ELECTRONIC SUPPORTING INFORMATION

Phenylalanine aminomutase-catalyzed addition of ammonia to substituted cinnamic acids – a route to enantiopure α- and β-amino acids

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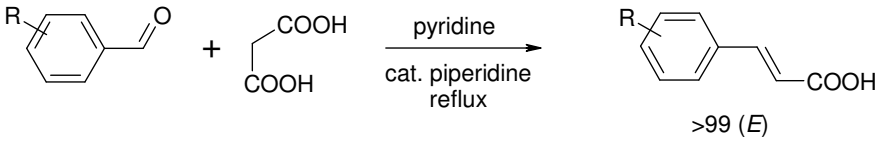
"PAM CATALYZED ADDITION OF AMMONIA TO CINNAMIC ACIDS"

General Remarks:

^{1}H-NMR spectra were recorded at 300 or 400 MHz with $CDCl_3$ as solvent. ^{13}C-NMR spectra were obtained at 75.4 or 100.59 MHz in $CDCl_3$. Chemical shifts were determined relative to the residual solvent peaks ($CHCl_3$, δ = 7.26 ppm for hydrogen atoms, δ = 77.0 for carbon atoms). The following abbreviations are used to indicate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad signal. Enantiomeric excess determination was performed by capillary GC analysis or HPLC analysis using flame ionization detector or UV-detection, respectively (all in comparison with racemic products, column and conditions further specified in relevant experimentals). Optical rotations were measured on a polarimeter with a 10 cm cell (*c* given in g/100 mL). Absolute configuration of the products was determined by comparison of the sign of the optical rotations with those of compounds previously published. Thin-layer chromatography (TLC) was performed using commercial Kieselgel 60, F_{254} silica gel plates, and components were visualized with $KMnO_4$ or phosphomolybdic acid reagent. Flash chromatography was performed on silica gel. Drying of solutions was performed with $MgSO_4$ or Na_2SO_4 and solvents were removed with a rotary evaporator.

Compounds (*R*)-phenylalanine ((***R*)-2a**), (*S*)-phenylalanine ((***S*)-2a**), (*S*)-2-fluoro-phenylalanine ((***S*)-2b**), (*S*)-2-chloro-phenylalanine ((***S*)-2c**), (*S*)-2-bromo-phenylalanine ((***S*)-2d**), (*S*)-2-methyl-phenylalanine ((***S*)-2e**), (*S*)-3-fluoro-phenylalanine ((***S*)-2h**), (*S*)-3-chloro-phenylalanine ((***S*)-2i**), (*S*)-3-bromo-phenylalanine ((***S*)-2j**), (*S*)-3-methyl-phenylalanine ((***S*)-2k**), (*S*)-4-fluoro-phenylalanine ((***S*)-2n**), (*S*)-4-chloro-phenylalanine ((***S*)-2o**), (*S*)-4-bromo-phenylalanine ((***S*)-2p**), (*S*)-4-methyl-phenylalanine ((***S*)-2q**), (*S*)-4-methoxy-phenylalanine ((***S*)-2r**), (*S*)-4-nitro-phenylalanine ((***S*)-2w**), (*R*)-*β*-phenylalanine ((***R*)-3a**), (*S*)-*β*-phenylalanine ((***R*)-3a**), (*R*)-3-amino-3-(2-fluoro-phenyl)-propionic acid ((***R*)-3b**), (*R*)-3-amino-3-(2-chloro-phenyl)-propionic acid ((***R*)-3c**), (*R*)-3-amino-3-(2-bromo-phenyl)-propionic acid ((***R*)-3d**), (*R*)-3-amino-3-(2-methyl-phenyl)-propionic acid ((***R*)-3e**), (*S*)-3-amino-3-(2-methyl-phenyl)-propionic acid ((***S*)-3e**), (*R*)-3-amino-3-(3-fluoro-phenyl)-propionic acid ((***R*)-3h**), (*R*)-3-amino-3-(3-chloro-phenyl)-propionic acid ((***R*)-3i**), (*R*)-3-amino-3-(3-bromo-phenyl)-propionic acid ((***R*)-3j**), (*R*)-3-amino-3-(3-methyl-phenyl)-propionic acid ((***R*)-3k**), (*R*)-3-amino-3-(4-fluoro-

phenyl)-propionic acid ((***R***)-**3n**), (*S*)-3-amino-3-(4-fluoro-phenyl)-propionic acid ((***S***)-**3n**), (*R*)-3-amino-3-(4-chloro-phenyl)-propionic acid ((***R***)-**3o**), (*S*)-3-amino-3-(4-chloro-phenyl)-propionic acid ((***S***)-**3o**), (*R*)-3-amino-3-(4-methyl-phenyl)-propionic acid ((***R***)-**3q**), (*S*)-3-amino-3-(4-methyl-phenyl)-propionic acid ((***S***)-**3q**), (*R*)-3-amino-3-(4-methoxy-phenyl)-propionic acid ((***R***)-**3r**), (*S*)-3-amino-3-(4-methoxy-phenyl)-propionic acid ((***S***)-**3r**), (*R*)-3-amino-3-(4-bromo-phenyl)-propionic acid ((***R***)-**3p**), (*R*)-3-amino-3-(4-nitro-phenyl)-propionic acid ((***R***)-**3w**) were synthesized by Peptech Corp.



1b, R = 2-F: 99%,
1c, R = 2-Cl: 93%,
1d, R = 2-Br: 73%,
1h, R = 3-F: 99%,
1i, R = 3-Cl: 96%,
1j, R = 3-Br: 92%,
1p, R = 4-Br: 88%,
1t, R = 4-Et: 96%,
1u, R = 4-Pr: 92%,
1w, R = 4-NO_2: 95%,
1x, R = 4-*t*-Bu: 99%,

General procedure for the synthesis of substituted cinnamic acids (1):

A mixture of substituted benzaldehyde (4.00 mmol), malonic acid (8.80 mmol) and piperidine (70 μL) in pyridine (1.80 mL) was stirred under gentle reflux for 80-180 min. The reaction mixture was cooled and slowly poured into ice-cold aqueous HCl (2N, 35 mL). The precipitate was filtered off and dried under vacuum.

1b: (*E*)-2-fluoro-cinnamic acid. Yield: 99%, >99% of (*E*) isomer. Light yellow solid. Mp. 173-174 °C (lit.[1] 175 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.56 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**), 7.10-7.59 (m, 4H, Ar**H**). 7.93 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**); ^{1}H NMR consistent with literature data.[2]

1c: (*E*)-2-chloro-cinnamic acid. Yield: 93%, >99% of (*E*) isomer. White solid. Mp. 210.9-211.2 °C (lit.[3] 208-210 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.59 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**), 7.86 (d, ^3J = 16.4 Hz, 1H, vinyl C**H**), 7.36-7.92 (m, 4H, Ar**H**).

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1d: (*E*)-2-bromo-cinnamic acid. Yield: 73%, >99% of (*E*) isomer. White solid. Mp. 219.5-219.7 °C (lit.[4] 217.5-218.5 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.54 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**), 7.82 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**), 7.32-7.90 (m, 4H, Ar**H**); ^{1}H NMR consistent with literature data.[5]

1h: (*E*)-3-fluoro-cinnamic acid. Yield: 99%, >99% of (*E*) isomer. White solid. Mp. 167.5-168.5 °C (lit.[6] 166.2-166.8 °C); ^{1}H NMR (400 MHz, DMSO-d6): 6.60 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**), 7.21-7.61 (m, 5H, Ar**H**, vinyl **H**). ^{1}H NMR consistent with literature data.[7]

1i: (*E*)-3-chloro-cinnamic acid. Yield: 96%, >99% of (*E*) isomer. White solid. Mp. 161.4-162.3 °C (lit.[6] 162.6-163.2 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.60 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**), 7.55 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**), 7.40-7.80 (m, 4H, Ar**H**); ^{1}H NMR consistent with literature data.[8]

1j: (*E*)-3-bromo-cinnamic acid. Yield: 92%, >99% of (*E*) isomer. White solid. Mp. 175.0-176.3 °C (lit.[9] 176-178 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.61 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**), 7.65 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**), 7.39-7.90 (m, 4H, Ar**H**).

