Large Steric Effect in the Substitution Reaction of Amines with Phosphoimidazolide-Activated Nucleosides

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Aliphatic amines react with phosphoimidazolide-activated derivatives of guanosine and cytidine (ImpN) by replacing the imidazole group. The kinetics of reaction of guanosine 5′-phospho-2-methylimidazolide (2-MeImpG) with glycine ethyl ester, glycinamide, 2-methoxyethylamine, n-butylamine, morpholine, dimethylamine (Me_2NH), ethylmethylamine (EtNHMe), diethylamine (Et_2NH), pyrrolidine, and piperidine were determined in water at 37 °C. With primary amines, a plot of the logarithm of the rate constant for attack by the amine on the protonated substrate, log k_{SH}^{A}, versus the pK_a of the amine exhibits a good linear correlation with a Brønsted slope, β_{nuc} = 0.48. Most of the secondary amines tested react with slightly higher reactivity than primary amines of similar pK_a. Interestingly, some secondary amines show substantially lower reactivity than might be expected: EtNHMe reacts about eight times, and Et_2NH at least 100 times, more slowly than Me_2NH although all three amines are of similar basicity. For comparison, the kinetics of reaction of guanosine 5′-phosphoimidazolide (ImpG) and cytidine 5′-phosphoimidazolide (ImpC) were determined with Me_2NH, EtNHMe, and Et_2NH, and similar results were obtained. These results establish that the increased steric hindrance observed with the successive addition of ethyl groups are not due to any special steric requirements imposed by the guanosine or the methyl on the 2-methylimidazole leaving group of 2-MeImpG. It is concluded that addition of ethyl and, perhaps, groups larger than ethyl dramatically increases the kinetic barrier for addition of aliphatic secondary amines to the P–N bond of ImpN. This study supports the observation (see ref 4b) that the primary amino groups on the natural polyamines are at least 2 orders of magnitude more reactive than the secondary amino groups in the reaction with ImpN.

Introduction

Phosphoimidazolide-activated derivatives of nucleosides (ImpN, $SH^{\pm}$, see eq 1) have been shown to be useful substrates for nonenzymatic, template-directed polymerizations.[1] In contrast to the natural triphosphate derivatives, which are extremely sluggish in the absence of polymerases, ImpN polymerize in aqueous solution within days.[2] These reactions work equally well in the presence of an RNA, DNA, or polynucleotide analog strand.[2,3] For these reasons, template-directed reactions have been used as models for the prebiotic synthesis of nucleic acid polymers and analogs.[4a,5] With respect to efficiency, however, they lag far behind their enzymatic counterparts. For example, the efficiency of incorporation of uridine into a growing oligomer chain is so poor that in some cases synthesis of the complementary strand is terminated at this point.[5c]

In an attempt to find compounds that facilitate template-directed reactions under prebiotically plausible conditions, we have tested the natural polyamines for their potential role in the prebiotic polymerizations of ImpN. Early on it became clear that the polyamines showed nucleophilic reactivity toward ImpN. When 2-MeImpG is the nucleoside derivative, it is found that the natural polyamines, spermidine, spermine, and putrescine, replace the 2-methylimidazolide group[4b] even at physiological pH where the amine groups are largely protonated. Although there is precedent to show that many secondary amines are more nucleophilic than primary amines of comparable basicity,[6] the study with the polyamines indicated that the substitution comes from reaction of the primary rather than the secondary amino groups.[4b]

In order to develop a better understanding of the above findings, we studied the kinetics of reaction of a series of primary and secondary aliphatic amines with 2-MeImpG, ImpG, and ImpC in water at 37 °C and μ = 1.0 M with NaCl. Furthermore, the reaction of ImpN with amines is interesting in its own right because it defines the limits regarding the extent of survivability of these materials in a prebiotic medium. The results described below show that secondary amino groups that carry ethyl or, perhaps, groups longer than ethyl such as are present in the polyamines, react at least 100 times more slowly than the primary amino groups. This reactivity difference explains why substitution products of the secondary amino groups of the polyamines could not be detected.[4b]

Results and Discussion

General Features. ImpN are good electrophiles. The imidazolide group is easily replaced by several types of nucleophiles. Nucleophiles studied so far include water,[7,8] hydroxide ion,[8] monobasic and dibasic phosphate,[9a] nucleoside 5′- and 3′-phosphates,[9b] and the ribose hy-

