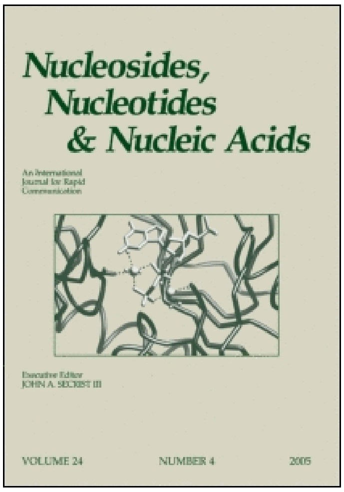

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N-Cyanoimidazole and Diimidazole Imine: Water-Soluble Condensing Agents for the Formation of the Phosphodiester Bond

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N-CYANOIMIDAZOLE AND DIIMIDAZOLE IMINE: WATER-SOLUBLE CONDENSING AGENTS FOR THE FORMATION OF THE PHOSPHODIESTER BOND

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Abstract: The reaction of BrCN with imidazole results in the formation of N-cyanoimidazole and diimidazole imine. These compounds were shown to be useful condensing agents for the formation of the phosphodiester bond in aqueous solution.

The synthesis of RNA and RNA analogs occupies a central place in current research in prebiotic chemistry, in part because of the recent discovery that RNA oligomers catalyze chemical transformations in biological systems.[1] We have been studying the prebiotic formation of the phosphodiester bond of RNA using the HCN derivative diiminosuccinonitrile (DISN) as the condensing agent.[2] Insight was gained in the mechanism of this process by comparing DISN with BrCN in the cyclization of 3'-adenylic acid (3'-AMP) to 2',3'-cyclic adenylic acid (2',3'-cAMP).[3] BrCN was suggested as a possible condensing agent by Orgel and Lohrmann.[4] We observed that BrCN is an efficient reagent for the formation of 2',3'-cAMP in aqueous solution and that transition metal ions enhance the yield of cyclic phosphate.[3] Imidazole buffer (0.2 M) was used routinely in these experiments because it effected higher yields in our previous studies with DISN although the role of imidazole in the condensation was not well understood. Transition metal ions enhanced the efficacy by which BrCN effected the formation of 2',3'-cAMP.[3]

We initiated an investigation of the mechanism of the BrCN mediated formation of 2,'3'-cAMP in 1984[5] and are prompted to publish these aspects of this study because of reports by Kanaya and Yanagawa[6,7] concerning the chemistry of BrCN.[a]

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[a] The results of studies in this laboratory by C.- H. Huang and W. J. Hagan, Jr. were communicated to Dr. H. Yanagawa in 1984 and 1985. Unfortunately he neglected to mention the priority of our studies in his papers[6,7].

The formation of N-cyanoimidazole (1) and diimidazole imine (2) will be reported together with data on the use of these compounds as condensing agents in aqueous solution.

2. Experimental

General Procedures. The procedures used in this research were described previously.[2] NMR spectra were obtained on a Varian XL-200. High performance liquid chromatography (HPLC) was performed with a Waters Microbondapak C-18 column using the Waters equipment described previously[3] or on a Beckman-Altex gradient system using an Altex Ultrasphere-ODS column (4.6 nm x 25 cm) eluted isocratically with 40% aqueous methanol at 1 ml/min. N-cyanoimidazole was prepared by the method of Giesemann,[8] mp. 60-61.5°C.

The Synthesis of Diimidazole Imine. To a solution of imidazole (6.8g, 0.1 mol) in 500 mL CH_2Cl_2 was added BrCN (3.73g, 0.033 mol) and the solution was heated at reflux for 30 min. The white precipitate of imidazolium bromide was filtered and the filtrate was concentrated to 50 mL and cooled at 4°C for 2 days. The crystalline product was filtered and dried to give 4.74g (88%) of N,N-diimidazole imine: mp. 103-104°C; IR (Nujol) 1660 cm^{-1} (C=N); ^1H NMR ($CDCl_3$) δ 7.25-7.27 (3H, m), 7.40 (1H, s), 7.91 (1H, s, N=CH-N), 8.03 (1H, s, N=CHN), 8.37 (1H, s, C=NH, exchanged D_2O); MS m/e 161 (14), 94 (41), 93 (30), 68 (100); high resolution MS 161.0705, Calcd. for $C_7H_7N_5$, 161.0705.

