Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supporting Information

Design, Synthesis, and Biological Evaluation of C_6-Difluoromethylenated Epoxymorphinan Mu Opioid Receptor Antagonists

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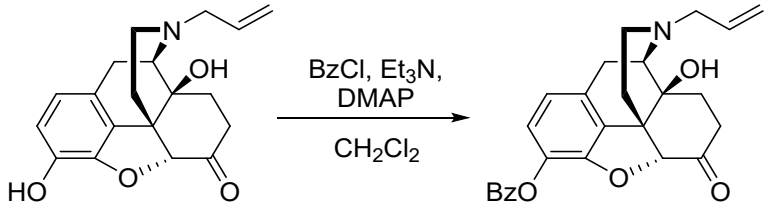
Synthesis and characterization of (6-difluoromethylene)naloxone (5)



2 **7**

3-Benzoyl-naloxone (7). To a 0 °C solution of naloxone (**2**, 0.300 g, 0.916 mmol) in 7 mL of anhydrous CH_2Cl_2 was added triethylamine (0.383 mL, 2.75 mmol) followed by a solution of benzoyl chloride (0.127 mL, 1.10 mmol) in 1 mL of CH_2Cl_2 slowly dropwise via syringe. The reaction was then warmed to ambient temperature and maintained for 3.5 h. TLC analysis (2% MeOH/CH_2Cl_2) showed the consumption of starting material. The reaction mixture was diluted with CH_2Cl_2 (60 mL) and washed with saturated aqueous $NaHCO_3$ and brine. The organics were dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on SiO_2 (42 g, 2% MeOH/CH_2Cl_2) afforded 377 mg (95%) of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.14-8.09 (m, 2H), 7.80-7.74 (m, 1H), 7.66-7.60 (m, 2H), 7.01 (d, J = 8.2 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 5.93-5.82 (m, 1H), 5.26 (dd, J = 1.7, 17.2 Hz, 1H), 5.19-5.14 (m, 1H), 5.09 (d, J = 1.2 Hz, 1H), 4.92 (s, 1H), 3.22-3.11 (m, 3H), 3.01 (d, J = 5.6 Hz, 1H), 2.92 (ddd, J = 5.2, 14.4, 14.4 Hz, 1H), 2.62 (dd, J = 5.8, 19.0 Hz, 1H), 2.57 (dd, J = 4.7, 12.0 Hz, 1H), 2.40 (ddd, J = 5.2, 12.6, 12.6 Hz, 1H), 2.11 (ddd, J = 3.0, 3.0, 14.3 Hz, 1H), 2.00 (ddd, J = 3.6, 12.0, 12.0 Hz, 1H), 1.79 (ddd, J = 3.0, 4.9, 13.6 Hz, 1H), 1.47 (ddd, J = 2.7, 14.2, 14.2 Hz, 1H), 1.34 (dd, J = 2.7, 13.0 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 207.6, 163.5, 147.4, 136.0, 134.2, 131.7, 131.4, 130.9, 129.8 (2C), 129.1 (2C), 128.4, 122.6, 119.3, 117.6, 90.3, 69.7, 61.4, 57.0, 50.3, 42.9, 35.7, 31.2, 29.9, 22.8. MS (ESI): m/z calcd for $C_{26}H_{25}NO_5$ 431.17, found 414.19 $[M+H-H_2O]^+$, 432.22 $[M+H]^+$, 454.23 $[M+Na]^+$.

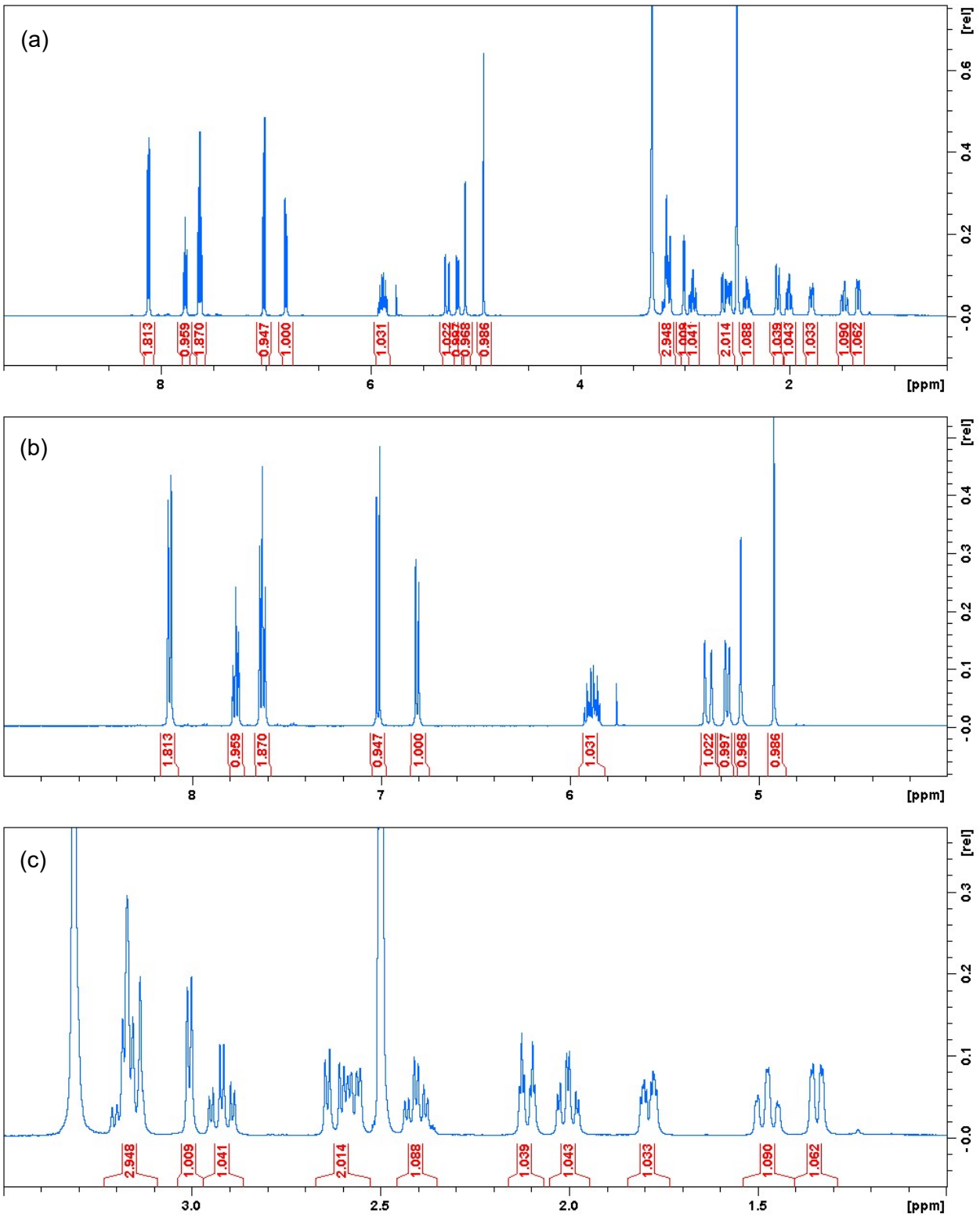


Figure S1. (a) ^{1}H NMR spectrum of 3-benzoyl-naloxone **7** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

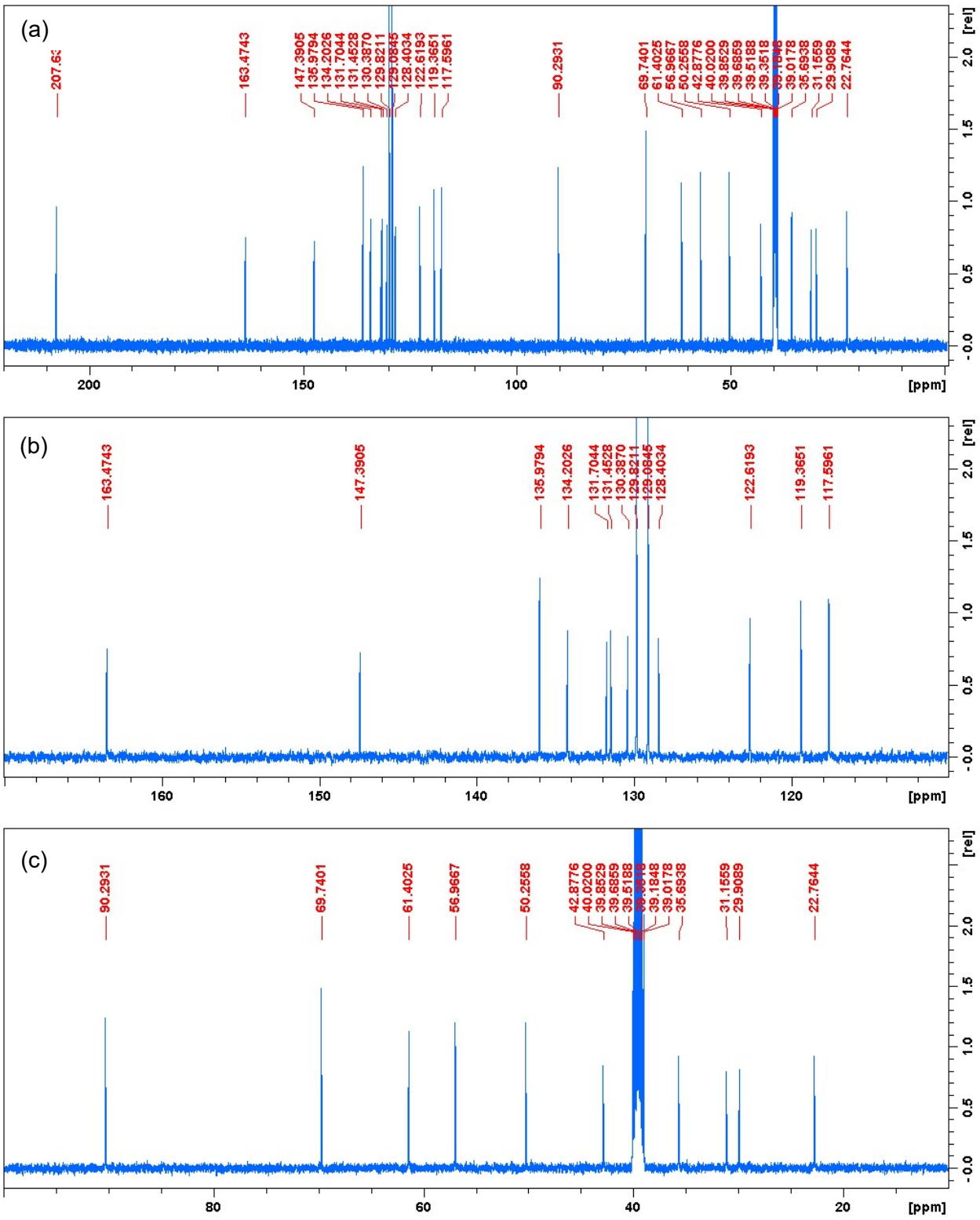
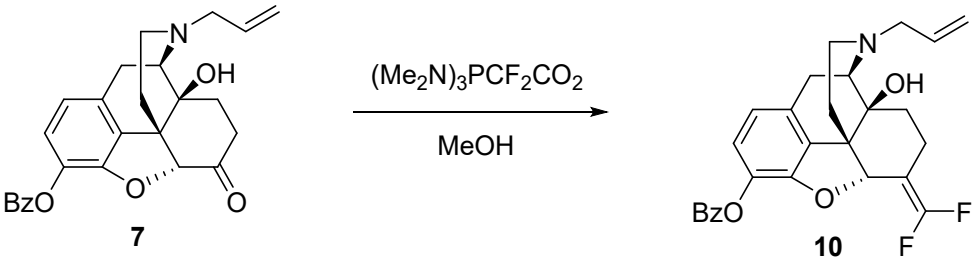


Figure S2. (a) ^{13}C NMR spectrum of 3-benzoyl-naloxone **7** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region



