Supporting Information

Improved peptide prodrugs of 5-ALA for PDT: rationalisation of cellular accumulation and protoporphyrin IX production by direct determination of cellular prodrug uptake and prodrug metabolisation

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Compound	Formula	Required	Found
3h	$C_{17}H_{30}N_2O_6$	C, 56.97; H, 8.44; N, 7.82	C, 57.00; H, 8.56; N, 8.08
3n	$C_{20}H_{28}N_2O_6$	C, 61.21; H, 7.19, N, 7.14	C, 61.20; H, 7.29; N, 7.21
3q	$C_{22}H_{29}N_3O_6$	C, 61.24; H, 6.77, N, 9.74	C, 60.90; H, 7.07; N, 9.54
3u	$C_{20}H_{36}N_2O_8$	C, 55.80; H, 7.96, N, 6.51	C, 55.40; H, 7.99; N, 6.51
3v	$C_{19}H_{32}N_2O_8$	C, 54.80; H, 7.74, N, 6.73	C, 54.30; H, 7.70; N, 6.74
4e	$C_{13}H_{22}N_2O_5$	C, 54.53; H, 7.74; N, 9.78	C, 54.53; H, 7.82; N, 9.78
4g	$C_{14}H_{24}N_2O_5$	C, 55.98; H, 8.05, N, 9.33	C, 56.10; H, 8.09; N, 9.26
4h	$C_{14}H_{24}N_2O_5$	C, 55.98; H, 8.05, N, 9.33	C, 55.90; H, 8.07; N, 9.42

***N*-Urethane-protected Dipeptides**

All the following compounds were synthesised on a 1.4 mmol scale, from **1a** (**1b** for **3o**) and the appropriate amino acid succinimido ester derivative, according to the general procedure.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-seryl-5-aminolaevulinic acid methyl ester (2a)**

White solid (668 mg, 91 %); mp 95-97 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.75 (2H, d, *J* 7.4, Fmoc Ar*H*), 7.73 (2H, d, *J* 7.4, Fmoc Ar*H*), 7.57-7.29 (5H m, Fmoc Ar*H*, amide N*H*), 5.75 (1H, d, *J* 5.2, urethane N*H*), 4.39 (2H, d, *J* 6.6, Fmoc CH_2), 4.30-4.19 (4H, m, Fmoc C*H*CH$_2$, C$H_A H_B$OBu^t, NHCH_2CO), 3.83-3.81 (1H, m, NHC*H*(R′)CO), 3.65 (3H, s, COOCH_3), 3.42-3.36 (1H, m, C$H_A H_B$OBu^t), 2.71-2.75 (4H, m, COCH_2CH_2COOCH$_3$), 1.20 (9H, s, C(CH_3)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.2, 172.9, 170.4, 156.2, 143.9, 143.8, 127.8, 127.2, 125.2, 120.1, 74.4, 67.3, 61.7, 49.5, 47.2, 34.6, 27.6, 27.4; ESI-HRMS$^+$: calcd. for $C_{28}H_{35}N_2O_7$: 511.2439; found: 511.2446 $[M+H]^+$.

***N*$^\alpha$-(9-Fluorenylmethoxycarbonyl)-*N*$^\varepsilon$-(*tert*-butoxycarbonyl)-L-lysyl-5-aminolaevulinic acid methyl ester (2b)**

White solid (774 mg, 93 %); mp 132-134 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.73 (2H, d, *J* 7.4, Fmoc Ar*H*), 7.57 (1H, d, *J* 7.1, Fmoc Ar*H*), 7.40-7.24 (4H, m, Fmoc Ar*H*), 6.95 (1H, br, amide N*H*), 5.72 (1H, br, urethane N*H*), 4.70 (1H, br, NHC*H*(R′)CO), 4.38-4.36 (2H, m, Fmoc CH_2), 4.21-4.15 (3H, m, Fmoc C*H*CH$_2$, NHCH_2CO), 3.63 (3H, s, COOCH_3), 3.10-3.05 (2H, m, CH_2NHBoc), 2.73-2.59 (4H, m, NHCH_2CH_2CO), 1.91-1.56 (2H, m, CHCH_2(CH$_2$)$_3$NHBoc), 1.56-1.25 (13H, m, CHCH$_2$CH_2CH_2CH$_2$NHBoc, C(CH_3)$_3$), ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.7, 173.0,

172.5, 172.3, 156.4, 141.3, 127.8, 127.2, 125.2, 120.0, 67.1, 52.1, 49.1, 47.2, 34.6, 28.5, 27.6, 24.5, 22.5, ESI-HRMS$^+$: calcd. for $C_{32}H_{42}N_3O_8$: 596.2971; found: 596.2961 $[M+H]^+$.

***tert*-Butoxycarbonyl-glycyl-5-aminolaevulinic acid methyl ester (3a)**[36]

Colourless oil. (304 mg, 72 %).

***tert*-Butoxycarbonyl-L-alanyl-5-aminolaevulinic acid methyl ester (3b)**[36]

Colourless oil (354 mg, 80 %).

***tert*-Butoxycarbonyl-D-alanyl-5-aminolaevulinic acid methyl ester (3c)**

Colourless oil (406 mg, 93 %); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 6.97 (1H, br, N*H* amide), 5.20 (1H, d, *J* 6.9, N*H* urethane), 4.19-4.12 (3H, m, NHC*H*(R′)CO, NHCH_2CO), 3.62 (3H, s, COOCH_3), 2.70 (2H, t, *J* 6.9, COCH_2CH$_2$COOCH$_3$), 2.59 (2H, t, *J* 6.9, COCH$_2$CH_2COOCH$_3$), 1.38 (9H, s, C(CH_3)$_3$), 1.31 (3H, d, *J* 7.2, CHCH_3); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 204.1, 173.0, 155.5, 80.1, 50.1, 49.1, 34.5, 28.5, 27.8, 18.6; ESI-HRMS$^+$: calcd. for $C_{14}H_{25}N_2O_6$: 317.1717; found: 317.1700 $[M+H]^+$.

***tert*-Butoxycarbonyl-L-valyl-5-aminolaevulinic acid methyl ester (3d)**[36]

White solid (371 mg, 77 %); mp 74-76 °C (lit[36], 74-77 °C).

***tert*-Butoxycarbonyl-D-valyl-5-aminolaevulinic acid methyl ester (3e)**

White solid (252 mg, 53 %); mp 72-75 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 6.74 (1H, br, amide N*H*), 5.10 (1H, d, *J* 7.4, urethane N*H*), 4.17 (2H, d, *J* 4.7, NHCH_2CO), 3.99-3.96 (1H, m, NHC*H*(R′)CO, 3.64 (3H, s, COOCH_3), 3.07 (2H, t, *J* 7.2, COCH_2CH$_2$), 2.77 (2H, t, *J* 7.2, COCH$_2$CH_2), 2.19-2.12 (1H, m, CHC*H*(CH$_3$)$_2$) 1.40 (9H, s, C(CH_3)$_3$), 0.93-0.90 (6H, m, CH(CH_3)$_2$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.7, 172.9, 172.0, 155.9, 80.3, 60.8, 52.0, 49.1, 34.6, 30.9, 28.4, 27.6, 19.3: ESI-HRMS$^+$: calcd. for $C_{16}H_{29}N_2O_6$: 345.2020; found: 345.2005 $[M+H]^+$.

