Supplementary Material (ESI) for Photochemical & Photobiological Sciences

Photophysics of Pyrene-labelled Compounds of Biophysical Interest

by

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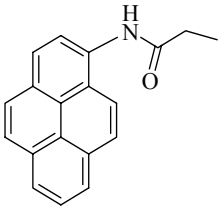
Synthesis of Compounds **4** - **12**



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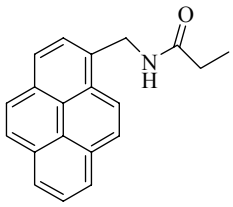
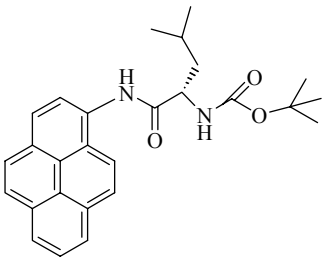


8

*Synthesis and Characterization of Py-$(CH_2)_n$ NH-CO-C_2H_5 (n = 0 (**4**), n = 1 (**8**)):* Solid HOBt (0.09 g, 0.6 mmol) and EDC (0.12 g, 0.6 mmol) were added successively to a 50 ml well-stirred CH_2Cl_2 solution of propionic acid (40µl, 0.5 mmol) in an ice-water bath at 0 °C. After 30 minutes, a solution of 1-aminopyrene (0.13 g, 0.6 mmol) treated by excess Et_3N in 20 ml CH_2Cl_2 was added into this reaction mixture. TLC was performed to monitor the progress of the reaction. The reaction solution was stirred vigorously in the dark overnight, at room temperature. Then, the reaction mixture was extracted consecutively with aqueous saturated $NaHCO_3$, 10% citric acid, saturated $NaHCO_3$ and distilled water (100 ml for each aqueous wash). The organic phase was dried over anhydrous Na_2SO_4 and then evaporated to dryness. After purification by FCC (SiO_2,

hexane/EtOAc 1:1, R_f = 0.44), compound **4** (0.09 g, 64%) was obtained as a yellow solid. Elem. anal. calc. for $C_{19}H_{25}NO$: C, 83.52; H, 5.49; N, 5.13; found: C, 83.25; H, 5.42; N, 5.13 %. HRMS (FAB-EI+) m/z for $C_{19}H_{25}NO$: calc. 273.1154, found 273.1157 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$): 8.19-8.02 (9H, m, CH of Py), 7.78 (1H, br, s, NH close to Py), 2.65, (2H, *J* = 7.5 Hz, CH_2), 1.42 (3H, t, *J* = 6.9 Hz, CH_3).

For compound **8**: Amounts: Propionic acid (75 µl, 1.0 mmol), HOBt (0.18 g, 1.2 mmol), EDC (0.23 g, 1.2 mmol), 1-pyrenemethylamine hydrochloride (0.29 g, 1.1 mmol). (hexane/ EtOAc/ MeOH 2: 0.5: 0.5, R_f = 0.34). Yellow solid, yield: 0.17 g (60 %). HRMS (FAB-EI+) m/z for $C_{20}H_{17}NO$: calc. 287.1310, found 287.1307 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$): 8.24-7.78 (9H, m, CH of Py), 5.76 (1H, br, NH), 5.15 (2H, d, *J* = 5.4 Hz, CH_2 close to Py), 2.25 (2H, q, *J* = 7.5 Hz, CH_2 close to C=O), 1.19 (3H, t, *J* = 7.5 Hz, CH_3). ^{13}C{^{1}H}-NMR (δ in ppm, $CDCl_3$): 173.6 (C=O), 131.4, 120.9, 129.2, 128.4, 127.7, 127.5, 127.4, 126.3, 125.6, 125.5, 124.9, 123.0 (C, CH of Py), 42.2 (CH_2 linking to Py), 29.9 (CH_2 close to C=O), 10.1 (CH_3).



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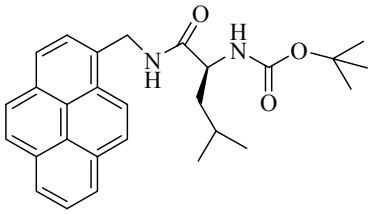


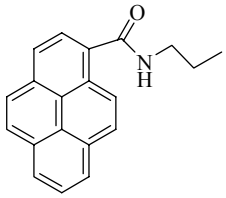
10

*Synthesis and Characterization of Py-$(CH_2)_n$-CO-Leu-Boc (n = 0 (**6**), n = 1 (**10**)):* To a well-stirred solution of Boc-Leu-OH (0.12 g, 0.5 mmol), HOBt (0.1 g, 0.6 mmol) and EDC (0.12 g, 0.6 mmol) in 50 ml CH_2Cl_2 at 0°C, was added a solution of 1-aminopyrene

