Electronic Supplementary Information

One-Pot and Sustainable Liquid-Phase Peptide Extension for Synthesis of C-terminal Amidation Peptides Aided by Small-molecular TAGs

Haidi Li*, Yuankui Jin, Lianjun Wang, Linyan Zhang, Tianyu Liu, Yu Liu and Taigang Liang*

Shanxi Provincial Key Laboratory of Drug Synthesis and Novel Pharmaceutical Preparation Technology, School of Pharmacy, Shanxi Medical University, Taiyuan, Shanxi, 030001, P. R. China.

*Corresponding authors: ltaigang@163.com; lihaidi@sxmu.edu.cn;

CONTENTS

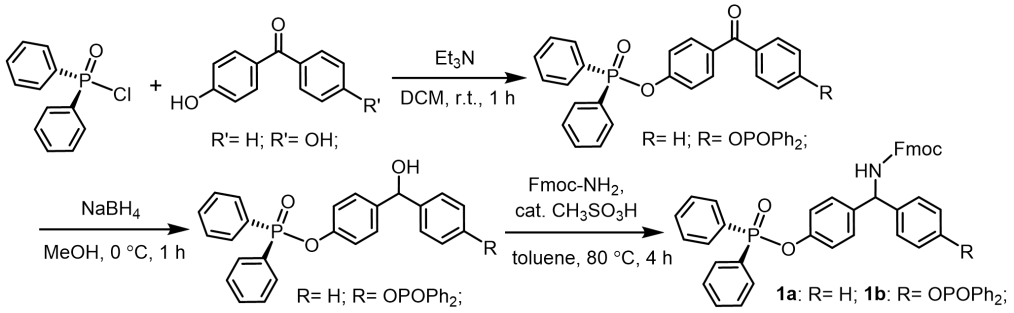
1. General Considerations

All the involved Fmocprotected amino acids were purchased from Shanghai Bide Pharmatech Ltd. All the chemical reagents and raw materials were purchased and used without further purification unless otherwise noted and remarked. All experiment procedures were carried out by standard experiment methods. The rotary evaporator (LKA RV 3, Germany) and circulating water multi-purpose vacuum pump (SHB-III, Zhengzhou) were employed to concentrate the samples. Magnetic stirrers (IKA, RCT, Germany) were used to completed liquid phase stirring reactions. The four-use UV analyzer (ZF-8, Shanghai) and silica gel GF_{254} (0.15mm thick, Qingdao) plates were used for TLC analysis. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and ^{31}P NMR (162 MHz) spectra were recorded on the Bruker NMR spectrometer (Bruker Avance 400 MHz, Germany). Spectra were obtained in $CDCl_3$ (δ_H 7.26 ppm, δ_C 77.16 ppm). HRMS data were recorded on a Thermo Scientific LTQ Orbitrap XL using ESI ionization. HPLC analyses for the peptide products were performed with SHIMADZU LC-2030, column, Globalsil 5 μm 200A C18BP; 250×4.6 mm, and HPLC preparation were performed for the peptide products purification with LC 3000 HPLC system with CXTH-3000 work station, column, Kunchen, 10×250 mm, C18, 5μm.

2. Abbreviations

AA':	Amino acid
ACN:	Acetonitrile
DCM:	Dichloromethane
DEA:	Diethylamine
DIPEA:	*N, N'*-diisopropylethylamine
DMAP:	4-dimethylaminopyridine
DODT:	3,6-dioxa-1,8-octanedithiol
EA:	Ethyl acetate
EDCl:	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
HOBt:	1-hydroxybenzotriazole
MeOH:	Methanol
Msa:	Mercaptosuccinic acid
Mpa:	mercaptopropionic acid
Mba:	2-mercaptobutanoic acid
PE:	Petroleum ether
TFA:	Trifluoroacetic acid
Rink Amide	2-(4-(((((9H-Fluoren-9-yl)methoxy)carbonyl)amino) (2,4-dimethoxyphenyl) methyl) phenoxy) acetic acid

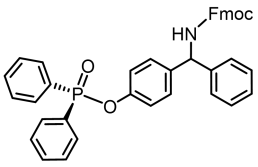
3. Synthesis of phosphonate based tags TAGs



Synthesis of 1a: 4-hydroxybenzophenone (1.98 g, 10 mmol) was added to a solution of Et_3N (1.7 mL, 12 mmol) in DCM (20 mL) at 0 °C and stirred for 10 min. The mixture was added with DPP-Cl (2.0 mL, 10.5 mmol) dropwise and stirred for 0.5 h. The mixture was quenched with 10 mL 0.1 mol/L H_2SO_4 and concentrated. The residue was then dissolved in 20 mL ethyl acetate and washed with H_2O and dried with $MgSO_4$. Concentration to afford the intermediate product 4-benzoylphenyl diphenylphosphinate.

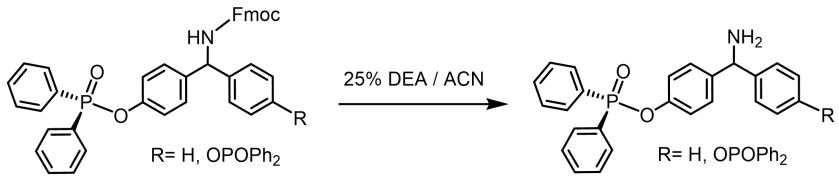
Then the $NaBH_4$ (912 mg, 24 mmol) was added to the solution of above intermediate product 4-benzoylphenyl diphenylphosphinate in CH_3OH (20 mL) at 0 °C and the mixture was sealed and stirred for 1 h. The mixture was quenched by adding 15 mL saturated NH_4Cl and concentrated to remove the CH_3OH. The residue was dissolved in 20 mL EA and washed with H_2O, dried with $MgSO_4$. 5 mL EA was added to dissolve the sample after concentration and 30 mL of petroleum ether was added dropwise and stirred. Precipitate appeared and filtered to afford the intermediate product 4-(hydroxy(phenyl)methyl)phenyl diphenylphosphinate for next use.

To the 4-(hydroxy(phenyl)methyl)phenyl diphenylphosphinate (2.0 g, 5 mmol) were added toluene (20 mL), 9-fluorenylmethyl carbamate (1.32 g, 5.5 mmol) and methanesulfonic acid (0.1 mL, 1.5 mmol) and the mixture was stirred at 80 °C for 5 h. After the stirring, the reaction mixture was cooled to 0 °C, the petroleum ether (50 mL) was added to the solvent mixture and oscillation to obtain the precipitated product. The precipitated product was dissolved in ethyl acetate (5 mL), then stired and added petroleum ether (30 mL) to get the purified product **1a** (3.01 g, 97%).

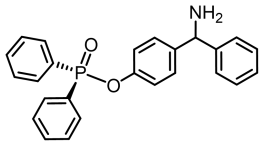


1a: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.85 (m, 4H), 7.75-7.73 (d, J= 8.0 Hz, 2H), 7.57-7.42 (m, 8H), 7.39-7.26 (m, 7H), 7.14-7.05 (m, 6H), 5.90-5.88 (d, J= 8.0 Hz 1H), 5.49-5.47 (d, J= 8.0 Hz 1H), 4.48-4.39 (m, 2H), 4.20-4.16 (m, 1H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.47 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 150.6, 150.2, 143.9, 141.4, 137.9, 132.6, 131.9, 131.8, 128.7, 128.6,

127.7, 127.4, 127.1, 125.1, 120.9, 120.0, 66.7, 58.2, 47.4 ppm. HRMS (ESI) m/z calcd for $C_{40}H_{32}NO_4PNa^+$ $(M+Na)^+$ 644.19612, found 644.19586.

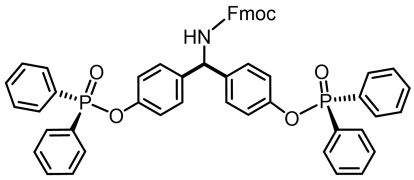


Preparation of 1a-deFmoc: (Remove of the Fmoc group of **1a**). The diethylamine (1.0 mL) was added to the solution of product **1a** (621 mg, 1.0 mmol) in acetonitrile (3.0 mL) and the mixture was stirred for 0.5 h. The mixture was concentrated to remove the diethylamine and acetonitrile. The residue was redissolved in 2.0 mL DCM, and 9.0 mL of petroleum ether was added dropwise and stirred. Precipitate appeared and filtered to afford the purifed deprotected product **1a-deFmoc**.



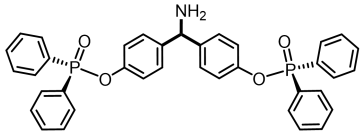
1a-deFmoc: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.84 (m, 4H), 7.52-7.47 (m, 2H), 7.44-7.40 (m, 4H), 7.29-7.16 (m, 7H), 7.13-7.11 (m, 2H), 5.09 (m, 1H), 1.76 (m, 2H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.47 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 149.7, 145.4, 141.9, 132.5, 131.9, 131.8, 130.4, 128.7, 128.6, 128.5, 128.3, 127.1, 126.9, 120.7, 59.1 ppm.

Synthesis of 1b: The synthesis of **1b** was according to the above procedures of **1a**.

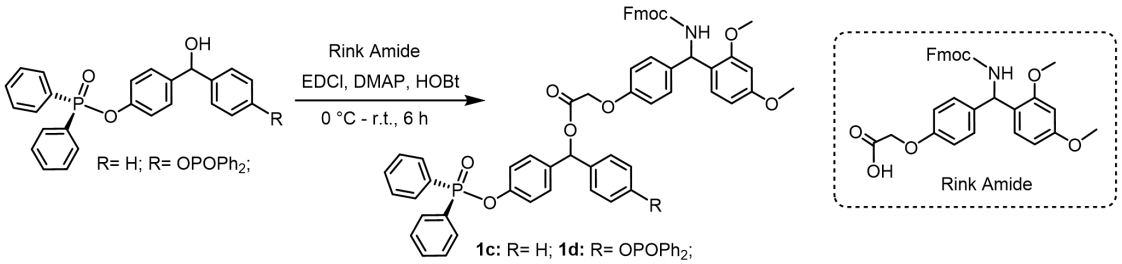


1b: yield 96%. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.88-7.83 (m, 8H), 7.72-7.70 (d, J= 8.0 Hz, 2H), 7.55-7.49 (m, 6H), 7.45-7.41 (m, 8H), 7.36-7.32 (m, 2H), 7.22-7.18 (m, 2H), 7.11-7.07 (m, 4H), 7.00-6.98 (m, 4H), 5.87-5.80 (m, 1H), 4.40-4.38 (d, J= 8.0 Hz 2H), 4.16-4.12 (m, 1H), 3.76 (s, 1H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.71 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 155.7, 150.2, 143.9, 141.3, 137.7, 132.6, 131.9, 131.8, 128.8, 128.6, 127.7, 127.1, 120.9, 120.8, 120.0, 66.6, 57.5, 47.4 ppm. HRMS (ESI) m/z calcd for $C_{52}H_{41}NO_6P_2Na^+$ $(M+Na)^+$ 860.23013, found 860.23047.

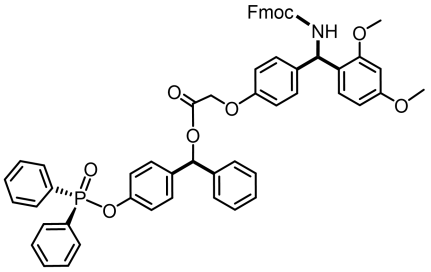
Preparation of 1b-deFmoc: The preparation of **1b-deFmoc** was according to the above procedures of **1a-deFmoc**.



1b-deFmoc: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.84 (m, 8H), 7.53-7.50 (m, 4H), 7.45-7.42 (m, 8H), 7.16-7.08 (m, 8H), 5.02 (s, 1H), 1.80 (s, 2H) ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.44 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 149.8, 141.6, 132.5, 131.9, 131.8, 130.3, 128.7, 128.6, 128.2, 120.7, 58.4 ppm. HRMS (ESI) m/z calcd for $C_{37}H_{31}NO_4P_2Na^+$ $(M+Na)^+$ 638.16205, found 638.16229.



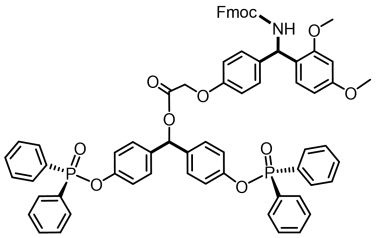
Synthesis of 1c: The 4-(hydroxy(phenyl)methyl) phenyl diphenylphosphinate (2.0 g, 5 mmol) were added DCM (20 mL) and stired at 0 °C for 10 min. Then the Rink Amide (2.83 g, 5.25 mmol), EDCl (1.15 g, 6.0 mmol), DMAP (75 mg, 0.6 mmol) and HOBt (810 mg, 6 mmol) were added to the solution, the mixture was stirred at room temperature for 6 h. The reaction mixture was then washed with 10% Na_2CO_3, dried with anhydrous $MgSO_4$. 7.0 mL ethyl acetate was added to dissolve the sample after concentration, and 30 mL of petroleum ether was added dropwise and stirred to obtain the purified product **1c** (4.37 g, 95%).



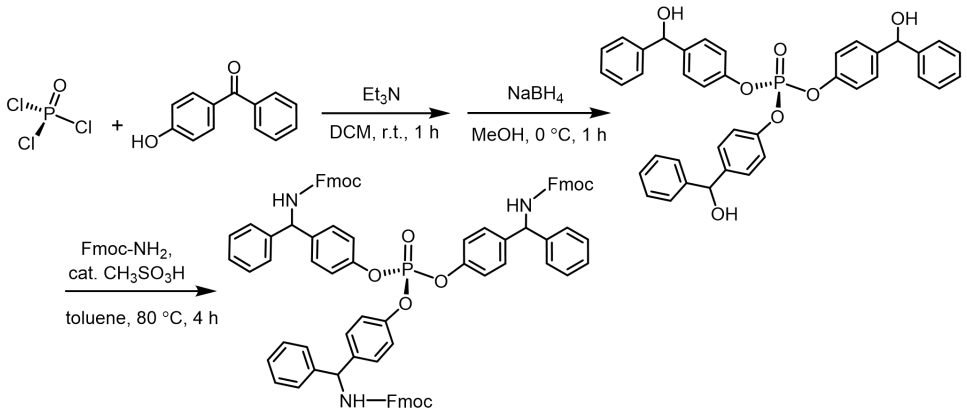
1c: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.84 (m, 4H), 7.74-7.72 (d, J= 8.0 Hz, 2H), 7.59-7.57 (m, 2H), 7.50-7.33 (m, 8H), 7.27-7.19 (m, 8H), 7.15-7.09 (m, 7H), 6.89 (m, 1H), 6.76-6.74 (m, 2H), 6.45-6.41 (m, 2H), 6.09-6.01 (m, 1H), 4.62 (s, 2H), 4.45-4.36 (m, 2H), 4.23-4.19 (m, 1H), 3.75 (s, 3H), 3.67 (s, 3H)ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.96 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 168.2, 160.6, 158.1, 156.7, 156.0, 150.7, 144.2, 144.1, 141.4, 139.2, 135.9, 135.6, 132.7, 131.9, 131.8, 131.6, 130.2, 129.5, 128.8, 128.7, 128.6, 128.3, 128.1, 127.7, 127.1, 125.2, 122.3, 120.9,

120.0, 114.5, 104.3, 99.4, 77.2, 66.7, 65.6, 55.6, 55.5, 54.6, 47.5 ppm. HRMS (ESI) m/z calcd for $C_{57}H_{48}NO_9PNa^+$ $(M+Na)^+$ 944.29589, found 944.29645.

Synthesis of 1d: The synthesis of **1d** was according to the above procedures of **1c**.



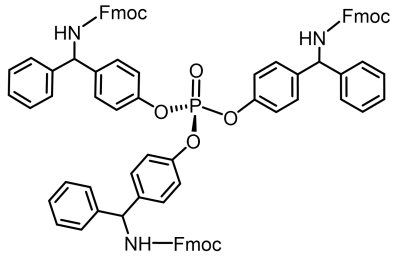
1d:^{1}H NMR (400 MHz, $CDCl_3$), δ 7.89-7.83 (m, 8H), 7.74-7.72 (d, J= 8.0 Hz, 2H), 7.59-7.34 (m, 18H), 7.13-7.07 (m, 12H), 6.82 (m, 1H), 6.73-6.71 (m, 2H), 6.45-6.42 (m, 2H), 6.15-6.07 (m, 1H), 4.59 (s, 2H), 4.44-4.35 (m, 2H), 4.23-4.19 (m, 1H), 3.75 (s, 3H), 3.68 (s, 3H)ppm; ^{31}P NMR (162 MHz, $CDCl_3$), δ 30.98 ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 168.1, 160.5, 158.0, 156.6, 156.0, 150.7, 144.2, 144.0, 141.3, 135.6, 135.5, 132.7, 132.6, 131.9, 131.8, 131.5, 130.1, 129.5, 128.8, 128.7, 128.6, 128.0, 127.7, 127.1, 125.2, 122.3, 120.9, 120.8, 120.0, 114.4, 104.2, 99.3, 76.5, 66.7, 65.5, 55.5, 55.4, 47.4 ppm. HRMS (ESI) m/z calcd for $C_{69}H_{57}NO_{11}P_2Na^+$ $(M+Na)^+$ 1160.32991, found 1160.33044.



Synthesis of 1e: 4-hydroxybenzophenone (6.2 g, 31 mmol) was added to a solution of Et_3N (5.0 mL, 36 mmol) in DCM (50 mL) at 0 °C and stirred for 10 min. The mixture was added with $POCl_3$ (0.93 mL, 10 mmol) dropwise and stirred for 1 h. The mixture was quenched with 10 mL 0.1 mol/L H_2SO_4 and concentrated. The residue was then dissolved in 40 mL ethyl acetate and washed with H_2O and dried with $MgSO_4$. Concentration to afford the intermediate product tris (4-benzoylphenyl) phosphate. Then the $NaBH_4$ (2.28 g, 60 mmol) was added to the solution of above intermediate product tris (4-benzoylphenyl) phosphate in CH_3OH (50 mL) at 0 °C and the mixture was sealed and stirred for 2 h. The mixture was quenched by adding 30 mL saturated NH_4Cl and concentrated to remove the CH_3OH. The residue was dissolved in 50 mL EA and washed with H_2O, dried with

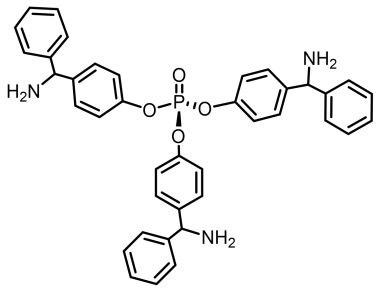
$MgSO_4$. 10 mL EA was added to dissolve the sample after concentration and 60 mL of petroleum ether was added dropwise and stirred. Precipitate appeared and filtered to afford the intermediate product tris (4-(hydroxy(phenyl)methyl)phenyl) phosphate for next use.

To the tris (4-(hydroxy(phenyl)methyl)phenyl) phosphate (3.22 g, 5.0 mmol) were added toluene (20 mL), 9-Fluorenylmethyl carbamate (3.77 g, 15.75 mmol) and methanesulfonic acid (0.34 mL, 1.5 mmol) and the mixture was stirred at 80 °C for 6 h. After the stirring, the reaction mixture was cooled to 0 °C, the petroleum ether (60 mL) was added to the solvent mixture and oscillation to obtain the precipitated product. The precipitated product was dissolved in ethyl acetate (10 mL), then stired and added petroleum ether (50 mL) to get the purified product **1e** (6.3 g, 96%).

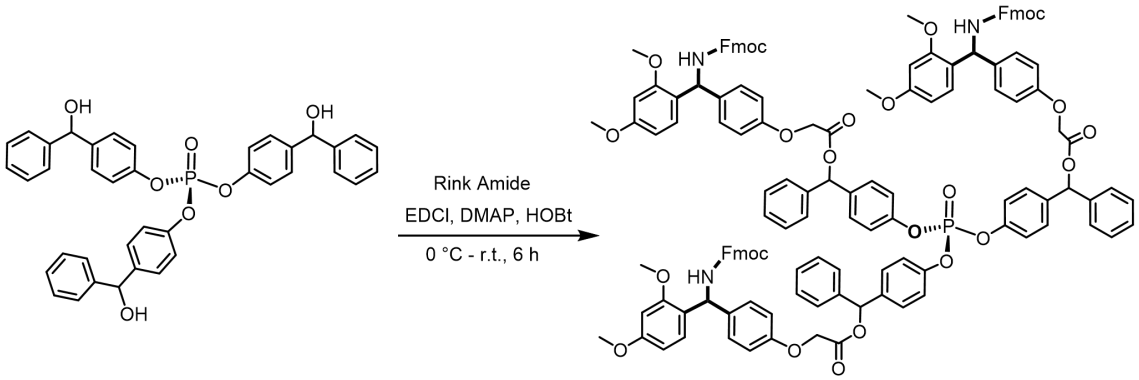


1e: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.72-7.52 (m, 9H), 7.36-7.10 (m, 42H), 5.92-5.53 (m, *3*H), 4.43-4.41 (m, 6H), 4.15-4.07 (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 155.7, 149.6, 143.9, 141.4, 141.2, 139.3, 128.9, 127.8, 127.4, 127.2, 125.2, 125.1, 120.3, 120.1, 66.9, 66.7, 58.3, 47.4, ppm. HRMS (ESI) m/z calcd for $C_{84}H_{66}N_3O_{10}PNa^+$ $(M+Na)^+$ 1330.43780, found 1330.43958.

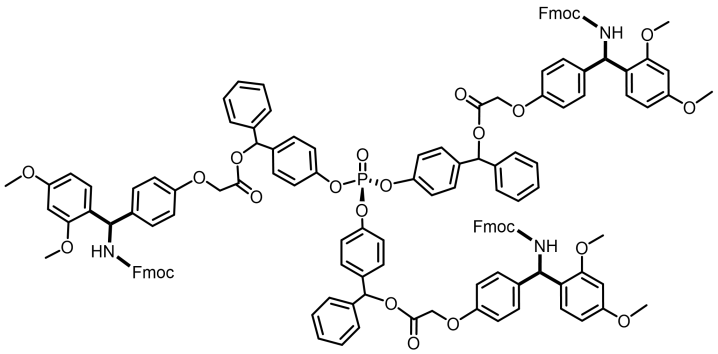
Preparation of 1e-deFmoc: The preparation of **1e-deFmoc** was according to the above procedures of **1a-deFmoc**.



1e-deFmoc: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.73-7.29 (m, 21H), 7.20-7.18 (m, 6H), 5.19 (m, 3H), 2.25 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 149.3, 149.2, 145.2, 143.0, 128.7, 128.5, 127.2, 126.9, 120.1, 77.6, 59.1 ppm. HRMS (ESI) m/z calcd for $C_{39}H_{36}N_3O_4PNa^+$ $(M+Na)^+$ 664.23356, found 664.23322.

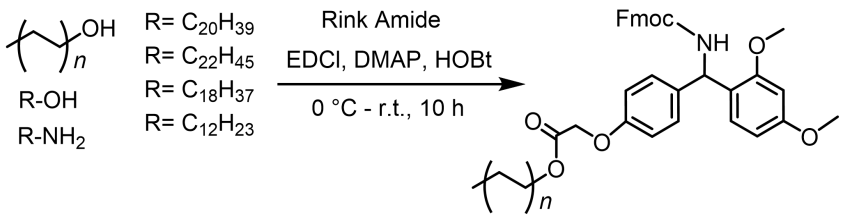


Synthesis of 1f: The tris (4-(hydroxy(phenyl)methyl)phenyl) phosphate (3.22 g, 5 mmol) were added DCM (20 mL) and stired at 0 °C for 10 min. Then the Rink Amide (8.5 g, 15.75 mmol), EDCl (3.44 g, 18.0 mmol), DMAP (225 mg, 1.8 mmol) and HOBt (2.43 g, 18 mmol) were added to the solution, the mixture was stirred at room temperature for 12 h. The reaction mixture was then washed with 10% Na_2CO_3, dried with anhydrous $MgSO_4$. 15.0 mL ethyl acetate was added to dissolve the sample after concentration, and 60 mL of petroleum ether was added dropwise and stirred to obtain the purified product **1f** (10.6 g, 96%).

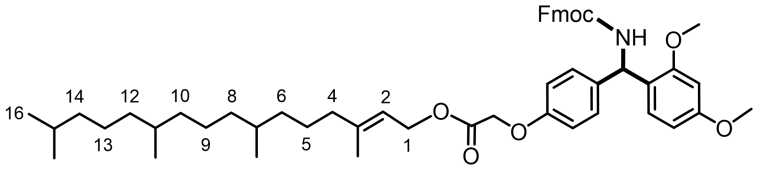


1f: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.72-7.55 (m, 9H), 7.35-7.05 (m, 48H), 6.91 (m, 3H), 6.77-6.75 (m, 6H), 6.44-6.40 (m, 6H), 6.07-5.88 (m, 3H), 4.63-4.59 (m, 6H), 4.44-4.38 (m, 6H), 4.20-4.17 (m, 3H), 3.73 (s, 9H), 3.64 (s, 9H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 188.2, 160.6, 158.1, 156.8, 155.9, 150.1, 150.0, 144.2, 144.1, 141.4, 139.1, 137.3, 135.7, 129.5, 128.9, 128.8, 128.4, 128.1, 127.8, 127.1, 125.2, 122.3, 120.4, 120.3, 120.1, 114.5, 104.4, 99.5, 77.5, 77.1, 71.7, 66.7, 65.6, 55.6, 55.5, 54.9, 54.6, 47.5 ppm. HRMS (ESI) m/z calcd for $C_{135}H_{114}N_3O_{25}PNa^+$ $(M+Na)^+$ 2230.73712, found 2230.73804.

4. Synthesis of aliphatic based tags TAGs

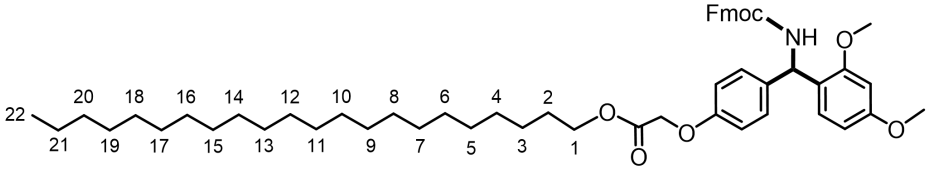


Synthesis of 2a: The 3,7,11,15-tetramethylhexadec-2-en-1-ol (1.48 g, 5 mmol) were added DCM (20 mL) and stired at 0 °C for 10 min. Then the Rink Amide (2.83 g, 5.25 mmol), EDCl (1.15 g, 6.0 mmol), DMAP (73 mg, 0.6 mmol) and HOBt (810 mg, 6 mmol) were added to the solution, the mixture was stirred at room temperature for 10 h. The reaction mixture was then washed with 10% Na_2CO_3, dried with anhydrous $MgSO_4$. The mixture was concentrated to remove the solvent and added 15.0 mL MeOH to the residue and white precipitates appeared. Centrifugation to obtain the purified product **2a** (3.9 g, 96%).



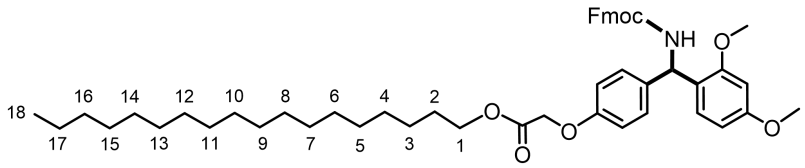
2a: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.72-7.55 (m, 3H), 7.37-7.06 (m, 7H), 6.80-6.78 (m, 2H), 6.44-6.40 (m, 2H), 6.07-5.87 (m, 1H), 5.36-5.32 (m, 1H), 4.70-4.69 (d, J= 4.0 Hz, 2H), 4.54 (m, 2H), 4.44-4.17 (m, 2H), 3.73 (s, 3H), 3.67 (s, 3H), 2.01-1.97 (m, 2 H), 1.67 (m, 3 H), 1.57-1.49 (m, 1 H), 1.41-1.03 (m, 20 H), 0.87-0.84 (m, 12 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 168.9, 160.6, 158.1, 156.9, 155.9, 144.2, 144.1, 143.8, 141.4, 135.5, 129.5, 128.0, 127.7, 127.1, 125.2, 122.3, 120.0, 117.5, 114.5, 104.3, 99.4, 77.5, 66.7, 65.6, 62.2, 55.5, 55.4, 54.7, 47.5, 39.9, 39.5, 37.5, 37.4, 36.7, 32.9, 32.8, 29.8, 28.1, 25.1, 24.9, 24.6, 22.9, 22.8, 19.9, 19.8, 16.5 ppm. HRMS (ESI) m/z calcd for $C_{52}H_{67}NO_7Na^+$ $(M+Na)^+$ 840.48097, found 840.48157.

Synthesis of 2b: The synthesis of **2b** was according to above procedure of **2a** by using the raw material of triacontan-1-ol (or 1-docosanol), Rink Amide and EDCl/HOBt/DMAP coupling reagents, **2b** yield 97%.



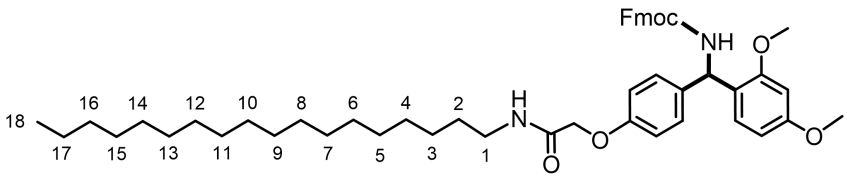
2b: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.76-7.57 (m, 3H), 7.40-7.25 (m, 4H), 7.12-7.04 (m, 3H), 6.82-6.80 (m, 2H), 6.46-6.44 (m, 2H), 6.05-5.80 (m, 1H), 4.57 (s, 2H), 4.46-4.37 (m, 2H), 4.24-4.16 (m, 2H), 3.78 (s, 3H), 3.71 (s, 3H), 3.63-3.59 (m, 1H), 1.65-1.53 (m, 2H), 1.31-1.25 (m, 40H), 0.89-0.86 (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.1, 160.6, 158.1, 156.8, 155.9, 144.1, 144.0, 141.4, 135.4, 129.5, 127.9, 127.7, 127.1, 125.2, 125.1, 122.1, 120.0, 114.5, 104.2, 99.5, 77.3, 66.7, 65.6, 55.5, 55.4, 54.8, 47.5, 32.9, 32.0, 29.8, 29.7, 29.6, 29.5, 29.3, 28.6, 25.9, 22.8,14.2 ppm. HRMS (ESI) m/z calcd for $C_{54}H_{73}NO_7Na^+$ $(M+Na)^+$ 870.52792, found 870.52893.

Synthesis of 2c: The synthesis of **2c** was according to the above procedure of **2c** by using the raw material of 1-hydroxyoctadecane, Rink Amide and EDCl/HOBt/DMAP coupling reagents, **2c** yield 97%.



2c: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.76-7.57 (m, 3H), 7.40-7.27 (m, 4H), 7.12-7.04 (m, 3H), 6.82-6.80 (m, 2H), 6.47-6.43 (m, 2H), 6.05-5.80 (m, 1H), 4.57 (s, 2H), 4.46-4.37 (m, 2H), 4.24-4.16 (m, 3H), 3.79 (s, 3H), 3.71 (s, 3H), 1.85-1.82 (m, 2H), 1.32-1.25 (m, 32H), 0.89-0.86 (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.1, 160.6, 158.1, 156.8, 155.9, 144.1, 144.0, 141.4, 135.4, 129.5, 127.9, 127.7, 127.1, 125.2, 125.1, 122.1, 120.0, 114.5, 104.2, 99.5, 77.3, 66.7, 65.5, 55.5, 55.4, 54.8, 47.4, 32.0, 29.8, 29.7, 29.6, 29.5, 29.3, 28.6, 25.9, 22.8,14.2 ppm. HRMS (ESI) m/z calcd for $C_{50}H_{65}NO_7Na^+$ $(M+Na)^+$ 814.46532, found 814.46515.

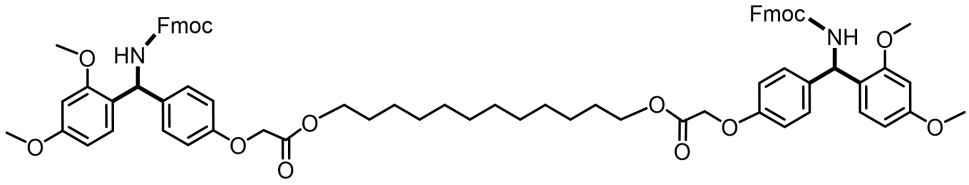
Synthesis of 2d: The synthesis of **2d** was according to the above procedure of **2a** by using the raw material of octadecan-1-amine and EDCl/HOBt/DIPEA coupling reagents, yield 96%.



2d: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.73-7.56 (m, 3H), 7.38-7.06 (m, 7H), 6.80-6.78 (m, 2H), 6.62-6.59 (m, 1H), 6.45-6.41 (m, 2H), 6.07-5.94 (m, 1H), 4.40 (s, 4H), 4.21-4.17 (m, 1H), 3.75 (s, 3H), 3.69 (s, 3H), 3.32-3.27 (m, 2H), 1.52-1.49 (m, 2H), 1.25 (m, 32H), 0.89-0.86 (m, 3H) ppm; ^{13}C

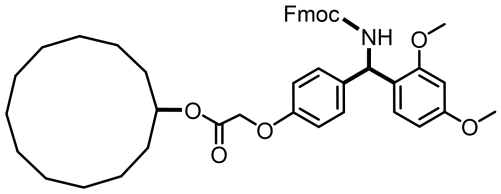
NMR (100 MHz, $CDCl_3$), δ 168.1, 160.2, 158.0, 156.2, 155.9, 144.1, 144.0, 141.4, 135.9, 129.5, 128.1, 127.7, 125.2, 125.1, 122.1, 120.0, 114.5, 104.3, 99.4, 67.6, 66.7, 55.5, 55.4, 54.6, 47.5, 39.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 27.0, 22.8, 14.2 ppm. HRMS (ESI) m/z calcd for $C_{50}H_{66}N_2O_6Na^+$ $(M+Na)^+$ 813.48131, found 813.48108.

Synthesis of 2e: The synthesis of **2e** was according to the above procedure of **2a** by using the raw material of 1,12-dodecanediol, Rink Amide and EDCl/HOBt/DMAP coupling reagents (2.0 equiv Tag), yield 88%.



