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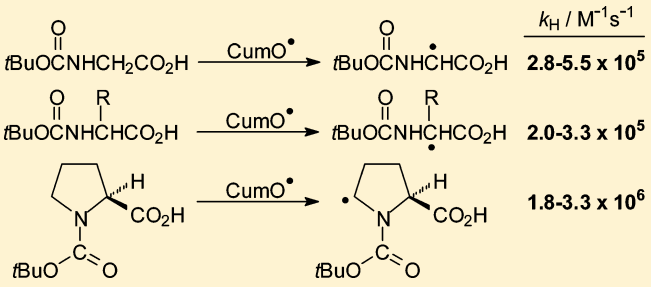
Reactivity and Selectivity Patterns in Hydrogen Atom Transfer from Amino Acid C−H Bonds to the Cumyloxyl Radical: Polar Effects as a Rationale for the Preferential Reaction at Proline Residues

Michela Salamone,* Federica Basili, and Massimo Bietti*

Dipartimento di Scienze e Tecnologie Chimiche, Università "Tor Vergata", Via della Ricerca Scientifica, 1, I-00133 Rome, Italy

Supporting Information

ABSTRACT: Absolute rate constants for hydrogen atom transfer (HAT) from the C−H bonds of *N*-Boc-protected amino acids to the cumyloxyl radical (CumO•) were measured by laser flash photolysis. With glycine, alanine, valine, norvaline, and *tert*-leucine, HAT occurs from the α-C−H bonds, and the stability of the α-carbon radical product plays a negligible role. With leucine, HAT from the α- and γ-C−H bonds was observed. The higher k_H value measured for proline was explained in terms of polar effects, with HAT that predominantly occurs from the δ-C−H bonds, providing a rationale for the previous observation that proline residues represent favored HAT sites in the reactions of peptides and proteins with •OH. Preferential HAT from proline was also observed in the reactions of CumO• with the dipeptides *N*-BocProGlyOH and *N*-BocGlyGlyOH. The rate constants measured for CumO• were compared with the relative rates obtained previously for the corresponding reactions of different hydrogen-abstracting species. The behavior of CumO• falls between those observed for the highly reactive radicals Cl• and •OH and the significantly more stable Br•. Taken together, these results provide a general framework for the description of the factors that govern reactivity and selectivity patterns in HAT reactions from amino acid C−H bonds.



INTRODUCTION

Free radical reactions of peptides and proteins are of fundamental importance and play an essential role in a variety of biochemical processes and physiological disorders.[1−3] Among these reactions, great attention has been devoted to the study of hydrogen atom transfer (HAT) reactions.[3c,4,5] HAT from peptides and proteins to free radicals can take place from the backbone and/or the side chains of amino acid residues, and depending on the abstraction site and on the reactions that follow the initial HAT step, a variety of structural and functional alterations can occur. These include fragmentations, side chain functionalization, cross-linking, unfolding, and loss of enzymatic activity. HAT reactions from proteins also have important practical applications, for example, in protein footprinting strategies, where HAT to the hydroxyl radical (•OH) is used to provide information on protein structure, folding events, and interactions that are essential to our understanding of the biological function of these biomolecules.[6]

Along these lines, a detailed understanding of the factors that govern the selectivity of HAT reactions from peptides and proteins appears to be of great importance, and accordingly, considerable efforts have been devoted to the study of this aspect. However, due to the complexity of protein substrates and to the multitude of reactive sites, simpler model substrates such as oligopeptides and amino acids have often been employed for this purpose. In this framework, it is also worth mentioning that the reactions of these model substrates with free radicals may provide access to side-chain-modified amino acids[4] that can be employed for a variety of purposes, such as in protein engineering through selective incorporation into peptide structures[7] or in alternative to proteinogenic amino acids as chiral auxiliaries in asymmetric reactions.[8]

The available results have been mostly obtained through product studies[9−13] and, more recently, computational studies.[14−19] On the other hand, limited direct kinetic information on these reactions is presently available.[20−22]

A number of experimental studies have clearly shown that in the reactions of amino acids and oligopeptides bearing aliphatic side chains with highly reactive radicals such as Cl• and •OH, HAT preferentially occurs from the C−H bonds of remote positions rather than from the α- and β-C−H bonds,[10a,12,13] despite the significantly lower bond dissociation enthalpy (BDE) of the α-C−H compared to the β and more remote C−H bonds.[14,18] For example, the following relative reactivities were obtained for HAT from amino acid α-, β-, and γ-tertiary C−H groups to Cl• (1.0, 8.4, and 93), and relative reaction rates showed that in HAT from N-acylated amino acids, a 48- and 9-fold increase in reactivity was observed going from glycine to leucine in their reactions with Cl• and •OH, respectively.[10a] This remarkable regioselectivity was rationalized by Easton and Radom on the basis of an early transition

