A Comparison of the Reactivity and Mutagenicity of *N*-(Benzoyloxy)-*N*-(benzyloxy)benzamides

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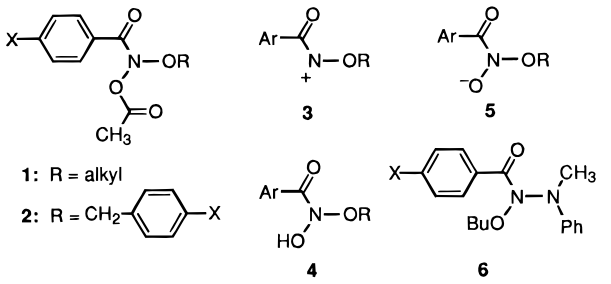
A new series of *N*-(acyloxy)-*N*-alkoxybenzamides, *N*-(benzoyloxy)-*N*-(benzyloxy)benzamides **7** have been synthesized and have been found to be direct acting mutagens in *Salmonella* TA100. They undergo $A_{Al}1$ solvolysis to give *N*-benzoyl-*N*-(benzyloxy)nitrenium ions **3** under conditions of acid catalysis as well as unusual $B_{Al}2$ reactions at nitrogen with hydroxide. The latter process affords as intermediates the anomeric hydroxamic esters **4** which rearrange intramolecularly to esters in a HERON reaction. Rates of acid-catalyzed solvolysis and reaction with hydroxide ions correlate with Hammett σ values with low sensitivity ($\rho = +0.32$ and $+0.55$, respectively) in accordance with the $A_{Al}1$ and $B_{Al}2$ mechanisms. Mutagenicity for the series also appears to correlate with Hammett σ values but with low, negative sensitivity ($\rho = -0.57$), and their biological activity may be attributable to their stability under conditions of the Ames assay and hydrophobic binding to DNA, rather than their chemical reactivity.

Introduction

N-Acetoxy-*N*-alkoxybenzamides **1** are a class of compounds that we have found to be universally mutagenic toward Salmonella TA100 without metabolic activation.[1−4] To date we have synthesized some 40 of these compounds with a variety of substituents on both the benzoyl and alkoxy side chains. They undergo acid-catalyzed solvolysis by the unusual $A_{Al}1$ mechanism which involves reversible protonation at the acetoxy carbonyl followed by unimolecular O−N cleavage to form alkoxynitrenium ions **3**.[3,4] Para substituents on the benzoyl ring in **1**



support this mechanism; rates of solvolyses correlate with Hammett σ^+ constants with a modest ρ value of -1.4 in accordance with incipient stabilization of positive charge in the transition states by electron donor groups.[3] Para substituents on the benzyloxy ring of **2** exert a similar effect since the charge deficiency in alkoxynitrenium ions is strongly delocalized onto oxygen. Thus positively inductive substituents facilitate nitrenium ion formation and substrates with para inductive groups correlate with Hammett σ constants with a ρ value of -1.61. Substrates with positively mesomeric groups, however, undergo concerted decomposition to benzyl cations and the rates for this process correlate with σ^+ with a ρ value of 2.0.[4] Under neutral conditions the mutagens are relatively stable, although in aqueous medium they ultimately decompose by an autocatalytic process.[2,3]

The fate of the *N*-alkoxynitrenium ions formed in these processes has been fully explored, and the products can all be accounted for by formation of the *N*-alkoxyhydroxamic acids **4** (from interception by water) which decompose by acid-catalyzed processes to give alcohols and aldehydes and a non-acid-catalyzed rearrangement to give esters.[4,5] Recent theoretical studies in these laboratories as well as experimental results suggest that the latter process proceeds via the conjugate anion of the hydroxamic acid **5** and is a special case of the so-called HERON[6] reaction of anomeric amides, amides that are geminally substituted at nitrogen with two heteroatoms (RCONXY, Figure 1).[7] A heteroatom bearing a high-energy pair of electrons such as nitrogen (Y = N), or in this case an oxyanion (Y = O^-), initiates rearrangement of X from N to C through a strong anomeric overlap with the σ^* orbital of the adjacent N−X bond. Anomeric interactions at nitrogen in bisheteroatom-substituted amides are enhanced when X is strongly electronegative resulting in a low-lying σ^* orbital.[8,9]

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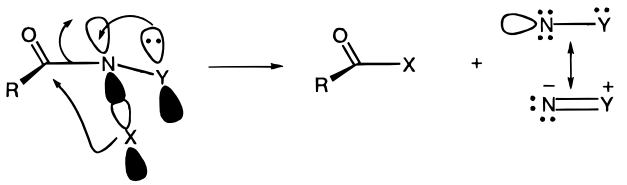
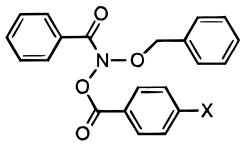


Figure 1. HERON reaction in an anomeric amide.

