A two-dimensional NMR study of the antimicrobial peptide magainin 2

Dominique Marion[+], Michael Zasloff* and Ad Bax

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, and **Human Genetics Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA*

Received 19 November 1987

Using two-dimensional NMR spectroscopy, a complete ^1H resonance assignment has been obtained for the peptide magaining 2 recently isolated from *Xenopus laevis*. It is demonstrated that this peptide adopts an α-helical structure with amphiphilic character when dissolved in a mixture of trifluoroethanol (TFE) and H_2O. The transition to the α-helical conformation occurs at very low concentrations of TFE.

Peptide; *α-Helix; Magainin; 2D NMR; NOESY*

1. INTRODUCTION

Amphibian skin has been a source of many biologically active alkaloids and peptides [1]. Recently, a new class of antimicrobial peptides called magainins [2] has been isolated from the skin of the African clawed frog *Xenopus laevis*. These peptides were recently shown to represent major components of the secretion released from the granular glands of *X. laevis* skin upon adrenergic stimulation [3]. By acting as sterilizing agents of the skin, they may be responsible for the extraordinary freedom from infection characteristic of wound healing in these frogs [2], even in a microbially contaminated habitat. At low concentration, these water-soluble peptides inhibit the growth of numerous species of bacteria and fungi and induce osmotic lysis of protozoa [2]. Here we present preliminary results of an NMR structural study of one of the most potent of these antibiotics, magainin 2. The sequence of its carboxyamide derivative, used in this study, is:

Gly-Leu-Gly-Lys-Phe[5]-Leu-His-Ser-Ala-
Lys[10]-Lys-Phe-Gly-Lys-Ala[15]-Phe-Val-Gly-
Glu-Ile[20]-Met-Asn-Ser-NH_2.

In view of the sequence of magainin 2, it has been postulated that it might form an α-helix with a large hydrophobic moment [2]. Because the conformation of the water soluble peptide may be dependent on the interaction with solvent, NMR is an ideal technique for investigating the conformations that can be adopted by this molecule.

Correspondence (present) address: D. Marion, Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA

[+] On leave from Centre de Biophysique Moléculaire, Centre National de la Recherche Scientifique, 45071 Orléans Cedex 2, France

Abbreviations: 2D, two-dimensional; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; HOHAHA, homonuclear Hartmann–Hahn spectroscopy; TFE, trifluoroethanol

2. EXPERIMENTAL

For all 2D NMR experiments, 15 mg of magainin 2 was dissolved in 0.125 ml TFE/0.375 ml H_2O (a molar ratio of 8:92), containing 3 mg NaCl (100 mM). A similar sample was made with D_2O instead of H_2O. For both samples, perdeuterated TFE was used and the pH of the sample (prior to addition of TFE) was adjusted to 4.1. All experiments reported

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

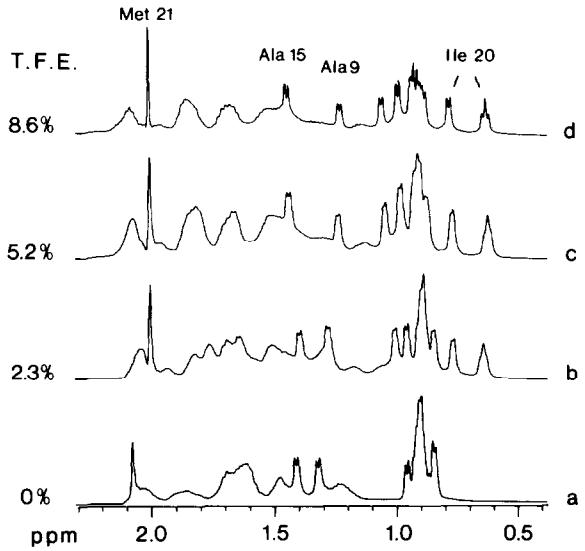

Fig.1. Methyl region of the ^1H spectra of magainin 2 for increasing molar ratios of TFE. Addition of 2.3% TFE (b) to the sample of spectrum (a) is sufficient to induce the conformational transition. Further addition of TFE (c,d) reduces self-association and reduces line width. 8.6 mol% corresponds to 27 volume %.

here were recorded on a Nicolet NT-500 spectrometer operating at 500 MHz ^1H frequency. Experiments were carried out at 18°C. Spectral assignments were verified by additional spectra (not shown) recorded at 36°C.

