Organic LETTERS

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# Reactions of the Cumyloxyl Radical with Secondary Amides. The Influence of Steric and Stereoelectronic Effects on the Hydrogen Atom Transfer Reactivity and Selectivity

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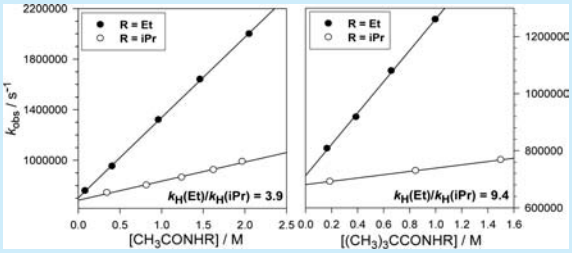
Supporting Information

**ABSTRACT:** A time-resolved kinetic study of the hydrogen atom transfer (HAT) reactions from secondary alkanamides to the cumyloxyl radical was carried out in acetonitrile. HAT predominantly occurs from the *N*-alkyl $\alpha$-C−H bonds, and a >60-fold decrease in $k_H$ was observed by increasing the steric hindrance of the acyl and *N*-alkyl groups. The role of steric and stereoelectronic effects on the reactivity and selectivity is discussed in the framework of HAT reactions from peptides.




Hydrogen atom transfer (HAT) from peptides and proteins to free radicals is a critical event that, depending on the site of attack and on follow-up reactions, can lead to cleavage of the peptide backbone or to side-chain modification.[1−3] Accordingly, considerable efforts have been devoted toward the understanding of the factors that govern the HAT selectivity for these processes, where model substrates such as oligopeptides, amino acids, and amides have been often employed for this purpose. Results in this respect have been mostly obtained through product studies[4−8] and, more recently, computational studies,[9−14] whereas limited direct kinetic information on these reactions is presently available.[15−17]

In this framework, recent time-resolved kinetic studies carried out in our group have provided quantitative information on the reactivity and selectivity patterns observed in HAT reactions from a variety of tertiary alkanamides to a reactive oxygen centered radical such as cumyloxyl ($PhC(CH_3)_2O^{\bullet}$, CumO$^{\bullet}$).[18,19] In these studies, the important role played by C−H bond strengths and stereoelectronic effects (i.e., orbital overlap between the C−H bond to be cleaved and the amide $\pi$ system) has been clearly pointed out.

In view of the importance of free radical reactions involving peptides and proteins and in keeping with our general interest in the role of structural effects on HAT reactions to alkoxyl radicals,[20] we have extended our study to secondary amides, substrates that, as compared to tertiary amides, can be considered more representative models for the peptide bond. Along this line, in order to provide an understanding of the role of substrate structure on the HAT reactivity and selectivity, we have carried out a detailed time-resolved kinetic study in acetonitrile solution on the reactions of CumO$^{\bullet}$ with three series of *N*-alkylalkanamides, namely *N*-alkylacetamides, *N*-alkyl-2,2-dimethylpropanamides (*N*-alkylpivalamides) and *N*-alkyl-3,3-dimethylbutanamides (where alkyl = methyl, ethyl, isopropyl, and *tert*-butyl), whose structures are displayed in Figure 1.[21]

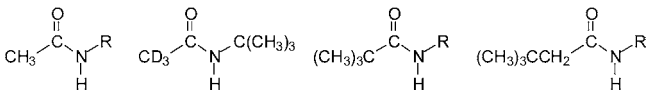



**Figure 1.** Structure of the secondary amides.

CumO$^{\bullet}$ was generated by 266 nm laser flash photolysis (LFP) of argon-saturated acetonitrile solutions ($T$ = 25 °C) containing dicumyl peroxide. CumO$^{\bullet}$ is characterized by a broad absorption band in the visible region of the spectrum centered at 485 nm[23,24] and, as a consequence of its electrophilic character,[25] displays an extremely low HAT reactivity toward the C−H bonds of acetonitrile ($k_{H(MeCN)} < 10^4$ M$^{-1}$ s$^{-1}$).[26,27] Accordingly, under these conditions CumO$^{\bullet}$ decays almost exclusively through C−CH$_3$ $\beta$-scission.[23,27]

Time-resolved kinetic studies on the reactions of CumO$^{\bullet}$ with the amides shown in Figure 1 were carried out by LFP following the decay of the CumO$^{\bullet}$ visible absorption band as a function of the amide concentration. When plotted against substrate concentration the observed rate constants ($k_{obs}$) gave excellent linear relationships and the second-order rate constants for HAT from the amides to CumO$^{\bullet}$ ($k_H$) were obtained from the slope of these plots.[28] As an example, Figure 2 shows the $k_{obs}$ vs [substrate] plots for the reactions of CumO$^{\bullet}$ with MA (black circles), EA (white circles), iPrA (gray circles), tBuA (white squares), and tBuA-$d_3$ (black squares), for measurements carried out in acetonitrile solution at $t$ = 25 °C.

