Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 110-112

Synthesis of novel mannopyranosyl betulinic acid phosphoniohexyl ester

Olga V. Tsepaeva, Andrey V. Nemtarev, Alexandra V. Kundina, Leysan R. Grigor'eva and Vladimir F. Mironov

Table of Contents

Experimental Section.

General remarks. The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded at 25 °C using a Bruker Avance-400 NMR spectrometer (400.0 MHz, ^{1}H; 100.6 MHz, ^{13}C; 162 MHz, ^{31}P). Chemical shifts are referenced to the residual solvent peak and reported in ppm (δ scale) and all coupling constant (*J*) values are given in Hz. IR spectra were recorded using a Bruker Tenzor 27 spectrometer for samples in KBr tablets. MALDI mass spectra were acquired on a Bruker MALDI-TOF Ultraflex III spectrometer. 2,5-Dihydroxybenzoic acid (5 mg/ml in methanol) was used as a matrix. Optical rotation was measured at 20 °C on an Atago POL-1/2 automatic polarimeter with an external Peltier module (concentration c is given as g/100mL). Melting points were determined on a Boetius compact heating table. Elemental analysis was accomplished with an automated EuroVector EA3000 CHNS-O elemental analyzer (Euro-Vector, Italy). The progress of reactions and the purity of products were monitored by TLC on Sorbfil plates (IMID Ltd., Russian Federation). The TLC plates were visualized by treatment with phosphotungstic acid in ethanol, followed by heating to 120 °C. The targeted compounds were isolated using column chromatography on silica gel (60A, 60-200 μm, Acros, Belgium). Solvents were purified and dried by standard protocols. 2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranosyl trichloroacetimidate **1** [S1], betulinic acid [S2], betulinic acid 6-bromohexyl ester [S3], phosphonium salt **9** [S3] were synthesized as reported.

1-(4-Bromobutyl) 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**2**)**.** A solution of compound **1** (206 mg, 0.28 mmol) and 4-bromobutanol (38 mg, 0.25 mmol) in dichloromethane (10 ml) was stirred for 30 min at room temperature over molecular sieves (4 Å, 300 mg) under argon atmosphere, then a solution TMSOTf (0.15 mmol) in dichloromethane (1 ml) was added. The mixture was stirred for 1 h, and the reaction was quenched with Et_3N (0.5 mL). The mixture was concentrated, and residue was purified by column chromatography (CH_2Cl_2/MeOH, 100:1) to give the compound **2** (95 mg, 52 %) as a colorless powder; mp 106-108 °C, $[\alpha]^{20}_D$ – 35.8 (*c* 0.6, $CHCl_3$), IR (KBr) ν_{max}: 1728, 1602, 1584, 1452, 1315, 1265, 1177, 1109, 1070, 1027, 975, 913, 709 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.21-2.32 (m, 4H, CH_2), 3.59-3.67 (m, 2H, CH_2Br), 3.72-3.75 (m, 1H, OCH_2), 4.06-4.10 (m, 1H, OCH_2), 4.50-4.52 (m, 1H, $H^{5'}$), 4.55 (dd, *J* = 12.0, 4.5, Hz 1H, $H_B{}^{6'}$), 4.76 (dd, *J* = 10.0, 2.2 Hz, 1H, $H_A{}^{6'}$), 5.15 (d, 1H, $H^{1'}$), 5.75 (m, 1H, $H^{2'}$), 5.93 (dd, *J* = 10.2, 3.2 Hz 1H, $H^{3'}$,), 6.16 (1H, $H^{4'}$), 7.27-8.15 (m, 20H, C_6H_5); ^{13}C NMR ($CDCl_3$, 100 MHz): 30.10, 32.19, 62.9 ($C^{6'}$), 65.9, 66.9 ($C^{4'}$), 69.1 ($C^{5'}$), 70.1 ($C^{3'}$), 70.5 ($C^{2'}$), 97.8 ($C^{1'}$), 165.3, 165.4, 165.5, 166.1; anal. C 62.1; H 4.7; Br 10.5 %, calcd for $C_{38}H_{35}BrO_{10}$, C 62.39; H 4.82; Br 10.92 %.

[4-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranos-1-yloxy)butyl]triphenylphosphonium bromide (**3**)**.** Triphenylphosphine (108 mg, 0.4 mmol) was added to a solution of compound **2** (100 mg, 0.14 mmol) in dry acetonitrile (7 ml) under argon, and the mixture was stirred under reflux for 12-18 h until the reaction was complete according to TLC detection. Acetonitrile was removed under reduced pressure, and the precipitate was washed with hot petroleum ether (3 × 5 ml) and dissolved in ethyl acetate. Petroleum ether (5 ml) was added to the reaction mixture. The resulting precipitate was washed with diethyl ether (3 ml) and dried in vacuum to give the pure TPP conjugate **3** (132 mg, 95%); mp 115 °C, $[\alpha]^{20}_D$ – 30.5 (*c* 0.8, $CHCl_3$), IR (KBr) ν_{max}: 3427, 1726, 1601, 1488, 1441, 1266, 1176, 1111, 1071, 1028, 974, 714 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.10-2.25 (m, 4H, CH_2), 3.89 (m, 1H,

OCH_2), 4.13 (m, 2H, CH_2P), 4.29 (m, 1H, OCH_2), 4.49-4.50 м (m, 2H, $H^{5'}$, $H_B^{6'}$), 4.70-4.72 (m, 1H, $H_A^{6'}$), 5.09 (m, 1H, $H^{1'}$), 5.67 (m, 1H, $H^{2'}$), 5.82-5.84 (dd, J = 10.1, 3.2 Hz, 1H, $H^{3'}$), 6.09 (t, J = 10.0 Hz, 1H, $H^{4'}$), 7.26-8.07 (m, 35H, C_6H_5); ^{13}C NMR ($CDCl_3$, 100 MHz): 19.7 (d, J = 52.2 Hz, CH_2P,), 23.1, 23.2, 62.8 ($C^{6'}$), 66.7 ($C^{4'}$), 67.1, 69.1 ($C^{5'}$), 70.2 ($C^{3'}$), 70.3 ($C^{2'}$), 97.6 ($C^{1'}$), 118.2 (d, J = 86.2 Hz, C^{ipso}), 128.3-135.0 (m, C_{Ar}), 165.4, 165.4, 165.7, 166.1; ^{31}P NMR ($CDCl_3$, 162 MHz): δ 26.0; anal. C 68.1; H 5.1; Br 7.9 %, calcd for $C_{56}H_{50}BrO_{10}$, C 67.68; H 5.07; Br 8.04 %.

3β-O-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl)-28-oxoallobetulin (**6**)**.** Starting from **4** (154 mg, 0.25 mmol), saponin **6** was obtained as a white foam. (38 mg, 15 %), IR (KBr) ν_{max}: 2958, 2937, 2872, 1769, 1724, 1602, 1585, 1451, 1270, 1178, 1113, 1063, 1027, 964, 706 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90, 0.91, 0.95, 0.96, 0.98, 1.05, 1.12 (s, 21H, $H^{23\text{-}27,29,30}$), 1.2-1.9 (m, 26H, lupane protons), 3.36 (dd, J = 11.6, 4.4 Hz, 1H, H^3), 3.95 (s, 1H, H^{19}), 4.49 (dd, J = 12, 4.4 Hz, 1H, $H^{6'}$), 4.54-4.58 (m, 1H, $H^{5'}$), 4.68 (dd, J = 12.0, 2.0 Hz, 1H, $H^{6'}$), 5.29 (d, J = 1.2 Hz, 1H, $H^{1'}$), 5.62 (dd, J = 3.2, 1.8 Hz, 1H, $H^{2'}$), 5.92 (dd, J = 10.2, 3.2 Hz, 1H, $H^{3'}$), 6.1 (t, J = 10.0 Hz, 1H, $H^{4'}$), 7.26-8.12 (m, 20H, C_6H_5); anal. C 75.3; H 7.6 %, calcd for $C_{64}H_{74}O_{12}$, C 74.25; H 7.20 %.

6-Bromhexyl 3β-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranos-1-yloxy)lup-20(29)-en-28-oate (**5**)**.** A solution of compound **1** (206 mg, 0.28 mmol) and compound **4** (154 mg, 0.25 mmol) in dichloromethane (15 ml) was stirred for 30 min at room temperature over molecular sieves (4 Å, 300 mg) under argon atmosphere, then cooled to –40 °C and solution TMSOTf (0.15 mmol) in dichloromethane (1 ml) was added. The mixture was stirred for 1 h, and the reaction was quenched with Et_3N (0.5 ml). The mixture was concentrated, and residue was purified by column chromatography (petroleum ether / EtOAc, 4:1) to give the compound **5** (212 mg, 70 %); mp 93 °C, $[\alpha]^{20}_D$ – 2.7 (*c* 0.8, $CHCl_3$), IR (KBr) ν_{max}: : 2945, 2869, 1729, 1642, 1602, 1585, 1452, 1377, 1362, 1316, 1267, 1177, 1109, 1069, 1027, 973, 881, 802, 711, 687 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89, 0.95, 0.99, 1.11 (s, 15H, $H^{23\text{-}27}$), 1.71 (s, 3H, H^{30}), 3.0 (m, 1H, H^{19}), 3.36 (dd, J = 11.6, 4.3 Hz, 1H, H^3), 3.43 (t, J = 6.8 Hz, 2H, CH_2Br,), 4.11 (m, 2H, $C(O)OCH_2$), 4.49 (dd, J = 12.0, 5.0 Hz, 1H, $H^{6'}$,), 4.54-4.58 (m, 1H, $H^{5'}$), 4.62 (s, 1H, H_A^{29}), 4.68 (dd, J = 12.0, 2.0 Hz, 1H, $H^{6'}$), 4.75 (m, 1H, H_B^{29}), 5.29 (d, J = 1.5 Hz, 1H, $H^{1'}$), 5.62 (dd, J = 3.2, 1.5 Hz, 1H, $H^{2'}$), 5.92 (dd, J = 10.1, 3.2 Hz, 1H, $H^{3'}$), 6.1 (t, J = 10.1 Hz, 1H, $H^{4'}$), 7.26-8.12 (m, 20H, C_6H_5); anal. C 70.3; H 7.35; Br 6.67 %, calcd for $C_{71}H_{89}BrO_{12}$, C 70.22; H 7.39; Br 6.58 %.

{6-[3β-(2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranos-1-yloxy)-28-oxolup-20(29)-en-28-yloxy]hexyl}triphenylphosphonium bromide (**7**)**.** Triphenylphosphine (168 mg, 0.64 mmol) was added to a solution of compound **5** (200 mg, 0.16 mmol) in dry acetonitrile (10 ml) under argon, and the mixture was stirred under reflux for 12-18 h until the reaction was complete according to TLC detection. Acetonitrile was removed under reduced pressure, and the precipitate was washed with hot petroleum ether (3 × 5 ml) and dissolved in ethyl acetate. Petroleum ether (10 ml) was added to the reaction mixture. The resulting precipitate was washed with diethyl ether (3 ml) and dried in vacuum to give pure conjugate **7** (198 mg, 85%); mp 135-145 °C, $[\alpha]^{20}_D$ – 7.4 (*c* 1.0, $CHCl_3$), IR (KBr) ν_{max}: 3431, 2941, 2867, 1727, 1641, 1602, 1586, 1452, 1439, 1377, 1316, 1267, 1177, 1110, 1069, 1027, 973, 749, 712, 689 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.85, 0.89, 0.95, 0.97, 1.11 (s, 15H, $H^{23\text{-}27}$), 1.70 (s, 3H, H^{30}), 1.1-2.2 (m, 31H, betulinic acid

scaffold and $(CH_2)_2$ fragment of linker), 3.0 (m, 1H, H^{19}), 3.36 (dd, *J* = 11.6, 4.0 Hz, 1H, H^3), 3.91-3.98 (m, 2H, CH_2P), 4.03 (m, 2H, $C(O)OCH_2$), 4.49 (dd, *J* = 11.6, 4.0 Hz, 1H, $H^{6'}$), 4.54-4.58 (m, 1H, $H^{5'}$), 4.61 (s, 1H, $H_A{}^{29}$), 4.69 (dd, *J* = 11.7, 1.8 Hz, 1H, $H^{6'}$), 4.72 (m, 1H, $H_B{}^{29}$), 5.29 (m, 1H, $H^{1'}$), 5.63 (dd, 1H, $H^{2'}$), 5.92 (dd, *J* = 10.2, 3.2 Hz, 1H, $H^{3'}$), 6.1 (t, *J* = 10.0 Hz, 1H, $H^{4'}$), 7.26-8.12 (m, 35 H, C_6H_5); ^{13}C NMR ($CDCl_3$, 100 MHz): 14.7, 16.0, 16.1, 16.4, 18.3, 19.3, 20.9, 22.2, 22.64, 22.69, 22.8 (d, *J* = 49.7 Hz, CH_2-P), 25.5, 25.7, 28.3, 28.7, 29.6, 29.9, 30.1, 30.6, 32.1, 34.3, 37.0, 37.1, 38.2, 38.6, 40.7, 42.4, 47.0, 49.4, 50.5, 55.7, 56.5, 63.1 ($C^{6'}$), 63.7 67.0 ($C^{4'}$), 69.4 ($C^{5'}$), 70.2 ($C^{3'}$), 71.6 ($C^{2'}$), 84.2 (C^3), 94.3 ($C^{1'}$), 109.5 (C^{29}), 118.4 (d, *J* = 85.8 Hz, C^{ipso}), 128.3-135.0 (m, C_{Ar}), 150.5, 165.54, 165.6, 166.1, 176.1; ^{31}P NMR ($CDCl_3$, 162 MHz): *δ* 24.3; MALDIMS *m*/*z* 1379.3 $[M - Br]^+$. Anal. Calcd for $C_{88}H_{100}BrO_{12}P$: C, 72.36; H, 6.90; Br, 5.47 %, Found; C, 72.2; H, 6.9; Br, 5.4 %.

