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Z-Ala-NH^tPr. The mixed anhydride coupling procedure¹ between Z-Ala (8.92 g, 40 mmol) and isopropylamine (3.40 mL, 40 mmol), followed by recrystallization from AcOEt gave 8.36 g (79%) of Z-Ala-NH^tPr; mp 153 °C; $[\alpha]_D^{25} -9.0$ (c 1.53; CH₂Cl₂); ¹H NMR (200 MHz, CDCl₃) δ 1.12 (d, *J* = 6.5 Hz, 6H), 1.36 (d, *J* = 7.0 Hz, 3H), 3.97-4.17 (m, 2H), 5.08 (s, 2H), 5.40 (br. s, 1H), 5.90 (br. s, 1H), 7.32 (m, 5H); Anal. (C₁₄H₂₀N₂O₃) C, H, N.

Boc-Ala-Ala-NH^tPr. The coupling of the activated ester² Boc-Ala-OSu (2.28 g, 8 mmol) with Ala-NHⁱPr (prepared by hydrogenolysis of Z-Ala-NH^tPr (2.12 g, 8 mmol)), followed by recrystallization from DME, gave 2.04 g (85 %) of Boc-Ala-Ala-NH^tPr, mp 202 °C; $[\alpha]_D^{25} -43.3$ (c 1.0; MeOH); ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.02 (d, *J* = 6.5 Hz, 6H, NH^tPr), 1.15 (d, *J* = 7.0 Hz, 6H, Ala1 and Ala2), 1.36 (s, 9H, Boc), 3.77 (m, 1H, CH^tPr), 3.91 (m, 1H, CH_α Ala1), 4.16 (m, 1H, CH_α Ala2), 7.00 (d, *J* = 7.0 Hz, 1H, NH Ala1), 7.64 (d, *J* = 7.0 Hz, 1H, NH^tPr), 7.69 (d, *J* = 7.0 Hz, 1H, NH Ala2); Anal. (C₁₄H₂₇N₃O₄) C, H, N.

Boc-Val-Ala-Ala-NHⁱPr. Similarly, coupling² of Boc-Val-OSu (1.82 g, 5.8 mmol) and with HCl, Ala-Ala-NHⁱPr (prepared by acidolysis of Boc-Ala-Ala-NH^tPr (1.75 g, 5.8 mmol), gave 1.67 g (72 %) of Boc-Val-Ala-Ala-NH^tPr, mp decomp. 230 °C (from DME); $[\alpha]_D^{25} -19.8$ (c 1.0; DMF); ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.79 (d, *J* = 8 Hz, 3H, CH₃ Val), 0.83 (d, *J* = 8 Hz, 3H, CH₃ Val), 1.00 (d, *J* = 8 Hz, 3H, CH₃^tPr), 1.03 (d, *J* = 8 Hz, 3H, CH₃ⁱPr), 1.14 (d, *J* = 8 Hz, 3H, CH₃ Ala3), 1.18 (d, *J* = 8 Hz, 3H, CH₃ Ala2), 1.36 (s, 9H, Boc), 1.92 (m, 1H, CH_β Val), 3.77 (m, 2H, CH_α Val and CH^tPr), 4.15 (quint, *J* = 6.8 Hz, 1H, CH_α Ala3), 4.26 (quint, *J* = 6.8 Hz, 1H, CH_α Ala2), 6.71 (d, *J* = 8.0 Hz, 1H, NH Val), 7.60 (d, *J* = 6.8 Hz, 1H, NH^tPr), 7.81 (d, *J* = 8.0 Hz, 1H, NH Ala3), 7.90 (d, *J* = 7.0 Hz, 1H, NH Ala2); Anal. (C₁₉H₃₆N₄O₅•H₂O) C, H, N.

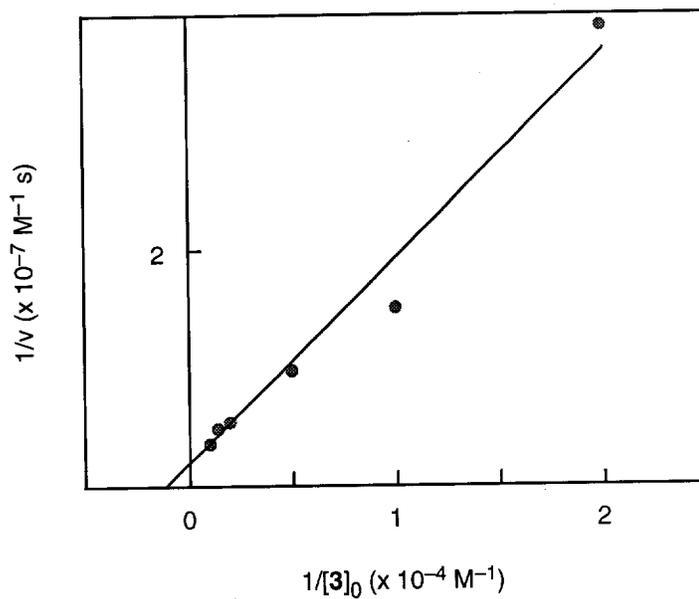
Boc-Ala-Pro-OBzl. Similarly, coupling² of Boc-Ala-OSu (2.86 g, 10 mmol) with HCl, ProOBzl (2.41g, 10 mmol), followed by flash chromatography [CH₂Cl₂/MeOH (98/2)] gave 3.29 g (87 %) of Boc-Ala-Pro-OBzl; oil, $[\alpha]_D^{25} -67.2$ (c 3.35, CH₂Cl₂); ¹H NMR (CDCl₃) (200 MHz) δ 1.27 (d, *J* = 7.0 Hz, 3H), 1.40 (s, 9H), 1.98 (m, 3H), 2.16 (m, 1H), 3.60 (m,

2H), 4.43 (quint, $J = 8$ Hz, 1H), 4.57 (dd, $J = 8$ Hz and $J = 4$ Hz, 1H), 5.07 and 5.18 (AB system, $J_{AB} = 7$ Hz, 2H), 5.32 (d, $J = 8.0$ Hz, 1H), 7.32 (br. s, 5H); Anal. ($C_{20}H_{28}N_2O_5$) C, H, N.

