Photoinduced switching of intramolecular hydrogen bond between amide NH and carboxyl oxygen†

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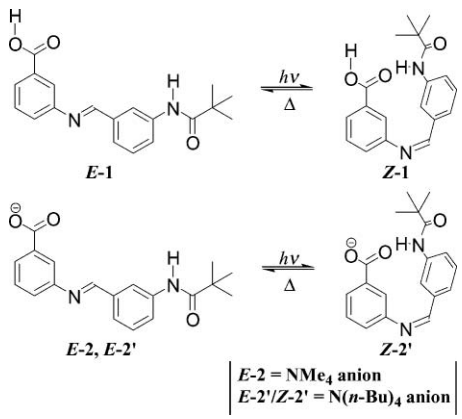
In this study, we synthesized two novel carboxylic acid and carboxylate compounds, both of which had an amide group linked with an azomethine moiety to introduce photoinduced switching of the intramolecular NH · · · O hydrogen bond. We suggest that the *cis*-carboxylate compound forms a stronger intramolecular NH · · · O hydrogen bond than the *cis*-carboxylic acid compound.

Introduction

During enzymatic reactions, rearrangement of the hydrogen bond network around the active site is believed to play a substantial role in regulating reactivity.[1] We have proposed that the hydrogen bond between the amide NH and the oxy-anion, such as that in carboxylate and phenolate, stabilizes and decreases the nucleophilicity of the anions.[2] If the switching of the intramolecular NH · · · O hydrogen bond by external stimulation (*e.g.* photoirradiation) is achieved, new functional small molecules are synthesized that can exhibit regulated reactivity like that of native enzymes. Arai and coo-workers have investigated the effect of intramolecular NH · · · N or NH · · · O hydrogen bonds in the *cis* form toward *cis–trans* photoisomerization of the C=C double bond.[3] Recently, photoisomerization has been investigated mainly as a photoswitching device.[4] In this study, we designed two novel compounds: carboxylic acid *E*-**1**/*Z*-**1** and carboxylate *E*-**2**/*Z*-**2**, both of which have an amide group linked with a photoinduced azomethine moiety. These compounds are expected to switch the intramolecular NH · · · O hydrogen bond, accompanied by *trans*-to-*cis* photoisomerization and by *cis*-to-*trans* thermal isomerization of the azomethine double bond (Scheme 1).



Scheme 1 Photoisomerization and thermal reversion of diarylazomethines *E*-**1**, *E*-**2** and *E*-**2**′.

Results and discussion

Molecular structure in solid state

The crystal structure of *E*-**2** is shown in Fig. 1. The intermolecular N′1 · · · O11 distance (2.89 Å) permitted sufficient hydrogen bond formation; this interaction stabilizes the packing structure [N′1 indicates N1 atom at equivalent position (1.5 − *x*, −0.5 + *y*, 1.5 − *z*)]. Both of the C · · · O distances in the carboxylate group, O11 · · · C01 (1.266[4] Å) and O12 · · · C01 (1.253[3] Å), are virtually identical. Solid Fourier transform infrared (FT-IR) spectra of *E*-**1** and *E*-**2** were measured to determine the existence of an intermolecular hydrogen bond. *E*-**1** exhibits an NH band at 3307 cm^{-1}, whereas the ν(NH) band appears at 3218 cm^{-1} in *E*-**2**, indicating the presence of a stronger NH · · · O hydrogen bond. These combined experimental results obtained by X-ray analysis indicate that *E*-**2** forms an intermolecular hydrogen bond in the solid state.

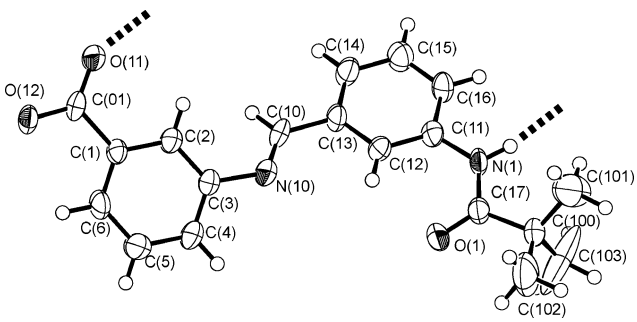


Fig. 1 Molecular structure of *E*-**2** (50% probability).

