The Pyridinone-Methide Elimination

Rotem Perry-Feigenbaum[a], Phil S. Baran[b] and Doron Shabat[a]*

[a]School of Chemistry, Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel Aviv 69978 Israel. [b]Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037.

Supporting Information

Experimental

General. All reactions requiring anhydrous conditions were performed under an Ar or N_2 atmosphere. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin layer chromatography (TLC): silica gel plates Merck 60 F_{254}; compounds were visualized by irradiation with UV light and/or by treatment with a solution of phosphomolybdic acid (20% wt. in ethanol), followed by heating. Flash chromatography (FC): silica gel Merck 60 (partical size 0.040-0.063 mm), eluent given in parentheses. ^{1}H NMR: Bruker AMX 200 or 400 instrument. The chemical shifts are expressed in δ relative to TMS (δ=0 ppm) and the coupling constants *J* in Hz. The spectra were recorded in $CDCl_3$, MeOD as a solvent at room temp. 400 Mesh copper grid SPI Supplies, West Chester, PA. All reagents, including salts and solvents, were purchased from Sigma-Aldrich.

Abbreviations. **ACN**- Acetonitrile, **DBTL**- Dibutyltin dilaurate**,** **DCM**- Dichloromethane, **DMAP**- Dimethylaminopyridine, **DMF**- Dimethylformamide, **DMSO**- Dimethyl sulfoxide, **DCC**- N,N'-Dicyclohexylcarbodiimide, **EtOAc**-

Ethylacetate, **Et_3N-** Triethylamine, **Hex** - Hexane, **MeOH**- Methanol, **PNA**- *p*-Nitroaniline, **PNPCl**- *p*-Nitrophenol chloroformate, **TBAF**- Tetrabutylammonium fluorid, **TBSCl**- t-Butyldimethylsillyl chloride, **TFA**- Trifluoroacetic acid, **THF** - Tetrahydrofuran, **TMSE**- Trimethylsillyl ethanol.

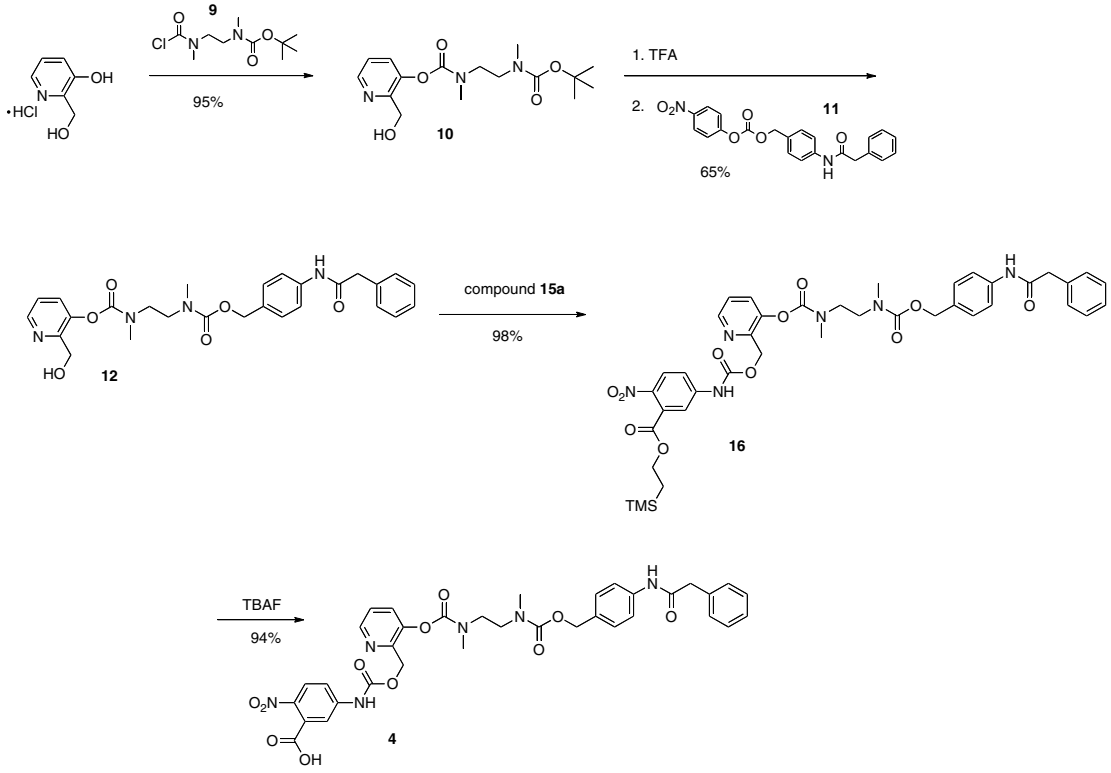


Figure 1. Chemical synthesis of compound **4**.

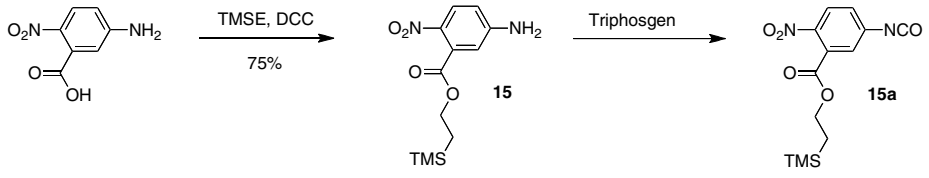


Figure 2. Chemical synthesis of compound **15**.

Compound 9

Compound **9** was synthesized according to the procedure described in *Chem. Eur. J.,* **2007**, *13,* 4523-4528.

Compound 10

Commercially available 3-hydroxy-2 (hydroxy methyl)-pyridine hydrochloride (150 mg, 0.92 mmol) was dissolved in 1 mL of pyridine, compound **9** (352 mg, 1.40 mmol) and catalytic amount of DMAP (5 mg) were added. The reaction was stirred in room temperature over night and was monitored by TLC (100% EtOAc). Upon completion, the reaction was diluted in EtOAc and washed twice with saturated NH_4Cl solution. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:MeOH 95:5) to give compound **10** (299 mg, 95%) as a colorless oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 8.39 (1H, d, *J* = 4.7 Hz); 7.50 (1H, d, *J* = 4.7 Hz); 7.25 (1H, t, *J* = 4.7 Hz); 4.70 (2H, s); 3.54-3.46 (4H, m); 3.12-3.03 (3H, m); 2.90 (3H, d, *J* = 3.0); 1.45 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 156.20, 154.10, 151.99, 145.94, 145.68, 130.95, 123.87, 80.82, 60.82, 48.27, 47.60, 36.19, 35.41, 29.11. MS (FAB): m/z calc. for $C_{16}H_{25}N_3O_5$: 339.1; found: 340.2 $[M+H]^+$.

Compound 12

Compound **10** (270 mg, 0.79 mmol) was dissolved in 1 mL of TFA and stirred in room temperature for 5 minutes. The excess of acid was removed under reduced pressure and the crude amine salt was dissolved in 2 mL of DMF. Compound **11** (370 mg, 0.91 mmol) was added followed by the addition of 200 μL Et_3N. The reaction mixture was stirred in room temperature for 4 hours and monitored by TLC

(EtOAc:MeOH 95:5). Upon completion, the reaction was diluted with EtOAc and washed with NH_4Cl solution followed by brine. The crude product was purified by using column chromatography on silica gel (EtOAc:MeOH 95:5) to give compound **12** (269 mg, 65%) as a yellowish gel.

^{1}H NMR (200MHz, MeOD): δ = 8.40 (1H, dd, *J* = 12 Hz, 2.16 Hz); 7.49-7.18 (11H, m); 5.03 (2H, m); 4.68 (2H, s); 3.74 (2H, s); 3.50-3.47 (4H, m); 3.15-2.89 (6H, m). ^{13}C NMR (100MHz, MeOD): δ = 170.18, 156.85, 154.16, 151.98, 145.87, 145.71, 138.64, 138.50, 135.34, 133.29, 132.95, 130.76, 130.62, 130.11, 129.48, 128.17, 124.33, 123.87, 119.65, 68.03, 67.69, 67.53, 60.81, 60.53, 48.05, 47.65, 47.45, 47.01, 36.16, 35.83. MS (FAB): m/z calc. for $C_{27}H_{30}N_4O_6$: 506.2; found: 507.3 $[M+H]^+$.

