Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Diaminodiacid-Based Solid-Phase Synthesis of all-Hydrocarbon Stapled α-helical Peptides

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1. General methods

1.1 Diaminodiacids synthesis and characterization

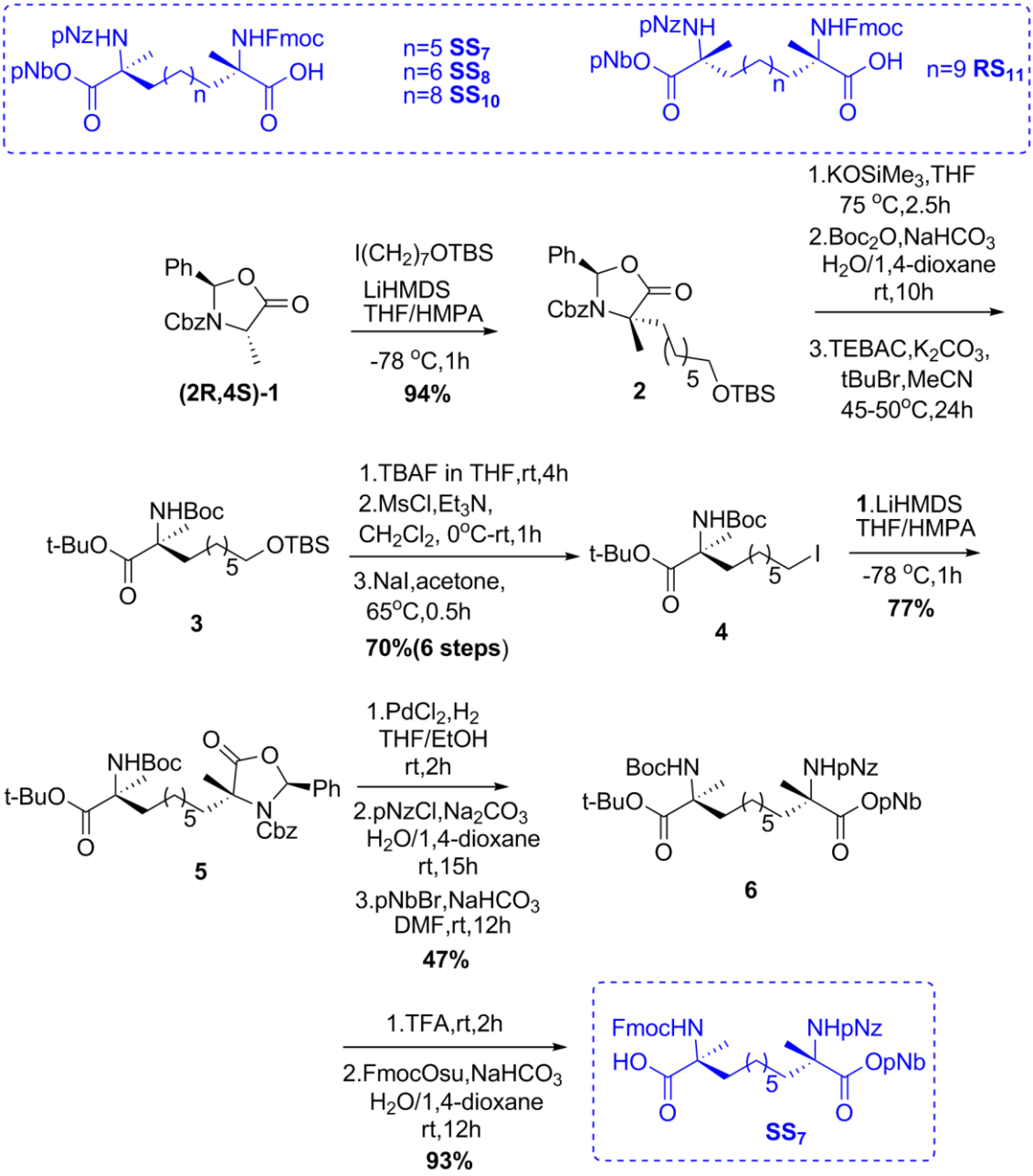
All starting materials and reagents were obtained from the commercial sources (Ouhe Technology, Sigmal-Aldrich, Alfa Aesar, Aladdin, Adamas, Sinopharm Chemical Reagent). Tetrahydrofuran (THF) was freshly distilled over sodium wire under argon atmosphere. Dichloromethane (CH_2Cl_2) were freshly distilled from calcium hydride (CaH_2) under argon atmosphere. Other solvents were used as purchased. All reactions vessels were purchased from Synthware Glass Co., Ltd. and oven-dried before use.Solvents used for isolation of products and chromatography were used as purchased. Reactions were monitored by thin-layer chromatography using pre-coated silica gel 60 glass plates and visualized by UV analyzer (254 nm) combined with ninhydrin solution, iodine vapor and potassium permanganate solution. Flash column chromatographic purification of products was finished using forced-flow chromatography on silica gel (300-400 mesh). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 400 MHz instrument at rt in $CDCl_3$. Chemical shifts (δ) were reported relative to TMS (0 ppm) or $CDCl_3$ (7.26 ppm) for ^{1}H-NMR and $CDCl_3$ (77.16 ppm) for ^{13}C-NMR spectra. The coupling constants (*J*) were given in Hertz (Hz), and the splitting patterns were designed as follows: s, singlet; s, br, broad singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet. ESI-MS was carried out with a Bruker Esquire-lcmass spectrometer. High resolution mass spectra were carried out on a Waters Xevo G2 QTOF mass spectrometer.

1.2 Peptides synthesis and characterization

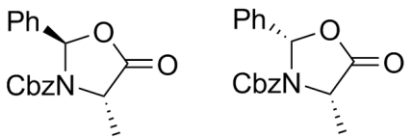
Peptide synthesis vessels were purchased from Synthware Glass Co., Ltd.. Fmoc amino acids, DIEA, HOBt, HCTU, HATU, HOAt, DIC, PyAOP and Rink amide-AM resin were from GL Biochem (Shanghai) Ltd. The side-chain AAs were Arg(Pbf), Asn(Trt), Asp(O^{t}Bu), Cys(Trt), His(Trt), Glu(O^{t}Bu), Gln(Trt), Lys(Boc), Ser(^{t}Bu), Thr(^{t}Bu), Trp(Boc), and Tyr(^{t}Bu). A double coupling strategy (10min/50min) was used for complete reaction. HCTU was chose as coupling reagent unless otherwise mentioned. For the coupling of diaminodiacids, HATU was used instead of HCTU. Automated peptide synthesis was conducted on a CS Bio Co. automated synthesizer. The deprotection of side chain protecting groups and final cleavage from the resin were achieved by TFA cocktails.The solutions were concentrated by blowing with N_2. The crude peptides were obtained by precipitation with cold ether. The crude peptides were dissolved with CH_3CN/H_2O and analyzed or purified by analytical or semi-preparative RP-HPLC, respectively. A Vydac C4 or C18 column (5 μm, 4.6 mm×250 mm) with a 1 mL/min flow rate was used for analytical RP-HPLC, and a Vydac C4 column (10 μm, 10 mm×250 mm or 22 mm×150 mm) with a 3-6 mL/min flow rate was used for semi-preparative RP-HPLC with different buffers: buffer A (0.1% TFA in water), buffer B (0.08% TFA in CH_3CN). Data were recorded and analyzed using the software system LC Solution. Peptides were identified by ESI-MS or HR-MS. ESI-MS was performed on Agilent 1200/6340 mass spectrometer and HR-MS was performed on a Waters Xevo G2 QTOF mass spectrometer.

2. Experimental section

2.1 Synthesis and characterizationof α-Me-diaminodiacids: To use diaminodiacids for SPPS of stapled peptides with i,i+4 or i,i+7 stapling, we synthesized the diaminodiacids building block shown in Scheme S1. Synthetic route of **SS_7** was also shown in Scheme S1.



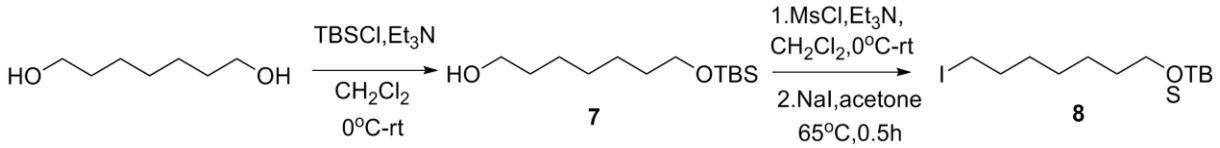
Scheme S1. Structures of diaminodiacids and the general synthetic route for α-Me-diamino-diacids (**SS_7** as an example).



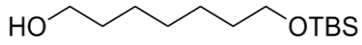
(2*R*,4*S*)-**1** (2*S*,4*S*)-**1**

(2*R*,4*S*)-**1** and (2*S*,4*S*)-**1**was synthesized as reported procedure.[1,2]

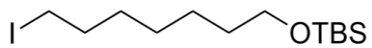
2.1.1 Synthesis and characterization data of SS_7



Scheme S2. Synthesis of alkylating linker **8**

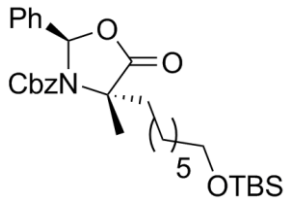


7-((tert-butyldimethylsilyl)oxy)heptan-1-ol(7): To a solution of1,7-Heptanediol (6.61 g, 50 mmol, 1 equiv) and dry CH_2Cl_2 (100 mL) was added triethylamine (7.59 g, 75 mmol,1.5 equiv). After stirring for 10 min under ice-bath, *tert*-butyldimethylsilyl chloride (8.29 g, 55 mmol, 1.1 equiv) in CH_2Cl_2 (50 mL) was added. The resulting solution was stirred overnight at ambient temperature. The reaction was quenched with H_2O and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, concentrated by rotary evaporation, and purified by flash column chromatography (10:1-5:1, petro ether/EtOAc) to yield **7** as a colorless oil (6.51g, 53%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.02 (s, 6H), 0.87 (s, 9H), 1.31 (m, 6H), 1.5 (m, 4H), 3.60 (m, 4H);
^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.16, 18.47, 25.88, 25.91, 26.09, 29.26, 32.83, 32.90, 62.96, 63.37.

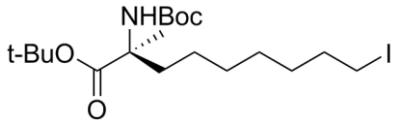


tert-butyl((7-iodoheptyl)oxy)dimethylsilane (8): To a solution of **7** (6.51 g, 26.41 mmol, 1 equiv) in dry CH_2Cl_2 (50 mL) was added triethylamine (4.01 g, 39.61 mmol,1.5 equiv) and the mixture was cooled to 0°C. Then methanesulfonyl chloride (4.54 g, 39.61 mmol, 1.5 equiv) was added dropwise. The mixture was warmed to rt and stirred for 1h. Then CH_2Cl_2 was evaporated, the residue was taken up by EtOAc, washed with 1 M HCl, sat. $NaHCO_3$ solution, brine. After concentrated by rotary evaporation, the resulting product was refluxed with NaI (19.79 g, 132.05 mmol, 5 eq) in dry acetone (100 mL) under 65°C. After 30 min, the reaction mixture was cooled to rt and diluted with EtOAc, which was washed with $Na_2S_2O_3$ solution, brine, dried over Na_2SO_4, concentrated by rotary evaporation, and purified by flash column chromatography (200:1 -100:1 petro ether/EtOAc) to yield **8** as a pale yellow oil (7.46 g, 79% two steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.04 (s, 6H), 0.89 (s, 9H), 1.32 (m, 4H), 1.40 (m, 2H), 1.51(m, 2H), 1.82 (m, 2H), 3.18 (t, 2H, *J*=6.4 Hz), 3.60 (t, 2H, *J*=7.0 Hz).
^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.11, 7.24, 18.49, 25.75, 26.12, 28.47, 30.62, 32.86, 33.64, 63.26.



(2*R*,4*S*)-benzyl-4-(3-((tert-butyldimethylsilyl)oxy)propyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (2): The solution of (2*R*,4*S*)-**1** (12.45 g, 40 mmol, 2.5equiv) and **8**(5.7 g, 16 mmol, 1 equiv) in dry THF/HMPA (4:1 ,100 mL) was added 1 M LiHMDS (48 mL, 48 mmol) in THF slowly under nitrogen at -78℃, and the slightly yellow solution was stirred at thistemperaturefor 1 h. Saturated NH_4Cl solution was added, and extracted with EtOAc twice. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. Purification by flash column chromatography (100:1-50:1 petro ether/EtOAc) gave compound **2** (8.16 g, 94 %) as a colorless oil.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.07 (s, 6H), 0.92 (s, 9H), 1.03-1.79 (m, 16H), 2.16, 2.53 (m, 1H), 3.61 (t, 2H, *J*=6.4 Hz), 4.99,5.32 (m, 2H), 6.38,6.44 (d, 1H, *J*=24.8 Hz), 6.88(d, 1H, *J*=6.8 Hz), 7.21-7.42 (m, 9H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.34, 18.19, 23.81, 24.17, 24.76, 25.55, 25.89, 29.00, 32.65, 35.59, 37.53, 62.35, 62.62, 62.94, 66.96, 67.41, 89.07, 126.62, 127.65, 127.90, 128.14, 128.50, 129.60, 135.30, 136.63, 137.09, 151.19, 152.71, 174.29, 174.70.
ESI-MS *m/z* calcd for $C_{31}H_{45}NO_5Si$ 539.3; found $[M+Na]^+$562.2.



(*S*)-tert-butyl 2-((tert-butoxycarbonyl)amino)-9-iodo-2-methylnonanoate (4): A mixture of **2** (8.16 g, 15.11 mmol, 1 equiv) and $KOSiMe_3$ (90% pure, 6.46 g, 45.33 mmol, 3 equiv) was suspended in dry THF (150 mL) and stirred at 75 ℃ for 2.5 h. MeOH (50 mL) was added and the reaction mixture was concentrated in vacuo. The residue was dissolved in H_2O/1,4-dioxane (1:1, 60ml). $NaHCO_3$ (6.35 g, 75.55 mmol, 5 equiv) and Boc_2O (6.60 g, 30.22 mmol, 2 equiv) was added successively. The mixture was stirred at rt for 10 h and after that 1,4-dioxane was evaporated off. The residue was dilute with H_2O, acidified with solid $KHSO_4$, extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. A mixture of the concentrated product, K_2CO_3 (31.32 g, 226.65 mmol, 15 equiv) and benzyltriethylammonium chloride (TEBAC, 3.78 g, 15.11 mmol, 1.1 equiv) in MeCN (100 mL) were stirred vigorously for 5 h rt. 2-Bromo-2-methyl propane (51.76 g, 377.75 mmol, 25 equiv) was then added and the reaction mixture was warmed to 45-50 ^oC and stirred in dark rapidly for 24 h. The reaction mixture was then cooled to R.T. and most of the CH_3CN was removed by

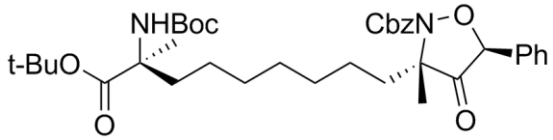
rotary evaporation. The reaction mixture was then partitioned between EtOAc and H_2O. The aqueous layer was extracted with EtOAc twice. The EtOAc layers were combined and washed with water, then with sat aq NaCl solution, and then dried over Na_2SO_4, filtered and concentrated to afford **3** which were used for the next step without purification.

A mixture of **3** and 1 M TBAF in THF (30 ml, 30 mmol, 2 equiv) were stirred at rt for 4h , and then THF was evaporated off. The residue was taken up by EtOAc and washed with brine. After concentrated by rotary evaporation, the resulting product was stirred with triethylamine (2.28 g, 22.5 mmol, 1.5 equiv) in dry CH_2Cl_2 (50 mL) for 10 min at 0^oC. Then methanesulfonyl chloride (2.58 g, 22.5 mmol, 1.5 equiv) was added dropwise. The mixture was warmed to R.T. and stirred for 1 h. Then CH_2Cl_2 was evaporated, the residue was taken up by EtOAc, washed with 1 M HCl, sat. $NaHCO_3$ solution, brine, succsively. After concentrated by rotary evaporation, the resulting product was refluxed with NaI (13.49 g, 90 mmol, 6 equiv) in dry acetone (100 mL) under 65^oC. After 30 min, the reaction mixture was cooled to rt and diluted with EtOAc, which was washed with $Na_2S_2O_3$ solution, brine , dried over Na_2SO_4, concentrated by rotary evaporation, and purified by flash column chromatography (50:1 petro ether/EtOAc) to yield **4** as a pale yellow oil (4.96 g, 70% 6 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.28-1.38 (m, 8H), 1.43 (s, 9H), 1.46 (s, 9H), 1.49 (s, 3H), 1.64, 2.09 (m, 2H), 1.80 (m, 2H), 3.17 (t, 2H, *J*=7.0 Hz), 5.33 (s, br, 1H),

^{13}C-NMR (400 MHz, $CDCl_3$): δ 7.13, 23.54, 23.93, 27.97, 28.37, 28.48, 29.37, 30.41, 33.54, 36.71, 59.81, 79.03, 81.50, 154.25, 173.73.

ESI-MS *m/z* calcd for $C_{19}H_{36}INO_4$ 469.2; found $[M+Na]^+$492.6, $[M+K]^+$508.3.

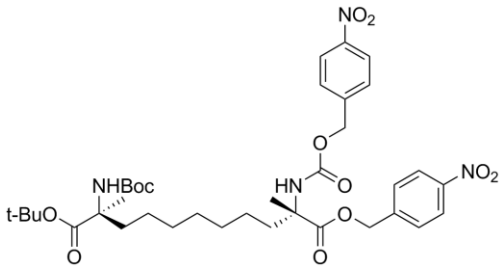


(3*S*,5*S*)-benzyl-3-((*S*)-9-(tert-butoxy)-8-((tert-butoxycarbonyl)amino)-8-methyl-9-oxononyl)-3-methyl-4-oxo-5-phenylisoxazolidine-2-carboxylate (5): To a solution of (2*R*,4*S*)-**1** (9.84 g, 30.15 mmol, 3 equiv) and **4** (4.95 g, 10.5 mmol, 1 equiv) in dry THF/HMPA (4:1 ,75 mL) was added 1 M LiHMDS (40 mL, 40 mmol) in THF slowly under nitrogen at -78 ℃, and the slightly yellow solution was stirred at this temperature for 1 h. Saturated NH_4Cl solution was added, and extracted with EtOAc twice. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. Purification by flash column chromatography (20:1 petro ether/EtOAc) gave compound **5** (5.27 g, 77 %) as a thick oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12-1.26 (m, 12H), 1.44 (s, 9H), 1.47 (s, 9H), 1.50 (s, 3H), 1.68-1.78 (m, 5H), 4.98, 5.31 (m, 2H), 5.34 (s, br, 1H), 6.37, 6.42 (d, 2H, *J*=22.4 Hz), 6.87 (d, *J*=6.4 Hz), 7.18-7.41 (m, 9H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.52, 24.02, 24.40, 24.96, 27.96, 28.48, 29.09, 29.26, 29.51, 29.75, 31.98, 35.82, 36.80, 37.73, 59.82, 62.68, 62.93, 67.30, 67.75, 79.04, 81.50, 89.37, 126.82, 127.90, 128.19, 128.40, 128.76, 129.91, 135.44, 136.69, 137.17, 151.48, 153.01, 154.27, 173.75, 174.72, 175.10.

ESI-MS *m/z* calcd for $C_{37}H_{52}N_2O_8$ 652.4; found $[M+Na]^+$ 653.4.

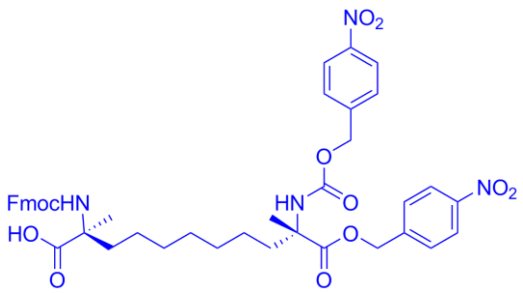


(2*S*,10*S*)-1-tert-butyl-11-(4-nitrobenzyl)-2-((tert-butoxycarbonyl)amino)-2,10-dimethyl-10-(((4-nitrobenzyl)oxy) carbonyl)amino)undecanedioate (6): a solution of **5** (2.84 g, 4.36 mmol, 1 equiv) in THF and EtOH **(**20 mL,1:1**)** was added palladium chloride (193 mg, 1.09 mmol, 0.25 equiv). The reaction mixture was hydrogenated with a hydrogen balloon for 2h at R.T.. The mixture was then filtered through Celite to remove the catalyst and concentrated. The product was dissolved in H_2O/1,4-dioxane (20 mL, 1:1) with Na_2CO_3 (1.39 g, 13.08 mmol, 3 equiv) and then a solution of 4-Nitrobenzyl chloroformate (1.88 g, 8.72 mmol , 2 equiv) in 1,4-dioxane (5 mL) was added dropwise. The resulting mixture was stirred for 15 h at R.T. after which 1,4-dioxane was removed and H_2O was added. The aqueous mixture was acidified with solid $KHSO_4$ and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated. Purification by flash column chromatography (4:1-2:1, petro ether/EtOAc, 1% AcOH) gave pNz protected diaminodiacid (1.36 g, 2.23 mmol, 1 equiv) as a white solid which was dissolved in DMF (10 mL). $NaHCO_3$ (222 mg, 2.64 mmol, 1.2 equiv) and 4-Nitrobenzyl bromide (0.58 g, 2.64 mmol, 1.2 equiv) were added successively. The resulting mixture was stirred in dark for at R.T.. After 12 h, the reaction mixture was diluted with EtOAc and washed with H_2O to remove DMF. The EtOAc layer was dried over Na_2SO_4 and concentrated. Purification by flash column chromatography (8:1 - 5:1, petro ether/EtOAc) afforded the pNz/pNb protected diaminodiacid **6** (1.55 g , 47% 3 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.06-1.21 (m, 10H), 1.42 (s, 9H), 1.45 (s, 9H), 1.48 (s, 3H), 1.60, (s, 3H), 1.63-2.01 (m, 4H), 5.16 (s, 2H), 5.26 (s, 2H), 5.30 (s, br, 1H), 5.54 (s, br, 1H), 7.49 (t, 3H, *J*=7.6 Hz), 8.20 (t, 3H, *J*=8.2 Hz).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.10, 23.47, 23.91, 27.91, 28.43, 29.22, 29.38, 29.46, 36.73, 37.32, 59.76, 60.10, 64.99, 65.78, 79.05, 81.48, 123.72, 123.81, 127.99, 128.44, 142.76, 143.99, 147.59, 147.82, 154.19, 154.24, 173.71, 173.76.

ESI-MS *m/z* calcd for $C_{37}H_{52}N_4O_{12}$ 744.4; found $[M+Na]^+$ 767.8.



(2*S*,10*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2,10-dimethyl-11-((4-nitrobenzyl)oxy)-10-((((4-nitrobenzyl)oxy)carbonyl)amino)-11-oxoundecanoic acid (SS_7): 6 (1.55g, 2.08 mmol, 1 equiv) was stirred in TFA at R.T. for 2h after which TFA was evaporated *in vacuo*. The

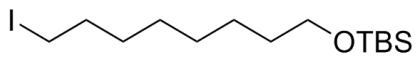
concentrated residue was treated with Fmoc-OSu (1.05 g, 3.11 mmol, 1.5 equiv) in the presence of $NaHCO_3$ (0.87 g, 10.4 mmol, 5 equiv) in H_2O/1,4-dioxane mixture (10 mL, 1:1) for 12 h. 1,4-dioxane was removed under reduced pressure and aqueous solution was acidifiedwith solid $KHSO_4$. The product was extracted with EtOAc and the combined EtOAc layers were dried over anhydrous Na_2SO_4. The product was purified by flash column chromatography (4:1~2:1, petro ether/EtOAc, 1% AcOH) to afford the target product diaminodiacid **SS_7** as a white solid (1.57 g, 93% 2 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.00-1.18 (m,10H), 1.59 (s, 6H), 1.78 (m, 2H), 2.06 (m, 2H), 4.22 (t, 2H, *J*=6.2 Hz), 4.39 (d, 2H, *J*=4 Hz), 5.16(s, 2H), 5.25 (s, 2H), 5.56 (s, br, 1H), 5.63 (s, br, 1H), 7.30 (t, 2H,*J*=7.4 Hz), 7.39 (t, 2H, *J*=7.2 Hz), 7.48 (t, 4H, *J*=6.2 Hz), 7.58 (d, 2H, *J*=7.2 Hz), 7.75(d, 2H, *J*=7.2 Hz), 8.19 (t, 4H, *J*=7.6 Hz).

^{13}C-NMR (400 MHz, $CDCl_3$): δ 21.40, 22.94, 23.21, 23.76, 29.04, 29.21, 29.28, 36.53, 37.15, 47.12, 59.68, 59.98, 64.92, 65.67, 66.56, 119.94, 123.58, 123.67, 125.00, 125.25, 127.03, 127.66, 127.86, 128.17, 128.32, 128.98, 137.76, 141.22, 142.66, 143.77, 143.86, 147.40, 147.61, 154.24, 154.73, 173.71, 178.53.

HR-MS *m/z* calcd for $C_{43}H_{46}N_4O_{12}$ 810.3112; found $[M+H]^+$ 811.3213

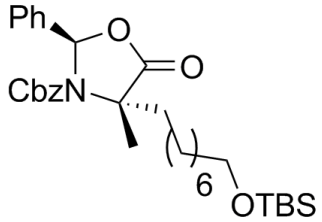
2.1.2 Synthesis and characterization data of SS_8



tert-butyl((8-iodooctyl)oxy)dimethylsilane (9): according to the procedure of synthesizing **8**, **9** was isolated as a pale yellow oil with 57 % yield (3 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.89 (s, 9H), 1.31 (m, 6H), 1.39 (m, 2H), 1.50 (m, 2H), 1.82 (m, 2H), 3.19 (t, 2H, *J*=7 Hz), 3.60 (t, 2H, *J*=6.6 Hz).

^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.12, 7.29, 18.48, 25.83, 26.11, 28.65, 29.33, 30.57, 32.92, 33.66, 63.32.

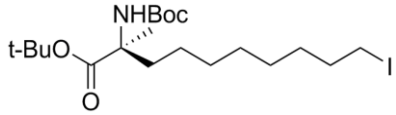


(2*R*,4*S*)-benzyl-4-(8-((tert-butyldimethylsilyl)oxy)octyl)-4-methyl-5-oxo-2-phenyloxazolidine -3-carboxylate (10): according to the procedure of synthesizing **2**, **10** was isolated as a colorless oil with 92% yield.

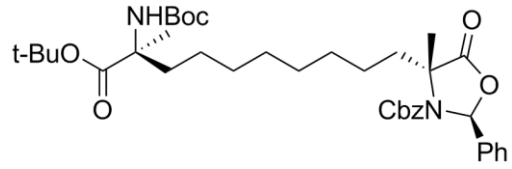
^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.90 (s, 9H), 1.05-1.78 (m, 18H), 2.14, 2.52 (m,1H), 3.61 (t, 2H, *J*=6.4 Hz), 4.98, 5.30 (m, 2H), 6.38,6.44 (d, 1H, *J*=24.0 Hz), 6.88(d, 1H, *J*=6.4 Hz), 7.18-7.42 (m, 9H).

^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.12, 18.49, 24.07, 24.44, 24.52, 25.02, 25.87, 26.12, 29.21, 29.42, 29.48, 32.95, 35.87, 37.78, 62.76, 63.02, 63.37, 67.34, 67.80, 89.43, 126.87, 127.93, 128.23, 128.44, 128.81, 129.95, 135.46, 135.50, 136.71, 137.19, 151.54, 153.06, 174.82, 175.21.

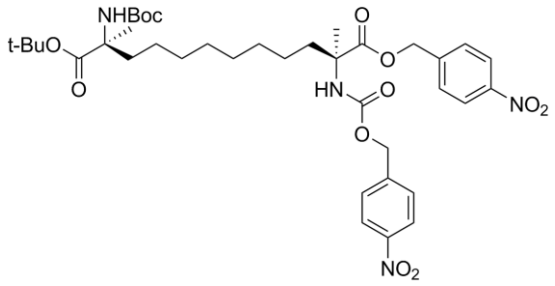
ESI-MS *m/z* calcd for $C_{32}H_{47}NO_5Si$ 553.3; found $[M+Na]^+$ 576.4.



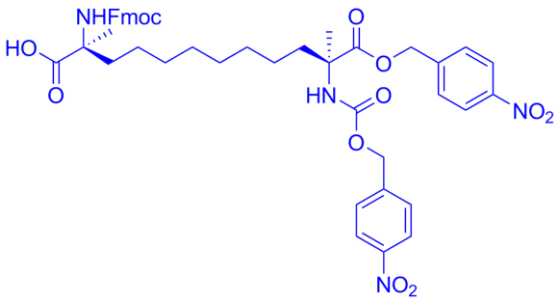
(*S*)-tert-butyl 2-((tert-butoxycarbonyl)amino)-10-iodo-2-methyldecanoate (11): according to the procedure of synthesizing **4**, **11** was isolated as a pale yellow oil with 48% yield (6 steps).
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.28-1.38 (m, 10H), 1.43 (s, 9H), 1.46 (s, 9H), 1.49 (s, 3H), 1.68, 2.06 (m, 2H), 1.81 (m, 2H), 3.18 (t, 2H, *J*=7.0 Hz), 5.33 (s, br, 1H),
^{13}C-NMR (400 MHz, $CDCl_3$): δ 7.13, 23.46, 23.89, 27.91, 28.37, 28.38, 28.43, 29.16, 29.41, 30.43, 33.53, 36.78, 59.75, 78.91, 81.37, 154.17, 173.67.
ESI-MS *m/z* calcd for $C_{20}H_{38}INO_4$ 483.2; found $[M+Na]^+$ 506.2.



(2*S*,4*R*)-benzyl-4-((S)-10-(tert-butoxy)-9-((tert-butoxycarbonyl)amino)-9-methyl-10-oxodecyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (12): according to the procedure of synthesizing **5**, **12** was isolated as thick oil with 90% yield.
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12-1.26 (m, 14H), 1.44 (s, 9H), 1.47 (s, 9H), 1.50 (s, 3H), 1.68-1.78 (m, 5H), 4.98, 5.30 (m, 2H), 5.33 (s, br, 1H), 6.37, 6.43 (d, 2H, *J*=22.8 Hz), 6.87 (d, *J*=6.8 Hz), 7.17-7.41 (m, 9H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.34, 23.84, 24.26, 24.83, 27.82, 28.34, 29.00, 29.12, 29.18, 29.41, 35.68, 36.79, 37.60, 59.67, 62.51, 62.76, 67.12, 67.58, 78.83, 81.23, 89.21,126.69, 127.74, 128.04, 128.25, 128.62, 129.75, 135.32, 136.61, 137.08, 151.32, 152.84, 154.14, 173.58, 174.52, 174.91.
ESI-MS *m/z* calcd for $C_{38}H_{54}N_2O_8$ 666.4; found $[M+Na]^+$ 689.5.



