Synthesis of Novel and Stable 5-Aminolevulinic Acid Derivatives for the Efficient Synthesis of 5-Aminolevulinic Acid Based Prodrugs

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Abstract: The efficient synthesis of two stable, easily handled precursors of 5-aminolevulinate is reported. These precursors are stable to the chemical transformation needed for the synthesis of bioconjugates and can be readily deprotected in good yields.

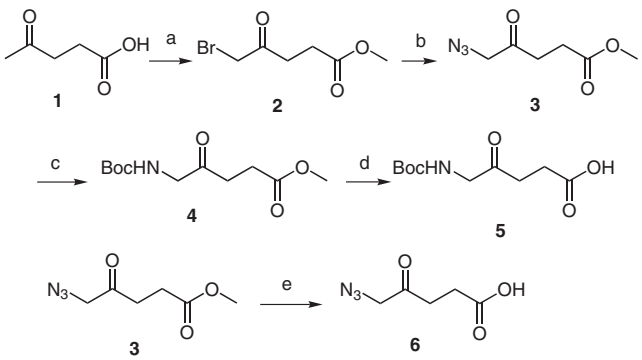
Key words: amino acid, activated ester, protecting group, prodrug, porphyrin

Using 5-aminolevulinic acid (ALA) and its derivatives in photodynamic therapy (PDT) as natural precursors of the photosensitizer has gained increasing interest for the treatment of different cancers.[1] Successful clinical trials for tumor-associated diseases such as actinic keratosis, basal cell carcinoma, Bowen's disease, and psoriasis have been reported.[2] 5-Aminolevulinic acid–photodynamic therapy involves administration of 5-aminolevulinic acid, or suitable derivatives thereof, which biosynthetically generate protoporphyrin IX (PpIX), a powerful photosensitizer. On application of light, protoporphyrin IX produces singlet oxygen, which then destroys tumor and tumor-associated cells. 5-Aminolevulinic acid is noninvasive and clears rapidly from the body. 5-Aminolevulinic acid induced protoporphyrin IX formation has been used not only in cancer therapeutics but also in the detection of cancer cells.[3]

5-Aminolevulinic acid, an unusual amino acid, possesses high water solubility and corresponding low lipid solubility and thereby has limited ability to cross biological membranes. Various 5-aminolevulinic acid esters have been synthesized to obtain more lipophilic prodrugs.[4] 5-Aminolevulinic acid hydrochloride (ALA·HCl) is used as the starting material for the synthesis of the corresponding 5-aminolevulinic acid prodrugs in most published reports. 5-Aminolevulinic acid hydrochloride is polar, difficult to purify, and highly hygroscopic; it is difficult to handle and forms side products at neutral pH, mostly due to reactions between the oxo and the amino function. 5-Aminolevulinic acid chloride has been used previously to derivatize 5-aminolevulinic acid; it is unstable and must be generated in situ and notoriously forms lactam-type impurities. We report the synthesis of an activated, stable, and storable form of 5-aminolevulinic acid that can be utilized for its efficient derivatization.

During our efforts to synthesize pseudopeptides containing 5-aminolevulinic acid, we tested several different protecting groups, such as the Fmoc or the Boc group.[5] The chemistry needed for deprotection of the Fmoc group was not compatible with the reactivity of 5-aminolevulinic acid and its derivatives in our hands. Boc-protected 5-aminolevulinic acid (Boc-ALA) is easy to cleave without affecting ester/amide functionalities. The Boc protection renders 5-aminolevulinic acid stable, easily handled, and assists purification.

Herein we report the efficient synthesis of Boc-ALA and various activated esters of Boc-ALA suitable for the synthesis of many different bioconjugates and other 5-aminolevulinic acid derivatives. We synthesized Boc-ALA in four steps from levulinic acid, an inexpensive and widely available starting material[6] in 20% overall yield (Scheme 1).



Scheme 1 *Reagents and conditions*: (a) Br_2, MeOH, r.t., overnight then reflux, 1.5 h, 45%; (b) NaN_3, THF–H_2O, r.t., 1 h, 95%; (c) H_2, Pd/C, Boc_2O, r.t., 24 h, 70%; (d) PLE, pH 8, 4 d, 64%; (e) PLE, pH 8, 10 h, 87%.

