Electronic Supplementary Information (ESI)

Copper(II)−dipicolylamine−coumarin sensor for maltosyltransferase assay

Wei-Li Lee,[a,‡] Tse-Wei Hsu,[a,‡] Wei-Cheng Hung,[a] and Jim-Min Fang*[ab]

[a] Department of Chemistry, National Taiwan University, Taipei 106, Taiwan.
[b] The Genomics Research Center, Academia Sinica, Taipei 115, Taiwan
[‡] W.-L. L and T.-W. H contributed equally.
* Corresponding author: jmfang@ntu.edu.tw

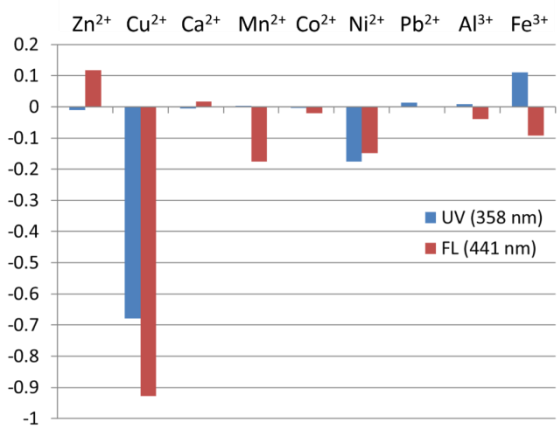


Figure S1. Changes of absorption (λ_{abs} = 358 nm, blue) and fluorescence intensity (λ_{em} = 441 nm, red), $[(I-I_0)/I_0]$, upon addition of metal ions (30 μM) to ligand **1a** (10 μM) in MeOH solution.

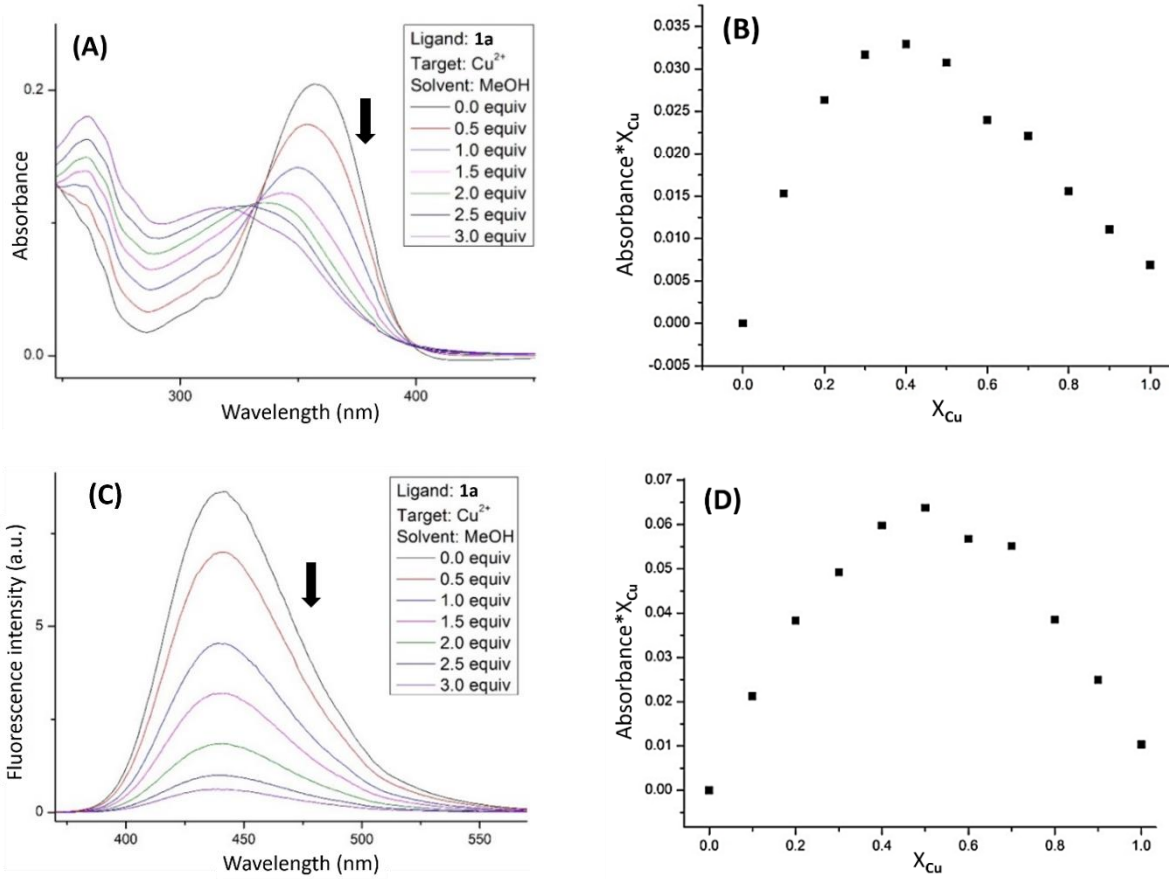


Figure S2. (A) UV-vis titration curve of ligand **1a** (10 μM solution in MeOH) upon incremental additions of Cu^{2+} ions. (B) Job plot using 10 μM MeOH solution of ligand **1a** and 10 μM aqueous solution of $Cu(NO_3)_2$ as the stock solutions. X_{Cu} is the molar ratio of $[Cu^{2+}]$:[**1a**]. (C) Fluorescence titration curve of **1a** (λ_{ex} = 358 nm). (D) Job plot using 10 μM MeOH solution of ligand **1b** and 10 μM aqueous solution of $Cu(NO_3)_2$ as the stock solutions. X_{Cu} is the molar ratio of $[Cu^{2+}]$:[**1b**]. Absorbance at 358 nm was taken for Job plots to determine the stoichiometry of the binding complexes.

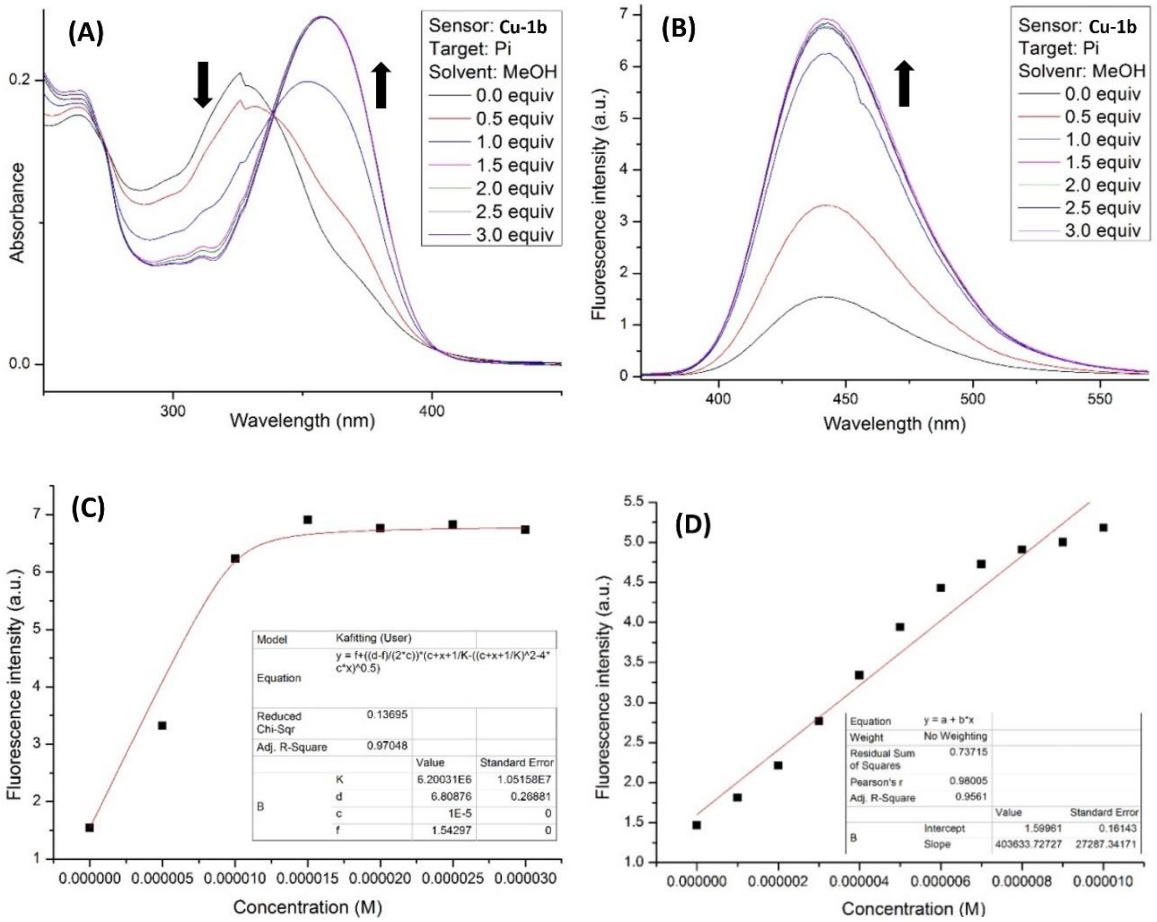


Figure S3. Titration of sensor Cu-**1b** (10 μM solution in MeOH) by incremental additions of Pi. (A) UV-vis titration curves, (B) fluorescence titration curves (λ_{ex} = 339 nm), (C) fluorescence K_{ass} fitting curves, and (D) fluorescence linear fitting (λ_{em} = 442 nm). Na_3PO_4 (10 mM in water) was used as the stock solution. The limit of detection (LOD) = 3.98×10^{-6} M as calculated by LOD = K × SD/S, where K = 3, SD is the standard deviation, and S is the slope of the calibration curve.

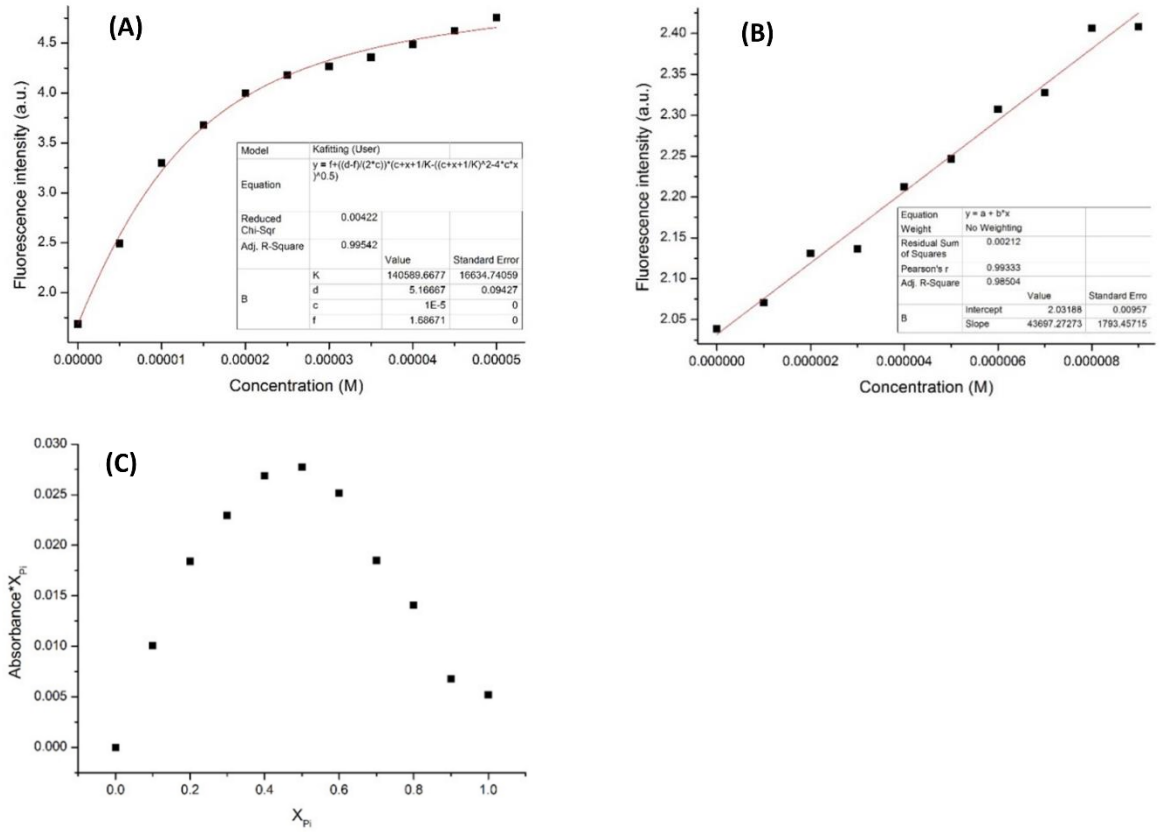


Figure S4. Titration of sensor Cu-**1b** (10 μM solution in HEPES buffer, pH 7.4) by incremental additions of Pi (referring to Figure 5A and 5B in text for the UV-vis and fluorescence titration curves). (A) Fluorescence K_{ass} fitting curves, and (B) fluorescence linear fitting (λ_{em} = 448 nm). The dash curves indicate the original absorption and fluorescence of free ligand **1b**. (C) Job plot with absorbance at 336 nm, X_{Pi} is the molar ratio of [Pi]:[Cu-**1b**]. Na_3PO_4 (10 mM in water) was used as the stock solution. The limit of detection (LOD) = 2.08 × 10^{-6} M as calculated by LOD = K × SD/S, where K = 3, SD is the standard deviation, and S is the slope of the calibration curve.

