

# Effects of Glycosylation of (2*S*,4*R*)-4-Hydroxyproline on the Conformation, Kinetics and Thermodynamics of Prolyl Amide Isomerization

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## Supporting Information

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## Experimental

### *General*

Thin-Layer chromatography was performed on Si250F precoated plates of silica gel (250 $\mu$ m).

Column chromatography was performed on SilicaFlash P60 silica gel (40-63 $\mu$ m). Reagent grade solvents were used without further purification. Spectra were assigned based on 2D COSY and 2D HSQC experiments. For  $^1\text{H}$  NMR, minor isomers are listed between square brackets. For  $^{13}\text{C}$  NMR, when assigned, carbon peaks for the minor isomer are listed in brackets.

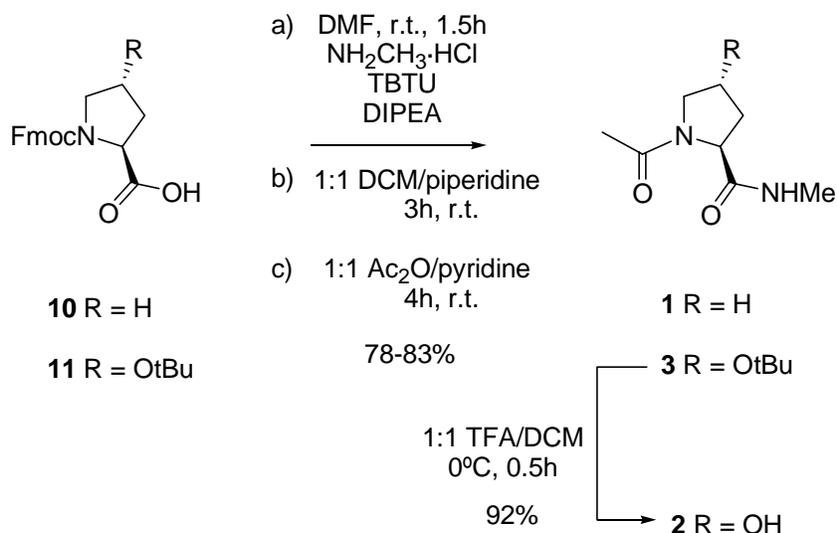
### *General Procedures*

#### **Preparation of *N*-Fmoc-*N'*-methylamide amino acids**

The amino acid (1.0 eq) was dissolved in anhydrous DMF, followed by addition of TBTU (1.5 eq), diisopropylethylamine (3.0 eq) and methylamine hydrochloride (1.5 eq). The solution was stirred at ambient temperature for 1.5 hrs. The reaction mixture was diluted with 20 mL water, followed by extraction (EtOAc), drying ( $\text{Na}_2\text{SO}_4$ ), and concentration under reduced pressure for purification by flash chromatography.

#### **Preparation of *N*-Acetyl-*N'*-methylamide amino acids**

The amino acid (1.0 eq) was dissolved in 1:1 (v/v) DCM/piperidine, stirred for 3 hours at ambient temperature, then co-distilled with toluene (3 x 10 mL). The crude product was then dissolved in 1:1  $\text{Ac}_2\text{O}$ /pyridine, and the mixture was stirred at ambient temperature for 4 hours. The solution was concentrated under reduced pressure, and then was co-distilled with toluene (3 x 10 mL) before purification by flash chromatography.



**Scheme 1.** Synthesis of Diamides **1**, **2** and **3**.

### *N*-Acetyl-*L*-proline *N'*-methylamide (**1**)

The general preparation methods were followed: *C*-terminal amidation of (2*S*)-Fmoc-Proline **10** (0.2 g, 1.175 mmol, 1.0 eq) using TBTU (0.566 g, 1.76 mmol, 1.5 eq), DIPEA (0.614 mL, 3.52 mmol, 3.0 eq), and methylamine hydrochloride (0.119 g, 1.76 mmol, 1.5 eq) in anhydrous DMF (5 mL). *N*-terminal acylation of the crude product was performed using 1:1 (v/v) DCM:piperidine (4 mL), then 1:1  $\text{Ac}_2\text{O}$ /pyridine (4 mL). The reaction mixture was purified by flash chromatography using first ethyl acetate, then 9:1 ethyl acetate/methanol to yield **1** as a clear oil (0.181 g, 1.063 mmol) (83.0%):  $[\alpha]_D^{25} = -1.4^\circ$  (*c* 1.0  $\text{CHCl}_3$ );  $^1\text{H NMR}$  (300 MHz,  $\text{D}_2\text{O}$ , 298K)  $\delta =$  [4.46, dd, 0.25H,  $\text{Pro}_\alpha^{cis}$ ,  $J = 3.0\text{Hz}$ ,  $J = 8.7\text{Hz}$ ], 4.30 (dd, 0.75H,  $\text{Pro}_\alpha^{cis}$ ,  $J = 4.1\text{Hz}$ ,  $J = 8.8\text{Hz}$ ), 3.52-3.70 (m, 1.5H,  $\text{Pro}_{\gamma 1}^{trans}$ ,  $\text{Pro}_{\gamma 2}^{trans}$ ), [3.39-3.54, m, 0.5H,  $\text{Pro}_{\gamma 1}^{cis}$ ,  $\text{Pro}_{\gamma 2}^{cis}$ ], [2.75, s, 0.75H,  $-\text{NHCH}_3^{cis}$ ], 2.70 (s, 2.25H,  $-\text{NHCH}_3^{trans}$ ), [2.26-2.35, m, 0.25H,  $\text{Pro}_{\beta 1}^{cis}$ ], 2.15-2.27 (m, 0.75H,  $\text{Pro}_{\beta 1}^{trans}$ ), 2.09 (s, 2.25H,  $-\text{COCH}_3^{trans}$ ), [1.95, s, 0.75H,  $-\text{COCH}_3^{cis}$ ], 1.85-2.01 (m, 3H,  $\text{Pro}_{\beta 1}^{trans}$ ,  $\text{Pro}_{\beta 1}^{cis}$ ,  $\text{Pro}_{\delta 1}^{trans}$ ,  $\text{Pro}_{\delta 1}^{cis}$ ,  $\text{Pro}_{\delta 2}^{trans}$ ,  $\text{Pro}_{\delta 2}^{cis}$ );  $^{13}\text{C NMR}$  (75 MHz,  $\text{D}_2\text{O}$ , 298K)  $\delta =$

175.3, (175.2), (173.9), 173.7, (62.3), 60.8, 49.1, (47.4), (31.9), 30.3, (26.3), 26.2, 24.5, (22.9), 21.8, (21.6).

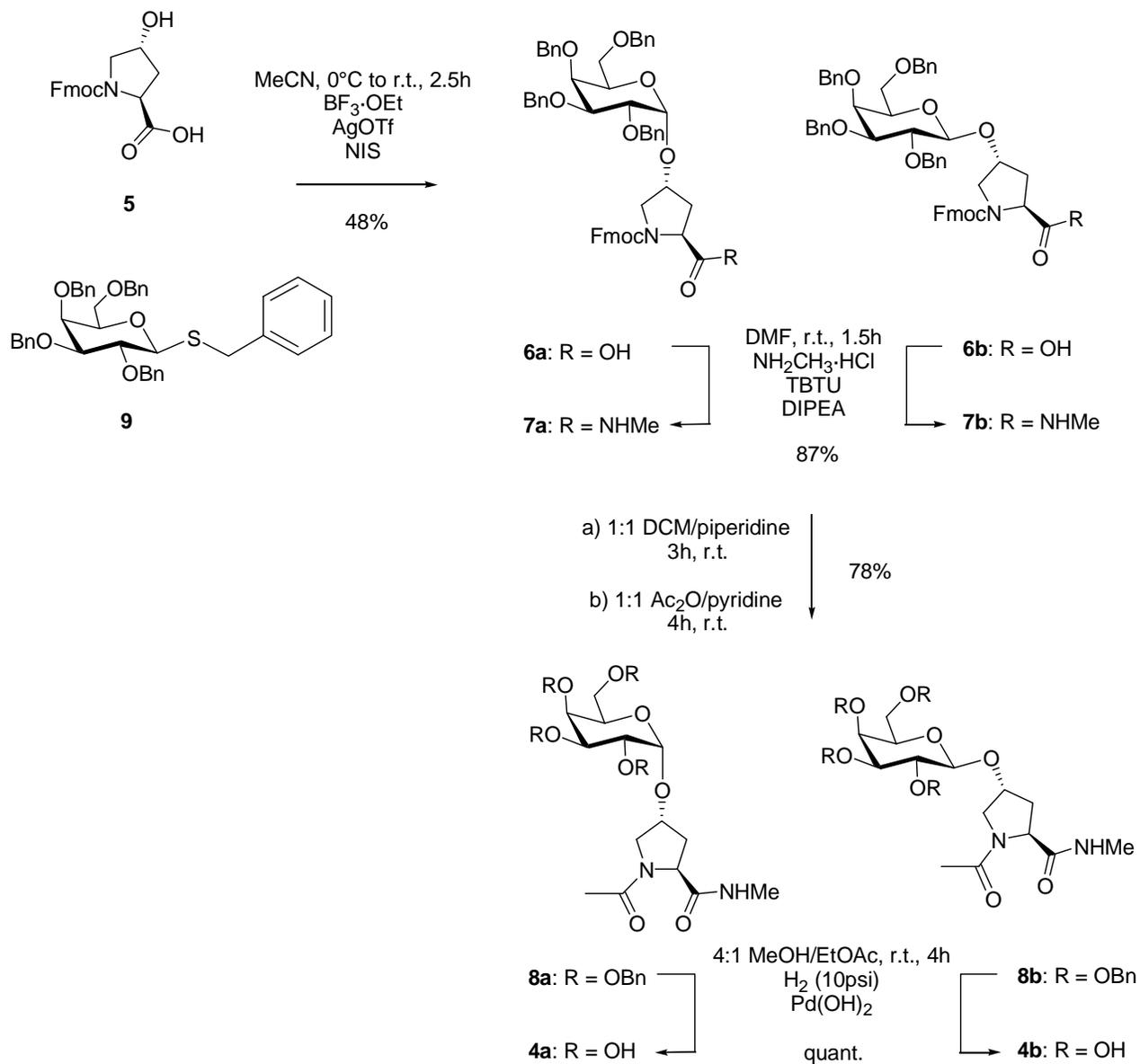
### ***N*-Acetyl-*trans*-4-hydroxy-*L*-proline *N'*-methylamide (2)**

Compound **3** (0.095 g, 0.392 mmol) was dissolved in 1:1 (v/v) TFA/DCM (4 mL), stirred for 30 minutes at 0°C, then co-distilled with toluene (3 x 6 mL). The product was purified by flash chromatography using 10:1 ethyl acetate/methanol to yield **2** as a clear oil (0.067 g, 0.36 mmol) (92.0%):  $[\alpha]_D^{25} = -26.7^\circ$  (*c* 0.3 CH<sub>3</sub>OH); <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O, 298K)  $\delta =$  [4.57, dd, 0.23H, Pro $_{\alpha}^{cis}$ ,  $J_{2,3a} = 8.1\text{Hz}$ ], 4.50-4.56 (m, 0.77H, Pro $_{\gamma}^{trans}$ ), [4.45-4.50, m, 0.23H, Pro $_{\gamma}^{cis}$ ], 4.40 (dd, 0.77H, Pro $_{\alpha}^{trans}$ ,  $J_{2,3a} = 8.4\text{Hz}$ ,  $J_{2,3b} = 8.5\text{Hz}$ ), 3.79 (dd, 0.77H, Pro $_{\delta 1}^{trans}$ ,  $J_{\gamma,\delta 1} = 4.1\text{Hz}$ ,  $J_{\delta 1,\delta 2} = 11.8\text{Hz}$ ), [3.69, dd, 0.23H, Pro $_{\delta 1}^{cis}$ ,  $J_{\gamma,\delta 1} = 3.9\text{Hz}$ ,  $J_{\delta 1,\delta 2} = 12.7\text{Hz}$ ], 3.61 (dd, 0.77H, Pro $_{\delta 2}^{trans}$ ,  $J_{\gamma,\delta 2} = 3.5\text{Hz}$ ), [3.53, dd, 0.23H, Pro $_{\delta 2}^{cis}$ ,  $J_{\gamma,\delta 2} = 4.5\text{Hz}$ ], [2.75, s, 0.69H, -NHCH<sub>3</sub><sup>*cis*</sup>], 2.71 (s, 2.31H, -NHCH<sub>3</sub><sup>*trans*</sup>), [2.35-2.48, m, 0.23H, Pro $_{\beta 1}^{cis}$ ], 2.29 (ddd, 0.77H, Pro $_{\beta 1}^{trans}$ ,  $J_{\beta 1,\gamma} = 4.4\text{Hz}$ ,  $J_{\beta 1,\beta 2} = 13.7\text{Hz}$ ), [2.17, 0.23H, Pro $_{\beta 2}^{cis}$ ,  $J_{\beta 2,\gamma} = 4.7\text{Hz}$ ,  $J_{\alpha,\beta 2} = 7.9\text{Hz}$ ,  $J_{\beta 1,\beta 2} = 13.7\text{Hz}$ ], 2.09 (s, 2.31H, -COCH<sub>3</sub><sup>*trans*</sup>), 2.17 (0.77H, Pro $_{\beta 2}^{trans}$ ,  $J_{\beta 2,\gamma} = 4.5\text{Hz}$ ), [1.96, s, 0.69H, -COCH<sub>3</sub><sup>*cis*</sup>]; <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O, 298K)  $\delta =$  174.7, (174.6), (174.3), 173.8, 69.9, (68.6), (60.5), 59.3, 56.5, (54.7), (39.6), 38.0, (26.3), 26.2, 21.9, (21.0).

### **4-*O*-(*tert*-Butyl)-*N*-acetyl-*trans*-4-hydroxy-*L*-proline *N'*-methylamide (3)**

The general preparation methods were followed: *C*-terminal amidation of (2*S*,4*R*)-Fmoc-*tert*-butyl-hydroxyproline **11** (0.263 g, 0.642 mmol, 1.0 eq) using TBTU (0.205 g, 0.963 mmol, 1.5

eq), DIPEA (0.336 mL, 1.926 mmol, 3.0 eq), and methylamine hydrochloride (0.119 g, 1.76 mmol, 1.5 eq) in anhydrous DMF (5 mL). *N*-terminal acylation of the crude product was performed using 1:1 DCM:piperidine (6 mL), then 1:1 Ac<sub>2</sub>O/pyridine (6 mL). The reaction mixture was purified by flash chromatography using 40:1 ethyl acetate/methanol to yield **3** as a clear oil (0.050 g, 0.206 mmol) (78.0%):  $[\alpha]_D^{25} = -21.6^\circ$  (*c* 0.9 CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O, 298K)  $\delta =$  [4.55, dd, 0.23H, Pro <sub>$\alpha$</sub> <sup>*cis*</sup>, *J* = 5.4Hz, *J* = 8.4Hz], 4.42-4.52 (m, 0.77H, Pro <sub>$\gamma$</sub> <sup>*trans*</sup>), [4.42-4.48, m, 0.23H, Pro <sub>$\gamma$</sub> <sup>*cis*</sup>], 4.39 (dd, 0.77H, Pro <sub>$\alpha$</sub> <sup>*trans*</sup>, *J* = 6.5Hz, *J* = 8.3Hz), 3.88 (dd, 0.77H, Pro <sub>$\delta_1$</sub> <sup>*trans*</sup>, *J* <sub>$\gamma,\delta_1$</sub>  = 5.8Hz, *J* <sub>$\delta_1,\delta_2$</sub>  = 11.1Hz), [3.68, dd, 0.23H, Pro <sub>$\delta_1$</sub> <sup>*cis*</sup>, *J* <sub>$\gamma,\delta_1$</sub>  = 6.0Hz, *J* <sub>$\delta_1,\delta_2$</sub>  = 12.3Hz], 3.43 (dd, 0.77H, Pro <sub>$\delta_2$</sub> <sup>*trans*</sup>, *J* <sub>$\gamma,\delta_2$</sub>  = 4.6Hz), [3.41, dd, Pro <sub>$\delta_2$</sub> <sup>*cis*</sup>, *J* <sub>$\gamma,\delta_2$</sub>  = 4.8Hz], [2.75, s, 0.69H, -NHCH<sub>3</sub>], 2.70 (s, 2.31H, -NHCH<sub>3</sub>), 2.09-2.37 (m, 2H, Pro <sub>$\beta_1$</sub> <sup>*trans*</sup>, Pro <sub>$\beta_1$</sub> <sup>*cis*</sup>, Pro <sub>$\beta_2$</sub> <sup>*trans*</sup>, Pro <sub>$\beta_2$</sub> <sup>*cis*</sup>), 2.07 (s, 2.31H, -COCH<sub>3</sub><sup>*trans*</sup>), [1.94, s, 0.69H, -COCH<sub>3</sub><sup>*cis*</sup>], 1.21 (s, 6.93H, *tert*-butyl<sup>*trans*</sup>), [1.20, s, 2.07H, *tert*-butyl<sup>*cis*</sup>]; <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O, 298K)  $\delta =$  174.7, (174.5), (174.0), 173.6, 76.6, (76.5), 70.1, (68.9), (60.6), 59.2, 55.4, (53.7), (39.1), 37.6, 27.6, (26.3), 26.2, 21.9, (20.9); HRMS (ES) calc. for C<sub>12</sub>H<sub>22</sub>N<sub>2</sub>NaO<sub>3</sub> (M + Na)<sup>+</sup>: 265.1528. Found (M + Na)<sup>+</sup>: 265.1530.



**Scheme 2.** Synthesis of Galactosylated Hydroxyproline Diamides **4a** and **4b**.

**4-O-(2,3,4,6-Tetra-O-benzyl- $\alpha,\beta$ -D-galactopyranosyl)-N-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline N'-methylamide (7a,b)**

(2S,4R)-Fmoc-hydroxyproline **5** (1.092 g, 3.08 mmol, 1.0 eq) was dissolved in MeCN (10 mL), followed by addition of 2,3,4,6-tetra-O-benzyl-thio- $\beta$ -D-galactopyranoside **9** (2.398 g, 3.70 mmol, 1.2 eq) and BF<sub>3</sub>·OEt (0.389 mL, 3.08 mmol, 1.0 eq). The solution was cooled to 0°C and stirred for ten minutes. Then, AgOTf (0.154 g, 0.617 mmol) and NIS (0.973 g, 4.32 mmol, 1.4 eq) were added, at which point the solution turned red. The mixture was allowed to return to ambient temperature, and stirred for an additional 2.5 hours before being concentrated under reduced pressure. The resulting residue was re-dissolved in ethyl acetate (20 mL). This solution was washed with 1:1 saturated sodium bicarbonate (aq)/saturated sodium thiosulphate (aq) (5 x 20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The product was purified by flash chromatography using 4:4:1 CH<sub>2</sub>Cl<sub>2</sub>/ethyl acetate/methanol to yield a mixture of diastereomers **6a,b** recovered as a white solid (1.284 g, 1.466 mmol) (48.0%).

The general procedure for C-terminal amidation was carried out on the mixture of diastereomers **6a,b** (0.200 g, 0.228 mmol, 1.0 eq) using TBTU (0.109 g, 0.342 mmol, 1.5 eq), DIPEA (0.120 mL, 0.684 mmol, 2.0 eq), and methylamine hydrochloride (0.022 g, 0.342 mmol, 1.0 eq) in anhydrous DMF (5 mL). The products were purified by flash chromatography using 2:1 ethyl acetate/hexanes to yield first **7a** (0.084 g, mmol) and then **7b** (0.088 g, mmol) (87.0%):

**7a**:  $[\alpha]_D^{25} = +14.2^\circ$  (c 2.0 CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 298K)  $\delta = 7.72$ -7.81 (m, 2H, aromatic), 7.47-7.64 (m, 2H, aromatic), 7.18-7.46 (m, 24H, aromatic), 6.31 (broad q, 0.65H, -

$NHCH_3$ ), [5.42, broad q, 0.35H,  $-NHCH_3$ ], 4.96 (d, 1H,  $-OCH_2Ph$ ,  $J = 11.6Hz$ ), 4.91 (broad d, 1H,  $H_1$ ), 4.67-4.87 (m, 3H,  $-OCH_2Ph$ ), 4.62 (d, 1H,  $-OCH_2Ph$ ,  $J = 12.3Hz$ ), 4.58 (d, 1H,  $-OCH_2Ph$ ,  $J = 11.6Hz$ ), 4.11-4.53 (m, 7H,  $-OCH_2Ph$ ,  $H_4$ ,  $H_5$ ,  $H_{6a}$ ,  $H_{6b}$ ,  $Pro_\gamma$ ,  $Pro_\alpha$ ), 4.05 (dd, 1H,  $H_2$ ,  $J = 3.6Hz$ ,  $J = 10.1Hz$ ), 3.94-4.01 (m, 2H,  $-C(O)OCH_2CH$ ), 3.90 (broad dd, 1H,  $H_3$ ), 3.44-3.84 (m, 4H,  $Pro_{\delta 1}$ ,  $Pro_{\delta 2}$ ,  $-C(O)OCH_2CH$ ), 2.73 (d, 2H,  $-NHCH_3^{trans}$ ,  $J = 4.3Hz$ ), [2.58, broad d, 1H,  $-NHCH_3^{cis}$ ], 2.42-2.54 (m, 1H,  $Pro_{\beta 1}$ ), 2.26-2.40 (m, 1H,  $Pro_{\beta 2}$ );  $^{13}C$  NMR (75 MHz,  $CDCl_3$ , 298K)  $\delta = 171.6, 155.9, 143.9, 143.7, 141.3, 138.8, 138.6, 137.9, 127.0-128.6$  (aromatic carbons), 125.1, 120.0, 97.5, 78.8, 76.4, 75.0, 74.8, 73.6, 73.3, 73.1, 70.0, 69.5, 67.7, 67.3, 59.4, 51.8, 47.2, 35.0, 26.3; HRMS (ES) calc. for  $C_{55}H_{56}N_2NaO_9$  ( $M + Na$ ) $^+$ : 911.3884. Found ( $M + Na$ ) $^+$ : 911.3887.

**7b**:  $[\alpha]_D^{25} = -23.7^\circ$  ( $c$  1.4  $CHCl_3$ );  $^1H$  NMR (300 MHz,  $CDCl_3$ , 298K)  $\delta = 7.70-7.81$  (m, 2H, aromatic), 7.47-7.64 (m, 2H, aromatic), 7.14-7.45 (m, 24H, aromatic), 6.28 (broad q, 0.75H,  $-NHCH_3$ ), [5.37, broad q, 0.25H,  $-NHCH_3$ ], 4.95 (d, 1H,  $-OCH_2Ph$ ,  $J = 11.7Hz$ ), 4.68-4.86 (m, apparent d, 4H,  $-OCH_2Ph$ ), 4.58 (d, 1H,  $-OCH_2Ph$ ,  $J = 11.7Hz$ ), 4.50-4.58 (m, 1H,  $Pro_\gamma$ ), 3.98-4.50 (m, 7H,  $-OCH_2Ph$ ,  $H_1$ ,  $H_5$ ,  $H_{6a}$ ,  $H_{6b}$ ,  $Pro_\alpha$ ), 3.87-4.96 (m, 1H,  $-C(O)OCH_2CH$ ), 3.73-3.86 (m, 2H,  $H_3$ ,  $Pro_{\delta 1}$ ), 3.45-3.72 (m, 5H,  $H_2$ ,  $H_4$ ,  $Pro_{\delta 2}$ ,  $-C(O)OCH_2CH$ ), 2.75 (d, 2.25H,  $-NHCH_3^{trans}$ ,  $J = 4.7Hz$ ), [2.61, broad d, 0.75H,  $-NHCH_3^{cis}$ ], 2.41-2.55 (m, 1H,  $Pro_{\beta 1}$ ), 2.08-2.30 (m, 1H,  $Pro_{\beta 2}$ );  $^{13}C$  NMR (75 MHz,  $CDCl_3$ , 298K)  $\delta = 171.5, 156.1, 143.8, 143.7, 141.3, 138.7, 138.5, 138.4, 137.8, 127.4-128.6$  (aromatic carbons), 127.2, 125.2, 120.0, 102.4, 82.2, 79.2, 75.2, 74.7, 73.5, 73.4, 73.3, 72.9, 68.6, 67.8, 58.9, 52.9, 47.1, 33.8, 26.4; HRMS (ES) calc. for  $C_{55}H_{56}N_2NaO_9$  ( $M + Na$ ) $^+$ : 911.3884. Found ( $M + Na$ ) $^+$ : 911.3880.

**4-*O*-(2,3,4,6-Tetra-*O*-benzyl- $\alpha,\beta$ -D-galactopyranosyl)-*N*-acetyl-*trans*-4-hydroxy-*L*-proline  
*N'*-methanamide (**8a,b**)**

The general preparation method was followed for *N*-terminal acylation of **7a** (0.080 g, 0.090 mmol) using 1:1 DCM:piperidine (4 mL), then 1:1 Ac<sub>2</sub>O/pyridine (4 mL). The reaction mixture was purified by flash chromatography using 10:1 ethyl acetate/methanol to yield **8a** as a clear oil (0.050 g, 0.071 mmol) (78.0%) (a similar procedure was used for **7b**):

**8a**:  $[\alpha]_D^{25} = +0.84^\circ$  (*c* 1.0 CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 298K)  $\delta = 7.22-7.40$  (m, 20H), 6.76 (broad q, 1H, -NHCH<sub>3</sub>, *J* = 4.6Hz), 4.94 (d, 1H, -OCH<sub>2</sub>Ph, *J* = 11.4Hz), 4.71-4.88 (m, 4H, H<sub>1</sub>, -OCH<sub>2</sub>Ph), 4.52-4.67 (m, 3H, Pro <sub>$\gamma$</sub> , -OCH<sub>2</sub>Ph), 4.35-4.52 (m, 3H, Pro <sub>$\alpha$</sub> , -OCH<sub>2</sub>Ph), 4.04 (dd, 1H, H<sub>2</sub>, *J* = 3.6Hz, *J* = 10.1Hz), 3.93-4.00 (m, 2H, H<sub>4</sub>, H<sub>5</sub>), 3.88 (dd, 1H, H<sub>3</sub>, *J* = 2.2Hz, *J* = 10.1Hz), 3.65 (dd, 1H, Pro <sub>$\delta 1$</sub> , *J* = 5.5Hz, *J* = 10.5Hz), 3.56-3.45 (m, 3H, H<sub>6a</sub>, H<sub>6b</sub>, Pro <sub>$\delta 2$</sub> ), 2.72 (d, 3H, -NHCH<sub>3</sub>, *J* = 4.6Hz), 2.58-2.62 (m, 1H, Pro <sub>$\beta 1$</sub> ), 2.11-2.15 (m, 1H, Pro <sub>$\beta 2$</sub> ), 1.99 (s, 3H, -COCH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>, 298K)  $\delta = 171.3, 170.8, 138.6, 138.5$  (2), 137.9, 127.3-128.6 (aromatic carbons), 98.1, 78.9, 76.9, 76.4, 74.9, 74.8, 73.8, 73.6, 72.9, 70.1, 69.2, 58.4, 53.2, 33.9, 26.2, 22.5; HRMS (ES) calc. for C<sub>42</sub>H<sub>48</sub>N<sub>2</sub>NaO<sub>8</sub> (M + Na)<sup>+</sup>: 731.3308. Found (M + Na)<sup>+</sup>: 731.3312.

**8b**:  $[\alpha]_D^{25} = -0.11^\circ$  (*c* 1.0 CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 298K)  $\delta = 7.20-7.40$  (m, 20H), 6.89 (broad q, 1H, -NHCH<sub>3</sub>, *J* = 4.7Hz), 4.94 (d, 1H, -OCH<sub>2</sub>Ph, *J* = 11.7Hz), 4.67-4.84 (m, 4H, -OCH<sub>2</sub>Ph), 4.62 (d, 1H, -OCH<sub>2</sub>Ph, *J* = 11.6Hz), 4.48-4.58 (m, 2H, Pro <sub>$\alpha$</sub> , Pro <sub>$\gamma$</sub> ), 4.36-4.47 (m, 3H, H<sub>1</sub>, -OCH<sub>2</sub>Ph), 3.87 (dd apparent d, 1H, H<sub>4</sub>, *J* = 3.0Hz, *J* = 3.0Hz), 3.77 (dd, 1H, H<sub>2</sub>, *J* = 7.8Hz, *J*

= 9.7Hz), 3.60-3.71 (m, 2H, Pro $_{\delta 1}$ , Pro $_{\delta 2}$ ), 3.47-3.60 (m, 4H, H $_3$ , H $_5$ , H $_{6a}$ , H $_{6b}$ ), 2.74 (d, 3H, -NHCH $_3$ , J = 4.6Hz), 2.57-2.68 (m, 1H, Pro $_{\beta 1}$ ), 1.97-2.08 (m, 1H, Pro $_{\beta 2}$ ), 2.04 (s, 3H, -COCH $_3$ );  $^{13}\text{C}$  NMR (75 MHz, CDCl $_3$ , 298K)  $\delta$  = 171.3, 170.9, 138.6, 138.5, 138.4, 137.8, 127.3-128.7 (aromatic carbons), 103.1, 82.2, 79.2, 77.5, 75.2, 74.6, 73.5 (2), 73.4, 73.1, 68.9, 57.7, 54.0, 33.0, 26.2, 22.5; HRMS (ES) calc. for C $_{42}\text{H}_{48}\text{N}_2\text{NaO}_8$  (M + Na) $^+$ : 731.3308. Found (M + Na) $^+$ : 731.3309.

