Supplementary Information

Effective Assay of Bacterial Transglycosylation by Molecular Turn-On Sensing and Secondary-Order Scattering Effect

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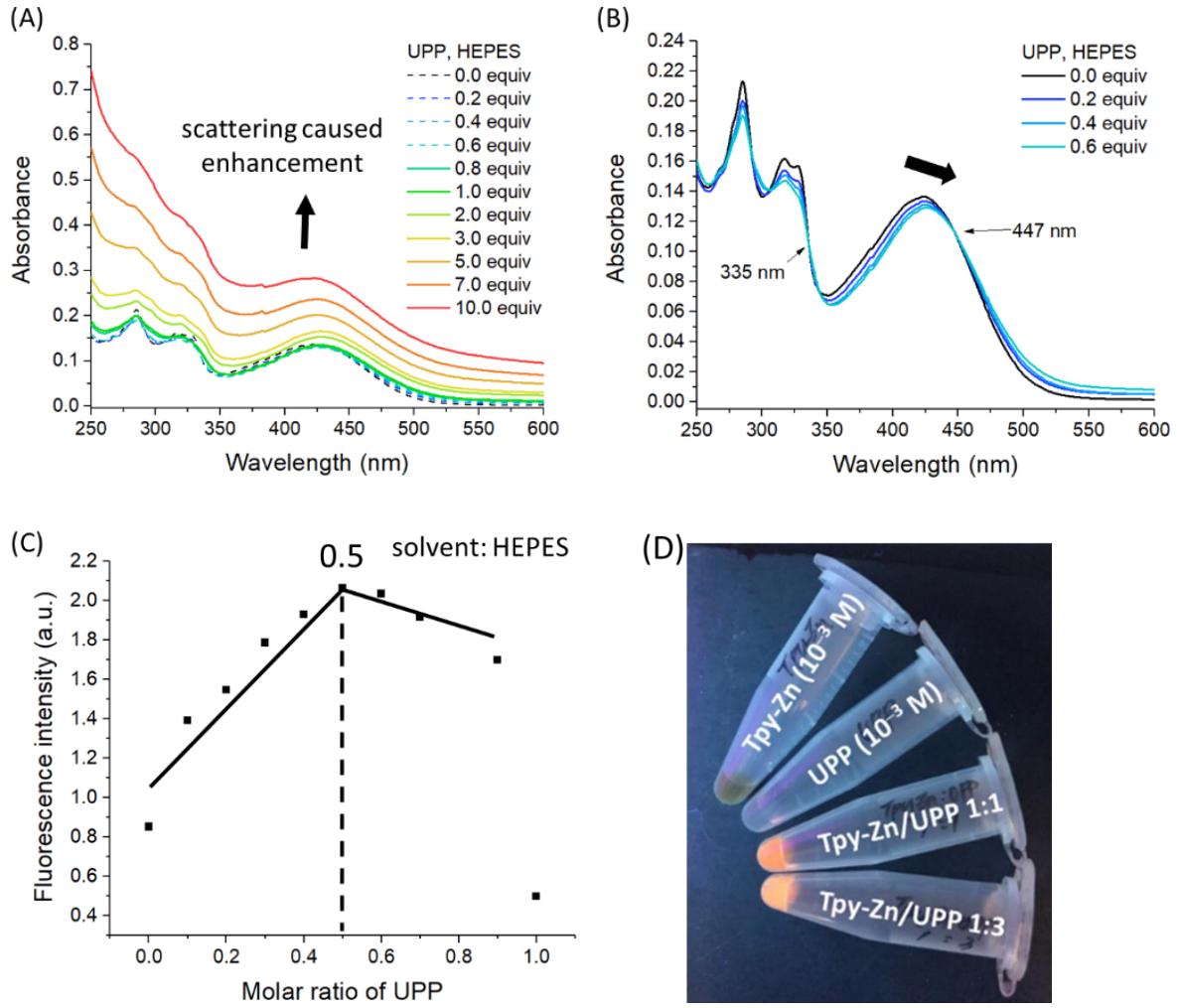


Figure S1. Absorption spectra of Tpy-Zn (**4**, 1.0×10^{-5} M) upon incremental addition of UPP (**3**) in HEPES buffer (10 mM, pH 7.4) containing 10 mM $CaCl_2$: (A) addition of 0–10.0 equiv UPP, (B) isosbestic point at 447 nm in the range of 0–0.6 equiv UPP. (C) Job plot for the complexation of Tpy-Zn and UPP (5.0×10^{-6} M total concentration) by monitoring the changes of fluorescence intensity at 597 nm, indicating 1:1 binding stoichiometry of the [Tpy-Zn]-UPP complex. (D) Pictures for mixing of Tpy-Zn (1.0×10^{-3} M) and UPP (1.0×10^{-3} M) in the ratio of 1:1 and 1:3 on irradiation of UV lamp (λ_{ex} = 365 nm).

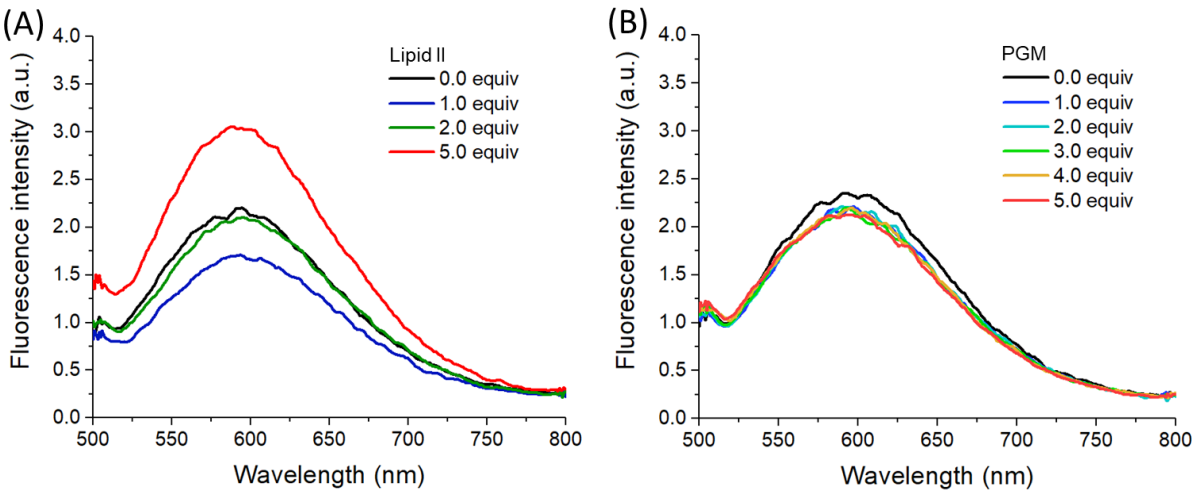


Figure S2. Fluorescence titration curves upon incremental addition of (A) lipid II (**1**, 0–5 equiv) and (B) PGM (**2**, 0–5 equiv) to Tpy-Zn sensor (**4**, 1.0×10^{-5} M) in HEPES buffer (10 mM, pH 7.4) containing 10 mM $CaCl_2$ and 0.08% decyl-PEG. λ_{ex} = 430 nm; λ_{em} = 597 nm.

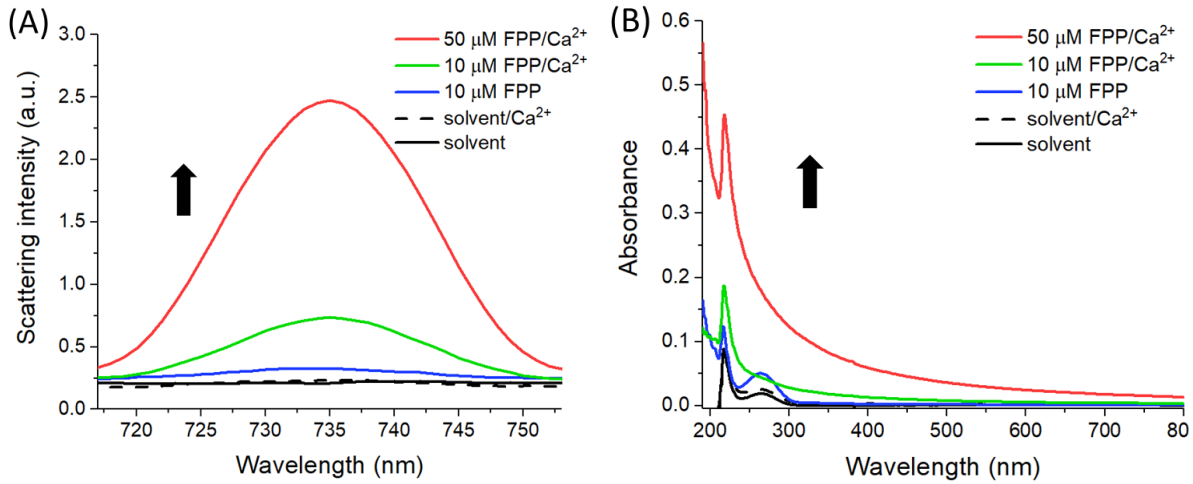


Figure S3. Secondary-order scattering spectra (A) and absorption spectra (B) of FPP (**5**, 10 or 50 μM) in HEPES buffer (10 mM, pH 7.4) in the absence and presence of 10 mM $CaCl_2$. λ_{ex} = 365 nm; λ_{SOS} = 735 nm.

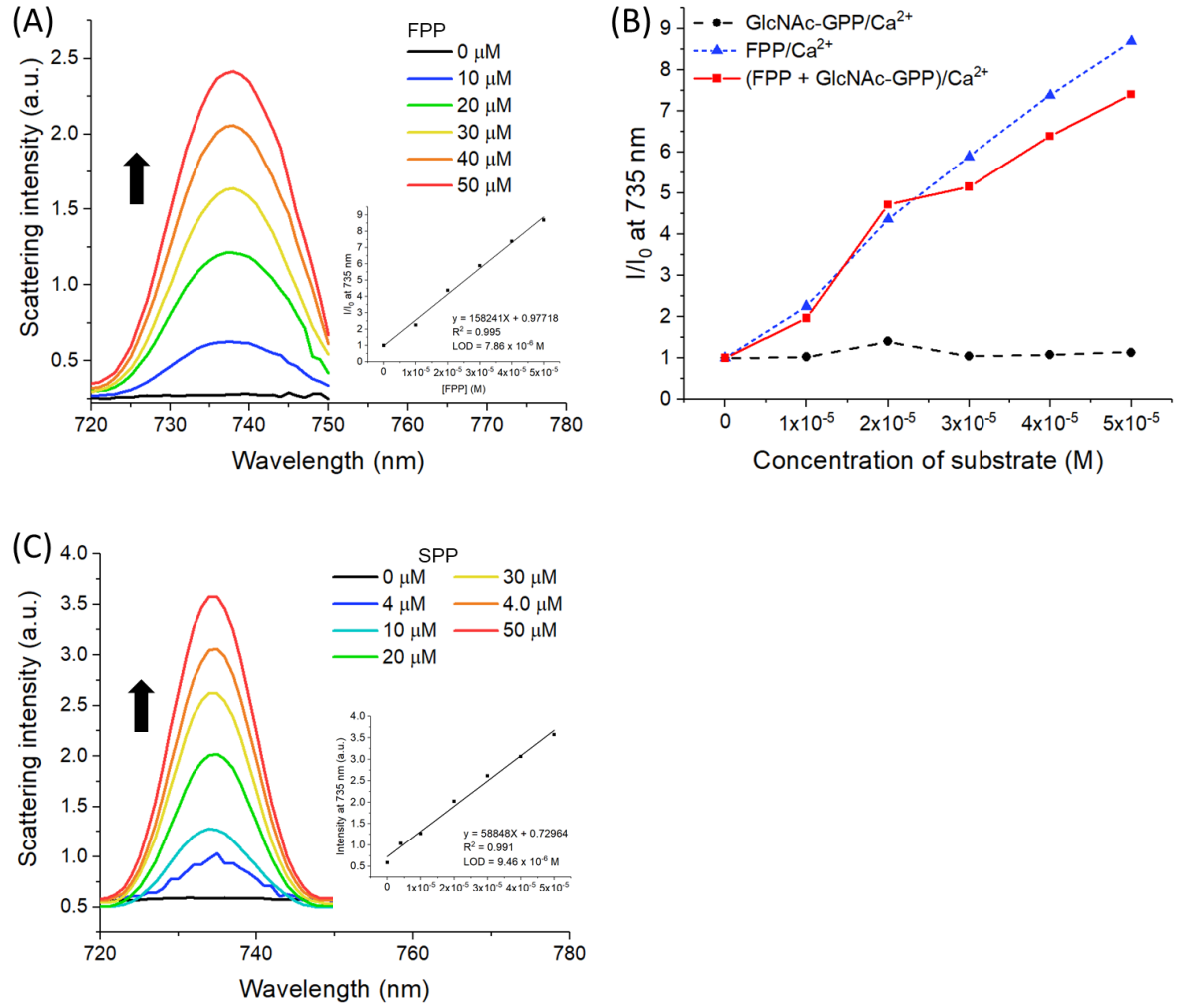


Figure S4. (A) SOS spectra of FPP (**5**, 0–50 μM) in HEPES buffer containing 10 mM $CaCl_2$. λ_{ex} = 365 nm; λ_{SOS} = 735 nm. Inset: Linear calibration line of FPP-Ca; the LOD was determined to be 7.86 μM. (B) The SOS signal of FPP-Ca increased as the concentration of FPP increased; and only slightly interfered with the presence of GlcNAc-GPP. (C) SOS spectra of SPP (**6**, 0–50 μM) in HEPES buffer (10 mM, pH 7.4) containing 10 mM $CaCl_2$ and 0.08% decyl-PEG. λ_{ex} = 365 nm; λ_{SOS} = 735 nm. Inset: Linear calibration line of SPP-Ca; the LOD value was determined to be 9.46 μM.

	diameter (nm)[a]					Avg. (nm)[b]
	1×10^{-5} M	2×10^{-5} M	3×10^{-5} M	4×10^{-5} M	5×10^{-5} M	
FPP (5)	1092	1466	1295	1154	1224	1246 ± 145
SPP[c] (6)	1035	1351	1318	1133	1503	1268 ± 185

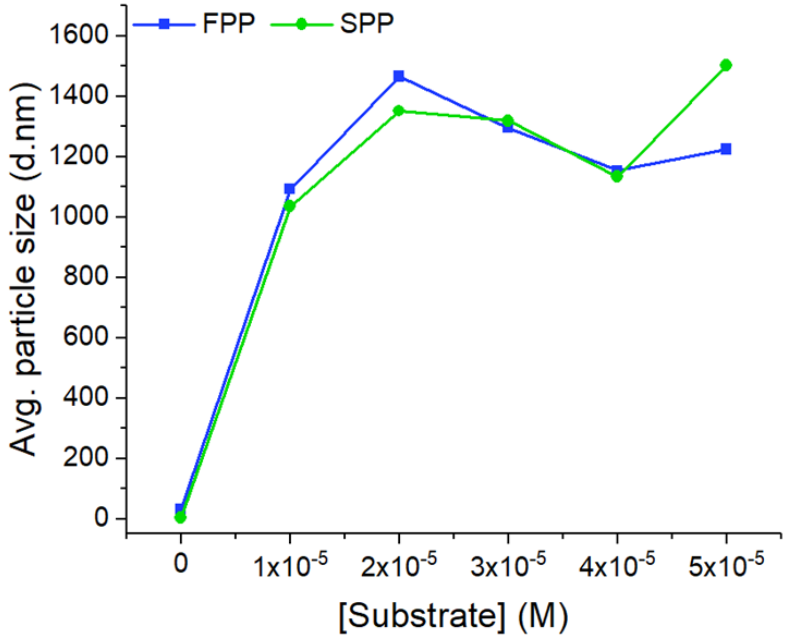


Figure S5. Average particle size versus concentration of FPP (**5**) and SPP (**6**) in HEPES buffer (10 mM, pH 7.4) containing 10 mM $CaCl_2$. In the experiments of SPP, the buffer also included decyl-PEG (0.08%).

[a] Each experiment was conducted at least three times in good reproducibility.

[b] The average diameter of the particles at five concentrations (1–5) × 10^{-5} M.

[c] Each sample contained 0.08% decyl-PEG.