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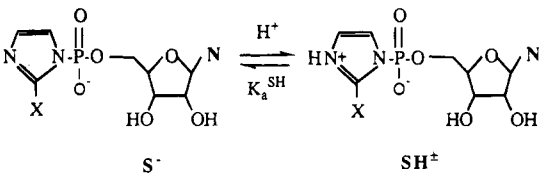
droxyls (2′-OH and 3′-OH) of oligonucleotides.[10] Nucleophilic attack at phosphorus with expulsion of imidazole has been confirmed by product identification. For example, 2-MeImpG reacts with water and hydroxide resulting in the formation of guanosine 5′-monophosphate (5′GMP),[8a] reaction with phosphate gives guanosine 5′-diphosphate,[9a] and reaction with a series of nucleotides acting as nucleophiles results in the formation of the corresponding dinucleoside pyrophosphate derivatives.[9b] The reaction of the 2′-OH or 3′-OH of the 3′ end of an oligonucleotide with 2-MeImpG has been the subject of many reports and results in the elongation of the oligonucleotide by one unit.[5a,b,10] Repetition of this reaction with 2-MeImpG or other ImpN represents nucleic acid synthesis or polymerization.

In the presence of an amine, disappearance of 2-MeImpG occurs concurrently with appearance of 5′-GMP, the hydrolysis product, and with another peak that exhibits guanosine spectral characteristics and elutes after 2-MeImpG. Plots of the percent [unknown peak] as a function of [amine] are linear with zero intercepts, confirming that the new peak is the substitution product. With morpholine the reaction was run on a larger scale and the product isolated and confirmed by negative FAB MS in a glycerol matrix. The amine derivatives proved to be very stable, much more so than the imidazolides. However, at pH ≈ 3 we could monitor, by HPLC, the disappearance of any one of the amine derivatives and the simultaneous appearance of 5′-GMP.

Kinetics of Reaction of Amines with 2-MeImpG. The kinetics of reaction of 2-MeImpG with glycine ethyl ester, glycinamide, 2-methoxyethylamine, *n*-butylamine, piperidine, morpholine, Me_2NH, EtNHMe, Et_2NH, and pyrrolidine were determined in amine solutions with buffer ratios $[AH^+]/[A] = 1$ in water at 37 °C at $\mu = 1.0$ M with NaCl. We were not able to test secondary amines with a propyl or longer alkyl chains because these amines are too weakly soluble for their reaction products to be measured against the competing hydrolysis reaction. Substrate concentration was held at about 5×10^{-4} M, and pseudo-first-order conditions prevailed at all times. Pseudo-first-order rate constants, k_{obsd}, were obtained from the slope of plots of ln (% area under 2-MeImpG peak) as a function of time. Table 1 lists rate constants with the secondary amines and Table 2 with the primary amines.

The mechanism we propose for the reaction of the amines with 2-MeImpG is analogous to the one postulated for hydrolysis.[8a] This study concluded that ImpN react mainly in their protonated form (eq 1, $SH^{\pm}$, neutral imidazole as the leaving group).[8a] Nevertheless, at pH > 10.5 the contribution of the reaction of the deprotonated species (eq 1, S^- with imidazolide anion as the leaving group) becomes detectable.[8] The pK_a^{SH} values of 7.09 for 2-MeImpG (eq 1, N = guanine and X = methyl) and 6.01 for ImpG (eq 1, N = guanine and X = H) were determined earlier at 37 °C and $\mu = 1.0$ M (with NaCl).[9a]

In order to account for the observations in the whole pH range both $SH^{\pm}$ and S^- species were assumed to be



(1)

reactive.[11] Thus, k_{obsd} is given by eq 2. In eq 2, A refers

$$k_{obsd} = \frac{K_a^{SH}}{K_a^{SH} + [H^+]}(k_S^{w} + k_S^{OH}[OH^-] + k_S^{A}[A]) + \frac{[H^+]}{K_a^{SH} + [H^+]}(k_{SH}^{w} + k_{SH}^{OH}[OH^-] + k_{SH}^{A}[A]) \quad (2)$$

to the free amine; k_S^w and k_{SH}^w are the rate constants for attack by water on S^- and $SH^{\pm}$, respectively, k_S^{OH} and k_{SH}^{OH} the rate constants for attack by hydroxide ion on S^- and $SH^{\pm}$, respectively, k_S^A and k_{SH}^A the rate constants for attack by the amine base on S^- and $SH^{\pm}$, respectively, and $[OH^-]$ and $[H^+]$ are the hydroxide and hydrogen ion activity, respectively.

