Supporting Information

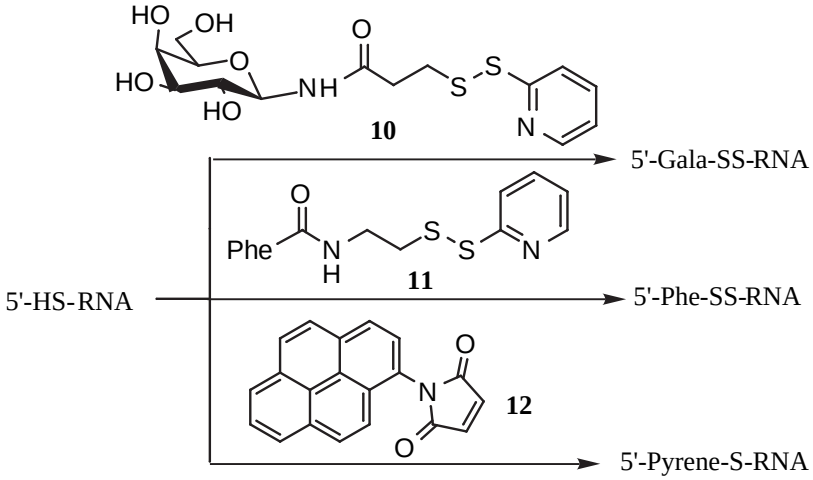
General procedure. All materials were obtained from commercial sources (Aldrich, Sigma, ACROS, Fisher, and VWR) and used without additional purification, unless otherwise noted. Solvents were all distilled before use. Methylene chloride, acetonitrile, and pyridine were dried by refluxing with CaH_2. 1H NMR spectra were carried out on Varian 300 MHz and 400 MHz spectrometers. Internal references used are TMS for ^{1}H and ^{13}C, and 85% H_3PO_4 for ^{31}P.

Synthesis of 2', 3'-Isopropylideneguanosine 2. To a suspension of guanosine (10 g, 35.31 mmol) in 600 ml of acetone was added 70% perchloric acid (4.1 ml, 47.54 mmol). After 70 minutes, concentrated ammonium hydroxide (6.7 ml, 49.79 mmol) was added to the reaction mixture and cooled down with ice-water bath. The solid was filtered out and dried over vacuum, 9.5 g (83.2%). ^{1}H-NMR (400MHz, DMSO-d6): δ10.67 (b, 1H, NH), 7.89 (s, 1H), 6.50 (b, 2H, NH_2), 5.90 (d, J=2.7Hz, 1H), 5.17 (dd, J=6.2Hz), 5.03 (t, J=5.1Hz, 1H, OH), 4.94 (dd, 1H), 4.09 (ddd, 1H), 3.50 (m, 2H), 1.49 (s, 3H, CH_3), 1.29(s, 3H, CH_3).

Synthesis of 5'-Deoxy-5'-Iodo-2',3'-isopropylideneguanosine 3. Methyltriphenoxyphosphonium iodide (0.86 g, 1.91 mmol) was added to a cooled (-78°C) suspension of 2',3'-O-isopropylideneguanosine (0.41 g, 1.27 mmol) in tetrahydrofuran (20 ml). The mixture was allowed to warm to room temperature after 10 minutes. After 4h the excess methyltriphenoxyphosphonium iodide was destroyed by addition of 1 ml of methanol and the solvent was removed by reduced pressure. The residue was suspended in a mixture of ethyl ether and hexane (1:1) and the solid was filtered and washed thoroughly by the mixture of ethyl ether and hexane. The crude product was purified by flash chromatography (gradient of methanol/chloroform). 0.34 g (61.8%) of the titled product was obtained. R_f=0.53 (chloroform/methanol = 4:1), ^{1}H-NMR (300MHz, DMSO-d6): δ7.88 (s, 1H), 6.55 (b, 2H, NH_2), 6.01(d, 1H), 5.30(dd, 1H), 5.04(dd, 1H), 4.25(ddd, 1H), 3.35(m, 2H), 1.50(s, 3H), 1.31(s, 1H).

