SUPPORTING INFORMATION

Large neutral amino acid transporter enables brain drug delivery via prodrugs

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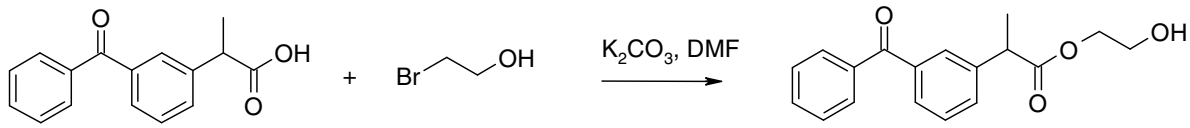
Contents:

Table S1. Combustion Analysis for Compounds **1-5**.

Compd	Formula	Anal. Calcd			Found		
		C	H	N	C	H	N
(**1**)	$C_{25}H_{23}NO_5$•HCl•H_2O	63.63	5.55	2.97	63.73	5.29	3.35
(**2**)	$C_{27}H_{27}NO_5$•HCl•1/4H_2O	66.79	2.88	5.90	66.48	3.10	6.08
(**3**)	$C_{25}H_{23}NO_4$•1/5H_2O	74.13	5.82	3.46	73.73	5.92	3.49
(**4**)	$C_{24}H_{29}NO_5$•HCl	63.71	6.79	3.10	63.87	6.82	3.33
(**5**)	$C_{22}H_{25}NO_4$	71.91	6.86	3.81	71.45	7.02	3.71

Experimental detail of synthesis of compounds 2-5

2-(3-Benzoyl-phenyl)-propionic acid 2-hydroxy-ethyl ester

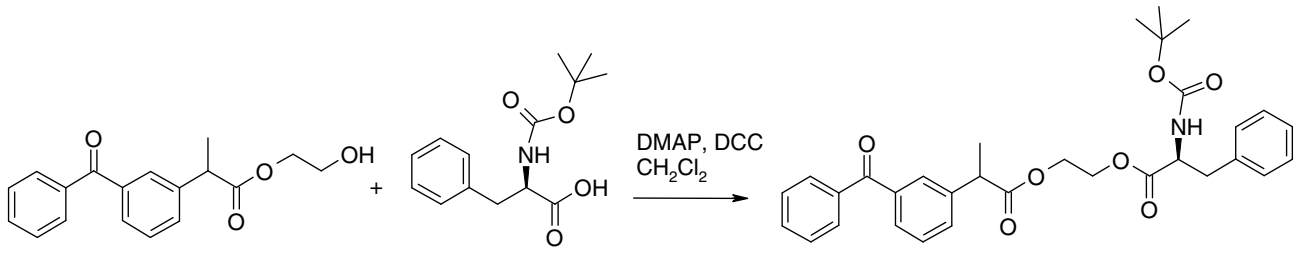


Ketoprofen (1.71 g, 6.70 mmol) and K_2CO_3 (1.20 g, 8.71 mmol) were stirred with DMF (40 mL) at 60°C. Bromoethanol (1.09 g, 8.71 mmol) was added and mixture was stirred over night at 60°C. Mixture was cooled and water (125 mL) was added and washed with ethyl acetate (5*25 mL). Combined organic phases was dried with Na_2SO_4 and purified by flash chromatography (petrolether:ethylacetate 1:1), R_f = 0.31. Yield 1.60 g (80 %).

^{1}H-NMR ($CDCl_3$) δ 1.54 (3H, d, *J* = 7.2 Hz, C*H*$_3$), 2.60 (1H, bs, O*H*), 3.77 (2H, t, *J* = 4.7 Hz, C*H*$_2$), 3.85 (1H, q, *J* = 7.2 Hz, C*H*$_3$), 4.17-4.25 (2H, m, C*H*$_2$), 7.42-7.50 (3H, m, Ar*H*), 7.54-7.60 (2H, m, Ar*H*), 7.65-7.66 (1H, m, Ar*H*), 7.78-7.80 (3H, m, Ar*H*). ^{13}C-NMR ($CDCl_3$) δ 18.5,

45.3, 60.9, 66.5, 128.3, 128.6, 129.1, 129.2, 130.1, 131.5, 132.6, 137.3, 137.9, 140.8, 174.4, 196.7. MS: *m/z* calcd. for $C_{18}H_{18}O_4$ $[M]^+$ = 298.3 Found 299.1 $[M + 1]^+$.

