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September 2014: The paragraphs above replace the section "Handling and Disposal of Hazardous Chemicals" in the originally published version of this article. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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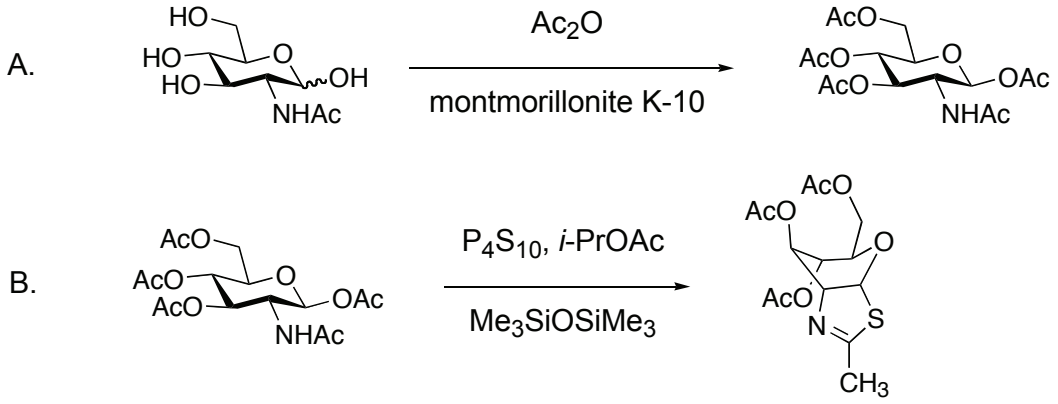
THIONATION: GlcNAc-THIAZOLINE TRIACETATE {(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-ACETOXYMETHYL-6,7-DIACETOXY-2-METHYL-5,6,7,7a-TETRAHYDRO-3a*H*-PYRANO[3,2-*d*]THIAZOLE}



Submitted by Spencer Knapp, Richard A. Huhn, and Benjamin Amorelli.[1]
Checked by Erikah E. Englund and Peter Wipf.[2]

1. Procedure

Caution! The following operations produce lachrymatory and corrosive vapors and should be carried out in a well-ventilated fume hood.

A. 2-Acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose. A dry, 2000-mL, three-necked, round-bottomed flask is equipped with a Teflon-coated magnetic stir bar (41 × 19 mm) and an ice-salt bath, and at the respective necks with a thermometer, a septum, and a rubber stopper. A nitrogen atmosphere is incorporated by a needle inserted through the septum. The stopper is temporarily removed, replaced with a funnel and acetic anhydride (900 mL, 9.52 mol, Note 1) is added from a bottle that had been stored in a refrigerator at 5 °C. The stopper is replaced and the acetic anhydride is cooled to 0 °C (internal temperature) with stirring, the stopper is again replaced by a funnel, and then 80.0 g (362 mmol) of *N*-acetyl-D-glucosamine and 270 g of montmorillonite K-10 (Note 1) are sequentially added over a 10-min period such that the internal temperature does not rise above ambient. The stopper is replaced, the ice bath is removed, and the suspension is stirred for 24 h.

The reaction mixture is filtered through a medium porosity sintered

glass funnel (600 mL capacity) pre-coated with a pad of 20 g of Celite moistened with methyl acetate (Note 1). The flask and filtered solids are washed with methyl acetate, about 1 L total, and then the combined filtrate is concentrated on a rotary evaporator (40 °C, 5 mmHg). The resulting orange residue is dislodged with a spatula, and then dissolved in 200 mL of hot methanol. The methanolic solution is placed in a 5 °C explosion proof refrigerator and a precipitate forms over 24 h. The solid is collected by filtration and washed with 100 mL of diethyl ether, then dried (23 °C, 1 mm) to afford 46.3 g (33%) of product as a white solid (mp 179–180 °C) (Notes 2 and 3).

