Synthesis and DNA Strand Breakage Activity of Some 1,4-Diazepines

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Reactions of 1,3-propanediamine with α-dicarbonyl compounds (1a—e) were examined and various condensed heterocyclic compounds such as 1,4-diazepines (2) and 3-pyrimidine derivatives (3) were obtained. Some of 1,4-diazepines (2) showed DNA strand breakage activity.

Key words 1,4-diazepine; 1,3-pyrimidine; DNA strand breakage; plasmid pBR322; α-dicarbonyl; 1,3-propanediamine

Study of compounds having DNA breakage activity is valuable because the DNA strand breakage process is involved in various biological stages such as inflammation, mutagenesis, carcinogenesis, or aging.[1] The elucidation of DNA breakage activity may contribute to the clarification of such important biological phenomena. Recently, we reported[2—5] that some pyrazine-related derivatives and other heterocyclic compounds derived from the reaction of 2,3-butanedione with ethylenediamine derivatives show significant DNA strand breakage activities.

In our recent report,[5] we obtained a 6,7-dihydro-5*H*-1,4-diazepine derivative (DHDA) (**2b**) having a 7-membered ring similar to that of the 6-membered ring system, and some of the products showed DNA breakage activity. The chemistry of 1,4-diazepine has attracted much attention because of the unique pharmacological activity toward central the nervous system (CNS) as observed in 1,4-benzodiazepines.[6] By contrast, simple monocyclic 1,4-diazepines have received less attention, with the exception of the 2,3-dihydro-1*H*-1,4-diazepine system.[7] Regarding the 6,7-dihydro-5*H*-1,4-diazepine (**2**) system as well as the corresponding unsaturated system, the preparation of these analogues is known to be difficult[8] and occasionally unsuccessful. To obtain further information on pyrazine analogues, we investigated the reaction of 1,3-propanediamine (PD) with α-dicarbonyl compounds. In this paper, we describe the synthesis of 6,7-dihydro-5*H*-1,4-diazepines (**2b**—**d**)[9,10] and a related 1,4-diazepine-type compound **2′e**[8] in a one-pot reaction and evaluate their DNA breakage activities.

Results and Discussion

Reactions of α-dicarbonyls (1a—e) with PD To synthesize a variety of the target DHDA derivatives, reactions of α-dicarbonyls (**1a**—**e**) with PD were attempted. The isolated products are shown in Charts 1 and 2.

The results are summarized in Table 1 and the physical data are listed in Table 2. The structures of the products were established by spectroscopic methods (^{1}H- and ^{13}C-NMR), the results of which are summarized in Table 3. Full assignments of these signals were confirmed by their ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra. The general procedure is as follows. The, α-dicarbonyl compound was added to a solution of PD in the proper solvent, the resulting mixture was heated or refluxed, and purification performed to give the products. As shown in Table 1, the re-