2e: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.75-7.73 (m, 4H), 7.59-7.57 (m, 3H), 7.40-7.26 (m, 8H), 7.18-7.08 (m, 7H), 6.82-6.80 (m, 4H), 6.47-6.45 (m, 4H), 6.05-5.81 (m, 3H), 4.57 (s, 4H), 4.46-4.34 (m, 4H), 4.24-4.16 (m, 6H), 3.78 (s, 6H), 3.71 (s, 6H), 3.63-3.59 (m, 1H), 1.66-1.61 (m, 4H), 1.32-1.25 (m, 18H) (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 169.1, 160.6, 158.1, 156.8, 155.9, 144.1, 144.0, 141.4, 135.4, 129.5, 129.1, 127.9, 127.7, 127.1, 125.2, 125.1, 122.1, 120.0, 114.5, 104.2, 99.5, 77.4, 66.7, 65.5, 55.5, 54.8, 47.4, 29.6, 29.5, 29.2, 28.6, 25.8 ppm. HRMS (ESI) m/z calcd for $C_{76}H_{80}N_2O_{14}Na^+$ $(M+Na)^+$ 1267.55018, found 1267.55090.

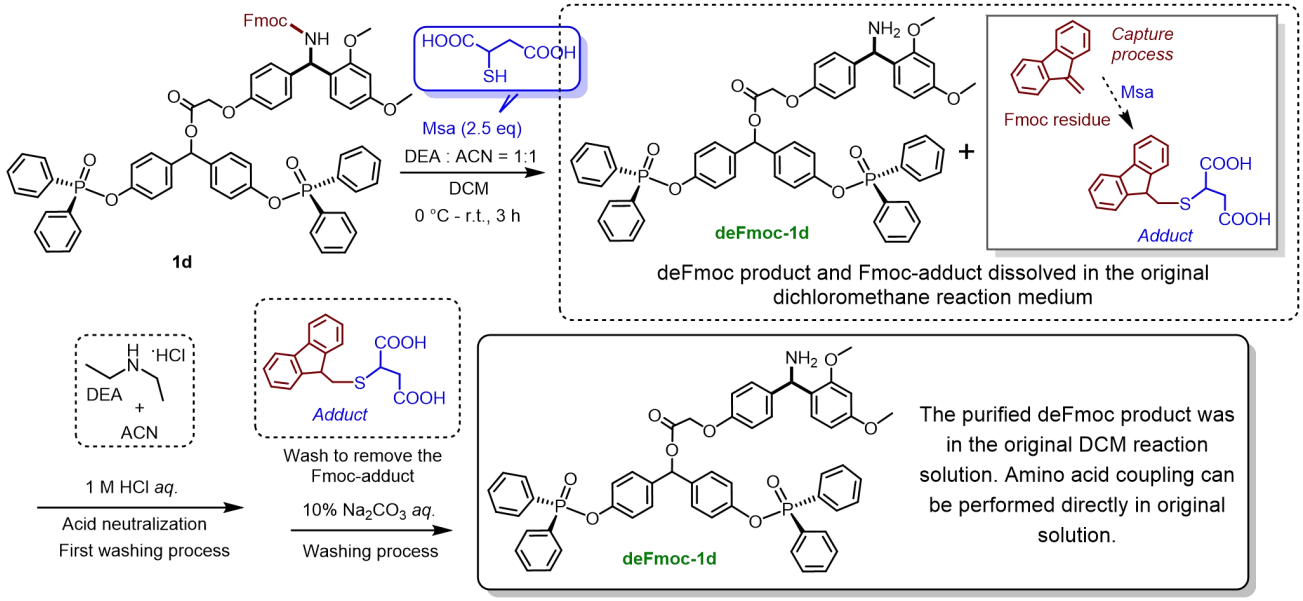
Synthesis of 2f: The synthesis of **2f** was according to the above procedure of **2a** by using the raw material of cyclododecanol, Rink Amide and EDCl/HOBt/DMAP coupling reagents, yield 96%.



2f: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.81-7.64 (m, 3H), 7.46-7.32 (m, 4H), 7.19-7.15 (m, 3H), 6.88-6.86 (m, 2H), 6.53-6.49 (m, 2H), 6.13-5.91 (m, 1H), 5.22-5.16 (m, 1H), 4.61 (s, 2H), 4.52-4.43 (m, 2H), 4.30-4.26 (m, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 1.81-1.73 (m, 2H), 1.61-1.55 (m, 2H), 1.46-1.33 (m, 19H), 0.96-0.90 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$), δ 168.8, 160.6, 158.1, 156.9, 155.9, 144.2, 144.0, 141.4, 135.3, 129.5, 127.9, 127.7, 127.1, 125.2, 122.2, 120.0, 114.4, 104.2, 99.4, 73.7, 66.7, 65.6, 55.5, 55.4, 54.7, 47.5, 29.2, 24.0, 23.9, 23.5, 23.3, 21.0 ppm. HRMS (ESI) m/z calcd for $C_{44}H_{51}NO_7Na^+$ $(M+Na)^+$ 728.35577, found 728.35559.

5. Study on the "one pot" strategy to remove the Fmoc protection.

(1) DEA/ACN/Msa deFmoc reagent system to remove the Fmoc group.



"One-pot" strategy for preparation of deFmoc-1d solution: (Remove of the Fmoc group of **1d**). The mercaptosuccinic acid (Msa, 375 mg, 2.5 mmol), diethylamine (DEA, 4.0 mL) and acetonitrile (4.0 mL) were added to the solution of product tag **1d** (1.14 g, 1.0 mmol) in DCM (15 mL) at 0 °C, and then the mixture was stirred at room temperature for 3 h. After the removal of Fmoc group, 1M HCl aqueous solution was added to the mixture at 0 °C to neutralize the DEA in mixed solution. The mixed solution was stratified, and the acetonitrile solvent and DEA base were washed away directly in the inorganic phase of the HCl solution. Next, the organic phase solution was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct.

The purified **deFmoc-1d** in original DCM solution was derived after washing with the 1M HCl aqueous solution and 10% Na_2CO_3 aqueous solution respectively. The **deFmoc-1d** in original DCM solution can be used for coupling the Fmoc amino acid (Fmoc-AA'-OH) directly, without concentrating and supplementing the DCM solution.

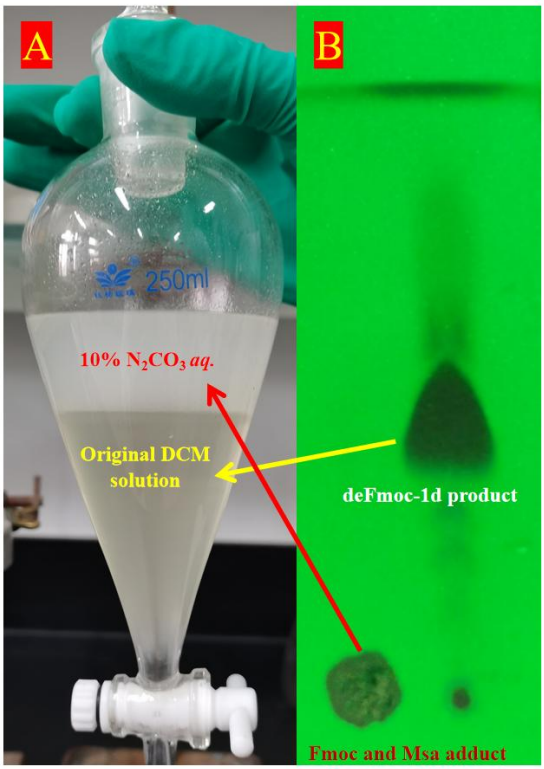
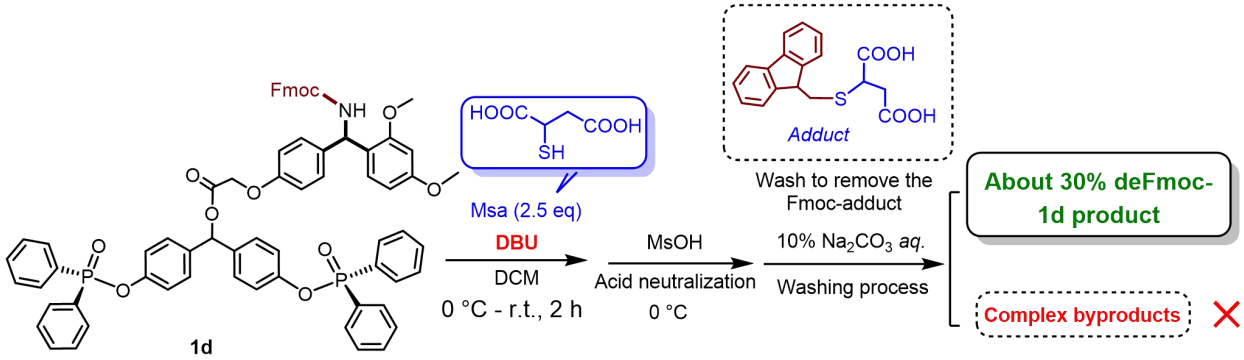


Figure S1. (A) The optical photograph of 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct of **deFmoc-1d** by using DEA/ACN/Msa deFmoc reagent system. The deFmoc product was dissolved in the original DCM solution (**Down**), the Fmoc-Msa adduct was dissolved in the 10% Na_2CO_3 aqueous solutionn (**Up**). **(B)** The optical photograph of TLC analysis for the Fmoc-Msa adduct (**Left**) and **deFmoc-1d** product (**Right**).

Other phosphonate based tags **(1a, 1b, 1c, 1e, 1f)** and aliphatic based tags (**2a, 2b, 2c, 2d, 2e, 2f**) produced the deFmoc results consistent with **1d** in the DEA/ACN/Msa deFmoc reagent system, with only slight differences in the solubility in DCM solvent. The process of Fmoc removal can be accelerated by adding acetonitrile reagent to the reaction system, and acetonitrile can be removed when washed in inorganic solvent due to the miscibility of acetonitrile and water.

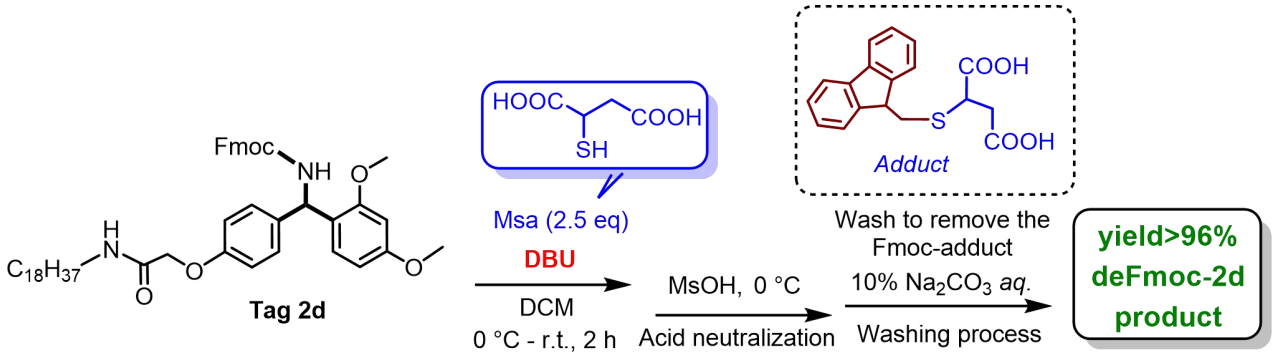
(2) DBU/Msa deFmoc reagent system to remove the Fmoc group.



The mercaptosuccinic acid (375 mg, 2.5 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 760 mg, 5 mmol) were added to the solution of product **1d** (1.14 g, 1.0 mmol) in DCM (15 mL) at 0 °C, and then the mixture was stirred at room temperature for 2 h. After the removal of Fmoc group, methanesulfonic acid (100 mg, 0.26 mmol) diluted in 1 mL DCM was added to the mixture at 0 °C to neutralize the DBU in mixed solution. Then the organic phase was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct.

After multiple repeated the above DBU/Msa system deFmoc experiments, in addition to some target products **deFmoc-1d (yield <30%)**, many complex byproducts were also generated in the deFmoc reaction solution. Therefore, the DBU/Msa deFmoc reagent system was not suitable for the phosphonate based tag **1d**.

Other phosphonate based tags **(1a, 1b, 1c, 1e, 1f)** produced the deFmoc results consistent with **1d** in the DBU/Msa deFmoc reagent system.



Preparations of deFmoc-2d: The preparation of aliphatic based tag ***deFmoc-2d*** was according to the above procedure of preparation of ***deFmoc-1d***, yield >96%. The deFmoc product **deFmoc-2d** in original DCM solution can be used for coupling the next Fmoc amino acid directly, without concentrating and supplementing the DCM solution.

Other aliphatic based tags (**2a, 2b, 2c, 2e, 2f**) produced the deFmoc results consistent with tag **2d**

in the above DBU/Msa deFmoc reagent system, with only slight differences in the solubility in the DCM solvent medium.

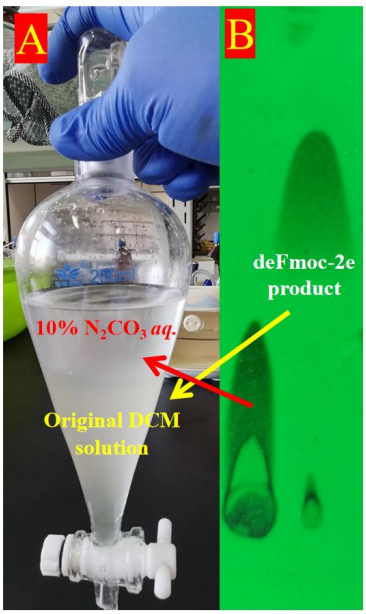
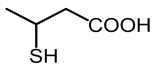
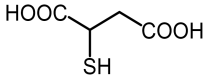
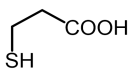
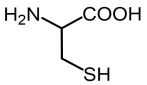


Figure S2. (A) The optical photograph of 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct of **deFmoc-2d** by using DBU/Msa deFmoc reagent system. The deFmoc product **deFmoc-2d** was dissolved in the original DCM solution (**Down**), the Fmoc-Msa adduct was dissolved in the 10% Na_2CO_3 aqueous solutionn (**Up**). **(B)** The optical photograph of TLC analysis for the Fmoc-Msa adduct (**Left**) and **deFmoc-2d** product (**Right**).

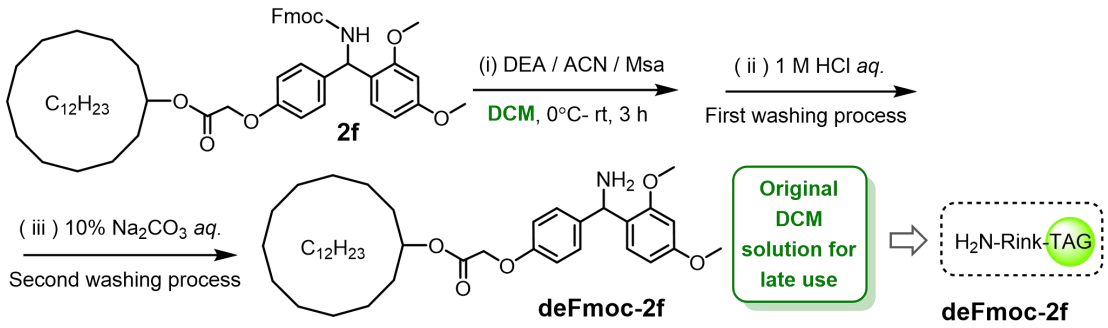
(3) Comparison of capturing and washing properties of Fmoc group capture reagents

Table S1. The capture reagents involved in the de-Fmoc process

Capture reagents	Structures	Hydrophilic group	Capture capacity	HCl and Na_2CO_3 *aq.* for washing removal capacity
Mba		COOH (1)	++++ (>95%)	++ (<50%)
Msa		COOH (2)	++++ (>95%)	+++++ (>95%)
Mpa		COOH (1)	++++ (>95%)	++ (<50%)
Cys		COOH (1) NH_2 (1)	++++ (>95%)	++++ (<90%)
"+" represents the strength of the capture or washing ability				

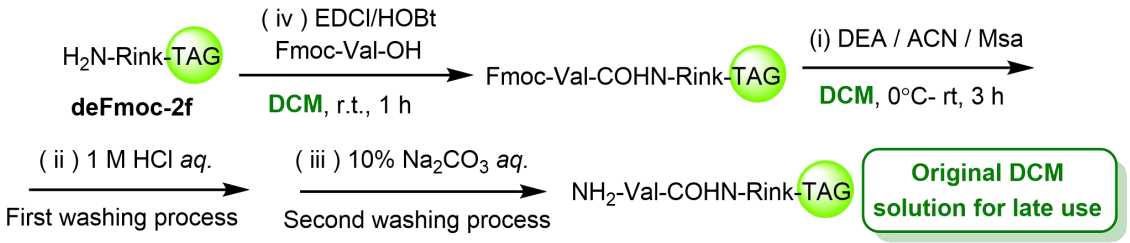
6. Study on the "one pot" strategy to synthesize the TREM-1 inhibitor LR12.

(1) "one pot" strategy to remove the Fmoc group of Tag 2f.



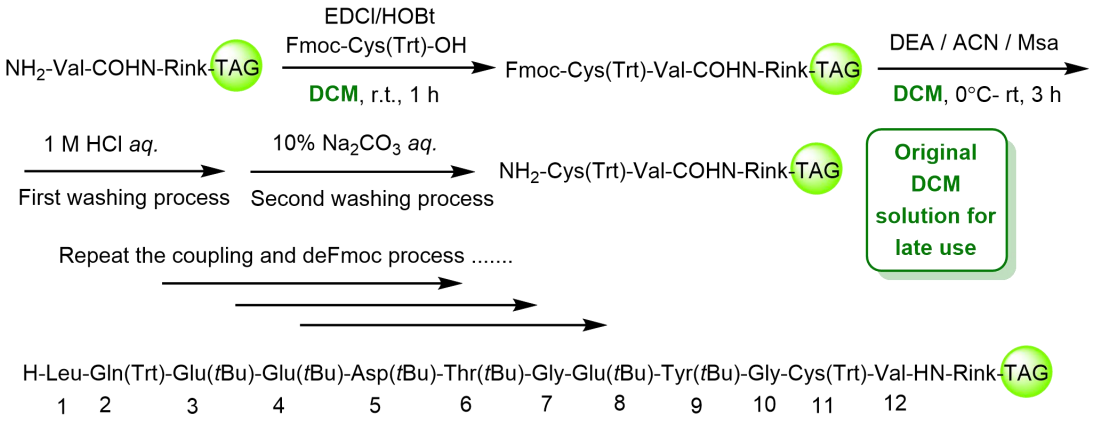
The mercaptosuccinic acid (375 mg, 2.5 mmol), diethylamine (4.0 mL) and acetonitrile (4.0 mL) were added to the solution of product **2f** (700 mg, 1.0 mmol) in DCM (15 mL) at 0 °C, and then the mixture was stirred at room temperature for 3 h. After the removal of Fmoc group, 1M HCl aqueous solution was added to the mixture at 0 °C to neutralize the DEA base in mixed solution. The mixed solution was stratified, and the acetonitrile solvent and DEA base were washed away directly in the inorganic phase of the HCl solution. Next, the organic phase solution was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct byproduct. The original DCM organic solution was then dried with anhydrous Na_2SO_3 to obtain the original DCM solution of **deFmoc-2f** for late use directly.

(2) Tag 2f assisted "one pot" strategy for TREM-1 inhibitor LR-12 chain extension.



The Fmoc-Val-OH (355 mg, 1.05 mmol), EDCl (230 mg, 1.2 mmol), HOBt (160 mg, 1.2 mmol) were added to the above original DCM solution of **deFmoc-2f**, the mixture was stirred at room temperature for 1 h. After the coupling reaction, the mercaptosuccinic acid (375 mg, 2.5 mmol), diethylamine (4.0 mL) and acetonitrile (4.0 mL) were added to the mixture directly at 0 °C, and then the mixture was stirred at room temperature for 3 h. After the removal of Fmoc group, 1M HCl aqueous solution was added to the mixture at 0 °C to neutralize the DEA base in mixed solution. The

mixed solution was stratified, and the acetonitrile solvent and DEA base were washed away directly in the inorganic phase of the HCl solution. Next, the organic phase solution was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct byproduct. The original DCM organic solution was then dried with anhydrous Na_2SO_3 to obtain the original DCM solution of **NH_2-Val-CONH-Rink-TAG(2f)** for late use directly.



The Fmoc-Cys(Trt)-OH (597 mg, 1.02 mmol), EDCl (230 mg, 1.2 mmol), HOBt (160 mg, 1.2 mmol) were added to the original DCM solution of **NH_2-Val-CONH-Rink-TAG(2f)**, the mixture was stirred at room temperature for 1 h. After the coupling reaction, the mercaptosuccinic acid (375 mg, 2.5 mmol), diethylamine (4.0 mL) and acetonitrile (4.0 mL) were added to the mixture directly at 0 °C, and then the mixture was stirred at room temperature for 3 h. After the removal of Fmoc group, 1M HCl aqueous solution was added to the mixture at 0 °C to neutralize the DEA base in mixed solution. The mixed solution was stratified, and the acetonitrile solvent and DEA base were washed away directly in the inorganic phase of the HCl solution. Next, the organic phase solution was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct byproduct. The original DCM organic solution was then dried with anhydrous Na_2SO_3 to obtain the original DCM solution of **NH_2-Cys(Trt)-Val-CONH-Rink-TAG(2f)** for late use.

The above one-pot procedures for Fmoc amino acid (Fmoc-AA'-OH) coupling and Fmoc group deprotection were repeated as the circular steps. The subsequent Fmoc amino acid 【Fmoc-Gly-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH】 coupling and deprotection were continued to obtain the **2f** tagged linear dodecapeptide LR-12 product **NH_2-Leu-Gln(Trt)-Glu(tBu)-Glu(tBu)-Asp(tBu)-Thr(tBu)-Gly-Glu(tBu) - Tyr(tBu)-Gly-Cys (Trt)-Val-CONH-Rink-TAG(2f).**

HRMS (ESI) m/z calcd for $C_{146}H_{199}N_{14}O_{28}S^+$ $(M+H)^+$ 2628.42935, found 2628.42969.

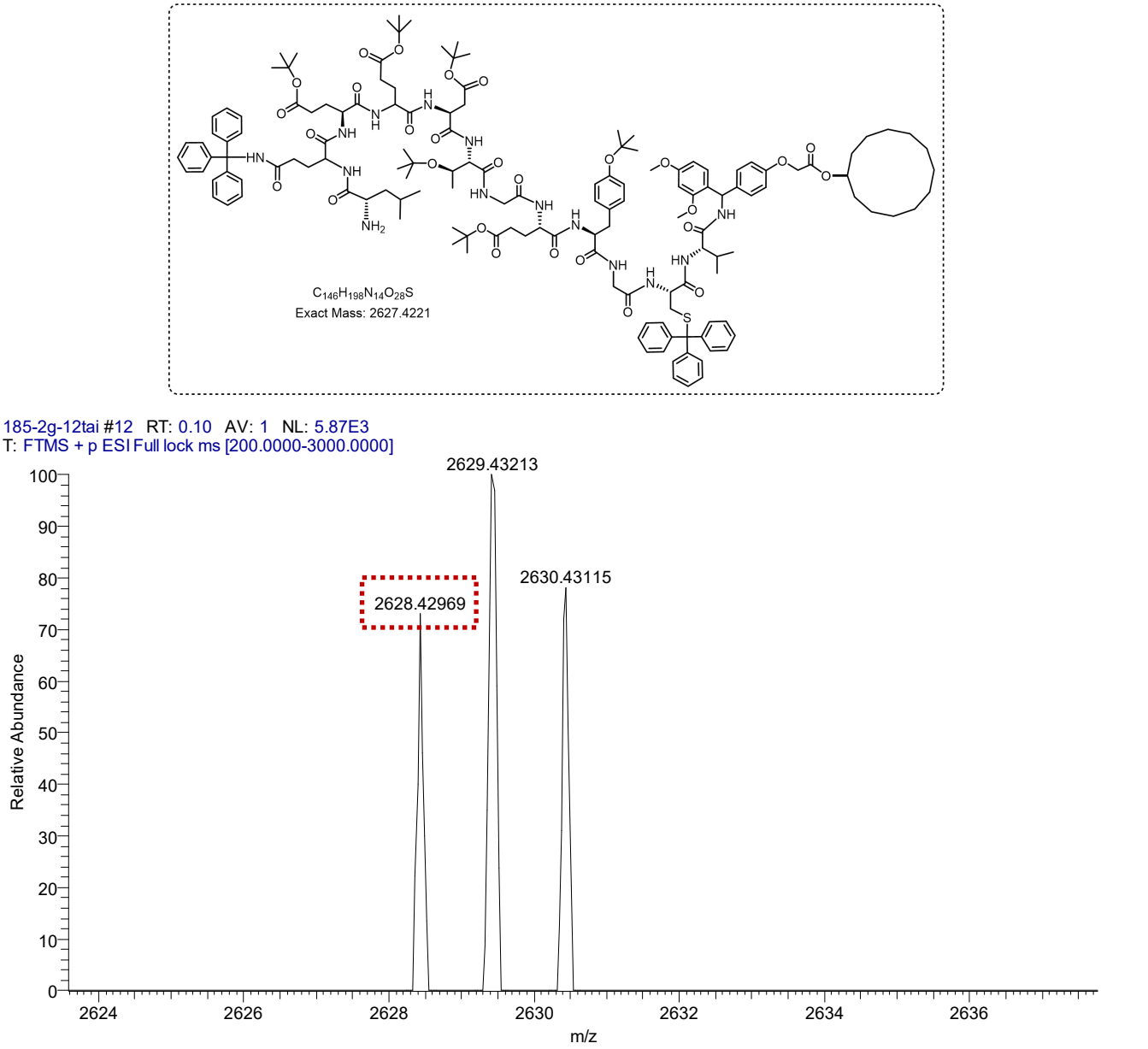


Figure S3. The HRMS (ESI) of H-Leu-Gln(Trt)-Glu(*t*Bu)-Glu(*t*Bu)-Asp(*t*Bu)-Thr(*t*Bu)-Gly-Glu(*t*Bu)-Tyr(*t*Bu)-Gly-Cys(Trt)-Val-CONH-Rink-TAG(**2f**).

(3) The cleavage/removal of Tag 2f and side chain protection groups

Shearing of TAG(2f) /Trt /-tBu group: The H-Leu-Gln(Trt)-Glu(*t*Bu)-Glu(*t*Bu)-Asp(*t*Bu)-Thr(*t*Bu)-Gly-Glu(*t*Bu)-Tyr(*t*Bu)-Gly-Cys(Trt)-Val-CONH-Rink-TAG(**2f**) was added to the mixed solution of TFA/Tis/H_2O/DODT (91/2.3/2.3/4.4, *v/v/v/v*) at room temperature and the reaction mixture was stirred at this temperature for 3 h. The reaction mixture was concentrated under reduced pressure to remove most of the TFA and H_2O. The residue was added with cold isopropyl ether and accompanied by ultrasound treatment, and then added isopropyl ether for three times repeatedly to derive the crude

linear LR-12 peptide precipitate, then the precipitate was centrifuged to obtain the target LR-12 peptide chain LQEEDTGEYGCV with 95% shearing yield. Then the purified LR-12 peptide product was prepared by RP HPLC prepartion.

TREM-1 inhibitor LR12: HRMS (ESI) m/z calcd for $C_{55}H_{85}N_{14}O_{23}S^+$ $(M+H)^+$ 1341.56272, found 1341.56272.

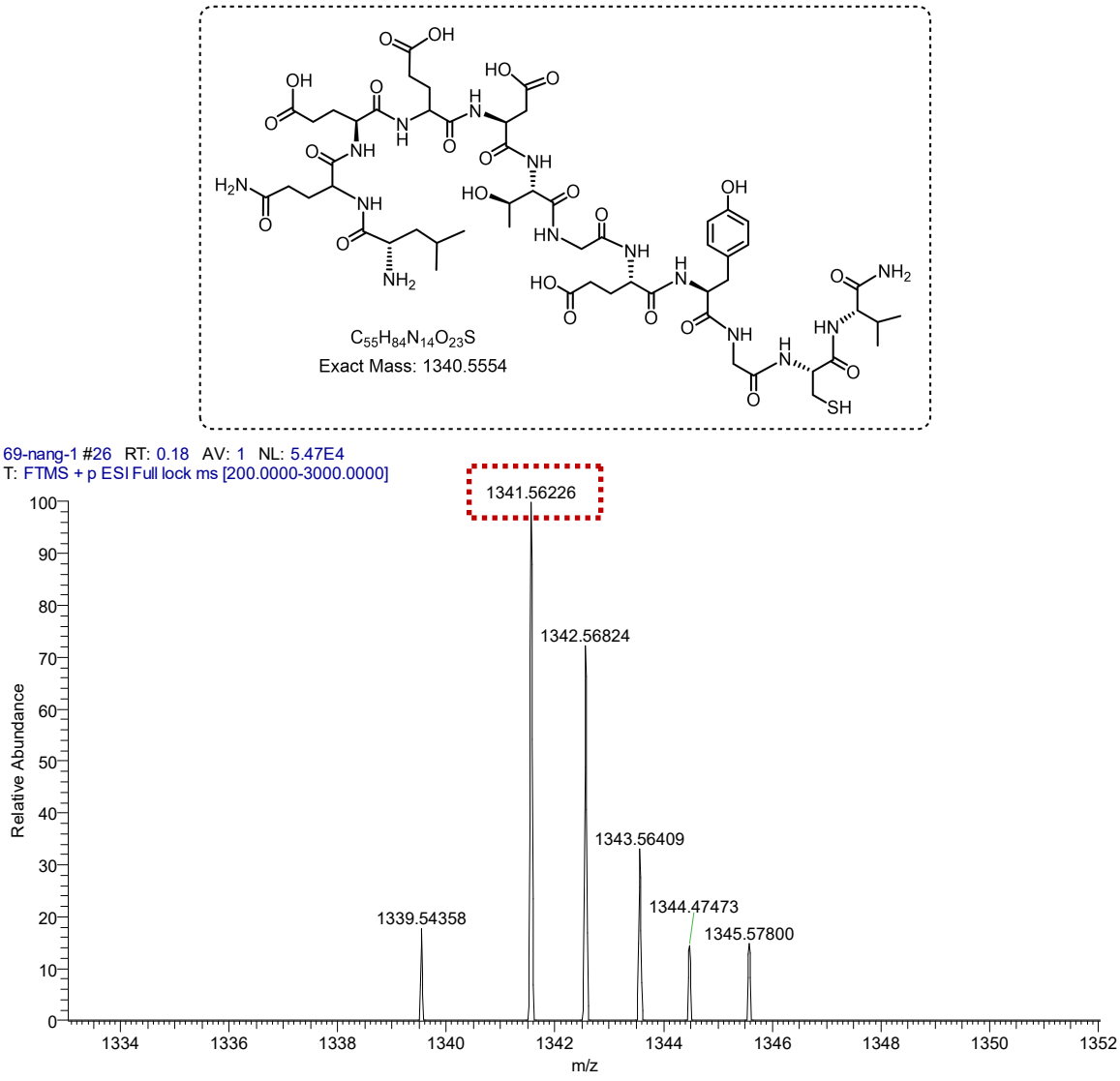


Figure S4. The HRMS (ESI) of LR-12 peptide chain LQEEDTGEYGCV.

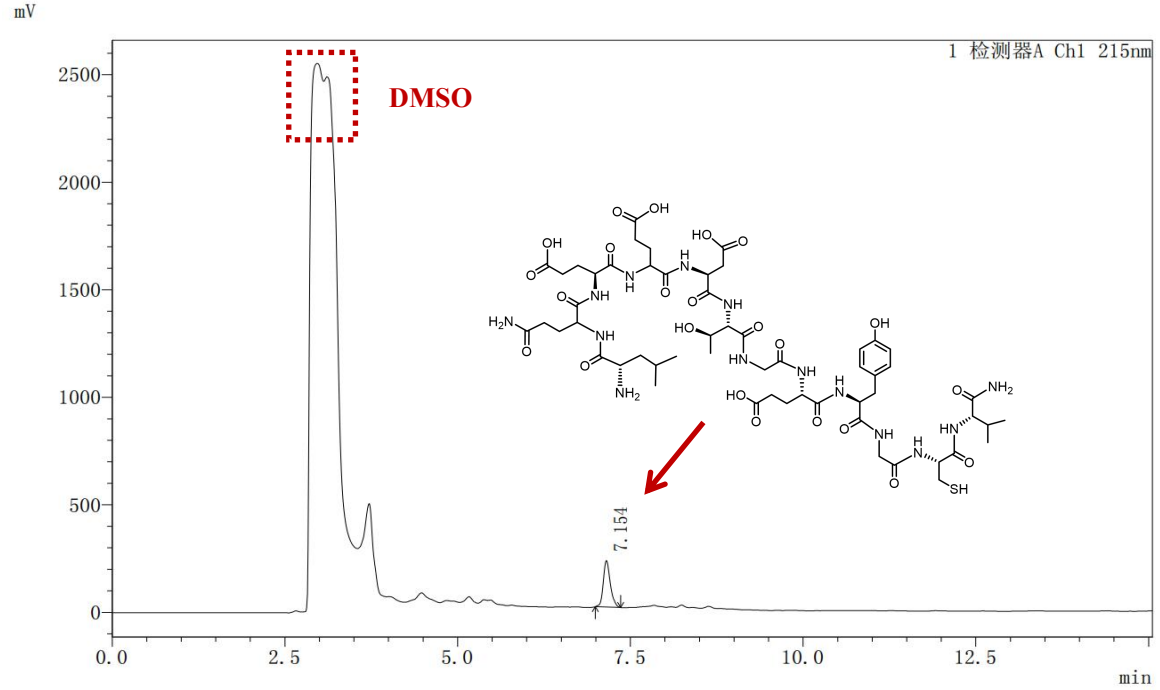


Figure S5. The HPLC analysis of LR-12 peptide chain LQEEDTGEYGCV.

