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Solvent Dependence of Carboxylic Acid Condensations with Dicyclohexylcarbodiimide[1]

Bruce J. Balcom and Nils O. Petersen*

Department of Chemistry, University of Western Ontario, London, Ontario, Canada, N6A 5B7

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The kinetics of a model dicyclohexylcarbodiimide (DCC)–carboxylic acid condensation have been studied in six organic solvents at low concentrations of DCC and acid. The first bimolecular rate constant for acid addition to DCC (k_1), to give an *O*-acylisourea, and the second bimolecular rate constant (k_3) for acid addition to this intermediate, to give an anhydride, are both dependent on the interaction between acid molecules and solvent. These rate constants correlate extremely well with measures of the solvent's hydrogen-bond accepting ability. The rate constant for intramolecular rearrangement from the *O*-acylisourea to the *N*-acylurea (k_2) is, by contrast, independent of the solvent. Formation of dimers of the acid is not important at these low concentrations, but it appears the reaction favors formation of anhydride whenever retardation of the k_1 and k_3 rate constants is minimized. This occurs in solvents in which the acid is least soluble. There is, therefore, a delicate interplay between the conditions that favor the pathway producing anhydride: on the one hand high concentrations are favorable while on the other solvents that offer limited solubility are desirable. For synthetic purposes, where anhydride is the more useful product, a careful optimization of solubility and fast k_1 and k_3 rate constants are required.

Introduction

Dicyclohexylcarbodiimide (DCC) has, over the past 25 years, proven to be an exceptionally useful reagent.[2-4] The carbodiimide coupling reaction we have investigated is widely used in the fields of synthetic organic chemistry,[4-8] peptide synthesis,[4,9-12] enzymology[3,13-17] and polymer chemistry.[4,18-22]

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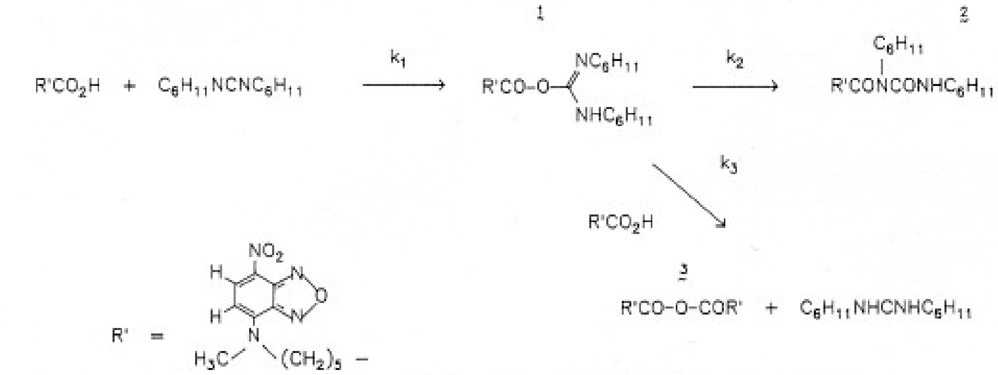
Scheme I



The reaction mechanism (Scheme I) was proposed by Khorana[2] in 1953. Results of subsequent investigators, based mostly on product data, have supported this proposal. Although the *O*-acylisourea (**1**) intermediate has not been isolated (an *O*-acylisourea has been observed in solution[23] and in a peptide synthesis[24]), studies on model intramolecular *O*-acylisoureas have supported its existence.[25]

The major imperfection in the reaction is a significant side reaction that yields, via an intramolecular O → N acyl migration, the undesirable *N*-acylurea (**2**) product. Additives such as *N*-hydroxysuccinimide, which trap the *O*-acylisourea, have ameliorated the problem of this rearrangement in certain cases. From a synthetic point of view, however, one still wishes the anhydride to effectively compete with this rearrangement. Scheme I shows that the efficiency of anhydride production is dependent on acid concentration.

Our original intent was to derivatize rare, biologically significant isoprenoid alcohols using DCC and *N*-methyl-*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-6-aminohexanoic acid (NBD-acid), a fluorescent carboxylic acid ($R'CO_2H$, Scheme I). Proceeding through the anhydride and utilizing a 4-pyrrolidinopyridine catalyst,[8] we hoped to produce these delicate esters in high yield and purity. Unfortunately, NBD-acid proved to have limited solubility in most of the organic solvents suggested in the literature.

