Supporting Information

Efficient one step syntheses of isoprenoid conjugates of nucleoside 5’-diphosphate

Youngha Ryu and A. Ian Scott*

General Methods

HPLC was performed on an Alltech Econosil C18 reverse phase column (220 mm x 22 mm) with flow rate of 10 mL/min of 0.1 M triethylammonium bicarbonate (TEAB)-MeCN mixture. Nuclear magnetic resonance spectra were recorded on a Bruker ARX-500 spectrometer. H_3PO_4 was used as external standard for ^{31}P NMR. High resolution ESI mass spectra were obtained on a MDS Sciex QStar Pulsar mass spectrometer.

General procedures for preparation of isoprenoid conjugates of NDPs.

Method A: Reaction of the imidazolide of nucleoside 5’-monophosphates with isoprenyl monophosphates.

To a solution of nucleoside 5’-monophosphate free acid form (1 mmol) in DMF (5 mL) was added CDI (0.65 g, 4 mmol). The reaction mixture was stirred for 3h and excess DMF was evaporated. The residue was dissolved in 9:1 water-triethylamine mixture (5 mL), stirred for 1h and evaporated *in vacuo* to afford the corresponding imidazolide. The imidazolide was dissolved in DMF (5 mL) and stirred with isoprenyl monophosphate dicyclohexylamine salt (1 mmol) for 1 month during which the imidazolide peak on ^{31}P NMR completely disappeared. The reaction mixture was evaporated *in vacuo* to an oily mixture, which was dissolved in 0.1 M TEAB (2 mL). The resulting solution was purified by HPLC with a gradient of 0.1M TEAB and MeCN (7:3 to 3:7 over 120 min).

Method B: Reaction of nucleoside 5’-diphosphates with isoprenyl chloride or tosylate.

Nucleoside 5’-diphosphate disodium salt (0.2 mmol) was converted into its acidic form by treatment of Dowex 50WX8-200 (H^+) ion exchange resin and the eluant was titrated with $^nBu_4NOH$ to pH 8. The solution was lyophilized to give a foamy product. To a solution of the resulting tetrabutylammonium salt in MeCN (0.5 mL) was added isoprenyl chloride or tosylate (0.2 mmol). The reaction mixture was stirred for 4h and evaporated. The residue was dissolved in 0.1M TEAB and purified by HPLC with a gradient of 0.1M TEAB and MeCN (7:3 to 3:7 over 120 min).

5’-O-Isopentenylpyrophosphoryl guanosine triethylammonium salt (3a).

Method A: GMP disodium salt was converted to its free acid by treatment with Dowex 50WX8-200 (H^+) ion exchange resin before it reacted with CDI. Isopentenyl monophosphate cyclohexylamine salt was prepared by the literature procedure. The reaction and purification is as decribed above. Yield: 76%

Method B: Isopentenyl tosylate was prepared from isopentenyl alcohol by the literature procedure. GDP was reacted with isopentenyl tosylate and the product was purified according to the general procedure. Yield: 73%

^{1}H NMR (500 MHz, D_2O) δ 7.67 (s, 1H, guanine CH), 5.81 (d, J= 4.9 Hz, 1H, anomeric CH), 4.79 (s, 1H, vinyl), 4.66 (s, 1H, vinyl), 4.58 (1H, CH), 4.42 (1H, CH), 4.20 (1H, CH), 4.06 (2H, CH_2O), 3.86(2H, CH_2O), 3.06 (12H, CH_2 of TEA), 2.15 (2H, CH_2), 1.55(s, 3H, CH_3) and 1.16 (18H, CH_3 of TEA); ^{31}P NMR(202.5 MHz, D_2O, proton-decoupled) δ -11.10 (d, J= 21.5 Hz) and -11.56 (d, J= 21.5 Hz); HRMS(ESI) calculated for $C_{15}H_{24}N_5O_{11}P_2$ $[M+H]^+$: 512.0948; found: 512.0936.

5’-O-Geranylpyrophosphoryl adenosine triethylammonium salt (3b).

Method A: AMP monohydrate was converted to its imidazolide. The imidazolide was reacted with geranyl monophosphate cyclohexylamine salt, which was prepared by the literature procedure. The product was purified according to the general procedure. Yield: 74%

Method B: ADP was reacted with geranyl chloride and the product was purified by HPLC. Yield: 71%

^{1}H NMR(500 MHz, D_2O) δ 8.57(s, 1H, adenine CH), 8.36 (s, 1H, adenine CH), 5.94(d, J= 5.5 Hz, 1H, anomeric CH), 5.00 (t, J= 6.7 Hz, 1H, vinyl CH), 4.75(t, J= 5.7 Hz, 1H, vinyl CH), 4.56(dd, J= 5.2, 5.2 Hz, 1H, CH), 4.37 (dd, J= 4.1, 4.2 Hz, 1H, CH), 4.21(3H, CH and CH_2O), 4.07 (2H, CH_2O), 2.79 (12H, CH_2 of TEA), 1.65 (m, 2H, CH_2), 1.59(m, 2H, CH_2), 1.44, 1.36, 1.32 (3s, 3H each, 3 CH_3) and 1.01 (18H, CH_3 of TEA); ^{31}P NMR(202.5 MHz, D_2O, proton-decoupled) δ -10.98 (d, J= 22.2 Hz) and -11.57 (d, J= 22.2 Hz); HRMS(ESI) calculated for $C_{20}H_{32}N_5O_{10}P_2$ $[M+H]^+$: 564.1624; found: 564.1634.

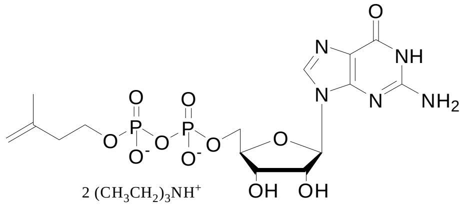
5’-O-*trans,trans*-Farnesylpyrophosphoryl guanosine (3c).

GDP was reacted with *trans,trans*-farnesyl chloride and two salt forms were purified by HPLC according to general method B.