All 2D NMR spectra were recorded in absorption mode [4]. NOESY spectra [5] were recorded for a mixing time of 100 ms, using 2 × 400 × 1024 data matrices, corresponding to acquisition times of 80 and 102 ms in the t_1 and t_2 dimensions, respectively. 64 scans were acquired per t_1 value and the measuring time was 13 h per spectrum. Through-bond connectivity was obtained from a 2D HOHAHA spectrum [6,7] recorded with the MLEV-17 mixing scheme, using a 32 μs 90° pulse and a 35 ms mixing period. For the sample containing H_2O, presaturation of the H_2O resonance was used during the delay between scans and during the mixing period (NOESY). H_2O presaturation did not give any noticeable decrease in intensity for the exchangeable protons, indicating that the exchange of the peptide NH protons is slow on the NMR time scale.

3. RESULTS AND DISCUSSION

Fig.1 shows the methyl region of the spectrum of magainin 2, recorded for increasing concentrations of TFE. Without TFE present (bottom trace) the chemical shift dispersion in the methyl region of the spectrum is very small. Moreover, under these

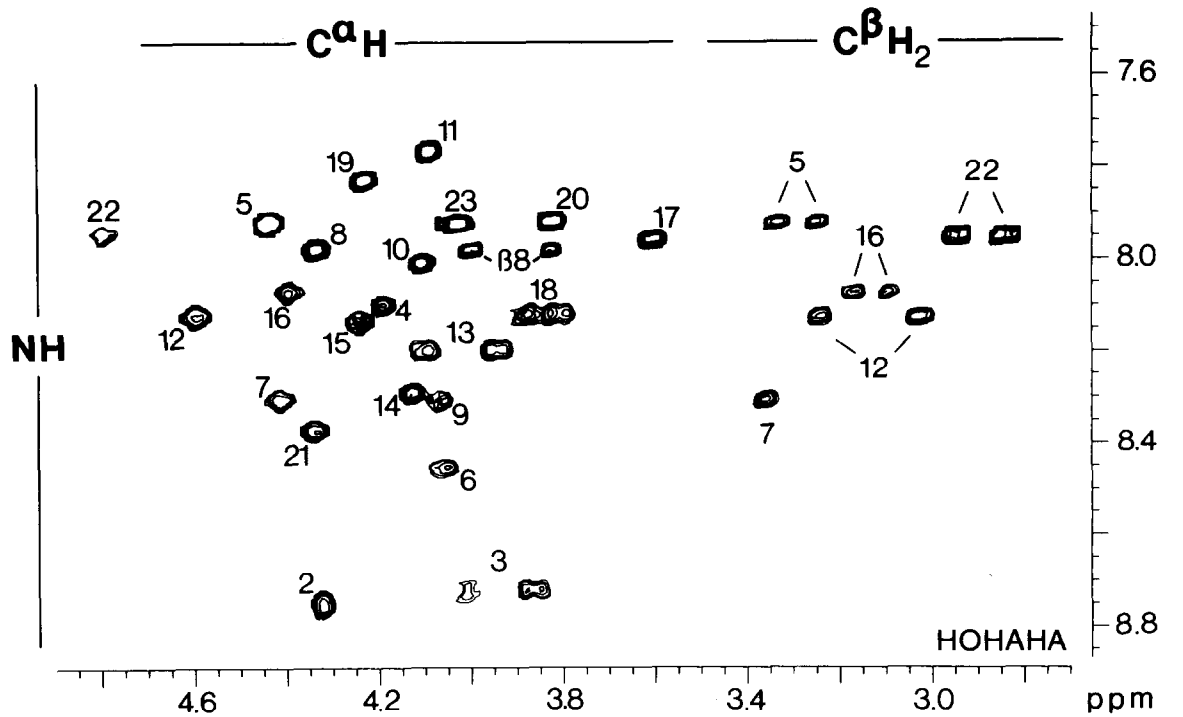

Fig.2. Fingerprint region of the 2D HOHAHA spectrum of magainin 2. Apart from the N-terminal residue all NH-$C_{\alpha}H$ connectivities are present.