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† Electronic supplementary information (ESI) available: crystallographic data, schematic illustration of low temperature ^{1}H NMR measurement, COSY NMR spectrum of *E*-**2**′/*Z*-**2**′ and first-order kinetics of thermal isomerization of *Z*-**1** and *Z*-**2**′. See DOI: 10.1039/b516049a

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Molecular structure in solution

The solubility of tetramethylammonium carboxylate (*E*-**2**) toward tetrahydrofuran (THF) was too low for spectroscopic analysis. Tetra-*n*-butylammonium carboxylate (*E*-**2**′), which had enhanced solubility by the lipophilic cation, was subjected to measurement by UV-vis and ^{1}H NMR spectra. The solution structures in THF-d_8 were determined by ^{1}H NMR spectra. ^{1}H NMR spectra of *E*-**1** and *E*-**2**′ in THF-d_8 are shown in Fig. 2. A positive nuclear Overhauser effect (NOE) between He and Ha′ or He and Hc′ coincided, indicating that the configurations of *E*-**1** and *E*-**2**′ are in the *trans* form. The chemical shifts of the amide NH signal are 8.52 ppm in *E*-**1** and 10.80 ppm in *E*-**2**′. The significant downfield shift ($\Delta\delta = 2.28$ ppm) of the NH proton suggests that *E*-**2**′ forms a strong NH $\cdots$ O hydrogen bond in THF-d_8 solution. However, the temperature dependency of the amide NH chemical shift for *E*-**2**′ was -10.9 ppb K^{-1}, whereas that of *E*-**1** was -5.0 ppb K^{-1} in the range of 173–303 K. In addition, the upfield shift ($\Delta\delta = 0.34$ ppm) of the NH proton was observed when the solution was diluted from 5 to 1 mM. The results show that *E*-**2**′ forms an intermolecular NH $\cdots$ O hydrogen bond in THF-d_8 solution.

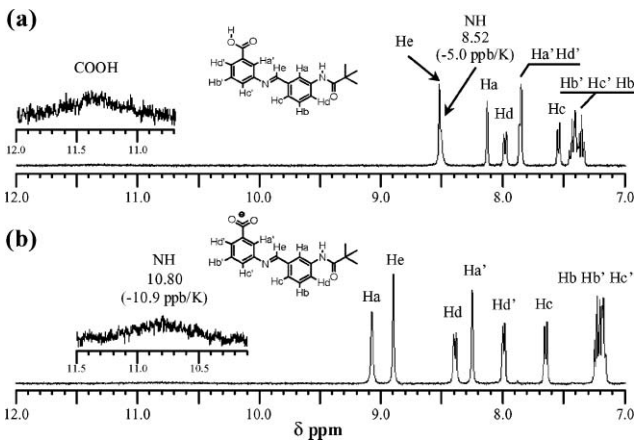

Fig. 2 ^{1}H NMR spectra of (a) *E*-**1**, and (b) *E*-**2**′, 5 mM in THF-d_8 solution at 303 K.

Direct photoisomerization of *E*-1 and *E*-2′

Although UV irradiation of *trans*-diarylazomethines results in extensive conversion into the corresponding *cis*-isomers, the *cis*-isomers are thermally unstable and cause thermal reversion to *trans*-isomers at high temperatures.[5] The *cis*-isomers were stable only at temperatures less than *ca.* 203 K.[6] Using UV-vis and ^{1}H NMR measurements at low temperature, the photoisomerization reactions of *E*-**1** and *E*-**2**′ were traced. UV-vis spectra of *E*-**1** and *E*-**2**′ were measured by a static method below 203 K. According to photoirradiation in THF solution at 173 K, changes occurred in the UV-vis spectra of *E*-**1** and *E*-**2**′, as shown in Fig. 3. *E*-**1** and *E*-**2**′ were isomerized and reached a photostationary state (PSS) at 313 and 365 nm UV light irradiation in the UV-vis spectrometer. Solid lines were those taken before irradiation, corresponding to *trans*-isomers, and dotted lines were those observed in PSS. The blue shift of λ_{max} in *cis*-compounds indicates that two phenyl rings are not in plane and that the conjugation is interrupted. These changes in the spectra were reversible toward thermal reversion at higher temperatures.

