
Supporting Information

Efficient discovery of novel antimicrobials through integration of synthesis and testing in crude ribosome extract

*Zitai Sang^{†,a}, Yongping Lu^{†,b}, Yuanzheng Zhou^{†,a}, Yuan Ju^a, Qi An^a, Silan Shen^c, Jianyou Shi^d, Jun He^a, Tao Yang^{*ae} and Youfu Luo^{*a}*

^a State Key Laboratory of Biotherapy and Cancer Center/Collaborative Innovation Center for Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, China

^b Pharmacy College, Chengdu university of Traditional Chinese Medicine, Chengdu, Sichuan 611137, China

^c Department of Gastroenterology, West China Hospital, Sichuan University, Chengdu, Sichuan, China

^d Individualized Medication Key Laboratory of Sichuan Province, Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu 610072, Sichuan, China

^e Laboratory of Human Diseases and Immunotherapies, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, China

Table of content	Pages
1. Chemistry section	S1
2. Biology section	S9
3. Supplementary tables and figures	S14
4. NMR and MS spectra of compounds	S24
References	S63

1. Chemistry section

1.1 General synthetic procedures

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. All the solvents were dried according to the standard methods before use. NMR spectra were recorded on a Bruker Avance (Varian Unity Inova) 400 MHz spectrometer in CDCl₃, DMSO-*d*₆, D₂O or CD₃OD with TMS as internal standard. NMR data were analyzed by using MestReNova Software. High resolution mass spectrometry (HRMS) was performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated plates (0.25 mm) from Qingdao Haiyang Inc., and components were visualized by ultraviolet light (254 nm). Silicycle silica gel 300-400 (particle size 40-63 μm) mesh was used for all flash column chromatography experiments.

1.2 Synthetic procedures of *N*-((3-(4-azido-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl) acetamide (**azide 1**)

To a solution of *N*-((3-(4-amino-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (100 mg), which was obtained as previous described¹, in 10 ml 15% hydrochloric acid aqueous solution was added the sodium nitrite aqueous solution by injection syringe at -5 °C. The mixture was allowed to stir for 0.5 hours, followed by adding the sodium azide aqueous solution by injection syringe. After completion of the reaction, the mixture was extracted with EA for three times. The combined organic layer was dried over anhydrous Na₂SO₄. Removal of solvent gave white solid (72.2 mg, 66%) that could be used for the next transformation without purification. The NMR spectra data was consistent with the reported data and was not shown here.

1.3 General synthesis procedure of *anti* (1,4)-**3a~3f**

To a solution of azide intermediate **1** (1.0 eq.), CuSO₄ (0.02 eq.) and (+)-sodium L-ascorbate (0.1 eq.) in 1:1 H₂O:t-BuOH was added the corresponding alkyne **2a~2f** (2.0 eq.), and the mixture was stirred at r.t. for 24 hours. Once the reaction complete, reaction mixture was poured into water, and extracted with EA for three times. The combined organic layer was washed with brine, dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography to give *anti* (1,4)-**3a~3f**.

1.4 Chemical and physical data of *anti* (1,4)-**3a~3f**

(S)-N-((3-(3-fluoro-4-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3a).

Following general procedure, product was purified by flash chromatography to afford desired compound **3a** as a white solid (yield of 72.5%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.03 (s, 1H), 8.65 (s, 1H), 8.26 (t, J = 5.6 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 7.94 (dd, J = 17.6, 8.8 Hz, 2H), 7.83 (dd, J = 13.2, 2.0 Hz, 1H), 7.57 (dd, J = 8.8, 2.0 Hz, 1H), 7.41 (t, J = 5.6 Hz, 1H), 4.85 – 4.72 (m, 1H), 4.22 (t, J = 9.2 Hz, 1H), 3.83 (m, 1H), 3.46 (t, J = 5.6 Hz, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ = 170.52, 155.76, 154.47, 153.29, 150.22, 149.85, 148.13, 141.27, 137.84, 126.93, 124.99, 123.86, 120.17, 114.52, 106.48, 72.41, 47.75, 41.85, 22.92. HRMS (Q-TOF): calculated for [M]⁺396.1346, found [M+H]⁺ 397.1461.

(S)-N-((3-(3-fluoro-4-(4-(pyridin-4-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3b).

Following general procedure, product was purified by flash chromatography to afford desired compound **3b** as a white solid (yield of 65.0%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.31 (d, J = 1.6 Hz, 1H), 8.70 (brs, 2H), 8.26 (t, J = 5.6 Hz, 1H), 7.96-7.92 (m, 3H), 7.86 (dd, J = 13.2, 2.4 Hz, 1H), 7.59 (dd, J = 8.8, 1.6 Hz, 1H), 4.86 – 4.73 (m, 1H), 4.22 (t, J = 8.8 Hz, 1H), 3.84 (m, 1H), 3.46 (t, J = 5.6 Hz, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.52, 155.61, 154.47, 153.13, 150.95 (2C), 145.12, 141.42, 137.63 (2C), 126.89, 125.30, 119.87, 114.59, 106.47, 72.43, 47.76, 41.85, 22.92. HRMS (Q-TOF): calculated for [M]⁺396.1346, found [M+H]⁺ 397.1460.

(S)-N-((3-(3-fluoro-4-(4-(pyridin-3-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3c).

Following general procedure, product was purified by flash chromatography to afford desired compound **3c** as a white solid (yield of 79.4%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.20 (d, J = 1.6 Hz, 1H), 9.17 (s, 1H), 8.60 (d, J = 4.0 Hz, 1H), 8.33 (dt, J = 8.0, 2.0 Hz, 1H), 8.26 (t, J = 6.0 Hz, 1H), 7.94 (t, J = 8.8 Hz, 1H), 7.86 (dd, J = 13.2, 2.0 Hz, 1H), 7.59 (dd, J = 11.2, 2.4 Hz, 1H), 7.54 (dd, J = 5.2, 8.0 Hz, 1H), 4.87 – 4.71 (m, 1H), 4.22 (t, J = 9.2 Hz, 1H), 3.84 (m, 1H), 3.46 (t, J = 5.6 Hz, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.52, 155.58, 154.48, 153.10, 149.77, 147.10, 144.62, 141.27, 133.17, 126.83, 124.59, 123.99, 119.98, 114.60, 106.49, 72.43, 47.76, 41.85, 22.93. HRMS (Q-TOF): calculated for [M]⁺ 396.1346, found [M+H]⁺ 397.1461.

(S)-N-((3-(3-fluoro-4-(4-(thiophen-2-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3d).

Following general procedure, product was purified by flash chromatography to afford desired compound **3d** as a white solid (yield of 81.0%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.97 (d, J = 1.6 Hz, 1H), 8.26 (t, J = 6.0 Hz, 1H), 7.91 (t, J = 8.8 Hz, 1H), 7.84 (dd, J = 13.2, 2.4 Hz, 1H), 7.60 (d, J = 4.8 Hz, 1H), 7.59

– 7.53 (m, 2H), 7.22 – 7.16 (m, 1H), 4.86 – 4.72 (m, 1H), 4.21 (t, J = 9.2 Hz, 1H), 3.83 (m, 1H), 3.46 (t, J = 5.6 Hz, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ = 170.52, 155.57, 154.47, 153.10, 142.78, 141.15, 132.58, 128.51, 126.64, 125.32, 122.44, 119.89, 114.54, 106.58, 72.41, 47.75, 41.85, 22.92. HRMS (Q-TOF): calculated for [M]⁺401.0958, found [M+H]⁺402.1117.

***(S)*-N-((3-(3-fluoro-4-(4-(pyrimidin-2-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3e).**

Following general procedure, product was purified by flash chromatography to afford desired compound **3e** as a white solid (yield of 85.0%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.18 (s, 1H), 8.93 (d, J = 4.8 Hz, 1H), 8.27 (t, J = 5.6 Hz, 1H), 7.95 (t, J = 8.8 Hz, 1H), 7.84 (d, J = 13.6 Hz, 1H), 7.58 (d, J = 9.2 Hz, 1H), 7.51 (t, J = 4.8 Hz, 1H), 4.80 (m, 1H), 4.22 (t, J = 8.8 Hz, 1H), 3.90 – 3.76 (m, 1H), 3.46 (t, J = 5.2, 2H), 1.85 (s, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.54, 158.68, 158.36, 155.78, 154.47, 153.31, 147.27, 141.29, 128.03, 126.97, 120.92, 119.93, 114.48, 106.45, 72.41, 47.75, 41.85, 22.92. HRMS (Q-TOF): calculated for [M]⁺ 397.1299, found [M+H]⁺398.1371.

***(S)*-N-((3-(3-fluoro-4-(4-(pyrimidin-5-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3f).**

Following general procedure, product was purified by flash chromatography to afford desired compound **3f** as a white solid (yield of 72.3%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.36 (s, 2H), 9.30 (d, J = 6.0 Hz, 1H), 9.22 (s, 1H), 8.27 (t, J = 5.6 Hz, 1H), 7.97 (t, J = 8.8 Hz, 1H), 7.89 (dd, J = 13.2, 5.2 Hz, 1H), 7.61 (dd, J = 9.6, 2.0 Hz, 1H), 4.80 (m, 1H), 4.24 (t, J = 9.2 Hz, 1H), 3.85 (m, 1H), 3.47 (t, J = 2.8 Hz, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.52, 158.38, 155.50, 154.47, 154.04, 153.03, 141.77, 141.37, 126.74, 124.96, 124.74, 119.79, 114.63, 106.49, 72.44, 47.75, 41.85, 22.92. HRMS (Q-TOF): calculated for [M]⁺397.1299, found [M+H]⁺398.1364.

1.5 General procedure for the synthesis of compounds *anti* (1, 4)-3g, 3h, 3i, 3j, 3k, 3p.

To a solution of azide intermediate (1) (1.0 eq.) in t-BuOH was added the copper (1.0 eq.), copper sulfate pentahydrate (0.5 eq.) and corresponding alkyne **2g**, **2h**, **2i**, **2j**, **2k**, **2p** (1.5 eq.). The reaction was stirred overnight at 65 °C. After completion of the reaction, the mixture was cooled to room temperature. Then, water was added into the mixture, and extracted with DCM for three times. The combined organic layer was washed with brine and dried over Na₂SO₄. After removing the solvents, the residue was stirred in the mixed solvents (PE: EA=4:1) for 10 min. Then, the mixture was filtered and the solid was collected to give the target compounds.

1.6 Chemical and physical data of *anti* (1,4)-3g, 3h, 3i, 3j, 3k, 3p.

(S)-N-((3-(3-fluoro-4-(4-(thiophen-3-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3g).

Following general procedure, product was collected by filtration to afford desired compound **3g** as a white solid (yield of 88.0%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.92 (d, J = 1.6 Hz, 1H), 8.26 (t, J = 5.6 Hz, 1H), 7.98 (dd, J = 2.8, 1.2 Hz, 1H), 7.92 (t, J = 8.8 Hz, 1H), 7.84 (dd, J = 13.2, 2.0 Hz, 1H), 7.70 (m, 1H), 7.62 (dd, J = 5.2, 1.2 Hz, 1H), 7.57 (dd, J = 8.8, 1.6 Hz, 1H), 4.80 (m, 1H), 4.21 (t, J = 9.0 Hz, 1H), 3.84 (m, 1H), 3.45 (t, J = 5.6 Hz, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ = 170.52, 155.46, 154.47, 152.99, 143.91, 141.10, 131.78, 127.84, 126.51, 122.87, 122.07, 120.17, 114.59, 106.60, 72.41, 47.75, 41.85, 22.92. HRMS (Q-TOF): calculated for [M]⁺401.0958, found [M+Na]⁺424.0881.

(S)-N-((3-(3-fluoro-4-(4-(4-nitrophenyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3h).

Following general procedure, product was collected by filtration to afford desired compound **3h** as a white solid (yield of 21.0%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.34 (s, 1H), 8.38 (d, J = 9.2 Hz, 2H), 8.25 (d, J = 8.8 Hz, 2H), 7.95 (t, J = 8.8 Hz, 1H), 7.86 (dd, J = 13.2, 2.0 Hz, 1H), 7.59 (dd, J = 8.4, 1.6 Hz, 1H), 6.50 (s, 1H), 4.81 (m, 1H), 4.22 (t, J = 7.2 Hz, 1H), 3.91 – 3.78 (m, 1H), 3.46 (t, J = 5.2 Hz, 2H), 1.85 (s, 3H). HRMS (Q-TOF): calculated for [M]⁺440.1244, found [M+Na]⁺463.1147.

(S)-N-((3-(3-fluoro-4-(4-(3-nitrophenyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3i).

Following general procedure, product was collected by filtration to afford desired compound **3i** as a white solid (yield of 78.5%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 9.37 (d, J = 1.6 Hz, 1H), 8.79 (t, J = 2.0 Hz, 1H), 8.43 (d, J = 8.0 Hz, 1H), 8.34 – 8.19 (m, 2H), 7.95 (t, J = 8.8 Hz, 1H), 7.84 (m, 2H), 7.60 (d, J = 9.6 Hz, 1H), 4.81 (m, 1H), 4.22 (t, J = 8.8 Hz, 1H), 3.85 (m, 1H), 3.46 (t, J = 5.2 Hz, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ = 170.54, 169.69, 155.58, 154.48, 153.24, 148.93, 144.82, 141.37, 132.13, 131.27, 126.82, 124.73, 123.36, 120.19, 114.68, 106.42, 72.33, 47.82, 41.81, 22.94. HRMS (Q-TOF): calculated for [M]⁺440.1244, found [M+Na]⁺463.1147.

(S)-N-((3-(3-fluoro-4-(4-((hydroxymethoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3j).

Following general procedure, product was collected by filtration to afford desired compound **3j** as a white solid (yield of 47.3%). ¹H NMR (400 MHz, CDCl₃) δ = 8.06 (d, J = 2.8 Hz, 1H), 7.93 (t, J = 8.8 Hz, 1H), 7.80 (dd, J = 13.2, 2.4 Hz, 1H), 7.30 (d, J = 13.2 Hz, 1H), 6.26 (t, J = 6.0 Hz, 1H), 4.84 (m, 1H), 4.79 (s, 2H), 4.11 (t, J = 8.8 Hz, 1H), 3.83 (m, 8H), 2.04 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ = 171.37, 154.59,

154.05, 152.11, 139.61, 125.02, 113.71, 106.56, 72.55, 72.16, 70.17, 64.33, 61.78, 47.35, 41.80, 23.06. HRMS (Q-TOF): calculated for [M]⁺ 393.1448, found [M+Na]⁺ 416.1393.

***(S)*-N-((3-(4-(4-(acetamidomethyl)-1H-1,2,3-triazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3k).**

Following general procedure, product was collected by filtration to afford desired compound **3k** as a white solid (yield of 91.0%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.40 (t, J = 6.0 Hz, 1H), 8.36 (d, J = 2.0 Hz, 1H), 8.25 (t, J = 6.4 Hz, 1H), 7.81 (m, 2H), 7.53 (dd, J = 9.6, 2.8 Hz, 1H), 4.79 (m, 1H), 4.38 (d, J = 5.6 Hz, 2H), 4.19 (t, J = 8.8 Hz, 1H), 3.81 (m, 1H), 3.44 (t, J = 5.6 Hz, 1H), 1.85 (s, 3H), 1.84 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.52, 169.68, 155.47, 154.46, 153.00, 146.06, 140.92, 126.64, 124.72, 120.21, 114.53, 106.45, 72.38, 47.72, 41.84, 34.48, 22.95. HRMS (Q-TOF): calculated for [M]⁺ 390.1452, found [M+Na]⁺ 413.1418.

***(S)*-N-((3-(3-fluoro-4-(4-(2-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3p).**

Following general procedure, product was collected by filtration to afford desired compound **3p** as a white solid (yield of 86.8%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.77 (s, 1H), 8.26 (t, J = 6.0 Hz, 1H), 7.93 (m, 2H), 7.84 (m, 2H), 7.70 (m, 1H), 7.58 (d, J = 10.4 Hz, 1H), 4.81 (m, 1H), 4.56 (m, 1H), 4.22 (t, J = 9.2 Hz, 1H), 3.83 (m, 1H), 3.45 (m, 2H), 1.85 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.52, 155.65, 154.48, 153.18, 144.45, 141.24, 133.21, 132.59, 129.75, 129.27, 127.31, 126.90 (2C), 125.78, 119.93, 114.56, 106.47, 72.42, 47.76, 41.85, 22.92. HRMS (Q-TOF): calculated for [M]⁺ 390.1452, found [M+Na]⁺ 413.1418.

1.7 General procedure for the synthesis of compounds anti (1, 4)-3l, 3m, 3n, 3o, 3q.

To a solution of azide intermediate **1** (1.0 eq.) in mixed solvents of tertiary butanol: water (1:3) was added the cupric oxide (0.05 eq.), sodium ascorbate (0.1 eq.) and corresponding alkyne **2l**, **2m**, **2n**, **2o**, **2q** (1.2 eq.). The reaction was allowed to stir at 65°C overnight. After the reaction completed, the mixture was cooled to room temperature. Then, water was added into the reaction mixture, and the mixture was extracted with DCM for three times. The combined organic layer was washed with brine and dried over Na₂SO₄. After removing the solvents, the residue was added into the mixture solvents (PE: EA=4:1), and the solid was collected by filtration to give target compounds.

1.8 Chemical and physical data of anti (1, 4)-3l, 3m, 3n, 3o, 3q.

***(S, E)*-N-((3-(3-fluoro-4-(5-(4-hydroxybut-2-en-2-yl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3l).**

Following general procedure, product was collected by filtration to afford desired compound **3l** as a white solid (yield of 68.9%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.52 (d, J = 2.0 Hz, 1H), 8.26 (t, J = 6.0 Hz, 1H), 7.86 (t, J = 8.4 Hz, 1H), 7.82 (dd, J = 13.2, 2.4 Hz, 1H), 7.54 (dd, J = 8.8, 1.6 Hz, 1H), 5.75 (t, J = 7.2 Hz, 1H), 4.78 (m, 2H), 4.33 (t, J = 5.6 Hz, 2H), 4.20 (t, J = 9.2 Hz, 1H), 3.82 (m, 1H), 3.45 (t, J = 5.6 Hz, 2H), 2.12 (s, 3H), 1.84 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ = 170.52, 155.66, 154.47, 153.19, 146.86, 141.11, 132.28, 126.91, 124.72, 123.74, 120.05, 114.51, 106.30, 72.40, 59.38, 47.75, 41.85, 22.91. HRMS (Q-TOF): calculated for [M]⁺389.1499, found [M+Na]⁺412.1415.

(S)-N-((3-(4-(4-((1,3-dioxoisindolin-2-yl)methyl)-1H-1,2,3-triazol-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3m).

Following general procedure, product was collected by filtration to afford desired compound **3m** as a white solid (yield of 70.5%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.55 (s, 1H), 8.24 (t, J = 4.0 Hz, 1H), 7.86 (m, 5H), 7.51 (dd, J = 7.6, 1.2 Hz, 1H), 6.51 (s, 1H), 4.95 (s, 2H), 4.77 (m, 1H), 4.18 (t, J = 9.2 Hz, 1H), 3.80 (m, 1H), 3.43 (t, J = 5.6 Hz, 2H), 1.83 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ = 170.50, 167.86, 155.49, 154.45, 153.02, 143.44, 141.07, 135.03 (2C), 132.14, 126.72, 125.10, 123.72 (2C), 120.00, 114.44, 106.25, 72.37, 47.71, 41.83, 33.31, 31.77, 22.90. HRMS (Q-TOF): calculated for [M]⁺478.1401, found [M+Na]⁺501.1303.

(S)-N-((3-(3-fluoro-4-(4-(1-hydroxycyclopentyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3n).

Following general procedure, product was collected by filtration to afford desired compound **3n** as a white solid (yield of 87.5%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.28 (d, J = 2.4 Hz, 1H), 8.26 (t, J = 6.0 Hz, 1H), 7.84 (t, J = 8.8 Hz, 1H), 7.80 (m, 1H), 7.53 (dd, J = 9.2, 2.0 Hz, 1H), 5.14 (s, 1H), 4.82 (m, 1H), 4.20 (t, J = 9.2 Hz, 1H), 3.82 (m, 1H), 3.45 (d, J = 5.6 Hz, 2H), 2.05 (m, 3H), 1.84 (s, 3H), 1.73 (m, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ = 170.51, 155.58, 154.47, 152.99, 140.81, 126.61, 123.01, 120.46, 114.50, 106.59, 77.87, 72.37, 47.72, 41.84, 41.20 (2C), 23.80 (2C), 22.91. HRMS (Q-TOF): calculated for [M]⁺403.1656, found [M+Na]⁺426.1541.

N-(((R)-3-(3-fluoro-4-(5-((S)-1-hydroxyethyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3o).

Following general procedure, product was collected by filtration to afford desired compound **3o** as a white solid (yield of 90.5%). ¹H NMR (400 MHz, DMSO-*d*₆) δ = 8.34 (d, J = 1.6 Hz, 1H), 8.26 (t, J = 5.8 Hz, 1H), 7.84 (m, 2H), 7.53 (dd, J = 8.8, 1.6 Hz, 1H), 5.39 (d, J = 5.2 Hz, 1H), 4.92 (m, 1H), 4.82 (m, 1H),

4.27 - 4.14 (t, $J = 5.2$ Hz, 1H), 3.82 (m, 1H), 3.45 (t, $J = 5.6$ Hz, 2H), 1.84 (s, 3H), 1.48 (d, $J = 6.8$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) $\delta = 170.52, 155.51, 154.05, 153.04, 140.77, 126.67, 123.28, 120.43, 114.50, 106.34, 72.37, 61.94, 47.72, 41.84, 24.10, 22.91$. HRMS (Q-TOF): calculated for $[\text{M}]^+$ 403.1656, found $[\text{M}+\text{Na}]^+$ 426.1541.

N-(((R)-3-(3-fluoro-4-(4-((R)-1-hydroxyethyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3q).

Following general procedure, product was collected by filtration to afford desired compound **3q** as a white solid (yield of 82.5%). ^1H NMR (400 MHz, DMSO- d_6) $\delta = 8.34$ (d, $J = 1.6$ Hz, 1H), 8.25 (t, $J = 5.6$ Hz, 1H), 7.81 (m, 2H), 7.53 (dd, $J = 8.8, 2.0$ Hz, 1H), 5.38 (d, $J = 4.8$ Hz, 1H), 4.92 (m, 1H), 4.78 (m, 1H), 4.20 (t, $J = 8.8$ Hz, 1H), 3.81 (m, 1H), 3.45 (t, $J = 5.6$ Hz, 2H), 1.84 (s, 3H), 1.48 (d, $J = 6.4$ Hz, 3H). HRMS (Q-TOF): calculated for $[\text{M}]^+$ 363.1343, found $[\text{M}+\text{Na}]^+$ 386.1215.

1.9 General procedure for the synthesis of compounds anti (1, 4)-5a~5i.

To a solution of azides **4a-4i** (50 mg, 1 eq.) in tert-butyl alcohol (5 mL) was added copper powder (1 eq.) and copper sulfate pentahydrate (0.5 eq.), then 2-ethynylpyridine (2 eq.) was added into the mixture. The mixture was stirred at 65°C overnight, then cooled to room temperature. Then 20 mL water was added into the mixture to quench the reaction, the mixture was extracted with DCM (20mL) for three times. The combined organic layer was evaporated under vacuum to give crude product, which was purified through silica gel thin layer chromatography to give compounds **5a-5i** (yield 10.98 ~ 38.45 %).

1.10 Chemical and physical data of anti (1, 4)-5a~5i.

2-(1-(2,4',6-trifluoro-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-4-yl)pyridine (5a)

Following general procedure, starting from 4-azido-2,4',6-trifluoro-1,1'-biphenyl (**4a**) and 2-ethynylpyridine to afford compound **5a** as a white solid (yield 28.41 %). ^1H NMR (400 MHz, DMSO- d_6) δ 9.51 (s, 1H), 8.68 (d, $J = 4.4$ Hz, 1H), 8.14 (m, 1H), 8.05 (m, 2H), 7.97 (td, $J = 7.6, 1.6$ Hz, 1H), 7.60 (m, 4H), 7.44 (m, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 161.30, 158.84, 150.25, 149.56, 149.00, 137.92, 137.51, 134.28, 132.44 (2C), 129.22 (2C), 126.94, 124.07, 122.07, 120.40, 104.90, 104.74, 104.58. HRMS (Q-TOF): calculated for $\text{C}_{19}\text{H}_{11}\text{F}_3\text{N}_4$ $[\text{M}]^+$: 352.0936. Found $[\text{M}+\text{H}]^+$: 353.1010.

1-(3'-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-4-yl)ethan-1-one (5b)

Following general procedure, starting from 1-(3'-azido-[1,1'-biphenyl]-4-yl)ethan-1-one (**4b**) and 2-ethynylpyridine to afford compound **5b** as a white solid (yield 32.66 %). ^1H NMR (400 MHz, DMSO- d_6) δ 9.55 (s, 1H), 8.68 (d, $J = 4.4$ Hz, 1H), 8.40 (m, 1H), 8.15 (m, 1H), 8.10 (m, 3H), 8.03 (m, 2H), 7.97 (td, $J = 7.6, 2.0$ Hz, 1H), 7.91 (m, 1H), 7.75 (t, $J = 8.0$ Hz, 1H), 7.42 (m, 1H), 2.65 (s, 3H). ^{13}C NMR (100

MHz, DMSO-*d*₆) δ 198.05, 150.16, 149.99, 148.75, 143.52, 141.07, 137.84, 137.74, 136.71, 131.16, 129.37 (2C), 127.76 (2C), 127.71, 123.85, 122.09, 120.44, 120.29, 119.00, 27.30. HRMS (Q-TOF): calculated for C₂₁H₁₆N₄O [M]⁺:340.1324. Found [M+H]⁺: 341.1400.

2-(1-(4-(benzo[d][1,3]dioxol-5-yl)-2-fluorophenyl)-1H-1,2,3-triazol-4-yl)pyridine (5c)

Following general procedure, starting from 5-(4-azido-3-fluorophenyl)benzo[d][1,3]dioxole (**4c**) and 2-ethynylpyridine to afford compound **5c** as a white solid (yield 10.98 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.06 (d, *J* = 2.0 Hz, 1H), 8.67 (m, 1H), 8.14 (m, 1H), 7.94 (m, 3H), 7.74 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.43 (m, 2H), 7.34 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 6.11 (s, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 150.25, 149.82, 148.69, 148.37, 148.19, 143.47, 137.87, 132.15, 126.66, 124.93, 123.89, 123.56, 123.53, 121.45, 120.35, 115.16, 109.29, 107.79, 101.94, 100.00. HRMS (Q-TOF): calculated for C₂₀H₁₃FN₄O₂ [M]⁺:360.1023. Found [M+H]⁺: 361.1101.

3-(1-(2,3',6-trifluoro-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-4-yl)pyridine (5d)

Following general procedure, starting from 4-azido-2,3',6-trifluoro-1,1'-biphenyl (**4d**) and 2-ethynylpyridine to afford compound **5d** as a white solid (yield 25.48 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.49 (m, 1H), 8.66 (m, 1H), 8.03 (m, 4H), 7.59 (s, 1H), 7.41 (m, 4H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 163.64, 161.27, 158.83, 150.22, 149.55, 149.00, 137.87, 137.53, 131.12, 130.18, 126.84, 124.04, 122.05, 120.38, 117.49, 116.29, 104.82, 104.67, 104.51. HRMS (Q-TOF): calculated for C₁₉H₁₁F₃N₄ [M]⁺:352.0936. Found [M+H]⁺: 353.1006.

1-(4'-(4-(pyridin-2-yl)-1H-1,2,3-triazol-1-yl)-[1,1'-biphenyl]-4-yl)ethan-1-one (5e)

Following general procedure, starting from 1-(4'-azido-[1,1'-biphenyl]-4-yl)ethan-1-one (**4e**) and 2-ethynylpyridine to afford compound **5e** as a yellow solid (yield 29.80 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.42 (s, 1H), 8.68 (d, *J* = 5.2 Hz, 1H), 8.16 (m, 3H), 8.08 (m, 2H), 8.02 (m, 2H), 7.96 (m, 3H), 7.42 (m, 1H), 2.64 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 197.98, 150.18, 149.94, 148.79, 143.51, 139.57, 137.84, 136.89, 136.52, 129.45 (2C), 128.91 (2C), 127.46 (2C), 123.87, 121.73, 121.12 (2C), 120.34, 27.29. HRMS (Q-TOF): calculated for C₂₁H₁₆N₄O [M]⁺:340.1324. Found [M+H]⁺: 341.1400.

2-(1-(3,5-difluoro-3',4'-dimethoxy-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-4-yl)Pyridine (5f)

Following general procedure, starting from 4-azido-3,5-difluoro-3',4'-dimethoxy-1,1'-biphenyl (**4f**) and 2-ethynylpyridine to afford compound **5f** as a white solid (yield 25.53 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.13 (s, 1H), 8.67 (d, *J* = 4.8 Hz, 1H), 8.15 (m, 1H), 7.97 (td, *J* = 7.6, 1.6 Hz, 1H), 7.89 (d, *J* = 10.0 Hz, 2H), 7.44 (m, 3H), 7.10 (m, 1H), 3.90 (s, 3H), 3.84 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 158.34, 155.84, 150.52, 150.29, 149.69, 149.66, 147.98, 144.97, 137.92, 129.52, 126.78, 123.98, 120.35, 120.15, 112.57, 111.10, 110.71, 110.51, 110.48, 56.25, 56.14. HRMS (Q-TOF): calculated for C₂₁H₁₆F₂N₄O₂ [M]⁺:394.1241. Found [M+H]⁺: 395.1318.

3-(1-(2,2',6-trifluoro-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-4-yl)pyridine (5g)

Following general procedure, starting from 4-azido-2,2',6-trifluoro-1,1'-biphenyl (**4g**) and 2-ethynylpyridine to afford compound **5g** as a white solid (yield 30.67 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.52 (s, 1H), 8.69 (d, *J* = 4.4 Hz, 1H), 8.12 (m, 3H), 7.97 (m, 1H), 7.60 (m, 2H), 7.43 (m, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 161.37, 158.90, 150.24, 149.57, 149.00, 138.14, 137.90, 132.80, 132.23, 125.25, 124.06, 122.17, 120.40, 116.38, 115.66, 112.15, 104.81, 104.65, 104.50. HRMS (Q-TOF): calculated for C₁₉H₁₁F₃N₄ [M] :352.0936. Found [M+H]⁺ : 353.1017.

3-(1-(3'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)-1H-1,2,3-triazol-4-yl)pyridine (5h)

Following general procedure, starting from 4'-azido-3-(trifluoromethoxy)-1,1'-biphenyl (**4h**) and 2-ethynylpyridine to afford compound **5h** as a white solid (yield 12.95 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.42 (s, 1H), 8.68 (d, *J* = 4.0 Hz, 1H), 8.16 (m, 3H), 7.97 (m, 3H), 7.84 (m, 1H), 7.77 (s, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.42 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 150.18, 149.93, 149.54, 149.52, 148.78, 141.60, 139.07, 137.84, 136.80, 131.52, 128.84 (2C), 126.41, 123.87, 121.73, 121.10 (2C), 120.79, 120.34, 119.89. HRMS (Q-TOF): calculated for C₂₀H₁₃F₃N₄O [M] :382.1041. Found [M+H]⁺ : 383.1122.

2-(1-(1,1'-biphenyl)-4-yl)-1H-1,2,3-triazol-4-yl)pyridine (5i)

Following general procedure, starting from 4-azido-1,1'-biphenyl (**4i**) and 2-ethynylpyridine to afford compound **5i** as a white solid (yield 38.45 %). ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.39 (s, 1H), 8.68 (d, *J* = 4.8 Hz, 1H), 8.14 (m, 3H), 7.95 (m, 3H), 7.77 (m, 2H), 7.52 (m, 2H), 7.43 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 150.16, 149.99, 148.73, 140.92, 139.25, 137.81, 136.24, 129.55 (2C), 128.48 (3C), 127.24 (2C), 123.82, 121.68, 121.07 (2C), 120.32. HRMS (Q-TOF): calculated for C₁₉H₁₄N₄ [M] :298.1218. Found [M+H]⁺ : 299.1300.

2. Biology section

2.1 Preparation for E. coli ribosome extracts (ERE)

This preparation for ERE was based on Kim et al. with some modifications.² For 3-liter culture, 20 to 25 ml of ribosome extracts was expected. The fermentation starter was incubated by growing *E. coli* BL21(DE3) strain (25% glycerol stock) to 100 ml in 2 xYT medium. For fermentation, 4 x 0.75 L of 2 x YT medium with vigorous agitation was performed (37 °C, 200 rpm). Cells were grown for 5 to 6 hours until mid-log phase (OD₆₀₀ of approximately 2) and centrifugation (6000 RCF, SLA-3000, 5950 rpm, 15 min, 4°C) was set to harvest cells. Then two other centrifugations (6000 RCF, SLA-3000, 5950 rpm or SLC-6000, 5230 rpm, 15 min, 4°C) were followed by the wash of homogenized cells. Wall-cracking was performed using two runs of french press (14,000 psi). In the end, ERE was obtained underdoing the last centrifugation (12,000 RCF, SS-34, 10,000 rpm, 10 min, 4 °C) and divided into small aliquots and frozen

in liquid nitrogen, stored at -80 °C. For each batch, A₂₈₀ and A₂₆₀ were measured using NanoDrop 2000C ultra- microspectrophotometry.

2.2 Preparation for R-free100 of *E. coli*

Centrifugation of ERE was employed at 100,000 RCF (48,000 RPM) for 2.5 h at 4 °C. Supernatant was collected followed by a same re-centrifugation.² The pellet was discarded and supernatant (R-free 100) was carefully collected. R-free 100 was divided into small aliquots and frozen in liquid nitrogen, stored at -80 °C.

2.3 *In situ* click experiment coupled with *in vitro* Transcription-Translation (T/T) system

ERE was incubated with azide **1** (5 μM) at 0°C (on an ice bucket) for 30min in 96-well plate. 5 mM of each alkyne was added to trigger *in situ* click reaction followed by 24h of incubation at rt. Then in each case, 2.5x *in vitro* Transcription-Translation Premix [500 mM potassium acetate, 87.5 mM Tris-acetate [pH 8.0], 67.5 mM ammonium acetate, 50 g/ml of folinic acid, 5 mM DTT, 87.5 mg/ml of polyethylene glycol, 5.0 mM ATP, 1.25 mM [each] additional ribonucleotide triphosphate, 50 mM phosphoenolpyruvate [trisodium salt], 2.5 mM cyclic AMP, 250 g/ml of each *E. coli* tRNA], amino acid mix (1.25 mM concentrations of each amino acid), luc-plasmid] was added to the incubation mixture. 1h of incubation at 37°C was set to perform the expression of luciferase. The amount of luciferase was measured using Luciferase Assay System Kit (Promega).³ In addition, stability test of ribosome at different temperature was performed and *in vitro* T/T assay results shown no much loss of ribosome activity after incubation at r.t for 24 h (Figure S1).

2.4 Ribosomes isolation and purification

E. coli strain BL21 (DE3) was used for purification of bacterial 70S ribosomes, 50S subunit and 30S subunit. Bacteria growth, cell wall disruption and ribosome purification were performed as Zohar described.⁴

2.5 Pure *E. coli* ribosomes-templated *in situ* click experiment

5 μM of ribosome template (*E. coli* 70S ribosome or 50S ribosome subunit), 5 μM azide **1**, and 5 mM alkyne were incubated at r.t for 24 h. The whole incubation system volume was 100 μL. Buffer-only control (without ribosomes in it) and bovine serum albumin (BSA) control (final concentration of 5 μM). 30S *E. coli* ribosomal subunit (5 μM) reaction was performed as another control group for investigating the nonspecific binding. Because 30S subunit does not possess an oxazolidone-binding site, it is supposed not capable to catalyze the cycloaddition reactions with azide **1** and alkyne **2a~2q**.

2.6 LC-MS analysis of *in situ* click reaction products

90 μ L of the above solutions were injected on an AB/MDS Sciex 5500QTRAP LC-MS instrument utilizing an XBridge™ BEH, 75 \times 2.1 mm, 2.5 μ m C18 reverse phase column with a flow rate of 0.3 mL/min and a 5-minute gradient from 0 % Methanol (0.1 % HCO₂H)/100 % water (0.1 % HCO₂H)-100 % Methanol (0.1 % HCO₂H)/0 % water (0.1 % HCO₂H). Extracted ion chromatograms (EIC's) were used to locate and quantify amounts of triazole products.

2.7 Ribosome Activity Assay.

Purified 70S *E. coli* ribosomes was used in translation reactions. Luc plasmid was gifted by Dr. Ada Yonath group (Department of Structural Biology, Weizmann Institute of Science). Bacterial ribosome activity was determined in a bacterial-coupled transcription/translation assay system, which measures the expression of the luciferase gene⁵. The IC₅₀ values represent the drug concentration that inhibits luciferase activity by 50 %.

2.8 Minimum inhibitory concentration (MIC) assay

S. aureus strains ATCC33591 and ATCC25923 were inoculated from freezer stock into Brain Heart Infusion (BHI) Broth 37 °C overnight. The culture was then diluted 1:100 and grown to an OD₆₀₀ = 0.6 (2-4 h). 75 μ L of BHI Broth was added to wells in rows 1-11 of 96-well plates. 150 μ L of a 256 μ g/mL antibiotic solution in BHI Broth was then added to row. Serial dilutions were made from row 12 to 1 for a final volume of 75 μ L in each well. The above-prepared *S. aureus* cultures were then diluted to OD₆₀₀ = 0.004 with BHI Broth and 75 μ L of the above-prepared *S. aureus* culture was then added to all wells. The last column of the 96-well plate was reserved for negative controls (BHI Broth only) and positive controls (OD₆₀₀ = 0.004). 96-well plates were covered and incubated at 37 °C for 18 h. Plates were visually inspected to determine MIC. MIC testing of compounds against other isolates utilized in this work was performed following similar method.

2.9 Reproducibility validation of ERE

Three parallel batches of ERE were prepared respectively as described previously on each day of DAY one, DAY three and DAY five. At last, 9-batch of ERE were obtained for robustness study of ERE as click reaction template catalyzing [2+3] Huisgen cycloaddition. Both ERE-templated *in situ* click experiments and *E. coli* *in vitro* T/T assay were carried out using 9-batch of ERE. Batch-to-batch variations of amount of luciferase generated in *in vitro* T/T system were calculated to value the reproducibility and robustness of different batches of *E. coli* ribosomal extracts. *In situ* click experiments using 9-batch ERE as template were performed as previously described and *E. coli* *in vitro* T/T assays were performed with some modifications as follow. 25 μ L of ERE was added to 75 μ L of buffer A followed by an incubation at r.t. for 24 hours. 15 μ L of the premix for *in vitro* Transcription-Translation

system (Murray et al., 2001) was added into the mixture.⁵ Another incubation of 1h at 37 °C was set to perform the expression of luciferase. The amount of luciferase was measured using Luciferase Assay system.³

2.10 *In cellulo click chemistry using S. aureus ATCC33591*

Alkyne control. 75 µL of alkyne solution (10 mM in BHI) was added to each well of the 96-well plate. Then 75 µL of 1:1000 v/v dilution of an overnight bacterial culture grown in BHI at 37 °C was added to each well.

Azide control. 205 µL (50 µM) of azide stock solution was diluted with 10 mL BHI into 5 µM solution. 75 µL of the mixture was added into each well of the 96-well plate. 75 µL of 1:1000 (v/v) overnight bacterial culture dilution was added to each well.

In cellulo click reaction. 75 µL of azide solution (20 µM) was added to columns 1-11 of the 96-well plate. 150 µL of alkyne solution (10 mM) was added and serial diluted into the remaining columns. 75 µL of 1:1000 (v/v) overnight bacterial culture dilution was added to each well.

Resazurin-based alamarBlue assay. AlamarBlue reagents were added to each well and the plate was incubated at 37°C for another 8 hours.

2.11 *RT-PCR and Western blot analysis*

Bacteria growth and treatment with tested compounds. *S. aureus* strains ATCC33591 was inoculated from freezer stock into Brain Heart Infusion (BHI) Broth 37 °C overnight. The culture was then diluted 1:100 and grown to an OD₆₀₀ = 0.6 (2-4 h). Cells were treated for 2 h with 1/2 and 1/4× MIC of **5c**, **5h**, **3a** and Chloramphenicol (CHL, positive control). MIC values of these compounds against ATCC33591 are 2.0, 4.0, 0.5 and 2.0 µg/mL respectively.