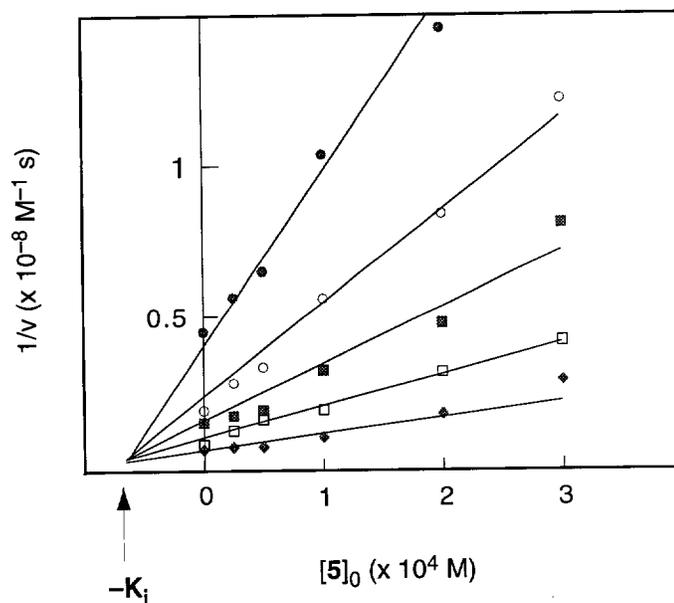
Boc-Ala-Ala-Pro-OBzl. Similarly, coupling² of Boc-Ala-OSu (1.82 g, 6.36 mmol) with HCl, Ala-Pro-OBzl (1.99g, 6.36 mmol, prepared by acidolysis of Boc-Ala-Pro-OBzl), followed by crystallization from diisopropyl ether, gave 1.95 g (69 %) of Boc-Ala-Ala-Pro-OBzl; mp 121 °C, $[\alpha]_D^{25} -123.9$ (c 1.01, MeOH); ¹H NMR (500 MHz, DMSO- d_6) (two conformers in a 90:10 ratio) δ 1.13 (d, $J = 8.0$ Hz, 6H), 1.35 (s, 9H), 1.78 (m, 1H), 1.88 (m, 2H), 2.16 (m, 1H), 3.48 (m, 1H), 3.61 (m, 1H), 3.92 (quint, $J = 8$ Hz, 1H), 4.33 (dd, $J = 9$ Hz and $J = 4$ Hz, 1H), 4.48 (quint, $J = 8$ Hz, 1H), 5.08 and 5.10 (AB system, $J_{AB} = 7$ Hz, 2H), 6.88 (d, $J = 8.0$ Hz, 1H), 7.34 (m, 5H) 7.88 (d, $J = 8.0$ Hz, 1H); Anal. ($C_{23}H_{33}N_3O_6 \cdot 0.25$ H₂O) C, H, N.

Boc-Ala-Ala-Pro-OSu. This compound was prepared³ from Boc-Ala-Ala-Pro-OH (1.02 g, 2.8 mmol, obtained by hydrogenolysis of Boc-Ala-Ala-Pro-OBzl); 0.80 g (63 %) of Boc-Ala-Ala-Pro-OSu was obtained after recrystallization from isopropanol; mp 179 °C, $[\alpha]_D^{25} -91.4$ (c 1.00, CH₂Cl₂); ¹H NMR (500 MHz, DMSO- d_6) (two conformers in a 88 : 12 ratio) δ 1.13 (d, $J = 8.5$ Hz, 3H), 1.18(d, $J = 7.5$ Hz, 3H), 1.35 (s, 9H), 1.92-2.06 (m, 3H), 2.34 (m, 1H), 2.76 (s, 4H), 3.56 (m, 1H), 3.68 (m, 1H), 3.96 (quint, $J = 8.5$ Hz, 1H), 4.52 (quint, $J = 8$ Hz, 1H), 4.69 (dd, $J = 9$ Hz and $J = 4$ Hz 1H), 6.88 (d, $J = 8.0$ Hz, 1H), 7.98 (d, $J = 7.0$ Hz, 1H); Anal. ($C_{20}H_{30}N_4O_8$) C, H, N.

Z-Ala-Ala-Pro-O'Bu. The coupling² of Z-Ala-OSu (2.87 g, 8.96 mmol) with Ala-Pro-O'Bu (obtained after treatment of 4.17 g Fmoc-Ala-Pro-O'Bu by diethylamine in DMF), gave after recrystallization from diisopropyl ether 2.52 g (63 %) of Z-Ala-Ala-Pro-O'Bu; mp 145 °C, $[\alpha]_D^{25} -65.0$ (c 1.48, CH₂Cl₂); ¹H NMR (200 MHz) (CDCl₃) δ 1.34 (m, 6H), 1.42 (s, 9H), 1.90-2.18 (m, 4H), 3.60 (m, 2H), 4.21 (m, 1H), 4.38 (m, 1H), 4.66 (m, 1H), 5.06 (m, 1H), 5.37 (br s, 1H), 6.88 (d, $J = 6.5$ Hz, 1H), 7.32 (br s, 5H); Anal. ($C_{23}H_{33}N_3O_6$) C, H, N.



