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Reactivity and Selectivity of Charged Phenyl Radicals Toward Amino Acids in a Fourier-Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometer

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Abstract

The reactivity of ten charged phenyl radicals toward several amino acids was examined in the gas phase in a dual-cell Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer. All radicals abstract a hydrogen atom from the amino acids, as expected. The most electrophilic radicals (with a greater calculated vertical electron affinity (EA) at the radical site) also react with these amino acids via NH_2 abstraction (a nonradical nucleophilic addition-elimination reaction). Both the radical (hydrogen atom abstraction) and nonradical (NH_2 abstraction) reaction efficiencies were found to increase with the electrophilicity (EA) of the radical. However, NH_2 abstraction is more strongly influenced by EA. In contrast to an earlier report, the ionization energies of the amino acids do not appear to play a general reactivity controlling role. Studies using several partially deuterium-labeled amino acids revealed that abstraction of a hydrogen atom from the α-carbon is only preferred for glycine; for the other amino acids, a hydrogen atom is preferentially abstracted from the side chain. The electrophilicity of the radicals does not appear to have a major influence on the site from which the hydrogen atom is abstracted. Hence, the regioselectivity of hydrogen atom abstraction appears to be independent of the structure of the radical but dependent on the structure of the amino acid. Surprisingly, abstraction of two hydrogen atoms was observed for the 3-nitro-5-dehydrophenyl pyridinium radical, indicating that substituents on the radical not only influence the EA of the radical but also can be involved in the reaction. In disagreement with an earlier report, proline was found to display several unprecedented reaction pathways that likely do not proceed via a radical mechanism but rather by a nucleophilic addition-elimination mechanism. Both NH_2 and $^{15}NH_2$ groups were abstracted from lysine labeled with ^{15}N on the side-chain, indicating that NH_2 abstraction occurs both from the amino terminus as well as from the side-chain. Quantum chemical calculations were employed to obtain insights into some of the reaction mechanisms.

Introduction

Attack of radicals on proteins is known to cause proteins to fragment, oxidize, denature and lose enzymatic activity.[1,2] The size and complexity of proteins make it difficult to obtain knowledge on these processes at the molecular level. Hence, many solution studies aimed at improving the understanding of the reactivity of radicals toward proteins have been carried out by using small peptides or individual amino acids as the substrates.[1,2]

One of the most thoroughly studied radicals is the OH radical. In aqueous solution, the OH radical reacts with most amino acids predominantly via hydrogen atom abstraction, and with

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Supporting Information Available. The absolute energies and the coordinates of atoms for all optimized structures, as well as the complete Reference 21. This information is available free of charge via the Internet at http://pubs.acs.org/.

aromatic amino acids via addition to the aromatic ring.[3–4] The hydrogen atom abstraction occurs from the α-carbon or from the side chain of the amino acid (abstraction of a hydrogen atom from the side chain is preferred over abstraction from the α-carbon for amino acids with large side-chains), and it corresponds to the major reaction pathway for amino acids with aliphatic non-sulfur containing side-chains.[5] For the sulfur-containing amino acids methionine and cysteine, the OH radical attacks the sulfur atom. Abstraction of a hydrogen atom from the SH group in cysteine by the OH radical has been also observed.[3–4]

While the reactions of the OH radical and other oxygen centered radicals in solution have been extensively investigated,[3] the reactivity of carbon-centered σ-type organic radicals, such as the phenyl radical, toward amino acids and proteins is nearly entirely unexplored.[6a] To the best of our knowledge, only one such study has appeared in the literature – and in this study, only glycine was examined.[6a] This lack of studies is due to the difficulty to cleanly generate carbon-centered organic radicals in solution.[7–9] However, these reactions are of great interest since organic radicals and biradicals released by some drugs and antitumor antiobiotics, such as the enediynes, are known to attack proteins, in addition to other biopolymers.[10]

Our laboratory has advanced the "distonic ion approach" for the investigation of the reactivity of phenyl radicals in the gas phase,[11,12] that is, via the addition of a chemically inert charged group (to form a distonic radical ion) that allows the manipulation of the radical in mass spectrometers, such as a Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR).[11,12] This approach was justified by results demonstrating that gaseous positively charged phenyl radicals undergo the same reactions (*e.g.,* hydrogen atom abstraction from thiophenol and addition to phenol, aniline, and toluene) and show similar reactivity trends as neutral phenyl radicals in solution.[11,12] The advantages associated with examining radical reactions by using the distonic ion approach include the ability to manipulate the radicals, carry out MS^n experiments, and trap the radicals for variable periods of time in order to measure their second-order reaction rate constants.[11,12] Further, it is possible to carry out experiments under clean conditions where only the desired radicals and reagent molecules are present. Finally, because of the lack of solvent effects, intrinsic radical reactivity can be explored.

Using the above approach, reactions of five phenyl radicals with glycine and glycine-(2,2-d_2), as well as with alanine, valine, proline, cysteine and methionine, were examined in the gas phase.[12b] These studies demonstrated that the α-carbon is not the only site in glycine and labeled glycine from which a hydrogen atom is abstracted.[12b] The less electrophilic radicals were found to react with the amino acids with no sulfur atoms only by hydrogen atom abstraction. However, the more electrophilic radicals also react via NH_2 group abstraction (the occurrence of this reaction demonstrates that the amino acids are not in zwitterionic forms in these experiments). NH_2 abstraction was proposed to occur via a nucleophilic addition-elimination mechanism.[12b] The electrophilicity of the radicals (quantified by calculated electron affinities (EA) of the radical site[12c]), as well as the ionization energies of the amino acids, were found to be the major reactivity controlling parameters.[12b] Various other possible reactivity controlling factors, such as the reaction enthalpy, were not found to play an important role here, in agreement with literature on polar radicals.[13,14]

In order to probe the generality of above findings, and to further explore the regioselectivity of the radicals, we have now extended this study to a more diverse group of phenyl radicals (Chart 1) and several previously unexamined amino acids (Chart 2), many of which are partially deuterium- or ^{15}N-labeled.

Experimental Section

All experiments were carried out in a Finnigan FTMS 2001 dual-cell Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR). The amino acids were introduced into the mass spectrometer by using a thermal solids probe. Depending on the identity of the amino acid, the manual probe was heated up to 140°C – 200°C. Observation of an abundant protonated amino acid upon reaction with protonated acetone (proton affinity[15] (PA): 194 kcal/mol) confirmed that the amino acids were introduced into the instrument without thermal decomposition. The proton affinities of the amino acids are 211.9 kcal/mol for L-glycine, 238 kcal/mol for L-lysine, 220 kcal/mol for L-proline, 218.6 kcal/mol for L-leucine, and 219.3 kcal/mol for L-isoleucine.[15] Only minor fragmentation was observed in the reactions of the amino acids with protonated acetone.

The radicals were generated as described previously.[11,12] For example, N-(2,3,5,6-tetrafluoro-4-dehydrophenyl)pyridinium ion (radical **a**; Chart 1) was formed by introducing pyridine and 1,4-diiodotetrafluorobenzene into the same cell through two variable leak valves. An electron beam of 20–25 eV kinetic energy was used to ionize both species; the filament current was 7 μA and the ionization time one second. This ionization yielded molecular ions as well as various fragment ions from both species. The 1,4-diiodotetrafluorobenzene radical cation was isolated by ejecting all other ions via the application of a stored-waveform inverse Fourier transform[16] (SWIFT) excitation pulse that is applied to the plates of the cell. The 1,4-diiodotetrafluorobenzene radical cation was then allowed to react for 1 – 20 s with pyridine to form the N-(2,3,5,6-tetrafluoro-4-iodophenyl)pyridinium cation by substitution of one of the iodine atoms in 1,4-diiodotetrafluorobenzene with pyridine. The N-(2,3,5,6-tetrafluoro-4-iodophenyl)pyridinium cation was transferred into the other cell by grounding the conductance limit plate for about 140 μs. The technique of sustained off-resonance irradiated collision-activated dissociation,[17] SORI-CAD, with argon target gas was used to homolytically cleave the remaining carbon-iodine bond. In SORI-CAD, the peak pressure of argon in the cell was about 1×10^{-5} torr. The ions were collisionally activated with argon for 0.5 – 1 s at a frequency 1000 Hz above the cyclotron frequency of the ions. All other radicals were generated using a similar procedure except that different precursors were used: 3-iodopyridine for **b**, 3-iodopyridine and bromobenzene for **c**, pyridine and 3,5-dinitroiodobenzene for **d**, pyridine and 3,5-dichloroiodobenzene for **e**, 3-fluoropyridine and 1,3-diiodobenzene for **f**, pyridine and 1,3-diiodobenzene for **g**, pyridine and 1,4-diiodobenzene for **h**, quinoline and 1,4-diiodobenzene for **i**, and pyridine and 4,4′-diiodobiphenyl for **j**.