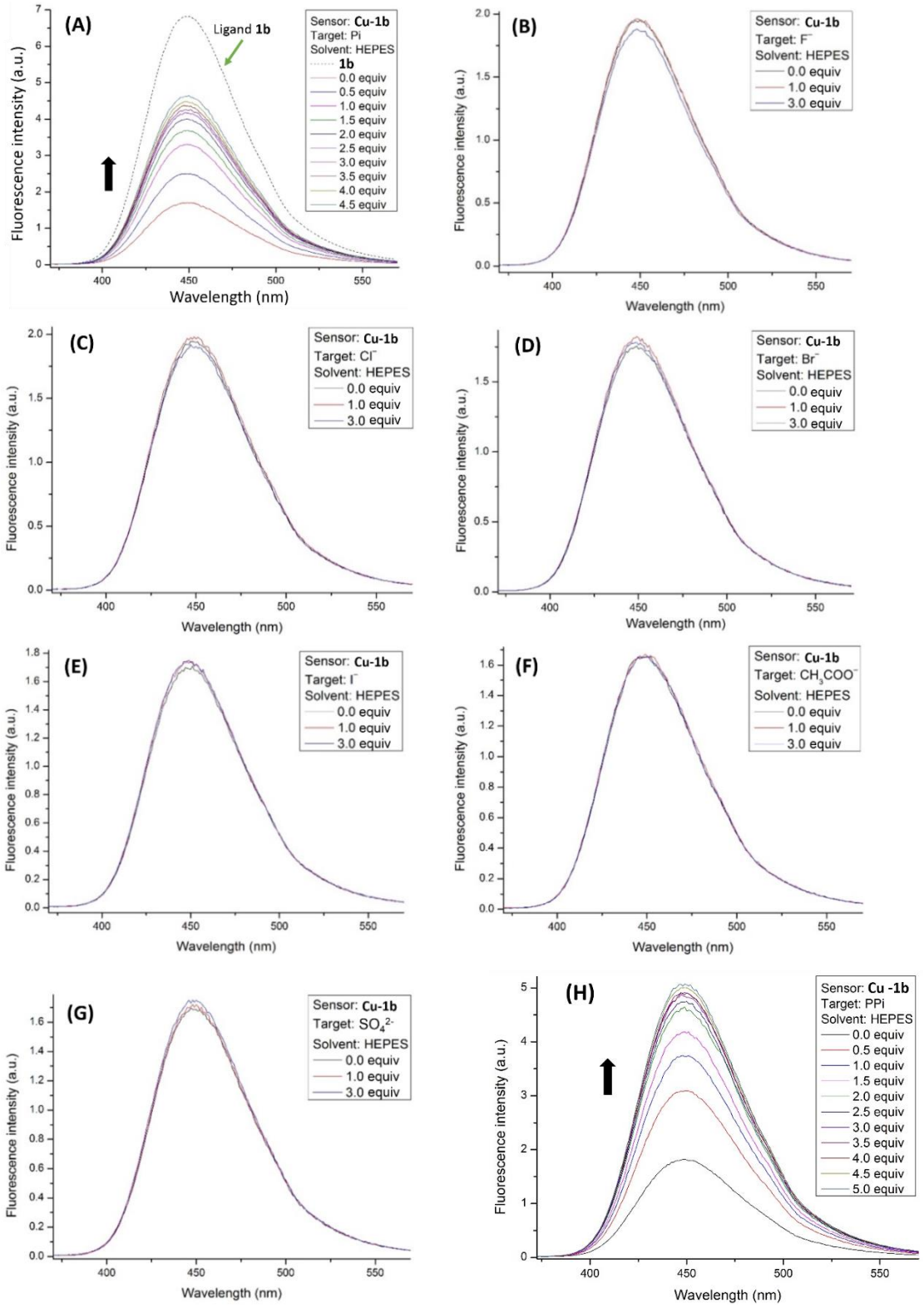


Figure S5. Fluorescence titration (λ_{ex} = 343 nm) of sensor Cu-**1b** (10 μM) with anions in HEPES buffer (pH 7.4). Titration with $H_2PO_4^-$ (A) and $H_2P_2O_7^{2-}$ (H) showed appreciable fluorescence change; however, no apparent fluorescence change was observed in titration with F^- (B), Cl^- (C), Br^- (D), I^- (E), $CH_3CO_2^-$ (F) and SO_4^{2-} (G). Stock solutions: $Na_4P_2O_7$ (10 mM), HEPES (20 mM), and $Cu(NO_3)_2$ (10 mM in water).

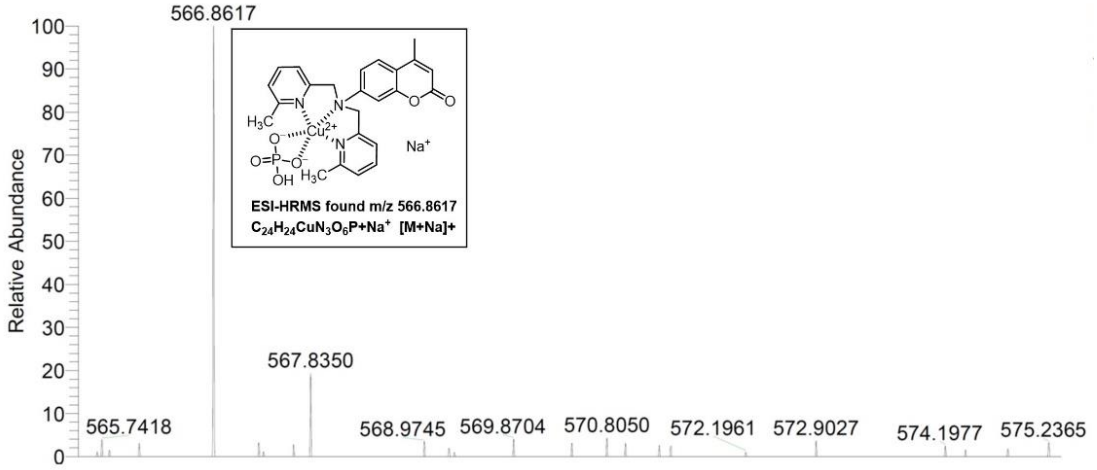


Figure S6. Electrospray ionization high-resolution mass spectrum (ESI−HRMS) of the Pi•(Cu-**1b**) 1:1 complex. The sample was obtained by addition of phosphate ions (3 equiv) to the freshly prepared methanolic solution of Cu-**1b**. The sodiated Pi•(Cu-**1b**) complex showed a signal at *m*/*z* 566.8617 attributable to the $[C_{24}H_{24}CuN_3O_6P+Na]^+$ ion (calcd. 567.0597).

Table S1A. Crystal data and structure refinement for compound **Cu-1b** (CCDC 1876683).

Identification code	Cu-1b	
Empirical formula	C48 H46 Cu2 N8 O12	
Formula weight	1054.01	
Temperature	150(2) K	
Wavelength	0.71075 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 11.7538(10) Å	= 118.519(3)°.
	b = 12.8198(12) Å	= 96.302(3)°.
	c = 13.1515(12) Å	= 110.063(3)°.
Volume	1543.1(2) $Å^3$	
Z	1	
Density (calculated)	1.134 Mg/m^3	
Absorption coefficient	0.744 mm^{-1}	
F(000)	544	
Crystal size	0.40 x 0.32 x 0.25 mm^3	
Theta range for data collection	2.465 to 24.999°.	
Index ranges	-13<=h<=13, -15<=k<=15, -15<=l<=15	
Reflections collected	41650	
Independent reflections	5378 [R(int) = 0.0456]	
Completeness to theta = 24.999°	99.0 %	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5378 / 0 / 316	
Goodness-of-fit on F^2	1.068	
Final R indices [I>2sigma(I)]	R1 = 0.0583, wR2 = 0.1576	
R indices (all data)	R1 = 0.0663, wR2 = 0.1645	
Extinction coefficient	n/a	
Largest diff. peak and hole	1.318 and -0.909 e.$Å^{-3}$	

Table S1B. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for compound **Cu-1b** (CCDC 1876683). U (eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
Cu(1)	3582(1)	9575(1)	4582(1)	13(1)
O(1)	8298(3)	12671(3)	1549(3)	30(1)
O(2)	6624(3)	11642(3)	1898(2)	19(1)
O(3)	5183(2)	11122(3)	5695(2)	19(1)
N(1)	3000(3)	9738(3)	2865(3)	14(1)
N(2)	2546(3)	10603(3)	5111(3)	15(1)
N(3)	2039(3)	7800(3)	3446(3)	12(1)
C(1)	7505(4)	11601(4)	1285(4)	20(1)
C(2)	7378(4)	10313(4)	401(4)	20(1)
C(3)	6439(4)	9166(4)	151(3)	17(1)
C(4)	6308(4)	7837(4)	-806(4)	27(1)
C(5)	5537(4)	9246(4)	815(3)	14(1)
C(6)	5663(3)	10497(4)	1673(3)	14(1)
C(7)	4852(3)	10705(4)	2360(3)	14(1)
C(8)	3850(3)	9595(4)	2198(3)	13(1)
C(9)	3717(4)	8316(4)	1356(3)	18(1)
C(10)	4532(4)	8146(4)	681(3)	18(1)
C(11)	3082(4)	11084(4)	3603(4)	19(1)
C(12)	2385(4)	11175(4)	4508(3)	17(1)
C(13)	1655(4)	11865(4)	4751(4)	24(1)
C(14)	1131(4)	12011(4)	5671(4)	24(1)
C(15)	1325(4)	11457(4)	6306(4)	22(1)
C(16)	2041(4)	10751(4)	6009(3)	20(1)
C(17)	2274(5)	10128(5)	6676(4)	32(1)
C(18)	1665(4)	8679(4)	2195(3)	16(1)
C(19)	1335(3)	7558(4)	2407(3)	14(1)
C(20)	298(4)	6343(4)	1545(4)	22(1)
C(21)	-65(4)	5341(4)	1767(4)	28(1)
C(22)	659(4)	5596(4)	2840(4)	23(1)
C(23)	1706(4)	6812(4)	3657(3)	16(1)
C(24)	2521(4)	7078(4)	4797(4)	23(1)

O(4)	8630(6)	7964(7)	1029(5)	90(2)
O(5)	7376(6)	5613(8)	-122(7)	114(3)
O(6)	8818(8)	6811(10)	-669(7)	131(3)
N(4)	8241(8)	6755(9)	35(7)	89(2)

Table S1C. Bond lengths [Å] and angles [°] for compound **Cu-1b** (CCDC 1876683).

Cu(1)-O(3)	1.918(3)
Cu(1)-O(3)#1	1.931(3)
Cu(1)-N(3)	2.007(3)
Cu(1)-N(2)	2.029(3)
Cu(1)-N(1)	2.413(3)
Cu(1)-Cu(1)#1	3.0110(9)
O(1)-C(1)	1.212(5)
O(2)-C(6)	1.376(4)
O(2)-C(1)	1.380(5)
O(3)-Cu(1)#1	1.931(3)
N(1)-C(8)	1.401(5)
N(1)-C(18)	1.475(5)
N(1)-C(11)	1.481(5)
N(2)-C(16)	1.341(5)
N(2)-C(12)	1.348(5)
N(3)-C(19)	1.349(5)
N(3)-C(23)	1.361(5)
C(1)-C(2)	1.435(6)
C(2)-C(3)	1.353(6)
C(2)-H(2)	0.9500
C(3)-C(5)	1.446(5)
C(3)-C(4)	1.497(5)
C(4)-H(4A)	0.9800
C(4)-H(4B)	0.9800
C(4)-H(4C)	0.9800
C(5)-C(6)	1.392(5)
C(5)-C(10)	1.411(5)
C(6)-C(7)	1.389(5)
C(7)-C(8)	1.401(5)
C(7)-H(7)	0.9500
C(8)-C(9)	1.411(5)
C(9)-C(10)	1.378(6)
C(9)-H(9)	0.9500
C(10)-H(10)	0.9500
C(11)-C(12)	1.499(5)
C(11)-H(11A)	0.9900

C(11)-H(11B)	0.9900
C(12)-C(13)	1.390(6)
C(13)-C(14)	1.381(6)
C(13)-H(13)	0.9500
C(14)-C(15)	1.374(6)
C(14)-H(14)	0.9500
C(15)-C(16)	1.392(6)
C(15)-H(15)	0.9500
C(16)-C(17)	1.501(6)
C(17)-H(17A)	0.9800
C(17)-H(17B)	0.9800
C(17)-H(17C)	0.9800
C(18)-C(19)	1.522(5)
C(18)-H(18A)	0.9900
C(18)-H(18B)	0.9900
C(19)-C(20)	1.380(5)
C(20)-C(21)	1.388(6)
C(20)-H(20)	0.9500
C(21)-C(22)	1.388(6)
C(21)-H(21)	0.9500
C(22)-C(23)	1.376(6)
C(22)-H(22)	0.9500
C(23)-C(24)	1.502(5)
C(24)-H(24A)	0.9800
C(24)-H(24B)	0.9800
C(24)-H(24C)	0.9800
O(4)-N(4)	1.330(9)
O(5)-N(4)	1.365(10)
O(6)-N(4)	1.220(9)

O(3)-Cu(1)-O(3)#1	77.09(12)
O(3)-Cu(1)-N(3)	171.27(11)
O(3)#1-Cu(1)-N(3)	95.13(11)
O(3)-Cu(1)-N(2)	93.09(12)
O(3)#1-Cu(1)-N(2)	170.12(12)
N(3)-Cu(1)-N(2)	94.59(12)
O(3)-Cu(1)-N(1)	106.06(11)
O(3)#1-Cu(1)-N(1)	105.77(11)

N(3)-Cu(1)-N(1)	79.68(11)
N(2)-Cu(1)-N(1)	77.77(11)
O(3)-Cu(1)-Cu(1)#1	38.70(8)
O(3)#1-Cu(1)-Cu(1)#1	38.39(8)
N(3)-Cu(1)-Cu(1)#1	133.40(9)
N(2)-Cu(1)-Cu(1)#1	131.78(9)
N(1)-Cu(1)-Cu(1)#1	110.52(8)
C(6)-O(2)-C(1)	121.5(3)
Cu(1)-O(3)-Cu(1)#1	102.91(12)
C(8)-N(1)-C(18)	115.2(3)
C(8)-N(1)-C(11)	117.2(3)
C(18)-N(1)-C(11)	112.8(3)
C(8)-N(1)-Cu(1)	111.0(2)
C(18)-N(1)-Cu(1)	102.0(2)
C(11)-N(1)-Cu(1)	95.8(2)
C(16)-N(2)-C(12)	119.5(3)
C(16)-N(2)-Cu(1)	124.4(3)
C(12)-N(2)-Cu(1)	116.0(3)
C(19)-N(3)-C(23)	118.7(3)
C(19)-N(3)-Cu(1)	118.6(2)
C(23)-N(3)-Cu(1)	122.6(2)
O(1)-C(1)-O(2)	115.6(4)
O(1)-C(1)-C(2)	127.2(4)
O(2)-C(1)-C(2)	117.2(3)
C(3)-C(2)-C(1)	122.9(4)
C(3)-C(2)-H(2)	118.6
C(1)-C(2)-H(2)	118.6
C(2)-C(3)-C(5)	118.4(4)
C(2)-C(3)-C(4)	121.5(4)
C(5)-C(3)-C(4)	120.1(3)
C(3)-C(4)-H(4A)	109.5
C(3)-C(4)-H(4B)	109.5
H(4A)-C(4)-H(4B)	109.5
C(3)-C(4)-H(4C)	109.5
H(4A)-C(4)-H(4C)	109.5
H(4B)-C(4)-H(4C)	109.5
C(6)-C(5)-C(10)	116.3(3)
C(6)-C(5)-C(3)	118.6(3)

C(10)-C(5)-C(3)	125.1(3)
O(2)-C(6)-C(7)	114.6(3)
O(2)-C(6)-C(5)	121.3(3)
C(7)-C(6)-C(5)	124.1(3)
C(6)-C(7)-C(8)	118.5(3)
C(6)-C(7)-H(7)	120.7
C(8)-C(7)-H(7)	120.7
C(7)-C(8)-N(1)	121.3(3)
C(7)-C(8)-C(9)	118.6(3)
N(1)-C(8)-C(9)	120.1(3)
C(10)-C(9)-C(8)	121.3(3)
C(10)-C(9)-H(9)	119.4
C(8)-C(9)-H(9)	119.4
C(9)-C(10)-C(5)	121.1(3)
C(9)-C(10)-H(10)	119.4
C(5)-C(10)-H(10)	119.4
N(1)-C(11)-C(12)	111.1(3)
N(1)-C(11)-H(11A)	109.4
C(12)-C(11)-H(11A)	109.4
N(1)-C(11)-H(11B)	109.4
C(12)-C(11)-H(11B)	109.4
H(11A)-C(11)-H(11B)	108.0
N(2)-C(12)-C(13)	121.7(4)
N(2)-C(12)-C(11)	116.7(3)
C(13)-C(12)-C(11)	121.5(3)
C(14)-C(13)-C(12)	118.6(4)
C(14)-C(13)-H(13)	120.7
C(12)-C(13)-H(13)	120.7
C(15)-C(14)-C(13)	119.5(4)
C(15)-C(14)-H(14)	120.3
C(13)-C(14)-H(14)	120.3
C(14)-C(15)-C(16)	119.6(4)
C(14)-C(15)-H(15)	120.2
C(16)-C(15)-H(15)	120.2
N(2)-C(16)-C(15)	121.0(4)
N(2)-C(16)-C(17)	117.7(4)
C(15)-C(16)-C(17)	121.4(4)
C(16)-C(17)-H(17A)	109.5