Initial experiments indicated a striking dependence of the reaction efficiency on both the acid concentration and the nature of the solvent. Through a systematic evaluation of the solvent dependence we hoped to learn more about the fundamental nature of the reaction, thereby permitting a rational choice of the best solvent for synthetic purposes.

Environmental effects on the DCC condensation reaction have taken on an added importance with the increasing use of DCC, and other carbodiimides, to modify or inhibit the function of carboxylic acid residues in proteins[15-17] and polymer resins.[20-22] The final product in most cases is uncertain and may be sensitive to the location of the carboxylic acid residue.

Using reverse phase HPLC and a programmable sample injector/dilutor, we observed the redistribution of the NBD probe amongst the acid, *N*-acylurea, and anhydride as a function of time and solvent. The intense absorption of the NBD moiety[26] (λ_{max} = 476 nm, ϵ_{max} = 3.44 × 10^4 mol^{-1} L cm^{-1}) makes it a convenient tag to follow the course of the reaction.

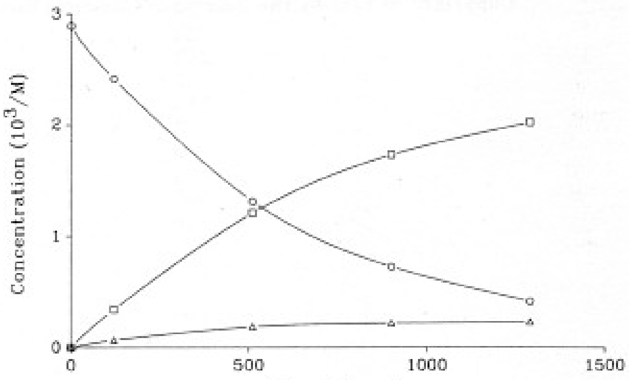


Figure 1. Time course of a sample reaction in acetonitrile. Concentrations are (○) [NBD-acid], (□) [*N*-acylurea], and (△) [anhydride]. The sum of [NBD-acid], [*N*-acylurea], and twice [anhydride] is a constant, independent of time. The factor 2 allows for the two NBD-acid molecules that form each anhydride molecule. Initial conditions $[\text{NBD-acid}]_0$ = 2.89 × 10^{-3} M, $[\text{DCC}]_0$ 5.26 × 10^{-2} M.

Results

Figure 1 shows an example of the disappearance of NBD–acid (A), with concomitant appearance of both anhydride (AA) and *N*-acylurea (NA), as the reaction proceeds. Concentrations are determined by the UV/vis absorption of NBD present in each species.

The rate of acid decay is analyzed in terms of the mechanism outlined in Scheme I. If one presumes that formation of *O*-acylisourea (AD*) is the rate-limiting step and DCC (D) is present in large excess, the result is

$$\frac{d[A]}{dt} = -k_1[A][D] - k_3[A][AD^*] \qquad (1)$$

With a steady state concentration of AD*, eq 1 integrates to yield

$$\ln [A] = \ln C^{1/2} + \ln [A]_0 - k_1[D]t \qquad (2)$$

In this equation the variable C is

$$C = (k_2 + 2k_3[A])/(k_2 + 2k_3[A]_0) \qquad (3)$$

From eq 2 it is possible to determine k_1, the second-order rate constant, by plotting the natural log of acid concentration versus time. A summary of these results is found in Table I for all solvents at a variety of initial concentrations. Good pseudo-first-order kinetics ($r^2 > 0.98$) are

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(26) Measured in ethanol.