conditions the NH-C_αH couplings are all in the vicinity of 7 Hz and the spectrum does not change appreciably when the temperature is increased from 10 to 50°C (data not shown). These features are indicative of an extended random coil polypeptide chain. Because the water is more polar than the environment experienced by a membrane-interacting peptide, we reduced the solvent polarity by adding TFE. Adding TFE stabilizes intramolecular hydrogen bonds in the peptide. In fact, it has been shown previously that glucagon analogs adopt similar conformations in a TFE–water mixture and in dodecylphosphocholine micelles [8,9], suggesting that such a solvent may be suitable for modeling peptide conformation at a lipid bilayer. Fig.1 shows the dramatic change in the ^{1}H spectrum of magainin 2 when TFE is added. On the basis of resonance assignments discussed below it is concluded that the entire chain is involved in a conformational transition. At low concentrations of TFE, a significant increase in line width is observed. This could be caused by rapid exchange between the random coil and the preferential ordered structure, or by self-association of the individual peptides. The rapid NOE build-up rates (see below) for this relatively small peptide and the short $T_{1\varrho}$ values (data not shown) indicate that association is the dominant cause of the line-broadening.

We have investigated the conformation of the ordered peptide structure existing in a TFE–water mixture (molar ratio 8:92). This TFE concentration provides a good compromise with respect to the effective correlation time of the peptide: it permits the observation of quite strong NOE interactions as well as scalar connectivities. Sequential resonance assignment strategies [10] require both these types of interactions. 2D HOHAHA spectra were recorded for several mixing times to delineate complete spin systems. Fig.2 shows the fingerprint region of the 2D HOHAHA spectrum recorded with a 35 ms mixing period. The corresponding region of the NOESY spectrum (fig.3) shows a large number of additional cross peaks which are caused by interresidue interactions. From the spectra of figs 2 and 3, a sequential resonance assignment can be made in the standard manner [10]. As indicated in fig.3, several NOE connectivities are observed between the NH protons of residue i+3 and the C_α protons of residue i. At contour levels lower than those shown, a large number of additional such NOEs are observed. This type of NOE connectivity is indicative of a helical structure [11]. Since the NH(i+3)-C_βH(i) NOE cross peaks (outside the spectral region shown in fig.3) are, on average, more intense than NOEs between the NH and C_α protons, the structure is most likely an α-helix and not a 3–10 helix [10]. The helical shape of the peptide is confirmed by the set of 19 NOEs between successive amide protons, starting at Gly-3 through Asn-22 (fig.4). The NH-NH connec-

Table 1

Resonance assignment of magainin 2 in water–TFE mixture (92:8)

