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Stabilization of the pesticide Fenitrothion toward *O* and *N* nucleophiles in the presence of cyclodextrins

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The reaction of Fenitrothion with *O* and *N* nucleophiles (H_2O_2, NH_2OH, *n*-butylamine and piperidine) was studied at 25 °C in water containing 2% 1,4-dioxane in the presence of native cyclodextrins (α-, β-, and γ-CD). For all the nucleophiles, the presence of CD produces reaction inhibition with saturation kinetics. The greatest effect in all cases is observed with β-CD, and the greatest inhibition was observed for the reaction of Fenitrothion with H_2O_2 (81%), which is the most efficient nucleophile in promoting Fenitrothion degradation in homogeneous media. In the absence of CD, competition between the $S_N2(P)$ and the $S_N2(C)$ pathways was observed with piperidine as was reported before for the reaction with NH_2OH and *n*-butylamine. The presence of β-CD does not modify product distribution in the case of the reaction with NH_2OH and *n*-butylamine, whereas there is an increase in $S_N2(C)$ pathway when the nucleophile is piperidine. Copyright © 2014 John Wiley & Sons, Ltd.

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INTRODUCTION

Fenitrothion [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl)phosphorothioate] (**1**) is a contact insecticide and selective acaricide of low ovicidal properties that belongs to the organophosphate family of insecticides. It is effective against a wide range of pests that damage forest and various crops and may also be used as a fly, mosquito, and cockroach residual contact spray for farms and public health programs.[1]

Cyclodextrins (CDs) have found many practical applications especially within the pharmaceutical industry, as well as the food, cosmetic and biotechnological industries, and in the field of analytical chemistry.[2] More recently, CD technology has been employed to the improvement of agrochemicals.[2,3]

For the last decade, we have been involved in the study of the reactivity of organophosphorus insecticides in the absence and presence of CDs.[4–8] Fenitrothion reacts in water with *O*- and *N*-based nucleophiles (Nu) through different pathways depending on the nucleophile (Scheme 1).[6] In this reaction media, the reactions of **1** with NH_2OH, $BuNH_2$, and NH_2NH_2 showed competition between the $S_N2(C)$ and $S_N2(P)$ pathways, while the $S_N2(P)$ was the only reaction taking place when the Nu was HO^- or HOO^-; no evidence of a S_NAr pathway was observed.[6] An important α-effect was observed with HOO^-, NH_2OH, and NH_2O^-.[6] Previously, we have studied the hydrolysis reactions of **1** in the presence of native (α-, β-, and γ-CD) and methylated CDs, all of them showed inhibition of the reaction with saturation kinetics because of the formation of complexes.[4,7,8]

We present now the results on the reaction of **1** with piperidine (Pip) in water and the effects of the presence of native CDs on the reactivity and regiochemistry of the reactions of **1** with H_2O_2, NH_2OH, $BuNH_2$, and Pip. As in the reaction of **1** with the other *N*-based nucleophiles studied, in the reaction with Pip, competition between the $S_N2(P)$ and $S_N2(C)$ pathways was also observed.

The occurrence of catalysis or inhibition in reactions where the substrate forms inclusion complexes with CDs depends, in many cases, on how these molecules are accommodated in the cavity.[9] The orientation of the molecule inside the cavity and the position of the reactive site is also an important factor that can affect the regio-, stereo-, or enantio-selectivity of a reaction.[9] For instance, in the presence of β-CD, the *para/ortho* ratio in the iodination of phenol and *o*-chlorophenol with I_2 in water solution increases.[10] The change of selectivity induced by β-CD was also found in the photo-Fries rearrangement of aromatic alkyl esters,[11,12] and on photo-Claisen rearrangement of allyl phenyl ether.[13] So, we expected that the presence of CDs would also affect the regiochemistry of the reactions of Fenitrothion with the *N*-based nucleophiles studied. The knowledge of the regiochemistry of the reactions of pesticides with nucleophiles is important if the reactions are to be used for their destruction because the toxicity of the products formed is different. To the best of our knowledge, there are no reports on the effects of CDs on the regiochemistry of organophosphorus compounds.

We found that the presence of β-CD produces inhibition of the reaction of **1** with all the nucleophiles studied, increase in the $S_N2(C)$ pathway in the reaction with Pip, and no effect on the product distribution in the reaction with the other nucleophiles.

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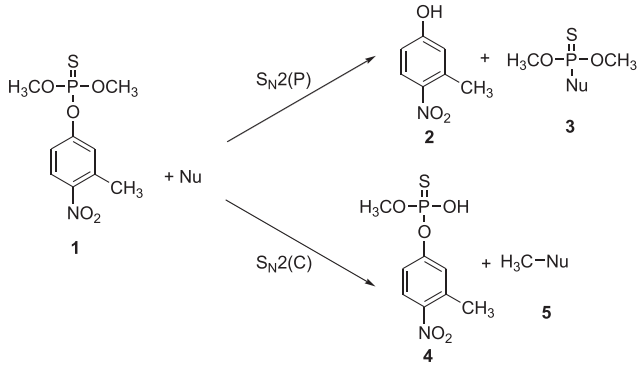

Scheme 1. Reaction pathways for the reaction of Fenitrothion with nucleophiles.

EXPERIMENTAL SECTION

Materials and reagents

Fenitrothion (**1**) was isolated from a commercial sample as described before.[6] A commercial sample of 3-methyl-4-nitrophenol characterized by ^{1}H NMR, GS-MS, and melting point was used as reference for product identification.

Aqueous solutions were prepared using water purified with a Millipore Milli-Q apparatus. $BuNH_2$, Pip,[14] and 1,4-dioxane[15] were purified as described previously. All of the inorganic reagents were of analytical-reagent grade and were used without further purification. Hydrogen peroxide solutions were titrated with $KMnO_4$ solution standardized by sodium oxalate. NH_2OH solutions were prepared from a commercial aqueous solution (50%) and potentiometrically titrated in triplicate with HCl.

The β-CD and sucrose were purchased from Sigma and used as received. α-CD and γ-CD were donated by Roquette (Argentina).

Ultraviolet–visible (UV–Vis) spectra were recorded on a double beam or a diode array spectrophotometer, and the change in absorbance during a kinetic run was measured on the same instruments.