RT-PCR. Total RNA was extracted using Trizol method and cDNA was obtained using reverse transcription kit (TransGen Biotech). Then PCR was conducted with cDNA. *HcaT* was used as internal control.

Primer sequences:

hcaT-F: GCTGCTCGGCTTTCTCATCC, hcaT-R: CCAACCACGCTGACCAACC.

EFTu-F: CAATCACCACCGTACTGGCT, EFTu-R: AAGAATCCAGGAAGCCAGCC.

Western blot

Total cellular proteins were extracted in *RIPA Lysis Buffer* (Beyotime Biotechnology). Protein concentrations were determined with BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein were run out on 10% SDS-PAGE gel and subsequently transferred onto PVDF membranes (Millipore). Membranes were blocked in 5% skimmed milk and incubated with *anti-EFTu antibody*

(Proteintech) at 4°C overnight. *Anti-Rabbit antibody* (Proteintech) was used as second antibodies and incubated at 37 °C for 1 h.

2.12 Microscopic observations

S. aureus strains ATCC33591 was inoculated from freezer stock into Brain Heart Infusion (BHI) Broth 37 °C overnight. The culture was then diluted 1:100 and grown to an OD₆₀₀ = 0.6 (2-4 h). Cells were treated for 6 h with 1/2 × MIC of 5c, 5h, 3a and Linezolid (LNZ, positive control). MIC values of these compounds against ATCC33591 are 2.0, 4.0, 0.5 and 2.0 μg/mL respectively. Then microscopic observation was conducted using Gram staining method.”

2.13 Screening for species-specific ribosome inhibitors

Based on the preparation methods of *E. coli* ribosome extracts (ERE) and 70S ribosomes, *S. aureus* ribosome extracts (SRE) and 70S ribosomes were obtained using *S. aureus* strain MRSA ATCC33591. The procedures of coupled assay and *in situ* click chemistry experiment with *S. aureus* materials were performed based on the those in *E. coli* experiments. The screening of alkyne **2a~2q** was against azide **1** and screening for azides **4a~4i** was against alkyne **2a**.

3. Supplementary tables and figures

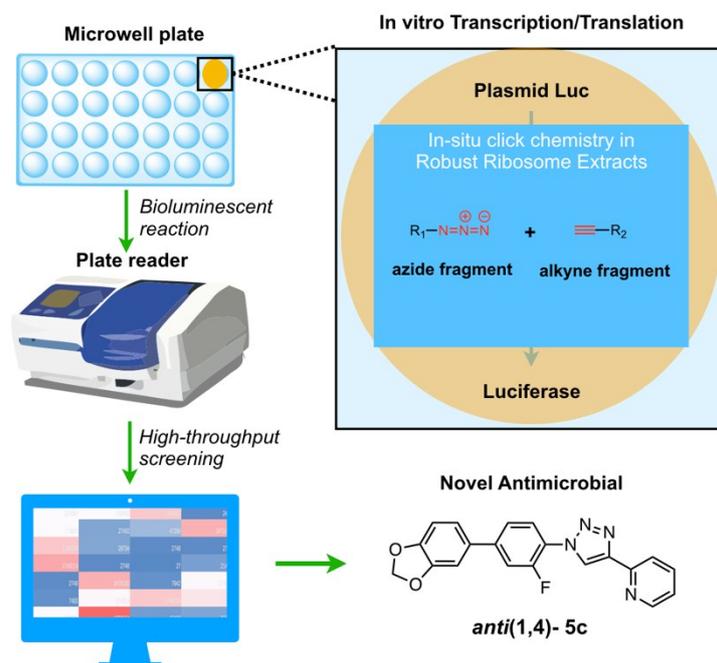


Figure S1. Illustration of the robust and accurate high-throughput platform developed in this work: the synthesis of the ribosomal inhibitors was driven by a very crude ribosome extract system (ERE) through *in situ* [2+3] Huisgen cycloaddition and integrated with an *in vitro* transcription/translation luminescence assay.

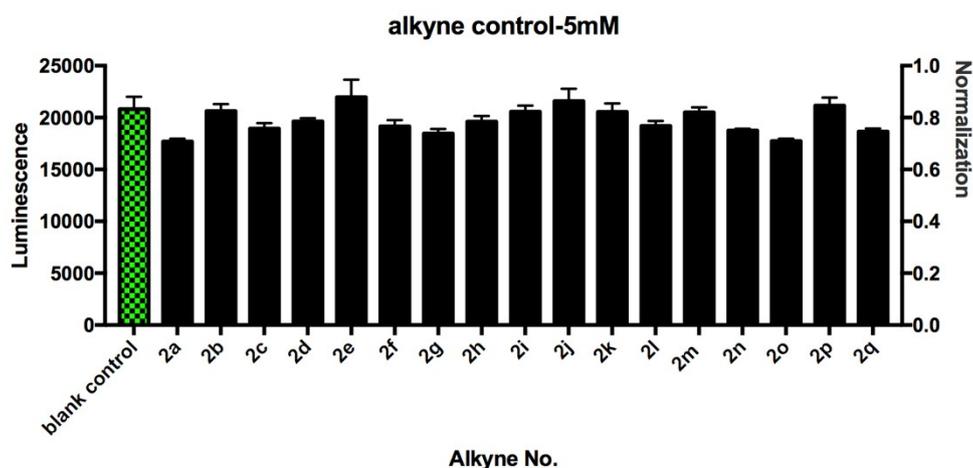


Figure S2. Alkyne control (5 mM) experiment of coupled assay established in this work (*in situ* click chemistry system coupled with *in situ* luciferase production and luminescence detection).

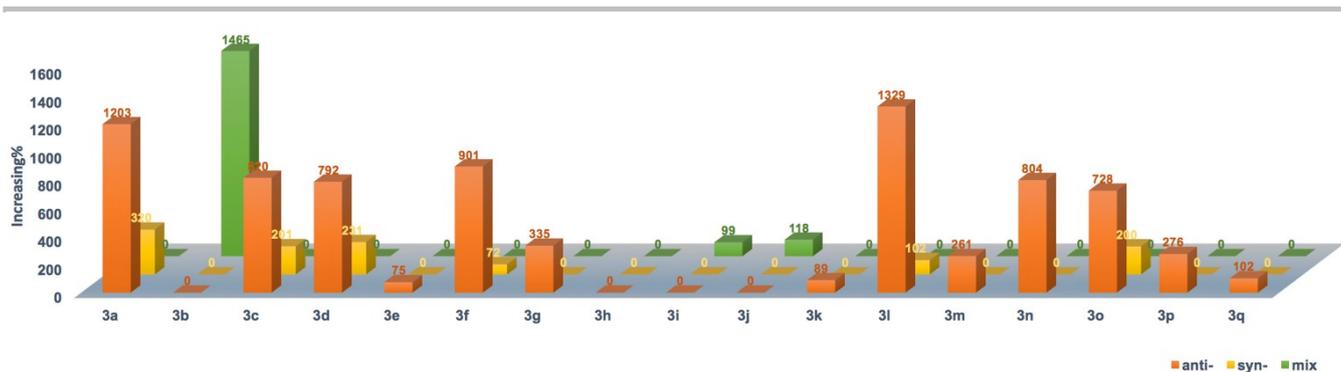


Figure S3. *In situ* click experiment (*E. coli* 70S subunits -templated) with **1** and 17 alkynes respectively. Mix represents unresolved *anti* (1, 4)- and *syn* (1, 5)- isomers. Increase % of mass counts are calculated from the ratio of the ribosome-templated reaction to the buffer control group. Results are an average of 3 independent experiments (see standard error in Table S1 below).

Table S1. LC-MS analysis of *E. coli* 70S subunits-templated *in situ* click experiments

Cmpd#	[mass counts of <i>anti</i> (1,4)-]	[mass counts of <i>syn</i> (1,5)-]	30S	BSA	Buffer	Increase%	S.D
3a	1147536	305247	227382	109291	90029	1524	123
3b		1452672 ^[a]	447278	437282	92839	1583	103
3c	723709	454579	444922	399945	98398	1195	34
3d	768722	224210	334883	300392	88372	1065	43
3e	78493	0	75882	57893	44893	51	8
3f	934633	74688	600923	465783	94883	1156	29
3g	885636	0	55564	66487	203483	335	109
3h	43799	0	58378	40095	45694	6	10
3i		99053 ^[a]	47937	57937	49683	75	88
3j		75847 ^[a]	65843	57833	34783	89	12
3k	58589	0	44589	46578	30942	151	19
3l	1474126	113138	246842	223984	100932	1366	133
3m	144532	0	54567	44795	39984	284	34
3n	888894	0	229302	222937	98348	910	59
3o	531552	146031	227563	374865	67578	1048	88
3p	167732	0	54737	56773	44657	246	39
3q	95883	0	47583	59489	47583	62	9

^[a] Unsolved mixture of *anti*- and *syn*- isomers. Results are an average of three independent experiments.

Table S2. MIC ($\mu\text{g/mL}$) values of *anti*-triazole products against *E.coli* and *P. aeruginosa*

Comp#	<i>Escherichia coli</i> ATCC25922	<i>Pseudomonas aeruginosa</i> ATCC27853	Comp#	<i>Escherichia coli</i> ATCC25922	<i>Pseudomonas aeruginosa</i> ATCC27853
3a	>64	>64	3o	>64	>64
3b	>64	>64	3p	>64	>64
3c	>64	>64	3q	>64	>64
3d	>64	>64	5a	>64	>64
3e	>64	>64	5b	>64	>64
3f	>64	>64	5c	>64	>64
3g	>64	>64	5d	>64	>64
3h	>64	>64	5e	>64	>64
3i	>64	>64	5f	>64	>64
3j	>64	>64	5g	>64	>64
3k	>64	>64	5h	>64	>64
3n	>64	>64	5i	>64	>64

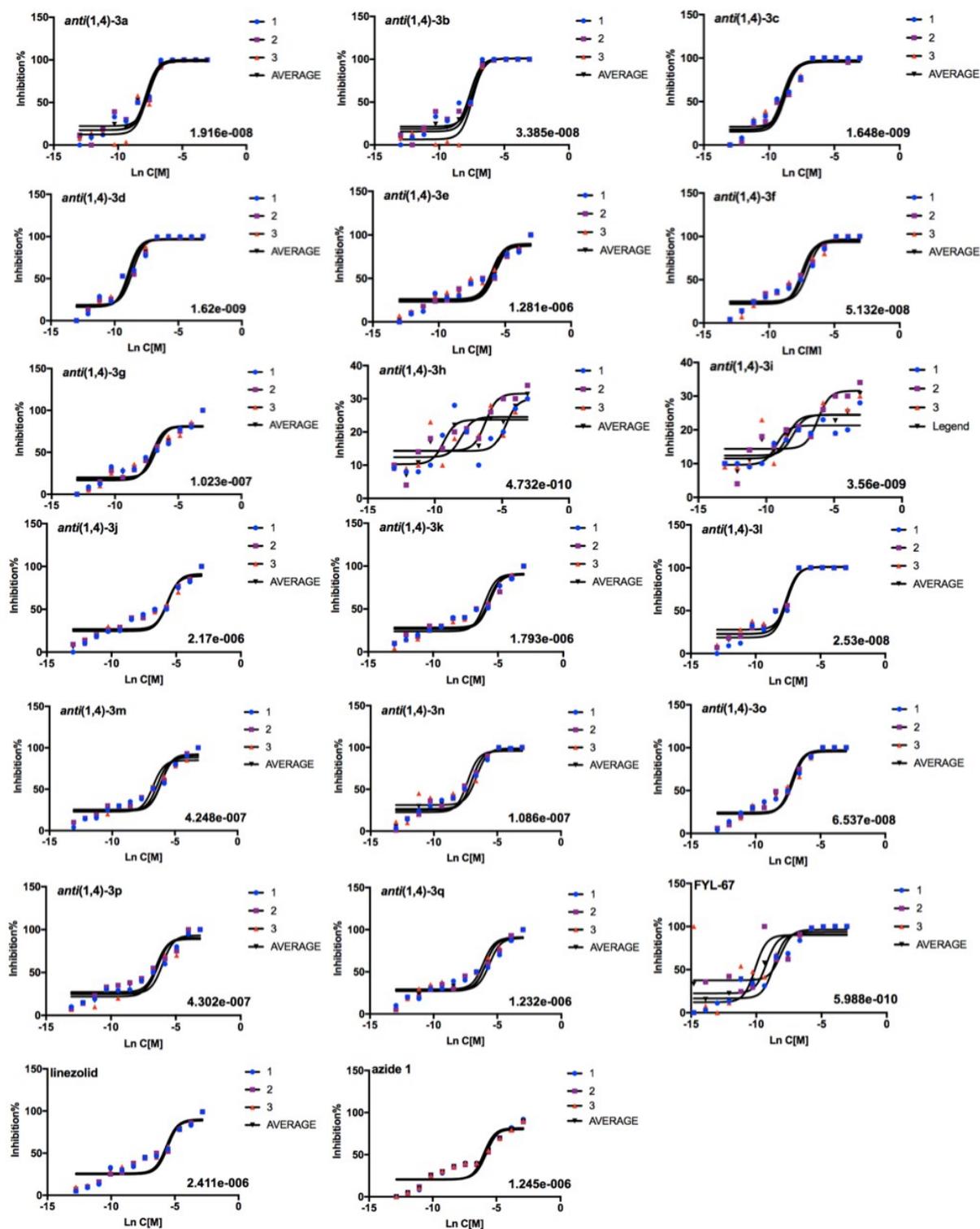


Figure S4. IC₅₀ values of *anti* (1, 4)- triazole products **3a~3q**, azide **1**, Linezolid and FYL-67 on *E. coli* ribosome.

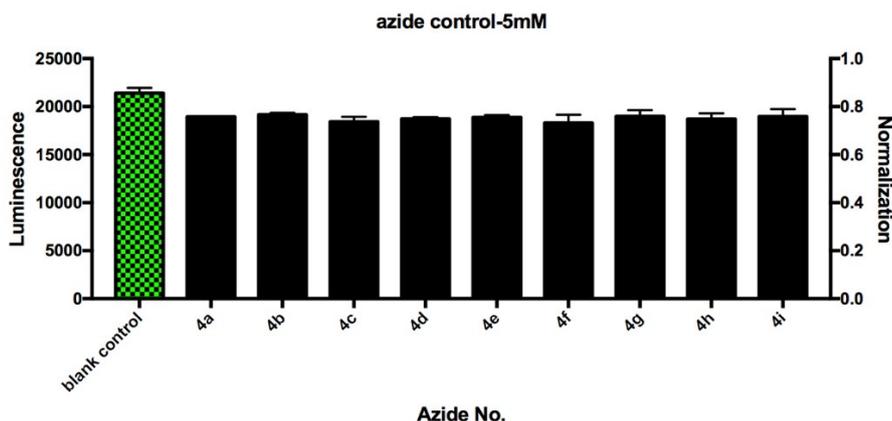


Figure S5. Azide control experiment (5mM) of coupled assay established in this work (*in situ* click chemistry coupled with *in situ* luciferase production and luminescence detection).

Table S3. Bacterial ribosomal inhibitory activity of *anti*-triazole products **5a**~**5i** at 100 μ M

Comp#	Inhibition ratio%	Comp#	Inhibition ratio%
5a	10	5f	8
5b	12	5g	7
5c	100	5h	100
5d	6	5i	10
5e	8	FYL-67	100

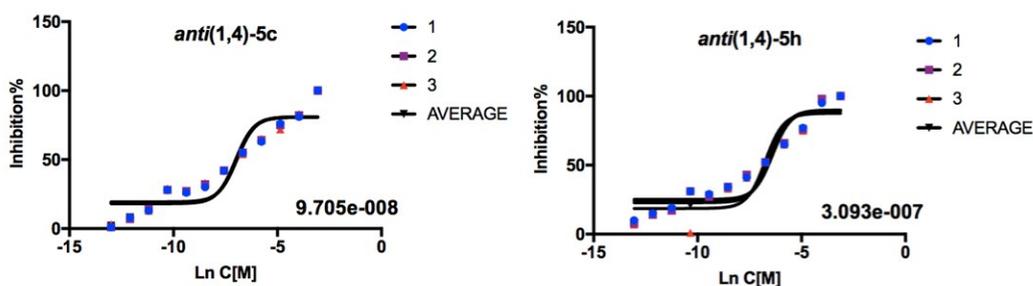


Figure S6. IC₅₀ values of *anti* (1, 4)- triazole products **5c** and **5h** on *E. coli* ribosome.

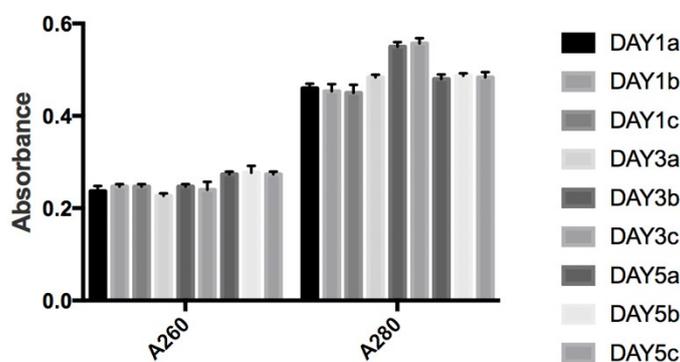


Figure S7. A₂₆₀ and A₂₈₀ measured using NanoDrop 2000C ultra-microspectrophotometer of 9-batch of ERE prepared on DAY one, DAY three and DAY five. Results are shown as mean \pm SD, n=9.

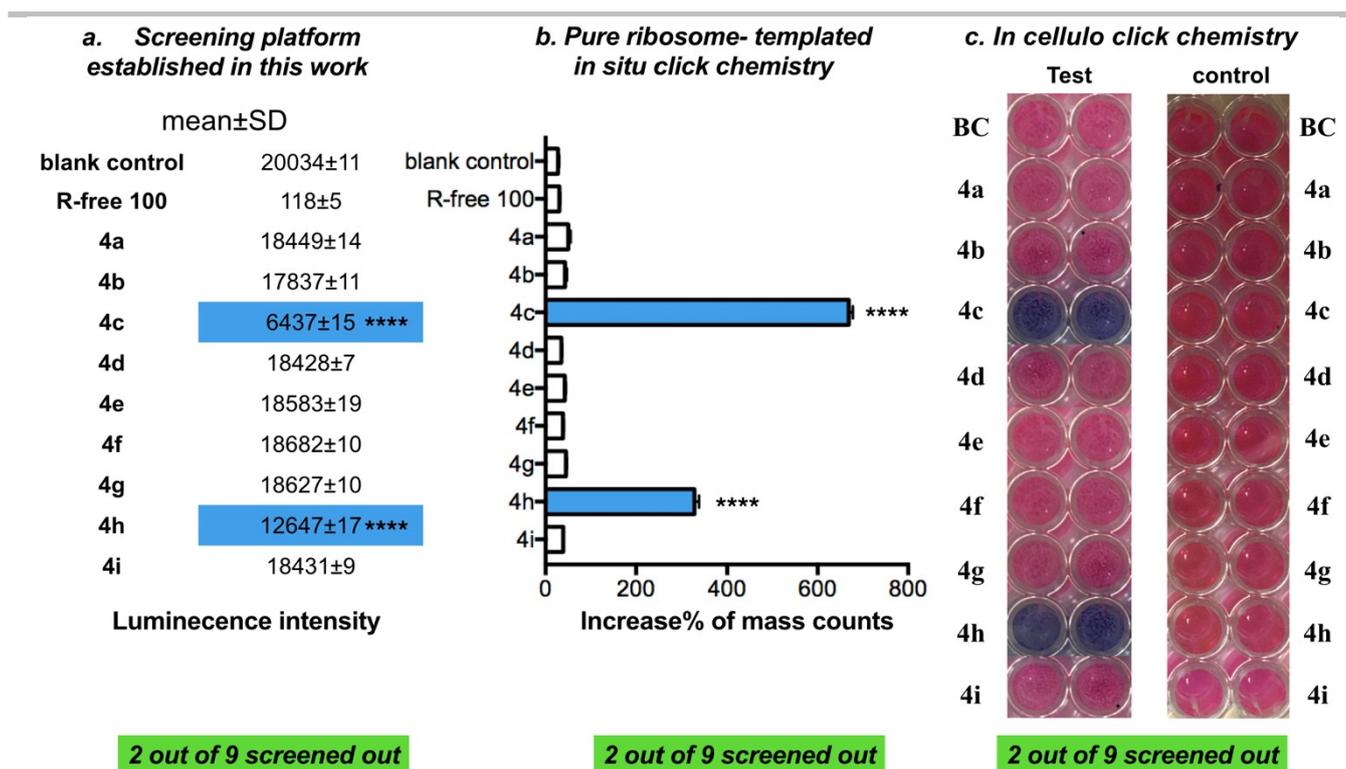


Figure S8. Screening results of 9-azide **4a~4i** against alkyne **2a** using three different screening strategies. **a)** The integration method established in this work. Results are shown as mean ±SD. 9 individual experiments using 9-batch of ERE prepared following the standard procedure on DAY 1, DAY 3 and DAY 5 (3 batches on each day) were respectively performed. Results are shown as mean ±SD (n=9; ****, $p < 0.0001$). **b)** Classical *in situ* click chemistry strategy using pure ribosomes to catalyze Huisgen cycloaddition reaction. Click products were analyzed using LC-MS and mass counts of each triazolles was recorded. Results are an average of three experiments. Increase% of mass counts are calculated from the ratio of the ribosome-templated reaction to the buffer control group. Results are shown as mean ±SD (n=3; ****, $p < 0.0001$). **c)** *In cellulo* click chemistry performed in *S. aureus* strain ATCC33591. Control groups were performed with only azide fragments. **BC**, blank control.

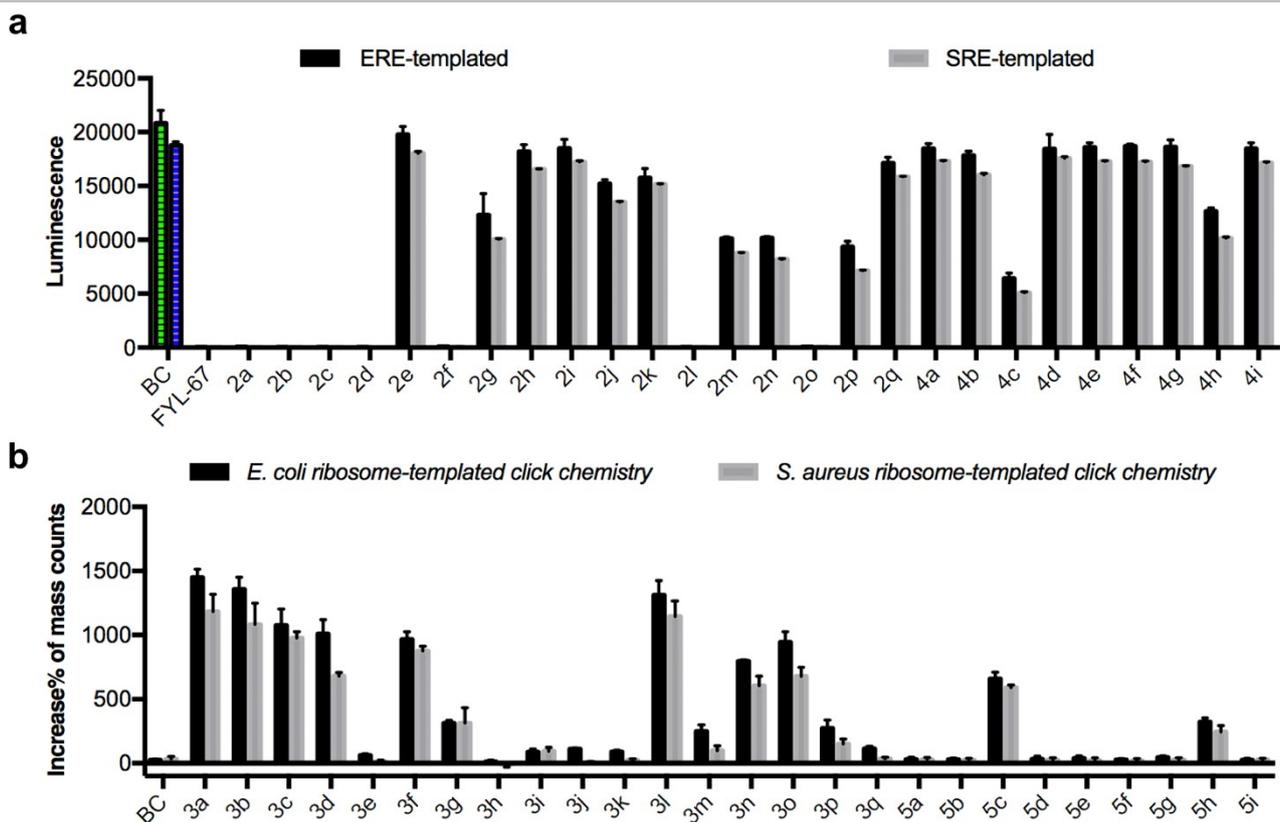


Figure S9. Screening results of species-specific inhibitor with (a) coupled assay in this work and (b) classical *in situ* click chemistry. (a) Preparation of *S. aureus* (MRSA, ATCC33591) ribosome extracts (SRE) is as same as it for ERE. Screening of building blocks alkynes **2a~2q** and azides **4a~4i** were against azide **1** and alkyne **2a** respectively. (b) Increase % of mass counts from LC-MS analysis of click products (total of *anti*- and *syn*- isomers). *E. coli* (BL21) 70S ribosome and *S. aureus* (MRSA, ATCC33591) 70S ribosome were used as click templates respectively. Increase% of mass counts are calculated from the ratio of the ribosome-templated reaction to the buffer control group. Results are shown as mean \pm SD (n=3, see standard error in Table S4).

Table S4. LC-MS analysis of *S. aureus* and *E. coli* 70S ribosomes-templated *in situ* click experiments

Cmpd#	<i>E. coli</i> 70S ribosomes				<i>S. aureus</i> 70S ribosomes			
	Increase%	Increase%	Increase%	S.D	Increase%	Increase%	Increase%	S.D
BSA	32	15	23	9	29	10	55	23
3a	1523	1399	1423	66	1300	1033	1211	136
3b	1465	1300	1309	93	1109	902	1233	167
3c	1021	988	1221	126	999	1011	920	49
3d	1023	888	1112	113	700	693	650	27
3e	75	50	59	13	-2	13	22	12
3f	973	902	1022	60	832	892	902	38
3g	335	290	310	23	293	440	203	120
3h	0	11	23	12	-31	11	0	22
3i	99	60	101	23	100	59	120	31
3j	118	110	101	9	-1	0	14	8
3k	89	80	102	11	21	34	10	12
3l	1431	1300	1203	114	1194	1002	1233	124
3m	261	192	290	50	102	56	133	39
3n	804	801	784	11	619	670	521	76
3o	928	877	1032	79	713	600	723	68
3p	276	202	334	66	168	103	177	40
3q	102	101	134	19	48	20	34	14
5a	50	20	22	17	45	29	10	18
5b	43	20	19	14	40	10	23	15
5c	669	703	603	51	609	593	566	22
5d	35	10	55	23	44	20	10	17
5e	43	20	55	18	47	10	2	24
5f	39	20	21	11	37	0	15	19
5g	45	30	57	14	44	23	10	17
5h	328	289	350	31	298	233	203	49
5i	39	10	23	15	40	20	19	12

^[a] Increase % of mass counts represent the sum of *anti*- and *syn*- isomers. BSA, bull serum albumin, negative control. Results are an average of 3 independent experiments.

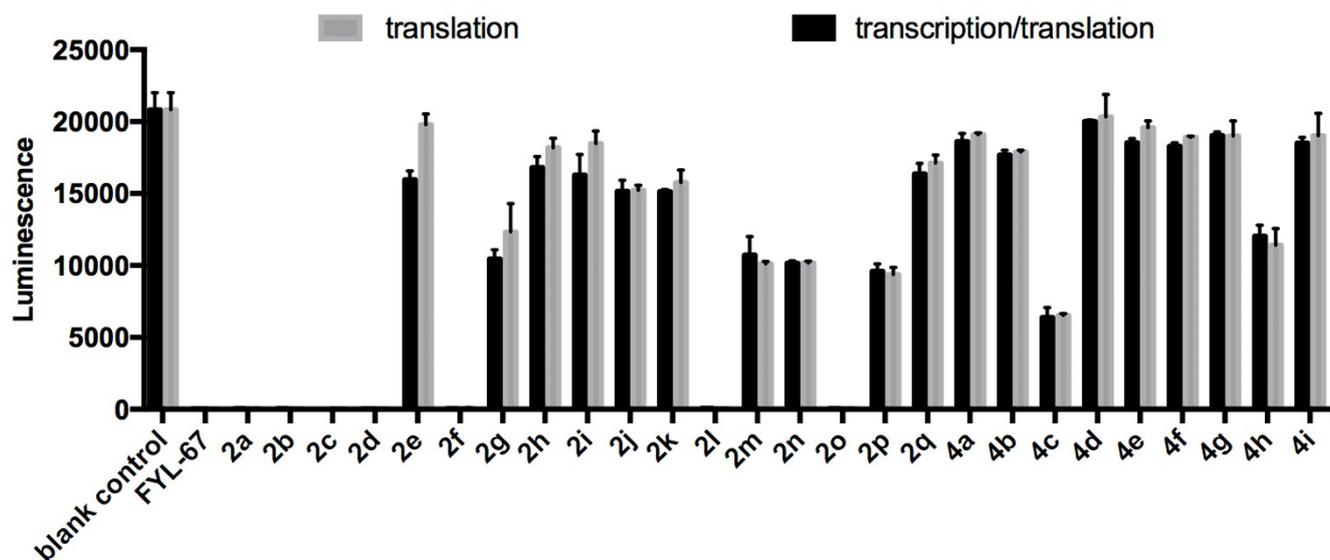


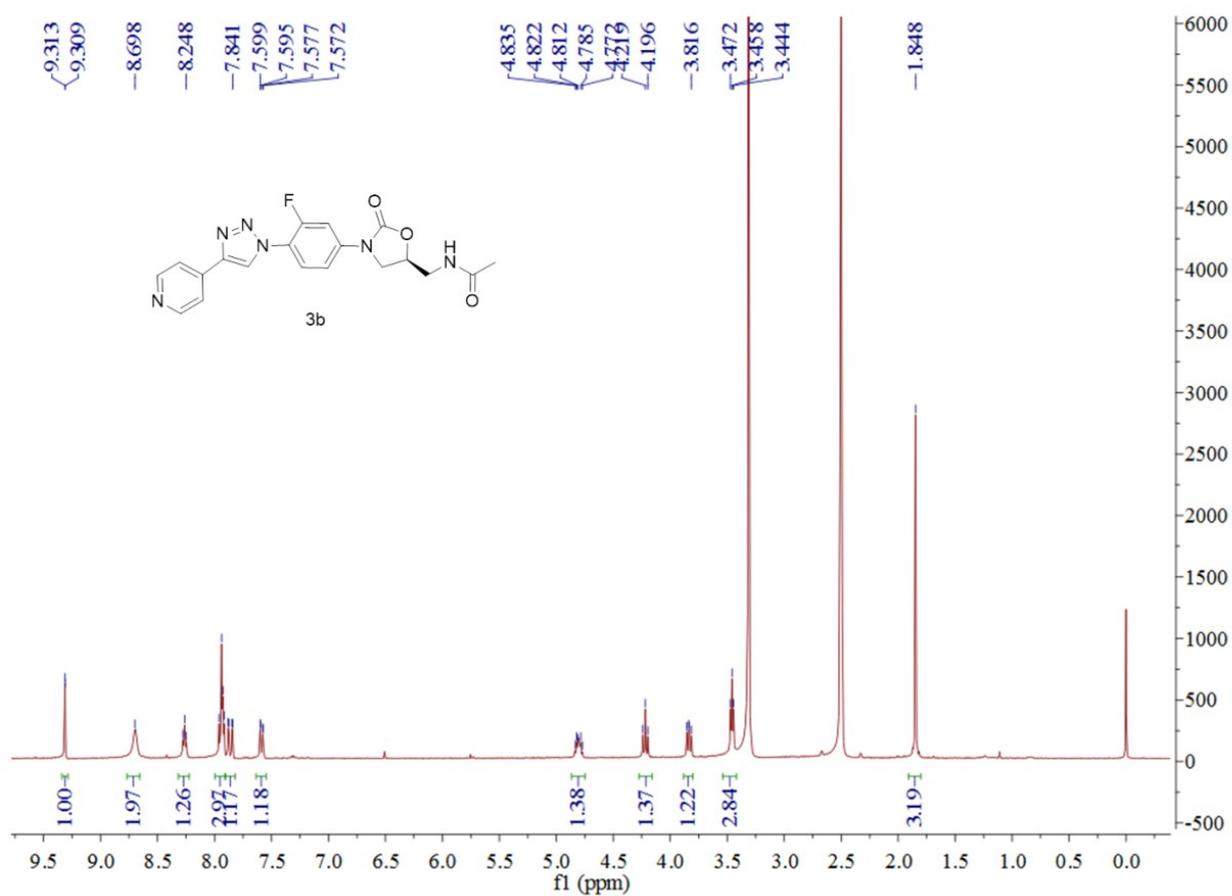
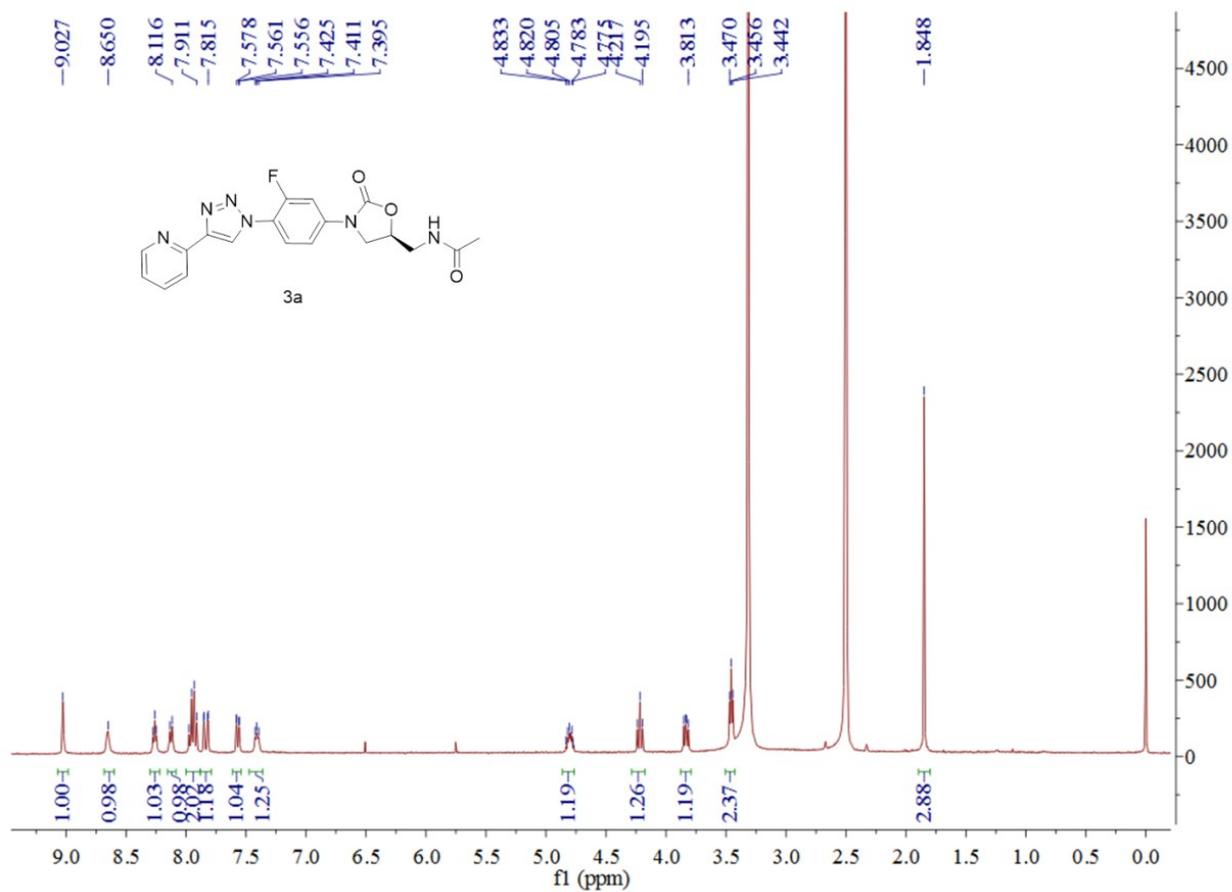
Figure S10. Screening results of alkynes **2a**~**2q** against azide **1** and azides **4a**~**4i** against alkyne **2a** using bacterial-coupled translation system⁵ and *in situ* click chemistry coupled with bacterial *in vitro* transcription/translation assay in this work. Firefly luciferase mRNA (produced *in vitro* using T7 RNA polymerase) instead of T7 RNA Polymerase was added in bacterial-coupled translation system,. Results are shown as mean \pm SD (n=3, see standard error in Table S5).

