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Synthesis of Opioid Biotin Probes

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Activity-based, protein-profiling molecular probes containing biotin were synthesized. The molecules included carfentanil and fentanyl analogs.

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PREFACE

The work described in this report was started in April 2014 and completed in July 2018. At the time this work was performed, the U.S. Army Combat Capabilities Command Chemical Biological Center was known as the U.S. Army Edgewood Chemical Biological Center.

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SCHEMES

SYNTHESIS OF OPIOID BIOTIN PROBES

1. INTRODUCTION

Potent synthetic opioids, such as 4-anilidopiperidines, are used worldwide as central nervous system analgesics.[1] Janssen and coworkers produced carfentanil, fentanyl, and many other potent and clinically useful compounds.[2–4] These compounds are agonists of the μ-opioid receptor (MOR; i.e., the receptor subtype responsible for analgesia, muscular rigidity, respiratory depression, and apnea).[5,6] It has been reported in literature that carfentanil is extremely specific to MORs; however, its low effective concentration at half optimal response indicates that it could have off-target effects at physiologically relevant concentrations.[7]

In this work, we describe the synthesis of molecular probes for activity-based protein profiling (ABPP) to discover protein-binding partners for carfentanil and fentanyl in biological media. The ABPP is applied for the identification of novel proteins that interact with *O*-ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX).[8] Carfentanil and fentanyl were chosen as the molecules of interest for the ABPP. Their inclusion in the carfentanil and fentanyl biotin probes (the Figure) required modifications, which allowed for aromatic amide formation para to the ethyl group attached to the piperidine ring. The aminated analogs were tethered to a 6–10 carbon chain, which incorporated an amide-linked biotin tail.