Kinetic procedures

Reactions were initiated by adding the substrate dissolved in 1,4-dioxane to a solution containing all the other constituents. The reaction temperature was 25.0 ± 0.1 °C, the ionic strength was 1.0 M, and NaCl was used throughout as compensating electrolyte. The solvent contained 2% 1,4-dioxane. In the reactions with H_2O_2, and NH_2OH, water and 1,4-dioxane were not only filtered but also degassed. The stock solution of NaCl was filtered by nylon membranes before preparing the reaction solutions. After the pH was adjusted to the desired value, the solution was sonicated for 5 min and thermostated to 25 °C before the substrate was added.

The native CDs have hydroxyl groups that can be deprotonated by hydroxide ions in basic media. Therefore, it is necessary to consider the pK_a values of these CDs when calculating the fraction of CD that is in neutral or ionized form. For all the reactions, the pH of the solutions was kept constant, and it was adjusted after the CDs were dissolved, so the changes in the observed rate constants, k_{obs}, cannot be attributed to a change in pH.

All kinetic runs were carried out protected from light, under pseudo-first-order conditions, with substrate concentrations of $4.76–6.60 \times 10^{-5}$ M. Fenitrothion concentration was at least 50 times smaller than that of the CDs and several orders of magnitude smaller than that of the nucleophile; therefore, pseudo-first-order conditions were held throughout the experiments.

The reactions were followed by measuring the increase in absorbance of the reaction mixture at 397 nm, the λ_{max} of 3-methyl-4-nitrophenoxide ion (**2**) in 2% 1,4-dioxane and $I = 1$ M (NaCl) ($\varepsilon = 17641\ cm^{-1}\ M^{-1}$). The concentration of **2** and demethylfenitrothion (**4**) was obtained as described in previous work.[6] The pseudo-first-order observed rate constants k_{obs} were dissected into k^P_{obs} and k^C_{obs}, according to Eqns (S1) and (S2).

Product analysis experiments

The ^{31}P NMR was used to identify the organophosphates products. The ^{31}P NMR spectra were recorded on a spectrometer operating at 161.97 MHz. Chemical shifts were measured with respect to the external standard of 85% H_3PO_4 in D_2O/H_2O (75:25). Spectra were acquired with a longitudinal relaxation time (D1) of 3 s with 128 accumulations and proton decoupling.

The ^{1}H and ^{31}P NMR spectra of the reaction of **1** with Pip were recorded in aqueous solution because the signals of 1,4-dioxane overlap with some signals of the reaction products. For sample preparation details, see SI.

RESULTS AND DISCUSSION

Reaction with H_2O_2

The reaction of **1** with H_2O_2 was studied previously in a pH range from 9.00 to 12.00 in 2% 1,4-dioxane/H_2O leading to the formation of 3-methyl-4-nitrophenoxide (**2**) and *O,O*-dimethylphosphorotioate (**3**).[6] We report now the results in the presence of β-CD, at pH 11.30 (Buffer Borax) at a constant analytical concentration of the nucleophile equal to 4.76×10^{-3} M. The individual plots of absorbance versus time were fitted using a non-linear simple exponential equation to obtain k_{obs} values.

In the presence of CDs, the UV–Vis spectrum of the product matches that of **2** at the expected concentration for a complete reaction; therefore, the only reaction taking place is that of P–O bond fission, as it was observed in the absence of CD.[6]

The observed rate constants obtained for the reactions in the presence of β-CD are presented in Table S1. Figure 1 shows the plot of k_{obs} as a function of the concentrations of the β-CD. The addition of β-CD produces a decrease in the observed rate constants showing a saturation effect, Fig. 1, as previously observed in the hydrolysis reaction of **1** in the presence of CDs.[7] The observed rate constant versus β-CD concentration was fitted to an equation of the form of Eqn (1) with $a = (2.20 \pm 0.08) \times 10^{-3}$, $b = (8 \pm 1) \times 10^{-2}$, and $c = (377 \pm 51)$.

$$k_{obs} = \frac{a + b[CD]}{1 + c[CD]} \quad (1)$$

As the reaction rate is affected in different extent by the size of the CD's cavity,[5,7,16,17] the effects of α-CD and γ-CD, at a concentration of 0.02 M, were determined. The observed inhibition was 14%, 81%, and 50% for α-, β-, and γ-CD, respectively, Table 1. The

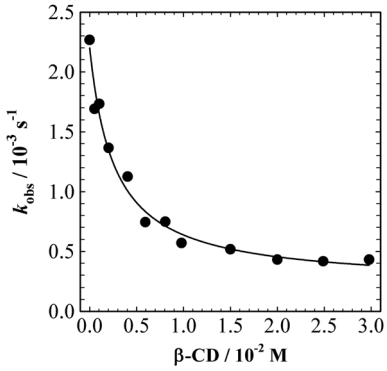

Figure 1. Plot of k_{obs} versus [β-CD] for the reaction of **1** with H_2O_2 at pH = 11.30 at 25 °C. $[\mathbf{1}]_0 = 6.00 \times 10^{-5}$ M, $[H_2O_2]0 = 4.76 \times 10^{-3}$ M, ionic strength $I = 1$ M (NaCl), solvent contains 2% 1,4-dioxane

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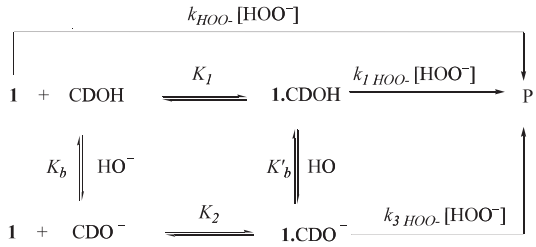
Table 1. Effects of α-, β-, and γ-CD and sucrose on the reaction of Fenitrothion with the studied nucleophiles at 25 °C[a]

	Reaction inhibition (%)				
CD	HO^- [b]	H_2O_2[c]	NH_2OH[d]	$BuNH_2$[e]	Pip[f]
α-	22	14	39	10	23
β-	38	81	61	38	30
γ-	39	50	29	14[g]	—
Sucrose[h]	13	30	—	0	2

