

Synthesis of a Dimer of β -(1,4)-L-Arabinosyl-(2*S*,4*R*)-4-hydroxyproline Inspired by Art v 1, the Major Allergen of Mugwort

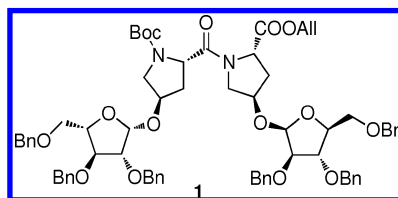
Ning Xie and Carol M. Taylor*

Department of Chemistry, Louisiana State University, Baton Rouge,
Louisiana 70803, United States

cmtaylor@lsu.edu

Received September 4, 2010

ABSTRACT



N α -*tert*-Butoxycarbonyl-L-trans-4-hydroxyproline allyl ester (Boc-Hyp-OAll) was glycosylated with 2,3,5-tri-*O*-benzyl-L-arabinose *p*-cresylthioglycoside in 60% yield with 4:1 β : α stereoselectivity. Deprotection of *N*- and *C*-termini independently gave a prolyl amine and prolyl carboxylate respectively that were coupled under standard conditions with 1-[*bis*-(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5,*b*]-pyridinium hexafluorophosphate 3-oxide (*N*-HATU) to give the dimer **1** in 46% yield. These results represent the first steps toward the production of homogeneous oligomers to determine the minimal epitope of the Art v 1 allergen.

The pollen of mugwort (*Artemisa vulgaris*) is a significant contributor to hay fever in Europe and North America. Altmann and co-workers have proposed a model for the structure of the major allergen, Art v 1 (Figure 1). Detailed degradation studies, in conjunction with molecular modeling¹ and characterization by NMR and mass spectrometry,² revealed two novel *O*-glycosides linked via *trans*-4-hydroxyproline (Hyp). One of these motifs, characterized by clusters of contiguous β -L-arabinofuranosides of Hyp, was found to be a key recognition element for antibodies generated in response to the natural protein. The identification of the minimal epitope and more detailed characterization requires access to homogeneous glycopeptide fragments. Exceedingly small quantities of Art v 1 glycoprotein can be isolated from pollen. Alkaline hydrolysis of the protein leads to complex mixtures of compounds containing the β -Araf-Hyp motif.

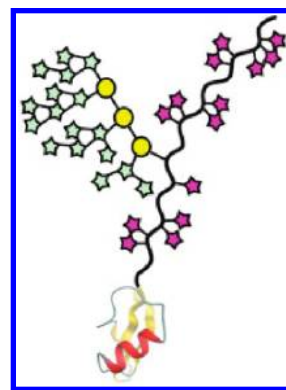


Figure 1. Model for the Art v 1 glycoprotein. Monosaccharide units are represented by: β -D-Gal (yellow circle), α -L-Ara (turquoise star), β -L-Ara (pink star). Copyright The American Society for Biochemistry and Molecular Biology, 2005.

The heterogeneous nature of this digest renders the isolation of even miniscule amounts of pure amino acids and oli-

(1) Himly, M.; Jahn-Schmid, B.; Dedic, A.; Kelemen, P.; Wopfner, N.; Altmann, F.; Van Ree, R.; Briza, P.; Richter, K.; Ebner, C.; Ferreira, F. *FASEB J.* **2003**, *17*, 106–18.

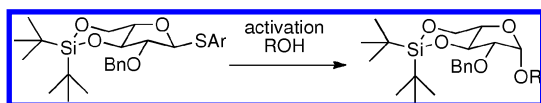
(2) Leonard, R.; Petersen, B. O.; Himly, M.; Kaar, W.; Wopfner, N.; Kolarich, D.; Van Ree, R.; Ebner, C.; Duus, J. Ø.; Ferreira, F.; Altmann, F. *J. Biol. Chem.* **2005**, *280*, 7932–7940.

gopeptides an unrealistic goal. The key to accessing useful amounts of high-purity oligomers is via chemical synthesis.

The repetitive β -Araf-Hyp motif of Art v 1 is unprecedented and poses a challenge to synthesis for several reasons. First, it is difficult to control the stereoselectivity of glycosylation with an arabinofuranosyl donor.³ The anomeric effect is weak due to multiple low energy conformations for furanoses and neighboring group participation favors an α (1,2-*trans*) arabinoside. Second, the hydroxy group of Hyp is a poor nucleophile, in part because of its axial orientation in the preferred *C_γ-exo* conformation of the pyrrolidine ring, and glycosylation rarely proceeds in good yield.⁴ Moreover, the best precedents for the assembly of such a glycoclustered peptide lie in the synthesis of the mucins, wherein glycosylated threonine residues have been coupled.⁵ The amalgamation of two glycosylated prolines is inherently more difficult since the prolyl amine is secondary and cyclic.

Fortunately, significant progress has been made in recent years vis-à-vis the synthesis of β -arabinosides. Ito's group has used intramolecular aglycone delivery (IAD) to good effect⁶ and Lowary has employed 2,3-anhydro sugars.⁷ We quickly focused our attention on the independent reports of conformationally restricted glycosyl donors from the Boons⁸ and Crich⁹ groups (Scheme 1). The 3,5-*O*-di-*tert*-butylsilyl

Scheme 1. Conformationally-Restricted Glycosyl Donor

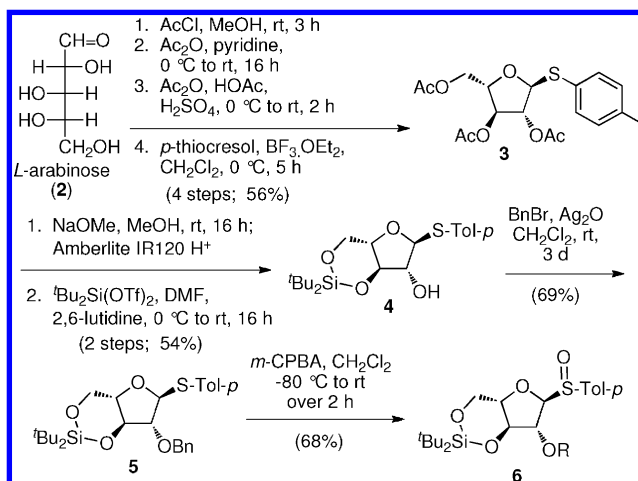


acetal protecting group locks the arabinofuranosyl ring in a conformation¹⁰ that favors nucleophilic attack from the β -face (Scheme 1). A number of subsequent oligosaccharide syntheses have utilized such donors, reporting excellent

stereoselectivity in some instances¹¹ and modest results in other cases.¹² To-date glycosyl acceptors have been predominantly primary and secondary alcohols of monosaccharides. Differences in the stereoselectivity of D- and L-donors has been explored by Zhu.¹³

Thioglycoside and sulfoxide glycosyl donors were prepared according to Scheme 2. We chose to work with the

Scheme 2. Synthesis of Glycosyl Donors



thiocresyl glycoside, as employed by Crich.⁹ We obtained mediocre yields of the silyl acetal **4** and experienced considerable difficulty forming the benzyl ether at C2 according to the originally reported, standard conditions (NaH, BnBr, DMF). We found that the nonbasic conditions reported more recently by Zhu's group¹³ gave more reproducible results. Oxidation of the thioglycoside gave the sulfoxide **6** as a mixture of diastereomers. We prepared this additional glycosyl donor, since Crich had shown that better β -selectivity was obtained via the sulfoxide method.⁹

Glycosylation of Boc-Hyp-OAll (**7**) with thioglycoside **5** and sulfoxide **6** gave complex mixtures of products (Scheme 3). Isolation of pure **8 β** , for characterization, required HPLC purification. Evidence for the stereochemistry of the glycosidic linkage was afforded by the coupling constant ($J = 5.2$ Hz) for H1 of the arabinose unit. The plethora of other products made it difficult to determine yields and α/β ratios. The outcome of the reaction was unsatisfactory in both regards.

Our hypothesis was that the poor results of the glycosylation reactions were due to the instability of the silyl acetal to the reaction conditions. To test this theory, we prepared perbenzylated thioglycoside **9** and the corresponding sul-

(3) Lowary, T. L. *Curr. Opin. Chem. Biol.* **2003**, *7*, 749–756.

(4) We have previously summarized these problems: (a) Taylor, C. M.; Weir, C. A.; Jorgensen, C. G. *Aust. J. Chem.* **2002**, *55*, 135–140. (b) Shaffer, K. J.; Taylor, C. M. *Org. Lett.* **2006**, *8*, 3959–3962.

(5) (a) Sames, D.; Chen, X.-T.; Danishefsky, S. J. *Nature* **1997**, *389*, 587–591. (b) Kuduk, S. D.; Schwarz, J. B.; Chen, X.-T.; Glunz, P. W.; Sames, D.; Ragupathi, G.; Livingston, P. O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 12474–12485. (c) Braun, P.; Davies, G. M.; Price, M. R.; Williams, P. M.; Tendler, S. J. B.; Kunz, H. *Bioorg. Med. Chem.* **1998**, *6*, 1531–1545. (d) Liu, M.; Barany, G.; Live, D. *Carbohydr. Res.* **2005**, *340*, 2111–2122. (e) Matsushita, T.; Hinou, H.; Kurogochi, M.; Shimizu, H.; Nishimura, S. *Org. Lett.* **2005**, *7*, 877–880. (f) Vepiek, P.; Hajduch, M.; Dzubak, P.; Kuklik, R.; Polakova, J.; Bezouska, K. *J. Med. Chem.* **2006**, *49*, 6400–6407. (g) Lo-Man, R.; Vichier-Guerre, S.; Bay, S.; Dériaud, E.; Cantacuzène, D.; Leclerc, C. *J. Immunol.* **2001**, *166*, 2849–2854.

(6) (a) Ishiwata, A.; Akao, H.; Ito, Y. *Org. Lett.* **2006**, *8*, 5525–5528. (b) Ishiwata, A.; Munemura, Y.; Ito, Y. *Eur. J. Org. Chem.* **2008**, 4250–4263.

(7) Gadikota, R. R.; Callam, C. S.; Wagner, T.; Del Fraino, B.; Lowary, T. L. *J. Am. Chem. Soc.* **2003**, *125*, 4155–4165.

(8) Zhu, X.; Kawatkar, S.; Rao, Y.; Boons, G.-J. *J. Am. Chem. Soc.* **2006**, *128*, 11948–11957.

(9) Crich, D.; Pedersen, C. M.; Bowers, A. A.; Wink, D. J. *J. Org. Chem.* **2007**, *72*, 1553–1565.

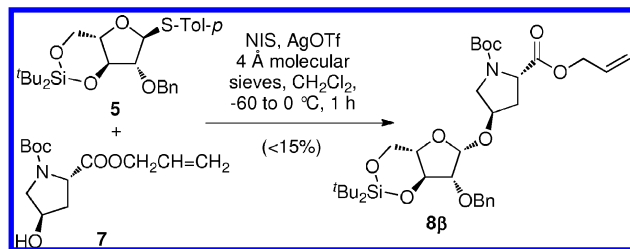
(10) The crystal structure of *p*-tolyl 1-*O*-benzyl-3,5-*O*-(di-*tert*-butylsilylanediyl)-1-thio- α -D-arabinofuranoside has been reported: Nacrio, R. C.; Lowary, T. L.; McDonald, R. *Acta Cryst. Sect. E* **2007**, *E63*, o498–o500.

(11) (a) Rao, Y.; Buskas, T.; Albert, A.; O'Neill, M. A.; Hahn, M. G.; Boons, G.-J. *ChemBioChem* **2008**, *9*, 381–388. (b) Joe, M.; Bai, Y.; Nacario, R. C.; Lowary, T. L. *J. Am. Chem. Soc.* **2007**, *129*, 9885–9901.

(12) (a) Sanki, A. K.; Boucau, J.; Ronning, D. R.; Sucheck, S. J. *Glycoconj. J.* **2009**, *26*, 589–596. (b) Akihiro, I.; Ito, Y. *Trends Glycosci. Glycotechnol.* **2009**, *21*, 266–289.

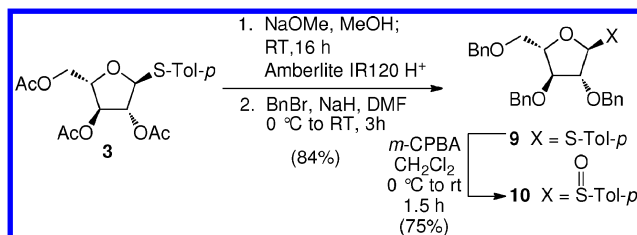
(13) Wang, Y.; Maguire-Boyle, S.; Dere, R. T.; Zhu, X. *Carbohydr. Res.* **2008**, *343*, 3100–3106.

Scheme 3. Glycosylation with Donor 5



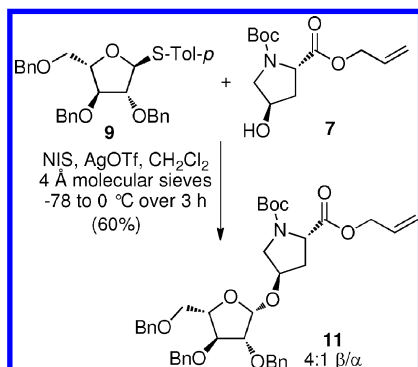
foxide **10** (Scheme 4). Lowary had previously obtained reasonable β -selectivity with *ent*-**9**.¹⁴

Scheme 4. Preparation of Perbenzylated Donors



To our delight, reaction of thioglycoside **9**¹² with hydroxyproline acceptor **7**, under standard conditions, gave a reasonable yield of glycoside **11** (Scheme 5) with a

Scheme 5. Formation of Glycoside 11β



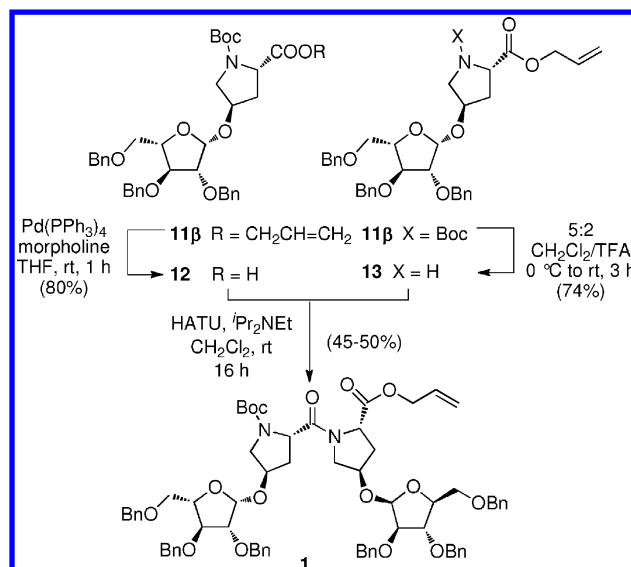
typical β/α ratio of 4:1. The product mixtures were less complex and the yields significantly improved relative to Scheme 3. Moreover, pure β -glycoside **11β** could be obtained by flash chromatography on gram-scale. There are further advantages to this route. The glycosyl donor **9** is available in two steps from **3** in 84% yield (compared to three steps in only 37% yield for donor **5**). The

(14) Yin, H.; D'Souza, F. W.; Lowary, T. L. *J. Org. Chem.* **2002**, *67*, 892–903.

glycoside **11** is more robust than **8** and, downstream, a single hydrogenolytic step will be required to remove all carbohydrate protecting groups. The use of the sulfoxide donor **10** gave similar yields and superior β -selectivity (25:1), as judged by integration of the anomeric proton signals in the ¹H NMR spectrum. Unfortunately, the chromatography to separate pure β -glycoside from assorted byproducts was more tedious. This practical consideration, in combination with the extra step to produce the sulfoxide donor **10**, led us to favor the thioglycoside donor **9** for large-scale preparations of **11β**.