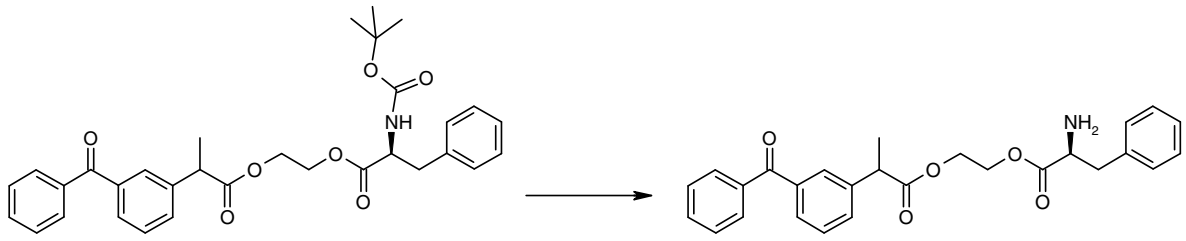
(S)-2-*tert*-Butoxycarbonylamino-3-phenyl-propionic acid 2-[2-(3-benzoyl-phenyl)-propionyloxy]-ethyl ester



Alcohol (0.60 g, 2.01 mmol), BOC-L-phenyl alanine (0.56 g, 2.11 mmol) and DMAP (0.02 g, 0.20mmol) were dissolved to dichloromethane (25 mL). DCC (0.54 g, 2.61 mmol) was added and mixture was stirred at R.T. under argon over night. Formed solid was filtered and filtrate was evaporated and purified by flash chromatography (hexane:ethyl acetate 4:1), R_f = 0.14. Combined fractions were evaporated and dried in vacuo. Yield of clear viscous oil was 1.02 g (93 %).

^{1}H-NMR ($CDCl_3$): δ 1.40 (9H, s, C*H*$_3$), 1.55 (3H, d, *J* = 7.2 Hz, C*H*$_3$), 2.92-3.07 (2H, m, C*H*$_2$-Bz), 3.82 (1H, q, *J* = 7.2 Hz, C*H*CH_3), 4.18-4.34 (4H, m, OC*H*$_2$C*H*$_2$O), 4.55 (1H, q, *J* = 6.6 Hz, C*H*CH_2), 4.97 (1H, d, *J* = 6.7 Hz, N*H*), 7.08-7.09 (2H, d, *J* = 9.0 Hz, Ar*H*), 7.20-7.31 (3H, m, Ar*H*), 7.41-7.49 (3H, m, Ar*H*), 7.53-7.55 (1H, m, Ar*H*), 7.57-7.60 (1H, m, Ar*H*), 7.64-7.66 (1H, m, Ar*H*), 7.76-7.78 (3H, m, Ar*H*). ^{13}C-NMR ($CDCl_3$) δ 18.5, 28.3, 38.2, 45.2, 54.3, 62.4, 62.7, 79.9, 127.1, 128.3, 128.5, 128.6, 129.15, 129.20, 129.3, 130.1, 131.5, 132.5, 135.9, 137.5, 138.0, 140.5, 155.1, 171.6, 173.8, 196.4. MS: *m/z* calcd. for $C_{32}H_{35}NO_7$ $[M]^+$ = 545.6 Found 545.8 $[M]^+$; $[M + Na]^+$ = 568.6. Found 568.1 $[M + Na]^+$.

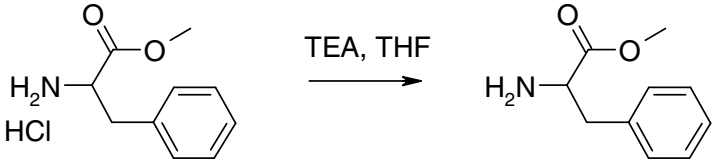
(S)-2-Amino-3-phenyl-propionic acid 2-[2-(3-benzoyl-phenyl)-propionyloxy]-ethyl ester (2)



Boc-protected derivative (0.94 g, 1.72 mmol) was dissolved to ethyl acetate (35 mL) and HCl gas was added to the mixture over 30 minutes. The solvent was evaporated and the product was dried in vacuo. Yield of white solid was 0.78 g (95 %).