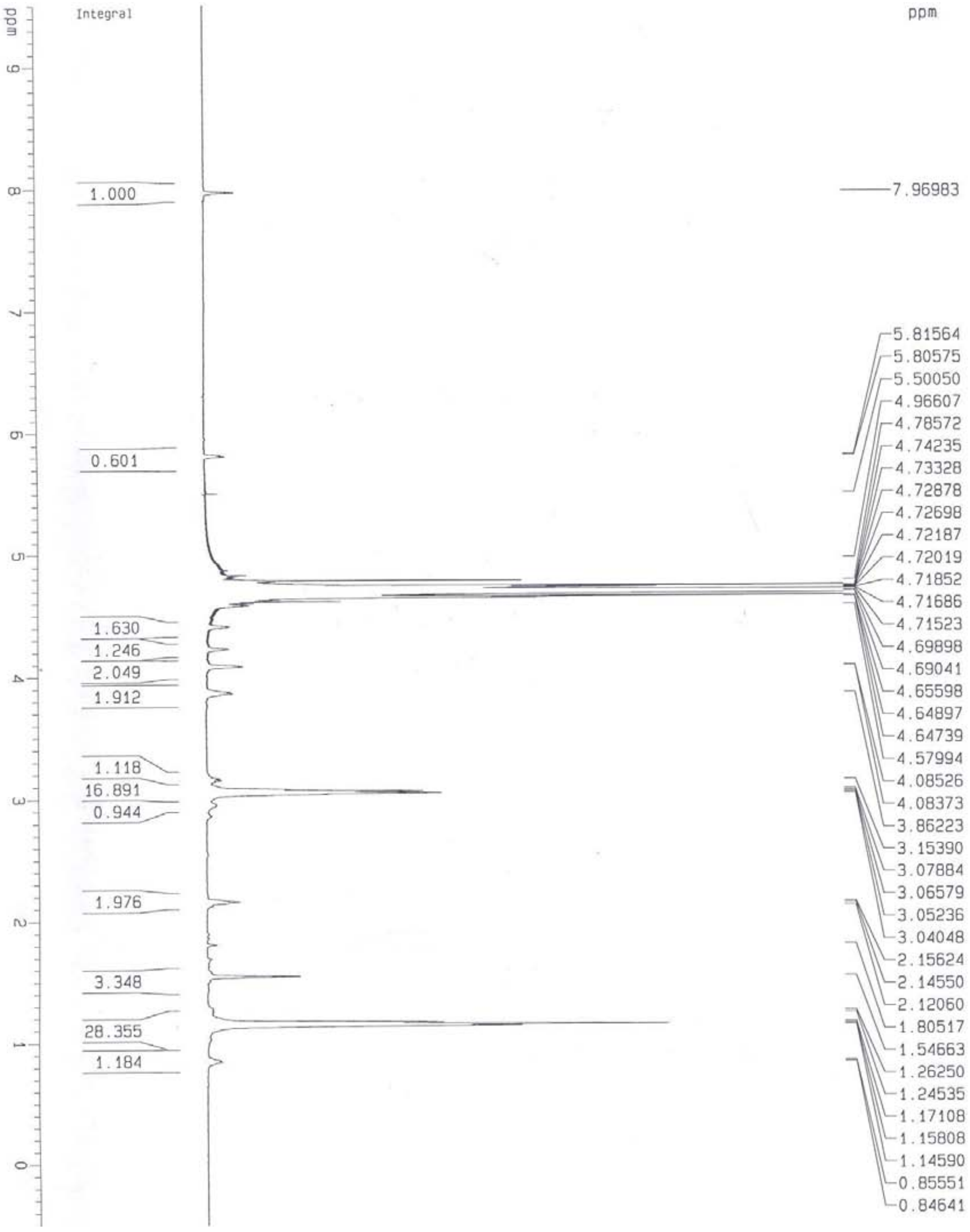
Dibasic TEA salt (RT= 48 min): Yield= 33%; ^{1}H NMR (500 MHz, D_2O plus ND_4OD) δ 7.81 (s, 1H, guanine CH), 5.68 (d, J= 6.0, 1H, anomeric CH), 5.05 (t, 1H, vinyl), 4.89 (t, 1H, vinyl), 4.81 (t, 1H, vinyl), 4.42 (1H, CH), 4.22 (1H, CH), 4.19 (2H, allylic CH_2), 4.05 (1H, CH), 3.96 (2H, 5’CH_2), 2.74 (12H, CH_2 of TEA), 1.80-1.69 (8H, CH_2 of farnesyl), 1.67, 1.43, 1.36, 1.29 (4s, 3H each, 4 CH_3) and 0.96 (18H, CH_3 of TEA); ^{31}P NMR(202.5 MHz, D_2O, proton-decoupled) δ -10.95(d, J= 21.9 Hz) and 11.50 (d, J= 21.9 Hz); HRMS(ESI) calculated for $C_{25}H_{40}N_5O_{11}P_2$ $[M+H]^+$: 648.2200; found: 648.2206.

Monobasic TEA salt (RT= 70 min): Yield= 42%; ^{1}H-NMR(D_2O) δ 7.88 (s, 1H, guanine CH), 5.66 (d, J= 5.0, 1H, anomeric CH), 4.89 (1H, vinyl), 4.79 (1H, vinyl), 4.69 (1H, vinyl), 4.47 (2H, 2 CH), 4.39 (2H, allylic CH_2), 4.08 (1H, CH), 3.96 (2H, 5’CH_2), 2.83 (6H, CH_2 of TEA), 1.87 (4H, 2 CH_2), 1.59 (7H, 2 CH_2 and CH_3), 1.32, 1.28, 1.22 (3s, 3H each, 3 CH_3) and 0.99 (9H, CH_3 of TEA); ^{31}P NMR(D_2O, proton-decoupled) δ -5.80 (d, J= 23.2) and -10.35 (d, J= 23.2); HRMS(ESI) calculated for $C_{25}H_{40}N_5O_{11}P_2$ $[M+H]^+$: 648.2200; found: 648.2207.

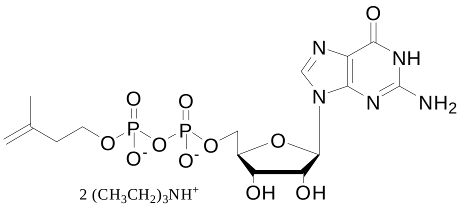
^{1}H NMR(500 MHz, D_2O)