The radicals of interest were isolated by ejecting all other ions via the application of a series of stored-waveform inverse Fourier transform (SWIFT) excitation pulses to the plates of the cell. The isolated charged phenyl radicals were allowed to react with each amino acid for a variable period of time (typically 0.5–1000 s). Detection was performed by using “chirp” excitation of 124 V amplitude, 2.7 MHz bandwidth, and 3.2 kHz/ms sweep rate. All of the spectra are the average of 10 transients, which were recorded as 64k data points and subjected to one zero fill prior to Fourier transformation. Each reaction spectrum was background corrected by using a procedure described previously.[18]

In the FT-ICR, the concentration of ions (in this case, charged monoradicals) inside the cell is much smaller than that of neutral molecules (amino acid molecules). Hence, the concentration of the amino acid can be assumed to be constant. Indeed, all the reactions studied followed pseudo-first order kinetics, which allowed for the derivation of the second-order reaction rate constant (k_{exp}) from a semilogarithmic plot of the relative abundance of the reactant ion versus reaction time, and the concentration of the amino acid. The pressure

readings inside the cell were measured by two ionization gauges located on each side of the dual cell. The ion gauge pressure readings were corrected for the sensitivity of the ion gauges toward each amino acid and for the pressure gradient between the ion gauge and the cell. The correction factors were obtained by measuring the reaction rate of an exothermic proton-transfer reaction from protonated acetone or protonated methanol to the given amino acid.[19] Such reactions can be expected to occur at collision rate.[20] The accuracy of the measured rate constants is estimated to be about 50%, with the precision estimated to be better than 20%. The theoretical collision rate constants (k_{coll}) were calculated using a parameterized trajectory theory.[21] The efficiency of each reaction (the fraction of collisions that leads to reaction) is given by k_{exp}/k_{coll}. The primary products' relative abundances are reported as branching ratios, which are given as the ratio of a primary product ions' abundance to the sum of all primary product ions' abundances.

All amino acids (purity ≥ 98.5%), except for DL-lysine-ε-^{15}N and DL-glycine-(2,2-d_2), were obtained from Fluka Biochemika. DL-Lysine-ε-^{15}N (purity ≥ 98.5%; isotopic purity (^{15}N) = 98%+) was obtained from Cambridge Isotope Laboratories, Inc., and DL-glycine-(2,2-d_2) (purity ≥ 98.5%: isotopic purity (D) = 98%) from Sigma-Aldrich. DL-Proline-(1-d_1) was synthesized from L-proline by using racemization via a Schiff base procedure.[22] This procedure involves selective exchange of the α-carbon hydrogen atom of L-proline with deuterium by racemization of this stereogenic center (α-carbon) in monodeuterated acetic acid (CH_3CO_2D). A vial with 500 mg of L-proline was refluxed with 0.04 ml of salicylaldehyde and 9.4 ml of monodeuterated acetic acid at 100°C for one hour. The deuterated amino acid was recrystallized. The product's structure was verified by ^{1}H-NMR and mass spectrometry (isotopic purity = 95 atom% D).

Quantum chemical calculations were performed with the Gaussian 98[23] electronic structure program suite. Molecular geometries for the radicals were calculated as previously described.[12c] Charge and spin densities for the radicals were calculated at the UBPW91/cc-pVDZ level of theory. Relative transition state energies for addition of ammonia to different positions in the 3-dehydropyridinium ion were calculated at the UHF/6–31G(d,p)//UHF/6–31G(d,p) level of theory.

Results and Discussion

The ten positively charged phenyl radicals used in this study (**a – j**) are shown in Chart 2. These radicals were generated by using literature methods.[11,12] The isolated charged phenyl radicals were allowed to react with L-glycine, DL-glycine-(2,2-d_2), L-lysine, ^{15}N-labeled DL-lysine, L-isoleucine, L-leucine, DL-leucine-(1-d_1), L-proline, and DL-proline-(1-d_1) (Chart 1) for variable periods of time in order to determine the reaction efficiencies and product branching ratios (Tables 1 – 5).

The ionic avoided curve crossing model[14b,c] predicts a strong relationship between the vertical electron affinity (EA; the energy released upon attachment of an electron to the radical site with no geometry change) of an electrophilic radical and its gas-phase radical reactivity.[24] The higher the EA of the radical, the faster are the reactions predicted to be. This has been found to be true for many reactions. Hence, favorable polarization of the transition state appears to be a crucial controlling factor in these reactions.[24] Consequently, radicals with widely different EA values, ranging from 3.31 eV to 6.18 eV (Chart 2), were chosen for this study. The vertical EAs of the radicals were calculated earlier at the (U)B3LYP/6–31+G(d) level of theory.[12c]

The amino acids L-glycine, L-lysine, L-isoleucine, and L-leucine react with the radicals predominantly by hydrogen atom transfer and addition-elimination pathways (e.g., NH_2

transfer). The less electrophilic radicals only exhibit hydrogen atom abstraction while the more electrophilic radicals also show NH_2 abstraction. The relative reactivity of the radicals was found to depend on their EA, and was independent of the types of reactions observed. This is intriguing as these reactions occur via drastically different mechanisms. While hydrogen atom transfer is a radical reaction, the mechanism for NH_2 transfer is thought to be a nucleophilic addition-elimination reaction rather than a radical reaction (illustrated for lysine in Scheme 1 and discussed in detail below).[12b] In general, for the radicals and amino acids studied here, more NH_2 abstraction and less hydrogen atom abstraction was observed as the EA of the radical increases. This finding is in agreement with a literature report that as the EA of a radical increases, its addition reactions become faster than hydrogen atom abstraction.[11] While NH_2 abstraction is the most common group abstraction observed, other group abstraction products were observed for reactions of L-proline and DL-proline-(1-d_1) with radical **c**. The reactivities observed for the different amino acids are discussed in detail below.

Based on studies on polar effects in radical reactions,[12c] amino acids with lower ionization energies (IE) are expected to react faster via hydrogen atom transfer than those with higher IE values. The preliminary study on a smaller group of amino acids supported this expectation.[12b] However, in the present study, only some of the radicals were found to follow this trend. For example, although radical **e** abstracts a hydrogen atom fastest from proline (IE = 8.3 eV) and slowest from glycine (IE = 8.9 eV), radical **a** reacts at similar efficiencies with glycine and lysine (IE = 8.6 eV), in spite of their quite different IE values.

L-Glycine and DL-Glycine-(2,2-d_2)

The more electrophilic radicals **a – c** display two pathways upon reaction with glycine, namely, hydrogen atom abstraction and NH_2 abstraction (Table 1). The greater the EA of the radical, the more is the NH_2 abstraction favored, and the greater is the total reaction efficiency. The less electrophilic radicals **d – i** react with L-glycine solely by hydrogen atom abstraction (Table 1). This finding is in agreement with previous studies[12b] that have shown that radicals **d** and **e** only react by hydrogen atom abstraction with glycine. The hydrogen atom abstraction efficiency of radicals **d – i** increases with increasing EA of the radical.