CD, cyclodextrins.
[a]$[\mathbf{1}]_0 = (4.76–6.00) \times 10^{-5}$ M, pH = 11.00 unless otherwise stated, [CD] = 0.020 M unless otherwise stated, I = 1.0 M (NaCl) in 2% dioxane/H_2O.
[b][NaOH] = 0.50 M, pH = 13.70
[c]$[H_2O_2]_0 = 4.76 \times 10^{-3}$ M, pH = 11.30, buffer Borax.
[d]$[NH_2OH]_0$ = 0.300 M, buffer NH_2OH, [CD] = 0.010 M.
[e]$[BuNH_2]_0$ = 0.161 M, buffer $BuNH_2$.
[f]$[Pip]_0$ = 0.101 M, buffer Pip
[g][γ-CD] = extrapolated from the plot k_{obs} versus [γ-CD] to 0.020 M.
[h]Sucrose in a weight amount equal to a solution 0.02 M in β-CD.

effect of a non-reducing disaccharide, sucrose, in a weight amount equal to a solution 0.02 M of β-CD was also determined, and an inhibition effect of 30% was observed. The inhibition of the reaction produced by the presence of α-, β-, and γ-CD is attributed to the formation of inclusion complexes of **1** with the CDs,[7] and that observed in the presence of sucrose to some type of unspecific association of the substrate with the sugar.[4] It is known that starch and other linear oligosaccharides can self-associate and form complexes with different types of compounds, and this could be responsible of the observed effect.[18] There are several examples in the literature of changes in reaction rates due to the presence of linear sugars or monosaccharides.[19–22] The inhibition produced by complexation with β-CD is greater for the reaction of **1** with HOO^- than for the hydrolysis reaction. If the reaction center is buried in the CD's cavity, the reaction with a bulkier nucleophile would lead to a greater inhibition as it is observed when we compared the reactions with HOO^- or HO^-.

In the absence of CDs at pH 9.00–12.00, the reaction of **1** with H_2O, HO^-, and H_2O_2, the other nucleophiles present in the reaction media, does not compete with the reaction of HOO^-.[6] Assuming the same behavior, the reaction in the presence of β-CD may take place as shown in Scheme 2. Here, k_{HOO^-}, k_{1HOO^-}, and k_{3HOO^-} represent the reactions of HOO^- with the free substrate, the substrate complexed with neutral CD (CDOH), and the reaction with the substrate complexed with ionized CD (CDO^-), respectively. The observed rate constant for Scheme 2 is shown in Eqn (2), where f represents the fraction of ionized CD as defined in Eqn (3) with $K_b = K_W/K_a$.



Scheme 2. General mechanism for the reaction of Fenitrothion with HOO^- in the presence of CDs.

$$f = \frac{[HO^-]}{[HO^-] + K_b} \tag{3}$$

As the pK_a of β-CD is 12.20,[23] in our reaction conditions f = 0.11, and we consider that the reaction pathways involving CDO^- in Scheme 2 can be neglected, then Eqn (2) simplifies to Eqn (4). This equation has the same mathematical form of Eqn (1) with parameters a, b, and c defined in Eqns (5), (6), and (7).

$$k_{obs} = \frac{k_{HOO^-}[HOO^-] + k_{1HOO^-}K_1[HOO^-][CD]}{1 + K_1[CD]} \tag{4}$$

$$a = k_{HOO^-}[HOO^-] \tag{5}$$

$$b = k_{1HOO^-}K_1[HOO^-] \tag{6}$$

$$c = K_1 \tag{7}$$

Parameter a represents the reaction of HOO^- with the free substrate, b is the product of the rate of reaction that occurs when **1** is included in the CD cavity and the association constant K_1, and parameter c considers only the equilibrium present in the system.

In Table 2, the calculated values of parameters a, b, and c are collected. It can be seen from the data in Table 2 that the value of k_{obs} obtained in the absence of β-CD is in good agreement, within experimental error, with the value of parameter a calculated by fitting the data to Eqn (1). The association constant of **1** with neutral β-CD, K_1, obtained from parameter c is $377 \pm 51\ M^{-1}$. This value is similar to that calculated before in the hydrolysis reaction for the association with ionized β-CD, that is, $K_2 = 417 \pm 118\ M^{-1}$.[7] It can be seen that K_1 and K_2 are not significantly different, as previously suggested.[4]

The reactions in the presence of α- and γ-CD were performed at only one CD concentration, then no value for K_1 could be calculated but we can assume that they will be comparable to those of K_2 determined previously.[7] Comparing the inhibition observed with the three CDs, we can see that, as it was the case in the hydrolysis reaction, the inhibition increases with the increase in the association constant of **1** with CDs.[7]

$$k_{obs} = \frac{k_{HOO^-}[HOO^-] + k_{1HOO^-}K_1[HOO^-](1-f)[CD] + k_{3HOO^-}[HOO^-]K_2f[CD]}{1 + K_1(1-f)[CD] + K_2f[CD]} \tag{2}$$

Table 2. Parameters of Eqn (1) for the reaction of Fenitrothion (**1**) with different nucleophiles in the presence of β-CD at 25 °C[a]

Nu		a (10^{-6} s^{-1})	b (10^{-3} M^{-1} s^{-1})	c (M^{-1})
HO^{-}[b]	k^{P}_{obs}	1260 ± 10 (1260)	301 ± 8	417 ± 118[c]
HOO^{-}	k^{P}_{obs}	2200 ± 80 (2180)	80 ± 10	377 ± 51
	k_{obs}	256 ± 8 (253)	13 ± 1	276 ± 53
NH_2OH	k^{P}_{obs}	220 ± 5 (218)	11 ± 1	276
	k^{C}_{obs}	36 ± 1 (35)	1.8 ± 0.2	276
	k_{obs}	61.9 ± 0.02 (62.0)	1.88 ± 0.03	67.1 ± 0.6
Pip	k^{P}_{obs}	6.1 ± 0.2 (6.31)	0.13 ± 0.02	67.1
	k^{C}_{obs}	55.8 ± 0.2 (55.7)	1.76 ± 0.03	67.1
	k_{obs}	37.7 ± 0.3 (38)	2.14 ± 0.05	121 ± 2
$BuNH_2$	k^{P}_{obs}	5.11 ± 0.05 (5.13)	3.04 ± 0.08	121
	k^{C}_{obs}	32.6 ± 0.3 (32.9)	1.83 ± 0.04	121

[a][NaOH] = 0.50 M, $[HOO^{-}] = 1.589 \times 10^{-3}$ M at pH = 11.30, $[NH_2OH]_0$ = 0.30 M, $[Pip]_0$ = 0.101 M, and $[BuNH_2]_0$ = 0.161 M at pH = 11.00. Values in parenthesis are the pseudo-first order rate constants for the reaction of **1** in the absence of β-CD. The uncertainties are the standard deviations of the non-linear regression.
[b]Data from Ref. 7.
[c]The value corresponds to K_2.