Table I

solvent[a]	[NBD-acid]$_0$ (10^4 M^{-1})	k_1 (10^2 M s)[b]	k_3/k_2 (M)[c]
methylene chloride	0.59	34.5	d
	1.18	41.8	691
	1.62	38.0	624
	1.77[e]	42.4	697
	2.30	37.5	590
	2.35	42.0	758
nitrobenzene	6.6	22.0	324
	13.2	22.4	306
	19.8	24.4	324
	26.4	23.6	320
nitropropane	2.36	15.1	223
	4.73	15.1	234
	7.09	14.0	236
	9.45	14.4	235
acetonitrile	14.4	2.64	65.7
	17.4[f]	2.83	72.4
	28.9	2.71	73.9
	43.4	2.97	73.1
	57.8	2.95	77.8
acetone	4.8	0.75	d
	11.3	1.03	15.9
	11.5	0.52	d
	23.0	0.95	17.0
	33.8	1.20	15.9
	37.6[g]	0.74	18.2
	45.0	1.21	17.8
	46.0[g]	1.00	21.2
	47.2[g]	0.94	18.7
	69.0	0.91	19.6
	92.0	0.89	20.0
tetrahydrofuran	27.7	0.45	9.3

[a] Solvents arranged in order of NBD-acid solubility. [b] Average values: methylene chloride, 41; nitrobenzene, 23; nitropropane, 15; acetonitrile, 2.8; acetone, 0.97; tetrahydrofuran, 0.45. [c] Average values; methylene chloride, 6.8×10^2; nitrobenzene, 3.2×10^2; nitropropane, 2.3×10^2; acetonitrile, 73; acetone, 19; tetrahydrofuran, 9.3. [d] Anhydride yield too meagre to allow determination of (k_3/k_2). [e] Average of six experiments, [NBD-acid]$_0$ identical. [f] Average of five experiments, [NBD-acid]$_0$ identical. [g] Average of two experiments, [NBD-acid]$_0$ identical.

found for all solvents. Using independently determined values of the ratio k_3/k_2 (vide infra), one may calculate the value of the $\ln C^{1/2}$ term. This term has no effect on the observed slope and intercept of the experimental ln [A] versus time plots for decays of 3 half-lives.

Since the reaction pathway branches after the rate-limiting step, it is impossible to measure independently the rate constants k_3 and k_2. However, by following the growth of anhydride (AA) relative to N-acylurea (NA), one may determine their ratio since

$$\frac{d[AA]}{d[NA]} = (k_3/k_2)[A] \tag{4}$$

Because the NBD absorbance is conserved (Figure 1), one knows that

$$[A] = [A]_0 - 2[AA] - [NA] \tag{5}$$

Substituting eq 5 into eq 4 yields, after applying an integrating factor

$$[AA] = \frac{(2(k_3/k_2)[A]_0 + 1)(\exp(-2(k_3/k_2)[NA]) - 1)}{-4(k_3/k_2)} - [NA]/2 \tag{6}$$

Expanding the exponential and regrouping by powers of (k_3/k_2) gives

$$[AA] = (k_3/k_2)([A]_0[NA]^2/2) + (k_3/k_2)^2([NA]^3/3 - [A]_0[NA]^2) + \ldots \tag{7}$$

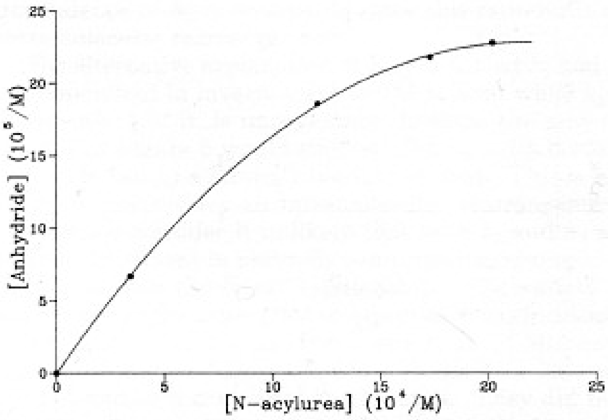

Figure 2. Growth of anhydride concentration relative to N-acylurea concentration for the sample reaction in Figure 1. These concentrations are implicit functions of time. The slope of the experimental curve is governed by eq 4. As the reaction progresses, less available NBD-acid means that the pathway producing anhydride becomes progressively less competitive. The solid line is the best fit result to eq 7.

The k_3/k_2 ratio (Table I) is determined by a fit to this equation using a single variable, nonlinear, least-squares algorithm[27] keeping up to the seventh power of k_3/k_2. The power series expansion accurately reproduces the experimental curve of anhydride versus N-acylurea (Figure 2) when the appropriate value of k_3/k_2 is determined.

