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From *N*-(dinitrophenyl) amino acids to benzimidazole *N*-oxides. Synthesis, kinetics and mechanism

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ABSTRACT: Several benzimidazole *N*-oxide derivatives were synthesized in very good yields by heating under reflux the corresponding *N*-(2,4- or 2,6-dinitrophenyl) amino acid derivative with NaOH in 60% 1,4-dioxane–H_2O. The *N*-oxides obtained from glycine and α- and β-alanine derivatives lost the carboxylic group. The observed rate constant for the reaction of *N*-(2,4-dinitrophenyl) glycine (**2a**) in 10% 1,4-dioxane–H_2O to give 5-nitro-1*H*-benzimidazole-3-oxide (**4a**) is first order on [NaOH]; the second-order rate constant is $k_N = 1.7 \times 10^{-3}\,\text{M}^{-1}\,\text{s}^{-1}$. The mechanism proposed includes the formation of an *N*-alkylidene 2-nitrosoaniline-type intermediate as the rate-determining step.

KEYWORDS: benzimidazole *N*-oxides; synthesis; kinetics; mechanism

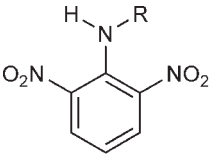
INTRODUCTION

Compounds derived from benzimidazole and benzimidazole *N*-oxide exhibit a wide range of biological properties, including insecticide and herbicide activity,[1] antiviral, antifungal and antibacterial activity,[2] antitumoral and antimicrobial activity,[3,4] anti-ulcerative, antihypertensive and antihistaminic activity,[5] antihelmintic activity in veterinarian medicine[5] and also anti HIV-1 activity.[6]

Benzimidazole *N*-oxides are not synthesized by direct oxidation of benzimidazoles, thus it is important to develop versatile synthethic methods to obtain a variety of benzimidazole *N*-oxide derivatives. Thermal and photochemical cyclizations of 2-nitroaniline derivatives having electron-withdrawing substituents α to the amino group are known to give benzimidazole *N*-oxides,[7–11] but the reaction failed to obtain 7-nitro-substituted compounds.[12]

We have reported previously on the synthesis of 7-substituted benzimidazole *N*-oxides by cyclization of several *N*-alkyl-2-nitroaniline derivatives.[13,14] The cyclization reaction requires two *ortho* substituents to the amino group and the presence of an NH proton. In some cases, substitution of the amino group leading to phenol competes with the cyclization reaction.[13,14] The yield of the *N*-oxide depends on the substituents on the aromatic ring and the concentration of the base.[13,14]

To determine the scope of the method with regard to the effect of an electron-withdrawing substituent on the alkylic chain, we carried out a study on the reaction of substrates **1a**–**1d**, **2a** and **2b** and the results are reported here.



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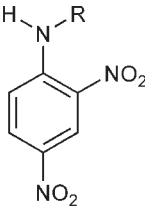

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a: R = -CH_2COOH
b: R = -$CH(CH_3)$COOH
c: R = -$(CH_2)_2$COOH
d: R = -$(CH_2)_3$COOH
e: R = -$(CH_2)_3CH_3$

RESULTS AND DISCUSSION

Synthesis

When compounds **1a**–**1d**, **2a** and **2b** were heated at reflux in 60% (v/v) 1,4-dioxane–H_2O with 0.2 M NaOH, they afforded the corresponding benzimidazole *N*-oxides

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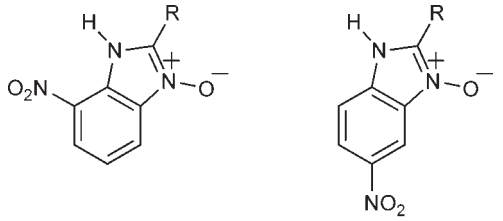
Table 1. Synthesis of benzimidazole *N*-oxides **3** and **4**[a]

Amino acid	[NaOH] (M)	Time (min)	Product	Yield[b] (%)	pK_{a1}	pK_{a2}	Product	Yield[b] (%)
1a	0.20	20	**3a**	70	2.20 ± 0.03	6.22 ± 0.01		
1b	0.20	15	**3b**	90	2.41 ± 0.04	6.83 ± 0.04	**5a**	<5[c]
1c	0.20	10	**3b**	79			**5a**	10
1d	0.20	20	**3c**	83	2.40 ± 0.03	7.18 ± 0.02	**5a**	7
2a	0.20	10	**4a**	71	2.23 ± 0.05[d]	5.90 ± 0.01[e]		
2a[f]	0.20	60	**4a**	84				
2b	0.20	120	**4b**	88	2.53 ± 0.02	6.56 ± 0.02	**5b**	13.5[c]
2c	0.20	300					**5b**	94
2c	0.01	2400					**5b**	6[c,g]

[a] In 60% 1,4-dioxane–H_2O at reflux.
[b] Percentage yield of isolated product.
[c] Quantified by ^1H NMR.
[d] Literature value of 2.2 (Ref. 9).
[e] Literature value of 5.9 (Ref. 9).
[f] Temperature = 25 °C.
[g] Unreacted **2c** was also found.

3a–**3c**, **4a** and **4b** in very good yields. The results are summarized in Table 1. It is important to notice that **1d**, which has the carboxylic acid in γ position with respect to the amino group, gave the *N*-oxide derivative **3c**, which retained the carboxylic group, whereas compounds **1a**–**1c**, **2a** and **2b**, which have the carboxylic group in α or β position, gave the cyclization product with elimination of the COOH group.