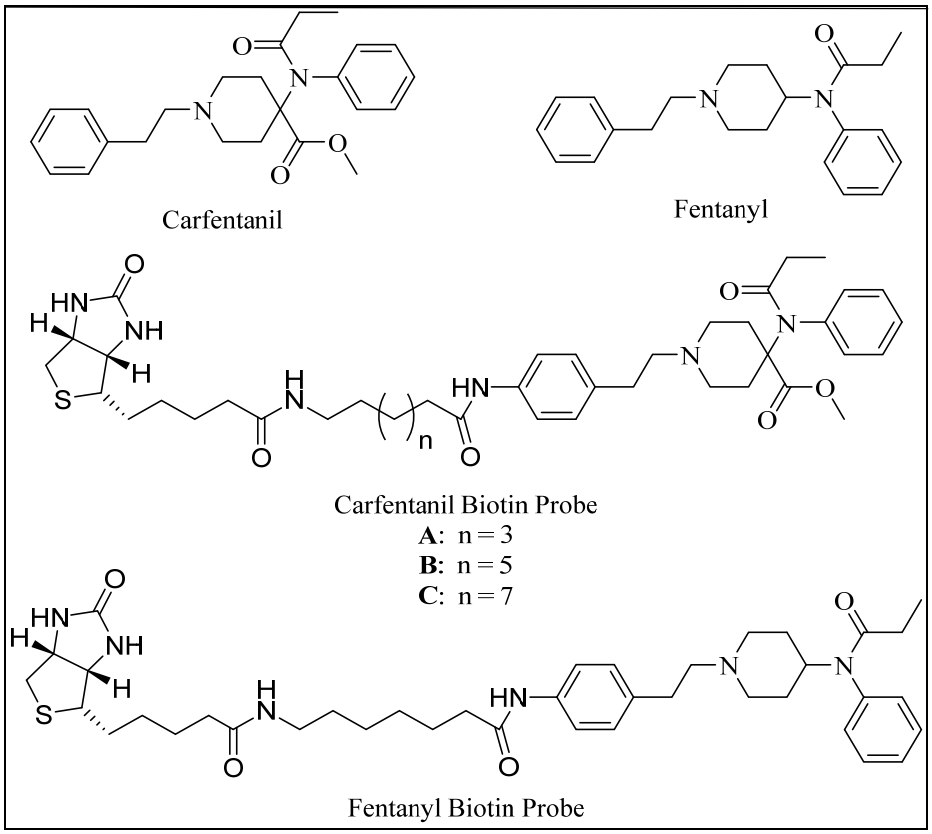
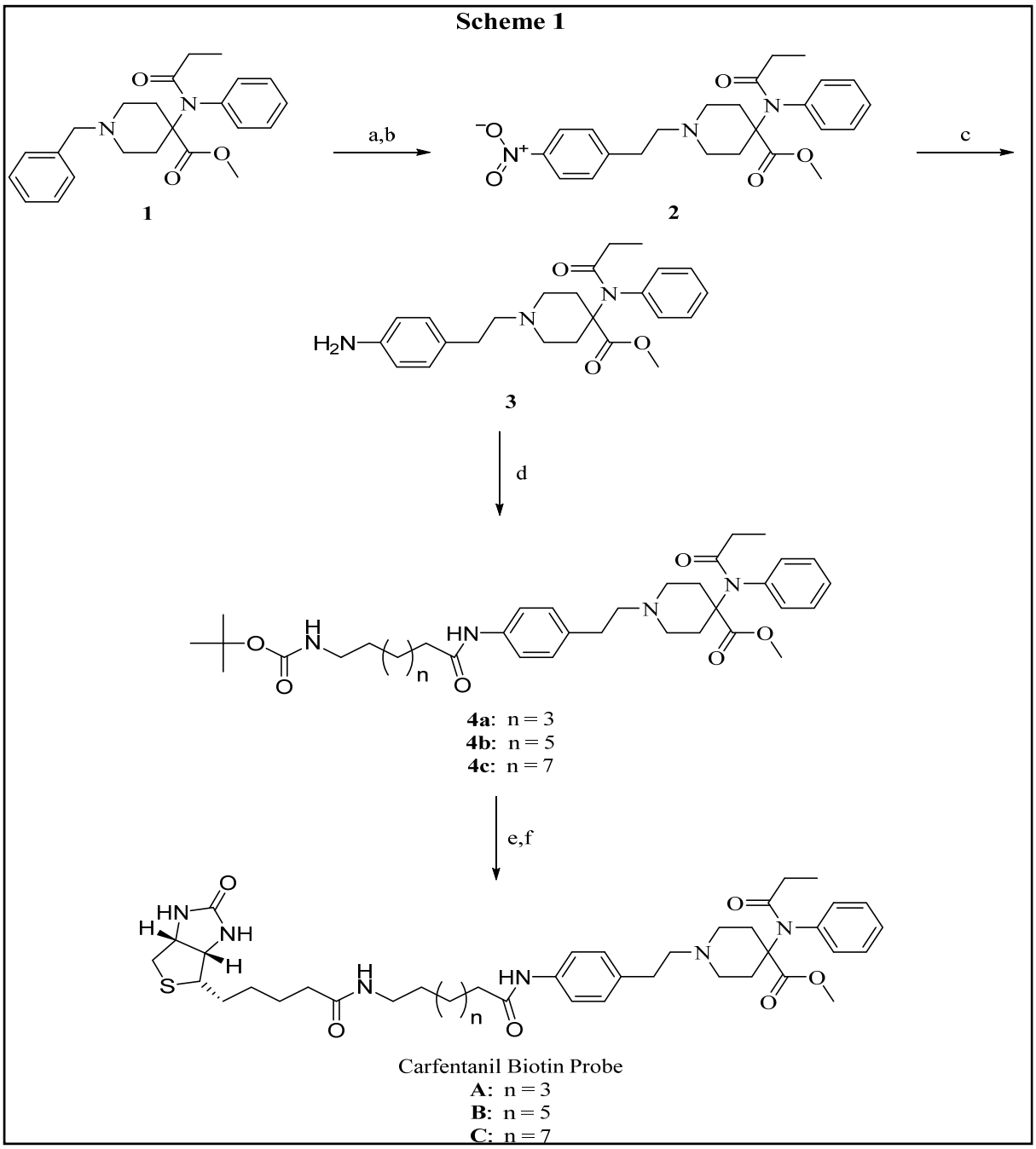


Figure. Carfentanil and fetanyl biotin probes.