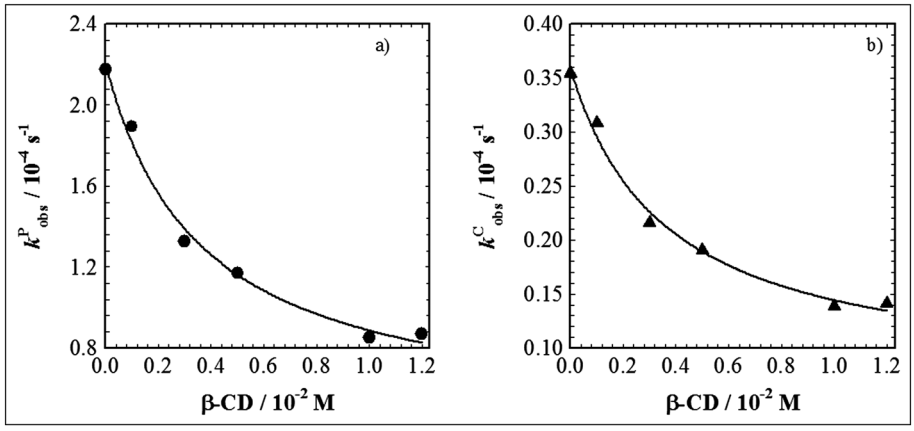


Figure 2. Plot of k^{P}_{obs} (a) and k^{C}_{obs} (b) versus [β-CD] for the reaction of **1** with NH_2OH at pH = 11.00 at 25 °C. $[\mathbf{1}]_0 = 5.88–6.02 \times 10^{-5}$ M, $[NH_2OH]_0$ = 0.30 M, buffer of NH_2OH, ionic strength, I = 1 M (NaCl), solvent contains 2% 1,4-dioxane

Reaction with NH_2OH

The reaction of **1** with NH_2OH was studied previously in a pH range from 9.00 to 12.00 in 2% 1,4-dioxane/H_2O.[6] In all cases, competition between $S_N2(P)$ and $S_N2(C)$ was observed, leading to the formation of phenol **2** and demethylfenitrothion, **4**, respectively. At constant pH and variable NH_2OH concentrations, the yield of products was constant, whereas the attack at P increases with pH.[6]

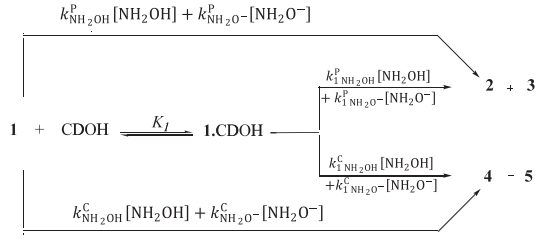
We report here a study of the reaction of **1** with NH_2OH in the presence of β-CD at pH 11.00 (buffer NH_2OH) at constant analytical concentration of the nucleophile, $[NH_2OH]_0$, equal to 0.30 M. The observed rate constants, k^{P}_{obs} and k^{C}_{obs}, and the yield of $S_N2(P)$ and $S_N2(C)$ pathways in the presence of β-CD were obtained as described before,[6] and are presented in Table S2.

For all CD concentrations used, the yield of **4** remained unchanged within experimental error and equal to that obtained in the absence of CD in the same conditions.[6] Figure 2 shows the plot of k^{P}_{obs} and k^{C}_{obs} as a function of β-CD concentration. These plots were fitted with Eqn (1) and show inhibition of the reaction with saturation kinetics.

It was previously observed that, although at the pH range studied, the predominant form of the nucleophile is neutral NH_2OH, both NH_2OH and NH_2O^{-} act as nucleophiles in the reaction with **1** and that the reaction of H_2O and HO^{-} does not compete with NH_2OH and NH_2O^{-}.[6] Considering that product distribution is not affected by the presence of β-CD, we assume that it does not modify the reactivity of these nucleophiles.

At pH 11.00, β-CD is only 6% in its ionized form (CDO^{-}), so the amount of complex of the substrate with ionized CD (**1**.β-CDO^{-}) is negligible. Therefore, the reaction of **1** with hydroxylamine at pH 11.00 in the presence of β-CD may take place as shown in Scheme 3 where $k^{P}_{NH_2OH}$, $k^{P}_{NH_2O^-}$, $k^{C}_{NH_2OH}$, and $k^{C}_{NH_2O^-}$ represent the reactions at the phosphorus and aliphatic carbon of the neutral and anionic species of hydroxylamine with the free substrate, whereas the reaction of NH_2OH and NH_2O^{-} with the complexed substrate is represented by $k^{P}_{1\ NH_2OH}$, $k^{P}_{1\ NH_2O^-}$, $k^{C}_{1\ NH_2OH}$, and $k^{C}_{1\ NH_2O^-}$, and K_1 the association constant of **1** with neutral CD.

The observed rate constant for the mechanism in Scheme 3 is shown in Eqn (8) where [CD] is the analytical concentration of CD, and it has the same mathematical form of Eqn (1) with *a*, *b*, and *c* given by Eqns (9)–(11).



Scheme 3. General mechanism for the reaction of Fenitrothion with NH_2OH in the presence of CDs at pH = 11.00.

the absorbance at infinite time of the aryloxide product (A_{inf}) was lower than that predicted for the reaction occurring solely via the S_N2(P) pathway. The yield of **2** calculated from A_{inf} as described before[6] varied from 5% at pH 10.57 to 18% at pH 11.42 and decreases with Pip concentration at constant pH. This fact indicates the competition of other reaction pathway: a S_N2 process involving nucleophilic attack at the aliphatic methoxy C atom and/or a S_NAr process in which the nucleophile attacks C-1 position of the aromatic moiety.