^{1}H-NMR ($CDCl_3$): δ 1.51 (3H, dd, *J* = 7.2 Hz, C*H*$_3$), 3.24-3.39 (2H, m, C*H*$_2$-Bz), 3.82 (1H, q, *J* = 7.2 Hz, C*H*CH$_3$), 4.06-4.37 (5H, m, OC*H*$_2$C*H*$_2$O + COC*H*CH$_2$), 7.17-7.25 (5H, bs, Ar*H*), 7.38-7.47(3H, m, Ar*H*), 7.51-7.62 (3H, m, Ar*H*), 7.73-7.75 (3H, m, Ar*H*), 8.82 (3H, bs, N*H*$_3$Cl$_3$). ^{13}C-NMR ($CDCl_3$) δ 18.4, 18.7, 36.1, 45.0, 45.1, 54.2, 62.1, 63,7, 63.8, 127.7, 128.3, 128.6, 128.8, 128.9, 129.0, 129.04, 129.2, 129.5, 130.1, 130.04, 131.56, 131.6, 132.5, 132.55, 133.65, 133.67, 137.3, 137.9, 140.54, 140.56, 168.40, 168.41, 173.7, 196.43, 196.47. MS: *m/z* calcd. for $C_{27}H_{27}NO_5$ $[M]^+$ = 445.52 Found 446.1 $[M+1]^+$.

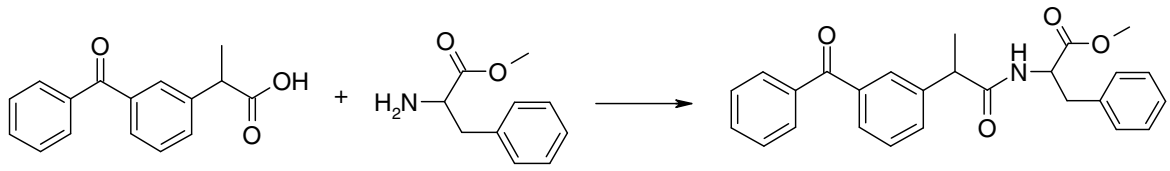
2-Amino-3-phenyl-propionic acid methyl ester



L-Phenylalanine methylester hydrochloride (1.00 g, 4.64 mmol) was dissolved to tetrahydrofurane (15 mL). Triethylamine (0.47 g, 4.64 mmol) was added and mixture was stirred at R.T. for 3h. Formed solid was filtered and filtrate was evaporated and dried in vacuo. Yield of clear viscous oil was quantitative.

^{1}H-NMR ($CDCl_3$): δ 1.49 (2H, s, NH_2), 2.84 (1H, dd, J = 7.9 Hz, 13.5 Hz, CH_a), 3.07 (1H, dd, J = 5.1 Hz, 13.5 Hz, CH_b), 3.69 (3H, s, OCH_3), 3.69-3.73 (1H, m, CH_2CHCO), 7.16-7.30 (5H, m, ArH). ^{13}C-NMR ($CDCl_3$) δ 41.0, 51.8, 55.7, 126.7, 128.4, 129.1, 137.1, 175.2.

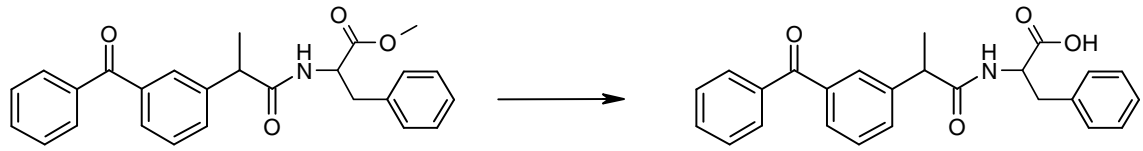
2-[2-(3-Benzoyl-phenyl)-propionylamino]-3-phenyl-propionic acid methyl ester



Ketoprofen (1.10 g, 4.34 mmol), L-phenylalanine methyl ester (0.74 g, 4.13 mmol) and DMAP (0.05 g, 0.413 mmol) were dissolved to dichloromethane (25 mL). DCC (0.98 g, 4.75 mmol) was added and mixture was stirred at R.T. under argon over night. Formed solid was filtered and filtrate was evaporated and purified by flash chromatography (hexane:ethyl acetate 3:1). Combined fractions were evaporated and dried in vacuo. Yield of clear viscous oil was 1.6 g (88 %).