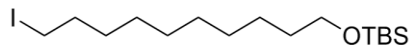
(2*S*,11*S*)-1-tert-butyl-12-(4-nitrobenzyl)-2-((tert-butoxycarbonyl)amino)-2,11-dimethyl-11-(((4-nitrobenzyl)oxy)carbonyl)amino)dodecanedioate (13): according to the procedure of synthesizing **6**, **13** was isolated with 30% yield.
ESI-MS *m/z* calcd for $C_{38}H_{54}N_4O_{12}$ 758.4; found $[M+Na]^+$ 781.8.



(2S,11S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2,11-dimethyl-12-((4-nitrobenzyl)oxy)-11-((((4-nitrobenzyl)oxy)carbonyl)amino)-12-oxododecanoic acid (SS_8): according to the procedure of synthesizing **SS_7**, **SS_8** was isolated as a white solid with 88% yield.

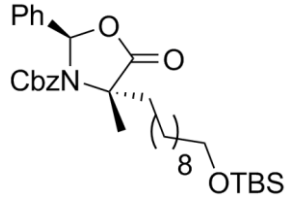
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.02-1.18 (m,12H), 1.60 (s, 6H), 1.79 (m, 2H), 2.09 (m, 2H), 4.22 (t, 2H, *J*=6.4 Hz), 4.39 (d, 2H, *J*=4 Hz), 5.17 (s, 2H), 5.26 (s, 2H), 5.58 (s, br, 1H), 5.67 (s, br, 1H), 7.30 (t, 2H, *J*=7.4 Hz), 7.39 (t, 2H, *J*=7.4 Hz), 7.48 (t, 4H, *J*=5.6 Hz), 7.58 (d, 2H, *J*=7.2 Hz), 7.75 (d, 2H, *J*=7.2 Hz), 8.19 (t, 4H, *J*=9 Hz).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.05, 23.20, 23.88, 29.18, 29.21, 29.26, 29.29, 29.39, 36.71, 37.23, 47.17, 59.85, 60.16, 65.14, 65.84, 66.76, 120.05, 123.72, 123.81, 125.05, 127.14, 127.78, 127.98, 128.46, 141.34, 142.68, 143.76, 147.56, 147.77, 154.42, 155.00, 173.86, 178.90.
HR-MS *m/z* calcd for $C_{44}H_{48}N_4O_{12}$824.3269; found $[M+H]^+$ 825.3343.

2.1.3 Synthesis and characterization data of SS_{10}



tert-butyl((10-iododecyl)oxy)dimethylsilane (14): according to the procedure of synthesizing **8**, **14** was isolated as a pale yellow oil with 47 % yield (3 steps).

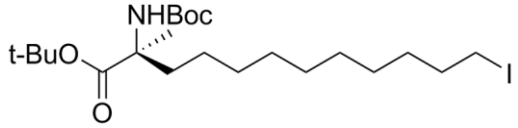
^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.05 (s, 6H), 0.89 (s, 9H), 1.28 (m, 6H), 1.38 (m, 2H), 1.50 (m, 2H), 1.82 (m, 2H), 3.19 (t, 2H, *J*=7 Hz), 3.60 (t, 2H, *J*=6.8 Hz).
^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.10, 7.28, 18.49, 25.91, 26.13, 28.66, 29.49, 29.51, 29.64, 30.64, 33.00, 33.70, 63.39.



(2*R*,4*S*)-benzyl-4-(10-((tert-butyldimethylsilyl)oxy)decyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (15): according to the procedure of synthesizing **2**, **15** was isolated as a colorless oil with 99% yield.

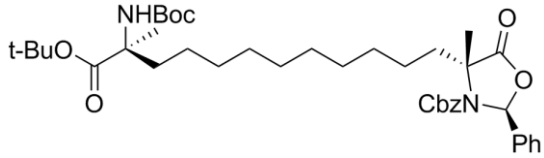
^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.06 (s, 6H), 0.90 (s, 9H), 1.14-1.78 (m, 22H), 2.16, 2,50 (m, 1H),

3.61 (t, 2H, *J*=6.6 Hz), 4.98, 5.29 (m, 2H), 6.37,6.43 (d, 1H, *J*=24.8 Hz), 6.88 (d, 1H, *J*=6.8 Hz), 7.21-7.41 (m, 9H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.16, 18.43, 24.03, 24.44, 24.97, 25.90, 26.08, 29.19, 29.41, 29.48, 29.51, 29.63, 32.96, 35.83, 37.75, 62.69, 62.95, 63.35, 67.27, 67.73, 89.36, 126.83, 127.88, 128.17, 128.39, 128.74, 129.88, 135.47, 136.73, 137.20, 151.48, 153.00, 174.71, 175.10.
ESI-MS *m/z* calcd for $C_{34}H_{51}NO_5Si$581.4; found $[M+Na]^+$ 604.4.



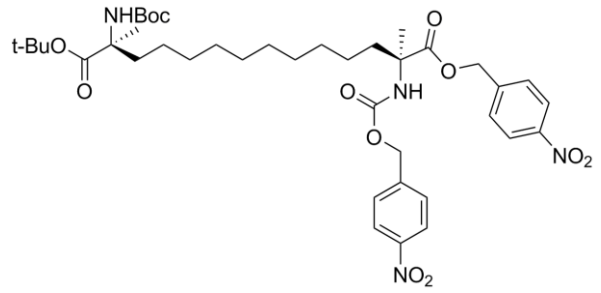
(*S*)-tert-butyl 2-((tert-butoxycarbonyl)amino)-12-iodo-2-methyldodecanoate (16): according to the procedure of synthesizing **4**, **16** was isolated as a pale yellow oil with 70% yield (6 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.26-1.38 (m, 14H), 1.43 (s, 9H), 1.46 (s, 9H), 1.49 (s, 3H), 1.67, 2.04 (m, 2H), 1.81 (m, 2H), 3.18 (t, 2H, *J*=7.0 Hz), 5.30 (s, br, 1H),
^{13}C-NMR (400 MHz, $CDCl_3$): δ 7.16, 23.49, 23.97, 27.95, 28.46, 28.56, 29.36, 29.39, 29.54, 30.53, 33.61, 36.88, 53.48, 59.83, 78.95, 81.39, 154.24, 173.73.
ESI-MS *m/z* calcd for $C_{22}H_{42}INO_4$ 511.2; found $[M+Na]^+$ 534.6.



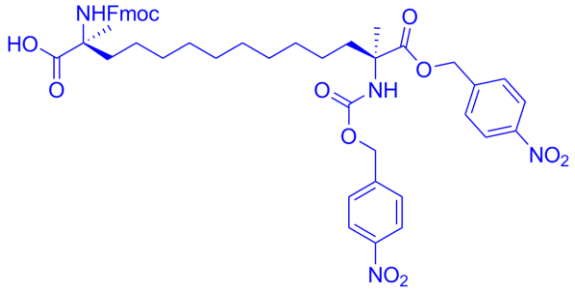
(2*S*,4*R*)-benzyl-4-((S)-12-(tert-butoxy)-11-((tert-butoxycarbonyl)amino)-11-methyl-12-oxodo decyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (17): according to the procedure of synthesizing **5**, **17** was isolated as a thick oil with 76% yield.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.12-1.26 (m, 18H), 1.44 (s, 9H), 1.46 (s, 9H), 1.50 (s, 3H), 1.68-1.78 (m, 5H), 4.98,5.29 (m, 2H), 5.33 (s, br, 1H), 6.37, 6.43 (d, 2H, *J*=23.6 Hz), 6.87 (d, *J*=6.4 Hz), 7.17-7.41 (m, 9H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.52, 24.05, 24.48, 25.00, 27.99, 28.50, 29.23, 29.45, 29.48, 29.54, 29.65, 35.87, 37.02, 37.76, 53.51, 59.89, 62.75, 63.00, 67.32, 67.79, 79.05, 81.48, 89.41, 126.86, 127.91, 128.23, 128.43, 128.79, 129.94, 135.48, 136.75, 137.21, 151.54, 153.06, 154.32, 173.81, 174.79, 175.17.
ESI-MS *m/z* calcd for $C_{40}H_{58}N_2O_8$ 694.4; found $[M+Na]^+$ 717.8.



(2*S*,13*S*)-1-tert-butyl-14-(4-nitrobenzyl)-2-((tert-butoxycarbonyl)amino)-2,13-dimethyl-13-(((4-nitrobenzyl)oxy)carbonyl)amino)tetradecanedioate (18): according to the procedure of synthesizing **6**, **18** was isolated with 48% yield (3 steps).

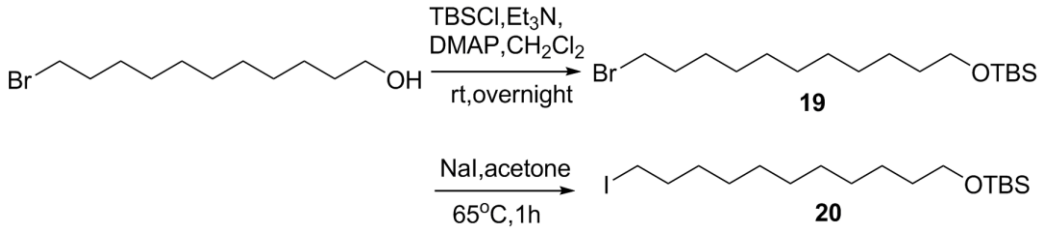
ESI-MS *m/z* calcd for $C_{40}H_{58}N_4O_{12}$ 786.4; found $[M+Na]^+$ 809.9.



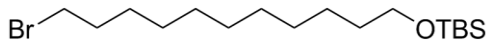
(2*S*,13*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2,13-dimethyl-14-((4-nitrobenzyl)oxy)-13-((((4-nitrobenzyl)oxy)carbonyl)amino)-14-oxotetradecanoic acid (SS_{10}): according to the procedure of synthesizing **SS_7**, **SS_{10}** was isolated as a white solid with 90% yield (2 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.00-1.16 (m,16H), 1.60 (s, 6H), 1.79 (m, 2H), 2.07 (m, 2H), 4.21 (t, 2H, *J*=6.4 Hz), 4.38 (d, 2H, *J*=4 Hz), 5.16(s, 2H), 5.26 (s, 2H), 5.55 (s, br, 1H), 5.65 (s, br, 1H), 7.31 (t, 2H, *J*=7.4 Hz), 7.39 (t, 2H, *J*=7.6 Hz), 7.48 (t, 4H, *J*=7.4 Hz), 7.58 (d, 2H, *J*=7.2 Hz), 7.75 (d, 2H, *J*=8.0 Hz), 8.19 (t, 4H, *J*=8.4 Hz).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 22.99, 23.16, 23.86, 29.31, 29.36, 29.41, 36.75, 37.24, 47.12, 59.80, 60.10, 65.03, 65.76, 66.64, 119.98, 123.65, 123.74, 125.02, 127.08, 127.71, 127.90, 128.39, 141.27, 142.64, 143.73, 143.84, 147.47, 147.67, 154.31, 154.89, 173.82, 178.91.
HR-MS *m/z* calcd for $C_{46}H_{52}N_4O_{12}$852.3582; found $[M+H]^+$ 853.3648.

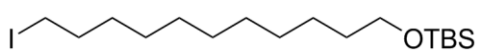
2.1.4 Synthesis and characterization data of RS_{11}



Scheme S3. Synthesis of alkylating linker **20**

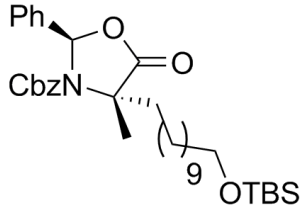


((11-bromoundecyl)oxy)(tert-butyl)dimethylsilane(19):[3] 19 was synthesized starting from 11-Bromo-1-undecanolfollowingliterature methodand used for the next step without purification.



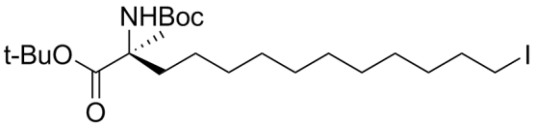
tert-butyl((11-iodoundecyl)oxy)dimethylsilane (20): according to the procedure of synthesizing **8**, **20** was isolated as a pale yellow oil with 94% yield (2 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.04 (s, 6H), 0.89 (s, 9H), 1.27 (m, 12H), 1.38 (m, 2H), 1.50 (m, 2H), 1.82 (m, 2H), 3.19 (t, 2H, *J*=7 Hz), 3.59 (t, 2H, *J*=6.6 Hz).
^{13}C-NMR (400 MHz, $CDCl_3$): δ -5.11, 7.30, 18.49, 25.92, 26.12, 28.67, 29.54, 29.61, 29.70, 30.64, 33.00, 33.71, 63.41.



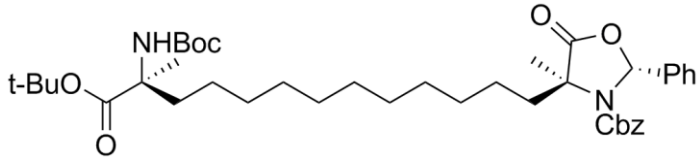
(2*S*,4*R*)-benzyl-4-(11-((tert-butyldimethylsilyl)oxy)undecyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (21): according to the procedure of synthesizing **2**, **21** was isolated as a colorless oil with 79% yield.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.06 (s, 6H), 0.91 (s, 9H), 1.14-1.78 (m, 24H), 2.16, 2,51 (m, 1H), 3.61 (t, 2H, *J*=6.6 Hz), 4.98, 5.26 (m, 2H), 6.37,6.43 (d, 1H, *J*=24.8 Hz), 6.87 (d, 1H, *J*=6.8 Hz), 7.20-7.43 (m, 9H).
ESI-MS *m/z* calcd for $C_{35}H_{53}NO_5Si$595.4; found $[M+Na]^+$ 618.5.



(*S*)-tert-butyl 2-((tert-butoxycarbonyl)amino)-13-iodo-2-methyltridecanoate (22): according to the procedure of synthesizing **4**, **22** was isolated as a pale yellow oil with 42% yield (6 steps).

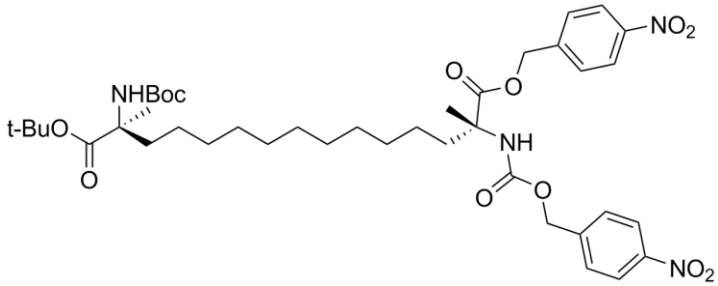
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.25-1.38 (m, 16H), 1.43 (s, 9H), 1.46 (s, 9H), 1.49 (s, 3H), 1.67, 2.04 (m, 2H), 1.81 (m, 2H), 3.19 (t, 2H, *J*=7.0 Hz), 5.34(s, br, 1H),
^{13}C-NMR (400 MHz, $CDCl_3$): δ 7.34, 14.24, 22.80, 23.52, 24.03, 27.99, 28.50, 28.64, 29.46, 29.51, 29.52, 29.58, 29.60, 30.60, 32.03, 33.67, 36.97, 53.53, 59.88, 79.01, 81.47, 154.28, 173.82.
ESI-MS *m/z* calcd for $C_{23}H_{44}INO_4$525.2; found $[M+Na]^+$ 548.4.



(2R,4S)-benzyl-4-((S)-13-(tert-butoxy)-12-((tert-butoxycarbonyl)amino)-12-methyl-13-oxotridecyl)-2,4-dimethyl-5-oxooxazolidine-3-carboxylate (23): 23 was synthesized according to the procedure of synthesizing **5** except that (2*S*,4*S*)-**1** was used instead of (2*R*,4*S*)-**1** and **23** was isolated as a thick oil with 67% yield.

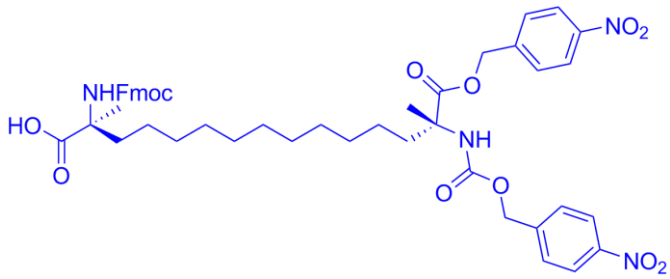
^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.13-1.26 (m, 20H), 1.43 (s, 9H), 1.46 (s, 9H), 1.50 (s, 3H), 1.68-1.78 (m, 5H), 4.98,5.30 (m, 2H), 5.33 (s, br, 1H), 6.37, 6.43 (d, 2H, *J*=24.0 Hz), 6.87 (d,

J=6.4 Hz), 7.19-7.42 (m, 9H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.42, 23.96, 24.35, 24.42, 24.93, 27.90, 28.43, 29.15, 29.38, 29.43, 29.48, 29.50, 29.55, 35.79, 36.91, 37.70, 59.73, 62.66, 62.92, 67.24, 67.70, 78.94, 81.36, 89.34, 126.79, 127.83, 128.14, 128.36, 128.72, 129.87, 135.39, 135.45, 136,65, 137.13, 151.45, 152.98, 154.22, 173.73, 174.71, 175.10.
ESI-MS *m/z* calcd for $C_{41}H_{60}N_2O_8$ 708.4; found $[M+Na]^+$ 731.7.



(2*S*,14*R*)-1-tert-butyl-15-(4-nitrobenzyl)-2-((tert-butoxycarbonyl)amino)-2,14-dimethyl-14-(((4-nitrobenzyl)oxy)carbonyl)amino)pentadecanedioate (24): according to the procedure of synthesizing **6**, **24** was isolated with 34% yield (3 steps).

ESI-MS *m/z* calcd for $C_{41}H_{60}N_4O_{12}$ 800.4; found $[M+Na]^+$ 823.8.



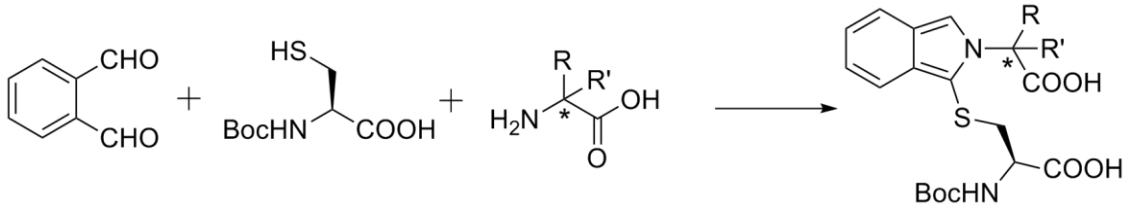
(2*S*,14*R*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2,14-dimethyl-15-((4-nitrobenzyl)oxy)-14-((((4-nitrobenzyl)oxy)carbonyl)amino)-15-oxopentadecanoic acid (RS_{11}): according to the procedure of synthesizing SS_7, RS_{11} was isolated as a white solid with 85% yield (2 steps).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.00-1.26 (m,18H), 1.61 (s, 6H), 1.80 (m, 2H), 2.07 (m, 2H), 4.22 (t, 2H, *J*=6.6 Hz), 4.39 (d, 2H, *J*=2 Hz), 5.15(s, 2H), 5.26 (s, 2H), 5.55 (s, br, 1H), 5.63 (s, br, 1H), 7.31 (t, 2H, *J*=7.4 Hz), 7.39 (t, 2H, *J*=7.4 Hz), 7.48 (t, 4H, *J*=7.6 Hz), 7.58 (d, 2H, *J*=7.2 Hz), 7.75 (d, 2H, *J*=7.2 Hz), 8.19 (t, 4H, *J*=8.6 Hz).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 23.05, 23.27, 23.92, 23.95, 29.38, 29.44, 36.79, 37.24, 47.20, 59.80, 60.10, 64.99, 65.75, 66.57, 119.98, 123.67, 123.76, 125.05, 127.08, 127.70, 127.92, 128.40, 141.30, 142.68, 143.84, 147.51, 147.72, 154.26, 154.78, 173.82, 178.71. .
HR-MS *m/z* calcd for $C_{47}H_{54}N_4O_{12}$ 866.3738; found $[M+H]^+$ 867.3813.

2.1.5 Experiment to verify the chiral purity of synthesized diaminodiacids

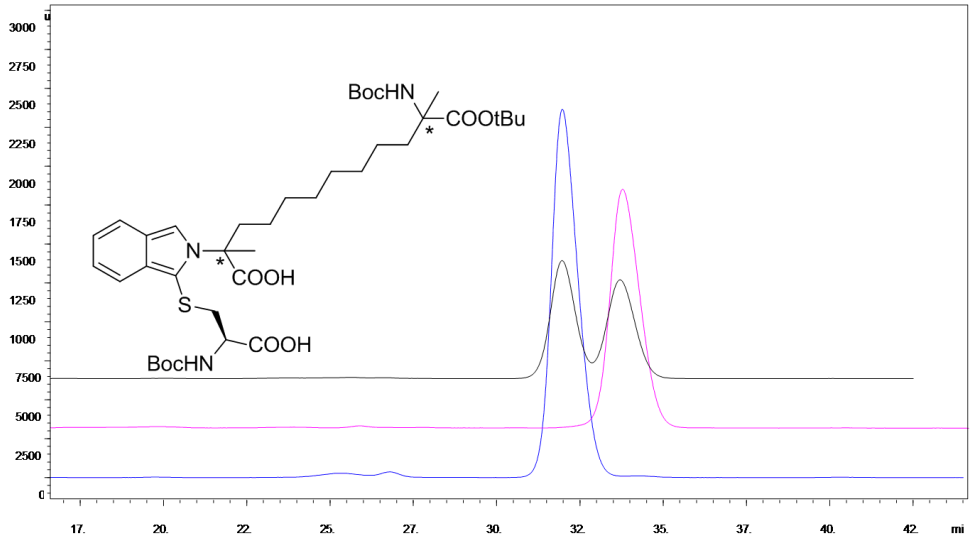
A derivatizingsolution (OPA/NBC) of 0.2 M o-phthalaldehydeand 0.2M L-N-Boc-cysteine

in methanol as well as anaqueous buffer solution (KHP) of 1.0 M pH 8.0 K_2HPO_4 were prepared. [4]100µl diaminodiacid with one terminus free (0.1 M), 100 µL OPA/NBC (0.2 M) and 100 µL KHP buffer were mixed and vibrated for 1 min at R.T. (Scheme S4). The derivative product was detected by HPLC at 344 nm (Figure S1).



Scheme S4. Reaction of OPA/NBC with a free amino acid

a).



b)

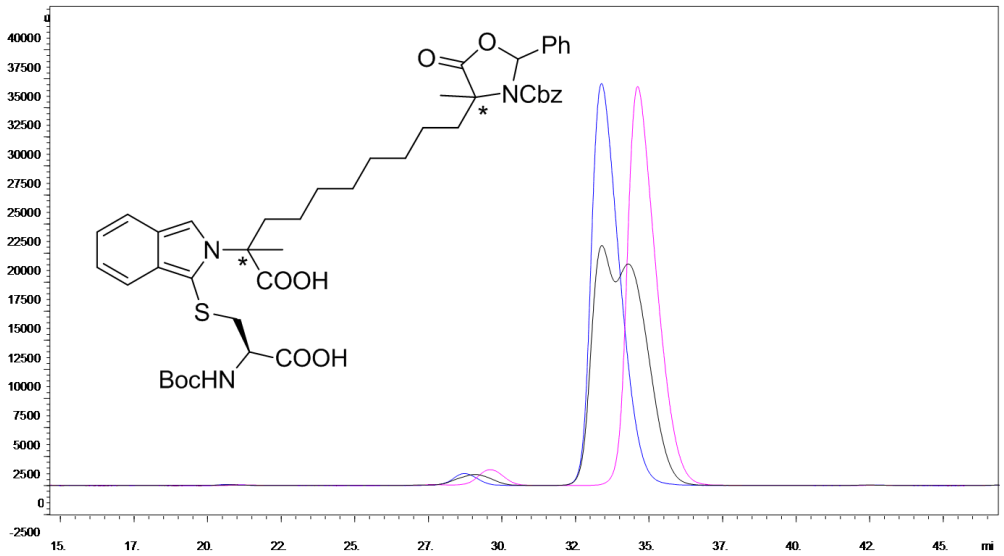


Figure S1. HPLC traces of OPA/NBC derivative diaminodiacids. For blue curve,*= (*R*); red curve, *= (*S*); black curve, *= (*R*) + (*S*). a) HPLC condition: 65 % of buffer B in 60 min with C18 (5 μm, 2.5 mm×250 mm). b) HPLC condition, 63% of buffer B in 60 min with C18 (5 μm, 2.5 mm×250 mm).

2.2 Synthesis and characterization of peptides

2.2.1 General procedures for the Fmoc solid phase peptide synthesis

The amino acid residues were attached to theresin with a double coupling procedure (10 min/50 min). All peptides were synthesized with a scale of 0.1 mmol.

(a) Standard pre-activation of resin protocol: The resin was swollen in CH_2Cl_2/DMF mixture solvent for 0.5 h.

(b) Standard Fmoc-deprotection protocol: After treatment with 20% piperidine/DMF (5 min, 10 min) the resin was washed (5×DMF, 5×CH_2Cl_2, 5×DMF).

(c) Standard coupling of natural amino acidsprotocol: After pre-activation of 4 equiv of Fmoc-protected amino acid in DMF for 5 min using 3.8 equiv of HCTU and 8 equiv of DIEA, the solution was added to the resin. After 10+50 min, the resin was washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×). The coupling reaction was monitored with the ninhydrin test.

(d) Standard coupling of diaminodiacids protocol: After pre-activation of 2 equiv of Fmoc-protected amino acid in DMF for 5 min using 1.9 equiv of HATU and 4 equiv of DIEA, the solution was added to the resin. After 2 h, the resin was washed with DMF (5×), CH_2Cl_2 (5×), and DMF (5×). The coupling reaction was monitored with the ninhydrin test.

(e) Standard deprotection of pNz/pNb protocol: After treatment with $SnCl_2$ (2.5 M in DMF)/HCl (cat.) for 1 h+2 h, the resin was washed (5×DMF, 5×CH_2Cl_2, 5×DMF).

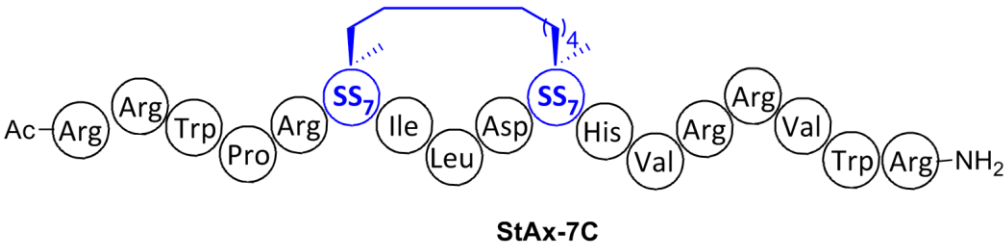
(f) Standard cyclization protocol: After removal of pNz/pNb and N-terminus Fmoc successively, a solution of PyAop (5eq), HOAt (5 eq) and NMM (10 eq) in NMP was added to resin. After overnight reaction, the resin was washed (5×DMF, 5×CH_2Cl_2, 5×DMF).

(g) Standard capping protocol: Ac_2O/DIEA/DMF (1:1:8) was added to the resin. After 5+5 min the resin was washed with DMF (5×) and CH_2Cl_2 (10×).

(h) Standard cleavage protocol: The cleavage cocktail (TFA: TIPS: H_2O= 95: 2.5: 2.5) was added at 30 ^oC. After 2 h, the cleavage cocktail was collected and the resin was washed with the TFA cleavage cocktail (3×).

(i) Workup: The collected TFA cocktails were pumped by N_2 and then the chilled diethyl ether was added to the concentrated TFA solution to precipitate the crude peptides. The peptide suspensions were centrifuged for 2 min at 5000 rpm and then the clear solution was decanted. The step of precipitation, centrifugation and decantation operations was repeated three times. The resulting white residues were dissolved in CH_3CN/H_2O, analyzed and purified by RP-HPLC, ESI-MS or HR-MS.

2.2.2 HPLC traces and MS spectrums of purified peptides



StAx-7C

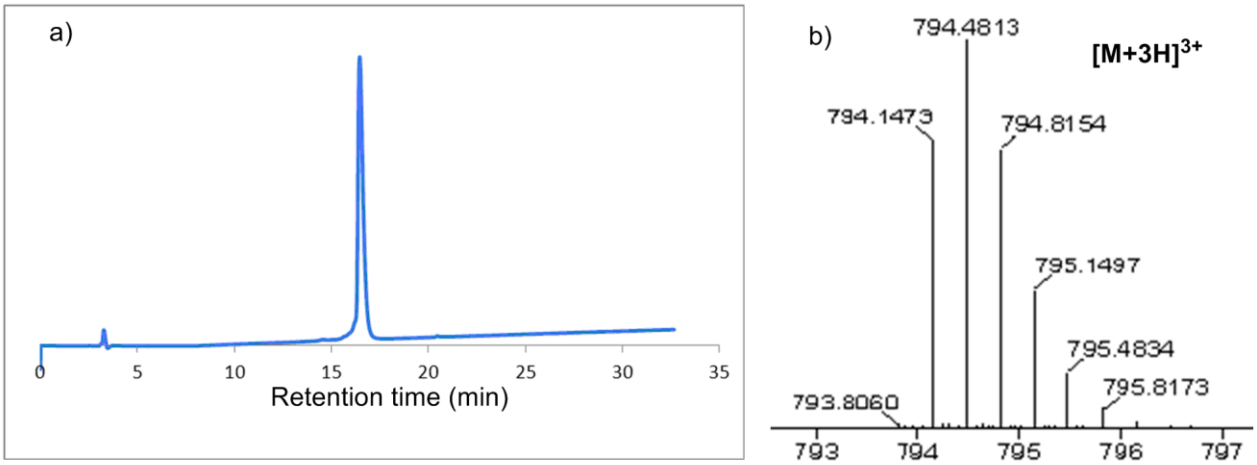
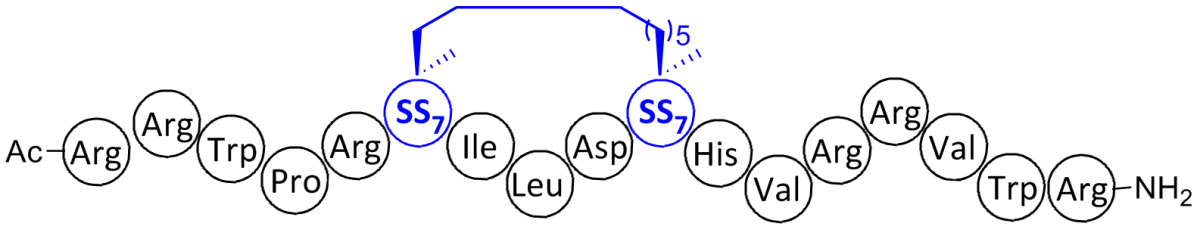


Figure S2. a) HPLC trace of purified **StAx-7C**. Gradient: 20-50% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm). b) HR-MS spectrum of **StAx-7C** (calc. for $C_{110}H_{178}N_{40}O_{20}$ 2379.4141, found $[M+3H]^{3+}$ 794.1473).



