group from the Mn^{2+} ion will result in a larger, positive value of $A/\hbar$[C(1)] as observed here, when the mixed glycine-Mn^{2+}-ATP^{4-} complex is compared with the corresponding binary glycine-Mn^{2+} complexes.

Finally the difference in the Mn^{2+}-NH_2 interaction of the binary and mixed-ligand complexes, just derived from the R_{1p} relaxation rates and the electron-nucleus hyperfine coupling, is paralleled by a difference in the glycine-ligand exchange rate which increases from $\tau_{MG}^{-1} = (3.9 \pm 1.2) \times 10^6\ s^{-1}$ and $\tau_{MG_2}^{-1} = (3.8 \pm 1.2) \times 10^6\ s^{-1}$ for the binary glycine complexes[15] to $\tau_{MAG}^{-1} = (7.8 \pm 1.5) \times 10^6\ s^{-1}$ in the ternary glycine-Mn^{2+}-ATP^{4-} complexes, all values referring to a temperature of 298 K. Again, this difference in exchange rates confirms the destabilizing effect of ATP^{4-} on the Mn^{2+}-glycine interaction.

According to model building, the difference between the glycine-Mn^{2+} interactions in the binary and the mixed-ligand complexes, thus established, can hardly be caused by steric hindrance. Rather, it is due to a direct interaction of the glycine with the ATP^{4-} ligand, most likely a polar interaction[4] between the positively charged ammonium group of the amino acid and the negatively charged phosphate groups of the nucleotide or a hydrogen bond between the carboxyl oxygen of the amino acid and the NH_2 group at the C(6) carbon of the adenine ring or a combination of such interactions as indicated in Figure 11. Both of these interactions may bring the two ligands in specific positions, or states, that are favorable for further reactions in enzymatic processes, in which they serve as substrates.

Conclusion

The present study shows that NMR relaxation studies can provide detailed information about the stability, structure, dynamics, and ligand exchange of paramagnetic metal complexes in solution, even in the case of a relatively complex system as the ternary glycine/Mn^{2+}/ATP^{4-} system investigated here. In particular, the study emphasizes the different glycine-Mn^{2+} interaction in the ternary glycine-Mn^{2+}-ATP^{4-} complexes and the binary glycine-Mn^{2+} complexes. Likewise, it reconciles the apparently conflicting results obtained previously[18-24,28,47] for systems with highly different values of the Mn^{2+}/ATP^{4-} ratio, by clarifying the difference in nature of the binary Mn^{2+}-ATP^{4-} complexes that dominate at these extremes.

The study also demonstrates that the paramagnetic relaxation of the ligand nuclei and the relaxation of the unpaired electrons of the Mn^{2+} complexes investigated can be described satisfactorily by the Solomon-Bloembergen-Morgan theories. This holds even if the Redfield limit for the spin-spin relaxation of the ligand nuclei is touched in part of the experimental region.

However, the data analyses strongly emphasize the necessity of providing a sufficiently versatile set of experimental data in order to obtain correct and unambiguous results. Hence, the experimental conditions must be varied over a range sufficiently large to allow each one of the involved parameters to influence the data set. This is clearly illustrated by the present analyses, where the tris complex, $Mn(ATP)_3^{10-}$, that reconciles the relaxation data obtained at the highly different Mn^{2+}/ATP^{4-} ratios is revealed only when the precise dependence of the relaxation rates on the ATP^{4-} concentration is incorporated in the data analysis.

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Supplementary Material Available: Tables (Tables III-VIII) containing the experimental R_{ka}, $R_{k,obsd}$, and R_{kp} data corresponding to Figure 1, 2, 3, and 8, (9 pages). Ordering information is given on any current masthead page.

Tandem Mass Spectrometry Methodology for the Sequence Determination of Cyclic Peptides

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Abstract: The unequivocal sequence determination of cyclic penta- and hexapeptides, including biologically active enkephaline and somatostatine analogues, is achieved by using the following procedure. Production of gas-phase $[M + H]^+$ by using fast atom bombardment is followed by investigating either unimolecular or collision-induced dissociations of mass selected $[M + H]^+$ and $[MH -$ amino acid residues$]^+$ fragments. The unimolecular and collisionally activated decompositions of these ions and of their fragments resulting from decarbonylation enable one to elucidate unequivocally the sequence of cyclic peptides. As an example, the sequence determination of an unknown synthetic enkephalin analogue is presented. The amount of sample required for the determination is in the range of 5-100 nmol. Unambiguous sequencing cannot normally be achieved by only analyzing the decompositions of $[M + H]^+$ ions.