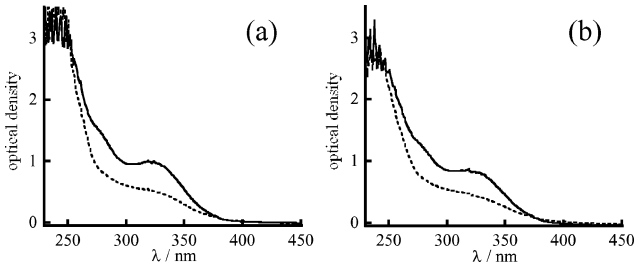

Fig. 3 UV-vis spectrum change of (a) *E*-**1**, and (b) *E*-**2**′, by UV irradiation of 313 and 365 nm toward 0.08 mM in THF solution at 173K. Before irradiation (solid lines) and after irradiation (dotted lines).

cis-Isomers were also observed in ^{1}H NMR spectra. Using a photoirradiation probe [designed for chemically induced dynamic nuclear polarization (CIDNP)], UV light was directly introduced into the NMR equipment. The spectra were taken before and after UV irradiation in THF-d_8 solution at 183 K, using a 200 W xenon-mercury arc passed through a liquid light guide. Irradiation was continued until the establishment of a PSS (about 30 min of irradiation). ^{1}H NMR spectra of *trans*-compounds (*E*-**1** and *E*-**2**′) and their PSS in THF-d_8 solution at 183 K are shown in Fig. 4. The signals that newly appeared after irradiation were assigned by correlation of NMR measurements. Exchange spectroscopy (EXSY) spectra under photoirradiation in THF-d_8 solution at 203 K are shown in Fig. 5. Because of the fast rate of photoisomerization, equilibrium between *E*-**1** and *Z*-**1** was achieved and positive cross-peaks based on the photochemical exchange process between the two compounds were observed in Hb, Hc, Hc′, Hd, and He [Fig. 5(a)]. The homo-decoupling measurement coincided with their expected coupling pattern, and the negative NOE between NH and Ha coincided with Ha and Ha′ signals. As a consequence, all of the signals derived from *Z*-**1** were assigned. In *cis*-form, the two rings of diarylazomethine are in close proximity to each other. Significant upfield shifts of Hc, Hc′ were observed in *Z*-**1** because the protons of one ring are in

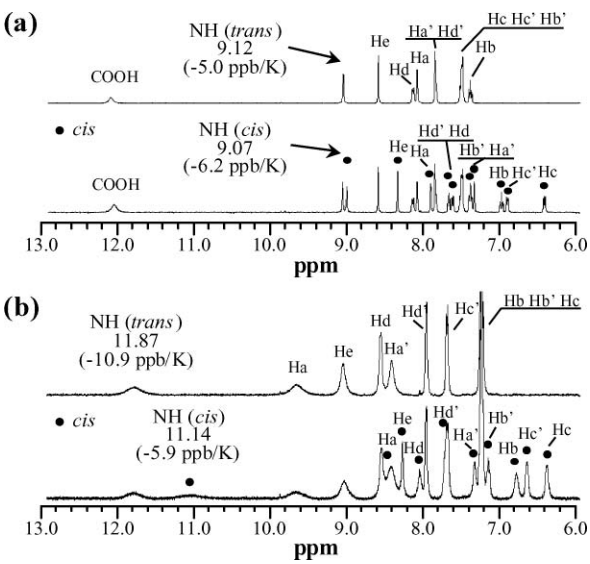

Fig. 4 ^{1}H NMR spectrum change of (a) *E*-**1**, and (b) *E*-**2**′, by UV irradiation of 313 nm and 365 nm 5 mM in THF-d_8 solution at 183 K. Before irradiation (upside) and after irradiation (downside).