^{1}H-NMR ($CDCl_3$): δ 1.51 (3H, d, J = 7.0 Hz, CH_3), 2.97 (2H, m, CH_2-Bn), 3.60 (1H, q, J = 7.1 Hz, CH-CH_3), 3.70 (3H, d, J = 18,5 Hz, OCH_3), 4.80-4.89 (1H, m, CH_2CHCO), 5.87 (1H, bs, NH), 6.77-7.22 (5H, m, ArH), 7.42-7.78 (9H, m, ArH). ^{13}C-NMR ($CDCl_3$) δ 18.2, 18.4, 37.61, 37.62, 46.81, 46.83, 52.31, 52.34, 52.9, 53.1, 127.04, 127.12, 128.33, 128.36, 128.46, 128.56, 128.77, 128.85, 129.10, 129.13, 129.15, 129.19, 129.24, 130.05, 130.07, 131.47, 131.61, 132.53, 132.56, 135.39, 135.65, 137.4, 138.04, 138.08, 140.97, 141.0, 171.75, 171.85, 172.79, 173.09, 196.37, 196.41. MS: m/z calcd. for $C_{26}H_{25}NO_4$ $[M]^+$ = 415.49 Found 416.0 $[M+1]^+$.

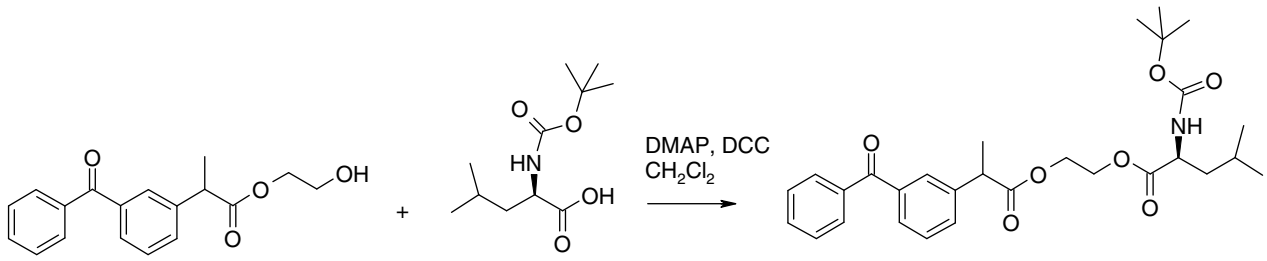
2-[2-(3-Benzoyl-phenyl)-propionylamino]-3-phenyl-propionic acid (3)



Methyl ester (0.2 g, 0.48 mmol), was dissolved to methanol water mixture (15 mL:15 mL) and 1 M LiOH (4 mL) was added. Mixture was stirred at R.T. until reaction was completed. Mixture was acidified with 12 M HCl and methanol was evaporated. Product was extracted with diethyl ether (3*10 mL) and a combined organic phase was dried with Na_2SO_4. Solvent was evaporated and formed solid was dried in vacuo. Yield of the white solid was 0.145 g (76%).

^{1}H-NMR ($CDCl_3$): δ 1.47 (3H, d, *J* = 7.0 Hz, C*H*$_3$), 2.92-3.21 (2H, m, C*H*$_2$-Bn), 3.49 (1H, q, *J* = 7.0 Hz, C*H*-CH$_3$), 3.64-3.72 (1H, m, CH$_2$C*H*CO), 4.83-4.94 (1H, m, N*H*), 6.60-7.16 (5H, m, Ar*H*), 7.34-7.76 (9H, m, Ar*H*), 10.65 (1H, bs, CO*OH*). ^{13}C-NMR ($CDCl_3$) δ 17.8, 18.1, 36.98, 37.0, 46.12, 46.21, 52.71, 52.94, 126.6, 126.73, 128.05, 128.10, 128.13, 128.2, 128.43, 128.47, 128.94, 128.99, 129.01, 129.07, 129.24, 129.88, 129.90, 131.34, 131.57, 132.41, 132.48, 135.34, 135.61, 137.0, 137.47, 137.51, 140.67, 141.20, 173.32, 173.44, 173.61, 173.97, 196.57, 196.71. MS: *m/z* calcd. for $C_{25}H_{23}NO_4$ $[M]^+$ = 401.47 Found 401.9 $[M]^+$; $[M + NH_4^+]^+$ = 419.52 Found 419.0 $[M + NH_4^+]^+$.