Compound 16

Toluene (3 mL) was heated to reflux (110° C) and triphosgen (75 mg, 0.25 mmol) was added. Then a solution of compound **15** (57 mg, 0.20 mmol) in 2 mL toluene was slowly added dropwise with an injector. The reaction was stirred for 30 minutes in reflux and was monitored by ^{1}H NMR (200MHz, $CDCl_3$). After the isocyanate derivative was observed, the solvent was removed under reduced pressure. A solution of compound **12** (87 mg, 0.16 mmol) in 2 mL toluene, followed by the addition of 20 μL DBTL, was added to the isocyanate residue. The reaction mixture was heated to reflux and was stirred for 1 hour. The reaction was monitored by TLC (EtOAc:Hex 1:1). The solvent was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (EtOAc:Hex 6:4) to give compound **16** (134 mg, 98%) as a yellow powder.

^{1}H NMR (200MHz, $CDCl_3$): δ = 8.39 (1H, m); 7.94-7.13 (13H, m); 6.99 (1H, s); 5.29 (2H, d, *J* = 7.8 Hz); 5.02 (2H, d, *J* = 10.6); 4.41 (2H, t, *J* = 4.4 Hz); 3.74-3.49 (6H,

m); 3.06-2.94 (6H, m); 1.10 (2H, t, *J* = 4.4 Hz); 0.06 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 170.29, 167.06, 157.93, 154.11, 152.23, 148.11, 144.71, 141.66, 138.28, 136.50, 135.03, 134.81, 132.95, 131.64, 130.26, 129.94, 129.53, 128.87, 126.5, 120.67, 119.60, 67.95, 67.70, 66.35, 65.76, 48.05, 47.25, 45.46, 35.59, 31.04, 17.73, -0.81. MS (FAB): m/z calc. for $C_{40}H_{46}N_6O_{11}Si$: 814.3; found: 815.2 $[M+H]^+$.

Compound 4

Compound **16** (115 mg, 0.14 mmol) was dissolved in dry THF (2.5 mL), and TBAF (0.282 mL, 1M in THF) was added. The reaction was stirred in room temperature for 2 hours and was monitored by TLC (EtOAc:MeOH:AcOH 97:2:1). Upon completion of the reaction, the solvent was removed under reduced pressure and the crude product was purified by using preperative C-18 RP-HPLC (eluent MeCN:H_2O 3:2) to give compound **4** (95 mg, 94%) as a white powder.

^{1}H NMR (200MHz, MeOD): δ = 9.27 (1H, s); 8.31-8.24 (2H, m); 7.7-7.06 (13H, m); 5.22-4.94 (4H, m); 3.60-3.43 (6H, m); 3.02-2.84 (6H, m). ^{13}C NMR (100MHz, MeOD): δ = 176.20, 170.80, 154.12, 153.48, 147.21, 144.37, 141.41, 138.70, 135.41, 133.55, 132.50, 130.07, 129.81, 128.88, 126.21, 120.73, 119.05, 118.37, 68.16, 47.66, 44.94, 34.86, 30.41. MS (FAB): m/z calc. for $C_{35}H_{34}N_6O_{11}$: 714.2; found: 715.1 $[M+H]^+$.

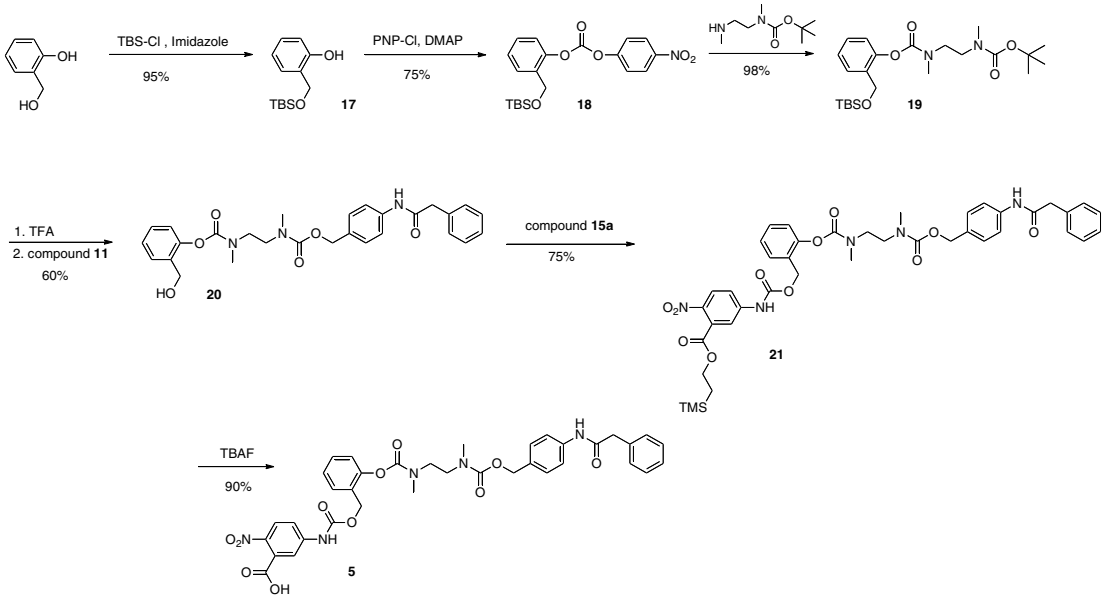


Figure 3. Chemical synthesis of compound **5**.

Compound 15

Commercially available 5-amino-2-nitrobenzoic acid (1 gr, 5.49 mmol) was dissolved in ACN (11 mL). Then TMSE (1.57 mL, 10.98 mmol) and pyridine (0.88 mL, 10.98 mmol) were added. The reaction mixture was cooled to 0° C and DCC (1.36 gr, 6.58 mmol) was added. The reaction was stirred in room temperature for 16 hours and monitored by TLC (EtOAc:Hex 1:1). Upon completion, the reaction was filtrated and washed with ACN. The solvent was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1:1) to give compound **15** (1.16 gr, 75%) as a yellow powder.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.95 (1H, d, J=10Hz); 6.67-6.61 (2H, m); 4.46 (2H, bs); 4.41 (2H, m); 1.13 (2H, t, *J* = 4.4Hz); 0.02 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 164.36, 151.55, 142.21, 132.27, 127.14, 114.11, 112.91, 64.81, 20.03, -5.36. MS MS (FAB): m/z calc. for $C_{12}H_{18}N_2O_4Si$: 282.1; found: 305.0 $[M+Na]^+$.

Compound 17

Commercially available 2-hydroxybenzylalcohol (500 mg, 4.02 mmol) was dissolved in DMF (2 mL) and cooled to 0° C. Imidazole (329 mg, 4.83 mmol) and TBSCl (729 mg, 4.83 mmol) were added. The reaction was allowed to warm to room temperature and was stirred for additional 3 hours. The reaction was monitored by TLC (EtOAc:Hex 1:19). Upon completion of the reaction, the reaction was diluted with diethyl ether and washed with NH_4Cl solution followed by brine. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1:19) to give compound **17** (912 mg, 95%) as a colorless oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 8.06 (1H, s); 7.19 (1H, dt, *J* = 1.07Hz, 8.46Hz); 6.96-6.83 (3H, m); 4.92 (2H, s); 0.98 (9H, s); 0.16 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 157.32, 129.65, 127.41, 124.90, 120.34, 118.57, 66.56, 26.75, 18.87,

-5.07. MS (CI): m/z calc. for $C_{13}H_{22}O_2Si$: 238.1; found: 239.2 $[M+H]^+$.