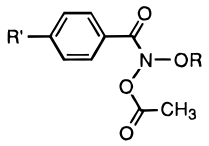
We have also shown that para-substituted *N*-acetoxy-*N*-butoxybenzamides react bimolecularly in methanol with *N*-methylaniline, a model we have used for the nucleosides bearing an exocyclic amino group such as guanosine.[10] These reactions involve rate-determining nucleophilic attack at the amide nitrogen, and the resultant *N*-butoxy-*N*-(*N*′-methylanilino)benzamides **6** rearrange to 1-methyl-1-phenyldiazene and butyl benzoates, again through the HERON process (Figure 1, Y = N(Me)Ph, X = OBu).[9]

Recent DNA damage studies have indicated that the mutagens react with guanine residues at N7[11] (its most nucleophilic center[12,13]) and that two mechanisms are possible; damage could initially be ascribed to their ability to generate electrophilic alkoxynitrenium ions, although, on the basis of the results we have to date, such formation, intracellularly, would of necessity require an acid or Lewis acid catalyst.[3] Alternatively, as indicated by their reaction with nucleophilic amines, the mutagens may themselves behave as electrophiles in reacting with nucleophilic centers on the nucleotide bases. These two processes have also been proposed for the interaction of the analogous metabolites of aromatic amines, *N*-aryl-*N*,*O*-diacetylhydroxylamines.[14−16]

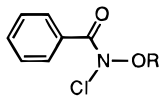
To shed further light on the process by which *N*-(acyloxy)-*N*-alkoxybenzamides interact with DNA, we have synthesized a new series of mutagens, *N*-(benzoyloxy)-*N*-(benzyloxy)benzamides **7a**−**h**, and have investigated their acid-catalyzed solvolysis, their reactions with organic and inorganic bases, and their mutagenicity.



7a: X = H
7b: X = MeO
7c: X = Me
7d: X = Cl
7e: X = CHO
7f: X = CF_3
7g: X = CN
7h: X = NO_2
7i: X = Bu^t



8a: R′ = Cl, R = Bu
8b: R′ =H, R =CH_2Ph



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Results and Discussion

N-(Benzoyloxy)-*N*-(benzyloxy)benzamides were conveniently prepared by a Finkelstein-type process from *N*-chloro-*N*-(benzyloxy)benzamide and the appropriate sodium benzoate in anhydrous acetone. Most were oils which could be purified by centrifugal chromatography but were only stable for long periods under dry conditions at low temperature. Accordingly they were characterized by ^{1}H and ^{13}C NMR and IR spectroscopies.

Acid-Catalyzed Solvolysis Reactions of 7. Like *N*-acetoxy-*N*-alkoxybenzamides **1** and **2**, *N*-(benzoyloxy)-*N*-(benzyloxy)benzamides **7** solvolyze in 25% D_2O−CD_3CN. The rates were obtained using 300 MHz NMR spectroscopy by monitoring the disappearance of the benzyloxy methylene singlet of the starting material. The reactions were pseudo-first-order over several half-lives and rate constants, evaluated for each mutagen at 10 K intervals over the temperature range of 298−338 K, and afforded excellent Arrhenius plots from which E_a and $\Delta S^{\ddagger}$ were obtained (Table 1).

The rate data at 308 K reveals that the reaction is promoted by electron withdrawing para substituents and analysis of Arrhenius parameters shows an excellent isokinetic relationship (Figure 2) confirming that a uniform mechanism is operative throughout. Substrates with electron-withdrawing substituents have the lowest enthalpies of activation and the tightest transition states. The converse is true for substrates with electron-donating groups. The positive $\Delta S^{\ddagger}$ values are in the range observed for $A_{Al}1$ hydrolysis of tertiary alkyl esters (+54 J K^{-1} mol^{-1} for *tert*-butyl acetate)[17] and in contrast to those reported for normal $A_{Ac}2$ hydrolysis of benzoate esters which are strongly negative.[18,19]

By analogy with those of their *N*-acetoxy analogues **1** and **2**,[3,4] the activation enthalpies and entropies are determined by two processes: protonation at the benzoyl carbonyl and N−OCOAr bond stretching as heterolysis proceeds. Both are influenced by the para substituent but in opposite senses.

Electron-donating substituents increase the basicity of the benzoyl carbonyl, which promotes protonation and hence shifts the equilibrium constant K_a, to the right (Scheme 1). However, electron-donating substituents, while increasing the concentration of the protonated intermediate, decrease the partial positive charge at the carbonyl carbon thereby reducing the ease of N−O bond heterolysis (and magnitude of k'_x). These opposing effects manifest themselves in low overall sensitivity to the electronic effects of the para substituents. Rate constants at 308 K (Table 1) correlate with Hammett σ substituent constants with positive slope of low sensitivity ($\rho = 0.32$, $r^2 = 0.87$, Figure 3). A similar sensitivity has been reported for normal acid-catalyzed solvolysis of alkyl benzoates where substituents also influence protonation and solvolysis steps in the opposite sense.[19] Reversible protonation of benzoic acids has a reported ρ^+ value of −1,[20] thus a similar sensitivity in the protonation of **7** would yield a ρ value for the heterolysis step in the region of +1.5 and entirely consistent with build up of negative charge at a center indirectly conjugated to the substituent.[21] Protonation to give the intermediate in Scheme 1

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Table 1. Bimolecular Rate Constants and Arrhenius Data for Acid-Catalyzed Solvolysis of 7 at 308 K