{6-[3β-(α-D-Mannopyranos-1-yloxy)-28-oxolup-20(29)-en-28-yloxy]hexyl}triphenylphosphonium bromide (8). A suspension of protected saponin **7** (145 mg, 0.1 mmol) and K_2CO_3 (25 mg) in MeOH (5 ml) was stirred for 1 h at room temperature, then neutralized with Amberlyst 15 resin (H^+ form), filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2/MeOH, gradient 10:1→1:0) to give unprotected saponin **8** as an amorphous powder (73 mg, 70 %); mp 126-130 °C, $[\alpha]^{20}_D$ 31 (*c* 0.4, MeOH), IR (KBr) ν_{max}: 3402, 2940, 2868, 1720, 1639, 1588, 1439, 1390, 1376, 1317, 1243, 1131, 1054, 1026, 976, 916, 882, 724, 691 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 0.8, 0.83, 0.9, 1.0, 1.02 (s, 15H, H^{23-27}), 1.7 (s, 3H, H^{30}), 0.89-2.2 (31H, m, betulinic acid scaffold and $(CH_2)_2$ fragment of linker), 3.0 (m, 1H, H^{19}), 3.2 (dd, *J* = 11.5, 4.0 Hz, 1H, H^3), 3.4-3.5 (m, 4H, $H^{2',5',6'}$) 3.7-3.8 (m, 4H, CH_2P, $H^{3'}$, $H^{4'}$), 4.0-4.1 (m, 2H, $C(O)OCH_2$), 4.6 (s, 1H, $H_A{}^{29}$), 4.7 (s, 1H, $H_B{}^{29}$), 4.97 (s, 1H, $H^{1'}$), 7.7-7.9 (m, 15 H, C_6H_5); ^{13}C NMR ($CDCl_3$, 100 MHz): 13.8, 15.4, 15.5, 15.6, 18.0, 18.2, 20.75, 21.4 (d, *J* = 51.2 Hz, CH_2-P), 21.5, 22.2, 22.25, 25.2, 25.4, 27.9, 28.1, 29.4, 29.7, 29.8, 30.3, 31.8, 34.2, 36.6, 36.9, 38.1, 38.15, 38.3, 40.6, 42.2, 49.1, 50.4, 55.5, 56.5, 61.5, 63.5 ($C^{6'}$), 67.1 ($C^{4'}$), 71.4 ($C^{2'}$), 71.7 ($C^{3'}$), 73.7 ($C^{5'}$), 81.9 (C^3), 96.4 ($C^{1'}$), 109.0 (C^{29}), 118.5 (d, *J* = 86.3 Hz, C^{ipso}), 130.-135.0 (m, C_{Ar}), 150.1, 176.7; ^{31}P NMR ($CDCl_3$, 162 MHz): *δ* 24.1; MALDIMS *m*/*z* 963.6 $[M - Br]^+$. Anal. Calcd for $C_{60}H_{84}BrO_8P$: C, 69.02; H, 8.11; Br, 7.65 %, Found; C, 69.5; H, 8.06; Br, 7.6 %.

References

[S1] T. Ikeda, T. Kajimoto, H. Kondo and C. H. Wong, *Bioorg. Med. Chem. Lett*., 1997, **7**, 2485.

[S2] D. S. H. L. Kim, Z. Chen, T. Nguyen, J. M. Pezzuto, S. Qiu and Z. Z. Lu, *Synth. Commun*., 1997, **27**, 1607.

[S3] O. V. Tsepaeva, A. V. Nemtarev, T. I. Salikhova, T. I. Abdullin, L. R. Grigor'eva, S. A. Khozyainova and V. F. Mironov, *Anti-Cancer Agents Med. Chem.*, 2020, **20** (3), 286.

