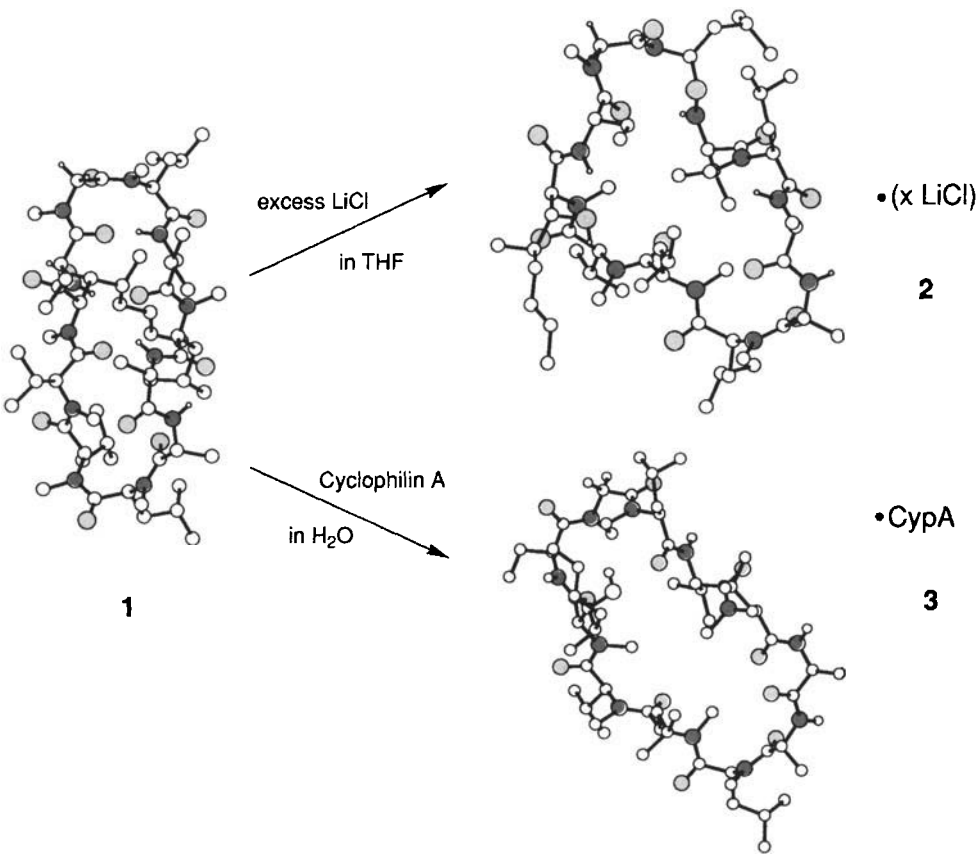
[2]) It is well known that the aprotic dipolar solvent dimethyl acetamide + LiCl is a unique system for dissolving polyamides and cellulose, being used on an industrial scale [6].

[3]) The affinity of alkali ions for peptides is also evident from the fact that water-free crystalline 1:1 complexes of small peptides, and alkali halides may separate from aqueous solution (*Pfeiffer* effect; these complexes have been described 80 years ago, and their structures were determined 20 years ago) [7] [8]. Thus, the large enthalpy of solvation of the components in water is more than compensated by their interaction in the crystal lattice.

[4]) For recent articles on salt effects, see the reviews by *Loupy* and *Tchoubar* [9a] and a publication on the assessment of *Lewis* acidity of $LiClO_4$ in ether [9b].

THF in the presence of 31 equiv. of LiCl [10] [11]: the four intramolecular H-bonds are all annihilated in the complex, and the *cis*-peptide bond between MeLeu[9] and MeLeu[10] has turned to *trans* (see **2** in *Scheme 1*). Although the number of Li ions actually binding to CS and their exact location has not been determined in this study, the changes brought about by LiCl can be interpreted as a consequence of the need to expose the carbonyl O-atoms to the Li ions in the solvent cage. Strikingly, the same gross changes (opening of transannular CO···HN bonds, *cis/trans*-isomerization) take place in the complexation of CS to its binding protein cyclophilin (NMR [14] [15] and X-ray [16] structure determination[5]); see **3** in *Scheme 1*). The originally proposed [1] possibility of studying protein-folding kinetics by returning a Li-salt-modified conformation from an organic solvent back into an aqueous medium[6]) has been elegantly realized [13].

Scheme 1. *Conformations of Cyclosporin A.* In an organic solvent such as THF or $CHCl_3$ (**1**), in THF/LiCl (**2**), and in the complex with its binding protein human cyclophilin A (**3**).

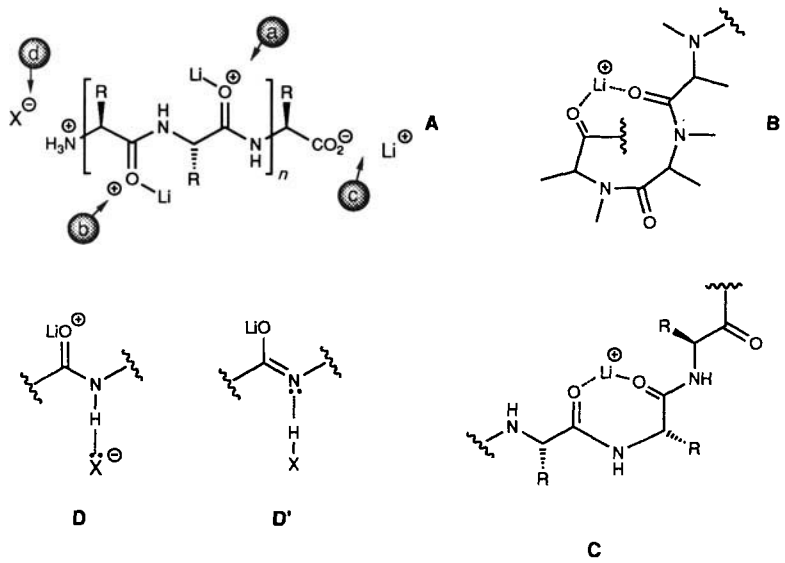


[5]) The structure of CS in watef is still unknown, due to very poor solubility in this medium. There are indications that several conformations may exist in aqueous media [17] [18].

[6]) See *Fig. 2* and the accompanying discussion in [1].

Crystal structures of Li salt complexes with amides, lactams, peptides, and ureas have been determined by X-ray diffraction analysis (see the literature cited in the review articles [2] [5a] [19] [20] and in [21] [22]). Of the two extreme geometries with dihedral angles Li–O=C–N 180° and 0°, the former one appears to occur preferentially [23] [24] (see ⓐ in *Formula* **A** of *Scheme 2*)[7]). There are chelate-type complexes with ten-membered rings as indicated in **B** [25]. Small cyclic peptides [4b] could possibly also form chelates containing a seven-membered ring **C**. The positive charge placed on the N-atom in the complexation of a peptide with an alkali ion increases the NH acidity[8]), and thus the ability to be a H-bond donor, possibly with the counter ion X^- (see **D** in *Scheme 2*).

Scheme 2. *Possible Complexations of* Li^+ *with Amides and Peptides.* **A**: Different coordination sites of Li^+ and X^- on a peptide, the Li^+ can take the place of a counter ion for CO_2^- (*c*), or it can sit on a carbonyl O-atom with (*E*)-(*a*) or (*Z*) geometry (*b*), the X^- may neutralize the NH_3^+ end of the peptide (*d*). **B**: Chelation of a Li ion by two peptide carbonyl O-atoms in a ten-membered ring. **C**: Seven-membered-ring chelate of Li^+ with a peptide. **D**: Acidification of the NH group upon complexation of a peptide bond with Li^+.