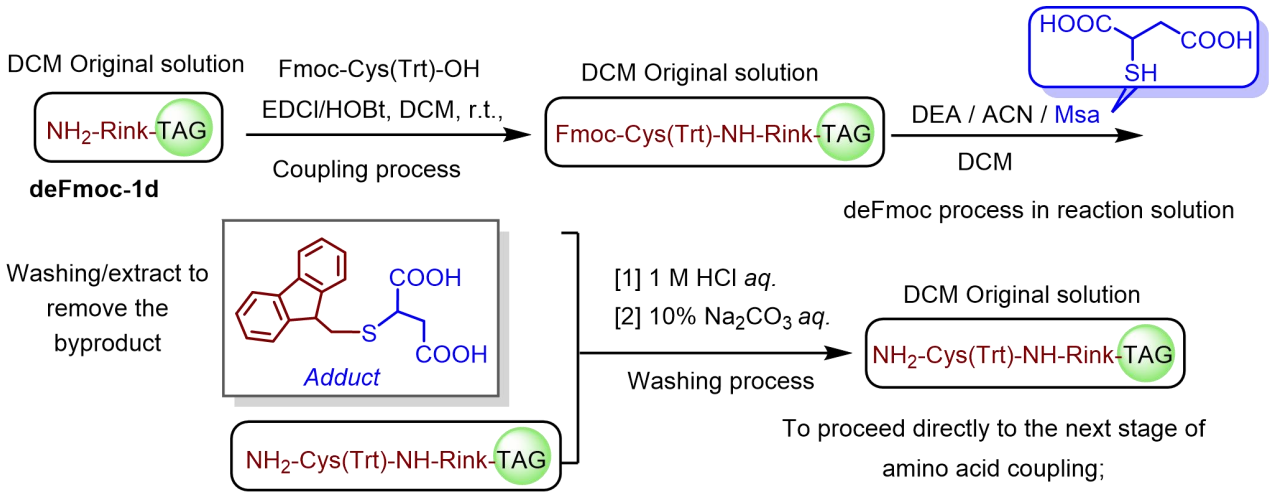
HPLC conditions: SHIMADZU LC-2030, column, Globalsil 5um 200A C18BP; 250×4.6 mm; 25 °C.

T	**Flow Rate**	**Elution**		***UV detection***
(min)	***(mL/min)***	**H_2O (0.1% TFA)**	**Acetonitrile**	***λ (nm)***
0.0	1.0	80	20	215
5.0	1.0	80	20	
30.0	1.0	40	60	
40.0	1.0	0	100	

7. One pot strategy to synthesize eptifibatide.

(1) One pot strategy for the synthesis of linear eptifibatide.

The mercaptosuccinic acid (375 mg, 2.5 mmol), diethylamine (4.0 mL) and acetonitrile (4.0 mL) were added to the solution of **Tag(1d)** (1.14 g, 1.0 mmol) in DCM (15 mL) at 0 °C. The mixture was then stirred at room temperature for 3 h. After the removal of Fmoc group, 1M HCl aqueous solution was added to the mixture at 0 °C to neutralize the DEA base. The mixed solution was stratified, and the acetonitrile solvent and DEA were washed away directly in the inorganic phase of the HCl solution. Next, the organic phase solution was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct byproduct, dried with $MgSO_4$. The purified **Tag(1d)-Rink-NH$_2$** in original DCM solution were obtained for late use directly.



The Fmoc-Cys(Trt)-OH (597 mg, 1.02 mmol), EDCl (230 mg, 1.2 mmol), HOBt (160 mg, 1.2 mmol) were added to the original DCM solution of **Tag(1d)-Rink-NH$_2$**, the mixture was stirred at room temperature for 1 h. After the coupling reaction, the mercaptosuccinic acid (375 mg, 2.5 mmol), diethylamine (4.0 mL) and acetonitrile (4.0 mL) were added to the mixture at 0 °C, and then the mixture was stirred at room temperature for 3 h. After the removal of Fmoc group, 1 M HCl aqueous solution was added to neutralize the DEA at 0 °C, the mixed solution was stratified, and the acetonitrile solvent and DEA were washed away directly in the inorganic phase of the HCl solution. Then the organic phase was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct. The original DCM organic solution was then dried with anhydrous $MgSO_4$ to obtain the depurated original DCM solution of **NH$_2$-Cys(Trt)-CONH-Rink-TAG(1d)** for late use directly.

Fmoc-Cys(Trt)-CONH-Rink-TAG(1d): HRMS (ESI) m/z calcd for $C_{91}H_{77}N_2O_{12}P_2S^+$ $(M+H)^+$ 1483.46670, found 1483.46960.

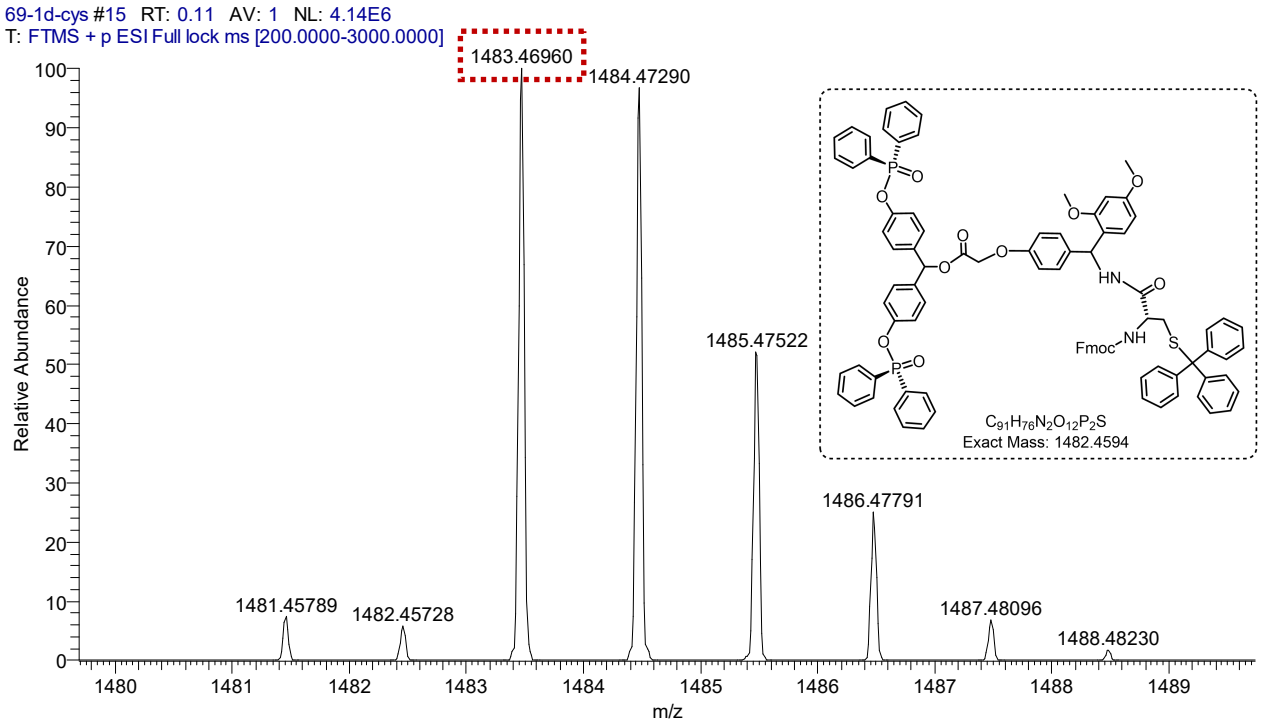
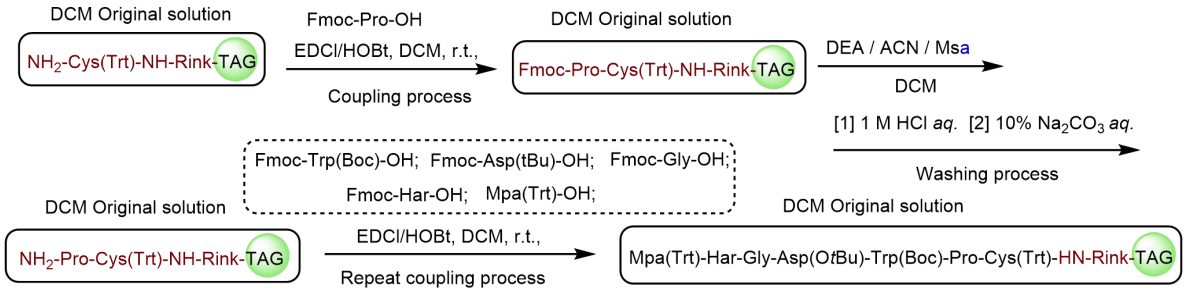


Figure S6. The HRMS (ESI) of Fmoc-Cys(Trt)-CONH-Rink-TAG(1d).



The Fmoc-Pro-OH (337 mg, 1.0 mmol), EDCl (230 mg, 1.2 mmol), HOBt (160 mg, 1.2 mmol) were added to the original DCM solution of **NH_2-Cys(Trt)-CONH-Rink-TAG(1d)**, the mixture was stirred at room temperature for 1 h. After the coupling reaction, the mercaptosuccinic acid (375 mg, 2.5 mmol), diethylamine (4.0 mL) and acetonitrile (4.0 mL) were added to the mixture at 0 °C, and then the mixture was stirred at room temperature for 3 h. After the removal of Fmoc group, 1 M HCl aqueous solution was added to neutralize the DEA at 0 °C, the mixed solution was stratified, and the acetonitrile solvent and DEA were washed away directly in the inorganic phase of the HCl solution. Then the organic phase was added with 10% Na_2CO_3 aqueous solution to wash and extract the Fmoc-adduct. The original DCM organic solution was then dried with anhydrous $MgSO_4$ to obtain the original DCM solution of **NH_2-Pro-Cys(Trt)-CONH-Rink-TAG(1d)** for late use.

Repeat the coupling and deprotection process to continue loading the remaining Fmoc amino acids to obtain the **TAG(1d)** tagged linear eptifibatide product **Mpa(Trt)-Har-Gly-Asp(tBu)-Trp**

(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG(1d). HRMS ESI of partial intermediates of etifibatide:

NH_2-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG(1d): HRMS (ESI) m/z calcd for $C_{107}H_{107}N_7O_{18}P_2SNa^+$ $(M+Na)^+$ 1894.67607, found 1894.67725.

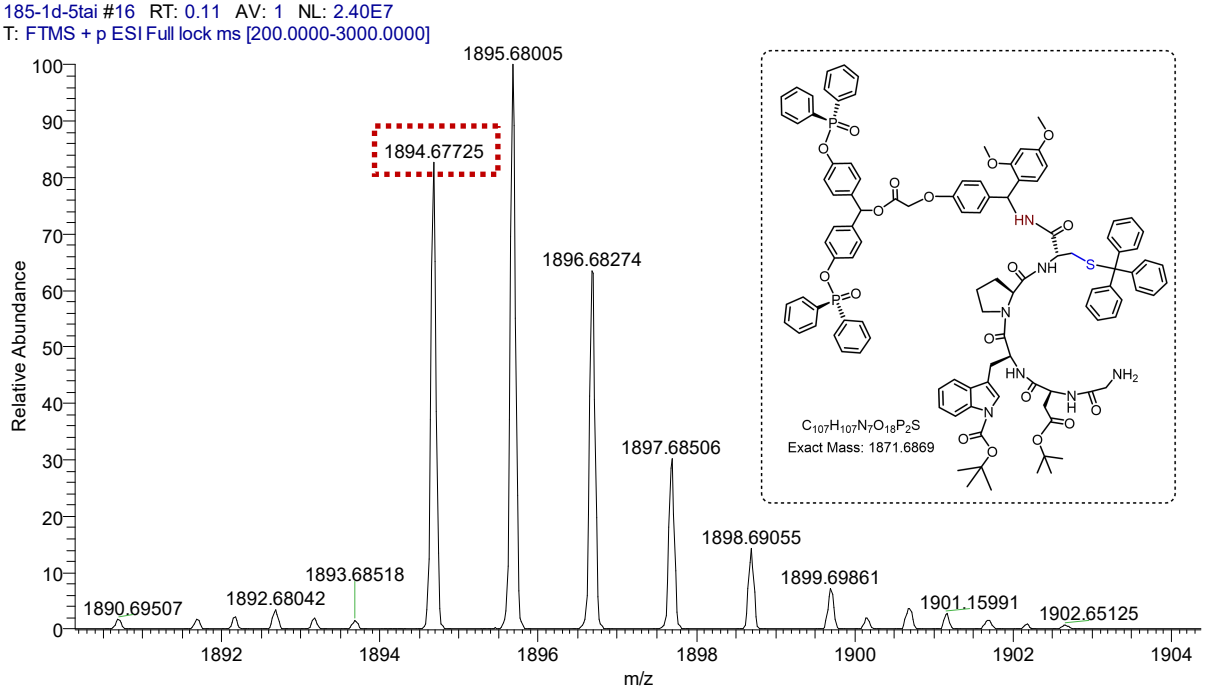


Figure S7. The HRMS (ESI) of NH_2-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG(1d).

NH_2-Har-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG(1d): HRMS (ESI) m/z calcd for $C_{114}H_{122}N_{11}O_{19}P_2S^+$ $(M+H)^+$ 2042.81089, found 2042.81384.

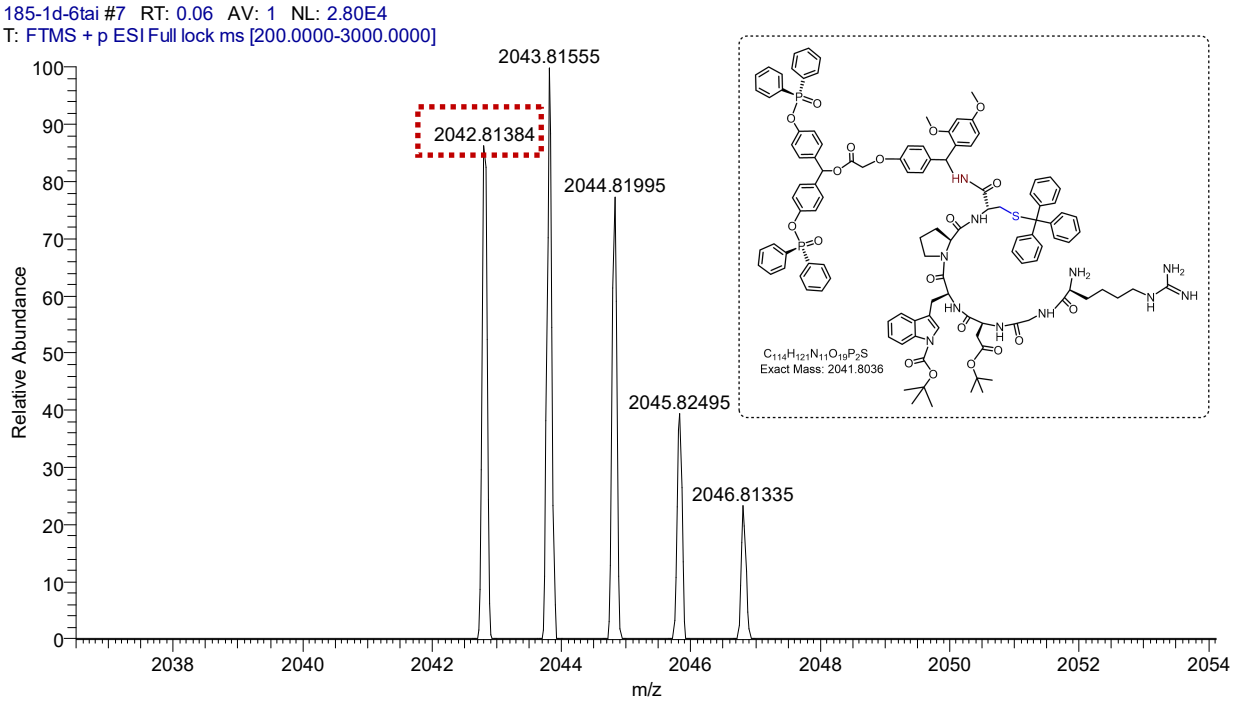


Figure S8. HRMS (ESI) of NH_2-Har-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG (1d)

Mpa(Trt)-Har-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG(1d): HRMS (ESI) m/z calcd for $C_{136}H_{140}N_{11}O_{20}P_2S_2^+$ $(M+H)^+$ 2372.91873, found 2372.92285.

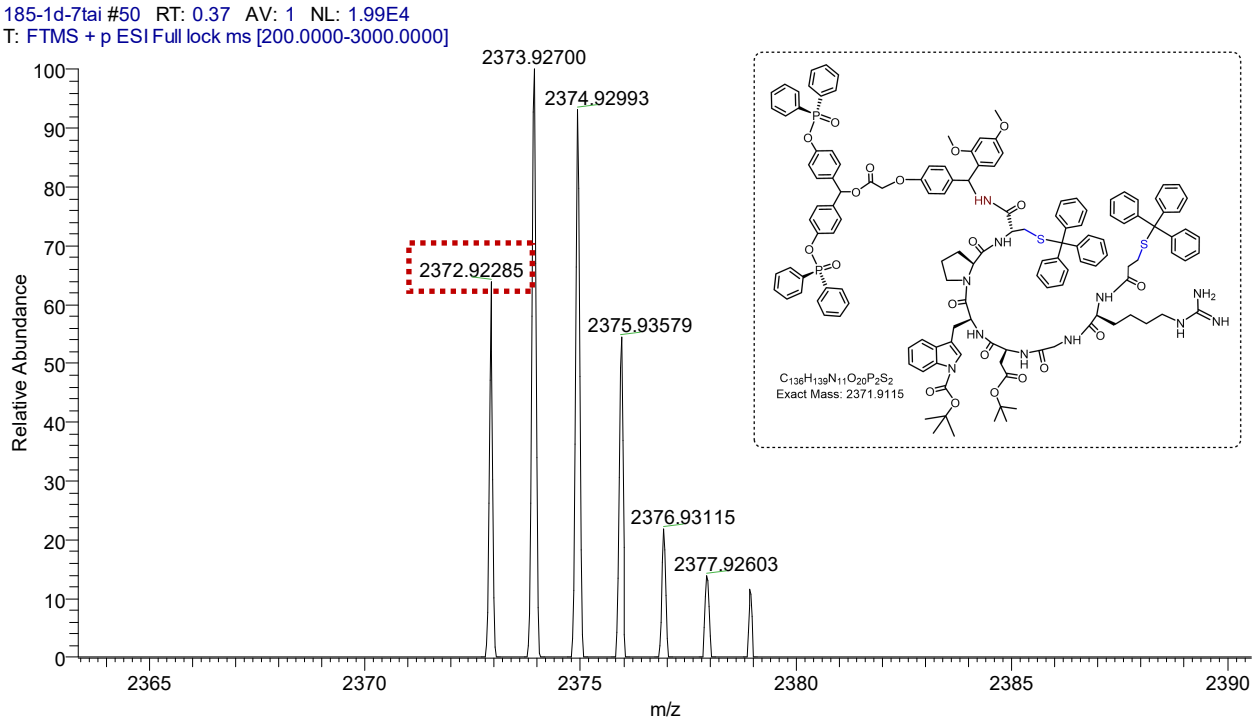


Figure S9. HRMS (ESI) of Mpa(Trt)-Har-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG(1d)

(2) Cleavage of the linear eptifibatide.

Shearing of TAG(1d) /Trt /-tBu group: The Mpa(Trt)-Har-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys (Trt)-CONH-Rink-TAG(1d) was added to the mixed solution of 10 mL TFA/Tis/H_2O/DODT (91/2.3/2.3/4.4, *v/v/v/v*) at room temperature and the reaction mixture was stirred at this temperature for 3 h. The reaction mixture was concentrated under reduced pressure to remove most of the TFA and H_2O. The residue was added with cold isopropyl ether and accompanied by ultrasound, and then centrifugation to obtain white solid. Next, the white solid was added with isopropyl ether for three times repeatedly to derive the crude linear eptifibatide precipitate, and the precipitate was centrifuged to obtain the linear eptifibatide Mpa(SH)-Har-Gly-Asp-Trp-Pro-Cys(SH) with 94% shearing yield, derived linear eptifibatide 0.50 g.

Linear eptifibatide: HRMS (ESI) m/z calcd for $C_{35}H_{51}N_{11}O_9S_2Na^+$ $(M+Na)^+$ 856.32048, found 856.32135.

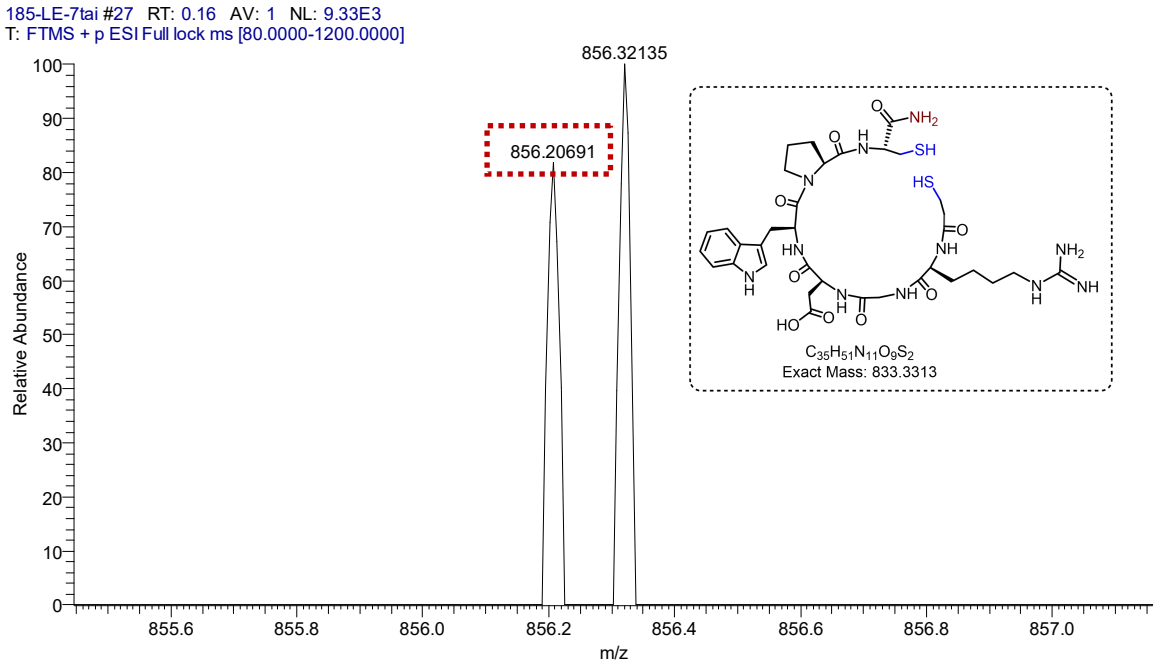


Figure S10. The HRMS (ESI) of Linear eptifibatide Mpa(SH)-Har-Gly-Asp-Trp-Pro-Cys(SH).

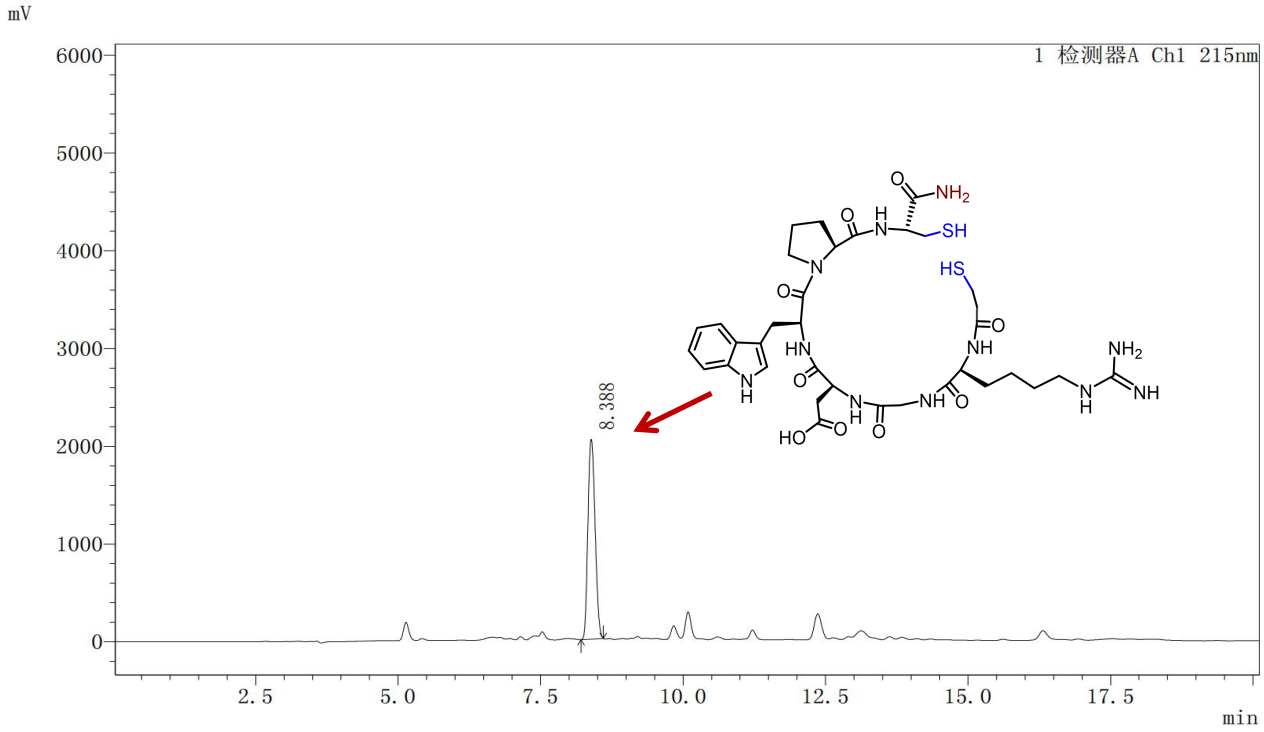


Figure S11. The HPLC analysis of linear eptifibatide.

HPLC conditions: SHIMADZU LC-2030, column, Globalsil 5um 200A C18BP; 250×4.6 mm; 25 °C.

T	Flow Rate	Elution		*UV detection*
(min)	*(mL/min)*	H_2O (0.1% TFA)	Acetonitrile	*λ (nm)*

0.0	1.0	90	10	215
5.0	1.0	90	10	
30.0	1.0	50	50	
40.0	1.0	0	100	

(3) Oxidation formation of disulfide bonds for preparation of eptifibatide

Cyclization strategy 1: DMSO oxidation for preparation of eptifibatide.

The linear eptifibatide Mpa(SH)-Har-Gly-Asp-Trp-Pro-Cys(SH) was dissolved in 100 mL DMSO/H_2O/ACN (V_{DMSO}:V_{H2O}/V_{ACN} = 0.05:0.65:0.30) (Concentration <10^{-3} M), and the pH was adjusted to 6.0 with dilute acetic acid. The reaction mixture was stirred for 12 h at room temperature. The mixture was then accompanied with vacuum concentration to remove the acetonitrile and part of H_2O. The samples were then analyzed by HPLC. And then lyophilized the mixture. The purified etifibatide product was prepared by preparative HPLC (CXTH, LC 3000 system).

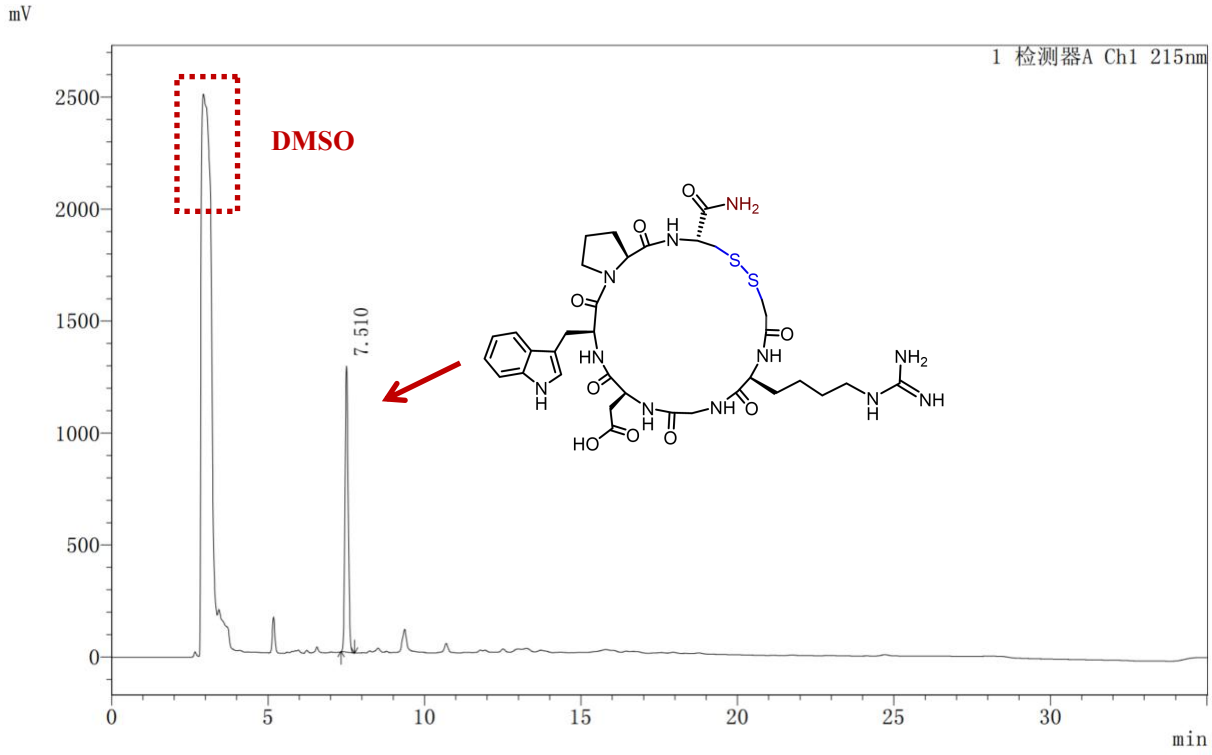


Figure S12. The HPLC analysis of eptifibatide cyclization solution by DMSO oxidation.

Cyclization strategy 2: Air oxidation for preparation of eptifibatide.

The linear eptifibatide Mpa(SH)-Har-Gly-Asp-Trp-Pro-Cys(SH) was dissolved in 100 mL H_2O/ACN (V_{H2O}/V_{ACN} = 0.60: 0.40) (Concentration <10^{-3} M), and the pH was adjusted to 10.0 with

dilute NH_4OH. The reaction mixture was stirred for 24 h at room temperature with air exposure and then lyophilized for HPLC analysis and preparation. The purified etifibatide product was prepared by preparative HPLC CXTH, LC 3000 system.

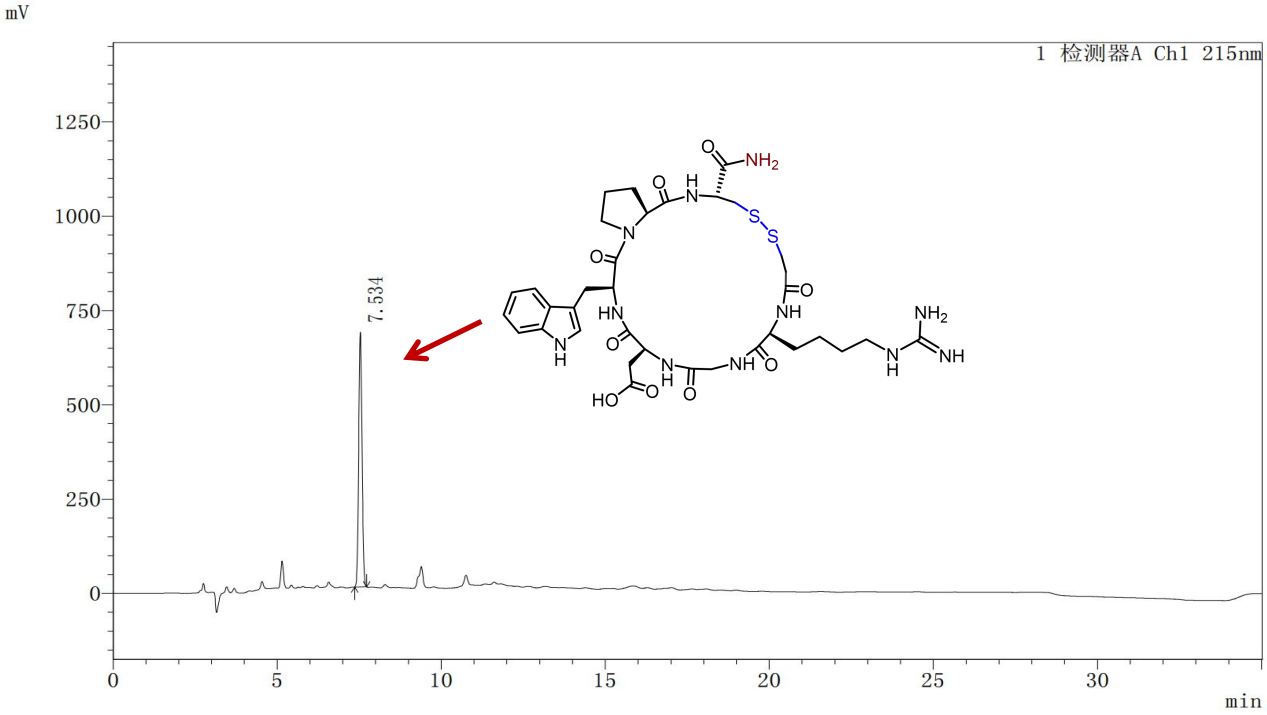


Figure S13. The HPLC analysis of eptifibatide cyclization solution by air oxidation.