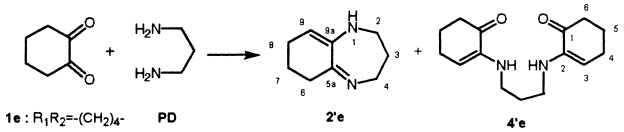

Chart 2

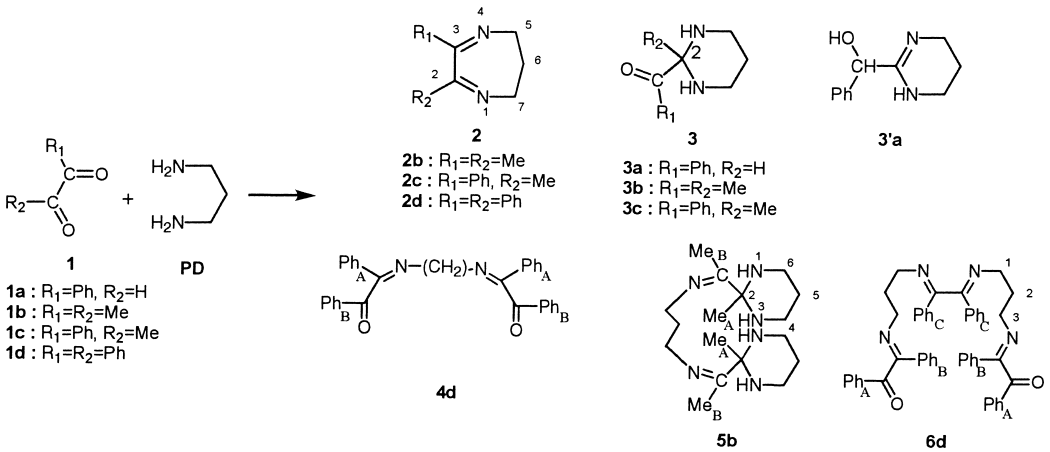

Chart 1

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Table 1. Reactions of PD with α-Dicarbonyls (**1a**—**e**)

Entry	1	Ratio of 1 : PD : (additive)	Solvent	Conditions	Products (Yield %)			
1	**1a**	1 : 1	Ether	Reflux 40 min		**3a** (89)		
2	**1a**	1 : 1	EtOH	65 °C 5 min, rt 1 d		**3′a** (17)		
3	**1b**	1 : 1	Ether	Reflux 1 h	**2b** (6),	**3b** (89)		
4	**1b**	1 : 2	Ether	Reflux 8 h			**5b** (80)	
5	**1b**	1 : 1	$CHCl_3$	Reflux 7 d	**2b** (15)			
6	**1c**	1 : 1	Ether	Reflux 3 h		**3c** (98)		
7	**1c**	1 : 1	CH_3CN	80 °C 1 h	**2c** (18),	**3c** (14)		
8	**1c**	1 : 1	CH_3CN	Reflux 1 d	**2c** (78)			
9	**1d**	1 : 1	Benzene	Reflux 1 d	**2d** (2),		**4d** (14),	**6d** (9)
10	**1d**	2 : 1	Benzene	Reflux 5 h			**4d** (25),	**6d** (7)
11	**1d**	1 : 1 : 0.04 (TsOH)	Benzene	Reflux 4 d	**2d** (70)			
12	**1d**	1 : 1 : 1 (AcOH)	EtOH	Reflux 1 d	**2d** (85)			
13	**1e**	1 : 1	CH_3CN	85 °C 20 min	**2′e** (64),		**4′e** (1)	
14	**1e**	2 : 1	H_2O	rt 7 h	**2′e** (trace),		**4′e** (37)	

a) The yield of **2b** (6%) was calculated by NMR analysis of the distilled fraction.

Table 2. Physical Data for Compounds (**2**—**4**)

Compd.	mp/bp (°C) (Recryst solvent)	Formula	Analysis (%) Calcd (Found)			Formula HR-MS *m/z* Calcd (Found)	IR (cm^{-1}) (KBr)
			C	H	N		
2b	60—65/ 1 mmHg	$C_7H_{12}N_2 \cdot H_2O$	59.12 (58.97	9.92 9.86	19.70 19.99)	$C_{14}H_{25}N_4$ $(2M+H)^+$ 249.2079 (249.2070)	1645 (C=N)
2c	Oil	$C_{12}H_{14}N_2 \cdot 0.25H_2O$	75.56 (75.50	7.66 7.45	14.69 14.42)	$C_{12}H_{15}N_2$ $(M+H)^+$ 187.1235 (187.1238)	1635 (C=N) 1605 (C=N) 1580 (C=C of Ph)
2d	114—116 (EtOH)	$C_{17}H_{16}N_2$	82.22 (82.14	6.49 6.52	11.28 11.18)	$C_{17}H_{17}N_2$ $(M+H)^+$ 249.1391 (249.1391)	1610 (C=N) 1575 (C=C of Ph)
2′e	174—178 (EtOH)	$C_9H_{14}N_2$	71.96 (71.71	9.39 9.19	18.65 18.66)	$C_9H_{13}N_2$ $(M-H)^+$ 149.1078 (149.1085)	3340 (NH) 1630 (C=C) 1610 (C=N)
3a	153—158 (Ether)	$C_{11}H_{14}N_2O$	69.45 (69.24	7.42 7.43	14.73 14.78)	$C_{11}H_{15}N_2O$ $(M+H)^+$ 191.1185 (191.1191)	3500 (NH) 1640 (C=O)
3′a	187—189 (EtOH)	$C_{11}H_{14}N_2O$	69.45 (69.42	7.42 7.38	14.73 14.69)	$C_{11}H_{15}N_2O$ $(M+H)^+$ 191.1185 (191.1181)	3365 (br, NH, OH) 1630 (C=N)
3c	90—92 (Ether)	$C_{12}H_{16}N_2O \cdot H_2O$	69.34 (69.36	7.95 7.76	13.48 13.54)	$C_{12}H_{17}N_2O$ $(M+H)^+$ 205.1341 (205.1342)	3315 (NH) 1675 (C=O) 1595 (C=C of Ph)
4d	114—116 (AcOEt)	$C_{31}H_{26}N_2O_2$	81.20 (81.17	5.72 5.81	6.11 6.06)	$C_{31}H_{27}N_2O_2$ $(M+H)^+$ 459.2073 (459.3069)	1665 (C=O) 1625 (C=N) 1580 (C=C of Ph)