(0.13 g, 0.6 mmol) in 20 ml CH_2Cl_2. The mixture was stirred in the dark at room temperature overnight, followed by an aqueous work up described above. The crude product was purified by column chromatography (SiO_2, hexane/ EtOAc 3: 1, R_f = 0.46) to give compound **6** as a yellow solid in 41% yield (0.09 g, 41%). Elem. Anal.: calc. for $C_{27}H_{30}N_2O_3 \cdot (0.5\ CH_3COOC_2H_5)$: C, 73.42; H, 7.17; N, 5.91; found: C, 73.95; H, 6.76; N, 6.43 %. HRMS (FAB-EI+) m/z for $C_{27}H_{30}N_2O_3$: calc. 430.2256, found 430.2259 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$): 9.06 (1H, br, NH close to Py), 8.51-7.96 (9H, m, CH of Py), 5.06 (1H, d, *J* = 6.6 Hz, NH of Leu), 4.48 (1H, m, CH^{α} of Leu), 2.04-1.66 (3H, m, CH_2^{β} and CH^{γ} of Leu), 1.55 (9H, br, s, $3CH_3$ of Boc), 1.06 (6H, t, *J* = 6.0 Hz, $2CH_3^{\delta}$ of Leu). ^{13}C{^{1}H}-NMR (δ in ppm, $CDCl_3$): 172.6, 156.8 (2 C=O), 131.2, 130.7, 130.2, 128.9, 127.5, 127.1, 126.6, 125.9, 125.2, 124.8, 124.7, 124.4, 123.7, 122.2, 120.7 (C, CH of Py), 80.5 (C of Boc), 54.0 (CH^{α} of Leu), 41.1 (CH_2^{β} of Leu), 28.8 ($3CH_3$ of Boc), 25.2, 23.3 ($2CH_3^{\delta}$ of Leu), 22 .5 (CH^{γ} of Leu).

For Compound **10:** Amounts: 1-pyrenemethylamine hydrochloride (0.29 g, 1.1 mmol), Boc-Leu-OH (0.23g, 1.0 mmol), HOBt (0.17 g, 1.1 mmol), EDC (0.21 g, 1.1 mmol). Column chromatography: SiO_2, CH_2Cl_2/ EtOAc 3: 0.5, R_f = 0.43. Yield: yellow solid (0.36 g, 82 %). Elem. Anal. calc. for $C_{28}H_{32}N_2O_3 \cdot (0.5\ CH_3COOC_2H_5)$: C 73.77, H 7.37, N 5.74; found C 73.76, H 7.18, N 6.30 %. HRMS (FAB-EI+) m/z for $C_{28}H_{32}N_2O_3$: calc. 444.2413, found 444.2407 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$): 8.17-7.95 (9H, m, CH of Py), 6.54 (1H, br, NH close to Py), 5.13 (2H, d, *J* = 4.8 Hz, CH_2 close to Py), 4.84 (1H, br, NH of Leu), 4.11 (1H, m, CH^{α} of Leu), 1.78-1.43 (3H, m, CH_2^{β} and of CH^{γ} Leu), 1.29 (9H, br, s, CH_3 of Boc), 0.90 (6H, d, *J* = 6.0 Hz, CH_3^{δ} of Leu). ^{13}C{^{1}H}-NMR (δ in ppm, $CDCl_3$): 173.0, 156.0 (2 C=O), 131.3, 131.1, 131.0, 130.8, 128.8, 128.0, 127.4,

126.6, 126.0, 125.3, 124.9, 124.8, 122.8 (C, CH of Py), 80.0 (C of Boc), 53.5 (CH^{α} of Leu), 41.7, 41.7 (CH_2 linking to Py and CH_2^{β} of Leu), 28.5 (CH_3 of Boc), 25.0, 23.1 ($2CH_3^{\delta}$ of Leu), 22.3 (CH^{γ} of Leu).



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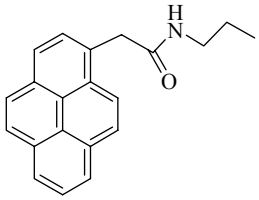
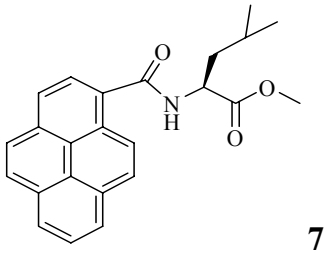