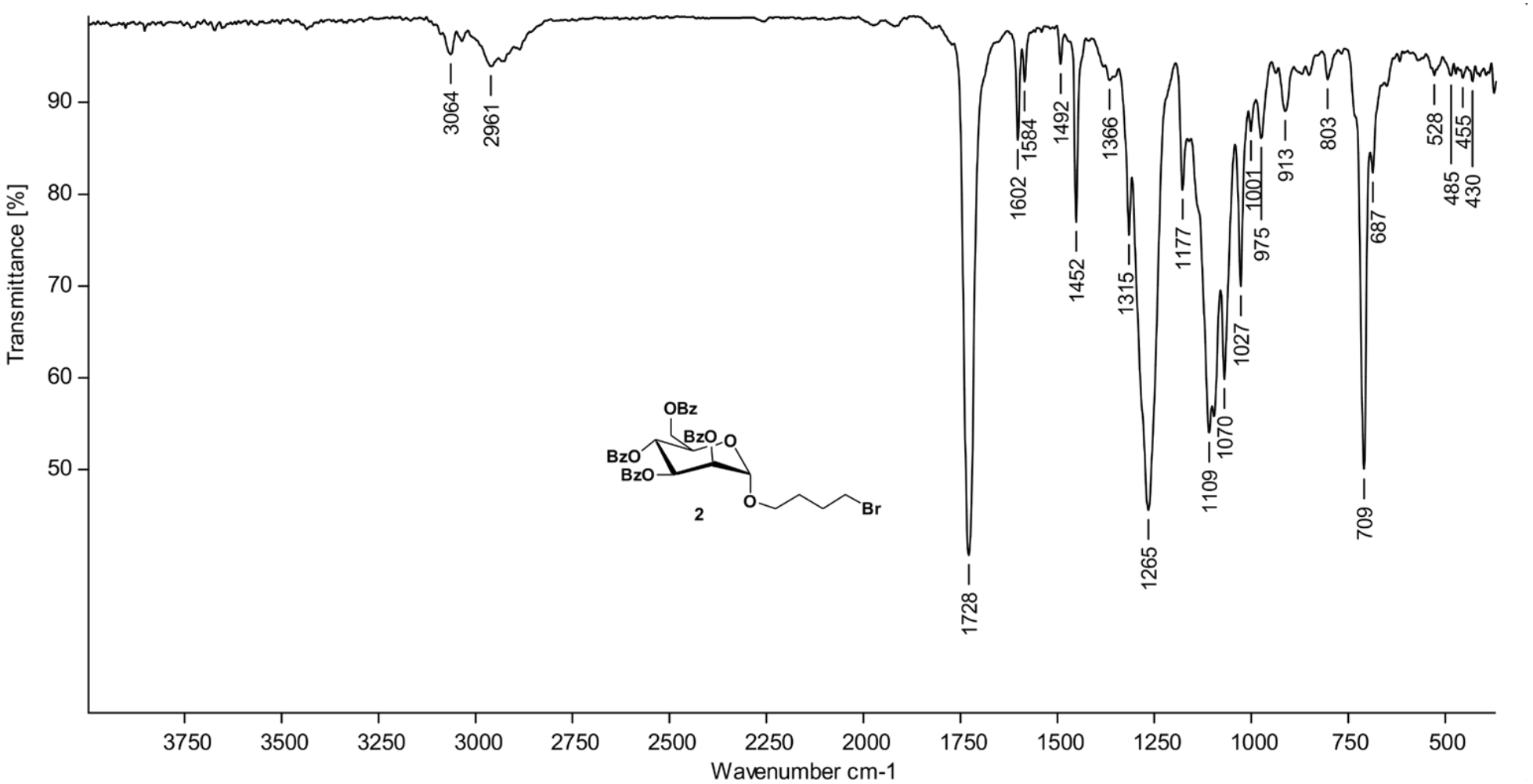

Figure S1. IR (film) spectrum of compound **2.**

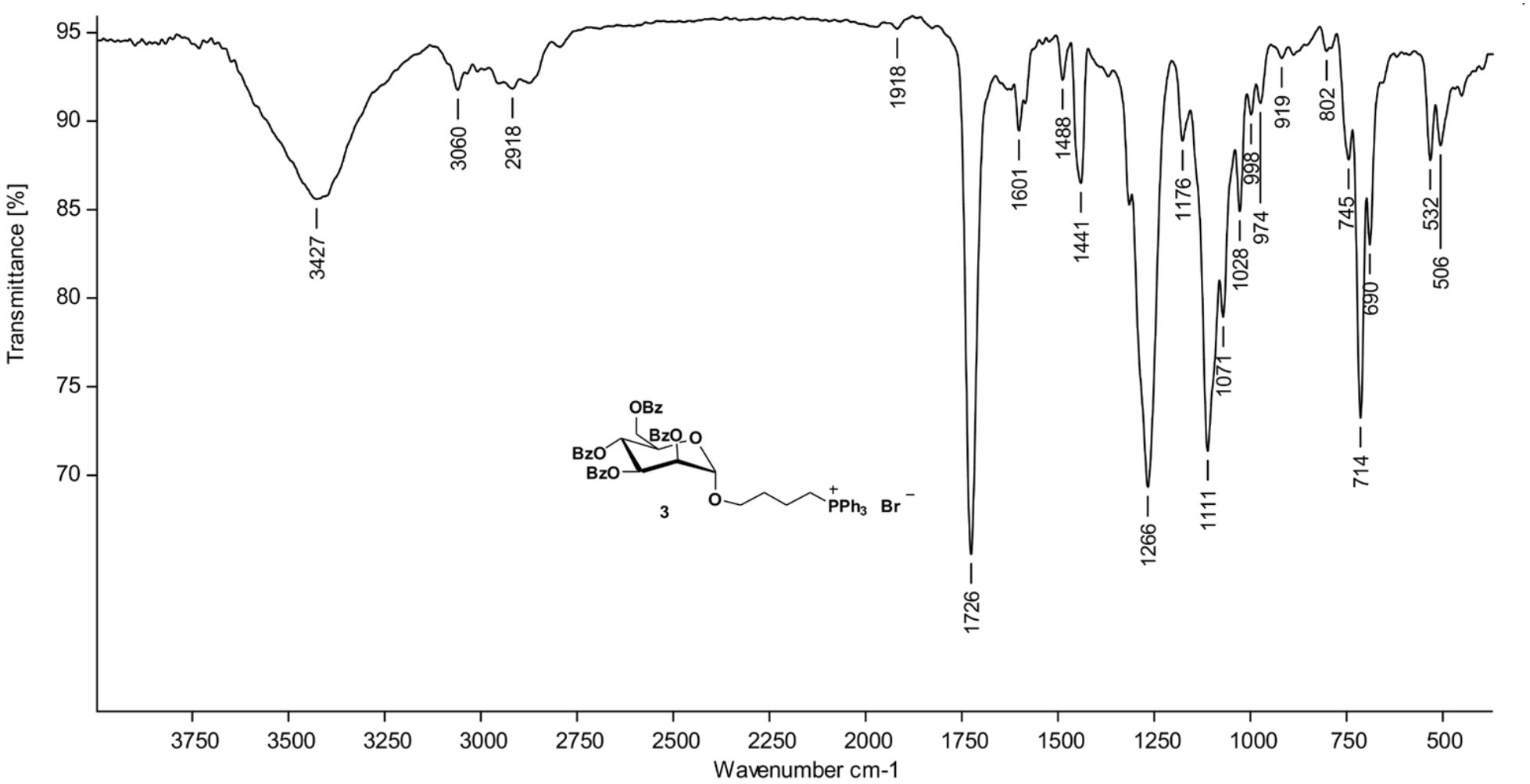

Figure S2. IR (film) spectrum of phosphonium salt **3.**

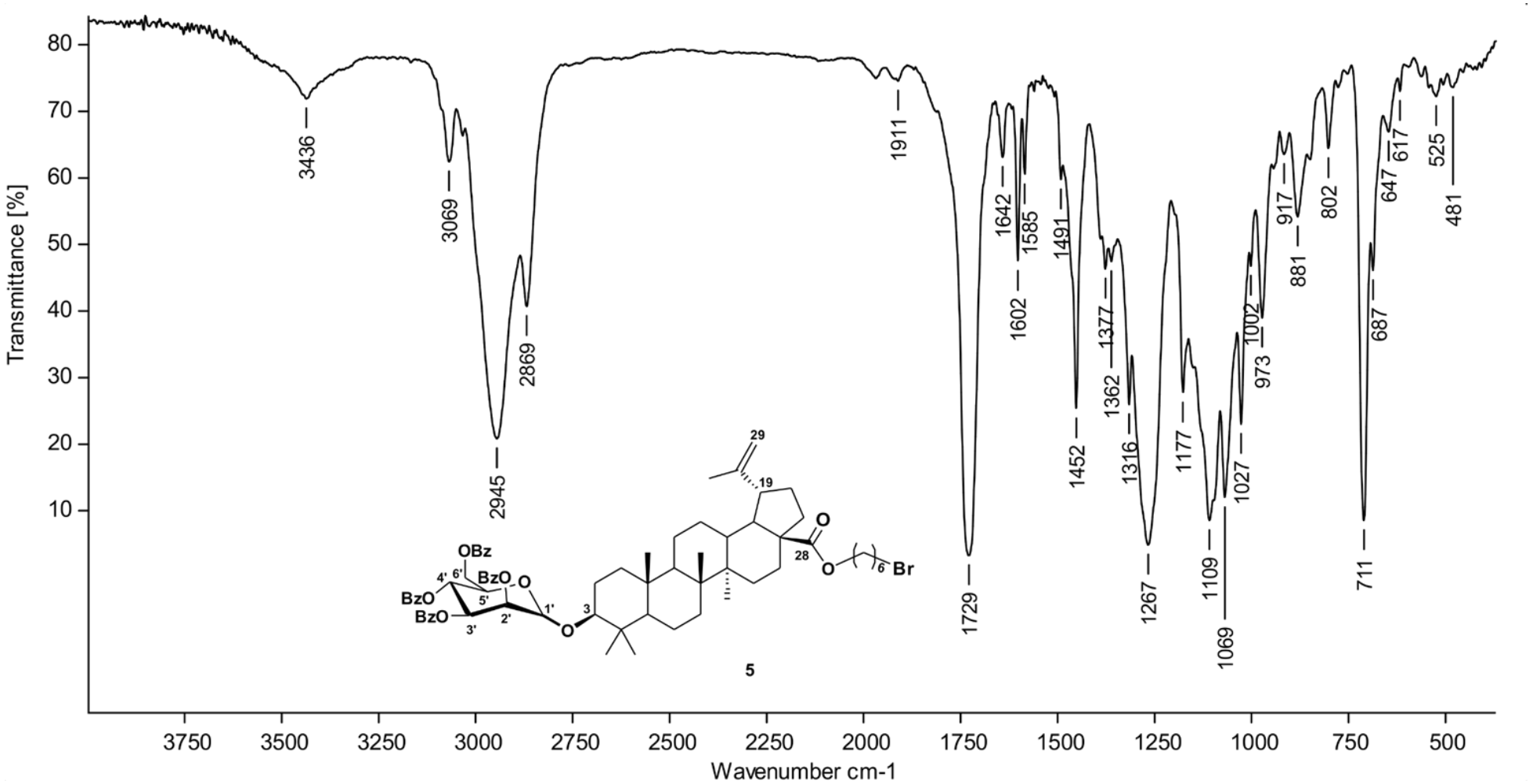

Figure S3. IR (KBr) spectrum of compound **5.**

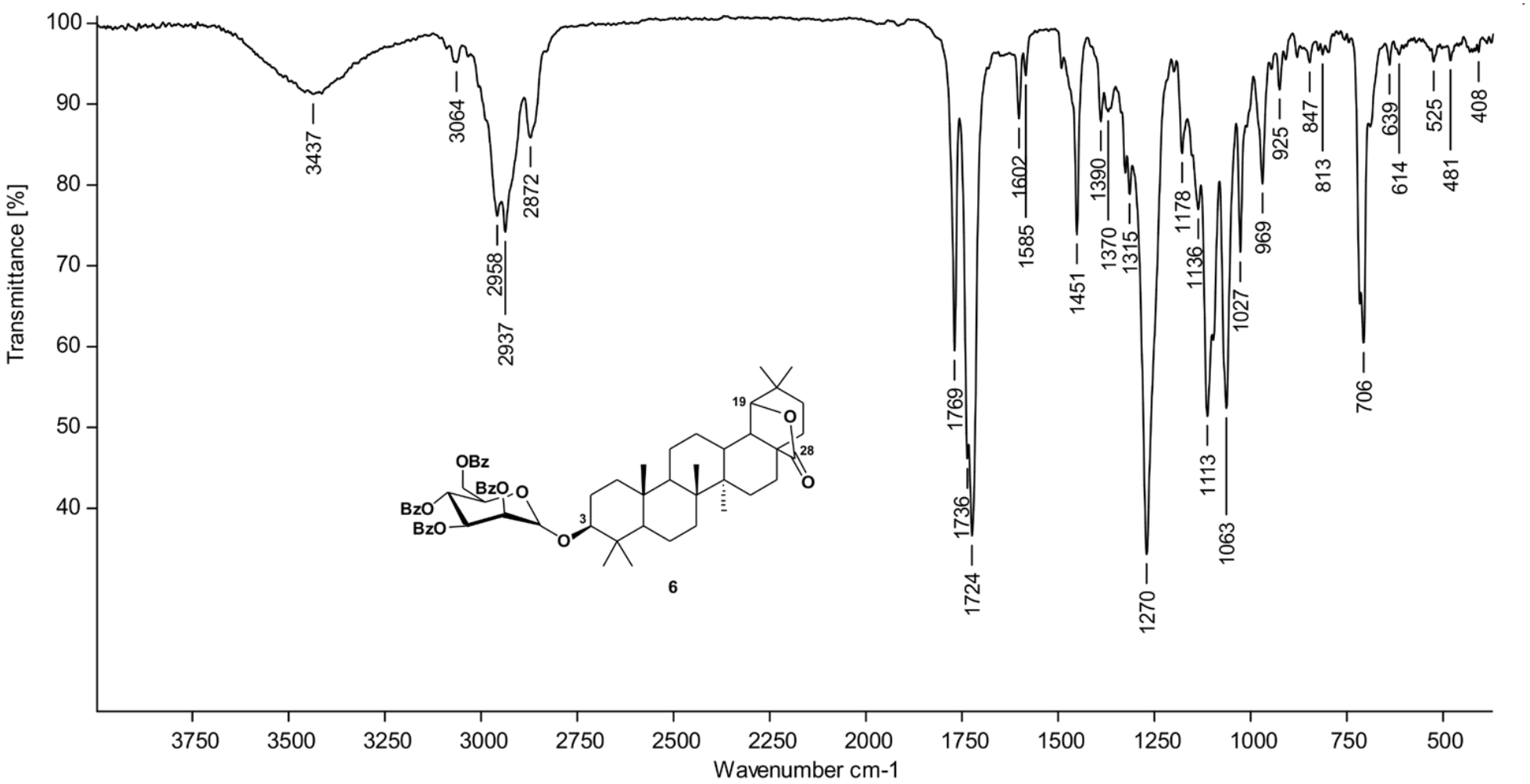

Figure S4. IR (KBr) spectrum of compound **6.**

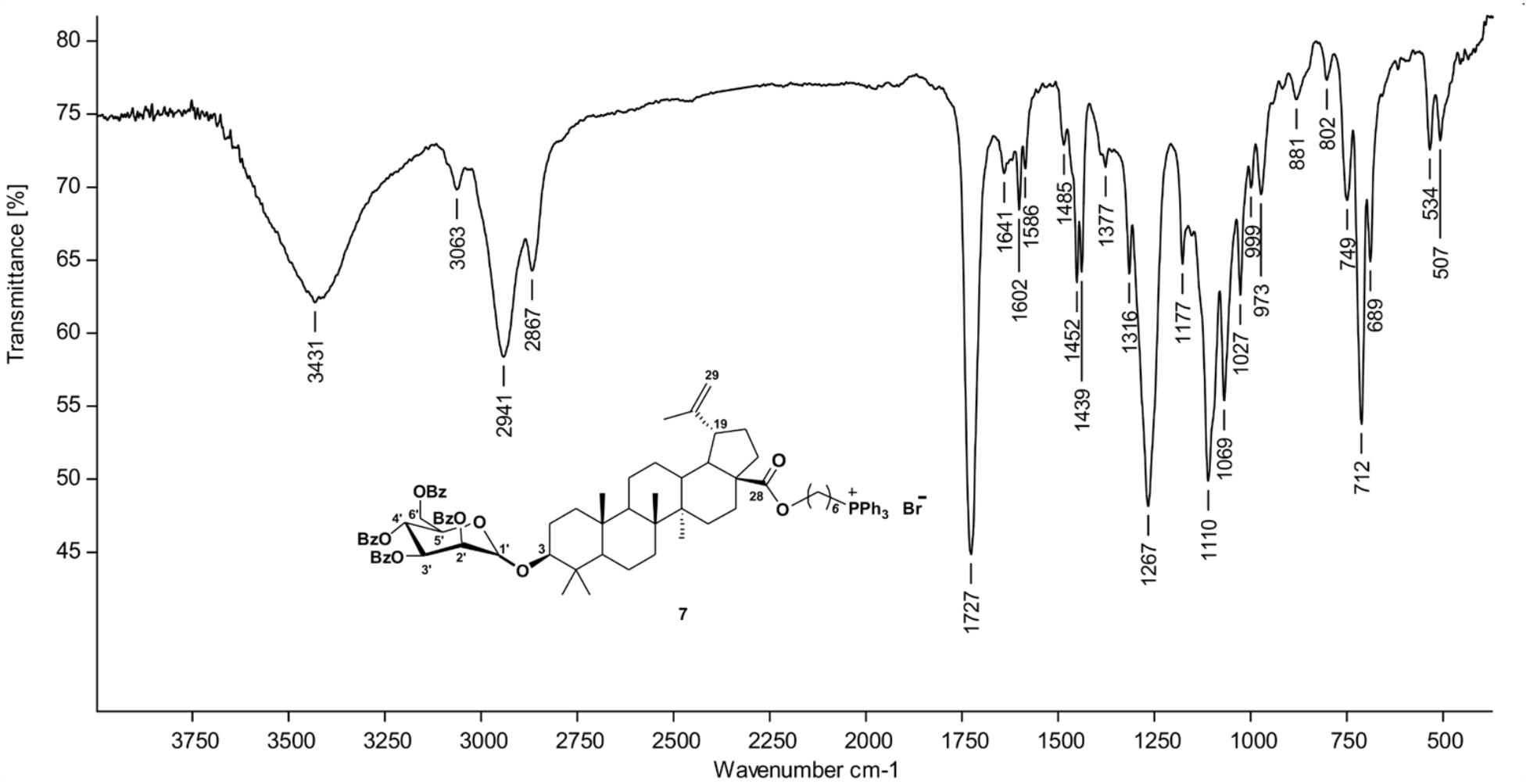