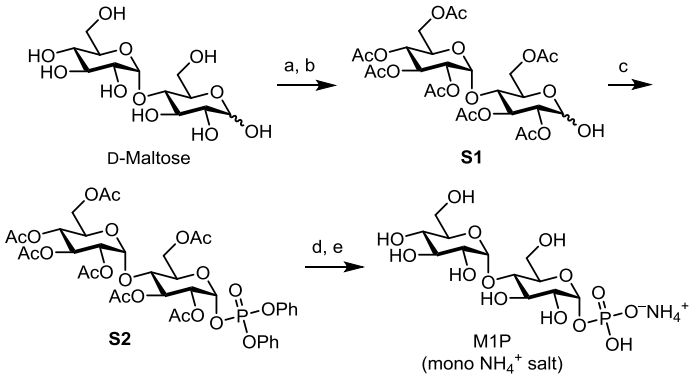
C(16)-C(17)-H(17B)	109.5
H(17A)-C(17)-H(17B)	109.5
C(16)-C(17)-H(17C)	109.5
H(17A)-C(17)-H(17C)	109.5
H(17B)-C(17)-H(17C)	109.5
N(1)-C(18)-C(19)	115.0(3)
N(1)-C(18)-H(18A)	108.5
C(19)-C(18)-H(18A)	108.5
N(1)-C(18)-H(18B)	108.5
C(19)-C(18)-H(18B)	108.5
H(18A)-C(18)-H(18B)	107.5
N(3)-C(19)-C(20)	122.5(3)
N(3)-C(19)-C(18)	118.9(3)
C(20)-C(19)-C(18)	118.6(3)
C(19)-C(20)-C(21)	119.1(4)
C(19)-C(20)-H(20)	120.4
C(21)-C(20)-H(20)	120.4
C(22)-C(21)-C(20)	118.2(4)
C(22)-C(21)-H(21)	120.9
C(20)-C(21)-H(21)	120.9
C(23)-C(22)-C(21)	120.5(4)
C(23)-C(22)-H(22)	119.7
C(21)-C(22)-H(22)	119.7
N(3)-C(23)-C(22)	120.9(3)
N(3)-C(23)-C(24)	118.3(3)
C(22)-C(23)-C(24)	120.8(3)
C(23)-C(24)-H(24A)	109.5
C(23)-C(24)-H(24B)	109.5
H(24A)-C(24)-H(24B)	109.5
C(23)-C(24)-H(24C)	109.5
H(24A)-C(24)-H(24C)	109.5
H(24B)-C(24)-H(24C)	109.5
O(6)-N(4)-O(4)	110.6(9)
O(6)-N(4)-O(5)	125.5(9)
O(4)-N(4)-O(5)	123.8(7)

Symmetry transformations used to generate equivalent atoms: #1 -x+1,-y+2,-z+1

Table S1D. Anisotropic displacement parameters ($Å^2 \times 10^3$) for compound **Cu-1b** (CCDC 1876683). The anisotropic displacement factor exponent takes the form: $-2p^2[h^2 a^{*2} U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Cu(1)	9(1)	13(1)	17(1)	7(1)	3(1)	5(1)
O(1)	23(2)	24(2)	40(2)	20(1)	17(1)	5(1)
O(2)	19(1)	16(1)	21(1)	10(1)	9(1)	6(1)
O(3)	11(1)	15(1)	23(1)	6(1)	3(1)	6(1)
N(1)	13(2)	14(2)	19(2)	11(1)	6(1)	6(1)
N(2)	12(2)	18(2)	15(2)	8(1)	2(1)	6(1)
N(3)	10(1)	13(2)	17(2)	10(1)	5(1)	5(1)
C(1)	15(2)	26(2)	22(2)	16(2)	7(2)	6(2)
C(2)	15(2)	31(2)	20(2)	17(2)	10(2)	12(2)
C(3)	18(2)	25(2)	12(2)	12(2)	4(2)	12(2)
C(4)	29(2)	26(2)	22(2)	9(2)	12(2)	14(2)
C(5)	15(2)	16(2)	11(2)	7(2)	2(1)	6(2)
C(6)	12(2)	15(2)	14(2)	10(2)	2(1)	3(1)
C(7)	15(2)	12(2)	15(2)	8(2)	4(1)	5(2)
C(8)	12(2)	16(2)	12(2)	9(2)	3(1)	6(2)
C(9)	19(2)	14(2)	18(2)	9(2)	5(2)	4(2)
C(10)	22(2)	13(2)	16(2)	6(2)	6(2)	7(2)
C(11)	23(2)	17(2)	25(2)	14(2)	14(2)	12(2)
C(12)	15(2)	13(2)	21(2)	9(2)	6(2)	5(2)
C(13)	25(2)	22(2)	33(2)	18(2)	13(2)	14(2)
C(14)	22(2)	22(2)	33(2)	14(2)	14(2)	16(2)
C(15)	20(2)	22(2)	22(2)	9(2)	11(2)	11(2)
C(16)	16(2)	22(2)	17(2)	9(2)	4(2)	8(2)
C(17)	38(3)	52(3)	28(2)	28(2)	19(2)	31(2)
C(18)	13(2)	17(2)	21(2)	12(2)	7(2)	6(2)
C(19)	12(2)	17(2)	18(2)	12(2)	7(1)	7(2)
C(20)	17(2)	25(2)	18(2)	13(2)	2(2)	2(2)
C(21)	27(2)	17(2)	23(2)	9(2)	-2(2)	-3(2)
C(22)	24(2)	18(2)	26(2)	16(2)	7(2)	4(2)
C(23)	16(2)	18(2)	22(2)	13(2)	10(2)	9(2)
C(24)	21(2)	26(2)	30(2)	21(2)	7(2)	10(2)
O(4)	93(4)	133(5)	50(3)	42(3)	30(3)	69(4)

O(5)	63(4)	154(7)	119(5)	100(5)	2(3)	13(4)
O(6)	190(8)	249(10)	117(5)	143(6)	122(6)	180(8)
N(4)	98(6)	127(7)	66(4)	55(5)	32(4)	73(5)



Scheme S1. Synthesis of α-maltosyl-1-phosphate (M1P). *Reagents and reaction conditions*: (a) Ac_2O, TsOH, 0−25 °C. (b) $BnNH_2$, THF, rt; 99% yield for two steps. (c) $(PhO)_2P(=O)Cl$, DMAP, CH_2Cl_2, −10 to −5 °C; 60%. (d) PtO_2, H_2 (~ 1 bar), EtOH, rt. (e) NH_3, MeOH, H_2O, rt; 93% yield for two steps.

The mono ammonium salt of α-maltosyl-1-phosphate (M1P) was synthesized according to the previously reported methods with slight modification.[s2−s4] D-Maltose was subject to peracetylation, followed by treatment with benzylamine to remove the acetyl group at C-1.[s1] Compound **S1** was reacted with diphenyl phosphoryl chloride in the presence of 4-(dimethylamino)pyridine to give phosphate **S2** as the α-anomer.[s3] After removal of the phenyl substituents by catalytic hydrogenation, the desired M1P product was obtained.

Experimental

General. All reagents and solvents were reagent grade and were used without further purification unless otherwise specified. All solvents were anhydrous grade unless indicated otherwise. CH_2Cl_2 was distilled from CaH_2. All non-aqueous reactions were performed in dried glassware under argon atmosphere. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plate using potassium permanganate or ninhydrin as visualizing agents. Silica gel (0.040–0.063 mm particle sizes) was used for column chromatography.

Melting points were recorded on a Yanaco apparatus. Infrared (IR) spectra were recorded on a Varian 640-IR FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Advance-400 (400 MHz) spectrometer. Chemical shifts (δ) are given in parts per million (ppm) relative to internal standards: $CHCl_3$ (δ_H = 7.24), $CDCl_3$ (δ_C = 77.0 for the central line of triplet), CH_3OD (δ_H = 3.31), CD_3OD (δ_C = 49.15), $(CD_2H)_2SO$ (δ_H = 2.49), $(CD_3)_2SO$ (δ_C = 39.5) and HDO (δ_H = 4.80). The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad), and coupling constant (*J*) are given in Hertz (Hz). The ESI−MS experiments were conducted on a Brucker Daltonsics BioTOF III high-resolution mass spectrometer. UV−Vis spectra were recorded on a Perkin Lambda 35 spectrometer. Fluorescence spectra were recorded on an AMINCO-Bowman Series 2 Luminescence spectrometer. SpectraMax i3x multimode microplate reader (Molecular Devices, Hong Kong) was used for maltosyltransferase assay.

Synthetic Procedure and Characterization of Compounds

The HBr salt 2-(bromomethyl)pyridine (**3a**) is commercially available.

3-(Trimethylsilyloxy)aniline (2).

Method A: A mixture of *m*-aminophenol (1.09 g, 10 mmol), trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.6 g, 2.7 mmol), and hexamethyldisilazazane (HMDS, 0.13 g, 7 mmol) was stirred at room temperature for 8 h, and then extracted with hexane. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give compound **2** (1.55 g, 86% yield).

Method B:[s2] A mixture of *m*-aminophenol (2.18 g, 20 mmol), hexamethyldisilazane (HMDS, 2.58 g, 16 mmol) and phosphotungstic acid (560 mg, 0.2 mmol) was heated at 55−60 ^oC for 45 min. After completion of the reaction as monitored by TLC, hexane (100 mL) was added, and the catalyst was removed by filtration. The filtrate was washed with water (4 × 50 mL) to remove excess HMDS. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give compound **2** (3.62 g, 99% yield).

$C_9H_{15}NOSi$; yellow oil; TLC (EtOAc/hexane = 2:1) R_f = 0.76; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (1 H, t, *J* = 8 Hz), 6.31–6.18 (3 H, m), 3.59 (2 H, s), 0.28–0.22 (9 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 147.7, 129.9, 110.4, 108.6, 107.1, 0.2 (3 ×); ESI–HRMS calcd for $C_9H_{15}NOSi$: 182.1001, found *m/z* 182.0992 $[M + H]^+$.

2-Bromomethyl-6-methylpyridine (3b). A mixture of 2-hydroxymethyl-6-methylpyridine (500 mg, 4.06 mmol) and H_2SO_4−HBr solution (v/v = 4:6, 10 mL) was heated at reflux and stirred for 17 h. The mixture was neutralized with $NaHCO_3$, and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the bromination product **3b** (689 mg, 91% yield). C_7H_8BrN; red solid; mp 36–37 ^{o}C; TLC (EtOAc/hexane = 1:1) R_f = 0.67; IR ν_{max} (neat) 3364, 2923, 2847, 1628, 1593, 1457, 1386, 1048, 798 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (1 H, t, *J* = 8.0 Hz), 7.21 (1 H, d, *J* = 8.0 Hz), 7.04 (1 H, d, *J* = 8.0 Hz), 4.49 (2 H, s), 2.52 (3 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 156.0, 137.1, 122.5, 120.4, 34.0, 24.3; ESI–HRMS C_7H_9NBr calcd for C_7H_9BrN: 185.9918, found *m/z* 185.9919 $[M + H]^+$.

3-(Bis(pyridin-2-ylmethyl)amino)phenol (4a). To a solution of aniline **2** (0.24 g, 1.32 mmol) and 2-(bromomethyl)pyridine (**3a**, as the HBr salt, 0.73 g, 2.9 mmol) in H_2O (0.11 mL), were added cetyltrimethylammonium chloride (CTAC, 4.6 mg), and NaOH (5 M, 1 mL). The mixture was stirred for 24 h at room temperature, and then extracted with CH_2Cl_2 and H_2O. The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by column chromatography (EtOAc/hexane = 1:2 to 2:1) to give compound **4a** (0.13g, 55% yield). $C_{18}H_{17}N_3O$; orange solid; mp 149–150 ^{o}C; TLC (EtOAc/hexane = 1:1) R_f = 0.09; IR ν_{max} (neat) 3068, 1589, 1509, 1474, 1195, 1175, 749 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (2 H, d, *J* = 4.4 Hz), 7.61 (2 H, td, *J* = 7.6, 1.6 Hz), 7.26 (2 H, d, *J* = 7.6 Hz), 7.11–7.08 (2 H, m), 7.03 (1 H, t, *J* = 8.2 Hz), 6.27－6.21 (2 H, m), 6.09 (1 H, d, *J* = 2.0 Hz), 4.79 (4 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 158.4 (2 ×), 149.3, 149.0 (2 ×), 137.3 (2 ×), 130.5, 122.2 (2 ×), 120.9 (2 ×), 105.2, 104.2, 99.5, 57.2 (2 ×); ESI–HRMS calcd for $C_{18}H_{18}N_3O$: 292.1450, found *m/z* 292.1450 $[M + H]^+$.

3-(Bis((6-methylpyridin-2-yl)methyl)amino)phenol (4b).

Method C: To a solution of aniline **2** (0.49 g, 2.72 mmol) and bromo compound **3b** (1.11 g, 5.98 mmol) in H_2O (0.5 mL) were added CTAC (8 mg) and NaOH (5 M, 2 mL). The mixture was stirred for 48 h at room temperature, and then extracted with CH_2Cl_2 and H_2O. The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by column chromatography (EtOAc/hexane = 1:2 to 2:1) to give compound **4b** (0.39 g, 45 % yield).

Method D: To a solution of aniline **2** (98 mg, 0.54 mmol) and bromo compound **3b** (202

mg, 1.08 mmol) were added cetyltrimethylammonium chloride (CTAC, 17 mg, 0.054 mmol) and sat. $NaHCO_{3(aq)}$ (1.8 mL). The mixture was stirred for 24 h at room temperature, and then extracted with CH_2Cl_2 and H_2O. The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by column chromatography (EtOAc/hexane = 1:2 to 2:1) to give compound **4b** (113 mg, 66% yield).

$C_{20}H_{21}N_3O$; solid; mp 212–214 °C; TLC (EtOAc/hexane = 1:1) R_f = 0.21; IR ν_{max} (neat) 3432, 2919, 1620, 1453, 1163, 1068, 782 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (2 H, t, *J* = 8.0), 7.09 (2 H, d, *J* = 8.0 Hz), 7.03 (1 H, t, *J* = 8.0 Hz), 6.92 (2 H, d, *J* = 8.0 Hz), 7.00 (1 H, d, *J* = 8.0 Hz), 6.27 (1 H, dd, *J* = 8.0, 2.0 Hz), 6.25 (1 H, dd, *J* = 8.0, 2.0), 6.10 (1 H, t, *J* = 2.0 Hz), 4.76 (4 H, s), 2.32 (1 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 158.1 (2 ×), 157.9 (2 ×), 149.3, 137.5 (2 ×), 130.5, 121.8 (2 ×), 117.7 (2 ×), 105.4, 103.8, 99.1, 57.2 (2 ×), 23.6 (2 ×); ESI–HRMS calcd for $C_{20}H_{22}N_3O$: 320.1763, found *m/z* 320.1758 $[M + H]^+$.