Additional plots for HAT from the *N*-alkyl-2,2-dimethylpropanamides and *N*-alkyl-3,3-dimethylbutanamides to CumO$^{\bullet}$

**Received:** November 11, 2014
**Published:** December 4, 2014





dx.doi.org/10.1021/ol503277r | *Org. Lett.* 2014, 16, 6444−6447

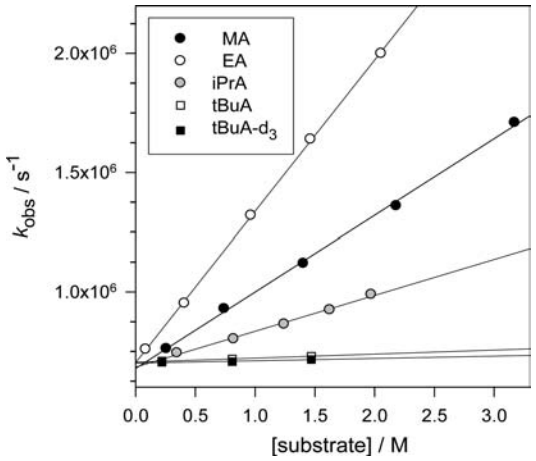



**Figure 2.** Plots of the observed rate constant ($k_{obs}$) against [substrate] for the reactions of the cumyloxyl radical (CumO$^\bullet$) with MA (black circles), EA (white circles), iPrA (gray circles), tBuA (white squares), and tBuA-$d_3$ (black squares) measured in argon-saturated MeCN solution at $t$ = 25 °C by following the decay of CumO$^\bullet$ at 490 nm. From the linear regression analysis: CumO$^\bullet$ + MA, intercept = $6.79 \times 10^5$ s$^{-1}$, $k_H = 3.21 \times 10^5$ M$^{-1}$ s$^{-1}$, $r^2 = 0.9986$. CumO$^\bullet$ + EA, intercept = $7.03 \times 10^5$ s$^{-1}$, $k_H = 6.35 \times 10^5$ M$^{-1}$ s$^{-1}$, $r^2 = 0.9998$. CumO$^\bullet$ + iPrA, intercept = $6.84 \times 10^5$ s$^{-1}$, $k_H = 1.51 \times 10^5$ M$^{-1}$ s$^{-1}$, $r^2 = 0.9943$. CumO$^\bullet$ + tBuA, intercept = $7.05 \times 10^5$ s$^{-1}$, $k_H = 1.68 \times 10^4$ M$^{-1}$ s$^{-1}$, $r^2 = 0.9977$. CumO$^\bullet$ + tBuA-$d_3$, intercept = $7.01 \times 10^5$ s$^{-1}$, $k_H = 9.7 \times 10^3$ M$^{-1}$ s$^{-1}$, $r^2 = 0.940$.

are displayed in the Supporting Information (SI, Figures S13 and S14). All the kinetic data thus obtained are collected in Table 1. Also included in this table are the rate constant ratios $k_H$(*N*-Me)/$k_H$(*N*-alkyl) for the three series.

Within the *N*-alkylacetamide series the lowest $k_H$ value was measured for the reaction of CumO$^\bullet$ with tBuA, for which $k_H = 1.8 \times 10^4$ M$^{-1}$ s$^{-1}$. With tBuA-$d_3$, only an upper limit to $k_H$ could be determined as $k_H \leq 10^4$ M$^{-1}$ s$^{-1}$. Comparison between these two values provides a lower limit to the kinetic deuterium isotope effect $k_H/k_D \geq 1.8$, indicating that in the reaction of tBuA with CumO$^\bullet$ HAT mostly occurs from the acetyl methyl group, in line with the very low HAT reactivity displayed by *tert*-butyl groups in their reactions with alkoxyl radicals (see below).[18] This relatively low $k_H$ value also reflects the operation of polar effects as the acetyl C−H bonds are known to be deactivated toward HAT to an electrophilic radical such as CumO$^\bullet$.[24] On the basis of this result, the 9- to 35-fold increase in $k_H$ measured on going from tBuA to MA, EA, and iPrA clearly indicates that with the latter three substrates HAT from the $\alpha$-C−H bonds of the *N*-alkyl groups is significantly faster than HAT from the acetyl methyl group, in full agreement with previous studies on the reactions of CumO$^\bullet$ with *N*,*N*-dimethylalkanamides,[18] and with the observation that in alkanamides these bonds are at least 5 kcal mol$^{-1}$ weaker than the C−H bonds that are adjacent to the carbonyl group.[18,19]

