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Supplementary information

Concurrent modifications of the C- terminus and side ring of thiostrepton and their synergistic effects with respect to improving antibacterial activities

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1. Supplementary Methods

1.1. General materials and methods

Biochemicals and media were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) unless otherwise stated. Phanta Super-Fidelity DNA Polymerases (cat. no. P501-d1/d2/d3) and Rapid T4 DNA Ligase (cat. no. N103-01) used for PCRs and molecular cloning were purchased from Vazyme Biotech Co.,Ltd (Nanjing, China). Restriction endonucleases were purchased from Thermo Fisher Scientific Co. Ltd. (USA). The synthesis of primers was performed at Shanghai Sangon Biotech Co. Ltd. (China). The DNA sequencing was conducted at Shanghai Majorbio Biotech Co. Ltd. or Shenzhen BGI in China. Bacterial strains and plasmids used in this study are summarized in Table S7. Primer sequences are listed in Table S8.

All commercially available reagents and solvents were used without further purification unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on DC-Alufolien Kieselgel $60F_{254}$ 0.2 mm plates (Merk) and compounds were visualized by UV fluorescence. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded on a Bruker AC-500 or Bruker AC-600 spectrometer, using deuterated solvents and were referenced internally to the residual solvent peak signal. Couple constants (*J*-values) are given in hertzs (Hz). NMR spectra assignation was aided by comparison with literature values for similar compounds. In this experimental section only clear identifiable peaks are assigned. High performance liquid chromatography (HPLC) analysis was carried out on an Agilent^{TM} 1260 HPLC system (Aglient Technologies Inc., USA). Electrospray ionization MS (ESI-MS) was performed on a Thermo Fisher LTQ Fleet ESI-MS spectrometer (Thermo Fisher Scientific Inc., USA). High resolution-MS (HRMS) analysis was carried out on an IonSpec 4.7 Tesla FTMS (IonSpec, Lake Forest, CA).

1.2. Construction of double mutant strain *S. laurentii* Δ*tsrB/T*

Cosmid pSL1001 served as the template for PCR amplification. A 2.19 kb fragment obtained by using the primers pTLf: 5’-CG GAA TTC GCG GTG ATC GAG TGG GTA CGG-3’ (*Eco*RI site underlined) and pTLr: 5’-GC TCT AGA GGG TGG ATC

TGG TTG GGG TTG-3' (*Xba*I site underlined) and a 2.18 kb fragment obtained by using the primers pTRf: 5'-GC TCT AGA GGG ATA CAC GGG CGC TTT CTG-3' (*Xba*I site underlined) and pTRr: C CCC AAG CTT CCC GTA CGT ATC TGG ACC ACC-3' (*Hin*dIII site underlined) were initially cloned into the pMD19-T vector, giving pSL12011 and pSL12012, respectively. After sequencing to confirm the fidelity, the 2.19 kb *Eco*RI/*Xba*I and 2.18 kb *Xba*I/*Hin*dIII fragments were recovered and then co-ligated into the *Eco*RI/*Hin*dIII site of pKC1139, yielding the recombinant plasmid pSL1201, in which a 1650 bp in-frame coding region (corresponding to AA_8-AA_{567} of the deduced product TsrT) of *tsrT* was deleted.

Introduction of pSL1201 into *S. laurentii* SL1051 was carried out by *E. coli-Streptomyces* conjugation, following the procedure described previously [1]. The apramycin-resistant colonies were subjected to a double-crossover recombination event, leading to the generation of the recombinant strains SL1201 (*ΔtsrB/T*). The genotype for each mutation was validated by PCR amplification by using primes pTf: 5'-CGG GCG GCT GCT GCT CTT CGG-3' and pTr: 5'-ATC CGC CAC CGC CAC GGC GAC-3' (Fig. S3).

1.3. Fermentation, chemical feeding, compound analysis and isolation

Fermentation. The Δ*tsrB/T* mutant was grown in flask containing seed medium [15 g L^{-1}solube starch, 15 g L^{-1} tryptic soy broth, 50 g L^{-1} sucrose, pH 7.2] (50 mL) for 2 days at 28 °C and 220 rpm. The seed culture (5 mL each) was used to inoculate ten flasks (500 mL) containing fermentation medium [15g L^{-1} tryptic soy broth, 15 g L^{-1} $CaSO_4$, 11 g L^{-1} yeast extra, 50 g L^{-1} glucose, pH 7.2] (100 mL). To produce CmTSR and its derivatives, the medium was fed with methyl 4-acetyl-quinoline-2-carboxylate and its analogues (2 mg dissolved in 1 mL DMSO) and incubated at 28 °C and 220 rpm for 3 days.

Compound analysis. After three days of incubation, the cultures were centrifuged and the mycelia cake was soaked with acetone overnight. The acetone was evaporated using rotary evaporator and the product was dissolved in chloroform. Samples were analyzed by HPLC with a

ZORBAX SB-C18 column (250 x 4.6 mm, 5 μm). The column was developed using solvents A (water) and B (acetonitrile) at a flow rate of 1 mL min^{-1} as follows: the mobile phase was developed with 15% solvent B for 3 min and then increased from 15% to 40% solvent B over 3 min, then held constant at 40% solvent B for 6 min, then increased from 40% to 55% solvent B over 7 min, the increased from 55% to 85% solvent B over 3 min, then held constant at 85% solvent B for 6 min, and finally decreased from 85% to 15% solvent B over 2 min. Absorbance was monitored at 254 nm.

Compound isolation. To obtain purified compound, the crude metabolites were subjected to a silica gel column and eluted with 100% $CHCl_3$ followed by $CHCl_3$-MeOH (100:1 to 100:10). Fractions containing CmTSR analogues were pooled and the organic solvent was evaporated to give crude products for further purification. For purification of CmTSR, the column was developed using solvents A (water) and B (acetonitrile) at a flow rate of 3 mL min-1 as follows: the mobile phase was developed with 15% solvent B for 3 min and then increased from 15% to 40 % solvent B over 3 min, then held constant at 40% solvent B for 6 min, then increased from 40% to 55 % solvent B over 7 min, the increased from 55% to 85% solvent B over 3 min, then held constant at 85% solvent B for 6 min, and finally decreased from 85% to 15% solvent B over 2 min. Purified samples were analyzed by HPLC-MS, HRMS and NMR and were stored at -20 °C.

1.4. Antibacterial activity of CmTSRs

The minimum inhibitory concentrations (MICs) were measured by broth dilution using a modified method described previously [3]. Each tested compound was dissolved in DMSO to produce a stock solution (100 μg/ml), which was serially diluted into 100 μL of Mueller-Hinton broth (Qingdao Hope Bio-Technology Co. Ltd., China) in a 96-well micro-titer plate to a final concentration ranging from 1 to 0 μg/ml. 100 μL of the testing strain (10^7-10^8 cfu/ml, calculated according to the 0.5 McFarland standard (McFarland, 1907)) was then added into each well of the

microtiter plate. After incubation at 37℃ for 18-24 hr, the MIC was determined to be the lowest concentration of compound that inhibited visible bacterial growth. For the determination of MICs of CmTSRs against *Clostridium difficile*, the bioassays were carried out by using a modified method described previously [4]. The manipulations and interpretations were in accordance with the descriptions of the Clinical and Laboratory Standard Institute (CLSI 2011) [5].

1.5. Synthesis of methyl quinoline-2-carboxylate and its analogues

Quinoline-2-carboxylic acid (10.0 mmol) was dissolved in the dry MeOH and placed in an ice-bath. $SOCl_2$ (0.92 mL, 12.0 mmol) was then added dropwise and the corresponding mixture refluxed overnight until the starting material was undetectable upon TLC analysis. The suspension cooling to room temperature was carefully poured into the aqueous and saturated $NaHCO_3$ solution. The resulting mixture was extracted twice with CH_2Cl_2. The organic extracts were combined, dried over anhydrous Na_2SO_4 and filtered, and the solvent was then removed by evaporation in vacuum. The resulting residue was purified by column chromatography to obtain the pure ester.

1.6. Synthesis of methyl 4-acetyl-quinoline-2-carboxylate and its analogues

$FeSO_4 \cdot 7H_2O$ (0.38 g, 1.37 mmol) was added to a solution of methyl quinoline-2-carboxylate (10 mmol) in TFA (0.97 mL) and acetaldehyde (50 mL) and the resulting mixture was cooled to 0 ℃. A first portion of 30% aqueous solution of H_2O_2 (5.5 mL) was slowly added dropwise and the mixture had been stirred for 30 min. And then, a second portion of 30% aqueous H_2O_2 (5.5 mL) was slowly added dropwise and the resulting mixture had been stirred for additional 90 min. After warming to 25 ℃, the reaction mixture was slowly quenched with 5% aqueous $Na_2S_2O_3$ solution (50 mL) followed by addition of saturated aqueous $NaHCO_3$ solution. The aqueous layer was

extracted with EtOAc and the combined organic layers were then washed with brine, dried over anhydrous Na_2SO_4, and filtered, and the solvent was then removed by evaporation in vacuum. The resulting residue was purified by column chromatography to obtain the pure product。

1.7. Synthesis of methyl 4-hydroxymethyl-quinoline-2-carboxylate

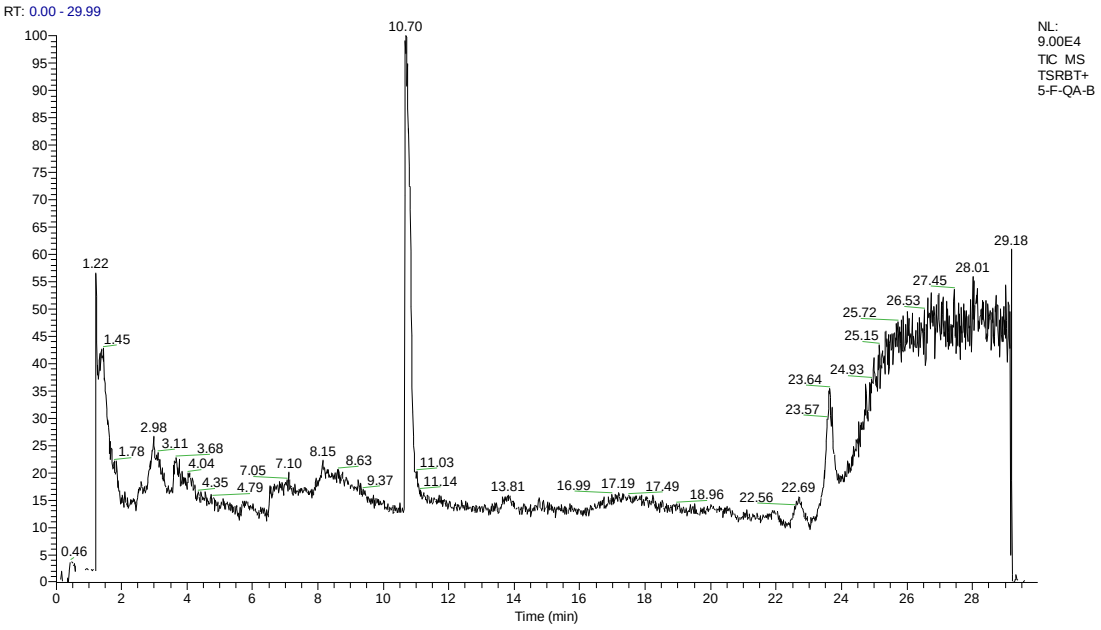
TFA was added carefully to a solution of $FeSO_4 \bullet 7H_2O$ and Methyl quinolone-2-carboxylate in MeOH and the solution brought to reflux. Aquenous H_2O_2 was carefully added over a period of 30 min to give a pale-brown solution which was heated at reflux for further 15 h. The solution was left to cool to room temperature and then concentrated in vacuo. The residue was diluted with water and extracted with EtOAc. The organic extracts were pooled, dried, filtered and the solvent evaporated in vacuo to give dark-brown oil. The product was obtained by chromatography on silica gel, eluting with EtOAc: hexanes.

2. Supplementary Results

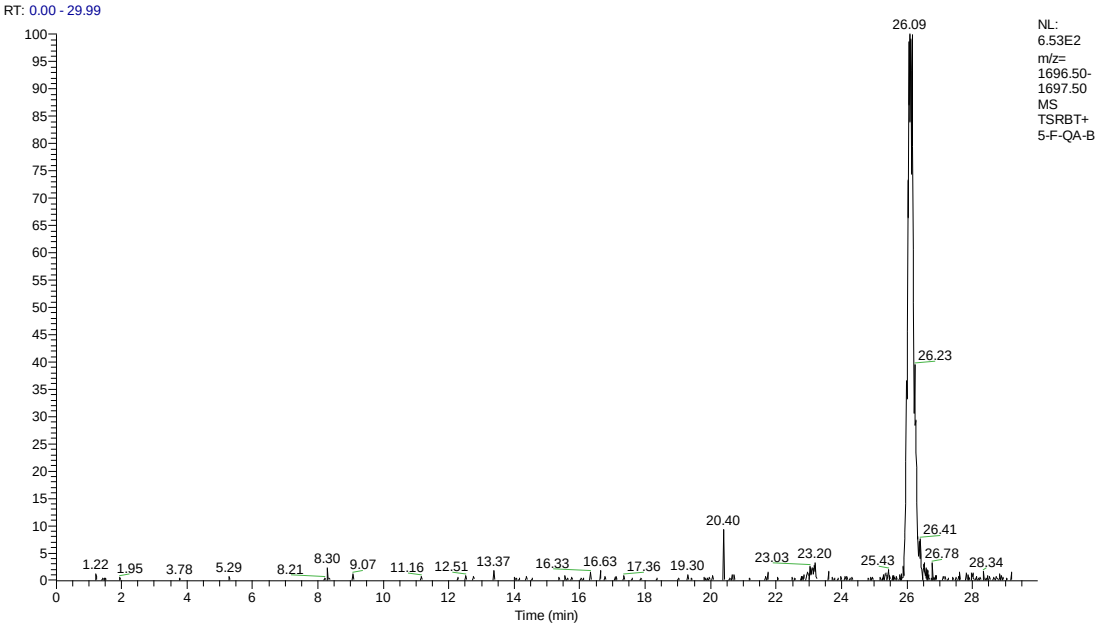
2.1. Characterization of 5’-F-CmTSR

HPLC-MS analysis of culture extracts from *S. laurentii* △*tsrB/T* mutant strains fed with **1**.

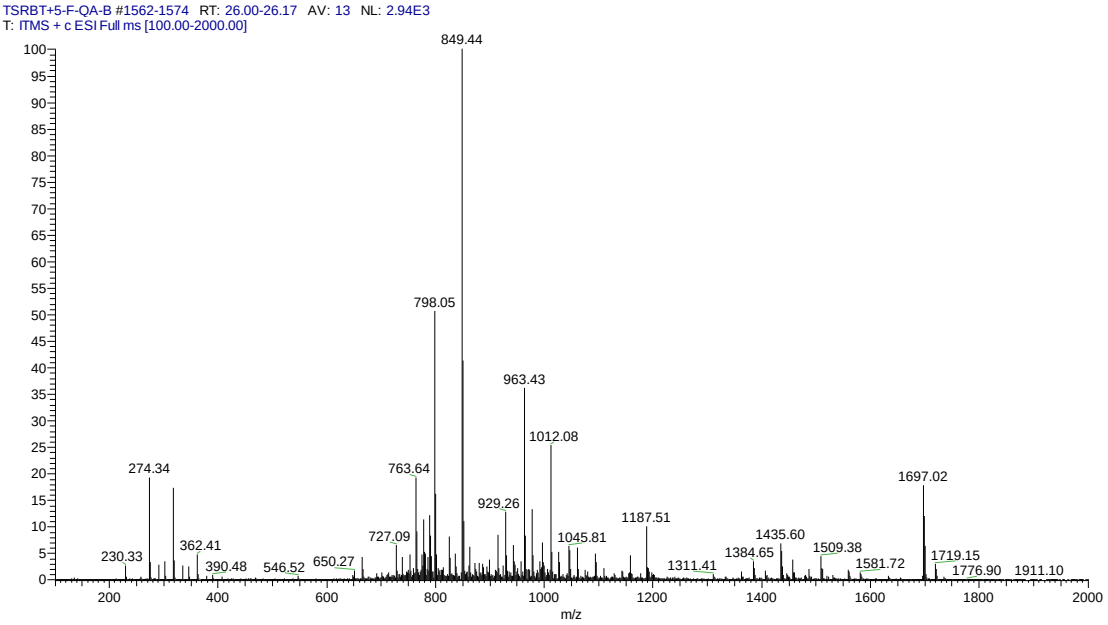
(1) Total ion chromatogram.



(2) Chromatogram extracted for m/z 1697.



(3) Mass spectrum of 5'-F-CmTSR from tsrBT mutant extract eluting at t_R = 26.10 min (calculated m/z 1697.49 [M+H]+, observed m/z 1697.02 [M+H]+).



Under the conditions used for HPLC-MS, **5'-F-CmTSR** elutes at a t_R of about 26.10 min providing ions at m/z 1697.02 $[M + H]^+$ and m/z 849.44 $[M + 2H]^{2+}$. The predominant ion for **5'-F-CmTSR** was $[M + 2H]^{2+}$, whereas $[M + H]^+$ was only a minor species.

^{1}H NMR (600 MHz, $CDCl_3$): 8.24(s, 1H), 8.23(s, 1H), 8.03(s, 1H), 7.52(s, 1H), 7.42(s, 1H), 6.75(d, *J* = 2.0 Hz, 1H), 6.66(s, 1H), 6.35(q, *J* = 7.2 Hz, 1H), 6.17(q, *J* = 7.2 Hz, 1H), 6.01(s, 1H), 5.85(m, 1H), 5.78(d, *J* = 9.8 Hz, 1H), 5.76(d, *J* = 9.0 Hz, 1H), 5.75(s, 1H), 5.61(q, *J* = 7.2 Hz, 1H), 5.51(s, 1H), 5.27(br, 1H), 5.18(s,1H), 4.95(dd, *J* = 12.5, 8.5 Hz, 1H), 4.74(m, 1H), 4.62(d, *J* = 6.3 Hz, 1H), 4.45(dd, *J* = 7.9, 3.0 Hz, 1H), 4.06(m, 1H), 3.87(s, 3H), 3.85(m 1H), 3.81(m, 1H), 3.67(m, 1H), 3.42(m, 1H), 3.38(m, 1H), 3.10(t, *J* = 12.5 Hz, 1H), 2.90(m, 1H), 2.94(d, *J* = 8.6 Hz, 1H), 2.26(m, 1H), 1.71(d. *J* = 7.2 Hz, 3H), 1.60(d, *J* =7.2 Hz, 3H), 1.43(m, 1H), 1.40(d, *J* = 6.9 Hz, 3H), 1.31(d, *J* = 6.9 Hz, 3H), 1.30(d, *J* = 7.2 Hz, 3H), 1.21(m, 1H), 1.21(s, 3H), 1.16(d, *J* = 6.8 Hz, 3H), 1.08(m, 1H), 0.95(d, *J* = 7.2 Hz, 3H), 0.91(t, *J* = 7.5 Hz, 3H), 0.88(d, *J* = 6.9 Hz, 3H);

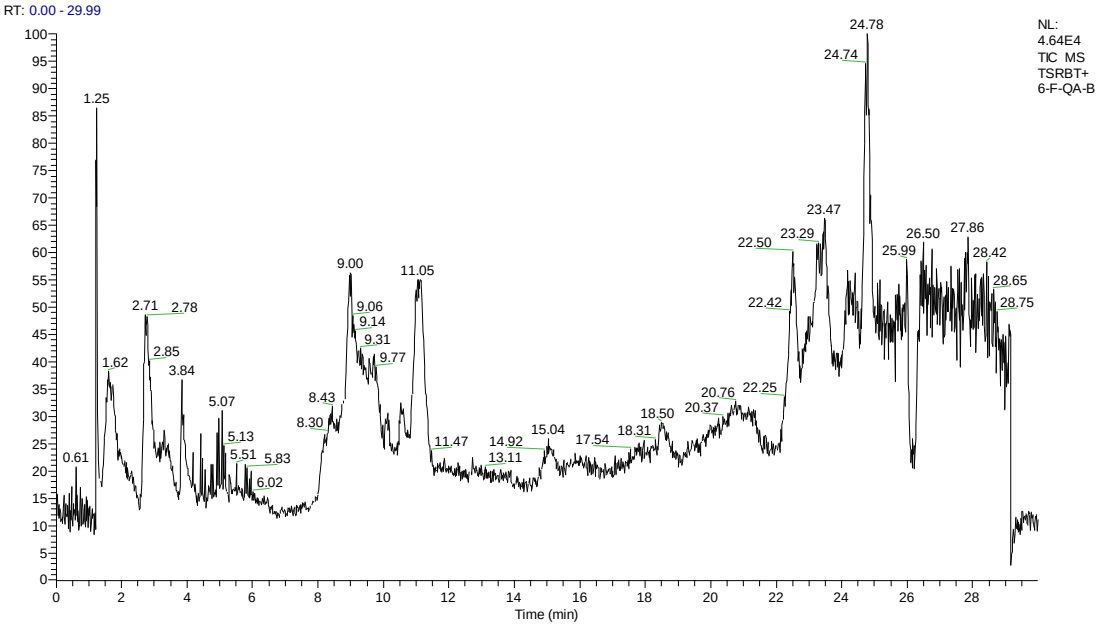
^{13}C NMR (150MHz, $CDCl_3$): 174.4, 172.9, 172.3, 170.9, 170.3, 169.6, 168.4, 168.3, 165.9, 165.5,

164.3, 163.3, 162.1, 162.0, 161.9, 161.6, 160.6, 159.5, 158.1(d, *J* = 312 Hz), 157.5, 156.4(d, *J* = 10.0 Hz), 155.6(d, *J* = 8.4 Hz), 150.4, 150.2, 146.8, 144.5, 134.3, 132.4, 132.2, 130.5, 128.7, 127.8, 125.2, 124.8, 123.3, 122.6(d, *J* = 29.1 Hz), 117.9, 109.8, 107.6(d, *J* = 21.8 Hz), 102.9, 102.3, 79.0, 77.8, 72.5, 67.5, 67.1, 67.0, 66.7, 65.5(d, *J* = 18.9 Hz), 64.4, 58.5(d, *J* = 12.8 Hz), 57.3, 55.7, 53.5, 53.3, 52.2, 48.8, 38.8, 35.5, 28.9, 25.8, 24.9, 23.3, 19.6, 19.5, 19.4, 19.0, 17.4, 16.3, 15.8, 15.7, 11.4 ; ^{19}F NMR (282 MHz, $CDCl_3$):-114.58; HRMS (m/z) $[M+H]^+$ calcd. For $C_{73}H_{86}FN_{18}O_{19}S_5$, 1697.4904; found 1697.4910.

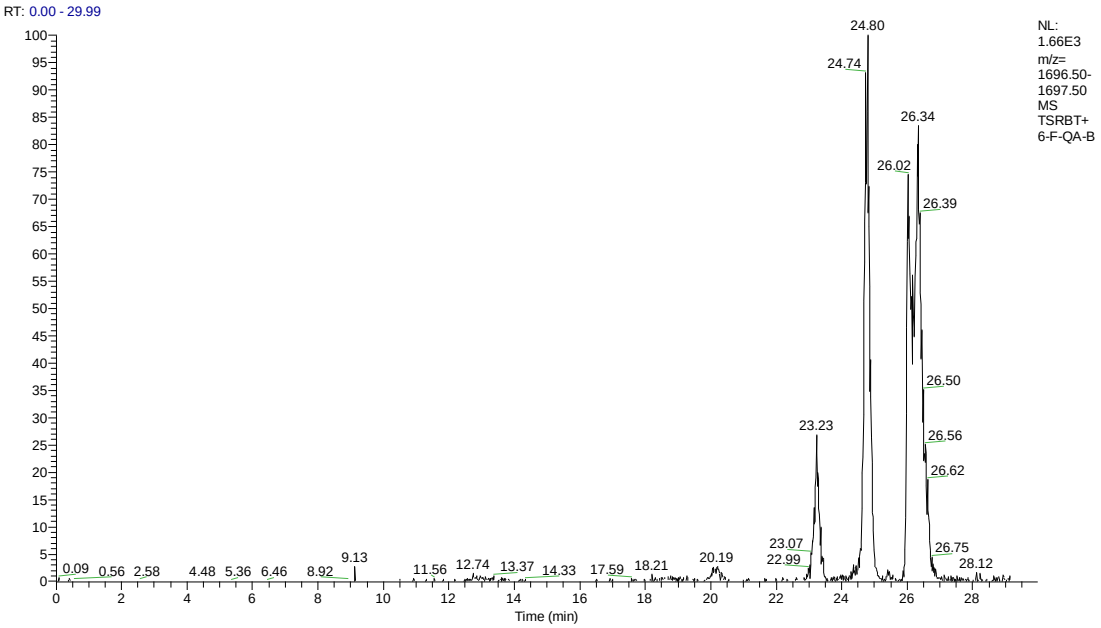
2.2. Characterization of 6’-F-CmTSR

HPLC-MS analysis of culture extracts from *S.Laurentii* tsrBT mutant strains fed with **2**.

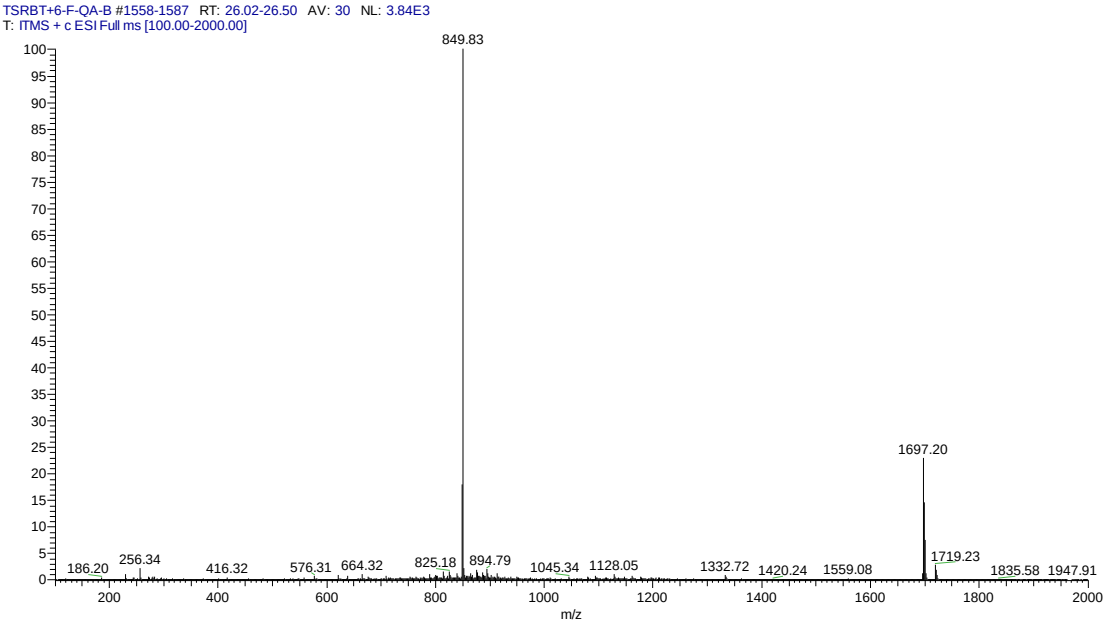
(1) Total ion chromatogram.



(2) Chromatogram extracted for m/z 1697.



(3) Mass spectrum of 6'-F-CmTSR from tsrBT mutant extract eluting at t_R = 26.30 min (calculated m/z 1697.49 [M+H]+, observed m/z 1697.20 [M+H]+).



Under the conditions used for HPLC-MS, **6'-F-CmTSR** elutes at a t_R of about 26.30 min providing ions at m/z 1697.20 $[M + H]^+$ and m/z 849.83 $[M + 2H]^{2+}$. The predominant ion for **6'-F-CmTSR** was $[M + 2H]^{2+}$, whereas $[M + H]^+$ was only a minor species.

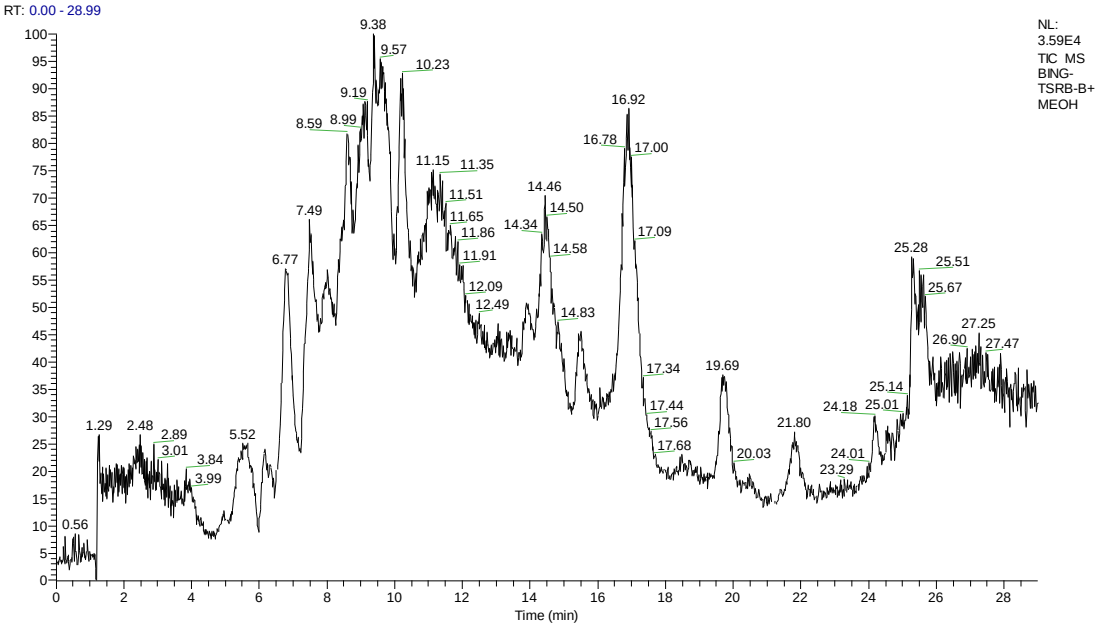
^{1}H NMR (500 MHz, $CDCl_3$): 8.29(s, 1H), 8.28(s, 1H), 8.11(s, 1H), 7.52(s, 1H), 7.47(s, 1H), 6.90(d, *J* = 13.5 Hz, 1H), 6.80(d, *J* = 1.5 Hz, 1H), 6.74(s, 1H), 6.40(m, 1H), 6.22(q, *J* = 7.2 Hz, 1H), 6.07(s, 1H), 5.84(d, *J* = 8.6 Hz, 1H), 5.78(d, *J* = 8.6Hz, 1H), 5.75(d, *J* = 2.0 Hz, 1H), 5.52(d, *J* = 1.5 Hz, 1H), 5.22(m, 1H), 5.15(q, *J* = 7.0 Hz, 1H), 5.11(br, 1H), 4.98(dd, *J* = 12.1, 6.5 Hz, 1H), 4.76(m, 1H), 4.75(m, 1H), 4.46(dd, *J* =8.1, 2.5Hz, 1H), 4.10(m, 1H), 3.92(s, 3H), 3.86(m, 1H), 3.82(m, 1H), 3.72(dd, *J* = 8.9, 3.6 Hz, 1H), 3.68(d, *J* = 7.5 Hz, 1H), 3.45(m, 1H), 3.13(dd, *J* = 8.3, 4.0 Hz, 1H), 3.02(d, *J* = 8.4 Hz, 1H), 2.94(m, 1H), 2.28(m, 1H), 1.75(d. *J* = 7.6 Hz, 3H), 1.69(m, 1H), 1.64(d, *J* = 7.2Hz, 3H), 1.50(m, 1H), 1.47(d, *J* = 7.0 Hz, 3H), 1.37(d, *J* = 7.2 Hz, 3H), 1.34(d, *J* = 7.0 Hz, 3H), 1.25(m, 1H), 1.20(d, *J* = 7.0Hz, 3H), 1.19(s, 3H), 1.13(m, 1H), 1.00(d, *J* = 7.2 Hz,

3H), 0.97(t, *J* = 7.2 Hz, 3H), 0.92(d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 174.4, 173.0, 172.5, 171.2, 170.2, 169.6, 168.5, 168.0, 165.9, 165.5, 164.4, 163.4(d, *J* = 274.1 Hz), 163.3, 162.2, 162.0, 161.8, 161.5, 160.9, 159.5, 157.6, 153.9(d, *J* = 8.9 Hz), 152.1, 150.6, 150.4, 146.9, 142.9, 134.5, 132.5, 132.3, 130.6, 128.8, 127.7, 127.2(d, *J* = 12.8 Hz), 125.2, 125.0, 122.3, 117.8, 109.7, 102.7, 101.2, 100.9(d, *J* = 22.5 Hz), 79.0, 78.0, 72.3, 68.1(d, *J* = 12.8 Hz), 67.1, 67.5, 66.5, 64.6, 64.5, 62.0(d, *J* = 27.5 Hz), 57.4, 55.8, 55.7, 53.4, 53.3, 52.4, 48.8, 38.9, 35.7, 29.0, 26.2, 24.9, 22.9, 19.8, 19.6, 19.5, 18.9, 17.4, 16.2, 15.9, 15.6, 11.3, ; ^{19}F NMR (282 MHz, $CDCl_3$):-101.48; HRMS (m/z) $[M+H]^+$ calcd. For $C_{73}H_{86}FN_{18}O_{19}S_5$, 1697.4904; found 1697.4909.