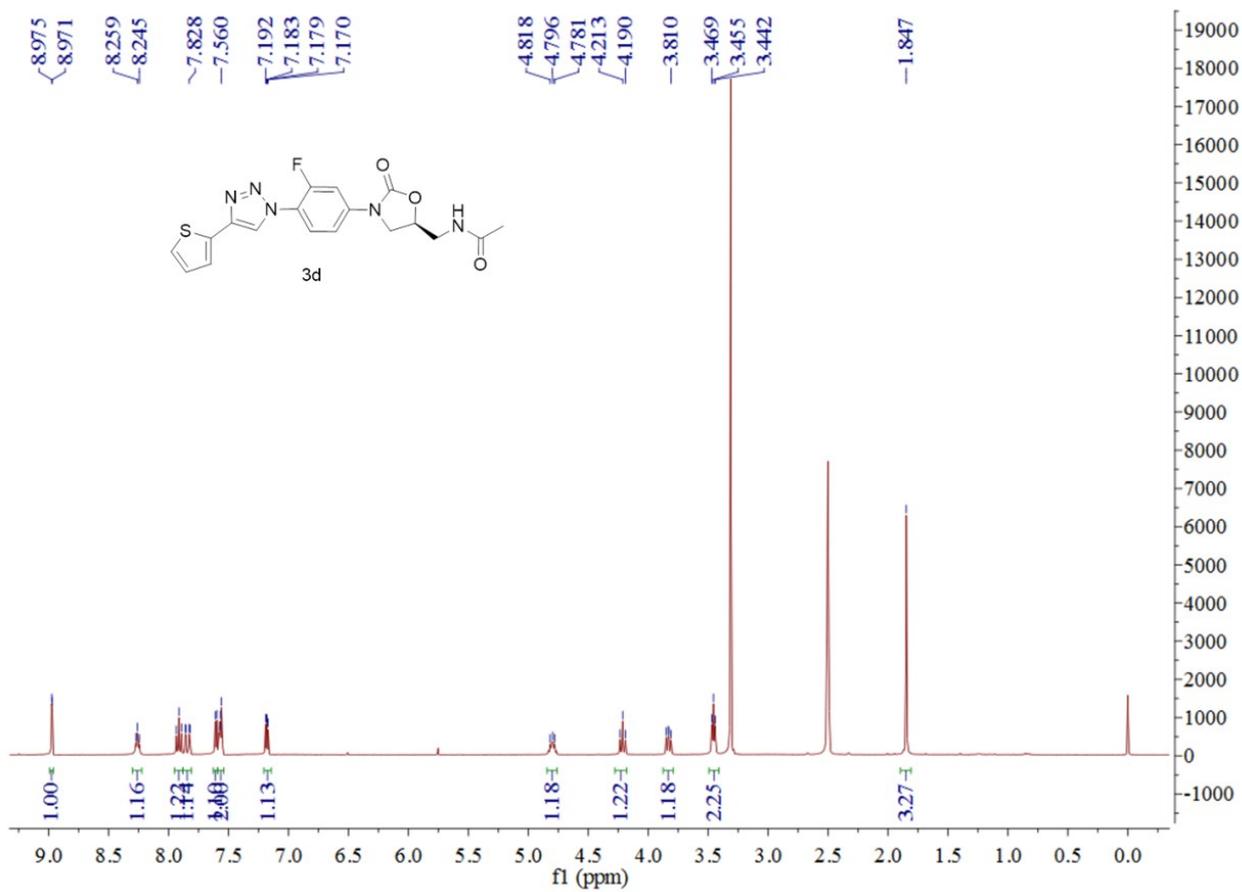
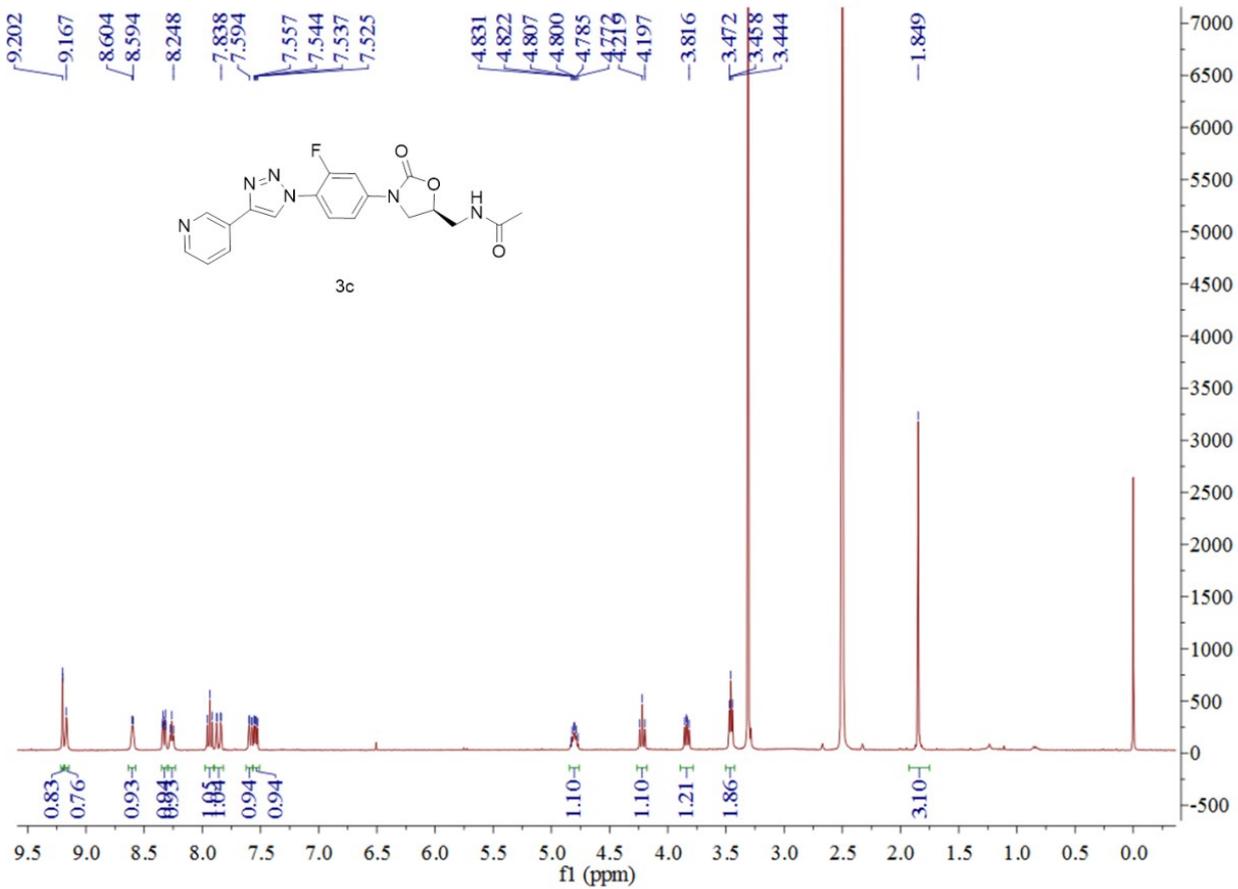
Table S5. Original data of luminescence intensity in bacterial-coupled translation assay and *in situ* click chemistry coupled with bacterial *in vitro* transcription/translation assay

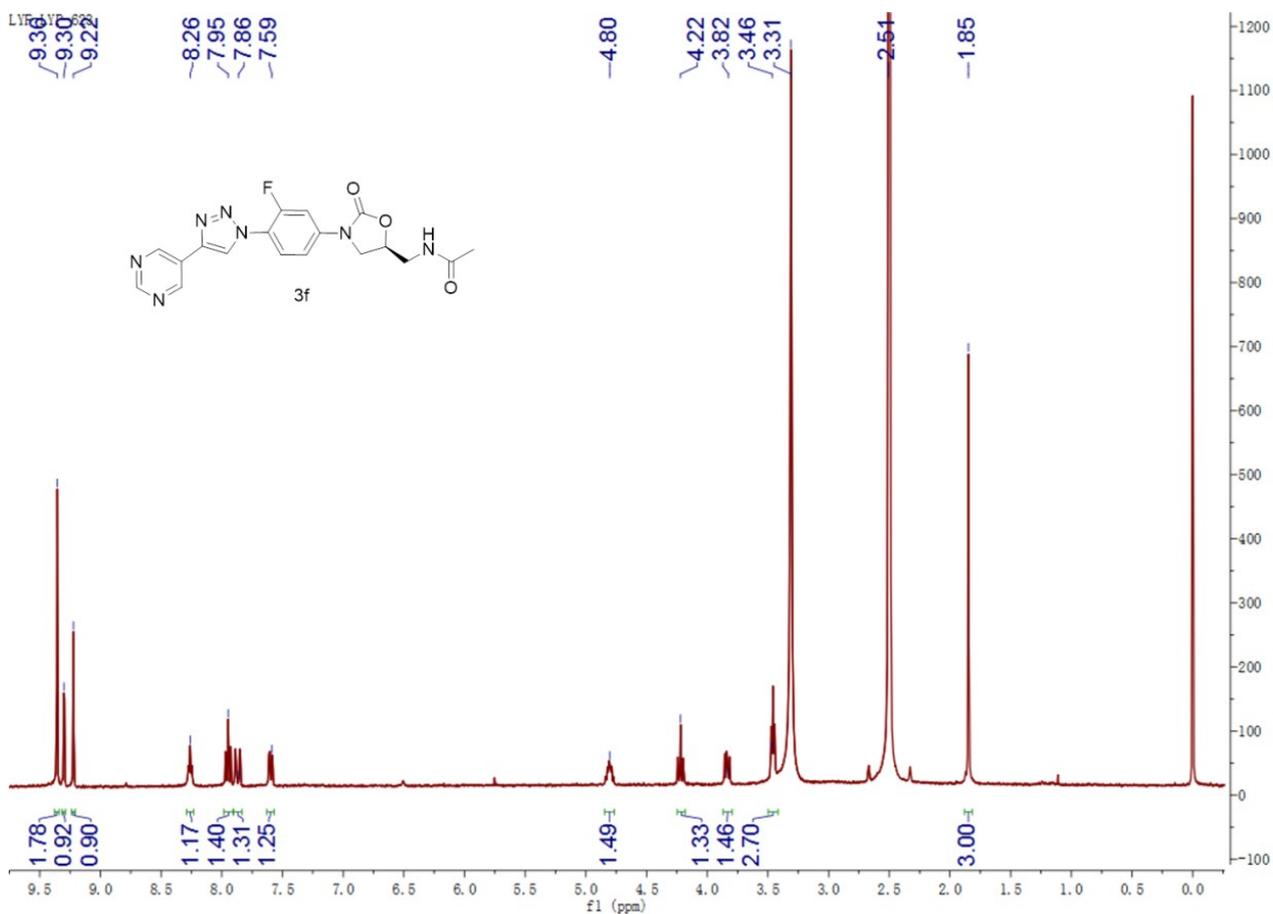
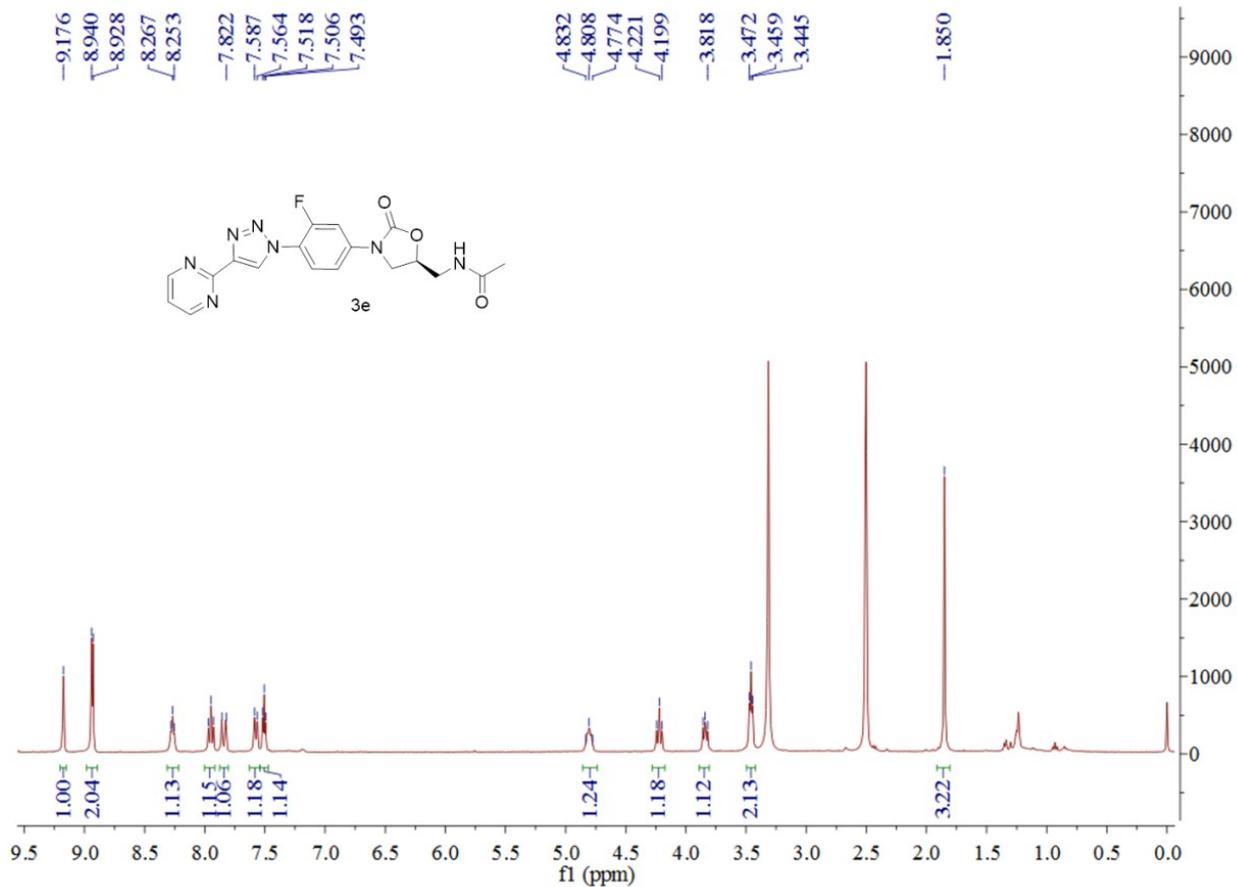
Cmpd#	<i>Transcription/Translation</i>				<i>Translation</i>			
	Increase%	Increase%	Increase%	S.D	Increase%	Increase%	Increase%	S.D
BC	22190	20190	20093	1184	22190	20190	20093	999
FYL-67	28	30	30	1	28	30	30	3
2a	49	55	38	9	67	48	39	14
2b	70	50	48	12	39	49	59	10
2c	20	15	10	5	48	40	40	5
2d	28	20	19	5	30	30	47	10
2e	15412	16620	15892	608	20399	18932	19983	756
2f	59	65	39	14	89	100	39	33
2g	11202	10092	10028	660	13647	13281	10039	1986
2h	16093	16690	17630	775	17839	18939	17782	652
2i	17820	15003	16049	1424	19389	18377	17663	867
2j	14456	15002	15992	779	15009	15002	15632	362
2k	15002	15102	15302	153	16732	15039	15493	876
2l	79	102	59	22	48	29	58	15
2m	9928	10020	12200	1286	10039	10020	10298	155
2n	10203	9950	10291	177	10201	10293	10039	129
2o	59	66	40	13	69	50	50	11
2p	9732	9002	10023	526	8932	9912	9230	502
2q	16620	16924	15539	728	17736	16998	16637	560
4a	18832	19032	18002	546	18993	19034	19234	129
4b	17322	17734	17983	334	17732	17893	18003	136
4c	5674	7032	6443	681	6394	6599	6601	119
4d	20133	19983	19890	123	19983	18932	22032	1577
4e	18732	18673	18211	285	19902	19802	19032	476
4f	18002	18343	18504	256	18834	18903	18993	80
4g	19324	18903	18873	252	17883	19093	19993	1059
4h	12098	11234	12763	767	12034	12112	10055	1166

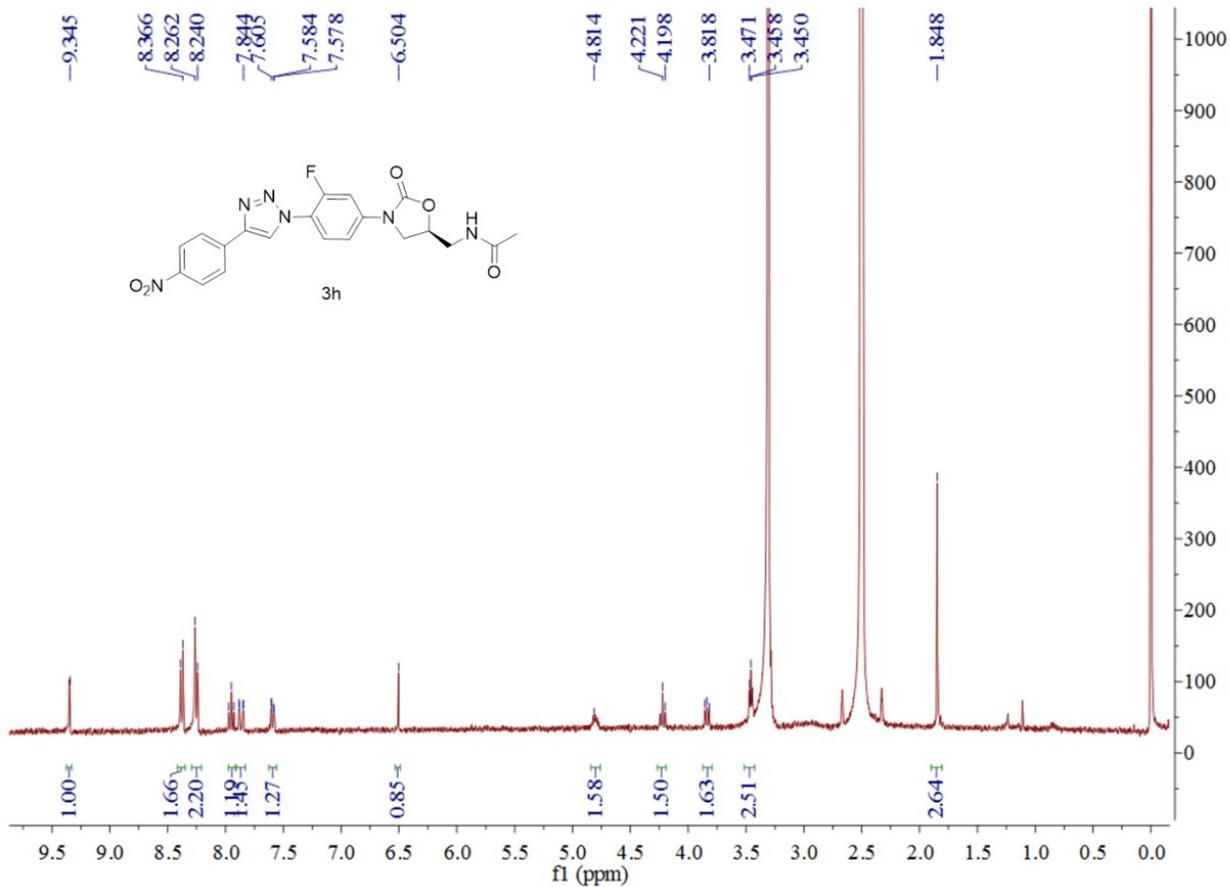
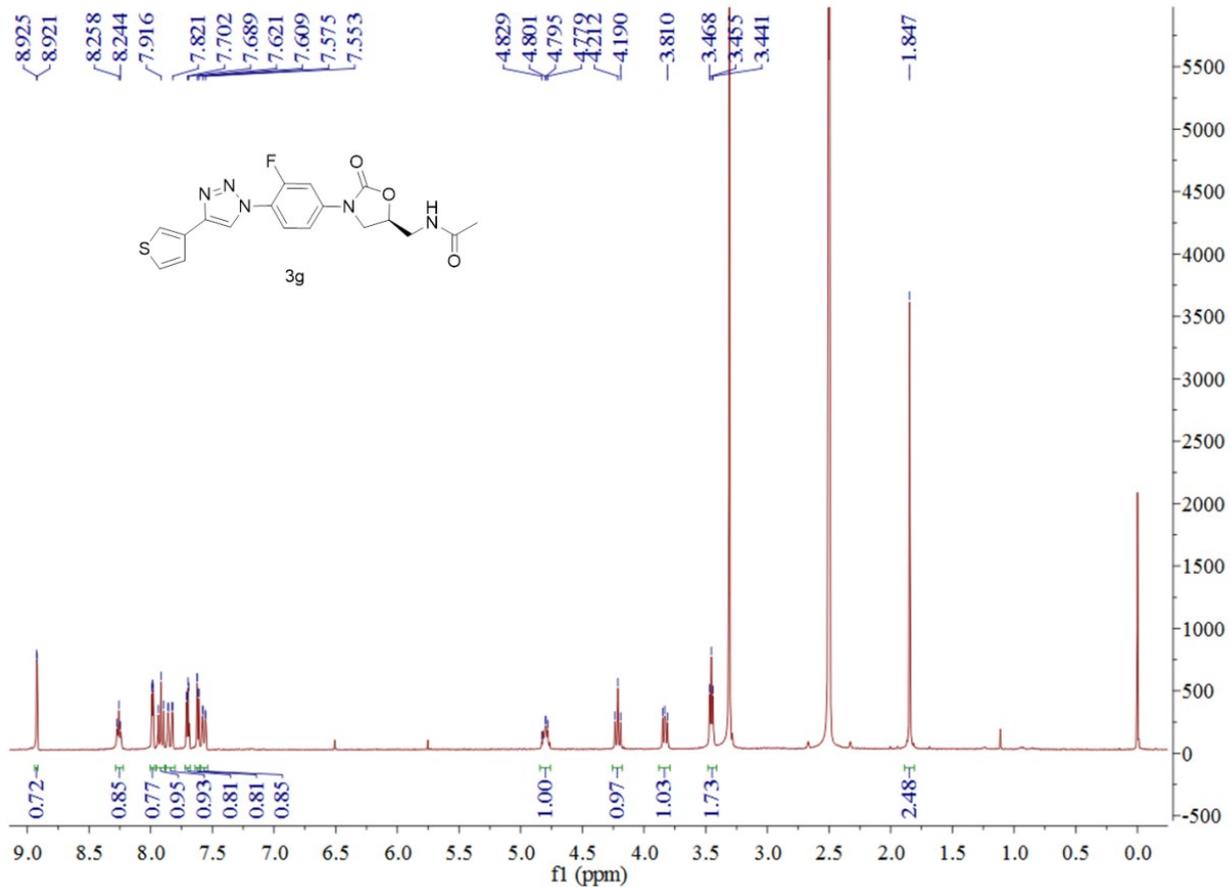
^[a] Results are an average of 3 independent experiments. Blank control, BC.

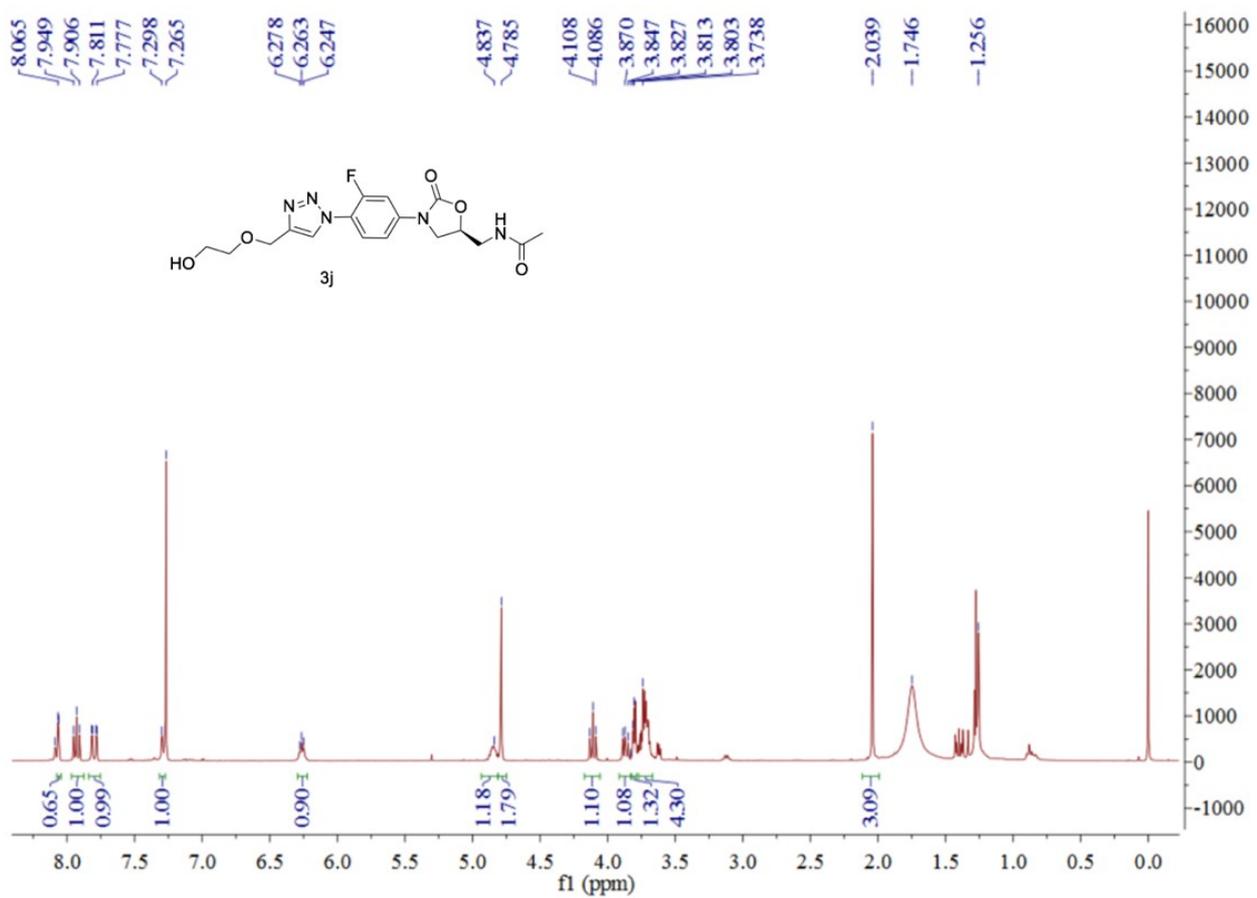
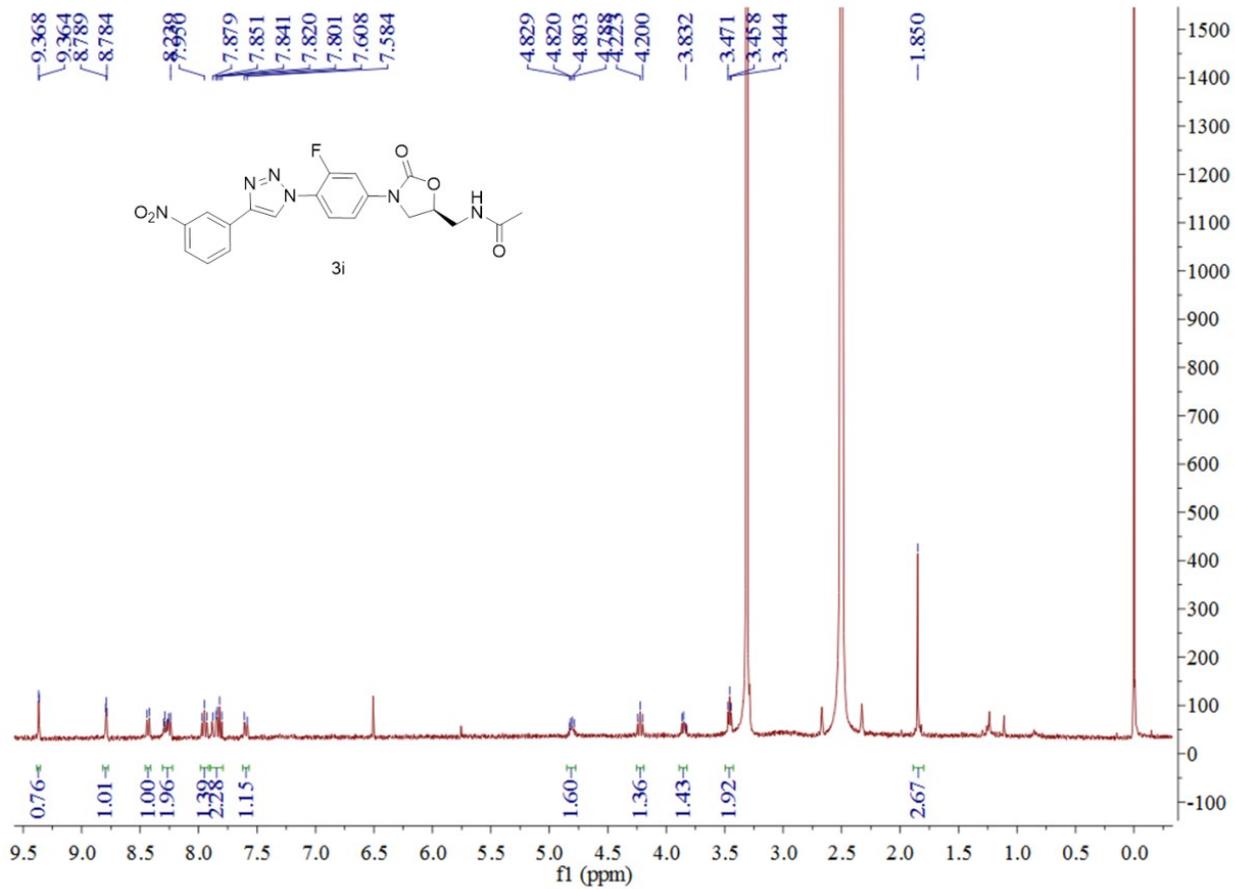
4. NMR and MS spectra

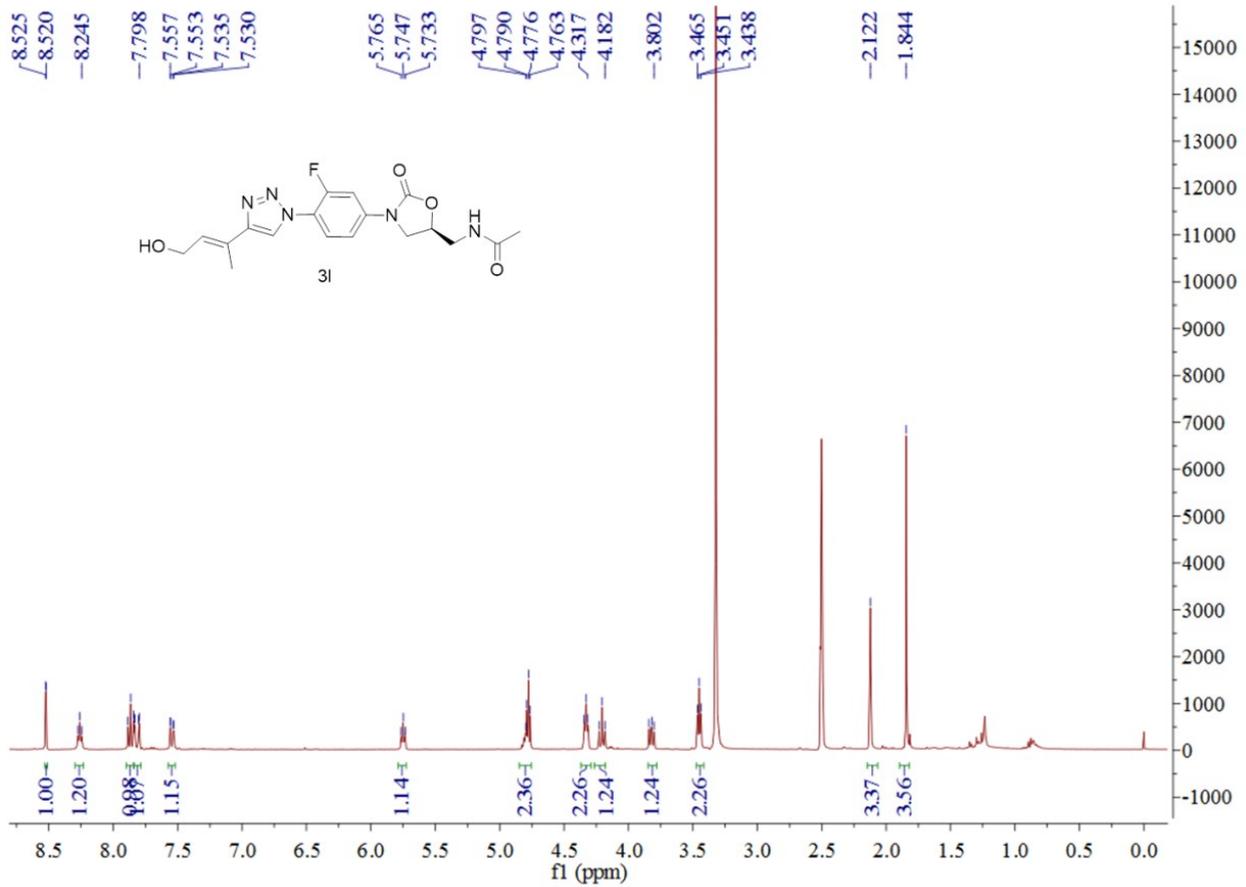
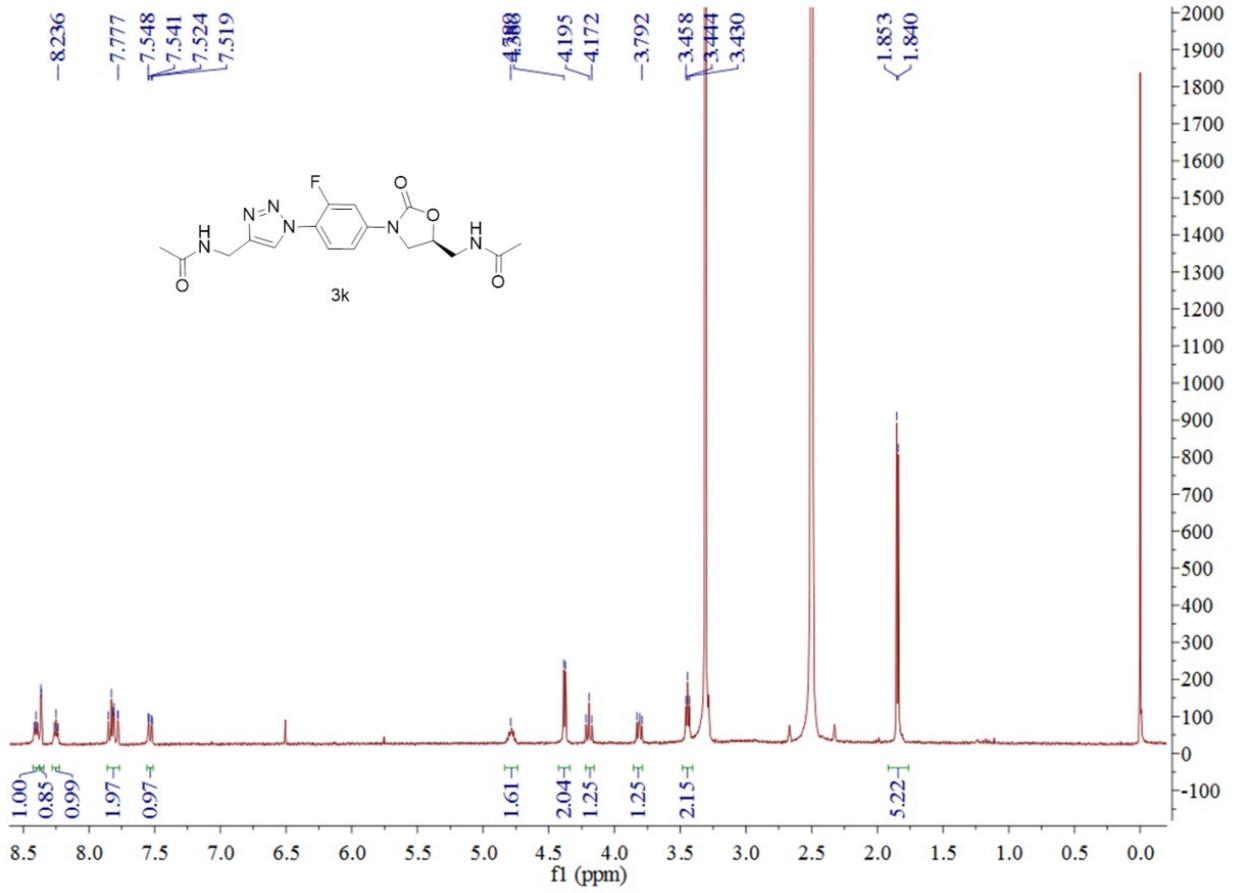


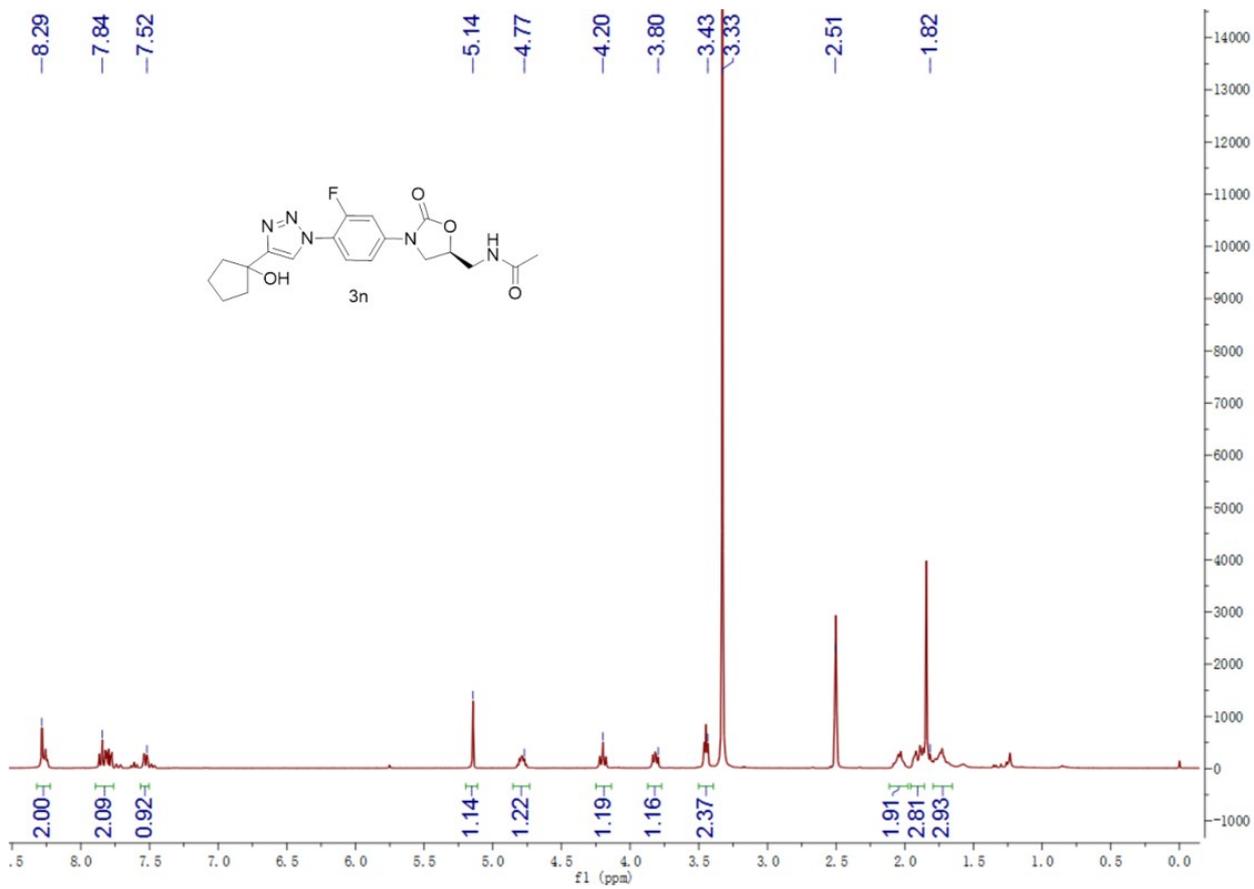
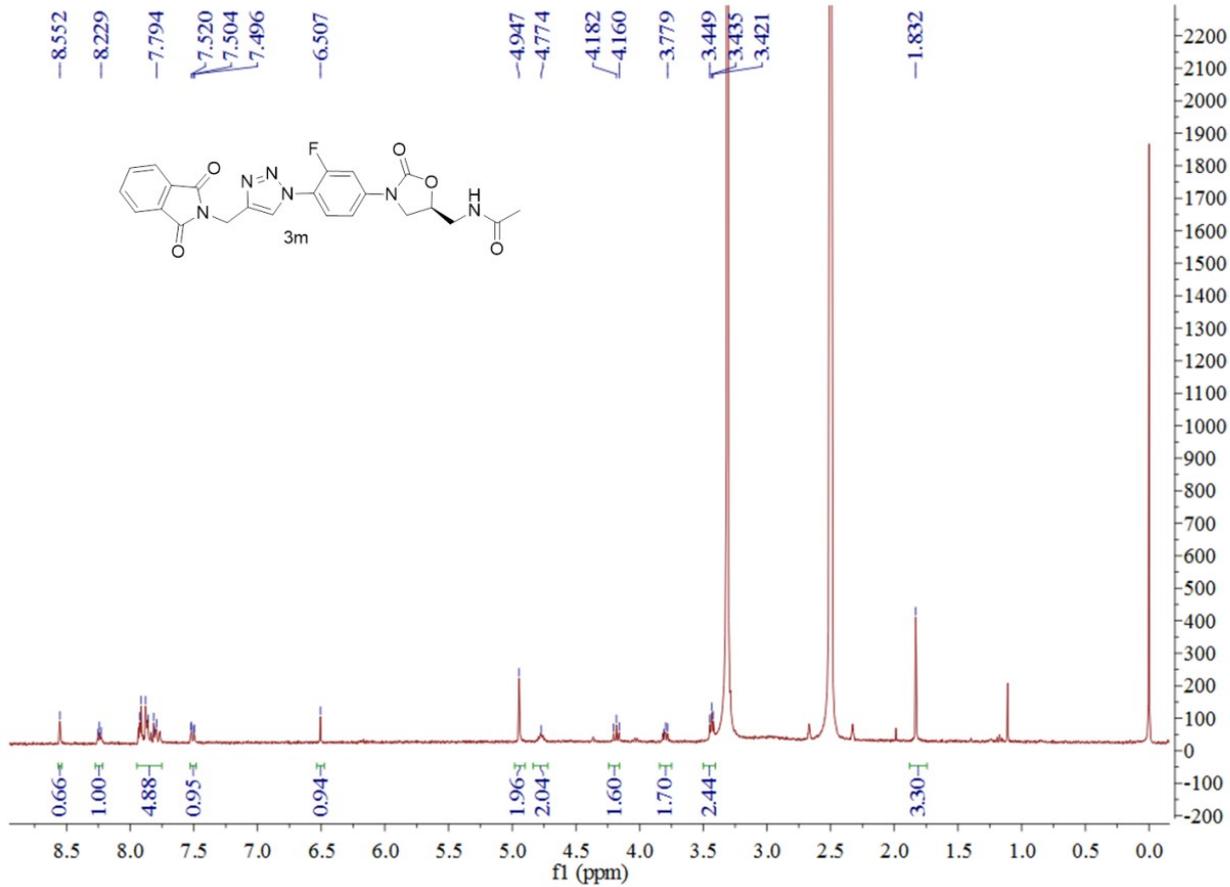