B. GlcNAc-thiazoline triacetate. A 1000-mL, two-necked, round-bottomed flask under a nitrogen atmosphere immersed in an oil bath is fitted with Teflon-coated magnetic stir bar (41 × 19 mm), a rubber septum and a reflux condenser capped with a septum, through which nitrogen is affixed by use of a needle. The septum on the flask is temporarily removed, a funnel inserted and the vessel is charged with 300 mL of isopropyl acetate and 18.3 g (40 mmol) of phosphorus pentasulfide (P_4S_{10}). The resulting suspension is stirred rapidly while 76.3 mL (360 mmol) of hexamethyldisiloxane (Note 4) is added via syringe over a 5-min period. The mixture is heated at reflux for 10 min while vigorous stirring is maintained. The solution is allowed to cool to just below reflux, the septum on the flask is replaced with a funnel and 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (40.0 g, 103 mmol) is added in four portions with continuous stirring over a 5-min period. The septum is replaced and the reaction mixture is then brought back to reflux, and held at that temperature for 2–3 h, during which period it becomes homogeneous and orange. TLC analysis (3:1, dichloromethane / ethyl acetate) indicates complete conversion to product (R_f = 0.40 on silica).

The reaction mixture is allowed to cool to room temperature and then stirred for an additional 12 h (Note 5). The septum is removed and acetone (150 mL) is added. The flask is cooled to 0 °C in an ice-salt bath (Note 6), and then 52 mL of 5.3 M aqueous K_2CO_3 is added. The quenched reaction mixture is stirred at 0 °C for 30 min, then water and isopropyl acetate are used to transfer the reaction mixture to a 2-L separatory funnel (total 300 mL of water and 200 mL of isopropyl acetate). The organic layer is separated and washed with 0.53 M aqueous K_2CO_3 (3 × 200 mL or until the aqueous layer is colorless), and then dried over 30 g of anhydrous magnesium sulfate. Filtration followed by concentration on the rotary evaporator (23 °C, 5 mmHg) gives an orange oil, which is mixed with 50 mL of isopropyl acetate

and 55 g of silica, and then concentrated to give an orange powder. A plug of 170 g of silica moistened with isopropyl acetate is layered onto a coarse porosity sintered glass funnel (600 mL capacity). The orange powder is placed on the silica plug and tamped with a piece of filter paper, which is left in place. A mixture of heptanes / isopropyl acetate (70:30, 1.25 L, then 40:60, 125 mL) is used to wash through high R_f impurities, which are discarded as waste. Elution with 1.5 L of 40:60 heptanes / isopropyl acetate is followed by pure isopropyl acetate (~1 L) until the product is washed off the silica according to TLC analysis. Concentration of this latter solution on the rotary evaporator (23 °C, 5 mmHg) followed by a high vacuum (27 °C, 0.05 mmHg), until a constant weight is obtained, provides 31.1 g (88%) of GlcNAc-thiazoline triacetate as an orange oil (Note 7).

Notes

1. Acetic anhydride, *N*-acetyl-D-glucosamine, and montmorillonite K-10 were purchased from Aldrich Chemical Company, Inc., and are used as received. All solvents throughout this procedure were reagent grade and were used as received. The submitters report that a nitrogen atmosphere is not required for either Step A or Step B. Similar results are obtained in the absence of a nitrogen atmosphere.

2. Lit. mp 187 °C (ethanol),[3] 185–186 °C (water),[4] 186–186.5 °C (methanol/ether),[5] 188–189 °C (ethanol, repeated).[6]

3. The product was >99% pure according to ^{1}H NMR analysis. The α-anomer, when present, gives a signal in the ^{1}H NMR spectrum at δ 6.17 (d, J = 3.2 Hz, 1 H). The spectral properties of the β-pentaacetate are as follows: $[\alpha]_D^{20}$ 7.2 (*c* 1.0, $CHCl_3$); IR (KBr) cm^{-1} 3257, 3074, 1747, 1656, 1555, 1444, 1370, 1220, 1069; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.93 (s, 3 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.09 (s, 3 H), 2.12 (s, 3 H), 3.81 (ddd, J = 2.4, 4.5, 9.0 Hz, 1 H), 4.12 (dd, J = 2.1, 12.5 Hz, 1 H), 4.27 (dd, J = 4.6, 12.6 Hz, 1 H), 4.26–4.35 (m, 1 H), 5.09–5.17 (m, 2 H), 5.56 (d, J = 9.5 Hz, 1 H), 5.69 (d, J = 8.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 20.5, 20.6, 20.7, 20.8, 23.1, 53.1, 61.7, 67.8, 72.6, 72.9, 92.6, 169.2, 169.5, 170.1, 170.6, 171.2; MS *m*/*z* (relative intensity) 413 (13), 412 (100, $[M+Na]^+$), 353 (5), 352 (40), 330 (3); HRMS Calcd for $C_{16}H_{23}NO_{10}Na$ (M+Na) 412.1220, Found 412.1216; Anal. Calcd for $C_{16}H_{23}NO_{10}$: C, 49.36; H, 5.95. Found: C, 48.98; H, 5.79.