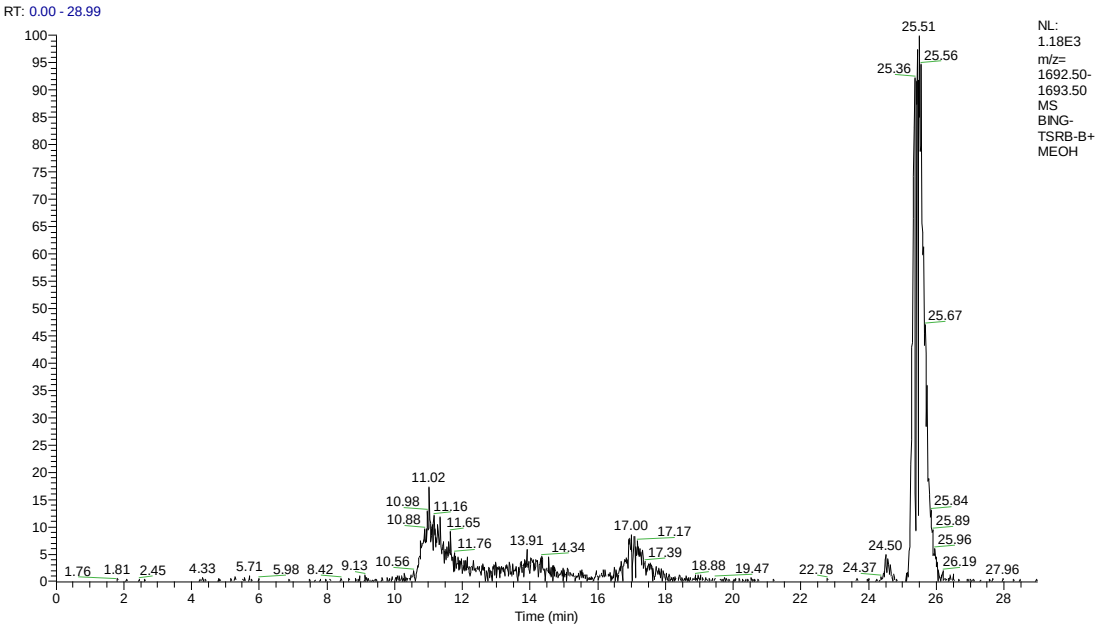
2.3. Characterization of 12’-Me-CmTSR

HPLC-MS analysis of culture extracts from *S. laurentii* △*tsrB/T* mutant strains fed with **3**.

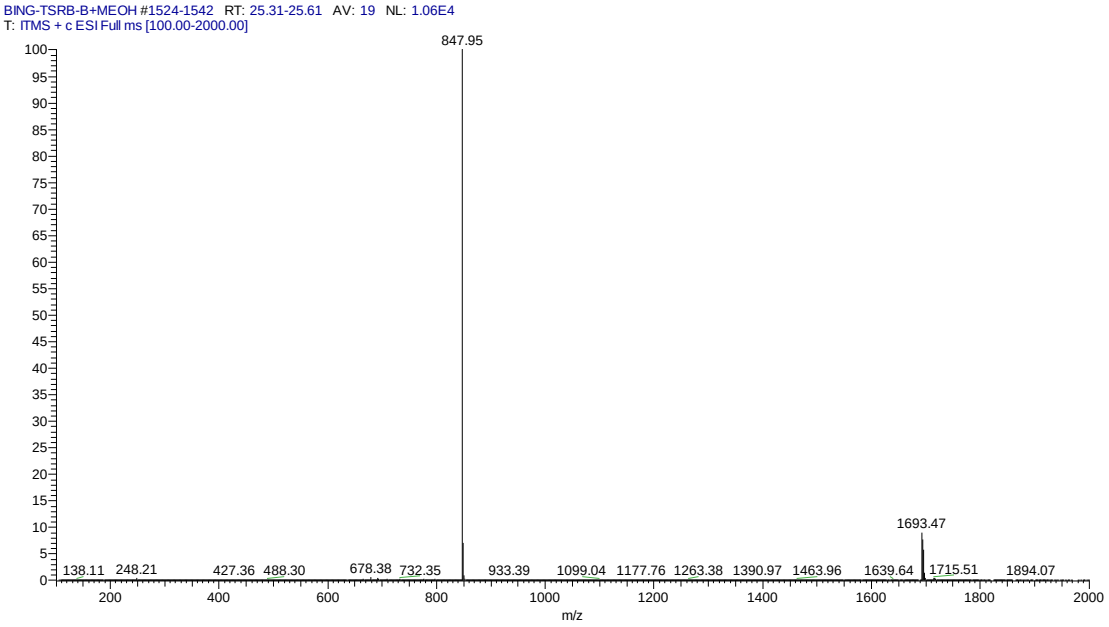
(1) Total ion chromatogram.



(2) Chromatogram extracted for m/z 1693.



(3) Mass spectrum of 12'-Me-CmTSR from tsrBT mutant extract eluting at t_R = 25.50 min (calculated m/z 1693.52 [M+H]+, observed m/z 1693.47 [M+H]+).



Under the conditions used for HPLC-MS, **12'-Me-CmTSR** elutes at a t_R of about 25.50 min providing ions at m/z 1693.47 $[M + H]^+$ and m/z 847.95 $[M + 2H]^{2+}$. The predominant ion for **12'-Me-CmTSR** was $[M + 2H]^{2+}$, whereas $[M + H]^+$ was only a minor species.

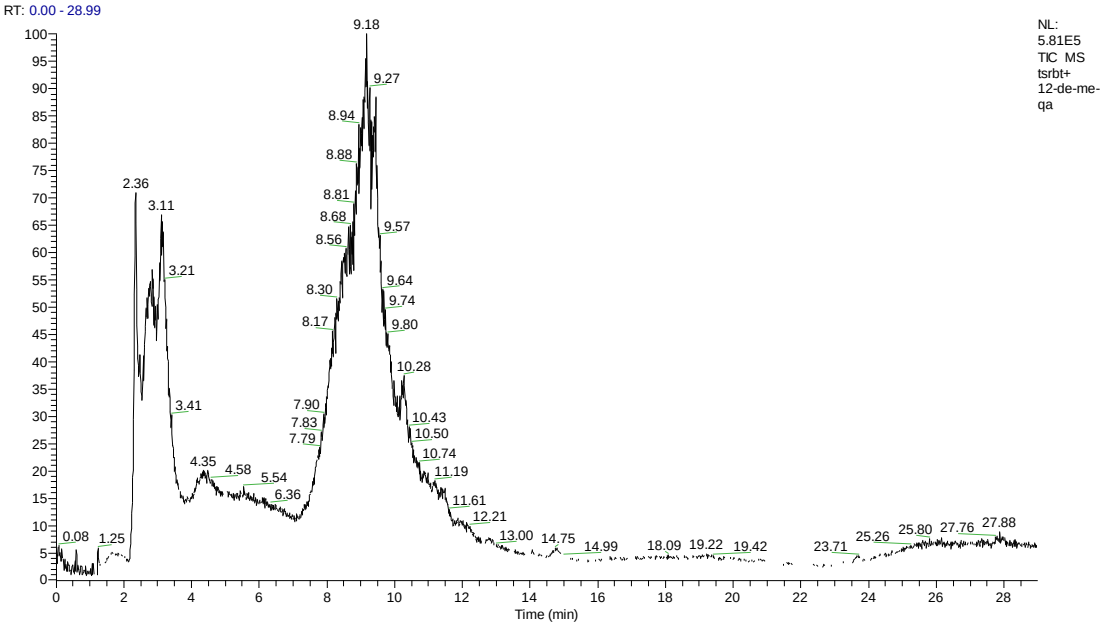
^{1}H NMR (500 MHz, $CDCl_3$): 8.28(s, 1H), 8.25(s, 1H), 8.15(s, 1H), 7.46(s, 1H), 7.29(s, 1H), 6.84(d, *J* = 8.4 Hz, 1H), 6.80(d, *J* = 2.0 Hz, 1H), 6.74(s, 1H), 6.40(m, 1H), 6.30(m, 1H), 6.20(q, *J* = 7.5 Hz, 1H), 6.07(s, 1H), 5.86(d, *J* = 9.0 Hz, 1H), 5.76(d, *J* = 9.6 Hz, 1H), 5.76(s, 1H), 5.52(s, 1H), 5.22(br, 1H), 5.12(s, 1H), 5.09 (t, *J* = 6.6 Hz, 1H), 4.95(dd, *J* = 12., 9.0 Hz, 1H), 4.78(m, 1H), 4.69(d, *J* = 7.0 Hz, 1H), 4.47(dd, *J* = 8.0, 2.7 Hz, 1H), 4.13(m, 1H), 3.93(s, 3H), 3.85(m, 1H), 3.82(m, 1H), 3.72(t, *J* = 12.0, 1H), 3.62(d, *J* = 7.2 Hz, 1H), 3.50(m, 1H), 3.15(t, *J* = 12.0 Hz, 1H), 2.99(d, *J* = 6.1 Hz, 1H), 2.95(m, 1H), 2.26(m, 1H), 1.75(d. *J* = 7.5 Hz, 3H), 1.60(m, 1H), 1.63(d, *J* = 7.5 Hz, 3H), 1.59(m, 2H), 1.47(m, 1H), 1.46(d, *J* = 7.2 Hz, 3H), 1.37(d, *J* = 7.2 Hz, 3H), 1.25(m, 1H), 1.20(d, *J* = 7.0 Hz, 3H), 1.19(s, 3H), 1.10(m, 1H), 1.05(t, *J* = 6.3 Hz, 3H), 1.02(d, *J* = 7.9 Hz, 3H), 0.96(t, *J* = 7.2 Hz, 3H), 0.90(d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$):

174.9, 173.0, 172.4, 171.1, 170.3, 169.5, 168.5, 168.1, 165.9, 165.6, 164.4, 163.5, 162.2, 162.1, 161.7, 161.5, 161.1, 159.5, 157.6, 154.3, 153.5, 150.6, 150.4, 146.9, 143.3, 134.5, 132.6, 132.2, 130.5, 129.6, 128.8, 127.8, 127.7, 125.1, 123.8, 123.1, 117.8, 109.7, 102.7, 101.4, 79.0, 77.9, 72.2, 69.4, 67.5, 67.4, 67.1, 66.9, 64.5, 59.8, 57.4, 55.8, 53.5, 53.3, 52.4, 48.9, 38.9, 35.7, 31.1, 29.0, 26.2, 24.9, 19.8, 19.5, 19.0, 17.3, 16.1, 15.9, 15.6, 11.3, 10.4 ; HRMS (m/z) $[M+H]^+$ calcd. For $C_{74}H_{89}N_{18}O_{19}S_5$, 1693.5155; found 1693.5159.

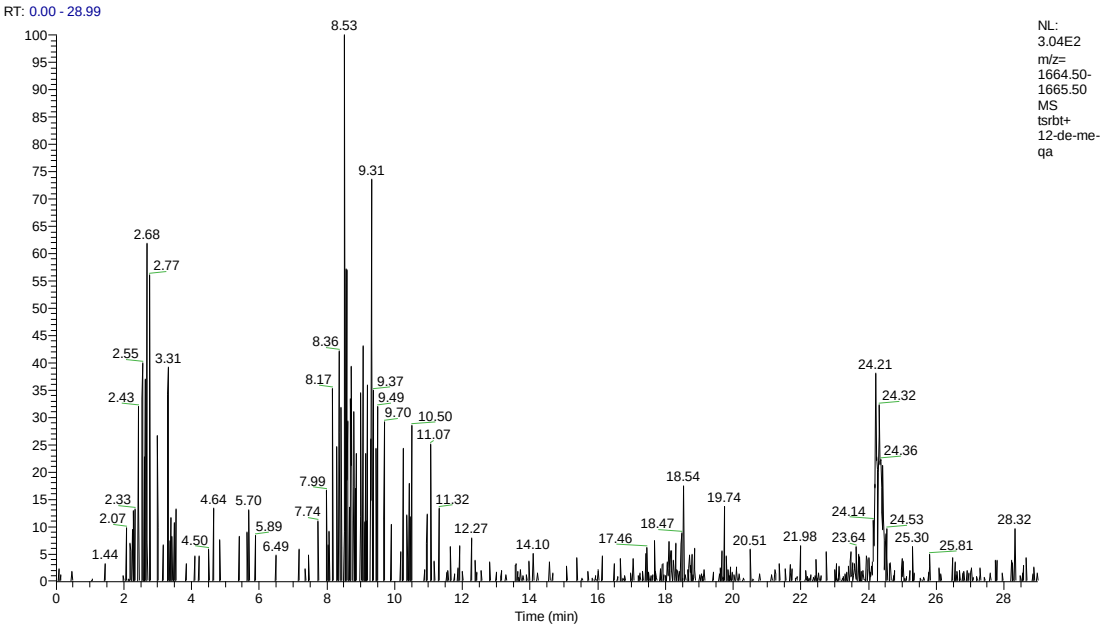
2.4. Characterization of 12’-de-Me-CmTSR

HPLC-MS analysis of culture extracts from *S.Laurentii* tsrBT mutant strains fed with **5**.

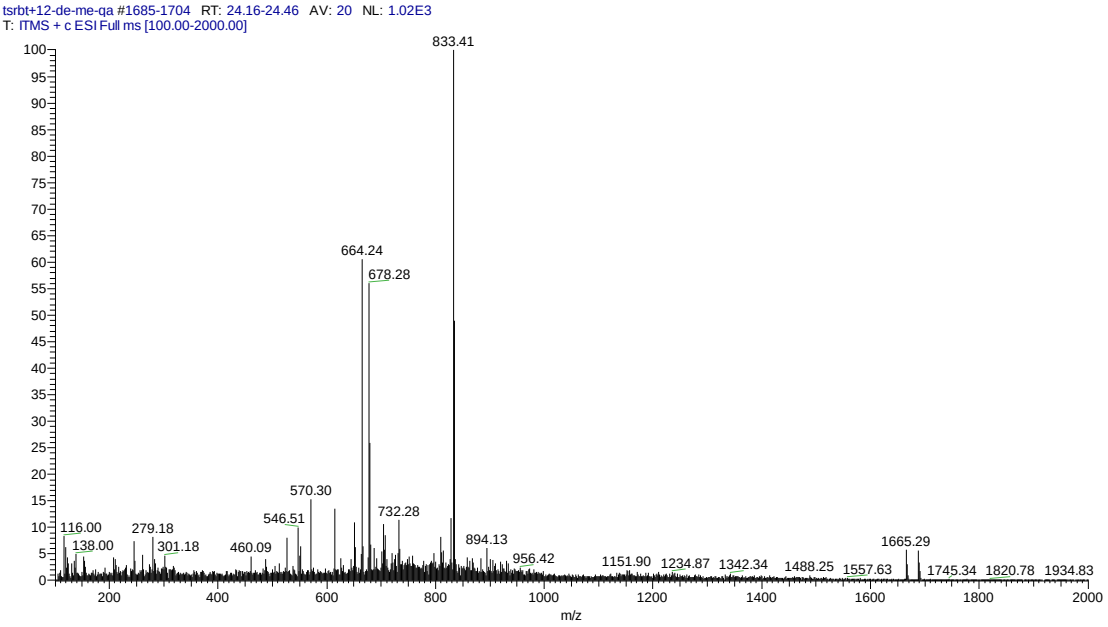
(1) Total ion chromatogram.



(2) Chromatogram extracted for m/z 1665.



(3) Mass spectrum of 12'-de-Me-CmTSR from tsrBT mutant extract eluting at t_R = 24.20 min (calculated m/z 1665.48 $[M+H]^+$, observed m/z 1665.29 $[M+H]^+$).



Under the conditions used for HPLC-MS, **12'-de-Me-CmTSR** elutes at a t_R of about 24.20 min providing ions at m/z 1665.29 $[M + H]^+$ and m/z 833.41$[M + 2H]^{2+}$. The predominant ion for **12'-de-Me-CmTSR** was $[M + 2H]^{2+}$, whereas $[M + H]^+$ was only a minor species.

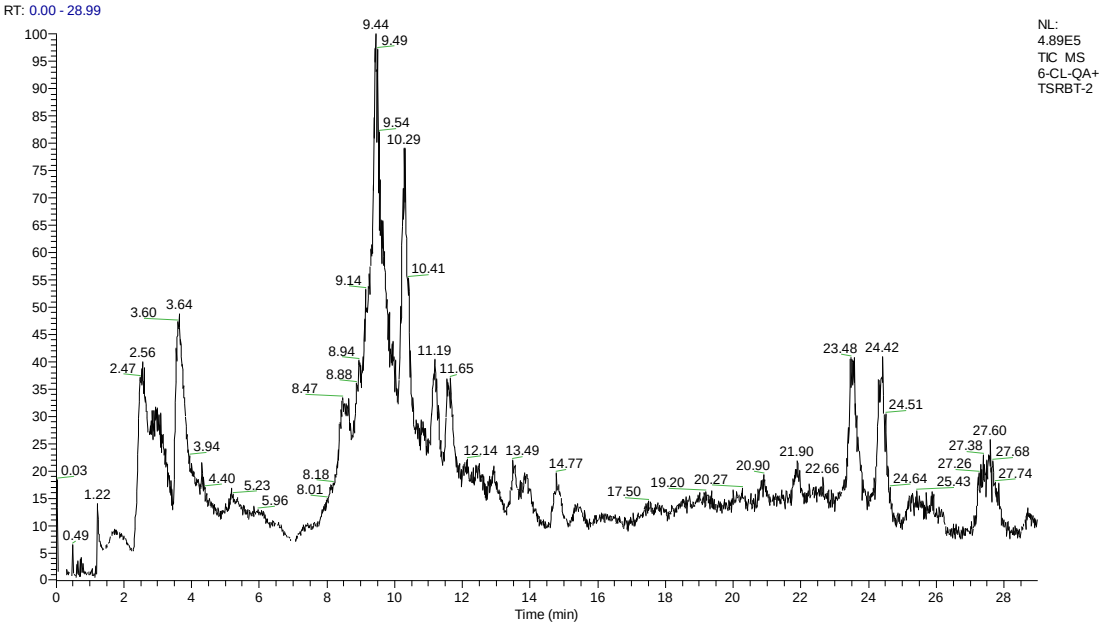
^{1}H NMR (600 MHz, $CDCl_3$): 8.28(s, 1H), 8.26(s, 1H), 8.10(s, 1H), 7.51(s, 1H), 7.47(s, 1H), 6.92(d, *J* = 9.0Hz, 1H), 6.79(s, 1H), 6.72(s, 1H), 6.39(q, *J* = 7.5 Hz, 1H), 6.31(m, 1H), 6.20(q, *J* = 7.2 Hz, 1H), 6.05(s, 1H), 5.85(d, *J* = 9.8 Hz, 1H), 5.77(d, *J* = 9.0 Hz, 1H), 5.71(d, *J* = 1.5 Hz, 1H), 5.51(s, 1H), 5.33(m, 2H), 5.19(br, 1H), 5.09(s, 1H), 4.94(m, 1H), 4.78(m, 1H), 4.66(d, *J* = 6.3 Hz, 1H), 4.46(dd, *J* = 7.9, 3.0 Hz, 1H), 4.09(m, 1H), 3.91(s, 3H), 3.82(m, 1H), 3.83(m, 1H), 3.71(t, *J* = 12.5, 1H), 3.62(d, *J* = 6.6Hz, 1H), 3.45(m, 1H), 3.12(t, *J* = 12.5 Hz, 1H), 2.99(d, *J* = 8.6 Hz, 1H), 2.94(m, 1H), 2.25(m, 1H), 1.75(d. *J* = 7.2 Hz, 3H), 1.65(m, 1H), 1.62(d, *J* = 7.2 Hz, 3H), 1.46(m, 1H), 1.46(d, *J* = 6.9Hz, 3H), 1.35(d, *J* = 6.9 Hz, 3H), 1.24(m, 1H), 1.19(d, *J* = 6.8 Hz, 3H), 1.18(s, 3H), 1.12(m, 1H), 0.98(d, *J* = 7.2 Hz, 3H), 0.93(t, *J* = 7.5 Hz, 3H), 0.86(d, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$):174.8, 173.2, 172.4, 171.0, 170.2, 169.9, 168.4, 168.2,

165.9, 165.6, 164.4, 163.3, 162.1, 161.9, 161.5, 161.2, 160.9, 159.5, 157.5, 154.6, 150.5, 150.3, 148.2, 147.2, 143.2, 134.4, 132.4, 132.2, 130.5, 130.2, 128.9, 128.8, 127.7, 125.1, 124.5, 124.4, 123.8, 117.9, 109.7, 102.8, 101.4, 79.0, 77.8, 72.2, 68.3, 67.5, 67.1, 66.7, 65.0, 64.4, 59.9, 57.5, 55.7, 53.4, 53.3, 52.3, 48.8, 38.9, 35.6, 29.1, 26.0, 24.9, 19.7, 19.4, 19.2, 17.4, 16.1, 15.9, 15.6, 11.4; HRMS (m/z) $[M+H]^+$ calcd. For $C_{72}H_{85}N_{18}O_{19}S_5$, 1665.4842; found 1665.4848.

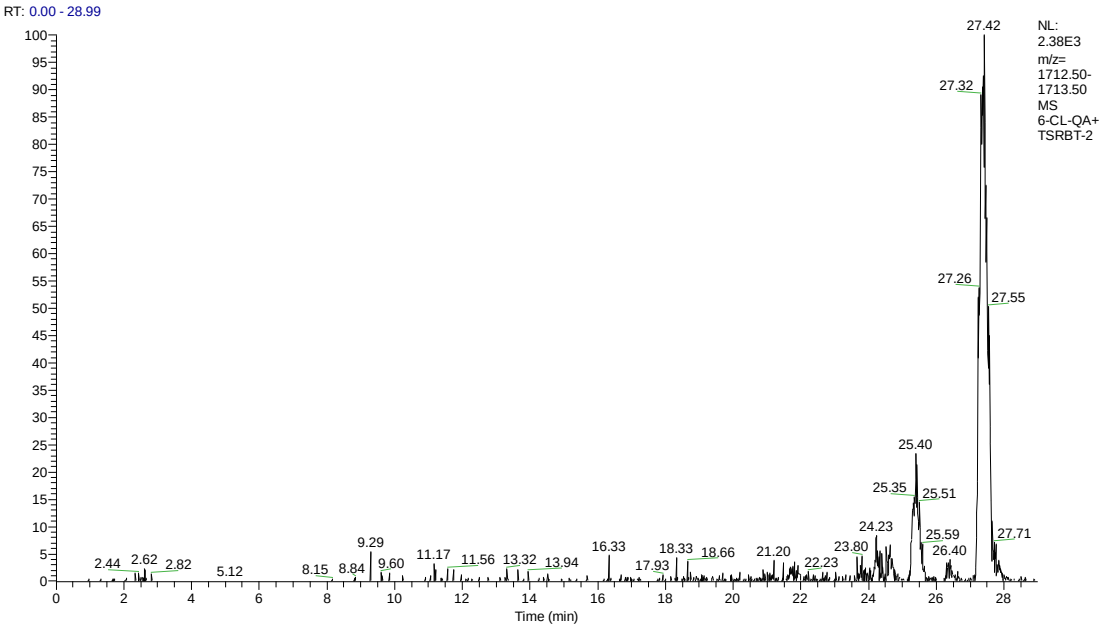
2.5. Characterization of 6’-Cl-CmTSR

HPLC-MS analysis of culture extracts from *S.Laurentii* tsrBT mutant strains fed with **4**.

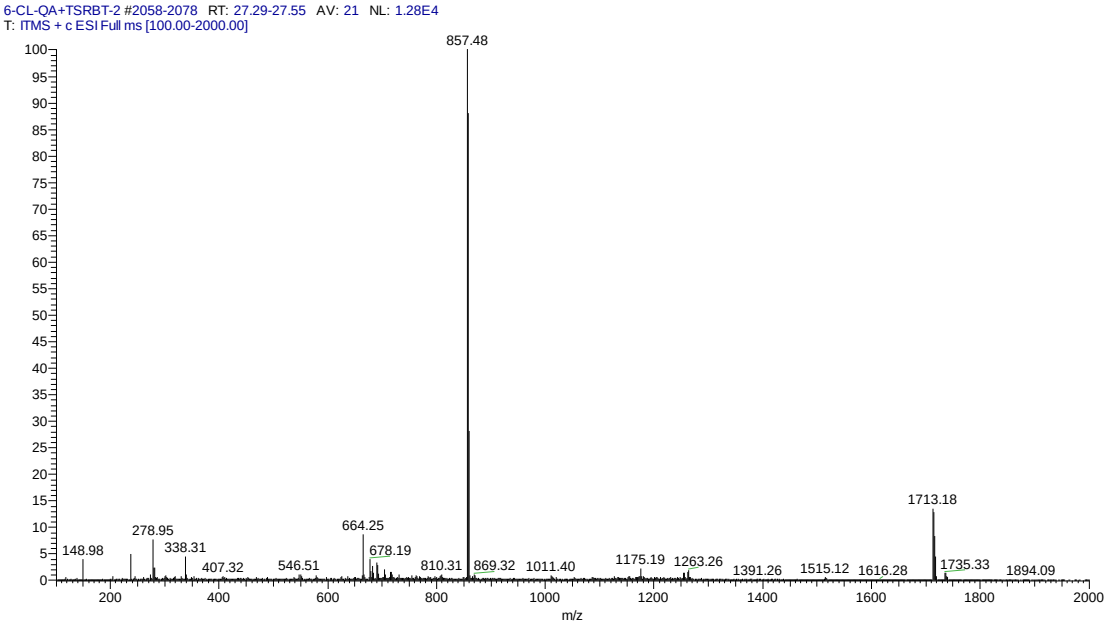
(1) Total ion chromatogram.



(2) Chromatogram extracted for m/z 1713.



(3) Mass spectrum of 6'-Cl-CmTSR from tsrBT mutant extract eluting at t_R = 27.30 min (calculated m/z 1713.46 [M+H]+, observed m/z 1713.18 [M+H]+).



Under the conditions used for HPLC-MS, **6'-Cl-CmTSR** elutes at a t_R of about 27.30 min providing ions at m/z 1713.18$[M + H]^+$ and m/z 857.48 $[M + 2H]^{2+}$. The predominant ion for **6'-Cl-CmTSR** was $[M + 2H]^{2+}$, whereas $[M + H]^+$ was only a minor species.

^{1}H NMR (500 MHz, $CDCl_3$): 8.29(s, 1H), 8.28(s, 1H), 8.11(s, 1H), 7.47(s, 1H), 7.31(s, 1H), 6.90(s, 1H), 6.81(d, *J* = 2.2 Hz, 1H), 6.73(s, 1H), 6.39(q, *J* = 6.3 Hz, 1H), 6.21(q, *J* = 7.0 Hz, 1H), 6.07(s, 1H),5.85(d, *J* = 9.1 Hz, 1H), 5.77(d, *J* = 8.6 Hz, 1H), 5.78(d, *J* = 1.5Hz, 1H), 5.53(s, 1H), 5.33(q, *J* = 6.6 Hz, 1H), 5.22(s, 1H), 5.15(s, 1H), 4.96(dd, *J* = 13.2, 8.8 Hz, 1H), 4.78(m, 1H), 4.765(m, 1H), 4.45(dd, *J* = 7.9, 3.1 Hz, 1H), 4.11(m, 1H), 3.93(s, 3H), 3.85(m, 1H), 3.81(m, 1H), 3.71(dd, *J* = 11.3, 8.7 Hz, 1H), 3.65(d, *J* = 5.0 Hz, 1H), 3.49(m, 1H), 3.15(dd, *J* = 13.2, 11.6 Hz, 1H), 2.99(d, *J* = 6.1Hz, 1H), 2.94(m, 1H), 2.28(m, 1H), 1.74(d. *J* = 6.5 Hz, 3H), 1.65(m, 1H), 1.61(m, 3H), 1.46(m, 1H), 1.45(d, *J* = 6.5 Hz, 3H), 1.37(d, *J* = 6.3Hz, 3H), 1.35(d, *J* = 6.5 Hz, 3H), 1.24(m, 1H), 1.19(d, *J* = 6.6 Hz, 3H), 1.19(s, 3H), 1.10(m, 1H), 0.99(d, *J* = 5.9 Hz, 1H), 0.95(t, *J* = 7.3 Hz, 3H), 0.88(d, *J* = 6.8 Hz, 3H); HRMS (m/z) $[M+H]^+$ calcd. For $C_{73}H_{86Cl}N_{18}O_{19}S_5$,

1713.4609; found 1713.4605.

