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Principles of Hydroxamate Inhibition of Metalloproteases: Carboxypeptidase A
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Synthesis of inhibitors. Our preferred intermediate for preparation of this class of inhibitor (**1**, in the form of racemic materials throughout), is benzyl 3-(*N*-benzyloxyamino)-2-benzylpropionate [$\text{C}_6\text{H}_5\text{CH}_2\text{ONHCH}_2\text{CH}(\text{CH}_2\text{C}_6\text{H}_5)\text{CO}_2\text{CH}_2\text{C}_6\text{H}_5$], obtained as follows. A mixture of 9.09 g (0.057 mol) of *O*-benzylhydroxylamine hydrochloride in 10.5 mL of water and 20.5 mL of methanol at 0 °C was adjusted to a pH of 4.7 with 5 N sodium hydroxide. Then 10.32 g (0.05 mol) of ethyl 2-hydroxymethylene-3-phenylpropionate (**45**) was added and the mixture was allowed to warm to room temperature. The pH was maintained by the addition of further 5 N sodium hydroxide. After 3 h, the reaction mixture was quenched with 40 mL of water, and it was then extracted with 3 × 25 mL of methylene chloride. The combined organic layer was washed with 20 mL of brine, dried over anhydrous sodium sulfate, filtered, and then was evaporated under reduced pressure to yield an oil. To a solution of the resulting benzyloxime in 37.2 mL of tetrahydrofuran and 74.5 mL of glacial acetic acid at 0 °C, 5.12 g (0.077 mol) of reducing agent 95% sodium cyanoborohydride was added portionwise (3 × 1.71 g) during 1 h. The reaction mixture was quenched with 500 mL of water and then was extracted with 4 × 100 mL of methylene chloride. The combined organic layer was washed with 150 mL of saturated sodium bicarbonate and 100 mL of brine, dried over anhydrous sodium sulfate, filtered, and then it was concentrated. Flash chromatography over silica gel (17–33% ethyl acetate in hexanes) gave 12.2 g (78%) of ethyl 3-benzyloxyamino-2-benzylpropionate as a colorless oil. A solution of 4 g (12.8 mmol) of this material in 25.3 mL of methanol was placed in a water bath at room temperature and 3.07 mL of 5 N sodium hydroxide was added with stirring. The mixture was maintained at room temperature for 2 h. The methanol was removed under reduced pressure, and 9 mL of water was added. The aqueous solution was washed with 3 × 7 mL of methylene chloride and was stirred during acidification to a pH of less than 3 (about 3 mL of 5 N hydrochloric acid). The resulting mixture was extracted with 4 × 7 mL of chloroform. The combined chloroform solution was washed with 15 mL of brine, dried over anhydrous sodium sulfate, filtered and then was evaporated. The resulting oil was crystallized from diethyl ether to afford 3.28 g (90%) of 3-benzyloxyamino-2-benzylpropionic acid as a white solid, mp 74–75 °C. To a mixture of 4.92 mL of freshly distilled benzyl alcohol and 43.8 mL of benzene in a 100 mL round-bottom flask, 3.2 g (0.0112 mol) of this material and 2.25 g (0.0118 mol) of *p*-toluenesulfonic acid monohydrate were added. The mixture was heated to reflux and the water formed was repeatedly separated from the distillate. When no more water appeared in the distillate, the mixture was allowed to cool to room temperature. It was diluted with 10 mL of benzene, washed with 40 mL of saturated sodium carbonate followed by 40 mL of water, dried over anhydrous sodium sulfate, filtered, and it then was concentrated. Flash chromatography over silica gel (17–33% of ethyl acetate in hexanes) afforded 3.40 g (81%) of benzyl 3-benzyloxyamino-2-benzylpropionate as a colorless oil. Neutralization with hydrochloric acid yielded the salt, benzyl 3-benzyloxyamino-2-benzylpropionate hydrochloride, recrystallized from chloroform and hexanes, mp 142–143 °C; 400 MHz ^1H NMR (CD_3OD) δ 7.42–7.37 (m, 5 H),

7.31–7.23 (m, 5 H), 7.16–7.14 (m, 5 H), 5.00–4.91 (m, 4 H), 3.72 (dd, 1 H, $J = 10.1$, 13.6 Hz), 3.42 (dd, 1 H, $J = 3.1$, 13.7 Hz), 3.25–3.17 (m, 1 H), 3.00 (dd, 1 H, $J = 7.6$, 13.6 Hz), and 2.93 (dd, 1 H, and $J = 7.3$, 13.8 Hz); IR (KBr) 1729 cm^{-1} . Anal. Calcd for $\text{C}_{24}\text{H}_{26}\text{NO}_3\text{Cl}$ (411.91): C, 69.98; H, 6.36; N, 3.40. Found: C, 69.80; H, 6.36; N, 3.38.

