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Oxidation of α-amino acids promoted by the phthalimide *N*-oxyl radical: A kinetic and product study

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ABSTRACT

A kinetic study of the hydrogen atom transfer (HAT) reaction from a series of *N*-Boc- or *N*-Acetyl-protected amino acids to the phthalimide *N*-oxyl radical (PINO) was carried out to obtain information about reactivity and selectivity patterns. With amino acids containing aliphatic side chains, the 2nd order rate constants are of the same order of magnitude, in agreement with a HAT process involving the Cα–H bond. Proline is the most reactive substrate suggesting that HAT process involves the C_δ–H bond instead of C_α–H bond. These results are confirmed by the product analysis of the aerobic oxidations of the corresponding *N*-Boc and *N*-Ac protected amino acids methyl esters promoted by *N*-hydroxyphthalimide. Comparison of our results with those reported for HAT reactions to other radical species indicates that PINO displays electrophilic characteristics that are intermediate between those observed for the more stable Br• radical and the more reactive cumyloxyl radical.

1. Introduction

Polypeptide chains represent one of the main targets for oxidative damage induced by free radicals and other reactive oxygen and nitrogen species (ROS and RNS), because of their abundance in cells and the relatively high reactivity of the amino acids components [1,2]. The oxidative damage of peptides and proteins determines significant structural changes of these biomolecules, with consequent loss of their functions through degradation and subsequent fragmentation of the proteins themselves [3]. The oxidative damage of proteins is of fundamental interest in biochemical and medicinal chemistry, being associated to a wide variety of pathological conditions such as aging, inflammatory processes, cancer, cardiovascular disorders and neurodegenerative diseases [4,5].

Hydrogen atom transfer (HAT) promoted by radical species represents one of the most important reactions occurring in the oxidative degradation of peptides and proteins [3]. This process may lead to the formation of a wide variety of radicals, due to the presence of multiple reaction sites on the backbone and amino acid side chains. Depending on the reactive site, the initial HAT may be followed by further processes such as side chain functionalization, fragmentations and cross-linking of the peptide. Previous studies, generally carried out using amino acids (either protected or not) as simple models of protein structures, pointed out how the type of radical formed after HAT depends on the nature of the abstracting radical: highly reactive electrophilic radicals such as Cl• and OH•, abstract a hydrogen atom preferentially from a C–H bonds of the side chains of amino acid residues [4,6], whereas more stable electrophilic radicals such as Br• [7,8], cumyloxyl radical (CumO•) [9] and active species of nonheme iron complexes [10,11], showed a marked selectivity for the Cα–H bonds abstraction. The growing interest in reactions promoted by free radicals and ROS toward peptides, coupled with the importance of the various diseases in which these reactions are involved, prompted us to analyze the reactivity and regioselectivity pattern of the HAT from a series of protected amino acids promoted by the phthalimide *N*-oxyl radical (PINO) (Scheme 1), a short-lived aminoxyl radical which has received a special attention in recent years for its involvement in aerobic oxidation of organic compounds catalyzed by *N*-hydroxyphthalimide (NHPI) [12,13].

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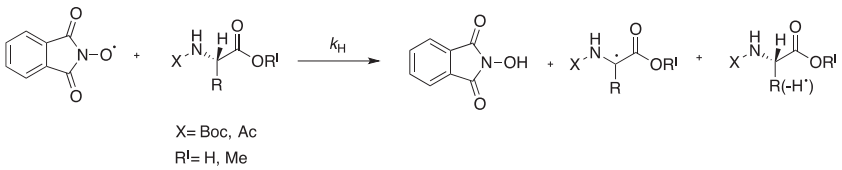


Scheme 1. Hydrogen atom transfer from protected amino acids to PINO.

The study of HAT process from amino acids to PINO is particularly relevant since recent studies have shown that PINO represents a good model of alkyl peroxyl radicals in view of the similar O—H bond dissociation energy (BDE) value in NHPI and alkylhydroperoxides (ca 87—88 kcal mol^{-1}) [14—17]. Therefore, the reaction of PINO with amino acids can be considered a useful model for the analysis of HAT processes from amino acids to peroxyl radicals at least considering the enthalpic effects based on the BDE difference between the C—H bonds in the substrates and the O—H bond in the NHPI/ROOH products [18].