#### 4-O-( $\alpha,\beta$ -D-Galactopyranosyl)-N-acetyl-*trans*-4-hydroxy-L-proline N'-methylamide (4a,b)

Compound **8a** (0.050 g, 0.048 mmol, 1.0 eq) was dissolved in 4:1 methanol/ethyl acetate (5 mL). After addition of Pearlman's catalyst (20% palladium hydroxide on carbon) (0.050 g, approx. 0.047 mmol, 1.0 eq), the reaction flask was flushed with N $_2$  for 5 minutes. The reaction mixture was then stirred vigorously under hydrogen atmosphere (10 psi) for 4 hours, after which it was flushed with nitrogen and filtered. The product was then concentrated under reduced pressure to provide **4a** as a clear oil (0.024 g, 0.069 mmol) (quant.) (a similar procedure was used for **8b**):

**4a**:  $[\alpha]_D^{25} = +82.0^\circ$  (c 0.3 CH $_3$ OH);  $^1\text{H}$  NMR (500 MHz, D $_2\text{O}$ , 298K)  $\delta$  = 4.93 (d, 0.8H, H $_1^{trans}$ , J = 3.2Hz), [4.89, d, 0.2H, H $_1^{cis}$ , J = 3.3Hz], [4.52, dd apparent t, 0.2H, Pro $_{\alpha}^{cis}$ , J = 7.7Hz, J = 7.9Hz], 4.38-4.42 (m, 0.8H, Pro $_{\gamma}^{trans}$ ), [4.33-4.37, m, 0.2H, Pro $_{\gamma}^{cis}$ ], 4.32 (dd apparent t, 0.8H, Pro $_{\alpha}^{trans}$ , J = 8.3Hz, J = 8.4Hz), 3.80-3.86 (m, 2H, H $_4^{trans}$ , H $_4^{cis}$ , H $_5^{trans}$ , H $_5^{cis}$ ), [3.72-3.77, m, 0.2H, Pro $_{\delta 1}^{cis}$ ], 3.63-2.71 (m, 3.6H, H $_2^{trans}$ , H $_2^{cis}$ , H $_3^{trans}$ , H $_3^{cis}$ , Pro $_{\delta 1}^{trans}$ , Pro $_{\delta 2}^{trans}$ ), 3.57-3.63 (m, 2H, H $_{6a}^{trans}$ , H $_{6a}^{cis}$ , H $_{6b}^{trans}$ , H $_{6b}^{cis}$ ), [3.45, dd, 0.2H, Pro $_{\delta 2}^{cis}$ , J = 4.4Hz, J = 12.8Hz], [2.64, s, 0.6H, -NHCH $_3^{cis}$ ], 2.60 (s, 2.4H, -NHCH $_3^{trans}$ ), [2.44-2.53, m, 0.2H, Pro $_{\beta 1}^{cis}$ ], 2.39 (ddd, 0.8H,

Pro $\beta_1$ <sup>trans</sup>, J = 2.5Hz, J = 8.2Hz, J = 13.4Hz), [2.14, ddd, 0.2H, Pro $\beta_2$ <sup>cis</sup>, J = 5.5Hz, J = 7.3Hz, J = 13.4Hz], 1.94-2.04 (m, 0.8H, Pro $\beta_2$ <sup>trans</sup>), 1.99 (s, 2.4H, -COCH<sub>3</sub><sup>trans</sup>), [1.85, s, 0.6H, -COCH<sub>3</sub><sup>cis</sup>]; <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O, 298K)  $\delta$  = 174.7, (174.6), (174.3), 173.8, 98.3, (98.1), 76.5, (74.8), 71.87, (71.85), 69.65, (69.62), 68.44, (68.38), 61.7, (60.8), 59.6, 53.9, (52.0), (38.2), 36.5, (26.3), 26.2, 21.9, (21.1); HRMS (ES) calc. for C<sub>14</sub>H<sub>24</sub>N<sub>2</sub>NaO<sub>8</sub> (M + Na)<sup>+</sup>: 371.1430. Found (M + Na)<sup>+</sup>: 371.1438.

**4b**: [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -22.5° (c 0.7 CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O, 298K)  $\delta$  = 4.62-4.67 (m, 0.8H, Pro $\gamma$ <sup>trans</sup>), [4.60, dd apparent t, 0.2H, Pro $\alpha$ <sup>cis</sup>, J = 7.9Hz, J = 8.1Hz], [4.57-4.61, m, Pro $\gamma$ <sup>cis</sup>], 4.48 (d, 0.8H, H<sub>1</sub><sup>trans</sup>, J = 8.1Hz), [4.46, d, 0.2H, H<sub>1</sub><sup>cis</sup>, J = 8.1Hz], 4.40 (dd apparent t, 0.8H, Pro $\alpha$ <sup>trans</sup>, J = 8.3Hz, J = 8.3Hz), [3.94-3.98, m, 0.2H, Pro $\delta_1$ <sup>cis</sup>], 3.89-3.94 (m, 1H, H<sub>4</sub><sup>trans</sup>, H<sub>4</sub><sup>cis</sup>), 3.79-3.89 (m, 1.6H, Pro $\delta_1$ <sup>trans</sup>, Pro $\delta_2$ <sup>trans</sup>), 3.66-3.78 (m, 3H, H<sub>5</sub><sup>trans</sup>, H<sub>5</sub><sup>cis</sup>, H<sub>6a</sub><sup>trans</sup>, H<sub>6a</sub><sup>cis</sup>, H<sub>6b</sub><sup>trans</sup>, H<sub>6b</sub><sup>cis</sup>), 3.63 (dd, 0.8H, H<sub>3</sub><sup>trans</sup>, J = 3.1Hz, J = 10.0Hz), [3.61-3.65, m, 0.2H, H<sub>3</sub><sup>cis</sup>], [3.58, dd, 0.2H, Pro $\delta_2$ <sup>cis</sup>, J = 4.3Hz, J = 12.9Hz], 3.46 (dd, 0.8H, H<sub>2</sub><sup>trans</sup>, J = 7.9Hz, J = 10.0Hz), [3.43-3.48, m, 0.2H, H<sub>2</sub><sup>cis</sup>], [2.77, s, 0.6H, -NHCH<sub>3</sub><sup>cis</sup>], 2.72 (s, 2.4H, -NHCH<sub>3</sub><sup>trans</sup>), [2.55-2.62, m, 0.2H, Pro $\beta_1$ <sup>cis</sup>], 2.44-2.51 (m, 0.8H, Pro $\beta_1$ <sup>trans</sup>), [2.18-2.25, m, 0.2H, Pro $\beta_2$ <sup>cis</sup>], 2.11 (s, 2.4H, -COCH<sub>3</sub><sup>trans</sup>), 2.03-2.10 (m, 0.6H, Pro $\beta_2$ <sup>trans</sup>), [1.96, s, 0.6H, -COCH<sub>3</sub><sup>cis</sup>]; <sup>13</sup>C NMR (75 MHz, D<sub>2</sub>O, 298K)  $\delta$  = 174.6, (174.5), (174.3), 173.7, (102.1), 102.0, 77.9, (76.7), (75.7), 75.6, (73.04), 73.02, (70.93), 70.90, 68.9, 61.3, (60.4), 59.2, 55.0, (53.2), (49.2), (37.5), 35.7, (26.3), 26.2, 21.9, (21.0); HRMS (ES) calc. for C<sub>14</sub>H<sub>24</sub>N<sub>2</sub> NaO<sub>8</sub> (M + Na)<sup>+</sup>: 371.1430. Found (M + Na)<sup>+</sup>: 371.1432.



**Table 3. Amide Isomer Equilibrium ( $K_{t/c}$ ) of 2-4b at Various Temperatures<sup>[a]</sup>**

Compound	Temperature (°C) <sup>[b]</sup>										
	24.8	30.0	37.0	46.0	51.3	56.6	62.0	67.3	72.6	78.0	83.3
<b>2</b>	3.89 ±0.11	3.73 ±0.09	3.52 ±0.05	3.39 ±0.13	3.29 ±0.11	3.22 ±0.09	3.16 ±0.09	2.94 ±0.08	2.87 ±0.08	2.74 ±0.08	2.70 ±0.04
<b>3</b>	3.66 ±0.15	3.50 ±0.07	3.34 ±0.15	3.15 ±0.20	3.03 ±0.17	2.96 ±0.18	2.85 ±0.2	2.77 ±0.01	2.72 ±0.05	2.65 ±0.03	2.55 ±0.07
<b>4a</b>	3.74 ±0.20	3.62 ±0.46	3.41 ±0.30	3.24 ±0.31	3.22 ±0.22	3.02 ±0.35	2.96 ±0.21	2.83 ±0.17	2.77 ±0.2	2.74 ±0.13	2.62 ±0.57
<b>4b</b>	3.71 ±0.21	3.62 ±0.21	3.37 ±0.28	3.14 ±0.25	3.07 ±0.17	2.99 ±0.17	2.91 ±0.25	2.79 ±0.19	2.72 ±0.18	2.66 ±0.18	2.61 ±0.11

<sup>[a]</sup>Carried out in D<sub>2</sub>O; ± SE determined by integration of two or more sets of *trans/cis* isomers; <sup>[b]</sup>Calibrated using an ethylene glycol standard;

**Table 4. Amide Isomer Equilibrium ( $K_{t/c}$ ) of 1 at Various Temperatures<sup>[a]</sup>**

Compound	Temperature (°C) <sup>[b]</sup>							
	3.5	14.2	24.8	37.0	46.0	56.6	72.6	83.3
<b>1</b>	3.61 ±0.11	3.39 ±0.11	3.19 ±0.06	3.03 ±0.08	2.87 ±0.11	2.73 ±0.11	2.56 ±0.11	2.45 ±0.11

<sup>[a]</sup>Carried out in D<sub>2</sub>O; <sup>[b]</sup>Calibrated using an ethylene glycol standard;

**Table 5. Thermodynamic Parameters for Amide Isomerization of 1-4b<sup>[a]</sup>**

Compound	$\Delta H^\circ$ (kcal/mol)	$\Delta S^\circ$ (cal/mol·K)
<b>1</b>	$-0.95 \pm 0.01$	$-0.87 \pm 0.02$
<b>2</b>	$-1.33 \pm 0.03$	$-1.76 \pm 0.09$
<b>3</b>	$-1.29 \pm 0.02$	$-1.72 \pm 0.06$
<b>4a</b>	$-1.27 \pm 0.02$	$-1.64 \pm 0.07$
<b>4b</b>	$-1.30 \pm 0.03$	$-1.77 \pm 0.10$

<sup>[a]</sup>Carried out in D<sub>2</sub>O; Error limits obtained by linear least-squares fitting the data in Figures 1-4 to equation  $\ln K_{ct} = (-\Delta H^\circ/R)(1/T) + \Delta S^\circ/R$ .

**Table 6. Rate of Prolyl Amide Isomerization of Compounds 1-4b at Various Temperatures**

Compound	Rate <sup>[a]</sup> (s <sup>-1</sup> )	Temperature (°C) <sup>[b]</sup>					
		62.0	67.3	72.6	78.0	83.3	37.0 <sup>[e]</sup>
<b>1</b>	$k_{ct}$	$0.461 \pm 0.012$	$0.810 \pm 0.022$	$1.262 \pm 0.035$	$2.000 \pm 0.071$	$3.171 \pm 0.081$	$0.039 \pm 0.024$
	$k_{tc}^{[d]}$	$0.180 \pm 0.009$	$0.312 \pm 0.015$	$0.507 \pm 0.022$	$0.815 \pm 0.046$	$1.314 \pm 0.053$	$0.013 \pm 0.010$
<b>2</b>	$k_{ct}$	$0.455 \pm 0.017$	$0.725 \pm 0.014$	$1.149 \pm 0.022$	$1.852 \pm 0.048$	$2.941 \pm 0.097$	$0.039 \pm 0.005$
	$k_{tc}^{[d]}$	$0.147 \pm 0.007$	$0.247 \pm 0.008$	$0.398 \pm 0.014$	$0.634 \pm 0.024$	$1.053 \pm 0.038$	$0.011 \pm 0.001$
<b>3</b>	$k_{ct}$	$0.455 \pm 0.024$	$0.769 \pm 0.022$	$1.395 \pm 0.029$	$2.071 \pm 0.036$	$3.414 \pm 0.054$	$0.032 \pm 0.009$
	$k_{tc}^{[d]}$	$0.163 \pm 0.009$	$0.272 \pm 0.008$	$0.532 \pm 0.015$	$0.792 \pm 0.016$	$1.302 \pm 0.040$	$0.010 \pm 0.004$
<b>4a</b>	$k_{ct}$	$0.476 \pm 0.042$	$0.833 \pm 0.047$	$1.428 \pm 0.089$	$2.149 \pm 0.075$	$3.124 \pm 0.077$	$0.043 \pm 0.015$
	$k_{tc}^{[d]}$	$0.150 \pm 0.016$	$0.271 \pm 0.021$	$0.429 \pm 0.047$	$0.728 \pm 0.043$	$0.988 \pm 0.181$	$0.013 \pm 0.005$
<b>4a<sup>[c]</sup></b>	$k_{ct}$		$0.820 \pm 0.059$				
	$k_{tc}^{[d]}$		$0.274 \pm 0.027$				
<b>4b</b>	$k_{ct}$	$0.385 \pm 0.038$	$0.606 \pm 0.037$	$1.064 \pm 0.075$	$1.682 \pm 0.053$	$3.071 \pm 0.066$	$0.022 \pm 0.007$
	$k_{tc}^{[d]}$	$0.127 \pm 0.021$	$0.207 \pm 0.018$	$0.350 \pm 0.032$	$0.578 \pm 0.040$	$1.113 \pm 0.051$	$0.007 \pm 0.004$
<b>4b<sup>[b]</sup></b>	$k_{ct}$		$0.571 \pm 0.052$				
	$k_{tc}^{[d]}$		$0.189 \pm 0.022$				

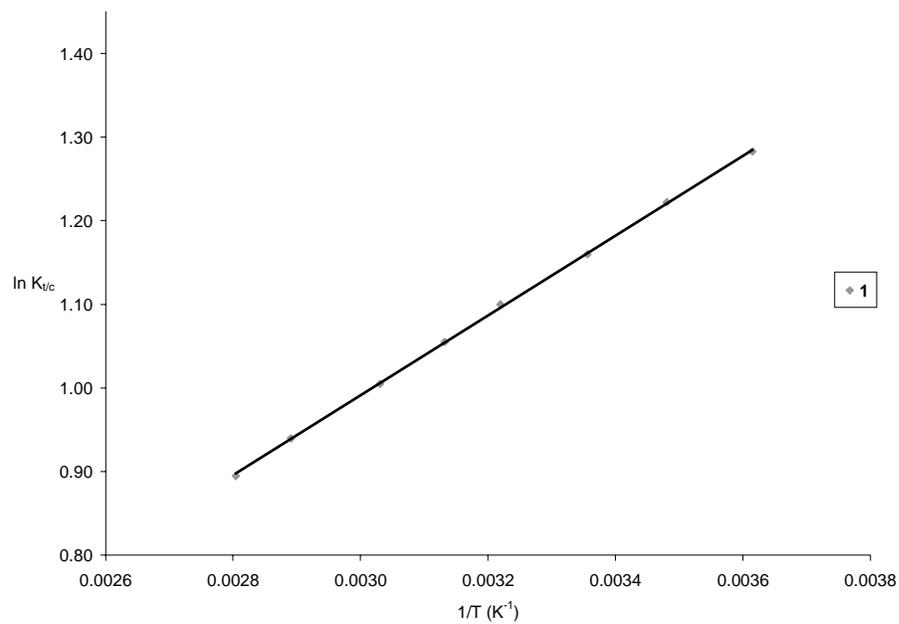
<sup>[a]</sup>Carried out in D<sub>2</sub>O. Error values obtained using the linear least-squares fit of the data in Figures 14-40; <sup>[b]</sup>Calibrated using an ethylene glycol standard; <sup>[c]</sup>Carried out in phosphate buffer pH = 7.4; <sup>[d]</sup>Calculated from  $k_{ct}$  and equilibrium ( $K_{t/c}$ ) at respective temperatures as described in the text; <sup>[e]</sup>Rate  $\pm$ SE calculated using equation  $\ln(k/T) = (-\Delta H^\ddagger/R)(1/T) + \Delta S^\ddagger/R + \ln(k_B/h)$  and values from Table 7.

**Table 7. Activation Enthalpy ( $\Delta H^\ddagger$ ) and Entropy ( $\Delta S^\ddagger$ ) of Isomerization of 1-4b<sup>[a]</sup>**

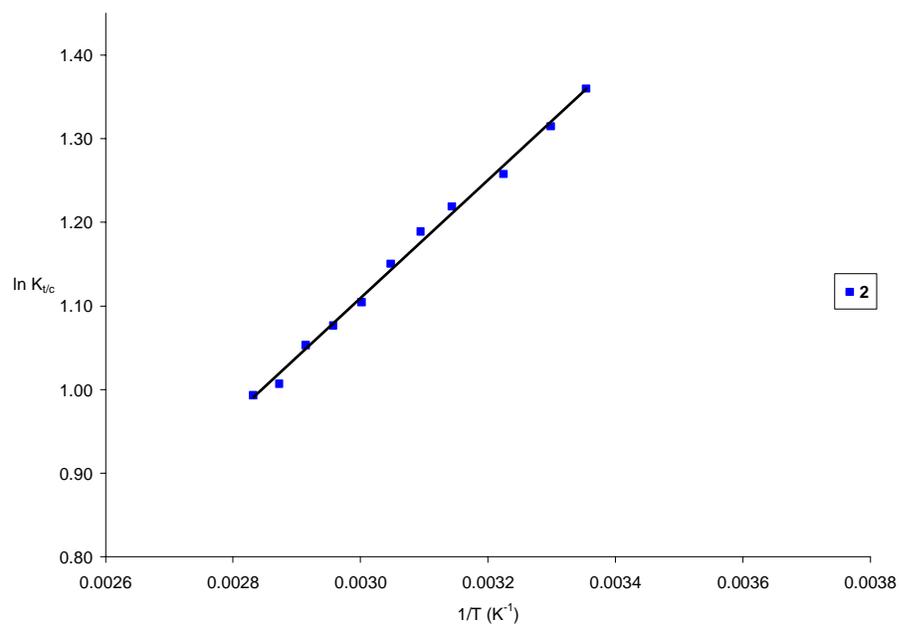
Compound	<i>cis to trans</i>			<i>trans to cis</i>		
	$\Delta H^\ddagger$ (Kcal/mol)	$\Delta S^\ddagger$ (cal/mol·K)	$\Delta G^\ddagger_{300K}$ (Kcal/mol)	$\Delta H^\ddagger$ (Kcal/mol)	$\Delta S^\ddagger$ (cal/mol·K)	$\Delta G^\ddagger_{300K}$ (Kcal/mol)
<b>1</b>	20.6 ±0.4	1.2 ±0.1	20.2 ±0.4	21.3 ±0.2	1.4 ±0.1	20.9 ±0.2
<b>2</b>	20.2 ±0.3	0.08 ±0.01	20.2 ±0.3	21.1 ±0.3	0.54 ±0.05	21.0 ±0.3
<b>3</b>	21.8 ±0.7	4.7 ±0.2	20.4 ±0.7	22.7 ±0.9	5.4 ±0.3	21.1 ±0.9
<b>4a</b>	20.4 ±0.9	0.60 ±0.03	20.2 ±0.9	20.6 ±1.0	1.0 ±0.1	20.3 ±1.0
<b>4b</b>	22.4 ±0.8	6.2 ±0.3	20.6 ±0.8	23.3 ±1.4	6.6 ±0.4	21.3 ±1.0

<sup>[a]</sup>Error values obtained using the linear least-squares fit of the data in Figures 6-10 to equation  $\ln(k/T) = (-\Delta H^\ddagger/R)(1/T) + \Delta S^\ddagger/R + \ln(k_B/h)$ ; Additionally the free energy of activation at 300 K ( $\Delta G^\ddagger_{300K}$ ) is given.

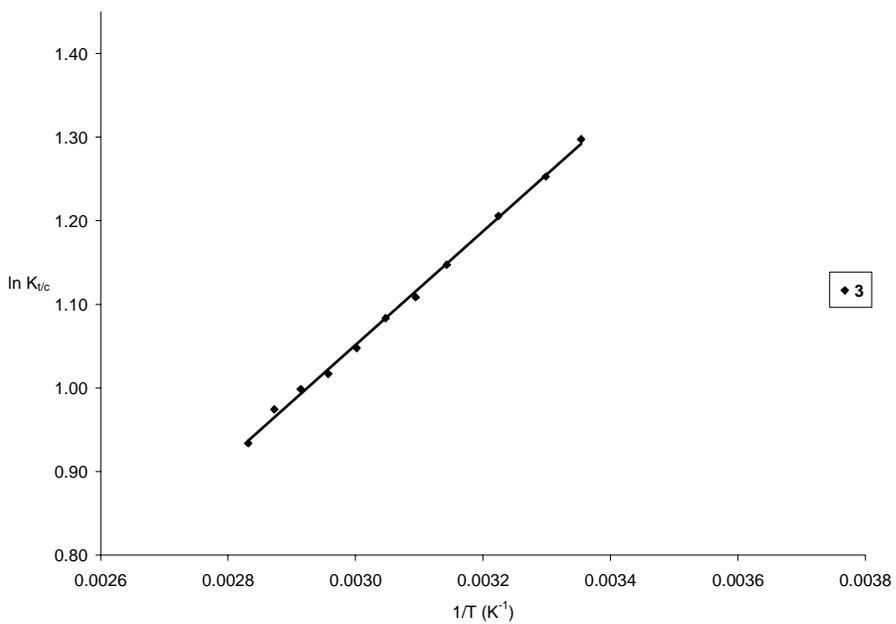
**Figure 1. Van't Hoff Plot for the *cis*-to-*trans* Isomerization of 1 in D<sub>2</sub>O**



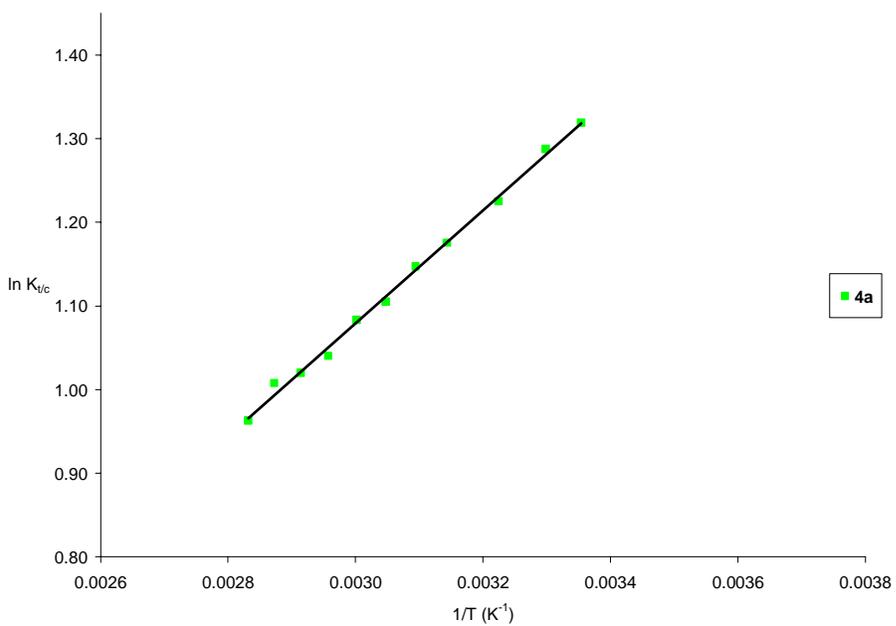
**Figure 2. Van't Hoff Plot for the *cis*-to-*trans* Isomerization of 2 in D<sub>2</sub>O**



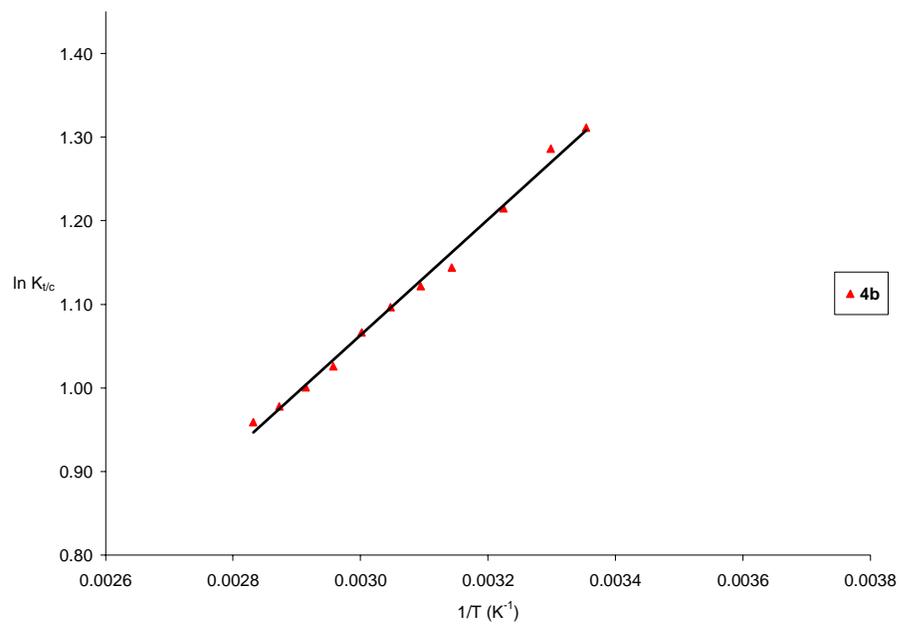
**Figure 2. Van't Hoff Plot for the *cis*-to-*trans* Isomerization of 3 in D<sub>2</sub>O**



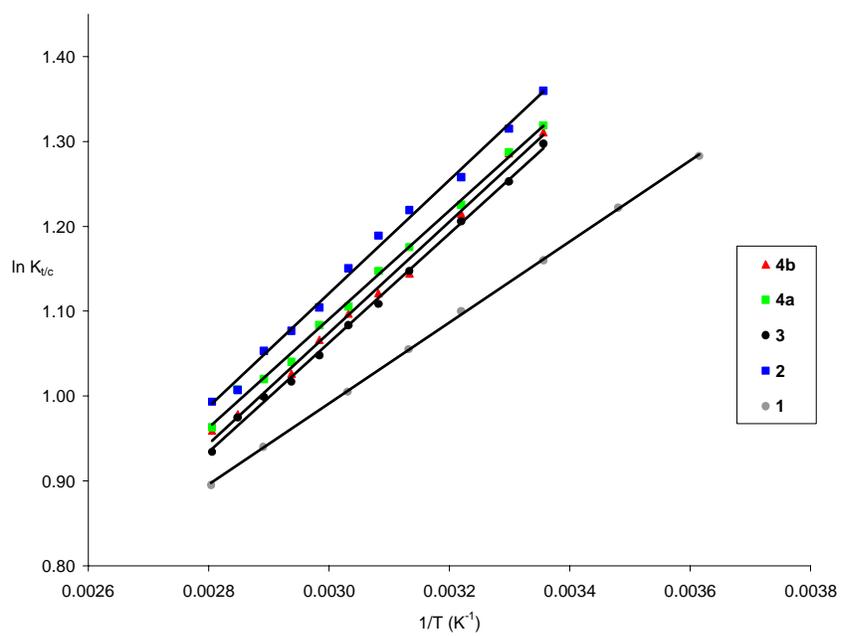
**Figure 3. Van't Hoff Plot for the *cis*-to-*trans* Isomerization of 4a in D<sub>2</sub>O**



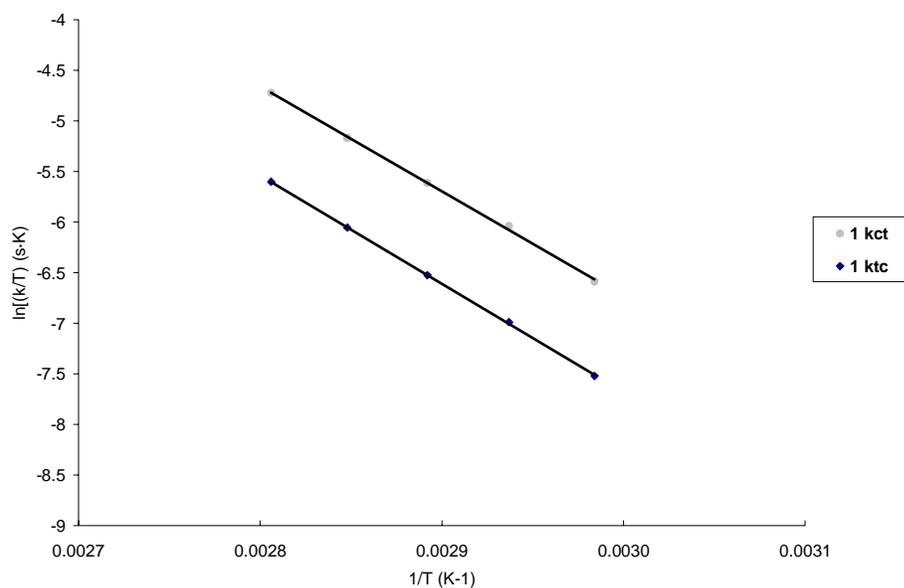
**Figure 4. Van't Hoff Plot for the *cis*-to-*trans* Isomerization of 4b in D<sub>2</sub>O**



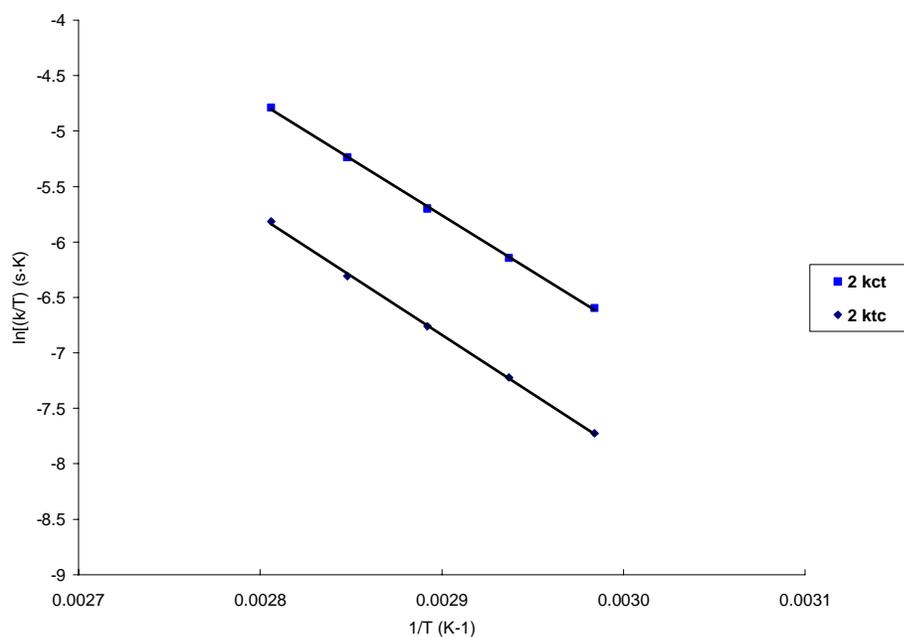
**Figure 5. Comparison of Van't Hoff Plots for 1-4b in D<sub>2</sub>O**