According to eq 2, a plot of k_{obsd} as a function of free amine concentration, [A], should exhibit an intercept (I) and a slope (S) given by eqs 3 and 4, respectively.

$$I = \frac{K_a^{SH}}{K_a^{SH} + [H^+]}(k_S^{w} + k_S^{OH}[OH^-]) + \frac{[H^+]}{K_a^{SH} + [H^+]}(k_{SH}^{w} + k_{SH}^{OH}[OH^-]) \quad (3)$$

$$S = \frac{K_a^{SH}k_S^{A} + [H^+]k_{SH}^{A}}{K_a^{SH} + [H^+]} \quad (4)$$

Plots of k_{obsd} as a function of the basic component of the buffer were in most cases linear; see, for example, Figure 1. For buffer systems with a total amine concentration of 0.2 M or more we observed curvature that was attributed to self-association of the buffer components. In these cases the initial slope, value for S in eq 4, was obtained using a second-order fit. S values are reported in Table 4. The intercepts of the buffer slopes were in good agreement with calculated values (I from eq 3) based on the published rate constants for the hydrolysis reaction. The agreement also means that amines do not catalyze the hydrolysis of the 2-MeImpG. This is in contrast to the observation that several metal ions[8a] as well as phosphate buffers[9a] catalyze the hydrolysis of ImpN.

Relative Contributions of k_S^A vs k_{SH}^A. A reasonable assumption is that $k_{SH}^A/k_S^A \approx k_{SH}^{OH}/k_S^{OH}$ and therefore a crude estimate of the ratio k_{SH}^A/k_S^A can be obtained from $k_{SH}^{OH}/k_S^{OH} = 4.5 \times 10^4$ (same value for 2-MeImpG and ImpG) determined earlier.[8a,9a] In order to obtain better values for k_{SH}^A and k_S^A and the corresponding ratio we performed experiments with one primary, *n*-butylamine, and one secondary amine, piperidine, at various buffer ratios. Rearranging eq 4 allows

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(11) A simpler mechanism has been proposed to account for the hydrolysis of uridine phosphoimidazolide derivatives (ref 8b). The reasonably good fit of the data in a Brønsted plot covering a pH range 7 < pH < 12 (see Figure 2) supports our mechanistic assignment with at least the tested substrates. It is unclear why uridine derivatives would react via a different mechanism.

Table 1. Kinetics of the Reaction of Secondary Amines with 2-MeImpG at 37 °C

amine	pH	[amine], M	$10^2 \times k_{obsd}$, h^{-1}
morpholine	8.51 ± 0.03	0.005	5.79
		0.01	10.4
		0.02	20.4
		0.025	25.8
		0.03	29.9
		0.04	39.2
		0.05	50.4
dimethylamine	10.64 ± 0.03	0.02	1.81
		0.04	3.41
		0.06	4.95
		0.08	6.23
		0.10	7.50
ethylmethylamine	10.66 ± 0.03	0.01	0.109
		0.02	0.204
		0.04	0.362
		0.06	0.502
		0.08	0.657
		0.10	0.793
diethylamine	10.82 ± 0.03	0.02	0.037
		0.04	0.044
		0.06	0.053
		0.08	0.066
		0.10	0.079
pyrrolidine	11.08 ± 0.03	0.02	1.38
		0.04	2.47
		0.06	3.41
		0.08	4.32
		0.10	5.17
piperidine	10.42 ± 0.02	0.01	1.04
		0.02	1.63
		0.04	2.95
		0.06	4.44
		0.08	5.01
		0.10	6.47
	10.90 ± 0.03	0.025	1.04
		0.05	1.71
		0.10	3.11
		0.15	4.38
		0.20	5.52
		0.25	6.50
	11.09 ± 0.02	0.0067	0.159
		0.013	0.336
		0.020	0.451
		0.027	0.579
		0.033	0.703
		0.040	0.821
		0.047	0.978
		0.053	1.09
		0.060	1.20
		0.067	1.28
		0.074	1.48
		0.080	1.50
	11.90 ± 0.03	0.010	0.21
		0.02	0.29
		0.04	0.45
		0.06	0.57
		0.08	0.67
		0.10	0.75