4. Phosphorus pentasulfide and hexamethyldisiloxane were purchased from Aldrich Chemical Company, Inc., and used as received. The

submitters utilized a drying tube as opposed to the nitrogen atmosphere and obtained similar results.

5. Cleaner reaction mixtures were obtained when the reaction mixture was allowed to rest for an additional period after reflux.

6. At quench temperatures higher than 0 °C, some hydrolysis of product during the carbonate treatment was observed by TLC.

7. The product was >95% pure according to ^{1}H NMR analysis. The spectral and analytical properties are as follows: $[\alpha]_D^{20}$ –28.3 (*c* 0.4, $CHCl_3$); IR (neat) cm^{-1} 3308, 2958, 1747, 1629, 1433, 1371, 1232, 1040; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.08 (s, 6 H), 2.13 (s, 3 H), 2.31 (d, *J* = 2.3 Hz, 3 H), 3.53 (dt, *J* = 9.5, 4.2 Hz, 1 H), 4.11 (app d, *J* = 4.1 Hz, 2 H), 4.44–4.48 (m, 1 H), 4.95 (dt, *J* = 9.5, 1.6 Hz, 1 H), 5.56 (dd, *J* = 1.7, 3.3 Hz, 1 H), 6.23 (d, *J* = 7.1 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 20.5, 20.6, 20.7, 20.8, 63.2, 68.3, 69.2, 70.5, 76.5, 88.6, 168.2, 169.2, 169.5, 170.5; MS *m*/*z* (relative intensity): 346 (9), 345 (23, $[M]^+$), 312 (15), 286 (29), 285 (24), 272 (25), 225 (25), 183 (26), 166 (44), 165 (100), 152 (53), 141 (60); HRMS Calcd for $C_{14}H_{19}NO_7S$ 345.0882; Found 345.0878; Anal. Calcd for $C_{14}H_{19}NO_7S$: C, 48.69; H, 5.55; N, 4.06; S, 9.29. Found: C, 48.44; H, 5.68; N, 4.00; S, 9.26.

Safety and Waste Disposal Information

All hazardous materials should be handled and disposed of in accordance with "Prudent Practices in the Laboratory"; National Academy Press; Washington, DC, 1995.

3. Discussion

The preparation of 2-acetamido-2-deoxy-tetra-*O*-acetyl-β-D-gluco-pyranose is based on the report by Bhaskar and Loganathan[7] that describes the use of montmorillonite K-10 clay as an acidic catalyst for per-*O*-acetylation of sugars. The conditions have been modified to facilitate scale-up and isolation of pure β-anomer. This product has most commonly been made from D-glucosamine by a four-step route involving the intermediacy of an *N*-(*p*-methoxybenzylidene) derivative,[3] and by acetylation with anhydrous zinc(II) chloride as promoter.[4] In our hands neither procedure proved as convenient on the 0.1 mole scale as the present one. The β-peracetate may also obtained commercially from Aldrich Chemical

