Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

β-Isocyanoalanine as an IR probe: comparison of vibrational dynamics between isonitrile and nitrile-derivatized IR probes

Michał Maj,[‡ab] Changwoo Ahn,[‡b] Dorota Kossowska,[ab] Kwanghee Park,[ab]
Kyungwon Kwak,[c] Hogyu Han*[b] and Minhaeng Cho*[ab]

[a] *Center for Molecular Spectroscopy and Dynamics, Institute for Basic Science (IBS), Seoul 136-701, Korea*

[b] *Department of Chemistry, Korea University, Seoul 136-701, Korea*

[c] *Department of Chemistry, Chung-Ang University, Seoul 156-756, Korea*

S1. Syntheses of compounds

General. ^{1}H and ^{13}C spectra were recorded on a Varian Unity Inova 500 NMR spectrometer. Chemical shifts (δ) and coupling constants (J) are reported in parts per million (ppm) and hertz (Hz), respectively. ^{1}H NMR spectra are referenced to TMS (tetramethylsilane in $CDCl_3$ and DMSO-d_6) as an internal standard. ^{13}C NMR spectra are referenced to solvent (^{13}C: $CDCl_3$, δ 77.00 ppm; DMSO-d_6, δ 39.50 ppm) as an internal standard. High-resolution mass spectra (HRMS) were recorded on a JEOL JMS-700 mass spectrometer using a chemical ionization (CI) technique. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} precoated plates (0.25 mm thickness, Merck, Darmstadt). Flash chromatography was carried out on silica gel 60 (230−400 mesh, Merck). Reagent-grade chemicals were purchased from Aldrich and TCI and used as received unless otherwise specified. Compounds **0** (Ac-L-Ala-NHMe)[S1] and **3** (Ac-L-Ala(N_3)-NHMe)[S2] were prepared as reported previously.

Ac-L-Dap(Boc)-OMe (5).[S2] To a suspension of **4**-HCl (H-Dap(Boc)-OMe·HCl, 3.00 g, 11.8 mmol, Bachem, Bubendorf) in CH_2Cl_2 (50 mL) were added triethylamine (5.0 mL, 35.9 mmol) and acetic anhydride (5.5 mL, 58.3 mmol). After stirring at room temperature for 3 h, the reaction mixture was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (100 mL × 2). The combined organic layers were washed with brine (100 mL × 1), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:100−1:10) to give **5** (2.98 g, 97%) as a colorless oil. TLC (MeOH/CH_2Cl_2 = 1:20) R_f = 0.34; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.73 (d, J = 4.0 Hz, 1H), 4.98 (t, J = 6.0 Hz, 1H), 4.60 (td, J = 6.0, 4.5 Hz, 1H), 3.76 (s, 3H), 3.56 (dt, J = 14.5, 7.1 Hz, 1H), 3.51 (dt, J = 14.5, 5.2 Hz, 1H), 2.04 (s, 3H), 1.44 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.91, 170.32, 156.65, 80.07, 53.71, 52.67, 42.10, 28.23, 23.05; HRMS (CI+) for $C_{11}H_{21}N_2O_5$ (MH$^+$), calcd 261.1450, found 261.1453.

Ac-L-Dap-OMe (6).[S2] To **5** (2.87 g, 11.0 mmol) was added trifluoroacetic acid (TFA, 15 mL). After stirring at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:100−1:5) to give **6**-TFA (2.83 g, 94%) as a colorless oil. TLC (MeOH/CH_2Cl_2 = 1:7 on a plate pretreated with triethylamine) R_f = 0.40; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.52 (d, J = 8.5 Hz, 1H), 8.12 (brs, 2H), 4.55 (td, J = 8.5, 4.8 Hz, 1H), 3.66 (s, 3H), 3.23 (dd, J = 13.0, 5.0 Hz, 1H),

3.06 (dd, J = 13.3, 9.3 Hz, 1H), 1.89 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.16, 169.69, 52.43, 49.93, 39.26, 22.48; HRMS (CI+) for $C_6H_{13}N_2O_3$ (MH$^+$), calcd 161.0926, found 161.0929.