one to plot $S(K_a^{SH} + [H^+])/K_a^{SH}$ as a function of $[H^+]$ and obtain k_S^A directly from the intercept and k_{SH}^A/K_a^{SH} from the slope. Indeed, $S(K_a^{SH} + [H^+])/K_a^{SH}$ values obtained from the piperidine and *n*-butylamine experiments exhibit a satisfactory linear correlation with $[H^+]$ and allow the determination of $k_{SH}^A = 1500\ M^{-1}\ h^{-1}$ and $k_S^A = 0.07\ M^{-1}\ h^{-1}$ for piperidine and $k_{SH}^A = 745\ M^{-1}\ h^{-1}$ and $k_S^A = 0.065\ M^{-1}\ h^{-1}$ for *n*-butylamine. From these rate constants one obtains k_{SH}^A/k_S^A equal to 2.1×10^4 for piperidine and to 1.1×10^4 for *n*-butylamine (average $\approx 1.5 \times 10^4$). These ratios are consistent with the value of 4.5×10^4 obtained earlier for hydroxide ion. The average value is considered more reliable because of the large reactivity difference between the $SH^{\pm}$ and S^- pathways and the experimental uncertainty associated with it. $k_{SH}^A/k_S^A = 1.5 \times 10^4$ is used in order to calculate rate constants for Me_2NH, EtNHMe, and pyrrolidine from the corresponding *S* values (Table 4). On

Table 2. Kinetics of the Reaction of Primary Amines with 2-MeImpG at 37 °C

amine	pH	[amine], M	$10^2 \times k_{obsd}$, h^{-1}
n-butylamine	9.89 ± 0.03	0.01	1.33
		0.02	2.55
		0.04	4.78
		0.06	6.71
		0.08	8.91
		0.10	10.17
	10.37 ± 0.03	0.025	1.59
		0.050	2.58
		0.075	3.84
		0.100	4.88
		0.125	5.81
		0.15	6.81
	11.07 ± 0.03	0.01	0.44
		0.02	0.54
		0.04	0.68
		0.06	0.86
		0.08	1.09
		0.10	1.42
	11.30 ± 0.02	0.0098	0.114
		0.0197	0.193
		0.0295	0.260
		0.0394	0.329
		0.0492	0.410
		0.0591	0.460
		0.0689	0.509
		0.0787	0.590
		0.0886	0.652
		0.0984	0.764
		0.1080	0.781
		0.1180	0.851
glycine ethyl ester	7.30 ± 0.04	0.005	7.1
		0.01	12.0
		0.02	25.0
		0.03	38.0
		0.04	47.0
		0.05	60.0
glycinamide	7.84 ± 0.01	0.01	6.57
		0.02	12.4
		0.03	18.0
		0.04	24.1
		0.05	29.3
2-methoxyethylamine	9.23 ± 0.02	0.01	2.10
		0.02	3.98
		0.03	5.36
		0.04	6.60
		0.05	7.37

Table 3. Kinetics of the Reactions of Dimethyl-, Ethylmethyl- and Diethylamine with ImpG and ImpC at 37 °C

amine	pH	[amine], M	$10^2 \times k_{obsd}$, h^{-1}	
			ImpG	ImpC
dimethylamine	10.64 ± 0.03	0.005		0.248
		0.01	0.82	0.460
		0.02	1.53	0.874
		0.03	2.07	1.31
		0.04	2.70	1.73
		0.05	3.33	2.03
ethylmethylamine	10.66 ± 0.02	0.005	0.071	0.054
		0.01	0.13	0.101
		0.02	0.23	0.125
		0.03	0.31	0.183
		0.04	0.39	0.246
		0.05	0.48	0.288
diethylamine	10.82 ± 0.02	0.01	0.055	0.067
		0.02	0.063	0.042
		0.03	0.044	0.048
		0.04	0.056	0.044

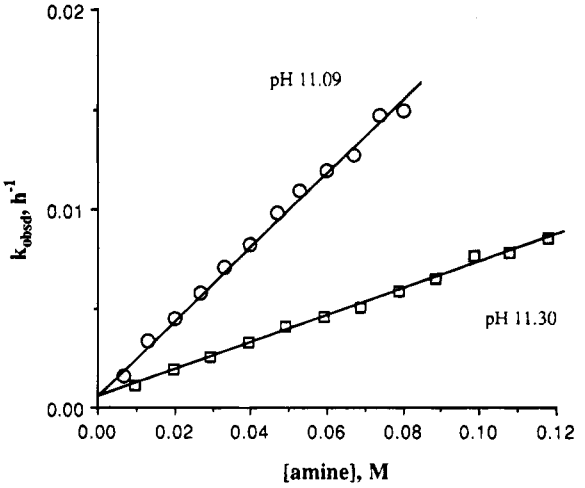

Figure 1. Observed pseudo-first-order rate constants, k_{obsd}, for the reaction of 2-MeImpG with piperidine at pH 11.09 (circles) and *n*-butylamine at pH 11.30 (squares) as a function of free amine concentration. Observed rate constants in general decrease with increasing pH (see text).