Sequencing of linear peptides by using either classical Edman degradation or various mass spectrometric methods can be viewed as a nearly routine procedure.[1-5] In distinct contrast, sequencing of cyclic peptides by means of the classical techniques is much more difficult because, except for special cases, chemical degradation via selective hydrolysis of specific peptide bonds is difficult to achieve. Unspecific hydrolysis followed by gas chromatogra-

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(1) Biemann, K. In "Biochemical Applications of Mass Spectrometry"; Waller, G. R., Dermer, O. C., Eds., Wiley: New York, 1980; p 469.
(2) Morris, H. R. *Nature (London)* **1980**, *286*, 447.
(3) Williams, D. H.; Bradley, C.; Bojesen, G.; Santikarn, S.; Taylor, L. C. E. *J. Am. Chem. Soc.* **1981**, *103*, 5700.

0002-7863/85/1507-6765$01.50/0

phy/mass spectrometry is sample and time-consuming.[1] There have been numerous reports on the use of fast atom bombardment (FAB) mass spectrometry[6] for elucidating the sequence of linear peptides.[3,7] Some of the shortcomings of FAB mass spectrometry, such as high background due to liquid matrix and limited fragmentation, can be overcome by coupling FAB with tandem mass spectrometry (often called mass spectrometry/mass spectrometry or MS/MS).[8,9] This combination permits the observation of fragment ions (produced unimolecularly or by collisional activation) arising from mass-selected peptide ions.[7,10-12] However, there are only four reports[13-15] of the successful direct sequencing of cyclic peptides by using mass spectrometry.

In a previous paper,[15] a study of the feasibility of structure determination of cyclic peptides by using MS/MS was reported. It was found that formation of the major ions can be interpreted in terms of protonation on a peptide nitrogen, followed by ring cleavage to form a linear acylium ion and then losses of amino acid residues (successively or competitively) from the C-terminus (see eq 1). If the cyclic peptide contains one amino acid with an amide nitrogen significantly more basic than the others, upon

$$H_2\overset{+}{N}\text{—}CHR_1CONHCHR_2CONHCHR_3CO \longrightarrow$$

$$H_2NCHR_1CONHCHR_2CONHCHR_3CO^+ \longrightarrow$$

$$H_2NCHR_1CONHCHR_2CO^+ \quad (1)$$

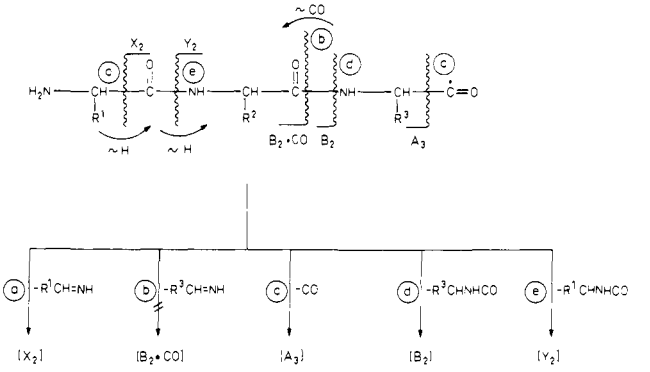
collisional activation of the protonated molecule ion, fragment ions resulting from protonation at that site dominate. The resulting CAD spectrum of the $[M + H]^+$ ion is simplified and relatively easy to interpret. If, however, no one nitrogen is significantly more basic, the resulting CAD spectrum is quite complex even though it was interpretable. When interpreting the CAD spectrum of an unknown, however, the increased complexity may hinder the determination.

Serious problems may arise because fragmentation of the ring-opened $[M + H]^+$ ion can also involve the N-terminus, thus causing problems for assigning an unambiguous sequence. This possibility is demonstrated by considering the fragmentations of the acylium ion m/z 375, obtained via loss of ProOH from the $[M + H]^+$ ion of the linear tetrapeptide Orn-Leu-Phe-Pro (eq 2). The mass selected m/z 375 ion undergoes both unimolecular

$$[\text{Orn-Leu-Phe-Pro}]H^+ \xrightarrow{-\text{ProOH}} \underset{m/z\ 490}{\text{Orn-Leu-Phe}} \xrightarrow{-\text{Phe}} \underset{m/z\ 228}{\text{Orn-Leu}^+} \quad (2)$$

$$\underset{m/z\ 375}{\text{Orn-Leu-Phe}} \xrightarrow{-\text{Orn}} \underset{m/z\ 261}{\text{Leu-Phe}^+}$$

Scheme I



and collision-induced losses of either Phe *or* Orn to a comparable extent, thus precluding a sequence determination. Losses of amino acid residues from both the C- and N-terminus of peptide acylium ions pertain to other cyclic peptides.[16] When this occurs, the unequivocal sequencing of acylium ions *cannot* be based on the observation of successive losses of amino acid residues.