3-Benzoyl-(6-difluoromethylene)naloxone (10). An oven dried microwave vial was charged with ketone **7** (0.200 g, 0.464 mmol) and phosphonium salt **9** (0.358 g, 1.39 mmol). The contents of the vial were purged with N_2 for 20 min, then anhydrous DMF (2.5 mL) was added under N_2. The slightly turbid reaction mixture was heated at 120 °C for 70 min. LC-MS analysis showed the starting material was mostly consumed. The reaction was allowed to cool to ambient temperature then poured into 25 mL of H_2O. This mixture was extracted with EtOAc (3 x 20 mL). The combined organics were washed with H_2O (2 x 20 mL) followed by brine then dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on SiO_2 (40 g, 0.5% MeOH/CH_2Cl_2) afforded 25 mg (12%) of the desired product as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.12-8.07 (m, 2H), 7.78-7.72 (m, 1H), 7.64-7.57 (m, 2H), 7.01 (d, J = 8.2 Hz, 1H), 6.78 (d, J = 8.2 Hz, 1H), 5.90-5.80 (m, 1H), 5.25 (J = 17.8 Hz, 1H), 5.22 (s, 1H), 5.15 (d, J = 9.9 Hz, 1H), 4.75 (s, 1H), 3.22-3.08 (m, 3H), 2.91 (d, J = 5.6 Hz, 1H), 2.68-2.60 (m, 1H), 2.60-2.52 (m, 1H), 2.35-2.26 (m, 1H), 2.23 (ddd, J = 4.8, 12.4, 12.4 Hz, 1H), 2.16-2.07 (m, 1H), 2.02-1.92 (m, 1H), 1.55-1.45 (m, 1H), 1.42 (d, J = 12.4 Hz, 1H), 1.23 (ddd, J = 7.0, 10.8, 14.1 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.7, 152.8 (dd, J = 286, 293 Hz), 147.3, 136.0, 134.1, 132.2, 131.7, 131.6, 129.8 (2C), 129.1 (2C), 128.5, 122.6, 119.1, 117.5, 86.5 (dd, J = 11.7, 19.0 Hz), 85.0 (d, J = 4.7 Hz), 69.4, 61.7, 57.0, 46.6, 43.1, 30.9, 28.4, 22.9, 15.6. ^{19}F NMR (470 MHz, DMSO-d_6): δ -85.9 (d, J = 40 Hz), -89.0 (d, J = 40 Hz). MS (ESI): m/z calcd for $C_{27}H_{25}F_2NO_4$ 465.18, found 448.26 $[M+H-H_2O]^+$, 466.26 $[M+H]^+$, 488.27 $[M+Na]^+$.

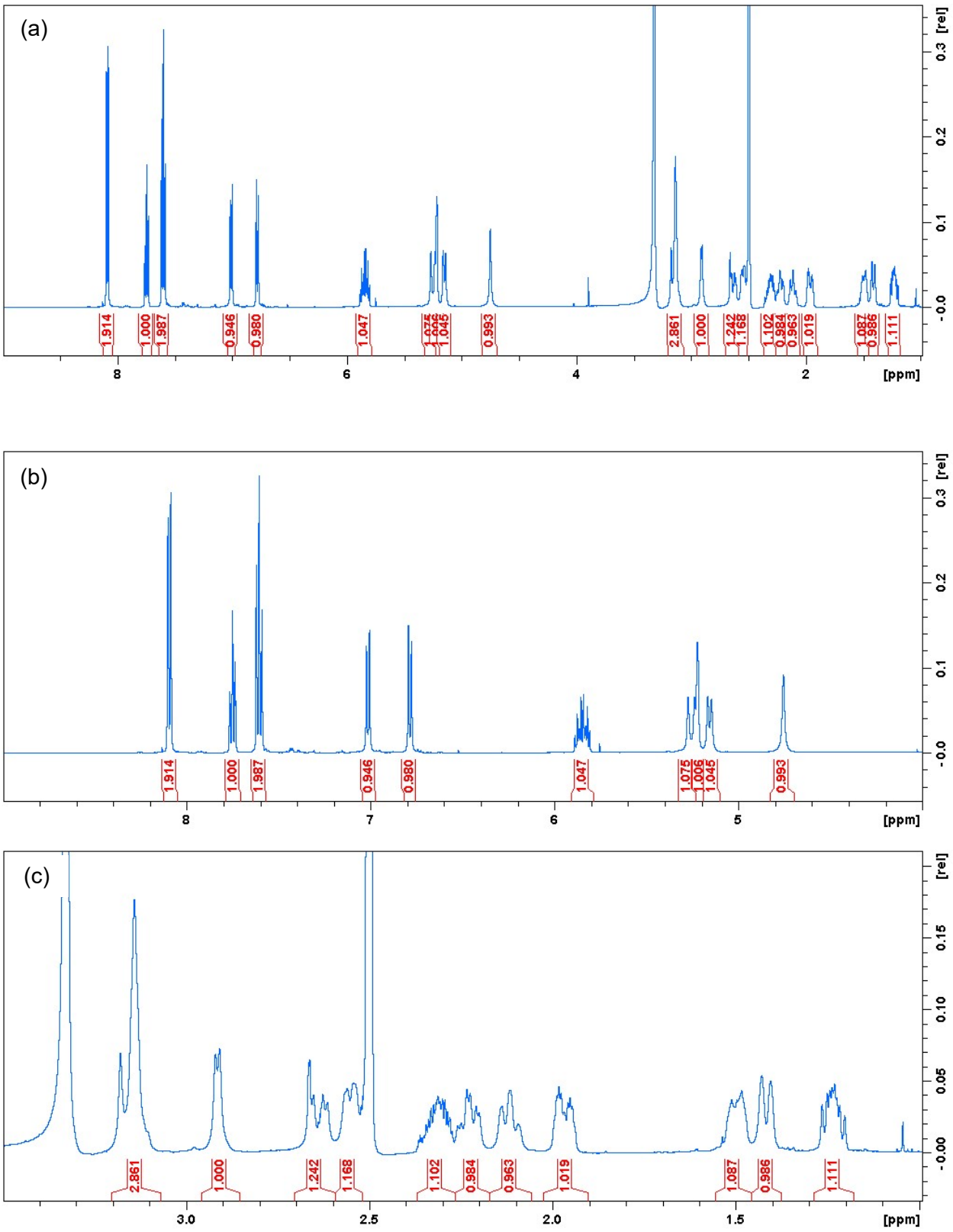


Figure S3. (a) ^{1}H NMR spectrum of 3-benzoyl-(6-difluoromethylene)naloxone **(10)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

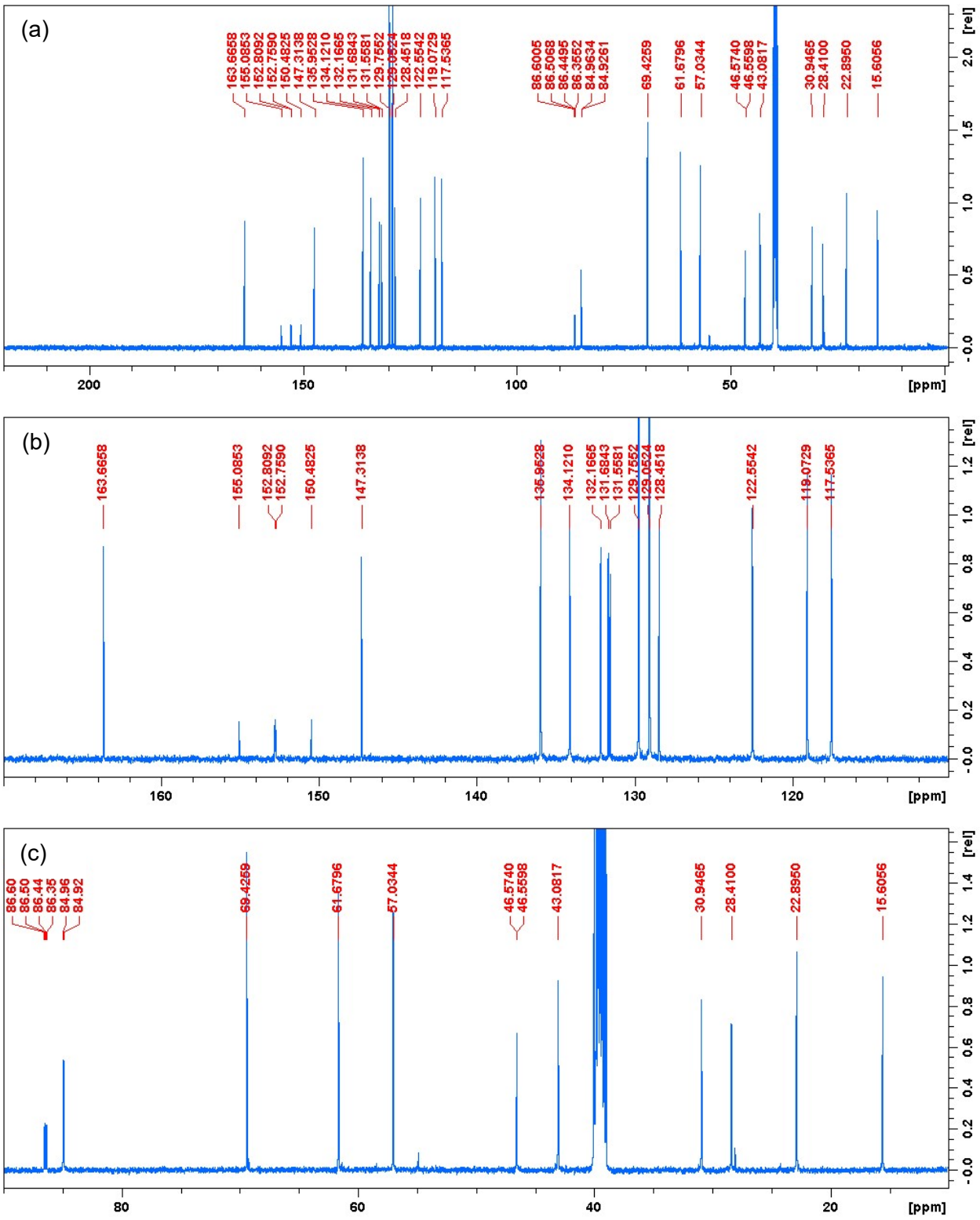


Figure S4. (a) ^{13}C NMR spectrum of 3-benzoyl-(6-difluoromethylene)naloxone **(10)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

