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## The radical scavenging activity of 1-methyl-1,4-dihydronicotinamide: theoretical insights into the mechanism, kinetics and solvent effects†

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1,4-Dihydronicotinamide derivatives, including 1-methyl-1,4-dihydronicotinamide (**MNAH**), are derivatives of the active center of nicotinamide coenzyme (NADH) and are therefore potent radical scavengers. **MNAH** serves as a useful model of NADH that allows for modeling studies to address the activity of this important biomolecule. In this work, **MNAH** activity was evaluated against typical free radicals using quantum chemical calculations in physiological environments, with a secondary aim of comparing activity against two physiologically relevant radicals of markedly different stability,  $\text{HO}^\cdot$  and  $\text{HOO}^\cdot$ , to establish which of these is a better model for assessing antioxidant capacity in physiological environments. The  $\text{HO}^\cdot + \text{MNAH}$  reaction exhibited diffusion-limited overall rate constants in all media, including the gas phase. The  $\text{HOO}^\cdot$  antiradical activity of **MNAH** was also good, with overall rate constants of  $2.00 \times 10^4$  and  $2.44 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ , in lipid and aqueous media, respectively. The calculated rate constant in water ( $k_{\text{overall}}(\text{MNAH} + \text{HOO}^\cdot) = 3.84 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ , pH = 5.6) is in good agreement with the experimental data ( $k_{\text{exp}}(\text{NADH} + \text{HOO}^\cdot) = (1.8 \pm 0.2) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ). In terms of mechanism, the H-abstraction of the C4-H bond characterized the  $\text{HOO}^\cdot$  radical scavenging activity of **MNAH**, whereas  $\text{HO}^\cdot$  could react with **MNAH** at several sites and following either of SET (in polar media), RAF, and FHT reactions, which could be ascribed to the high reactivity of  $\text{HO}^\cdot$ . For this reason the results suggest that activity against  $\text{HOO}^\cdot$  is a better basis for comparison of anti-radical potential. In the broader context, the  $\text{HOO}^\cdot$  scavenging activity of **MNAH** is better than that of reference antioxidants such as *trans*-resveratrol and ascorbic acid in the nonpolar environment, and Trolox in the aqueous physiological environment. Therefore, in the physiological environment, **MNAH** functions as a highly effective radical scavenger.

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### 1. Introduction

Reactive oxygen species (ROS) are molecules that are extremely reactive and are primarily produced by the mitochondrial electron transport chain.<sup>1</sup> Under normal circumstances, ROS are generated as natural biproducts of normal metabolic processes and also serve a variety of functions in healthy physiological processes. For example, they activate signaling pathways to initiate biological processes as “secondary messengers”.<sup>2</sup> Oxidative stress is the result of an imbalance between the antioxidant defense system and ROS production.<sup>3</sup> It is also crucial to preserve the equilibrium of ROS in bone homeostasis and pathology.<sup>4</sup> Nicotinamide coenzyme (**NADH**) is a natural redox factor that is crucial for the reduction of ROS; it is a ubiquitous hydride and electron source that participates

in a diverse array of biochemical processes that occur *in vivo*.<sup>5-7</sup> The mechanism of oxidoreductase primarily relies on the cycling of nicotinamide adenine dinucleotide (NAD) and its reduced form, **NADH**. The NAD redox pair ( $\text{NAD}^+/\text{NADH}$ ) serves as a coenzyme essential for oxidoreductase metabolism.<sup>8-12</sup>

Since the active center of **NADH** is dihydronicotinamide (Fig. 1), which contains two weak C4-H bonds; the radical scavenging could occur directly there following the formal hydrogen transfer mechanism.<sup>13</sup> The nicotinamide component may also react with highly-reactive ROS, such as  $\text{HO}^\cdot$  radicals, through the radical adduct formation (RAF) and either the hydrogen transfer pathway or single electron transfer (SET). Nevertheless, this matter has not yet been thoroughly investigated.