Figure S5. IR (KBr) spectrum of phosphonium salt **7.**

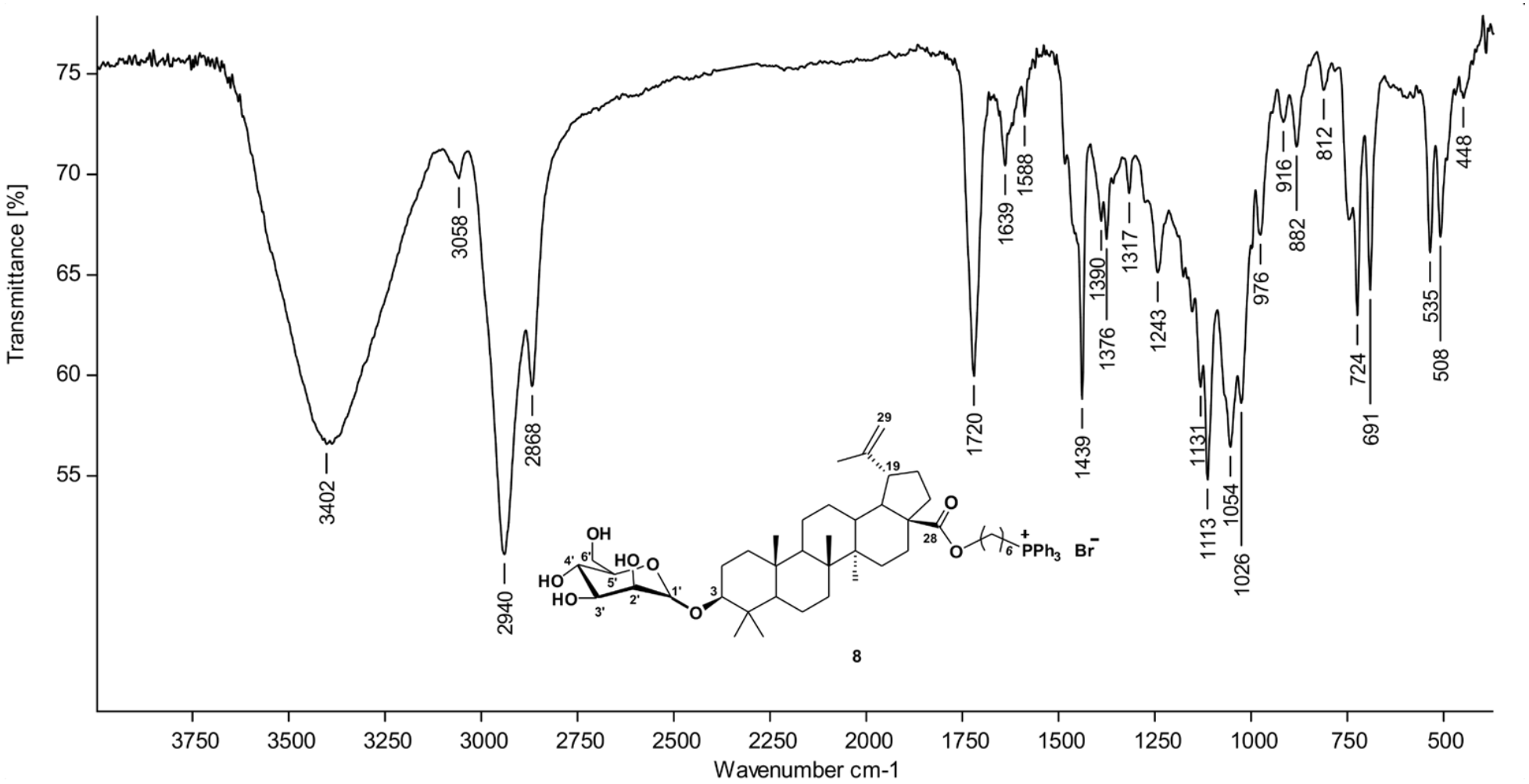

Figure S6. IR (KBr) spectrum of phosphonium salt **8.**

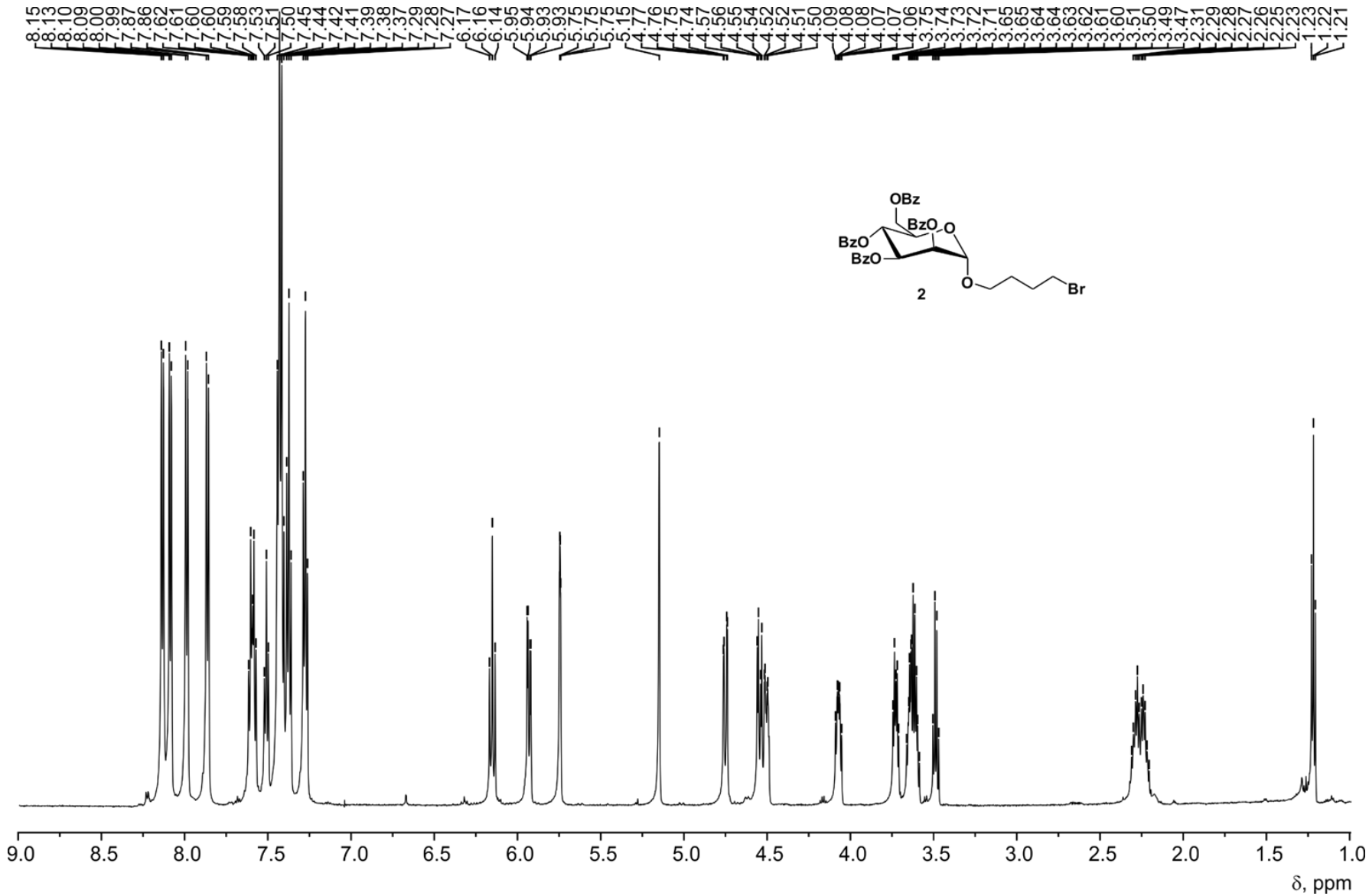

Figure S7. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **2.**

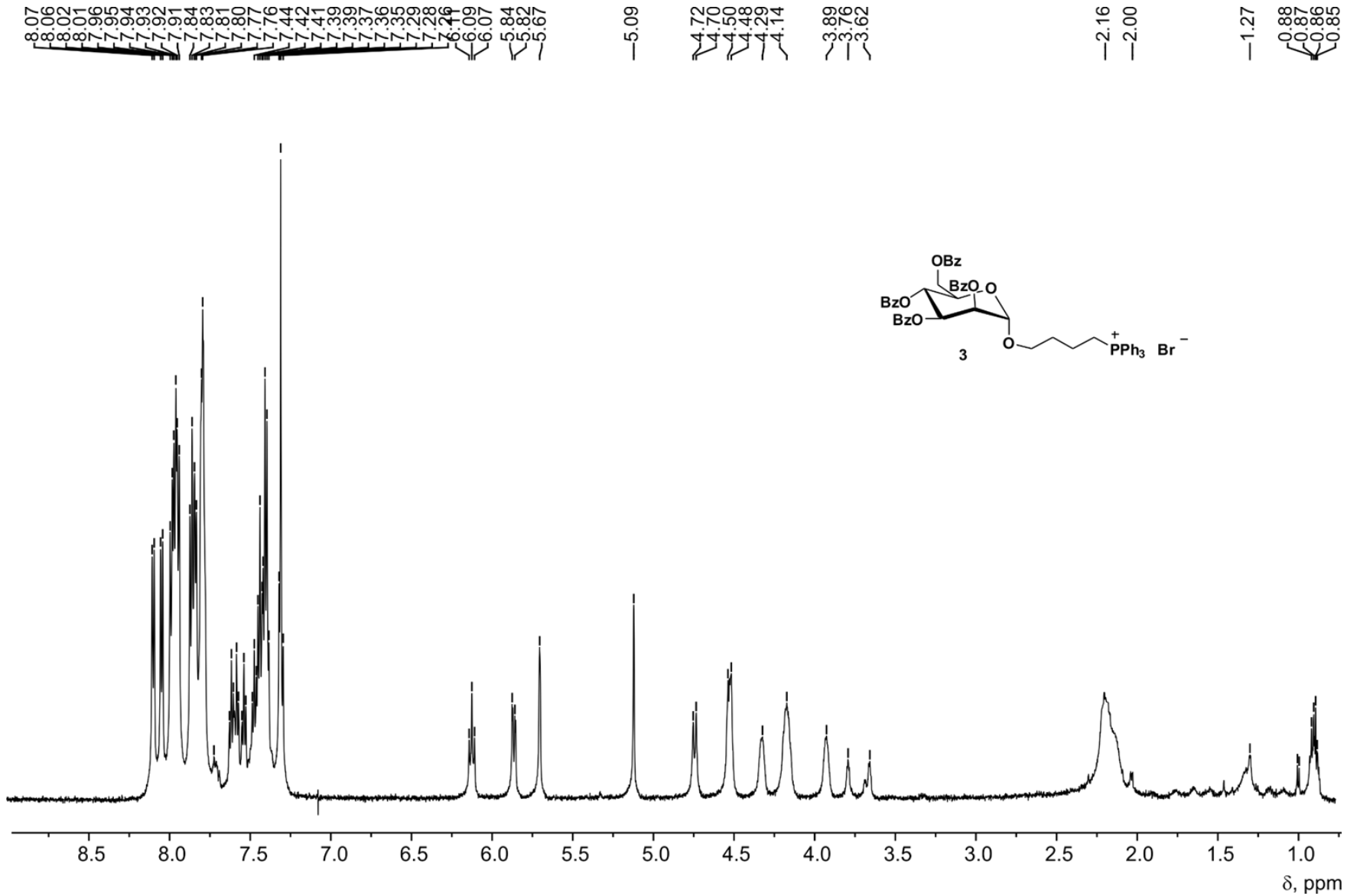

Figure S8. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of phosphonium salt **3.**