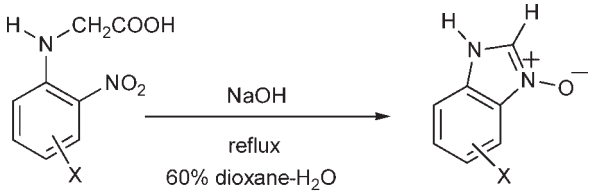


3 **4**

a : R = H
b : R = -CH_3
c : R = -$(CH_2)_2$COOH

The two pK_a values of the benzimidazole *N*-oxides **3a**–**3d**, **4a** and **4b** were determined by spectrophotometric titration and are summarized in Table 1; they are similar to those reported before for **4a**[9] and other related *N*-oxides.[13,14] These pK_a values correspond to deprotonation of $^+NH_2$ in conjugate acid of benzimidazole *N*-oxide (pK_{a1}) and deprotonation of NH in benzimidazole *N*-oxide (pK_{a2}).[14,15]

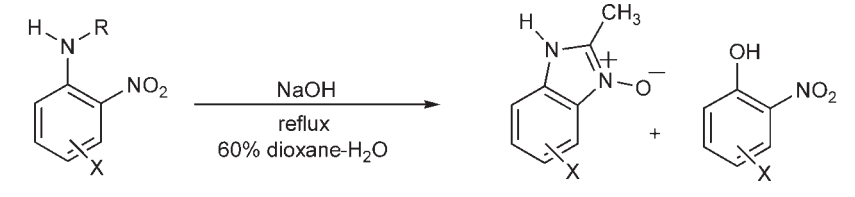
The cyclization reaction leading to the formation of *N*-oxide **3a** or **4a** was the only reaction observed with glycine derivatives **1a** and **2a** (Scheme 1). Substitution of the amino group to give 2,6-dinitrophenol (**5a**) or 2,4-dinitrophenol (**5b**) competes with cyclization in the reaction of compounds **1b**–**1d** and **2b** (Schemes 2 and 3). Formation of the substitution product and cyclization product was also reported for the reaction of *N*-*n*-butyl-2,4,6-trinitroaniline (**6**),[13] *N*-*n*-butyl-2,6-dinitro-4-trifluoromethylaniline (**7a**) and *N*-*n*-propyl-2,6-dinitro-4-trifluoromethylaniline (**7b**)[14] in 60% 1,4-dioxane–H_2O at reflux. On the other hand, *N*-(2,4-dinitrophenyl)



1a X = 6-nitro
2a X = 4-nitro
9 X = 4,6-dinitro

3a X = 7-nitro
4a X = 5-nitro
10 X = 5,7-dinitro

Scheme 1

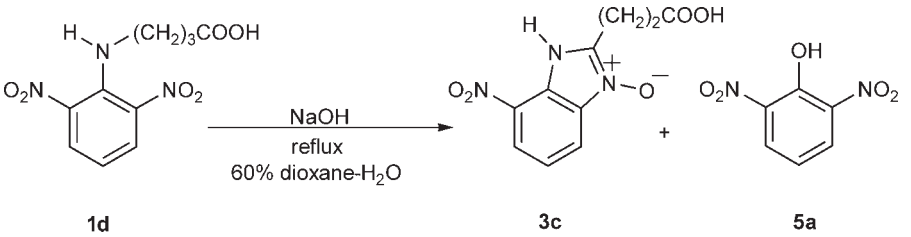


1b X = 6-nitro, R = -$CH(CH_3)$COOH
1c X = 6-nitro, R = -CH_2CH_2COOH
2b X = 4-nitro, R = -$CH(CH_3)$COOH

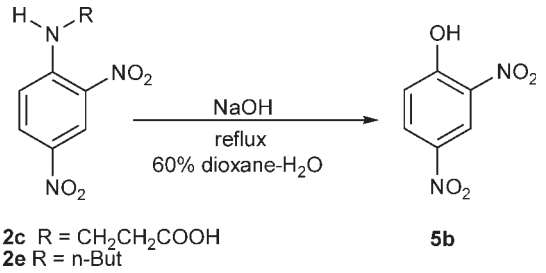
3b X = 7-nitro
4b X = 5-nitro

5a X = 6-nitro
5b X = 4-nitro

Scheme 2



Scheme 3



Scheme 4

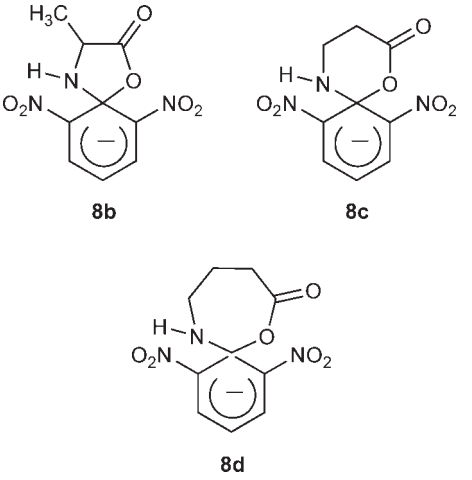
β-alanine (**2c**) gave only 2,4-dinitrophenol (**5b**) even with 0.01 M NaOH (Scheme 4). In previously studied reactions[16] it was found that the relative yield of *N*-oxide/phenol increases as the HO^- concentration decreases.

Effect of COOH group

The presence of a carboxylic group in the alkylic substituent on the amino nitrogen has an important effect on the reaction. With the 2,4-dinitro derivatives, although *N*-*n*-butyl-2,4-dinitroaniline (**2e**) gave only 2,4-dinitrophenol (**5b**) (Scheme 4),[13,16] **2a** gave only *N*-oxide **4a** (Scheme 1) and **2b** gave *N*-oxide **4b** as the main product together with a minor amount of phenol **5b** (Scheme 2). In order to obtain the cyclization product, proton abstraction from the CH_2 group is proposed in the generally accepted mechanism for these reactions.[11,12] The presence of an electron-withdrawing substituent close to the reaction center facilitates this process. As the distance of the carboxylic group from the reaction center increases, the stabilization of the negative charge diminishes and only the substitution reaction takes place, as in **2c** (Scheme 4).