In contrast, the sequence of the acylium ions can be determined by searching the unimolecular or CAD spectra of the $[M + H]^+$ for three ionic doublets A-B, B-C, and C-D, where A, B, C, and D are amino acid residues (-HNCHRCO-), and/or for losses of neutral amino acid doublets.[17] If the doublets are found, *two* possible sequences emerge: A-B-C-D$^+$ and D-C-B-A$^+$. Having established the connectivities of the amino acids, the N- or C-terminus of the linear acylium ions needs to be determined so that the sequence c(A-B-C-D) can be distinguished from the *retro* sequence c(D-C-B-A). This distinction can be easily made for cases in which the linear acylium ion **1** undergoes α-cleavage of the C–C bond adjacent to the N-terminal carbonyl group accompanied by hydrogen migration (Scheme I, process a).[18] Since there is no evidence that this reaction ever involves the C-terminus of peptide acylium ions (process b)[16], the observation of reaction a can be used to define positively the N-terminus of an unknown peptide acylium ion. In fact, cleavage of the amide bond accompanied by CO migration (Scheme 1, process b) is highly unlikely because of the small migrating aptitude of CO. There exists, to the best of our knowledge, no precedent for such a reaction.

As part of our continuing interest in this problem and in view of the need for a widely applicable, sensitive, and rapid methodology for the sequence determination of cyclic peptides by using mass spectrometry, we have investigated the MS/MS spectra of some linear acylium ions and their decarbonylated analogues both generated as fragments from cyclic and acyclic peptides. During the investigation, several reactions of these ionic species that provide useful structural information have been noted. These are reported first. Then, for the first time, we describe a methodology which appears to be extremely valuable for the unequivocal sequence determination of cyclic penta- and hexapeptides,[19] including biologically active enkephaline and somatostatine analogues.

(4) Rinehart, K. L., Jr.; Cook, J. C., Jr.; Pandey, R. C.; Gaudioso, L. A.; Meng, H.; Moore, M. L.; Gloer, J. B.; Wilson, G. R.; Gutowsky, R. E.; Zierath, P. D.; Shield, L. S.; Li, L. H.; Renis, H. H.; McGovern, J. P.; Coconico, P. G. *Pure Appl. Chem.* **1982**, *54*, 2409.

(5) Dell, A.; Morris, H. R. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 1456.

(6) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325.

(7) Lippstreu-Fischer, D. L.; Gross, M. L. *Anal. Chem.* **1985**, *57*, 1174 and references cited therein.

(8) For the methodology of MS/MS see: McLafferty, F. W., Ed. "Tandem Mass Spectrometry"; Wiley-Interscience: New York, 1983.

(9) For a recent review see: Levsen, K.; Schwarz, H. *Mass Spectrom. Rev.* **1983**, 77 and references cited therein.

(10) Hunt, P. F.; Buko, A. M.; Ballard, J. M.; Shabanowitz, J.; Giordani, A. B. *Biomed. Mass Spectrom.* **1981**, *8*, 397.

(11) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *Biomed. Mass Spectrom.* **1982**, *9*, 208.

(12) Heerma, W.; Kamerling, J. P.; Slotboom, A. J.; v. Scharrenberg, G. J. M.; Green, B. N.; Lewis, I. A. S. *Biomed. Mass Spectrom.* **1983**, *10*, 13.

(13) Aubagnac, J. L.; Devienne, F. M.; Cambarieu, R. *Tetrahedron Lett.* **1983**, 2263.

(14) (a) Gross, M. L.; McCrery, D.; Crow, F. W.; Tomer, K. B.; Pope, M. R.; Cicufetti, L. M.; Knoche, H. W.; Daly, J. M.; Dunkle, L. D. *Tetrahedron Lett.* **1982**, *23*, 5381. (b) Kim, S.-D.; Knoche, H. W.; Dunkle, L. D.; McCrery, D. A.; Tomer, K. B. *Tetrahedron Lett.* **1985**, *26*, 969.

(15) Tomer, K. B.; Crow, F. W.; Gross, M. L.; Kopple, K. D. *Anal. Chem.* **1984**, *56*, 880.

(16) Eckart, K.; Schwarz, H., unpublished results.

(17) Fragment ions also decompose to give amino acid doublet ions or to expel neutral amino acid doublets. This information should be used with caution for determining the sequence of the original peptide because of the possibility of cyclization of the fragment acylium ions.

(18) The recently introduced nomenclature of peptide fragment ions used in this article is described by the following: Roepstorff, P.; Fohlhammer, J. *Biomed. Mass Spectrom.* **1984**, *11*, 601.

