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A multi-component reaction to 5-cyanouracils: Synthesis and mechanistic study

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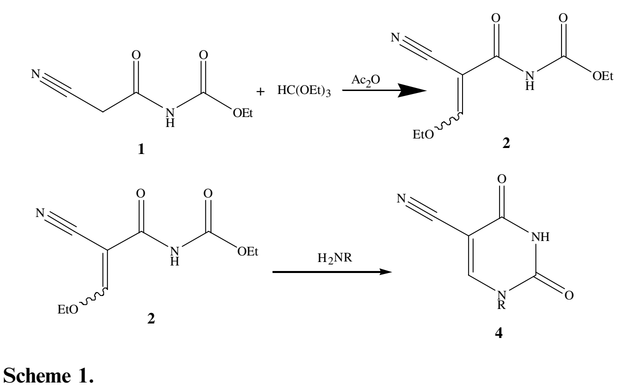
Abstract—Acetonitrile as a solvent, excess of primary amines as general bases, and a reflux condition make the multi-component reactions of (2-cyanoacetyl) carbamate **1**, ethyl orthoformate, and primary amines form 5-cyanouracils **4a**–**c** feasibly. Mechanistic studies of the multi-component reaction were carried out by proton NMR spectrometer. Acetonitrile as a solvent makes the reaction of **1** with ethyl orthoformate produce *E*-ethyl (2-cyano-3-ethoxyacryloyl) carbamate *E*-**2** without a catalyst of acetic anhydride. The reactions of *E*-**2** with primary amines produce the corresponding *Z*-**3a**–**c** as the only stable isomers eventually in $CDCl_3$ or CD_3CN. General-base-catalyzed intramolecular cyclizations of *Z*-**3a**–**c** at a reflux condition in CD_3CN generated the corresponding 5-cyanouracils **4a**–**c**.

1. Introduction

Modified nucleosides are widely distributed naturally. All species of tRNA contain unusual minor bases and many bacteria and fungi provide rich sources of nucleosides modified in the base, in the sugar, or in both base and sugar residues.[1] Modification of nucleosides sometimes shows a wide and useful biological activity due to inhibition of nucleic acid metabolism. For example, 5-fluorouracil kills cancer cells, AZT acts as an inhibitor of HIV reverse transcriptase, and 5-iodouridine is valuable for treating Herpes simplex infections.[1] Therefore, modern syntheses have been aimed at producing nucleoside analogues.[2]

Uracil is one of bases in nucleosides. Its analogues are usually prepared by base-promoted condensation of urea or its analogues with β-ketoesters, 3-alkoxyacrylates, or 3-dialkylaminoacrylates.[3] Shaw's method[4a,b] for synthesis of 5-cyanouracils involved two steps: (1) reaction of ethyl (2-cyanoacetyl) carbamate, **1**, with ethyl orthoformate in the presence of acetic anhydride produced ethyl (2-cyano-3-ethoxyacryloyl) carbamate, **2**, whose configuration was not characterized. (2) Reaction of **2** with a primary amine generated a 5-cyanouracil, **4** (Scheme 1). The drawback of Shaw's method is that the reaction of **2** with a primary amine sometimes generates a non-cyclized product, instead of 5-cyanouracil, **4**.[4c] In this article, we try to solve this problem and search for optimal conditions of a multi-component reaction (MCR)[5] for synthesis of 5-cyanouracils.

5-Fluorouracil (5-FU) is an effective antitumor agent with a strong toxicity and poor tumor affinity.[6a] A lot of efforts have been made to chemically modify 5-FU by adding lipophilic substituents in order to reduce toxicity and increase antitumor activity.[6b] It was reported that 5-FU was prepared from 5-cyanouracil by one step easily.[7] Therefore, the MCR to 5-cyanouracil derivatives in this article provides an alternative way for synthesis of the masked 5-FU.



Scheme 1.

Keywords: Multi-component reaction; Uracil.

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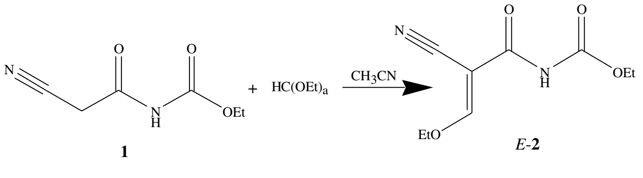
Development of synthetic oligonucleotides in the recognition of specific DNA and RNA sequences for the antisense or antigene therapy has grown explosively in last 10 years.[8] In addition to that, polyamide nucleic acids (PNA), where a polyamide replaced DNA backbone, successfully recognized their complementary double-stranded DNA by strand displacement, and this opened up a new era in molecular biology.[9] The MCR to 5-cyanouracil derivatives in this article may provide an alternative way to prepare the modified bases in oligonucleotides or PNA.