Synthesis of N-Cyanoimidazole in Aqueous Solution. To a 1 mL solution of 0.2 M imidazole which had been adjusted to pH 6.15 ± 0.15 with HNO_3 was added 10 µL of 1.0 M aqueous BrCN. Samples were heated at 41° for 15 to 180 min and aliquots were analyzed by HPLC. N-cyanoimidazole was identified by the development of an HPLC peak with the same retention time as the authentic sample (Giesemann, 1955). The optimal yield occurred between 30-45 min.

Synthesis of Diimidazole Imine in Aqueous Solution. To 10 ml of 2.0 M imidazole which had been adjusted to pH 6.15 ± 0.15 with HNO_3 was added 0.098g (0.9 mmol) of BrCN and the mixture was heated at 41°C for 2 h. The HPLC of reaction solution exhibited one peak with the same retention time as an authentic sample of 2. The aqueous solution was extracted with 10 mL of ethyl acetate, dried and concentrated to dryness to give a solid which had the same HPLC retention time as an authentic sample of diimidazole imine.

Synthesis of Amino-1-Imidazo-N-Phenylimine (4). To a solution of aniline (0.183g, 2 mmol) in 4 mL of methanol was added diimidazole imine (2) (0.326g, 2mmol) and the reaction was allowed to proceed at room temperature for 2 h before it was concentrated to dryness. The product was chromatographed on silica gel and eluted with ethyl acetate. This fraction was concentrated to dryness, washed with methylene chloride and filtered to give 0.178g (48%) of 4: mp 128-129°C; ^{1}H NMR ($CDCl_3$) δ 4.86 (2H, s(b), exchange with D_2O, NH_2), 7.00-7.52 (7H, m, phenyl H and imidazole-4,5-H), 8.19 (1H, s, N=CHN); UV (CH_3OH) λ_{max} 260 nm (shoulder); MS m/e 186 (40), 120 (10), 119 (100), 118 (42), 91 (28), 77 (69), 69 (43); high resolution MS m/e 186.0902, calculated for $C_{10}H_{10}N_4$ 186.0905.

Synthesis of 1-Imidazo-N-Benzylaminoimine (5). A solution of benzylamine (0.217g, 2 mmol) and diimidazole imine (0.329g, 2.05 mmol) in 2 mL of methanol was allowed to react for 2 h at room temperature and was then concentrated to dryness. The product was eluted from a silica gel column with 1:1 acetone:ethyl acetate and crystallized from benzene to give 0.061g (15%) of 5: mp 65-85; ^{1}H NMR ($CDCl_3$) δ 4.44 (2H, s, CH_2), 5.0-6.0 (2H, broad, exchanges with D_2O, NH) 6.99 (1H, s), 7.35 (6H, m), 7.96 (1H, s, N=CH-N); MS m/e 200 (14), 158 (5), 100 (10), 91 (100), 68 (18); high resolution mass spectrum 200.1062, calculated for $C_{11}H_{12}N_4$ 200.1062.

Comparison of the Effectiveness of (1) and (2) for the Conversion of 3'-AMP to 2',3'-cAMP. Compound (1) or (2) (0.05 mmol) was added to 3'-AMP (0.01 mmol in 1 mL 2.0 M imidazolium nitrate buffer (pH 6.2) and the formation of 2',3'-cAMP at 25°C was monitored by HPLC. The results are given in the Figure.

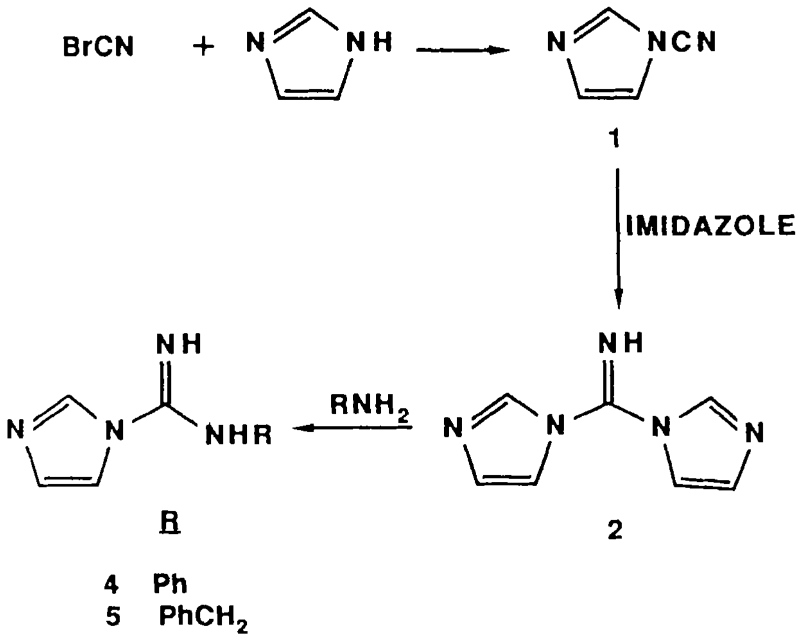
Reaction of 3'-AMP with N-Cyanoimidazole. To a 1 mL solution containing 0.01 M 3'-AMP, 0.01 M metal ion and 0.2 M imidazole buffer was added sufficient N-cyanoimidazole to give a 0.01 M solution. The pH was adjusted to 6.15 ± 0.15 as required by the addition of 1-10 µL of 2 M imidazole and the mixture was heated in a 8 mL screw cap test tube for 3 h at 41°C. EDTA was added to give a final concentration of 0.1 M, the solution was analyzed by HPLC and the results are given in the Table.

