Electronic Supplementary Material (ESI) for RSC Advances.


**Supplementary Data**

# Design and Synthesis of Uracil Urea Derivatives as Potent and Selective Fatty Acid Amide Hydrolase Inhibitors

Yan Qiu[a,ł], Jie Ren[a,ł], Hongwei Ke[b,c], Yang Zhang[a], Qi Gao[a], Longhe Yang[d], Canzhong Lu[b], and Yuhang Li[a,b,*]

Supplementary Data Table of Contents

## Supplemental Experimental Procedures

### *Chemistry*

**General.** The $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker 400 spectrometer. $^{1}$H-NMR spectra were registered in $CDCl_3$, $d_6$-DMSO, or $CD_3OD$, and chemical shifts are expressed in parts per million ($\delta$) relative to internal $Me_4Si$. IR spectra were recorded on a Nicolet Avatar 360 RT-IR spectrophotometer. Mass spectra were recorded on an Applied Biosystems MDS SCIEX 3200Q TRAP mass spectrometry (MS) system with electrospray ionization (ESI) and direct injection. The HRFABMS spectra were recorded on a Bruker APEX-FTMS apparatus. Elemental analyses were performed using a Vario RL analyzer. Melting points were determined on a Yanaco MP-500 melting point apparatus and uncorrected.

**Materials.** All reagents used in the present study were purchased from Sigma-Aldrich (Shanghai, China), seeking the highest grade commercially available unless otherwise indicated. Tetrahydrofuran (THF) was distilled prior to use from sodium benzophenone ketyl. Methylene chloride ($CH_2Cl_2$) was distilled from phosphorus pentoxide. Dimethylformamide (DMF) was distilled from calcium hydride. Silica gel (300–400 mesh) from Yantai Athy Chemical Technology Co. Ltd. (Zhifu, China) was used for column chromatography, and compounds were eluted with an ethyl acetate/petroleum ether (PE) (60–90 °C) mixture (unless otherwise stated).

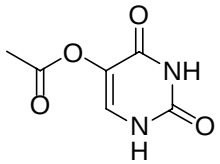
**General Procedure A for the synthesis of arylalkyl isocyanate**. To a stirred and ice–cold solution of the suitable acid (0.5 mmol) in $CH_2Cl_2$ (5 mL), DMF (0.01 mL) and $(COCl)_2$ (0.6 mmol, 0.05 mL) were added. After being stirred at 0°C for 1 h, the mixture was concentrated under reduced pressure, keeping the temperature below 30°C. The residue was diluted with dry acetone (5 mL), and added dropwise to a

stirred and ice–colded solution of $NaN_3$ (1.0 mmol, 33 mg) in $H_2O$ (1 mL). The resulting mixture was stirred at 0°C for 30 min, diluted with EtOAc, and washed with brine. The combined organic layers were dried over $Na_2SO_4$, and concentrated under reduced pressure, keeping the temperature below 30°C. The residue was diluted with toluene (5 mL), and reflux for 2 h. The solution obtained was used as such for the next reaction due to their high instability.

**General Procedure B for the synthesis of uracil ureas**

To a mixture of the corresponding uracil derivates (1.0 mmol) and DMAP (0.5 mmol, 61 mg) in pyridine (5 mL), a solution of isocyanate (0.5 mmol) in toluene (3 mL) was added dropwise under a nitrogen atmosphere at room temperature. After being stirred at 50°C for 2 h, the reaction mixture was filtered through celite and concentrated. The residue was purified by flash chromatography on silica gel to afford **1a**, **1c**, **2a**, **2d**, **2i-2k**, **3a-3n**.

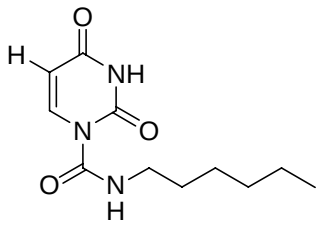
**5-Acetoxyuracil**




To a mixture of 5-hydroxyuracil (2.0 mmol, 256 mg) and DMAP (24 mg, 0.2 mmol) in pyridine (10 mL), a solution of acetic anhydride (4.0 mmol, 0.39 mL) was added dropwise under a nitrogen atmosphere at room temperature. After being stirred room temperature for 8 h, the reaction mixture was filtered through celite and concentrated. The residue was washed with water (3 × 5 mL) and acetone (3 × 5 mL), and dry under

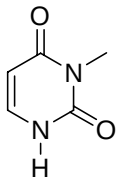
reduced pressure. The 5-acetoxyuracil obtained (about 50% purity) were used as such for the next reaction due to its poor solubility.

**N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (1a)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with uracil afforded **1a** (25 mg; yield: 21%) as white crystals; IR (film) $\nu_{max}$: 2914, 2844, 1777, 1701, 1582, 1486, 1387 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.8 Hz, 3 H), 1.23-1.28 (m, 6 H), 1.47-1.52 (m, 2 H), 3.24-3.29 (m, 2 H), 5.79 (d, *J* = 8.4 Hz, 1 H), 8.20 (d, *J* = 8.4 Hz, 1 H), 9.11 (t, *J* = 5.2 Hz, 1 H), 11.70 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.3, 22.5, 26.3, 29.1, 31.3, 40.8, 104.0, 139.2, 150.4, 152.0, 163.3 ppm; MS (ESI, m/z): 238 $(M - H)^-$; Anal. calcd for $C_{11}H_{17}N_3O_3$: C, 55.22; H, 7.16; N, 17.56; Found: C, 55.06; H, 7.15; N, 17.59.

**3-Methyluracil (1b-1)**

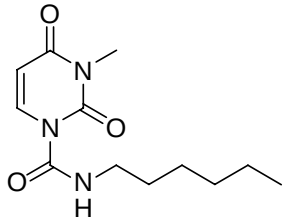


To a mixture of N1-Boc protected uracil (1.0 mmol) and cesium carbonate (1.2 mmol) in THF (15 mL), a solution of iodomethane (0.5 mmol) in THF (3 mL) was added dropwise under a nitrogen atmosphere at –10 °C. The reaction mixture was stirred at –10 °C for 0.5 h and allowed to warm slowly to room temperature over 5 h. The reaction was quenched with a saturated aqueous solution of $Na_2S_2O_3$ (1.0 mL) and

extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The N1-Boc-3-methyluracil obtained (about 50% purity) was used as such for the next reaction.

To a stirred and ice–cold solution of the $CH_3OH$ (10 mL), acetyl chloride (1 mL) was added. After being stirred at 0°C for 1 h, a solution of N1-Boc-3-methyluracil in $CH_3OH$ (5 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 0.5 h and allowed to warm slowly to room temperature over 2 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to afford **1b-1** (eluent: EtOAc) as yellow solid (19 mg, yelid: 15%). $^1$H NMR (400 MHz, $d_6$-DMSO) δ 3.12 (s, 3 H), 5.62 (d, *J* = 7.8 Hz, 1 H), 7.38–7.50 (m, 1 H), 11.15 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $d_6$-DMSO) δ 27.0, 100.2, 141.1, 150.9, 163.4 ppm; MS (ESI, m/z): 125 $(M - H)^-$.

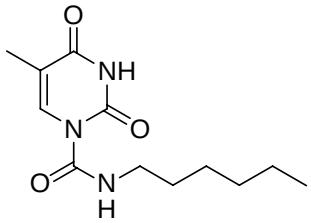
**N-hexyl-3-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (1b)**




Following the **General Procedure B** (eluent: EtOAc/PE 1:6), the addition of hexyl isocyanate with **1b-1** afforded **1b** (47 mg; yield: 37%) as colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.8 Hz, 3 H), 1.23-1.28 (m, 6 H), 1.52-1.31 (m, 2 H), 3.21 (s, 3 H), 3.34-3.39 (m, 2 H), 5.98 (d, *J* = 8.4 Hz, 1 H), 8.40 (d, *J* = 8.4 Hz, 1 H), 9.31 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.5, 26.3, 28.1, 29.1, 31.3, 41.8,

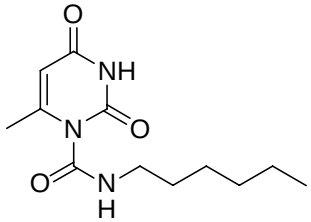
103.8, 137.2, 150.4, 152.6, 163.1 ppm; MS (ESI, m/z): 252 $(M - H)^-$.

**N-hexyl-5-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (1c)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with thymine afforded **1c** (25 mg; yield: 20%) as white crystals; IR (film) $\nu_{max}$: 3079, 2914, 2844, 1750, 1700, 1570, 1486, 1387 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.29-1.38 (m, 6 H), 1.59-1.62 (m, 2 H), 1.99 (d, *J* = 1.2 Hz, 3 H), 3.35-3.40 (m, 2 H), 8.24 (q, *J* = 1.2 Hz, 1 H), 8.60 (s, 1 H), 9.09 (br, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 12.4, 14.0, 22.5, 26.5, 29.2, 31.4, 41.2, 112.3, 134.6, 150.0, 151.5, 163.0 ppm; MS (ESI, m/z): 252 $(M - H)^-$; Anal. calcd for $C_{12}H_{19}N_3O_3$: C, 56.90; H, 7.56; N, 16.59; Found: C, 57.08; H, 7.55; N, 17.02.

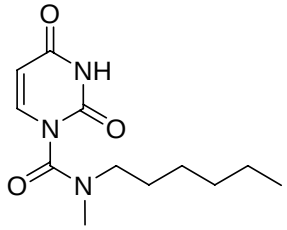
**N-hexyl-6-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (1d)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with 6-methyluracil afforded **1d** (5 mg; yield: 4%) as colorless oil; $^1H$ NMR (400 MHz, $CDCl_3$/pyridine-d5) δ 0.90 (t, *J* = 6.8 Hz, 3H), 1.29–1.38 (m, 6 H), 1.55–1.65 (m, 2 H), 1.76 (br, 3 H), 6.25 (m, 1 H), 8.24 (br, 1 H), 9.25 (br, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$/pyridine-d5) δ 9.4, 14.0, 22.5, 26.5, 29.2, 31.4, 41.2,

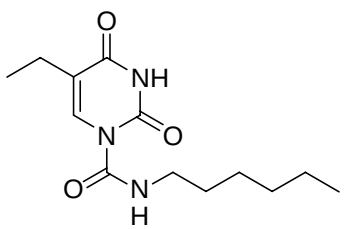
102.5, 145.3, 150.0, 155.1, 163.1 ppm; MS (ESI, m/z): 252 $(M - H)^-$.

**N-hexyl-N-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (1e)**



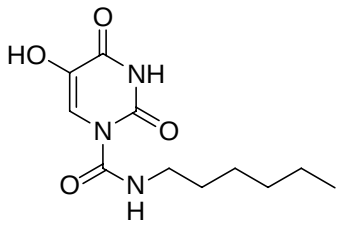
To a mixture of uracil (5.0 mmol) and DMAP (1 mmol) in pyridine (50 mL), a solution of triphosgene (5.0 mmol) in toluene (50 mL) was added dropwise under a nitrogen atmosphere at –20°C. The reaction mixture was stirred at –20 °C for 0.5 h and allowed to warm slowly to room temperature over 5 h, and cooled to 0 °C before adding N-methylhexylamine (5.0 mmol). After being stirred at room temperature for 8 h, the reaction mixture was filtered through celite and concentrated. The residue was purified by flash chromatography on silica gel to afford **1e** (eluent: EtOAc/PE 1:1) as colorless oil (63 mg; yield: 5%); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.78-0.86 (m, 3 H), 1.07–1.35 (m, 6 H), 1.44–1.55 (m, 2 H), 2.85-2.96 (s, 3 H), 3.08–3.52 (m, 2 H), 5.65-5.69 (m, 1 H), 7.64-7.69 (m, 1 H), 11.39-11.45 (m, 1 H) ppm; $^{13}$C NMR (100 MHz, DMSO-d6) δ13.8, 13.9, 21.9, 22.1, 25.4, 25.5, 26.1, 26.9, 30.8, 31.0, 34.2, 35.6, 48.9, 50.0, 102.4, 102.4, 141.5, 141.6, 148.4, 148.8, 151.1, 151.2, 163.2, 163.3 ppm; MS (ESI, m/z): 252 $(M - H)^-$.

**5-ethyl-N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2a)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with 5-ethyluracil afforded **2a** (11 mg; yield: 8%) as white crystals; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.17 (t, *J* = 7.6 Hz, 3 H), 1.32-1.37 (m, 6 H), 1.57-1.62 (m, 2 H), 2.43 (q, *J* = 7.2 Hz, 2 H), 3.37-3.42 (m, 2 H), 8.24 (s, 1 H), 9.21 (br, 1 H), 10.21 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 12.5, 13.9, 20.0, 22.4, 26.4, 29.0, 31.3, 41.1, 117.8, 134.0, 150.1, 151.6, 163.7 ppm; MS (ESI, m/z): 266 $(M - H)^-$; Anal. calcd for $C_{13}H_{21}N_3O_3$: C, 58.41; H, 7.92; N, 15.72; Found: C, 58.63; H, 7.91; N, 15.74.

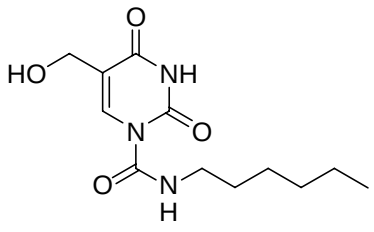
**N-Hexyl-5-hydroxy-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2b)**




To a mixture of 5-hydroxyuracil (5.0 mmol, 640 mg) and DMAP (1 mmol, 122 mg) in pyridine (20 mL), a solution of t-Butyldi-methylsilyl chloride (6.0 mmol, 900 mg) in $CH_2Cl_2$ (20 mL) was added under a nitrogen atmosphere at room temperature. After being stirred room temperature for 8 h, the reaction mixture was concentrated. The residue was washed with water (3 × 8 mL), acetone (3 × 8 mL) and $CH_2Cl_2$ (3 × 10 mL) and dry under reduced pressure. The 5-(tert-butyldimethylsilyloxy)uracil obtained was used as such for the next reaction. To a mixture of 5-(tert-butyldimethylsilyloxy)uracil and DMAP (1 mmol, 122 mg) in pyridine (15 mL), a solution of hexyl isocyanate (5 mmol, 635 mg) was added dropwise under a nitrogen atmosphere at room temperature. After being stirred at 50°C for 2 h, the

reaction mixture was filtered through celite and concentrated. The residue was dissolved in THF (10 mL), and a solution of Tetrabutylammonium fluoride (4 mmol) in THF (4 mL) was added under a nitrogen atmosphere at 0°C. The reaction was allowed to warm to room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered through celite and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/PE 1:1) on silica gel to afford **2b** (95 mg; yield: 7%) as white crystals; IR (film) $\nu_{max}$: 3307, 3254, 3112, 2955, 2919, 2850, 1730, 1663, 1546, 1476, 1384, 1277, 1258, 1097 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.8 Hz, 3 H), 1.27-1.31 (m, 6 H), 1.48-1.51 (m, 2 H), 3.23-3.28 (m, 2 H), 7.70 (s, 1 H), 9.21 (s, 1 H), 9.24 (t, *J* = 5.6 Hz, 1 H), 11.88 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.3, 22.5, 26.4, 29.2, 31.3, 40.7, 117.0, 133.9, 150.6, 150.9, 160.5 ppm; MS (ESI, m/z): 254 $(M-H)^-$; Anal. calcd for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46; Found: C, 51.90; H, 6.70; N, 16.49.

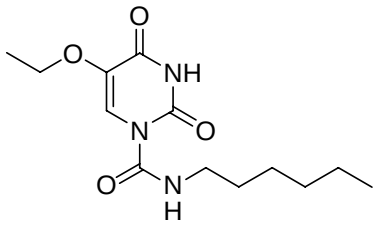
**N-Hexyl-5-(hydroxymethyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2c)**




To a mixture of 5-(hydroxymethyl)uracil (5.0 mmol, 710 mg) and DMAP (1 mmol, 122 mg) in pyridine (20 mL), a solution of t-Butyldi-methylsilyl chloride (6.0 mmol, 900 mg) in $CH_2Cl_2$ (20 mL) was added under a nitrogen atmosphere at room temperature. After being stirred room temperature for 8 h, the reaction mixture was

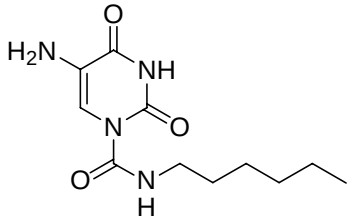
concentrated. The residue was washed with water (3 × 8 mL), acetone (3 × 8 mL) and $CH_2Cl_2$ (3 × 10 mL) and dry under reduced pressure. The 5-((tert-butyldimethylsilyloxy)methyl) uracil obtained was used as such for the next reaction. To a mixture of 5-((tert-butyldimethylsilyloxy)methyl)uracil and DMAP (1 mmol, 122 mg) in pyridine (15 mL), a solution of hexyl isocyanate (5 mmol, 635 mg) was added dropwise under a nitrogen atmosphere at room temperature. After being stirred at 50°C for 2 h, the reaction mixture was filtered through celite and concentrated. The residue was dissolved in THF (10 mL), and a solution of Tetrabutylammonium fluoride (4 mmol) in THF (4 mL) was added under a nitrogen atmosphere at 0°C. The reaction was allowed to warm to room temperature. After being stirred at room temperature for 1 h, the reaction mixture was filtered through celite and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/PE 1:1) on silica gel to afford **2c** (4 mg; yield: 2%) as white crystals; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3 H), 1.30 −1.40 (m, 6 H), 1.56−1.65 (m, 2 H), 2.40 (br, 1 H), 3.37-3.42 (m, 2 H), 4.50 (d, *J* = 6.8 Hz, 2 H), 8.33 (s, 1 H), 8.45 (s, 1 H), 8.98 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 26.6, 29.3, 31.5, 41.5, 58.9, 115.2, 136.2, 149.7, 151.1, 162.5 ppm; MS (ESI, m/z): 268 $(M - H)^-$; Anal. calcd for $C_{12}H_{19}N_3O_4$: C, 53.52; H, 7.11; N, 15.60; Found: C, 53.36; H, 7.12; N, 15.58.

**5-Ethoxy-N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2d)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with 5-ethoxyuracil afforded **2d** (53 mg; yield: 37%) as white crystals; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.20 (t, *J* = 7.5 Hz, 3 H), 1.31-1.38 (m, 6 H), 1.56-1.63 (m, 2 H), 3.26-3.31 (m, 2 H), 3.68 (dd, *J* = 7.5, 7.5 Hz, 3 H), 8.20 (s, 1 H), 9.18 (t, *J* = 5.3 Hz, 1 H), 10.03 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.8, 15.5, 22.4, 26.3, 29.0, 31.2, 41.3, 64.5, 115.2, 135.2, 149.2, 150.6, 158.2 ppm; MS (ESI, m/z): 282 $(M - H)^-$. Anal. calcd for $C_{13}H_{21}N_3O_4$: C, 55.11; H, 7.47; N, 14.83; Found: C, 55.18; H, 7.46; N, 14.83.

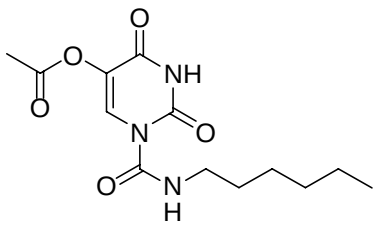
**5-Amino-N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2e)**



To a mixture of 5-aminouracil (5.0 mmol, 635 mg) and DMAP (1 mmol, 122 mg) in pyridine (20 mL), a solution of benzyl carbonochloridate (6.0 mmol, 1020 mg) in $CH_2Cl_2$ (20 mL) was added under a nitrogen atmosphere at room temperature. After being stirred room temperature for 8 h, the reaction mixture was concentrated. The residue was washed with water (3 × 8 mL), acetone (3 × 8 mL) and $CH_2Cl_2$ (3 × 10 mL) and dry under reduced pressure. The Cbz-protected uracil derivate obtained was used as such for the next reaction. To a mixture of Cbz-protected uracil derivate and

DMAP (1 mmol, 122 mg) in pyridine (15 mL), a solution of hexyl isocyanate (5 mmol, 635 mg) was added dropwise under a nitrogen atmosphere at room temperature. After being stirred at 50°C for 2 h, the reaction mixture was filtered through celite and concentrated. The residue was dissolved in $CH_2Cl_2$ (10 mL), and a solution of trimethylsilyl iodide (1.4 mmol, 280 mg) in $CH_2Cl_2$ (2 mL) was added under a nitrogen atmosphere at 0°C. The reaction was allowed to warm to room temperature. After being stirred at room temperature for 1 h, the reaction was quenched with a saturated solution of $Na_2S_2O_3$ and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent: EtOAc) on silica gel to afford **2e** (50 mg; yield: 4%) as white crystals; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.8 Hz, 3 H), 1.23-1.31 (m, 6 H), 1.46-1.51 (m, 2 H), 3.23-3.28 (m, 2 H), 4.48 (s, 2 H), 7.57 (s, 1 H), 9.30 (t, *J* = 5.6 Hz, 1 H), 11.81 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ14.3, 22.5, 26.4, 29.2, 31.3, 40.7, 112.2, 124.5, 150.8, 151.0, 160.9 ppm; MS (ESI, m/z): 253 $(M - H)^-$; HRMS (ESI) calcd for $[C_{11}H_{17}N_4O_3]^+$ $(M - H)^-$ : 253.1301; found: 253.1295.

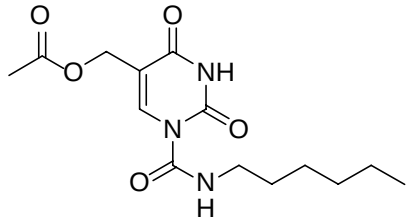
**1-(Hexylcarbamoyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl acetate (2f)**




Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with 5-acetoxyuraci afforded **2f** (31 mg; yield: 21%) as white crystal; IR

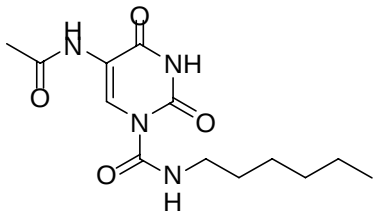
(film) $\nu_{max}$: 3313, 3253, 2953, 2917, 2849, 1727, 1693, 1577, 1541, 1466, 1383, 1266, 1199 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.31-1.38 (m, 6 H), 1.56-1.63 (m, 2 H), 2.30 (s, 3 H), 3.36-3.41 (m, 2 H), 8.39 (s, 1 H), 9.08 (t, *J* = 5.2 Hz, 1 H), 10.03 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 13.8, 20.0, 22.4, 26.3, 29.0, 31.2, 41.3, 128.1, 130.2, 149.2, 150.6, 158.2, 168.3 ppm; MS (ESI, m/z): 296 $(M - H)^-$; Anal. calcd for $C_{13}H_{19}N_3O_5$: C, 52.52; H, 6.44; N, 14.13; Found: C, 52.33; H, 6.43; N, 14.15.

**(1-(Hexylcarbamoyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl acetate (2g)**



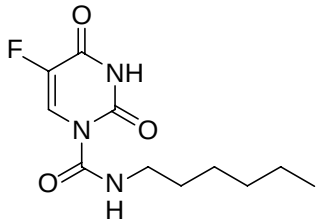

Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with (5-acetoxymethyl)uraci afforded **2g** (35 mg; yield: 23%) as white crystals; IR (film) $\nu_{max}$: 3263, 3182, 3068, 2950, 2917, 2849, 1758, 1735, 1704, 1690, 1543, 1536, 1352, 1287, 1101 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.29-1.38 (m, 6 H), 1.57-1.62 (m, 2 H), 2.09 (s, 3 H), 3.37-3.42 (m, 2 H), 4.93 (s, 2 H), 8.56 (s, 1 H), 9.09 (t, *J* = 5.2 Hz, 1 H), 9.81 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 13.9, 20.8, 22.4, 26.4, 29.0, 31.3, 41.3, 58.5, 111.1, 138.6, 149.5, 151.3, 162.2, 170.6 ppm; MS (ESI, m/z): 310 $(M - H)^-$; Anal. calcd for $C_{14}H_{21}N_3O_5$: C, 54.01; H, 6.80; N, 13.50; Found: C, 54.20; H, 6.81; N, 13.48.

**5-Acetamido-N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2h)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with (5-acetylamino)uracil afforded **2h** (22 mg; yield: 15%) as white crystals; IR (film) $\nu_{max}$: 3295, 2917, 2849, 1743, 1735, 1572, 1546, 1263, 1094 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.26-1.38 (m, 6 H), 1.56-1.64 (m, 2 H), 2.19 (s, 3 H), 3.37-3.42 (m, 2 H), 7.56 (s, 1 H), 9.00 (br, 1 H), 9.48 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 24.1, 26.5, 29.1, 31.4, 41.4, 115.4, 125.2, 149.4, 149.7, 159.3, 168.2 ppm; MS (ESI, m/z): 295 $(M - H)^-$; Anal. calcd for $C_{13}H_{20}N_4O_4$: C, 52.69; H, 6.80; N, 18.91; Found: C, 52.49; H, 6.79; N, 18.89.

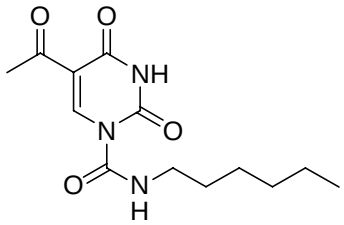
**5-Fluoro-N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2i)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with 5-fluorouracil afforded **2i** (42 mg; yield: 33%) as white crystals; IR (film) $\nu_{max}$: 3321, 3260, 3079, 2917, 2850, 1725, 1694, 1539, 1514, 1450, 1342, 1267, 1195 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$) δ 0.91 (t, *J* = 6.8 Hz, 3 H), 1.33-1.42 (m, 6 H), 1.59-1.66 (m, 2 H), 3.39-3.44 (m, 2 H), 8.50 (d, *J* = 6.8 Hz, 1 H), 9.02 (br, 1 H), 9.20 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 13.9, 22.5, 26.4, 29.1, 31.3, 41.5, 123.2 (d, *J* = 40 Hz), 140.8 (d, *J* = 240 Hz), 149.0, 149.9, 156.0, 156.2 (d, *J* = 40 Hz)

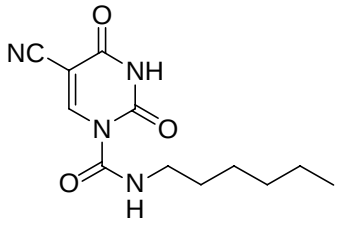
ppm; MS (ESI, m/z): 256 $(M - H)^-$; Anal. calcd for $C_{11}H_{16}FN_3O_3$: C, 51.36; H, 6.27; N, 16.33; Found: C, 51.19; H, 6.28; N, 16.26.