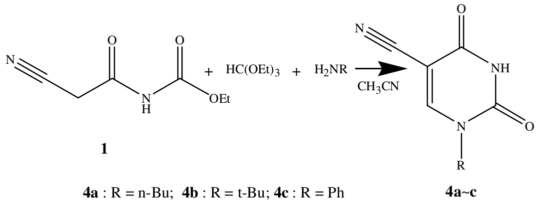
2. Results and discussion

We found that **1** hardly reacts with ethyl orthoformate in chloroform but acetic anhydride may catalyze the reaction to get *E*-**2** exclusively.[10a] We also found that *E*-**2** is much more stable than *Z*-**2** due to resonance effect and steric hindrance.[10b] When *E*-**2** was treated with excess *n*-BuNH$_2$ at room temperature, a 5-cyanouracil **4a** was generated. Then we attempted to combine these two reactions together to become a multi-component reaction (MCR) for synthesis of 5-cyanouracils **4**, so we mixed **1**, ethyl orthoformate, and primary amine together in the presence of acetic anhydride. What we found was that acetic anhydride reacted with primary amines at first before it could catalyze the reaction. To solve this problem, we discarded acetic anhydride and used a better solvent for this reaction. Due to good solubility of **1** in acetonitrile, we replaced chloroform with acetonitrile as a solvent for the reaction of **1** with ethyl orthoformate without any catalyst at a reflux condition, and it worked very well (Scheme 2). Presumably the polar acetonitrile solvent helps stabilize the polar transition state, and that makes this reaction work without a catalyst of acetic anhydride. Then acetonitrile was also used as a solvent for this MCR involving **1**, ethyl orthoformate, and primary amines without any catalyst. Strangely, sometimes cyclized products of 5-cyanouracils **4** were generated, but sometimes non-cyclized products *Z*-**3** were produced (Scheme 3). This inspired us to study its reaction mechanism and find out the key point to control this MCR.

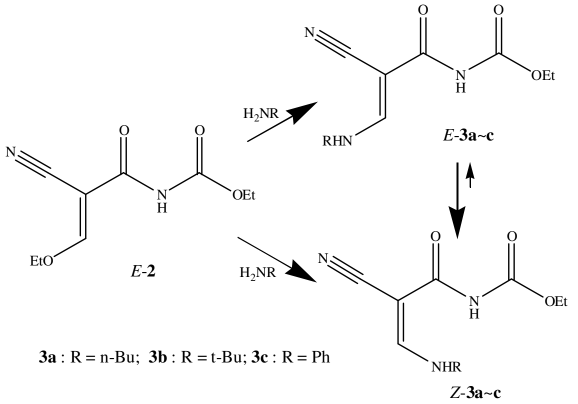
When *E*-**2** was treated with one equivalent of *n*-BuNH$_2$ in CDCl$_3$, both *E*-**3a** and *Z*-**3a** were formed at a ratio of 50:50 at 5 min, 25:75 at 30 min, and 0:100 at 24 h after the reaction (Scheme 4). This reaction was monitored by a proton NMR spectrometer, and the results are shown in Figure 1. Eventually, *Z*-**3a**, instead of *E*-**3a**, is the only stable isomer in CDCl$_3$, indicating that there is an intramolecular hydrogen bonding in *Z*-**3a**, which makes *Z*-**3a** more stable than *E*-**3a**. Similar result was found with the same reaction in CD$_3$CN. Reaction of *E*-**2** with one equivalent of *t*-BuNH$_2$ in CDCl$_3$ or CD$_3$CN generated *Z*-**3b** at a much slower rate, because bulky *t*-butylamine retarded its nucleophilic attack. Similarly, due to less nucleophilicity of aniline, reaction of *E*-**2** with one equivalent of aniline in CDCl$_3$ or CD$_3$CN produced *Z*-**3c** at a slow rate, too.



Scheme 2.



Scheme 3.



Scheme 4.