StAx-8C

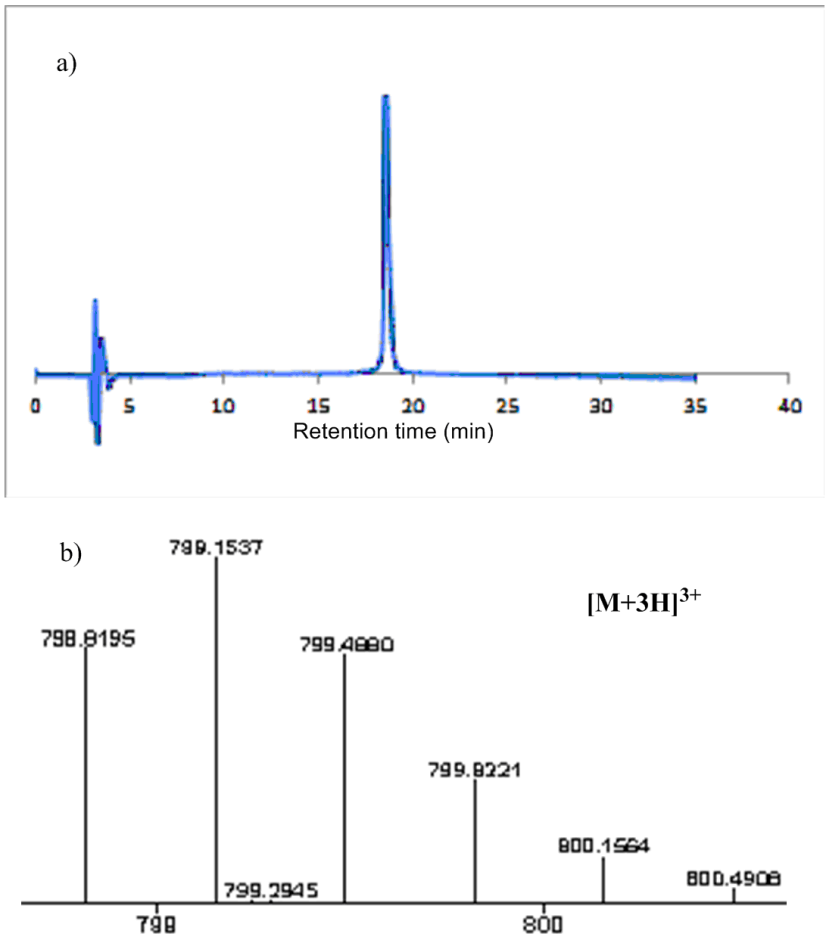
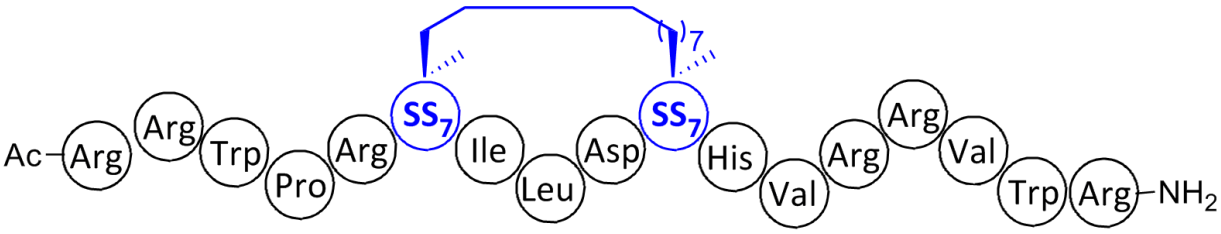


Figure S3. a) HPLC trace of purified **StAx-8C**. Gradient: 20-50% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm). b) HR-MS spectrum of **StAx-8C** (calc. for $C_{111}H_{180}N_{40}O_{20}$ 2393.4298, found $[M+3H]^{3+}$ 798.8195).



StAx-10C

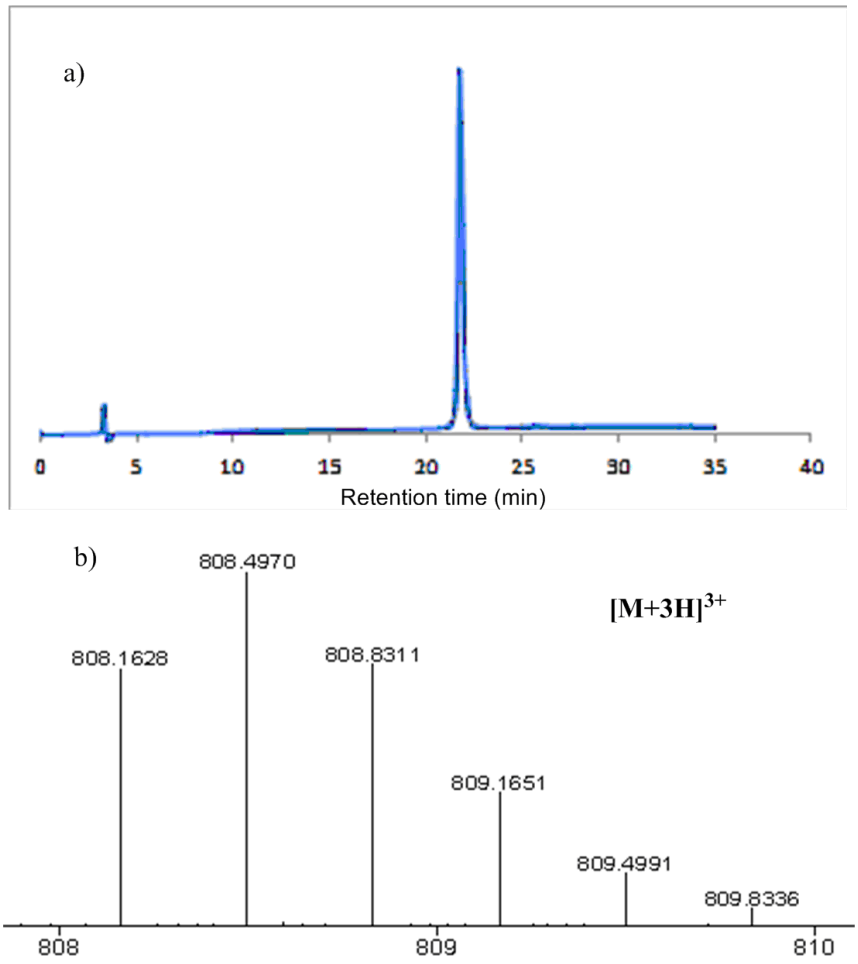
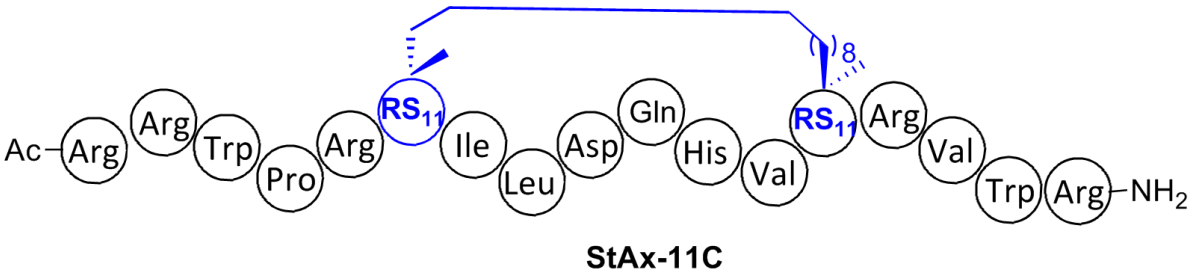


Figure S4. a) HPLC trace of purified **StAx-10C**. Gradient: 10-60% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm). b) HR-MS spectrum of **StAx-10C** (calc. for $C_{113}H_{184}N_{40}O_{20}$ 2421.4611, found $[M+3H]^{3+}$ 808.1628).



StAx-11C

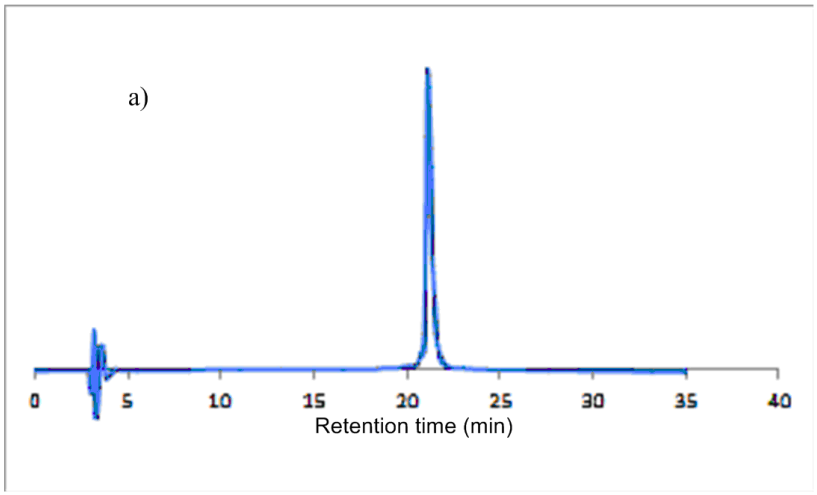
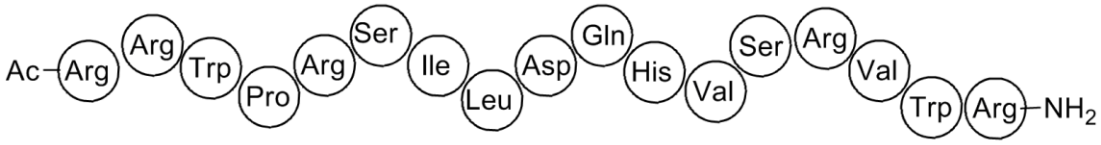


Figure S5. a) HPLC trace of purified **StAx-11C**. Gradient: 20-50% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm). b) HR-MS spectrum of **StAx-11C** (calc. for $C_{113}H_{182}N_{38}O_{21}$ 2407.4342, found $[M+3H]^{3+}$ 803.4866).



N-35R

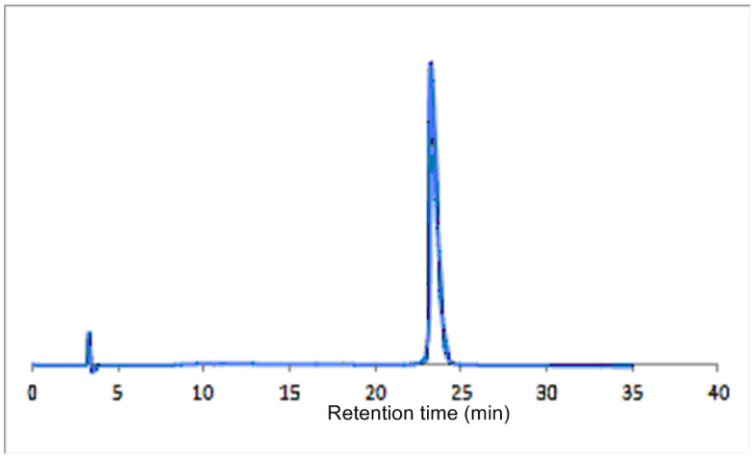
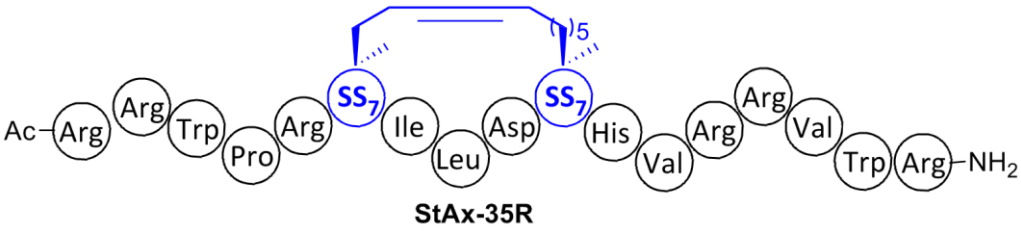


Figure S6. HPLC trace of purified **N-35R**. Gradient: 10-40% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm). ESI-MS calc. For $C_{99}H_{157}N_{37}O_{21}$ 2200.2, found $[M+3H]^{3+}$ 735.1.



StAx-35R

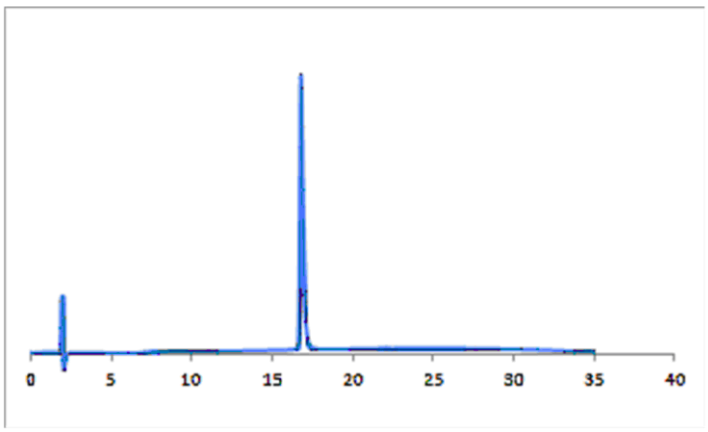


Figure S7. HPLC trace of purified **StAx-35R**. Gradient: 10-70% of buffer B in 30 min with C18 column (5 μm, 2.5 mm×250 mm). ESI-MS calc. for $C_{111}H_{178}N_{40}O_{20}$ 2391.4, found $[M+3H]^{3+}$ 798.4.

2.2.3 Automated synthesis of stapled peptide on automated peptide synthesizer

StAx-7C was successfully synthesized by using a CS136XT synthesizer running with a scale of 0.1 mmol.

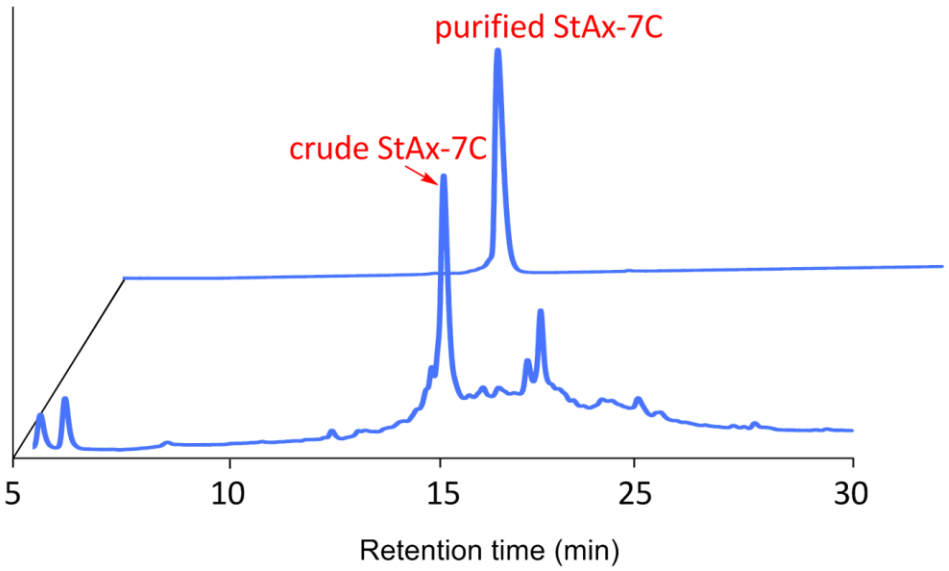


Figure S8. HPLC traces of crude and purified **StAx-7C** synthesized on automated chemical synthesizer.

2.2.4 CD spectra

The helicity of peptides was investigated by circular dichroism spectroscopy. Peptides were dissolved in a mixture of water and trifluoroethanol (9:1,v/v) to a final concentration of 100 μM. CD spectra were acquired with a Applied Photophysics Chirascan Spectrometer at rt. between 260 nm and 180 nm in a 0.1 cm path length cell. The percent helicity of peptides was calculated with the equation described by Fairlie.[5]

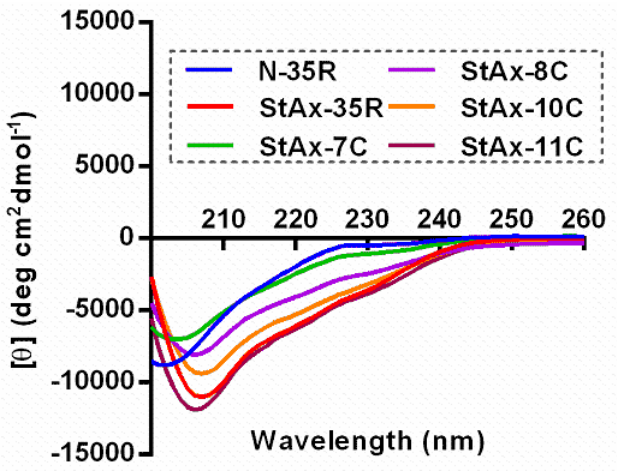


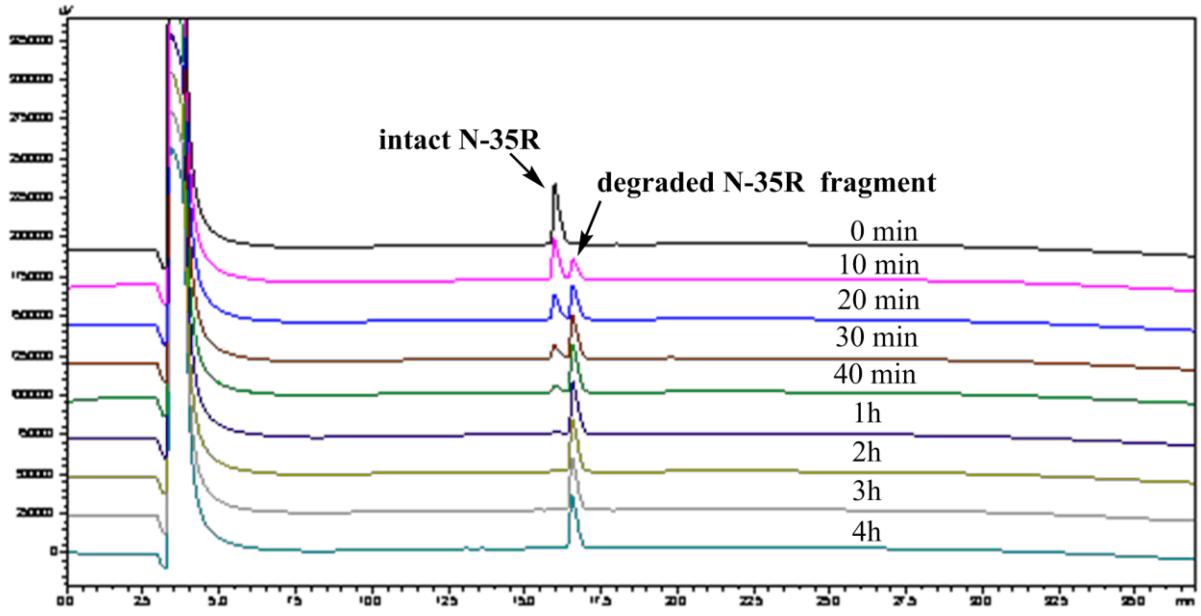
Figure S9. Circular dichroism spectra of each peptide.

2.2.5 Wnt pathway inhibiting test

HeLa cells containing Topflash reporter were treated with stapled peptides (40 μM) for 24 h in the presence of the ligand Wnt3a or not, then followed by luciferase activity measurement.[6]

2.2.6 Protease stability experiment[7]

50 mM PBS buffer (pH 7.4) containing 2 mM $CaCl_2$ was first prepared and centrifuged to remove precipitate. Then chymotrypsin was dissolved in this buffer to a final concentration of 0.5 ng/μL. Peptide (25 μL) in DMSO (1 mM stock) and 975 μL of above buffer containing chymotrypsin were mixed for protease reaction. Kinetic degradation of peptides was monitored by HPLC (5-95% of buffer B in 30 min with C4 column). Degradation experiment for every peptide was conducted twice.



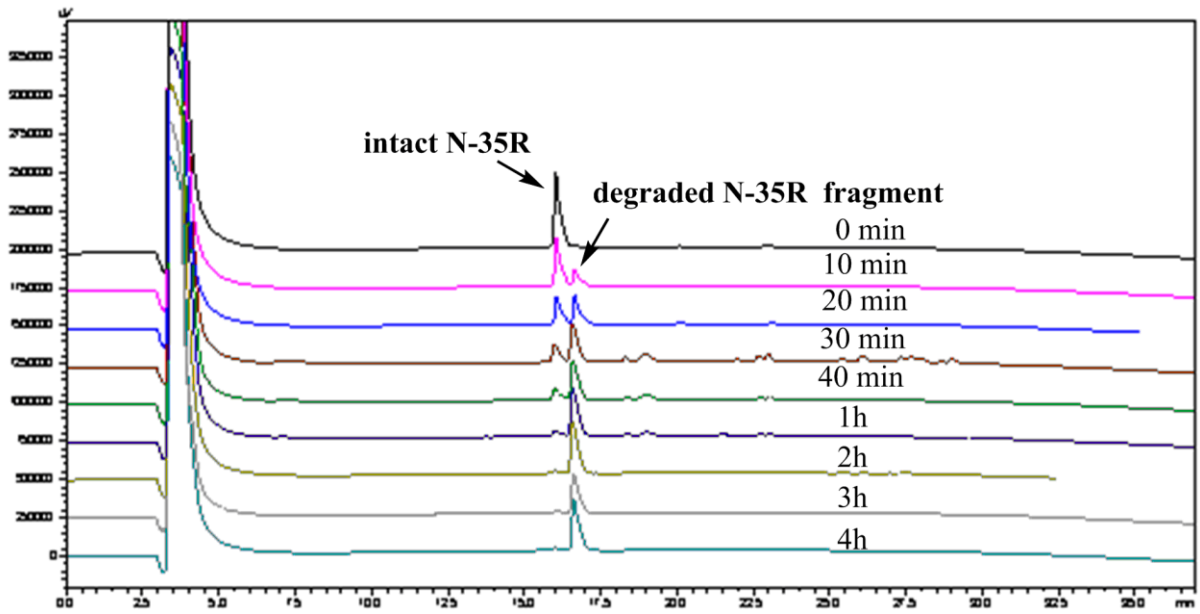
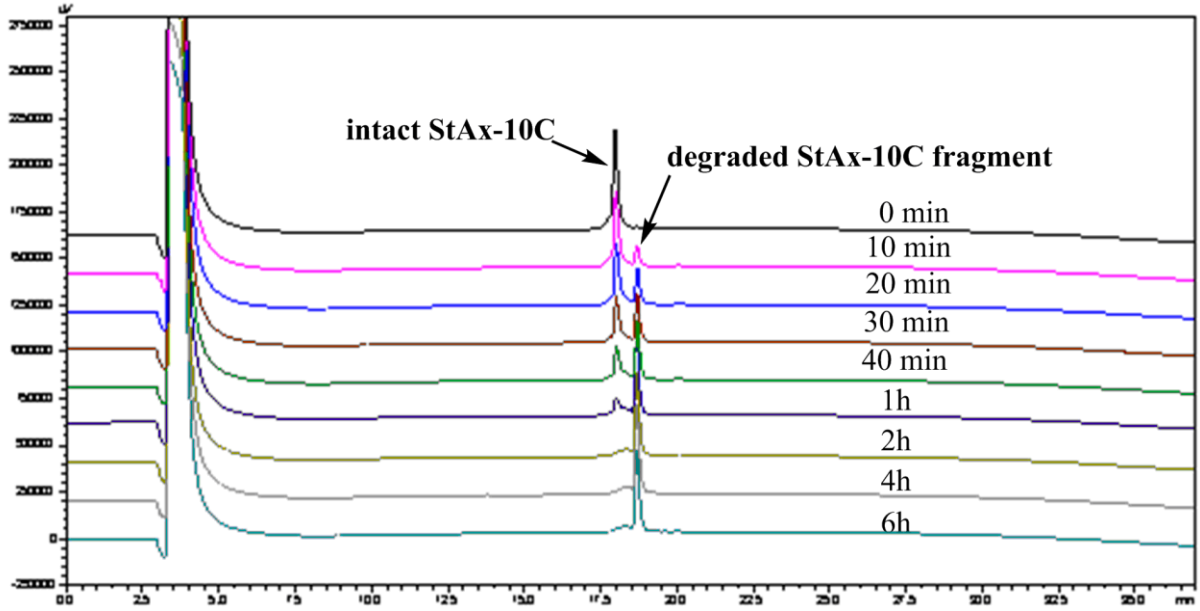


Figure S10. HPLC trace of degradation of **N-35R** between 0 min-4 h. Gradient: 5-95% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm).



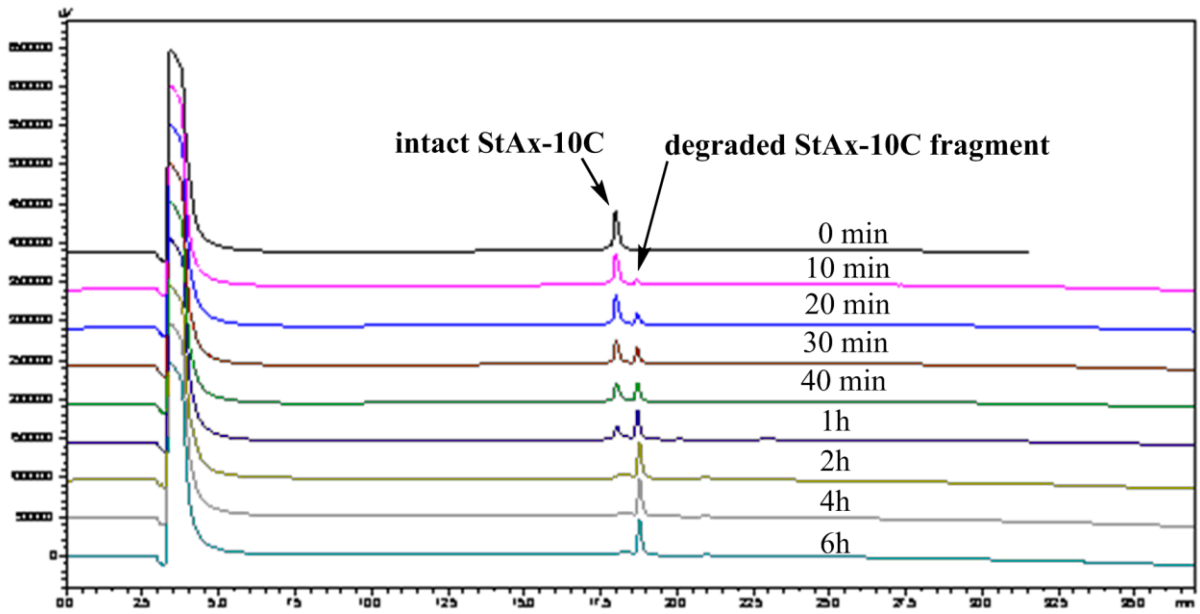
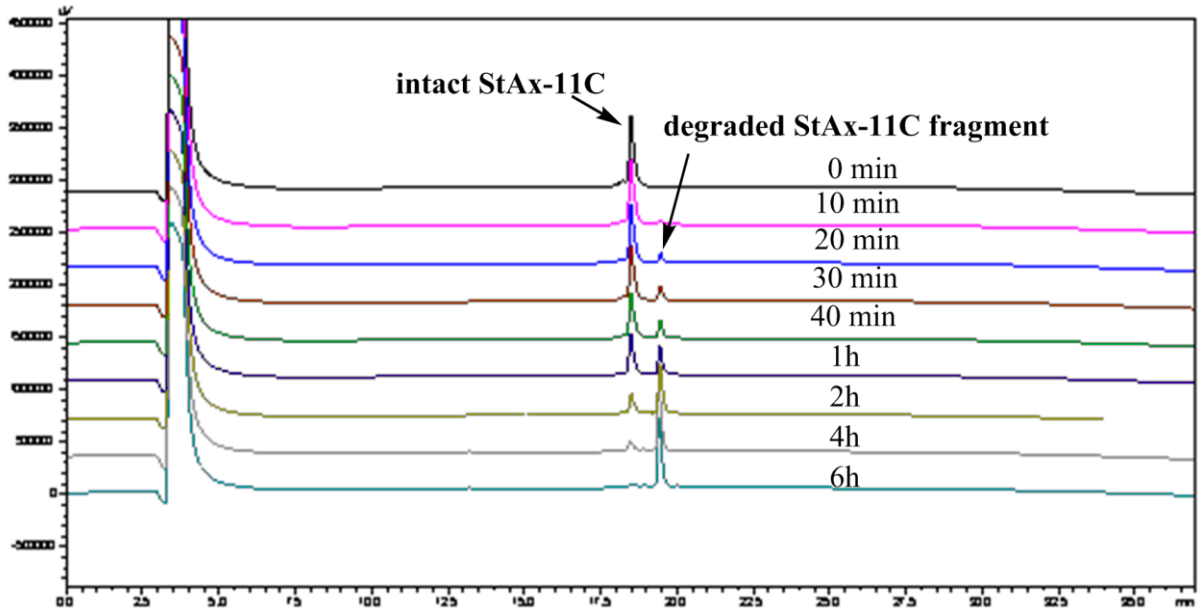


Figure S11. HPLC trace of degradation of **StAx-10C** between 0 min-4 h. Gradient: 5-95% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm).



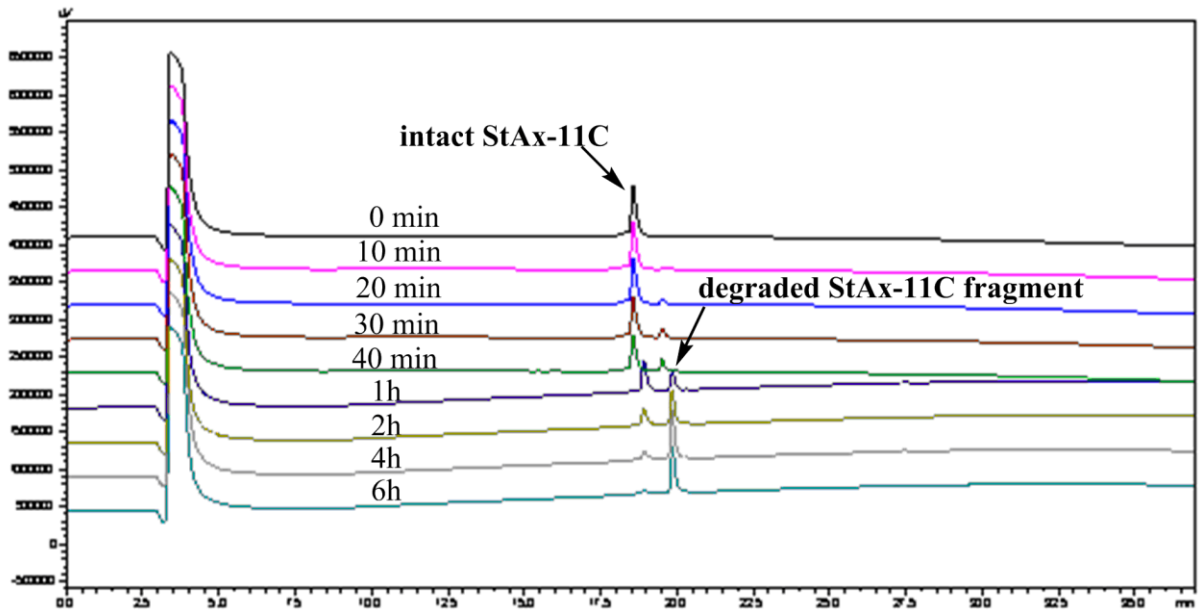


Figure S12. HPLC trace of degradation of **StAx-11C** between 0 min-4 h. Gradient: 5-95% of buffer B in 30 min with C4 column (5 μm, 2.5 mm×250 mm).

