Supporting Information

Carbohydrate-Conjugated 4-(1,3,2-Dithiarsolan-2-yl) Aniline as a Cytotoxic Agent Against Colorectal Cancer

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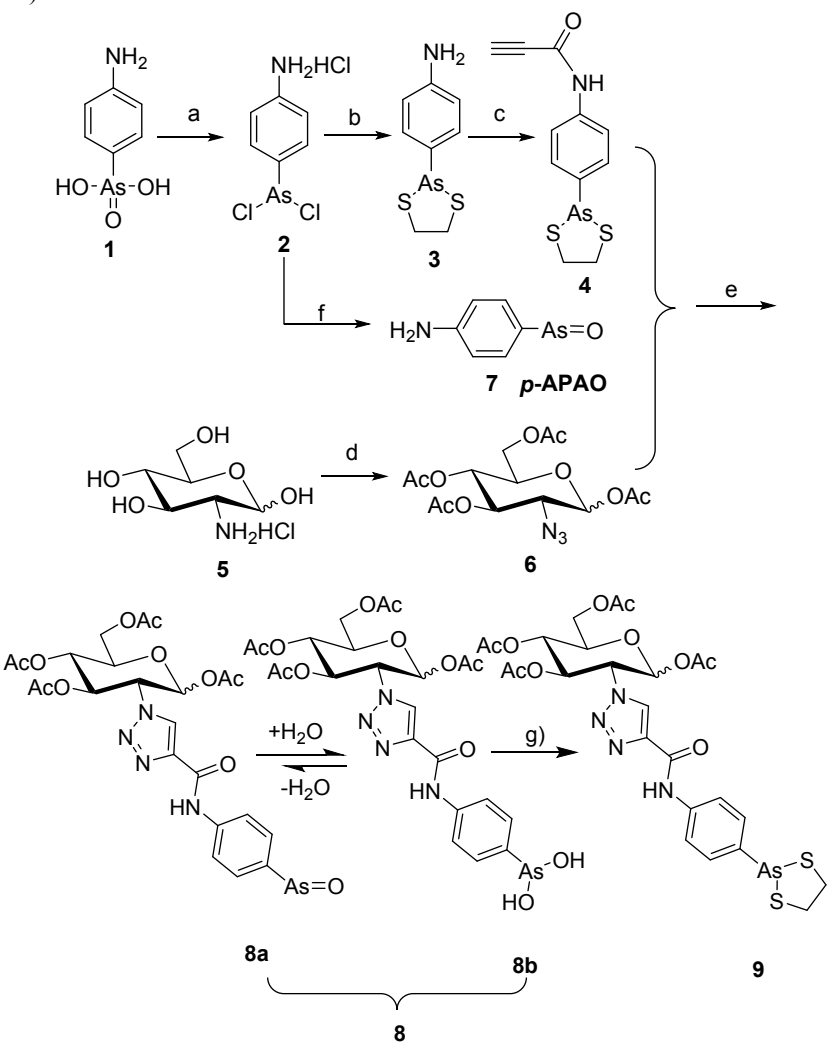
Materials and methods

All the chemical reagents and solvents were purchased from Sinopharm Group Company limited， and used without further purification, unless specified otherwise. 4-Aminophenylarsonic acid was bought from Tokyo Chemical Industry (Shanghai) Development Co., Ltd. All anhydrous reactions were performed under nitrogen atmosphere. Organic phases during work-up were dried over anhydrous Na_2SO_4 and removed by evaporation under reduced pressure.

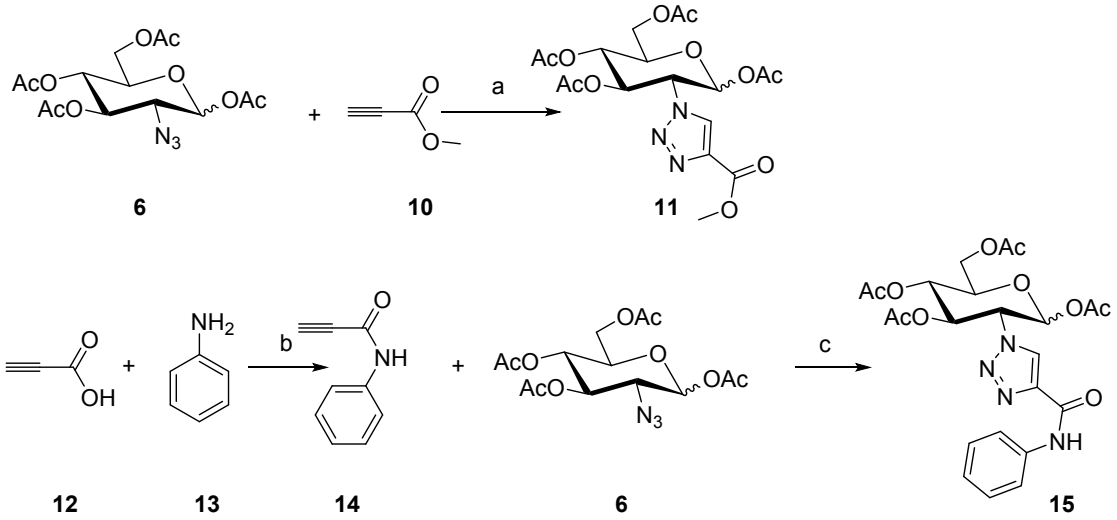
Equipment

Purities of the intermediates were established by thin-layer chromatography (TLC). Thin Layer chromatography was carried out by silica gel F254 and column chromatography was conducted over silica gel (200- 300 mesh), both of which were obtained from Qingdao Ocean Chemicals (Qingdao, China). In all experiments, water used was distilled and purified by a Milli-Q system (Millipore, USA). ^1H NMR and ^{13}C NMR spectra of final compounds were recorded on a Bruker Ultrashied 400MHZ Plus spectrometer using TMS as internal standard. All chemical shifts are reported in the standard δ notation of parts per million. High Resolution Mass Spectra were performed using Waters UPLC Class I/XevoG2Q-Tof. Microwave reactions were performed using AntonPaar Monowave 300 microwave reactor (Austria).



Reaction conditions: a) SO_2, KI, HCl, MeOH; b) ethane-1,2-dithiol, $NaHCO_3$, MeOH, 82%; c) propiolic acid, dicyclohexyl carbodiimide, CH_2Cl_2, r.t, 64%; d)(i)TfN_3, $CuSO_4{\cdot}5H_2O$,CH_3CN; (ii) Ac_2O, pyridine; e) sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, microwave, 100 °C, 38.3%; f) $NH_3{\cdot}H_2O$, N_2, 53.9%; g) ethane-1,2-dithiol, MeOH, 69%.