9

*Synthesis and Characterization of Py-$(CH_2)_n$ CO-NH-C_3H_7 (n = 0 (**5**), n = 1 (**9**)):* 1-pyrenecarboxylic acid (0.25 g, 1.0 mmol), HOBt (0.17 g, 1.1 mmol) and EDC (0.21 g, 1.1 mmol) were added to 50 ml CH_2Cl_2. *N*-propylamine (100 µl, 1.2 mmol) was dissolved in 20 ml CH_2Cl_2, and added into the reaction mixture and stirred overnight. The work up procedure was identical to that described for compound **4**. The organic residue was then purified by FCC (SiO_2, hexane/ EtOAc/ MeOH 4: 1.5: 0.5, R_f = 0.46) to give compound **5** as a yellow solid. Yield: 0.14 g (48 %). HRMS (FAB-EI+) m/z for $C_{20}H_{17}NO$: calc. 287.1310, found 287.1306 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$): 8.50-7.95 (9H, m, CH of Py), 6.25 (1H, br.s, NH), 3.56 (2H, q, *J* = 6.3, 13.2 Hz, CH_2 close to NH), 1.75 (2H, m, CH_2 close to CH_3), 1.10 (3H, t, *J* = 7.4 Hz, CH_3). $^{13}C\{^1H\}$-NMR (δ in ppm, $CDCl_3$): 170.2 (C=O), 132.5, 131.6, 131.3, 130.9, 128.7, 127.3, 126.5, 125.9, 125.8, 124.9, 124.5 (C, CH of Py), 42.2, 23.3 (2 CH_2), 11.8 (CH_3).

For compound **9**: Amounts: 1-pyreneacetic acid (0.26 g, 1.0 mmol), HOBt (0.17 g, 1.1 mmol), EDC (0.21 g, 1.1 mmol), *N*-propylamine (100 µl, 1.2 mmol). Purification by column chromatography: SiO_2, hexane/ EtOAc 1: 1, R_f = 0.24). Yield: yellow solid,

71 % yield; 0.21g. Elem. Anal. calc. for $C_{21}H_{19}NO$: C, 83.72; H, 6.31; N, 4.65; found: C, 83.79; H, 5.96; N, 4.44 %. HRMS (FAB-EI+) m/z for $C_{21}H_{19}NO$: calc. 301.1467, found 301.1469 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$): 8.20-7.92 (9H, m, CH of Py), 5.23 (1H, br., NH), 4.30 (2H, s, CH_2 close to Py), 3.09 (2H, q, J = 6.6, 13.8 Hz, CH_2 close to NH), 1.27 (2H, m, CH_2 close to CH_3), 0.66 (3H, t, J = 7.2, 7.5 Hz, CH_3). ^{13}C{^{1}H}-NMR (δ in ppm, $CDCl_3$): 171.2 (C=O), 131.5, 131.1, 131.0, 129.7, 128.8, 128.7, 128.4, 127.7, 127.5, 126.4, 125.7, 125.6, 125.2, 124.8, 123.2 (C, CH of Py), 42.2 (CH_2), 41.6 (CH_2), 22.9 (CH_2), 11.4 (CH_3).



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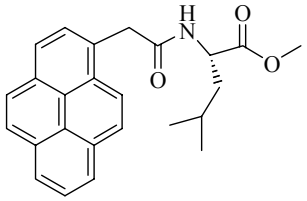

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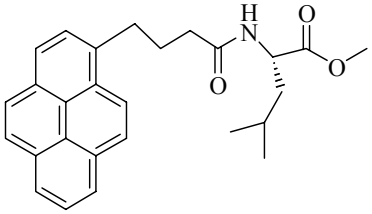

12

*Synthesis and Characterization of Preparation of Py-$(CH_2)_n$-CO-NH-Leu-OMe (n = 0 (**7**), n = 1 (**11**), n = 3 (**12**):* To a well-stirred solution of 1-pyrenecarboxylic acid (0.25 g, 1.0 mmol) in 50 ml CH_2Cl_2, was added HOBt (0.17 g, 1.1 mmol) and EDC (0.21 g, 1.1 mmol). After 30 minutes, a solution of H-Leu-OMe·HCl (0.20 g, 1.1 mmol) treated with NEt_3 (0.5 mL) in CH_2Cl_2 (10 mL) was added to the reaction mixture. After stirring overnight, the reaction mixture was worked up using the same procedure as described