1p: (*E*)-4-bromo-cinnamic acid. Yield: 88%, >99% of (*E*) isomer. White solid. Mp. 264.0-265.0 °C (lit.[10] 264-266 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.55 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**), 7.55 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**), 7.58-7.65 (m, 4H, Ar**H**); ^{1}H NMR consistent with literature data.[10]

1t: (*E*)-4-ethyl-cinnamic acid. Yield: 96%, >99% of (*E*) isomer. White solid. Mp. 143 °C (lit.[11] 143 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (t, ^3J =7.6 Hz, 3H, C**H$_3$**), 2.68 (q, ^3J =7.6 Hz, 2H, C**H$_2$**), 6.42 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**), 7.22-7.49 (m,

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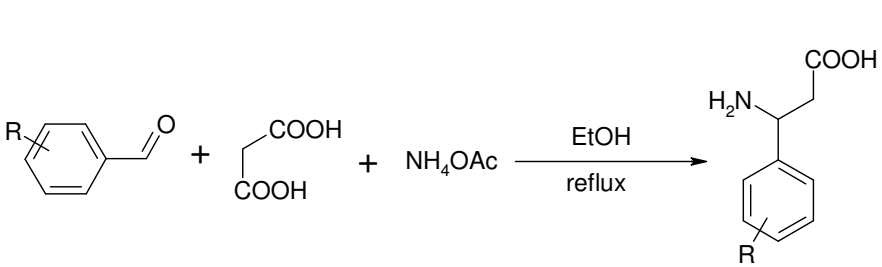
4H, Ar**H**). 7.78 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**); ^{1}H NMR consistent with literature data.[12]

1u: (*E*)-4-*n*-propyl-cinnamic acid. Yield: 92%, >99% of (*E*) isomer. White solid. Mp. 176.0-177.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (t, ^3J = 7.2 Hz, 3H, C**H$_3$**), 1.61-1.70 (m, 2H, CH_3C**H$_2$**), 2.62 (t, ^3J = 8.0 Hz, 2H, C**H$_2$**Ar), 6.42 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**), 7.21-8.49 (m, 4H, Ar**H**), 7.78 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**); ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.3, 24.5, 37.7, 118.9, 128.9, 129.6, 132.5, 144.6, 145.4, 168.4; MS (EI) *m/z* 190 (M^+, 40), 161 (100), 115 (75); HRMS (EI+) calc. for $C_{12}H_{14}O_2$: 190.0994, found: 190.0996.

1w: (*E*)-4-nitrocinnamic acid. Yield: 95%, >99% of (*E*) isomer. Yellow solid. Mp. 292-293 °C (lit.[9] 293 °C); ^{1}H NMR (400 MHz, DMSO-d6): δ 6.74 (d, ^3J = 16.4 Hz, 1H, vinyl C**H**), 7.68 (d, ^3J = 16.4 Hz, 1H, vinyl C**H**), 7.96-8.24 (m, 4H, Ar**H**). ^{1}H NMR consistent with literature data.[13]

1x: (*E*)-4-*tert*-butyl-cinnamic acid. Yield: 99%, >99% of (*E*) isomer. White solid. Mp. 202.4-204.2 °C (lit.[2] 201-203 °C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 9H, (C**H$_3$**)$_3$); 6.43 (d, ^3J = 15.6 Hz, 1H, vinyl C**H**), 7.42-8.51 (m, 4H, Ar**H**), 7.78 (d, ^3J = 16.0 Hz, 1H, vinyl C**H**); ^{13}C NMR (50 MHz, $CDCl_3$): δ 31.4, 35.2; 116.4, 126.2, 128.5, 131.5, 147.1, 172.0. MS (EI) *m/z* 204 (M^+, 24), 189 (100); HRMS (EI+) calc. for $C_{13}H_{16}O_2$: 204.1150, found: 204.1159.



3b, R = 2-F: 41%
3c, R = 2-Cl: 31%
3d, R = 2-Br: 13%
3h, R = 3-F: 67%
3i, R = 3-Cl: 56%
3j, R = 3-Br: 55%
3k, R = 3-Me: 56%
3p, R = 4-Br: 29%
3t, R = 4-Et: 60%
3u, R = 4-Pr: 31%
3v, R = 4-*i*-Pr: 60%

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[13] Fukuyama, T.; Arai, M.; Matsubara, H.; Ryu L. *J. Org. Chem.* **2004**, *69*, 8105.

General procedure for the synthesis of β-amino acids (3):[14]

A suspension of substituted benzaldehyde (2.00 mmol), malonic acid (2.00 mmol) and ammonium acetate (4.0 mmol) in ethanol was heated under reflux for 3 h. The solid product was filtered off and titrated with boiling methanol.

3b: 3-Amino-3-(2-fluoro-phenyl)-propionic acid. Yield: 41%. White solid. Mp. 219.0-219.5 °C (lit.[15] 234-236 °C); ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.46-2.53 (m, 2H, C**H$_2$**COOH), 4.36 (t, ^3J = 7.2 Hz, 1H, C**H**NH$_2$), 6.96-7.31 (m, 4H, ArH). ^{1}H NMR consistent with literature data.[15]

3c: 3-Amino-3-(2-chloro-phenyl)-propionic acid. Yield: 31%. White solid. Mp. 230.2-231.8 °C (lit.[16] 219 °C); ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.41 (dd, ^2J = 15.2 Hz ^3J = 8.0 Hz, 1H, C**H$_2$**COOH), 2.54 (dd, ^2J = 15.2 Hz ^3J = 6.0 Hz, 1H, C**H$_2$**COOH), 4.54-4.60 (m, 1H, C**H**NH$_2$), 7.13-7.36 (m, 4H, ArH).

3d: 3-Amino-3-(2-bromo-phenyl)-propionic acid. Yield: 13%. White solid. Mp. 229.2-229.5 °C; ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.38 (dd, ^2J = 14.8 Hz ^3J = 8.0 Hz, 1H, C**H$_2$**COOH), 2.54 (dd, ^2J = 14.8 Hz ^3J = 6.0 Hz, 1H, C**H$_2$**COOH), 4.51-4.54 (m, 1H, C**H**NH$_2$), 7.05-7.52 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 45.2, 51.8, 118.8, 123.0, 127.3, 128.2, 129.0, 133.1, 167.4; NMR data is identical for the one obtained for commercial enantiopure sample; (ESI+) calc. for $C_9H_{11}O_2NBr$: 243.9968, found: 243.9967.

3h: 3-Amino-3-(3-fluoro-phenyl)-propionic acid. Yield: 67%. White solid. Mp. 217.8-218.4 °C; ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.40-2.46 (m, C**H$_2$**COOH), 4.12 (t, ^3J = 6.8 Hz, 1H, C**H**NH$_2$), 6.91-7.26 (m, 4H, ArH). NMR data is identical for the one obtained for commercial enantiopure sample.

3i: 3-Amino-3-(3-chloro-phenyl)-propionic acid. Yield: 56%. White solid. Mp. 221.8-222.0 °C; ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.41-2.45 (m, C**H$_2$**COOH), 4.10

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[16] Tan, C. Y. K.; Weaver, D. F. *Tetrahedron* **2002**, *58*, 7449.

(t, ^3J = 7.2 Hz, 1H, C**H**NH_2), 7.16-7.28 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 46.9, 52.8, 124.9, 126.5, 127.4, 130.3, 133.8, 146.2, 167.3; NMR data is identical for the one obtained for commercial enantiopure sample. HRMS (ESI+) calc. for $C_9H_{11}O_2NCl$: 200.0473, found: 200.0473.