When *Z*-**3a** was heated at a reflux condition in CD$_3$CN, no intramolecular cyclization occurred. The solvent of CDCl$_3$ was not used in the reaction because of limited solubility of **4a–c** in CDCl$_3$. Similarly, we did not obtain any intramolecular cyclization product when *Z*-**3b** or *Z*-**3c** was heated at a reflux condition in CD$_3$CN.

When *Z*-**3a** was treated with excess NEt$_3$ in CD$_3$CN at room temperature, the doublet signal for the ^{1}H NMR absorption of the vinyl hydrogen of *Z*-**3a** turned to a singlet immediately and 5-cyanouracil **4a** was generated in 4 h (Scheme 5). This reaction was monitored by a proton NMR spectrometer and the result is shown in Figure 2. This result implies that NEt$_3$ works as a general base[11] to remove the proton on enamine nitrogen and enhances nucleophilicity of the enamine for the intramolecular attack on carbonyl group of ester. During this general-base-promoted intramolecular cyclization, a small amount of *E*-**3a** was found because of NEt$_3$-promoted isomerization.

On the other hand, when *Z*-**3b** was treated with excess NEt$_3$ in CD$_3$CN at room temperature, we found that the doublet signal for the ^{1}H NMR absorption of the vinyl hydrogen of *Z*-**3b** became a singlet. However, no intramolecular cyclization occurred at all until the reaction temperature was increased to 80 °C. Clearly the bulky *t*-Bu group retarded the ring-closure reaction.

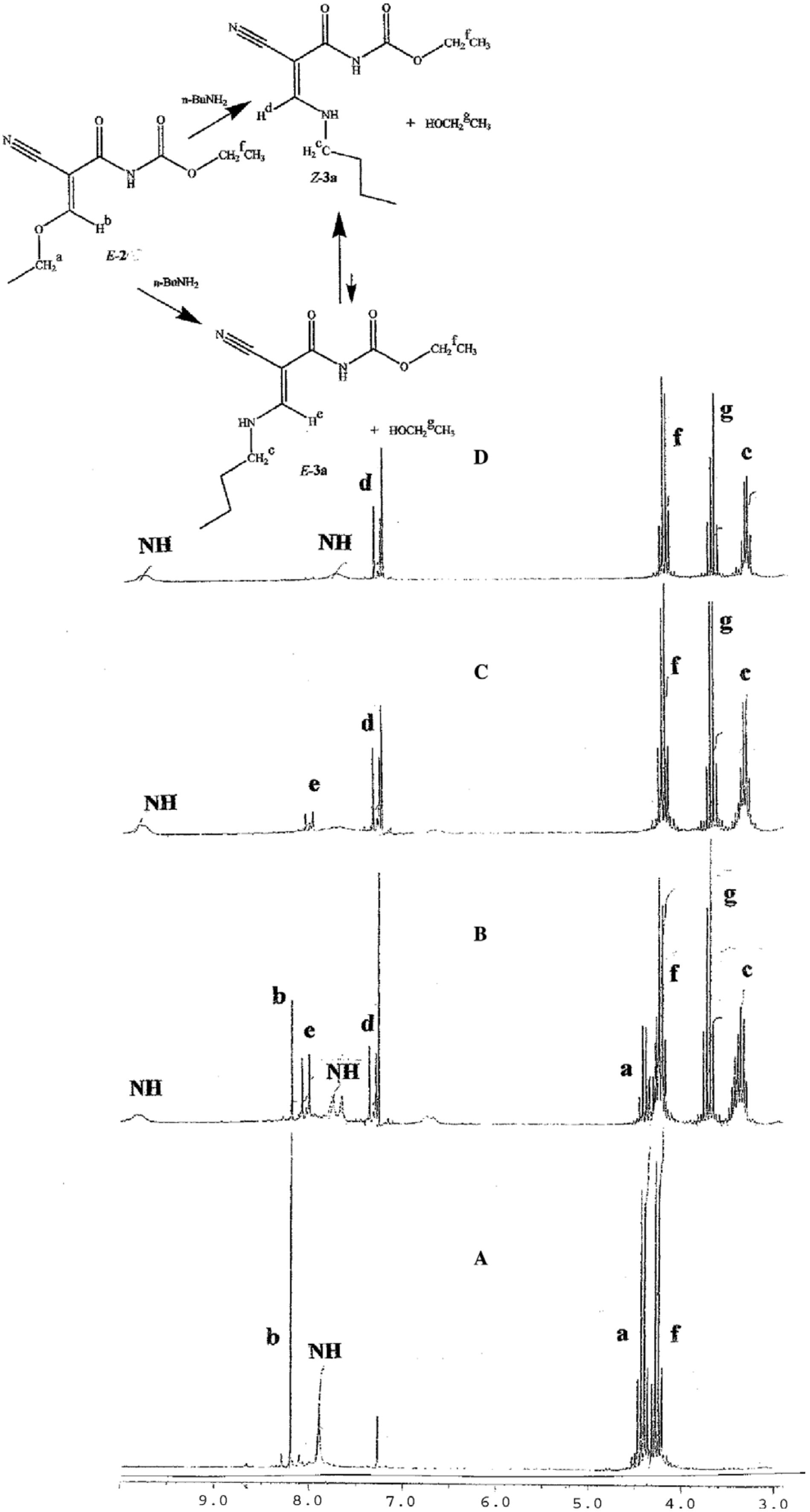
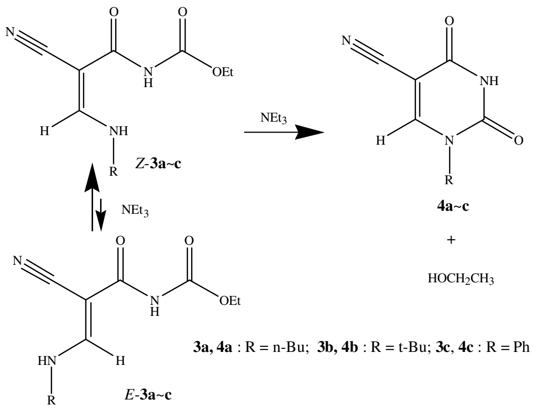