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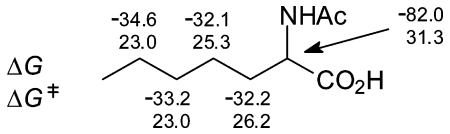
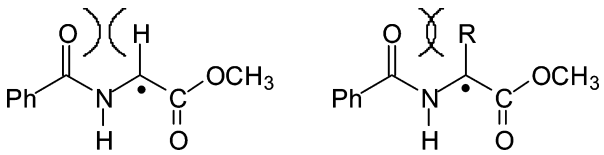
structure where polar effects[23] resulting from the electron-withdrawing character of the α-substituents deactivate the C−H bonds that are closer to the amino acid backbone toward HAT to electrophilic radicals such as Cl• and •OH.[10a] It was also pointed out that the extent of these effects is sufficient to override the thermodynamic preference for formation of the more stable α-carbon-centered radical. Strong support for this picture was provided by computational studies showing that while HAT from the α-C−H of *N*-acetyl amino acids to Cl• is associated with the most negative reaction free energy, abstraction from this position is characterized by the largest free energy barrier for any position along the chain (Scheme 1 displays the calculated reaction free energies (ΔG) and free energy barriers ($\Delta G^{\ddagger}$) in kJ mol^{-1} for HAT from the C−H bonds of 2-acetylaminoheptanoic acid to Cl• in acetic acid at T = 25 °C).[14c]

Scheme 1



With the significantly less reactive bromine radical (Br•), it was observed that HAT from amino acids occurs exclusively from the α-C−H bond, in line with a relatively late transition structure where C−H bond cleavage has progressed substantially and the stability of the α-carbon-centered radical formed following HAT plays a major role.[10b,c] In these reactions, from large to very large decreases in reactivity were observed going from glycine to valine (k_H(Gly)/k_H(Val) = 25) and *tert*-leucine (k_H(Gly)/k_H(Tle) > 2500), that is, with increasing side chain bulkiness. This behavior was explained on the basis of steric interactions that, compared to glycine, prevent planarization of the α-carbon-centered radical and thus captodative stabilization exerted by the adjacent amido and carboxy groups (Scheme 2; R = CH(CH$_3$)$_2$ (Val), R = C(CH$_3$)$_3$ (Tle)).

Scheme 2



The dependence of the abstracting radical on the HAT reactivity and selectivity was also examined in detail by means of computational studies, comparing reaction free energies (ΔG) and free energy barriers ($\Delta G^{\ddagger}$) for HAT from the α-, β-, γ-, δ-, and ε-C−H bonds of a model substrate such as 2-acetylaminoheptanoic acid to •OH, Cl•, •OOH, and Br• in acetic acid.[14a] Full support for the mechanistic picture discussed above was obtained, showing that the influence of favorable thermodynamic effects on the barrier for HAT from the α-position is greatest for Br• with its later transition structure and smallest for Cl• and •OH with their earlier transition structures. On the other hand, deactivating polar effects are fully operative in the reactions involving Cl• and •OH where the barriers decrease with increasing distance between the site of reaction and the α-substituents, whereas with Br•, these effects are overshadowed by the favorable thermodynamic effect of the captodatively stabilized α-carbon radical.

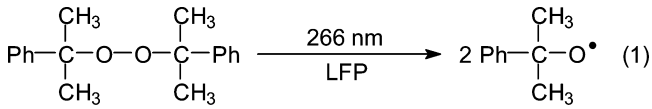
As pointed out in previous studies, HAT reactions to alkoxyl radicals are characterized by transition stuctures that are intermediate between those described for HAT to Cl• and Br•,[10c,14a,26,28] and accordingly, compared to these radicals (and to •OH and •OOH), different reactivity and selectivity patterns can be reasonably expected in the reactions of alkoxyl radicals with amino acids. Among these radicals, cumyloxyl (PhC(CH$_3$)$_2$O•, CumO•) has attracted considerable interest. CumO• can be conveniently generated from commercially available dicumyl peroxide by UV photolysis and, most importantly, displays an absorption band in the visible region of the spectrum[29,30] that, differently from •OH, Cl•, •OOH, and Br•, allows the direct measurement of HAT rate constants by means of time-resolved techniques such as laser flash photolysis (LFP).[31]

Along these lines, due to the great importance of HAT reactions from peptides and proteins to free radicals and in order to obtain direct kinetic information on the reactivity and selectivity patterns observed in these reactions, we have carried out a detailed time-resolved kinetic study on the reactions of CumO• with a series of *N*-*tert*-butoxycarbonyl (*N*-Boc)-protected proteinogenic (glycine (*N*-BocGlyOH), alanine (*N*-BocAlaOH), valine (*N*-BocValOH), leucine (*N*-BocLeuOH), proline (*N*-BocProOH)) and nonproteinogenic (norvaline (*N*-BocNvaOH), *tert*-leucine (*N*-BocTleOH), α-aminoisobutyric acid (*N*-BocAibOH)) amino acids bearing aliphatic side chains, whose structures are displayed in Chart 1.

In addition, information on the reactivity and selectivity observed in the reaction of proline with CumO• appears to be of great interest because this amino acid has been suggested to provide a favored site for HAT in the reactions of peptides and proteins with •OH,[13,32] where selective fragmentation of the protein structure at proline residues was observed. Other studies have also suggested that proline is involved in the antioxidant defense of plants where, by acting as a •OH scavenger, it contributes in preventing enzyme inactivation.[15] Accordingly, in order to expand the information obtained from *N*-BocProOH, the reaction of CumO• with the *N*-Boc-protected dipeptide *N*-BocProGlyOH has also been studied with a comparison to the *N*-BocGlyGlyOH dipeptide, the structures for which are also included in Chart 1.