3j: 3-Amino-3-(3-bromo-phenyl)-propionic acid. Yield: 55%. White solid. Mp. 225.4-225.6 °C (lit.[17] 243-245 °C (dec)); ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.40-2.44 (m, C**H$_2$**COOH), 4.03-4.10 (m, 1H, C**H**NH_2), 7.13-7.43 (m, 4H, ArH). ^{1}H NMR consistent with literature data.[17]

3k: 3-Amino-3-(3-methyl-phenyl)-propionic acid. Yield: 56%. White solid. Mp. 219.0-219.2 °C (lit.[18] 221-222 °C); ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.20 (s, 3H, C**H$_3$**), 2.41-2.44 (m, 2H, C**H$_2$**COOH), 4.09 (t, ^3J = 7.2 Hz, 1H, C**H**NH_2), 7.02-7.19 (m, 4H, ArH); ^{1}H NMR consistent with literature data.[16]

3p: 3-Amino-3-(4-bromo-phenyl)-propionic acid. Yield: 29%. White solid. Mp. 228.0-228.7 °C (lit.[16] 234 °C); ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 2.37-2.50 (m, 2H, C**H$_2$**COOH), 4.08 (t, ^3J = 6.8 Hz, 1H, C**H**NH_2), 7.14-7.17 (m, 2H, ArH), 7.39-7.42 (m, 2H, ArH); ^{1}H NMR consistent with literature data.[16]

3t: 3-Amino-3-(4-ethyl-phenyl)-propionic acid. Yield: 60%. White solid. Mp. 219.6-220.7 °C; ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 1.04 (t, ^3J = 7.6 Hz, 3H, C**H$_3$**), 2.40-2.43 (m, 2H, C**H$_2$**COOH), 2.48 (q, ^3J = 8.0, CH_3C**H$_2$**), 4.05-4.10 (m, 1H, ArC**H**), 7.13-7.19 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2, 28.0, 46.9, 52.8, 166.1, 126.7, 128.3, 144.1, 167.7; MS (EI) *m/z* 193 (M^+, 16), 134 (100); Anal. calc. for $C_{11}H_{15}NO_2$: C 68.37, H 7.82, N 7.25; found: C 68.20, H 7.88, N 7.19.

3u: 3-Amino-3-(4-propyl-phenyl)-propionic acid. Yield: 31%. White solid. Mp. 217.8-218.3 °C; ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 0.75 (t, ^3J = 7.2 Hz, 3H, C**H$_3$**CH_2), 1.42-1.50 (m, 2H, CH_3C**H$_2$**), 2.41-2.47 (m, 4H, C**H$_2$**Ar, C**H$_2$**COOH), 4.10 (t, ^3J = 7.2Hz, 1H, ArC**H**), 7.12-7.20 (m, 4H, ArH); ^{13}C NMR (125 MHz, D_2O +

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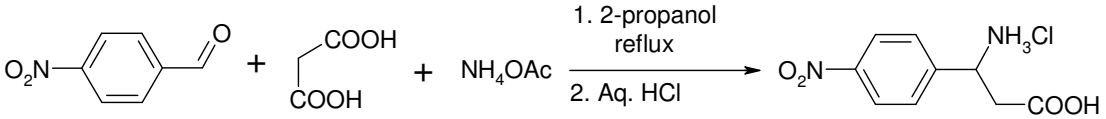
K_2CO_3): δ 13.2, 24.3, 37.0, 46.7, 52.8, 126.5, 129.0, 142.6, 166.5, 180.2; Anal. calc. for $C_{12}H_{17}NO_2$: C 69.54, H 8.27, N 6.76; found: C 69.46, H 8.27, N 6.72. HRMS (EI+) calc. for $C_{12}H_{18}O_2N$: 208.1332, found: 208.1333.

3v: 3-Amino-3-(4-*iso*-propyl-phenyl)-propionic acid. Yield: 60%. White solid. Mp. 243.1-243.4 °C; ^{1}H NMR (400 MHz, D_2O + K_2CO_3): 1.05 (d, ^3J = 6.8 Hz, 6H, (C**H**$_3$)$_2$CH), 2.39 (d, ^3J = 7.2 Hz, 2H, C**H**$_2$COOH), 2.75 (sept, ^3J = 6.6, $(CH_3)_2$C**H**), 4.07 (t, ^3J = 7.2Hz, 1H, ArC**H**), 7.15-7.20 (m, 4H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.3, 36.7, 47.0, 51.2, 121.5, 124.2, 126.6, 126.8, 168.1; MS (EI) *m/z* 207 (M^+, 16), 148 (100); Anal. calc. for $C_{12}H_{17}NO_2$: C 69.54, H 8.27, N 6.76; found: C 69.40, H 8.25, N 6.74.



3w, R = NO_2: 21%

3w: 3-Amino-3-(4-nitrophenyl)-propionic acid (modification of literature procedure[16]). A suspension of 4-nitrobenzaldehyde (6.65 mmol, 1.00 g), malonic acid (6.70 mmol, 0.70 g) and ammonium acetate (14.2 mmol, 1.09 g) in 2-propanol was heated under reflux for 22 h. The solid was filtered off, redissolved in aqueous HCl (1N, 10 mL) and washed with Et_2O (3 x 10 ml). The aqueous phase was concentrated to give 0.30 g (1.42 mmol, 21 %) of a yellow solid. ^{1}H NMR (400 MHz, D_2O + K_2CO_3): δ 2.44-2.50 (m, 2H, C**H**$_2$COOH), 4.21-4.32 (m, 1H, C**H**NH), 7.41-8.10 (m, 4H, Ar**H**).

Racemic α-amino acids (2) were synthesized by literature procedure,[19] and spectral data were found to be identical with commercial enantiopure samples. Analytical data for commercially unavailable α-amino acids **2t**, **2u** and **2v** are presented below:

2t x HCl: 4-Ethylphenylalanine hydrochloride. White solid; ^{1}H NMR (300 MHz, CD_3OD): δ 1.21 (t, ^3J=7.6 Hz, 3H; C**H**$_3$), 2.62 (q, ^3J=7.6 Hz, 2H; C**H**$_2$), 3.15 (dd, ^4J=14.7 Hz, ^3J=7.4 Hz, 1H; C**H**$_2$), 3.28 (dd, ^4J=14.8 Hz, ^3J=5.8 Hz, 1H; C**H**$_2$), 4.23

[19] Panella, L.; Marco Aleixandre, A.; Kruidhof, G. J.; Robertus, J.; Feringa, B. L.; de Vries, J. G.; Minnaard, A. J. *J. Org. Chem.* **2006**, *71*, 2026.

(dd, ^3J=7.2 Hz, ^3J=6.0 Hz, 1H; C**H**), 7.18-7.24 (m, 4H; Ar**H**). ^{13}C NMR (100 MHz, CD_3OD): δ=15.0 (CH_3), 28.3 (CH_2), 35.7 (CH_2) 15.1 (CH), 128.4 (CH), 129.4 (CH), 131.5 (C), 143.9 (C), 170.8 (CO). HR-ESI-MS: m/z calcd for $C_{11}H_{16}NO_2$ $[M+H]^+$ 194.1176, found 194.1174.

2u x HCl: 4-Propylphenylalanine hydrochloride. White solid. ^{1}H NMR (400 MHz, D_2O + K_2CO_3): δ 0.73 (t, J = 7.6 Hz, 3H, C**H$_3$**), 1.41-1.47 (m, 2H, CH_3C**H$_2$**), 2.42 (t, J = 7.6 Hz, 2H, PhC**H$_2$**), 2.61-2.83 (m, 2H, PhC**H$_2$**), 3.25-3.46 (m, 1H, C**H**), 7.02-7.09 (m, 4H, Ar**H**). ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.1 (CH_3), 24.2 (CH_2), 35.3 (CH_2), 37.0 (CH_2), 54.2 (CH), 116.1 (CH), 118.8 (C), 124.3 (CH), 126.5 (CH), 131.2 (CH), 143.2 (C), 171.5 (CO). HR-ESI-MS: m/z calcd for $C_{12}H_{18}NO_2$ $[M+H]^+$ 208.1332, found 208.1334.