2.6. Characterization of methyl quinoline-2-carboxylate and its analogues

Methyl quinoline-2-carboxylate (1.59 g, 85.1%). ^{1}H NMR(500 MHz, $CDCl_3$) δ 8.32(d, *J* = 8.5 Hz, 1H), 8.21(d, *J* = 8.5 Hz, 1H), 7.89(dd, *J* = 8.0, 1.0 Hz, 1H), 7.80(ddd, *J* = 8.5, 7.0, 1.5 Hz, 1H), 7.66(ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 4.10(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 166.0, 147.9, 147.6, 137.4, 130.8, 130.4, 129.4, 128.7, 127.6, 121.1, 53.3； LC-MS (EI^+) m/z 188.09 $[M+H]^+$.

Methyl 5-fluoro-quinoline-2-carboxylate (1.65 g, 80.5%).^{1}H NMR(500 MHz, $CDCl_3$) δ 8.59(d, *J* = 9.0 Hz, 1H), 8.25(d, *J* = 8.5 Hz, 1H), 8.13(d, *J* = 9.0 Hz, 1H), 7.75~7.71(m, 1H), 7.34~7.30(m, 1H), 4.11(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 165.58, 157.51(d, *J* = 255 Hz), 148.74, 148.16(d, *J* = 2.9 Hz), 130.79(d, *J* = 3.7 Hz), 129.82(d, *J* = 8.5 Hz), 126.54(d, *J* = 4.7 Hz), 121.08(d, *J* = 2.9 Hz), 120.13(d, *J* = 17.1 Hz), 122.03(d, *J* = 18.9 Hz), 53.29; ^{19}F NMR(282 MHz, $CDCl_3$) δ -122.42(dd, *J* = 9.3, 6.1 Hz)； LC-MS (EI^+) m/z 206.09 $[M+H]^+$.

Methyl 6-fluoro-quinoline-2-carboxylate (1.66 g, 80.9%).^{1}H NMR(500 MHz, $CDCl_3$) δ 8.32(dd, *J* = 9.5, 5.5 Hz, 1H), 8.26(d, *J* = 9.0 Hz, 1H), 8.22(d, *J* = 9.0 Hz, 1H), 7.59~7.55(m, 1H), 7.50(dd, *J* = 8.5, 3.0 Hz, 1H), 4.09(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 165.7, 161.7(d, *J* = 251 Hz), 147.4(d, *J* = 2.9 Hz), 144.6, 136.6(d, *J* = 5.6 Hz), 133.4(d, *J* = 9.5 Hz), 130.2(d, *J* = 10.5 Hz), 121.8, 120.9(d, *J* = 25.6 Hz), 110.6(d, *J* = 21.7 Hz), 53.2; ^{19}F NMR(282 MHz, $CDCl_3$) δ -109.8(d, *J* = 4.7 Hz)； LC-MS (EI^+) m/z 206.08 $[M+H]^+$.

Methyl 6-chloro-quinoline-2-carboxylate (1.88 g, 85.1%). ^{1}H NMR(500 MHz, $CDCl_3$) δ 8.18(d, *J* = 9.0 Hz, 1H), 8.16(s, 2H), 7.80(d, *J* = 2.3 Hz, 1H), 7.67(dd, *J* = 9.0,2.3 Hz, 1H), 4.05(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 165.5, 148.0, 145.8, 135.3, 134.6, 132.2, 131.4, 129.8, 125.2, 121.9, 53.2; LC-MS (EI^+) m/z 222.03 $[M+H]^+$.

Methyl 6-Bromo-quinoline-2-carboxylate (2.19 g, 82.9%). ^{1}H NMR(500 MHz, $CDCl_3$) δ 8.21(s, 2H), 8.17(d, *J* = 9.0 Hz, 1H), 8.05(d, *J* = 2.1 Hz, 1H), 7.85(dd, *J* = 9.0, 2.2 Hz, 1H), 4.09(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 165.6, 148.2, 146.1, 136.3, 133.9, 132.3, 130.3, 129.6, 123.0, 121.9, 53.3; LC-MS (EI^+) m/z 265.98 $[M+H]^+$.

2.7. Characterization of methyl 4-acetyl-quinoline-2-carboxylate and its analogues

Methyl 4-acetyl-quinoline-2-carboxylate (2.03 g, 88.6%). ^{1}H NMR(500 MHz, $CDCl_3$) δ 8.52(td, *J* = 8.6, 2.6 Hz, 1H), 8.42(s, 1H), 8.34(ddd, *J* = 8.6, 1.2, 0.6 Hz, 1H), 7.83(m, 1H), 7.73(m, 1H), 4.11(s, 3H), 2.80(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 200.6, 165.4, 148.7, 147.5, 143.6, 131.2, 130.7, 130.5, 125.5, 119.9, 53.4, 30.0; LC-MS (EI^+) m/z 230.08 $[M+H]^+$.

Methyl 4-acetyl-5-fluoro-quinoline-2-carboxylate (**1**) (2.12 g, 85.9%). ^{1}H NMR(500 MHz, $CDCl_3$) δ 8.18(d, *J* = 8.5 Hz, 1H), 8.04(s, 1H), 7.78(tt, *J* = 11.3, 5.7 Hz, 1H), 7.39(ddd, *J* = 10.8, 7.8, 0.9 Hz, 1H), 4.09(s, 3H), 2.66(d, *J* = 2.6 Hz, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 201.9, 164.8, 156.7(d, *J* = 256.0 Hz), 148.7, 148.5, 146.4, 130.6(d, *J* = 9.2 Hz), 127.3(d, *J* = 4.0 Hz), 117.3, 115.2(d, *J* = 14.7 Hz), 113.8(d, *J* = 20.4 Hz), 53.5, 30.9(d, *J* = 7.3 Hz); ^{19}F NMR(282 MHz, $CDCl_3$) δ -109.9; LC-MS (EI^+) m/z 248.14 $[M+H]^+$.

Methyl 4-acetyl-6-fluoro-quinoline-2-carboxylate (**2**) (2.05 g, 82.9%). ^{1}H NMR(500 MHz, $CDCl_3$) δ 8.50(s, 1H), 8.34(m, 2H), 7.59(m, 1H), 4.10(s, 3H), 2.80(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 199.9, 165.2, 163.4(d, *J* = 253.7 Hz); 147.0(d, *J* = 3.2 Hz), 146.1, 141.9(d, *J* = 6.4 Hz), 133.8(d, *J* = 9.8 Hz), 126.2(d, *J* = 11.5 Hz), 121.4, 121.4(d, *J* = 26.1 Hz), 109.9(d, *J* = 25.0 Hz), 53.6, 29.7; ^{19}F NMR(282 MHz, $CDCl_3$) δ -106.1(dd, *J* = 17.0, 6.8 Hz); LC-MS (EI^+) m/z 248.12 $[M+H]^+$.

Methyl 4-propionyl-quinoline-2-carboxylate (**3**) (1.95 g, 79.9%). ^{1}H NMR(500

MHz, $CDCl_3$) δ 8.35~8.33(m, 2H), 7.82(ddd, *J* =8.3, 6.9, 1.4 Hz, 1H), 7.73~7.70(m, 1H), 4.10(s, 3H), 3.11(q, *J* = 5 Hz, 2 H), 1.29(t, *J* = 5 Hz, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 203.9, 165.4, 148.5, 147.47, 144.6, 131.1, 130.6, 130.2, 128.7, 125.3, 118.9, 53.4, 35.7, 7.9 ; LC-MS (EI^+) m/z 244.20 $[M+H]^+$.

Methyl 4-acetyl-6-chloro-quinoline-2-carboxylate (**4**) (2.28 g, 86.6%). ^1H NMR(500 MHz, $CDCl_3$) δ 8.56(d, *J* = 2.3 Hz, 1H), 8.41(s, 1H), 8.17 (d, *J* = 9.0 Hz, 1H), 7.69(dd, *J* = 9.0, 2.3 Hz, 1H), 4.06(s, 3H), 2.76(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 199.8, 165.0, 147.6, 146.9, 141.7, 137.0, 132.4, 131.7, 125.3, 124.7, 121.1, 53.5, 29.6; LC-MS (EI^+) m/z 264.31 $[M+H]^+$.

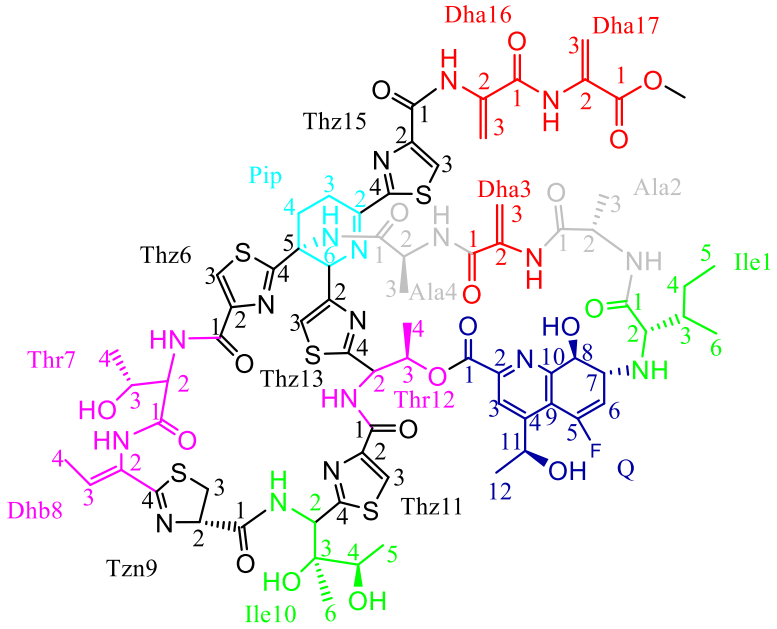
Methyl 4-(hydroxymethyl)quinoline-2-carboxylate (**5**) (1.55 g, 82.3%).^1H NMR(500 MHz, $CDCl_3$) δ 8.31(s, 1H), 8.25(d, J = 8.5 Hz, 1H), 7.98(d, J = 8.5 Hz, 1H), 7.77(t, J = 7.7 Hz, 1H), 7.65(dd, J = 8.1, 7.2 Hz, 1H), 5.18(s, 2H), 4.04(s, 3H) ; ^{13}C NMR(125 MHz, $CDCl_3$) δ 165.8, 149.7, 147.3, 146.8, 130.4, 130.2, 128.7, 125.9, 122.8, 117.6, 60.5, 52.7 ; LC-MS (EI^+) m/z 218.10 $[M+H]^+$.

Methyl 4-acetyl-6-bromo-quinoline-2-carboxylate (**6**) (2.57 g, 83.7%). ^1H NMR(500 MHz, $CDCl_3$) δ 8.74(d, *J* = 2.1 Hz, 1H), 8.41(s, 1H), 8.11(d, *J* = 9.0 Hz, 1H), 7.84(dd, *J* = 9.0, 2.2 Hz, 1H), 4.07(s, 3H), 2.77(s, 3H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 199.8, 165.0, 147.7, 147.2, 141.7, 134.3, 132.4, 128.0, 125.7, 121.0, 53.5, 29.7; LC-MS (EI^+) m/z 308.34 $[M+H]^+$.

Methyl 4-butyrylquinoline-2-carboxylate (**7**) (1.75 g, 75.9%).^1H NMR(500 MHz, $CDCl_3$) δ 8.37(m, 3H), 7.85(ddd, J = 8.5, 5.2, 1.3 Hz, 1H), 7.74(ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 4.13(s, 3H), 3.08(t, J = 7.2 Hz, 2H), 1.85(m, 2H), 1.06(t, J = 7.2 Hz, 3H) ; ^{13}C NMR(125 MHz, $CDCl_3$) δ 203.6, 165.5, 148.5, 147.4, 144.8, 130.2, 130.7, 130.2, 125.3, 125.0, 118.9, 53.4, 44.4, 17.5, 13.7 ; LC-MS (EI^+) m/z 258.09 $[M+H]^+$.

3. Supplementary Tables

Table S1. ^{1}H and ^{13}C NMR assignments of **5'-F-CmTSR**



Position	C ppm	H ppm	HMBC	COSY
Ile 1				
Ile 1-1	C q 174.41			
Ile 1-2	CH 66.66	2.94(d, 8.6)	Ile 1-3	Ile 1-3
Ile 1-3	CH 38.85	1.65(m)	Ile 1-1	Ile 1-6
Ile 1-4	CH2 25.85	a-1.21(m)		
		b-1.43(m)		
Ile 1-5	CH3 11.45	0.91(t, 7.5)	Ile 1-3, Ile 1-4	Ile 1-4
Ile 1-6	CH3 15.70	0.88(d, 6.9)	Ile 1-2, Ile 1-3, Ile 1-4	Ile 1-3
Ala 2				
Ala 2-1	Cq 168.25			
Ala 2-2	CH 48.84	3.67(m)	Ala 2-3	Ala 2-3, Ala 2-NH
Ala 2-3	CH3 19.54	1.16(d, 6.8)	Ala 2-1, Ala 2-2	Ala 2-2

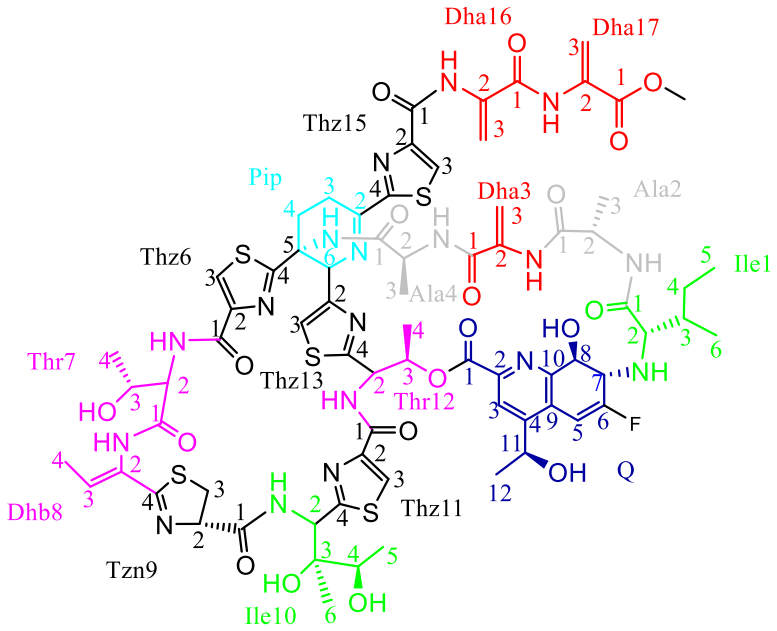
Ala 2-NH		7.61(d, 9.0)		Ala 2-2
Dha 3				
Dha 3-1	Cq 163.29			
Dha 3-2	Cq 132.43			
Dha 3-3	CH2 102.33	Ha: 5.75(s)	Dha 3-1, Dha 3-2	Dha 3-3-Hb
		Hb: 5.18(s)	Dha 3-1	Dha 3-3-Ha
Ala 4				
Ala 4-1	Cq 172.98			
Ala 4-2	CH 52.17	4.74(m)	Dha 3-1, Ala 4-1	Ala 4-3, Ala 4-NH
Ala 4-3	CH3 19.64	1.31(d, 6.9)		Ala 4-2
Ala 4-NH		6.62(d, 9.0)		Ala 4-2
Pip				
Pip-2	Cq 162.10			
Pip-3	CH2 24.88	Ha: 2.90(m)		Pip-4-Hb
		Hb: 3.38(m)		Pip-4-Hb
Pip-4	CH2 28.90	Ha: 2.26(m)		Pip-4-Hb
		Hb:4.06(m)		Pip-3, Pip-4-Ha
Pip-5	Cq 57.29			
Pip-6	CH 64.41	5.27(br)	Pip-2, Pip-5, Thz 13-2	
Thz 6				
Thz 6-1	Cq 161.64			
Thz 6-2	Cq 146.87			
Thz 6-3	CH 124.87	8.03(s)	Thz 6-2, Thz 6-4	
Thz 6-4	Cq 169.59			
Thr 7				
Thr 7-1	Cq 165.53			
Thr 7-2	CH 55.73	4.45(dd, 7.9,3.0)	Thz 6-1	Thr 7-3, Thr 7-NH
Thr 7-3	CH 67.01	1.08(m)		Thr 7-4
Thr 7-4	CH3 19.36	0.95(d, 7.2)	Thr 7-2, Thr 7-3	Thr 7-3

Thr 7-NH		7.00(d, 6.9)		Thr 7-2
Dhb 8				
Dhb 8-2	Cq 128.74			
Dhb 8-3	CH 132.20	6.17(q, 7.2)	Dhb 8-2, Tzn 9-4	Dhb 8-4
Dhb 8-4	CH3 16.25	1.60(d, 7.2)	Dhb 8-2, Tzn 9-4	Dhb 8-3
Tzn 9				
Tzn 9-1	Cq 170.90			
Tzn 9-2	CH 79.01	4.95(dd, 12.5, 8.5)	Tzn 9-1, Tzn 9-4	Tzn 9-3
Tzn 9-3	CH2 35.51	Ha: 3.10(t, 12.5)	Tzn 9-2, Tzn 9-4	Tzn 9-2, Tzn 9-3-Hb
		Hb: 3.81(m)	Tzn 9-1, Tzn 9-4	Tzn 9-2, Tzn 9-3-Ha
Tzn 9-4	Cq 172.29			
Ile 10				
Ile 10-2	CH 53.47	5.76(d, 9.0)	Tzn 9-1, Ile 10-3	Ile 10-NH
Ile 10-3	Cq 77.84			
Ile 10-4	CH 67.46	3.85(m)	Ile 10-2, Ile 10-3, Ile 10-5, Ile 10-6	Ile 10-5
Ile 10-5	CH3 15.80	1.40(d, 6.9)	Ile 10-3, Ile 10-4, Ile 10-6	Ile 10-4
Ile 10-6	CH3 17.45	1.21(s)		
Ile 10-NH		7.57(d, 8.6)		Ile 10-2
Thz 11				
Thz 11-1	Cq 161.88			
Thz 11-2	Cq 150.41			
Thz 11-3	CH 125.24	8.23(s)	Thz 11-1, Thz 11-2, Thz 11-4	
Thz 11-4	Cq 165.92			
Thr 12				
Thr 12-2	CH 55.73	5.78(d, 9.8)	Thz 11-1, Thz 13-4	Thr 12-NH
Thr 12-3	CH 72.48	6.35(q, 7.2)	Q-1	Thr 12-4

Thr 12-4	CH3 18.99	1.71(d, 7.2)	Thr 12-2, Thr 12-3	Thr 12-3
Thr 12-NH		8.39(d, 9.0)		Thr 12-2
Thz 13				
Thz 13-2	Cq 170.28			
Thz 13-3	CH 117.88	7.42(s)	Pip-6, Thz 13-2, Thz 13-4	
Thz 13-4	Cq 157.52			
Thz 15				
Thz 15-1	Cq 159.49			
Thz 15-2	Cq 168.43			
Thz 15-3	CH 127.80	8.24(s)	Thz 15-1, Thz 15-2, Thz 15-4	
Thz 15-4	Cq 150.18			
Dha 16				
Dha 16-1	Cq 161.99			
Dha 16-2	Cq 134.32			
Dha 16-3	CH2 102.92	Ha: 6.75(d,)	Dha 16-1, Dha 16-2	Dha 16-3-Hb
		Hb: 5.51(s)	Dha 16-1, Dha 16-2	Dha 16-3-Ha
Dha 17				
Dha 17-1	Cq 164.34			
Dha 17-2	Cq 130.52			
Dha 17-3	CH2 109.81	Ha: 6.66(s)	Dha 17-1, Dha 17-2	Dha 17-3-Hb
		Hb: 6.01(s)	Dha 17-1, Dha 17-2	Dha 17-3-Ha
Dha 17-4	CH3 53.30	3.87(s)	Dha 17-1	
Q				
Q-1	Cq 160.56			
Q-2	Cq 144.54			
Q-3	CH 123.32	7.52(s)	Q-1，Q-9，Q-11	
Q-4	Cq 155.60(d, 8.4)			
Q-5	Cq 158.09(d, 312)			
Q-6	CH 107.60(d, 21.8)	5.85(m)	Q-8, Q-9	Q-7

Q-7	CH 58.50(d, 12.8)	3.42(m)	Q-5，Q-10	Q-6, Q-8
Q-8	CH 67.14	4.62(d, 6.3)	Q-6，Q-9	Q-7
Q-9	Cq 122.60 (d, 29.1)			
Q-10	Cq 156.45(d, 10.0)			
Q-11	CH 65.50(d, 18.9)	5.61(q, 7.2)	Q-9, Q-12	Q-12
Q-12	CH2 23.35	1.30(d, 7.2)	Q-4，Q-11	Q-11

Table S2. ^{1}H and ^{13}C NMR assignments of **6'-F-CmTSR**



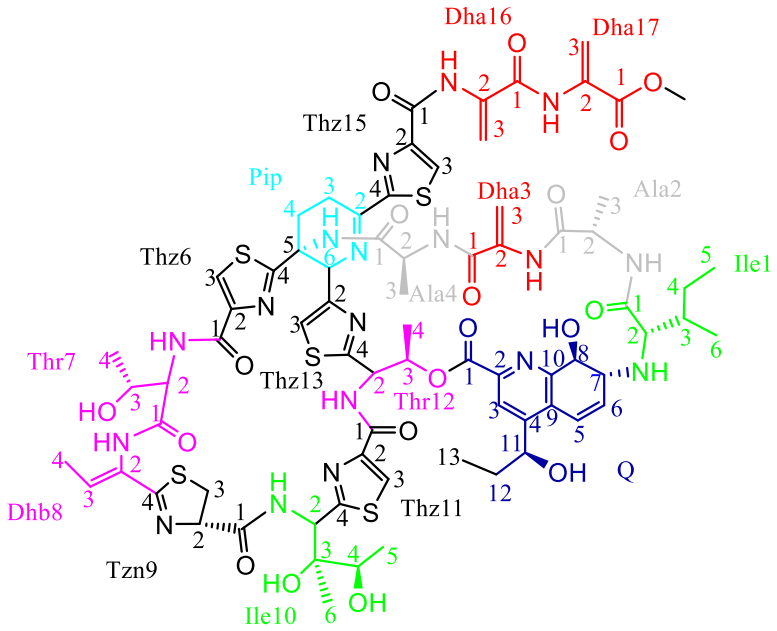
Position	C ppm	H ppm	HMBC	COSY
Ile 1				
Ile 1-1	C q 174.42			
Ile 1-2	CH 66.49	3.02(d,8.4)	Ile 1-3	Ile 1-3
Ile 1-3	CH 38.89	1.69(m)	Ile 1-1	Ile 1-1
Ile 1-4	CH2 26.17	a-1.25(m)		
		b-1.50(m)		
Ile 1-5	CH3 11.28	0.97(t, 7.2)	Ile 1-3	Ile 1-4
Ile 1-6	CH3 15.61	0.92(d,6.9)	Ile 1-3, Ile 1-4	Ile 1-3
Ala 2				
Ala 2-1	Cq 168.04			
Ala 2-2	CH 48.82	3.82(m)	Ala 2-3	Ala 2-3, Ala 2-NH
Ala 2-3	CH3 19.63	1.20(d,7.0)	Ala 2-1, Ala 2-2	Ala 2-2
Ala 2-NH		7.60(d,9.0)		Ala 2-2
Dha 3				

Dha 3-1	Cq 163.31			
Dha 3-2	Cq 132.50			
Dha 3-3	CH2 101.20	Ha: 5.75(d, 2.0)	Dha 3-1, Dha 3-2	Dha 3-3-Hb
		Hb: 5.11(br)	Dha 3-1	Dha 3-3-Ha
Ala 4				
Ala 4-1	Cq 173.03			
Ala 4-2	CH 52.45	4.76(m)	Dha 3-1, Ala 4-1	Ala 4-3
Ala 4-3	CH3 19.51	1.47(d,7.0)		Ala 4-2
Pip				
Pip-2	Cq 162.16			
Pip-3	CH2 24.89	Ha: 2.94(m)		Pip-4-Hb
		Hb: 3.45(m)		
Pip-4	CH2 29.00	Ha: 2.28(m)		Pip-4-Hb
		Hb: 4.10(m)		
Pip-5	Cq 57.36			
Pip-6	CH 64.50	5.22(m)	Pip-2, Pip-5	
Thz 6				
Thz 6-1	Cq 161.76			
Thz 6-2	Cq 146.91			
Thz 6-3	CH 124.98	8.11(s)	Thz 6-2, Thz 6-4	
Thz 6-4	Cq 169.60			
Thr 7				
Thr 7-1	Cq 165.51			
Thr 7-2	CH 55.84	4.46(dd,8.1, 2.5)	Thz 6-1	Thr 7-3
Thr 7-3	CH 67.08	1.13(m)		Thr 7-2, Thr 7-4
Thr 7-4	CH3 19.84	1.00(d,7.2)	Thr 7-2, Thr 7-3	Thr 7-3
Dhb 8				
Dhb 8-2	Cq 128.81			
Dhb 8-3	CH 132.34	6.22(q, 7.2)	Dhb 8-2, Tzn 9-4	Dhb 8-4
Dhb 8-4	CH3 15.88	1.64(d,7.2)	Dhb 8-2, Tzn 9-4	Dhb 8-3

Tzn 9				
Tzn 9-1	Cq 171.20			
Tzn 9-2	CH 79.04	4.98(dd,12.0,6.5)	Tzn 9-1, Tzn 9-4	Tzn 9-3
Tzn 9-3	CH2 35.70	Ha:3.13(dd,8.3,4.0)	Tzn 9-2, Tzn 9-4	Tzn 9-2
		Hb:3.72(dd,8.9,3.6)	Tzn 9-1, Tzn 9-4	Tzn 9-2
Tzn 9-4	Cq 172.46			
Ile 10				
Ile 10-2	CH 55.76	5.78(d, 8.6)	Tzn 9-1, Ile 10-3	Ile 10-NH
Ile 10-3	Cq 78.01			
Ile 10-4	CH 67.46	3.86(m)	Ile 10-2, Ile 10-3, Ile 10-5, Ile 10-6	Ile 10-5
Ile 10-5	CH3 16.21	1.37(d, 7.2)	Ile 10-3, Ile 10-4, Ile 10-6	Ile 10-4
Ile 10-6	CH3 17.42	1.19(s)		
Ile 10-NH		7.56(d,8.5)		Ile 10-2
Thz 11				
Thz 11-1	Cq 161.50			
Thz 11-2	Cq 150.58			
Thz 11-3	CH 125.20	8.28(s)	Thz 11-2, Thz 11-4	
Thz 11-4	Cq 165.88			
Thr 12				
Thr 12-2	CH 53.44	5.84(d,8.6)	Thz 11-1, Thz 13-4	Thr 12-3
Thr 12-3	CH 72.31	6.40(m)	Q-1	Thr 12-2, Thr 12-4
Thr 12-4	CH3 18.94	1.75(d,7.5)	Thr 12-2, Thr 12-3	Thr 12-3
Thz 13				
Thz 13-2	Cq 170.25			
Thz 13-3	CH 117.85	7.47(s)	Thz 13-2, Thz 13-4	
Thz 13-4	Cq 157.62			
Thz 15				

Thz 15-1	Cq 159.52			
Thz 15-2	Cq 168.48			
Thz 15-3	CH 127.68	8.29(s)	Thz 15-1, Thz 15-2, Thz 15-4	
Thz 15-4	Cq 150.37			
Dha 16				
Dha 16-1	Cq 162.05			
Dha 16-2	Cq 134.48			
Dha 16-3	CH2 102.74	Ha: 6.80(d, 1.5)	Dha 16-1, Dha 16-2	Dha 16-3-Hb
		Hb: 5.52(d,)	Dha 16-1, Dha 16-2	Dha 16-3-Ha
Dha 17				
Dha 17-1	Cq 164.41			
Dha 17-2	Cq 130.55			
Dha 17-3	CH2 109.67	Ha: 6.74(s)	Dha 17-1, Dha 17-2	Dha 17-3-Hb
		Hb: 6.07(s)	Dha 17-1, Dha 17-2	Dha 17-3-Ha
Dha 17-4	CH3 53.27	3.92(s)	Dha 17-1	
Q				
Q-1	Cq 160.91			
Q-2	Cq 142.88			
Q-3	CH 122.26	7.52(s)	Q-1，Q-9，Q-11	
Q-4	Cq 153.86(d, 8.9)			
Q-5	CH 100.93(d, 22.5)	6.90(d,13.5)	Q-7, Q-9，Q-10	
Q-6	CF 163.38(d, 274.1)			
Q-7	CH 62.03(d, 27.5)	3.68(d, 7.5)	Q-5，Q-8	Q-8
Q-8	CH 68.15(d, 12.8)	4.75(m)	Q-6，Q-9	Q-7
Q-9	Cq 127.19(d, 12.8)			
Q-10	Cq 152.07			
Q-11	CH 64.61	5.15(q,7.0)	Q-3,Q-9， Q-12	Q-12
Q-12	CH2 22.96	1.34(d,7.0)	Q-4，Q-11	Q-11