**5-Acetyl-N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2j)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with 5-acetyluracil afforded **2j** (14 mg; yield: 10%) as white crystals; IR (film) $\nu_{max}$: 3288, 3189, 3085, 2915, 2846, 1734, 1713, 1681, 1408, 1333, 1211 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.92 (t, *J* = 6.8 Hz, 3 H), 1.28-1.44 (m, 6 H), 1.60-1.65 (m, 2 H), 2.64 (s, 3 H), 3.41-3.46 (m, 2 H), 8.39 (s, 1 H), 8.93 (br, 1 H), 9.27 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.0, 22.5, 26.5, 29.1, 30.6, 31.3, 41.6, 114.2, 145.4, 148.7, 150.6, 159.8, 192.9 ppm; MS (ESI, m/z): 280 $(M - H)^-$; Anal. calcd for $C_{13}H_{19}N_3O_4$: C, 55.50; H, 6.81; N, 14.94; Found: C, 55.64; H, 6.80; N, 14.95.

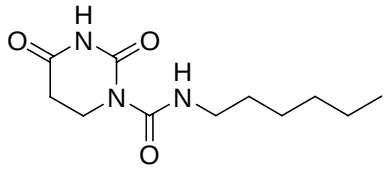
**5-Cyano-N-hexyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2k)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of hexyl isocyanate with 5-cyanouracil afforded **2k** (15 mg; yield: 11%) as white crystals; IR (film) $\nu_{max}$: 3309, 3068, 2914, 2844, 1756, 1719, 1687, 1633, 1571, 1536, 1331, 1294, 1084, 1046 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.88-0.92 (m, 3 H), 1.26-1.38 (m, 6

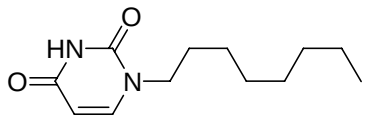
H), 1.58-1.65 (m, 2 H), 3.39-3.44 (m, 2 H), 5.01 (s, 1H), 8.83 (br, 1 H), 9.02 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.5, 26.4, 29.0, 31.3, 41.8, 93.0, 111.5, 147.0, 147.8, 149.7, 157.6 ppm; MS (ESI, m/z): 263 $(M - H)^-$; Anal. calcd for $C_{12}H_{16}N_4O_3$: C, 54.54; H, 6.10; N, 21.20; Found: C, 54.70; H, 6.11; N, 21.18.

**N-Hexyl-2,4-dioxo-tetrahydropyrimidine-1(2H)-carboxamide (2l)**



The **1a** (24 mg, 0.1 mmol) in EtOAc (10 mL) was treated with 10% Pd/C (100 mg) and purged with $H_2$. After stirring for 12 h at room temperature, the reaction mixture was filtered through celite and concentrated. The residue was purified by flash chromatography on silica gel (eluent: EtOAc/PE 1:5) to afford **2l** (24 mg; yield: 98%) as white crystals; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, $J$ = 6.8 Hz, 3 H), 1.26-1.38 (m, 6 H), 1.53-1.60 (m, 2 H), 2.67-2.70 (m, 2 H), 3.29-3.34 (m, 2 H), 4.08-4.12 (m, 2 H), 8.30 (s, 1 H), 8.61 (br, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.5, 26.5, 29.3, 30.8, 31.4, 37.7, 40.8, 152.8, 153.2, 170.0 ppm.

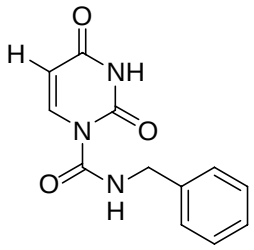
**1-Octyl uracil (2m)**



To a mixture of uracil (1 g, 8.9 mmol) and $K_2CO_3$ (6.2 g, 45 mmol) in DMF (10 mL), a solution of 1-bromooctane (2.1 g, 11 mmol) in DMF (5 mL) was added under a nitrogen atmosphere at 0°C. After being stirred room temperature for 12 h, the reaction mixture was concentrated. The residue was poured into water (150 mL) and

extracted with EtOAc (5 × 20 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent: EtOAc/PE 1:2) on silica gel to afford **2m** (880 mg; yield: 44%) as white crystals; IR (film) $\nu_{max}$: 2920, 2852, 1667, 1461 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.28-1.32 (m, 10 H), 1.67-1.71 (m, 2 H), 3.73 (t, *J* = 7.6 Hz, 2 H), 5.71 (d, *J* = 7.6 Hz, 1 H), 7.16 (d, *J* = 7.6 Hz, 1 H), 9.56 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 26.4, 29.0, 29.1, 29.1, 31.7, 48.9, 102.1, 144.5, 151.0, 164.0 ppm; MS (ESI, m/z): 223 $(M - H)^-$; Anal. calcd for $C_{12}H_{20}N_2O_2$: C, 64.26; H, 8.99; N, 12.49; Found: C, 64.45; H, 9.01; N, 12.55.

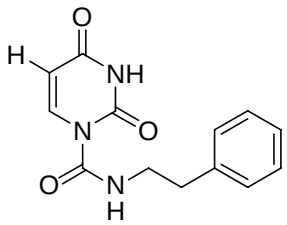
**N-benzyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3a)**




Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of benzyl isocyanate with uracil afforded **3a** (71 mg; yield: 58%) as white crystals; IR (film) $\nu_{max}$: 2955, 2917, 2849, 1725, 1691, 1620, 1579, 1546, 1537 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-d6):δ 4.50 (d, *J* = 5.8 Hz, 2 H), 5.81 (dd, *J* = 2.2 Hz, 8.4 Hz,1 H), 7.25 -7.30 (m, 1 H), 7.35 (m, 4 H), 8.22 (d, *J* =8.4 Hz, 1 H), 9.57 (t, *J* = 5.8 Hz, 1 H), 11.76 (br, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6): δ 44.4, 104.1, 127.6, 127.8, 128.9, 138.7, 139.2, 150.8, 151.9,163.3 ppm; MS (ESI, m/z): 244.2 $(M - H)^-$; Anal. calcd for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13; Found: C, 58.85; H, 4.51; N,

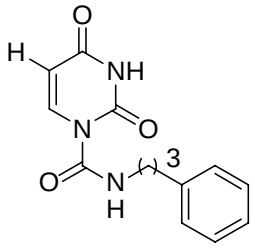
17.12.

**N-phenethyl -2,4-dioxo -3,4-dihydropyrimidine-1(2H)-carboxamide (3b)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:4), the addition of phenethyl isocyanate with uracil afforded **3b** (88 mg; yield: 68%) as white crystals; IR (film) $\nu_{max}$: 2950, 2918, 2849, 1725, 1580, 1547,1402, 1383 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6): δ 2.84 (t, *J* = 7.0 Hz, 2 H), 3.50-3.55 (m, 2 H), 5.80 (d, *J* = 8.4 Hz,1 H), 7.22-7.31 (m, 5 H), 8.21 (d, *J* = 8.4 Hz, 1 H), 9.20 (br, 1 H), 11.72 (br, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6): δ 35.2, 42.4, 104.1, 126.8, 128.9, 129.2, 139.1, 139.3, 150.4, 152.0, 163.2; MS (ESI, m/z): 258.2 $(M - H)^{-}$. Anal. calcd for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21; Found: C, 60.07; H, 5.05; N, 16.20.

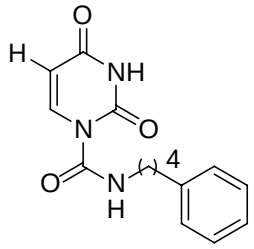
**N-(3-phenylpropyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3c)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:4), the addition of phenylpropyl isocyanate with uracil afforded **3c** (66 mg; yield: 48%). IR (film) $\nu_{max}$: 2953, 2917, 2849, 1727, 1575, 1541, 1383 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 1.98 (m, 2 H), 2.73 (t, *J* = 7.6 Hz, 2 H), 3.44 (m, 2 H), 5.90 (d, *J* = 8.5 Hz, 1 H), 7.20-7.23 (m, 3 H), 7.29-7.33 (m, 2 H), 8.42 (d, *J* = 8.5 Hz, 1 H), 9.15 (br, 2 H) ppm; $^{13}C$

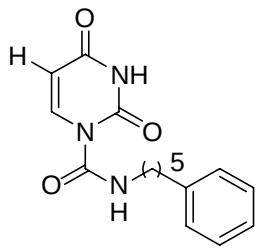
NMR(100 MHz,$CDCl_3$): δ 30.6, 33.0, 40.7, 103.9, 126.1, 128.3, 128.5, 138.9, 140.8, 149.7, 151.5, 162.5 ppm; MS (ESI, m/z): 272.1 $(M - H)^-$; Anal. calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38; Found: C, 61.53; H, 5.53; N, 15.38.

**N-(4-phenylbutyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3d)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of phenylbutyl isocyanate with uracil afforded **3d** (74 mg; yield: 52 %). Mp: 84.0–84.6 °C; IR (film) $\nu_{max}$: 3438, 2955, 2917, 2849, 1724, 1579, 1468, 1381 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 1.67-1.73 (m, 4 H), 2.68 (t, *J* = 7.3 Hz, 2 H), 3.44 (m, 2 H), 5.90 (d, *J* = 8.5 Hz, 1 H), 7.18-7.23 (m, 3 H), 7.28-7.32 (m, 2 H), 8.42 (d, *J* = 8.5 Hz, 1 H), 9.10-9.15 (br, 2 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 28.5, 28.8, 35.4, 41.1, 103.9, 125.9, 128.4, 139.0, 141.8, 149.7, 151.5, 162.4 ppm; MS (ESI, m/z): 286.1 $(M - H)^-$; Anal. calcd for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63; Found: C, 62.58; H, 5.97; N, 14.63.

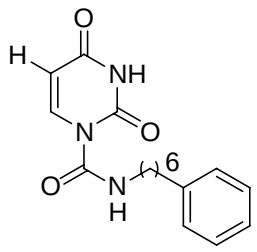
**N-(5-phenylpentyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3e)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of

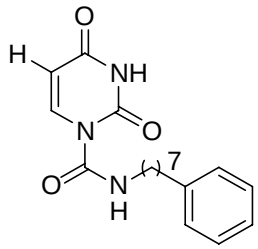
phenylpentyl isocyanate with uracil afforded **3e** (87 mg; yield: 58 %). IR (film) $\nu_{max}$: 2955, 2917, 2849, 1726, 1577, 1463, 1382 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 1.39-1.47 (m, 2 H), 1.63-1.71 (m, 4 H), 2.65 (t, *J* = 7.6 Hz, 2 H), 3.41 (m, 2 H), 5.90 (d, *J* = 8.5 Hz, 1 H), 7.18-7.22 (m, 3 H), 7.28-7.31 (br , 2 H), 8.42 (d, *J* = 8.5 Hz, 1 H), 8.56 (br, 1 H), 9.06 (br, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 26.4, 29.0, 31.0, 35.7, 41.2, 103.9, 125.8, 128.3, 128.4, 139.2, 142.3, 149.8, 151.7, 163.4 ppm; MS (ESI, m/z): 300.1 $(M - H)^-$ ; Anal. calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94; Found: C, 63.83; H, 6.36; N, 13.92.

**N-(6-phenylhexyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3f)**



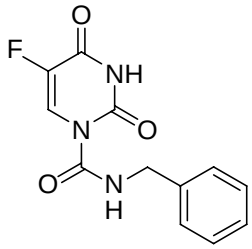

Following the **General Procedure B** (eluent: EtOAc/PE 1:7), the addition of phenylhexyl isocyanate with uracil afforded **3f** (68 mg; yield: 43 %). IR (film) $\nu_{max}$: 3423, 2957, 2917, 2849, 1724, 1579, 1403, 1384 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): δ 1.38 (m, 4 H), 1.59-1.65 (br, 4 H), 2.61 (t, *J* = 7.6 Hz, 2 H), 3.38 (m, 2 H), 5.90 (d, *J* = 8.5 Hz, 1 H), 7.15-7.19 (m, 3 H), 7.25-7.29 (br , 2 H), 8.42 (d, *J* = 8.5 Hz, 1 H), 9.08 (br, 1 H), 9.17 (br, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 26.7, 28.8, 29.1, 31.3, 35.8, 41.2, 103.8, 125.7, 128.3, 128.4, 139.0, 142.5, 149.7, 151.4, 162.4 ppm; MS (ESI, m/z): 314.4 $(M - H)^-$ ; Anal. calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32; Found: C, 64.61; H, 6.70; N, 13.33.

**N-(7-phenylheptyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3g)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:7), the addition of phenylheptyl isocyanate with uracil afforded **3g** (64 mg; yield: 39 %). IR (film) $\nu_{max}$: 3425, 2953, 2917, 2849, 1726, 1579, 1415, 1380 $cm^{-1}$; $^{1}H$ NMR (400 MHz, $CDCl_3$): δ 1.35 (br, 6 H), 1.59-1.63 (m, 4 H), 2.60 (t, *J* = 7.6 Hz, 2 H), 3.38 (m, 2 H), 5.90 (d, *J* = 8.5 Hz, 1 H), 7.15-7.17 (m, 3 H), 7.25-7.27 (br , 2 H), 8.41 (d, *J* = 8.5 Hz, 1 H), 9.09 (br, 1 H), 9.60 (br, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 26.7, 29.1, 29.1, 31.4, 35.9, 41.2, 103.8, 125.6, 128.2, 128.4, 139.1, 142.7, 149.7, 151.6, 162.9 ppm; MS (ESI, m/z): 328.4 $(M - H)^-$ ; Anal. calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76; Found: C, 65.49; H, 7.04; N, 12.77.

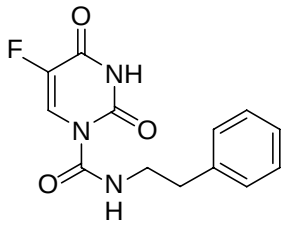
**N-benzyl-5-fluoro-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3h)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of benzyl isocyanate with uracil afforded **3h** (43 mg; yield: 33%) as white crystals; IR (film) $\nu_{max}$: 2917, 2849, 1738, 1692, 1673, 1578, 1537, 1335, 1268, 1204 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 4.49 (d, *J* = 5.6 Hz, 2 H), 7.27-7.37 (m, 5 H), 8.38 (d, *J* = 7.6 Hz, 1 H), 9.64 (br, 1 H), 12.28 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ

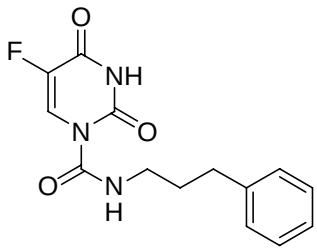
44.5, 123.0 (d, *J* = 35 Hz), 127.6, 127.8, 128.9, 138.7, 141.0 (d, *J* = 230 Hz), 150.0, 150.6, 157.5 (d, *J* = 30 Hz) ppm; MS (ESI, m/z): 262 $(M - H)^-$; Anal. calcd for $C_{12}H_{10}FN_3O_3$: C, 54.75; H, 3.83; N, 15.96; Found: C, 54.58; H, 3.83; N, 15.98.

**5-Fluoro-2,4-dioxo-N-phenethyl-3,4-dihydropyrimidine-1(2H)-carboxamide (3i)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of phenethyl isocyanate with uracil afforded **3i** (43 mg; yield: 39%) as white crystals; IR (film) $\nu_{max}$: 3275, 2917, 2849, 1731, 1579, 1536, 1463, 1273, 1099 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-d6) δ 2.84 (t, *J* = 7.2 Hz, 2 H), 3.50-3.54 (m ,2 H), 7.20-7.26 (m, 3 H), 7.31 (t, *J* = 7.2 Hz, 2 H), 8.38 (d, *J* = 7.2 Hz, 1 H), 9.19 (br, 1 H), 12.26 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 35.2, 42.5, 123.1 (d, *J* = 38 Hz), 126.8, 128.9, 129.1, 139.2, 141.1 (d, *J* = 233 Hz), 149.9, 150.6, 157.4 (d, *J* = 27 Hz) ppm; MS (ESI, m/z): 276 $(M - H)^-$; Anal. calcd for $C_{13}H_{12}FN_3O_3$: C, 56.32; H, 4.36; N, 15.16; Found: C, 56.49; H, 4.36; N, 15.18.

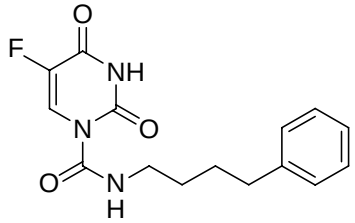
**5-Fluoro-2,4-dioxo-N-(3-phenylpropyl)-3,4-dihydropyrimidine-1(2H)-carboxamide (3j)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of

phenylpropyl isocyanate with uracil afforded **3j** (52 mg; yield: 36%) as white crystals; IR (film) $\nu_{max}$: 3320, 3077, 2917, 2849, 1736, 1578, 1537, 1334, 1265, 1211 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.84-1.87 (m, 2 H), 2.62 (t, *J* = 7.6 Hz, 2 H), 3.29-3.32 (m, 2 H), 7.18-7.40 (m, 5 H), 8.36-8.50 (m, 1 H), 9.17-9.29 (m, 1 H), 12.30 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 30.6, 33.0, 40.8, 123.3 (d, *J* = 45 Hz), 126.2, 128.7, 128.8, 140.8 (d, *J* = 233 Hz), 142.4, 149.9, 150.6, 157.4 (d, *J* = 30 Hz)ppm; MS (ESI, m/z): 290 $(M - H)^-$; Anal. calcd for $C_{14}H_{14}FN_3O_3$: C, 57.73; H, 4.84; N, 14.43; Found: C, 57.96; H, 4.84; N, 14.42.

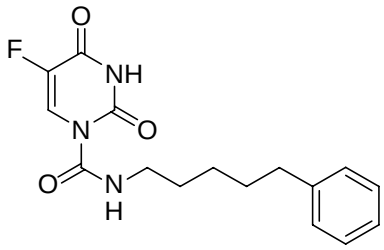
**5-Fluoro-2,4-dioxo-N-(4-phenylbutyl)-3,4-dihydropyrimidine-1(2H)-carboxamide (3k)**




Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of phenylbutyl isocyanate with uracil afforded **3k** (62 mg; yield: 41%) as white crystals; IR (film) $\nu_{max}$: 3297, 3082, 2917, 2849, 1745, 1694, 1537, 1336, 1267, 1200, 1096 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.53-1.60 (m, 4 H), 2.59 (t, *J* = 7.6 Hz, 2 H), 3.30-3.31 (m, 2 H), 7.17-7.21 (m, 3 H), 7.27 (t, *J* = 6.8 Hz, 2 H), 8.37 (d, *J* = 7.6 Hz, 1 H), 9.12 (br, 1 H), 12.26 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 28.6, 28.8, 35.2, 40.8, 123.3 (d, *J* = 40 Hz), 126.2, 128.7, 128.7, 141.1 (d, *J* = 230 Hz), 142.4, 149.9, 150.6, 157.4 (d, *J* = 30 Hz) ppm; MS (ESI, m/z): 304 $(M - H)^-$; Anal. calcd for $C_{15}H_{16}FN_3O_3$: C, 59.01; H, 5.28; N, 13.76; Found: C, 59.19; H, 5.29; N,

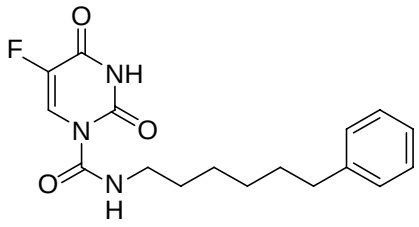
13.74.

**5-Fluoro-2,4-dioxo-N-(5-phenylpentyl)-3,4-dihydropyrimidine-1(2H)-carboxamide (3l)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of phenylpentyl isocyanate with uracil afforded **3l** (49 mg; yield: 31%) as white crystals; IR (film) $\nu_{max}$: 3300, 3083, 2817, 2849, 2808, 1724, 1688, 1536, 1439, 1337, 1273, 1097 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.28-1.35 (m, 2 H), 1.51-1.63 (m, 4 H), 2.57 (t, *J* = 8.0 Hz, 2 H), 3.24-3.29 (m, 2 H), 7.14-7.19 (m, 3 H), 7.24-7.28 (m, 2 H), 8.37 (d, *J* = 7.6 Hz, 1 H), 9.11 (t, *J* = 5.6 Hz, 1 H), 12.25 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 26.3, 28.9, 31.0, 35.5, 40.9, 123.3 (d, *J* = 40 Hz), 126.1, 128.7, 128.7, 141.3 (d, *J* = 240 Hz), 142.5, 149.9, 150.6, 157.5 (d, *J* = 30 Hz) ppm; MS (ESI, m/z): 318 $(M - H)^-$; Anal. calcd for $C_{16}H_{18}FN_3O_3$: C, 60.18; H, 5.68; N, 13.16; Found: C, 60.23; H, 5.69; N, 13.16.

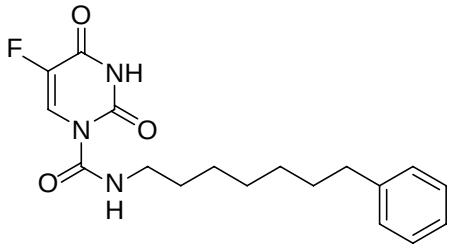
**5-Fluoro-2,4-dioxo-N-(6-phenylhexyl)-3,4-dihydropyrimidine-1(2H)-carboxamide (3m)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of

phenylhexyl isocyanate with uracil afforded **3m** (69 mg; yield: 42%) as white crystals; IR (film) $\nu_{max}$: 2917, 2849, 1745, 1690, 1578, 1537, 1389, 1278, 1028 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.30-1.36 (m, 4 H), 1.47-1.60 (m, 4 H), 2.56 (t, *J* = 8.0 Hz, 2 H), 3.24-3.28 (m, 2 H), 7.14-7.19 (m, 3 H), 7.24-7.28 (m, 2 H), 8.37 (d, *J* = 7.6 Hz, 1 H), 9.12 (t, *J* = 5.6 Hz, 1 H), 12.26 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 26.5, 28.7, 29.1, 31.3, 35.5, 40.9, 123.3 (d, *J* = 38 Hz), 126.0, 128.7, 128.7, 141.1 (d, *J* = 233 Hz), 142.7, 149.9, 150.7, 157.4 (d, *J* = 27 Hz) ppm; MS (ESI, m/z): 332 $(M-H)^-$; Anal. calcd for $C_{17}H_{20}FN_3O_3$: C, 61.25; H, 6.05; N, 12.61; Found: C, 61.03; H, 6.04; N, 12.63.

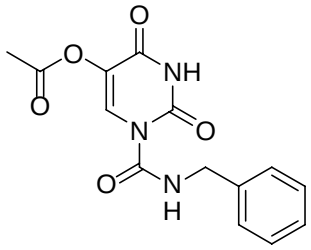
**5-Fluoro-2,4-dioxo-N-(7-phenylheptyl)-3,4-dihydropyrimidine-1(2H)-carboxamide (3n)**




Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of phenylheptyl isocyanate with uracil afforded **3n** (95 mg; yield: 55%) as white crystals; IR (film) $\nu_{max}$: 3301, 3075, 2820, 2850, 2800, 1722, 1685, 1540, 1440, 1338, 1275, 1100 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.29-1.35 (m, 6 H), 1.45-1.61 (m, 4 H), 2.55 (t, *J* = 7.6 Hz, 2 H), 3.23-3.27 (m, 2 H), 7.15-7.20 (m, 3 H), 7.25-7.29 (m, 2 H), 8.38 (d, *J* = 7.8 Hz, 1 H), 9.12 (t, *J* = 5.7 Hz, 1 H), 12.26 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 26.5, 28.5, 29.0, 29.2, 31.4, 35.5, 41.0, 123.4 (d, *J* = 33 Hz), 126.0, 128.7, 128.8, 141.2 (d, *J* = 230 Hz), 142.7, 149.9, 150.7, 157.5 (d, *J* = 30 Hz)

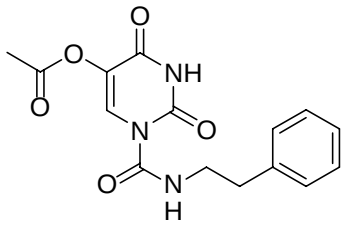
ppm; MS (ESI, m/z): 346 $(M - H)^-$; Anal. calcd for $C_{18}H_{22}FN_3O_3$: C, 62.23; H, 6.38; N, 12.10; Found: C, 62.11; H, 6.37; N, 12.11.

**1-(Benzylcarbamoyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3o)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:2), the addition of benzyl isocyanate with uracil afforded **3o** (20 mg; yield: 13%) as white crystals; IR (film) $\nu_{max}$: 3313, 2917, 2849, 1774, 1743, 1702, 1536, 1452, 1269, 1189 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.23 (s, 3 H), 4.50 (d, *J* = 5.6 Hz, 2 H), 7.21-7.32 (m, 4 H), 8.29 (s, 1 H), 9.56 (t, *J* = 6.0 Hz, 1 H), 12.19 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 20.5, 43.4, 44.5, 127.0, 127.5, 127.6, 127.8, 128.1, 128.7, 128.9, 130.4, 138.6, 141.3, 150.3, 150.9, 158.6, 158.7, 168.8 ppm; MS (ESI, m/z): 302 $(M - H)^-$; Anal. calcd for $C_{14}H_{13}N_3O_5$: C, 55.45; H, 4.32; N, 13.86; Found: C, 55.26; H, 4.32; N, 13.88.