***tert*-Butoxycarbonyl-L-leucyl-5-aminolaevulinic acid methyl ester (3f)**[36]

White solid (451 mg, 90 %); mp 56-59 °C (lit[36], oil).

***tert*-Butoxycarbonyl-D-leucyl-5-aminolaevulinic acid methyl ester (3g)**

White solid (445 mg, 90 %); mp 55-57 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 6.94 (1H, br, amide N*H*), 5.15 (1H, br, amide N*H*), 4.13-4.12 (3H, m, NHC*H*(R′)CO, NHCH_2CO), 3.64 (3H, s, COOCH_3), 2.70 (2H, t, *J* 6.6, COCH_2CH$_2$COOCH$_3$), 2.59 (2H, t, *J* 6.6, COCH$_2$CH_2COOCH$_3$), 1.63-1.60 (2H, m, CHCH_2CH(CH$_3$)$_2$), 1.38 (9H, m, C(CH_3)$_3$), 0.89-0.87 (6H, m, CHCH$_2$CH(CH_3)$_2$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.8, 172.9, 143.6, 80.1, 52.0, 49.1, 41.5, 34.5, 34.0, 28.3, 27.6, 24.8, 23.1; ESI-HRMS$^+$: calcd. for $C_{17}H_{31}N_2O_6$: 359.2177; found: 359.2161 $[M+H]^+$.

***tert*-Butoxycarbonyl-L-isoleucyl-5-aminolaevulinic acid methyl ester (3h)**

White solid (455 mg, 92 %); mp 77-80 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 8.21 (1H, br, amide N*H*), 6.75 (1H, br, urethane N*H*), 4.07 (2H, d, *J* 10.2, NHCH_2CO), 3.98-3.95 (1H, m, NHC*H*(R′)CO), 3.64 (3H, s, COOCH_3), 3.08 (1H, t, *J* 7.1, C*H*(CH$_3$)CH$_2$CH$_3$), 2.80-2.74 (2H, m, CHCH_2CH$_3$, COCH_2CH$_2$COOCH$_3$), 2.58 (2H, t, *J* 6.9, COCH$_2$CH_2COOCH$_3$), 1.45 (9H, s, C(CH_3)$_3$), 0.93-0.90 (6H, m, CH(CH_3)CH$_2$CH_3); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.8, 172.9, 172.0, 152.9, 143.6, 79.9, 59.2, 51.9, 49.0, 37.3, 32.5, 29.5, 28.3, 27.6, 24.7, 15.6, 11.5; ESI-HRMS$^+$: calcd. for $C_{17}H_{31}N_2O_6$: 381.1996; found: 381.1977 $[M+H]^+$; Anal. ($C_{17}H_{30}N_2O_6$) C, H, N.

***tert*-Butoxycarbonyl-L-prolyl-5-aminolaevulinic acid methyl ester (3i)**

Colourless oil (443 mg, 94 %); ^{1}H NMR (270 MHz, CD_3OD) δ: 4.23-4.21 (1H, m, BocNC*H*(R′)CO), 4.06 (2H, br, NHCH_2CO), 3.64 (3H, s, COOCH_3), 3.51-3.35 (2H, m, BocNCH_2), 2.76 (2H, t, *J* 6.61, COCH_2CH$_2$COOCH$_3$), 2.60-2.58 (2H, m, COCH$_2$CH_2COOCH$_3$), 2.24-2.21, 2.01-1.93 (2H each, m, BocNCH$_2$CH_2CH_2), 1.38

(9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.3, 174.8, 173.5, 154.7, 80.3, 60.6, 33.9, 31.2, 30.2, 27.4, 27.1, 25.1, 24.0, 23.3; ESI-HRMS$^+$: calcd. for $C_{16}H_{27}N_2O_6$: 343.1864; found: 343.1854 $[M+H]^+$.

***tert*-Butoxycarbonyl-D-prolyl-5-aminolaevulinic acid methyl ester (3j)**

Colourless oil (378 mg, 80 %); ESI-HRMS$^+$: calcd. for $C_{16}H_{27}N_2O_6$: 343.1864; found: 343.1852 $[M+H]^+$.

***tert*-Butoxycarbonyl-L-methionyl-5-aminolaevulinic acid methyl ester (3k)**

White solid (493 mg, 95 %); mp 50-53 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.12 (1H, br, amide N*H*), 5.49 (1H, br, urethane N*H*), 4.27-4.22 (1H, m, NHC*H*(R′)CO), 4.11-4.08 (2H, m, NHC*H*$_2$CO), 3.57 (3H, s, COOC*H*$_3$), 2.68-2.62 (2H, m, COC*H*$_2$CH$_2$COOCH$_3$,), 2.57-2.45 (4H, m, COCH$_2$C*H*$_2$COOCH$_3$, CH$_2$C*H*$_2$S), 2.15-1.95 (5H, m, CH$_2$SC*H*$_3$, C*H*$_A$*H*$_B$CH$_2$SCH$_3$), 1.89-1.81 (1H, m, C*H*$_A$*H*$_B$CH$_2$SCH$_3$), 1.39 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.7, 172.9, 173.0, 155.6, 80.2, 53.5, 49.1, 34.5, 31.9, 30.2, 28.3, 27.6, 15.2; ESI-HRMS$^+$: calcd. for $C_{16}H_{28}N_2O_6SNa$: 399.1560; found: 399.1549 $[M+Na]^+$.

***tert*-Butoxycarbonyl -L-seryl-5-aminolaevulinic acid methyl ester (3l)**

Colourless oil (446 mg, 96 %); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.40 (1H, br, amide N*H*), 5.78 (1H, d, *J* 5.7, urethane N*H*), 4.18-4.12 (4H, m, NHC*H*$_2$CO, NHC*H*(R′)CO, C*H*$_A$*H*$_B$OH), 3.97-3.87 (1H, m, C*H*$_A$*H*$_B$OH), 2.69 (2H, t, *J* 4.7, COC*H*$_2$CH$_2$COOCH$_3$), 2.59 (2H, t, *J* 4.7, COC*H*$_2$COOCH$_3$), 1.37 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ 204.4, 173.1, 171.7, 156.0, 80.4, 58.2, 55.7, 52.0, 49.2, 34.4, 28.3, 27.6: ESI-HRMS$^+$: calcd. for $C_{24}H_{25}N_2O_7$: 333.1662; found 333.1655 $[M+H]^+$.