Synthesis of 5'-Deoxy-5'-thioguanosine-5'-monophosphorothioate 4. A suspension of 5'-deoxy-5'-iodo-2',3'-isopropylidene guanosine (2.88 g, 6.65 mmol) in 50% aqueous formic acid (100ml) was stirred for 2.5 days and then evaporated. The crude product (2.83 g) was without further purification used in the next reaction. R_f = 0.78 (i-propyl alcohol:NH_3:H_2O = 6:3:1). To a suspension of 5'-deoxy-5'-iodoguanosine (2.83 g, 7.2 mmol) in 140 ml of water added trisodium thiophosphate (4.8 g, 26 mmol). The reaction mixture was stirred for 3 days at room temperature under argon atmosphere. After filtration to remove any precipitate, the filtrate was evaporated under reduced pressure. The residue was dissolved in 100 ml of water and precipitated by addition of 200 ml of methanol. After removing the precipitate by filtration, filtrate was evaporated and dissolved in small amount of water and applied to reverse phase chromatography. The desired product was collected and dried by lyophilizer (1.9 g, 68% for two steps). R_f = 0.36 (isopropylalcohol:NH_3:H_2O = 6:3:1). ^{1}H NMR (400MHz, DMSO-d6+D_2O): δ7.82 (s, 1H), 5.63 (d, J=, 5.9Hz, 1H), 4.28 (dd, J=3.9Hz 1H), 4.08 (ddd, 2H), 2.83 (m, 2H). ^{31}P NMR (D_2O): δ 16.4. Mass spectrum ESI: calculated 379, found 378 (negative ion).