A series of amino acids, proteinogenic and nonproteinogenic, bearing aliphatic or aromatic side chains, shown in Fig. 1, have been used as substrates. All the amino acids are N-protected with *N*-*tert*-butoxycarbonyl (*N*-Boc)- or *N*-Acetyl (*N*-Ac)- groups. Some of them are also C-protected as methyl esters.

In order to obtain information on the reactivity of amino acids with different structure and to investigate the regioselectivity of HAT process, we have carried out a detailed kinetic and product study on the reactions of PINO with the amino acids series reported in Fig. 1. Kinetic studies for the HAT promoted by PINO can be performed quite easily by following the decay of PINO spectrophotometrically in the UV—vis region.

Product analysis of the aerobic oxidations catalyzed by NHPI have been performed with the Ishii system, consisting in the use of NHPI in combination with a cobalt salt cocatalyst [12,15,16,19]. The substrates are all protected on the amino group. N-protected amino acids are required in order to avoid the degradation of the catalyst NHPI in the presence of the free amino group (Scheme 2) [20]. To evaluate the possible role played by the amino protecting group both *N*-Ac-a.a. and *N*-Boc-a.a. have been analyzed. In some case carboxyl groups are protected as methyl esters to facilitate the analysis of the oxidation products.

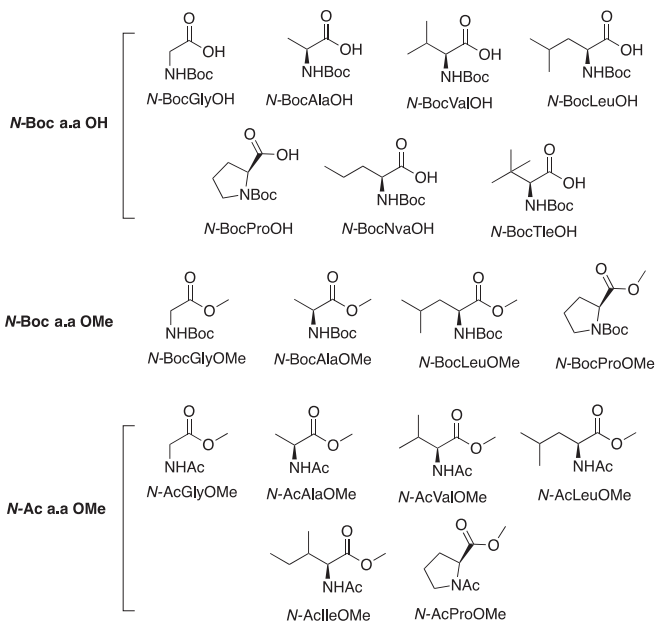


Fig. 1. Panel of substrates studied in this work.

2. Results

In kinetic studies PINO was generated by the oxidation of NHPI with the monoelectronic oxidizing agent cerium (IV) ammonium nitrate (CAN) at 25 °C in CH_3CN, as described in the literature [21]. Rate constants for the HAT reactions from a.a. to PINO (k_H) can be spectrophotometrically obtained by following the decay of PINO at its maximum absorption wavelength ($\lambda_{max} = 380$ nm, $\varepsilon = 1.46 \times 10^3$ L mol^{-1} cm^{-1}, in CH_3CN) [22]. In the absence of substrate, PINO is relatively stable and decays by a mixed first-second order process ($k_d = 0.4$ M^{-1} s^{-1} in CH_3CN at 25 °C) [23].

In the presence of an excess of a.a. substrates displayed in Fig. 1 (at least 10 fold with respect to PINO concentration), pseudo-first order decay of PINO is observed. By plotting the pseudo-first order rate constants (k_{obs}) as function of substrate concentration, excellent linear relationships were observed and the second-order rate constants (k_H) were obtained from the slopes of these plots (see Figs. S1—S17 in the SI).

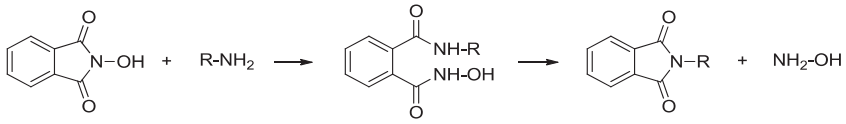
All the k_H values determined for the HAT from N-protected α-amino acids, and the corresponding methyl esters to PINO are reported in Table 1.