X	ln A	$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	E_a (kJ mol^{-1})	$10^2 k_X$ (308 K)[a]	r^2
MeO	41.2 (1.3)	89.2 (11.1)	119.6 (3.5)	0.395 (0.018)	0.9974
Me	40.5 (1.2)	83.9 (10.0)	118.2 (3.0)	0.369 (0.015)	0.9980
H	39.44 (0.58)	74.7 (4.8)	115.1 (1.6)	0.411 (0.008)	0.9992
Cl	38.83 (0.84)	69.8 (7.0)	113.4 (2.2)	0.424 (0.010)	0.9988
CHO	39.1 (1.3)	71.4 (10.8)	113.2 (3.5)	0.578 (0.031)	0.9972
CF_3	40.1 (1.2)	80.2 (10.0)	115.4 (3.3)	0.689 (0.030)	0.9976
CN	36.2 (1.3)	47.4 (10.8)	105.6 (3.3)	0.630 (0.035)	0.9970
NO_2	37.0 (1.7)	54.4 (14.1)	106.9 (4.4)	0.863 (0.076)	0.9950

[a] Interpolated rate constants at 308 K.

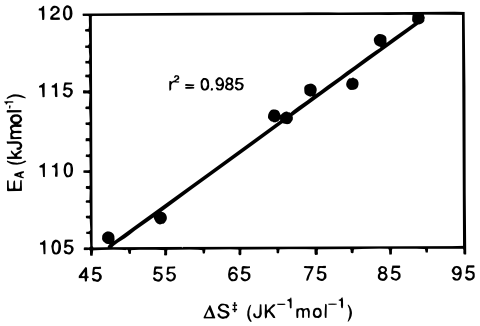


Figure 2. Isokinetic relationship for the acid-catalyzed solvolysis of **7a**−**h**.

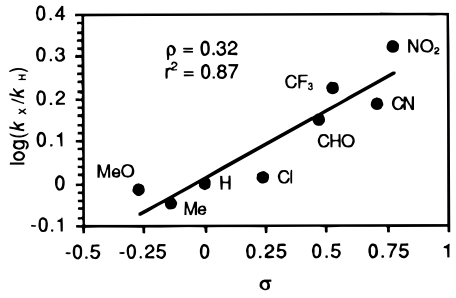
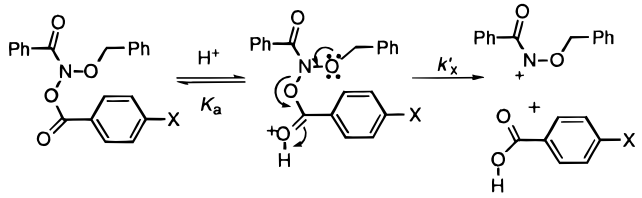


Figure 3. Hammett plot for acid-catalyzed solvolysis of **7a**−**h** at 308 K.

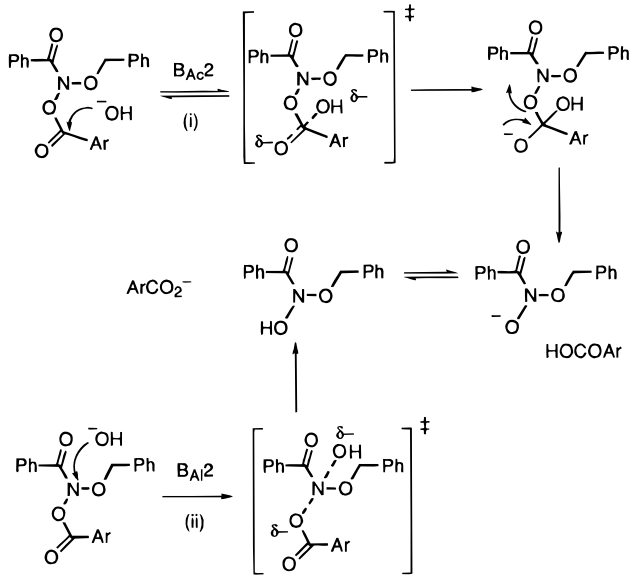
Scheme 1



would result in a significant lowering in energy of the N−OCO$^+$HAr σ^* orbital and the strong n_O−σ^*_{NO} anomeric interaction at nitrogen leads, in this case, to elimination of benzoic acid.[9] The effect can be viewed as being similar to that observed when thermally stable *N*-alkoxy-*N*-chlorobenzamides **9** are treated with Lewis acids. Complexation of chlorine with silver ions also results in the formation of *N*-alkoxy-*N*-acylnitrenium ions, and we and others have utilized this reaction in the formation of novel benzoxazines and benzoxazepines as well as *N*-alkoxybenzolactams by intramolecular cyclization of the electrophilic nitrenium ions onto aromatic rings.[22−27]