2. CHEMISTRY

The syntheses of the carfentanil biotin probes **A**, **B**, and **C** are shown in Scheme 1. The route began with intermediate **1,** which was prepared as described in the literature.[9] Debenzylation of the tertiary amine was performed before alkylation to provide compound **2,** and then, nitro group reduction was performed to provide compound **3.**[10] Inclusion of the *tert*-butyloxycarbonyl (Boc)-protected linker provided intermediates **4a, b,** and **c,** which were deprotected under acidic conditions and linked to the biotin tail, which provided the carfentanil biotin probe molecules **A**, **B**, and **C**.[8]

The syntheses of the fentanyl biotin probe are shown in Scheme 2. The route began with intermediate **5,** which was prepared as described in the literature.[11] Debenzylation of the tertiary amine was performed before alkylation to provide compound **6,** and then nitro group reduction was performed to provide compound **7**.[12] Inclusion of the Boc-protected linker provided intermediate **8,** which was acid deprotected and linked to the biotin tail. The fentanyl biotin probe molecule was produced in this manner.[8]

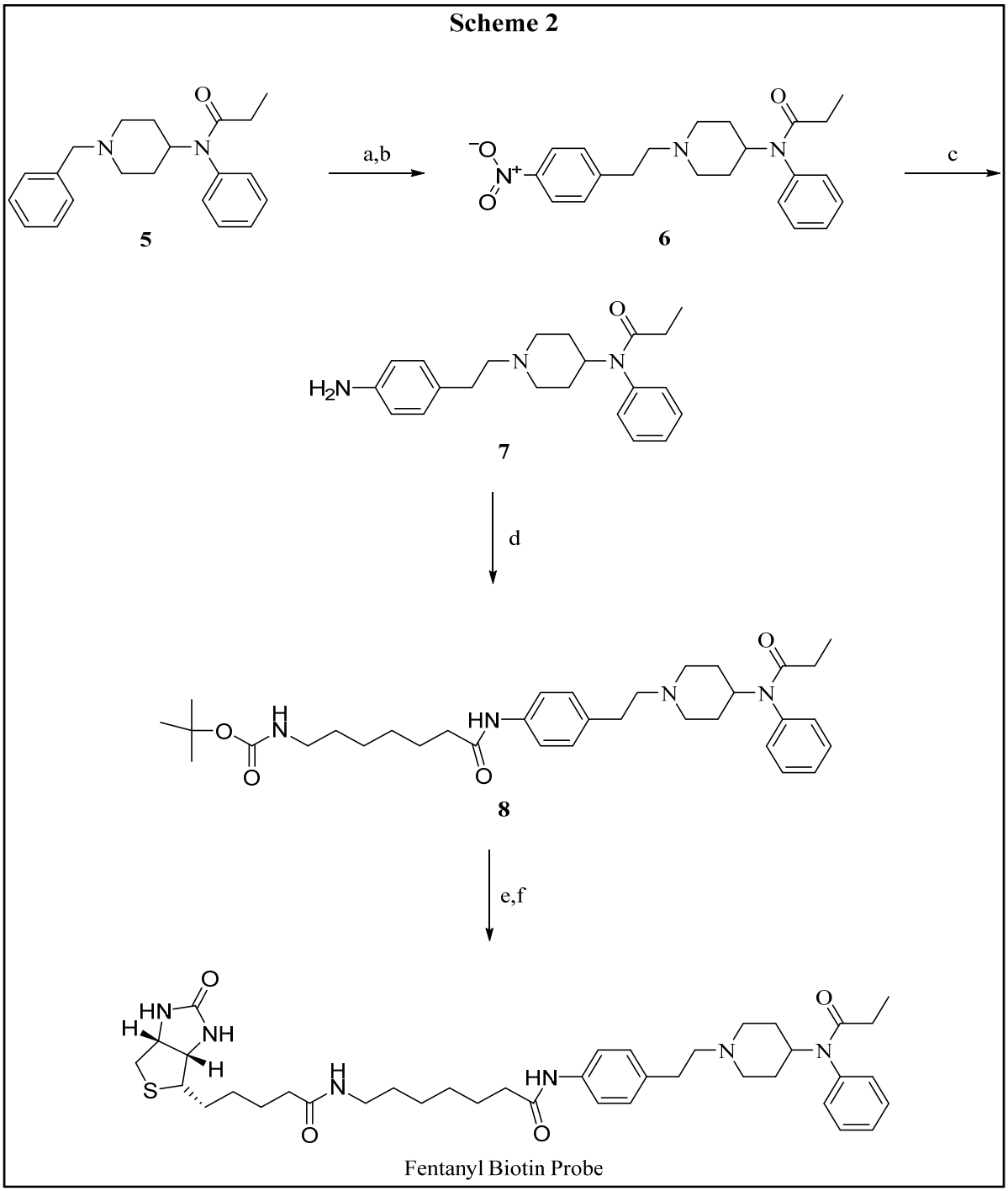


Scheme 1. Reagents and conditions: (a) ammonium formate, 10% palladium on carbon, and methanol at 65 °C; (b) 4-nitrophenylethyl bromide, sodium carbonate, 4-methyl-2-pentanone, and reflux; (c) H_2, 10% palladium on carbon, and ethanol; (d) *tert*-butyloxycarbonyl (Boc)-7-aminoalkanoic acid, N-hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), and dimethylformamide (DMF); (e) trifluoroacetyl (TFA) and dichloromethane (DCM); and (f) Biotinyl N-hydroxysuccinimide ester (NHS-Biotin), 4-dimethylaminopyridine (DMAP), and DMF.

3. EXPERIMENTAL PROCESS

3.1 Compound **2**

Compound **1** (1.69 g and 3.75 mmol) was dissolved in 40 mL of nitrogen-sparged methanol. After 10% of palladium on 0.17 g of carbon was added, ammonium formate (1.44 g, 2.28 mmol) was added. The reaction was stirred under a nitrogen atmosphere in a 65 ºC oil bath for 4 h, allowed to cool, and then filtered through celite. The celite pad was washed with methanol, and the filtrate was concentrated and taken up in chloroform-saturated aqueous sodium hydrogen