(19) Cyclic peptides successfully sequenced by using the method described in this paper include the following. Tetrapeptides: c[Gly-NMeAla-Leu-ΔPhe]. Pentapeptides: c[Pro-Pro-Phe-Phe-Gly], c[Pro-Ser-Gly-Phe-Gly], c[Phe-Gly-Aib-Val-Ala], c[Ser-Gly-Gly-Phe-Leu], c[Thr-Gly-Gly-Phe-Leu], c[(gln)Thz-(gly)Thz-Pro-Leu-Val], and c[Val-Leu-Pro-(gly)Thz-(gln)Thz]. Hexapeptides: c[Trp-Phe-Pro-Phe-Thr-Lys(Z)], c[Lys(Z)-Thr-Phe-Pro-Phe-Trp], c[Trp-Lys(Z)-Thr-Gly-Pro-Phe], and c[Trp-Lys(Z)-Thr-Pro-Gly-Phe]. Studies of larger cyclic peptides are underway; preliminary results indicate that the strategy is also applicable to systems containing up to 8–10 amino acids.

Experimental Section

The acylium ion Orn-Leu-Phe$^+$ was generated from Orn-Leu-Phe-Pro [kindly provided by Dr. Z. Palacz, Technical University (TU), Berlin] and from decomposition of Gramicidin S (kindly provided by Dr. C. Costello, Massachusetts Institute of Technology, Boston). A sample of pGlu-His-ProNH$_2$ was provided by Dr. K. Bauer, TU Berlin. The remaining cyclic peptides were synthesized and provided by Prof. Horst Kessler, University of Frankfurt, and purified by using Sephadex chromatography.[20,21] FAB mass spectra were recorded at the TU Berlin with a Varian MAT 311A mass spectrometer equipped with a Ion Tech atom gun, at the following operating conditions: 6 keV Xenon, emission current 0.1–0.2 mA; ion source conditions: accelerating voltages 3 kV, temperature 20 °C; pressure 10^{-5} torr.

Metastable ion (MI) and CAD spectra were recorded with use of mass analyzed ion kinetic energy spectroscopy (MIKES) with a scan rate of approximately 30 s/scan; filter 10 Hz; approximately 15–50 full scans were averaged with a Varian 620f NMR computer; air was used as the collision gas. MI spectra recorded on the Vacuum Generators mass spectrometer of BEB configuration (ZAB-3F) were obtained at the following conditions: accelerating voltage 8 kV, temperature 20 °C, pressure 5×10^{-5} torr. The ions of interest were selected with magnet B_1, and their unimolecular dissociations were recorded by using a linked scan (E/B_2 = constant) of the electrostatic analyzer and magnet B_2 at a resolution for daughter ions of approximately 600. The scan rate was 15 s/scan; filter 300 Hz; 20 full scans were averaged with use of the VG 11/250 data system.

Mass spectra recorded at the Midwest Center for Mass Spectrometry (MCMS) were obtained with a Kratos (Manchester, England) MS-50 triple-analyzer mass spectrometer which has been described elsewhere.[22] The FAB ion source is a standard Kratos design equipped with an Ion Tech atom gun (Teddington, England). Samples were dissolved in glycerol or acidified glycerol, and 1 μL of the solution was placed on the copper FAB probe tip of a direct insertion probe. The sample was bombarded by 7–8 keV xenon atoms (emission current was 0.2 to 0.4 mA). The CAD spectra were obtained by selecting the sample ion with MS-I (a double focusing mass spectrometer of EB configuration) followed by collisionally activating with helium gas (reducing the primary beam by 50%) and scanning MS-II (an electrostatic analyzer). MI mass spectra were obtained by selecting the sample ion with MS-I and scanning MS-II without introduction of collision gas. CAD and MI mass spectra were acquired, signal-averaged, and output with use of software written at MCMS for the Kratos DS-55 data system. Full-scan FAB mass spectra were acquired and processed with use of the standard DS-55 software available from Kratos.

Results and Discussion

As the major impetus for this work was to establish a strategy for the sequence determination of cyclic peptides by analyzing their mass selected $[M + H]^+$ and $[M + H - \text{amino acid residue}]^+$ fragment ions, it is necessary to understand the unimolecular (metastable) and collisionally activated decompositions of relatively simple acylium ions containing approximately three or four amino acid residues. The ions undergo three principal reactions: (1) C-terminus fragmentation (two fragments A_n and B_n[18]); (2) N-terminus fragmentation (X_n and Y_n fragments[18]) (see Scheme I); and (3) elimination of NH_3.

C-Terminus Fragmentation. As observed in the MI and CAD mass spectra of fragments from the protonated molecules of cyclic and linear peptides, a major fragmentation sequence involves loss of CO and/or amino acid residues from the C-terminus.[13–15,21] This is illustrated in Figure 1 for the collision-induced decomposition of Pro-Pro-Phe$^+$ from c(Pro-Pro-Phe-Phe-Gly). The resulting fragment ions (designated as A_n and B_n in the figure) can be used to derive the amino acid sequence of the acylium ion.