actions of α-dicarbonyls (**1b**—**e**) gave the desired DHDA-type compounds (**2b**—**d**) and the similar compound **2′e** in good yields. However, in the case of phenylglyoxal (**1a**) with ether as a solvent, we could not obtain the DHDA derivative **2a** (R_1=Ph, R_2=H), and the hexahydropyrimidine (HHP) derivative (**3a**) was the sole product (89%) (see entry 1). Using EtOH as a solvent, a tetrahydropyrimidine derivative (**3′a**) was isolated in 17% yield (see entry 2). In a similar manner, **3b** was isolated in excellent yield (89%) (entry 3). In this entry, a small amount of **2b** (6%) was also obtained. In the reaction of mole ratio (**1b** : PD=1 : 2), the propylene-bridged HHP derivative (**5b**) was obtained in good yield as unstable crystals (entry 4). Recently,[5] we reported the isolation of **2b** with the reaction of **1b** and PD after 7-d refluxing in $CHCl_3$ (entry 5, see Method A in Experimental). Although the formation rate was estimated at more than 95% yield by monitoring of the reaction mixture with the ^{1}H-NMR spectrum, the isolated yield (15%) was quite low because of the difficulty of trapping it in distillation. Alternatively, refluxing a solution of the isolated **3b** in $CHCl_3$ gave **2b** by a ring-expansion reaction in 26% yield (see Method B in Experimental).

The reaction of 1-phenyl-1,2-propanedione (**1c**) and PD was similar to that of **1b**. Eggleston and Jackels[11] reported the formation of **3c** in the reaction of **1c** and PD in CCl_4 or CD_3OD. However, they did not mention the formation of the diazepine derivative **2c**. The reaction under the conditions of low temperature with ether as a solvent afforded HHP-type **3c** as the sole product (entry 6), and we found that the condition of higher temperature under prolonged heating resulted in the predominant formation of DHDA-type **2c** (entries 7

Table 3. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds (**2**—**6**)