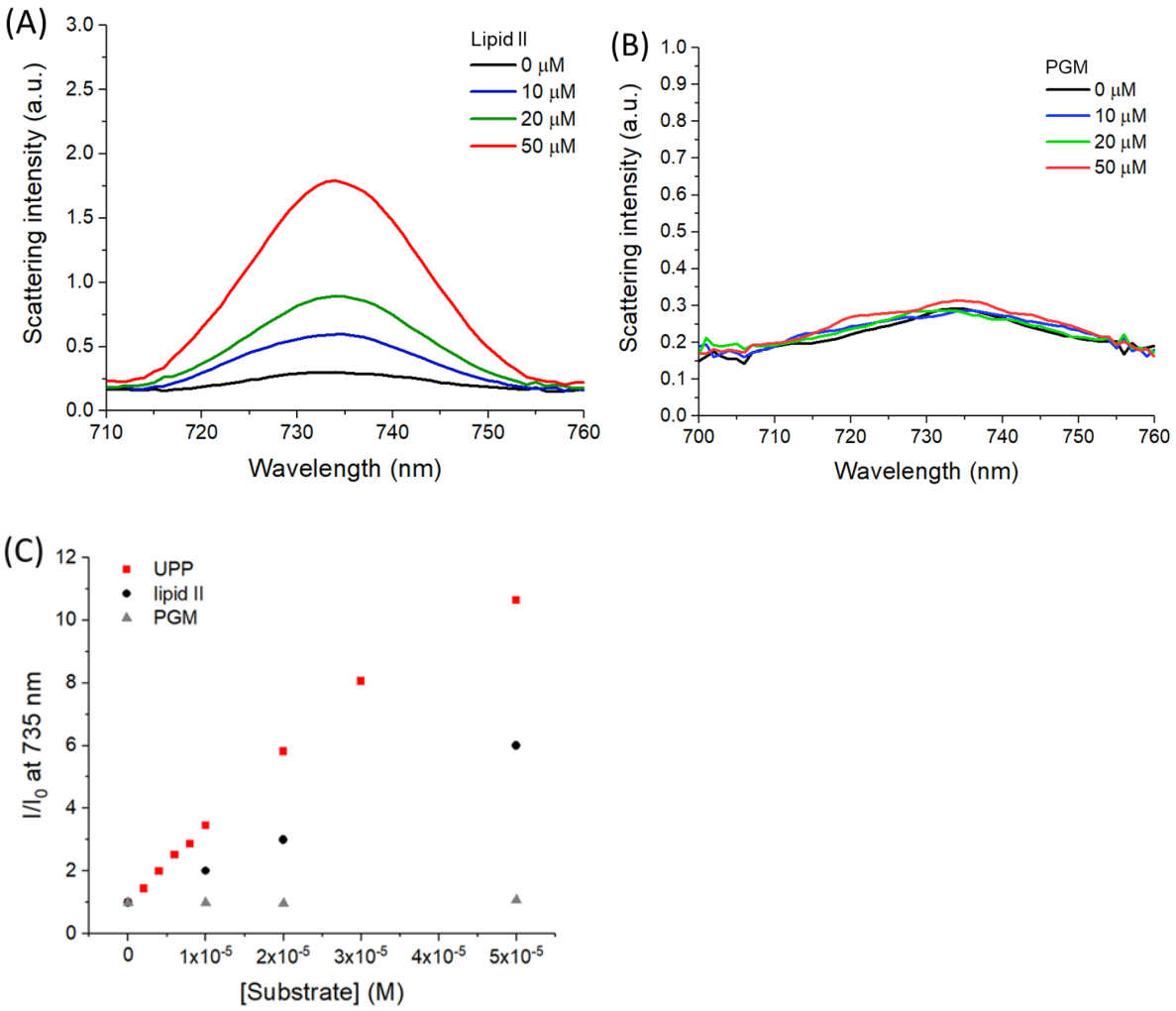
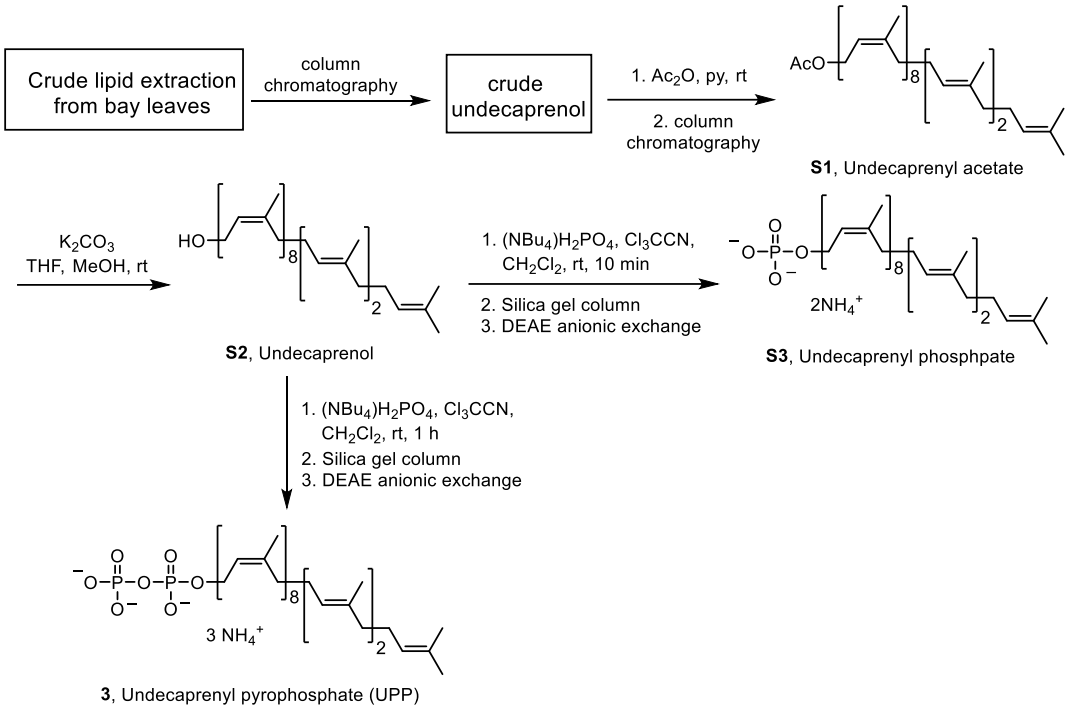
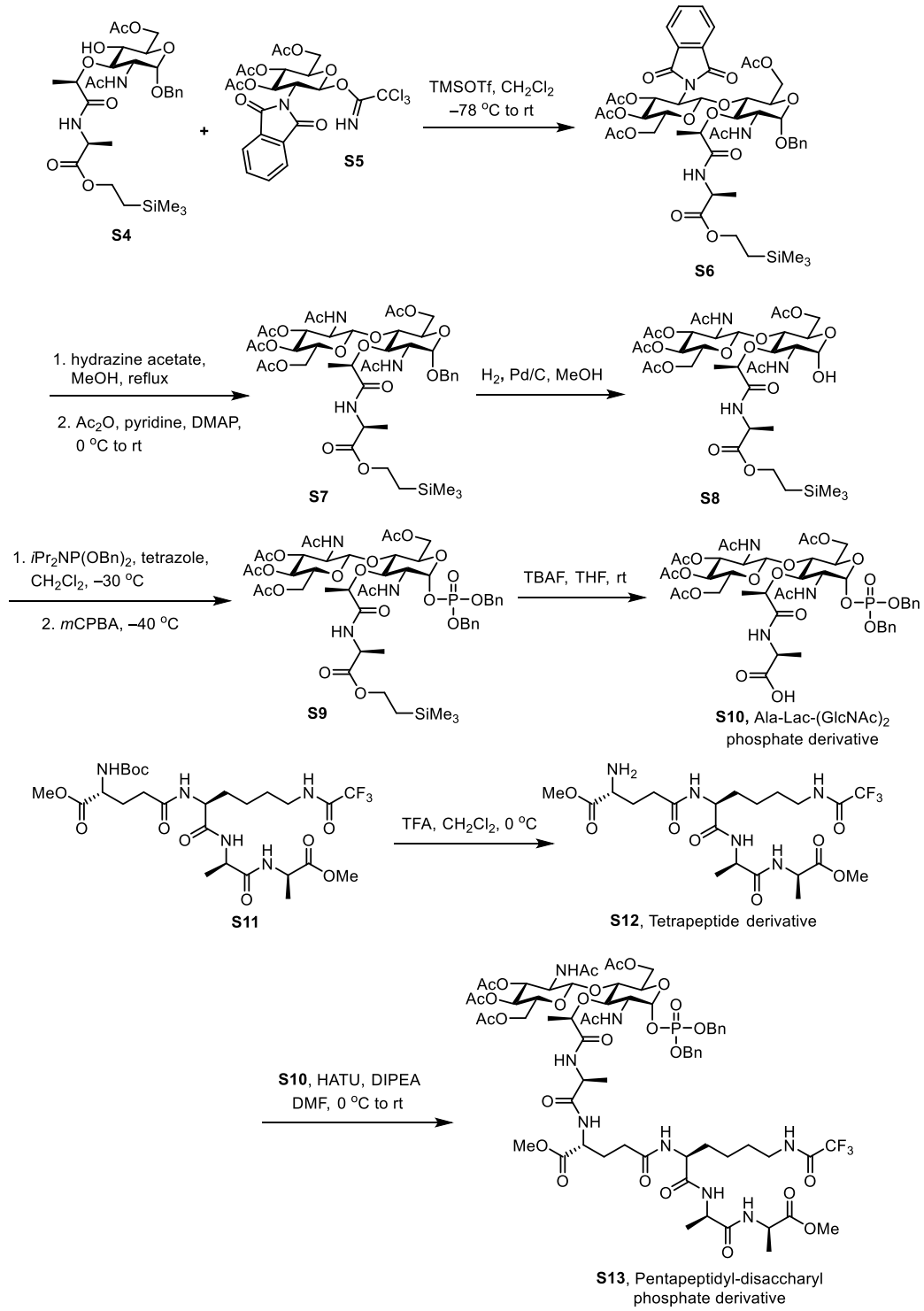


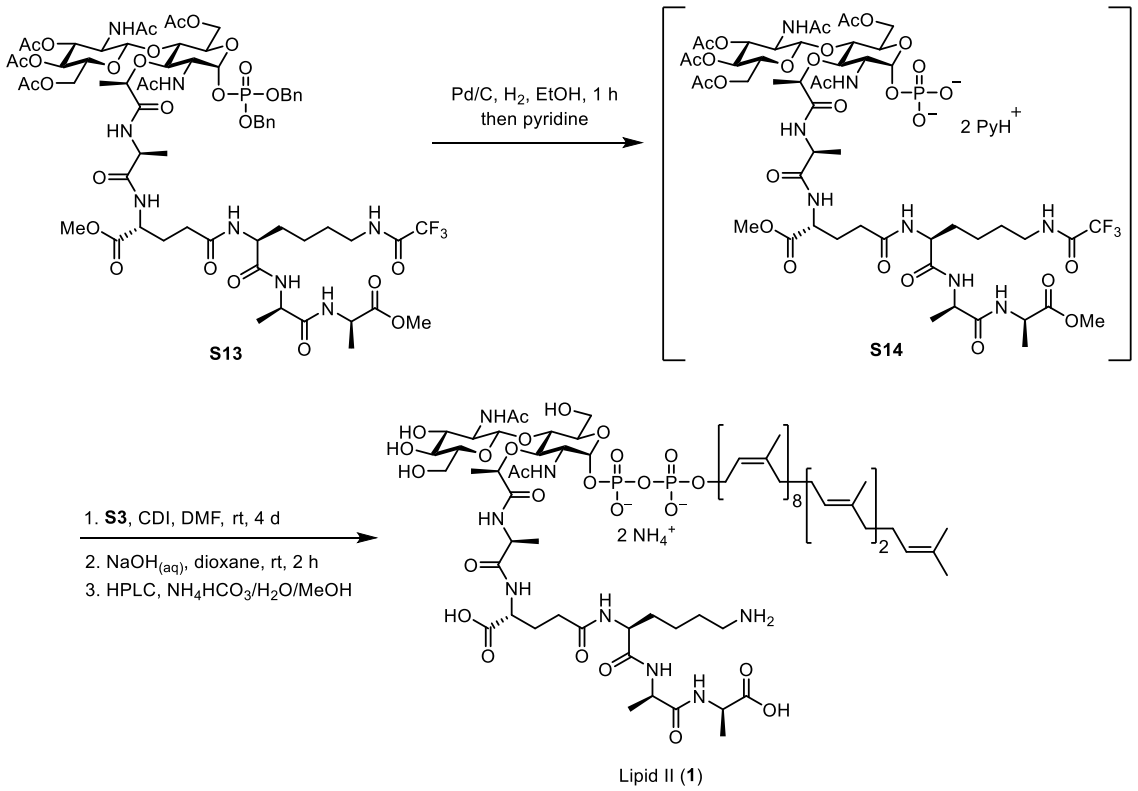
Figure S6. Secondary-order scattering spectra of (A) lipid II (**1**, 0–50 μM) and (B) PGM (**2**, 0–50 μM) in HEPES buffer (10 mM, pH 7.4) containing 10 mM $CaCl_2$ and 0.08% decyl-PEG. λ_{ex} = 365 nm; λ_{SOS} = 735 nm. (C) Ratio of SOS intensity (I/I_0) at λ = 735 nm at various concentrations (1–5) × 10^{-5} M of UPP, lipid II and PGM. I_0 and I represent the scattering intensity of the solution before and after addition of substrate.



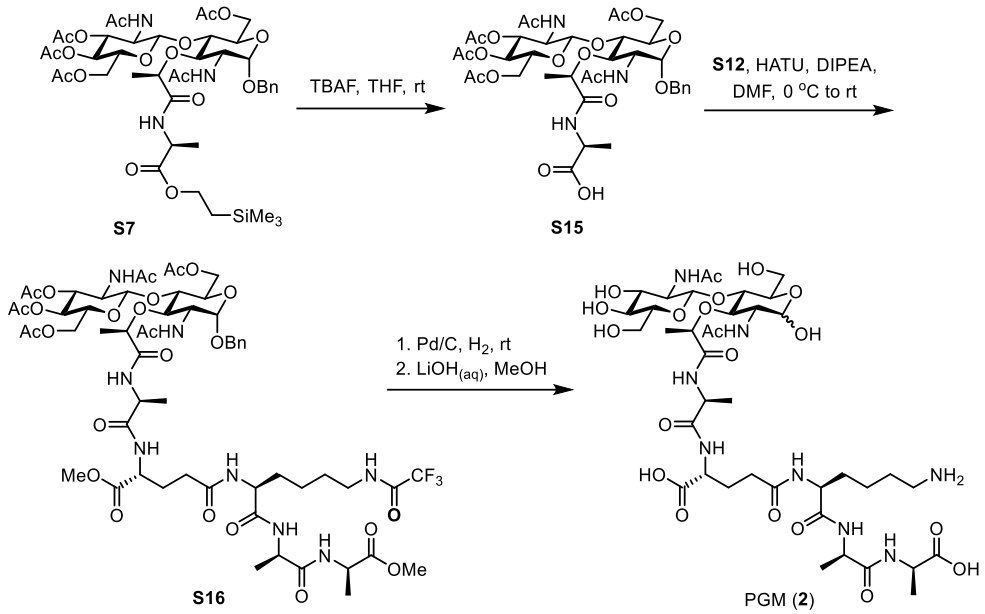
Scheme S1. Preparation of undecaprenyl phosphate (**S3**) and undecaprenyl pyrophosphate (**3**, UPP).[20, 21, S1]



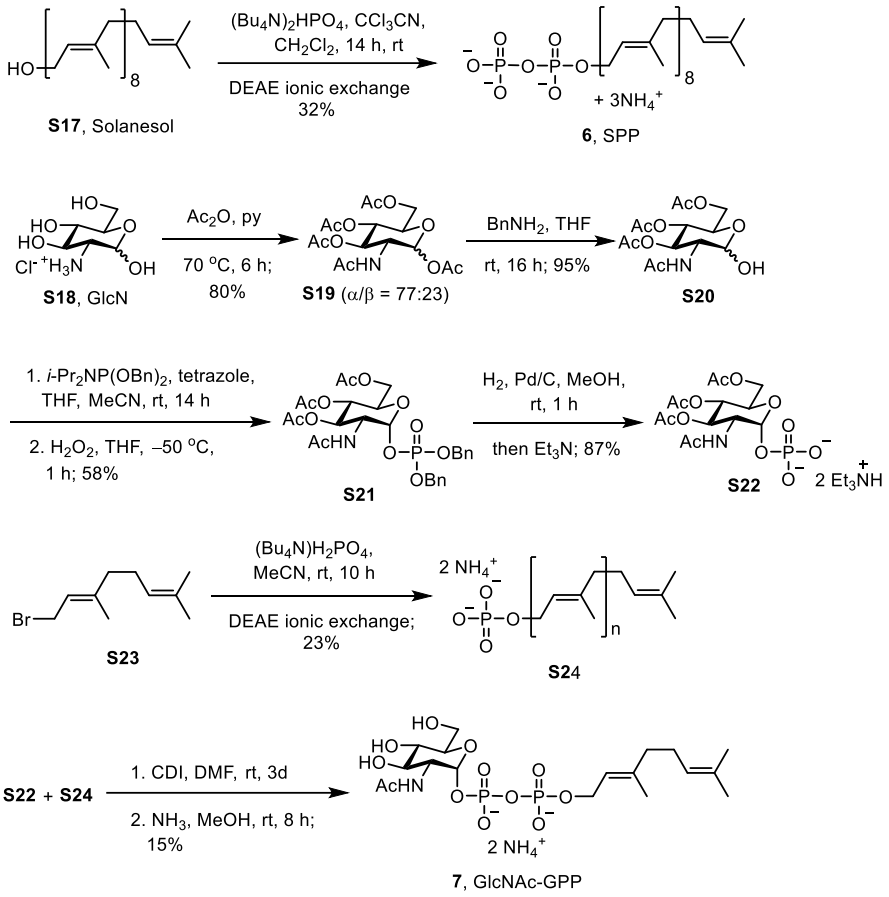
Scheme S2. Preparation of pentapeptidyl-disaccharyl phosphate derivative (**S13**).[13]



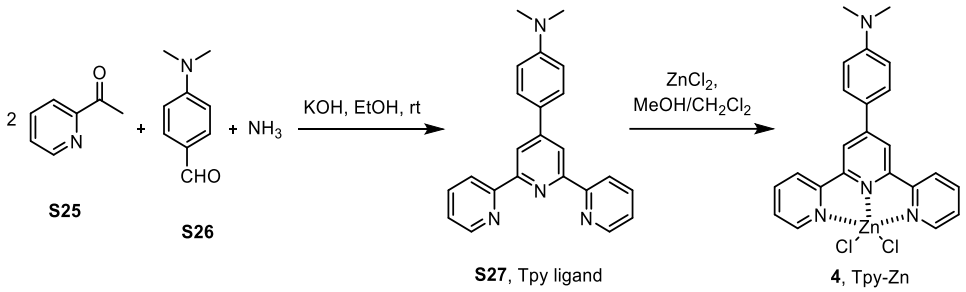
Scheme S3. Synthesis of lipid II (**1**, as the ammonium salt).[22]



Scheme S4. Synthesis of PGM (**2**).[22]



Scheme S5. Synthesis of solanesyl pyrophosphate (SPP, **6**)[20] and GlcNAc-geranyl pyrophosphate (GlcNAc-GPP, **7**).[17]



Scheme S6. Synthesis of Tpy-Zn sensor (**4**).[17, 23]

Experimental

General

All solvents and reagents were reagent grade and were used as purchased without further purification unless otherwise specified. Dichloromethane (CH_2Cl_2) was distilled from CaH_2, and tetrahydrofuran (THF) was distilled from sodium. Farnesyl pyrophosphate (2.3×10^{-3} M in MeOH/ammonia solution), and octaethylene glycol monodecyl ether (decyl-PEG) was purchased from Sigma-Aldrich. All solvents of spectroscopic grade (Merck; Acros Organics) and deionized water were used in UV-vis and fluorescence titration experiments. Moenomycin A and *Acinetobacter baumannii* PBP1b are gifts of Dr. Wei-Chieh Cheng and Dr. Ting-Jen Cheng at the Genomics Research Center, Academia Sinica.

All air or moisture sensitive experiments were conducted under an atmosphere of argon or nitrogen. Merck silica gel 60 F_{254} plates (0.25 mm thickness) were used in thin-layer chromatography (TLC). Compounds were visualized by using UV lamp, or by staining with ninhydrin, *p*-anisaldehyde, phosphomolybdic acid (PMA) or ceric ammonium molybdate. E. Merck silica gel 60 (0.040–0.063 mm particle size) and LiChroprep RP-18 (0.040–0.063 mm particle size) were used for column chromatography. DEAE anionic exchange resin (DE-52, Whatman) was purchased form Merck Co. (New Jersey, USA).

UV–vis spectra were recorded on a PerkinElmer Lambda 35 spectrometer. Fluorescence spectra and secondary-order scattering spectra were recorded on an Aminco-Bowman Series 2 luminescence spectrometer (Thermo Electron Corp., MA, USA). Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker Avance-III NMR (400 MHz) or a Varian Advance-400 (400 MHz) spectrometer. Chemical shifts (δ) were recorded in parts per million (ppm) relative to internal standards: $CHCl_3$ ($\delta_H = 7.24$), $CDCl_3$ ($\delta_C = 77.0$ for the central line of the triplet), CD_2HOD ($\delta_H = 3.31$), CD_3OD ($\delta_C = 49.0$), HDO ($\delta_H = 4.81$), $(CH_3)_2SO$ ($\delta_H = 2.50$), and $(CD_3)_2SO$ ($\delta_C = 39.5$). Coupling constants (*J*) are given in hertz (Hz), and the splitting patterns are reported as singlet (s), doublet (d), triplet (t), quartet (quart), quintet (quint),

multiplet (m), dd (double of doublets), and broad (br). Electrospray mass spectra (ESI–MS) were recorded on a Bruker Daltonics BioTOF III high-resolution mass spectrometer. Lyophilization were performed on an EYELA FDU-1200 freeze dryer.

Fluorescence and UV–vis titration

Stock solution of analyte were freshly prepared in deionized water and stored in 4 ºC. All titration experiments were performed by addition of the analyte solution (1 mL total volume) at various concentrations in a quartz cell (1 cm pathlength). The fluorescence and absorption spectra were recorded at 298 K.

Job plot

Stock solutions of Tpy-Zn sensor and analyte (UPP) were prepared in HEPES buffer (10 mM, pH 7.4). Eleven sample solutions containing the sensor and analyte in different ratio (0:10 to 10:0) were prepared and diluted with appropriate solvent to maintain the total volume of 1 mL. The fluorescence intensity (I) were monitored as a function of molar ratio (X) of the analyte. The complex concentration was calculated as [complex] = $\Delta I \times X$, where ΔI is the fluorescence intensity after adding analyte minus the fluorescence intensity before adding any analyte, and X is the molar ratio of [analyte]:[receptor].

Secondary-order scattering (SOS) spectra

Stock solutions of pyrophosphate analyte (1.0×10^{-2} M) were freshly prepared and stored in HEPES buffer (10 mM, pH 7.4) at 4 ºC. Stock solution of calcium chloride (1.0 M) and decyl-PEG (20%) were prepared in deionized water prior to use. All experiments were performed by incremental addition of the pyrophosphate solution at various concentrations (1 mL total volume) in a quartz cell (1 cm pathlength). The pyrophosphate substrates include FPP,

SPP, UPP, FPP/GlcNAc-GPP and UPP/PGM/lipid II. The SOS spectra were recorded at 298 K using incident light at 365 nm.

Transglycosylation assay using fluorometric method with Tpy-Zn sensor

A mixture comprising lipid II (5.0×10^{-5} M), decyl-PEG (0.08%), $CaCl_2$ (10 mM), DMSO (10%) in 100 μL HEPES buffer (10 mM, pH 7.4) was placed in an Eppendorf tube. *A. baumannii* PBP (114 nM) was added, and the system was incubated at room temperature with shaking (1000 rpm) for 2 h in dark. The mixture was heated at 95 ºC for 5 min to quench the enzymatic reaction. The solution was cooled to room temperature, and Tpy-Zn sensor (**4**, 1.0×10^{-5} M) was added. The solution was well-mixed and transferred to a Hellma precision cell (Type No. 105.254-QS, quartz, 3 × 15 mm), and the fluorescence intensity at λ = 597 nm (λ_{ex} = 430 nm) was recorded with an Aminco-Bowman Series 2 luminescence spectrometer (Thermo Electron Corp., MA, USA) to determine the released UPP from transglycosylation reaction.

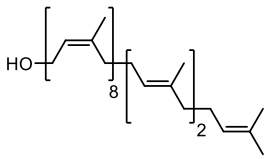
Transglycosylation assay using secondary-order scattering method

A mixture comprising lipid II (5.0×10^{-5} M), decyl-PEG (0.08%), $CaCl_2$ (10 mM), DMSO (10%) in 100 μL HEPES buffer (10 mM, pH 7.4) was placed in an Eppendorf tube. *A. baumannii* penicillin binding protein (PBP, 114 nM) was added, and the system was incubated at room temperature with shaking (1000 rpm) for 2 h in dark. The mixture was heated at 95 ºC for 5 min to quench the enzymatic reaction. The solution was cooled to room temperature, and transferred to a Hellma precision cell (Type No. 105.254-QS, quartz, 3 × 15 mm). The solution was irradiated at λ_{ex} = 365 nm, and the secondary-order scattering intensity at λ_{SOS} =735 nm was recorded on an Aminco-Bowman Series 2 luminescence spectrometer (Thermo Electron Corp., MA, USA) to determine the released UPP from transglycosylation reaction.