On the other hand, although the *N*-alkyl-2,6-dinitro derivatives, like **1e** in 60% dioxane–H_2O, gave only the *N*-oxides irrespective of the length of the alkylic chain,[13,14,16] in the case of compounds **1b**–**1d** the substitution reaction competes with *N*-oxide formation to give phenol **5a** (Schemes 2 and 3) in yields of 5–10%.

The fact that a substitution reaction was found with compounds **1b**–**1d** indicates that a parallel pathway for the substitution of the amino group may operate when a carboxylic group is present in the alkylic chain. One possibility is the intramolecular reaction shown in **8b**–**8d**. The formation of spiro complexes is a process thermodynamically and kinetically favored over that of analogous complexes arising from intermolecular processes.[17,18] There are also reports on Smiles rearrangements where N displaces O and others where O displaces N.[19] The presence of the carboxylic group in substrates **1b**–**1d** enables the formation of the spiro Meisenheimer complexes **8b**–**8d**; the phenol could be obtained from these complexes via C—N bond-breaking and hydrolysis of the intermediate ester.



The five-membered complex **8b** is expected to be more stable than the others[18] and to display a greater neighboring group effect.[20] It is well known that the rate of cyclization increases when geminal dialkyl groups are located in the alkyl chain, in-between the neighbouring group and the reaction center.[20] Thus the absence of an electron-donating CH_3 group in **1a** may favor cyclization to give *N*-oxide over substitution via spiro complex formation.

Kinetics of *N*-oxide formation

We undertook a kinetic study of the reaction of **2a** in 10% dioxane–H_2O at 25 °C with 0.01–1.00 M NaOH, which leads to the formation of *N*-oxide **4a**. The formation of **4a** was first order on [NaOH] with a second-order rate constant $k_N = 1.57 \times 10^{-3}\ M^{-1}\ s^{-1}$ (Table 2 and Fig. 1). This rate constant can be compared with the value of the observed rate constant for the reaction of **2e** in 20%

Table 2. Rate constants for the reaction of *N*-(2,4-dinitrophenyl) glycine **(2a)** with NaOH at 25 °C in 10% 1,4-dioxane–H2O[a]

[NaOH] (M)	$k_{obs} \times 10^4$ (s^{-1})
0.204	2.95 ± 0.02
0.407	5.88 ± 0.03
0.610	8.47 ± 0.05
0.814	12.9 ± 0.02
1.001	16.3 ± 0.2

[a] Ionic strength $I = 1$ M (NaCl when needed); $[\mathbf{2a}]_0 = 6.93 \times 10^{-5}$M.

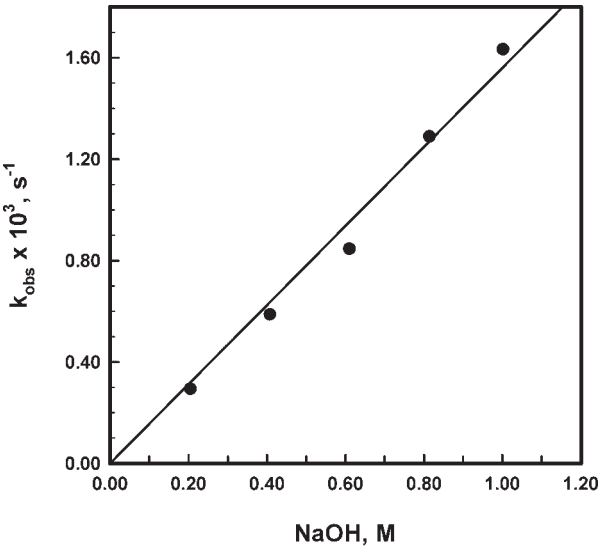


Figure 1. Plot of k_{obs} vs. [NaOH] for the formation of **4a** from **2a** at 25 °C in 10% 1,4-dioxane–H_2O. Ionic strength $I = 1$ M (NaCl); $[\mathbf{2a}]_0 = 7.00 \times 10^{-5}$ M

1,4-dioxane–H_2O, which gave only the substitution product **5b**, and for 1 M NaOH it is $k_P = 8 \times 10^{-7}\ s^{-1}$;[16] substrate **2a** is about 2000 times more reactive than **2e**. These results are consistent with the proposed mechanism for the cyclization reaction that requires deprotonation of the CH_2 α to the amino group,[11,12] because it is well known that a carboxylate group decreases the acidity of an α carbon by about 25 pK_a units.[21]

Kinetics of phenol formation

We also performed a kinetic study of the reaction of **2c** to give phenol **5b** in 20% 1,4-dioxane–H_2O at 25 °C with 1 M NaOH. The observed rate constant for the formation of **5b** is $k_P = 2.2 \times 10^{-6}\ s^{-1}$, which is 2.75 times higher than that for the formation of the same phenol from **2e**.[16] These results support the assumption that, along with the expected aryl nucleophilic substitution pathway proposed for the formation of **5b** from **1e**,[16] a parallel reaction pathway is available when there is a COOH group in the alkyl chain, as suggested above for the reaction of compounds **1b**–**1d**.