Reactions of 7 with Hydroxide Ions. Treatment of **7a**−**h**, with dilute aqueous sodium hydroxide at room temperature resulted in the rapid formation of benzyl benzoates. Similarly, *N*-acetoxy-*N*-butoxy-*p*-chlorobenzamide (**8a**) and *N*-acetoxy-*N*-(benzyloxy)benzamide (**8b**) afforded butyl *p*-chlorobenzoate and benzyl benzoate, respectively. A crossover experiment using both mutagens resulted in the exclusive formation of butyl *p*-chlorobenzoate (46%) and benzyl benzoate (43.3%) esters along with the hydrolysis products, *p*-chlorobenzoic and benzoic acids. This result indicates that ester formation involves an intramolecular process. The formation of esters in the acid-catalyzed solvolyses described above involves the intermediate hydroxamic acids **4**.[5,7] These hydroxamic acids can be formed from the reaction of the mutagen with base by two processes (Scheme 2, pathways i and ii) and the presence of excess base would ensure conversion to the conjugate anion **5** resulting in the HERON reaction. Pathway ii involves a $B_{Al}2$ rearside attack at the nitrogen, and while reactions of this type are rare, they would in this case release para-substituted benzoate anions and should proceed under moderate influence of the para substituents on the leaving group. Anomeric weakening of the N−OCOAr bond would be expected with electron-withdrawing para substituents since these would lower the energy of the

Scheme 2



(21) $\log(k_X/k_H) = \log(K_X/K_H) + \log(k'_X/k'_H) = -1.0\sigma^+ + \rho\sigma$ and a plot of $(\log(k_X/k_H) + \sigma^+)$ vs σ yields a better correlation ($r^2 = 0.94$) and a ρ value for the heterolysis step in the region of +1.5.

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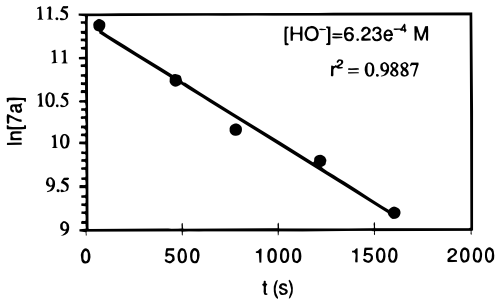

Figure 4. Pseudo-first-order kinetic plot for the reaction of **7a** with excess base at 275.4 K.

Table 2. Bimolecular Rate Constants for the Reaction of Hydroxide with 7 at 275.4 K

X	k_2^X (L mol^{-1} s^{-1})[a]	r^2	$\log(k_2^X/k_2^H)$
Me	2.27 (0.39)	0.9716	−0.144 (0.135)
H	3.16 (0.65)	0.9998	0.000 (0.149)
Cl	3.82 (0.69)	0.9990	0.082 (0.105)
CHO	4.91 (0.59)	0.9860	0.191 (0.118)
CN	6.97 (0.09)	0.9998	0.343 (0.100)
NO_2	8.14 (1.26)	0.9541	0.410 (0.129)

[a] Bimolecular rate constants were obtained from reactions at different concentrations of hydroxide and were determined by linear regression analysis of plots of k' vs [HO$^-$].

σ* orbital and $B_{Al}2$ substitution by base rather than rate-determining attack at the benzoyloxy carbonyl (the $B_{Ac}2$ process) may be more favorable in these cases.[9]

Pathway i involves the normal $B_{Ac}2$ mechanism and attack at the carbonyl of the benzoyloxy moiety, a process which should be under significantly greater control of a para substituent on the ring. Rates of base-catalyzed solvolyses of benzoate esters correlate with Hammett σ values with $\rho = 2.0-2.4$ and reflect the greater importance of attack at the carbonyl carbon.[19] In these systems, anomeric effects at nitrogen should disfavor this process since negative charge would be delocalized onto the acyloxy group.

To distinguish between pathways i and ii, the rates of base solvolyses were determined for members of series **7**.

The bimolecular reaction of **7a** at low temperature (275.4 K) and at low hydroxide concentration was monitored by analytical HPLC, and pseudo-first-order kinetics was obtained by ensuring that the relative concentration of base was significantly greater than the concentration of mutagen (Figure 4). Under these conditions d[**7a**]/dt $= -k'$[**7a**] where $k' = k_2$ [HO$^-$] and is the pseudo-first-order reaction rate constant.

The pseudo-first-order rate constant k' was linearly dependent upon the concentration of base and the bimolecular rate constants for the reaction of hydroxide with **7a**,**c**−**e**,**g**,**h**, k_2^X, at 275.4 K are given in Table 2.

The least-squares fit of $\log(k_2^X/k_2^H)$ versus Hammett σ values gave $\rho = 0.55$ (Figure 5) which is inconsistent with normal $B_{Ac}2$ hydrolysis process but in accordance with a $B_{Al}2$ mechanism (Scheme 2, pathway ii), an S_N2 process at nitrogen where substituents on the leaving group exert only a weak influence on the developing negative charge in the transition state. Bimolecular rate constants for the reaction of *N*-methylaniline with series **7** in methanol have recently been determined and also yield a σ correlation with modest, positive sensitivity ($\rho = 1.8$).[28] In those reactions, as was the case for the reaction between

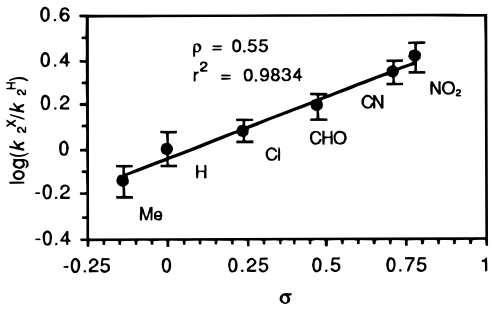

Figure 5. Hammett correlation for the base hydrolysis of **7a**,**c**−**e**,**g**,**h**.