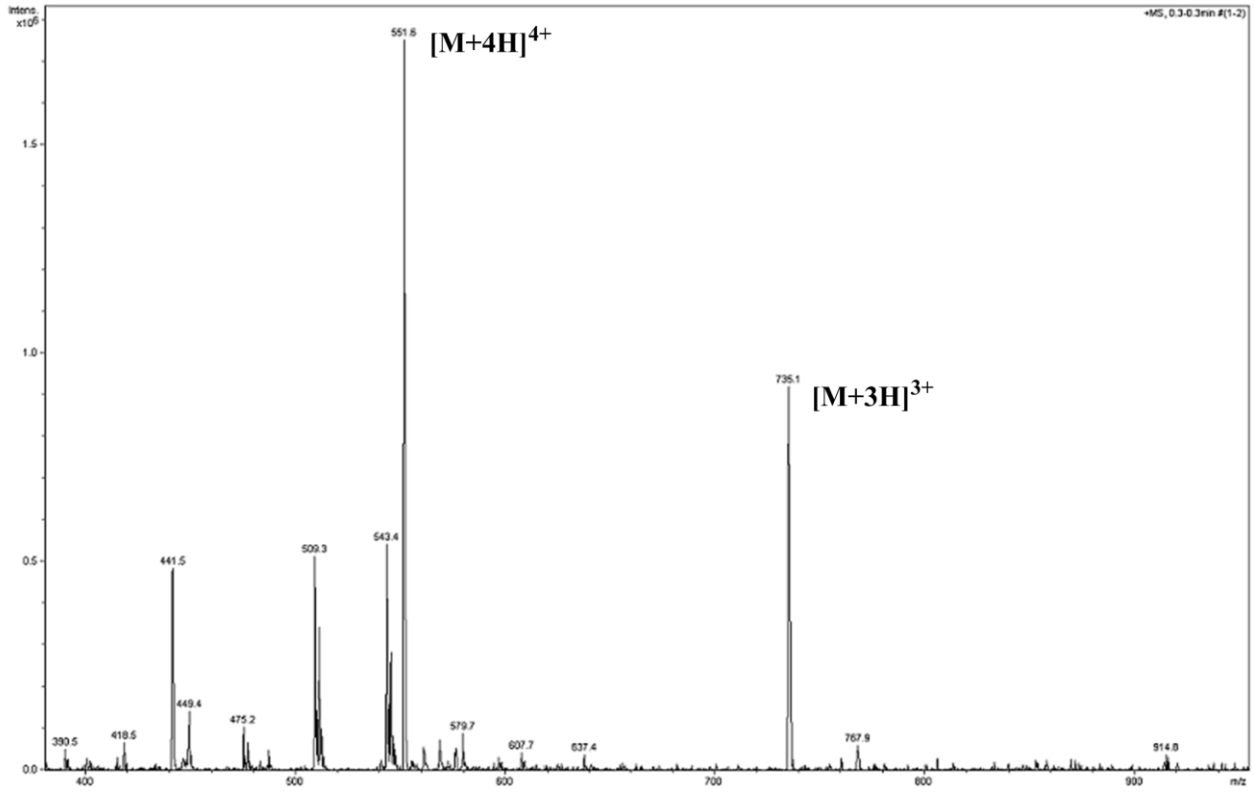


Figure S13. ESI spectrum of degraded peptide fragments of **N-35R** (cleaved at N-terminus Trp-Arg site) (calc. for $C_{99}H_{156}N_{36}O_{22}$ 2201.2, found $[M+3H]^{3+}$ 735.1, $[M+4H]^{4+}$ 551.6).

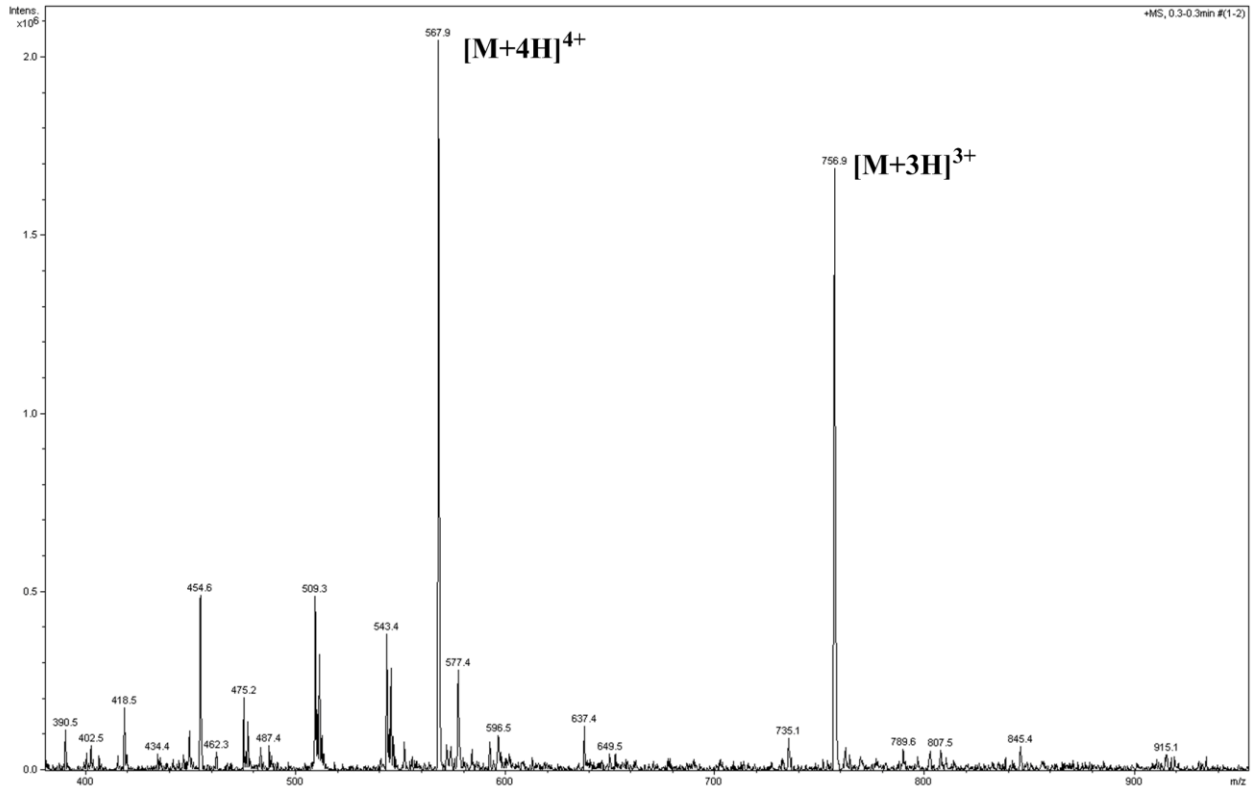


Figure S14. ESI spectrum of degraded peptide fragments of **StAx-10C** (cleaved at N-terminus Trp-Arg site) (calc. for $C_{107}H_{171}N_{35}O_{20}$ 2266.3, found $[M+3H]^{3+}$ 756.9, $[M+4H]^{4+}$ 567.9).

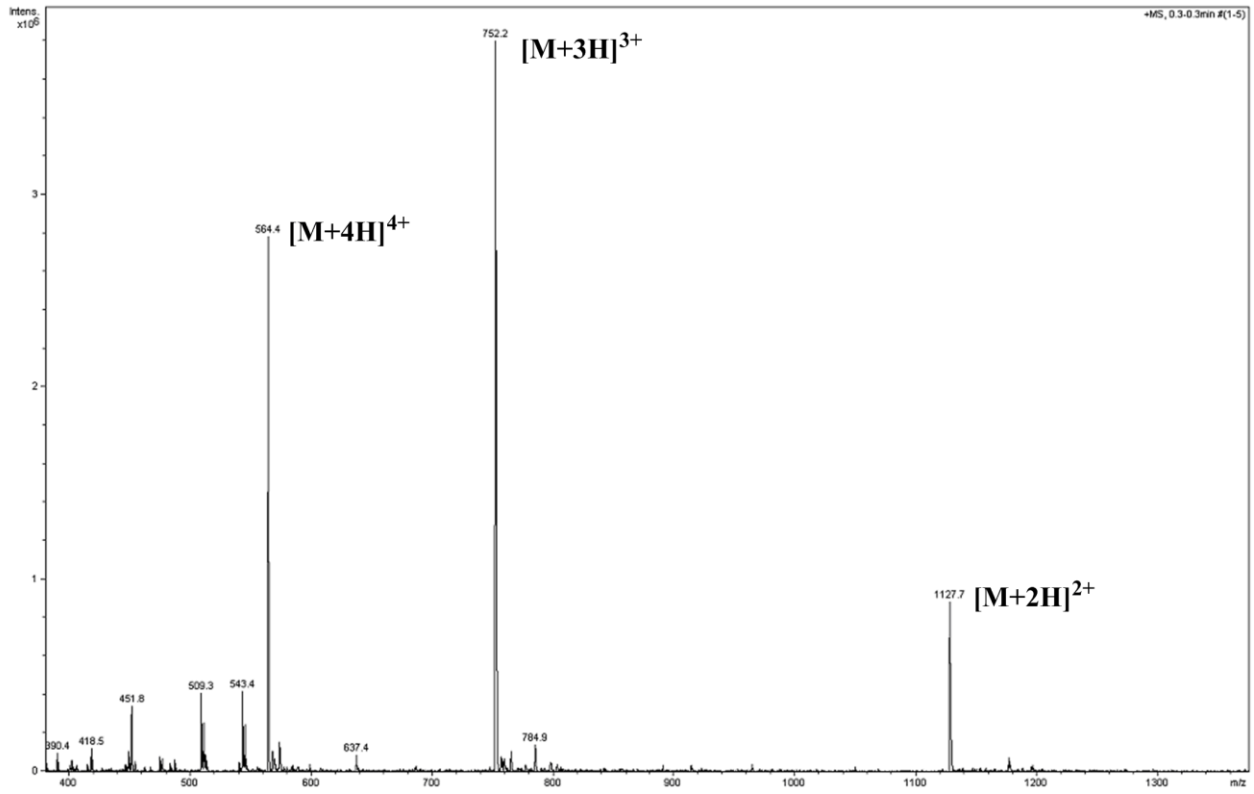


Figure S15. ESI spectrum of degraded peptide fragments of **StAx-11C** (cleaved at N-terminus Trp-Arg site) (calc. for $C_{107}H_{169}N_{33}O_{21}$ 2252.3, found $[M+2H]^{2+}$ 1127.7, $[M+3H]^{3+}$ 752.2, $[M+4H]^{4+}$ 564.4).

Reference:

1. Long, Y. Q.; Xue, T.; Song, Y. L.; Liu, Z. L.; Huang, S. X.; Yu, Q. *J. Med. Chem.* **2008,** 51, 6371.
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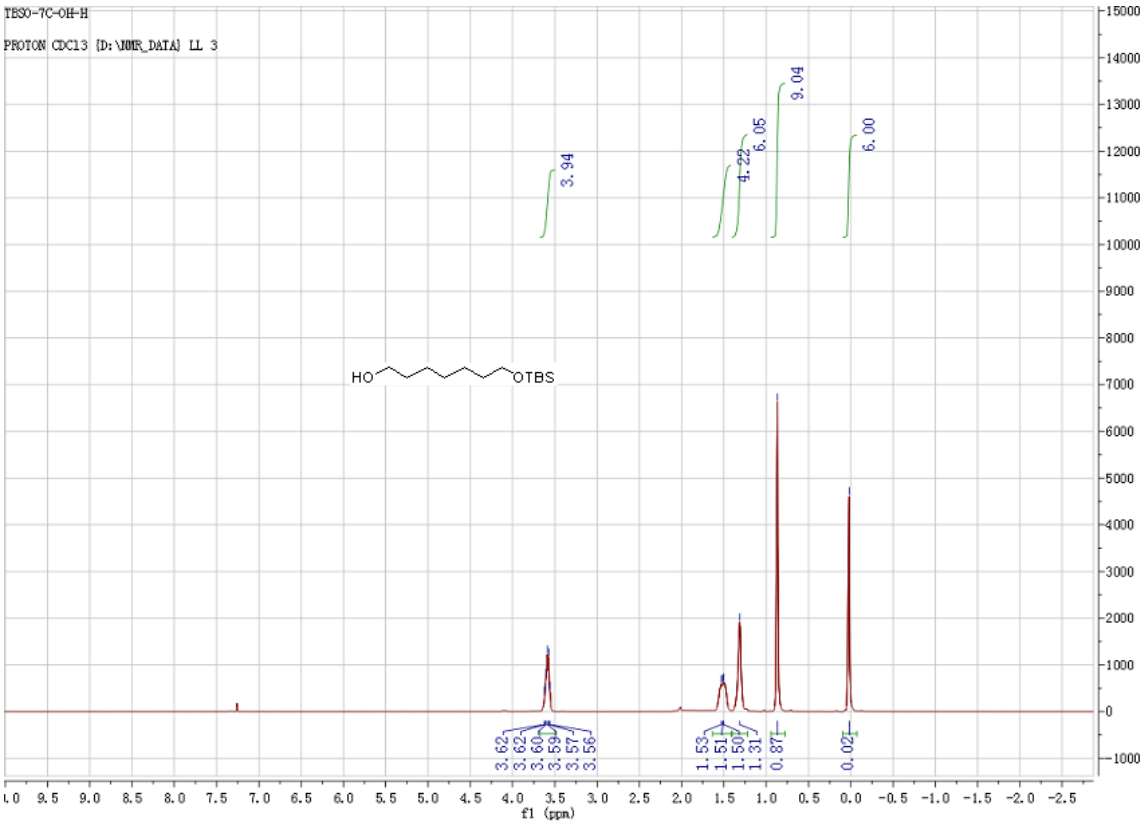
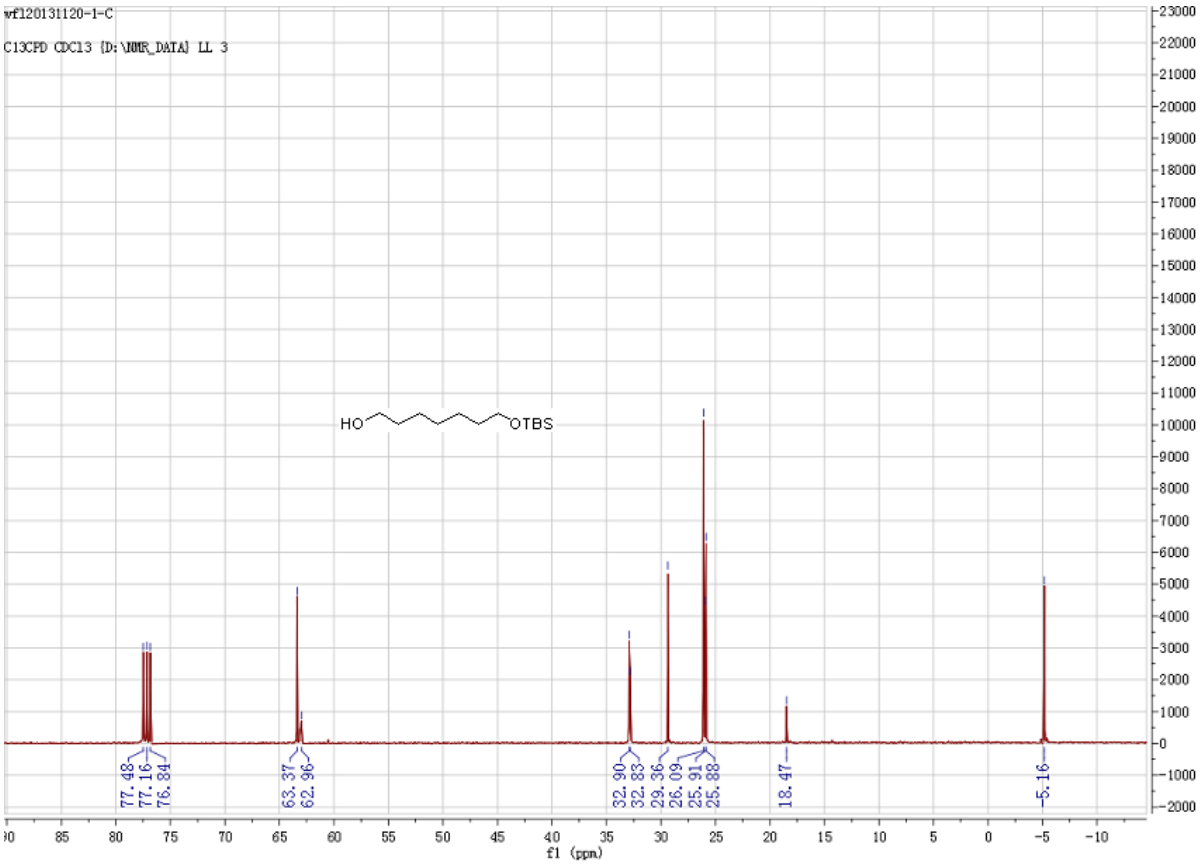
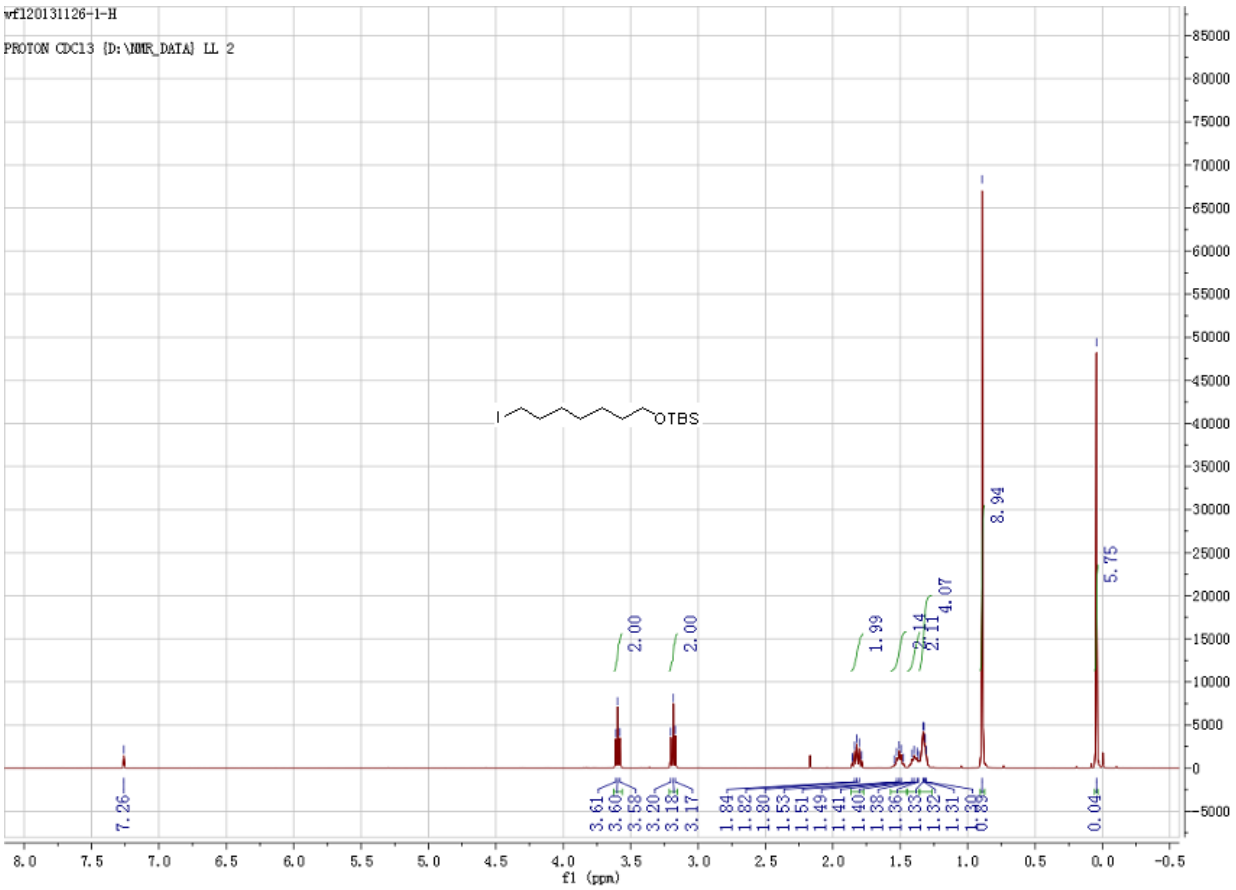
^{1}H and ^{13}C NMR spectra of compounds