carbonate. The mixture was shaken, and the organic layer was separated. The aqueous layer was washed two times with chloroform. The combined organic extracts were dried over sodium sulfate, filtered, and the volatiles were evaporated. The residue was taken up in 40 mL of 4-methyl-2-pentanone under a nitrogen atmosphere, and then 4-nitrophenylethyl bromide (1.15 g, 5.02 mmol) and sodium carbonate (1.76 g, 16.6 mmol) were added. The mixture was heated at reflux for 16 h with an oil bath, allowed to cool to room temperature, and then concentrated. The residue was taken up in chloroform-saturated aqueous sodium hydrogen carbonate, the solution was shaken, and the organic layer was separated. The aqueous layer was washed two times with chloroform. The combined organic extracts were dried over sodium sulfate, filtered, and the volatiles were evaporated. The residue was purified by column chromatography using 0–2.5% methanol in chloroform as the eluent, which provided 0.944 g of compound **2** as an amorphous white solid in a 47% yield. The spectral data matched the results reported in the literature.[10]



Scheme 2. Reagents and conditions: (a) Ammonium formate, 10% palladium on carbon, and methanol at 65 °C; (b) 4-Nitrophenylethyl bromide, sodium carbonate, 4-methyl-2-pentanone, and reflux; (c) H_2, 10% palladium on carbon, and ethanol; (d) Boc-7-aminoheptanoic acid, HOBt, EDC-HCl, and DMF; (e) TFA and DCM; and (f) NHS-Biotin, DMAP, and DMF.

3.2 Compound **4a**

Compound **2** (0.192 g, 0.437 mmol) was dissolved in 16 mL of ethanol in a Parr hydrogenation bottle (Parr Instrument Company; Moline, IL). Palladium (10%) on carbon (0.040 g) was added, and the reaction was placed on a Parr hydrogenator at 40 psi for 8 h. The mixture was filtered through celite, and the celite pad was washed with ethanol. The filtrate was concentrated, and compound **3** was used in the next step without purification. The residue exhibited spectral data consistent with the literature.[2] The following reagents were added in the order they are listed to compound **3** in 4 mL of DMF at room temperature under a nitrogen atmosphere:

- Boc-7-aminoheptanoic acid (0.107 g, 0.437 mmol),
- HOBt (0.059 g, 0.437 mmol), and
- EDC-HCl (0.092 g, 0.437 mmol).