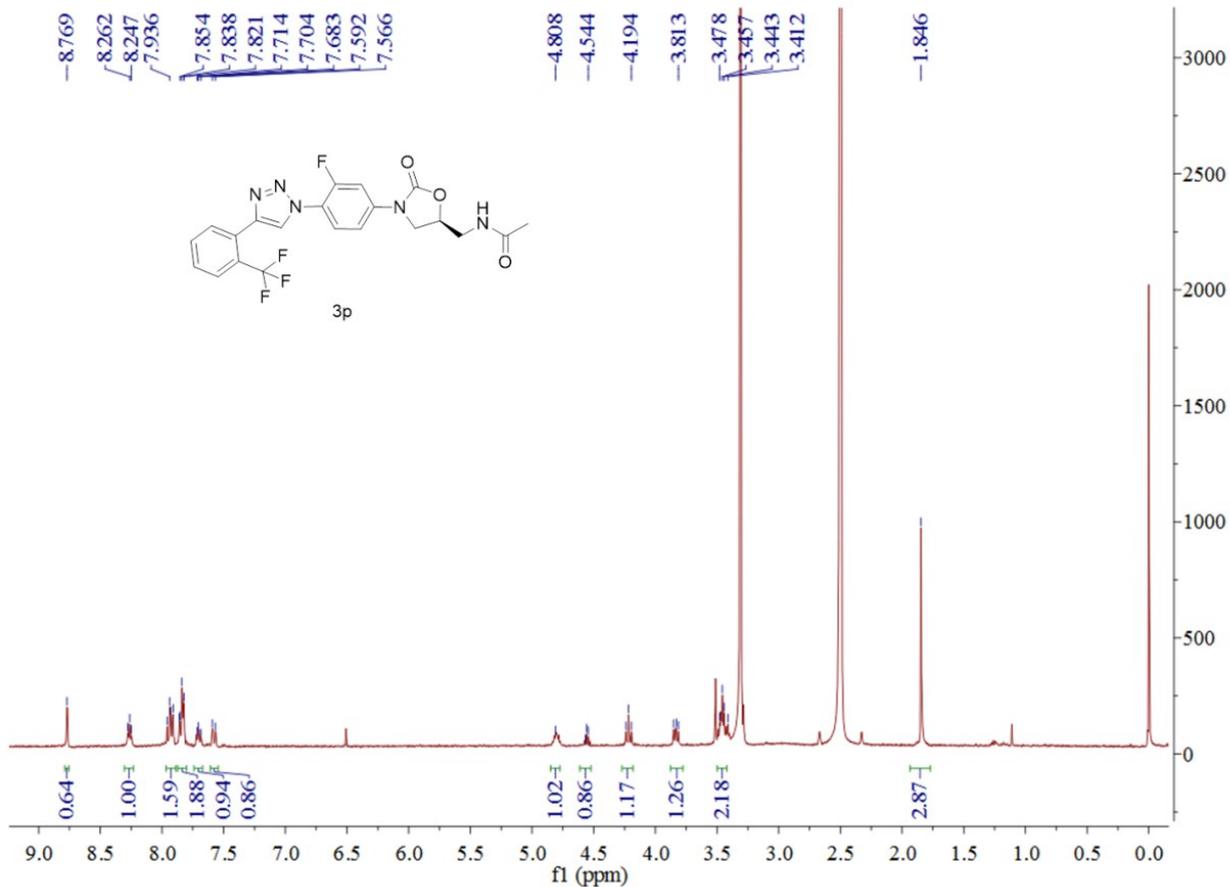
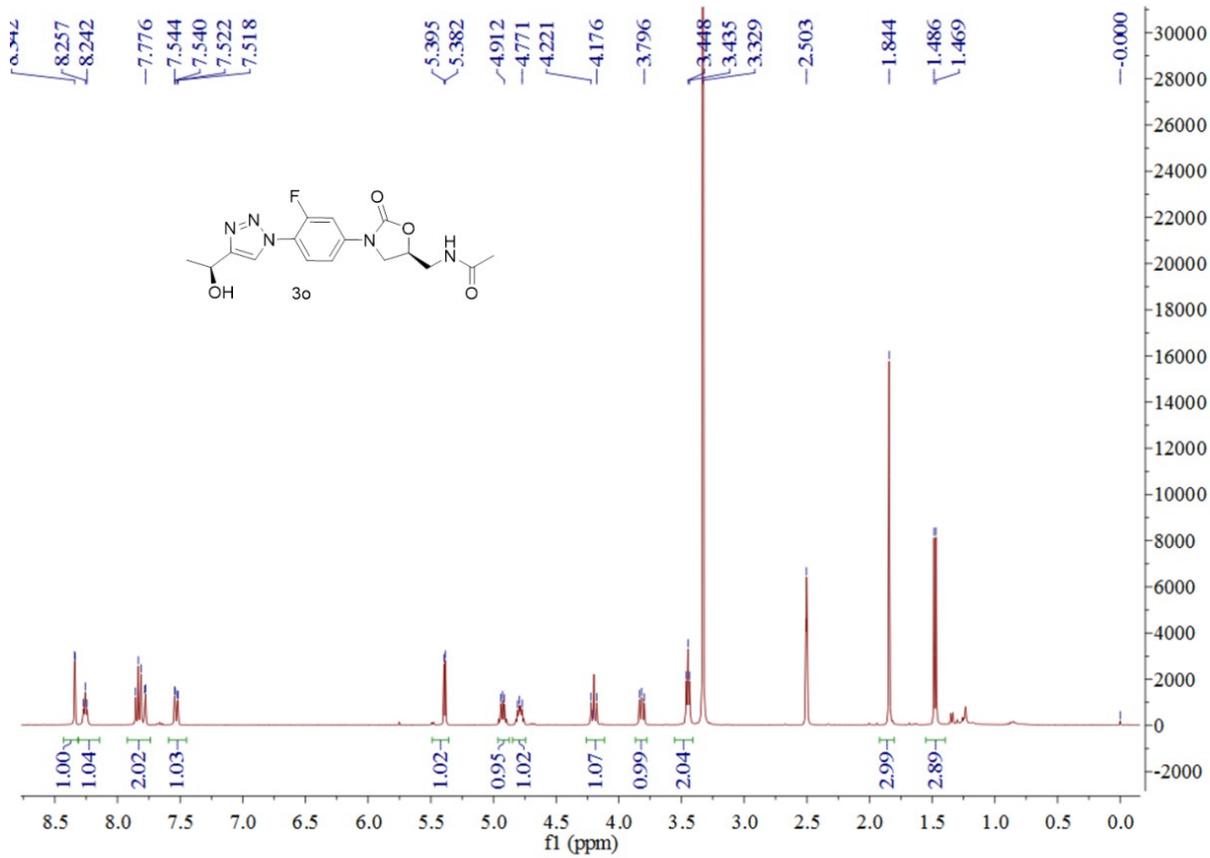


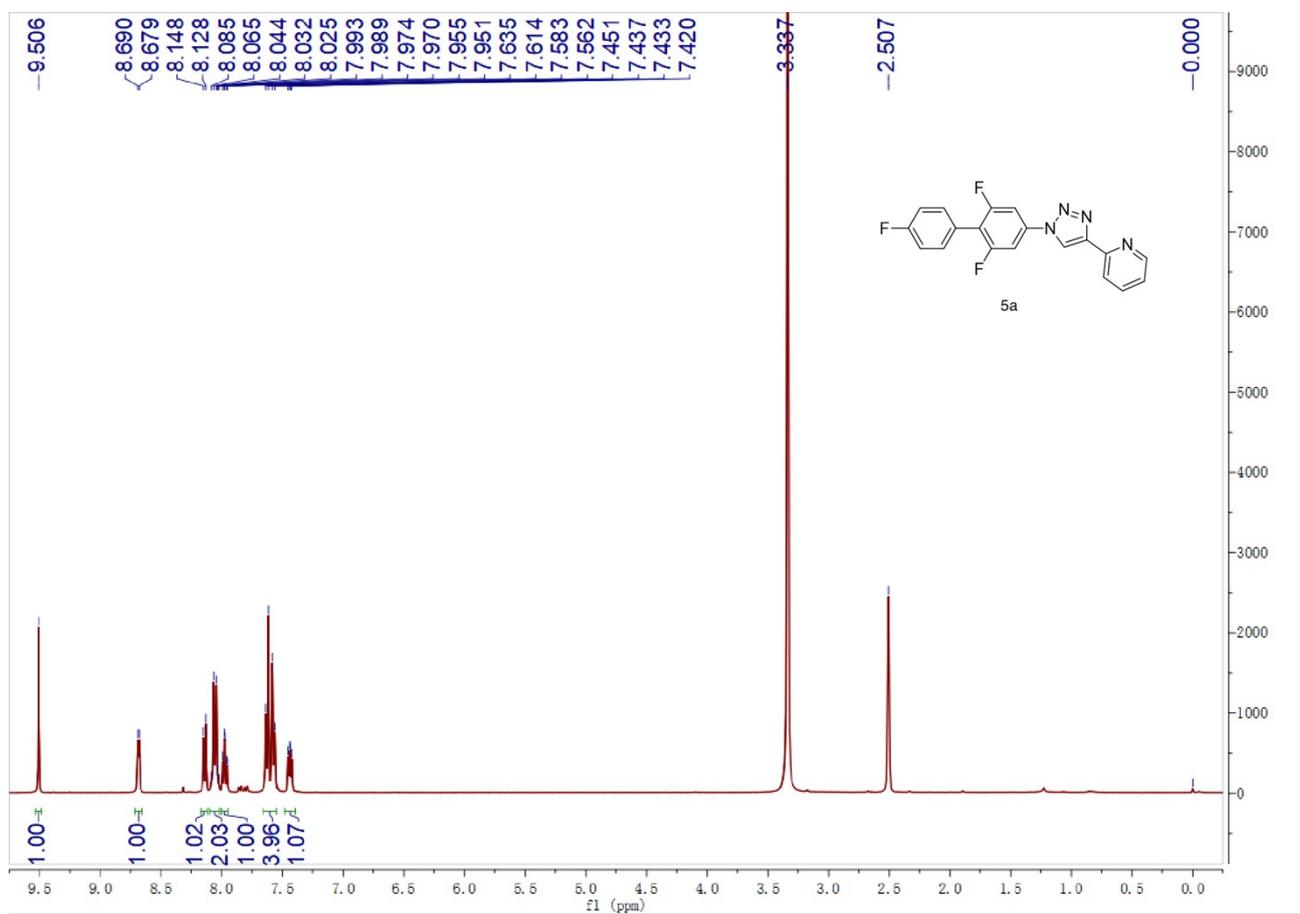
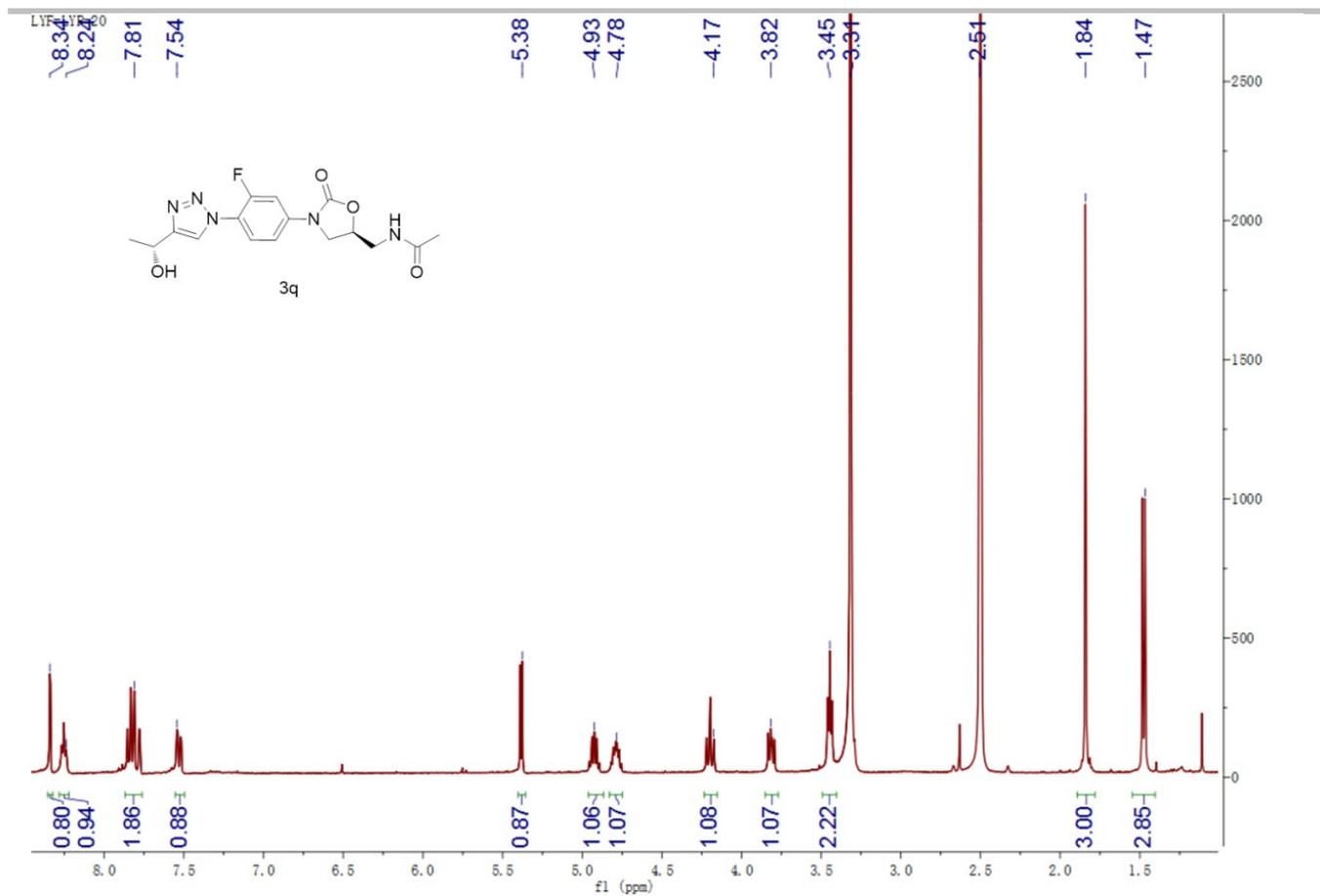


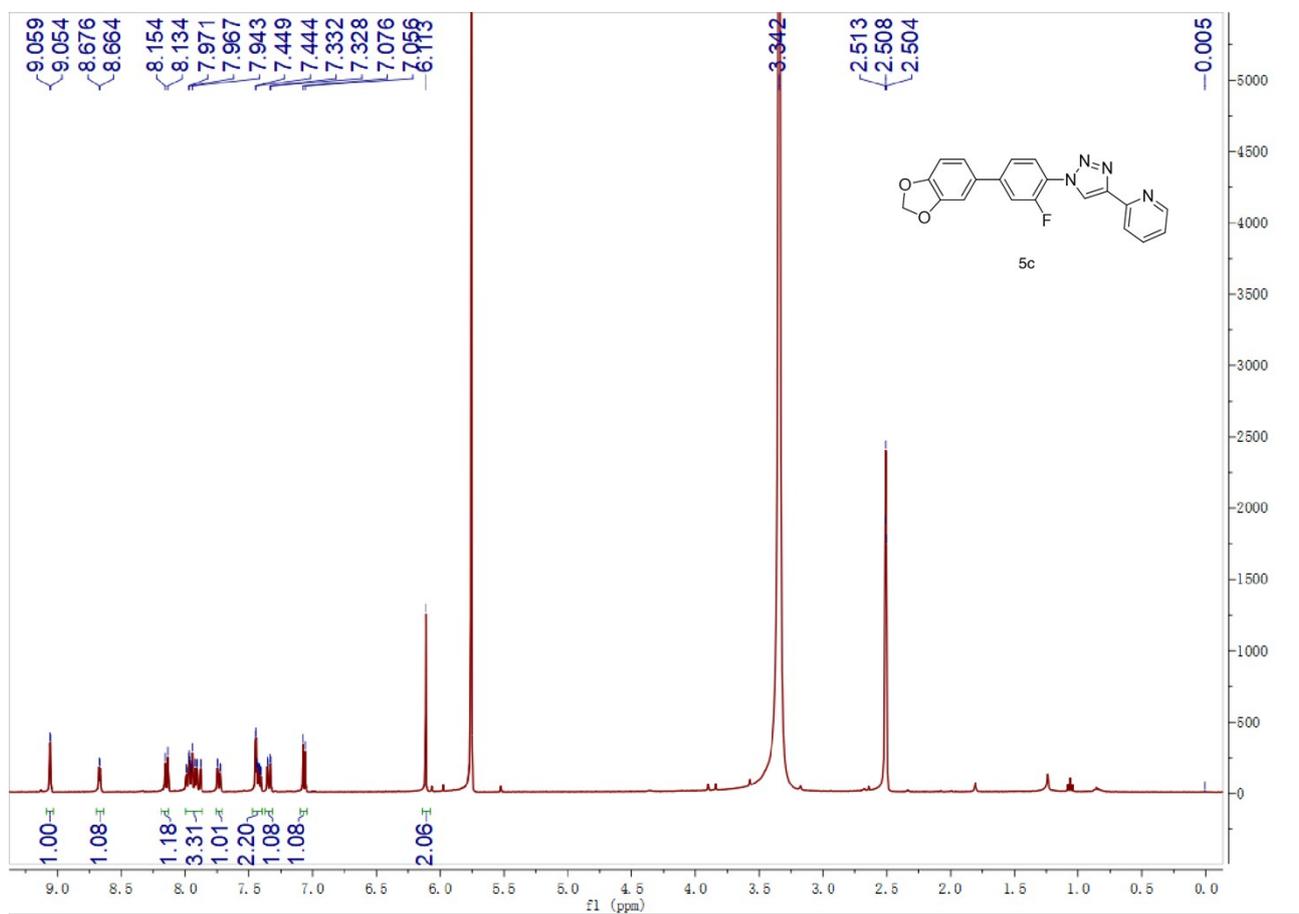
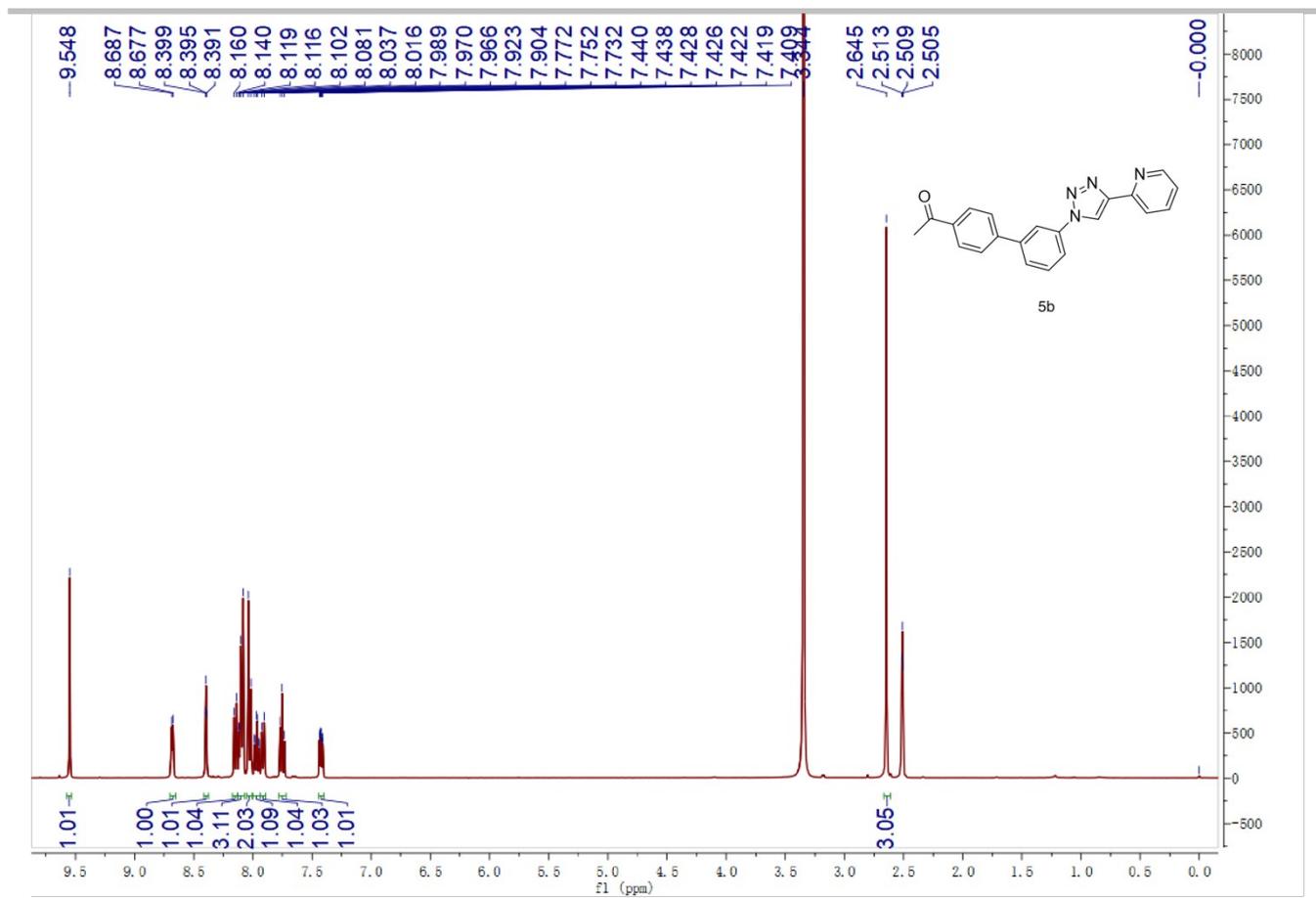


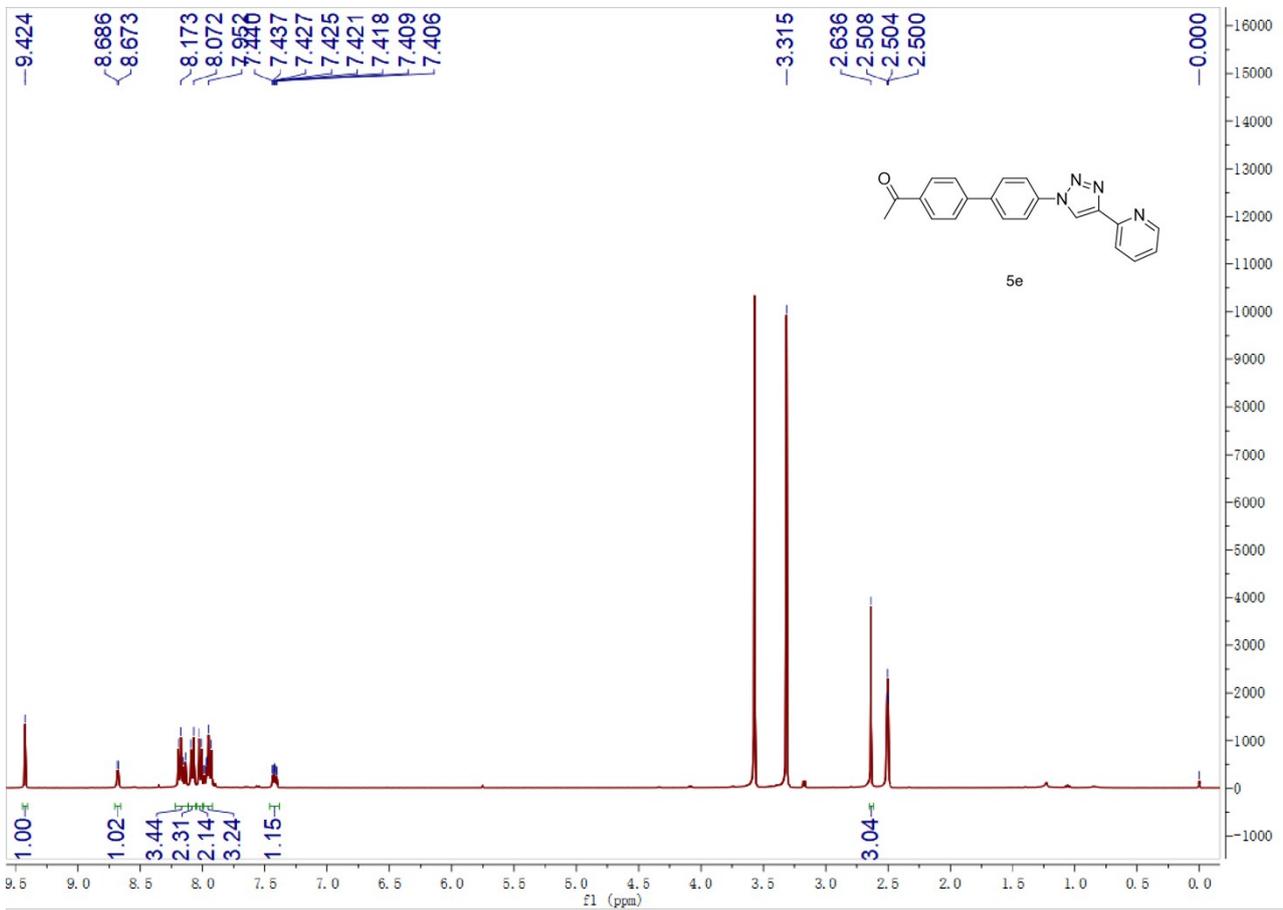
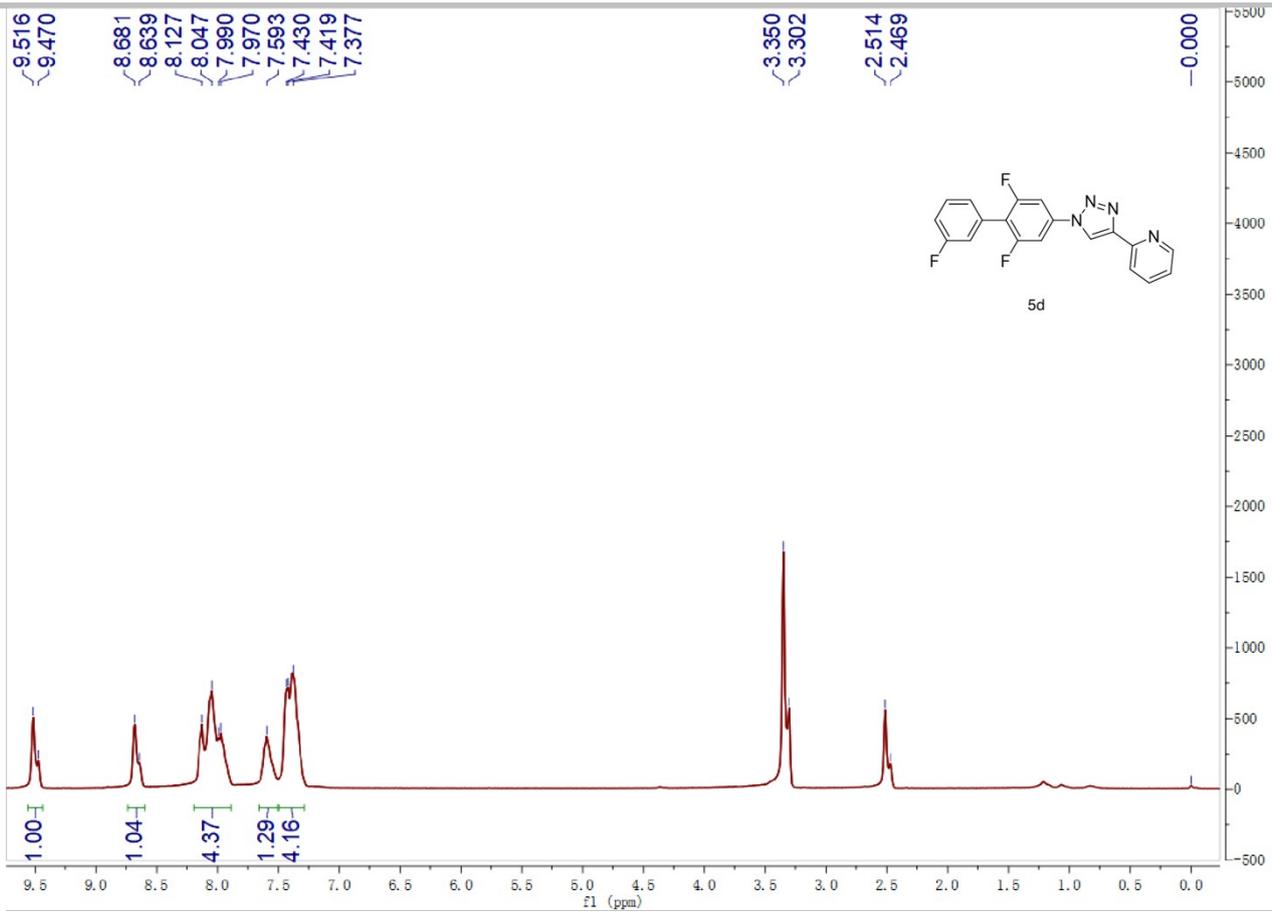


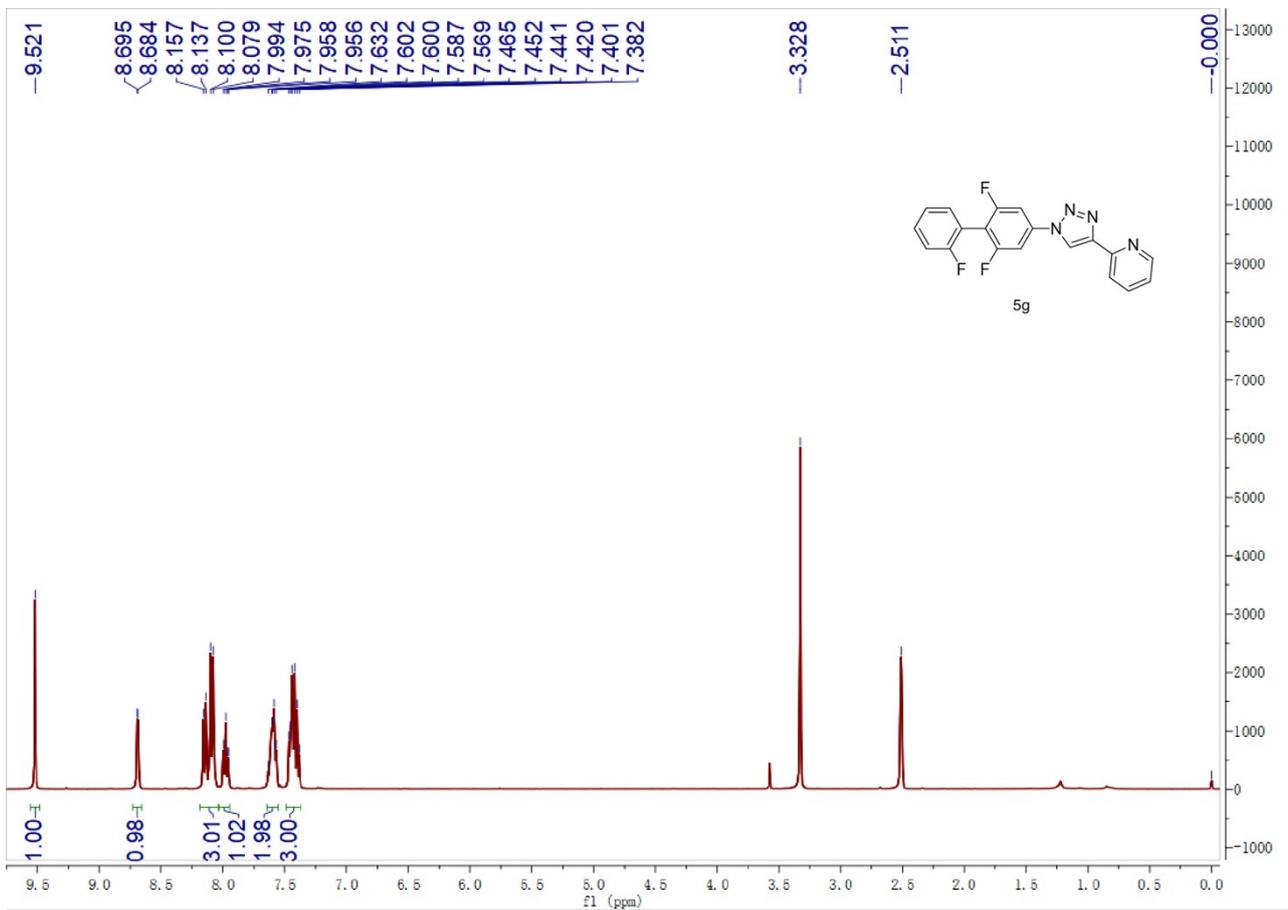
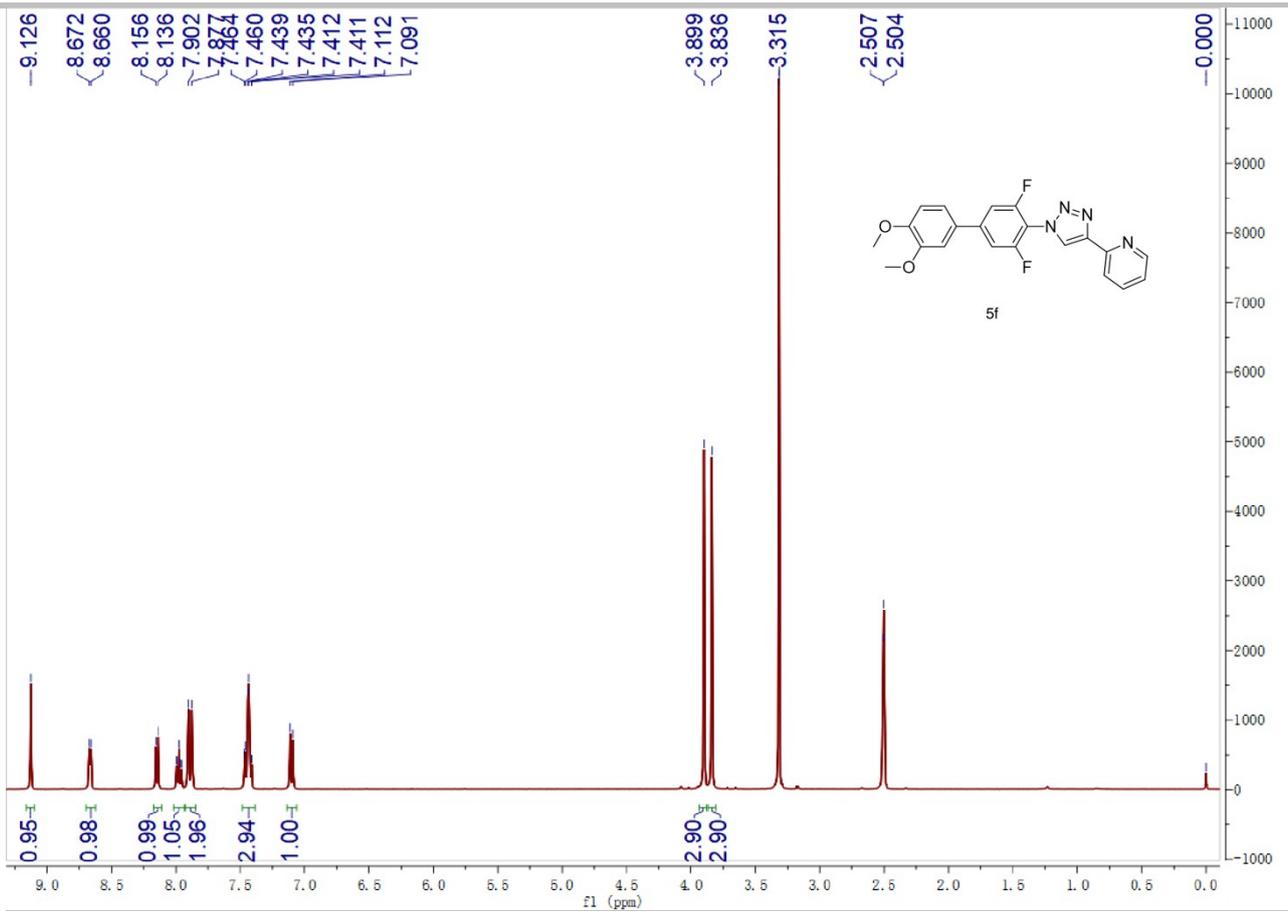


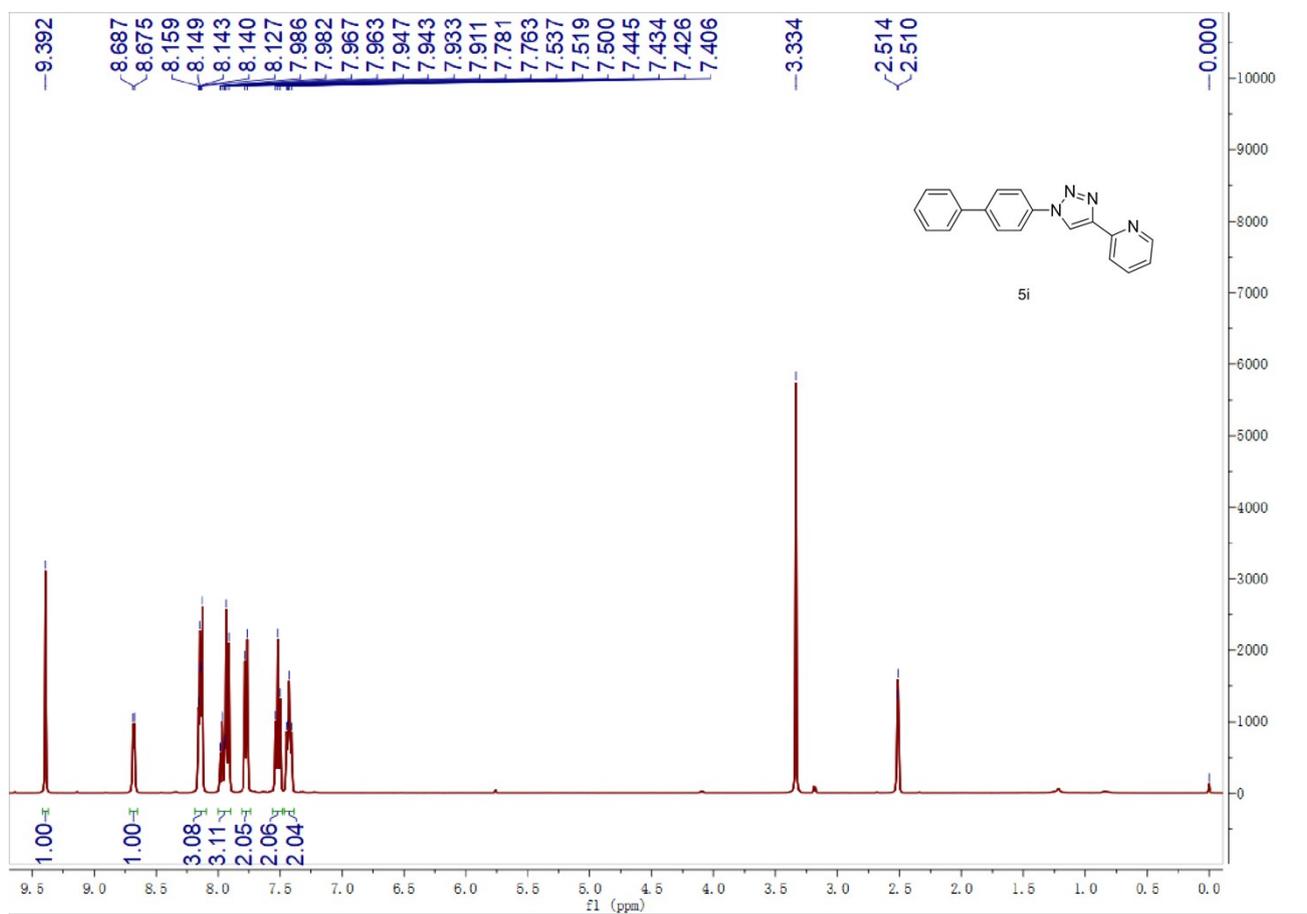
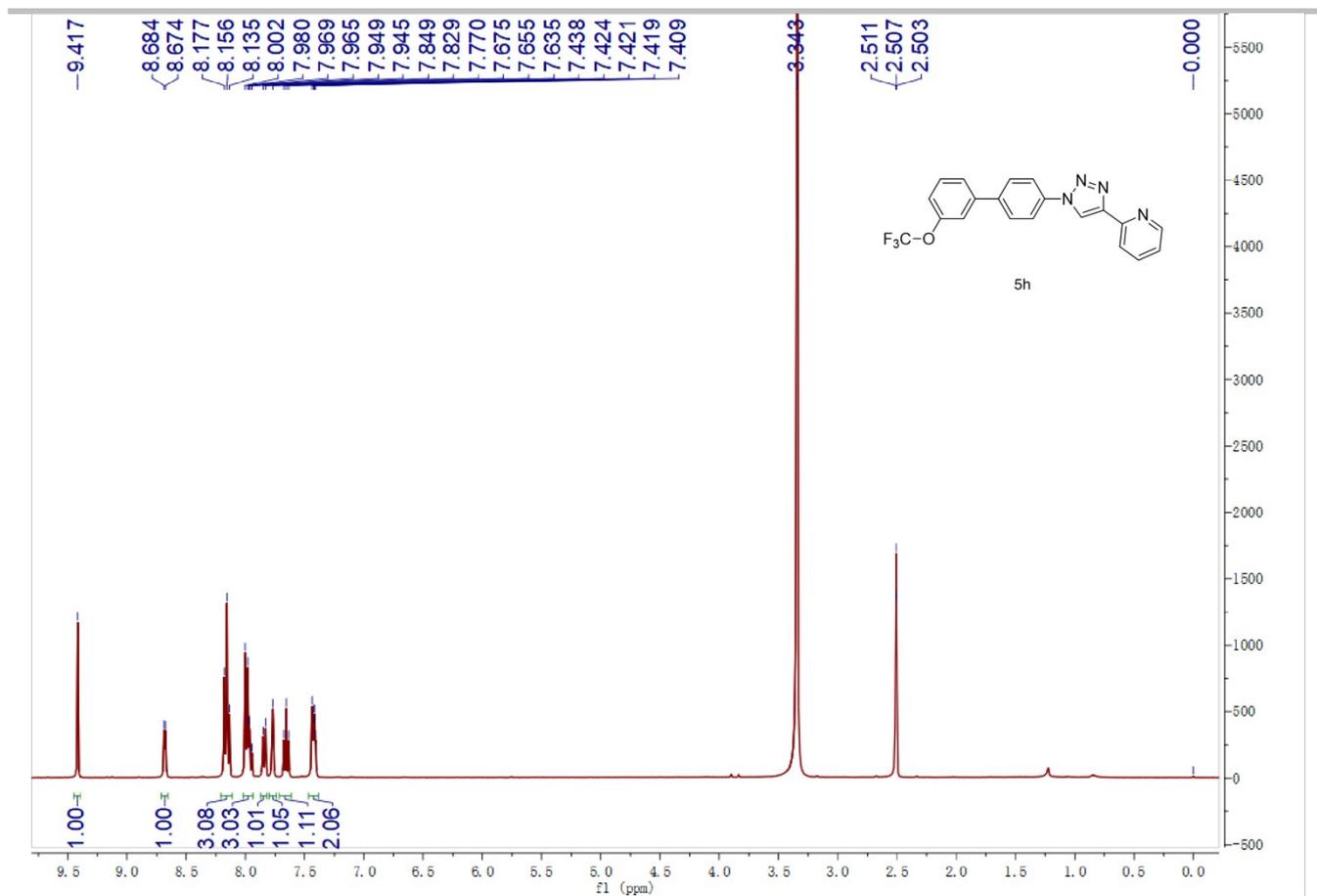