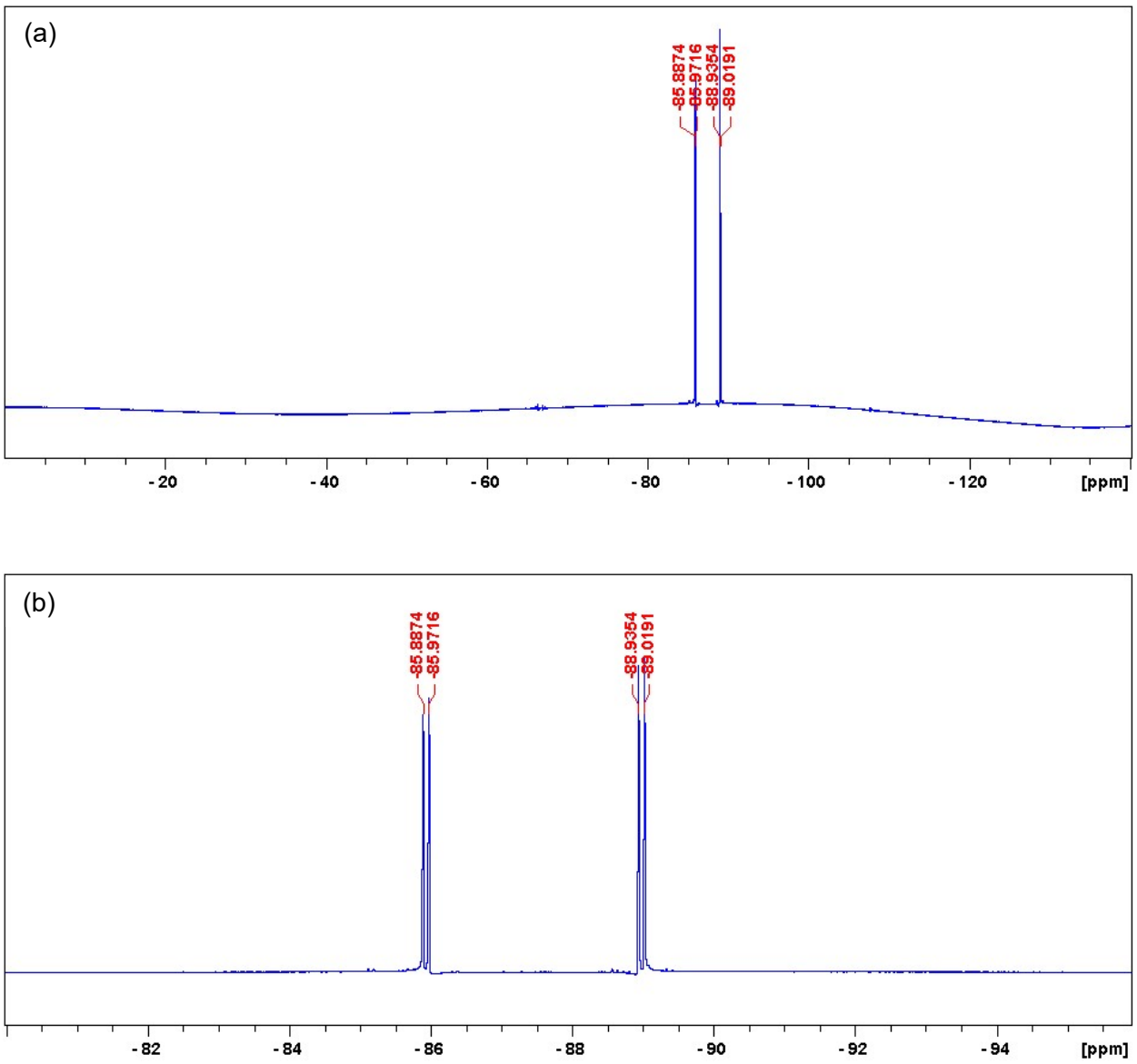
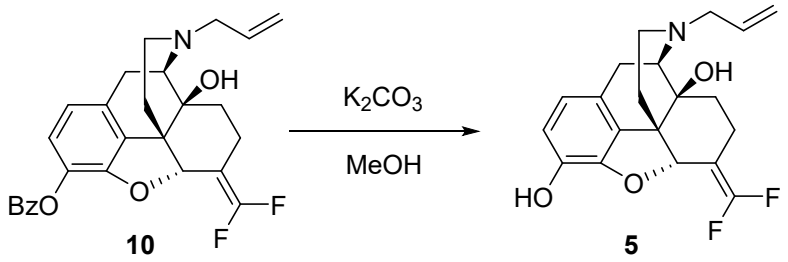


Figure S5. (a) ^{19}F NMR spectrum of 3-benzoyl-(6-difluoromethylene)naloxone **(10)** in d_6-DMSO (b) Expansion for clarity.



(6-Difluoromethylene)naloxone (5). To a solution of benzoate **10 (**45 mg, 0.097 mmol) in 2 mL of MeOH was added K_2CO_3 (20 mg, 0.145 mmol) in one portion. The mixture was maintained at ambient temperature for 3 h. LC-MS analysis showed the reaction was complete. The reaction was diluted with 30 mL of CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (15 mL). The aqueous layer was further extracted with CH_2Cl_2 (10 mL). The combined organics were washed with brine, dried over anhydrous MgSO4, filtered, and concentrated. Purification by flash chromatography on SiO_2 (12 g, 0.5% MeOH/CH_2Cl_2) afforded 25 mg (71%) of the desired product as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.03 (s, 1 H), 6.58 (d, J = 8.0 Hz, 1H), 6.50 (d, J = 8.0 Hz, 1H), 5.88-5.77 (m, 1H), 5.23 (dd, J = 1.7, 17.2 Hz, 1H), 5.13 (dd, J = 1.7, 10.2 Hz, 1H), 5.07 (d, J = 2.5 Hz, 1H), 4.67 (s, 1H), 3.15-3.05 (m, 2H), 3.02 (d, J = 18 Hz, 1H), 2.84 (d, J = 6.1 Hz, 1H), 2.54-2.45 (m, 2H), 2.35-2.25 (m, 1H), 2.16 (ddd, J = 4.9, 12.5, 12.5 Hz, 1H), 2.09 (ddd, J = 3.3, 12.4, 12.4 Hz, 1H), 1.98-1.89 (m, 1H), 1.47-1.39 (m, 1H), 1.38-1.32 (m, 1H), 1.20 (ddd, J = 6.8, 10.8, 13.7, Hz 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 153.4 (dd, 287, 293 Hz), 143.8, 139.6, 136.5, 130.9, 124.5, 119.1, 117.9, 117.5, 87.4 (dd, J = 12.2, 17.7 Hz), 83.7 (d, J = 5.0 Hz), 69.9, 62.4, 57.5, 46.8, 43.7, 31.8, 28.7, 22.9, 16.2. ^{19}F NMR (470 MHz, DMSO-d_6): δ -86.3 (d, J = 40 Hz), -89.3 (d, J = 40 Hz). MS (ESI): m/z calcd for $C_{20}H_{21}F_2NO_3$ 361.15, found 344.00 $[M+H-H_2O]^+$, 362.02 $[M+H]^+$.

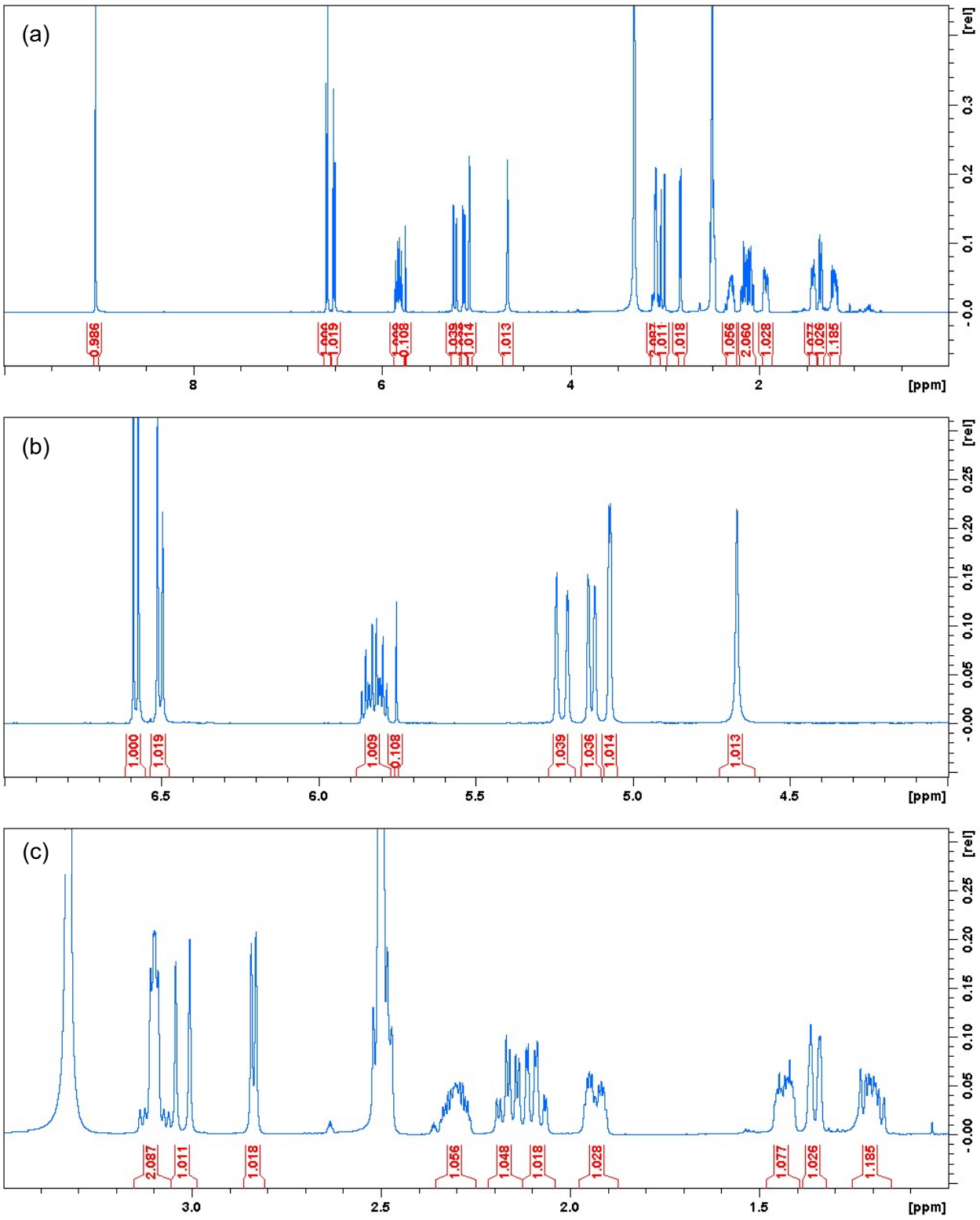


Figure S6. (a) ^{1}H NMR spectrum of (6-difluoromethylene)naloxone **(5)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

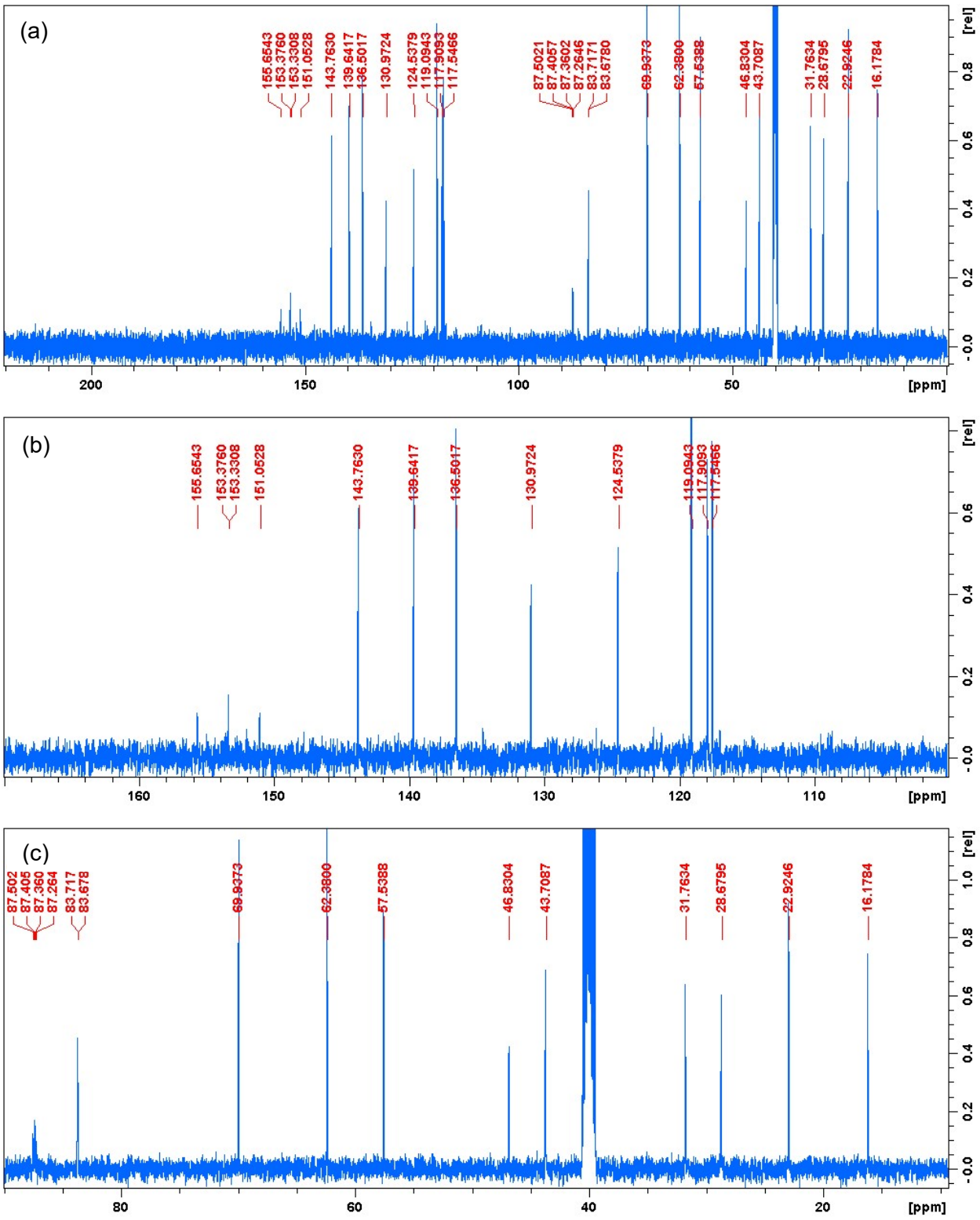


Figure S7. (a) ^{13}C NMR spectrum of (6-difluoromethylene)naloxone **(5)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