Table 4. Kinetic Parameters for the Reaction of Amines with 2-MeImpG, ImpG, and ImpC at 37 °C

amine	pK_a	pH	S,[d] M^{-1} h^{-1}	k_{SH}^A, M^{-1} h^{-1}	k_S^A, M^{-1} h^{-1}
n-butylamine[a]	10.37	9.89	1.222	745	0.065
		10.37	0.542		
		11.07	0.128		
		11.37	0.119		
piperidine[a]	10.90	10.42	0.769	1500	0.07
		10.90	0.331		
		11.09	0.184		
		11.90	0.104		
glycine ethyl ester	7.30	7.30	11.8	31	
glycinamide	7.84	7.84	6.05	40	
morpholine	8.51	8.51	9.87	252	
2-methoxy-ethylamine	9.23	9.23	2.26	314	
dimethylamine[b]	10.64	10.64	0.911	1850	0.12
			0.62 (ImpG)	6990	0.46
			0.40 (ImpC)	4440	0.30
ethylmethyl-amine[b]	10.66	10.66	0.08	240	0.016
			0.09 (ImpG)	1010	0.067
			0.05 (ImpC)	560	0.037
diethylamine[c]	10.82	10.82	<0.004	<19	
pyrrolidine[b]	11.08	11.08	0.644	3810	0.254

[a] Values k_{SH}^A calculated from the slope of a plot of S as a function of α_H and k_S^A directly from the intercept of such plot. [b] Rate constants calculated based on eq 4 and assuming $k_{SH}^A/k_S^A = 1.5 \times 10^4$. [c] k_{SH}^A not corrected for a possible contribution by k_S^A. [d] S is the slope of the plots of k_{obsd} as a function of free amine concentration.

the basis of the ratio of $k_{SH}^A/k_S^A = 1.5 \times 10^4$, a standard deviation of ±5% for S values, and the corresponding pK_a^{SH}'s and by means of eq 4, one can calculate that the k_S^A pathway becomes important for 2-MeImpG at pH ≥ 10.4 and for ImpG at pH ≥ 9.4 (the lower pH range for ImpG is due to its lower pK_a^{SH}). Hence, for the amines examined at a lower pH than 10.4 with 2-MeImpG, the k_S^A pathway is negligible and k_{SH}^A values were obtained from $k_{SH}^A = S(K_a^{SH} + [H^+])/[H^+]$ and are listed in Table 4.

Steric Hindrance to P–N Bond Attack. A plot of log k_{SH}^A (2-MeImpG) as a function of amine pK_a (Figure 2) shows that the four primary amines exhibit a linear correlation spanning a 3 unit pK_a range. The line drawn through the primary amines has a slope, β_{nuc}, of 0.48 and

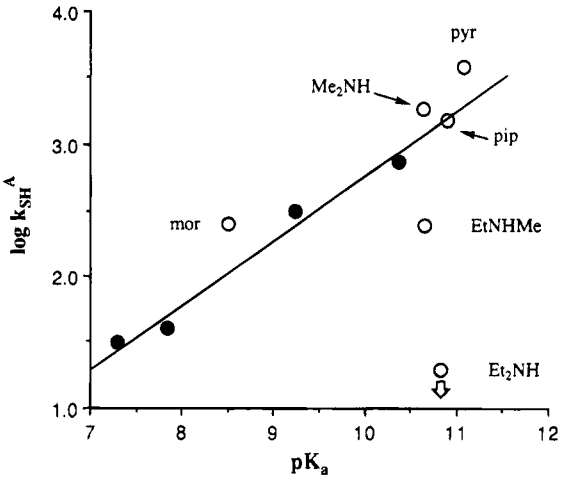

Figure 2. Brønsted plot (log k_{SH}^A as a function of amine pK_a) for the reaction of amines with 2-MeImpG; primary amines (filled circles), secondary amines (open circles), pyr, pip, and mor stand for pyrrolidine, piperidine, and morpholine, respectively.