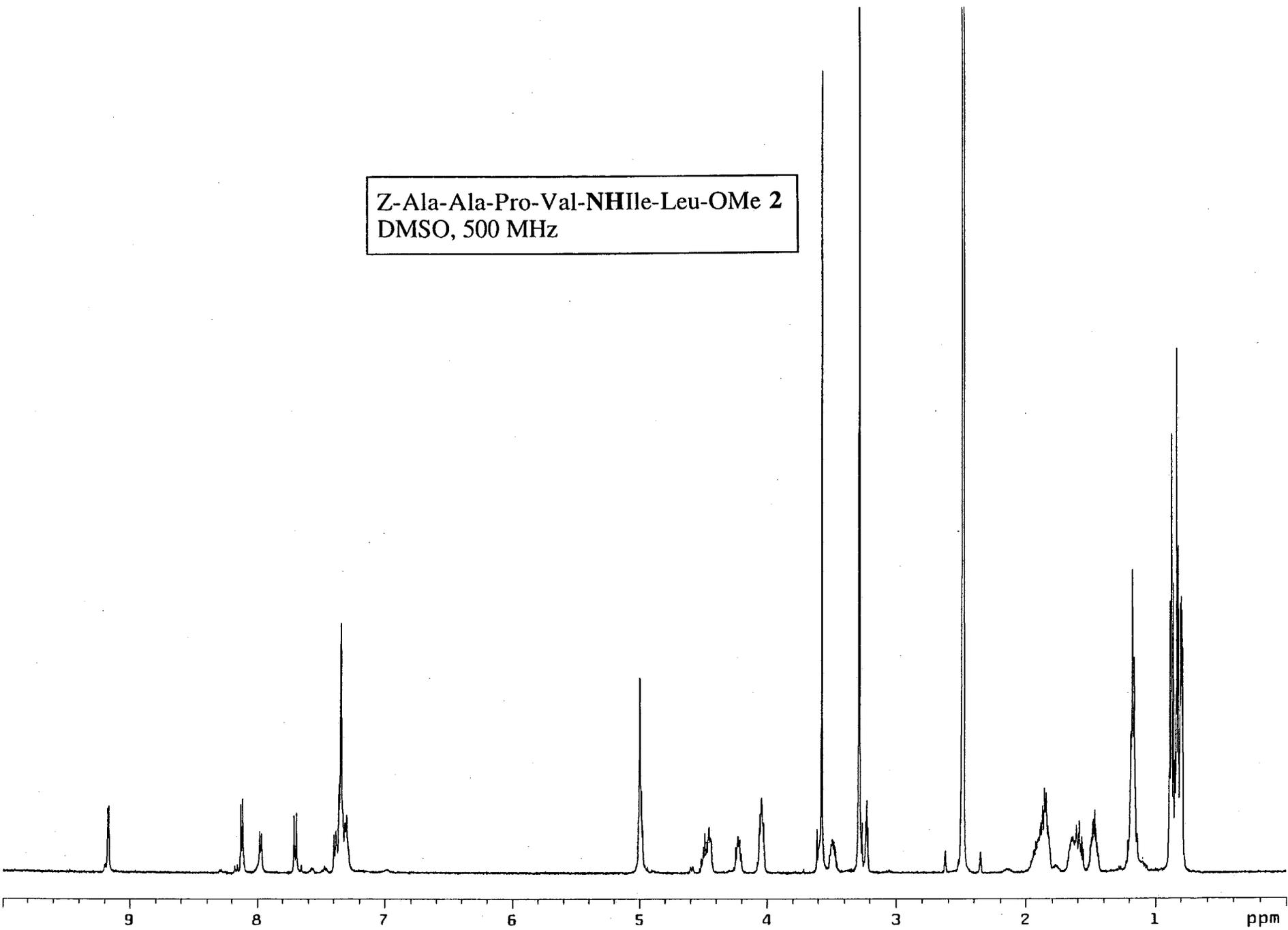
Lineweaver-Burk representation of the HLE-catalyzed hydrolysis of hydrazinopeptide **3** at pH 8.0 and 25 °C : (●) $[3]_0 = 5 \times 10^{-5}$ to 5×10^{-4} M and $[\text{HLE}] = 200$ nM.