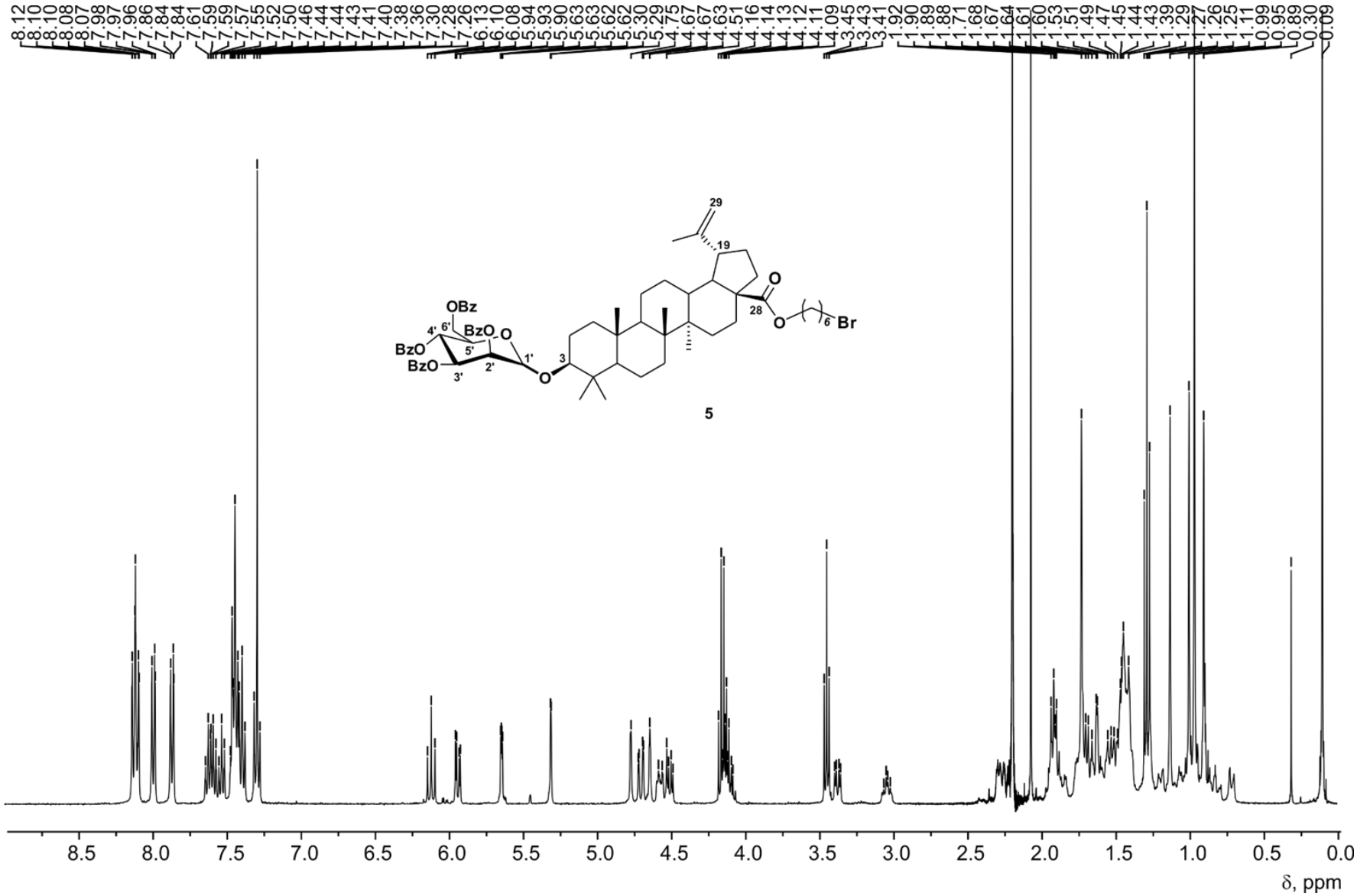

Figure S9. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **5.**

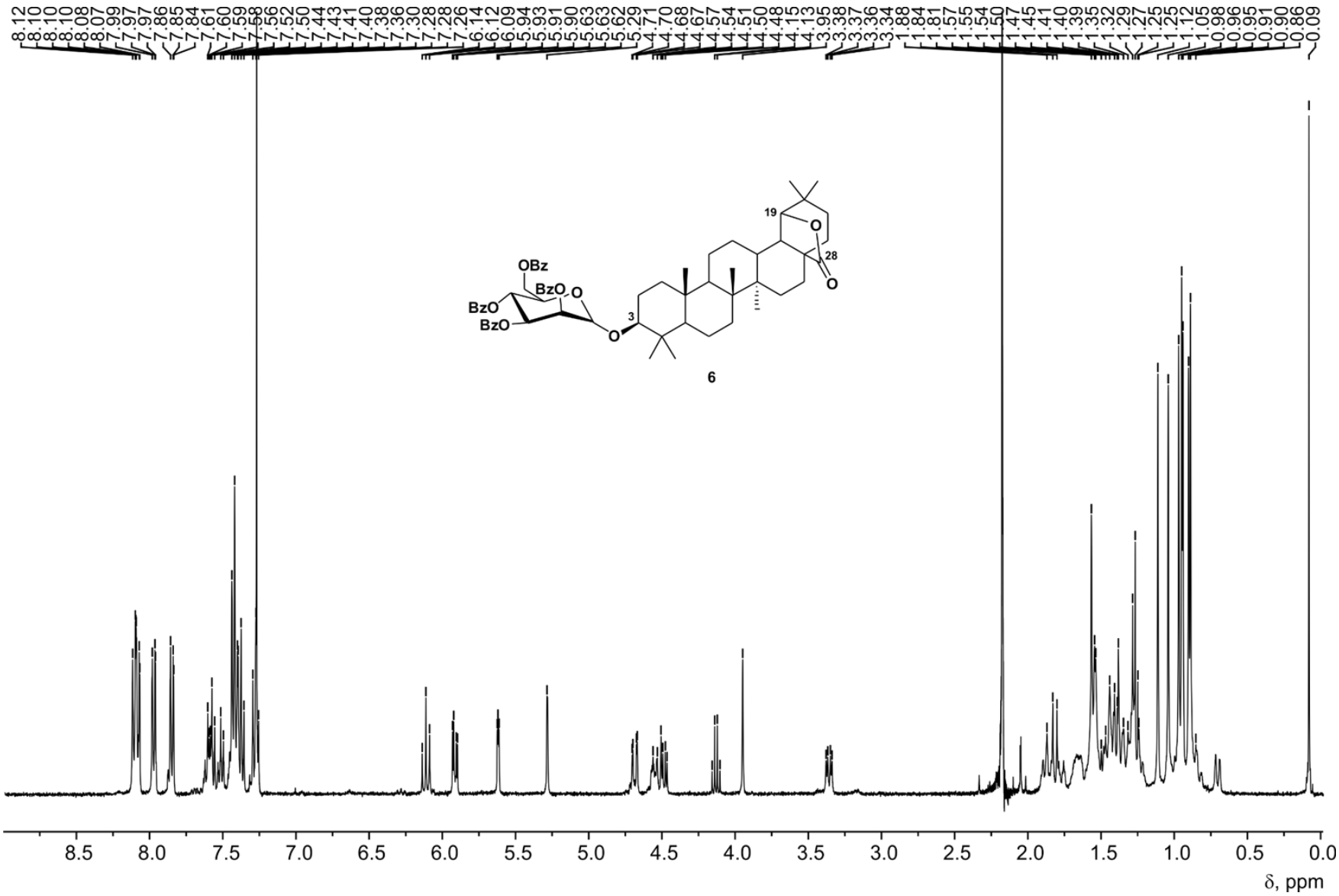

Figure S10. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **6.**

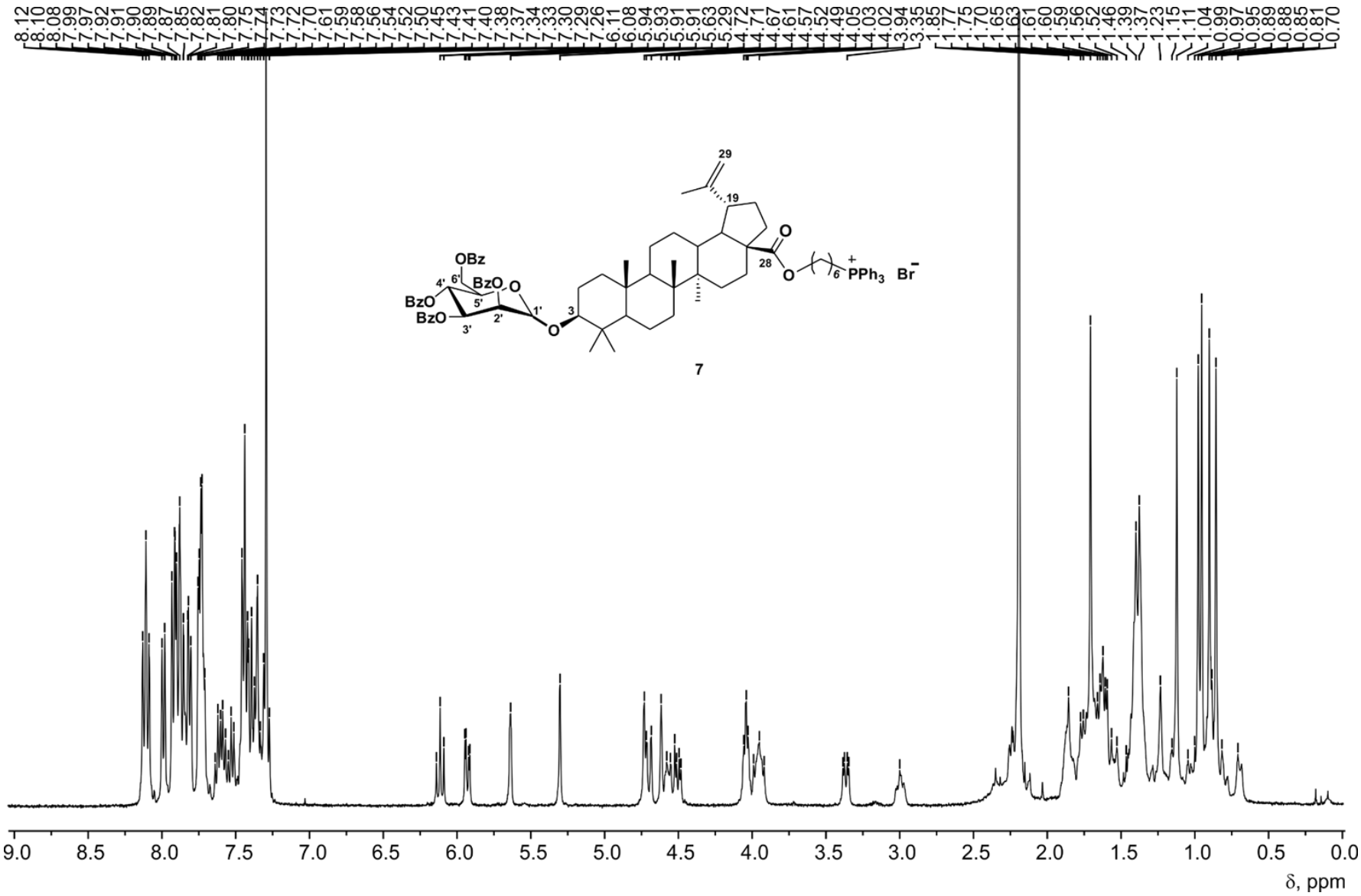

Figure S11. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of compound **7.**

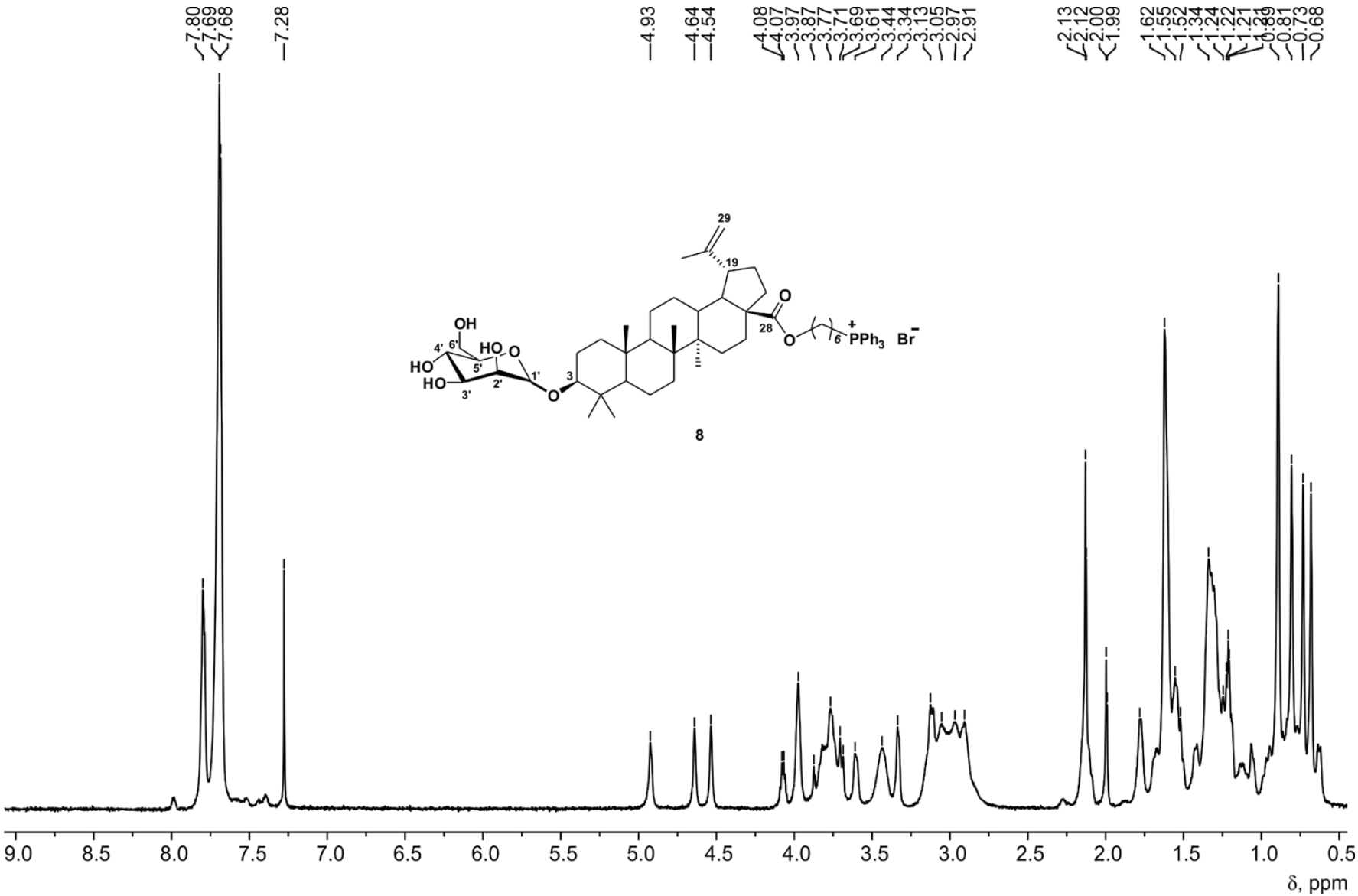

Figure S12. ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of phosphonium salt **8.**