$$k_{obs} = \frac{\left(k^{P}_{NH_2OH} + k^{C}_{NH_2OH}\right)[NH_2OH] + \left(k^{P}_{NH_2O^-} + k^{C}_{NH_2O^-}\right)[NH_2O^-]}{1 + K_1[CD]} + \frac{\left\{\left(k^{P}_{1NH_2OH} + k^{C}_{1\ NH_2OH}\right)[NH_2OH] + \left(k^{P}_{1\ NH_2O^-} + k^{C}_{1\ NH_2O^-}\right)[NH_2O^-]\right\}K_1[CD]}{1 + K_1[CD]} \quad (8)$$

$$a = \left(k^{P}_{NH_2OH} + k^{C}_{NH_2OH}\right)[NH_2OH] + \left(k^{P}_{NH_2O^-} + k^{C}_{NH_2O^-}\right)[NH_2O^-] \quad (9)$$

$$b = \left\{\left(k^{P}_{1NH_2OH} + k^{C}_{1\ NH_2OH}\right)[NH_2OH] + \left(k^{P}_{1\ NH_2O^-} + k^{C}_{1\ NH_2O^-}\right)[NH_2O^-]\right)K_1 \quad (10)$$

$$c = K_1 \quad (11)$$

Parameter *a* represents the reaction of the nucleophiles with the free substrate, *b* is the product of the rate of the reaction that occurs when Fenitrothion is included in the CD cavity with the association constant K_1, and parameter *c* considers only the equilibrium present in the system.[4,8] Table 2 shows the calculated values for the mentioned parameters. The parameter ratios a^P/a^C and b^P/b^C are equal to 6.11, whereas the relationship of the observed rate constants in the absence of β-CD[6] k^{P}_{obs}/k^{C}_{obs} is equal to 6.16 at the same conditions ($[NH_2OH]_0 = 0.30$, pH = 11.00). Hence, the relative reactivity of NH_2OH toward the P and aliphatic C reaction centers is not affected by the complexation.

For comparative purposes, the effect of 0.01 M α-CD and γ-CD under the same conditions used with β-CD was determined, Table S3. It can be seen from data in Table S3 that the percentage yield of pathways S_N2(P) and S_N2(C) is independent of the presence of any type of CD. On the other hand, the observed inhibition was 39%, 61%, and 29% for α-, β-, and γ-CD, respectively (Table 1). The values of the association constant between **1** and α-CD or γ-CD, K_1, could not be calculated because the reactions were performed at only one concentration of CD.

Reaction with piperidine

The reaction of **1** with piperidine was studied in 2% 1,4-dioxane/H_2O at 25°C and I = 1.00 M (NaCl) in a pH range from 10.57 to 11.42 (pK_a = 11.42)[14]; buffers of Pip were used to control the pH of the solutions. The reaction was followed by measuring the increase in absorbance at 397 nm corresponding to the formation of **2**. As it was observed in the reaction with $BuNH_2$,[6]

In order to identify the reaction products, NMR experiments were conducted. The degradation of a 0.01 M solution of **1** with an analytical concentration of Pip 0.02 M at pH = pK_a = 11.42 in D_2O at room temperature was followed by ^1H and ^{31}P NMR. The initial reading in the ^{31}P NMR spectrum showed a single peak at 65.6 ppm, corresponding to the chemical shift of **1** (Fig. 3).[6,24,25] As the reaction proceeded, the signal corresponding to **1** decreased, while one peak was observed at 53.4 ppm. The reaction was followed until no signal corresponding to **1** was observed, and only the peak at 53.4 ppm remained. This last signal was attributed to demethylfenitrothion (**4**), by comparison with our previous work[6] and others reported in the literature.[24,25] The formation of **4** is attributed to the reaction of Pip at the aliphatic C atom by a S_N2(C) pathway (Scheme 4). No other phosphorus signal was observed. Nevertheless, the other phosphorus compounds that can be formed simultaneously with compound **2** that is detected by UV–Vis technique are **3a** and **3b**. Compound **3a** can be formed by hydrolysis and/or by S_NAr of Pip in the aromatic carbon of **1** (pathway not shown). On the other hand, compound **3b** can be formed by the attack of Pip on P leading to an *N*-phosphorylated product. As the highest yield of the sum of **3a** and **3b** determined indirectly by UV–Vis analysis of **2** in our experimental conditions was 18% (Table S4), this value represents a low quantity to be detected by ^{31}P NMR technique suggesting that S_NAr does not occur or it occurs in a very low amount.

The ^1H NMR spectrum of the reaction at infinite time showed only signals of products **3b** and **5b** corresponding to (S_N2(P) and S_N2(C) pathways, respectively, Figure S1, confirming that they occur.

Values of k_{obs} for the reaction of **1** with Pip were obtained at different pH values as a function of the analytical concentration of the nucleophile ($[Pip]_0$) (Table S4).

The second-order rate constants for the attack of Pip on P and on the aliphatic C atom were calculated from plots of k^{P}_{obs} and k^{C}_{obs} versus $[Pip]_0$ according to Eqns (12) and (13) (Figures S4 and S5, respectively), and the results are collected in Table S5.

$$k^{P}_{obs} = k^{P}_{HO}[HO^-] + k^{P}_{Pip}[Pip] = k^{P}_{HO}[HO^-] + k^{P}_{Pip}X_{Pip}[Pip]_0 \quad (12)$$