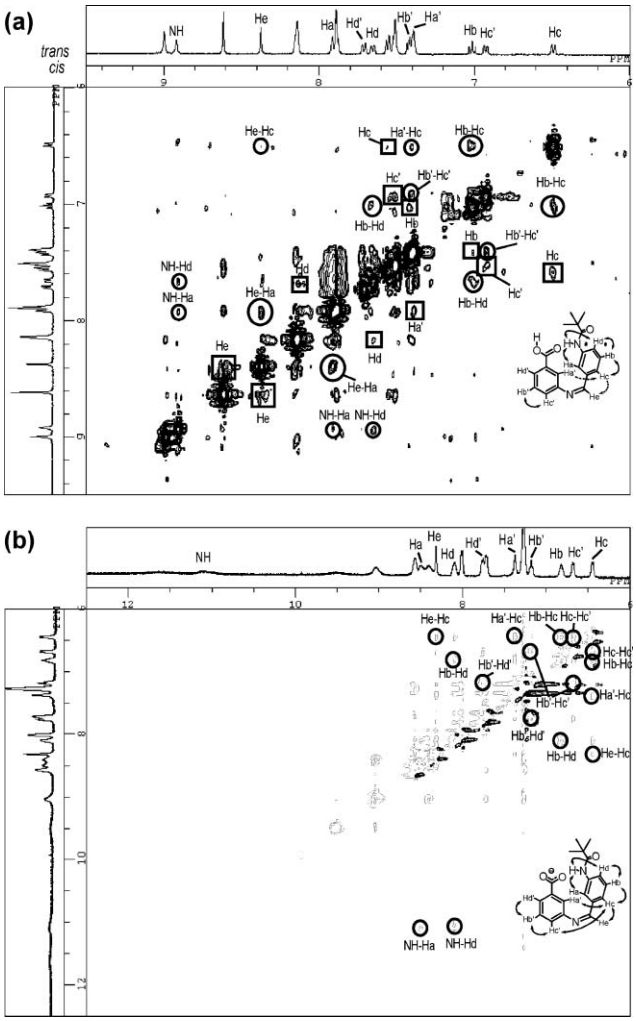

Fig. 5 Photoirradiating NOESY NMR spectra of (a) carboxylic acid *E*-**1**/*Z*-**1**, and (b) carboxylate *E*-**2**′/*Z*-**2**′, 5 mM in THF-d_8 solution at 203 K. The cross-peaks in circles indicate the intramolecular NOE signals in the *Z*-isomers, and those in the squares indicate the EXSY signals between *E*- and *Z*-isomers.

the shielding cone of another ring. The solution structure of *Z*-**1** was estimated based on NOE correlations. NOE correlations of He–Hc′ or He–Ha′, which were observed characteristically in *E*-**1**, disappeared in *Z*-**1**. Instead, the NOE correlation was observed between Ha′ and Hc in *Z*-**1**. These results confirm that the configuration of the photoproduct *Z*-**1** is exactly in *cis*-form. In *Z*-**1**, NOE correlations of He–Ha and He–Hc are observed at the same time, which indicates that the amide-side aromatic ring of *Z*-**1** rotates easily.

In contrast to carboxylic acid, the correlation between the two compounds is not clearly observed in the carboxylate [Fig. 5(b)]. It was also difficult to assign the signals using the homo-decoupling method because of broadening of signals at low temperatures. To assign the signals of *Z*-**2**′, the correlation spectroscopy (COSY) NMR spectrum in PSS was measured (ESI†). Two singlets, NH and He, do not have a COSY. One broad signal at 11.10 ppm, which has a large temperature coefficient, is assigned to the NH signal. Another sharp signal at 8.31 ppm is assigned to the He signal. In the nuclear Overhauser effect spectroscopy (NOESY) spectrum [Fig. 5(b)], positive NOE of NH–Ha and NH–Hd confirmed the Ha and Hd signals. The COSY NMR spectrum coincided with the Ha′, Hb, Hb′, and Hc signals. Of two residual signals, an upfield-shifted signal is assigned to Hc′ because Hc′ is closer to the amide-side aromatic ring than Hd′, and Hc′ is more affected by the shielding effect of the aromatic ring. A positive NOE was observed in Ha′–Hc and in Hc–Hc′ of *Z*-**2**′, which confirms that the configuration of the *Z*-**2**′ is in the *cis*-form.

When the temperature was raised, the new signals disappeared and only the signals of the *trans*-isomer were observed. This reaction cycle (*i.e.* photoisomerization and thermal reversion) is completely reversible. The ratios of *cis* : *trans* isomers were 47 : 53 (*E*-**1**/*Z*-**1**) and 40 : 60 (*E*-**2**′/*Z*-**2**′) at PSS (173 K), respectively. The chemical shifts of the amide NH signals of *cis*-isomers at 183 K were 9.07 ppm in *Z*-**1** and 11.14 ppm in *Z*-**2**′. The downfield shift of 2.07 ppm suggests that *Z*-**2**′ forms an NH···O hydrogen bond in the THF-d_8 solution. The decrease in the temperature coefficient during the *trans*-to-*cis* isomerization of *E*-**2**′ (−10.9 ppb K^{-1}) and *Z*-**2**′ (−6.2 ppb K^{-1}) indicates that the intramolecular hydrogen bond is predominant in *Z*-**2**′, whereas the intermolecular interaction is formed in *E*-**2**′.

In contrast to the sharp signals observed in carboxylic acids (*E*-**1**, *Z*-**1**), the signals of carboxylates (*E*-**2**′, *Z*-**2**′) were broadened at low temperature. Because of the intermolecular hydrogen bond formed in THF-d_8 solution, *E*-**2**′ assumes an origomeric structure easily because exchange reactions occur more slowly at low temperature. It is thought that only signals of carboxylates were broadened at low temperatures because of this equilibrium. Decreasing the temperature also seemed to cause the signals of the aromatic ring protons to shift to a low magnetic field; these signals were observed as a result of the intermolecular deshielding effect produced by the aromatic ring of the other molecule, which became adjacent through the presence of the intermolecular NH···O hydrogen bond. The intermolecular interaction of *Z*-**2**′ is estimated to be small because the signals those in *E*-**2**′ are broader than those in *Z*-**2**′.