RESULTS

CumO• was generated by 266 nm LFP of argon-saturated solutions (T = 25 °C) containing dicumyl peroxide as described in eq 1. As mentioned previously, in aprotic solvents, CumO• is characterized by a visible absorption band centered at 485 nm.[29,30] Under these conditions, the most important decay pathway of CumO• is represented by C−CH$_3$ β-scission.[31]



(1)

The time-resolved kinetic study on the reactions of CumO• with the amino acids displayed in Chart 1 was carried out by LFP in acetonitrile solution following the decay of the CumO• visible absorption band as a function of the concentration of added substrate. The relatively low solubility of *N*-BocTleOH and *N*-BocAibOH in MeCN did not allow the kinetic study of their reactions with CumO•, which, accordingly, was carried out

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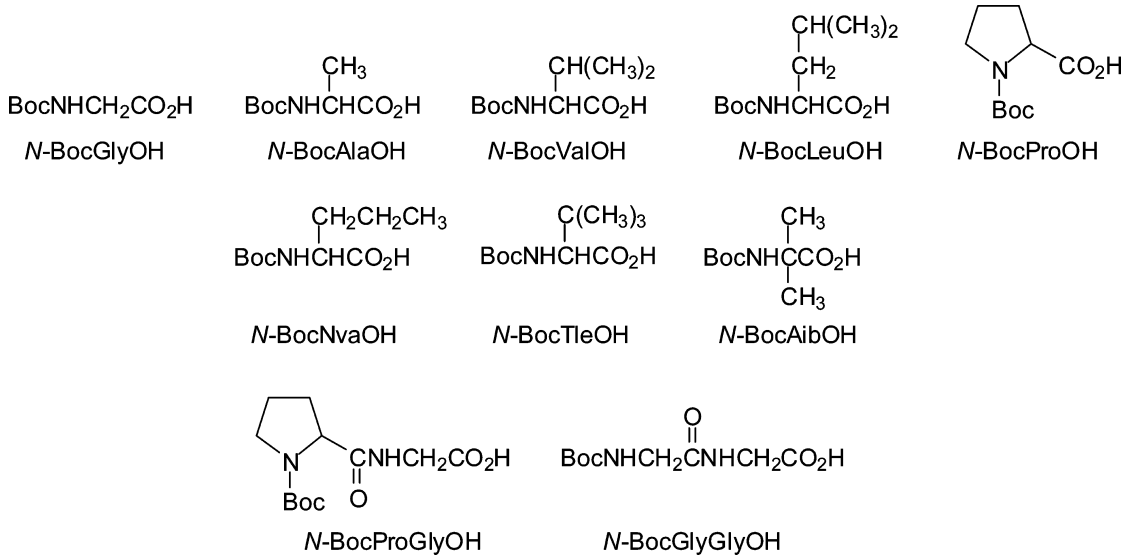
Chart 1



in dichloromethane. As a matter of comparison, the reactions of CumO• with *N*-BocGlyOH, *N*-BocAlaOH, *N*-BocValOH, and *N*-BocProOH were also studied in this solvent. When the observed rate constants (k_{obs}) were plotted against substrate concentration, excellent linear relationships were observed and the second-order rate constants for HAT to CumO• (k_H) were obtained from the slopes of these plots. The plots for HAT from the amino acids to CumO• are displayed in the Supporting Information (SI, Figures S1−S7). The k_H values obtained from these plots are collected in Table 1. Also included in this table is the k_H value measured for the reaction of CumO• with *N*-Boc-pyrrolidine, the plot for which is displayed in the SI (Figure S8).

Table 1. Second-Order Rate Constants (k_H) for Reaction of the Cumyloxyl Radical (CumO•) with *N*-Boc-Protected Amino Acids, Measured in Different Solvents at T = 25 °C[a]

substrate	solvent	k_H (M^{-1} s^{-1})
N-BocGlyOH	MeCN	$(3.96 \pm 0.05) \times 10^5$
	CH_2Cl_2	$(5.5 \pm 0.2) \times 10^5$
N-BocAlaOH	MeCN	$(2.76 \pm 0.02) \times 10^5$
	CH_2Cl_2	$(2.68 \pm 0.09) \times 10^5$
N-BocAibOH[b]	CH_2Cl_2	$\leq 6 \times 10^4$
N-BocValOH	MeCN	$(1.99 \pm 0.02) \times 10^5$
	CH_2Cl_2	$(2.26 \pm 0.03) \times 10^5$
N-BocNvaOH	MeCN	$(3.3 \pm 0.2) \times 10^5$
N-BocLeuOH	MeCN	$(5.9 \pm 0.2) \times 10^5$
N-BocTleOH	CH_2Cl_2	$(3.1 \pm 0.3) \times 10^5$
N-BocProOH	MeCN	$(2.51 \pm 0.08) \times 10^6$
	CH_2Cl_2	$(1.81 \pm 0.05) \times 10^6$
N-Boc-pyrrolidine	MeCN	$(1.4 \pm 0.2) \times 10^7$

[a]Argon-saturated solution, [dicumyl peroxide] = 0.010 M. The k_H values have been determined from the slope of the k_{obs} vs [substrate] plots, where the k_{obs} values have been measured following the decay of the CumO• visible absorption band at 490 nm. Average of at least two determinations. [b]Because of the low reactivity and limited solubility (≤1.2 M) displayed by this substrate under the experimental conditions employed, the measured k_H value should be taken as an upper limit.