$$k^{C}_{obs} = k^{C}_{Pip}[Pip] = k^{C}_{Pip}X_{Pip}[Pip]_0 \quad (13)$$

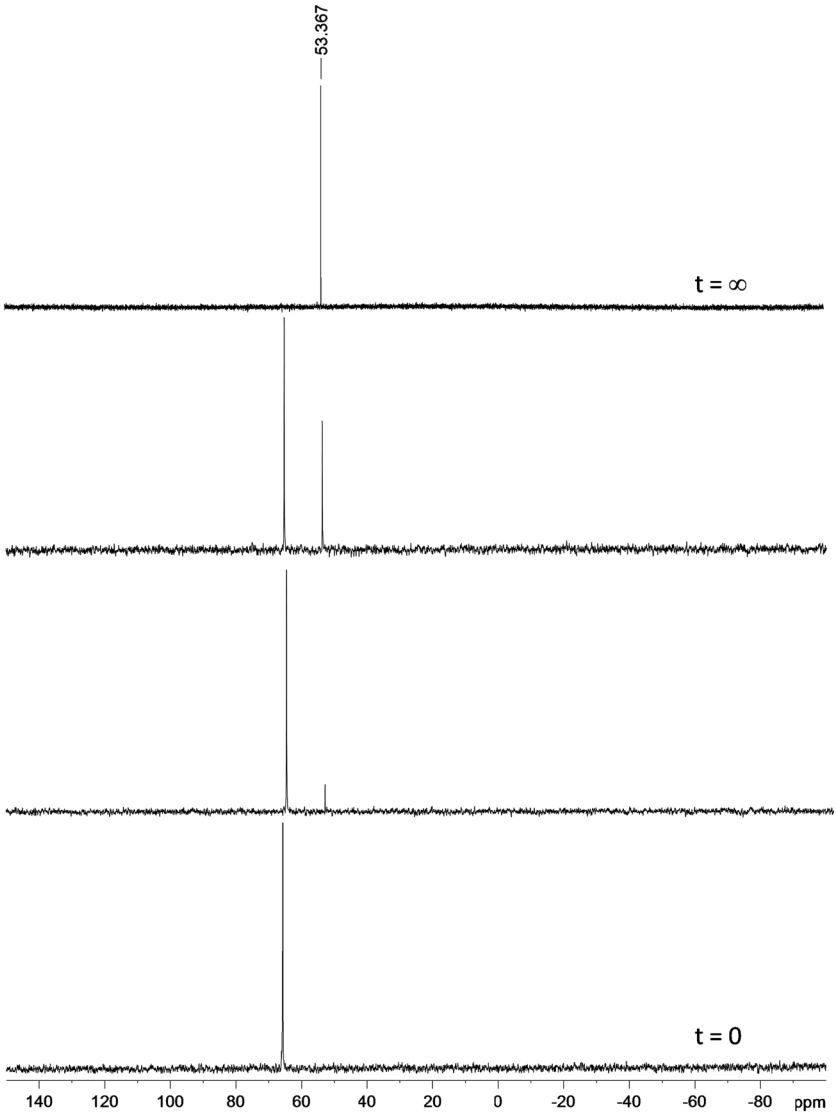
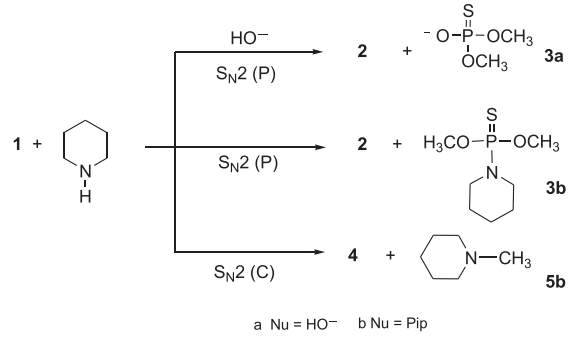


Figure 3. ^{31}P NMR spectra of **1** (0.010 M) in $D_2O:H_2O$ (70:30) at room temperature in the presence of $[Pip]_0 = 0.020$ M at pH 11.41 at different reaction times



Scheme 4. Reaction pathways for the reaction of Fenitrothion with Pip

$$X_{Pip} = \frac{[Pip]}{[Pip]_0} \quad (14)$$

By plotting the intercepts of the plots in Figure S4 as a function of $[HO^-]$ (Figure S6), we obtained from its slope a value of $(3.4 \pm 0.2) \times 10^{-3}\,M^{-1}\,s^{-1}$ for the second-order rate constant for the hydrolysis reaction, in agreement with that calculated in the absence of Pip.[6] In order to calculate the second-order rate constants for the reaction of Pip free base with P and aliphatic C atom, we plotted the slopes of the plots of k^P_{obs} versus $[Pip]_0$ ($k^P_{[Pip]_0}$), and the slopes of the plots of k^C_{obs} versus $[Pip]_0$ ($k^C_{[Pip]_0}$), versus X_{Pip} according to Eqns (15) and (16) (Figures S7 and S8, respectively). From the intercepts of these plots at $X_{pip} = 1$, the values of $k^P_{Pip} = (2.9 \pm 0.2) \times 10^{-5}\,M^{-1}\,s^{-1}$ and $k^C_{Pip} = (1.62 \pm 0.08) \times 10^{-3}\,M^{-1}\,s^{-1}$ were calculated. The intercept at $X_{Pip} = 0$ was zero, within experimental error; therefore, the only nucleophile is the free base, Pip.

$$k^P_{[Pip]_0} = k^P_{Pip} X_{Pip} \quad (15)$$

$$k^C_{[Pip]_0} = k^C_{Pip} X_{Pip} \quad (16)$$

The reactivity of Pip on the C atom is greater than on P atom of **1** as it has been observed before with $BuNH_2$.[6] Considering the bulkiness of Pip, this could be attributed to the fact that the attack on the C atom of **1** has less steric hindrance than the attack on the P atom, which gives a pentacoordinated P in the transition state. Additionally, Pip is 5.1 and 2.6 times more reactive than $BuNH_2$ at C and P centers, respectively. This is in agreement with the fact that secondary amines are in general more effective nucleophiles than primary ones.[26]

We have studied the effect of the CDs on the reaction of **1** with Pip. The reaction in the presence of increasing concentrations of β-CD was conducted at pH 11.00 in 2% 1,4-dioxane/H_2O at 25 °C and ionic strength 1.00 M (NaCl) at a constant concentration of the amine of 0.101 M. As we mentioned before, at these pH, the formation of complexes with ionized CD (CDO^-) can be neglected. The observed rate constants obtained for the reactions were dissected into k^P_{obs} and k^C_{obs} (Table S6). The percentages of $S_N2(P)$ and $S_N2(C)$ and the yield of **2** obtained for the reactions with the amines in the presence of β-CD are presented in Table S6. The presence of β-CD reduces the reaction rate in comparison with the same reaction in the absence of the receptor. The percentage yields of **2** decrease as β-CD concentration increases from 0 to 0.0177 M.