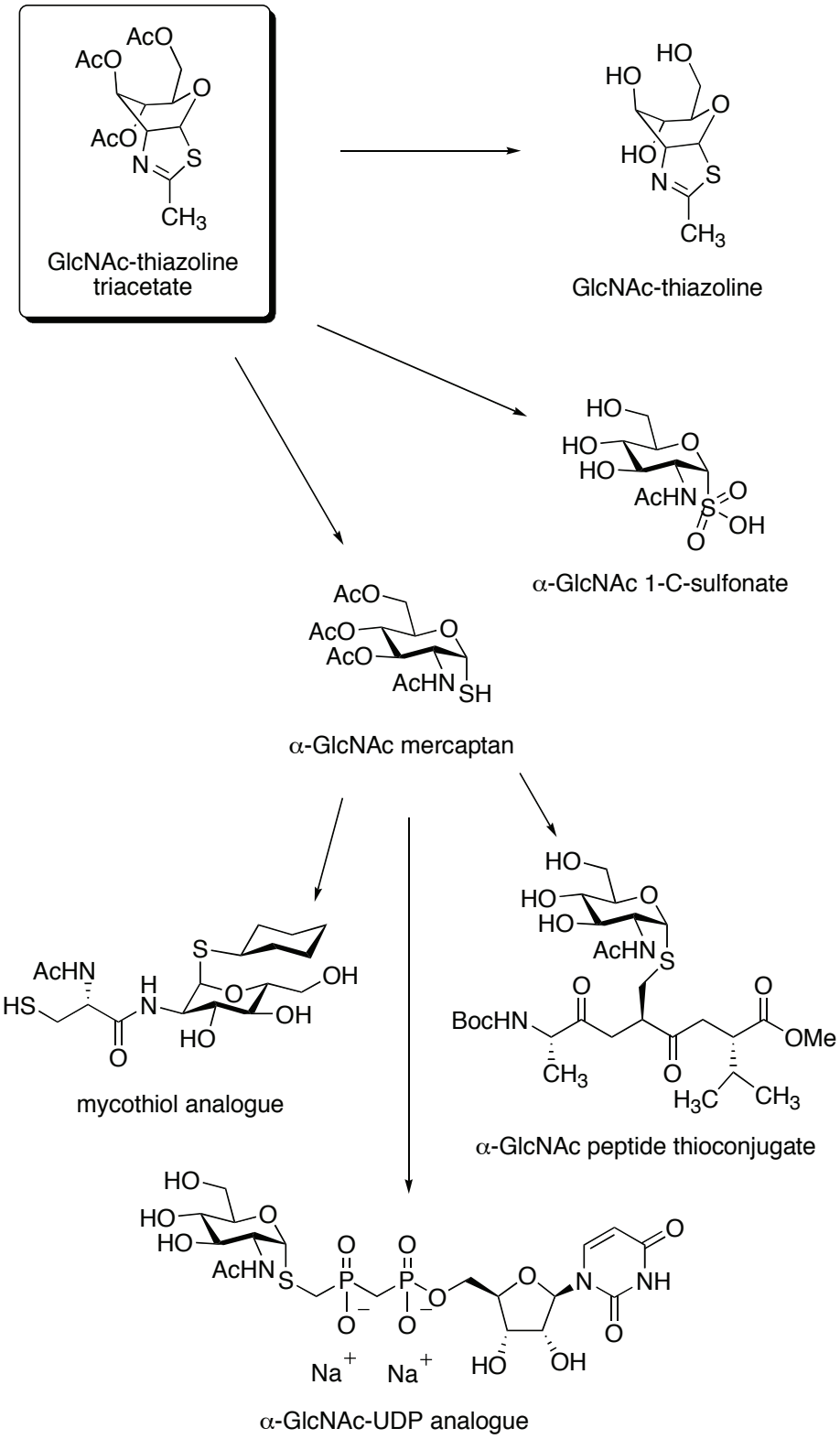
Company ($87 for 5 g in 2005).

GlcNAc-thiazoline triacetate may be synthesized from 2-acetamido-2-deoxy-tetra-*O*-acetyl-β-D-glucopyranose in quantitative yield on a 2 g scale by using freshly prepared Lawesson's reagent, 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide, followed by column chromatography.[8] The corresponding GalNAc-thiazoline triacetate has been synthesized analogously.[9] The thionation/cyclization reaction described herein is based on the P_4S_{10}/hexamethyldisiloxane procedure of Curphey,[10] which minimizes the formation of P/S byproducts that must be removed chromatographically. The amount of P_4S_{10} relative to starting material is increased to 0.4 equivalents in this procedure to accommodate the thionation of the byproduct, acetic acid, and to ensure that the reaction runs to completion.

GlcNAc-thiazoline triacetate has been hydrolyzed to the corresponding triol (Scheme 1), which is an effective inhibitor[11] and mechanistic/structural probe [12,13,14,15] of a family of 20 *N*-acetylhexosaminidases and related enzymes.[16,17,18] Alternatively, hydrolysis of the thiazoline ring gives the α-mercaptan stereoselectively,[8] and this has been used in turn to prepare α-GlcNAc thioconjugates,[8,19] including glycopeptide analogues,[20,21] mycothiol analogues,[22,23] and α-GlcNAc-UDP analogues.[24] Oxidation of the thiazoline in the presence of ethanol leads to the α-GlcNAc 1-C-sulfinate and 1-C-sulfonate *O*-ethyl esters.[25]