Compound	^{1}H-NMR		
	N–C<u>H</u>$_2$–	N–CH$_2$–C<u>H</u>$_2$–	Me or Ph or other
2b[a]	3.21 (4H, t, *J*=6.9)	2.18 (2H, qu, *J*=6.9)	2.08 (3H, s, Me)
2c[a]	3.35 (2H, t, *J*=6.7, H-7) 3.44 (2H, t, *J*=6.7, H-5)	2.28 (2H, qu, *J*=6.7)	2.12 (3H, s, Me), 7.41—7.45 (3H, m, *m*-, *p*-PhH), 7.57—7.59 (2H, m, *o*-PhH)
2d[a]	3.55 (4H, m)	2.36 (2H, m)	7.28—7.32 (4H, m, *m*-PhH), 7.33—7.37 (2H, m, *p*-PhH), 7.60—7.62 (4H, m, *o*-PhH)
2′e[a]	3.05—3.08 (2H, m, H-2) 3.45 (2H, t, *J*=5.2, H-4)	2.02—2.06 (2H, m, H-3)	1.76 (2H, qu, *J*=6.4, H-7), 2.21—2.24 (2H, m, H-8), 2.34 (2H, t, *J*=6.4, H-6), 4.93 (1H, t, *J*=4.6, H-9), 6.57 (1H, br s, NH)
3a[a]	3.02—3.09 (2H, m) 3.28—3.34 (2H, m)	1.48—1.60 (2H, m)	1.5 (1H, br s, NH), 5.03 (1H, s, H-2), 7.44—7.48 (2H, m, *m*-PhH), 7.54—7.58 (1H, m, *p*-PhH), 8.04—8.08 (2H, m, *o*-PhH)
3′a[b]	3.27 (4H, t, *J*=5.8)	1.72 (2H, q, *J*=5.8)	5.03 (1H, s, PhCH–), 7.25 (1H, tm, *J*=7.3, *p*-PhH), 7.31 (2H, tm, *J*=7.3, *m*-PhH), 7.46 (2H, dm, *J*=7.6, *o*-PhH)
3c[a]	2.62—2.68 (2H, m) 2.92—2.97 (2H, m)	1.35—1.45 (1H, m) 1.55—1.60 (1H, m)	1.42 (3H, s, Me), 2.15 (2H, br s, NH), 7.41 (2H, tm, *J*=7.9, *m*-PhH), 7.52 (1H, tm, *J*=7.3, *p*-PhH), 8.49 (2H, dm, *J*=7.3, *o*-PhH)
4d[a]	3.55 (4H, t, *J*=6.7)	2.06 (2H, qu, *J*=6.7)	7.30—7.34 (4H, m, *m*-Ph$_A$H), 7.37—7.40 (2H, m, *p*-Ph$_A$H), 7.41—7.44 (4H, m, *m*-Ph$_B$H), 7.54—7.58 (2H, m, *p*-Ph$_B$H), 7.61—7.63 (4H, m, *o*-Ph$_A$H), 7.86—7.88 (4H, m, *o*-Ph$_B$H)
4′e[a]	2.93 (4H, t, *J*=6.9)	1.82 (2H, qu, *J*=6.9)	1.92—1.97 (4H, m, H-5), 2.35—2.38 (4H, m, H-4), 2.45—2.48 (4H, m, H-6), 5.42 (2H, t, *J*=4.7, H-3)
5b[c]	3.40 (4H, t, *J*=6.7, C=N–C<u>H</u>$_2$–) 2.57—2.64, 2.75—2.80 (4H, m, H-4,6)	1.99 (2H, qu, *J*=6.7, C=N–CH$_2$ C<u>H</u>$_2$–), 1.22—1.31, 1.50—1.62 (2H, m, H-5)	1.05 (6H, s, Me$_A$), 1.90 (6H, s, Me$_B$)
6d[a]	3.38 (4H, t, *J*=6.7, H-3) 3.50—3.59 (4H, m, H-1)	1.97—2.08 (4H, m)	7.24—7.28 (4H, m, *m*-Ph$_B$H), 7.28—7.35 (6H, m, *p*-Ph$_B$H, *m*-Ph$_C$H), 7.35—7.39 (2H, m, *p*-Ph$_C$H), 7.39—7.44 (4H, m, *m*-Ph$_A$H), 7.54—7.58 (2H, m, *p*-Ph$_A$H), 7.59—7.64 (8H, m, *o*-Ph$_B$H, -Ph$_C$H), 7.84—7.88 (4H, m, *o*-Ph$_A$H)