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1.51
1.49
1.41
1.40
1.38
1.36
1.33
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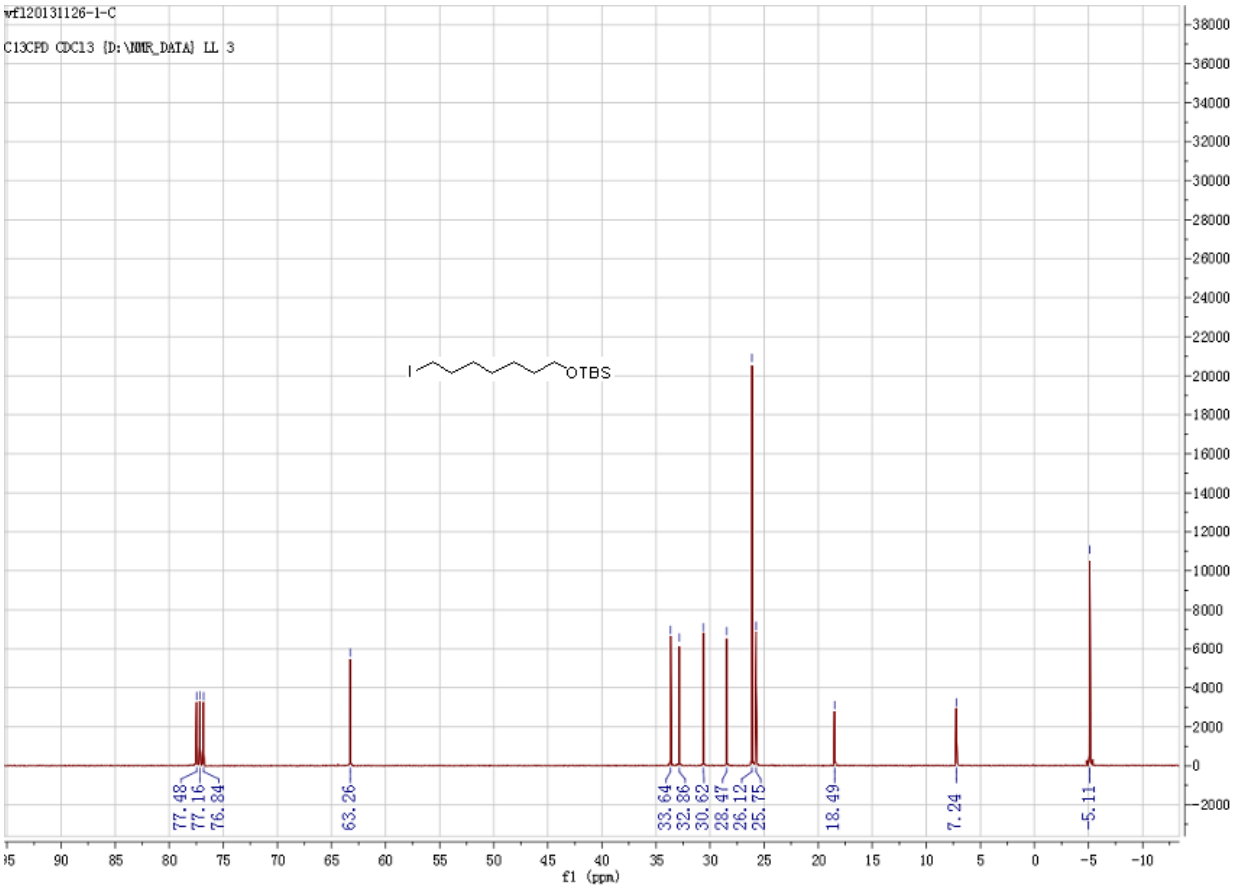
f1 (ppm)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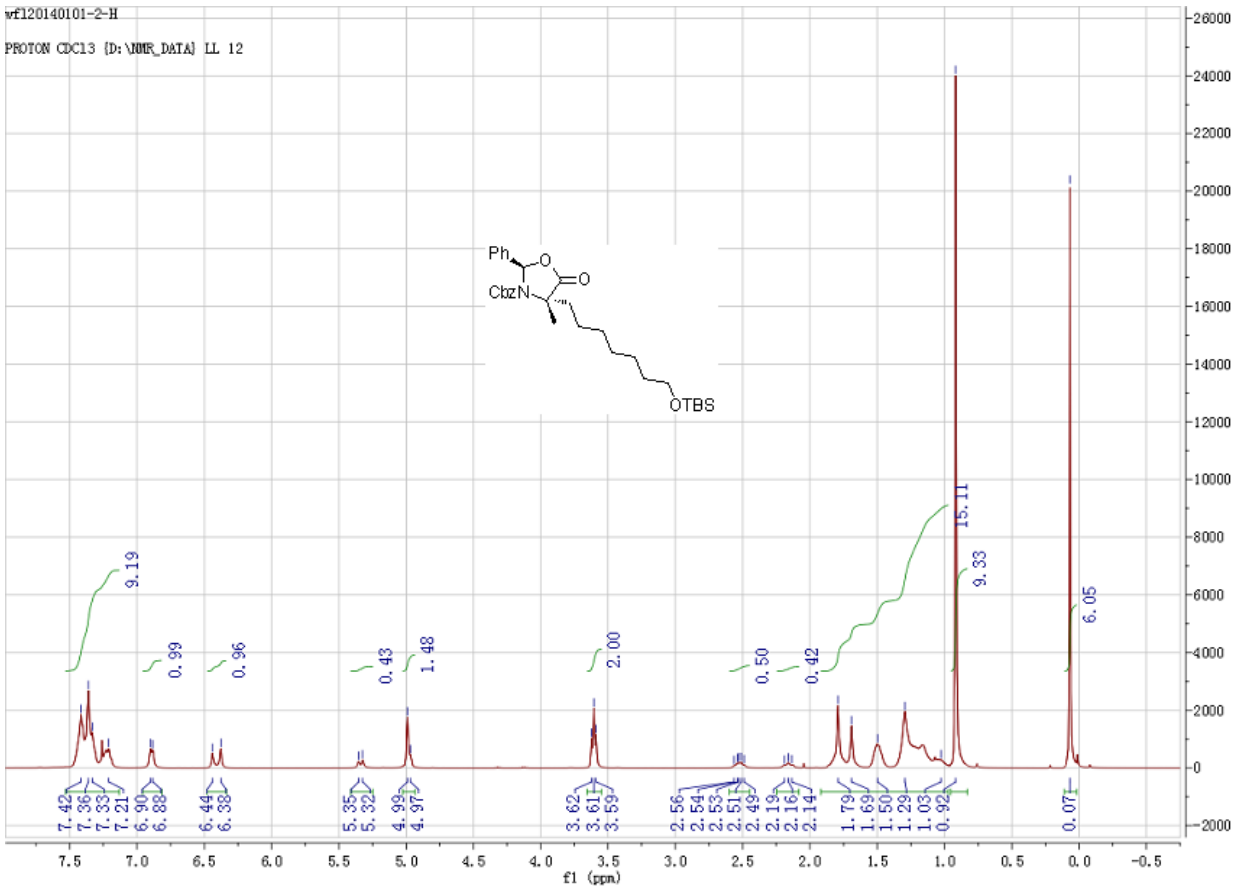
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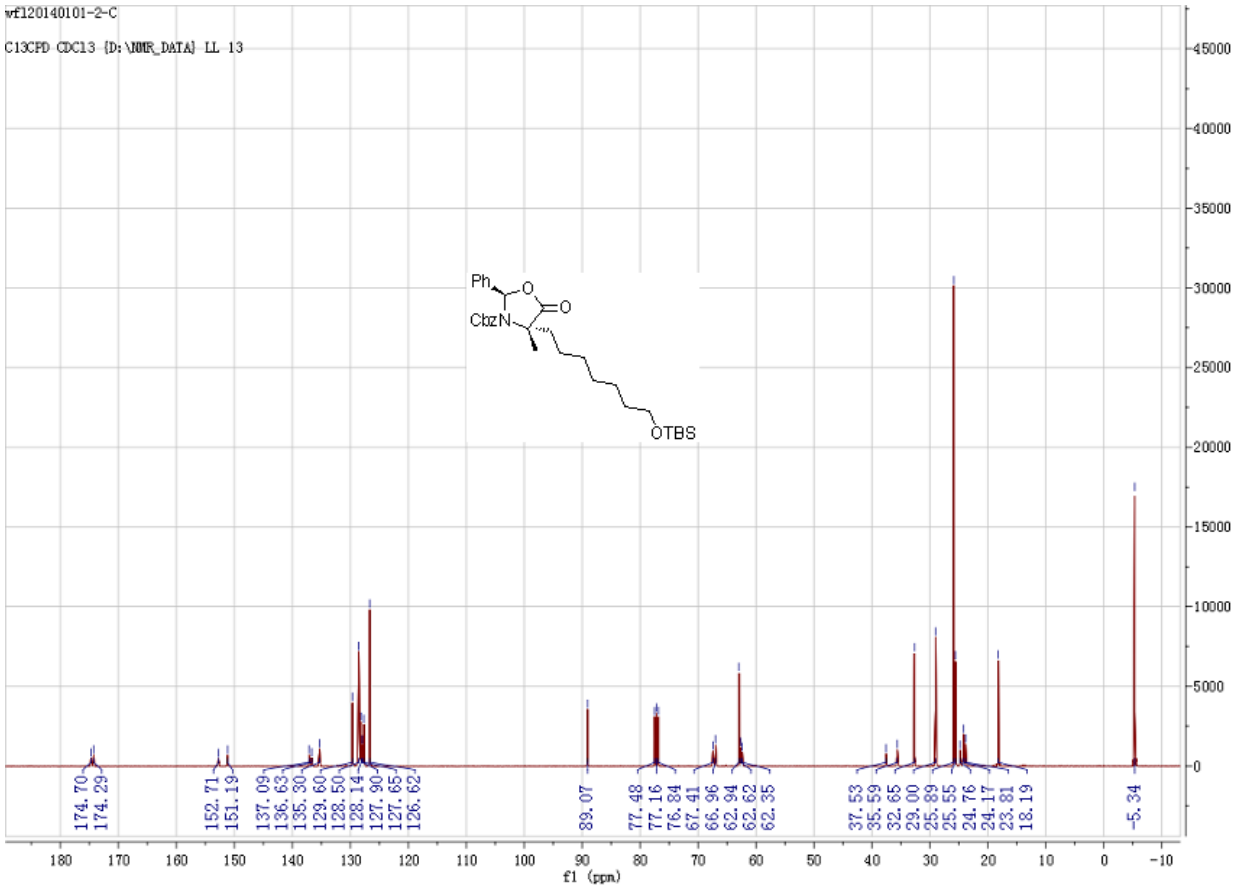
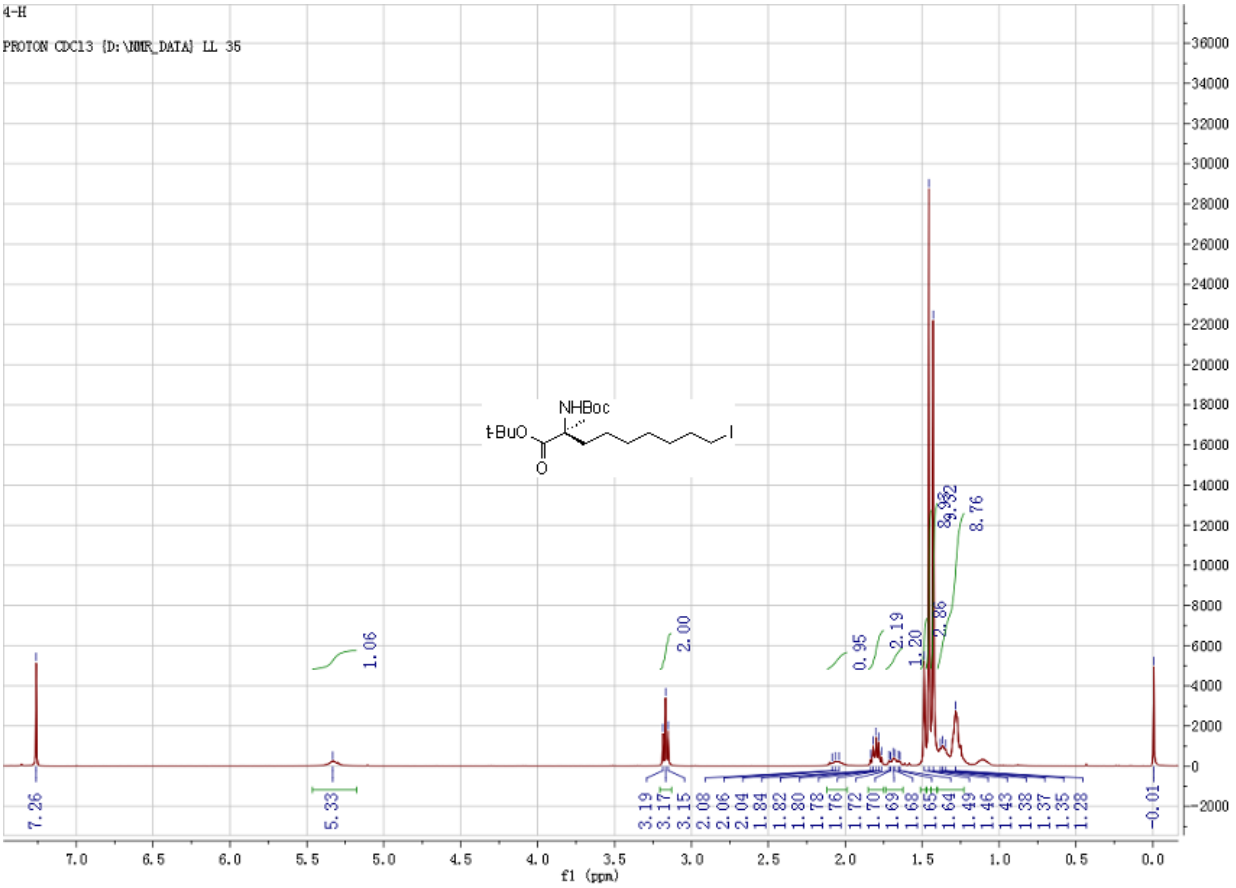
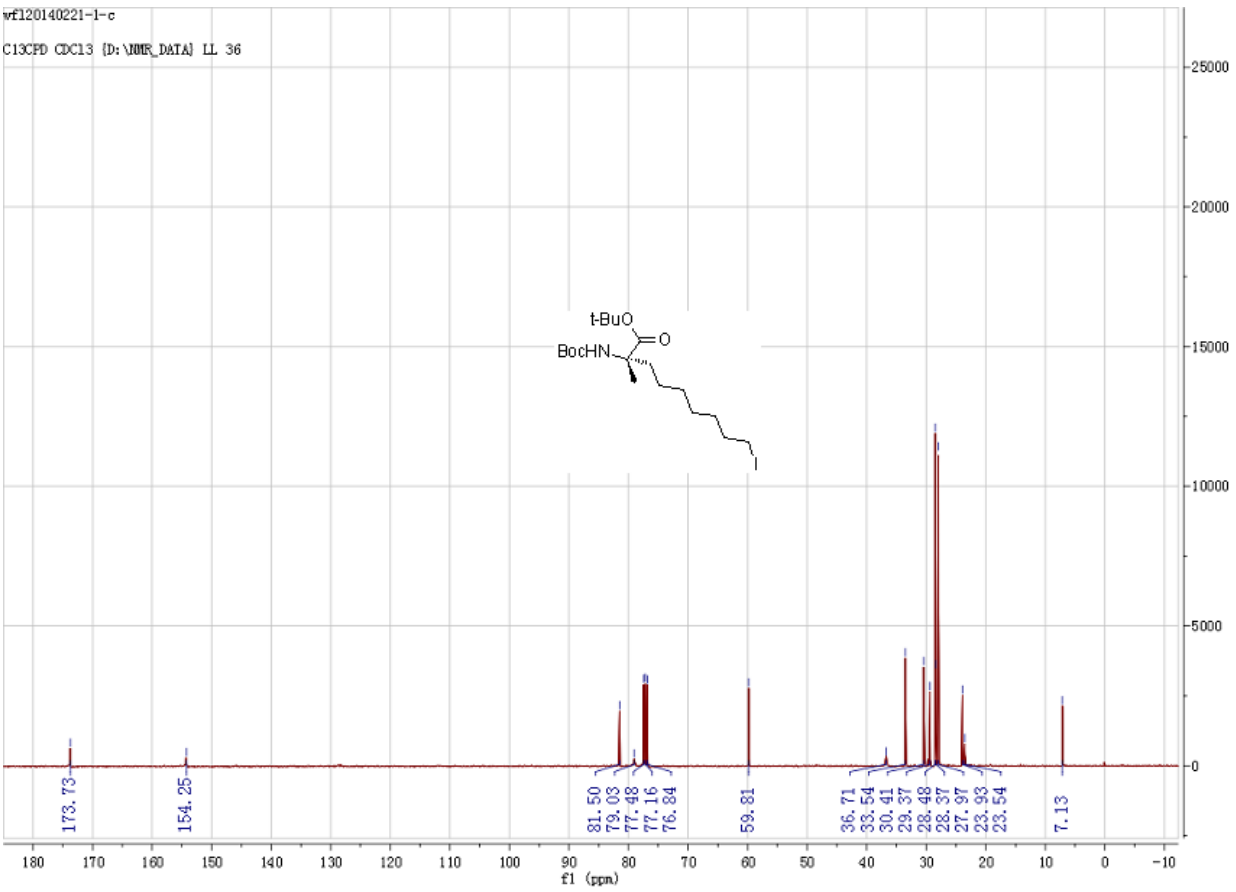
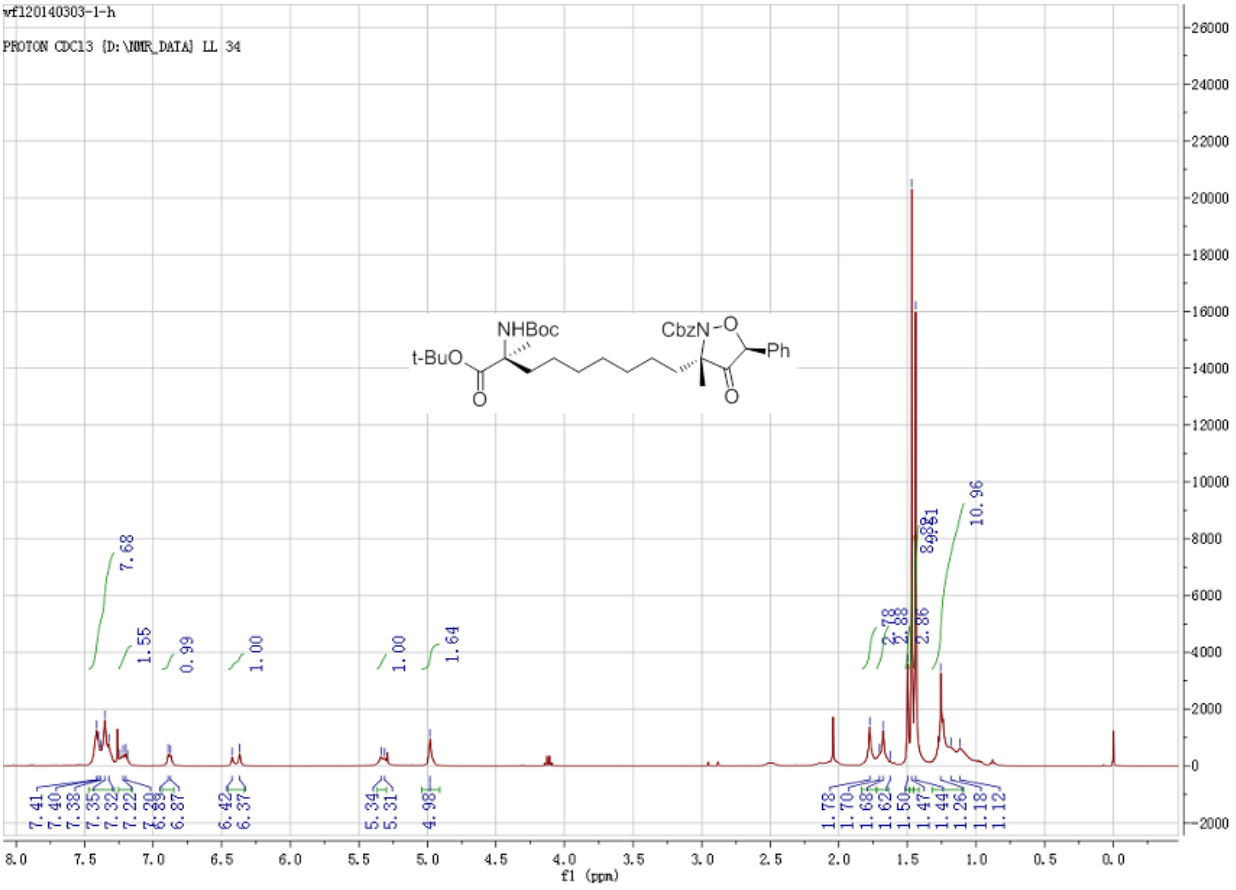
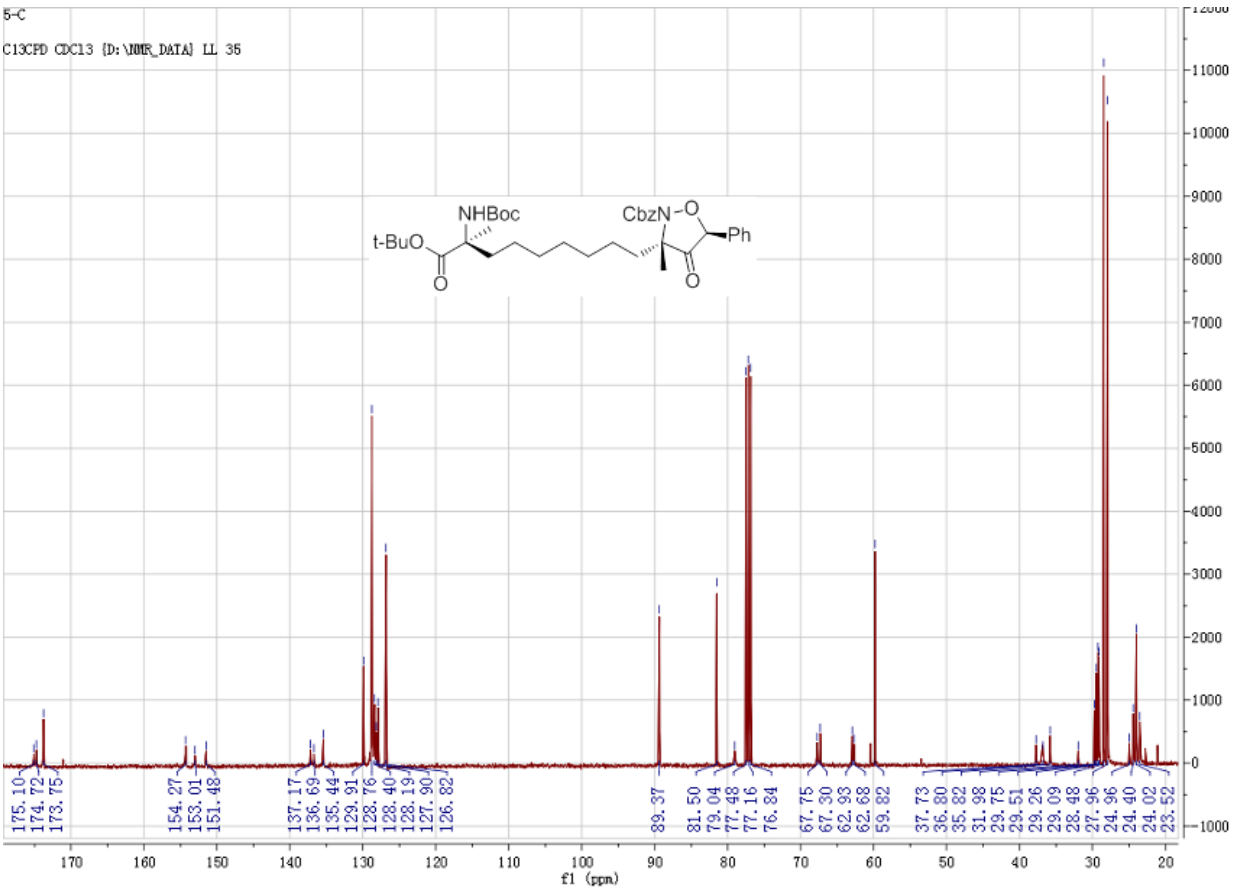
9.33
6.05
f1 (ppm)

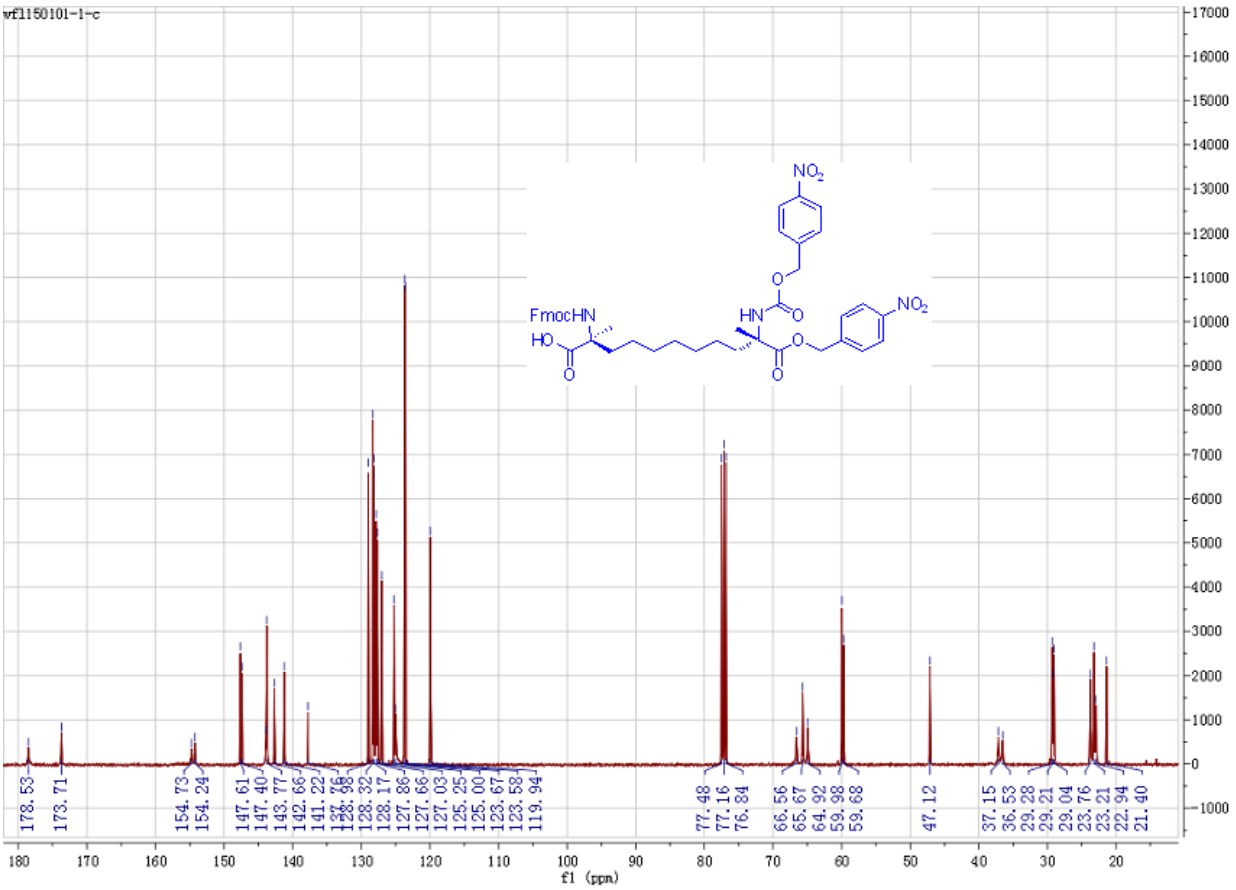

wf120140101-2-C
C13CPD CDCl3 (D:\NMR_DATA) LL 13
Ph
O
CbzN
O
OTBS
f1 (ppm)

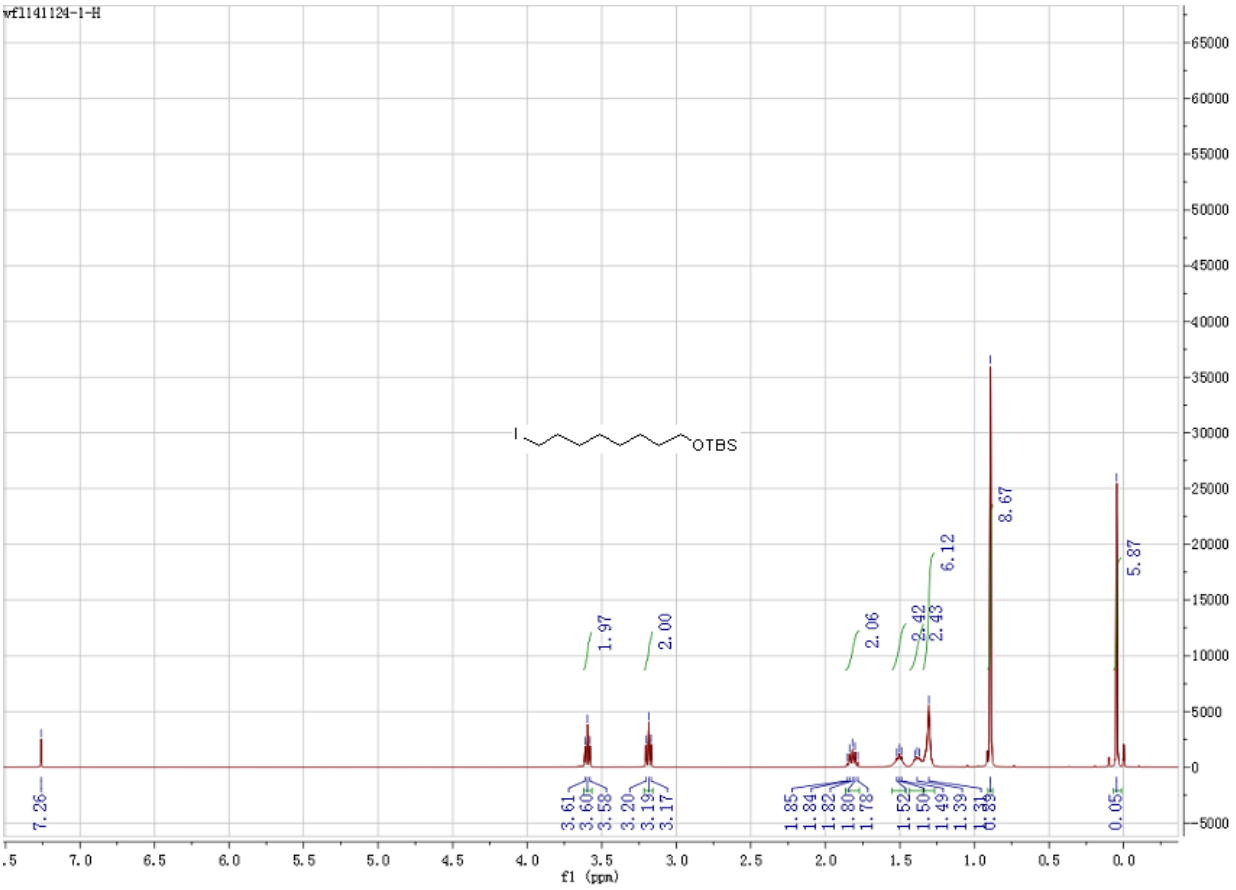

4-H
PROTON CDCl3 [D:\NMR_DATA] LL 36
NHBoc
t-BuO
O
I
7.26
1.06
5.33
2.00
3.19
3.17
3.15
0.95
2.19
1.20
2.86
8.76
0.01
f1 (ppm)

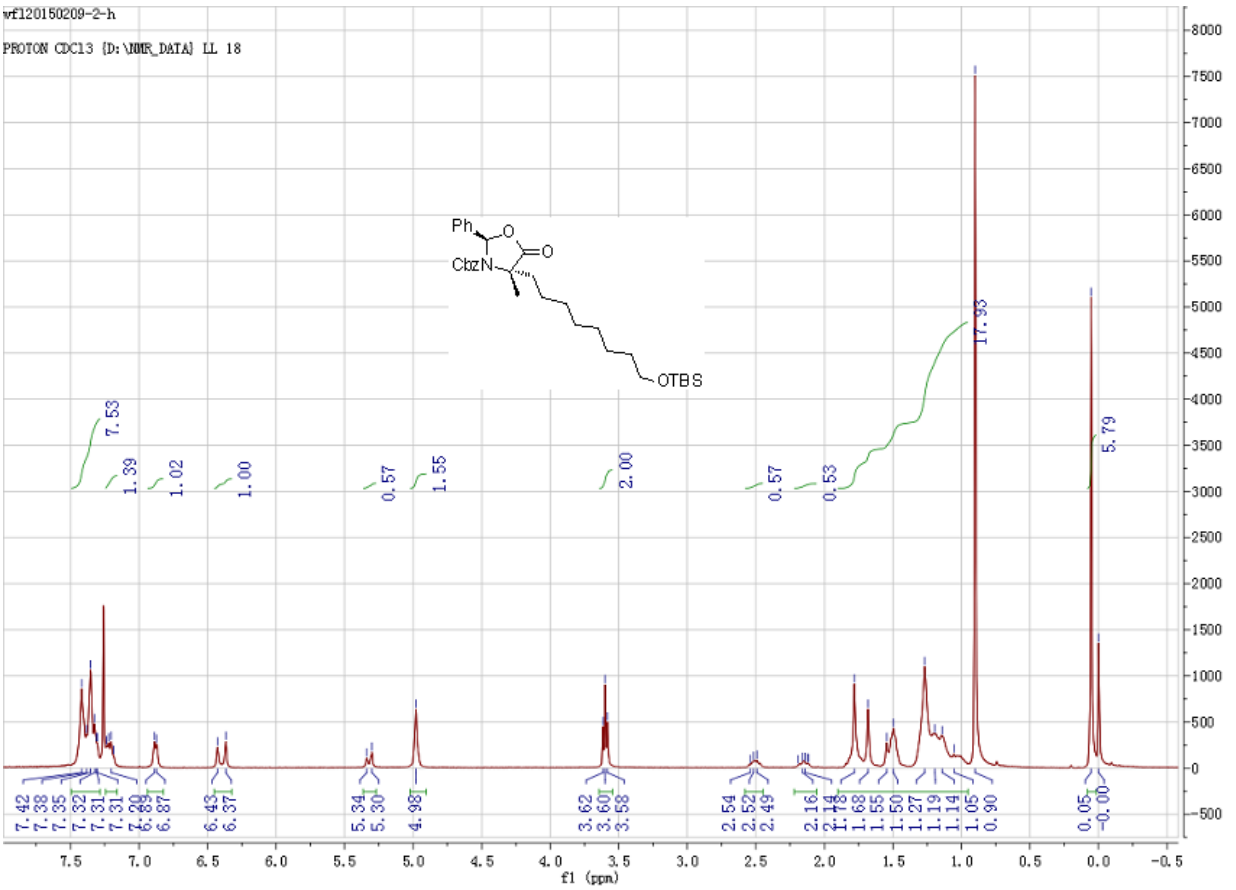

wfl20140221-1-c
C13CPD CDCl3 [D:\NMR_DATA] LL 36
t-BuO
O
BocHN
I
173.73
154.25
81.50
79.03
77.48
77.16
76.84
59.81
36.71
33.54
30.41
29.37
28.48
28.37
27.97
23.93
23.54
7.13
f1 (ppm)

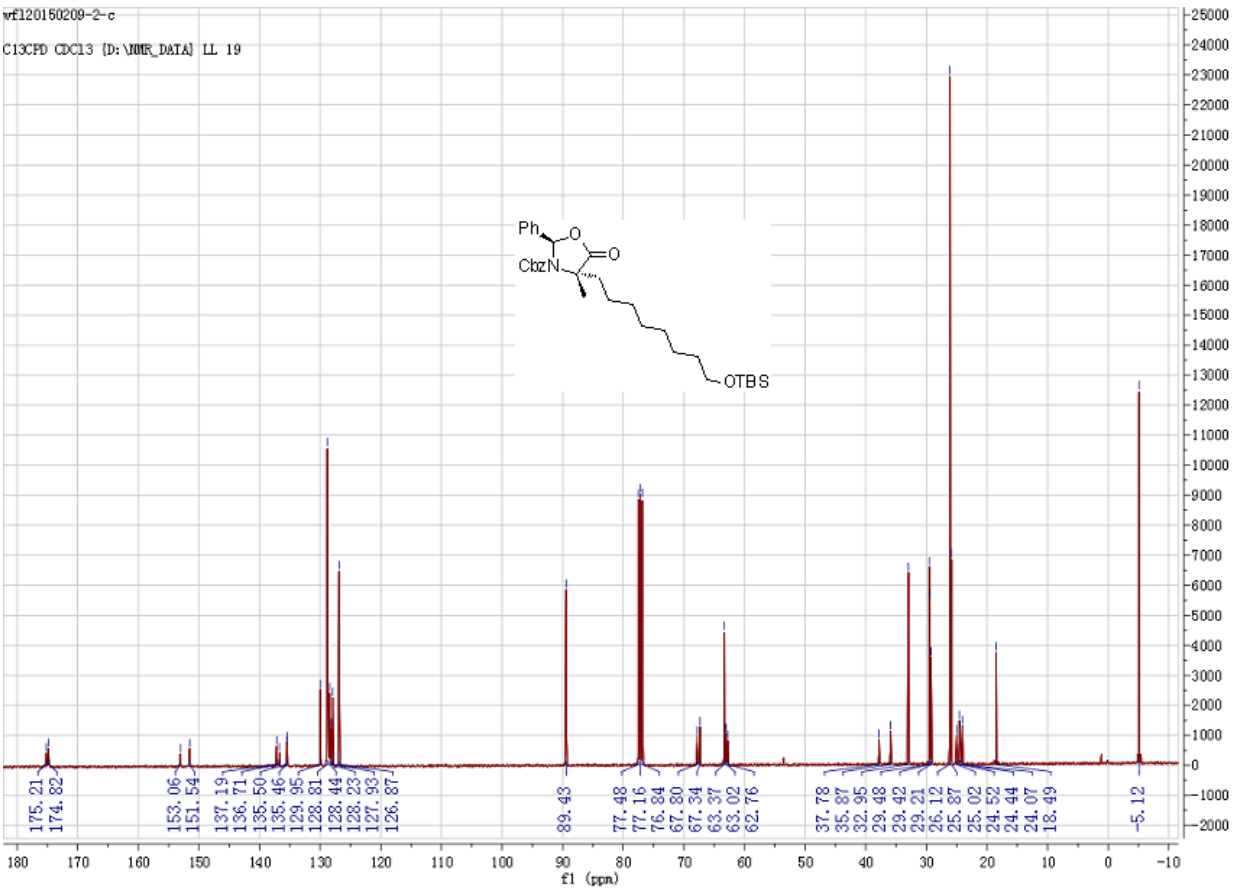

wf120140303-1-h
PROTON CDCl3 [D:\NMR_DATA] LL 34
NHBoc
CbzN
O
t-BuO
Ph
O
O
7.68
1.55
0.99
1.00
1.00
1.64
10.96
f1 (ppm)