No reactions were observed for radical **j**. This lack of reactivity is rationalized by its very low EA of 3.31 eV (the other radicals have EAs ranging from 4.50 to 6.18 eV). In agreement with the present findings, previous studies[12c] have shown that radical **j** abstracts a hydrogen atom from cyclohexane (IE = 10.32 eV) and isopropyl alcohol (IE = 10.44 eV) only at very low reaction efficiencies of 0.0034 and 0.0029, respectively.

Previous examination of the reactions of OH radicals with glycine in solution has shown that hydrogen atoms can be abstracted from either the NH_2 or OH group in glycine, in addition to the α-carbon, which is thermodynamically favored.[25] This occurs in spite of the fact that the homolytic C-H bond dissociation energy in glycine (79.2 kcal/mol) is lower than that of the N-H bond (102.6 kcal/mol), which is substantially lower than that of the O-H bond (112.9 kcal/mol).[26]

Glycine-(2,2-d_2) was allowed to react with radicals **a – j** to obtain a better understanding on which position is preferred for hydrogen atom abstraction from glycine. All these reactions, except those of radicals **a** – **c**, occur via both hydrogen and deuterium atom abstraction (NH_2 abstraction was also observed for radicals **a** – **c**; Table 1), and hydrogen atom abstraction dominates over deuterium atom abstraction in most cases. Hence, hydrogen atom abstraction occurs also from other sites besides the α-carbon. Since abstraction of a deuterium atom is likely to be slower than of a hydrogen atom from the same site due to the primary kinetic isotope effect,[24b] abstraction from the α-carbon is seemingly less favored than it actually is.

For the more electrophilic monoradicals **a – c,** a trend was observed between the EA of the radical and hydrogen/deuterium atom vs. NH_2 abstractions (as the EA of the radical decreases, the extent of both deuterium and hydrogen atom abstractions increases relative to NH_2 abstraction; however, the total reaction efficiency goes down). This competition between hydrogen/deuterium abstraction and NH_2 abstraction is not present for radicals **d – j** where hydrogen and deuterium atom abstractions are the only observed reaction pathways. When NH_2 group abstraction is not observed, there is no correlation between EA and deuterium vs. hydrogen atom abstraction. A similar finding was reported earlier for ethanol.[24a] However, a correlation has been reported in the literature for isopropanol (less electrophilic radicals are more selective for hydrogen atoms bound to the α-carbon).[24b]

For a given radical, abstraction of a hydrogen atom from the α-carbon rather than from the NH_2 group is not only thermodynamically but also kinetically favored. Previously calculated transition state energies for radicals **a** – **c**, **e**, and **g** show that the transition state for hydrogen atom abstraction from the α-carbon in glycine is 2–3 kcal/mol[12b] lower than from the NH_2 group (calculations were not performed for the OH group). However, both present and previous results show that the α-carbon in glycine is not the only site from which a hydrogen atom is abstracted.[12b]

The site at which the initial attack occurs in the NH_2 abstraction reaction pathway is likely the radical site since this readily leads to a homolytic C-N bond cleavage in the amino acid (Scheme 1), and charged aromatic compounds with no radical sites do not undergo this reaction.[12b] This is in spite of the fact that for different radicals, the radical site may or may not be the most electrophilic site. This was explored by calculating the charge densities for 2-, 3- and 4-dehydropyridinium cations at the UBPW91/cc-pVDZ//UBPW91/cc-pVDZ level of theory (Figure 1). For 2-dehydropyridinium cation (radical site charge density = 0.162) and 4-dehydropyridinium cation (radical site charge density = 0.126), the radical sites are the most electrophilic sites. Thus, for these two isomers, the radical site is the preferred site for nucleophilic attack. For 3-dehydropyridinium cation, the radical site (charge density = 0.033) is less electrophilic than the 2-position (charge density = 0.076) or the 4-position (charge density = 0.087; in spite of a smaller positive charge, the 2-position may be favored over the 4-position for nucleophiles that can form a stabilizing hydrogen bond with the NH^+ group). Although the 2- and 4-positions are calculated to be the most electrophilic sites, addition also must occur at the 3-position since a homolytic C-N bond cleavage was observed to occur for the adduct.

The relative transition state energies calculated at the UHF/6–31G(d,p)//UHF/6–31G(d,p) level of theory for addition of ammonia (instead of an amino acid in order to simplify the calculations) to 3-dehydropyridinium cation are 0.5 kcal/mol (C-2), 0.0 kcal/mol (C-3), 1.4 kcal/mol (C-4), 19.5 kcal/mol (C-5), and 2.5 kcal/mol (C-6). Thus, nucleophilic attack at the 3-position has the lowest transition state energy, making addition of ammonia to the 3-position kinetically favored for radical **b**, although additions to the other sites may also occur. Figure 2 shows the calculated charge and spin densities for the ammonia adduct of 3-dehydropyridinium cation. Thus, it seems likely that most of the positive charge resides on the hydrogen atoms bound to the nitrogen atom, and the odd spin is delocalized over the pyridinium ring, in agreement with the Lewis structure presented for an analogous adduct in Scheme 1. These findings suggest that the nucleophilic attack of amino acids to the charged phenyl radicals, leading to NH_2 group abstraction, occurs at the radical site, and yields an adduct with the odd spin delocalized over the pyridine ring.

L-Leucine, DL-Leucine-(1-d_1), and L-Isoleucine

The difference in the alkyl side chain for isomeric L-leucine and L-isoleucine does not substantially influence their reactivity (Table 2). Just as for glycine, both NH_2 abstraction and hydrogen atom abstraction reactions were observed when this group of amino acids was allowed to react with the highly electrophilic charged radicals **a** and **c** (Table 2). Radical **a** shows more NH_2 abstraction than radical **c**. Radical **b** is an exception to the above behavior. This radical reacts by proton transfer with these amino acids (L-leucine: PA = 218.6 kcal/mol;[15] L-isoleucine: PA = 219.3 kcal/mol[15]) because the amino acids are more basic than the conjugate base of radical **b**.[12b] The proton transfer is so facile that it suppresses all radical reactions. Radicals **d** – **g,** with relatively low EA, react exclusively by hydrogen atom abstraction (Table 2). The lower the EA of the radical, the slower the hydrogen atom abstraction.

DL-Leucine-(1-d_1) was allowed to react with radicals **a – g** to obtain a better understanding of the selectivity of hydrogen atom abstraction from leucine. The radicals (with the exception of radical **c**) did not abstract a deuterium atom from the α-carbon of the partially labeled leucine, as expected, but instead a hydrogen atom from somewhere else in the molecule. Although abstraction of the deuterium atom is slowed down by the primary kinetic isotope effect, this finding nevertheless suggests that the α-carbon is not the kinetically preferred site for hydrogen atom abstraction from this amino acid. This finding is in sharp contrast to the behavior of partially labeled glycine where extensive deuterium atom abstraction from the α-carbon was observed. Apparently, the presence of several hydrogen atoms in the side-chain of leucine, as opposed to only one at the α-cabon, makes hydrogen atom abstraction from the side-chain kinetically favored. Furthermore, the sidechain may sterically hinder abstraction of the hydrogen atom from the α-carbon.

Reactions of Phenyl Radicals with L-Lysine and DL-Lysine-ε-^{15}N

As observed for leucine and isoleucine, radical **b** (PA = 216 kcal/mol), the only Brönsted acid among the radicals studied, reacts exclusively by proton transfer with L-lysine (PA = 238 kcal/mol[15]). Further, as reported for glycine, leucine and isoleucine above, NH_2 and hydrogen atom abstractions were observed for the most electrophilic radicals **a** and **c** with lysine (Table 3), while the less reactive radicals **d – h** exhibit only hydrogen atom abstraction.