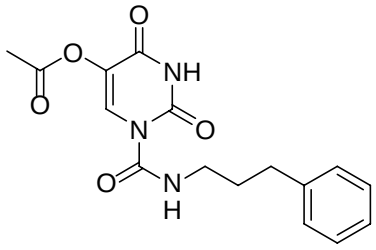
**2,4-Dioxo-1-(phenethylcarbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3p)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of phenethyl isocyanate with uracil afforded **3p** (23 mg; yield: 15%) as white crystals; IR (film) $\nu_{max}$: 2917, 2849, 1774, 1745, 1536, 1269, 1098 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 3 H), 2.93 (t, *J* = 7.2 Hz, 2 H), 3.64-3.69 (m, 2 H), 7.23-7.35 (m, 5

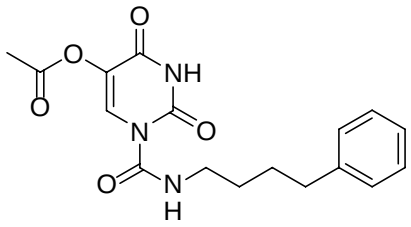
H), 8.39 (s, 1 H), 9.11 (t, $J$ = 5.6 Hz, 1 H), 9.65 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 20.2, 35.4, 42.7, 126.8, 128.3, 128.7, 130.2, 138.1, 149.3, 150.5, 158.0, 168.4 ppm; MS (ESI, m/z): 316 $(M - H)^-$; Anal. calcd for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24; Found: C, 56.95; H, 4.77; N, 13.22.

**2,4-Dioxo-1-((3-phenylpropyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3q)**



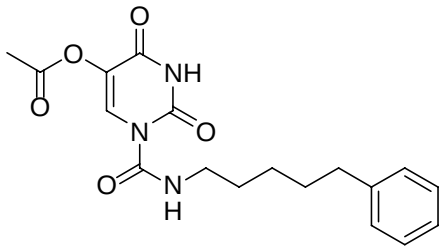

Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of phenylpropyl isocyanate with uracil afforded **3q** (14 mg; yield: 9%) as white crystals; IR (film) $\nu_{max}$: 2953, 2917, 1745, 1577, 1540, 1267, 1181, 1099 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 1.90-1.98 (m, 2 H), 2.30 (s, 3 H), 2.68 (t, $J$ = 7.6 Hz, 2 H), 3.38-3.43 (m, 2 H), 7.17-7.20 (m, 3 H), 7.26-7.30 (m, 2 H), 8.37 (s, 1 H), 9.09 (t, $J$ = 5.6 Hz, 1 H), 9.70 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 20.2, 30.6, 33.0, 40.8, 126.1, 128.3, 128.4, 128.5, 130.2, 140.9, 149.3, 150.6, 158.1, 168.4 ppm; MS (ESI, m/z): 330 $(M - H)^-$; Anal. calcd for $C_{16}H_{17}N_3O_5$: C, 58.00; H, 5.17; N, 12.68; Found: C, 58.22; H, 5.18; N, 12.65.

**2,4-Dioxo-1-((4-phenylbutyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3r)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of phenylbutyl isocyanate with uracil afforded **3r** (19 mg; yield: 11%) as white crystals; IR (film) $\nu_{max}$: 3086, 2918, 2849, 1779, 1747, 1696, 1537, 1436, 1334, 1268, 1188, 1096 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.52-1.63 (m, 4 H), 2.23 (s, 3 H), 2.60 (t, *J* = 7.6 Hz, 2 H), 3.28-3.33 (m, 2 H), 7.14-7.21 (m, 3 H), 7.24-7.29 (m, 2 H), 8.26 (br, 1 H), 9.12 (t, *J* = 6.0 Hz, 1 H), 12.17 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 20.5, 28.6, 28.8, 35.2, 40.8, 126.1, 128.0, 128.7, 128.7, 130.4, 142.4, 150.0, 151.0, 158.7, 168.8 ppm; MS (ESI, m/z): 344 $(M - H)^{-}$; Anal. calcd for $C_{17}H_{19}N_3O_5$: C, 59.12; H, 5.55; N, 12.17; Found: C, 59.33; H, 5.54; N, 12.15.

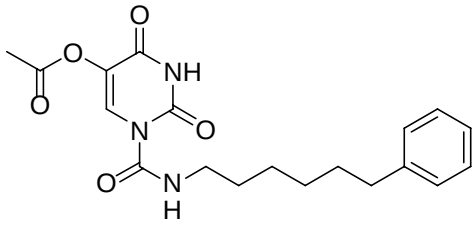
**2,4-Dioxo-1-((5-phenylpentyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3s)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:5), the addition of phenylpentyl isocyanate with uracil afforded **3s** (18 mg; yield: 10%) as white crystals; IR (film) $\nu_{max}$: 2918, 2849, 1747, 1696, 1537, 1436, 1334, 1268, 1188, 1096 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.24-1.39 (m, 2 H), 1.52-1.62 (m, 4 H), 2.23 (s, 3 H), 2.57 (t, *J* = 7.6 Hz, 2 H), 3.25-3.29 (m, 2 H), 7.14-7.19 (m, 3 H), 7.24-7.28 (m, 2 H),

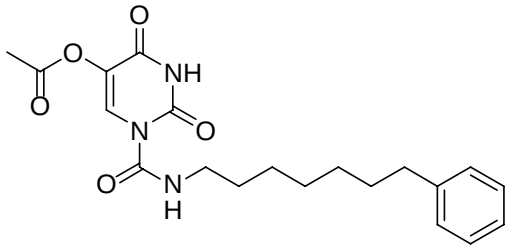
8.27 (br, 1 H), 9.10 (t, *J* = 5.6 Hz, 1 H), 12.17 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, DMSO-d6) δ 20.5, 26.2, 28.9, 31.0, 35.5, 40.9, 126.1, 128.1, 128.7, 128.7, 130.4, 142.6, 150.0, 151.0, 158.7, 168.8 ppm; MS (ESI, m/z): 358 $(M - H)^-$; Anal. calcd for $C_{18}H_{21}N_3O_5$: C, 60.16; H, 5.89; N, 11.69; Found: C, 60.34; H, 5.90; N, 11.67.

**2,4-Dioxo-1-((6-phenylhexyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3t)**



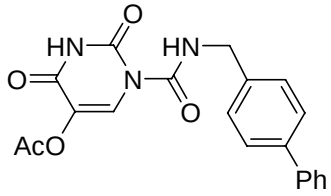
Following the **General Procedure B** (eluent: EtOAc/PE 1:7), the addition of phenylhexyl isocyanate with uracil afforded **3t** (29 mg; yield: 16%) as white crystals; IR (film) $\nu_{max}$: 3025, 2929, 2855, 1747, 1698, 1536, 1452, 1339, 1269, 1189, 1097 $cm^{-1}$; $^1$H NMR (400 MHz, DMSO-d6) δ 1.27-1.32 (m, 4 H), 1.48-1.60 (m, 4 H), 2.23 (s, 3 H), 2.56 (t, *J* = 7.6 Hz, 2 H), 3.24-3.29 (m, 2 H), 7.14-7.19 (m, 3 H), 7.26 (t, *J* = 7.6 Hz, 2 H), 8.27 (s, 1 H), 9.10 (t, *J* = 5.6 Hz, 1 H), 12.18 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, DMSO-d6) δ 20.5, 26.5, 28.7, 31.3, 35.5, 41.0, 126.0, 128.1, 128.7, 128.7, 130.4, 142.7, 150.0, 151.0, 158.7, 168.8 ppm; MS (ESI, m/z): 372 $(M - H)^-$; Anal. calcd for $C_{19}H_{23}N_3O_5$: C, 61.11; H, 6.21; N, 11.25; Found: C, 60.92; H, 6.22; N, 11.23.

**2,4-Dioxo-1-((7-phenylheptyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3u)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:7), the addition of phenylheptyl isocyanate with uracil afforded **3u** (46 mg; yield: 24%) as white crystals; IR (film) $\nu_{max}$: 3300, 3025, 2930, 2855, 1750, 1700, 1533, 1450, 1340, 1270, 1190, 1097 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.27-1.32 (m, 6 H), 1.45-1.57 (m, 4 H), 2.24 (s, 3 H), 2.57 (t, *J* = 7.8 Hz, 2 H), 3.25-3.32 (m, 2 H), 7.15-7.20 (m, 3 H), 7.30 (t, *J* = 7.8 Hz, 2 H), 8.30 (s, 1 H), 9.11 (t, *J* = 5.8 Hz, 1 H), 12.20 (s, 1 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 20.4, 26.5, 28.6, 29.2, 31.5, 35.5, 41.0, 126.1, 128.2, 128.8, 128.9, 130.4, 142.7, 149.8, 151.2, 159.1, 168.9 ppm; MS (ESI, m/z): 386 $(M-H)^-$; Anal. calcd for $C_{20}H_{25}N_3O_5$: C, 62.00; H, 6.50; N, 10.85; Found: C, 61.90; H, 6.5z; N, 10.84.

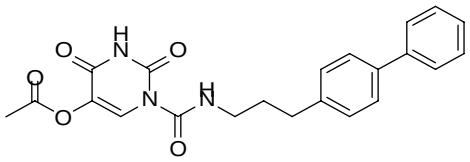
**N-phenylbenzyl-5-fluoro-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (4a)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:3), the addition of phenylbenzyl isocyanate with uracil afforded **4a** (15 mg; yield: 8%) as white crystals; IR (film) $\nu_{max}$: 2917, 2849, 1738, 1692, 1673, 1578, 1537, 1335, 1268, 1204 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 2.23 (s, 3 H), 4.54 (d, *J* = 6.0 Hz, 2 H), 7.36 (dddd, *J*

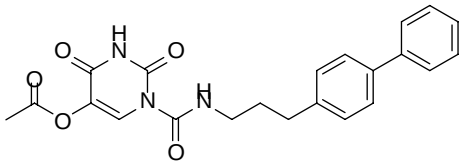
= 7.6, 7.6, 2.0, 2.0 Hz, 1 H), 7.43-7.48 (m, 4 H), 7.63-7.66 (m, 4 H), 8.30 (s, 1 H), 9.59 (t, *J* = 5.6 Hz, 1 H), 12.20 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, DMSO-d6) δ 20.6, 44.3, 127.1, 127.2, 127.9, 128.2, 128.4, 129.4, 130.4, 137.9, 139.6, 140.4, 150.3, 150.9, 158.7, 168.8 ppm; MS (ESI, m/z): 378 $(M - H)^-$; Anal. calcd for $C_{20}H_{17}N_3O_5$: C, 63.32; H, 4.52; N, 11.08; Found: C, 63.45; H, 4.52; N, 11.09.

**2,4-Dioxo-1-(biphenethylcarbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (4b)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:6), the addition of biphenethyl isocyanate with uracil afforded **4b** (24 mg; yield: 12%) as white crystals; IR (film) $\nu_{max}$: 3282, 2917, 2849, 1777, 1744, 1696, 1673, 1537, 1268, 1188, 1095 $cm^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) δ 2.23 (s, 3 H), 2.89 (t, *J* = 7.2 Hz, 2 H), 3.55-3.58 (m, 2 H), 7.35-7.37 (m, 3 H), 7.46 (t, *J* = 8.0 Hz, 2 H), 7.61 (t, *J* = 8.0 Hz, 2 H), 7.65 (t, *J* = 8.0 Hz, 2 H), 8.29 (s, 1 H), 9.22 (t, *J* = 5.6 Hz, 1 H), 12.19 (s, 1 H) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 20.6, 34.8, 42.5, 127.0, 127.2, 127.7, 128.1, 129.4, 129.8, 130.2, 138.6, 138.7, 140.4, 150.0, 151.0, 158.6, 168.8 ppm; MS (ESI, m/z): 392 $(M - H)^-$; Anal. calcd for $C_{21}H_{19}N_3O_5$: C, 64.12; H, 4.87; N, 10.68; Found: C, 64.01; H, 4.87; N, 10.68.

**2,4-Dioxo-1-((3-biphenylpropyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (4c)**



Following the **General Procedure B** (eluent: EtOAc/PE 1:6), the addition of biphenylpropyl isocyanate with uracil afforded **4c** (33 mg; yield: 16%)) as white crystals; IR (film) $\nu_{max}$: 3279, 2917, 2849, 1745, 1692, 1664, 1537, 1335, 1268, 1189 $cm^{-1}$; $^{1}H$ NMR (400 MHz, DMSO-d6) δ 1.95-2.02 (m, 2 H), 2.31 (s, 3 H), 2.73 (t, *J* = 8.0 Hz, 2 H), 3.41-3.46 (m, 2 H), 7.25-7.27 (m, 2 H), 7.32 (dddd, *J* = 7.4, 7.4, 1.2, 1.2 Hz, 1 H), 7.42 (t, *J* = 7.2 Hz, 2 H), 7.52 (d, *J* = 8.0 Hz, 2 H), 7.57 (d, *J* = 7.2 Hz, 2 H), 8.38 (br, 1 H), 9.08-9.11 (m, 2 H) ppm; $^{13}C$ NMR (100 MHz, DMSO-d6) δ 20.2, 30.5, 32.6, 40.8, 126.9, 127.1, 127.2, 128.2, 128.7, 128.8, 130.1, 139.1, 139.9, 140.9, 149.2, 150.4, 157.6, 168.3 ppm; MS (ESI, m/z): 406 $(M - H)^{-}$; Anal. calcd for $C_{22}H_{21}N_3O_5$: C, 64.86; H, 5.20; N, 10.31; Found: C, 64.73; H, 5.20; N, 10.30.

***Cell culture.***

HEK293 cells overexpressing or rat FAAH (HEK293–rFAAH), human FAAH (HEK293–hFAAH), rat MAGL (HEK293–rMAGL), rat AC (HEK293–rAC), or rat NAAA (HEK293–rNAAA) were kind gifts from Dr. Daniele Piomelli of the University of California, Irvine, CA, USA. The stable overexpressing cell lines were maintained in Dulbecco's modified Eagle medium (DMEM, Hyclone, Beijing, China) supplemented with 10% fetal bovine serum (FBS, Gibco®, Shanghai, China) containing 0.3 mg/mL G418 in a humidified 5% $CO_2$ atmosphere at 37 °C. RAW264.7 cells were plated and cultured overnight until 80% confluence and then incubated with a series of different concentrations of compounds for 30 min before being challenged with lipopolysaccharide.

***Protein Preparation and Enzymatic Assay.***

HEK293–rNAAA, HEK293–rFAAH, HEK293–rMAGL or HEK293–rAC cells were harvested, washed with phosphate-buffered saline, sonicated in 20 mM Tris–HCl (pH 7.5) containing 0.32 M sucrose, and centrifuged at 800 × *g* for 15 min at 4 °C. The supernatants were collected, and the protein concentrations were measured by a BCA protein assay kit (Pierce, Shanghai, China). NAAA activity was measured by incubating 30 μg of recombinant rNAAA protein at 37 °C for 30 min in 0.2 mL of phosphate buffer (50 mM, pH 5.0) containing 0.1% Triton X-100 and 3 mM dithiothreitol (DTT), 25 μM $d_4$-PEA as the substrate, and the test compound. FAAH activity was measured by incubating 30 μg of recombinant rFAAH protein at 37 °C for 30 min in 0.2 mL of Tris–HCl buffer (50 mM, pH 8.0) containing fatty acid-free bovine serum albumin (0.05%), 25 μM anandamide as the substrate, and the test compound. MAGL activity was measured by incubating 30 μg of recombinant rMAGL protein at 37 °C for 30 min in 0.2 mL of Tris–HCl buffer (50 mM, pH 8.0) containing fatty acid-free bovine serum albumin (0.05%), 100 μM 2-AG as the substrate, and the test compound. AC activity was measured by incubating 100 μg of recombinant rAC protein at 37 °C for 30 min in 0.2 mL of phosphate buffer (50 mM, pH 5.0) containing 0.1% Triton X-100 and 3 mM DTT, 100 μM N-lauroylceramide as the substrate, and the test compound. The reactions were terminated by adding 0.2 mL of methanol containing 1 nmol heptadecanoic acid, and the products were analyzed by LC/MS.

***LC/MS***

We used an Agilent 1200-LC system coupled to a 3200Q TRAP-MS detector equipped with an ESI interface (Agilent Technologies, Shanghai, China). Fatty acids were eluted isocratically on an XDB Eclipse C18 column (4.6 × 50 mm i.d., 1.8 μm, Agilent Technologies) at 0.6 mL/min for 4 min with a solvent mixture of 95%

methanol and 5% water, both containing 0.25% acetic acid and 5 mM ammonium acetate. The column temperature was set at 40 °C. ESI was set in the negative mode, and capillary voltage was set at –4.5 kV. Heptadecanoic acid was used as an internal standard (*m/z* = 267 for heptadecenoic acid, *m/z* = 303 for arachidonic acid, and *m/z* = 269 for heptadecanoic acid). Lipids were separated using an XDB Eclipse C18 column and eluted with a gradient of methanol in water (from 85% to 100% methanol over 5 min, held at 100% methanol for 10 min) at a flow rate of 1 mL/min. The column temperature was kept at 25 °C. MS detection was carried out in positive-ion atmospheric pressure chemical ionization mode and monitored in multiple reaction monitoring mode. The parameters were set as follows: curtain gas at 30 psi; nebulizer pressure at 60 psi; and temperature at 275 °C. The molecular ions were monitored at the transitions of *m/z* 300.20–62.00 for PEA, *m/z* 304.10–66.00 for $d_4$-PEA, *m/z* 348.00-62.00 for AEA, *m/z* 379.10-287.10 for 2-AG. Quantifications were based on chromatographic peak areas calculated by using Analyst® version 1.4.1. software (Applied Biosystems).

***Dialysis assay***

The dialysis assay was performed using Slide-A-Lyzer Dialysis Cassettes (Pierce, Shanghai, China). Briefly, 2 mg of NAAA protein was incubated with **4c** or DMSO in 4 mL of Tris–HCl buffer (50 mM, pH 8.0) for 10 min at 37 °C. The mixed reaction solution was loaded onto a dialysis cartridge using a syringe and incubated in Tris–HCl buffer (50 mM, pH 8.0) at 4 °C for 8 h. The samples were removed from the dialysis cassettes by syringes for the NAAA enzymatic assay.

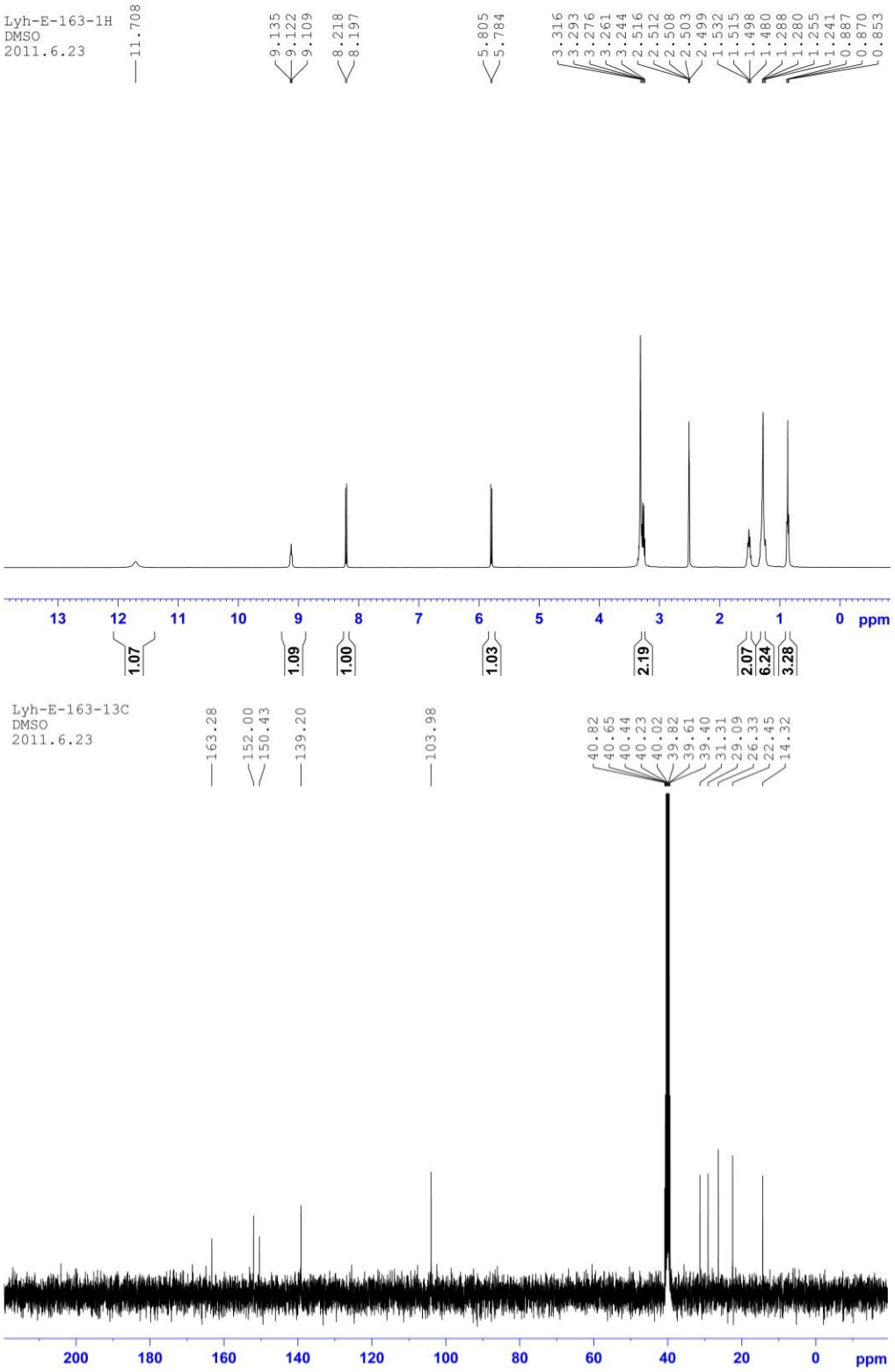
***Rapid dilution assay***

The rapid dilution assay was performed as described previously[24]. Briefly, the samples containing 100-fold-concentrated rFAAH protein were pre-incubated with

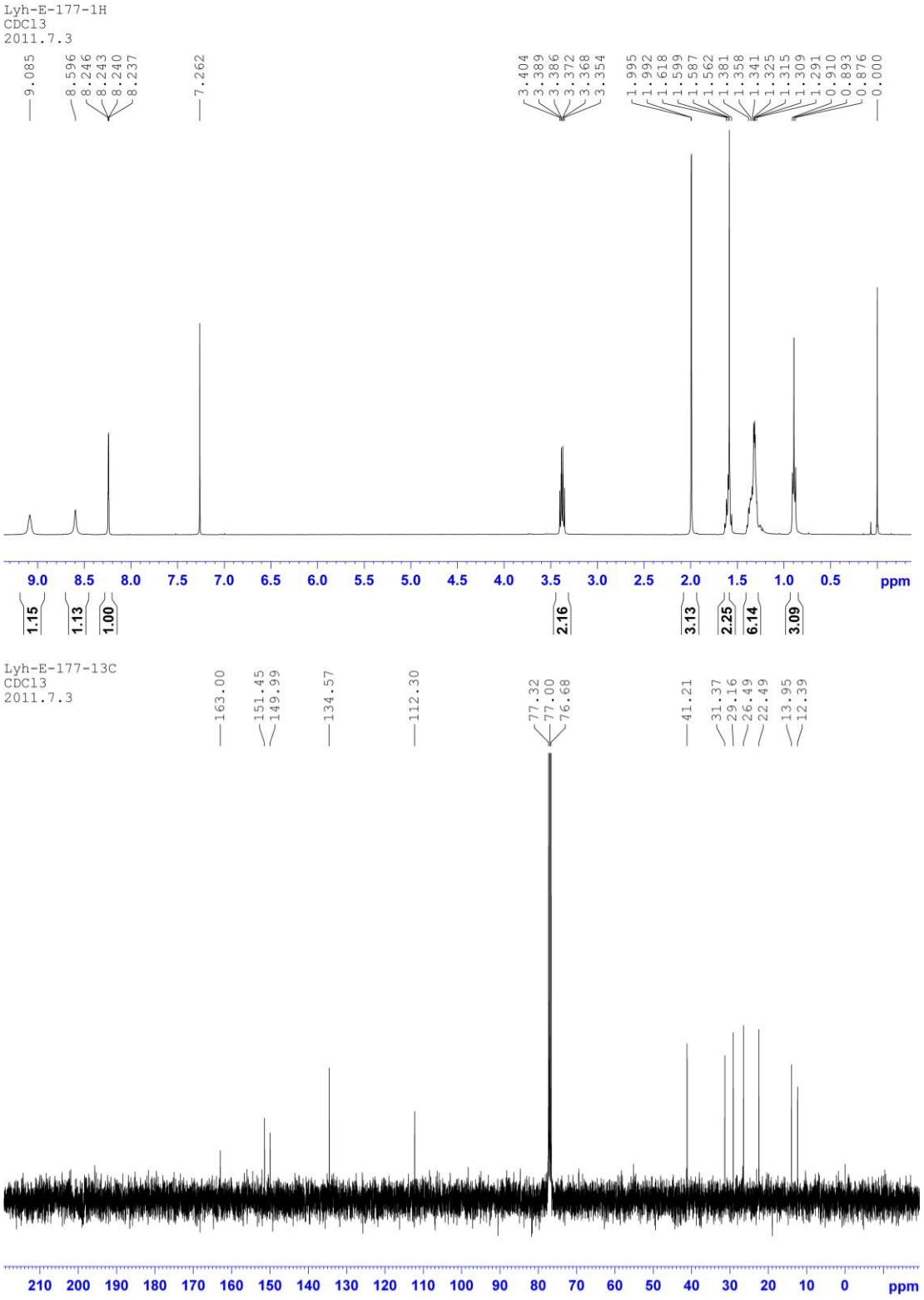
10-fold of the $IC_{50}$-equivalent concentration of compounds or vehicle (1% DMSO) for 10 min at 37 °C. The samples were then diluted 100-fold with assay buffer containing substrate to initiate the reactions, and the time course of product formation was measured by LC/MS.