Table S3. ^{1}H and ^{13}C NMR assignments of **12'-Me-CmTSR**



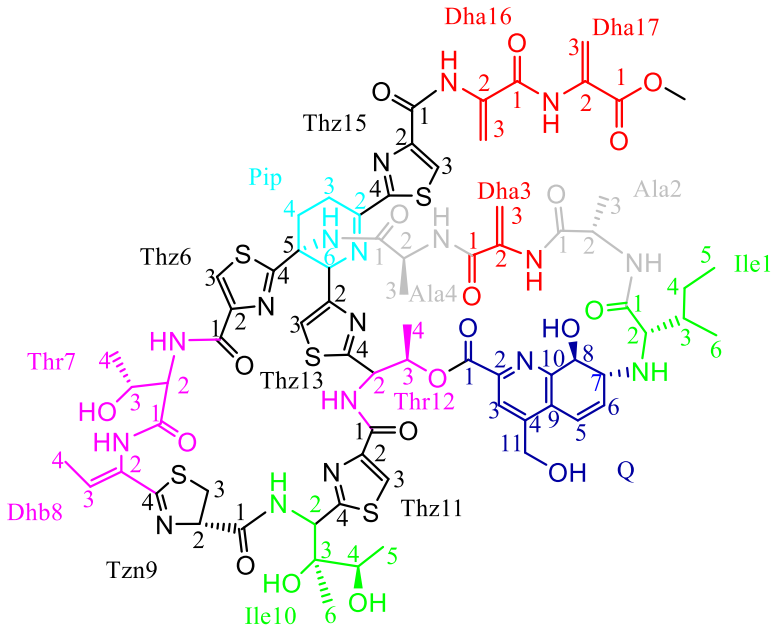
Position	C ppm	H ppm	HMBC	COSY
Ile 1				
Ile 1-1	C q 174.95			
Ile 1-2	CH 67.08	2.99(d, 6.1)	Ile 1-3	Ile 1-3
Ile 1-3	CH 38.94	1.60(m)	Ile 1-1	Ile 1-6
Ile 1-4	CH2 26.22	1.25(m)		
		1.47(m)		
Ile 1-5	CH3 11.28	0.96(t, 7.2)	Ile 1-3, Ile 1-4	Ile 1-4
Ile 1-6	CH3 15.62	0.90(d, 6.9)	Ile 1-2, Ile 1-3, Ile 1-4	Ile 1-3
Ala 2				
Ala 2-1	Cq 168.18			
Ala 2-2	CH 48.91	3.82(m)	Ala 2-3	Ala 2-3, Ala 2-NH
Ala 2-3	CH3 19.54	1.20(d,7.0)	Ala 2-1, Ala 2-2	Ala 2-3
Ala 2-NH		7.59(d,6.8)		Ala 2-2
Dha 3				

Dha 3-1	Cq 163.50			
Dha 3-2	Cq 132.56			
Dha 3-3	CH2 101.36	Ha: 5.76(s)	Dha 3-1, Dha 3-2	Dha 3-3-Hb
		Hb: 5.12(s)	Dha 3-1	Dha 3-3-Ha
Ala 4				
Ala 4-1	Cq 173.03			
Ala 4-2	CH 52.41	4.78(m)	Dha 3-1, Ala 4-1	Ala 4-3
Ala 4-3	CH3 19.50	1.47(d,7.2)	Ala 4-2	Ala 4-2
Pip				
Pip-2	Cq 162.06			
Pip-3	CH2 24.92	Ha: 2.95(m)		Pip-4-Hb
		Hb: 3.50(m)		Pip-4-Hb
Pip-4	CH2 29.05	Ha: 2.26(m)		Pip-4-Hb
		Hb: 4.13(m)		Pip-4-Ha
Pip-5	Cq 57.37			
Pip-6	CH 64.50	5.22(br)	Pip-2, Pip-5	
Thz 6				
Thz 6-1	Cq 161.72			
Thz 6-2	Cq 146.89			
Thz 6-3	CH 125.13	8.15(s)	Thz 6-2, Thz 6-4	
Thz 6-4	Cq 169.51			
Thr 7				
Thr 7-1	Cq 165.63			
Thr 7-2	CH 55.82	4.47(dd,8.0,2.7)	Thz 6-1	Thr 7-3, Thr 7-NH
Thr 7-3	CH 67.36	1.10(m)		Thr 7-4
Thr 7-4	CH3 19.87	1.02(d,7.9)	Thr 7-2, Thr 7-3	Thr 7-3
Thr 7-NH		7.29(d, 3.0)		Thr 7-2
Dhb 8				
Dhb 8-2	Cq 128.85			
Dhb 8-3	CH 132.20	6.20(q, 7.5)	Dhb 8-2	Dhb 8-4

Dhb 8-4	CH3 15.89	1.63(d, 7.5)	Tzn 9-4	Dhb 8-3
Tzn 9				
Tzn 9-1	Cq 171.12			
Tzn 9-2	CH 79.06	4.95(dd,12.0, 9.0)	Tzn 9-1, Tzn 9-4	Tzn 9-3
Tzn 9-3	CH2 35.68	Ha: 3.15(t,12.0)	Tzn 9-2, Tzn 9-4	Tzn 9-2, Tzn 9-3-Hb
		Hb: 3.72(t,12.0)	Tzn 9-1, Tzn 9-4	Tzn 9-2, Tzn 9-3-Ha
Tzn 9-4	Cq 172.45			
Ile 10				
Ile 10-2	CH 53.47	5.76(d,)	Tzn 9-1, Ile 10-3	
Ile 10-3	Cq 77.90			
Ile 10-4	CH 67.55	3.85(m)	Ile 10-2, Ile 10-3, Ile 10-5	Ile 10-5
Ile 10-5	CH3 16.14	1.37(d, 7.2)	Ile 10-3, Ile 10-4	Ile 10-4
Ile 10-6	CH3 17.39	1.19(s)		
Thz 11				
Thz 11-1	Cq 161.55			
Thz 11-2	Cq 150.57			
Thz 11-3	CH 125.13	8.25(s)	Thz 11-1, Thz 11-2, Thz 11-4	
Thz 11-4	Cq 165.92			
Thr 12				
Thr 12-2	CH 55.81	5.86(d,9.0)	Thz 11-1, Thz 13-4	Thr 12-NH
Thr 12-3	CH 72.18	6.40(m)	Q-1	Thr 12-4
Thr 12-4	CH3 19.05	1.75(d,7.5)	Thr 12-2, Thr 12-3	Thr 12-3
Thr 12-NH		8.31(d,9.0)		Thr 12-2
Thz 13				
Thz 13-2	Cq 170.29			
Thz 13-3	CH 117.80	7.46(s)	Thz 13-2, Thz 13-4	

Thz 13-4	Cq 157.64			
Thz 15				
Thz 15-1	Cq 159.53			
Thz 15-2	Cq 168.49			
Thz 15-3	CH 127.78	8.28(s)	Thz 15-2, Thz 15-4	
Thz 15-4	Cq 150.36			
Dha 16				
Dha 16-1	Cq 162.16			
Dha 16-2	Cq 134.49			
Dha 16-3	CH2 102.74	Ha: 6.80(d,)	Dha 16-1, Dha 16-2	Dha 16-3-Hb
		Hb: 5.52(s)	Dha 16-1, Dha 16-2	Dha 16-3-Ha
Dha 17				
Dha 17-1	Cq 164.42			
Dha 17-2	Cq 130.55			
Dha 17-3	CH2 109.67	Ha: 6.74(s)	Dha 17-1, Dha 17-2	Dha 16-3-Hb
		Hb: 6.07(s)	Dha 17-1, Dha 17-2	Dha 16-3-Ha
Dha 17-4	CH3 53.27	3.93(s)	Dha 17-1	
Q				
Q-1	Cq 161.06			
Q-2	Cq 143.32			
Q-3	CH 123.06	7.29(s)	Q-1，Q-9，Q-11	
Q-4	Cq 153.47			
Q-5	CH 123.75	6.84(d,8.4)	Q-7, Q-10	Q-6
Q-6	CH 129.61	6.30(m)	Q-8，Q-9	Q-5, Q-7
Q-7	CH 59.79	3.62(d,7.2)	Q-5，Q-10	Q-6, Q-8
Q-8	CH 66.89	4.69(d,7.0)	Q-6，Q-9	Q-7
Q-9	Cq 127.68			
Q-10	Cq 154.34			
Q-11	CH 69.45	5.09(t,6.6)	Q-3, Q-13	Q-12
Q-12	CH2 31.07	1.59(m)	Q-4	Q-11, Q-13
Q-13	CH3 10.41	1.05(t, 6.3)	Q-11	Q-12

Table S4. ^{1}H and ^{13}C NMR assignments of **12'-de-Me-CmTSR**



Position	C ppm	H ppm	HMBC	COSY
Ile 1				
Ile 1-1	C q 174.79			
Ile 1-2	CH 66.74	2.99(d, 8.6)	Ile 1-3	Ile 1-3
Ile 1-3	CH 38.90	1.65(m)	Ile 1-1	Ile 1-6
Ile 1-4	CH2 26.04	a-1.24(m)		
		b-1.46(m)		
Ile 1-5	CH3 11.38	0.93(t, 7.5)	Ile 1-3, Ile 1-4	Ile 1-4
Ile 1-6	CH3 15.59	0.86(d, 6.9)	Ile 1-2, Ile 1-3, Ile 1-4	Ile 1-3
Ala 2				
Ala 2-1	Cq 168.22			
Ala 2-2	CH 48.88	3.83(m)	Ala 2-3	Ala 2-3, Ala 2-NH
Ala 2-3	CH3 19.44	1.19(d, 6.8)	Ala 2-1, Ala 2-2	Ala 2-2
Ala 2-NH		7.61(d, 9.0)		Ala 2-2
Dha 3				

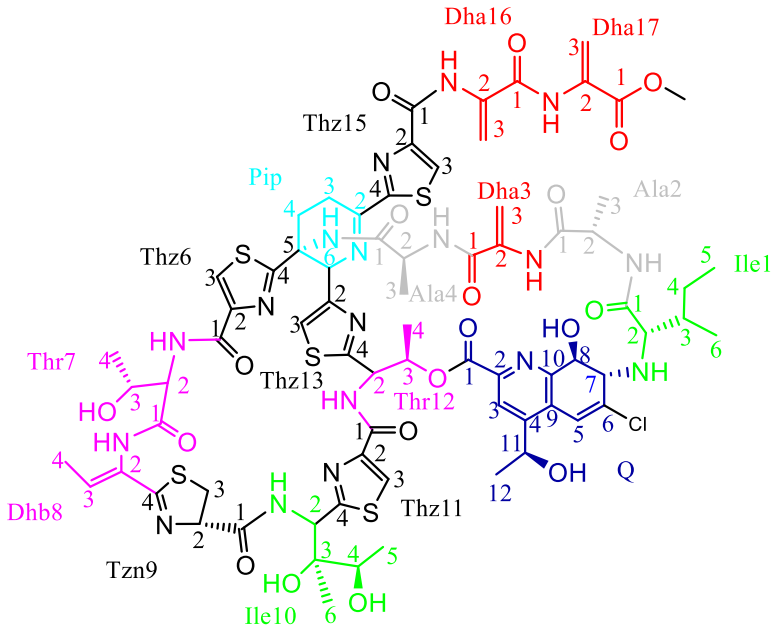
Dha 3-1	Cq 163.29			
Dha 3-2	Cq 132.39			
Dha 3-3	CH2 101.45	Ha: 5.71(d,)	Dha 3-1, Dha 3-2	Dha 3-3-Hb
		Hb: 5.09(s)	Dha 3-1	Dha 3-3-Ha
Ala 4				
Ala 4-1	Cq 173.17			
Ala 4-2	CH 52.32	4.78(m)	Dha 3-1, Ala 4-1	Ala 4-3, Ala 4-NH
Ala 4-3	CH3 19.44	1.46(d, 6.9)		Ala 4-2
Ala 4-NH		6.62(d, 9.0)		Ala 4-2
Pip				
Pip-2	Cq 161.98			
Pip-3	CH2 24.91	Ha: 2.94(m)		Pip-4-Hb
		Hb: 3.45(m)		Pip-4-Hb
Pip-4	CH2 29.09	Ha: 2.25(m)		Pip-4-Hb
		Hb:4.09(m)		Pip-3, Pip-4-Ha
Pip-5	Cq 57.46			
Pip-6	CH 64.42	5.19(br)	Pip-2, Pip-5, Thz 13-2	
Thz 6				
Thz 6-1	Cq 161.19			
Thz 6-2	Cq 147.16			
Thz 6-3	CH 124.53	8.10(s)	Thz 6-2, Thz 6-4	
Thz 6-4	Cq 169.89			
Thr 7				
Thr 7-1	Cq 165.56			
Thr 7-2	CH 55.75	4.46(dd, 7.9,3.0)	Thz 6-1	Thr 7-3, Thr 7-NH
Thr 7-3	CH 67.10	1.12(m)		Thr 7-4
Thr 7-4	CH3 19.69	0.98(d, 7.2)	Thr 7-2, Thr 7-3	Thr 7-3
Thr 7-NH		7.00(d, 6.9)		Thr 7-2
Dhb 8				

Dhb 8-2	Cq 128.77			
Dhb 8-3	CH 132.21	6.20(q, 7.2)	Dhb 8-2, Tzn 9-4	Dhb 8-4
Dhb 8-4	CH3 15.86	1.62(d, 7.2)	Dhb 8-2, Tzn 9-4	Dhb 8-3
Tzn 9				
Tzn 9-1	Cq 171.05			
Tzn 9-2	CH 79.00	4.94(m)	Tzn 9-1, Tzn 9-4	Tzn 9-3
Tzn 9-3	CH2 35.63	Ha: 3.12(t, 12.5)	Tzn 9-2, Tzn 9-4	Tzn 9-2, Tzn 9-3-Hb
		Hb: 3.71(t, 12.5)	Tzn 9-1, Tzn 9-4	Tzn 9-2, Tzn 9-3-Ha
Tzn 9-4	Cq 172.40			
Ile 10				
Ile 10-2	CH 53.41	5.77(d, 9.0)	Tzn 9-1, Ile 10-3	Ile 10-NH
Ile 10-3	Cq 77.81			
Ile 10-4	CH 67.54	3.82(m)	Ile 10-2, Ile 10-3, Ile 10-5, Ile 10-6	Ile 10-5
Ile 10-5	CH3 16.08	1.35(d, 6.9)	Ile 10-3, Ile 10-4, Ile 10-6	Ile 10-4
Ile 10-6	CH3 17.38	1.18(s)		
Ile 10-NH		7.57(d, 8.6)		Ile 10-2
Thz 11				
Thz 11-1	Cq 161.55			
Thz 11-2	Cq 150.46			
Thz 11-3	CH 125.15	8.26(s)	Thz 11-1, Thz 11-2, Thz 11-4	
Thz 11-4	Cq 165.90			
Thr 12				
Thr 12-2	CH 55.72	5.85(d, 9.8)	Thz 11-1, Thz 13-4	Thr 12-NH
Thr 12-3	CH 72.25	6.39(q,)	Q-1	Thr 12-4
Thr 12-4	CH3 19.19	1.75(d, 7.2)	Thr 12-2, Thr 12-3	Thr 12-3
Thr 12-NH		8.39(d, 9.0)		Thr 12-2

Thz 13				
Thz 13-2	Cq 170.22			
Thz 13-3	CH 117.94	7.47(s)	Pip-6, Thz 13-2, Thz 13-4	
Thz 13-4	Cq 157.54			
Thz 15				
Thz 15-1	Cq 159.50			
Thz 15-2	Cq 168.42			
Thz 15-3	CH 127.72	8.28(s)	Thz 15-1, Thz 15-2, Thz 15-4	
Thz 15-4	Cq 150.26			
Dha 16				
Dha 16-1	Cq 162.13			
Dha 16-2	Cq 134.39			
Dha 16-3	CH2 102.80	Ha: 6.79(s)	Dha 16-1, Dha 16-2	Dha 16-3-Hb
		Hb: 5.51(s)	Dha 16-1, Dha 16-2	Dha 16-3-Ha
Dha 17				
Dha 17-1	Cq 164.36			
Dha 17-2	Cq 130.48			
Dha 17-3	CH2 109.66	Ha: 6.72(s)	Dha 17-1, Dha 17-2	Dha 17-3-Hb
		Hb: 6.05(s)	Dha 17-1, Dha 17-2	Dha 17-3-Ha
Dha 17-4	CH3 53.26	3.91(s)	Dha 17-1	
Q				
Q-1	Cq 160.94			
Q-2	Cq 143.17			
Q-3	CH 124.43	7.51(s)	Q-1，Q-9，Q-11	
Q-4	Cq 154.59			
Q-5	CH 123.84	6.92(d, 9.0)	Q-7, Q-10	Q-6
Q-6	CH 130.24	6.31(m)	Q-8,Q-9	Q-5, Q-7
Q-7	CH 59.94	3.62(d, 6.6)	Q-5，Q-10	Q-6, Q-8
Q-8	CH 65.00	4.66(d, 6.3)	Q-6，Q-9	Q-7
Q-9	Cq 128.94			

Q-10	Cq 148.23			
Q-11	CH 68.30	5.33(m)	Q-3, Q-9	

Table S5. ^{1}H NMR assignments of **6'-Cl-CmTSR**



Position	H ppm	COSY
Ile 1		
Ile 1-1		
Ile 1-2	2.99(d, 6.1)	Ile 1-3
Ile 1-3	1.65(m)	Ile 1-6
Ile 1-4	a-1.24(m)	
	b-1.46(m)	
Ile 1-5	0.95(t, 7.3)	Ile 1-4
Ile 1-6	0.88(d, 6.8)	Ile 1-3
Ile 1-NH	8.51(s)	
Ala 2		
Ala 2-1		
Ala 2-2	3.85(m)	Ala 2-3, Ala 2-NH
Ala 2-3	1.19(d, 6.8)	Ala 2-2
Ala 2-NH	7.62(d, 9.0)	Ala 2-2

Dha 3		
Dha 3-1		
Dha 3-2		
Dha 3-3	Ha: 5.78(d,)	Dha 3-3-Hb
	Hb: 5.15(s)	Dha 3-3-Ha
Dha 3-NH	7.81(s)	
Ala 4		
Ala 4-1		
Ala 4-2	4.78(m)	Ala 4-3, Ala 4-NH
Ala 4-3	1.46(d, 6.5)	Ala 4-2
Ala 4-NH	6.62(d, 7.4)	Ala 4-2
Pip		
Pip-2		
Pip-3	Ha: 2.94(m)	Pip-4-Hb
	Hb: 3.49(m)	Pip-4-Hb
Pip-4	Ha: 2.28(m)	Pip-4-Hb
	Hb:4.11(m)	Pip-3, Pip-4-Ha
Pip-5		
Pip-6	5.22(s)	
Pip -NH	9.85(s)	
Thz 6		
Thz 6-1		
Thz 6-2		
Thz 6-3	8.11(s)	
Thz 6-4		
Thr 7		
Thr 7-1		
Thr 7-2	4.45(dd, 7.9,3.1)	Thr 7-3
Thr 7-3	1.10(m)	Thr 7-4
Thr 7-4	0.99(d, 5.9)	Thr 7-3
Dhb 8		

Dhb 8-2		
Dhb 8-3	6.21(q, 7.0)	Dhb 8-4
Dhb 8-4	1.61(m)	Dhb 8-3
Dhb 8-NH	8.12(s)	
Tzn 9		
Tzn 9-1		
Tzn 9-2	4.96(dd, 13.2, 8.8)	Tzn 9-3
Tzn 9-3	Ha: 3.15(dd, 13.2, 11.6)	Tzn 9-2, Tzn 9-3-Hb
	Hb: 3.71(dd, 11.3, 8.7)	Tzn 9-2, Tzn 9-3-Ha
Tzn 9-4		
Ile 10		
Ile 10-2	5.77(d, 8.6)	Ile 10-NH
Ile 10-3		
Ile 10-4	3.81(m)	Ile 10-5
Ile 10-5	1.37(d, 6.3)	Ile 10-4
Ile 10-6	1.19(s)	
Ile 10-NH	7.56(d, 10.1)	Ile 10-2
Thz 11		
Thz 11-1		
Thz 11-2		
Thz 11-3	8.28(s)	
Thz 11-4		
Thr 12		
Thr 12-2	5.85(d, 9.1)	Thr 12-NH
Thr 12-3	6.39(q, 6.3)	Thr 12-4
Thr 12-4	1.74(d, 6.5)	Thr 12-3
Thr 12-NH	8.39(d, 9.0)	Thr 12-2
Thz 13		

Thz 13-2		
Thz 13-3	7.47(s)	
Thz 13-4		
Thz 15		
Thz 15-1		
Thz 15-2		
Thz 15-3	8.29(s)	
Thz 15-4		
Dha 16		
Dha 16-1		
Dha 16-2		
Dha 16-3	Ha: 6.81(d, 2.2)	Dha 16-3-Hb
	Hb: 5.53(s)	Dha 16-3-Ha
Dha 16-NH	9.96(s)	
Dha 17		
Dha 17-1		
Dha 17-2		
Dha 17-3	Ha: 6.73(s)	Dha 17-3-Hb
	Hb: 6.07(s)	Dha 17-3-Ha
Dha 17-4	3.93(s)	
Dha 17-NH	8.57(s)	
Q		
Q-1		
Q-2		
Q-3	7.31(s)	
Q-4		
Q-5	6.90(s)	
Q-6(Cl)		
Q-7	3.65(d, 5.0)	Q-8
Q-8	4.76(m)	Q-7
Q-9		

Q-10		
Q-11	5.33(q, 6.6)	Q-12
Q-12	1.35(d, 6.5)	Q-11

Table S6. Antibacterial activity of **CmTSRs**

	TSR	CmTSR	5'-F-CmTSR	6'-F-CmTSR	12'-Me-CmTSR	12'-de-Me-CmTSR	6'-Cl-CmTSR	VAN
PRSP-1063	0.001	0.000125	< 0.000125	< 0.000125	< 0.000125	0.000125	0.5	0.25
PRSP-2831	0.001	0.000125	< 0.000125	< 0.000125	< 0.000125	0.000125	0.25	0.25
PRSP-224588	0.008	0.002	< 0.000125	< 0.000125	< 0.000125	0.001	0.5	0.25
MRSA-s1	0.032	0.008	0.00025	0.0005	0.004	0.008	1	0.5
MRSA-SAU3	0.064	0.008	0.00025	0.0005	0.002	0.004	2	0.5
MRSA- SAU5	0.064	0.008	0.0005	0.001	0.004	0.008	1	1.0
VRE-3	0.032	0.008	0.00025	0.0005	0.008	0.008	1	> 256
VRE-73	0.064	0.016	0.0005	0.001	0.008	0.016	1	> 256
VRE-83	0.064	0.016	0.00025	0.0005	0.008	0.008	1	> 256
CD-70057	0.05	0.0125	0.00025	0.001	0.0125	0.008	0.5	0.4
CD-10070702	0.05	0.008	0.00025	0.001	0.008	0.008	0.5	0.4
CD-10071903	0.025	0.008	0.0005	0.001	0.004	0.008	0.5	0.4
CD-10070701	0.05	0.008	0.00025	0.002	0.008	0.004	1	0.4
CD-10072001	0.025	0.008	0.0005	0.001	0.008	0.016	0.5	0.4
CD-10072905	0.025	0.008	0.00025	0.001	0.008	0.008	0.5	0.2
CD-10072904	0.025	0.008	0.000125	0.001	0.008	0.008	0.5	0.4
CD-10081001	0.05	0.0125	0.0005	0.002	0.008	0.008	1	0.4
CD-10081207	0.025	0.008	0.00025	0.001	0.004	0.004	0.5	0.4
CD-10081604	0.05	0.008	0.0005	0.001	0.008	0.004	1	0.4
CD-11110306	0.025	0.008	0.0005	0.001	0.004	0.008	0.5	0.2

Table S7. Strains and plasmids used in this study

Strains/ plasmids	***Characteristic(s)***	***Source/ reference***
SL1051	*tsrB* mutant, intermediate **CmTSR** producing	[1]
SL1201	Derivative of SL1051, *tsrB/T* double mutant	This study
Streptococcus pneumoniae PRSP1063	Penicillin resistance and for MIC test	Clinical isolates
Streptococcus pneumoniae PRSP2831	Penicillin resistance and for MIC test	Clinical isolates
Streptococcus pneumoniae PRSP224588	Penicillin resistance and for MIC test	Clinical isolates
Staphylococcus aureus MRSA-s1	Methicillin resistance and for MIC test	Clinical isolates
Staphylococcus aureus MRSA-SAU3	Methicillin resistance and for MIC test	Clinical isolates
Staphylococcus aureus MRSA-SAU5	Methicillin resistance and for MIC test	Clinical isolates
Enterococcus	Vancomycin resistance and for MIC test	Clinical isolates

faecium VRE3		
Enterococcus faecium VRE73[a]	Vancomycin resistance and for MIC test	Clinical isolates
Enterococcus faecium VRE83[a]	Vancomycin resistance and for MIC test	Clinical isolates
Clostridium difficile CD-70057	for MIC test	Clinical isolates
Clostridium difficile CD-10070702	for MIC test	Clinical isolates
Clostridium difficile CD-10071903	for MIC test	Clinical isolates
Clostridium difficile *Clostridium difficile* CD-10070701	for MIC test	Clinical isolates
Clostridium difficile CD-10072001	for MIC test	Clinical isolates
Clostridium difficile CD-10072905	for MIC test	Clinical isolates
Clostridium difficile CD-10072904	for MIC test	Clinical isolates
Clostridium difficile CD-10081001	for MIC test	Clinical isolates

Clostridium difficile CD-10081207	for MIC test	Clinical isolates
Clostridium difficile CD-10081604	for MIC test	Clinical isolates
Clostridium difficile CD-11110306	for MIC test	Clinical isolates
E. coli		
DH5α	Host for general cloning	Invitrogen
ET12567 (pUZ8002)	Donor strain for conjugation between *E. coli* and *Streptomyces*	[1]
Plasmids		
pMD19-T	*E. coli* subcloning vector	TaKaRa
pKC1139	*E. coli-Streptomyces* shuttle vector containing the *aac(3)IV* gene, and a temperature-sensitive replicon	[2]
pSL12011	pMD19-T derivative carrying the upstream fragment of *tsrT*	This study
pSL12012	pMD19-T derivative carrying the downstream fragment of *tsrT*	This study
pSL1201	pKC1139 derivative for in-frame deletion within *tsrT*	This study

Table S8. Primers used in this study

Name	***Sequence***	***Description***
pTLf	CG GAA TTC GCG GTG ATC GAG TGG GTA CGG (*Eco*RI)	Cloning of the upstream region of *tsrT* for in-frame deletion
pTLr	GC TCT AGA GGG TGG ATC TGG TTG GGG TTG (*Xba*I)	
pTRf	GC TCT AGA GGG ATA CAC GGG CGC TTT CTG (*Xba*I)	Cloning of the downstream region of *tsrT* for in-frame deletion
pTRr	C CCC AAG CTT CCC GTA CGT ATC TGG ACC ACC (*Hind*III)	

4. Supplementary Figures

Figure S1. Structures of thiostrepton-series thiopeptides

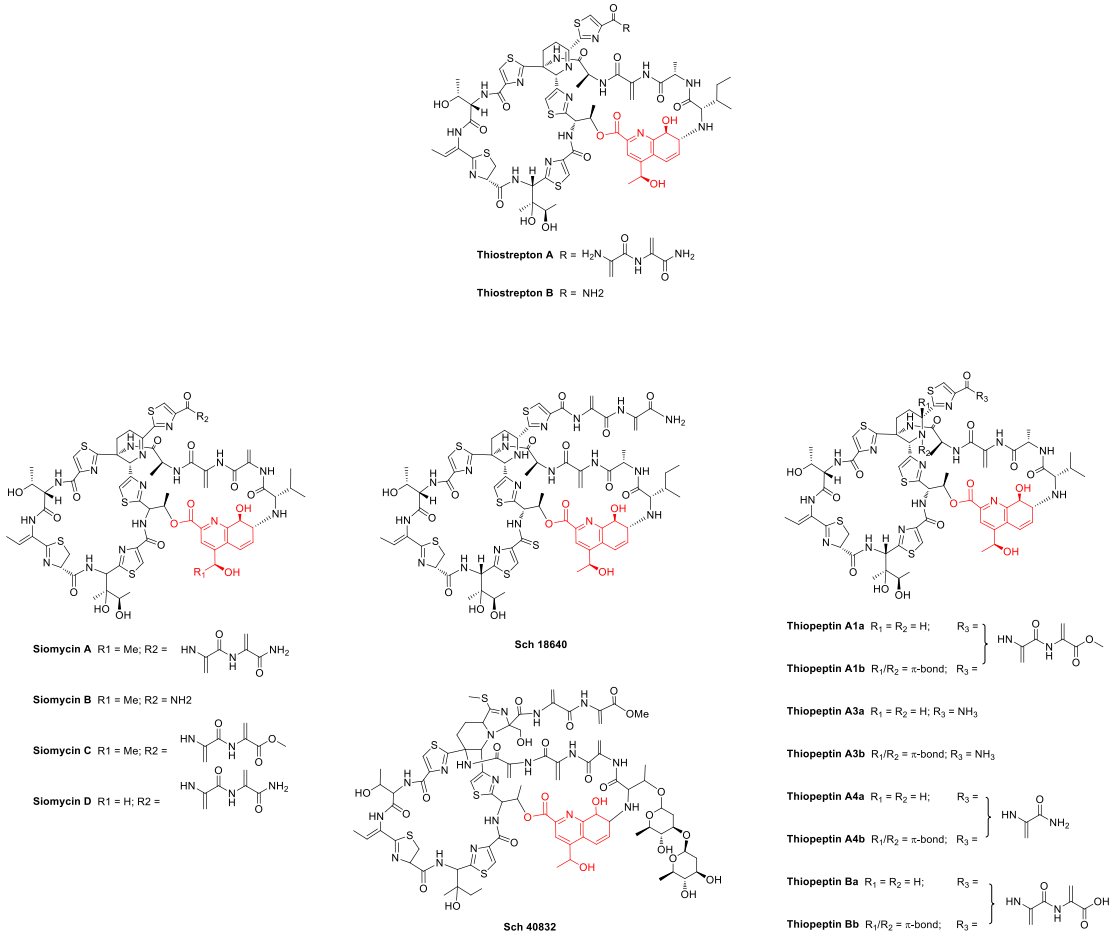


Figure S2. Biosynthesis of the quinaldic acid side ring of thiostrepton

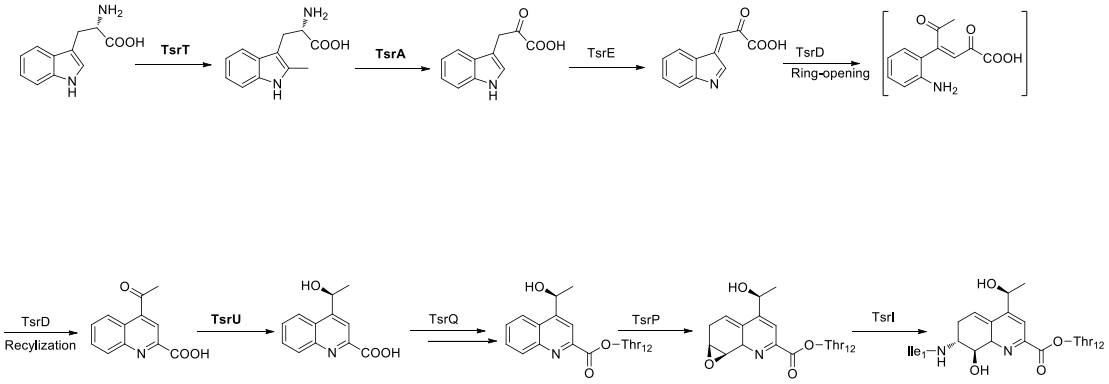


Figure S3. Construction and genotype verification of the double mutant, *Streptomyces laurentii* SL1201 (Δ*tsrB/T*). A) *Streptomyces laurentii* Δ*tsrB/T* with a 1650 bp deletion within tsrT was constructed via a double-crossover event. B) Gel electrophoresis analysis of the PCR products were amplified from the genomic DNAs of S. sioyaensis SL1201 (lane 1, 1.19 kb), SL1051 (lane 2, 2.83 kb), using primer pair pTf/pTr.