Determination of inhibitory activity (IC_{50}) of moenomycin A in transglycosylation

The experiments were performed using *A. baumannii* PBP (114 nM) and lipid II (50 μM) with various concentrations of moenomycin A (0–2000 nM). A mixture of lipid II (5.0×10^{-5} M) and moenomycin A (at a designated concentration of 0–2000 nM) in 100 μL HEPES buffer (10 mM, pH 7.4) containing decyl-PEG (0.08%), $CaCl_2$ (10 mM)and DMSO (10%) was placed in an Eppendorf tube. *A. baumannii* penicillin binding protein (PBP, 114 nM) was added, and the system was incubated at room temperature with shaking (1000 rpm) for 2 h in dark. The mixture was heated at 95 ºC for 5 min to quench the enzymatic reaction. The solution was cooled to room temperature, and transferred to a Hellma precision cell (Type No. 105.254-QS, quartz, 3 × 15 mm). The extent of transglycosylation was derived from the amount of the released UPP, either by the SOS effect or by the change of fluorescence intensity using Tpy-Zn sensor. The half maximal inhibitory concentration (IC_{50}) values are defined as the compound concentration required to reduce the binding capacity of lipid II toward PBP by 50%. All curve fitting was performed by OriginPro 2017, and calibrated with dose-response relationship to determine the inhibitory activity.

Extraction and purification of undecaprenol (S2) from bay leaves[21, S1]



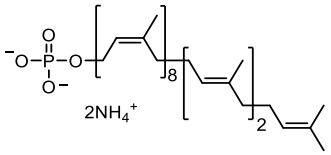
A Soxhlet system containing 100 g of bay leaves powder (purchased from Wu-Feng Co. Ltd., Taipei City, Taiwan) was extracted with hexane (400 mL) for 48 h. The collection flask was cooled to room temperature, and concentrated to obtain a deep-green mixture. The mixture was added hexane/EtOH/15% $KOH_{(aq)}$ (500 mL, v/v = 3:15:2), and stirred at 90 ºC for 1 h. The mixture was cooled to room temperature, added H_2O (250 mL) and extracted with Et_2O (250 mL × 2). The combined organic phase was dried over $MgSO_4$, filtered and concentrated under

reduced pressure. The crude product was subjected to silica gel chromatography (hexene/Et_2O = 17:3, R_f = 0.24) to yield semi-finished undecaprenol (1.03 g, 1% w/w bay leaves).

To the semi-finished undecaprenol (727 mg) in anhydrous pyridine (5 mL) was added Ac_2O (10 mL). The mixture was stirred at room temperature for 5 h, concentrated under reduced pressure, and extracted with EtOAc and brine. The combined organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by silica gel chromatography (hexene/EtOAc = 30:1, R_f = 0.46) to yield undecaprenyl acetate (**S1**, 699 mg).

Compound **S1** (699 mg, 0.86 mmol) was redissolved in THF/MeOH (7.6 mL, v/v = 3:2), and added K_2CO_3 (635 mg, 4.58 mmol). The mixture was stirred at room temperature for 18 h, diluted with hexane, and washed with H_2O. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give pure undecaprenol (**S2**, 646 mg, 97%). $C_{55}H_{90}O$; TLC (hexene/Et_2O = 17:3) R_f = 0.24. The ^1H, ^{13}C NMR and ESI–HRMS spectra were in accordance with the assigned structure.[21]

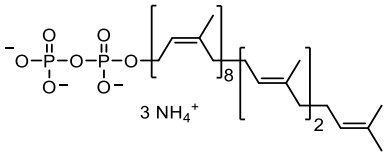
(2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*E*,30*E*,34*E*,38*E*)-3,7,11,15,19,23,27,31,35,39,43-undecamethyltetratetraconta-2,6,10,14,18,22,26,30,34,38,42-undecaen-1-yl dihydrogen phosphate (S3, undecaprenyl phosphate as the ammonium salt)



To a solution of undecaprenol (**S2**, 303 mg, 0.394 mmol) in anhydrous CH_2Cl_2 (21.8 mL) was added tetrabutylammonium dihydrogen phosphate (536 mg, 1.58 mmol). The mixture was stirred at room temperature until all solids dissolved. Then, trichloroacetonitrile (200 μL, 1.97 mmol) was added in one portion. The mixture was stirred in dark for 10 min at room temperature, and then concentrated under reduced pressure. To the resulting yellow syrup were added THF (21.6 mL) and concentrated NH_4OH aqueous solution (30%, 1.1 mL). The mixture

was stirred at room temperature for 30 min, and a toluene/MeOH solution (108 mL, v/v = 1:1) was added. The mixture was stirred for another 30 min, and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a crude product, which was subjected to silica gel chromatography (H_2O/*i*-PrOH/EtOAc = 1:2:4) to remove impurities. The fractions containing crude **S3** were collected, and subjected to ion-exchange chromatography on DEAE anionic exchange resin by successive elution with $CHCl_3$/MeOH/H_2O (10:10:3) up to $CHCl_3$/MeOH/50 mM $CH_3CO_2NH_{4(aq)}$ (10:10:3). The fractions containing the desired product **S3** were collected and lyophilized to furnish undecaprenyl phosphate (as the ammonium salt, 73 mg, 21%). $C_{55}H_{91}O_4P$; TLC (H_2O/*i*-PrOH/EtOAc = 1:2:4) R_f = 0.73; The ^1H, ^{13}C, ^{31}P NMR and ESI–HRMS spectra were in accordance with the assigned structure.[S1, S2]

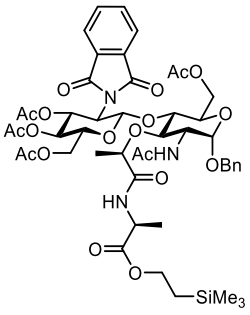
(2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*E*,30*E*,34*E*,38*E*)-3,7,11,15,19,23,27,31,35,39,43-undecamethyltetratetraconta-2,6,10,14,18,22,26,30,34,38,42-undecaen-1-yl trihydrogen diphosphate (3, undecaprenyl pyrophosphate (UPP) as the ammonium salt)



To a solution of undecaprenol (**S2**, 197 mg, 0.257 mmol) in anhydrous CH_2Cl_2 (14.3 mL) was added tetrabutylammonium dihydrogen phosphate (349 mg, 1.03 mmol). The mixture was stirred at room temperature until all solids dissolved. Then, trichloroacetonitrile (128 μL, 1.28 mmol) was added in one portion. The mixture was stirred in dark for 1 h at room temperature, and then concentrated under reduced pressure. To the resulting yellow syrup were added THF (14 mL) and concentrated NH_4OH aqueous solution (30%, 0.7 mL). The mixture was stirred at room temperature for 30 min, and a toluene/MeOH solution (70 mL, v/v = 1:1) was added. The mixture was stirred for another 20 min, and the precipitate was removed by filtration. The

filtrate was concentrated under reduced pressure to give a crude product, which was subjected to silica gel chromatography (H_2O/*i*-PrOH/EtOAc = 1:2:4) to remove impurities. The fractions containing crude product **3** were collected, and subjected to ion-exchange chromatography on DEAE anionic exchange resin by successive elution with $CHCl_3$/MeOH/H_2O (10:10:3) up to $CHCl_3$/MeOH/0.5 M $CH_3CO_2NH_{4(aq)}$ (10:10:3). The fractions containing the desired product **3** were collected and lyophilized to furnish UPP (as the ammonium salt, 10 mg, 4%). $C_{55}H_{92}O_7P_2$; TLC (H_2O/*i*PrOH/EtOAc = 1:2:4) R_f = 0.1; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.36 (1 H, br), 5.09 (10 H, br), 4.39 (1 H, br), 2.05–2.01 (40 H, m), 1.69 (1 H, s), 1.64 (21 H, s), 1.57 (12 H, s). ^{31}P{^{1}H} NMR (162 MHz, $CHCl_3$) δ –7.76, –9.08; ESI–HRMS calcd for $C_{55}H_{91}O_7P_2$: 925.6246, found: *m/z* 925.6251 $[M - H]^-$.

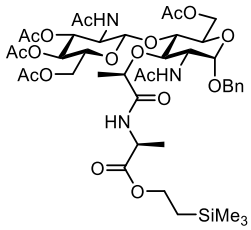
(2*R*,3*S*,4*R*,5*R*,6*S*)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-5-Acetamido-2-(acetoxymethyl)-6-(benzyloxy)-4-(((*R*)-1-oxo-1-(((*S*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)-2-(acetoxymethyl)-5-(1,3-dioxoisoindolin-2-yl)tetrahydro-2*H*-pyran-3,4-diyl diacetate (S6)[13]



TMSOTf (0.14 mL, 0.78 mmol) was added dropwise to a CH_2Cl_2 solution (78 mL) containing glycosyl acceptor **S4**[S3] (9.04 g, 15.6 mmol), glycosyl donor **S5** (2.33 g, 3.9 mmol) and 4Å MS (3.96 g) at –78 ºC. The mixture was stirred at –78 ºC for 30 min, gradually warmed to room temperature, and then stirred for 40 h at room temperature. The mixture was quenched with diisopropyl ethylamine (DIPEA, 0.67 mL, 3.9 mmol), filtered through a pad of Celite, and washed with CH_2Cl_2. The filtrate was concentrated and purified by silica gel chromatography

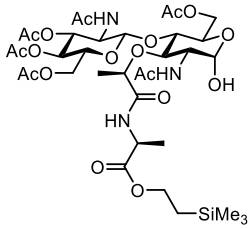
(EtOAc/toluene = 3:2) to yield compound **S6** (2.61 g, 66%). $C_{48}H_{63}N_3O_{19}Si$; TLC (EtOAc/toluene = 3:2) R_f = 0.45. The ^1H ,^{13}C NMR and ESI–HRMS spectra were in accordance with the assigned structure.[13]

(2*R*,3*S*,4*R*,5*R*,6*S*)-5-Acetamido-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-5-acetamido-2-(acetoxymethyl)-6-(benzyloxy)-4-(((*R*)-1-oxo-1-(((*S*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)-2-(acetoxymethyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate (S7)[13]



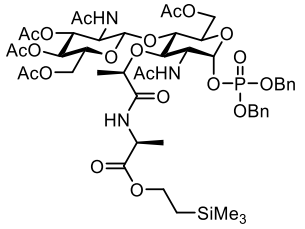
Hydrazine acetate (221 mg, 2.4 mmol) was added to a solution of compound **S6** (737.5 mg, 0.73 mmol) in MeOH (14.5 mL). The mixture was heated under reflux for 3 h. A second portion of hydrazine acetate (221 mg, 2.4 mmol) was added, and mixture was heated under reflux for additional 15 h. The mixture was concentrated under reduced pressure, subjected to azeotropic distillation with toluene twice, and dried under reduced pressure for 1 h. To the crude glycosylamine product were added anhydrous pyridine (9 mL), acetic anhydride (3.3 mL) and 4-dimethylaminopyridine (DMAP, 9 mg, 0.07 mmol) at 0 ºC. The mixture was gradually warmed to room temperature and stirred for 4 h. The mixture was concentrated under reduced pressure, and then redissolved in EtOAc. The organic phase was washed with 1 M $HCl_{(aq)}$, saturated $NaHCO_{3(aq)}$ and brine. The combined organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (EtOAc/toluene = 3:2 to 1:0) to yield compound **S7** (559 mg, 83%). $C_{42}H_{63}N_3O_{18}Si$; TLC (EtOAc) R_f = 0.58. The ^1H, ^{13}C NMR and ESI–HRMS spectra were in accordance with the assigned structure.[13]

(2*R*,3*S*,4*R*,5*R*,6*S*)-5-Acetamido-6-(((2*R*,3*S*,4*R*,5*R*)-5-acetamido-2-(acetoxymethyl)-6-hydroxy-4-(((*R*)-1-oxo-1-(((*S*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)-2-(acetoxymethyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate (S8)[13]



To a solution of compound **S7** (558 mg, 0.6 mmol) in MeOH (18 mL) was added 10% Pd/C (178 mg). The mixture was hydrogenated under an atmosphere of hydrogen for 9 h at room temperature. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel chromatography (EtOAc elution) to yield compound **S8** (466 mg, 93%). $C_{35}H_{57}N_3O_{18}Si$; TLC (EtOAc) R_f = 0.23. The ^{1}H NMR and ESI–HRMS spectra were in accordance with the assigned structure.[13]

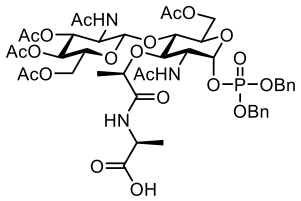
(2*R*,3*S*,4*R*,5*R*,6*S*)-5-Acetamido-6-(((2*R*,3*S*,4*R*,5*R*,6*R*)-5-acetamido-2-(acetoxymethyl)-6-((bis(benzyloxy)phosphoryl)oxy)-4-(((*R*)-1-oxo-1-(((*S*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)-2-(acetoxymethyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate (S9)[13]



Dibenzyl *N*,*N*-diisopropyl phosphoramidite (366 μL, 1.11 mmol) was added to a solution of **S8** (465 mg, 0.556 mmol) and 1*H*-tetrazole (117 mg, 1.67 mmol) in anhydrous CH_2Cl_2 (17

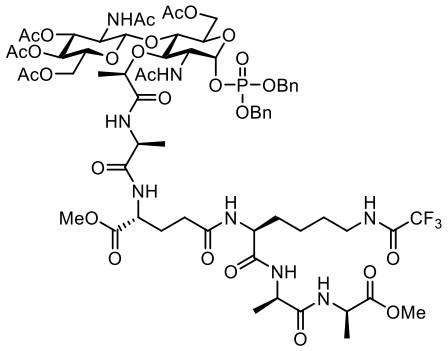
mL) at –30 °C. The mixture was gradually warmed to room temperature and stirred for 1.5 h. The mixture was cooled to –40 °C and *m*CPBA (75%, 703 mg, 3.06 mmol) was added. The mixture was slowly warmed over 1 h to 0 °C in ice-bath. The mixture was diluted with CH_2Cl_2 and washed successively with saturated $Na_2S_2O_{3(aq)}$, saturated $NaHCO_{3(aq)}$ and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (EtOAc elution) to yield compound **S9** (378 mg, 62%). $C_{49}H_{70}N_3O_{21}PSi$; TLC (EtOAc) R_f = 0.47. The ^1H, ^{13}C, ^{31}P NMR and ESI–HRMS spectra were in accordance with the assigned structure.[13]

((*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-Acetamido-5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-6-(acetoxymethyl)-2-((bis(benzyloxy)phosphoryl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanine (S10)[13]



To a solution of compound **S9** (317 mg, 0.289 mmol) in anhydrous THF (8.3 mL) was added tetrabutylammonium fluoride (TBAF, 1.1 mL of 1 M THF solution). The mixture was stirred for 75 min at room temperature, and then concentrated under reduced pressure. The crude product was dissolved in EtOAc and washed with 1 M $HCl_{(aq)}$ and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The carboxylic acid **S10** (266 mg, 93%) was obtained without further purification. $C_{44}H_{58}N_3O_{21}P$; TLC (CH_2Cl_2/MeOH = 9:1) R_f = 0.39. The ^1H, ^{13}C, ^{31}P NMR and ESI–HRMS spectra were in accordance with the assigned structure.[13]

(2*R*,3*S*,4*R*,5*R*,6*S*)-5-Acetamido-6-(((2*R*,3*S*,4*R*,5*R*,6*R*)-5-acetamido-2-(acetoxymethyl)-6-((bis(benzyloxy)phosphoryl)oxy)-4-(((4*R*,7*R*,10*S*,15*R*,18*S*,21*R*)-15-(methoxycarbonyl)-4,7,18-trimethyl-3,6,9,12,17,20-hexaoxo-10-(4-(2,2,2-trifluoroacetamido)butyl)-2-oxa-5,8,11,16,19-pentaazadocosan-21-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)-2-(acetoxymethyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate (S13)[13]

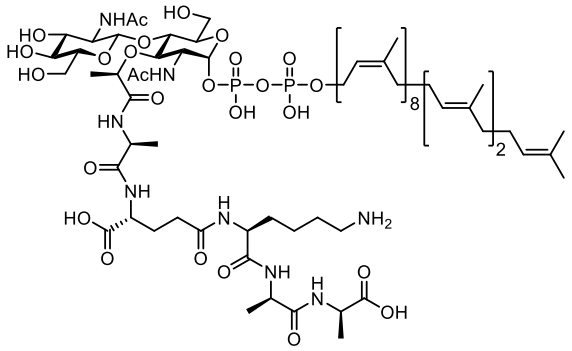


To a solution of tetrapeptide **S11**[S4] (71 mg, 0.11 mmol) in anhydrous CH_2Cl_2 (1.1 mL) was added trifluoroacetic acid (TFA, 1.1 mL). The mixture was stirred for 3 h at room temperature, concentrated under reduced pressure, azeotropically distilled with toluene for three times, and then dried under reduced pressure for 1 h to obtain a tetrapeptide derivative **S12**. On the other hand, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU, 42 mg, 0.11 mmol) and DIPEA (19.2 μL, 0.11 mmol) were added to a suspension of acid **S10** (100 mg, 0.1 mmol) in anhydrous DMF (0.5 mL) at 0 °C in an ice-bath. The mixture was stirred for 10 min at 0 °C to obtain an activated carboxylate. A solution of tetrapeptide **S12** and DIPEA (19.2 μL, 0.11 mmol) in anhydrous DMF (0.6 mL) was transferred to the solution of activated carboxylic acid. Another portion of DIPEA (35 μL, 0.2 mmol) was added at 0 °C. The ice-bath was removed and the mixture was stirred for 24 h. The mixture was concentrated under reduced pressure, and the residue was dissolved in $CHCl_3$. The organic phase was washed with 1 M $HCl_{(aq)}$ and saturated $NaHCO_{3(aq)}$. The aqueous phase was back-extracted with $CHCl_3$. The combined organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel

chromatography (CH_2Cl_2/MeOH = 20:1) to yield compound **S13** (117 mg, 77%). $C_{65}H_{90}F_3N_8O_{28}P$; TLC ($CH_2Cl_2$/MeOH = 9:1) R_f = 0.39; ^{1}H NMR (500 MHz, $CDCl_3$, a mixture of atropisomers) δ 8.18 (1 H, br), 7.97–7.90 (1 H, m), 7.68 (1 H, m), 7.56 (1 H, d, *J* = 6.4 Hz), 7.43–7.39 (2 H, m), 7.31–7.28 (10 H, m), 6.57 (1 H, br), 5.69–5.64 (1 H, m), 5.18 (1 H, t, *J* = 8.2 Hz), 5.05–4.91 (6 H, m), 4.60–4.41 (6 H, m), 4.30–4.21 (3 H, m), 4.10 (2 H, br), 4.05–4.01 (1 H, m), 3.87–3.81 (3 H, m), 3.70 (1 H, t, *J* = 9.8 Hz), 3.65 (3 H, s), 3.62 (3 H, s), 3.30–3.26 (2 H, m), 2.29–2.21 (3 H, m), 2.08 (3 H, s), 2.01–1.97 (12 H, m), 1.89–1.80 (6 H, m), 1.45–1.33 (16 H, m); ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$, a mixture of atropisomers) δ 174.4, 174.1, 173.3, 173.2, 173.1, 172.7, 172.4, 172.3, 172.2, 172.19, 172.12, 171.9, 171.6, 171.3, 170.97, 170.92, 170.8, 170.79, 170.72, 170.6, 170.5, 169.5, 169.4, 157.5 (quart, $J_{C\text{-}F}$ = 36 Hz), 135.4, 135.3, 135.27, 135.21, 135.1, 128.8, 128.7, 128.6, 128.4, 128.3, 128.0, 127.9, 127.7, 116.0 (quart, $J_{C\text{-}F}$ = 286 Hz), 100.1, 99.8, 96.2, 78.4, 77.5, 75.9, 74.7, 72.2, 72.0, 71.8, 71.2, 71.1, 69.9, 69.85, 69.82, 69.78, 69.7, 69.6, 68.9, 68.8, 68.2, 61.9, 61.7, 54.8, 54.6, 54.1, 55.5, 53.1, 52.9, 52.4, 52.32, 52.30, 52.2, 51.6, 50.7, 50.6, 49.2, 48.9, 48.4, 48.1, 48.0, 39.5, 39.3, 31.6, 31.5, 31.0, 28.2, 28.1, 27.5, 23.0, 22.9, 22.7, 22.6, 22.5, 20.8, 20.7, 20.6, 20.56, 20.54, 20.52, 20.4, 19.1, 19.0, 17.7, 17.6, 17.5, 17.4, 17.3; ^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$, a mixture of atropisomers) δ –2.98/–3.12; ESI–HRMS calcd for $C_{65}H_{90}F_3N_8NaO_{28}P$: 1541.5466, found: *m/z* 1541.5408 $[M + Na]^+$.