Inspite of all this knowledge about interactions between peptides and Li salts, no thermodynamic data are hitherto available. To learn about the energies involved, we have now undertaken calorimetric measurements. These gave such surprising results that we employed not only peptides and Li salts but extended the investigation to macrocyclic immunosuppressants and to interactions with the immunophilin cyclophilin.

---

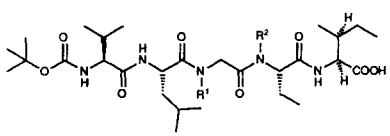
[7]) The arrangement with a dihedral angle of 0° (ⓑ in *Formula* **A** of *Scheme 2*) would be stereoelectronically favored.

[8]) The barrier to rotation around the OC–N bond in DMF is raised appreciably in the presence of LiCl, as determined by NMR spectroscopy [26].

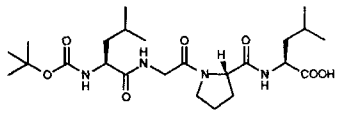
2. Calorimetric Measurements of Enthalpies of Interaction of Lithium Salts with Peptides in THF. – First, we chose some linear and cyclic peptides which we had prepared for our current investigations of peptide enolates. The open-chain tetra- and pentapeptides **4–9** [27] have a free COOH group at the C-terminus, and are Boc-protected at the N-terminus. The cyclic tetrapeptides **10** and **11** have been described recently [4b]. The decapeptolide **12** (SDZ 090.215)[9]), containing eight 'normal' (proteinogenic) amino-acids, pipecolic acid, and an (*R*)-lactate residue, was also included in the series of measurements.

An 0.03–0.05M solution of the corresponding peptide in THF was added to a given volume of an 0.5M solution of LiCl in THF (21.2 g/l), and the heat of interaction was measured. The values in kcal/mol of peptide are listed in *Table 1*. As can be seen, the heat

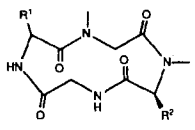
Table 1. *Linear* (**4–8**) *and Cyclic* (**10, 11**) *Oligopeptide Derivatives, and the Peptolide* **12**, *and Their Enthalpies of Interaction with LiCl in THF at 25°*. Peptide solutions in THF of the given concentrations were titrated into a 40-ml volume of 0.5M LiCl in THF. Usually, at least five calorimetric measurements were performed with each compound.



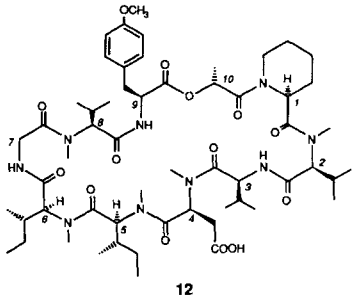
4 – 8



9



10, 11



12

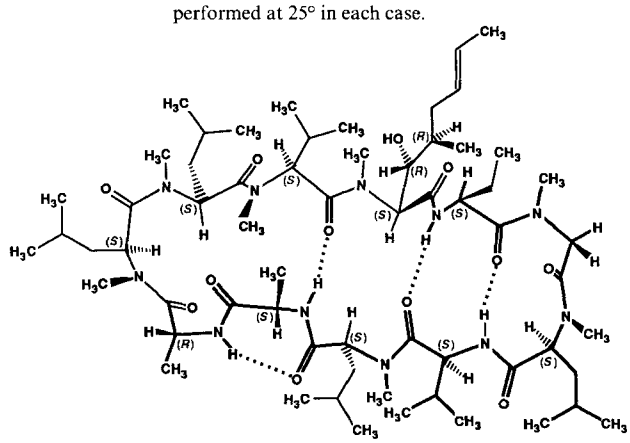
Peptide	R^1	R^2	Concentration [M]	$\Delta H°$ [kcal/mol]
4	H	H	0.036	−11.48 ± 0.41
5	H	Me	0.05	−12.77 ± 0.64
6	Me	Me	0.039	−13.15 ± 0.49
7	H	$PhCH_2$	0.05	−9.89 ± 0.56
8	$PhCH_2$	$PhCH_2$	0.034	−8.89 ± 0.41
9	–	–	0.05	−10.36 ± 0.31
10	D-CH_3	i-Bu	0.046	−7.89 ± 0.50
11	i-Bu	H	0.037	−10.50 ± 0.49
12	–	–	0.02	−13.04 ± 0.48

[9]) Peptolide **12** has been described in a patent; full papers are in preparation [28]. Compound **12** has *antifungal activities* (for instance against *Candida*) and can be used to treat *multidrug resistance*. We thank Dr. *K. Baumann* and Dr. *M.A. Grassberger* (Department of Dermatology, *Sandoz Research Institute*, A-1235 Vienna) and Dr. *R.M. Wenger* (*Sandoz Pharma AG*, CH-4002 Basel) for a sample of **12** and for pertaining information. For a discussion of cyclo-depsipeptides, see Chapt. 2.3 in [29].

of interaction is in the range of −7.9 to −13.2 kcal/mol, a surprisingly large number if compared with the heat of neutralization in aqueous solution ($H^+ + OH^- \rightarrow H_2O$, $\Delta H_R^\circ = -13.3$ kcal/mol)! The excess of LiCl is 10–30 equiv. per mole of peptide in these experiments, an amount of salt found to be sufficient for the maximum conformational change by NMR analysis [1] [10–12].

The peptide with which we performed most calorimetric measurements was cyclosporin A (CS; **1**)[10]). The results are collected in *Entries 1–11* of *Table 2*. We first made sure that dilution of THF solutions of cyclosporin (*Entry 1*) and of LiCl (*Entry 2*) is essentially thermoneutral in the concentration range chosen. The *Entries 3* and *4* contain a puzzling result: 10:1 combination of LiCl with CS shows −0.26 kcal/mol, when a LiCl solution (2 ml, 0.4M; 0.8 mmol) is added to a CS solution (40 ml, 0.002M; 0.08 mmol), and −3.53 kcal/mol (14-fold!), when a CS solution (2 ml, 0.03M; 0.06 mmol) is added to a LiCl solution (40 ml, 0.015M; 0.6 mmol) in THF; with a 31:1 ratio 11.6 kcal/mol are liberated using the normal addition mode (peptide to salt), see *Entry 5*. The different

Table 2. *Enthalpies of Interaction between Cyclosporin A* (**1**) *and Li Salts.* Solutions of **1** in THF were titrated into 40 ml of Li salt solutions in the same solvent within the calorimeter. Five to seven replica measurements were performed at 25° in each case.



Entry	Solution (2.00 ml THF)	Titrated into (40.00 ml THF)	ΔH° [kcal/mol]
1	1.01M **1**	THF	−0.20 ± 0.02
2	0.74M LiCl	THF	−0.19 ± 0.01
3	10 equiv. of LiCl	0.002M **1**	−0.26 ± 0.03
4	0.030M **1**	10 equiv. of LiCl	−3.53 ± 0.19
5	0.019M **1**	30.9 equiv. of LiCl	−11.62 ± 0.32
6	0.022M **1**	10 equiv. of $LiClO_4$	−3.50 ± 0.04
7	0.017M **1**	30.9 equiv. of $LiClO_4$	−8.59 ± 0.63
8	0.030M **1**	1600 equiv. of $LiClO_4$	−27.49 ± 0.86
9	0.030M **1**	3000 equiv. of $LiClO_4$	−37.38 ± 1.37
10	0.018M **1**	10 equiv. of LiBr	−9.32 ± 0.85
11	0.022M **1**	30.9 equiv. of LiBr	−18.69 ± 0.85

[10]) We thank the *Sandoz Pharma AG*, CH–4002 Basel (Dr. *R. M. Wenger*) for generous supplies of **1** for these and other studies.

heats of interaction measured, when the mode of addition is reversed, might be caused by the fact that a LiCl solution, added in portions to a THF solution of a peptide, may generate different new conformations, the ratio of which depends upon the number of equivalents of the salt and the concentration of the components [1] [11]. Thus, for instance cyclosporin **1** is converted to a mixture of several conformers equilibrating slowly on the NMR time scale with 3 equiv. of LiCl, while only one new conformer is present with 30 equiv. LiCl [11]. It was also shown that the different conformers may equilibrate [1] even slowly on the 'human time scale' (hour(s)) [13], and, therefore, inverse addition in the experiments described here could lead to different conformations and thus different heats of interaction[11]).