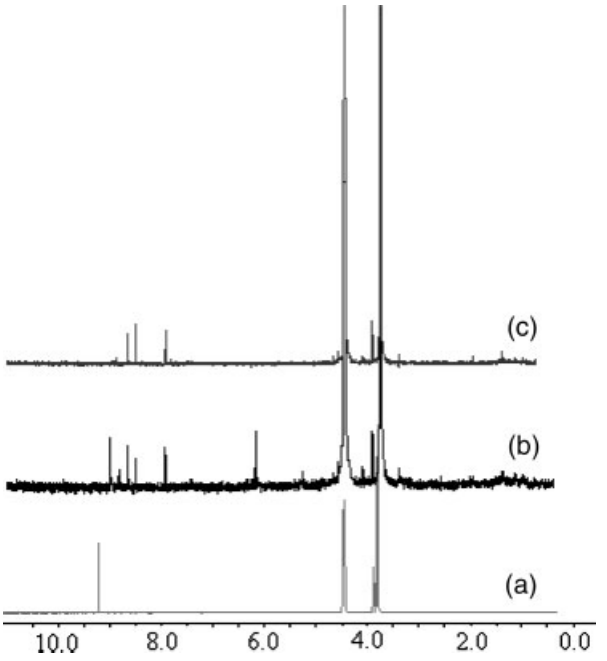


Figure 2. 200 MHz ^{1}H NMR spectra of: (a) **9** recorded in 60% dioxane–d_8-D_2O at room temperature, (b) 15 min after addition of 0.2 M NaOD (c) at infinity; $[\mathbf{9}]_0 = 3.08 \times 10^{-2}$ M

NMR studies

The reaction of *N*-(2,4,6-trinitrophenyl) glycine (**9**) was followed by ^{1}H NMR in 60% dioxane–d_8-D_2O at room temperature with 0.2 M NaOD; only the formation of 5,7-dinitro-1*H*-benzimidazole-3-oxide (**10**) was detected (Scheme 1). The NMR spectrum of **9** has a single peak in the aromatic region at $\delta = 9.16$ (Fig. 2a). In the spectrum taken 15 min after the addition of NaOD (Fig. 2b), three signals attributed to *N*-oxide **10** by comparison with the spectrum taken at infinite (Fig. 2c) appeared at 8.67 (d), 8.52 (d) and 7.94 (d), along with signals at $\delta = 9.00$ (d), 8.97, 8.85 (d), 6.19 (d), 6.15 (d) and 5.26 which may be attributed to the presence of scomplexes formed by addition of nucleophiles to unsubstituted ring positions[17] and the dianion of **9**.[22] The addition of nucleophiles to unsubstituted ring positions of trinitrobenzene derivatives is a well-documented reaction.[17,22,23] Intermediates of this type are formed in the hydrolysis reaction of 1-amino-2,4-dinitro[24] and 1-amino-2,4,6-trinitrobenzenes[25] with pyrrolidine, piperidine or morpholine as the amino group.

We followed the reaction of **2a** by ^{1}H NMR with NaOD in 10% 1,4-dioxane–d_8-D_2O at room temperature. When the initial concentration of **2a** was 4.8×10^{-2} M and NaOD was 1 M the spectrum of the solution at the end of the reaction indicates that an unknown product was formed along with *N*-oxide **4a**. This was confirmed by the

UV–visible spectrum. When the reaction was conducted with 0.2 M NaOD the only product observed by ^{1}H NMR and UV–visible spectrophotometry was **4a**, as was found in the kinetic study with $[\mathbf{2a}]_0 = 7.00 \times 10^{-5}$ M. These results may indicate that the formation of the unknown product depends on substrate concentration as well as on [NaOH]. Smith and co-workers reported previously the formation of azoxybenzene derivatives in the reaction of some substituted *o*-nitroanilines in basic media.[12,26] No further attempts to identify the product were made.

Mechanism of *N*-oxide formation

Considering the results of the kinetic study of the reaction of **2a** in 10% 1,4-dioxane–H_2O, and the mechanism previously proposed,[16] we suggest the mechanism shown in Scheme 5 for the formation of *N*-oxides **4a** and **4b**.

The first step of the mechanism is the ionization of substrate. The absence of the formation of *N*-oxide in the reactions of *N*-butyl-*N*-methyl-2,6-dinitroaniline in 10% and 60% 1,4-dioxane–H_2O,[16] *N*,*N*-di-*n*-propyl-2,6-dinitro-4-trifluoromethylaniline in 60% 1,4-dioxane–H_2O[14] and *N*,*N*-diethyl-2,4-dinitro-6-trifluoromethylaniline in basic ethanol[11] is a good indication of the need to generate a negative charge over the nitrogen of the amino group in order to give the cyclization product. However, it may be that for compounds **2a** and **2b**, which bear a carboxylate group in the lateral chain, this is not true. A reviewer suggested that an alternative mechanism could involve ionization of the α-carbon of compounds **2a** and **2b** as the first step in the mechanism and then cyclization through intramolecular attack of this carbanion on the nitrogen of the *o*-nitro group. However, we think that the mechanism shown in Scheme 5 is the one that better represents the complete set of data.

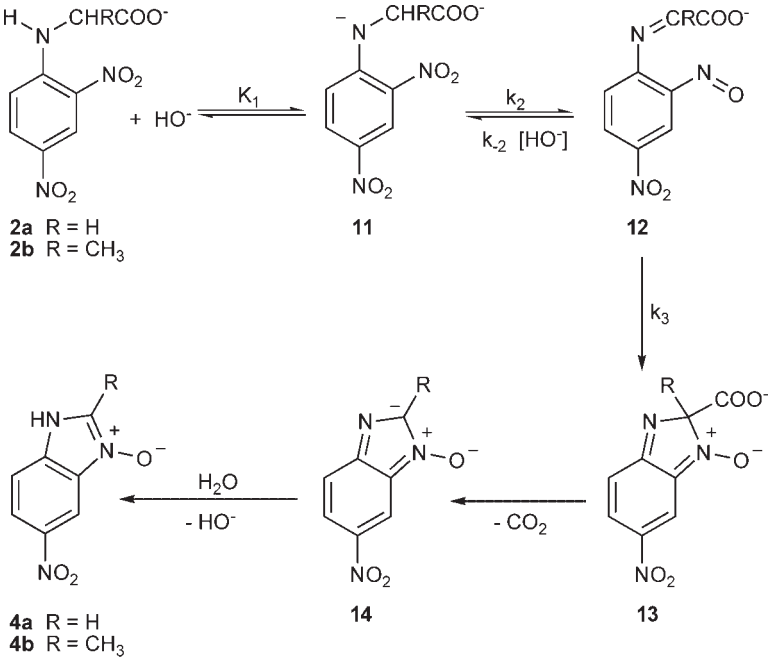
We suggest the formation of intermediate **12** in the reaction pathway to benzimidazole *N*-oxide on the following basis: the reduction of nitro to nitroso groups of benzene derivatives in basic solution is a well-documented reaction;[27] the reaction of formaldehyde with *N*-methyl-4-nitro-2-nitrosoaniline in 9 N sulfuric acid gave 1-methyl-5-nitro-1*H*-benzimidazole-3-oxide;[7] 2-aryl-5-nitrobenzimidazole-3-oxides were formed by reaction of 4-nitro-2-nitrosoaniline and aromatic aldehydes in acid media;[28] reductive cyclization of *N*-benzylidene-2-nitroanilines prepared from *o*-nitroaniline and benzaldehydes is described as a synthethic route to substituted benzimidazoles;[5] and in the reactions of some ester and nitrile derivatives of *N*-(*o*-nitrophenyl) glycine with and without *N*-alkyl substituents, structures of the type of **12** were proposed as intermediates in benzimidazole *N*-oxide formation.[12]