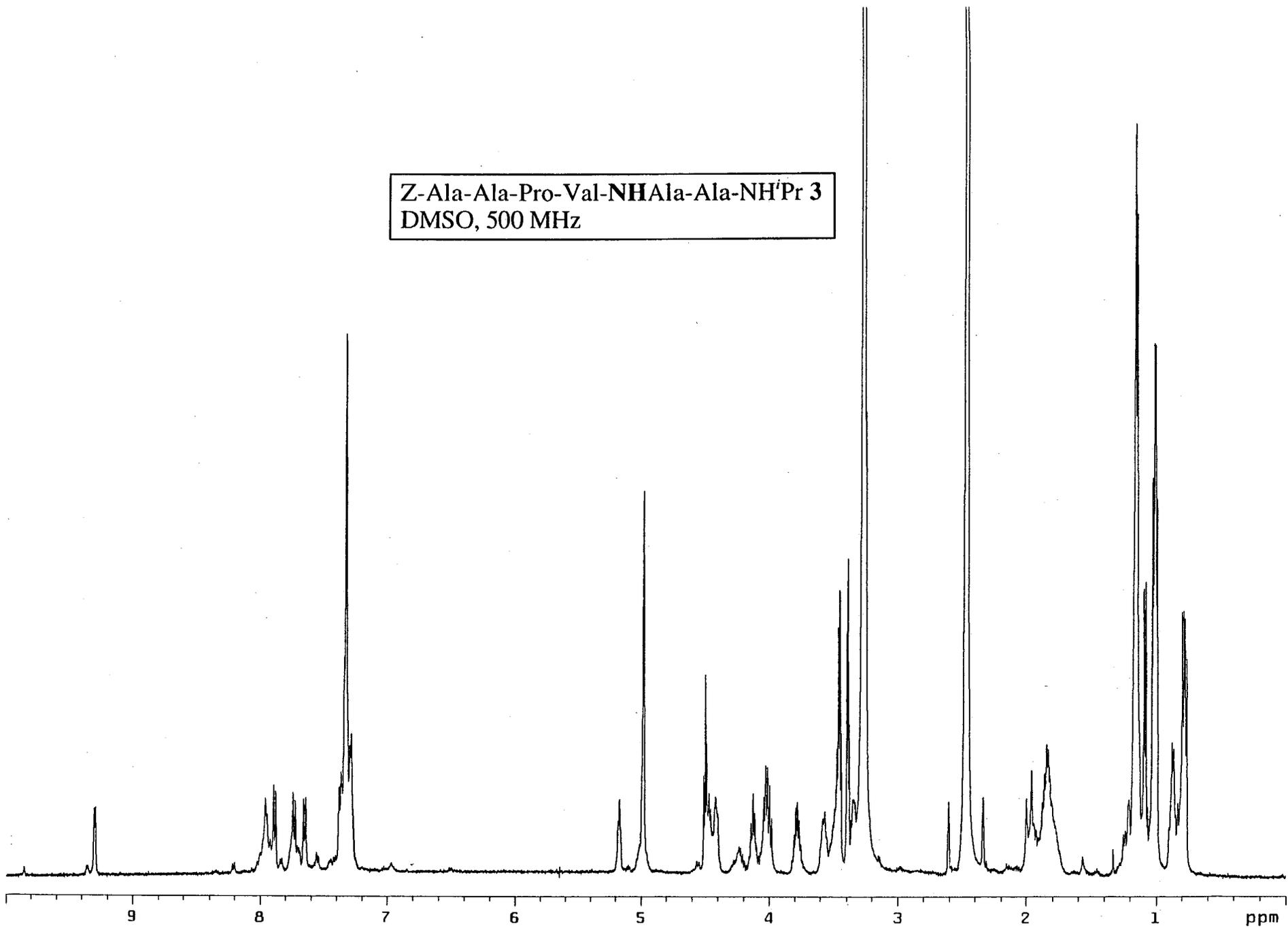
Dixon plot of the inhibition of HLE (20 nM) at pH 8.0 and 25 °C by **5** with the substrate MeO-Suc-Ala-Ala-Pro-Val-*p*-NA at concentrations (μM) of 20 (●); 50 (○); 100 (■); 200 (□); 500 (◆)

- ¹ Bodanszky, M.; Bodanszky, A. *The practice of Peptide Synthesis*; 2nd ed; Springer Verlag: Berlin, 1994, pp 93-94.
- ² Bodanszky, M.; Bodanszky, A. *The practice of Peptide Synthesis*; 2nd ed; Springer Verlag: Berlin, 1994, pp 110-111.
- ³ Bodanszky, M.; Bodanszky, A. *The practice of Peptide Synthesis*; 2nd ed; Springer Verlag: Berlin, 1994, pp 104-105.