Z-to-*E* Thermal isomerization

The intramolecular hydrogen bond can influence the ground-state equilibrium between configurational and conformational isomers as well as photophysical and photochemical behavior.[3,4] If the intramolecular hydrogen bond is formed in a *cis*-compound, it may decrease the *cis*-to-*trans* thermal reversion rate. Kinetic studies of thermal reversion of diarylazomethine derivatives from *cis*-to-*trans* isomers were reported in a wide temperature range and found to follow first-order kinetics.[5,6] *Z*-**1** and *Z*-**2**′ also produce *cis*-to-*trans* thermal reversion in accordance with first-order kinetics. Using the time-course experiments of UV-visible spectra in THF solution, the thermal reversion rates of *Z*-**1** and *Z*-**2**′ in the temperature range of 213–243 K were measured.

The thermal reversion of *Z*-**1** is faster than that of *Z*-**2**′ in the temperature range 213–243 K. This result also suggests that the NH···O intramolecular hydrogen bond forming at *Z*-**2**′ is stronger than the one formed at *Z*-**1**. Activation energies of *cis*-to-*trans* thermal isomerization were determined using the Arrhenius equation. Arrhenius plots of *Z*-**1**/*E*-**1** and *Z*-**2**′/*E*-**2**′ are shown in Fig. 6. The activation energies of thermal reversion of *Z*-**1** and *Z*-**2**′ are 15.1 and 16.9 kcal mol^{-1}, respectively. *Z*-**2**′ has an

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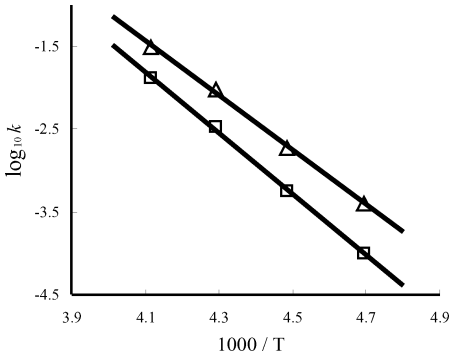

Fig. 6 Arrhenius plots of *Z*-to-*E* thermal isomerization for *Z*-**1** (△) and *Z*-**2′** (□).

activation energy that is 1.8 kcal mol^{-1} higher for this reaction. This difference in the level of activation energy is thought to arise from the formation of an intramolecular NH · · · O hydrogen bond in *Z*-**2′**.

Conclusion

In conclusion, carboxylic acid *E*-**1** and carboxylate *E*-**2′**, both of which have an amide group linked to an azomethine moiety that switches the intramolecular NH · · · O hydrogen bond through photoirradiation, were synthesized and characterized. *E*-**2′** was determined to form intermolecular, rather than intramolecular, NH · · · O hydrogen bonds in the solid state and in THF solution. Using UV light irradiation, the generation of a *cis*-isomer was observed by UV-visible and ^{1}H NMR spectra. The temperature coefficient for the chemical shift of amide NH signals and *cis*-to-*trans* thermal reversion rates of their compounds show that the intramolecular NH · · · O hydrogen bond of *Z*-**2′** is stronger than that of *Z*-**1**. Switching of the intramolecular NH · · · O hydrogen bond toward the carboxylate group by external stimulations (*e.g.* photoirradiation and heating) will achieve the regulation of nucleophilicity of carboxylate-like native enzymes by low molecular weight compounds.

Experimental

General procedures

All manipulations involving air- and moisture-sensitive compounds were carried out by the use of standard Schlenk techniques under an argon atmosphere. 3-Nitrobenzaldehyde, 2,2-dimethylpropionyl chloride were purchased from Tokyo-Kasei Co. 3-Aminobenzaldehyde, stannous chloride were purchased from Nacalai tesque Co. Inc. Dichloromethane was distilled over CaH_2. Tetrahydrofuran was distilled over CaH_2 and dried over Na. Methanol, ethanol, and acetonitrile were distilled over CaH_2 and dried over molecular sieves (3Å). ^{1}H (400 and 270 MHz) and ^{13}C (100 MHz) NMR spectra were measured on a JEOL JNM-GSX400 or a JEOL JNM-GSX270 spectrometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a JEOL LA500 spectrometer. When THF-d_8, CD_3CN, and $CDCl_3$ were used as solvent, the ^{1}H NMR and ^{13}C NMR spectra were referenced to the tetramethylsilane protons at δ 0.00, and to the tetramethylsilane carbons at δ 0.00, respectively. When DMSO-d_6 was used as solvent, ^{1}H NMR and ^{13}C NMR spectra were referenced to the residual solvent protons at δ 2.49, and to the solvent carbons at δ 39.5. Elemental analysis was performed at the Elemental Analysis Center, Faculty of Science, Osaka University. All melting points of the compounds were measured on a micro melting point apparatus of YANAGIMOTO Co. ESI-MS measurements were performed on a Finnigan MAT LCQ ion trap mass spectrometer. FT-IR spectra were measured on a JASCO FT/IR-8300 spectrometer.