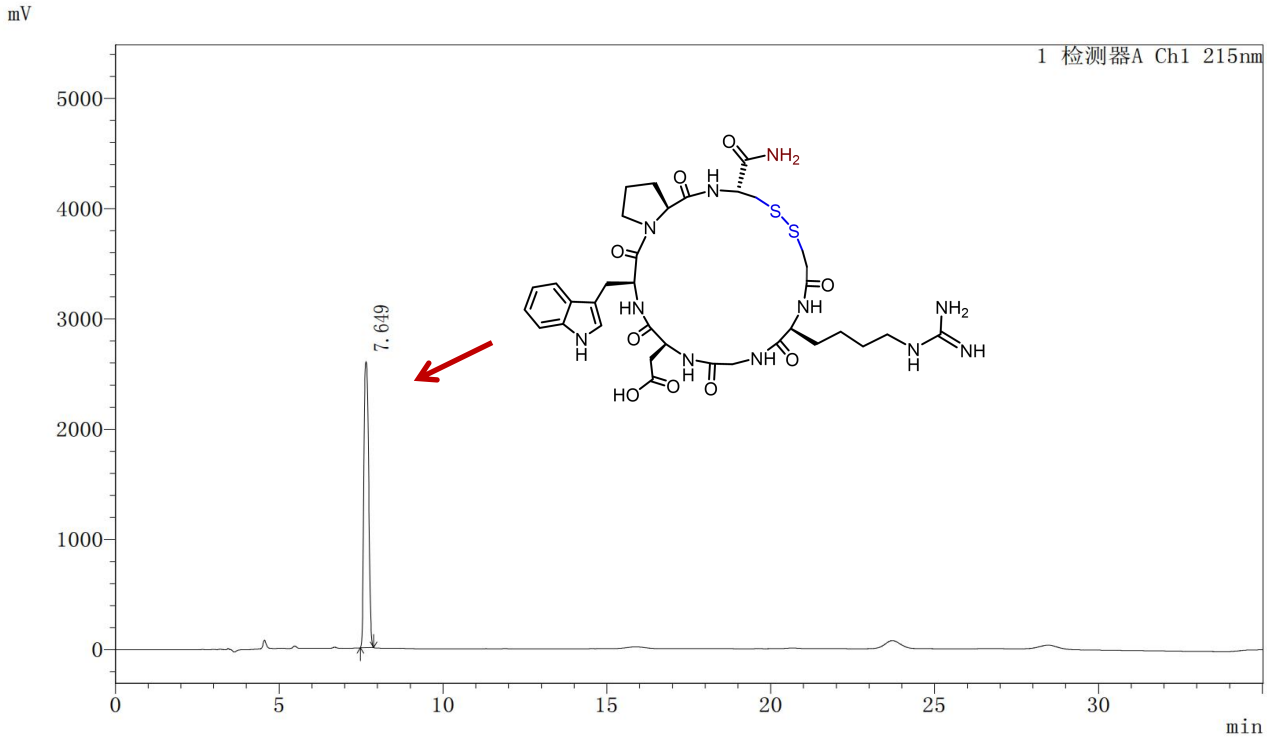


Figure S14. The The HPLC analysis of eptifibatide.

HPLC conditions: SHIMADZU LC-2030, column, Globalsil 5um 200A C18BP; 250×4.6 mm; 25 °C.

T	Flow Rate	Elution		*UV detection*
(min)	*(mL/min)*	H_2O (0.1% TFA)	Acetonitrile	*λ (nm)*
0.0	1.0	80	20	215
5.0	1.0	80	20	
30.0	1.0	40	60	
40.0	1.0	0	100	

Eptifibatide: HRMS (ESI) m/z calcd for $C_{35}H_{50}N_{11}O_9S_2^+$ $(M+H)^+$ 832.32289, found 832.32385.

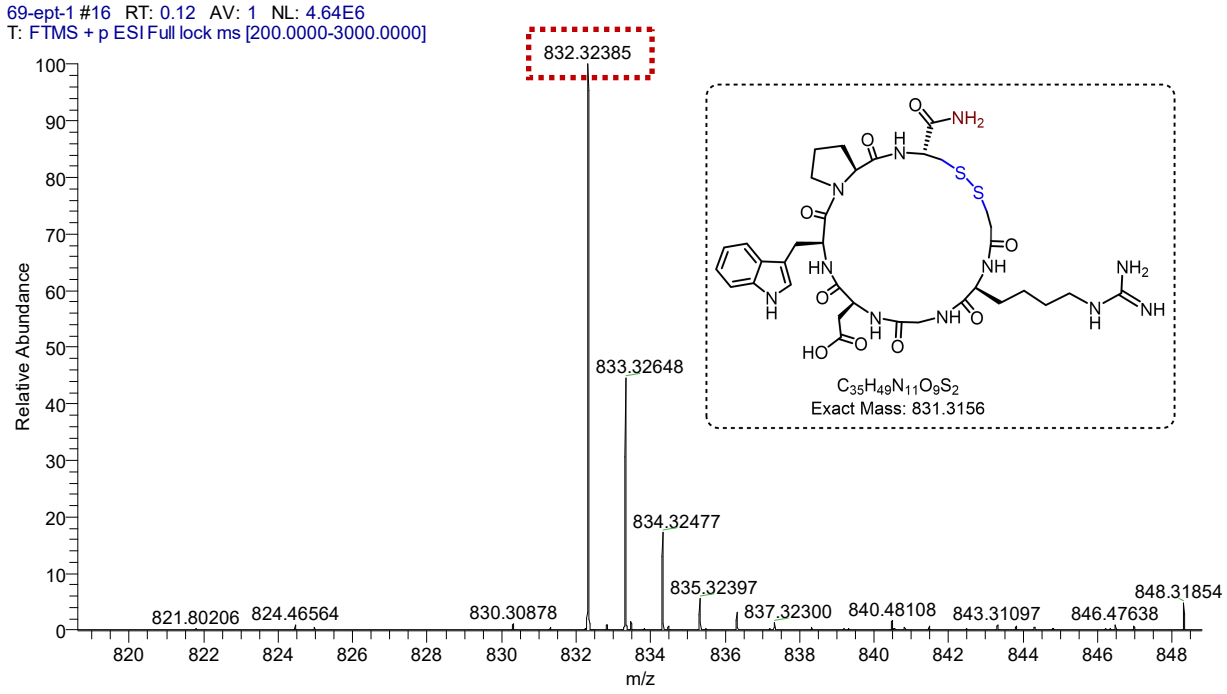
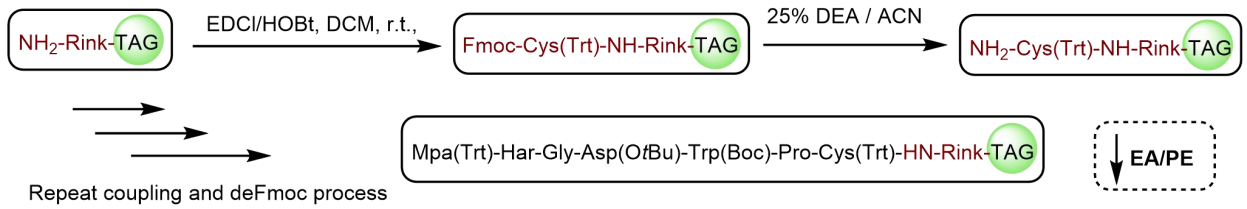


Figure S15. The HRMS (ESI) of target eptifibatide.

8. Precipitation strategy to synthesize the eptifibatide.

Tag(1d) assisted precipitation strategy to synthesize the linear eptifibatide, the key steps are as follows.



***Preparation of* Fmoc-Cys(Trt)-CONH-Rink-TAG(1d)**

The Fmoc-Cys(Trt)-OH (597 mg, 1.02 mmol), EDCl (230 mg, 1.2 mmol), HOBt (160 mg, 1.2 mmol), **Tag(1d)-Rink-NH$_2$** (915 mg, 1.0 mmol) were added to DCM (15 mL), the mixture was stirred at room temperature for 1 h. The reaction mixture was then washed with 10 mL 10% Na_2CO_3, dried with 1.0 g anhydrous $MgSO_4$. 4.0 mL ethyl acetate was added to dissolve the sample after concentration, and 15 mL of petroleum ether was added dropwise and stirred, repeat the preceding steps once to obtain the purified product **Fmoc-Cys(Trt)-CONH-Rink-TAG(1d)**.

Preparation of* H$_2$N-Cys(Trt)-CONH-Rink-TAG(1d)*: (Remove of the Fmoc group). The diethylamine (2.0 mL) was added to the solution of **Fmoc-Cys(Trt)-CONH-Rink-TAG(1d)** in acetonitrile (6.0 mL) and the mixture was stirred for 0.5 h. The mixture was concentrated to remove the diethylamine and acetonitrile. The residue was redissolved in 4.0 mL EA, and 20.0 mL of petroleum ether was added dropwise and stirred repeat the preceding steps once, precipitate appeared and filtered to afford the purifed deprotected product **H$_2$N-Cys(Trt)-CONH-Rink-TAG(1d)**.

TAG(1d) loaded linear etifibatide Mpa(Trt)-Har-Gly-Asp(tBu)-Trp(Boc)-Pro-Cys(Trt)-CONH-Rink-TAG(1d) was obtained by repeating the above coupling and deprotection procedures.

The cleavage and cyclization of linear etifibatide according to the “one-pot” strategy for cleavage and cyclization of inear etifibatide, Mpa-Har-Gly-Asp-Trp-Pro-Cys-CONH$_2$, derived linear eptifibatide 0.49 g.

9. Total input raw materials of synthesis the linear eptifibatide

Table S2. Total input materials of "One-pot" strategy to synthesize the linear eptifibatide

Material	TAG-1d	Cys[1]	Pro[2]	Trp[3]	Asp[4]	Gly[5]	Har[6]	Mpa[7]	Input (g)
Fmoc amino acid	1137 mg	600 mg	350 mg	540 mg	420 mg	310 mg	420 mg	360 mg	4.14 g
DCM	15	0	0	0	0	0	0	0	15 mL = 20 g
EA	0	0	0	0	0	0	0	0	0
PE	0	0	0	0	0	0	0	0	0
DEA	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	0	28 mL = 20 g
ACN	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	0	28 mL = 22.2 g
Msa	375 mg	375 mg	375 mg	375 mg	375 mg	375 mg	375 mg	0	2.63 g
EDCl	0	230 mg	230 mg	230 mg	230 mg	230 mg	230 mg	230 mg	1.6 g
HOBt	0	160mg	160mg	160mg	160mg	160mg	160mg	160mg	1.2 g
$MgSO_4$	1 g	1g	1g	1g	1g	1g	1g	1g	8 g
1 M HCl	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	0	70 mL = 70 g
10% Na_2CO_3	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10	80 mL = 88.5 g
TFA/DODT /Tis/H_2O	-	-	-	-	-	-	-	-	10mL = 14 g
Isopropyl ether									30 mL= 21.8 g
Total input (g)									274.07 g

"One-pot strategy" **PMI**= 274.07g / 0.5g = **548.14**

Organic solvents consumption during peptide chain extension process by "One-pot strategy" (mL):

Organic Solvents (mL) = 71 mL

DCM（15 mL）+EA（0 mL）+PE（0 mL）+DEA（28 mL）+ACN（28 mL）=71 mL

Table S3. Total input materials of "Precipitation" strategy to synthesize the linear eptifibatide

Material	TAG-1d	Cys[1]	Pro[2]	Trp[3]	Asp[4]	Gly[5]	Har[6]	Mpa[7]	Input (g)
Fmoc amino acid	1137 mg	600 mg	350 mg	540 mg	420 mg	310 mg	420 mg	360 mg	4.14 g
DCM	0	15 mL	15 mL	15 mL	15 mL	15 mL	15 mL	15 mL	105 mL = 139 g
EA	4x2= 8 mL	[4x2]x2 =16 mL	[4x2]x2 =16 mL	[4x2]x2 =16 mL	[4x2]x2 =16 mL	[4x2]x2 =16 mL	[4x2]x2 =16 mL	4x2= 8 mL	112 mL = 101 g
PE	20x2= 40 mL	20x2x2 =80 mL	[20x2]x2 =80 mL	[20x2]x2 =80 mL	[20x2]x2 =80 mL	[20x2]x2 =80 mL	[20x2]x2 =80 mL	20x2 40 mL	560 mL = 370 g
DEA	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	2 mL	0	14 mL = 10 g
ACN	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	6 mL	0	42 mL = 34 g
EDCl	0	230 mg	230 mg	230 mg	230 mg	230 mg	230 mg	230 mg	1.6 g
HOBt	0	160mg	160mg	160mg	160mg	160mg	160mg	160mg	1.2 g
$MgSO_4$	0	1g	1g	1g	1g	1g	1g	1g	7 g
10% Na_2CO_3	0	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL	70 mL = 77.5 g
TFA/DODT /Tis/H_2O	-	-	-	-	-	-	-	-	10 mL = 14 g
Isopropyl ether	-	-	-	-	-	-	-	-	30 mL = 21.8 g
Total input (g)									781.24 g

"Precipitation strategy" **PMI**= 781.24g / 0.49g = **1594.37**

Organic solvents consumption during peptide chain extension process by "Precipitation strategy" (mL):

Solvents (mL) = 743 mL

DCM（15 mL）+EA（112 mL）+PE（560 mL）+DEA（14 mL）+ACN（42 mL）= 743 mL

Remark: PMI (ratio of the total mass of all input materials to the mass of isolated product)

References

[1] D. Takahashi, T. Inomata, T. Fukui. AJIPHASEU®: A highly efficient synthetic method for one-pot peptide elongation in the solution phase by an Fmoc strategy. *Angew. Chem., Int. Ed.* 2017, **56**, 7803-7807.

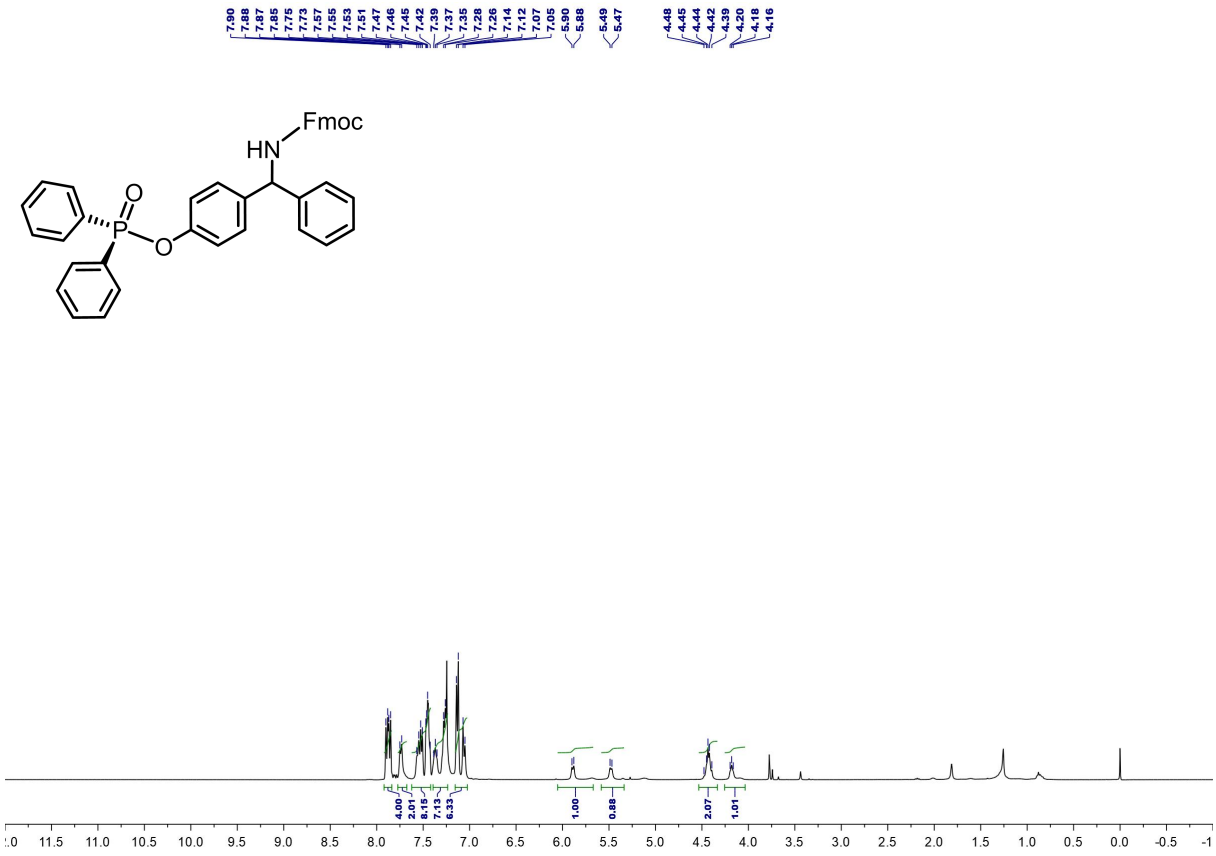
[2] J. Yeo, L. Peeva, S. Chung, P. Gaffney, D. Kim, C. Luciani, S. Tsukanov, K. Seibert, M. Kopach, F. Albericio, A. Livingston. Liquid phase peptide synthesis via one-pot nanostar sieving (PEPSTAR). *Angew. Chem. Int. Ed.* 2021, **60**, 7786-7795.

[3] J. P. Tam, C. R. Wu, W. Liu, J W. Zhang. Disulfide bond formation in peptides by dimethyl sulfoxide. Scope and applications. *J. Am. Chem.Soc.* 1991, **113**, 6657-6662.

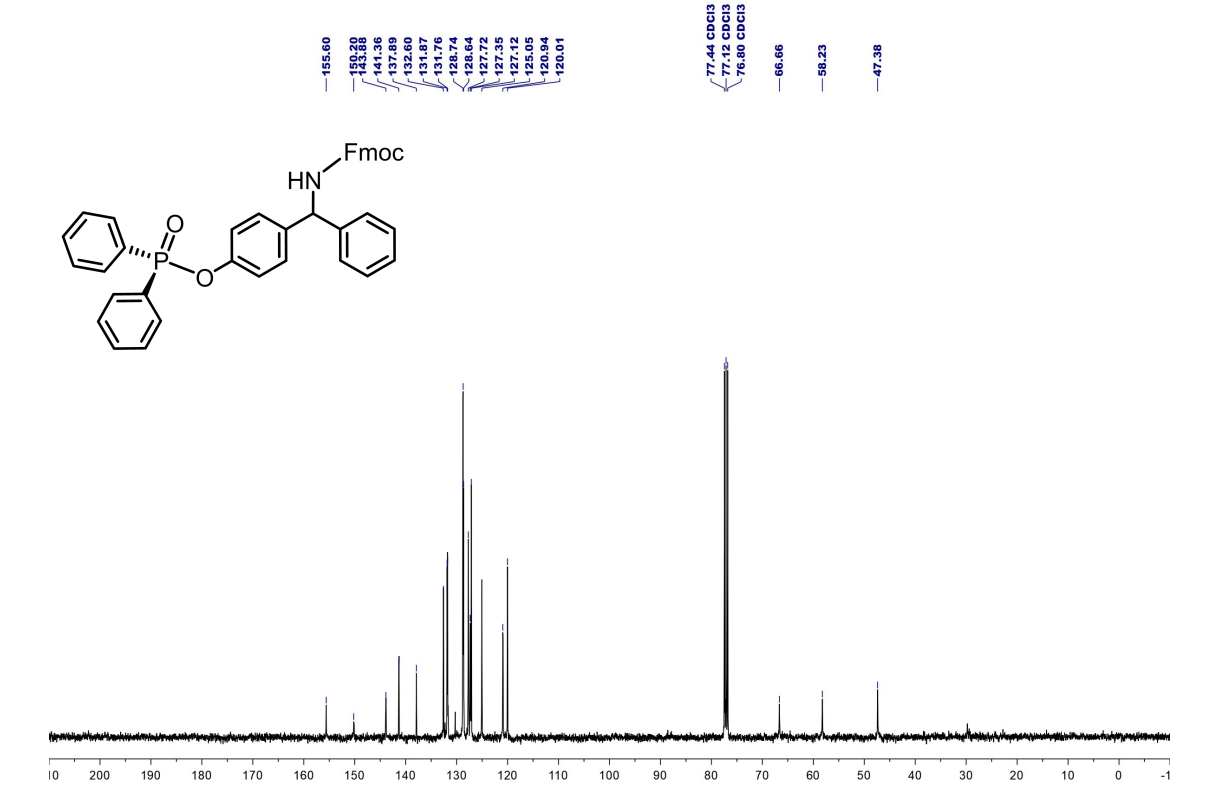
[4] M. Mochizuki, S. Tsuda, K. Tanimura, Y. Nishiuchi, Regioselective Formation of Multiple Disulfide Bonds with the Aid of Postsynthetic S‐Tritylation. *Org. Lett*. 2015, **17**, 2202-2205.

[5] T. M. Postma, M. Giraud, F. Albericio. Trimethoxyphenylthio as a highly labile replacement for tert butylthio cysteine protection in Fmoc solid phase synthesis. *Org. Lett*. 2012, **14**, 5468-5471.

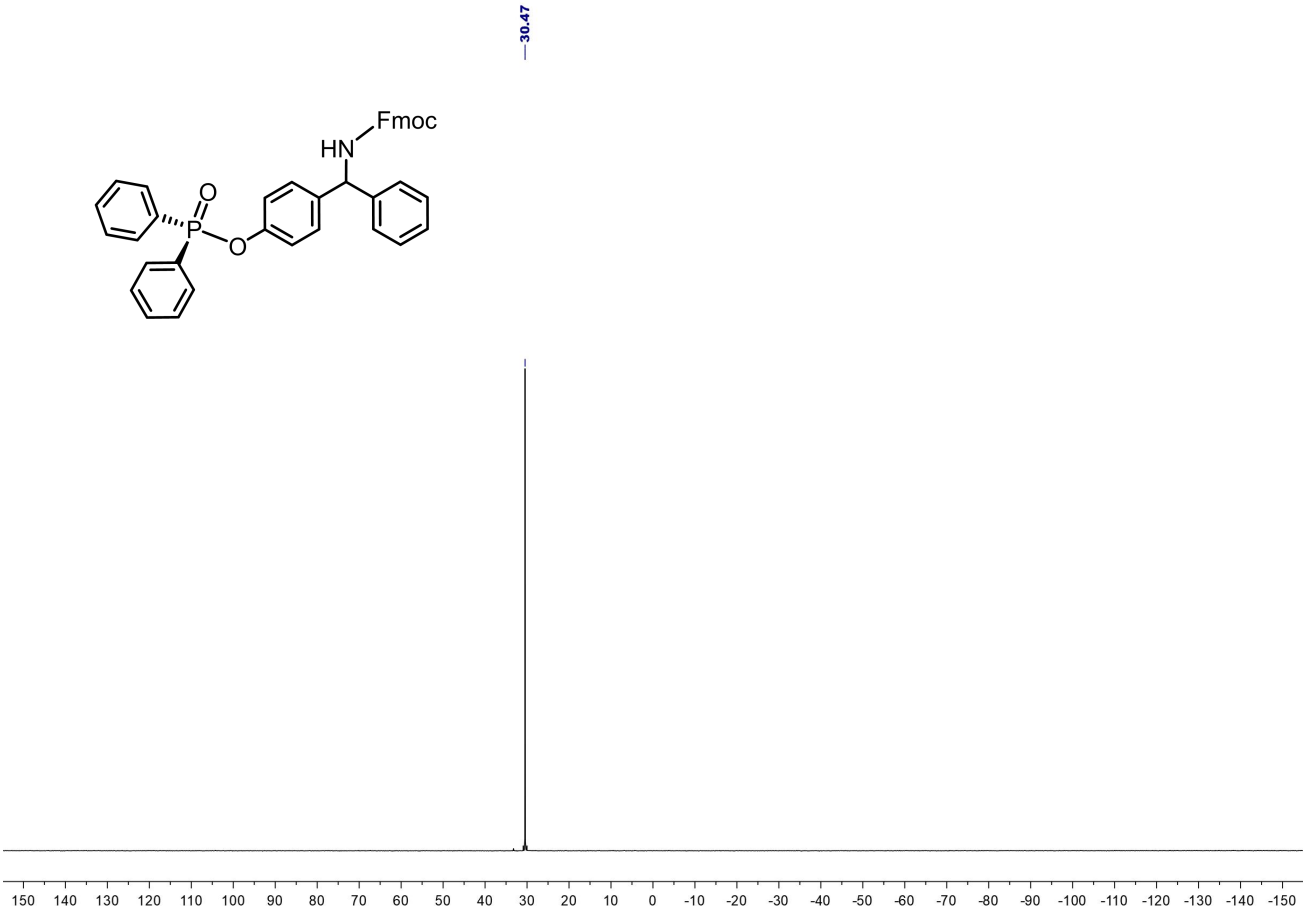
NMR Spectra and HRMS (ESI) Spectra of TAGs



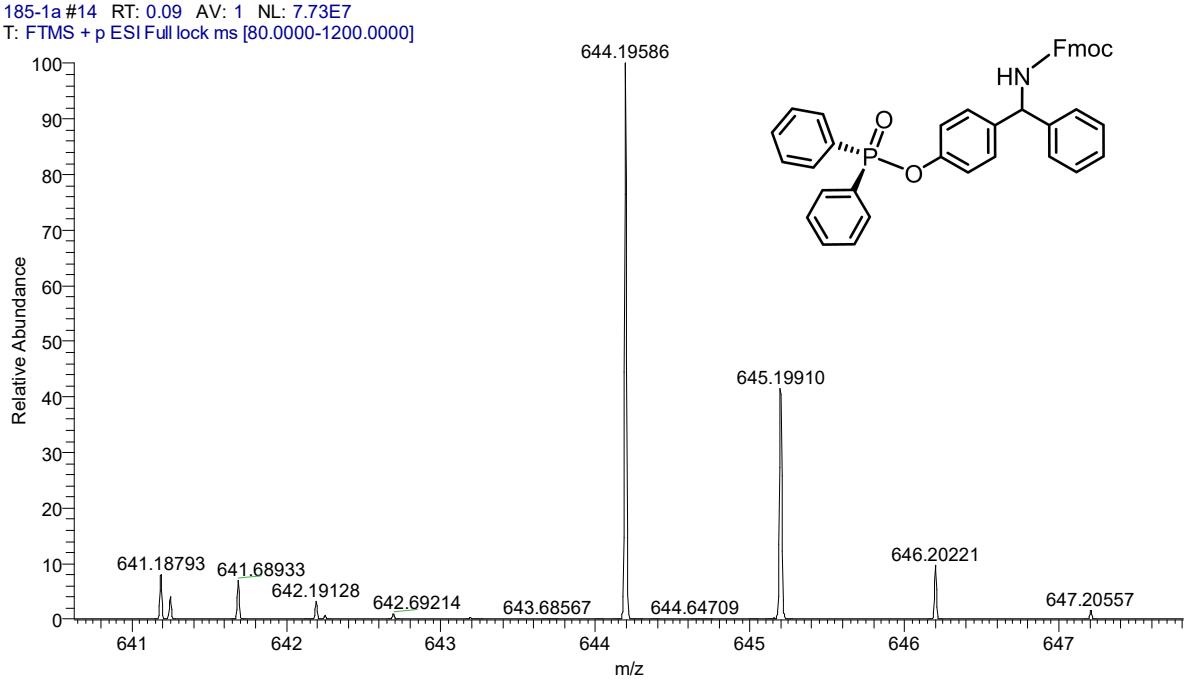
1a: ^{1}H NMR (400 MHz, $CDCl_3$)



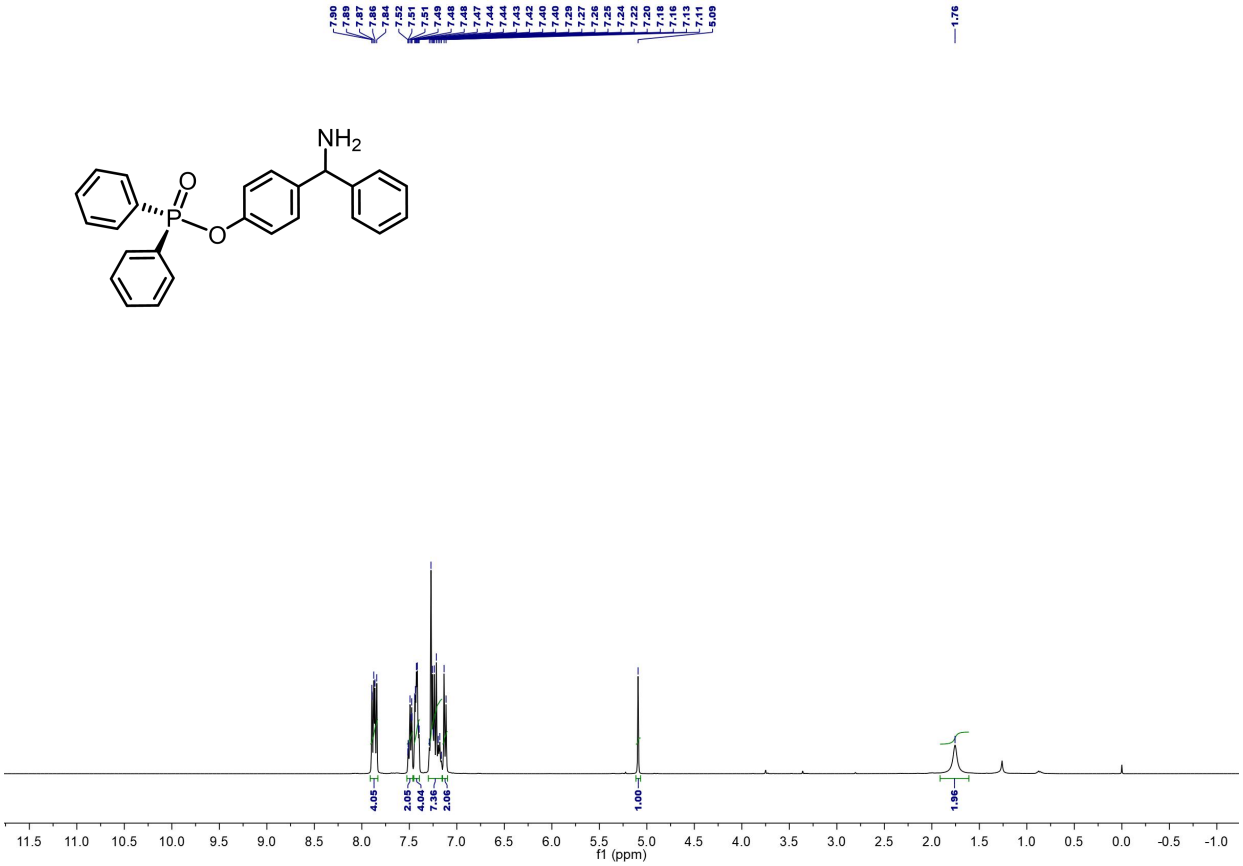
1a: ^{13}C NMR (100 MHz, $CDCl_3$)



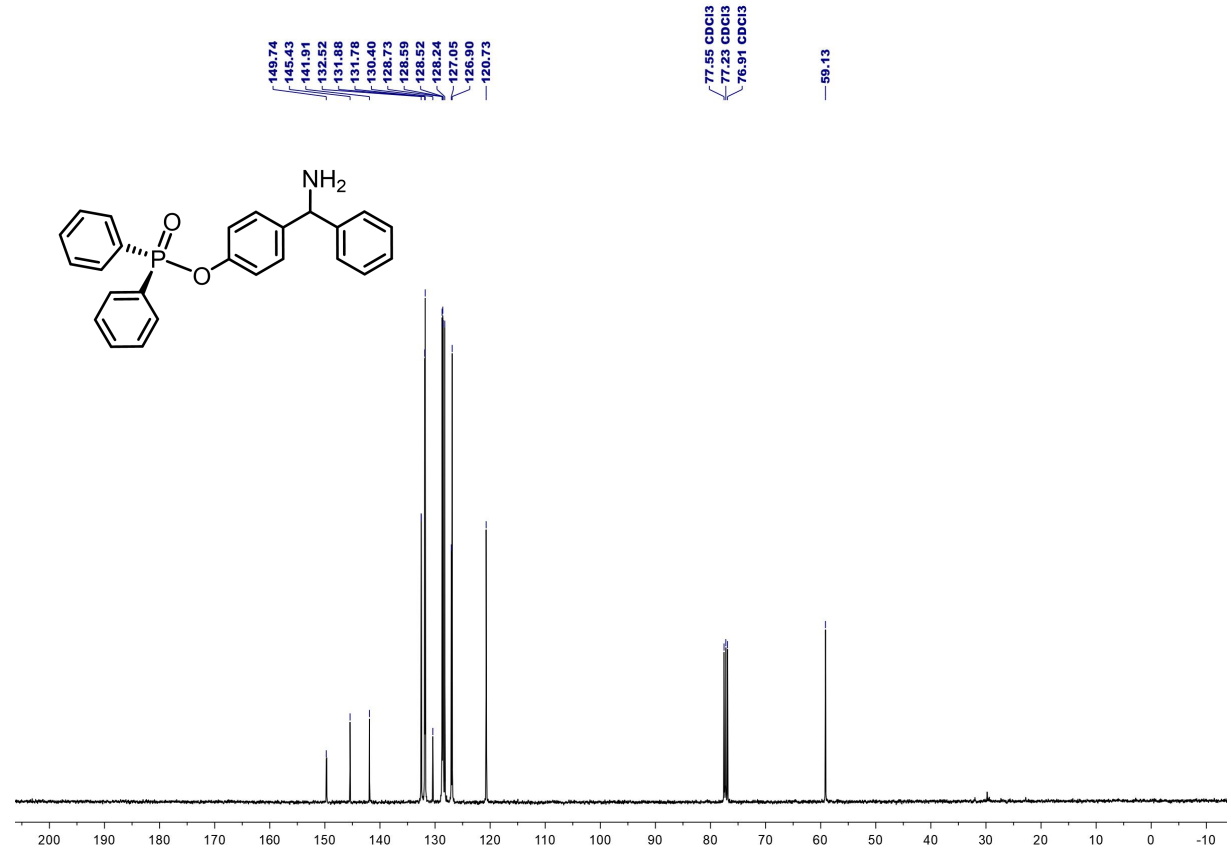
1a: ^{31}P NMR (162 MHz, $CDCl_3$)



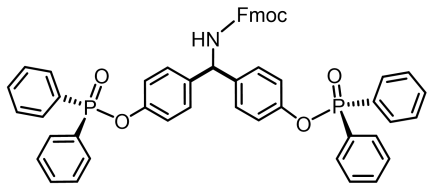
1a: HRMS (ESI) m/z calcd for $C_{40}H_{32}NO_4PNa^+$ $(M+Na)^+$ 644.19612, found 644.19586.

