Synthesis of Photoswitchable Hemithioindigo-Based ω-Amino Acids and Application in Boc-Based Peptide Assembly

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Abstract: Efficient procedures for the preparation of the *N*-Boc-protected aldehydes **5a** and **5b** are described which are valuable precursors for the synthesis of hemithioindigo-based ω-amino acids and peptides.

Key words: hemithioindigos, amino acids, peptides, photoswitches

Driven by the development of new experimental techniques, the investigation and modulation of fast processes of peptide and protein folding gain increasing interest.[1] In addition, the reversible photocontrol of the conformation and activity of biomolecules is intensively being explored.[2,3] In an attempt to develop modular and tunable photoswitches for these applications, we are exploring the synthesis of novel hemithioindigo-derived compounds.[4,5] Our design is based on significant light-induced end-to-end distance changes and changes in the dipole moment. Recently, we have demonstrated that a hemithioindigo-based α-amino acid can be applied as a light switch for the photomodulation of ionic current through modified gramicidin ion channels.[6] Based on this promising approach, the Boc-protected hemithioindigo-derived pseudo-ω-amino acids **1a** and **1b** (Figure 1) are developed in our laboratories for their incorporation into cyclic peptides.

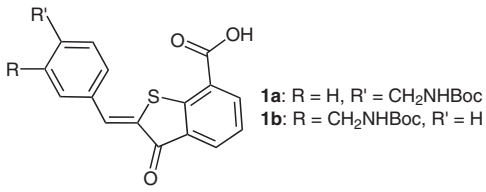
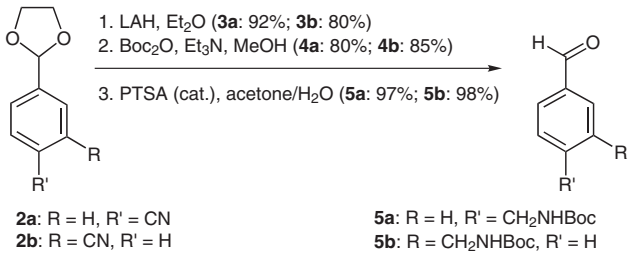


Figure 1

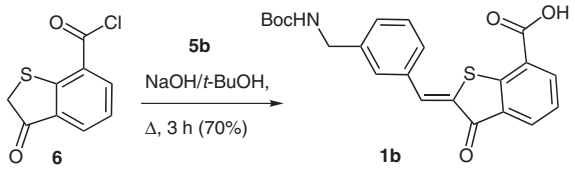
So far, these pseudo-amino acids have been synthesized and used as valuable building blocks in the synthesis of di- and tripeptides via Boc-based peptide assembly in solution.

For the synthesis of the hemithioindigo-based ω-amino acids **1a** and **1b**, the Boc-protected aldehydes were prepared starting from 3-cyano- or 4-cyanobenzaldehyde. Protection of the aldehydes to give the dioxolanes **2a** and **2b**,[7,8] reduction to the benzylamines **3a** and **3b**,[7] Boc-protection to the carbamates **4a** and **4b**, and removal of the acetal protecting group provided the *N*-Boc-protected aldehydes **5a** and **5b** in excellent overall yields (Scheme 1). For the synthesis of the *meta*-substituted ω-amino acid **1b**, the aldehyde **5b** and the literature-known thioindoxyl carboxylic acid chloride **6** were condensed using 1% sodium hydroxide/*tert*-butyl alcohol (Scheme 2) as previously described by us.[4] These conditions provided the hemithioindigo **1b** in 70% isolated yield and >95% purity after purification by chromatography on Florisil.



Scheme 1

In Boc-based solution-phase peptide synthesis, trifluoroacetic acid in combination with scavengers (e.g., H_2O, DMS, ethanedithiol, phenols, cresols, thiocresols, or thioanisols) is commonly used for deprotection strategies. However, we found treatment with hydrogen chloride in dioxane to be superior. Thus, Boc-deprotection of the dipeptide **7**[4] (Scheme 3), derived from **1a**[4] by EDC coupling, was carried out with 4 M hydrogen chloride in dioxane (r.t.) and gave the hydrochloride **8** quantitatively after evaporation of the solvent.