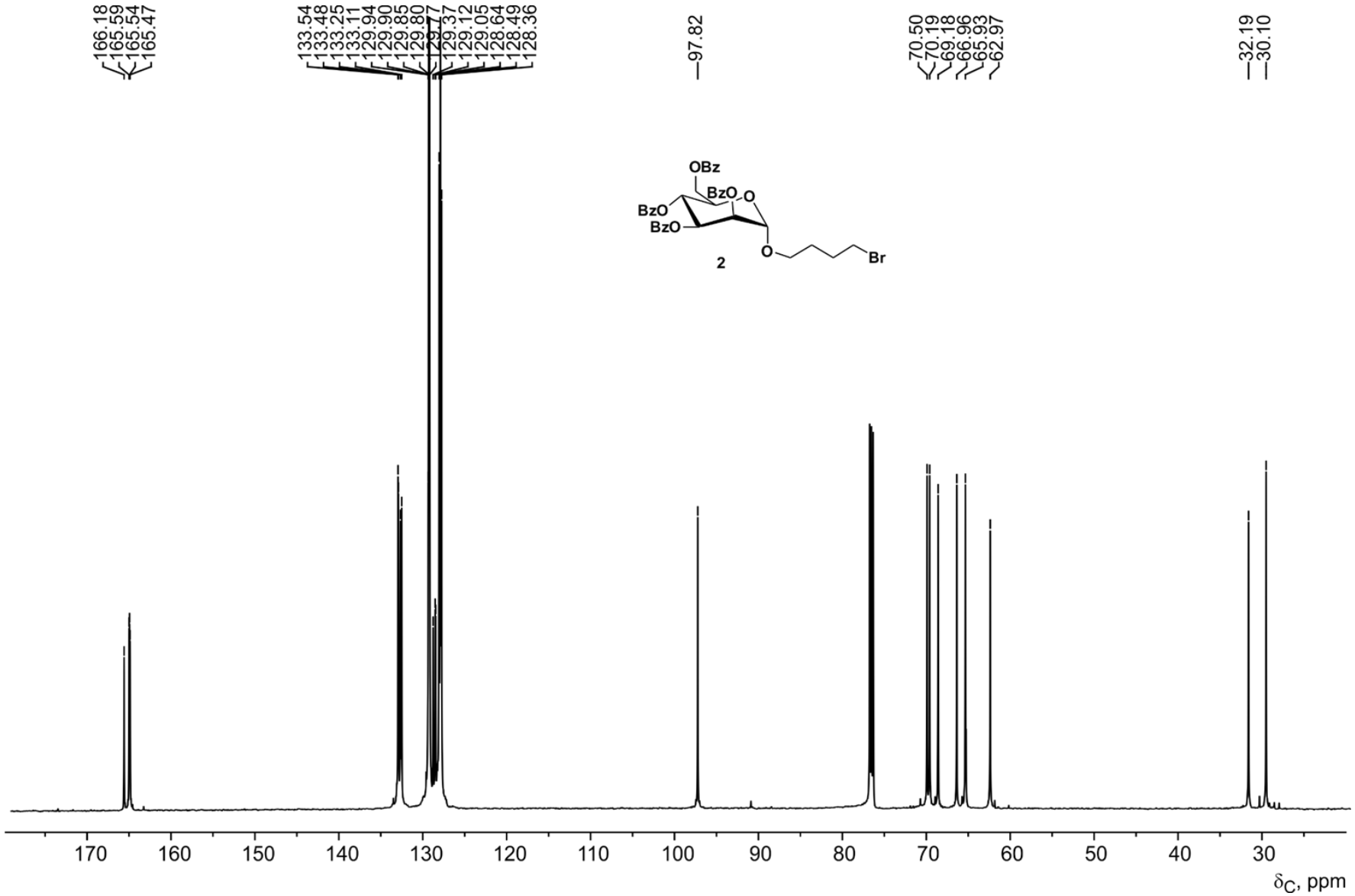

Figure S13. ^{13}C-{^{1}H} NMR ($CDCl_3$, 100.6 MHz) spectrum of compound **2.**

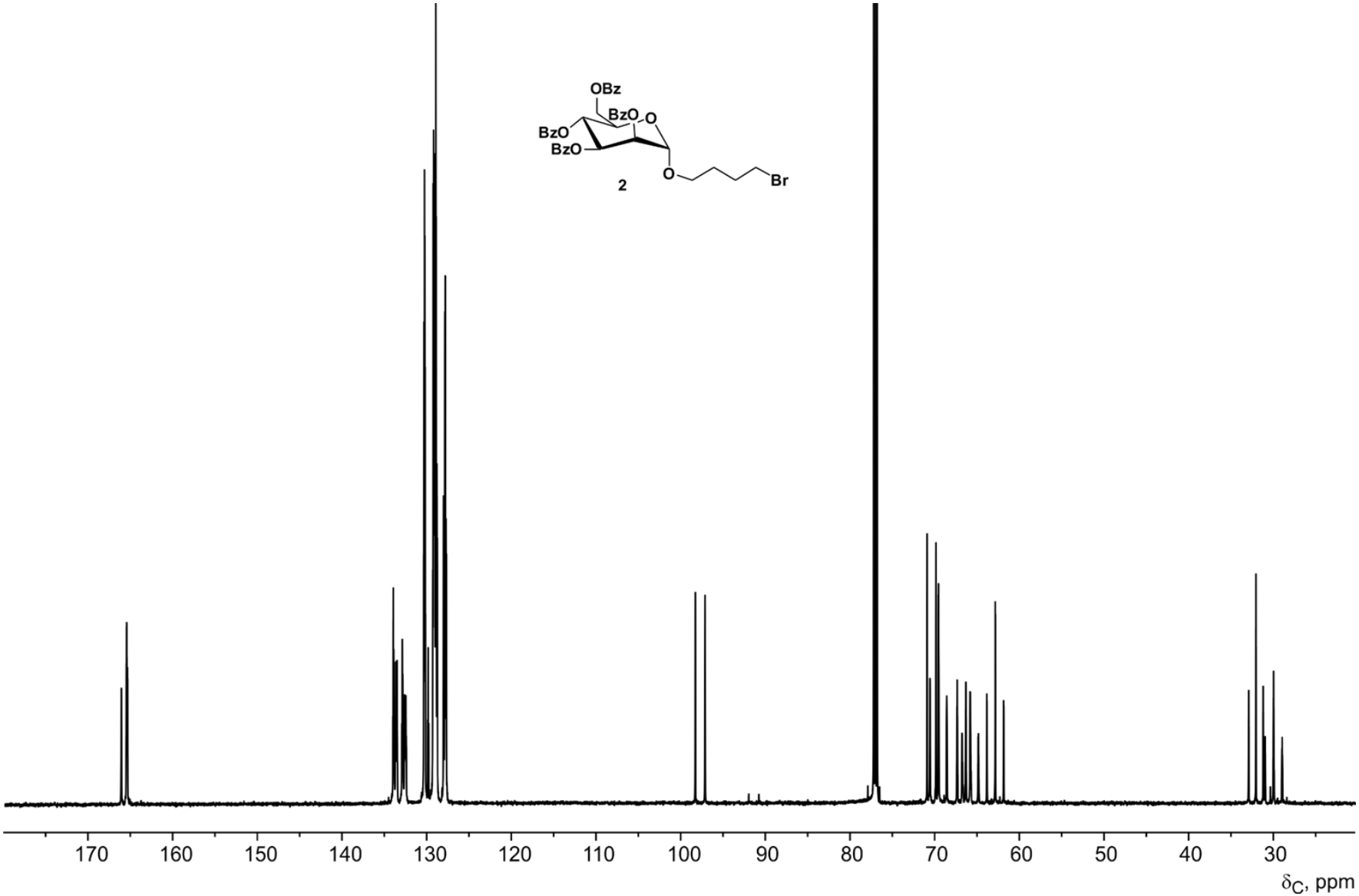

Figure S14. ^{13}C NMR ($CDCl_3$, 100.6 MHz) spectrum of compound **2.**

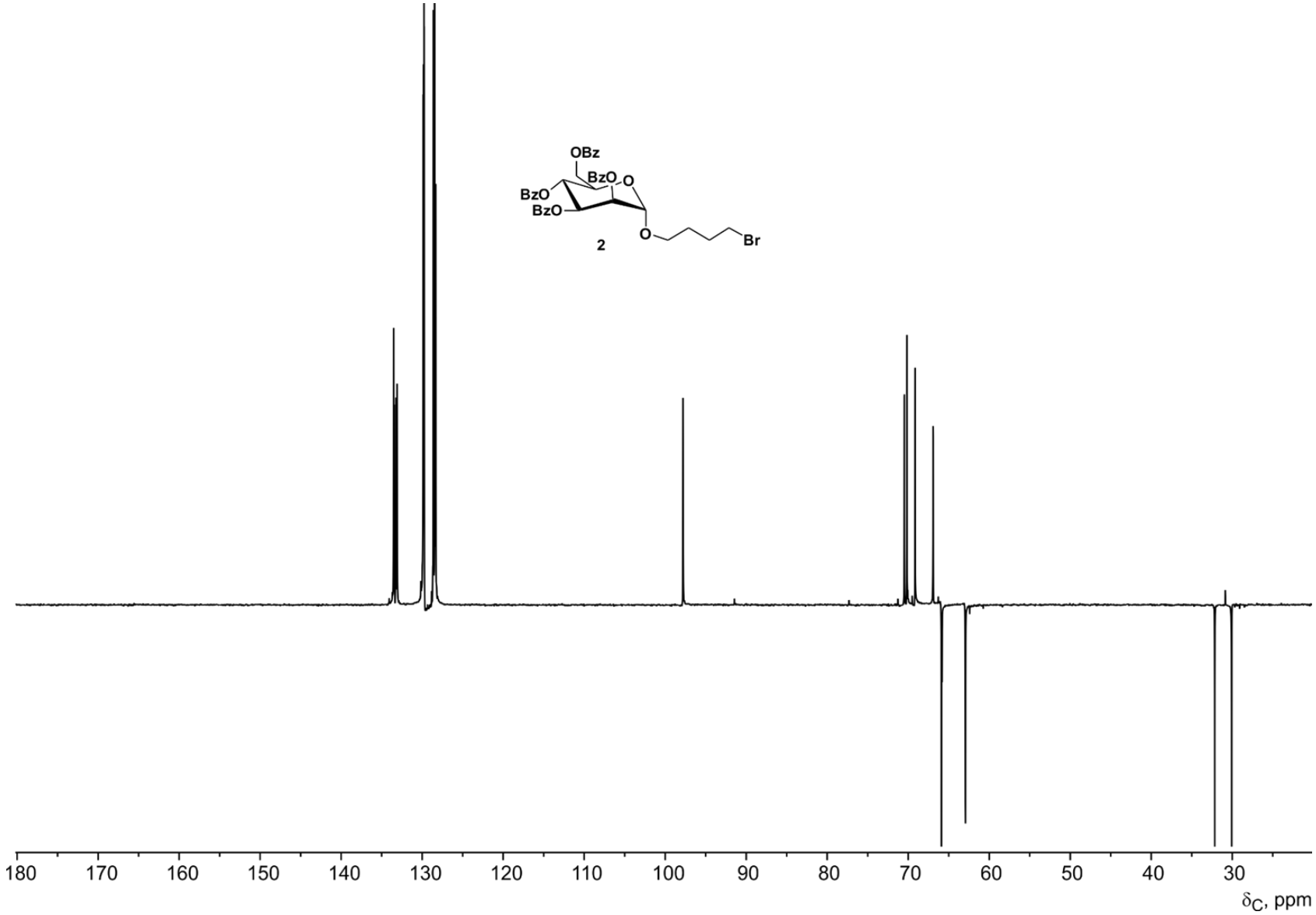

Figure S15. ^{13}C DEPT NMR ($CDCl_3$, 100.6 MHz) spectrum of compound **2.**

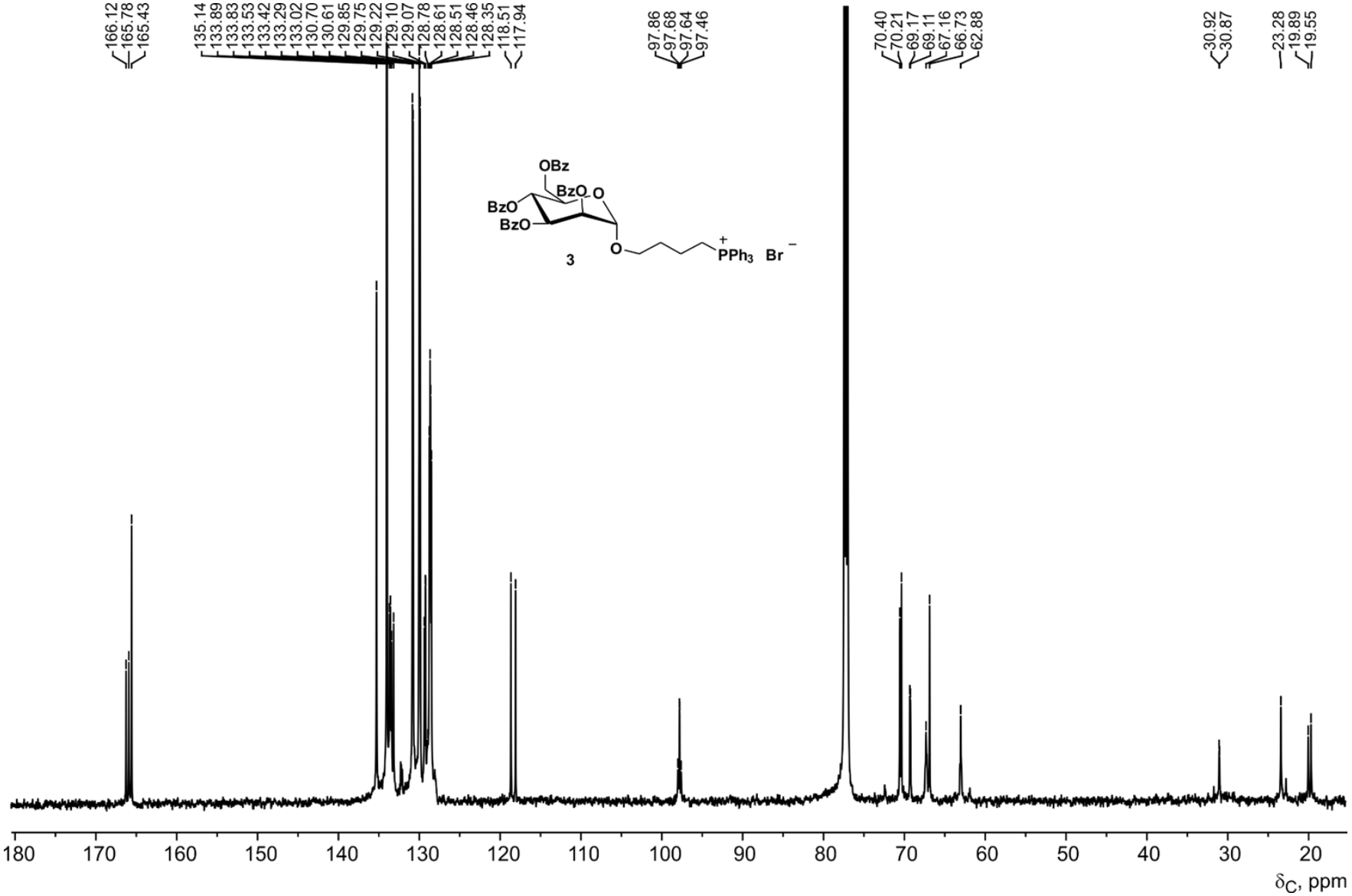

Figure S16. ^{13}C-{^{1}H} NMR ($CDCl_3$, 100.6 MHz) spectrum of phosphonium salt **3.**

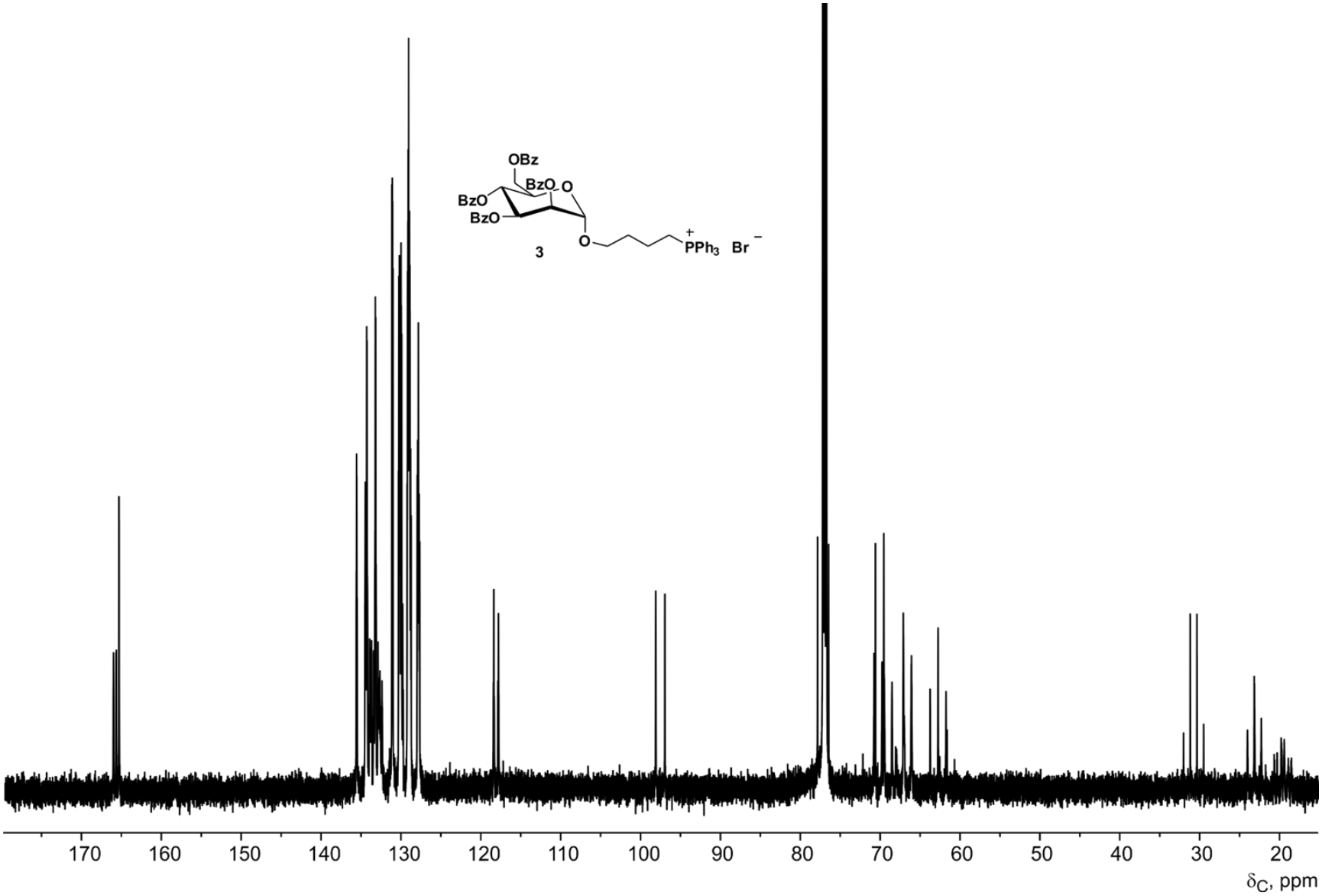

Figure S17. ^{13}C NMR ($CDCl_3$, 100.6 MHz) spectrum of phosphonium salt **3.**

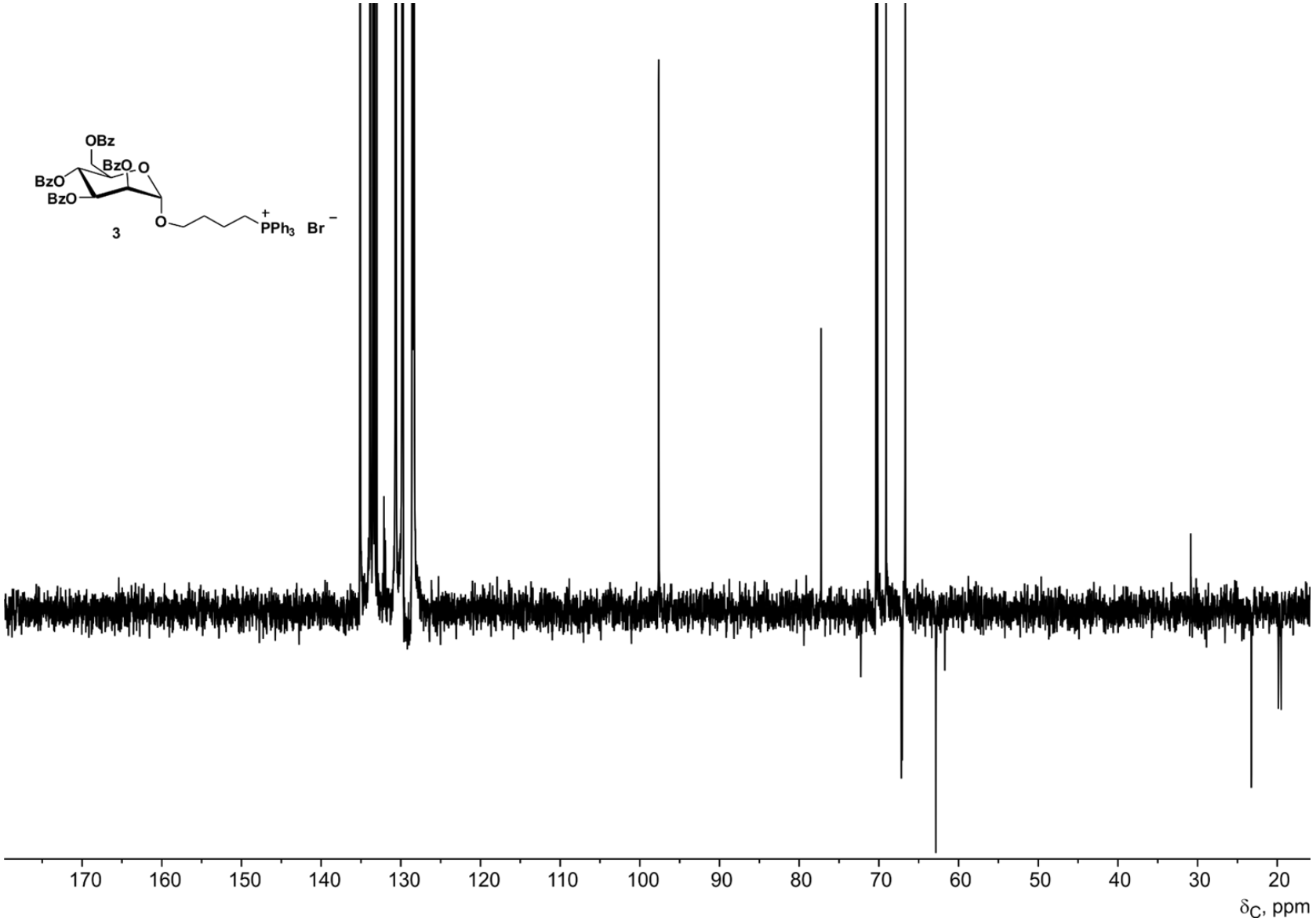

Figure S18. ^{13}C DEPT NMR ($CDCl_3$, 100.6 MHz) spectrum of phosphonium salt **3.**