The dipeptides *N*-BocGlyGlyOH and *N*-BocProGlyOH were found to be relatively insoluble in MeCN and CH_2Cl_2 but sufficiently soluble in dimethyl sulfoxide (DMSO). Accordingly, the kinetic study of their reactions with CumO• was carried out in this solvent. As a matter of comparison, the corresponding reactions of *N*-BocGlyOH and *N*-BocProOH with CumO• were also studied in DMSO, the plots for which are displayed in Figure 1.

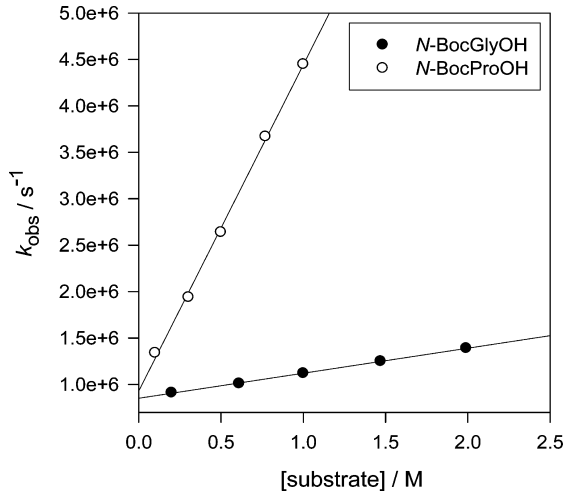


Figure 1. Plots of the observed rate constant (k_{obs}) against substrate concentration for the reactions of the cumyloxyl radical (CumO•) with *N*-BocGlyOH (filled circles) and *N*-BocProOH (empty circles), measured in Ar-saturated DMSO solution at T = 25 °C following the decay of CumO• at 490 nm. From the linear regression analysis: CumO• + *N*-BocGlyOH, intercept = 8.52×10^5 s^{-1}, $k_H = 2.70 \times 10^5$ M^{-1} s^{-1}, r^2 = 0.9994; CumO• + *N*-BocProOH, intercept = 9.29×10^5 s^{-1}, $k_H = 3.51 \times 10^6$ M^{-1} s^{-1}, r^2 = 0.9985.

The plots for HAT from *N*-BocGlyGlyOH and *N*-BocProGlyOH to CumO• in DMSO solution are displayed in the SI (Figure S9). The k_H values obtained from these plots are collected in Table 2.

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Table 2. Second-Order Rate Constants (k_H) for Reaction of the Cumyloxyl Radical (CumO•) with *N*-Boc-Protected Amino Acids and Dipeptides, Measured in DMSO at T = 25 °C[a]

substrate	k_H (M^{-1} s^{-1})
N-BocGlyOH	$(2.8 \pm 0.1) \times 10^5$
N-BocProOH	$(3.3 \pm 0.2) \times 10^6$
N-BocGlyGlyOH	$(5.8 \pm 0.2) \times 10^5$
N-BocProGlyOH	$(3.36 \pm 0.02) \times 10^6$

[a]Argon-saturated solution, [dicumyl peroxide] = 0.010 M. The k_H values have been determined from the slope of the k_{obs} vs [substrate] plots, where the k_{obs} values have been measured following the decay of the CumO• visible absorption band at 490 nm. Average of at least two determinations.

DISCUSSION

The data displayed in Table 1 clearly show that among the amino acids studied the least reactive one is *N*-BocAibOH, for which only an upper limit to k_H could be determined ($k_H \leq 6 \times 10^4$ M^{-1} s^{-1}). This upper limit is in line with previous findings and confirms that nonactivated methyl groups display a very low reactivity toward alkoxyl radicals.[33,34] Compared to *N*-BocAibOH, which lacks hydrogen atoms bound to the α-carbon, the significantly higher k_H values measured for *N*-BocGlyOH and *N*-BocAlaOH are indicative of HAT from the α-C−H bonds to CumO• as the most important pathway for these two amino acids. The observation of a 1.4−2-fold increase in k_H going from *N*-BocAlaOH to *N*-BocGlyOH can be explained on the basis of the double number of α-C−H bonds displayed by *N*-BocGlyOH compared to *N*-BocAlaOH, suggesting that in the reactions of these substrates with CumO•, differences in the stability of the two α-carbon-centered radicals formed following HAT are relatively unimportant.[35] On the basis of these results, the very similar k_H values measured for *N*-BocAlaOH, *N*-BocValOH, *N*-BocNvaOH, and *N*-BocTleOH strongly support the hypothesis that, with these amino acids, HAT from the α-C−H bond represents the most important pathway, indicating at the same time that the side chain primary, secondary, and tertiary C−H bonds of these substrates display a very low reactivity toward CumO•.

Along these lines, the higher k_H value measured for *N*-BocLeuOH ($k_H = 5.9 \times 10^5$ M^{-1} s^{-1}) compared to the other acyclic amino acids (*N*-BocAlaOH, *N*-BocValOH, *N*-BocNvaOH, and *N*-BocTleOH, for which $k_H = 1.99-3.3 \times 10^5$ M^{-1} s^{-1}) clearly indicates that, in addition to the α-C−H bond, HAT from the side chain of *N*-BocLeuOH now plays an important role. Analysis of the side chain structures of these amino acids points toward the tertiary γ-C−H bond of *N*-BocLeuOH as the additional reactive site, suggesting that the corresponding tertiary β-C−H bond of *N*-BocValOH that is sterically more hindered and closer to the carboxylic group is deactivated toward HAT by a combination of steric and electronic effects.