The thiazoline has been modified on the methyl group by buffer- or acylation-induced imine-to-enamine conversion and then electrophile or radical addition. Several of the methyl-functionalized GlcNAc-thiazolines show highly selective inhibition of human *O*-GlcNAcase.[26] The α-mercaptan has been converted to an α-GlcNAc thiolsulfonate, which shows distinctive inhibition of the short isoform, and irreversible inhibition of the long isoform, of human *O*-GlcNAcase.[27]

Scheme 1


GlcNAc-thiazoline triacetate
GlcNAc-thiazoline
α-GlcNAc 1-C-sulfonate
α-GlcNAc mercaptan
mycothiol analogue
α-GlcNAc peptide thioconjugate
α-GlcNAc-UDP analogue

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Appendix
Chemical Abstracts Nomenclature; (Registry Number)

Acetic anhydride; (108-24-7)

N-Acetyl-D-glucosamine: D-Glucose, 2-(acetylamino)-2-deoxy-; (7512-17-6)

2-Acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose: β-D-Glucopyranose, 2-(acetylamino)-2-deoxy-, 1,3,4,6-tetraacetate; (7772-79-4)

Phosphorus pentasulfide; (1314-80-3)

GlcNAc-thiazoline triacetate. (3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-Acetoxymethyl-6,7-diacetoxy-2-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole: 5*H*-Pyrano[3,2-*d*]thiazole-6,7-diol, 5-[(acetyloxy)methyl]-3a,6,7,7a-tetrahydro-2-methyl-, diacetate (ester), (3a*R*,5*R*,6*S*,7*R*,7a*R*)-; (67109-74-4)

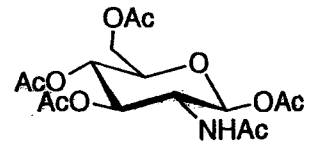
Hexamethyldisiloxane; (107-46-0)

Spencer Knapp was born in Baytown, Texas, and raised in Tallmadge, Ohio. As a Fellow of the Ford Foundation Six-Year BA-PhD Program, he received his respective degrees in 1972 and 1975 from Cornell University, both under the mentorship of Jerrold Meinwald. Following an NIH Postdoctoral Fellowship with E. J. Corey, he joined the faculty of Rutgers University. His research interests include the synthesis of natural products, enzyme inhibitors, and complex ligands, and the development of new synthetic methods. He has received an NCI Young Investigator Award, an American Cyanamid Faculty Award, and a Hoechst-Celanese Innovative Research Award.

Richard Huhn was born in Providence, Rhode Island and was raised mostly in Chester, New Jersey. He received his bachelor's degree in 2005 with highest honors from Rutgers University, where he double-majored in chemistry and physics and worked in the laboratories of Professor Spencer Knapp. He is currently a graduate student working for Professor Stephen Buchwald in the chemistry department of the Massachusetts Institute of Technology. His interests include organometallic chemistry, computational chemistry, and mechanistic investigations into organic and organometallic processes.

Benjamin Amorelli was born in Livingston, New Jersey in 1974. He graduated from Rutgers University with a BS in Environmental Science in 1997 and was employed as a geologist until beginning graduate study in 1999 at Montclair State University, where he received a MS in chemistry. He is currently a Thomas Reid Graduate Fellow at Rutgers University under the guidance of Professor Spencer Knapp, and is interested in synthetic chemistry, molecular design, and the chemistry and biology of natural products.

Erikah E. Englund received her B.S. degree at the University of Wisconsin, Madison in 2001 and performed research under Prof. Steven Burke. She is currently pursuing her PhD degree at the University of Pittsburgh under the supervision of Prof. Peter Wipf. Her graduate studies have included the synthesis of palmarumycin analogues and the continuation of studies towards the total synthesis of the spiroxins.