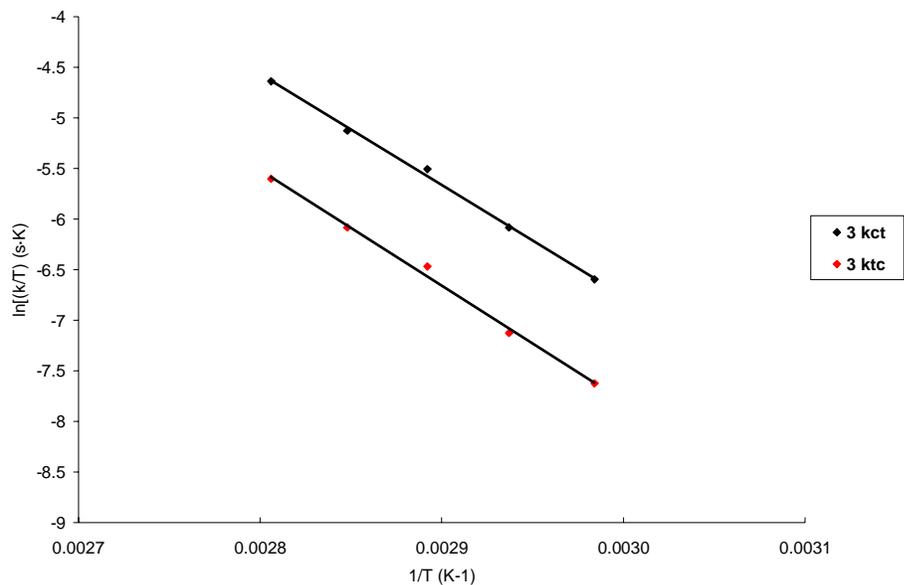
**Figure 6. Eyring Plot for the *cis-to-trans* Isomerization ( $k_{ct}$ ) and *trans-to-cis* Isomerization ( $k_{tc}$ ) for 1 in D<sub>2</sub>O**



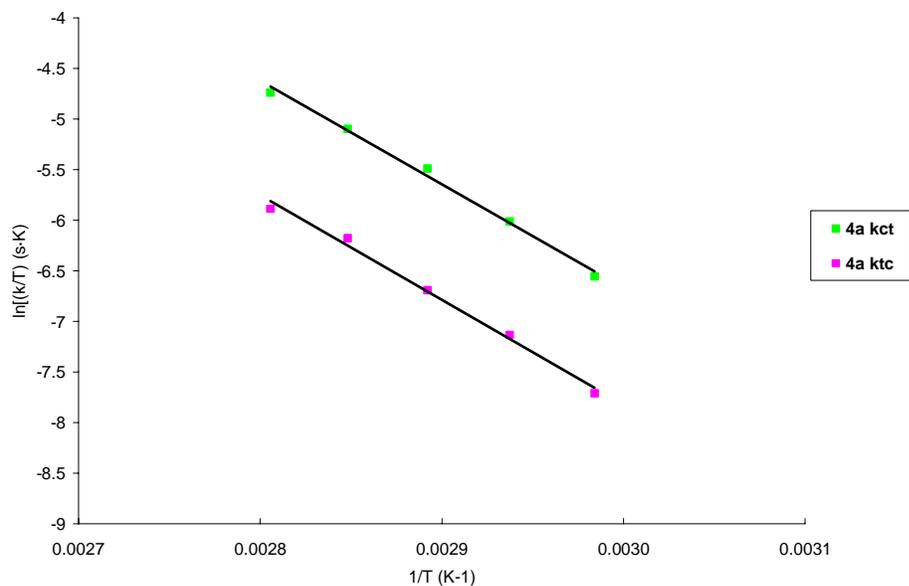
**Figure 7. Eyring Plot for the *cis-to-trans* Isomerization ( $k_{ct}$ ) and *trans-to-cis* Isomerization ( $k_{tc}$ ) for 2 in D<sub>2</sub>O**



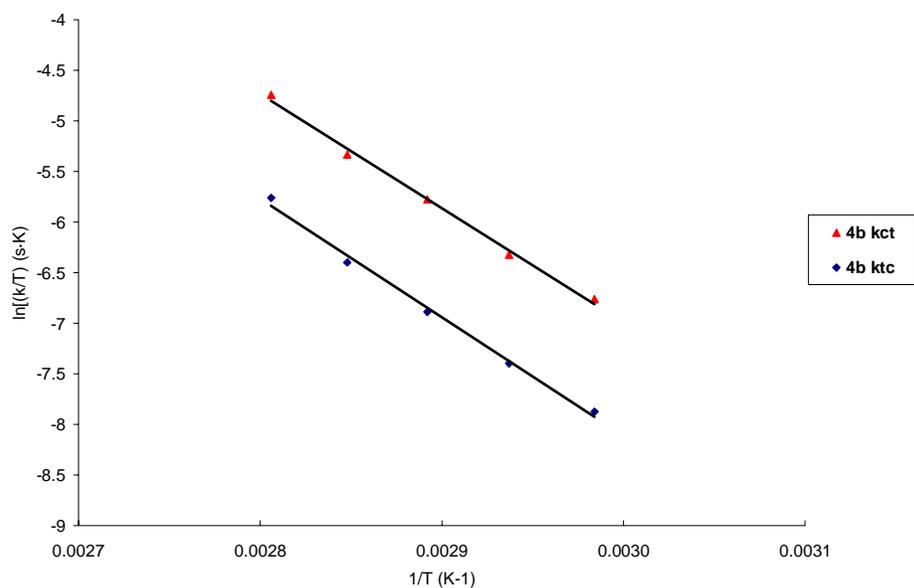
**Figure 8. Eyring Plot for the *cis*-to-*trans* Isomerization ( $k_{ct}$ ) and *trans*-to-*cis* Isomerization ( $k_{tc}$ ) for 3 in D<sub>2</sub>O**



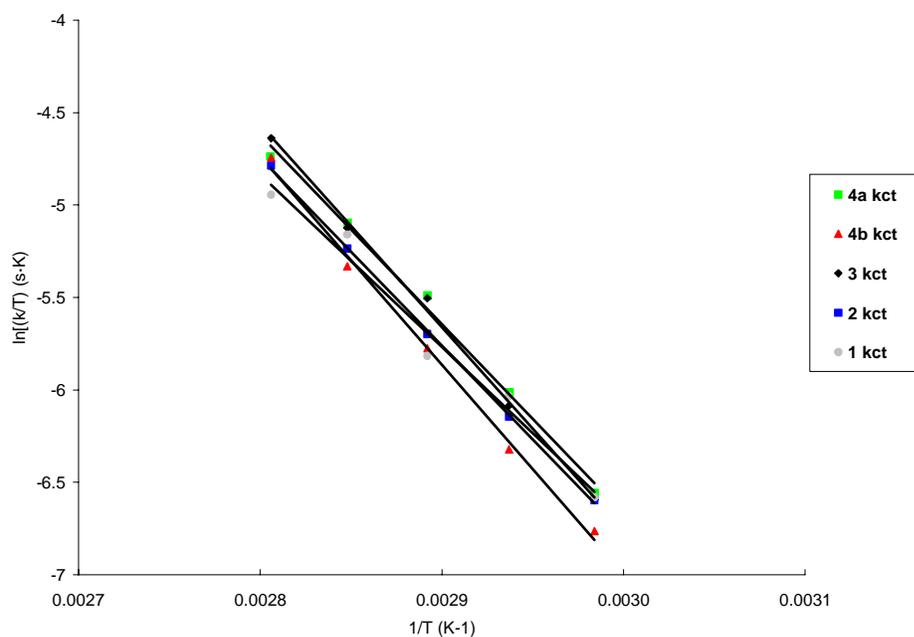
**Figure 9. Eyring Plot for the *cis*-to-*trans* Isomerization ( $k_{ct}$ ) and *trans*-to-*cis* Isomerization ( $k_{tc}$ ) for 4a in D<sub>2</sub>O**



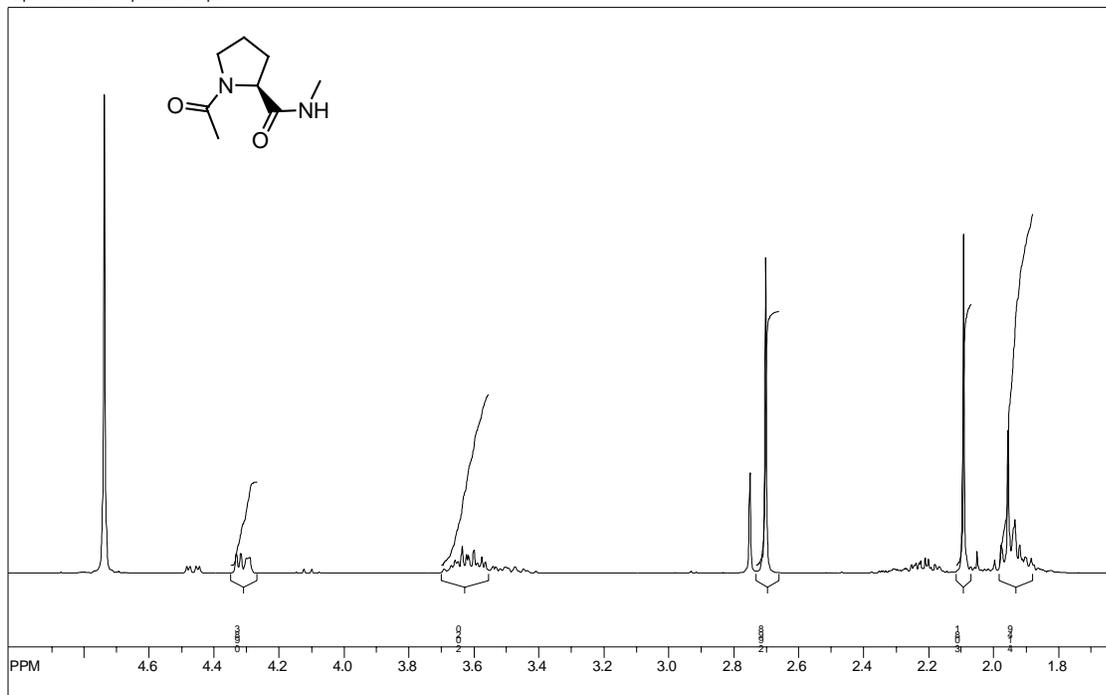
**Figure 10. Eyring Plot for the *cis*-to-*trans* Isomerization ( $k_{ct}$ ) and *trans*-to-*cis* Isomerization ( $k_{tc}$ ) for 4b in D<sub>2</sub>O**



**Figure 11. Comparison of Eyring Plot of *cis*-to-*trans* isomerization for 1-4b**

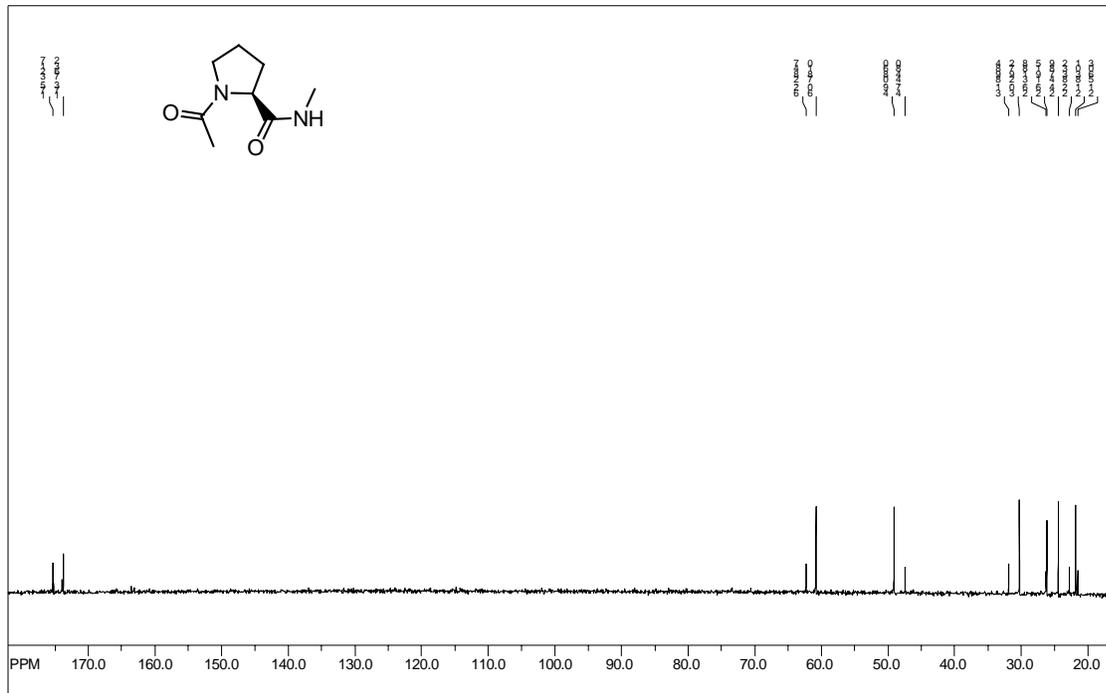


SpinWorks 2.5: repurified sample



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number of scans: 16

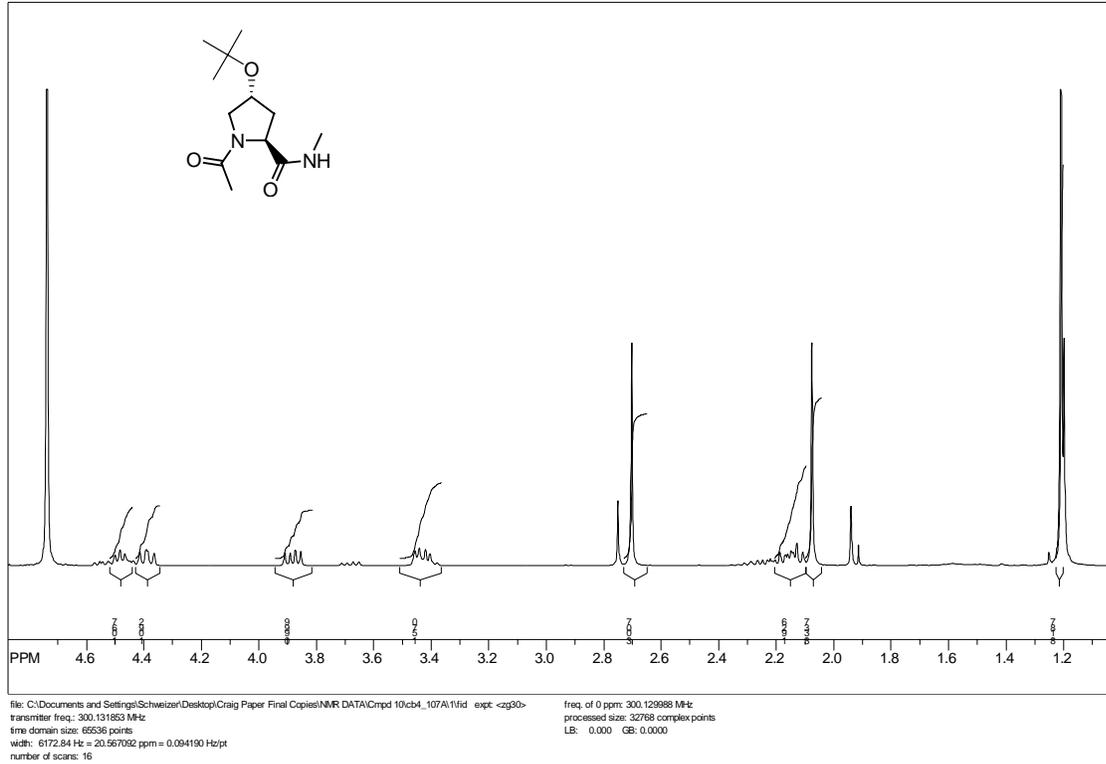
SpinWorks 2.5: more scans c13



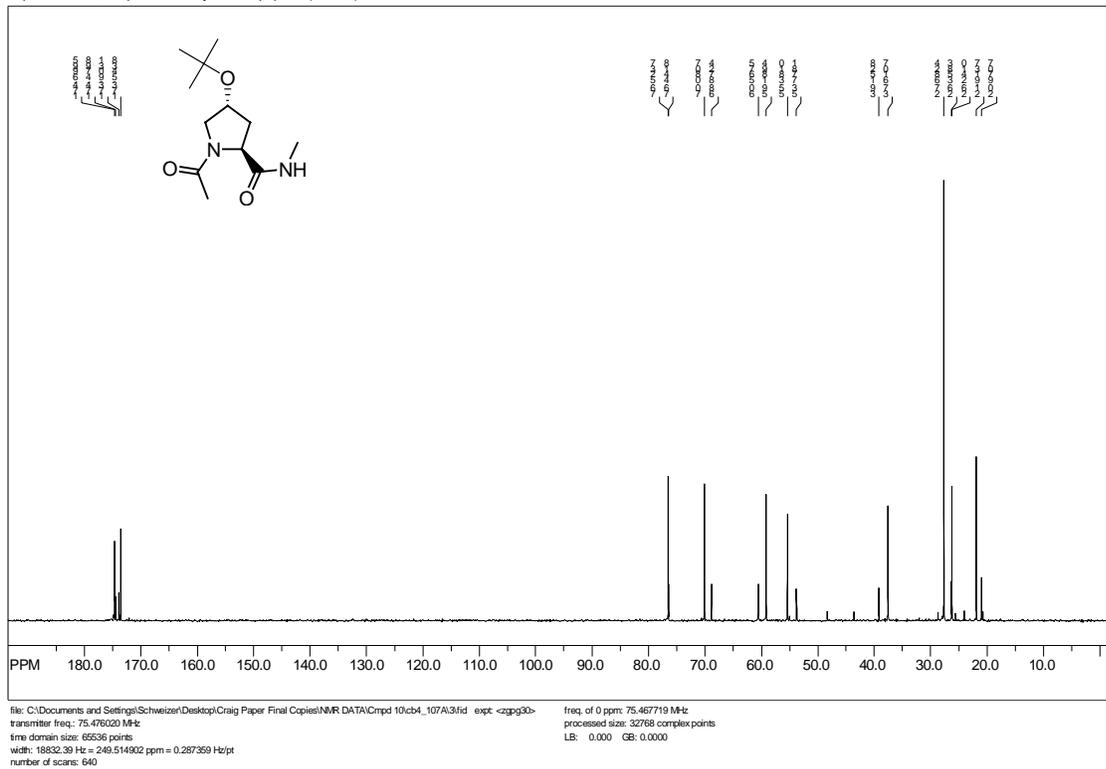
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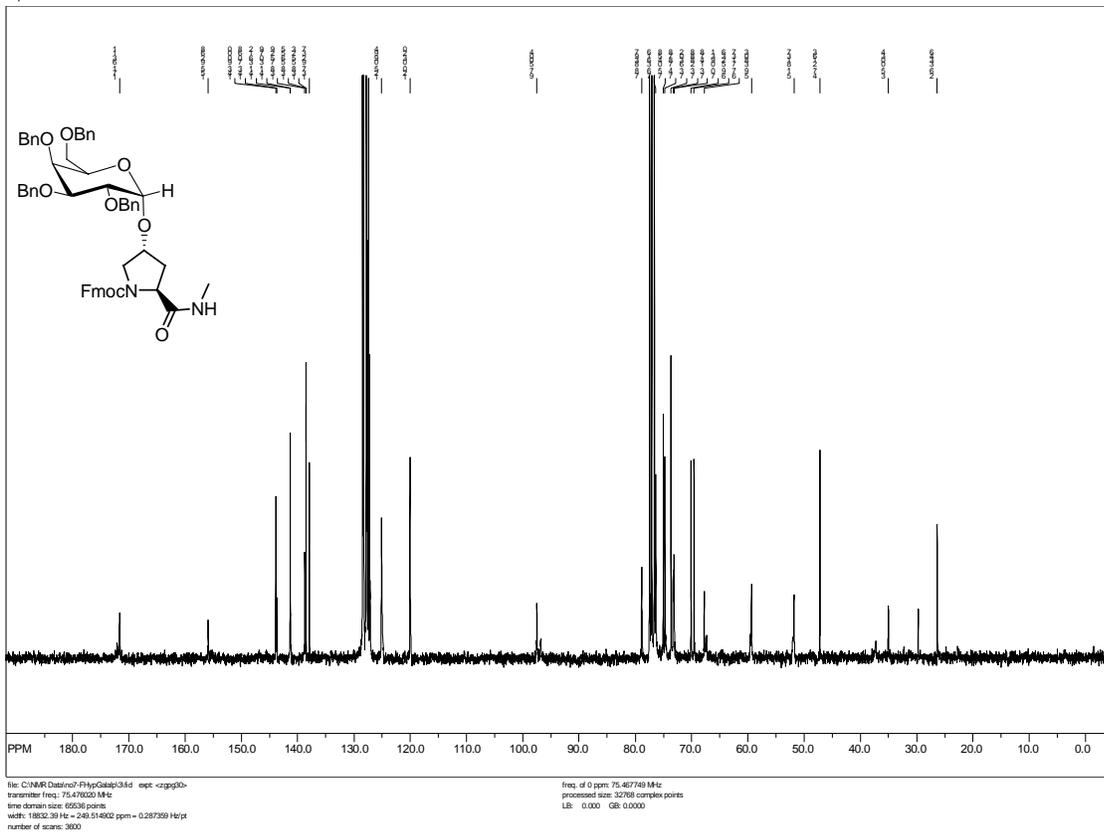
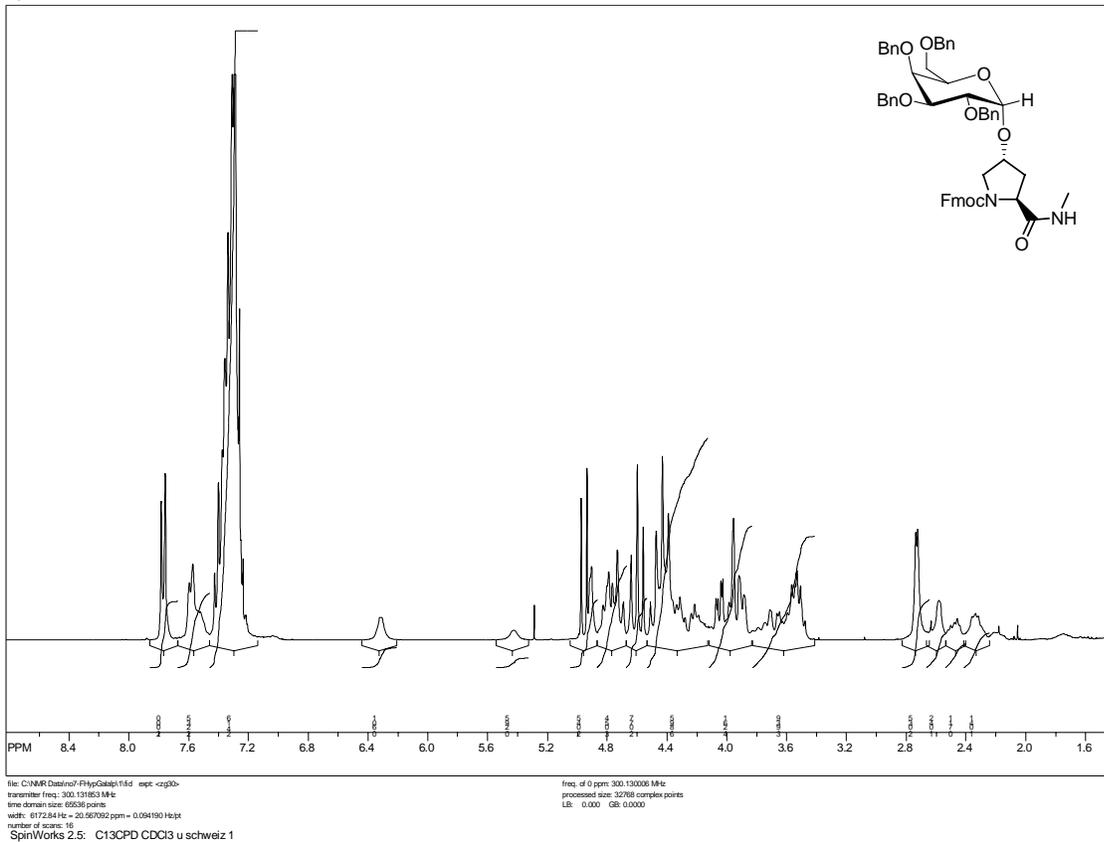


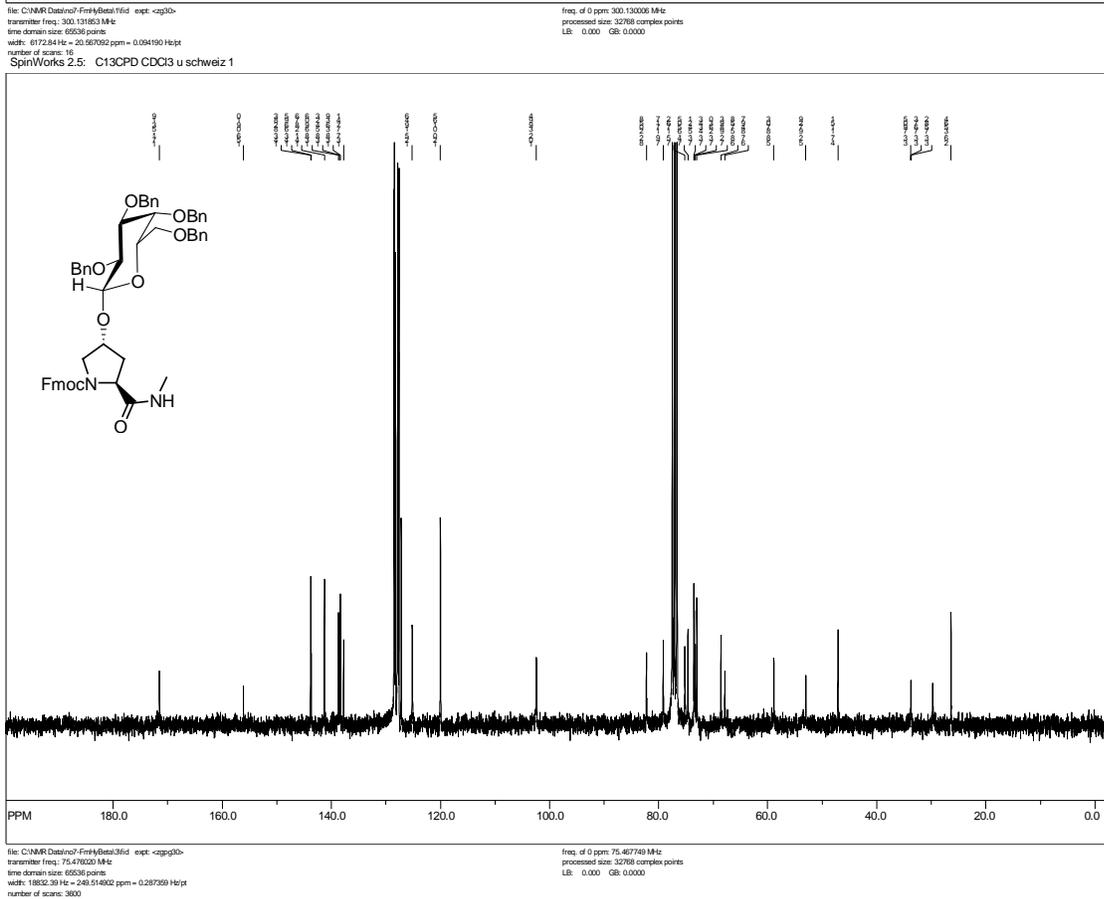
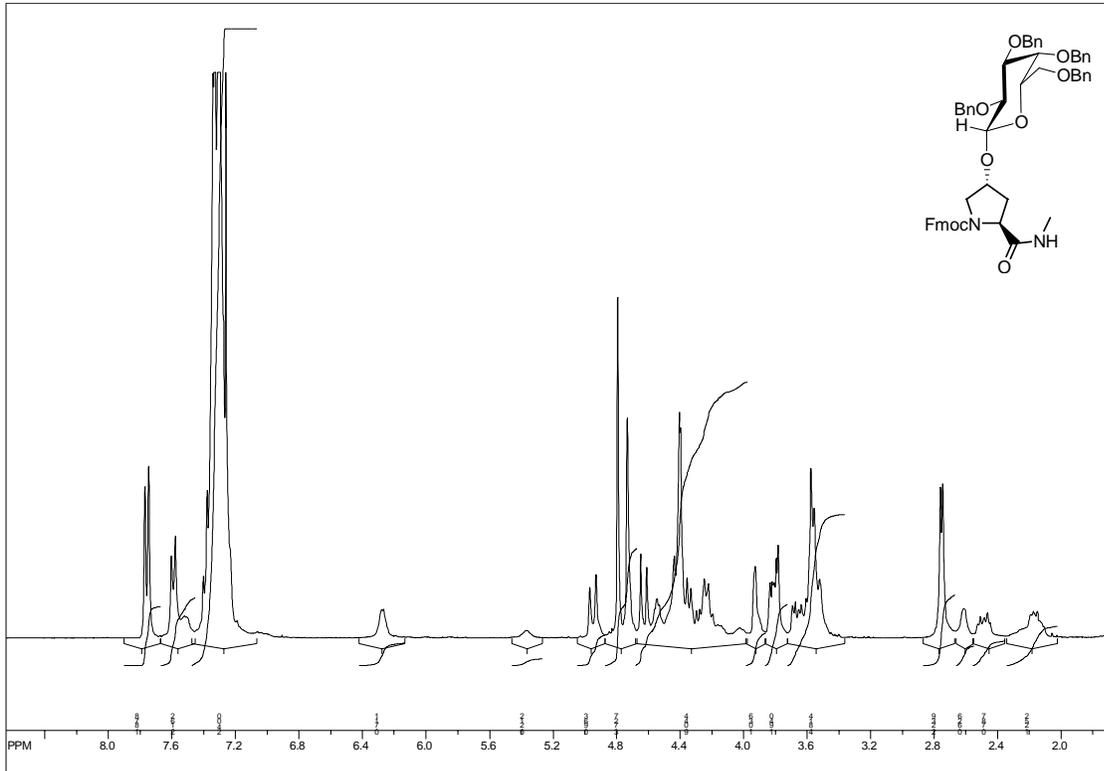
SpinWorks 2.5: purified t-butyl model peptide (in D2O)