The hydroxyl radical is a prevalent and highly reactive species among free radicals. It is identified as the primary effector of tissue damage caused by ionizing radiation and oxidative damage to DNA.<sup>14,15</sup> Because of its high reactivity its physiological lifetime is short, therefore the ideal way of reducing oxidative stress due to  $\text{HO}^\cdot$  would be to inhibit the production of hydroxyl radicals.<sup>16</sup> Due to its dominant role in pathologic processes it is quite common in the literature to

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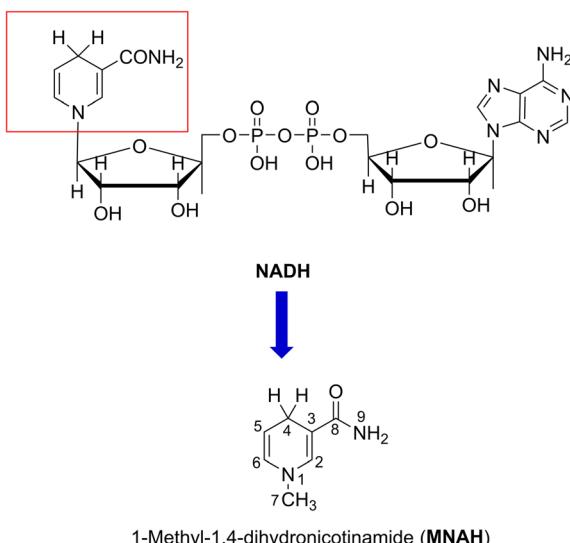


Fig. 1 The structure of MNAH.

investigate radical scavenging activity against the hydroxyl radical, and it is indeed crucial if the focus is on evaluating the degradation of organic compounds.<sup>17–19</sup> On the other hand, the HO<sup>·</sup> model may not be an effective way to compare the radical scavenging activity of organic compounds due to the inherent high reactivity of this radical. A more representative model of the typical less reactive radicals is the HOO<sup>·</sup> radical, and thus it is a better alternative for computational studies to evaluate the yet unknown free radical scavenging activity of compounds.<sup>16,17,19</sup> To highlight this issue in this study we examine and compare activity against HO<sup>·</sup> and HOO<sup>·</sup>.

Previous studies demonstrated that the HO<sup>·</sup>/HOO<sup>·</sup> radical scavenging activity of organic compounds can be accurately modeled by quantum chemical methods.<sup>20–22</sup> Using this method, we modeled the kinetics and mechanism of the HO<sup>·</sup>/HOO<sup>·</sup> scavenging activity of 1-methyl-1,4-dihydronicotinamide (**MNAH**) (Fig. 1), the active center of **NADH**, in physiological environments.

## 2. Computational methods

The kinetic calculations in this study were conducted in accordance with the quantum mechanics-based test for overall free radical scavenging activity (QM-ORSA) protocol, combined with the SMD solvation model<sup>23</sup> procedure for pentyl ethanoate and water solvents.<sup>17,24–27</sup> The traditional transition state theory (TST) at a temperature of 298.15 K and a standard state of 1 M was used to compute the rate constant (*k*) as outlined in eqn (1) by using the Eyringpy code.<sup>26</sup> (more information method in Table S1, ESI†):<sup>28–33</sup>

$$k = \sigma \kappa \frac{k_B T}{h} e^{-(\Delta G^\ddagger)/RT} \quad (1)$$

where  $\sigma$  is the reaction symmetry number,<sup>34,35</sup>  $\kappa$  stands for tunneling corrections that were calculated using Eckart barrier,<sup>36</sup>  $k_B$  is the Boltzmann constant,  $h$  is the Planck constant,  $\Delta G^\ddagger$  is Gibbs free energy of activation.

Gaussian 16 software<sup>37</sup> was employed to conduct all calculations at the M06-2X/6-311++G(d,p) level of theory, which was previously identified as an appropriate model chemistry for this application.<sup>38,39</sup>

## 3. Results and discussions

### 3.1. The radical scavenging in the gas phase

**3.1.1. Thermodynamic study.** Under conditions of non-polar media such as in the gas phase, the antiradical activity can follow either of three primary mechanisms: sequential electron transfer proton transfer (SETPT),<sup>40,41</sup> formal hydrogen transfer (FHT),<sup>25</sup> or radical adduct formation (RAF) in the case of molecules with double bonds.<sup>42</sup> To identify the most effective antioxidant mechanisms, we calculated the Gibbs free energy changes ( $\Delta G^\circ$ ) in the gas phase for each reaction of **MNAH** with HO<sup>·</sup> and HOO<sup>·</sup> radicals in one of the following reactions: FHT, RAF, or single electron transfer (SET) for the SETPT reaction. The results are shown in Table 1.