***tert*-Butoxycarbonyl-D-phenylalanyl-5-aminolaevulinic acid methyl ester (3n)**

White solid (941 mg, 87 %); mp 70-72 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.30-7.15 (5H, m, Ph), 6.69 (1H, s, amide N*H*), 4.95 (1H, d, *J* 6.6, urethane N*H*), 4.39 (1H, br,

NHC*H*(R′)CO), 4.22-4.03 (2H, m, NHCH_2CO), 3.65 (3H, s, COOCH_3), 3.13-3.03 (2H, m, CH_2Ph), 2.68-2.61 (4H, m, COCH_2CH_2COOCH$_3$), 1.37 (9H, s, C(CH_3)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.3, 172.7, 171.4, 136.5, 129.2, 128.6, 126.9, 79.9, 55.6, 49.0, 38.3, 34.4, 28.2, 27.5; ESI-HRMS$^+$: calcd. for $C_{20}H_{29}N_2O_6$: 393.2020; found: 393.2010 $[M+H]^+$. Anal. ($C_{20}H_{28}N_2O_6$) C, H, N.

***tert*-Butoxycarbonyl-L-phenylalanyl-5-aminolaevulinic acid ethyl ester (3o)**

White solid (455 mg, 80 %); mp 83-85 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.25-7.14 (5H, m, Ph), 6.80-6.77 (1H, m, amide N*H*), 5.13 (1H, d, *J* 8.3, urethane N*H*), 4.33-4.44 (1H, m, NHC*H*(R′)CO), 4.11-4.06 (4H, m, NHCH_2CO, OCH_2CH$_3$), 3.10-2.91 (2H, m, CH_2Ph), 2.68-2.51 (4H, m, COCH_2CH_2COOCH$_2$CH$_3$), 1.34 (9H, s, C(CH_3)$_3$), 1.20 (3H, t, *J* 7.7, OCH$_2$CH_3); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 206.6, 172.4, 171.6, 136.7, 129.3, 128.6, 126.9, 80.2, 60.9, 49.1, 34.4, 28.2, 27.9, 14.2; ESI-HRMS$^+$: calcd. for: $C_{20}H_{31}N_2O_6$: 407.2182; found: 407.2171 $[M+H]^+$.

***tert*-Butoxycarbonyl-L-tryptophyl-5-aminolaevulinic acid methyl ester (3p)**

White solid (597 mg, 97%); mp 77-80 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 8.56 (1H, br, indole N*H*), 7.56 (1H, d, *J* 7.7, indole H-4), 7.30 (1H, d, *J* 7.7, indole H-7), 7.13-7.03 (3H, m, indole H-2, H-5, H-6), 6.68 (1H, br, amide N*H*), 5.28 (1H, d, *J* 6.6, urethane N*H*), 4.46 (1H, br, NHC*H*(R′)CO), 3.96 (2H, br, NHCH_2CO), 3.62 (3H, s, COOCH_3), 3.29-3.18 (2H, m, CH_2-indolyl), 2.57-2.53 (4H, m, COCH_2CH_2COOCH$_3$) 1.40 (9H, s, C(CH_3)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.3, 173.0, 172.3 0, 155.7, 136.3, 127.56, 123.4, 122.1, 119.6, 118.8, 111.4, 110.3, 80.1, 69.9, 55.6, 51.9, 37.4, 29.4, 28.2, 27.4; ESI-HRMS$^+$: calcd. for $C_{22}H_{30}N_3O_6Na$: 454.1949; found: 454.1943 $[M+Na]^+$.

***tert*-Butoxycarbonyl-D-tryptophyl-5-aminolaevulinic acid methyl ester (3q)**

White solid (565 mg, 95 %); mp 76-78 °C; ESI-HRMS$^+$: calcd. for $C_{22}H_{30}N_3O_6$: 432.2129; found: 432.2119 $[M+H]^+$. Anal. ($C_{22}H_{29}N_3O_6$) C, H, N.

N$^{\alpha}$-*tert*-Butoxycarbonyl-*O*-(benzyl)-L-tyrosyl-5-aminolaevulinic acid methyl ester (3r)

White solid (674 mg, 98 %); mp 103-105 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.42-7.30 (5H, m, Ph), 7.09 (2H, d, *J* 8.5, Tyr Ar*H*), 6.89 (2H, d, *J* 8.5, Tyr Ar*H*), 6.56 (1H, br, amide N*H*), 5.02 (2H, s, OCH_2Ph), 4.91 (1H, br, urethane N*H*), 4.34 (1H, br, NHC*H*(R′)CO), 4.15-4.10 (2H, m, NHCH_2CO), 3.65 (3H, s, COOCH_3), 3.00 (2H, d, *J* 6.3, CH_2Ar), 2.72-2.58 (4H, m, COCH_2CH_2COOCH$_3$), 1.39 (9H, s, C(CH_3)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.5, 172.9, 171.7, 157.9, 155.5, 137.1, 130.4, 128.9, 128.6, 128.0, 127.5, 115.0, 80.27, 70.1, 55.8, 52.0, 49.1, 37.6, 34.5, 28.4, 27.6; ESI-HRMS$^+$: calcd. for $C_{27}H_{34}N_2O_7Na$: 521.2258; found: 521.2254 $[M+Na]^+$.

N$^{\alpha}$-*tert*-Butoxycarbonyl-*N*$^{\tau}$-(trityl)-L-histidyl-5-aminolaevulinic acid methyl ester (3s)

White solid (707 mg, 82 %); mp 70-72 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.33-7.24 (11H, m, trityl Ar*H*, imidazole H-5, imidazole H-2), 7.12-7.07 (6H, m, trityl Ar*H*), 6.61 (1H, br, amide N*H*), 5.87-5.84 (1H, urethane N*H*, d, *J* 7.1), 4.54-4.40 (1H, m, NHC*H*(R′)CO), 4.12-4.09 (2H, s, NHCH_2CO), 3.64 (3H, s, COOCH_3), 3.08-2.59 (6H, m, CHCH_2-imidazolyl, COCH_2CH_2COOCH$_3$), 1.38 (9H, s, C(CH_3)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.7, 173.8, 172.9, 142.0, 138.5, 135.9, 129.8, 128.3, 128.2, 119.9, 75.7, 52.0, 49.2, 34.5, 28.4, 27.5, 26.7: ESI-HRMS$^+$: calcd. for $C_{36}H_{41}N_4O_6$: 625.3031; found: 625.3032 $[M+H]^+$.

N$^{\alpha}$-(*tert*-Butoxycarbonyl)-N$^{\varepsilon}$-(benzyloxycarbonyl)-L-lysyl-5-aminolaevulinic acid methyl ester (3t)[36]

White solid (651 mg, 93 %); mp 141-143 °C (lit[36], 144-146 °C).

N$^{\alpha}$-*tert*-Butoxycarbonyl-γ-(*tert*-butoxy)-L-glutamyl-5-aminolaevulinic acid methyl ester (3u)

Colourless oil (578 mg, 94 %); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.15 (1H, br, N*H* amide), 5.68 (1H, d, *J* 8.5, N*H* urethane), 4.11 (2H, d, *J* 4.7, NHCH_2CO), 4.10-4.02 (1H, m, NHC*H*(R′)CO), 3.62 (3H, s, COOCH_3), 2.72-2.57 (4H, m, COCH_2CH_2COOCH$_3$), 2.40-2.27 (2H, m, CH$_2$CH_2COOH), 2.11-2.00 (1H, m, CH_2CH$_2$COOH), 1.92-1.78 (1H, m, CH_2CH$_2$COOH), 1.40 (9H, s, COOC(CH_3)$_3$), 1.38 (9H, s, NHCOOC(CH_3)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.8, 173.0, 172.2, 155.7, 80.9, 53.9, 50.0, 49.1, 34.5, 31.7, 28.3, 27.6; ESI-HRMS^{+}: calcd. for $C_{20}H_{35}N_2O_8$: 431.2388; found: 431.2367 $[M+H]^{+}$. Anal. ($C_{20}H_{36}N_2O_8$) C, H, N.