Radical **d** deviates from the other radicals in that it displays an additional reaction pathway - abstraction of two hydrogen atoms from L-lysine and DL-lysine-ε-^{15}N (Table 3). This reaction is as fast as a single hydrogen atom abstraction. Apparently, after hydrogen atom abstraction by the radical site, the NO_2 group can abstract another hydrogen atom from the lysine radical. The same reaction was observed for radical **d** upon interaction with proline (as discussed below) but not for other amino acids. A possible mechanism for proline is shown in Scheme 2.

Radical **c** forms a stable adduct with L-lysine and DL-lysine-ε-^{15}N, with branching ratios of 32% and 23%, respectively (Table 3). The formation of a stable adduct is most likely due to: 1) the presence of two amino groups in lysine (since only this amino acid yields an abundant stable adduct), and 2) the intrinsic properties of radical **c** (the only radical that forms a stable adduct). With the exception of radical **b** that only undergoes proton transfer reactions, radical **c** is the only radical studied here wherein the radical site is in the same ring as the charge site. Hence, lysine can interact with both these sites of the radical. A possible mechanism for the formation of a stable adduct (m/z 301) for radical **c** with lysine only is shown in Scheme 3. The initial addition is proposed to occur like in Scheme 1, and involve the amino group in the lysine side chain since this group should be more nucleophilic than

that in the amino acid moiety (the adjacent electron-withdrawing carbonyl group reduces its nucleophilicity). However, addition of either amino group should be followed by the same reaction sequence. After addition, the second amino group may abstract the proton from the protonated amino group attached to the aromatic ring. This reversible step would explain why this reaction was only observed for lysine. An irreversible hydrogen atom transfer to the radical site (now delocalized over the ring) would generate a resonance stabilized radical cation. Some support for this mechanism was obtained when the stable adduct ion (m/z 301) was subjected to collision-activated dissociation. It does not fragment to yield back the reactant monoradical **c** via loss of intact lysine, which demonstrates that the adduct has not retained the initially formed structure (shown left in the middle row in Scheme 3). It also does not fragment via loss of NH_3, which indicates that it does not contain a protonated amino group formed upon proton transfer to the second amino functionality (shown right in the middle row in Scheme 3). Instead, the stable adduct (m/z 301) fragments by loss of an NH_2 group (to yield an ion of m/z 285) and loss of CO_2 (to yield an ion of m/z 257), in support of the final structure proposed for the adduct in Scheme 3.

DL-Lysine-ε-^{15}N was studied to provide support for the mechanism shown in Scheme 3. Indeed, radical **c** was found to abstract either one of the two NH_2 groups in DL-lysine-ε-^{15}N, with branching ratios for $^{15}NH_2$ and NH_2 group abstractions of 8% and 21%, respectively. Radical **a** also abstracts $^{15}NH_2$ and NH_2 groups, with branching ratios of 18% and 53%, respectively. Based on these four values, it can be concluded that the electrophilicity of the radical does not have a major influence on the radicals' preference towards side-chain NH_2 abstraction versus NH_2 abstraction from the amino acid moiety. Scheme 1 shows possible mechanisms for NH_2 and $^{15}NH_2$ abstraction from DL-lysine-ε-^{15}N. Abstraction of the NH_2 group from the amino acid moiety is more facile than the abstraction of the $^{15}NH_2$ group (Table 3), likely due to the different stabilities of the radicals produced in these reactions. The radical produced upon the abstraction of the NH_2 group of the amino acid moiety is resonance stabilized, and hence more stable than the radical produced in the abstraction of the NH_2 group from the side chain.

L-Proline and DL-Proline-(1-d_1)

Radical **b** again reacts only by proton transfer when allowed to react with proline (Table 4). In contrast, radicals **a** and **e-h** react with proline via exclusive hydrogen atom abstraction (Table 4). NH_2 abstraction from proline does not occur because the nitrogen atom is a part of a five-membered ring. DL-proline-(1-d_1) was allowed to react with radicals **a – h** to obtain a better understanding of the selectivity of hydrogen atom abstraction from proline. Only a minor amount of deuterium atom abstraction was observed (Tables 4 and 5). Hence, hydrogen atom abstraction from the unlabelled proline mostly occurs from positions other than the α-carbon.

Radicals **c** and **d** (and **b**, as discussed above) are the only radicals that undergo another reaction with proline besides hydrogen atom abstraction. Radical **d** abstracts two hydrogen atoms from L-proline. The abstraction of two hydrogen atoms by **d** was also observed for lysine. The mechanism for this reaction is likely the same for proline and lysine (a possible mechanism is shown in Scheme 2). Steric hindrance due to the methyl branch may slow down this reaction for leucine and isoleucine. As opposed to an earlier study,[12b] wherein only hydrogen atom abstraction was reported for the reaction of radical **c** with proline, additional reaction pathways were observed in the present study. Although hydrogen atom abstraction was found to dominate, HO abstraction, C_2H_4N abstraction, C_5H_7NO abstraction, and stable adduct formation were also observed. This discrepancy between the two studies may arise from the greater sensitivity of the more recent measurements. The different reactivity of radical **c**, when compared to the other radicals studied, may again be

explained by the fact that radical **c** is the only radical studied (with the exception of acidic radical **b**) that has the radical site and the positive charge in the same ring, hence allowing interactions of proline with both. For example, C_2H_4N abstraction from proline by radical **c** may be initiated by nucleophilic addition of the proline nitrogen to the radical carbon, as shown in Scheme 1 for lysine. For lysine, N-C bond cleavage leads to the NH_2 abstraction product. For proline, this is not possible. Instead, the radical (now delocalized over the ring) may abstract a hydrogen atom from the carboxylic acid group of proline, leading to the elimination of CO_2 (Scheme 4). Finally C_2H_4 may be eliminated from the ring in proline, resulting in a resonance-stabilized radical cation (the C_2H_4N abstraction product).

Conclusions

Examination of the gas-phase reactions of ten different phenyl radicals with several amino acids, many partially isotope labeled, revealed the commonly observed hydrogen atom abstraction, but also a number of other reactions. Results obtained for partially deuterium-labeled leucine and proline demonstrate that only a very small amount of hydrogen atoms, if any, are abstracted from the α-carbon, and a large amount from elsewhere (likely the alkyl side-chain). This is in sharp contrast to glycine whose α-carbon is the predominant hydrogen atom donor site. The regioselectivity of hydrogen atom abstraction appears to be independent of the structure of the radical but dependent on the structure of the amino acid.

The mechanism of the previously reported NH_2 abstraction (proposed to occur via nucleophilic addition-elimination mechanism[12b]) was examined computationally. The relative transition state energies for addition of ammonia to different positions in 3-dehydropyridinium cation revealed that addition to the radical carbon is kinetically favored, and hence the most likely addition site for amino acids in charged phenyl radicals. The calculated charge and spin densities in the ammonia adduct of 3-dehydropyridinium cation support the Lewis structure chosen to illustrate the amino acid adducts of the radicals.

Several unprecedented reaction pathways were also observed. These include the abstraction of two hydrogen atoms from proline and lysine by the nitro-substituted radical **d**. Further, abstraction of C_2H_4N, C_5H_7NO, and OH groups by radical **c** were observed for proline. These reactions are likely to occur by nucleophilic addition-elimination pathways, similar to that leading to NH_2 abstraction from all the other amino acids but proline where the nitrogen atom is part of a ring structure. Finally, both NH_2 and $^{15}NH_2$ groups were abstracted from lysine labeled with ^{15}N on the side-chain, indicating that NH_2 abstraction occurs both from the amino terminus as well as from the side-chain of lysine.