Considering that Pip forms inclusion complexes with CDs,[27] the nucleophilic attack to organic substrates in the presence of CD may take place by different pathways. In the case of Fenitrothion and an amine (A) as Pip, these reactions are as follows: (i) the free **1** with the free amine; (ii) the free amine with the complexed **1**; (iii) the complexed amine with the free **1**;[27–29] and (iv) the complexed amine with the complexed **1**(each of them complexed by one CD).[27] Figure 4 illustrates the possible reactions

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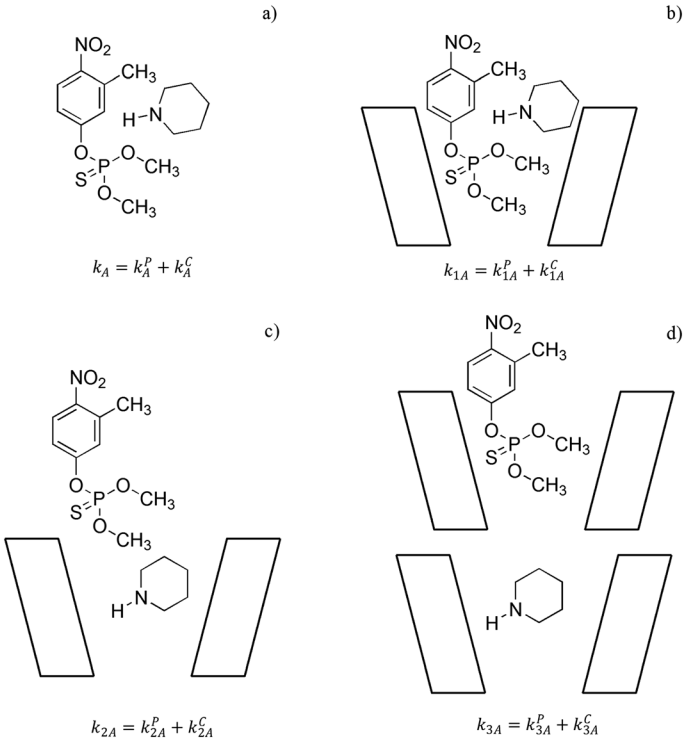


Figure 4. Schemes of the possible reaction pathways of the reaction of Fenitrothion with Pip in the presence of a cyclodextrin

of **1** when the amine is Pip with its second-order rate constant expression, respectively.

At pH = 11.00, the molar fraction of Pip is 0.275, and the molar fraction of $PipH^+$ is 0.725. It has been observed that the ammonium species of the amine does not react with **1**. Nevertheless, the amine acid dissociation constant (K_{HA}) changes by the inclusion of the amine in the CD[27] (Scheme S1). Because A (Pip) as well as AH ($PipH^+$) form inclusion complexes with β-CD, in a buffer solution of amine and ammonium ion, the amount of total amine, free and complexed ([A] + [AH] and [A.CD] + [AH.CD]), changes as the CD concentration increases.[27] From the association equilibrium constant of Pip with β-CD ($K_{CD}^A = 53\ M^{-1}$),[27] it can be calculated that under our reaction conditions, the concentration of Pip freebase (A) decreases 9.7% (from 0.0279 to 0.0252 M) as [β-CD] increases from 0 to 0.018 M, whereas the concentration of total effective amine, $[A]_T = [A] + [A.CD]$, increases 21% (from 0.0279 to 0.0338 M). This effect is illustrated in Table S7 where the calculated concentrations of the involved species are shown.

The values of k_{obs}^P and k_{obs}^C were plotted against the concentration of β-CD (Figure S9). As it was previously observed for the hydrolysis of **1**,[7] the reaction with Pip is inhibited by β-CD showing saturation kinetics (Figure S9). The plots shown in Figure S9 were fitted to an equation of the form of Eqn (1) where *a* represents the reaction in the absence of CD and *b* and *c* are adjustable parameters (Table 2).

Reaction pathway d) in Scheme 4 that implies that the aminolysis reaction occurs when the substrate and the amine are complexed each of them with one CD can be discarded on the following bases: (i) crystallographic data,[30] molecular docking calculations[31] and kinetic studies,[7] indicate that Fenitrothion includes in the cavity of one CD, preferably through the thiophosphate group (reaction center); (ii) a second-order dependence on CD concentration, $[CD]^2$, is not observed from the plots of k_{obs}^P and k_{obs}^C versus $[\beta\text{-CD}]_0$.

The reaction may take place as indicated in Scheme 5, where k_{HO^-} and k_1 represent the reactions of HO^- with the free and complexed substrate, respectively; k_A^P and k_A^C represent the reaction of the amine on P and aliphatic C atom of the free substrate; k_{1A}^P and k_{1A}^C represent the reaction of the amine on P and aliphatic C atom of the complexed substrate; k_{2A}^P and k_{2A}^C represent the reaction of the complexed amine on P and aliphatic C atom of the free substrate; K_1 is the association constant of **1** with β-CD.

The observed rate constant for the mechanism in Scheme 5 is given by Eqn (17) that has the same mathematical form of Eqn (1) with *a* and *b* given by Eqns (18) and (19). In that equation, k_A is the sum of k_A^P and k_A^C, K_{CD}^A is the association constant of the amine with CDOH, where [*CD*] is the analytical concentration of CD, *f b* is the fraction of the amine free base, $[A]_0$ is the analytical concentration of the amine, and K_1 is the association constant of the substrate with the CD (Eqn (1)).

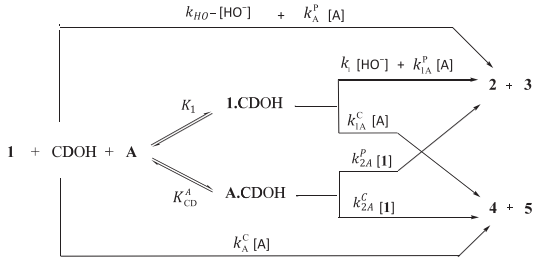
$$k_{obs} = \frac{\left(k_{HO^-}[HO^-] + k_A\left(1 + K_{CD}^A[CD]\right)^{-1}\right) fb[A]_0}{1 + K_1[CD]} + \frac{\left(k_1K_1[HO^-] + \left(k_{1A}K_1 + k_{2A}K_{CD}^A\right)\left(1 + K_{CD}^A[CD]\right)^{-1} fb[A]_0\right)[CD]}{1 + K_1[CD]} \quad (17)$$

$$a = k_{HO^-}[HO^-] + k_A fb[A]_0 \quad (18)$$

$$b = k_1K_1[HO^-] + \left(k_{1A}K_1 + k_{2A}K_{CD}^A\right)\left(1 + K_{CD}^A[CD]\right)^{-1} fb[A]_0 \quad (19)$$

$$c = K_1 \quad (20)$$

The parameters obtained for the reaction of **1** with Pip are shown in Table 2. The ratios a^C/a^P and b^C/b^P weight the nucleophilic attack preference to the reactions centers in the absence and in the presence of β-CD, respectively. If the ratios a^C/a^P and b^C/b^P are equal or similar, it implies that the presence of



Scheme 5. General mechanism for the reaction of Fenitrothion (**1**) with Pip (A) in the presence of CD at pH = 11.00

the CD does not affect the nucleophile reactivity over the reactions centers of **1**. Although there is a weak increase in the percentage yield of **2** (Table S6), the difference in the ratios b^C/b^P (13.60) and a^C/a^P (9.15) may indicate that there is a change in the selectivity of the attack of the nucleophile in the presence of CD.