Scheme 1 Synthesis routes of Compounds **7** and **9**



Reaction conditions: a) sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, microwave, 100 °C, 61.1%; c) propiolic acid, dicyclohexyl carbodiimide, CH_2Cl_2, r.t, 70%; c) sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, microwave, 100 °C, 32.5%.

Scheme 2 Synthesis routes of Compounds **11** and **15**

Synthesis of 4-(1,3,2-dithiarsolan-2-yl)aniline (3) [3]

Compound 3 was synthesized in yield of 87% as described as previuos literature[3].It was characterized with ^{1}H NMR ^{13}C NMR and HRMS. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 7.43-7.40 (m, 2H, aromatic), 6.67–6.64(m, 2H, aromatic), 3.77(br, 2H, amine), 3.36-3.30 (m, 2H, CH_2), 3.24-3.18 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100MHz) δ: 147.59, 132.12, 114.91, 41.59. HRMS (ESI) m/z calcd for $C_8H_{11}AsNS_2$ $[M+H]^+$ 259.9549, found 259.9549.

Synthesis of N-(4-(1,3,2-dithiarsolan-2-yl)phenyl) propiolamide (4)

Compound **3** (0.761 g, 2.93 mmol) and dicyclohexylcarbodiimide (0.726 g, 3.52mmol) were dissolved in anhydrous dichloromethane (10 mL) at 0 °C for 30 min. Then propiolic acid (216 μL, 3.52 mmol) was added into the reaction mixture at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. The reaction mixture was stirred for 4 h at room temperature. It was monitored by TLC (R_f=0.55, petroleum/ethyl acetate = 4/1). Removal the solvent under reduced pressure afforded the residue, which was purified through silica gel chromatography with 20% ethyl acetate in petroleum ether to obtain the product as pale yellow solid (584.5 mg, 64%). Compound **4** was characterized by ^{1}H NMR, ^{13}C NMR and HRMS. ^{1}H NMR (400MHz,$CDCl_3$) δ: 7.80(brs, 1H), 7.61(d, J = 8.4 Hz, 2H), 7.51(d, J = 8.4 Hz, 2H), 3.39-3.33(m, 2H), 3.18-3.12(m, 2H), 2.95(s, 1H).^{13}C NMR (100MHz, $CDCl_3$) δ:149.64,140.12, 137.74, 131.65,119.75,74.39, 41.85.HRMS (ESI) m/z calcd for$C_{11}H_{11}AsNOS_2$ $[M+H]^+$ 311.9498, found 311.9487.

Synthesis of 1,3,4,6-tetra-O-acetyl-2-azido-2-deoxy-D-glucopyranose(6)

Compound **6** was synthesized according to the literature[1-2]. The NMR and mass data of **6** were in agreement with the data reported in the literature.[1-2]

Synthesis of *p*-aminophenylarsine oxide (*p*-APAO) ***(7)***

Compound **7** was synthesized in yield of 53.9% as described previously. [4]

Synthesis of 1-(1-(1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranos-2-yl) -1H-1, 2, 3-triazole-4-carboxamido)phenyl arsonous acid (8)

Compound **6** (70.5 mg, 0.19 mmol) and compound **4** (64.7 mg, 0.21 mmol) were dissolved in *N*, *N*-dimethylforamide (1 mL). The solution of copper sulfate pentahydrate (106.3 mg, 0.43 mmol) in water (0.1 mL) was added to the above solution. Sodium L-ascorbate (3.8 mg, 0.019 mmol) was added to the above mixture solution under nitrogen. The reaction mixture was sealed in 10 mL vial and was heated to 100 °C for 30 min in the microwave reactor. The reaction was monitored by TLC (R_f = 0.26,

dichloromethane/methanol = 20/1). After completion of the reaction, the solvent was removed under reduced pressure. The resultant residue was dissolved in the mixture solvent of ethyl acetate (20 mL) and water(10 mL). It was extraced with ethyl acetate (20 mL) for three times. The organic layers were combined and washed with 10 mL brine. It was dried over anhydrous sodium sulfate. Filtration and removal of the solvent afforded the residue, which was purified through silica gel column chromatography with gradient eluent of the mixture of dichloromethane and methanol (100/1, 80/1, 40/1, 20/1) to obtain the compound **8** as white solid (44 mg, 38.3%). Compound **8** was characterized with ^{1}H NMR and HRMS. ^{1}H NMR (400 MHz, DMSO-d_6+MeOH): (α/β=1/2) β: δ 8.98(s, 1H), 8.21(s,1H, NH), 7.82-7.75(m, 4H, aromatic), 6.28(d, J = 9.2 Hz, 1H), 5.91-5.87 (m, 1 H, CH), 5.34-5.21 (m, 1 H, CH), 4.43-4.37(m, 2H, CH_2O), 4.27-4.10(m, 2H, CH), 2.16-1.90 (m, 12 H, 4AcO) ppm; α: δ 9.02(s, 1H), 8.27(s,1H, NH).65 (s, 1H), 7.82-7.75(m, 4H), 6.44(d, J = 1.2 Hz, 1H), 6.03-5.98 (t, 1 H, CH), 5.34-5.21 (m, 1 H, CH), 4.72-4.67(m, 2H, CH_2O), 4.27-4.10(m, 2H, CH), 2.16-1.90 (m, 12 H, 4AcO) ppm; HRMS m/z calcd for (**8a**) $C_{23}H_{26}AsN_4O_{11}$ $[M+H]^+$ 609.0814, found 609.0816; calcd for (**8b**) $C_{23}H_{28}AsN_4O_{12}$ $[M+H]^+$ 627.0920, found 627.0925.

Synthesis of 1-(1-(1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranos-2-yl)-1H-1,2,3-triazole-4 -carboxamidophenyl)-1,3,2-dithiaarsolane (9)