Scheme 4. Reactions of 5'-HS-RNA with thiol-reactive reagents



zyc-I-104

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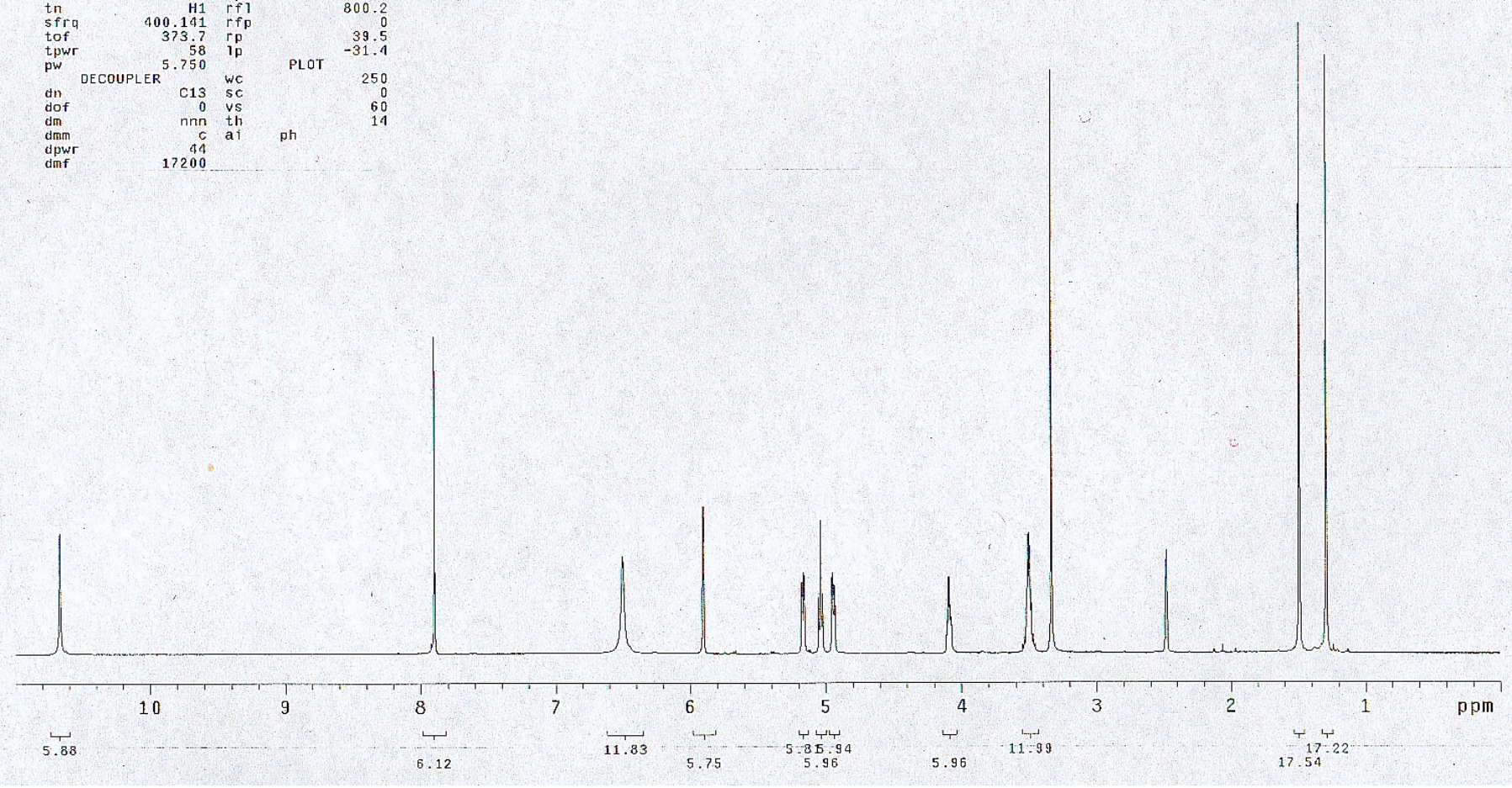
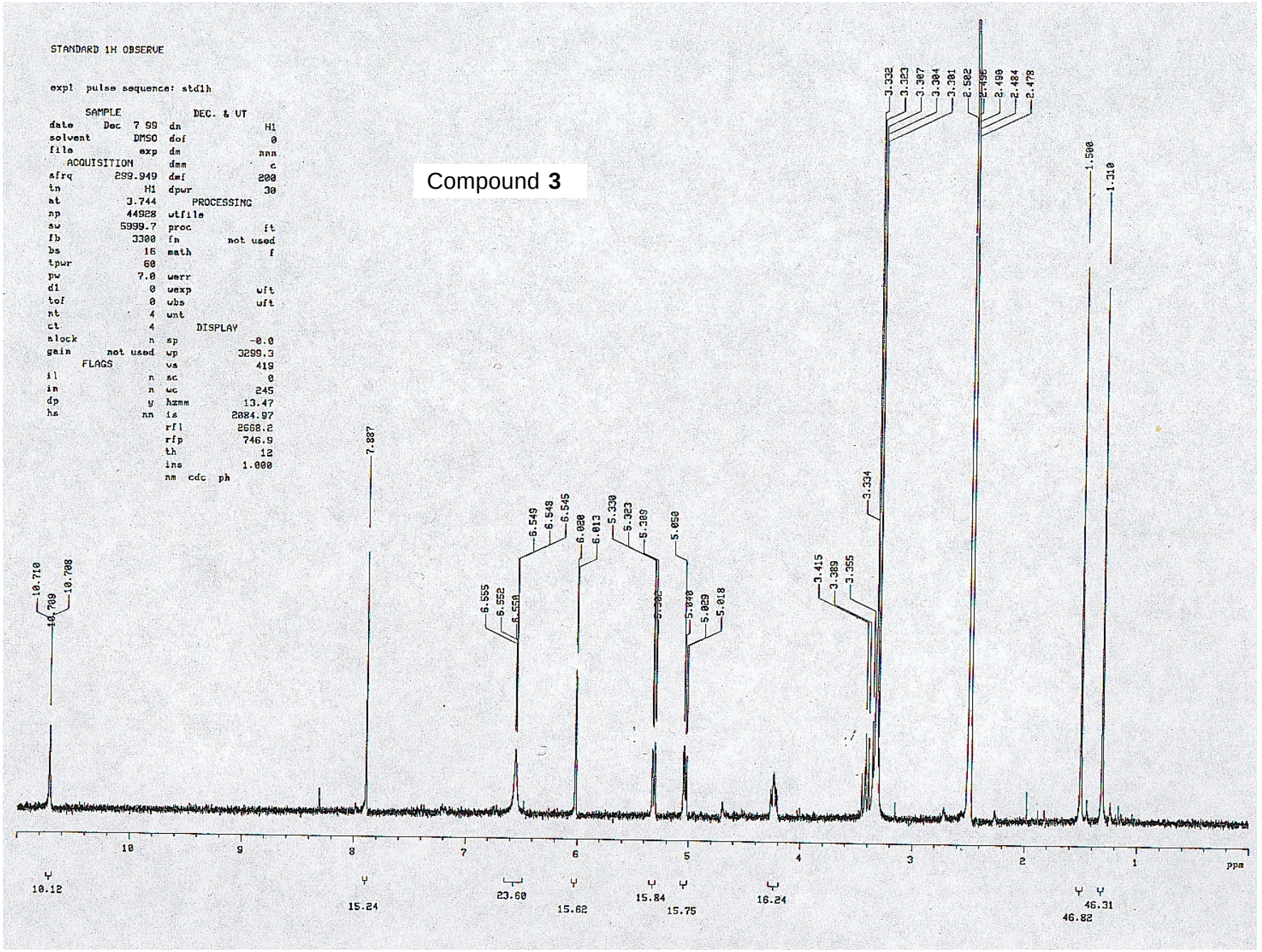
Compound **2**

