Strain Effects in Acyl Transfer Reactions. Part II.[1] The Kinetics of Aminolysis of Some *N*-Aryl-lactams

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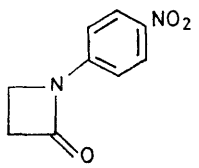
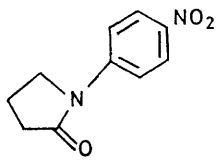
The reactions between aliphatic amines and 1-*p*-nitrophenylazetidin-2-one (I) show marked general base catalysis while hydroxide ion catalysis becomes dominant at high pH. The effect of strain in the β-lactam is manifest in the overall rate of reaction of piperidine with (I) which is at least 10^3 times greater than that with the corresponding γ-lactam (II). The Brønsted coefficient of 1·05 for the uncatalysed reactions of (I) with alkylamines shows that the transition state for these reactions is advanced along the reaction co-ordinate as a result of this ring strain and involves less C–N bond stretching than expected for the aminolysis of unstrained anilides.

AMINOLYSES of amides usually proceed with difficulty, a situation generally attributed to resonance stabilisation of the amide.[2] Consequently mechanistic studies have been restricted to cases which involve either activated amides [2-4] or amines of enhanced nucleophilicity.[5] The majority of cases investigated exhibit general and sometimes specific acid–base catalysis which commonly has the greatest influence on reactions with the largest activation energy. Only in the cases of the acylpyridinium [6,7] and 1-acetyl-3-methylimidazolium cations [3] is catalysis not observed to augment nucleophilic reaction, a consequence of the greatly enhanced reactivity which results from the imposition of unit positive charge on the heterocyclic ring. As a broad generalisation, it appears that amides activated in the acyl group, as in the case of trifluoroacetanilide, show general base catalysis of aminolysis while those activated in the amino-group, such as acetylimidazole, display kinetic general acid catalysis.[3]

The simple aminolysis of penicillins under physiological conditions is known to be a slow process [8] though the principal antigenic determinant in penicillin allergy is the binding of the penicilloyl group through an imide



(I)



(II)

linkage to protein [9] which results from aminolysis of the penicillin β-lactam function.[10,11] It thus appears that catalysis is likely to play a considerable part in the biochemical antigenicity reactions of antibiotics. In this respect, Bundgaard has demonstrated that imidazole accelerates the isomerisation of penicillins into penicillenic acids and has suggested that this process is assisted both by general acid and by general base catalysis.[12]

The activity of the penicillins is thought to originate partly in angle strain and partly in torsional strain of the β-lactam ring.[13,14] It is thus desirable to evaluate these effects separately in the aminolyses of amides. This paper reports data on the aminolyses of β- and γ-lactams by moderately basic amines and it analyses the nature of the catalytic processes which assist these reactions.

EXPERIMENTAL

Materials.—1-*p*-Nitrophenylazetidin-2-one, 1-*p*-nitrophenyl-2-pyrrolidone, and *N*-methyl-*p*-nitroacetanilide were prepared as before.[1] Butylamine, ethanolamine, morpholine, piperidine, and propylamine were distilled and converted into their hydrochlorides which were crystallised from 95% ethanol or ethanol–ether and dried *in vacuo* before use. Glass-distilled water was used throughout.

Apparatus.—A Radiometer PHM 26 in conjunction with G202B or G2222B glass electrodes was used for measurement of pH. U.v. spectral data were recorded using a Gilford 240 or Unicam SP 1800 spectrophotometer, both having cell temperature controlled by water circulated from a Haake model F thermostat.

Kinetic Measurements.—All reactions were run in glass-distilled water at 298 ± 0·1 K with ionic strength maintained at 0·1 with AR grade potassium chloride in 10 mm quartz cuvettes. Reactions were initiated by the addition of substrate in pure dioxan (0·05 ml) to the appropriate amine buffer (3 ml) containing 10^{-5}M-ethylenediaminetetraacetic acid to give a final substrate concentration of 0·3—1×10^{-4}M. The appearance of product was monitored continuously by increase in optical density at 410 nm and pH values of reaction solutions were determined at the beginning and end of each run.