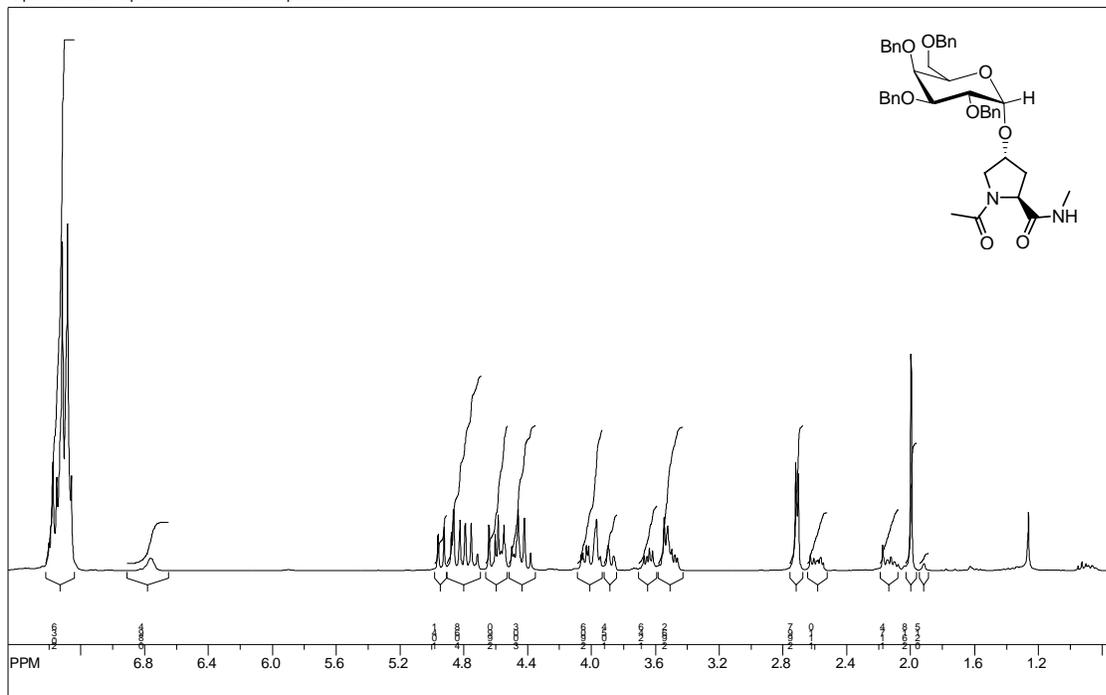
SpinWorks 2.5: purified t-butyl model peptide (in D2O)





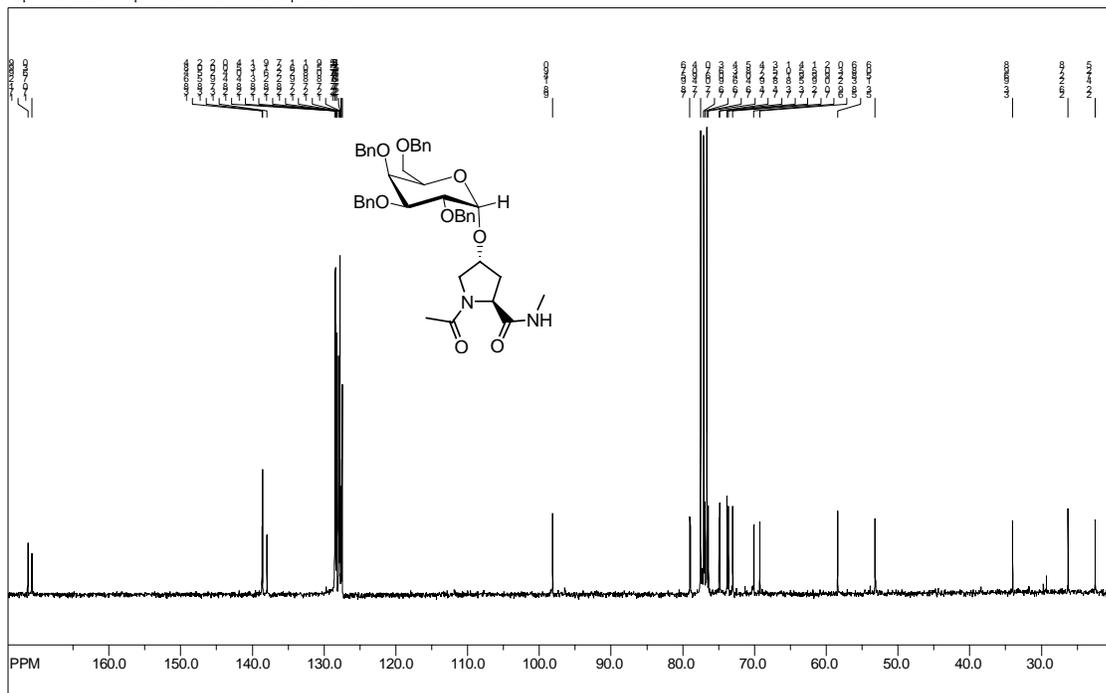


SpinWorks 2.5: alpha anomer after second purification



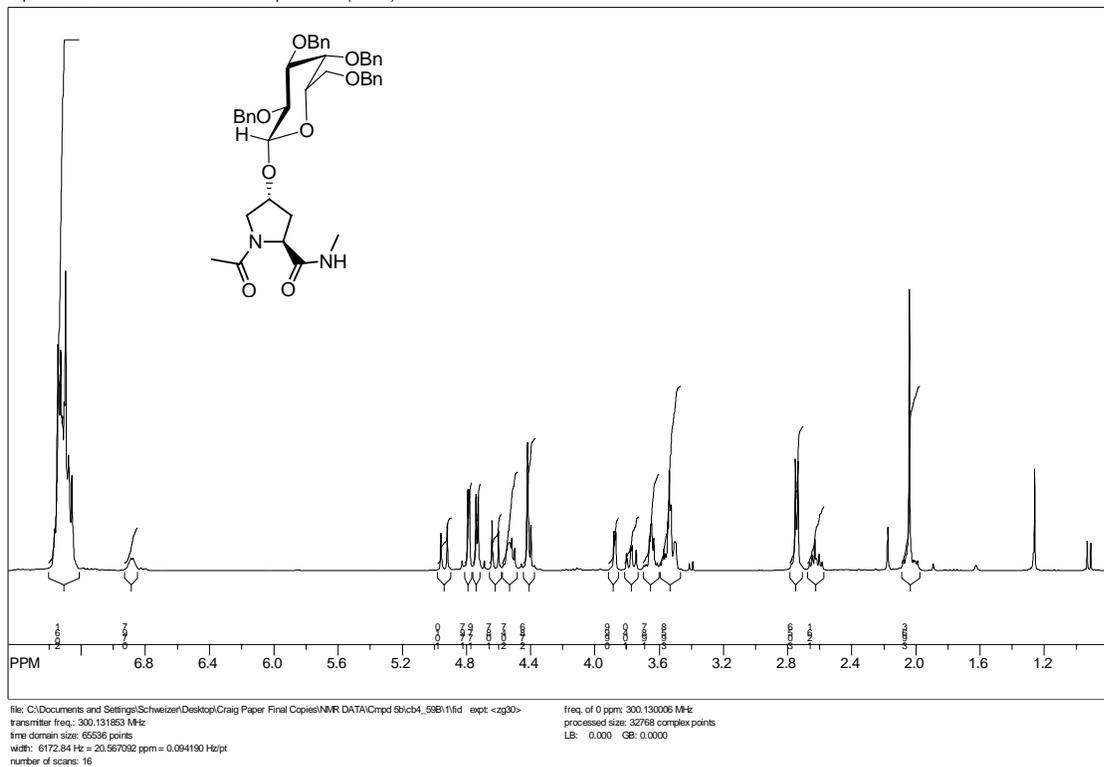
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number of scans: 16  
freq: 0 ppm: 300.130006 MHz  
processed size: 32768 complex points  
LB: 0.000 GB: 0.0000

SpinWorks 2.5: alpha anomer after second purification

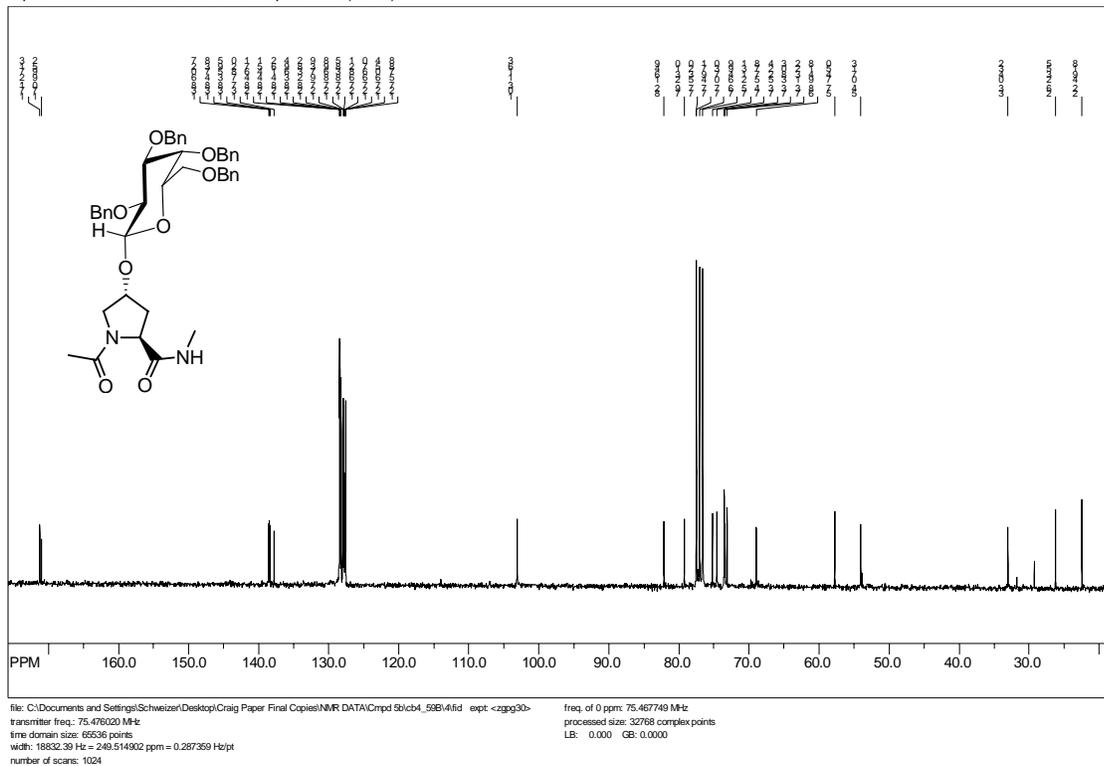


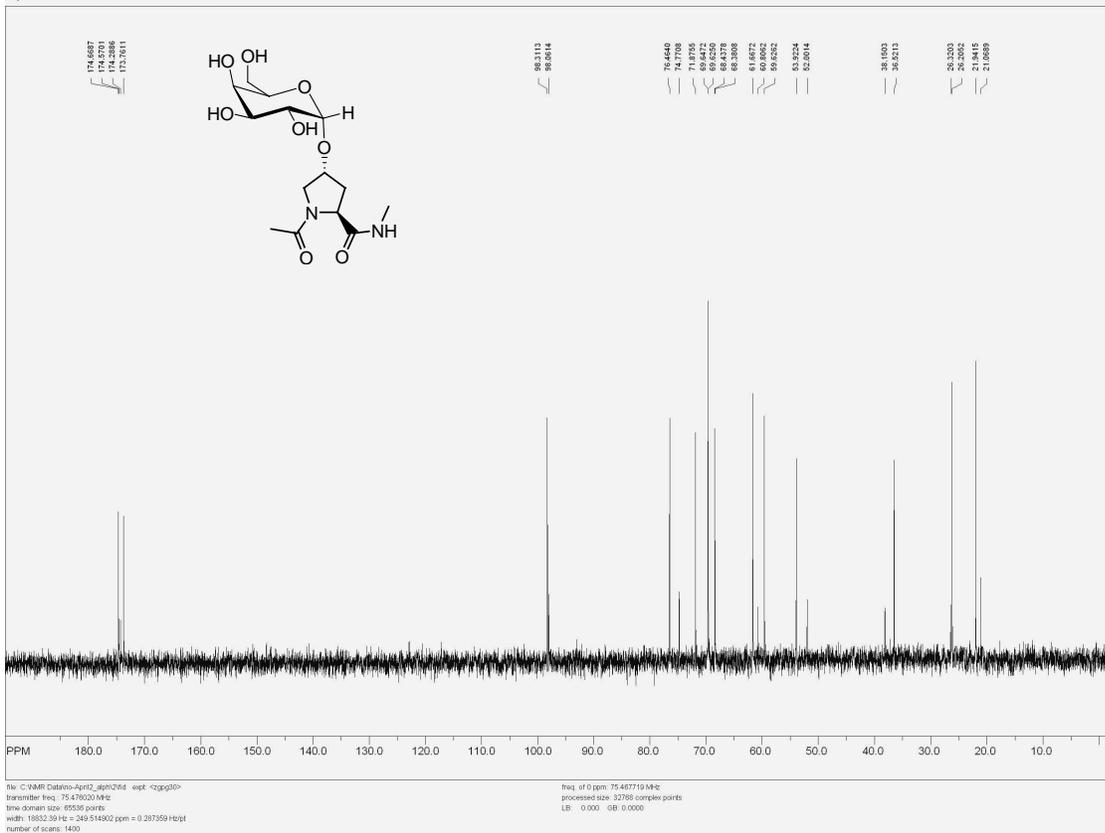
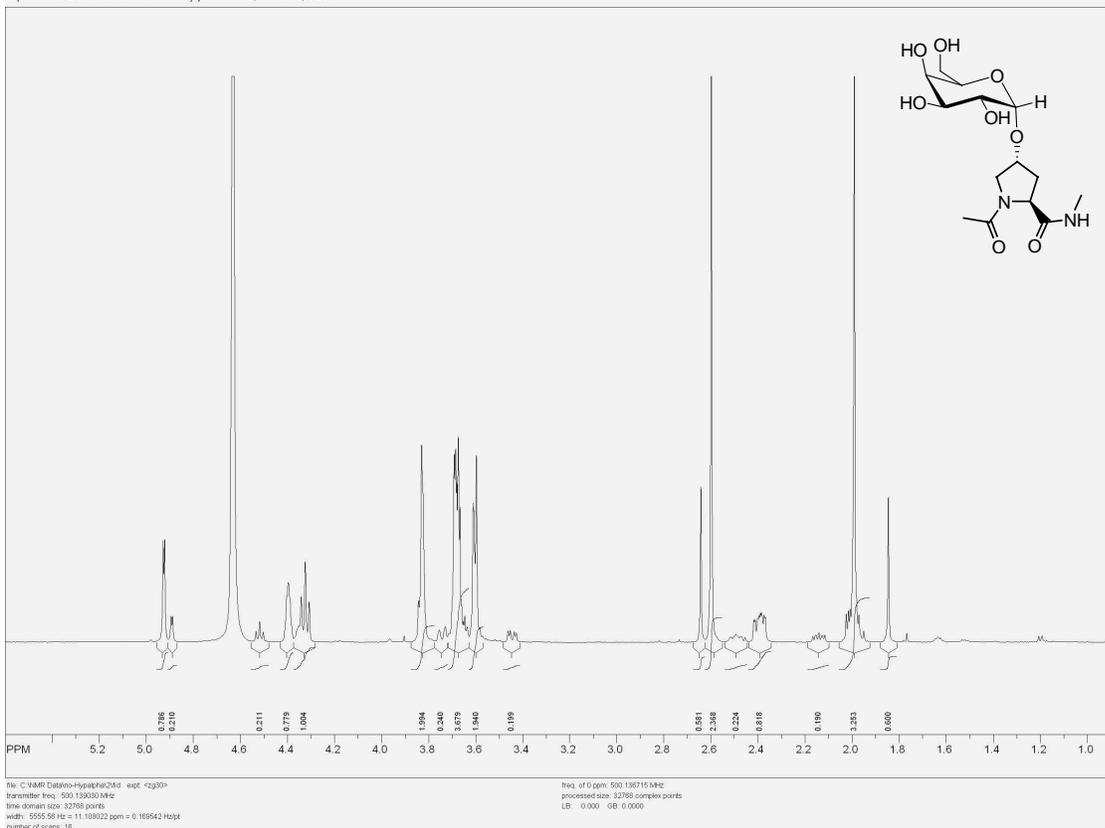
file: C:\Documents and Settings\Schweizer\Desktop\Craig Paper Final Copies\NMR DATA\Cmpd 56\cb4\_49A\4\fid exp: <zgg30>  
transmitter freq: 75.476020 MHz  
time domain size: 65536 points  
width: 19832.39 Hz = 249.514902 ppm = 0.287359 Hz/pt  
number of scans: 1536  
freq: 0 ppm: 75.467749 MHz  
processed size: 32768 complex points  
LB: 0.000 GB: 0.0000

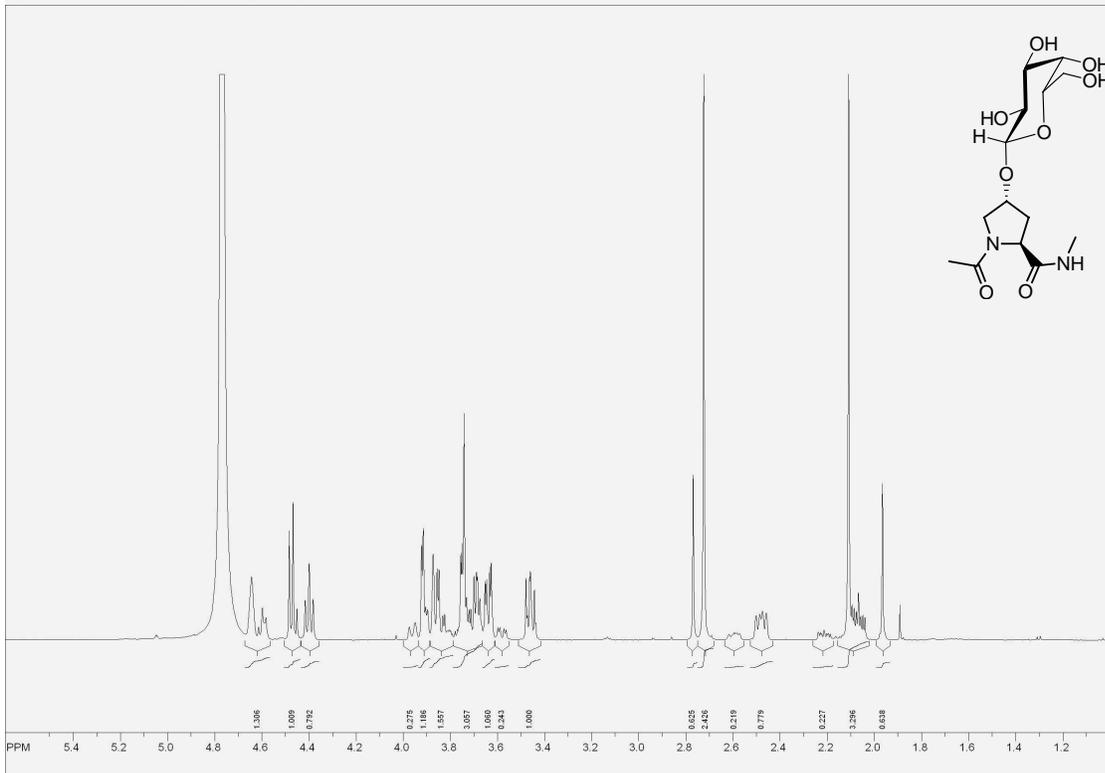
SpinWorks 2.5: beta anomer after second purification (CDCl3)



SpinWorks 2.5: beta anomer after second purification (CDCl3)

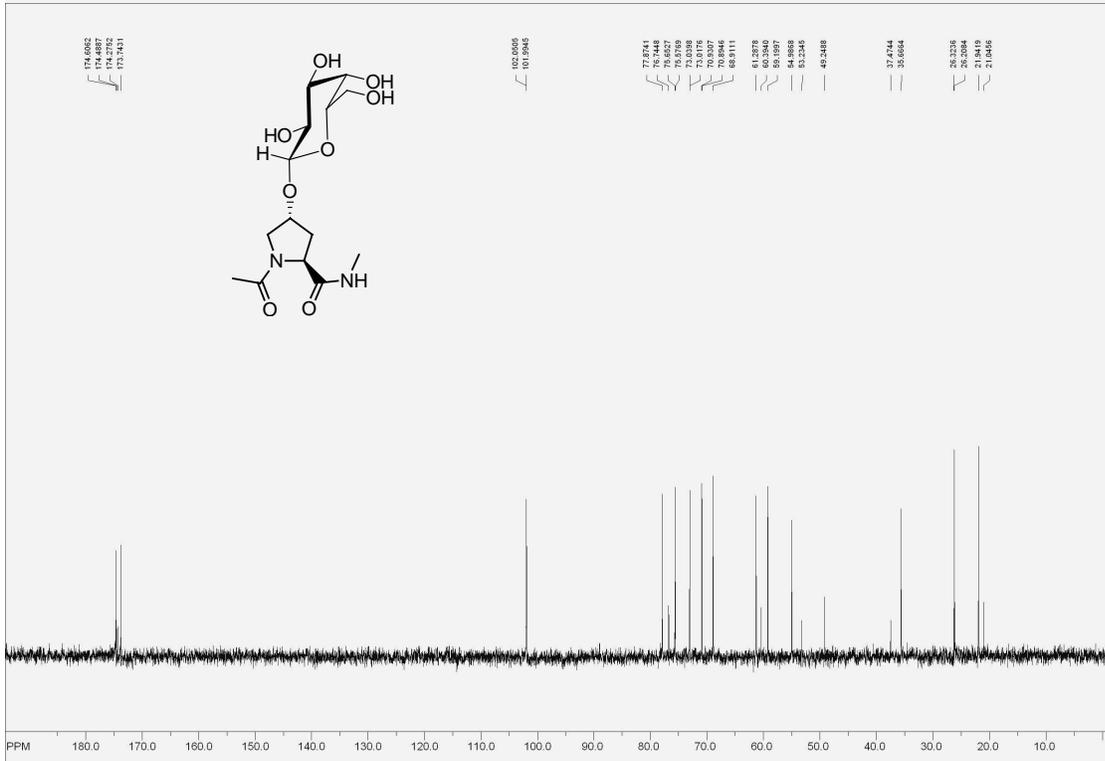






File: C:\NMR Data\No-Hydrate\CDCl3\18d -ept -c3g310  
 transmitter freq: 500.130030 MHz  
 time domain size: 32768 points  
 width: 5555.50 Hz = 11.108022 ppm = 0.169542 Hz/pt  
 number of scans: 16  
 SpinWorks 2.5: C13CPD D2O u schweiz 1

freq: 0 ppm: 500.130040 MHz  
 processed size: 32768 complex points  
 LB: 0.0000 0B: 0.0000

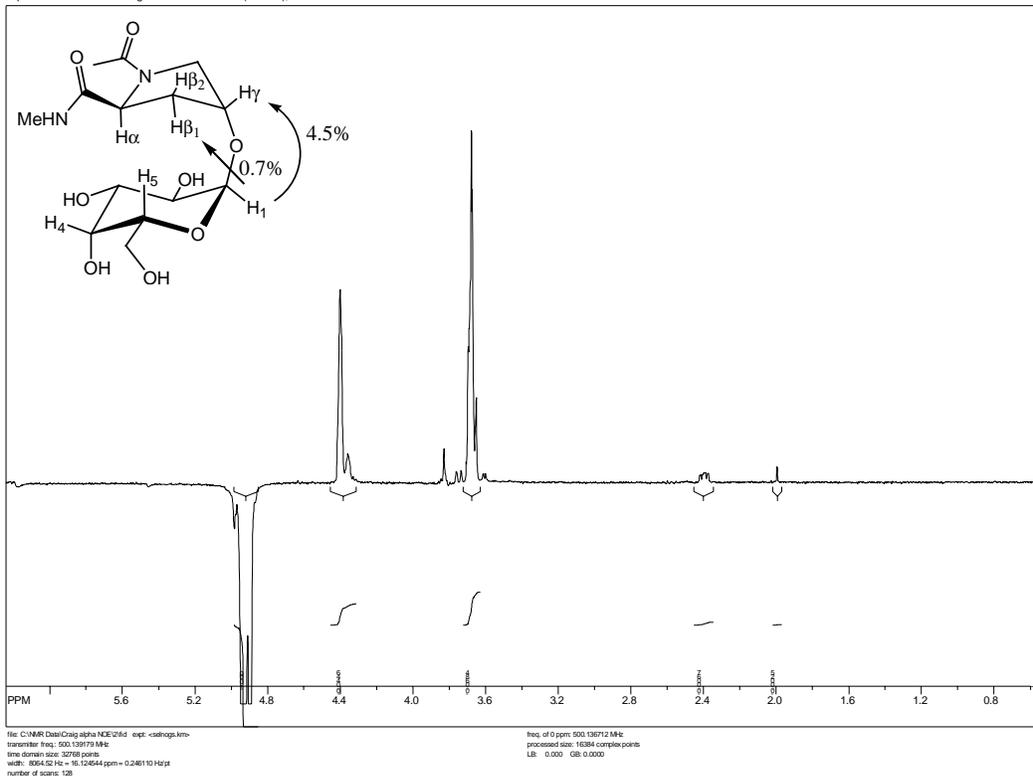


File: C:\NMR Data\No-Hydrate\CDCl3\18d -ept -c3g310  
 transmitter freq: 75.476020 MHz  
 time domain size: 65536 points  
 width: 19532.30 Hz = 340.514802 ppm = 0.287350 Hz/pt  
 number of scans: 3600

freq: 0 ppm: 75.487710 MHz  
 processed size: 32768 complex points  
 LB: 0.0000 0B: 0.0000

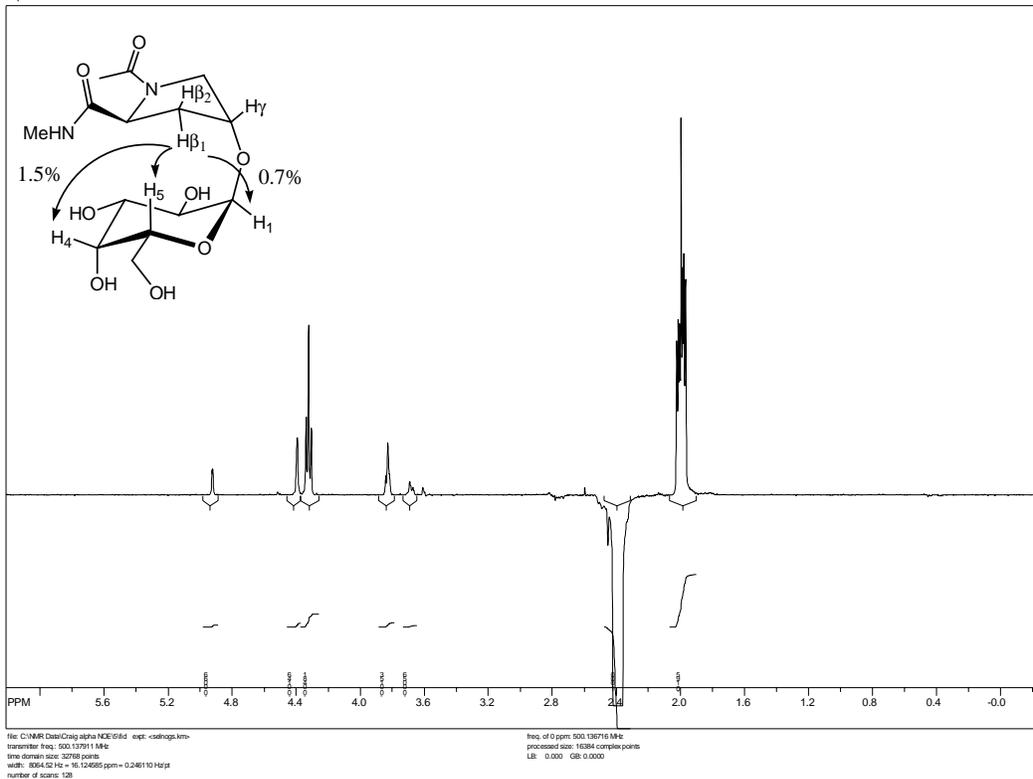
A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the H<sub>1</sub><sup>trans</sup> (4.93 ppm, 0.8H) [H<sub>1</sub><sup>cis</sup>, 4.89 ppm, 0.2H] in **4a** showed inter-proton effect to Pro<sub>γ</sub><sup>trans</sup> (4.40 ppm, 0.8H) (4.5%), Pro<sub>β1</sub><sup>trans</sup> (2.39 ppm, 0.8H) (0.7%) and the *N*-acetyl methyl singlet -COCH<sub>3</sub><sup>trans</sup> (1.99 ppm, 2.4H) (0.3%).

SpinWorks 2.5: Selective gradient selected NOE (GOESY), D2O



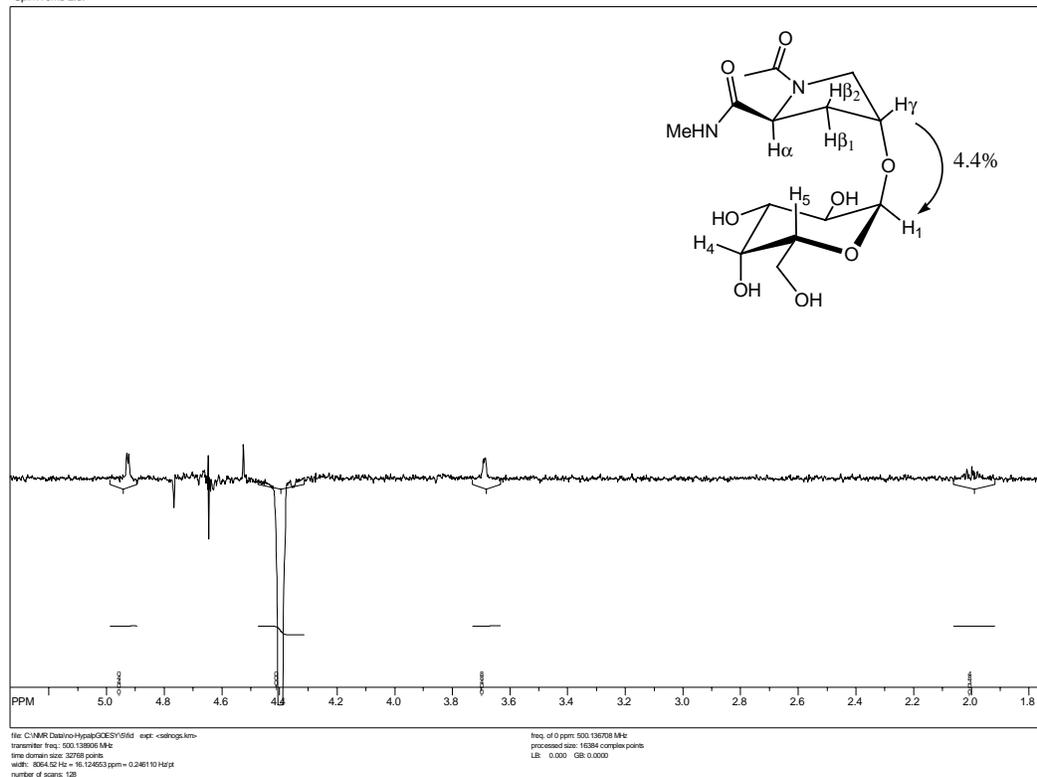
A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the Pro<sub>β1</sub><sup>trans</sup> (2.39 ppm, 0.8H) in **4a** showed inter-proton effect to H<sub>1</sub><sup>trans</sup> (4.93 ppm, 0.8H) (0.7%) and the H<sub>4</sub><sup>cis,trans</sup>/H<sub>5</sub><sup>cis,trans</sup> peak (3.82 ppm, 2H) (1.5%).