The reaction mixture was stirred at room temperature for 16 h. The volatiles were removed under reduced pressure. The residue was taken up in ethyl actetate saturated aqueous sodium hydrogen carbonate and shaken, and the organic layer was separated. The aqueous layer was washed two times with ethyl acetate. The combined organic extracts were dried over sodium sulfate, filtered, and the volatiles were evaporated. The residue was purified by column chromatography using 0–7% methanol in chloroform as the eluent, which provided 0.248 g of compound **4a** as an amorphous white solid in an 89% yield. ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.42–7.38 (m, 5H), 7.31–7.29 (m, 2H), 7.07 (d, 2H, J = 8.24 Hz), 4.53 (br s, 1H), 3.79 (s, 3H), 3.22–3.01 (m, 2H), 2.71–2.65 (m, 4H), 2.52–2.29 (m, 8H), 1.86 (q, 2H), J = 7.33Hz), 1.70–1.67 (m, 6H), 1.48–1.33 (m, 13H), 0.95 (t, 3H, J = 7.33 Hz); ^{13}C NMR ($CDCl_3$) δ 174.22, 174.10, 171.38, 156.21, 139.45, 136.18, 130.76, 129.42, 129.17, 128.78, 119.97, 79.23, 62.95, 60.42, 52.19, 50.00, 40.29, 37.47, 33.66, 33.14, 30.03, 29.17, 28.52, 26.21, 25.53, 9.26.

3.3 Compound **4b**

The same procedure used for compound **4a** was repeated for compound **4b**. The residue was purified by column chromatography using 0–5% methanol in chloroform as the eluent, which provided 0.308 g of compound **4b** as an amorphous white solid in an 81% yield. ^{1}H NMR ($CDCl_3$) δ 7.45–7.29 (m, 7H), 7.07 (d, 2H, J = 8.24 Hz), 4.51 (br s, 1H), 3.78 (s, 3H), 3.22–2.85 (m, 2H), 2.74–2.03 (m, 12H), 1.86 (q, 2H, J = 7.33 Hz), 1.70–1.21 (m, 23H), 0.95 (t, 3H, J = 7.33 Hz); ^{13}C NMR ($CDCl_3$) δ 174.24, 174.06, 171.43, 156.12, 139.42, 136.19, 130.76, 136.05, 130.73, 129.43, 129.17, 128.80, 119.99, 79.15, 62.86, 60.29, 52.21, 49.92, 40.54, 37.70, 33.52, 33.00, 30.04, 29.16, 29.10, 28.96, 28.52, 26.65, 25.59, 9.25.

3.4 Compound **4c**

The same procedure used for compounds **4a and 4b** was used for compound **4c**. The residue was purified by column chromatography using 0–5% methanol in chloroform as the eluent, which provided 0.400 g of compound **4c** as an amorphous white solid in an 83% yield. ^{1}H NMR ($CDCl_3$) δ 7.44–7.23 (m, 4H), 7.31–7.20 (m, 3H), 7.07 (d, 2H, J = 8.24 Hz), 4.51 (br s, 1H), 3.78 (s, 3H), 3.09–2.87 (m, 2H), 2.74–2.14 (m, 12H), 1.86 (q, 2H, J = 7.33 Hz),

1.73–1.21 (m, 27H), 0.95 (t, 3H, J = 7.33 Hz); ^{13}C NMR ($CDCl_3$) δ 174.24, 174.04, 171.43, 156.08, 139.40, 136.21, 135.95, 130.71, 129.43, 129.18, 128.81, 119.99, 79.10, 62.80, 60.19, 52.22, 49.86, 40.69, 37.83, 33.40, 32.88, 30.11, 29.47, 29.38, 29.34, 29.27, 29.25, 29.16, 28.52, 26.80, 25.69, 9.25.