Ac-L-Dap(CHO)-OMe (7).[S3] To a suspension of **6**-TFA (2.0 g, 7.29 mmol) in THF (100 mL) were added sodium formate (520 mg, 7.65 mmol) and 2,2,2-trifluoroethyl formate (TFEF, 2.13 mL, 21.9 mmol). After stirring at room temperature for 48 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:100−1:10) to give **7** (1.08 g, 79%) as a colorless oil. TLC (MeOH/CH_2Cl_2 = 1:10) R_f = 0.42; ^{1}H NMR (500 MHz, $CDCl_3$) two rotamers at 25 °C (4.8:1), major rotamer δ 8.19 (d, J = 1.0 Hz, 1H), 6.83 (d, J = 7.0 Hz, 1H), 6.57 (t, J = 5.5 Hz, 1H), 4.66 (dt, J = 7.0, 5.5 Hz, 1H), 3.78 (s, 3H), 3.70 (t, J = 6.0 Hz, 2H), 2.05 (s, 3H), minor rotamer δ 7.93 (d, J = 11.0 Hz, 1H), 7.06 (d, J = 7.5 Hz, 1H), 6.87 (dt, J = 11.8, 5.8 Hz, 1H), 4.75 (dt, J = 7.3, 3.8 Hz, 1H), 3.82 (s, 3H), 3.69 (dt, J = 13.3, 3.8 Hz, 1H), 3.64 (dt, J = 13.3, 4.6 Hz, 1H), 2.08 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) major rotamer δ 170.85, 170.58, 162.21, 52.96, 52.87, 40.18, 23.01, minor rotamer δ 170.55, 170.40, 165.36, 53.07, 53.03, 43.39, 22.84; HRMS (CI+) for $C_7H_{13}N_2O_4$ (MH$^+$), calcd 189.0875, found 189.0873.

Ac-L-Ala(NC)-OMe (8).[S4] To a solution of **7** (973 mg, 5.17 mmol) in dry CH_2Cl_2 (50 mL) was added triethylamine (3.6 mL, 25.8 mmol) under Ar. The resulting mixture was cooled to −30 °C and a solution of phosphorus(V) oxychloride (0.72 mL, 7.72 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise over 30 min with stirring. After stirring at −30 °C for 2 h, the reaction mixture was quenched with cold saturated aqueous $NaHCO_3$ (100 mL), warmed to room temperature and extracted with CH_2Cl_2 (100 mL × 2). The combined organic layers were washed with brine (100 mL × 1), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:100−1:50) to give **8** (448 mg, 51%) as a white solid. TLC (MeOH/CH_2Cl_2 = 1:20) R_f = 0.39; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.57 (d, J = 4.5 Hz, 1H), 4.75 (dt, J = 6.0, 3.0 Hz, 1H), 3.94 (dd, J = 15.3, 3.8 Hz, 1H), 3.89 (dd, J = 15.5, 3.5 Hz, 1H), 3.87 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.17, 168.67, 159.53 (t, J = 3.8 Hz), 53.42, 51.49, 43.52 (t, J = 6.2 Hz), 22.94; HRMS (CI+) for $C_7H_{11}N_2O_3$ (MH$^+$), calcd 171.0770, found 171.0768.