	NH	C_αH	C_βH	Others
Gly 1	?	4.08 3.95		
Ile 2	8.76	4.32	2.02	$C_\gamma H_2$ 1.59 1.38 $C_\gamma H_3$ $C_\delta H_3$ 1.05
Gly 3	8.74	4.01 3.86		
Lys 4	8.12	4.19	1.92 ?	$C_\gamma H_2$ 1.47 $C_\delta H_2$ 1.47 $C_\epsilon H_2$ 2.98
Phe 5	7.94	4.43	3.34 3.26	$C_{2,6}H$ 7.24 $C_{3,4,5}H$ 7.29
Leu 6	8.47	4.05	1.87 1.87	$C_\gamma H$ 1.51 $C_\delta H_3$ 0.95 0.95
His 7	8.31	4.41	3.38 3.38	C_2H 8.60 C_4H 7.33
Ser 8	7.98	4.33	4.02 3.84	
Ala 9	8.32	4.06	1.32	
Lys 10	8.02	4.11	1.88 1.73	$C_\gamma H_2$ 1.48 $C_\delta H_2$ 1.56 $C_\epsilon H_2$ 3.00
Lys 11	7.77	4.09	1.69 1.69	$C_\gamma H_2$ 1.08 $C_\delta H_2$ 1.23 $C_\epsilon H_2$ 2.90
Phe 12	8.13	4.59	3.27 3.06	$C_{2,6}H$ 7.20 $C_{3,4,5}H$ 7.26
Gly 13	8.21	4.10 3.95		
Lys 14	8.30	4.12	1.93 1.73	$C_\gamma H_2$ 1.50 $C_\delta H_2$ 1.60 $C_\epsilon H_2$ 3.03
Ala 15	8.15	4.23	1.53	
Phe 16	8.07	4.40	3.19 3.11	$C_{2,6}H$ 7.13 $C_{3,4,5}H$ 7.18
Val 17	7.96	3.61	2.15	$C_\gamma H_3$ 1.12 1.00
Gly 18	8.13	3.87 3.82		
Glu 19	7.83	4.22	2.15 2.13	$C_\gamma H_2$ 2.44
Ile 20	7.93	3.82	1.90	$C_\gamma H_2$ 1.35 1.05 $C_\gamma H_3$ 0.84 $C_\delta H_3$ 0.70
Met 21	8.38	4.33	2.13 2.13	$C_\gamma H_2$ 2.58 2.71 $C_\epsilon H_3$ 2.07
Asn 22	7.91	4.80	2.95 2.85	$N_\gamma H_2$ 6.79 7.66
Ser 23	7.91	4.03	4.05 4.05	terminal NH_2 7.46 7.13