suggests that P–N bond formation in the transition state is about 50% complete.[12] Figure 2 includes the log k_{SH}^A values for the secondary amines. They can be separated into two groups. One group has rate constants that either fall slightly above the Brønsted line defined by the primary amines (pyrrolidine, Me_2NH, and morpholine) or are on the line (piperidine). The other group, comprised of EtNHMe and Et_2NH, is characterized by log k_{SH}^A values that fall significantly below the line. The observed patterns are best understood in terms of steric effects of varying magnitude. In the absence of steric effects, alicyclic secondary amines as well as Me_2NH generally display a substantially higher nucleophilicity than primary amines of the same basicity;[6] k(secondary amine)/k(primary amine) ratios of 3 or larger are not uncommon.[6a] On the other hand, these ratios strongly decrease when there is significant steric hindrance either in the substrate[15] or the amine[6a,b] (e.g., noncyclic secondary amines larger than Me_2NH). Hence, the fact that none of the secondary amines in our study displays a significantly higher reactivity than the primary amines is most easily accounted for by steric crowding in the transition state.

With Me_2NH and the alicyclic amines, the steric effect just about offsets the intrinsically higher reactivity of secondary amines. However, as the steric bulk of the amine is increased (EtNHMe and especially Et_2NH), the steric effect becomes more dramatic, as seen by the large negative deviations from the Brønsted line for these two amines.[16] In as much as the secondary amino groups in spermine and spermidine have a similar or even more crowded steric environment than in Et_2NH, it is not

(12) This is the traditional interpretation of β_{nuc},[13] which, however, is not universally accepted.[14]

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(16) The $k(Me_2NH)/k(Et_2NH)$ ratio of ≥100 observed in our system is not out of line with the $k(Me_2NH)/k(Et_2NH)$ ratio of 184 for the reaction of 1-chloro-2,4-dinitrobenzene in ethanol.[6a]

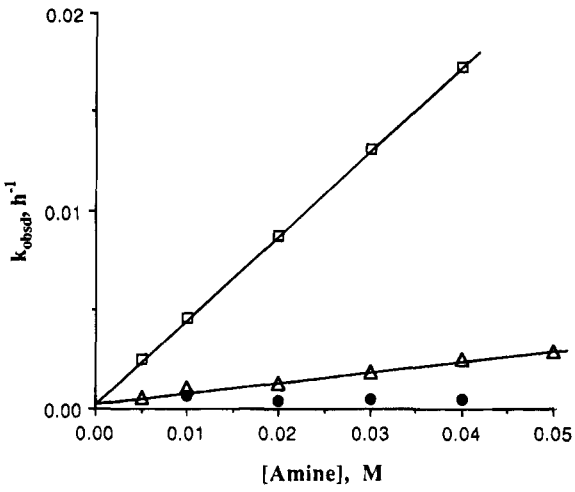
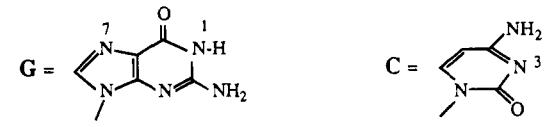


Figure 3. Observed pseudo-first-order rate constants, k_{obsd}, for the reaction of ImpC with Me_2NH at pH 10.64 (squares), with EtNHMe at pH 10.66 (triangles), and with Et_2NH at pH 10.82 (filled circles) as a function of free amine concentration. The corresponding plots for 2-MeImpG and ImpG are very similar to the one in the figure.

surprising that the only products of the reaction of the polyamines with 2-MeImpG are those derived from nucleophilic attack by the primary amino groups.[4b]

Reaction of Dimethyl-, Ethylmethyl-, and Diethylamine with ImpG and ImpC. The most notable result of our study, illustrated in Figure 2, is that Me_2NH, EtNHMe, and Et_2NH, all three of similar basicity, exhibit dramatically different reactivity toward 2-MeImpG. Because guanosine derivatives have a tendency to be present in the syn-conformation[17] which places the nucleobase moiety closer to the P–N bond, we wanted to establish whether or not the different reactivity might be attributed to interference from the guanosine portion of the molecule. Another question was whether or not the 2-methyl group on the 2-methylimidazole is partially responsible for the steric effect. Both these questions were resolved by examining the P–N bond reactivity of ImpG, where there is no 2-methyl on the imidazole leaving group, and of ImpC, which has no 2-methyl group but also a different nucleobase present in the anti-conformation with lesser steric requirements.[17] Kinetic data are summarized in Table 3. Both substrates react in a manner similar to that of 2-MeImpG (see Figure 3 for an illustration of the reactions of Me_2NH, EtNHMe, and Et_2NH with ImpC). Reaction of Et_2NH with ImpG was too slow for determination of a rate constant, but the formation of the corresponding Et_2NH–pG was detectable by HPLC, whereas we have no evidence for the formation of a Et_2NH–pC derivative from ImpC.