For product studies NHPI, $Co(OAc)_2$ and the substrate (10:1:200 M ratio) were mixed in CH_3CN and stirred at room temperature under oxygen atmosphere, for 24 h-48 h. At the end of the reaction, after workup, reaction products were identified by GC-MS and ^{1}H NMR analysis. Products and yields (referred to the initial amount of substrate) are reported in Table 2. In all cases the mass balance was satisfactory.

Product analysis carried out using the substrate *N*-BocValOMe indicated that no products were formed and *N*-BocValOMe was recovered almost quantitatively. The oxidation of *N*-BocGlyOMe afforded *tert*-butyl *N*-formyl carbamate as the exclusive reaction product (30%). Oxidation of *N*-AcLeuOMe furnished *N*-isovalerylacetamide as major product (15%) accompanied by methyl 4-methyl-2-oxopentanoate (3%). *N*-BocProOMe was the most reactive substrate with formation of *N*-Boc-δ-hydroxyproline methyl esters (mixture of diastereomers) (31%) and *N*-Boc-δ-oxoproline methyl ester (24%) as oxidation products.

3. Discussion

We start the analysis of the kinetic data reported in Table 1 with the simplest amino acid *N*-BocGlyOH where HAT necessarily involves the Cα-H bonds in view of the absence of a side-chain. The k_H value measured for *N*-BocGlyOH (0.1 M^{-1}s^{-1}) is significantly higher than those determined for the HAT from methylenic C—H bonds in aliphatic hydrocarbons to PINO. As an example, the k_H measured in the same conditions for cyclohexane is 3.9×10^{-3} M^{-1}s^{-1} (ca 150 time less reactive than *N*-BocGlyOH considering the reactivity per H atom) [16,24]. The higher reactivity of *N*-BocGlyOH is not surprising and can be rationalized on the basis of polar effects *i.e.* the



Scheme 2. Reaction of a primary amine with *N*-hydroxyphthalimide.

Table 1
Second-Order Rate Constants k_H ($M^{-1}s^{-1}$) for HAT from amino acids to PINO measured at T = 25 °C in CH_3CN.

Substrate	k_H ($M^{-1}s^{-1}$)[a]	
	R = H	R = CH_3
N-BocGly-OR	0.10(1)	0.15(1)
N-AcGlyOMe	n.d.	0.20(1)
N-BocAla-OR	0.075(4)	0.044(2)
N-AcAla-OMe	n.d	0.032(1)
N-BocVal-OR	0.019(1)	n.d.
N-AcValOMe	n.d.	0.011(1)
N-BocNva-OR	0.060(7)	n.d.
N-BocTle-OR	0.013(1)	n.d.
N-BocLeu-OR	0.079(5)	0.077(4)
N-AcLeuOMe	n.d.	0.050(4)
N-AcIleOMe	n.d.	0.016(1)
N-BocPro-OR	0.56(1)	0.50(5)
N-AcPro-OMe	n.d	0.72(4)

[a] The error in the last significant digit is given in parentheses.

stabilization of the partial positive charge which develops in the substrate in the HAT transition state (Fig. 2) [20,25].

With *N*-Boc amino acids, Cα–H bonds benefit from the activation provided by the lone pair of the adjacent carbamate nitrogen of the Boc protecting group which stabilize the partial positive charge which develops in the HAT TS. Clearly, the higher k_H value measured for *N*-BocGlyOH, with respect to those measured for aliphatic hydrocarbons, suggests that this effect overrides the deactivating effect of the electron-withdrawing carboxylic group.

It is interesting to note that the esterification of the carboxylic function does not significantly alter the reactivity of *N*-BocGlyOH

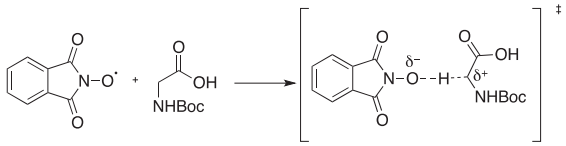


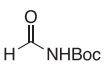
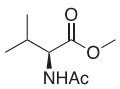
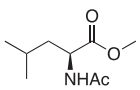
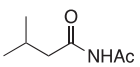
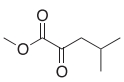
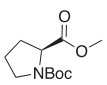
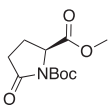
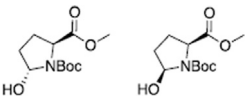
Fig. 2. Transition state for the hydrogen atom transfer from N-BocGlyOH to PINO.

and, as also observed with other amino acids, similar k_H values were measured for amino acids and the corresponding methyl esters. In a similar way, kinetic studies carried out using *N*-Ac protected α-amino acids showed that the substitution of the carbamate group with the amide group does not significantly alter the reactivity of these substrates.