Very interestingly, an analogous explanation was put forward to account for the side chain selectivities observed for leucine and valine in the reaction of *N*-Boc-protected amino acids with 3,3-dimethyldioxirane (DMDO).[36] Exclusive hydroxylation of the tertiary γ-C−H bond was observed with *N*-BocLeuOMe, while under identical experimental conditions, *N*-BocValOMe was found to be unreactive.[37] As C−H bond hydroxylation by DMDO has been recently rationalized on the basis of a mechanism that involves HAT from a substrate C−H bond to DMDO followed by in cage collapse of the first formed radical pair (Scheme 3),[40] this indication is in full agreement with the observation of analogous selectivities from remote tertiary C−H bonds in the reactions of CumO• and DMDO with leucine and valine derivatives.

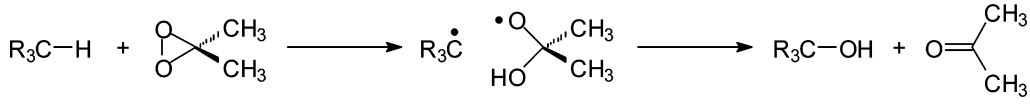
Among the amino acids displayed in Table 1, the highest k_H value was measured for *N*-BocProOH, $k_H = 2.51 \times 10^6$ M^{-1} s^{-1}, a value that is up to 12 times higher than the values measured for the reactions of the other amino acids bearing α-C−H bonds and >30 times higher than the upper limit to k_H determined for *N*-BocAibOH. A similar reactivity pattern was also observed in an indirect kinetic study on the reactions of the *tert*-butoxyl radical ($(CH_3)_3CO$•, *t*BuO•) with *N*-benzoyl-protected amino acid methyl esters, where a 10-fold increase in reactivity was observed going from valine to proline.[10c] By comparing the reactivity of *N*-benzoylproline methyl ester with those measured for the corresponding α- and δ-deuterated derivatives (Scheme 4, structures **A**, **B**, and **C**, respectively), it was concluded that the most important reaction pathway is represented by HAT from the substrate δ-C−H bonds to give the δ-carbon radical **D**.

The reaction selectivity was explained on the basis of severe nonbonding interactions in the α-carbon radical that prevent planarization and thus captodative stabilization by the adjacent amido and carboxy groups (Scheme 5, structure **E**),[35b] directing the reaction toward the formation of radical **D** following HAT from the δ-C−H bonds.[10c]

An analogous selectivity was also observed in the reactions of *N*-BocProOH, *N*-BocProOMe, and *N*-CbzProOEt with DMDO, where in all cases exclusive hydroxylation of the δ-C−H bond was observed,[36] and, most importantly, in the reaction of •OH with peptides where, among the proline residues, selective hydroxylation and carbonylation of the δ-C−H bonds were detected.[6a,41] In these studies, however, no clear explanation for the reaction selectivity was given.

Taken together, these results clearly indicate that the reaction of CumO• with *N*-BocProOH can be explained accordingly on the basis of preferential HAT from the δ-C−H bonds of this substrate. However, as mentioned above, the stability of the α-carbon radical formed following HAT plays a negligible role in the reactions of CumO• with acyclic amino acids, as clearly shown by the very similar k_H values measured for HAT from the α-C−H bonds of *N*-BocAlaOH, *N*-BocValOH, *N*-BocNvaOH, and *N*-BocTleOH. Because the CumO• and *t*BuO• display a very similar behavior in HAT reactions,[42] this observation rules out the explanation given above for the selectivity observed in the reaction of *t*BuO• with proline

Scheme 3



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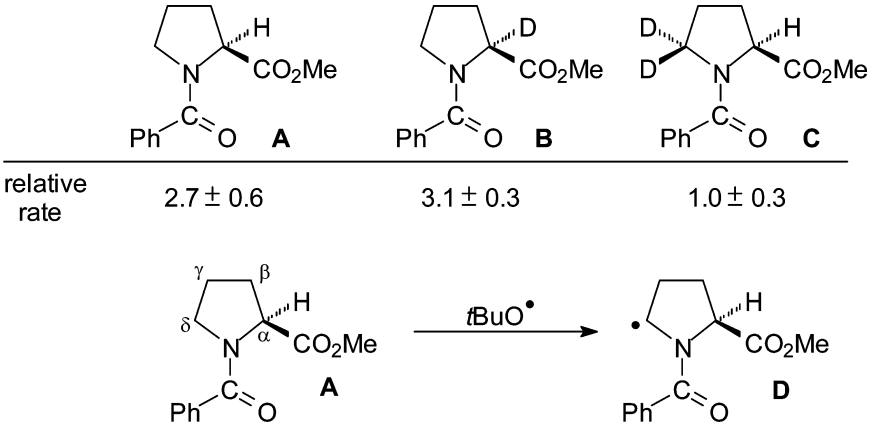
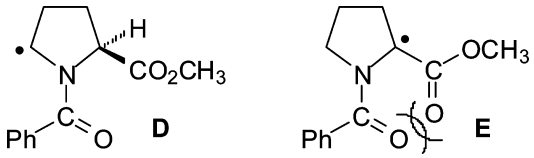
Scheme 4