With a practical synthesis of monomer **11β** in hand, the next objective became formation of the dimer (Scheme 6).

Scheme 6. Dimerization of Glycoside 11β



Selective cleavage of the Boc-group afforded secondary amine **12**. Flash chromatography of amine **12**, prior to the coupling reaction, was beneficial. Deallylation with Pd(PPh₃)₄ in the presence of morpholine generated the acid **13**, but we were unable to remove traces of the catalyst.

We investigated a number of peptide coupling reagents, renowned for their effectiveness in hindered couplings,¹⁵ including HATU,¹⁶ bromo-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBroP)¹⁷ and tetramethylfluoroformamidinium hexafluorophosphate (TFFH).¹⁸ Our best results to-date are given in Scheme 6. The doubly glycosylated dipeptide exists as four conformations, presumably varying about the two central peptide bonds, in approximately equal

(15) (a) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243–2266. (b) Han, S.-Y.; Kim, Y.-A. *Tetrahedron* **2004**, *60*, 2447–2467.

(16) Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, J. K.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 442–445.

(17) (a) Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2439. (b) Walker, M. A.; Heathcock, C. H. *J. Org. Chem.* **1992**, *57*, 5566.

(18) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401–5402.

amounts. Further efforts to improve the efficiency of dimer formation, the assembly of larger oligomers, and conformational studies are underway.

Acknowledgment. We thank the Department of Chemistry at LSU for financial support, Dr. Thomas Wedeghiorghis (LSU NMR Facility) for his expertise in acquiring NMR data, and Professor Friedrich Altmann

(Universität für Bodenkultur Wein, BOKU, Vienna, Austria) for helpful discussions.

Supporting Information Available: Experimental procedures and ^1H and ^{13}C NMR spectra for compounds **1** and **3–11**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL102112Z

Synthesis of a Dimer of β -(1,4)-L-Arabinosyl-(2*S*,4*R*)-4-hydroxyproline Inspired by Art v 1, the Major Allergen of Mugwort

Ning Xie and Carol M. Taylor *

Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803.

cmtaylor@lsu.edu

SUPPORTING INFORMATION

Experimental procedures for the preparation of compounds and their ^1H and ^{13}C NMR spectra. Compounds are listed in the order that they appear in the manuscript, with page references:

Scheme	Compound	Procedure	^1H NMR	^{13}C NMR
Scheme 2	3	S3	S12	S13
	4	S4	S14	S15
	5	S5	S16	S17
	6	S5	6A : S18	S19
			6B : S20	S21
Scheme 3	7	S6	S22	S23
	8β	S7	S24	S25
Scheme 4	9	S7	S26	S27
	10	S8	S28	S29
Scheme 5	11β	S9	S30	S31
	1	S10	S32	S33

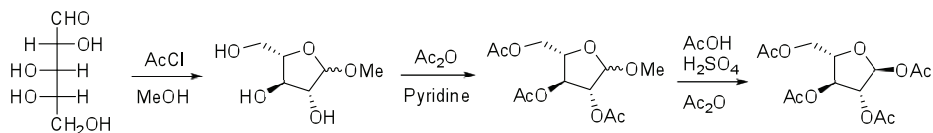
General Experimental Methods:

All reactions were performed under a dry nitrogen atmosphere unless otherwise noted. Reagents were obtained from commercial sources and used directly, with the following exceptions. Pyridine, 2,6-lutidine, morpholine and diisopropylethylamine were dried and distilled from CaH_2 and stored over KOH pellets. Methanol was distilled from magnesium turnings and stored over 3 Å molecular sieves. Powdered 4 Å molecular sieves were flame-dried immediately prior to use.

Reactions were followed by TLC on pre-coated silica plates (200 μm , F-254). Flash chromatography was performed using flash silica gel (32-63 μ). The compounds were visualized by UV fluorescence or by staining with a 10% solution of sulfuric acid in ethanol or an acidic, ethanolic solution of ninhydrin.

NMR spectra were recorded at either 400 or 700 MHz. Proton NMR data is reported in ppm downfield from TMS as an internal standard. High resolution mass spectra were recorded using either time-of-flight MS, or LC-ion trap MS with electrospray ionization. Optical rotations were recorded under the specified conditions.

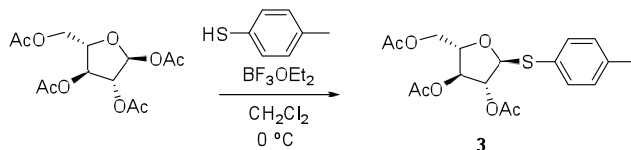
Peracetylated- α -L-Arabinofuranoside



L-Arabinose (5.0 g, 33.3 mmol, 1 equiv.) was dissolved in methanol (100 mL) and treated with a solution of acetyl chloride (2.5 mL) in methanol (30 mL) at rt under N_2 . The mixture was stirred for 3 h, during which time the solid completely dissolved. The reaction was quenched dropwise with pyridine. The solvent was evaporated, followed by azeotropeing with CH_2Cl_2 to give the methyl arabinoside as a mixture of anomers. The crude product was dissolved in pyridine (40 mL) and cooled to 0 °C, after which acetic anhydride (20 mL) was added and the reaction was stirred overnight at rt. The solvent was evaporated and the mixture was diluted with CH_2Cl_2 (250 mL), washed with water (250 mL), 1 M HCl (250 mL), sat. NaHCO_3 (250 mL), and brine (250 mL). The organic phase was filtered through MgSO_4 and concentrated to give the triacetate. The crude product was dissolved in acetic anhydride (80 mL) and cooled to 0 °C. Acetic acid (20 mL) was added dropwise. After 15 min, sulfuric acid (5 mL) was added dropwise. The

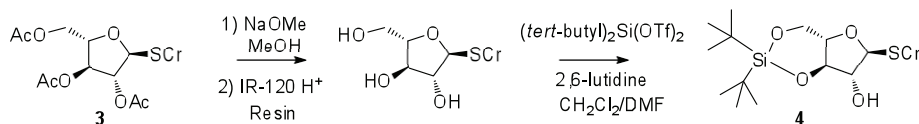
mixture was warmed to rt while stirring for approximately 2 h. The solution was poured over a mixture of ice (50 g), CH₂Cl₂ (250 mL) and sat'd aq. NaHCO₃ (200 mL). The organic layer was separated and washed again with several volumes of sat'd aq. NaHCO₃, filtered through MgSO₄, and concentrated to give the crude peracetylated furanoside as a light oil (9.071 g, 85%; 3 steps).

p*-Cresyl 2,3,5-*O*-acetyl-1-thio- α -*L*-arabinofuranoside (**3**)

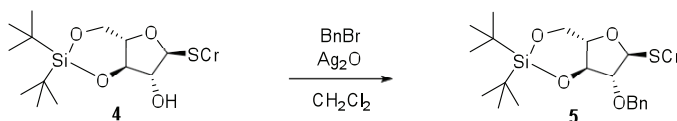


A solution of peracetylated arabinofuranoside (4.47 g, 14 mmol, 1.0 equiv.) and *p*-thiocresol (2.64 g, 21 mmol, 1.5 equiv.) in dry CH₂Cl₂ (60 mL) was cooled to 0 °C under N₂. Boron trifluoride diethyl etherate (1.0 mL, 1.17 g, 8.1 mmol, 0.5 equiv.) was added dropwise and the mixture stirred for 5 h under N₂ at 0 °C. The reaction was quenched with Et₃N (4 mL) and concentrated. The mixture was diluted with EtOAc (150 mL) and washed with H₂O (150 mL) and brine (150 mL). The organic layer was filtered through MgSO₄ and concentrated. The residue was purified by flash chromatography eluting with 4:1 Hex/EtOAc to give **3** as a light colored oil (3.54 g, 66 %). *R*_f 0.25 (3:1 Hexanes/EtOAc). [α]_D²⁵ -170.2 (*c* 1, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 2.10 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 2.34 (s, 3H), 4.28 (dd, *J* = 12.1, 5.5 Hz, 1H), 4.39 (dd, *J* = 12.1, 3.6 Hz, 1H), 4.48 (app. q, *J* = 4.7 Hz, 1H), 5.07 (d, *J* = 5.5 Hz, 1H), 5.27 (s, 1H), 5.47 (s, 1H), 7.13 (d, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 20.7 (3C), 21.1, 62.8, 77.2, 79.9, 81.4, 91.2, 129.6, 129.8, 132.7, 138.1, 169.6, 170, 170.5. HRMS (ESI) calcd for C₁₈H₂₁O₇SN⁺ (M+Na)⁺: 405.0978; obsd: 405.0985.

**The synthesis of ent-3 has been previously reported by Crich and co-workers, however, without purification and characterization of this intermediate.*

***p*-Cresyl 3,5-*O*-(Di-*tert*-butylsilylene)-1-thio- α -*L*-arabinofuranoside (**4**)**

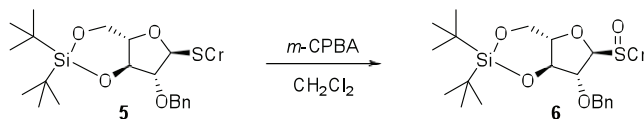
Thioglycoside triacetate (**3**) (694 mg, 1.8 mmol) was dissolved in MeOH (15 mL). Sodium methoxide (200 μ L, 25% in MeOH) was added and stirred under N₂ overnight. Amberlite® IR-120 acid resin was added portionwise while stirring until solution was neutralized, after which it was filtered and rinsed with MeOH, and concentrated. A portion of the crude triol (363 mg, 1.4 mmol, 1.0 equiv.) was suspended in a mixture of dry CH₂Cl₂ (13 mL) and DMF (2.5 mL) and cooled to 0 °C under N₂. 2,6-Lutidine (762 μ L, 705 mg, 6.6 mmol, 4.7 equiv.) and di-*tert*-butylsilyl *bis*-(trifluoromethanesulfonate) (545 μ L, 741 mg, 1.7 mmol, 1.2 equiv.) were then added sequentially. The mixture was stirred overnight under N₂ at rt. The mixture was concentrated and the residue diluted with EtOAc (30 mL) and washed with H₂O (30 mL) and brine (30 mL). The organic layer was filtered through MgSO₄ and concentrated. The residue was purified by flash chromatography eluting with 15:1 Hex/EtOAc to give **4** as an amorphous colorless solid (297 mg, 54%, 2 steps). *R*_f 0.49 (3:1 Hexanes/EtOAc). [α]_D²⁵ -181.0 (c 1, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 1.00 (s, 9H), 1.08 (s, 9H), 2.33 (s, 3H), 3.58 (d, *J* = 4.2 Hz, 1H), 3.87-3.98 (m, 2H), 4.02 (app. t, *J* = 8.3 Hz, 1H), 4.14 (app. q, *J* ~ 5 Hz, 1H), 4.35 (dd, *J* = 8.3, 4.2 Hz, 1H), 5.27 (d, *J* = 5.9 Hz, 1H), 7.12 (d, *J* = 7.8 Hz, 2H), 7.42 (d, *J* = 7.9 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 20.1, 21.1, 22.7, 27.1, 27.5, 67.4, 73.7, 80.6, 77.4, 91.5, 129.8, 130.3, 132.3, 137.9. HRMS (ESI) calcd for C₂₀H₃₃O₄SSi (M+H)⁺: 397.1863; obsd: 397.1856.

***p*-Cresyl 2-*O*-Benzyl-3,5-*O*-(di-*tert*-butylsilylene)-1-thio- α -*L*-arabinofuranoside (**5**)**

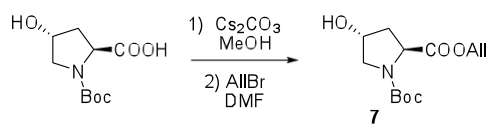
Compound **4** (400 mg, 1.1 mmol, 1 equiv.) was dissolved in dry CH₂Cl₂ (11 mL) and stirred under N₂. Benzyl bromide (240 μ L, 345 mg, 2.0 mmol, 2 equiv.) was added, followed by Ag₂O (714 mg, 3.1 mmol, 3 equiv.). The mixture was stirred for 3 d, filtered through an inch of silica and washed with CHCl₃, and concentrated. The residue was purified by flash chromatography eluting with 120:1 Hex/Ether $\rightarrow$ 60:1 Hex/EtOAc to give **5** as a colorless solid (336 mg, 64%). *R*_f 0.62 (3:1 Hexanes/EtOAc). [α]_D²⁵ -129.93 (c 1,

CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 0.97 (s, 9H), 1.06 (s, 9H), 2.28 (s, 3H), 3.84-3.98 (m, 3H), 4.12 (app. t, *J* = 8.4 Hz, 1H), 4.31 (dd, *J* = 8.2, 4.1 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.81 (d, *J* = 12.0 Hz, 1H), 5.34 (d, *J* = 5.2 Hz, 1H), 7.05 (d, *J* = 7.9 Hz, 2H), 7.26-7.40 (m, 7H); ¹³C NMR (100 MHz, CDCl₃) δ 20.2, 21.2, 22.7, 27.2, 27.6, 67.4, 72.2, 73.8, 81.4, 86.8, 90.3, 127.9, 128.1, 128.5, 129.8, 130.6, 132.3, 137.71, 137.74. HRMS (ESI) calcd for C₂₇H₃₉O₄SSi (M+H)⁺: 487.2333; obsd: 487.2324.