***N*^{2}-(((2*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-2-((hydroxy((hydroxy(((2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*E*,30*E*,34*E*,38*E*)-3,7,11,15,19,23,27,31,35,39,43-undecamethyltetratetraconta-2,6,10,14,18,22,26,30,34,38,42-undecaen-1-yl)oxy)phosphoryl)oxy)phosphoryl)oxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-*N*^{5}-((*S*)-6-amino-1-(((*R*)-1-(((*R*)-1-carboxyethyl)amino)-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)-D-**

glutamine (1, lipid II)[22]

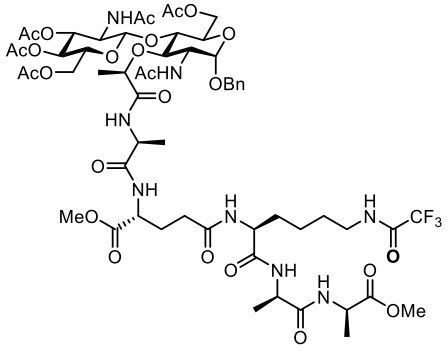


To a solution of **S13** (139 mg, 91.2 μmol) in EtOH (6.2 mL) was added 10% Pd/C (52 mg). The mixture was hydrogenated under an atmosphere of hydrogen for 1 h. The catalyst was filtered through a pad of Celite, and pyridine (0.8 mL) was added to the filtrate. The mixture was concentrated under reduced pressure to give a crude phosphate **S14** without further purification. The ESI–HRMS analysis of **S14** showed a molecular ion at *m/z* 1339.4680 (calcd for $C_{51}H_{79}F_3N_8O_{28}P$: 1339.4680, $[M - 2\times C_5H_5N + H]^+$).

To a solution of above-prepared disaccharyl phosphate **S14** in anhydrous DMF/THF solution (5.4 mL, v/v = 1:1) was added carbonyldiimidazole (CDI, 74 mg, 456 μmol). The mixture was stirred for 2 h at room temperature. After the reaction was complete, anhydrous MeOH (19.3 μL, 477 mmol) was added and stirred for 45 min at room temperature to destroy excess CDI. The mixture was concentrated under reduced pressure and dried in vacuum for 1 h. To the activated phosphate was added undecaprenyl phosphate (**S3**, 73.1 mg, 82.9 μmol) in anhydrous THF (5.4 mL). The mixture was stirred for 4 days at room temperature, and then concentrated under reduced pressure. The ESI-HRMS analysis of this product showed a molecular ion at *m/z* 2166.1102, which was attributable to the preacetylation derivative of protected lipid II. (calcd for $C_{106}H_{166}F_3N_8O_{31}P_2$: 2166.1092, $[M - H]^-$). The protected lipid II derivative was dissolved in CH_2Cl_2 (30 mL), washed with H_2O (6 mL) twice and then concentrated to dryness under reduced pressure. The mixture was dissolved in dioxane (3.5 mL) and 1 M $NaOH_{(aq)}$ (3.5 mL) was added. The mixture was stirred for 2 h at room temperature,

carefully neutralize to pH 7–8 with 1 M $HCl_{(aq)}$, and filtered through a polyvinylidene difluoride (PVDF) pad. The filtrate was concentrated under reduced pressure and purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O/30% $NH_4OH_{(aq)}$ = 88:48:10:1). The fraction containing product was further purified by reverse-phase HPLC on a ZORBAX RX-C8 column (5 μm particle, 9.4 × 250 mm) using a gradient elution with A/MeOH solution (15:85 to 0:100) over 60 min at a flow rate of 1 mL/min, where A solution is 50 mM $NH_4HCO_{3(aq)}$. The retention time of the desired product was 26 min (detection at 214 nm wavelength). Lyophilization of this pure fraction gave lipid II (**1**, 4.5 mg, 3%). $C_{94}H_{156}N_8O_{26}P_2$; TLC ($CHCl_3$/MeOH/$H_2O$/$NH_4OH$ = 88:48:10:1) R_f = 0.28; ESI–HRMS calcd for $C_{94}H_{154}N_8O_{26}P_2$: 936.5230, found: *m/z* 936.5230 $[M - 2H]^{2-}$.

(2*R*,3*S*,4*R*,5*R*,6*S*)-5-Acetamido-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-5-acetamido-2-(acetoxymethyl)-6-(benzyloxy)-4-(((4*R*,7*R*,10*S*,15*R*,18*S*,21*R*)-15-(methoxycarbonyl)-4,7,18-trimethyl-3,6,9,12,17,20-hexaoxo-10-(4-(2,2,2-trifluoroacetamido)butyl)-2-oxa-5,8,11,16,19-pentaazadocosan-21-yl)oxy)tetrahydro-2*H*-pyran-3-yl)oxy)-2-(acetoxymethyl)tetrahydro-2*H*-pyran-3,4-diyl diacetate (S16)[22]



By a procedure similar to that for compound **S13**, TBAF (1.0 M in THF, 0.22 mL) was added to a solution of **S7** (100 mg, 0.108 mmol) in anhydrous THF (10 mL). The mixture was stirred for 2 h at room temperature and then evaporated to dryness. The crude product was dissolved in EtOAc and washed with 1 M $HCl_{(aq)}$ and brine. The organic phase was dried over

$MgSO_4$, filtered and concentrated under reduced pressure. The carboxylic acid **S15** (74 mg, 83%) was obtained without further purification.

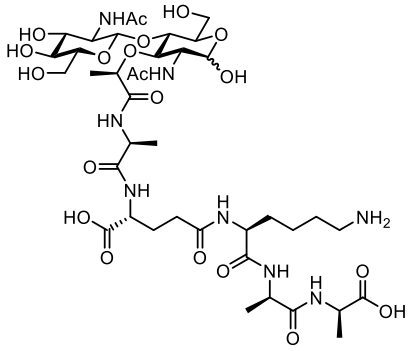
HATU (31 mg, 81 μmol) and DIPEA (14.2 μL, 81 μmol) were added to a suspension of acid **S15** (60 mg, 72.6 μmol) in anhydrous DMF (0.43 mL) at 0 °C in an ice-bath. The mixture was stirred for 10 min at 0 °C to give an activated carboxylate. The deprotected tetrapeptide derivative **S12** in anhydrous DMF (0.43 mL) and DIPEA (14.2 μL, 81 μmol) was transferred to the solution of activated carboxylate. Another portion of DIPEA (25.4 μL, 145 μmol) was added at 0 °C. The ice-bath was removed, and the mixture was stirred for 24 h. The mixture was concentrated under reduced pressure, and the crude product was dissolved in $CHCl_3$. The organic phase was washed with 1 M $HCl_{(aq)}$ and saturated $NaHCO_{3(aq)}$. The aqueous phase was back-extracted with $CHCl_3$. The combined organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Compound **S16** (97 mg, 99%) was obtained without further purification.

Compound **S15**: $C_{37}H_{51}N_3O_{18}$; TLC (EtOAc) R_f = 0.19; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (1 H, br), 1.29–7.22 (5 H, m), 6.69 (1 H, br), 5.09–4.99 (3 H, m), 4.57 (1 H, d, J = 12.0 Hz), 4.49–4.42 (4 H, m), 4.28 (1 H, d, J = 9.2 Hz), 4.22–4.12 (2 H, m), 4.02–3.97 (2 H, m), 3.93–3.88 (1 H, m), 3.74–3.70 (2 H, m), 3.59–3.55 (2 H, m), 2.08 (3 H, s), 1.97–1.92 (12 H, m), 1.88 (3 H, s), 1.41 (3 H, d, J = 6.8 Hz), 1.35 (3 H, d, J = 6.4 Hz); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 174.3, 174.1, 171.5, 171.4, 171.3, 170.8, 170.6, 169.3, 137.2, 129.4, 127.8, 127.6, 99.9, 96.3, 72.5, 71.7, 69.9, 69.4, 68.1, 62.4, 61.6, 54.5, 53.6, 48.1, 22.9, 22.8, 20.9, 20.8, 20.5, 18.8, 17.7; ESI–HRMS calcd for $C_{37}H_{52}N_3O_{18}$: 826.3240, found: *m/z* 826.3276 $[M + H]^+$.

Compound **S16**: $C_{58}H_{83}F_3N_8O_{25}$; TLC (EtOAc/MeOH = 9:1) R_f = 0.69; ^{1}H NMR (500 MHz, 1:1 $CD_3OD/CDCl_3$) δ 7.33–7.24 (5 H, m), 5.23–5.16 (1 H, m), 5.00 (1 H, t, J = 7.6 Hz), 4.97–4.92 (1 H, m), 4.64–4.59 (1 H, m), 4.55 (1 H, d, J = 6.8 Hz), 4.51–4.26 (8 H, m), 4.21–4.14 (1 H, m), 4.06–4.01 (2 H, m), 3.96–3.86 (2 H, m), 3.78–3.77 (2 H, m), 3.72–3.65 (7 H, m), 3.60–3.53 (1 H, m), 3.24 (2 H, br), 2.36–2.20 (4 H, m), 2.10 (3 H, s), 2.02–1.97 (9 H, m), 1.80-1.88

(6 H, m). 1.78–1.72 (1 H, m), 1.69–1.62 (1 H, m), 1.57–1.52 (2 H, m), 1.40–1.33 (14 H, m); $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$) δ 175.8, 175.6, 174.3, 174.1, 174.0, 173.97, 173.95, 173.8, 173.6, 172.9, 172.8, 172.6, 172.2, 171.8, 171.47, 171.45, 170.79, 170.76, 158.6 (quart, $J_{C\text{-}F}$ = 37.5 Hz), 137.8, 137.7, 129.12, 129.1, 128.7, 116.9 (quart, $J_{C\text{-}F}$ = 285 Hz), 100.9, 100.8, 96.8, 96.6, 77.8, 77.5, 77.2, 77.1, 76.4, 73.3, 73.2, 72.3, 72.2, 70.4, 70.1, 69.9, 69.5, 69.4, 63.3, 63.2, 65.6, 62.5, 55.4, 55.3, 54.8, 54.7, 54.5, 54.4, 52.9, 52.8, 52.7, 52.3, 51.9, 50.3, 40.1, 40.0, 32.1, 31.9, 31.7, 30.3, 30.0, 29.0, 28.9, 23.6, 23.0, 22.9, 22.8, 21.1, 21.0, 20.9, 20.88, 20.82, 19.4, 19.3, 18.4, 18.1, 17.7, 17.6, 17.5, 17.4; ESI–HRMS calcd for $C_{58}H_{84}F_3N_8O_{25}$: 1349.5494, found: *m/z* 1349.5503 $[M + H]^+$.

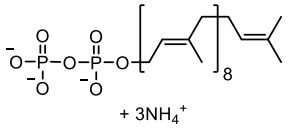
N^2-(((*R*)-2-(((3*R*,4*R*,5*S*,6*R*)-3-Acetamido-5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-2-hydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((*S*)-6-amino-1-(((*R*)-1-(((*R*)-1-carboxyethyl)amino)-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)-D-glutamine (2, PGM)[22]



To a solution of **S16** (57 mg, 42.3 μmol) in MeOH (1.3 mL) was added 10% Pd/C (50 mg). The mixture was hydrogenated under an atmosphere of hydrogen for 9 h. The catalyst was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to obtain a lactol intermediate. The lactol intermediate was dissolved in 9:1 MOH/H_2O solution (2.1 mL), and LiOH (17.7 mg, 0.42 mmol) was added. The mixture was stirred for 3 h at room

temperature, and neutralized to pH 7.0 with 1 M of $HCl_{(aq)}$. The mixture was concentrated under reduced pressure and purified by silica gel chromatography (*i*PrOH/35% $NH_4OH_{(aq)}$ = 2:1) to yield PGM (**2**, 24.2 mg, 59%). $C_{39}H_{66}N_8O_{20}$, TLC (*i*PrOH/35% $NH_4OH_{(aq)}$ = 2:1) R_f = 0.42; ^{1}H NMR (400 MHz, D_2O) δ 5.21–5.10 (1 H, m), 4.60–4.53 (1 H, m), 4.36–4.26 (4 H, m), 4.20–4.04 (3 H, m), 3.93–3.65 (7 H, m), 3.53–3.40 (4 H, m), 2.97 (2 H, t, *J* = 7.0 Hz), 2.36–2.23 (2 H, m), 2.16–1.97 (7 H, m), 1.94–1.86 (1 H, m), 1.80–1.74 (2 H, m), 1.67 (2 H, quint, *J* = 7.6 Hz), 1.41–1.30 (14 H, m); ESI–HRMS calcd for $C_{39}H_{67}N_8O_{20}$: 967.4466, found: *m/z* 967.4462 $[M + H]^+$.

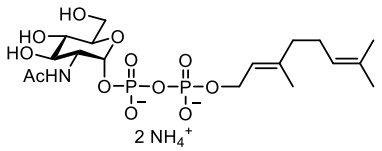
(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*)-3,7,11,15,19,23,27,31,35-nonamethyl-hexatriaconta-2,6,10,14,18,22,26,30,34-nonaen-1-yl diphosphate (6, solanesyl pyrophosphate (SPP) as the ammonium salt).[20]



Tetrabutylammonium dihydrogen phosphate (286 mg, 0.84 mmol) was added to a solution of solanesol (**S17**, 133 mg, 0.21 mmol) in anhydrous CH_2Cl_2 (2.7 mL). The mixture was stirred at room temperature until all solids dissolved. Then, trichloroacetonitrile (105 μL, 1.05 mmol) was added in one portion. The mixture was stirred in dark at room temperature for 14 h, and then concentrated under reduced pressure. To the resulting yellow syrup were added THF (2.0 mL) and concentrated NH_4OH aqueous solution (30%, 0.42 mL). The mixture was stirred at room temperature for 30 min, and a toluene/MeOH solution (11 mL, v/v = 1:1) was added. The mixture was stirred for another 30 min, and the precipitate was removed by filtration. The filtrate was concentrated under reduced pressure to give a crude product, which was purified by ion-exchange chromatography on DEAE anionic exchange resin. The crude product was obtained by successive elution with $CHCl_3$/MeOH/H_2O (10:10:3), $CHCl_3$/MeOH/5 mM aqueous $CH_3CO_2NH_4$ (10:10:3) and $CHCl_3$/MeOH/100 mM aqueous $CH_3CO_2NH_4$ (10:10:3).

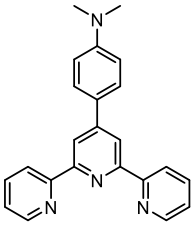
The fractions containing SPP (**6**, as the ammonium salt) were collected and lyophilized to furnish white solids (55 mg, 32% yield). $C_{45}H_{85}N_3O_7P_2$; TLC (H_2O/*i*-PrOH/EtOAc = 1:2:4) R_f = 0.18. The ^1H, ^{31}P NMR and ESI–HRMS spectra were in accordance with the assigned structure.[17, 20]

P^1-2-Acetamido-2-deoxy-α-D-glucopyranosyl-P^2-geranyl diphosphate (7, GlcNAc-GPP as the ammonium salt).



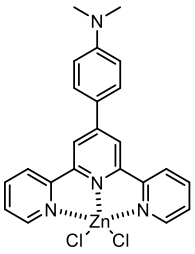
The peracetylated GlcNAc phosphate (**S22**) was prepared from glycosamine (**S18**) according to the known procedure (Scheme S5).[17] To a solution of geranyl phosphate (**S24**, 111 mg, 0.41 mmol) in anhydrous DMF (5.0 mL) was added carbonyldiimidazole (CDI, 335 mg, 2.07 mmol) in anhydrous DMF (5.0 mL). The mixture was stirred at room temperature for 3 h. Anhydrous MeOH (67 μL) was added to destroy excess CDI. The mixture was stirred for additional 30 min, and then concentrated under reduced pressure. A solution of **S22** (392 mg, 0.74 mmol) in DMF (5 mL) was added. The mixture was stirred at room temperature for 3 days, concentrated under reduce pressure, and purified by silica gel chromatography (H_2O/*i*-PrOH/EtOAc = 1:2:4). The eluate was concentrated under reduced pressure. The residue was dissolved in ammonia (33% $NH_4OH_{(aq)}$, 1 mL) and stirred at room temperature for 8 h. After concentration under reduced pressure, the residue was subjected to silica gel chromatography (H_2O/*i*-PrOH/EtOAc = 1:2:4) to afford GlcNAc-GPP (compound **7**, 56 mg, 15%). $C_{18}H_{39}N_3O_{12}P_2$; TLC (H_2O/*i*-PrOH/EtOAc = 1:2:4) R_f = 0.13. The ^1H, ^{13}C, ^{31}P NMR and ESI–HRMS spectra were in accordance with the assigned structure.[17]

4′-(4-Dimethylamino)phenyl-2,2′:6′,2″-terpyridine (Tpy ligand, S27)[17, 23]



To a solution of 4-(dimethylamino)benzaldehyde (2.98 g, 20 mmol) in EtOH (100 mL) was added 2-acetylpyridine (4.84 g, 40 mmol). KOH pellets (3.30 g, 85%, 50 mmol) and aqueous ammonia (18 M, 58 mL) were subsequently added. The mixture was stirred for 18 h at room temperature. The precipitate was filtered, washed three times with EtOH, and dissolved in $CHCl_3$. Excess hexane was added, and the greenish solids were collected by filtration. The solids were rinsed with hexane, and dried in air to afford the Tpy ligand **S27** (2.16 g, 31%). The ^1H, ^{13}C NMR and ESI–HRMS spectra were in accordance with the assigned structure.[17, 23]

4′-(4-Dimethylamino)phenyl-2,2′:6′,2″-terpyridine zinc complex (4, Tpy-Zn)[17, 23]



To a solution of Tpy ligand **S27** (2.16 g, 6.13 mmol) in CH_2Cl_2 (20 mL) was added a solution of $ZnCl_2$ in MeOH (490 mM, 15 mL). The mixture was stirred at room temperature for 30 min. The precipitate was collected by filtration, and rinsed with water, MeOH and Et_2O to give Tpy-Zn complex (**4**) ($C_{23}H_{20}N_4ZnCl_2$, 1.95 g, 65%). The ^1H NMR and ESI–HRMS spectra were in accordance with the assigned structure.[17, 23]

Supplementary References

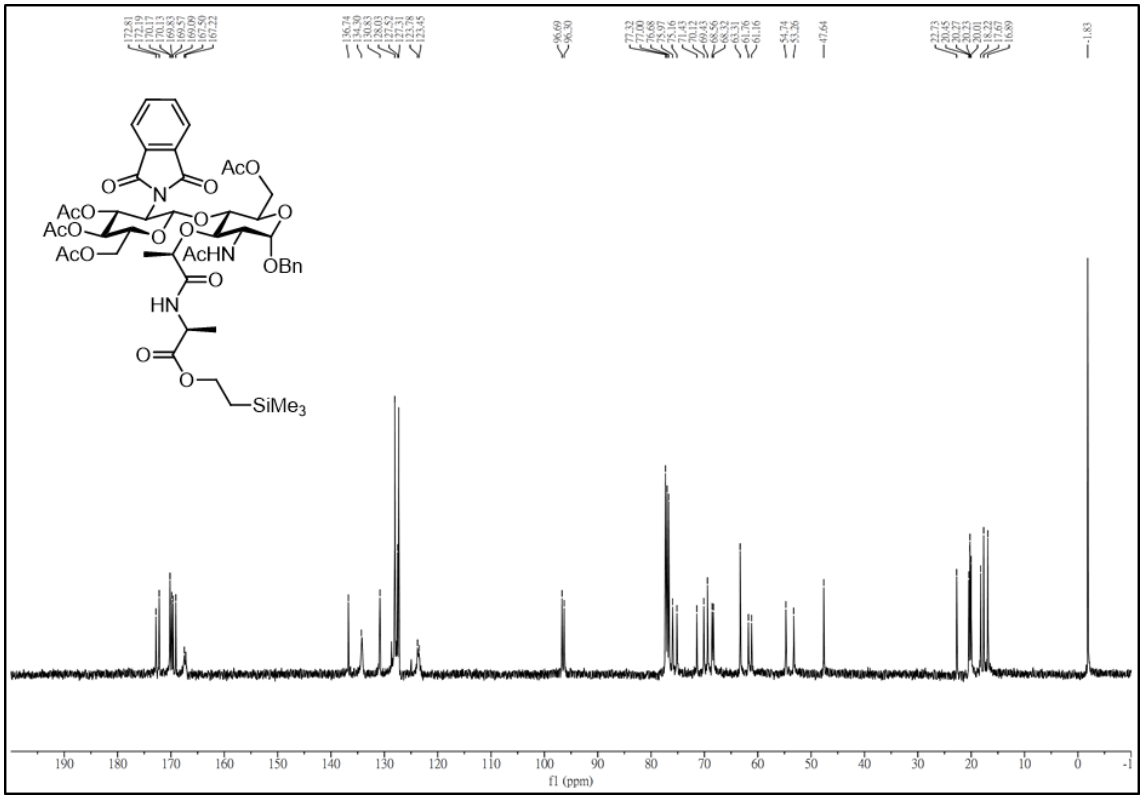
[S1] R. T. Gale, E. W. Sewell, T. A. Garrett, E. D. Brown, *Chem. Sci.*, 2014, **5**, 3823–3830.