An analysis identical to the one presented for 2-MeImpG allowed the determination of rate constants from the slopes (S, reported in Table 4) of the buffer plots. Assuming K_a^{SH} (ImpC) = K_a^{SH} (ImpG) = 9.77×10^{-7} and $k_{SH}^A/k_S^A \approx 1.5 \times 10^4$, the reported S values in conjunction with eq 4 give $k_{SH}^A = 6900\ M^{-1}\ h^{-1}$ and $k_S^A = 0.46\ M^{-1}\ h^{-1}$ for the reaction of Me_2NH with ImpG and $k_{SH}^A = 4440\ M^{-1}\ h^{-1}$ and $k_S^A = 0.30\ M^{-1}\ h^{-1}$ for the reaction of Me_2NH with ImpC (Table 4). Corresponding rate constants for the reaction with EtNHMe are also included in Table 4. Although there is some uncertainty associated with the obtained rate constants, it is notable that the rate constants for reaction of Me_2NH with ImpG and EtNHMe with ImpG are substantially larger than the corresponding rate constants with 2-MeImpG as electrophile. This, seemingly, contradicts the observation that for the reaction with a specific amine the slope, S, of the plots of k_{obsd} as a function of free amine concentration S (2-MeImpG) > S (ImpG) as can be seen in Table 4. However, the higher reactivity of ImpG as compared with 2-MeImpG has been observed with other nucleophiles, such as water and hydroxide ion, and can be easily understood as a consequence of their pK_a difference; pK_a^{SH} (2-MeImpG) – pK_a^{SH} (ImpG) = $\Delta pK_a^{SH} \approx 1$, which makes ImpG a better electrophile than 2-MeImpG. The reason that the higher electrophilicity of ImpG cannot be observed directly from the S values is attributed to the fact that for the same pH the relative contribution of k_{SH}^A and k_S^A favors more the k_S^A pathway with ImpG and this results in a smaller absolute value for S. The observation that ImpC is somewhat less reactive than ImpG has been reported already with phosphate as the nucleophile[9a] and may be partially due to the different nucleobase.



Stability/Reactivity of ImpN in the Presence of Amines. It is known that ImpN slowly hydrolyze to form the corresponding free nucleotide which by themselves are poor substrates for template-directed reactions. Hence, hydrolysis competes with and is counter-productive for template-directed polymer synthesis. Our study indicates that amines would also react with ImpN and form amine derivatives that may or may not be good substrates for template-directed polymerizations. It is plausible that in a prebiotic medium concentrations of organics, including amines, would not exceed a few mM. Assuming pH ≈ 8.5 and [morpholine] = 1×10^{-3} M, one calculates from a direct comparison between k_{SH}^w and k_{SH}^A[A], the two major pathways for reaction in this pH range, that decomposition of 2-MeImpG by aminolysis will be increased by almost a factor of 8 over hydrolysis. Hence, amines, excluding secondary acyclic amines with side chains as long or longer than ethyl, are very potent nucleophiles for phosphoimidazolides.

Conclusions

This study shows that most amines react with ImpN by substitution of the imidazole group. We find that dimethylamine and the alicyclic secondary amines react somewhat faster than the primary amines, but the presence of one ethyl group results in about an 8-fold and the presence of a second ethyl group in at least a 100-fold decrease in reactivity. These observations support experiments[4b] suggesting that under our conditions substitution products come from the primary amino group of the polyamines spermidine and spermine and that products from the secondary amino groups are negligible.

Experimental Section

Materials and Syntheses. Solvents used were HPLC quality. Amines (Aldrich) were distilled or recrystallized prior to use. The sodium salts of 2-MeImpG, ImpG, and ImpC at

(17) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, Inc., 1984; p 77 and references therein.

purity ≥99% were synthesized by a modification of a literature procedure.[18] 2-Methylimidazole (Aldrich) was recrystallized twice from toluene before use. Triphenylphosphine (Aldrich) was recrystallized from 2-propanol before use. Guanosine 5′-monophosphate (5′-GMP, Sigma) was better than 99% pure by HPLC analysis. 5′-GMP was dried overnight at reduced pressure. Dimethyl sulfoxide was dried over CaH_2 and distilled at reduced pressure. 2,2′-Dipyridyl disulfide and anhydrous sodium perchlorate (Aldrich) were used without further purification.