7-(Bis(pyridin-2-ylmethyl)amino)-4-methylcoumarin (1a). To a mixture of phenol **4a** (50 mg, 0.16 mmol) and ethyl acetoacetate (32 mg, 0.32 mmol) was added H_2SO_4 (70%, 0.2 mL) at 0 °C. The mixture was stirred for 3 h at room temperature, neutralized with $NaHCO_3$, and then extracted with CH_2Cl_2 and H_2O. The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by column chromatography (EtOAc/hexane = 1:1 to 1:0) to give compound **1a** (55 mg, 94 % yield). $C_{22}H_{19}N_3O_2$; yellow solid; mp 67–69 °C; TLC (EtOAc/hexane = 1:1) R_f = 0.09; UV-Vis (MeOH) λ_{abs} = 358 nm; FL (MeOH) λ_{em} = 441 nm (λ_{ex} = 358 nm); IR ν_{max} (neat) 3412, 3070, 3007, 2927, 1720, 1612, 1525, 1406, 1175, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (2 H, d, *J* = 4.8 Hz), 7.62 (2 H, td, *J* = 7.6, 1.9 Hz), 7.33 (1 H, d, *J* = 8.8 Hz), 7.21–7.17 (4 H, m), 6.64 (1 H, dd, *J* = 8.8, 2.4 Hz), 6.57 (1 H, d, *J* = 2.4 Hz), 5.95 (1 H, d, *J* = 0.8 Hz), 4.87 (4 H, s), 2.29 (3 H, d, *J* = 1.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 157.3 (2 ×), 155.5, 152.6, 151.3, 149.9 (2 ×), 136.9 (2 ×), 125.5, 122.4 (2 ×), 120.7 (2 ×), 110.7, 109.9, 109.4, 99.4, 57.3 (2 ×), 18.4.; ESI–HRMS calcd for $C_{22}H_{20}N_3O_2$: 358.1556, found *m/z* 358.1531 $[M + H]^+$.

7-(Bis((6-methylpyridin-2-ylmethyl)amino)-4-methylcoumarin (1b). To a mixture of phenol **4b** (0.183 g, 0.57 mmol) and ethyl acetoacetate (0.112 g, 0.86 mmol) was added H_2SO_4 (70%, 1 mL) at 0°C. The mixture was stirred for 3 h at room temperature, neutralized with $NaHCO_3$, and then extracted with CH_2Cl_2 and H_2O. The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by column chromatography (EtOAc/hexane = 1:1 to 1:0) to give coumarin compound **1b** (0.198 g, 90% yield). $C_{24}H_{23}N_3O_2$; yellow sticky liquid; TLC (EtOAc/hexane = 1:1) R_f = 0.16; UV-Vis (MeOH) λ_{abs} = 358 nm; FL (MeOH) λ_{em} = 442 nm (λ_{ex} = 358 nm); IR ν_{max} (neat) 3476, 2927, 2844, 1716, 1612, 1577, 1530, 1457, 1413, 1330, 1270, 1171 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (2 H, t, *J* = 7.6 Hz), 7.31 (1 H, d, *J* = 8.8), 7.01 (2 H, d, *J* = 7.6 Hz), 6.96 (2 H,

d, *J* = 7.6 Hz), 6.61 (1 H, dd, *J* = 8.8, 2.4 Hz), 6.52 (1 H, d, *J* = 2.4), 5.92 (1 H, d, *J* = 0.8 Hz), 4.81 (4 H, s), 2.52 (6 H, s), 2.26 (3 H, d, *J* = 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 158.7 (2 ×), 156.5 (2 ×), 155.5, 152.6, 151.3, 137.1 (2 ×), 125.4, 121.9 (2 ×), 117.3 (2 ×), 110.5, 109.8, 109.3, 99.3, 57.4 (2 ×), 24.4 (2 ×), 18.4.; ESI–HRMS calcd for $C_{24}H_{24}N_3O_2$: 386.1863, found *m/z* 386.1848 $[M + H]^+$.

7-(Bis(pyridin-2-ylmethyl)amino)-4-methylcoumarin copper complex (Cu-1a). A solution of **1a** in MeOH (2 mL, 10^{-5} M) was added $Cu(NO_3)_2 \cdot 3H_2O$ (6×10^{-2} M) in H_2O (1 μL). The mixture was stirred for 30 min at room temperature to afford the stock solution of Cu-**1a** complex (2 mL, 10^{-5} M in MeOH). ESI–HRMS calcd for $[C_{22}H_{19}CuN_3O_2 \bullet NO_3]^+$: 482.0661, found *m/z* 482.0630 $[(\text{Cu-}\mathbf{1a}) \bullet NO_3]^+$.

7-(Bis((6-methylpyridin-2-ylmethyl)amino)-4-methylcoumarin copper complex (Cu-1b). To a solution of **1b** in MeOH (2 mL, 10^{-5} M) was added $Cu(NO_3)_2 \cdot 3H_2O$ (6×10^{-2} M) in H_2O (1 μL). The mixture was stirred for 30 min at room temperature to afford the stock solution of Cu-**1b** complex (2 mL, 10^{-5} M in MeOH). ESI–HRMS calcd for $[C_{24}H_{23}CuN_3O_2 \bullet NO_3]^+$: 510.0964, found *m/z* 510.0949 $[(\text{Cu-}\mathbf{1b}) \bullet NO_3]^+$.

A crystal of Cu-**1b** complex was obtained as follows. To a solution of ligand **1b** in MeOH (0.5 mL, 29.7 mM) was added an aqueous solution of $Cu(NO_3)_2$ (44.6 μL, 1.0 M). The mixture was stirred for 30 min at room temperature. After complexation completed, MeOH was removed under reduced pressure, and the residual aqueous solution was kept in the dark for three days to give as dark-blue crystals of Cu-**1b** complex. The X-ray diffraction analysis indicated that the complex existed as a dimeric form $[H_2O \bullet \text{Cu-}\mathbf{1b}]_2$ with two water molecules coordinated to the two Cu(II) centers.

2,3,6,2',3',4',6'-Heptaacetyl-maltose (S1). To a well-stirred suspension of D-maltose monohydrate (500 mg, 1.39 mmol) in Ac_2O (1.4 mL, 15.0 mmol) at 0 °C was added TsOH monohydrate (27 mg, 0.14 mmol) under N_2.[s2] The mixture was stirred at 0 °C for 0.5 h, slowly warmed to room temperature, and stirred until peracetylation completed (4 h). The mixture was quenched by adding H_2O, and stirred for another 15 min. The mixture was partitioned between EtOAc and water. The organic layer was washed successively with water, 1 M $NaOH_{(aq)}$ (×3), water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dried under reduced pressure for 2 h to give a crude product of peracetylated maltose, which was used for the next step without further purification.

To a well-stirred solution of the above-prepared peracetylated maltose in anhydrous THF (4.6 mL) at room temperature was added benzylamine (0.23 mL, 2.08 mmol) under N_2.[s3] The mixture was stirred for 16 h, and then concentrated under reduced pressure. The residue was diluted with EtOAc, washed successively with 1 M $HCl_{(aq)}$ (× 2), $NaHCO_{3(satd.)}$, H_2O and brine.

The organic phase was dried over Na_2SO_4, concentrated under reduced pressure, and subject to flash chromatography over silica gel (hexane/EtOAc gradients, 4:1 to 2:3) to afford compound **S1** (875 mg, 1.37 mmol, 99% yield).

Diphenylphosphoryl 2,3,6,2',3',4',6'-heptaacetyl-maltoside (S2). To a well-stirred solution of **S1** (875 mg, 1.37 mmol) in anhydrous CH_2Cl_2 (14 mL) at room temperature was added DMAP (502 mg, 4.11 mmol) under N_2. The mixture was stirred for 0.5 h, cooled to −10 ^oC, and diphenyl phosphoryl chloride (0.71 mL, 3.43 mmol) was added dropwise. After which, the mixture was slowly warmed to −5 ^oC, and kept stirring for 1 h until phosphorylation completed.[s4] The mixture was quenched by adding $NaHCO_{3(satd.)}$, and stirred at room temperature for 15 min. The organic layer was separated, diluted with EtOAc, and washed successively with $NaHCO_{3(satd.)}$, H_2O and brine. The organic phase was dried over Na_2SO_4, concentrated under reduced pressure, and subject to flash chromatography over silica gel (hexane/EtOAc/CH_2Cl_2 gradients, 4:1:1 to 2:1:1) to give compound **S2** (714 mg, 60% yield). $C_{38}H_{45}O_{21}P$; colorless oil; TLC (hexane/EtOAc/CH_2Cl_2 = 2:1:1) R_f = 0.18; $[\alpha]^{23}_D$ = +106.1 (*c* = 1.35, $CHCl_3$); IR (KBr, neat) ν_{max} 1759, 1595, 1483, 1370, 1232, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33−7.29 (4 H, m, ArH), 7.24−7.14 (6 H, m, ArH), 5.90 (1 H, dd, *J* = 6.6 Hz, 3.2 Hz, H-1), 5.50 (1 H, dd, *J* = 10.0, 8.4 Hz), 5.35 (1 H, d, *J* = 4.0 Hz, H-1'), 5.28 (1 H, t, *J* = 10.0 Hz), 4.99 (1 H, t, *J* = 9.6 Hz), 4.83−4.77 (2 H, m), 4.19−4.14 (2 H, m), 4.04 (1 H, dd, *J* = 12.4, 3.2 Hz), 3.97−3.93 (3 H, m), 3.82 (1 H, d, *J* = 10.2, 2.8 Hz), 2.01−2.00 (9 H, m, CH_3 × 3), 1.95−1.93 (9 H, s, CH_3 × 3), 1.74 (3 H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.3, 170.1 (2×), 169.7, 169.5, 169.2, 150.14, 150.07, 129.8, 129.7, 125.6, 120.42, 120.38, 120.0, 119.9, 95.5 (C-1'), 94.7 (C-1, J_{CP} = 5.2 Hz), 71.6, 71.4, 70.00, 69.95, 69.90, 69.1, 68.4, 67.8, 61.8, 61.2, 20.7, 20.53, 20.48, 20.4, 20.0; ^{31}P NMR (162 Hz, $CDCl_3$) δ −13.5; HRMS (ESI) calcd for $C_{38}H_{45}NaO_{21}P$: 891.2089, found: *m*/*z* 891.2068 $[M + Na]^+$.

***α*-Maltosyl-1-phosphate (M1P).** A suspension of compound **S2** (250 mg, 0.29 mmol) and $PtO_2 \cdot H_2O$ (18 mg, 0.07 mmol) in EtOH (6 mL) was stirred at room under H_2 (balloon) atmosphere for 20 h until the hydrogenolysis completed.[s4] The mixture was filtered through a pad of Ceilite, and the filtrate was concentrated under reduced pressure. The residue was dried under reduced pressure for 2 h to give a crude product, which was dissolved in a cosolvent system [(7 M NH_3 in MeOH)/(30% aqueous NH_3) = 1:1, 6 mL], and stirred at room temperature for 12 h. The mixture was concentrated under reduced pressure, and the residue was dissolved in 30% $NH_{3(aq)}$ (16 mL). The mixture was stirred at room temperature for another 12 h, and concentrated under reduced pressure. The residue was directly purified by DE-52 anionic ion exchange gel with elution of H_2O, and then 0.5 M $NH_4HCO_{3(aq)}$, to give M1P as the mono-ammonium salt (119 mg, 93% yield). $C_{12}H_{26}NO_{14}P$; white hydroscopic solid; $[\alpha]^{24}_D$ = +155.5 (*c* = 0.5, H_2O), lit.[s5] $[\alpha]_D$ = +147; IR (KBr, neat) ν_{max} 3390, 1640, 1401,

1150,1028, 922 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 5.45 (1 H, dd, *J* = 6.8 Hz, 3.2 Hz, H-1), 5.42 (1 H, d, *J* = 4 Hz, H-1´), 4.04−4.00 (2 H, m), 3.89−3.83 (2 H, m), 3.80−3.71 (3 H, m), 3.69 (1 H, d, *J* = 9.6 Hz), 3.65 (1 H, t, *J* = 9.6 Hz), 3.56 (1 H, dd, *J* = 9.8, 3.6 Hz), 3.52 (1 H, dd, *J* = 8.8, 2.4 Hz), 3.41 (1 H, t, *J* = 9.2 Hz); ^{13}C NMR (100 MHz, D_2O) δ 99.4, 93.4, 76.7, 73.4, 72.7, 72.5, 71.8, 71.7, 70.4, 69.2, 60.5, 60.3; ^{31}P NMR (162 MHz, D_2O) δ 2.2; HRMS (ESI, negative mode) calcd for $C_{12}H_{22}O_{14}P$: 421.0747, found: *m*/*z* 421.0761 $[M - NH_4]^-$.

UV−Vis titration. Spectroscopic grade methanol, deionized water and HEPES buffer (20 mM, pH 7.4) were used to prepare stock solutions: ligand (**1a** or **1b**, 10 μM in MeOH or HEPES); metal ion, e.g. $Cu(NO_3)_2$, (10 mM in water); analyte (Na_3PO_4, $Na_4P_2O_7$, maltose-1-phosphate or maltotetraose, 10 mM in water). All titration experiments were carried out with 2 mL of the ligand stock solution (10 μM) in a quartz cell (1 cm length), added the indicated amount (1.0−3.0 equiv) of Cu^{2+} stock solution (10 mM), and the absorption spectra were recorded at 298 K. The freshly prepared stock solution of analyte (10 mM, 2 μL corresponds to 1.0 equiv) was then added incrementally with a micropipette, and the absorption change was monitored.

Fluorescence titration. The fluorescence spectra were taken by using the same samples employed in the UV−vis study, i.e., transferring the same cuvette from the UV−vis spectrophotometer to the fluorescence spectrophotometer for each incremental addition. Fluorescence titration curves were analyzed with the nonlinear least-squares curve-fitting method on the basis of 1:1 stoichiometry to evaluate the apparent association constant (*K*, M^{-1}) of the complex.

$$y = f + \left[\frac{d-f}{2c}\right]\{\frac{1}{K} + c + x - \left[\left(\frac{1}{K} + c + x\right)^2 - 4cx\right]^{0.5}\}$$

Where *c* is the receptor concentration, *d* is the maximum change of fluorescence intensity at saturation, *f* is the initial fluorescence intensity, *K* is the association constant, *x* is the substrate concentration, and *y* is the fluorescence intensity.

Job plots. Stock solutions of receptor compound (Cu-**1a** and Cu-**1b**) and analyte (Pi and PPi) were prepared in the same concentration (10 μM) by using spectroscopic grade methanol and deionized water, respectively. Eleven sample solutions containing the receptor and analyte ion in different ratios (0:10 to 10:0) were made to maintain total volume of 2 mL. Changes of the absorbance (ΔA) or fluorescence intensity (ΔI) were monitored as a function of molar ratio (X) of the analyte. The complex concentration was calculated as [complex] = ΔA (or ΔI) × X, where ΔA (or ΔI) is the absorbance (or fluorescence intensity) after adding analyte minus the absorbance (or fluorescence intensity) before adding any analyte, and X is

the molar ratio of [analyte]:[receptor].