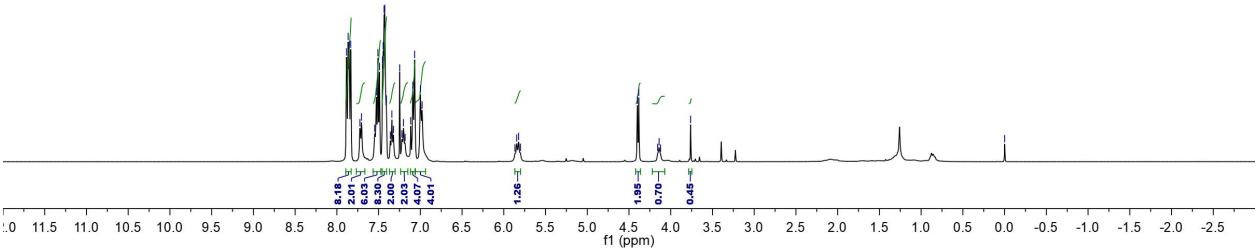


1a-deFmoc: ^{1}H NMR (400 MHz, $CDCl_3$)

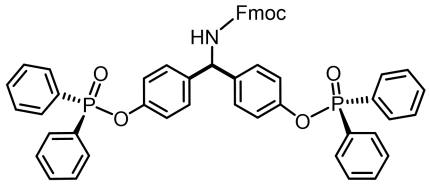


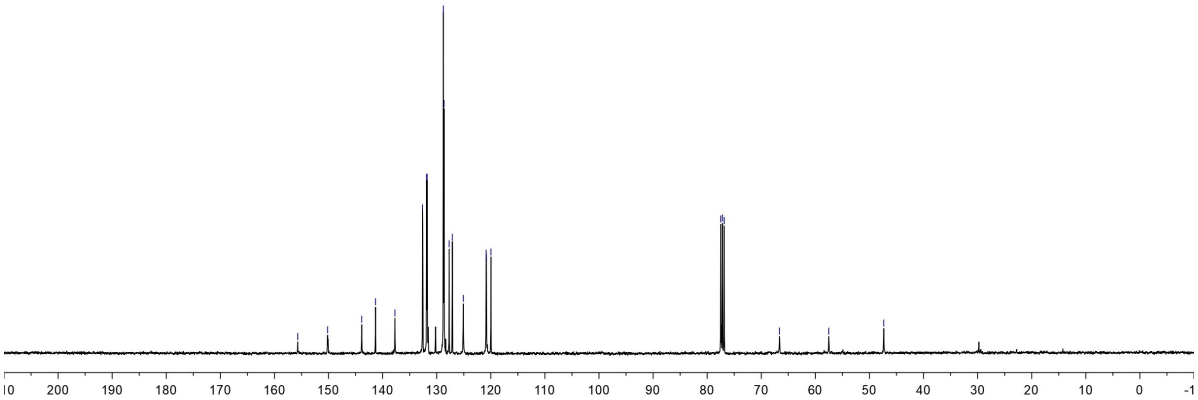
1a-deFmoc: ^{13}C NMR (100 MHz, $CDCl_3$)



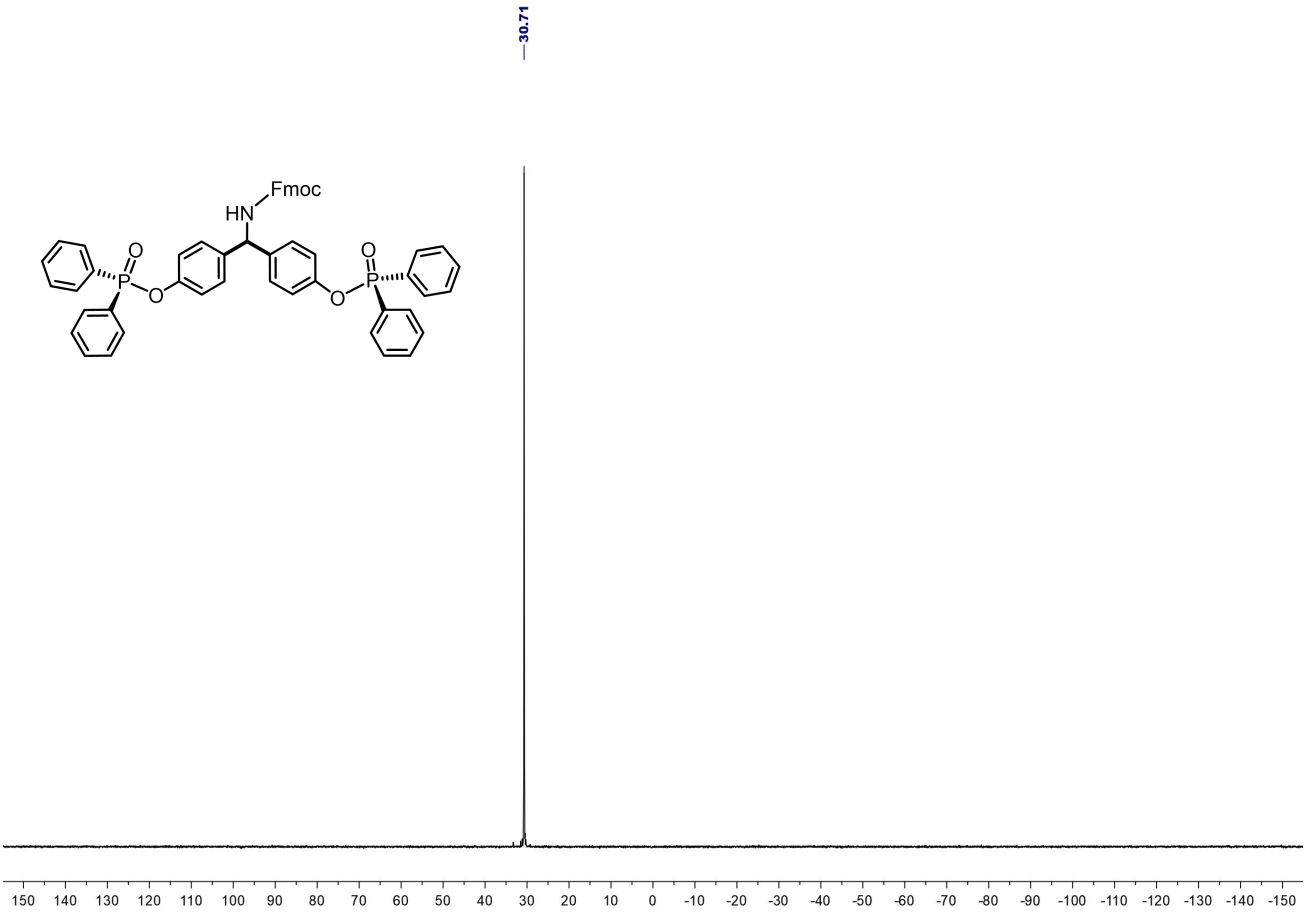


1b: ^{1}H NMR (400 MHz, $CDCl_3$)

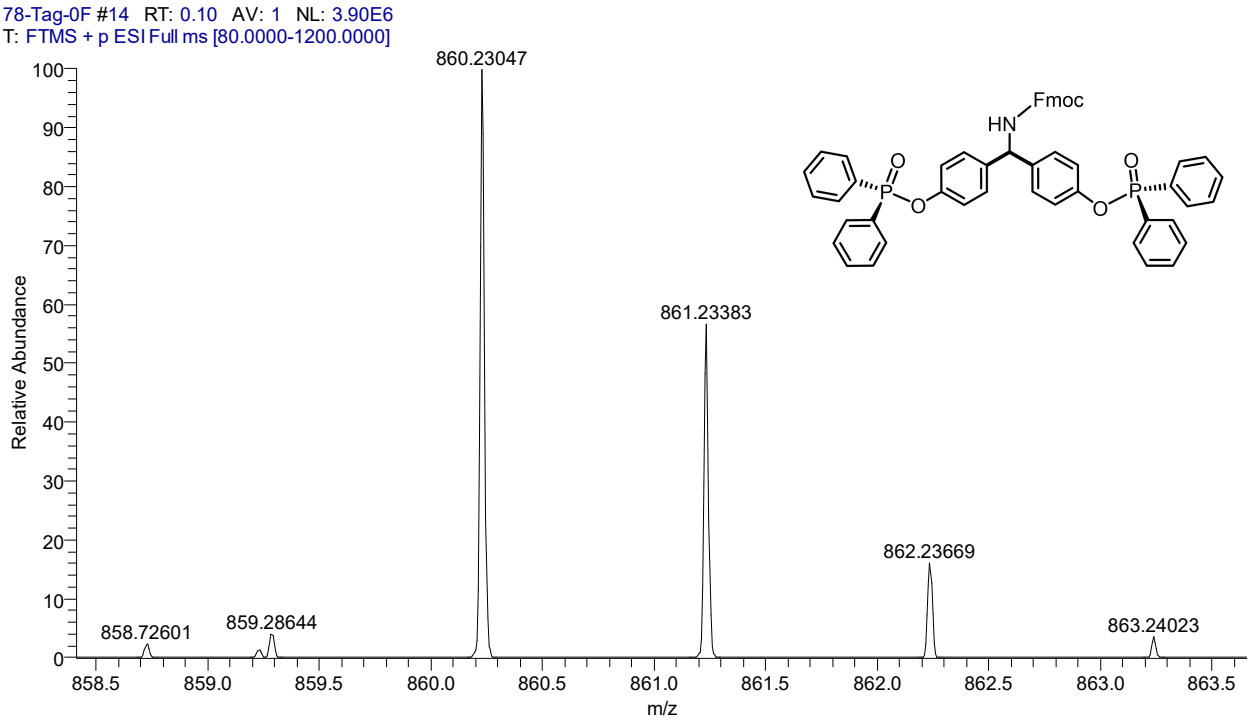




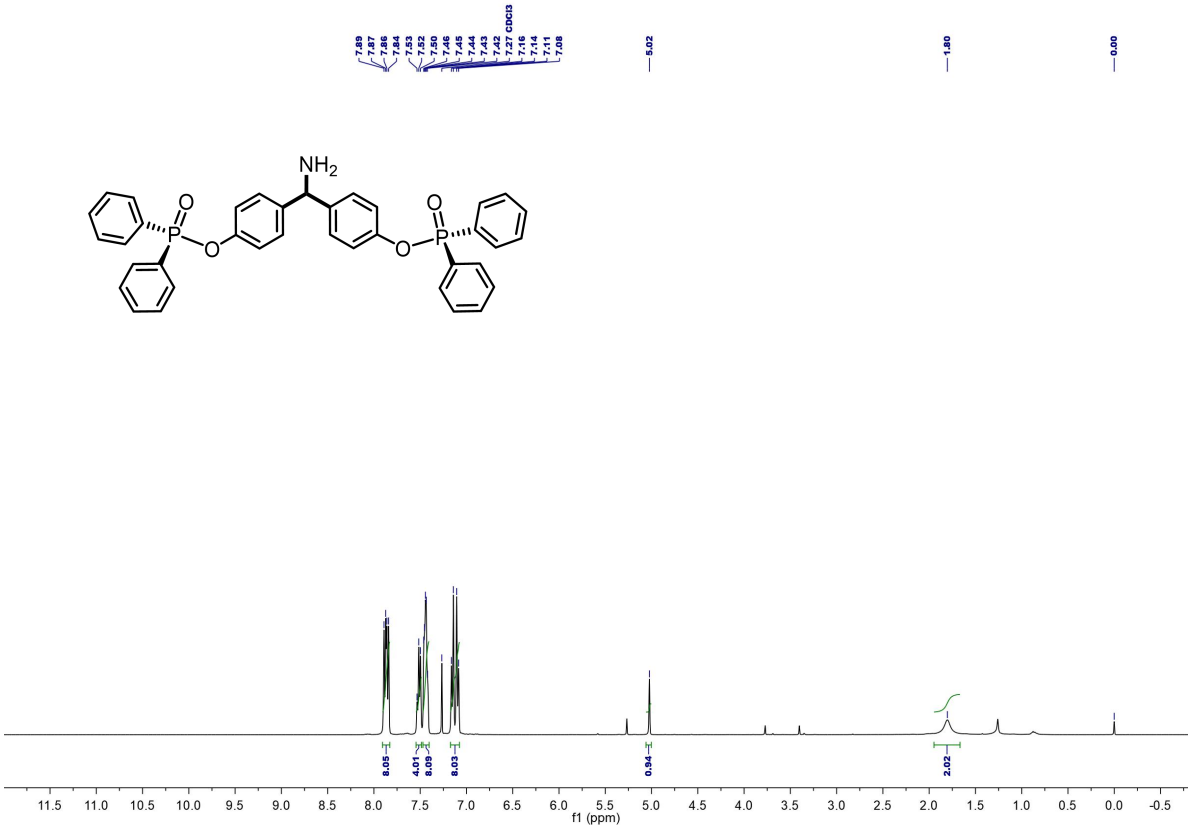
1b: ^{13}C NMR (100 MHz, $CDCl_3$)



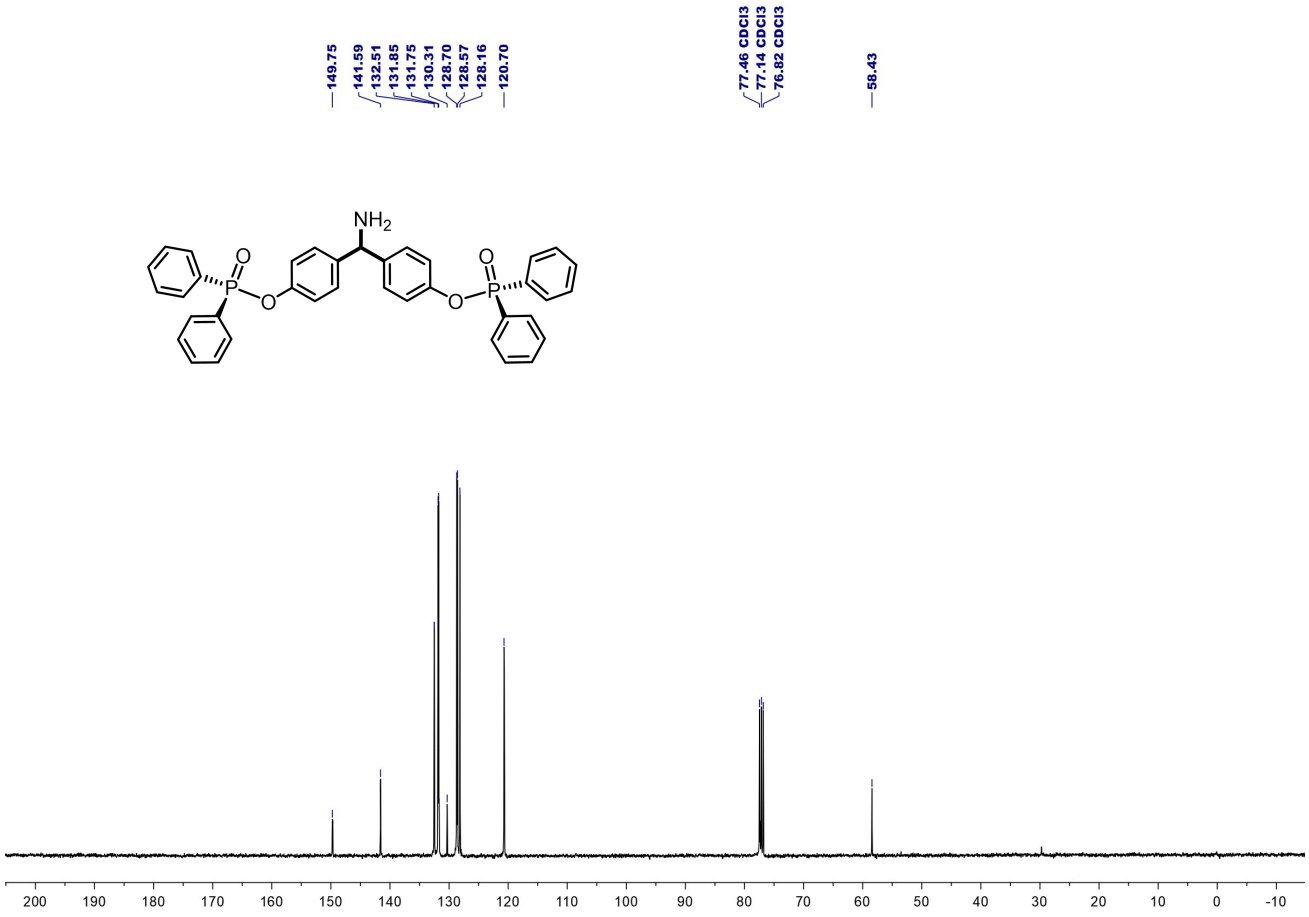
1b: ^{31}P NMR (162 MHz, $CDCl_3$)



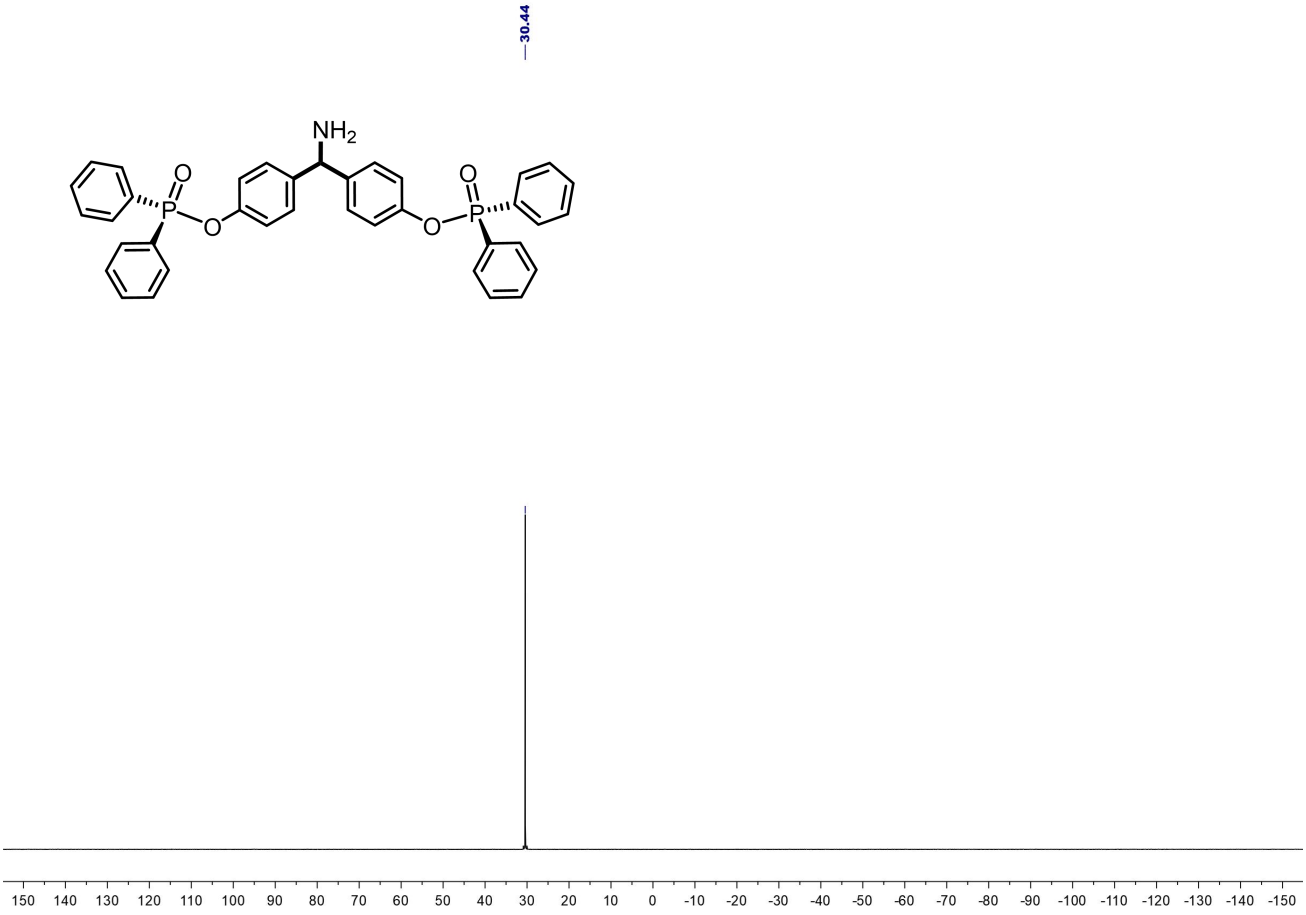
1b: HRMS (ESI) m/z calcd for $C_{52}H_{41}NO_6P_2Na^+$ $(M+Na)^+$ 860.23013, found 860.23047.



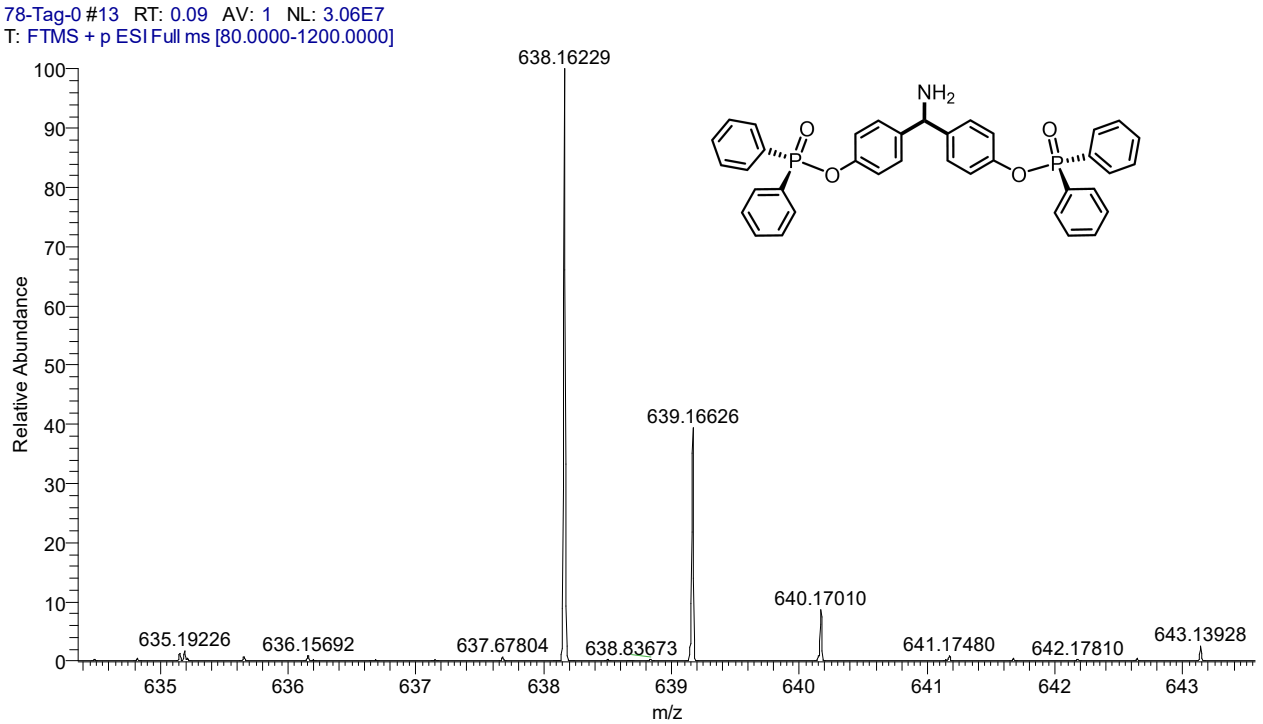
1b-deFmoc: ^{1}HNMR (400 MHz, $CDCl_3$)



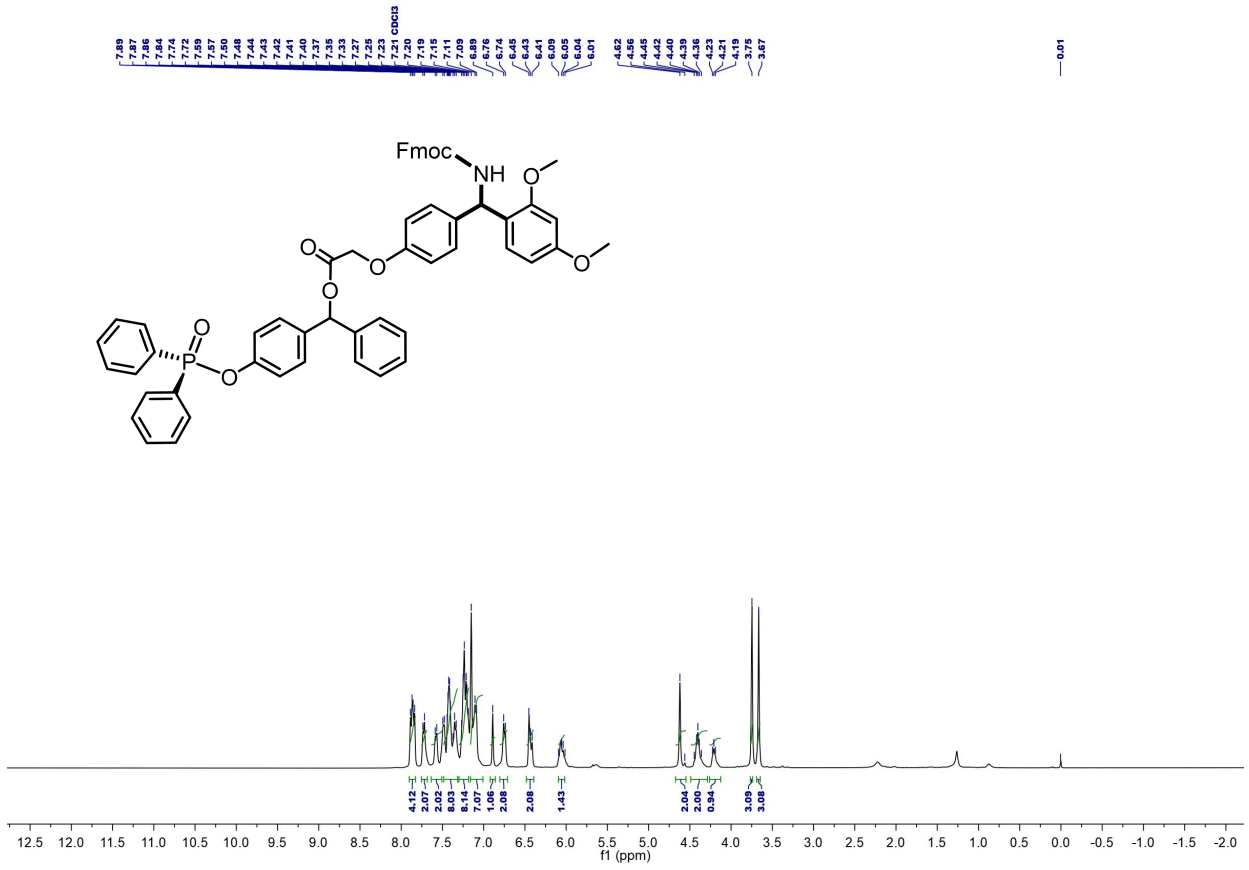
1b-deFmoc: ^{13}C NMR (100 MHz, $CDCl_3$)



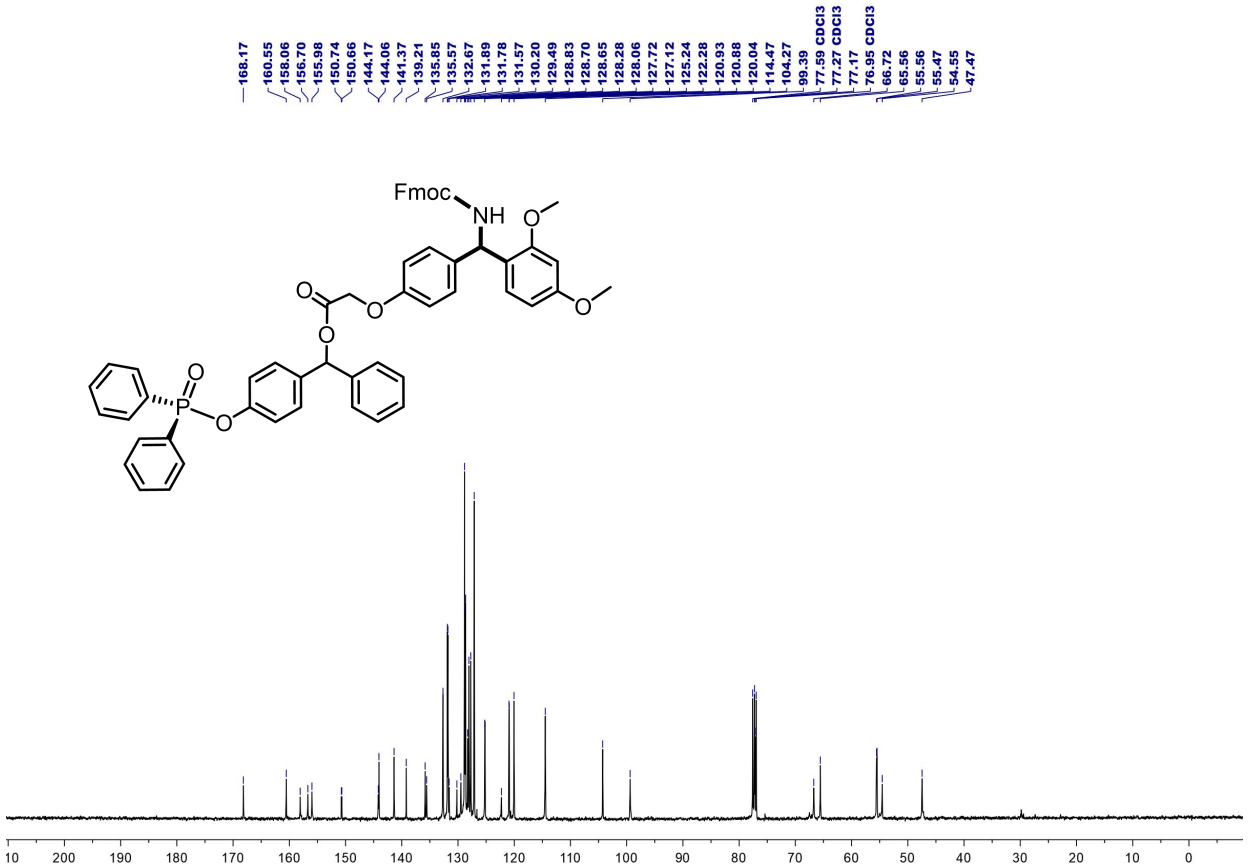
1b-deFmoc: ^{31}P NMR (162 MHz, $CDCl_3$)



1b-deFmoc: HRMS (ESI) m/z calcd for $C_{37}H_{31}NO_4P_2Na^+$ $(M+Na)^+$ 638.16205, found 638.16229.