To the solution of compound **8** (100 mg, 0.16 mmol) in 10 mL anhydrous methanol, ethane-1,2-dithiol (14 μL, 0.1660 mmol) was added under nitrogen at room temperature. The reaction mixture was stirred at room temperature and was monitored with TLC (R_f= 0.5, dichloromethane/methanol = 20/1). Upon completion of the reaction, the solution was filtered to harvest the solid product, which was washed with cold methanol (2 mL× 3) and dried in vacuum chamber overnight to obtain the compound **9** as white solid (77.7 mg, 69%). The product was characterized by ^{1}H NMR, ^{13}C NMR and HRMS. ^{1}H NMR (400 MHz, $CDCl_3$): (α/β = 1/3), α-isomer: δ 8.94 (brs, 1H, NH), 8.29 (s, 1H, CH), 7.85-7.64 (m, 4H, aromatic), 6.44 (d, J = 3.2Hz, 1H, CH), 6.03-5.98 (m, 1H, CH), 5.34-5.29(m, 1H, CH), 4.73-4.68 (m, 1H, CH), 4.28-4.21 (m, 2H, CH_2O), 3.78(s, 1H, CH), 3.40- 3.34(m, 2H, CH_2-S), 3.21-3.15(m, 2H, CH_2-S), 2.16-1.89(m, 12H, 4-AcO); β-isomer: δ 8.96 (brs, 1H, NH), 8.23 (s, 1H, CH), 7.85-7.64 (m, 4H, aromatic), 6.30 (d, J = 8.4Hz, 1H, CH), 5.93-5.88 (m, 1H, CH), 5.28-5.23 (m, 1H, CH), 4.43-4.36 (m, 1H, CH), 4.28-4.11 (m, 2H, CH_2O), 3.49(s, 1H, CH), 3.40-3.34(m, 2H, CH_2-S), 3.21-3.15 (m, 2H, CH_2-S), 2.16-1.89 (m, 12H, 4AcO); ^{13}C NMR ($CDCl_3$,100MHz) α+β δ 170.47, 169.71, 169.57, 169.33, 169.04, 167.91, 157.37, 143.64, 143.37, 139.51, 138.14, 131.72, 128.87, 125.21, 119.69, 119.65, 99.99, 91.53, 89.78, 73.10, 72.08, 69.99, 68.75, 68.17, 68.03, 63.33, 61.64, 61.30, 41.87, 49.07, 20.70, 20.66, 20.52, 20.49, 20.26, 20.21. HRMS (ESI) m/z calcd for $C_{25}H_{30}AsN_4O_{10}S_2$ $[M+H]^+$, 685.0620, found 685.0628.

Synthesis of 1-(1-(1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranos-2-yl)-1H-1,2,3-triazole-4-methoxy carbonyl (11)

Compound **6** (300 mg, 0.8036 mmol) and methyl propiolate (75 mg, 0.8839 mmol) were dissolved in *N*,*N*-dimethylforamide (2 mL). The solution of copper sulfate pentahydrate (10 mg, 0.04018 mmol) in water (0.1 mL) was added to the above solution. Sodium L-ascorbate (16 mg, 0.08036 mmol) was added to the reaction mixture under nitrogen. The reaction mixture was sealed in 10 mL vial and was heated to 100 ^{o}C for 30 min in the microwave reactor. The reaction was monitored by TLC (R_f= 0.49, petroleum/ethyl acetate = 1/1). After completion of the reaction, the solvent was removed under reduced pressure. The resultant residue was dissolved in the mixture solvent of ethyl acetate (50 mL) and 10 mL brine. It was extracted with ethyl acetate (20 mL) for three times. The organic layers were combined and dried over anhydrous sodium sulfate. Filtration and removal of the solvent afforded the residue, which was purified through silica gel column chromatography with gradient eluent of the mixture of dichloromethane and methanol (2/1) to obtain the compound **11** as white solid (224.6 mg, 61.1%). Compound **11** was characterized with ^{1}H NMR, ^{13}C NMR and HRMS. α/β = 10/1. ^{1}H NMR (400 MHz, $CDCl_3$) α-isomer: δ 8.19(S, 1H, H-triazole), 6.40(d, J = 4Hz, 1H, anomeric H), 5.99-5.94(m, 1H, CH), 5.32-5.27(m, 1H, CH), 4.75-4.70(m, 1H, CH), 4.43-4.34(m, 1H, CH), 4.19-4.05(m, 2H, CH_2), 3.95(s, 3H, CH_3), 2.15-1.88(m, 12H, CH_3O).β-isomer:δ 8.13(S, 1H, H-triazole), 6.20(d, J = 8Hz, 1H, anomeric H), 5.83-5.78(m, 1H, CH), 5.23-5.21(m, 1H, CH), 4.75-4.70(m, 1H, CH), 4.43-4.34(m, 1H, CH), 4.19-4.05(m, 2H, CH2), 3.97(s, 3H, CH3), 2.15-1.88(m, 12H, CH_3O). ^{13}C NMR (100 MHz, $CDCl_3$) α+β δ 169.53, 168.83, 168.61, 168.31, 168.07, 166.98, 166.68, 159.71, 159.64,139.35,139.11, 126.97, 125.81, 90.40, 88.71, 72.00, 70.96, 68.85, 67.67, 66.86, 61.99, 60.34, 51.43, 19.71, 19.55, 19.49,19.29,19.21. HRMS (ESI): calcd for $C_{18}H_{24}N_3O_{11}$ $[M+H]^+$, 458.1411; found 458.1404. $C_{18}H_{23}N_3NaO_{11}$[M+Na]$^+$,480.1230; found 480.1224.

***Synthesis of N-phenylpropiolamide*(14)**

Compound **12** (1.504 g, 21.48 mmol) and dicyclohexylcarbodiimide (5.319 g, 25.78 mmol) were dissolved in anhydrous dichloromethane (250 mL) at 0 °C for 30 min. Then aniline **12** (2.00 g, 21.48 mmol) was added into the reaction mixture at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. Then it was stirred for 4 h at room temperature. The reaction was monitored by TLC (R_f = 0.25, petroleum/ethyl acetate = 10/1). Removal the solvent under reduced pressure afforded the residue, which was purified through silica gel chromatography with 20% ethyl acetate in petroleum ether to get the product as pale yellow solid (2.1826 g, 70%). Compound **14** was characterized by ^{1}H NMR, ^{13}C NMR and GCMS. ^{1}H NMR (400MHz,$CDCl_3$) δ: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16(brs, 1H, NH), 7.47(d, *J* = 8.0 Hz, 2H, H-Phenyl), 7.26-7.22(t, *J* = 8.0, 8.0 Hz, 2H, H-Phenyl), 7.08-7.04(t, *J* = 8.0, 8.0 Hz, 2H, H-Phenyl), 2.83(s, 1H, H-proparagyl).^{13}C NMR (100MHz, $CDCl_3$) δ:149.00,136.02, 128.05, 124.15,119.17,76.57, 73.30.GC-MS m/z calcd forC_9H_7NO,145.05, found 145.10.

