washed with saturated brine (1 × 15 mL). The ether solution was dried ($MgSO_4$) and evaporated. The residue was an oil and had α^{22}_D +21.1° (neat, 1 dm). Analysis of the product by GC showed that the hydrogenolysis was essentially complete. A peak with a retention time of 3.8 min was present, corresponding to α-phenylethyl alcohol (**2a**), but the peak corresponding to the retention time of **2f** (5.9 min) was absent.

Hydrogenolysis of (*R*)-α-(*o*-Chlorophenyl)ethyl Alcohol [(*R*)-2j]. The hydrogenolysis of (*R*)-**2j** was accomplished by using the same general procedure used for the hydrogenolysis of (*R*)-**2f**. The product oil has α^{22}_D +24.3° (neat, 1 dm), and GC analysis showed that the hydrogenolysis was complete. The peak corresponding to the retention time of α-phenylethyl alcohol (**2a**) was present, but the peak corresponding to the retention time of **2j** (5.5 min) was absent.

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Neutralization–Chemical Reionization Mass Spectrometry

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Abstract: A neutralization–reionization scheme for tandem mass spectrometry has been developed which uses proton-transfer rather than electron-transfer reactions. The neutralization reaction is an endothermic transfer of a proton from protonated peptides to ammonia carried out at beam energies close to threshold. The ammonia collision gas also serves as a reagent gas in a collision chamber that is also a chemical ionization source. As the kinetic energies of neutralized molecules are reduced by additional collisions, they are reprotonated in the same chamber by the reverse, exothermic reaction. The method has the advantages that the neutralization and reionization reactions can be carried out in a single chamber, that proton-transfer reactions are compatable with the protonated species produced by soft ionization techniques, and that additional fragmentation is observed. The method is referred to as neutralization–chemical reionization mass spectrometry (NCRMS).

Recently, research groups at Cornell[1,2] introduced a technique for tandem mass spectrometry known as *neutralization–reionization mass spectrometry* (NRMS), in which a mass selected high energy ion beam from MS1 is neutralized by collision with a metal vapor and then reionized and mass analyzed in MS2. The collision chamber consists of two regions.[3] Collision with vaporized metals in the first chamber favors *charge exchange* over *collision induced dissociation* (CID). Reionization by collision with O_2, the most efficient of the target gases tested,[4,5] occurs in the second chamber, producing fragment ions as well for structural information. Both charge-transfer processes (neutralization and reionization) take place at high kinetic energy and involve electron transfer. In McLafferty's experiments, mass analyses in MS2 were performed with an electric sector only.

Both positive and negative ions may be used in neutralization–reionization schemes.[6] Thus, McLafferty et al.[7] used a (+NR+) scheme to study the interconversion (isomerization) of gas-phase neutral C_6H_6O isomers (phenol and cyclohexa-2,4-dienone), prepared from their radical cations by 9.9 keV collision with mercury vapor. Alternatively, Harrison and co-workers[8] have obtained (+NR+) and (+NR−) spectra of $[SF_5]^+$ and (−NR+) and (−NR−) spectra of $[SF_5]^-$. In their experiments, O_2 was used as both the neutralization and reionization gas. The processes were, however, carried out in separate chambers and deflector electrodes were used between the chambers to remove ions surviving the neutralization collisions.

Recently we introduced the use of *endothermic ion–molecule reactions* as an alternative to both *low-energy* and *high-energy* CID for inducing fragmentation in peptides and proteins.[9] In that scheme we assumed that in the protonated, even-electron molecular ions (MH^+) of peptides produced by *fast atom bombardment* the protons were localized primarily at the amide bonds. Such ions could then be reacted with ammonia in a collision chamber, where the proton-transfer reaction

$$MH^+ + NH_3 \rightarrow M + NH_4^+ \quad (1)$$

would occur at a relative energy in the center-of-mass frame, E_{cm}

$$E_{cm} = (E_{lab}M_n)/(M_{ion} + M_n) \quad (2)$$

M_n is the mass of the (target) neutral

M_{ion} is the mass of the (projectile) ion

whose threshold could be estimated from the reaction

$$NH_4^+ + CH_3CONH_2 \rightarrow NH_3 + CH_3CONH_3^+ \quad (3)$$

which is exothermic (ΔH = −2.2 kcal/mol = −0.10 eV).[10] The reaction between peptides and ammonia proceeds via a long-lived, proton-bound *collision complex*, MNH_4^+, and for leucine enkephalin thresholds for the appearance of both the MNH_4^+ and NH_4^+ ions were measured at relative kinetic energies of 0.18 eV.[9] The assumption that protons were transferred from an amide bond