The electron affinity of the radical appears to be the major factor in controlling the radical's reaction rates with the amino acids. Both the radical (hydrogen atom abstraction) and nonradical (NH_2 abstraction) reaction efficiencies were found to depend on the electrophilicity of the radical, although the nonradical reactions are influenced more strongly. However, in contrast to an earlier report,[12b] the ionization energies of the amino acids do not appear to have a general reactivity controlling role.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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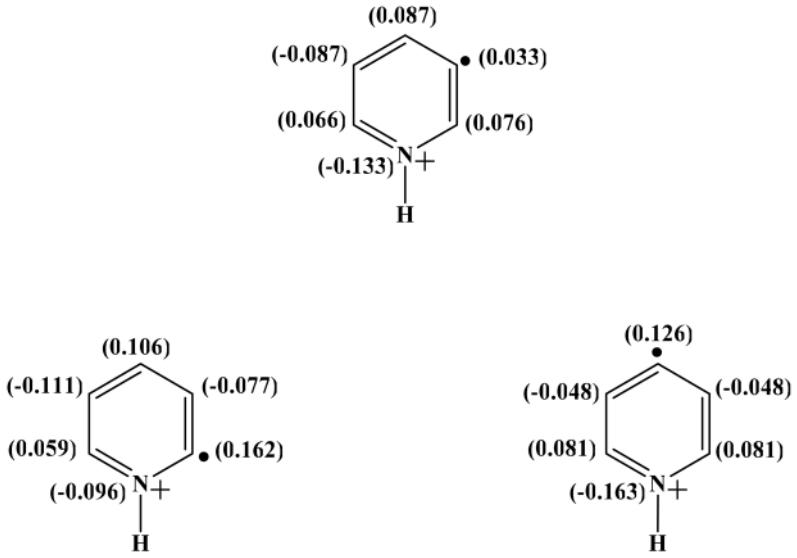


Figure 1.
Calculated (UBPW91/cc-pVDZ//UBPW91/cc-pVDZ) charges for heavy atoms of 2-, 3- and 4-dehydropyridinium cations.

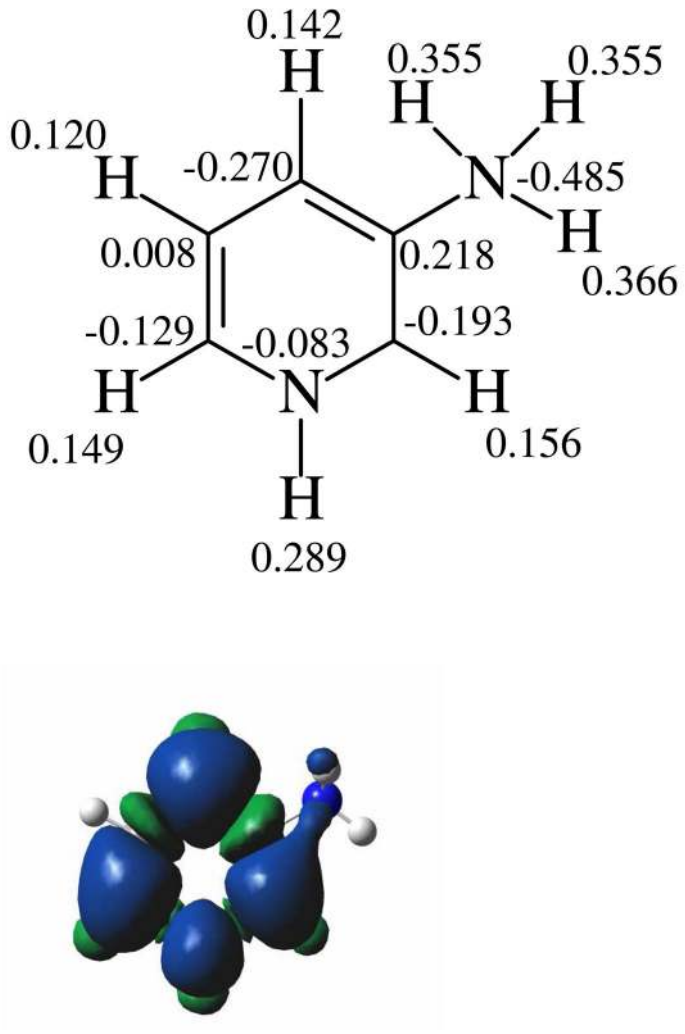
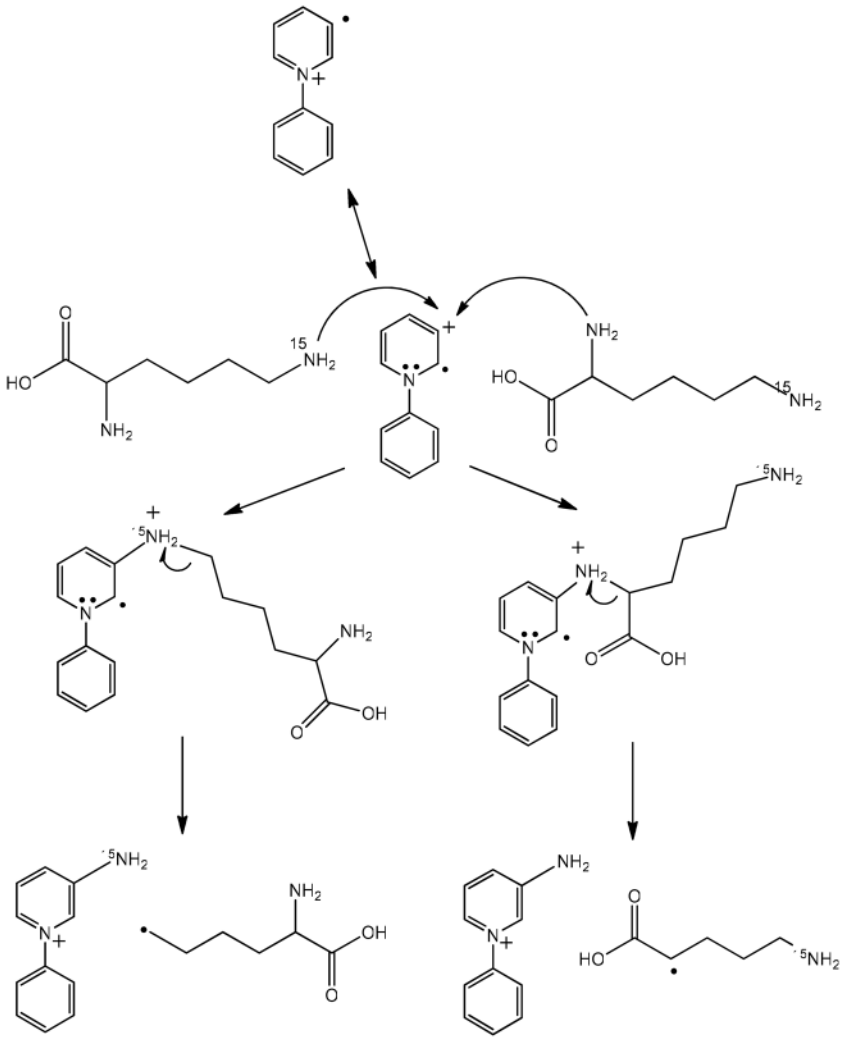
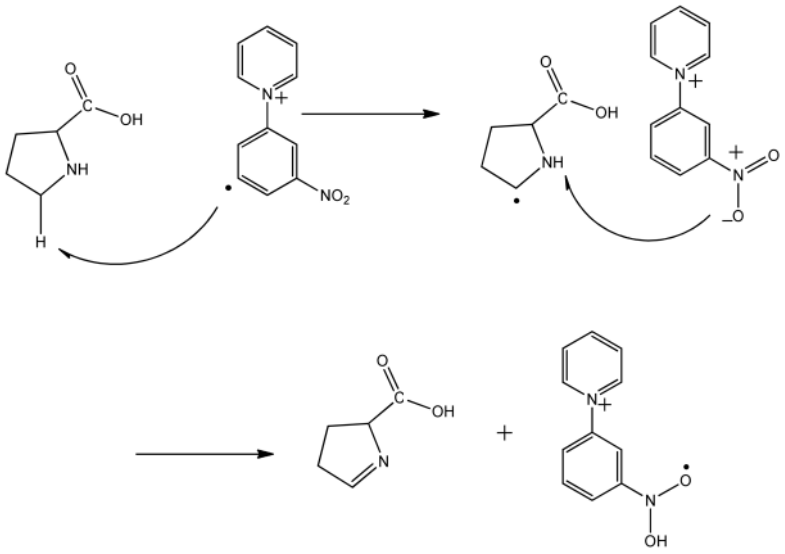