Compound	^{13}C-NMR		
	N–<u>C</u>H$_2$–	N–CH$_2$–<u>C</u>H$_2$–	Me or Ph or other
2b[a]	47.91	32.14	22.9
2c[a]	48.34 (C-7) 48.58 (C-5)	32.15	23.98 (Me), 127.19 (*o*-C of Ph), 128.63 (*m*-C of Ph), 130.49 (*p*-C of Ph), 135.56 (C-Ph), 167.63 (C-3), 168.74 (C-2)
2d[a]	48.93	31.99	127.43 (*o*-C of Ph), 128.55 (*m*-C of Ph), 130.45 (*p*-C of Ph), 136.24 (C-Ph), 166.76 (C-2,3)
2′e[a]	45.19 (C-2) 51.53 (C-4)	28.63 (C-3)	22.77 (C-7), 23.87 (C-8), 26.65 (C-6), 101.28 (C-9), 141.21 (C-9a), 162.32 (C-5a)
3a[a]	41.29	28.17	71.78 (C-2), 128.59 (*m*-C of Ph), 129.06 (*o*-C of Ph), 133.49 (*p*-C of Ph), 134.79 (C-Ph), 196.70 (C=O)
3′a[b]	41.91	21.59	75.30 (Ph<u>C</u>H–), 127.51 (*o*-C of Ph), 128.74 (*p*-C of Ph), 129.23 (*m*-C of Ph), 143.10 (C-Ph), 162.29 (C-2)
3c[a]	42.45	26.09	29.28 (Me), 76.75 (C-2), 127.91 (*m*-C of Ph), 132.61 (*p*-C of Ph), 130.19 (*o*-C of Ph), 135.84 (C-Ph), 204.35 (C=O)
4d[a]	51.18	32.22	127.24 (*o*-C of Ph$_A$), 128.53 (*m*-C of Ph$_A$), 129.17, 129.19 (*o*-, *m*-C of Ph$_B$), 130.70 (*p*-C of Ph$_A$), 134.50 (*p*-C of Ph$_B$), 134.71 (C-Ph$_B$), 135.34 (C-Ph$_A$), 166.68 (C=N), 198.86 (C=O)
4′e[a]	40.97	28.35	23.50 (C-5), 24.55 (C-4), 37.93 (C-6), 111.09 (C-3), 140.59 (C-2), 195.80 (C-1)
5b[c]	42.61 (C-4,6) 57.31 (C=N–<u>C</u>H$_2$–)	27.09 (C-5) 32.92 (C=N–CH$_2$–<u>C</u>H$_2$–)	13.28 (Me$_B$), 29.80 (Me$_A$), 73.72 (C-2), 173.06 (C=N)
6d[a]	51.60 51.72	32.16	127.18, 127.24 (*o*-C of Ph$_B$, Ph$_C$), 128.55, 128.60 (*m*-C of Ph$_B$, *p*-C of Ph$_C$), 129.15, 129.19 (*o*-, *m*-C of Ph$_A$), 130.54, 130.69 (*p*-C of Ph$_B$, Ph$_C$), 134.51 (*p*-C of Ph$_A$), 134.74 (C-Ph$_A$), 135.31 (C-Ph$_C$), 135.91 (C-Ph$_B$), 165.42 (Ph$_B$–C=N–), 166.59 (Ph$_C$–C=N–), 198.85 (C=O)

a) Measured in $CDCl_3$. *b*) Measured in CD_3OD. *c*) Measured in ether-d_{10}.

and 8). The reaction of **1d** and PD resulted in a trace formation of cyclized products, and intermolecular polymeric condensation was accelerated to give mainly multiple condensed compounds **4d**[12] and **6d** (entries 9 and 10). In this reaction, the addition of an acid catalyst such as *p*-toluenesulfonic acid (TsOH) or acetic acid (AcOH), prolongation of reaction time, or employment of a solvent having higher polarity enhanced cyclization to give DHDA (**2d**) in good yields (entries 11, 12).

Our recent observation[5] in the reaction of **1b** and PD by ^{1}H-NMR spectra suggested that an initial intermediate is a common imino ketone **I** (diastereomers *E* and *Z*) (Chart 3).