As compared to MA, for which $k_H = 3.18 \times 10^5$ M$^{-1}$ s$^{-1}$ was measured, a 2-fold increase in $k_H$ was observed for *N*-ethylacetamide (EA). This behavior can be explained on the basis of the ∼1 kcal mol$^{-1}$ lower BDE for the $\alpha$-C−H bonds of an *N*-ethyl group as compared to an *N*-methyl group.[18] On the other hand, the 2-fold decrease in $k_H$ observed on going from MA to iPrA and, in particular, the 4-fold decrease in $k_H$ observed on going from EA to iPrA, reflect, at least to a certain extent, the operation of stereoelectronic effects. Replacement of the *N*-methyl and *N*-ethyl groups in MA and EA with isopropyl increases the energy barrier required to achieve the most suitable

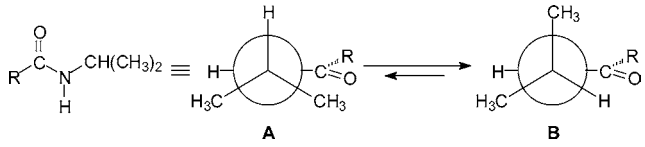
**Table 1. Second-Order Rate Constants ($k_H$) for the Reactions of the Cumyloxyl Radical (CumO$^\bullet$) with Secondary Amides**

| | $k_H$/M$^{-1}$ s$^{-1}$ [a] | $k_H$(*N*-Me)/$k_H$(*N*-alkyl) |
|---|---|---|
| *N*-alkylacetamides | | |
| MA | $(3.18 \pm 0.03) \times 10^5$ | 1.0 |
| EA | $(6.2 \pm 0.2) \times 10^5$ | 0.5 |
| iPrA | $(1.6 \pm 0.1) \times 10^5$ | 2.0 |
| tBuA | $(1.8 \pm 0.1) \times 10^4$ | 17.7 |
| tBuA-$d_3$ [b] | $\leq 10^4$ | ≥32 |
| $k_H/k_D$ | ≥1.8 | |
| *N*-alkyl-2,2-dimethylpropanamides | | |
| MPVA | $(2.99 \pm 0.03) \times 10^5$ | 1.0 |
| EPVA | $(5.43 \pm 0.07) \times 10^5$ | 0.6 |
| iPrPVA | $(5.8 \pm 0.1) \times 10^4$ | 5.2 |
| tBuPVA [c] | $<10^4$ | >30 |
| *N*-alkyl-3,3-dimethylbutanamides | | |
| MDMBA | $(2.9 \pm 0.1) \times 10^5$ | 1.0 |
| EDMBA | $(7.1 \pm 0.1) \times 10^5$ | 0.4 |
| iPrDMBA | $(7.3 \pm 0.1) \times 10^4$ | 4.0 |
| tBuDMBA [c] | $<10^4$ | >29 |

[a]Measured in argon-saturated acetonitrile solution at $t$ = 25 °C employing 266 nm LFP: [dicumyl peroxide] = 10 mM. $k_H$ values were determined from the slope of the $k_{obs}$ vs [substrate] plots, where in turn $k_{obs}$ values were measured following the decay of the CumO$^\bullet$ visible absorption band at 490 nm. Average of at least two determinations. [b]Because of the very low reactivity and limited solubility (≤1.5 M) displayed by this substrate under the experimental conditions employed, the measured $k_H$ value should be taken as an upper limit. [c]No significant change in $k_{obs}$ was observed up to the highest concentration of substrate employed (≤0.9 M), and accordingly only an upper limit to $k_H$ could be obtained.

conformation for HAT, where the $\alpha$-C−H bond is perpendicular to the plane of the amide group (Scheme 1, R = $CH_3$: conformation A) thus preventing optimal orbital overlap between the $\alpha$-C−H bond and the amide $\pi$-system.[18]