N-Terminus Cleavage. As mentioned above (eq 2), linear acylium ions also often undergo an elimination involving an amino acid residue from the N-terminus of the ion. This fragmentation produces a Y_n daughter ion (see Figure 1) which may interfere in assigning a correct sequence. Both the direct or stepwise loss of HNCHRCO from the C-terminus (Scheme I, process d) or the loss of HNCHRCO, combined with hydrogen migration, from the N-terminus (Scheme I, process e) involve elimination of amino acid residues. Because the loss of HNCHRCO may be from either terminus, it may be difficult to predict a priori the origin of the loss. For example, the acylium ion of composition Pro-Ala-Ala$^+$ produced in the fragmentation of the $[M + H]^+$ of *Helminthosporium carbonum* (HC) toxin[14] does not undergo rearrangement to give loss from the N-terminus to any significant extent, whereas Orn-Leu-Phe$^+$ expels HNCHRCO from both termini (eq 2).

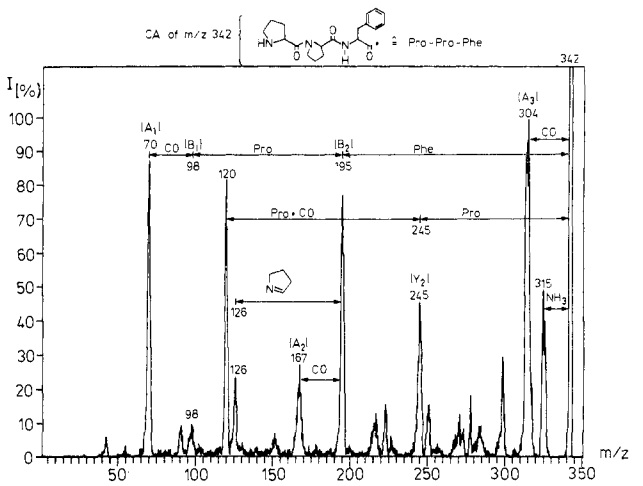


Figure 1. CAD spectrum (Kratos MS-50) of the Pro-Pro-Phe$^+$ ion from c(Pro-Pro-Phe-Phe-Gly).

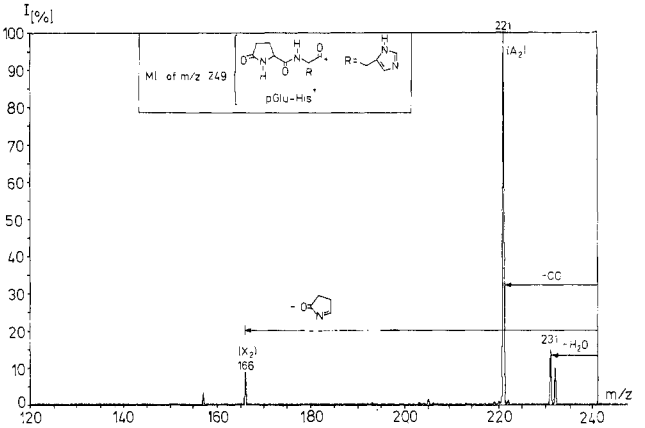


Figure 2. MI spectrum (ZAB-3F) of the pGlu-His$^+$ ion obtained via loss of ProNH$_2$ from $[M + H]^+$ of pGlu-His-ProNH$_2$.

Another useful N-terminus fragmentation of linear acylium ions is cleavage of the C–C bond adjacent to the N-terminal carbonyl group accompanied by hydrogen migration (Scheme I, process a) leading to the fragment X_2. This fragmentation, when observed, can be used to define the N-terminus of an unknown acylium ion. An example is provided by the acylium ion pGlu-His$^+$ (see Figure 2) which fragments to a significant extent in this manner.

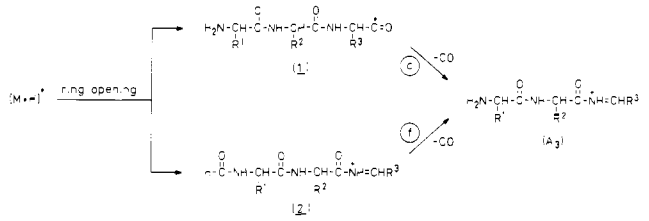
Both of the N-terminus losses described above can result in charge retention on the N-terminus to give amino acid specific fragments in the low mass region. The ion m/z 120 (Phe-CO)$^+$ (see Figure 1) results from such a process.

Elimination of NH_3. Loss of NH_3 is a generally observed decomposition of a linear acylium ion. For some acylium ions, especially those with an N-terminal histidine or tyrosine, this process is dominant possibly because of neighboring aromatic group participation which serves to disperse the charge. The mechanistic details of this rearrangement/dissociation process are not yet available.