Compound 18

Compound **17** (813 mg, 3.41 mmol) was dissolved in dry THF (2 mL), Et_3N (1.42 mL, 10.23 mmol) and a catalytic amount of DMAP (10 mg) were added. The reaction was cooled to 0° C, and a solution of 4-nitrophenol chloroformate (1.37 gr, 6.82 mmol) in 1.5 mL dry THF was added dropwise. The reaction was stirred for 2 hours in room temperature and monitored by TLC (EtOAc:Hex 1:9). Upon completion of the reaction, the reaction mixture was diluted with EtOAc and washed with saturated NH_4Cl solution. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1:9) to give compound **18** (1.04 gr, 75%) as a colorless oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 8.33 (2H, d, *J* = 9.2Hz); 7.51-7.47 (3H, m); 7.33-7.30 (3H, m); 4.80 (2H, s); 0.94 (9H, s); 0.12 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 156.10, 151.35, 148.62, 146.29, 133.62, 129.17, 128.69, 127.61, 126.15, 122.33, 121.81, 61.06, 26.62, 19.11, -4.57. MS (FAB): m/z calc. for $C_{20}H_{25}NO_6Si$: 403.1; found: 402.2 $[M-H]^-$.

Compound 19

Compound **18** (613 mg, 1.52 mmol) was dissolved in 3 mL of DMF and mono-Boc-protected *N,N'*-dimethyl-ethylenediamine (343 mg, 1.82 mmol) was added. The reaction was stirred in room temperature for 1 hour and was monitored by TLC (EtOAc:Hex 1:9). Upon completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1:17) to give compound **19** (680 mg, 98%) as a viscous oil. ^{1}H NMR (200MHz, $CDCl_3$): δ = 7.52 (1H, d, *J* = 3.6Hz); 7.27-7.22 (2H, m); 7.08 (1H, d, *J* = 3.6); 4.71 (2H, s); 3.6-3.36 (4H, m); 3.11 (3H, s); 2.92 (3H, m); 1.47 (9H, s); 0.94 (9H, s); 0.09 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 163.40, 148.85, 134.09, 128.40, 126.77, 126.30, 122.61, 116.26, 80.03, 60.96, 47.74, 46.68, 35.54, 32.64, 30.08, 26.62, 19.08,-4.52. MS (FAB): m/z calc. for $C_{23}H_{40}N_2O_5Si$: 452.2; found: 451.3 $[M-H]^-$.

Compound 20

Compound **19** (258 mg, 0.57 mmol) was dissolved in 1 mL of TFA and stirred in room temperature for 7 minutes. The excess of acid was removed under reduced pressure and the crude amine salt was dissolved in 2 mL of DMF. Compound **15** (255 mg, 0.62 mmol) was added followed by the addition of 200 μL Et_3N. The reaction

mixture was stirred in room temperature for 2 hours and monitored by TLC (EtOAc:MeOH 95:5). Upon completion, the reaction was diluted with EtOAc and washed with NH_4Cl solution followed by brine. The crude product was purified by using column chromatography on silica gel (EtOAc:MeOH 95:5) to give compound **20** (172 mg, 60%) as a yellowish gel.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.70 (1H, m); 7.41-7.18 (11H, m); 6.98 (1H, dd, *J* = 15.6Hz, 3.8Hz); 5.04 (2H, d, *J* = 8.4); 4.52 (2H, s); 3.67-3.45 (6H, m); 3.12-2.77 (6H, m). ^{13}C NMR (100MHz, $CDCl_3$): δ = 170.10, 163.33, 155.80, 149.67, 138.44, 135.28, 134.31, 130.97, 130.67, 129.95, 129.65, 128.23, 120.60, 67.74, 67.53, 60.86, 48.09, 47.74, 45.32, 36.19, 32.64. MS (FAB): m/z calc. for $C_{28}H_{31}N_3O_6$: 505.2; found: 506.1 $[M+H]^+$.

Compound 21

Toluene (3 mL) was heated to reflux (110° C) and triphosgen (89 mg, 0.30 mmol) was added. Then a solution of compound **15** (70 mg, 0.24 mmol) in 2 mL toluene was slowly added dropwise with an injector. The reaction was stirred for 30 minutes in reflux and was monitored by ^{1}H NMR (200 MHz, $CDCl_3$). After the isocyanate derivative was observed, the solvent was removed under reduced pressure. A solution of compound **20** (102 mg, 0.20 mmol) in 2 mL toluene, followed by the addition of 10 μL DBTL, was added to the isocyanate residue. The reaction mixture was heated to reflux and was stirred for 40 minutes. The reaction was monitored by TLC (EtOAc:Hex 1:1). The solvent was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (EtOAc:Hex 3:2) to give compound **21** (122 mg, 75%) as a yellow powder.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.97 (1H, m); 7.72-6.98 (15H, m); 5.11-4.94 (4H,

m); 4.39 (2H, t, *J* = 4.4Hz); 3.77-3.52 (6H, m); 3.08-2.83 (6H, m); 1.13 (2H, t, *J* = 4.4Hz); 0.02 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 170.08, 167.21, 158.37, 157.42, 155.08, 153.64, 152.24, 150.78, 145.24, 141.57, 138.53, 136.51, 130.46, 130.21, 130.05, 129.53, 128.98, 128.27, 126.44, 120.65, 120.56, 119.65, 68.69, 67.94, 65.63, 63.09, 46.34, 45.47, 30.92, 30.42, 17.71, -0.80. MS (FAB): m/z calc. for $C_{41}H_{47}N_5O_{11}Si$: 813.3; found: 814.2 $[M+H]^+$.

Compound 5

Compound **21** (89 mg, 0.10 mmol) was dissolved in dry THF (2.5 mL) and TBAF (0.220 mL, 1M in THF) was added. The reaction was stirred in room temperature for 3 hours and was monitored by TLC (EtOAc:MeOH:AcOH 97:2:1). Upon completion of the reaction, the solvent was removed under reduced pressure and the crude product was purified by using preperative C-18 RP-HPLC (eluent MeCN:H_2O 3:2) to give compound **5** (70 mg, 90%) as a white powder.

^{1}H NMR (200MHz, $CDCl_3$): δ = 9.13 (1H, m); 7.79-7.07 (16H, m); 5.09-4.96 (4H, m); 3.62-3.44 (6H, m); 3.05-2.90 (6H, m). ^{13}C NMR (100MHz, $CDCl_3$): δ = 171.00, 157.68, 155.32, 153.96, 150.85, 146.32, 144.50, 137.79, 134.92, 130.86, 130.24, 129.89, 129.53, 128.79, 126.35, 123.54, 121.27, 119.76, 118.72, 67.82, 64.16, 48.24, 47.45, 45.11, 36.18, 36.08, 31.05. MS (ESI): m/z calc. for $C_{36}H_{35}N_5O_{11}$: 713.2; found: 736.0 $[M+Na]^+$.

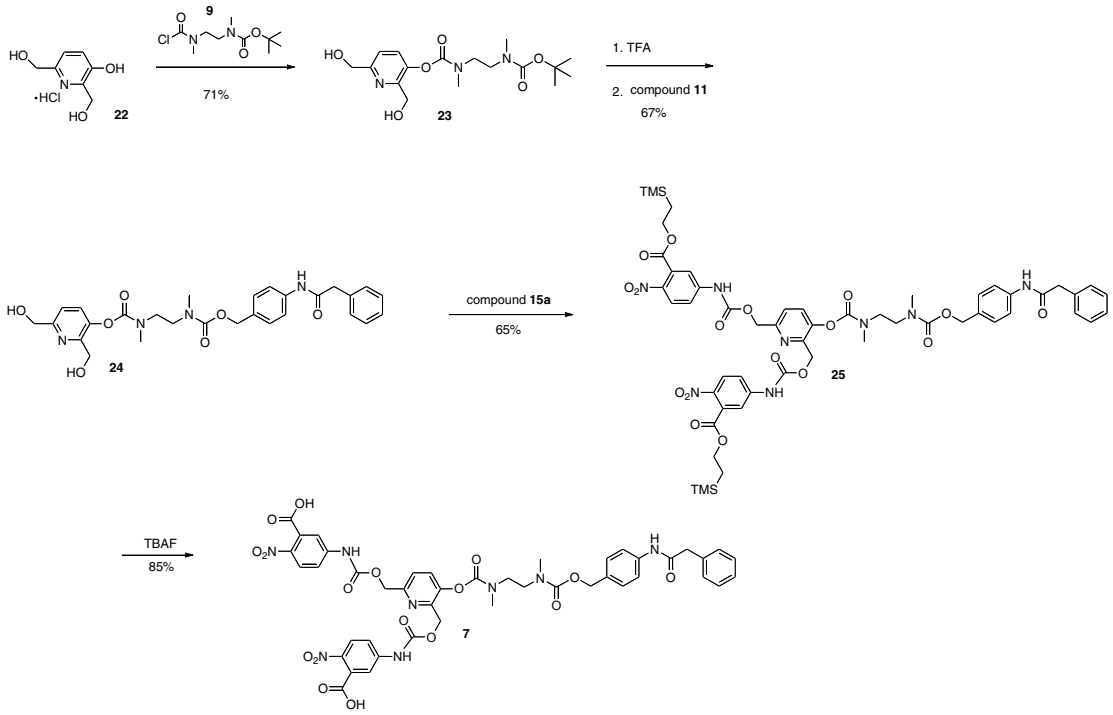


Figure 4. Chemical synthesis of compound **7**.