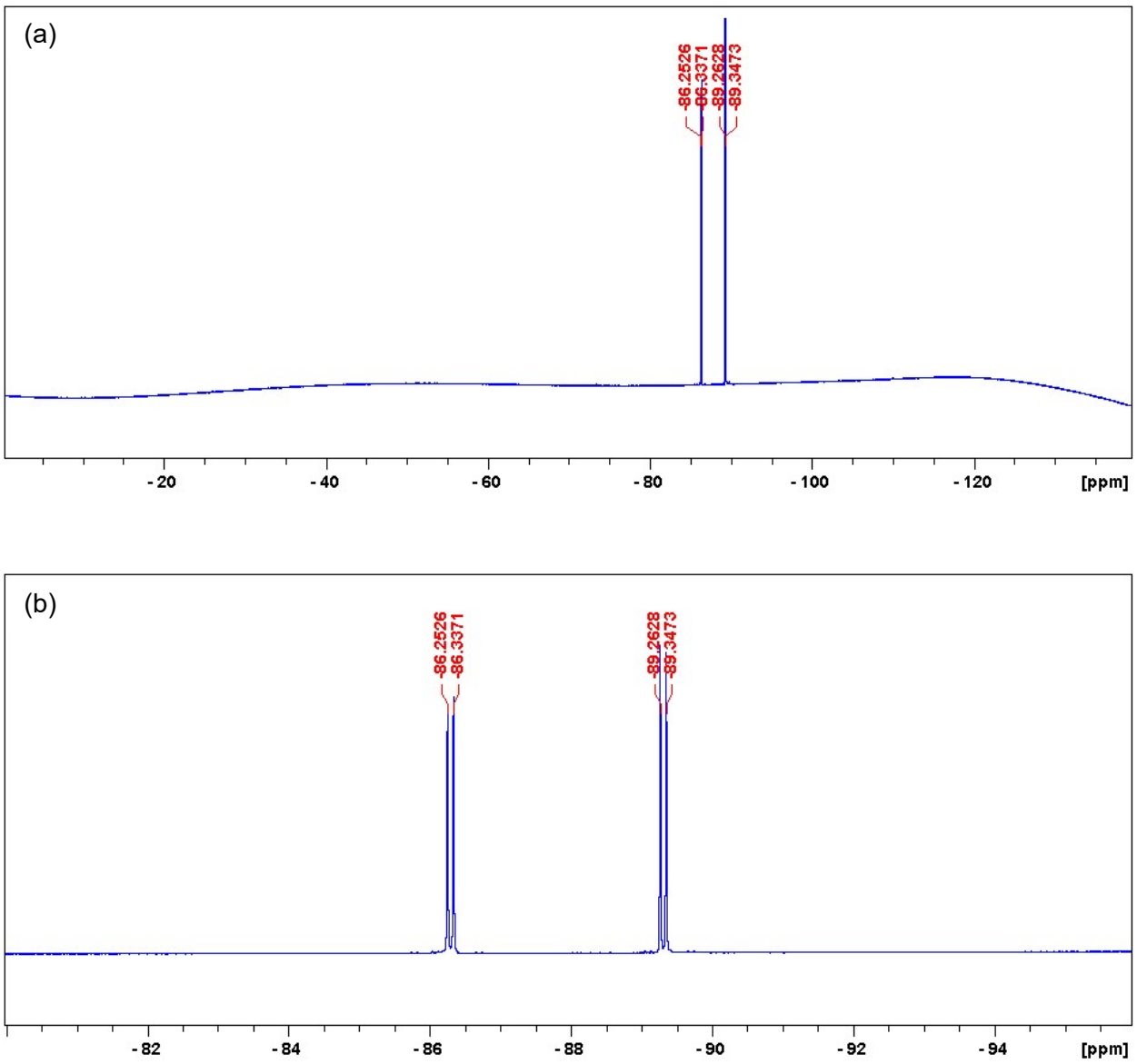


Figure S8. (a) ^{19}F NMR spectrum of (6-difluoromethylene)naloxone **(5)** in d_6-DMSO (b) Expansion for clarity.

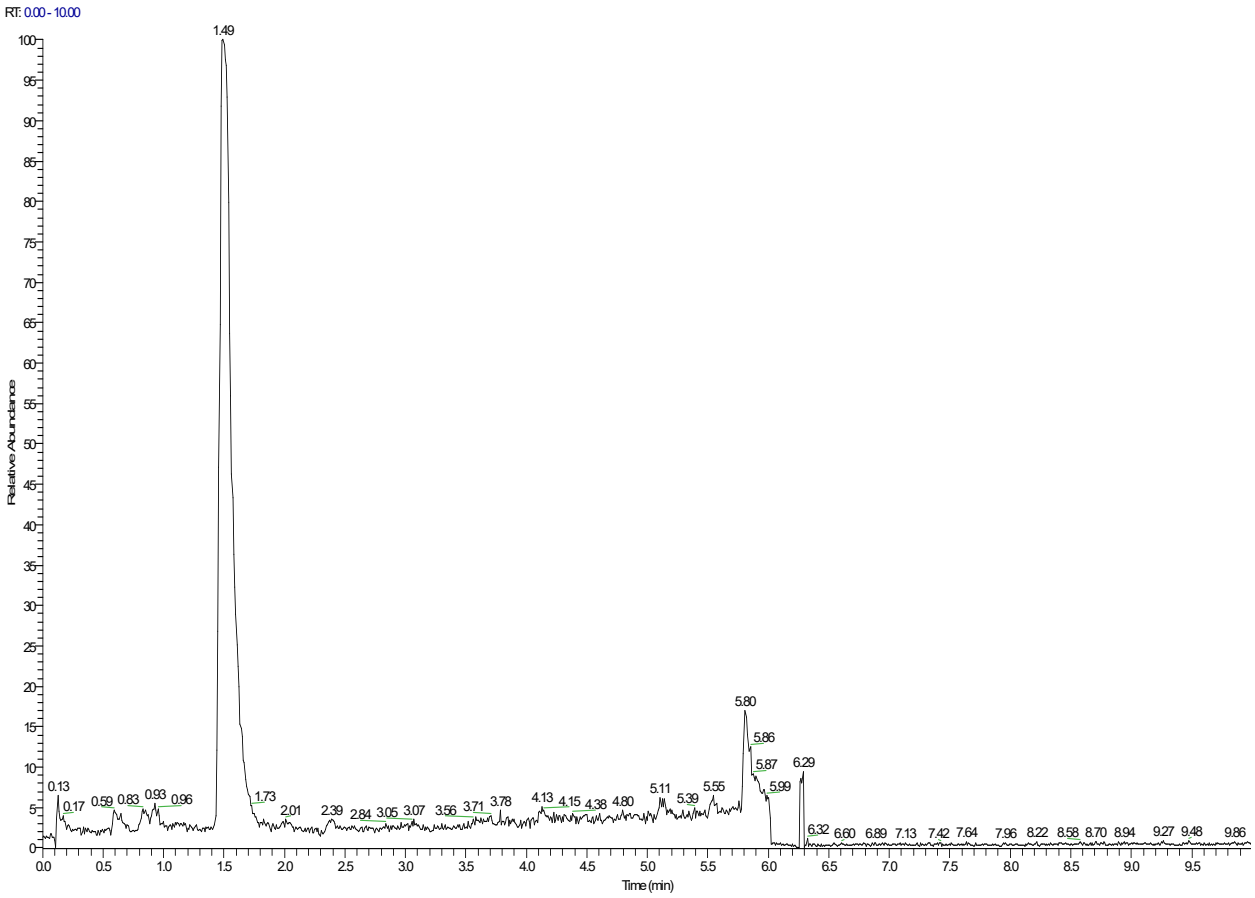


Figure S9. LC-MS chromatogram of (6-difluoromethylene)naloxone **(5)**.

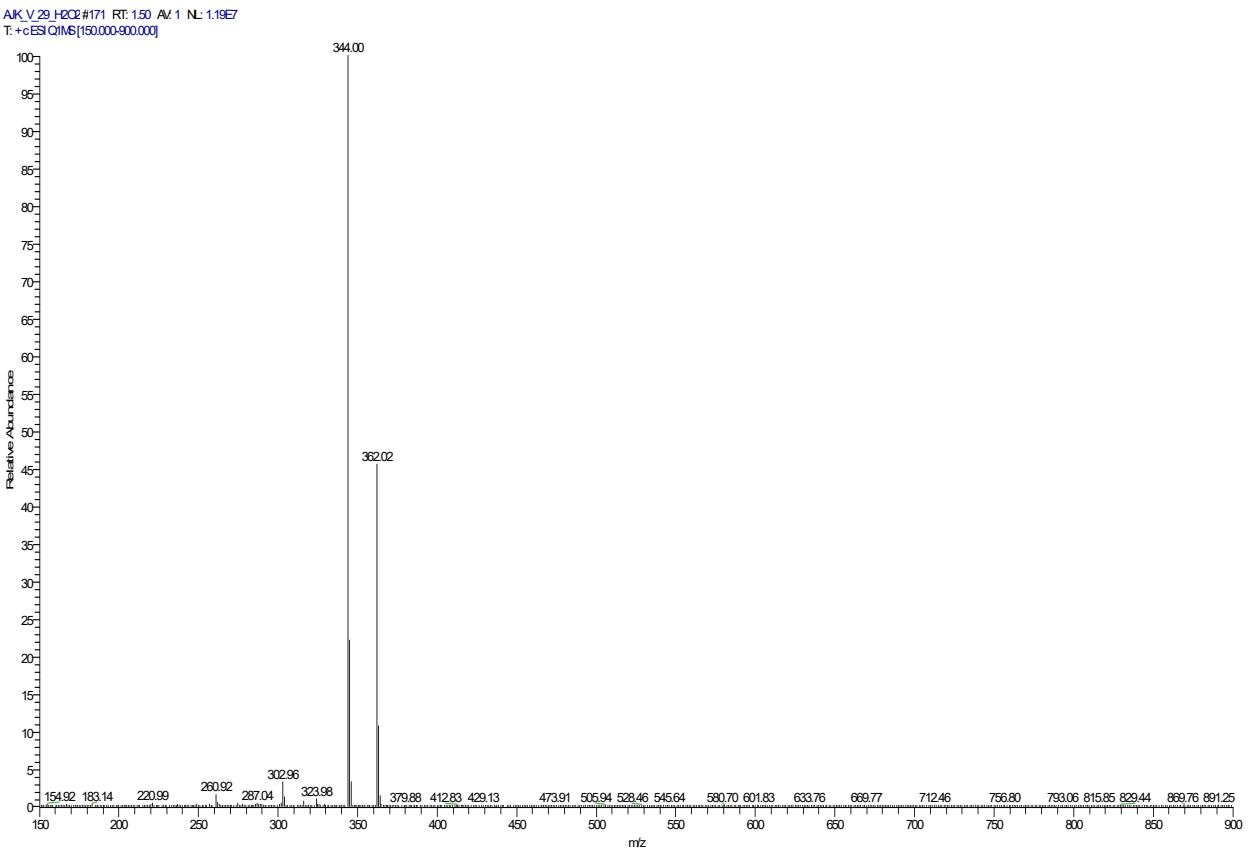
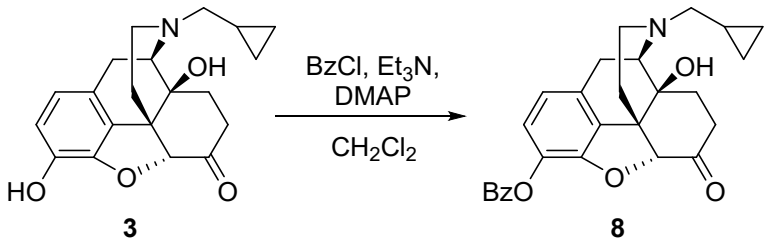


Figure S10. LC-MS mass spectrum of (6-difluoromethylene)naloxone **(5)**.