Preparation of 3-(2,2-dimethylpropionylamino)benzaldehyde

To a concentrated HCl (60 mL) suspension of 3-nitrobenzaldehyde (10.0 g, 66 mmol) was added stannous chloride (45.1 g, 240 mmol). The temperature rose rapidly, 3-nitrobenzaldehyde dissolved, and a clear, orange solution was obtained. The solution was refluxed for 2 h, and the solution color turned to red. The solution was cooled in an ice bath, and a pasty orange-red suspension resulted. The precipitate was collected and washed with the diethyl ether, dried overnight in an NaOH desiccator under reduced pressure, yielding an orange powder (17.3 g). All of the orange powder was suspended in CH_2Cl_2 (70 mL) and cooled in an ice bath (A). 2,2-Dimethylpropionyl chloride and triethylamine were added to CH_2Cl_2 (40 mL) and cooled in an ice bath (B). (A) was slowly added to (B). After the mixture had been stirred for 90 min, the reactant was washed with 2% HCl aq., 4% $NaHCO_3$ aq., water, and with saturated NaCl aq. in a dropping funnel. The organic layer was dried over anhydrous Na_2SO_4. Removal of solvents under reduced pressure followed by recrystallization from ethyl acetate–diethyl ether (1 : 3) gave colorless crystals (4.9 g, 36%). Mp 104–105 °C; Found: C, 70.07; H, 7.34; N, 6.73. Calc. for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82%; δ_H(270 MHz; DMSO-d_6) 1.23 (9H, s, *tert*-butyl), 7.52 (1H, t, *J* 7.6 Hz, aryl), 7.59 (1H, dt, *J* 7.6 and 1.4, aryl), 7.95 (1H, dt, *J* 7.6 and 1.4, aryl), 8.22 (1H, t, *J* 1.4, aryl), 9.45 (1H, br s, NH) and 9.96 (1H, s, CHO); δ_C(125 MHz; DMSO-d_6) 27.06, 39.18, 120.14, 124.80, 125.96, 129.25, 136.52, 140.15, 176.81 and 192.97; *m/z* (ESI) 204.4 ([M − H]$^+$ requires 204.1).

Preparation of (*E*)-3-{[3-(2,2-dimethylpropionylamino)-benzylidene]amino}benzoic acid (*E*-1)

3-(2,2-Dimethylpropionylamino)benzaldehyde (1.0266 g, 5.00 mmol) and 3-aminobenzoic acid (1.3720 g, 10.0 mmol) was dissolved in methanol (70 mL) and stirred overnight at room temperature. Removal of solvents under reduced pressure followed by recrystallization from ethyl acetate–hexane (1 : 3) gave a white powder (0.5471 g, 33.7%). Mp 185 °C; Found: C, 70.35; H, 6.10; N, 8.62. Calc. for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64%; ν_{max}(KBr pellet)/cm^{-1} 3307 (NH), 1700 (C=O), 1655 (C=O) and 1565 (C=N); δ_H(400 MHz; DMSO-d_6) 1.24 (9H, s, *tert*-butyl), 7.44 (1H, t, *J* 7.8 Hz, aryl), 7.49 (1H, dt, *J* 7.8 and 1.8, aryl), 7.53 (1H, t, *J* 7.8, aryl), 7.59 (1H, dt, *J* 7.8 and 1.8, aryl), 7.76 (1H, t, *J* 1.8, aryl), 7.81 (1H, dt, *J* 7.8 and 1.8, aryl), 7.84 (1H, dt, *J* 7.8 and 1.8, aryl), 8.29 (1H, t, *J* 1.8, aryl), 8.63 (1H, s, –CH=N–), 9.39 (1H, s, NH) and 13.02 (1H, br s, COOH); δ_C(150 MHz; THF-d_8) 27.74, 40.23, 120.08, 122.67, 123.88, 125.15, 126.02, 127.74, 129.53, 129.75, 133.04, 137.80, 141.31, 153.27, 161.72, 167.38 and 176.79; *m/z* (ESI) 324.3 ([M + H]$^+$ requires 325.2), 346.3 ([M + Na]$^+$ requires 347.1).