Kinetic data were collected for at least three half-lives and first-order rate constants obtained from plots of log $(A_t - A_0)$ against time where possible. Rate constants for very slow reactions were determined either by Guggenheim's method [15] or from initial slopes. All the reactions observed displayed good first-order kinetic behaviour.

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Product Analysis.—A sample of 1-*p*-nitrophenylazetidin-2-one was allowed to react with 1M-butylamine solution (90% free base) at room temperature. After several hours the solution was diluted with water, acidified to pH 6 with hydrochloric acid, and thrice extracted with ether. Evaporation of the dried ether extracts gave a yellow powder which was shown to be homogeneous by t.l.c. Its i.r. spectrum showed strong absorption at 1640 cm^{-1} (aliphatic amide) and the n.m.r. spectrum showed significant differences from, though a similar pattern of signals to, that of *N*-(3-bromopropionyl)-*p*-nitroaniline [1] in addition to the signals associated with the C_4H_9N group. Potentiometric titration of this product revealed no titrateable function in the pH range 6—11.

TABLE 1

Experimental data for the aminolyses of 1-*p*-nitrophenylazetidin-2-one

Amine	pH	Number of runs	[$Amine_{tot}$]/M	% Free base	$k_2'/l^2\ mol^{-2}\ s^{-2}$
Morpholine	8·76—8·83	5	0·2—1·0	41	$3·88 \times 10^{-6}$
	9·93—10·0	5	0·2—1·0	91	$8·0 \times 10^{-6}$
	13·37—13·54	7	0—0·8	100 [a]	*c*
Ethanolamine	9·80—9·90	6	0·1—1·0	45·5	$7·45 \times 10^{-5}$
	10·95—10·98	4	0·2—0·8	91	$1·50 \times 10^{-4}$
Butylamine	9·94—9·82	6	0·1—1·0	10	$1·70 \times 10^{-4}$
	10·41—10·53	5	0·2—1·0	30	$4·15 \times 10^{-4}$
	10·87—10·92	6	0·1—1·0	50	$8·15 \times 10^{-4}$
	11·20—11·27	6	0·1—1·0	70	$1·13 \times 10^{-3}$
	11·37—11·49	8	0·05—1·0	80	$1·28 \times 10^{-3}$
	11·70—11·78	6	0·1—1·0	90	$1·53 \times 10^{-3}$
Propylamine	10·80—10·98	7	0·05—1·0	40	$5·60 \times 10^{-4}$
	11·59—11·68	6	0·1—1·0	80	$1·21 \times 10^{-3}$
Piperidine	11·52—11·59	6	0·1—1·0	50	$1·29 \times 10^{-3}$
	12·00—12·15	6	0·1—1·0	80	$2·0 \times 10^{-3}$
	13·72—13·85	8	0—0·7	100 [b]	*c*
[Dioxan]	13·75—13·80	4	0·1—0·75	*b*	

[a] In the presence of 0·2N-potassium hydroxide. [b] In the presence of 0·5N-potassium hydroxide. [c] Not detectable.

TABLE 2

Calculated rate constants for aminolysis reactions of 1-*p*-nitrophenylazetidin-2-one

Amine	pK_a'	$k_1/l\ mol^{-1}\ s^{-1}$	$k_2/l^2\ mol^{-2}\ s^{-1}$	$k_3/l^2\ mol^{-2}\ s^{-1}$
Morpholine	8·95 ± 0·04	$3·5 \pm 1·2 \times 10^{-7}$	$8·80 \pm 0·25 \times 10^{-6}$	3×10^{-3}
Ethanolamine	9·95 ± 0·1	$2·2 \pm 0·4 \times 10^{-5}$	$1·64 \pm 0·13 \times 10^{-4}$	$1·7 \times 10^{-2}$
Butylamine	10·87 ± 0·05	$1·47 \pm 0·33 \times 10^{-4}$	$0·25 \pm 0·02 \times 10^{-3}$	0·25 ± 0·02
Propylamine	10·97 ± 0·07	$2·5 \pm 1·0 \times 10^{-4}$	$1·46 \pm 0·10 \times 10^{-3}$	0·08 ± 0·04
Piperidine	11·57 ± 0·05	*ca.* 3×10^{-4}	$2·56 \pm 0·14 \times 10^{-3}$	0·31 ± 0·01