Assuming that the steps after cyclization are faster than the others and considering **12** as a steady-state intermediate, k_{obs} for the mechanism of Scheme 5 is given by

$$k_{obs} = \frac{K_1 k_2 k_3 [HO^-]}{k_{-2}[HO^-] + k_3} \quad (1)$$

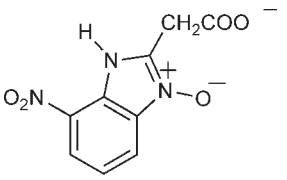
If $k >>_3 k_{-2}[HO^-]$, Eqn (1) is reduced to Eqn (2), which has the same mathematical form of the experimentally observed expression for the reaction of **2a** in 10% 1,4-dioxane–H_2O. This suggests that the rate-determining step for the reaction is the formation of intermediate **12**

$$k_{obs} = K_1 k_2 [HO^-] \quad (2)$$



Scheme 5

It was observed that compounds with a COOH group α or β to NH (**1a**–**1c**, **2a** and **2b**) lost the carboxylate to give the *N*-oxides (**3a**, **3b**, **4a** and **4b**) whereas that with COOH γ to NH (**1d**) retained the COOH (**3c**). This could be explained by the mechanism proposed in Scheme 5; loss of CO_2 is posible in those compounds in which the carboxy group is adjacent to an *N*-oxide system[8] where the negative charge generated could be stabilized by resonance. In the case of compound **1c**, which has the COOH group β to NH, we suggest that CO_2 elimination could occur after the formation of compound **15**, leading to the formation of a stable anion.



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Conclusions

The method described here allowed the synthesis of 7-nitro-1*H*-benzimidazole *N*-oxide derivatives that were not formed by previously described methods.[12] Besides, the presence of an electron-withdrawing group in the alkylic chain α to the NH that facilitates proton abstraction from the CH_2 group enables cyclization in the absence of a second substituent in the aromatic ring *ortho* to the amino group that was not possible in previously studied *N*-alkyl derivatives.[13] The results presented give support to the mechanism proposed before for *N*-oxide formation.[16]

EXPERIMENTAL

Materials

1,4-Dioxane was purified as described previously.[29] Water purified in a Millipore Milli-Q apparatus was used throughout. All of the inorganic reagents were of analytical-reagent grade and were used without further purification. The NaOH concentrations are expressed in terms of total solvent volume (1,4-dioxane–H_2O). Melting points were determined on an electrothermal apparatus. Proton (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on a Bruker ACE 200 spectrometer. Chemical shifts in $CDCl_3 + (CD_3)_2SO$ are reported as δ values downfield from $(CH_3)_4Si$ and those in $(CD_3)_2SO$ are referenced to the solvent signal. The mass spectra (EI) and HRMS (EI) were done at Unidad de Espectrometría de Masas, Universidad de Santiago de Compostela, Spain. Infrared (IR) spectra in KBr were obtained on a Nicolet 5SXC FT-IR spectrometer and UV–visible spectra and kinetic measurements were recorded on a Shimadzu UV 2101 spectrophotometer. Thin-layer chromatography (TLC) was carried out using silica gel 60 F_{254} (Macherey-Nagel) and spots were visualized by UV. Silica gel 60 mesh size (0.063–0.200 nm, Merck) was used for column chromatography.

Determination of pK_a

The pK_a values of compounds **3a**–**3d**, **4a** and **4b** were determined by spectrophotometric titration in 10% (v/v) 1,4-dioxane–H_2O at 25 °C. Solutions for the various pH ranges were prepared using HCl (pH 0.7–3.0), acetic acid–sodium hydroxide (pH 3.5–5.5) and Na_2HPO_4–KH_2PO_4 (pH 5.8–8.0).

Synthesis of *N*-dinitrophenyl amino acids

Compounds **1a**–**1d**, **2a**–**2c** and **9** were synthesized and isolated by the method previously described.[8]

*N-(2,6-Dinitrophenyl) glycine (**1a**).* Yellow solid, m.p. 172–174 °C (lit.[12] 173–175 °C), yield 60%. ^1H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 3.65 (d, 2H, $J = 4.38$ Hz, CH_2), 6.76 (t, 1H, $J = 8.4$ Hz, Ar), 8.14 (d, 2H, $J = 8.4$ Hz, Ar) and 8.99. ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 46.81, 114.87, 132.14, 138.15, 139.10 and 170.50. IR (KBr, ν_{max} cm^{-1}): 1723.9 (s). MS *m/z*: 241.8 ($[M+1]^+$, 2.2), 240.75 (M^+, 16.25), 196 (100), 168, 138, 105, 91 and 75. HRMS (EI): *m/z* calcd. for $C_8H_7N_3O_6$: 241.0335; found: 241.0338.