SpinWorks 2.5:

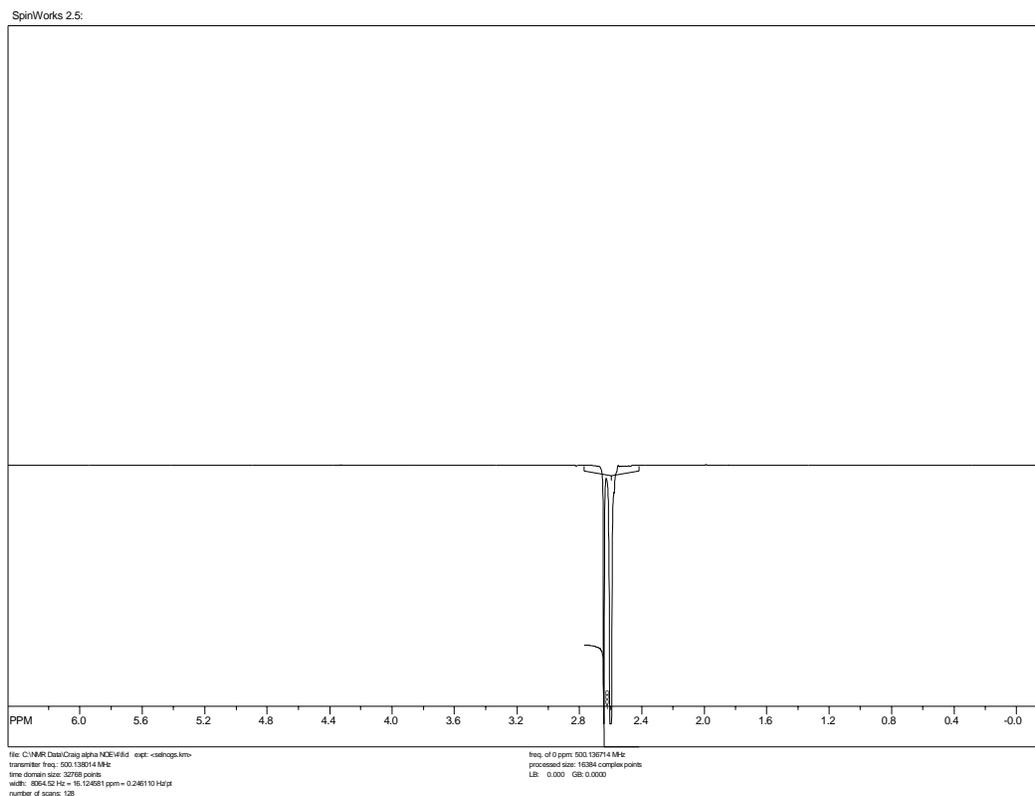


A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the Pro<sub>γ</sub><sup>trans</sup> (4.40 ppm, 0.8H) in **4a** showed inter-proton effect to H<sub>1</sub><sup>trans</sup> (4.93 ppm, 0.8H) (4.4%).

SpinWorks 2.5:

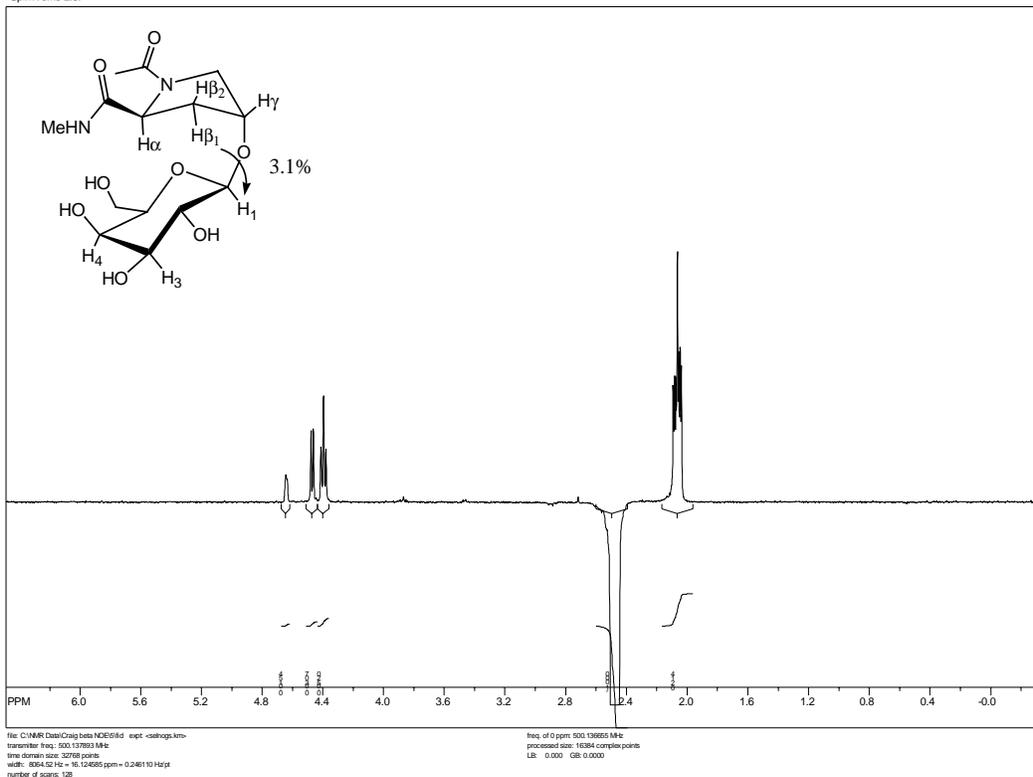


A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the *N*-methylamide singlet - NHCH<sub>3</sub><sup>trans</sup> (2.60 ppm, 2.4H) [-NHCH<sub>3</sub><sup>cis</sup>, 2.64 ppm, 0.6H] in **4a** showed no inter-proton effects.



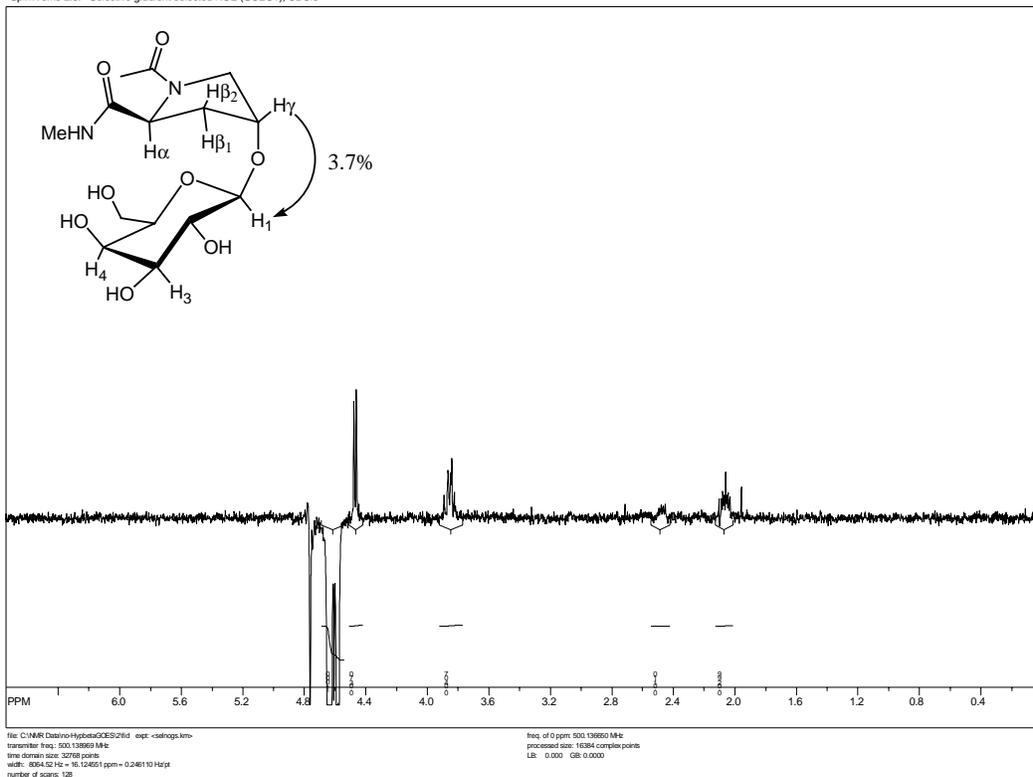
A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the Pro<sub>β1</sub><sup>trans</sup> (2.46 ppm, 0.8H) in **4b** showed inter-proton effect to H<sub>1</sub><sup>trans</sup> (4.48 ppm, 0.8H) (3.1%).

SpinWorks 2.5:



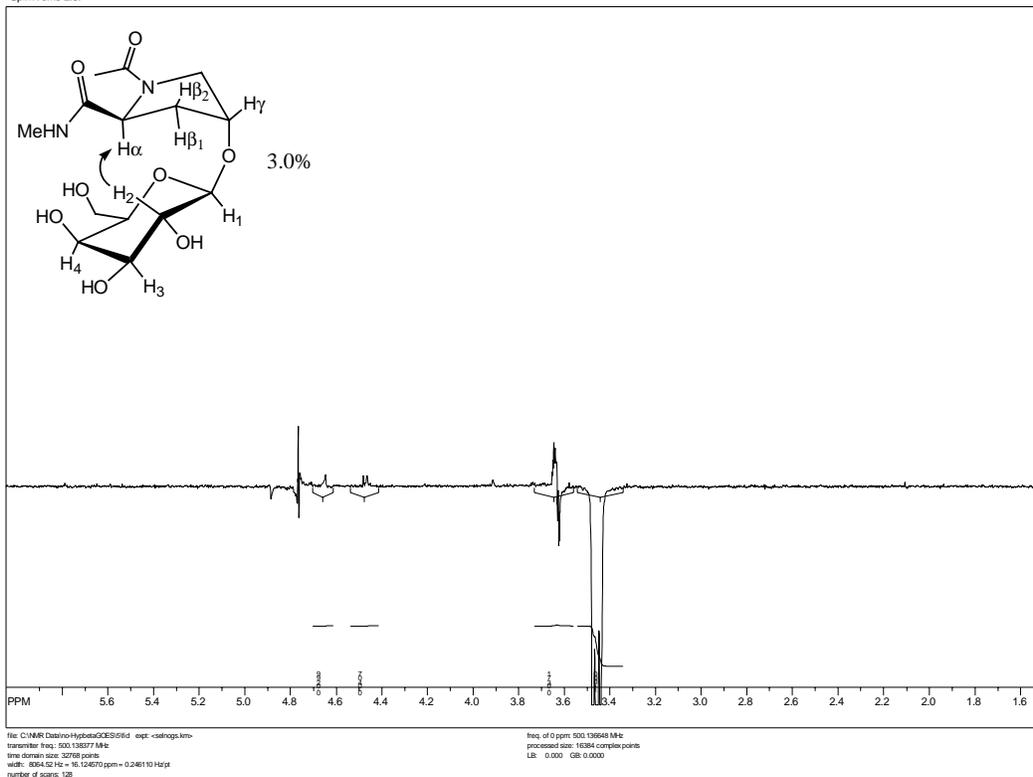
A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the Pro<sub>γ</sub><sup>trans</sup> (4.65 ppm, 0.8H) [Pro<sub>γ</sub><sup>cis</sup>, 4.59 ppm 0.2H] in **4b** showed inter-proton effect to H<sub>1</sub><sup>trans</sup> (4.48 ppm, 0.8H) (3.7%).

SpinWorks 2.5: Selective gradient selected NOE (GOESY), CDC13



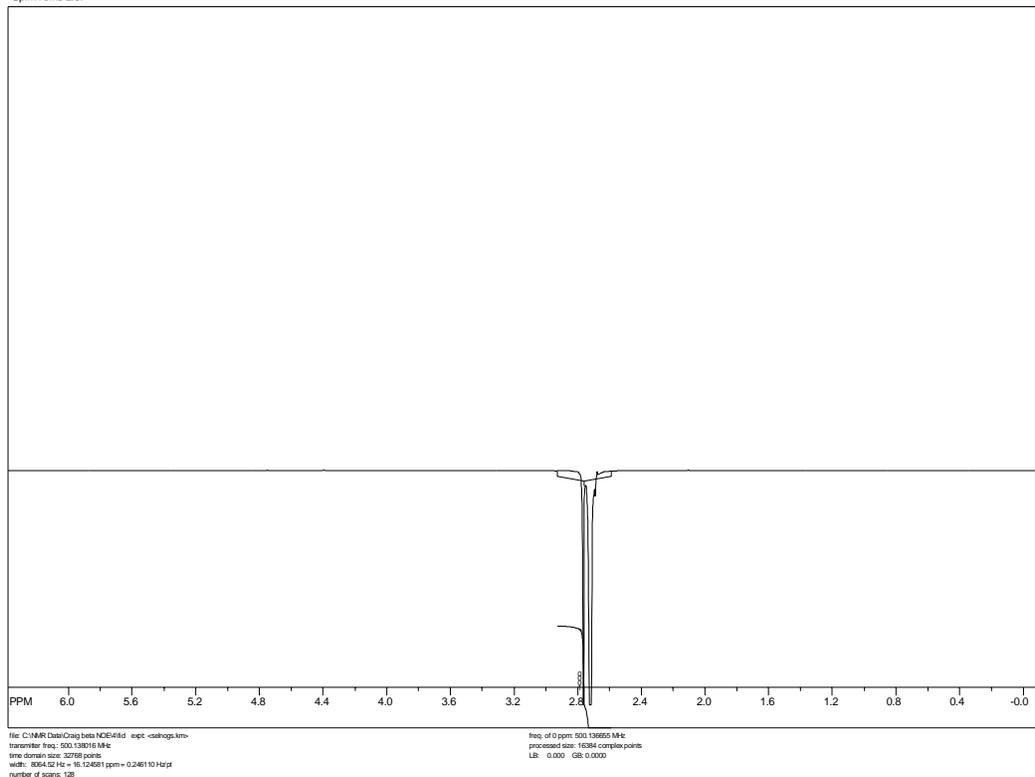
A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the H<sub>2</sub><sup>trans</sup> (3.46 ppm, 0.8H) in **4b** showed inter-proton effect to Pro<sub>α</sub><sup>trans</sup> (4.65 ppm, 0.8H) (3.0%).

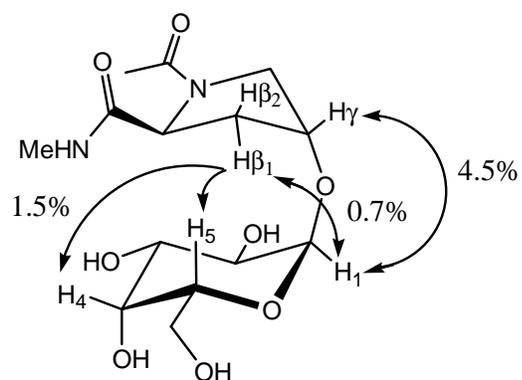
SpinWorks 2.5:



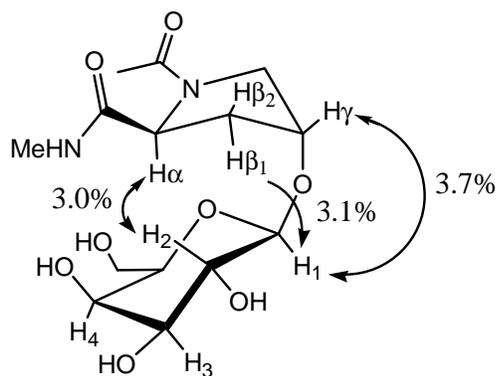
A one-dimensional GOESY experiment in D<sub>2</sub>O irradiating the *N*-methylamide singlet - NHCH<sub>3</sub><sup>trans</sup> (2.72 ppm, 2.4H) [-NHCH<sub>3</sub><sup>cis</sup>, 2.77 ppm, 0.6H] in **4b** showed no inter-proton effects.

SpinWorks 2.5:





**Figure 12. Summary of nOe Interactions Between the Prolyl and Galactosyl Rings in 4a**



**Figure 13. Summary of nOe Interactions Between the Prolyl and Galactosyl Rings in 4b**

## Magnetization Transfer NMR Experiments

Samples of **1-4b** were prepared by dissolving compounds in D<sub>2</sub>O at concentrations between 0.05 M and 0.1 M. All experiments were performed on a Bruker AMX500 NMR spectrometer equipped with a triple resonance (<sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N) gradient inverse probehead.

Selective inversion of the *N*'-methylamide singlet of the *trans* isomer was done using an 80 msec pulse centered on resonance. Relaxation delays of 20 s and inversion recovery delays between 1 ms and 15 s were used. Experiments were performed over several temperatures from 333.15 to 353.15 K for each compound. The temperature was calibrated using an ethylene glycol standard. For each compound at an individual temperature, inversion-recovery data were collected with selective inversion of the <sup>1</sup>H *N*'-methylamide singlet of the major *trans* peak. <sup>1</sup>H NMR spectroscopy was used in preference to <sup>13</sup>C NMR since over the course of an experiment, the signal/noise ratio is much higher for <sup>1</sup>H than <sup>13</sup>C, and heating effects caused by decoupling for <sup>13</sup>C causes uncertainty in the temperature of the sample.

The time-dependent magnetization transfers of the *cis* (M<sub>c</sub>(t)) and *trans* (M<sub>t</sub>(t)) NMR signals as a function of the inversion transfer time (t) were simultaneously fit to equations 1 and 2 (Alger and Prestegard, 1977; Mariappan and Rabenstein, 1992) below for compounds **1-4b** using *Mathematica* (v. 5.0). In the following pulse sequence, the <sup>1</sup>H *trans* resonance is selectively inverted using a shaped pulse. Its recovery during t is determined by its intrinsic T<sub>1t</sub>, magnetization transfer to and from the *cis* resonance, and the T<sub>1c</sub> of the *cis* resonance:

$$\pi(x)\text{sel}-----t-----\pi/2(x,y.-x.-y)---\text{acquire}$$

The resonances of the *trans* and *cis* isomers show the following time dependences:

$$M_t(t) = (c_1)(\tau_t)(\lambda_1 + 1/\tau_{1c})\exp(\lambda_1 * t) + (c_2)(\tau_t)(\lambda_2 + 1/\tau_{1c})\exp(\lambda_2 * t) + M_{c\infty}----- 1$$

$$M_c(t) = (c_1)\exp(\lambda_1 * t) + (c_2)\exp(\lambda_2 * t) + M_{t\infty}----- 2$$

T<sub>1c</sub> and T<sub>1t</sub> are the longitudinal relaxation times of the resonances in the absence of exchange.

τ<sub>c</sub> and τ<sub>t</sub> are the lifetimes of the *cis* and *trans* conformers and k<sub>ct</sub> and k<sub>tc</sub> are the corresponding rate constants.

τ<sub>1c</sub> and τ<sub>1t</sub> are the effective relaxation times of the *cis* and *trans* resonances when relaxation and exchange are both occurring and are defined below in terms of T<sub>1c</sub> and τ<sub>c</sub>, T<sub>1t</sub> and τ<sub>t</sub>.

λ<sub>1</sub> and λ<sub>2</sub> are related to the time constants τ<sub>c</sub>, τ<sub>t</sub>, τ<sub>1c</sub>, and τ<sub>1t</sub>, and are defined below.

c<sub>1</sub> and c<sub>2</sub> are defined below.

M<sub>c∞</sub> and M<sub>t∞</sub> are determined experimentally from the magnetization measured after 5 T<sub>1</sub> periods for the *cis* and *trans* resonances, respectively.

*Mathematica* then calculates τ<sub>t</sub> from τ<sub>c</sub>, M<sub>c∞</sub>, and M<sub>t∞</sub> as: τ<sub>t</sub> = τ<sub>c</sub> \* M<sub>t∞</sub>/M<sub>c∞</sub>

Thus,

$$k_{ct} = 1/\tau_c$$

$$k_{tc} = 1/\tau_t$$

$$K_{eq} = M_{t\infty}/M_{c\infty}$$

$$\tau_{1c} = (T_{1c} * \tau_c) / (\tau_c + T_{1c})$$

$$\tau_{1t} = (T_{1t} * \tau_t) / (\tau_t + T_{1t})$$

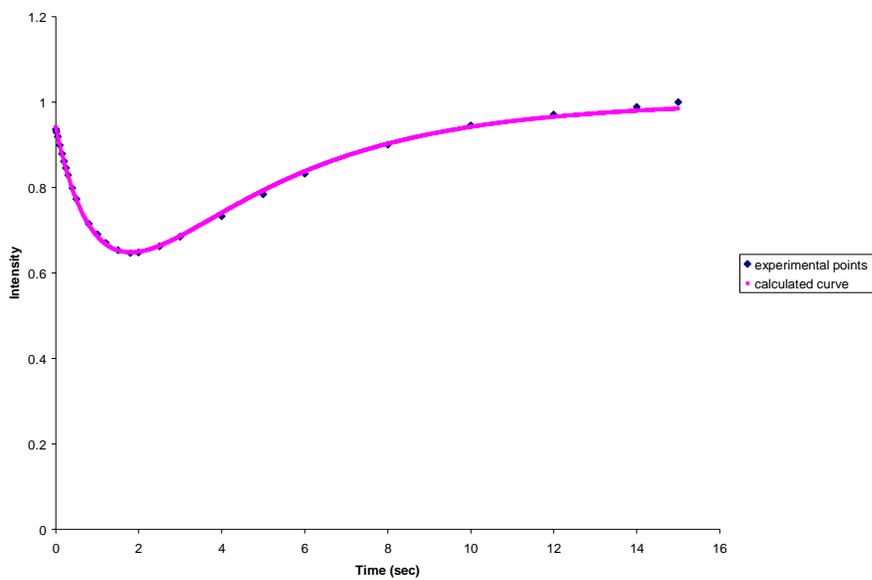
$$\lambda_1 = (0.5) * (-((1/\tau_{1c}) + (1/\tau_{1t})) + (((((1/\tau_{1c}) + (1/\tau_{1t})) / 2) - 4) * (((1/\tau_{1c}) * (1/\tau_{1t})) - ((1/\tau_c) * (1/\tau_t)))))) / 2)$$

$$\lambda_2 = (0.5) * (-((1/\tau_{1c}) + (1/\tau_{1t})) - (((((1/\tau_{1c}) + (1/\tau_{1t})) / 2) - 4) * (((1/\tau_{1c}) * (1/\tau_{1t})) - ((1/\tau_c) * (1/\tau_t)))))) / 2)$$

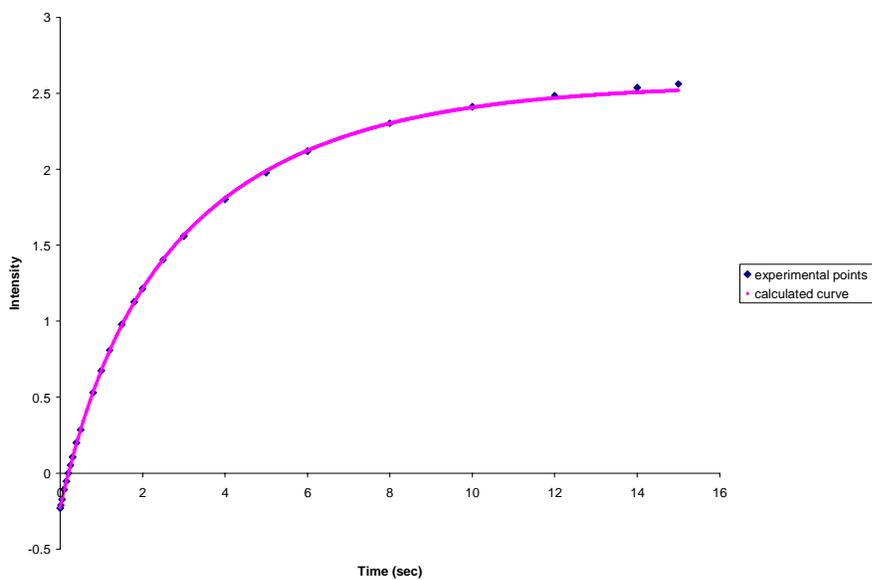
$$c_2 = ((1/(\tau_t)) * (\lambda_1 - \lambda_2)) * ((\tau_t * (\lambda_1 + (1/\tau_{1c}))) * ((M_{0c} - M_{c\infty}) + (M_{t\infty} - M_{0t})))$$

$$c_1 = M_{0c} - M_{c\infty} - c_2$$

**Figure 14. Inversion recovery of non-inverted (*cis*) *N'*-methyamide singlet (A) and inverted (*trans*) *N'*-methyamide singlet (B) of 1 at 62.0°C in D<sub>2</sub>O**

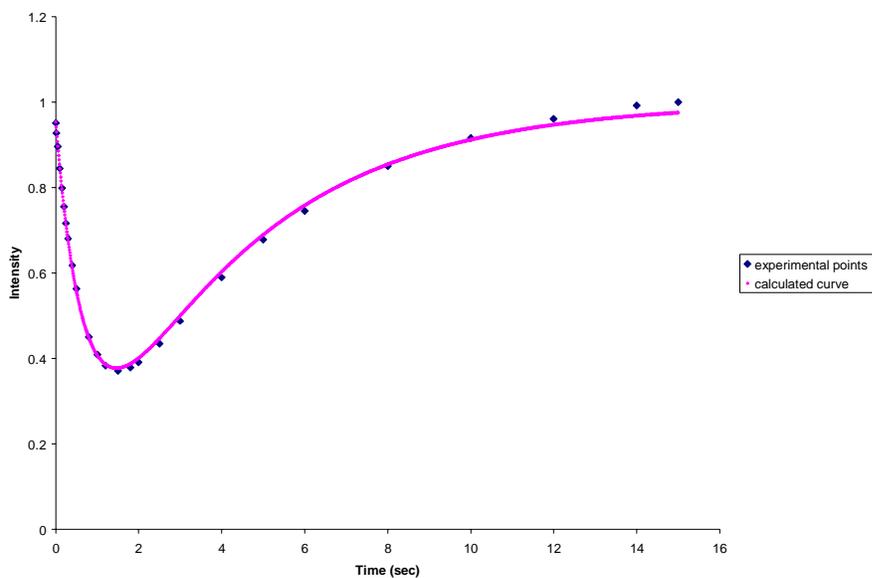


A

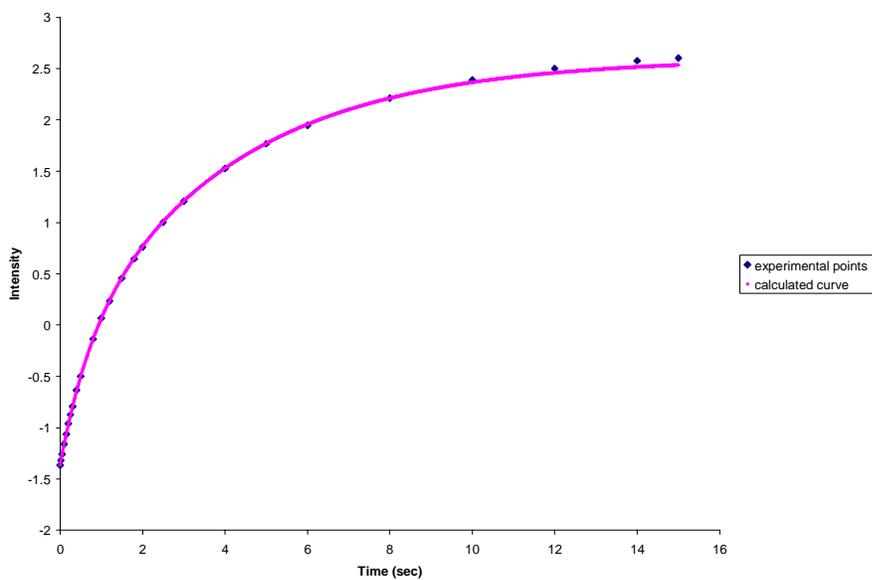


B

**Figure 15. Inversion recovery of non-inverted (*cis*) *N'*-methanamide singlet (A) and inverted (*trans*) *N'*-methanamide singlet (B) of 1 at 67.3°C in D<sub>2</sub>O**