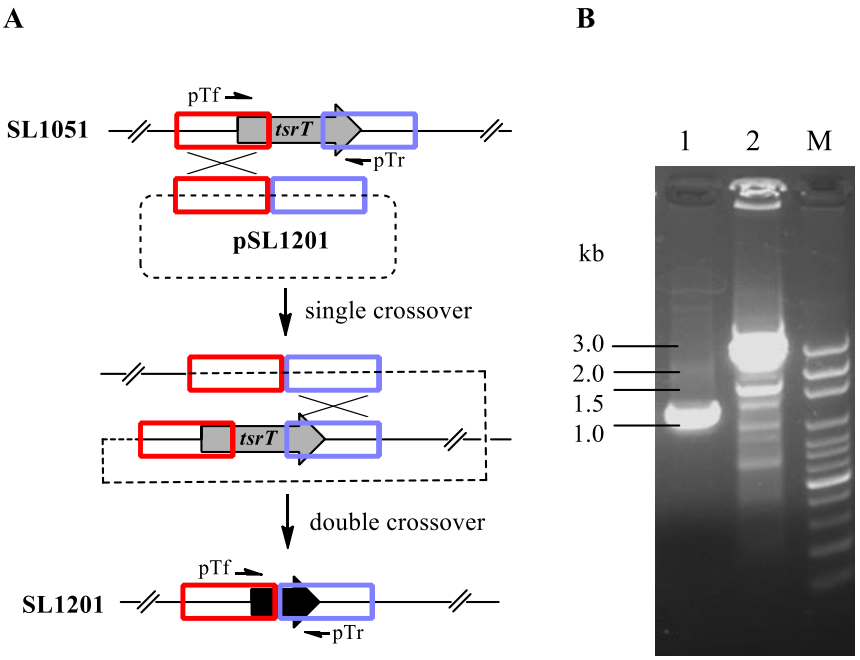
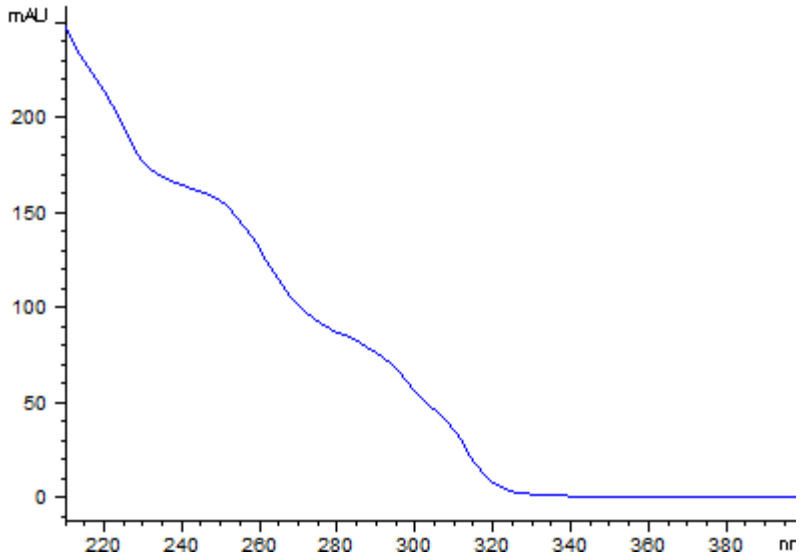
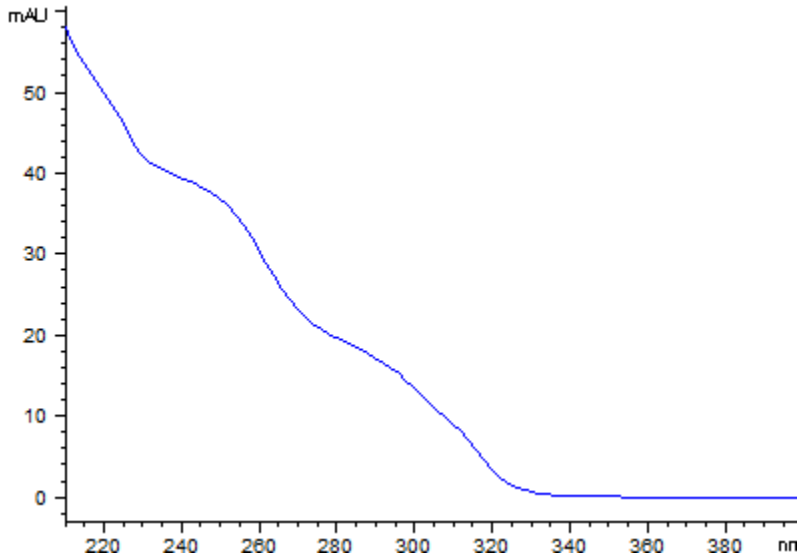


Figure S4. Ultraviolet absorption of CmTSR and its analogs

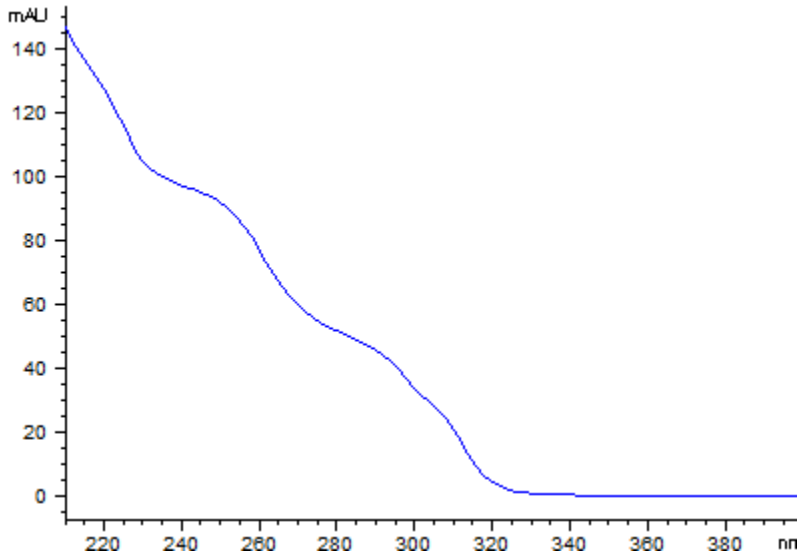
(1) CmTSR



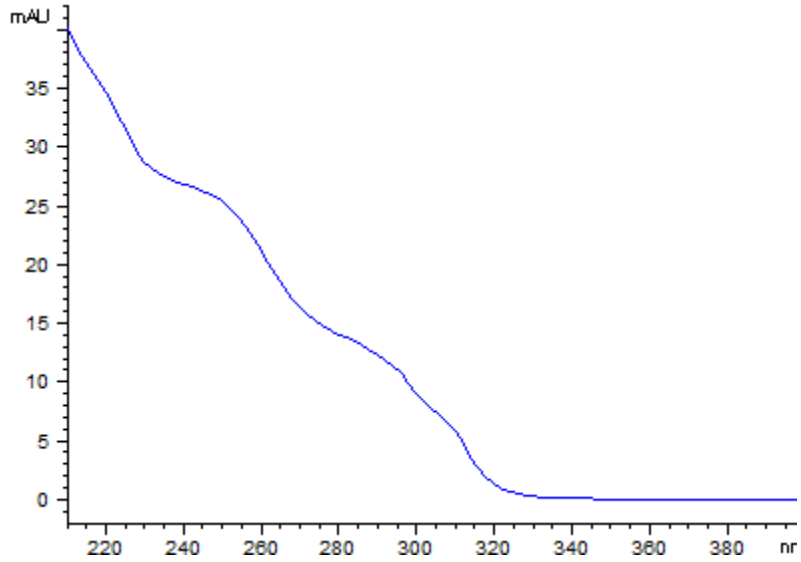
(2) **5’-F-CmTSR**



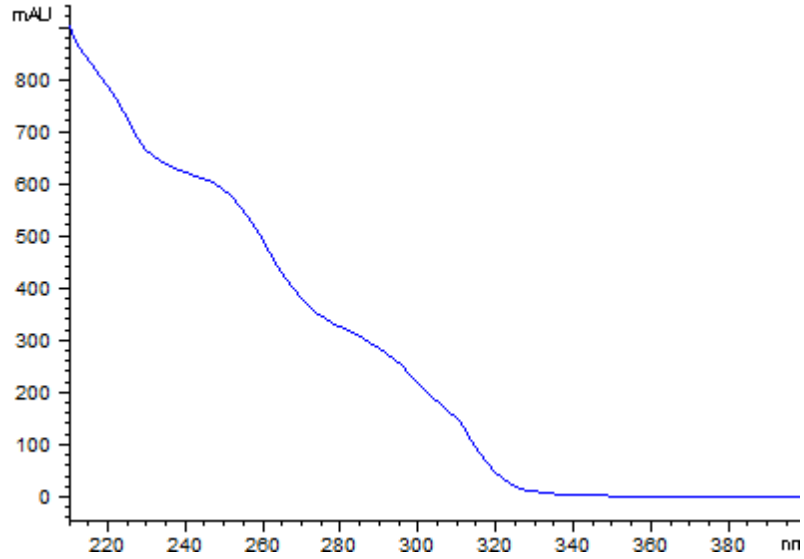
(3) **6’-F-CmTSR**



(4) **12’-Me-CmTSR**



(5) **12’-de-Me-CmTSR**



(6) **6’-Cl-CmTSR**

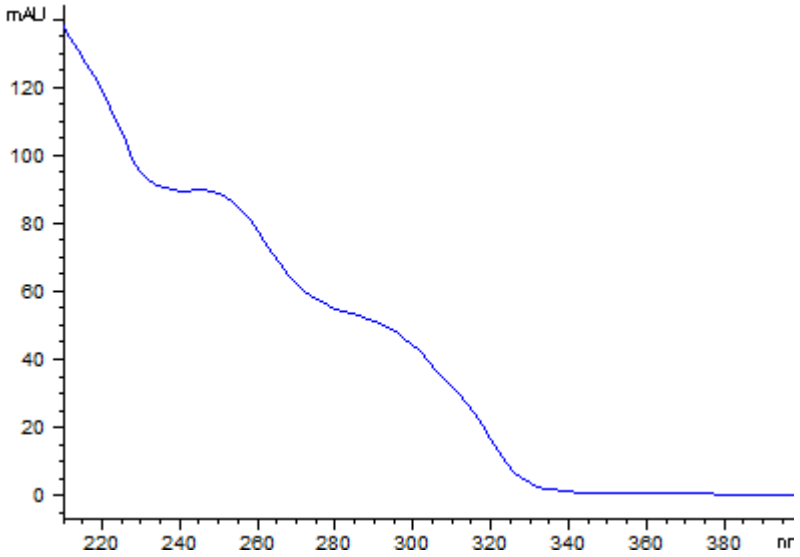
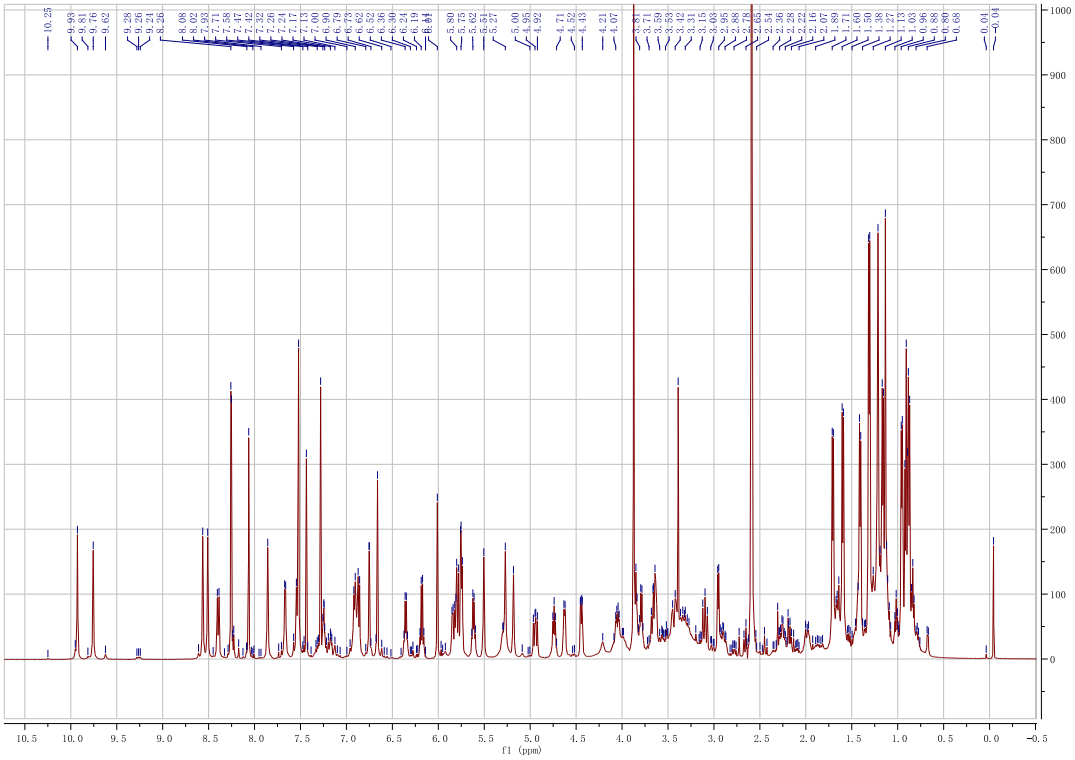
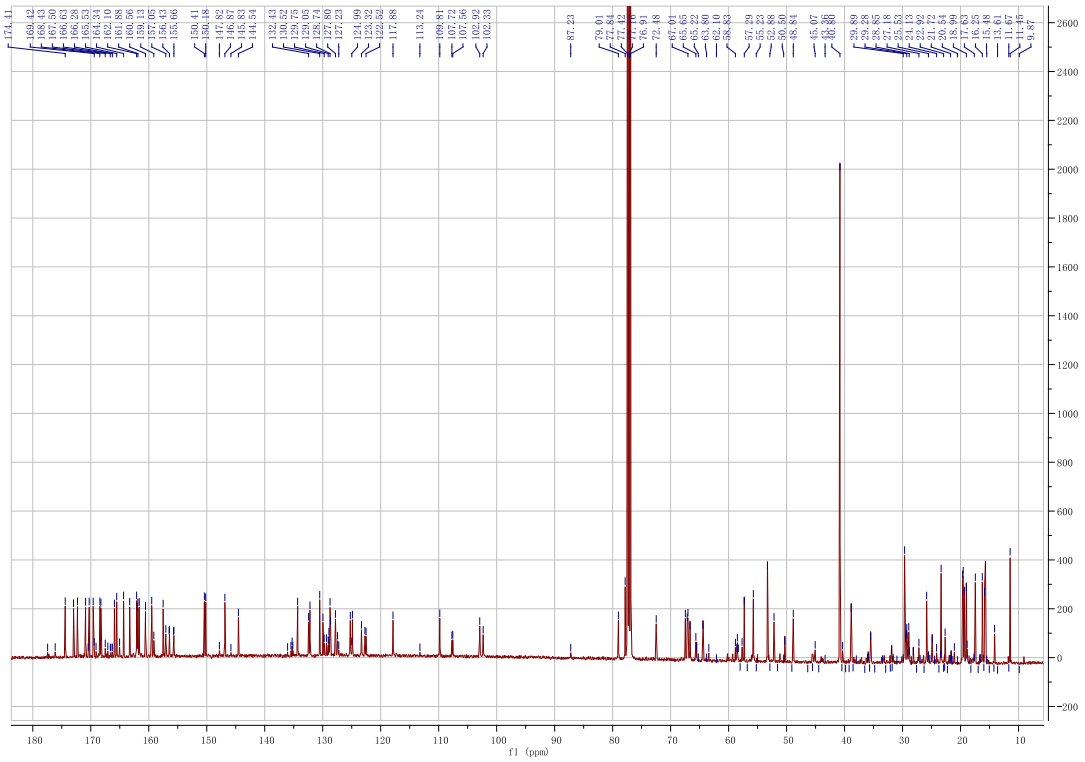


Figure S5. 1D and 2D NMR spectra of **5'-F-CmTSR**

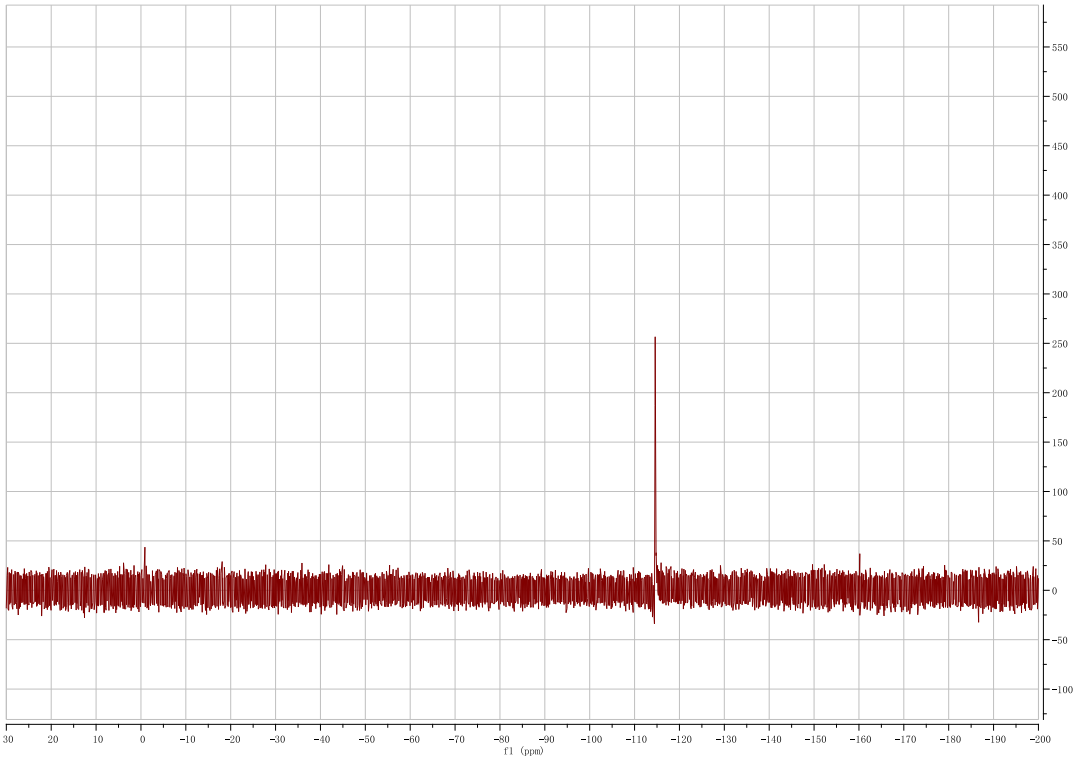
^{1}H NMR of 5'-F-CmTSR



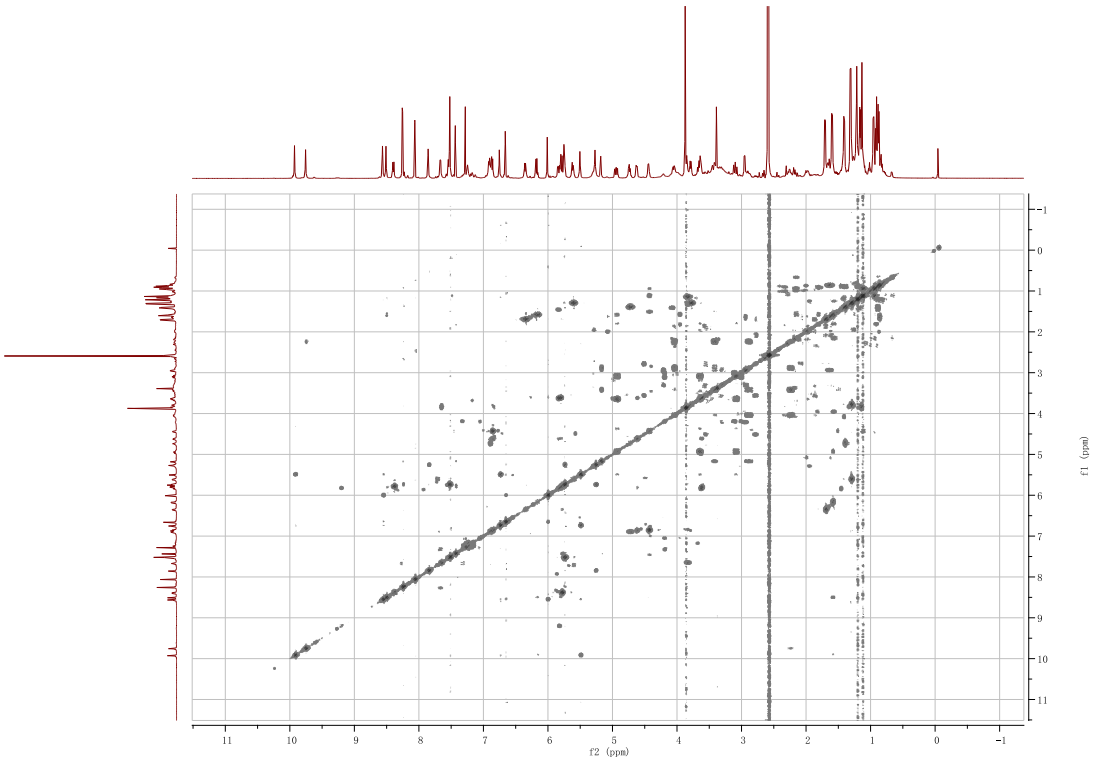
^{13}C NMR of 5’-F-CmTSR



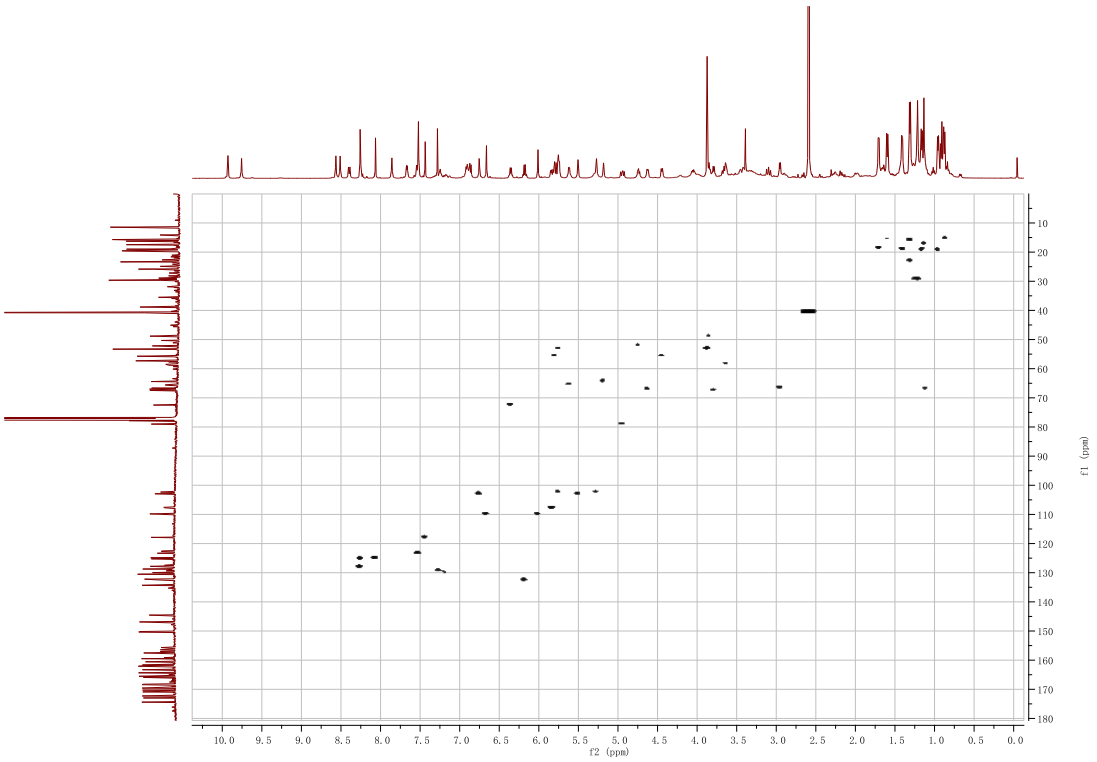
^{19}F NMR of 5’-F-CmTSR



gCOSY of 5’-F-CmTSR



gHSQC of 5’-F-CmTSR



gHMBC of 5’-F-CmTSR

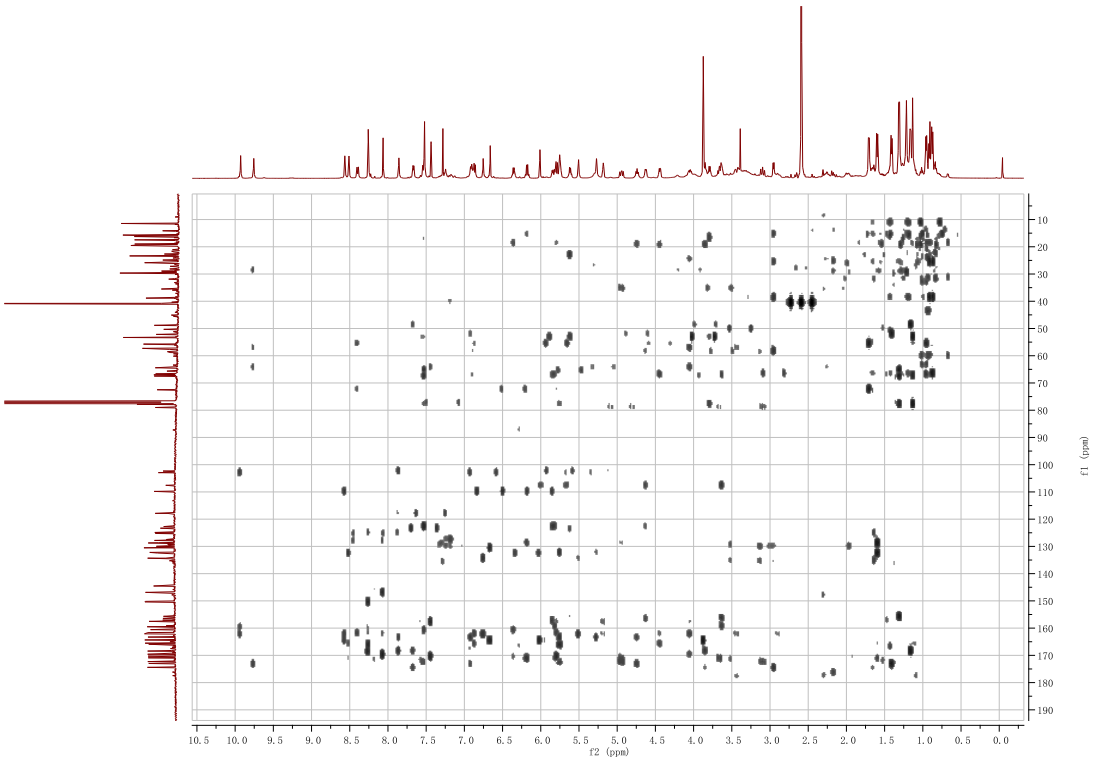
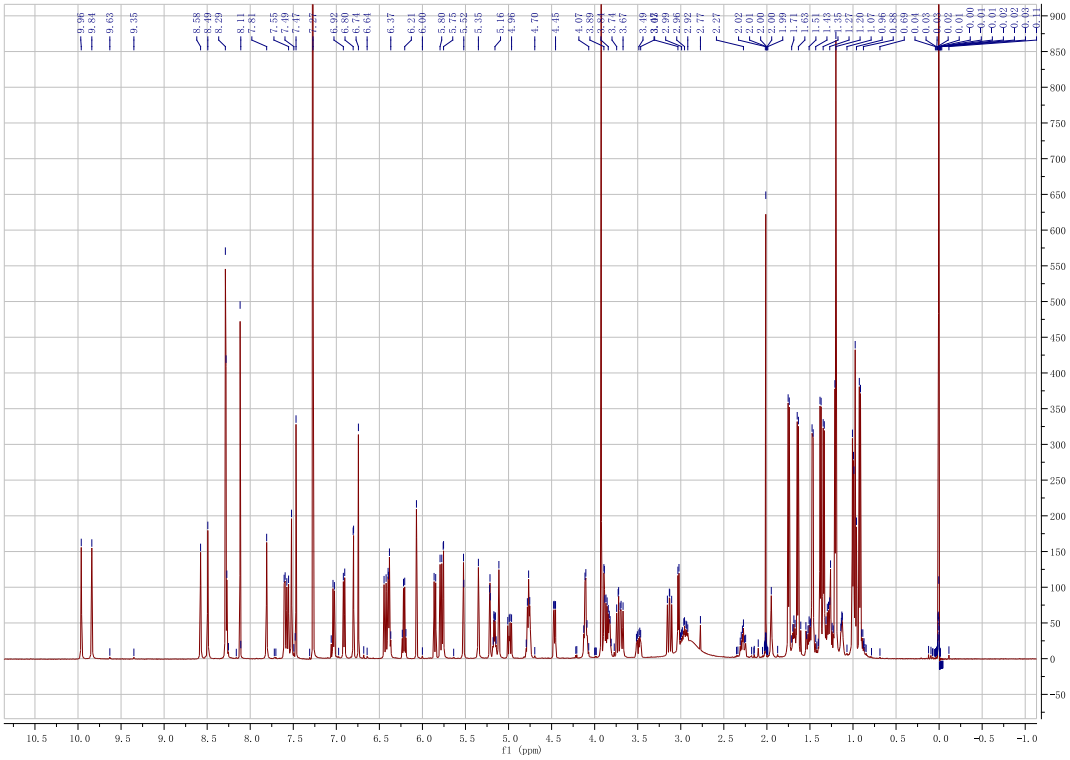
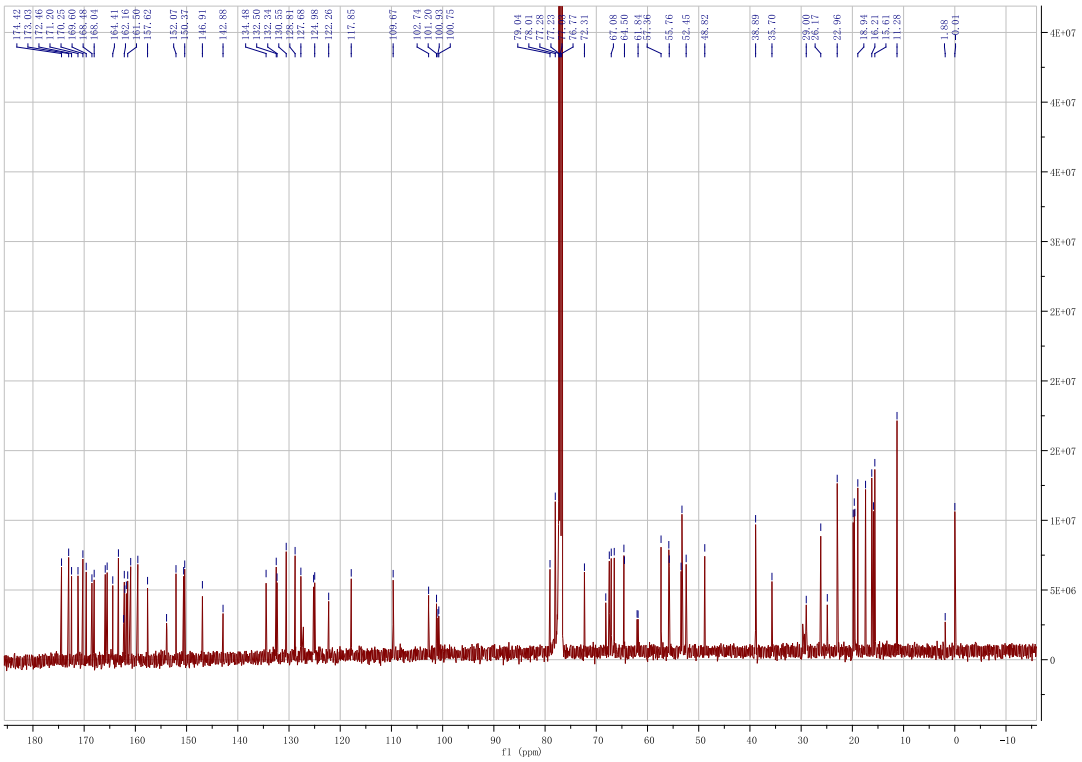