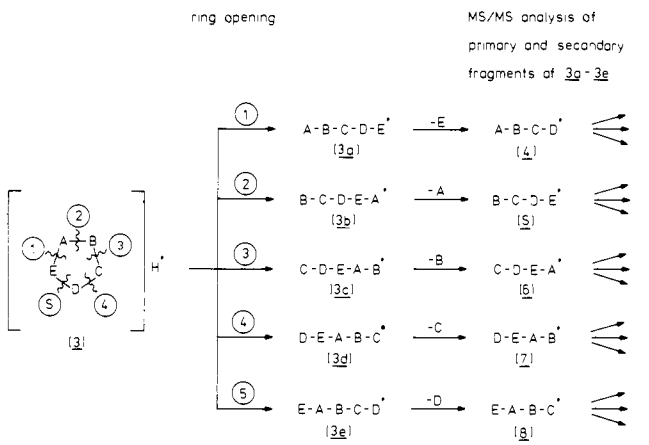
Proposed Sequencing Method. When loss of the N-terminal amino acid residue is observed, unequivocal sequencing of the acylium ions is no longer straightforward. One can, however, determine the sequence of the residues from the tetrapeptide fragment (A-B-C-D) by looking for mass spectral evidence for

(20) Kogler, H. Dissertation, Universität Frankfurt/Main, 1984.
(21) Zechel, C. Dissertation, Universität Frankfurt/Main, 1985.
(22) Gross, M. L.; Chess, E. K.; Lyon, P. A.; Crow, F. W.; Evans, S.; Tudge, H. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *42*, 243.

Scheme II

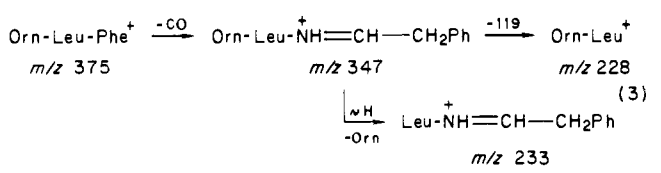


Scheme III



three ionic doublets A-B, B-C, and C-D.[17] If these doublets are observed, *two* sequences are defined; i.e. A-B-C-D$^+$ and D-C-B-A$^+$. The question, whether A or D represent the N-terminus, may then be answered by searching for products formed according to reaction a (Scheme I). Unfortunately, this important reaction does not always occur. For example, Pro-Pro-Phe$^+$ (Figure 1) does not fragment in this manner. There are at least two reasons for this process not occurring. (i) The acylium ions **1** prefer to dissociate via energetically more favored competing reactions (processes c, d, and e, Scheme I); (ii) Ring opening of a protonated cyclic peptide may not generate **1** but instead isomeric *N*-formyl immonium ions **2** (Scheme II). From the latter, loss of $R^1CH—NH$ is as unlikely to occur as is loss of $R^3CH{=}NH$ from **1**. However, CO loss from **1** and **2** would result in the formation of an identical species, A_n, which may be subjected to further analysis (see below).

Studying the immonium ions A_n has some advantages in comparison with the study of the acylium ions **1**. The most important advantage is that loss of an amino acid fragment $R^1CHNHCO$ occurs from the N-terminus, whereas the loss of $R^1CH{=}NH$ is not observed. An example is the tripeptide acylium ion Orn-Leu-Phe$^+$ (see eq 3). This ion undergoes decarbonylation to give *m/z* 347 which, in turn, produces just two abundant fragments (*m/z* 228 and 233). The *m/z* 228 arises from loss of 119 amu ($PhCH_2CH{=}NH$), which defines the C-terminus as Phe. The *m/z* 233 is formed by loss of the N-terminus amino acid which permits identification of this residue. On the basis of the peptides studied thus far, it appears that loss of $R^1CH{=}NH$ from the C-terminus is favored over the elimination of $R^1CHNHCO$ from the N-terminus.



What emerges from these results and considerations is a promising procedure for sequencing cyclic peptides. The procedure is outlined in Scheme III for a pentapeptide. N-protonation of **3** followed by ring-opening can, in principle, result in the formation of five isomeric linear peptide acylium ions (**3a–3e** Scheme III) or their isomeric N-formyl immonium ions, which are not shown in the scheme. Evidence should be sought for amino acid doublets either as ions appearing in the CAD spectra or as entities lost from the ring-opened $[M + H]^+$ ion. Alternately, the MS/MS spectra of the primary fragmentation products **4–8** and, if needed, those of their decarbonylated analogues should be obtained (provided the eliminated residues A, B, C, D, and E, respectively, are different in mass). From an investigation of the primary ions, **3**, the fragment ions **4–8**, and also their decarbonylated species, the doublets required for establishing the connectivities of the amino acids should be found. Thus, two sequences for the linear peptide acylium ions can be defined. The sequences, in conjunction with the elucidation of the N- or C-terminus, as determined on the basis of any of the reactions described above, can be used to define the sequence in an unequivocal fashion.