Ac-L-Ala(NC)-NHMe (1).[S5] To **8** (301 mg, 1.77 mmol) was added methylamine solution (40% in MeOH, 2.8 mL, 36.1 mmol). After stirring at room temperature for 4 h, the reaction

mixture was concentrated *in vacuo*. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:100−1:10) to give **1** (213 mg, 71%) as a white solid. TLC (MeOH/CH_2Cl_2 = 1:10) R_f = 0.48; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 (d, J = 8.5 Hz, 1H), 8.08 (q, J = 4.5 Hz, 1H), 4.54 (td, J = 7.7, 5.8 Hz, 1H), 3.75 (dd, J = 14.8, 5.3 Hz, 1H), 3.65 (dd, J = 15.0, 7.5 Hz, 1H), 2.61 (d, J = 4.5 Hz, 3H), 1.90 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.68, 168.21, 157.44 (t, J = 3.9 Hz), 51.38, 42.84 (t, J = 5.8 Hz), 25.69, 22.50; HRMS (CI+) for $C_7H_{12}N_3O_2$ (MH$^+$), calcd 170.0930, found 170.0928.

Boc-L-Ala(CN)-OMe (10).[S6] To a solution of **9** (Boc-β-cyano-Ala-OH, 3.00 g, 14.0 mmol, Bachem, Bubendorf) in DMF (20 mL) was added potassium carbonate (1.93 g, 14.0 mmol) and then slowly iodomethane (3.5 mL, 56.2 mmol) at 0 °C. After stirring at room temperature for 12 h, the reaction mixture was quenched with H_2O (200 mL) and extracted with EtOAc (200 mL × 2). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (200 mL × 1) and brine (200 mL × 1), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc/*n*-hexane = 1:5−1:2) to give **10** (2.68 g, 84%) as a white solid. TLC (EtOAc/*n*-hexane = 1:2) R_f = 0.46; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.47 (d, J = 5.0 Hz, 1H), 4.53 (dt, J = 6.0, 5.0 Hz, 1H), 3.85 (s, 3H), 3.01 (dd, J = 17.0, 5.0 Hz, 1H), 2.93 (dd, J = 17.0, 4.5 Hz, 1H), 1.46 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.36, 154.77, 116.12, 81.02, 53.34, 50.26, 28.19, 21.87; HRMS (CI+) for $C_{10}H_{17}N_2O_4$ (MH$^+$), calcd 229.1188, found 229.1192.

H-L-Ala(CN)-OMe (11).[S7] To **10** (2.50 g, 11.0 mmol) was added hydrogen chloride solution (4.0 M in 1,4-dioxane, 30 mL, 120 mmol). After stirring at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography (MeOH/CH_2Cl_2 = 1:100−1:10) to give **11**-HCl (1.66 g, 92%) as a colorless oil. TLC (MeOH/CH_2Cl_2 = 1:20) R_f = 0.52; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.81 (dd, J = 7.0, 5.5 Hz, 1H), 3.80 (s, 3H), 2.81 (dd, J = 16.8, 4.8 Hz, 1H), 2.72 (dd, J = 17.0, 7.0 Hz, 1H), 1.81 (brs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.50, 116.82, 52.84, 51.17, 23.79; HRMS (CI+) for $C_5H_9N_2O_2$ (MH$^+$), calcd 129.0664, found 129.0662.

Ac-L-Ala(CN)-OMe (12).[S2] To a suspension of **11**-HCl (1.29 g, 7.84 mmol) in CH_2Cl_2 (50 mL) were added triethylamine (3.3 mL, 23.7 mmol) and acetic anhydride (3.7 mL, 39.2 mmol). After stirring at room temperature for 2 h, the reaction mixture was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (100 mL × 2). The combined organic layers were

washed with brine (100 mL × 1), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$ = 1:100−1:20) to give **12** (626 mg, 47%) as a white solid. TLC ($MeOH/CH_2Cl_2$ = 1:20) R_f = 0.52; ^1H NMR (500 MHz, $CDCl_3$) δ 6.66 (d, J = 5.5 Hz, 1H), 4.77 (dt, J = 6.8, 5.1 Hz, 1H), 3.86 (s, 3H), 3.08 (dd, J = 17.0, 5.5 Hz, 1H), 2.96 (dd, J = 16.8, 4.8 Hz, 1H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.27, 169.28, 116.12, 53.43, 49.00, 22.88, 21.26; HRMS (CI+) for $C_7H_{11}N_2O_3$ (MH$^+$), calcd 171.0770, found 171.0769.