3.5 Carfentanil Biotin Probe **A**

Compound **4a** (0.243 g, 0.322 mmol) was dissolved in 3 mL of DCM. TFA (3 mL) was added, and the reaction was stirred for 16 h at room temperature under a nitrogen atmosphere. The volatiles were evaporated. The residue was taken up in a mixture of chloroform-saturated aqueous sodium hydrogen carbonate. The organic layer was separated and the aqueous solution was extracted two times with chloroform. The combined organic extracts were dried over sodium sulfate and filtered, and the volatiles were evaporated. The residue was dissolved in 3 mL of DMF and 0.17 g of DMAP before NHS-Biotin (0.124 g, 0.393 mmol) was added. The reaction was stirred at room temperature for 48 h under nitrogen. The solvent was evaporated, and the residue was purified by column chromatography using 5–7.5% 95/5 methanol/37% aqueous ammonium hydroxide in chloroform as the eluent, which provided 0.226 g of carfentanil biotin probe **A** as an amorphous solid in an 82% yield. ^{1}H NMR (CD_3OD) δ 7.51–7.36 (m, 7H), 7.09 (d, 2H, J = 8.24 Hz), 4.44 (dd, 1H, J = 4.81 and 7.56 Hz), 4.26 (dd, 1H, J = 4.58 and 7.79 Hz), 3.75 (s, 3H), 3.18–3.13 (m, 3H), 2.89 (dd, 1H, J = 4.81 and 12.60 Hz), 2.77–2.65 (m, 6H), 2.53–2.45 (m, 4H), 2.34–2.25 (m, 4H), 2.16 (d, 2H, J = 7.10 Hz), 1.90 (q, 2H, J = 7.33 Hz), 1.71–1.37 (m, 18H), 0.91 (t, 3H, J = 7.33 Hz); ^{13}C NMR (CD_3OD) δ 175.18, 174.62, 173.66, 173.17, 164.77, 138.96, 136.74, 135.55, 130.35, 129.39, 128.95, 128.61, 120.08, 62.63, 62.04, 60.27, 59.86, 55.69, 51.33, 49.45, 39.70, 38.92, 36.52, 35.48, 32.56, 32.12, 28.92, 28.62, 28.60, 28.45, 28.18, 26.37, 25.61, 25.53, 8.25.

3.6 Carfentanil Biotin Probe **B**

The procedure for carfentanil biotin probe **A** was repeated for carfentanil biotin probe **B**. The residue was purified by column chromatography using 5–7.5% 95/5 methanol/37% aqueous ammonium hydroxide in chloroform as the eluent, which provided 0.287 g of carfentanil biotin probe **B** as an amorphous solid in an 83% yield. ^{1}H NMR (CD_3OD) δ 7.52–7.36 (m, 7H), 7.10 (d, 2H, J = 8.70 Hz), 4.45 (dd, 1H, J = 4.81 and 8.02 Hz), 4.26 (dd, 1H, J = 4.12 and 7.78 Hz), 3.75 (s, 3H), 3.18–3.10 (m, 3H), 2.89 (dd, 1H, J = 4.81 and 12.60 Hz), 2.79–2.64 (m, 5H), 2.55–2.45 (m, 4H), 2.34–2.24 (m, 4H), 2.16 (t, 2H, J = 7.33 Hz), 1.90 (q, 2H, J = 7.33 Hz), 1.74–1.25 (m, 22H), 0.91 (t, 3H, J = 7.33 Hz); ^{13}C NMR (CD_3OD) δ 175.18, 174.61, 173.63, 173.27, 164.77, 138.94, 136.77, 135.45, 130.35, 129.40, 128.97, 128.60, 120.09, 62.58, 62.04, 60.27, 59.81, 55.70, 51.33, 39.71, 38.98, 36.60, 35.48, 32.50, 29.04, 29.02, 28.89, 28.62, 28.59, 28.45, 28.19, 26.57, 25.63, 25.57, 8.24.

3.7 Carfentanil Biotin Probe **C**

The procedure for carfentanil biotin probe **A** was repeated for carfentanil biotin probe **C**. The residue was purified by column chromatography using 5–7.5% 95/5 methanol/37% aqueous ammonium hydroxide in chloroform as the eluent, which provided 0.314 g of carfentanil biotin probe **B** as an amorphous solid in a 57% yield. ^{1}H NMR (CD_3OD) δ 7.50–7.36

(m, 7H), 7.09 (d, 2H, *J* = 8.66 Hz), 4.46 (dd, 1H, *J* = 4.58 and 7.79 Hz), 4.27 (dd, 1H, *J* = 4.58 and 7.79 Hz), 3.75 (s, 3H), 3.19–3.10 (m, 3H), 2.89 (dd, 1H, *J* = 5.04 and 12.83 Hz), 2.77–2.66 (m, 5H), 2.53–2.44 (m, 4H), 2.34–2.25 (m, 4H), 2.16 (t, 2H, *J* = 7.33 Hz), 1.90 (q, 2H, *J* = 7.33 Hz), 1.74–1.25 (m, 26H), 0.91 (t, 3H, *J* = 7.33 Hz); ^{13}C NMR (CD_3OD) δ 175.18, 174.07, 173.65, 173.29, 164.77, 139.95, 136.75, 135.51, 130.35, 129.39, 128.96, 128.60, 120.08, 62.62, 62.04, 60.28, 59.86, 55.69, 51.33, 49.45, 39.71, 39.01, 36.61, 35.49, 32.55, 32.11, 29.25, 29.18, 29.08, 29.02, 28.97, 28.62, 28.60, 28.45, 28.18, 26.63, 25.63, 25.61, 8.24.