## Supplementary $^{1}$H and $^{13}$C NMR spectra

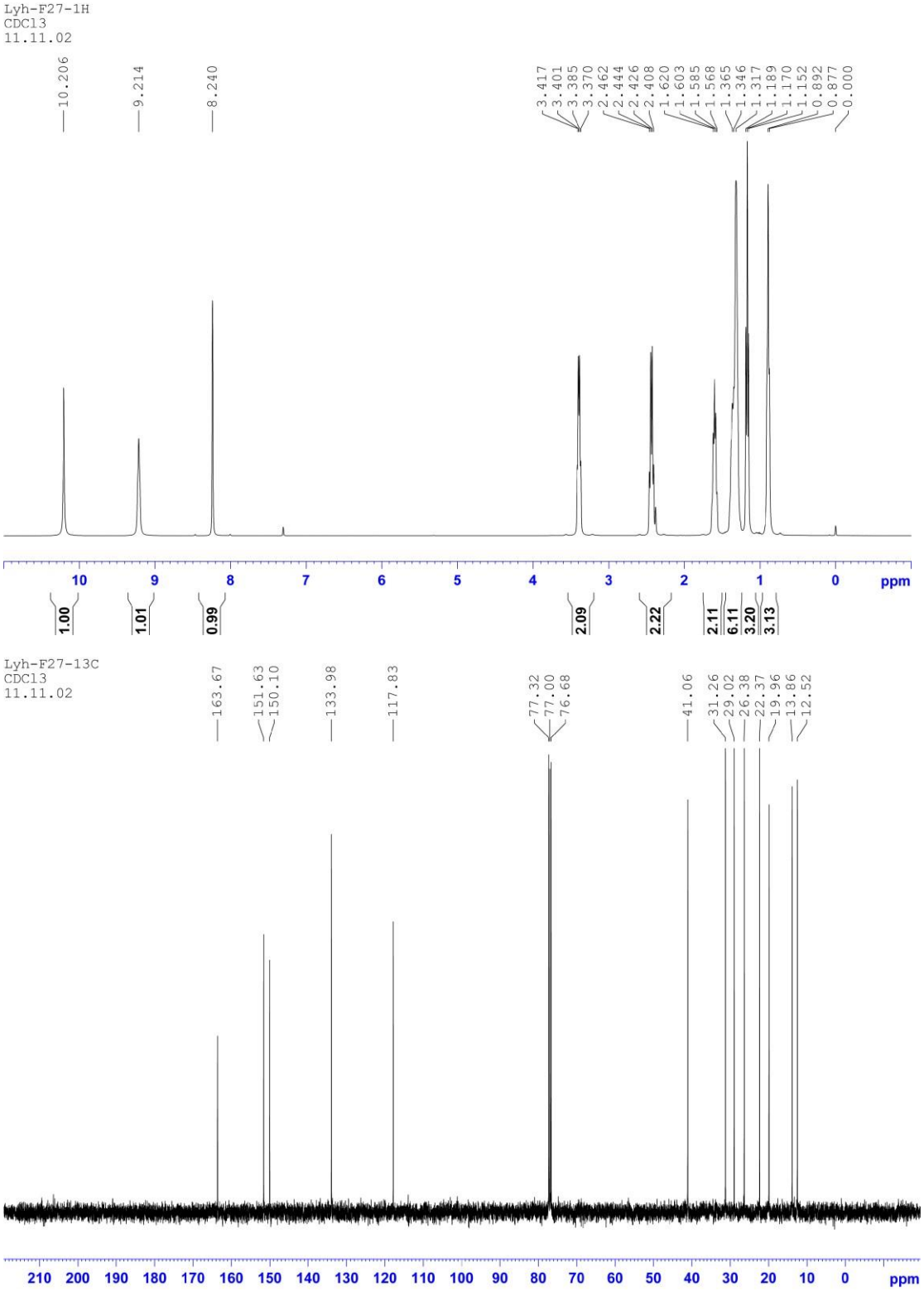
### Hexyl-**2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (1a)**



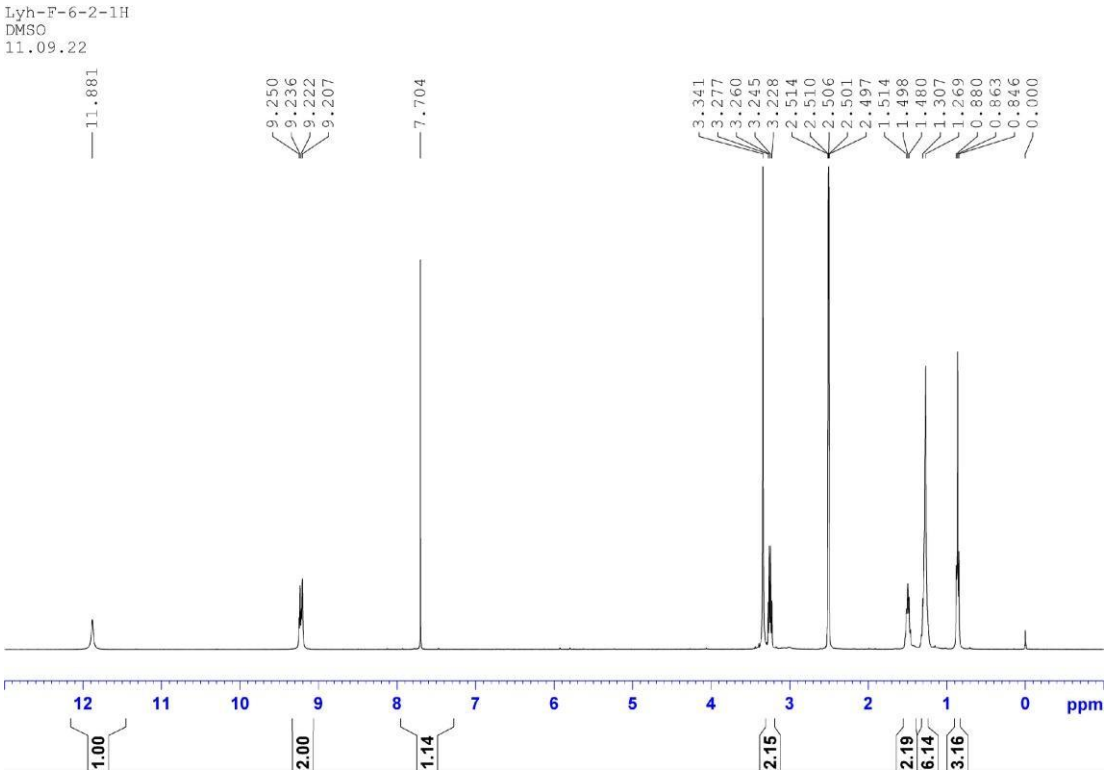
Hexyl-**5-methyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (1c)**

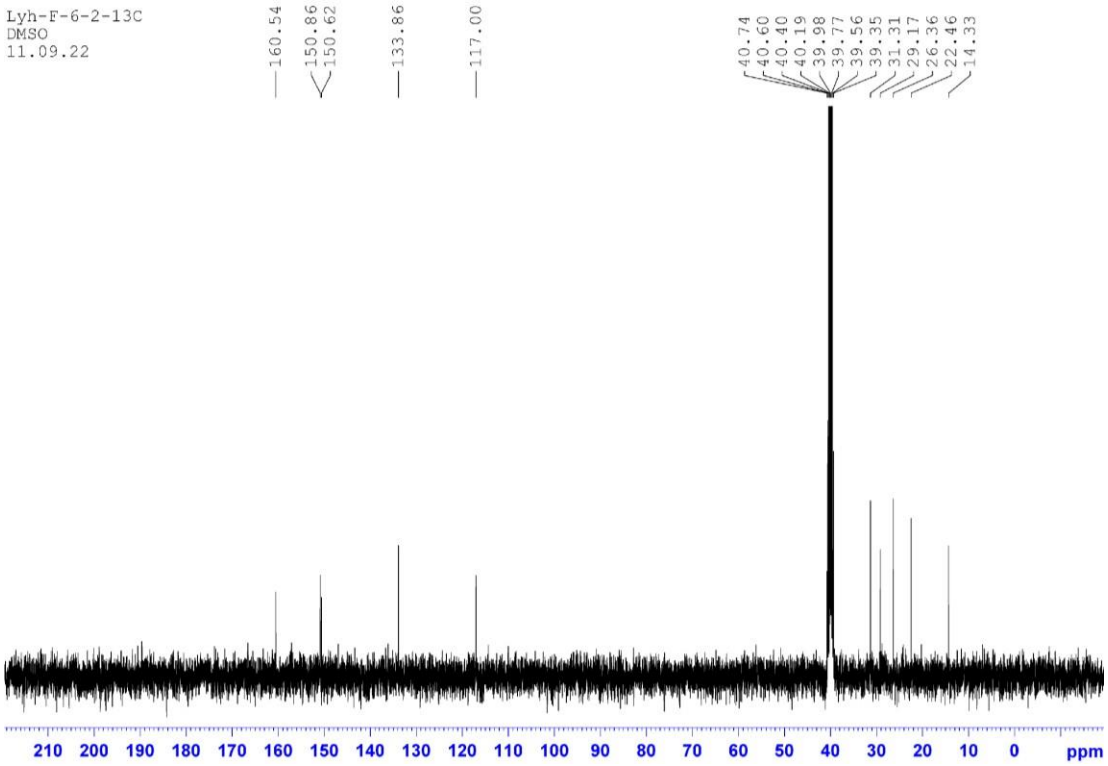


**5-ethyl-**Hexyl-**2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2a)**

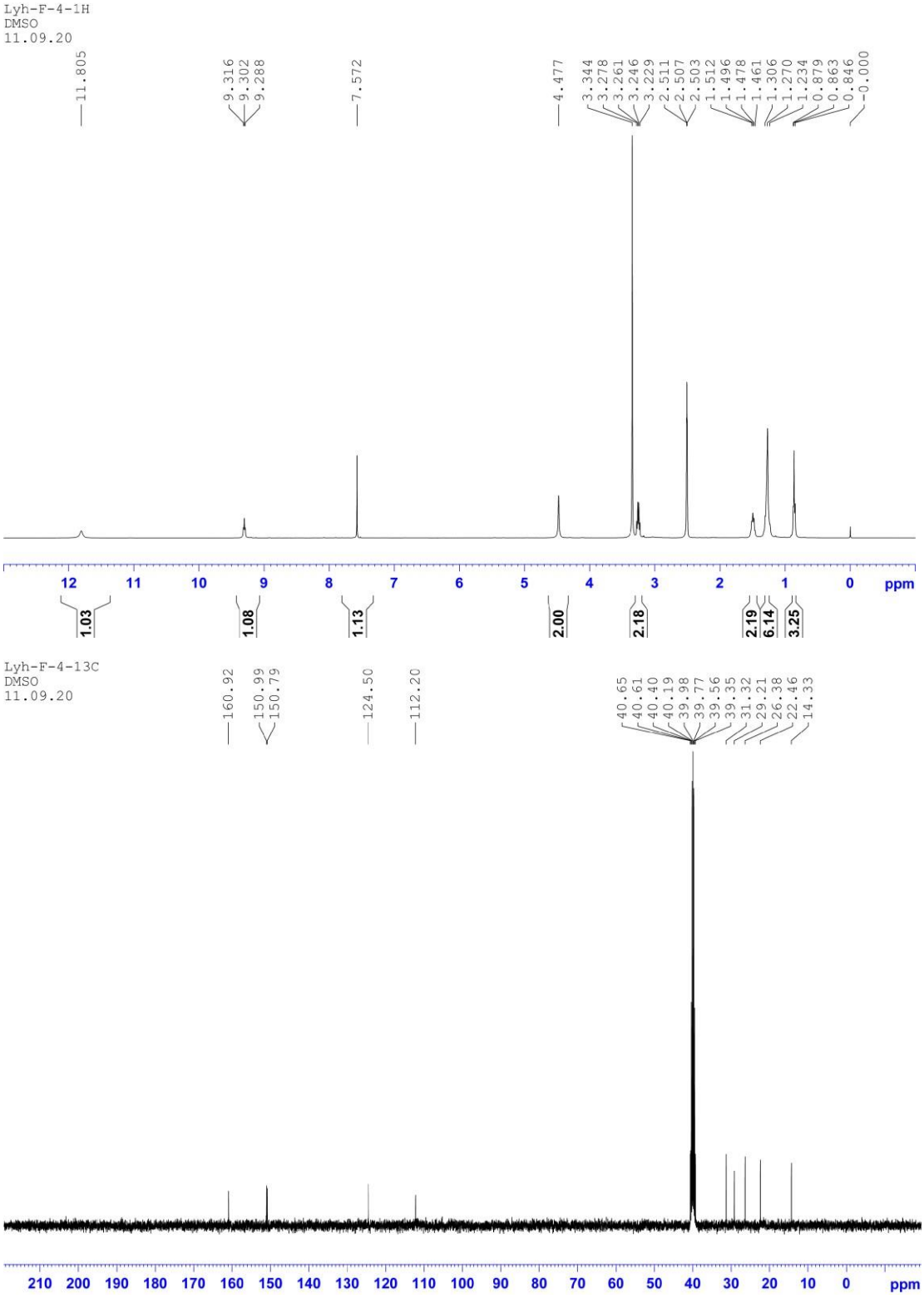


Hexyl-**5-hydroxy-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2b)**

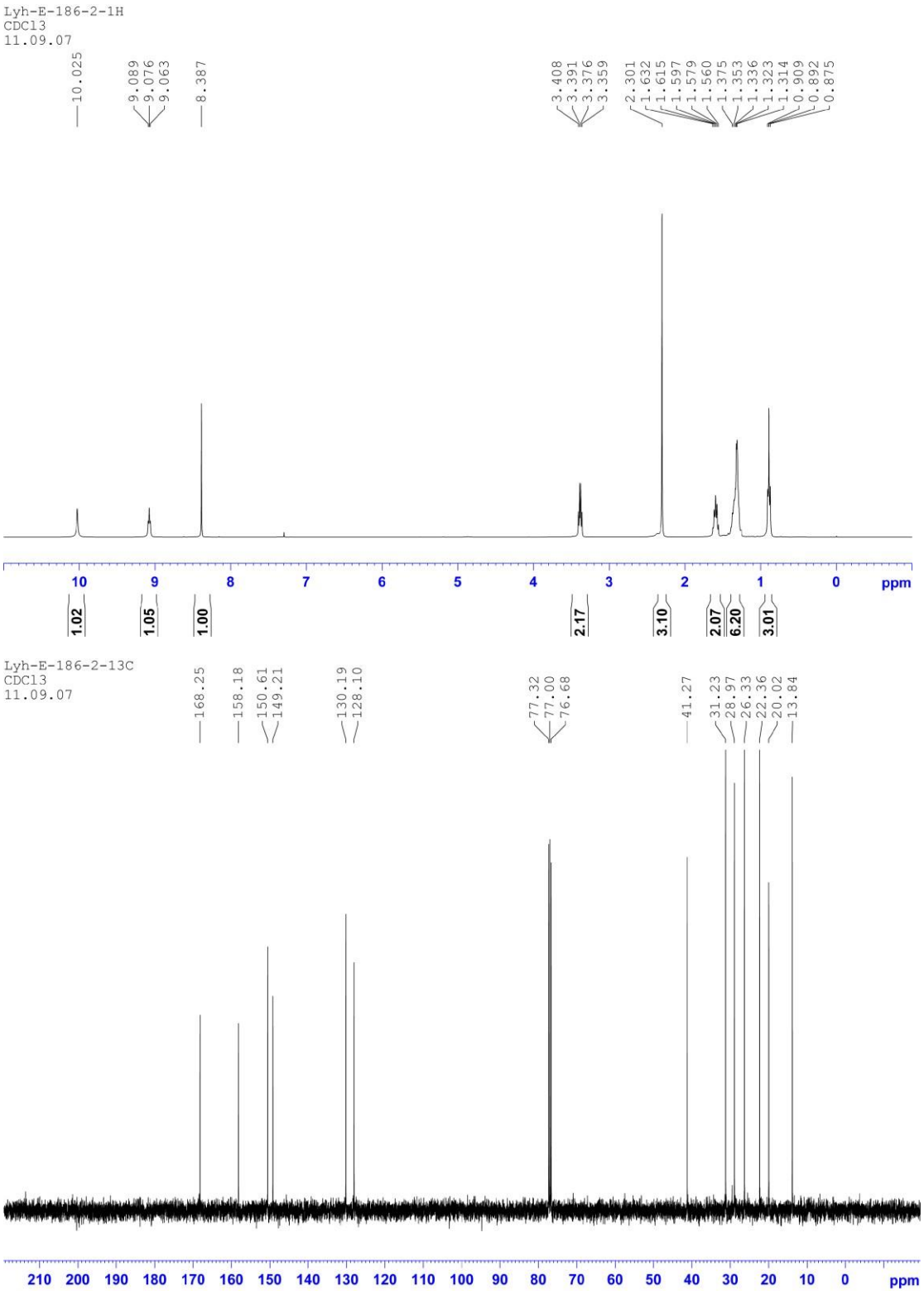


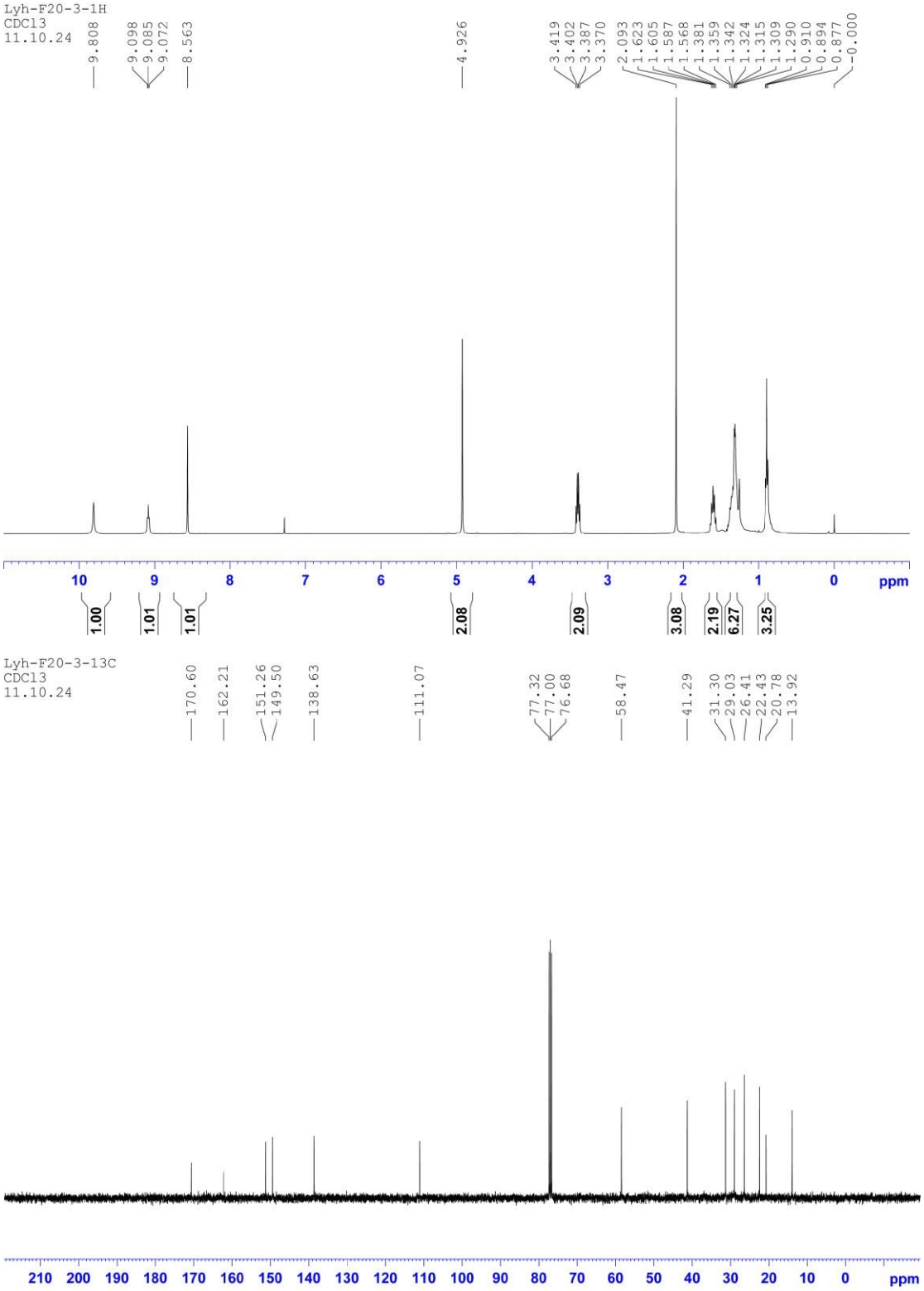
**5-amino-**Hexyl-**2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2e)**



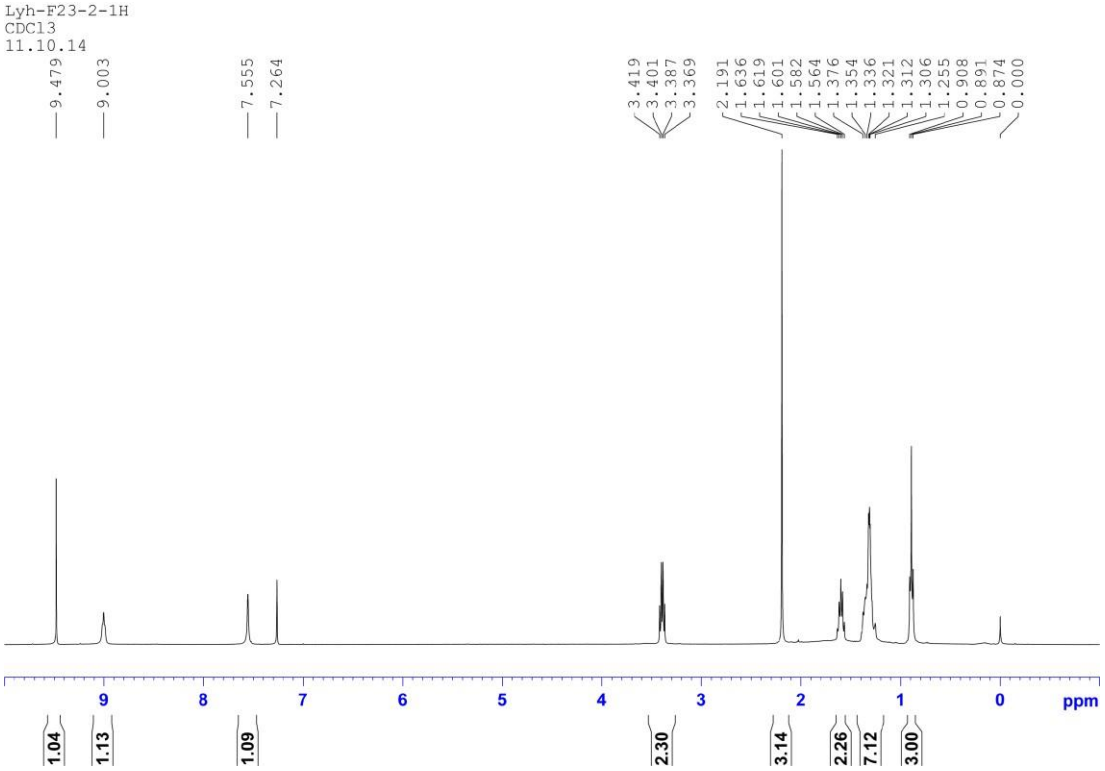
## 1-(hexylcarbamoyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl acetate (2f)