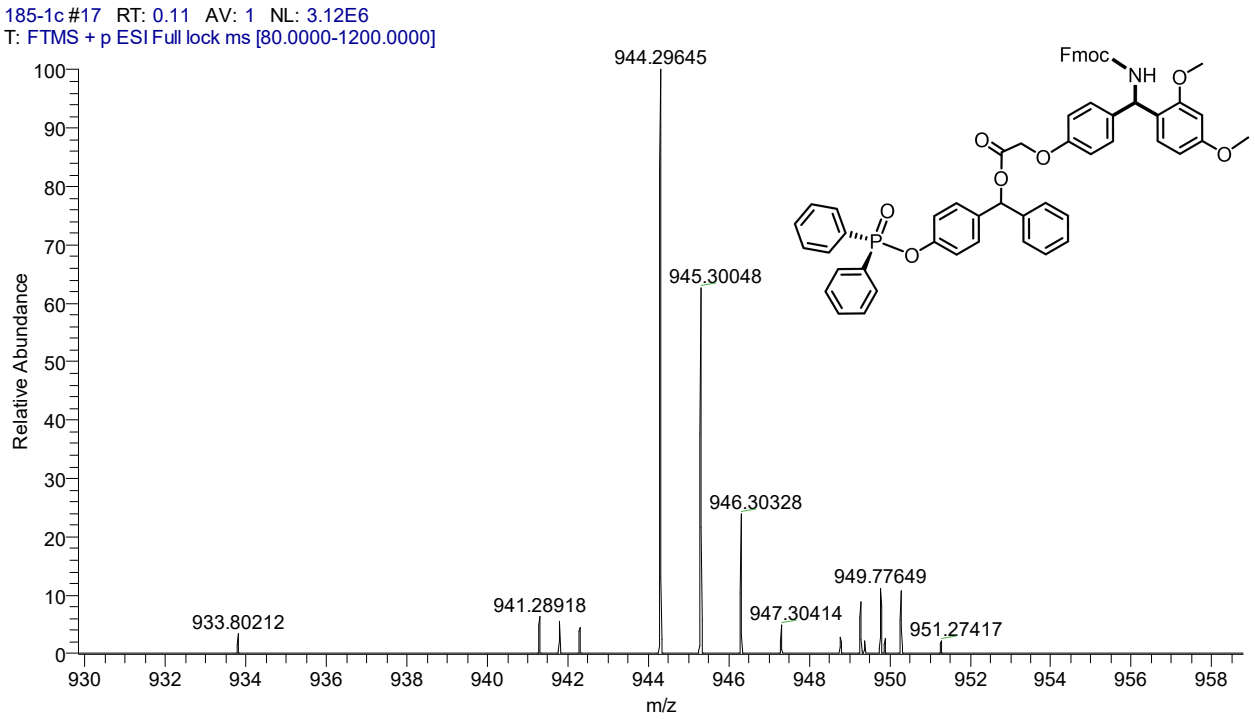


1c: ^{1}H NMR (400 MHz, $CDCl_3$)

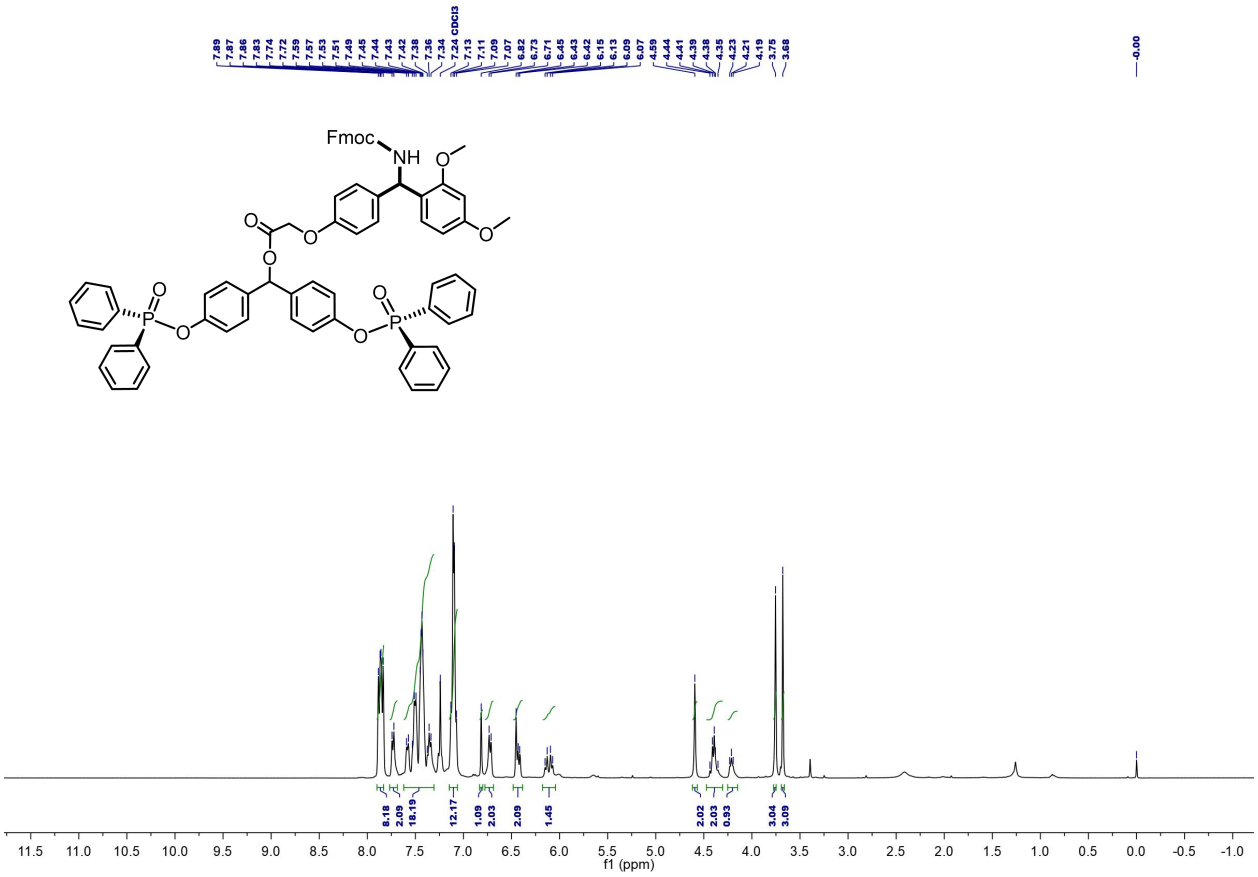


1c: ^{1}H NMR (400 MHz, $CDCl_3$)

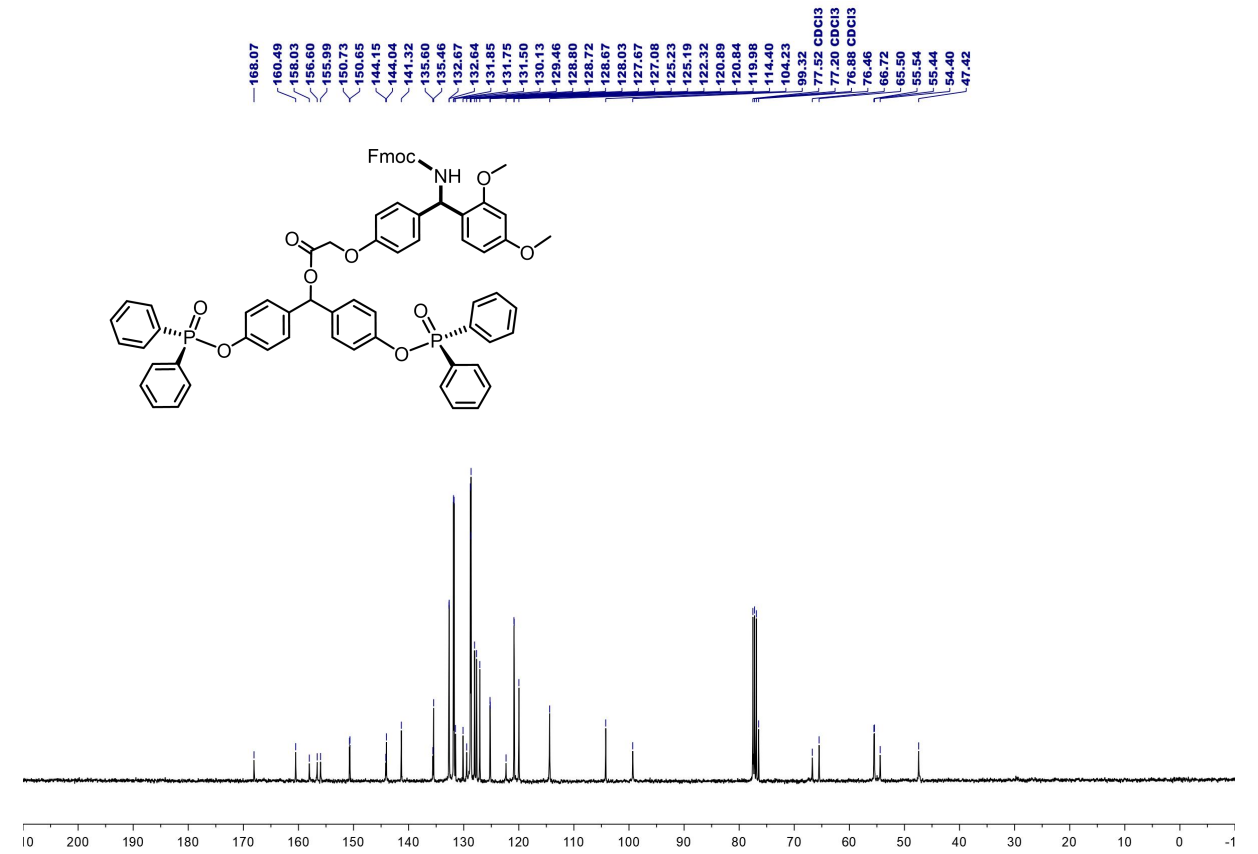
1c: ^{31}P NMR (162 MHz, $CDCl_3$)



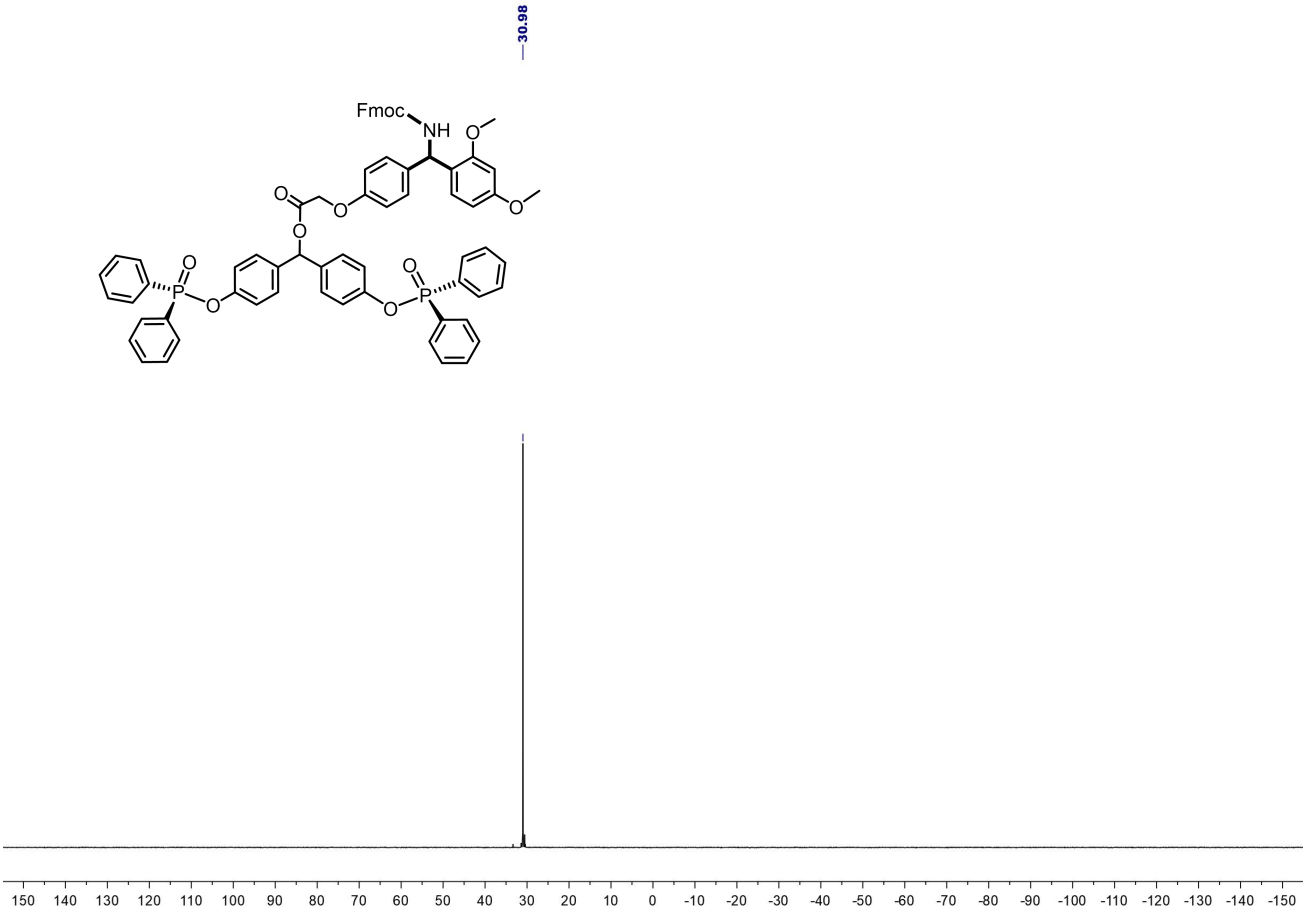
1c: HRMS (ESI) m/z calcd for $C_{57}H_{48}NO_9PNa^+$ $(M+Na)^+$ 944.29589, found 944.29645.



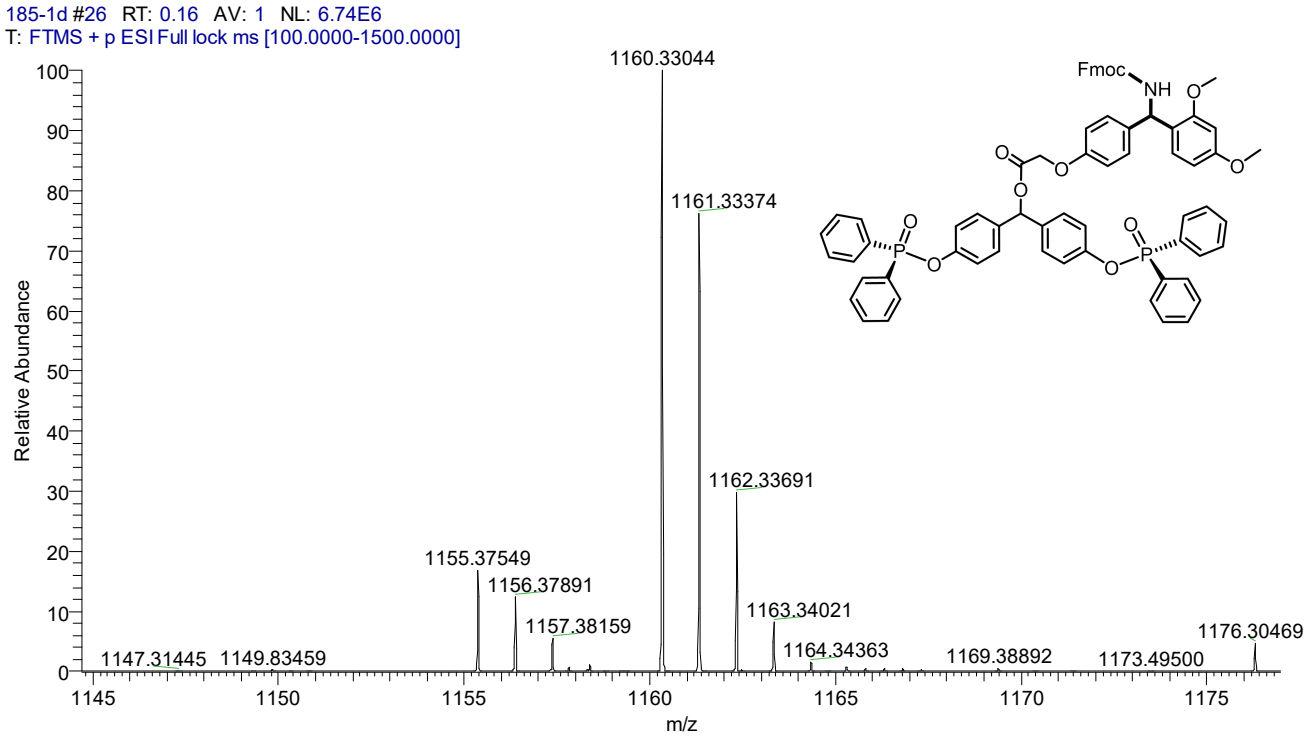
1d: ^{1}H NMR (400 MHz, $CDCl_3$)



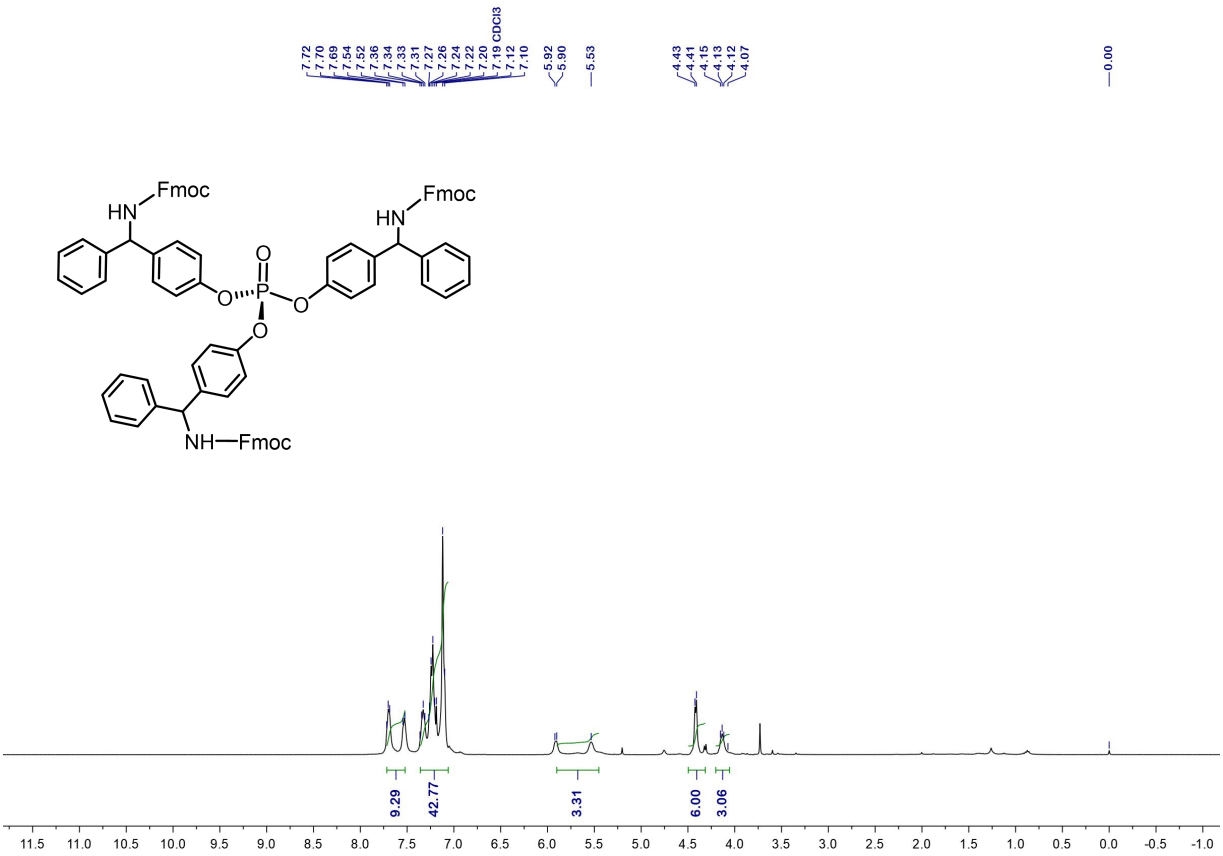
1d: ^{13}C NMR (100 MHz, $CDCl_3$)



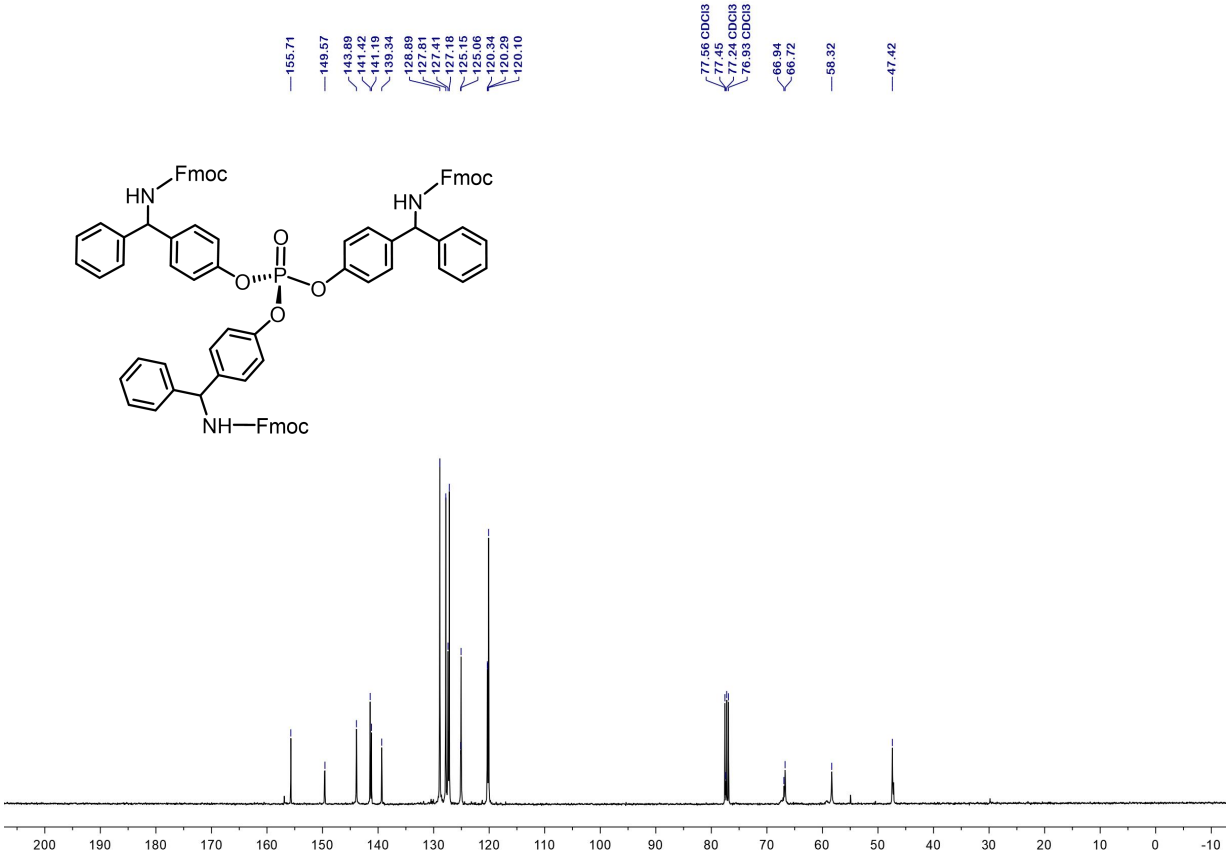
1d: ^{31}P NMR (162 MHz, $CDCl_3$)



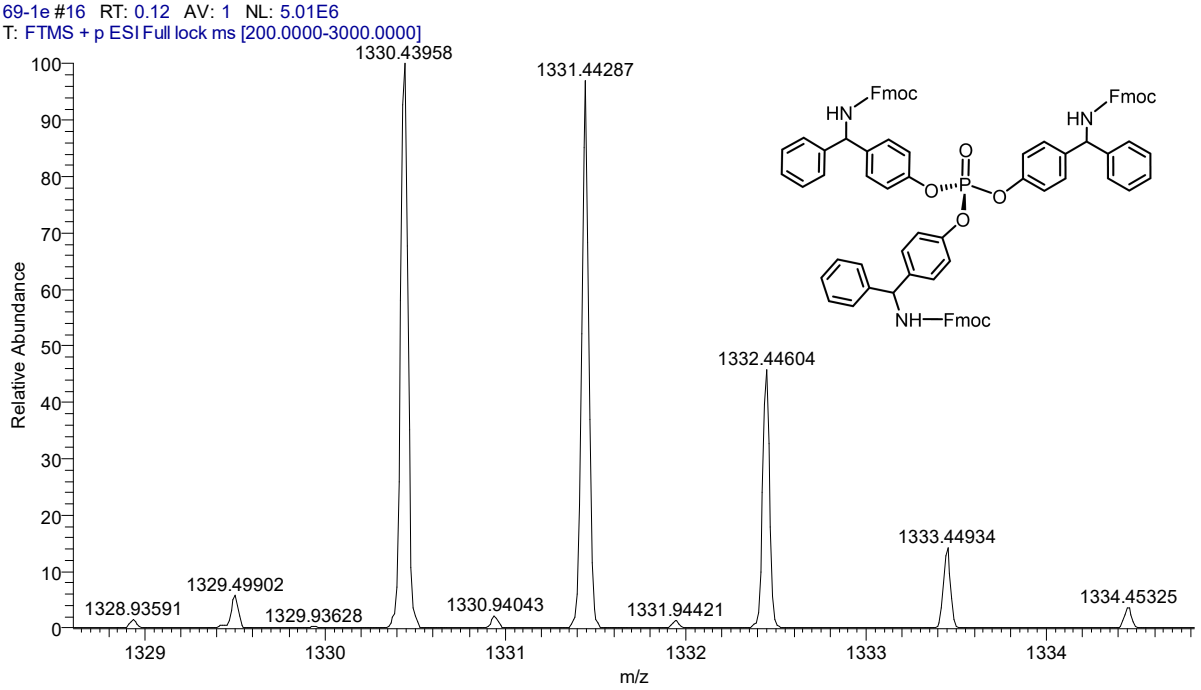
1d: HRMS (ESI) m/z calcd for $C_{69}H_{57}NO_{11}P_2Na^+$ $(M+Na)^+$ 1160.32991, found 1160.33044.



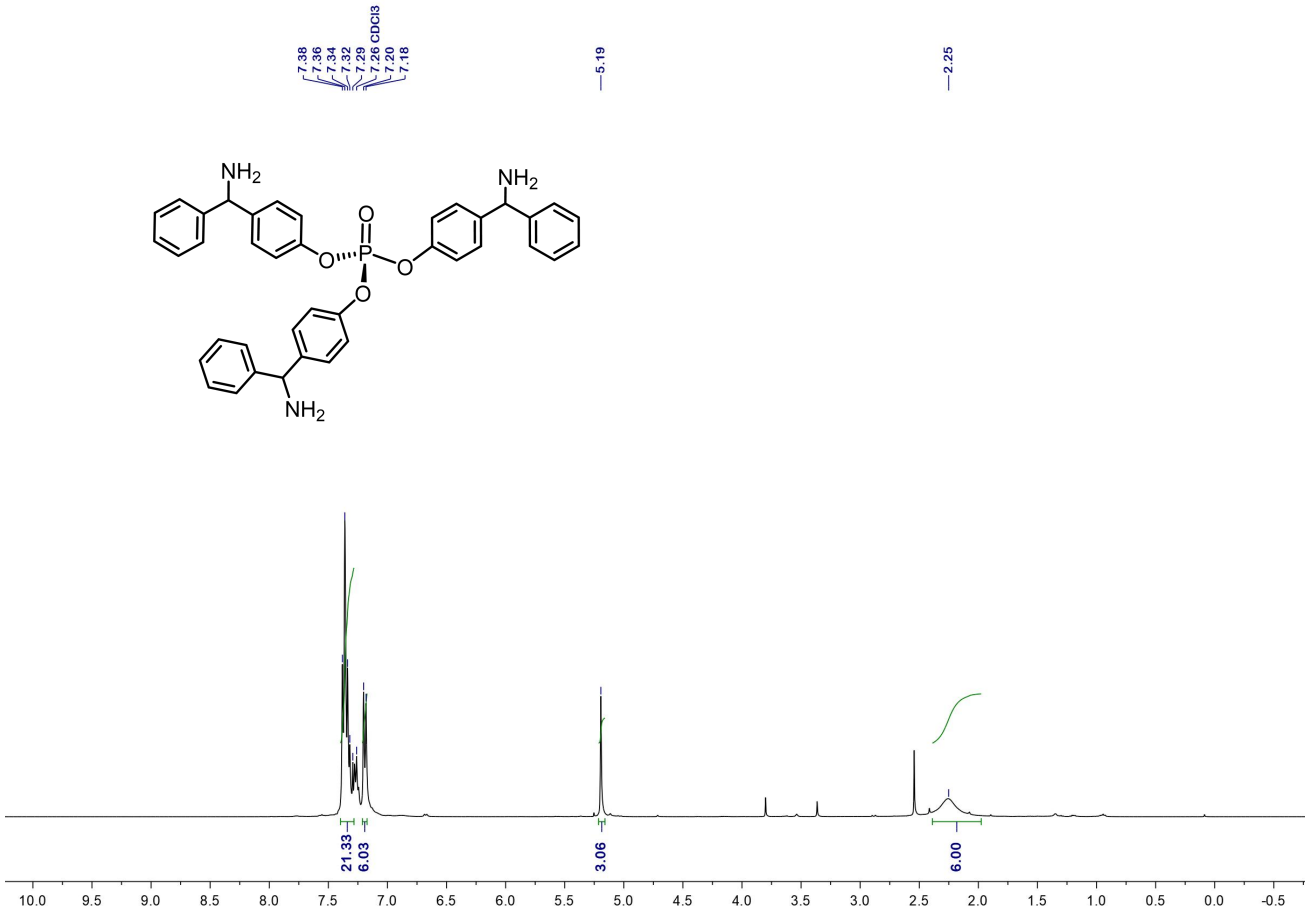
1e: ^{1}H NMR (400 MHz, $CDCl_3$)



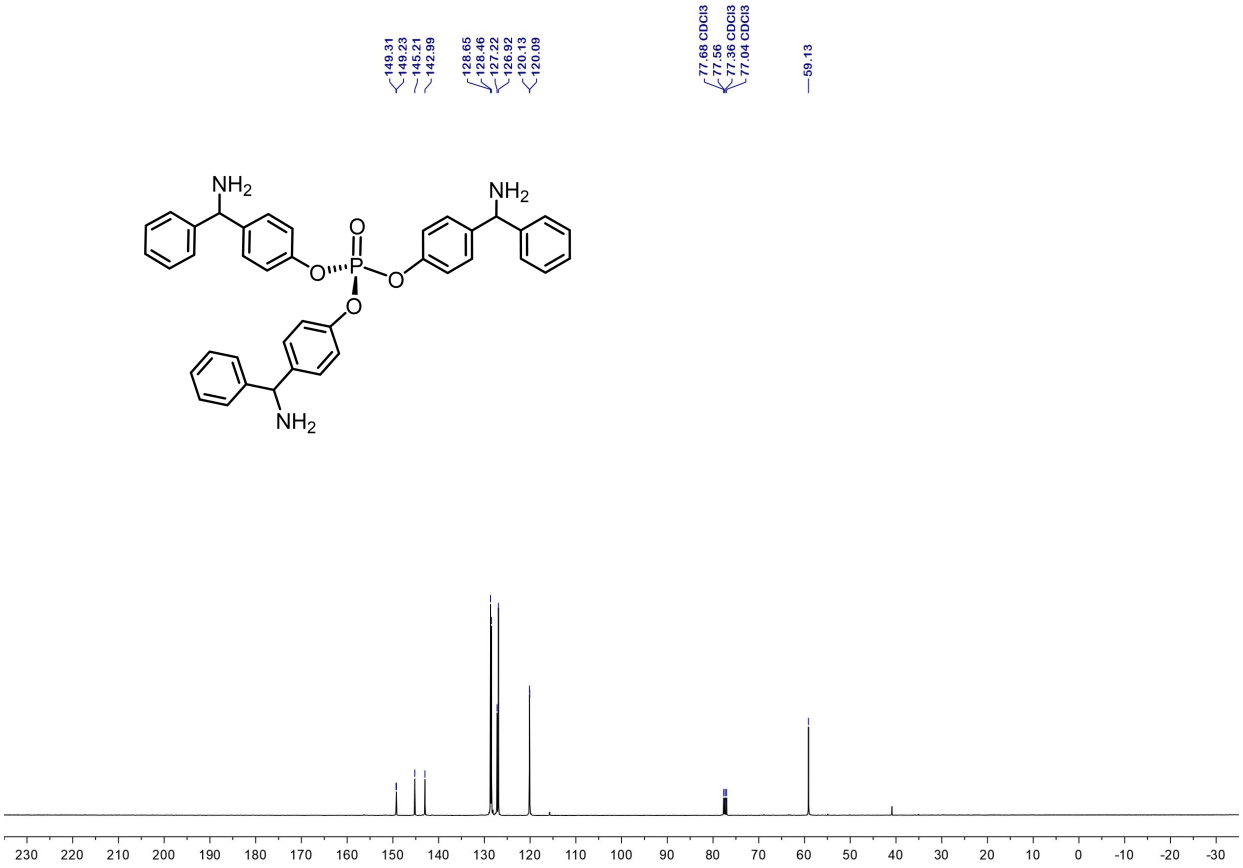
1e: ^{13}C NMR (100 MHz, $CDCl_3$)



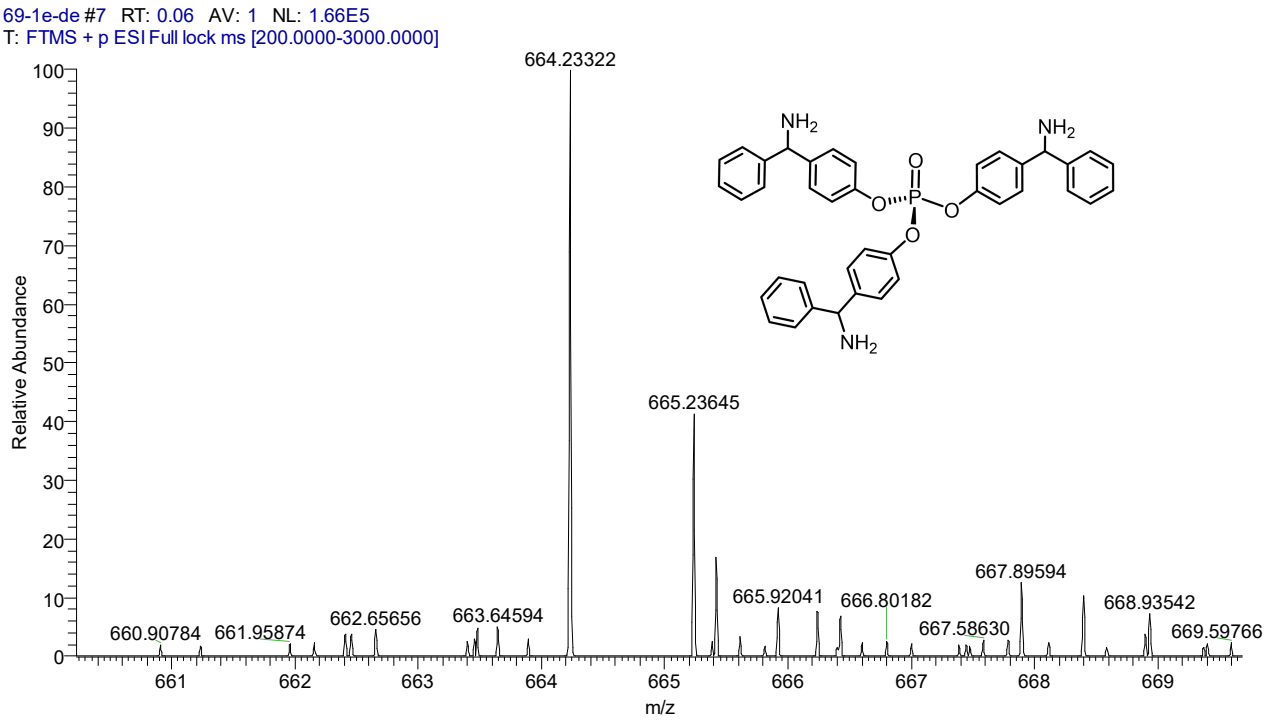
1e: HRMS (ESI) m/z calcd for $C_{84}H_{66}N_3O_{10}PNa^+$ $(M+Na)^+$ 1330.43780, found 1330.43958.