*N-(2,6-Dinitrophenyl) α-alanine (**1b**).* Yellow solid, m.p. 137–139 °C, yield 70%. ^1H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 1.46 (d, 3H, $J = 6.94$ Hz, CH_3), 4.02 (m, 1H, $J = 7.3$ Hz, $J = 6.94$ Hz, CH), 6.86 (t, 1H, $J = 7.68$ Hz, $J = 8.76$ Hz, Ar), 8.18 (d, 2H, $J = 8.04$ Hz, Ar) and 8.66 (d, 1H, $J = 7.68$ Hz, NH). ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 18.70, 53.42, 115.65, 131.90, 138.37, 139.39 and 173.89. IR (KBr, ν_{max} cm^{-1}): 1734.1 (s), 1713.7 (s). MS *m/z*: 255.0 (M^+, 14.18), 209.9 (100), 163.9, 134.0, 117.0 and 106.0. HRMS (EI): *m/z* calcd. for $C_9H_9N_3O_6$: 255.0491; found: 255.0496.

*N-(2,6-Dinitrophenyl) β-alanine (**1c**).* Orange solid, m.p. 137–139 °C, yield 88%. ^1H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 2.65 (t, 2H, $J = 5.84$ Hz, CH_2CH_2), 3.26 (q, 2H, $J = 5.86$ Hz, CH_2), 6.78 (t, 2H, $J = 8.4$ Hz, Ar), 8.17 (d, 2H, $J = 8.4$ Hz, Ar) and 8.57 (s, 1H, NH). ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 34.01, 42.44, 114.35, 132.06, 138.02, 139.02 and 172.95. IR (KBr, ν_{max} cm^{-1}): 1734.1 (s). MS *m/z*: 254.9 (M^+, 57.33), 196.0, 191.9, 175.9 (100), 133.0 and 105.0. HRMS (EI): *m/z* calcd. for $C_9N_9N_3O_6$: 255.0491; found: 255.0487.

*N-(2,6-Dinitrophenyl) aminobutyric acid (**1d**).* Yellow solid, m.p. 174–175 °C, yield 76.6%. ^{1}H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 1.99 (m, 2H, $J =$ 6.94 Hz, CH_2CH_2), 2.37 (t, 2H, $J = 7.12$ Hz, $CH_2CH_2CH_2$), 3.05 (td 2H, $J = 4.74$ Hz, $J = 2.2$ Hz, CH_2), 6.80 (t, 2H, $J = 8.04$ Hz, Ar), 8.18 (d, 2H, $J = 8.04$ Hz, Ar) and 8.30 (s, 1H, NH). ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 24.71, 30.59, 45.46, 113.84, 131.63, 137.29, 139.31 and 173.62. IR (KBr, ν_{max} cm^{-1}): 1708.6 (s). MS *m/z*: 271.2 ([M + 1]$^+$, 3.22), 270.1 (M$^+$, 27.39), 154.0, 87.1 (100) and 85.1. HRMS (CI): *m/z* calcd. for $C_{10}N_{12}N_3O_6$: 270.0726; found: 270.0724.

*N-(2,4-Dinitrophenyl) glycine (**2a**).* Yellow solid, m.p. 189–191 °C, yield 83%. ^{1}H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 4.24 (d, 2H, $J = 5.48$ Hz, CH_2) 6.98 (d, 1H, $J = 9.5$ Hz, Ar), 8.29 (dd, 1H, $J = 2.56$, 6.94 Hz, Ar) and 9.04 (d, 1H, $J = 2.56$ Hz, Ar). ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 43.79, 114.14, 122.66, 129.12, 129.47, 135.03, 146.59 and 168.99. IR (KBr, ν_{max} cm^{-1}): 1718.8 (s). MS *m/z*: 241.75 ([M + 1]$^+$, 4.5), 240.75 (M$^+$, 55.09), 195.9 (100), 168.0, 138.0, 105.0, 91.0 and 75.0. HRMS (EI): *m/z* calcd. for $C_8N_7N_3O_6$: 241.0335; found: 241.0334.

*N-(2,4-Dinitrophenyl) α-alanine (**2b**).* Yellow solid, m.p. 178–180 °C, yield 73%. ^{1}H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 1.66 (d, 3H, $J = 6.94$ Hz, CH_3), 4.37 (m, 1H, CH), 6.87 (d, 1H, $J = 9.88$ Hz, Ar), 8.28 (dd, 2H, $J = 7.3$; 2.2 Hz, Ar), 8.99 (d, 1H, NH) and 9.14 (d, 1H, $J = 2.4$ Hz, Ar). ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 18.24, 51.47, 114.22, 124.30, 130.36, 130.93, 136.40, 147.07 and 173.24. IR (KBr, ν_{max} cm^{-1}): 1708.6 (s). MS *m/z*: 256.9 ([M + 1]$^+$, 0.41), 255.8 (M$^+$, 2.68), 209.9 (100), 164.0, 149.0, 118.0, 91.0 and 75.0. HRMS (EI): *m/z* calcd. for $C_9H_9N_3O_6$: 255.0491; found: 255.0496.