[S2] L.-Y. Huang, S.-H. Huang, Y.-C. Chang, W.-C. Cheng, T.-J. R. Cheng, C.-H. Wong, *Angew. Chem. Int. Ed.*, 2014, **53**, 8060–8065.

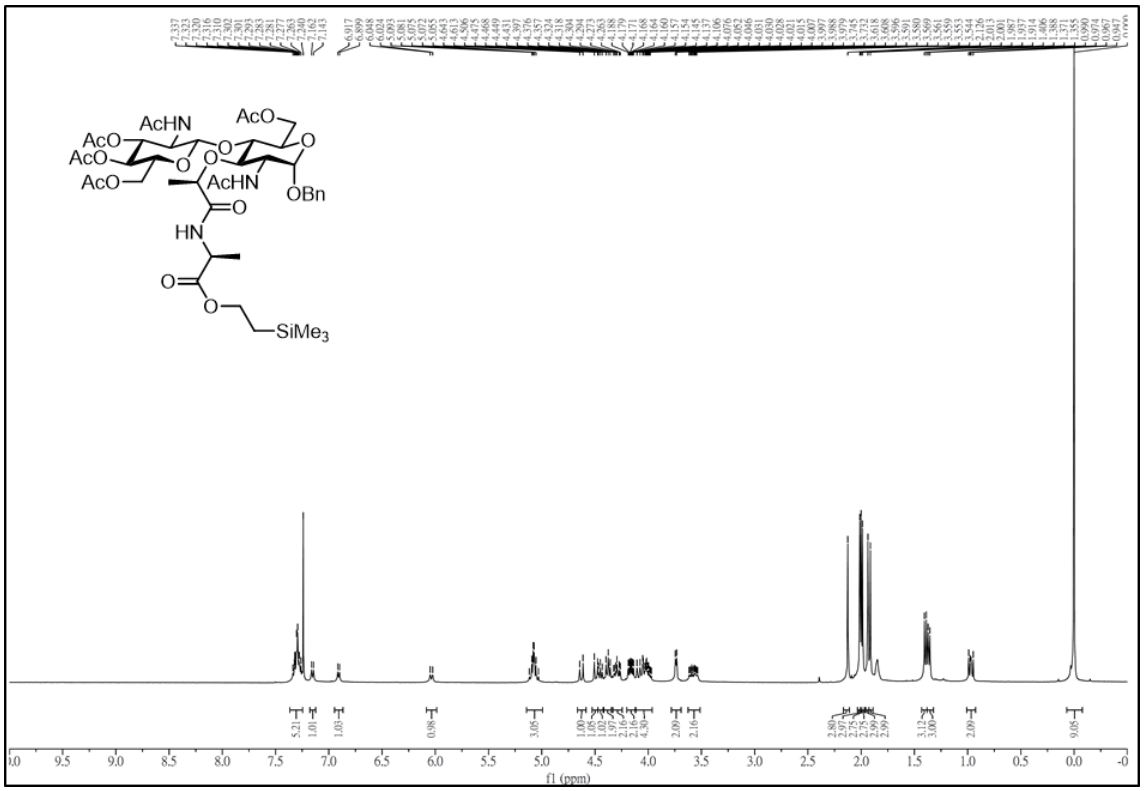
[S3] B. Schwartz, J. A. Markwalder, Y. Wang, *J. Am. Chem. Soc.*, 2001, **123**, 11638–11643.

[S4] M. S. VanNieuwenhze, S. C. Mauldin, M. Zia-Ebrahimi, J. A. Aikins, L. C. Blaszczak, *J. Am. Chem. Soc.*, 2001, **123**, 6983–6988.

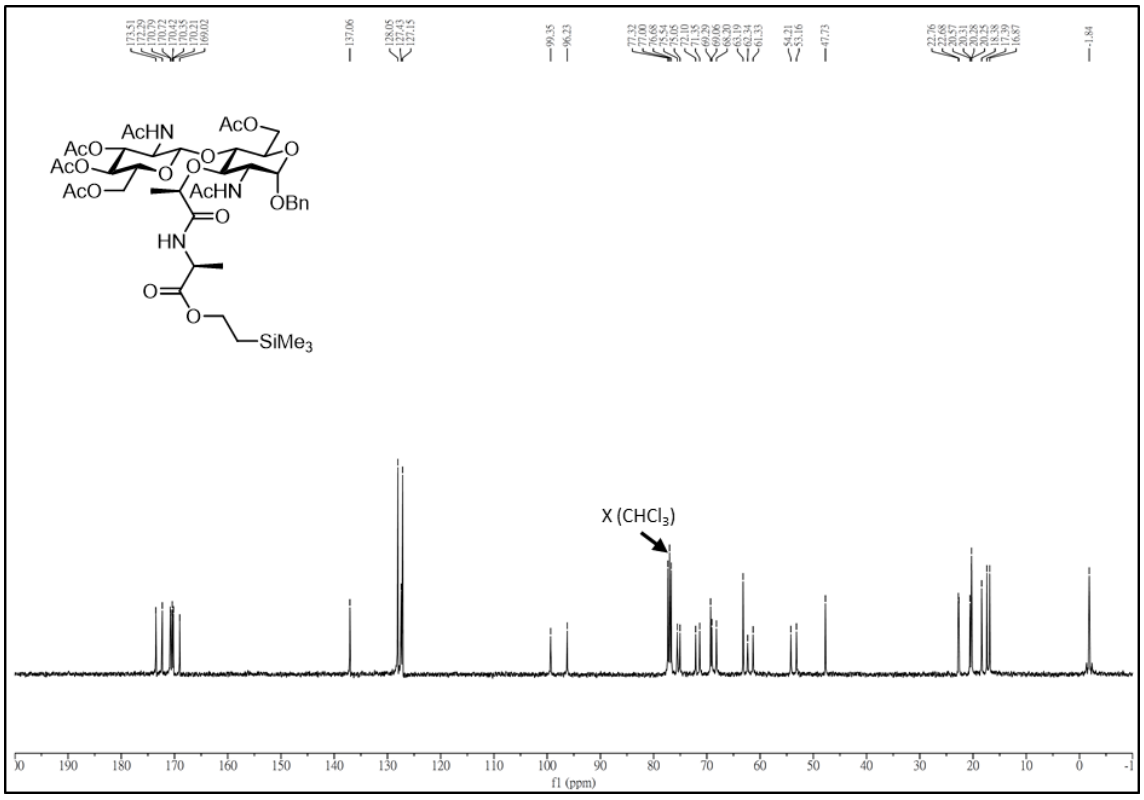
^{1}H NMR spectrum of compound **S6** (400 MHz, $CDCl_3$)



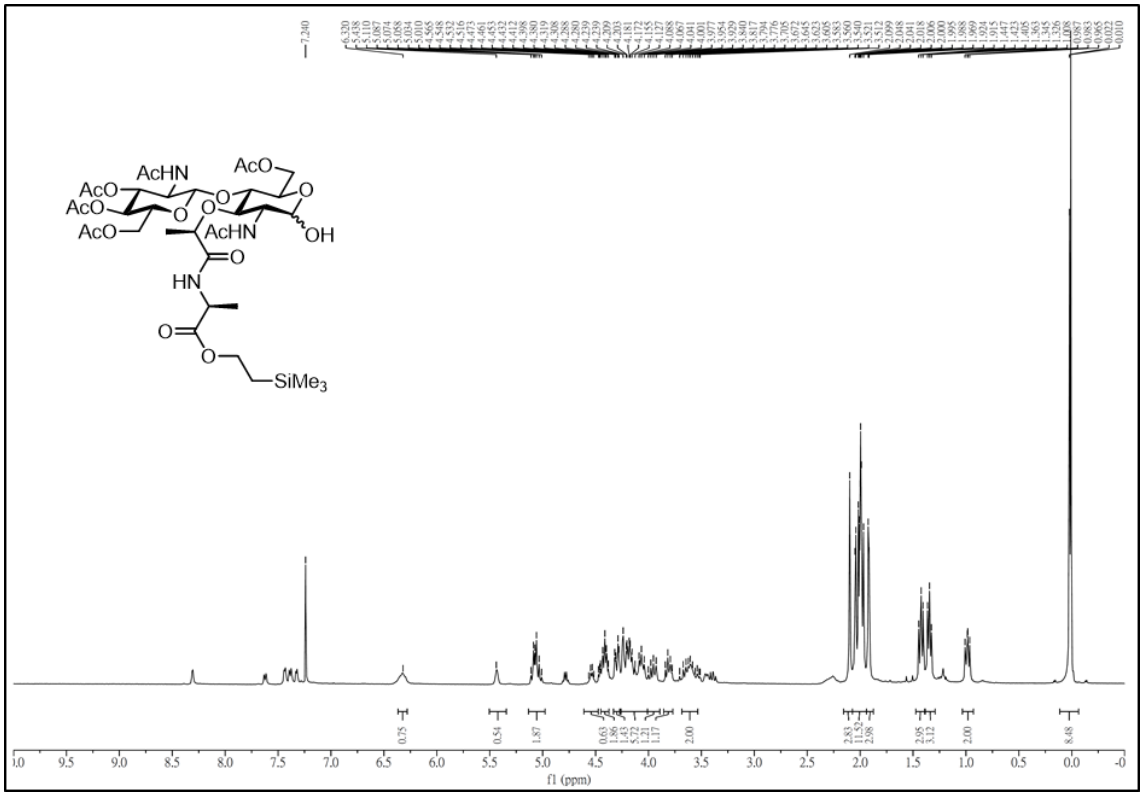
^{13}C NMR spectrum of compound **S6** (100 MHz, $CDCl_3$)



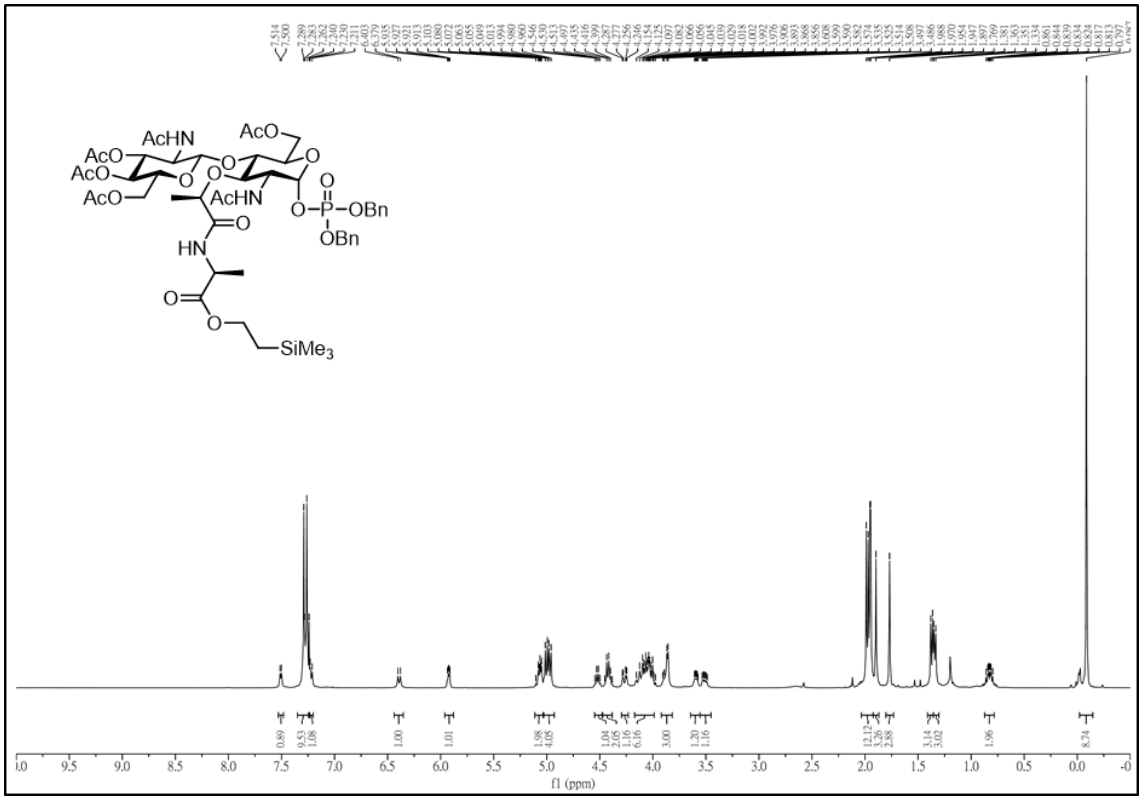
^{1}H NMR spectrum of compound **S7** (400 MHz, $CDCl_3$)



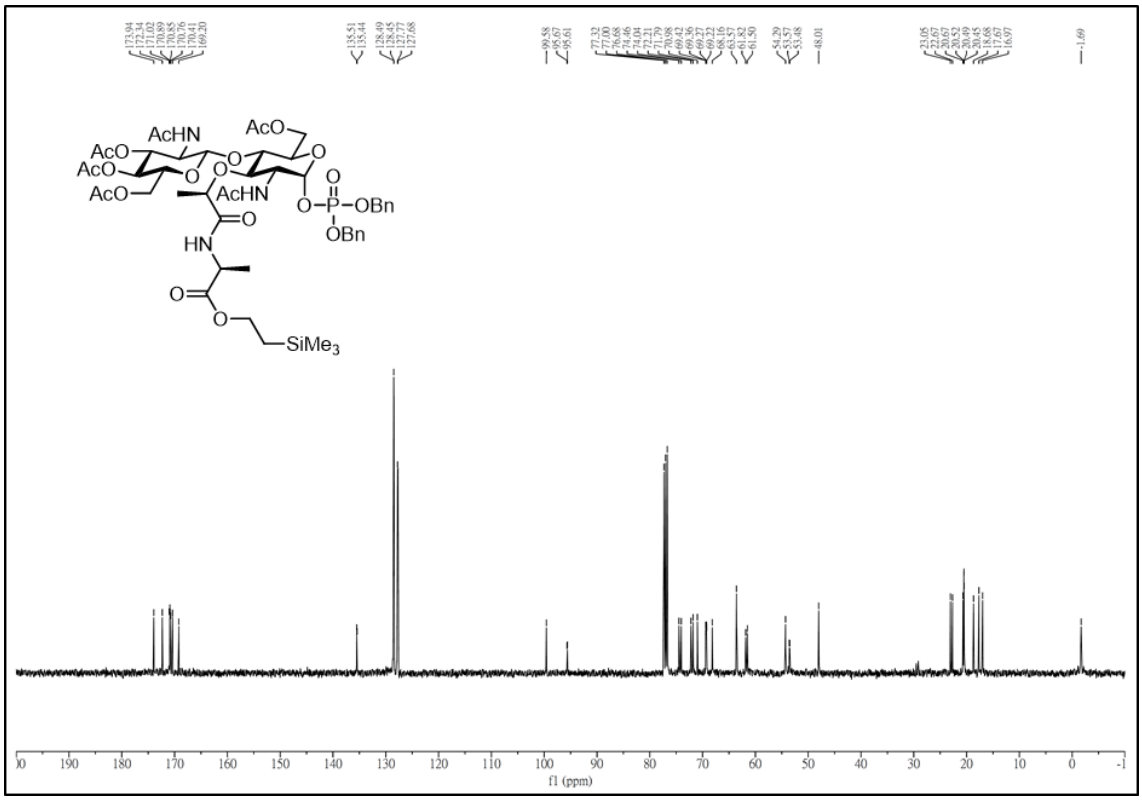
^{13}C NMR spectrum of compound **S7** (100 MHz, $CDCl_3$)



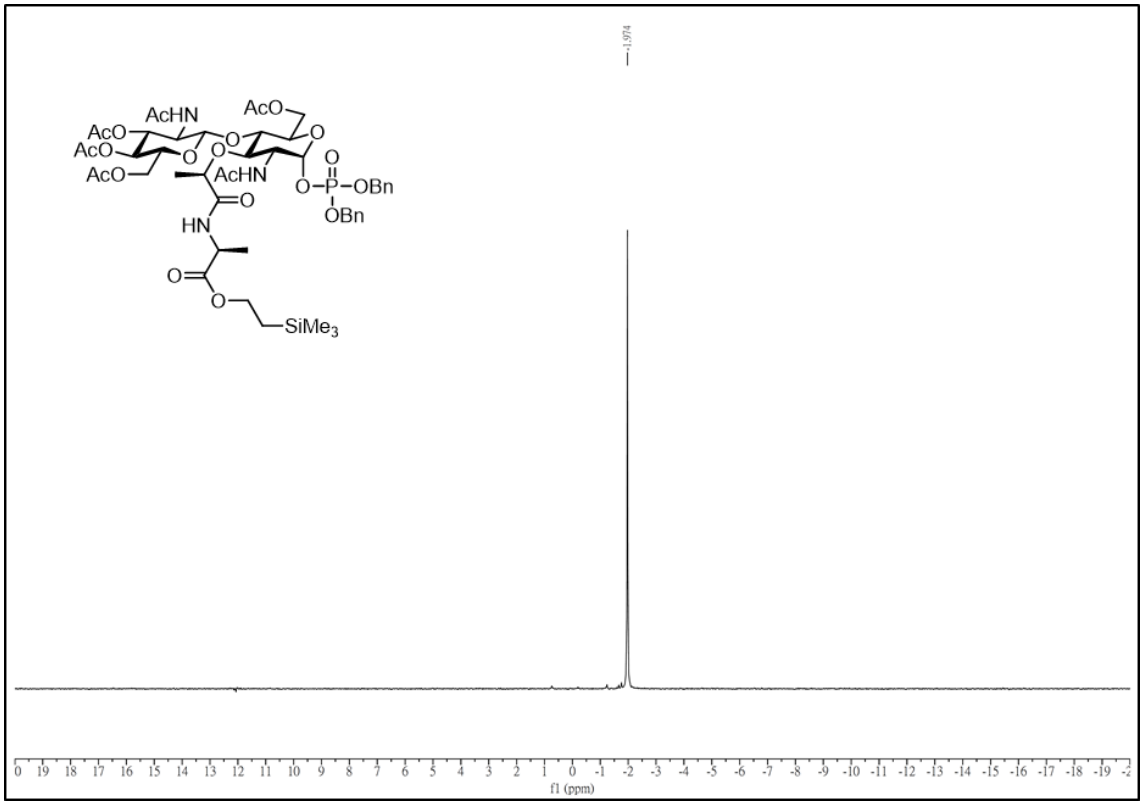
^{1}H NMR spectrum of compound **S8** (400 MHz, $CDCl_3$)



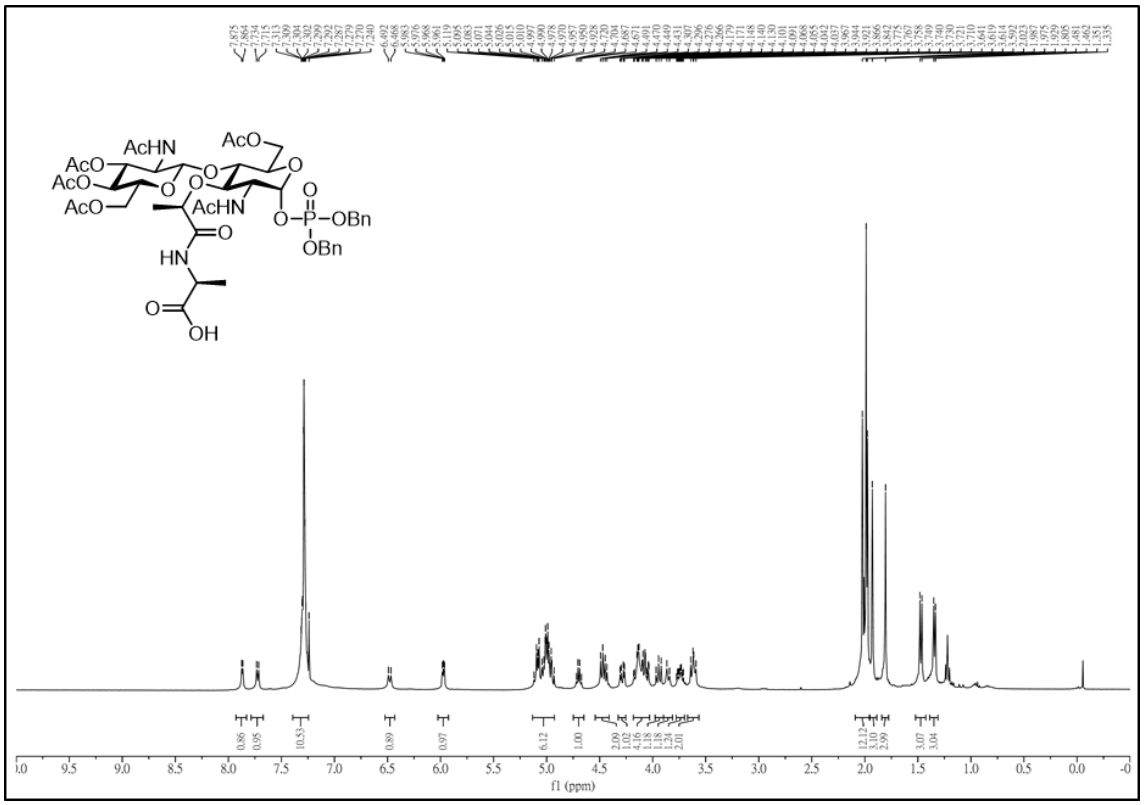
^{1}H NMR spectrum of compound **S9** (400 MHz, $CDCl_3$)