N$^{\alpha}$-*tert*-Butoxycarbonyl-γ-(*tert*-butoxy)-L-aspartyl-5-aminolaevulinic acid methyl ester (3v)

Colourless oil (563 mg, 98 %); ^{1}H NMR (270 MHz, $CDCl_3$) δ: 7.15 (1H, br, N*H* amide), 5.68 (1H, d, *J* 8.5, N*H* urethane), 4.47-4.44 (1H, m, NHC*H*(R′)CO), 4.11 (2H, d, *J* 4.7, NHCH_2CO), 3.64 (3H, s, COOCH_3), 2.85-2.83 (6H, m, CH_2COOBu^{t} and COCH_2CH_2COOCH$_3$), 1.40 (9H, s, COOC(CH_3)$_3$), 1.38 (9H, s, NHCOOC(CH_3)$_3$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 203.4, 172.9, 171.2, 155.5, 81.6, 80.4, 52.0, 50.8, 49.3, 37.3, 34.5, 28.3, 28.0, 27.6; ESI-HRMS^{+}: calcd. for $C_{19}H_{33}N_2O_8$: 417.2231; found: 317.2211 $[M+H]^{+}$. Anal. ($C_{19}H_{32}N_2O_8$) C, H, N.

N-Acetylated Dipeptides

Unless stated, all the following compounds were obtained as white solids.

Acetyl-glycyl-5-aminolaevulinic acid methyl ester (4a)[36]

Scale: 0.14 mmol. Yield: 18.1 mg, 52%; mp 190-193 °C (lit[36], 192-194 °C).

Acetyl-L-alanyl-5-aminolaevulinic acid methyl ester (4b)[36]

Scale: 0.12 mmol. Yield: 27.2 mg, 84%; mp 100-102 °C (lit[36], 102-106 °C).

Acetyl-D-alanyl-5-aminolaevulinic acid methyl ester (4c)[36]

Scale: 0.32 mmol. Yield: 60.7 mg, 74 %; mp 100-101 °C (lit[36], 102-104 °C).

Acetyl-L-valyl-5-aminolaevulinic acid methyl ester (4d)[36]

Scale: 0.20 mmol. Yield: 50 mg, 85%; mp 147-149 °C (lit[36], 147-148 °C).

Acetyl-D-valyl-5-aminolaevulinic acid methyl ester (4e)

Scale: 0.30 mmol. Yield: 62.3 mg, 75 %; mp 150-152 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 4.21 (1H, d, *J* 6.9, NHC*H*(R′)CO), 4.14-3.98 (2H, m, NHCH_2CO), 3.64 (3H, s, COOCH_3), 2.78-2.73 (2H, m, COCH_2CH$_2$), 2.60-2.56 (2H, m, COCH$_2$CH_2), 2.08-2.01 (4H, m, CHC*H*(CH$_3$)$_2$, CH_3CO), 0.99-0.95 (6H, m, CH(CH_3)$_2$); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.6, 173.5, 172.9, 172.2, 58.9, 33.9, 30.4, 27.1, 18.4, 17.2; ESI-HRMS$^+$: calcd. for $C_{13}H_{23}N_2O_5$: 287.1601; found: 287.1590 $[M+H]^+$. Anal. ($C_{13}H_{22}N_2O_5$) C, H, N.

Acetyl-L-leucyl-5-aminolaevulinic acid methyl ester (4f)[36]

Scale: 1.33 mmol. Yield: 372 mg, 94%; mp 75-77 °C (lit[36], 74-76 °C).

Acetyl-D-leucyl-5-aminolaevulinic acid methyl ester (4g)

Scale: 0.28 mmol scale. Yield: 77.9 mg, 93 %; mp 94-97 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ: 4.44-4.39 (1H, m, NHC*H*(R′)CO), 4.04 (2H, AB q, *J* 18.4, NHCH_2CO), 3.65 (3H, s, COOCH_3), 2.57 (2H, t, *J* 6.7, COCH_2CH$_2$COOCH$_3$), 2.57 (2H, t, *J* 6.7,

$COCH_2CH_2COOCH_3$), 1.99 (3H, s, CH_3CO), 1.74-1.56 (3H, m, $CHCH_2CH(CH_3)_2$, $CHCH_2CH(CH_3)_2$), 0.97-0.93 (6H, m, $CHCH_2CH(CH_3)_2$); ^{13}C NMR (68 MHz, $CDCl_3$) δ: 204.8, 174.1, 173.5, 172.1, 52.0, 51.9, 40.6, 33.8, 27.1, 24.6, 22.1, 20.5; ESI-HRMS$^+$: calcd. $C_{14}H_{24}N_2O_5$: 301.1758; found: 301.1754 $[M+H]^+$. Anal. ($C_{14}H_{24}N_2O_5$) C, H, N.

Acetyl-L-isoleucyl-5-aminolaevulinic acid methyl ester (4h)

Scale: 0.28 mmol. Yield: 54.5 mg, 65 %; mp 172-174 °C; ^1H NMR (270 MHz, CD_3OD) δ: 4.25 (1H, d, *J* 7.4, $NHCH(R')CO$), 4.06 (2H, AB q, *J* 18.4, $NHCH_2CO$), 3.64 (3H, s, $COOCH_3$), 2.76 (2H, t, *J* 6.9, $COCH_2CH_2COOCH_3$), 2.58 (2H, t, *J* 6.9, $COCH_2CH_2COOCH_3$), 1.99 (3H, s, CH_3CO), 1.89-1.80 (1H, m, $CH(CH_3)CH_2CH_3$), 1.62-1.49, 1.28-1.14 (1H each, m, $CHCH_2(CH_3)CH_3$), 0.97-0.91 (6H, m, $CH(CH_3)CH_2CH_3$); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.5, 173.5, 172.9, 58.0, 36.6, 33.9, ESI-HRMS$^+$: calcd. for $C_{14}H_{24}N_2O_5Na$: 323.1577; found: 323.1572 $[M+Na]^+$. Anal. ($C_{14}H_{24}N_2O_5$) C, H, N.