Probing GlgE catalyzed reaction.

A. Conventional colorimetric method using MESG.[s6] A mixture comprising MESG (1 mM, 20 μL), PNP (0.2 U, 1 μL), 20× reaction buffer (1.0 M Tris−HCl, 20 mM $MgCl_2$, pH 7.5, containing 2 mM NaN_3) (5 μL), glycogen (0.5 μL), GlgE (50 nM, 5 μL) and M1P (250 μM, 3.125 μL) was placed in an Eppendorf tube. Distilled water was added to make an overall reaction volume of 100 μL. The mixture was incubated at 22 ^oC under atmospheric pressure, and then transferred to a 96-well microtiter plate. The absorption at 360 nm for AMMP product was recorded to determine the concentration of the released phosphate ions.

B. Fluorescence method of this study. A mixture comprising Cu-**1b** (1.0×10^{-5} M), maltotetraose (1.25×10^{-4} M), GlgE (6.0×10^{-6} M) and M1P (1.0×10^{-4} M) in 100 μL HEPES buffer (20 mM, pH 7.53, containing 10 mM $MgCl_2$) was placed in an Eppendorf tube. The mixture was incubated at 37 ^oC with shaking (800 rpm) for 18 h, and then transferred to a 96-well microtiter plate. The fluorescence intensity at 448 nm for the Pi•(Cu-**1b**) complex was recorded with a SpectraMax i3x multimode microplate reader to determine the concentration of the released phosphate ions.

Supplementary References:

[s1] Chao, C.-S.; Chen, M.-C.; Lin, S.-C.; Mong, T. K.-K. Versatile acetylation of carbohydrate substrates with bench-top sulfonic acids and application to one-pot syntheses of peracetylated thioglycosides. *Carbohydr. Res.* **2008**, *343*, 957–964.

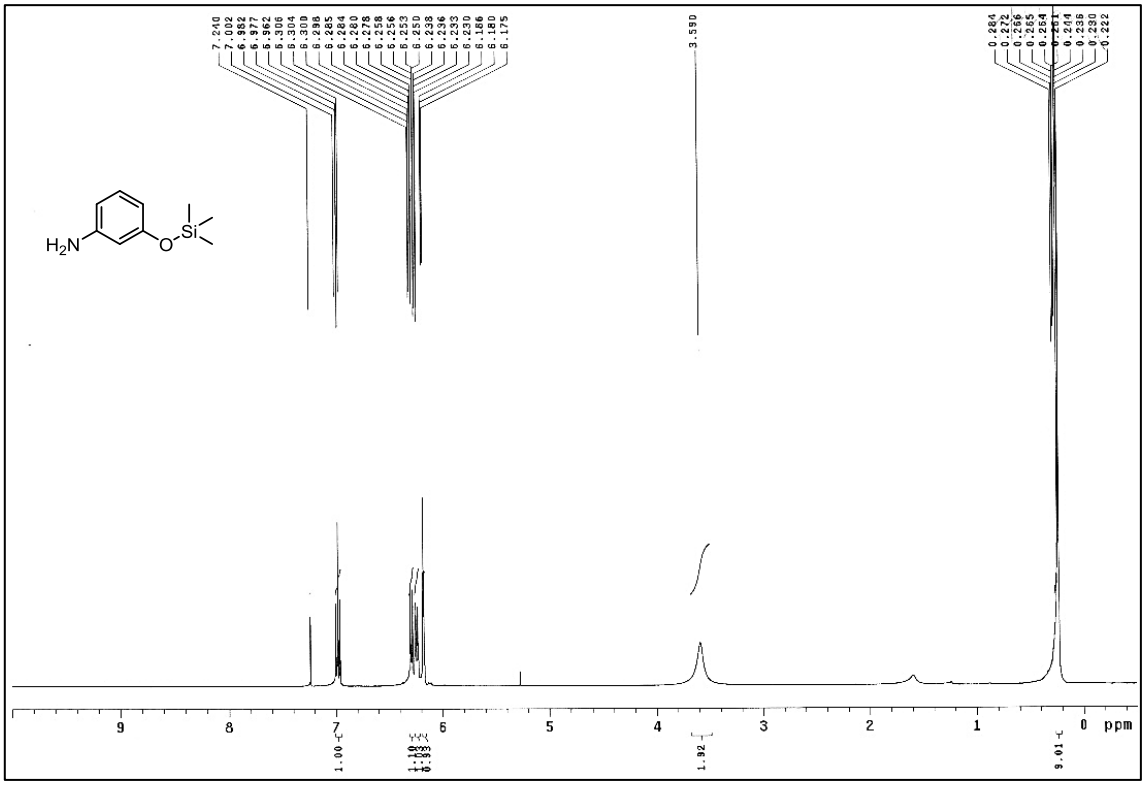
[s2] Firouzabadi, H.; Iranpoor, N.; Amani, K.; Nowrouzi, F. Tungstophosphoric acid ($H_3PW_{12}O_{40}$) as a heterogeneous inorganic catalyst. Activation of hexamethyldisilazane (HMDS) by tungstophosphoric acid for efficient and selective solvent-free *O*-silylation reactions. *J. Chem. Soc., Perkin Trans.* **2002**, *1*, 2601−2604.

[s3] Sim, M. M.; Kondo, H.; Wong, C.-H. Synthesis of dibenzyl glycosyl phosphites using dibenzyl *N,N*-diethylphosphoramidite as phosphitylating reagent: an effective route to glycosyl phosphates, nucleotides, and glycosides. *J. Am. Chem. Soc.* **1993**, *115*, 2260−2267.

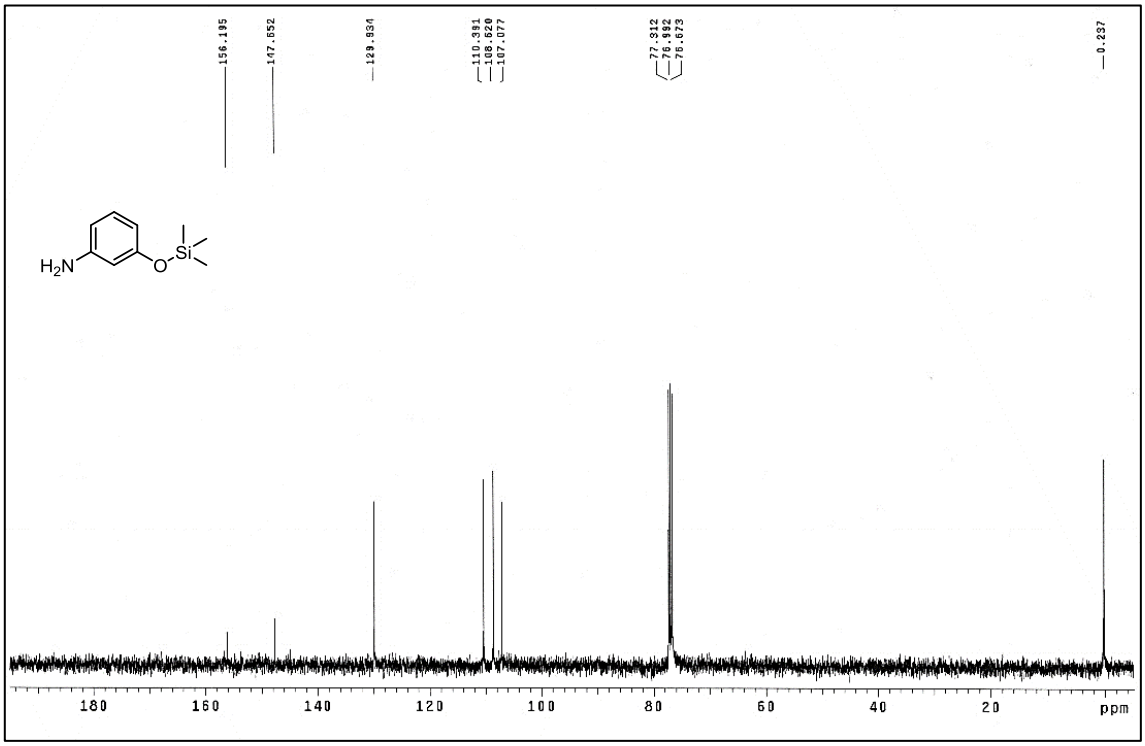
[s4] Sabesan, S.; Neira, S. Synthesis of glycosyl phosphates and azides. *Carbohydr. Res.* **1992**, *223*, 169–185.

[s5] Narumi, K.; Tsumita, T. Identification of α,α-trehalose 6,6-dimannosylphosphate and α-maltose 1-phosphate of *Mycobacteria*. *J. Biol. Chem.* **1967**, *242*, 2233–2239.

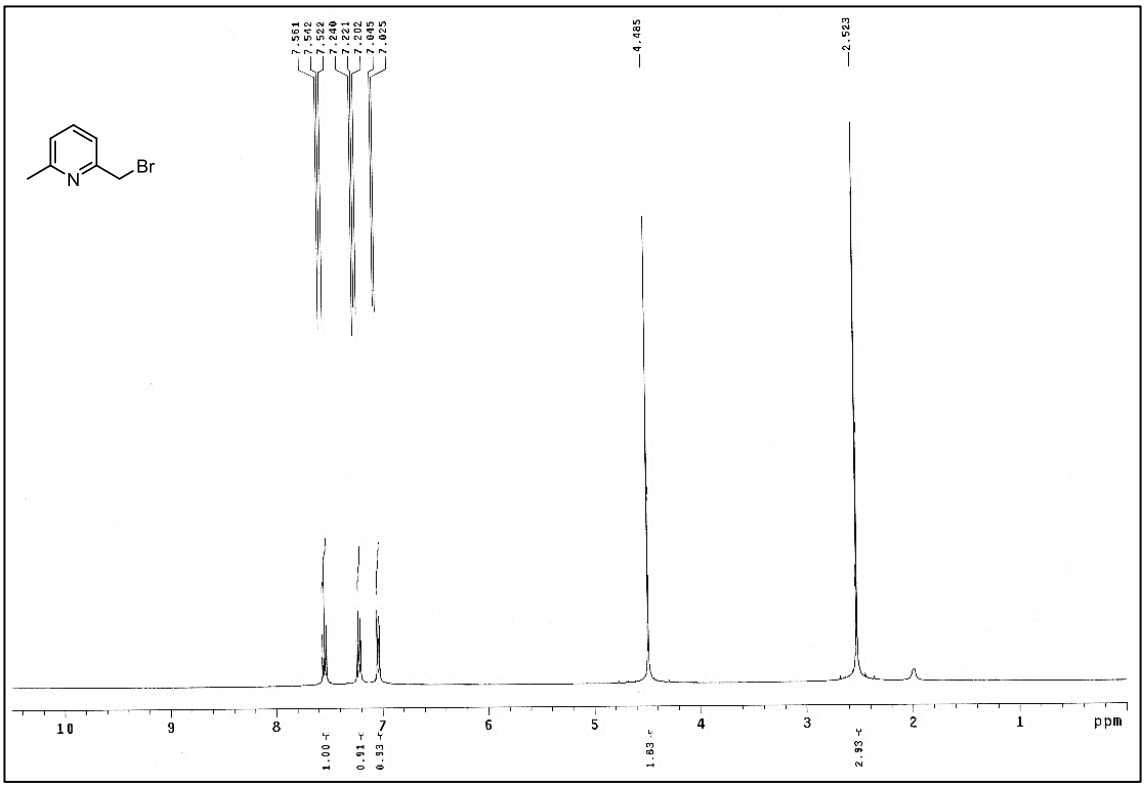
[s6] Veleti, S. K.; Lindenberger, J. J.; Ronning, D. R.; Sucheck, S. J. Synthesis of a *C*-phosphonate mimic of maltose-1-phosphate and inhibition studies on *Mycobacterium tuberculosis* GlgE *Bioorg. Med. Chem.* **2014**, *22*, 1404–1411.



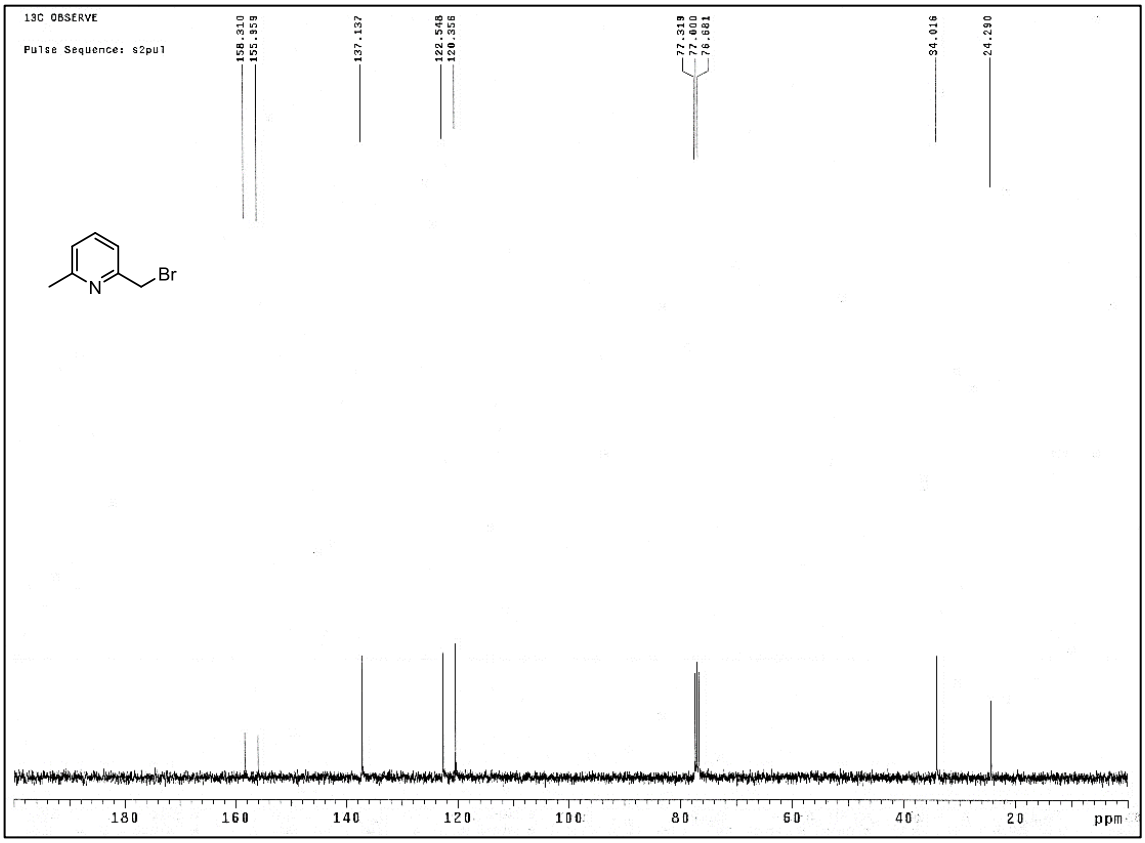
^{1}H NMR spectrum of compound **2** (400 MHz, $CDCl_3$)