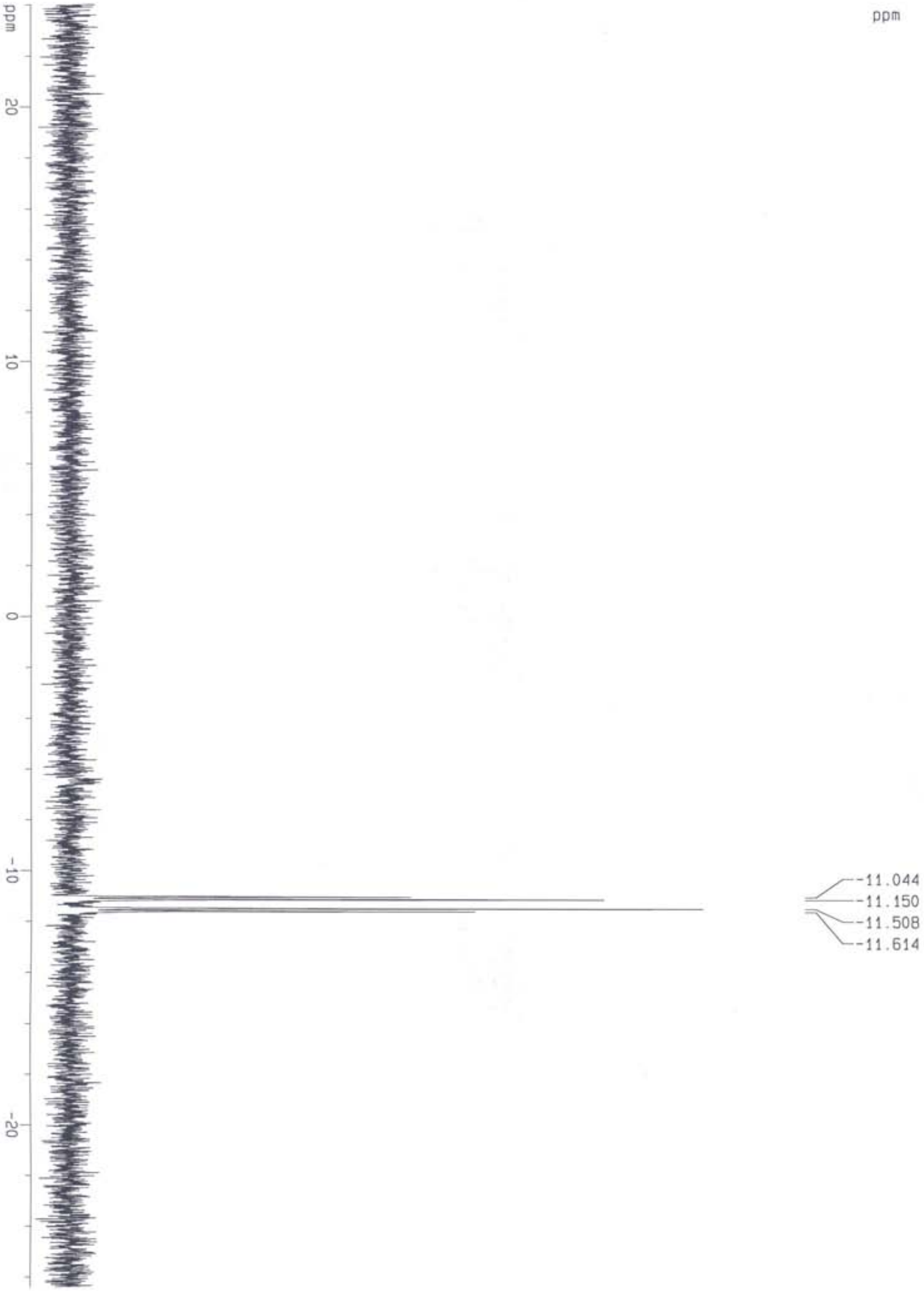
3a



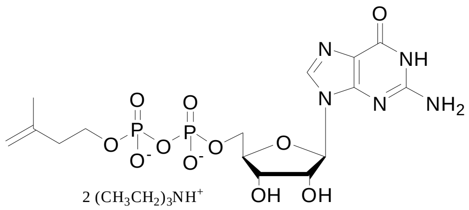
^{31}P NMR(202.5 MHz, D$_2$O, ^{1}H-decoupled)