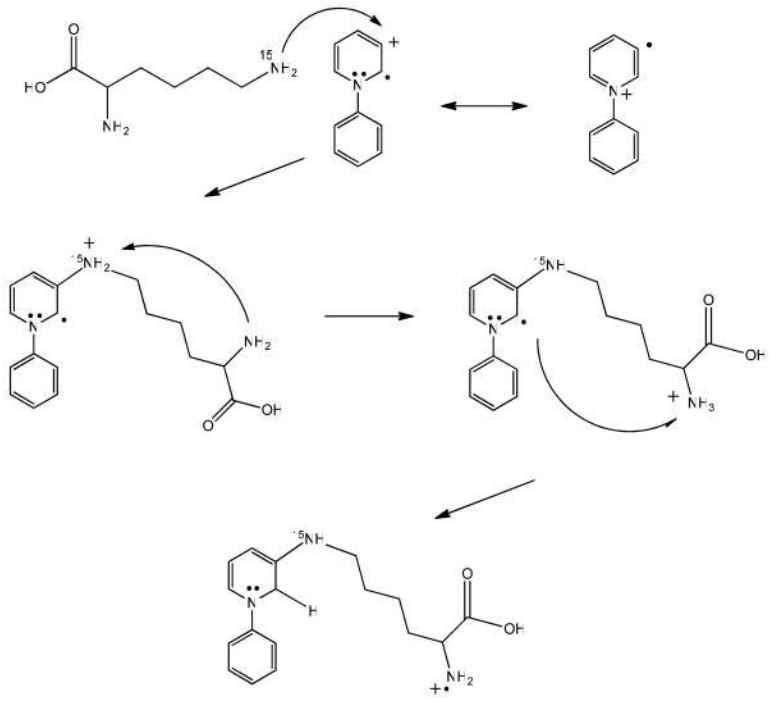
Figure 2.
Calculated (UBPW91/cc-pVDZ//UBPW91/cc-pVDZ) charges (top) and spin density (bottom) for the NH_3 adduct of 3-dehydropyridinium cation.



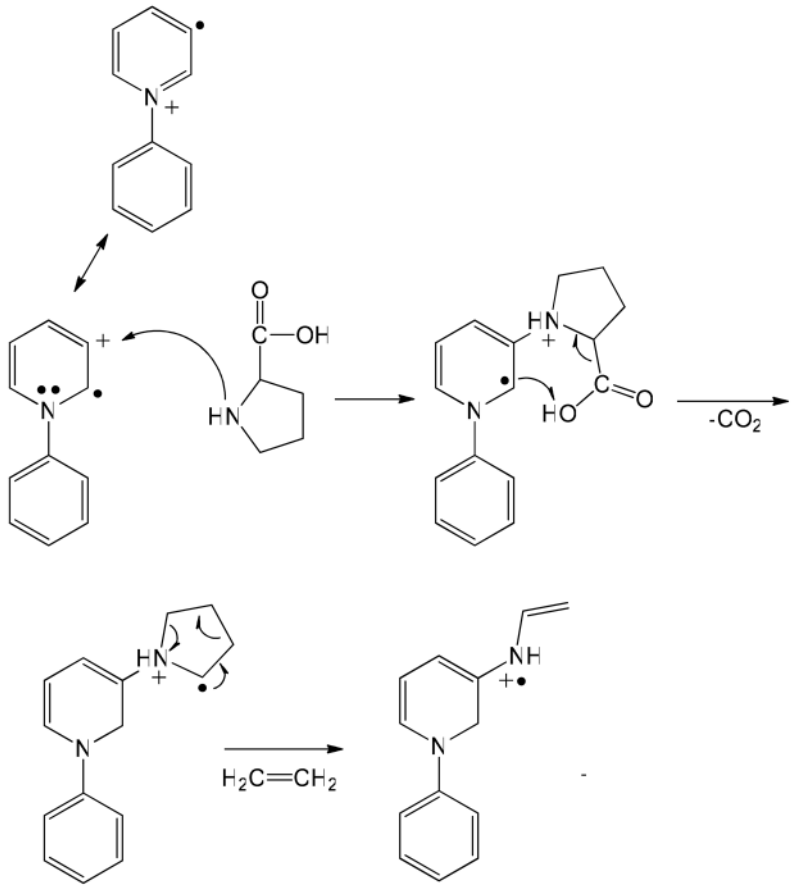
Scheme 1.
Possible mechanisms for the NH_2 abstraction reactions of radical **c** with lysine-ε-^{15}N.



Scheme 2.
Possible mechanism for the abstraction of two hydrogen atoms by radical **d** from proline.



Scheme 3.
Possible mechanism for the formation of a stable adduct between radical **c** and lysine.



Scheme 4.
Possible mechanism of C_2H_4N abstraction upon the reaction of radical **d** with proline.

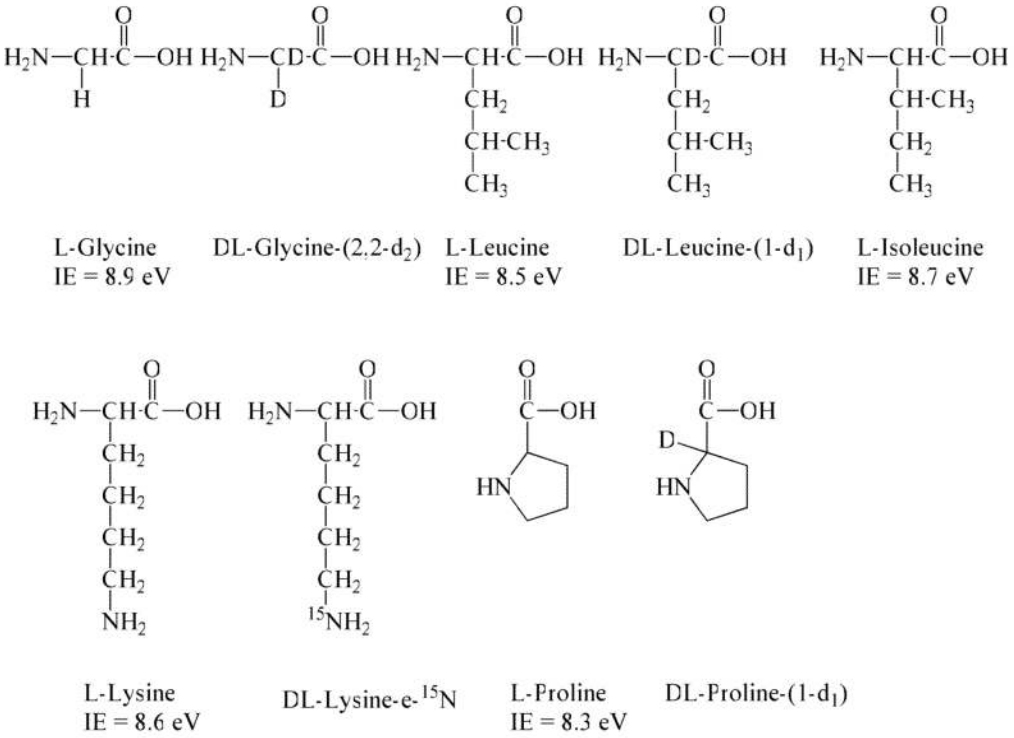


Chart 1.
Structures of the amino acids studied and their ionization energies.[13]