Ac-L-Ala(CN)-NHMe (2).[S5] To **12** (379 mg, 2.23 mmol) was added methylamine solution (40% in MeOH, 3.5 mL, 45.1 mmol). After stirring at room temperature for 1 h, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography ($MeOH/CH_2Cl_2$ = 1:100−1:10) to give **2** (329 mg, 87%) as a white solid. TLC ($MeOH/CH_2Cl_2$ = 1:20) R_f = 0.28; ^1H NMR (500 MHz, $CDCl_3$) δ 6.87 (d, J = 8.5 Hz, 1H), 6.85 (brs, 1H), 4.84 (dt, J = 8.0, 6.5 Hz, 1H), 2.91 (dd, J = 17.0, 6.5 Hz, 1H), 2.85 (d, J = 5.0 Hz, 3H), 2.81 (dd, J = 17.0, 6.5 Hz, 1H), 2.08 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.77, 168.94, 116.81, 49.24, 26.48, 23.02, 21.12; HRMS (CI+) for $C_7H_{12}N_3O_2$ (MH$^+$), calcd 170.0930, found 170.0930.

S2. Wobbling-in-a-cone model[S8,S9]

To extract detailed information on restricted orientational motion from biexponential anisotropy decay, the experimentally measured orientational relaxation lifetimes are analysed by a wobbling-in-a-cone model. The long time component is associated with full orientational randomization, whereas the short time component results from restricted wobbling motion, with the transition dipoles undergoing orientational diffusion within a cone of semiangle θ_c. The anisotropy decay within the wobbling-in-a-cone model is given as

$$r(t) = 0.4\left[Q^2 + \left(1 - Q^2\right)\exp\left(-t/\tau_w\right)\right]\exp\left(-t/\tau_1\right) \tag{S1}$$

where Q^2 ($0 \le Q^2 \le 1$) is the generalized order parameter describing the degree of restriction on the orientational diffusion, and τ_w and τ_1 are time constant for the restricted (fast) and complete relaxation, respectively. The τ_w time constant is determined from the experimentally measured orientational relaxation lifetimes as

$$\tau_w = \left(\tau_{or2}^{-1} - \tau_{or1}^{-1}\right)^{-1} \tag{S2}$$

Note that for $Q^2 = 1$, the equation simplifies to a single exponential decay with long time component τ_1, whereas for $Q^2 = 0$ the orientational motion is fully restricted, and complete randomization occurs mostly due to the wobbling motion since $\tau_1 > \tau_w$. The parameter Q^2 is related to the cone semiangle by the following formula:

$$Q^2 = \left[0.5\cos\theta_c\left(1 + \cos\theta_c\right)\right]^2 \tag{S3}$$

The wobbling-in-a-cone diffusion constant can be then calculated by

$$D_w = \frac{x_w^2\left(1 + x_w\right)^2\left\{\ln\left[\left(1 + x_w\right)/2\right] + \left(1 - x_w\right)/2\right\}}{\tau_w\left(1 - Q^2\right)\left[2\left(x_w - 1\right)\right]} + \frac{\left(1 - x_w\right)\left(6 + 8x_w - x_w^2 - 12x_w^3 - 7x_w^4\right)}{24\tau_w\left(1 - Q^2\right)} \tag{S4}$$

where $x_w = \cos\theta_c$. The diffusion constant of slow, complete orientational randomization can be calculated by

$$D_1 = \frac{1}{6\tau_{or1}} \tag{S5}$$

Table S1 Vibrational properties of the NC stretching mode of **1** in various solvents[a]

Solvent	ω_1 (cm^{-1})	FWHM[b] (cm^{-1})	ω_2 (cm^{-1})	FWHM[b] (cm^{-1})
DMF	2148.4	10.7	—	—
THF	2148.7	9.6	—	—
MeOH	2150.2	10.3	2172.7	18.5
MeOAc	2151.0	10.0	—	—
$CHCl_3$	—	—	2154.0	17.4
D_2O	—	—	2169.5	24.1
CF_3CH_2OH	2152.6	14.6	2183.8	22.0