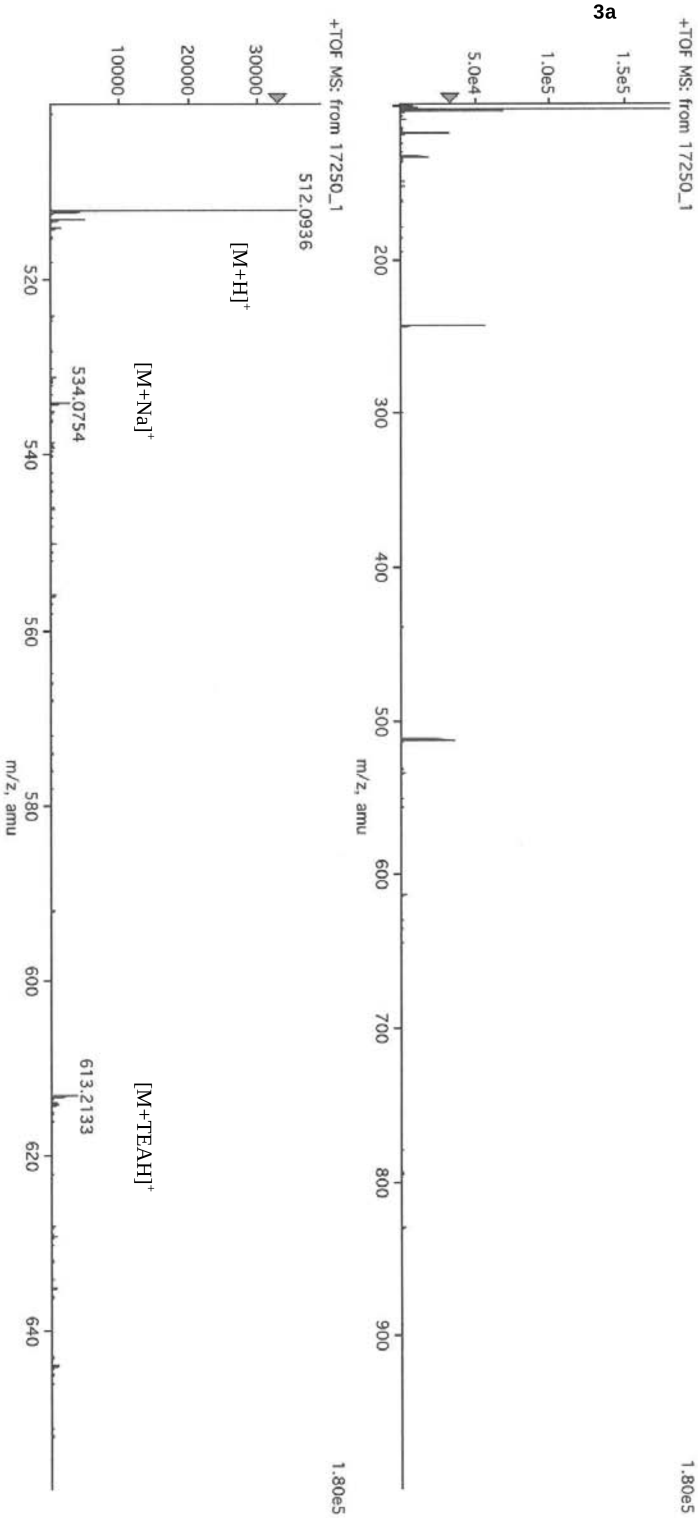
3a



ESI-HRMS

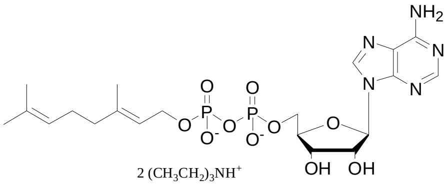

O
N
NH
N
N
NH2
O
P
O
O-
O
O
P
O
O-
O
O
OH
OH
2 (CH3CH2)3NH+

3a

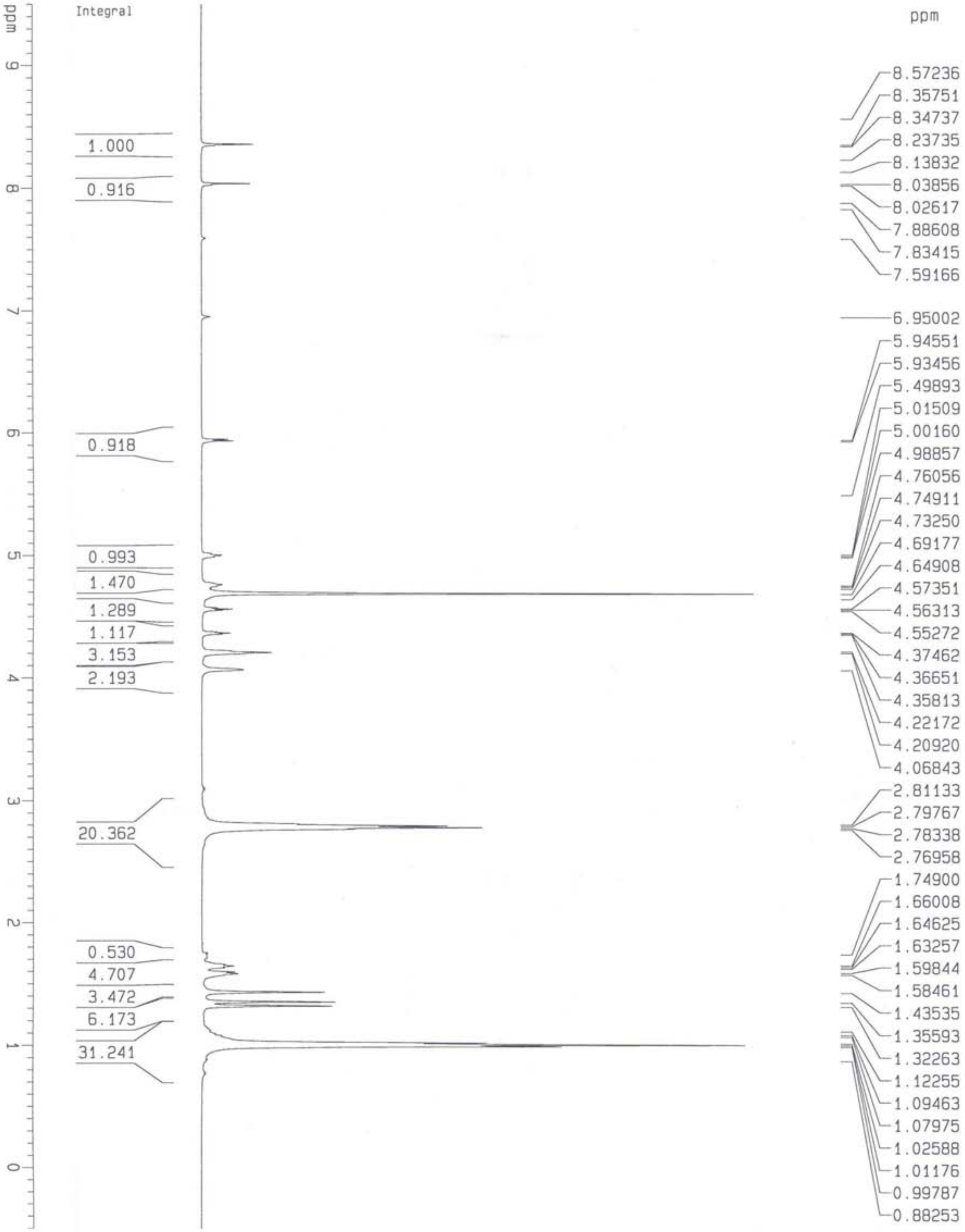

+TOF MS: from 17250_1
5.0e4
1.0e5
1.5e5
200
300
400
500
600
700
800
900
m/z, amu
1.80e5
+TOF MS: from 17250_1
10000
20000
30000
512.0936
[M+H]+
534.0754
[M+Na]+
613.2133
[M+TEAH]+
520
540
560
580
600
620
640
m/z, amu
1.80e5

.

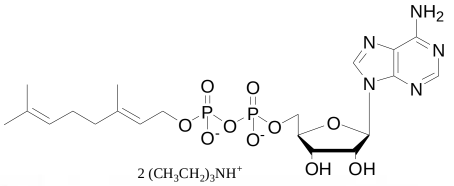
^{1}H NMR(500 MHz, D$_2$O)