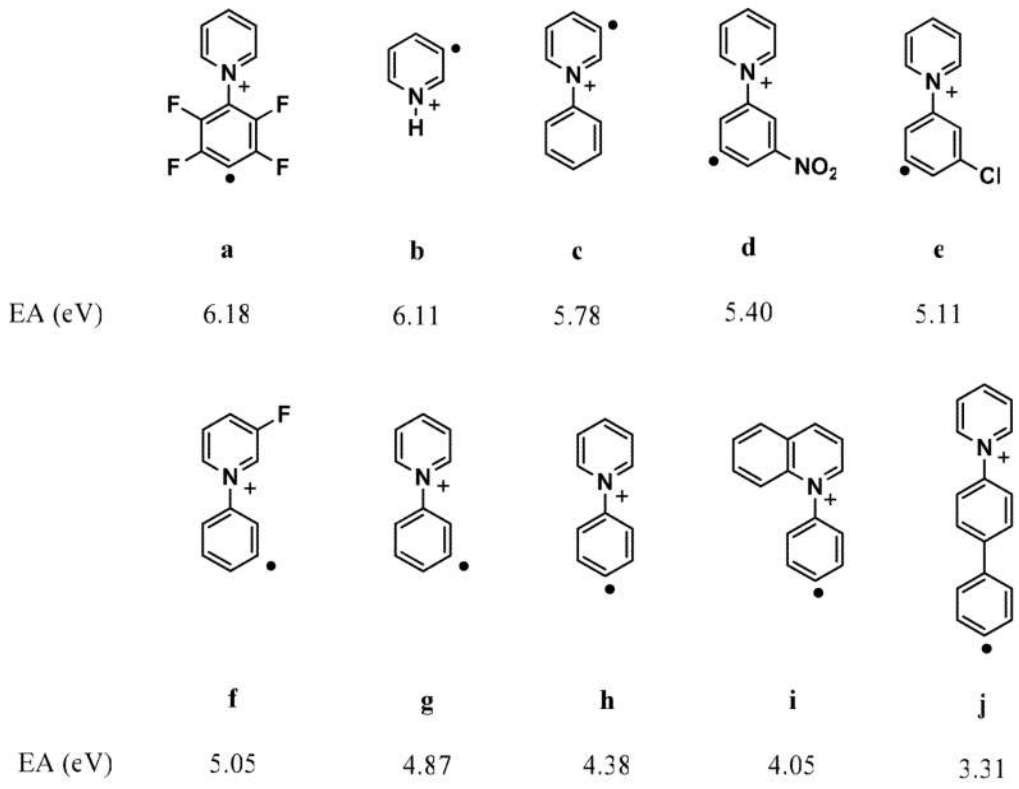
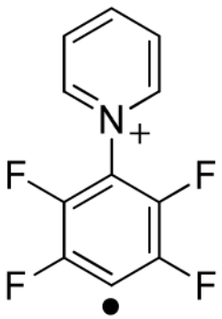
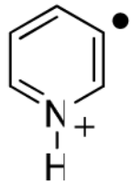
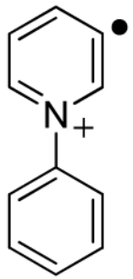
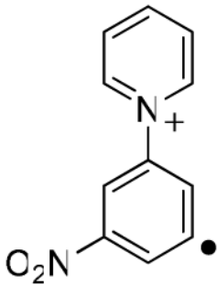
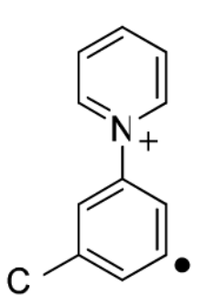
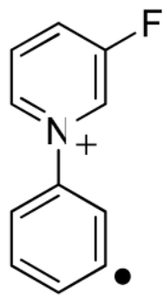
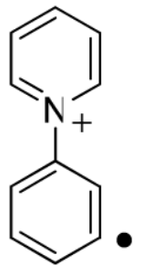
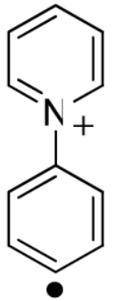
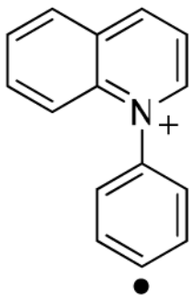
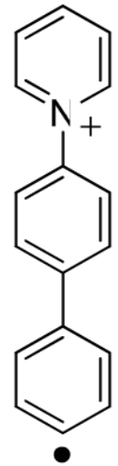


Chart 2.
Structures and calculated vertical electron affinities[20] (EA) of the positively charged phenyl radicals studied.

Table 1

Reaction efficiencies (Eff.) and product branching ratios for reactions of monoradicals **a – j** with glycine and glycine-(2,2-d_2)

		 **a**	 **b**	 **c**	 **d**	 **e**	 **f**	 **g**	 **h**	 **i**	 **j**
	EA[a] (eV)	**6.18**	**6.11**	**5.78**	**5.40**	**5.11**	**5.05**	**4.87**	**4.50**	**4.05**	**3.31**
L-glycine MW = 75	NH_2 abs.[b] (%)	96[c]	79[c]	57[c]	0	0	0	0	0	0	0
	H abs. (%)	4[c]	21[c]	43[c]	100	100	100	100	100	100	0
	Eff. (%)	50[c]	22[c]	8.5[c]	0.9	0.5	0.2	0.1	0.1	0	0
DL-glycine-(2,2-d_2) MW = 77	NH_2 abs. (%)	98[c]	87[c]	63.5[c]	0	0	0	0	0	0	0
	H abs. (%)	2[c]	6[c]	18.5[c]	72	83	54	71	59	100	0
	D abs. (%)	0.5[c]	7[c]	17.5[c]	28	17	46	29	41	0	0
	Eff. (%)	53[c]	25[c]	10[c]	0.9	0.4	0.2	0.1	0.1	0[d]	0

[a] Calculated vertical electron affinities (EA) from Reference 12c;

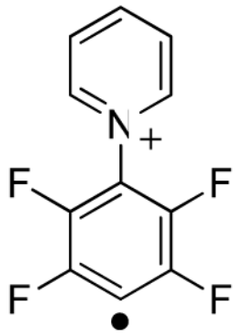
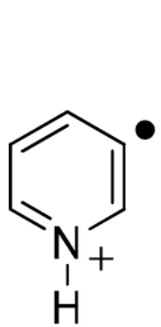
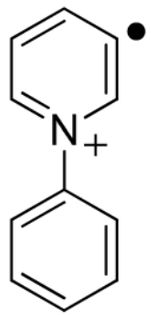
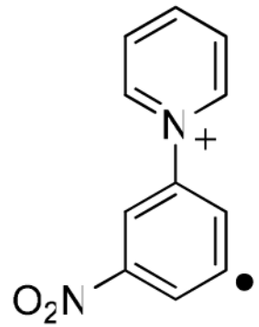
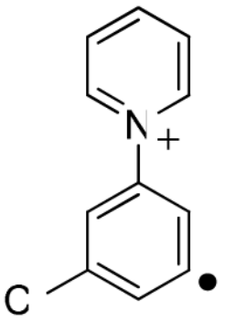
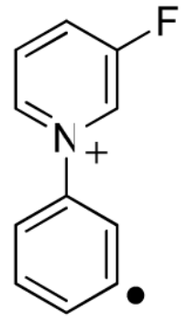
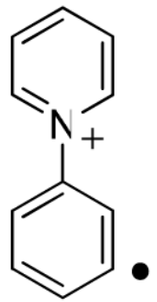
[b] abs. = abstraction;

[c] Data taken from References 12b and 12c;

[d] Efficiency (Eff. (%)) is actually 0.00003.