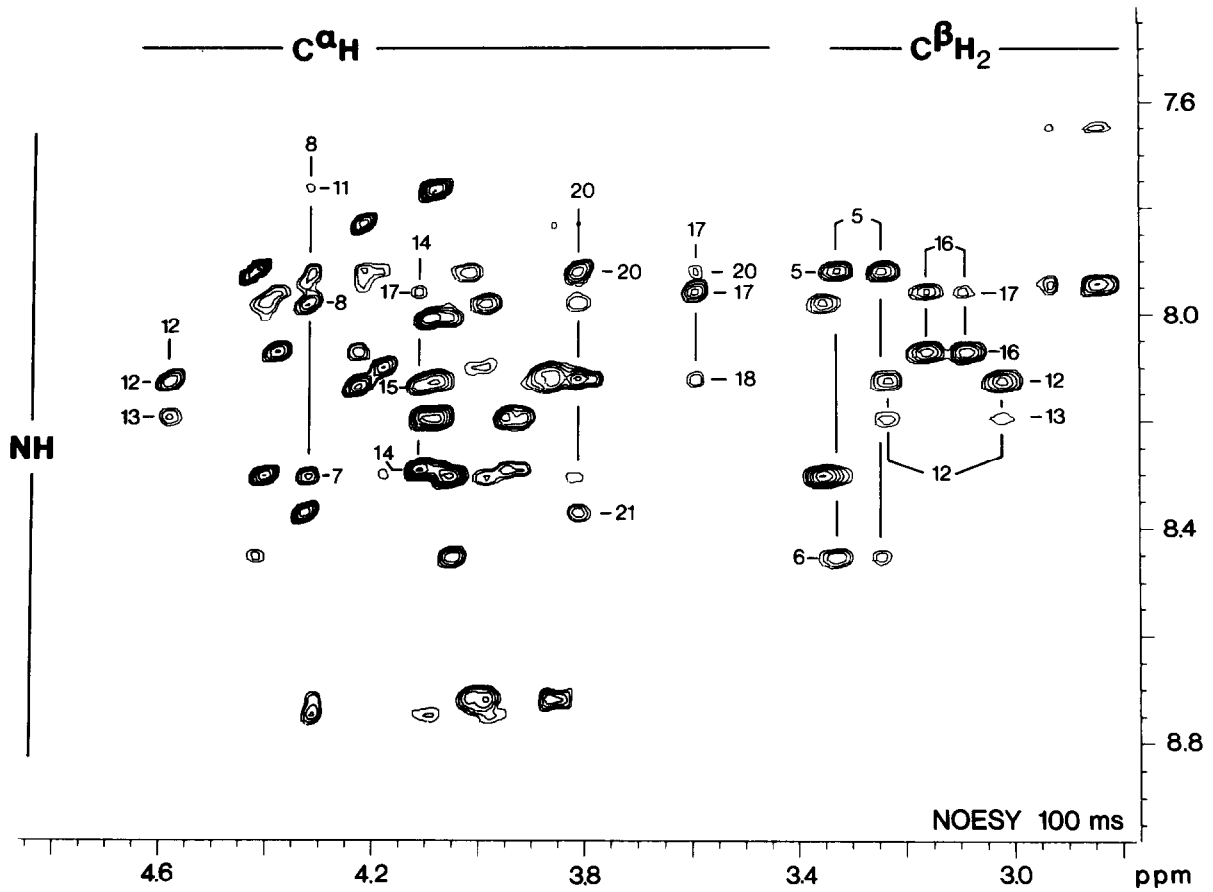

Fig.3. Fingerprint region of the NOESY spectrum of magainin 2. Some interresidue $C_{\alpha}H(i)$-NH(i+3) connectivities, indicative of helical structure, are labeled.

tivity to Ser-23 is probably present but could not be observed because the chemical shifts of the NH protons of Asn-22 and Ser-23 are degenerate. Similarly, NOE connectivity between the overlapping NH resonances of Ile-2 and Gly-3 cannot be observed but may be present. The complete assignment of the ^1H spectrum is given in table 1.

4. CONCLUSION

Results reported here present experimental evidence that the conformation of magainin 2 is solvent dependent, changing from a random coil in H_2O to an α-helix upon addition of a small amount of TFE. The primary structure of the peptide is such that, as an α-helix, it exhibits a distribution of lipophilic and hydrophilic side-chains along the length of the helical structure characteristic of amphiphilic peptides [12,13]. Molecular modeling of this peptide in α-helical conformation has suggested that several monomers could organize to form a transmembrane anion-specific channel (R. Feldmann, unpublished results). Indeed, conductance studies utilizing synthetic membranes have recently confirmed these predictions (R. Cruciani et al., in preparation). Refinement of the structure of magainin 2 is in progress, relying on J couplings with stereospecifically assigned side chain protons and on a large number of NOE interactions between side chains. A complete three-dimensional structure of magainin 2 and comparison with analogs may provide new insights into the structure–activity relationships of this class of peptide.