This intermediate is a convenient precursor for inhibitors **1**. For example, acetylation with acetic anhydride in chloroform gave the benzyloxyacetamide-benzyl ester (**46**), which with subsequent hydrogenolysis (10% Pd on charcoal, methanol, H_2 at atmospheric pressure) provided a 93% yield of 3-(*N*-acetyl-*N*-hydroxyamino)-2-benzylpropionic acid, **1b**, (**8**, **46**) as a white solid from chloroform and hexanes, mp 128–129 °C; 400 MHz ^1H NMR (CD_3OD) δ 7.28–7.18 (m, 5 H), 3.83 (d, 2 H, $J = 7.0$ Hz), 3.01–3.07 (m, 1 H), 2.90 (dd, 1 H, $J = 8.6$, 13.8 Hz), 2.80 (dd, 1 H, $J = 6.0$, 13.7 Hz), and 2.09 (s, 3 H); IR (KBr) 1682 and 1590 cm^{-1} . Anal. Calcd for $\text{C}_{12}\text{H}_{15}\text{NO}_4$ (237.26): C, 60.75; H, 6.37; N, 5.90. Found: C, 60.58; H, 6.29; N, 5.82. The doubled acetyl NMR signal of this substance in CDCl_3 exhibited a coalescence temperature of ~ 28 °C, for syn-anti rotation about the amide linkage (Figure 1S).