Variation of the Li salts leads to quite different values for the heats of interaction, with an increase going from perchlorate to chloride to bromide (−8.6/−11.6/−18.7 kcal/mol, see *Entries 7/5/11*)[12]). Finally, a strong exotherm results, when CS (0.06 mmol in 2 ml) and a highly concentrated solution of $LiClO_4$ (40 ml, 4.5M; 180 mmol) are combined: −37.4 kcal/mol (*Entry 9*). In this case of a highly concentrated $LiClO_4$ solution in THF, the contribution from the dilution effect may be sizeable, and the peptide competes more successfully with THF for Li^+ ions [9b].

These enthalpies of interaction are a measure of the affinity of amide as compared to ether groups (peptide *vs.* THF) for Li^+ (and for the corresponding anions, see **D** in *Scheme 2*, above)[13]). By comparison, the heat of deaggregation of BuLi (calorimetric titration of a hydrocarbon solution into THF [35] is −38.1 kcal/mol [$(BuLi)_6$ + 12 THF → 3 $(BuLi)_2 \cdot 4$ THF]. For CS we know the structural changes brought about by the addition of 30 equiv. of LiCl in THF [11]; thus, the now measured enthalpy of interaction corresponds to the sum of enthalpies for the conformational rearrangement (see **1**→**2** in *Scheme 1*) and the complexation of LiCl. It is clear from the solubility measurements [1] and from NMR investigation [12] that other, biologically more important ions such as Na^+, Mg^{2+}, Ca^{2+}, Zn^{2+} have similar effects (even in more polar solvents [5c] [13]) to those demonstrated for Li^+[14]). In view of the differences we found between different Li salts (*Table 2*), it appears that the role of the *gegenions* in this case has not been appropriately appreciated, so far.

3. Enthalpy of Complexation of Cyclosporin A with Its Binding Protein Cyclophilin A, and of Ascomycin, Fujimycin, and Rapamycin with LiCl. – Cyclosporin A is a selective immunosuppressive agent which has played the central role in revolutionizing the surgical success of organ transplantations [37]. Its binding protein, of which several subtypes

---

[11]) Normally, the time lapsed between two consecutive additions in the calorimetric titrations was ≤ 15 min. There was no evidence for a thermochemical change during 0.5 h in those cases checked. See also the time-dependent changes in binding of CS to cyclophilin and to a FAB immunoglobuline (antibody) fragment alluded to in [30] below.

[12]) This may have to do with different degrees of aggregation of these salts in THF before addition of the peptide. We are not aware of work in which these numbers were determined in a comparative study. For LiCl/THF see [31], for LiBr/acetone see [22].

[13]) For leading references see the books by *Cram, Lehn, Loupy, Schlosser,* and *Szwarc* [9] [29] [32a], the review articles [32b], and the discussion in [33]. For gas-phase investigations (mass spectroscopy) of complexes between alkali and alkaline-earth-metal ions with peptides, see the most recent papers by *Adams* and coworkers [34a], and *Grese* and *Gross* [34b], and earlier publications cited therein.

[14]) For the psychopharmacological effects of Li^+, see [36].

exist, is the *cis/trans*-peptidyl-prolyl isomerase cyclophilin; the affinity has so far been determined by a biosensor assay [30][15]). The human cyclophilin A (165 amino acids) can be produced by gene-technological methods[16]), opening the possibility of measuring the interaction with cyclosporin **1** (→**3**) calorimetrically, and thus determine the thermodynamic parameters. The results are shown in *Scheme 3*. The free enthalpy of complex formation $\Delta G°$ is −10 kcal/mol which corresponds to a binding constant of $2 \cdot 10^7$ l/mol, in good agreement with the values derived from the biosensor assay ($2.6 \pm 0.7 \cdot 10^7$ l/mol) [30a] and from fluorescence measurements ($0.5 \cdot 10^7$ l/mol) [30b]. It is an amazing coincidence that the heat of formation $\Delta H°$ of the complex **3** in an aqueous medium is −16 kcal/mol, a value which falls in the same range as the enthalpy of complexation of cyclosporin (→**2**) and other small proteins with Li salts in THF solvent, described in the previous section. The entropy change for CS/CypA (**3**) formation, $\Delta S° = -20$ cal/mol·degree, is not unusual for a bimolecular process. The enthalpy and entropy of binding are of course the sums of many increments: the peptide **1** and the binding protein must be at least partially desolvated, and maybe they have to change conformations before forming the complex **3** (see the discussion about time-dependent inhibition of cyclophilin by LiCl-modified cyclosporin [13]). Thus, the calorimetric measurement provides information which will become more valuable as more details about the conformational equilibrium of CS in aqueous solution emerge[5]).

Scheme 3. *Thermodynamic Parameters for the Reaction of Cyclosporin A* (**1**) *with Its Binding Protein (Human) Cyclophilin A*. For details of the determination of these values, see *Exper. Part.*

$$\text{Cyclosporin A (1)} + \text{Cyclophilin A} \underset{}{\overset{K}{\rightleftharpoons}} \text{Complex 3}$$

$\Delta H° = -15.9$ kcal/mol $\quad \Delta G° = -10$ kcal/mol
$\Delta S° = -20$ cal/mol·degree $\quad K = 2 \cdot 10^7$ l/mol
$T\Delta S° = -6$ kcal/mol

Following more whim than logic, we also included the three other prominent *cis/trans*-peptidyl-prolyl isomerase (immunophilin) inhibitors besides cyclosporin: the pipecolic acid containing macrolides fujimycin (FK-506, **13**), ascomycin (**14**), and rapamycin (**15**); the similarities and differences of these four drugs targeted to a ubiquitous family of intracellular binding proteins, as drugs which inhibit T-cell activation (immunosuppression), and which are being tested for a variety of new applications, have been discussed in recent review articles [38]. Although there is no apparent structural similarity between CS (**1**) and the macrolides **13**–**15**, all four have an immunophilin-binding domain and an effector domain (the structures of the complexes CS/Cyp (**3**), FK-506/macrophilin, and rapamycin/macrophilin are known from X-ray and NMR investigations [14–17] [38]). It turns out that the macrolides **13**–**15**[17]), with or without water

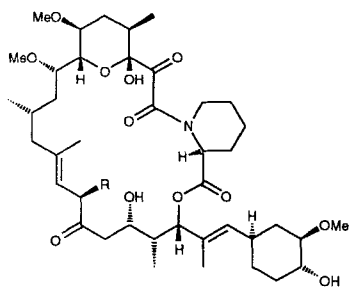
[15]) For a detailed description of the method see [30a]; we thank the authors for providing us with a preprint of this paper.