These two diastereomers are apparently convertible through **3**, and both the *E* and *Z* isomer could afford HHP (**3**). On the other hand, cyclization to DHDA (**2**) proceeds only *via* the *Z* isomer because of its steric demand. In some benzil-monoimines (for example, *N*-(1-phenylethyl)-1-benzoyl-benzylidene-imine) assigned the *Z* configuration around the C=N bond it has been established that the planes containing C=O and C=N groups (conjugated with two phenyl groups bonded to them) take nearly orthogonal features to minimize the dipolar repulsion between them.[13] The structural resemblance of imino ketone **Id** (R_1=R_2=Ph) and the above benzoyl-imines may be responsible for the predominant formation of **Id**(*Z*) and no formation of HHP (**3d**). Although ^{1}H-NMR studies on the 1 : 1 condensation of **1d** with PD were undertaken, this reaction was quite complicated and many condensation products prevented the assignment of **Id**(*Z*) and **Id**(*E*) in the spectra of the reaction mixture. Therefore we tried to analyze the reaction using a simplified model system. Thus we synthesized 2-(3-dimethylaminopropylimino)-1,2-diphenyl-ethanone (**7**) as a model for an imino ketone structure to reveal the configuration around the C=N bond (see Chart 4). The NMR spectra of **7** indicated the presence of two diastereomers in a proportion of 95 : 5.[14] In addition, in the nuclear Overhauser effect spectroscopy (NOESY) experiment on the predominant isomer of **7**, a significant NOE between the *ortho*-aromatic HB of the benzoyl group and H1′ of =N–CH_2– was observed, but no NOE between the *ortho*-aromatic HA of benzylidene-imine and H1′ of =N–CH_2– was observed. This apparently indicates that the predominant isomer of imino ketone **7** exists in the *Z* form. The steric energy and population of **Id**(*Z*) and **Id**(*E*) were calculated by molecular dynamics (MD)[15] using the Merck molecular force field (MMFF)[16] parameters. The results of calculation showed that the steric energy of **Id**(*Z*) is lower than that of **Id**(*E*) by an energy difference of 5.64 kcal/mol and the population of **Id**(*Z*) is estimated at almost 100%. The failure of the isolation of **3c′** (R_1=Me, R_2=Ph) can also be attributable to the predominant formation of the imino ketone **Ic** (R_1=Ph, R_2=Me) rather than the imino ketone **Ic′** (R_1=Me, R_2=Ph).[17] Cyclic α-dicarbonyl compound 1,2-cyclohexanedione (**1e**) easily reacted with PD to produce a diazepine-type compound **2′e** in 64% yield together with formation of a trace of trimethylene-bridged dimeric **4′e** (1%) (entry 13). Our further trial of the reaction of mole ratio (**1e** : PD=2 : 1) in water afforded **4′e** predominantly, and a trace of **2′e** was detected by TLC, but the trial of isolation was unsuccessful (entry 14). We observed the tendency for intermolecular multiple condensation to take place and give polymeric compound **4d** or **6d** in the case of the reactions with **1d**. Details of these reaction conditions and experimental results are summarized in Table 1.

Evaluation of DNA strand breakage activities by reaction products (2—4) The DNA strand breakage activity data are shown in Table 4. For comparison, the activities of 2,3-dihydropyrazine derivatives (DHP1 and DHP2) are also listed in Table 4. These DNA strand breakage activities were remarkably accelerated by the addition of cupric ion (Cu^{2+}), which may stimulate the production of active radicals, resulting in DNA strand breakage.[18] The activity was based on the

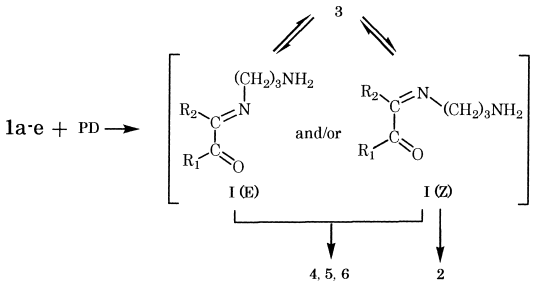

Chart 3

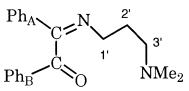

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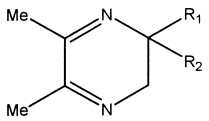
Chart 4



DHP1 : R_1=R_2=H
DHP2 : R_1=R_2=Me

Chart 5

Table 4. DNA Single-strand Breakage Activities of the Condensation Products (**2**—**4**) and 2,3-Dihydropyrazine Derivatives (DHP)

Compound	Real amounts of remaining ccc-DNA (%)[a]							
	Without Cu^{2+}, incubation for 3 h				With Cu^{2+} (1 mM), incubation for 1 h			
	0.1 mM	1 mM	5 mM	10 mM	0.1 mM	1 mM	5 mM	10 mM
2b	99	96	94	93	93	65	59	51
2c	100	100	100	99	99	97	87	78
2d	100	100	100	99	86	88	86	77
2′e	96	88	71	62	97	75	67	66
3a				96				78
3′a				96				87
3b				94				94
3c				94				95
4d				100				100
DHP1[b]		94		84		76		6
DHP2[b]	97	91		71	64	0		0

a) The values of remaining ccc-DNA listed here were estimated by the control experiments with or without Cu^{2+}. *b*) Data were taken from the ref. 4). DHP1: 2,3-dihydro-5,6-dimethylpyrazine, DHP2: 2,3-dihydro-2,2,5,6-tetramethylpyrazine.