The findings revealed that most reactions between HO<sup>·</sup> and **MNAH** were thermodynamically favorable ( $\Delta G^\circ < 0$ ), except for the SET reaction ( $\Delta G^\circ = 139.6$  kcal mol<sup>-1</sup>). The **MNAH** + HOO<sup>·</sup> reaction was only spontaneous at the FHT (C4–H,  $\Delta G^\circ = -14.6$  kcal mol<sup>-1</sup>), whereas those of other mechanisms, such as the SET and FHT (C7–H and N9–H), are not thermodynamically spontaneous ( $\Delta G^\circ = 5.1$ –144.3 kcal mol<sup>-1</sup>). The H-abstraction of C4–H is the most preferred thermodynamically **MNAH** + HO<sup>·</sup> reaction ( $\Delta G^\circ = -45.8$  kcal mol<sup>-1</sup>, BDE = 71.2 kcal mol<sup>-1</sup>). Thus, this could make a significant contribution to the HO<sup>·</sup> radical scavenging activity of **MNAH**. Nevertheless, the **MNAH** + HO<sup>·</sup> reaction could also follow the RAF reactions at C2, C3, C5, and C6 and the FHT (C7–H) due to the low negative  $\Delta G^\circ$  values ( $\Delta G^\circ = -17.4$  to  $-26.1$  kcal mol<sup>-1</sup>). The HO<sup>·</sup>/HOO<sup>·</sup> radical scavenging activity of **MNAH** may not involve the H-abstraction of N9–H due to the high BDE and  $\Delta G^\circ$  values (BDE = 109.9 kcal mol;  $\Delta G^\circ = -6.2$  and 25.1 kcal mol<sup>-1</sup> for HO<sup>·</sup> and HOO<sup>·</sup> radicals, respectively). Consequently, the kinetics of the HO<sup>·</sup>/HOO<sup>·</sup> radicals scavenging activity of **MNAH** were evaluated at all of the sites of spontaneous reactions ( $\Delta G^\circ < 0$ ).

**3.1.2. Kinetic study.** In the initial kinetic evaluation, the potential energy surfaces (PES) were first computed; the findings are presented in Fig. 2. The highest reaction barrier

Table 1 The computed BDE and  $\Delta G^\circ$  (in kcal mol<sup>-1</sup>) following the RAF, FHT, and SET mechanisms of the **MNAH** + HO<sup>·</sup>/HOO<sup>·</sup> reactions

Mechanisms	Positions	BDE	$\Delta G^\circ$	
			HO <sup>·</sup>	HOO <sup>·</sup>
FHT	C4–H	71.2	-45.8	-14.6
	C7–H	90.9	-26.1	5.1
	N9–H	109.9	-6.2	25.1
RAF	C2		-23.1	
	C3		-17.4	
	C5		-25.7	
	C6		-20.2	
SET			139.6	144.3



(12.5 kcal mol<sup>-1</sup>) for the **MNAH** + HO<sup>·</sup> reaction is observed at the FHT of the N9–H bond, while the C4 position of **MNAH** presented the lowest reaction barrier value (0.1 kcal mol<sup>-1</sup>). The RAF at C2, C3, and C5 positions had reaction barriers of 2.7, 2.0, and 3.5 kcal mol<sup>-1</sup>, respectively, whereas those of C6 and C7–H were 4.6 and 8.3 kcal mol<sup>-1</sup>, respectively. Based on these results, the dominant **MNAH** + HO<sup>·</sup> reactions are the addition of the HO<sup>·</sup> radical at the C2, C3, or C5 positions and the FHT reaction of the C4–H bonds, while the H-abstraction of **MNAH** by HO<sup>·</sup> radicals *via* the C7–H and N9–H bonds would not contribute to the activity. The HOO<sup>·</sup> radical scavenging reaction is defined by the H-abstraction at the C4–H bond with the low reaction barrier at 0.1 kcal mol<sup>-1</sup>.