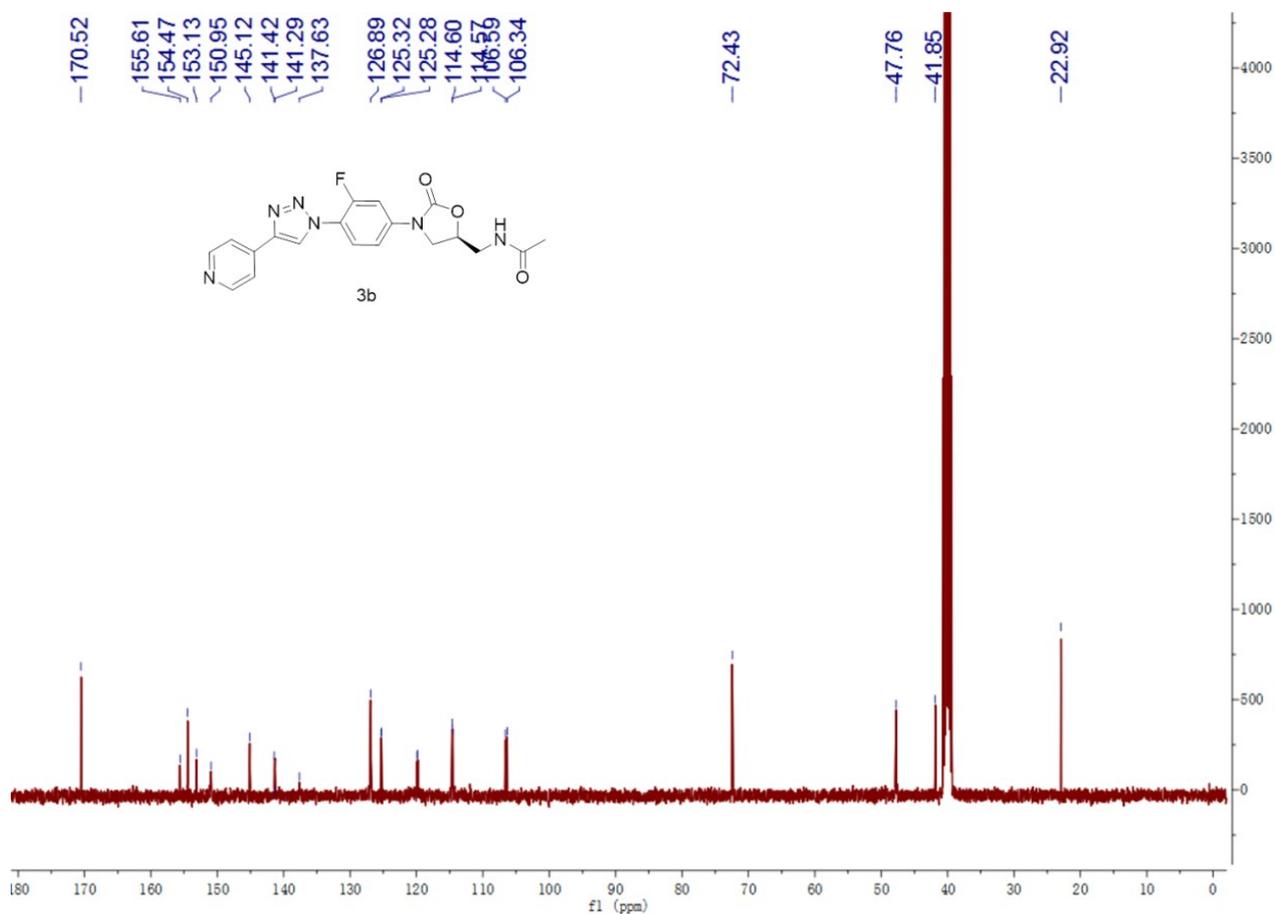
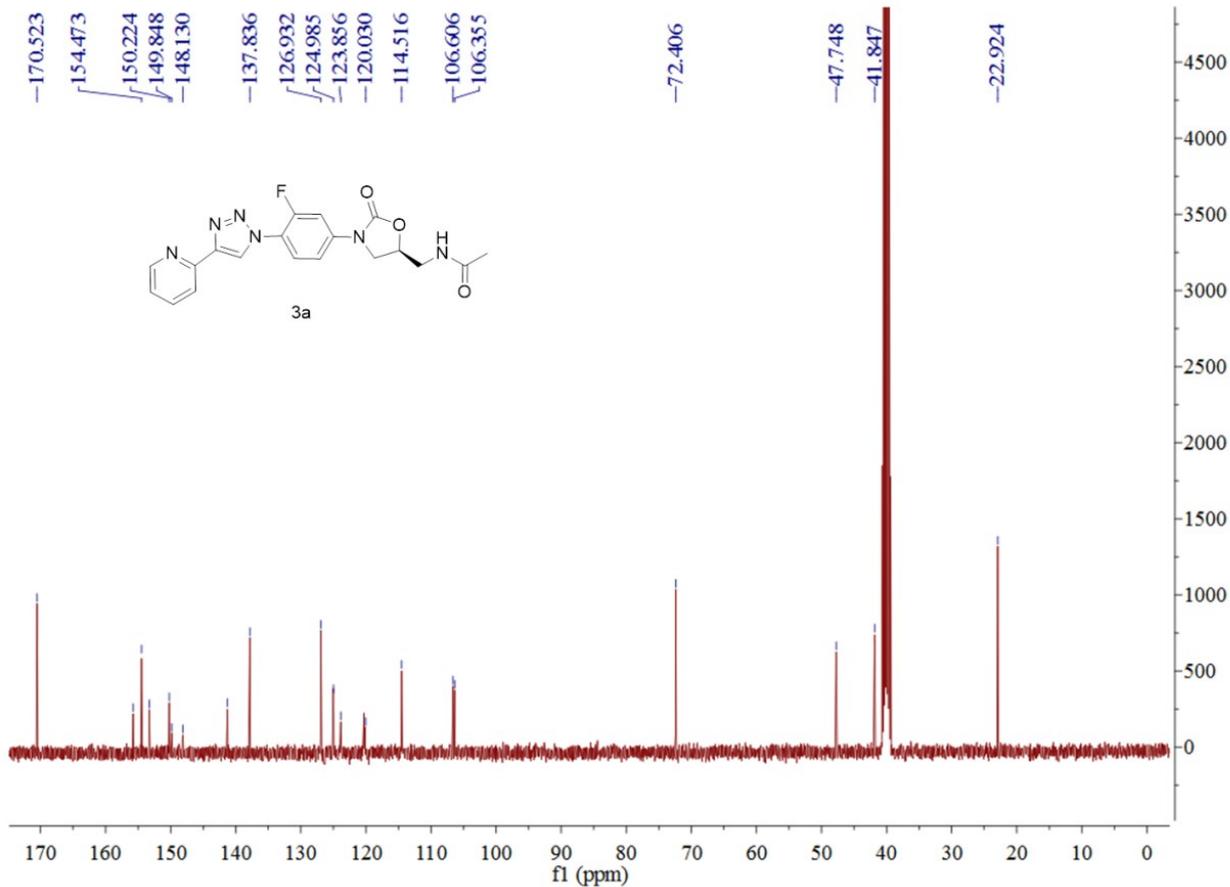


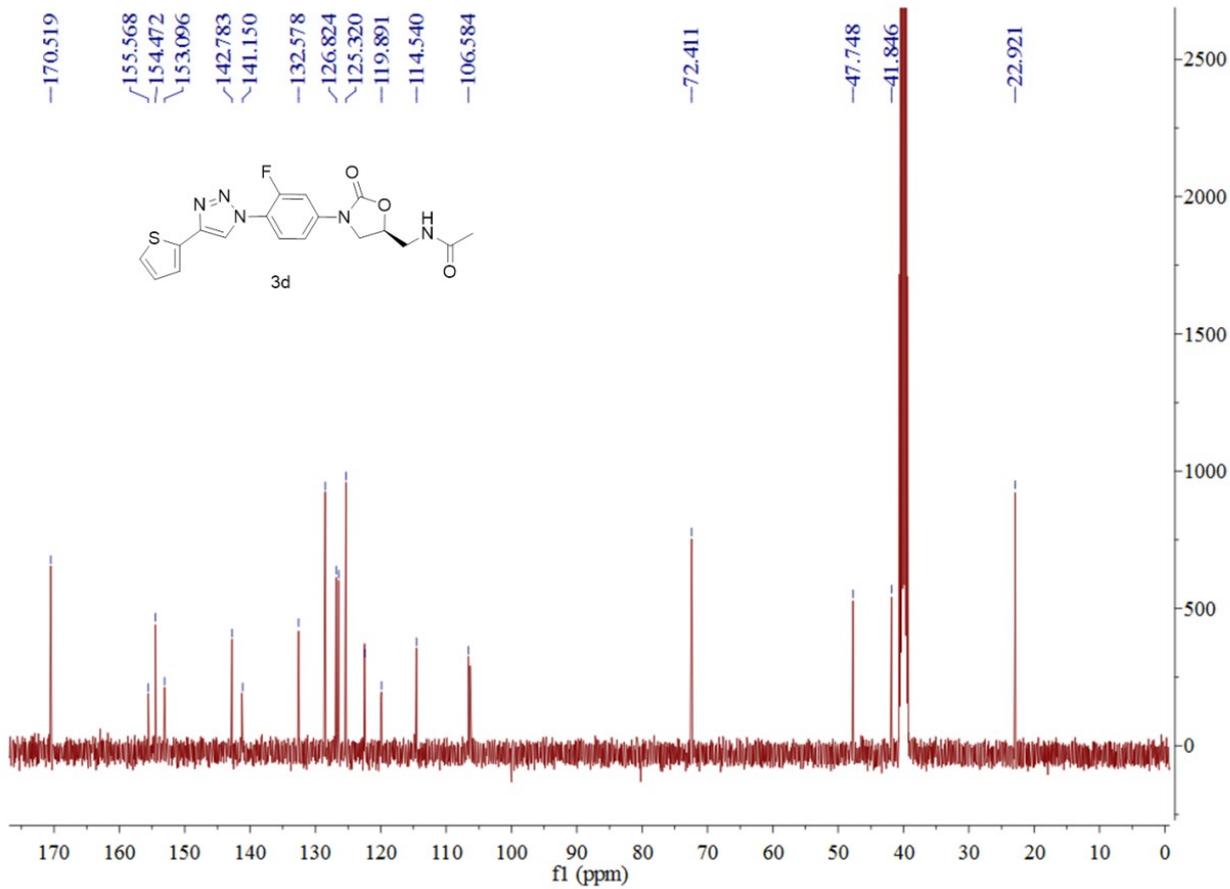
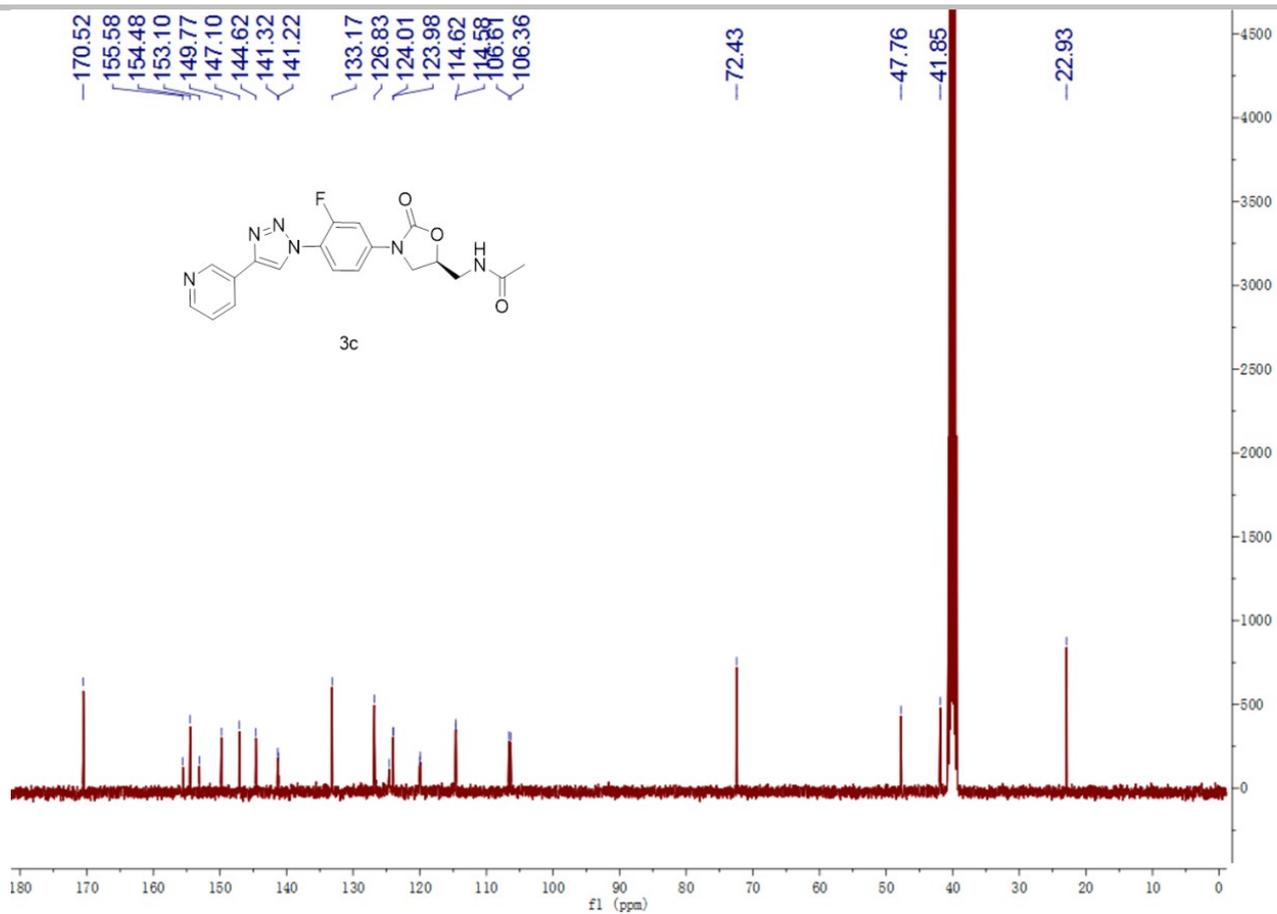


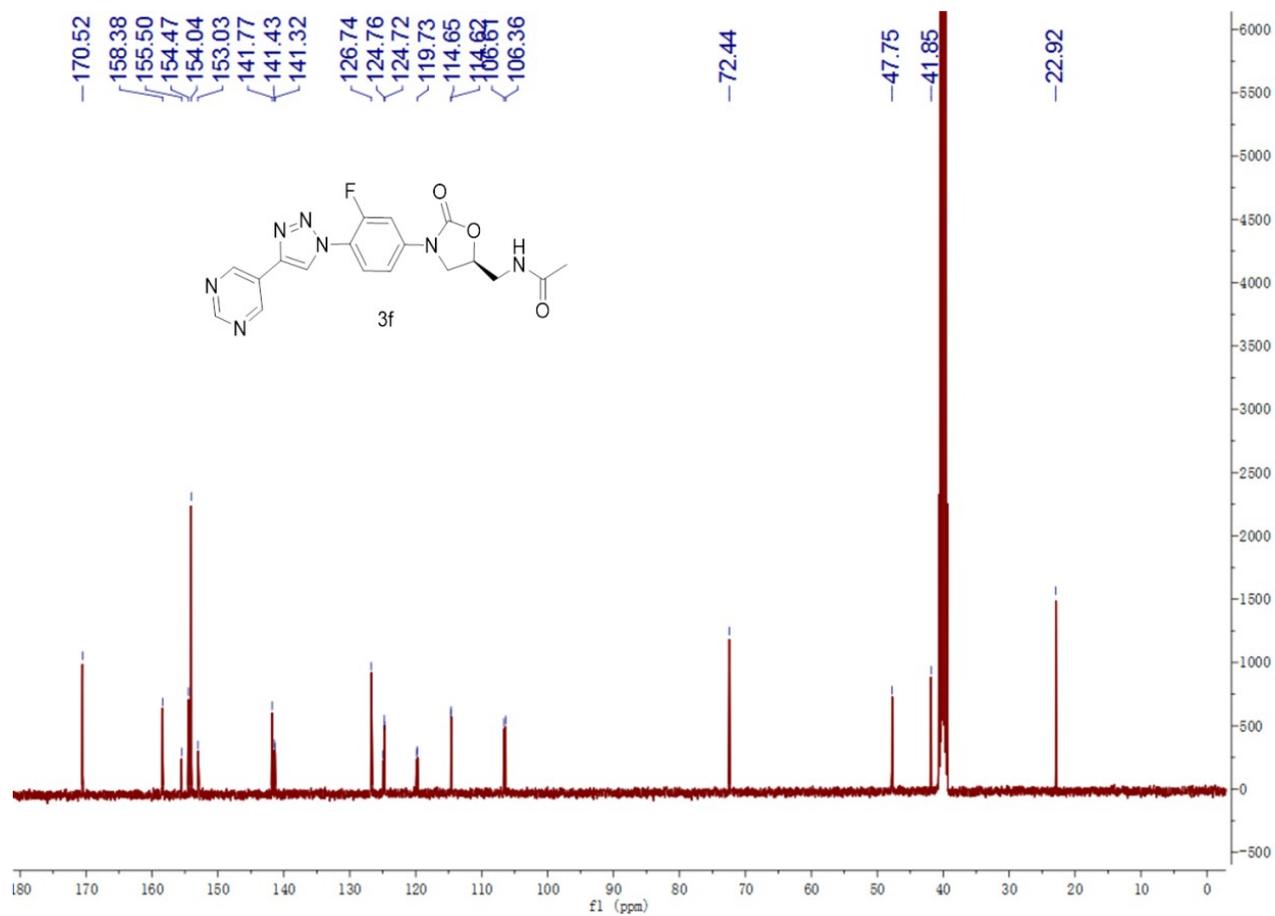
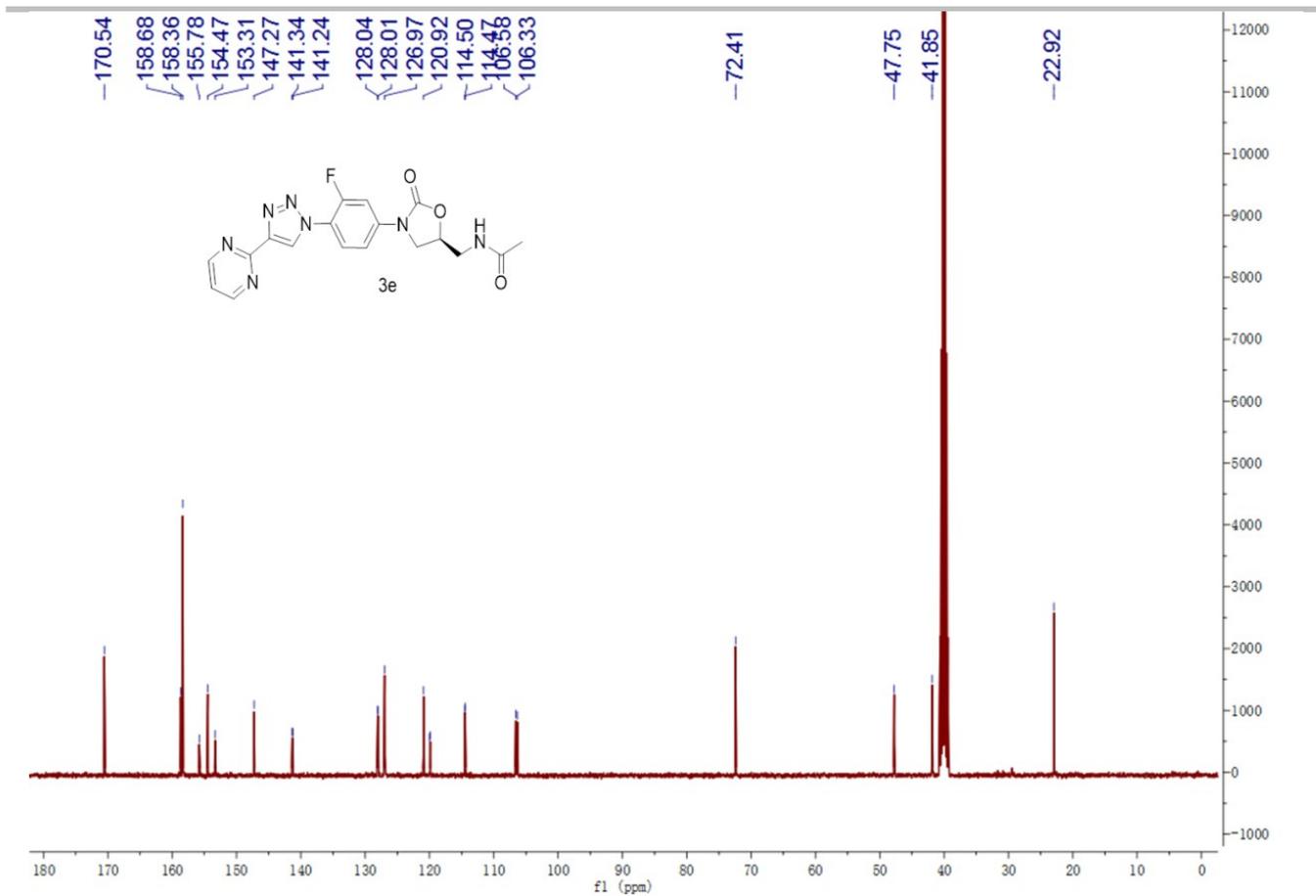


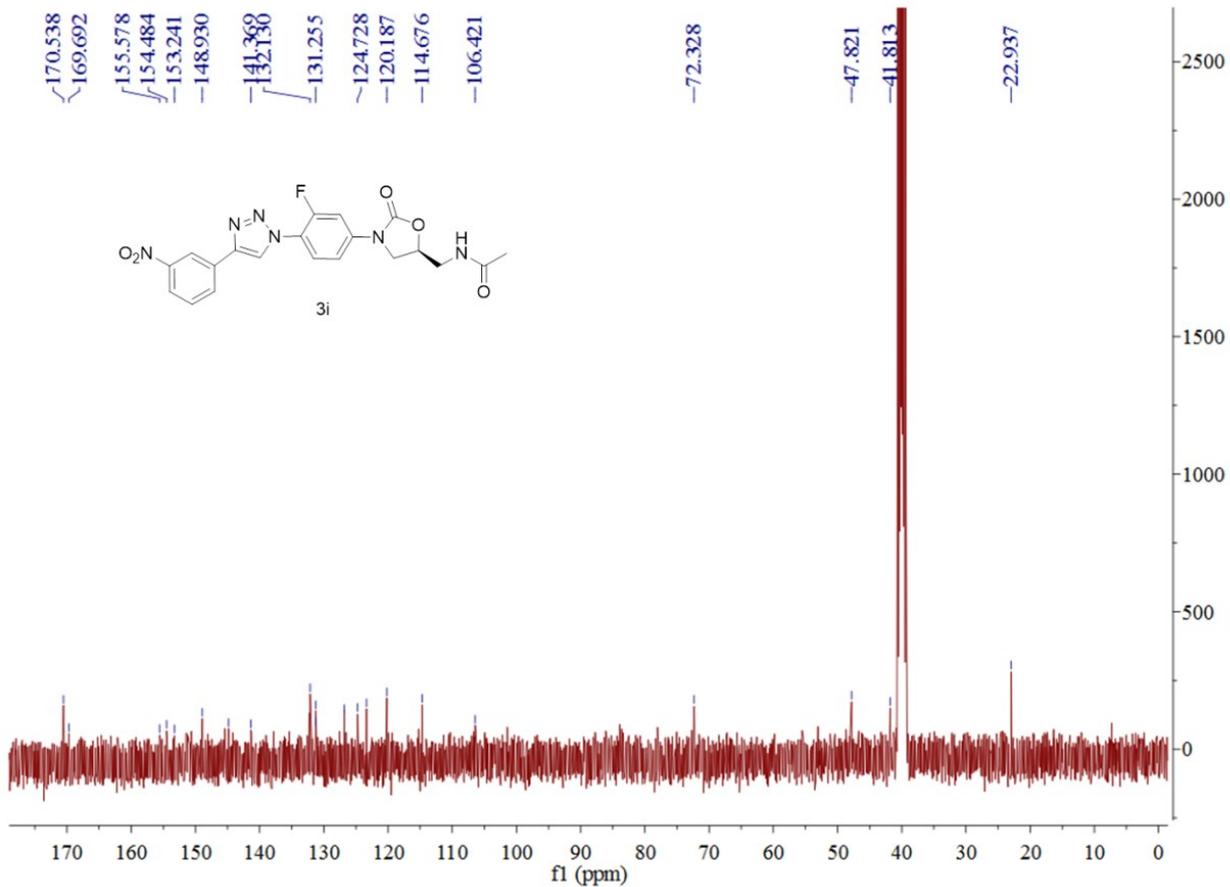
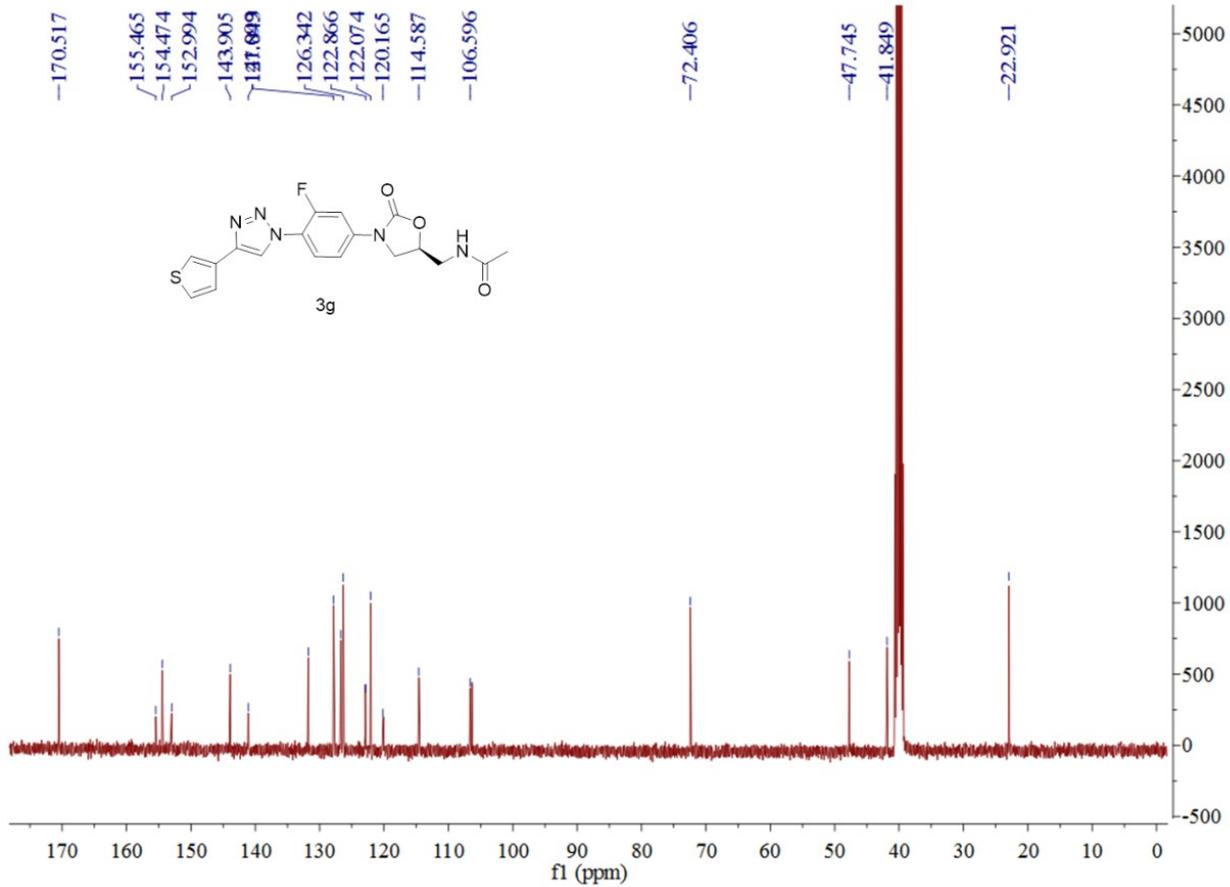


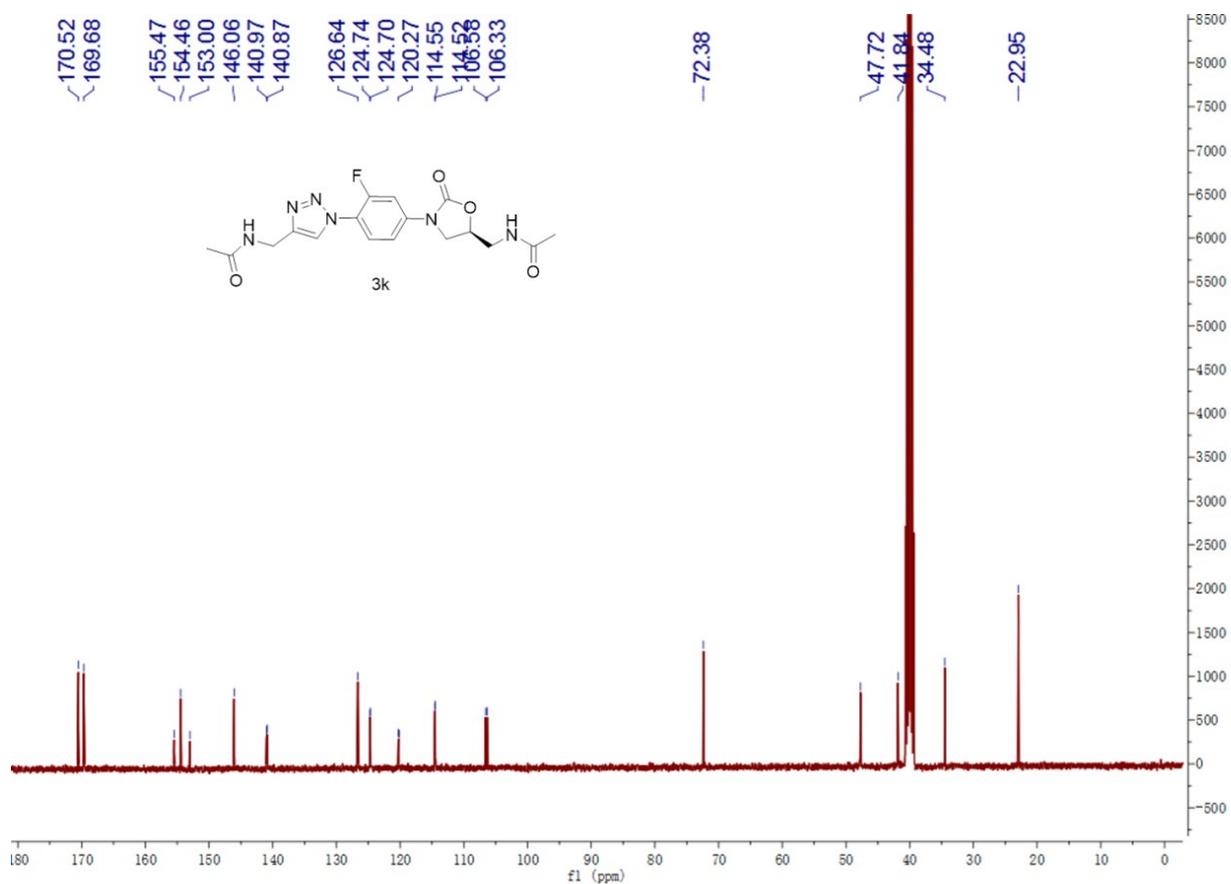
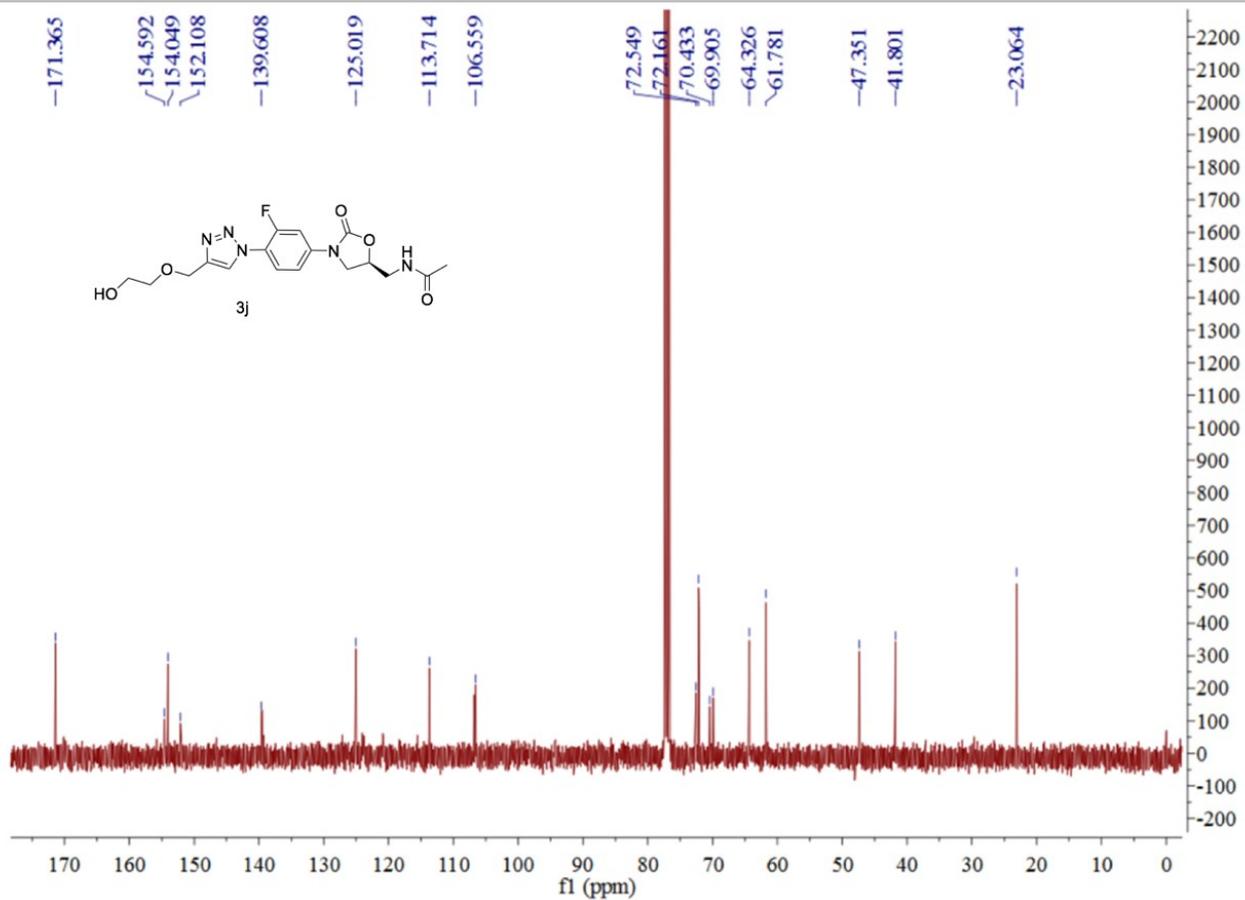