***p*-Cresyl 2-*O*-Benzyl-3,5-*O*-(di-*tert*-butylsilylene)-1-thio- α -*L*-arabinofuranoside S-Oxide (**6**)**

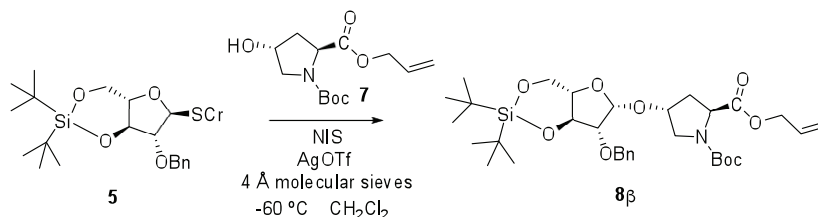


A solution of 3-chloroperoxybenzoic acid (63.5 mg, 77 wt %, 0.28 mmol, ~1.2 equiv.) in dry CH₂Cl₂ (1 mL) was added dropwise to a solution of compound **5** (116 mg, 0.24 mmol, 1 equiv.) in dry CH₂Cl₂ (4 mL) at -80 °C under Argon and stirred. The reaction mixture was gradually warmed to rt over 2 h. The solution was diluted with CH₂Cl₂ (30 mL), washed with sat'd aq. NaHCO₃ (30 mL), filtered through MgSO₄, and concentrated. The residue was purified by column chromatography, eluting with 10:1 Hex/EtOAc to afford **6** as a colorless gel (68 mg, 57%) and a mixture of diastereomers. Diastereomer **6A**: *R_f* 0.42 (3:1 Hexanes/EtOAc). [α]_D²⁵ 101.4 (*c* 1, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 1.00 (s, 9H), 1.05 (s, 9H), 2.40 (s, 3H), 3.89 (app. t, *J* = ~10 Hz, 1H), 3.99-4.05 (td, *J* = 10, 5.0 Hz, 1H), 4.22 (dd, *J* = ~10, 7.2 Hz, 1H), 4.30 (d, *J* = 11.4 Hz, 1H), 4.35 (dd, *J* = 9.0, 5.0 Hz, 1H), 4.57-4.59 (m, 1H), 4.58 (d, *J* = 11.4 Hz, 1H), 4.65 (d, *J* = 5.1 Hz, 1H), 6.98 (app. t, *J* = 3.6 Hz, 2H), 7.25-7.27 (m, 3H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 20.2, 21.4, 22.6, 27.1, 27.4, 67.4, 72.0, 77.0, 78.5, 82.0, 99.3, 124.4, 127.7, 127.9, 128.2, 129.9, 136.2, 137.3, 141.7. HRMS (ESI) calcd for C₂₇H₃₉O₅SSi (M+H)⁺: 503.2282; obsd: 503.2298. Diastereomer **6B**: *R_f* 0.33 (3:1 Hexanes/EtOAc). [α]_D²⁵ -178.6 (*c* 1.35, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 0.95 (s, 9H), 1.05 (s, 9H), 2.40 (s, 3H), 3.78-3.84 (m, 1H), 3.86 (app. t = ~9.4 Hz, 1H), 4.20 (app. t, *J* = 8.2 Hz, 1H), 4.30 (dd, *J* = 8.6, 4.5 Hz, 1H), 4.35 (app. t, *J* = 6.4 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.63 (d, *J* = 5.6 Hz, 1H), 4.76 (d, *J* = 11.7 Hz, 1H), 7.23-7.36 (app. m, 7H), 7.52 (d, *J* = 8.1 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 20.0, 21.5, 22.6, 27.0, 27.4, 67.3, 72.3, 76.9, 81.1, 81.5, 98.2, 125.6, 128.0, 128.1, 128.4, 129.9, 136.4, 137.3, 142.3. HRMS (ESI) calcd for C₂₇H₃₉O₅SSi (M+H)⁺: 503.2282; obsd: 503.2294.

***N*-tert-butoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester (7)**

To a suspension of Boc-Hyp-OH (1.77 g, 7.7 mmol, 1.0 equiv.) in dry MeOH (16 mL) was added cesium carbonate (1.37 g, 4.2 mmol, 0.55 equiv.). The mixture was stirred under N₂ for 1.5 h during which time the reaction mixture became a homogeneous solution. The solvent was evaporated, and the residue dissolved in dry DMF (10 mL) and treated immediately with allyl bromide (0.86 mL, 1.2 g, 9.9 mmol, 1.3 equiv.). The mixture was stirred overnight at RT under N₂. The mixture was diluted with EtOAc (150 mL) and washed with H₂O (150 mL) and brine (150 mL). The organic layer was filtered through MgSO₄ and concentrated. The residue was purified by flash column chromatography, eluting with 2:1 EtOAc/Hex to give the title compound as a light oil (1.86 g, 89%). *R*_f 0.34 (2:1 EtOAc/Hex). [α]_D²⁵ -65.0 (*c* 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 1.40 (1.46)* (s, 9H), 2.03-2.10 (m, 1H), 2.25-2.35 (m, 1H), 2.96 (s, 1H), 3.44-3.65 (m, 2H), 4.40-4.48 (m, 2H), 4.56-4.71 (m, 2H), 5.25 (app. t, *J* = ~11.3 Hz, 1H), 5.35 (dt, *J* = 17.1, 3.8, 1.2 Hz, 1H), 5.87-5.95 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 28.2 (28.3), 39.1 (38.3), 54.6, 58.0 (57.7), 65.6, 69.1 (69.8), 80.5 (80.2), 118.8 (118.3), 131.6 (131.8), 154.1 (154.6), 172.9 (172.6). HRMS (ESI) calcd for C₁₃H₂₁NO₅Na (*M*+Na)⁺: 294.1312, obs'd: 294.1320.

* values in parentheses signify a second signal due to a minor rotamer

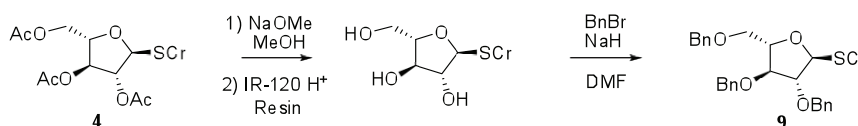
***N*α-*tert*-butyloxycarbonyl-*trans*-4-hydroxy-4-*O*-[2-*O*-Benzyl-3,5-*O*-(di-*tert*-butylsilylene)-*L*-arabinofuranosyl]-*L*-proline Allyl Ester (8β)**

A solution of compound **5** (219 mg, 0.45 mmol, 1.0 equiv.) and **7** (250 mg, 0.92 mmol, 2.0 equiv.) in dry CH₂Cl₂ (30 mL) was added to a flask containing activated 4 Å crushed molecular sieves under N₂. The

suspension was stirred for ~20 min at RT, then cooled in a bath of ethylene glycol and dry ice. The temperature lowered to -30 °C and NIS (173 mg, 0.68 mmol, 1.5 equiv.) was added, followed by a solution of AgOTf (57 mg, 0.22 mmol, 0.5 equiv) in toluene (0.6 mL) as the temperature continued to drop to -60 °C. The suspension was stirred for 1 h during which it was allowed to gradually warm to rt. Solution continued to stir until room temperature was reached. After ~20 mins at rt, the reaction was quenched with a few drops of Et₃N, filtered, and concentrated. The residue was diluted with EtOAc (50 mL) and washed with sat'd aq. Na₂S₂O₃ (50 mL) and brine (50 mL), filtered through MgSO₄, and concentrated. The residue was purified by column chromatography, eluting with 10:1 Hex/EtOAc to afford crude **8β** as a colorless oil (147 mg, 51%) as a mixture of anomers and rotamers. Crude **8β** was subjected to HPLC, eluting with 20% EtOAc, 80% hexanes at 13.0 mL min⁻¹ on an Econosil 10μ silica column (21 x 250 mm). R_T (**8β**) = 23 min; β-anomer: R_f 0.30 (3:1 Hexanes/EtOAc). [α]_D²⁵ +57.3 (c 1, CH₂Cl₂), ¹H NMR (400 MHz, CDCl₃) δ 0.99 (s, 9H), 1.07 (s, 9H), 1.38 (1.45) (1 s, 9H), 2.08-2.15 (m, 1H), 2.35-2.42 (2.43-2.49)* (m, 1H), 3.58-3.69 (m, 3H), 3.86-3.91 (m, 2H), 4.26-4.32 (m, 3H), 4.36-4.44 (m, 1H), 4.56-4.73 (m, 3H), 4.79 (d, *J* = 12.2 Hz, 1H), 4.97 (4.93) (d, *J* = ~5.3 Hz, 1H), 5.24 (app. t, *J* = 11.6 Hz, 1H), 5.32 (ddd, *J* = 17.2, 5.9, 1.3 Hz, 1H), 5.85-5.96 (m, 1H), 7.29-7.40 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 20.1, 22.6, 27.1, 27.5, 28.2 (28.4)*, 37.6 (36.8), 51.1 (51.6), 58.1 (57.7), 65.5 (65.6), 68.4, 71.8 (71.9), 73.6 (73.4), 75.2, 78.4, 80.2 (80.1), 80.7, 99.2 (100.0), 118.7 (118.3), 127.7, 127.8, 127.9, 128.0, 128.4, 131.7 (131.9), 137.7, 153.7 (154.2), 172.7 (172.4). HRMS (ESI) calcd for C₃₃H₅₂NO₉Si (M+H)⁺: 634.3406; obsd: 634.3408.

* values in parentheses signify a second signal due to a minor rotamer

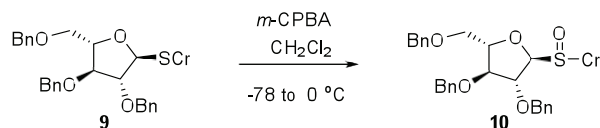
***p*-Cresyl 2,3,5-*O*-Benzyl-1-thio- α -*L*-arabinofuranoside (**9**)**



Thioglycoside triacetate **4** (2.86 g, 7.5 mmol, 1.0 equiv.) was dissolved in MeOH (35 mL). Sodium methoxide (0.5 mL, 25% in MeOH, 4.1 mmol, 0.55 equiv.) was added and stirred under N₂ overnight. Amberlite® IR-120 acid resin was added in portions, stirring until the solution was neutralized. The resin

was filtered, rinsed with MeOH, and the filtrate concentrated. The crude triol (1.91 g, 7.5 mmol, 1.0 equiv.) was dissolved in dry DMF (15 mL) and cooled to 0 °C under N₂. Benzyl bromide (5.3 mL, 7.61 g, 44.5 mmol, 6.0 equiv.) and NaH (1.78 g, 60% dispersion, 44.5 mmol, 6.0 equiv.) were added sequentially. The reaction mixture was allowed to warm to RT while it stirred under N₂ for 3 h. The reaction was quenched with sat'd aq. NaHCO₃ (150 mL) and extracted with CH₂Cl₂ (150 mL). The organic layer was washed with H₂O (150 mL) and brine (150 mL), filtered through MgSO₄, and concentrated. The residue was purified by flash chromatography eluting with 20:1 → 10:1 → 1:2 Hex/EtOAc to give **9** as a clear gel (3.26 g, 84%; 2 steps). *R*_f 0.46 (3:1 Hexanes/EtOAc). [α]_D²⁵ -107.9 (c 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 2.32 (s, 3H), 3.63 (dd, *J* = 10.8, 4.7 Hz, 1H), 3.68 (dd, *J* = 10.8, 3.9 Hz, 1H), 4.03 (app. q, *J* = 3.3 Hz, 1H), 4.11 (t, *J* = 2.9 Hz, 1H), 4.36-4.38 (m, 1H), 4.48-4.65 (m, 6H), 5.53 (d, *J* = 2.0 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.26-7.35 (m, 15H), 7.41 (d, *J* = 7.8 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 21.2, 69.1, 72.7, 72.3, 73.4, 80.5, 83.5, 88.4, 90.6, 127.6, 127.7, 127.8, 127.9, 127.9, 128.0, 128.3, 128.4, 128.5, 129.7, 131.0, 132.0, 137.4, 137.8, 138.2. HRMS (ESI) calcd for C₃₃H₃₄NaO₄S (M+Na)⁺: 549.2070; obsd: 549.2067.

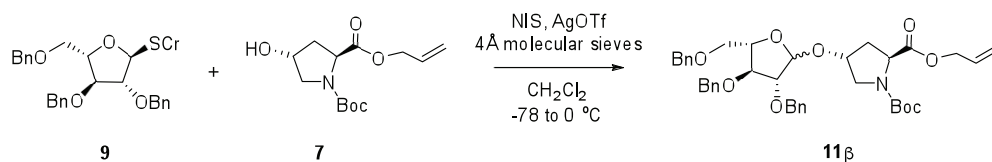
***p*-Cresyl 2,3,5-*O*-Benzyl-1-thio- α -L-arabinofuranoside S-Oxide (**10**)**



A solution of 3-chloroperoxybenzoic acid (283 mg, 77 wt %, 1.3 mmol, 1.2 equiv.) in dry CH₂Cl₂ (4 mL) was added dropwise to a solution of compound **9** (555 mg, 1.1 mmol, 1.0 equiv.) in dry CH₂Cl₂ (16 mL) at -78 °C under N₂ and stirred. The reaction mixture was gradually warmed to RT over 1.5 h. The solution was diluted with CH₂Cl₂ (70 mL), washed with sat'd aq. NaHCO₃ (70 mL), filtered through MgSO₄, and concentrated. The residue was purified by column chromatography, eluting with 3:1 Hex/EtOAc to afford **10** as a colorless gel (429mg, 75%), essentially a single diastereomer. Major diastereomer: *R*_f 0.19 (3:1 Hexanes/EtOAc). [α]_D²⁵ +30.1 (c 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 2.39 (s, 3H), 3.53 (dd, *J* = 10.6, 5.5 Hz, 1H), 3.60 (dd, *J* = 10.6, 4.8 Hz, 1H), 4.15 (dd, *J* = 5.5, 2.8 Hz, 1H), 4.37-4.51 (m, 6H), 4.59 (d, *J* = 11.8 Hz, 1H), 4.63 (d, *J* = 1.8 Hz, 1H), 4.74 (app. t, *J* = ~2.4 Hz, 1H), 7.20-7.32 (m, 17H), 7.57 (d, *J* = 8.1 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 21.5, 69.1, 71.9, 72.2, 73.3, 83.3, 83.9, 84.4, 102.0, 124.8,

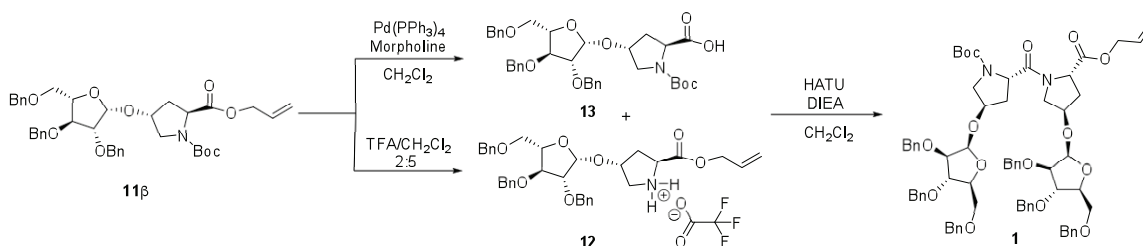
127.7, 127.9, 128.0 (2 signals), 128.4 (2 signals), 128.5, 129.9, 137.3, 137.4, 137.9, 138.7, 141.8. HRMS (ESI) calcd for $C_{33}H_{35}O_5S$ ($M+H$)⁺: 543.2160; obsd: 543.2206.