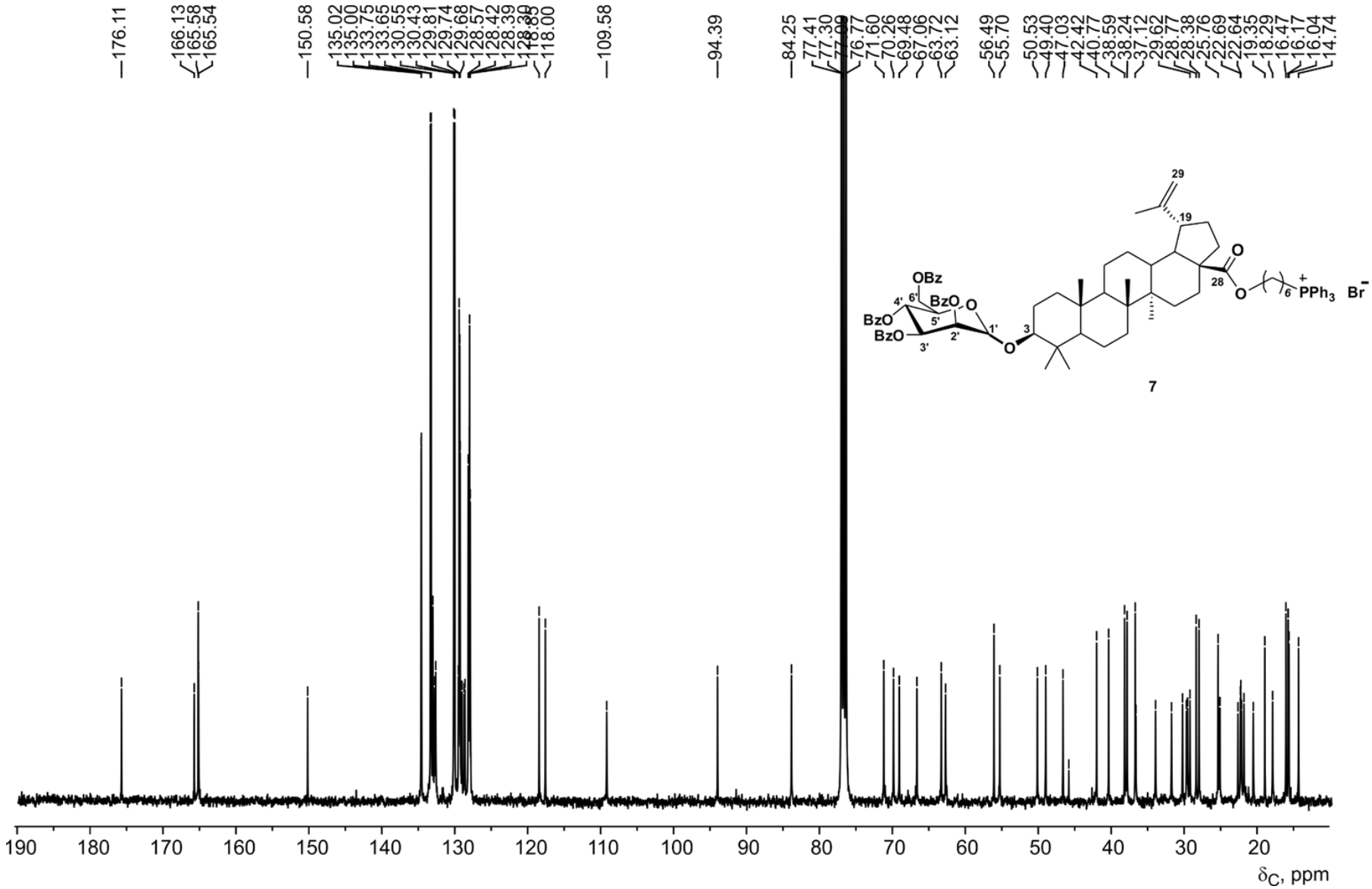

Figure S19. ^{13}C-{^{1}H} NMR ($CDCl_3$, 100.6 MHz) spectrum of phosphonium salt **7.**

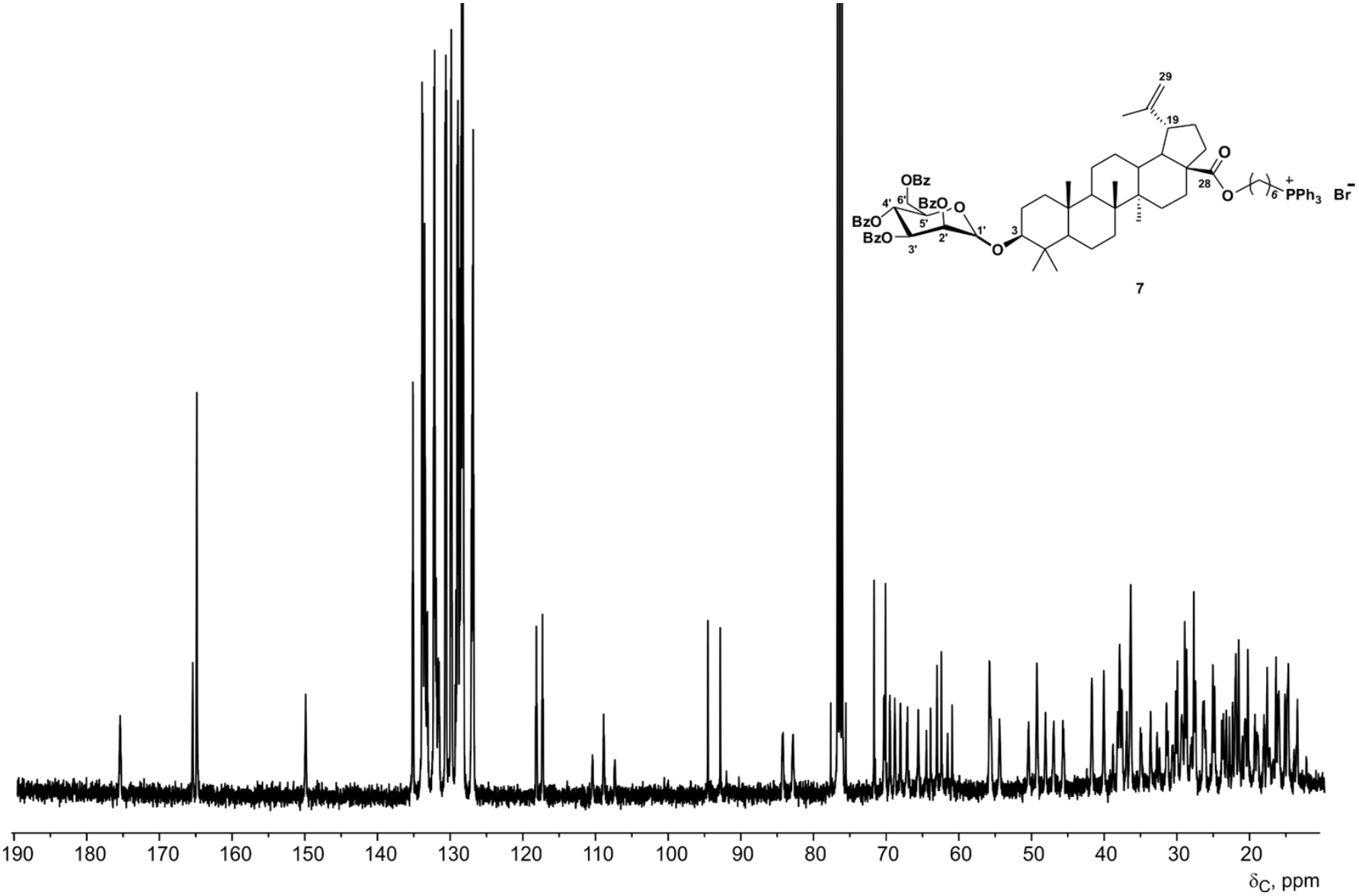

Figure S20. ^{13}C NMR ($CDCl_3$, 100.6 MHz) spectrum of phosphonium salt **7.**

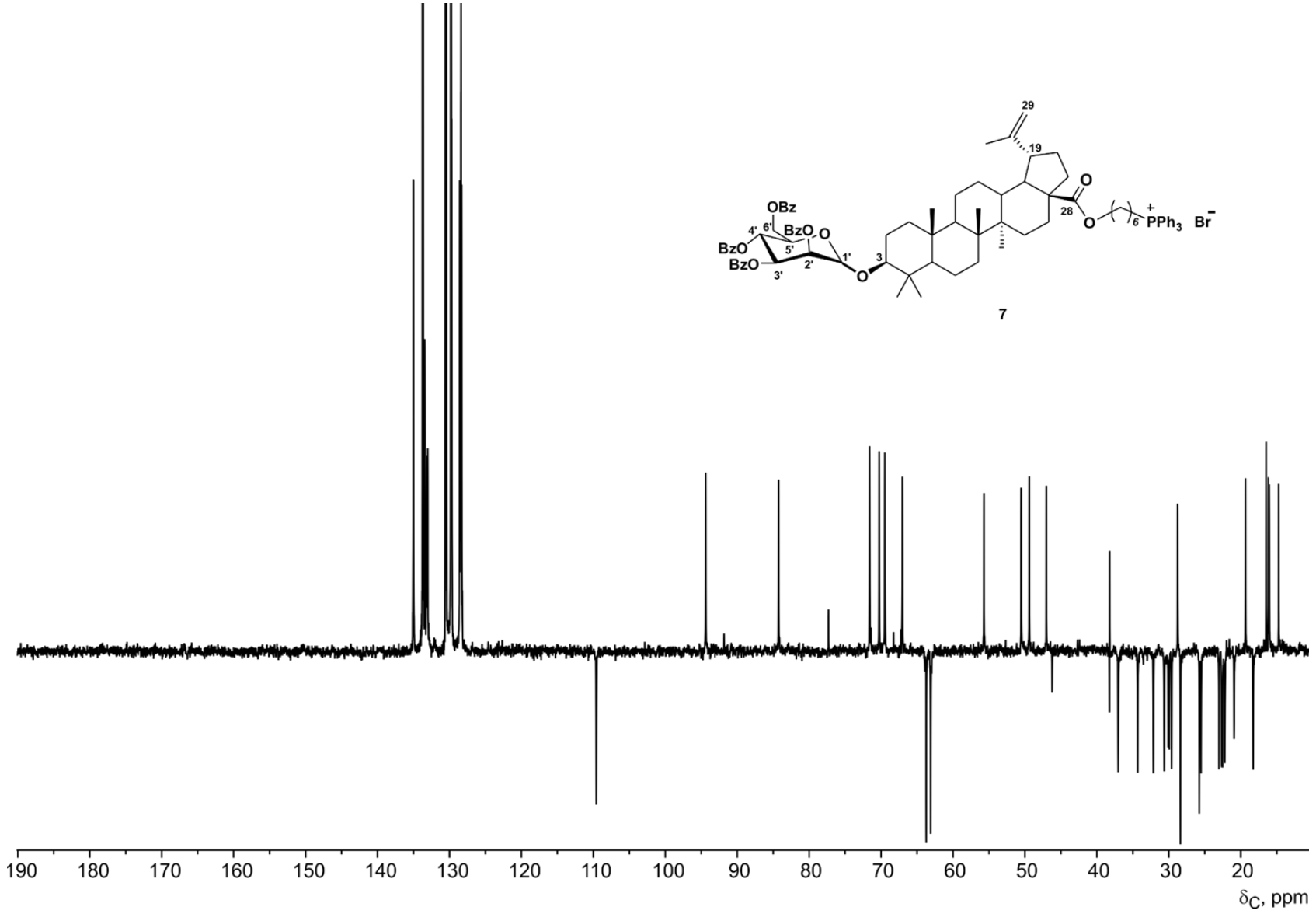

Figure S21. ^{13}C DEPT NMR ($CDCl_3$, 100.6 MHz) spectrum of phosphonium salt **7.**