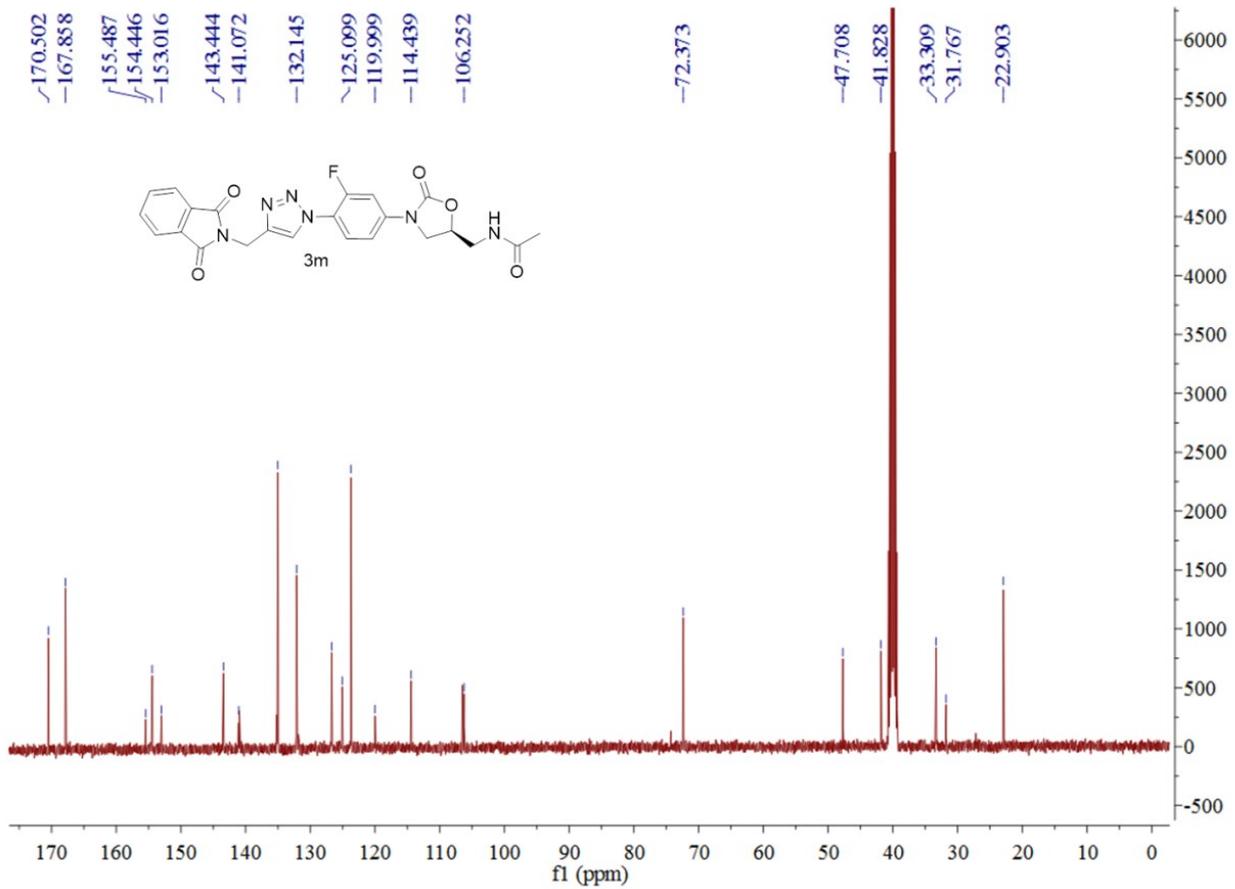
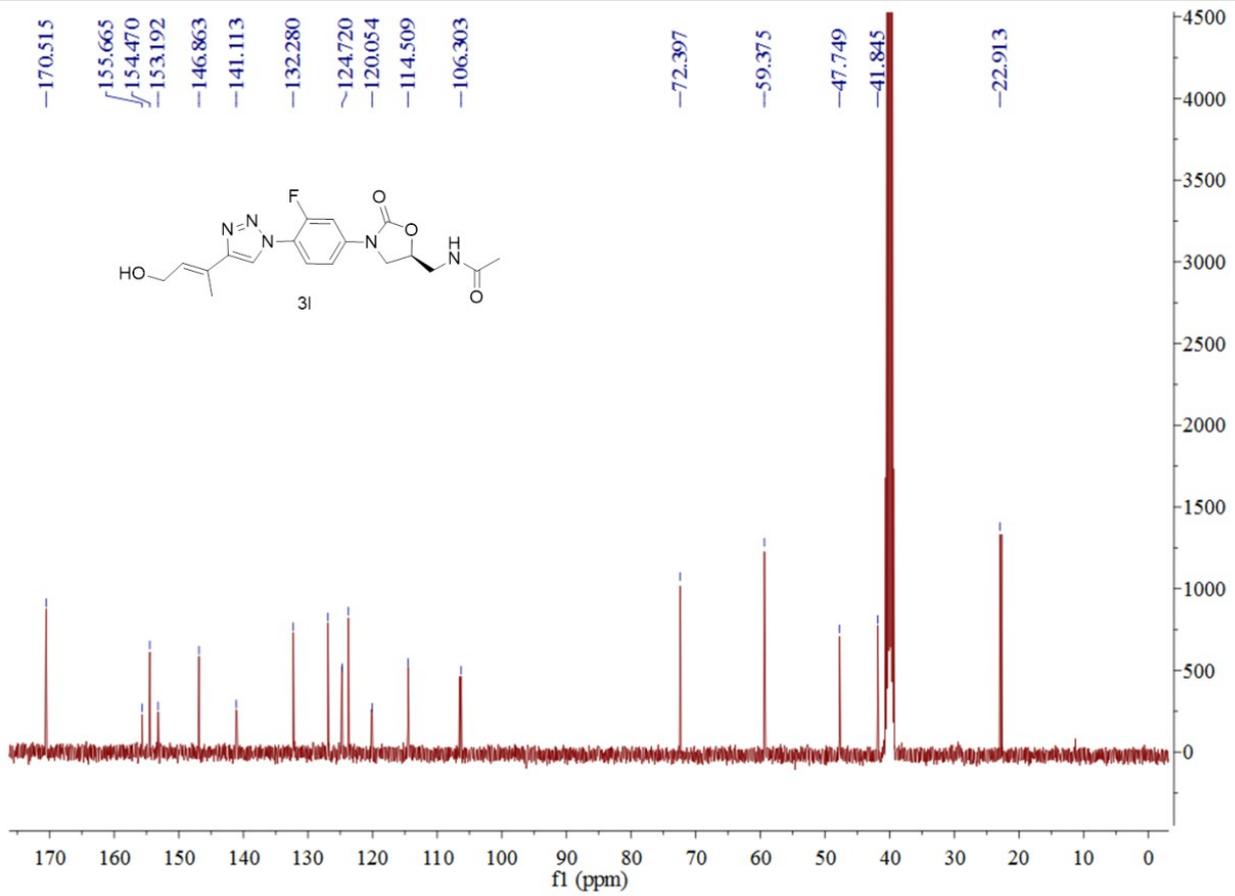


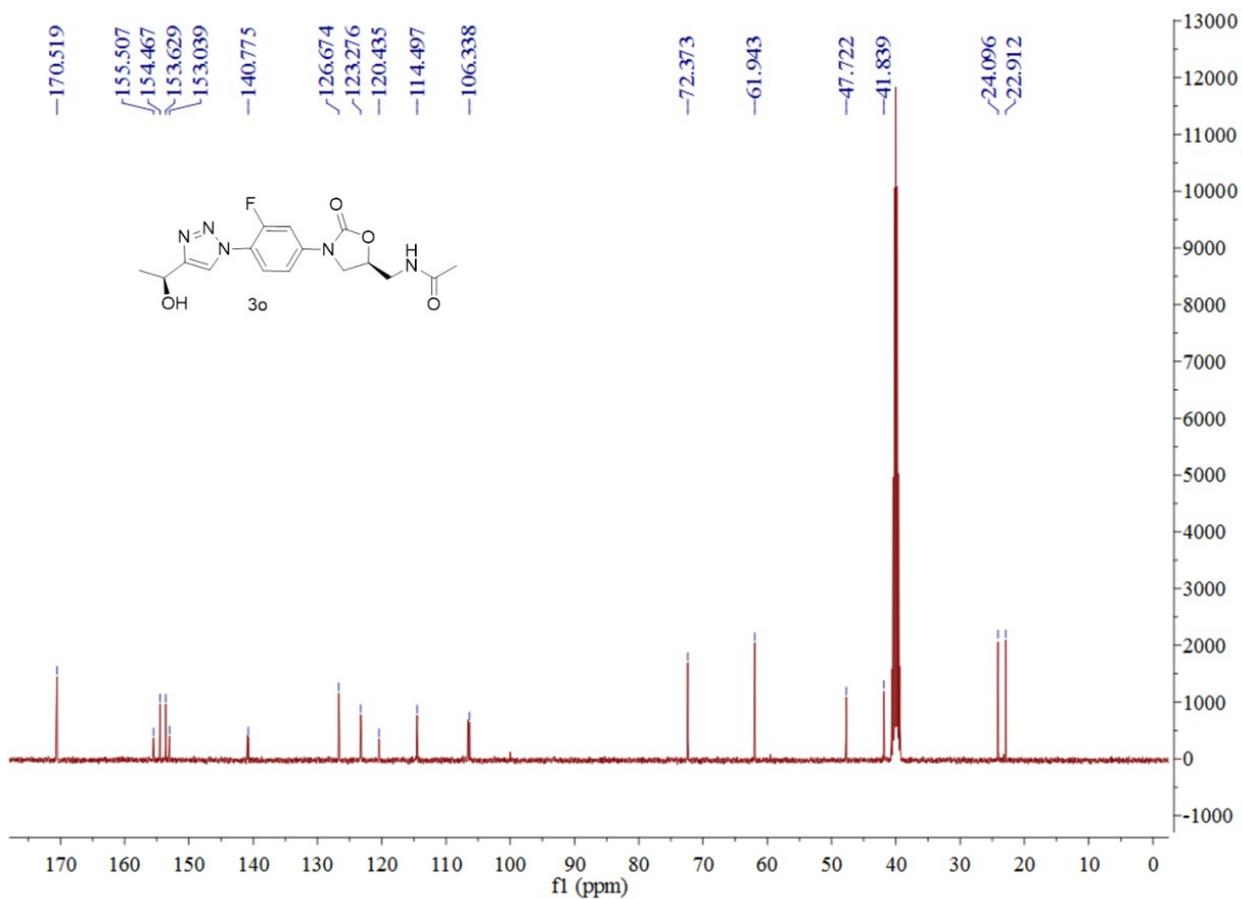
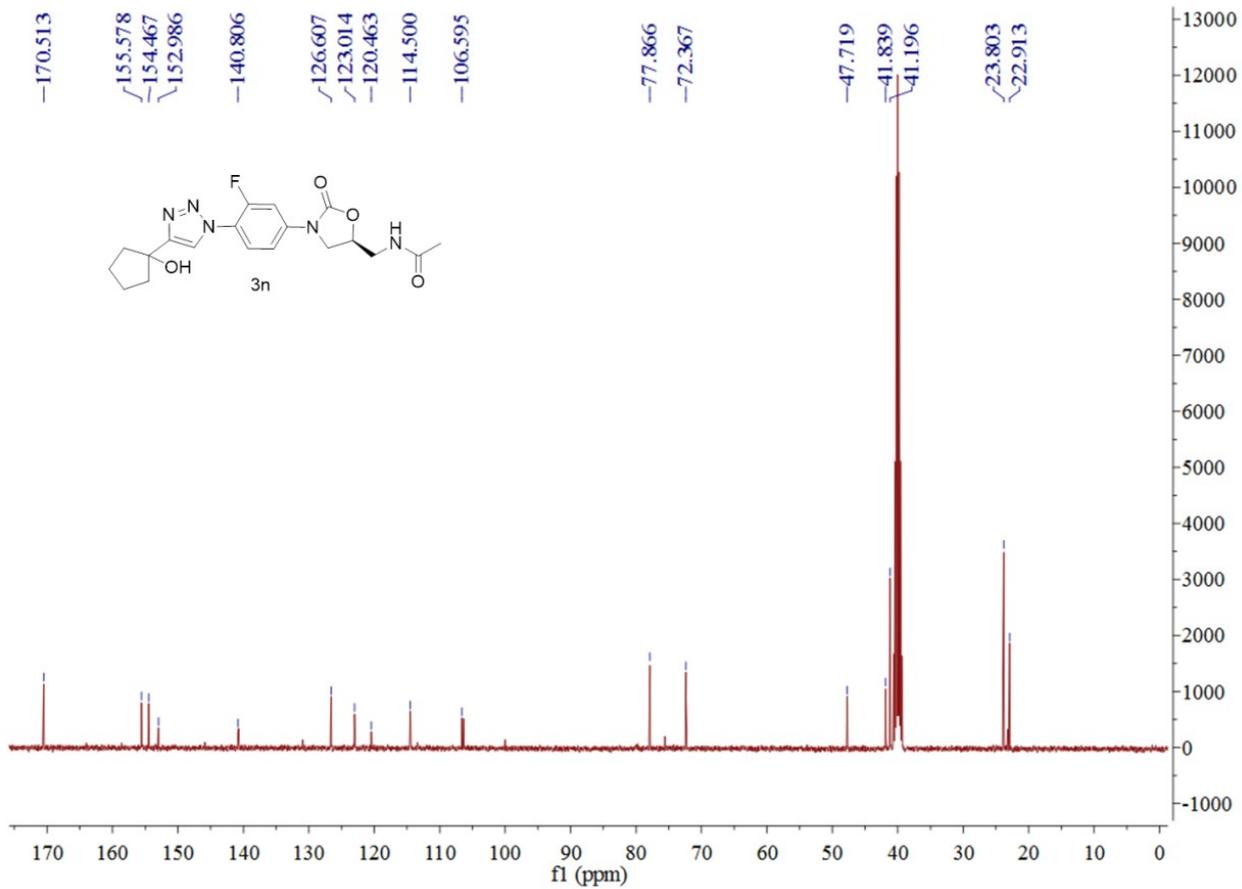


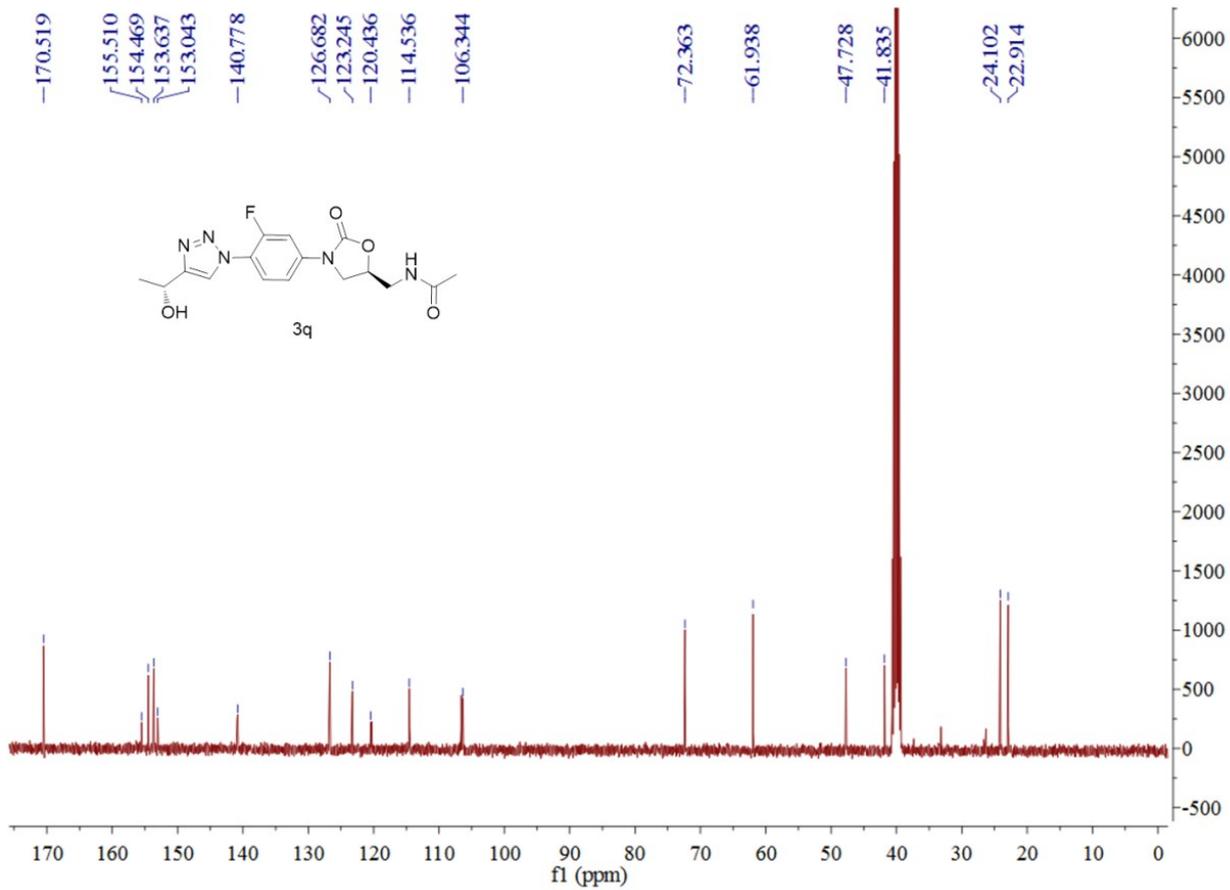
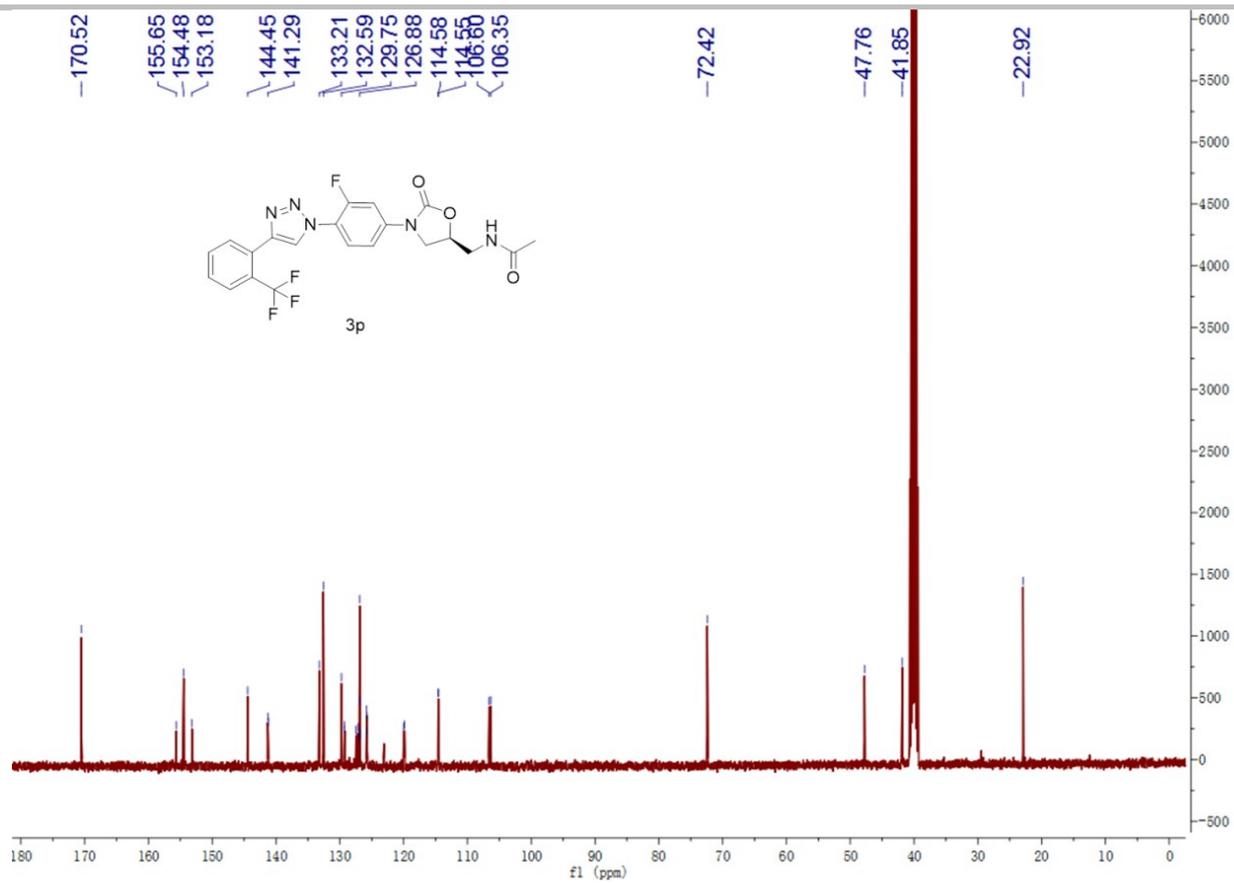


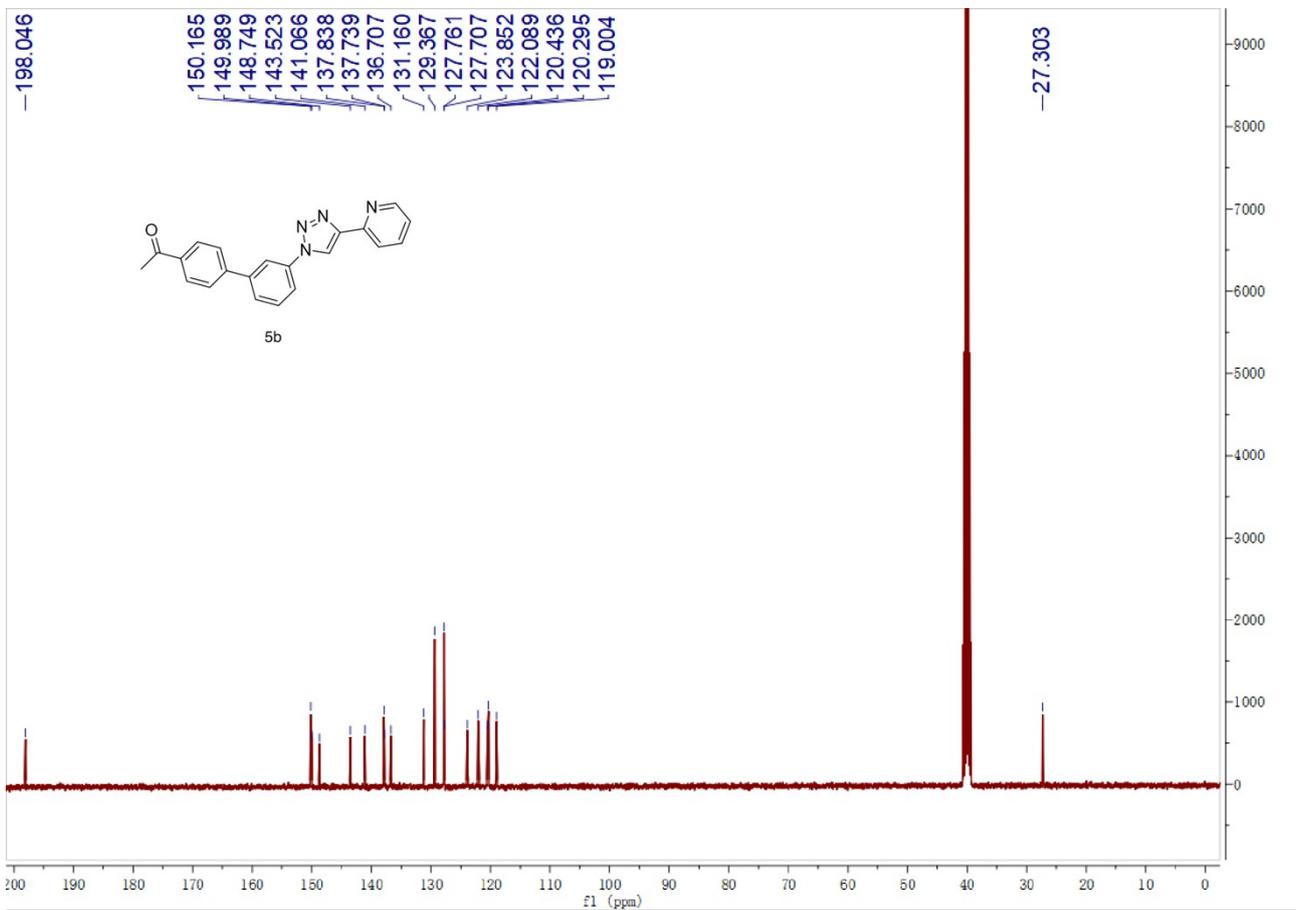
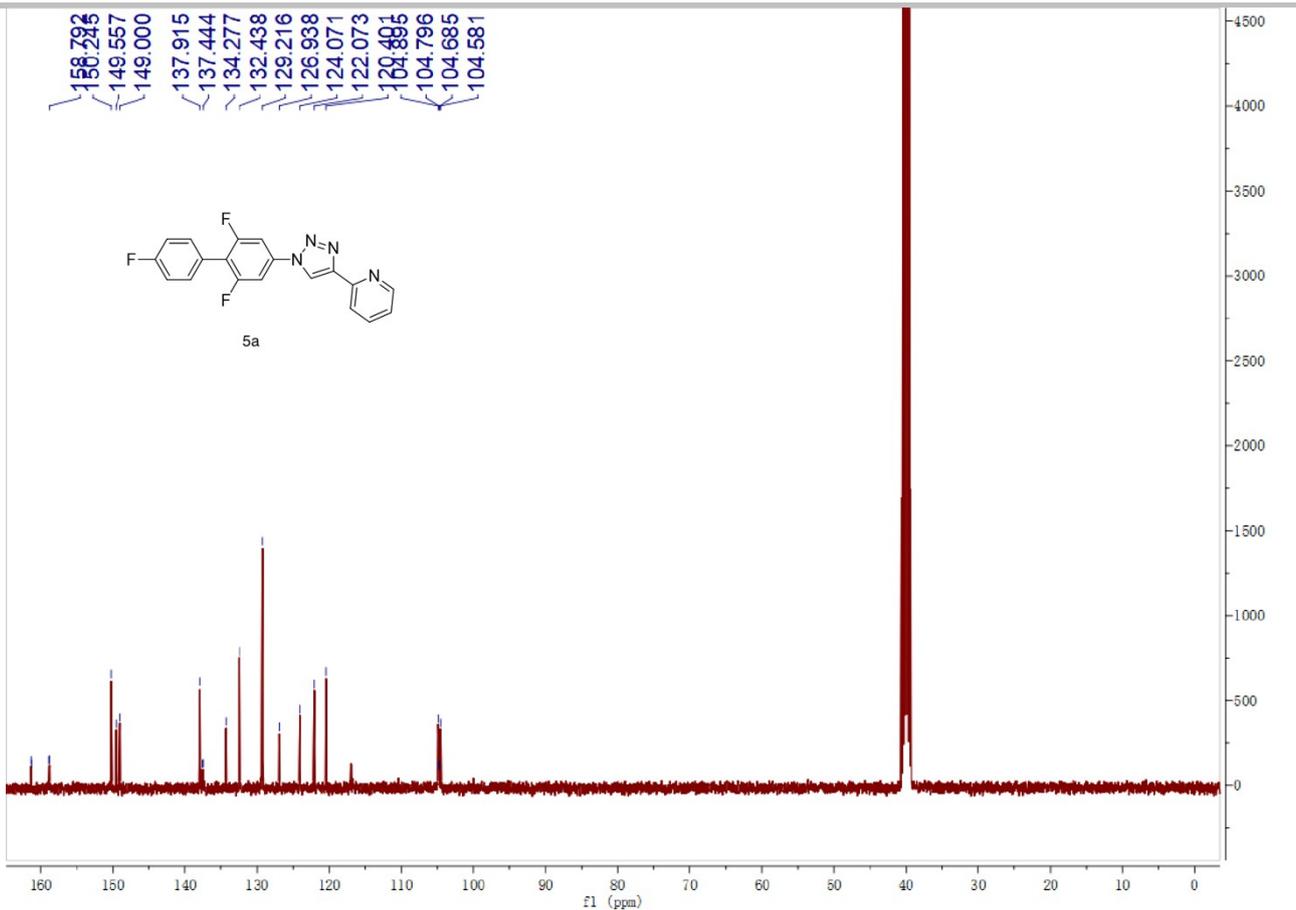


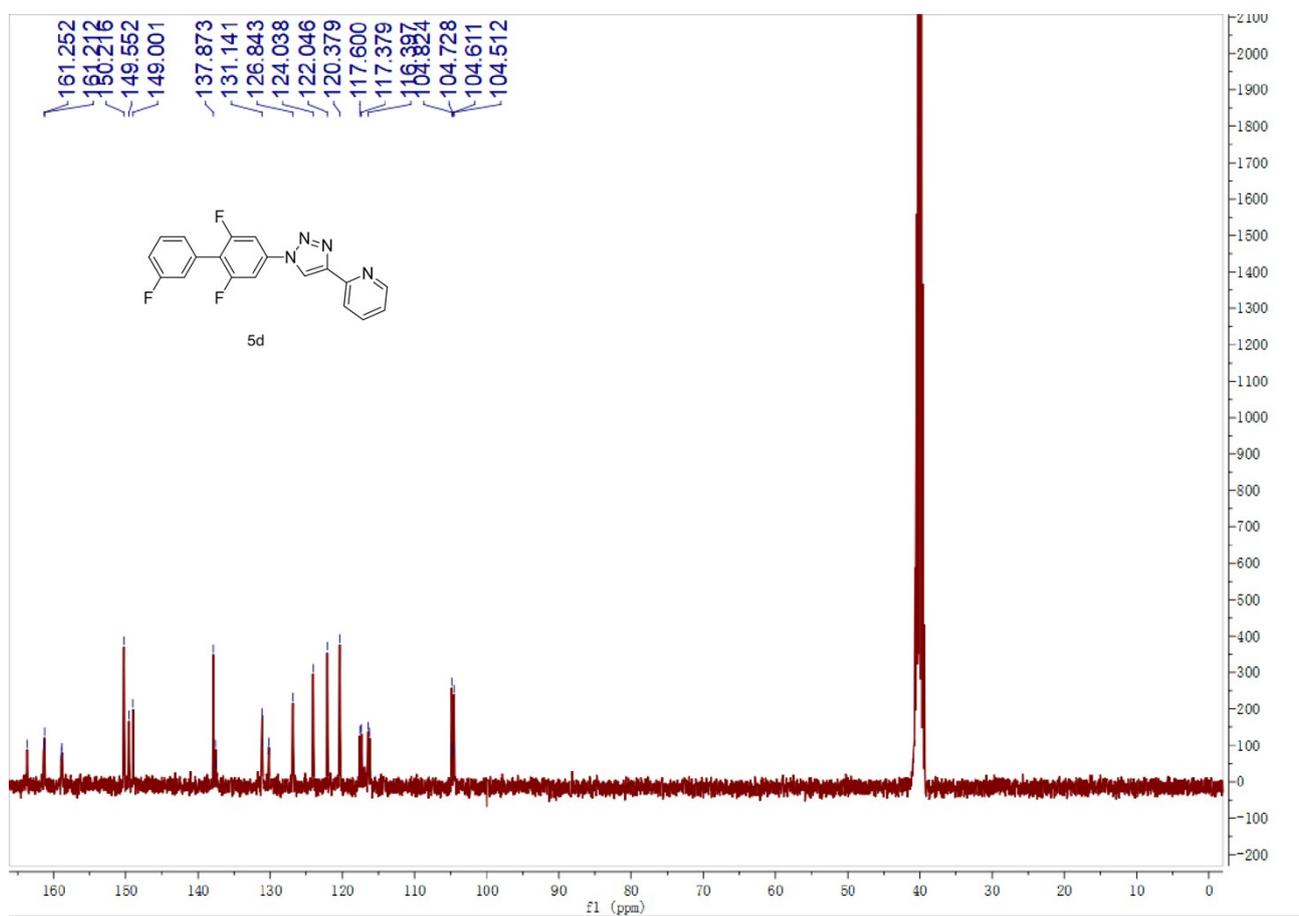
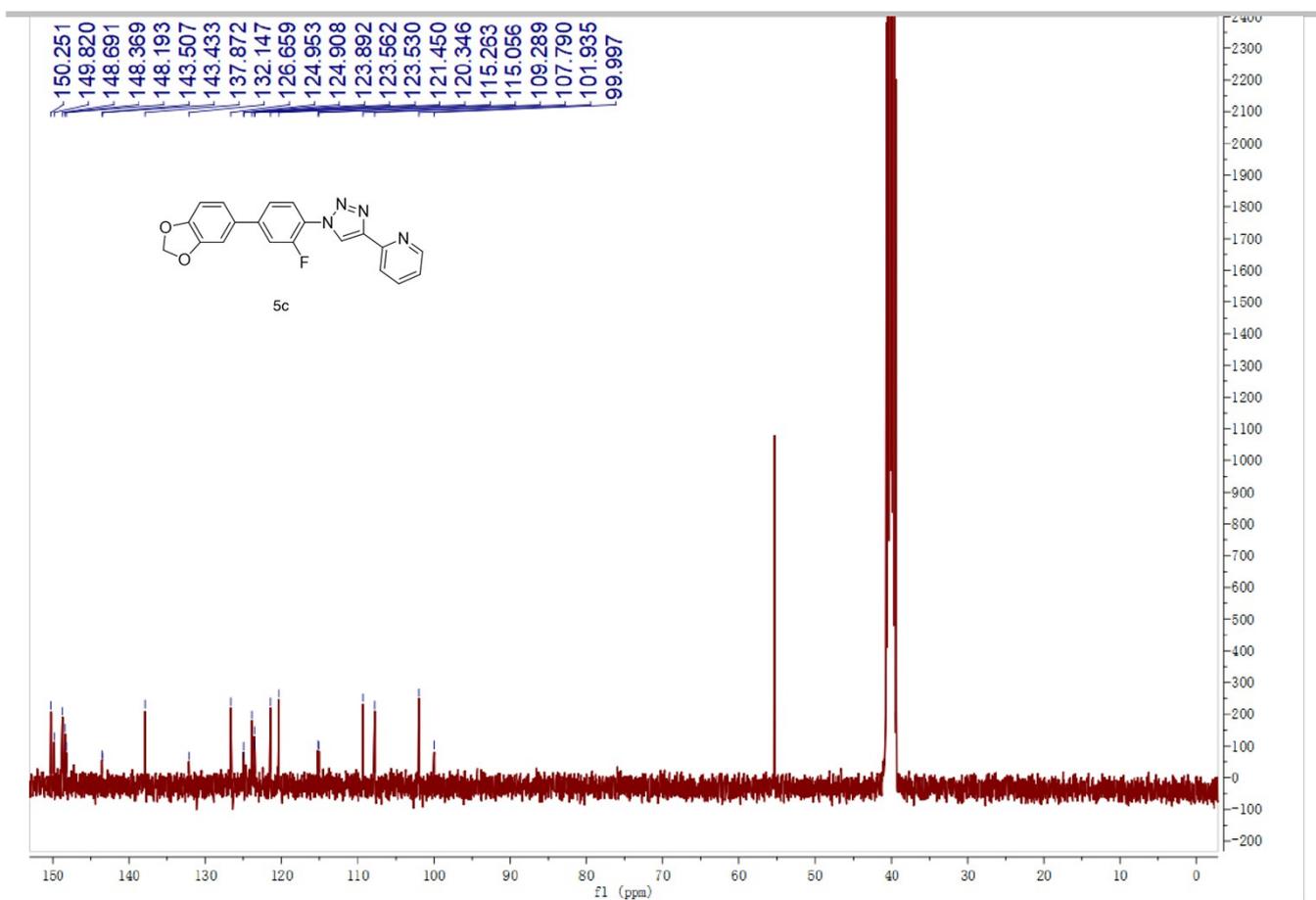


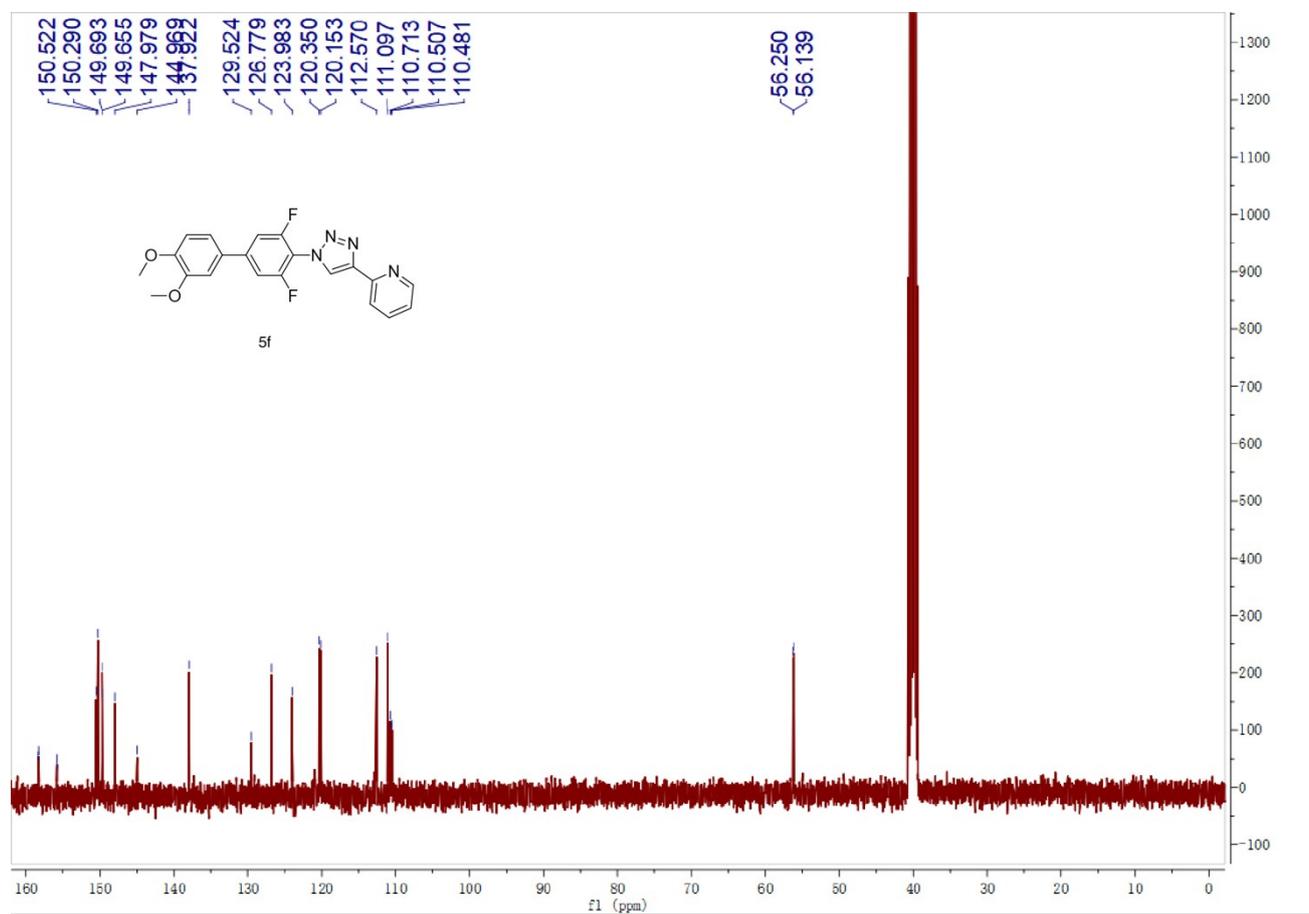
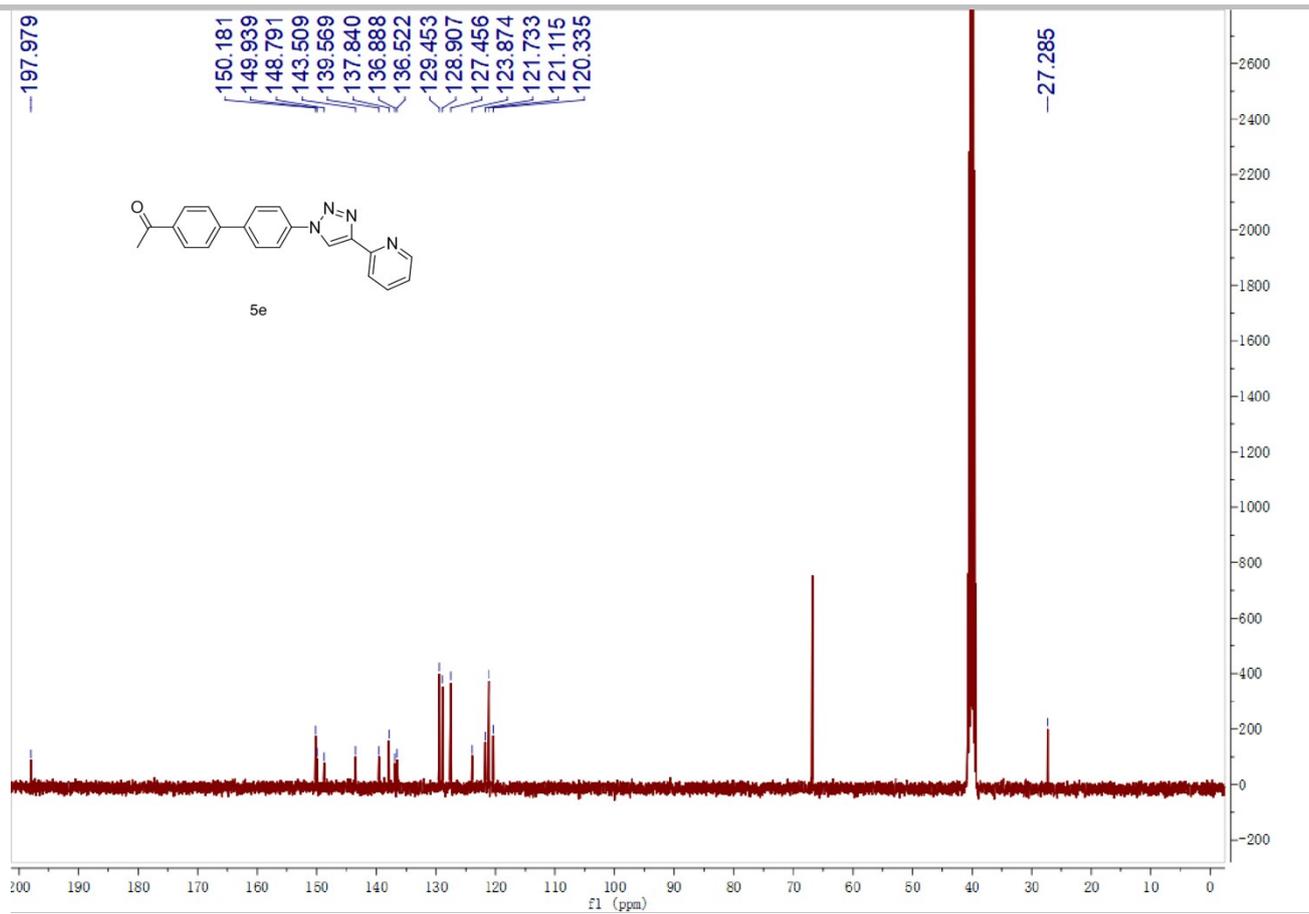