Acetyl-L-prolyl-5-aminolaevulinic acid methyl ester (4i)

Scale: 0.31 mmol. Yield: 70.6 mg, 80 %; mp 113-115 °C; ^1H NMR (270 MHz, CD_3OD) δ: 4.49-4.41 (1H, m, $AcNCH(R')CO$), 4.04 (2H, s, $NHCH_2CO$), 3.64 (3H, s, $COOCH_3$), 3.61-3.51 (2H, m, $N(Ac)CH_2$), 2.76 (2H, t, *J* 5.8, $COCH_2CH_2CO_2CH_3$), 2.59 (2H, t, *J* 5.8, $COCH_2CH_2CO_2CH_3$), 2.20-1.97 (4H, m, $NAcCH_2CH_2CH_2$); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.8, 173.8, 173.5, 171.3, 61.4, 59.9, 50.8, 33.8, 31.8, 29.8, 27.0, 24.3, 20.9; ESI-HRMS$^+$: calcd. for $C_{13}H_{21}N_2O_5$: 285.1445; found: 285.1432 $[M+H]^+$.

Acetyl-D-prolyl-5-aminolaevulinic acid methyl ester (4j)

Scale: 0.31 mmol. Yield: 75.9 mg, 86 %; mp 114-116 °C; ESI-HRMS$^+$: calcd. for: $C_{13}H_{21}N_2O_5$: 285.1445; found: 285.1440 $[M+H]^+$.

Acetyl-L-methionyl-5-aminolaevulinic acid methyl ester (4k)

Scale: 0.26 mmol. Yield: 59.2 mg, 70 %; mp 125-127 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 4.49 (1H, d, *J* 5.9, NHC*H*(R′)CO), 4.51-3.98 (AB q, *J* 18.2, NHCH_2CO), 3.64 (3H, s, COOCH_3), 2.76 (2H, t, *J* 6.9, COCH_2CH$_2$COOCH$_3$), 2.61-2.57 (4H, m, COCH$_2$CH_2COOCH$_3$, CH$_2$CH_2S), 1.99 (3H, s, CH_3CO), 1.95-1.83 (5H, m, CH_2CH$_2$S, CH$_2$CH$_2$SCH_3); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.6, 173.5, 173.1, 172.2, 52.2, 33.8, 31.3, 29.7, 27.1, 21.7, 13.9; ESI-HRMS^{+}: calcd. for $C_{13}H_{23}N_2O_5S$: 567.2602; found: 567.2592 $[M+H]^{+}$.

Acetyl-L-seryl-5-aminolaevulinic acid methyl ester (4l)

Scale: 0.30 mmol. Yield: 60.0 mg, 72 %; mp 133-135 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 4.45 (1H, t, *J* 5.2, NHC*H*(R′)CO), 4.08 (2H, s, NHCH_2CO), 3.79 (1H, d, *J* 5.5, CH_2OH), 3.64 (3H, s, COOCH_3), 2.77 (2H, t, *J* 6.6, COCH_2CH$_2$CO$_2$CH$_3$), 2.58 (2H, t, *J* 6.6, COCH$_2$CH_2CO$_2$CH$_3$), 2.04 (3H, s, CH_3CO); ^{13}C NMR (68 MHz, CD_3OD) δ: 205.1, 173.5, 172.3, 171.6, 61.7, 55.6, 50.9, 33.8, 27.1, 21.3; ESI-HRMS^{+}: calcd. for $C_{11}H_{19}N_2O_6$: 275.1238; found: 275.1211 $[M+H]^{+}$.

Acetyl-D-phenylalanyl-5-aminolaevulinic acid methyl ester (4n)[36]

Scale: 0.25 mmol. Yield: 73.3 mg, 86 %; mp 130-131 °C (lit[36], 126-128 °C).

Acetyl-L-phenylalanyl-5-aminolaevulinic acid ethyl ester (4o)

Scale: 0.17 mmol scale. Yield: 47 mg, 81 %; mp 101-103 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.30-7.19 (5H, m, Ph), 4.69-4.62 (1H, m, NHC*H*(R′)CO), 4.13-4.00 (4H, m, OCH_2CH$_3$, NHCH_2CO), 3.21-3.12, 2.91-2.81 (1H each, m, C$H_A H_B$Ph), 2.71-2.64 (2H, m, COCH_2CH$_2$CO$_2$CH$_2$CH$_3$), 2.57-2.50 (2H, m, COCH$_2$CH_2CO$_2$CH$_2$CH$_3$), 1.89 (3H, s, CH_3CO), 1.20 (3H, t, *J* 6.1, OCH$_2$CH_3); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.8, 172.9, 172.7, 171.8, 137.2, 129.0, 128.1, 126.4, 60.3, 54.7, 37.4, 33.7, 27.3,

21.0, 13.12; ESI-HRMS$^+$: calcd. for $C_{18}H_{25}N_2O_5$: 349.1758; found: 349.1743 $[M+H]^+$.

Acetyl-L-tryptophyl-5-aminolaevulinic acid methyl ester (4p)

Scale: 0.23 mmol. Yellow oil. Yield: 79.6 mg, 92 %; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.58 (1H, d, *J* 7.7, indole H-4), 7.31 (1H, d, *J* 7.7, indole H-7), 7.12-7.00 (3H, m, , indole H-2, H-5, H-6), 4.70 (1H, t, *J* 6.6, NHC*H*(R′)CO), 3.93 (2H, AB q, *J* 18.0, NHCH_2CO), 3.62 (3H, s, COOCH_3), 3.32-3.26 (1H, m, CH_AH_B-indolyl), 3.09 (1H, dd, *J* 14.6, 7.7, CH_AH_B-indolyl), 2.58-2.48 (4H, m, COCH_2CH_2COOCH$_3$), 1.91 (3H, s, CH_3CO); ^{13}C NMR (68 MHz, CD_3OD) δ: 205.1, 173.5, 173.2, 171.9, 136.7, 127.5, 123.2, 121.1, 118.5, 118.0, 110.9, 109.6, 54.4, 58.9, 33.6, 27.6, 27.0, 21.2; ESI-HRMS$^+$: calcd. for $C_{19}H_{24}N_3O_5$: 374.1710; found: 374.1707 $[M+H]^+$.

Acetyl-D-tryptophyl-5-aminolaevulinic acid methyl ester (4q)

Scale: 0.23 mmol. Yellow oil. Yield: 74.4 mg, 86 %; ESI-HRMS$^+$: calcd. for $C_{19}H_{24}N_3O_5$: 374.1710; found: 374.1695 $[M+H]^+$.

***N*$^{\alpha}$-Acetyl-O-(benzyl)-L-tyrosyl-5-aminolaevulinic acid methyl ester (4r)**

Scale: 0.20 mmol. Yield: 75.1 mg, 85 %; mp 141-143 °C; ^{1}H NMR (270 MHz, CD_3OD), δ: 7.43-7.28 (5H, m, Ph), 7.16 (2H, d, *J* 8.8, Tyr Ar*H*), 6.90 (2H, d, *J* 8.8, Tyr Ar*H*), 5.03 (2H, s, OCH_2Ph), 4.59 (1H, dd, *J* 8.8, 6.1, NHC*H*(R′)CO), 3.99 (2H, s, NHCH_2CO), 3.64 (3H, s, COOCH_3), 3.09 (1H, dd, *J* 14.0, *J* 6.0, CHCH_AH_BAr), 2.82 (1H, dd, *J* 14.0, *J* 9.0, CHCH_AH_BAr)), 2.70-2.65 (2H, m, COCH_2CH$_2$COOCH$_3$), 2.57-2.52 (2H, m, COCH$_2$CH_2COOCH$_3$), 1.90 (3H, s, CH_3CO); ^{13}C NMR (68 MHz, CD_3OD), δ: 204.9, 173.4, 172.8, 181.9, 157.8, 137.5, 130.0, 129.4, 128.2, 127.5, 127.2, 114.5, 69.6, 54.9, 36.8, 33.7, 27.0; ESI-HRMS$^+$: calcd. for $C_{24}H_{29}N_2O_6$: 441.1980; found: 441.1998 $[M+H]^+$.