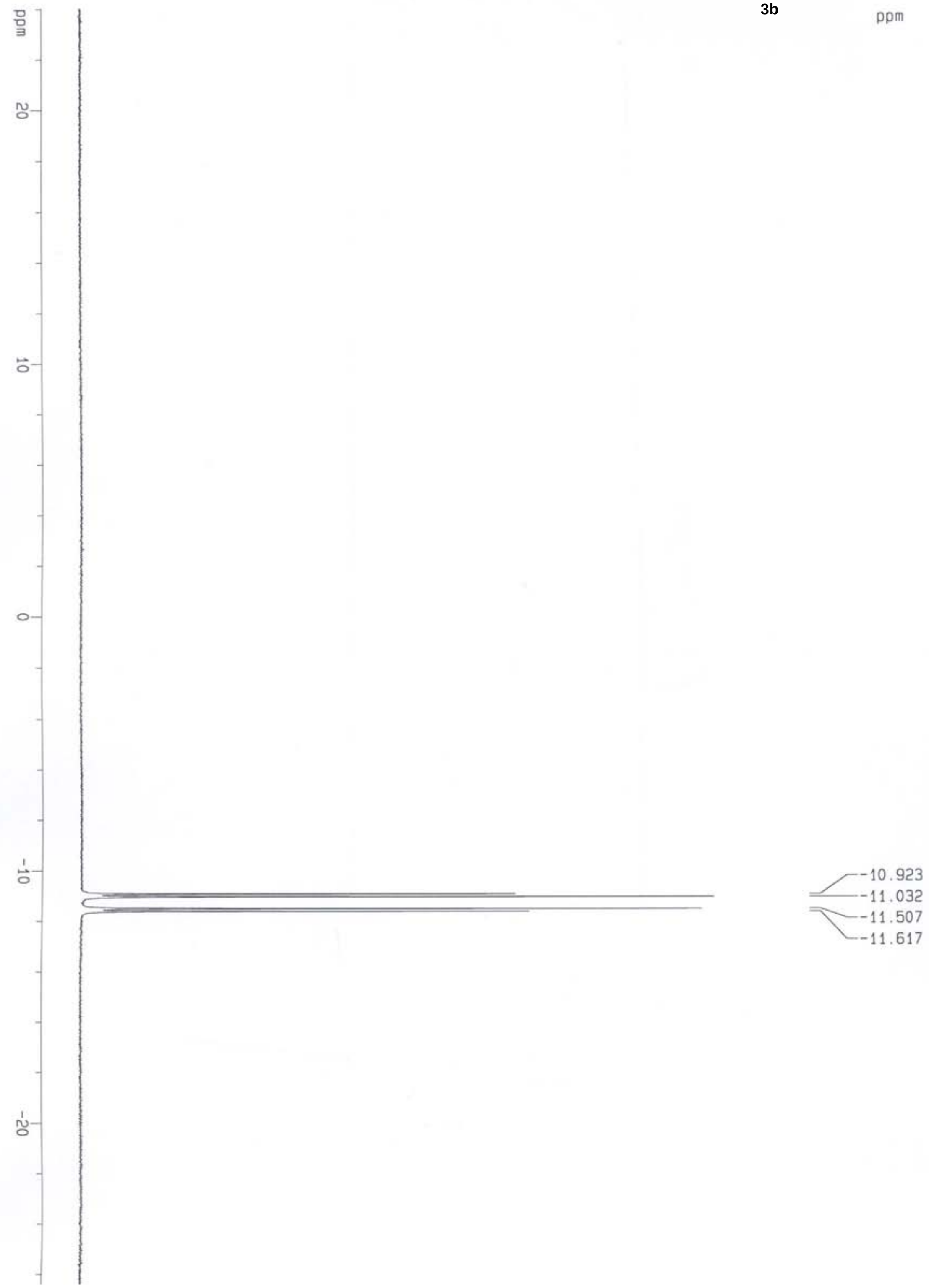
3b



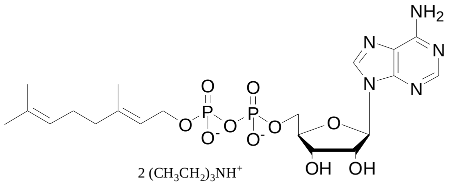
^{31}P NMR(202.5 MHz, D_2O, ^1H-decoupled)