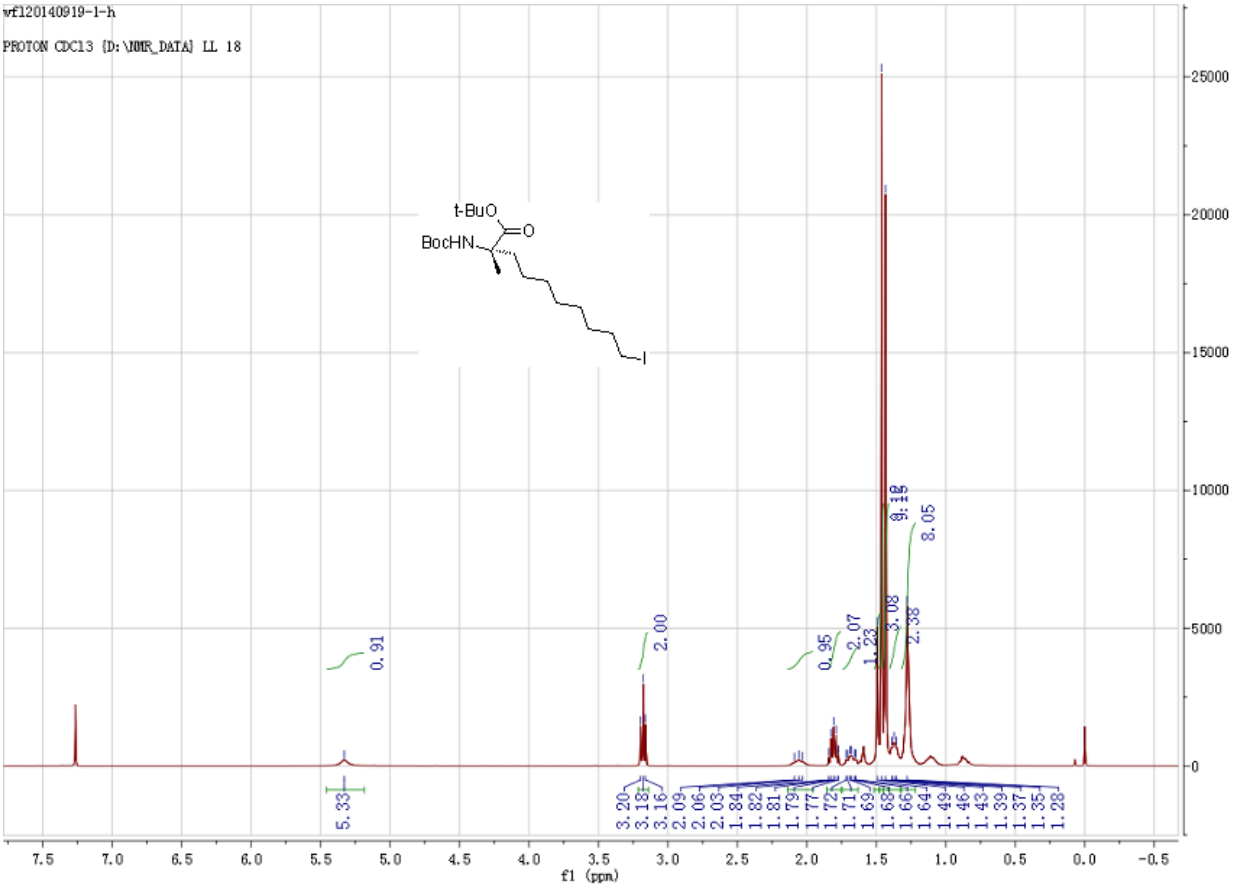

5-C
C13CPD CDCl3 [D:\NMR_DATA] LL 35
NHBoc
CbzN
O
t-BuO
Ph
O
O
175.10
174.72
173.75
154.27
153.01
151.48
137.17
136.69
135.44
129.91
128.76
128.40
128.19
127.90
126.82
89.37
81.50
79.04
77.48
77.16
76.84
67.75
67.30
62.93
62.68
59.82
37.73
36.80
35.82
31.98
29.75
29.51
29.26
29.09
28.48
27.96
24.96
24.40
24.02
23.52
f1 (ppm)

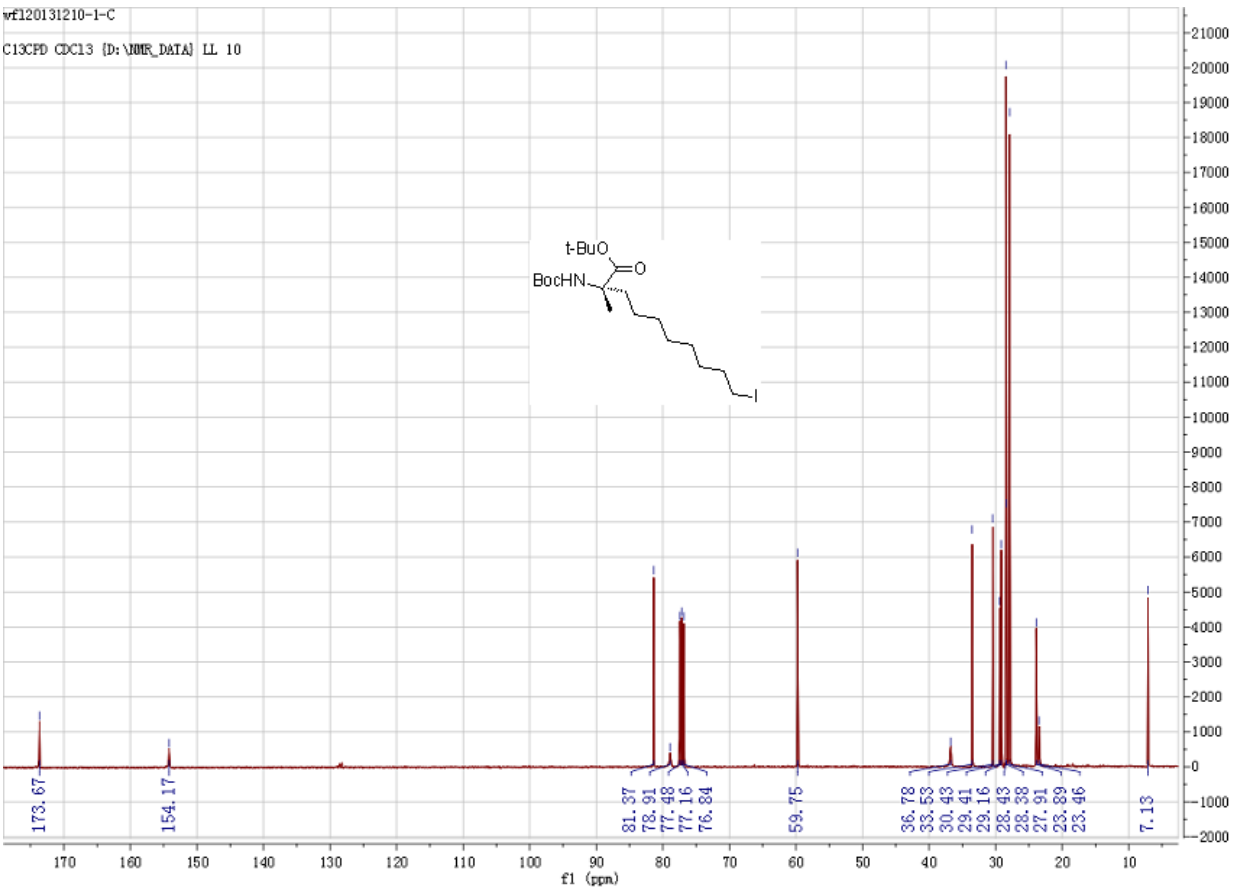

wf1150101-1-c
FmocHN
HO
HN
NO2
NO2
178.53
173.71
154.73
154.24
147.61
147.40
143.77
142.66
141.22
137.76
128.98
128.32
128.17
127.86
127.66
127.03
125.25
125.00
123.67
123.58
119.94
77.48
77.16
76.84
66.56
65.67
64.92
59.98
59.68
47.12
37.15
36.53
29.28
29.21
29.04
23.76
23.21
22.94
21.40
f1 (ppm)

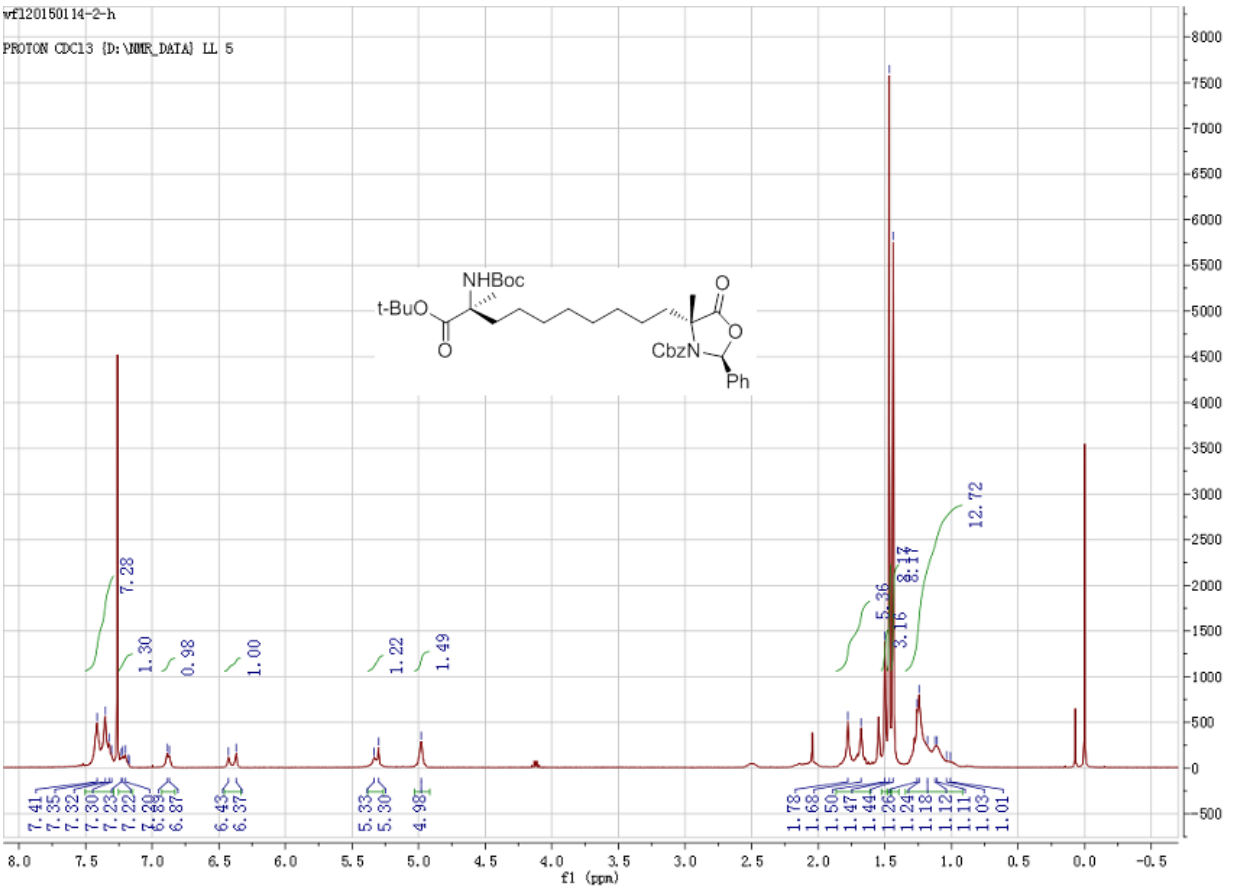

wf1141124-1-H
I
OTBS
7.26
1.97
2.00
2.06
2.42
2.43
6.12
8.67
5.87
3.61
3.60
3.58
3.20
3.19
3.17
1.85
1.84
1.82
1.80
1.78
1.52
1.50
1.49
1.39
1.31
0.88
0.05
f1 (ppm)

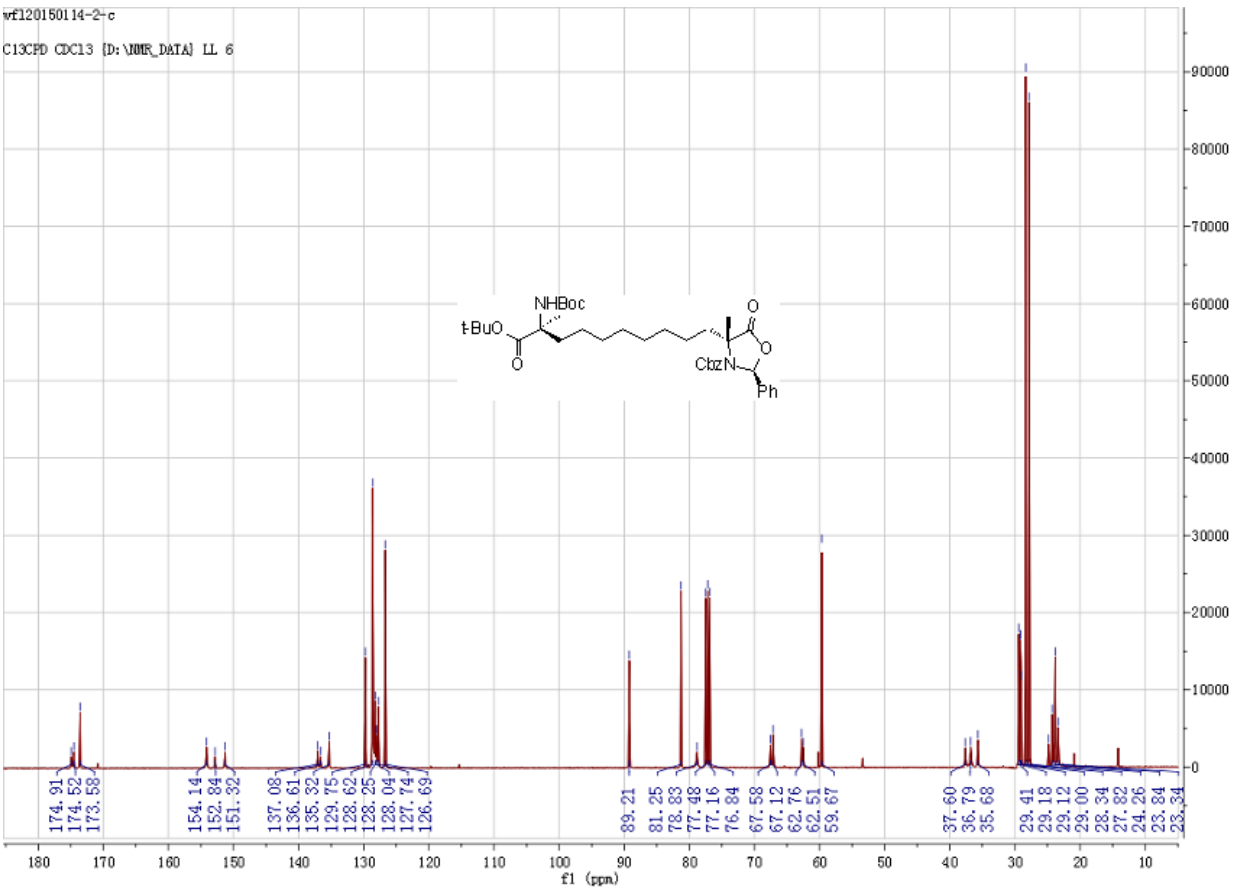

wf120150209-2-h
PROTON CDCl3 [D:\NMR_DATA] LL 18
Ph
O
CbzN
O
OTBS
7.53
1.39
1.02
1.00
0.57
1.55
2.00
0.57
0.53
17.93
5.79
f1 (ppm)

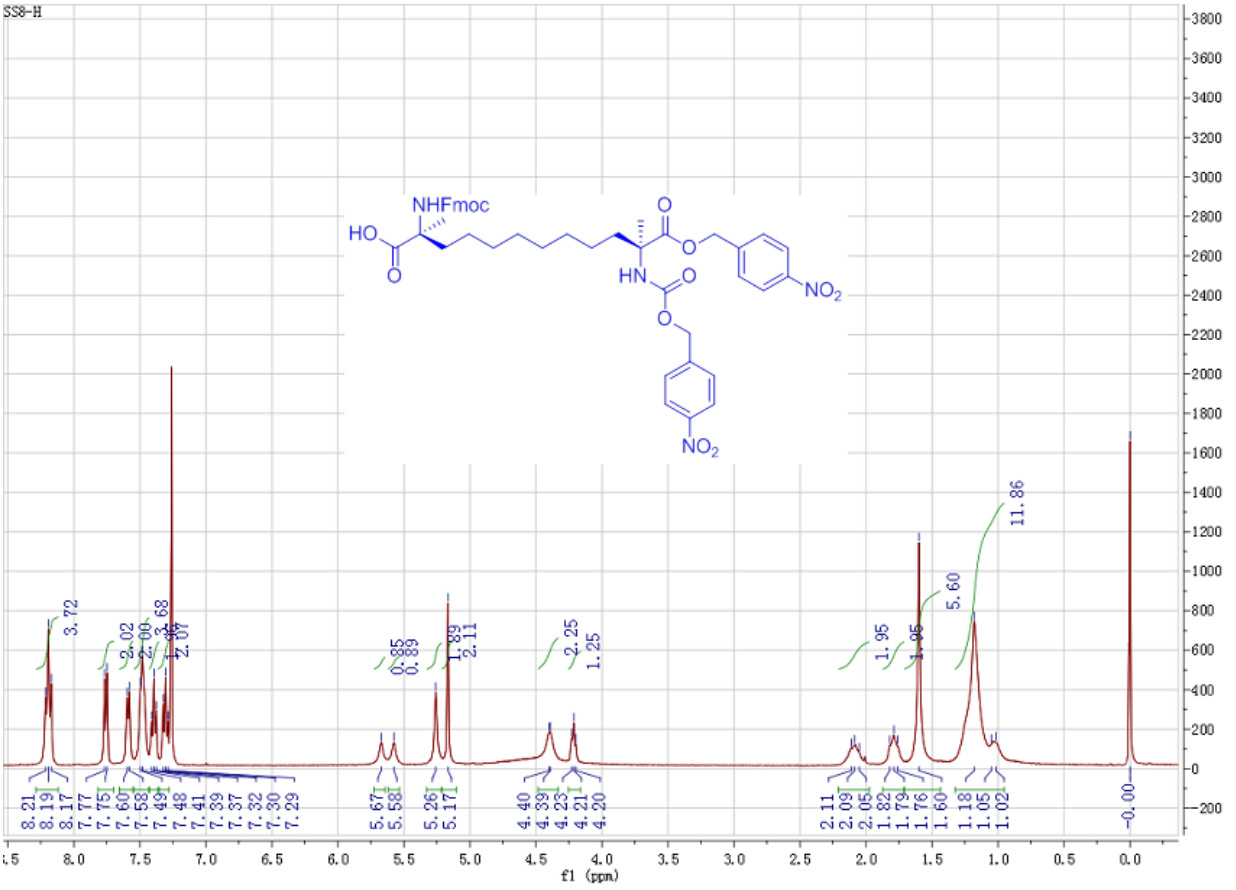

wf120150209-2-c
C13CPD CDCl3 [D:\NMR_DATA] LL 19
Ph
O
CbzN
O
OTBS
f1 (ppm)

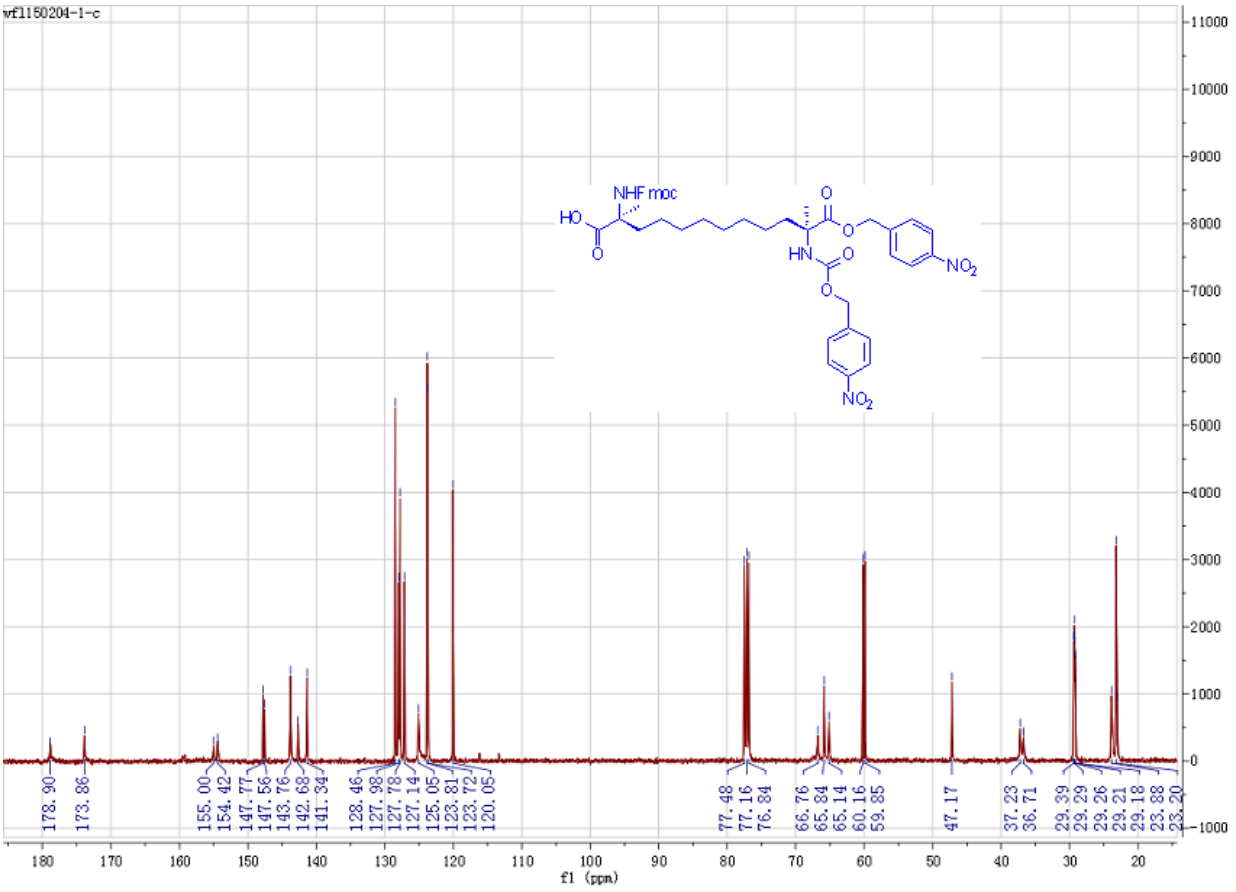

wfl20140919-1-h
PROTON CDCl3 [D:\NMR_DATA] LL 18
t-BuO
BocHN
f1 (ppm)

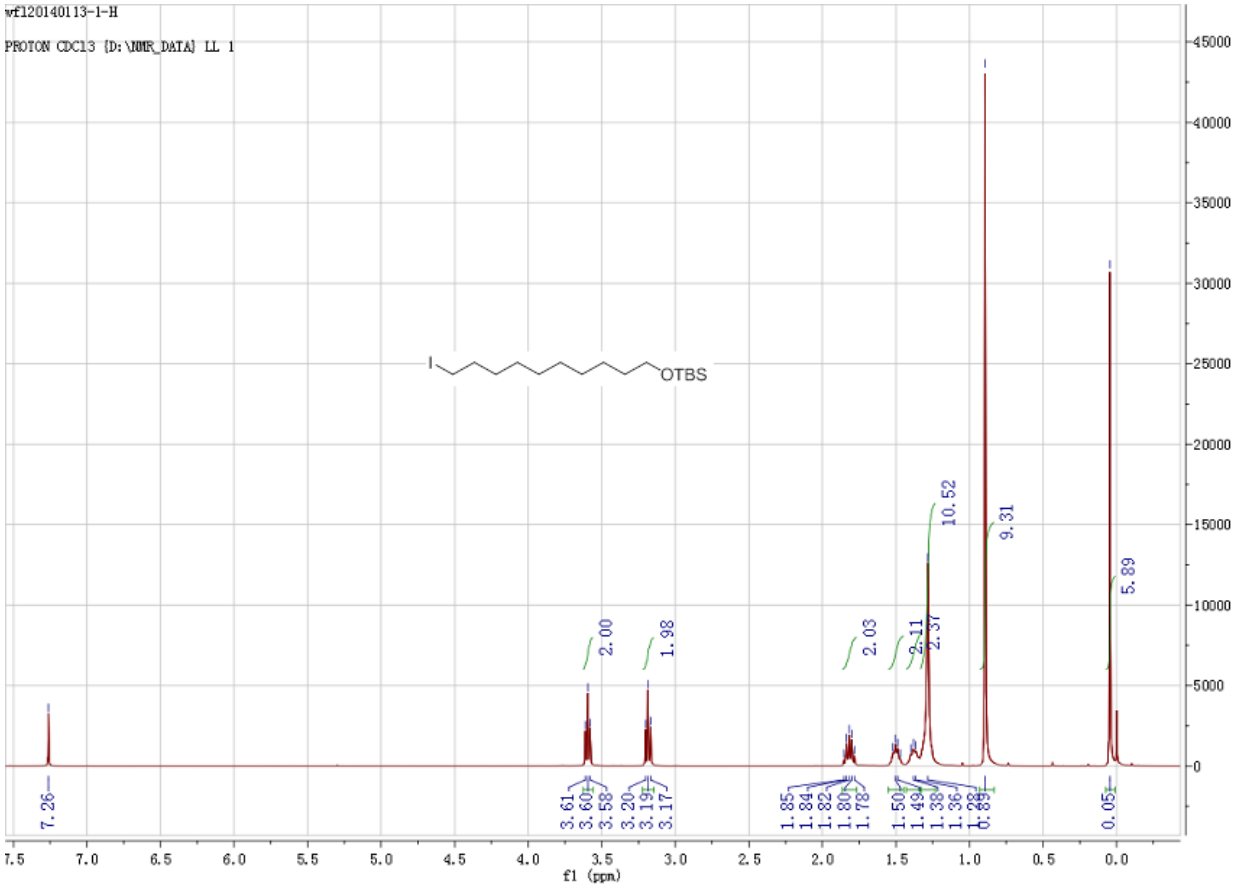

wfl20131210-1-C
C13CPD CDCl3 [D:\NMR_DATA] LL 10
t-BuO
BocHN
f1 (ppm)

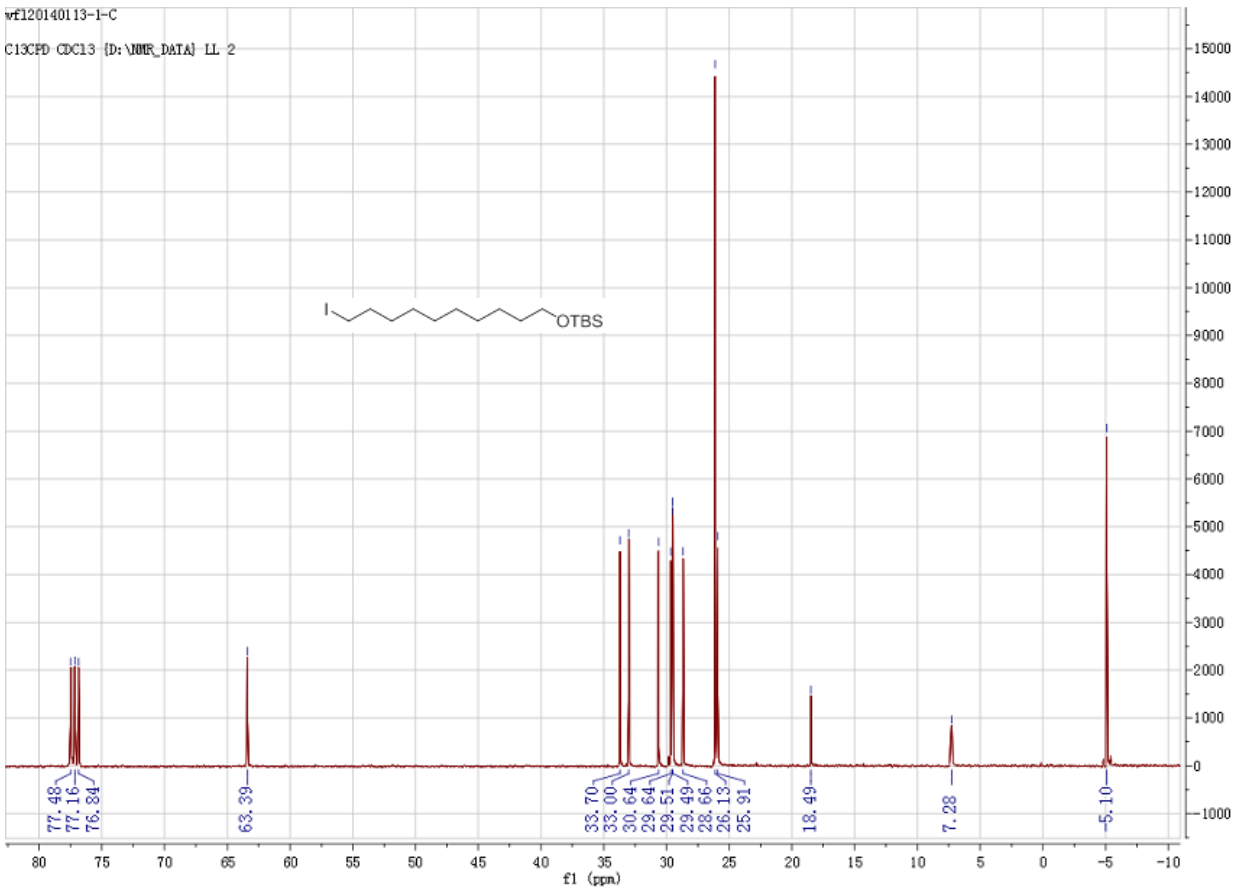

wf120150114-2-h
PROTON CDCl3 [D:\NMR_DATA] LL 5
NHBoc
t-BuO
O
CbzN
Ph
f1 (ppm)

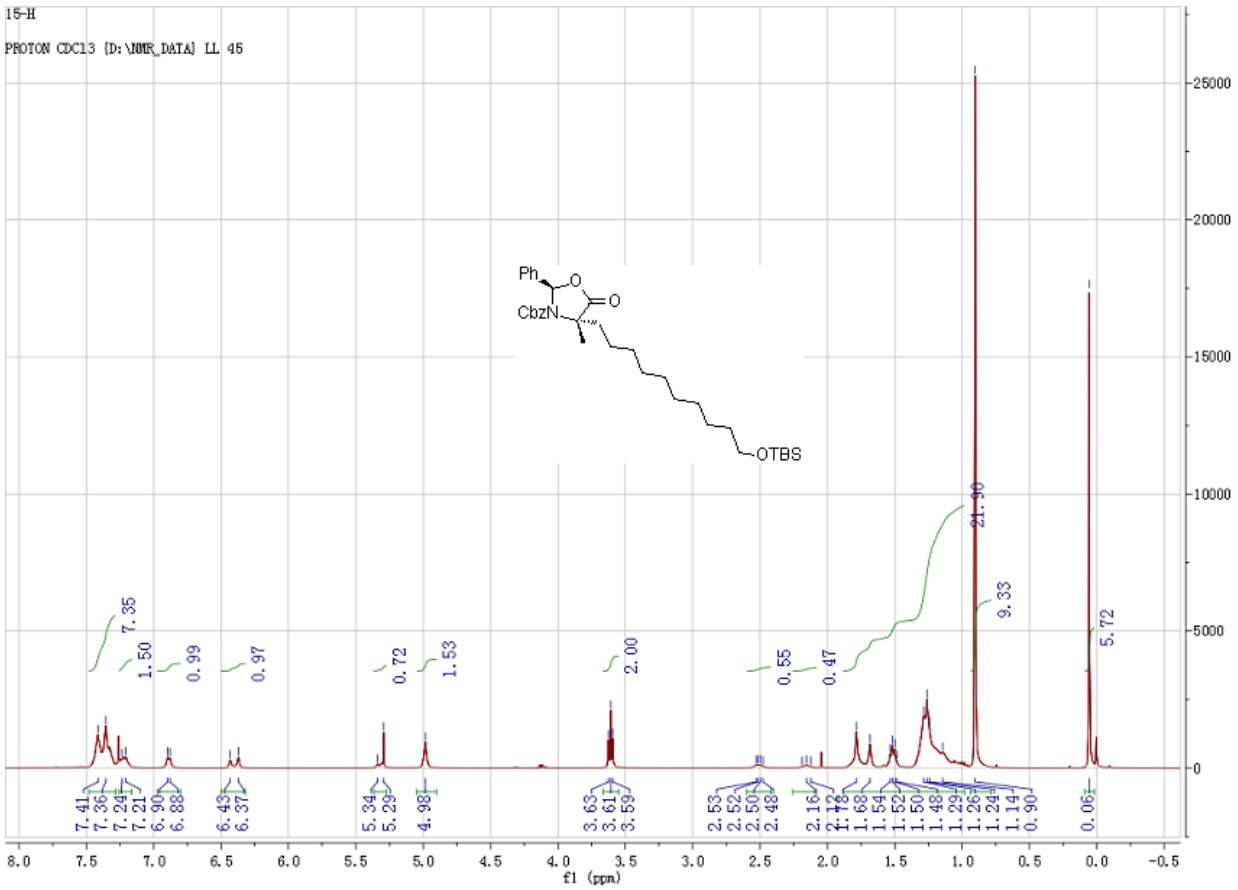

wf120150114-2-c
C13CPD CDCl3 [D:\NMR_DATA] LL 6
NHBoc
t-BuO
O
CbzN
Ph
f1 (ppm)