Levulinic acid (**1**) was brominated to give methyl 5-bromolevulinate (**2**) as the major regioisomer.[7] Then, methyl 5-bromolevulinate (**2**) was converted into methyl 5-azidolevulinate (**3**) by an S_N2 reaction using sodium azide. The reduction of the azide and in situ protection of the amino group with the Boc group was initially attempted by applying a Staudinger procedure, but this gave only

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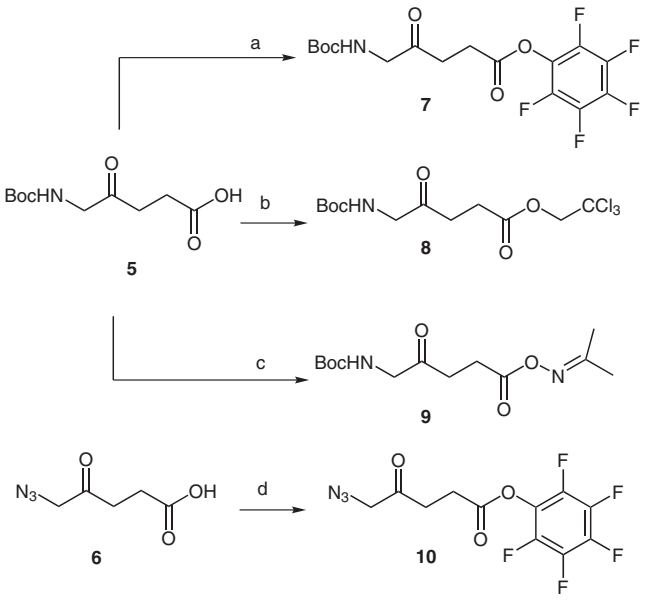
poor yields of **4**.[8a] Hydrogenation of the azido group of methyl 5-azidolevulinate (**3**) using hydrogen and palladium on carbon in the presence of di-*tert*-butyl dicarbonate afforded cleanly methyl 5-(*tert*-butoxycarbonylamino)levulinate (**4**) in a satisfactory 70% yield.[8b] Using other 'Boc' reagents such as 2-[(*tert*-butoxycarbonyloxy)imino]-2-phenylacetonitrile (Boc-ON),[9a] *tert*-butyl aminocarbonate,[9b] or *tert*-butyl carbonate (Boc_2O) in the presence of 4-(dimethylamino)pyridine did not improve the yield of **4**. Hydrolysis of methyl 5-(*tert*-butoxycarbonylamino)levulinate (**4**) was, in our hands, best performed in the presence of the enzyme pig liver esterase (PLE) to provide 5-(*tert*-butoxycarbonylamino)levulinic acid (5-Boc-ALA, **5**) in 64% yield as a stable, crystalline solid. Using our procedure 5-Boc-ALA **5** can be made in an easily handled free-flowing form. 5-Boc-ALA **5** can be stored at room temperature for months without any significant decomposition, unlike ALA, which must be stored in the freezer to prevent its degradation.

Methyl 5-azidolevulinate (**3**) can also be hydrolyzed using the same conditions to provide crystalline 5-azidolevulinic acid (**6**), a masked form of ALA that can also be used for the synthesis of various ALA prodrugs.

Starting from 5-Boc-ALA **5** we efficiently synthesized various activated esters of 5-(*tert*-butoxycarbonylamino)levulinic acid suitable for further transformations (Scheme 2). Pentafluorophenyl 5-(*tert*-butoxycarbonylamino)levulinate (**7**), trichloroethyl 5-(*tert*-butoxycarbonylamino)levulinate (**8**), and 5-(*tert*-butoxycarbonylamino)levulinic acid acetone oxime ester (**9**) were synthesized in good to excellent yields by coupling[10] 5-Boc-ALA **5** with pentafluorophenol, trichloroethanol, and acetone oxime, respectively. Pentafluorophenyl 5-azidolevulinate (**10**) was synthesized from 5-azidolevulinic acid (**6**) in almost quantitative yield. These activated esters are commonly used for the esterification of biomolecules such as monosaccharides.[11]



Scheme 2 *Reagents and conditions*: (a) pentafluorophenol, DCC, CH_2Cl_2, r.t., overnight, 98%; (b) Cl_3CCH_2OH, EDC, DMAP, CH_2Cl_2, r.t., overnight, 85%; (c) Me_2C=NOH, EDC, DMAP, CH_2Cl_2, r.t., overnight, 84%; (d) pentafluorophenol, DCC, CH_2Cl_2, r.t., overnight, 98%.