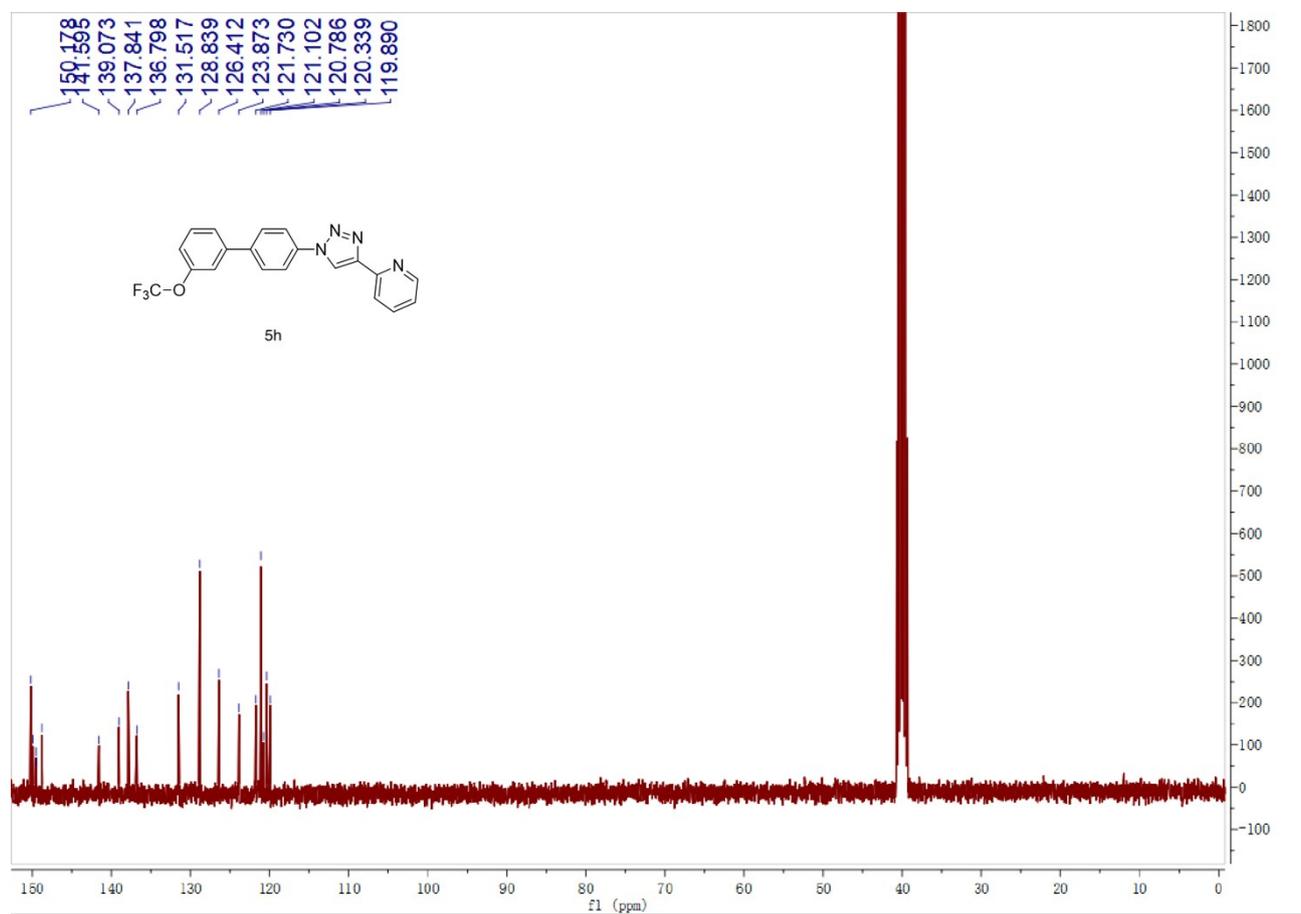
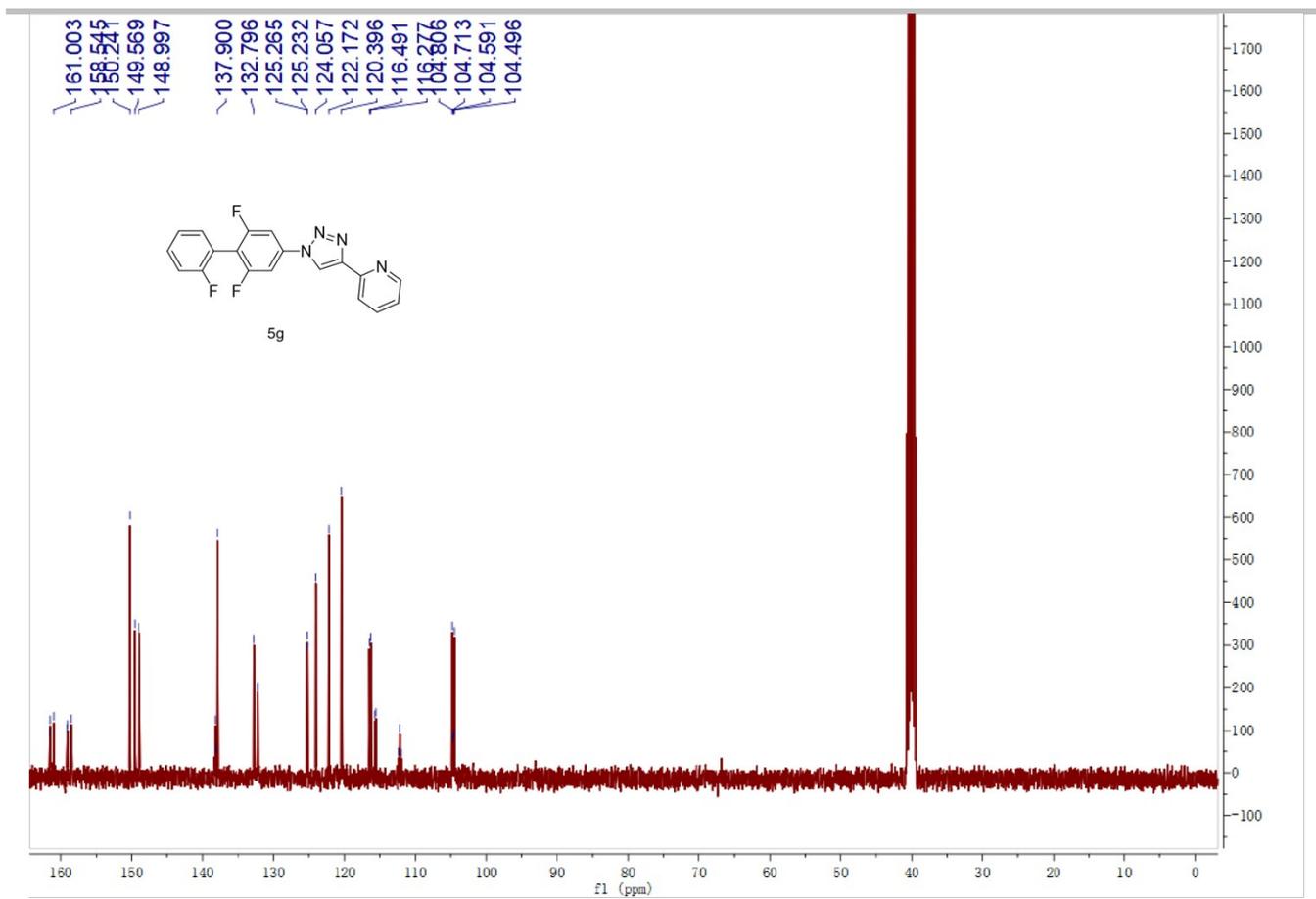


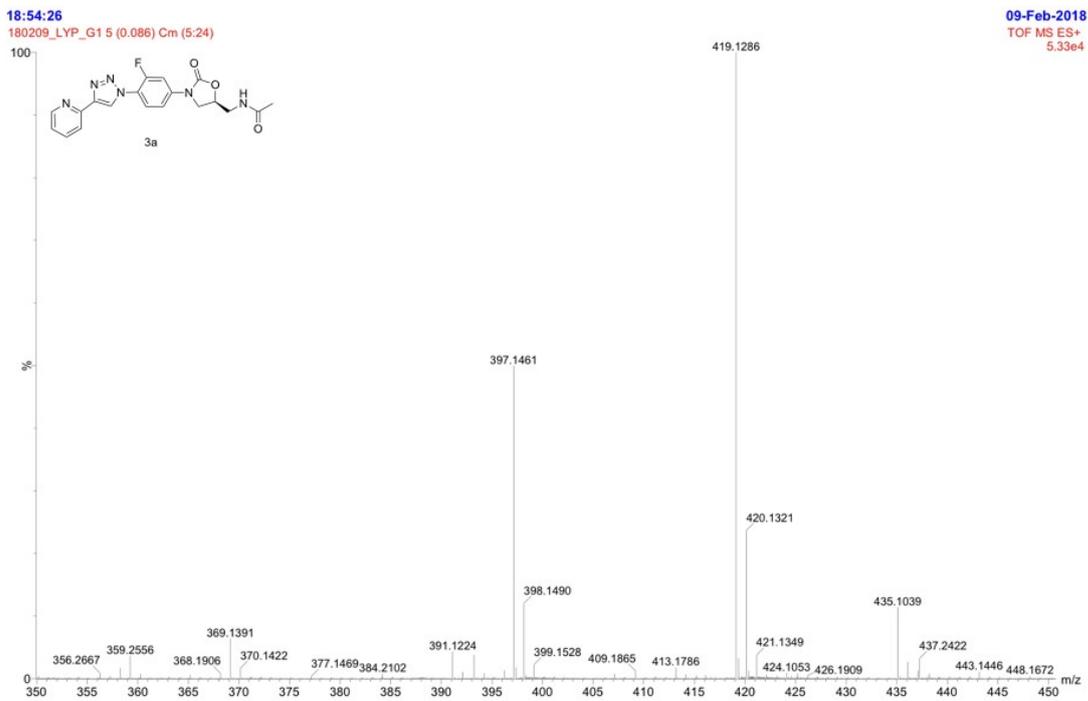
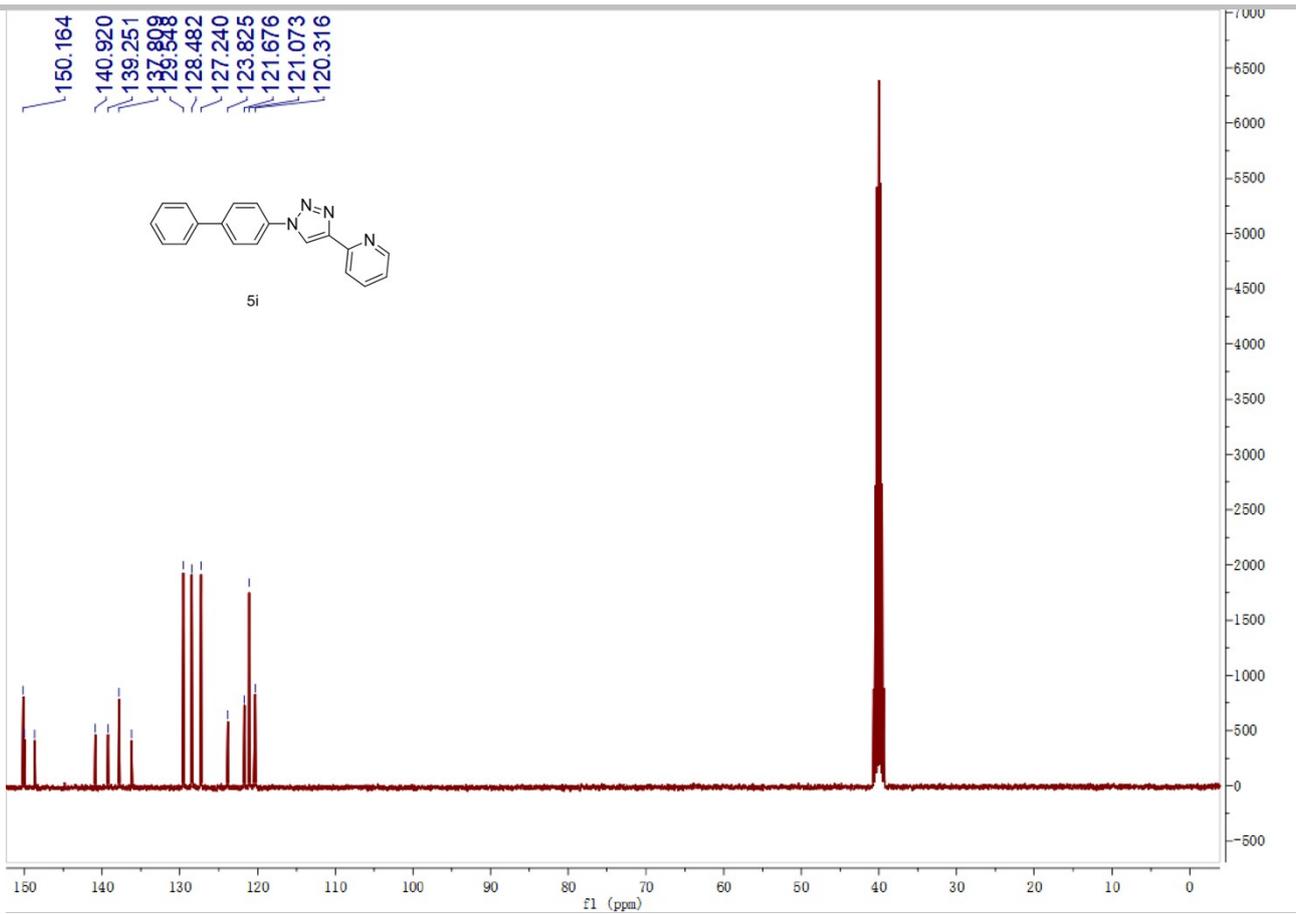










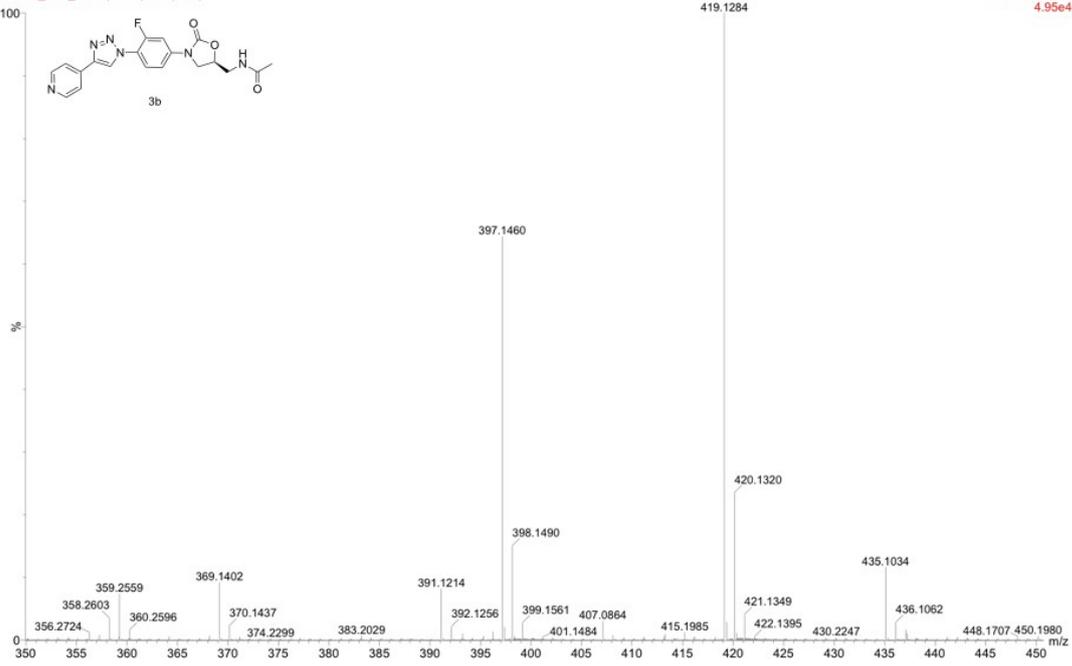


18:57:07

180209_LYP_G2 3 (0.051) Cm (3:23)

09-Feb-2018

TOF MS ES+
4.95e4

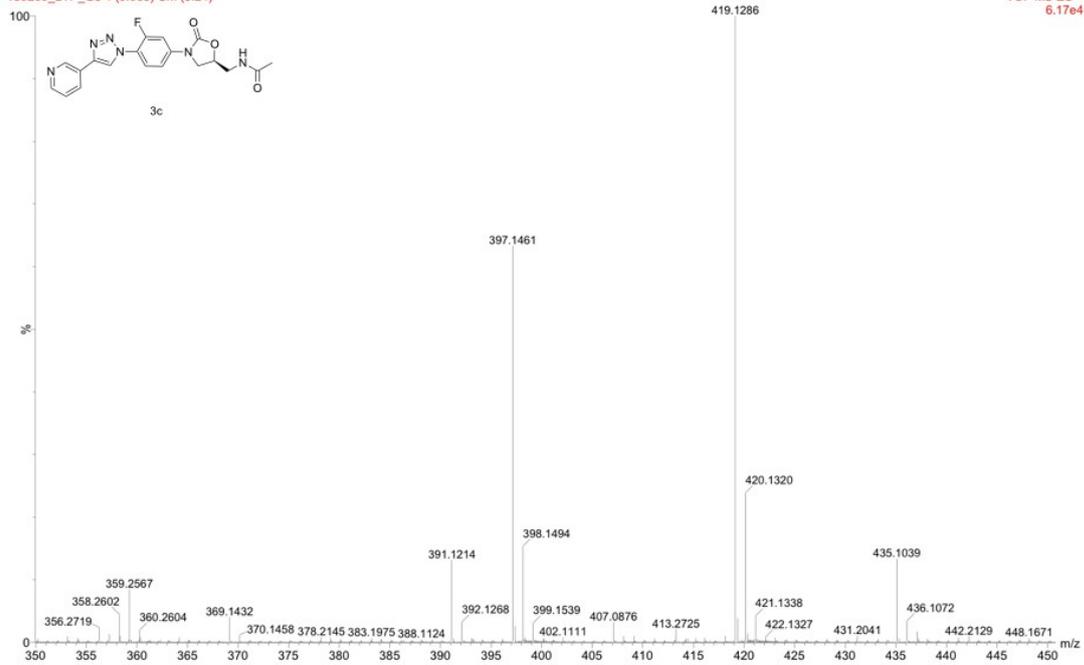


18:59:32

180209_LYP_G3 4 (0.068) Cm (3:24)

09-Feb-2018

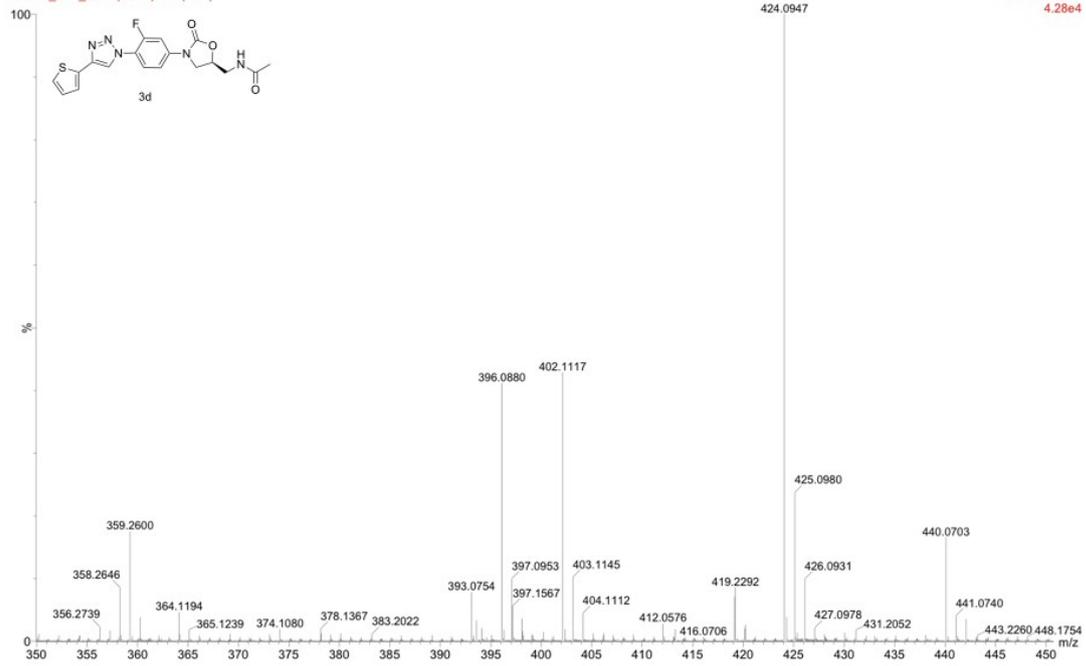
TOF MS ES+
6.17e4



19:02:50

180209_LYP_G4 5 (0.086) Cm (4:24)

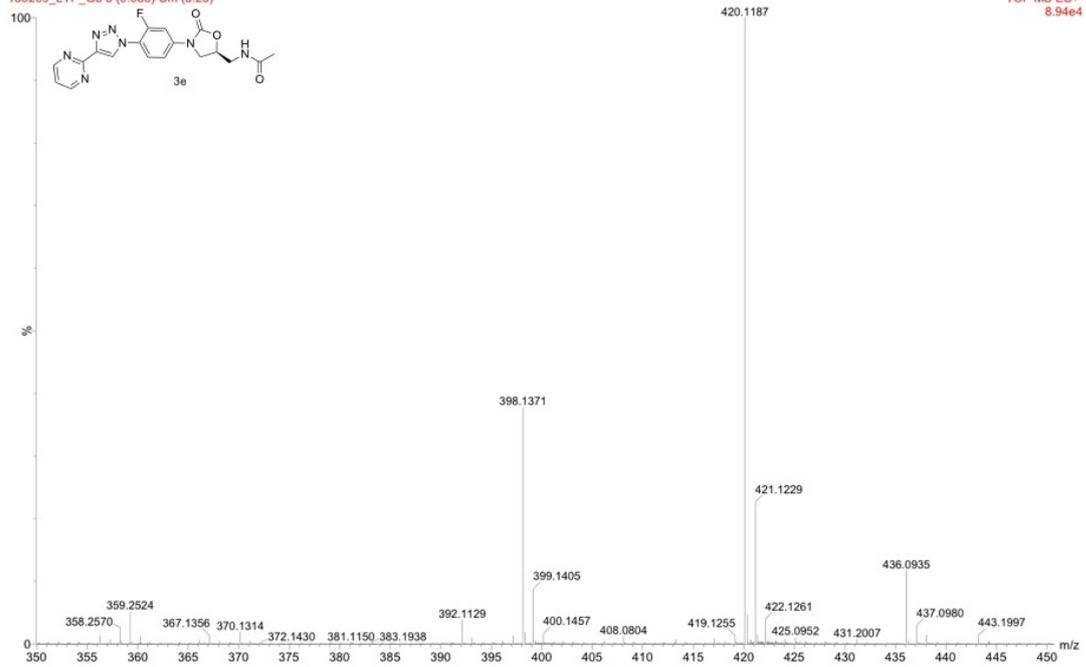
09-Feb-2018
TOF MS ES+
4.28e4



19:04:56

180209_LYP_G5 5 (0.086) Cm (3:23)

09-Feb-2018
TOF MS ES+
8.94e4

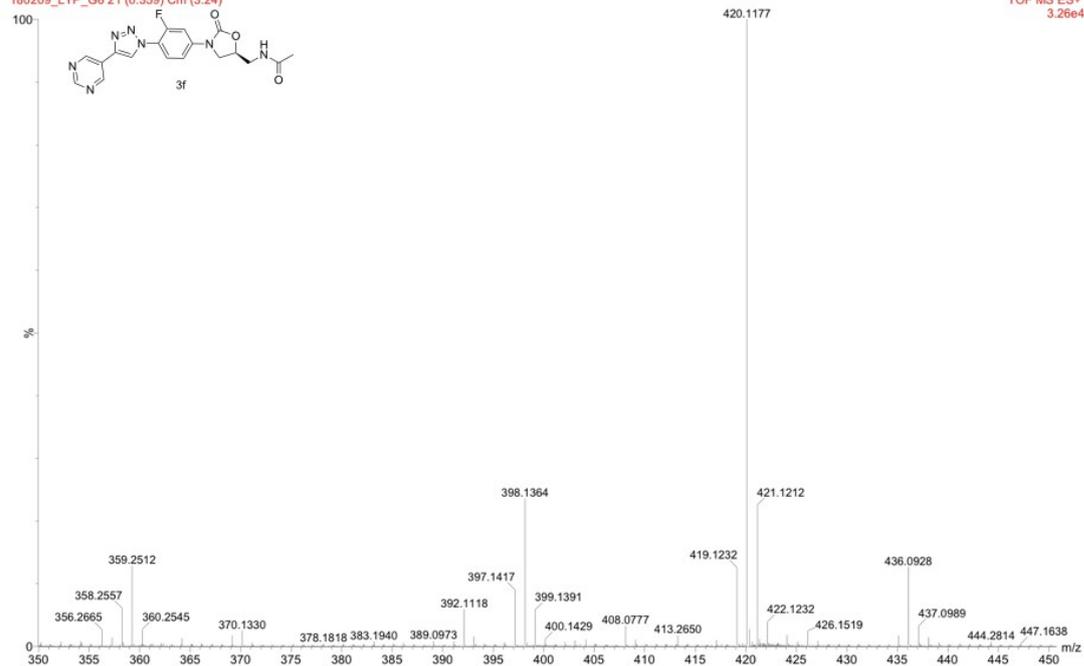


19:06:54

180209_LYP_G6 21 (0.359) Cm (3:24)

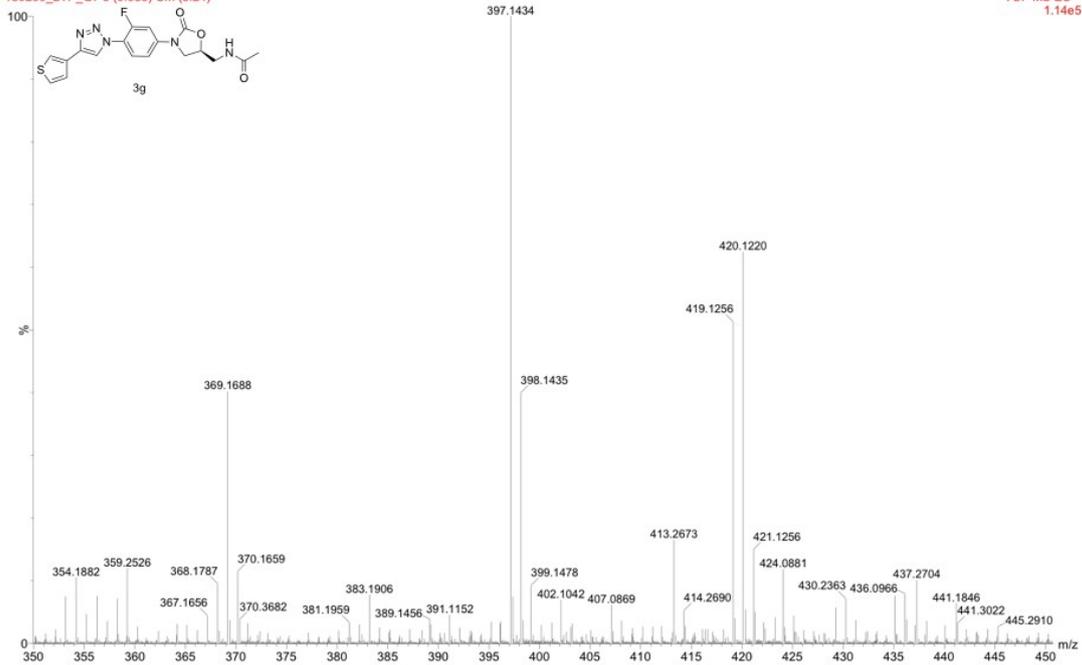
09-Feb-2018

TOF MS ES+
3.26e4



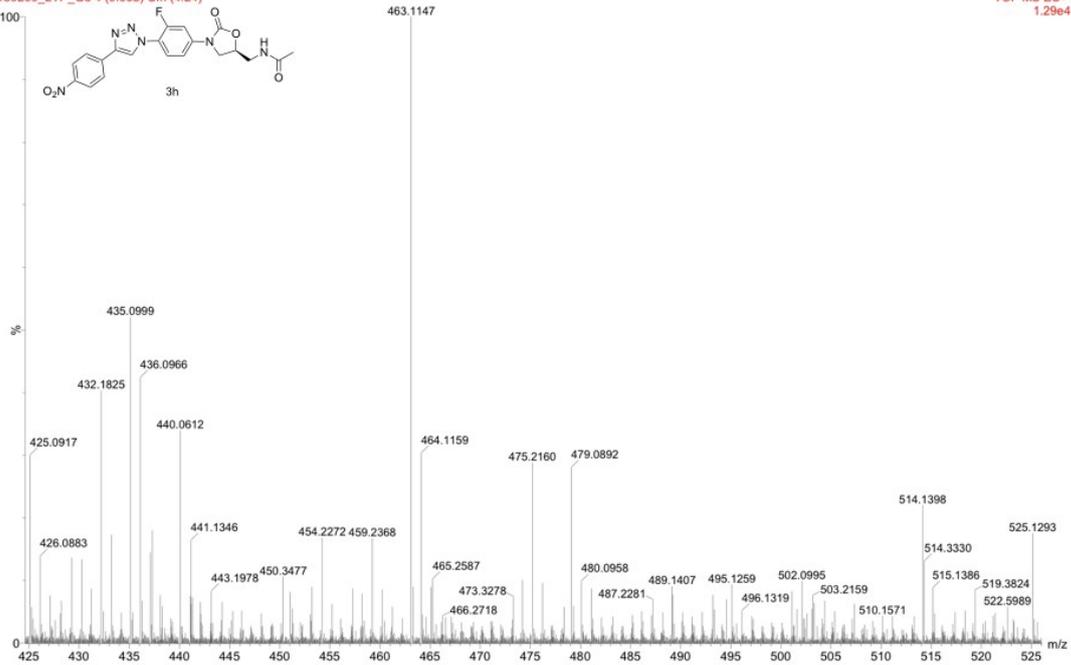
19:11:09
180209_LYP_G7 5 (0.086) Cm (5:24)

09-Feb-2018
TOF MS ES+
1.14e5

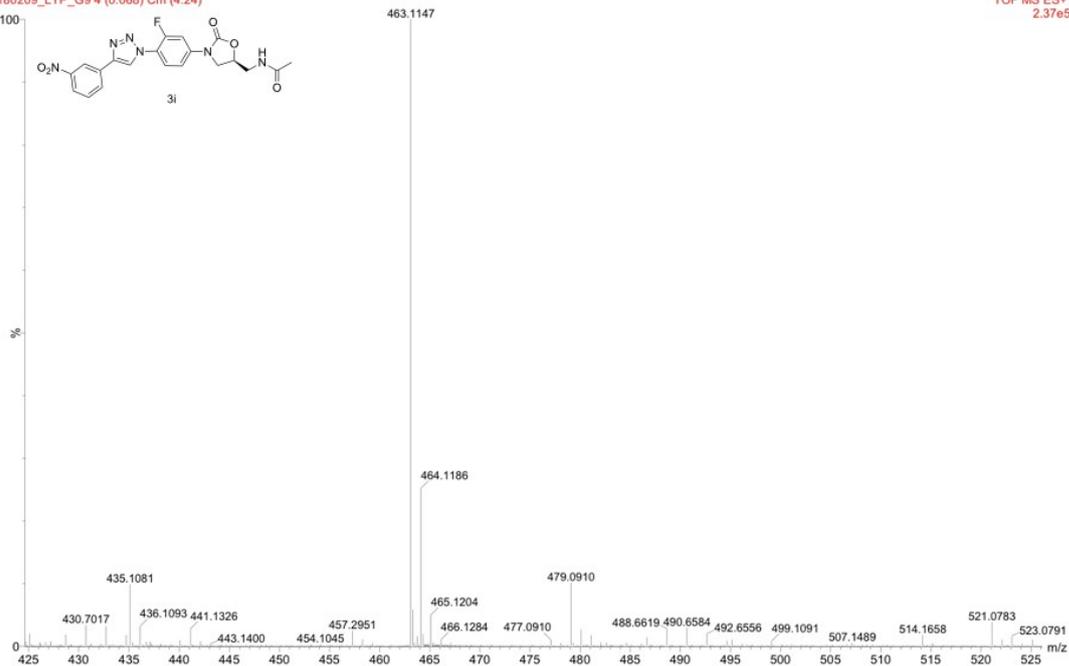


19:14:20
180209_LYP_G8 4 (0.068) Cm (4:24)

09-Feb-2018
TOF MS ES+
1.29e4

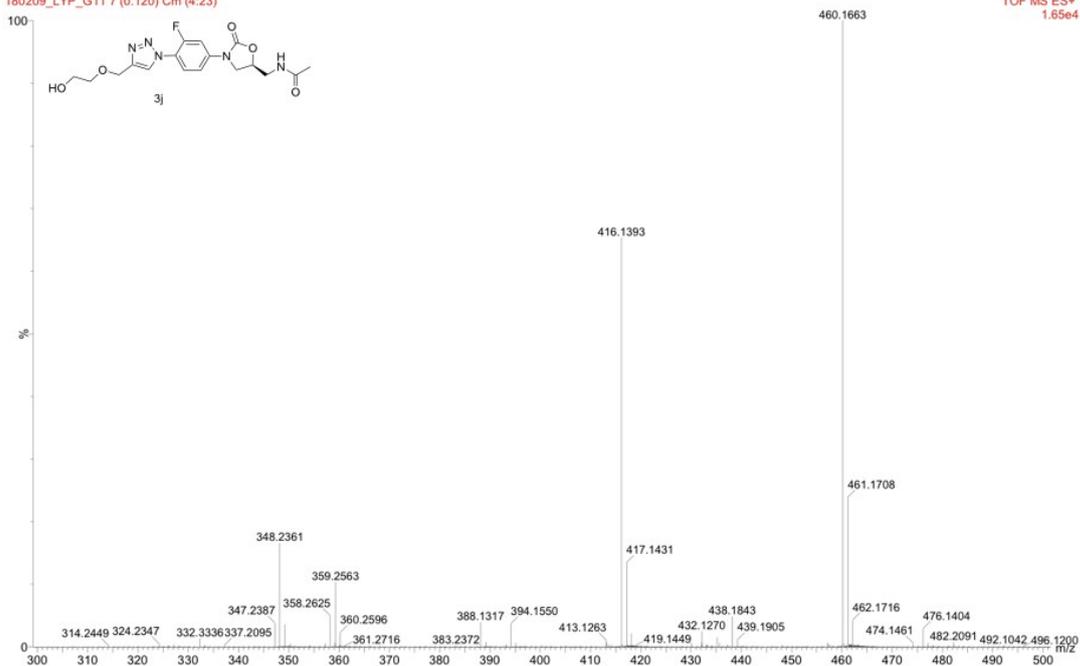


19:16:29
180209_LYP_G9 4 (0.068) Cm (4:24)



09-Feb-2018
TOF MS ES+
2.37e5

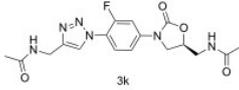
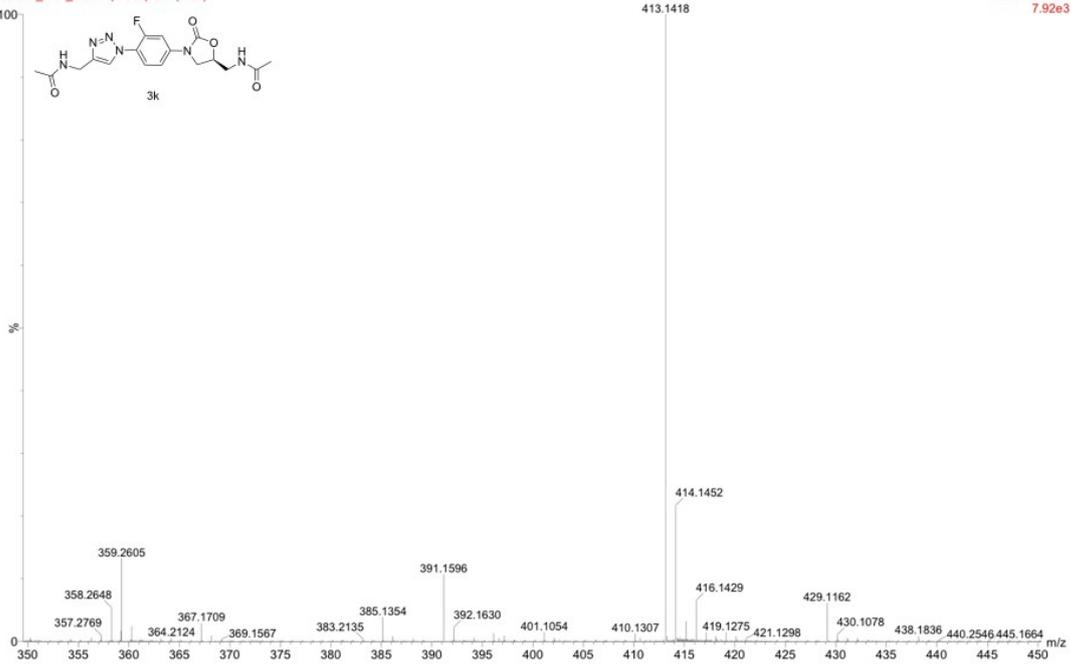
19:19:44
180209_LYP_G11 7 (0.120) Cm (4:23)