The k_H value measured for HAT from *N*-BocAlaOH, ($7.5 \times 10^{-2}\,M^{-1}\,s^{-1}$) is comparable with that of *N*-BocGlyOH. In consideration of the relatively high BDE of the C_β-H bond in *N*-BocAlaOH it derives that, also with this substrate, HAT reaction takes place from the Cα–H bond rather than from the methyl group [9]. Taking into account the statistical factor (two equivalent Cα–H bonds are present in *N*-BocGlyOH) we observe a slight increase of HAT reactivity of *N*-BocAlaOH with respect to *N*-BocGlyOH. This is not surprising in consideration of the greater stability of the tertiary radical formed in HAT process from *N*-BocAlaOH (Fig. 3a).

Moreover, k_H value determined for *N*-BocAlaOH is very similar to those measured for the other amino acids containing aliphatic side chains: *N*-BocNvaOH and *N*-BocLeuOH, ($k_H = 6.0 \times 10^{-2}\,M^{-1}\,s^{-1}$ and $7.9 \times 10^{-2}\,M^{-1}\,s^{-1}$, respectively). These results clearly suggest that even with these amino acids HAT reaction involves the Cα-H bonds instead of the C–H bonds in β, γ

Table 2
Products and yields in the aerobic oxidation of N and C-protected amino acids under Ishii conditions.[a]

entry	substrate	Products	
1	 *N*-BocGlyOMe	 30	
2	 *N*-AcValOMe		n.r.
3	 *N*-AcLeuOMe	 15	 3
4	 *N*-BocProOMe	 24	 31

[a] Yield (%), determined by GC and ^1H NMR analysis, are referred to the initial amount of substrate. Reaction conditions: 0.5 mol % $Co(OAc)_2$, 5 mol % NHPI CH_3CN at 25 °C, reaction time 24–48 h. The reported results are the average of at least two runs (error ± 5%).

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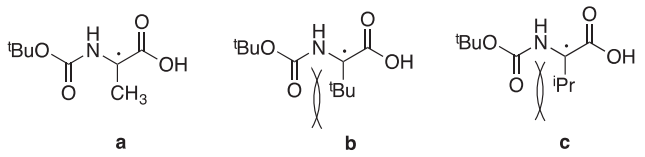


Fig. 3. Steric hindrance in the Cα radicals of *N*-BocAlaOH (a), *N*-BocTleOH (b) and *N*-BocValOH (c).

and δ positions of the side chain. This marked regioselectivity can be explained on the basis of polar effects, since both NHBoc and CO_2H groups exert a deactivating effect on HAT processes from C—H bonds of the side chain to PINO radical. The lower k_H values determined with *N*-BocValOH and *N*-BocTleOH can be explained by the presence of steric effects. With these substrates a drop of reactivity of about 4–6 times is observed when compared to *N*-BocAlaOH. The steric interaction of the bulky isopropyl and the *tert*-butyl side chains of *N*-BocValOH and *N*-BocTleOH with the carboxylic and carbamate groups hinders the planarization of the carbon centered radical generated in the HAT process and therefore the captodative stabilization of these radicals (Fig. 3b and c). Under these conditions it is not possible to have an optimal overlap between the *p*-orbital on Cα and the π orbitals of the carbamate and carbonyl groups in the radical product [8,26].

In order to obtain information about the role of the hydrogen-abstracting radical nature in the HAT process from amino acids, it is interesting to compare the k_H values measured in this work with those determined for HAT process promoted by other radical species. Comparing the relative reactivity $k_H(X)/k_H(Gly)$ displayed in Table 3, it is possible to note that the results obtained in our kinetic studies follow the same behavior of those recently obtained for the study of HAT process from *N*-Boc protected α-amino acids to cumyloxyl radical (CumO•) with the exception of *N*-BocLeuOH for which a significantly higher relative reactivity was observed with CumO• likely indicating an involvement of the Cγ—H bond of the side chain in the HAT process [9]. This behavior is different from that found in HAT process promoted by PINO where a regioselective HAT from Cα-H bond is observed. Such a different behavior might be due to the much greater stability of PINO with respect to CumO•.