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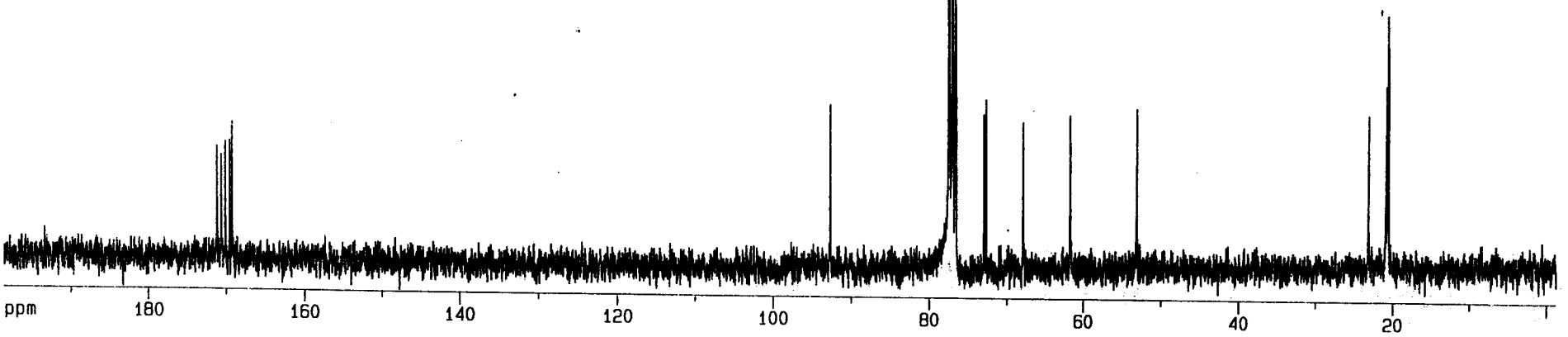