Table I. Unimolecular and Collisionally Activated Decomposition of *m/z* 462 (Protonated **10**)[a]

	rel intensities		
m/z	MI	CA	assignment
444	100	100	$(M + H)^+ - H_2O$
434	42	82	$(M + H)^+ - CO$
405	1.0	8.0	$(M + H)^+ -$ Gly
387	0.6	3.2	$(M + H)^+ -$ (Gly + H_2O)
375	1.9	8.8	$(M + H)^+ -$ Ser
349	11	52	$(M + H)^+ -$ Leu
321		16	$(M + H)^+ -$ (Leu + CO)
318	1.1	*b*	$(M + H)^+ -$ (Gly + Ser)
301	0.8	5.2	$(M + H)^+ -$ (Gly + Ser + NH_3)
279	1.0	*b*	$(GlyGlyPheNH_3)^+$
262	0.9	13	$(M + H)^+ -$ (Ser + Leu)
258	0.9	*b*	$(M + H)^+ -$ (Gly + Phe)
202	1.4	22	$(M + H)^+ -$ (Leu + Phe)
145		11	$(Gly + Ser)^+$
120	0.3	64	$(Phe - CO)^+$
86		43	$(Leu - CO)^+$
60		10	$(Ser - CO)^+$
30		5.6	$(Gly - CO)^+$

[a] The signal-to-noise ratio was >5:1. [b] Overlapping of broadened peaks prevents exact intensity measurements.

Table II. Unimolecular Decomposition of *m/z* 321 [Protonated "Unknown" - (Leu + CO)]

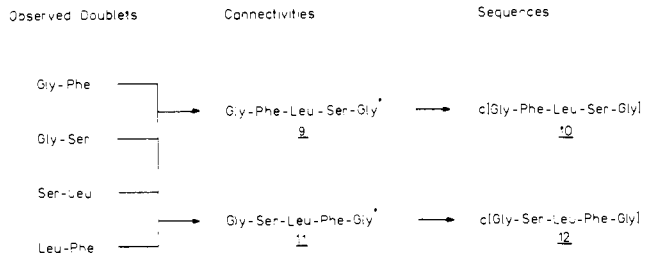
m/z	rel intensity	assignment
304	88	*m/z* 321 - NH_3
286	11	*m/z* 321 - (NH_3 + H_2O)
234	6	*m/z* 321 - (Ser)
202	100	*m/z* 321 - (C_8H_9N)
177	23	*m/z* 321 - (Gly + Ser)
145	4	$(GlySer)^+$
120	70	$(Phe - CO)^+$

Application. We have applied this strategy to sequence a dozen structurally different cyclic penta- and hexapeptides.[19] The sequence determination of an unknown peptide will serve as an example. The synthetic peptide was provided with no structural information. The peptide gave upon FAB abundant *m/z* 462 ions; addition of NaCl to the glycerol matrix caused a shift to *m/z* 484. Thus, the molecular weight is 461 daltons. The unimolecular and collision-induced decompositions of $[M + H]^+$ are listed in Table I. In addition, collision-induced dissociation of *m/z* 462 generated abundant fragments *m/z* 30, 60, and 144 which correspond to decarbonylated Gly$^+$ and Ser$^+$ and the acylium ion Gly-Ser$^+$ or Ser-Gly$^+$, respectively.

The molecular weight and fragmentations, taken together, are consistent with a pentapeptide containing the amino acids Ser, Leu, Phe, and two Gly. Moreover, the required doublets can be deduced from the data in Table I and the results of the CA experiment. These doublets are Gly-Ser, Ser-Leu, Leu-Phe, and Gly-Phe. Two and only two possible pentapeptide acylium ions can be defined on the basis of these results, i.e., the species **9** and **11** (Scheme IV), from which two isomeric cyclic enkephaline peptide analogues **10** and **12** emerge by head-to-tail connections.

Unambiguous differentiation of **10** and **12** is achieved by studying *m/z* 321 $[M + H - Leu - CO]^+$ (Table II). This

Scheme IV



decarbonylated fragment ion undergoes a facile unimolecular decomposition to give m/z 202 (100%), which arises from the loss of 119 daltons (C_8H_9N). This process has already been referred to in eq 3 and is indicative of the presence of a peptide $-^+NH{=}CH{-}CH_2Ph$ unit in an immonium ion. Consequently, the tetrapeptide immonium ion m/z 321 generated via loss of Leu and CO from the protonated pentapeptide must have the sequence Ser-Gly-Gly-$^+NH{=}CH{-}CH_2Ph$ and *not* Phe-Gly-Gly-$^+NH{=}CH{-}CH_2OH$. This result, in turn, is taken to rule out sequence **11** and leaves **9** as the only remaining option. Head-to-tail connection yields **10** as the correct sequence of the cyclic pentapeptide. This conclusion is in agreement with the independent sequence assignment of this peptide (based on synthesis and NMR studies) by Kessler et al.[20,21] c[Gly-Phe-Leu-Ser-Gly] (**10**) is an important enkephaline analogue whose conformational properties and biological activity have been reported elsewhere.[21]

By applying the strategy outlined in this paper, various cyclic tetra- to hexapeptides (including somatostatine analogues)[19] have been successfully sequenced. The amount of sample used in all cases was in the 5–100 nmol range.