Thus, we have described the efficient and simple synthesis of two stable, easily handled 5-aminolevulinic acid precursors **5** and **6**. Both precursors can be conveniently transformed into activated derivatives **7**–**10** that can be used in place of 5-aminolevulinic acid hydrochloride for the synthesis of 5-aminolevulinic acid prodrugs avoiding most of the practical problems associated with the use of this sensitive compound.

All moisture-sensitive reactions were carried out under argon or N_2 using oven-dried glassware. All reagents were of the highest commercial quality available if not specifically mentioned. Pig liver esterase (PLE, 24 units/mg) was purchased from Sigma-Aldrich. Solvents were freshly distilled prior to use. Flash chromatography (FC): SDS silica gel A C.C. Chromagel (35–70 μm); under positive pressure, 0.5–0.9 bar. TLC: precoated silica gel thin-layer sheets 60 F 254 from Merck, detection by UV, basic $KMnO_4$ soln or/and vanillin soln. Refractive index (n_D): Carl Zeiss; IR spectra: Perkin Elmer FT-IR Spectrum One version B; with a resolution of 2 cm^{-1}. Solids and oils were analyzed as KBr pellets, liquids as films between KBr plates. NMR Spectra: Bruker Avance-400 (400 and 100 MHz) or Varian Gemini XL-2000 (200 and 50 MHz), at r.t., if not specified; Reference was TMS or the solvent peak as reference. The sign denotes the calculated average value of *J* in cases where the measured values varied up to 0.4 Hz. HETCOR (short range), COSY and DEPT 135 were systematically measured for all compounds to allow the attribution of all signals. MS: ESI and APCI: Finnigan LCQ; the postulated formula of the peaks are indicated in parenthesis; HRMS: Bruker FTMS 4.7T BioAPEXII measured at University of Fribourg (Switzerland) by Mr. F. Nydegger.

Methyl 5-Bromolevulinate (2)

To a soln of levulinic acid (**1**, 100 g, 861 mmol, 1.0 equiv) in MeOH (860 mL) at 0 °C was added dropwise over 3 h Br_2 (44 mL, 861 mmol, 1.0 equiv); the temperature did not exceed 30 °C. The mixture was brought to r.t. and stirred overnight and then the colorless soln was refluxed for 90 min. MeOH was evaporated and the yellow residue was dissolved in CH_2Cl_2 (400 mL). The organic phase was washed with H_2O (400 mL) followed by further washing with sat. $NaHCO_3$ (200 mL) and then with H_2O (3 × 200 mL). The CH_2Cl_2 soln was dried ($MgSO_4$), filtered through filter paper, and evaporated. The yellow residue (156 g) was subjected to fractional distillation using a Widmer column (40 cm) under reduced pressure (0.04 mbar) to give **2** (81 g, 45%); 98% purity (^{1}H NMR); bp ~80 °C/ 0.045 mbar; R_f = 0.50 (hexane–EtOAc, 1:1).

n_D^{20} 1.4820.

IR (film): 3000 (m), 2953 (m), 2849 (w), 1734 (s), 1722 (s), 1439 (s), 1411 (s), 1359 (s), 1322 (m), 1207 (s), 1177 (s), 1079 (m), 1025 (m), 988 (m), 862 (w), 768 (vw), 694 (w), 595 (vw), 496 (vw), 464 (w) cm^{-1}.

^{1}H NMR (200 MHz, $CDCl_3$, 298 K): δ = 3.95 (s, 2 H, H5), 3.67 (s, 3 H, CO_2CH_3), 2.95 (t, $^3J_{3,2}$ = 6.4 Hz, 2 H, H3), 2.64 (t, $^3J_{2,3}$ = 6.4 Hz, 2 H, H2).

^{13}C NMR (50 MHz, $CDCl_3$, 298 K): δ = 201.3 (C4), 173.4 (C1), 52.6 (CO_2CH_3), 35.1 (C3), 34.7 (C2), 28.8 (C5).

MS (ESI+): *m*/*z* = 231.1 [M(^{79}Br) + Na]$^+$, 233.0 [M(^{81}Br) + Na]$^+$.