Scheme 2

Coupling of **8** with (*S*)-Boc-Lys(Cbz)-OH proved to be a challenge. By coupling with EDC/HOBt/DIPEA (1.1:1.1:1) in *N*,*N*-dimethylformamide or HBTU/HOBt/DIPEA (1:1:1) in *N*-methyl-2-pyrrolidinone, compound formation varied and occasionally decomposition was observed. A retro-aldol reaction could be ruled out based on the analysis of the ^1H NMR spectra of the crude material

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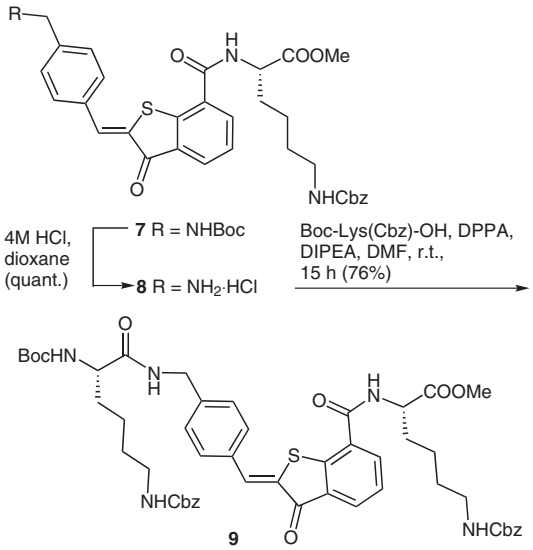
isolated. Similar results were obtained for the coupling of (*S*)-Boc-Lys(Cbz)-OSu with DIPEA or of (*S*)-Boc-Lys(Aloc)-OH with EDC/HOBt and DIPEA in dimethylformamide. Fortunately, tripeptide **9** was obtained using (*S*)-Boc-Lys(Cbz)-OH and dipeptide salt **8** with DPPA and DIPEA in *N*,*N*-dimethylformamide. Treatment of the crude product with methanol followed by centrifugation gave **9** in 76% isolated yield and >95% purity.



Scheme 3

In summary, for the Boc-protected ω-amino acid building blocks **1a** and **1b** syntheses and coupling strategies for the solution-phase peptide synthesis of di- and tripeptides were developed. The synthesis of **5a** and **5b** should have general applicability to other *N*-Boc-protected aldehydes.

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometers AC 200 and DRX 500. Solvents are mentioned for the particular substances. The MS and HRMS spectra were recorded on a Finnigan MAT 95 SQ or Varian MAT 711. The MS (EI) or HRMS (EI) samples were ionized at an ionization potential of 70 eV. Compound **6** was prepared following the literature procedure.[4]

4-(1,3-Dioxolan-2-yl)benzonitrile (2a); Typical Procedure[7]
4-Cyanobenzaldehyde (10.00 g, 0.08 mol) was dissolved in toluene (150 mL) and treated with ethylene glycol (17.0 mL, 0.31 mol, 4 equiv) followed by PTSA (10 mg, 0.06 mmol). The mixture was refluxed in a Dean Stark apparatus overnight. After cooling to r.t., the solution was washed with 5% aq $NaHCO_3$ (80 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The light yellow residue was recrystallized (Et_2O–pentane, 3:1) to give **2a** as a colorless solid.

Yield: 12.1 g (90%).

Mp 39 °C.

R_f = 0.41 (EtOAc–pentane, 1:4).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.67–7.63 (m, 2 H), 7.58–7.53 (m, 2 H), 5.82 (s, 1 H), 4.13–4.01 (m, 4 H).

^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 142.2, 131.3, 126.3, 117.7, 112.0, 101.5, 64.5.

3-(1,3-Dioxolan-2-yl)benzonitrile (2b)[8]
Colorless solid.

Yield: 12.0 g (90%).

Mp 26 °C.

R_f = 0.67 (EtOAc–pentane, 1:4).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.75–7.73 (m, 1 H), 7.65 (dt, *J* = 7.8, 1.6 Hz, 1 H), 7.62 (dt, *J* = 7.8, 1.6 Hz, 1 H), 7.44 (t, *J* = 7.8 Hz, 1 H), 5.77 (s, 1 H), 4.12–3.96 (m, 4 H).