***N*α-*tert*-Butyloxycarbonyl-*trans*-4-hydroxy-4-*O*-[2,3,5-*O*-Benzyl-*L*-arabinofuranosyl]-*L*-proline Allyl Ester (11)**



A solution of compound **9** (1.17 g, 2.2 mmol, 1.0 equiv.) and **7** (0.688 g, 2.5 mmol, 1.1 equiv.) in dry CH_2Cl_2 (40 mL) was stirred with activated 4 Å molecular sieves (3.5 g) under N_2 for ~30 min at RT. The suspension was cooled to -78 °C (acetone/dry ice) and then NIS (0.747 g, 3.2 mmol, 1.5 equiv.) and AgOTf (0.285 g, 0.55 mmol, 0.5 equiv.) were added. The reaction was allowed to gradually reach 0 °C over 3 h, at which time it was quenched with Et_3N (3 mL) and filtered. The filtrate was diluted with EtOAc (150 mL) and washed with 10% aqueous $Na_2S_2O_3$ (2 x 200 mL) and brine (200 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue, determined to be a 4:1 β:α ratio by NMR, was purified by column chromatography, eluting with 3:1 Hex/EtOAc to afford **11β** (0.732 g, 50%, 2:1 ratio of conformations) as an orange oil. R_f 0.56 (1:1 Hexanes/EtOAc). $[\alpha]_D^{25} +17.9$ (c 1.0, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$) δ 1.37 (1.44)* (s, 9H), 1.96-2.07 (m, 1H), 2.27-2.37 (m, 1H), 3.44-3.67 (m, 4H), 4.03-4.12 (m, 3H), 4.25-4.71 (m, 10H), 4.96 (4.92) (d, J = 3.2 Hz, 1H), 5.20-5.34 (m, 2H), 5.86-5.91 (m, 1H), 7.28-7.33 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3 (28.4), 37.4 (36.5), 50.7 (51.4), 58.1 (57.8), 65.6 (65.7), 72.0, 72.4, 73.3, 75.5, 80.1, 80.3, 82.7, 83.8, 83.9, 99.0 (99.9), 118.8 (118.4), 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5; 131.7 (131.9), 137.4, 137.9, 138.1, 153.7 (155.4), 172.7 (172.4). HRMS (ESI) calcd for $C_{39}H_{48}NO_9$ ($M+H$)⁺: 674.3324, obs'd: 674.3343.

* values in parentheses signify a second signal due to a minor rotamer

Diglycopeptide (1)***N*-*tert*-Butyloxycarbonyl-*trans*-4-hydroxy-4-*O*-[2,3,5-*O*-Benzyl- β -*L*-arabinofuranosyl]-*L*-proline (13).**

(13). A solution of compound **11 β** (383 mg, 0.57 mmol, 1.0 equiv.) and Pd(PPh₃)₄ (66.0 mg, 0.05 mmol, 0.1 equiv.) in dry THF (10 mL) was treated with morpholine (345 μ L, 347 mg, 4.0 mmol, 7.0 equiv.) and stirred at RT under N₂ for 1 h. The solvent was evaporated and the residue dissolved in CH₂Cl₂ (60 mL), washed with 1M HCl (3 x 50 mL), filtered through MgSO₄, and concentrated. The crude acid **13** (350 mg, 97%) was used in the next reaction without further purification.

***trans*-4-hydroxy-4-*O*-[2,3,5-*O*-Benzyl- β -*L*-arabinofuranosyl]-*L*-proline Allyl Ester (12).** Compound **11 β** (144 mg, 0.21 mmol, 1.0 equiv.) was dissolved in dry CH₂Cl₂ (2.5 mL) and stirred under N₂. The mixture was cooled to 0 °C and treated with TFA (1 mL) and thioanisole (25 μ L, 27 mg, 0.21 mmol, 1.0 equiv.). The mixture was stirred at 0 °C for 30 min, warmed to RT over the next 2.5 h, and concentrated. The residue was purified by flash column chromatography, starting with 2:1 EtOAc/Hex and flushing with 4:1 CH₂Cl₂/MeOH to give **12** as a light brown oil (107 mg, 73%).

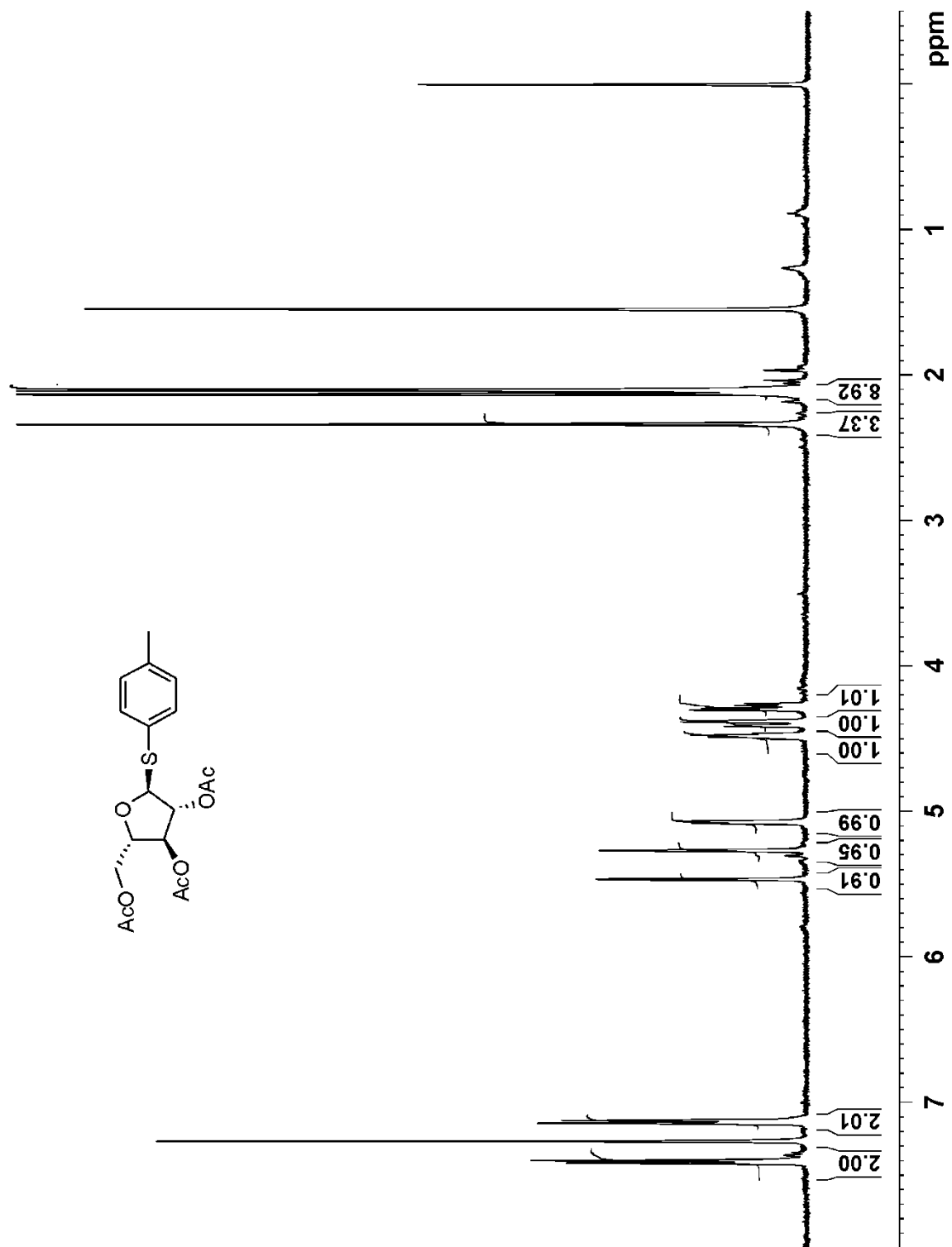
Compound **12** (53 mg, 0.08 mmol, 1.0 equiv.) and compound **13** (68 mg, 0.11 mmol, 1.4 equiv.) were suspended in dry CH₂Cl₂ (3 mL). The mixture was cooled to 0 °C and DIEA (40 μ L, 30 mg, 0.23 mmol, 3.0 equiv.) and HATU (35 mg, 0.10 mmol, 1.2 equiv.) were added successively. The reaction was allowed to gradually warm to rt overnight while stirring under N₂. The mixture was diluted with CH₂Cl₂ to 25 mL total volume, washed with 1M HCl (2 x 20 mL), sat'd NaHCO₃ (20 mL), and brine (20 mL). The organic layer was filtered through MgSO₄ and concentrated. The residue was purified by flash column chromatography, eluting with 1.5:1 Hex/EtOAc $\rightarrow$ 1.5:1 EtOAc/Hex to give **1** as a light oil (45 mg, 49%). *R*_f 0.56 (2:1 EtOAc/Hex). [α]_D²⁵ +30.1 (c 1.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 1.35 (1.30)* (s, 9H), 1.96-2.13 (m, 2H), 2.17-2.37 (m, 2H), 3.40-3.75 (m, 8H), 3.90-4.27 (m, 7H), 4.38-4.72 (m, 19H), 4.88 (5.08) (s {d, *J* = 4.0 Hz}, 1H), 4.99 (app. t, *J* = ~4.3 Hz, 1H), 5.20 (app. d, *J* = 10.4 Hz, 1H), 5.29 (app. d, *J* = 16.9 Hz, 1H), 5.82-5.91 (m, 1H), 7.25-7.33 (m, 30H); ¹³C NMR (100 MHz, CDCl₃) δ *t*-Boc (28.4, 28.5)*,