Table 3. Ames Mutagenicity Levels of 7 at 1 μmol/plate

X	revertants[a]
MeO	1661.7
Me	2758.5
Bu^t	2511.1
H	2949.6
Cl	1528.8
CHO	841.1
CN	1104.3
NO_2	606.9

[a] Relative mutagenicities at 1 μmol/plate. The mutagenicity data was obtained over the concentration range 0−0.25 μmol, and the slopes (mutagenicities at 1 μmol/plate) were calculated from the linear dose−response region for each component. Results in different sets of assays were scaled relative to the mutagenicity of **7a**. Mutagen **7i** (X = Bu^t), not synthesized for the original rate studies, was available for Ames testing and is included as a member of the series.

N-methylaniline and *N*-acetoxy-*N*-butoxybenzamides **1**,[10] there is unequivocal evidence for attack of *N*-methylaniline through nitrogen at the benzamide nitrogen. Products are esters formed from the HERON reaction.[7,29]

The susceptibility of *N*-(acyloxy)-*N*-alkoxybenzamides to nucleophilic attack at nitrogen suggests that this process could play a role in the mutagenesis of this class of compounds; DNA nucleotides are inherently nucleophilic at nitrogen and oxygen centers.[13,30−33]

Mutagenicity of 7. The mutagenicity levels for **7** were obtained in *Salmonella* TA100 without metabolic activation. All substrates gave satisfactory dose response curves from which induced revertants at 1 μmol/plate were derived (Table 3). Relative mutagenicities show a possible correlation with Hammett σ constants with low sensitivity (Figure 6). A line of best fit ($r^2 = 0.79$) has a negative gradient with $\rho = -0.55$. While sensitivities to substituents in reactivity studies are similar, nucleophilic substitution at nitrogen (with HO$^-$, methylaniline[28]) and acid-catalyzed solvolysis are both favored by electron-withdrawing substituents resulting in Hammett plots of positive slope. This structure−activity relationship therefore suggests that the ease of neither process plays a significant role in determining mutagenicity.

The activity of chemical mutagens is a function of several properties: lipophilicity, which plays a role in transport across cell walls; stability toward side reactions

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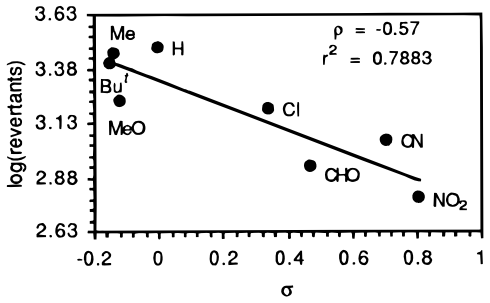

Figure 6. Correlation of mutagenicities of **7** at 1 μmol/plate with Hammett σ constants.

with adventitious, intracellular reagents the substrate encounters en route to the target DNA; residence time at the DNA and their reactivity with nucleotides when bound to DNA. If the putative correlation in Figure 6 is real, it suggests that mutagenicity may be controlled either by the basicity of the benzoate carbonyl oxygen, which would be enhanced with para electron-releasing groups or, alternatively, mutagenic activity may be inversely related to overall chemical reactivity. In other words it may be dependent upon their survival in the aqueous environment of the Ames assay.

Conclusions

N-(Benzyloxy)-*N*-(benzoyloxy)benzamides **7** are alkoxynitrenium ion sources under acidic aqueous/organic conditions. Like their *N*-acetoxy counterparts, they undergo solvolysis by the $A_{Al}1$ rather than the $A_{Ac}2$ mechanism. They are also susceptible to S_N2 reactions at nitrogen in base yielding the reactive hydroxamic esters **4**. Both processes are facilitated by electron-withdrawing para substituents on the benzoyloxy ring, and in the case of acid-catalyzed solvolysis, the heterolysis step (characterized by k' in Scheme 1) is more important than the protonation step (characterized by K_a in Scheme 1). The anomeric effect at nitrogen can be viewed as favoring both reaction processes.