Synthesis and characterization of (6-difluoromethylene)naltrexone (6)



3-Benzoyl-naltrexone (8). To a 0 °C solution of naltrexone (**3**, 0.350 g, 1.03 mmol) in 8 mL of anhydrous CH_2Cl_2 was added triethylamine (0.430 mL, 3.08 mmol) followed by a solution of benzoyl chloride (0.143 mL, 1.23 mmol) in 1 mL of CH_2Cl_2 slowly dropwise via syringe. The reaction was warmed to ambient temperature and maintained for 3 h. TLC analysis (2% MeOH/CH_2Cl_2) showed the consumption of starting material. The reaction was diluted with CH_2Cl_2 (70 mL) and washed with saturated aqueous $NaHCO_3$ and brine. The organics were dried over anhydrous $MgSO_4$, filtered and concentrated. Purification by flash chromatography on SiO_2 (42 g, 2% MeOH/CH_2Cl_2) afforded 421 mg (92%) of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.14-8.09 (m, 2H), 7.79-7.74 (m, 1H), 7.66-7.59 (m, 2H), 7.00 (d, J = 8.2 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H), 5.17 (br s, 1H), 4.93 (s, 1H), 3.20 (d, J = 5.7 Hz, 1H), 3.11 (d, J = 19.0 Hz, 1H), 2.93 (ddd, J = 5.0, 14.3, 14.3 Hz, 1H), 2.70 (dd, J = 4.9, 12.0 Hz, 1H), 2.63 (dd, J = 6.0, 19.0 Hz, 1H), 2.46-2.34 (m, 3H), 2.12 (ddd, J = 3.0, 3.0, 14.2 Hz, 1H), 2.00 (ddd, J = 3.8, 12.0, 12.0 Hz, 1H), 1.81 (ddd, J = 3.0, 4.9, 13.5 Hz, 1H), 1.49 (ddd, J = 3.1, 14.2, 14.2 Hz, 1H), 1.35 (dd, J = 2.7, 12.8 Hz, 1H), 0.95-0.85 (m, 1H), 0.56-0.45 (m, 2H), 0.20-0.10 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 207.6, 163.5, 147.4, 134.2, 131.7, 131.5, 130.4, 129.8 (2C), 129.1 (2C), 128.4, 122.6, 119.4, 90.3, 69.6, 61.2, 58.4, 50.3, 43.1, 35.7, 31.2, 30.2, 22.6, 9.2, 3.7, 3.6. MS (ESI): m/z calcd for $C_{27}H_{27}NO_5$ 445.19, found 428.31 $[M+H-H_2O]^+$, 446.33 $[M+H]^+$, 468.31 $[M+Na]^+$.

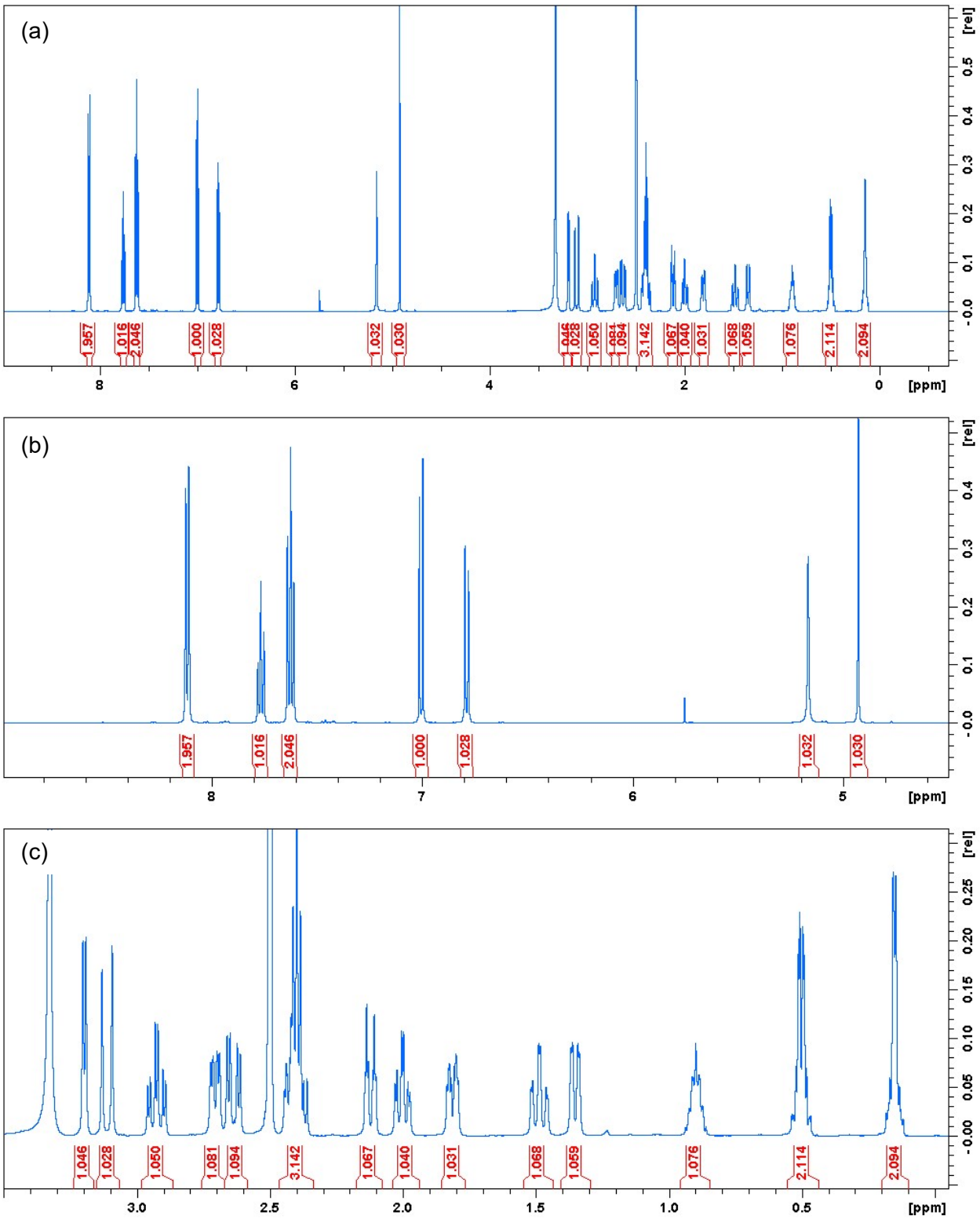


Figure S11. (a) ^{1}H NMR spectrum of 3-benzoyl-naltrexone **8** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

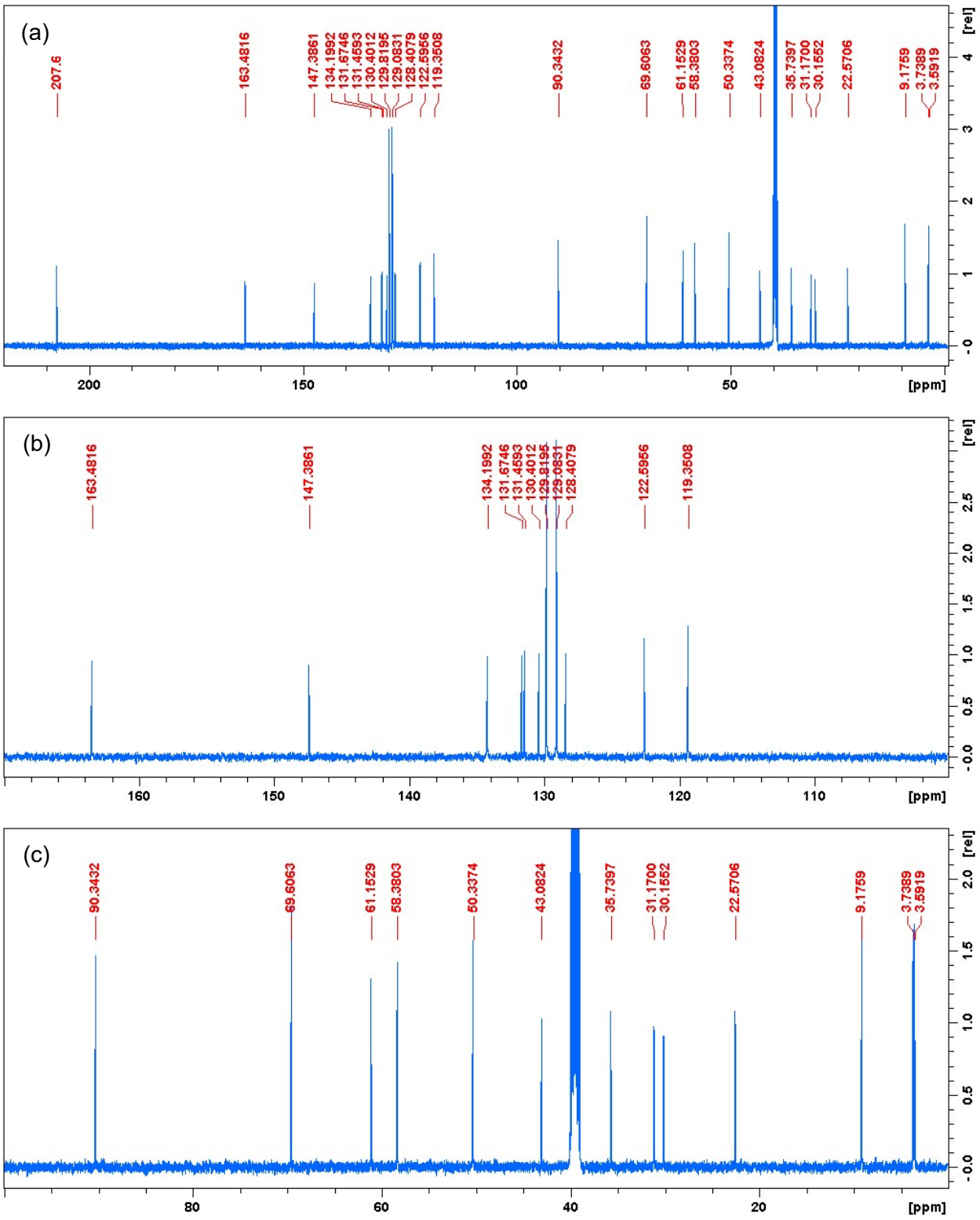
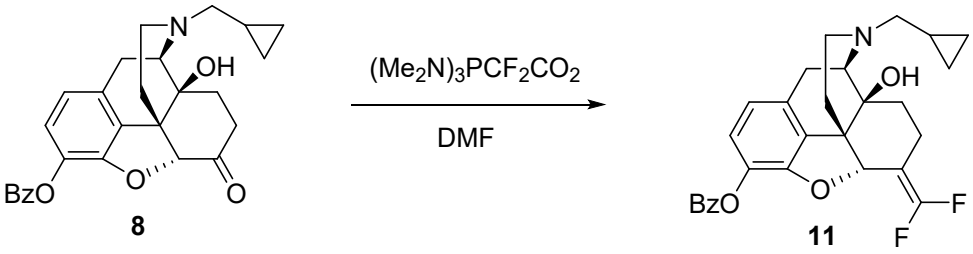


Figure S12. (a) ^{13}C NMR spectrum of 3-benzoyl-naltrexone **8** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region