RESULTS

The observed rates of reaction of 1-*p*-nitrophenyl-2-piperidone and *N*-methyl-*p*-nitroacetanilide in piperidine or butylamine buffers do not differ significantly from the calculated rates for hydrolysis at low amine concentrations and become slower at higher buffer concentration. A similar retardation of hydrolysis of 1-*p*-nitrophenyl-2-pyrrolidone results from the addition either of dioxan or of piperidine to solutions in 0·5N-potassium hydroxide (Figure 1). The differences between the piperidine reaction rates and the linear-regression-derived slope for dioxan inhibition of hydrolysis lead to an estimated maximum value for the apparent second-order rate constant of 10^{-4} l mol^{-1} s^{-1} for piperidine reaction with the γ-lactam. There is no significant inhibition of the alkaline hydrolysis of the β-lactam (I) by dioxan in concentrations up to 0·75M.

The rates of aminolysis of (I) were observed in aqueous solutions of butylamine, ethanolamine, morpholine, piperidine, and propylamine at various concentrations of amine and different buffer ratios as set out in Table 1. The apparent aminolysis rate constants were obtained as usual as the difference between the observed rate constant and the calculated rate for hydrolysis at the same hydroxide activity (derived from the alkaline hydrolysis rate constant of $4·42 \times 10^{-2}$ l^2 mol^{-2} s^{-1}). In the majority of experiments for all amine buffers at concentrations less than 0·2M, the rate of hydrolysis exceeds the rate of aminolysis.

For the most weakly basic amine, morpholine, the aminolysis rate constants showed greater than first-order dependence on the concentration of morpholine and the apparent second-order rate constants exhibit linear dependence on the concentration of free amine. From these data

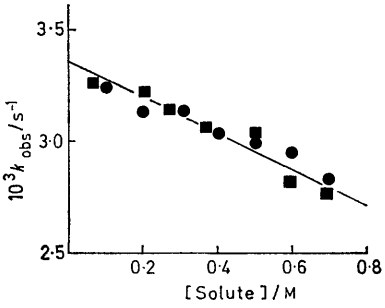

FIGURE 1 Variation in rate constants for the alkaline hydrolysis of 1-*p*-nitrophenyl-2-pyrrolidone in 0·5N-potassium hydroxide at unit ionic strength as a function of the concentration of 1,4-dioxan, ■; and of piperidine, ●. Slope obtained by linear regression analysis of the data for the dioxan reactions

the rate constants for water-catalysed and general base-catalysed reactions were computed by linear regression analysis (Table 2).

Aminolyses of (I) by butylamine, ethanolamine, and

propylamine are similar to the morpholine reactions but the intercepts of plots of the apparent second-order rate constants against concentration of free amine increase with increasing pH (Figure 2). The slopes of these plots for butylamine are simply proportional to the mole fraction of free amine in the buffers used while the intercepts show a linear dependence on hydroxide concentration from which can be derived the rate constants for hydroxide- and water-catalysed aminolysis. Thus for these three amines, the rate law is established as that described in equation (1).

$$k_{obs} - k_{hyd} = k_1[\text{Amine}] + k_2[\text{Amine}]^2 + k_3[OH^-][\text{Amine}] \quad (1)$$

Multiple linear regression analysis of the data from all the reactions for which the concentration of free butylamine did not exceed 0·5M permitted the computation of the rate constants given in Table 2 and the calculation of the theoretical curves in Figure 2. Similar analyses were performed to evaluate the rate constants for the reactions of (I) with ethanolamine and propylamine.

The deviations from predicted behaviour seen for butylamine (Figure 2) and for propylamine are most marked for the former and result at high concentration of free amine in the attainment of a limiting value for the apparent first-order aminolysis rate constant with a consequent fall in that of the apparent second-order rate constant.

The reaction of piperidine with (I) shows greater than first-order dependence on the concentration of free amine when the buffer ratio [amine]/[amine cation] is <5 but at high pH the reaction exhibits simple first-order dependence on amine and on hydroxide concentration (Figure 3). The hydroxide catalytic rate constant was computed by analysis of the data obtained at high pH and employed to evaluate the pH-independent aminolysis term, ($k_{obs} - 0{\cdot}0442[OH^-] - k_3[OH^-][\text{Piperidine}_{free}]$), from which data were computed the rate constants for the water- and amine-catalysed reactions (Table 2).