All members of series **7** have been found to be mutagenic in *Salmonella* TA100 without metabolic activation. Para substituents on the benzoyloxy ring appear to affect mutagenicity in the opposite sense which suggests that their biological activity may be related either to their basicity or stability, rather than their ease of conversion to electrophilic alkoxynitrenium ions or susceptibility to nucleophilic attack at nitrogen. Studies to be published elsewhere have also pointed to a significant hydrophobic effect and residence time on DNA may thus be a significant factor.[5,34] In both respects, the mutagenic activity of *N*-(benzoyloxy)-*N*-(benzyloxy)benzamides **7** bears some resemblance to the antitumor activity of the duocarmycins where cytotoxicity is inversely correlated with rates of acid-catalyzed solvolysis and reactivity with DNA appears to be driven by hydrophobic binding.[35]

Experimental Section

General Methods. Ames tests were carried out by Dr. A. Bonin in the Department of Environmental Toxicology at the National Institute of Occupational Health and Safety in Sydney. Acetonitrile used was HiPerSolv, far UV grade (BDH). Ether refers to anhydrous diethyl ether stored over sodium wire. Dichloromethane (DCM) and acetone were distilled and dried over 4 Å molecular sieves. Ethyl acetate (EtOAc) and methanol (MeOH) were distilled before use. Anhydrous sodium sulfate was used for drying all organic mixtures. Flash chromatography was executed on columns loaded with Kieselgel 60 (Merck). Thin-layer chromatography was performed on aluminum sheets precoated with 0.2 mm of silica gel 60 F_{254} (Merck). Para-substituted benzoic acids and deuterioacetonitrile (99.5%) were purchased from Aldrich. The syntheses of butyl *N*-acetoxy-*p*-chlorobenzohydroxamate (**8a**), benzyl *N*-acetoxybenzohydroxamate (**8b**), and benzyl benzohydroxamate have been described previously.[3,4,36] ^{1}H NMR coupling constants (J) are reported in hertz.

Sodium Para-Substituted Benzoate Salts. The appropriate benzoic acid was treated with slightly less than a molar equivalent of aqueous sodium carbonate at room temperature. The filtrate was collected from the suspension and evaporated to dryness in an oven. Dried salts were used without further purification.

Benzyl *N*-Chlorobenzohydroxamate. Benzyl benzohydroxamate (3.41 g, 15 mmol) and *tert*-butyl hypochlorite (4.87 g, 45 mmol) in DCM were stirred for 5 h in the dark. Removal of solvent in vacuo provided the title compound which was used immediately without further purification: IR ($CDCl_3$) 1718 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.09 (2H, s), 7.26 (2H, m), 7.30 (3H, m), 7.40 (2H, t), 7.54 (1H, t), 7.68 (2H, d).

General Synthesis of Benzyl *N*-(4-Substitutedbenzoyloxy)benzohydroxamates. Benzyl *N*-chlorobenzohydroxamates were stirred in the dark at room temperature, with 1.4 molar equiv of the appropriate anhydrous sodium benzoate salts in dry acetone, for 12−72 h. The reaction was monitored by ^{1}H NMR, and filtration and concentration provided the benzyl *N*-(benzoyloxy)benzohydroxamate derivatives in high yields. Yields were determined by analytical HPLC analysis.

Benzyl *N*-(Benzoyloxy)benzohydroxamate (7a). Sodium benzoate (0.91 g, 6.3 mmol) was stirred at room temperature with benzyl *N*-chlorobenzohydroxamate (1.18 g, 4.5 mmol) in acetone for 48 h. Purification by flash chromatography (88% hexane:12% EtOAc) provided the title compound (60%): IR ($CDCl_3$) 1758, 1731 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.26 (2H, s), 7.26−7.40 (9H, m), 7.46 (1H, t, J = 8, *p*-Ar), 7.54 (1H, t, *p*″-Ar), 7.77 (2H, d, J = 8), 7.91 (2H, d, J = 8); ^{13}C NMR ($CDCl_3$) δ 77.44 (t), 127.07 (s), 128.18 (d), 128.35 (d), 128.49 (d), 128.54 (d), 128.97 (d), 129.11 (d), 129.85 (dt), 131.51 (s), 132.67 (dt), 133.92 (dt), 134.64 (s), 164.13 (s), 174.28 (s).

Benzyl *N*-(4-Methoxybenzoyloxy)benzohydroxamate (7b). Purification by flash chromatography (84% hexane:16% EtOAc) provided the title compound (39%): IR ($CDCl_3$) 1750 (ester CO), 1718 (amide CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.73 (3H, s), 5.26 (2H, s), 6.81 (2H, d), 7.25−7.47 (8H, m), 7.75 (2H, d, J = 8), 7.86 (2H, d, J = 8); ^{13}C NMR ($CDCl_3$) δ 55.18 (q), 77.31 (t), 113.73 (d), 118.92 (s), 128.01 (d), 128.20 (d), 128.36 (d), 128.80 (d), 128.98 (d), 131.55 (s), 131.94 (d), 132.43 (d), 134.69 (s), 163.70 (s), 164.04 (s), 174.25 (s).

Benzyl *N*-(4-Methylbenzoyloxy)benzohydroxamate (7c). Purification by flash chromatography (88% hexane:12% EtOAc) provided the title compound (47%): IR ($CDCl_3$) 1756 (ester CO), 1733 (amide CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.38 (3H, s), 5.26 (2H, s), 7.29 (2H, d), 7.3−7.4 (8H, m), 7.47 (1H, t, J = 8), 7.77 (2H, d, J = 8), 7.81 (2H, d, J = 8); ^{13}C NMR ($CDCl_3$) δ 21.61 (q), 77.58 (t), 124.27 (st), 128.15 (d), 128.34 (d), 128.50 (d), 128.99 (d), 129.11 (d), 129.23 (d), 129.94 (d), 131.64 (dt), 132.59 (s), 134.75 (s), 144.94 (s), 164.21 (s), 174.36 (s).