Methyl 5-Azidolevulinate (3)

To a soln of NaN_3 (17.68 g, 272 mmol, 2 equiv) in deionized H_2O (68 mL) at 0 °C was added dropwise a soln of **2** (28.43 g. 136 mmol, 1 equiv) in THF (70 mL) . The yellow-colored mixture was brought to r.t. and stirred for 1 h. The resulting two phases were separated. The aqueous phase was extracted with EtOAc (3 × 200 mL). The organic phases were combined and washed with H_2O (2 × 200 mL), dried ($MgSO_4$), filtered, and evaporated to give **3** (22.05 g, 95%) as a yellow oil; R_f = 0.50, 0.33 (UV_{254} active, vanillin) (hexane–EtOAc, 2:1).

IR (film): 2956 (m), 2914 (m), 2850 (w), 2543 (vw), 2202 (m), 2106 (vs), 1732 (vs), 1619 (w), 1438 (s), 1417 (s), 1361 (s), 1280 (s), 1210 (s),1172 (s), 1096 (s), 1028 (m), 1008 (m), 988 (m), 921 (m), 885 (w), 843 (w), 556 (m) cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$, 298 K): δ = 4.03 (s, 2 H, H5), 3.69 (s, 3 H, CO_2CH_3), 2.76–2.72 (m, AA′ part of a AA′BB′ system, 2 H, H3), 2.70–2.66 (m, BB′ part of a AA′BB′ system, 2 H, H2).

^{13}C NMR (50 MHz, $CDCl_3$, 298 K): δ = 202.9 (C4), 173.0 (C1), 57.3 (C5; 51.9 (CO_2CH_3), 34.3 (C3), 27.4 (C2).

MS (ESI+): *m*/*z* = 194 $[M + Na]^+$.

Methyl 5-(*tert*-Butoxycarbonylamino)levulinate (4)

A soln of **3** (23 g, 134.37 mmol, 1 equiv) in EtOAc (250 mL) was charged in a hydrogenation autoclave, followed by Boc_2O (29.63 g, 135.76 mmol, 1.01 equiv) and 10% Pd/C (5 g, 4.7 mmol, 0.035 equiv). The mixture was stirred mechanically during hydrogenation at r.t. under 4.14 bar H_2. After 24 h, the H_2 pressure was released and the mixture was filtered through Celite, which was washed with EtOAc (3 × 25 mL); the filtrate was evaporated to give a yellow oily. This crude mixture was purified by flash chromatography (gradient, CH_2Cl_2–EtOAc 95:5 to CH_2Cl_2–EtOAc 85:15) to give pure **4** (23.05 g, 70%) as a yellow oil; R_f = 0.25–0.30 (UV_{254} active, $KMnO_4$) (CH_2Cl_2–EtOAc, 9:1).

IR (film): 2979 (s), 2932 (s), 1735 (s), 1719 (s),1701 (s), 1697 (s), 1523 (s), 1518 (s), 1508 (s), 1500 (s), 1438 (s), 1412 (s), 1392 (s), 1252 (s), 1209 (s),1165 (s), 1098 (m), 1060 (m), 1024 (m), 983 (m), 737 (m) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K): δ = 5.24 (br s, 1 H, NH), 4.03 (d, $^3J_{5,NH}$ = 3.6 Hz, 2 H, H5), 3.64 (s, 3 H, CO_2CH_3), 2.71–2.68 (m, 2 H, H3), 2.63–2.59 (m, 2 H, H2), 1.40 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 204.5 (C4), 173.0 (C1), 155.7 [*C*(O)*t*-Bu], 79.9 [*C*(CH_3)$_3$], 52.0 (CO_2CH_3), 50.3 (C5), 34.4 (C3), 28.4 [C(CH_3)$_3$], 27.6 (C2).

MS (APCI+): *m*/*z* (%) = 269.1 (15, $[M + Na]^+$).

5-(*tert*-Butoxycarbonylamino)levulinic Acid (5)

To a 0.1 M phosphate buffer soln (500 mL) of pH 8 was added with stirring **4** (22.85 g, 93.2 mmol, 1 equiv). Then pig liver esterase (PLE) (300 mg, 24 units per mg) was added. The pH of the mixture was maintained at pH 8 by addition of 5 M NaOH. The reaction was followed by TLC (CH_2Cl_2–EtOAc, 90:10). After 4 d the mixture was extracted with EtOAc (3 × 200 mL), the aqueous phase was acidified to pH 2 with 6 M HCl, and extracted with EtOAc (4 × 500 mL). The organic phases were combined and washed with sat. NaCl (500 mL) followed by deionized water (500 mL). The organic phase was dried ($MgSO_4$) and evaporated. A yellow crude solid product was obtained by trituration with Et_2O at 0 °C to give pure white **5** (13.7 g, 64%); R_f = 0.47 ($KMnO_4$, tailing) (EtOAc).