[a] Fitting parameters obtained from Fig. 2. [b] Full width at half-maximum

Table S2 Amide I band properties of **0**−**3** in D_2O[a]

	0	**1**	**2**	**3**
ω_{center} (cm^{-1})	1635.8	1650.0	1651.3	1642.7
FWHM[b] (cm^{-1})	37.7	39.2	42.5	39.7

[a] Fitting parameter obtained from Fig. 3a. [b] Full width at half-maximum.

Table S3 Wobbling-in-a-cone model analysis results for the biexponential anisotropy decay of **1** in D_2O[a]

Q	θ_c (°)	τ_w (ps)	τ_1 (ps)	D_w^{-1} (ps)	D_1^{-1} (ps)
0.53	36.5	0.55	10.3	5.3	61.8

[a] See eqn (S1)−(S5) in Section S1 of the ESI.†

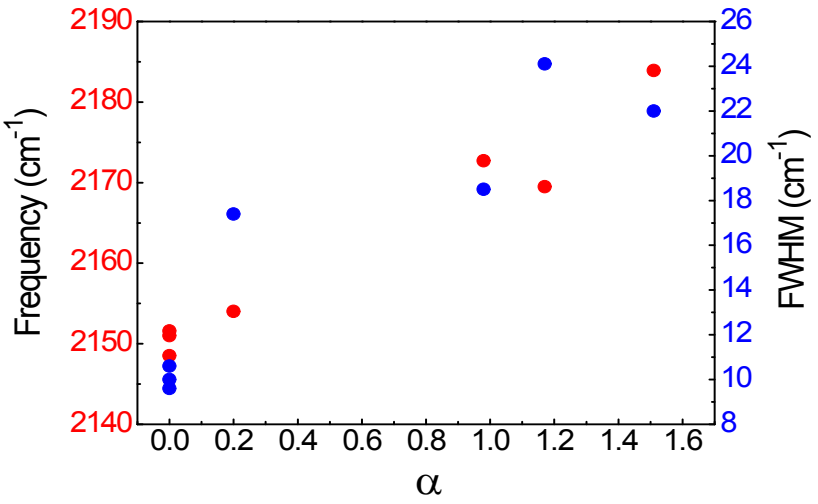

Fig. S1 Kamlet-Taft plot for **1**. The frequency and full width at half-maximum (FWHM) of the NC stretching mode are plotted against the Kamlet-Taft solvent parameter α^{S10} for H-bond donor strength.