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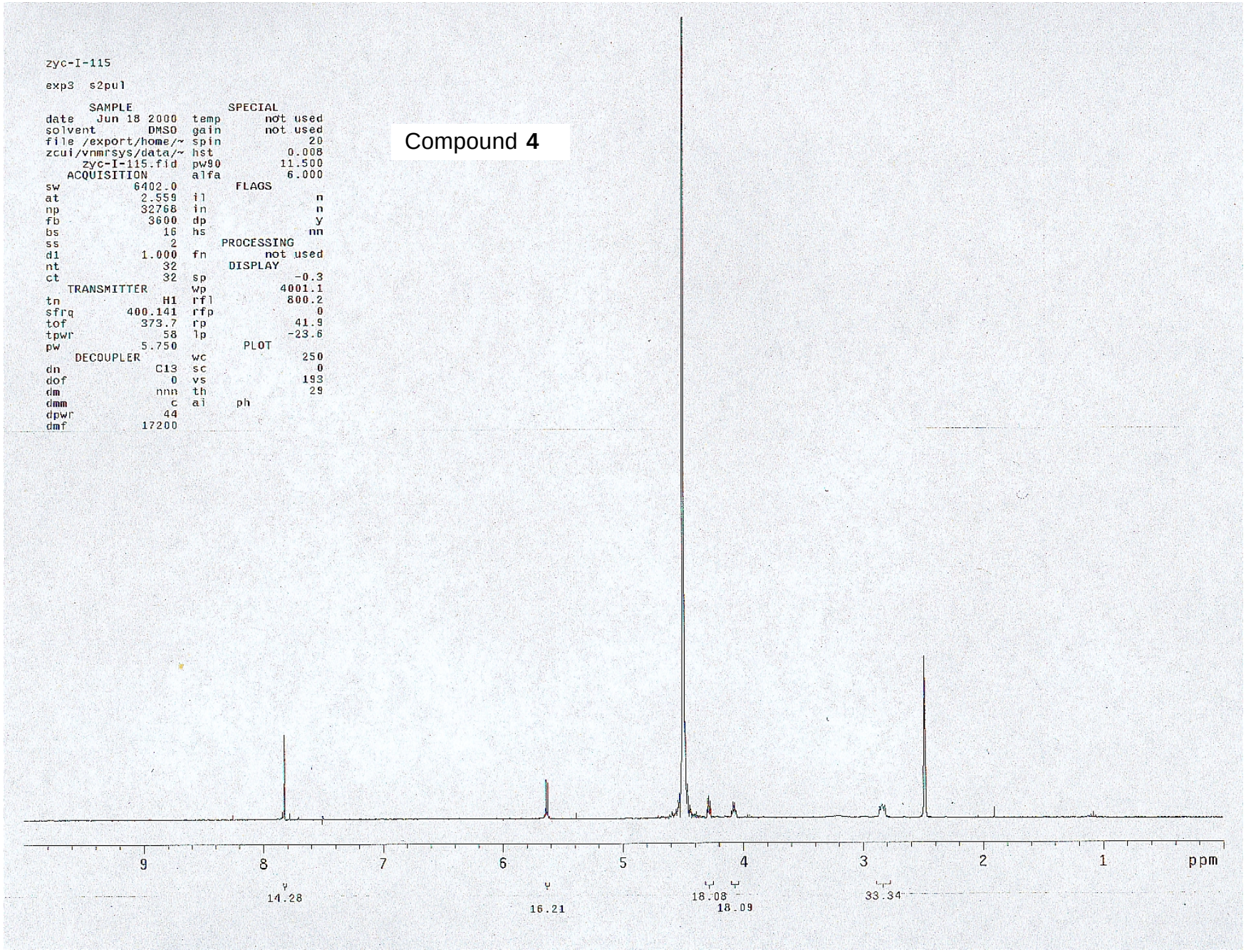
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