Analogously prepared were the following: 3-(*N*-Formyl-*N*-hydroxyamino)-2-benzylpropionic acid, **1a**, (**8**) as a colorless oil; 400 MHz ^1H NMR (CD_3OD) (NCHO syn:anti = 2:1, Figure 2S) δ 8.30 (s, 1/3 H), 7.87 (s, 2/3 H), 7.30–7.19 (m, 5 H), 3.84–3.78 (m, 1 H), 3.76–3.71 (dd, 1/3 H, $J = 6.2$, 14.1 Hz), 3.51 (dd, 2/3 H, $J = 4.5$, 14.6 Hz), 3.20–3.17 (m, 2/3 H), 3.12–3.08 (m, 1/3 H), 2.99 (dd, 2/3 H, $J = 7.5$, 13.8 Hz), 2.93 (dd, 1/3 H, $J = 8.4$, 13.7 Hz), and 2.84 (dd, 1 H, $J = 6.9$, 13.5 Hz); IR (CHCl_3) 1715 and 1673 cm^{-1} . Anal. Calcd for $\text{C}_{11}\text{H}_{13}\text{NO}_4$ (223.22): C, 59.19; H, 5.87; N, 6.27. Found: C, 59.16; H, 6.01; N, 6.17. 3-(*N*-Fluoroacetyl-*N*-hydroxyamino)-2-benzylpropionic acid, **1c**, as white crystals, mp 106–107 °C; 200 MHz ^1H NMR (CD_3OD) δ 7.28–7.12 (m, 5 H), 5.08 (d, 2 H, $J = 48.0$ Hz), 3.95–3.79 (m, 2 H), 3.12–3.02 (m, 1 H), 2.95 (dd, 1 H, $J = 8.5$, 13.6 Hz), and 2.81 (dd, 1 H, $J = 6.8$, 13.5 Hz); IR (KBr) 1701 and 1640 cm^{-1} . Anal. Calcd for $\text{C}_{12}\text{H}_{14}\text{NO}_4\text{F}$ (255.24): C, 56.47; H, 5.53; N, 5.49. Found: C, 56.10; H, 5.42; N, 5.38. 3-(*N*-Difluoroacetyl-*N*-hydroxyamino)-2-benzylpropionic acid, **1d**, as white crystals, mp 107–108 °C; 200 MHz ^1H NMR (CD_3OD) δ 7.26–7.20 (m, 5 H), 6.45 (t, 1 H, $J = 53.8$ Hz), 3.92 (dd, 1 H, $J = 7.6$, 16.5 Hz), 3.80 (dd, 1 H, $J = 6.0$, 14.0 Hz), 3.20–3.06 (m, 1 H), 2.96 (dd, 1 H, $J = 7.8$, 13.6 Hz), and 2.83 (dd, 1 H, $J = 6.5$, 13.5 Hz); IR (KBr) 1707 and 1645 cm^{-1} . Anal. Calcd for $\text{C}_{12}\text{H}_{13}\text{NO}_4\text{F}_2$ (273.23): C, 52.75; H, 4.80; N, 5.13. Found: C, 52.55; H, 4.77; N, 5.03. 3-(*N*-Trifluoroacetyl-*N*-hydroxyamino)-2-benzylpropionic acid, **1e**, as a colorless oil by evaporation of an ether solution; 400 MHz NMR (CD_3OD) δ 7.33–7.18 (m, 5 H), 3.97 (dd, 1 H, $J = 8.0$, 14.0 Hz), 3.76 (dd, 1 H, $J = 5.9$, 14.0 Hz), 3.18–3.11 (m, 1 H), 2.97 (dd, 1 H, $J = 8.1$, 13.8 Hz), and 2.83 (dd, 1 H, $J = 6.5$, 13.8 Hz); 100 MHz ^{13}C NMR (CD_3OD) δ 176.6, 157.9 (q), 139.6, 130.1, 129.8, 127.7, 117.9 (q), 51.4, 45.7, and 36.6; IR (CHCl_3) 1699 cm^{-1} [**1e** is susceptible to alkaline hydrolysis, the $\text{RN}(\text{OH})\text{COCF}_3$ group must be handled accordingly]. Anal. Calcd for $\text{C}_{12}\text{H}_{12}\text{NO}_4\text{F}_3$ (291.23): C, 49.49; H, 4.15; N, 4.81. Found: C, 50.38; H, 4.71; N, 4.53; $\text{C}_{12}\text{H}_{12}\text{NO}_4\text{F}_3 \cdot 0.2(\text{C}_2\text{H}_5)_2\text{O}$ requires C, 50.23; H, 4.61; N, 4.58. 3-(*N*-Methoxyacetyl-*N*-hydroxyamino)-2-benzylpropionic acid, **1f**, as white crystals, mp 125–126 °C; 200 MHz ^1H NMR (CD_3OD) δ 7.31–7.12 (m, 5 H), 4.21 (s, 2 H), 3.88–3.80 (m, 2 H), 3.38 (s, 3 H), 3.13–3.02 (m, 1 H), 2.95 (dd, 1 H, $J = 7.6$, 13.8 Hz), and 2.83 (dd, 1 H, $J = 6.8$, 13.7 Hz); IR (KBr) 1699 and 1610 cm^{-1} . Anal. Calcd for $\text{C}_{13}\text{H}_{17}\text{NO}_5$ (267.28):

C, 58.42; H, 6.41; N, 5.24. Found: C, 58.36; H, 6.36; N, 5.21. 3-(*N*-Methoxycarbonyl-*N*-hydroxyamino)-2-benzylpropionic acid, **1g**, as a colorless oil; 400 MHz ^1H NMR (CD_3OD) δ 7.28–7.18 (m, 5 H), 3.79 (dd, 1 H, $J = 7.5, 14.2$ Hz), 3.70 (s, 3 H), 3.64 (dd, 1 H, $J = 6.0, 14.1$ Hz), 3.10–3.03 (m, 1 H), 2.91 (dd, 1 H, $J = 8.6, 13.7$ Hz), and 2.83 (dd, 1 H, $J = 6.1, 13.7$ Hz); IR (CHCl_3) 1711 cm^{-1} ; converted to a crystalline dicyclohexylammonium salt for analysis, mp 169–170 $^\circ\text{C}$. Anal. Calcd for $\text{C}_{24}\text{H}_{38}\text{N}_5\text{O}_5$ (434.58): C, 66.33; H, 8.81; N, 6.45. Found: C, 65.94; H, 8.72; N, 6.45.