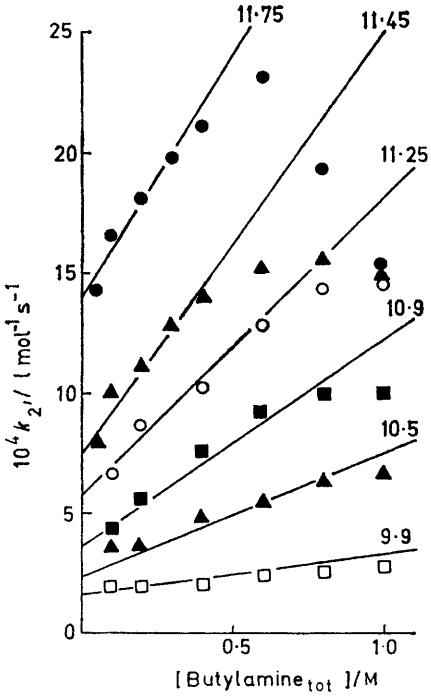

FIGURE 2 Variation of the apparent second-order rate constants for the reaction of (I) with butylamine as a function of butylamine concentration at the pH values indicated. Curves calculated from equation (1) and rate constants in Table 2

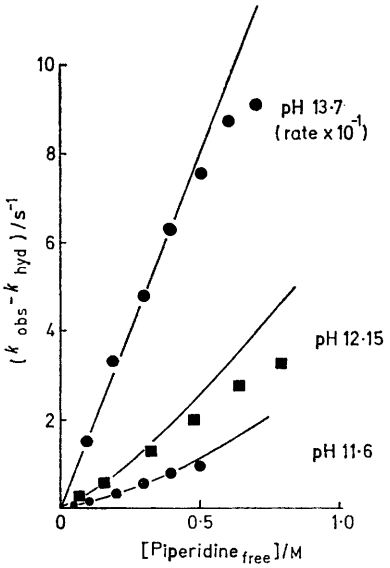

FIGURE 3 Variation of the apparent first-order rate constants for the reaction of (I) with piperidine as a function of piperidine concentration. Slopes calculated from equation (1) and rate constants in Table 2 (the ordinate scale for reactions at pH 13·7 is 10-fold larger than that inscribed)

DISCUSSION

Analysis of the data for all amines leads to a common rate law, equation (1), for their reaction with 1-*p*-nitrophenylazetidin-2-one (I) which contains water-, amine-, and hydroxide-catalysed terms.

The relative contribution of each of these terms depends on the basicity of the amine. Thus in the cases of butylamine and propylamine evaluation of the buffer catalysis at several ratios of amine : amine hydrochloride showed that the whole of the catalysis is donated by the free amine and none is contributed by the ammonium cation. The same conclusion emerges from analysis of the more limited data obtained for catalysis by other amine buffers, and for these two amines the reactions at high pH proceed predominantly *via* the specific base-catalysed pathway while at low pH and low amine concentration the major part of the reaction involves the uncatalysed aminolysis of (I) (Figure 2). However, for the most basic amine studied, piperidine, the reaction at pH 13·7 is almost exclusively a hydroxide-catalysed aminolysis (Figure 3) and this process makes a significant contribution to all reactions even at the lowest pH at which a measurable aminolysis process is detectable. In consequence, the apparent rate constant for the water-catalysed piperidinolysis lies within the error for the first-order rate constant calculated for the hydroxide-catalysed reaction, $\Delta k_3[OH^-]$. Consequently, the latter value was adopted as a more realistic estimate of k_1 for piperidine (Table 2). In addition, it was not possible to make an accurate evaluation of k_3 for

morpholine because at the high pH values where this term would become dominant, the major part of the reaction of (I) involved simple alkaline hydrolysis. The problems attendant on the alkaline ionisation of ethanolamine also impeded evaluation of k_3 for this amine.

The accuracy of the apparent aminolysis rate constant for the reactions of (I) with the basic amines at high pH depends much on the independence of the rate of alkaline hydrolysis of (I) of the concentration of free amine present. Although the alkaline hydrolysis of the γ-lactam (II) is clearly retarded both by dioxan and by piperidine (Figure 1), there is no comparable effect of dioxan on the hydrolysis of (I) up to 0·75M-dioxan and, in any case, any small effect of piperidine on this hydrolysis would be negligible compared to the large rate of the hydroxide-catalysed aminolysis (Figure 3). The disparity in response of (I) and (II) to the presence of dioxan in the solution may result from a greater degree of charge dispersion in the two transition states.