**Scheme 1**




It is also very interesting to compare the $k_H$ values measured for the reaction of CumO$^\bullet$ with MA and EA ($k_H = 3.18 \times 10^5$ and $6.2 \times 10^5$ M$^{-1}$ s$^{-1}$, respectively) with those measured previously for the corresponding reactions of CumO$^\bullet$ with *N*,*N*-dimethylacetamide (DMA) and *N*,*N*-diethylacetamide (DEA), for which $k_H = 1.24 \times 10^6$ and $6.64 \times 10^5$ M$^{-1}$ s$^{-1}$, respectively.[18,19] The decrease in $k_H$ measured on going from DMA to DEA has been explained on the basis of the above-mentioned stereoelectronic effects, where, despite the decrease in the *N*-alkyl $\alpha$-C−H bond BDE, replacement of the methyl groups by ethyl increases the barrier required to achieve the most suitable conformation for HAT.[18] Computational studies have shown that in DMA and DEA HAT to CumO$^\bullet$ preferentially occurs from the $\alpha$-C−H bonds of the *trans* *N*-alkyl group, as evidenced by the observation that the BDEs of the $\alpha$-C−H bonds of the *N*-alkyl group that is in a *trans* relationship to the carbonyl group are about 2 kcal mol$^{-1}$ lower than those of the


dx.doi.org/10.1021/ol503277r | *Org. Lett.* 2014, 16, 6444−6447

corresponding *N*-alkyl group in the *cis* arrangement,[19] and by the calculated rate constant ratios for HAT from the *trans* and *cis* methyl groups of DMA that is around 2.

As mentioned above, the increase in $k_H$ measured on going from MA to EA can be explained on the basis of the lower BDE for the α-C−H bonds of an *N*-ethyl group as compared to an *N*-methyl group. At room temperature, MA and EA mostly exist in the *cis* form,[21] and the measured $k_H$ values now reflect HAT from the α-C−H bonds of the *cis* *N*-alkyl groups. Accordingly, the 4-fold decrease in $k_H$ measured on going from DMA to MA, despite a 2-fold decrease in the number of α-C−H bonds, reflects the intrinsically lower reactivity of a *cis* *N*-methyl group as compared to a *trans* *N*-methyl group. On the other hand, the very similar $k_H$ values measured for DEA and EA are a result of the combination of stereoelectronic and enthalpic effects, showing that it is relatively easier for the secondary amide as compared to the tertiary one to achieve the conformation that allows for optimal overlap between the α-C−H bonds and the amide π-system.

When comparing the $k_H$ values measured for the reaction of CumO$^\bullet$ with the *N*-alkylacetamides with those measured for the corresponding reactions with *N*-alkyl-2,2-dimethylpropanamides and *N*-alkyl-3,3-dimethylbutanamides, only an upper limit to the rate constant ($k_H < 10^4$ M$^{-1}$ s$^{-1}$) could be obtained for tBuPVA and tBuDMBA, as compared to $k_H = 1.8 \times 10^4$ M$^{-1}$ s$^{-1}$ measured for the reaction of CumO$^\bullet$ with tBuA that, as mentioned above, mostly undergoes HAT from the acetyl methyl group. The extremely low HAT reactivity displayed by these substrates is again indicative of the relative inertness of *tert*-butyl groups toward alkoxyl radicals, suggesting that the decrease in $k_H$ observed on going from tBuA to tBuDMBA can be accounted for on the basis of steric effects.

An almost identical behavior was observed within the three series for the *N*-methyl and *N*-ethyl derivatives. The $k_H$ values for HAT from MA, MPVA, and MDMBA vary between 2.9 and 3.18 $\times 10^5$ M$^{-1}$ s$^{-1}$ and increase by a factor 1.8−2.4 on going to the corresponding *N*-ethylalkanamides. These findings, together with the extremely low reactivity displayed by tBuPVA and tBuDMBA, indicate that, with MPVA, EPVA, MDMBA, and EDMBA, HAT almost exclusively occurs from the α-C−H bonds of the *N*-alkyl groups, indicating moreover that with *N*-methyl and *N*-ethylalkanamides $k_H$ is essentially unaffected by steric hindrance at the acyl moiety.

On the other hand, the sterics associated with the acyl group appear to play an important role in the reactions of the *N*-isopropylalkanamides, as evidenced by the significantly larger decreases in $k_H$ observed for the 2,2-dimethylpropanamides and 3,3-dimethylbutanamides as compared to the acetamides, on going from the *N*-ethyl to the *N*-isopropyl derivatives. These effects can be quantified on the basis of the rate constant ratios $k_H$(*N*-Et)/$k_H$(*N*-iPr) that increase from ∼4 for the acetamides to ∼10 for the 2,2-dimethylpropanamides and 3,3-dimethylbutanamides. It appears that in the presence of an *N*-isopropyl group the stereoelectronic requirements for HAT become more stringent, where the greater steric bulk associated with the pivaloyl and 3,3-dimethylbutanoyl groups as compared to acetyl increases the energy barrier required to achieve optimal overlap between the α-C−H bond and the amide π-system (Scheme 1, R = $C(CH_3)_3$, $CH_2C(CH_3)_3$).