2-MeImpG was synthesized by the following procedure: 5′-GMP free acid, 0.46 g (1.25 mmol), and 1.64 g of 2-methylimidazole were dissolved with stirring in approximately 10 mL of DMSO in a 50 mL round bottom flask. Four hundred μL of triethylamine was added, followed by 1.05 g (4 mmol) of triphenylphosphine and 0.88 g (4 mmol) of 2,2′-dipyridyl disulfide. The mixture was stirred for 1.5 h and quenched by adding dropwise with stirring to a 250 mL Erlenmeyer flask cooled at 0 °C containing 80 mL of acetone, 50 mL of anhydrous ether, and 4 mL of saturated sodium perchlorate/acetone. This was stirred for 20 min to allow precipitation and the product collected by vacuum filtration and washed three times with 1:1 acetone:ether and three times with acetone. The product was dried under reduced pressure, redissolved in 10 mL of DMSO, and purified as before.

The above procedure cannot be used for the synthesis of other phosphoramidates because in organic solvents most amines form a precipitate with guanylic acid. It has been reported that exposure of phosphorimidazolides to amine-containing molecules in aqueous solution results in the production of a wide range of stable phosphoramidates in good yield.[19] Using this method we obtained piperidine-pG, morpholine-pG, and pyrrolidine-pG in up to 80% yields. We were able to get yields of 95% or better by dissolving the lithium salt of 2-MeImpG (0.3 mmol) in a solution containing both the amine and dry DMF or dry DMSO. The sodium salt of ImpG can be used in place of the lithium salt of 2-MeImpG. The amine was at a 50- to 100-fold excess over 2-MeImpG. Optimal reaction conditions for pyrrolidine-pG were 20 h at 37 °C and for morpholine-pG 12 h at 75 °C. The higher temperature does not result in a higher percentage of impurities. The product of the reaction was dissolved in dry DMSO and precipitated out as the sodium salt by dropwise addition under stirring to a cool mixture of 40 mL of acetone, 25 mL of diethyl ether, and 2 mL of a saturated solution of sodium perchlorate in acetone. Morpholine–pG was isolated as the lithium salt by using a saturated solution of lithium perchlorate in acetone.

(18) Joyce, G. F.; Inoue, T.; Orgel, L. E. *J. Mol. Biol.* **1984**, *176*, 279.
(19) Chu, B. C. F.; Wahl, G. M.; Orgel, L. E. *Nucl. Acids Res.* **1983**, *11*, 6513.

HPLC Analysis and Kinetics. pH of solutions was measured at room temperature either in mock solutions excluding the substrate or directly in the test tubes using a microelectrode (Microelectrodes MI 410). At the basic end of the pH range KCl was used as the compensating electrolyte. Samples of 1000 μL prepared in 1.5 mL polypropylene test tubes were transferred directly into the HPLC vials. ImpN concentration was 0.5–1 mM, and the amine was always in excess, so that pseudo-first-order conditions applied. The vials were stoppered and placed inside the thermostated (37 ± 0.5 °C) autosampler of a Hewlett-Packard 1090M liquid chromatograph in which the analyses were carried out. Reactions were monitored for approximately 1–2 half-lives with the exception of the very slow ones. UV absorbances were monitored at 254 nm with the G-derivatives and at 270 nm with the C-derivative. The absorbance observed in this region comes from the nucleotide moiety; amines including imidazoles do not absorb. Ten to twenty μL aliquots of each sample were analyzed automatically, and from the HPLC reports the percent unreacted ImpN was calculated. Percent product distribution was determined from total HPLC areas of a sample taking into consideration that all derivatives of a single nucleoside have identical extinction coefficients. Plots of ln(% unreacted ImpN) as a function of time were perfectly linear, and k_{obsd} was obtained directly from the slope using least-squares analysis. Values of k_{obsd} are accurate to ±5%, unless otherwise noted.

HPLC analysis was performed using a 20 cm reversed-phase Hypersil ODS 5 μm column. Chromatographic conditions were modified from a previous method[8a] and were as follows: mobile phase, solvent A, 0.02 M potassium dihydrogen phosphate (pH 6 to 6.5); solvent B, acetonitrile–water (30:70); gradient elution, 0–65% B in 13 min; isocratic elution at 65% B for 4 min. Under our analytical conditions retention times (in min) of the nucleoside derivatives are as follows: ImpG, 8.8; 2-MeImpG, 9.5; ImpC, 8.3; 5′-GMP, 3.7. All amine–nucleoside derivatives exhibit different retention times and elute after the corresponding ImpN. Amine–pC elutes earlier than the corresponding amine–pG derivative.

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