The kinetics of the **MNAH** + HO<sup>·</sup>/HOO<sup>·</sup> reactions were calculated by using the QM-ORSA methodology.<sup>17</sup> The results are presented in Table 2, whereas the optimized structures and the SOMO orbitals of transition states (TS) are shown in Fig. 3 and S1, ESI,† respectively. In the gas phase, the FHT reaction of the C4–H with HO<sup>·</sup> radicals was barrierless ( $\Delta G^\ddagger \approx 0$  kcal mol<sup>-1</sup>,  $k_{\text{Eck}} = 6.02 \times 10^{12}$  M<sup>-1</sup> s<sup>-1</sup>,  $\Gamma = 27.1\%$ ), whereas that of C7–H and N9–H bonds had no contribution to the radical scavenging activity with  $k_{\text{Eck}} = 1.39 \times 10^{10}$  M<sup>-1</sup> s<sup>-1</sup> ( $\Gamma = 0.1\%$ ) and  $5.60 \times 10^7$  M<sup>-1</sup> s<sup>-1</sup> ( $\Gamma = 0.0\%$ ), respectively. At the same time, the RAF reactions at C2, C3, and C5 form a substantial part of the overall **MNAH** + HO<sup>·</sup> reaction with  $\Delta G^\ddagger \approx 0$  kcal mol<sup>-1</sup>,  $k_{\text{Eck}} = 6.02 \times 10^{12}$  M<sup>-1</sup> s<sup>-1</sup>,  $\Gamma = 27.1\%$  for each position. The addition reaction at the C6 location contributed only about 1.1% to the overall rate constant. Thus in the gas phase, the **MNAH** + HO<sup>·</sup> reaction was rapid and defined by the FHT(C4–H) and RAF(C2, C3, and C5) mechanisms with the overall rate constant  $k_{\text{overall}} = 2.22 \times 10^{13}$  M<sup>-1</sup> s<sup>-1</sup>, whereas the **MNAH** + HOO<sup>·</sup> reaction was moderate and characterized by the

Table 2 Computed  $\Delta G^\ddagger$  (in kcal mol<sup>-1</sup>),  $\kappa$ ,  $k_{\text{Eck}}$  (M<sup>-1</sup> s<sup>-1</sup>) and branching ratios ( $\Gamma\%$ ) for the **MNAH** + HO<sup>·</sup>/HOO<sup>·</sup> reactions<sup>a</sup>

Radicals	Mechanisms	Positions	$\Delta G^\ddagger$	$\kappa$	$k_{\text{Eck}}$	$\Gamma$
HO <sup>·</sup>	FHT	C4–H	0.0	1.0	$6.02 \times 10^{12}$	27.1
		C7–H	3.9	1.6	$1.39 \times 10^{10}$	0.1
		N9–H	8.9	30.2	$5.60 \times 10^7$	0.0
	RAF	C2	0.0	1.0	$6.02 \times 10^{12}$	27.1
		C3	0.0	1.0	$6.02 \times 10^{12}$	27.1
		C5	0.3	1.0	$3.91 \times 10^{12}$	17.6
HOO <sup>·</sup>	FHT	C6	2.0	1.0	$2.35 \times 10^{11}$	1.1
		C4–H	9.1	2.1	$2.83 \times 10^6$	100.0

<sup>a</sup>  $\Gamma = k_{\text{Eck}} \cdot 100/k_{\text{overall}}$ .

FHT(C4–H) with  $k_{\text{overall}} = 2.83 \times 10^6$  M<sup>-1</sup> s<sup>-1</sup>. The main products of the **MNAH** + HO<sup>·</sup> reaction in the gas phase were **P-C2** (27.1%), **P-C3** (27.1%), **P-C4** (27.1%), and **P-C5** (17.6%), whereas for the **MNAH** + HOO<sup>·</sup> reaction **P-C4(HOO)** was the only product (100%) (Fig. 2 and Table 2).