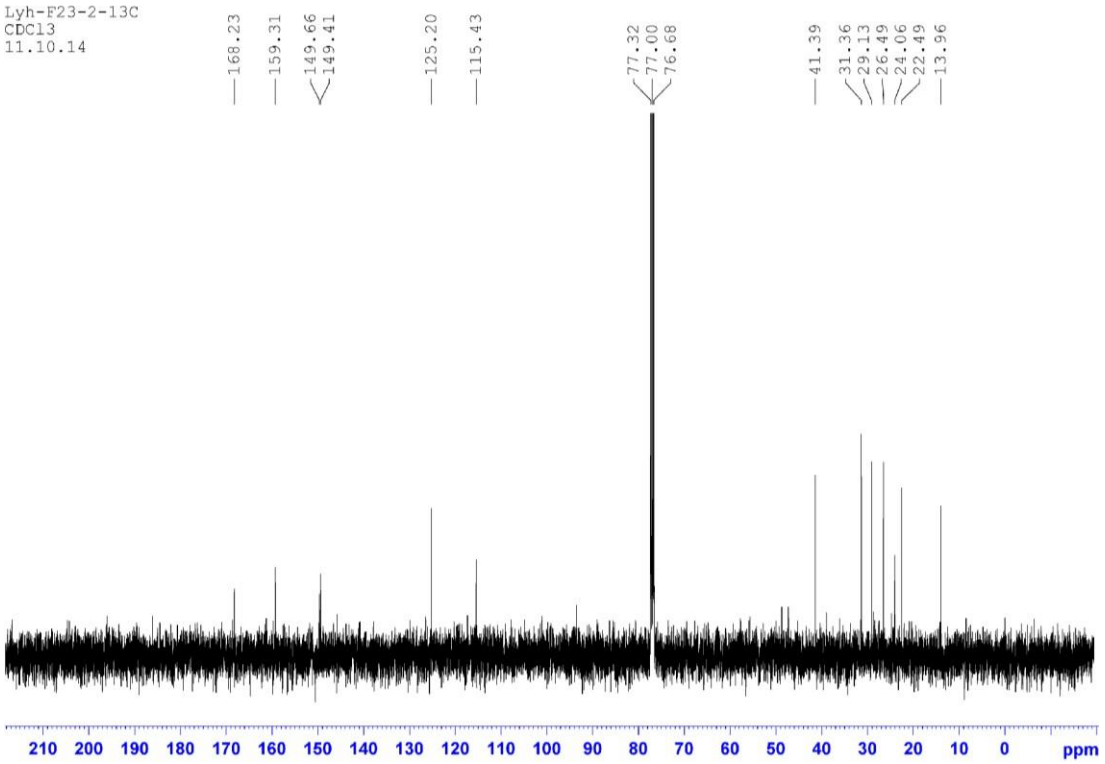


**(1-(hexylcarbamoyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl acetate (2g)**

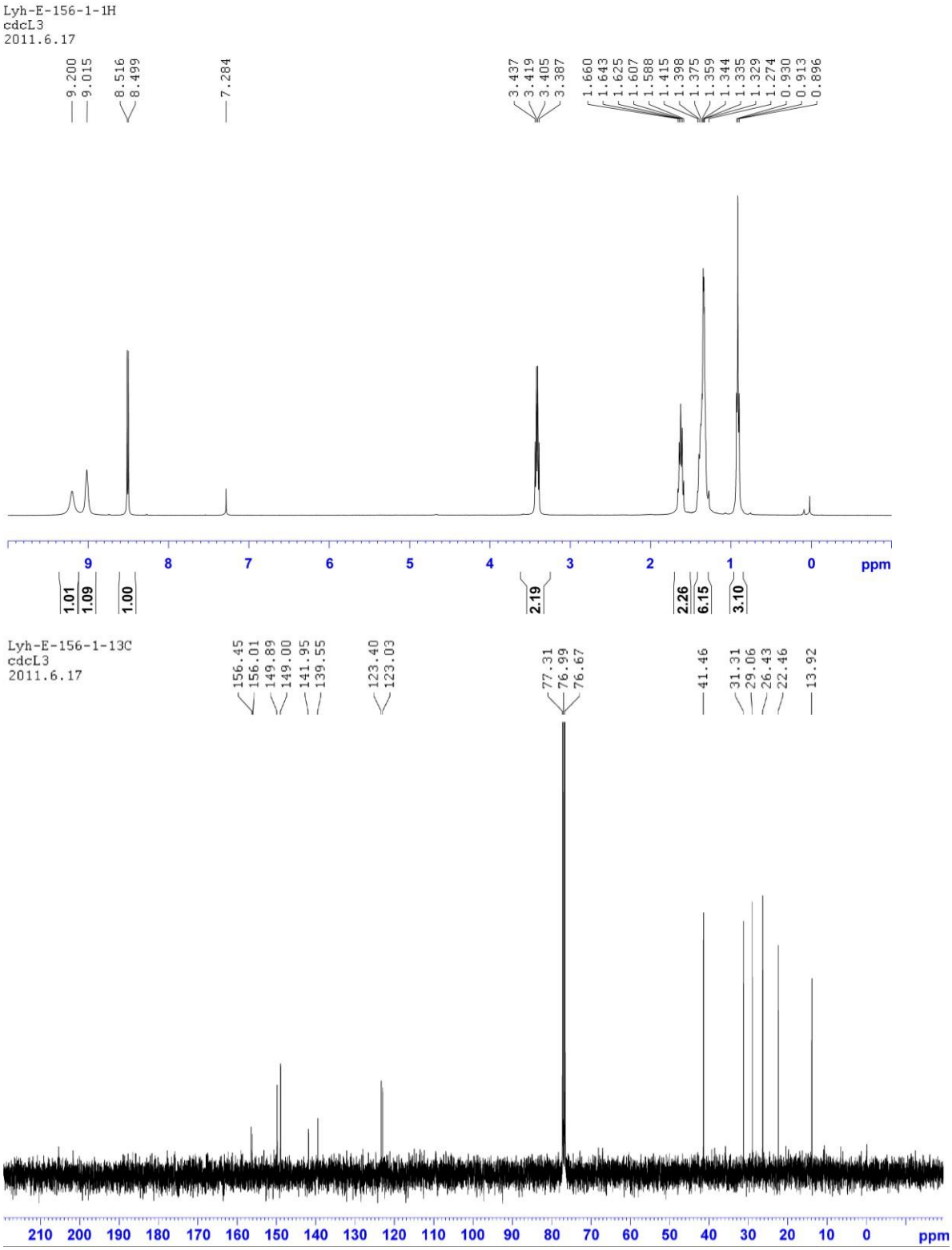


**5-acetamido-**Hexyl-**2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2h)**

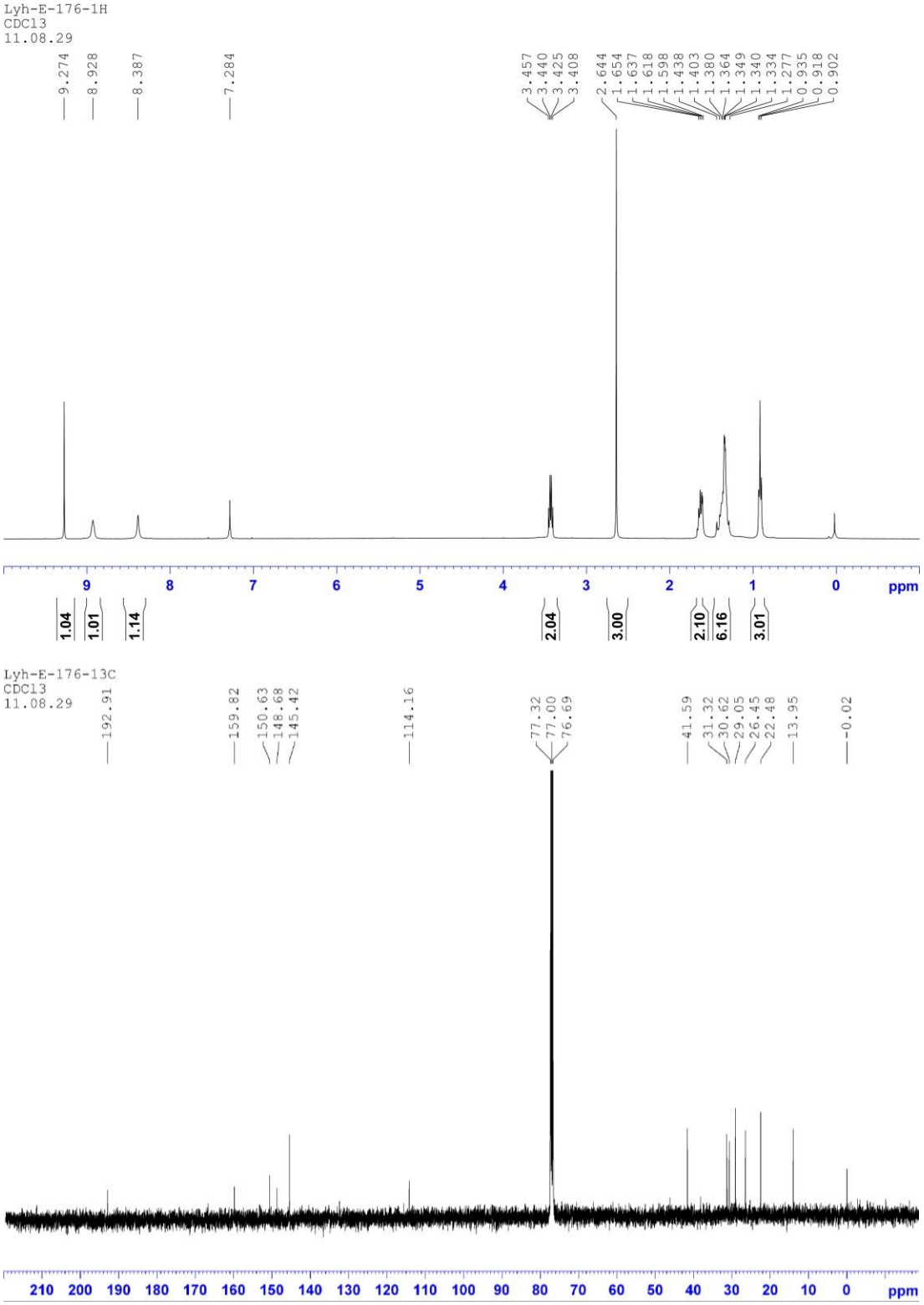


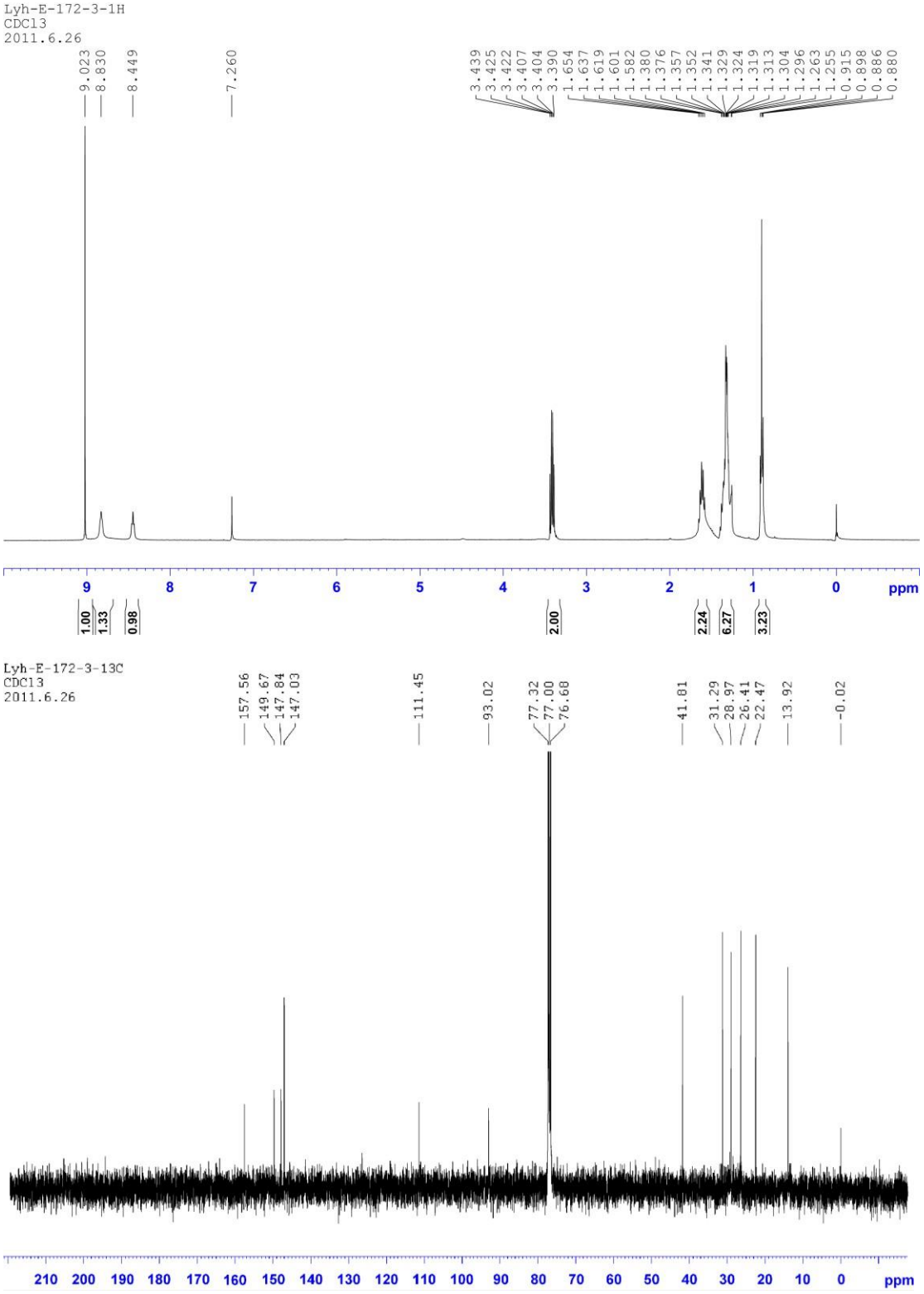
**5-fluoro-**Hexyl-**2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2i)**



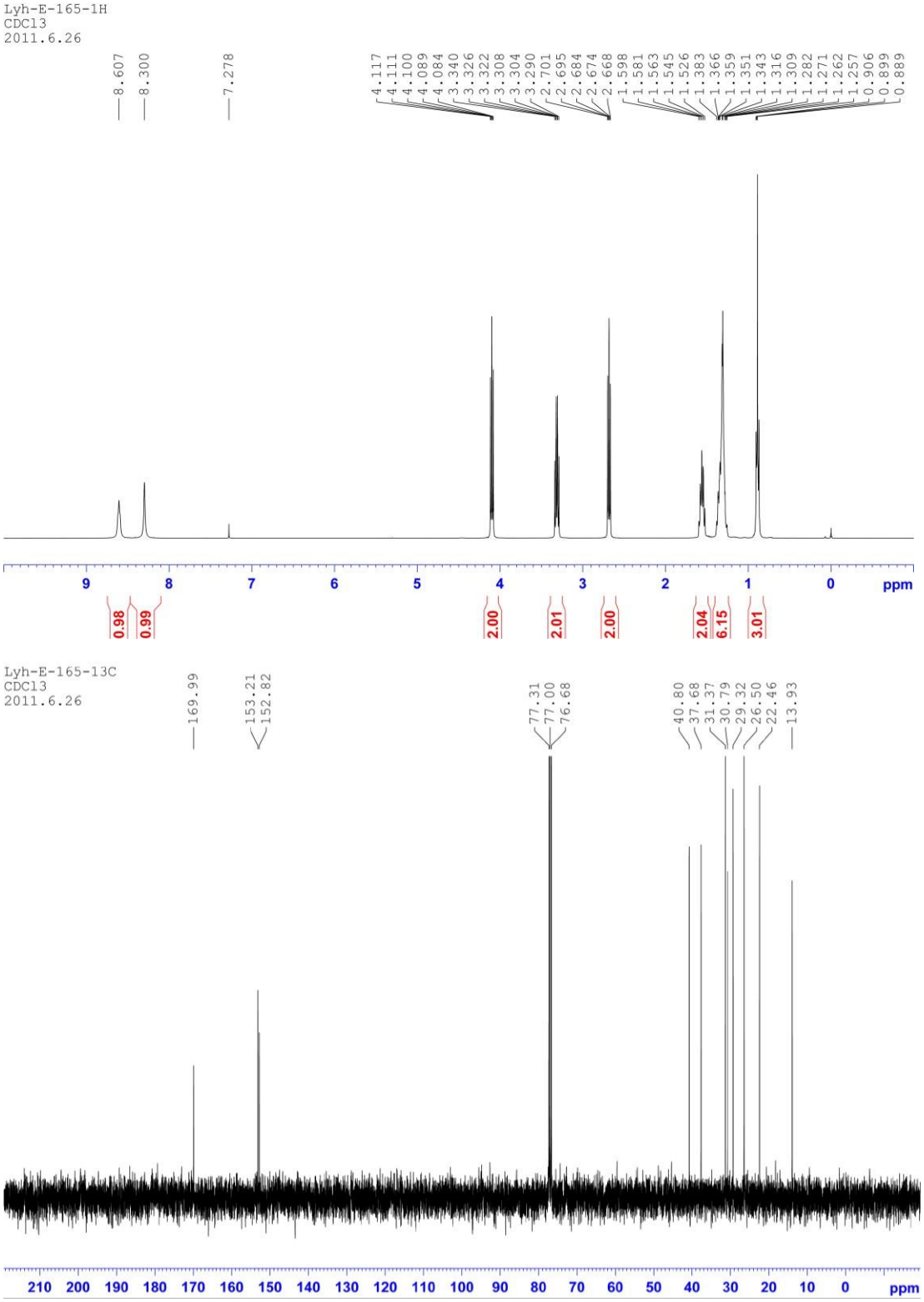
**5-acetyl-**Hexyl-**2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2j)**



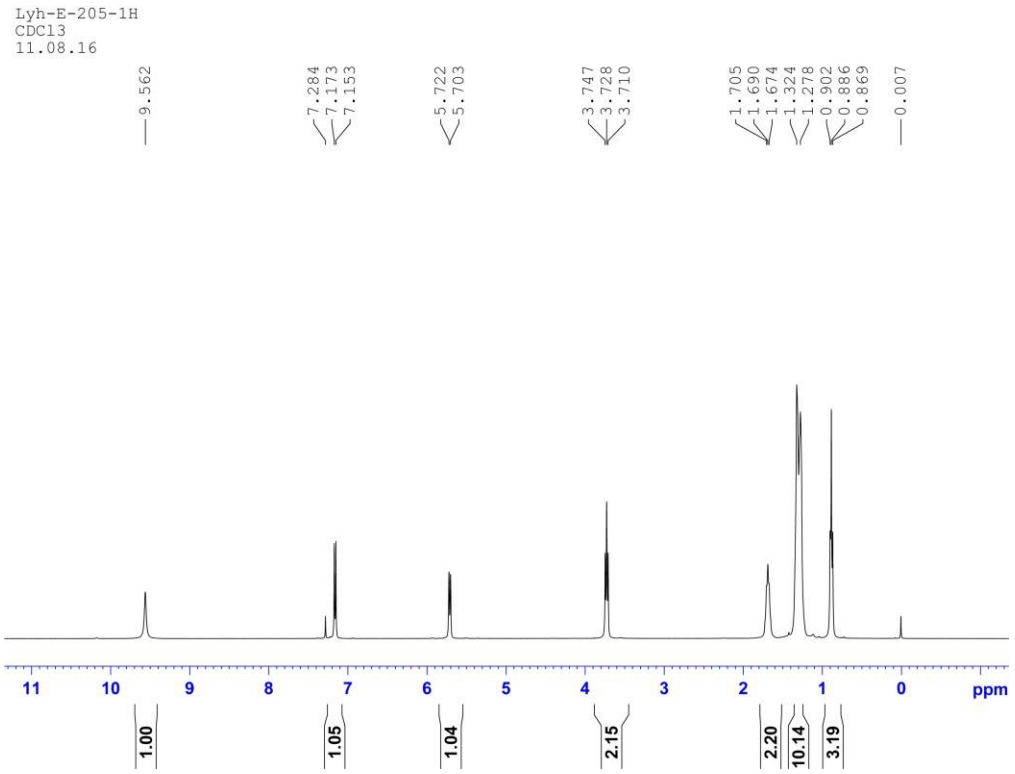
**5-cyano-**Hexyl-**2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (2k)**

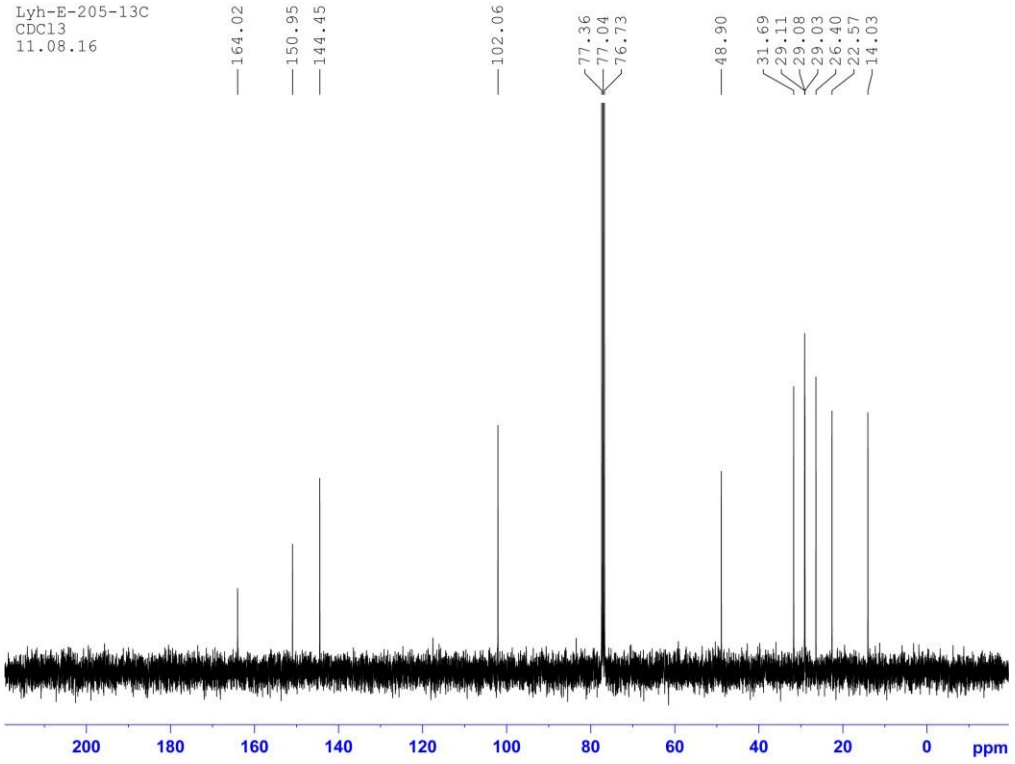


Hexyl-**2,4-dioxo-tetrahydropyrimidine-1(2H)-carboxamide (2l)**

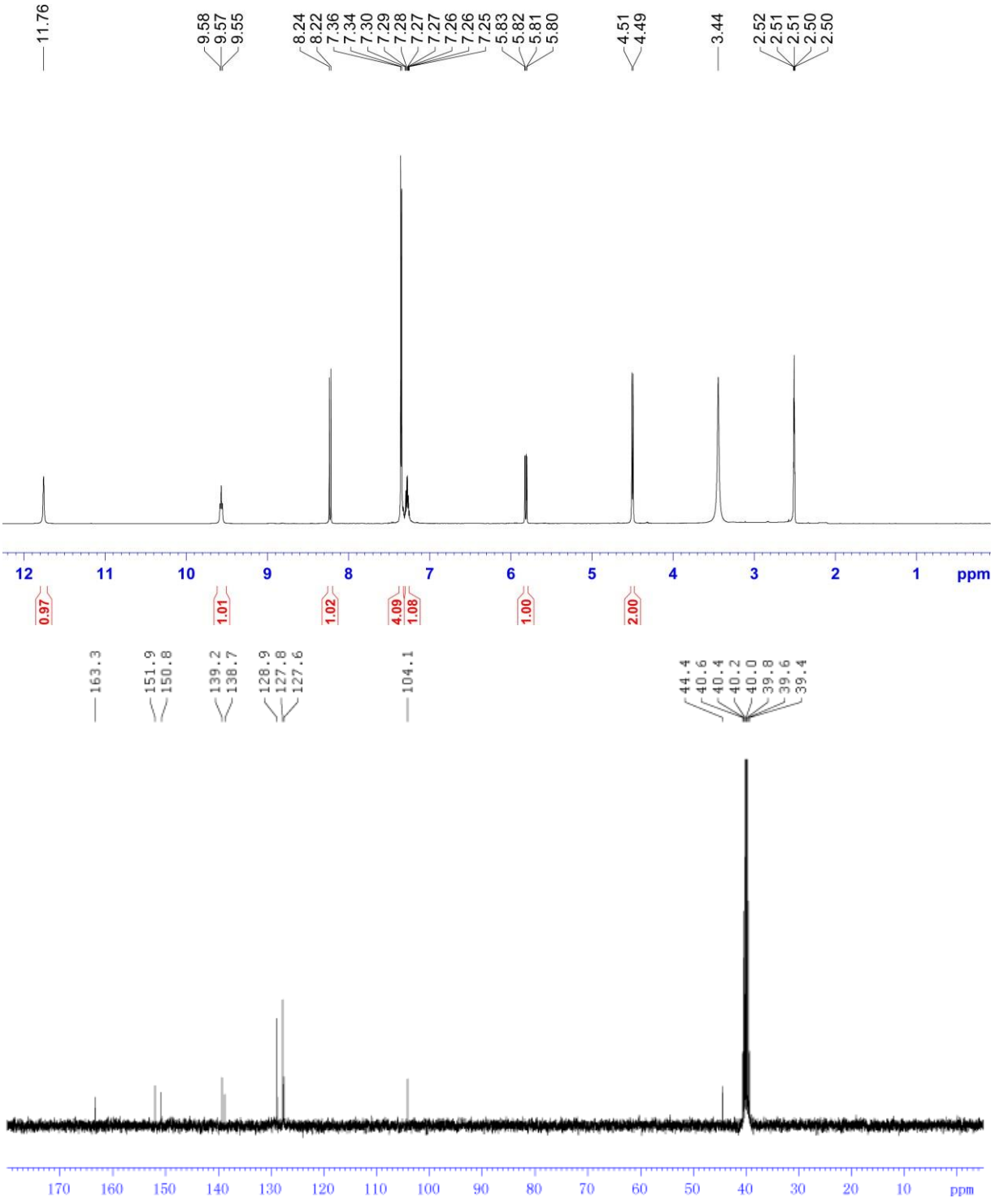


## 1-heptylpyrimidine-2,4(1H,3H)-dione (2m)

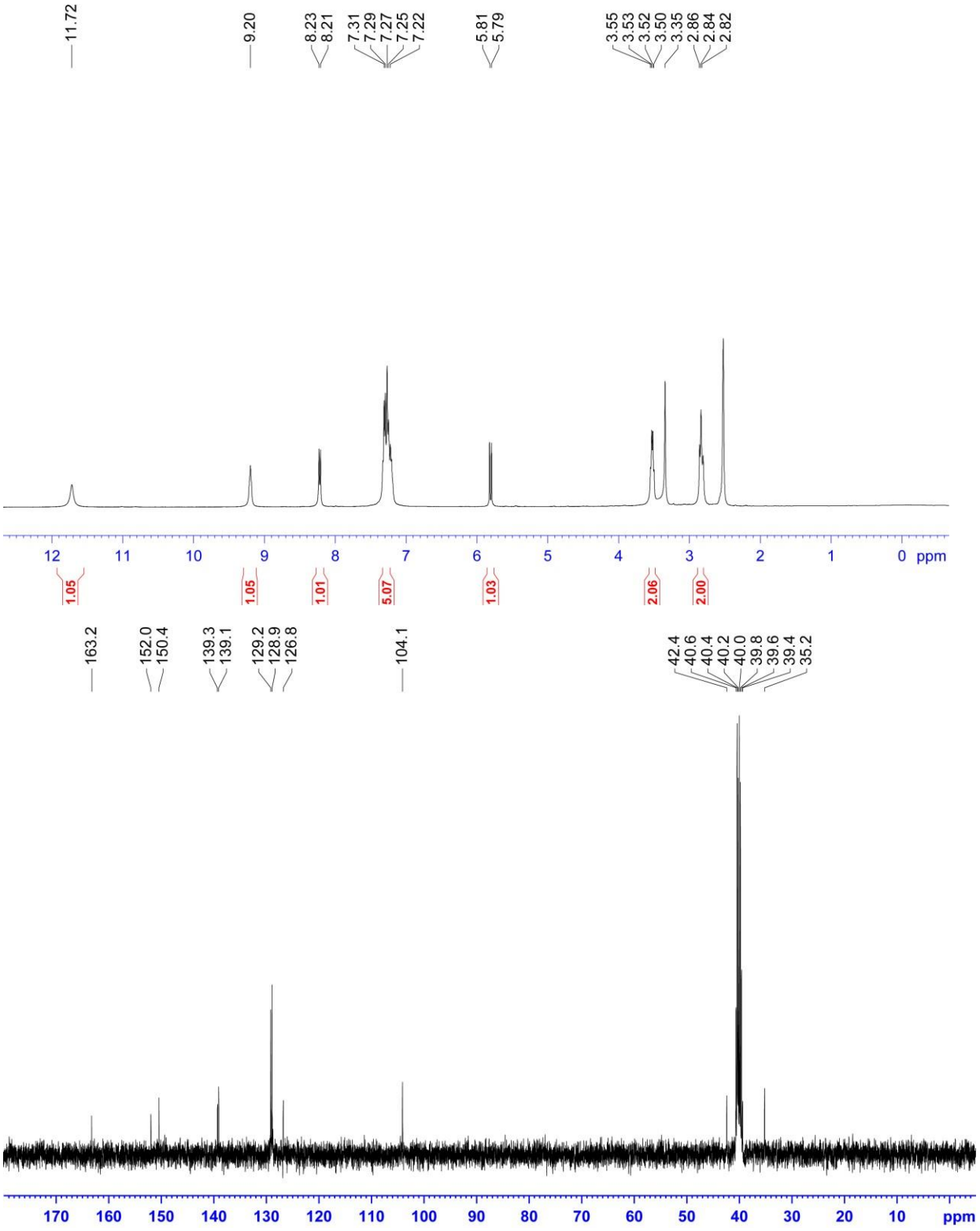


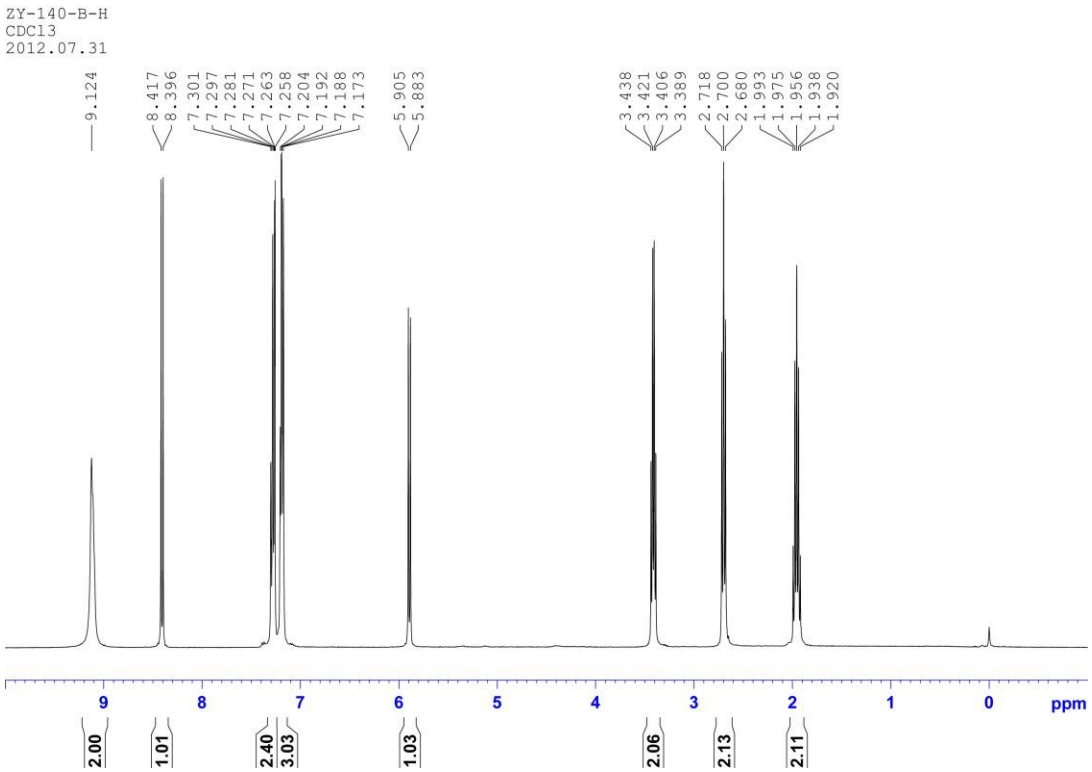
**N-benzyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3a)**