3-Benzoyl-(6-difluoromethylene)naltrexone (11). An oven dried microwave vial was charged with ketone **8** (0.305 g, 0.685 mmol) and phosphonium salt **9** (0.528 g, 2.05 mmol). The contents of the vial were purged with N_2 for 20 min, then anhydrous DMF (3.6 mL) was added under N_2. The slightly turbid reaction mixture was heated at 120 C for 75 min. LC-MS analysis showed the starting material was mostly consumed. The reaction was allowed to cool to ambient temperature then poured into 35 mL of H_2O. The mixture was extracted with EtOAc (3 x 30 mL). The combined organics were washed with H_2O (2 x 30 mL) followed by brine. Dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on SiO_2 (42 g, 0.6% MeOH/CH2Cl2) afforded 42 mg (13%) of the desired product as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.13-8.07 (m, 2H), 7.79-7.72 (m, 1H), 7.65-7.58 (m, 2H), 7.01 (d, J = 8.2 Hz, 1H), 6.77 (d, J = 8.2 Hz, 1H), 5.25-5.21 (m, 1H), 4.83 (s, 1H), 3.16-3.08 (m, 2H), 2.72-2.62 (m, 2H), 2.38 (d, J = 6.5 Hz, 2H), 2.36-2.28 (m, 1H), 2.24 (ddd, J = 5.2, 12.7, 12.7 Hz, 1H), 2.12 (ddd, J = 3.4, 12.0, 12.0 Hz, 1H), 2.03-1.94 (m, 1H), 1.57-1.48 (m, 1H), 1.43 (dd, J = 2.2, 12.5 Hz, 1H), 1.25 (ddd, J = 6.5, 10.6, 13.7 Hz, 1H), 0.93-0.83 (m, 1H), 0.56-0.44 (m, 2H), 0.20-0.09 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.7, 152.8, (dd, J = 286, 293 Hz), 147.3, 134.1, 132.2, 131.7, 131.6, 129.8 (2C), 129.1 (2C), 128.5, 122.5, 119.1, 86.5 (dd, J = 11.7, 19.0 Hz), 85.0 (d, 4.7 Hz), 69.3, 61.4, 58.5, 46.6, 43.3, 31.2, 28.4, 22.7, 15.7, 9.1, 3.8, 3.5. ^{19}F NMR (470 MHz, DMSO-d_6): δ -85.9 (d, J = 39.0 Hz), -88.9 (d, J = 39.0 Hz). MS (ESI): m/z calcd for $C_{28}H_{27}F_2NO_4$ 479.19, found 462.25 $[M+H-H_2O]^+$, 480.25 $[M+H]^+$, 502.28 $[M+Na]^+$.

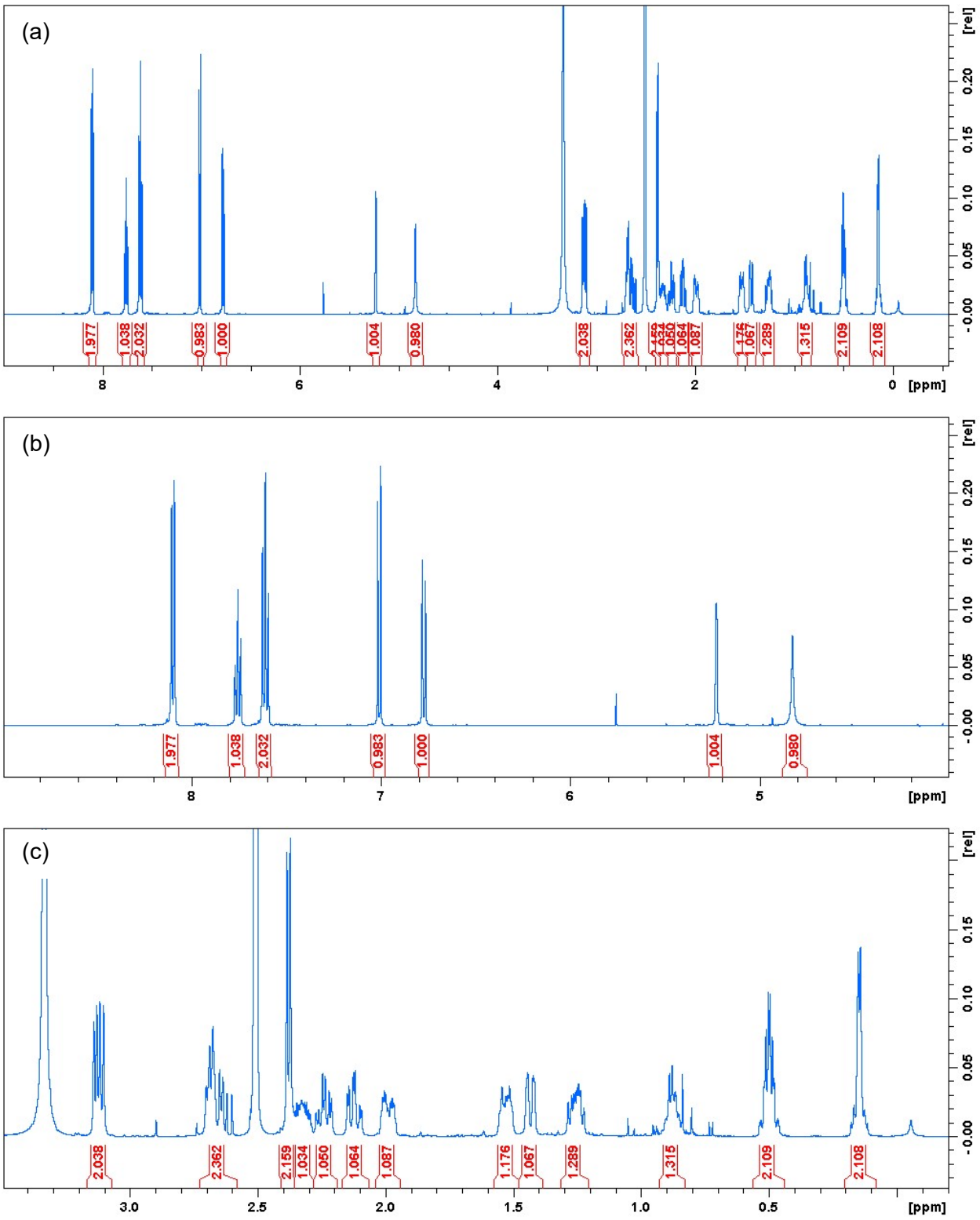


Figure S13. (a) ^{1}H NMR spectrum of 3-benzoyl-(6-difluoromethylene)naltrexone **(11)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

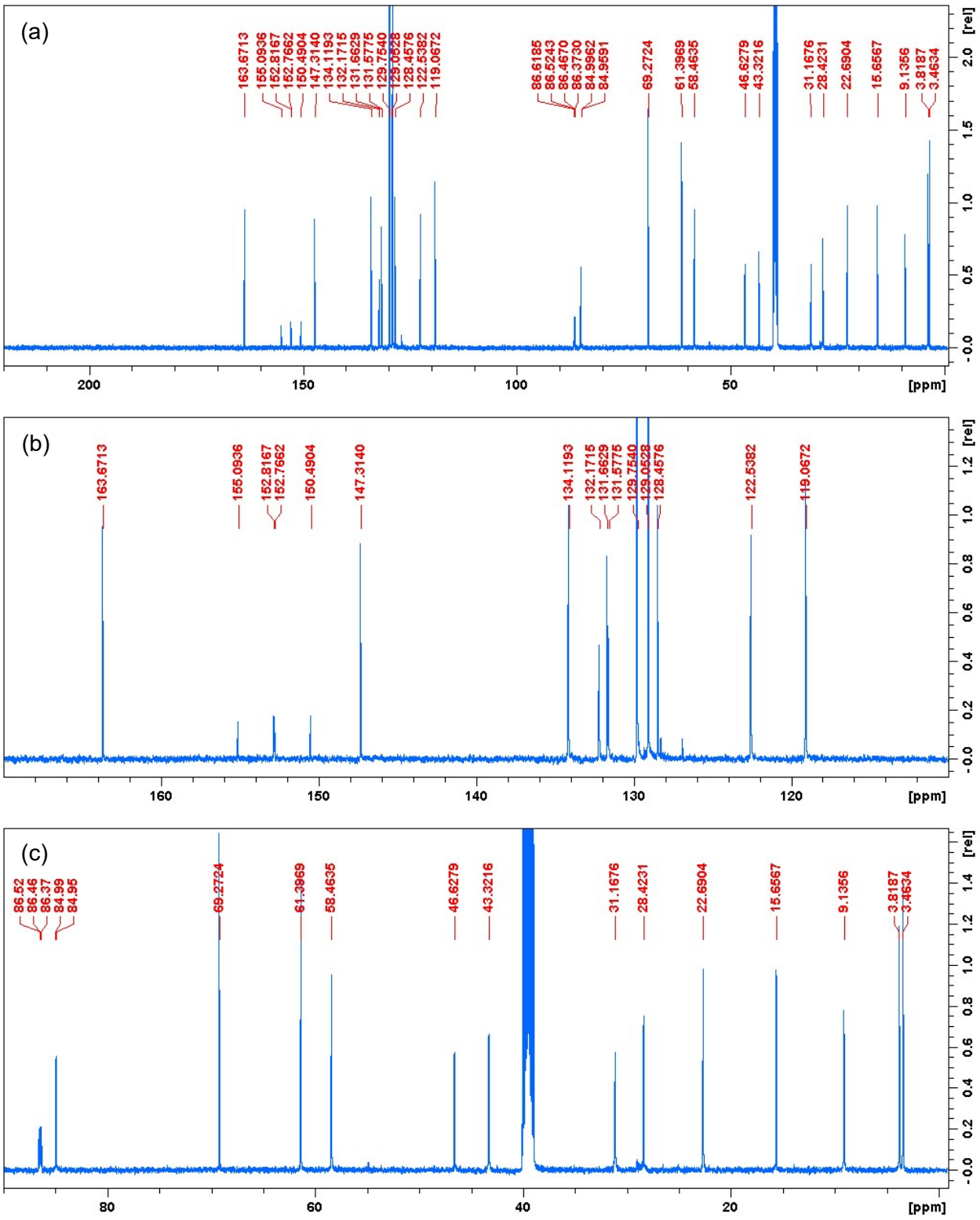


Figure S14. (a) ^{13}C NMR spectrum of 3-benzoyl-(6-difluoromethylene)naltrexone **(11)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