The extremely high theoretical rate constant for the **MNAH** + HO<sup>·</sup> reaction ( $k_{\text{overall}} = 2.22 \times 10^{13}$  M<sup>-1</sup> s<sup>-1</sup>) suggests that the reaction is diffusion-limited even in the gas phase where the collision rate at the given temperature would limit the reaction to  $10^9$ – $10^{10}$  M<sup>-1</sup> s<sup>-1</sup>. Hence the activity against HO<sup>·</sup> radical is not a useful basis for comparison. On the other hand, the HOO<sup>·</sup> radical scavenging activity of **MNAH** is comparable to the reference antioxidant Trolox ( $k_{(\text{HOO}^\cdot + \text{Trolox})} = 1.87 \times 10^7$  M<sup>-1</sup> s<sup>-1</sup>).<sup>33</sup> This suggests that **MNAH** may exhibit a good radical scavenging activity in the physiological environment that warrants further investigation.

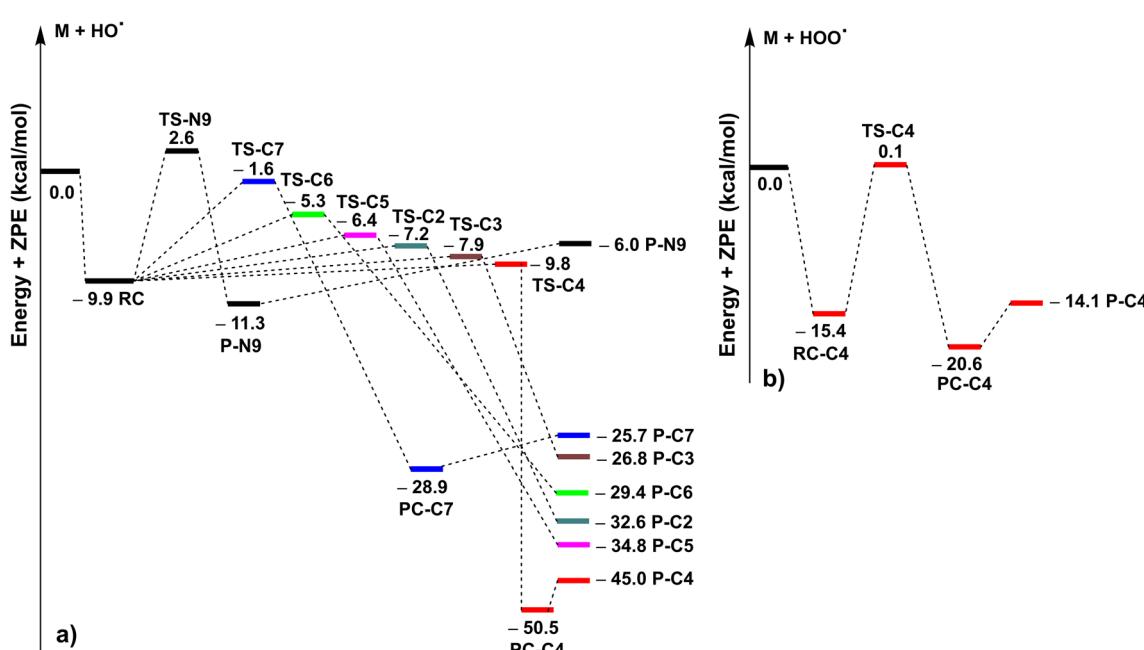


Fig. 2 The PES of the **MNAH** + HO<sup>·</sup> (a)/HOO<sup>·</sup> (b) reactions at the spontaneous reactions (RC: pre-complex; TS: transition state; PC: post-complex; P: product).