Benzyl *N*-(4-Chlorobenzoyloxy)benzohydroxamate (7d). Purification by flash chromatography (88% hexane:12% EtOAc) provided the title compound (83%): IR ($CDCl_3$) 1759 (ester CO), 1734 (amide CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.26 (2H, s), 7.26−7.40 (9H, m), 7.47 (1H, t, J = 8), 7.77 (2H, d, J = 8),

(34) Mutagenicity appears to increase with increasing aromatic character, i.e., *N*-acetoxy-*N*-alkoxybenzamides **1** < *N*-acetoxy-*N*-benzyloxybenzamides **2** < *N*-benzoyloxy-*N*-benzyloxybenzamides **7**. In addition, *N*-acetoxy-*N*-butoxy-2-naphthamide (5500 revertants) is more than 10 times more mutagenic than *N*-acetoxy-*N*-butoxy-2-benzamide (477 revertants) in TA100 at 1 μmol/plate.

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Table 4. Acid-Independent Rate Constants for Acid-Catalyzed Solvolysis of 7a−h at Various Temperatures

	ln *k*							
T (K)	NO_2	CN	CF_3	CHO	Cl	H	Me	MeO
298	−6.210	−6.368	−6.423	−6.5989	−6.9093	−6.9466	−7.0311	−7.0759
308	−4.571	−5.231	−4.971	−5.3054	−5.5661	−5.5069	−5.7158	−5.6648
318	−3.535	−3.671	−3.747	−3.6629	−3.9811	−4.1918	−4.2095	−3.8927
328	−2.363	−2.607	−2.173	−2.3597	−2.7931	−2.7380	−2.8198	−2.7562
338	−0.932	−1.377	−0.924	−1.3213	−1.5270	−1.5083	−1.4166	−1.3925
338						−1.4561		

Table 5. Pseudo-Unimolecular Rate Constants for Reaction of 7 with Hydroxide at 275.4 K

H		NO_2		Cl		Me		CN		CHO	
$10^4[HO^-]$ (mol L^{-1})	$10^3k'$ (s^{-1})	$10^4[HO^-]$ (mol L^{-1})	$10^3k'$ (s^{-1})	$10^4[HO^-]$ (mol L^{-1})	$10^3k'$ (s^{-1})	$10^4[HO^-]$ (mol L^{-1})	$10^3k'$ (s^{-1})	$10^4[HO^-]$ (mol L^{-1})	$10^3k'$ (s^{-1})	$10^4[HO^-]$ (mol L^{-1})	$10^3k'$ (s^{-1})
9.12	2.422	4.80	3.484	5.40	1.524	18.00	4.646	5.54	2.585	7.38	2.943
7.54	1.418	3.55	2.505	4.48	1.225	11.72	2.449	3.69	1.275	5.54	2.225
6.23	1.380	2.67	2.141	3.58	0.657	8.03	1.406	2.21	0.271	3.69	1.132
3.51	0.508	2.03	1.080	2.72	0.579	5.35	1.024				

7.82 (2H, d, $J = 8$); ^{13}C NMR ($CDCl_3$) δ 77.62 (t), 125.51 (st), 128.16 (d), 128.32 (d), 128.54 (d), 128.80 (d), 128.94 (d), 129.08 (d), 131.15 (dd), 131.32 (s), 132.73 (dt), 134.52 (s), 140.32 (s), 163.25 (s), 174.2 (s).

Benzyl *N*-(4-Formylbenzoyloxy)benzohydroxamate (7e). Purification by flash chromatography (85% hexane:15% EtOAc) provided the title compound (50%): IR ($CDCl_3$) 1761 (ester CO), 1735 (amide CO), 1707 (CHO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.27 (2H, s), 7.26 (3H, m), 7.39 (4H, m), 7.49 (1H, t), 7.78 (2H, d, $J = 8$), 7.89 (2H, d, $J = 8$), 8.06 (2H, d, $J = 8$), 10.06 (H, s); ^{13}C NMR ($CDCl_3$) δ 77.87 (t), 128.31 (d), 128.45 (d), 128.70 (d), 129.08 (d), 129.19 (d), 129.45 (d), 130.45 (dd), 131.26 (st), 132.10 (st), 132.97 (dt), 134.48 (s), 139.63 (s), 163.21 (s), 174.07 (s), 191.28 (d).

Benzyl *N*-(4-(Trifluoromethyl)benzoyloxy)benzohydroxamate (7f). Purification by flash chromatography (85% hexane:15% EtOAc) provided the title compound (78%): IR ($CDCl_3$) 1765 (ester CO), 1734 (amide CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.27 (2H, s), 7.26 (3H, m), 7.39 (4H, m), 7.52 (1H, t), 7.66 (2H, d, $J = 8$), 7.78 (2H, d, $J = 8$), 8.02 (2H, d, $J = 8$); ^{13}C NMR ($CDCl_3$) δ 77.91 (t), 125.55 (dq), 128.35 (d), 128.49 (d), 128.74 (d), 129.12 (d), 129.23 (d), 129.64 (s), 130.33 (dd), 130.59 (s), 131.32 (s), 133.0 (dt), 134.54 (s), 163.07 (s), 174.12 (s).