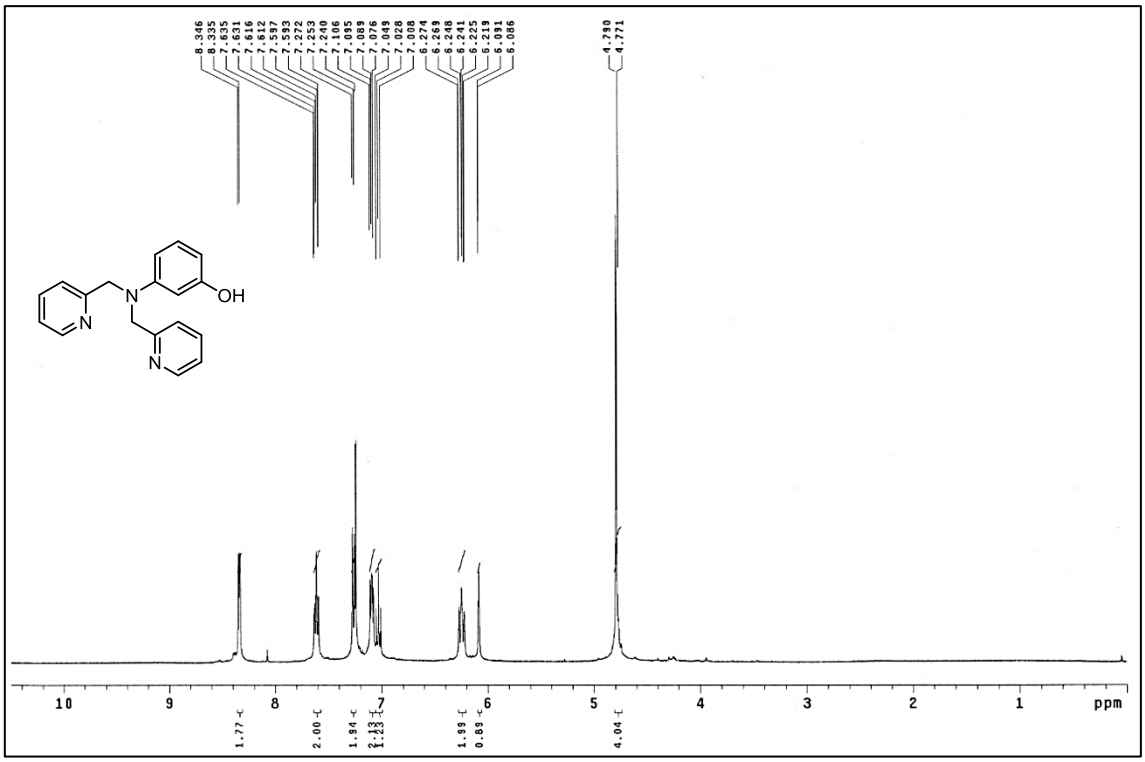
^{13}C NMR spectrum of compound **2** (100 MHz, $CDCl_3$)



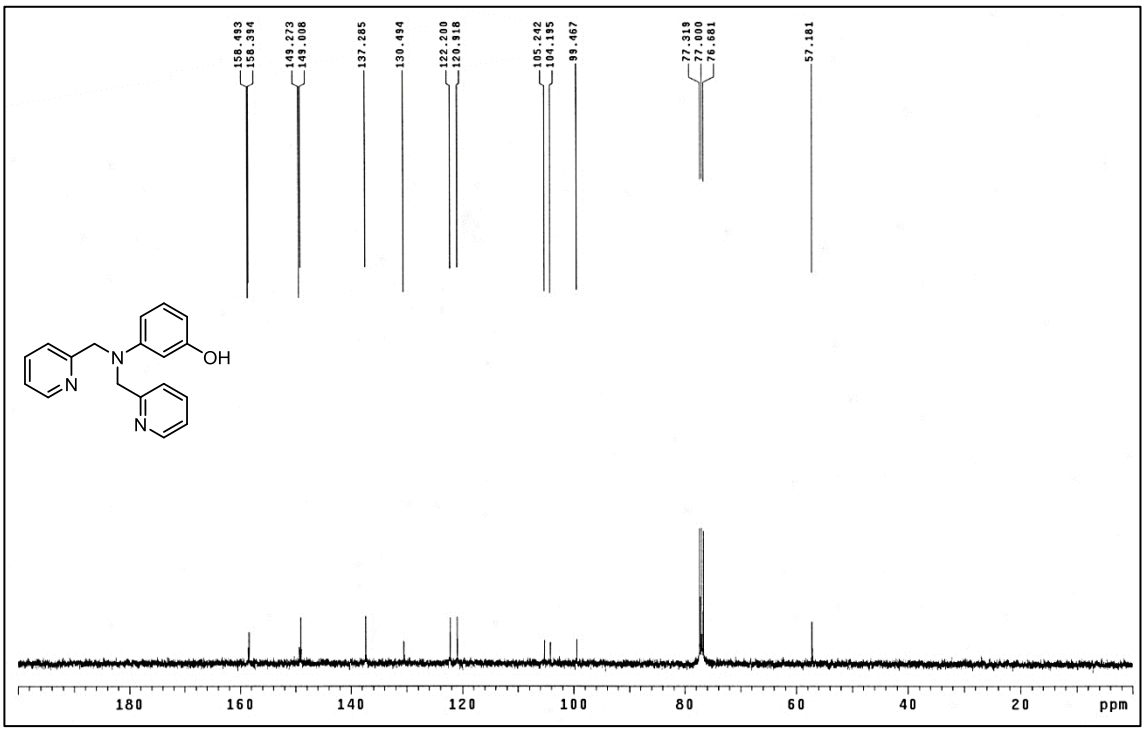
^{1}H NMR spectrum of compound **3b** (400 MHz, $CDCl_3$)



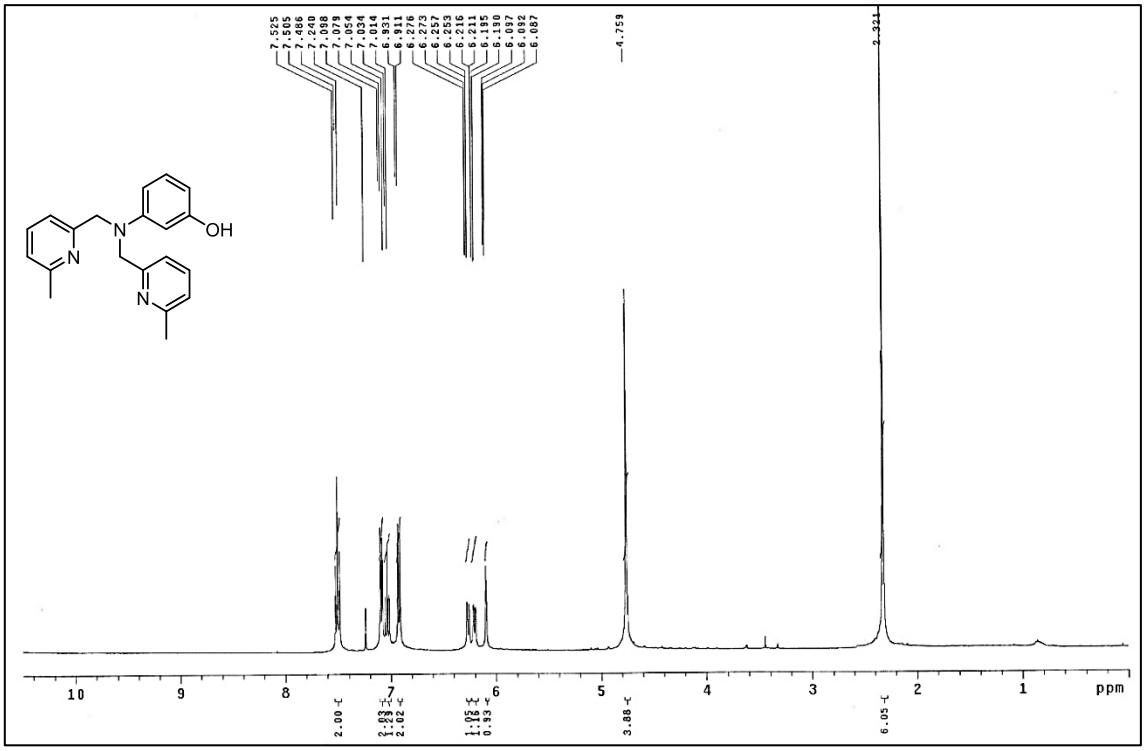
^{13}C NMR spectrum of compound **3b** (100 MHz, $CDCl_3$)



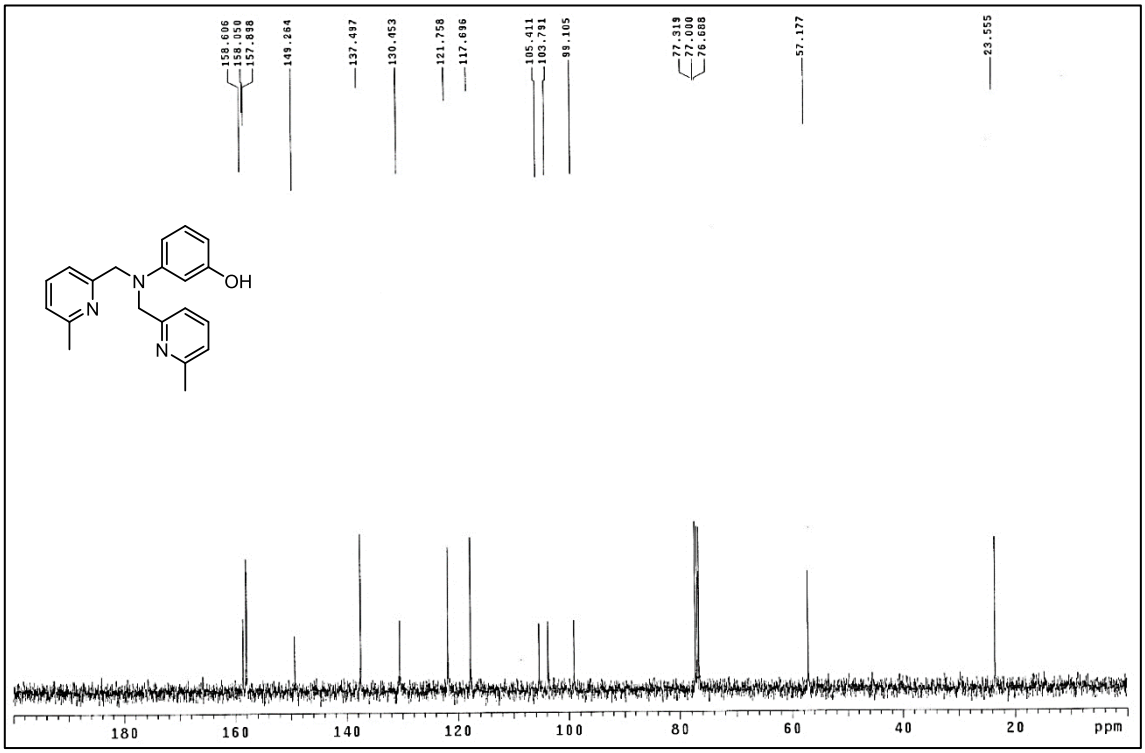
^{1}H NMR spectrum of compound **4a** (400 MHz, $CDCl_3$)



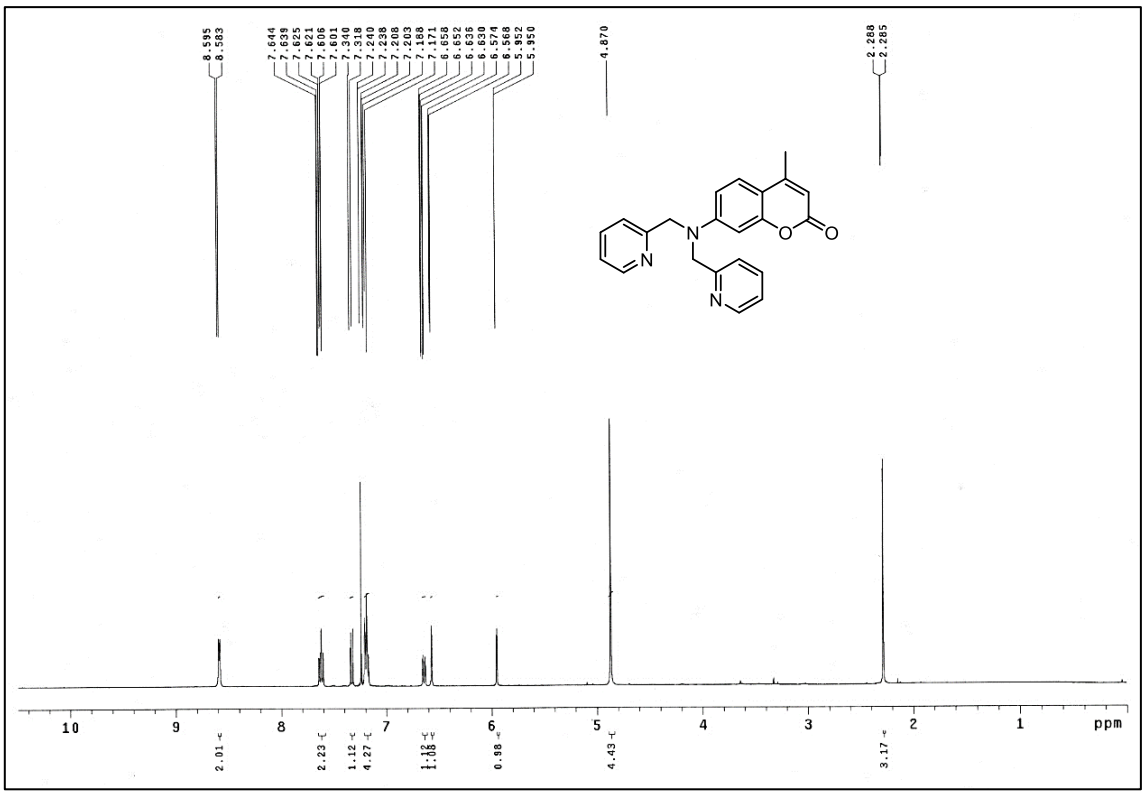
^{13}C NMR spectrum of compound **4a** (100 MHz, $CDCl_3$)