remaining amounts of covalently closed circular duplex DNA (ccc-DNA) of plasmid pBR322, and the smaller value in Table 4 represents higher activity. As shown in Table 4, **2b** showed high activity in the compounds tested by this procedure (in the presence of Cu^{2+}). In the absence of Cu^{2+}, **2′e** resulted in the highest activity and this activity was not accelerated by the addition of 1 mM Cu^{2+}, the reason of which is ambiguous at this moment. To the best of our knowledge, no report has dealt with the DNA strand breakage activity of such 1,4-diazepine derivatives. Further studies of these simple heterocycles are under investigation.

Experimental

Melting points were determined using a micro melting point apparatus (Yanagimoto MP-S3) without correction. IR spectra were measured with a Shimadzu FTIR-8100 IR spectrophotometer. Low- and high-resolution mass spectra (LR-MS and HR-MS) were taken with a JEOL JMS HX-110 double-focusing model equipped with a FAB ion source interfaced with a JEOL JMA-DA 7000 data system. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM α-500. Chemical shifts were expressed in δ ppm downfield from an internal tetramethylsilane (TMS) signal for ^{1}H-NMR and the carbon signal of the corresponding solvent [$CDCl_3$ (77.0 ppm), and CD_3OD (49.0 ppm), and ether-d_{10} (65.3 ppm)] for ^{13}C-NMR. Microanalyses were performed with a Yanaco MT-6 CHN Corder. Routine monitoring of reactions was carried out using precoated Kieselgel 60F_{254} plates (E. Merck). Flash column chromatography was performed on silica gel (Fuji Silysia FL-60D) with a UV detector. Commercially available starting materials were used without further purification.

General Procedure for the Reaction of α-Dicarbonyls (1a—e) with PD To a stirred solution of PD (46 mmol) in the solvent (20 ml) mentioned in Table 1 was added a solution of the selected α-dicarbonyl compound (**1**) (46 mmol) in the same solvent (30 ml) dropwise at 0 °C, and the resulting mixture was stirred for 30 min. Then about 1.5 g of solid KOH was added for dehydration. In the case of **1b** and **1c**, the resulting solution was concentrated and allowed to stand at −10 °C overnight, and then the precipitated material was collected by filtration, washed with the solvent used above, and dried in a vacuum. In the other case (**1a**, **1d**, or **1e**), the solvent was removed under a vacuum and the residue was purified by flash chromatography (CH_2Cl_2/*n*-hexane–EtOAc as solvent). The structures of the products were determined by elemental analysis and spectroscopic methods. The yields and physical data of the compounds (**2**—**6**) are summarized in Tables 1, 2, and 3. Some additional data on multiple condensed compounds **4′e**, **5b**, and **6d** are shown below.

2,2-(Trimethylenediamino)dicyclo-hex-2-enone (**4′e**): Yellow needles, mp 55—56 °C (ether). IR (KBr) cm^{-1}: 3375 (NH), 1675 (C=O), 1620 (C=C). Positive HR-FAB-MS *m/z*: 263.1763 $[M+H]^+$ (Calcd for $C_{15}H_{23}N_2O_2$: 263.1760). The data of ^{1}H- and ^{13}C-NMR ($CDCl_3$) are shown in Table 3.

N,*N*′-Bis-[1-(2-methylhexahydropyrimidin-2-yl)ethylidene]propane-1,3-diamine (**5b**): Unstable colorless crystals. IR (KBr) cm^{-1}: 3300 (NH), 1630 (C=N). Positive HR-FAB-MS *m/z*: 345.2738 $[M+Na]^+$ (Calcd for $C_{17}H_{34}N_6Na$: 345.2743). The data of ^{1}H- and ^{13}C-NMR ($CDCl_3$) are shown in Table 3.