above, followed by purification by (SiO_2, hexane/ EtOAc 3: 1, R_f = 0.35). Compound **7** was obtained as a yellow solid in 57 % yield (0.21 g). Elem. Anal. calc. for $C_{24}H_{23}NO_3$: C, 77.21; H, 6.17; N, 3.75; found: C, 76.99; H, 5.96; N, 3.82 %. HRMS (FAB-EI+) m/z for $C_{24}H_{23}NO_3$: calc. 373.1678, found 373.1679 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$):8.21-8.06 (9H, m, CH of Py), 6.51 (1H, d, *J* = 8.7 Hz, NH of Leu), 5.06 (1H, m, CH^{α} of Leu), 3.84 (3H, s, OCH_3), 1.88-1.71 (3H, m, CH_2^{β} and CH^{γ} of Leu), 1.10, 1.03 (3H, 3H, d, d, *J* = 6.0, 6.0 Hz, 2 CH_3^{δ} of Leu).^{13}C{^{1}H}-NMR (δ in ppm, $CDCl_3$): 173.9, 170.0 (2C=O), 132.9, 131.3, 130.9, 130.5, 129.0, 128.9, 127.3, 126.5, 126.0, 125.9, 124.9, 124.6, 124.4 (C, CH of Py), 52.9 (CH^{α} of Leu), 51.7 (OCH_3), 41.9(CH_2^{β} of Leu), 25.4 (CH^{γ} of Leu), 23.2, 22.3 (2 CH^{δ} of Leu).

For compound **11**: Amounts: 1-pyreneacetic acid (0.26 g, 1.0 mmol), HOBt (0.17 g, 1.1 mmol), EDC (0.21 g, 1.1 mmol), H-Leu-OMe·HCl (0.20 g, 1.1 mmol). CH_2Cl_2/ EtOAc 3:0.5, R_f = 0.39. Yellow solid. Yield: 62 %, 0.24 g. Elem. Anal. calc. for $C_{25}H_{25}NO_3$: C, 77.52; H, 6.46; N, 3.62; found: C, 77.41; H, 6.45; N, 3.30 %. HRMS (FAB-EI+) m/z for $C_{25}H_{25}NO_3$: calc. 387.1834, found 387.1835 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$):8.20-7.96 (9H, m, CH of Py), 5.59 (1H, d, *J* =7.2 Hz, NH), 4.62 (1H, m, CH of Leu close to NH), 4.33 (2H, d, *J* = 4.5 Hz, CH_2 near Py), 3.65 (3, s, OCH_3), 1.46-1.19 (3H, m, CH_2^{β} and CH^{γ} of Leu), 0.76, 0.67 (3H, 3H, d, d, *J* = 6.3, 6.4 Hz, 2CH_3^{δ} of Leu). ^{13}C{^{1}H}-NMR (δ in ppm, $CDCl_3$): 173.4, 171.1 (2 C=O), 131.5, 131.2, 131.0, 128.6, 128.4, 128.3, 127.7, 127.5, 126.3, 125.6, 125.5, 125.3, 124.8, 123.3 (C, CH of Py), 52.4 (CH^{α} of Leu), 51.1 (OCH_3), 41.8, 41.3 (CH_2 near Py and CH_2^{β} of Leu), 25.0 (CH^{γ} of Leu), 22.9, 22.0 (2 CH_3^{δ} of Leu).

For compound **12**: Amounts: 1-pyrenebutyric acid (0.29 g, 1.0 mmol), HOBt (0.17 g, 1.1 mmol), EDC (0.21 g, 1.1 mmol), H-Leu-OMe·HCl (0.20 g, 1.1 mmol). hexane/ EtOAc/ MeOH 2:0.5:0.5, R_f = 0.67. Yellow solid. Yield: 37 %, 0.15 g. Elem. anal. calc. for $C_{27}H_{29}NO_3$: C, 78.07; H, 6.99; N, 3.37; found: C, 77.86; H, 7.08; N, 3.49 %. HRMS (FAB-EI+) m/z for $C_{27}H_{29}NO_3$: calc. 415.2147, found 415.2149 (M^+). ^{1}H-NMR (δ in ppm, $CDCl_3$):8.15-7.80 (9H, m, CH of Py), 5.84 (1H, d, *J* = 8.1 Hz, NH of Leu), 4.69 (1H, m, CH□ of Leu), 3.73 (3H, s, OCH_3), 3.40 (2H, t, *J* = 6.6, 7.2 Hz, CH_2 linked to Py), 2.36-2.22 (4H, m, 2 CH_2 close to Py), 1.69-1.48 (3H, m, CH_2^{β} and CH^{γ} of Leu), 0.94 (6H, dd, *J* = 6.3, 7.2 Hz, $2CH_3^{\delta}$ of Leu). ^{13}C{^{1}H}-NMR (δ in ppm, $CDCl_3$): 174.0, 172.9 (2 C=O), 136.1, 131.6, 131.1, 130.1, 129.0, 127.7, 127.6, 127.0, 126.1, 125.3, 125.1, 125.0, 125.0, 123.6 (C, CH of Py), 52.5, 50.9 (CH^{α} of Leu and OCH_3), 41.8, 36.0, 32.9, 27.6 (CH_2), 25.2 (CH^{γ} of Leu), 23.1, 22.2 ($2CH_3^{\delta}$ of Leu).