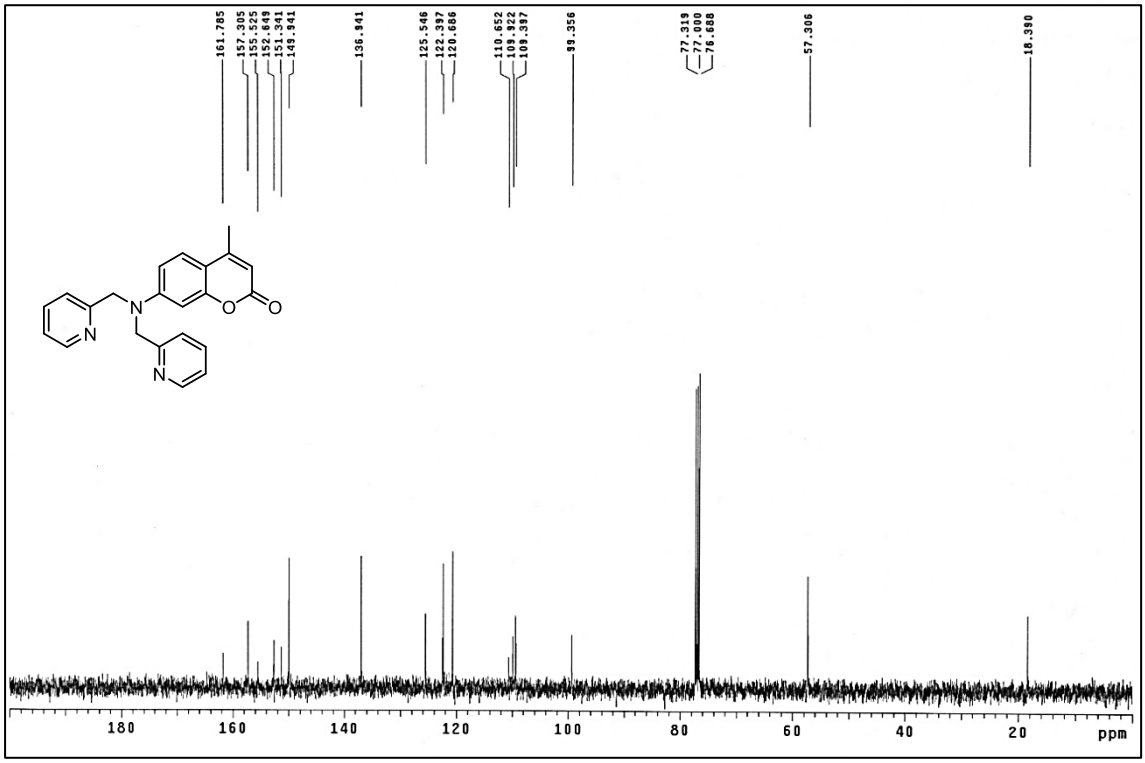
^{1}H NMR spectrum of compound **4b** (400 MHz, $CDCl_3$)



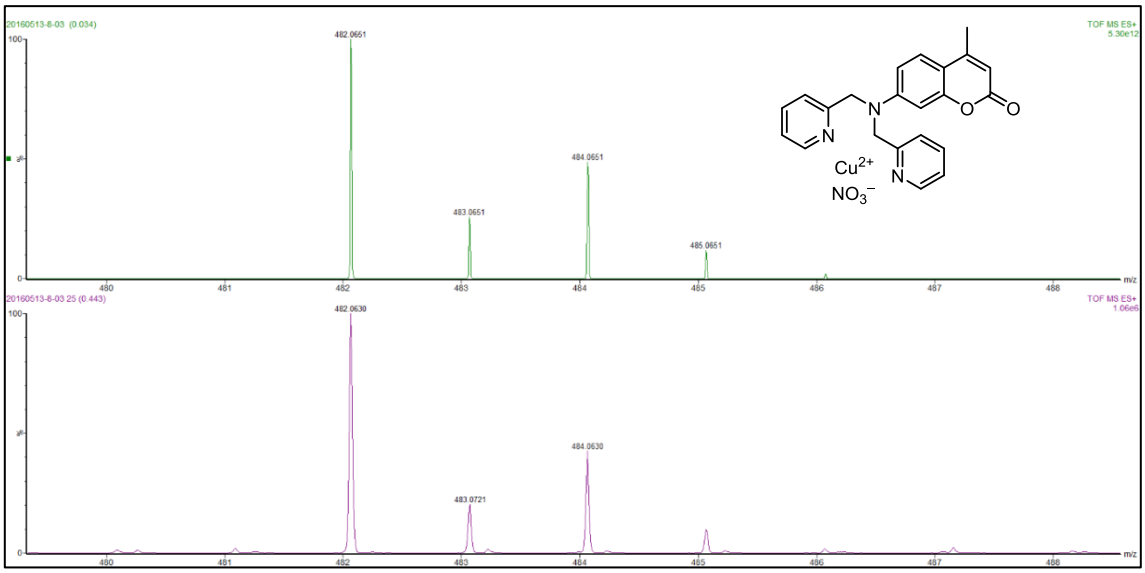
^{13}C NMR spectrum of compound **4b** (100 MHz, $CDCl_3$)



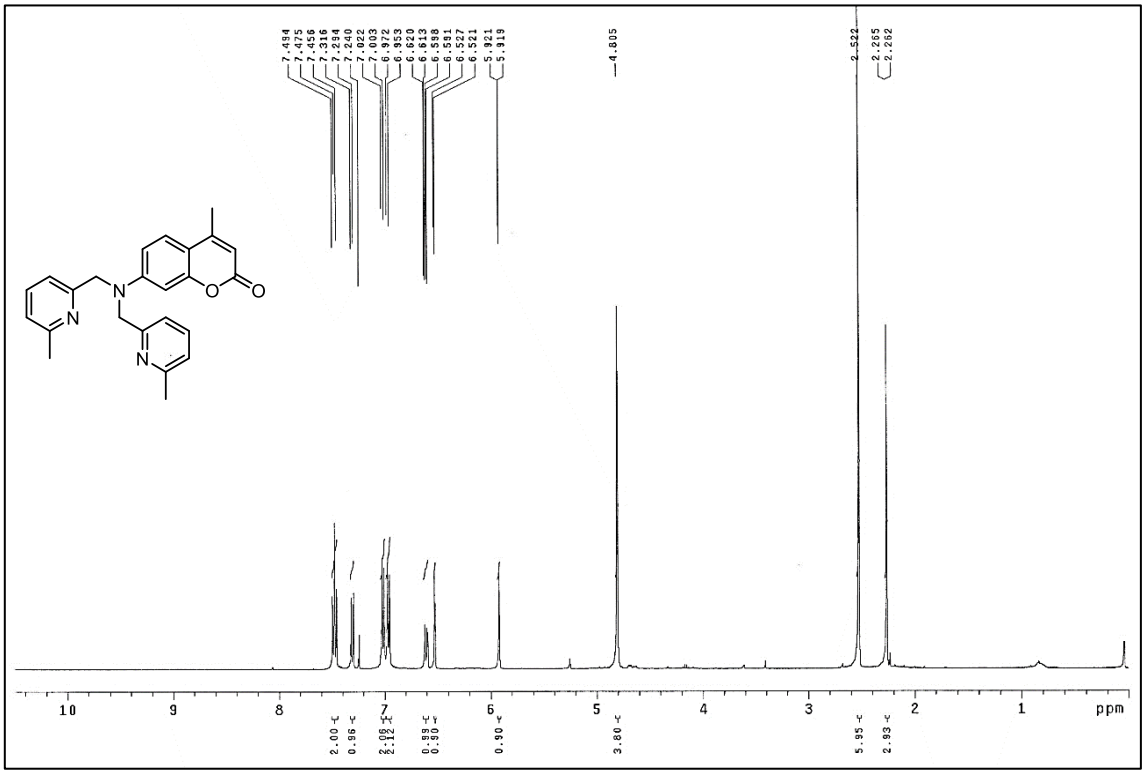
^{1}H NMR spectrum of compound **1a** (400 MHz, $CDCl_3$)



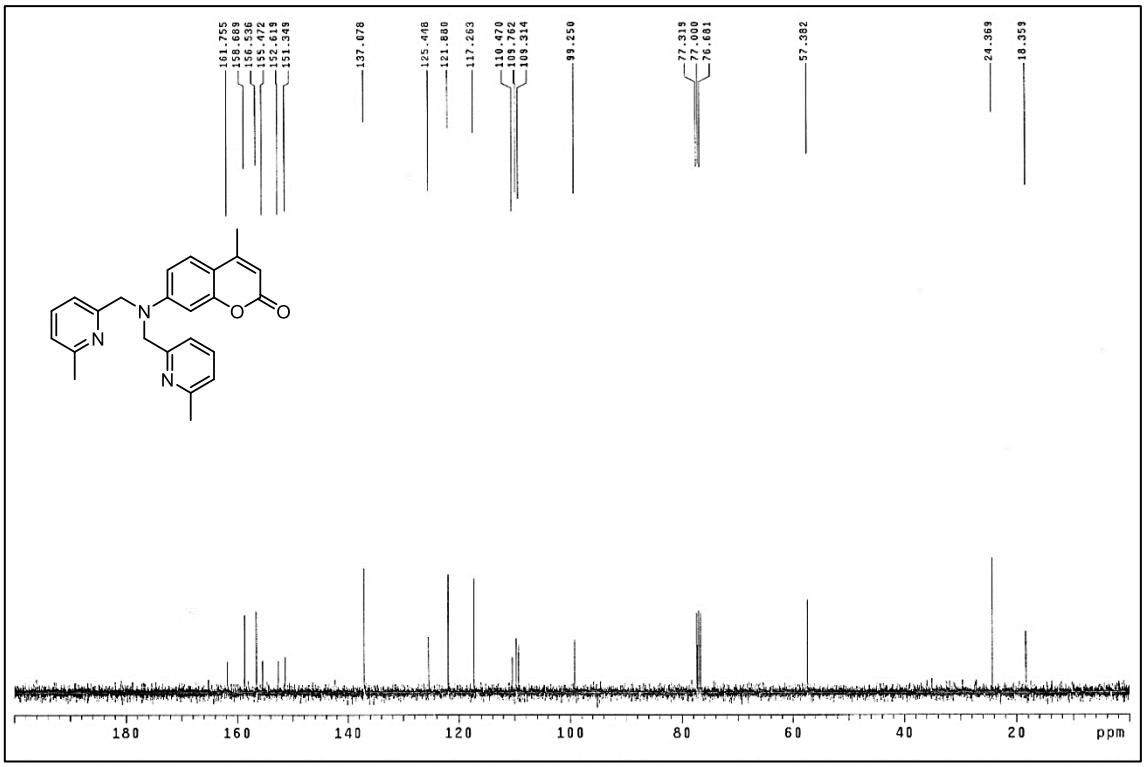
^{13}C NMR spectrum of compound **1a** (100 MHz, $CDCl_3$)



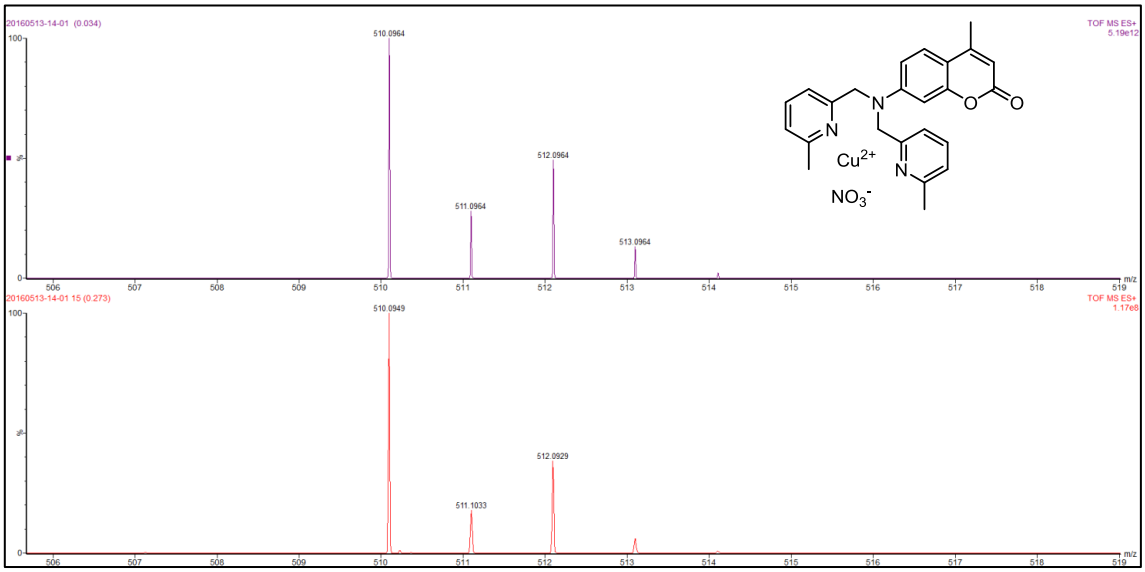
MS analysis of compound **Cu-1a**: calculated data (upper) and experimental result (lower).



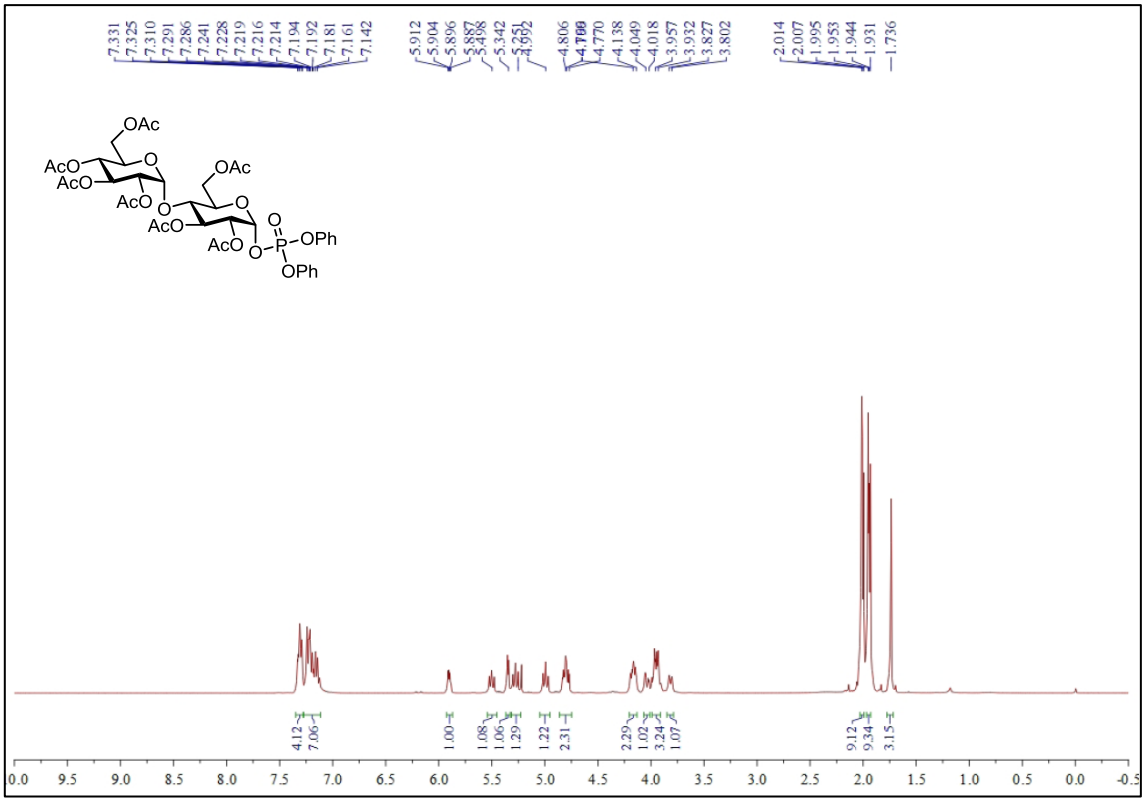
^{1}H NMR spectrum of compound **1b** (400 MHz, $CDCl_3$)