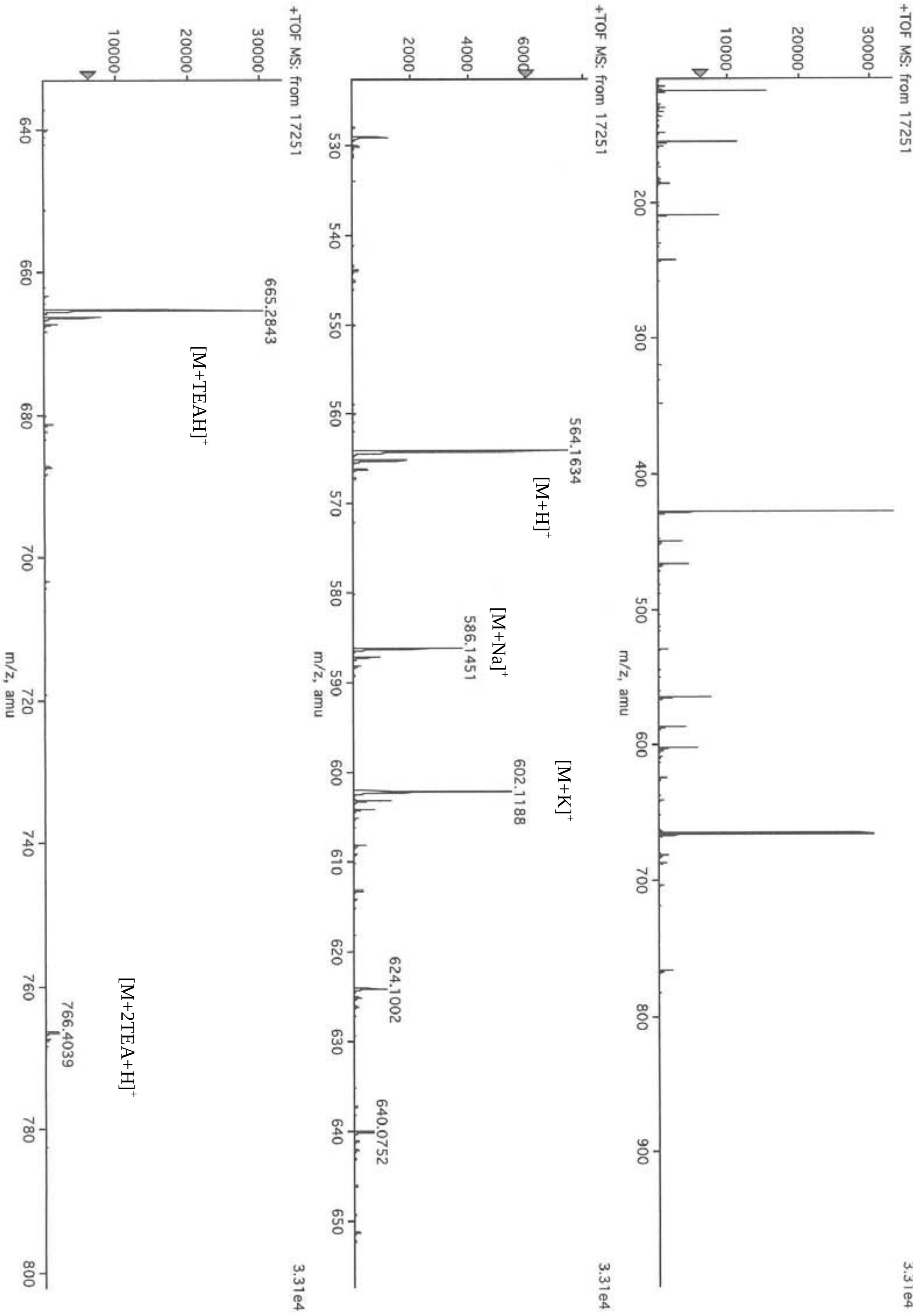
3b



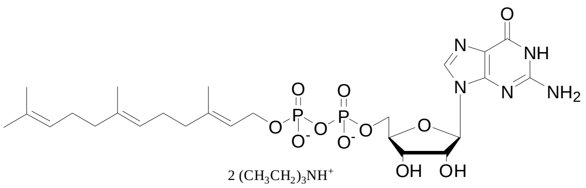
ESI-HRMS


NH2
N
N
N
N
O
O
O
P
P
O
O
O
O-
O-
OH
OH
2 (CH3CH2)3NH+

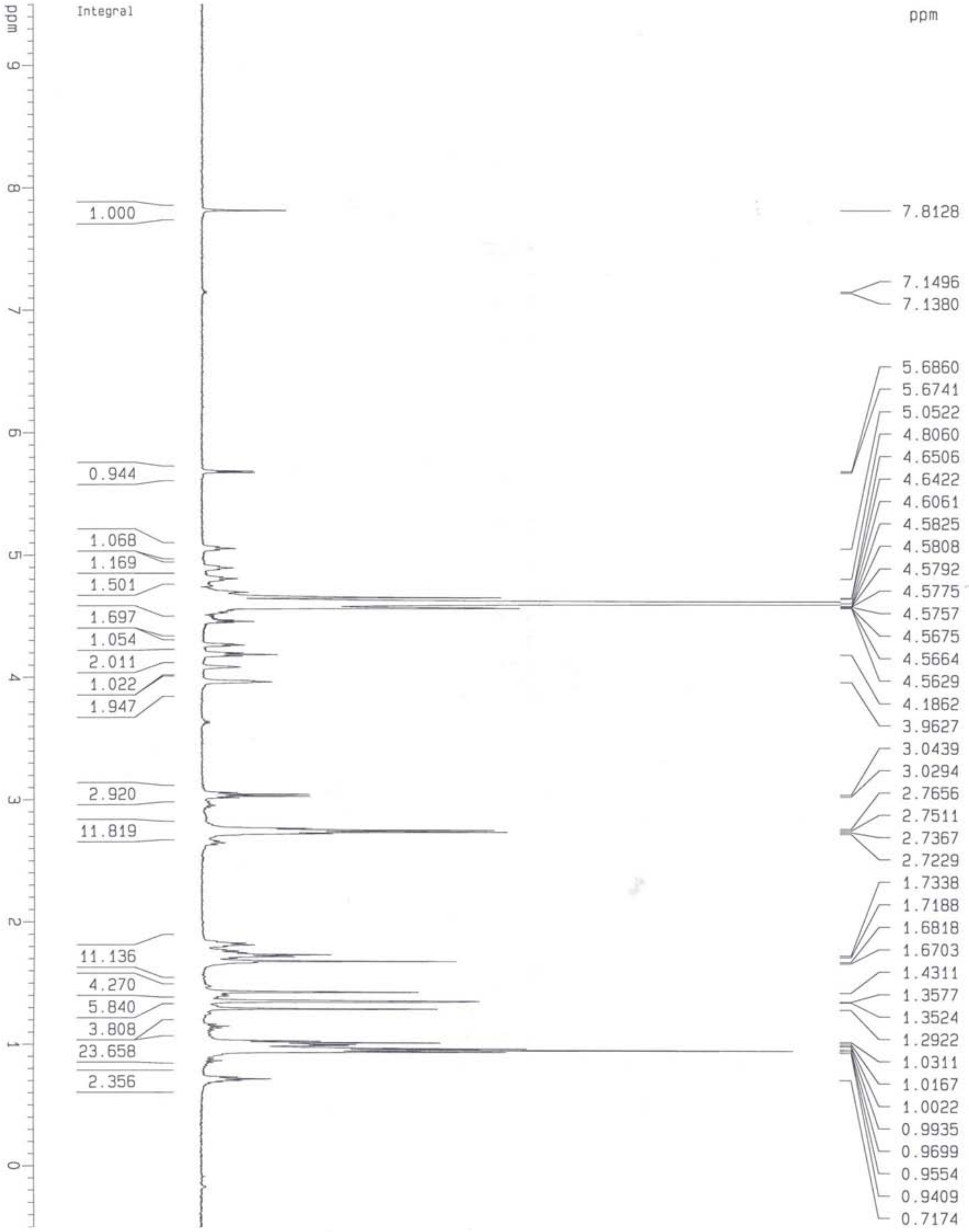
3b


+TOF MS: from 17251
30000
20000
10000
200
300
400
500
600
700
800
900
m/z, amu
3.31e4
+TOF MS: from 17251
6000
4000
2000
530
540
550
560
570
580
590
600
610
620
630
640
650
564.1634
[M+H]+
586.1451
[M+Na]+