Compound 22

Compound **22** was synthesized according to the procedure described in *Tetrahedron Lett.,* **2007**, *48,* 3463-3466.

Compound 23

Compound **22** (302 mg, 1.86mmol) was dissolved in 1mL of pyridine, compound **9** (606 mg, 2.42 mmol) and a catalytic amount of DMAP (10 mg) were added. The reaction was stirred in room temperature over night and was monitored by TLC (100% EtOAc). Upon completion, the reaction was diluted in EtOAc and washed twice with saturated NH_4Cl solution followed by brine. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:MeOH 95:5) to give compound **23** (490 mg, 71%) as a white viscous oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.45 (1H, d, *J* = 8.3); 7.25 (1H, d, *J* = 8.3); 4.67 (2H, s); 3.54-3.37 (4H, m); 3.09-2.85 (6H, m); 1.42 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 157.07, 156.56, 154.63, 151.26, 144.49, 131.84, 131.58, 120.99, 65.05, 60.91, 51.02, 46.97, 45.73, 36.06, 35.95, 29.05. MS (FAB): m/z calc. for $C_{17}H_{27}N_3O_6$: 369.1; found: 370.2 $[M+H]^+$.

Compound 24

Compound **23** (76 mg, 0.20 mmol) was dissolved in 1 mL of TFA and stirred in room temperature for 5 minutes. The excess of acid was removed under reduced pressure and the crude amine salt was dissolved in 1.5 mL DMF. Then the reaction was cooled to 0° C and compound **15** (100 mg, 0.24 mmol) was added followed by the addition of 100 µL Et_3N. The reaction mixture was stirred in room temperature for 3 hours and monitored by TLC (EtOAc:MeOH 95:5). Upon completion, the reaction was diluted with EtOAc and washed with NH_4Cl solution followed by brine. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:MeOH 95:5) to give compound **24** (72 mg, 67%) as a yellowish gel.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.41-7.15 (11H, m); 5.08 (2H, s); 4.75-4.67 (4H, m); 3.74 (2H, s); 3.53-3.49 (4H, m); 3.04-2.96 (6H, m). ^{13}C NMR (100MHz, $CDCl_3$): δ = 170.33, 157.49, 154.19, 153.63, 148.83, 139.73, 135.35, 134.29, 132.09, 130.48, 130.04, 128.49, 122.38, 120.03, 67.45, 65.02, 60.66, 48.15, 47.46, 45.92, 34.82, 30.41. MS (FAB): m/z calc. for $C_{28}H_{32}N_4O_7$: 536.2; found: 537.4 $[M+H]^+$.

Compound 25

Toluene (5 mL) was heated to reflux (110° C) and triphosgen (91 mg, 0.30 mmol)

was added. Then a solution of compound **15** (73 mg, 0.25 mmol) in 3 mL toluene was slowly added dropwise with an injector. The reaction was stirred for 30 minutes in reflux and was monitored by ^{1}H NMR (200 MHz, $CDCl_3$). After the isocyanate derivative was observed, the solvent was removed under reduced pressure. A solution of compound **24** (55 mg, 0.10 mmol) in 2 mL toluene, followed by the addition of 10 μL DBTL, was added to the isocyanate residue. The reaction mixture was heated to reflux and was stirred for 2 hours. The reaction was monitored by TLC (EtOAc:Hex 1:1). The solvent was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (EtOAc:Hex 7:3) to give compound **25** (77 mg, 65%) as a yellow powder.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.97-7.01 (17H, m); 5.28 (2H, d, *J* = 4.8Hz); 5.04 (4H, m); 4.44 (4H, t, *J* = 4.2 Hz); 3.77-3.52 (6H, m); 3.09-2.97 (6H, m); 1.12 (4H, t, *J* = 4.2 Hz); 0.03 (18H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 171.13, 167.17, 154.08, 152.26, 147.93, 146.79, 141.76, 139.34, 136.52, 134.87, 131.65, 130.20, 129.75, 126.50, 125.26, 68.03, 65.87, 48.34, 45.20, 36.36, 35.26, 32.67, 32.16, 30.10, 26.1021.92, 17.75, -0.80. MS (FAB): m/z calc. for $C_{54}H_{64}N_8O_{17}Si_2$: 1152.3; found: 1175.4 $[M+Na]^+$.

Compound 7

Compound **25** (76 mg, 0.06 mmol) was dissolved in dry THF (2 mL), and TBAF (0.263 mL, 1M in THF) was added. The reaction was stirred in room temperature for 3 hours and was monitored by TLC (EtOAc:MeOH:AcOH 97:2:1). Upon completion of the reaction, the solvent was removed under reduced pressure and the crude product was purified by using preperative C-18 RP-HPLC (eluent MeCN:H_2O 3:2) to give compound **7** (48 mg, 85%) as a white powder.

^{1}H NMR (200MHz, $CDCl_3$): δ = 9.6 (2H, m); 7.94 (2H, t, *J* = 8.8Hz); 7.74-7.18 (15H,

m); 5.26 (4H, m); 5.05 (2H, s); 3.72-3.50 (6H, m); 3.10-2.92 (6H, m). ^{13}C NMR (100MHz, $CDCl_3$): δ = 176.32, 170.03, 168.72, 147.95, 146.69, 141.76, 139.35, 136.55, 134.87, 131.65, 130.20, 129.75, 126.50, 125.26, 70.03, 68.83, 66.62, 65.32, 50.42, 48.32, 45.25, 43.22, 35.47, 30.06. MS (ESI): m/z calc. for $C_{44}H_{40}N_8O_{17}$: 952.2; found: 953.0 $[M+H]^+$.