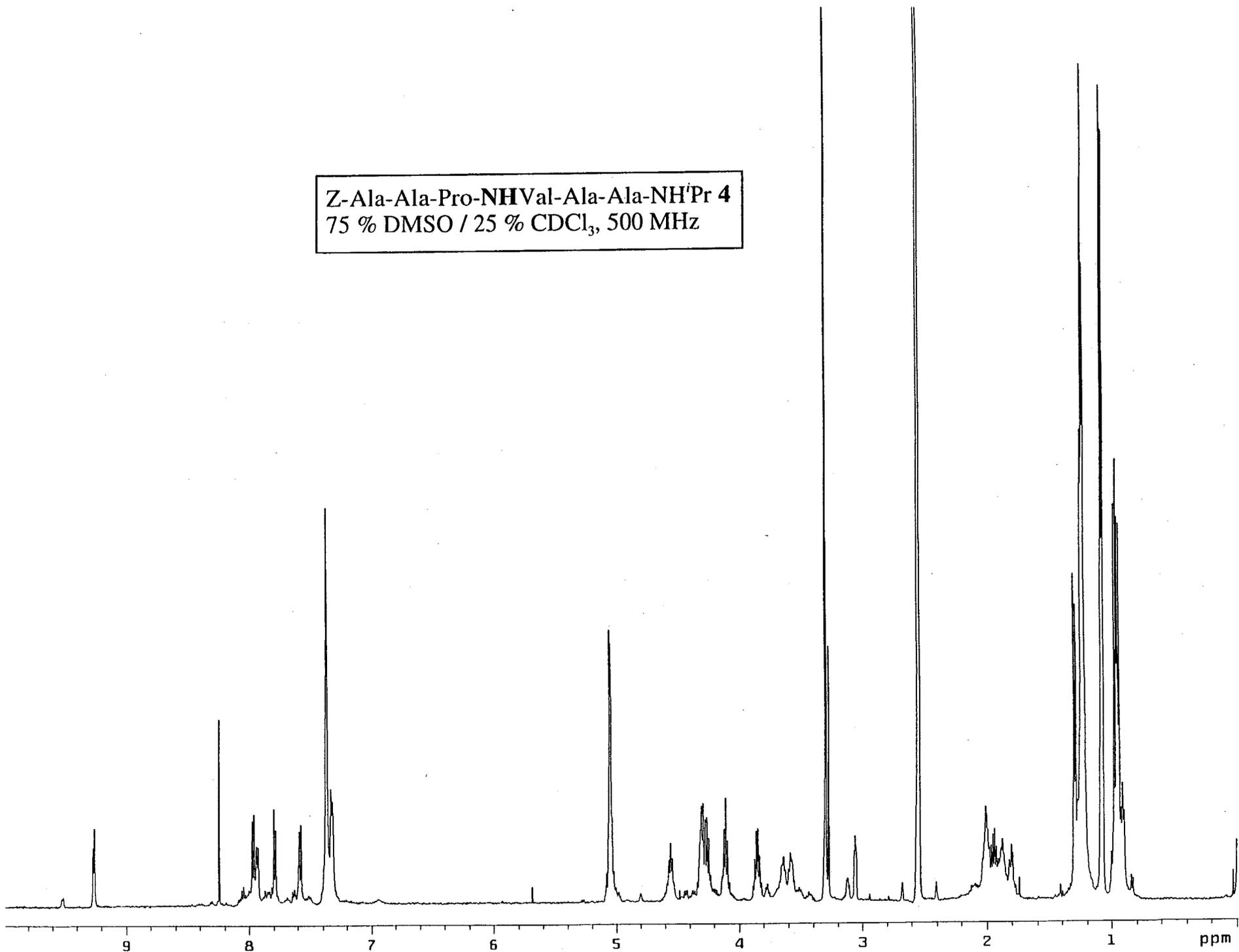
Z-Ala-Ala-Pro-Val-NHile-Leu-OMe 2
DMSO, 500 MHz