^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 139.8, 132.8, 131.1, 130.3, 129.3, 118.7, 112.6, 102.4, 65.5.

4-(1,3-Dioxolan-2-yl)benzylamine (3a)[7]
Hygroscopic colorless solid.

Yield: 14.2 g (92%).

Mp 108 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.39 (d, *J* = 8.2 Hz, 2 H), 7.26 (d, *J* = 7.9 Hz, 2 H), 5.75 (s, 1 H), 4.07–3.95 (m, 4 H), 3.81 (s, 2 H), 1.41 (s, 2 H).

^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 144.2, 136.4, 127.0, 126.6, 103.6, 65.2, 46.2.

3-(1,3-Dioxolan-2-yl)benzylamine (3b)
Hygroscopic colorless oil.

Yield: 6.4 g (80%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.41 (s, 1 H), 7.33–7.29 (m, 3 H), 5.77 (s, 1 H), 4.12–3.99 (m, 4 H), 3.85 (s, 2 H), 1.46 (s, 2 H).

^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 143.7, 138.2, 128.7, 128.0, 125.1 (2C), 103.8, 65.4, 46.5.

***tert*-Butyl [4-(1,3-Dioxolan-2-yl)benzyl]carbamate (4a); Typical Procedure**
Benzylamine **3a** (9.07 g, 0.05 mol) was dissolved in anhyd MeOH (80 mL). While stirring, Et_3N (7.02 mL, 0.05 mol) was added followed by Boc_2O (14.37 g, 0.07 mol, 1.3 equiv). After 2.5 h, Et_2O (250 mL) was added and the organic layer was separated and washed with sat. aq NH_4Cl (300 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was recrystallized (Et_2O–pentane, 20:1) to give **4a** as a colorless solid.

Yield: 11.34 g (80%).

Mp 101 °C.

R_f = 0.76 (EtOAc–pentane, 2:1).

IR (film): 3355, 2881, 1679, 1527 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.38 (d, *J* = 8.0 Hz, 2 H), 7.38 (s, 1 H), 7.26 (d, *J* = 7.9 Hz, 2 H), 5.70 (s, 1 H), 4.15 (d, *J* = 5.8 Hz, 2 H), 4.07–3.90 (m, 4 H), 1.41 (s, 9 H).

^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 155.8, 141.2, 136.5, 126.7, 126.5, 102.8, 77.8, 64.8, 43.2, 28.2.

MS (EI, 70 eV): *m*/*z* (%) = 279 (<5) [M^+], 278 (<5) [M^+ – H], 222 (100), 149 (33), 73 (25), 57 (59).

HRMS (EI): *m*/*z* calcd for $C_{15}H_{20}NO_4$ [M^+ – H]: 278.1392; found: 278.1399.

Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.59; H, 7.65; N, 4.78.

***tert*-Butyl [3-(1,3-Dioxolan-2-yl)benzyl]carbamate (4b)**
Yield: 9.1 g (85%).

Mp 68 °C.

R_f = 0.79 (EtOAc–pentane, 2:1).

IR (film): 3354, 2976, 1710, 1697 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 7.41 (br s, 1 H), 7.35–7.32 (m, 2 H), 7.29 (d, *J* = 7.3 Hz, 1 H), 7.26 (d, *J* = 7.2 Hz, 1 H), 5.71 (s, 1 H), 4.15 (d, *J* = 6.0 Hz, 2 H), 4.05–3.93 (m, 4 H), 1.41 (s, 9 H).

^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 155.8 (C-10), 140.3 (C-7), 138.1 (C-3), 128.1 (C-8), 127.7 (C-5), 125.0 (C-4, C-6), 102.9 (C-2), 77.9 (C-11), 64.8 (C-1), 43.3 (C-9), 28.2 (C-12). The assignment was confirmed by HMQC measurements.

HRMS (EI): *m*/*z* calcd for $C_{15}H_{21}NO_4$: 279.1471; found: 279.1472.

Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.68; H, 7.65; N, 4.62.

tert-Butyl (4-Formylbenzyl)carbamate (5a); Typical Procedure

Carbamate **4a** (11.34 g, 0.04 mol) was dissolved in acetone (190 mL). After addition of H_2O (23 mL) and PTSA (10 mg, 0.06 mmol) the solution was stirred at r.t. overnight. The mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was separated and washed with 5% aq $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to give **5a** as colorless crystals.

Yield: 9.25 g (97%).

Mp 81 °C.

R_f = 0.91 (EtOAc–pentane, 2:1).

IR (film): 3351, 2978, 1693, 1168 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 9.97 (s, 1 H), 7.86 (d, *J* = 7.7 Hz, 2 H), 7.51 (s, 1 H), 7.45 (d, *J* = 7.7 Hz, 2 H), 4.22 (d, *J* = 5.8 Hz, 2 H), 1.40 (s, 9 H).

^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 192.7, 155.8, 147.3, 135.0, 129.6, 127.4, 78.0, 43.3, 28.2.

Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.09; H, 7.26; N, 5.72.

tert-Butyl (3-Formylbenzyl)carbamate (5b)

Yield: 7.0 g (98%).

R_f = 0.89 (EtOAc–pentane, 2:1).

Mp 59 °C.

IR (film): 3350, 2978, 1605, 1163 cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6): δ = 10.00 (s, 1 H), 7.79–7.77 (m, 2 H), 7.57–7.54 (m, 2 H), 7.50 (s, 1 H), 4.22 (d, *J* = 5.6 Hz, 2 H), 1.40 (s, 9 H).

^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 193.1, 155.8, 141.4, 136.3, 133.1, 129.1, 128.5, 127.2, 77.9, 43.0, 28.2.

HRMS (EI): *m*/*z* calcd for $C_{13}H_{16}NO_3$ $[M - H]^+$: 234.1130; found: 234.1120.

Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.21; H, 7.29; N, 5.55.

(2*Z*)-2-(3-{[(*tert*-Butoxycarbonyl)amino]methyl}benzylidene)-3-oxo-2,3-dihydrobenzo[*b*]thiophene-7-carboxylic Acid (1b)

Yield: 795 mg (70%).

Mp 223 °C.

R_f = 0.66 (EtOAc–AcOH, 80:1).

IR (film): 3336, 1700, 1682, 1274 cm^{-1}.

^1H NMR (500 MHz, CD_3OD): δ = 8.28 (dd, *J* = 7.5, 1.1 Hz, 1 H), 7.95 (dd, *J* = 7.5, 1.3 Hz, 1 H), 7.86 (s, 1 H), 7.77 (d, *J* = 7.7 Hz, 1 H), 7.71 (s, 1 H), 7.49–7.47 (m, 1 H), 7.40–7.37 (m, 2 H), 4.31 (s, 2 H), 1.47 (s, 9 H).

^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 187.6, 166.5, 155.9, 147.0, 143.2, 136.9, 133.5, 132.4, 131.3, 131.1, 131.0, 129.9, 129.3, 128.9, 127.8, 127.0, 126.1, 77.9, 43.3, 28.3.

MS (EI, 70 eV): *m*/*z* (%) = 412 (10) $[M^+ + H]$, 295 (60), 57 (100).

HRMS (EI): *m*/*z* calcd for $C_{22}H_{21}NO_5S$: 411.1141; found: 411.1144.

Methyl *N*-{(2*Z*)-2-[4-(Aminomethyl)benzylidene]-3-oxo-2,3-dihydrobenzo[*b*]thiophen-7-yl}carbonyl-*N*^{6}-[(benzyloxy)carbonyl]lysinate Hydrochloride (8)

Compound **7** (0.19 g, 0.28 mmol) was dissolved in 4 M HCl in dioxane (10 mL). The solution was stirred for 1 h at r.t. under a nitrogen atmosphere. Then, the solvent was removed in vacuo to give **8** as a light-yellow solid.

Yield: 170 mg (quant).

Mp 194 °C.

IR (film): 3323, 2952, 1684, 1534 cm^{-1}.