The major source of errors in this study appears to be the day to day handling and preparation of samples. Values of k_1 and k_3/k_2 determined from a common set of solutions on the same day generally showed relative uncertainties of less than 5%. When a larger number of experiments were attempted over several days, relative uncertainties increased to somewhat over 10%. Hence, error bars that reflect relative errors of 10% were assigned to the kinetic results in all solvents.

Additives had minimal effects on the reaction. Equimolar amounts (with respect to acid) of either water, triethylamine, or pyridine were added at a number of initial acid concentrations with no deviation from the kinetics observed in their absence. A large excess, relative to acid, of triethylamine or pyridine was required to slow the reaction to any measurable extent.

Control experiments exclude the possibility that the NBD moiety is affecting the kinetics of the reaction. Addition of NBD-amine, up to equimolar acid, did not change the rate or extent of acid decay in a series of reactions in methylene chloride.

Discussion

Few investigations have considered the effect of solvent on the DCC coupling reaction. DeTar and Silverstein[28] examined the DCC and acetic acid reaction in acetonitrile and carbon tetrachloride. They postulated that the reaction in carbon tetrachloride was faster due to reactive acetic acid dimers in this solvent. Less reactive monomers, they believed, predominated in acetonitrile. Similarly the higher k_3/k_2 ratio, and therefore larger anhydride yield, was attributed to a "cellular effect" of the dimer in carbon tetrachloride. That is, the local concentration of acid in the vicinity of the O-acylisourea is higher because of the residual acid molecule from the just disrupted dimer. Despite a dissenting report from Mironova et al.,[29] these

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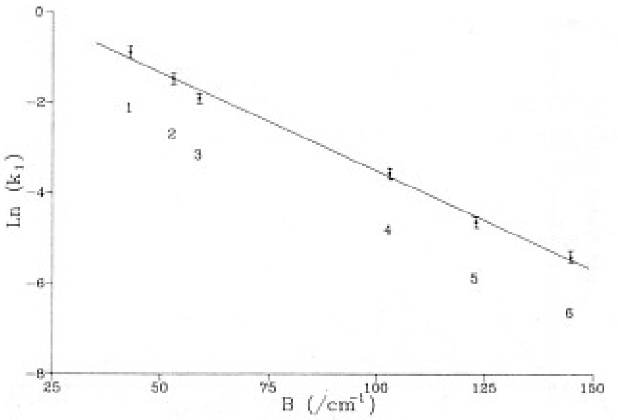

Figure 3. Correlation of ln (k_1) with Shorter's B parameter. Solvents are numbered as follows: (1) methylene chloride, (2) nitrobenzene, (3) nitropropane, (4) acetonitrile, (5) acetone, and (6) tetrahydrofuran. The B value for nitropropane is approximated by the literature value for nitromethane. The best fit result is indicated by the solid line. Error bars represent relative uncertainties of 10%.

results have been widely quoted.

DeTar and Silverstein[28] chose to analyze their experimental information with a kinetic scheme that required fitting to many rate constants at once. We simplify the analysis by making the k_1 step pseudo first order with respect to acid and working at acid concentrations that minimize dimer concentration (vide infra). This permits us to use analytic rate equations (eq 2 and 7) that require fits to only one variable, k_1 and k_3/k_2, respectively.

The results in Table I show that, even with minimal dimer concentration, a large range of k_1 and k_3/k_2 values are possible. We therefore attempted to correlate the rate constants with a variety of empirical and semiempirical solvent scales[30,31] and found, except with parameters that measured the solvent's hydrogen-bond acceptor ability,[30,32] poor or no correlation. The hydrogen-bond acceptor ability refers to the ease with which a Lewis base solvent accepts a hydrogen bond from a donor Lewis acid. One common measure of this is the wavenumber difference between the OD stretching vibration of deuterated methanol (MeOD) in a test solvent and a reference solvent or MeOD in the gas phase. As shown in Figure 3 the correlation of ln (k_1) versus B, the Shorter[33] hydrogen-bonding parameter, is very good ($r^2 = 0.99$).