Preparation of (tetramethylammonium) (*E*)-3-{[3-(2,2-dimethylpropionylamino)benzylidene]amino}benzoate (*E*-2)

To a solution of (*E*)-3-{[3-(2,2-dimethylpropionylamino)benzylidene]amino}benzoic acid in CH_3CN was added a solution of an equivalent amount of tetramethylammonium acetate in CH_3CN. The solution was stirred and concentrated to give an orange oil. The oil was washed with diethyl ether to give a pale orange powder. The powder was recrystallized from hot CH_3CN to give colorless crystals (yield was not certain). Mp 245 °C; Found: C, 68.47; H, 7.89; N, 10.40. Calc. for $C_{23}H_{31}N_3O_3$: C, 69.49; H, 7.86; N, 10.57%; ν_{max}(KBr)/cm^{-1} 3218 (br, NH) and 1668 (CO); δ_H(400 MHz; DMSO-d_6) 1.24 (9H, s, *tert*-butyl), 3.09 (12H, s, NMe_4), 7.12 (1H, dt, *J* 7.8 and 1.6 Hz, aryl), 7.23 (1H, t, *J* 7.8, aryl), 7.42 (1H, t, *J* 7.8, aryl), 7.56 (1H, dt, *J* 7.8 and 1.6, aryl), 7.65 (1H, t, *J* 1.6, aryl), 7.66 (1H, dt, *J* 7.8 and 1.6, aryl), 7.86 (1H, dt, *J* 7.8 and 1.6, aryl), 8.22 (1H, t, *J* 1.6, aryl), 8.55 (1H, s, –CH=N–) and 9.39 (1H, s, NH).

X-Ray crystallography. A suitable, single, colorless crystal of *E*-**2** was mounted on a fine nylon loop with Nujol and immediately frozen at 200 K. All measurements were performed on a Rigaku RAXIS-RAPID Imaging Plate diffractometer with graphite monochromated MoKα radiation. The structures were solved by direct methods (SIR 92) and the following refinements were performed using SHELXL-97 and teXsan crystallographic software packages. All non-hydrogen atoms were refined anisotropically. H1 was placed by reflection and hydrogen atoms without H1 were placed in the calculated position and including least-squares refinement.

Crystal data for *E*-**2**. $C_{23}H_{31}N_3O_3$, $M_r = 397.52$, monoclinic, $a = 16.08(2)$, $b = 8.501(8)$, $c = 17.00(3)$ Å, $\beta = 104.5(1)°$, $V = 2249(16)$ Å^3, $T = 200 \pm 1$ K, space group $P2_1/n$, $Z = 4$, μ(MoKα) = 0.8 cm^{-1}, total number of reflections measured 21 943, unique reflections 5009 ($R_{int} = 0.161$), final *R* indices: $R_1 = 0.059$, $wR_2 = 0.103$ for all data. CCDC reference number 267875. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b516049a.

Preparation of (tetra-*n*-butylammonium) 3-{[3-(2,2-dimethylpropionylamino)benzylidene]amino}benzoate (*E*-2′)