[16]) We are most grateful to Drs. *H. Fliri, M. D. Walkinshaw, R. M. Wenger*, and *M. Zurini* of the *Sandoz Pharma Ltd.*, Preclinical Research, Department of Immunology, CH-4002 Basel, for giving permission and supplying the valuable CypA sample so that we could do the experiment described herein.

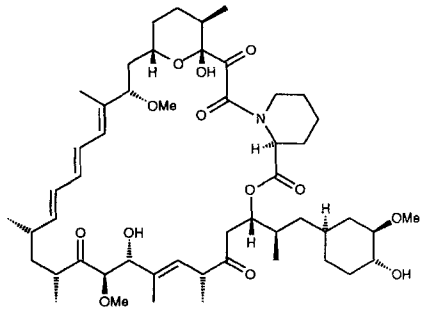
[17]) We thank Drs. *S. Cottens, R. Sedrani*, and *M. Grassberger* of the Immunology Departments of *Sandoz Pharma Ltd.*, CH-4002 Basel, and of the Department of Dermatology of the *Sandoz Research Institute*, A-1235 Vienna, for providing us with samples of the macrolides **13**–**15**.

content, have even larger heats of interaction with LiCl in THF (−16.9 to −18.6 kcal/mol) than cyclosporin **1** (−11.6 kcal/mol) under comparable conditions (see *Table 3*). This is again a puzzling result, because these macrolides contain only one amide bond which is probably not a good donor, because the amide C=O group is part of a 1,2-dicarbonyl system; otherwise ascomycin and fujimycin contain three OH, three MeO groups, an acetal-type O-atom, a keto and an ester group, and rapamycin one more C=O group. One would, therefore, not expect that they can compete successfully with the bulk solvent THF present in *ca.* 8000-fold molar excess. Note that addition of a THF solution containing H_2O (*ca.* 1M) to the LiCl solution does not lead to an appreciable liberation of heat (*Table 3, Entry 1*).

Table 3. *Heats of Interaction of Fujimycin, Ascomycin, and Rapamycin, and of H_2O, with LiCl in THF at 25°.* The measurements were done as before: 2 ml of *ca.* 0.03M **13**–**15** in THF was added to 40 ml of 0.5M LiCl in the same solvent within the calorimeter.



Fujimycin (FK506, **13**, R = $CH_2CH{=}CH_2$)
Ascomycin (**14**, R = CH_2CH_3)



Rapamycin (**15**)

Solution in 2.0 ml THF		$\Delta H°$ [kcal/mol] with 0.5M LiCl in THF
Compound	Concentration [M]	
H_2O	0.98	−2.00 ± 0.35
13·2 H_2O	0.03	−16.98 ± 0.97
14	0.034	−16.88 ± 1.07
15·3 H_2O	0.022	−18.55 ± 0.67
15	0.020	−18.32 ± 1.28

We figured that the complexation of the macrolides with LiCl might have a similarly drastic effect on the conformation of these compounds as in the case of cyclosporin [1] [10–13]. Therefore, we did some preliminary NMR measurements in (D_8)THF.

4. NMR Spectra of the Macrocycles 12–15 in the Presence of LiCl. – Solutions of the macrocycles in (D_8)THF (20–30 mg/ml) were combined with increasing amounts of anhydrous LiCl until up to saturated solutions were obtained (solubility of LiCl in THF at room temperature *ca.* 50 mg/ml). ^{1}H-NMR Spectra were measured with various concentrations of LiCl. As can be seen from the spectra in *Figs. 1–4*, there are dramatic shift effects in certain parts of the spectra. With up to 60 equiv. of LiCl per mole of the peptolide **12**, the signals from the peptide NH and CH protons α to the C=O groups

undergo the strongest changes (downfield shifts and line broadening, see *Fig. 1*). A careful analysis of the spectra shows that there are at least three conformers present (only two in pure THF)[18][19]). With the macrocyclic lactams/lactones **13**–**15** which, under comparable conditions, showed larger heats of interaction with LiCl than the peptolide (and than the peptides in general) there are also pronounced changes in the NMR spectra obtained in the presence of LiCl (up to 20 equiv./mol of macrolide; see *Figs. 2–4*). At the beginning of LiCl addition, there is mainly line broadening, strongest with *ca.* 5 equiv. of LiCl in the case of fujimycin and *ca.* 20 equiv. in the case of rapamycin[20]). With up to 20

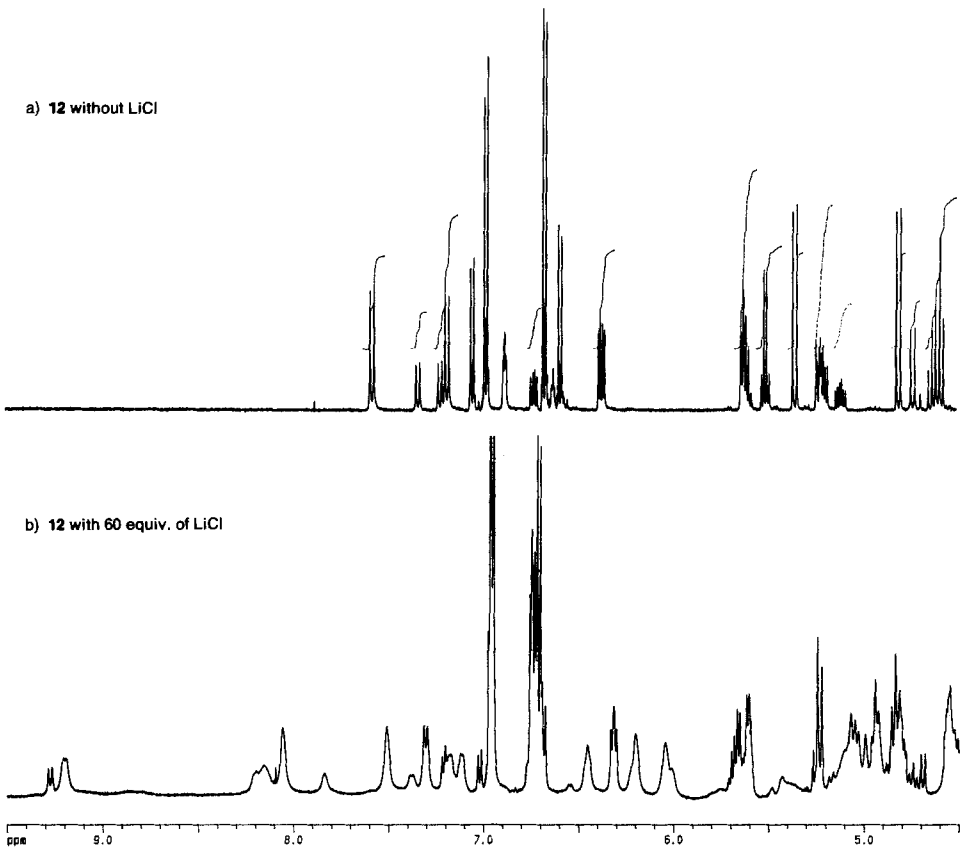


Fig. 1. *500-MHz ^{1}H-NMR Spectra of the peptolide* **12** *in (D_8)THF without* (a) *and with* (b) *LiCl.* The solution for obtaining spectrum *b* was essentially saturated with LiCl which corresponds to a *ca.* 60-fold molar excess over **12**. In the upfield part of the spectrum, not shown here, the changes caused by LiCl are smaller. The spectra of **12** were measured by Dr. *G. Schulz* of the *Sandoz Research Institute,* Vienna. We are grateful to *G. Schulz* for allowing us to reproduce the spectra herein.