3.8 Compound **6**

Compound **5** (1.00 g, 3.10 mmol) was dissolved in 25 mL of nitrogen sparged methanol. Palladium (10%) on carbon (0.25 g) was added followed by ammonium formate (0.98 g, 15.50 mmol). The reaction was stirred under a nitrogen atmosphere in a 65 ºC oil bath for 4 h. The mixture was allowed to cool and was filtered through a celite pad. The celite pad was washed with methanol. The filtrate was concentrated and taken up in chloroform-saturated aqueous sodium hydrogen carbonate. The mixture was shaken and the organic layer was separated. The aqueous layer was washed two times with chloroform. The combined organic extracts were dried over sodium sulfate, filtered, and the volatiles were evaporated. The residue was taken up in 40 mL of 4-methyl-2-pentanone under a nitrogen atmosphere. 4-nitrophenylethyl bromide (0.79 g, 3.41mmol) and sodium carbonate (1.64 g, 15.50 mmol) were added. The mixture was heated at reflux for 16 h with an oil bath, allowed to cool to room temperature, and was concentrated. The residue was taken up in chloroform-saturated aqueous sodium hydrogen carbonate and shaken, and the organic layer was separated. The aqueous layer was washed two times with chloroform. The combined organic extracts were dried over sodium sulfate, filtered, and the volatiles were evaporated. The residue was purified by column chromatography using 0–3% methanol in chloroform as the eluent, which provided 0.65 g of compound **6** as an amorphous white solid in a 55% yield. ^{1}H NMR ($CDCl_3$) δ 8.10 (d, 2H, *J* = 8.70 Hz), 7.42–7.26 (m, 5H), 7.31–7.29 (m, 2H), 7.07 (dd, 2H, *J* = 7.55 and 1.60 Hz), 4.67 (tt, 1H, *J* = 12.14 and 3.89 Hz), 2.99–2.91 (m, 2H), 2.84–2.78 (m, 2H), 2.57–2.52 (m, 2H), 2.16 (t, 2H, *J* = 11.91), 1.91 (q, 2H, *J* = 7.33Hz), 1.84–1.76 (m, 2H), 1.39 (qd, 2H, *J* = 12.36 and 3.66 Hz), 1.00 (t, 3H, *J* = 7.33 Hz); ^{13}C NMR ($CDCl_3$) δ 173.68, 148.34, 146.57, 138.89, 130.47, 129.53, 129.42, 128.41, 123.72, 59.56, 53.16, 52.15, 33.76, 30.62, 28.61, 9.70.

3.9 Compound **8**

Compound **6** (0.25 g, 0.655 mmol) was dissolved in 15 mL of ethanol in a Parr hydrogenation bottle. Palladium (10%) on carbon (0.025 g) was added, and the reaction was placed on a Parr hydrogenator at 40 psi for 16 h. The mixture was filtered through a celite pad, and the celite pad was washed with ethanol. The filtrate was concentrated, and compound **7** was used in the next step without purification. The following reagents were added in the order they are listed to compound **8** in 4 mL of DMF at room temperature under a nitrogen atmosphere:

- Boc-7-aminoheptanoic acid (0.177 g, 0.721 mmol);
- HOBt (0.097 g, 0.721 mmol); and
- EDC-HCl (0.138 g, 0.721 mmol).