Figure S6. 1D and 2D NMR spectra of **6'-F-CmTSR**

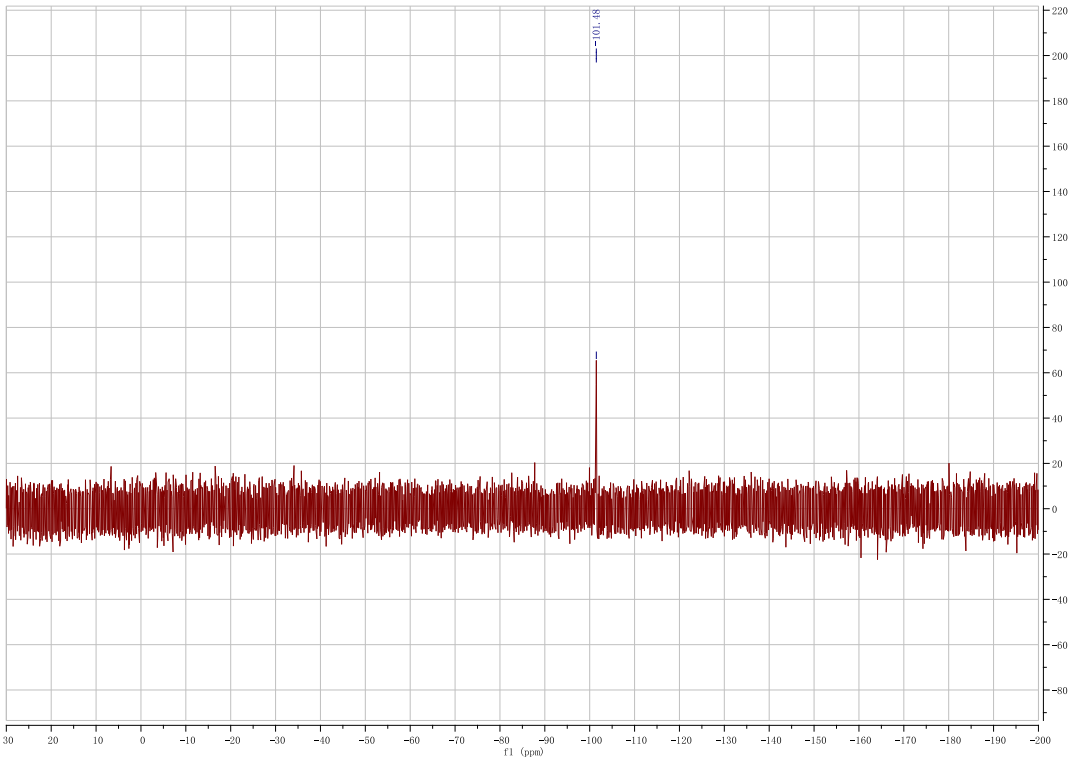
^{1}H NMR of 6'-F-CmTSR



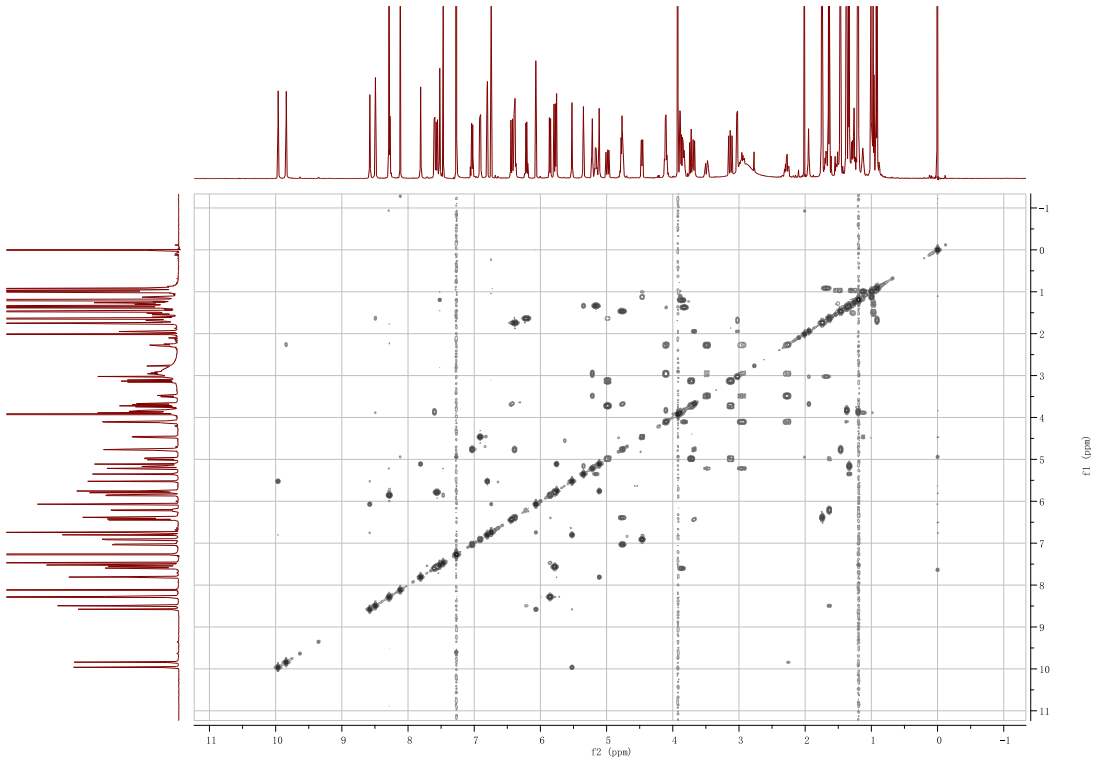
^{13}C NMR of 6’-F-CmTSR



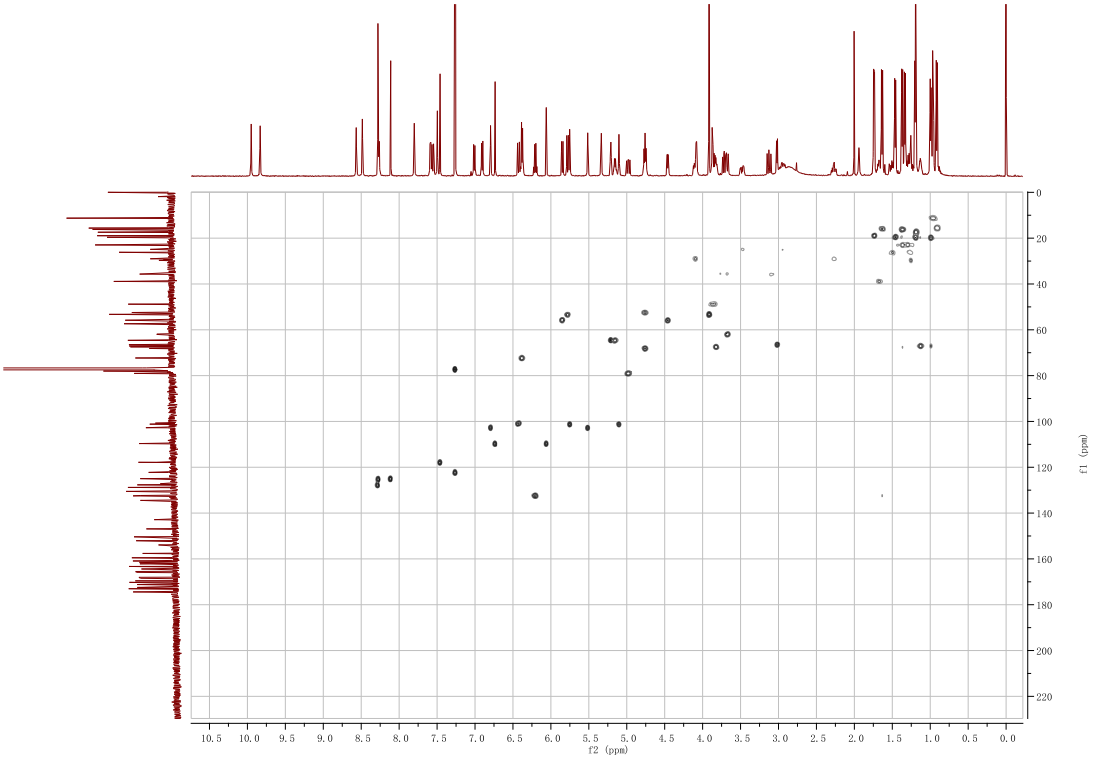
^{19}F NMR of 6’-F-CmTSR



gCOSY of 6’-F-CmTSR



gHSQC of 6’-F-CmTSR



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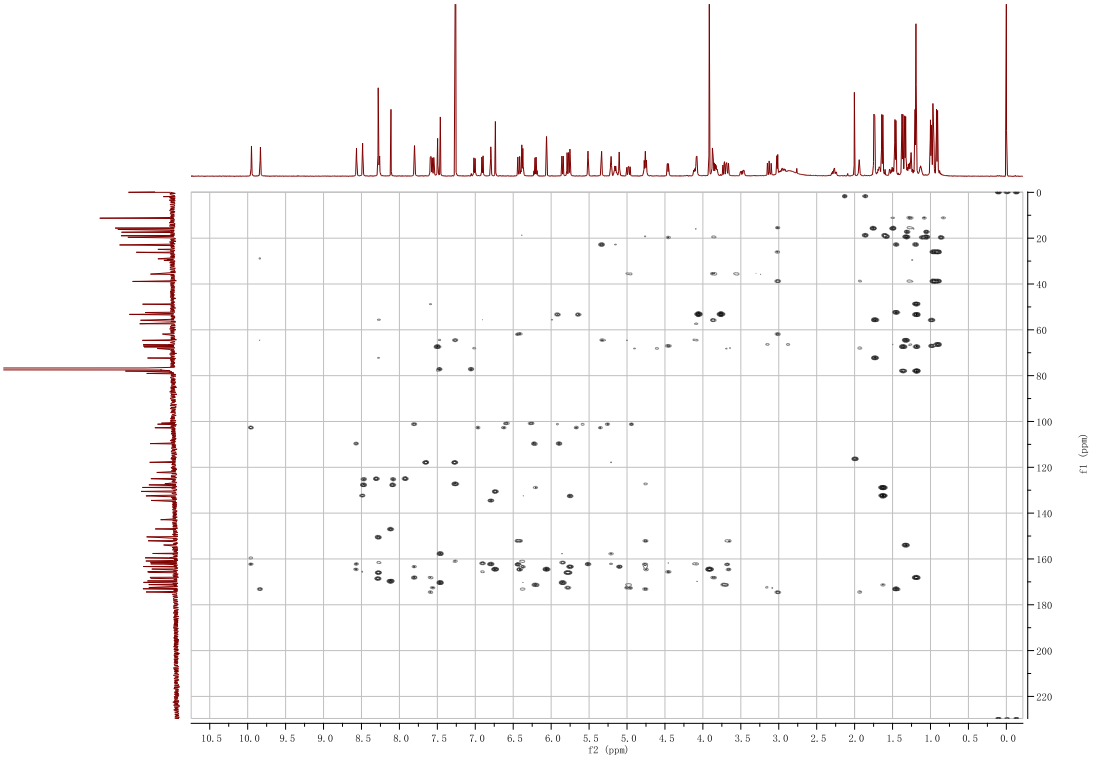
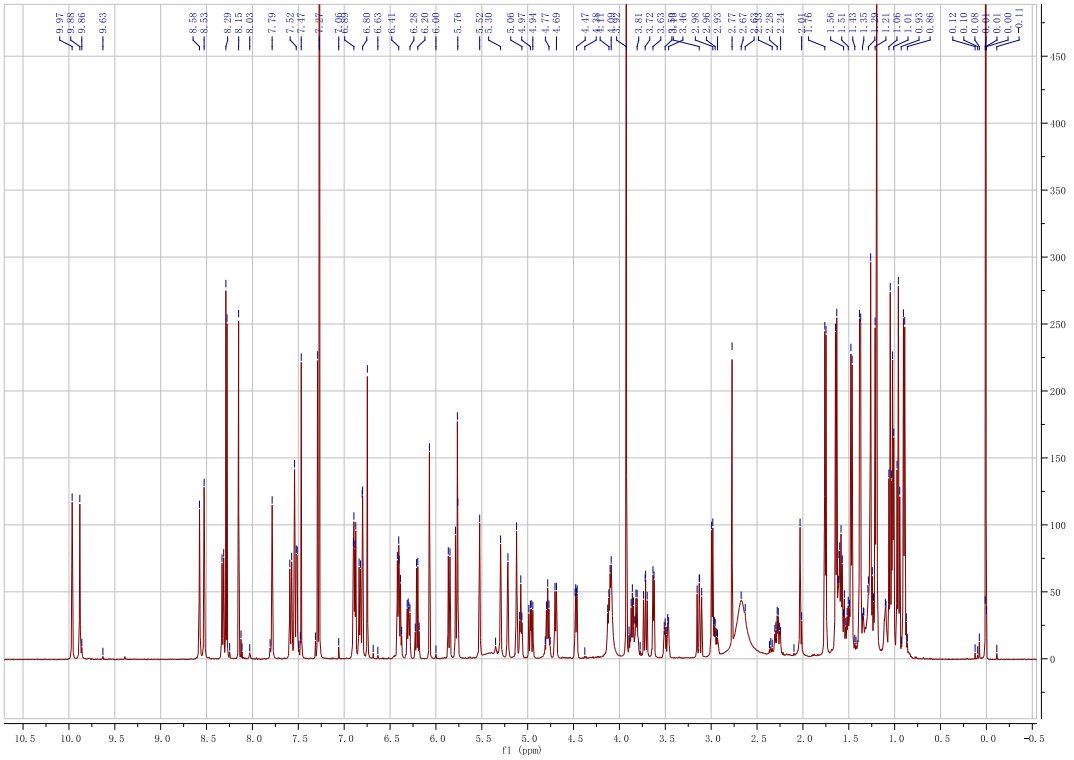


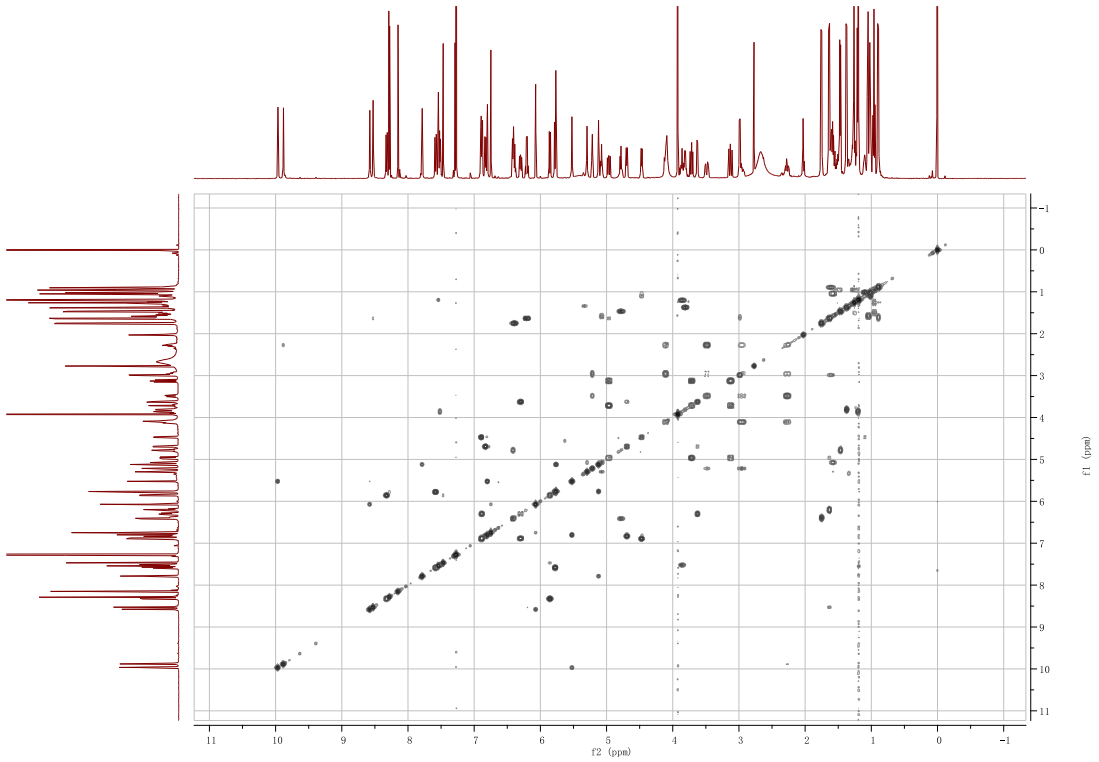
Figure S7. 1D and 2D NMR spectra of **12'-Me-CmTSR**

^{1}H NMR of 12'-Me-CmTSR

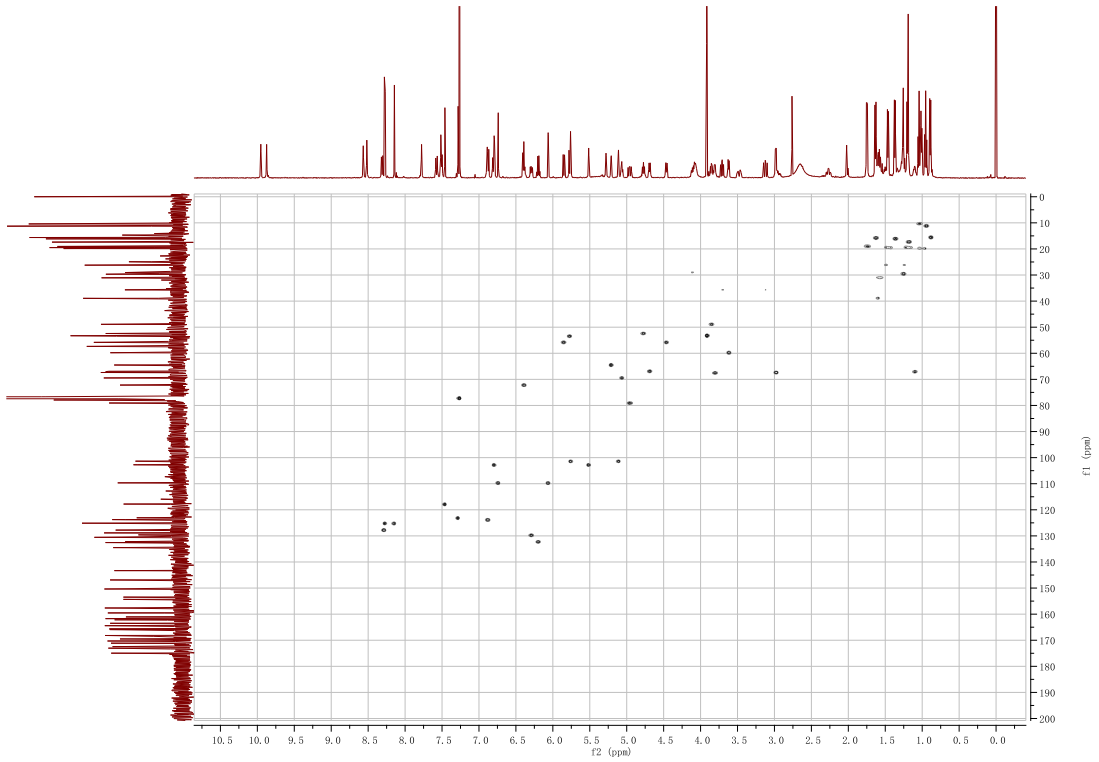


^{13}C NMR of 12’-Me-CmTSR

gCOSY of 12’-Me-CmTSR



gHSQC of 12’-Me-CmTSR



gHMBC of 12’-Me-CmTSR

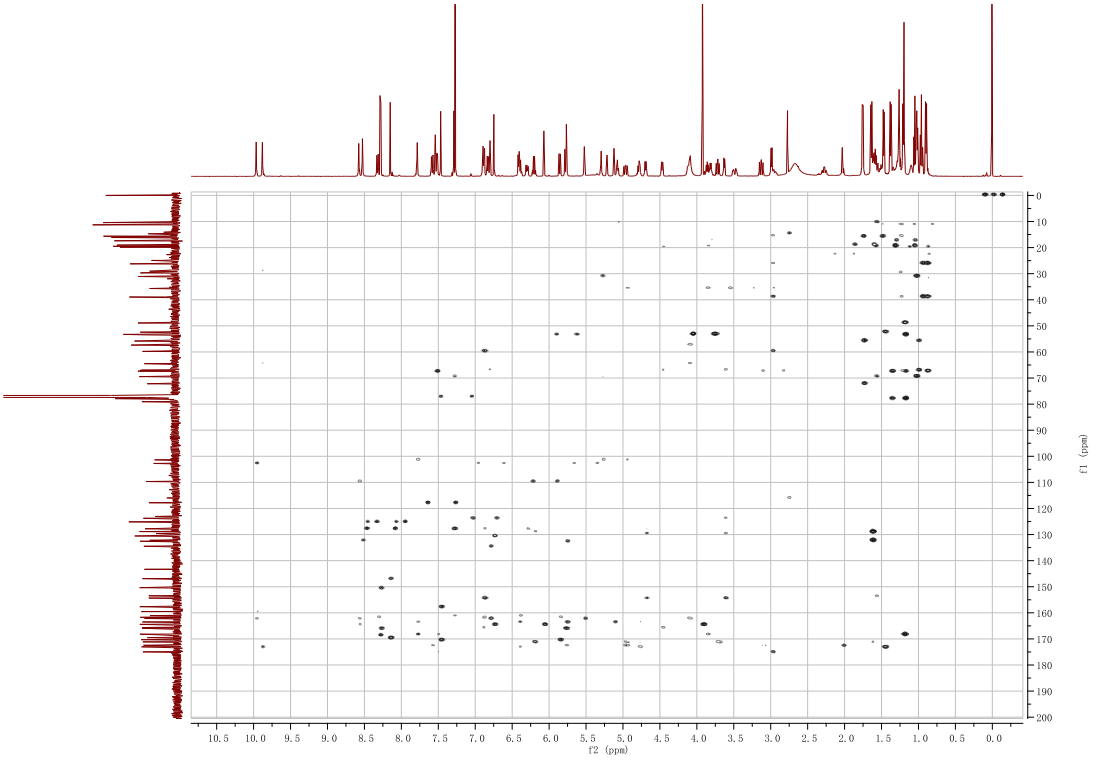
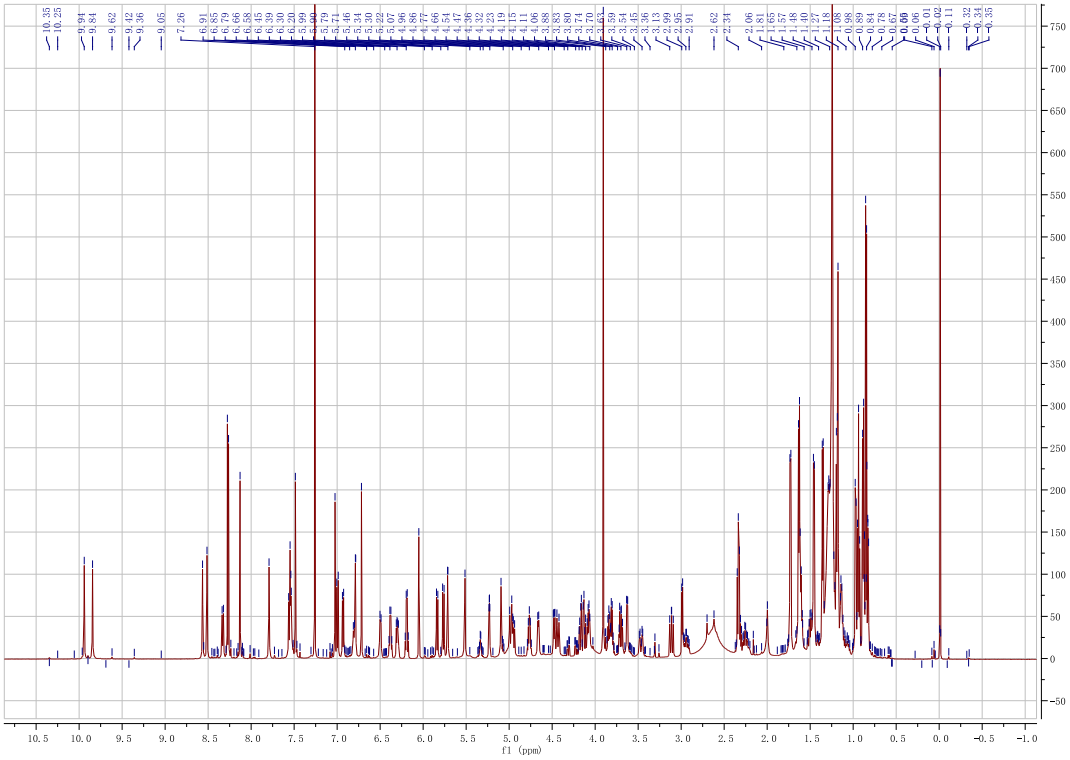
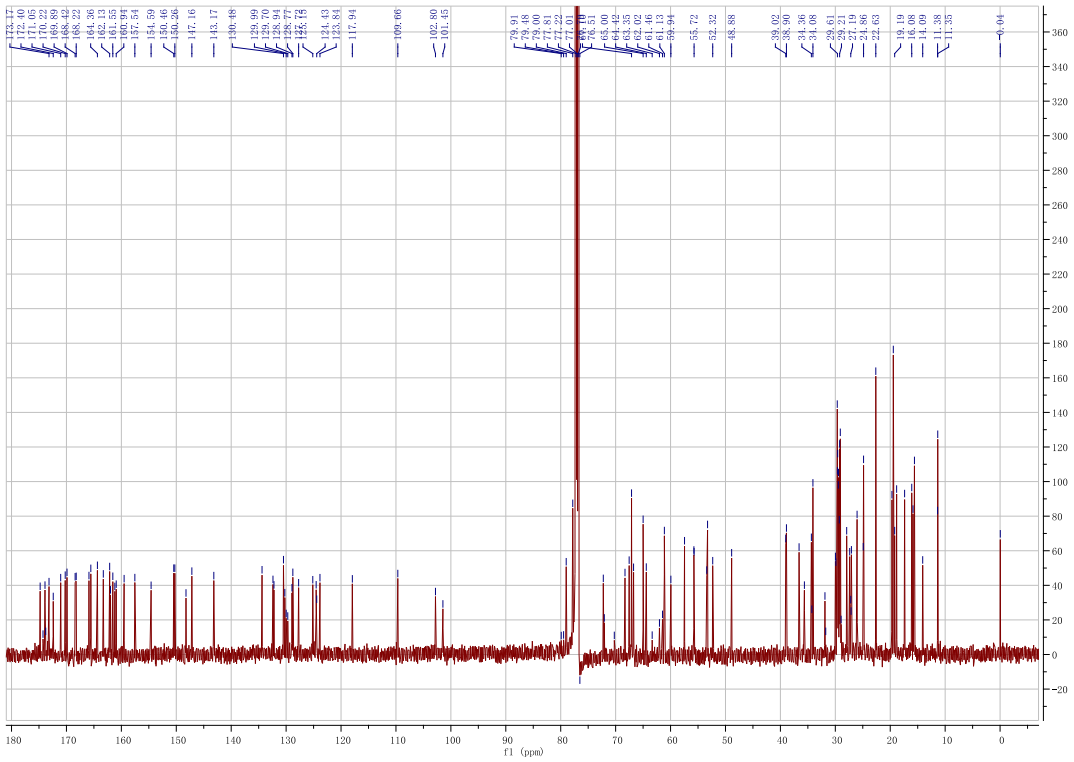