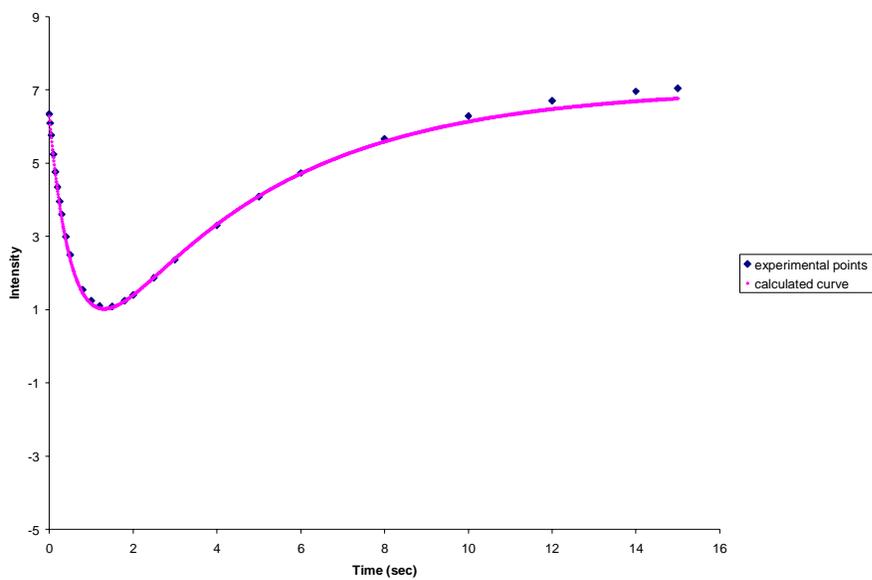


A

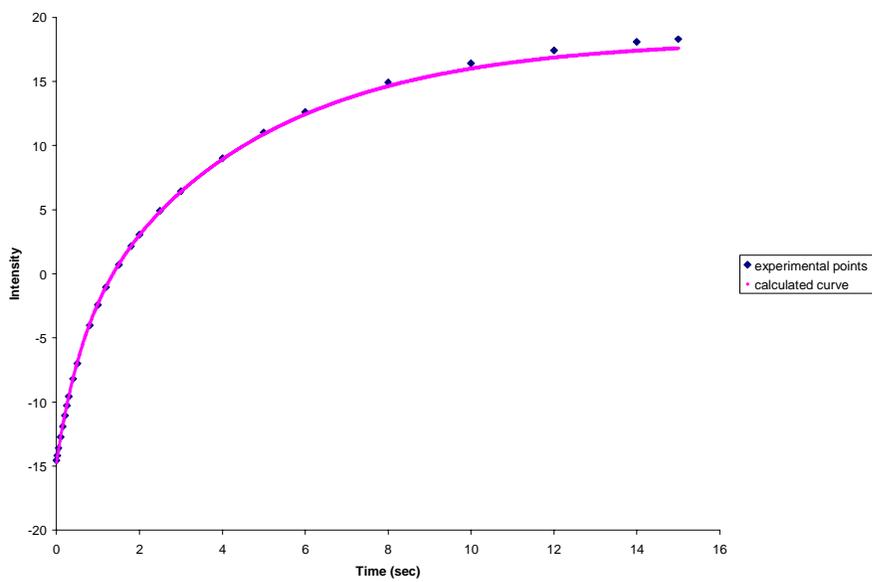


B

**Figure 16. Inversion recovery of non-inverted (*cis*) *N'*-methyamide singlet (A) and inverted (*trans*) *N'*-methyamide singlet (B) of 1 at 72.6°C in D<sub>2</sub>O**

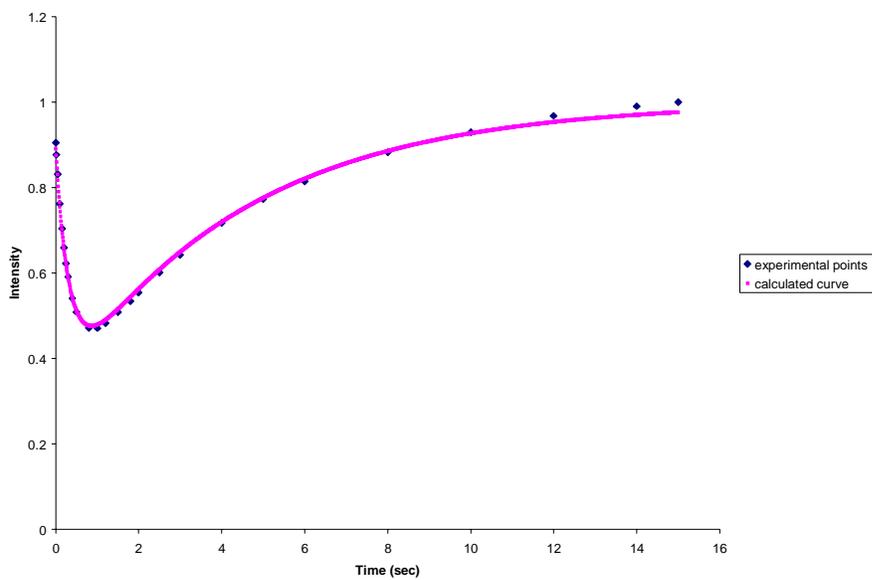


A

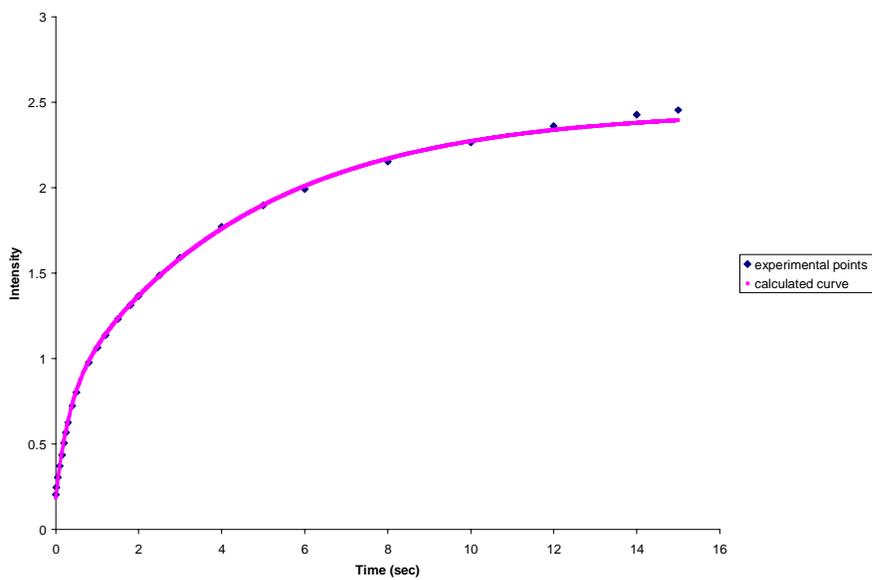


B

**Figure 17. Inversion recovery of non-inverted (*cis*) *N'*-methamide singlet (A) and inverted (*trans*) *N'*-methamide singlet (B) of 1 at 78.0°C in D<sub>2</sub>O**

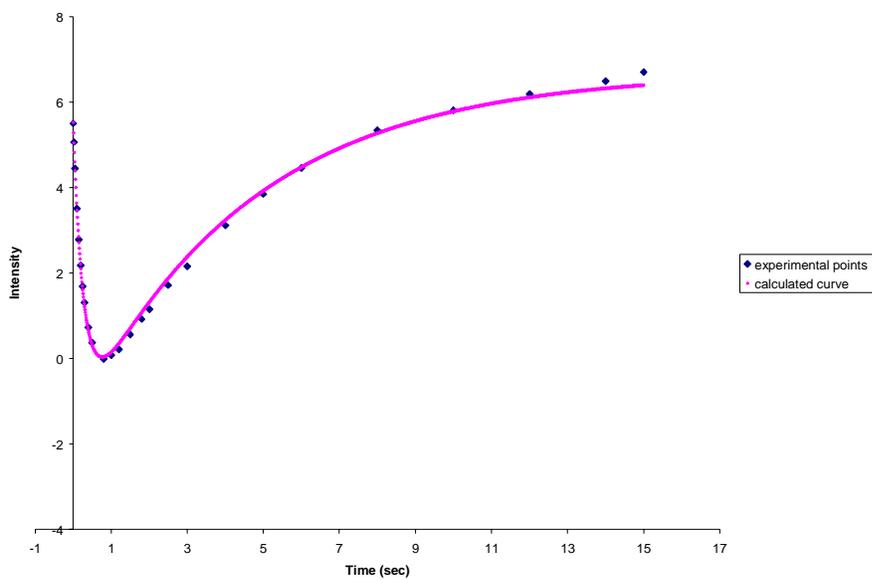


A

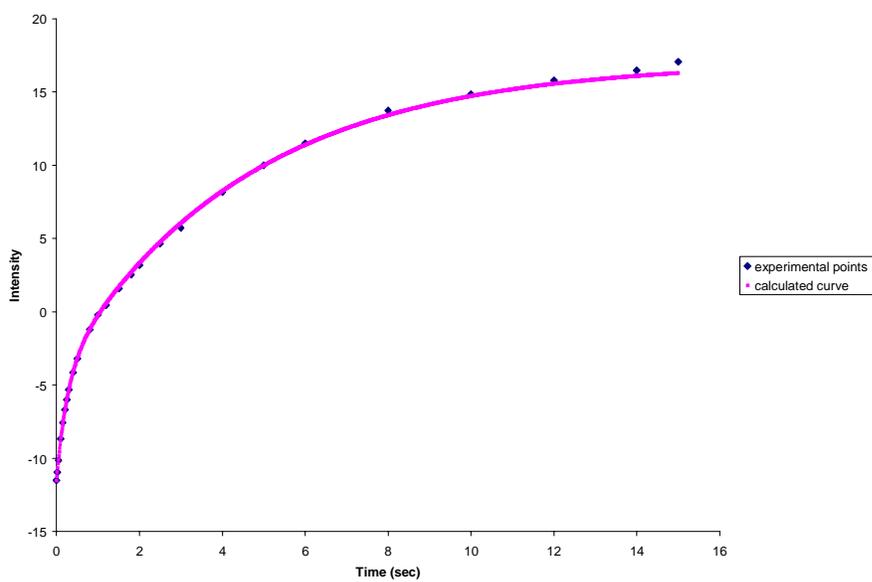


B

**Figure 18. Inversion recovery of non-inverted (*cis*) *N'*-methyamide singlet (A) and inverted (*trans*) *N'*-methyamide singlet (B) of 1 at 83.0°C in D<sub>2</sub>O**