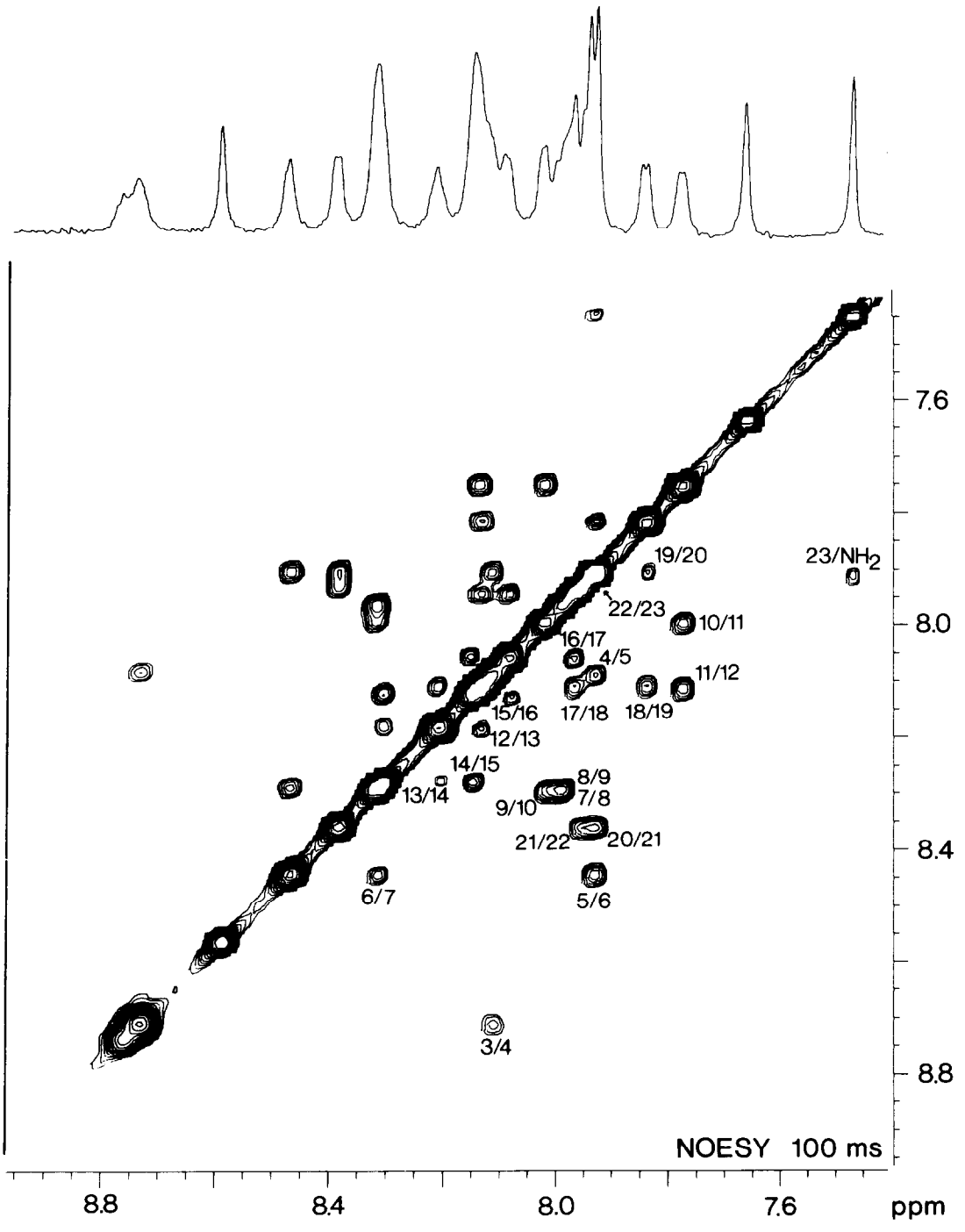

Fig.4. NH(i)-NH(i + 1) NOE connectivities of magainin 2. Contours are drawn at the same levels as in fig.3.

Acknowledgements: D.M. acknowledges financial support from Centre National de la Recherche Scientifique and from the CNRS–NIH exchange agreement. We thank Rolf Tschudin for technical support.

REFERENCES

[1] Erspamer, V. and Melchiorri, P. (1980) Trends Pharmacol. Sci. 1, 391–395.
[2] Zasloff, M. (1987) Proc. Natl. Acad. Sci. USA 84, 5449–5453.
[3] Giovannini, M.G., Poulter, L., Gibson, B.W. and Williams, D.H. (1987) Biochem. J. 243, 113–120.
[4] States, D.J., Haberkorn, R.A. and Reuben, D.J. (1982) J. Magn. Reson. 48, 286–292.
[5] Jeener, J., Meier, B.H., Bachmann, P. and Ernst, R.R. (1979) J. Chem. Phys. 71, 4546–4553.
[6] Braunschweiler, L. and Ernst, R.R. (1983) J. Magn. Reson. 53, 521–528.
[7] Bax, A. and Davis, D.G. (1985) J. Magn. Reson. 65, 355–360.
[8] Braun, W., Wider, G., Lee, K.H. and Wuethrich, K. (1983) J. Mol. Biol. 169, 921–948.
[9] Clore, G.M., Martin, S.R. and Gronenborn, A.M. (1986) J. Mol. Biol. 191, 553–561.
[10] Wuethrich, K. (1986) NMR of Proteins and Nucleic Acids, John Wiley & Sons, New York.
[11] Billeter, M., Braun, W. and Wuethrich, K. (1982) J. Mol. Biol. 155, 321–346.
[12] Kaiser, E.T. and Kezdy, F.J. (1987) Annu. Rev. Biophys. Biophys. Chem. 16, 561–581.
[13] Eisenberg, D. (1984) Annu. Rev. Biochem. 53, 595–623.