The preparation of **2** was as follows. In a 100 mL round-bottomed flask fitted with a refluxing water-benzene separator were placed 7.57 g (40.2 mmol) of dimethyl 3-oxohexanedioate (**47**), 4.7 g (44.3 mmol) of benzaldehyde, 47 mL of benzene, 0.16 mL of piperidine, and 0.48 mL of glacial acetic acid. After heating for 3 h, the reaction solution was cooled and diluted with 57 mL of ether, and was then washed successively with 50 mL portions of 1 N hydrochloric acid, saturated sodium bicarbonate, 5% acetic acid, and twice with water. The extract was dried over anhydrous sodium sulfate, filtered, and then was concentrated under reduced pressure, yielding a benzylidene derivative of the ketodiester. To a solution of the resulting oil in 114 mL of ethyl acetate was added 0.62 g of 10% palladium on charcoal, and the mixture was shaken under 3 atm of hydrogen for 45 minutes. After filtration, the solution was evaporated under reduced pressure. Flash chromatography over silica gel (17–50% ethyl acetate in hexanes) gave 9.96 g (89%) of dimethyl 2-benzyl-3-oxohexanedioate as a colorless oil, 200 MHz ^1H NMR (CDCl_3) δ 7.35–7.13 (m, 5 H), 3.84 (t, 1 H, $J = 7.6$ Hz), 3.67 (s, 3 H), 3.62 (s, 3 H), 3.17 (d, 2 H, $J = 7.6$ Hz), 2.99–2.64 (m, 2 H), and 2.60–2.50 (m, 2 H); IR (CHCl_3) 1742 and 1717 cm^{-1} .

A solution of 8.09 g (29.0 mmol) of this material plus 5.10 g (31.9 mmol) of *O*-benzyl hydroxylamine hydrochloride in 19.1 mL of water and 38.3 mL of methanol at 0 $^\circ\text{C}$, adjusted to a pH of 4.7 with sodium hydroxide, was allowed to warm to room temperature over 3 h, with pH maintained by the addition of further 5 N sodium hydroxide. The reaction mixture was then quenched with 200 mL of water, followed by extraction with 3 \times 150 mL of methylene chloride. The combined organic layer was washed with 100 mL of brine, dried over anhydrous sodium sulfate, filtered, and then was evaporated under reduced pressure. Flash chromatography over silica gel (17–50% ethyl acetate in hexanes) gave 4.65 g of (*E*)-dimethyl 2-benzyl-3-benzyloxyiminohexanedioate and 4.62 g of the (*Z*)-isomer (84% total) as colorless oils, 400 MHz ^1H NMR (CDCl_3) (*E*) δ 7.34–7.10 (m, 10 H), 5.08 (s, 2 H), 3.64 (s, 3 H), 3.61 (s, 3 H), 3.67–3.58 (m, 1 H), 3.20 (dd, 1 H, $J = 13.8, 7.6$ Hz), 3.03 (dd, 1 H, $J = 13.9, 7.8$ Hz), and 2.62–2.34 (m, 4 H); (*Z*) δ 7.34–7.10 (m, 10 H), 5.04 and 5.03 (AB system, 2 H, $J = 12.3$ Hz), 3.99 (dd, 1 H, $J = 8.1, 7.3$ Hz), 3.58 (s, 6 H), 3.23 (dd, 1 H, $J = 13.8, 7.1$ Hz), 3.00 (dd, 1 H, $J = 13.8, 8.2$ Hz), 2.52–2.38 (m, 3 H), and 2.30–2.22 (m, 1 H); IR (CHCl_3) (*E*) 1733 cm^{-1} and (*Z*) 1735 cm^{-1} .

To a solution of 6.15 g (16.0 mmol) of the benzyloxime geometrical isomers plus 4.8 g (35.2 mmol) of reducing agent dimethylphenylsilane in 30.8 mL of trifluoroacetic acid was added 1.86 g (32.0 mmol) of potassium fluoride (**48**, **49**). After the mixture was stirred for 36 h at room temperature, it was quenched with 500 mL of saturated sodium bicarbonate, and then was extracted with 4 \times 250 mL of methylene chloride. The combined organic layer was washed with brine, dried over sodium sulfate, filtered, and then was concentrated under reduced pressure. Flash chromatography over silica gel (17–100% ethyl acetate in hexanes) gave two