Concerning HAT process from amino acids to Br•, data in Table 3 show that it is strongly influenced by steric effects: increasing the steric hindrance of the amino acid side chain a decrease of relative rates was observed. With this radical, HAT process exclusively involves Cα-H bond and a "late" transition state (TS), in which C—H bond cleavage is advanced, has been proposed. With both Br• and PINO the relative stability of the α-carbon centered radical determines the reactivity of the different amino acidic substrates [7,8]. The lower selectivity observed with PINO compared to that observed with Br•, could be explained with a lower degree of C—H bond cleavage in the TS with the former radical with respect to the latter [7,8].

An opposite regioselectivity is instead observed in HAT promoted by HO• as compared to that found with PINO. Highly reactive OH• was found to promote HAT preferentially from C—H bonds of the side chain. This regioselectivity was rationalized on the basis of an "early" TS. In this scenario, a hypothetical HAT from the Cα-H bond is less influenced by the thermodynamic stability of the incoming radical but is affected by the presence of the adjacent electron-withdrawing groups which deactivate the Cα-H bond towards a HAT process promoted by the electrophilic OH• radical [2,4,27].

Among all the *N*-Boc-protected amino acids investigated, *N*-BocProOH showed the highest k_H value for the HAT process to PINO ($0.56\ M^{-1}\ s^{-1}$), about 6 times higher than that obtained with *N*-BocGlyOH. Differently from the other amino acids, *N*-BocProOH has two different reactive sites adjacent to the nitrogen atom indicating that the HAT process can occur not only at C_α-H bond, but also at C_δ-H bonds leading to the formation of two radicals as shown in Fig. 4.

Both the C_α—H and the C_δ—H bonds benefit from the activation provided by the adjacent carbamate group, however, at the same time, C_α—H bond is deactivated by the electron withdrawing character of the carboxylic group. The remarkable increase in reactivity observed with *N*-BocProOH suggests that, unlike the other acyclic amino acids seen so far, the HAT process may involve the C_δ—H bond rather than the C_α—H bond.

In addition to the polar effects discussed above, steric effects may also be responsible for the observed HAT regioselectivity in *N*-BocProOH. Steric effects prevent the planarization of the α carbon centered radical (Fig. 4B) and does not allow its stabilization by delocalization of the unpaired electron on the adjacent carbamate and carbonyl groups, which is instead possible for the δ carbon centered radical (Fig. 4A) [8]. In support to this hypothesis, previous studies concerning HAT processes from C—H bonds of amides have confirmed that the result obtained with *N*-BocProOH can be reasonably explained on the basis of stereoelectronic effects. Accordingly, HAT reactions from C—H bonds of amides to PINO were found to be faster when the Cα-H bond is collinear with the π amide system. This orbital overlap weakens the adjacent C—H bond and stabilizes the carbon radical generated in HAT process [21]. With *N*-BocProOH the steric restrictions imposed by the 5-membered heterocyclic ring force the C—H bonds in δ position to the nitrogen to assume a collinear position to the π system of the carbamate group, making them more activated by stereoelectronic effect towards HAT process with respect to the Cα-H bond of the other acyclic α-amino acids described above (Fig. 5).

As previously mentioned, a significant advantage of HAT processes promoted by PINO resides in the possibility to demonstrate the regioselectivity hypotheses based on kinetic data, by investigating the product distribution in the corresponding aerobic oxidation under Ishii conditions. Thus, to confirm that HAT process promoted by PINO involves the Cα—H bond with acyclic amino

Table 3
Relative Rates ($k_H(X)/k_H(Gly)$) for HAT processes from amino acids to different radical species.

Amino acid	PINO[a]	CumO•[b]	Br•[c]	OH•[d]
Gly	1.0	1.0	1.0	1.0
Ala	0.8	0.7	0.33	1.1
Val	0.2	0.5	0.04	3.7
Nva	0.6	0.8		9.7
Tle	0.1	0.6	$<4 \times 10^{-4}$	4.3
Leu	0.8	1.5		9.1
Pro	5.7	6.3	1.4	

[a] This work.
[b] *N*- tert-butoxycarbonyl protected amino acids, 266 nm laser flash photolysis, T = 25 °C, MeCN, dicumyl peroxide 10 mM [6].
[c] *N*-Benzoylated amino acids, steady-state photolysis, T = 25 °C in CCl_4 containing *N*-bromosuccinimide [5a,b].
[d] *N*-Acetylated amino acids, steady-state photolysis, T = 25 °C in D_2O acidified with TFA [4,8].