IR (KBr): 3379 (s), 3500–2500 (br s), 2986 (m), 2919 (m), 1707 (vs), 1686 (vs), 1506 (s), 1429 (s), 1407 (m), 1392 (m), 1383 (m), 1371 (m), 1363 (m), 1349 (m), 1295 (w), 1278 (m), 1240 (s), 1209 (s),1170 (s), 1098 (w), 1065 (m), 1039 (vw), 1015 (m), 943 (m), 894 (m), 872 (m), 803 (w), 787 (w), 759 (w), 741 (w), 653 (w), 575 (m) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K): δ = 11.30 (s, 1 H, COOH), 5.35 (br s, 1 H, NH), 4.03 (d, $^3J_{5,NH}$ = 4.8 Hz, 2 H, H5), 2.69–2.62 (m, 4 H, H2, H3), 1.40 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 204.5 (C4), 177.4 (C1), 156.0 [*C*(O)*t*-Bu], 80.2 [*C*(CH_3)$_3$], 50.3 (C5), 34.2 (C3), 28.4 [C(CH_3)$_3$], 27.7 (C2).

MS (APCI–): *m*/*z* (%) = 230.1 (95, $[M - H]^-$).

5-Azidolevulinic Acid (6)

To a 0.1 M phosphate buffer soln (500 mL) was added, while stirring the mixture, **3** (22.05 g, 128.8 mmol 1 equiv). Then pig liver esterase (PLE) (300 mg, 24 units per mg) was added. The pH of the mixture was maintained at pH 8 adding 5 M NaOH. The reaction was followed by TLC (hexane–EtOAc, 1:1) and after 10 h was extracted with EtOAc (3 × 200 mL). The aqueous phase was acidified to pH 2 with 6 M HCl. The aqueous phase was extracted with EtOAc (4 × 500 mL). The combined organic phases were washed with sat. NaCl (500 mL) followed by deionized H_2O (500 mL). The organic phase was dried ($MgSO_4$) and evaporated. The crude solid was triturated with Et_2O at 0 °C. The pale brown pure solid thus obtained was filtered and dried to give pure white **6** (17.61 g, 87%); R_f = 0.35 (EtOAc), 0.91 (CH_2Cl_2–MeOH, 4:1) (UV_{254} active, $KMnO_4$).

IR (KBr): 3500–2500 (br s), 2914 (s), 2221 (m), 2110 (vs), 1720 (vs), 1411 (s), 1399 (s), 1374 (s), 1344 (m), 1288 (s), 1262 (s), 1235 (s), 1224 (s), 1173 (m), 1088 (s), 1046 (m), 1005 (w), 927 (s), 887 (m), 828 (w), 689 (w), 648 (w), 633 (m), 557 (m), 526 (w) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K): δ = 9.48 (s, 1 H, COOH), 4.02 (s, 2 H, H5), 2.77–2.69 (m, 4 H, H2, H3).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 203.0 (C4), 178.4 (C1), 57.6 (C5), 34.3 (C3), 27.7 (C2).

MS (APCI+): *m*/*z* (%) = 156.8 (60, $[M - H]^+$).

Pentafluorophenyl 5-(*tert*-Butoxycarbonylamino)levulinate (7)

To a soln of pentafluorophenol (2.7 g, 14.67 mmol, 1.1 equiv) in CH_2Cl_2 (50 mL) at 0 °C, was added **5** (3 g, 12.97 mmol, 1 equiv) followed by DCC (3 g, 14.54 mmol, 1.12 equiv). The reaction was brought to r.t. and kept overnight. The mixture was filtered over Celite and the solvent was evaporated. The thick yellow oily mass obtained was triturated with hexane to remove excess pentafluorophenol and the white solid obtained was filtered. The white solid was dried to give pure **7** (5.1 g, 98%); R_f = 0.33 (CH_2Cl_2–EtOAc, 95:5) (UV_{254} active; $KMnO_4$).