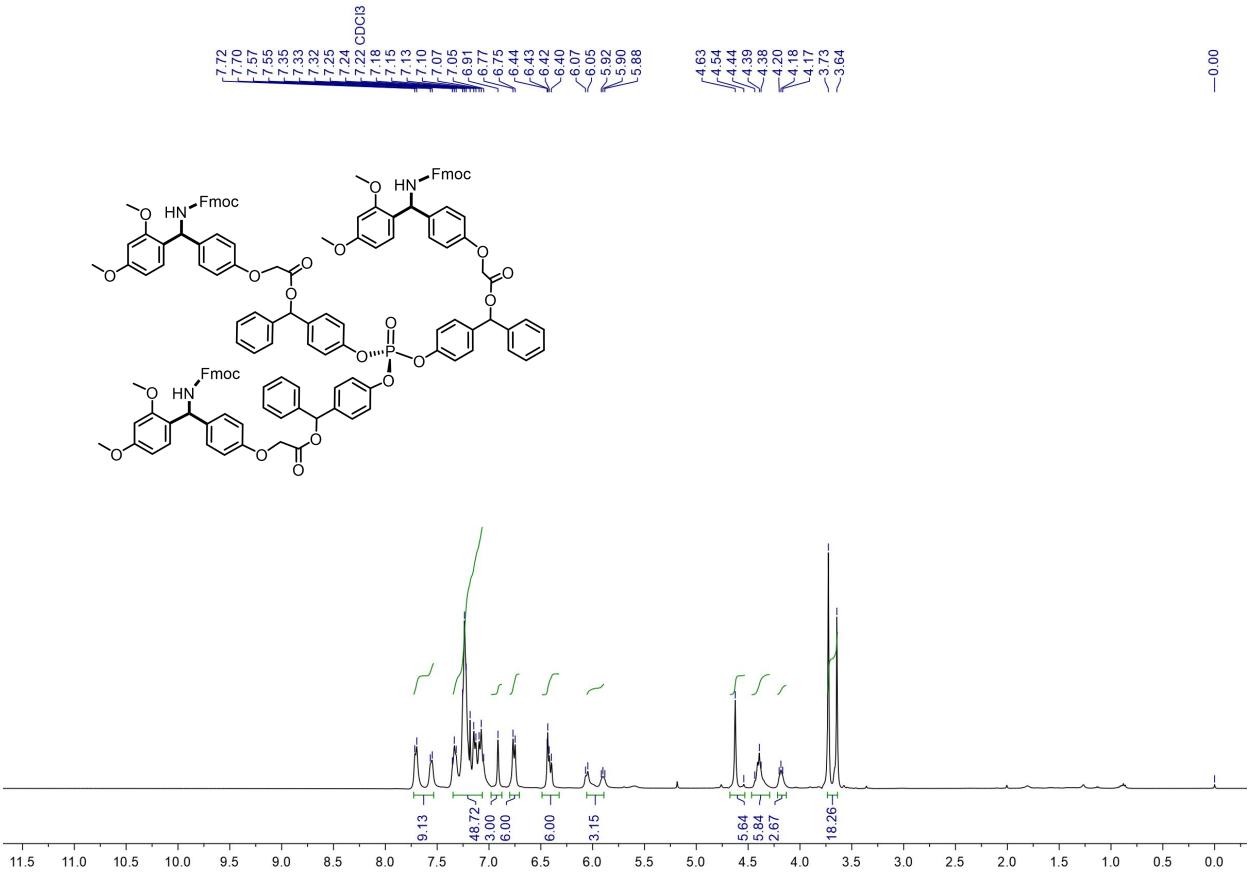
1e-de Fmoc ^{1}H NMR (400 MHz, $CDCl_3$)



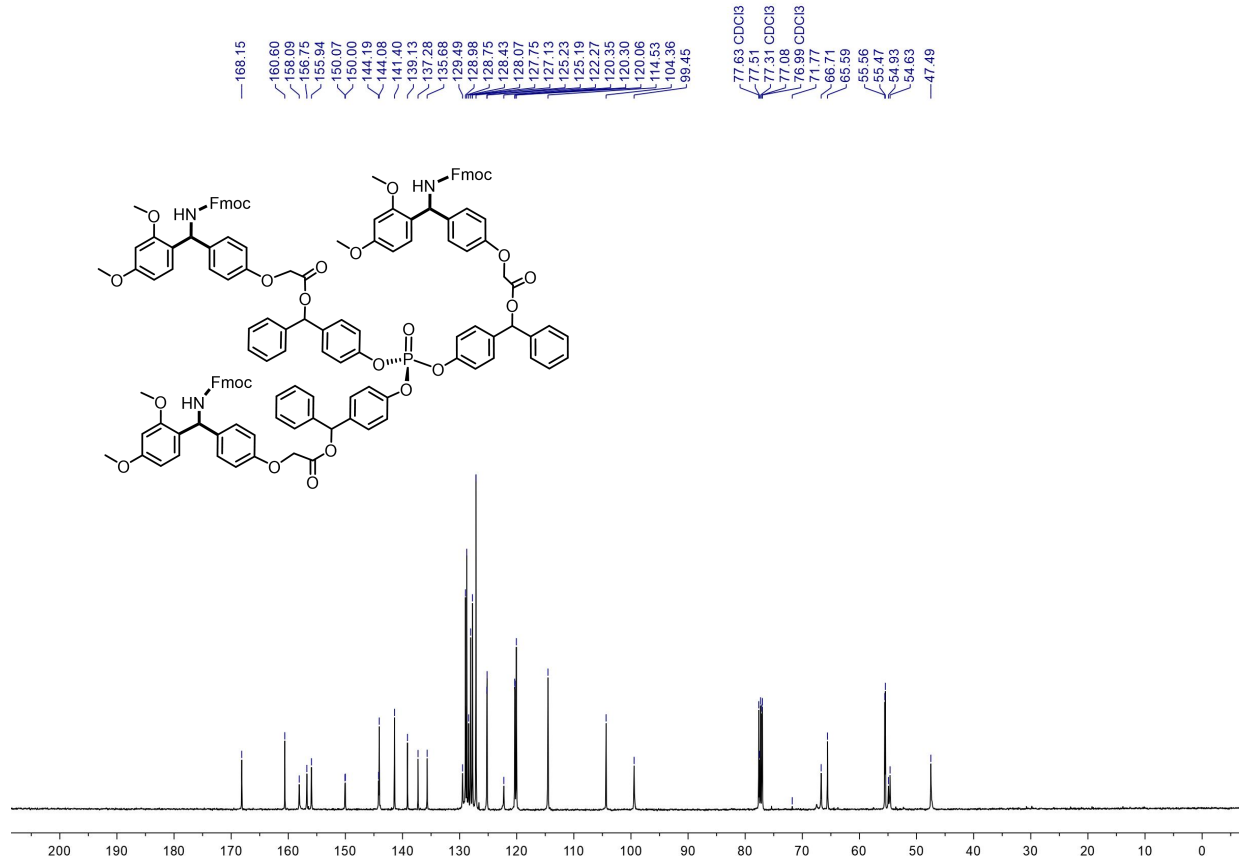
1e-de Fmoc ^{13}C NMR (100 MHz, $CDCl_3$)



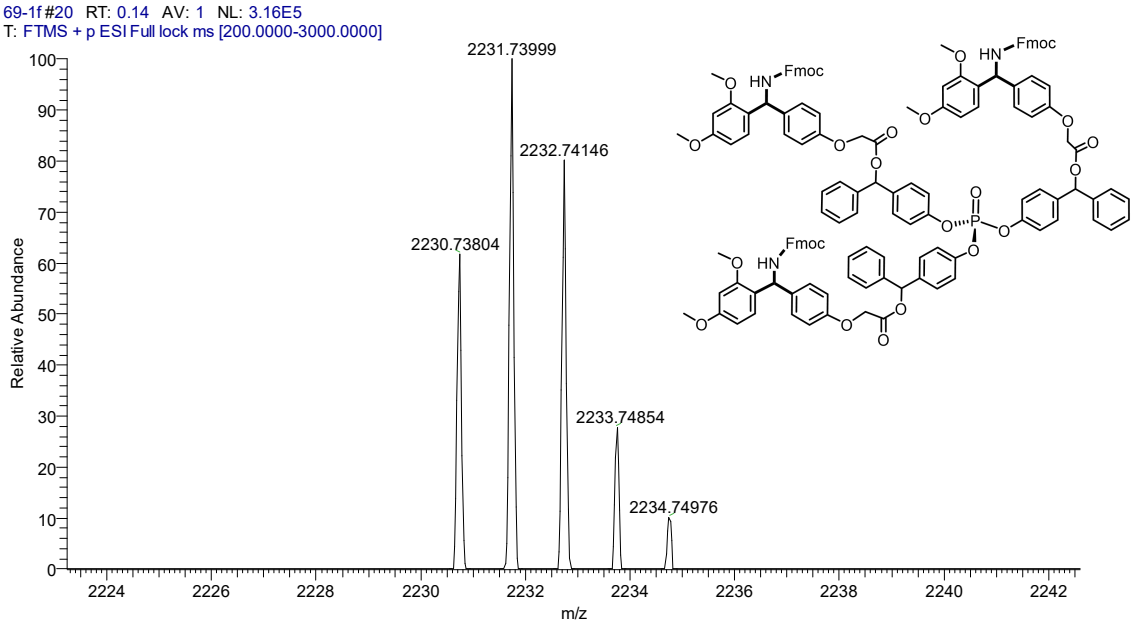
1e-de: HRMS (ESI) m/z calcd for $C_{39}H_{36}N_3O_4PNa^+$ $(M+Na)^+$ 664.23356, found 664.23322.



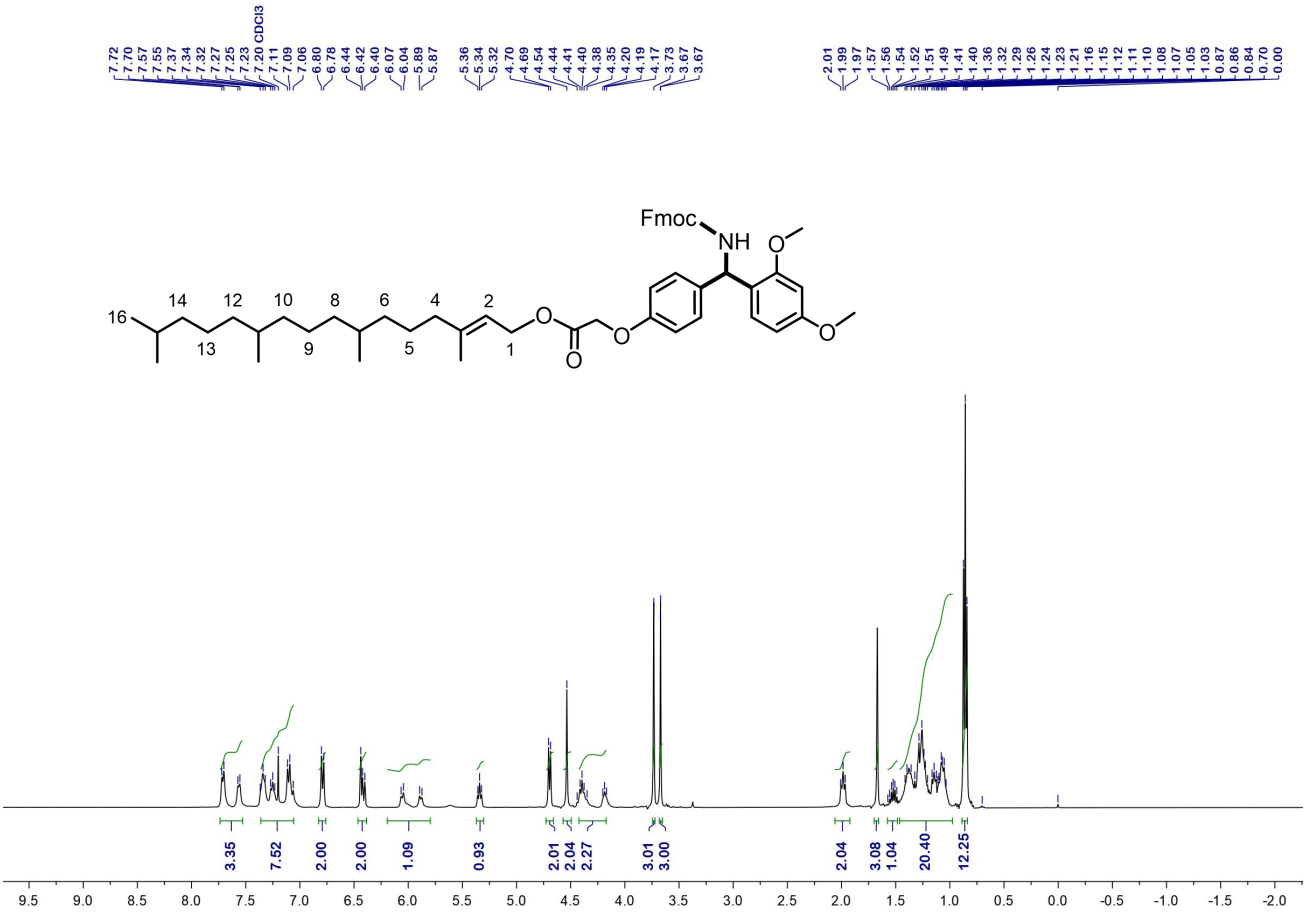
1f: ^{1}H NMR (400 MHz, $CDCl_3$)



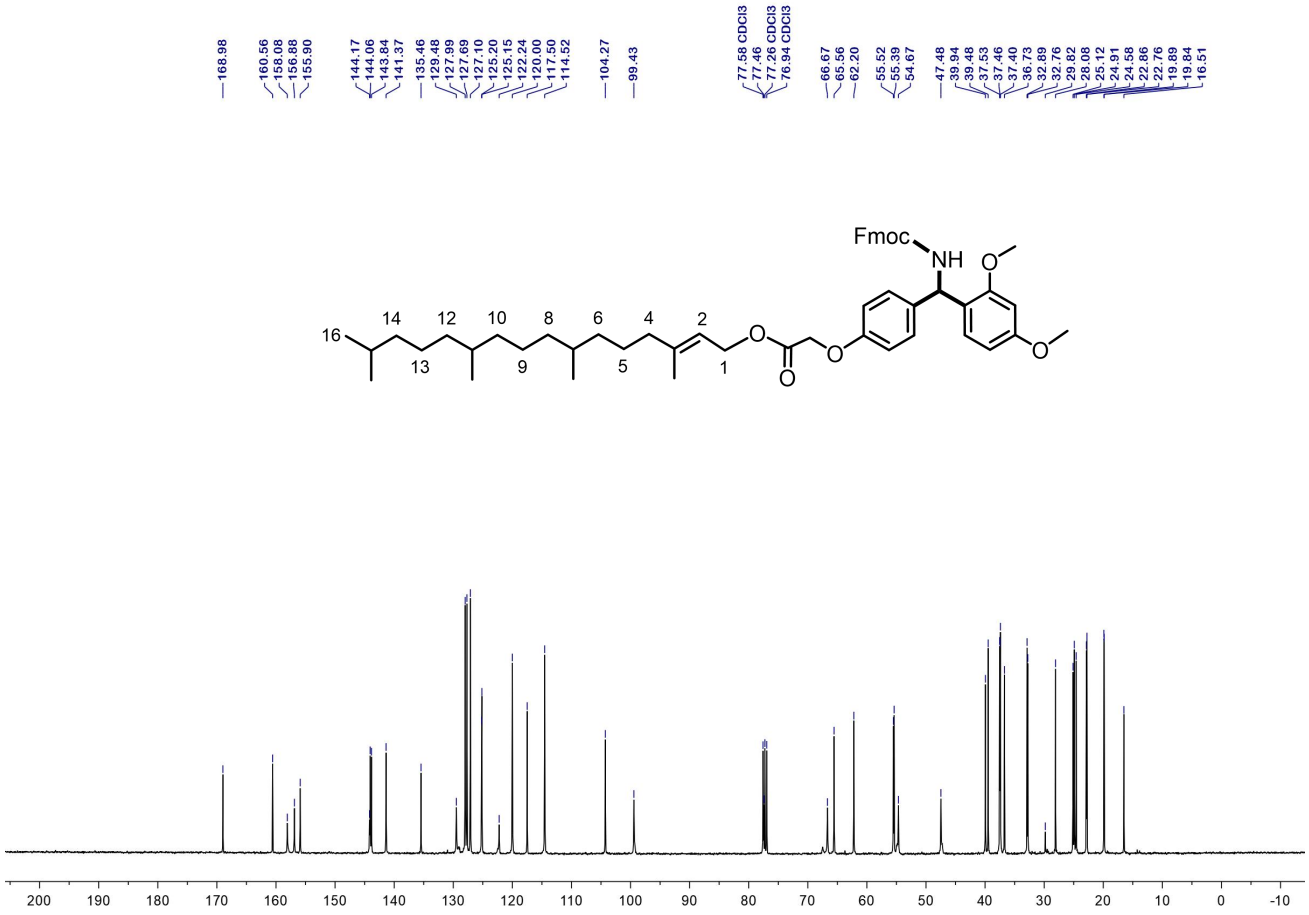
1f: ^{13}C NMR (100 MHz, $CDCl_3$)



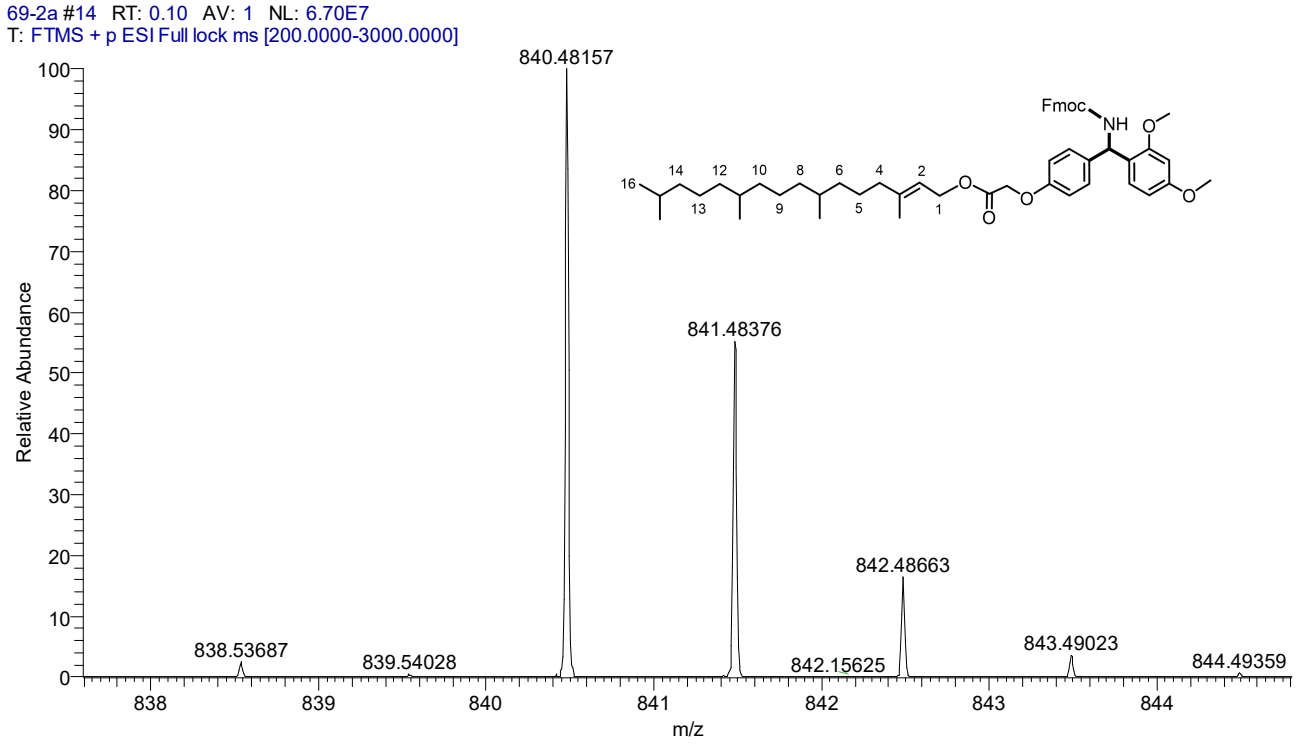
1f: HRMS (ESI) m/z calcd for $C_{135}H_{114}N_3O_{25}PNa^+$ $(M+Na)^+$ 2230.73712, found 2230.73804.



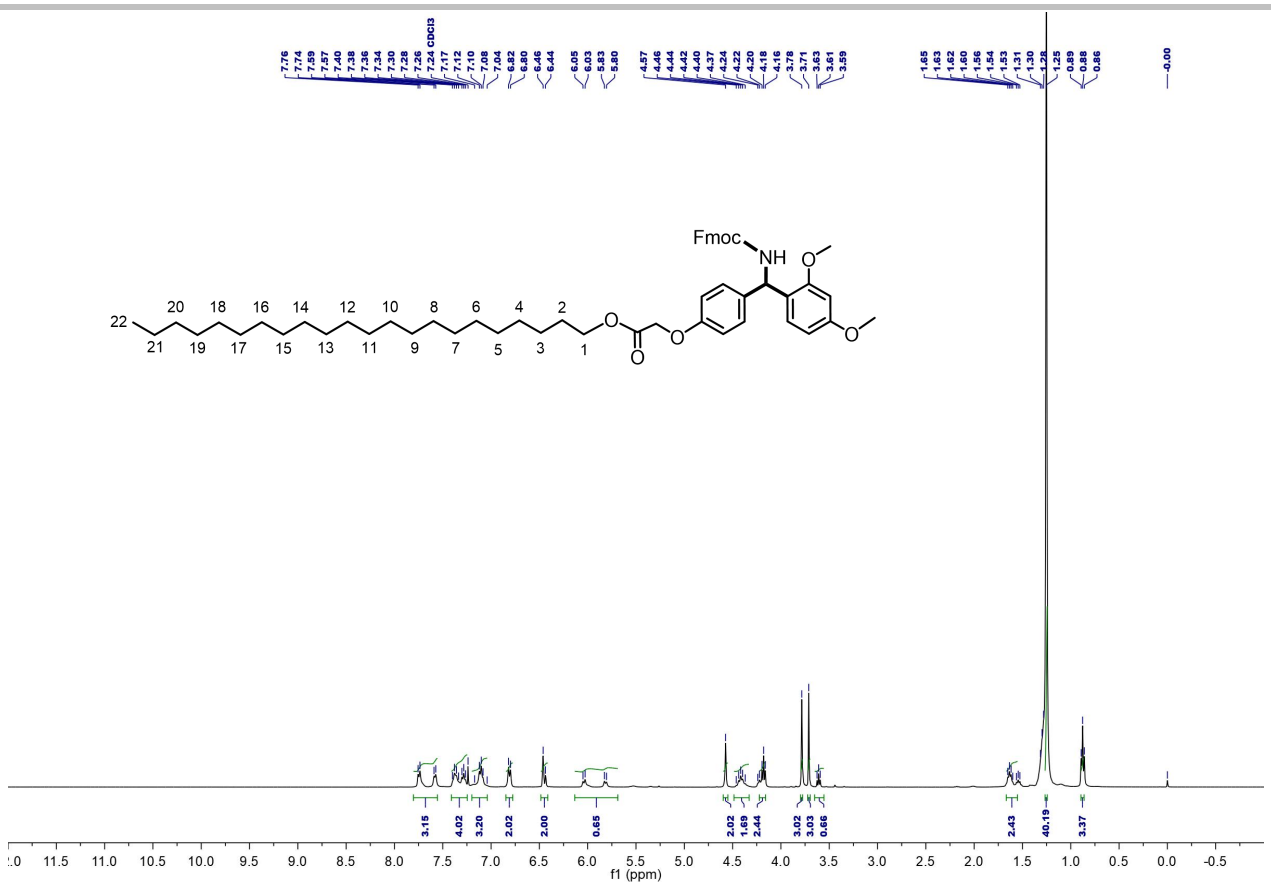
2a: ^{1}H NMR (400 MHz, $CDCl_3$)



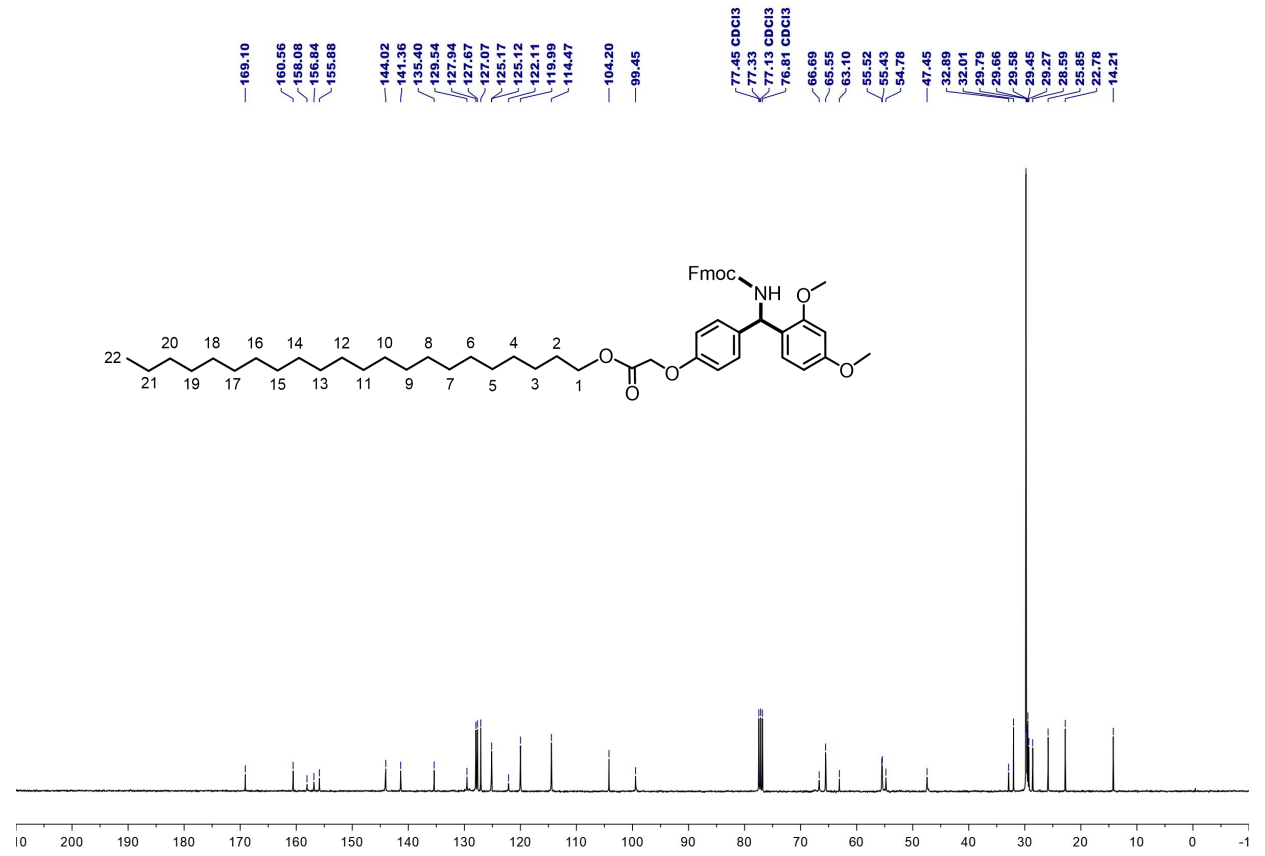
2a: ^{13}C NMR (100 MHz, $CDCl_3$)



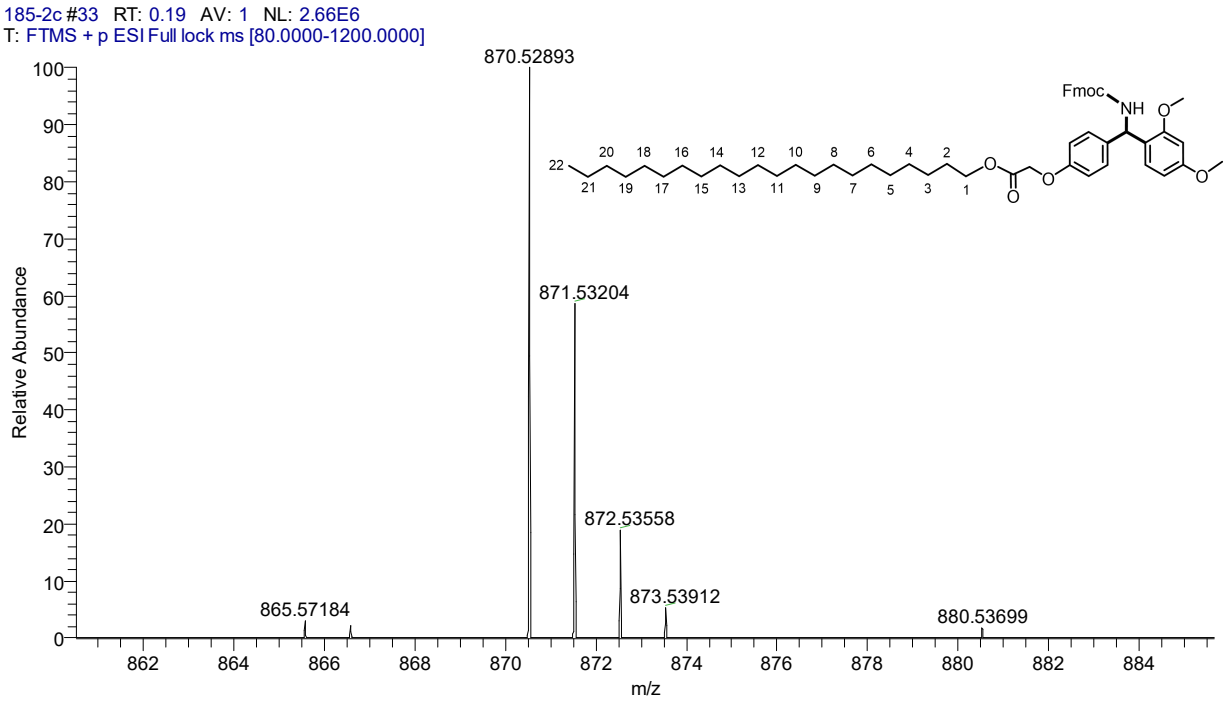
2a: HRMS (ESI) m/z calcd for $C_{52}H_{67}NO_7Na^+$ $(M+Na)^+$ 840.48097, found 840.48157.



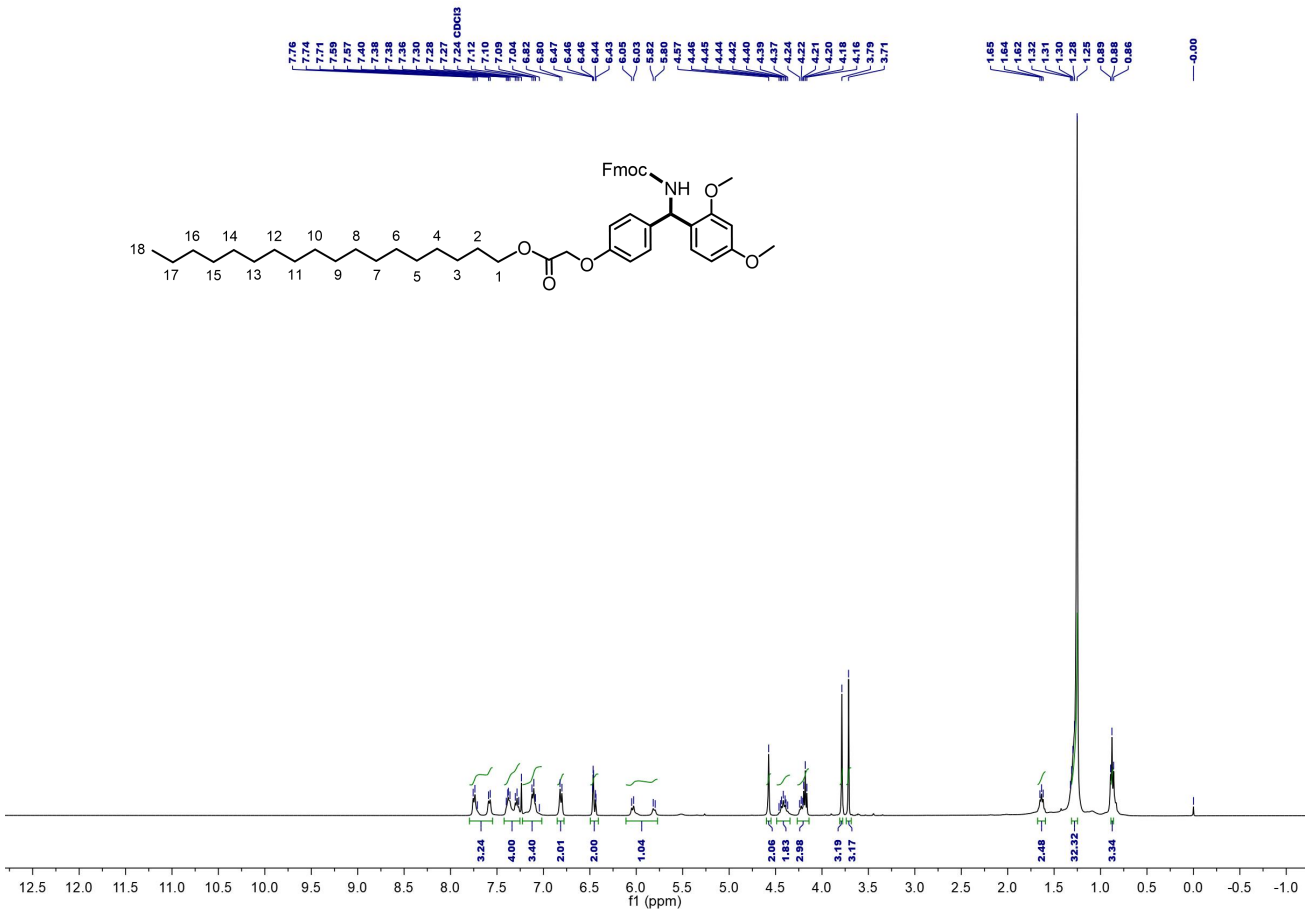
2b: ^{1}H NMR (400 MHz, $CDCl_3$)



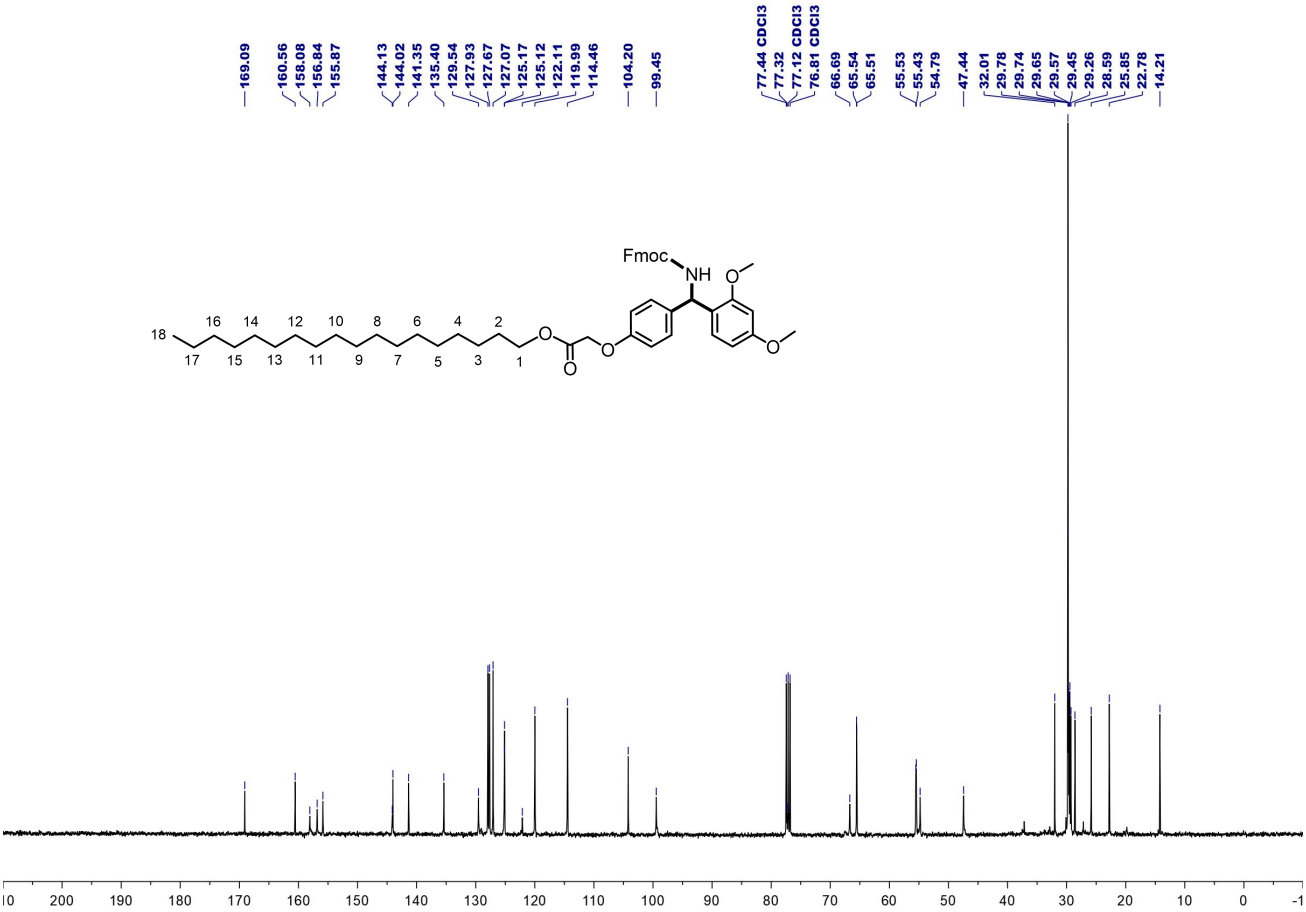
2b: ^{13}C NMR (100 MHz, $CDCl_3$)



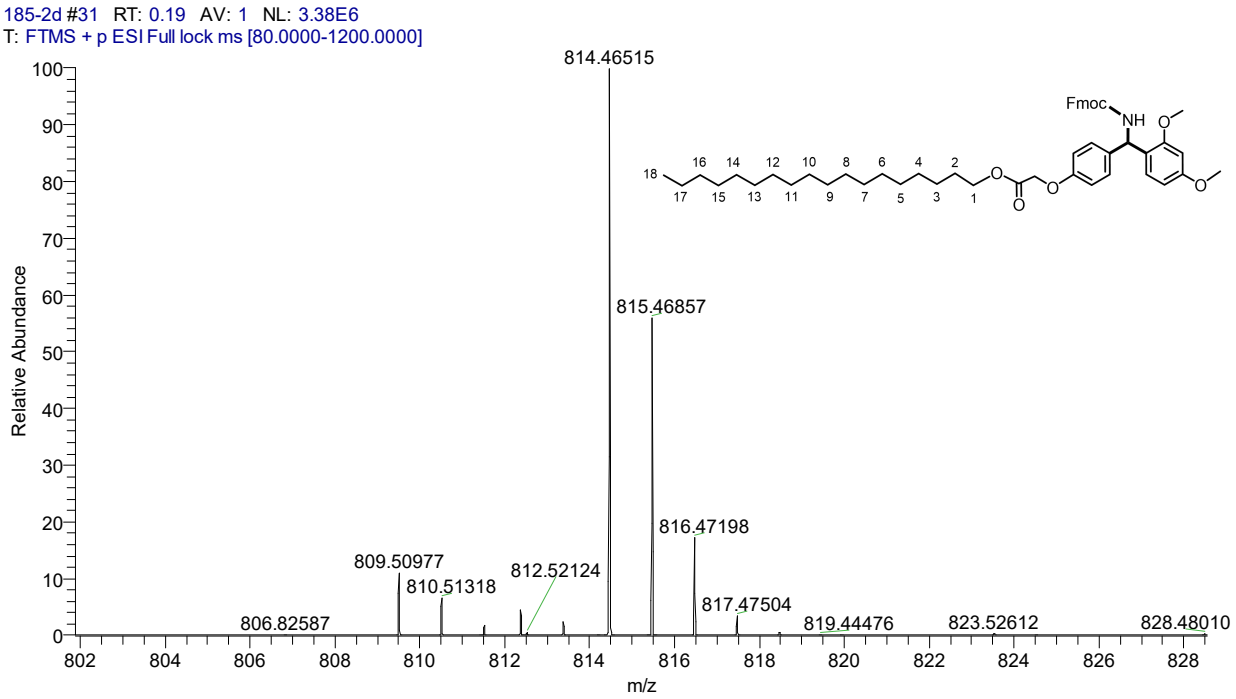
2b: HRMS (ESI) m/z calcd for $C_{54}H_{73}NO_7Na^+$ $(M+Na)^+$ 870.52792, found 870.52893.