Scheme 5



derivatives (Schemes 4 and 5), based on the relative stability of the product α- and δ-carbon radicals.[10c] The remarkable increase in reactivity observed for *N*-BocProOH compared to that of the other amino acids displayed in Chart 1 in its reaction with CumO$^{\bullet}$ can be explained on the basis of the influence of polar effects on the HAT selectivity. The electron-withdrawing character of the carboxylic group deactivates the α-C−H bond toward reaction with an electrophilic radical such as CumO$^{\bullet}$, directing HAT to the δ-C−H bonds, which benefit from a certain extent of activation provided by the adjacent carbamate nitrogen. A similar activation is clearly not possible for the acyclic amino acids discussed above, where, despite of the unfavorable polar effect determined by the carboxylic group, HAT preferentially occurs from the α-C−H bond.

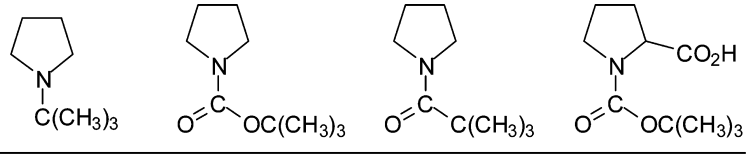
Additional support for this mechanistic picture is provided by the observation that removal of the carboxylic group (i.e., going from *N*-BocProOH to *N*-Boc-pyrrolidine, for which a value k_H = 1.4 × 10^7 M^{-1} s^{-1} was measured) leads to an almost 6-fold increase in k_H. This finding clearly indicates that, in addition to the α-C−H bond, the deactivation exerted by this group extends up to the δ-C−H bonds, in keeping with the results of computational studies on acyclic amino acids.[14] Within this framework, comparison between the k_H value measured for reaction of CumO$^{\bullet}$ with *N*-BocProOH and those measured for the corresponding reactions of other pyrrolidine derivatives (namely, *N*-*tert*-butylpyrrolidine,[43] *N*-Boc-pyrrolidine, and *N*-pivaloylpyrrolidine[44]) allows moreover a quantitative evaluation of the role of deactivating polar effects on HAT from the C−H bonds that are adjacent to the nitrogen atom in these substrates (Scheme 6).

The highest k_H value was measured for *N*-*tert*-butylpyrrolidine,[43] where the four α-C−H bonds fully benefit from the activation provided by the lone pair of electrons on the adjacent nitrogen atom. Replacement of *tert*-butyl with *tert*-butoxycarbonyl and pivaloyl groups reduces the electron density at nitrogen, leading to significant decreases in HAT reactivity. The 2.6-fold increase in k_H measured going from *N*-pivaloylpyrrolidine[44] to *N*-Boc-pyrrolidine is in line with the stronger electron-withdrawing character of an acetyl group compared to an alkoxycarbonyl group. The lowest k_H value measured for *N*-BocProOH is the result of the combined effect exerted by the *N*-Boc and α-carboxylic groups where, as mentioned above, HAT to CumO$^{\bullet}$ mostly involves the δ-C−H bonds.

Taken together, these results point toward the δ-C−H bonds as the preferential site for HAT from proline derivatives to electrophilic hydrogen-atom-abstracting species such as alkoxyl radicals, $^{\bullet}$OH and DMDO, with the observed selectivity that can be explained in all cases on the basis of deactivating polar effects. Most importantly, these results provide a possible explanation for the observations that proline residues represent favored HAT sites in the reactions of peptides and proteins with $^{\bullet}$OH[13,32] and of proline accumulation in plants exposed to oxidative stress.[15]

In order to expand these findings and to obtain information on the preferential reactivity of proline residues within peptide structures, we have studied the reactions of CumO$^{\bullet}$ with the two dipeptides, *N*-BocGlyGlyOH and *N*-BocProGlyOH, in DMSO solution, the rate constants for which are collected in Table 2. As a matter of comparison, the reactions of CumO$^{\bullet}$ with *N*-BocGlyOH and *N*-BocProOH have been studied under

Scheme 6



k_H(CumO$^{\bullet}$)/M^{-1}s^{-1}	3.0 x 10^8	1.4 x 10^7	5.2 x 10^6	2.5 x 10^6
k_{rel}	120	5.6	2.1	1.0

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the same conditions. An 11.8-fold increase in k_H was measured going from *N*-BocGlyOH to *N*-BocProOH. The values measured for the reactions of the two dipeptides show that k_H increases by a factor 6 going from *N*-BocGlyGlyOH to *N*-BocProGlyOH. This result is in agreement with the greater HAT reactivity displayed by proline residues toward CumO•, where the decrease in the rate constant ratio observed going from the amino acids to the dipeptides can be explained on the basis of the relatively greater contribution that an additional glycine residue has on the reactivity of *N*-BocGlyGlyOH compared to *N*-BocProGlyOH. This observation suggests, on the other hand, that HAT rate constants from peptides to alkoxyl radicals (and possibly other electrophilic radicals) should be significantly influenced by the number of proline residues.