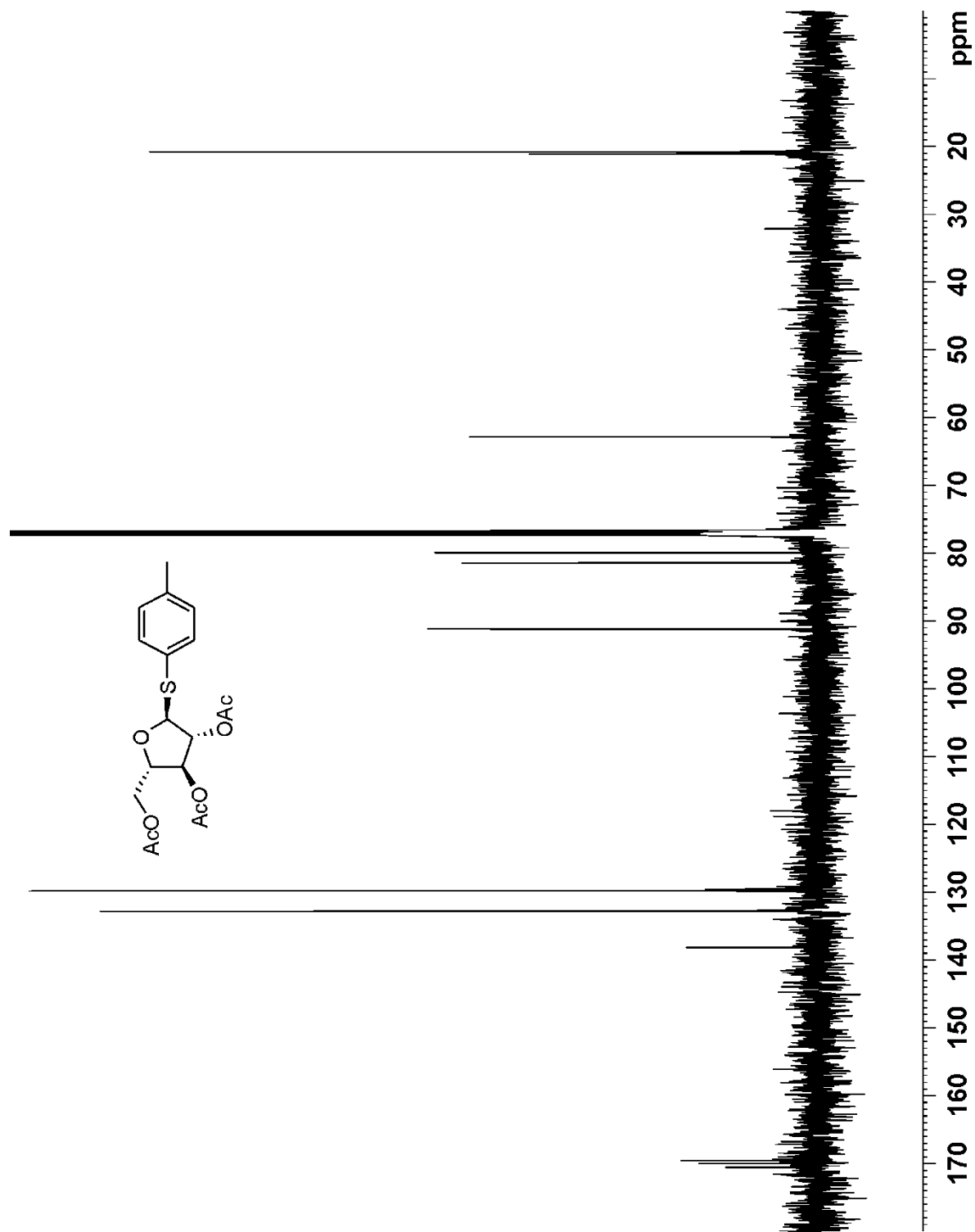
S11

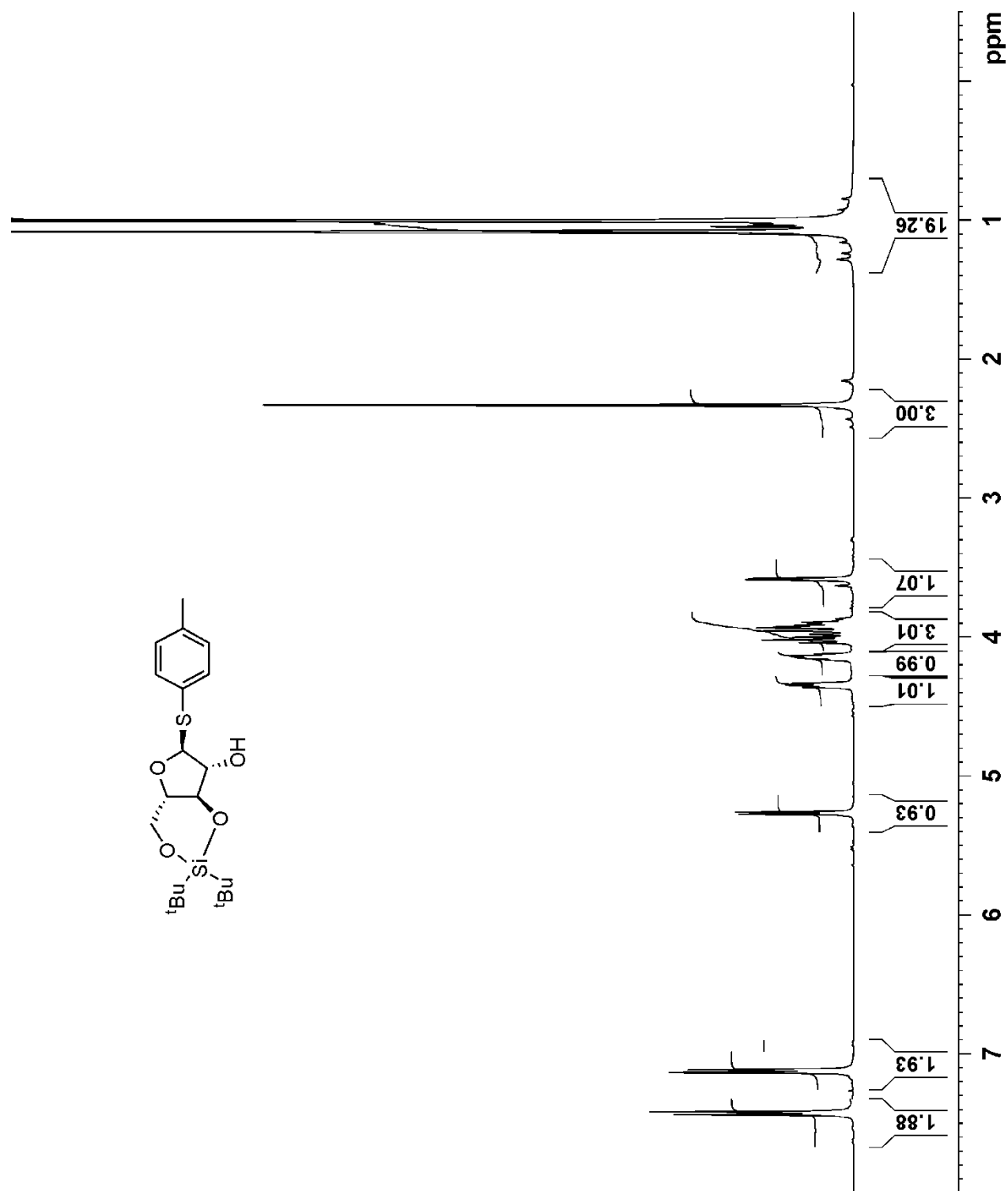
S11

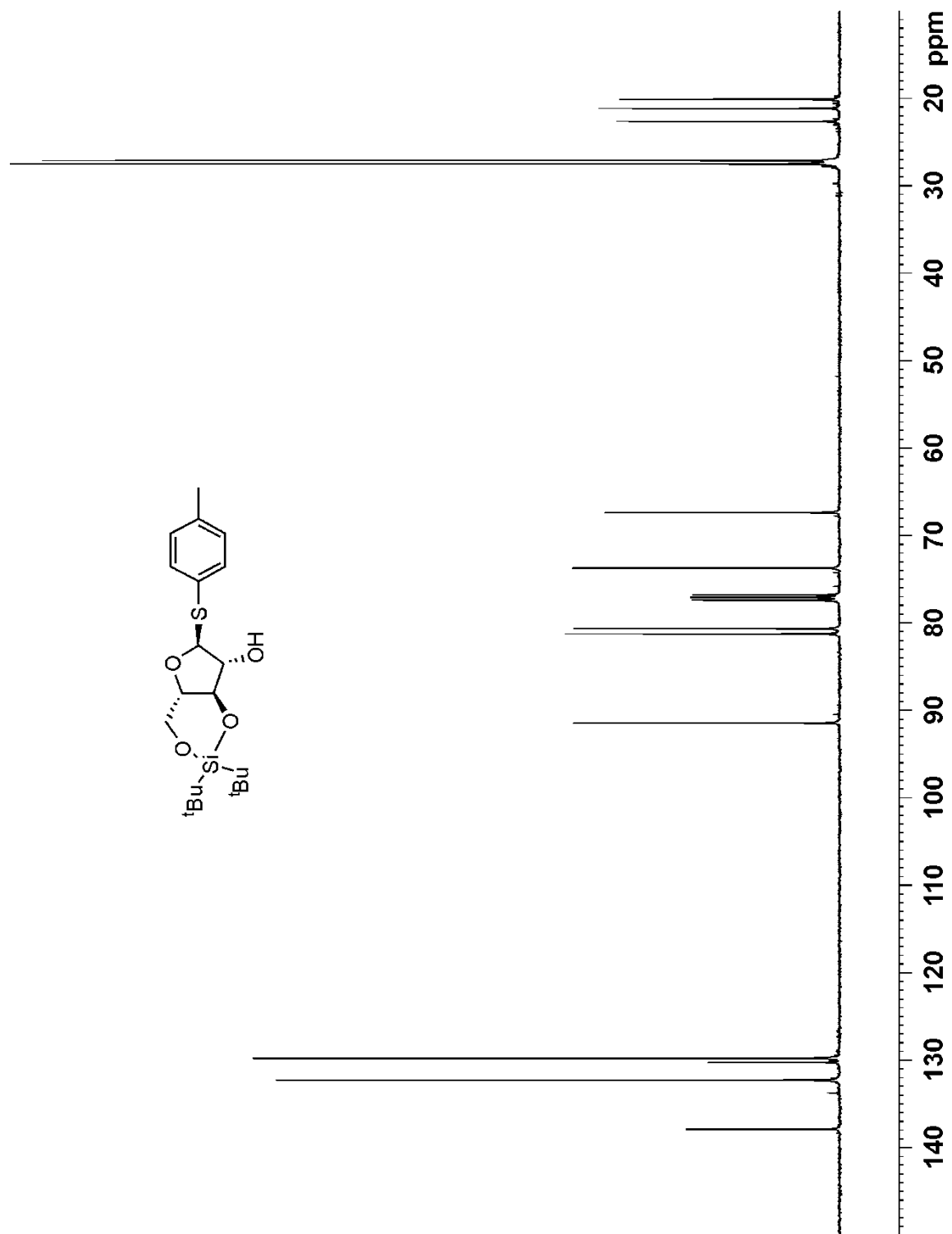
C- β (35.3, 35.5, 36.0, 36.6), C- δ (50.3, 51.0, 51.6, 51.7), C- α (56.6, 56.8, 57.8, 58.0), **CH**₂CH=CH₂ (65.7, 65.9), OCH₂Ph (72.0-72.8, 73.4, 73.5), furanose C5 73.3, C- γ (73.8, 74.3), (CH₃)₃**C**- (75.5, 76.8), furanose C2,3,4 (79.8-84.2), furanose C1 (98.5, 99.3, 98.9, 101.3), CH₂CH=**CH**₂ (118.4, 118.7), aromatic CH (127.7-128.6), CH₂**CH**=CH₂ (131.8, 131.9), aromatic -C- (137.4-138.2), NCOOR (153.7, 154.2), **COR** (171.4, 171.6, 171.4, 171.5); HRMS (ESI) calcd C₇₀H₈₀N₂O₁₅Na for (M+Na)⁺: 1211.5456; obsd: 1211.5412.

*signals in parentheses signify pairs of rotamers.

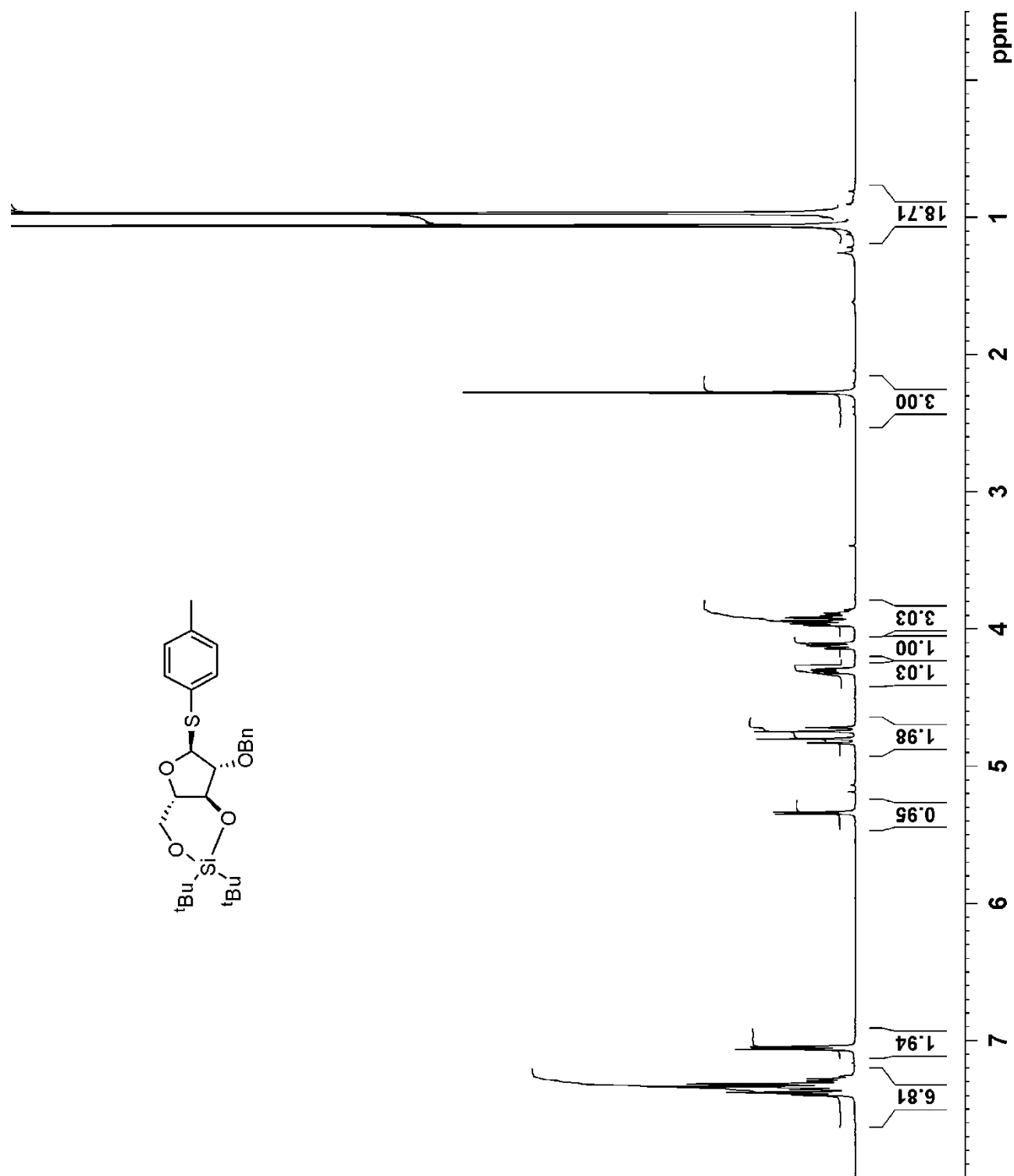
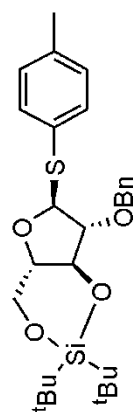
Compound **3** – thioglycoside triacetate – ^1H NMR spectrum - CDCl_3 , 400 MHz

Compound **3** – thioglycoside triacetate – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

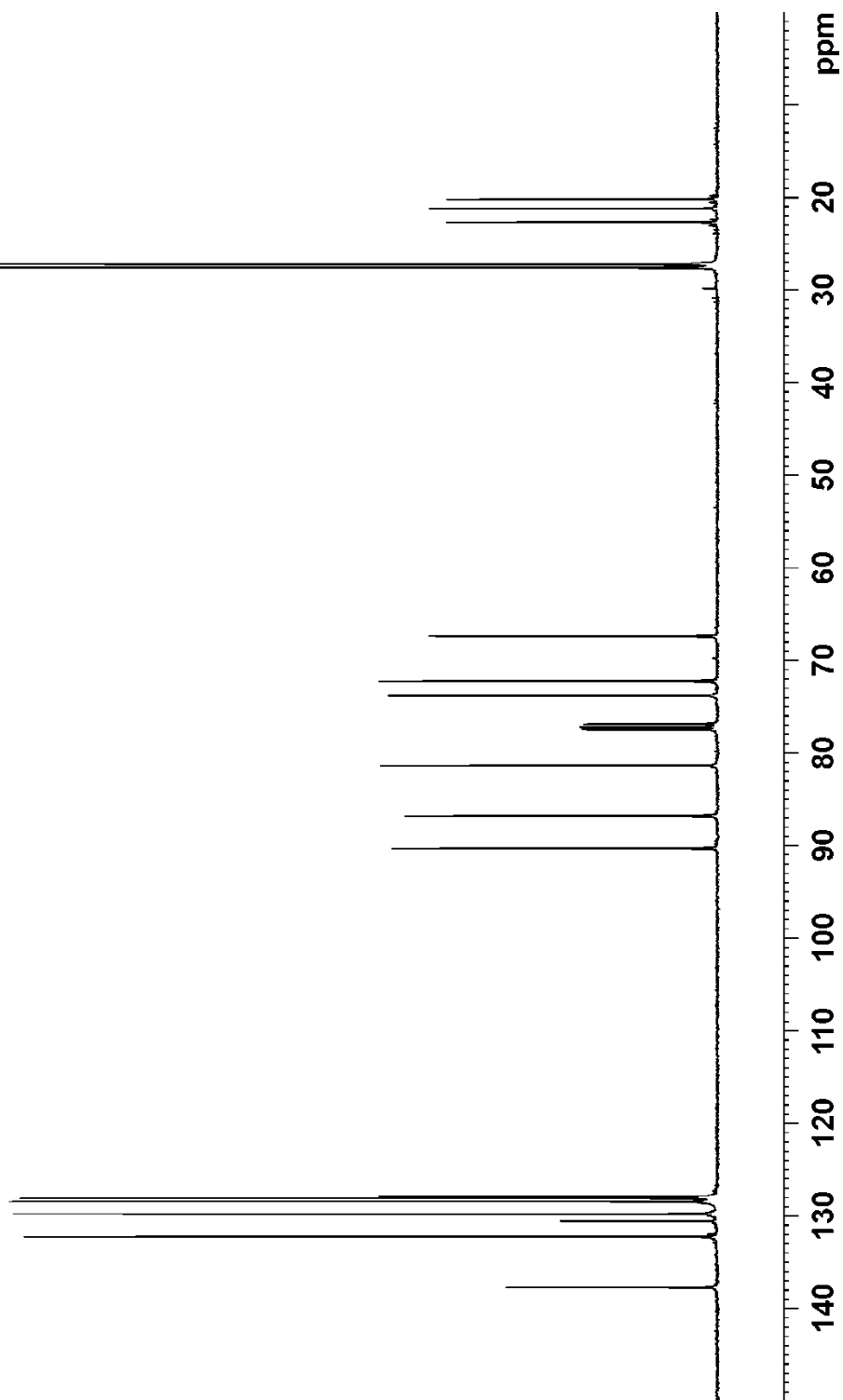
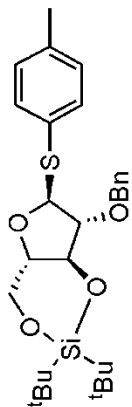
Compound **4** – silyl acetal – ^1H NMR spectrum - CDCl_3 , 400 MHz