*N-(2,4-Dinitrophenyl) β-alanine (**2c**).* Yellow solid, m.p. 146–148 °C, yield 81%. ^{1}H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 2.74 (t, 2H, CH_2CH_2), 3.74 (q, 2H, $J = 6.22$ Hz, CH_2), 7.00 (d, 1H, $J = 7.04$ Hz, Ar), 8.29 (dd, 2H, $J = 2.56$, 7.3 Hz, Ar), 8.87 (s, 1H, NH) and 9.06 (d, 1H, $J = 2.56$ Hz, Ar). ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 33.01, 38.86, 113.73, 123.92, 130.09, 130.23, 135.67, 147.94 and 172.79. IR (KBr, ν_{max} cm^{-1}): 1723.9 (s). MS *m/z*: 256.9 ([M + 2]$^+$, 0.41), 255.8 ([M + 1]$^+$, 12.68), 254.8 (M$^+$, 100), 195.9, 190.9, 175.9, 149.9, 119.95, 104.0 and 91.95. HRMS (EI): *m/z* calcd. for $C_9H_9N_3O_6$: 255.0491; found: 255.0491.

*N-(2,4,6-Trinitrophenyl) glycine (**9**).* Orange solid. ^{1}H NMR ($CDCl_3 + (CD_3)_2SO$, 200 MHz) δ: 3.63 (d, 2H, $J = 4.02$ Hz, CH_2), 8.9 (s, 2H, Ar) and 9.8 (s, 1H). ^{13}C NMR ($CDCl_3 + (CD_3)_2SO$, 50 MHz) δ: 45.98, 125.86, 132.38, 135.38, 140.77 and 168.50.

Synthesis of benzimidazole *N*-oxides; general procedure

The corresponding *N*-dinitrophenyl amino acid derivative was heated at reflux in 60% (v/v) 1,4-dioxane–H_2O with NaOH at the concentration indicated in Table 1. The reaction was followed by TLC; when no reactant was observed, the heating was stopped; the solution was cooled to room temperature and acidified to pH < 5 with 3.2 M HCl.

*7-Nitro-1H-benzimidazole-3-oxide (**3a**).* A solution prepared by dissolving 105 mg (0.436 mmol) of **1a** in 50 ml of solvent was heated at reflux with NaOH (0.2 M) for 20 min. The reaction mixture was acidified to pH 4 and the solvent was evaporated in a rotavapor. The salts were separated from the product by column chromatography on alumina (Aluminumoxid G type E, Merck) by eluting with acetone and acetone–methanol (90:10). The *N*-oxide was isolated as an orange powder that decomposed at 198 °C.

^{1}H NMR ($(CD_3)_2SO$, 200 MHz) δ: 7.14 (t, 1H, $J =$ 7.8 Hz, Ar), 7.61 (d, 2H, $J = 8.04$ Hz, Ar), 7.89 (d, 1H, $J = 8.04$ Hz, Ar) and 8.22 (s, 1H, Ar). ^{13}C NMR ($(CD_3)_2SO$, 50 MHz) δ: 116.58, 117.52, 119.49, 132.10, 135.33, 137.89 and 142.53. HRMS (FAB) *m/z*: calcd. for $C_7H_4N_3O_3$: 178.0247; found: 178.0254.

*2-Methyl-7-nitro-1H-benzimidazole-3-oxide (**3b**) from **1b**.* A solution prepared by dissolving 199.9 mg (0.784 mmol) of **1b** in 100 ml of solvent was heated at reflux with NaOH (0.2 M) for 15 min. The reaction mixture was acidified to pH 3 and filtrated with an ionic interchange resin (DIAION) with H_2O, MeOH and acetone. The products were treated with acetone, in which the phenol is completely soluble and the *N*-oxide precipitates. The *N*-oxide was isolated by vacuum filtration as a yellow solid that decomposed at 186 °C.

^{1}H NMR ($(CD_3)_2SO$, 200 MHz) δ: 2.58 (s, 3H, CH_3), 7.37 (t, 1H, $J = 8.04$ Hz, Ar), 7.84 (d, 1H, $J = 8.04$ Hz, Ar) and 7.98 (d, 1H, $J = 8.04$ Hz, Ar). ^{13}C NMR ($(CD_3)_2SO$, 50 MHz) δ: 12.16, 115.58, 117.92, 120.75, 131.21, 135.17, 137.44 and 152.26. MS *m/z*: 192.95(M$^+$, 43.16), 150.9 (100), 133.0 and 103.88. HRMS (EI): *m/z* calcd. for $C_8H_7N_3O_3$: 193.0487; found: 193.0493.

*2-Methyl-7-nitro-1H-benzimidazole-3-oxide (**3b**) from **1c**.* A solution prepared by dissolving 204 mg (0.80 mmol) of **1c** in 100 ml of solvent was heated at reflux with NaOH (0.2 M) for 10 min. The reaction mixture was acidified to pH 5 and filtrated with an ionic interchange resin (DIAION) with H_2O, MeOH and acetone. The products were isolated by column chromatography on silica gel by eluting with variable amounts of acetone–*n*-PrOH. The *N*-oxide was a yellow solid that decomposed at 184 °C.

^{1}H NMR ($(CD_3)_2SO$, 200 MHz) δ: 2.58 (s, 3H, CH_3), 7.36 (t, 1H, J = 8.04 Hz, Ar), 7.83 (d, 1H, J = 8.04 Hz, Ar) and 7.98 (d, 1H, J = 8.04 Hz, Ar). ^{13}C NMR ($(CD_3)_2SO$, 50 MHz) δ: 12.14, 115.50, 118.00, 120.92, 131.18, 135.07, 137.50 and 152.21. MS *m/z*: 192.9 (M^+, 31.2), 177.0, 150.95 (100) and 103.05. HRMS (EI): *m/z* calcd. for $C_8H_7N_3O_3$: 193.0487; found: 193.0491.

*2-Ethylcarboxylic-7-nitro-1H-benzimidazole-3-oxide (**3c**).* A solution prepared by dissolving 200 mg (0.743 mmol) of **1d** in 100 ml of solvent was heated at reflux with NaOH (0.2 M) for 20 min. The reaction mixture was acidified to pH 4. The solvent was evaporated untill the products precipitated. The *N*-oxide was a yellow solid that decomposed at 196 °C.