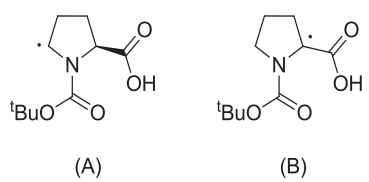


Fig. 4. Possible radicals formed in the HAT process from *N*-BocProOH.

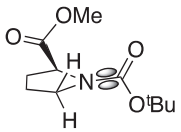


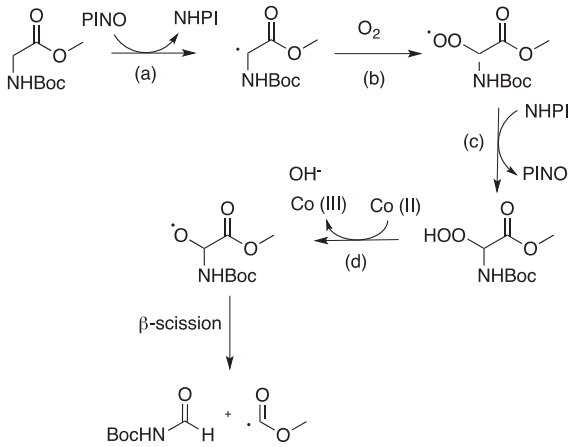
Fig. 5. Schematic representation of the collinearity between the carbamate group π system and the δ C-H bond of *N*-BocProOH.

acids and Cδ−H bonds with proline, we carried out product analysis of selected *N*-Boc and *N*-Ac protected amino acids methyl esters, catalyzed by *N*-hydroxyphthalimide, following the Ishii protocol. Oxidations were carried out in acetonitrile by using 10 mol % of the catalyst NHPI and 1 mol% of the co-catalyst $Co(OAc)_2$ under oxygen atmosphere.

Product analysis of the aerobic oxidation carried out with *N*-BocGlyOMe showed the formation of *tert*-butyl *N*-formyl carbamate. According to the mechanism proposed by Ishii, PINO (generated in the initiation step involving NHPI, the cocatalyst and oxygen) abstracts a hydrogen atom from Cα-H bond of glycine leading to the formation of a α-carbon centered radical (Scheme 3, path a). The latter reacts with O_2 generating a peroxyl radical (path b), which promotes HAT from NHPI, regenerating PINO and a hydroperoxide (path c). The reduction of hydroperoxide by Co(II) leads to the formation of an α-oxyl radical (path d) from which the product *tert*-butyl *N*-formyl carbamate is obtained through β-scission of a C−C bond.

A similar mechanism has been reported for the formation of *N*-acetyl-isopentyl amide in the oxidation of *N*-AcLeuOMe, which also leads to the formation of methyl 4-methyl-2-oxopentanoate. These two products derive from the α-oxyl radical generated in the HAT reaction from Cα-H of leucine to PINO. In this case the α-oxyl radical may undergo two competing decomposition pathways as previously proposed for the oxidation of similar compounds [10,11]: β-scission of C−C bond (Scheme 4, path a) leading to the formation of *N*-Ac-isopentyl amide, or further HAT reaction from NHPI (Scheme 4, path b) generating an α-hydroxy carbamate, which subsequently decomposes leading to the formation of methyl 4-methyl-2-oxopentanoate. Product analysis are in full agreement with kinetic data indicating the exclusive formation of the oxidation products deriving from HAT involving the Cα-H bond. This result excludes a competitive side chain HAT process, which is instead observed with other electrophilic radicals, such as CumO• and the most reactive Cl• and OH•.

No oxidation products were observed in the oxidation of *N*-AcValOMe, which was recovered almost quantitatively (>98%). The lack of reactivity of this substrate is in line with the kinetic data showing this substrate as the least reactive in HAT promoted by PINO.



Scheme 3. Mechanism of the aerobic oxidation of *N*-BocGlyOMe promoted by NHPI.