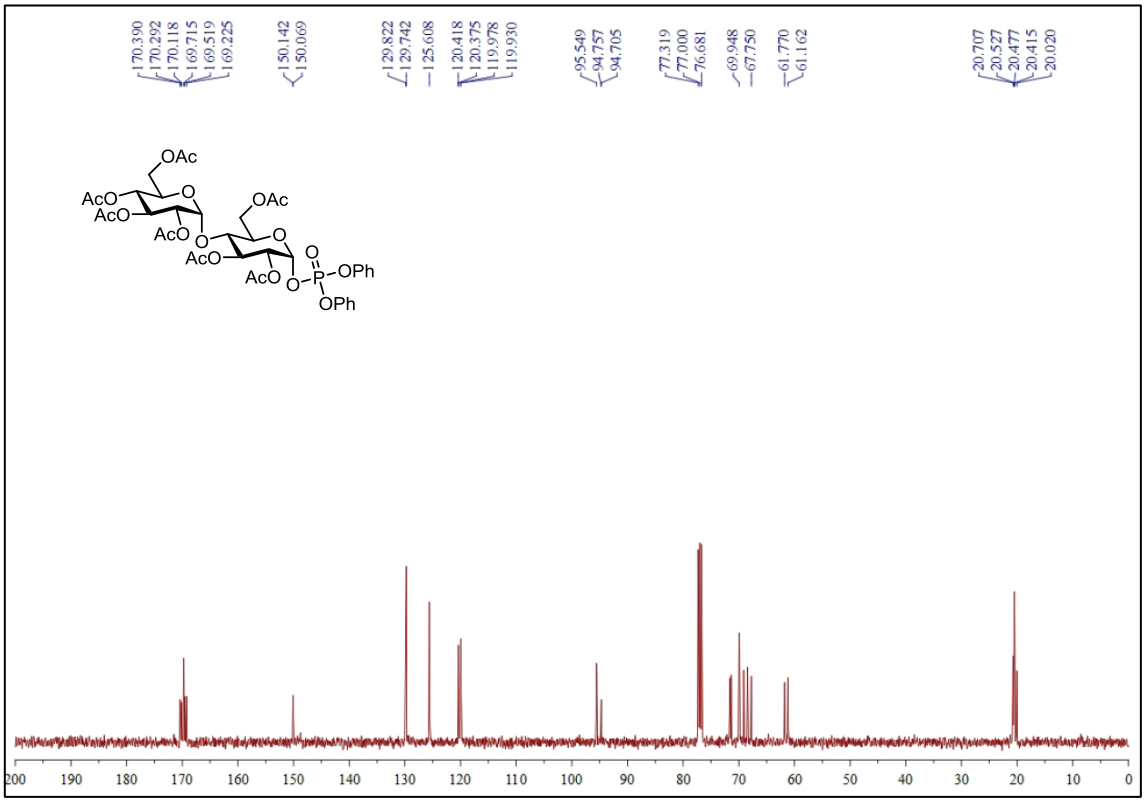
^{13}C NMR spectrum of compound **1b** (100 MHz, $CDCl_3$)



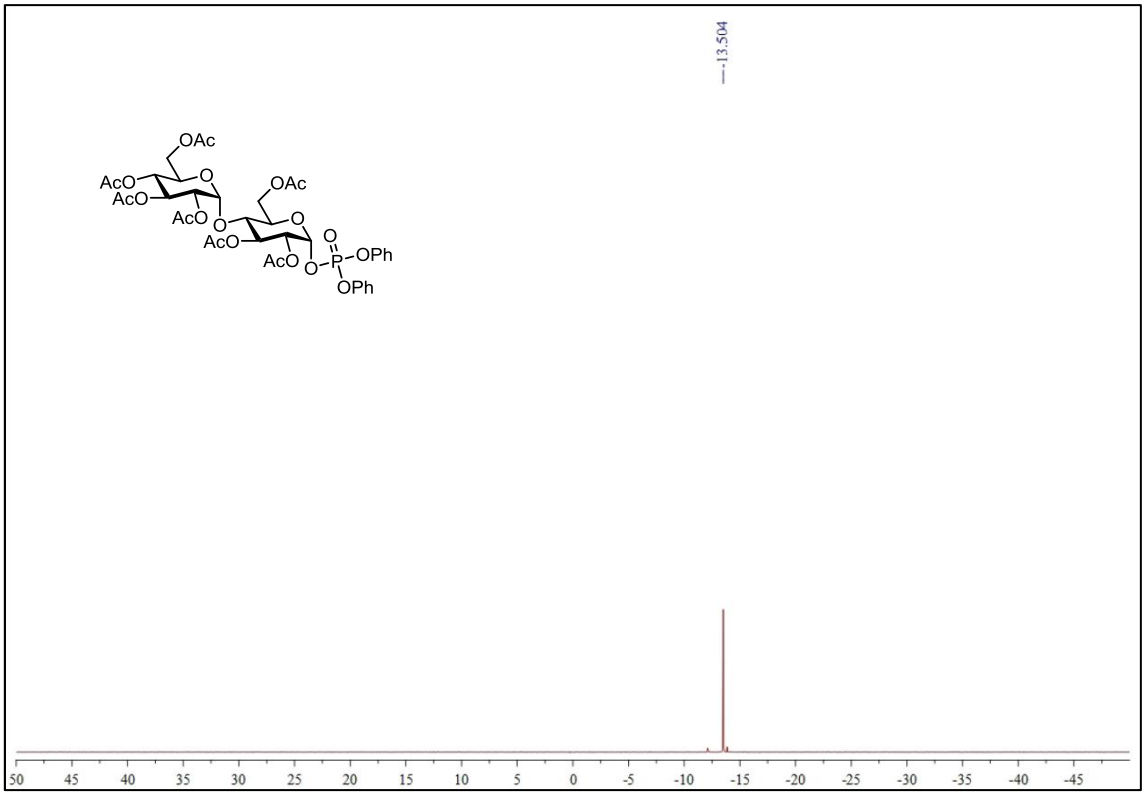
MS analysis of compound **Cu-1b**: calculated data (upper) and experimental result (lower).



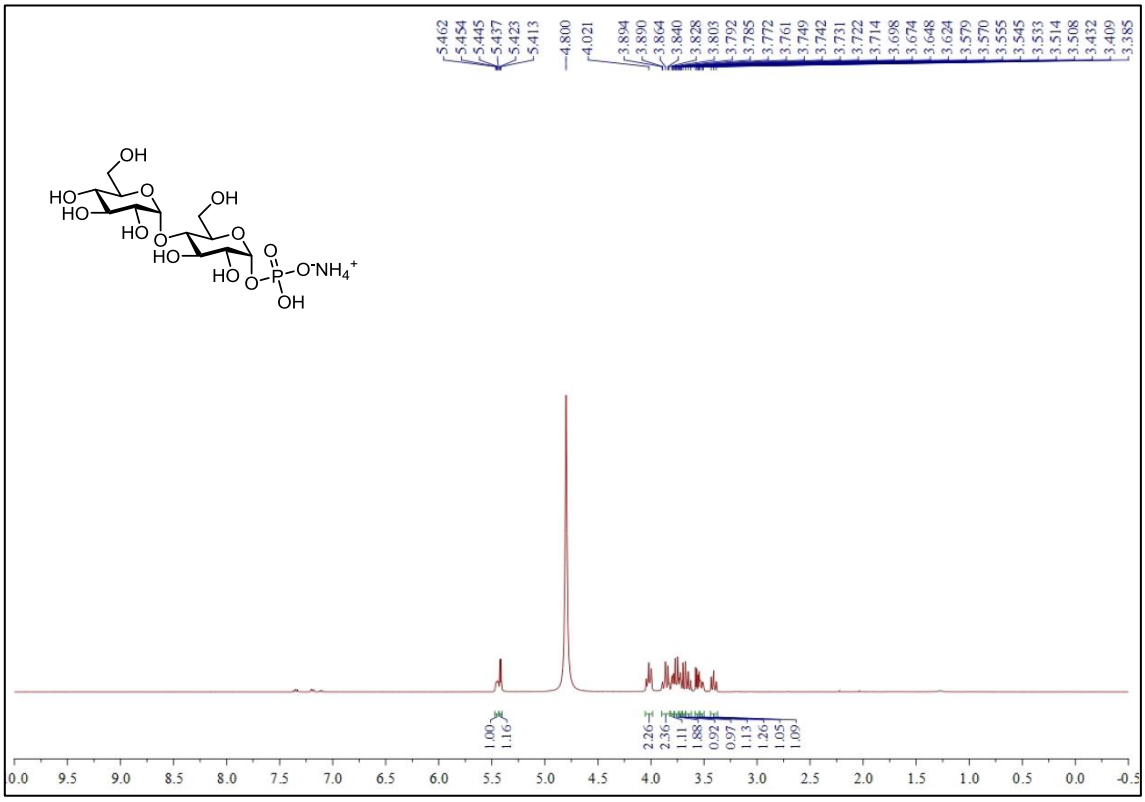
^{1}H NMR spectrum of compound **S2** (400 MHz, $CDCl_3$)



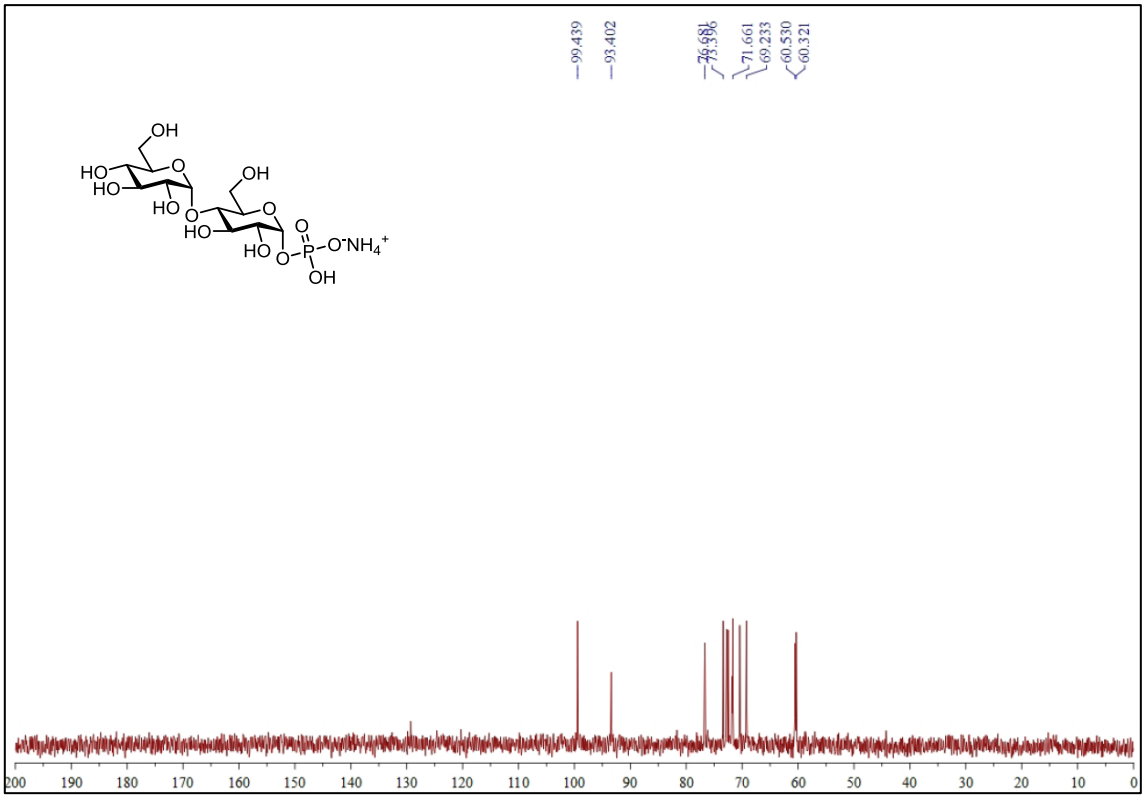
^{13}C NMR spectrum of compound **S2** (100 MHz, $CDCl_3$)



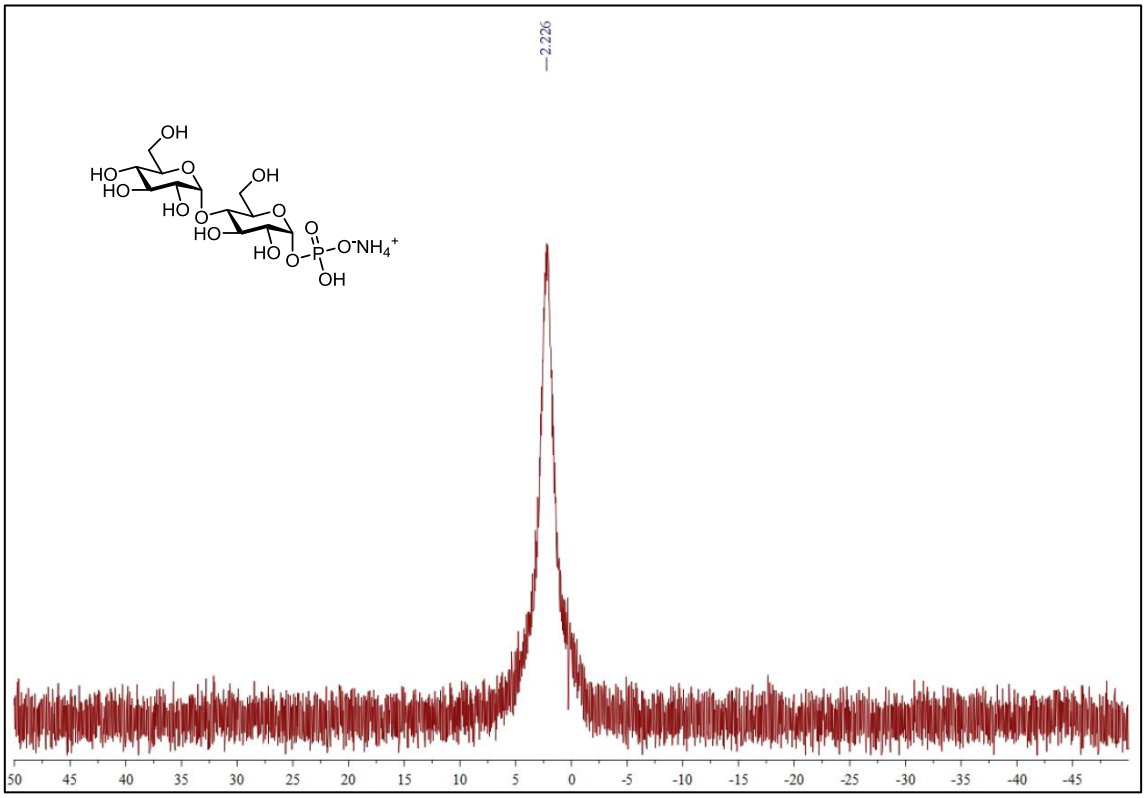
^{31}P NMR spectrum of compound **S2** (162 MHz, $CDCl_3$)



^{1}H NMR spectrum of compound M1P (mono NH_4^+ salt, 400 MHz, D_2O)



^{13}C NMR spectrum of compound M1P (mono NH_4^+ salt, 100 MHz, D_2O)



^{31}P NMR spectrum of compound M1P (mono NH_4^+ salt, 162 MHz, D_2O)