2v x HCl: 4-*iso*-propylphenylalanine hydrochloride. White solid. Mp. 260-262. °C ^{1}H NMR (300 MHz, D_2O): δ 1.09 (d, ^3J = 6.6 Hz, 6H, (C**H$_3$**)$_2$CH), 2.81 (sept, ^3J = 6.6, $(CH_3)_2$C**H**), 3.03 (dd, ^2J = 14.7 Hz ^3J = 7.8 Hz, 1H, ArC**H**$_2$), 3.16 (dd, ^2J = 14.7 Hz ^3J = 5.4 Hz, 1H, ArC**H**$_2$), 4.07 (dd, ^3J = 7.5 Hz ^3J = 5.4 Hz, 1H, NHC**H**), 7.13-7.24 (m, 4H, ArH); ^{1}H NMR consistent with literature data.[20]

Determination of kinetic parameters for the amination activity of PAM

UV-Vis spectroscopy was used to determine the kinetic parameters of the PAM-catalyzed ammonia addition reaction. A 6 M ammonia solution was prepared and the pH was adjusted to 10 by passing CO_2 into the solution. In a typical assay, (*E*)-cinnamic acid or a derivative at various concentrations was incubated with 0.06 mg of purified PAM in ammonia solution (300 µl). The reaction mixture was incubated at 30 ^{o}C. The ammonia addition activity was monitored by UV-Vis spectroscopy. The initial rates were plotted against the substrate concentration and these data were fitted to the Michaelis-Menten equation to obtain the kinetic constants.

Stereochemical analysis of the phenylalanine products by chiral HPLC.

Purified PAM (0.02 mg) was added to 5 mM of (*E*)-cinnamic acid or a derivative in ammonia solution (6M, pH 10, 200 µl). The reaction mixture was incubated for 24 h

[20] Wadia, M. S.; Mali, R. S.; Tilve, S. G.; Yadav, V. J. *Synthesis* **1987**, *4*, 401.

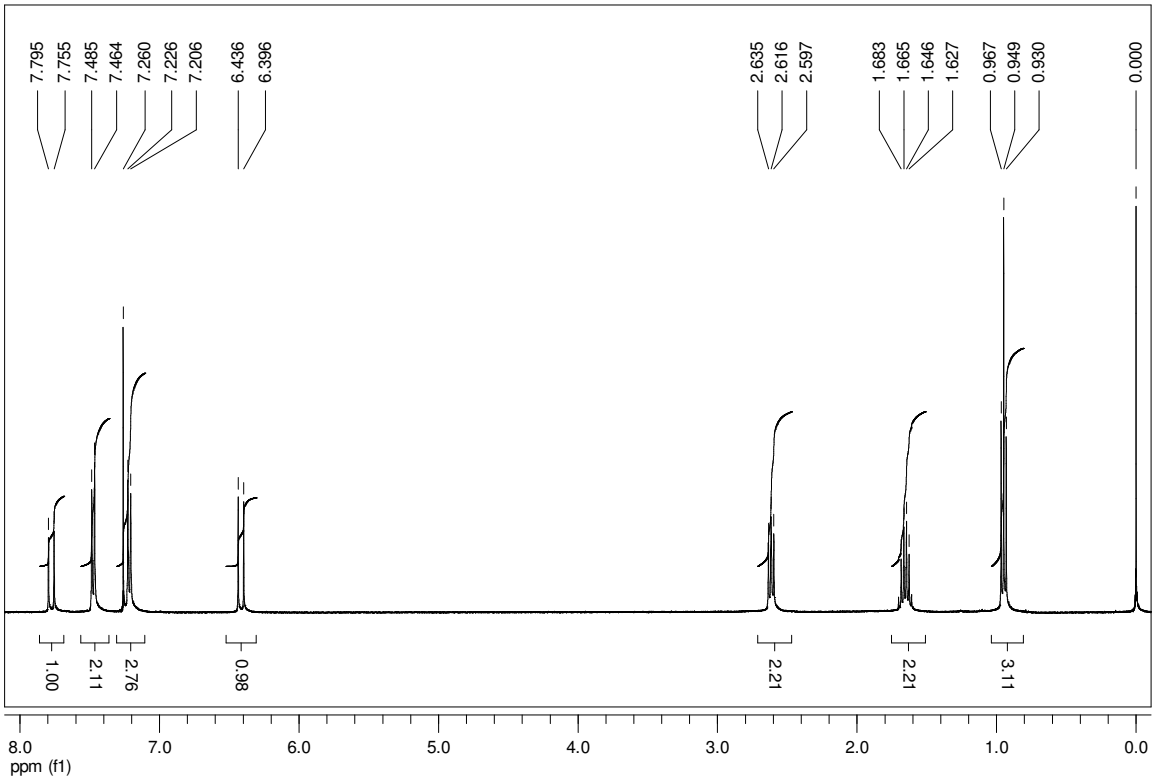
at 30 °C. Subsequently, a 20-µl portion was taken and the reaction was quenched by heating for 5 min at 99 °C. A 40-µl portion of 2 M aqueous NaOH was added to remove the excess of ammonia. The sample was then frozen in liquid nitrogen. Subsequently, the sample was lyophilized and dissolved in 55 µl of 2 M aqueous $HClO_4$. Analysis was carried out on a Crownpak CR(+) (4 mm x 150 mm) column. Compounds were eluted isocratically with aqueous $HClO_4$ in 15% MeOH (pH given in table) and with UV detection at 210 nm. Retention times and other resolution parameters are given in table.