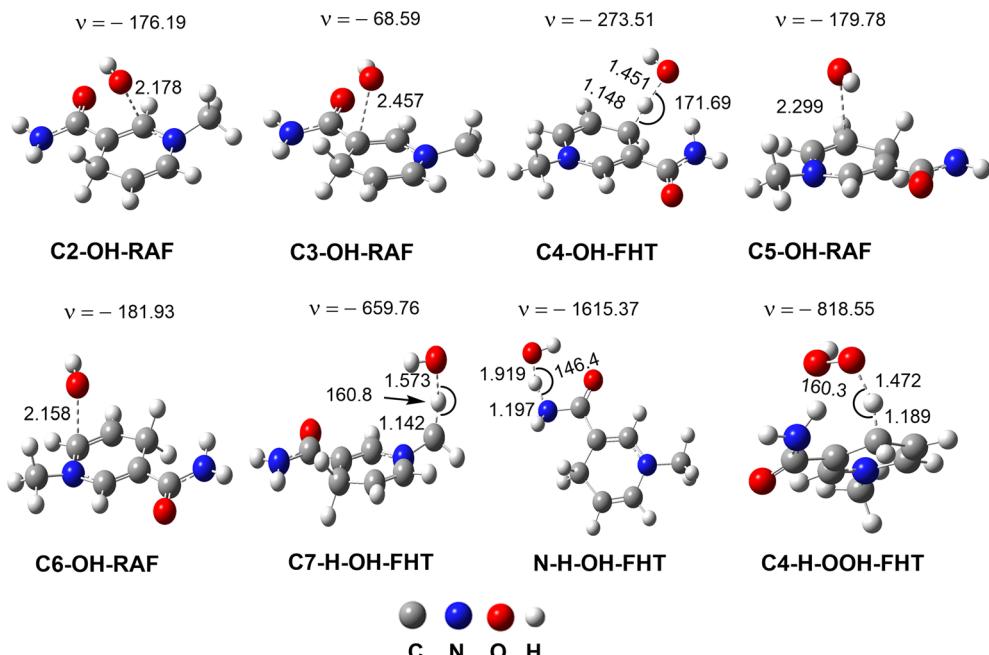


Fig. 3 The optimized transition states of the RAF and FHT mechanisms in the **MNAH** +  $\text{HO}^\bullet/\text{HOO}^\bullet$  reactions ( $\nu$  in  $\text{cm}^{-1}$ , bond length in  $\text{\AA}$ ).

### 3.2. The $\text{HO}^\bullet/\text{HOO}^\bullet$ scavenging activity of MNAH in the physiological environments

**3.2.1. Acid-base equilibrium of MNAH in water.** Sequential proton loss electron transfer (SPLET) is the principal antioxidant mechanism that controls the radical scavenging efficacy of nitrogenous substances in aqueous solutions.<sup>43</sup> This is mainly attributable to the spontaneous deprotonation, which removes the potential barrier of the initial phase. Therefore, this section evaluated the deprotonation equilibria of **MNAH**. The  $\text{p}K_a$  value was determined using a literature method,<sup>44</sup> as illustrated in Fig. 4. It was determined that the  $\text{p}K_a$  value of **MNAH** in an aqueous solution was  $-1.45$  (N1-H). **MNAH** molecule is exclusively present in a neutral state (**MNAH**, 100%) in the physiological aqueous solution. Thus, the neutral state was assessed in the  $\text{HO}^\bullet/\text{HOO}^\bullet$  scavenging activity of **MNAH** in the physiological environments (water and pentyl ethanoate).

**3.2.2. Kinetic study.** The  $\text{HO}^\bullet/\text{HOO}^\bullet$  antiradical activity of **MNAH** in physiological media was calculated using the QM-ORSA protocol.<sup>17,19</sup> The overall rate constants of the  $\text{HO}^\bullet/\text{HOO}^\bullet + \text{MNAH}$  reaction were calculated using eqn (2)–(5). The findings are presented in Table 3.

In pentyl ethanoate:

$$k_{\text{overall}}(\text{HO}^\bullet) = \Sigma k_{\text{app}}(\text{RAF}) + \Sigma k_{\text{app}}(\text{FHT}) \quad (2)$$

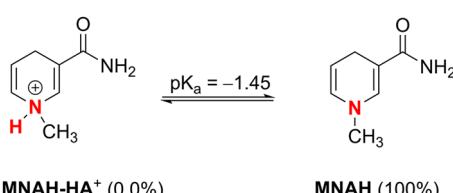


Fig. 4 Dissociation equilibria of **MNAH** at  $\text{pH} = 7.4$ .

$$k_{\text{overall}}(\text{HOO}^\bullet) = k_{\text{app}}(\text{FHT}(\text{C4-H})) \quad (3)$$

In water:

$$k_{\text{overall}}(\text{HO}^\bullet) = k_{\text{f}}(\text{SET}) + \Sigma k_{\text{f}}(\text{RAF}) + \Sigma k_{\text{f}}(\text{FHT}) \quad (4)$$

$$k_{\text{overall}}(\text{HOO}^\bullet) = k_{\text{f}}(\text{FHT}) + k_{\text{f}}(\text{SET}) \quad (5)$$