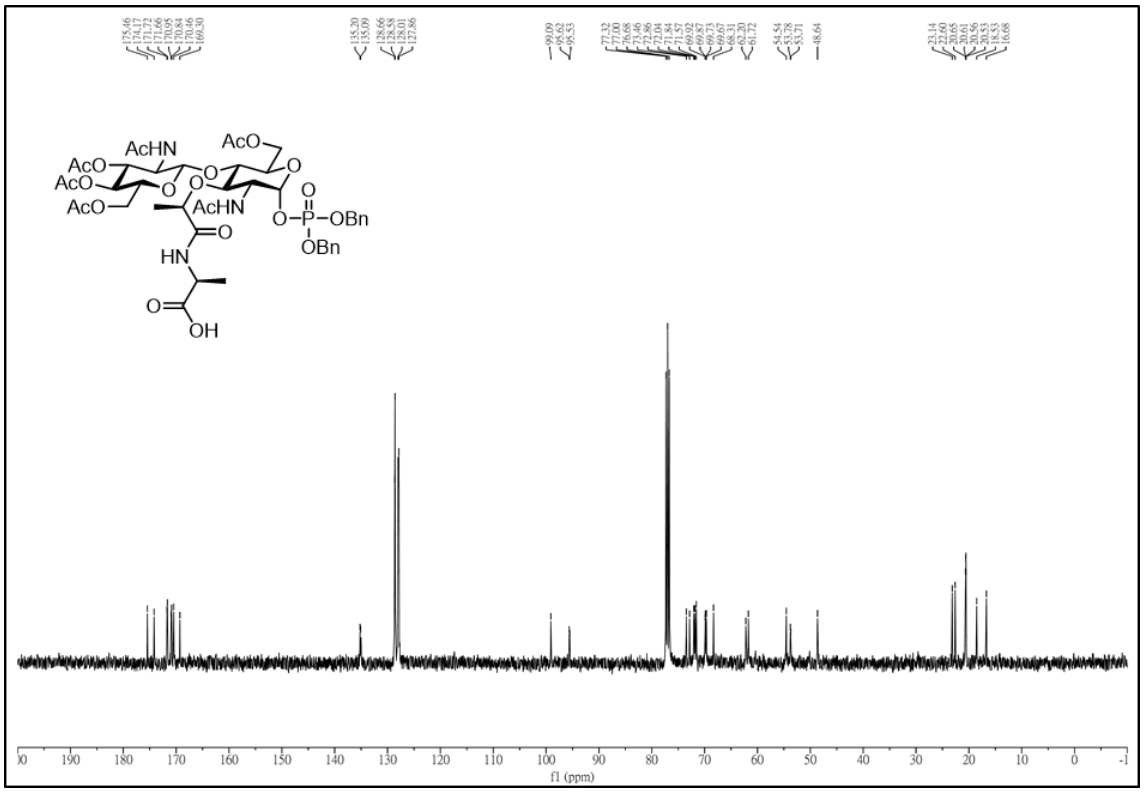
^{13}C NMR spectrum of compound **S9** (100 MHz, $CDCl_3$)



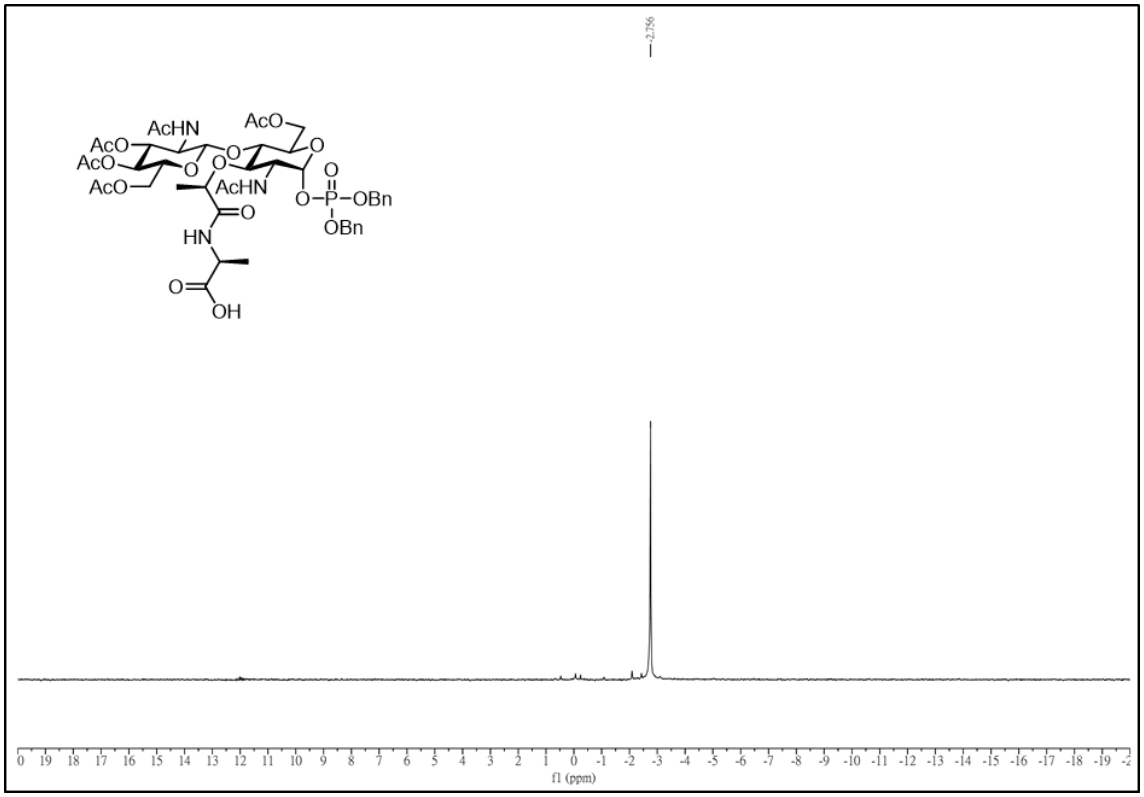
^{31}P NMR spectrum of compound **S9** (162 MHz, $CDCl_3$)



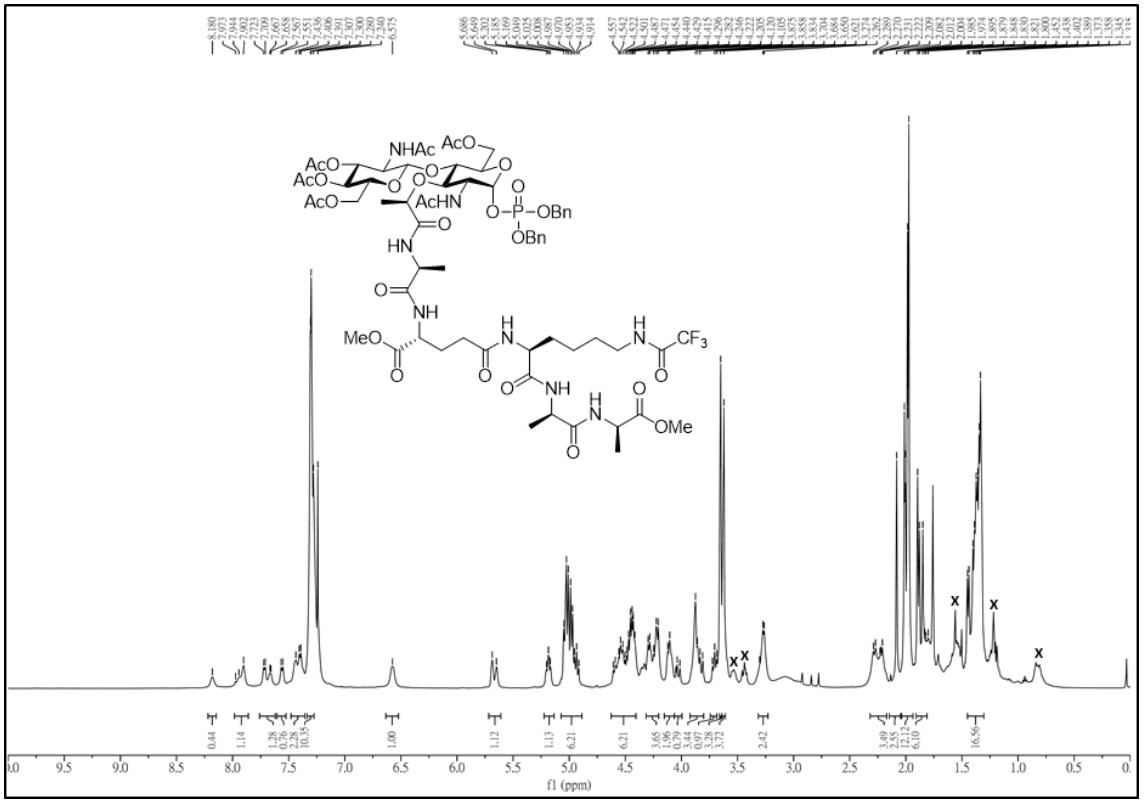
^{1}H NMR spectrum of compound **S10** (400 MHz, $CDCl_3$)



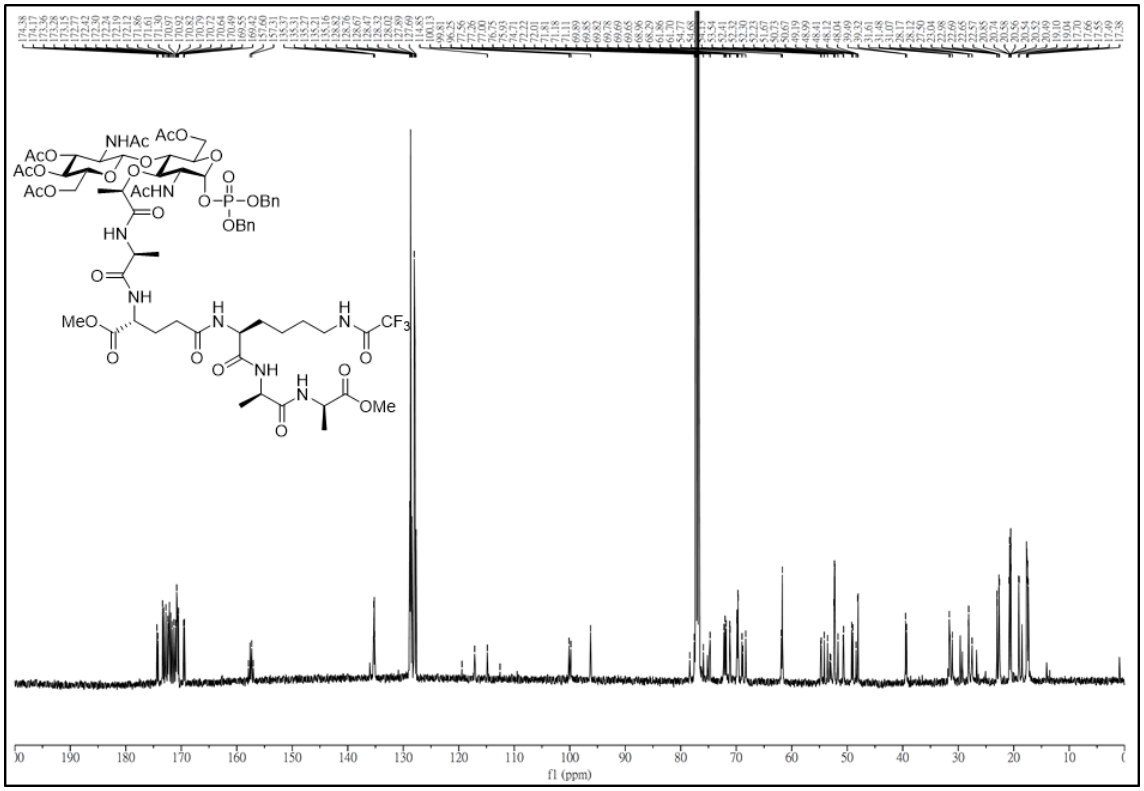
^{13}C NMR spectrum of compound **S10** (100 MHz, $CDCl_3$)



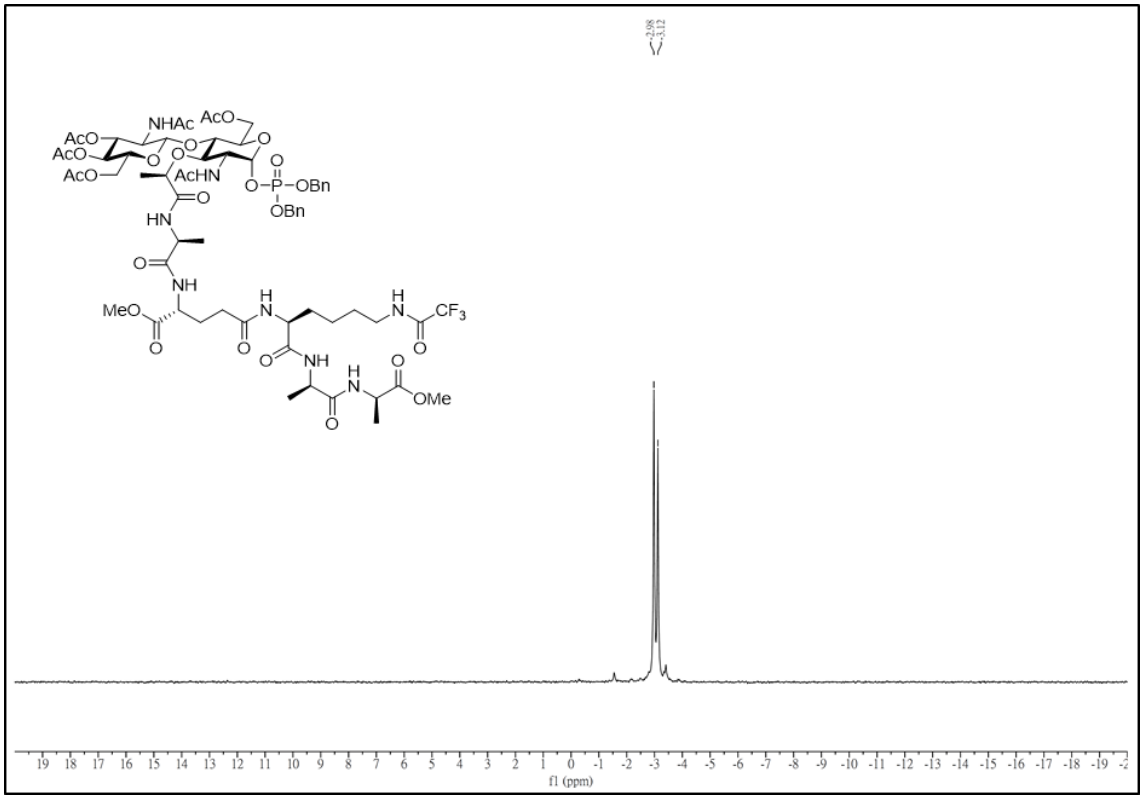
^{31}P NMR spectrum of compound **S10** (162 MHz, $CDCl_3$)



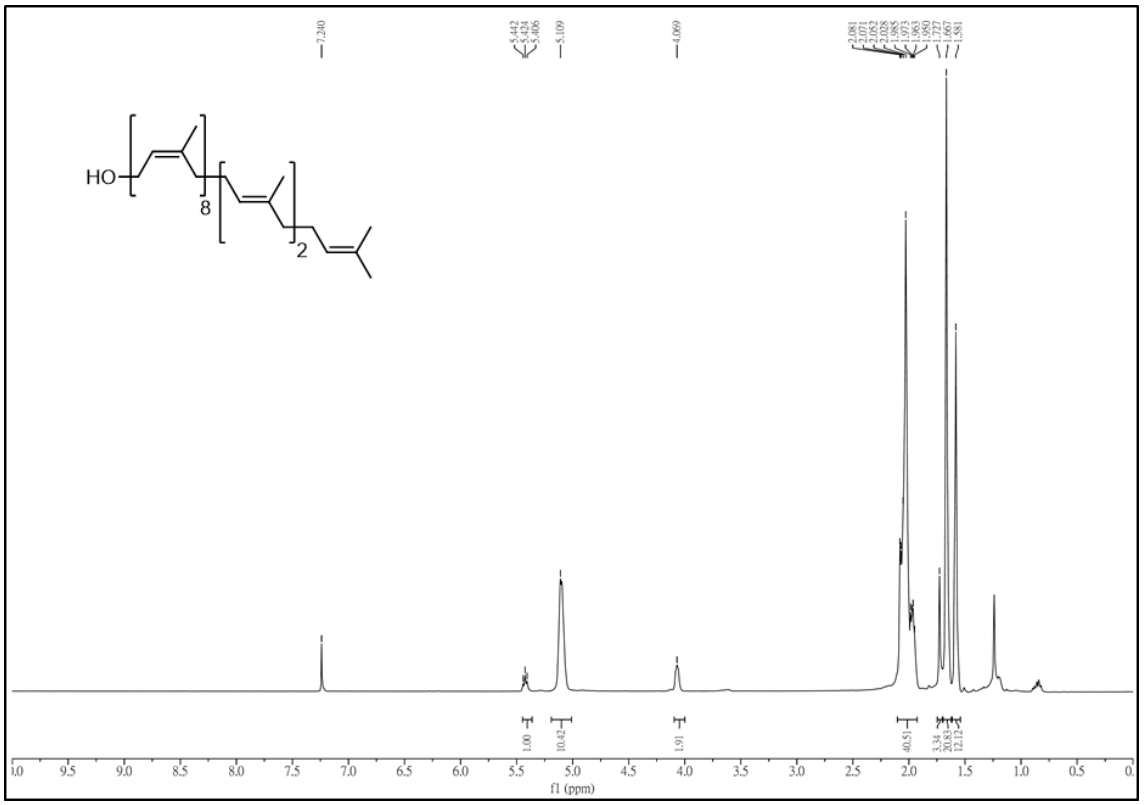
^{1}H NMR spectrum of compound **S13** (500 MHz, $CDCl_3$)



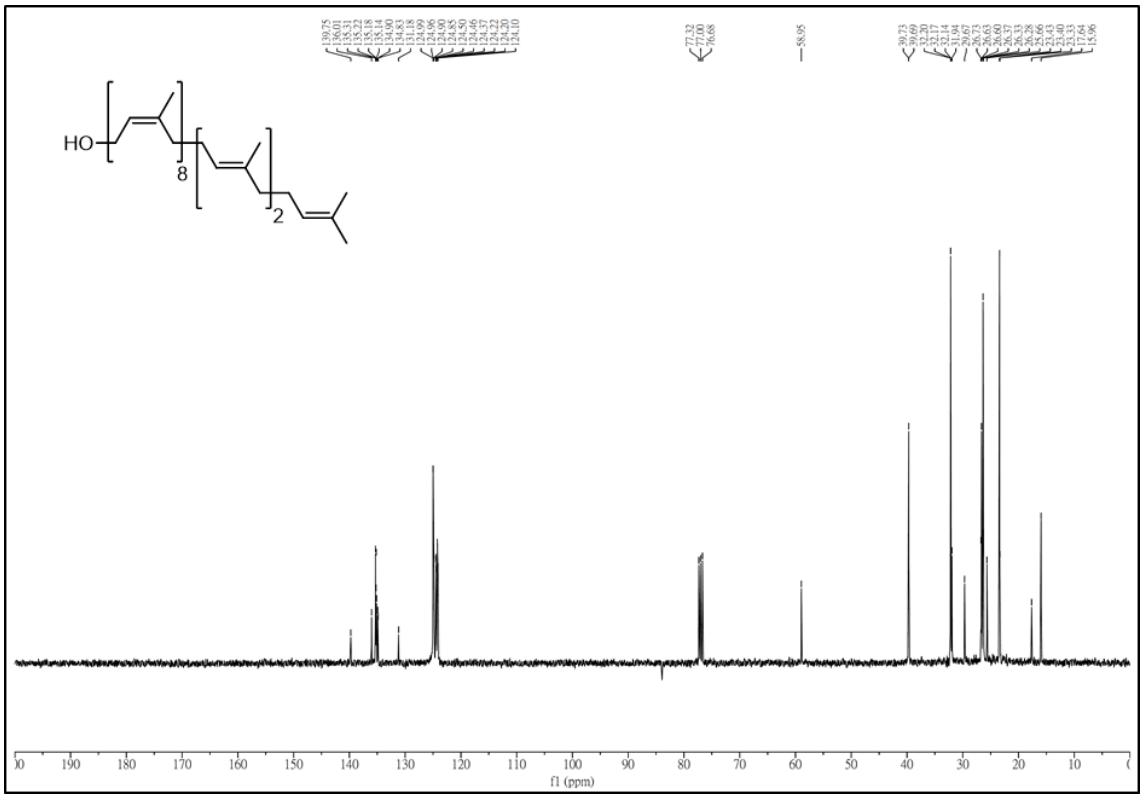
^{13}C NMR spectrum of compound **S13** (125 MHz, $CDCl_3$)