The oxidation of *N*-BocProOMe was the most efficient in term of product yields in accordance with the kinetic data showing that this substrate is the most reactive among all the investigated ones. The analysis of the final reaction mixture showed the presence of *N*-Boc-δ-oxoproline methyl ester and *N*-Boc-δ-hydroxyproline methyl esters (mixture of two diastereomers). Formation of these products clearly indicates that the initial step involves a HAT occurring selectively from Cδ-H bonds of *N*-BocProOMe to PINO (Scheme 5, path a) according to what suggested in the kinetic studies.

The δ-oxyl radical intermediate cannot undergo a C−C β-scission, as instead observed with glycine and leucine, because it would lead to a primary alkyl radical. It then abstracts a hydrogen atom from NHPI (Scheme 5, path b) leading to the formation of the mixture of the two *N*-Boc-5-hydroxyproline methyl esters diastereomers, which can be further oxidized to the *N*-Boc-5-oxoproline methyl ester.

4. Conclusions

Kinetic studies of the HAT process from a.a. to the phthalimide *N*-oxyl radical (PINO) coupled with the results of product analysis of the aerobic oxidations of the same substrate catalyzed by NHPI allowed us to obtain information about reactivity and selectivity pattern of this process. With amino acids containing aliphatic side chains HAT involves the Cα−H bonds activated by the adjacent nitrogen atom. HAT from C_δ−H bond instead occurs with the a.a. proline. Comparison of our results with those reported for HAT reactions from a.a. to other radical species clearly indicates that PINO displays electrophilic characteristics that are intermediate between those observed for more stable radicals such as Br• and more reactive radicals as the cumyloxyl radical.

5. Experimental

5.1. Instruments and general methods

NMR spectra were recorded on a 300 MHz spectrometer and were internally referenced to the residual proton solvent signal. GC-MS analyses were performed with a mass detector (EI at 70 eV) coupled with a gas chromatograph equipped with a melted silica capillary column (30 m 0.2 mm 25 mm) covered with a methylsilicone film (5% phenylsilicone, OV5).

5.2. Materials

HPLC grade acetonitrile was employed in the kinetic experiments. *N*-hydroxyphthalimide (NHPI), cerium (IV) ammonium nitrate (CAN) and *N*-Boc protected amino acids (*N*-BocGlyOH, *N*-BocAlaOH, *N*-BocValOH, *N*-BocNvaOH, *N*-BocTleOH, *N*-BocLeuOH, *N*-BocProOH) were of the highest purity available and used without further purification. Methyl esters of *N*-Boc amino acids and methyl esters of *N*-acetylated amino acids were prepared by standard methods and characterized by comparison of their spectroscopic data with those reported in the literature [28,29].

5.3. Kinetic studies

PINO was generated by oxidation of NHPI (1 mM) with cerium(IV) ammonium nitrate (CAN, 0.5 mM) in CH_3CN at 25 °C. After the generation of PINO, an excess of substrate was added under

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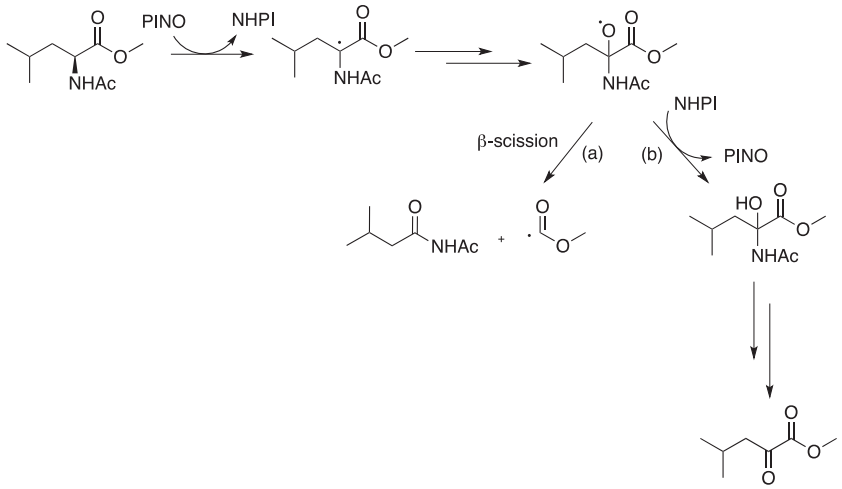
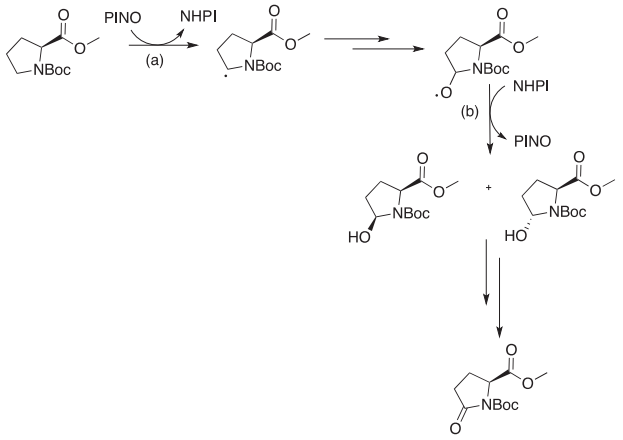