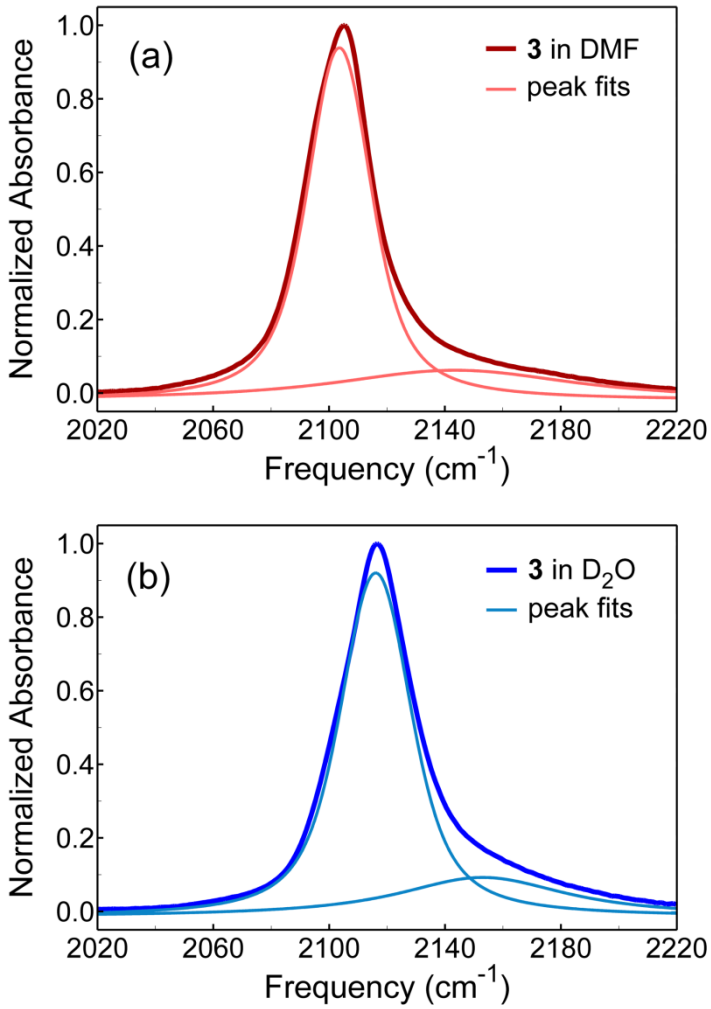

Fig. S2 Factor analyses of the azido (N_3) FTIR spectra of **3** in DMF (a) and D_2O (b). The azido FTIR spectra, which are taken from Fig. 3d, can be fitted to two Voigt functions. Asymmetry in the high frequency region is observed regardless of the solvent used.

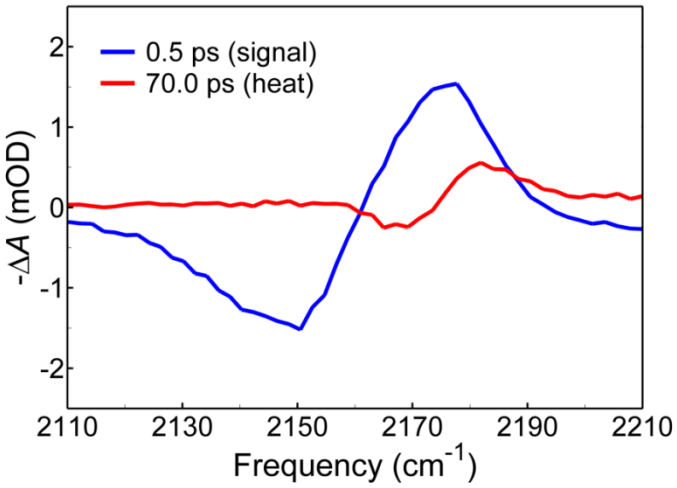

Fig. S3 Isotropic IR pump–probe spectra of **1** in D_2O at short (0.5 ps) and long (70 ps) delay times.