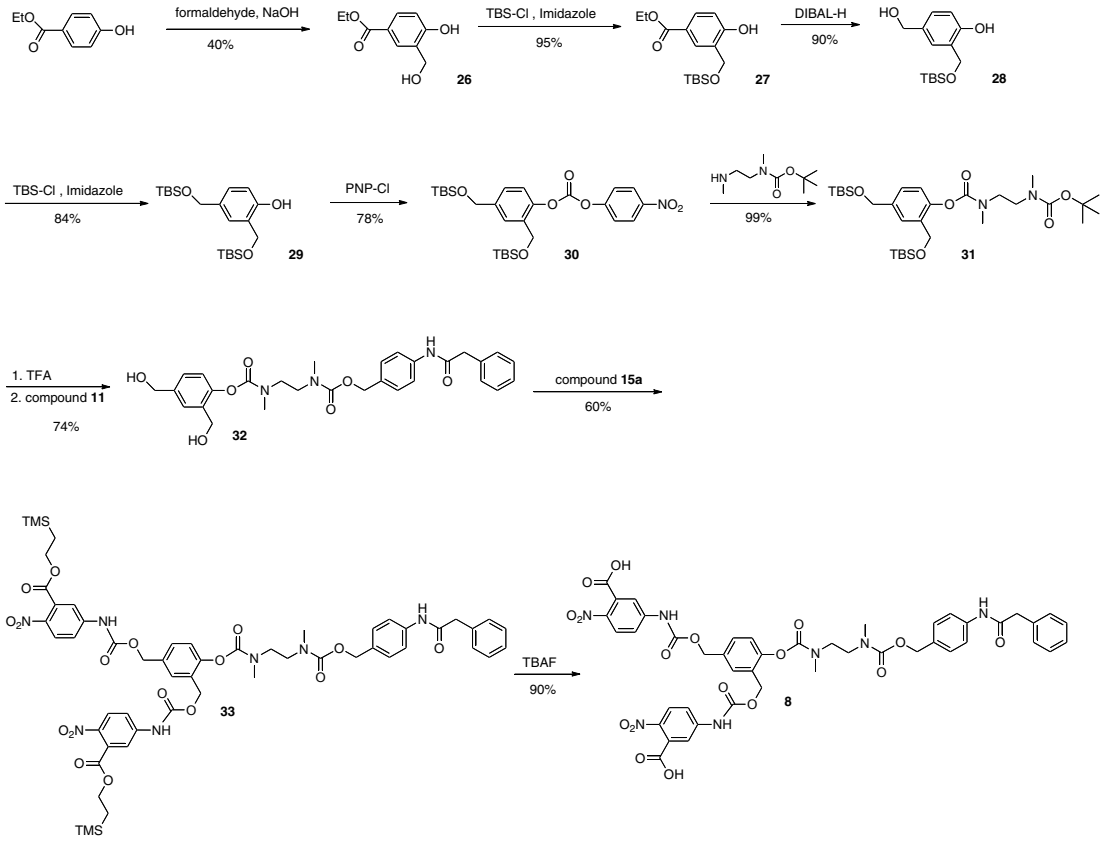


Figure 5. Chemical synthesis of compound **8**.

Compound 26

Commercially available Ethyl 4-hydroxybenzoate (2 gr, 12 mmol) was added to a cool solution (0° C) of 12% NaOH (10 mL, 30 mmol). Formaldehyde 37% in water (8 mL, 0.28 mol) was added. The reaction was stirred at 55° C for 12 hours and was monitored by TLC (EtOAc:Hex 3:1). Upon completion the reaction was diluted with

EtOAc and washed with saturated NH_4Cl solution. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 3:1) to give compound **26** (941 mg, 40%) as a white solid.

^{1}H NMR (200MHz, MeOD): δ = 7.86 (2H, d, *J* = 9.2Hz); 6.83 (1H, d, *J* = 9.2Hz); 4.77 (2H, s); 4.38 (2H, q, *J* = 7.2Hz); 3.46 (1H, bs); 1.37 (3H, t, *J* = 7.2). ^{13}C NMR (100MHz, MeOD): δ = 167.09, 161.70, 131.40, 129.27, 125.93, 124.36, 117.33, 62.21, 60.95, 14.13.

MS (CI): m/z calc. for $C_{10}H_{12}O_4$: 196.0; found: 197.1 $[M+H]^+$.

Compound 27

Compound **26** (395 mg, 2.01 mmol) was dissolved in DMF (2 mL) and cooled to 0° C. Imidazole (150 mg, 2.2 mmol) and TBSCl (334 mg, 2.2 mmol) were added. The reaction was allowed to warm to room temperature and was stirred for additional 1 hour. The reaction was monitored by TLC (EtOAc:Hex 1: 9). Upon completion of the reaction, the reaction was diluted with diethyl ether and washed with NH_4Cl solution. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1: 9) to give compound **27** (592 mg, 95%) as a colorless oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 8.67 (1H, s); 7.88 (1H, dd, *J* = 8.6Hz, 2Hz); 7.68 (1H, d, *J* = 2Hz); 6.90 (1H, d, *J* = 8.6Hz); 4.94 (2H, s); 4.33 (2H, q, *J* = 7.2Hz); 1.37 (3H, t, *J* = 7.2Hz); 0.93 (9H, s); 0.16 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 167.09, 161.70, 131.63, 129.27, 124.36, 122.65, 117.32, 66.49, 61.35, 32.65, 30.91, 30.41, 30.08, 26.40, 18.82, 15.10, 14.84, -4.55, -5.12. MS (CI): m/z calc. for

$C_{16}H_{26}N_8O_4Si$: 310.1; found: 311.3 $[M+H]^+$.

Compound 28

Compound **27** (462 mg, 1.49 mmol) was dissolved in dry THF, under argon and cooled to -78° C. DIBAL-H (1M in toluene, 5.95 mmol) was added dropwise and the reaction was stirred for additional 2 hours under these conditions, and monitored by TLC (EtOAc:Hex 3:7). Upon completion, the reaction was quenched with 2 mL of NH_4Cl solution and diluted with diethyl ether. Celite was added and the reaction mixture was stirred at room temperature for 30 minutes. After filtration, the organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 3: 7) to give compound **28** (361 mg, 90%) as a colorless oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 8.11 (1H, s); 7.19 (1H, dd, *J* = 8.6Hz, 2Hz); 6.98 (1H, d, *J* = 2Hz); 6.87 (1H, d, *J* = 8.6Hz); 4.92 (2H, s); 4.58 (2H, s); 0.95 (9H, s); 0.16 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 156.22, 131.94, 127.95, 125.79, 124.00, 116.64, 65.77, 65.00, 29.59, 25.60, 26.44, 18.02, -5.02. MS (CI): m/z calc. for $C_{14}H_{24}O_3Si$: 268.1; found: 267.2 $[M-H]^+$.

Compound 29

Compound **28** (355 mg, 1.32 mmol) was dissolved in DMF (2 mL) and cooled to 0° C. Imidazole (135 mg, 1.98 mmol) and TBSCl (298 mg, 1.98 mmol) were added. The reaction was allowed to warm to room temperature and was stirred for additional 2 hours. The reaction was monitored by TLC (EtOAc:Hex 1: 9). Upon completion of the reaction, the reaction was diluted with diethyl ether and washed with NH_4Cl

solution. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1: 9) to give compound **29** (423 mg, 84%) as a yellowish oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.98 (1H, s); 7.10 (1H, dd, 1H, dd, *J* = 8.6Hz, 2Hz); 6.92 (1H, d, *J* = 2Hz); 6.83 (1H, d, *J* = 8.6Hz); 4.89 (2H, s); 4.63 (2H, s); 0.93 (9H, s); 0.15 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 156.35, 133.23, 127.76, 125.61, 124.49, 117.10, 66.71, 65.49, 30.42, 26.71, 26.44, 19.16, -4.43. MS (CI): m/z calc. for $C_{20}H_{38}O_3Si_2$: 382.2; found: 383.3 $[M+H]^+$.

Compound 30

Compound **29** (400 mg, 1.04 mmol) was dissolved in dry THF (1 mL), Et_3N (423 μL, 3.12 mmol) and a catalytic amount of DMAP (5 mg) were added. The reaction was cooled to 0° C, and a solution of 4-nitrophenol chloroformate (421 mg, 2.09 mmol) in 1 mL dry THF was added dropwise. The reaction was stirred for 30 minutes in room temperature and monitored by TLC (EtOAc:Hex 1:9). Upon completion of the reaction, the reaction mixture was diluted with EtOAc and washed with saturated NH_4Cl solution. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1:9) to give compound **30** (450 mg, 78%) as a yellow oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 8.31 (2H, d, *J* = 9.2Hz); 7.50 (2H, d, *J* = 9.2Hz); 7.27-7.17 (3H, m); 4.78 (4H, d, *J* = 5.4); 0.95 (9H, s); 0.13 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 156.10, 151.35, 148.62, 147.08, 146.29, 133.62, 129.17, 128.69, 127.61, 126.15, 122.33, 61.06, 60.56, 26.62, 18.98, -4.38. MS (CI): m/z calc.

for $C_{27}H_{41}NO_7Si_2$: 547.2; found: 548.4 $[M+H]^+$.