Interestingly, the rate constant ratio measured in DMSO for the reactions of *N*-BocProOH and *N*-BocGlyOH (k_H(Pro)/k_H(Gly) = 11.8) is significantly larger than the corresponding values measured in MeCN and CH_2Cl_2: k_H(Pro)/k_H(Gly) = 6.3 and 3.3, respectively. These sizable kinetic solvent effects reflect opposite solvent-dependent reactivity trends for the two amino acids, with k_H values for the reaction of *N*-BocGlyOH that decrease and those for the reaction of *N*-BocProOH that increase going from CH_2Cl_2 to DMSO, that is, with increasing solvent polarity (see Tables 1 and 2). As mentioned above, *N*-BocGlyOH has been shown to undergo HAT from the α-C−H bonds, whereas *N*-BocProOH mostly undergoes HAT from the δ-C−H bonds, and accordingly, the observed behavior may be in line with these different selectivity patterns.[45]

The k_H values measured in the present study for the reactions of CumO• can be compared with those obtained previously in a number of different studies on HAT reactions from amino acid derivatives. This comparison appears to be of great importance because it can provide a general framework for the description of the role of structural effects and of the nature of the abstracting radical on the reactivity and selectivity patterns observed in HAT reactions from amino acid C−H bonds.[47] For this purpose, Table 3 compares the relative rates obtained in the reactions of CumO• with amino acids bearing aliphatic side chains with those obtained for the corresponding reactions involving a variety of hydrogen-atom-abstracting species, namely, Cl•,[10a] •OH,[10a] *t*BuO•,[10b] Br•,[10b,c] and an iron(IV)oxo complex ($[Fe^{IV}(O)(N4Py)]^{2+}$).[11a]

As mentioned previously, in the reactions with Cl• and •OH, HAT preferentially occurs from remote (γ-, δ-, and ε-) C−H bonds rather than from the α- and β-C−H bonds, with the relative rates that increase with increasing side chain length and number of remote C−H bonds.[10a] This behavior was explained on the basis of an early HAT transition state where deactivating polar effects are fully operative, with the stability of the carbon radical product playing a negligible role.[10a,14] The electron-withdrawing character of the α-substituents deactivates the C−H bonds that are closer to the amino acid backbone toward HAT to these highly reactive electrophilic radicals. The reactions of proline with Cl• and •OH were not studied under these experimental conditions. However, radical probe mass spectrometry experiments on the reactivity of peptide amino acid side chains toward •OH confirm the significantly higher reactivity of proline residues compared to other aliphatic amino acids.[6a,41] As mentioned previously, in this study, selective HAT from the δ-C−H bonds of proline residues was observed.

Table 3. Relative Rates for Reaction of Amino Acid Derivatives with Different Hydrogen-Atom-Abstracting Species[a]

amino acid	Cl•[a]	•OH[b]	CumO•[c]	*t*BuO•[d]	Br•[e]	$Fe^{IV}(O)$[f]
Nle	55	10.3				
Leu	47.5	9.1	1.5			0.13
Nva	27.5	9.7	0.8			
Ile	16.5	8.9				0.08
Tle	11.3	4.3	0.6[g]		<0.0004	
Val	8.8	3.7	0.5	0.19	0.04	0.22
Gly	1.0	1.0	1.0	1.0	1.0	1.0
Ala	0.3	1.1	0.7	0.24	0.33	0.42
Pro			6.3	1.9	1.4	0.08

[a]N-Acetylated amino acids. Steady-state photolysis at room temperature in chlorine-saturated trifluoroacetic acid (TFA).[10a] [b]N-Acetylated amino acids. Steady-state photolysis at room temperature in deuterium-labeled water acidified with TFA.[10a] [c]This work. *N*-Boc-protected amino acids; 266 nm LFP at T = 25 °C in MeCN containing 10 mM dicumyl peroxide (see Table 1). [d]N-Benzoylated amino acids. Steady-state photolysis at room temperature in 2-methyl-2-propanol containing di-*tert*-butyl peroxide.[10b] [e]N-Benzoylated amino acids. Steady-state photolysis at room temperature in CCl_4 containing *N*-bromosuccinimide.[10b,c] [f]N-Acetylated and *C*-*tert*-butylamidated amino acids. Kinetic study at T = 25 °C following the decomposition of the ferryl species $[Fe^{IV}(O)(N4Py)]^{2+}$ in 1:1 H_2O/MeCN.[11a] [g]In CH_2Cl_2. The relative rate has been obtained by comparison with the k_H value measured for reaction of CumO• with *N*-BocGly in this solvent (see Table 1).

With Br•, exclusive HAT from the α-C−H bond was observed, with relative rates that decrease with decreasing radical stability, in line with a relatively late transition state characterized by a pronounced radical character at the α-carbon.[10b,c] In these reactions, steric effects were observed to play a dramatic role as indicated by the greater than 3 orders of magnitude decrease in reactivity measured going from glycine to *tert*-leucine. The increased steric hindrance of the amino acid side chain prevents planarization of the α-carbon radical and thus captodative stabilization by the adjacent amido and carboxy groups (Scheme 2). With proline, preferential HAT from the δ-C−H bonds was observed, and this selectivity was explained accordingly on the basis of severe nonbonding interactions in the α-carbon radical that prevent planarization (Scheme 5).[10c]