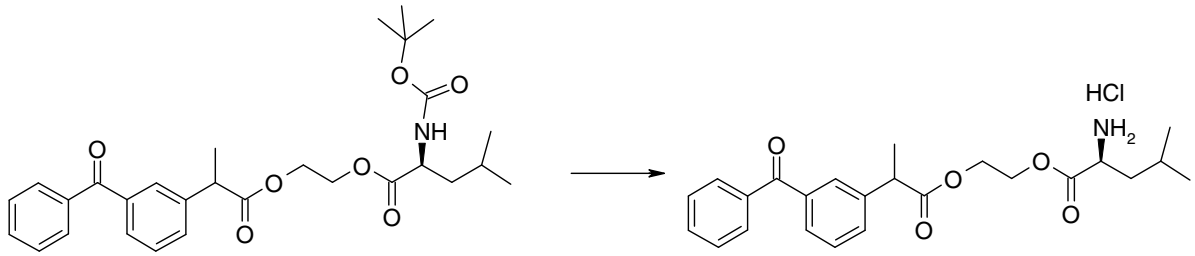
(S)-2-*tert*-Butoxycarbonylamino-4-methyl-pentanoic acid 2-[2-(3-benzoyl-phenyl)-propionyloxy]-ethyl ester



Alcohol (0.60 g, 2.01 mmol), BOC-L-leucine (0.49 g, 2.11 mmol) and DMAP (0.02 g, 0.20 mmol) were dissolved to dichloromethane (25 mL). DCC (0.54 g, 2.61 mmol) was added and mixture was stirred at R.T. under argon over night. Formed solid was filtered and filtrate was evaporated and purified by flash chromatography (petrol ether:ethyl acetate 4:1), R_f = 0.15. Combined fractions were evaporated and dried in vacuo. Yield of clear viscous oil was 0.97 g (94 %).

^{1}H-NMR ($CDCl_3$): δ 0.91 (3H, d, *J* = 3.5 Hz, C*H*$_3$), 0.92 (3H, d, *J* = 3.4 Hz, C*H*$_3$), 1.43 (9H, s, C*H*$_3$), 1.55 (3H, d, *J* = 7.2 Hz, C*H*$_3$), 1.50-1.58 (1H, m, C*H*), 1.65-1.73 (2H, m, C*H*$_2$), 3.83 (1H, q, *J* = 7.1 Hz, C*H*CH$_3$), 4.21-4.38 (4H, m, OC*H*$_2$C*H*$_2$O), 4.91 (1H, t, *J* = 7.0 Hz, N*H*), 7.43-7.51 (3H, m, Ar*H*), 7.54-7.56 (1H, m, Ar*H*), 7.59-7.62 (1H, m, Ar*H*), 7.67-7.69 (1H, m, Ar*H*), 7.76-7.82 (3H, m, Ar*H*). ^{13}C-NMR ($CDCl_3$): δ 18.5, 21.8, 22.8, 24.8, 28.3, 41.6, 45.2, 52.0, 62.5, 62.6, 79.9, 128.3, 128.6, 129.2, 130.1, 131.5, 132.5, 137.5, 138.0, 140.6, 155.1, 173.2, 173.8, 196.5. MS: *m*/*z* calcd. for $C_{29}H_{37}NO_7$ $[M]^+$ = 511.6 Found 511.8 $[M]^+$; $[M + Na]^+$ = 534.6. Found 534.1 $[M + Na]^+$.

(S)-2-Amino-4-methyl-pentanoic acid 2-[2-(3-benzoyl-phenyl)-propionyloxy]-ethyl ester hydrochloride (4)

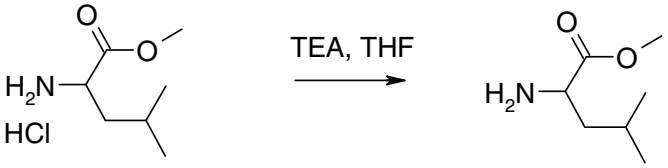


Boc-protected compound (0.895 g, 1.75 mmol) was dissolved to ethyl acetate (35 mL) and HCl gas was added to the mixture over 30 minutes. The solvent was evaporated and the product was dried in vacuo. Yield of white solid was 0.66 g (92 %).