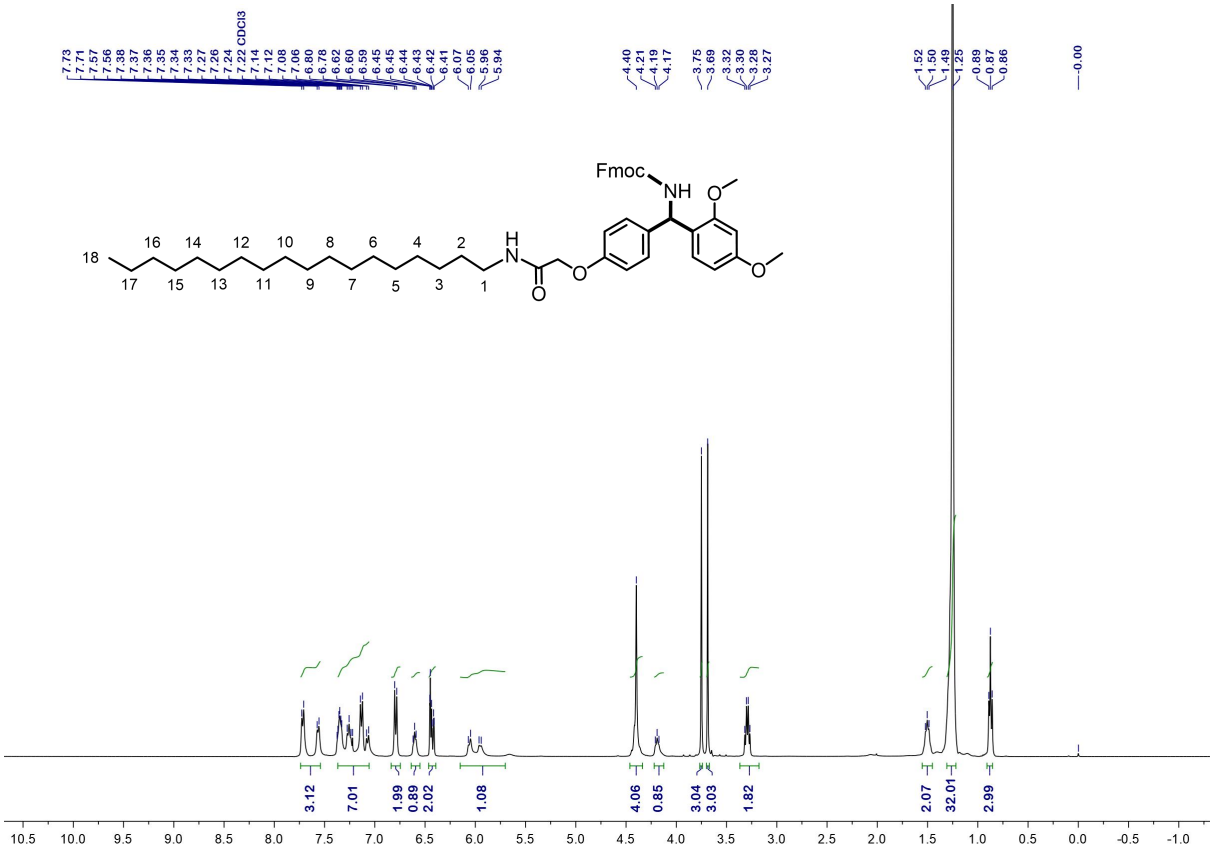
2c: ^{1}H NMR (400 MHz, $CDCl_3$)



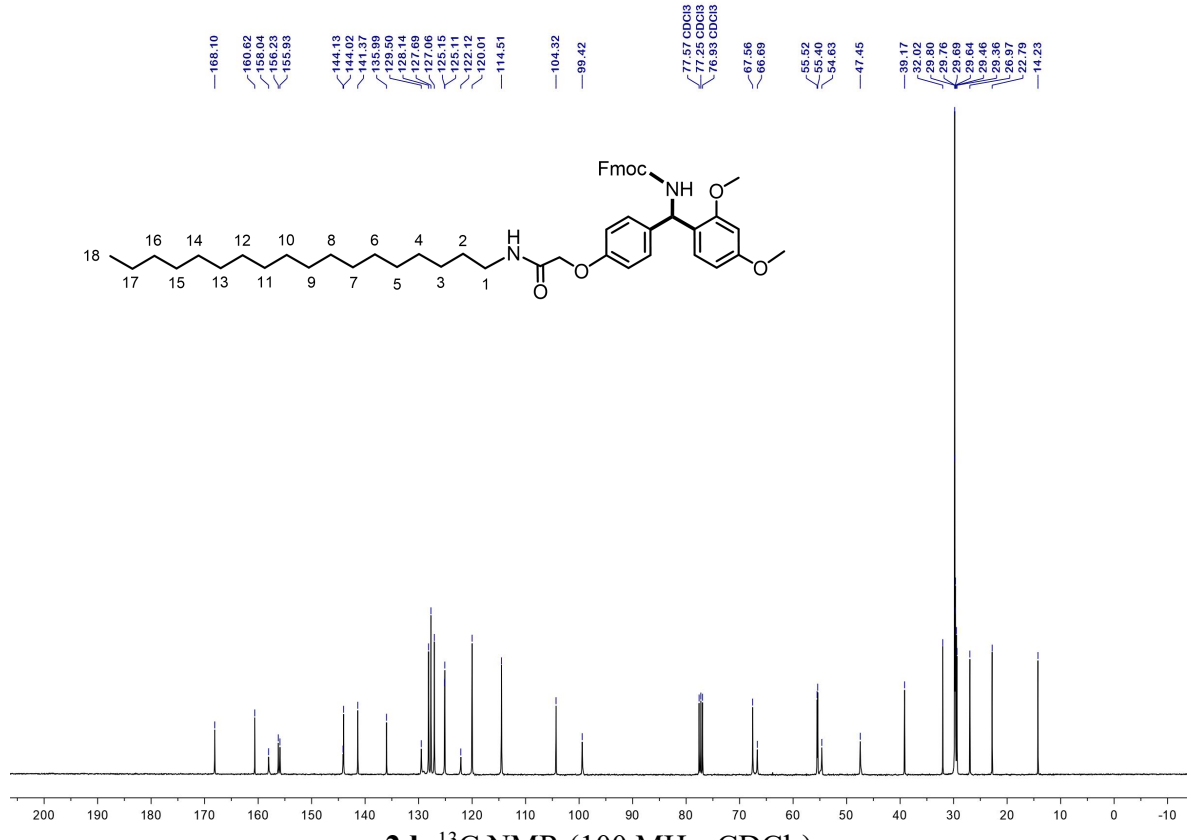
2c: ^{13}C NMR (100 MHz, $CDCl_3$)



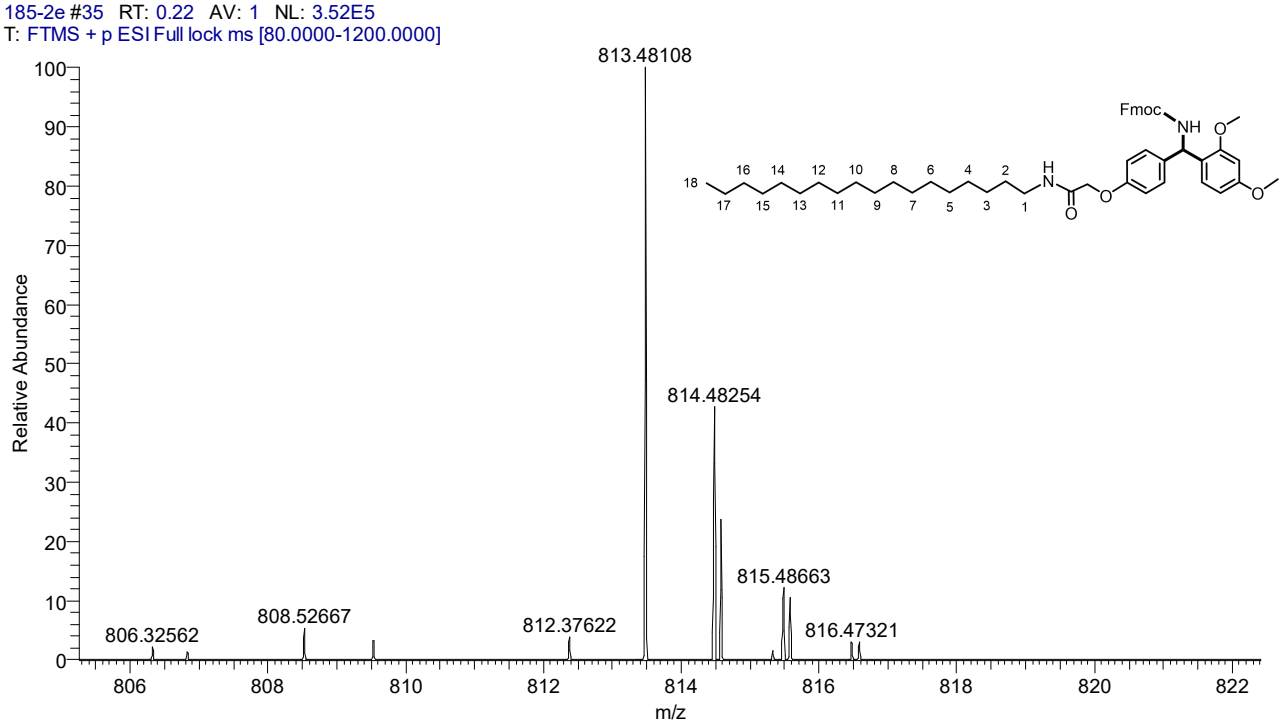
2c: HRMS (ESI) m/z calcd for $C_{50}H_{65}NO_7Na^+$ $(M+Na)^+$ 814.46532, found 814.46515.



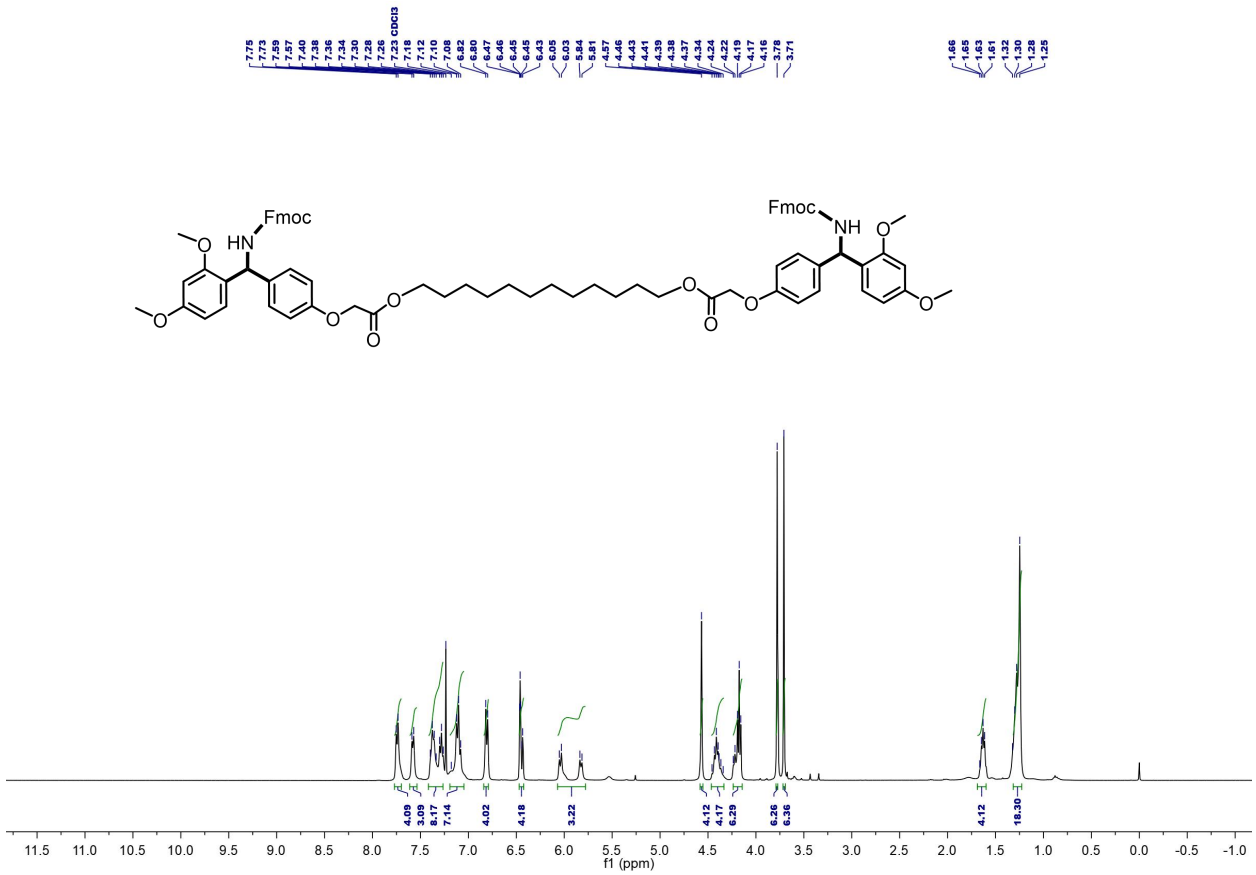
2d: ^{1}H NMR (100 MHz, $CDCl_3$)



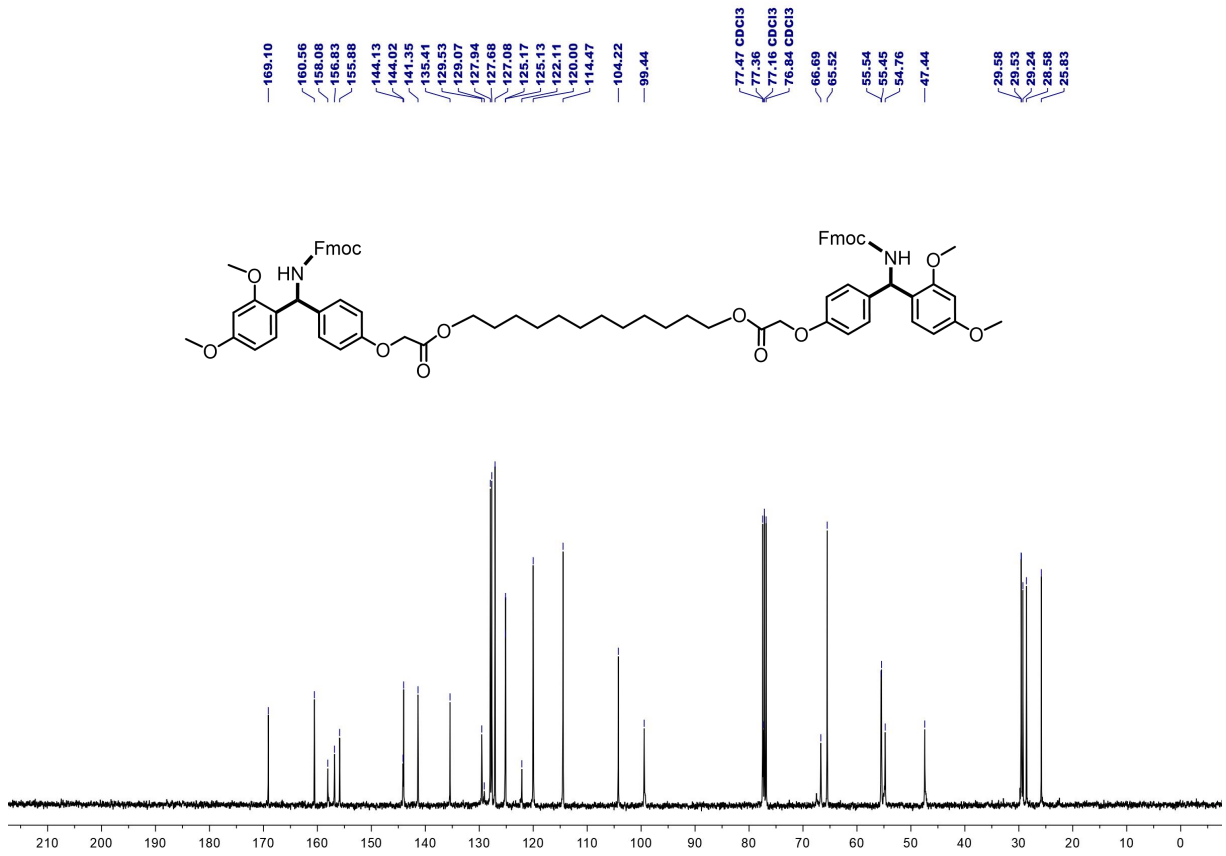
2d: ^{13}C NMR (100 MHz, $CDCl_3$)



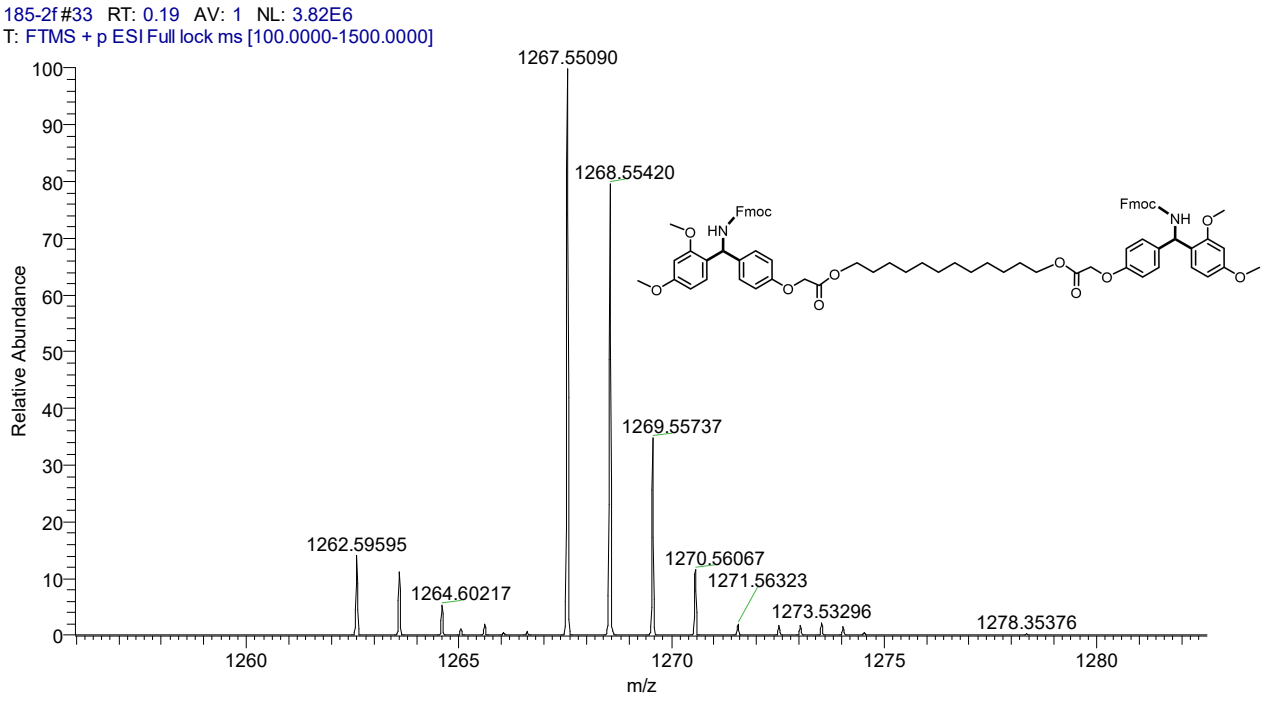
2d: HRMS (ESI) m/z calcd for $C_{50}H_{66}N_2O_6Na^+$ $(M+Na)^+$ 813.48131, found 813.48108.



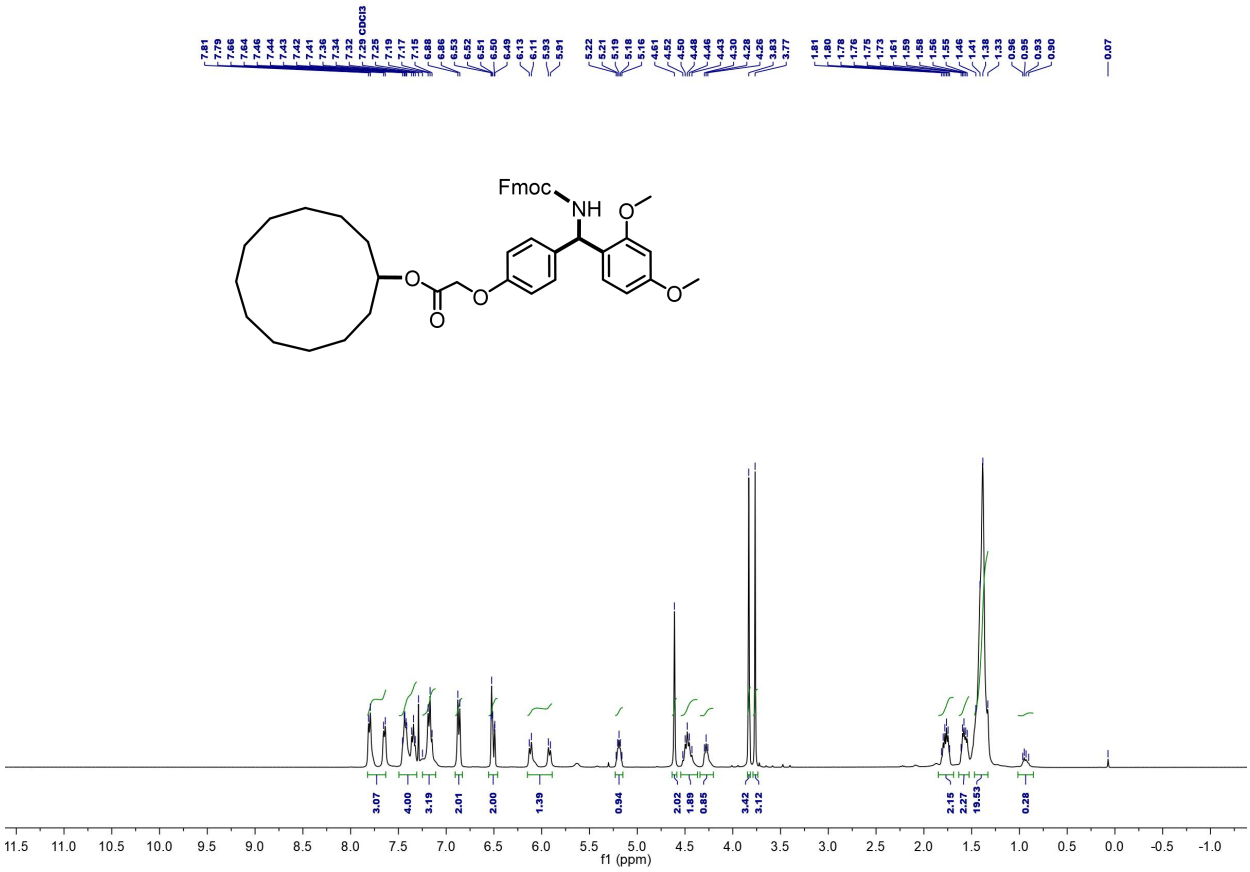
2e: ^1H NMR (400 MHz, $CDCl_3$)



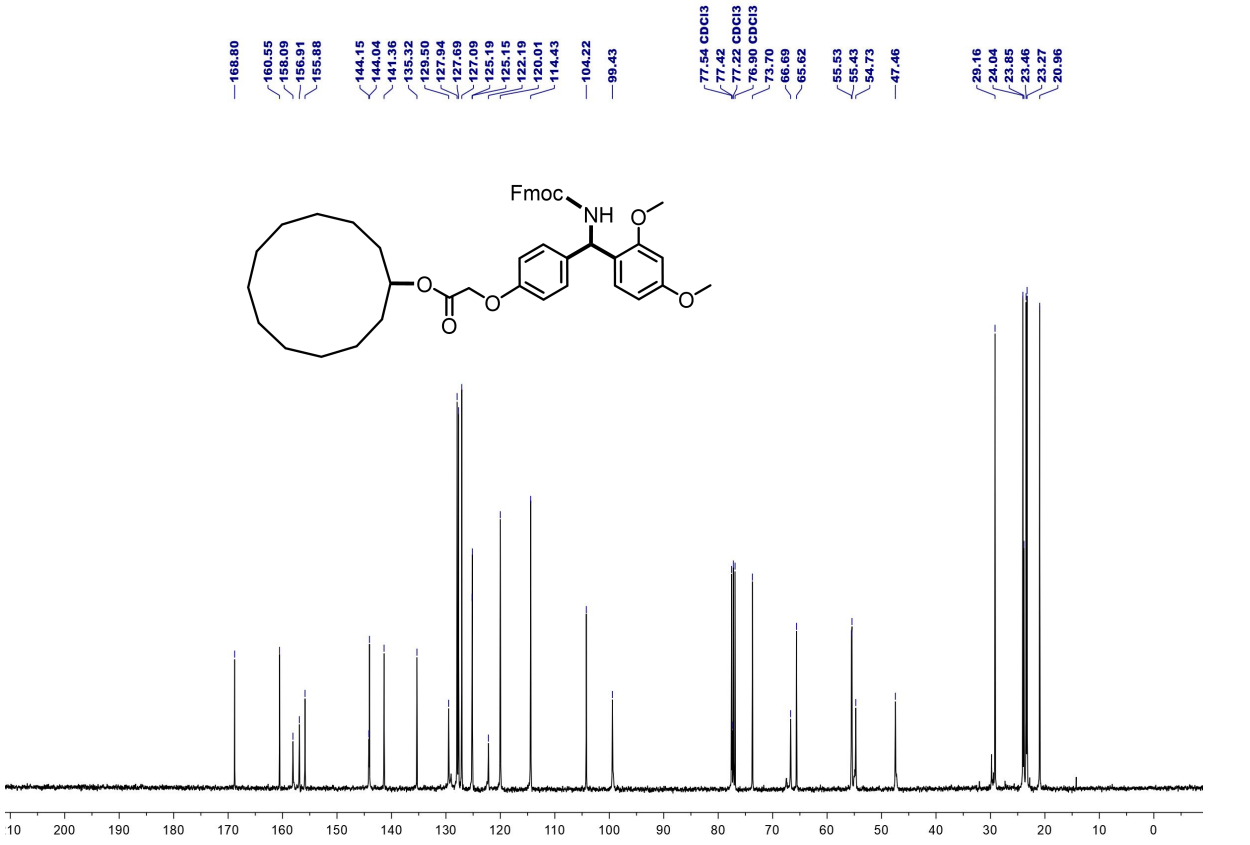
2e: ^{13}C NMR (100 MHz, $CDCl_3$)



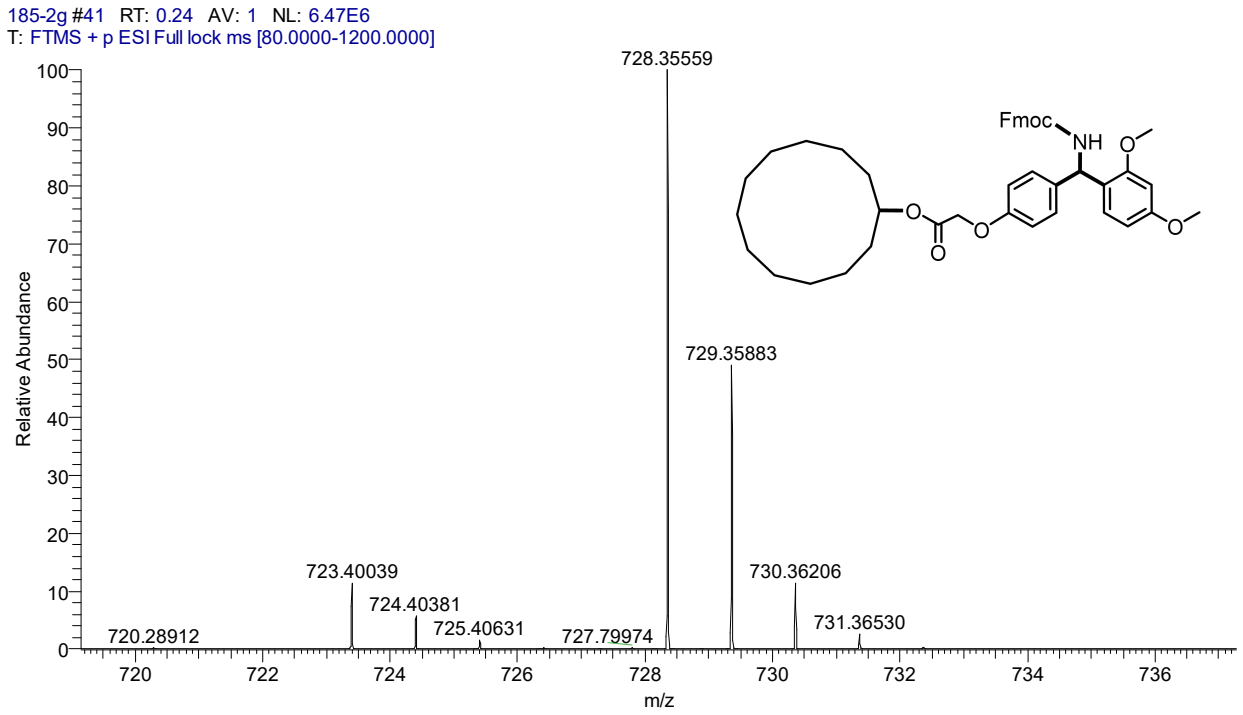
2e: HRMS (ESI) m/z calcd for $C_{76}H_{80}N_2O_{14}Na^+$ $(M+Na)^+$ 1267.55018, found 1267.55090.



2f: ^{1}H NMR (400 MHz, $CDCl_3$)



2f: ^{13}C NMR (100 MHz, $CDCl_3$)



2f: HRMS (ESI) m/z calcd for $C_{44}H_{51}NO_7Na^+$ $(M+Na)^+$ 728.35577, found728.35559.