602.1188
[M+K]+
624.1002
640.0752
m/z, amu
3.31e4
+TOF MS: from 17251
30000
20000
10000
640
660
680
700
720
740
760
780
800
665.2843
[M+TEAH]+
766.4039
[M+2TEA+H]+
m/z, amu
3.31e4

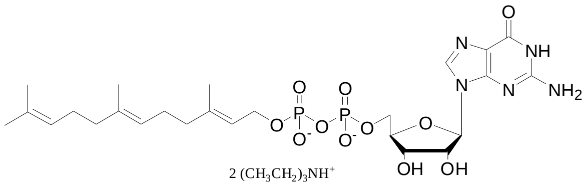
^{1}H NMR(500 MHz, D_2O plus ND_4OD)



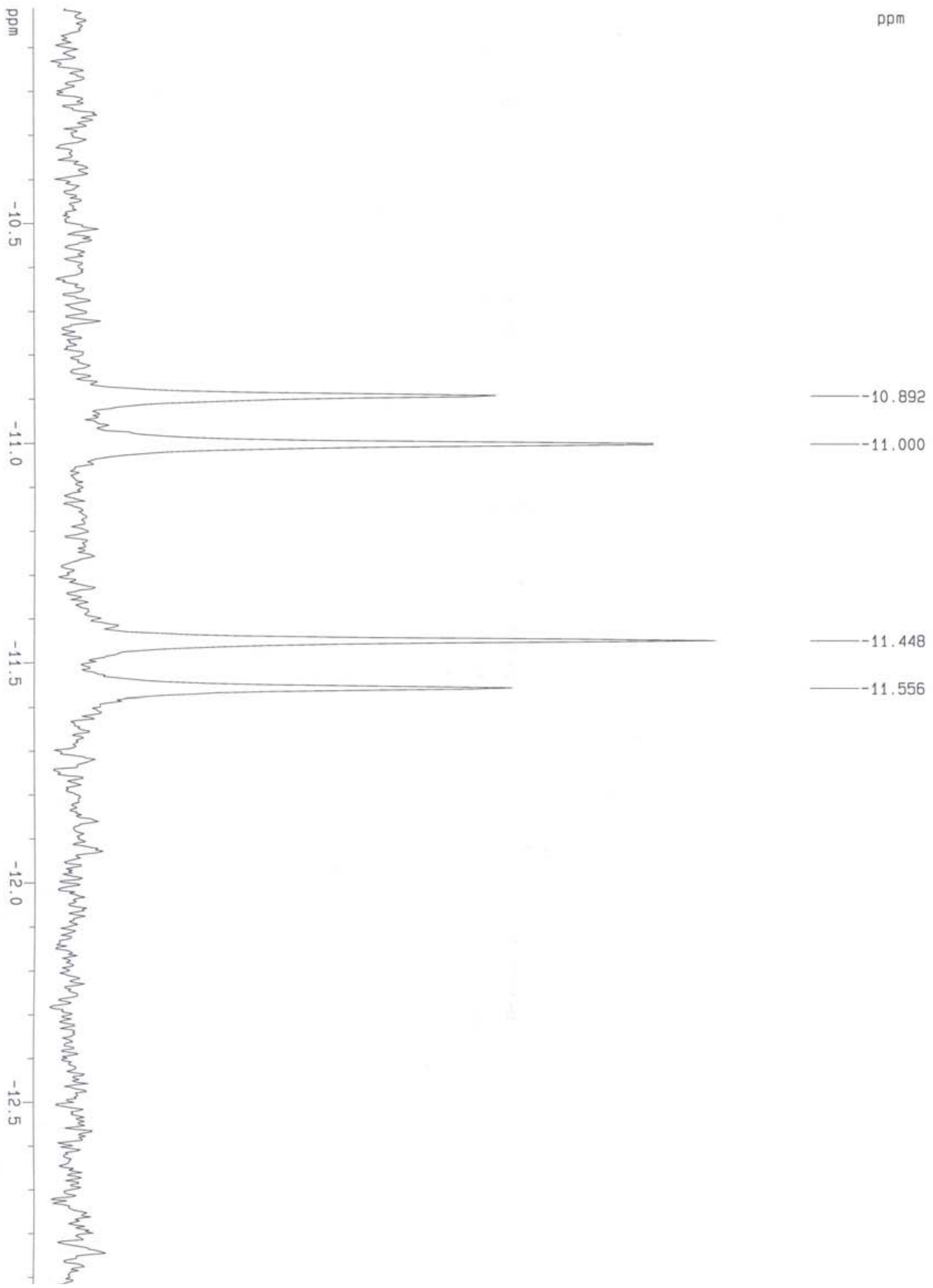
3c dibasic



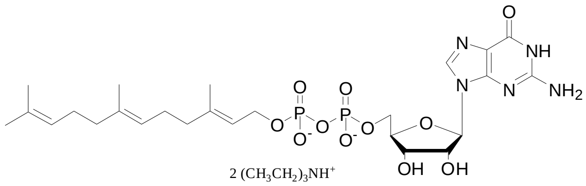
^{31}P NMR(202.5 MHz, D_2O, ^{1}H-decoupled)