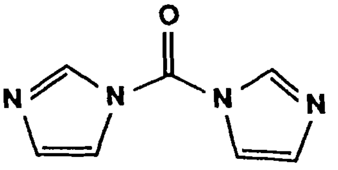
3. Results and Discussion

Diimidazole imine (2) was detected as a transient intermediate in the reaction of 3'-AMP with BrCN in imidazole buffer to form 2',3'-cAMP by HPLC. The same HPLC peak was detected in the reaction of 2 M aqueous imidazole (pH 6.2) with BrCN. Diimidazole imine was synthesized in 88% yield by the reaction of imidazole and BrCN in methylene chloride.

N-Cyanoimidazole (1) was also detected as the product of the reaction of imidazole and BrCN when 0.2 M aqueous imidazole (pH 6.2) was used in place of 2 M imidazole. It was identified by comparison with an authentic sample prepared by the procedure of Giesemann.[8] The reaction of N-cyanoimidazole with 2 M imidazole resulted in the formation of 2, a result which established that N-cyanoimidazole is an intermediate in the formation of diimidazole imine from BrCN.

Both 1 and 2 effect the conversion of 3'-AMP to 2',3'-cAMP in aqueous solution. The yield of 2',3'-cAMP from the reaction of N-cyanoimidazole with 3'-AMP was optimal in





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the presence of 10 mM Cu^{+2} (Table). This result is consistent with the observation that Cu^{+2} forms the most stable complex with 3'-AMP of any of the cations of the first row transition elements.[3] The yields observed with N-cyanoimidazole are appreciably higher than those observed with mixtures of BrCN and imidazole.[3] These higher yields may reflect the loss of some of the BrCN by hydrolysis when the reaction of 3'-AMP is performed in aqueous 0.2 M imidazole buffer. There is no decrease in the yield of 2',3'-cAMP when imidazole is omitted from the N-cyanoimidazole reaction solution (Table) demonstrating that imidazole buffer has no special catalytic role in the reactions.

The relative efficacies of N-cyanoimidazole (1) and diimidazole imine (2) as condensing agents was evaluated by comparison of the rates of cyclization of 3'-AMP to 2',3'-cAMP in 0.2 M imidazole buffer. (Fig.). The faster rate of N-cyanoimidazole reaction indicates that it is the more efficient condensing agent under the reaction conditions of the BrCN reaction.[3] The determination of the time

Table

Conversion of 3'-AMP to 2',3'-cAMP with N-Cyanoimidazole[a]

Metal Ion	Yield Cyclic 2',3'-cAMP (%)
--	52
Mg^{+2}	50
Mn^{+2}	72
Ni^{+2}	74
Cu^{+2}	79
Zn^{+2}	71
b	51

[a] Reaction of 10 mM 3'-AMP, 10 mM M^{2+} and 10 mM cyanoimidazole in 0.20 M imidazole buffer (pH 6.15 ± 0.15) for 3 h at 41°C. The results are averages of 2 determinations using HPLC analysis.

[b] Same conditions as in other reactions except no buffer was used and the pH was in the 7.0-7.3 range.

course for the formation and loss of both 1 and 2 has not been determined to see which reagent is more important for the formation of 2'3'-cAMP under the conditions of the BrCN reaction.