---

[18]) Unpublished work by *G. Schulz* of the *Sandoz Research Institute,* Vienna.

[19]) The formation of new conformers in the presence of Li salts is typical for peptides [1] [11–13].

[20]) For a masterful NMR analysis of the solution structure of rapamycin in DMSO (two conformers in a 10:1 ratio), see the recent paper by *Kessler et al.* [39].

equiv. of LiCl, a strong downfield shift of the signals due to the CH–O and protons α to the C=O groups is observed. From the MeO signals, we conclude that the *ca.* 3:1 ratio of two conformers present in the THF solution of **13** and **15** does not change upon addition of LiCl[21]).

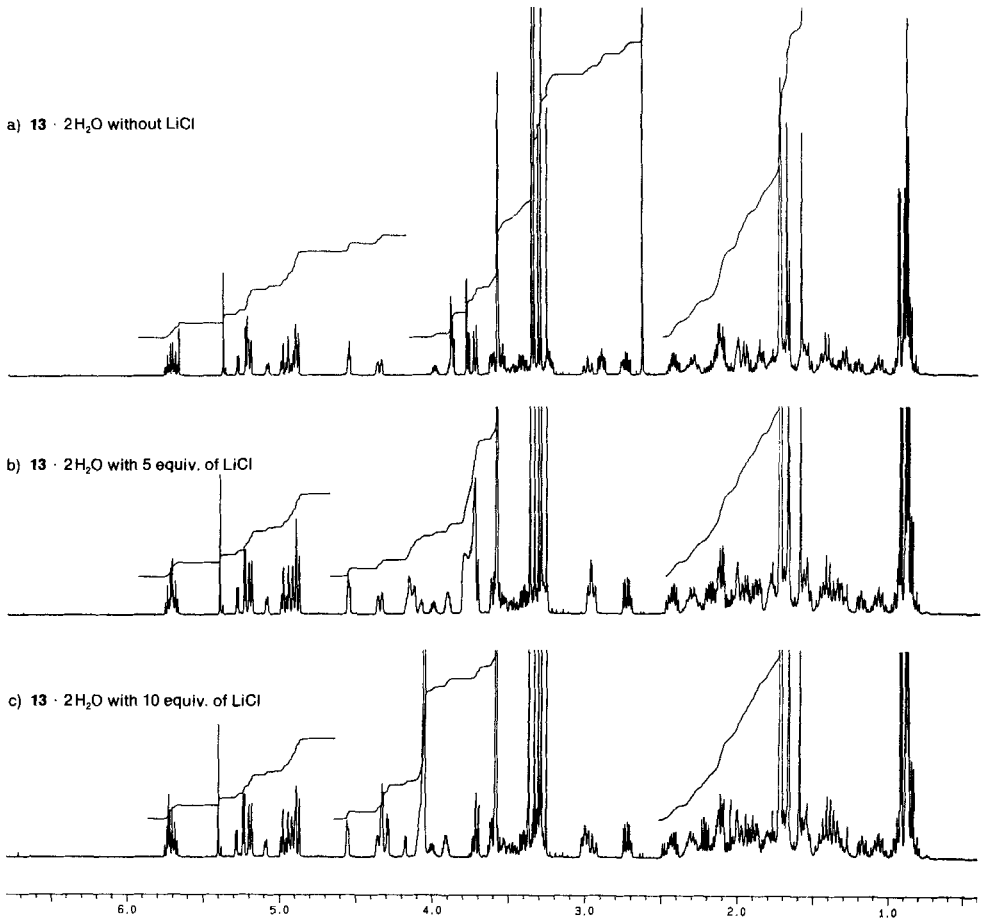


Fig. 2. *500-MHz ^{1}H-NMR Spectra of fujimycin* (**13**) *in (D_8)THF without* (a) *and with* (b,c) *LiCl added.* The sample used for the measurement contained 2 equiv. of H_2O which could not be removed by warming the sample to 60° *in vacuo* (*ca.* 10^{-2} Torr). Spectra were measured with 0.2, 1.0, 2.0, 5.0, 10.0, and 15 equiv. of LiCl. In addition to signal shifts occurring from the very first portion of LiCl, there was a peak broadening with 5 equiv. (see *b*) which disappeared when larger amounts of LiCl were added (10 equiv., see *c*).

5. Conclusions and Outlook. – The surprisingly large heats of interaction between simple and more complex open-chain and cyclic peptides as well as of the macrocyclic immunosuppressive lactones with Li salts in THF suggest that there are strong and specific interactions. The effect is especially intriguing with the immunosuppressive

[21]) The LiCl effects on the spectra observed with **13** and **15** in the presence or absence of 2–3 equiv. of H_2O (see *Table 3*) did not differ much.

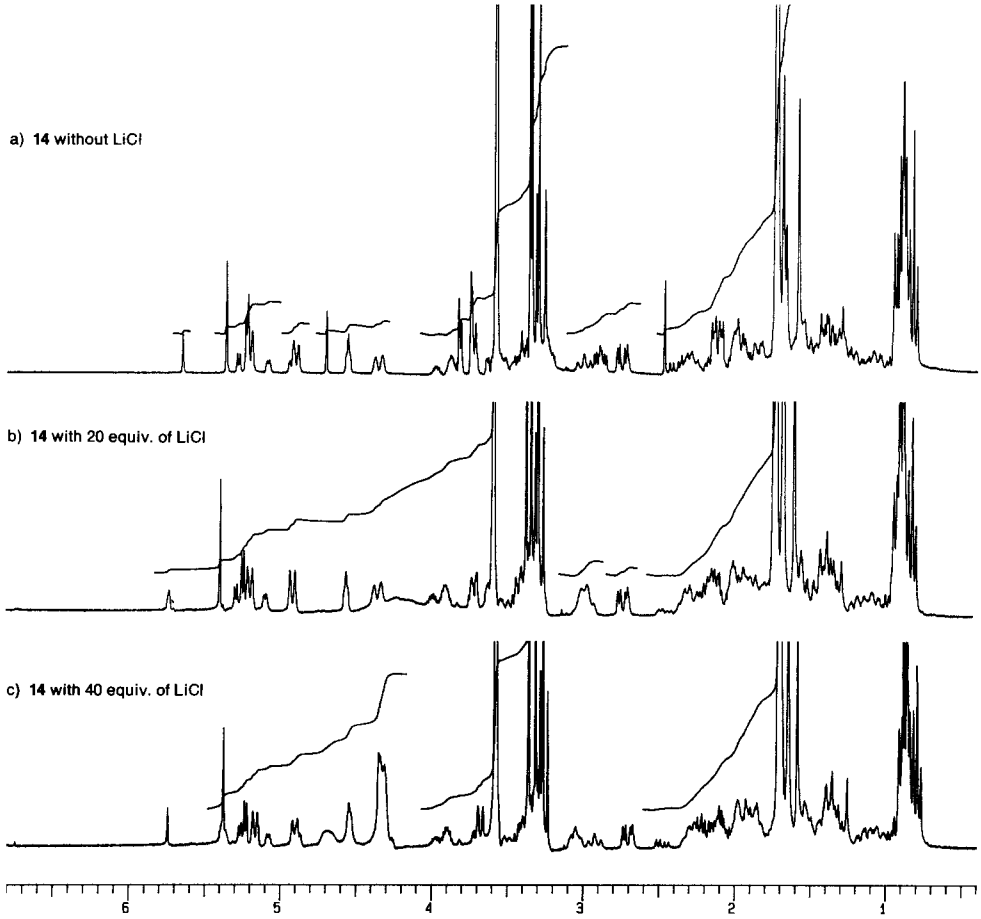


Fig. 3. *300-MHz ^{1}H-NMR Spectra of ascomycin* (**14**) *in* (D_8)*THF without* (a) *and with* (b,c) *LiCl added.* As with **13**, there is a peak broadening in the low-field part of the spectrum with intermediate amounts of LiCl (20 equiv., see *b*). With 40 equiv. LiCl (see *c*), the lines sharpen again, and the pattern of the entire spectrum has changed considerably as compared to the one observed in the absence of LiCl.

compounds cyclosporin, ascomycin, fujimycin, and rapamycin, because they have, as far as we know, hitherto, not been recognized as being ionophoric[22]); on the other hand, it is well known that some reactions of the immune system with which they interfere are calcium-dependent. There are intriguing similarities between the structures of the immuno-suppressive macrocycles **13**–**15** and some of the macrolides isolated as secondary metabolites from marine organisms; it has been suggested in an excellent recent review article by *Michael* and *Pattenden* that the function of these macrolides may be ionophoric in nature [42]!