We have also studied the effect of α-CD on the reaction of **1** with Pip. In the presence of α-CD, there was an inhibition of the reaction. The presence of this CD changed the product distribution with an increase on product **2** relative to product **4** (Table S8). This could be attributed to a major protection of the carbon center by α-CD or a major expose of the P center to the bulk.

The effect of sucrose, a non-reducing disaccharide, on the reactions of **1** with Pip was also investigated; almost no effect on the observed rate constants was found. Instead, the yield of **2** increases from 10.2% to 13.8% for the reaction with Pip as the concentration of sucrose increases from 0 to 0.020 M (Table S9). The observed effect with sucrose can be attributed to a medium effect or to some type of unspecific association of the substrate with the sugar, as it was mentioned before.

Reaction with *n*-butylamine

The reaction of **1** with $BuNH_2$ was previously studied in a pH range from 10.57 to 11.42 in 2% 1,4-dioxane/H_2O.[6] In all cases, competition between $S_N2(P)$ and $S_N2(C)$ was observed, leading to the formation of the aromatic products **2** and demethylfenitrothion, **4**, respectively. At constant pH and increasing $BuNH_2$ concentrations, the yield of product **4** increases. On the other hand, the attack at P leading to product **2** increases with the pH.[6]

We report now a study of the effects of CDs on the reaction of **1** with $BuNH_2$. The reaction in the presence of increasing concentrations of β-CD was conducted at pH 11.00 in 2% 1,4-dioxane/H_2O at 25 °C and ionic strength 1.00 M (NaCl) at a constant concentration of the amine of 0.161 M. At this pH, the complexes of ionized CD (CDO^-) can be neglected.

The reaction was followed in the same way as it was performed with Pip, by measuring the increase in absorbance of product **2** at 397 nm. The data of the observed rate constants, k_{obs}, were obtained and dissected into k^P_{obs} and k^C_{obs} as shown before for Pip. The percentages of $S_N2(P)$ and $S_N2(C)$ and the yield of **2** obtained for the reactions with the $BuNH_2$ in the presence of β-CD are presented in Table S10.

At pH = 11.00, the molar fraction of $BuNH_2$ is 0.59, and the molar fraction of $BuNH_3^+$ is 0.41. From the association equilibrium constants of $BuNH_2$ with β-CD ($K^A_{CD} = 3\ M^{-1}$),[27] it can be calculated that at the maximum β-CD concentration used (0.02 M), only 4.3% of the amine is complexed, Table S7.

In the reaction of **1** with $BuNH_2$, the presence of β-CD reduces the reaction rate in comparison with the same reaction in the absence of the receptor, and the yields of reaction products remain unchanged. Therefore, in Scheme 5, the pathways that imply the complexed amines can be discarded; the observed rate constant for the mechanism of the reaction of **1** with $BuNH_2$ is given by Eqn (21), which has the same mathematical form of Eqn (1) with parameters *a* and *b* given by Eqns (22) and (23), and parameter *c* is given by Eqn (20).

$$k_{obs} = \frac{\left(k_{HO^-}[HO^-] + k_A fb[A]_0\right) + \left(K_1\left(k_1[HO^-] + k_{1A} fb[A]_0\right)\right)[CD]}{1 + K_1[CD]} \quad (21)$$

$$a = k_{HO^-}[HO^-] + k_A fb[A]_0 \quad (22)$$

$$b = K_1\left(k_1[HO^-] + k_{1A} fb[A]_0\right) \quad (23)$$

For $BuNH_2$, the ratio a^C/a^P is in good agreement with the ratio k^C_{obs}/k^P_{obs} (6.40) obtained in the absence of CDs under the same conditions ($[BuNH_2]_0 = 0.161$ and pH = 11.00)[6] (Table 2). The ratios a^C/a^P and b^C/b^P are nearly the same, 6.37 and 6.02, respectively. Therefore, the presence of β-CD does not affect the relative reactivity of $BuNH_2$ toward the P and aliphatic C reaction centers of the complexed substrate, as it was observed when the Nu is NH_2OH.

The effects of α-CD and γ-CD on the reaction of **1** with $BuNH_2$ were also studied. The presence of α- or γ-CD reduces the reaction rate slightly in comparison to the effect of β-CD (Table 1, Table S11), and as it was observed in the reactions with Pip, there was a change in product distribution increasing product **2** in detriment to **4**.

When the reactions of **1** with $BuNH_2$ were performed in the presence of sucrose, there was no effect on the reactions rates. Nevertheless, the yield of **2** increases from 13.5% to 19.6% as the concentration of sucrose increases from 0 to 0.020 M (Table S11). The observed effect with sucrose can be attributed to a medium effect or to some type of unspecific association of the substrate with the sugar, as it was mentioned before.

CONCLUSIONS

Fenitrothion (**1**) reacts with *O*-nucleophiles by $S_N2(P)$ pathways, whereas the reaction with *N*-based nucleophiles occurs with competition between $S_N2(P)$ and $S_N2(C)$ pathways. Pip is more reactive than $BuNH_2$, as it is generally observed.

In all cases, the highest inhibition is produced by β-CD, the one that has the highest association constant with **1**.

The reactivity of the nucleophiles toward the P centers decreases in the order $HOO^- > NH_2O^- > HO^-$ as it is the inhibition produced by β-CD.

The reactivity toward C and P is not affected by the presence of β-CD in the reaction of **1** with NH_2OH or $BuNH_2$, whereas in the reaction with Pip, the reactivity toward C increases. This result may be a consequence of the larger size of piperidine than the primary amines, which is more evident in a constrained system as is the substrate included in the cavity of CDs.

The formation of a complex that stabilizes **1** toward the nucleophilic attack could be the reason of the observed inhibition of the reaction of **1** with the studied nucleophiles.

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