Synthesis of 1-(1-(1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranos-2-yl)-1H-1,2,3-triazole-4-carbox amido phenyl (15)

Compound **6** (267 mg, 0.7139 mmol) and *N*-phenylpropiolamide (114 mg, 0.7853 mmol) were dissolved in *N*, *N*-dimethylforamide (2 mL). The solution of copper sulfate pentahydrate (9 mg, 0.03570 mmol) in water (0.1 mL) was added to the above solution. Sodium L-ascorbate (14 mg, 0.07139 mmol) was added to the reaction mixture under nitrogen. The reaction mixture was sealed in 10 mL vial and was heated to 100 °C for 30 min in the microwave reactor. The reaction was monitored by TLC (R_f = 0.21, petroleum/ethyl acetate = 2/1). After completion of the reaction, the solvent was removed under reduced pressure. The resultant residue was dissolved in the mixture solvent of ethyl acetate (20 mL) and water (10 mL). It was extracted with ethyl acetate (20 mL) for three times. The organic layers were combined and washed with 10 mL brine .The organic phase was separated and dried over anhydrous sodium sulfate. Filtration and removal of the solvent afforded the residue, which was purified through silica gel column chromatography with gradient eluent of the mixture of dichloromethane and methanol (4/1, 2/1) to yield the compound **15** as white solid (120.4 mg, 32.5%). Compound **15** was characterized with ^{1}H NMR, ^{13}C NMR and HRMS. α/β = 100/13. ^{1}H NMR (400 MHz, $CDCl_3$) α-isomer: δ 8.93(brs, 1H, NH), 8.27(s, 1H, H- triazole),7.68(d, *J* = 8.0 Hz, 2H, H-Ph), 7.41-7.37(t, *J* = 8.0, 8.0 Hz, 2H, H-Ph), 7.19-7.15(t, *J* = 8.0, 8.0 Hz, 2H, H-Ph), 6.43(d, *J* = 4.0 Hz, 1H, anomeric H), 6.03-5.97(m, 1H, CH), 5.34-5.29(m, 1H, CH), 4.72-4.67(m, 1H, CH), 4.38-4.37(m, 1H, CH), 4.19-4.08(m, 2H,CH_2), 2.18-1.90(12H, CH_3O); β-isomer:δ 8.89(brs, 1H, NH), 8.23(s, 1H, H-triazole),7.68(d, *J* = 8.0 Hz, 2H, H-Ph), 7.41-7.37(t, *J* = 8.0, 8.0 Hz, 2H, H-Ph), 7.19-7.15(t, *J* = 8.0, 8.0 Hz, 2H, H-Ph), 6.28(d, *J* = 8.0 Hz, 1H, anomeric H), 5.92-5.87(m, 1H, CH), 5.28-5.23(m, 1H, CH), 4.72-4.67(m, 1H, CH), 4.43-4.39(m, 1H, CH), 4.19-4.08(m, 2H,CH_2), 2.18-1.90(12H,CH_3O); ^{13}C NMR (100 MHz, $CDCl_3$) α+β δ 169.53, 168.67,168.60, 168.37, 168.05, 166.97,166.85, 156.37, 156.29, 142.79, 142.40, 136.15, 128.16, 125.84, 123.78, 118.93, 90.48, 88.74, 71.90, 70.98, 68.89, 67.64, 67.04, 66.88, 62.21, 60.49, 60.21, 20.22, 19.74, 19.71, 19.55,19.53, 19.30,19.25. HRMS (ESI): calcd for $C_{23}H_{27}N_4O_{10}$ $[M+H]^+$, 519.1727; found 519.1728.

Cell culture and cell proliferation MTS assay

Cell line

The colon cancer cell line HCT116 was purchased from the Culture Collection of Chinese Academy of Science (Shanghai, China). DLD1 and RKO were purchased from the American type culture collection. NCM460 was afforded by Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Diseases.

Cell proliferation MTS assay[5]

The MTS cell proliferation assay was performed according to a published protocol.[5] Briefly, the cells were grown at 37°C in a humidified atmosphere (5% CO_2) in the specified media (i.e. RPMI 1640 for HCT116 and RKO.), supplemented with 10% fetal bovine serum, 100 μg/mL penicillin and 100 μg/mL streptomycin. All media and supplements were obtained from Gibco (ThermoFisher Scientific). Cells (HCT116, DLD1, RKO and NCM460) were seeded in 96-well, clear bottom plates (Corning) seeded with 1000 cells in 200 μL of growth media per well. The cells were then treated with either 0.5% DMSO in media alone (used as vehicle control) or with a solution of the compound to be tested in specified growth media in a 2-fold dilution range (i.e. 0.0195 μM to 5 μM for the compound **3** and **4**; 0.195 μM to 50 μM for the compound **8**,**9**,**11** and **15**; and 0.39 μM to 100 μM for 5-FU). After incubation for 72 h, the drug solutions were removed and replaced with 100 μL of fresh growth media

and 20 μL of CellTiter 96®AQueous One Solution Reagent (Promega, China). After incubation for 2 to 4 hours, the absorbance was measured at 490 nm using the Varioskan Flash plate reader (Thermo Scientific). The IC_{50} data were analyzed using Prism 5.0 (Graphpad) and reported as the mean ± SD of three independent experiments.

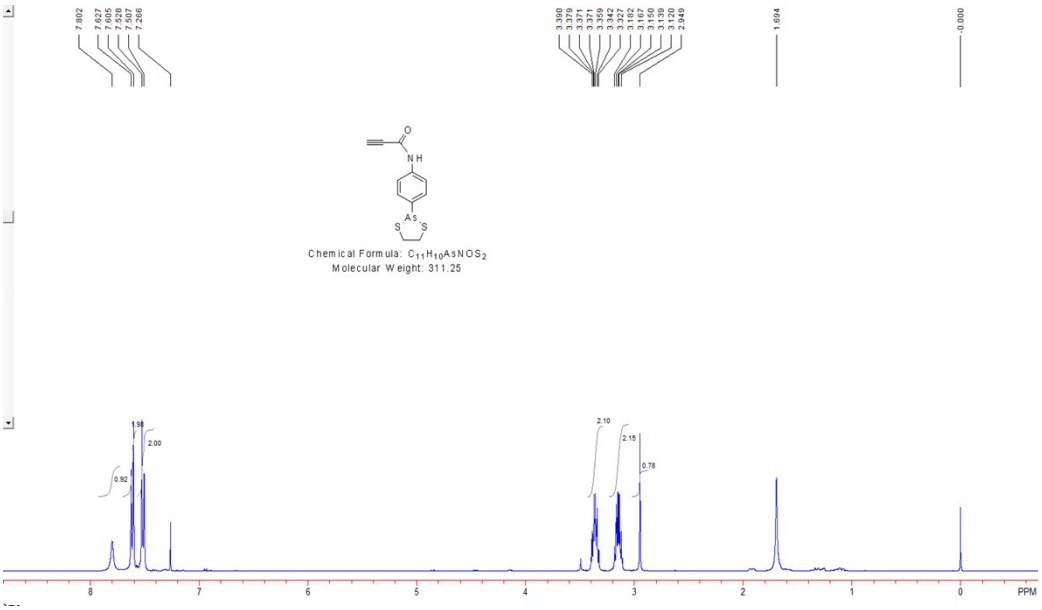


Figure S1.^{1}H NMR of Compound 4(400 MHz, $CDCl_3$)