^{1}H NMR ($(CD_3)_2SO$, 200 MHz) δ: 2.85 (t, 2H, J = 7.32 Hz, CH_2), 3.16 (t, 2H, J = 7.32 Hz, CH_2CH_2), 7.41 (t, 1H, J = 7.82 Hz, Ar), 7.94 (dd, 2H, J = 8.0, 17.1 Hz, Ar) and 12.22 (s, 1H, NH). ^{13}C NMR ($(CD_3)_2SO$, 50 MHz) δ: 21.08, 30.19, 115.39, 118.22, 121.40, 130.94, 135.04, 137.81, 154.17 and 173.12. MS *m/z*: 251.9 ($[M+1]^+$, 4.88), 250.85 (M^+, 67.99) 234.8, 205.85, 189.9 (100), 176.9, 142.95, 116.0 and 101.0. HRMS (EI): *m/z* calcd. for $C_{10}H_9N_3O_5$: 251.0542; found: 251.0536.

*5-Nitro-1H-benzimidazole-3-oxide (**4a**).* A solution prepared by dissolving 50 mg (0.207 mmol) of **2a** in 25 ml of solvent was heated at reflux with NaOH (0.2 M) for 10 min. Only the *N*-oxide was formed; it precipitated upon acidification of the solution with HCl and was separated by vacuum filtration as a yellow solid that decomposed at 215 °C (lit.[9] 269 °C).

^{1}H NMR ($(CD_3)_2SO$, 200 MHz) δ: 7.81 (d, 1H, J = 9.12 Hz, Ar), 8.07 (d, 1H, J = 8.8 Hz, Ar), 8.34 (s, 1H, Ar) and 8.71 (s, 1H, Ar). MS *m/z*: 178.9 (M^+, 100), 163.0, 149.0, 133.0, 110.0, 105.0, 89.0 and 78.0. HRMS (EI): *m/z* calcd. for $C_7H_4N_3O_3$: 179.0331; found: 179.0336.

*2-Methyl-5-nitro-1H-benzimidazole-3-oxide (**4b**).* A solution prepared by dissolving 199.7 mg (0.783 mmol) of **2b** in 100 ml of solvent was heated at reflux with NaOH (0.2 M) for 2 h. The *N*-oxide precipitated upon acidification of the solution with HCl and was separated by vacuum filtration. It was a yellow solid that decomposed at 199 °C. The filtrate was evaporated to dryness, and acetone was added to precipitate the salts. The acetone was evaporated giving a mixture of phenol **5b** and *N*-oxide **4b**.

^{1}H NMR ($(CD_3)_2SO$, 200 MHz) δ: 2.58 (s, 3H, CH_3) 7.70 (d, 1H, J = 9.12 Hz, Ar) 8.06 (dd, 1H, J = 2.2, 6.6 Hz, Ar) and 8.27 (d, 1H, J = 2.2 Hz, Ar). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 12.24, 104.98, 116.98, 118.95, 131.32, 142.26, 142.34 and 153.85. MS *m/z*: 193.85($[M+1]^+$, 12.26), 192.9 (M^+, 100), 177.0, 163.0, 147.0, 130.0, 104.0, 89.0 and 78.0. HRMS (EI): *m/z* calcd. for $C_8H_7N_3O_3$: 193.0487; found: 193.0490.

*Reaction of N-(2,4-dinitrophenyl) β-alanine (**2c**).* A solution prepared by dissolving 103.2 mg (0.405 mmol) of **2c** in 50 ml of solvent was heated at reflux with NaOH (0.2 M) for 6 h. The reaction mixture was acidified to pH 2 and the solvent was evaporated in a rotavapor. The salts were separated from the product by column chromatography on celite by eluting with acetone. After evaporation a yellow solid was isolated and identified as 2,4-dinitrophenol (**5b**) by comparison of the ^{1}H NMR spectrum with that of an authentic sample.

Because it is known that *N*-oxide yield increases at a lower base concentration,[13,14,16] a solution prepared by dissolving 101.1 mg (0.396 mmol) of **2c** in 50 ml of solvent was heated at reflux with 0.01 M NaOH for 40.5 h. After work-up, the solid obtained was identified and quantified by ^{1}H NMR as a mixture of unreacted substrate and phenol **5b**.

^{1}H NMR ($(CD_3)_2SO + ((CH_3)_2SO$, 200 MHz) δ: 2.74 (t, 2H, CH_2), 3.74 (q, 2H, J = 6.22 Hz, CH_2) 7.00 (d, 1H, J = 9.5 Hz, Ar), 8.29 (dd, 1H, J = 2.56 Hz, Ar), 8.43 (dd, 1H, J = 2.96 Hz, Ar), 8.86 (s, 1H, NH), 9.01 (d, 1H, J = 2.96 Hz, Ar) and 9.12 (d, 1H, J = 2.96 Hz, Ar).

Kinetic procedures

Reactions were initiated by adding the substrate dissolved in 1,4-dioxane to a solution containing all the other constituents. The total 1,4-dioxane concentration was 10% or 20% v/v; the reaction temperature was 25 ± 0.01 °C. The ionic strength was kept constant at 1 M by adding NaCl as compensating electrolyte when needed.

All kinetic runs were carried out under pseudo-first-order conditions, with substrate concentrations of about 7.00–7.15 × 10^{-5} M. The reaction of **2a** was followed by measuring the decrease in absorbance of the reaction mixture at 360 nm, which is the wavelength maximum of the substrate. The spectra of the solutions at infinity were compared with solutions of the corresponding *N*-oxide under the same reaction conditions. The reaction of **2c** was followed by measuring the decrease in absorbance of 3 ml aliquots of the reaction solution taken at different reaction times and poured over 1 ml of 3 M HCl at 358 nm, which is the wavelength maximum of the substrate.

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