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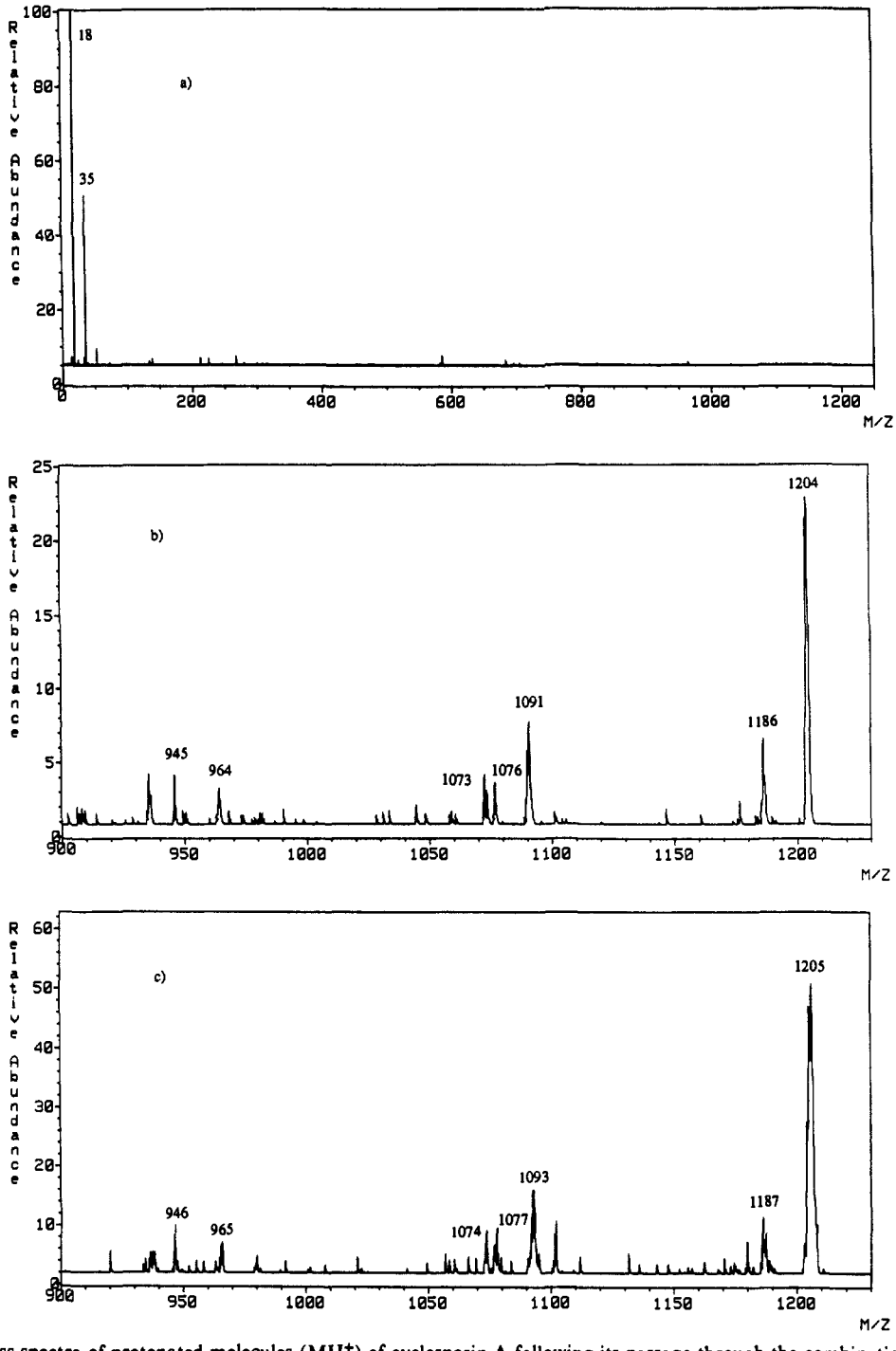

Figure 1. Tandem mass spectra of protonated molecules (MH^+) of cyclosporin A following its passage through the combination collision chamber/chemical ionization source, CI2: (a) electron beam OFF, (b) electron beam ON, with NH_3 as the collision/reagent gas (11 scans signal averaged), and (c) electron beam ON, with ND_3 as the collision/reagent gas (14 scans signal averaged). Collision energy was 20 eV (laboratory frame); collision/reagent gas pressure was approximately 0.3 Torr at 180 °C; mass resolution was 1/500.

in the peptide to ammonia was further corroborated by comparative investigations of the depsipeptide *valinomycin* (which contains alternate amide and ester linkages) and *luteinizing hormone releasing hormone*, LHRH (which contains a basic arginine residue).[11] In the former, mixed exothermic and endothermic behavior was observed, consistent with attachement of protons to ester and amide bonds, respectively.[11] In the latter, the localization of charge on the basic arginine residue resulted in a substantial increase in observed endothermicity.[11]