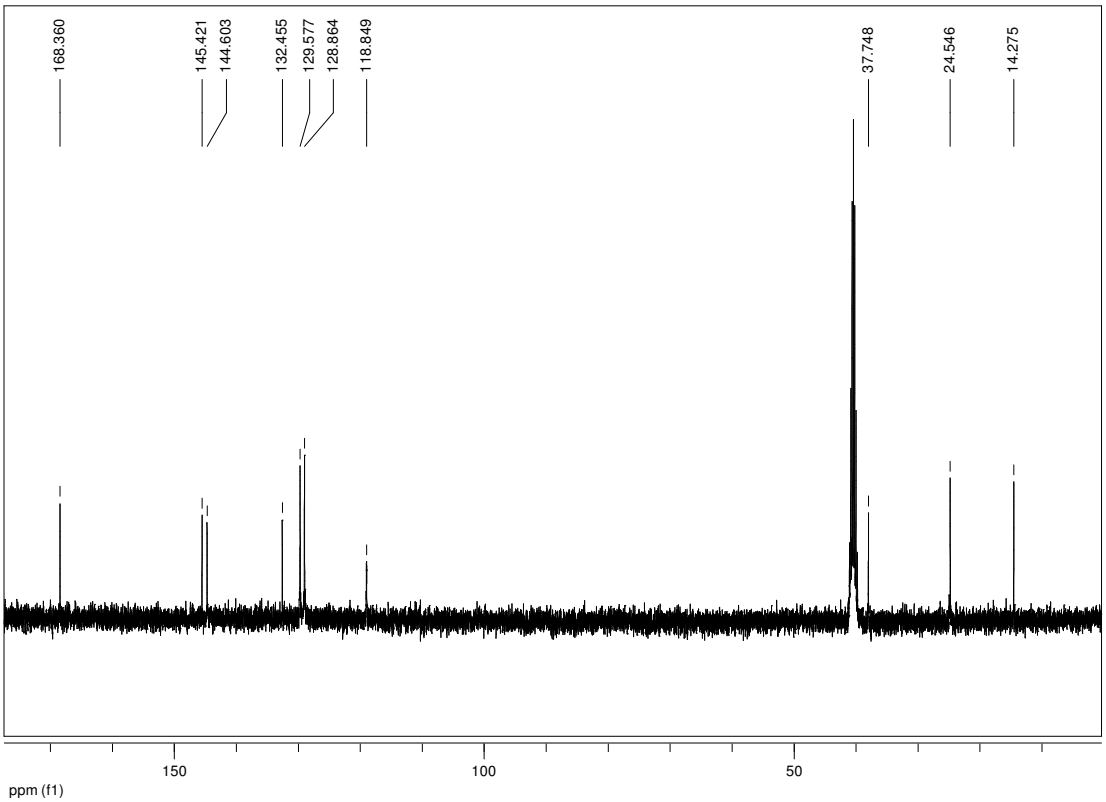
				Rt (min)			
R	Eluent Ph	Flow	Temp	(*R*)-**2**	(*S*)-**2**	(*S*)-**3**	(*R*)-**3**
H	pH 1.8	0.3 mL/min	-7 °C	17.5	32.3	45.4	56.3
2-F	pH 2.4	0.3 mL/min	-7 °C	29.8	40.6	-	-
2-Cl	pH 2.4	0.3 mL/min	-7 °C	165.6	103.8	-	-
2-Br	pH 2.6	0.3 mL/min	-7 °C	77.6	94.3	-	-
2-Me	pH 2.3	0.3 mL/min	-7 °C	86.6	100.5	-	-
3-F	pH 2.0	0.3 mL/min	-7 °C	57.1	74.0	-	-
3-Cl	pH 2.0	0.3 mL/min	-7 °C	118.6	160.8	-	-
3-Br	pH 2.6	0.3 mL/min	-7 °C	114.4	197.8	-	-
3-Me	pH 2.3	0.3 mL/min	-7 °C	155.8	111.1	150.8	123.1
	pH 2.0	0.3 mL/min	-7 °C	217.6	151.0	187.7	151.8
4-F	pH 2.5	0.3 mL/min	-5 °C	22.0	35.6	53.8	68.3
4-Cl	pH 2.7	0.3 mL/min	-6 °C	45.8	73.9	136.6	155.1
4-Br	pH 2.6	0.3 mL/min	-7 °C	122.5	163.3	198.5	219.7
4-Me	pH 2.6	0.3 mL/min	-5 °C	34.7	71.5	109.8	126.3
4-MeO	pH 2.7	0.3 mL/min	-6 °C	24.7	41.5	74.4	106.2
4-Et	pH 2.6	0.3 mL/min	-7 °C	-	-	175.0*	231.6*
4-Pr	pH 3.0	0.3 mL/min	-7 °C	-	-	250.1*	387.3*
4-iPr	pH 1.8	0.5 mL/min	20 °C	-	-	280*	294*
4-NO_2**	pH 2.2	0.3 mL/min	-6 °C	58.6	72.1	-	-

* Absolute configuration not determined
** 10% methanol used in the eluent

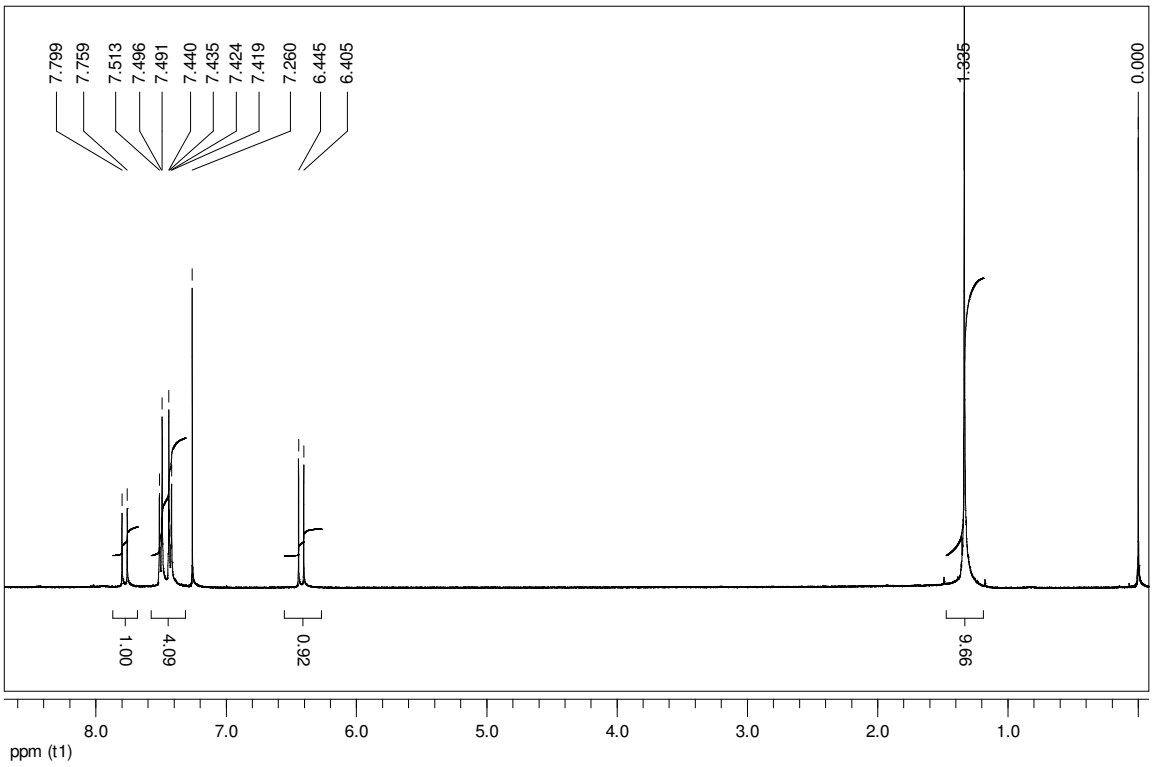
NMR-spectra of new compounds.

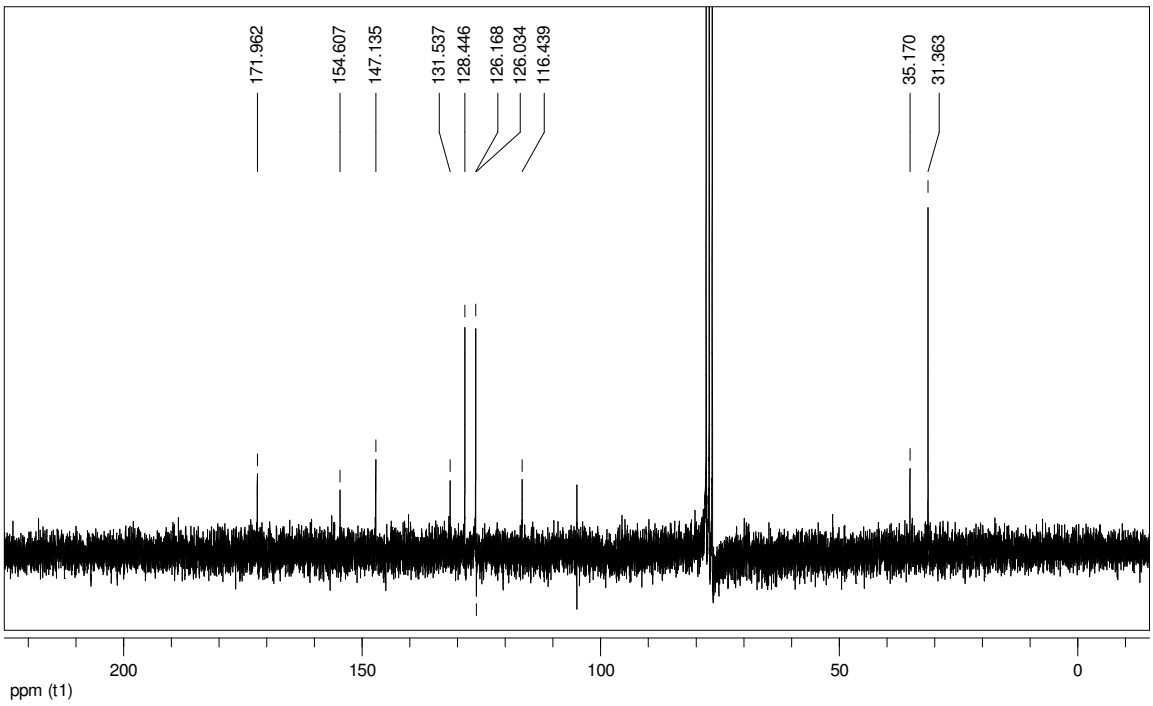
(*E*)-4-*n*-propyl-cinnamic acid (1u):