Table 2

Reaction efficiencies (Eff.) and product branching ratios for reactions of monoradicals **a** – **g** with L-leucine, DL-leucine-(1- d_1) and L-isoleucine

	EA[a] (eV)	 **a** **6.18**	 **b** **6.11**	 **c** **5.87**	 **d** **5.40**	 **e** **5.11**	 **f** **5.05**	 **g** **4.87**
L-leucine MW = 131	NH_2abs.[b] (%)	73	proton transfer only	28	0	0	0	0
	H abs. (%)	27		72	100	100	100	100
	Eff. (%)	55		45	33	16	16	15
DL-leucine-(1-d_1) MW = 132	NH_2abs. (%)	60	proton transfer only	27	0	0	0	0
	H abs. (%)	31		70	100	100	100	100
	D abs. (%)	0		3	0	0	0	0
	unknown (%)(abs. 17 Da)	9		0	0	0	0	0
	Eff. (%)	55		48	36	17	15	10
L-isoleucine MW = 131	NH_2abs. (%)	64	proton transfer only	25	0	0	0	0
	H abs. (%)	36		75	100	100	100	100
	Eff. (%)	57		43	36	16	13	19

[a] Calculated vertical electron affinities (EA) from Reference 12c.

[b] abs. = abstraction

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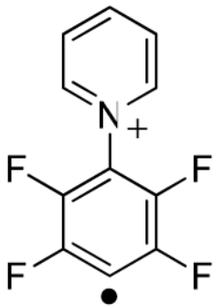
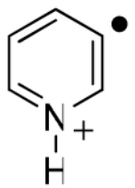
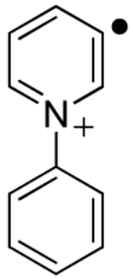
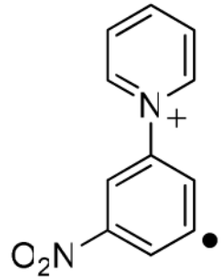
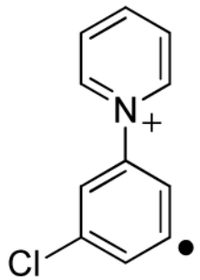
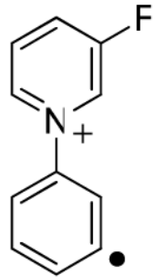
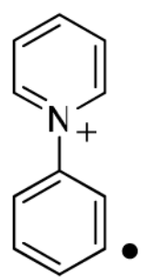
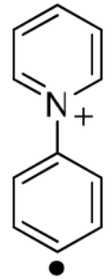
Table 3

Reaction efficiencies (Eff.) and product branching ratios for reactions of monoradicals **a** – **h** with L-lysine and DL-lysine-ε-^{15}N

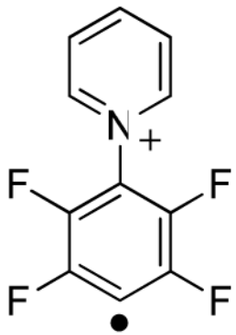
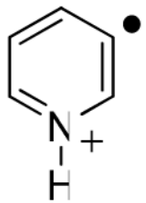
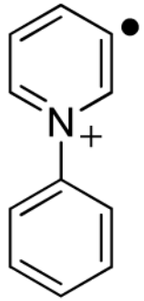
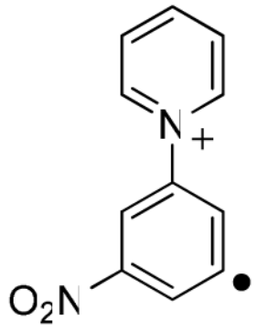
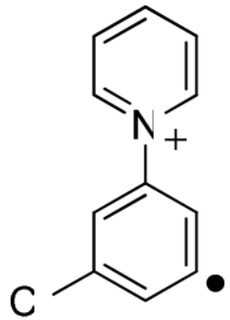
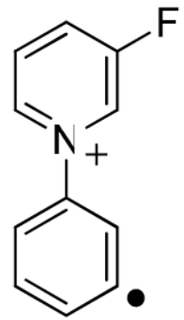
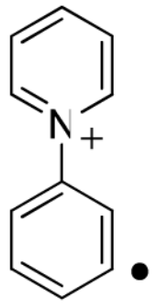
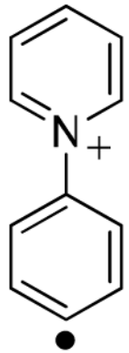
	EA[a] (eV)	 **a** **6.18**	 **b** **6.11**	 **c** **5.87**	 **d** **5.4**	 **e** **5.11**	 **f** **5.05**	 **g** **4.87**	 **h** **4.50**
L-lysine MW = 146	NH_2 abs.[b] (%)	62	proton transfer only	24	0	0	0	0	0
	H abs. (%)	38		44	45	100	100	100	100
	2 H abs. (%)	0		0	55	0	0	0	0
	addition (%)	0		32	0	0	0	0	0
	Eff. (%)	46		49	40	21	25	22	8
DL-lysine-e-^{15}N MW = 147	NH_2 abs. (%)	53	proton transfer only	21	0	0	0	0	0
	$^{15}NH_2$ abs.(%)	18		9	0	0	0	0	0
	H abs. (%)	29		47	66	100	100	100	100
	2 H abs. (%)	0		0	34	0	0	0	0
	addition (%)	0		23	0	0	0	0	0
	Eff. (%)	42		39	38	19	24	24	10

[a] Calculated vertical electron affinities (EA) from Reference 12c.

[b] abs. = abstraction

Table 4

Reaction efficiencies (Eff.) and product branching ratios for reactions of monoradicals **a – h** with L-proline and DL-proline- (1-d_1)

	EA[a] (eV)	 **a** **6.18**	 **b** **6.11**	 **c** **5.87**	 **d** **5.4**	 **e** **5.11**	 **f** **5.05**	 **g** **4.87**	 **h** **4.50**
L-proline MW = 115	NH_2 abs.[b] (%)	0	proton transfer only	see table 5	0	0	0	0	0
	H abs. (%)	100			64	100	100	100	100
	2 H abs. (%)	0			36	0	0	0	0
	Eff. (%)	92			44	22	21	19	1.3
DL-proline-(1-d_1) MW = 116	NH_2 abs. (%)	0	proton transfer only	see table 5	0	0	0	0	0
	H abs. (%)	100			58	100	100	98	93
	2 H abs. (%)	0			42	0	0	0	0
	D abs. (%)	0			0[c]	0	0	2	8
	Eff. (%)	92			46	24	22	18	2.1

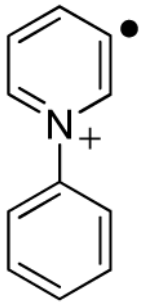
[a] Calculated vertical electron affinities (EA) from Reference 12c.

[b] abs. = abstraction

[c] Distinguishing between D atom abstraction (2.014102 amu) and 2 H atom abstraction (2.01588 amu) is difficult due to the closeness of their masses and the detection limits of the mass spectrometer.

Table 5

Reaction efficiencies (Eff.) and product branching ratios for reactions of monoradical c with L-proline and DL-proline-(1-d_1)

		
	EA[a] (eV)	**c** **5.87**
L-proline MW = 115	NH_2 abs.[b] (%)	0
	H abs. (%)	73
	HO abs. (%)	4
	C_2H_4N abs. (%)	12
	addition-OH (C_5H_7NO abs.) (%)	6
	addition (%)	5
	Eff. (%)	42
DL-proline-(1-d1) MW = 116	NH_2 abs.[b] (%)	0
	H abs. (%)	56
	D abs. (%)	6
	HO abs. (%)	12
	C_2H_4N abs. (%)	12
	addition-OH (C_5H_7NO abs.) (%)	7
	addition (%)	7
	Eff. (%)	46

[a] Calculated vertical electron affinity from Reference 12c.

[b] abs. = abstraction