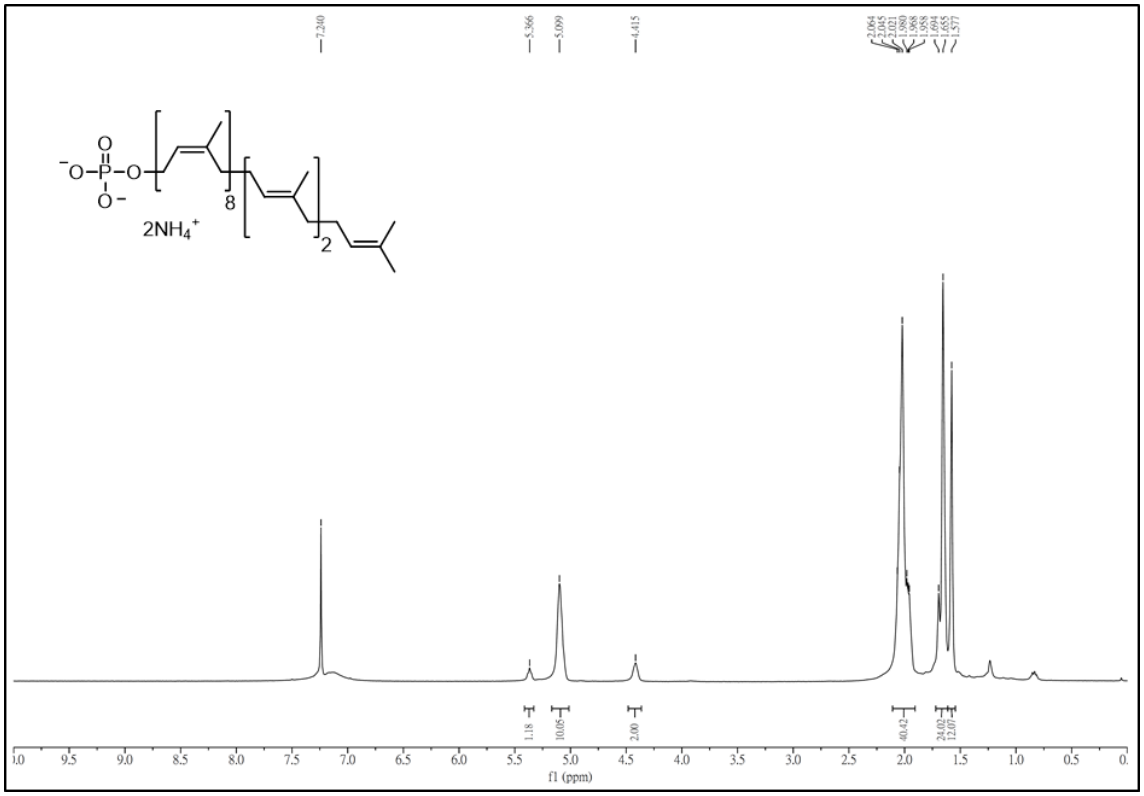
^{31}P NMR spectrum of compound **S13** (162 MHz, $CDCl_3$)



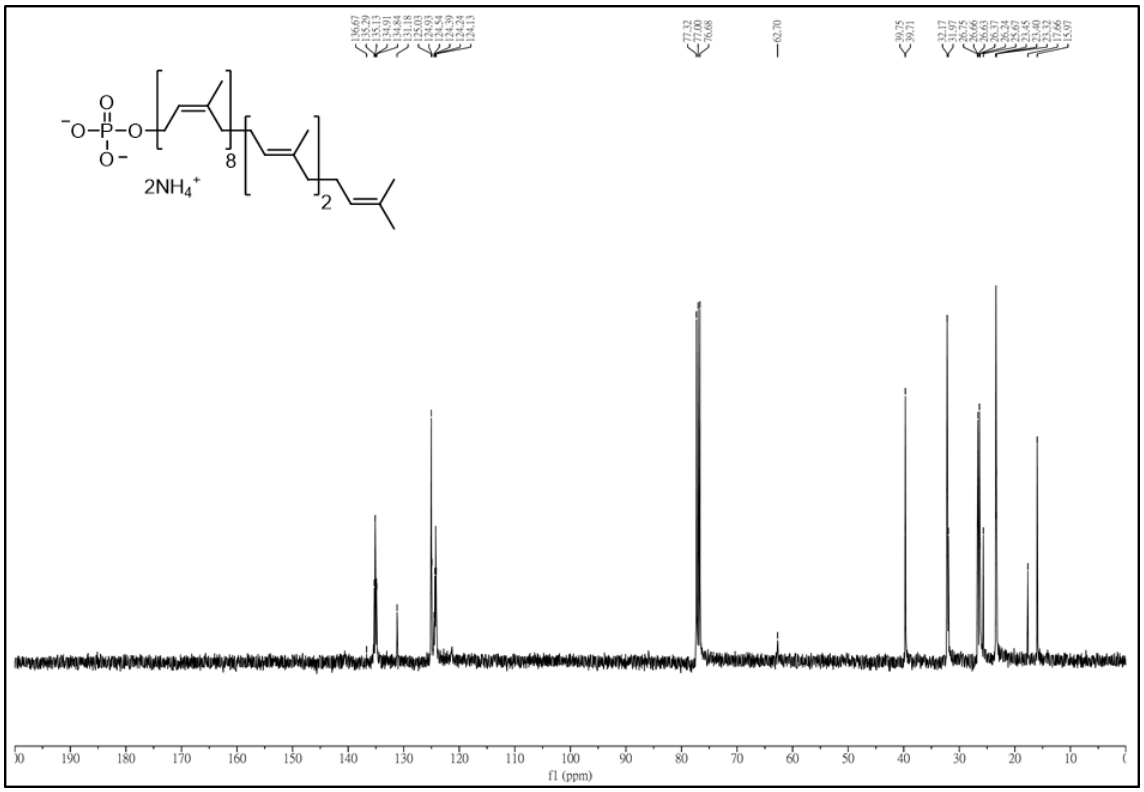
^{1}H NMR spectrum of compound undecaprenol (**S2**) (400 MHz, $CDCl_3$)



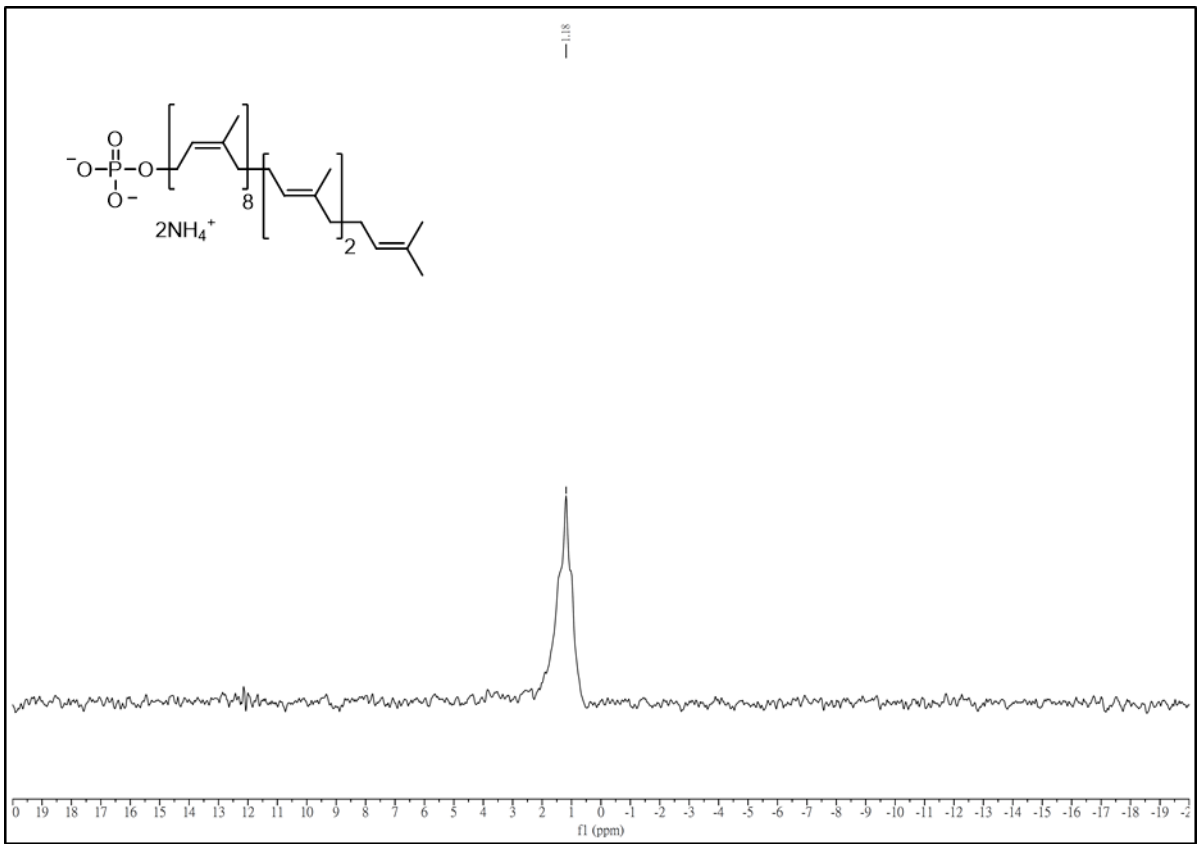
^{13}C NMR spectrum of compound undecaprenol (**S2**) (100 MHz, $CDCl_3$)



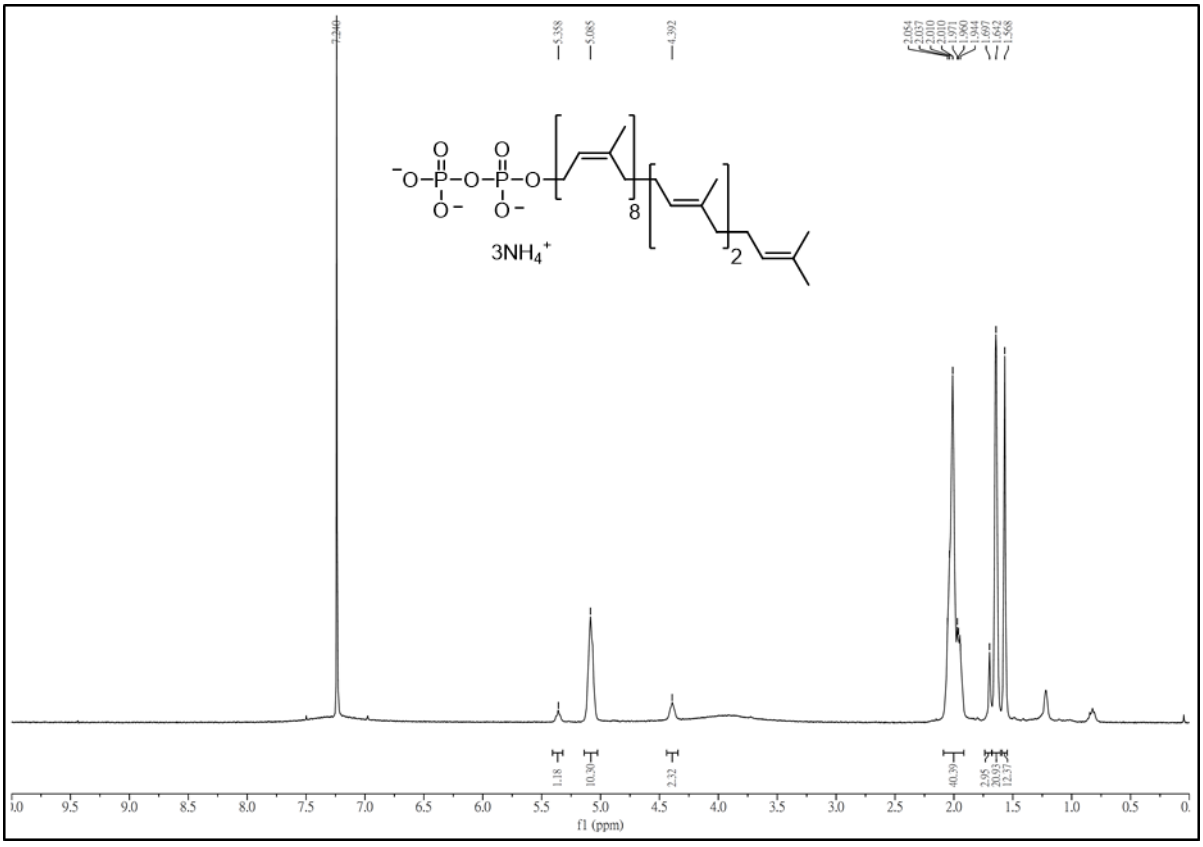
^{1}H NMR spectrum of compound UP (**S3**) (400 MHz, $CDCl_3$)



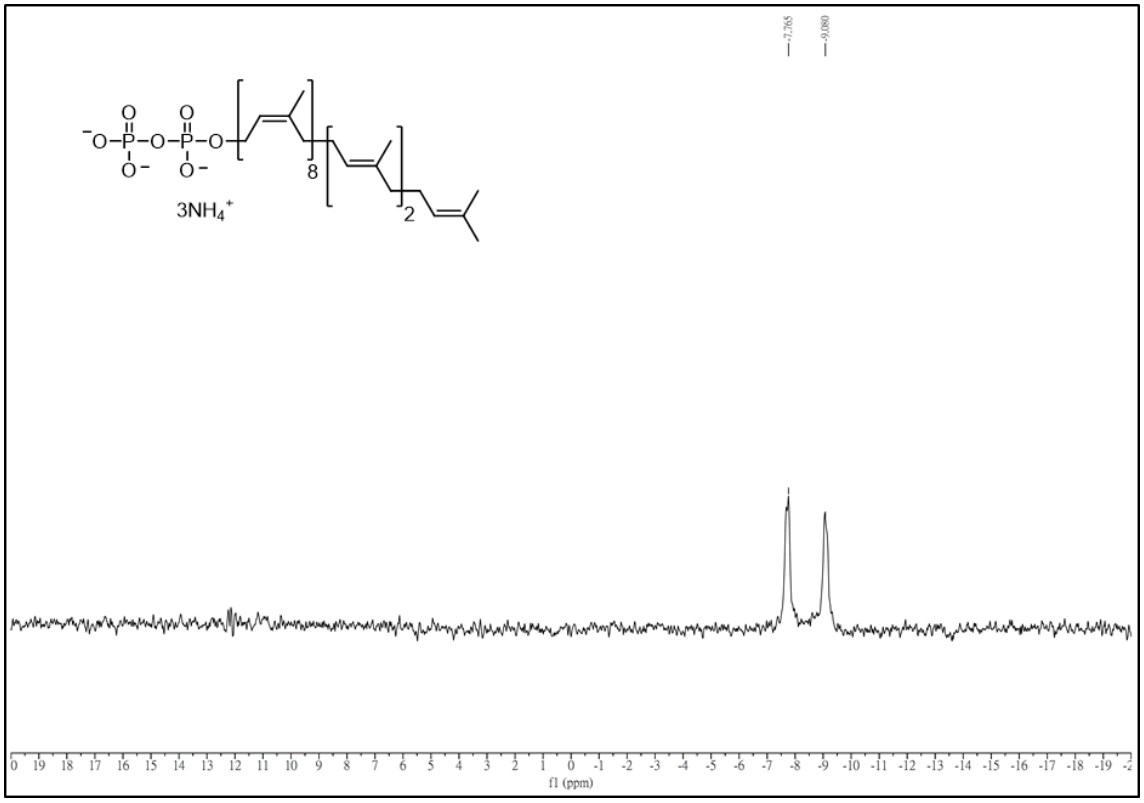
^{13}C NMR spectrum of compound UP (**S3**) (100 MHz, $CDCl_3$)



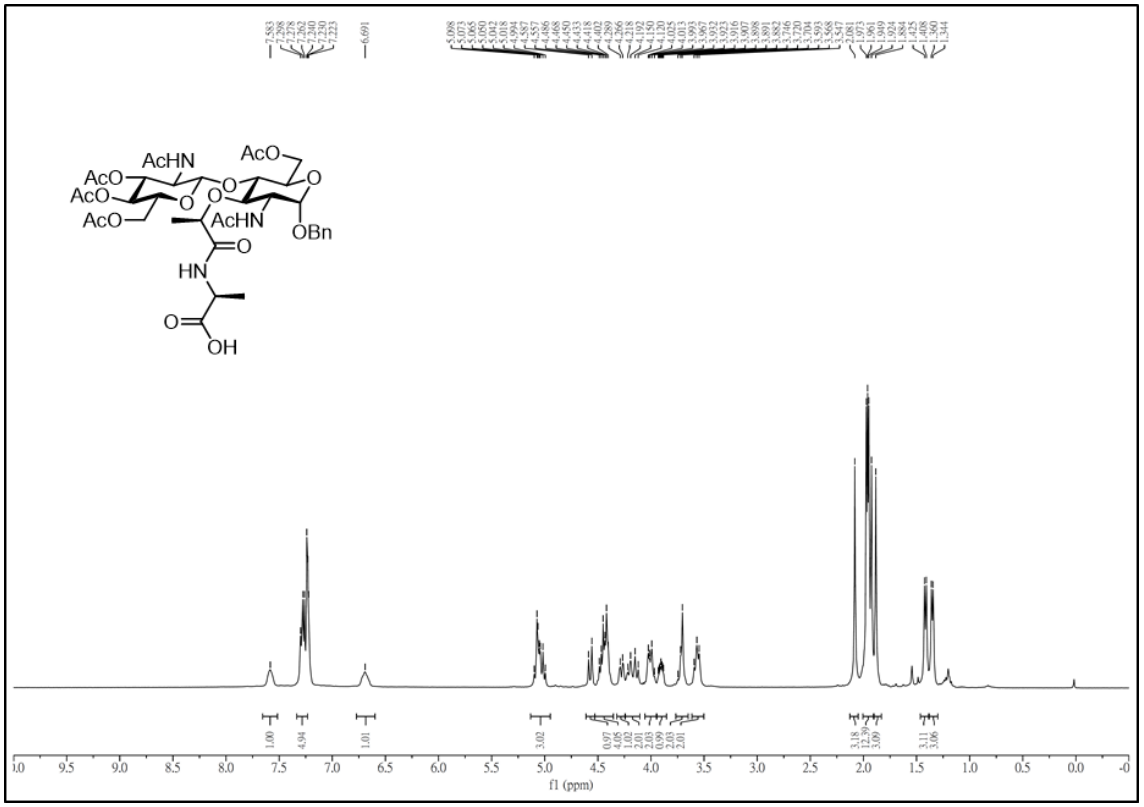
^{31}P NMR spectrum of compound UP (**S3**) (162 MHz, CD_3OD)