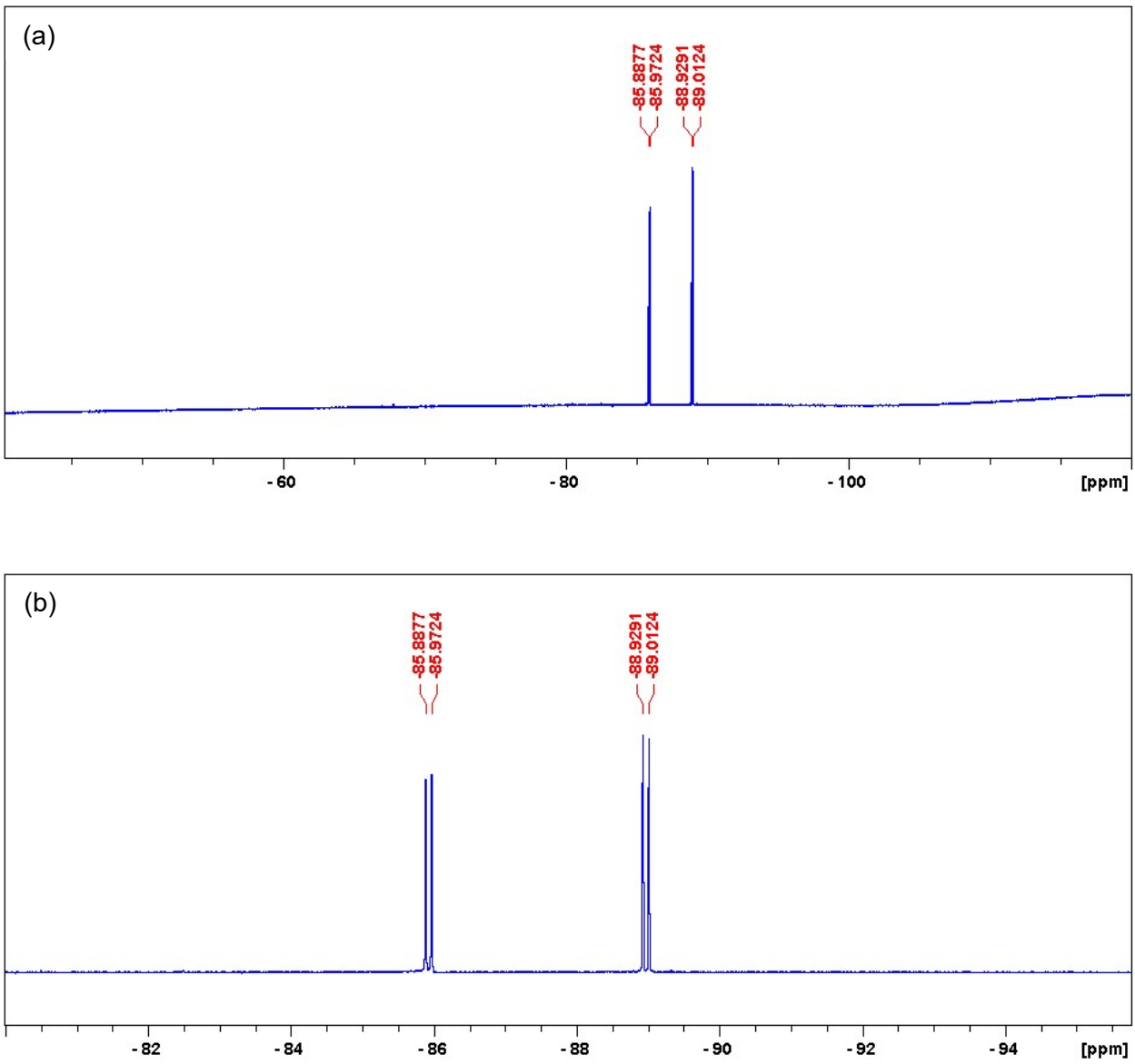
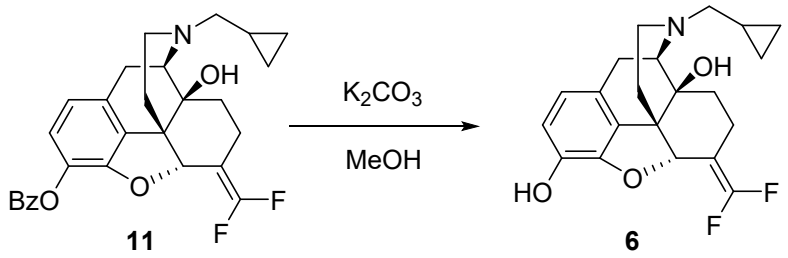


Figure S15. (a) ^{19}F NMR spectrum of 3-benzoyl-(6-difluoromethylene)naltrexone **(11)** in d_6-DMSO (b) Expansion for clarity.



6-Difluoromethylene-naltrexone (6). To a white suspension of benzoate **11** (42 mg, 0.088 mmol) in MeOH (2 mL) was added K_2CO_3 (18 mg, 0.131 mmol) in one portion. The mixture was maintained at ambient temperature for 2.5 h. LC-MS analysis showed the reaction was complete. Diluted with 30 mL of CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (15 mL). The aqueous layer was further extracted with CH_2Cl_2 (10 mL). The combined organics were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification by flash chromatography on SiO_2 (12 g, 0.8% MeOH/CH_2Cl_2 then 2% MeOH/CH_2Cl_2) afforded 22 mg (67%) of the desired product as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.02 (s, 1H), 6.58 (d, J = 8.0 Hz, 1H), 6.49 (d, J = 8.0 Hz, 1H), 5.08 (d, J = 2.4 Hz, 1H), 4.75 (s, 1H), 3.03 (d, J = 6.1. Hz, 1H), 2.98 (d, J = 18.5 Hz, 1H), 2.62 (dd, J = 3.9, 11.3 Hz, 1H), 2.55-2.50 (m, 1H), 2.36-2.26 (m, 3H), 2.17 (ddd, J = 5.0, 12.6, 12.6 Hz, 1H), 2.09 (ddd, J = 3.4, 12.2, 12.2 Hz, 1H), 1.99-1.91 (m, 1H), 1.50-1.41 (m, 1H), 1.40-1.33 (m, 1H), 1.21 (ddd, J = 6.5, 10.7, 13.4 Hz, 1H), 0.90-0.79 (m, 1H), 0.52-0.42 (m, 2H), 0.16-0.07 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 152.9 (dd, 286, 292 Hz), 143.3, 139.1, 130.5, 124.1, 118.6, 117.1, 86.9 (dd, J = 12.2, 18.0 Hz), 83.3 (d, J = 4.7 Hz), 69.3, 61.6, 58.5, 46.4, 43.4, 31.5, 28.2, 22.2, 15.8, 9.2, 3.8, 3.5. ^{19}F NMR (470 MHz, DMSO-d_6): δ -86.3 (d, J = 39.8 Hz), -89.3 (d, J = 39.8 Hz). MS (ESI): m/z calcd for $C_{21}H_{23}F_2NO_3$ 375.16, found 358.04 $[M+H-H_2O]^+$, 376.07 $[M+H]^+$.

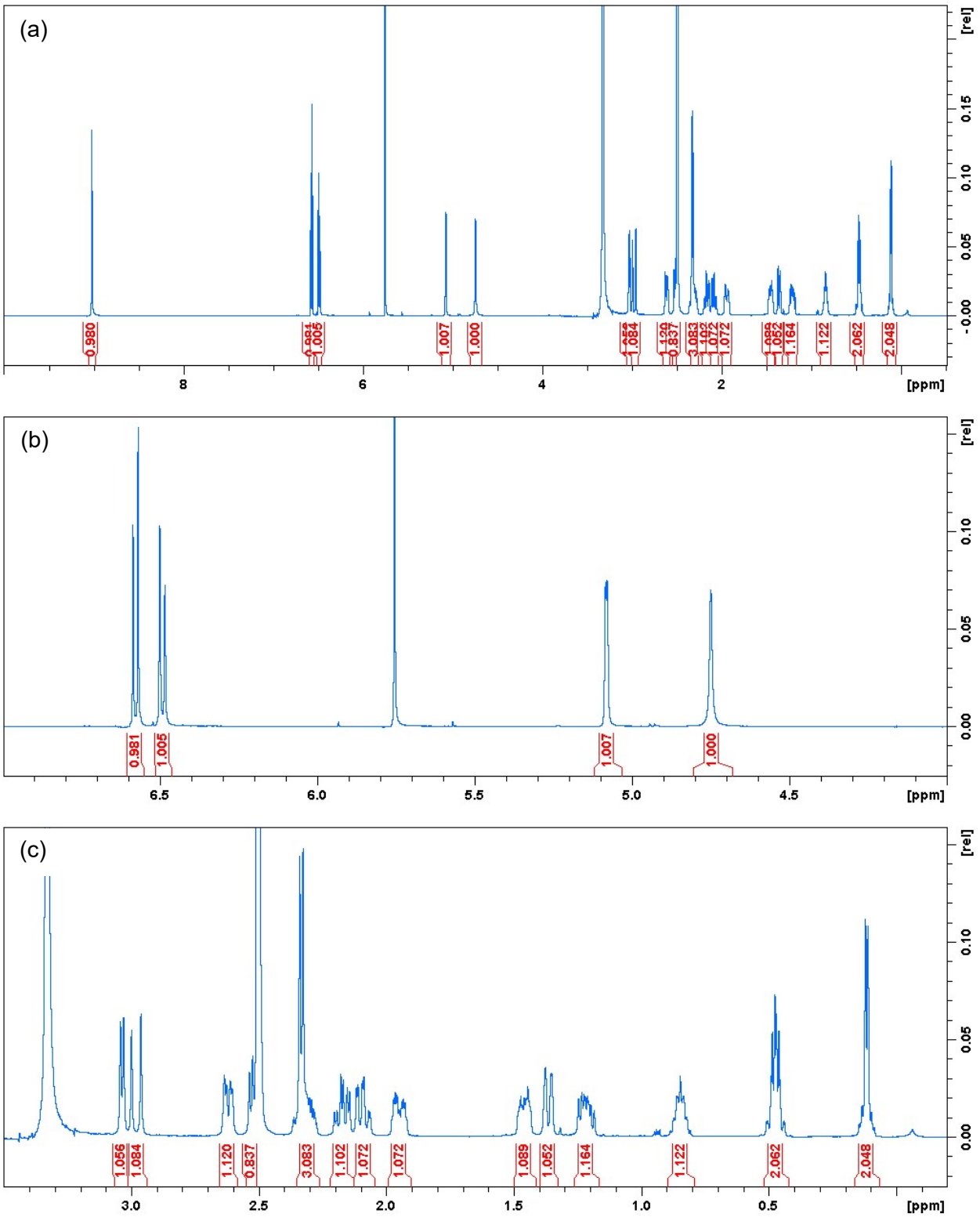


Figure S16. (a) ^{1}H NMR spectrum of (6-difluoromethylene)naltrexone **(6)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

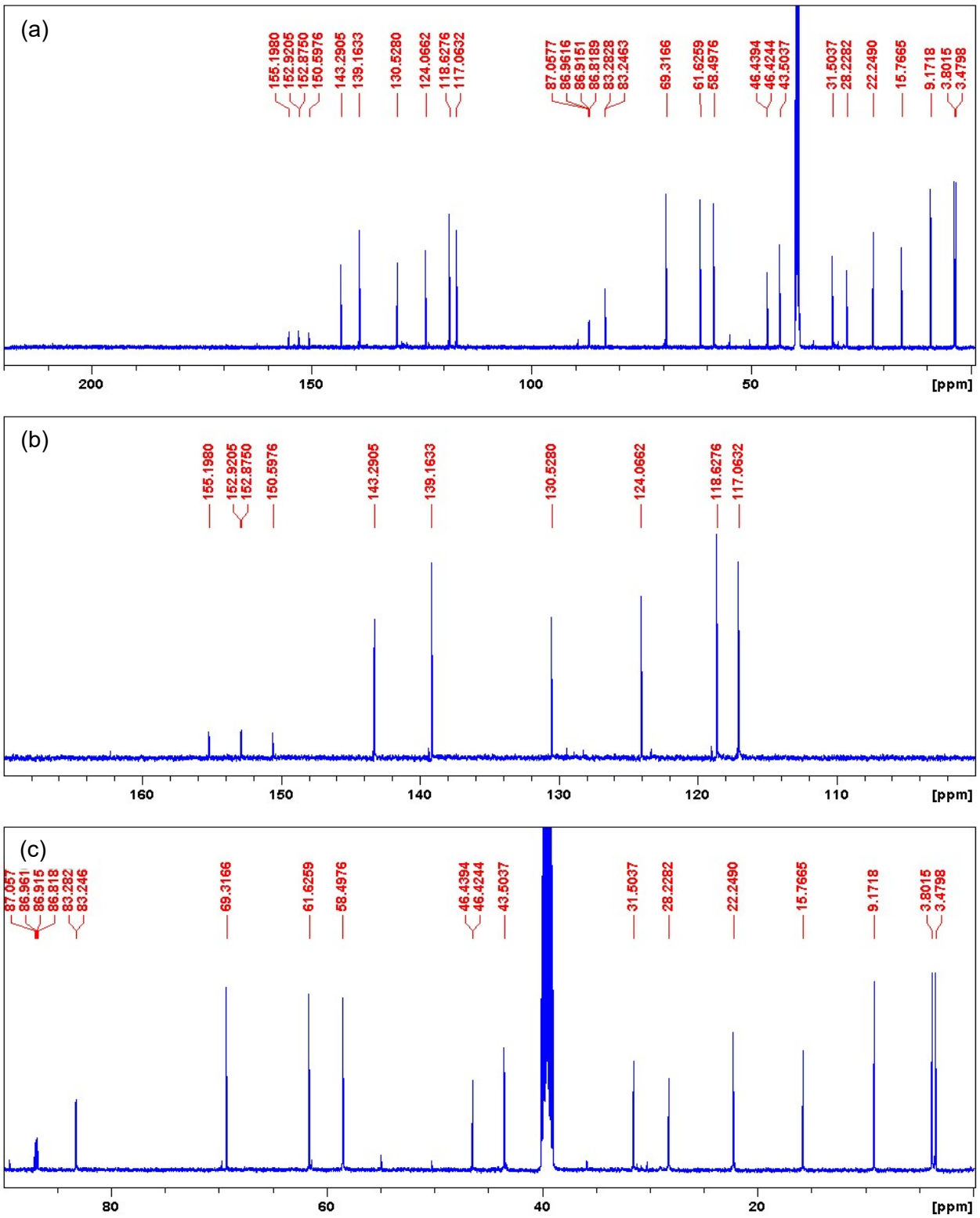


Figure S17. (a) ^{13}C NMR spectrum of (6-difluoromethylene)naltrexone **(6)** in d_6-DMSO (b) Expanded downfield region (c) Expanded upfield region