09-Feb-2018
TOF MS ES+
1.65e4

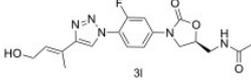
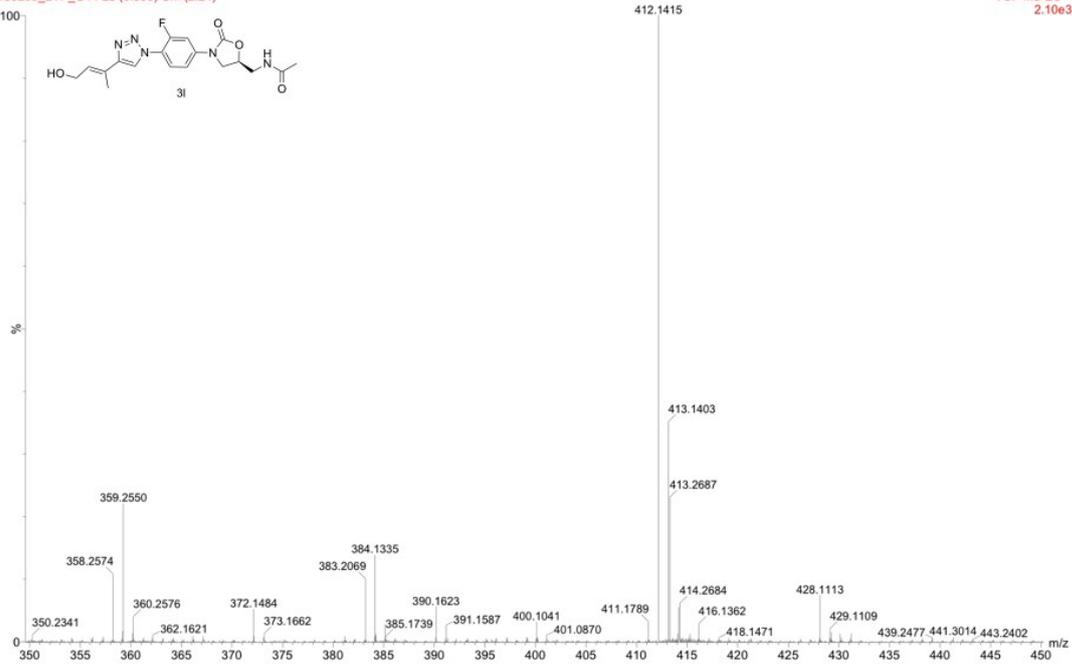
19:22:01
180209_LYP_G13 4 (0.068) Cm (3:24)

09-Feb-2018
TOF MS ES+
7.92e3



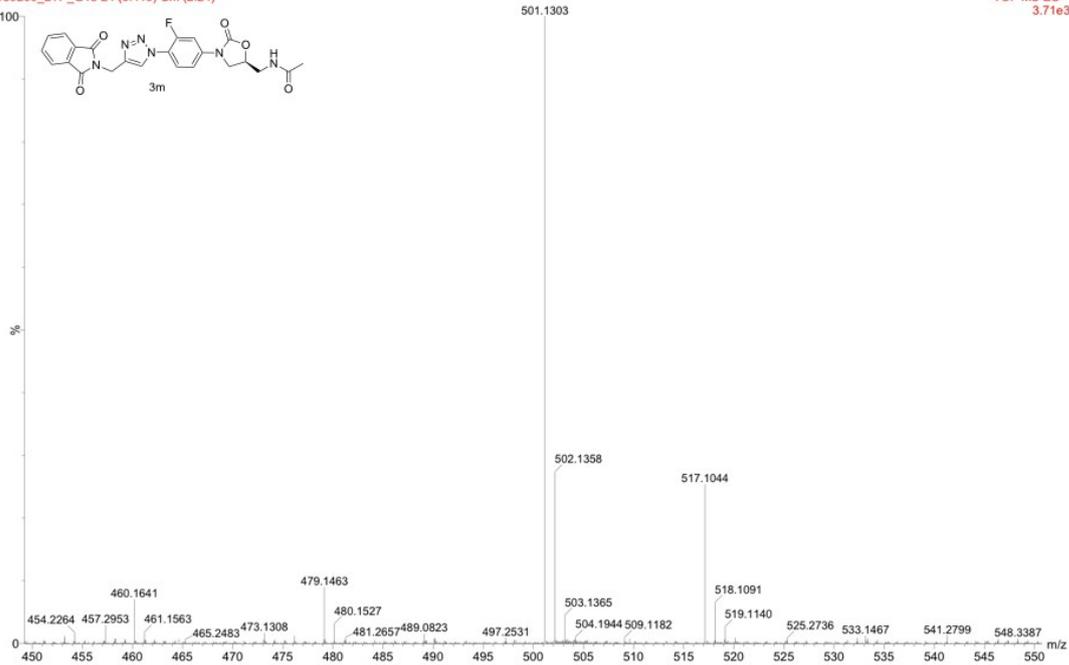
19:24:11
180209_LYP_G14 23 (0.393) Cm (2:24)

09-Feb-2018
TOF MS ES+
2.10e3



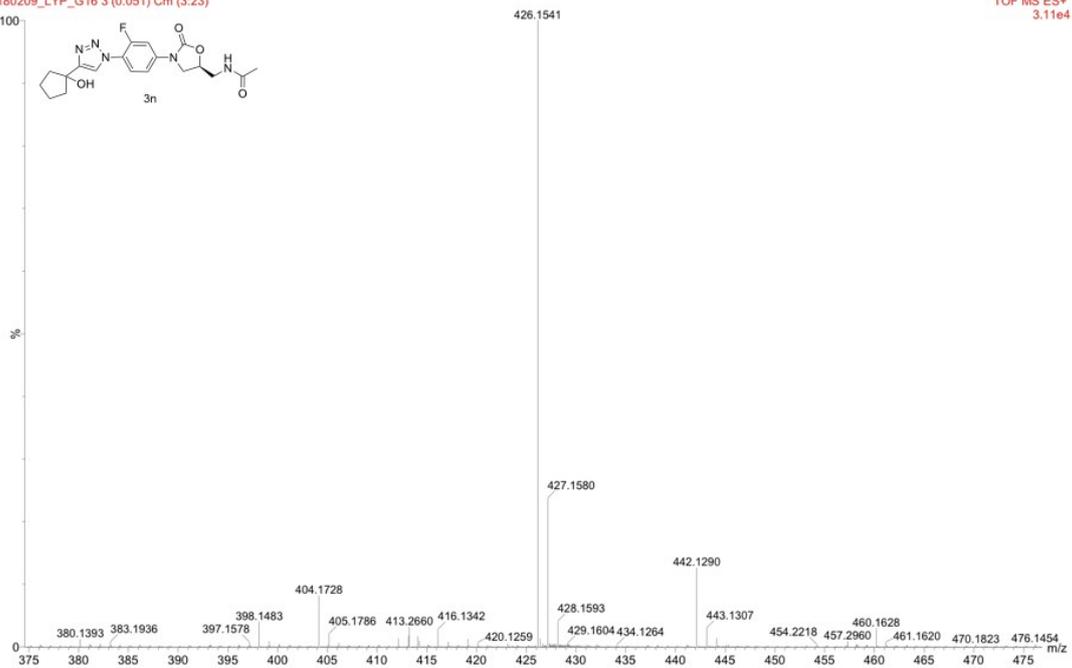
19:27:51
180209_LYP_G15 24 (0.410) Cm (2:24)

09-Feb-2018
TOF MS ES+
3.71e3



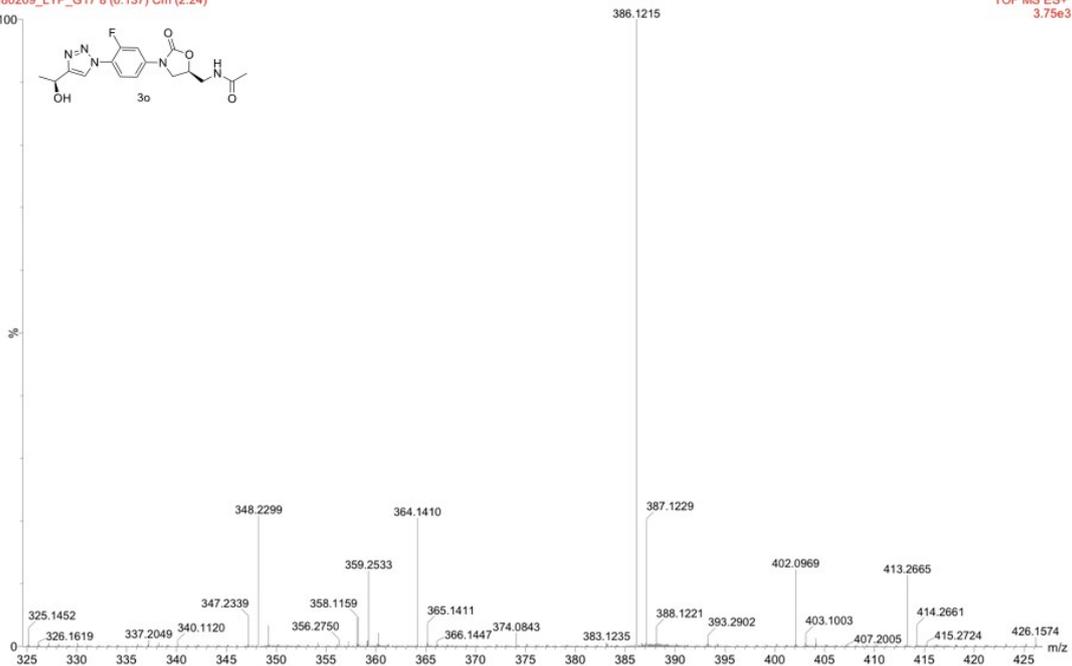
19:30:52
180209_LYP_G16 3 (0.051) Cm (3:23)

09-Feb-2018
TOF MS ES+
3.11e4



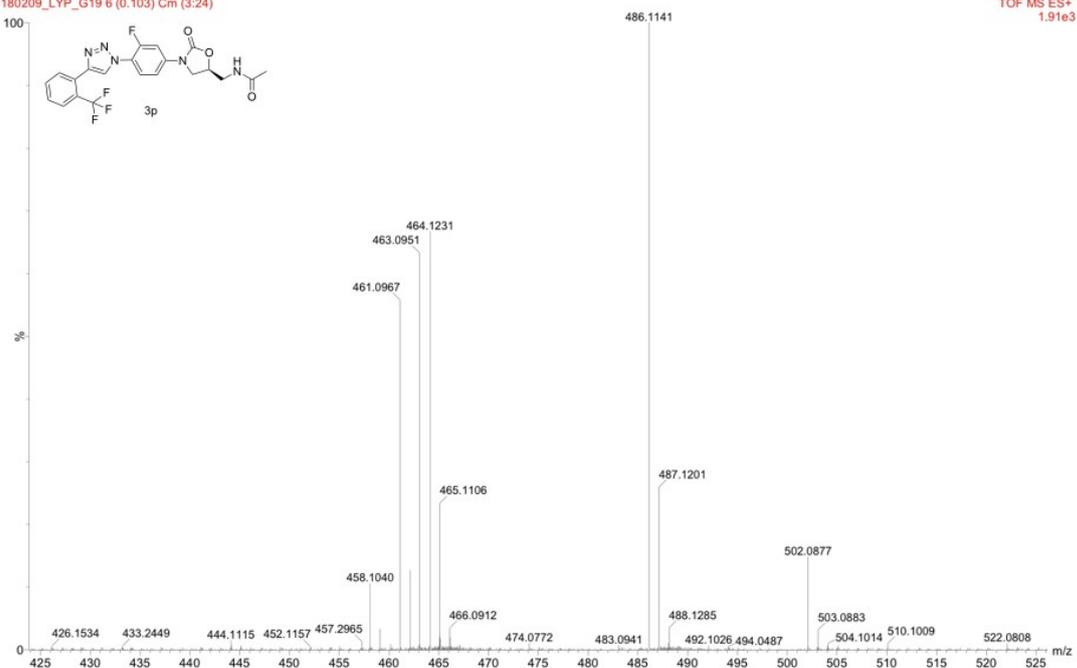
19:33:02
180209_LYP_G17 8 (0.137) Cm (2:24)

09-Feb-2018
TOF MS ES+
3.75e3



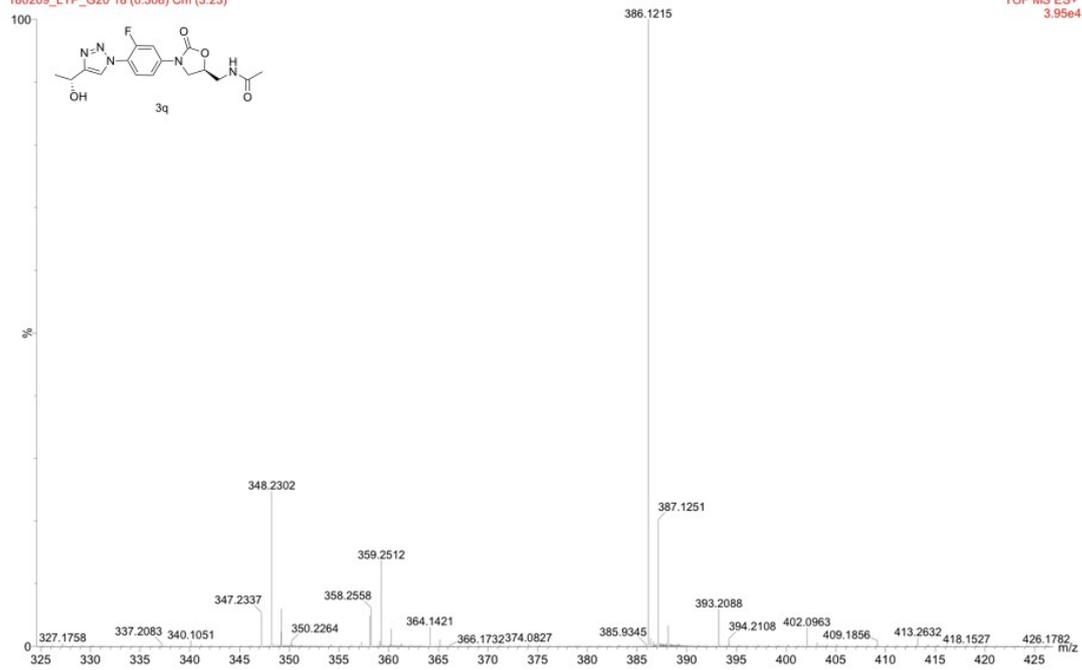
19:35:13
180209_LYP_G19 6 (0.103) Cm (3:24)

09-Feb-2018
TOF MS ES+
1.91e3



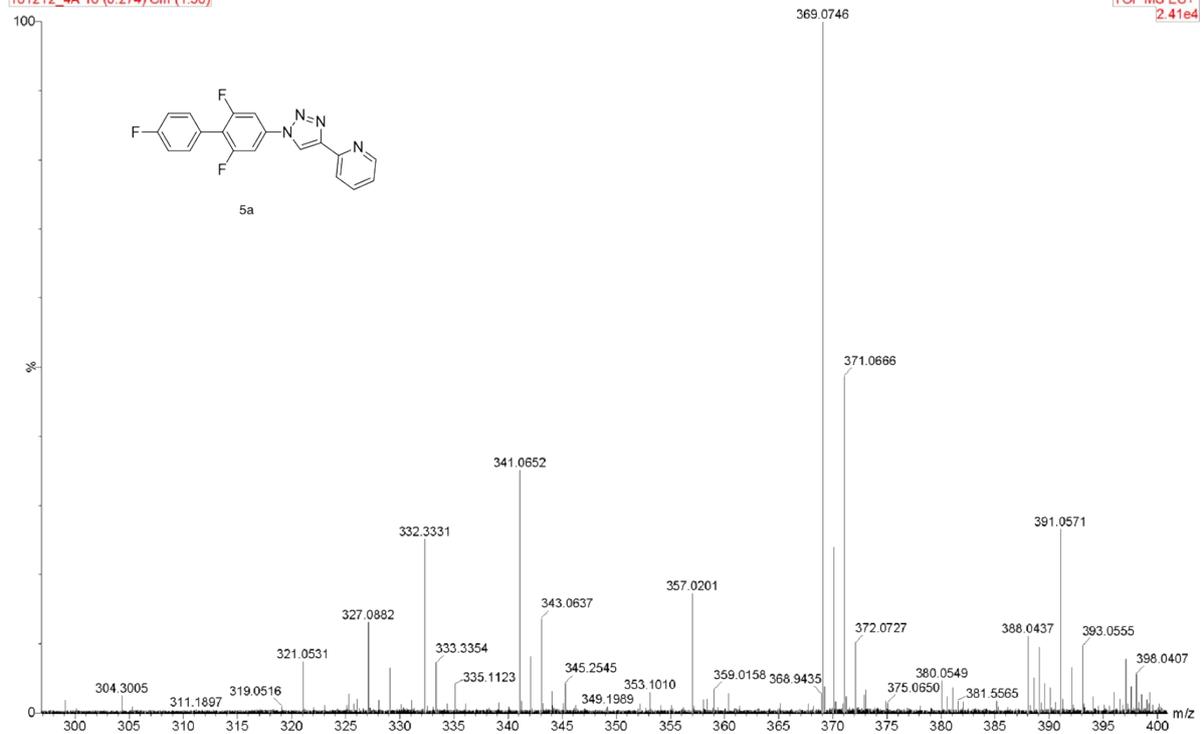
19:37:22
180209_LYP_G20 18 (0.308) Cm (3:23)

09-Feb-2018
TOF MS ES+
3.95e4



09:16:09
181212_4A 16 (0.274) Cm (1:30)

13-Dec-2018
TOF MS ES+
2.41e4

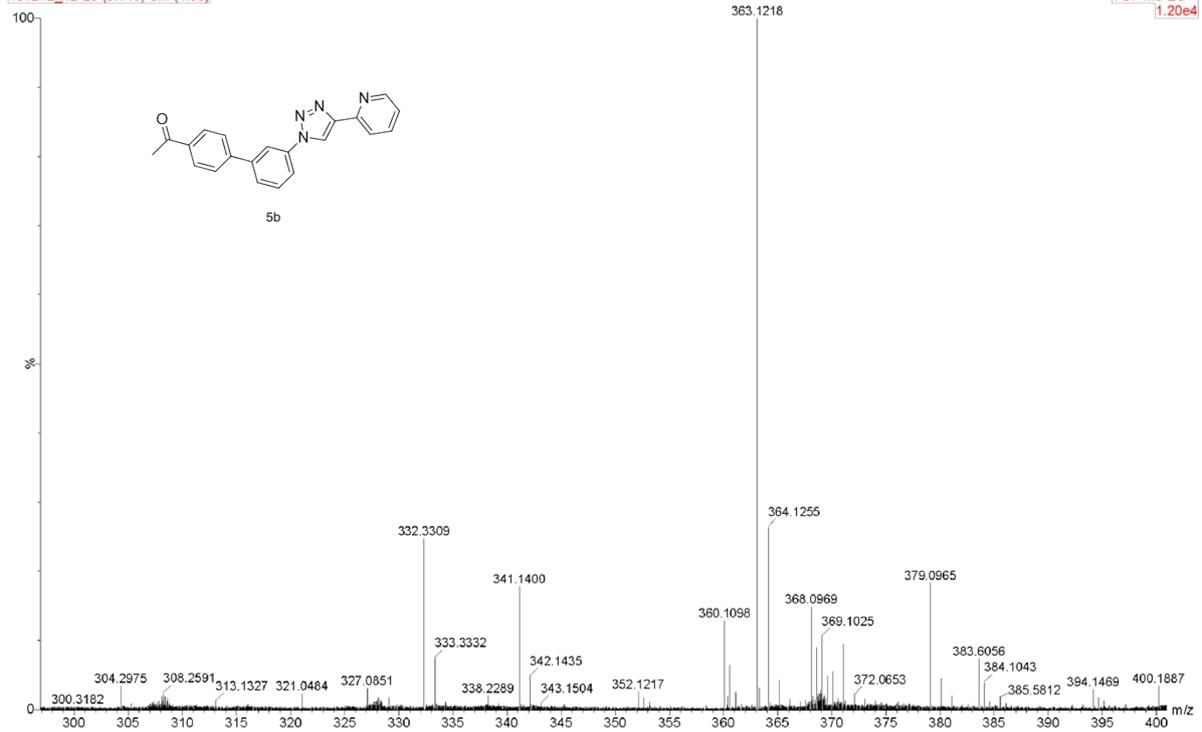


09:18:31

181212_4B 26 (0.445) Cm (1:30)

13-Dec-2018

TOF MS ES+
1.20e4

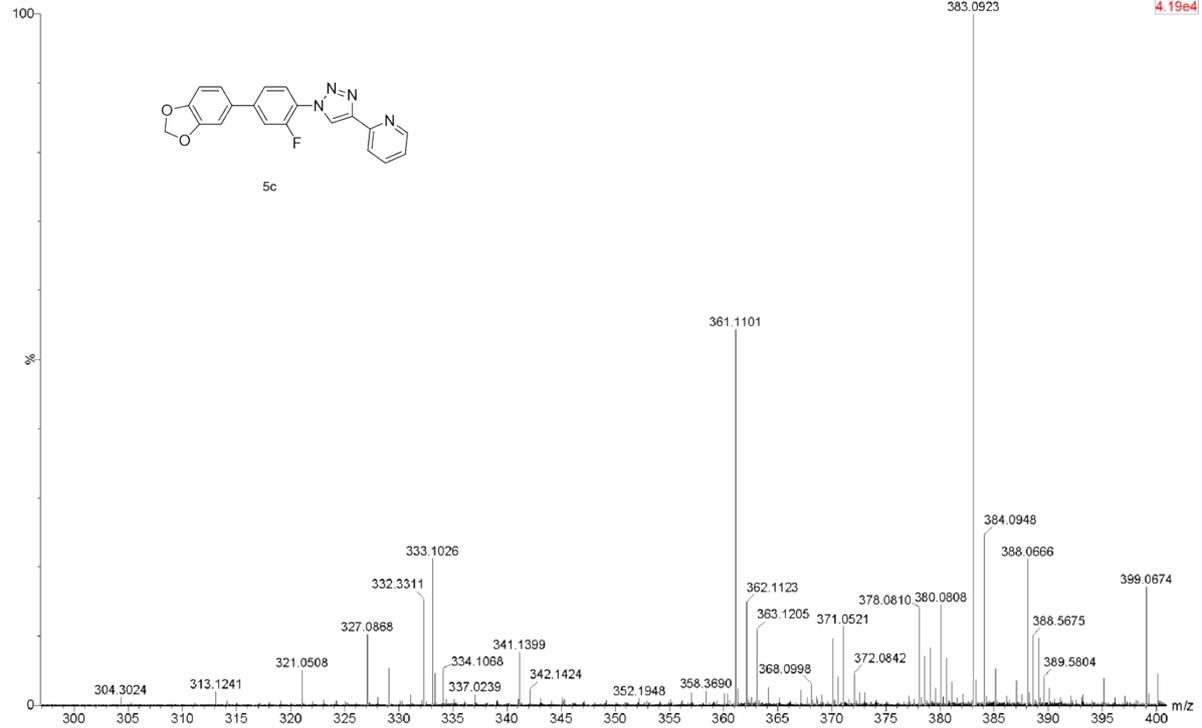


09:20:52

181212_4C 28 (0.479) Cm (1:30)

13-Dec-2018

TOF MS ES+
4.19e4

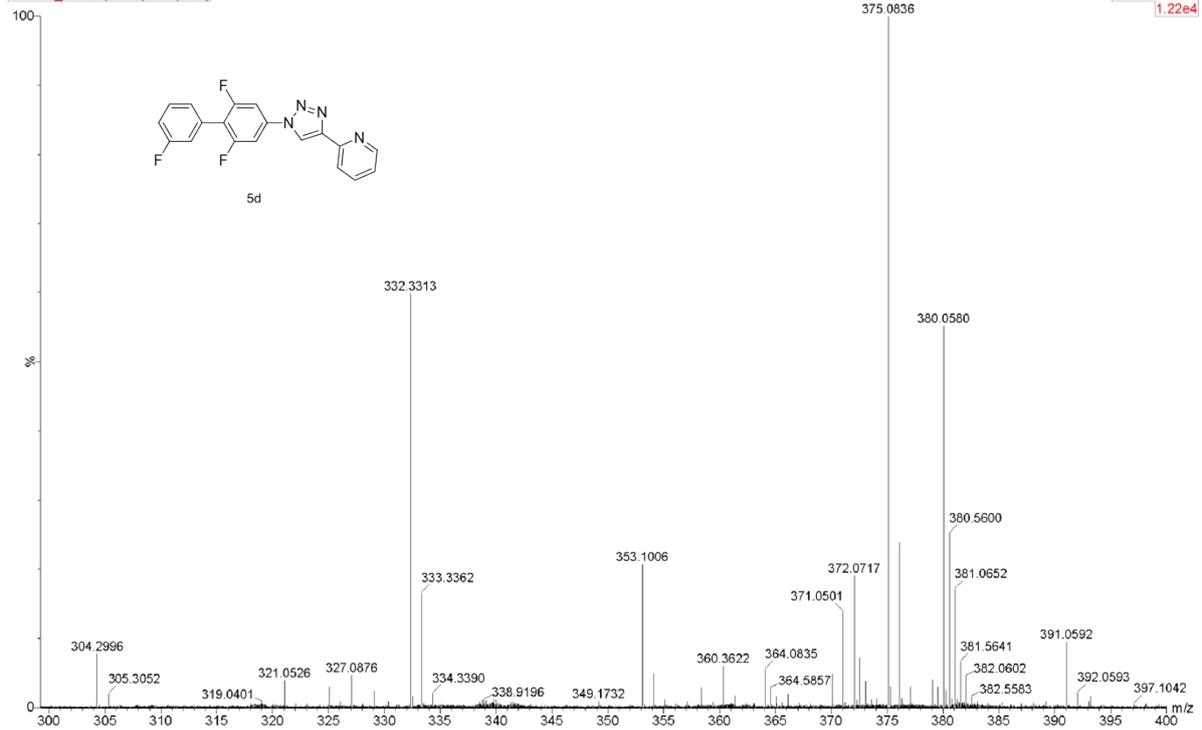


09:23:56

181212_4D 27 (0.462) Cm (1:30)

13-Dec-2018

TOF MS ES+
1.22e4

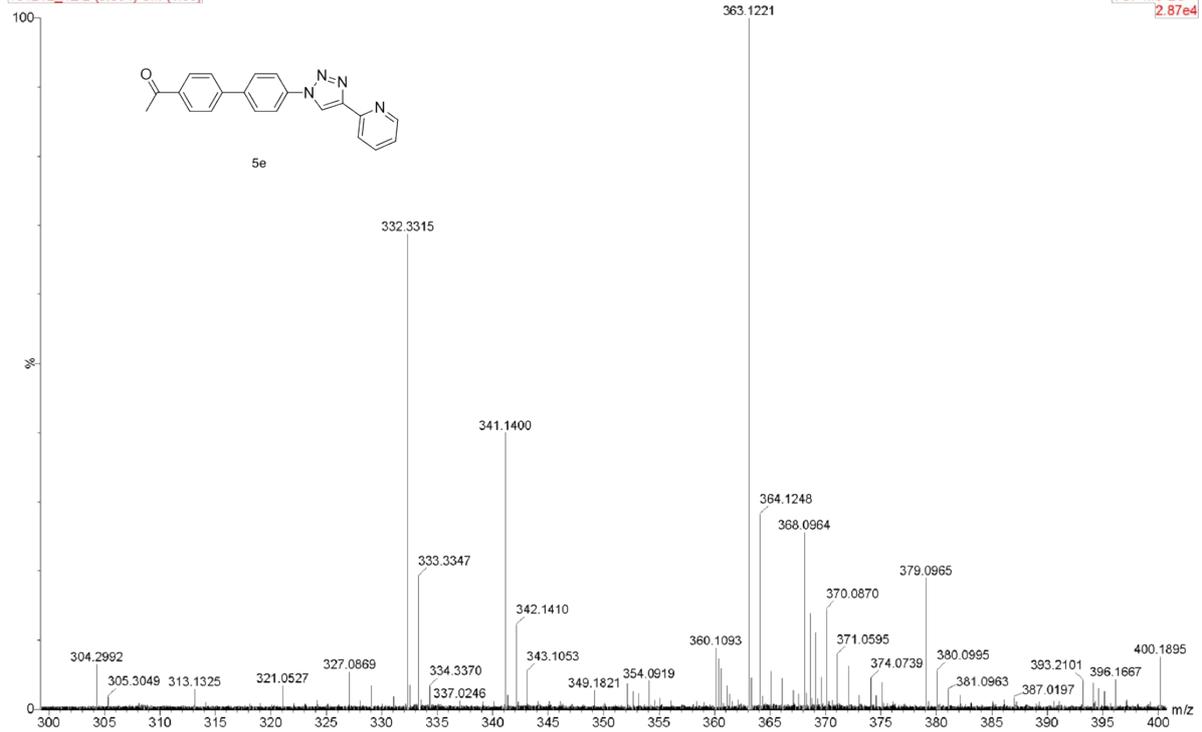


09:27:24

181212_4E 2 (0.034) Cm (1:30)

13-Dec-2018

TOF MS ES+
2.87e4

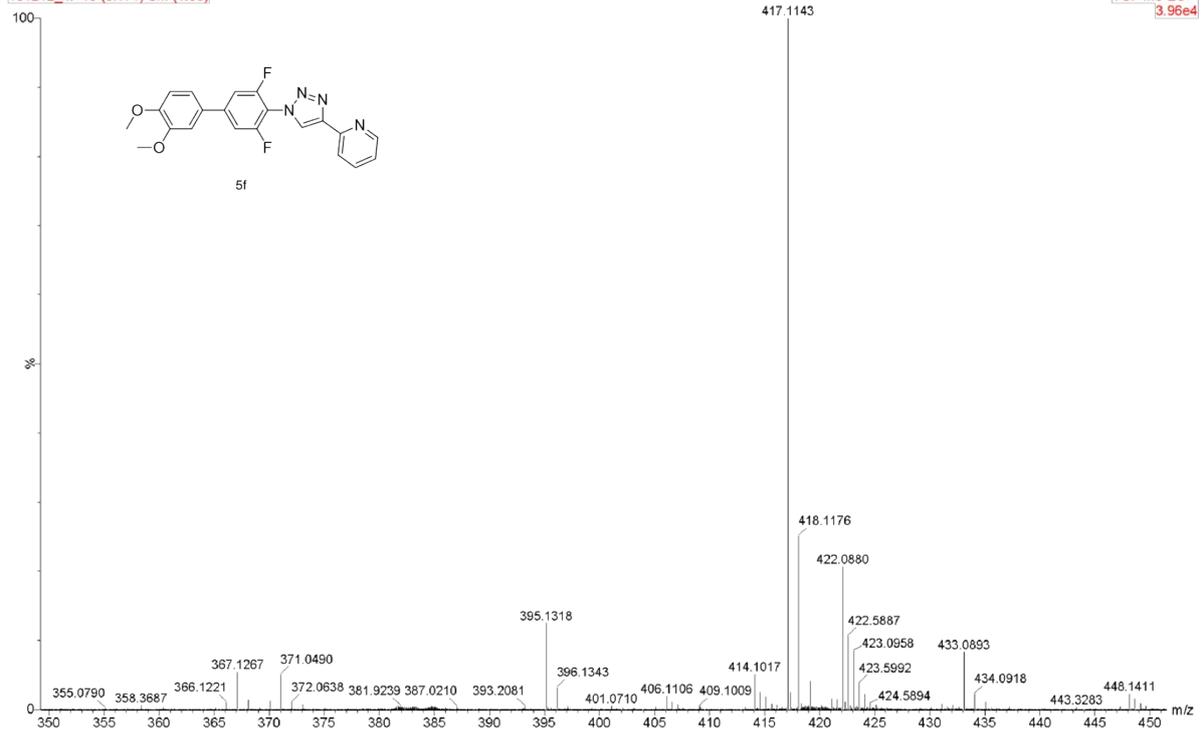


09:29:08

181212_4F 10 (0.171) Cm (1:30)

13-Dec-2018

TOF MS ES+
3.96e4

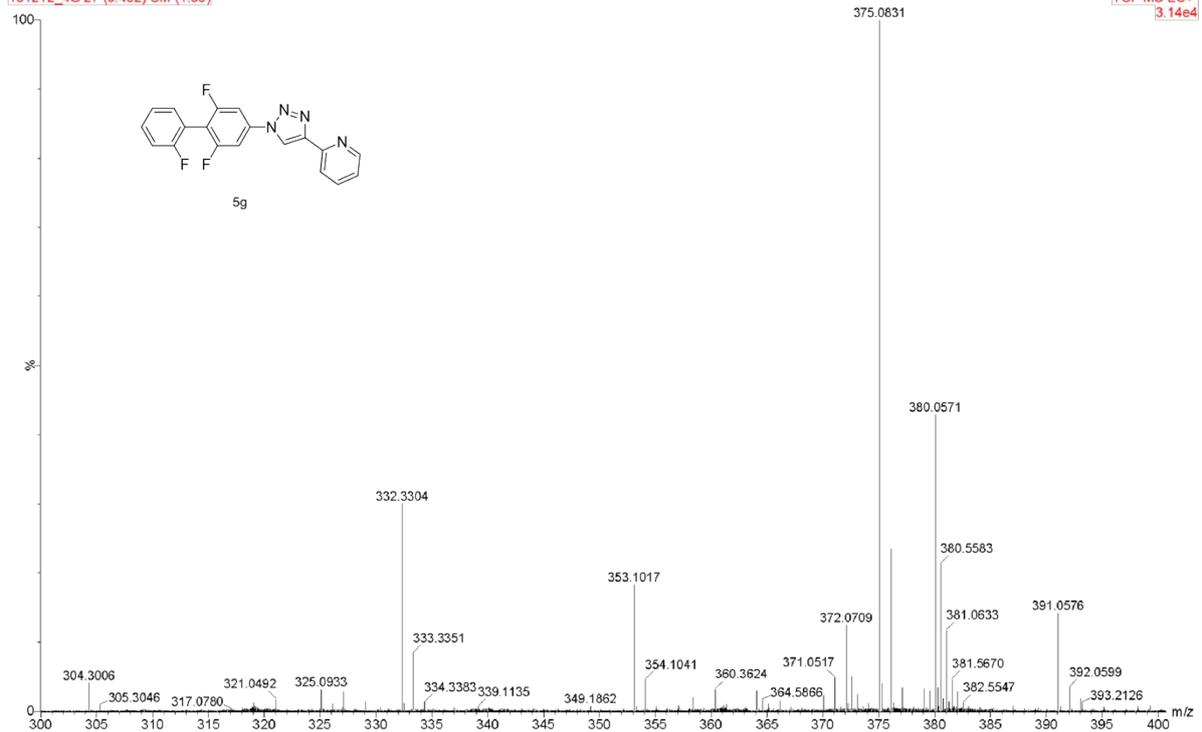


09:31:25

181212_4G 27 (0.462) Cm (1:30)

13-Dec-2018

TOF MS ES+
3.14e4

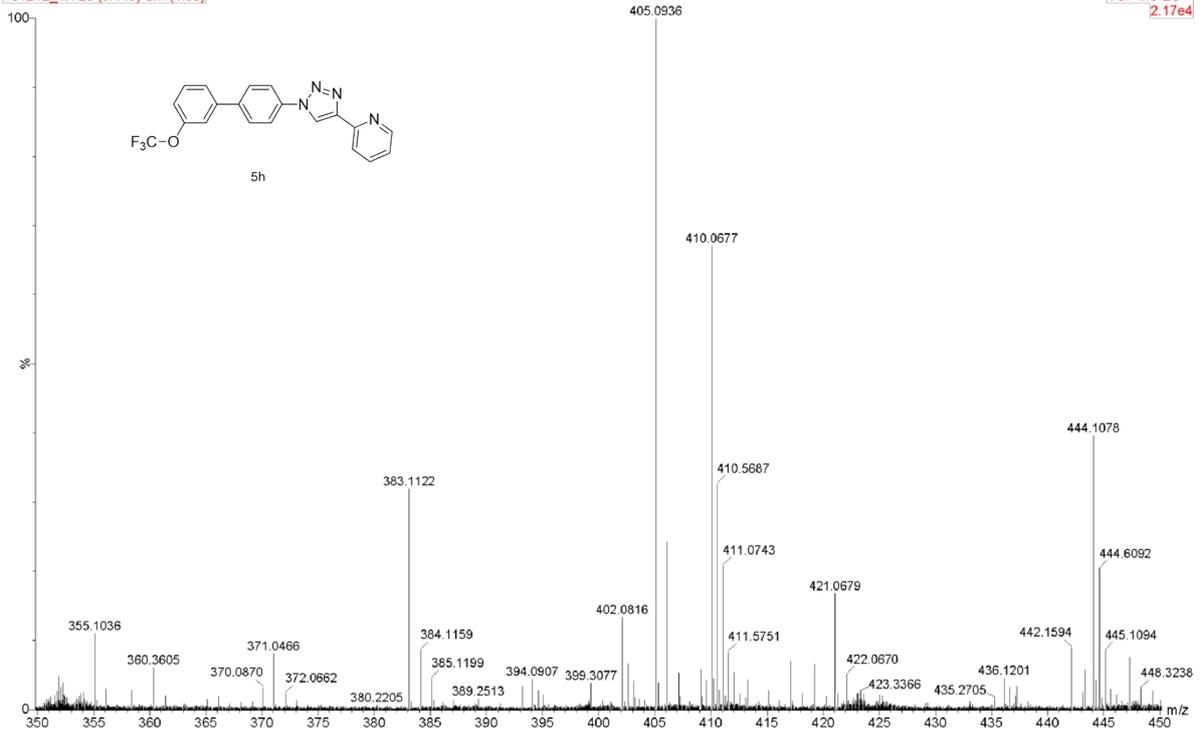


09:33:24

181212_4H 26 (0.445) Cm (1:30)

13-Dec-2018

TOF MS ES+
2.17e4

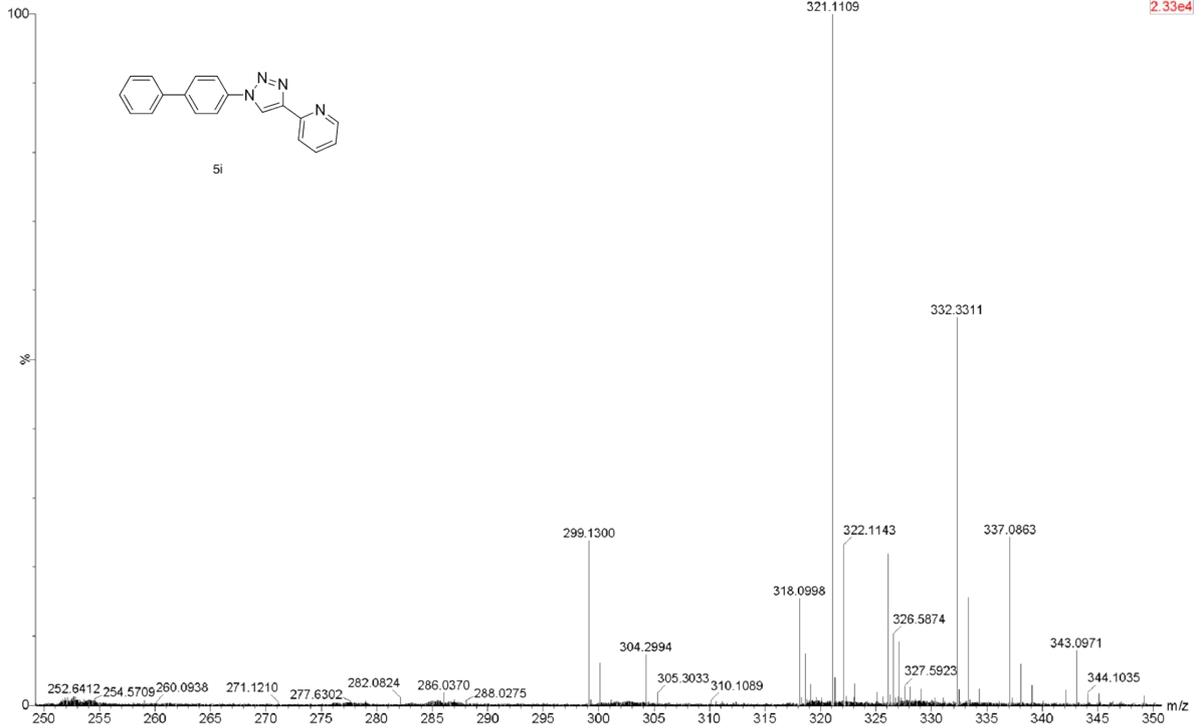


09:35:14

181212_4I 25 (0.428) Cm (1:30)

13-Dec-2018

TOF MS ES+
2.33e4



Reference

1. P. C. Patil, F. A. Luzzio, J. M. Ronnebaum, *Tetrahadron. Lett.*, 2017, **58**, 3730-3733
2. T. M. Kim, J. M. Keum, I. S. Oh, *J. Biotechnol.*, 2006, **126**, 554-561.
3. K. V. Wood, *Promega Notes*, 1990, **28**, 1-3.
4. Z. Eyal, D. Matzov, M. Krupkin, I. Wekselman, S. Paukner, E. Zimmerman, H. Rozenberg, A. Bashan, A. Yonath. *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, E5805-14.
5. R. W. Murray, E. P. Melchior, J. C. Hagadorn, K. R. Marotti., *Antimicrob. Agents. Chemother.*, 2001, **45**, 1900-1904.