Figure 1. Part of ^{1}H NMR ($CDCl_3$) spectra for the reaction of *E*-**2** with *n*-$BuNH_2$ at 25 °C (A) before adding *n*-$BuNH_2$, (B) at 5 min, (C) at 30 min, and (D) at 24 h after mixing the solution.



Scheme 5.

Similarly, the reaction of *Z*-**3c** with excess NEt_3 in CD_3CN at room temperature made the doublet signal for the ^1H NMR absorption of the vinyl hydrogen of *Z*-**3c** become a singlet. The ring-closure product *Z*-**4c** was not generated until the reaction temperature was raised to 80 °C, indicating that *N*-phenyl group reduced nucleophilicity of the enamine.

According to the mechanistic studies we just mentioned, there are three key points in carrying out the MCR of **1**, ethyl orthoformate, and primary amines to form the 5-cyanouracils **4**. (1) Acetonitrile as a solvent in the MCR may eliminate the use of acetic anhydride as a catalyst. (2) A general base like NEt_3 is needed for the intramolecular cyclization to form the 5-cyanouracils **4**, and primary amines in the MCR can be used for this purpose. (3) To overcome the barrier of the ring-closure reactions, reflux is needed for some of this type of reactions. Therefore, to do the MCR to 5-cyanouracils **4**, we mixed **1** and ethyl orthoformate with excess of primary amine like *n*-$BuNH_2$, *t*-$BuNH_2$, or aniline in acetonitrile and heated the reaction mixture at a reflux condition for 4 h. By following this method, isolated yields of 5-cyanouracils **4a**–**c** were good. Aliphatic, aromatic, and bulky primary amines are suitable for this MCR. However, primary amines with other active functional groups like aldehyde, carboxylic acid, etc., are not suitable for this MCR, so these active functional groups need to be protected before running this MCR.

Configuration assignment of *E*-**3a**–**c** and *Z*-**3a**–**c** is based on two findings. (1) Proton NMR absorption of the vinyl hydrogen (δ 8.17) of *E*-**2** is 0.37 ppm more downfield than that (δ 7.80) of *Z*-**2**.[10a] The vinyl hydrogen of **3** is subjected to almost the same electronic environment as that of **2**, so proton NMR absorption of the vinyl hydrogen of *E*-**3a**–**c** should be a more downfield than that of *Z*-**3a**–**c**. (2) Theoretically, only *Z*-**3a**–**c** can undergo intramolecular cyclization to form the corresponding 5-cyanouracils **4a**–**c**. In these proton NMR experiments (Figs. 1 and 2), proton NMR absorption of the vinyl hydrogen of the one that does intramolecular cyclization is more upfield. It means that proton NMR absorptions of the vinyl hydrogens of *Z*-**3a**–**c** are more upfield than those of *E*-**3a**–**c**, and this is consistent with the first finding.