Compound 31

Compound **30** (728 mg, 0.78 mmol) was dissolved in 1.5 mL DMF and mono-Boc-protected *N,N'*-dimethyl-ethylenediamine (294 mg, 1.56 mmol) was added. The reaction was stirred in room temperature for 3 hours and was monitored by TLC (EtOAc:Hex 1:4). Upon completion of the reaction, the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc:Hex 1:9) to give compound **31** (466 mg, 99%) as a viscous oil.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.48 (1H, s); 7.21 (1H, d, *J* = 8Hz); 7.03 (1H, m); 4.72 (4H, d, *J* = 11.2); 3.48-3.35 (4H, m); 3.14-2.89 (6H, m); 1.47 (9H, s); 0.95 (9H, s); 0.16 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 163.65, 155.09, 148.01, 139.42, 133.92, 133.75, 129.46, 126.73, 126.08, 124.70, 122.34, 116.26, 80.55, 65.40, 61.01, 52.79, 47.72, 46.67, 35.54, 35.31, 29.16, 27.08, 26.66, 24.08, 19.96, 19.11, -4.53.

MS (CI): m/z calc. for $C_{30}H_{56}N_2O_6Si_2$: 596.3; found: 597.5 $[M+H]^+$.

Compound 32

Compound **31** (200 mg, 0.33 mmol) was dissolved in 1 mL of TFA and stirred in room temperature for 7 minutes. The excess of acid was removed under reduced pressure and the crude amine salt was dissolved in 1 mL of DMF. Compound **3** (163 mg, 0.40 mmol) was added followed by the addition of 200 μL Et_3N. The reaction mixture was stirred in room temperature for 2 hours and monitored by TLC (100% EtOAc). Upon completion, the reaction was diluted with EtOAc and washed with NH_4Cl solution followed by brine. The crude product was purified by using column chromatography on silica gel (EtOAc:MeOH 95:5) to give compound **32** (133 mg,

74%) as a white solid.

^{1}H NMR (200MHz, MeOD): δ = 7.38-7.14 (11H, m); 6.88 (1H, m); 4.99 (2H, s); 4.52-4.30 (4H, m); 3.61-3.27 (6H, m); 3.05-2.80 (6H, m). ^{13}C NMR (100MHz, MeOD): δ = 170.33, 157.01, 155.81, 148.83, 139.73, 135.35, 132.52, 130.11, 129.72, 128.28, 128.94, 128.27, 127.93, 120.64, 120.48, 68.01, 67.45, 65.01, 60.66, 48.10, 47.42, 45.19, 35.07, 30.41. MS (FAB): m/z calc. for $C_{29}H_{33}N_3O_7$: 535.2; found: 558.3 $[M+Na]^+$.

Compound 33

Toluene (10 mL) was heated to reflux (110° C) and triphosgen (173 mg, 0.58 mmol) was added. Then a solution of compound **15** (137 mg, 0.48 mmol) in 5 mL toluene was slowly added dropwise with an injector. The reaction was stirred for 30 minutes in reflux and was monitored by ^{1}H NMR (200MHz, $CDCl_3$). After the isocyanate derivative was observed, the solvent was removed under reduced pressure. A solution of compound **32** (104 mg, 0.19 mmol) in 4 mL toluene, followed by the addition of 10 μL DBTL, was added to the isocyanate residue. The reaction mixture was heated to reflux and was stirred for 1 hour. The reaction was monitored by TLC (EtOAc:Hex 1:1). The solvent was removed under reduced pressure and the crude product was purified by using column chromatography on silica gel (EtOAc:Hex 4:1) to give compound **33** (134 mg, 60%) as a yellow powder.

^{1}H NMR (200MHz, $CDCl_3$): δ = 7.86-6.87 (18H, m); 5.11-4.97 (6H, m); 4.42 (4H, t, *J* = 4.2Hz); 3.72-3.50 (6H, m); 3.03-2.94 (6H, m); 1.11 (4H, t, *J* = 4.2Hz), 0.03 (18H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 171.13, 169.97, 167.21, 158.37, 157.65, 155.56, 153.91, 153.64, 152.24, 151.26, 150.78, 145.24, 141.57, 139.34, 136.52, 134.87, 133.02, 131.65, 131.48, 130.46, 130.21, 130.05, 129.95, 129.53, 128.98, 128.43,

126.44, 123.45, 68.69, 68.26, 67.94, 65.72, 64.28, 63.09, 47.47, 45.20, 35.26, 34.59, 30.94, 30.43, 30.10, 17.75, -0.80. MS (FAB): m/z calc. for $C_{55}H_{65}N_7O_{17}Si_2$: 1151.4; found: 1174.0 $[M+Na]^+$.

Compound 8

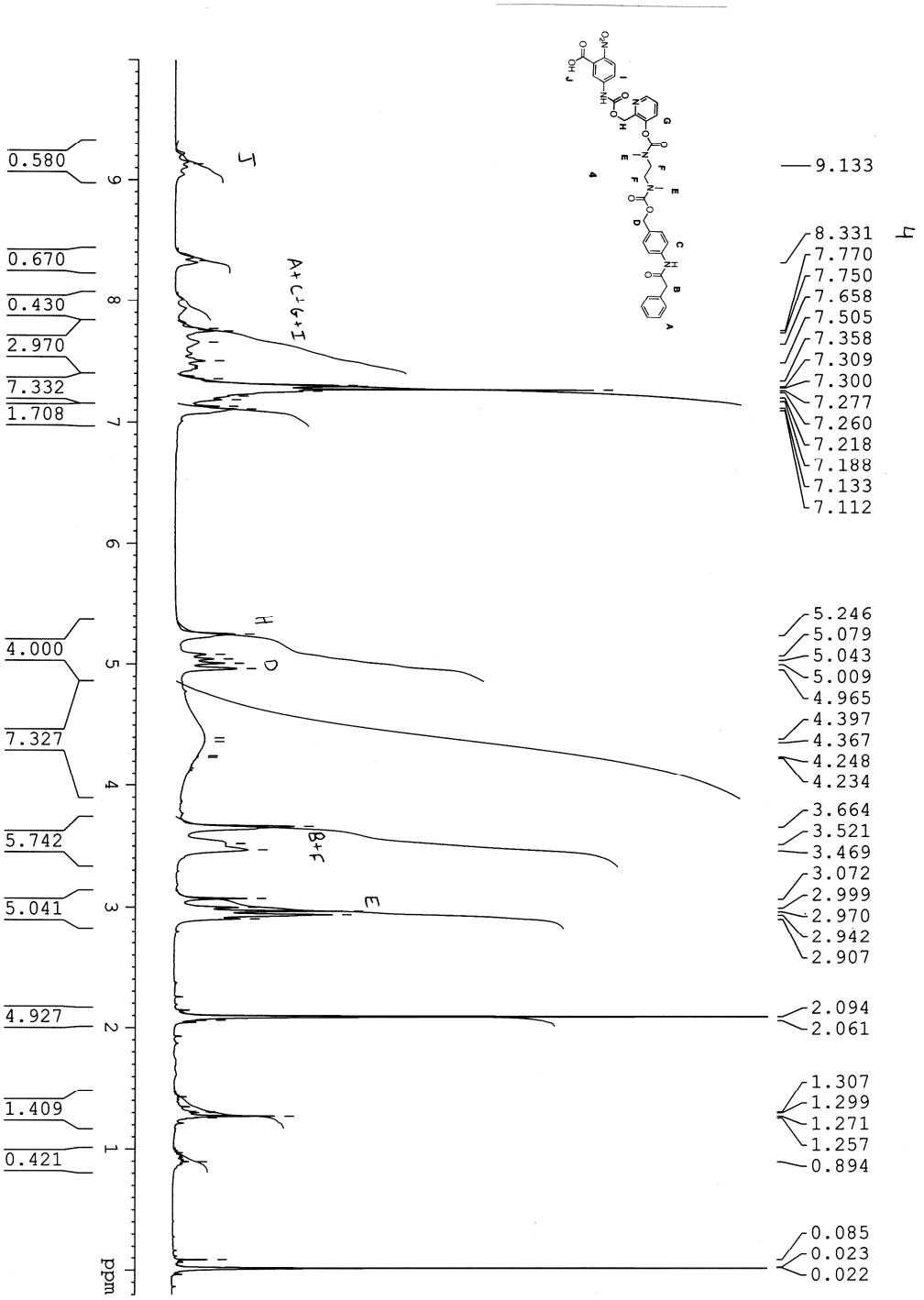
Compound **33** (90 mg, 0.07 mmol) was dissolved in dry THF (2 mL), and TBAF (0.313 mL, 1M in THF) was added. The reaction was stirred in room temperature for 2 hours and was monitored by TLC (EtOAc:MeOH:AcOH 97:2:1). Upon completion of the reaction, the solvent was removed under reduced pressure and the crude product was purified by using preperative C-18 RP-HPLC (eluent MeCN:H_2O 3:2) to give compound **8** (67 mg, 90%) as a white powder.