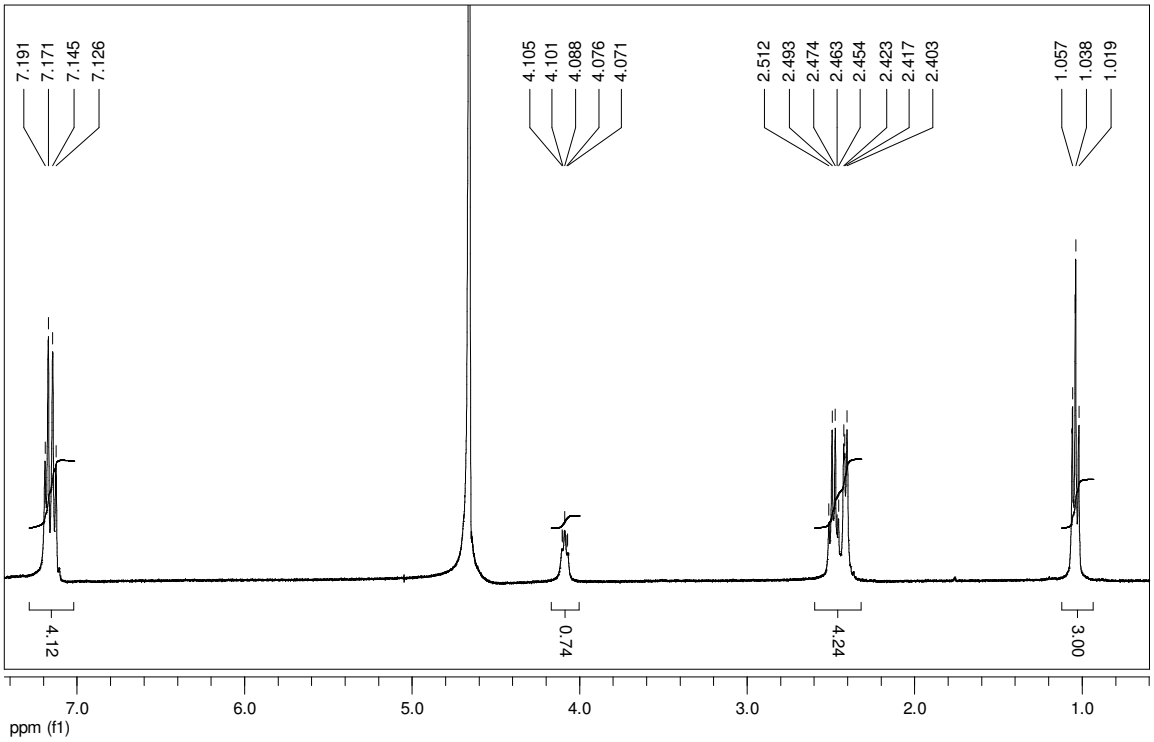


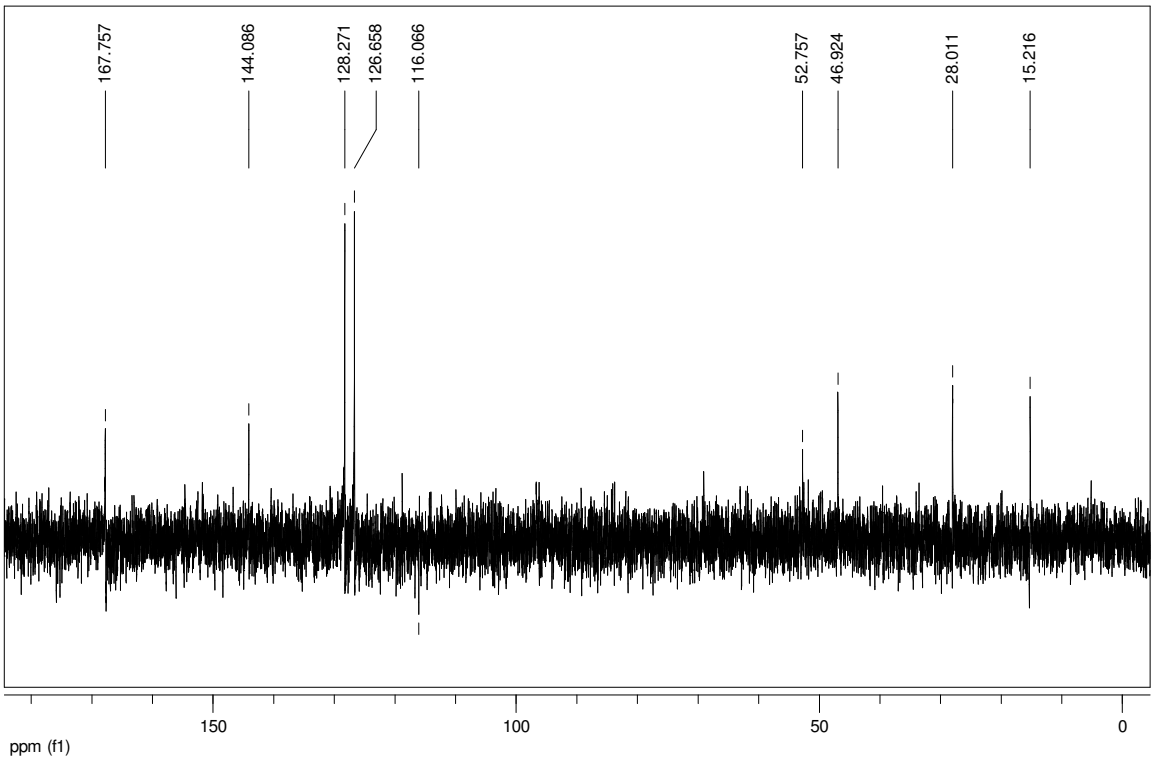
(*E*)-4-*tert*-butyl-cinnamic acid (1x):