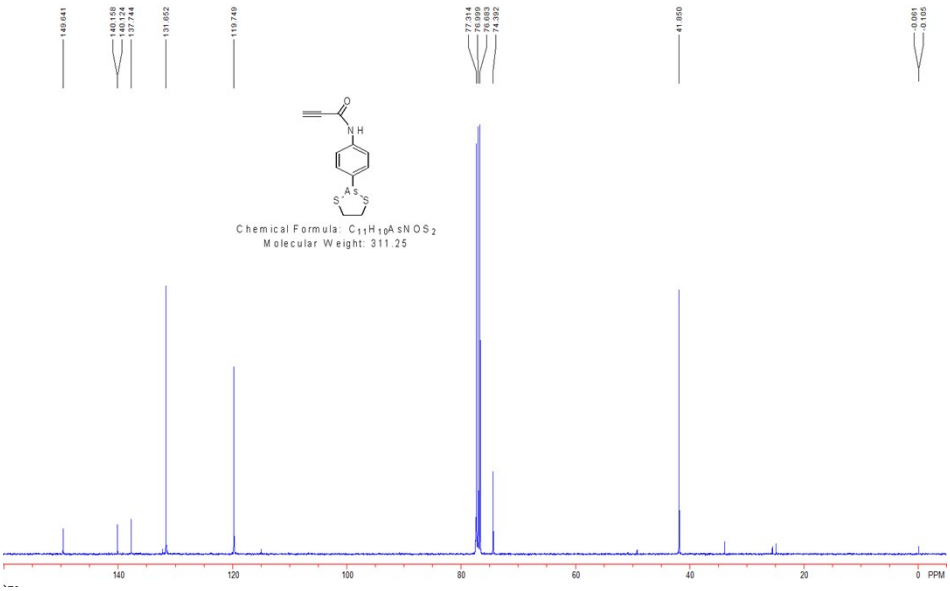


Figure S2.^{13}C NMR of Compound 4 (100 MHz, $CDCl_3$)

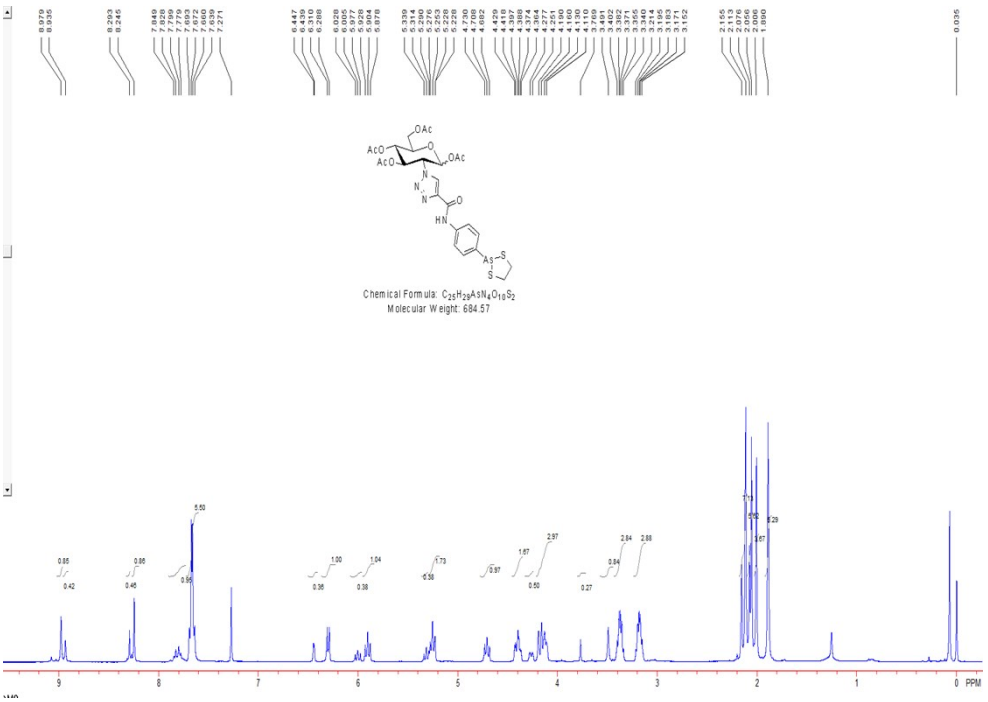


Figure S3. ^{1}H NMR of Compound 9(400 MHz, $CDCl_3$)

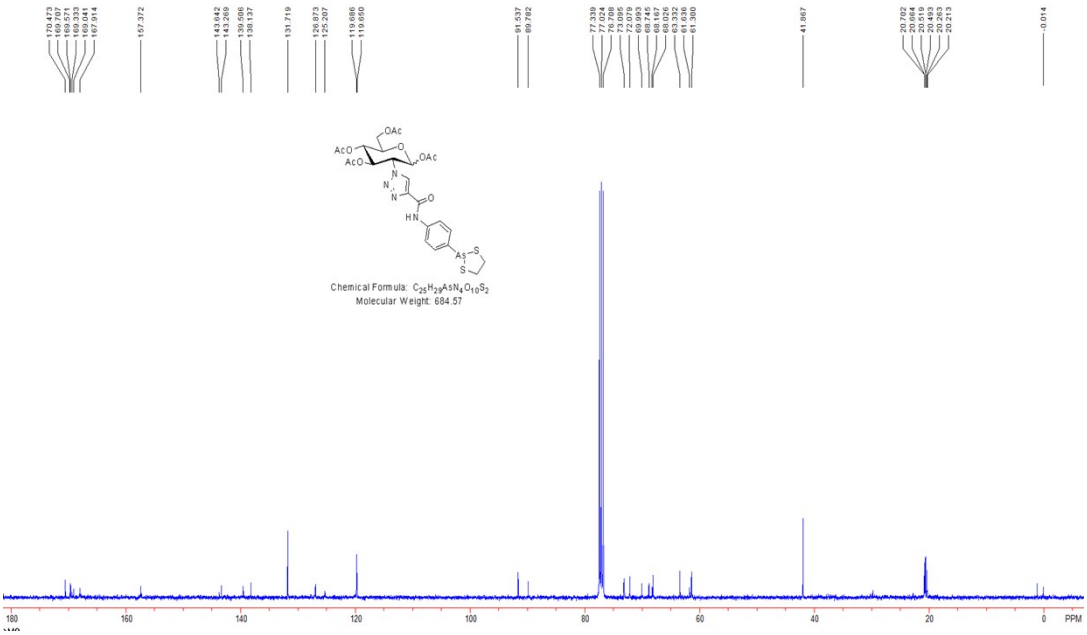


Figure S4. ^{13}C NMR of Compound 9 (100 MHz, $CDCl_3$)