***N*$^{\alpha}$-Acetyl-*N*$^{\tau}$-(trityl)-L-histidyl-5-aminolaevulinic acid methyl ester (4s)**

Scale: 0.16 mmol. Yield: 63.5 mg, 70 %; mp 163-165 °C;

^{1}H NMR (270 MHz, CD_3OD) δ: 7.38-7.37 (11H, m, trityl Ar*H*, imidazole H-5), 7.15-7.13 (6H, m, trityl Ar*H*), 6.77 (1H, s, imidazole H-2) 4.65-4.62 (1H, m, NHC*H*(R′)CO), 4.01 (2H, s, NHCH_2CO), 3.05 (1H, dd, *J* 14.7, 5.4, CHCH_AH_B-imidazolyl), 2.82 (1H, dd, *J* 14.7, 8.8, CHCH_AH_B-imidazolyl), 2.71 (2H, t, *J* 6.3, COCH_2CH$_2$COOCH$_3$), 2.51 (2H, t, *J* 6.3, COCH$_2$CH_2COOCH$_3$), 1.90 (3H, s, CH_3CO); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.6, 172.5, 171.7, 142.3, 138.1, 136.3, 129.6, 128.1, 120.0, 53.3, 51.0, 33.8, 27.1, 21.4; ESI-HRMS$^+$: calcd. for $C_{33}H_{35}N_4O_5$: 567.2602; found: 567.2592 $[M+H]^+$.

N^{α}-Acetyl-N^{ε}-(benzyloxycarbonyl)-L-lysyl-5-aminolaevulinic acid methyl ester (4t)

Scale: 0.30 mmol scale. Yield: 101 mg, 75%); mp: 141-143 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.33 (5H, br, Ph), 7.00 (1H, br, N*H* urethane), 5.06 (2H, s, OCH_2Ph), 4.31 (1H, t, *J* 7.4, NHC*H*(R′)CO), 4.12-4.03 (2H, m, NHCH_2CO), 3.64 (3H, s, COOCH_3), 3.18-3.12 (2H, m, CH_2NHZ), 2.74 (2H, t, *J* 6.5, COCH$_2$CH_2COOCH$_3$), 2.57 (2H, t, *J* 6.5, COCH_2CH$_2$COOCH$_3$), 1.98 (3H, s, CH_3CO), 1.81-1.64 (2H, m, CHCH_2(CH$_2$)$_3$NHZ), 1.52-1.44 (4H, m, CHCH$_2$CH_2CH_2CH$_2$NHZ); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.8, 173.5, 128.1, 127.6, 127.4, 66.0, 53.4, 50.9, 39.8, 33.8, 31.7, 28.5, 27.1, 24.6, 22.7, 21.1; ESI-HRMS$^+$: calcd. for $C_{22}H_{32}N_3O_7$: 450.2240; found: 450.2235 $[M+H]^+$.

Acetyl-L-glutamyl-5-aminolaevulinic acid methyl ester (4u)

Scale: 0.25 mmol. Yield: 39.7 mg, 54 %; mp 130-132 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 4.42-4.39 (1H, m, NHC*H*(R′)CO), 4.07-4.04 (2H, m, NHCH_2CO), 3.64, (3H, s, COOCH_3), 2.75 (2H, t, *J* 4.9, COCH_2CH$_2$COOCH$_3$), 2.58 (2H, t, *J* 4.9, COCH$_2$CH_2COOCH$_3$), 2.46 (2H, t, *J* 7.4, CHCH$_2$CH_2COOH), 2.19-2.09 (1H, m,

CHCH_AH_BCH_2COOH), 2.00 (3H, s, CH_3CO), 1.96-1.89 (1H, m, CHCH_AH_BCH_2COOH); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.6, 175.8, 172.8, 172.1, 52.6, 50.9, 48.3, 34.7, 34.0, 27.0, 20.8; ESI-HRMS$^+$: calcd. for $C_{13}H_{21}N_2O_7$: 303.1192; found: 303.1170 $[M+H]^+$.

Acetyl-L-aspartyl-5-aminolaevulinic acid methyl ester (4v)

Scale: 0.23 mmol. Yield: 53.7 mg, 75 %; mp 119-121 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 4.59 (1H, dd, *J* 9.1, 5.5, NHC*H*(R′)CO), 4.42 (2H, AB q, *J* 17.6, NHCH_2CO), 3.64 (3H, s, COOCH_3), 3.10 (1H, dd, *J* 17.6, 9.1, CHCH_AH_BCOOH), 2.84 (2H, t, *J* 6.0, COCH_2CH_2COOCH$_3$), 2.73 (1H, dd, *J* 17.6, *J* 5.5, CHCH_AH_BCOOH), 2.59 (2H, t, *J* 6.0, COCH_2CH_2COOCH$_3$), 1.99 (3H, s, CH_3CO); ^{13}C NMR (68 MHz, CD_3OD) δ: 201.8, 175.8, 173.2, 171.3, 51.0, 49.1, 34.7, 34.0, 27.0, 20.8; ESI-HRMS$^+$: calcd. for $C_{12}H_{19}N_2O_7$: 317.1349; found: 317.1320 $[M+H]^+$.

Acetyl-L-tyrosyl-5-aminolaevulinic acid methyl ester (5r)

A solution of **4r** (56.3 mg, 0.11 mmol) in ethanol (6 mL) was purged with N_2, and 10% Pd/C (11 mg) was added. The flask was connected to a balloon of H_2, and the reaction mixture was stirred at room temperature for 3 h, then it was filtered through a pad of celite and the solvent was evaporated. Purification by flash chromatography (10:1 ethyl acetate/CH_3OH = 10/1) gave **5r** as a white solid (35.8 mg, 89 %); mp 103-105 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.05 (2H, d, *J* 8.5, Ar*H*), 6.68 (2H, d, *J* 8.5, Ar*H*), 4.57 (1H, dd, *J* 8.8, 6.0, NHC*H*(R′)CO), 3.99 (2H, s, NHCH_2CO), 3.64 (3H, s, COOCH_3), 3.05 (1H, dd, *J* 13.9, 6.0, CHCH_AH_BAr), 2.78 (1H, dd, *J* 13.9, 8.8, CHCH_AH_BAr), 2.67 (2H, t, *J* 6.3, COCH_2CH_2COOCH$_3$), 2.55 (2H, t, *J* 6.3, COCH$_2$CH_2COOCH$_3$), 1.90 (3H, s, CH_3CO); ^{13}C NMR (100 MHz, CD_3OD) δ: 206.3, 174.8, 174.2, 173.2, 157.3, 131.3, 129.1, 116.2, 56.3, 52.2, 38.1, 35.0, 28.4, 22.4; ESI-HRMS$^+$: calcd. for $C_{17}H_{23}N_2O_5Na$: 573.1376; found: 573.1366 $[M+Na]^+$.