2-(3-{2-[3-(2-Oxo-1,2-diphenylethylideneamino)propylimino]-1,2-diphenylethylidenamino}propylimino)-1,2-diphenylethanone (**6d**): Pale yellow solid. Positive HR-FAB-MS: 707.3382 $[M+H]^+$ (Calcd for $C_{48}H_{43}N_4O_2$: 707.3386). The data of ^{1}H- and ^{13}C-NMR ($CDCl_3$) are shown in Table 3.

6,7-Dihydro-2,3-dimethyl-5*H*-1,4-diazepine (2b). Method A A solution of PD (740 mg, 10 mmol) in $CHCl_3$ (10 ml) was added to an ice-cooled solution of **1b** (860 mg, 10 mmol) in $CHCl_3$ (20 ml) under stirring and allowed to stand at room temperature. After being stirred for 30 min at room temperature and then refluxed for 7 d, the resulting solution was concentrated under slightly reduced pressure. The residual reddish oil was distilled using a Kugel Role distillation instrument and the distillate at 60—65 °C (oven temperature)/1 mmHg was collected to give **2b** (187 mg, 15.1%) as a colorless oil. The physical data are summarized in Tables 2 and 3.

Method B A solution of PD (764 mg, 10.3 mmol) in CH_2Cl_2 (10 ml) was added dropwise to an ice-cooled solution of **1b** (860 mg, 10 mmol) in CH_2Cl_2 (20 ml) under stirring and allowed to stand at room temperature. After being stirred for 30 min at room temperature, the solution became an opaque yellow color with separated water. The next day the CH_2Cl_2 layer was separated by decantation and was dried with anhydrous Na_2SO_4. After evaporation of CH_2Cl_2 under reduced pressure, the residual yellow solid was sublimed at 80—90 °C (oven temperature)/1 mmHg using a Kugel Role distillation instrument to give **3b** (918 mg, 64.6%) as a colorless bar: mp <25 °C. A solution of **3b** (918 mg) in $CHCl_3$ (20 ml) was refluxed for 7 d followed by treating the resulting solution as described above in Method A to give **2b** (205 mg, 26%).

2-(3-Dimethylamino-propylimino)-1,2-diphenyl-ethanone (7) Under a nitrogen stream, a solution of benzil (**1d**) (3 mmol) and *N*,*N*-dimethyl-1,3-propanediamine (3 mmol) in MeOH (25 ml) was refluxed for 6 h, the solvent was removed *in vacuo*, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH as solvent) to give **7** as a reddish-brown oil (348 mg, 39%). IR (neat) cm^{-1}: 1680 (C=O), 1630 (C=N), 1595 and 1580 (aromatic C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.86 (2H, m, H2′), 2.18 (6H, s, Me), 2.33 (2H, m, H3′), 3.48 (2H, t, *J*=7.0, H1′), 7.35 (2H, m, *meta*-PhA), 7.40 (1H, m, *para*-PhA), 7.47 (2H, m, *meta*-PhB), 7.60 (1H, m, *para*-PhB), 7.71 (2H, m, *ortho*-PhA), 7.91 (2H, m, *ortho*-PhB). ^{13}C-NMR ($CDCl_3$) δ_C: 29.00 (C-2′), 45.34 (Me), 51.95 (C-1′), 57.52 (C-3′), 127.20 (*ortho*-PhA), 128.57 (*meta*-PhA), 129.17 (*ortho*-, *meta*-PhB), 130.71 (*para*-PhA), 134.51 (*para*-PhB), 134,75 (C-PhB), 135.33 (C-PhA), 166.43 (C=N), 198.90 (C=O). Positive HR-FAB-MS: 295.1805 $[M+H]^+$ (Calcd for $C_{19}H_{23}N_2O$: 295.1810). *Anal.* Calcd for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.74; H, 7.58; N, 9.53.

Assay of DNA Strand Breakage Activity The assay of the DNA strand breakage activity of some compounds synthesized above were carried out using ccc-DNA of plasmid pBR322 as a substrate as described in a previous paper.[3] The results of this assay of the condensation products including 1,4-diazepines are summarized in Table 4.

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18) Some amines have been shown to cause DNA strand breakage in the presence of Cu^{2+} by generation of hydroxyl radicals. For example see: Hadi N., Singh S., Ahmad A., Zaidi R., *Neurosci. Lett.*, **308**, 83—86 (2001).