In comparison with the original Shaw's method for the synthesis of **4**, our modified Shaw's method has the following advantages. (1) Shaw's method used acetic anhydride to catalyze the first step of reaction. Our modified method discarded acetic anhydride as a catalyst but used acetonitrile as a solvent. (2) Shaw's method is a two-pot reaction. Our modified method is a one-pot MCR with high yield. (3) We found that the second step of the reaction in Shaw's method is general-base catalyzed, so excess amine is needed to accelerate the ring-closure reaction to **4**.

3. Conclusion

Acetonitrile as a solvent makes the reaction of **1** with ethyl orthoformate produce *E*-**2** without a catalyst of acetic anhydride. The reactions of *E*-**2** with primary amines produce the corresponding *Z*-**3a**–**c** as the only stable isomers eventually in $CDCl_3$ or CD_3CN. Intramolecular cyclizations of *Z*-**3a**–**c**, which form the corresponding 5-cyanouracils **4a**–**c**, work in CD_3CN at a reflux condition and in the presence of a general base like NEt_3. Acetonitrile as a solvent, excess of primary amines as general bases, and a reflux condition make the MCR of **1**, ethyl orthoformate, and primary amines form 5-cyanouracils **4a**–**c** feasibly.

4. Experimental

4.1. General

All the reagents were obtained from commercial suppliers and used as received. Ethyl (2-cyanoacetyl) carbamate **1** was prepared according to the literature method.[4]

4.2. *E*-Ethyl (2-cyano-3-ethoxyacryloyl) carbamate (*E*-2)

To a solution of **1** (0.312 g, 2 mmol) in 2 mL of acetonitrile was added ethyl orthoformate (1 mL, 6 mmol). The mixture was refluxed at 80 °C under nitrogen atmosphere for 4 h. After the reaction was complete, the reaction mixture was cooled down and concentrated by rotary evaporator. Ether was poured into the reaction mixture and the mixture kept in a refrigerator for 12 h. After filtration of the mixture, white powder was collected and recrystallized from chloroform/ether. Yield: 92%; its characterization is the same as in the literature.[10a]

4.3. General method to prepare *Z*-3a–c

To a solution of **2** (0.2 g, 1 mmol) in 1 mL of $CDCl_3$ was added 1 mmol of *n*-butylamine, *t*-butylamine, or aniline. The mixture was monitored by a proton NMR spectrometer. At the beginning, both *cis* and *trans* isomers of **3a**–**c** were found, but 1 day later the *cis* isomer is the only product. The solvent and byproducts were removed from the solution by rotary evaporator to get crude *Z*-**3a**–**c**, which were purified by column chromatography with a mobile phase of hexane/ethyl acetate (3:1).