Correlation of ln (k_1) with IR absorbance shifts relates changes in reactivity to changes in the acidic OH bond. One might argue, however, that such changes are not *solely* due to the hydrogen-bond "basicity" of the solvents. To clarify this point, we plotted the natural logarithms of the k_1 rate constants versus the Taft et al.[32,34,35] hydrogen-bond acceptor basicity (Figure 4). Unlike most of other solvent property scales, which are based on changes of some indicator with solvent, the parameter β is arrived at by averaging multiple normalized solvent effects on a variety of properties involving many diverse types of indicators.

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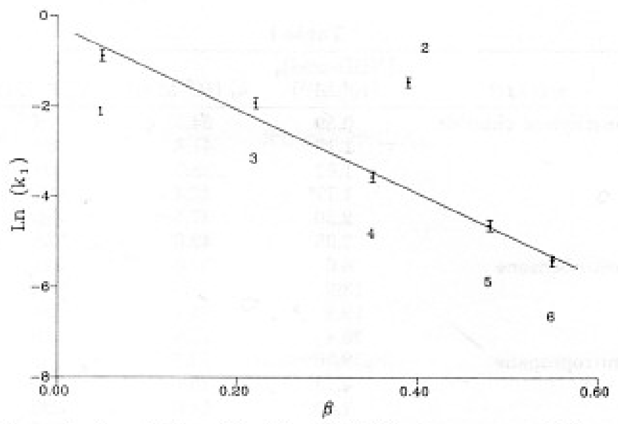

Figure 4. Correlation of ln (k_1) with Taft's β parameter. Solvent numbering as in Figure 3. Nitrobenzene from two sources gave identical values for k_1. The nitropropane β value is approximated by the literature value for nitromethane. The solid line is the best fit result excluding nitrobenzene. Error bars represent relative uncertainties of 10%.

Table II

solvent	K_{eq}[a] (M)	[NBD-acid]$_0$ (10^4 M^{-1})	mole fraction dimer[a,b]
acetone	3[c]	4.84	0.003
		92.0	0.05
acetonitrile	0.5[d]	57.8	0.006
		17.4	0.002
nitrobenzene	5.8[e]	26.4	0.03
		6.60	0.007

[a] These numbers should be considered estimates only. [b] Mole fraction estimate calculated as [monomers in form of dimer]/[total monomers]. [c] Reference 38. [d] Reference 28. [e] Reference 37.

The correlation, except for nitrobenzene, is excellent ($r^2 = 0.96$). The β value for nitrobenzene, however, is based solely on the UV/vis absorbance of one indicator[36] and therefore is not as reliable as averaging many experimental cases. Since nitrobenzene clearly falls on the regression line for Shorter's B value, we believe that the value given by Taft et al.[34] may be in error. The results of our kinetic experiments (Figure 4) suggest a β value of approximately 0.15, not 0.39 as reported.[36]

Based on equilibrium constants for carboxylic acids in nitrobenzene,[37] acetone,[38] and acetonitrile,[28] plus the fact monomer–dimer equilibrium constants change little in the hierarchy of linear aliphatic carboxylic acids,[37,39] one may calculate that in the concentration ranges studied, little of the acid will exist as dimer (Table II). An equilibrium constant for carboxylic acids in methylene chloride is not available in the literature. Consistent kinetic results, however, suggest that even in methylene chloride an insignificant portion of the acid will be in dimer form. Equilibrium constants for carboxylic acids in a related solvent more favorable to dimer, carbon tetrachloride,[40,41] suggest that at most 25% of the acid in our concentration range is dimerized.

Since acid dimerization is likely to be a function of solvent basicity, one might argue that the linearity of the

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(37) Rublo, F. C.; Rodriguez, V. B.; Alameda, E. J. *Ind. Eng. Chem. Fundam.* **1986**, *25*, 142.
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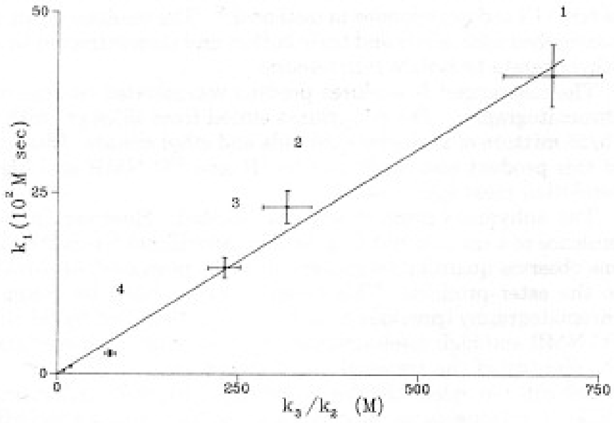