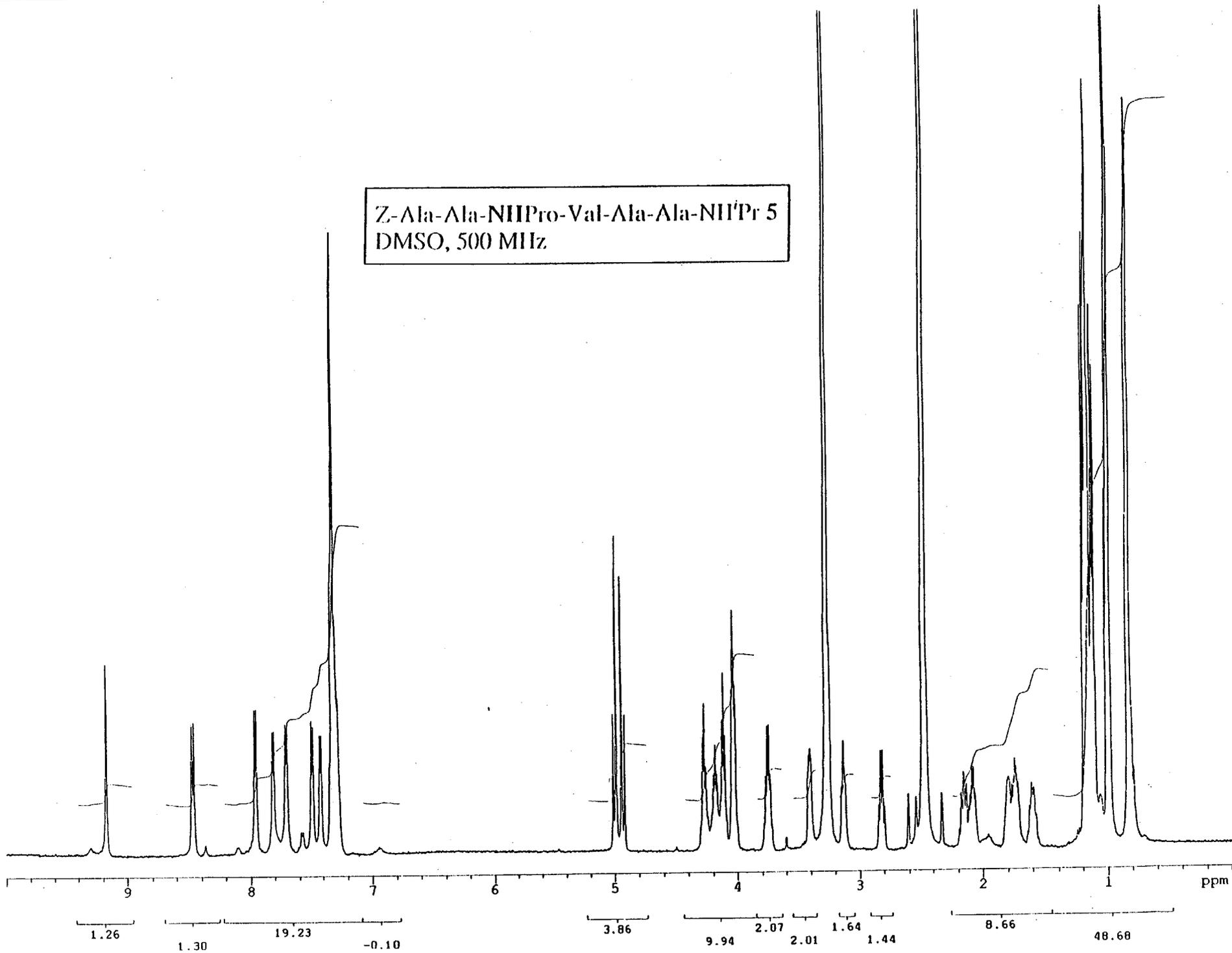


Z-Ala-Ala-Pro-Val-NHAla-Ala-NHⁱPr 3
DMSO, 500 MHz

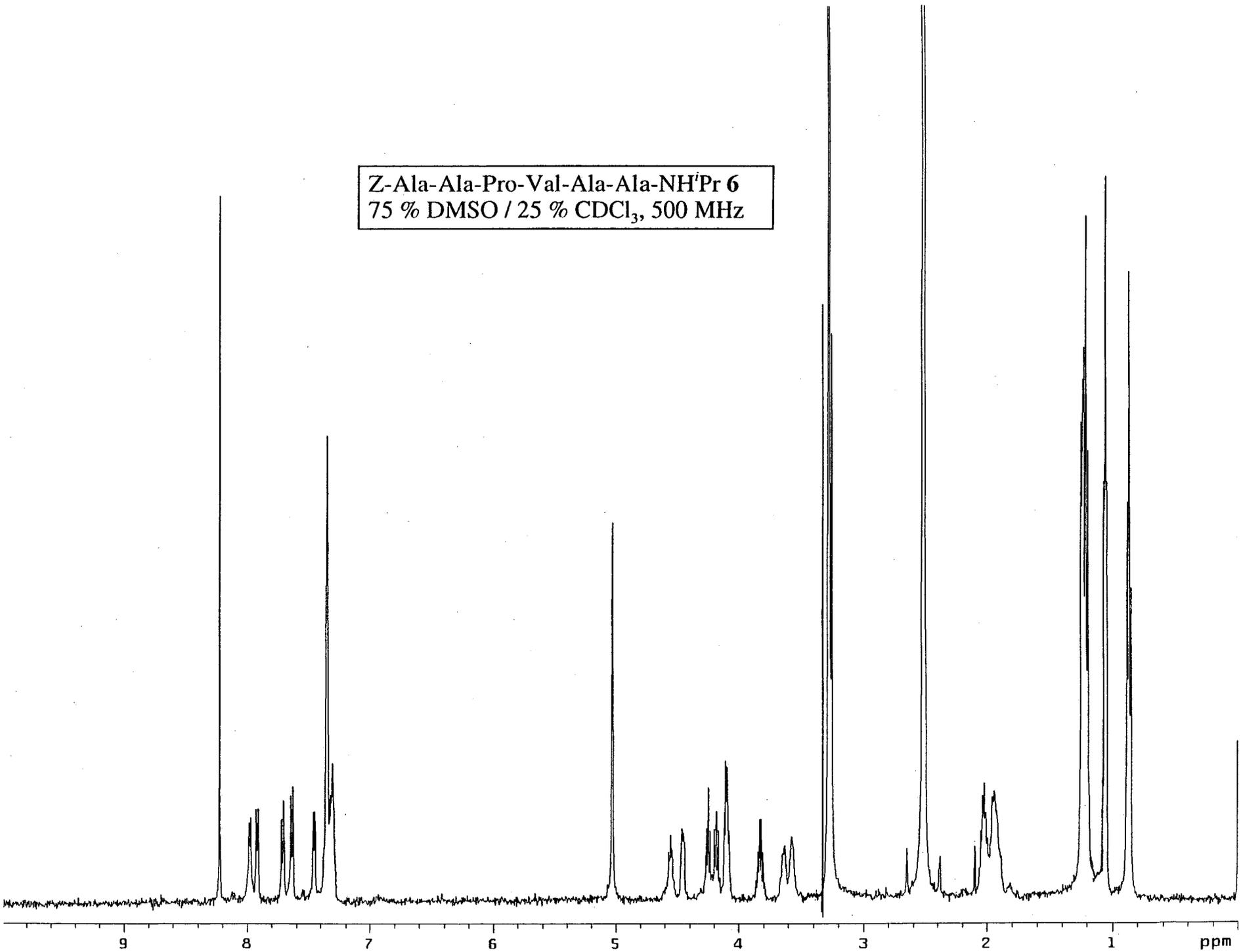