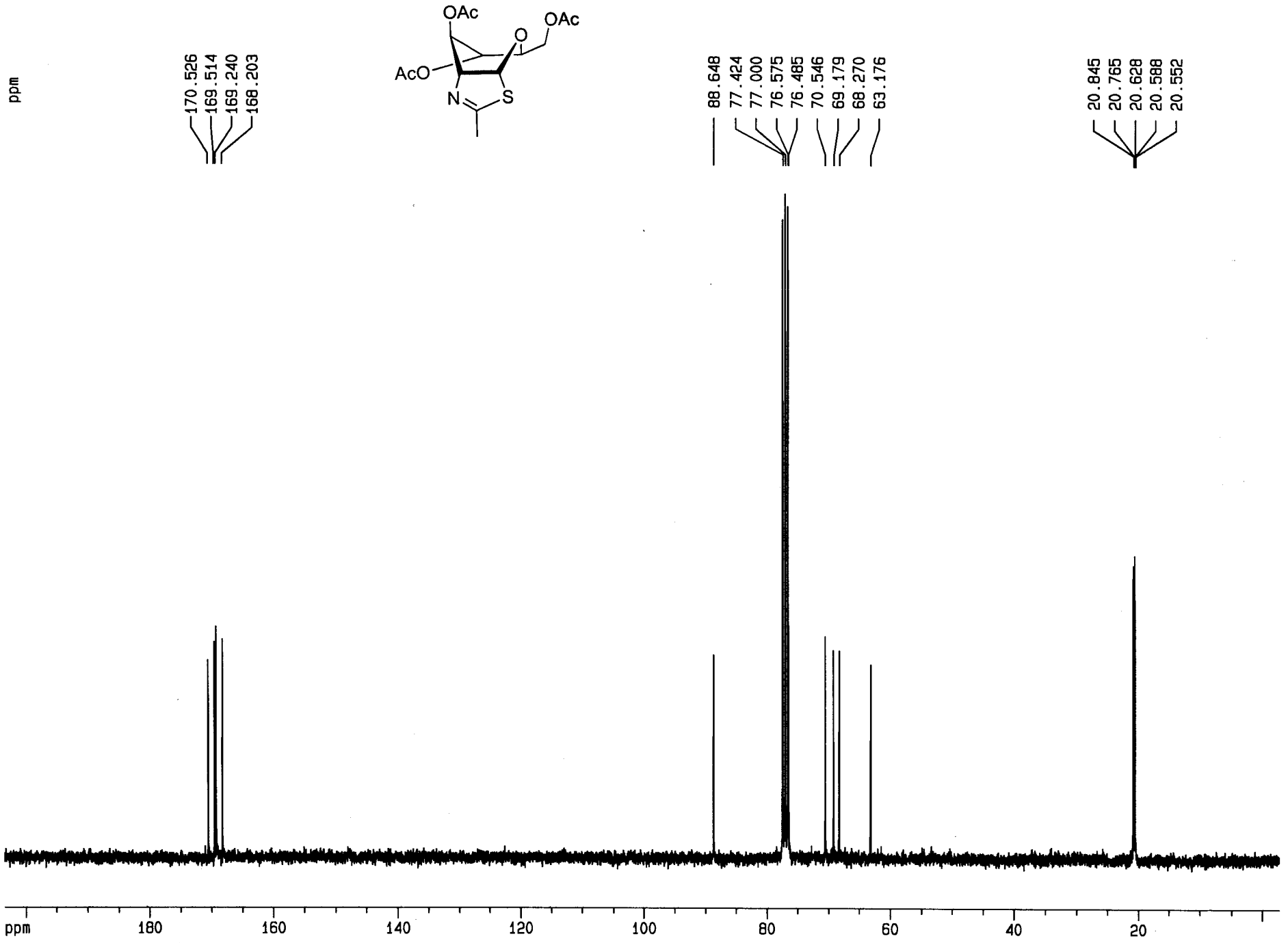
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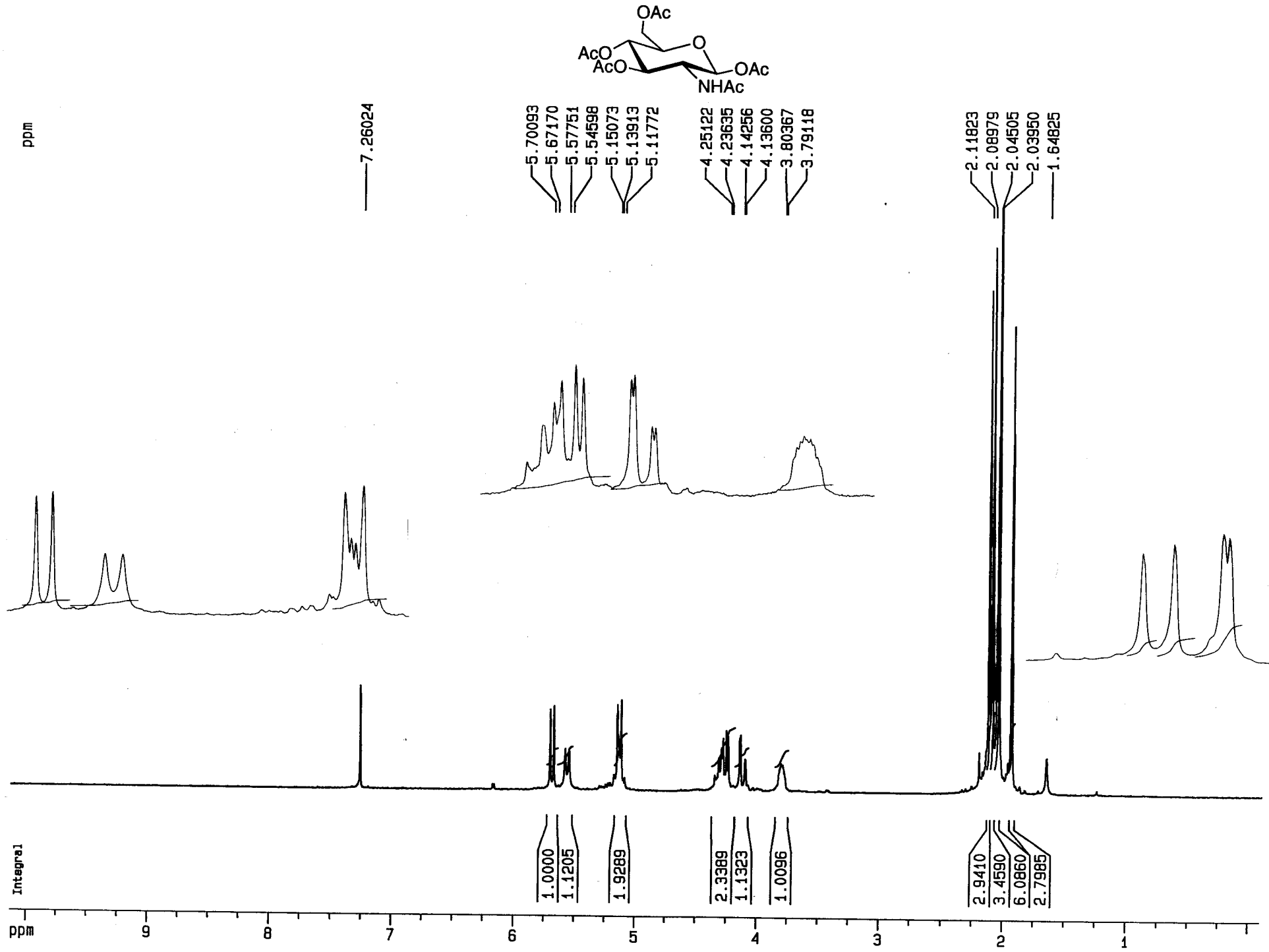