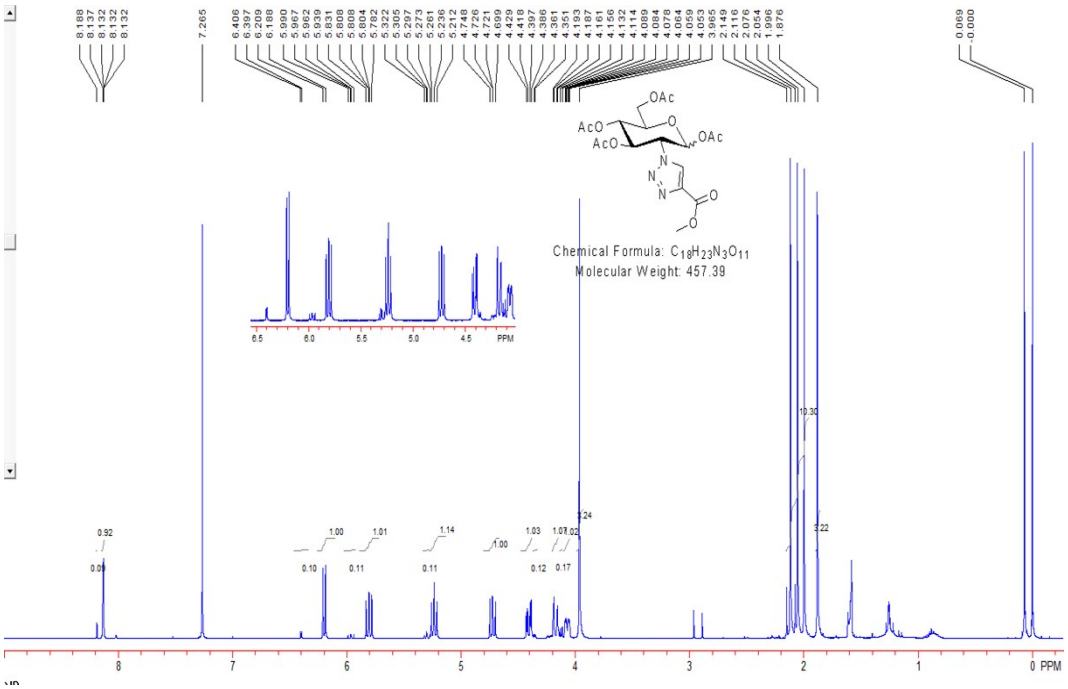


Figure S5. ^{1}H NMR of Compound 11(400 MHz, $CDCl_3$)

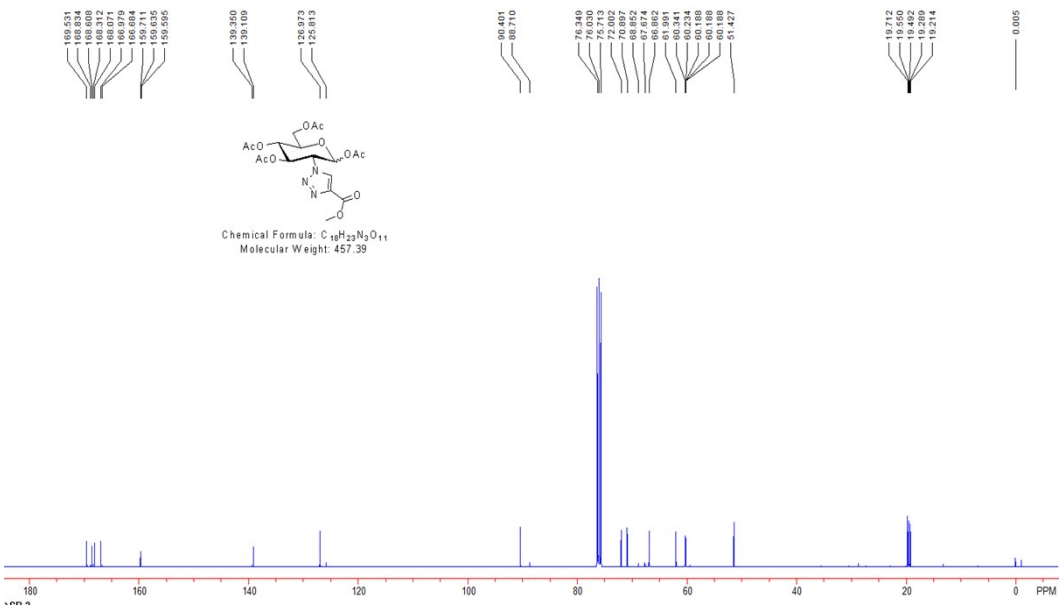


Figure S6. ^{13}C NMR of Compound 11(100 MHz, $CDCl_3$)

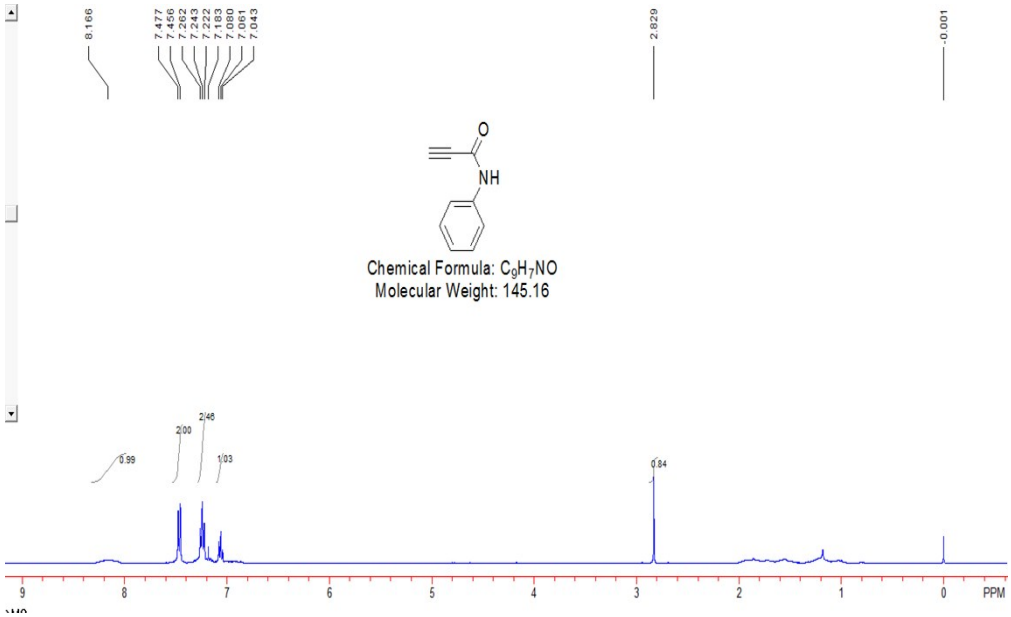


Figure S7. ^{1}H NMR of Compound 14 (400 MHz, $CDCl_3$)