^{1}H-NMR ($CDCl_3$): δ 0.93 (3H, s, C*H*$_3$), 0.94 (3H, s, C*H*$_3$), 1.53 (3H, d, *J* = 7.1 Hz, C*H*$_3$), 1.76-1.81 (1H, m, C*H*), 1.89-1.98 (2H, m, C*H*$_2$), 3.84 (1H, q, *J* = 7.0 Hz, C*H*CH$_3$), 4.02-4.06

(1H, m, COC*H*(NH_3Cl)CH_2), 4.21-4.45 (4H, m, OCH_2CH_2O), 7.42-7.49 (3H, m, Ar*H*), 7.53-7.66 (3H, m, Ar*H*), 7.75-7.79 (3H, m, Ar*H*), 8.91 (3H, bs, CHNH_3Cl). ^{13}C-NMR ($CDCl_3$) δ 18.5, 22.1, 22.4, 24.5, 39.6, 45.3, 51.8, 62.2, 63.9, 128.5, 128.7, 129.2, 129.3, 130.2, 131.7, 132.7, 137.5, 138.1, 140.7, 169.4, 173.9, 196.6. MS: *m/z* calcd. for $C_{24}H_{29}NO_5$ $[M]^+$ = 411.5 Found 412.1 $[M+1]^+$.

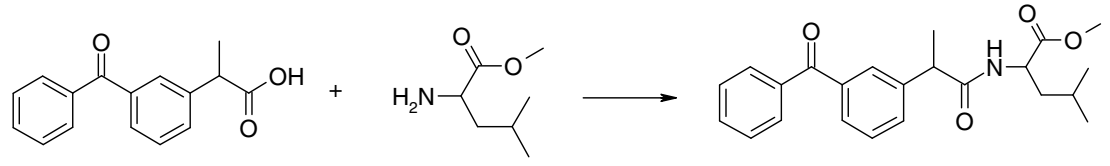
2-Amino-4-methyl-pentanoic acid methyl ester



L-Leucine methylester hydrochloride (1.00 g, 5.50 mmol) was dissolved to tetrahydrofurane (15 mL). Triethylamine (0.56g, 5.50 mmol) was added and mixture was stirred at R.T. for 3h. Formed solid was filtered and filtrate was evaporated and dried in vacuo. Yield of clear viscous oil was 0.70 g (88%).

^{1}H-NMR ($CDCl_3$): δ 0.85 (3H, d, *J* = 6.6 Hz, CH_3), 0.87 (3H, d, *J* = 6.7 Hz, CH_3), 1.32-1.38 (1H, m, CH_a), 1.46-1.52 (1H, m, CH_b), 1.66 (2H, bs, NH_2), 1.66-1.74 (1H, m, C*H*), 3.41 (1H, dd, *J* = 4.8 Hz, C*H*), 3.64 (3H, s, CH_3). ^{13}C-NMR ($CDCl_3$) δ 21.7, 22.8, 24.6, 43.9, 51.7, 52.6, 176.9.

2-[2-(3-Benzoyl-phenyl)-propionylamino]-4-methyl-pentanoic acid methyl ester

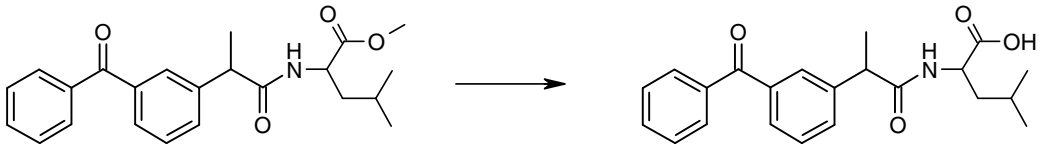


Ketoprofen (0.88 g, 3.31 mmol), L-leucine methyl ester (0.48 g, 3.47 mmol) and DMAP (0.04 g, 0.33 mmol) were dissolved to dichloromethane (25 mL). DCC (0.78 g, 3.80 mmol) was

added and mixture was stirred at R.T. under argon over night. Formed solid was filtered and filtrate was evaporated and purified by flash chromatography (hexane:ethyl acetate 3:1). Combined fractions were evaporated and dried in vacuo. Yield of clear viscous oil was 1.18 g (94%).