With CumO• (and *t*BuO•), predominat HAT from the α-C−H bonds was observed for all the acyclic amino acids, with the exclusion of leucine where competition with HAT from the tertiary γ-C−H bond was observed. The reactivity of the α-position of these amino acids was shown to be essentially unaffected by side chain bulkiness, indicating that, in the reactions with alkoxyl radicals, the stability of the α-carbon radical is relatively unimportant. Also with this radical, HAT preferentially occurs from the δ-C−H bonds of proline, with the k_H value measured for this amino acid observed to be up to 12 times higher than the values measured for the other acyclic amino acids. The reaction selectivity was explained on the basis of deactivating polar effects exerted by the carboxylic group. The different explanations for the analogous selectivities observed in the reactions of proline derivatives with Br• and CumO• are in full agreement with the significantly earlier HAT transition structure described for the reactions of alkoxyl radicals compared to those involving Br•.[10c,26]

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With the iron(IV)oxo complex ($[Fe^{IV}(O)(N4Py)]^{2+}$), the relative rates displayed in Table 3 show, for glycine, alanine, and valine, a trend that is very similar to those observed for the corresponding reactions of CumO$^{\bullet}$ and *t*BuO$^{\bullet}$.[11a] In view of the biological relevance of these species that represent models of mononuclear non-heme iron enzymes,[48] which are involved in a variety of oxidative transformations, this observation appears to be of great interest because it suggests that iron(IV)oxo complexes and alkoxyl radicals display similar mechanistic features in HAT reactions. Significantly lower relative rates were instead measured for leucine and proline in their reactions with $[Fe^{IV}(O)(N4Py)]^{2+}$ compared to CumO$^{\bullet}$. This different behavior may be tentatively explained on the basis of the greater steric demand associated with the former hydrogen-abstracting species compared to the latter one, indicating that additional studies on the reactions of metal-oxo species with amino acids and peptides are needed in order to improve our understanding of the role of structural effects on these reactions.

The relative rates displayed in Table 3, together with the associated selectivities, clearly show that in these reactions CumO$^{\bullet}$ displays a behavior that falls between those observed for highly reactive radicals such as Cl$^{\bullet}$ and $^{\bullet}$OH and that observed with the significantly more stable radical Br$^{\bullet}$. These different behaviors are nicely exemplified by the results obtained with *tert*-leucine derivatives that accordingly show up as extremely sensitive mechanistic probes for the study of these reactions. The nine primary γ-C−H bonds account for the relatively high reactivity observed in the reactions of *tert*-leucine derivatives with Cl$^{\bullet}$ and $^{\bullet}$OH.[10a] The lower intrinsic reactivity of CumO$^{\bullet}$ prevents reaction at these positions, and HAT now exclusively occurs from the α-C−H bond, with a k_H value that is very similar to those measured for alanine, valine, and norvaline derivatives (k_H(Ala)/k_H(Tle) = 0.86, k_H(Val)/k_H(Tle) = 0.73, k_H(Nva)/k_H(Tle) = 1.06). With the significantly less reactive Br$^{\bullet}$, exclusive HAT from the α-C−H bond was observed. In the reaction with this radical, however, the steric bulk determined by the *tert*-butyl group leads to very significant decreases in reactivity compared to amino acid derivatives characterized by less sterically hindered side chains such as alanine and valine (k_H(Ala)/k_H(Tle) > 825, k_H(Val)/k_H(Tle) > 100).[10b,c]

EXPERIMENTAL SECTION

Materials. Spectroscopic grade dichloromethane, acetonitrile, and DMSO were used in the kinetic experiments. Dicumyl peroxide, *N*-BocGlyOH, *N*-BocAlaOH, *N*-BocAibOH, *N*-BocValOH, *N*-BocNvaOH, *N*-BocLeuOH, *N*-BocTleOH, *N*-BocProOH, *N*-Boc-pyrrolidine, *N*-BocGlyGlyOH, and *N*-BocProGlyOH were of the highest commercial quality available and were used as received.

Laser Flash Photolysis Studies. LFP experiments were carried out with a laser kinetic spectrometer using the fourth harmonic (266 nm) of a Q-switched Nd:YAG laser, delivering 8 ns pulses. The laser energy was adjusted to ≤10 mJ/pulse by the use of the appropriate filter. A 3.5 mL Suprasil quartz cell (10 mm × 10 mm) was used in all experiments. Argon-saturated dichloromethane, MeCN, or DMSO solutions of dicumyl peroxide (10 mM) were employed. All the experiments were carried out at T = 25 ± 0.5 °C under magnetic stirring. The observed rate constants (k_{obs}) were obtained following the decay of the cumyloxyl radical at 490 nm as a function of the concentration of added substrate. The k_{obs} values obtained from the decay traces are the average of 2−5 individual values and were reproducible to within 5%.

Second-order rate constants for the reactions of the cumyloxyl radical with the *N*-Boc-protected amino acids and peptides displayed in Chart 1 were obtained from the slopes of the k_{obs} versus [substrate] plots. Fresh solutions were used for every concentration. Correlation coefficients were in all cases >0.99. The rate constants displayed in Tables 1 and 2 are the average of at least two independent experiments, with typical errors being ≤10%.

ASSOCIATED CONTENT

Supporting Information

Plots of k_{obs} vs [substrate] for the reactions of CumO$^{\bullet}$. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: michela.salamone@uniroma2.it.
*E-mail: bietti@uniroma2.it.

Notes

The authors declare no competing financial interest.

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