Figure 5. Correlation of k_1 with (k_3/k_2). Solvent numbering as in Figure 3. The solid line gives the best fit result. Error bars reflect relative uncertainties of 10%. Solvents 5 and 6 (unlabeled) have error bars smaller than the data symbols used.

ln (k_1) versus basicity plots merely reflects the *extent*, however limited, of dimerization in the different solvents. Kinetic arguments, however, show that the initial rate of acid decay in such a system should not be simply proportional to the starting acid concentration.

The equilibrium between monomer and dimer is goverened by the equilibrium constant K_{eq}. If one presumes that the "cellular effect" is operational, then the rate of acid decay will be

$$\frac{d[A]}{dt} = -2k_d K_{eq}[A]^2 \tag{8}$$

In eq 8 k_d is the bimolecular rate constant for reaction of NBD-acid dimer and DCC.

If the "cellular effect" is not operational but dimer is the active species in the second stage of reaction, then eq 8 contains a second term that has a fourth power dependence on monomer concentration. Thus, for either mechanism, the initial rate of acid decay should depend nonlinearly on the initial NBD-acid concentration, thereby reflecting the different relative amounts of dimer in solution (Table II). This is contrary to our experimental evidence. We observe a simple first-order dependence on acid,[42] and rate constants independent of concentration, in all solvents at all concentrations.

The k_3/k_2 ratio (Table I) determined from eq 7 shows a dependence, similar to that of k_1, on solvent basicity. In fact if one plots k_1 versus the ratio k_3/k_2 for all solvents (Figure 5), a straight line with intercept zero is obtained.

The linear relationship between k_1 and k_3/k_2 is best explained by postulating that k_3 and k_1 depend in similar ways on solvent basicity while k_2 is independent of solvent. Since addition of acid to DCC resembles addition to the *O*-acylisourea species, a priori one might expect similar solvent dependencies of the rate constants k_1 and k_3. If the dominant effect of solvent on k_1 and k_3 is the change in strength of the acid to solvent hydrogen bond, a precise consideration of their mechanisms is unnecessary. It suffices to say they are similar and that breaking the hydrogen bond is involved in the rate-limiting step for each. It's contribution, therefore, to their free energies of activation will be identical and k_1 and k_3 will be a constant multiple in all solvents. This contention is supported by the observation that NBD-acid solubility correlates qualitatively with the solvent basicity, indicating specific binding of the acid to a basic solvent. The lack of solvent dependence of k_2 is reasonable since this represents an intramolecular rearrangement.

The alternative explanation of Figure 5, that k_2 and k_1 are dependent in inverse ways on the solvent while k_3 is independent of it, is unreasonable because the zero intercept of Figure 5 would suggest that k_2 must become infinitely fast in a strongly binding solvent. This is not likely to be true for an intramolecular rearrangement. Finally, we consider it unlikely that both k_2 and k_3 are solvent dependent in mutually compensating manners so as to maintain the linear relationship. The variety of solvent properties is too great to expect such a coincidence.

Our conclusions are consistent with those of Mironova et al.[29] who argued that a specific interaction between solvent and acid controlled the kinetics. They did not, however, determine the nature of this interaction nor did they attempt to treat it quantitatively.

The rate constant k_1 is known to increase with acid strength.[43] By analogy, k_3 should also increase with acid strength. In our system one decreases the effective acid strength versus DCC by binding to solvent. While Hegarty et al.[44] have found, in aqueous solution, a pH dependence of the k_2 rate constant for model *O*-acylisoureas, such does not appear to be the case here.

Conclusion

The second-order rate constants k_1 and k_3 are related to the solvent basicity. Desolvation of the acid, for steps k_1 and k_3, is the energetic restriction that results in a free energy of activation dependent on the strength of the hydrogen bond between solvent and acid. The rate constant k_2 for intramolecular N $\rightarrow$ O acyl transfer is solvent independent.