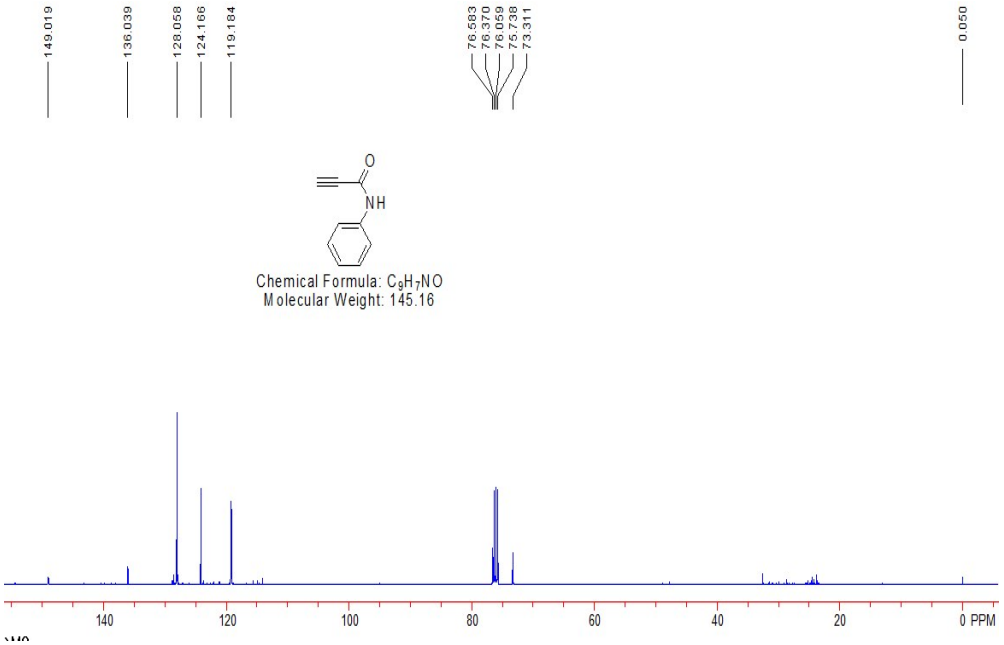


Figure S8. ^{13}C NMR of Compound 14 (100 MHz, $CDCl_3$)

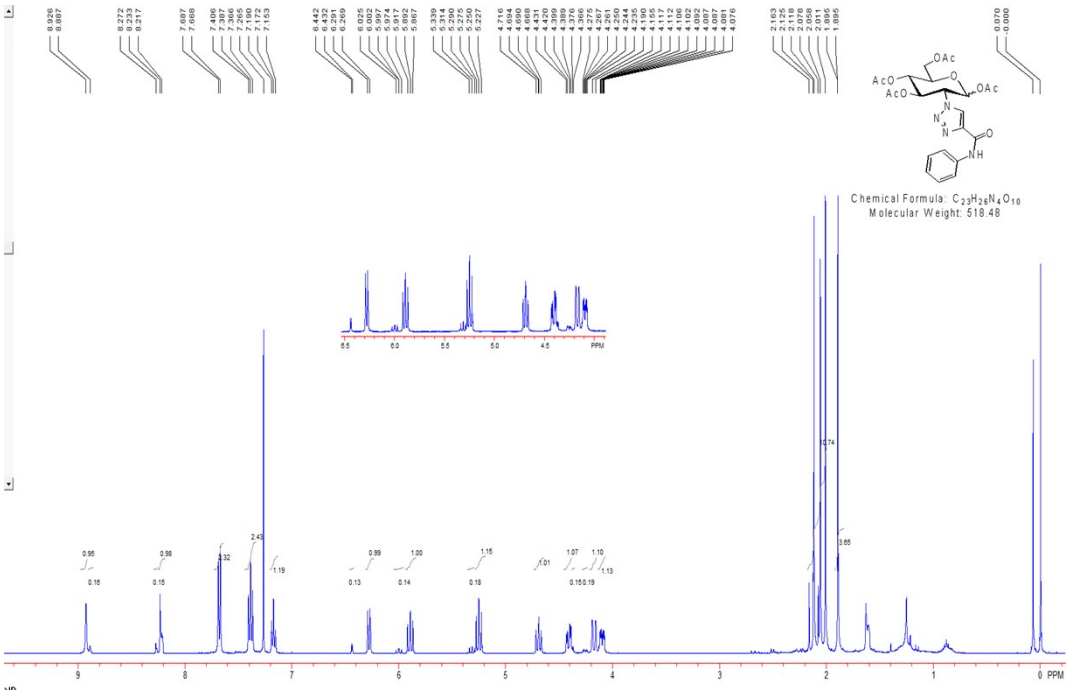


Figure S9. ^{1}H NMR of Compound 15 (400 MHz, $CDCl_3$)

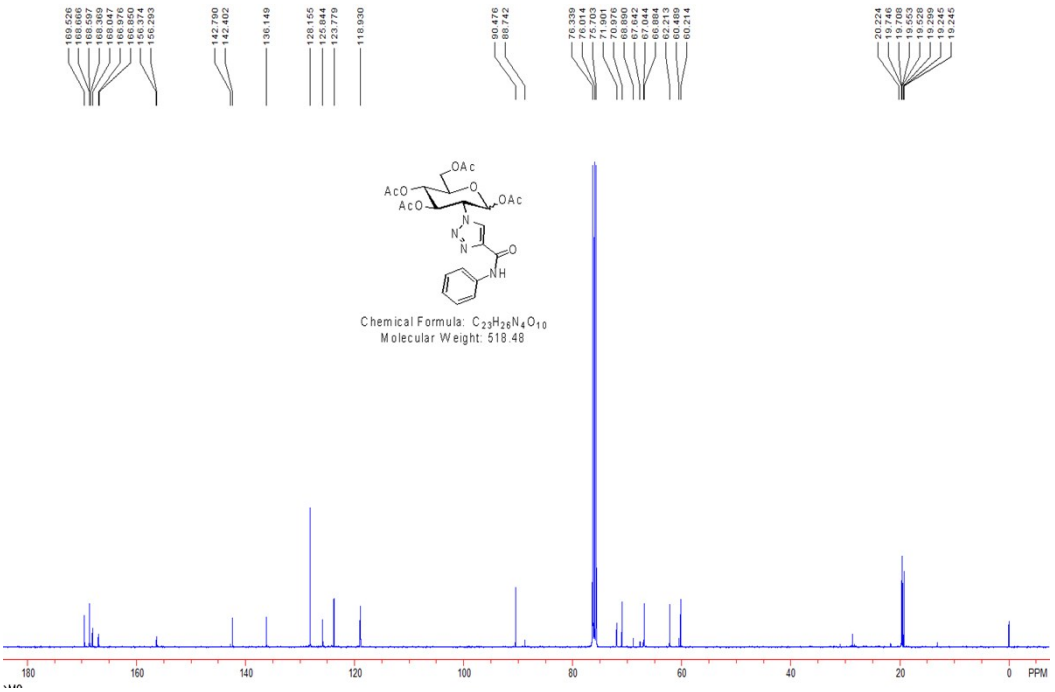


Figure S10. ^{13}C NMR of Compound 15 (100 MHz, $CDCl_3$)