The  $k_{\text{overall}}$  values for the  $\text{MNAH} + \text{HO}^\bullet$  reaction in the nonpolar and aqueous environments were  $1.69 \times 10^{10}$  and  $2.77 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ , respectively. The  $k_{\text{overall}}$  of the  $\text{MNAH} + \text{HOO}^\bullet$  reaction is slower compared to the hydroxyl radical, with values of  $2.00 \times 10^4$  and  $2.44 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  in the pentyl ethanoate and water solvents, respectively. It is important to notice that the  $\text{p}K_a$  value of the  $\text{HOO}^\bullet$  radical is 4.88. Consequently, the molar fraction of  $\text{HOO}^\bullet$  is 0.137 at  $\text{pH} = 5.6$ , resulting in a  $k_{\text{overall}}(\text{MNAH} + \text{HOO}^\bullet)$  of  $3.84 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ . This is in excellent agreement with the experimental data ( $k_{\text{exp}}(\text{NADH} + \text{HOO}^\bullet) = (1.8 \pm 0.2) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ).<sup>45</sup> It was found that the  $\text{HO}^\bullet$  scavenging activity of **MNAH** was defined by the FHT of C4-H and C7-H positions (31.9%) and RAF (68.0%) reactions in the nonpolar environment. Conversely, the H-abstraction of N9-H bond did not contribute to the  $\text{MNAH} + \text{HO}^\bullet$  reaction. On the other hand, the H-abstraction of the C4-H bond dominated the activity against  $\text{HOO}^\bullet$  (100%).

The  $\text{HO}^\bullet$  antiradical activity in the polar environment is a combination of all analyzed mechanisms (FHT (32.9%), SET (26.7%) and RAF (40.4%)). Formal hydrogen transfer was the driving force behind the activity against the  $\text{HOO}^\bullet$  radical, where the SET reaction contributed only 1.8% of the overall rate constant. Based on the findings, the FHT and RAF reactions with  $\text{HO}^\bullet$  radicals in the aqueous physiological environment were barrierless ( $\Delta G^\ddagger \approx 0 \text{ kcal mol}^{-1}$ ). Consequently, the  $k_{\text{app}}$  values of these processes were diffusion-limited (cannot exceed



**Table 3** The computed  $\Delta G^\#$  (in kcal mol<sup>-1</sup>), branching ratios ( $\Gamma$ %), and  $k_{app}$ ,  $k_f$ ,  $k_{overall}$  (M<sup>-1</sup> s<sup>-1</sup>) of the reaction between **MNAH** and HO<sup>·</sup>/HOO<sup>·</sup> in the physiological environment

Radicals	Mechanism	Pentyl ethanoate				Water				
		$\Delta G^\#$	$\kappa$	$k_{app}$	$\Gamma$	$\Delta G^\#$	$\kappa$	$k_{app}$	$k_f$	$\Gamma$
HO <sup>·</sup>	SET					2.5	4.6 <sup>a</sup>	$7.40 \times 10^9$	$7.40 \times 10^9$	26.7
	FHT	C4-H	~0	1.0	$3.20 \times 10^9$	18.9	~0	1.0	$3.10 \times 10^9$	$3.10 \times 10^9$
		C7-H	4.4	1.8	$2.20 \times 10^9$	13.0	~0	1.0	$3.10 \times 10^9$	$3.10 \times 10^9$
		N9-H	12.0	37.6	$3.80 \times 10^5$	0.0	~0	1.0	$2.90 \times 10^9$	$2.90 \times 10^9$
	RAF	C2	1.7	1.0	$2.60 \times 10^9$	15.4	~0	1.0	$2.60 \times 10^9$	2.60 × 10 <sup>9</sup>
		C3	1.5	1.0	$3.20 \times 10^9$	18.9	~0	1.0	$3.00 \times 10^9$	3.00 × 10 <sup>9</sup>
		C5	1.0	1.0	$3.10 \times 10^9$	18.3	~0	1.0	$3.00 \times 10^9$	3.00 × 10 <sup>9</sup>
		C6	2.0	1.0	$2.60 \times 10^9$	15.4	~0	1.0	$2.60 \times 10^9$	2.60 × 10 <sup>9</sup>
		$k_{overall}$			<b><math>1.69 \times 10^{10}</math></b>					<b><math>2.77 \times 10^{10}</math></b>
HOO <sup>·</sup>	SET					11.1	16.6 <sup>a</sup>	$4.30 \times 10^4$	$4.30 \times 10^4$	1.8
	FHT	C4-H	11.9	1.6	$2.00 \times 10^4$		9.3	2.4	$2.40 \times 10^6$	$2.40 \times 10^6$
$k_{overall}$					<b><math>2.00 \times 10^4</math></b>					<b><math>2.44 \times 10^6</math></b>