Benzyl *N*-(4-Cyanobenzoyloxy)benzohydroxamate (7g). Purification by flash chromatography (86% hexane:14% EtOAc) provided the title compound (34%): IR ($CDCl_3$) 2234, 2236 (CN),1763 (ester CO), 1732 (amide CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.26 (2H, s), 7.25−7.44 (8H, m), 7.55 (1H, t, $J = 8$), 7.73 (2H, d, $J = 8$), 7.78 (2H, d, $J = 8$), 8.00 (2H, d, $J = 8$); ^{13}C NMR ($CDCl_3$) δ 78.05 (t), 117.29 (st), 117.62 (s), 128.42 (d), 128.54 (d), 128.92 (d), 129.18 (d), 129.56 (d), 130.39 (d), 131.21 (s), 132.30 (d), 133.15 (d), 134.46 (s), 162.70 (s), 174.04 (s).

Benzyl *N*-(4-Nitrobenzoyloxy)benzohydroxamate (7h). Purification by flash chromatography (88% hexane:12% EtOAc) provided the title compound (55%): IR ($CDCl_3$) 1763 (ester CO), 1732 (amide CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.27 (2H, s), 7.27−7.43 (8H, m), 7.54 (1H, t, $J = 8$), 7.79 (2H, d, $J = 8$), 8.05 (2H, d, $J = 8$), 8.21 (2H, d); ^{13}C NMR ($CDCl_3$) δ 78.01 (t), 123.55 (d), 128.32 (d), 128.43 (d), 128.71 (d), 129.08 (d), 129.18 (d), 130.98 (d), 131.07 (dd), 132.67 (s), 133.12 (dt), 134.39 (s), 150.84 (s), 162.37 (s), 173.93 (s).

Benzyl *N*-(4-*tert*-Butylbenzoyloxy)benzohydromamate (7i). Purification by flash column chromatography (85% hexane:15% EtOAc) afforded pure benzyl *N*-(*p*-*tert*-butylbenzoyloxy)benzohydroxamate (44%): IR ($CDCl_3$) 1756 (ester CO), 1738 (amide CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (9H, s), 5.26 (2H, s), 7.25−7.44 (8H, m), 7.55 (1H, t), 7.73 (2H, d), 7.78 (2H, d), 8.00 (2H, d); ^{13}C NMR ($CDCl_3$) δ 30.99 (q), 34.89 (s), 77.58 (t), 124.27 (st), 128.15 (d), 128.34 (d), 128.50 (d), 128.99 (d), 129.11 (d), 129.23 (d), 129.94 (d), 131.64 (dt), 132.59 (s), 134.75 (s), 144.94 (s), 164.21 (s), 174.36 (s).

Kinetic Studies. Acid-Catalyzed Solvolysis. The acid-catalyzed solvolysis of the substrates was monitored in the variable-temperature probe of the Bruker AC300P NMR spectrometer (298−338 K). Substrate (10−40 mg) in CD_3CN (400 μL) was diluted with D_2O such that, after addition of an appropriate volume of a solution of sulfuric acid in D_2O (typically 0.5−1.5 molar), the ratio of CD_3CN:D_2O was constant at 3.81:1. The acid solution was mixed into the mixture to initiate reaction immediately prior to insertion in the probe. ^1H NMR spectra were acquired automatically at preprogrammed intervals, and the peak areas for the benzylic methylene singlet (and acetic acid) were obtained by integration. Arrhenius studies were carried out at an appropriate acid concentration to enable data collection at each temperature. A minimum of five temperatures between 298 and 338 K were used for each substrate, and rate data is presented in Table 4.

Reaction with Hydroxide. The progress of the reaction between hydroxide ion and mutagenic substrate was monitored by analytical HPLC. Typically, a 0.4×10^{-6} M solution of mutagen in 25% aqueous acetonitrile was treated with a 10−30 M excess of sodium hydroxide such that pseudo-unimolecular kinetics were valid for the disappearance of mutagen. The temperature for all solvolysis reactions was held at 275.4 ± 0.1 K by circulating water around the reaction vessel supplied from a large reservoir. Approximately 10 μL of sample was analyzed by HPLC injection as the reaction progressed. Naphthalene was used as the internal reference. Pseudo-unimolecular rate constants at various concentrations of base are presented in Table 5.

Crossover Experiment. A solution of butyl *N*-acetoxy-*p*-chlorobenzohydroxamate (0.013 mmol) and benzyl *N*-acetoxybenzohydroxamate (0.012 mmol) in 25% aqueous acetonitrile (50 mL) was equilibrated for 1 h at 298 K. Dilute aqueous NaOH (170 μL, 0.9 M) was added, and the reaction was complete within 60 s. Analytical HPLC analysis of the reaction mixture revealed the presence of benzyl benzoate (43.3%) and butyl *p*-chlorobenzoate (46.3%) and the complete absence of the crossover esters, benzyl *p*-chlorobenzoate and butyl benzoate.

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Supporting Information Available: 300 MHz ^1H NMR spectra for new compounds **7a−i** prepared in this work (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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