^1H NMR (DMSO-d_6, 200 MHz): δ = 9.16 (d, *J* = 7.2 Hz, 1 H), 8.46 (d, *J* = 7.4 Hz, 1 H), 8.40 (br s, 3 H), 8.08 (d, *J* = 7.2 Hz, 1 H), 7.92 (s, 1 H), 7.88 (d, *J* = 8.4 Hz, 2 H), 7.68 (d, *J* = 8.4 Hz, 2 H), 7.58 (t, *J* = 7.6 Hz, 1 H), 7.33–7.23 (m, 6 H), 4.98 (s, 2 H), 4.47 (q, *J* = 5.4 Hz, 1 H), 4.11 (br d, *J* = 5.0 Hz, 2 H), 3.68 (s, 3 H), 3.01–2.99 (m, 2 H), 1.86–1.76 (m, 2 H), 1.43–1.35 (m, 4 H).

^{13}C NMR (DMSO-d_6, 50.3 MHz): δ = 187.5, 172.7, 169.3, 165.1, 156.0, 146.1, 137.2, 137.1, 136.3, 133.9, 132.6, 132.2, 131.3, 131.0, 129.7, 129.2, 128.6, 128.2, 127.6, 127.1, 65.0, 51.8, 51.6, 41.8, 30.5, 28.9, 22.3 (one C atom overlapped by the adjacent DMSO signal).

FAB-MS: *m*/*z* (%) = 588 (100) $[M^+ - HCl]$.

Tripeptide 9

To a solution of compound **8** (152 mg, 0.24 mmol) in DMF (5 mL) were gradually added Boc-Lys(Cbz)-OH (92 mg, 0.24 mmol), DPPA (74 mg, 0.26 mmol), and DIPEA (26 mg, 0.24 mmol). After the solution was stirred under nitrogen for 15 h, a mixture of EtOAc–benzene (4:1, 100 mL) was added. The organic layer was separated and washed with 2% citric acid (2 × 50 mL) followed by H_2O (2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed in vacuo. The residue was treated with MeOH and isolated by centrifugation (3 ×) to give tripeptide **9** as a light-yellow solid.

Yield: 175 mg (76%); 95% purity.

Mp 128 °C.

R_f = 0.34 (CH_2Cl_2–MeOH, 1:1).

IR (film): 3324, 2935, 1700, 1532, 1251 cm^{-1}.

^1H NMR ($CDCl_3$, 500 MHz): δ = 8.01 (d, *J* = 7.5 Hz, 1 H), 7.97 (d, *J* = 7.0 Hz, 1 H), 7.84 (s, 1 H), 7.68 (d, *J* = 7.5 Hz, 2 H), 7.39 (t, *J* = 7.5 Hz, 1 H), 7.35–7.20 (m, 13 H), 7.19–7.14 (m, 1 H), 6.88 (br s, 1 H), 5.31–5.26 (m, 1 H), 5.01–4.98 (m, 5 H), 4.80–4.79 (m, 1 H), 4.44 (br s, 2 H), 4.12–4.01 (m, 1 H), 3.80 (s, 3 H), 3.18 (br s, 4 H), 2.00–1.98 (m, 1 H), 1.92–1.85 (m, 2 H), 1.69–1.66 (m, 1 H), 1.60–1.48 (m, 5 H), 1.46–1.30 (m, 3 H), 1.41 (s, 9 H).

^{13}C NMR ($CDCl_3$, 50.3 MHz): δ = 188.4, 172.8, 172.3, 165.5, 156.8, 156.7, 156.0, 140.6, 136.5, 136.3, 134.0, 133.3, 132.5, 132.4, 131.6, 130.0, 129.7, 128.5, 128.1, 128.0, 127.9, 127.5, 126.1, 125.2, 120.7, 120.2, 120.1, 77.2, 66.7, 52.8, 52.7, 43.0, 40.3, 40.2, 31.7, 31.4, 29.6, 29.4, 28.3, 22.5, 22.4 (two C atoms are overlapped by adjacent signals).

FAB-MS: *m*/*z* (%) = 951 (26) $[M^+ + H]$, 950 (2) $[M^+]$, 850 (100).

Anal. Calcd for $C_{51}H_{59}N_5O_{11}S$: C, 64.47; H, 6.26; N, 7.37; S, 3.37. Found: C, 64.38; H, 6.22; N, 7.27; S, 3.41.

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