<sup>a</sup> The nuclear reorganization energy ( $\lambda$ , in kcal mol<sup>-1</sup>).

diffusion rates  $k_D$ ) and accounted for approximately 73.3% of the overall rate constant.

According to the results the **MNAH** + HO<sup>·</sup> reaction is practically diffusion-limited under all conditions, including the gas phase. On the other hand, the activity against HOO<sup>·</sup> was more nuanced. **MNAH** exhibits a higher HOO<sup>·</sup> antiradical activity in the lipid medium than *trans*-resveratrol (~1.5 times,  $k = 1.31 \times 10^4$  M<sup>-1</sup> s<sup>-1</sup>)<sup>46</sup> and ascorbic acid (~3.5 times,  $k = 5.71 \times 10^3$  M<sup>-1</sup> s<sup>-1</sup>)<sup>17</sup>, but it is inferior to Trolox (~5.0 times,  $k = 1.00 \times 10^5$  M<sup>-1</sup> s<sup>-1</sup>).<sup>33</sup> In the polar medium **MNAH** exhibits a higher activity than Trolox (~18.5 times,  $k = 1.30 \times 10^5$  M<sup>-1</sup> s<sup>-1</sup>),<sup>33</sup> but it is weaker than ascorbic acid and *trans*-resveratrol. The markedly different activity against the two radicals is arguably the result of the high reactivity of HO<sup>·</sup> and not the exceptional specific activity of **MNAH** in targeting and eliminating HO<sup>·</sup>. Thus our results underscore the importance of comparing antioxidant activity against the less reactive free radicals that have longer lifetimes under physiological conditions. Nevertheless, our results suggest that **MNAH** is an efficient radical scavenger in key physiological environments.

## 4. Conclusions

The radical scavenging activity of **MNAH** against HO<sup>·</sup> and HOO<sup>·</sup> was assessed through density functional theory calculations. In the lipid and water media, the  $k_{overall}$  values of the HO<sup>·</sup> + **MNAH** reaction were  $1.69 \times 10^{10}$  and  $2.77 \times 10^{10}$  M<sup>-1</sup> s<sup>-1</sup>, respectively. The HOO<sup>·</sup> radical scavenging activity was measured at  $2.00 \times 10^4$  and  $2.44 \times 10^6$  M<sup>-1</sup> s<sup>-1</sup>. In water, at pH = 5.6, the calculated rate constant ( $k_{overall}(\text{MNAH} + \text{HOO}^\cdot) = 3.84 \times 10^5$  M<sup>-1</sup> s<sup>-1</sup>) is in good agreement with the experimental data ( $k_{exp}(\text{NADH} + \text{HOO}^\cdot) = (1.8 \pm 0.2) \times 10^5$  M<sup>-1</sup> s<sup>-1</sup>) and could verify the accuracy of the computing method. In both nonpolar and polar media, the HOO<sup>·</sup> antiradical activity of **MNAH** was defined by the H<sup>·</sup> abstraction of the C4-H bond, whereas the HO<sup>·</sup> antiradical activity was determined by a combination of the SET (in polar media), RAF, and FHT reactions. The hydroperoxyl radical

scavenging activity of **MNAH** is greater than that of *trans*-resveratrol and ascorbic acid in the lipid medium, and Trolox in the aqueous physiological environment. The results have verified the potent radical scavenger role of **MNAH** in physiological environments, also highlighting that HOO<sup>·</sup> is a better model for comparing antiradical activity than the highly reactive HO<sup>·</sup>.

## Data availability

The data supporting this article have been included as part of the ESI.†

## Conflicts of interest

There are no conflicts to declare.

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