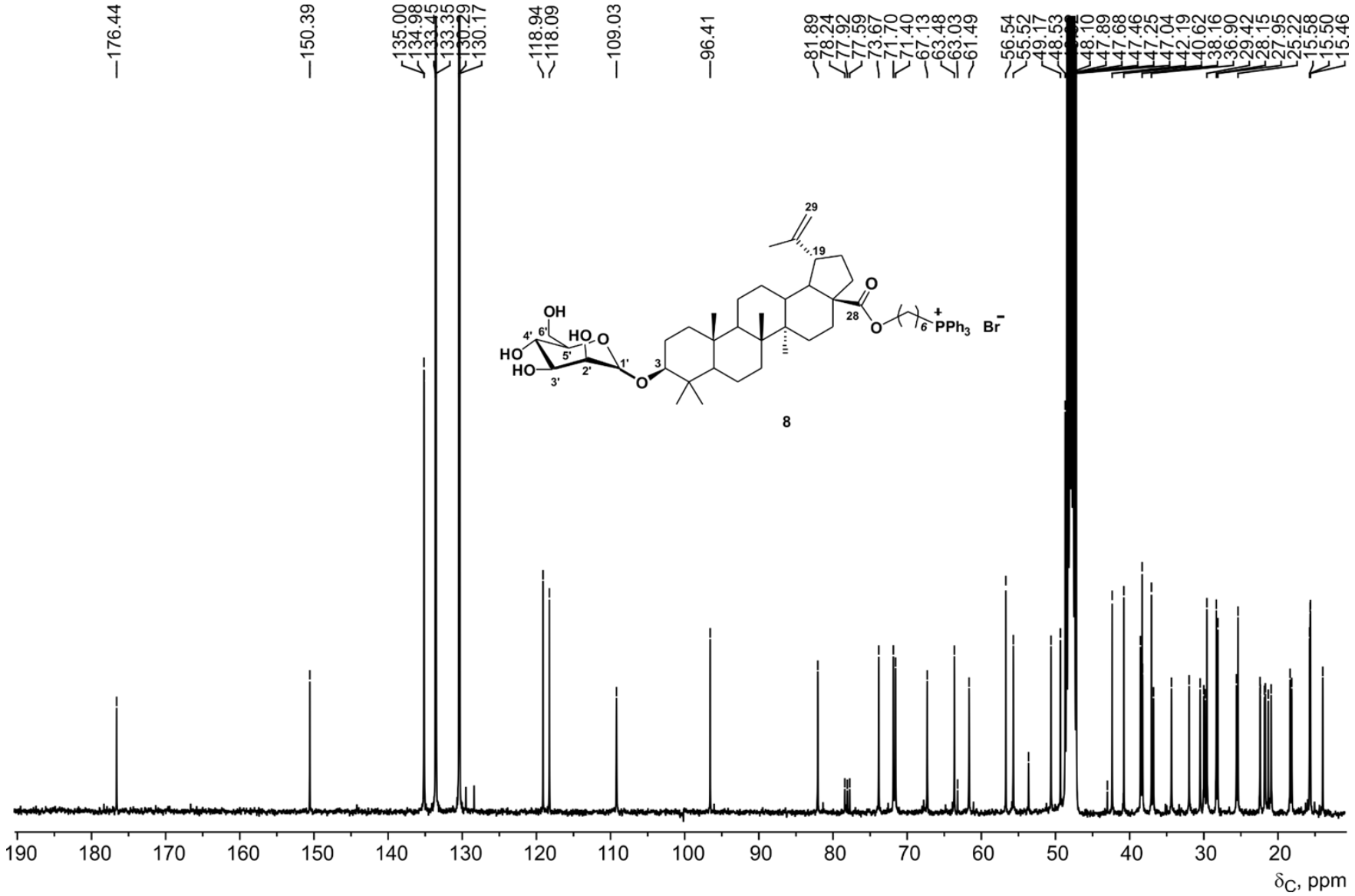

Figure S22. ^{13}C-{^{1}H} NMR (DMSO-d_6, 100.6 MHz) spectrum of phosphonium salt **8.**

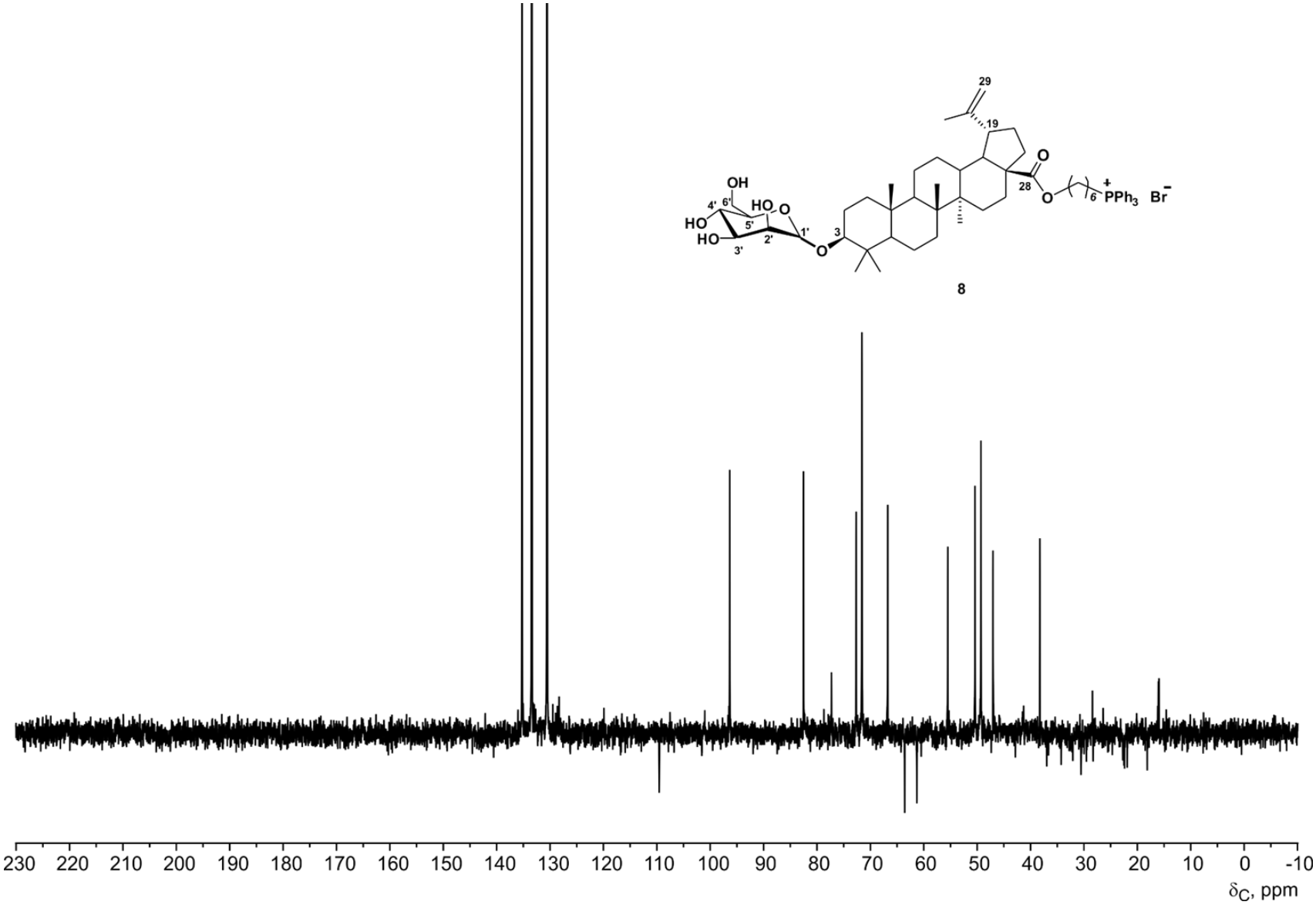

Figure S23. ^{13}C DEPT NMR (DMSO-d_6, 100.6 MHz) spectrum of phosphonium salt **8.**

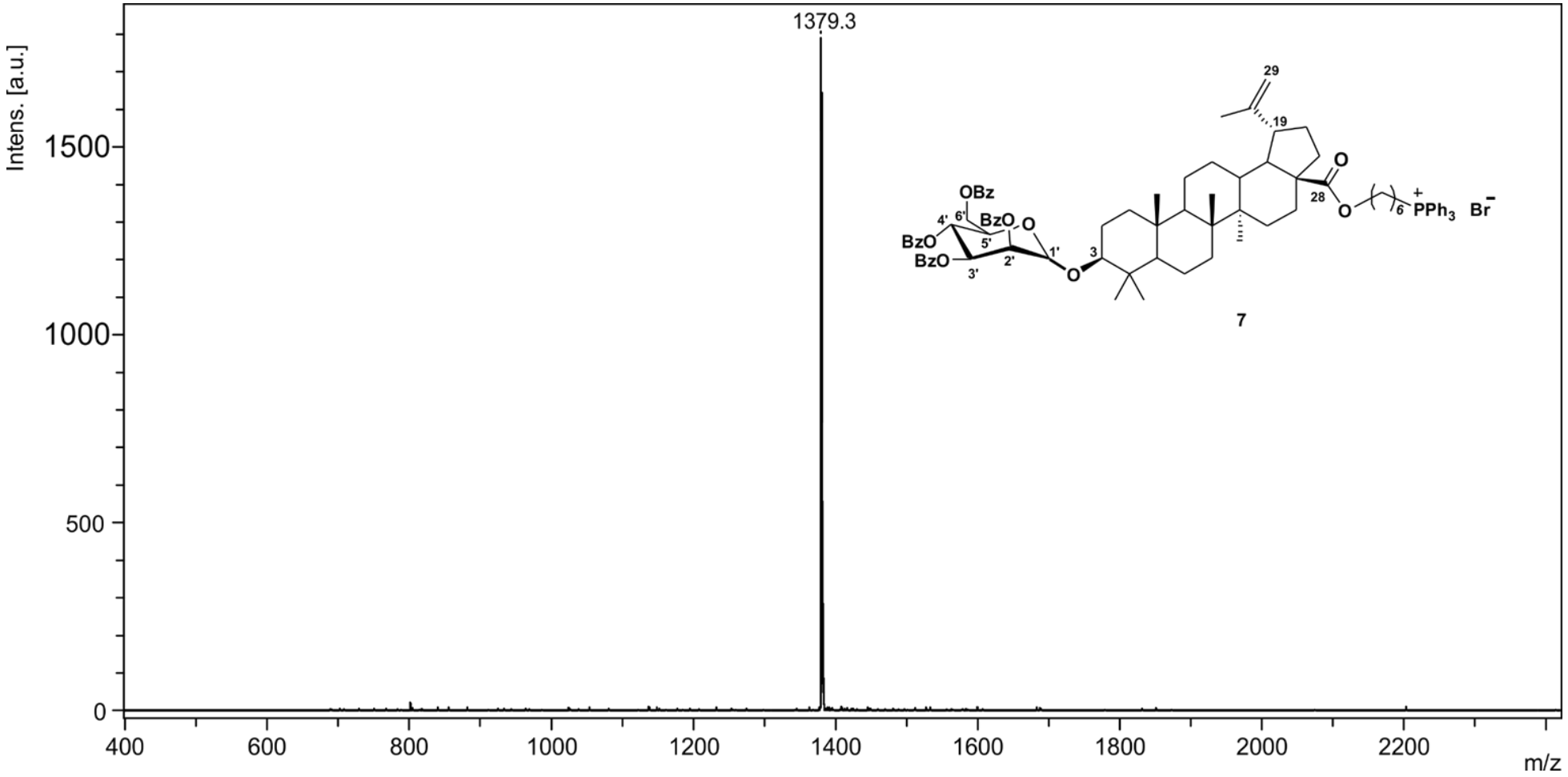

Figure S24. MALDI MS spectrum of phosphonium salt **7.**

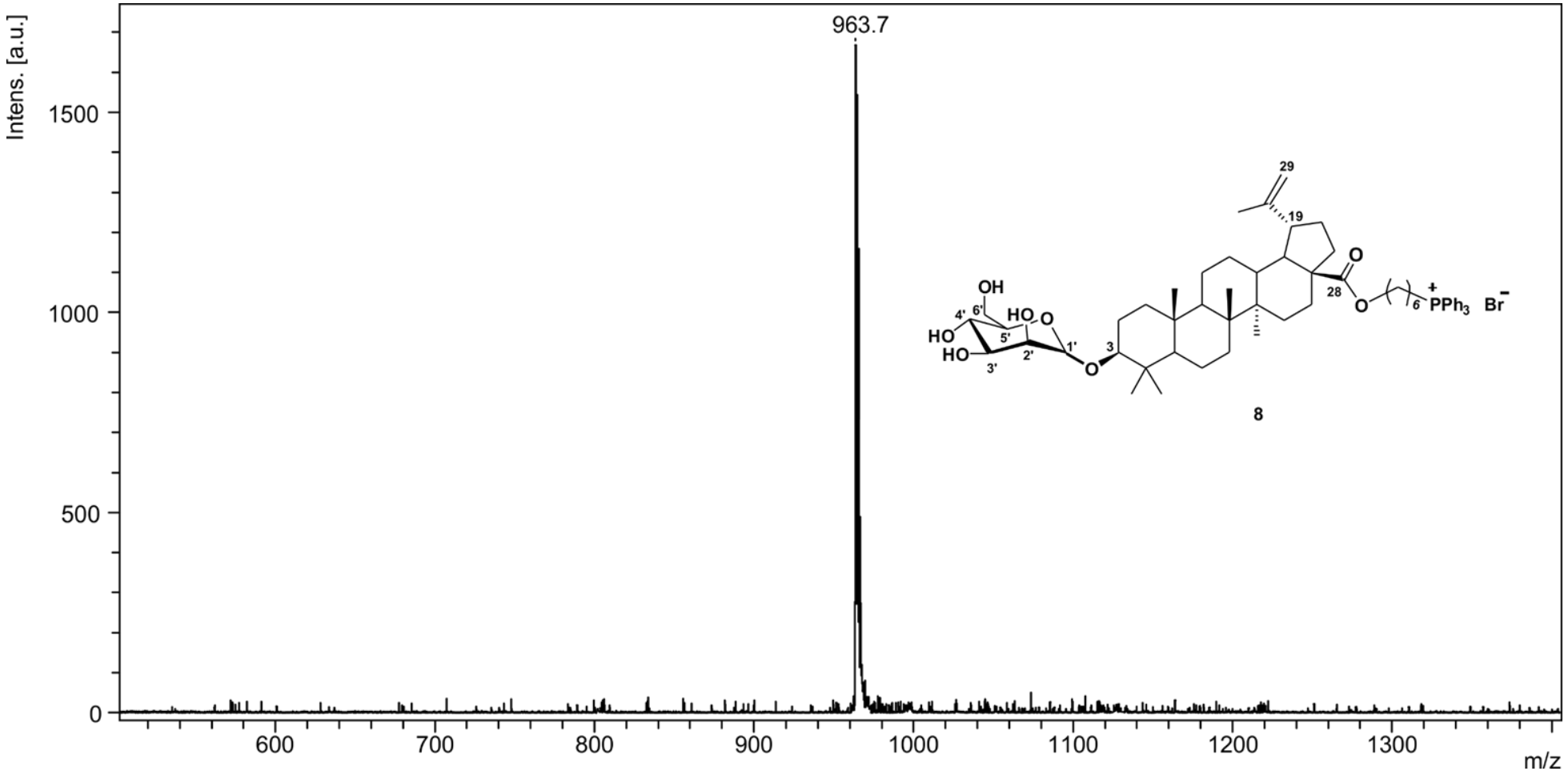

Figure S25. MALDI MS spectrum of phosphonium salt **8.**