Scheme 4. Mechanism of the aerobic oxidation of *N*-AcLeuOMe promoted by NHPI.



Scheme 5. Mechanism of the aerobic oxidation of *N*-BocProOMe promoted by NHPI.

pseudo-first-order conditions (final concentration of substrate 7.5–75 mM). The decay of PINO was monitored at 380 nm and follows a first-order kinetic. Plotting the measured pseudo-first order rate constants (k_{obs}) as function of substrate concentration, second-order rate constants (k_H) were obtained from the slopes of these plots. Rate constants are reported as an average of at least three independent determinations with an error ±5%.

5.4. Product analysis of the oxidation of N-protected methyl ester amino acids with the Ishii system

In a typical oxidation experiment $Co(OAc)_2$ (1.2 mg, 0.004 mmol) and *N*-hydroxyphthalimide (NHPI) (6.6 mg, 0.04 mmol) were mixed in a vial in CH_3CN (2 mL). Substrate (0.8 mmol) was then added and the reaction mixture was stirred at room temperature, under oxygen atmosphere, for 24 h (*N*-Boc-ProOMe, *N*-BocGlyOMe) or 48 h (*N*-AcLeuOMe, *N*-AcValOMe). An internal standard (4-methoxybenzophenone) was then added and the reaction mixture was filtered over a short pad of SiO_2 and eluted with AcOEt. The filtered solution was subjected to GC and GC-MS analysis or evaporated to furnish the product mixture for the ^1H NMR analysis. The following oxidation products were identified by comparison with authentic specimens or by comparison of their spectral data with those reported in the literature: (2S,5S)- and (2S,5R)-*N*-Ac-5-hydroxyproline methyl esters (mixture of diastereomers) [30], (2S)–*N*-acetyl-5-oxoproline methyl ester [31], 4-methyl-2-oxo-pentanoic acid methyl ester [32].

N-isovalerylacetamide and *tert*-butyl *N*-formyl carbamate were isolated from the crude reaction mixture by column chromatography (silica gel, gradient dichloromethane/ethyl acetate from 98:2 to 95:5) and characterized as follows:

5.4.1. N-isovalerylacetamide

^1H NMR ($CDCl_3$, 300 MHz) δ: 8.92 (br, 1H, NH), 2.36 [m, 5H, CH_3CO, $CH_2CH(CH_3)_2$], 2.18–2.09 [m, 1H, $CH_2CH(CH_3)_2$], 0.97 [m, 6H, $CH_2CH(CH_3)_2$]. ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 173.8, 173.1, 46.8, 25.9, 25.7, 22.9. GC-MS (EI, 70 eV) *m/z* (relative intensity): 143 (1) $[M]^{+\cdot}$, 128 (22), 101 (100), 86 (21), 85 (17), 73 (18), 69 (21), 60 (36), 59 (50), 57 (40), 43 (76), 42 (13), 41 (24) [33]. (See SI for full NMR spectra).

5.4.2. tert-butyl N-formyl carbamate

^1H NMR ($CDCl_3$, 300 MHz) δ: 9.03 (d, 1H, *H*C=O), 7.55 (br, 1H, N*H*), 1.52 (s, 9H, *t*-Bu). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 163.6, 151.7, 84.3, 28.5. GC-MS (EI, 70 eV) *m/z* (relative intensity): 130 (1), 59 (62), 57 (100), 56 (20), 44 (10), 43 (10), 41(34). (See SI for full NMR spectra).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2019.05.026.

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