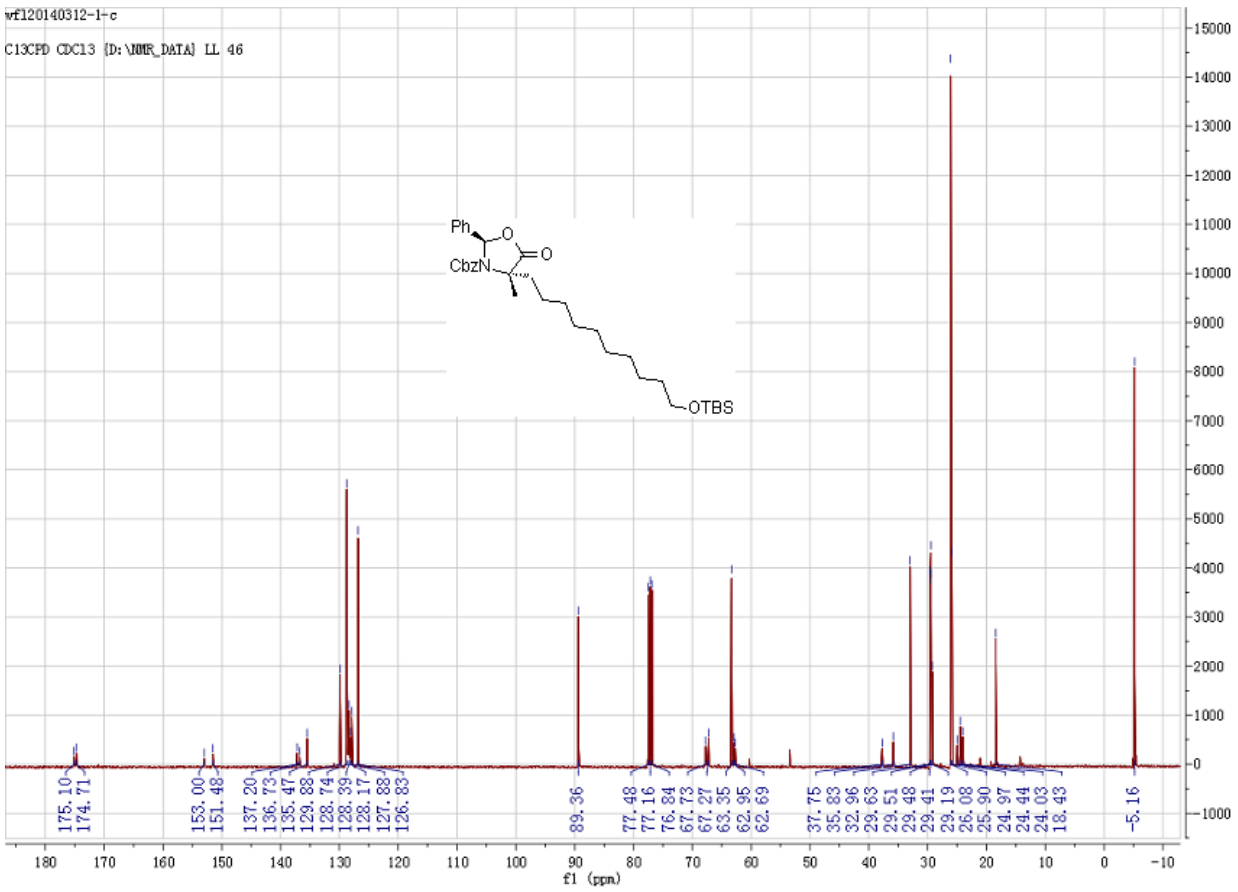

SS8-H
NHFmoc
HO
O
HN
NO2
f1 (ppm)

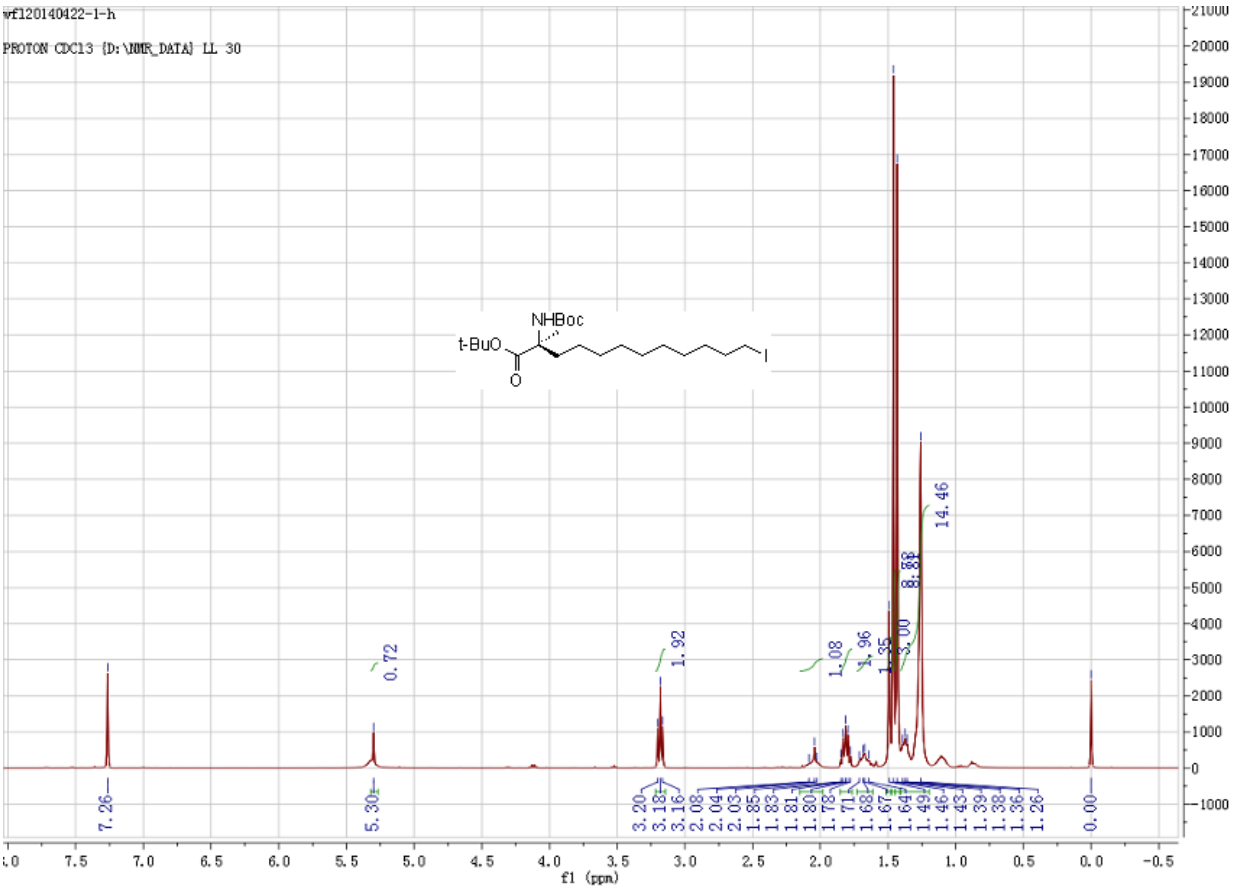

wf1150204-1-c
NHFmoc
HO
O
HN
NO2
f1 (ppm)

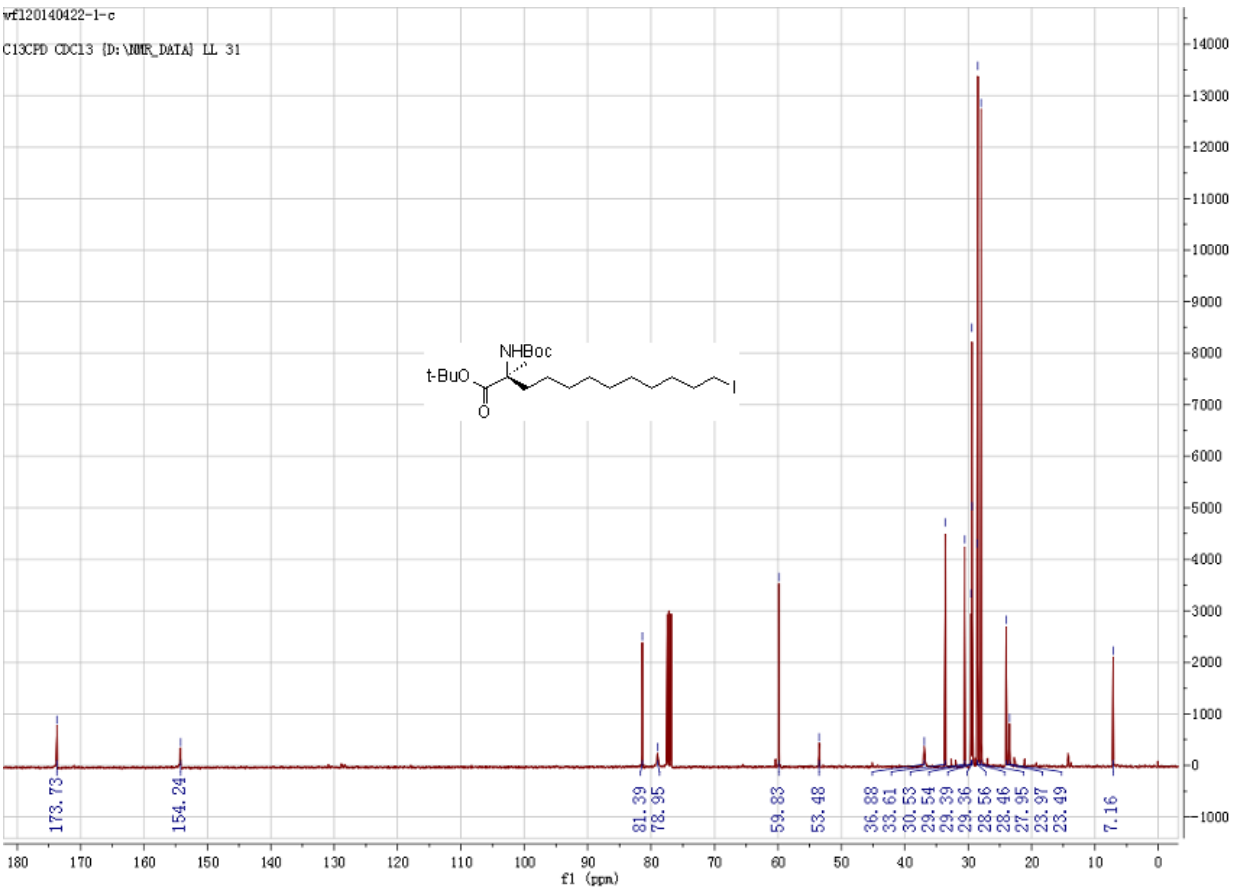

wf120140113-1-H
PROTON CDCl3 [D:\NMR_DATA] LL 1
I
OTBS
7.26
3.61
3.60
3.58
3.20
3.19
3.17
2.00
1.98
2.03
2.11
2.37
1.85
1.84
1.82
1.80
1.78
1.50
1.49
1.38
1.36
10.52
9.31
5.89
0.05
f1 (ppm)

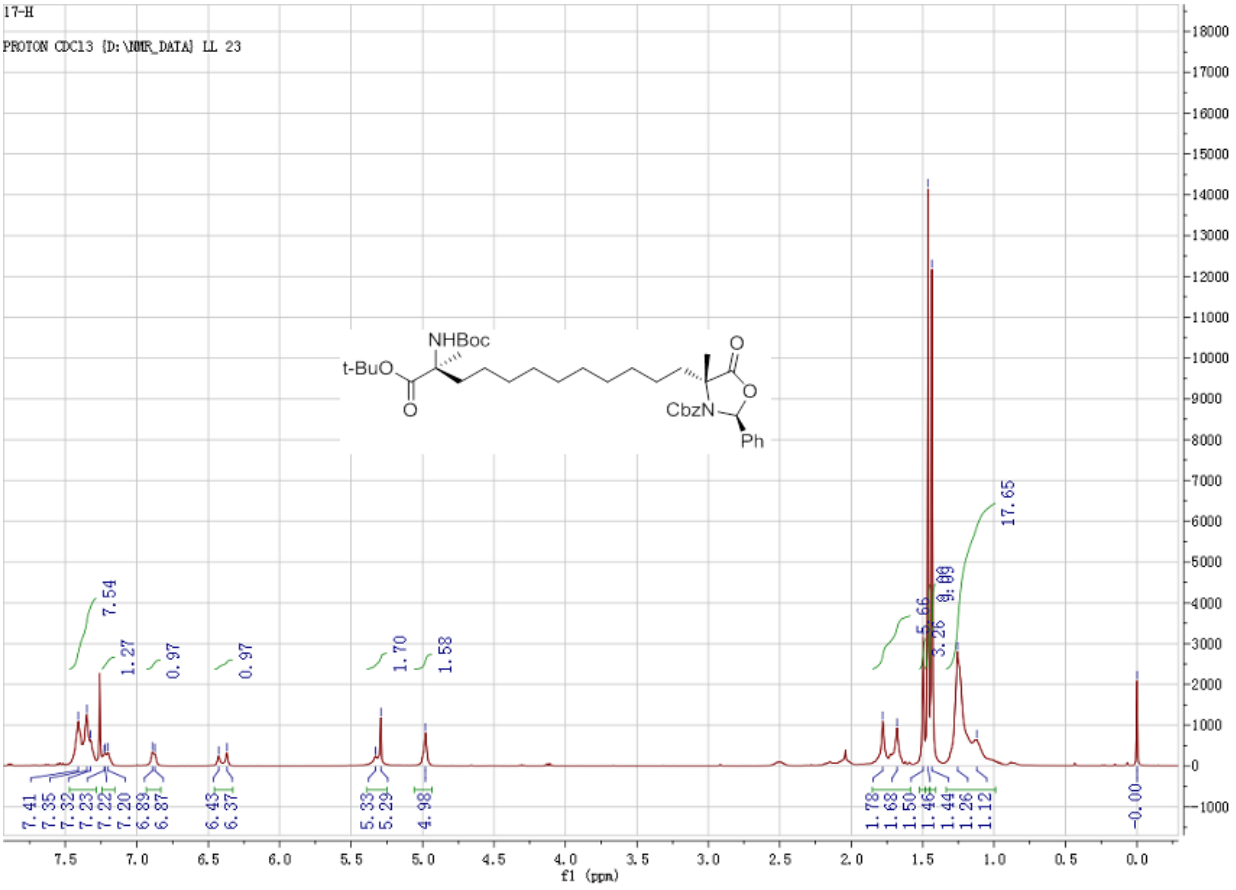

wf120140113-1-C
C13CPD CDCl3 [D:\NMR_DATA] LL 2
I
OTBS
77.48
77.16
76.84
63.39
33.70
33.00
30.64
29.64
29.51
29.49
28.66
26.13
25.91
18.49
7.28
-5.10
f1 (ppm)

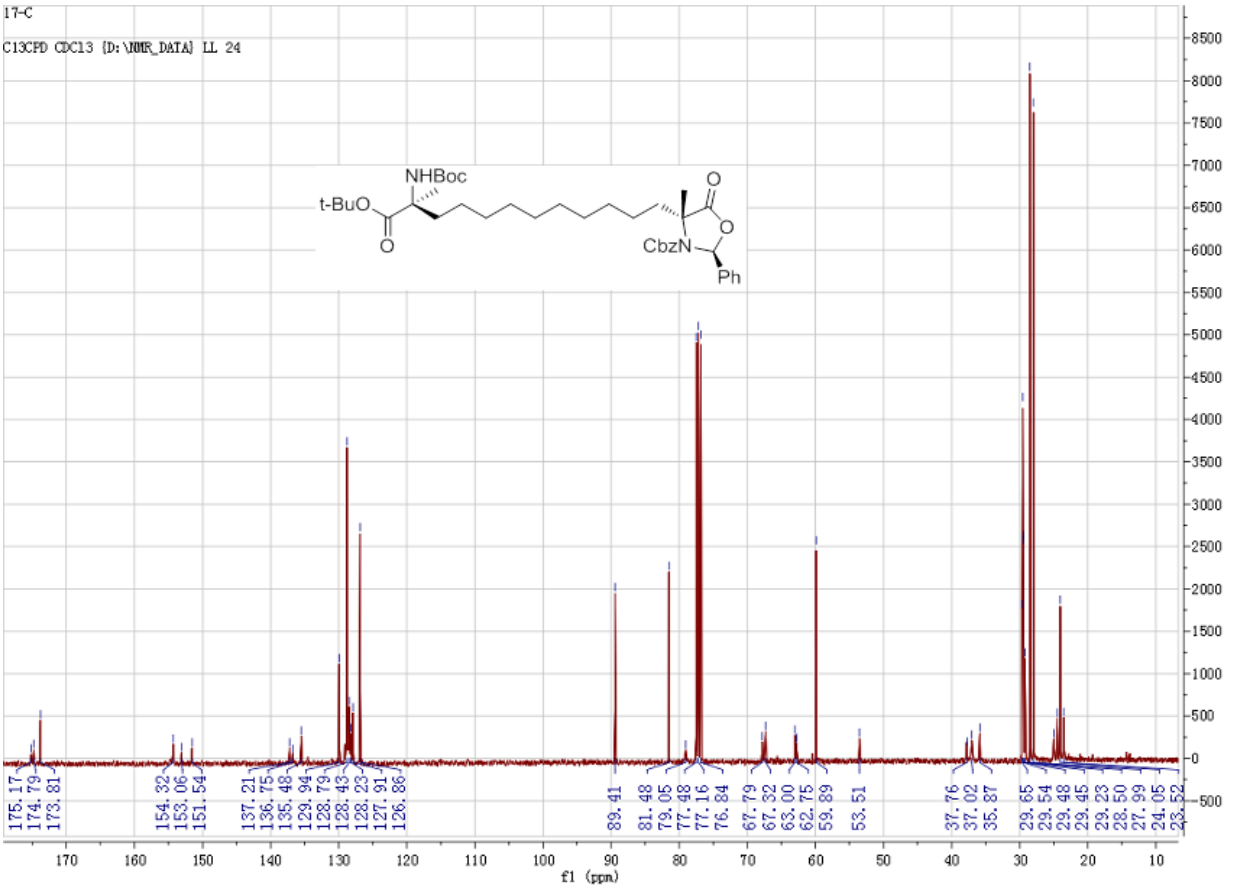

15-H
PROTON CDCl3 [D:\NMR_DATA] LL 45
Ph
O
CbzN
O
OTBS
7.35
1.50
0.99
0.97
0.72
1.53
2.00
0.55
0.47
21.90
9.33
5.72
f1 (ppm)

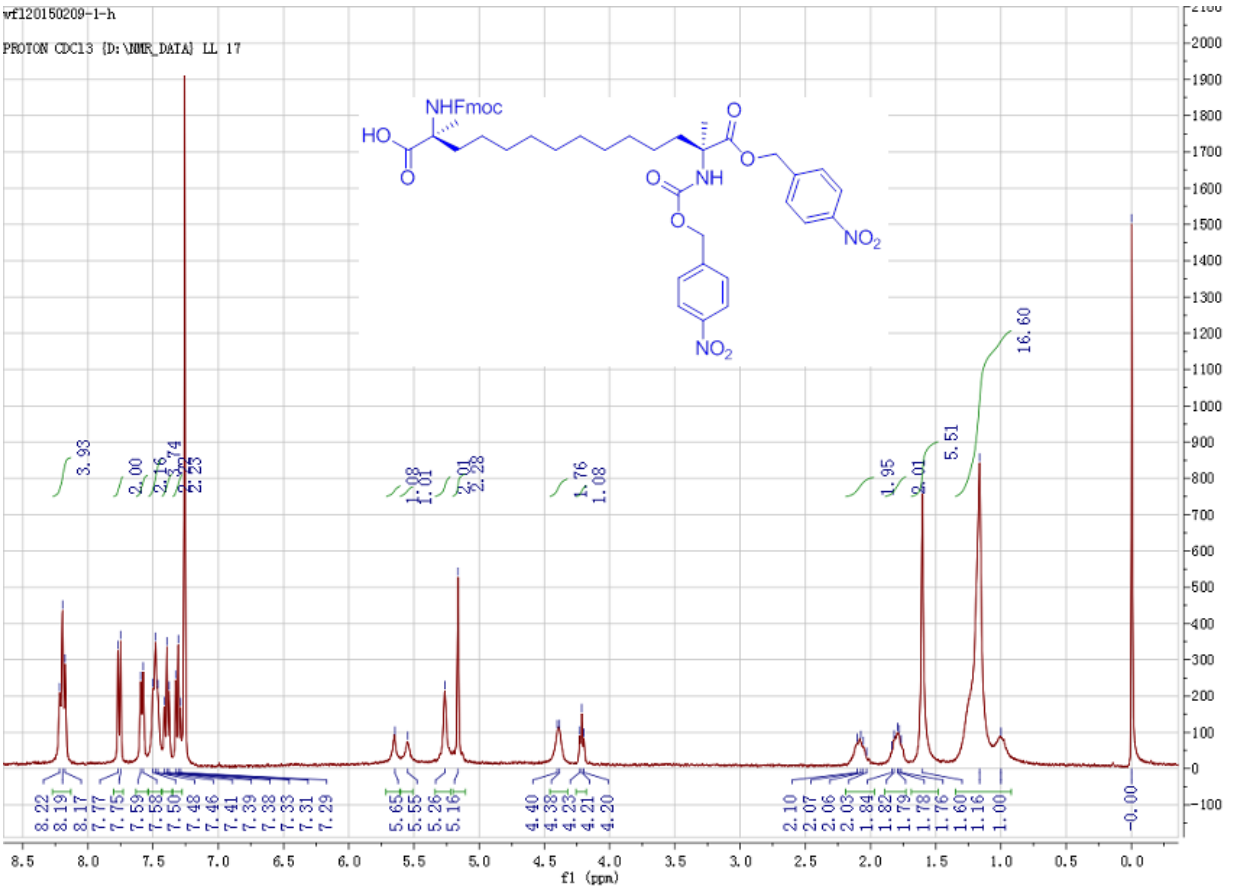

wfl20140312-1-c
C13CPD CDCl3 [D:\NMR_DATA] LL 46
Ph
O
CbzN
O
OTBS
f1 (ppm)

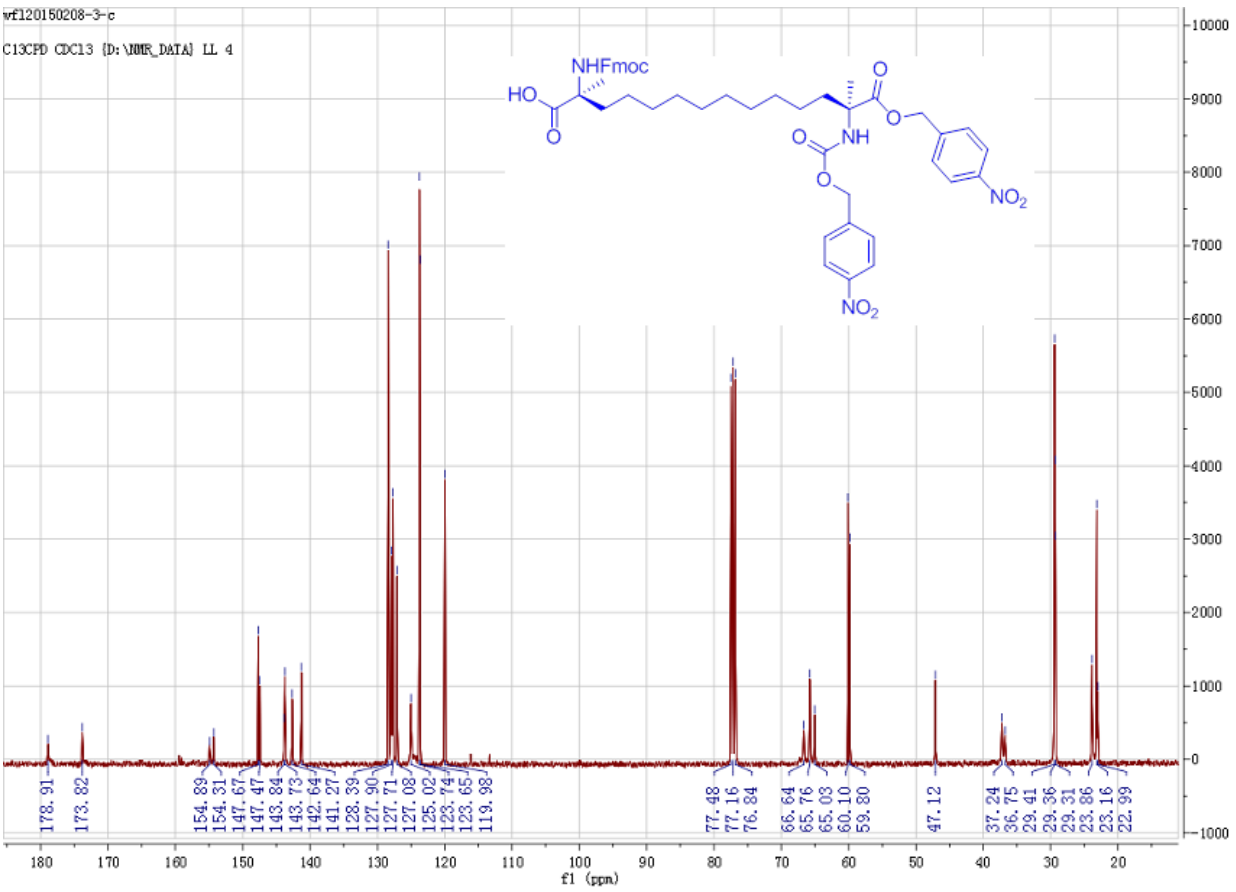

wf120140422-1-h
PROTON CDCl3 [D:\NMR_DATA] LL 30
NHBoc
t-BuO
O
I
7.26
0.72
5.30
1.92
3.20
3.18
3.16
2.08
2.04
2.03
1.85
1.83
1.81
1.80
1.78
1.71
1.68
1.67
1.64
1.49
1.46
1.43
1.39
1.38
1.36
1.26
1.08
0.96
1.35
3.00
8.88
14.46
0.00
f1 (ppm)

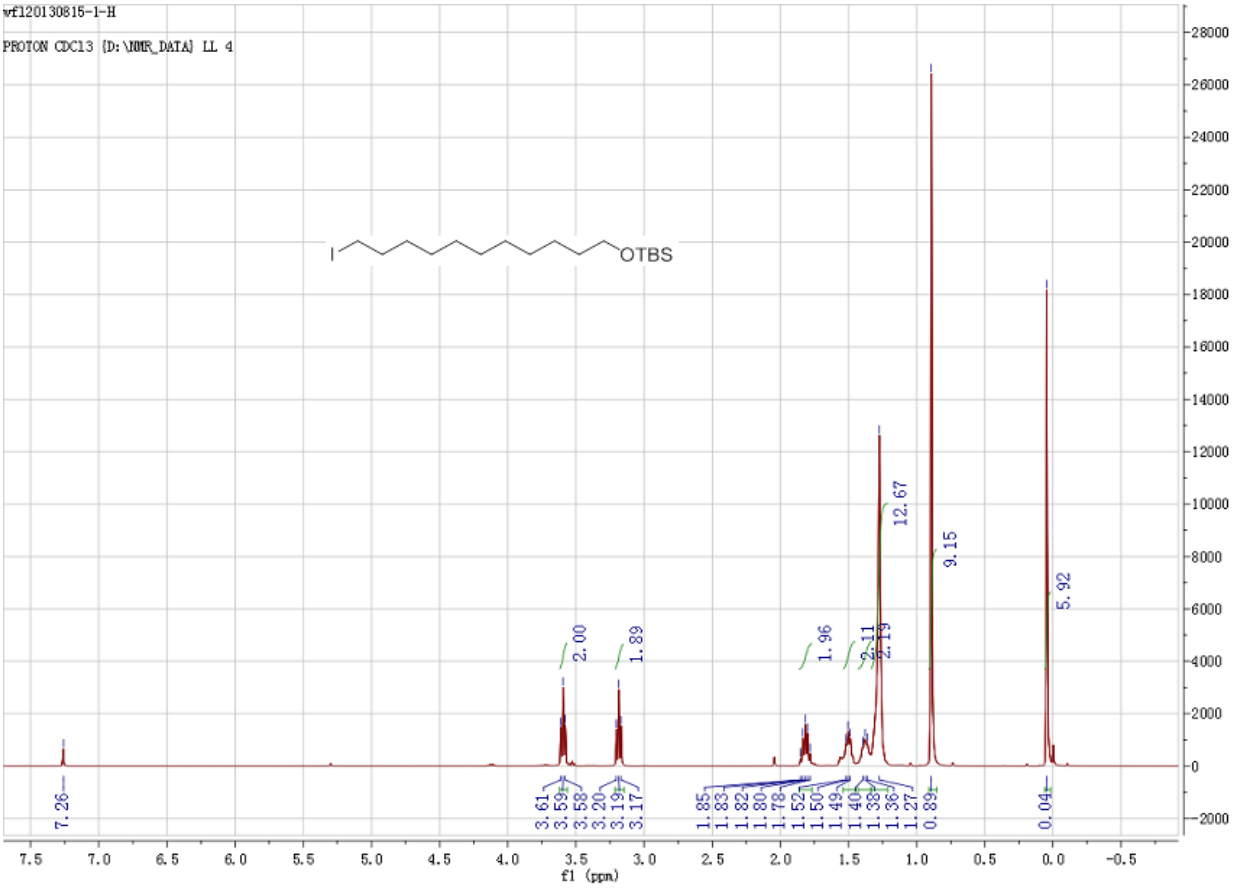

wf120140422-1-c
C13CPD CDCl3 [D:\NMR_DATA] LL 31
NHBoc
t-BuO
O
I
173.73
154.24
81.39
78.95
59.83
53.48
36.88
33.61
30.53
29.54
29.39
29.36
28.56
28.46
27.95
23.97
23.49
7.16
f1 (ppm)