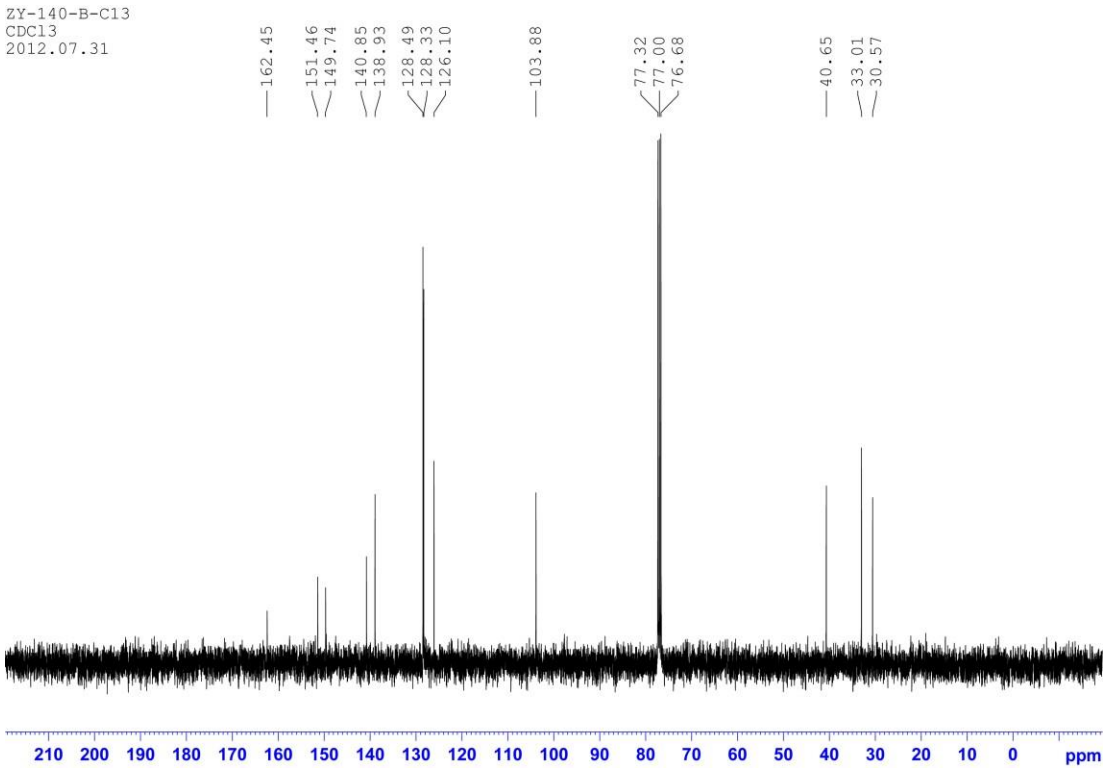


**N-phenethyl -2,4-dioxo -3,4-dihydropyrimidine-1(2H)-carboxamide (3b)**

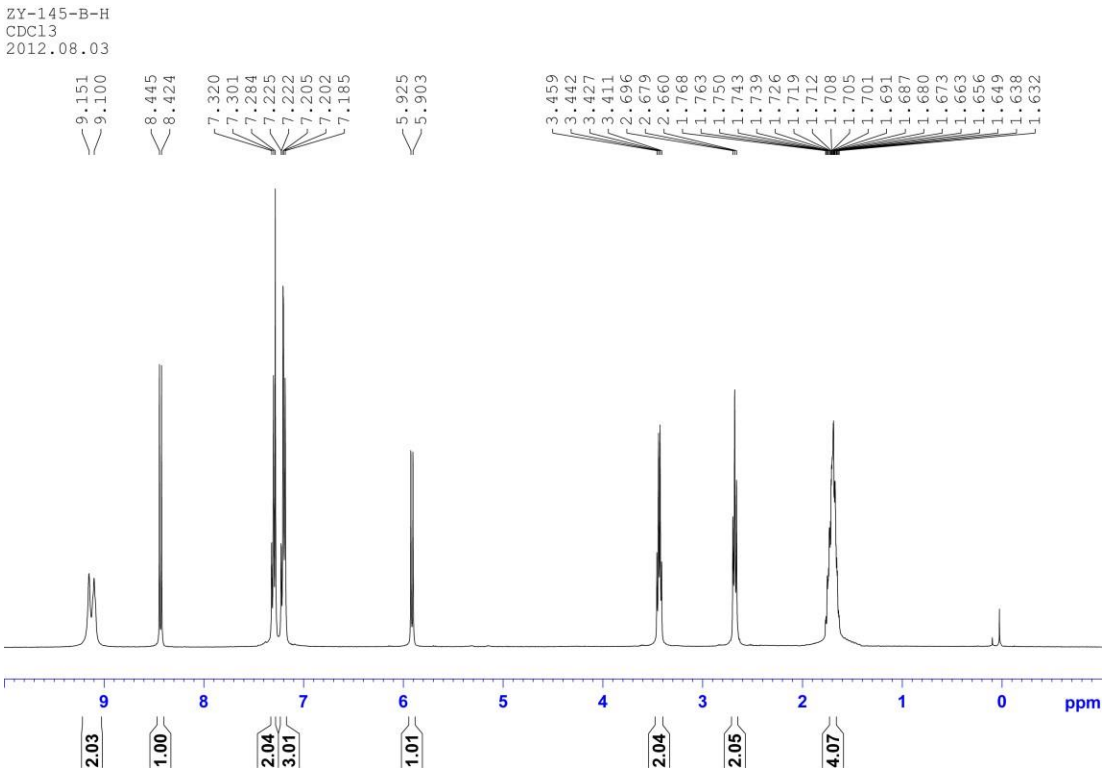


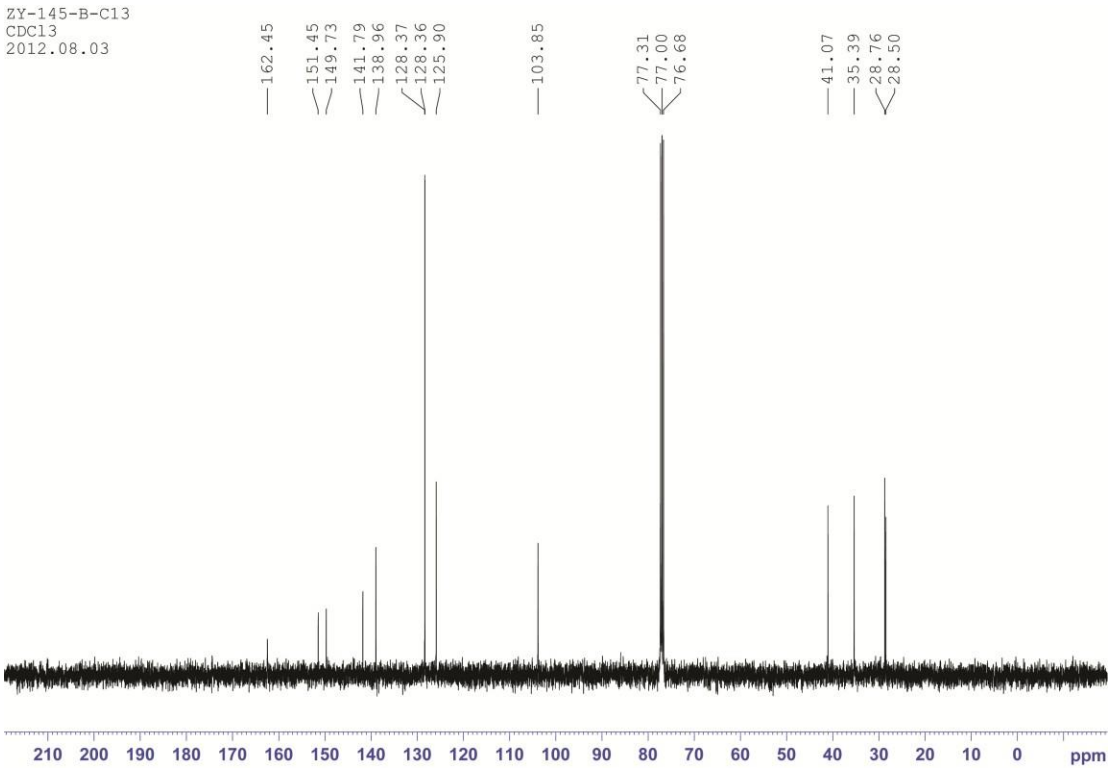
## N-(3-phenylpropyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3c)



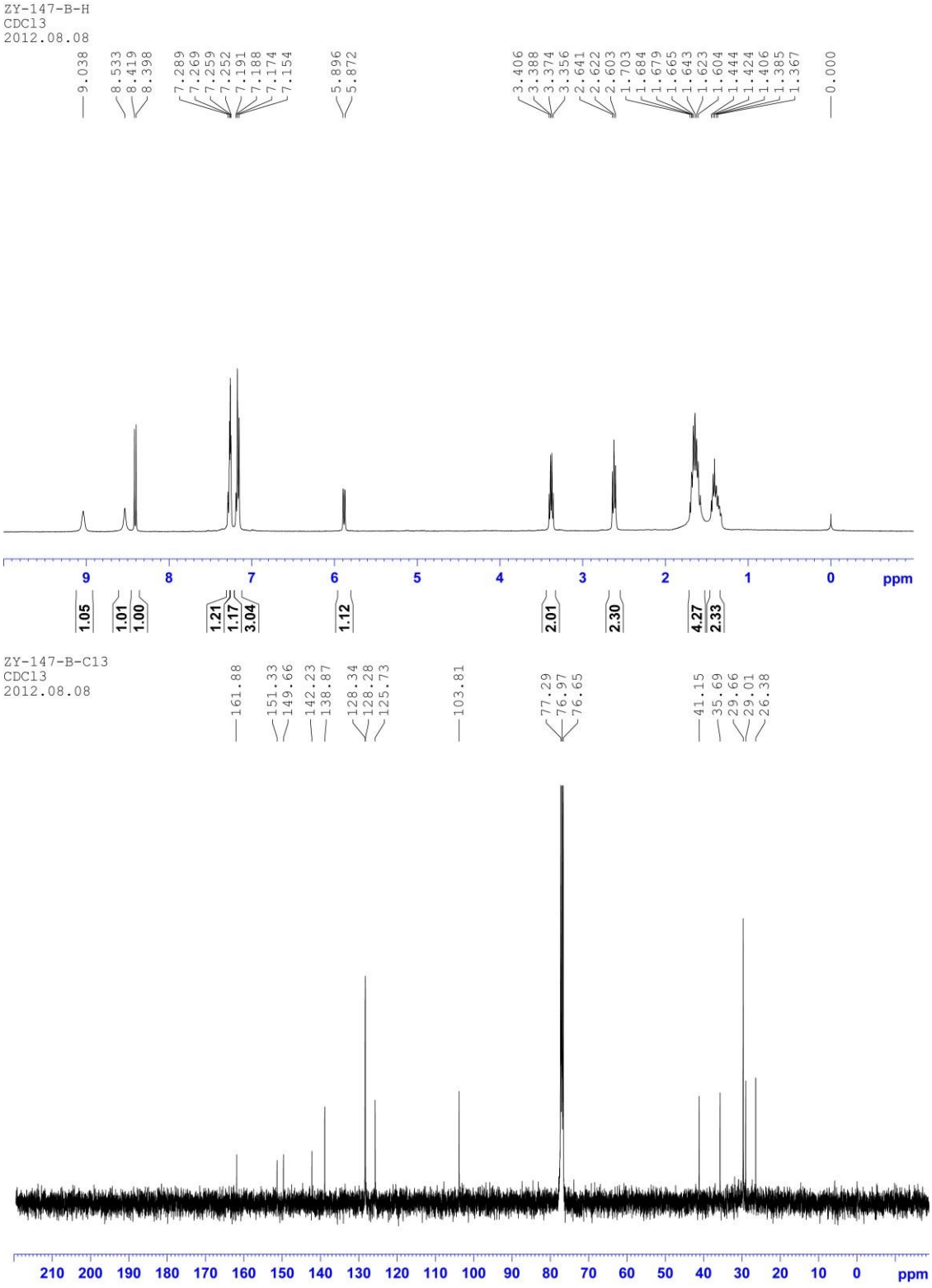



## N-(4-phenylbutyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3d)

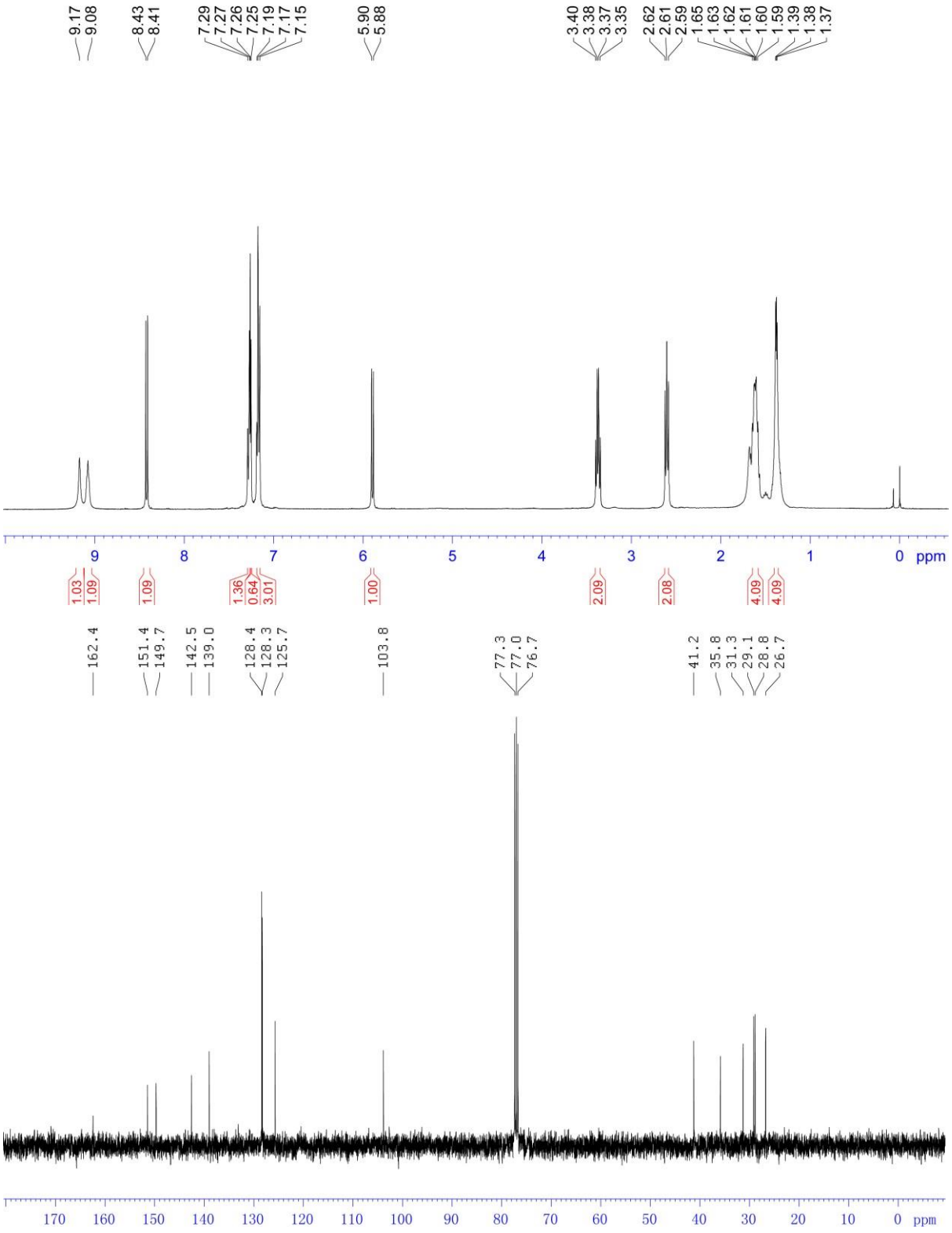


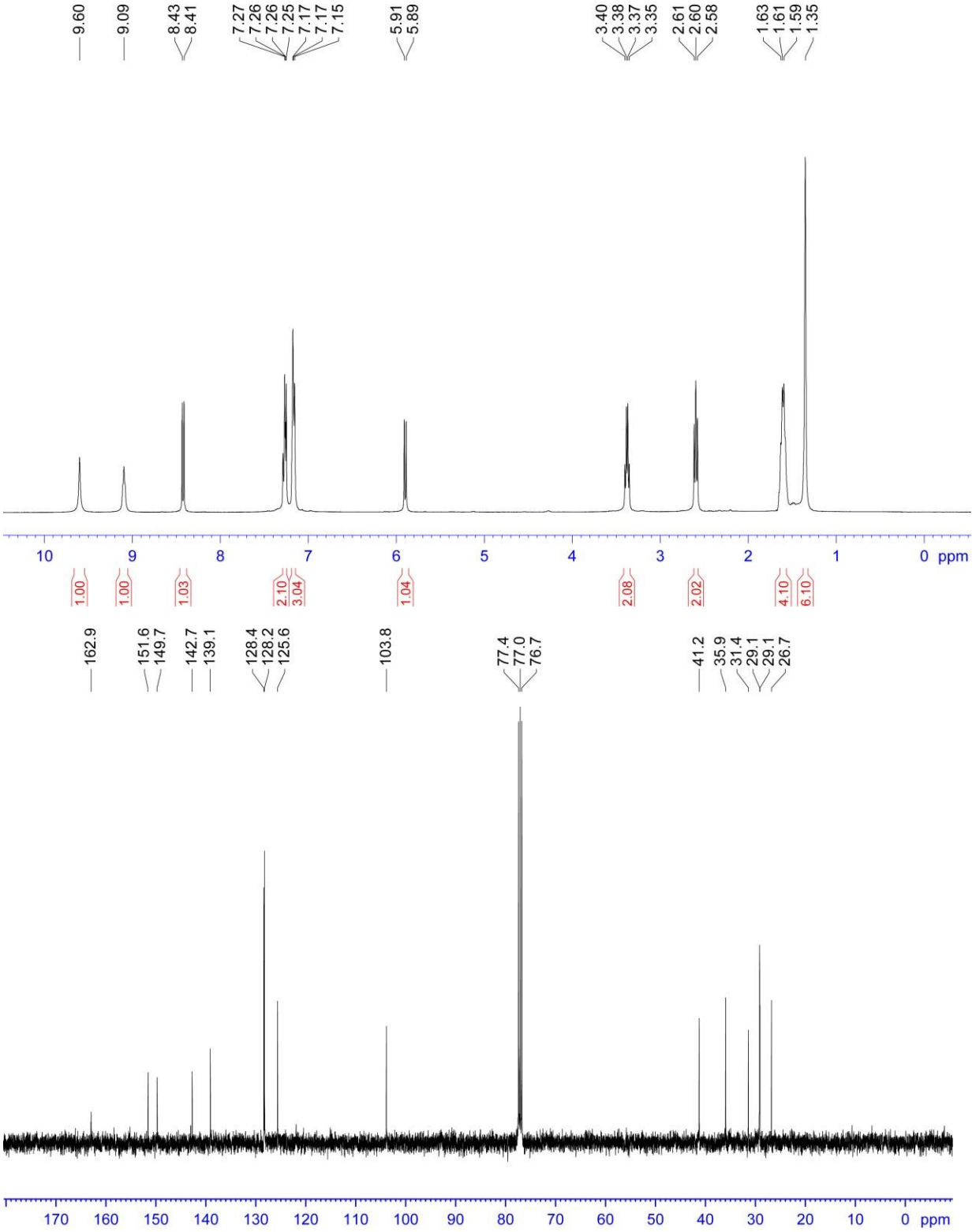
## N-(5-phenylpentyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3e)



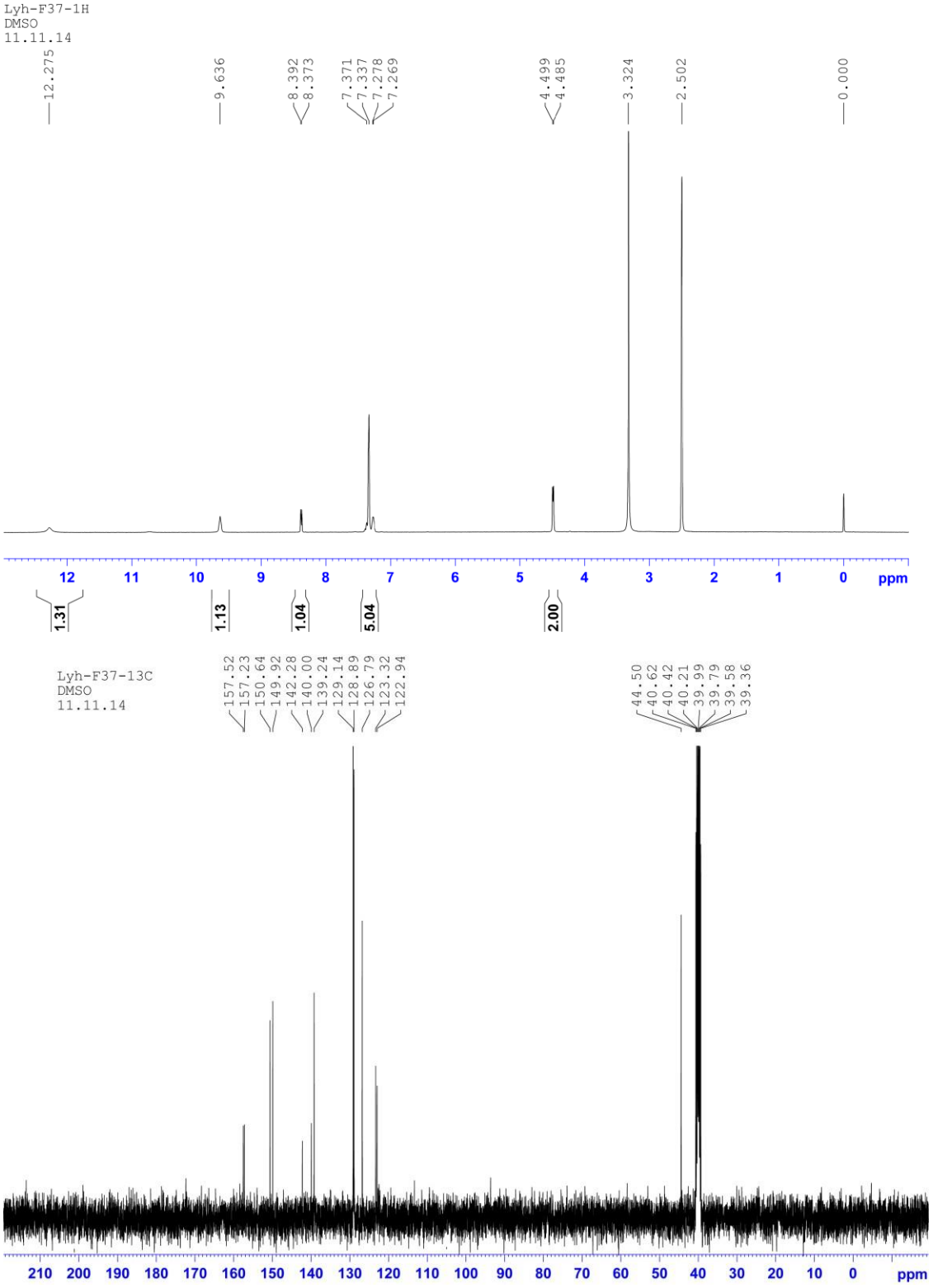
**N-(6-phenylhexyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3f)**