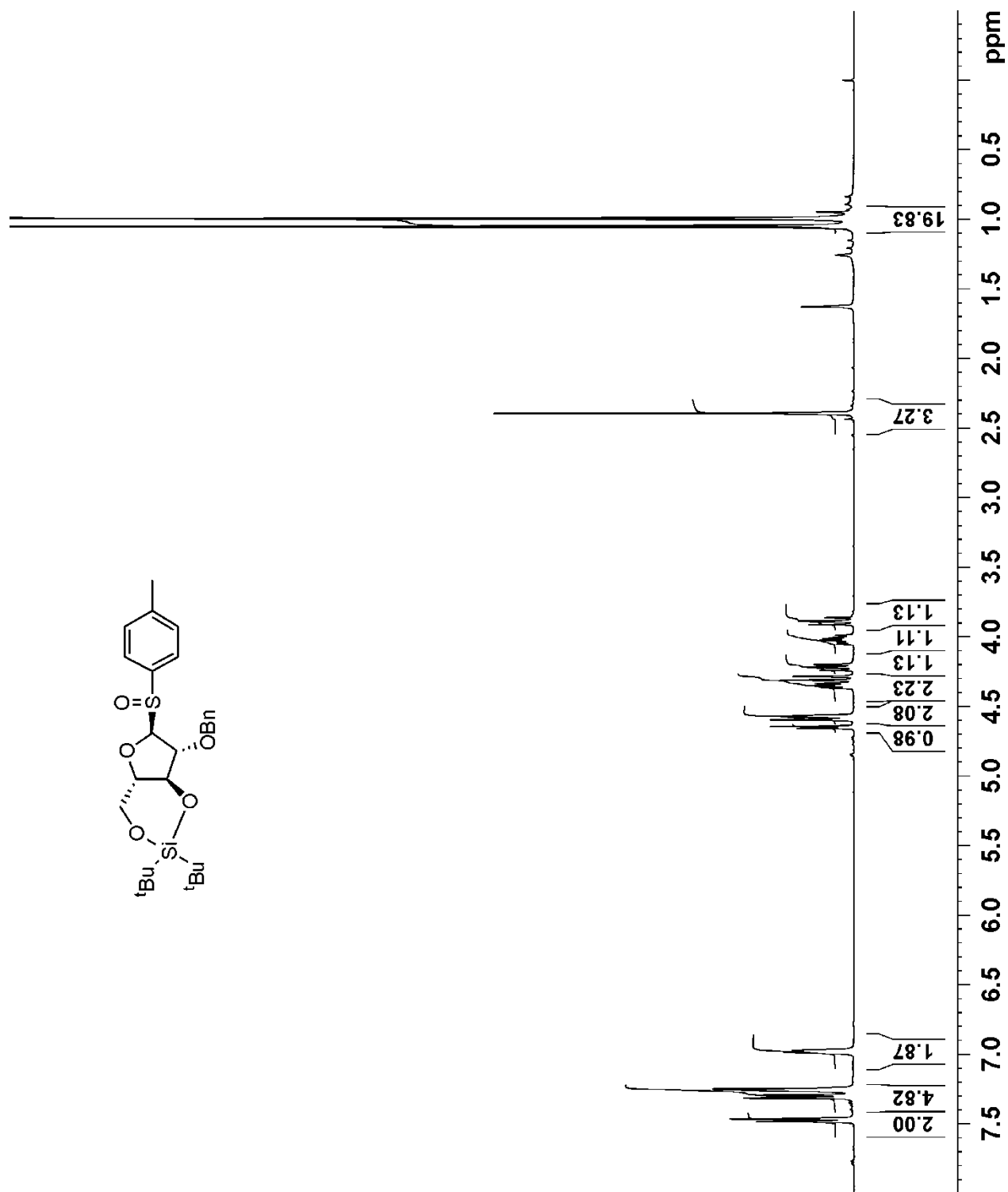
Compound **4** – silyl acetal – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

Compound **5** – silyl acetal, benzyl ether – ^1H NMR spectrum - CDCl_3 , 400 MHz

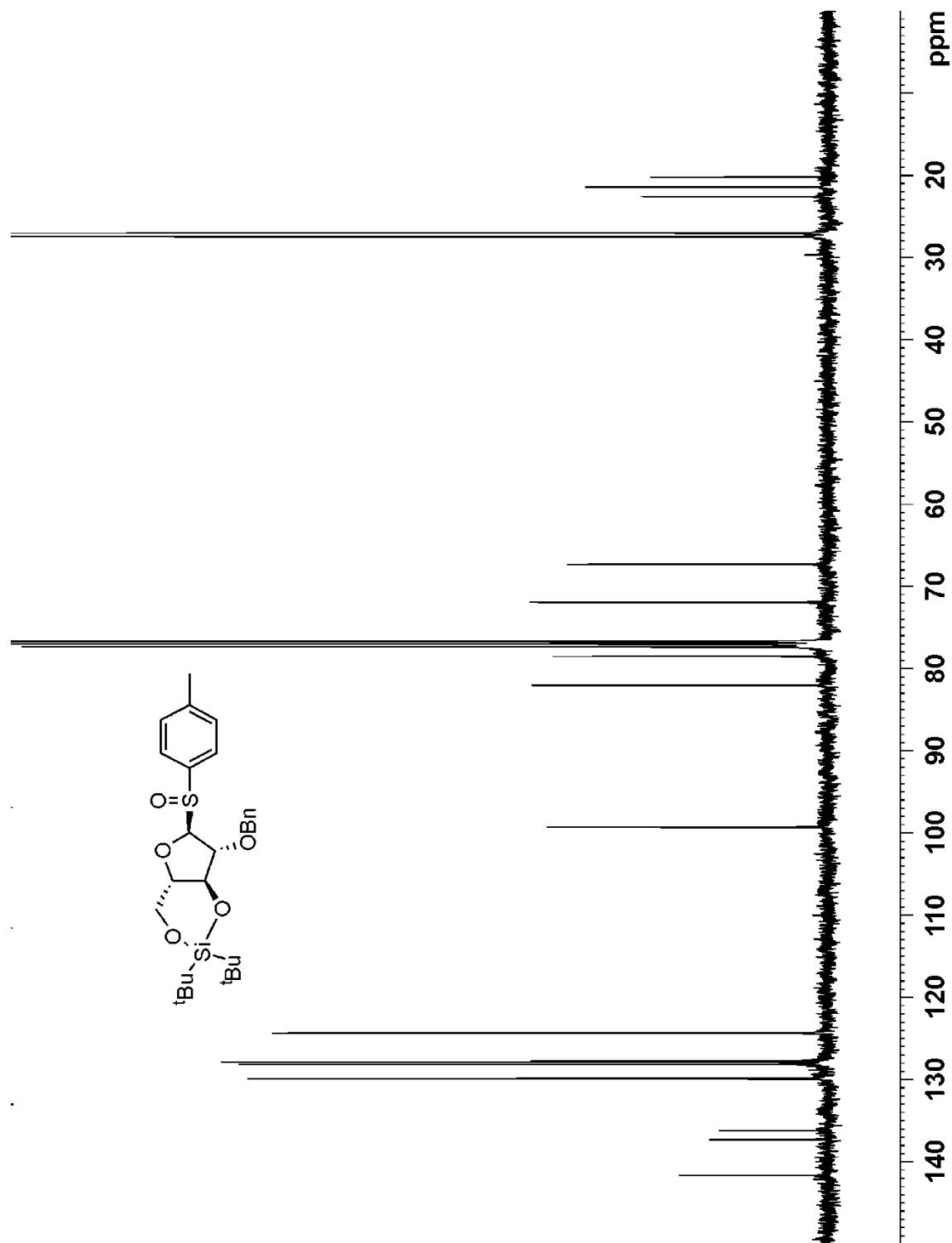


Compound **5** – silyl acetal, benzyl ether – ^{13}C NMR spectrum – CDCl_3 , 100 MHz

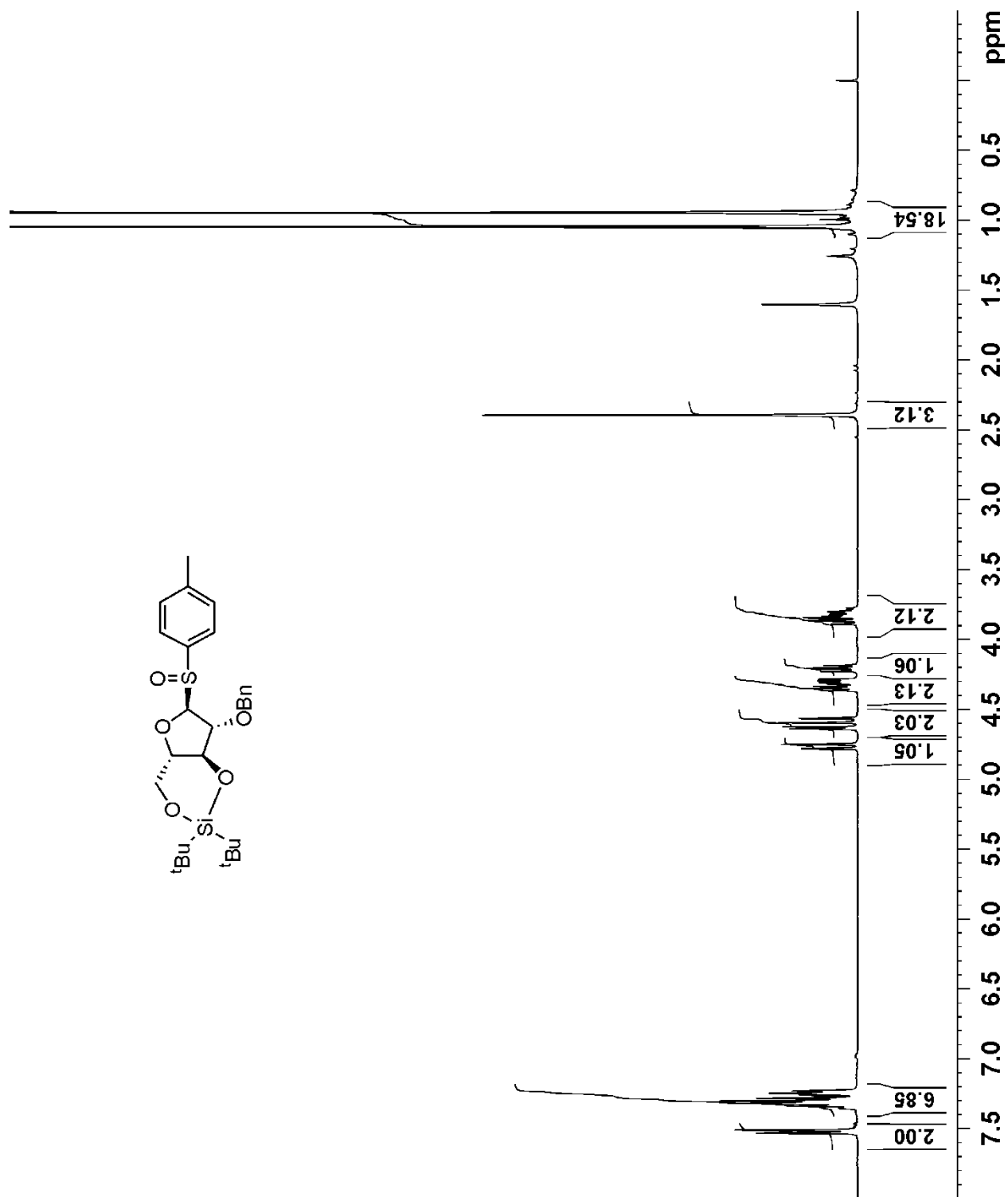
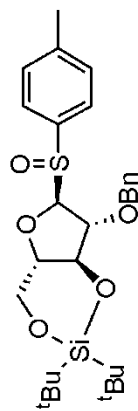


Compound **6A** – sulfoxide, first-eluting diastereomer – ^1H NMR spectrum – CDCl_3 , 400 MHz

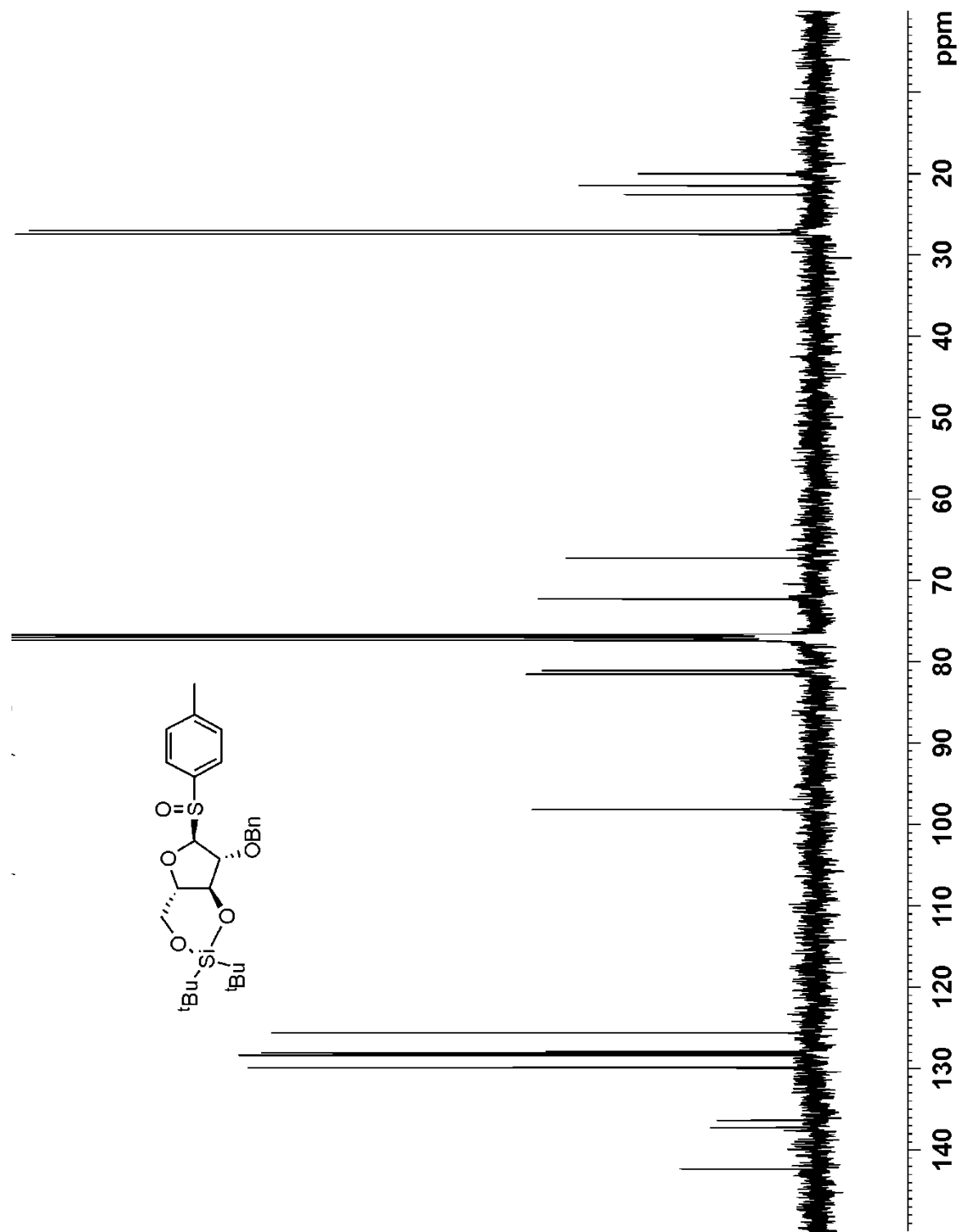
Compound **6A** – sulfoxide, first-eluting diastereomer – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