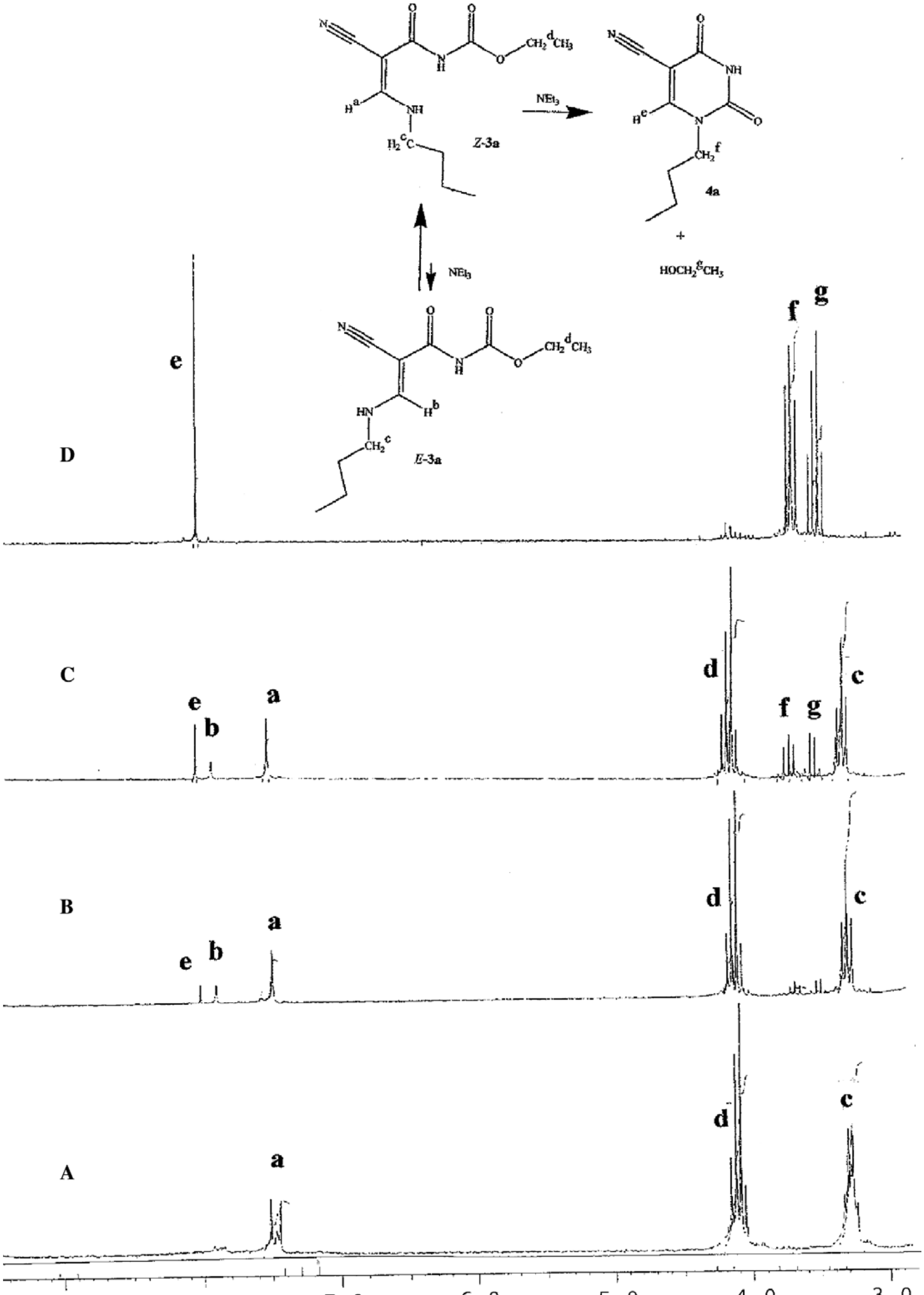


Figure 2. Part of ^{1}H NMR (CD_3CN) spectra for the intramolecular cyclization of *Z*-**3a** at 25 °C (A) before adding excess NEt_3, (B) at 5 min, (C) at 10 min, and (D) at 4 h after adding excess NEt_3.

4.3.1. *Z*-Ethyl [2-cyano-3-(*n*-butylamino) acryloyl] carbamate (*Z*-3a). Yield: 95%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, t, $J = 7.3$ Hz, CH_3), 1.31 (3H, t, $J = 7.1$ Hz, CH_3), 1.38 (2H, m, CH_2), 1.57 (2H, m, CH_2), 3.36 (2H, t, $J = 6.7$ Hz, CH_2), 4.23 (2H, t, $J = 7.1$ Hz, CH_2), 7.32 (1H, d, $J = 13.9$ Hz, CH_2), 7.74 (1H, s, NH), 9.81 (1H, s, NH); ^{13}C NMR (CD_3CN) δ 13.41, 14.14, 19.41, 32.17, 50.04, 62.09, 71.26, 118.77, 150.31, 159.42, 165.03; IR (thin film) 2199 (CN), 1762, 1671 (C=O) cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{11}H_{17}N_3O_3$-Na ($[M + Na]^+$) 262.1168, found 262.1170.

4.3.2. *Z*-Ethyl [2-cyano-3-(*t*-butylamino) acryloyl] carbamate (*Z*-3b). Yield: 92%; yellow oil; ^{1}H NMR ($CDCl_3$) δ

1.31 (3H, t, $J = 7.0$ Hz, CH_3), 1.35 (9H, s, CH_3), 4.24 (2H, q, $J = 7.0$ Hz, CH_2), 7.46 (2H, d, $J = 13.9$ Hz, CH_2), 10.17 (1H, d, $J = 13.9$ Hz, NH); ^{13}C NMR (CD_3CN) δ 14.13, 29.50, 54.55, 62.05, 70.86, 119.11, 150.32, 155.29, 165.06; IR (thin film) 2200 (CN), 1766, 1667 (C=O) cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{11}H_{17}N_3O_3Na$ ($[M + Na]^+$) 262.1168, found 262.1167.