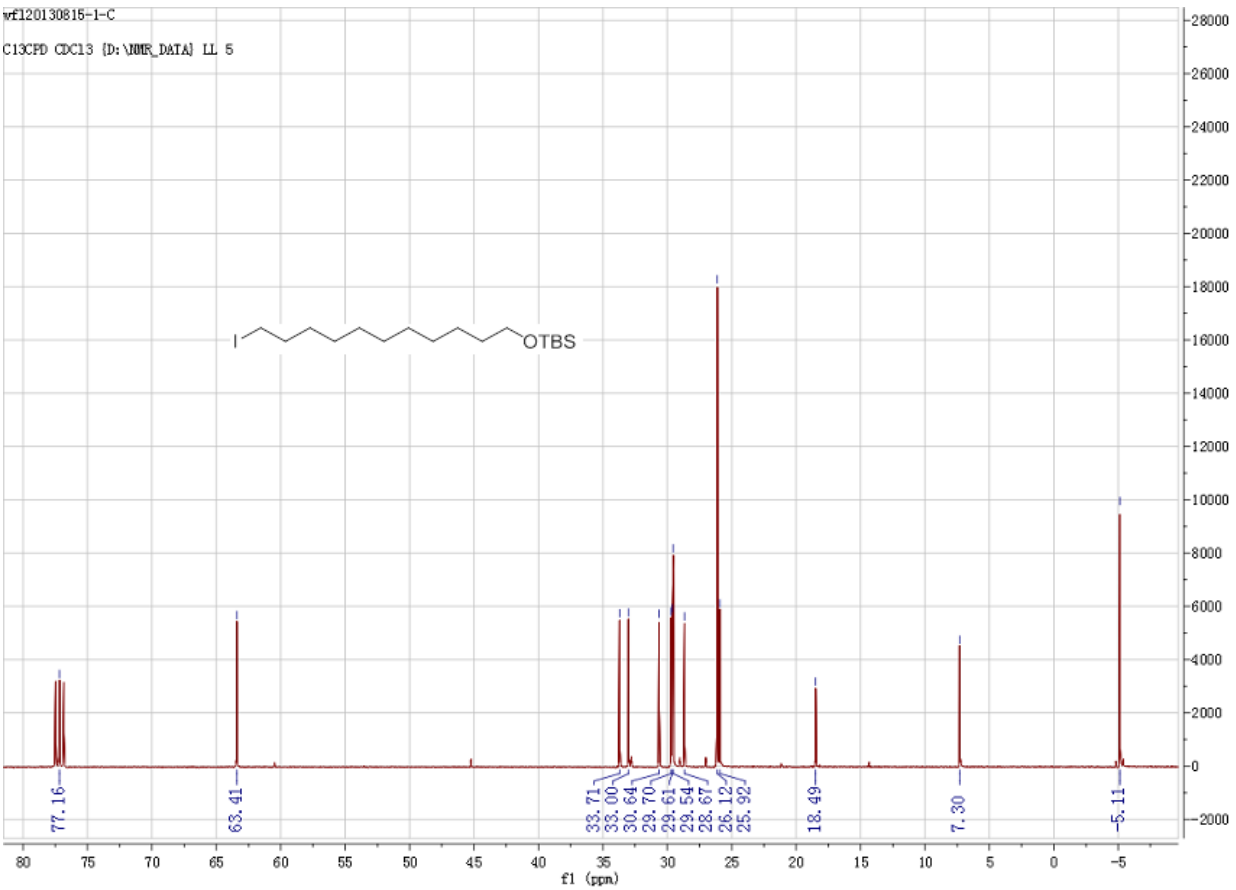

17-H
PROTON CDCl3 [D:\NMR_DATA] LL 23
NHBoc
t-BuO
O
CbzN
Ph
f1 (ppm)

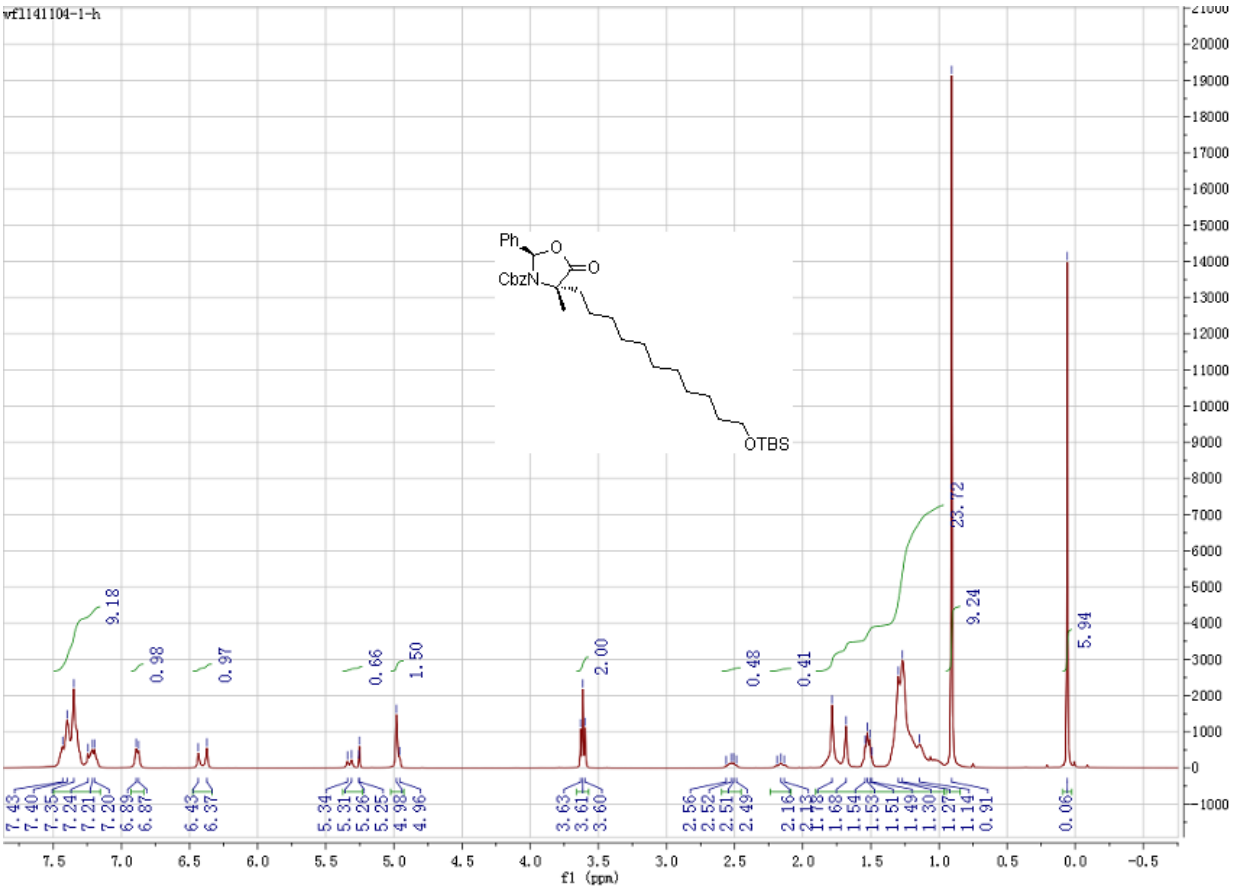

17-C
C13CPD CDCl3 [D:\NMR_DATA] LL 24
NHBoc
t-BuO
O
CbzN
Ph
f1 (ppm)

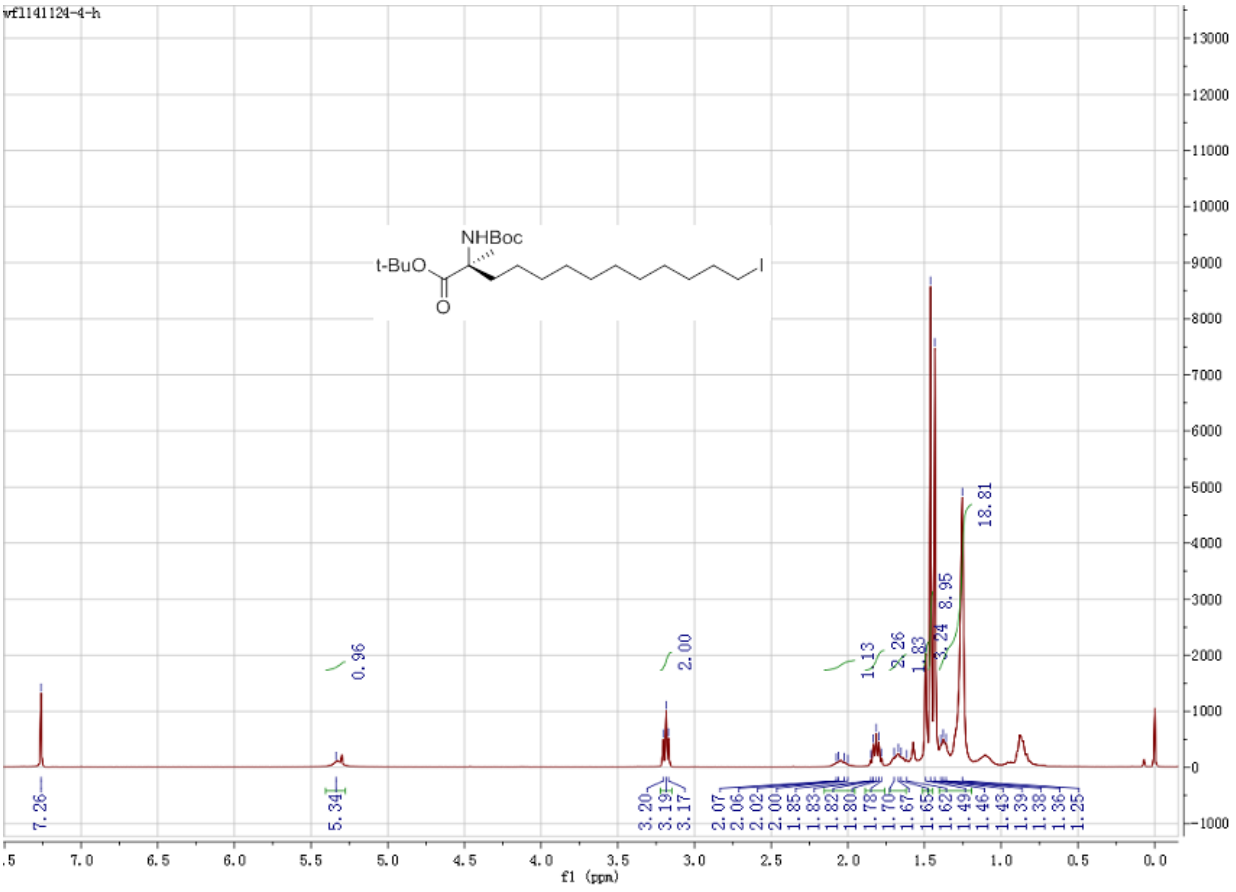

wf120150209-1-h
PROTON CDCl3 [D:\NMR_DATA] LL 17
NHFmoc
HO
O
NH
O
O
NO2
NO2
f1 (ppm)

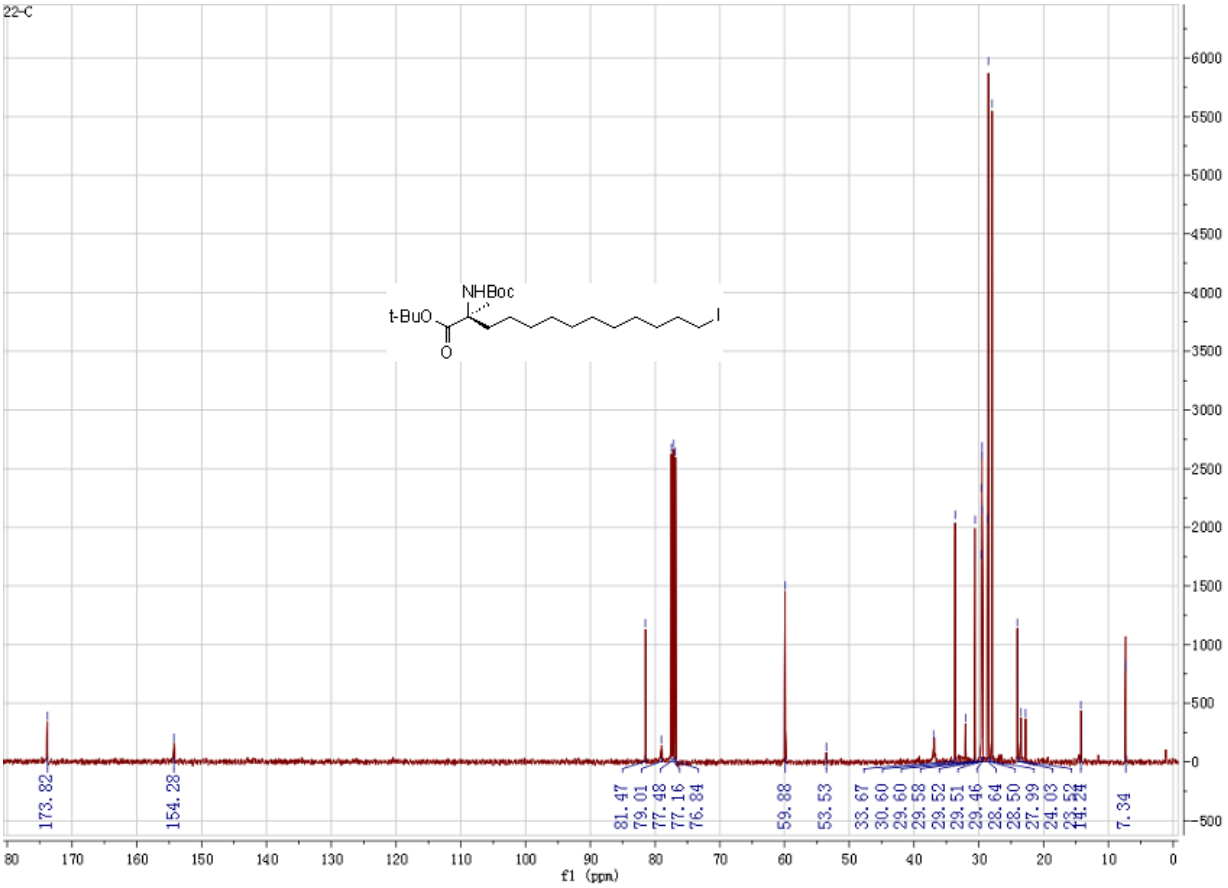

wf120150208-3-c
C13CPD CDCl3 [D:\NMR_DATA] LL 4
NHFmoc
HO
O
NH
O
O
NO2
NO2
f1 (ppm)

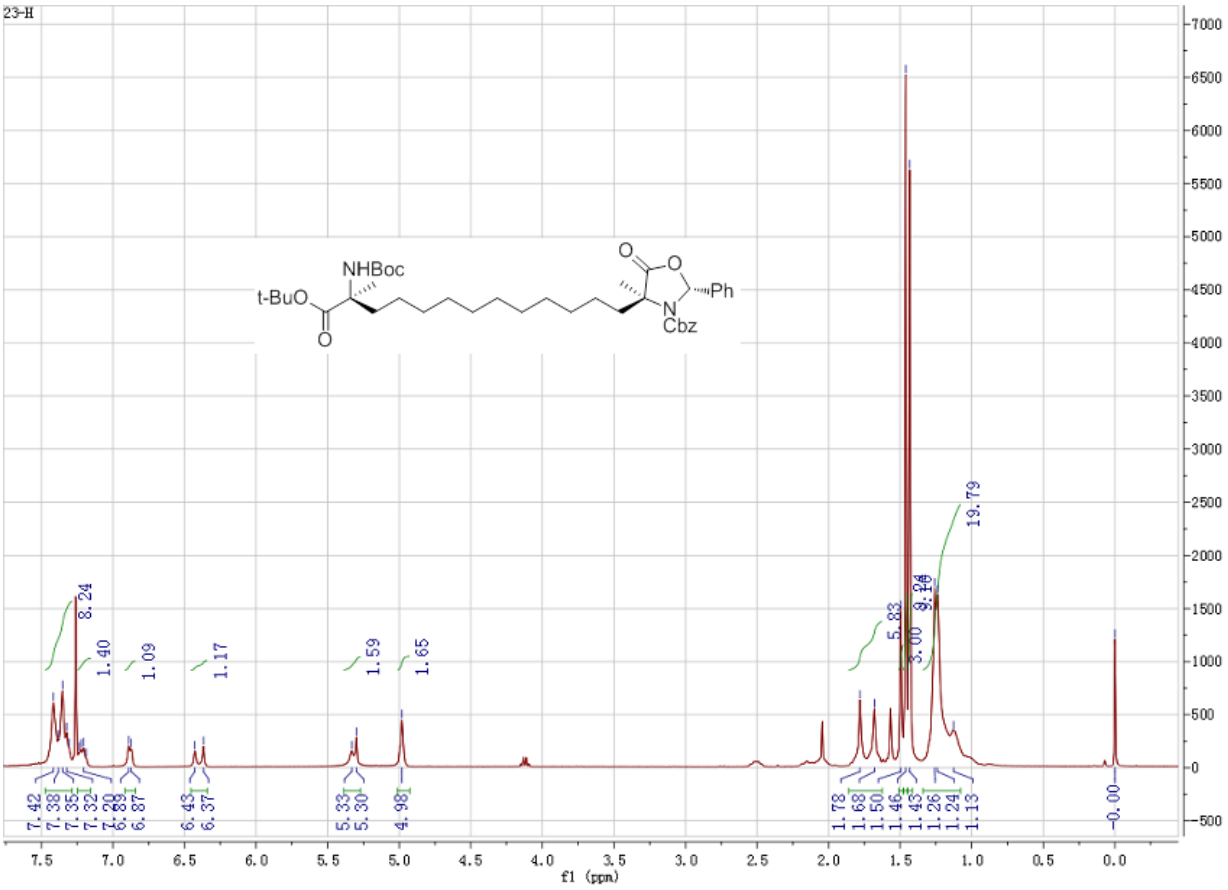

wf120130815-1-H
PROTON CDCl3 [D:\NMR_DATA] LL 4
OTBS
f1 (ppm)

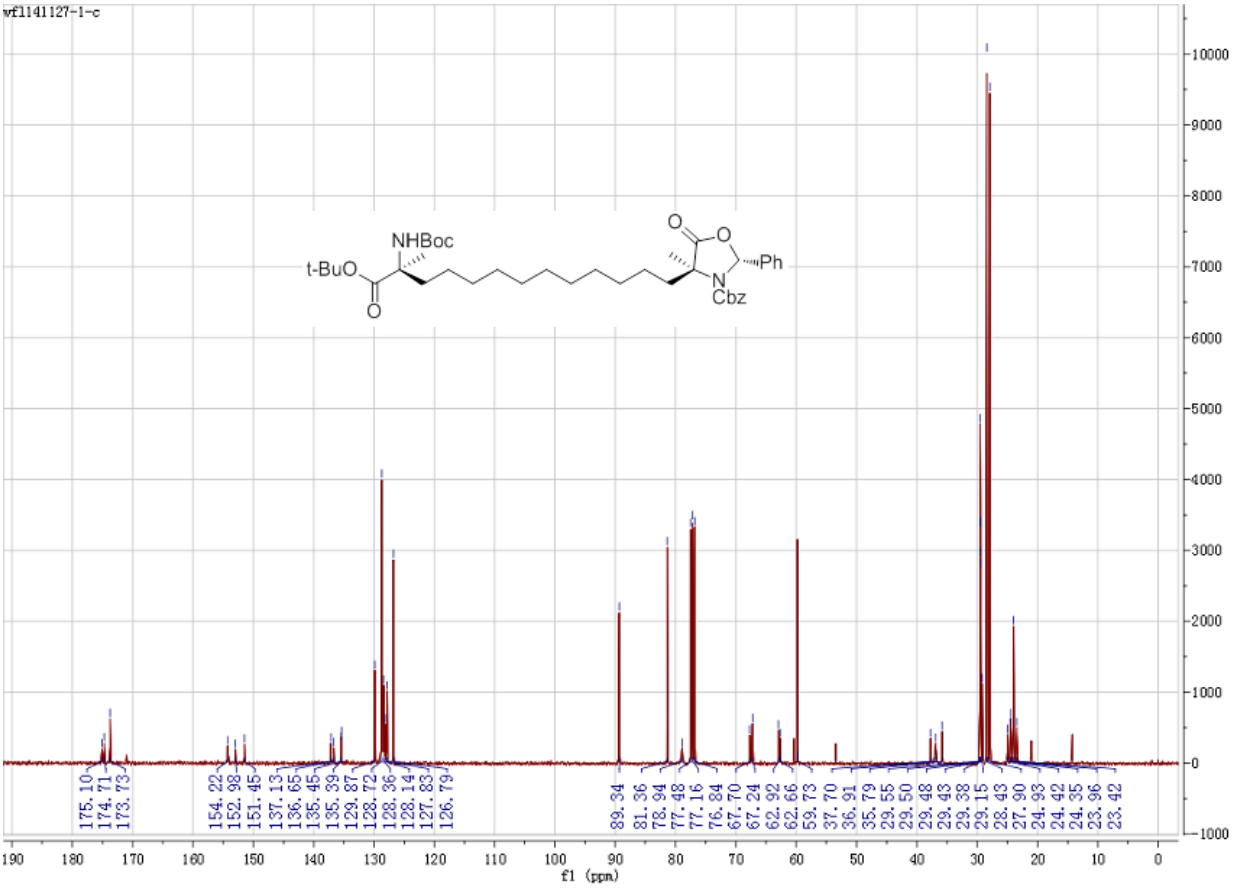

wf120130815-1-C
C13CPD CDCl3 [D:\NMR_DATA] LL 5
OTBS
f1 (ppm)

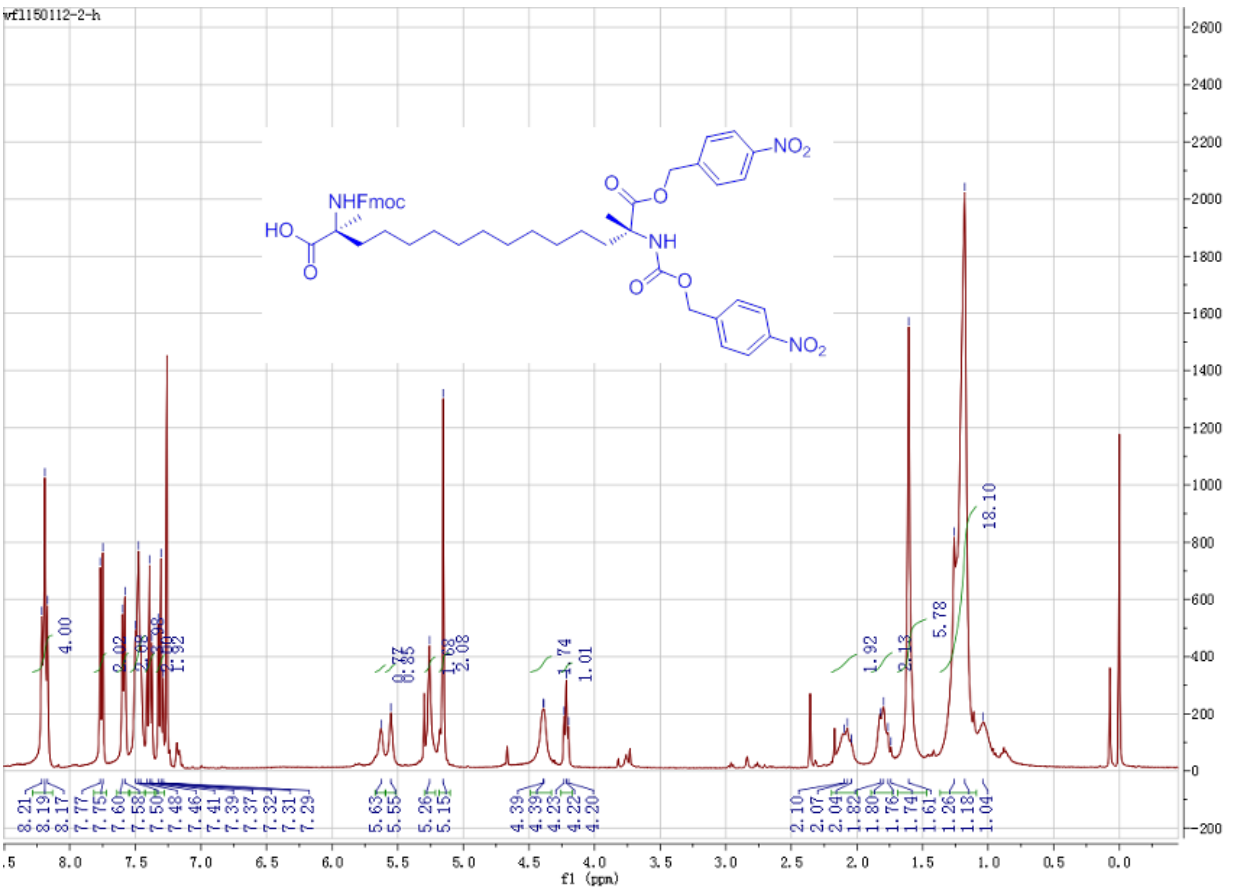

wfl141104-1-h
Ph
O
CbzN
O
OTBS
9.18
0.98
0.97
0.66
1.50
2.00
0.48
0.41
23.72
9.24
5.94
f1 (ppm)

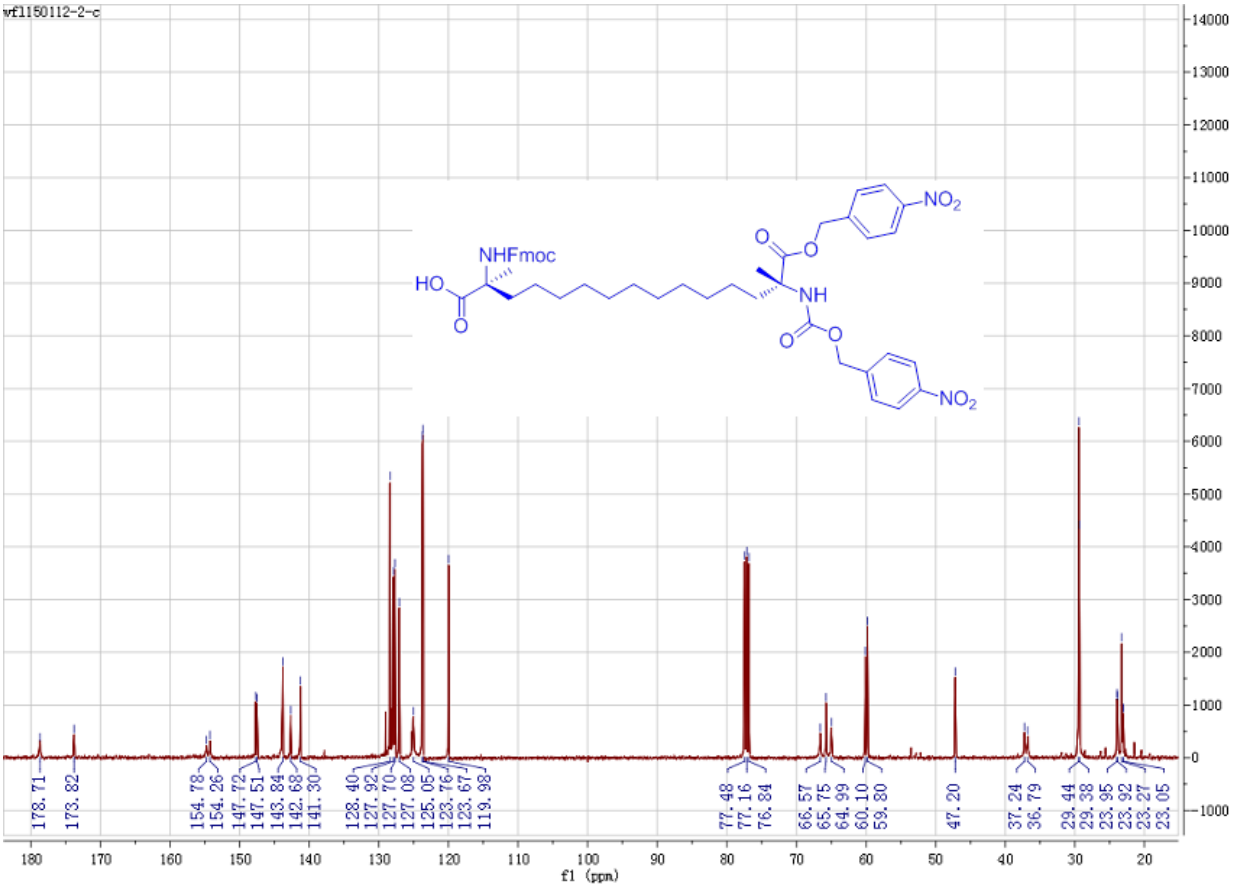

wfl141124-4-h
NHBoc
t-BuO
O
I
0.96
2.00
1.13
2.26
1.83
3.24
8.95
18.81
7.26
5.34
f1 (ppm)


22-C
NHBoc
t-BuO
O
I
f1 (ppm)


23-H
NHBoc
t-BuO
O
O
O
Ph
N
Cbz
f1 (ppm)


wf1141127-1-c
NHBoc
t-BuO
O
O
O
Ph
N
Cbz
175.10
174.71
173.73
154.22
152.98
151.45
137.13
136.65
136.45
135.39
129.87
128.72
128.36
128.14
127.83
126.79
89.34
81.36
78.94
77.48
77.16
76.84
67.70
67.24
62.92
62.66
59.73
37.70
36.91
35.79
29.55
29.50
29.48
29.43
29.38
29.15
28.43
27.90
24.93
24.42
24.35
23.96
23.42
f1 (ppm)


wf1150112-2-h
NHFmoc
HO
O
NH
O
O
O
O
NO2
NO2
4.00
2.02
1.92
0.85
1.68
2.08
1.74
1.01
1.92
2.13
5.78
18.10
f1 (ppm)


wf1150112-2-c
NHFmoc
HO
O
NH
O
O
O
O
NO2
NO2
178.71
173.82
154.78
154.26
147.72
147.51
143.84
142.68
141.30
128.40
127.92
127.70
127.08
125.05
123.76
123.67
119.98
77.48
77.16
76.84
66.57
65.75
64.99
60.10
59.80
47.20
37.24
36.79
29.44
29.38
23.95
23.92
23.27
23.05
f1 (ppm)