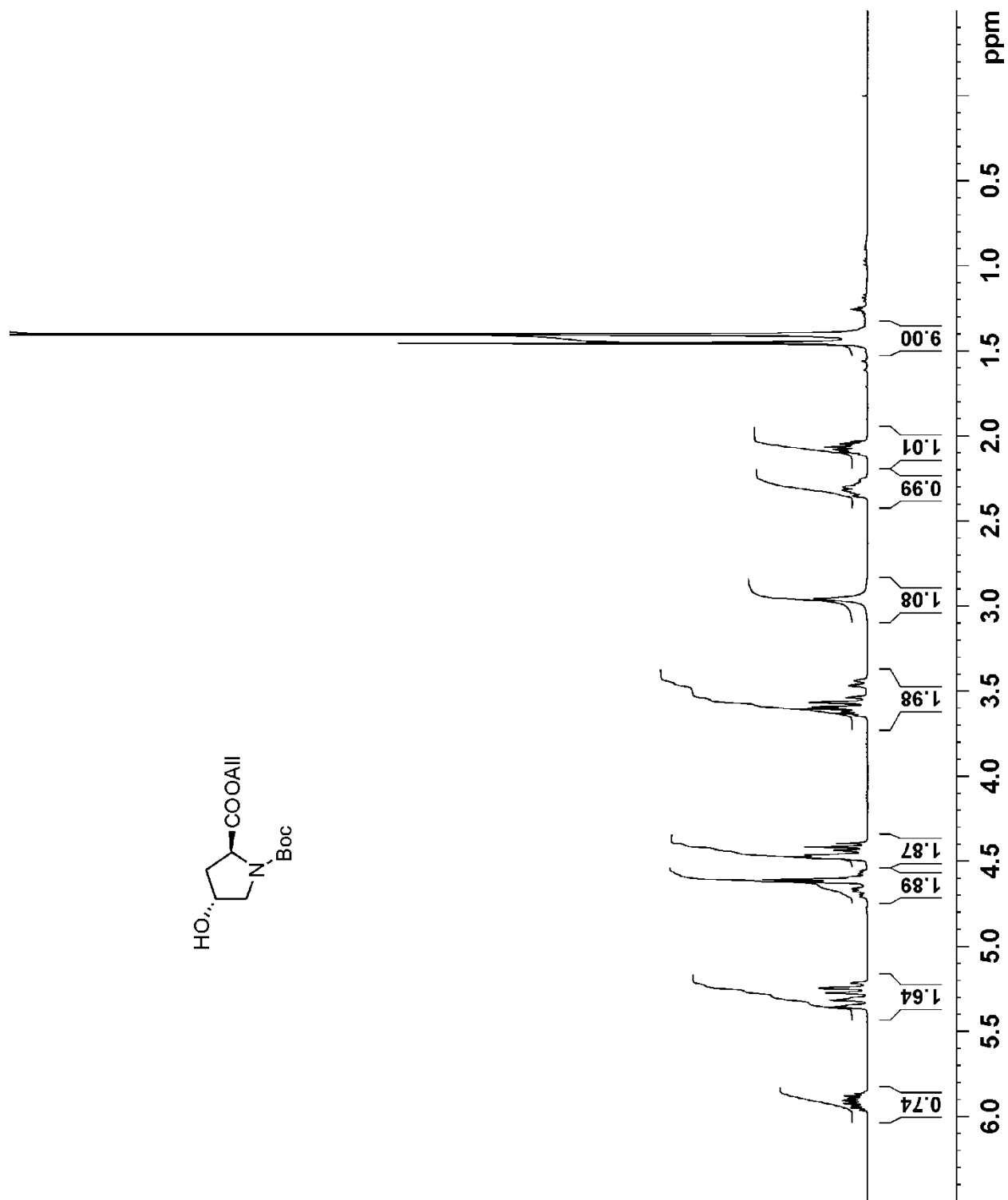


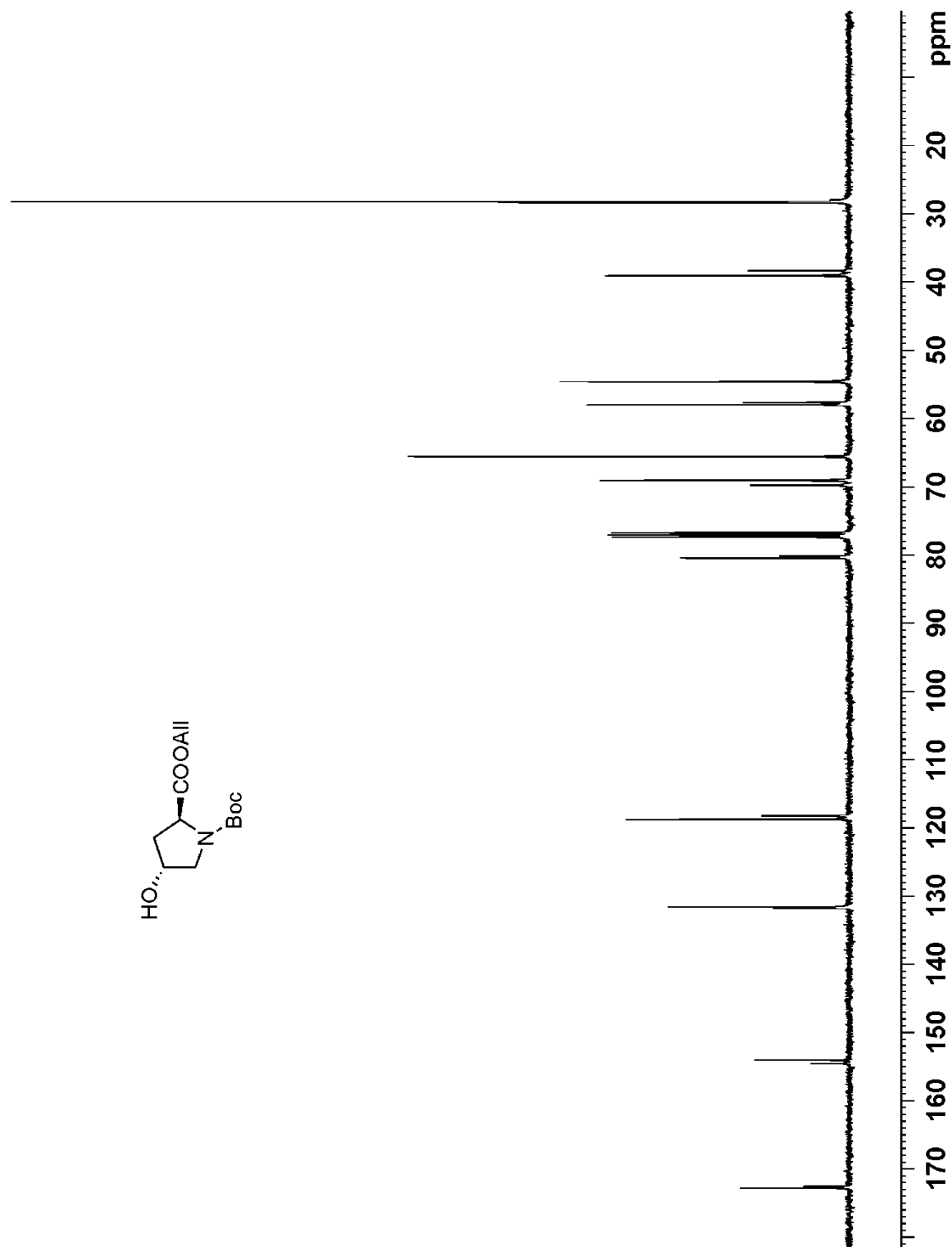
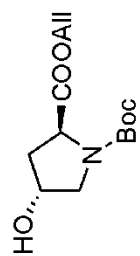
Compound **6B** – sulfoxide, second-eluting diastereomer – ^1H NMR spectrum – CDCl_3 , 400 MHz

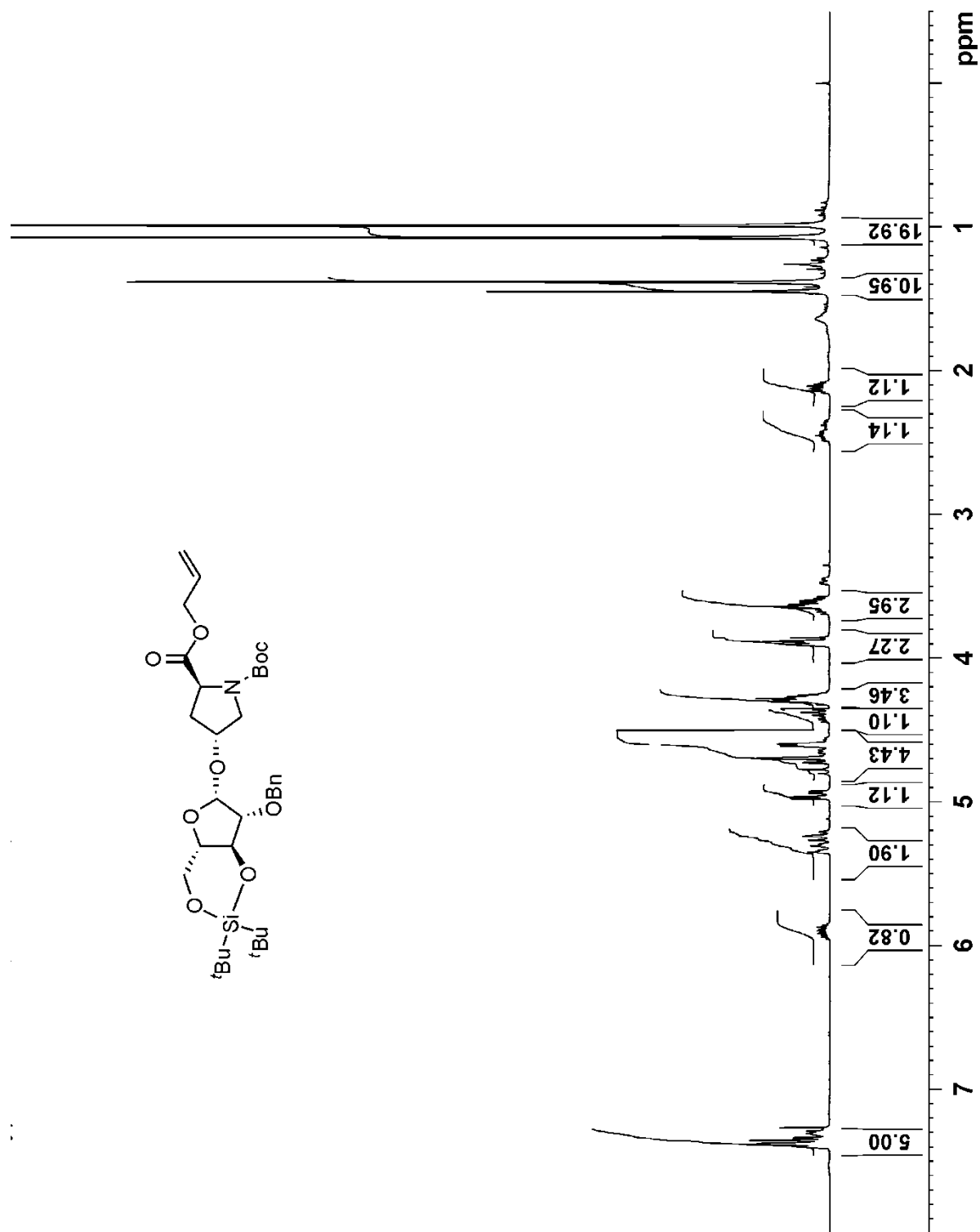


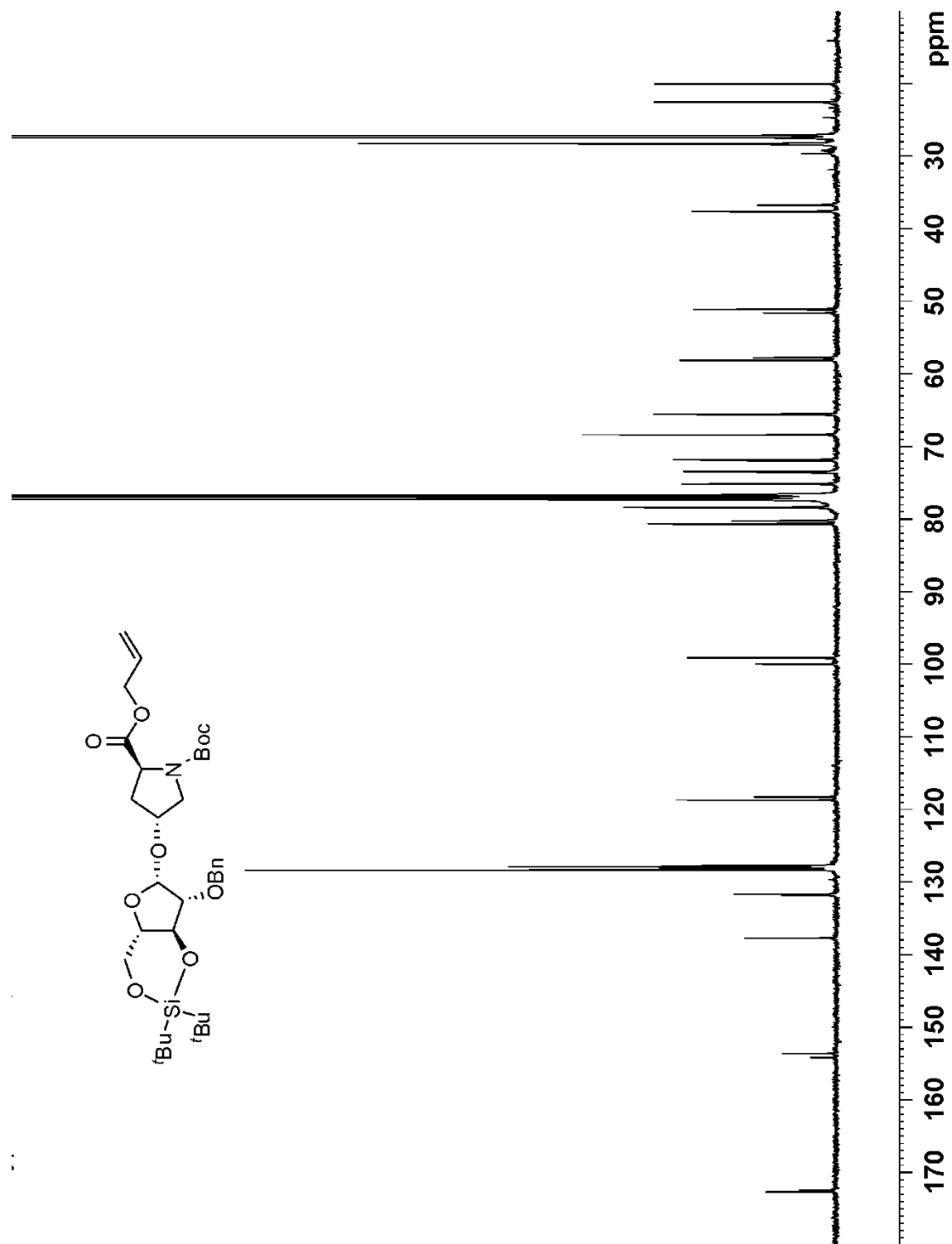
Compound **6B** – sulfoxide, second-eluting diastereomer – ^{13}C NMR spectrum - CDCl_3 , 100 MHz