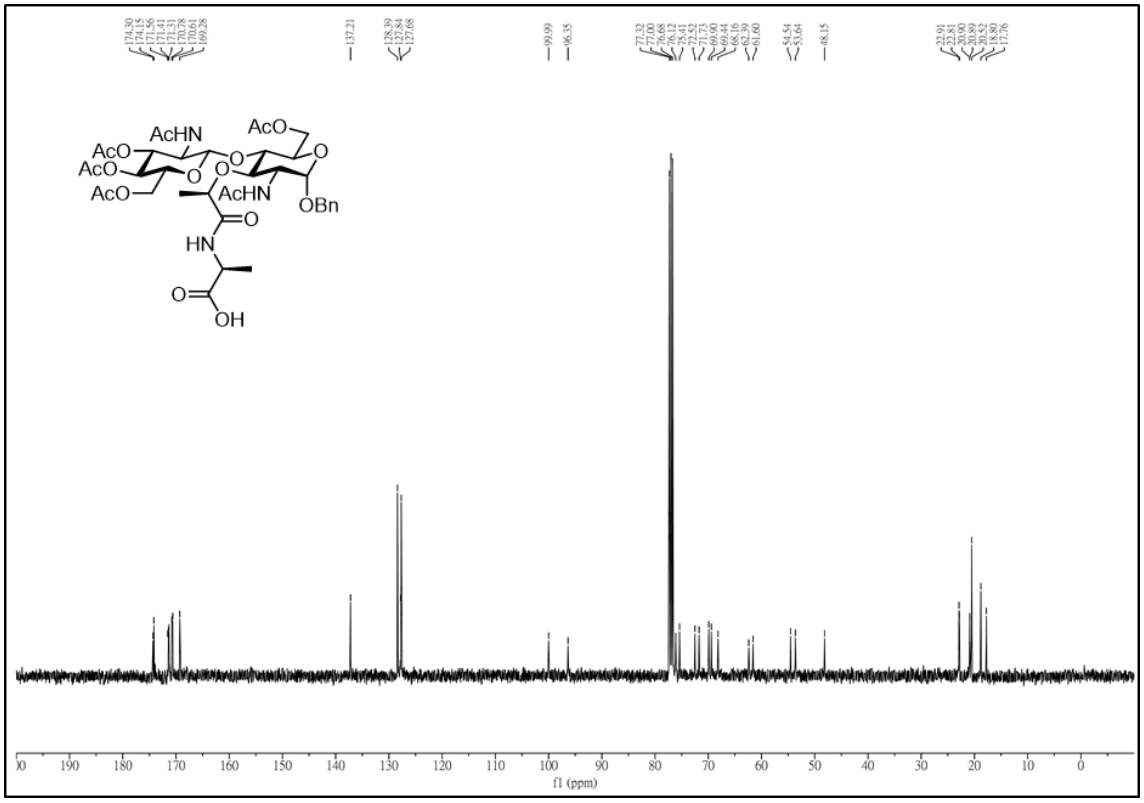
^{1}H NMR spectrum of compound UPP (**3**) (400 MHz, $CDCl_3$)



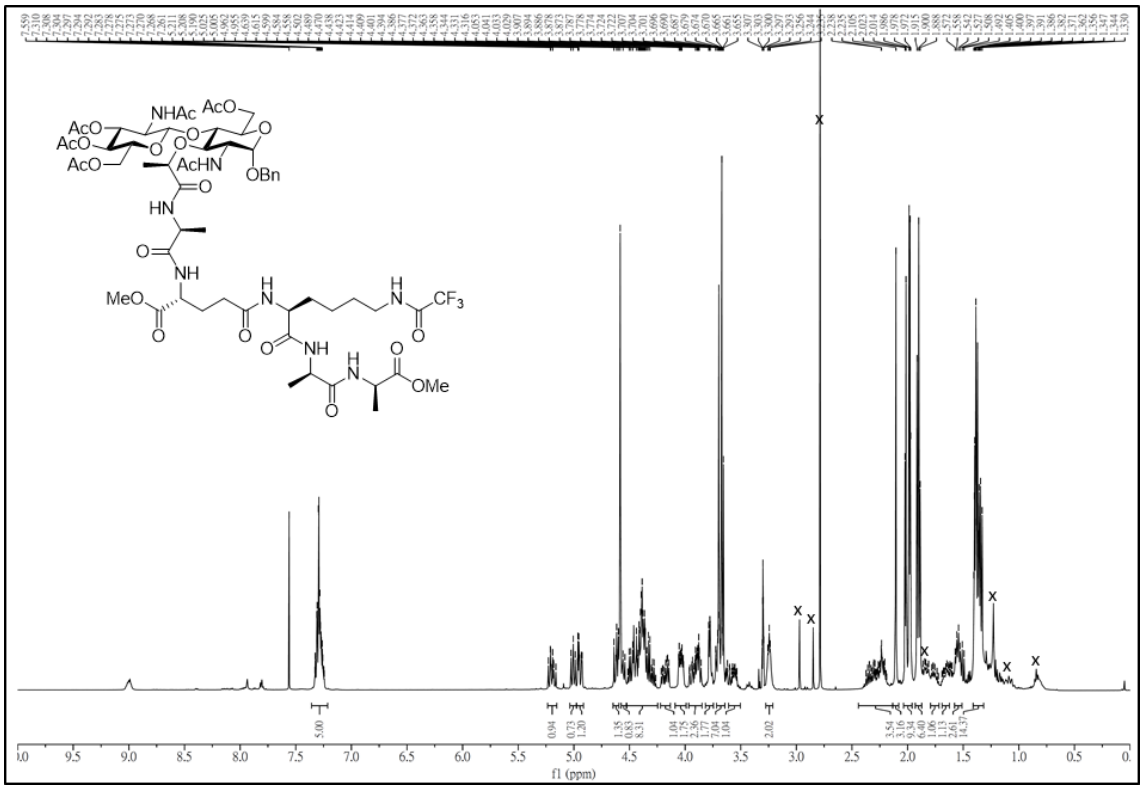
^{31}P NMR spectrum of compound UPP (**3**) (162 MHz, CD_3OD)



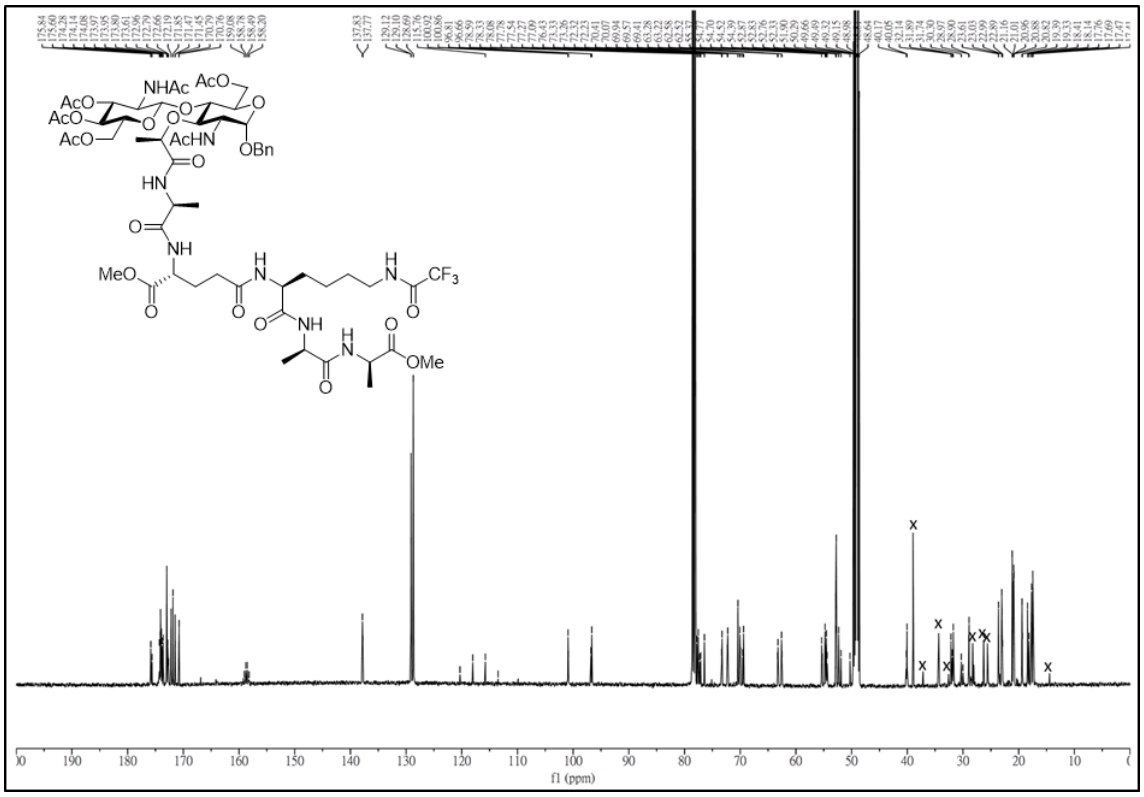
^{1}H NMR spectrum of compound **S15** (400 MHz, $CDCl_3$)



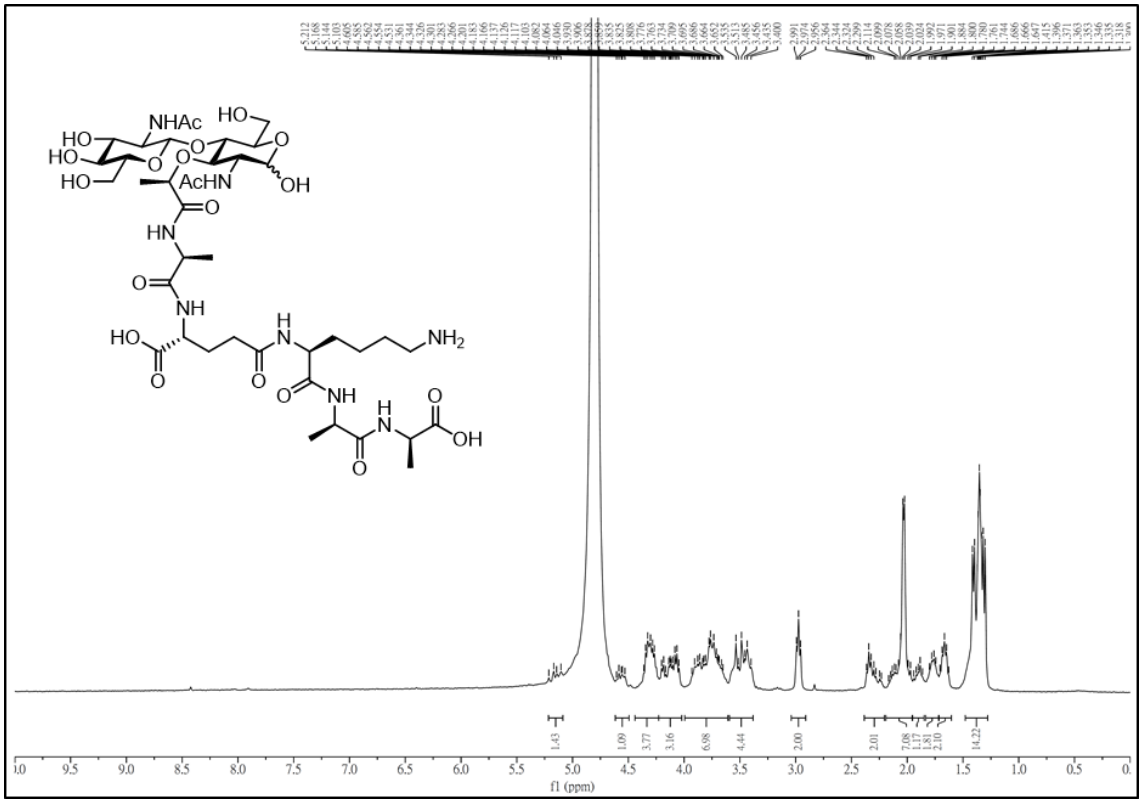
^{13}C NMR spectrum of compound **S15** (100 MHz, $CDCl_3$)



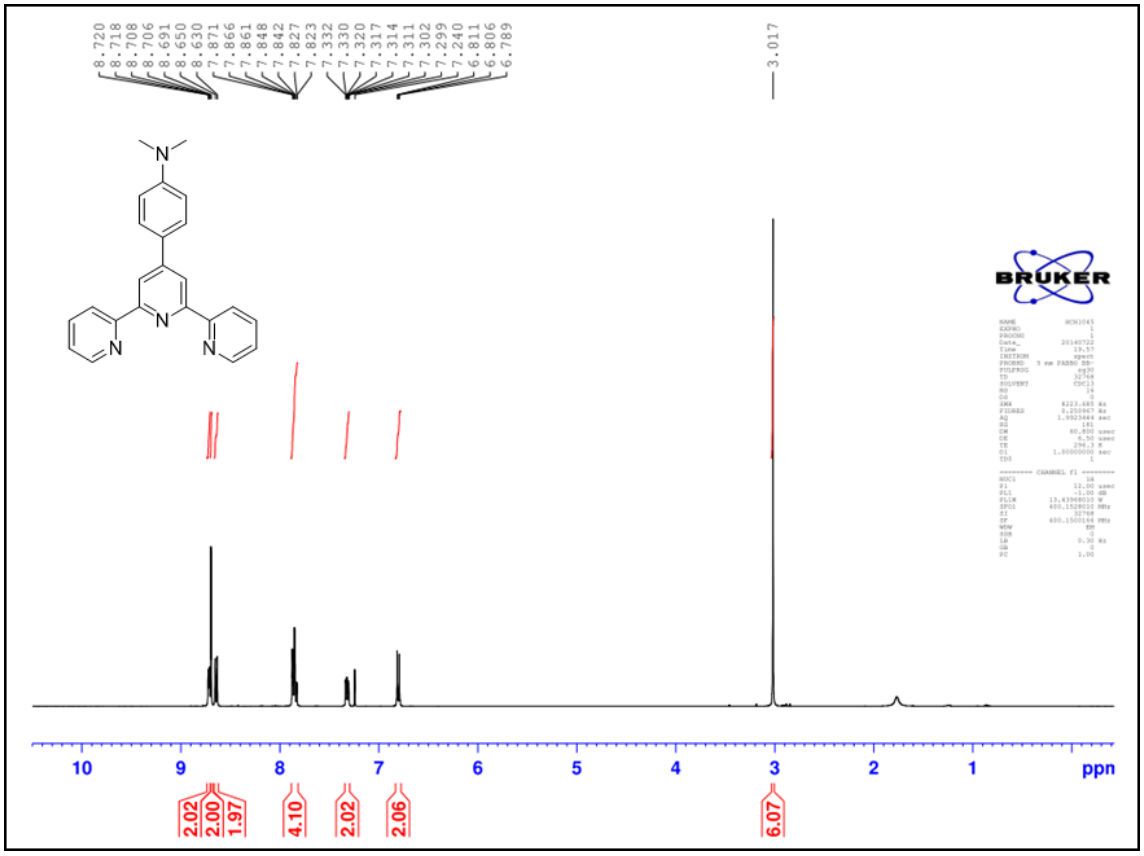
^{1}H NMR spectrum of compound **S16** (500 MHz, 1:1 $CD_3OD/CDCl_3$)



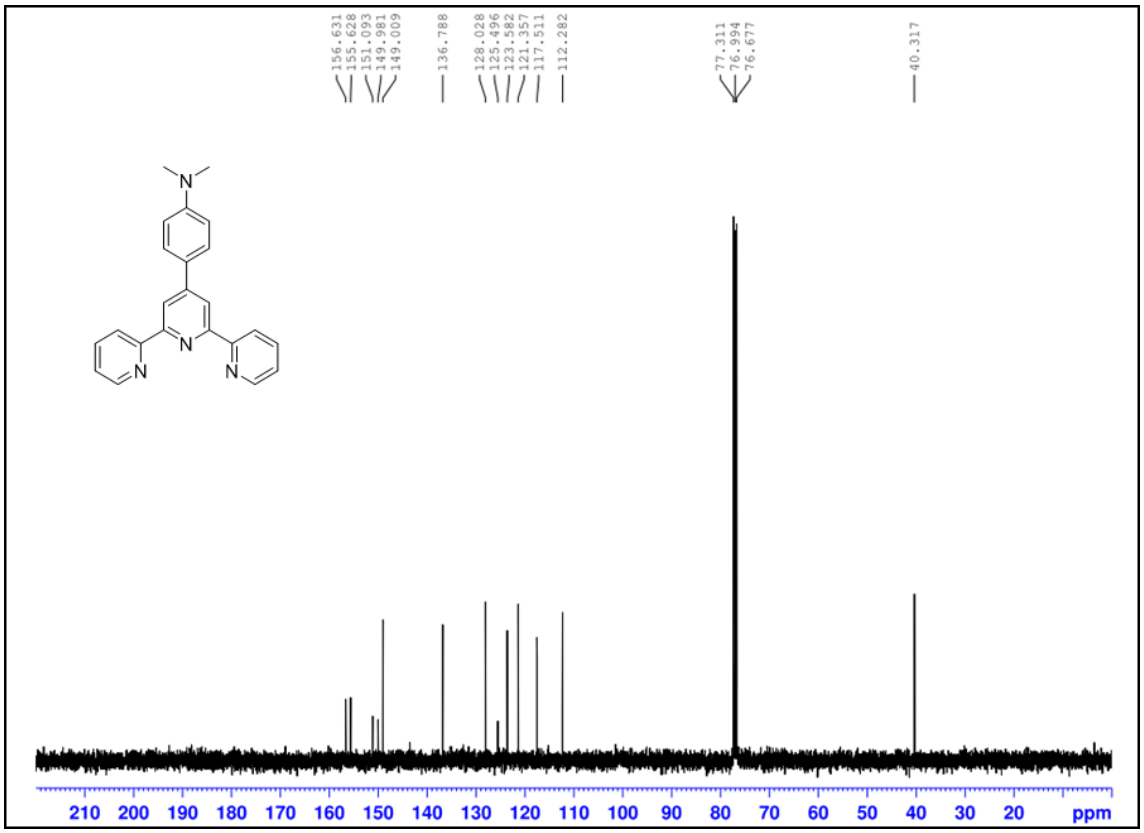
^{13}C NMR spectrum of compound **S16** (125 MHz, 1:1 $CD_3OD/CDCl_3$)



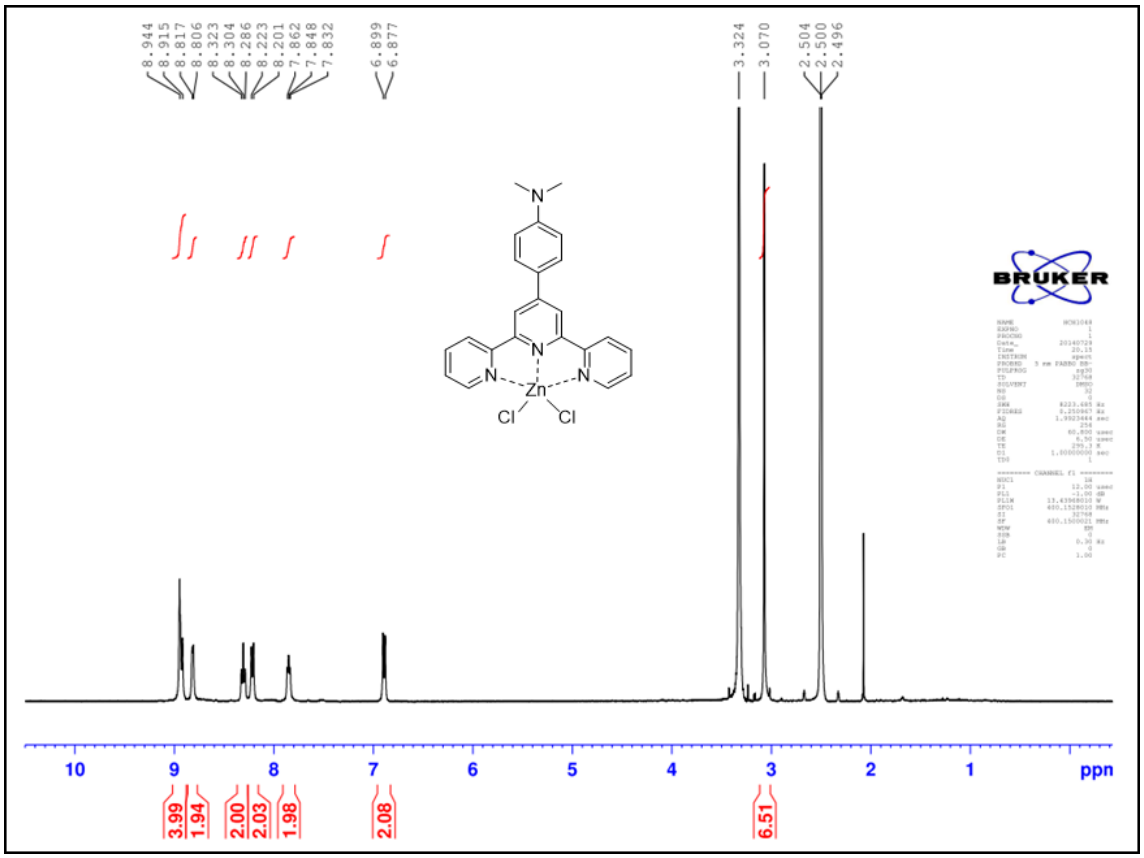
^{1}H NMR spectrum of PGM (compound **2**, 400 MHz, D_2O)



^{1}H NMR spectrum of Tpy ligand **S27** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of Tpy ligand **S27** (100 MHz, $CDCl_3$)



^{1}H NMR spectrum of Tpy-Zn complex (**4**) (400 MHz, DMSO-d_6)