To a solution of (*E*)-3-{[3-(2,2-dimethylpropionylamino)benzylidene]amino}benzoic acid (0.0909 g, 2.8×10^{-4} mol) in ethanol (4.0 mL) was added a 2.8 M ethanol solution of sodium ethoxide (0.10 mL, 2.8×10^{-4} mol). The solution was stirred overnight and concentrated to give a white-yellow powder. The powder was washed with diethyl ether, and then dissolved in ethanol again. A solution of tetra-*n*-butylammonium chloride (0.0778 g, 2.8×10^{-4} mol) in EtOH was added. After stirring for a few minutes, the solvent was removed under reduced pressure to give a pale white-orange powder. The powder was washed by diethyl ether. Recrystallization from THF–hexane (1 : 2) gave pale white-orange powder (yield was not certain). Found: C, 67.23; H, 9.72; N, 6.54. Calc. for $C_{35}H_{55}N_3O_3 + (H_2O)_{0.3}$: C, 67.23; H, 9.93; N, 6.72%; δ_H(400 MHz; DMSO-d_6) 0.91 (12H, t, *J*, $-CH_2CH_3 \times 4$), 1.23 (9H, s, *tert*-butyl), 1.30 (8H, multiplet, $-CH_2CH_3 \times 4$), 1.56 (8H, multiplet, $-CH_2CH_2CH_3 \times 4$), 3.15 (8H, multiplet, $N-CH_2- \times 4$), 7.12 (1H, dt, *J* 7.8 and 1.6, aryl), 7.23 (1H, t, *J* 7.8, aryl), 7.42 (1H, t, *J* 7.8, aryl), 7.56 (1H, dt, *J* 7.8 and 1.6, aryl), 7.67 (1H, t, *J* 1.6, aryl), 7.68 (1H, dt, *J* 7.8 and 1.6, aryl), 7.87 (1H, dt, *J* 7.8 and 1.6, aryl), 8.22 (1H, t, *J* 1.6, aryl), 8.55 (1H, s, –CH=N–) and 9.38 (1H, s, NH); δ_C(150 MHz; THF-d_8) 14.01, 20.54, 24.75, 59.19, 120.43, 120.65, 122.46, 125.14, 125.46, 127.81, 128.34, 128.71, 138.23, 142.94, 144.41, 151.64, 160.98, 170.09 and 177.67; *m*/*z* (ESI) 313.2 ([M − N(*n*-butyl)$_4$]$^-$ requires 323.1) and 564.4([M − H]$^-$ requires 564.4).

UV light irradiation technique for UV-vis spectrum measurement at 173 K

The temperature was controlled in the UV cell using the DN1704 liquid nitrogen cryostat. A Xe/Hg lamp (MUV-202U, Moritex Co.) was used for UV light irradiation. The UV light was directed using a liquid light guide. The sample was prepared under Ar atmosphere and dissolved in the degassed solvent. The sample in the UV cell was irradiated after the temperature was lowered. After irradiation, the spectrum was measured. During irradiation and spectrum measurements, the sample was always kept at the desired temperature.

UV light irradiation technique for ^{1}H NMR spectrum measurement at low temperatures

A Xe/Hg lamp was used for UV light irradiation. The UV light was directed using a liquid light guide. The sample was dissolved in the degassed solvent and sealed in an NMR tube under an Ar atmosphere. After the temperature was lowered, UV light was irradiated in the NMR spectrometer using a CIDNP probe. After irradiation, the spectrum was measured. During irradiation and spectrum measurements, the sample was always kept at the desired temperature. A schematic illustration of the low temperature ^{1}H NMR spectrum measurement is given in the ESI†.

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References

1 G. O. Borgstahl, D. R. Williams and E. D. Getzoff, *Biochemistry*, 1995, **34**, 6278; A. Warshel, *Biochemistry*, 1981, **20**, 3167; Y. Imamoto, M. Kataoka, F. Tokunaga, T. Asahi and H. Masuhara, *Biochemistry*, 2001, **40**, 6047; Y. Imamoto, Y. Shirahige, F. Tokunaga, T. Kinoshita, K. Yoshihara and M. Kataoka, *Biochemistry*, 2001, **40**, 8997.
2 A. Onoda, Y. Yamada, J. Takeda, Y. Nakayama, T. Okamura, M. Doi, H. Yamamoto and N. Ueyama, *Bull. Chem. Soc. Jpn.*, 2004, **77**, 321; A. Onoda, Y. Yamada, Y. Nakayama, K. Takahashi, H. Adachi, T. Okamura, A. Nakamura, H. Yamamoto, N. Ueyama, D. Vyprachticky and Y. Okamoto, *Inorg. Chem.*, 2004, **43**, 4447.
3 F. D. Lewis, B. A. Yoon, T. Arai, T. Iwasaki and K. Tokumaru, *J. Am. Chem. Soc.*, 1995, **117**, 3029; T. Arai, M. Moriyama and K. Tokumaru, *J. Am. Chem. Soc.*, 1994, **116**, 3171.
4 M. Irie, T. Fukaminato, T. Sasaki, N. Tamai and T. Kawai, *Nature*, 2002, **420**, 759; M. Irie, O. Miyatake, K. Uchida and T. Eriguchi, *J. Am. Chem. Soc.*, 1994, **116**, 9894.
5 T. Asano, H. Furuta, H. J. Hofmann, R. Cimaraglia, Y. Tsuno and M. Fujino, *J. Org. Chem.*, 1993, **58**, 4418.
6 K. Maeda, K. A. Muszkat and S. S. Ozeri, *J. Chem. Soc., Perkin Trans. 2*, 1980, 1282; K. Maeda and E. Fischer, *Isr. J. Chem.*, 1977, **16**, 294.

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