3c dibasic

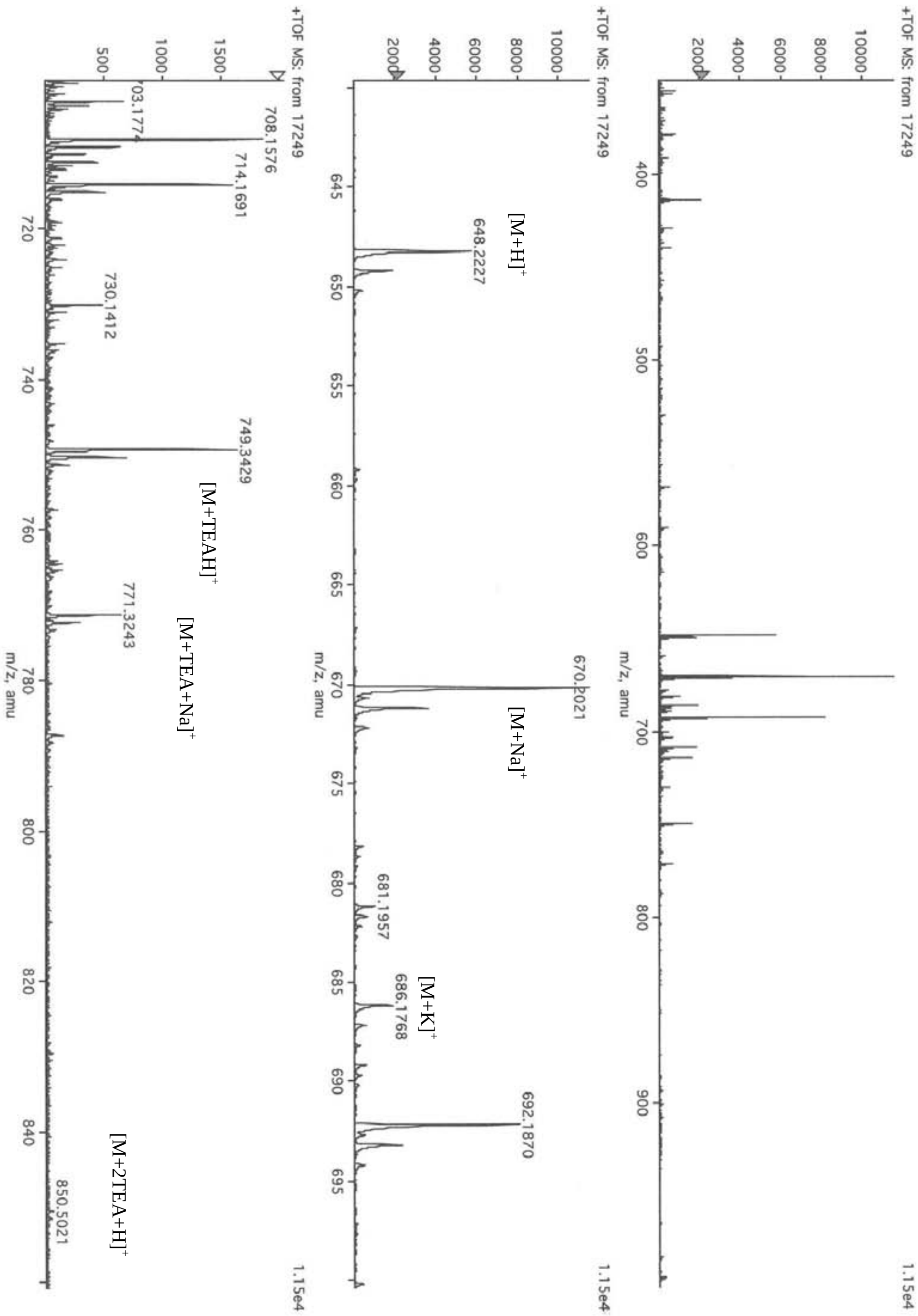


ESI-HRMS

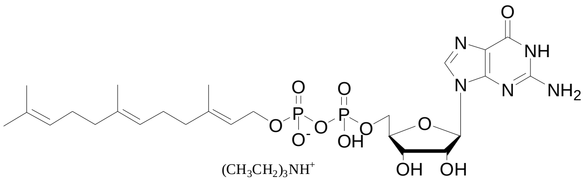


2 (CH$_3$CH$_2$)$_3$NH$^+$

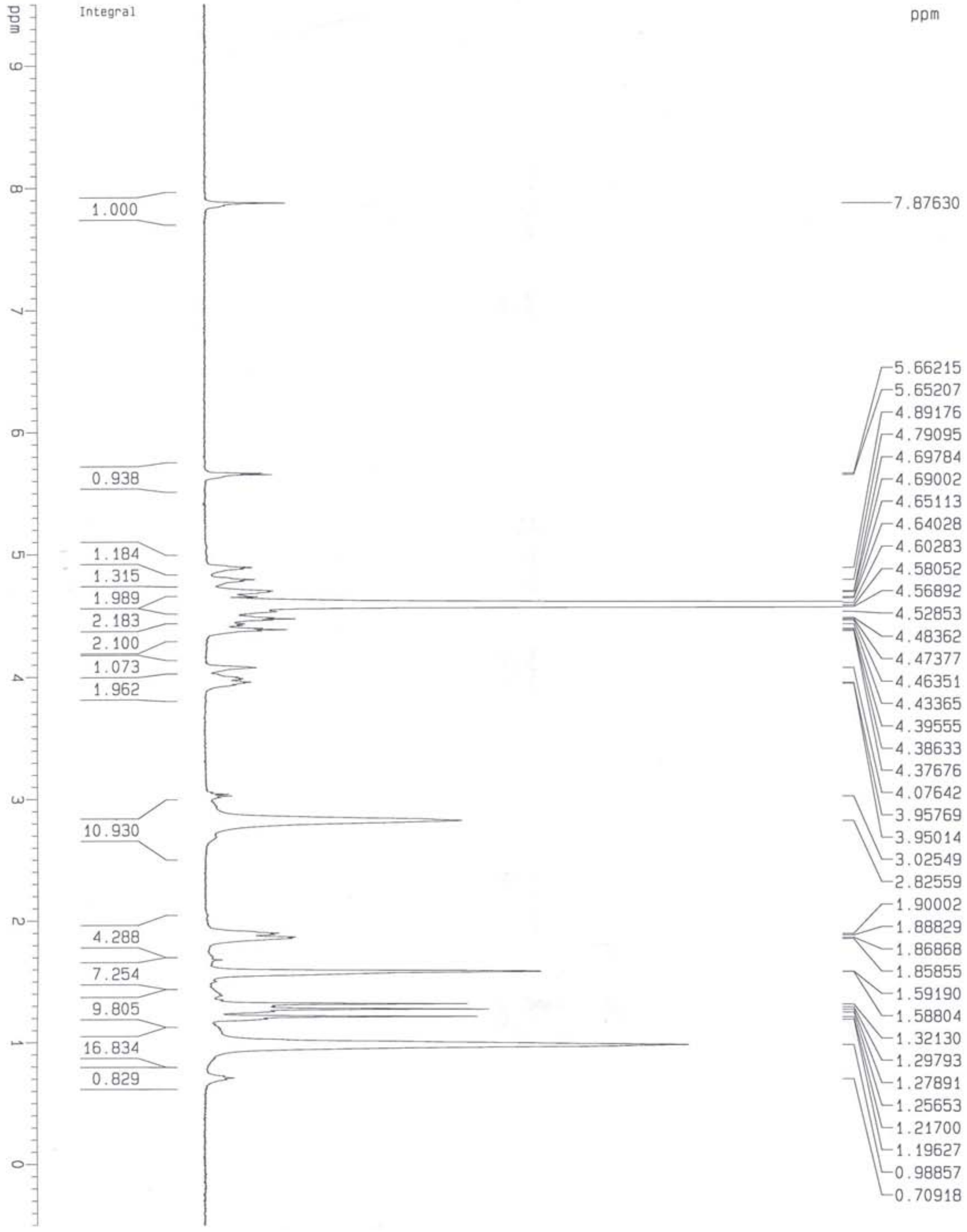
3c dibasic



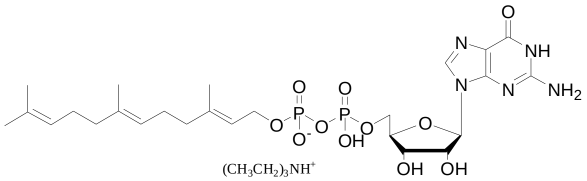
^{1}H NMR(500 MHz, D_2O)



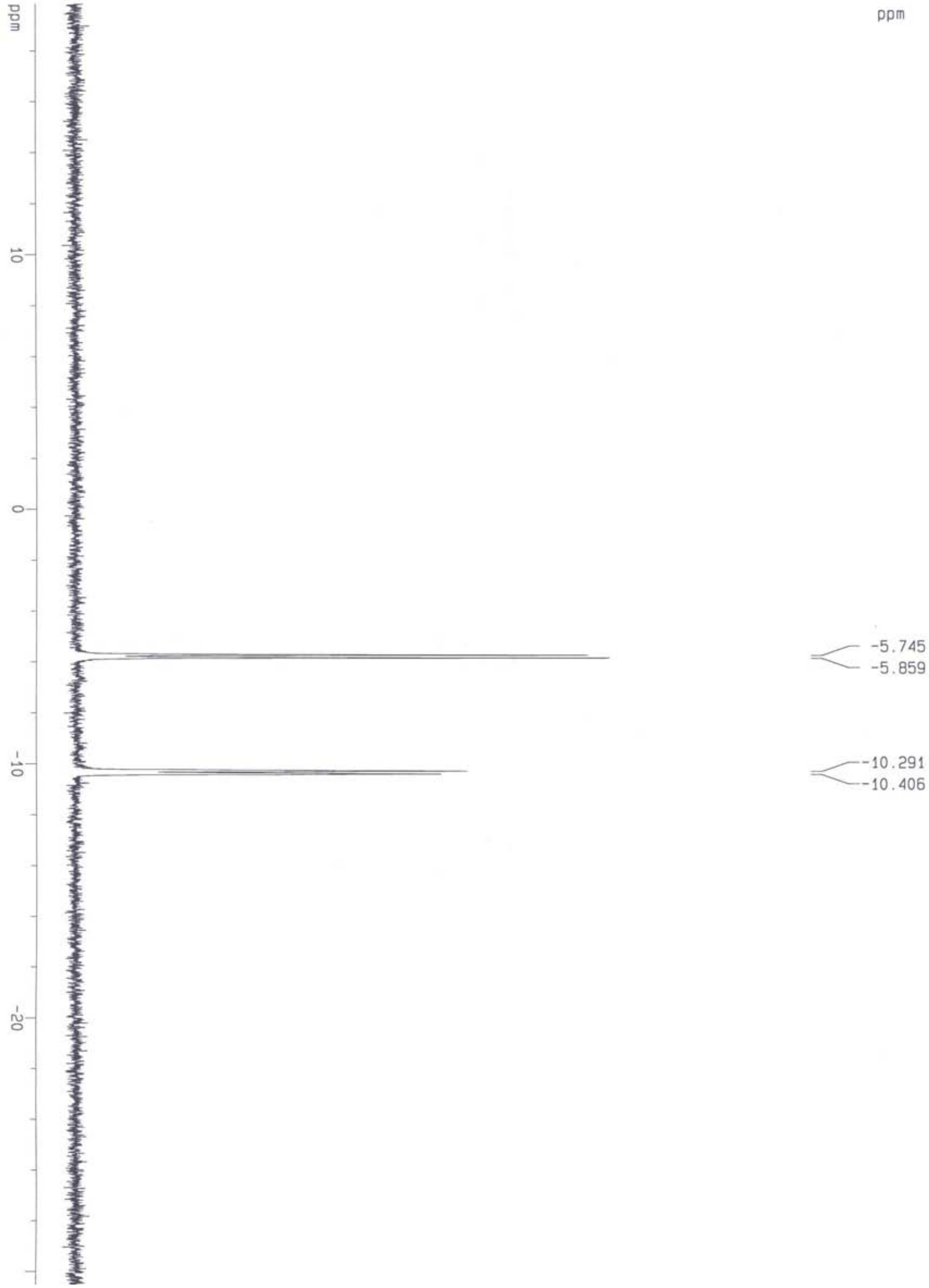
3c monobasic



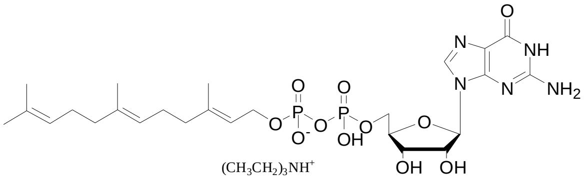
^{31}P NMR(202.5 MHz, D_2O, ^{1}H-decoupled)



3c monobasic



ESI-HRMS



3c monobasic