Z-Ala-Ala-Pro-NHVal-Ala-Ala-NH^tPr 4
75 % DMSO / 25 % CDCl₃, 500 MHz

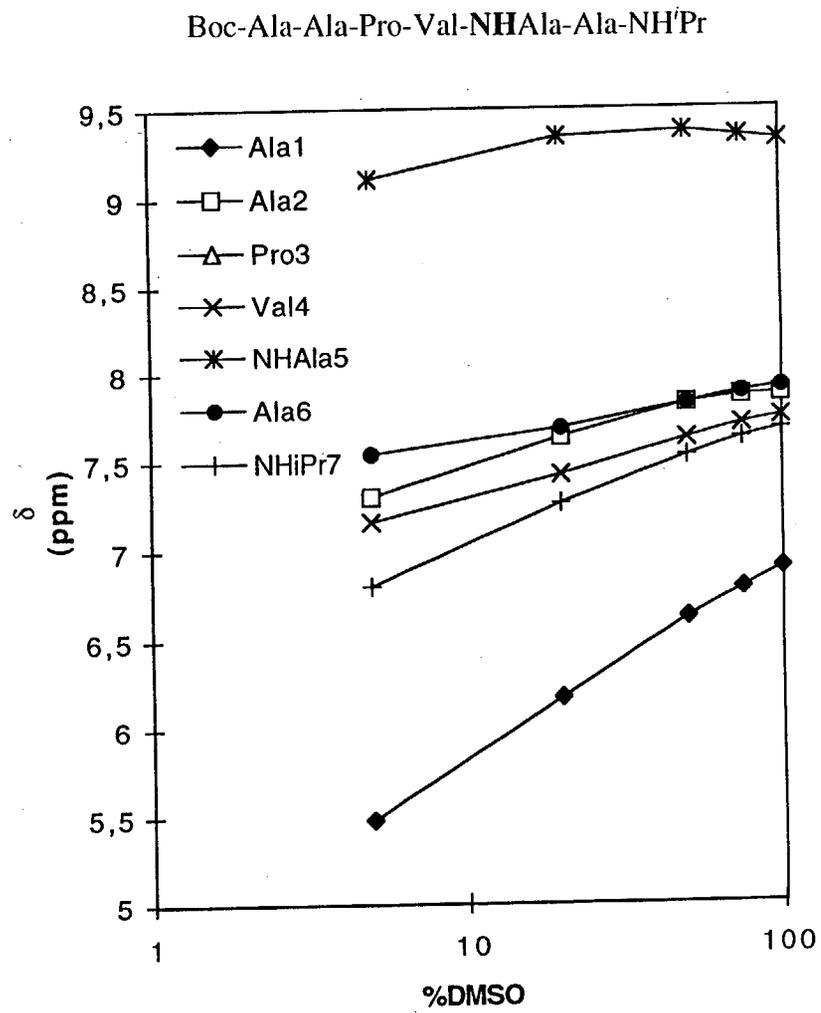
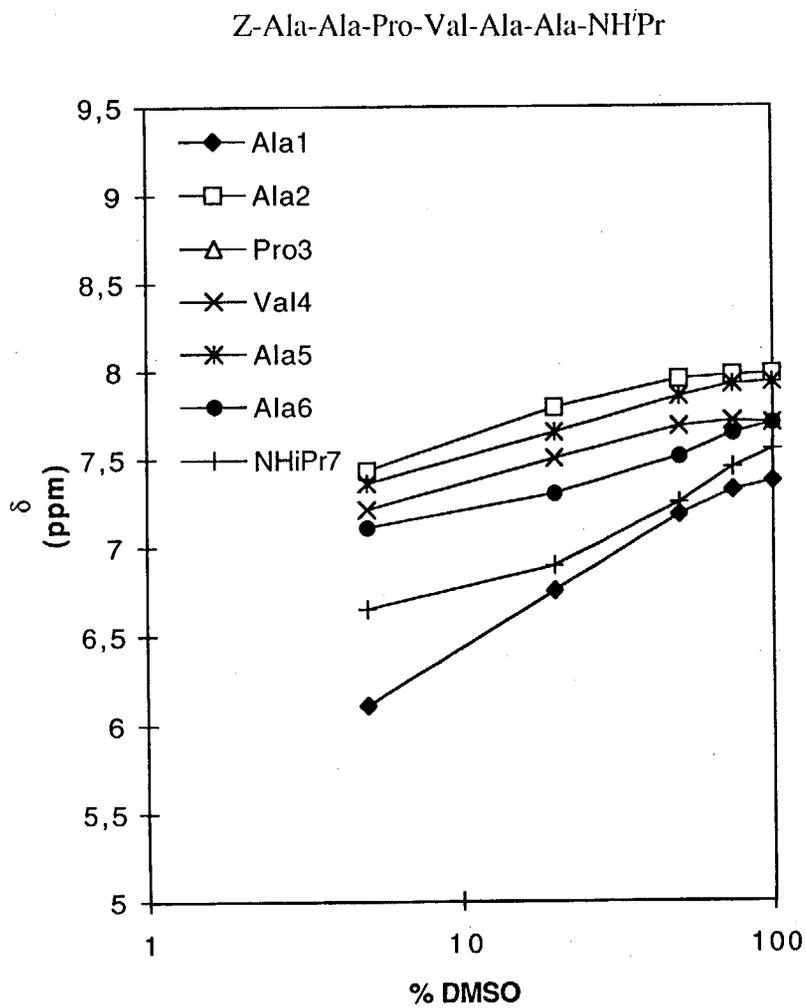