The cellular effect of DeTar and Silverstein[28] is not the basis for enhanced anhydride yields in solvents such as methylene chloride and carbon tetrachloride. While extensive acid dimerization may complicate the kinetics in a higher concentration range, changes in the ratio k_3/k_2 do not require dimerization. The extent of acid dimerization and values of k_3/k_2 appear to be independent manifestations of the solvent's hydrogen-bonding ability. While this may be a subtle distinction, it is fundamental to the nature of this reaction.

Synthetic work requires a compromise between acid solubility and retardation of the k_1 and k_3 rate constants. Solvents in which the acid is the most soluble are those with the slowest k_1 and k_3 rate constants. This relation between solubility and rate constants suggests a useful rule of thumb. For a given DCC and acid concentration, other things being equal, the reaction will be faster and the anhydride yield better in the solvent for which the acid is least soluble.

Experimental Section

HPLC/Reaction Conditions. The HPLC system comprised two Waters 510 pumps controlled by a Waters automated gradient controller. The reaction was followed at 480 nm and 210 nm with a Waters 490 multiwavelength detector. Peak areas at 480 nm were quantified with a Waters 740 integrator. A Gilson 231 programmable sample injector and dilutor were interfaced to the Waters system.

For HPLC analyses we used a Waters C_{18} reverse phase radial compression column. Elution required a 70/30 mixture of acetonitrile and water with a flow rate of 2.0 mL/min. These conditions gave retention times of 1.3 min for NBD-acid, 4.3 min for anhydride, and 5.6 min for *N*-acylurea. Integrated NBD intensity

(42) The reaction is also first order with respect to DCC. In methylene chloride the order is 1.17 ± 0.11, in acetone 1.10 ± 0.05.

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(44) Hegarty, A. F.; McCormack, M. T.; Brady, K.; Ferguson, G.; Roberts, P. J. *J. Chem. Soc., Perkin Trans. 2* **1980**, 867.

remained constant with time; only the distribution changed. Concentrations were calculated by multiplying the normalized intensity for each species by the initial concentration of NBD-acid present. Allowance was made for the two NBD species present in each anhydride molecule.

The dilutor/injector was programmed to initiate the reaction by mixing suitable aliquots of pure solvent and solutions of DCC and NBD-acid. In all cases DCC concentration was maintained in 10-fold excess. The dilutor/injector automatically sampled the reaction mixture at appropriate intervals and injected these samples onto the column. Reactions took place in a 2-mL screw top vial sealed with a septum and were quenched upon injection by dilution and separation on the column. No hydrolysis or other reaction of the products occurs while on the column. Mixtures were stirred during reaction with a small stir bar. Temperatures were controlled to within ±1 °C of 30 °C.

Chemicals. NBD-acid was prepared by reacting *N*-methyl-6-aminohexanoic acid and 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) as previously described.[45]

The NBD-acid analogue *N*-(nitrobenz-2-oxa-1,3-diazol-4-yl)-5-pentylamine (NBD-amine) was prepared by the direct reaction of NBD-Cl and pentylamine in methanol.[46] The resulting solution was washed with acidic and basic buffers and then extracted with ethyl acetate to isolate NBD-amine.

The rearranged *N*-acylurea product was isolated by column chromatography. The *N*-acylurea eluted from silica gel with a 75/25 mixture of methylene chloride and ethyl acetate. Identity of this product was confirmed by ^{1}H and ^{13}C NMR and high resolution mass spectroscopy.

The anhydride product was not isolated. However, in the presence of a catalyst and 1-undecanol (esterification conditions), one observes quantitative conversion of the presumed anhydride to the ester product. This product was isolated by column chromatography (previous conditions) and identified by ^{1}H and ^{13}C NMR and high resolution mass spectroscopy. This confirms the identity of the carboxylic acid anhydride.

Solvents methylene chloride, tetrahydrofuran and nitrobenzene (Baker), nitropropane (Kodak), and acetone and acetonitrile (BDH) were distilled or vacuum distilled and stored over molecular sieves in sealed vessels.

DCC and pentylamine were purchased from Kodak. NBD-Cl was purchased from Sigma.

(45) Petersen, N. O. *Spectrosc. Int. J.* **1983**, *2*, 408.

(46) Gosh, P. B.; Whitehouse, M. W. *Biochem. J.* **1968**, *108*, 155.