---

[22]) Experiments testing **1** and **12**–**15** as carriers for ion transport through organic phases are being carried out in our laboratories (*cf.* [40] and the measurements of *Simon* and coworkers [41] with peptides for ion-selective membranes). We also plan to investigate the possible transport of ions through liposomes by these compounds.

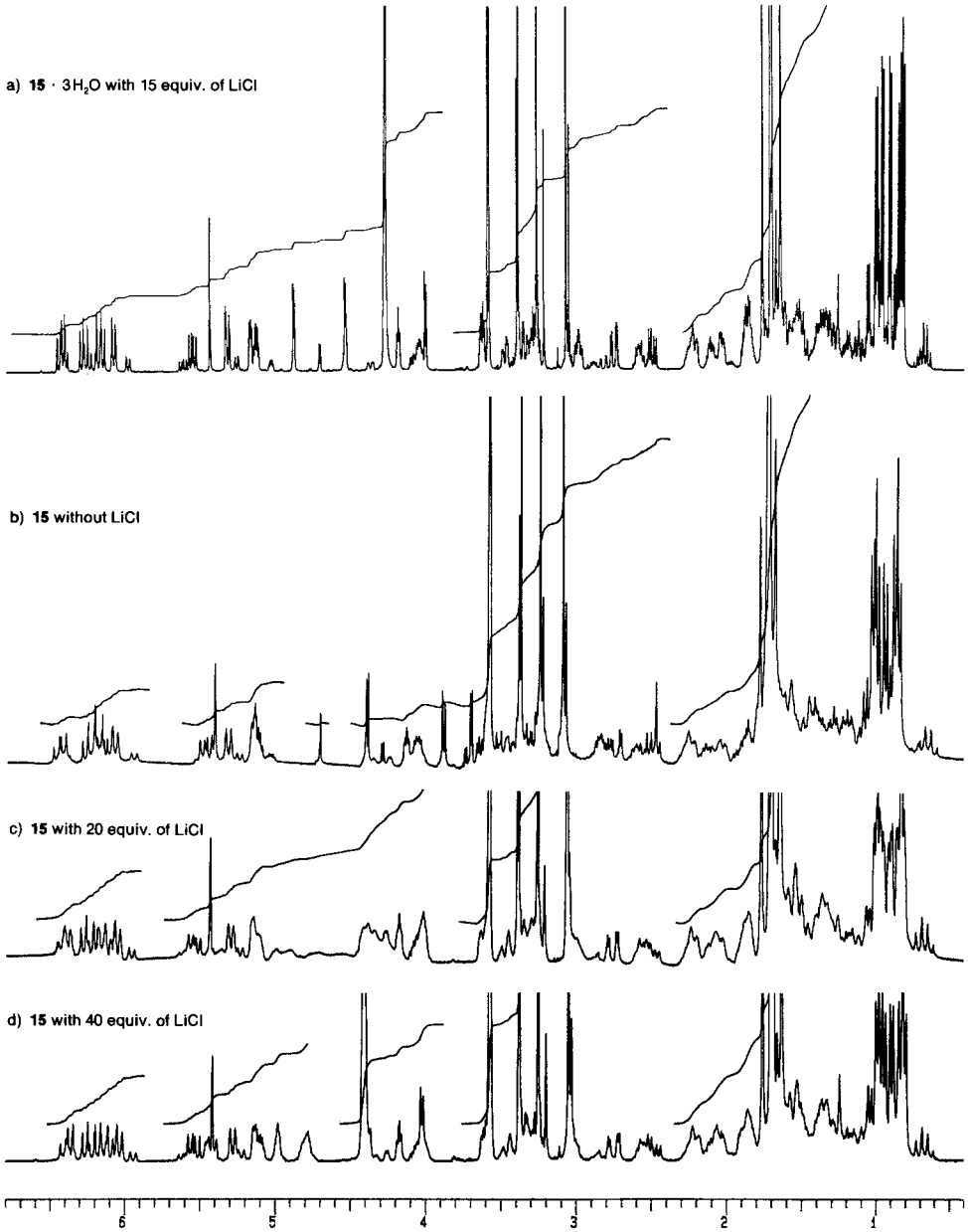


Fig. 4. *300- and 500-MHz ^{1}H-NMR Spectra of rapamycin* (**15**) *in* (D_8)*THF in the presence and absence of H_2O and LiCl* (a) *and of LiCl alone* (b–d). The sample used for spectrum *a* (15 equiv. of LiCl, 500-MHz machine) contained 3 equiv. of H_2O/equiv. of **15**; as with **13**, this H_2O could not be pumped off. The spectra of anhydrous rapamycin without (*b*) and with 20 (*c*) and 40 equiv. of LiCl (*d*) show the same general change of pattern as those of the other two immuno-suppressive macrocycles **13** and **14**, except that the broadening is more pronounced, and that the spectrum with the largest excess LiCl present shows sharper lines throughout than that obtained in the absence of the salt! The ratio of the two conformers does not seem to be altered under the influence of LiCl, as judged from the MeO signals. For a detailed NMR analysis of **15**, see [39].

The strong interactions of the macrocycles **13–15** with Li salts, reported herein, suggest that, as with peptides, there might be a pronounced effect of alkali and alkaline-earth-metal salts upon their reactivity in chemical transformations. The exact nature of the structural changes caused by the Li salts, as indicated by the NMR experiments, can not be delineated at this stage of the investigations.

Experimental Part

General. Inorg. salts were dried at 180° under high vacuum and stored in a drybox equipped with a *VAC HE-493* purification system. THF (*Fisher*) was distilled from sodium-benzophenone/ketyl [43], then heated at reflux over a column containing molecular sieves. The dry THF was then degassed using three-freeze thaw cycles and transferred to the drybox and stored over molecular sieves. The THF was tested for H_2O content before each experiment by a *Karl Fisher* titration. THF containing more than 50 ppm of H_2O was discarded.

Calorimetry. Heats of interaction (ΔH_{int}) between Li salts and peptides were determined with a *Tronac 458* isoperibol soln. calorimeter. The operation of the calorimeter was checked periodically by measuring the heat of neutralization of an aq. soln. of NaOH with a standard aq. HCl soln.