The reaction mixture was stirred at room temperature for 16 h. The volatiles were removed under reduced pressure. The residue was taken up in ethyl actetate saturated aqueous sodium hydrogen carbonate and shaken, and the organic layer was separated. The aqueous layer was washed two times with ethyl acetate. The combined organic extracts were dried over sodium sulfate, filtered, and the volatiles were evaporated. The residue was purified by column chromatography using 0–7% methanol in DCM as the eluent, which provided 0.336 g of compound **8** as an amorphous white solid in an 89% yield. ^{1}H NMR ($CDCl_3$) δ 7.53 (br s, 1H), 7.43–7.41 (m, 5H), 7.08–7.05 (m, 4H), 4.66 (tt, 1H, *J* = 12.14 and 3.89 Hz), 4.54 (br s, 1H), 3.14–2.93 (m, 4H), 2.69–2.64 (m, 2H), 2.51–2.46 (m, 2H), 2.31 (br t, 2H, *J* = 7.33 Hz), 2.12 (br t, 2H, *J* = 11.40 Hz), 1.95–1.66 (m, 6H), 1.47–1.29 (m, 13H), 0.99 (t, 3H, *J* = 7.33 Hz); ^{13}C NMR ($CDCl_3$) δ 173.69, 171.42, 156.21, 138.86, 136.27, 136.01, 130.48, 129.40, 129.16, 128.38, 120.00, 79.24, 60.49, 53.11, 52.18, 40.30, 37.46, 33.20, 30.55, 30.01, 28.61, 28.52, 26.23, 25.53, 9.71.

3.10 Fentanyl Biotin Probe

Compound **8** (0.313 g, 0.540 mmol) was dissolved in 4 mL of DCM. TFA (4 mL) was added, and the reaction was stirred for 16 h at room temperature under a nitrogen atmosphere. The volatiles were evaporated. The residue was taken up in a mixture of chloroform-saturated aqueous sodium hydrogen carbonate. The organic layer was separated and the aqueous solution was extracted two times with chloroform. The combined organic extracts were dried over sodium sulfate, filtered, and the volatiles were evaporated. The residue was dissolved in 4 mL of DMF and 0.03 g of DMAP before NHS-Biotin (0.176 g, 0.514 mmol) was added. The reaction was stirred at room temperature for 48 h under nitrogen. The solvent was evaporated, and the residue was purified twice by column chromatography using 0–11% 95/5 methanol/37% aqueous ammonium hydroxide in DCM as the eluent. The residue was dissolved in the least amount of methanol and precipitated with ethyl acetate. The mixture was centrifuged, and the liquid was decanted. The solids were suspended in ethyl acetated and filtered, which provided 0.222 g of fentanyl biotin probe as an amorphous solid in a 61% yield. High-resolution mass spectrometry electrospray ionization time-of-flight, mass-to-charge ratio: $[M+H]^+$ calculated for $C_{41}H_{59}N_8O_8S$ 728.4217, found 728.4253; ^{1}H NMR (CD_3OD) δ 7.49–7.40 (m, 5H), 7.20 (d, 2H, *J* = 6.87 Hz), 7.10 (d, 2H, *J* = 8.70 Hz), 4.57 (tt, 1H, *J* = 12.14 and 3.89 Hz), 4.41 (dd, 1H, *J* = 5.04 and 7.79 Hz), 4.26 (dd, 1H, *J* = 4.58 and 7.79 Hz), 3.19–3.10 (m, 3H), 3.07–2.99 (m, 2H), 2.89 (dd, 1H, *J* = 4.58 and 12.37 Hz), 2.71–2.64 (m, 3H), 2.53–2.49 (m, 2H), 2.37 (t, 2H, *J* = 7.56 Hz), 2.22–2.12 (m, 4H), 1.95 (q, 2H, *J* = 7.56Hz), 1.84–1.80 (m, 18H), 1.75–1.33 (m, 0.97 (t, 3H, *J* = 7.33 Hz); ^{13}C NMR (CD_3OD) δ 174.63, 173.55, 173.17, 164.77, 138.50, 136.74, 135.53, 130.13, 129.30, 128.61, 128.54, 120.07, 62.04, 60.27, 59.91, 55.69, 52.63, 52.42, 39.69, 38.92, 36.52, 35.48, 32.12, 29.75, 28.92, 28.62, 28.45, 28.18, 28.07, 26.37, 25.61, 25.52, 8.67.

4. CONCLUSION

Three carfentanil biotin probes and a fentanyl biotin probe were synthesized for ABPP.

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ABBREVIATIONS AND ACRONYMS

ABPP	activity-based protein profiling
Boc	*tert*-butyloxycarbonyl
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
EDC-HCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HOBt	N-hydroxybenzotriazole
MOR	μ-opioid receptor
NHS-Biotin	Biotinyl N-hydroxysuccinimide ester
TFA	trifluoroacetyl
VX	*O*-ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate

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