IR (KBr): 3009 (w), 2982 (w), 2948 (w), 2920 (w), 1798 (m), 1725 (s), 1694 (vs), 1656 (w), 1626 (w), 1527 (vs), 1518 (vs), 1468 (w), 1446 (w), 1426 (w), 1401 (w), 1392 (w), 1370 (m), 1359 (w), 1328 (w), 1271 (s), 1253 (m), 1235 (w), 1170 (m), 1146 (m), 1111 (s), 1042 (m), 1031 (m), 1007 (s), 989 (s), 977 (m), 895 (w), 864 (w), 609 (w), 556 (w) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K): δ = 5.25 (br s, 1 H, NH), 4.06 (d, $^3J_{5,NH}$ = 4.9 Hz, 2 H, H5), 2.99 (t, $^3J_{2,3}$ = 6.5 Hz, 2 H, H3), 2.87 (t, $^3J_{3,2}$ = 6.5 Hz, 2 H, H2), 1.43 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 203.6 (C4), 168.8 (C1), 155.8 [*C*(O)*t*-Bu], 142.4 (d m, $^1J_{C-F}$ = 248 Hz, $C2_{Pfp}$, $C3_{Pfp}$, or $C4_{Pfp}$), 139.9 (d m, $^1J_{C-F}$ = 244 Hz, $C2_{Pfp}$, $C3_{Pfp}$, or $C4_{Pfp}$), 137.5 (d m, $^1J_{C-F}$ = 240 Hz, $C2_{Pfp}$, $C3_{Pfp}$, or $C4_{Pfp}$), 125.0 ($C1_{Pfp}$), 80.2 [*C*(CH_3)$_3$], 50.3 (C5), 34.2 (C3), 28.4 [C(CH_3)$_3$], 27.0 (C2).

^{19}F NMR (188 MHz, $CDCl_3$, 298 K): δ = –152.9 (m, 2 F, $C2_{Pfp}$, $C2'_{Pfp}$), –158.2 (t, $^3J_{F-F} \approx 21.0$ Hz, 1 F, ($C4_{Pfp}$), –162.6 (m, 2 F, $C3_{Pfp}$, $C3'_{Pfp}$).

MS (ESI+): *m*/*z* (%) = 419.9 $[M + Na]^+$.

2,2,2-Trichloroethyl 5-(*tert*-Butoxycarbonylamino)levulinate (8)

To a soln of 2,2,2-trichloroethanol (1.267 g, 8.48 mmol, 2 equiv) in CH_2Cl_2 (80 mL) at 0 °C, DMAP (0.528 g, 4.32 mmol, 1 equiv) was added followed by EDC (0.912 g, 4.75 mmol, 1.1 equiv) and **5** (1 g, 4.32 mmol, 1 equiv). The mixture was brought to r.t. and kept overnight. The solvent was evaporated and the mixture was dissolved in EtOAc (100 mL). The organic phase was extracted with H_2O (200 mL).The organic phase was washed with cold 1 M citric acid (170 mL), followed by sat. $NaHCO_3$ (200 mL) and sat. NaCl (200 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated. The remaining crude product was purified by trituration with hexane to remove excess alcohol and the white solid obtained was filtered. The white solid was dried to yield **8** (1.33 g, 85%); R_f = 0.17 (UV_{254} active; $KMnO_4$) (hexane–EtOAc, 7:3).

IR (KBr): 3000 (w), 2981 (w), 2932 (w), 1759 (vs), 1721 (vs), 1688 (vs), 1528 (vs), 1477 (w), 1452 (w), 1408 (m), 1387 (m), 1369 (s), 1357 (m), 1333 (w), 1283 (vs), 1252 (m), 1195 (m), 1175 (s), 1156 (vs), 1134 (vs), 1084 (w), 1070 (w), 1043 (m), 1029 (w), 1006 (vw), 968 (w), 904 (w), 867 (vw), 831 (m), 797 (m), 716 (s), 681 (w), 654 (w), 571 (w) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K): δ = 5.24 (br s, 1 H, NH), 4.70 (s, 2 H, CH_2CCl_3), 4.04 (d, $^3J_{5,NH}$ = 5.0 Hz, 2 H, H5), 2.77 (s, 4 H, H2, H3), 1.41 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 204.0 (C4), 170.9 (C1), 155.7 [*C*(O)*t*-Bu], 94.8 (CH_2CCl_3), 80.0 [$C(CH_3)_3$], 74.2 (CH_2CCl_3), 50.3 (C5), 34.2 (C3), 28.4 [$C(CH_3)_3$], 27.5 (C2).