The solns. of peptides were prepared inside the Ar filled drybox with an anal. balance and volumetric flask. Before each calorimetric run, the calibrated motor-driven buret, filled with peptide soln., and the reaction vessel, containing *ca.* 40 ml of the Li-salt soln. in THF, were connected to the calorimeter insert assembly. A dry Ar atmosphere was maintained at the top of the reaction vessel. The basic operation of the instrument has been described previously [44] [45]. All enthalpy measurements were conducted in the isoperibolic mode at 25°. ΔH Values were measured by titrating 2 ml of a peptide soln. (0.01 to 0.03M) into 40 ml of a soln. containing the Li salt (10–30.9 equiv. based on the peptide). ΔH Values were calculated (in kcal/mol) by the standard method [44] [45]. The heats obtained are from a minimum of 2 runs, containing 6 titrations per run. Reproducibility of the ΔH results were verified by using different samples of the peptides and Li salts prepared on different days.

Titration Calorimetry of Cyclosporin A and Cyclophilin. The enthalpy of binding was determined by titrating 40 μM cyclosporin A with 0.56 mM cyclophilin in an *Omega* titration calorimeter (*MicroCal*, Northampton, MA). A CS soln. was made by addition of 5 μl of the CS stock soln. (40 mM) in DMSO to 5 ml of buffer (20 mM HEPES, 100 mM NaCl); followed by brief sonication. The enthalpy of binding between CS and cyclophilin was determined from heats of multiple single injections. Injection volumes were 10 μl, and 5 min of equilibration time was allowed between each injection. The heat of dilution of protein into buffer was determined. The protein-drug titration heat was adjusted by this small contribution.

The binding constant K was determined from the calorimetric data employing the *Origin*^{TM} (*MicroCal*, Northampton, MA) data analysis software for the *Omega* titration calorimeter. One binding site was assumed in the determination of K. The free energy of binding was determined from K.

Procedure 1. All samples were weighed in the drybox using a high-grade anal. balance. *Ca.* 150.0 ± 0.1 mg of cyclosporin A (*Sandoz*)[10]) was weighed into a 5-ml volumetric flask and dissolved into THF. The concentration of CS was always between 10 and 30 mmol. The number of equivs. of Li salt desired was weighed into a calorimeter dewar (based on CS in 2 ml of standard soln.) and dissolved into THF.

Procedure 2. Ca. 50.0 ± 0.1 mg of each peptide was weighed separately into a 5-ml volumetric flask and dissolved into THF. Stock solns. of 0.5M LiCl were prepared and transferred to the calorimeter dewar.

The anh. macrocyclic lactams/lactones **14** and **15** displayed a gradual increase in enthalpy with increasing addition of the peptide soln. to the Li-salt soln.

REFERENCES

[1] D. Seebach, A. Thaler, A.K. Beck, *Helv. Chim. Acta* **1989,** *72,* 857.

[2] Review articles: a) D. Seebach, *Angew. Chem.* **1988,** *100,* 1685; *ibid. Int. Ed.* **1988,** *27,* 1624; b) *Aldrichim. Acta* **1992,** *25,* 59.

[3] E. Oliveira, R. Marchetto, G.N. Jubilut, A.C.M. Paiva, C.R. Nakaie, in 'Peptides, Chemistry and Biology', Eds. J.A. Smith and J.E. Rivier, Escom, Leiden, 1992, p. 569; J.M. Stewart, W.A. Klis, in 'Innovation and Perspectives in Solid Phase Synthesis', Ed. R. Epton, SOCC, Birmingham, 1990, p. 1.

[4] a) D. Seebach, H. Bossler, H. Gründler, S.-i. Shoda, R. Wenger, *Helv. Chim. Acta* **1991,** *74,* 197; b) S.A. Miller, S.L. Griffiths, D. Seebach, *ibid.* **1993,** *76,* 563; c) D. Seebach, A.K. Beck, H.G. Bossler, C. Gerber, S.Y. Ko, C.W. Murtiashaw, R. Naef, S.-i. Shoda, A. Thaler, M. Krieger, R. Wenger, *ibid.* **1993,** *76,* 1564.

[5] a) D. Seebach, *Angew. Chem.* **1990,** *102,* 1363; *ibid. Int. Ed.* **1990,** *29,* 1320; b) J.C. Hendrix, K.J. Halverson, J.T. Jarrett, P.T. Lansbury, Jr., *J. Org. Chem.* **1990,** *55,* 4517; c) A. Thaler, D. Seebach, F. Cardinaux, *Helv. Chim. Acta* **1991,** *74,* 617; *ibid.* **1991,** *74,* 628; d) D. Seebach, A. Thaler, D. Blaser, S.Y. Ko, *ibid.* **1991,** *74,* 1102.

[6] M. Panar, L.F. Beste, *Polymer Preprints* **1976,** *17,* 65; D.C. Johnson, in 'Cellulose Chemistry and its Applications', Eds. T.P. Nevell and S.H. Zeronian, Wiley, New York, 1985, Chapt. 7.

[7] P. Pfeiffer, J. von Modelski, *Hoppe-Seyler's Z. Physiol. Chem.* **1913,** *81,* 329; P. Pfeiffer, F. Wittka, *Chem. Ber.* **1915,** *48,* 1289; P. Pfeiffer, *Hoppe-Seyler's Z. Physiol. Chem.* **1924,** *133,* 22.

[8] R. Meulemans, P. Piret, M. v. Meerssche, *Bull. Soc. Chim. Belg.* **1971,** *80,* 73.

[9] a) A. Loupy, B. Tchoubar, 'Effets de sels en chimie organique et organométallique', Dunod, Paris, 1988, 'Salt Effects in Organic and Organometallic Chemistry', VCH, Weinheim, 1992; A. Loupy, B. Tchoubar, D. Astruc, *Chem. Rev.* **1992,** *92,* 1141; b) R.M. Pagni, G.W. Kabalka, S. Bains, M. Plesco, J. Wilson, J. Bartmess, *J. Org. Chem.* **1993,** *58,* 3130.

[10] H. Kessler, M. Gehrke, J. Lautz, M. Köck, D. Seebach, A. Thaler, *Biochem. Pharmacol.* **1990,** *40,* 169; *erratum, ibid.* **1990,** *40,* 2185.

[11] M. Köck, H. Kessler, D. Seebach, A. Thaler, *J. Am. Chem. Soc.* **1992,** *114,* 2676.

[12] K.A. Carver, M.J. Rees, D.L. Turner, S.J. Senior, B.Z. Chowdhry, *J. Chem. Soc., Chem. Commun.* **1992,** 1682.

[13] J.L. Kofron, P. Kuzmic, V. Kishore, G. Gemmecker, S.W. Fesik, D.H. Rich, *J. Am. Chem. Soc.* **1992,** *114,* 2670; C. Garcia-Echeverria, J.L. Kofron, P. Kuzmic, V. Kishore, D.H. Rich, *ibid.* **1992,** *114,* 2758.

[14] C. Weber, G. Wider, B. von Freyberg, R. Traber, W. Braun, H. Widmer, K. Wüthrich, *Biochemistry* **1991,** *30,* 6563.

[15] S.W. Fesik, R.T. Game, Jr., H.L. Eaton, G. Gemmecker, E.T. Olejniczak, P. Neri, T.F. Holzman, D.A. Egan, R. Edalji, R. Simmer, R. Helfrich, J. Hochlowski, M. Jackson, *Biochemistry* **1991,** *30,* 6574; S.W. Fesik, R.T. Gambe, Jr., T.F. Holzman, D.A. Egan, R. Edalji, J.R. Luly, R. Simmer, R. Helfrich, V. Kishore, D.H. Rich, *Science* **1990,** *250,* 1406.

[16] G. Pflügl, J. Kallen, T. Schirmer, J.N. Jansonius, M.G.M. Zurini, M.D. Walkinshaw, *Nature (London)* **1993,** *361,* 91.