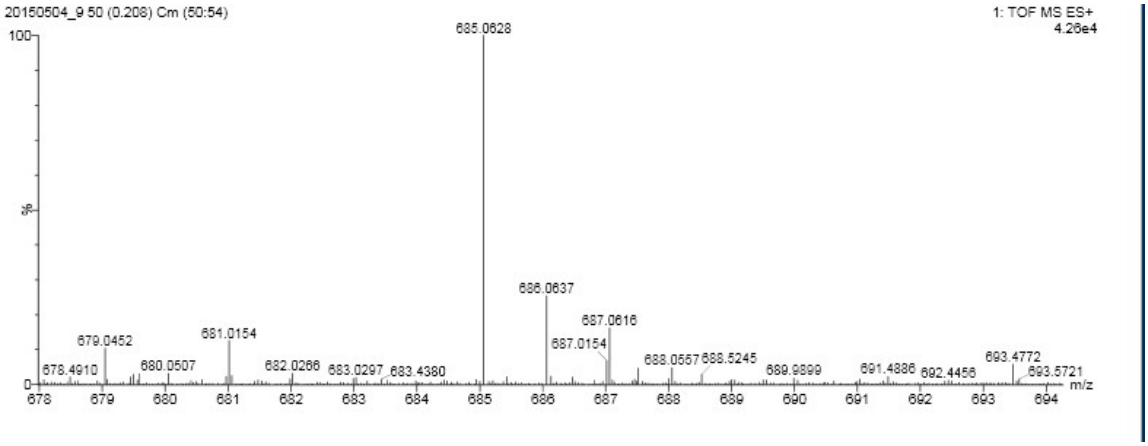


Figure S11. HR-MS of Compound 9

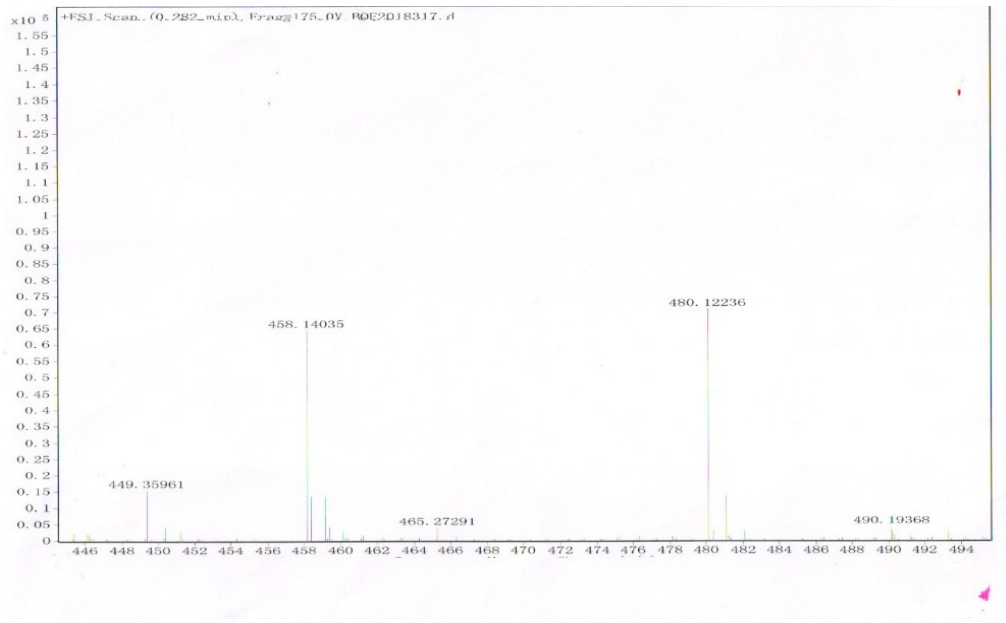


Figure S12. HR-MS of Compound 11

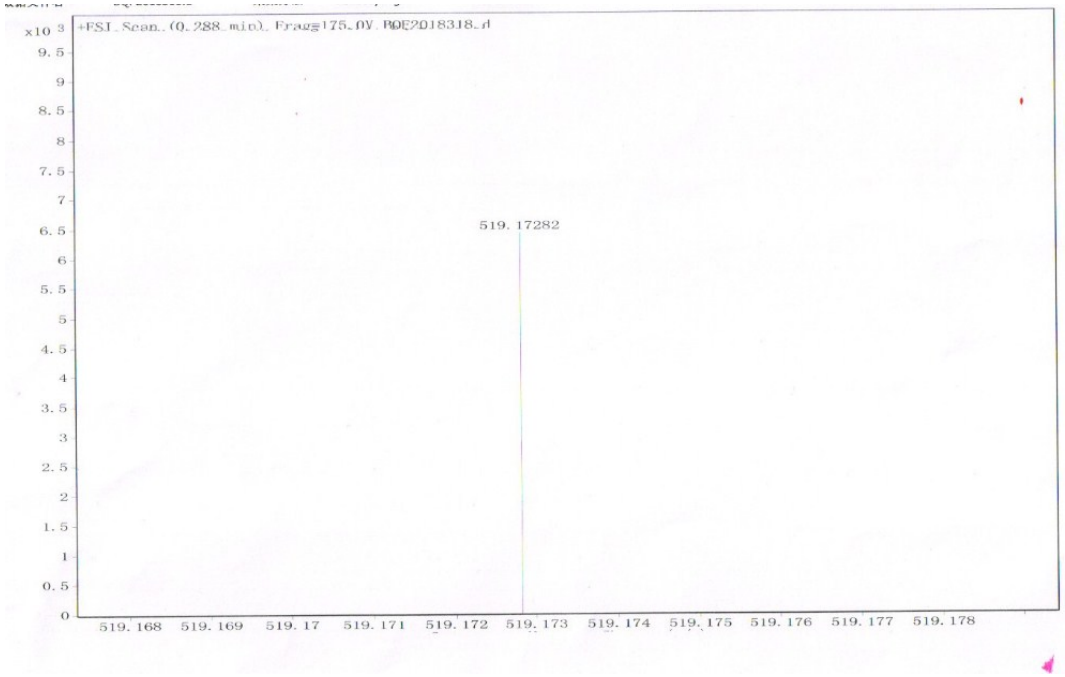


Figure S13. HR-MS of Compound 15

References:

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