Figure S8. 1D and 2D NMR spectra of **12'-de-Me-CmTSR**

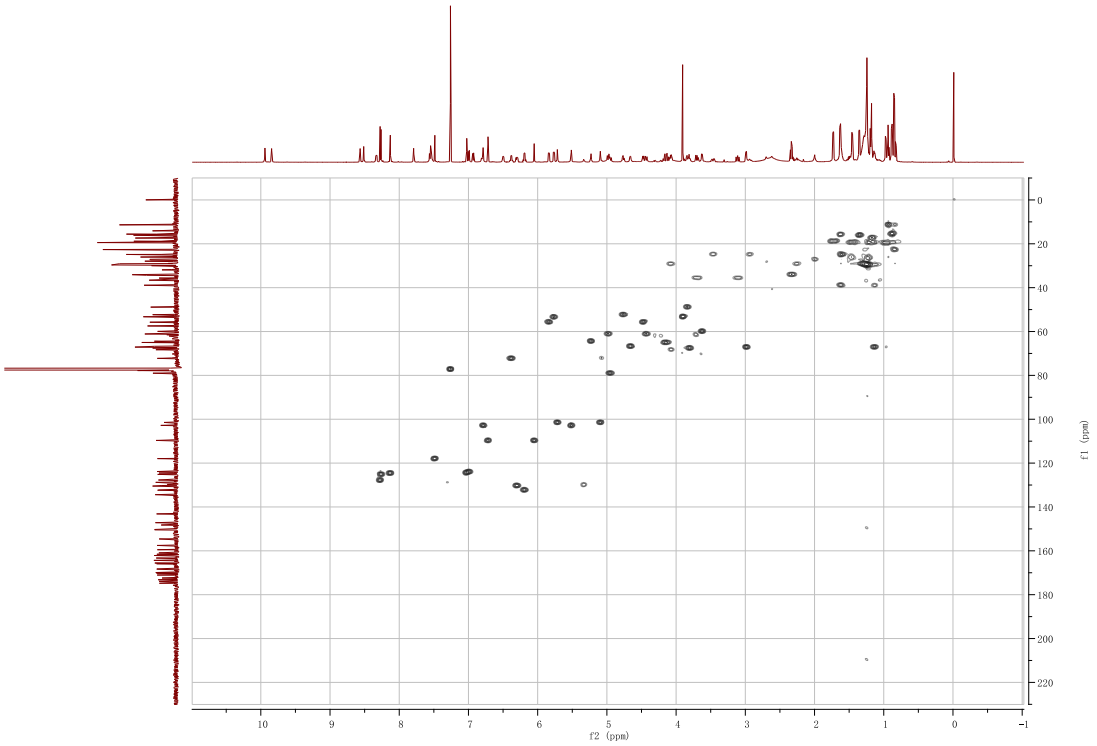
^{1}H NMR of 12'-de-Me-CmTSR



^{13}C NMR of 12’-de-Me-CmTSR



gCOSY of 12’-de-Me-CmTSR



gHMBC of 12’-de-Me-CmTSR

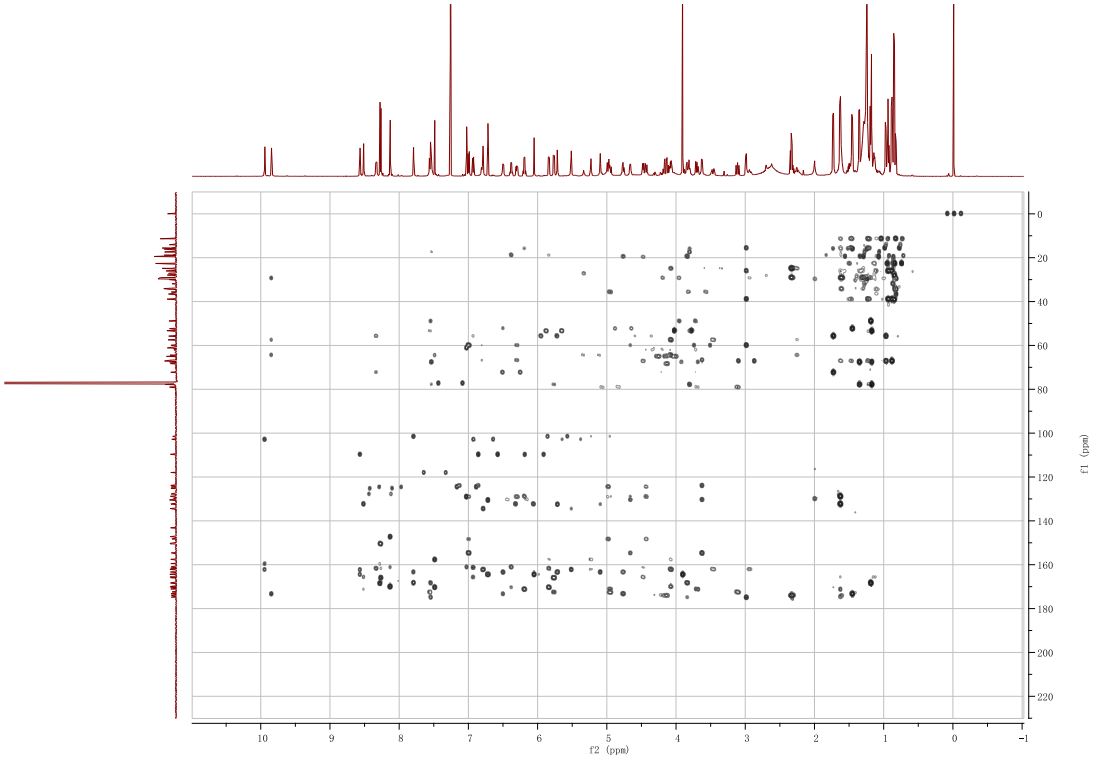
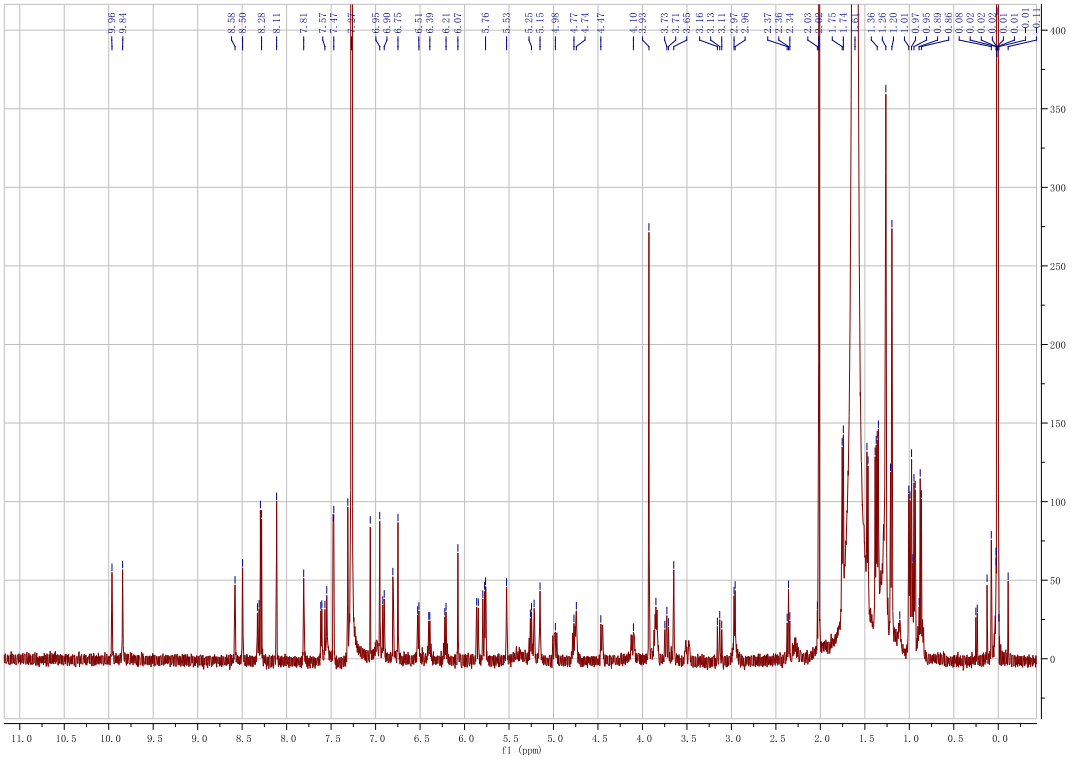


Figure S9. 1D and 2D NMR spectra of **6'-Cl-CmTSR**

^{1}H NMR of 6'-Cl-CmTSR



gCOSY of 6’-Cl-CmTSR

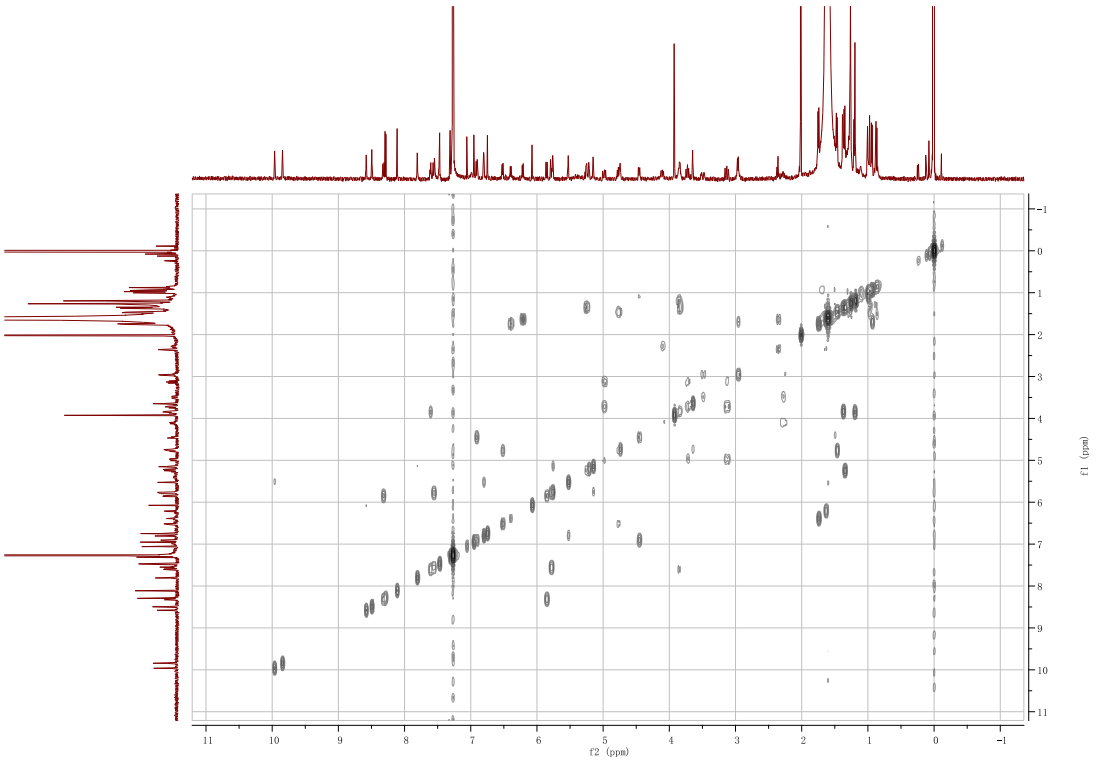
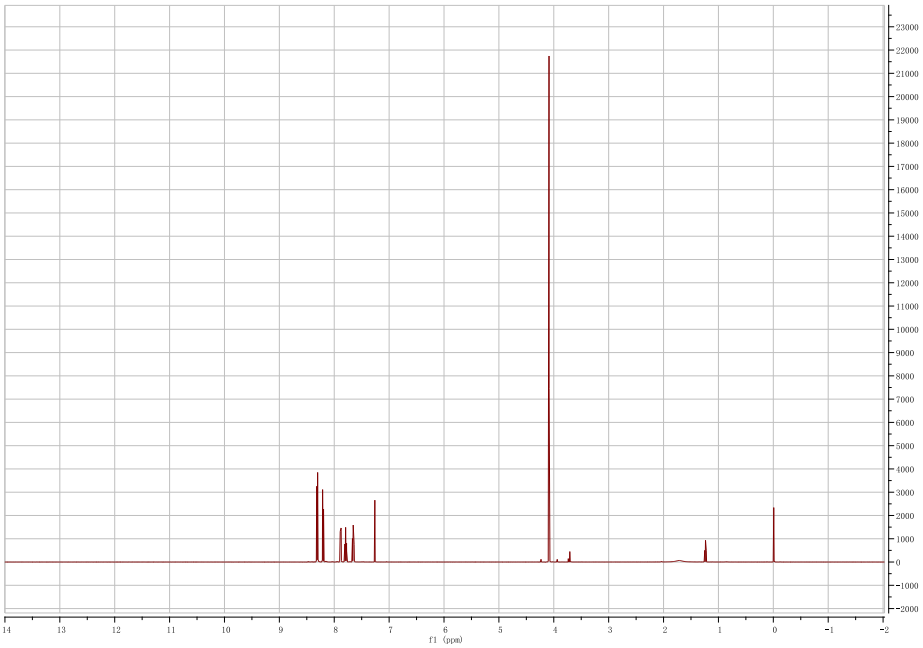
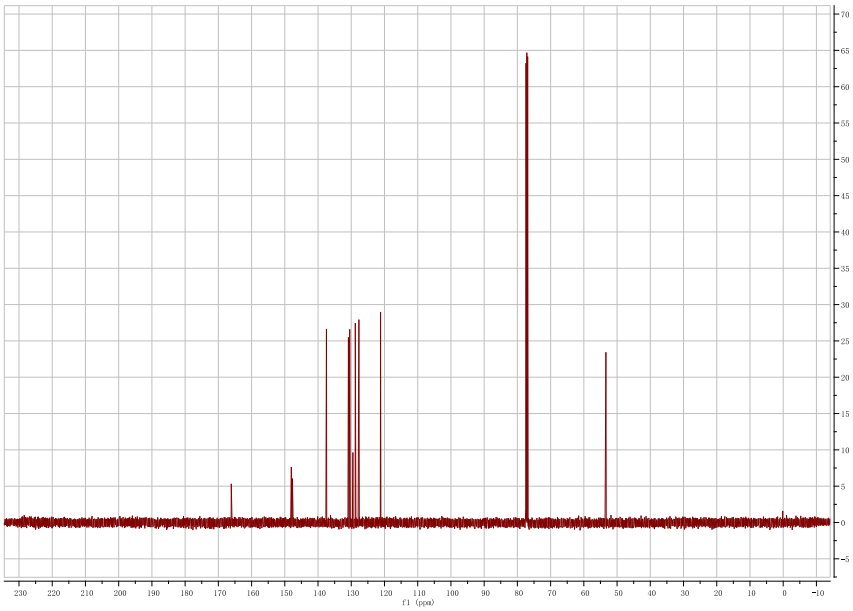


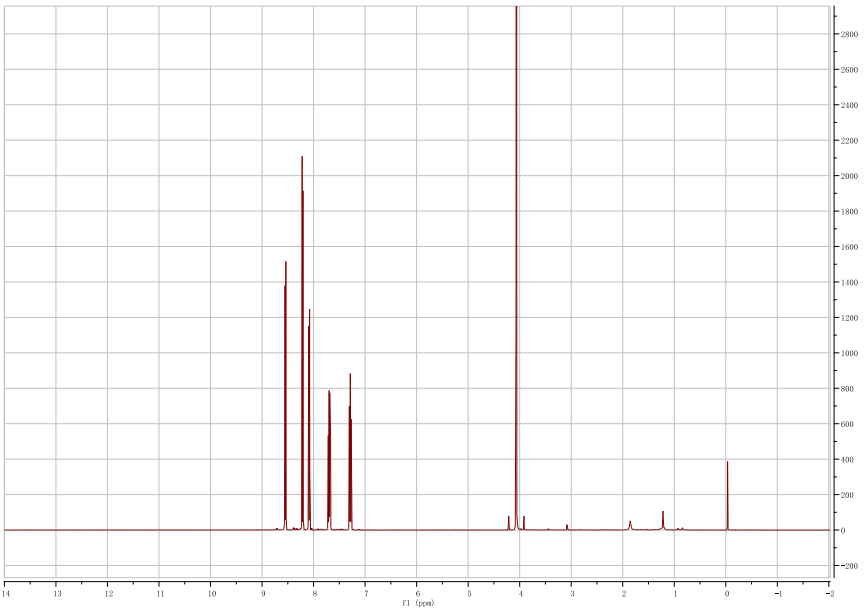
Figure S10. NMR analysis of methyl quinoline-2-carboxylates and its analogues

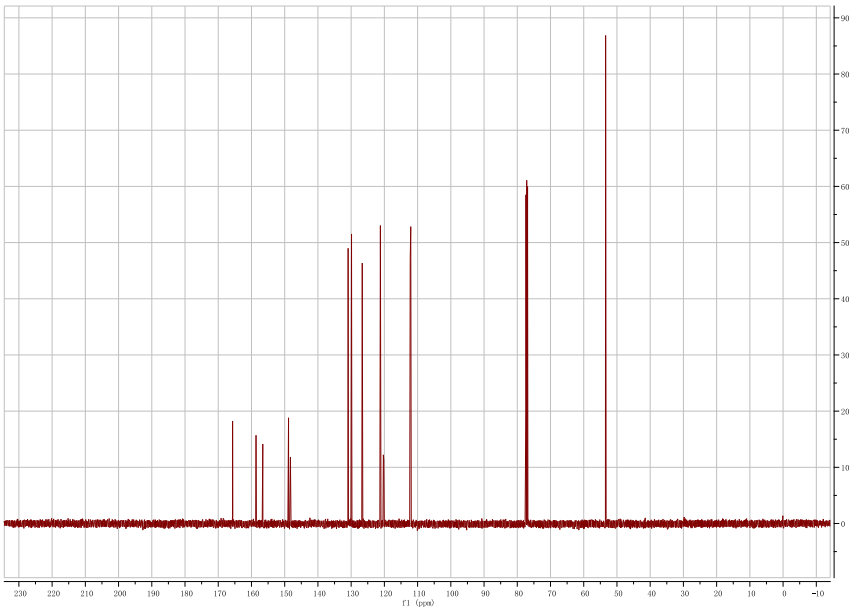
Methyl quinoline-2-carboxylate

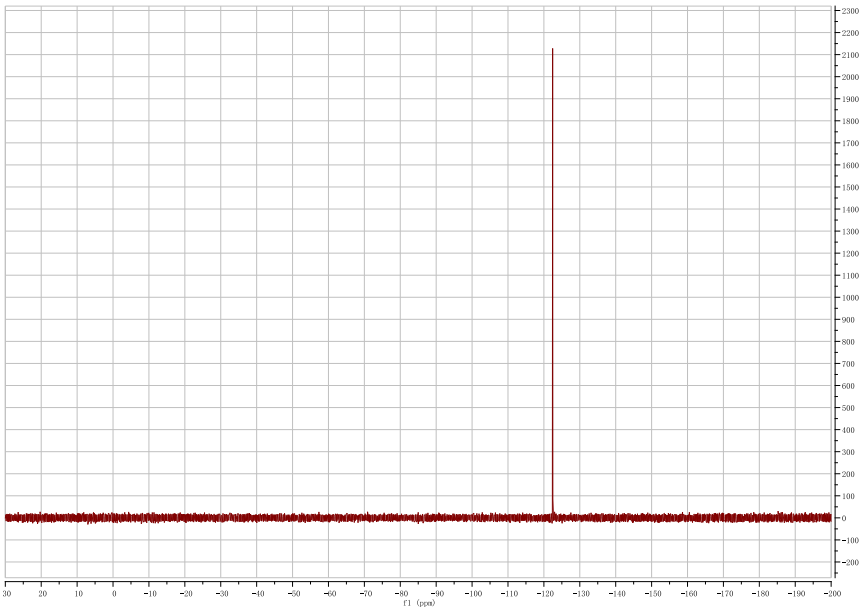




Methyl 5-fluoro-quinoline-2-carboxylate



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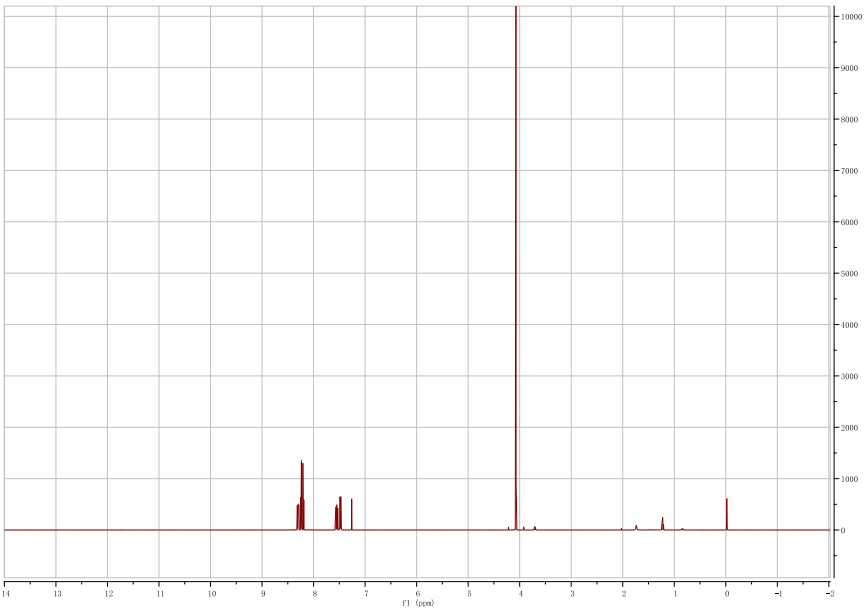
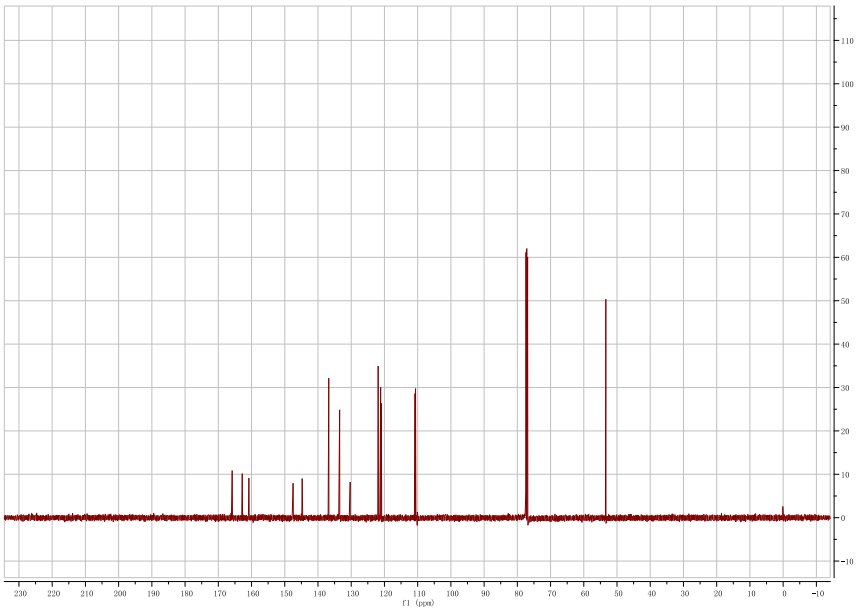
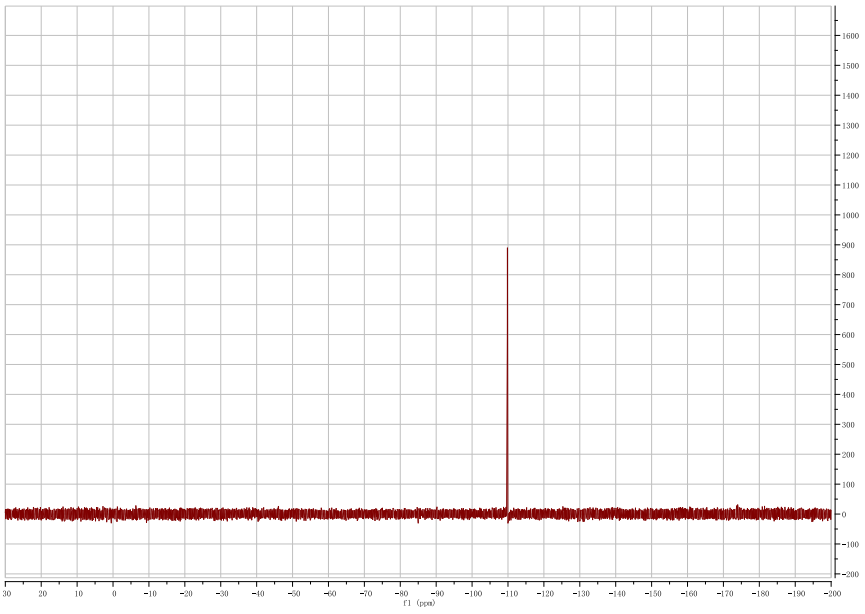
f1 (ppm)

Methyl 6-fluoro-quinoline-2-carboxylate



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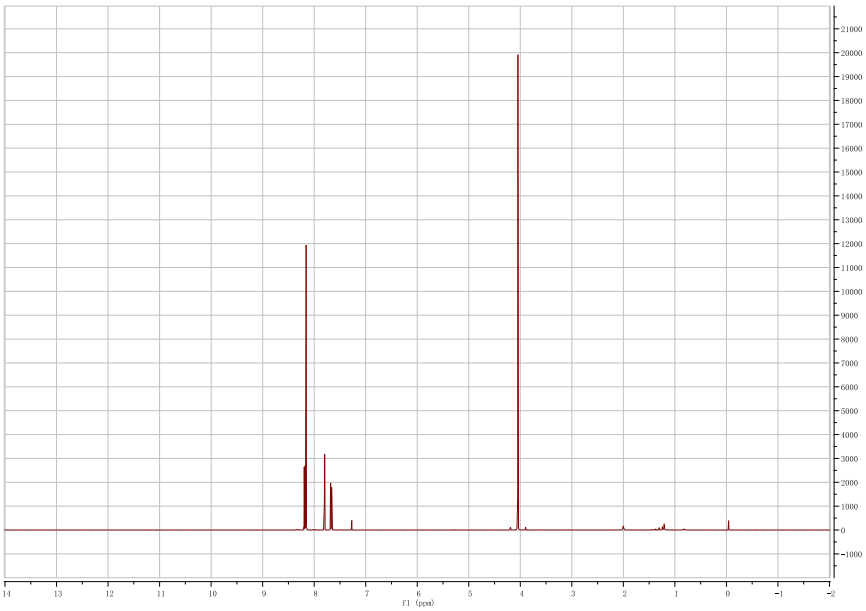
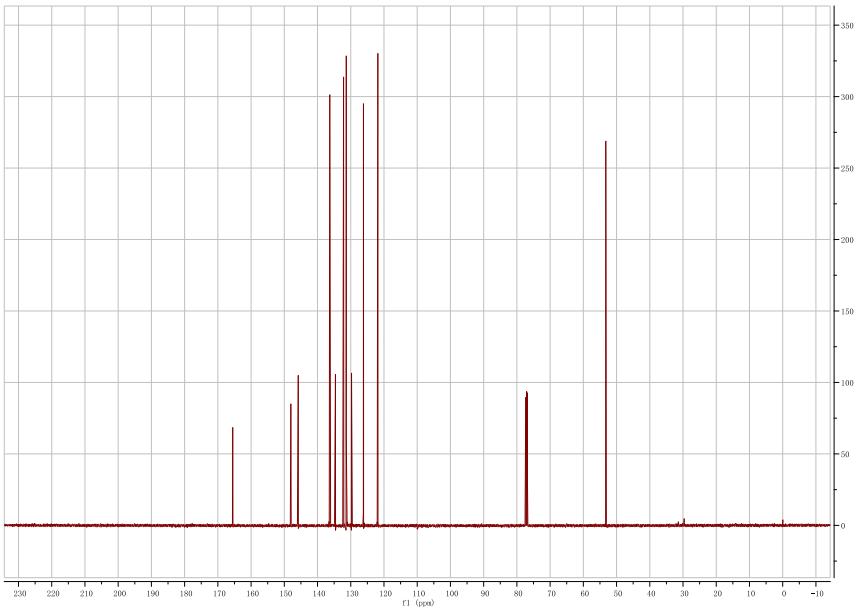
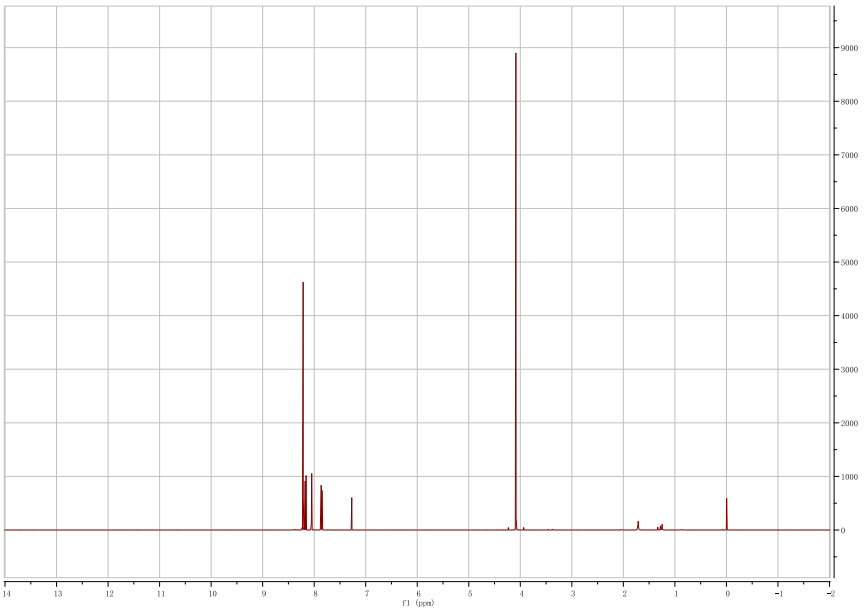
f1 (ppm)