N^{α}-Acetyl-L-histidyl-5-aminolaevulinic acid methyl ester trifluoroacetate salt (5s)

4s (30.6 mg, 0.05 mmol) was treated with a 10 % solution of TFA in dry CH_2Cl_2 (3 mL). The resulting bright yellow solution was then treated with triisopropylsilane (8 x 25 μL), added every 20 minutes. The mixture was allowed to stir at room temperature for 30 min, then the solvent was evaporated. Purification by flash chromatography (10:1 EtOAc/CH_3OH = 10/1) gave **5s** as a colourless oil (14.6 mg, 85 %); ^{1}H NMR (270 MHz, CD_3OD) δ: 8.80 (1H, s, imidazole H-5), 7.37 (1H, s, imidazole H-2), 4.78 (1H, t, *J* 6.0, NHC*H*(R′)CO), 4.18-4.01 (2H, AB q, *J* 18.2, NHC*H*$_2$CO), 3.64 (3H, s, COOC*H*$_3$), 3.11 (1H, dd, *J* 15.4, 5.2, CHC*H*$_2$-imidazolyl), 3.09 (1H, dd, *J* 15.4, 8.0, CHC*H*$_2$-imidazolyl), 2.76 (2H, t, *J* 6.9, COC*H*$_2$CH$_2$COOCH$_3$), 2.58 (2H, t, *J* 6.9, COCH$_2$C*H*$_2$COOCH$_3$), 1.99 (3H, s, C*H*$_3$CO); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.8, 172.0, 171.1, 129.7, 128.6, 127.9, 125.0, 117.2, 52.1, 50.9, 33.9, 27.1, 26.8, 21.2; ESI-HRMS$^+$: calcd. for $C_{14}H_{20}N_4O_5Na$: 347.1326; found: 347.1310 $[M+H]^+$.

N^{α}-Acetyl-L-lysyl-5-aminolaevulinic acid methyl ester hydrochloride salt (5t)

A solution of **4t** (20.2 mg, 0.04 mmol) in EtOH (5 mL) was treated with 2M HCl in diethyl ether (5 μL), and the resulting mixture was purged with N_2. 10% Pd/C (4 mg) was added, and the flask was connected to a balloon of H_2. The reaction mixture was stirred at room temperature for 2 h, then it was filtered through a pad of celite and the solvent was evaporated. The crude product was purified by precipitation from CH_3OH/diethyl ether to give **5t** as sticky, hygroscopic solid (14 mg, 96 %); ^{1}H NMR (270 MHz, CD_3OD) δ: 4.36 (1H, br, NHC*H* (R′)CO), 4.12-4.02 (2H, m, NHC*H*$_2$CO), 3.63 (3H, s, COOC*H*$_3$), 2.95-2.90 (2H, m, C*H*$_2$NHZ), 2.76 (2H, br, COC*H*$_2$CH$_2$COOCH$_3$), 2.57 (2H, br, COCH$_2$C*H*$_2$COOCH$_3$), 2.03 (3H, s, C*H*$_3$CO), 1.85-1.51 (6H, m, CHC*H*$_2$C*H*$_2$C*H*$_2$CH$_2$NHZ); ^{13}C NMR (68 MHz, CD_3OD) δ: 205.0,

174.3, 173.5, 65.6, 53.3, 51.0, 39.2, 33.9, 31.1, 28.1, 26.8, 22.4, 21.3; ESI-HRMS$^+$: calcd. for $C_{14}H_{26}N_3O_5$: 316.1872; found: 316.1853 $[M+H]^+$.

Acetyl-L-phenylalanyl-5-aminolaevulinic acid 2-methoxyethyl ester. (7b) Scale: 0.16 mmol. White solid. Yield: 17.2 mg, 30 %; mp 107-110 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.27-7.23 (5H, m, Ph), 4.68-4.61 (1H, m, NHC*H*(R′)CO), 4.19 (2H, t, *J* 4.7, COOCH_2CH$_2$OCH$_3$), 4.03-4.01 (2H, m, NHCH_2CO), 3.57 (2H, t, *J* 4.7, COOCH$_2$CH_2OCH$_3$), 3.35 (3H, s, OCH_3), 3.17 (1H, dd, *J* 14.0, 5.5, CH_AH_BPh), 2.87 (1H, dd, *J* 14.0, 9.6, CH_AH_BPh), 2.91 (2H, t, *J* 6.6, COCH_2CH$_2$CO$_2$R), 2.59 (2H, t, J 6.6, COCH$_2$CH_2CO$_2$R), 1.89 (3H, s, CH_3CO); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.7, 172.7, 171.7, 170.5, 137.2, 128.9, 128.1, 126.2, 70.0, 62.7, 57.7, 54.4, 20.7; ESI-HRMS$^+$: calcd. for $C_{19}H_{27}N_2O_6$: 379.1864; found: 369.1847 $[M+H]^+$.

Acetyl-L-phenylalanyl-5-aminolaevulinic acid 2-(2-methoxyethyl)ethyl ester. (7c) Scale: 0.15 mmol. Colourless oil. Yield: 25.2 mg, 40 %; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.27-7.17 (5H, m, Ph), 4.67 (1H, dd, *J* 9.1, *J* 5.1, NHC*H*(R′)CO), 4.21-4.16 (2H, m, COOCH_2CH$_2$O), 4.02 (2H, s, NHCH_2CO), 3.57-3.59 (4H, m, CH_2OCH_2), 3.54-3.50 (2H, m, CH_2OCH$_3$), 3.17 (1H, dd, *J* 13.5, 5.6, CH_AH_BPh), 2.87 (1H, dd, *J* 13.5, 8.8, CH_AH_BPh), 2.72-2.66 (2H, m, COCH_2CH$_2$CO$_2$R), 2.60-2.58 (2H, m, COCH$_2$CH_2CO$_2$R), 1.87 (3H, s, CH_3CO); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.6, 172.8, 172.7, 171.8, 154.4, 137.3, 128.7, 127.9, 126.3, 71.7, 69.4, 68.7, 63.3, 57.4, 54.6, 37.5, 33.7, 27.3, 21.1; ESI-HRMS$^+$: calcd. for $C_{21}H_{31}N_2O_7$: 423.2126; found: 423.2106 $[M+H]^+$.