It would be expected that the chemical reactivity of 1 and 2 parallels that of diimidazole carbonyl(3). Diimidazole carbonyl reacts with nucleotides to form phosphoimidazolides,[9] compounds which are known to react with alcohols to form phosphate esters.[10] Thus the reactions of 3'-AMP with 1 and 2 probably proceeds via the phosphoimidazolide derivative. The analogous formation of N-acetylimidazole by the reaction of acetic acid with N-cyanoimidazole has been reported.[11] Diimidazole carbonyl (3) reacts with ribonucleosides to form the corresponding 2',3'-carbonate.[12] Consequently, the facile formation of 2,2'-anhydrouridine 3'-carbonate by the reaction of uridine with BrCN probably proceeds by the initial formation of N-cyanoimidazole and/or diimidazole imine and the subsequent reaction of these intermediates with uridine to form a 2',3'-imidocarbonate.[13]

The reaction of diimidazole imine with amines was investigated to confirm its structure and compare its reactivity with diimidazole carbonyl. Since the NMR spectrum of 2 is consistent with different environments for the imidazole groups its conversion to 4 and 5 was performed to provide an unambiguous structure

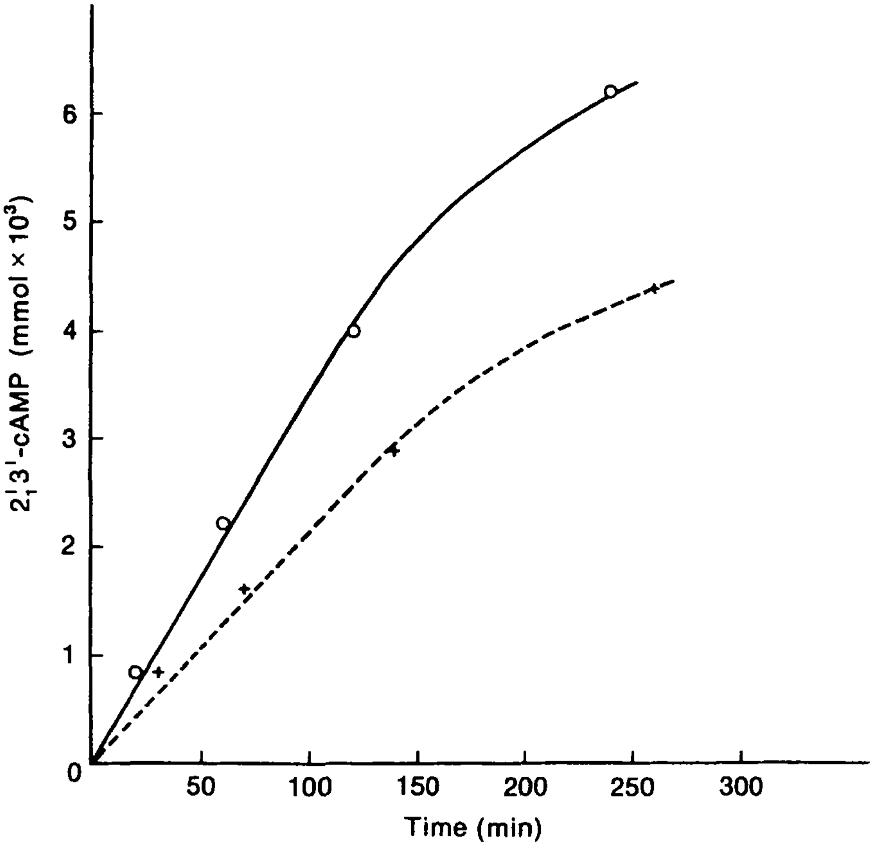

FIG. The yield of 2',3'-cAMP from the reaction of 3'-AMP with 1 (o-o) and 2 (x---x). Details in Experimental.

assignment. The reaction of amines with 2 is comparable to the first step in the reaction of glycine with diimidazole carbonyl.[14]

Compounds 1 and 2 may find application as condensing agents in both aqueous and anhydrous media. They appear to be especially well suited for use in aqueous solution because they are water soluble. In addition, they are more stable in water than diimidazole carbonyl which is reported[15] to be hydrolyzed in less than 15 min at 0° C and pH 7. The half life for hydrolysis of diimidazole imine in 0.01 M HEPES at pH 7.5 is 10 min at room temperature.[16] Although only one molar equivalent of the condensing agent was used in the cyclization of 3'-AMP (Table), the optimal yields of 2',3'-cAMP compare favorably with those obtained using a conventional condensing agent (dicyclohexylcarbodiimide) in 30-fold excess.[17] Higher yields could likely be realized if more than one molar equivalent of N-cyanoimidazole were used. The only by-product of the reaction, imidazole, is water soluble and thus readily separable from the desired reaction products. The condensing agents do not cause the pH to deviate significantly from neutrality, so no buffers are required. The likelihood that acid catalysis may enchance the reactivity of both of these reagents by protonation of the imidazole group remains to be explored.

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