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Prediction of Silicon-29 Nuclear Magnetic Resonance Chemical Shifts Using a Group Electronegativity Approach: Applications to Silicate and Aluminosilicate Structures

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Abstract: Linear relations between group electronegativeity (EN) sums of ligands bonded to tetravalent silicon and silicon-29 nuclear magnetic resonance (NMR) chemical shift (δ_{Si}) are shown to exist for both type P silicon (all ligands have lone-pair electrons available for (d–p) π-bonding, e.g., in $(MeO)_4Si$) and type S silicon (all ligands have only σ-bonding electrons available, e.g., in $(CH_3)_4Si$). For type P silicon having group electronegativity sums greater than 11, a range encompassing all minerals, we have used previously reported EN and δ_{Si} values (for aryl-, halo-, and alkoxysilanes) to describe the observed silicon-29 NMR chemical shift as $\delta(Si,P) = -24.336\sum EN(P) + 279.27$. We then apply this correlation to a wide range of silicates and aluminosilicates (containing insular (Q^0) to framework (Q^4) Si sites) to predict silicon-29 NMR chemical shifts by means of a group fragment electronegativity sum approach, in which all fragments (e.g., OAl, OLi, OCa) attached to Si are assigned, on the basis of experiments on a series of model silicates and the above equation, a characteristic group (or fragment) electronegativity value. OSi group electronegativities are scaled linearly with bridging bond angle. As an example of the use of the method, the electronegativity sum value for the cyclosilicate (Q_2) beryl ($Al_2Be_3(SiO_3)_6$) is derived as EN(OBe) + EN(OAl) + 2(EN(OSi) (168.2°)) = 15.67, which predicts a silicon-29 chemical shift of −102.1 ppm (from Me_4Si), that compares favorably with the value from experiment, −102.6 ppm. On the basis of a total of 99 sites in 51 different compounds, the mean absolute deviation between theory and experiment is 1.96 ppm (correlation coefficient = 0.979). When all types of silicon are considered (Q^0–Q^4), this empirical approach is the most accurate method of predicting silicon-29 chemical shifts found to date.

Since 1980, there has been very considerable interest in using solid-state silicon-29 nuclear magnetic resonance (NMR) spectroscopy, with "magic-angle" sample-spinning (MASS), to investigate the structures of a wide variety of silicate and aluminosilicate materials of interest in chemistry and geochemistry.[1–5] In order to interpret the silicon-29 shifts observed in structural terms, various correlations based on bond length,[6] bridging bond angle,[7,8] bond strength,[9,10] mean TOT distance,[11] and σ-orbital hybridization[12] have been presented, together with a recent calculation of the paramagnetic shift term (σ_p) based on band-gap and refractive index dispersion data.[13]

To date, when *all* silicate phases are examined, the best correlation between experiment and prediction is by use of the

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(1) E. Lippmaa, M. Magi, A. Samoson, G. Engelhardt, and A.-R. Grimmer, *J. Am. Chem. Soc.*, **102**, 4889 (1980).
(2) E. Lippmaa, M. Magi, A. Samoson, M. Tarmak, and G. Engelhardt, *J. Am. Chem. Soc.*, **103**, 4992 (1981).
(3) C. A. Fyfe, J. M. Thomas, J. Klinowski, and G. C. Gobbi, *Angew. Chem., Int. Ed. Engl.*, **22**, 259 (1983).
(4) C. A. Fyfe, G. C. Gobbi, J. Klinowski, J. M. Thomas, and S. Ramdas, *Nature (London)*, **296**, 530 (1982).
(5) E. Oldfield and R. J. Kirkpatrick, *Science*, **227**, 1537 (1985).
(6) J. B. Higgins and D. E. Woessner, *EOS (Trans. Am. Geophys. Union)*, **63**, 1139 (1982). A.-R. Grimmer, R. Peter, E. Fechner, and G. Molgedey, *Chem. Phys. Lett.*, **77**, 331 (1981). A.-R. Grimmer and R. Radeglia, *Chem. Phys. Lett.*, **106**, 262 (1984).

0002-7863/85/1507-6769$01.50/0