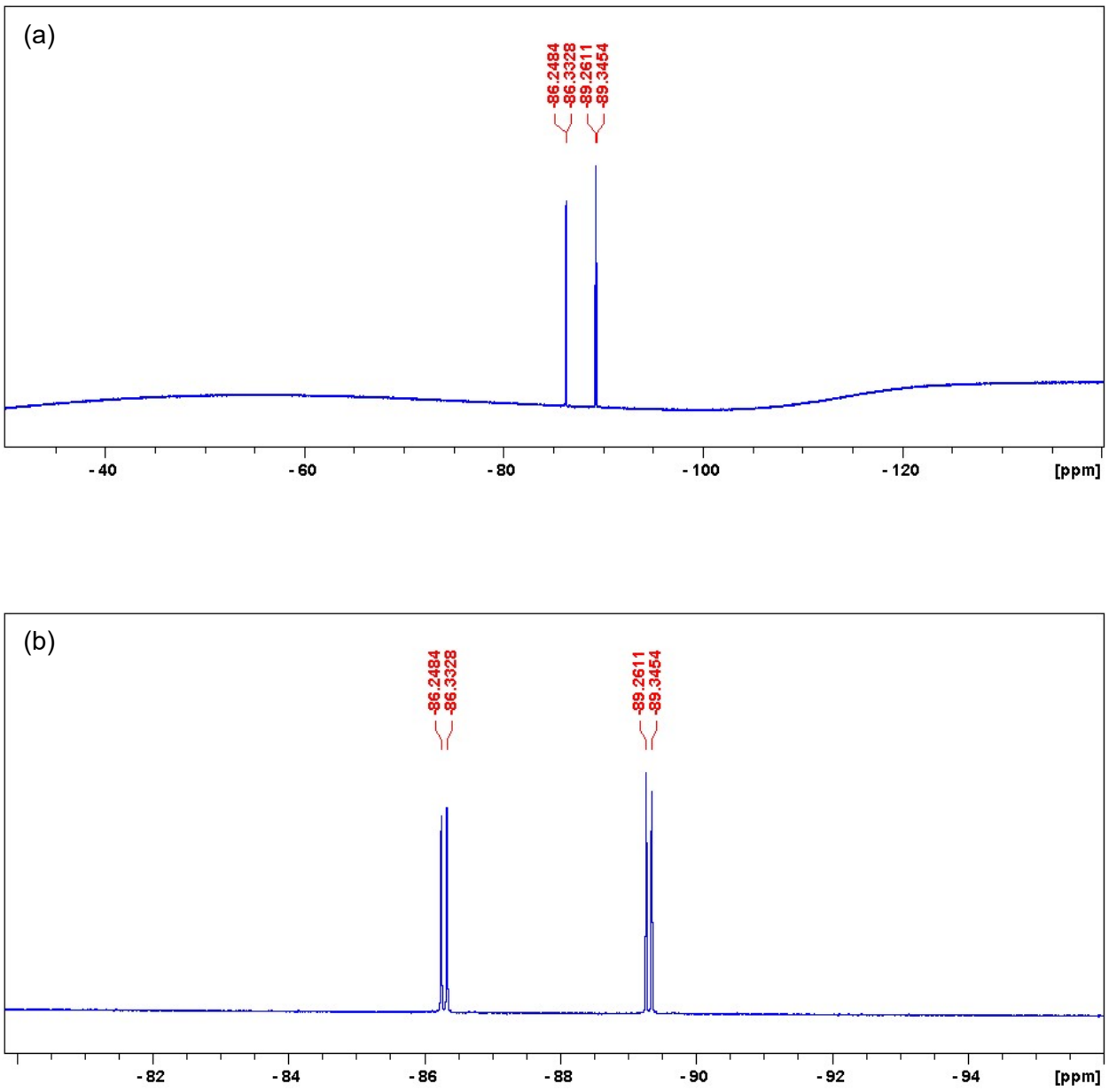


Figure S18. (a) ^{19}F NMR spectrum of (6-difluoromethylene)naltrexone **(6)** in d_6-DMSO (b) Expansion for clarity.

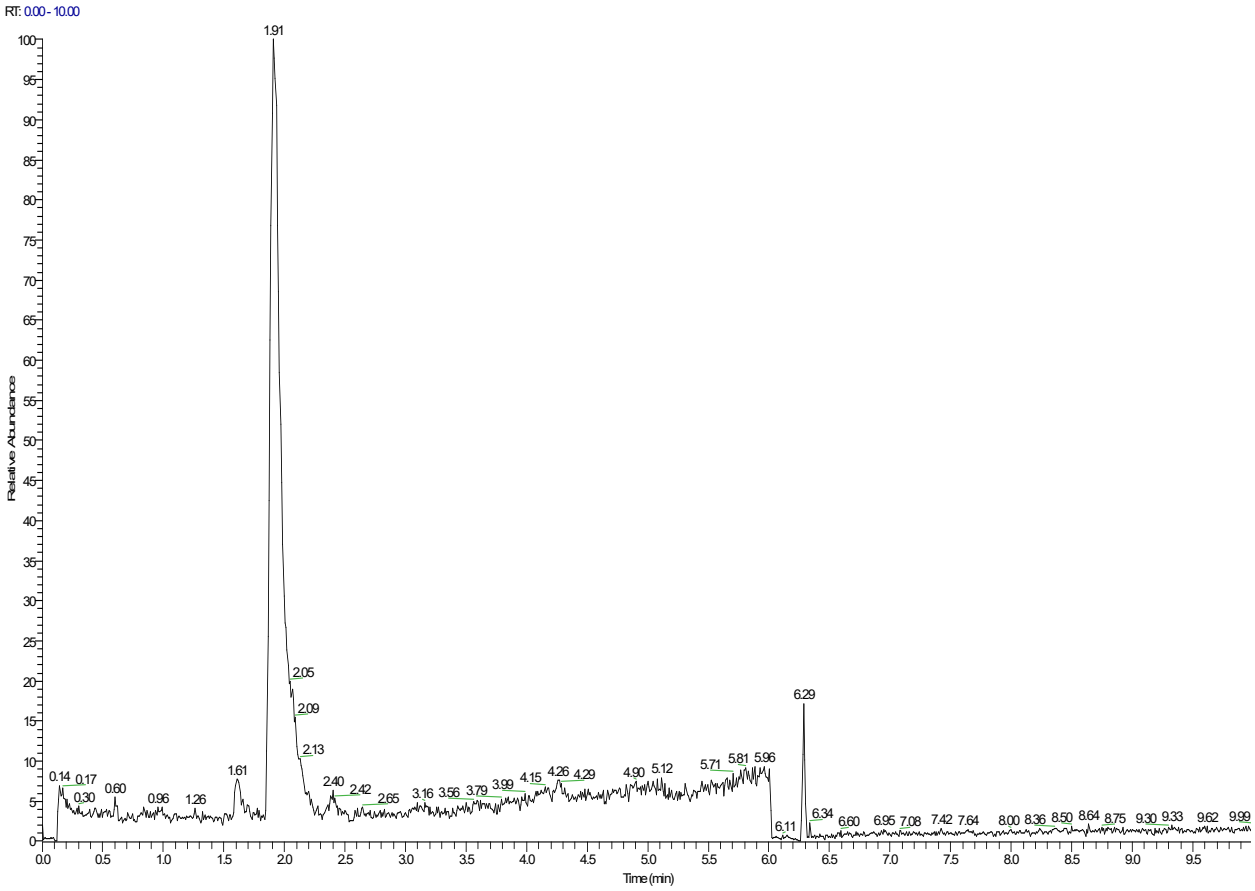


Figure S19. LC-MS chromatogram of (6-difluoromethylene)naltrexone **(6)**.

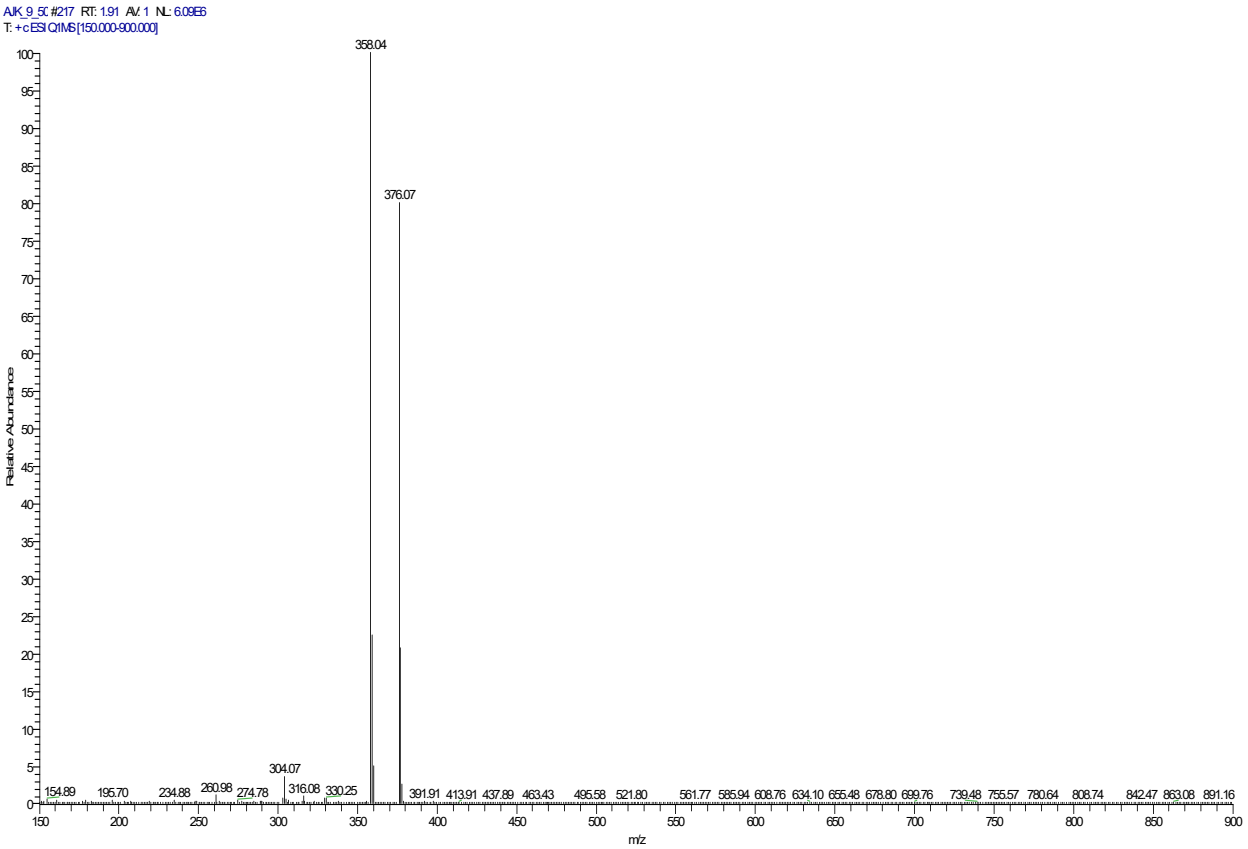


Figure S20. LC-MS mass spectrum of (6-difluoromethylene)naltrexone **(6)**.