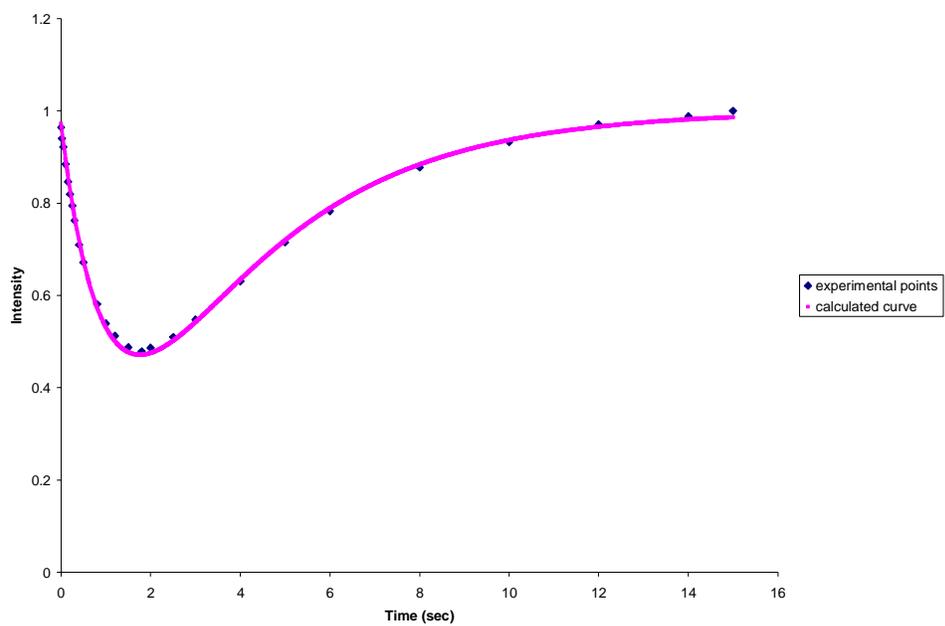


A

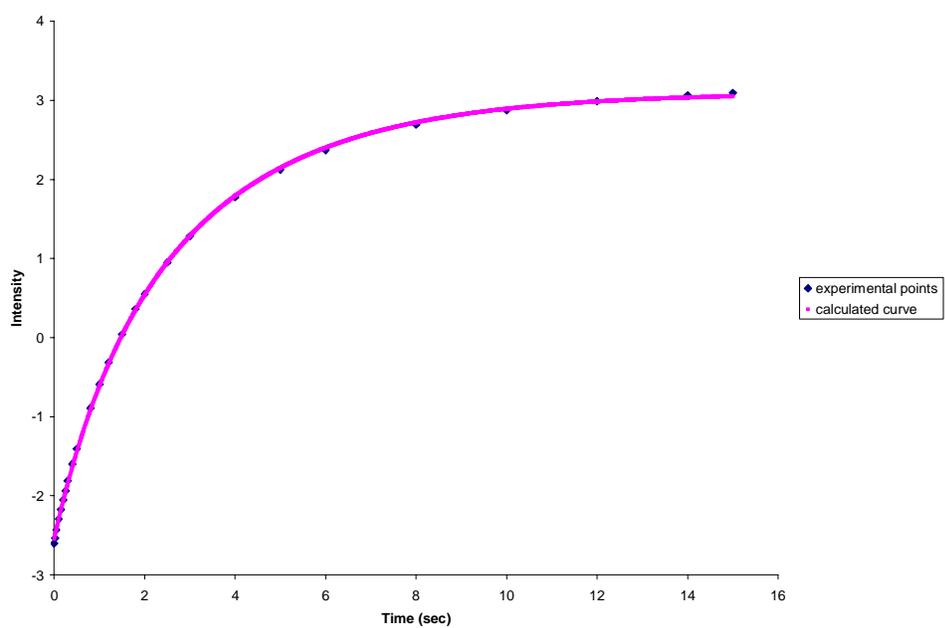


B

**Figure 19. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 2 at 62.0°C in D<sub>2</sub>O**

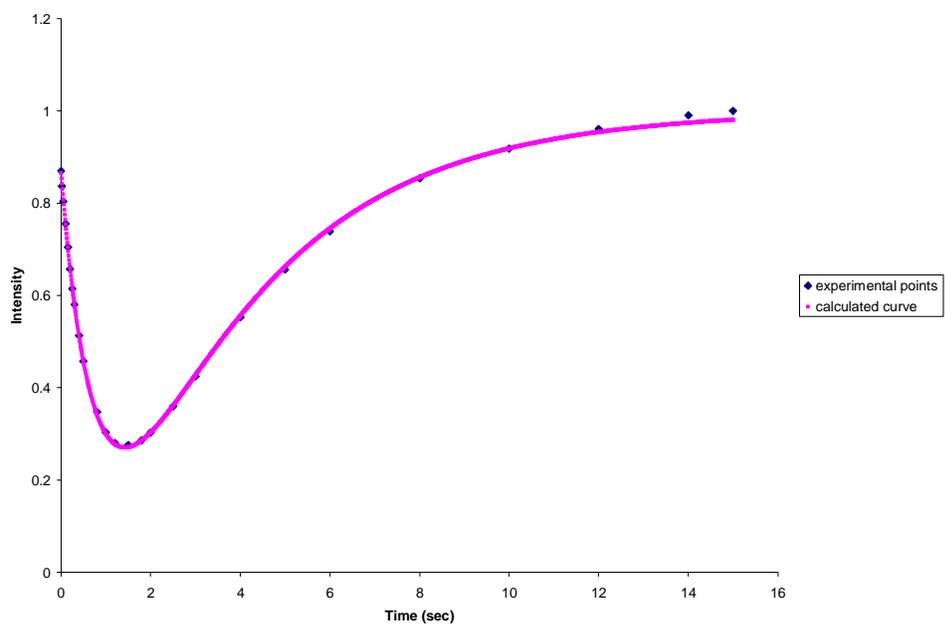


A

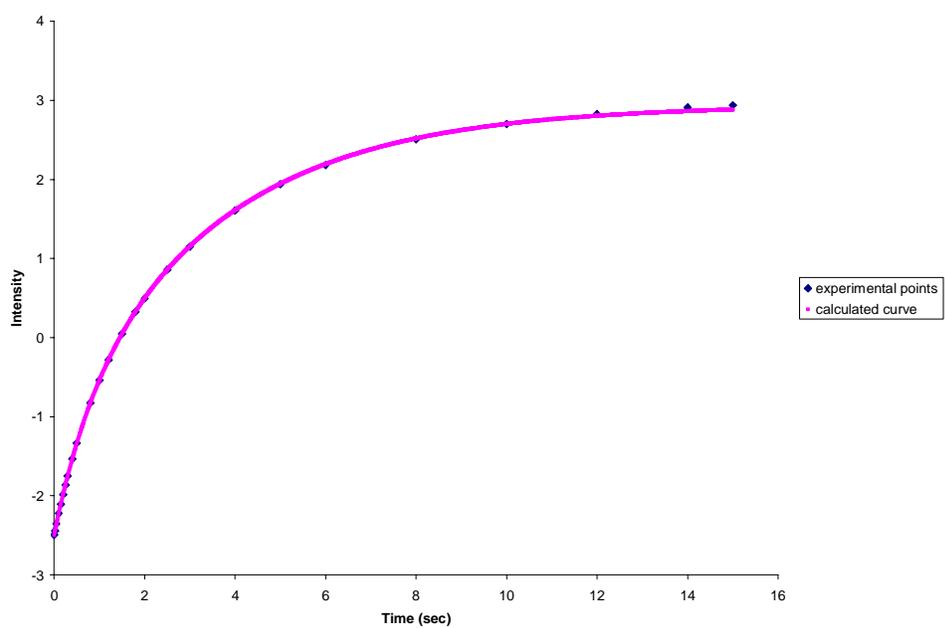


B

**Figure 20. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 2 at 67.3°C in D<sub>2</sub>O**

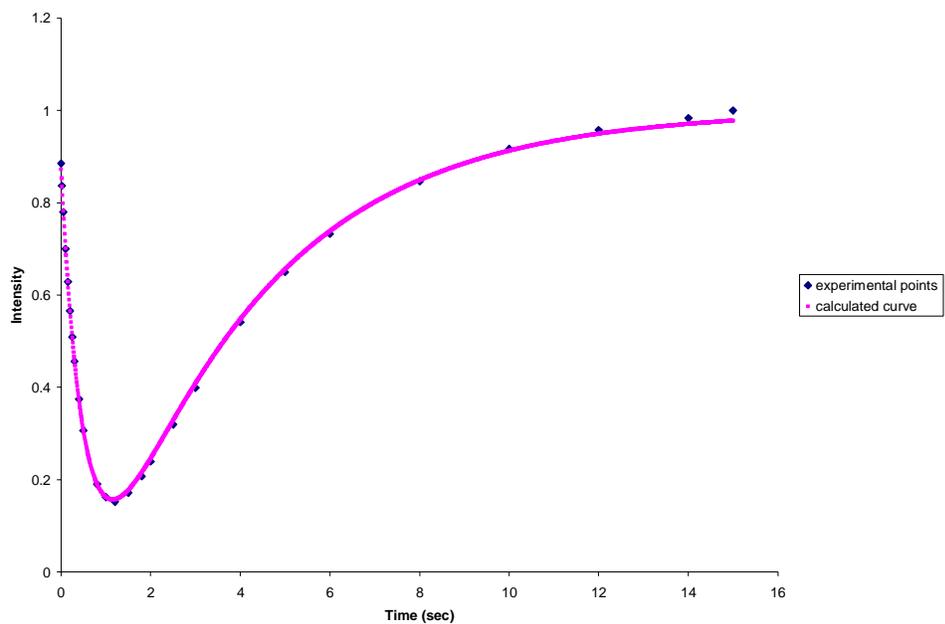


A

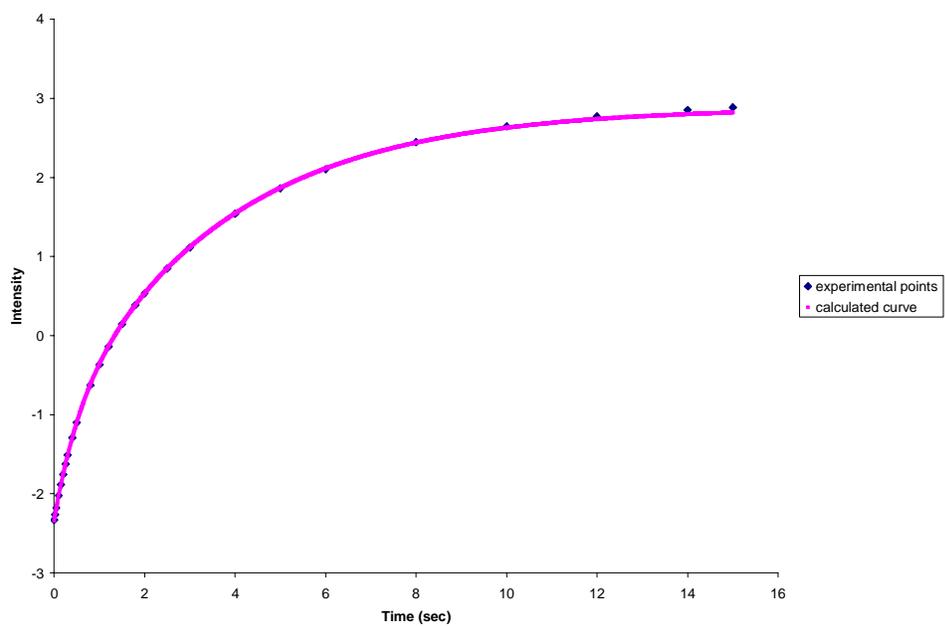


B

**Figure 21. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 2 at 72.6°C in D<sub>2</sub>O**

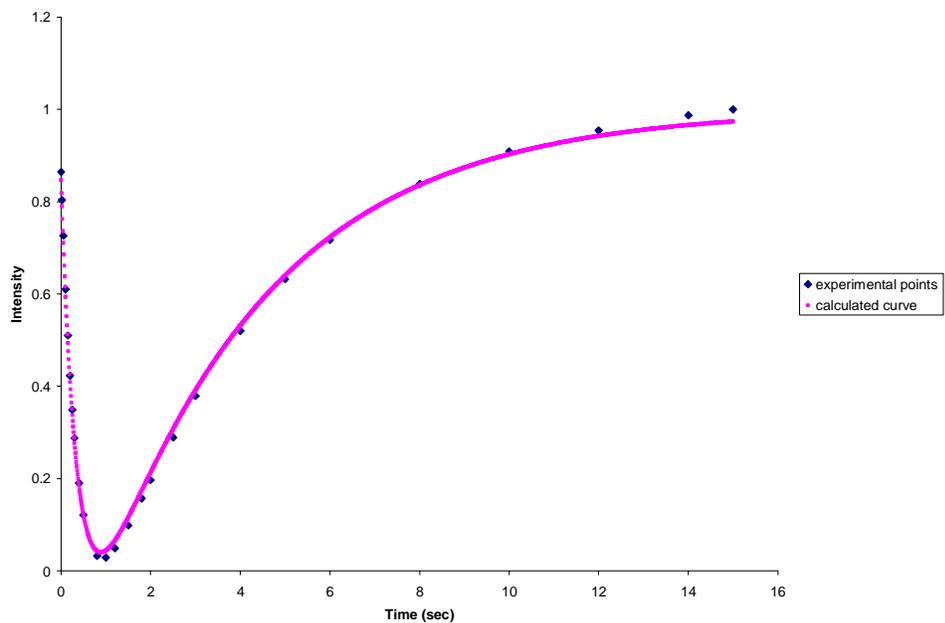


A

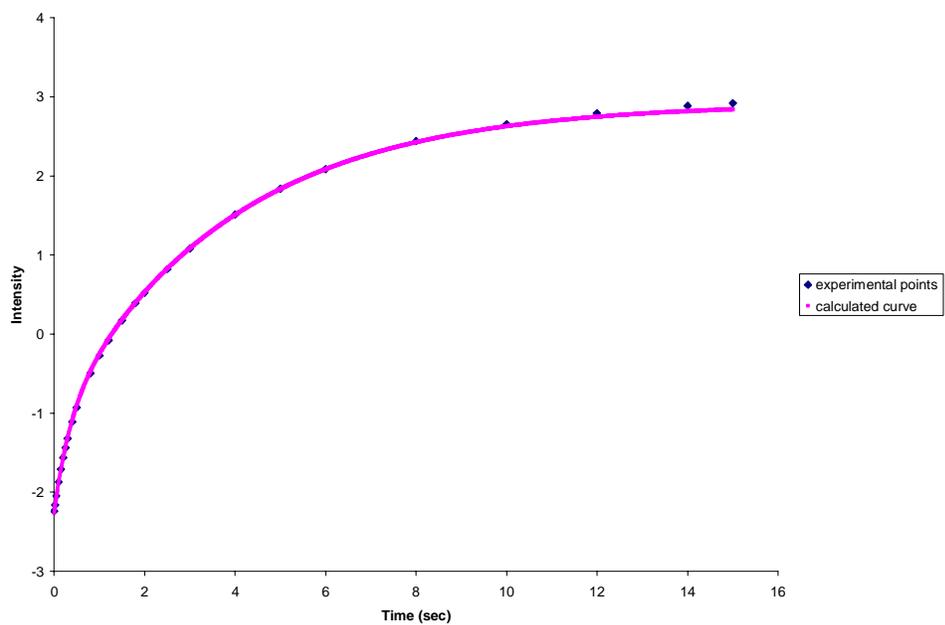


B

**Figure 22. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 2 at 78.0°C in D<sub>2</sub>O**

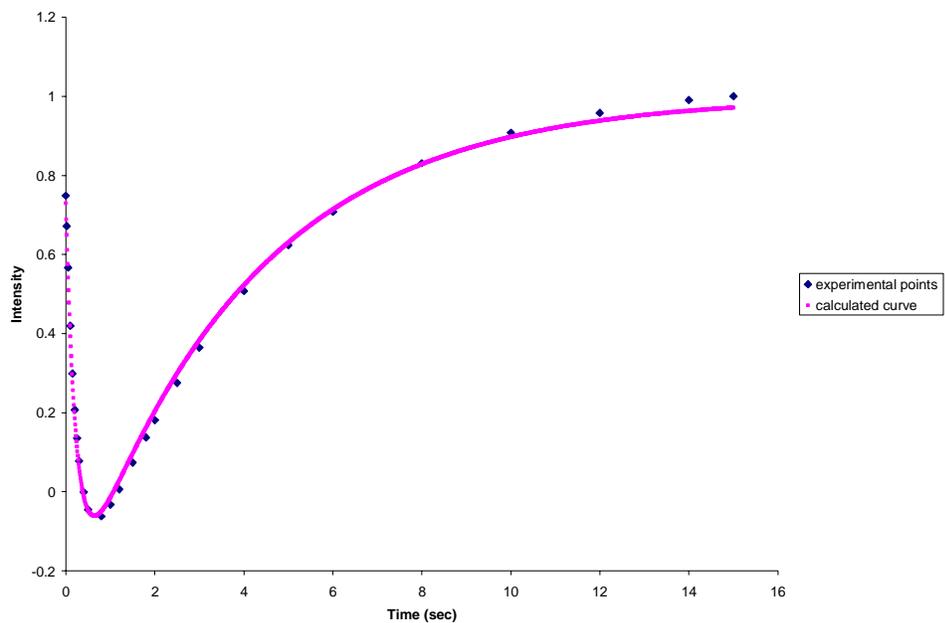


A

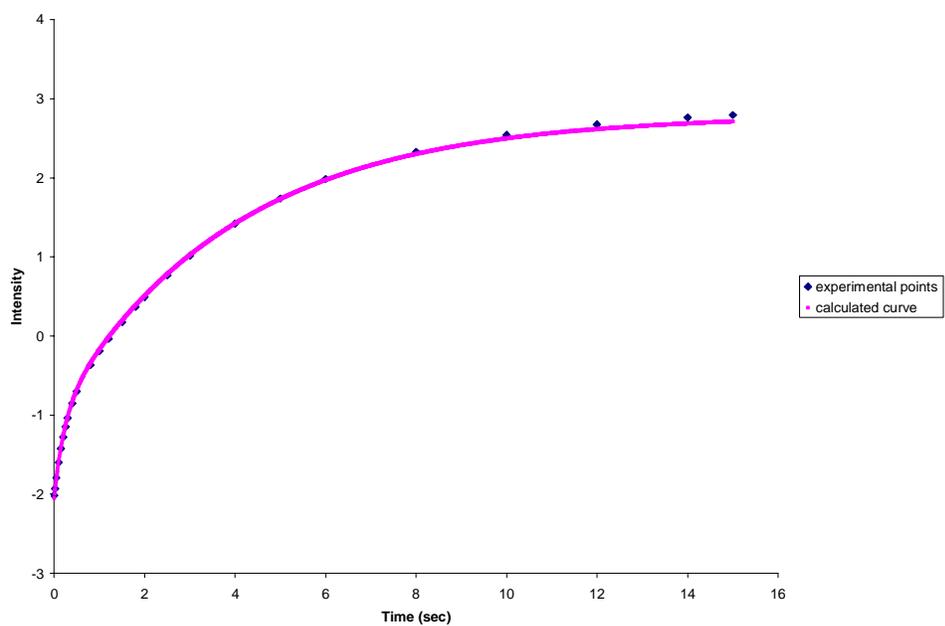


B

**Figure 23. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 2 at 83.0°C in D<sub>2</sub>O**

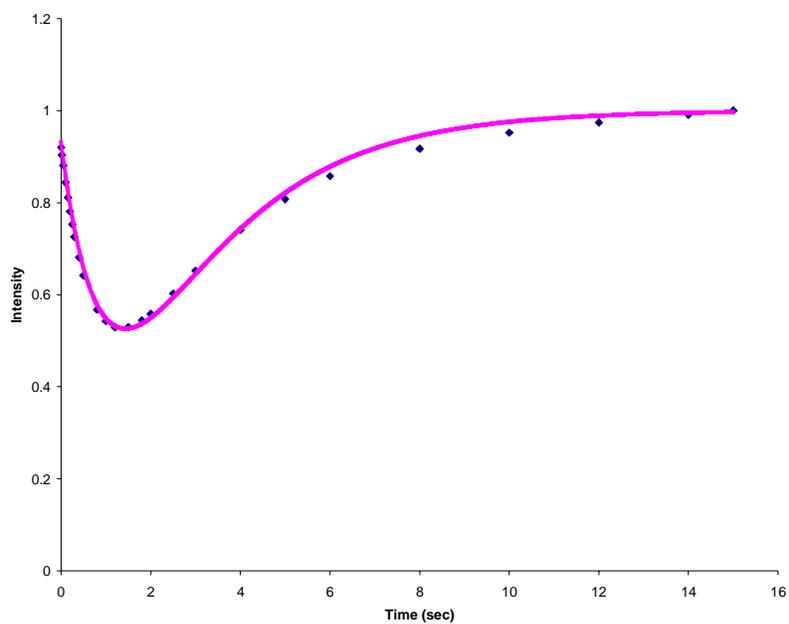


A

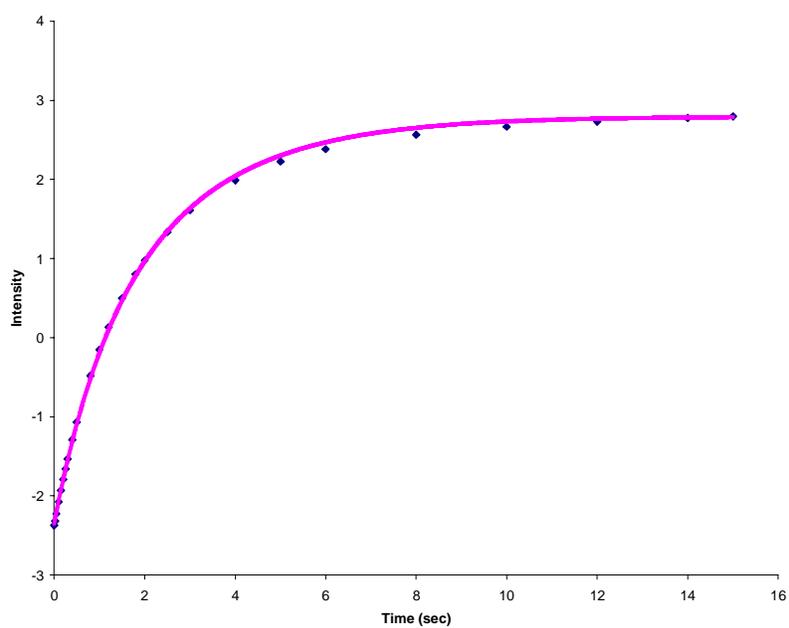


B

**Figure 24. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 3 at 62.0°C in D<sub>2</sub>O**

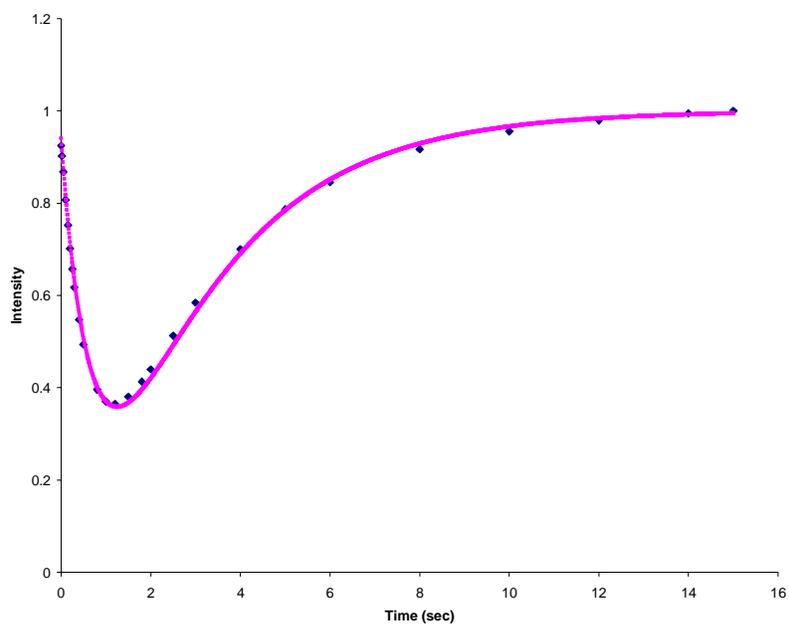


A

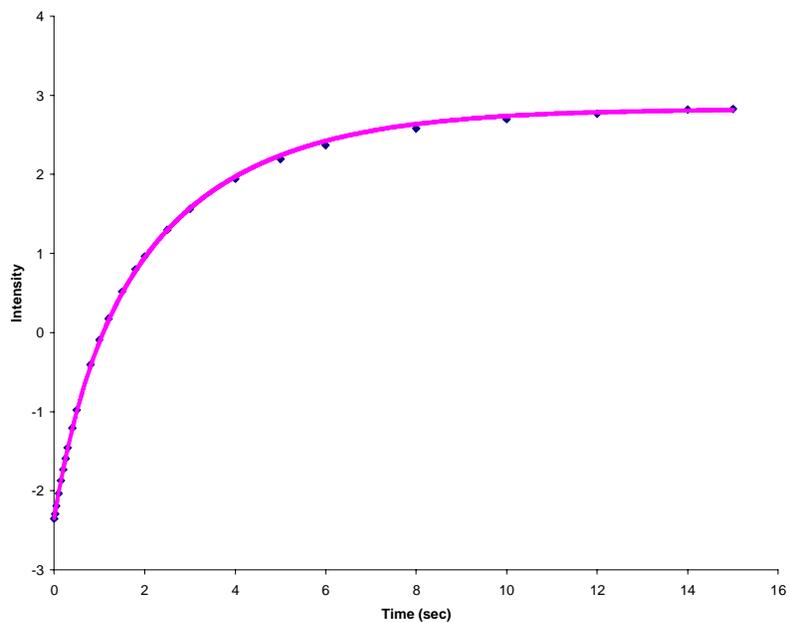


B

**Figure 25. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 3 at 67.3°C in D<sub>2</sub>O**

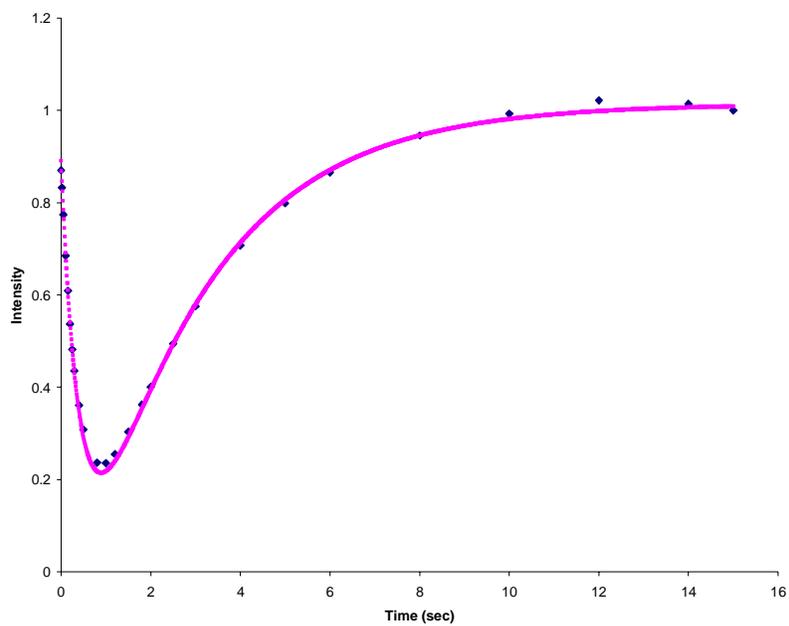


A

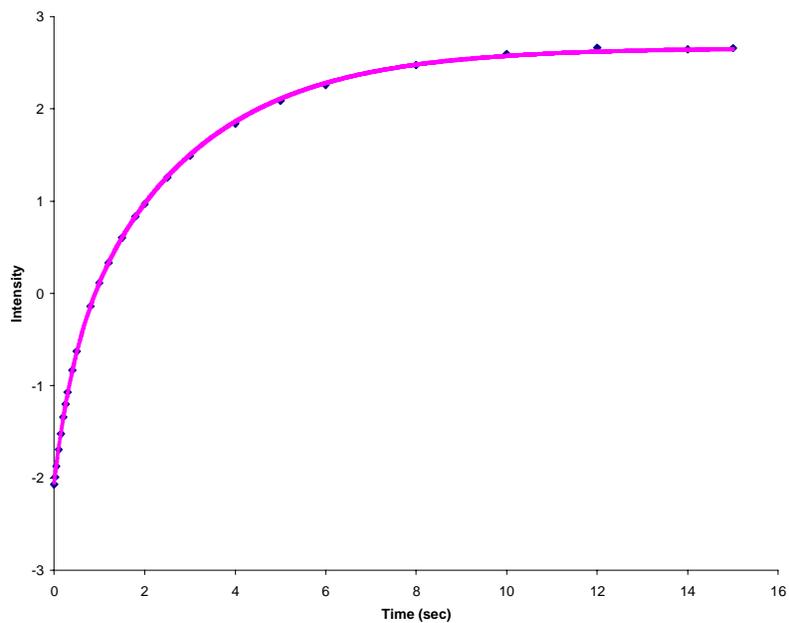


B

**Figure 26. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 3 at 72.6°C in D<sub>2</sub>O**

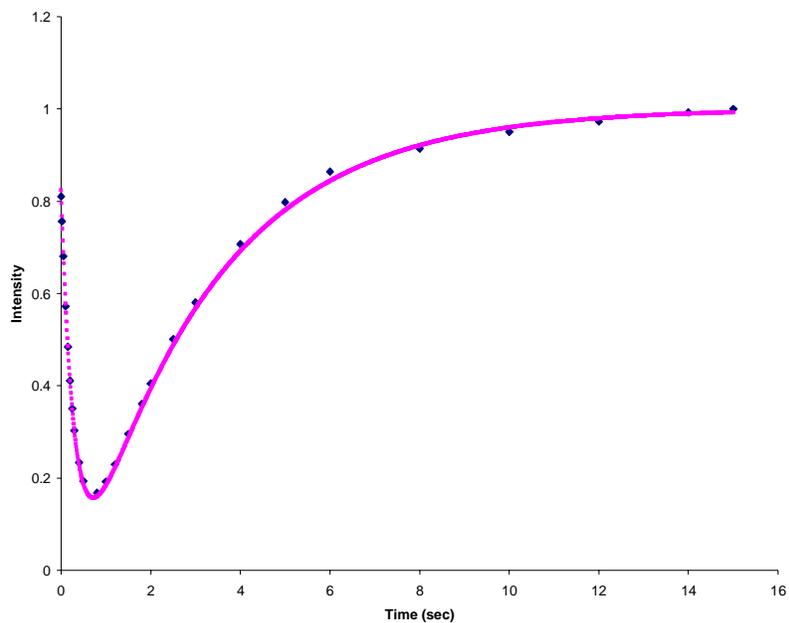


A

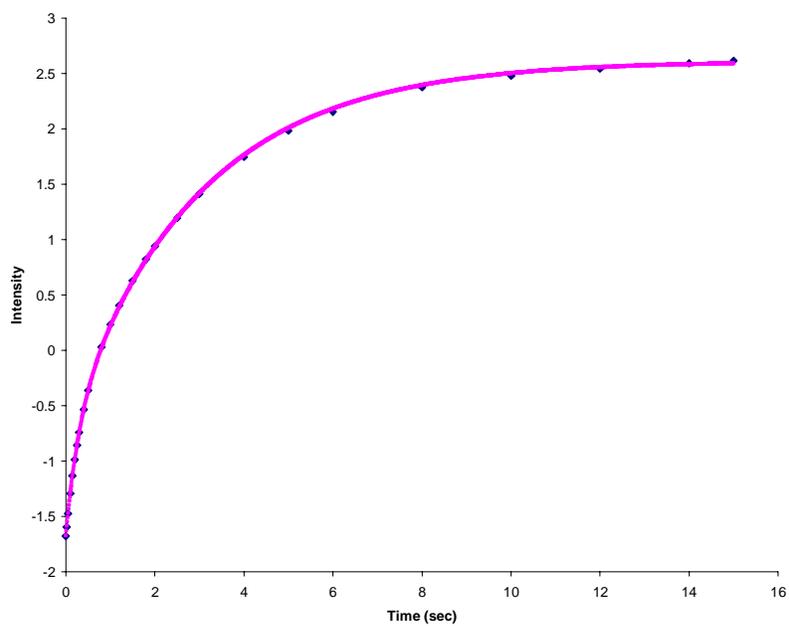


B

**Figure 27. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 3 at 78.0°C in D<sub>2</sub>O**

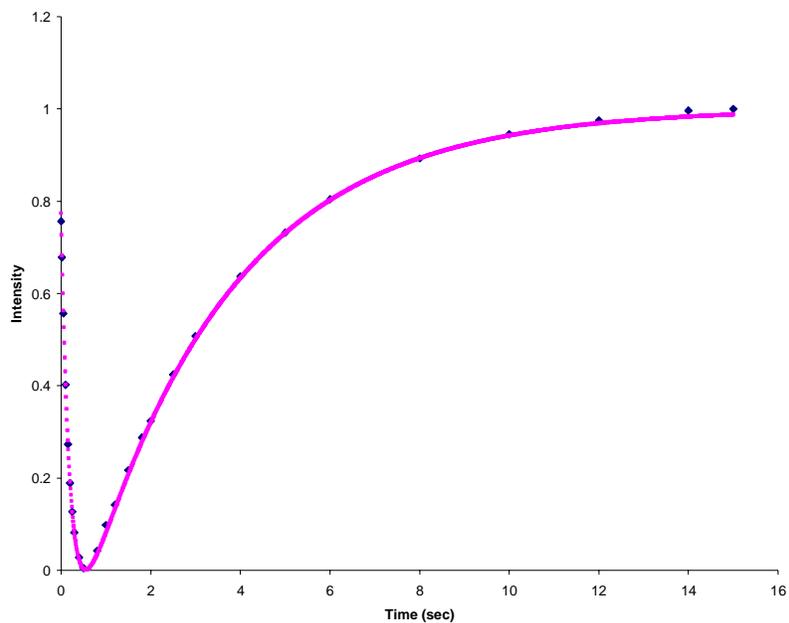


A

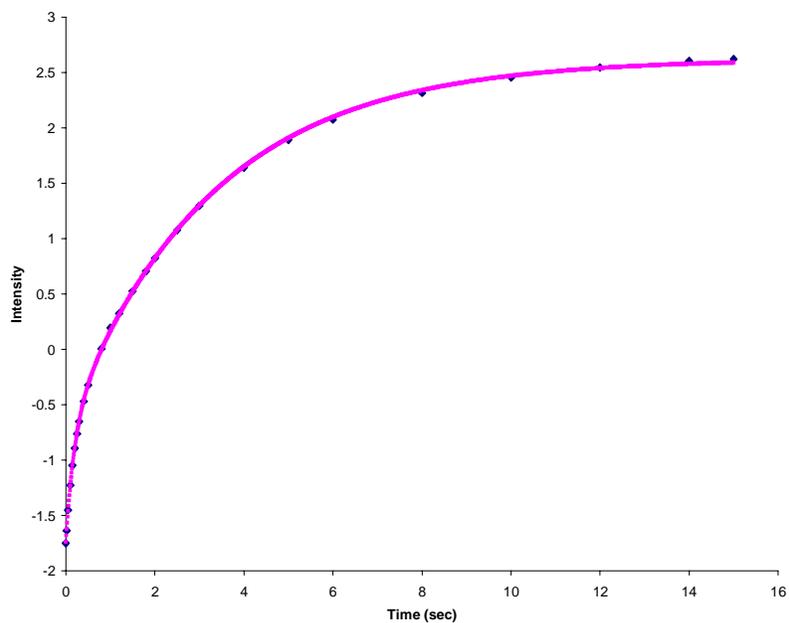


B

**Figure 28. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 3 at 83.0°C in D<sub>2</sub>O**

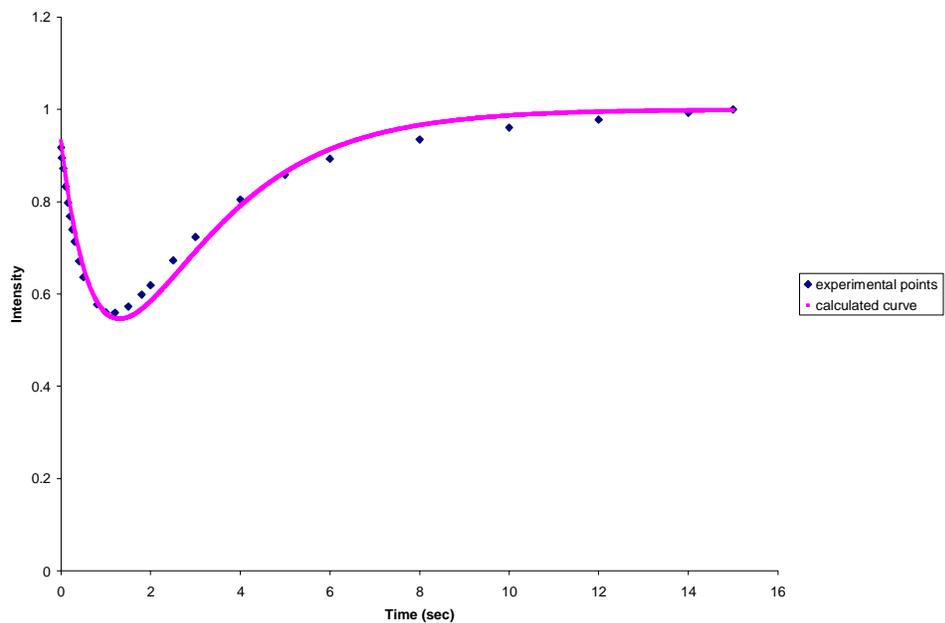


A

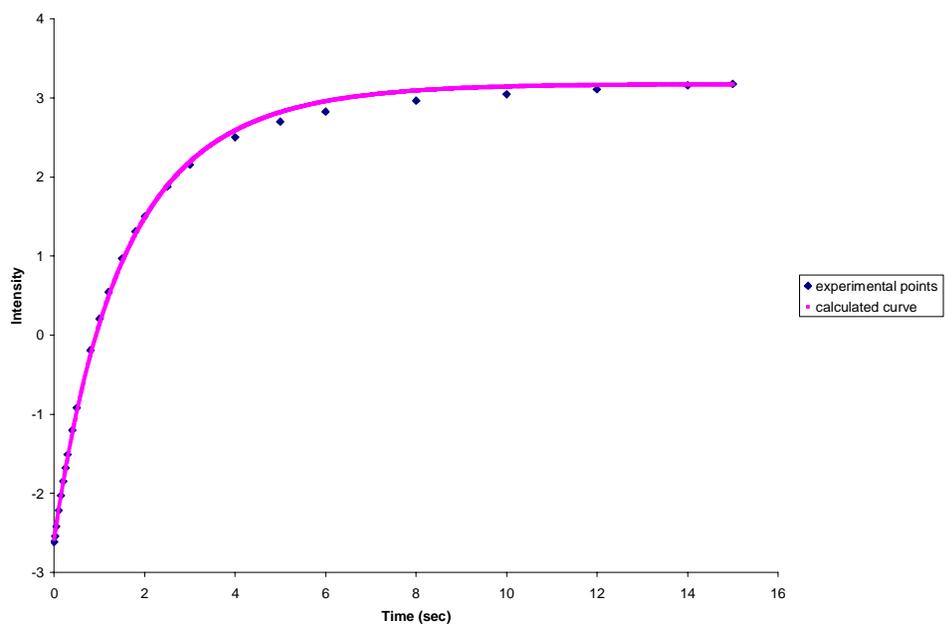


B

**Figure 29. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4a at 62.0°C in D<sub>2</sub>O**

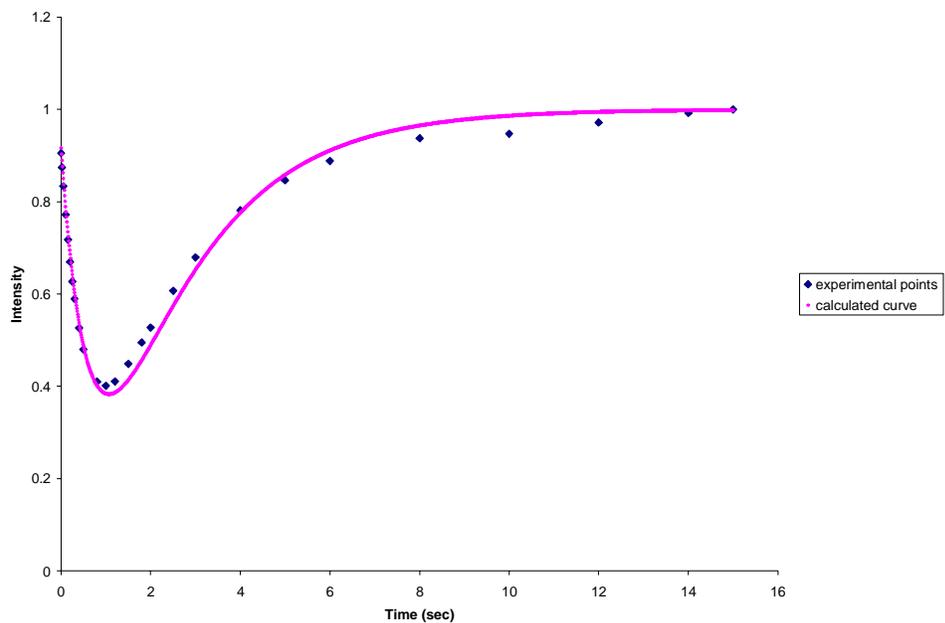


A