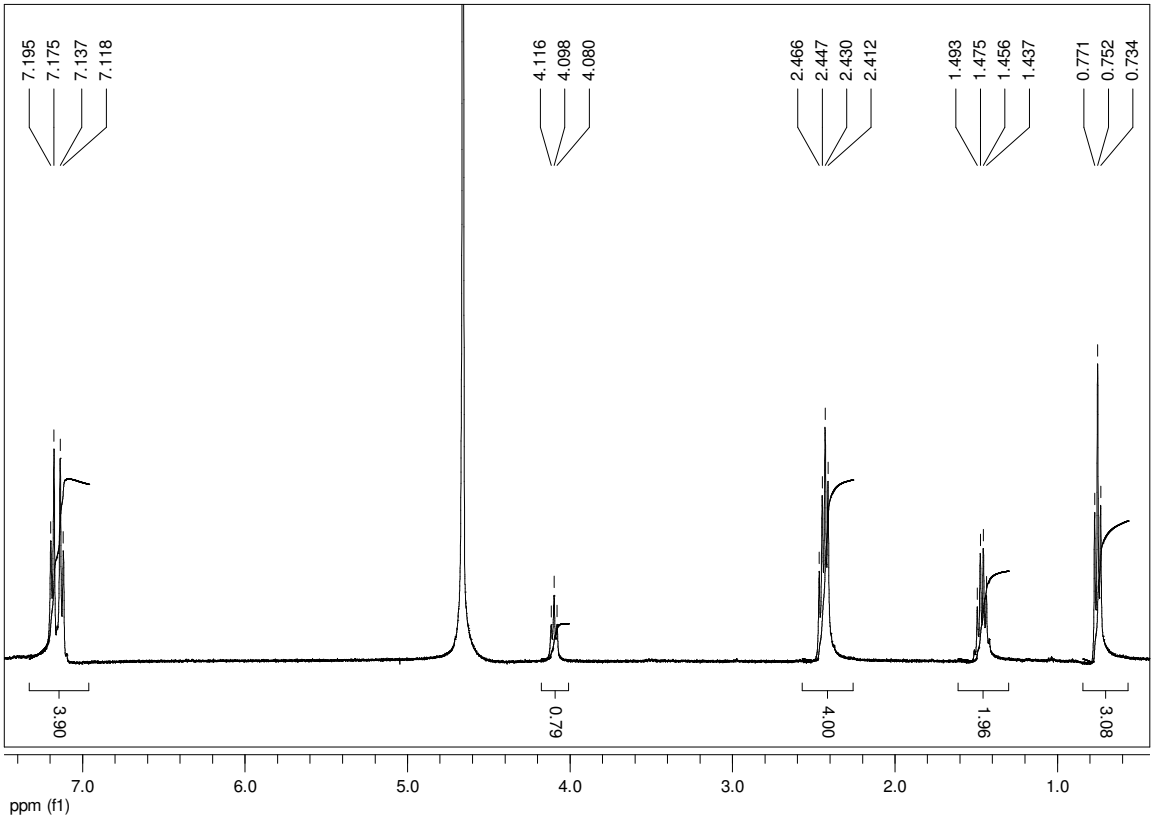


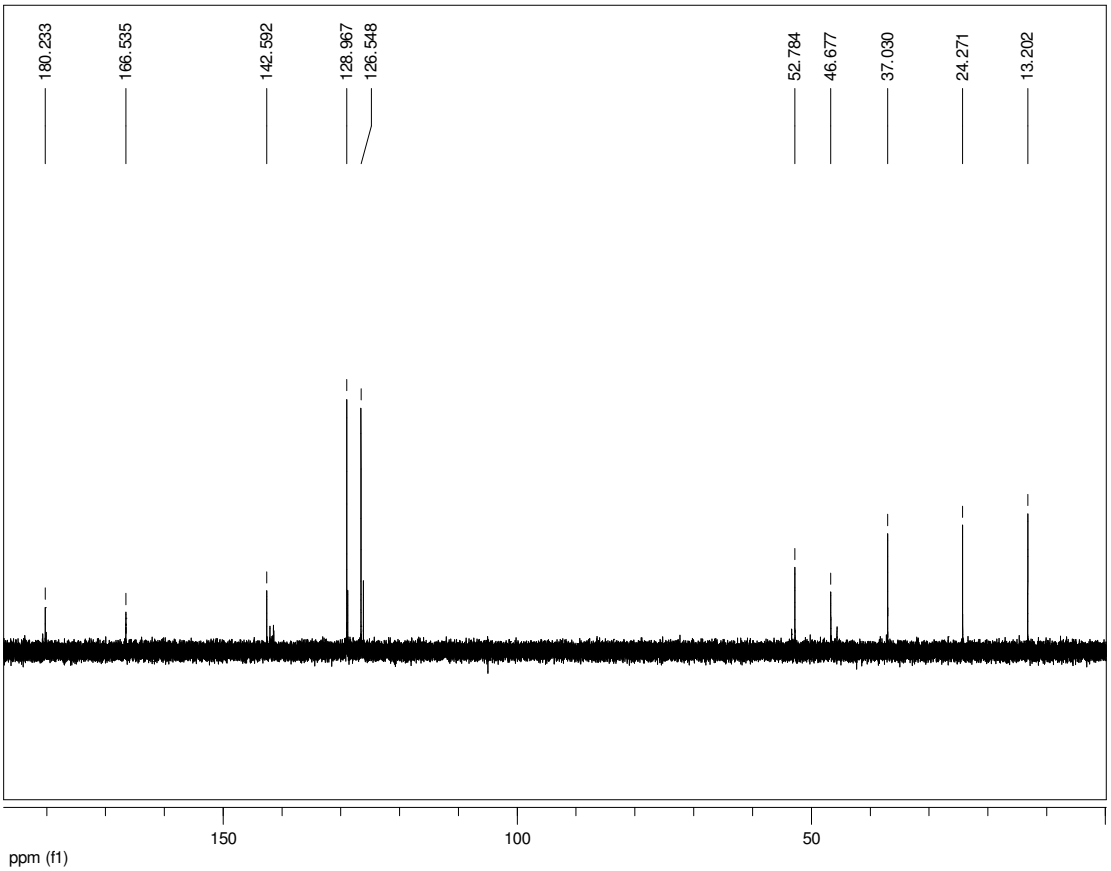
3-Amino-3-(4-ethyl-phenyl)-propionic acid (3t):