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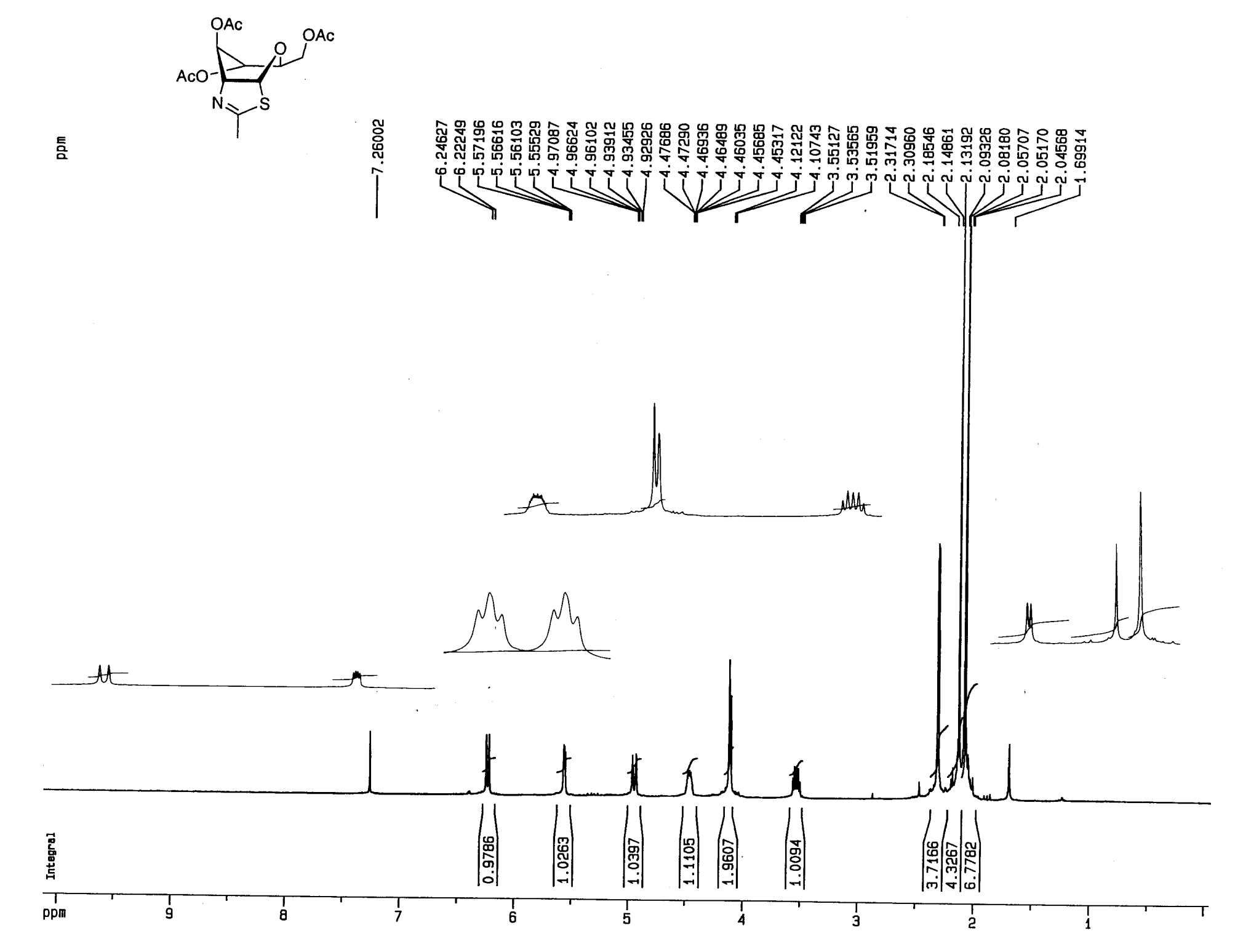
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