In our previous studies[9,11] we exploited the fragmentation that occurs above threshold, as excess energy in the collision complex leads to dissociation. In the present work, we have used neutralization by *proton transfer* as a first step in a neutralization–reionization scheme, the latter being achieved by *reprotonation*. This scheme, which we have called *neutralization–chemical reionization mass spectrometry* (NCRMS), has several attractive features: (1) The *deprotonation–reprotonation* reactions used may be more appropriate than electron-transfer reactions for the even-electron (protonated) species produced by soft ionization techniques. (2) Both proton transfers may be carried out in the same chamber, which serves as both a collision chamber and a chemical ionization (CI) source. Protonated molecules enter the chamber with kinetic energies that are low but sufficient to overcome the endothermicity for transfer of a proton to ammonia. Multiple collisions then reduce the kinetic energies of the neutral products, permitting them to be reionized by the reverse (exothermic) transfer of a proton from the ammonium ion to the

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neutrals. (3) Neutral fragments resulting from dissociation of the collision complex will also be protonated, thus increasing the amount of structural information available from the mass spectrum.

Experimental Section

The NCRMS studies reported here were carried out on a JEOL (Tokyo, Japan) Model HX110/HX110 tandem mass spectrometer with four-sector (EBEB)[12] geometry. The collision chamber/chemical ionization source (CI2), operated at 0.3 Torr, was specially fabricated by JEOL and is located in the field-free region between MS1 and MS2. Protonated molecules were produced in the ion source by fast atom bombardment, accelerated to 10 keV and mass selected by MS1. These ions were then decellerated to 20 eV and focussed at the aperture of CI2, which was placed a few volts below 10 kV (corresponding to E_{lab}) and equipped with an electron beam. Neutralization could be observed, i.e. the disappearance of MH^+, and the appearance of NH_4^+, by recording the mass spectrum in MS2 with the electron filament OFF, while the regeneration of MH^+ could be observed with the filament ON. Cyclosporin (1 nM) was used to obtain the averaged spectra presented in each section of Figure 1.

Results and Discussion

Cyclosporin A (MW 1203) produces almost exclusively MH^+ ions, and (with the exception of side-chain losses) little fragmentation, when ionized by fast atom bombardment. With the electron filament in CI2 OFF (Figure 1a) the molecular ion at a collision energy of 20 eV undergoes efficient transfer of a proton to ammonia (NH_4^+ at $m/z = 18$). Fragment ions, resulting from dissociation of the collision complex (MNH_4^+),[9,11] are not observed at relative energies (around 0.27 eV) for which there is insufficient excess energy for the complex to dissociate. This spectrum was obtained by tuning the collision energy to maximize neutralization relative to fragmentation, and therefore to optimize the conditions for reprotonation when the electron beam was turned ON. In Figure 1b, with the electron filament turned ON (generating NH_4^+ ions), neutral cyclosporin is reprotonated ($m/z = 1204$). In addition, a number of fragment ions are observed. Some of these may arise from protonation of neutral fragments produced in the collision; however, the absence of ionic fragments in Figure 1a implies that there are few neutral fragments as well and we suggest that these ions are formed directly from the reprotonated molecule. In Figure 1c, deuterated ammonia (ND_3) was used as the reagent gas to firmly establish that the ions in Figure 1b were reprotonated species and *not* surviving MH^+ ions. In general, ion masses are increased by 1 amu, suggesting that they were formed by transfer of a deuterium ion from ND_4^+ to the neutral molecule. In one case (the peak at 1093) the mass has been increased by 2 amu. In this cyclic peptide, fragmentation occurs by loss of side chains[13] (e.g. m/z 1091 in Figure 1b) and from the cleavage of two in-ring bonds (eg. m/z 964 in Figure 1b). In the former case a more complex mechanism apparently requires transfer of both a neutral deuterium and a deuterium ion.

Conclusions

The consequences of eq 2 are that molecular ions of large mass will have low relative energies in their collisions with inert gases, even while their laboratory energies may be substantial. This results in reduced fragmentation and in the structural (sequence) information this provides. We have noted previously[9,11] that the fragmentation resulting from dissociation of collision complexes can be obtained at reasonable laboratory energies. In this work, however, we have outlined a scheme for neutralization–chemical reionization that can also be carried out at reasonable laboratory energies, is appropriate for soft ionization techniques, and has the simplicity of using a single collision chamber/CI source that can be located (and retrofitted) at the same position as the conventional collision chamber in a tandem instrument. Because the mass of the ion selected by MS1 is always known, it is a simple task to choose a collision energy (corresponding to about 0.1 eV above threshold in the center of mass frame) that will optimize the neutralization reaction. The particular reaction scheme that we have used is appropriate for peptides that do not contain very basic residues; however, more basic reagent gases can also be envisioned which can be more generally applied.

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(12) In this configuration, E refers to the *electrostatic energy analyzer* (ESA) and B refers to the *magnetic* (or mass) analyzer.

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