Methyl 6-chloro-quinoline-2-carboxylate





Methyl 6-Bromo-quinoline-2-carboxylate



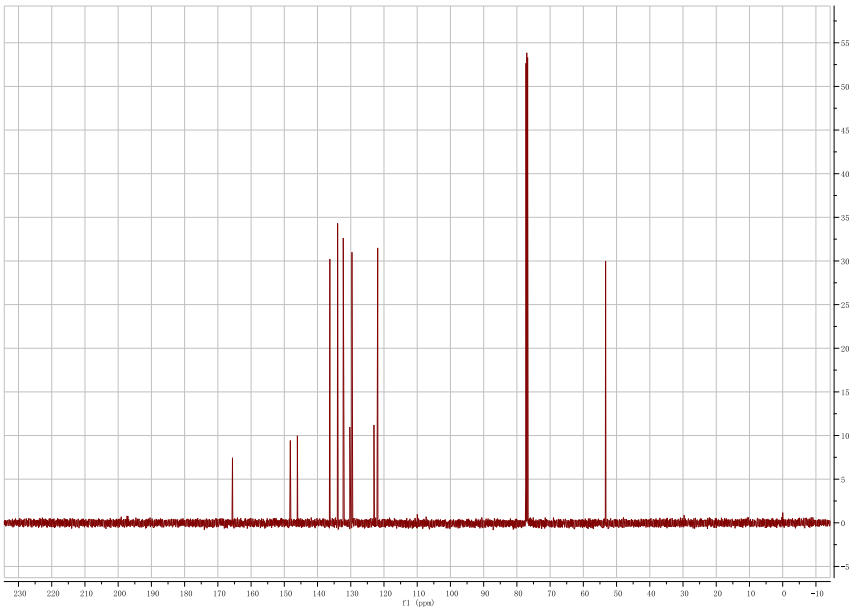
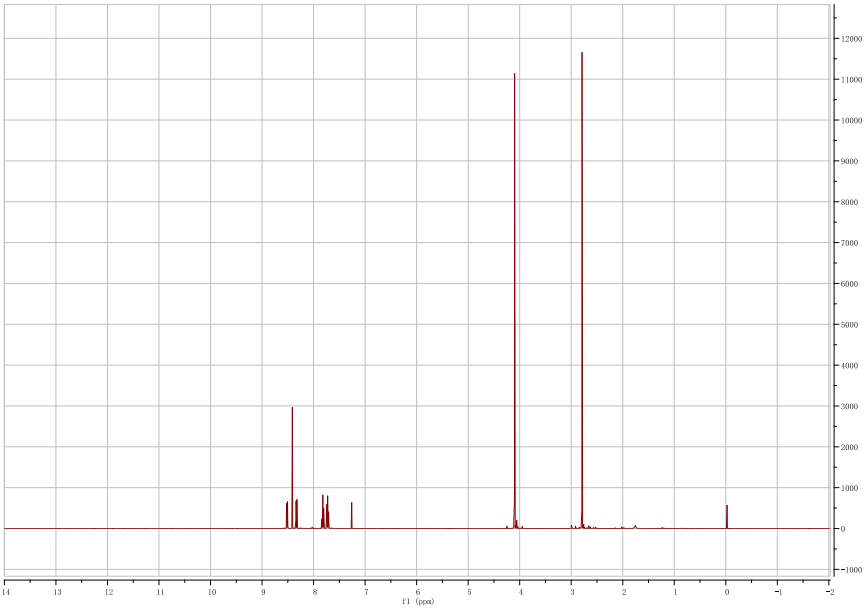
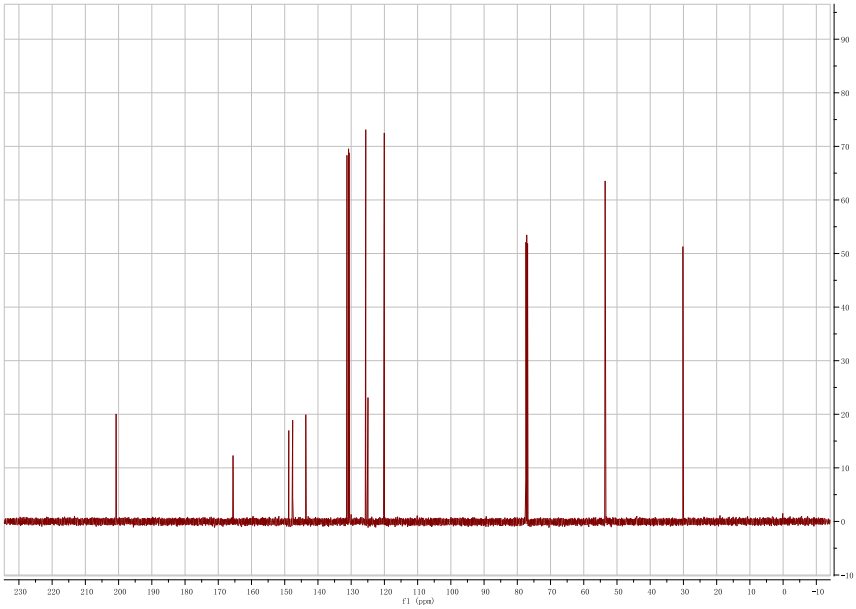


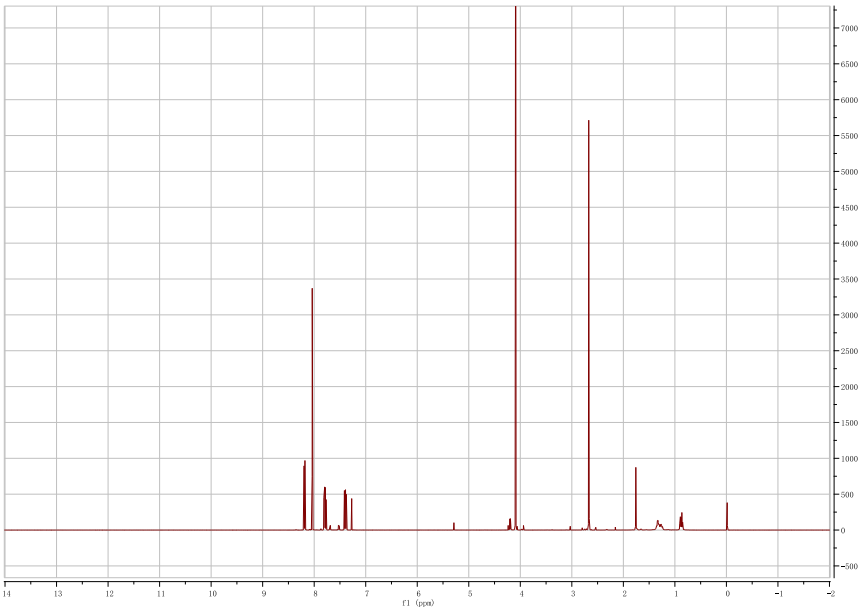
Figure S11. NMR analysis of methyl 4-acetyl-quinoline-2-carboxylate and its analogues

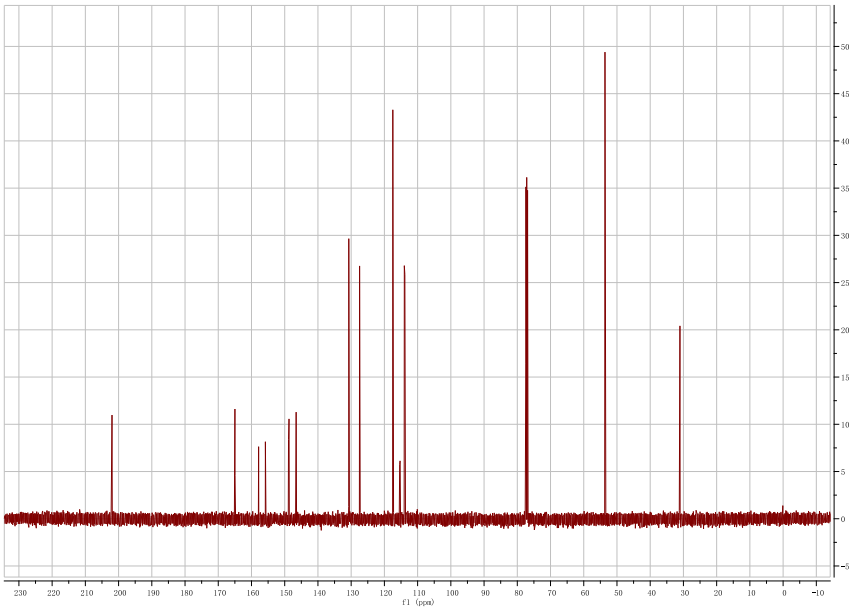
Methyl 4-acetyl-quinoline-2-carboxylate

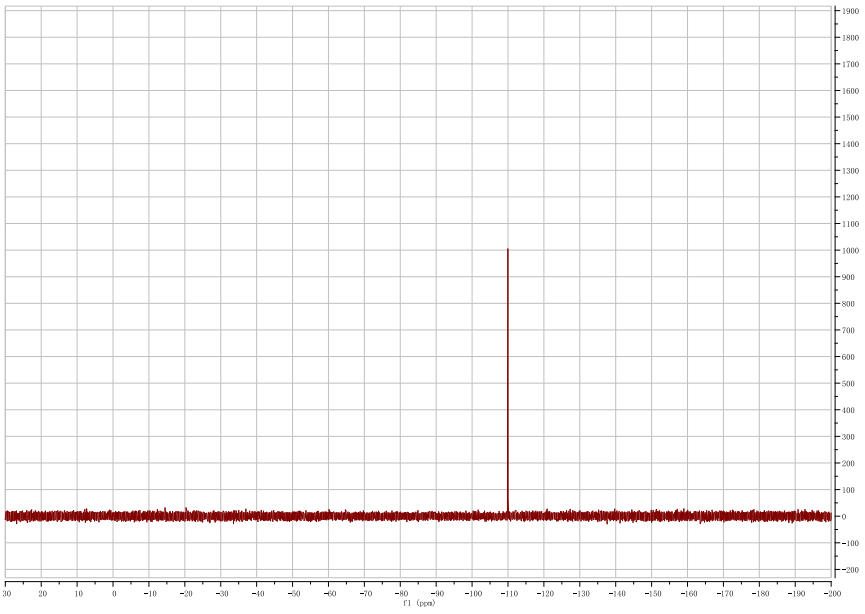




Methyl 4-acetyl-5-fluoro-quinoline-2-carboxylate (**1**)



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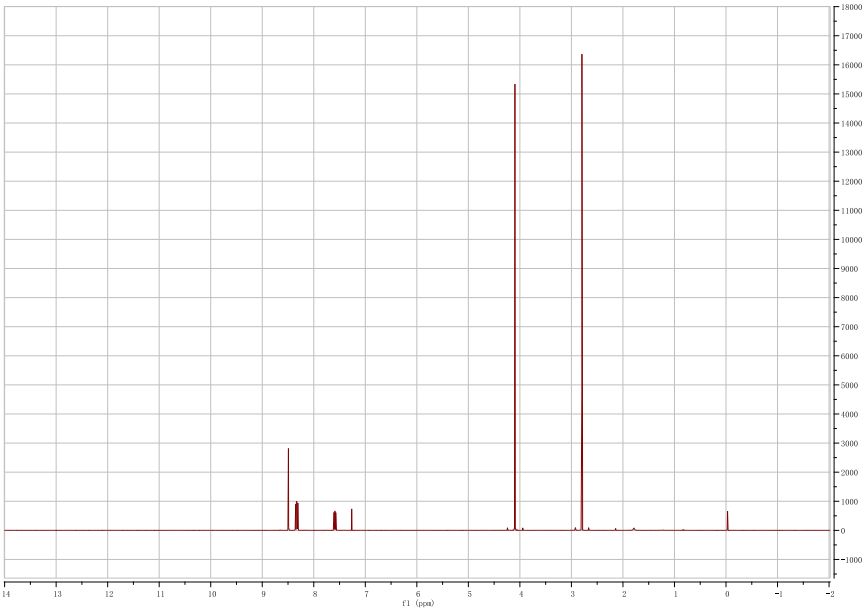
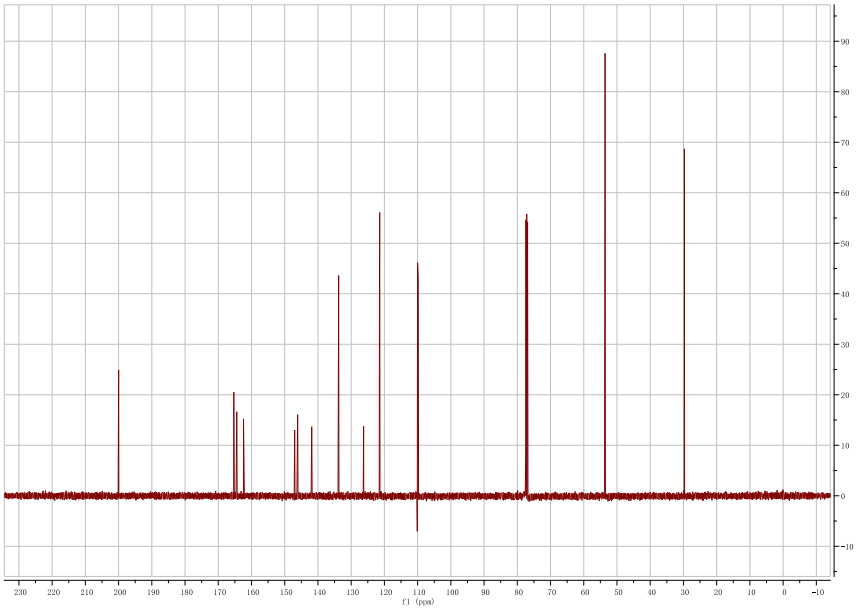
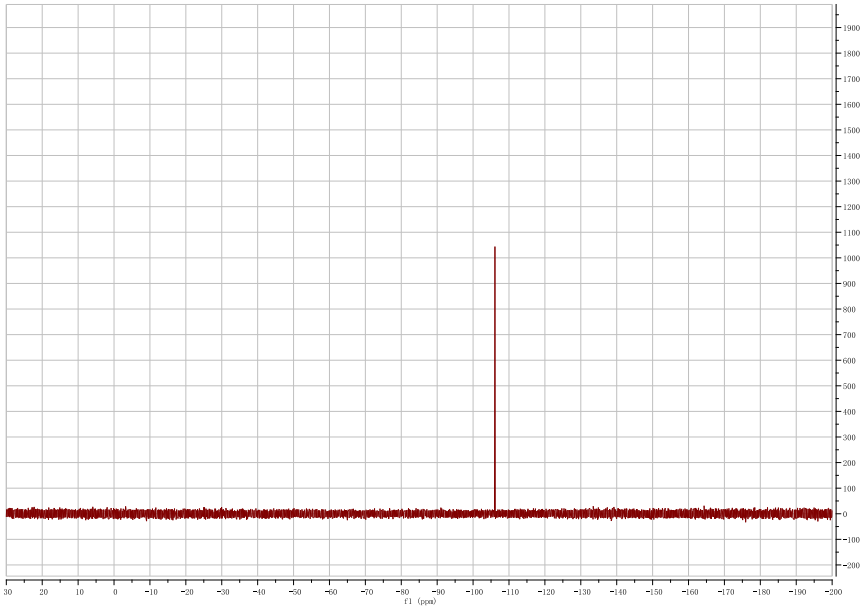
f1 (ppm)

Methyl 4-acetyl-6-fluoro-quinoline-2-carboxylate (**2**)



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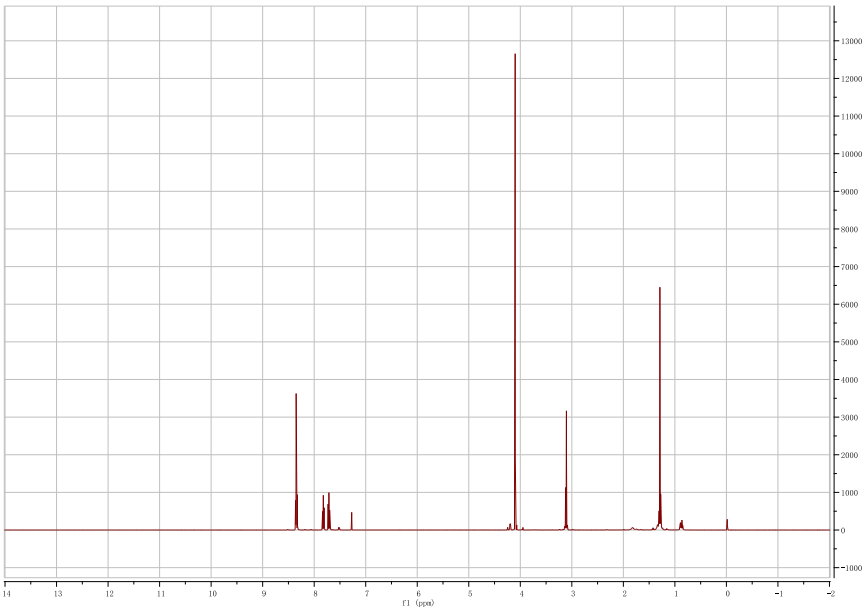
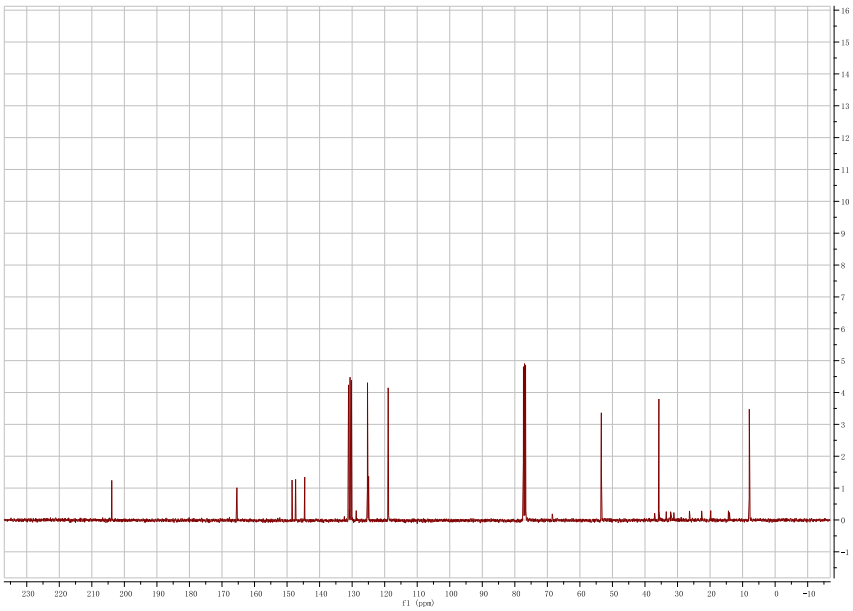
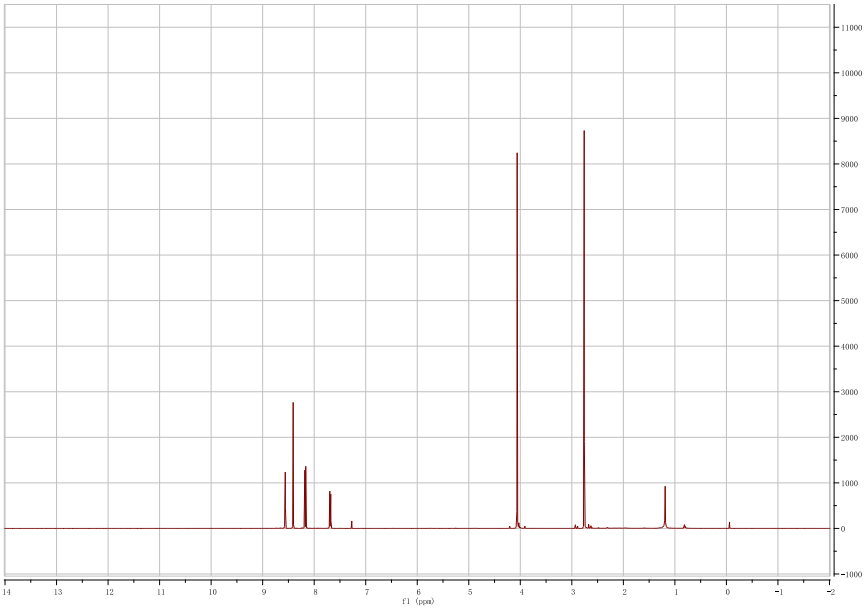
f1 (ppm)

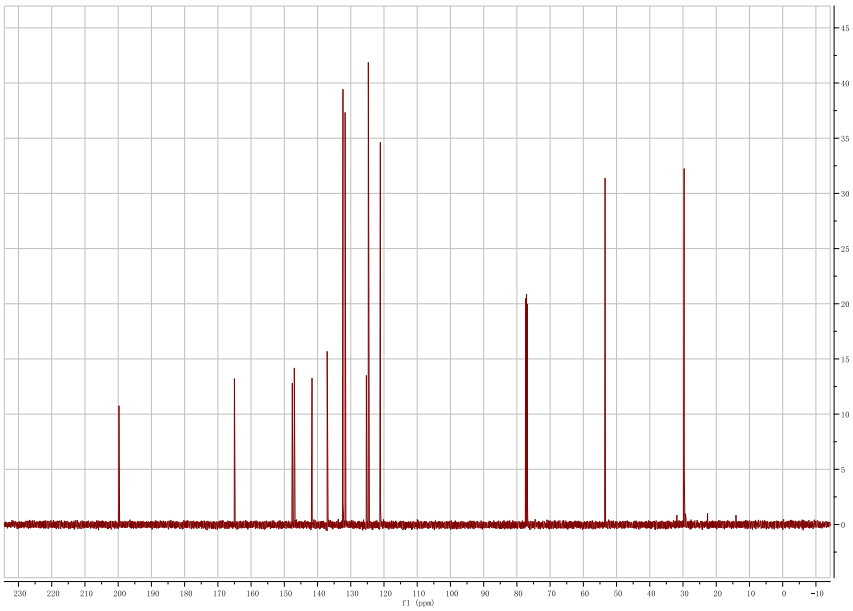
Methyl 4-propionyl-quinoline-2-carboxylate (**3**)



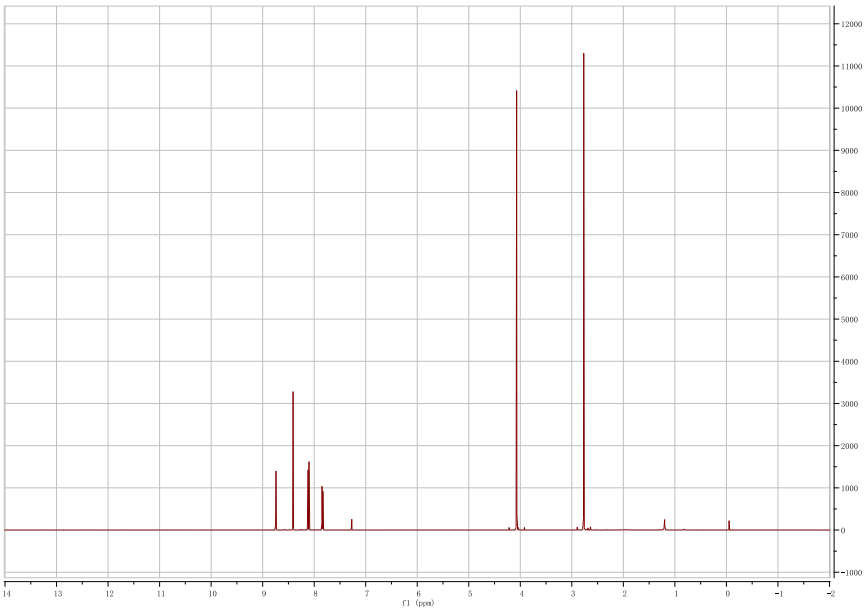


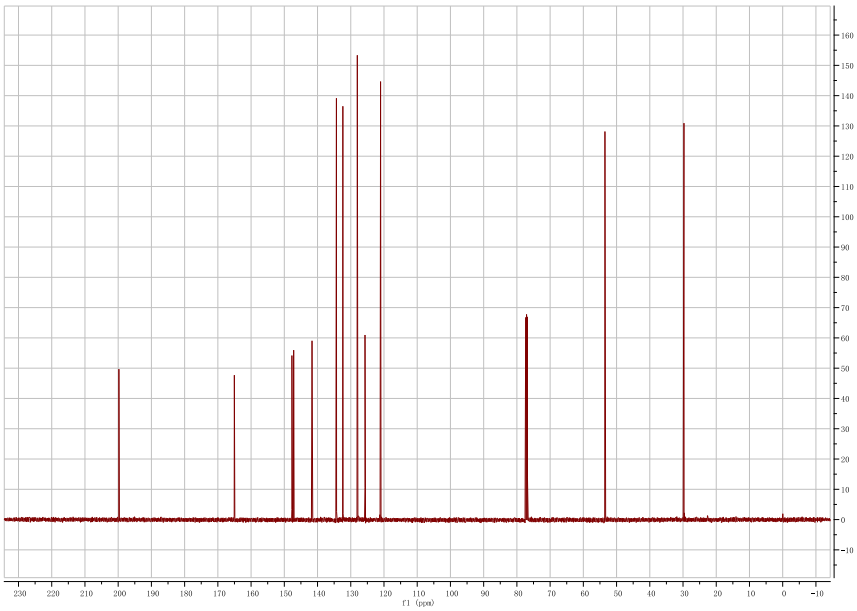
Methyl 4-acetyl-6-chloro-quinoline-2-carboxylate (**4**)



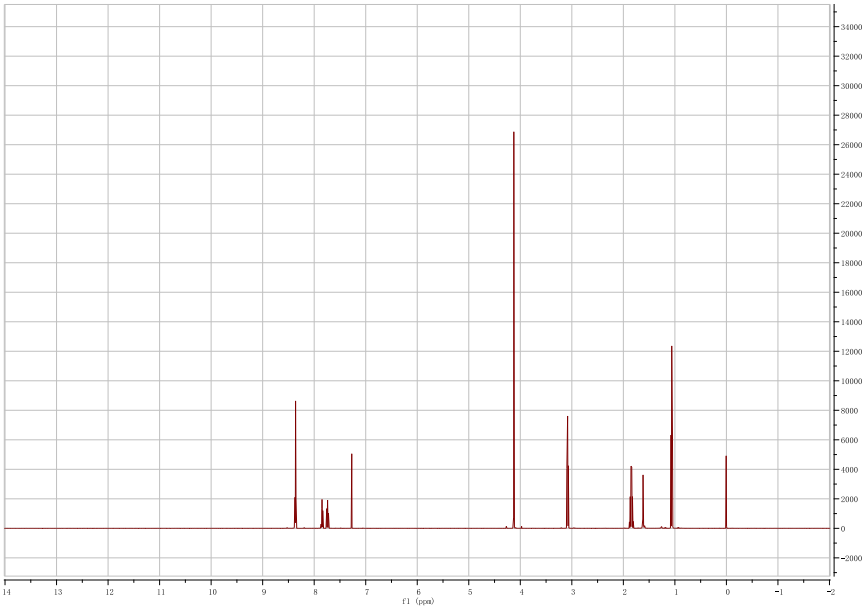


Methyl 4-acetyl-6-bromo-quinoline-2-carboxylate (**6**)





Methyl 4-butyrylquinoline-2-carboxylate (**7**)



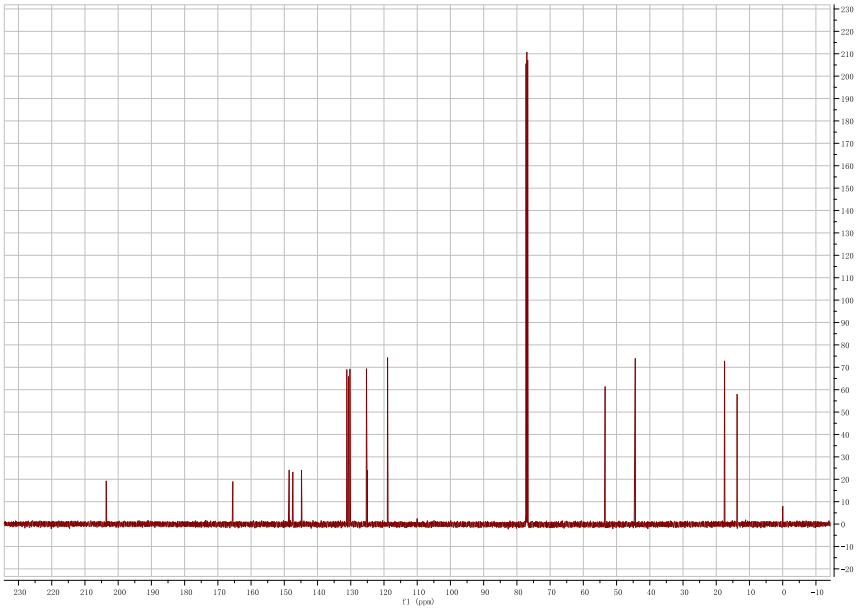
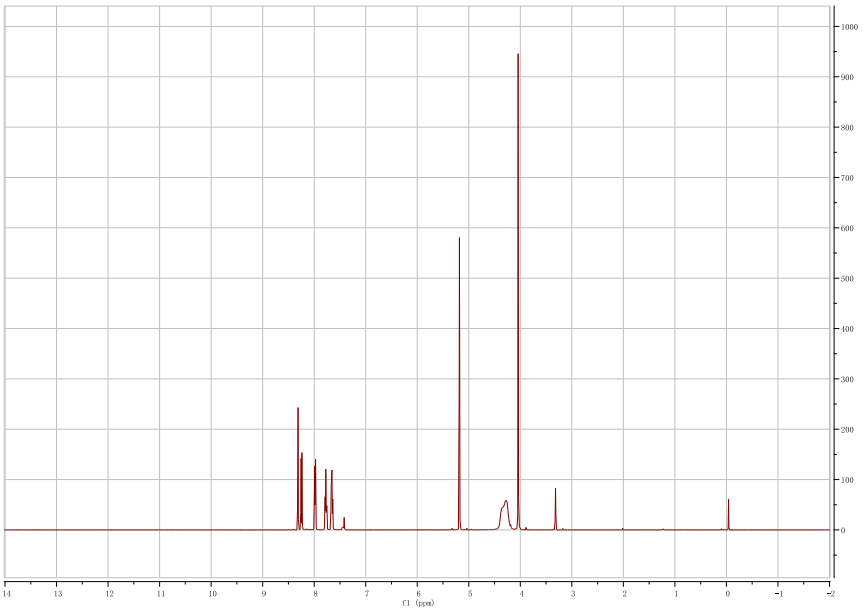
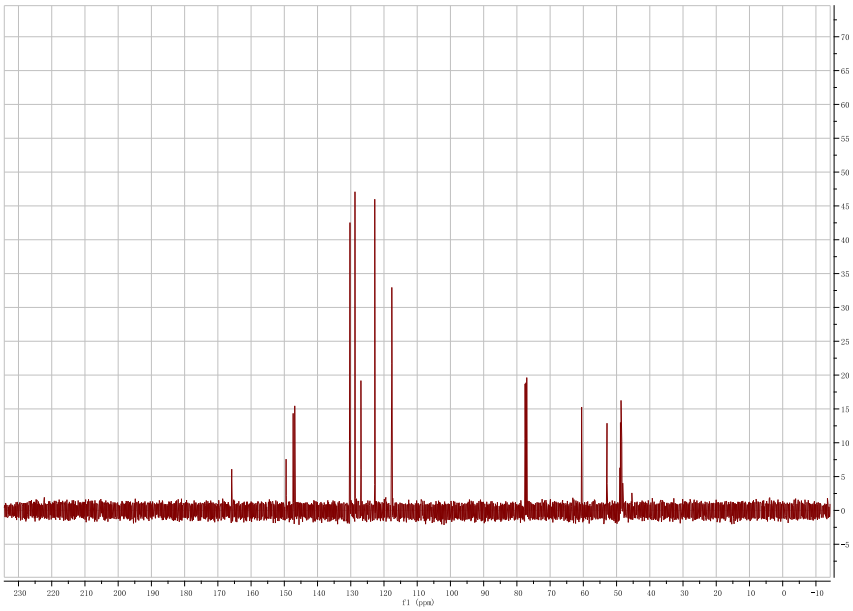


Figure S12. NMR analysis of methyl 4-(hydroxymethyl)quinoline-2-carboxylate(**5**)





5. Reference

[1]. R. Liao, and W. Liu, *J. Am. Chem. Soc.*, 2011, **133**, 2852.
[2]. M. Bierman, R. Logan, K. O'Brien, E. T. Seno, R. N. Rao, and B. E. Schoner, *Gene*, 1992, **116**, 43.
[3]. S. Wang, Q. Zheng, J. Wang, Z. Zhao, Q. Li, Y. Yu, R. Wang, and W. Liu, *Org. Chem. Front.*, 2015, **2**, 106.
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