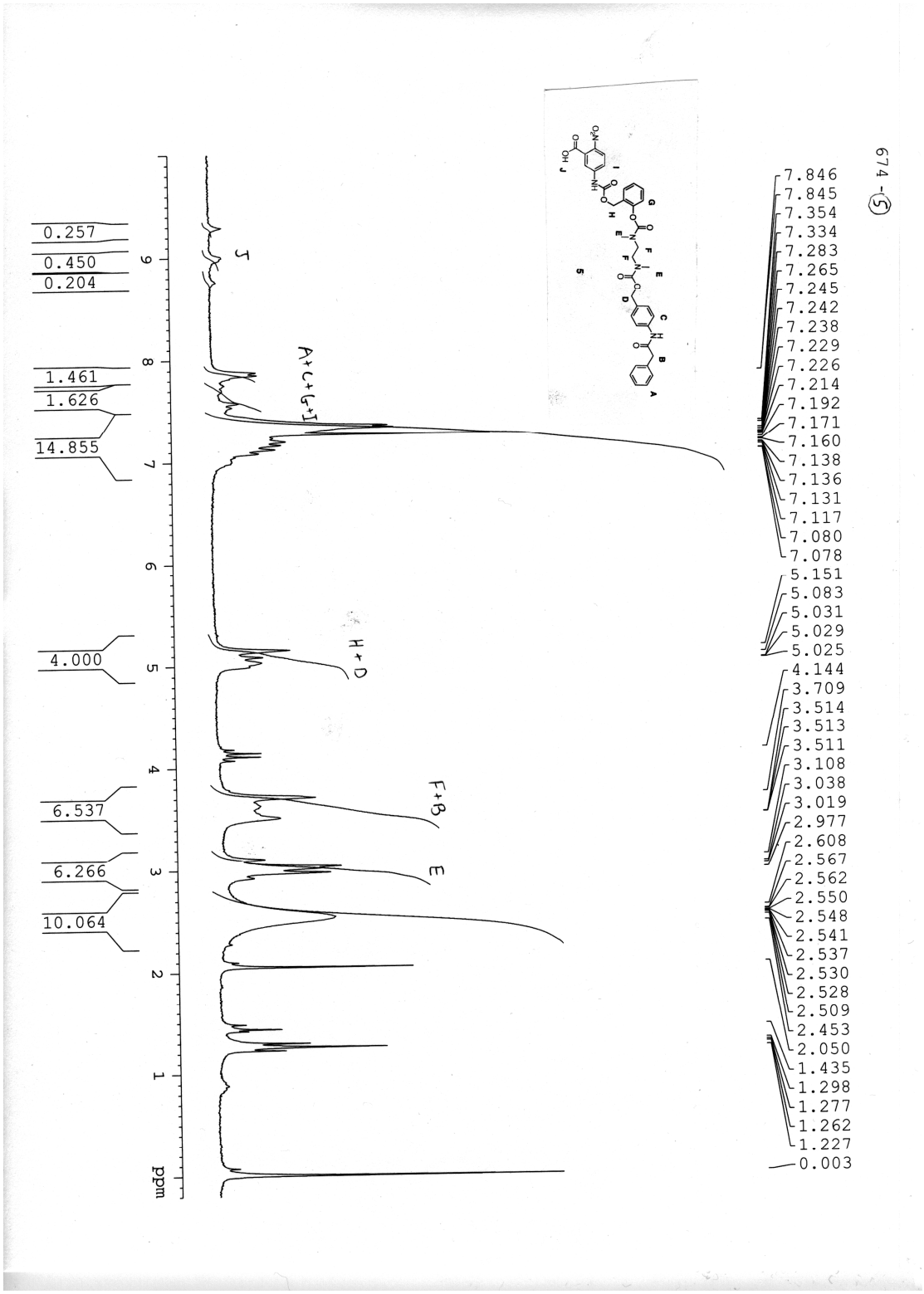
^{1}H NMR (200MHz, $CDCl_3$): δ = 7.93-7.78 (2H, m); 7.70-7.51 (4H, m); 7.44-6.96 (12H, m); 5.17-4.99 (6H, m); 3.71-3.47 (6H, m); 3.36-2.99 (6H, m).^{13}C NMR (100MHz, $CDCl_3$): δ = 171.23, 168.80, 154.04, 144.45, 141.80, 138.53, 135.33, 131.58, 130.37, 129.88, 129.46, 129.31, 127.93, 126.25, 123.78, 120.73, 120.45, 119.58,118.54, 118.36, 66.97, 63.30, 50.34, 50.13, 49.70, 49.27, 44.74, 36.14, 35.70, 30.30. MS (FAB): m/z calc. for $C_{45}H_{41}N_7O_{17}$: 951.2; found: 974.0 $[M+Na]^+$.

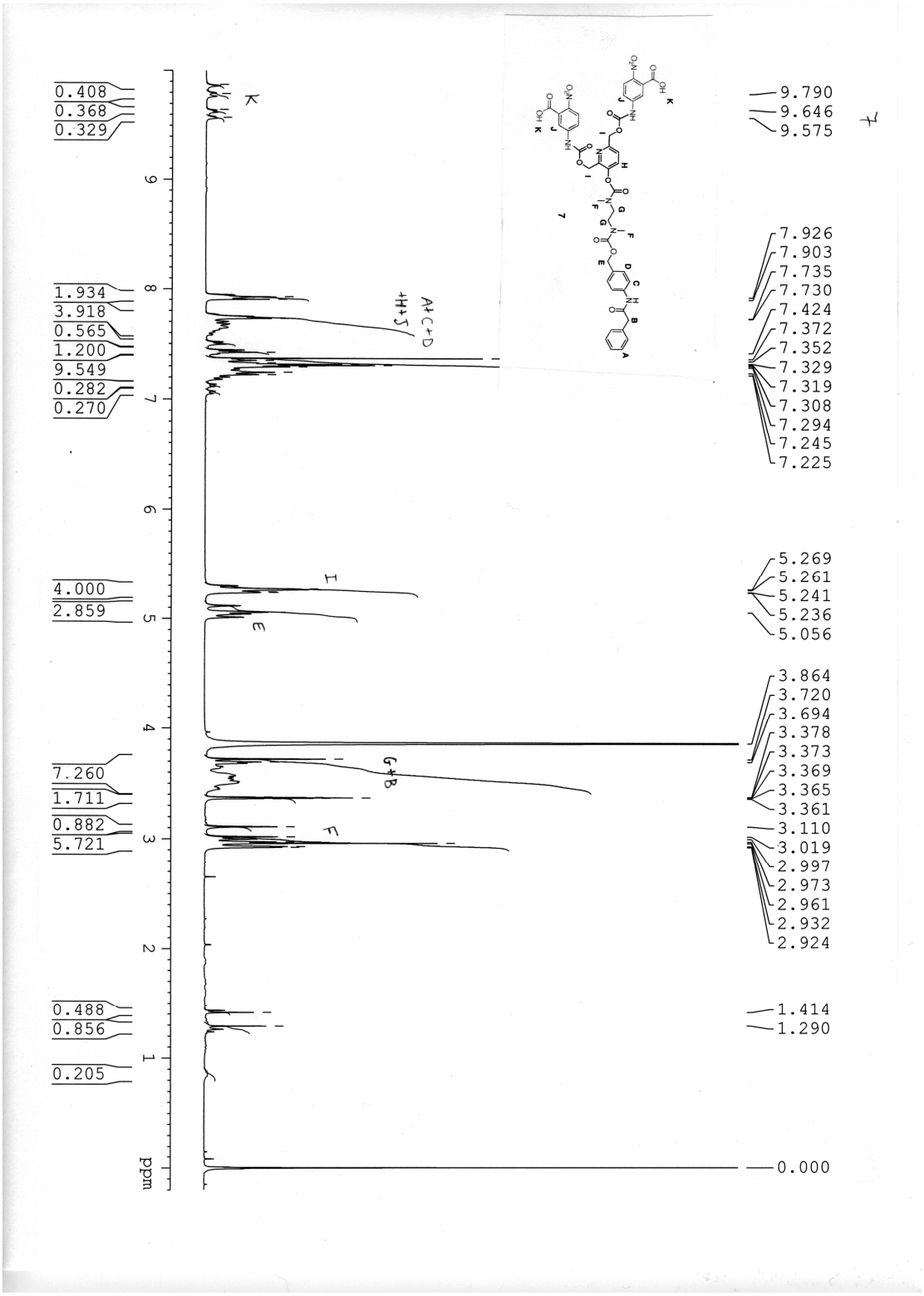
HPLC assay conditions:

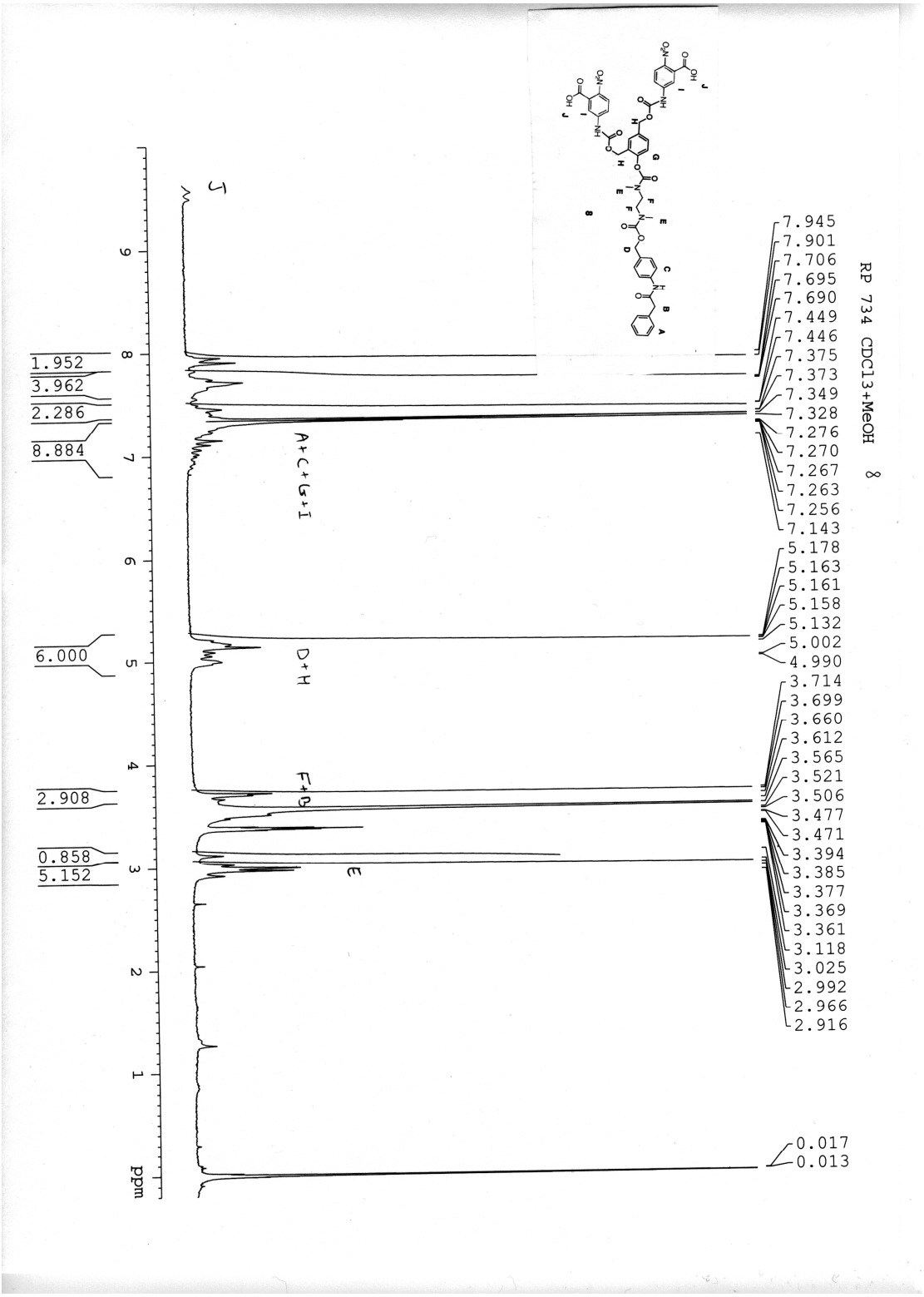
Compounds **4**, **5**, **7** and **8** were dissolved in DMSO to form stock solutions of 2 mM. The stock solutions were diluted in phosphate buffer saline (pH 7.4; PBS) in the presence of PGA (0.1 mg/mL) to give final solutions concentration of 100 μM. The release of 5-amino-2-nitrobenzoic acid was monitored by an HPLC assay using C-18 reverse-phase analytical column; λ= 348 ; flow: 1 mL min^{-1}; eluent: MeCN-H_2O

gradient progrem: t = 0 (10% ACN-90% H_2O), t = 20 (100% ACN); t_R = 9.50 min (5-amino-2-nitrobenzoic acid) t_R = 16.11 min (compound **4**); t_R = 17.94 min (compond **5**); t_R = 18.86 min (compound **7**); t_R = 19.31 min (compound **8**). The relative peak areas (in %) were used for the conversion calculation.


0.580
0.670
0.430
2.970
7.332
1.708
4.000
7.327
5.742
5.041
4.927
1.409
0.421
ppm
9.133
8.331
7.770
7.750
7.658
7.505
7.358
7.309
7.300
7.277
7.260
7.218
7.188
7.133
7.112
5.246
5.079
5.043
5.009
4.965
4.397
4.367
4.248
4.234
3.664
3.521
3.469
3.072
2.999
2.970
2.942
2.907
2.094
2.061
1.307
1.299
1.271
1.257
0.894
0.085
0.023
0.022
4


674 -(5)
7.846
7.845
7.354
7.334
7.283
7.265
7.245
7.242
7.238
7.229
7.226
7.214
7.192
7.171
7.160
7.138
7.136
7.131
7.117
7.080
7.078
5.151
5.083
5.031
5.029
5.025
4.144
3.709
3.514
3.513
3.511
3.108
3.038
3.019
2.977
2.608
2.567
2.562
2.550
2.548
2.541
2.537
2.530
2.528
2.509
2.453
2.050
1.435
1.298
1.277
1.262
1.227
0.003
0.257
0.450
0.204
1.461
1.626
14.855
4.000
6.537
6.266
10.064
J
A+C+G+I
H+D
F+B
E
ppm


0.408
0.368
0.329
1.934
3.918
0.565
1.200
9.549
0.282
0.270
4.000
2.859
7.260
1.711
0.882
5.721
0.488
0.856
0.205
ppm
K
A+C+D
+H+J
I
E
G+B
F
9.790
9.646
9.575
7.926
7.903
7.735
7.730
7.424
7.372
7.352
7.329
7.319
7.308
7.294
7.245
7.225
5.269
5.261
5.241
5.236
5.056
3.864
3.720
3.694
3.378
3.373
3.369
3.365
3.361
3.110
3.019
2.997
2.973
2.961
2.932
2.924
1.414
1.290
0.000


RP 734 CDCl3+MeOH 8
7.945
7.901
7.706
7.695
7.690
7.449
7.446
7.375
7.373
7.349
7.328
7.276
7.270
7.267
7.263
7.256
7.143
5.178
5.163
5.161
5.158
5.132
5.002
4.990
3.714
3.699
3.660
3.612
3.565
3.521
3.506
3.477
3.471
3.394
3.385
3.377
3.369
3.361
3.118
3.025
2.992
2.966
2.916
0.017
0.013
1.952
3.962
2.286
8.884
6.000
2.908
0.858
5.152
A+C+G+I
D+H
F+B
E
J
ppm