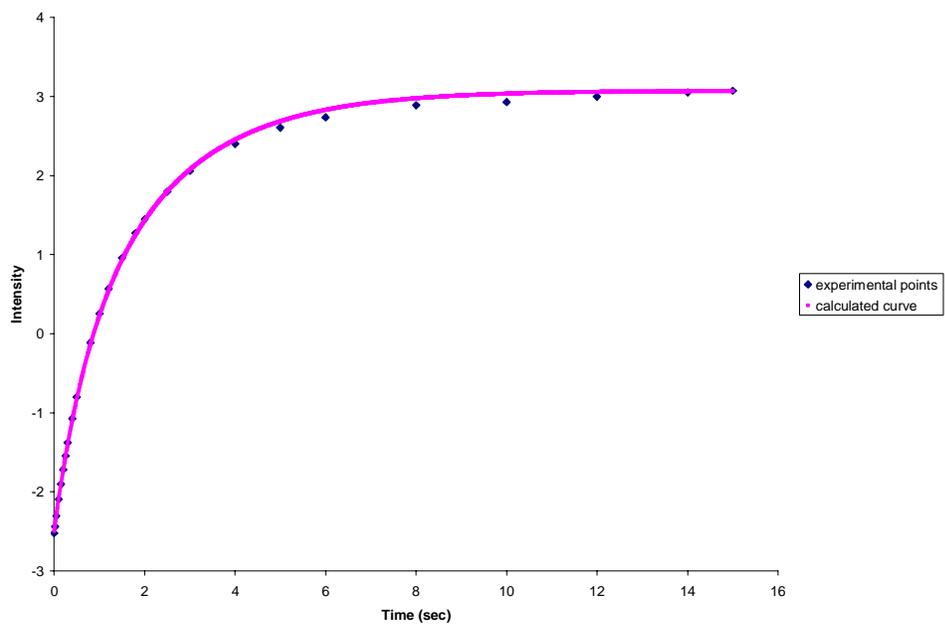


B

**Figure 30. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4a at 67.3°C in D<sub>2</sub>O**

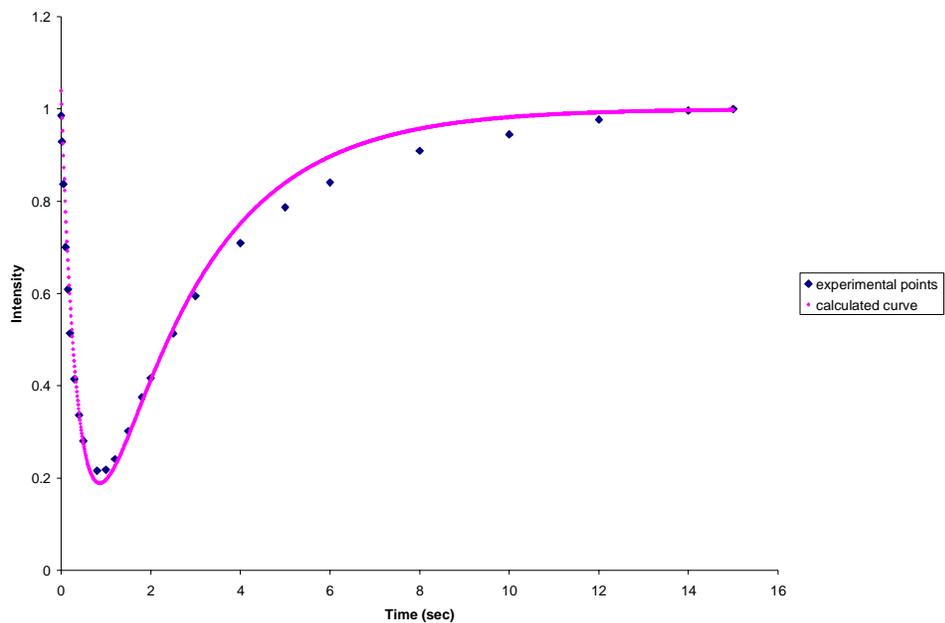


A

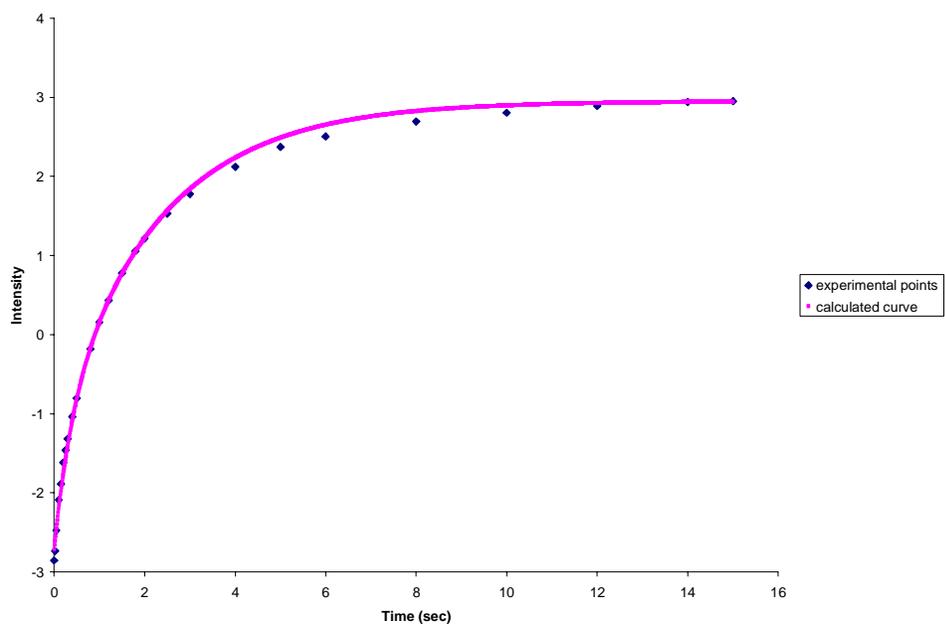


B

**Figure 31. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4a at 72.6°C in D<sub>2</sub>O**

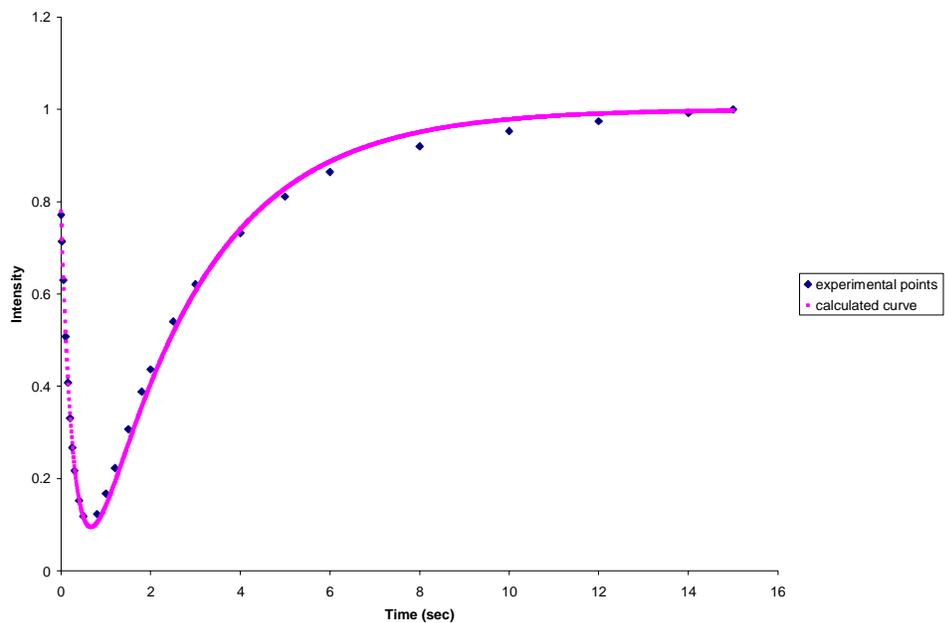


A

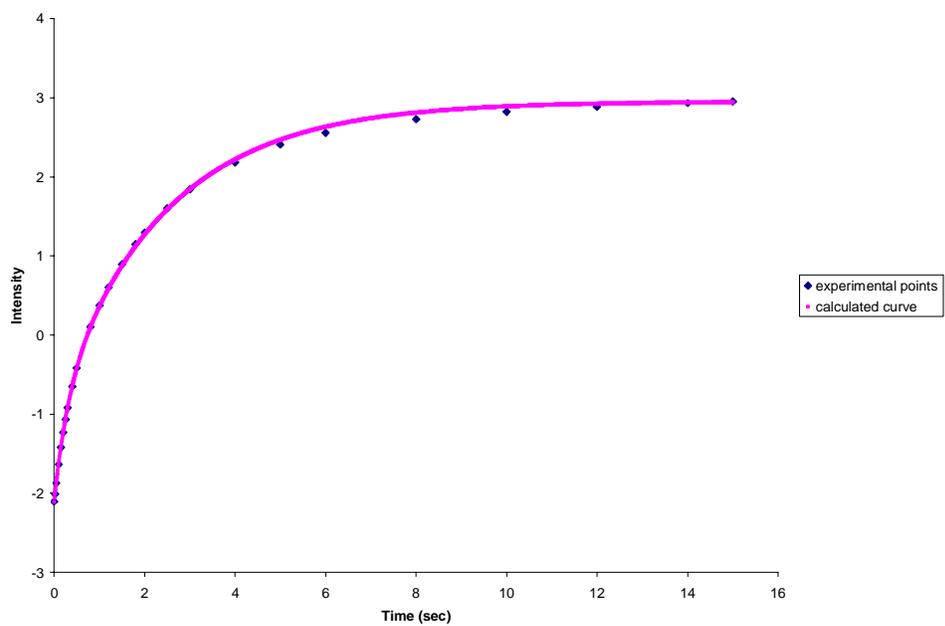


B

**Figure 32. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4a at 78.0°C in D<sub>2</sub>O**

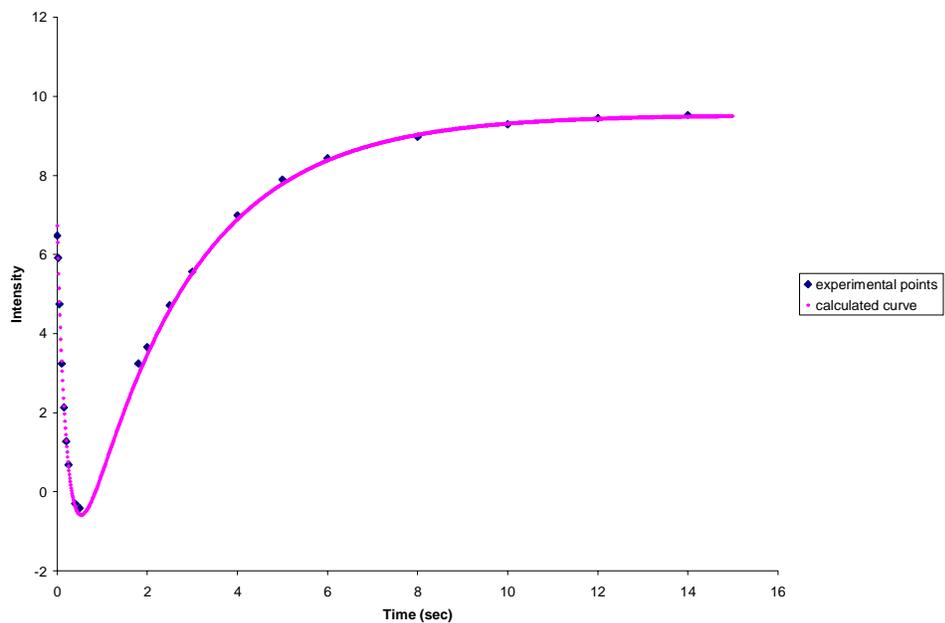


A

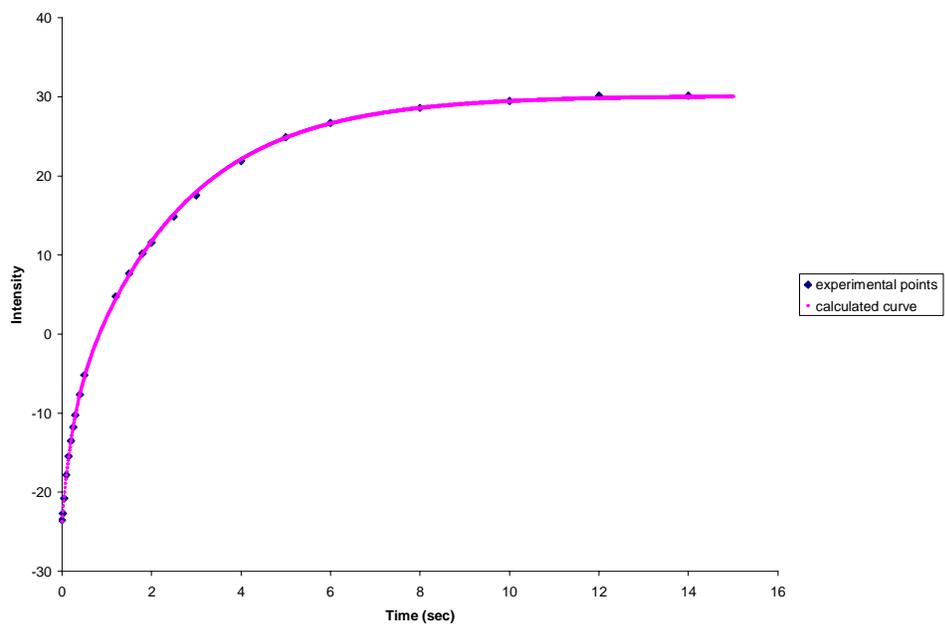


B

**Figure 33. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4a at 83.0°C in D<sub>2</sub>O**

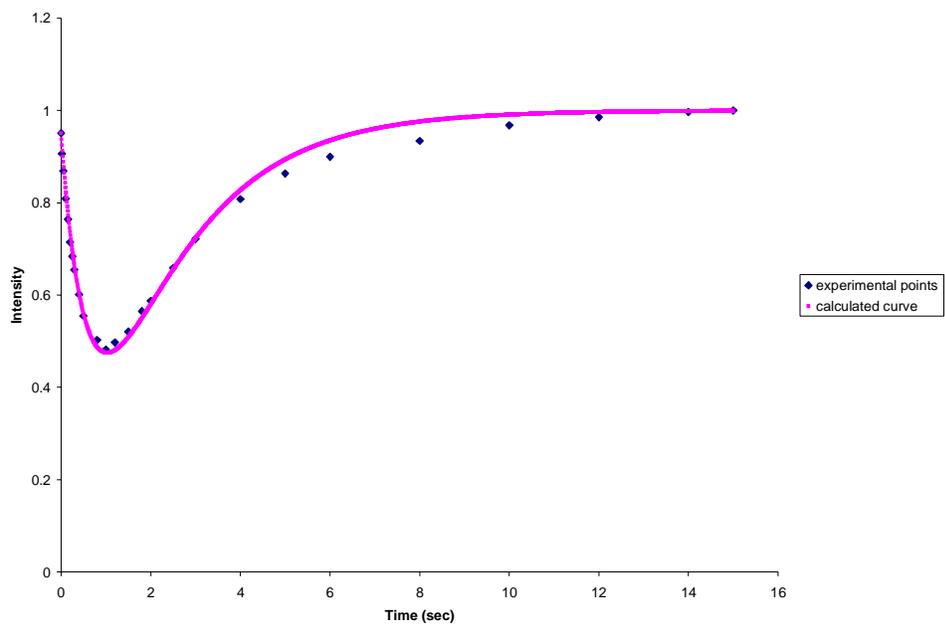


A

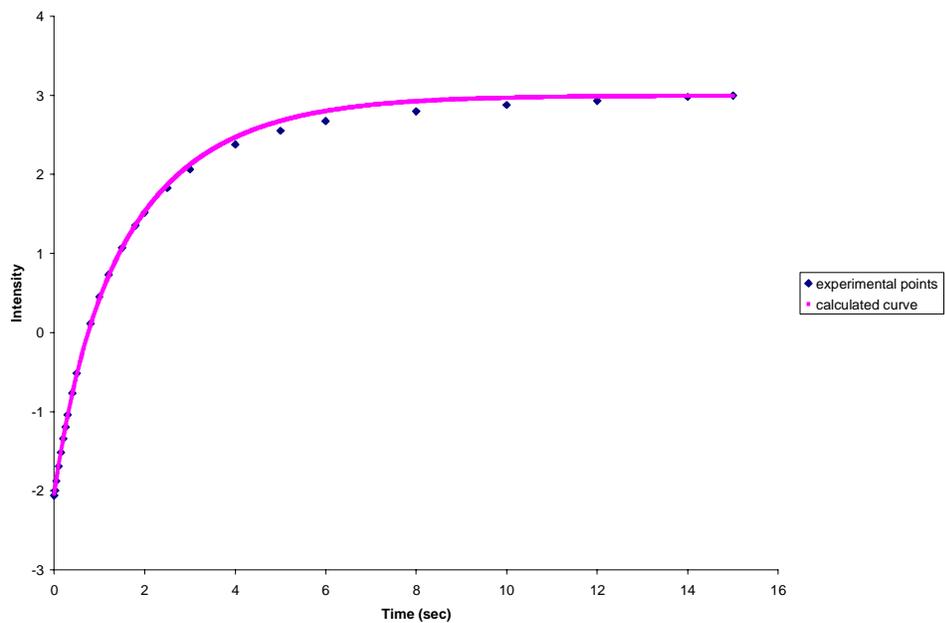


B

**Figure 34. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4a at 67.3°C in phosphate buffer pH 7.4**

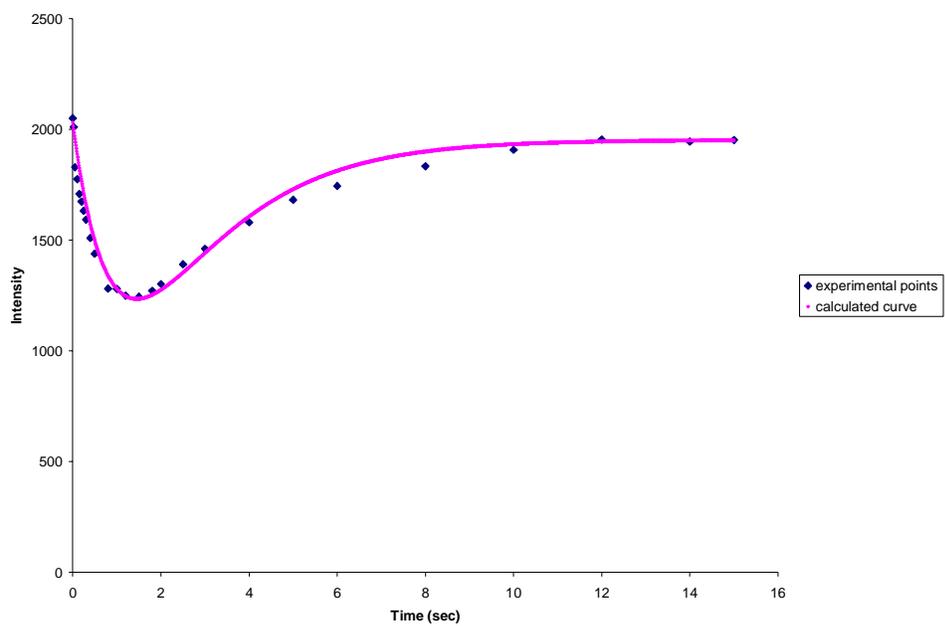


A

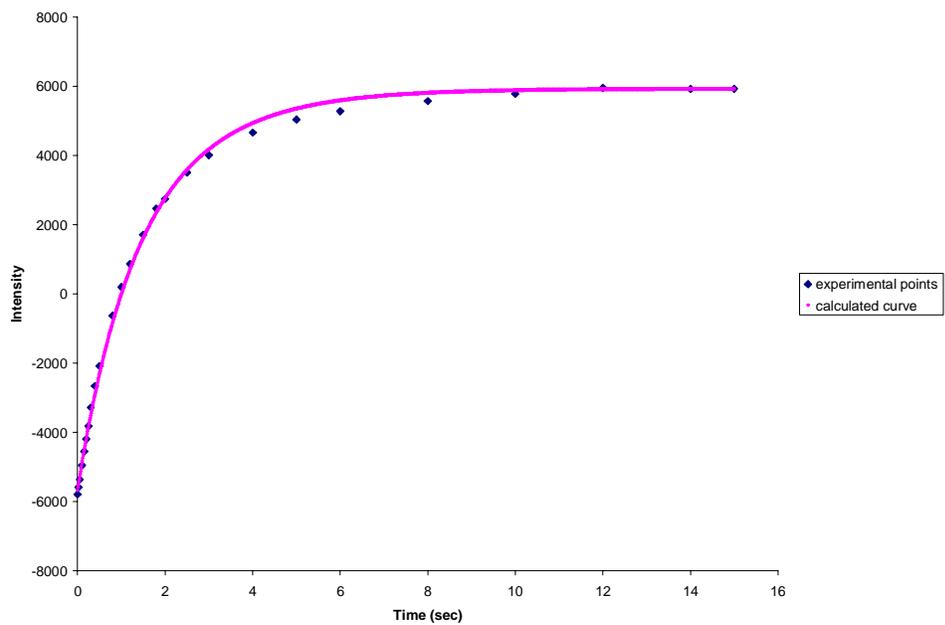


B

**Figure 35. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4b at 62.0°C in D<sub>2</sub>O**

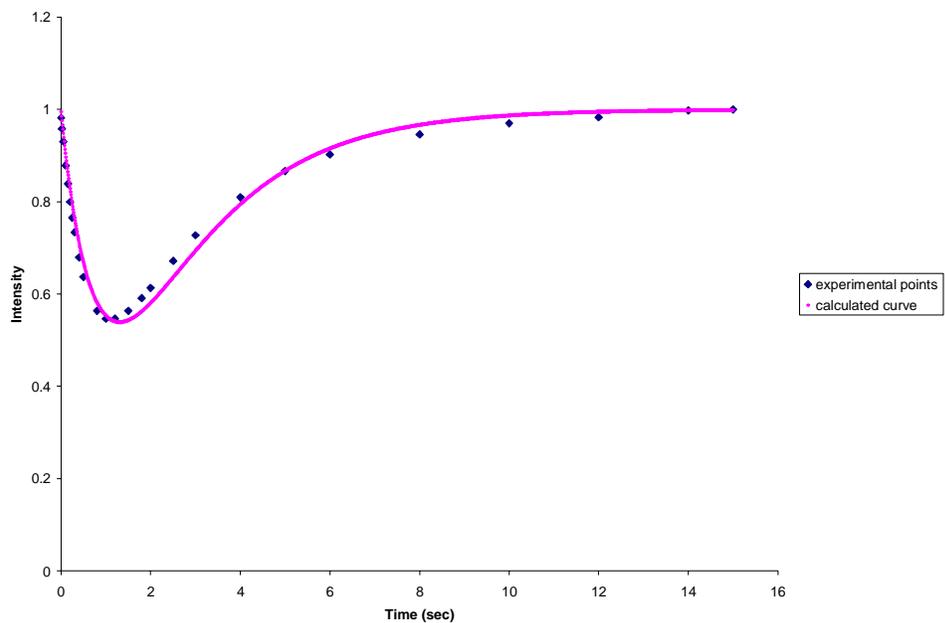


A

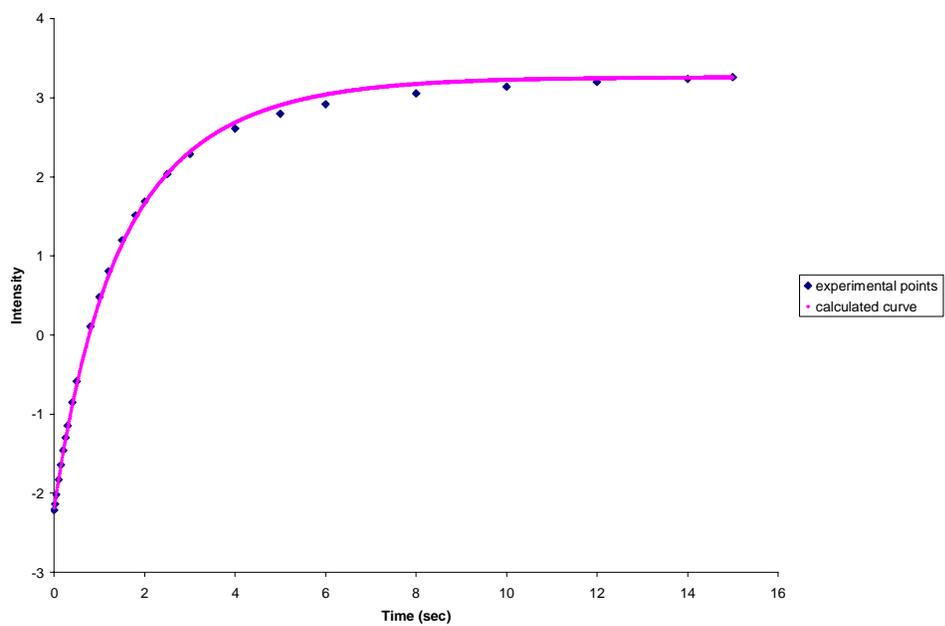


B

**Figure 36. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4b at 67.3°C in D<sub>2</sub>O**

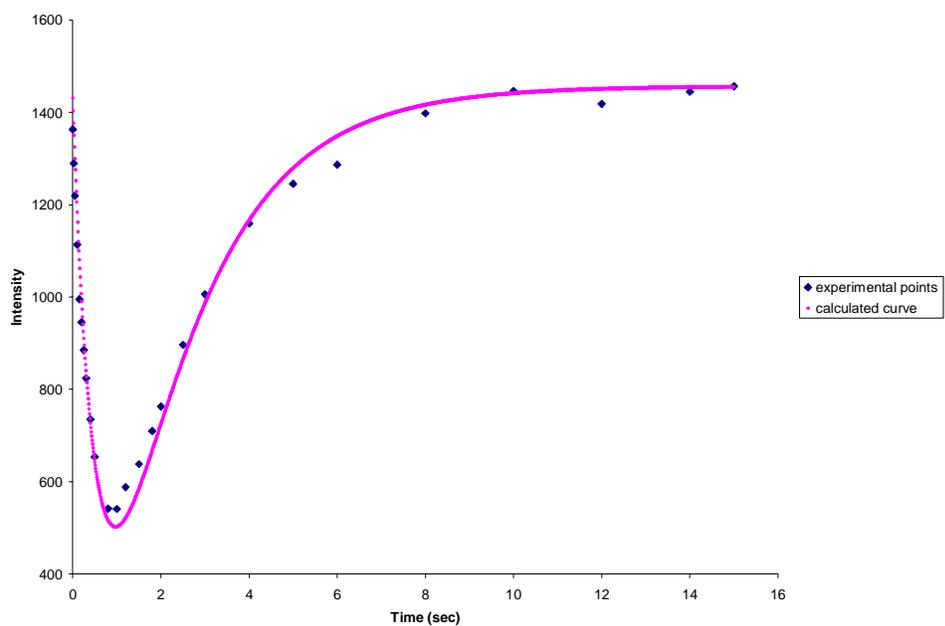


A

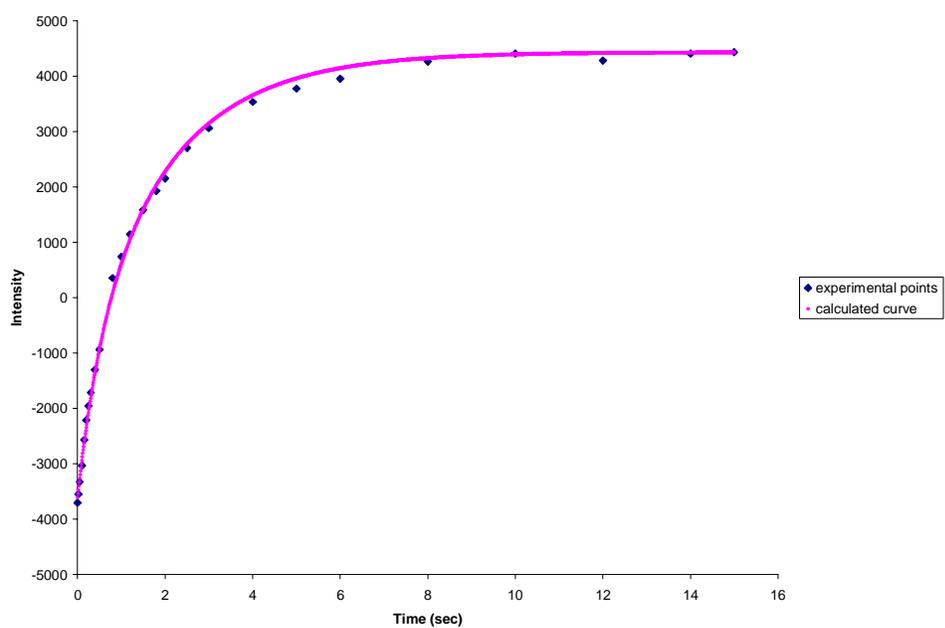


B

**Figure 37. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4b at 72.6°C in D<sub>2</sub>O**

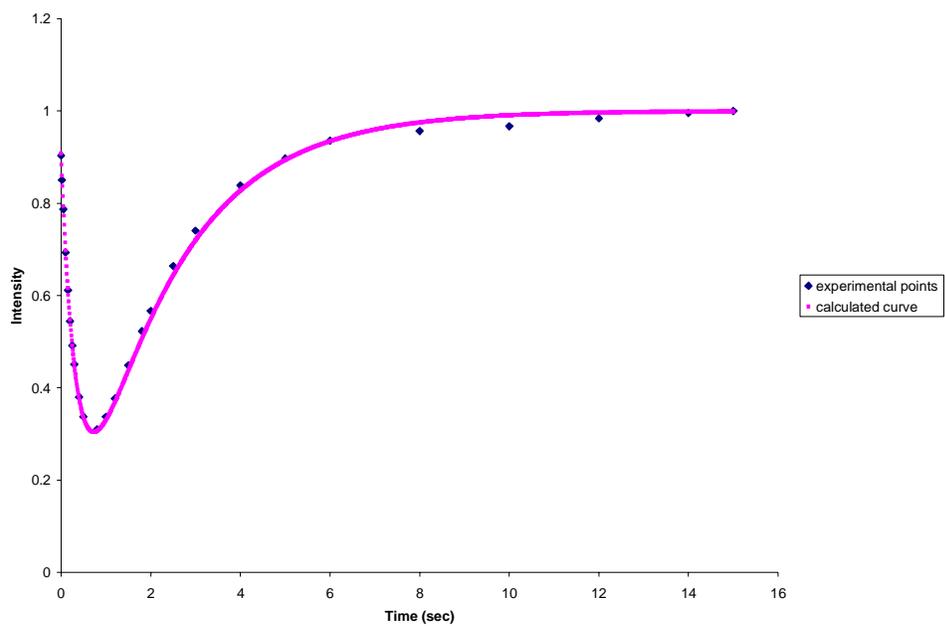


A

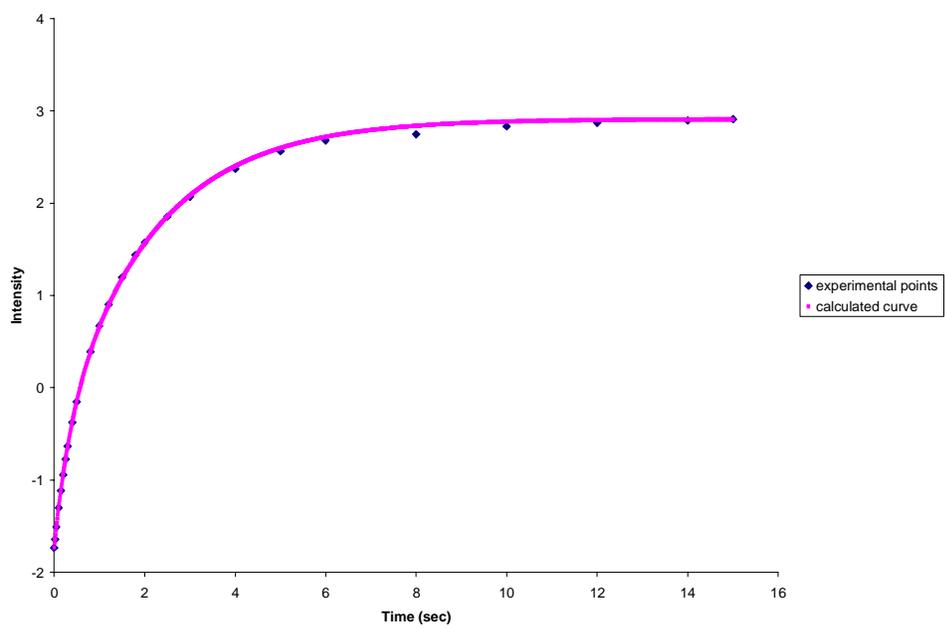


B

**Figure 38. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4b at 78.0°C in D<sub>2</sub>O**

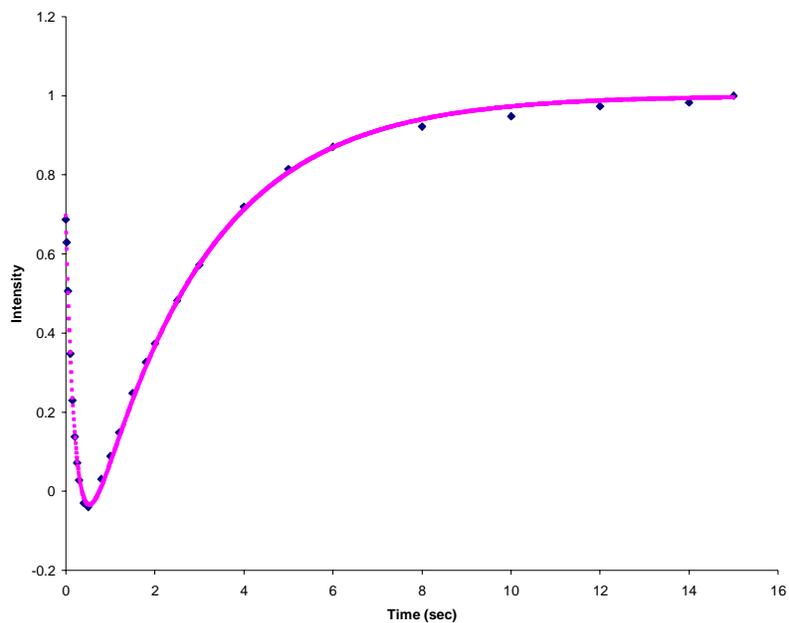


A

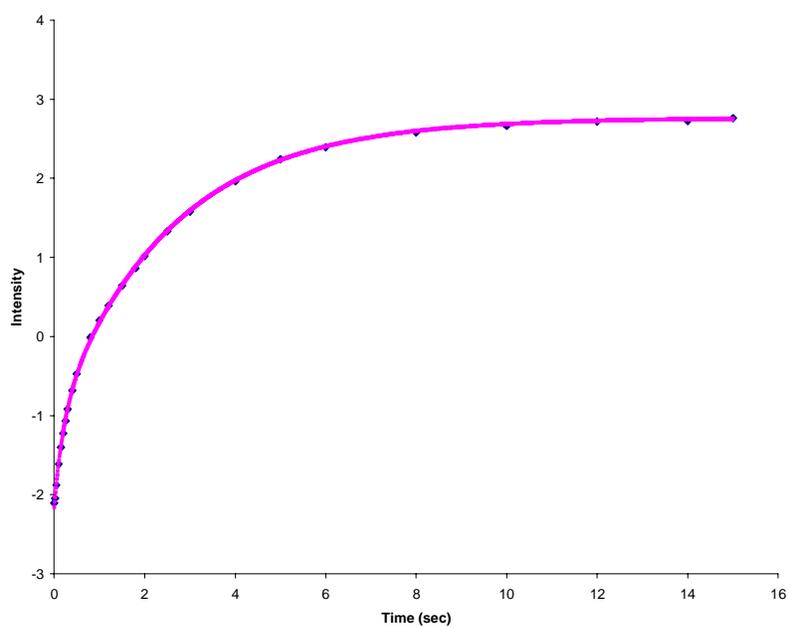


B

**Figure 39. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4b at 83.0°C in D<sub>2</sub>O**

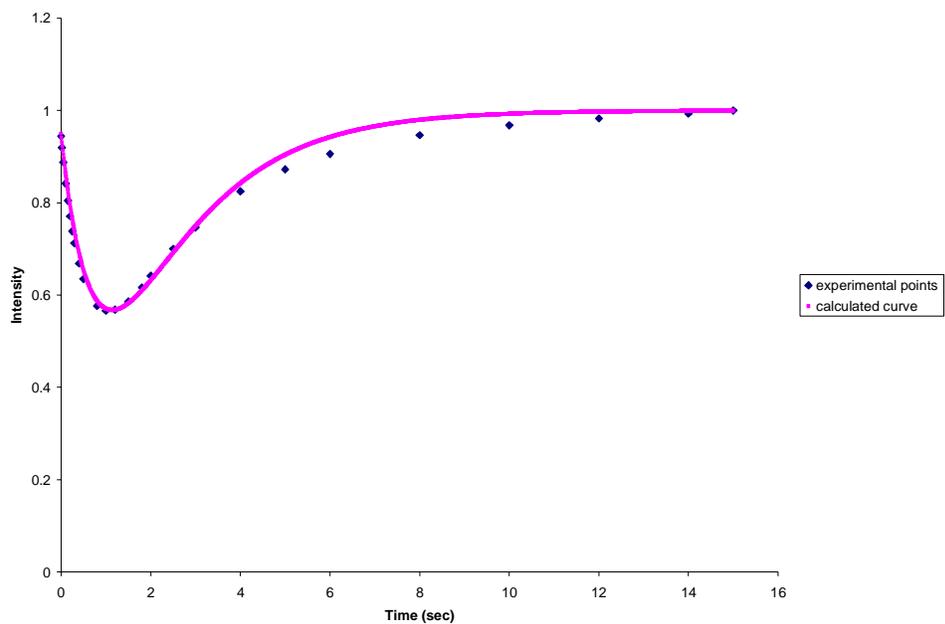


A

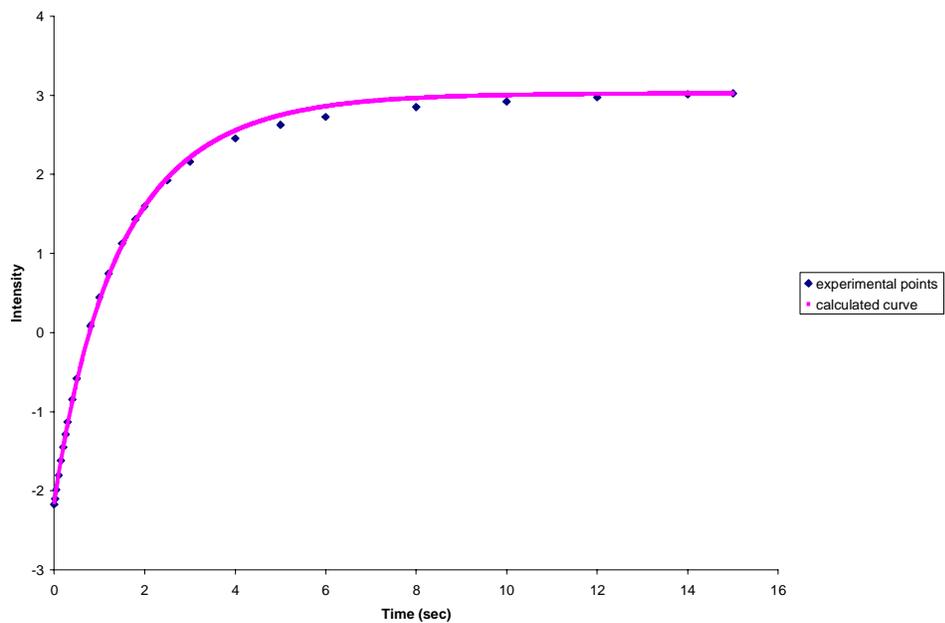


B

**Figure 40. Inversion recovery of non-inverted (*cis*) methylamide singlet (A) and inverted (*trans*) methylamide singlet (B) of 4b at 67.3°C in phosphate buffer pH 7.4**



A



B