substances: the initial reduction product, 3.8 g (61%) of dimethyl 2-benzyl-3-benzyloxyamino-hexanedioate as a colorless oil, 200 MHz ^1H NMR (CDCl_3) δ 7.32–7.16 (m, 10 H), 4.67 and 4.61 (AB system, 2 H, $J = 12.1$ Hz), 3.64 (s, 3 H), 3.58 (s, 3 H), 3.31–3.21 (m, 1 H), 3.11–2.95 (m, 2 H), 2.85 (dd, 1 H, $J = 6.6, 13.8$ Hz), 2.57–2.28 (m, 2 H), 1.95–1.78 (m, 1 H), 1.70–1.49 (m, 1 H); IR (CHCl_3) 1730 cm^{-1} ; plus a cyclization product, 1.21 g (21%) of methyl 3-phenyl-2-(1-benzyloxy-2-oxo-5-pyrrolidinyl)propionate as a white solid, mp 79–83 °C. The first substance could be converted to the second: A solution of 0.2 g (0.52 mmol) of the diester in 5 mL of *N,N*-dimethylcyclohexylamine was heated at 150 °C overnight. Then the *N,N*-dimethylcyclohexylamine was removed under reduced pressure. Flash chromatography over silica gel (ethyl acetate) gave 0.13 mg (71%) of methyl 3-phenyl-2-(1-benzyloxy-2-oxo-5-pyrrolidinyl)propionate as a white solid (mixed diastereomers, chromatographically inseparable), 400 MHz ^1H NMR (CDCl_3) δ 7.47–7.11 (m, 10 H), 5.03–4.94 (m, 2 H), 3.99–3.92 (m, 0.5 H), 3.60 (s, 1.5 H), 3.50–3.44 (m, 0.5 H), 3.37 (s, 1.5 H), 3.26–3.20 (m, 0.5 H), 3.00–2.91 (m, 1 H), 2.87–2.78 (m, 1 H), 2.68 (dd, 0.5 H, $J = 7.1, 13.8$ Hz) 2.45–2.21 (m, 2 H), and 2.11–1.89 (m, 2 H); IR (CHCl_3) 1728 and 1703 cm^{-1} .

Deprotection was accomplished by hydrogenolysis of the benzyl group (10% Pd on charcoal, methanol, H_2 at atmospheric pressure) followed by saponification (1 N sodium hydroxide in methanol-water, 2 h at 25 °C), with a work-up consisting of neutralization and extraction of by-products (with CH_2Cl_2), followed by acidification (pH <3) and recovery of final product (extraction with CHCl_3) in 40% overall yield: 3-phenyl-2-(1-hydroxy-2-oxo-5-pyrrolidinyl)propionic acid, **2**, obtained as a white froth (mixed diastereomers, chromatographically inseparable), 400 MHz ^1H NMR (CD_3OD) δ 7.30–7.17 (m, 5 H), 4.18–4.11 (m, 0.5 H), 3.93–3.89 (m, 0.5 H), 3.22–3.19 (m, 0.5 H), 3.13–3.08 (m, 1 H), 2.94–2.85 (m, 1 H), 2.77–2.71 (m, 0.5 H), 2.39–2.24 (m, 2 H), 2.20–2.10 (m, 1.5 H), and 2.08–1.98 (m, 0.5 H); IR (KBr) 1709 and 1673 cm^{-1} ; 100 MHz ^{13}C NMR (CD_3OD): δ 176.7, 176.1, 173.3, 172.5, 140.5, 140.3, 130.1, 130.0, 129.6, 129.4, 127.5, 127.4, 62.1, 61.7, 51.2, 35.1, 32.9, 28.0, 27.9, 19.4, and 18.8 (doubled ^{13}C resonances confirm two diastereomers); HRMS of mixture: Calcd for $\text{C}_{13}\text{H}_{15}\text{NO}_4$ (M^+) m/z 249.10011. Found: 249.10052. Deliberate lactonization of the carboxyl onto the hydroxamic-OH to give a more rigid bicyclic structure did not render feasible a separation of diastereomers for **2**.