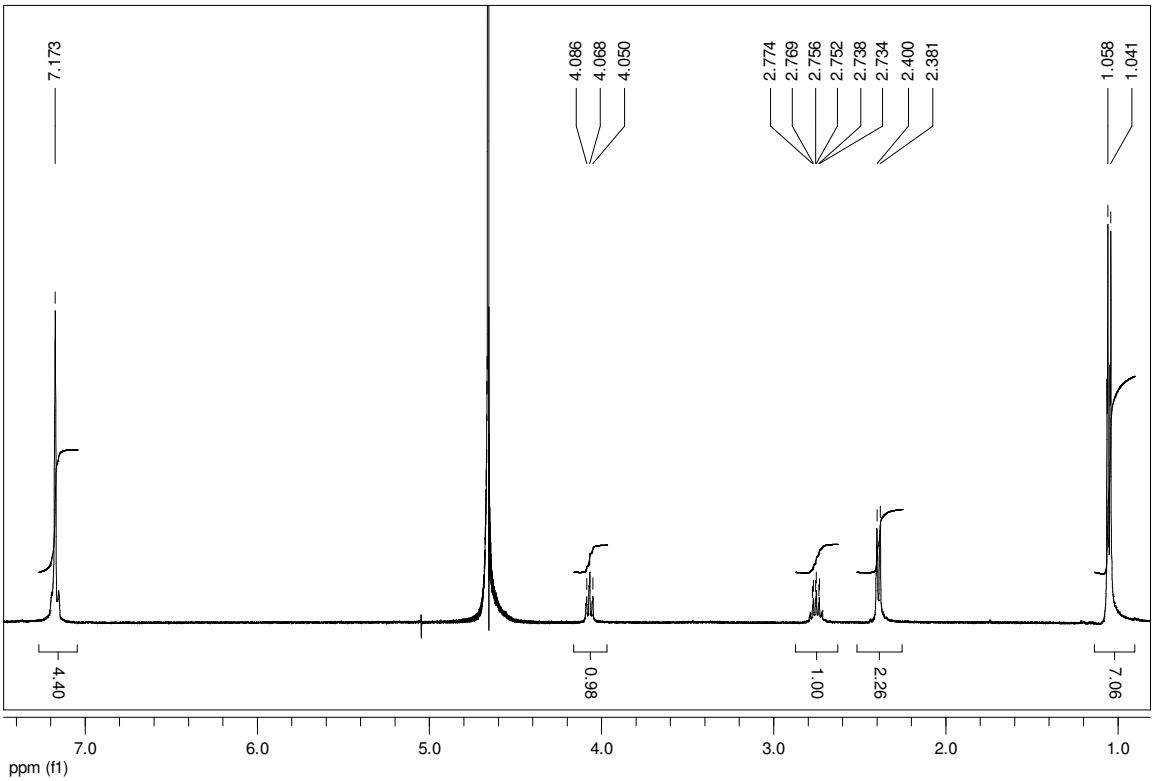


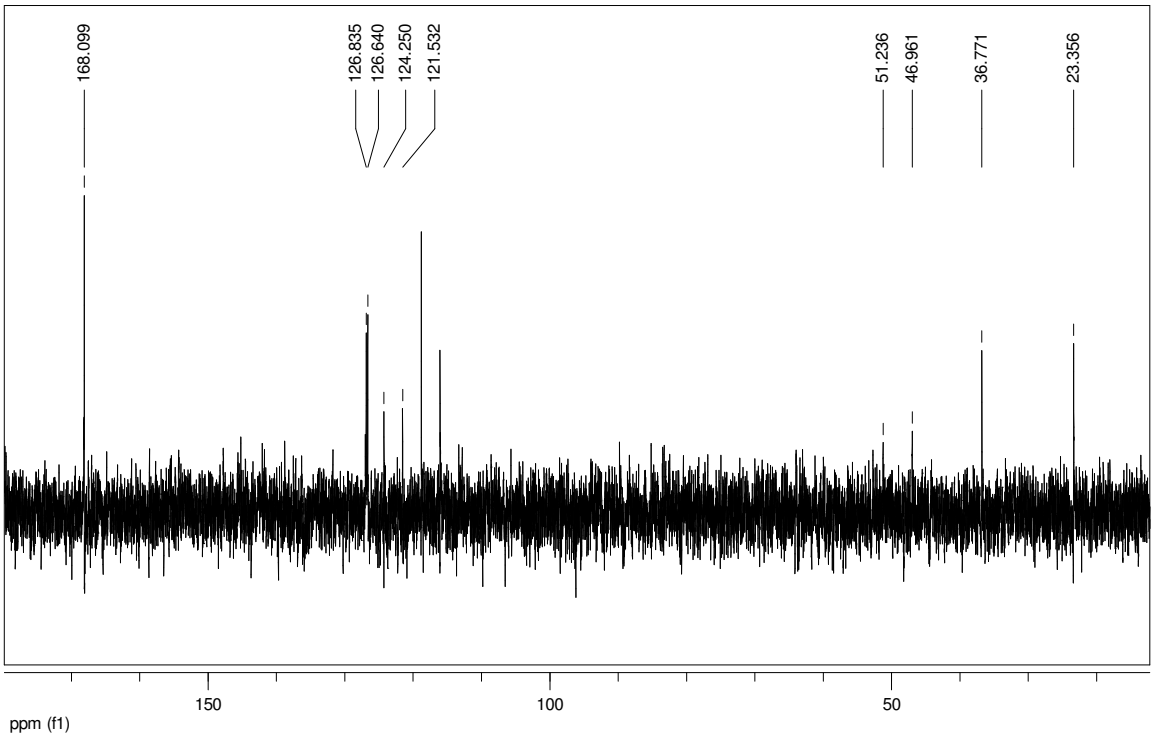
3-Amino-3-(4-propyl-phenyl)-propionic acid (3u):



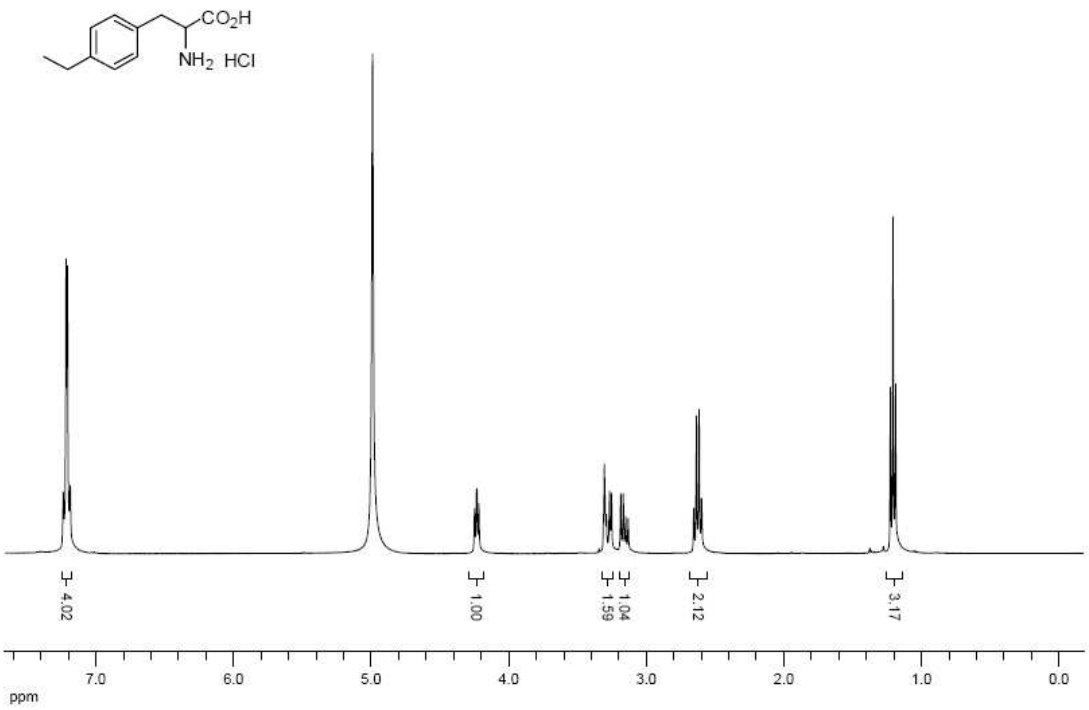


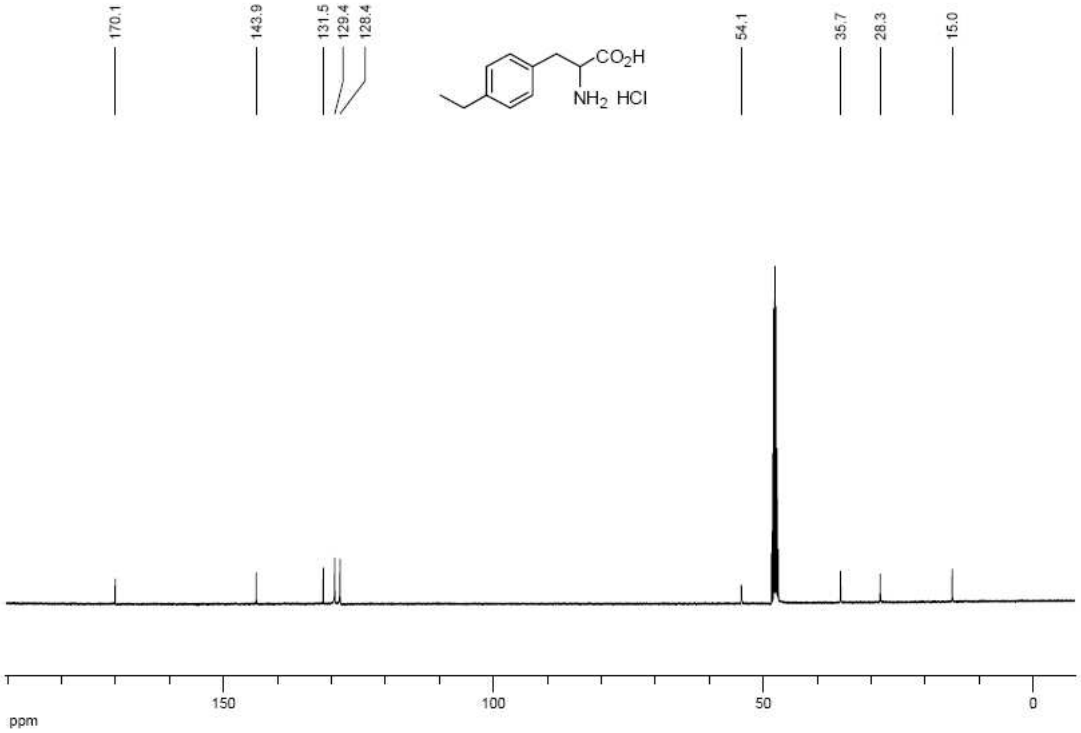
3-Amino-3-(4-*iso*-propyl-phenyl)-propionic acid (3v):





4-Ethylphenylalanine hydrochloride (2t):





4-Propylphenylalanine hydrochloride (2u):