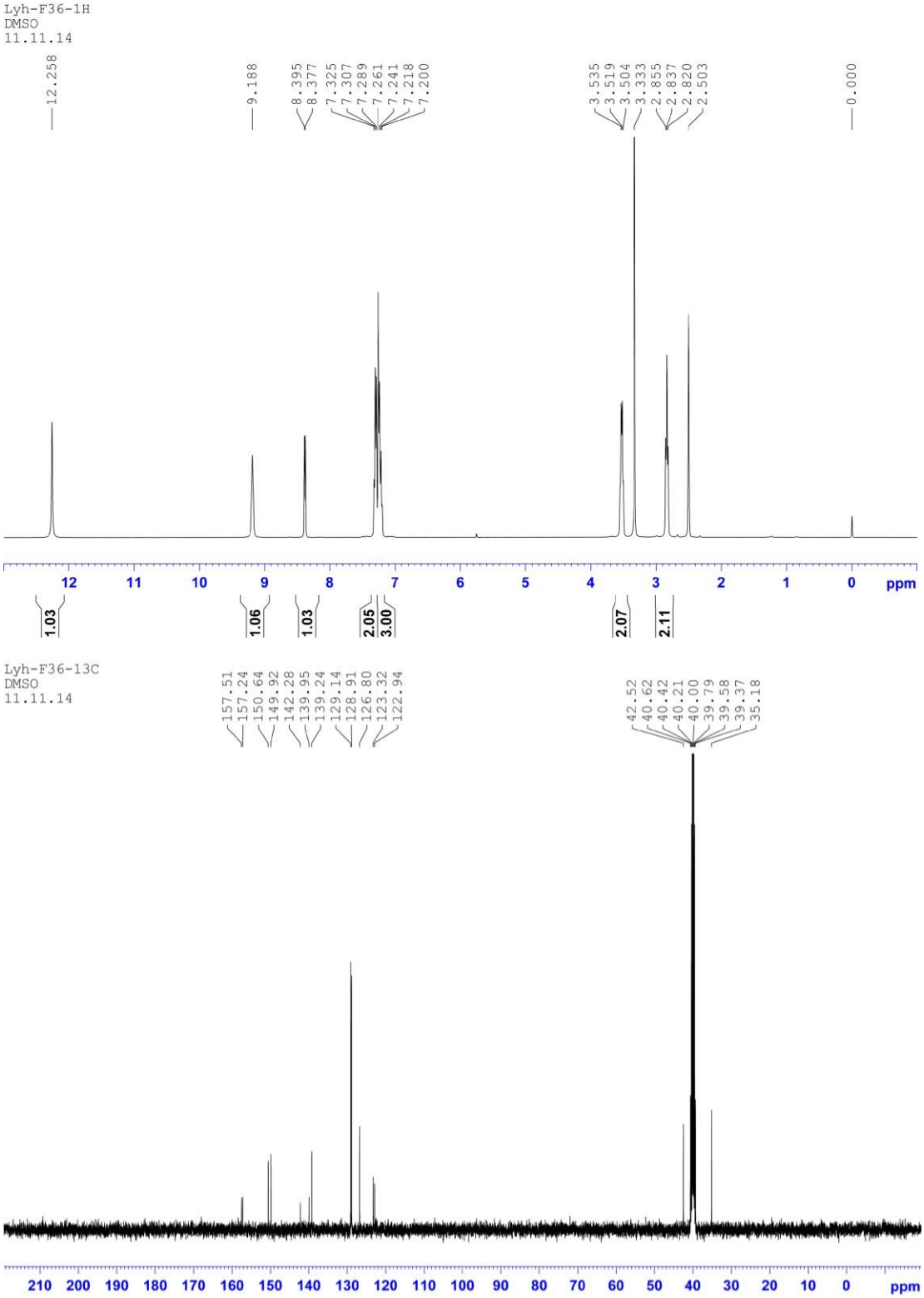
**N-(7-phenylheptyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3g)**



## N-benzyl-5-fluoro-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide (3h)

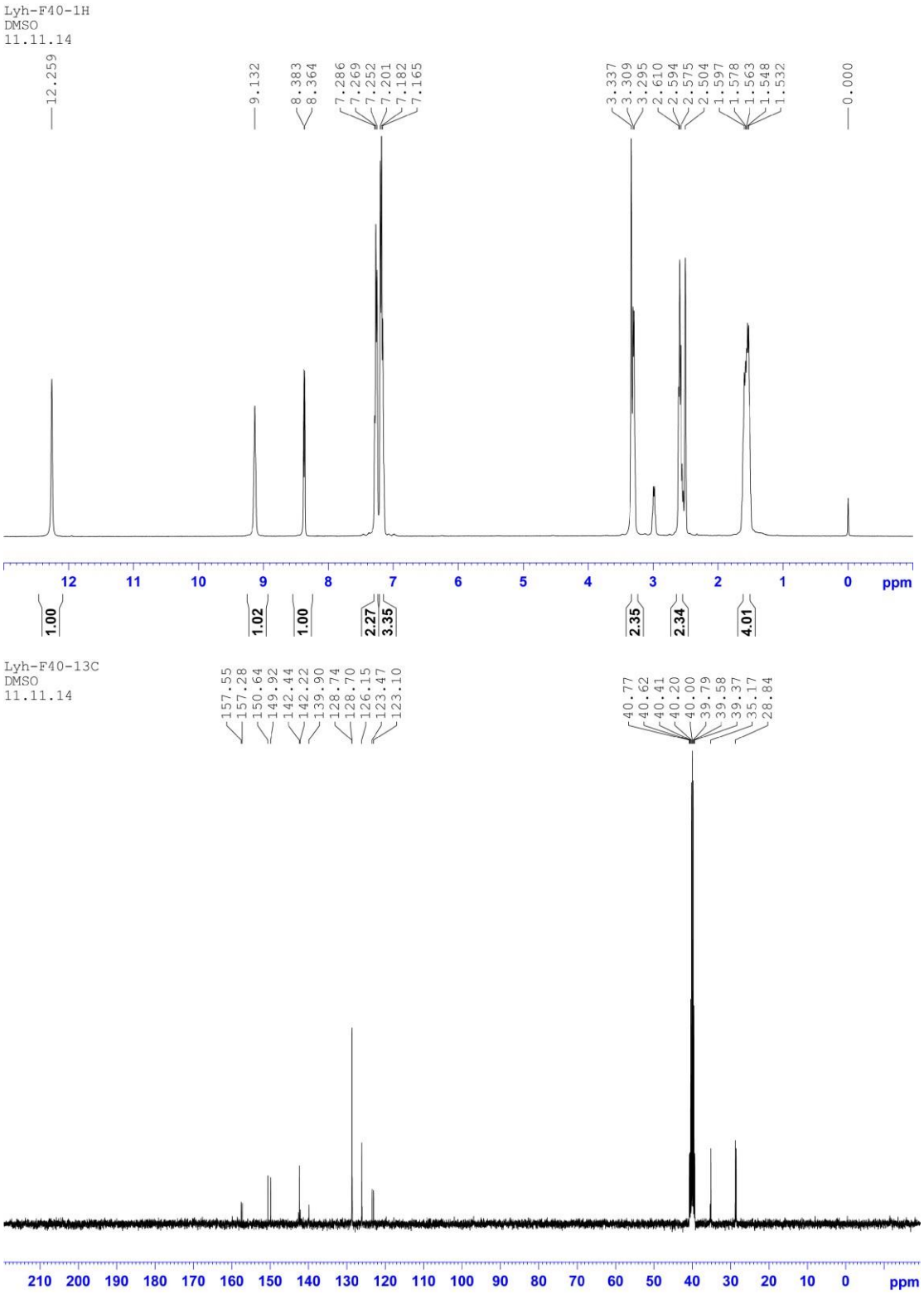


## 5-fluoro-2,4-dioxo-N-phenethyl-3,4-dihydropyrimidine-1(2H)-carboxamide (3i)



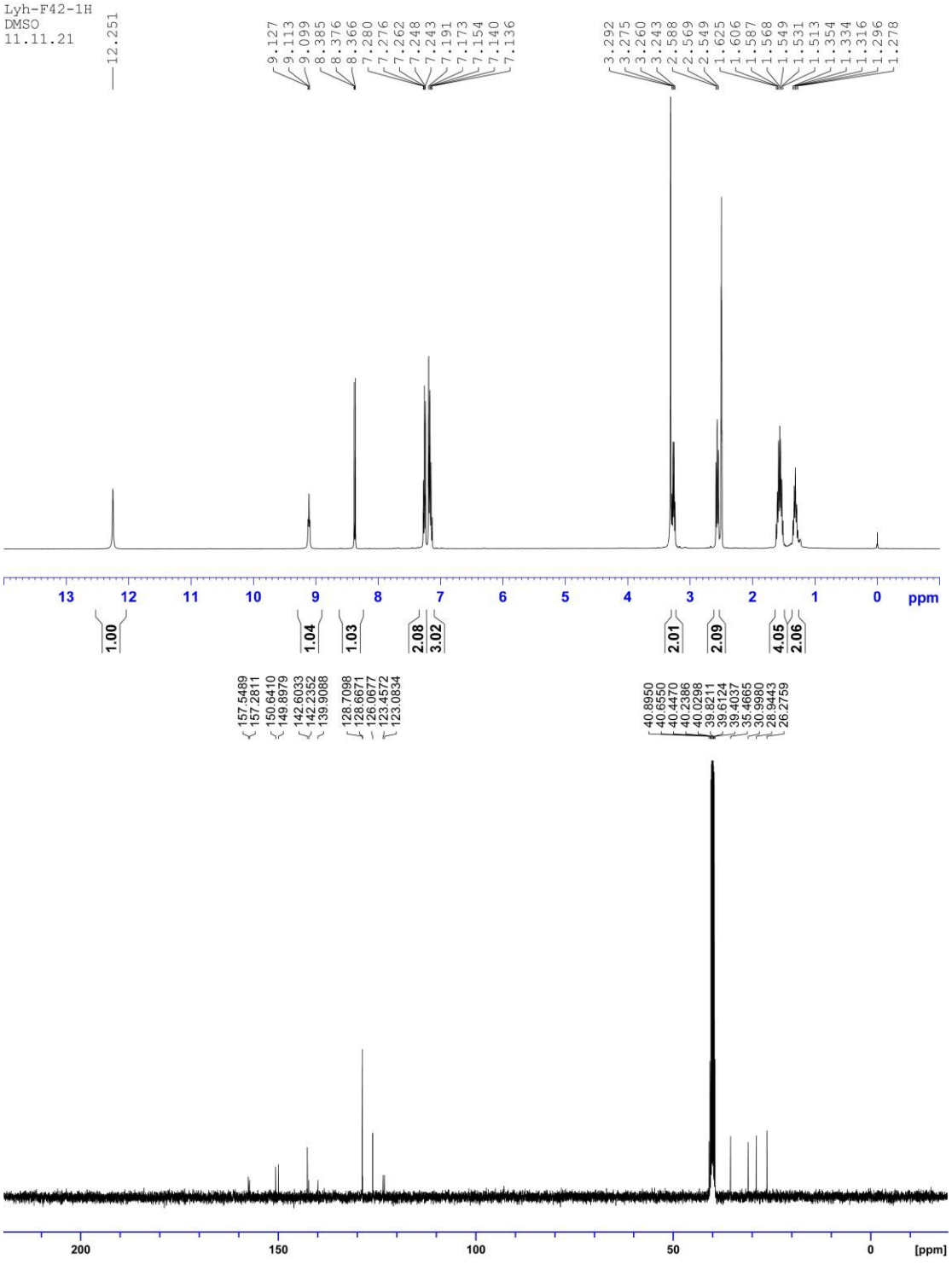
**5-fluoro-2,4-dioxo-N-(4-phenylbutyl)-3,4-dihydropyrimidine-1(2H)-carboxamide**

**(3k)**



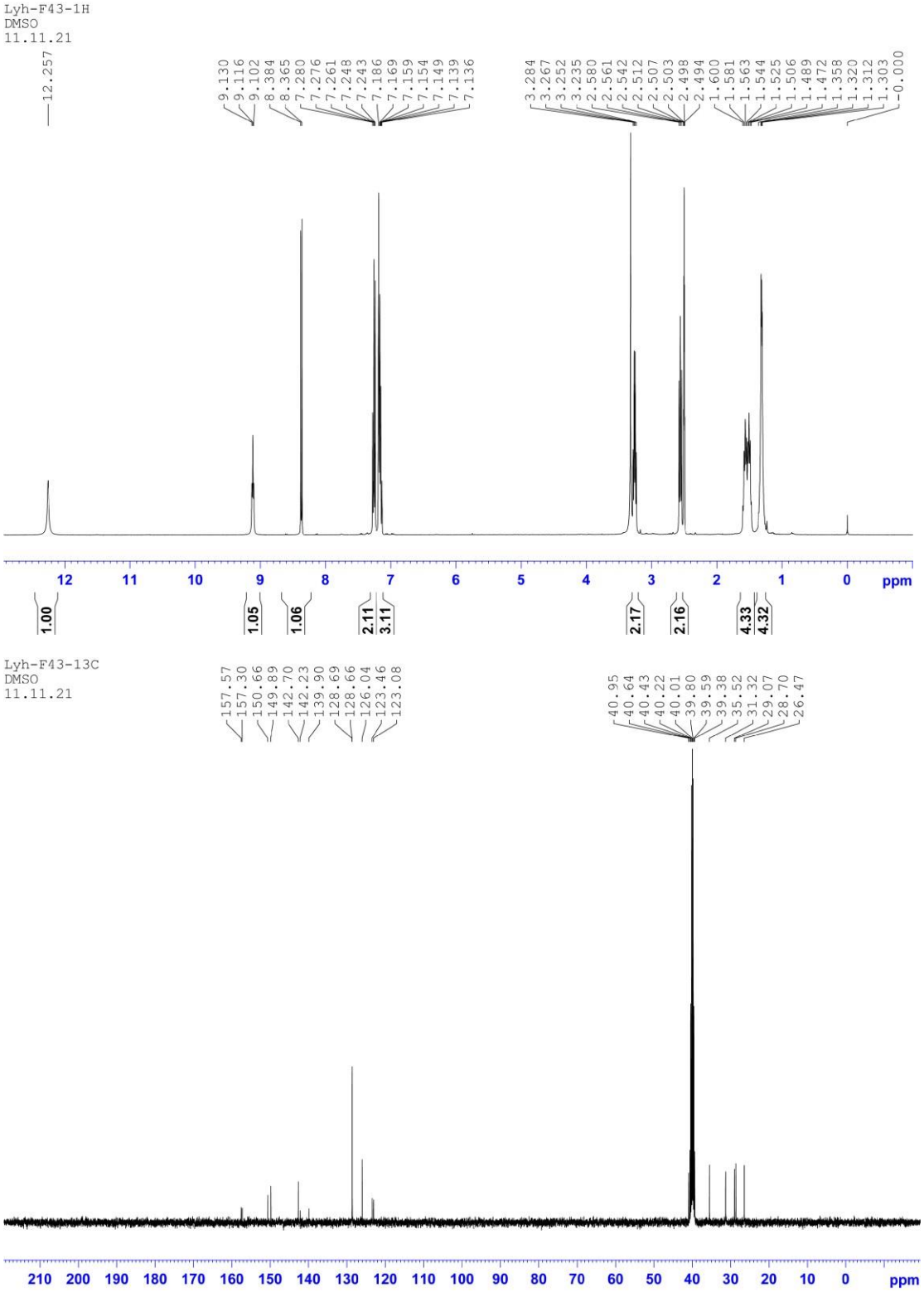
**5-fluoro-2,4-dioxo-N-(5-phenylpentyl)-3,4-dihydropyrimidine-1(2H)-carboxamid**

**e (3l)**

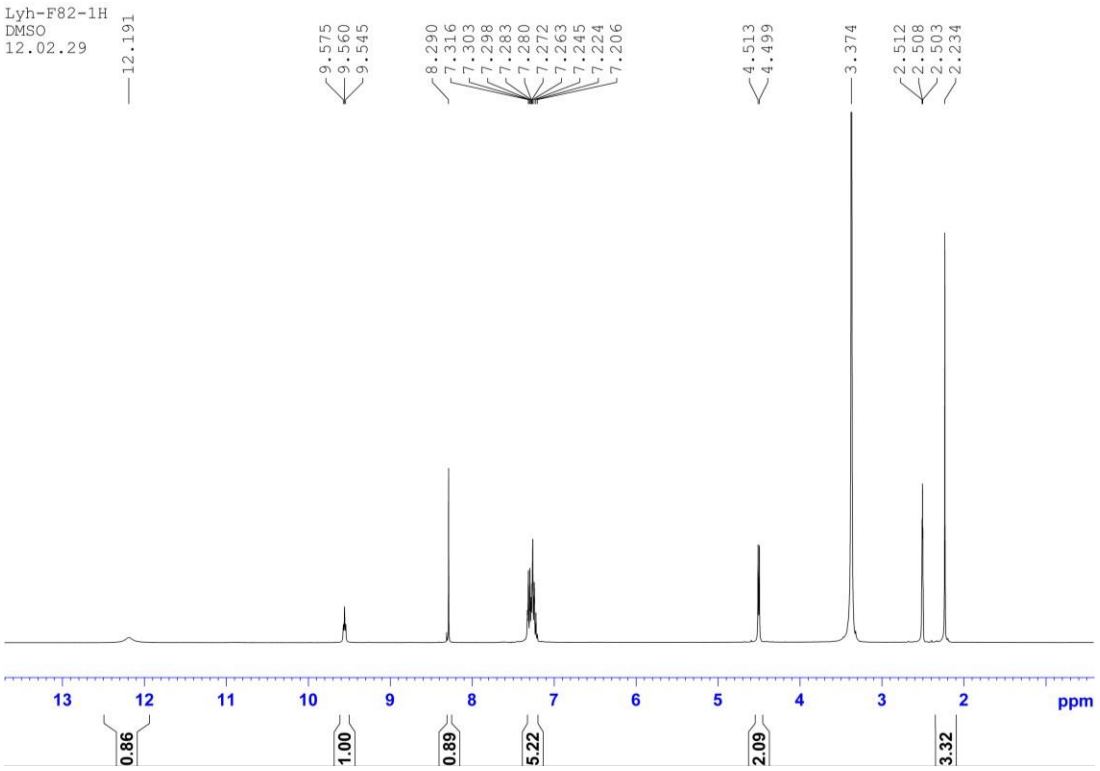


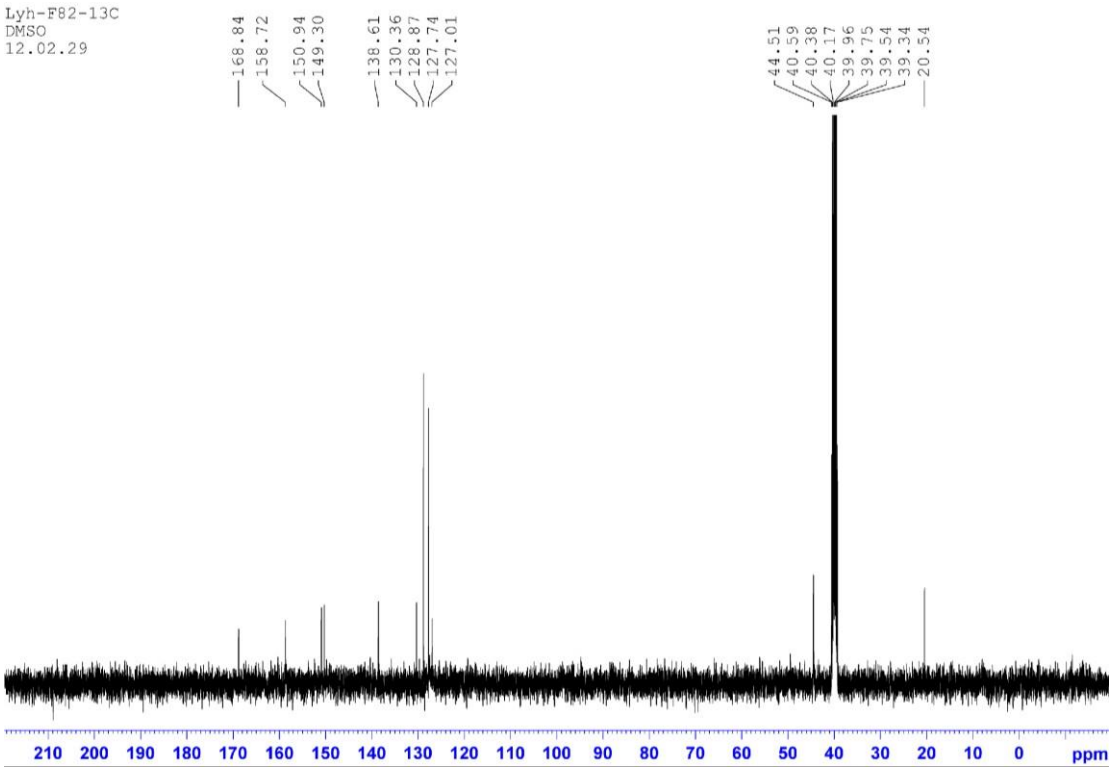
**5-fluoro-2,4-dioxo-N-(6-phenylhexyl)-3,4-dihydropyrimidine-1(2H)-carboxamide**