These findings indicate that, in the framework of HAT reactions from peptides to free radicals, steric and stereoelectronic effects can play an important role. Neglecting for the sake of simplicity the possible role of the peptide secondary structure and of solvent effects,[29] these results suggest that the reactivity of the α-C−H bond of a given amino acid residue can vary as a function of the steric hindrance exerted by the side chain of an adjacent residue ($R_1$ in Figure 3), indicating that these

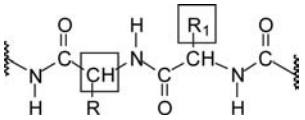



**Figure 3.** Representative structure of a peptide.

effects can also influence the HAT selectivity. In other words, the presence of an amino acid residue characterized by a bulky side chain such as for example valine or isoleucine ($R_1$ = isopropyl and *sec*-butyl, respectively) could significantly decrease the rate constant for HAT from the α-C−H bond of an adjacent residue, thus providing to a certain extent protection toward hydrogen abstraction.

This intriguing hypothesis, however, would require dedicated experiments because, to the best of our knowledge, no kinetic data in this respect is presently available. It is also worth mentioning that this picture should apply to HAT reactions that predominantly occur from the α-C−H bonds and proceed through relatively early transition states such as those involving alkoxyl radicals, reactions for which stereoelectronic effects should be operative.[4c,30] The observation of similar relative reactivities and selectivity patterns in the reaction of the *tert*-butoxyl radical[30] and of a nonheme ferryl complex[6] with aliphatic amino acids indicates that a similar picture may also apply to the reactions that involve the latter oxidants. With less reactive radicals such as Br$^\bullet$ and peroxyl radicals, that are also known to undergo HAT from the α-C−H bonds, the stability of the product radical plays an important role in line with the relatively late transition states described for these reactions,[4b,c,9a,30] and a negligible role for stereoelectronic effects can be envisaged. A different picture can be instead expected with highly reactive radicals such as HO$^\bullet$ and Cl$^\bullet$, because these radicals have been shown to undergo predominant HAT from the side-chain C−H bonds of amino acids and peptides through very early transition states,[4a,7−9] a behavior that has been rationalized on the basis of the important role played by polar effects in these reactions.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental details. 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.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

Financial support from the Ministero dell'Istruzione dell'Università e della Ricerca (MIUR) - project 2010PFLRJR (PRIN 2010-2011) is gratefully acknowledged. We thank Prof. Miquel Costas (Universitat de Girona, Spain) for helpful discussions and Prof. Lorenzo Stella (Università "TorVergata", Italy) for the use of LFP equipment.

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(21) *N*-Methylacetamide is characterized by a free energy barrier for rotation around the OC−NC bond of 21.3 kcal $mol^{-1}$, and the *Z* isomer (*cis*) is more stable than the *E* isomer (*trans*) by 2.1−2.5 kcal $mol^{-1}$, leading, at room temperature, to a composition of the isomeric mixture of 97:3.[22] Bulkier alkyl groups bound to the carbonyl and/or the NH group are expected to increase the *Z*/*E* ratio, indicating that under the experimental conditions employed all the substrates investigated predominantly exist in the *Z* isomeric form.

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(28) An important contribution of HAT from the amides to the methyl radical, formed after C−$CH_3$ cleavage in CumO$^{\bullet}$,[23,26,27] can be ruled out because the methyl radical is produced in competition with HAT from the amides to CumO$^{\bullet}$, in amounts that decrease with increasing substrate concentration. Moreover, the methyl radical is expected to be scavenged by the solvent acetonitrile that is present in large excess and is characterized by electron poor C−H bonds.[25]

(29) It is important to point out that our $k_H$ values have been measured in MeCN while HAT from peptides and proteins generally occurs in aqueous media. However, as it is well established that proteins possess hydrophobic domains, kinetic data measured in organic solvents may also be of relevance in the framework of these reactions. Studies of kinetic solvent effects on HAT reactions from amides to CumO$^{\bullet}$ are currently underway in our laboratories, and the results of these studies will be published in due course.

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