MS (APCI+): *m*/*z* (%) = 263.9 (99, $[M(^{35}Cl) – Boc + H]^+$), 265.9 (32, $[M(^{37}Cl) – Boc + H]^+$).

5-(*tert*-Butoxycarbonylamino)levulinic Acid Acetone Oxime Ester (9)

To a soln of acetone oxime (0.643 g, 8.8 mmol, 1 equiv) in CH_2Cl_2 (85 mL) at 0 °C, DMAP (1.18 g, 9.68 mmol, 1.1 equiv) was added followed by EDC (2.05 g, 10.69 mmol, 1.2 equiv) and **5** (2.03 g, 8.80 mmol, 1 equiv). The mixture was brought to r.t. and was kept overnight. The solvent was evaporated and the residue was purified by flash chromatography (Et_2O) to give **9** (2.13 g, 84%); R_f = 0.19 (UV_{254} active; $KMnO_4$) (Et_2O).

IR (KBr): 3005 (m), 2978 (s), 2922 (m), 1771 (vs), 1705 (vs), 1651 (m), 1519 (vs), 1457 (m), 1440 (m), 1411 (s), 1397 (s), 1367 (vs), 1287 (vs), 1251 (s), 1164 (vs), 1127 (vs), 1070 (s), 1054 (m), 1944 (m), 1027 (m), 935 (m), 952 (w), 935 (w), 885 (vs), 784 (m), 771 (w), 747 (w), 588 (m), 577 (w), 548 (w), 464 (w) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, 298 K): δ = 5.24 (br s, 1 H, NH), 4.03 (d, $^3J_{5,NH}$ = 5.0 Hz, 2 H, H5), 2.76–2.69 (m, 4 H, H2, H3), 1.99 [s, 3 H, $N{=}C(CH_3)_2$], 1.96 [s, 3 H, $N{=}C(CH_3)_2$], 1.45 [s, 9 H, $C(CH_3)_3$].

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 204.4 (C4), 170.4 (C1), 164.2 (CO_2CH_3), 155.7 [*C*(O)*t*-Bu], 80.0 [$C(CH_3)_3$], 50.3 (C5), 34.2 (C3), 28.4 [$C(CH_3)_3$], 26.6 (C2), 21.9, 17.0 [$N{=}C(CH_3)_2$].

Pentafluorophenyl 5-Azidolevulinate (10)

To a soln of pentafluorophenol (2.2 g, 11.9 mmol, 1.1 equiv) in CH_2Cl_2 (40 mL) at 0 °C, was added **6** (1.72 g, 11 mmol, 1 equiv) followed by DCC (2.5 g, 12.3 mmol, 1.12 equiv). The reaction was brought to r.t. and kept overnight. The mixture was filtered over Celite and the solvent was evaporated. The thick yellow oil obtained was triturated with hexane to remove excess pentafluorophenol and the white solid was filtered to give pure **10** (3.47 g, 98%) as a white solid. R_f = 0.36 (UV_{254} active; $KMnO_4$) (CH_2Cl_2–EtOAc, 95:5).

^1H NMR (400 MHz, $CDCl_3$, 298 K): δ = 4.06 (s, 2 H, H5), 3.06 (t, $^3J_{2,3}$ = 6.5 Hz, 2 H, H3), 2.94 (t, $^3J_{3,2}$ = 6.5 Hz, 2 H, H2).

^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 202.3 (C4), 168.9 (C1), 141.5 (d m, $^1J_{C–F}$ = 252 Hz, $C2_{Pfp}$, $C3_{Pfp}$, or $C4_{Pfp}$), 140.0 (d m, $^1J_{C–F}$ = 254 Hz, $C2_{Pfp}$, $C3_{Pfp}$, or $C4_{Pfp}$), 138.2 (d m, $^1J_{C–F}$ = 250 Hz, $C2_{Pfp}$, $C3_{Pfp}$, or $C4_{Pfp}$), 125.0 ($C1_{Pfp}$), 57.8 (C5), 27.0 (C2).

MS (APCI–): *m*/*z* (%) = 322.2 (95, $[M – H]^-$).

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