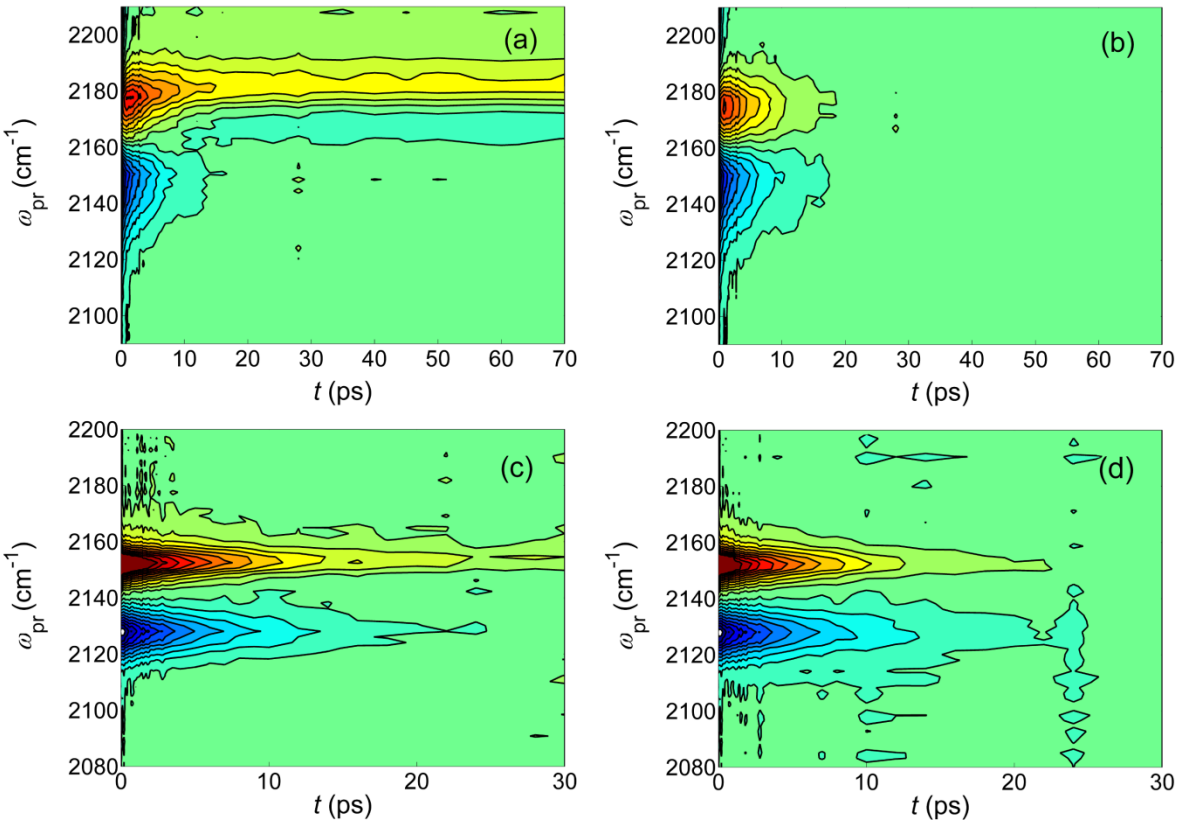

Fig. S4 Time- and frequency-resolved isotropic IR pump–probe signals at the delay time t for **1** in D_2O (upper) and DMF (lower) before (left) and after (right) subtraction of the heat contribution. Fig. S4b and d were used to obtain Fig. 5.

References

S1 K.-K. Lee, K.-I. Oh, H. Lee, C. Joo, H. Han and M. Cho, *ChemPhysChem.*, 2007, **8**, 2218−2226.

S2 K.-I. Oh, J.-H. Lee, C. Joo, H. Han and M. Cho, *J. Phys. Chem. B*, 2008, **112**, 10352−10357.

S3 D. R. Hill., C.-N. Hsiao, R. Kurukulasuriya and S. J. Wittenberger, *Org. Lett.*, 2002, **4**, 111−113.

S4 G. Zhao, C. Bughin, H. Bienaymé and J. Zhu, *Synlett*, 2003, 1153−1154.

S5 G. A. Reichard, C. Stengone, S. Paliwal, I. Mergelsberg, S. Majmundar, C. Wang, R. Tiberi, A. T. McPhail, J. J. Piwinski and N.-Y. Shih, *Org. Lett.*, 2003, **5**, 4249−4251.

S6 D. Kand, D. P. Chauhan, M. Lahiri and P. Talukdar, *Chem. Commun.*, 2013, **49**, 3591−3593.

S7 A. J. Pearson and D. V. Ciurea, *J. Org. Chem.*, 2008, **73**, 760−763.

S8 S. Park, D. E. Moilanen and M. D. Fayer, *J. Phys. Chem. B*, 2008, **112**, 5279−5290.

S9 G. Lipari and A. Szabo, *Biophys. J.*, 1980, **30**, 489−506.

S10 M. J. Kamlet, J.-L. M. Abboud, M. H. Abraham and R. W. Taft, *J. Org. Chem*., 1983, **48**, 2877−2887.