Acetyl-L-phenylalanyl-5-aminolaevulinic acid geranyl ester. (7d) Scale: 0.15 mmol. White solid. Yield: 30.0 mg, 44 %; mp 78-80 °C; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.26-7.25 (5H, m, Ph), 5.32 (1H, t, *J* 6.9, OCH$_2$C*H*), 5.10-5.05 (1H, m, C*H*C(CH$_3$)$_2$), 4.66 (1H, dd, *J* 9.3, 4.8, NHC*H*(R′)CO), 4.57 (2H, d, *J* 8.1, OCH_2CH), 4.02 (2H, s, NHCH_2CO), 3.17 (1H, dd, *J* 14.3, 6.0, CH_AH_BPh), 2.87 (1H, dd, *J* 14.3, 9.4, CH_AH_BPh), 3.16 (2H, t, *J* 6.8, COCH_2CH$_2$CO$_2$R), 2.54 (2H, d, *J* 6.8,

$COCH_2CH_2CO_2R$), 2.15-2.00 (4H, m, CH_2CH_2 geranyl), 1.89 (3H, s, CH_3CO), 1.69 (3H, s, CH_3 geranyl), 1.66 (3H, s, CH_3 geranyl), 1.60 (3H, s, CH_3 geranyl); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.8, 172.9, 172.7, 171.9, 141.9, 137.2, 131.3, 130.0, 128.1, 126.4, 123.6, 118.4, 61.1, 54.7, 39.3, 37.6, 33.8, 27.4, 26.1, 21.1, 16.5, 15.2; ESI-HRMS$^+$: calcd. for $C_{26}H_{37}N_2O_5$: 457.2697; found: 457.2675 $[M+H]^+$.

Acetyl-L-phenylalanyl-5-aminolaevulinic acid menthyl ester. (7e) Scale: 0.15 mmol. Colourless oil. Yield: 29.3 mg, 42 %; ^{1}H NMR (270 MHz, CD_3OD) δ: 7.27-2.20 (5H, m, Ph), 4.70-4.64 (m, 2H, NHC*H*(R′)CO and OC*H* menthyl), 4.01 (2H, s, $NHCH_2CO$), 3.18 (1H, dd, *J* 14.0, 5.5, CH_AH_BPh), 2.87 (1H, dd, *J* 14.0, 9.1, CH_AH_BPh), 2.69 (2H, t, *J* 6.9, $COCH_2CH_2CO_2R$), 2.53 (2H, t, *J* 6.9, $COCH_2CH_2CO_2R$), 1.89 (4H, br, CH_3CO, C*H* menthyl), 1.70-1.67 (2H, m, C*H* menthyl, CH_2 menthyl)**,** 1.43-1.32, (3H, m, C*H* menthyl, CH_2 menthyl), 1.10-0.95 (3H, m, C*H* menthyl, CH_2 menthyl), 0.84-0.92 (6H, m, $(CH_3)_2CH$ menthyl), 0.75 (3H, d, *J* 6.9, CH_3CH menthyl); ^{13}C NMR (68 MHz, CD_3OD) δ: 204.8, 172.7, 172.6, 171.9, 137.2, 128.9, 128.1, 126.4, 74.3, 54.7, 47.0, 40.6, 37.6, 34.1, 33.8, 31.4, 27.6, 21.1, 19.8; ESI-HRMS$^+$: calcd. for $C_{26}H_{39}N_2O_5$: 459.2853; found: 459.2831 $[M+H]^+$.

Measured cellular PpIX and ALA content in PAM212 cells after incubation with 0.1 or 1 mM concentrations of prodrugs (ALA and dipeptides). Values of φ_0 are included for comparison.

		0.1 mM		**1 mM**	
	φ_0 (Clog *P*)	ALA (μg/mg protein)	PpIX (ng/mg protein)	ALA (μg/mg protein)	PpIX (ng/mg protein)
ALA	(-1.12)	0.81 ± 0.06	108.19 ± 13.67	3.41 ± 0.29	309.54 ± 1.78
ALA-Hex	(2.00)	1.21 ± 0.16	274.68 ± 17.69	5.87 ± 0.81	234.44 ± 19.70
4m	34.71	2.90 ± 0.06	312.67 ± 36.67	13.72 ± 0.31	294.52 ± 4.72
7a	56.64	4.14 ± 0.14	283.85 ± 5.39	18.92 ± 0.68	247.00 ± 7.62
4f	36.67	2.80 ± 0.06	275.73 ± 16.58	12.23 ± 0.25	289.97 ± 17.60
4k	28.89	3.28 ± 0.10	314.60 ± 20.31	13.25 ± 0.42	294.82 ± 1.31
4b	14.18	0.88 ± 0.14	10.92 ± 4.02	3.93 ± 0.62	12.88 ± 10.56
4n	37.71	1.07 ± 0.09	4.57 ± 0.64	4.35 ± 0.36	-2.99 ± 2.71
5t	8.48	0.68 ± 0.04	13.20 ± 4.60	3.06 ± 0.35	20.17 ± 2.14
4v	19.62	0.41 ± 0.08	19.07 ± 11.25	1.94 ± 0.19	20.17 ± 2.14
4l	14.61	0.50 ± 0.09	33.94 ± 5.31	2.17 ± 0.44	221.65 ± 8.78
4h	37.14	1.64 ± 0.09	40.19 ± 4.14	7.10 ± 0.72	22.30 ± 13.88
4p	37.35	2.41 ± 0.12	26.63 ± 16.40	10.28 ± 0.51	38.92 ± 5.90
4u	23.14	1.20 ± 0.11	165.11 ± 9.11	5.04 ± 0.43	275.65 ± 1.49

Measured cellular PpIX and ALA content in A549 and Caco-2 cells after incubation with 0.1 or 1 mM concentrations of prodrugs (ALA and dipeptides).

		A549		**Caco-2**	
		0.1 mM		**1 mM**	
	φ_0 **(Clog *P*)**	ALA (μg/mg protein)	PpIX (ng/mg protein)	ALA (μg/mg protein)	PpIX (ng/mg protein)
ALA	(-1.12)	0.08 ± 0.01	39.88 ± 3.02	2.66 ± 0.40	603.45 ± 39.21
4m	34.71	0.38 ± 0.56	4.42 ± 0.91	11.87 ± 2.14	594.28 ± 17.02
7a	56.64	1.63 ± 0.55	10.06 ± 1.63	14.82 ± 2.21	479.64 ± 46.25
4f	37.14	0.20 ± 0.03	5.91 ± 1.29	6.54 ± 0.98	612.45 ± 13.43
4p	37.35	0.23 ± 0.03	-0.02 ± 0.88	8.35 ± 1.25	-5.90 ± 3.63

Validation parameters for the HPLC-fluorescence method for quantitative ALA determination.

Range 0.6-65 µM (0.1-10 µg/mL)				
Calibration curve properties				
Slope	Intercept	R^2	LOD (µM)	LOQ (µM)
14.4 ± 0.63	-0.31 ± 0.02	0.992	4.2×10^{-3}	0.14
Inter-day variability				
Accuracy (%)	91.8-106.0			
Precision (RSD%)	< 6.3			
Intra-day variability				
Nominal concentration (µM)	Intra-day variability measured concentration			
	Mean	SD		
6.0	5.5	0.08		
30.2	30.8	0.31		
48.4	49.0	0.13		

References

(36) Rogers, L. M. A.; McGivern, P. G.; Butler, A. R.; MacRobert, A. J.; Eggleston, I. M. An efficient synthesis of 5-aminolaevulinic acid (ALA)-containing prodrugs for use in photodynamic therapy. *Tetrahedron* **2005**, *61*, 6951-6958.