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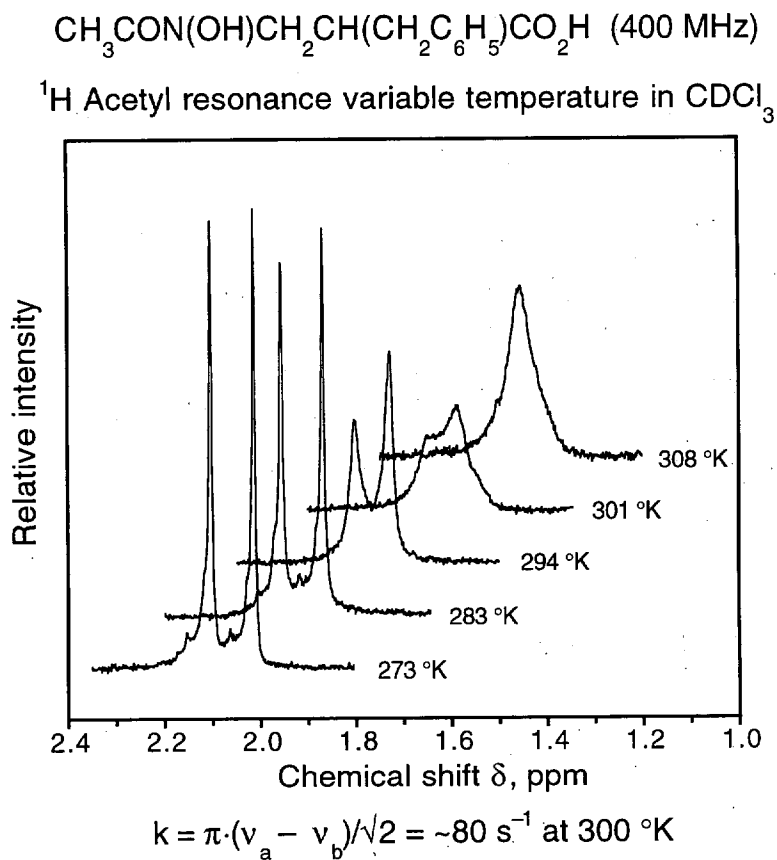


FIGURE 1S. ^1H NMR spectra (stacked plots) of acetyl group within **1b** as a function of temperature. Signal coalescence occurs due to increasing rate of rotation about the $\text{CH}_3\text{CO}-\text{N}(\text{OH})\text{R}$ linkage.

$\text{HCON(OH)CH}_2\text{CH(CH}_2\text{C}_6\text{H}_5\text{)CO}_2\text{H}$ (400 MHz)
 ^1H Formyl resonance in CDCl_3 plus CD_3OD (25 °C)

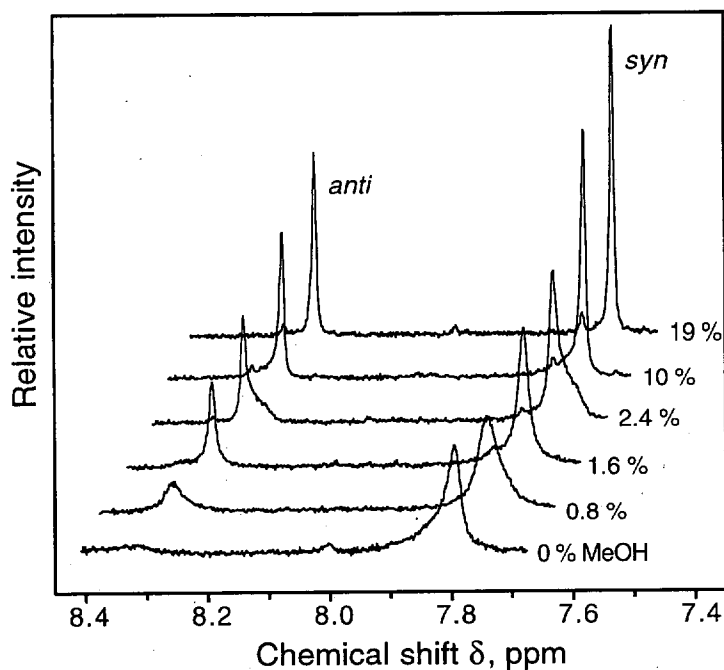


FIGURE 2S. ^1H NMR spectra (stacked plots) of formyl group within **1a** as a function of solvent composition (CDCl_3 – CD_3OD). Intermolecular H-bonding involving methanol favors anti rotamer (sharpened signals may also reflect slowed rotation).