The rates calculated from the rate constants in Table 2 and equation (1) give a satisfactory fit to the experimental data for reactions spanning a 10^5 fold range of rate with the exception of those involving a high concentration of the most basic amines used, which show a marked depression in rate below that predicted. In the extreme case observed the effect for butylamine is so pronounced as to correspond to a change in rate law from second- to zero-order in butylamine.* Such a rate depression is unlikely to indicate a change in mechanism but probably originates in changes in the properties of the solvent resulting from the exchange of the potassium for the ammonium cation.[16,17] Alternatively, it is possible that the buffer catalysis is a diffusion-controlled proton transport which would be materially retarded by any increase in the solvent viscosity which would be consequent upon changes in the concentration of organic solute.[18]

It can be concluded that for all the amines examined, the pattern of their reactivity towards the β-lactam (I) does not involve a change in rate-determining step under the conditions explored. In this light, it is possible to analyse the pattern of the catalysis which assists these aminolyses and to attempt to uncover the nature and the magnitude of the contribution made by ring strain in the β-lactam.

1-*p*-Nitrophenylazetidin-2-one (I) is a very weakly electrophilic acylating agent and is considerably less reactive than alkyl carboxylates.[16,17] Nonetheless, it is unusually reactive for a neutral amide † and its reactions thus permit the observation of the relatively symmetrical acyl group transfer between two amino-functions, comparable to those between the heterocyclic nitrogens of imidazole,[2,3] pyridine,[6] and triazole[19] functions and amines.

For the general base-catalysed aminolyses, the correlation of reactivity with basicity, corrected in the usual way for statistical effects,[17] gives a Brønsted coefficient of 0·97 with a lower β value of 0·75 for the hydroxide-assisted aminolyses (Figure 4). The latter process cannot result from attack of the amide anion in (I) since such a reaction would have to exceed the limit of diffusion in order to give the observed rate. Thus hydroxide must be acting either as a general base or be involved in a diffusion-controlled proton transport. The criteria set out by Jencks[18] for the operation of concerted general acid–base catalysis are satisfied with

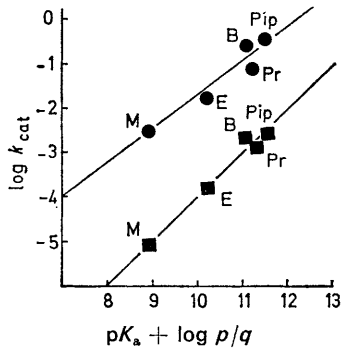

FIGURE 4 Brønsted plot for the amine-catalysed, ■, and hydroxide-catalysed, ●, reactions of (I) with amines as a function of their basicity: B, butylamine; E, ethanolamine; M, morpholine; Pip, piperidine; Pr, propylamine

respect to both hydroxide- and amine-catalysed aminolyses of the lactam (I). Moreover, the Brønsted coefficient of 0·75 means that the negative charge on the hydroxide catalyst has diminished by some 0·75 units in the transition state, significantly less than would be expected for the complete transfer of a proton in a diffusion-controlled encounter. Consequently, it seems reasonable to consider both the catalytic processes as cases of concerted general base catalysis.

The complete analysis of the mechanism of aminolysis of the β-lactam (I) requires a knowledge of the partitioning of the corresponding tetrahedral addition intermediate of amide and lactam, as described for the aminolysis of methyl formate.[16] Since the 2-imino-1-*p*-nitrophenylazetidine needed for such a study is unknown, the assignment of mechanism must hinge on the interpretation of linear free energy relationships.

Jencks has established a classification of acyl transfer reactions into three types according to their dependence on the basicities of attacking and leaving groups and without taking a stance concerning the existence of a stable intermediate.[6] These correspond to early, inter-

* This behaviour cannot result from the rapid conversion of the β-lactam into a non-productive intermediate since rapid scanning of the spectrum between 230 and 510 nm of the reaction mixture at high amine concentration throughout the duration of the reaction shows the existence of two isobestic points at 274 and 358 nm. This is behaviour characteristic of a binary system relating reactant to product.