4.3.3. *Z*-Ethyl [2-cyano-3-anilinoacryloyl] carbamate (*Z*-3c). Yield: 96%; yellow oil; ^1H NMR ($CDCl_3$) δ 1.32 (3H, t, $J = 7.1$ Hz, CH_3), 4.27 (2H, q, $J = 7.1$ Hz, CH_2), 7.12 (2H, m, PhH), 7.25 (1H, m, PhH), 7.41 (2H, m, PhH), 7.89 (1H, d, $J = 13.3$ Hz, CH), 7.91 (1H, s, NH), 11.62 (1H, d, $J = 13.3$ Hz, NH); ^{13}C NMR (CD_3CN) δ 14.13, 62.38, 75.17, 117.34, 117.96, 126.29, 130.06, 137.85, 150.06, 152.37, 164.56; IR (thin film) 2203 (CN), 1765, 1665 (C=O) cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{13}H_{13}N_3O_3Na$ ($[M + Na]^+$) 282.0855, found 282.0855.

4.4. General method for intramolecular cyclizations of *Z*-3a–c

To a solution of *Z*-**3a**–**c** (0.2 mmol) in 1 mL of CD_3CN was added triethylamine (0.2 g, 2 mmol). The mixture was kept for 4 h at room temperature for *Z*-**3a** and at 80 °C for *Z*-**3b** and *Z*-**3c**. The whole process was monitored by a proton NMR spectrometer. The solution was concentrated by a rotary evaporator and the solid product was recrystallized by chloroform/acetonitrile to afford **4a**–**c**.

4.4.1. 1-*n*-Butyl-5-cyanouracil (4a). Yield: 93%; white solid; mp: 181 °C; ^1H NMR (CD_3CN) δ 0.93 (3H, t, $J = 7.3$ Hz, CH_3), 1.34 (2H, m, CH_2), 1.63 (2H, m, CH_2), 3.74 (2H, t, $J = 7.3$ Hz, CH_2), 8.12 (1H, s, CH); ^{13}C NMR (CD_3CN) δ 13.84, 20.13, 31.48, 50.16, 89.14, 114.73, 153.00, 154.92, 161.29; IR (thin film) 2235 (CN), 1726, 1704 (C=O) cm^{-1}; HRMS (ESI) *m/z* calcd for $C_9H_{11}N_3O_2$ ($[M]^+$) 194.0929, found 194.0931.

4.4.2. 1-*t*-Butyl-5-cyanouracil (4b). Yield: 95%; white solid; mp: 241 °C; ^1H NMR (CD_3CN) δ 1.52 (9H, s, CH_3), 8.18 (1H, s, CH); ^{13}C NMR (CD_3CN) δ 28.49, 64.41, 88.41, 115.21, 150.41, 152.59, 161.14; IR (thin film) 2234 (CN), 1734, 1704 (C=O) cm^{-1}; HRMS (ESI) *m/z* calcd for $C_9H_{11}N_3O_2Na$ ($[M + Na]^+$) 216.0749, found 216.0751.

4.4.3. 5-Cyano-1-phenyluracil (4c). Yield: 96%; white solid; mp: 278 °C; ^1H NMR (CD_3CN) δ 7.35 (2H, m, PhH), 7.54 (3H, m, PhH), 8.22 (1H, s, CH); ^{13}C NMR (CD_3CN) δ 90.25, 114.43, 118.37, 127.79, 130.51, 138.90, 150.13, 154.88, 161.21; IR (thin film) 2238 (CN), 1721, 1713 (C=O) cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{11}H_7N_3O_2Na$ ($[M + Na]^+$) 236.0436, found 236.0437.

4.5. Multi-component reaction to 5-cyanouracils (4a–c)

To a solution of **1** (0.156 g, 1 mmol) and ethyl orthoformate (1 mL, 6 mmol) in 1.5 mL of acetonitrile was added 7 mmol of *n*-butylamine, *t*-butylamine, or aniline. The mixture was refluxed at 60 °C under nitrogen atmosphere for 4 h. The reaction mixture was cooled down and concentrated by rotary evaporator. The solid residue was recrystallized by chloroform/acetonitrile to get **4a**–**c**. Yields to **4a**, **4b**, and **4c** were 87%, 90%, and 95%, respectively.

Acknowledgment

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