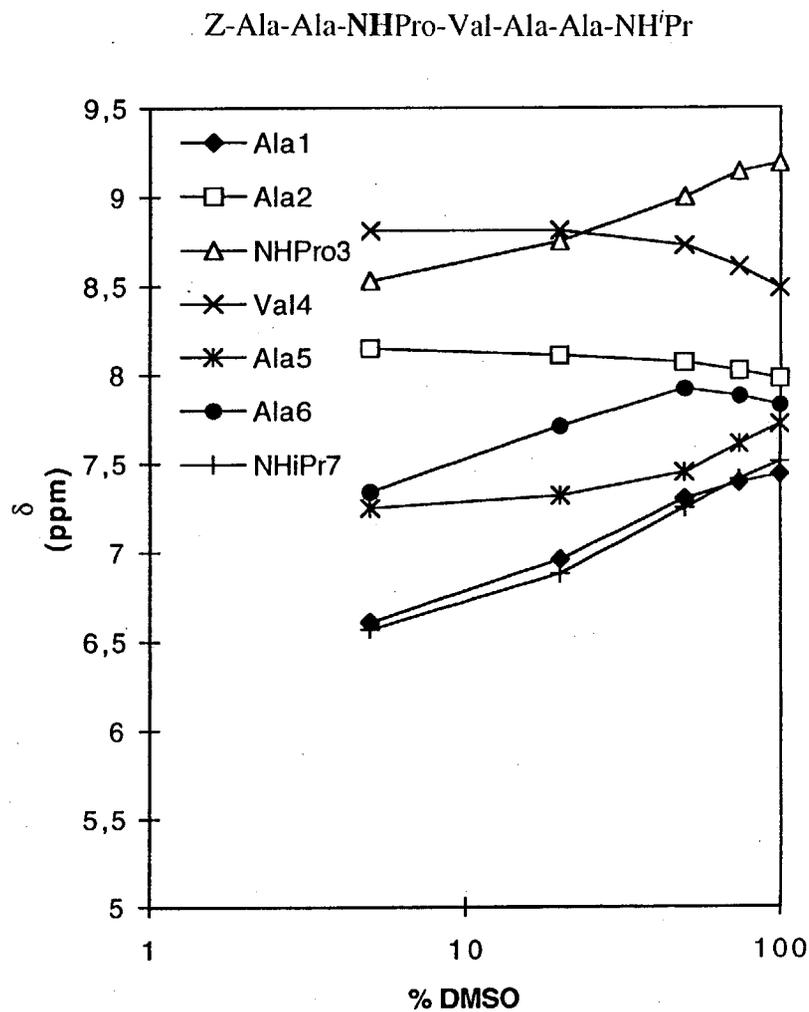
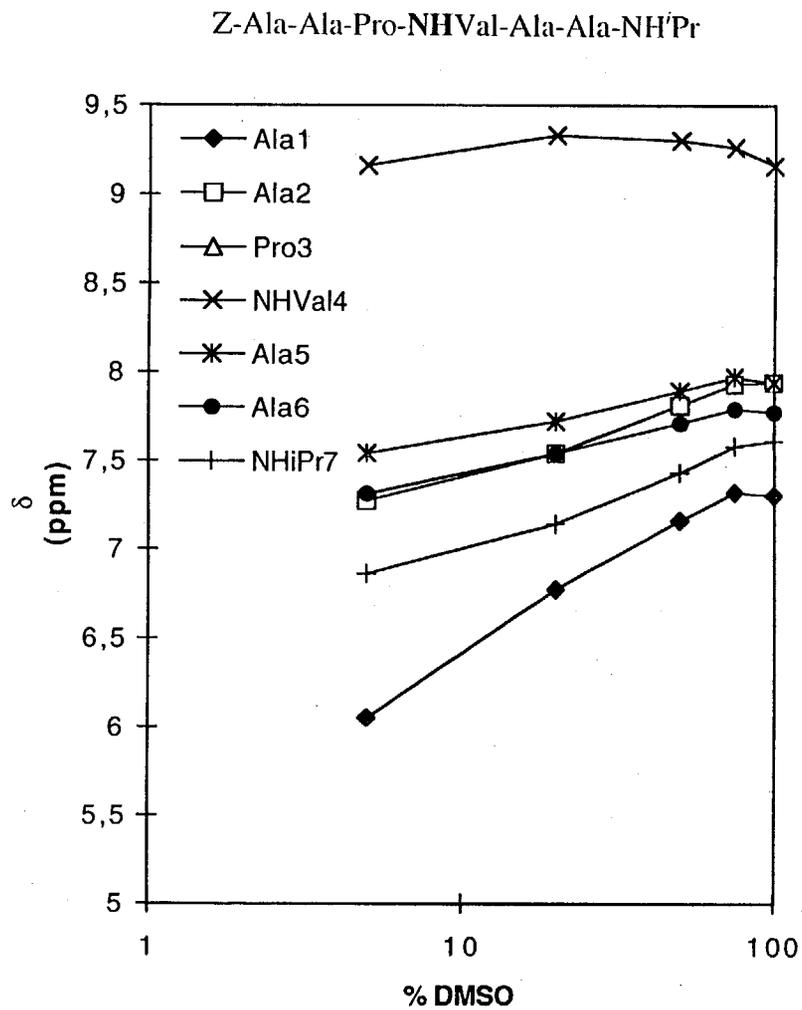
Z-Ala-Ala-Pro-Val-Ala-Ala-NHⁱPr **6**
75 % DMSO / 25 % CDCl₃, 500 MHz



Chemical shift (δ in ppm) of NH in hydrazinohexapeptides **3-5** and peptide **6** as a function of DMSO- d_6 content in $CDCl_3$ / DMSO- d_6 mixtures
 ($c = 0.005 \text{ mol l}^{-1}$, $T = 30 \text{ }^\circ\text{C}$, 500 MHz)



Chemical shift (δ in ppm) of NH in hydrazinohexapeptides 3-5 and peptide 6 as a function of DMSO- d_6 content in $CDCl_3$ / DMSO- d_6 mixtures ($c = 0.005 \text{ mol l}^{-1}$, $T = 30 \text{ }^\circ\text{C}$, 500 MHz)



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