^{1}H-NMR ($CDCl_3$): δ 0.91 (3H, d, *J* = 6.1 Hz, C*H*$_3$), 0.92 (3H, d, *J* = 5.5 Hz, C*H*$_3$), 1.45-1.51 (1H, m, C*H*), 1.55 (3H, d, *J* = 7.2 Hz, C*H*$_3$), 1.58.64 (2H, m, C*H*$_2$), 3.67 (3H, s, C*H*$_3$), 3.67 (1H, q, *J* = 7.1 Hz, C*H*), 4.58-4.63 (1H, m, C*H*), 5.80 (1H, d, *J* = 7.9 Hz, N*H*), 7.46-7.51 (3H, m, Ar*H*), 7.58-7.62 (2H, m, Ar*H*), 7.68-7.70 (1H, m, Ar*H*), 7.75-7.81 (3H, m, Ar*H*). ^{13}C-NMR ($CDCl_3$) δ 18.6, 21.9, 22.7, 24.8, 41.5, 46.8, 50.8, 52.1, 128.3, 128.7, 129.1, 129.2, 130.0, 132.5, 137.4, 138.0, 141.3, 173.2, 176.3, 196.5. MS: *m*/*z* calcd. for $C_{23}H_{27}NO_4$ $[M+1]^+$ = 382.48 Found 382.1 $[M]^+$.

2-[2-(3-Benzoyl-phenyl)-propionylamino]-4-methyl-pentanoic acid (5)



Methyl ester (0.48 g, 1.26 mmol), was dissolved to methanol water mixture (15 mL:15 mL) and 1 M LiOH (4 mL) was added. Mixture was stirred at R.T. until reaction was completed. Mixture was acidified with 12 M HCl and methanol was evaporated. Product was extracted with diethyl ether (3*10 mL) and a combined organic phase was dried with Na_2SO_4. Solvent was evaporated and formed solid was dried in vacuo. Yield of white solid was 0.28 g (61%).

^{1}H-NMR ($CDCl_3$): δ 0.89 (3H, d, *J* = 2.5 Hz, C*H*$_3$), 0.90 (3H, d, *J* = 2.6 Hz, C*H*$_3$), 1.45-1.51 (1H, m, C*H*), 1.54 (3H, d, *J* = 7.2 Hz, C*H*$_3$), 1.57-1.67 (2H, m, C*H*$_2$), 3.70 (1H, q, *J* = 7.1 Hz, C*H*), 4.55-4.59 (1H, m, C*H*), 6.07 (1H, d, *J* = 8.0 Hz, N*H*), 7.41-7.49 (3H, m, Ar*H*), 7.57-7.60 (2H, m, Ar*H*), 7.64-7.66 (1H, m, Ar*H*), 7.74-7.78 (3H, m, Ar*H*). ^{13}C-NMR ($CDCl_3$) δ 18.5, 21.8, 22.8, 24.9, 40.9, 46.7, 51.0, 128.4, 128.8, 129.26, 129.29, 130.1, 131.7, 132.7, 137.3,

138.0, 141.2, 174.2, 176.3, 196.8. MS: *m/z* calcd. for $C_{22}H_{25}NO_4$ $[M]^+$ = 367.45, Found 385.0 $[M + NH_4^+]^+$.

In situ inhibition studies for compounds 2-5. The ability of the prodrug to bind into LAT1 was studied with the in situ rat brain perfusion technique. The 100% PA product of [^{14}C]L-leucine was determined after 30 s perfusion of 0.2 µCi/mL [^{14}C]L-leucine solution. In a competition study [^{14}C]L-leucine (0.2 µCi/mL) was co-perfused with 70 µM concentration of the prodrugs for 30 s. In the presence of **2-5** the PA product of [^{14}C]L-leucine was 0.02815 ± 0.0059, 0.01910 ± 0.0063, 0.02117 ± 0.0072, 0.02655 ± 0.0018, respectively (mean ± s.d., n=2). The addition of the prodrugs did not significantly decrease the uptake of [^{14}C]L-leucine.