[17] D. Altschuh, O. Vix, B. Rees, J.-C. Thierry, *Science* **1992,** *256,* 92.

[18] S.Y. Ko, C. Dalvit, *Int. J. Peptide Protein Res.* **1992,** *40,* 380.

[19] C. Schade, P. von Ragué Schleyer, *Adv. Organomet. Chem.* **1987,** *27,* 169.

[20] K. Gregory, P. von Ragué Schleyer, R. Snaith, *Adv. Inorg. Chem.* **1991,** *37,* 47.

[21] T. Maetzke, D. Seebach, *Organometallics* **1990,** *9,* 3032; T. Maetzke, C.P. Hidber, D. Seebach, *J. Am. Chem. Soc.* **1990,** *112,* 8248.

[22] R. Amstutz, J.D. Dunitz, T. Laube, W.B. Schweizer, D. Seebach, *Chem. Ber.* **1986,** *119,* 434.

[23] N. Takahashi, K. Tanaka, T. Yamane, T. Ashida, *Acta Crystallogr., Sect. B* **1977,** *33,* 2132.

[24] R. Meulemans, P. Piret, M. van Meerssche, *Acta Crystallogr., Sect. B* **1971,** *27,* 1187.

[25] I.L. Karle, *J. Am. Chem. Soc.* **1974,** *96,* 4000, and ref. in M. Dobler, 'Ionophores and Their Structures', Wiley, New York, 1981.

[26] V. Madison, M. Atreyi, C.M. Deber, E.R. Blout, *J. Am. Chem. Soc.* **1974,** *96,* 6725; V. Madison, C.M. Deber, E.R. Blout, *ibid.* **1977,** *99,* 4788; K.G. Rao, E.D. Becker, C.N.R. Rao, *J. Chem. Soc., Chem. Commun.* **1977,** 350; M. Feigel, *ibid.* **1980,** 456; H. Kessler, W. Hehlein, R. Schuck, *J. Am. Chem. Soc.* **1982,** *104,* 4534.

[27] H.G. Bossler, unpublished results, Ph.D. thesis, ETH-Zürich, 1990–1993; a full paper for publication in *Helv. Chim. Acta* is in preparation.

[28] M.M. Dreyfuss, G. Emmer, M. Grassberger, K. Ruedi, H. Tscherter, *Sandoz* Pat., Ger 3, 832, 362, 1990 (*CA.* **1990,** *113,* 172754s); F. Loor, D. Boesch, C. Gavériaux, B. Jachez, A. Pourtier-Manzanedo, G. Emmer, *Br. J. Cancer* **1992,** *65,* 11; G. Emmer, M.A. Grassberger, G. Schulz, M. Schaude, *J. Med. Chem.* **1993,** in preparation; G. Emmer, M.A. Grassberger, G. Schulz, D. Boesch, C. Gavériaux, F. Loor, *ibid.* **1993,** in preparation.

[29] B. Dietrich, P. Viout, J.-M. Lehn, 'Macrocyclic Chemistry. Aspects of Organic and Inorganic Supramolecular Chemistry', VCH Verlagsgemeinschaft GmbH, Weinheim, 1993.

[30] a) G. Zeder-Lutz, R. Wenger, M.H.V. v. Regenmortel, D. Altschuh, *FEBS Lett.* **1993,** *326,* 153; b) R.E. Handschumacher, M.W. Harding, J. Rice, R.J. Drugge, *Science* **1984,** *226,* 544.

[31] F.E. Hahn, S. Rupprecht, *Z. Naturforsch., B* **1991,** *46,* 143.

[32] a) D.J. Cram, 'Fundamentals of Carbanion Chemistry', Academic Press, New York, 1965; M. Szwarc, 'Carbanions, Living Polymers, and Electron Transfer Processes', Interscience, New York, 1968; M. Schlosser, 'Struktur und Reaktivität polarer Organometalle', Springer Verlag, Berlin, 1973; M. Szwarc, 'Ions and Ion Pairs in Organic Chemistry', Interscience, New York, 1972, Vol. 1, *ibid.* 1974, Vol. 2; b) E.A. Kovrizhnykh, A.I. Shatenshtein, *Russ. Chem. Rev.* **1969,** *38,* 840; J.M. Mallan, R.L. Bebb, *ibid.* **1969,** *69,* 693; J.M. Brown, *Chem. Ind. (London)* **1972,** 454.

[33] J. Heinzer, J.F.M. Oth, D. Seebach, *Helv. Chim. Acta* **1985,** *68,* 1848.

[34] a) H. Zhao, A. Reiter, L.M. Teesch, J. Adams, *J. Am. Chem. Soc.* **1993,** *115,* 2854; b) R.P. Grese, M.L. Gross, *ibid.* **1990,** *112,* 5098.

[35] a) R.P. Quirk, D.E. Kester, R.D. Delaney, *J. Organomet. Chem.* **1973,** *59,* 45; b) R.P. Quirk, D.E. Kester, *ibid.* **1974,** *72,* C23.

[36] F. Neil Johnson, 'The History of Lithium Therapy', Macmillan, London, 1984; F. Neil Johnson, 'The Psychopharmacology of Lithium', Macmillan, London, 1984; R.O. Bach, 'Lithium Current Applications in Science, Medicine, and Technology', Wiley, New York, 1985.

[37] J.F. Borel, F.D. Padova, J. Mason, V. Quesniaux, B. Ryffel, R. Wenger, *Pharmacol. Rev.* **1989,** *41,* 239; H. Fliri, G. Baumann, A. Enz, J. Kallen, M. Luyten, V. Mikol, R. Movva, V. Quesniaux, M. Schreiber, M. Walkinshaw, R. Wenger, G. Zenke, M. Zurini, *Ann. N. Y. Acad. Sci.* **1993,** *696,* 47.

[38] A. Stütz, M.A. Grassberger, K. Baumann, A.J.F. Edmunds, P. Hiestand, J.G. Meingassner, P. Nussbaumer, W. Schuler, G. Zenke, 'Immunophilins as Drug Targets' in 'Perspectives in Medicinal Chemistry', Eds. B. Testa, E. Kyburz, W. Fuhrer, and R. Giger, Verlag Helv. Chim. Acta, Basel and VCH, Weinheim, 1993, Chapt. 27, pp. 427–443, and ref. cit. therein; M.K. Rosen, S.L. Schreiber, *Angew. Chem.* **1992,** *104,* 413; *ibid. Int. Ed.* **1992,** *31,* 384; S.L. Schreiber, *Chem. Eng. News* **1992,** *26,* 22.

[39] H. Kessler, R. Haessner, W. Schüler, *Helv. Chim. Acta* **1993,** *76,* 117.

[40] H.M. Bürger, D. Seebach, *Helv. Chim. Acta* **1993,** *76,* 2570.

[41] F. Behm, D. Ammann, W. Simon, K. Brunfeldt, J. Halstrøm, *Helv. Chim. Acta* **1985,** *68,* 110.

[42] J.P. Michael, G. Pattenden, *Angew. Chem.* **1993,** *105,* 1; *ibid. Int. Ed.* **1993,** *32,* 1.

[43] D.D. Perrin, W.L.F. Armarego, D.R. Perrin, 'Purification of Laboratory Chemicals', 2nd edn., Pergamon Press, New York, 1980, p. 426–7.

[44] J.K. Grime, 'Analytical Solution Calorimetry', Wiley, New York, 1985.

[45] D.J. Eatough, J.J. Christensen, R.M. Izatt, 'Experiments in Thermometric Titrimetry and Titration Calorimetry', Brigham Young University Press, Provo, 1974.