**(3m)**

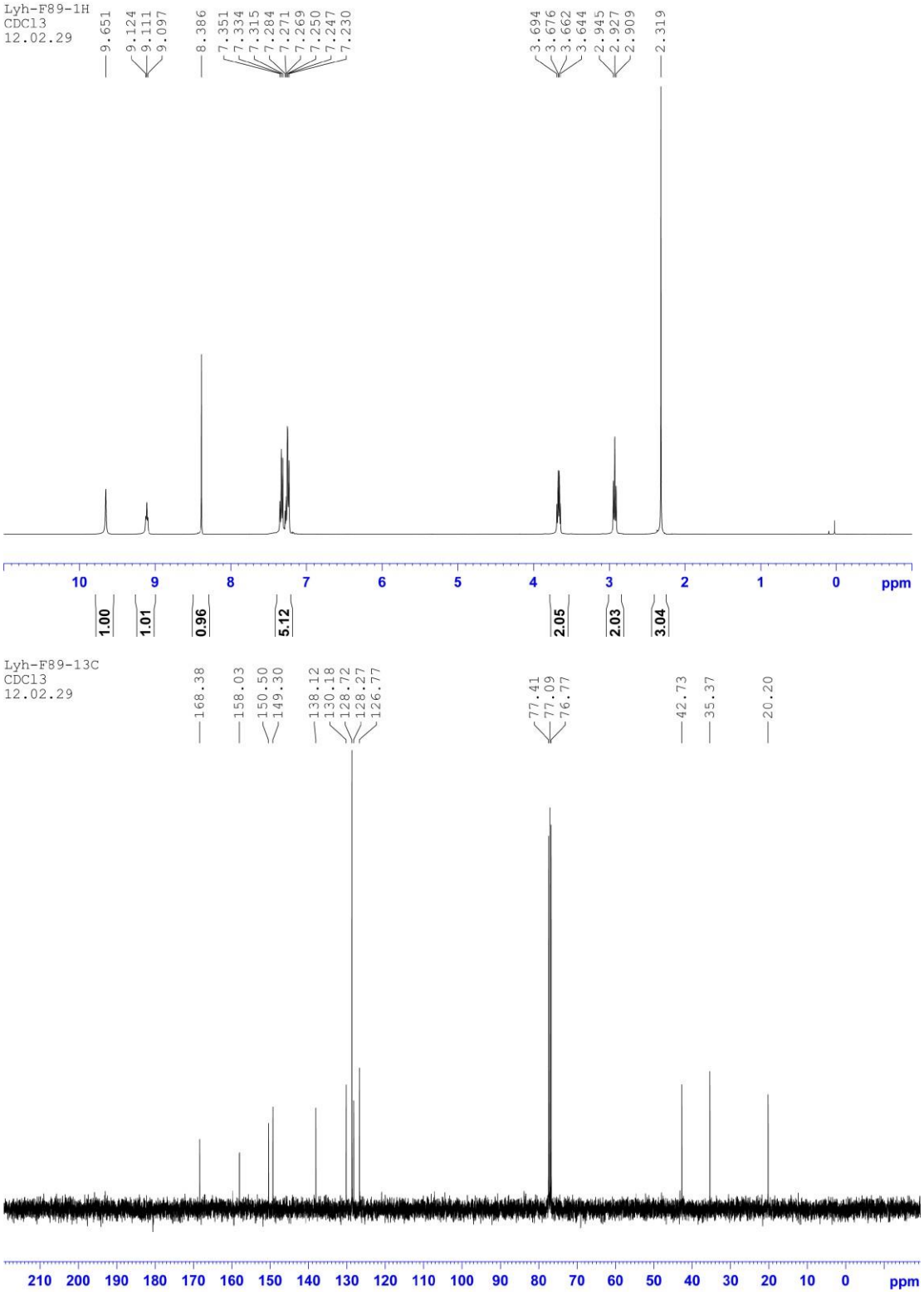


**1-(benzylcarbamoyl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3o)**

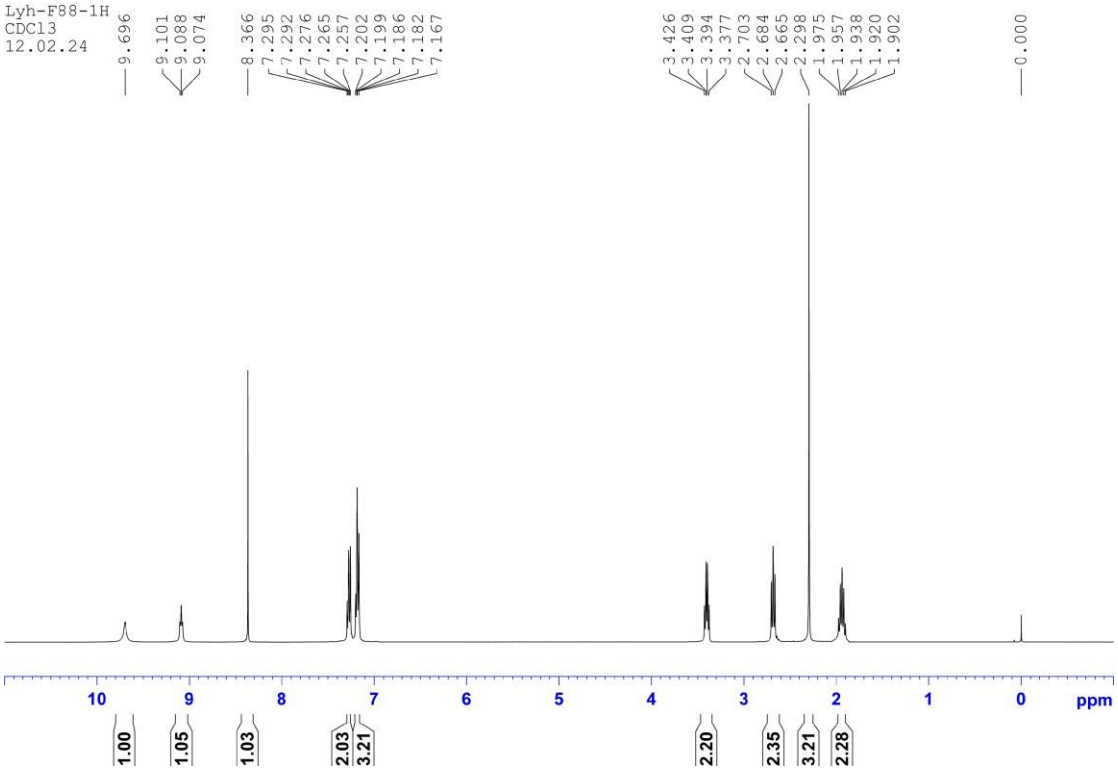


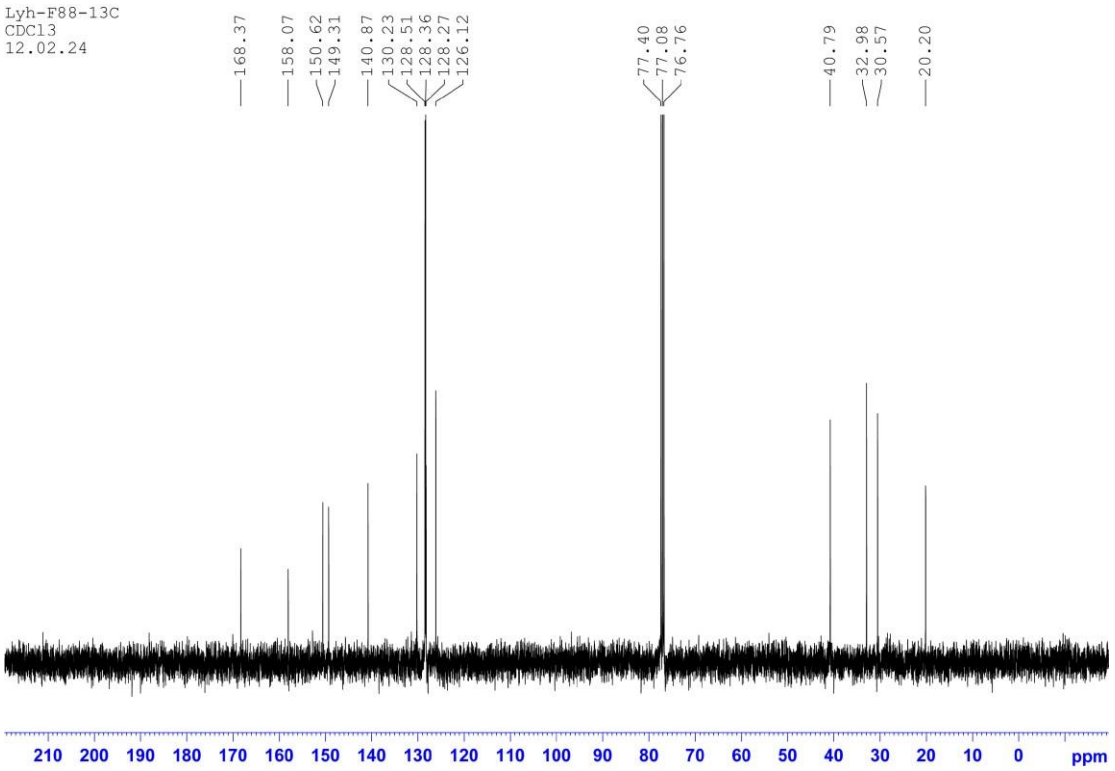



## 2,4-dioxo-1-(phenethylcarbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3p)

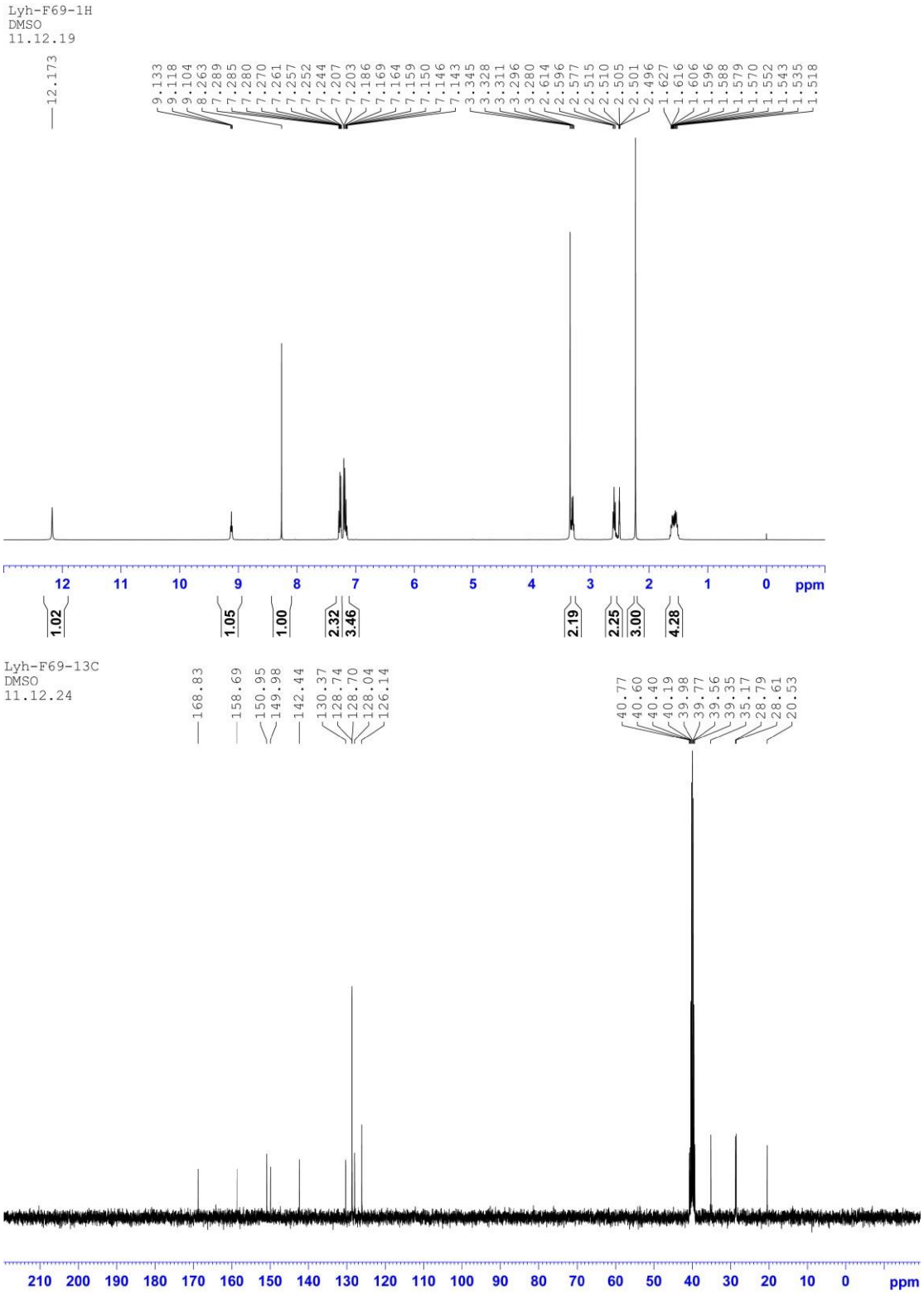


**2,4-dioxo-1-((3-phenylpropyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3q)**

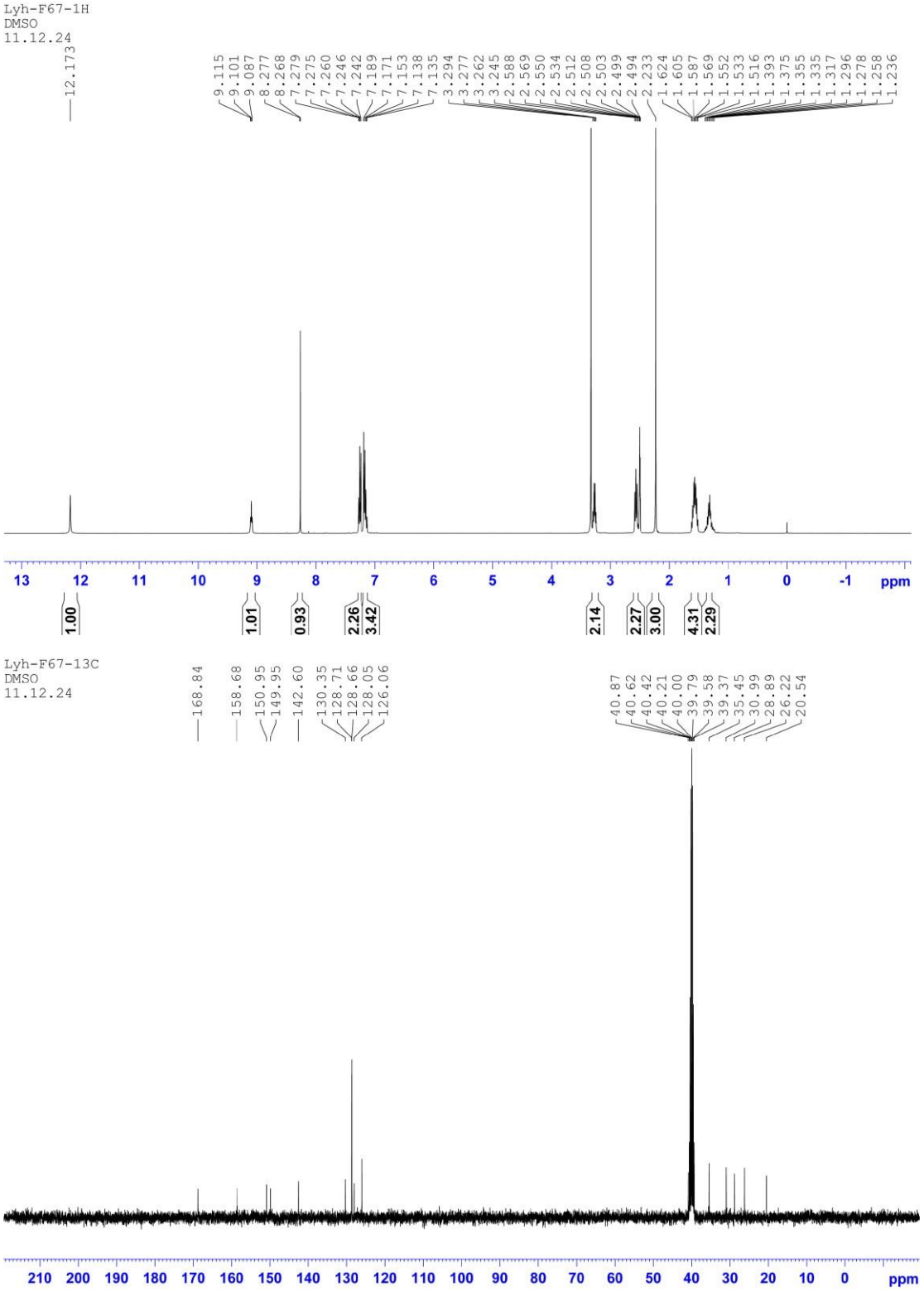


**2,4-dioxo-1-((4-phenylbutyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3r)**

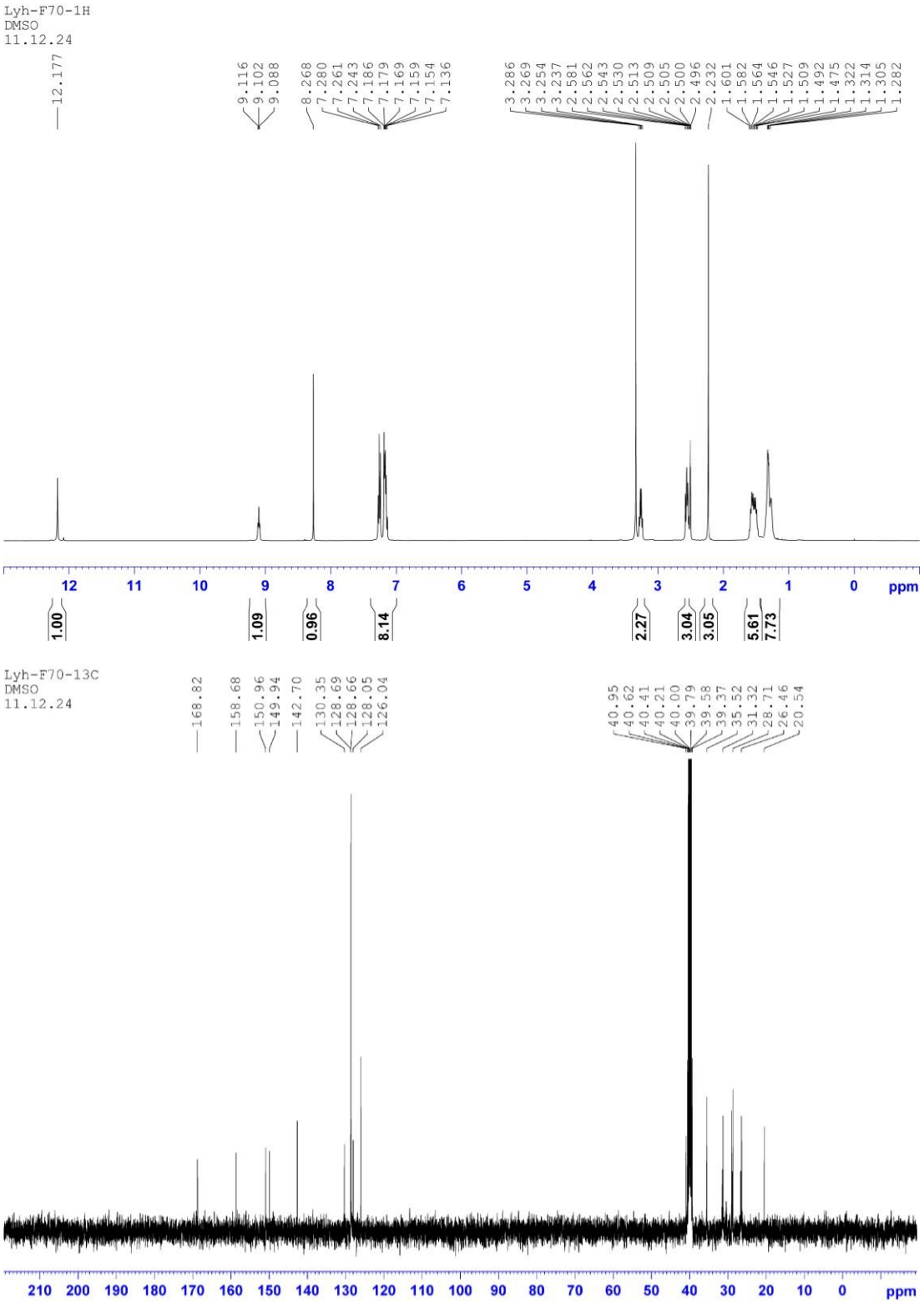


**2,4-dioxo-1-((5-phenylpentyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (3s)**



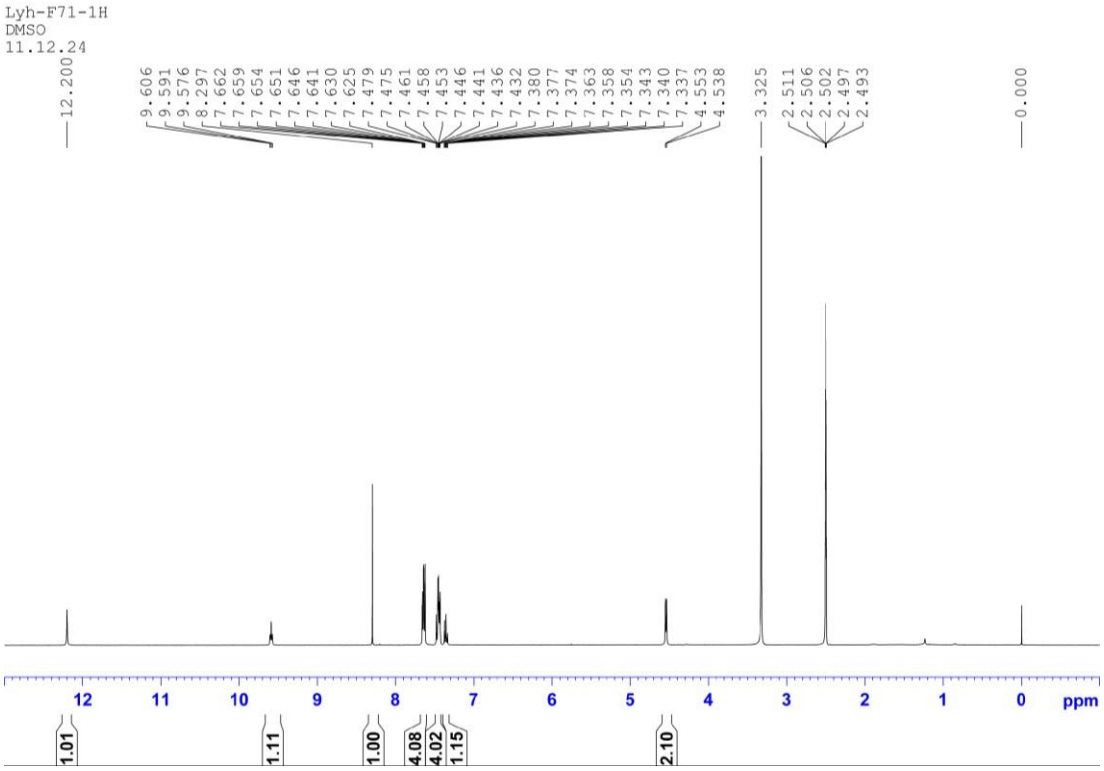
**2,4-dioxo-1-((6-phenylhexyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate**

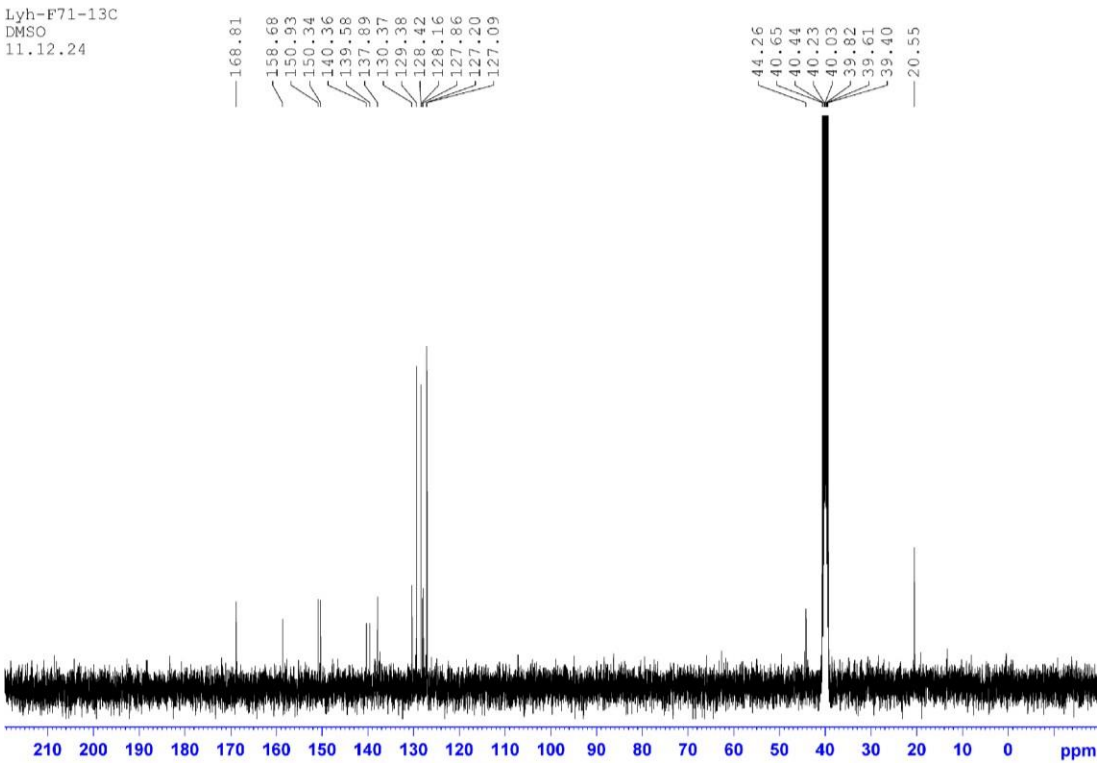
**(3t)**



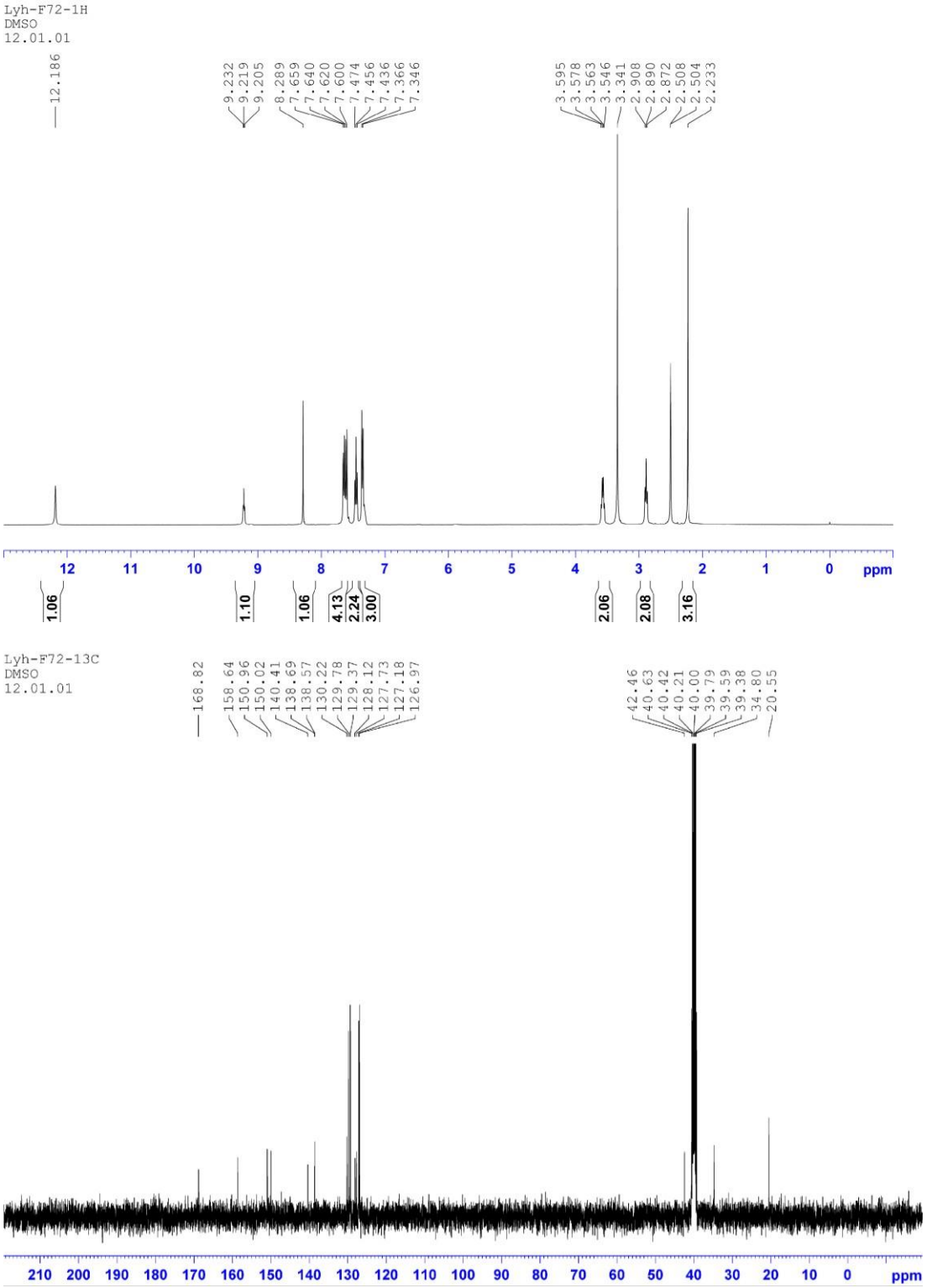
**N-phenylbenzyl-5-fluoro-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxamide**

**(4a)**



**2,4-dioxo-1-(biphenethylcarbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (4b)**



**2,4-dioxo-1-((3-biphenylpropyl)carbamoyl)-1,2,3,4-tetrahydropyrimidin-5-yl acetate (4c)**