† Acetylimidazole is far more reactive than (I) but owes its activity [3] to the relatively low basicity of the imidazole anion leaving group.

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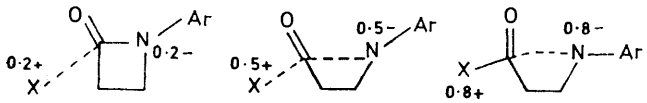
mediate, and late transition states with respect to bond-forming and bond-breaking processes. For the aminolysis of (I) they are represented as (III), (IV), and



(III) (IV) (V)

(V) respectively. It is the intermediate of the three which is characterised by a Brønsted coefficient in the range 0·7—1·0, out of a possible range of 0—1·7, and is typical of the reactions of amines with phenyl carboxylates and with acetylimidazolium and acetylpyridinium cations where the entering and leaving groups have similar pK_a values. However, the aminolysis of *p*-nitroacetanilide, where the attacking and leaving groups have very different basicities, would be expected to exhibit a β value of 1·2—1·6 corresponding to a transition state like (V).

In fact, the water-catalysed aminolyses of (I) show a Brønsted coefficient of 1·05 for the attacking group (Figure 5) which clearly identifies the transition state as

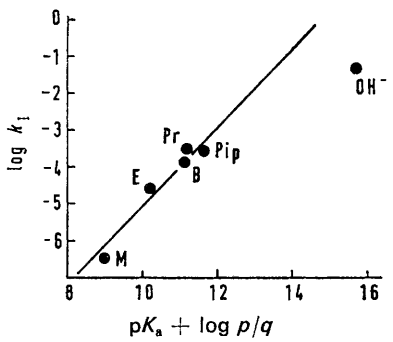


FIGURE 5 Brønsted plot for the nucleophilic reactions of amines with (I) as a function of their basicity: B, butylamine; E, ethanolamine; M, morpholine; Pip, piperidine; Pr, propylamine

resembling (IV). It is thus apparent that the strain in the β-lactam ring offsets the disparity in basicity of the attacking and leaving groups in the ring-opening reaction and advances the transition state to an earlier point on the reaction co-ordinate, with corresponding lesser charge development on both attacking and leaving groups (IV). A precise evaluation of this strain effect is made difficult by the very low reactivity of the corresponding pyrrolidone (II) but it must be not less than a factor of 10,[3] corresponding to the ratio of reaction rates of (I) and (II) with piperidine under identical conditions.

While the foregoing analysis presupposes a *linear* free energy relationship for nucleophilic attack of amines on (I), it is evident (Figure 5) that hydroxide is an unusually weak nucleophile towards this β-lactam. Indeed, this experimental data appears to support better a non-linear free energy relationship in which the sensitivity of (I) to the basicity of the nucleophile is large for weaker amines but small for strong bases. This conclusion is given independent support from the fact that comparison of the alkaline hydrolyses of (I) and 1-(3-trifluoromethylphenyl)azetidin-2-one shows that a 10^6 difference in the basicities of the leaving groups [20] corresponds to only a 10-fold factor in reactivity. This gives a Brønsted coefficient of 0·17 for the leaving group and indicates an early transition state like (III). These two considerations therefore suggest that for the strongest nucleophiles, the strain in the β-lactam ring advances the transition state even further until it involves very little stretching of the C–N bond in the ring.

It can be thus seen that the contribution of ring strain to the enhancement of alkaline hydrolysis of (I) results almost solely from the effects of rehybridisation at C-2 in the transition state and is qualitatively similar to the bond-angle effect characteristic of cyclobutanone activation.[21] On the other hand, strain acceleration for aminolysis of (I) also involves relief of the ring strain by considerable stretching of the ring C–N bond in the transition state (IV). It seems likely that this latter effect will increase in relative importance for attack by weak nucleophiles until breakdown of a tetrahedral intermediate becomes the rate-determining feature of the reaction and external catalysis can only be effective if applied to the leaving group.

It is evident, therefore, that this ring-strain catalysis can play a significant part in the reactions of β-lactams with moderately weak nucleophiles in biological systems and yet does not restrict the opportunity for additional general acid–base catalysis.

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