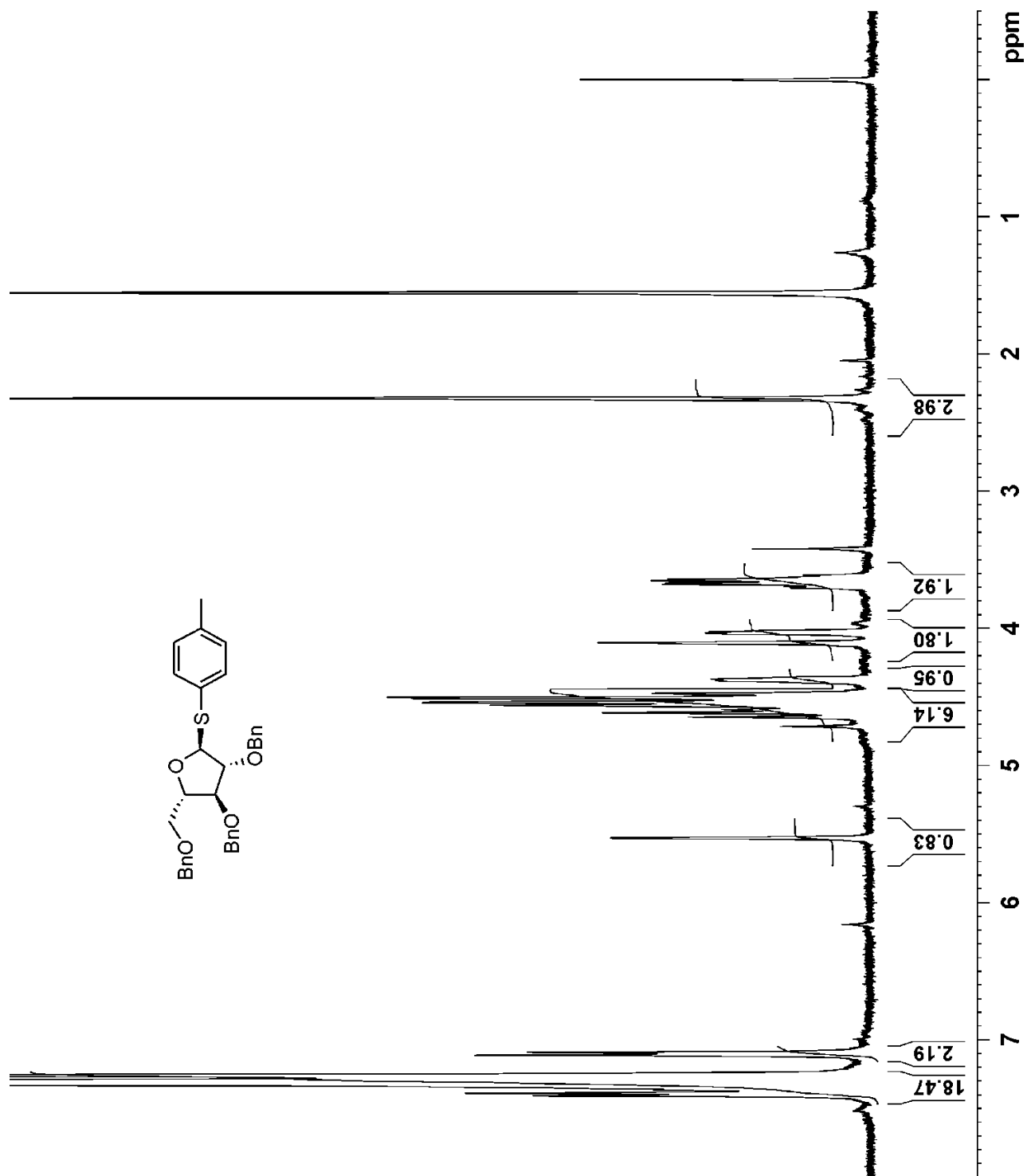
Compound **7** – Boc-Hyp-OAll – ^1H NMR spectrum - CDCl_3 , 400 MHz

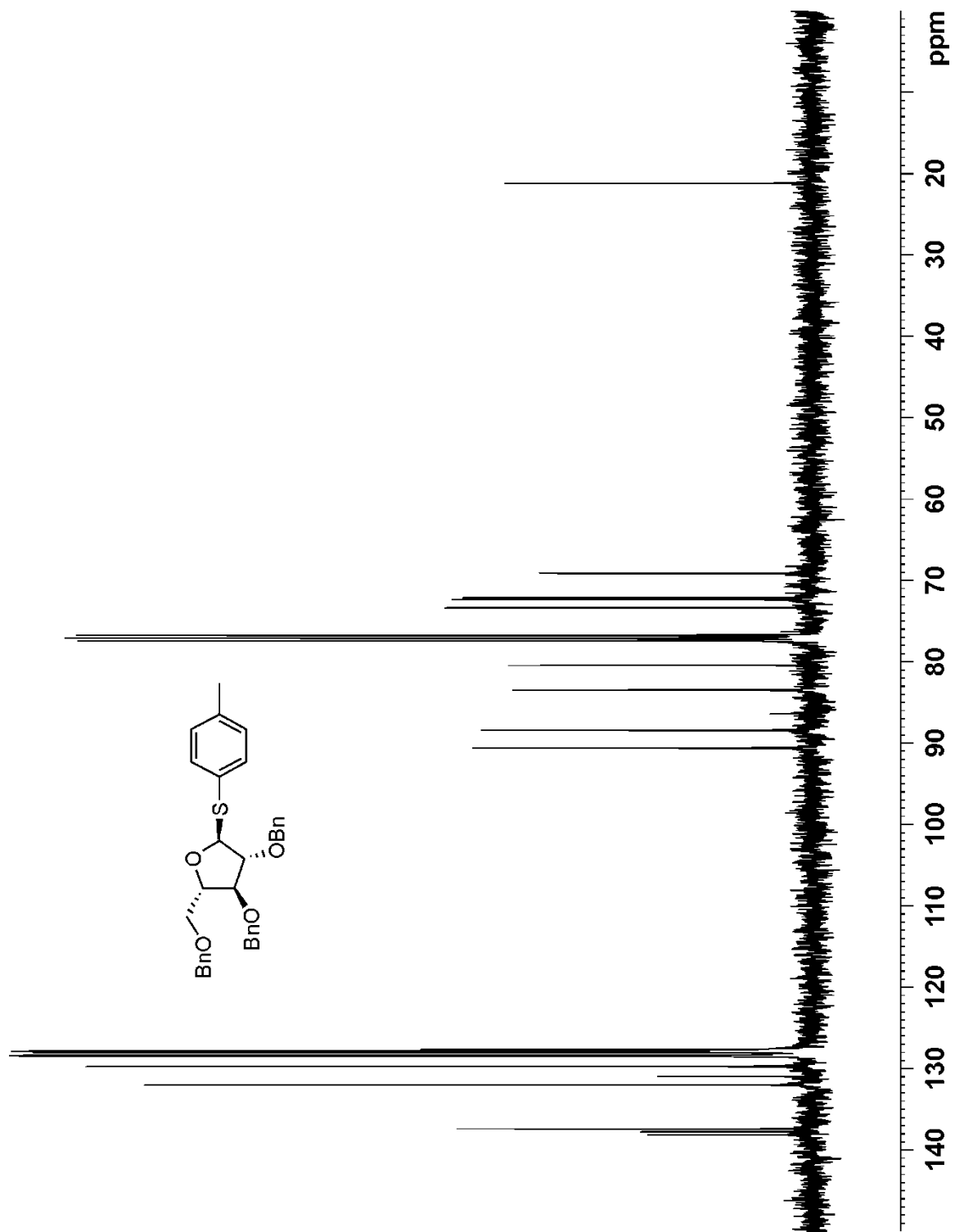
Compound **7** – Boc-Hyp-OAll – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

Compound **8 β** – beta-glycoside – ^1H NMR spectrum - CDCl_3 , 400 MHz

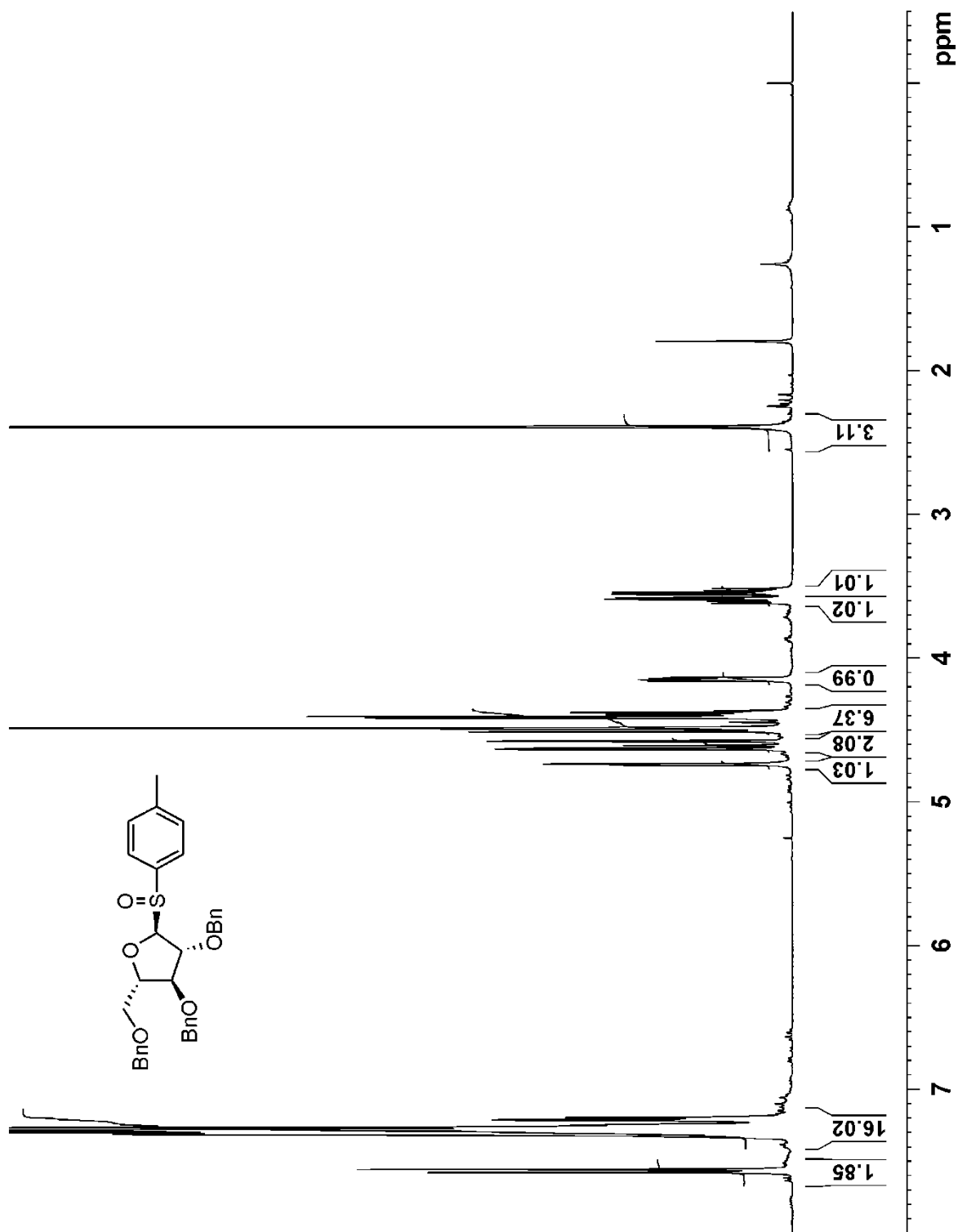
Compound **8 β** – beta-glycoside – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

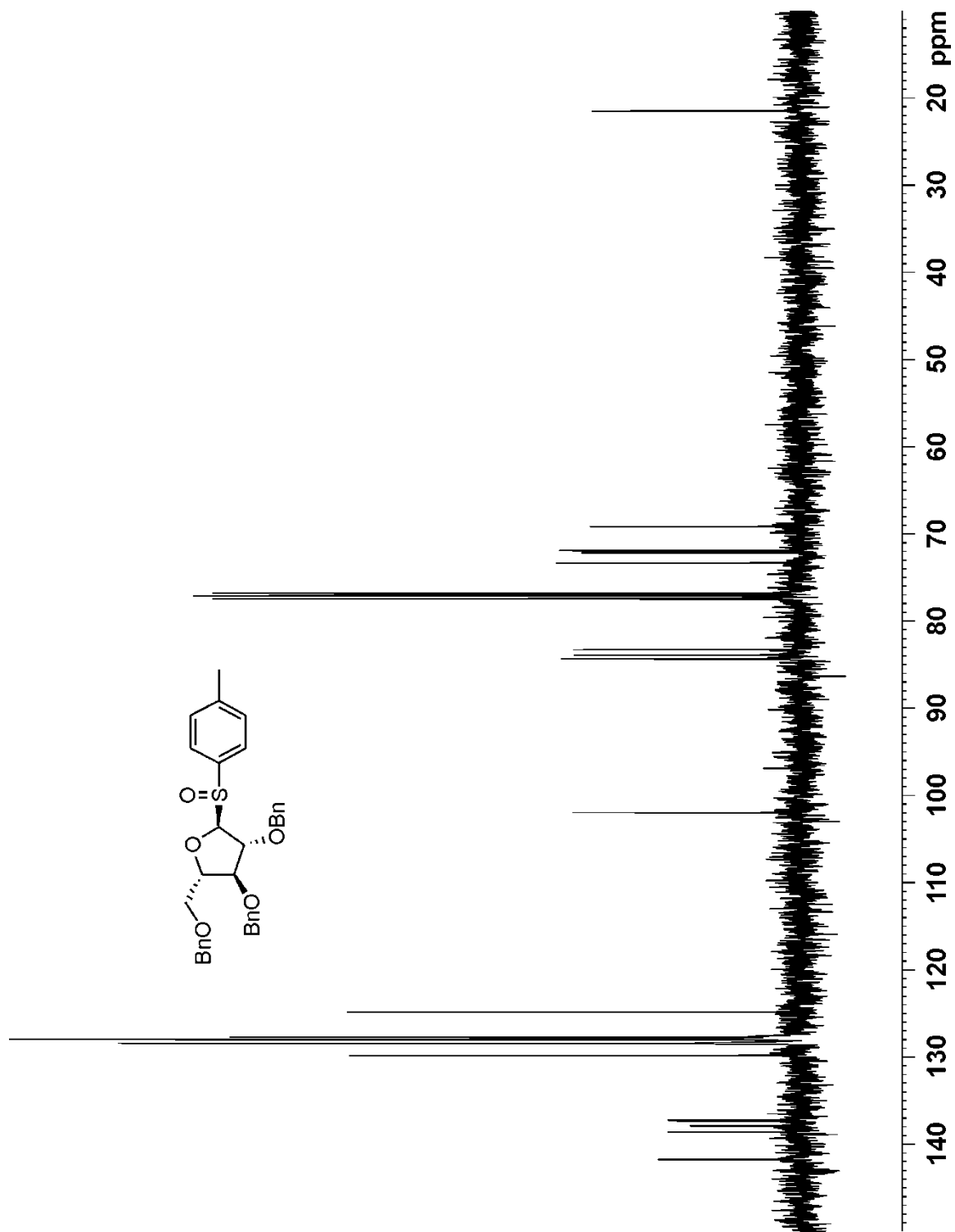
Compound **9** – perbenzylated thioglycoside – ^1H NMR spectrum - CDCl_3 , 400 MHz



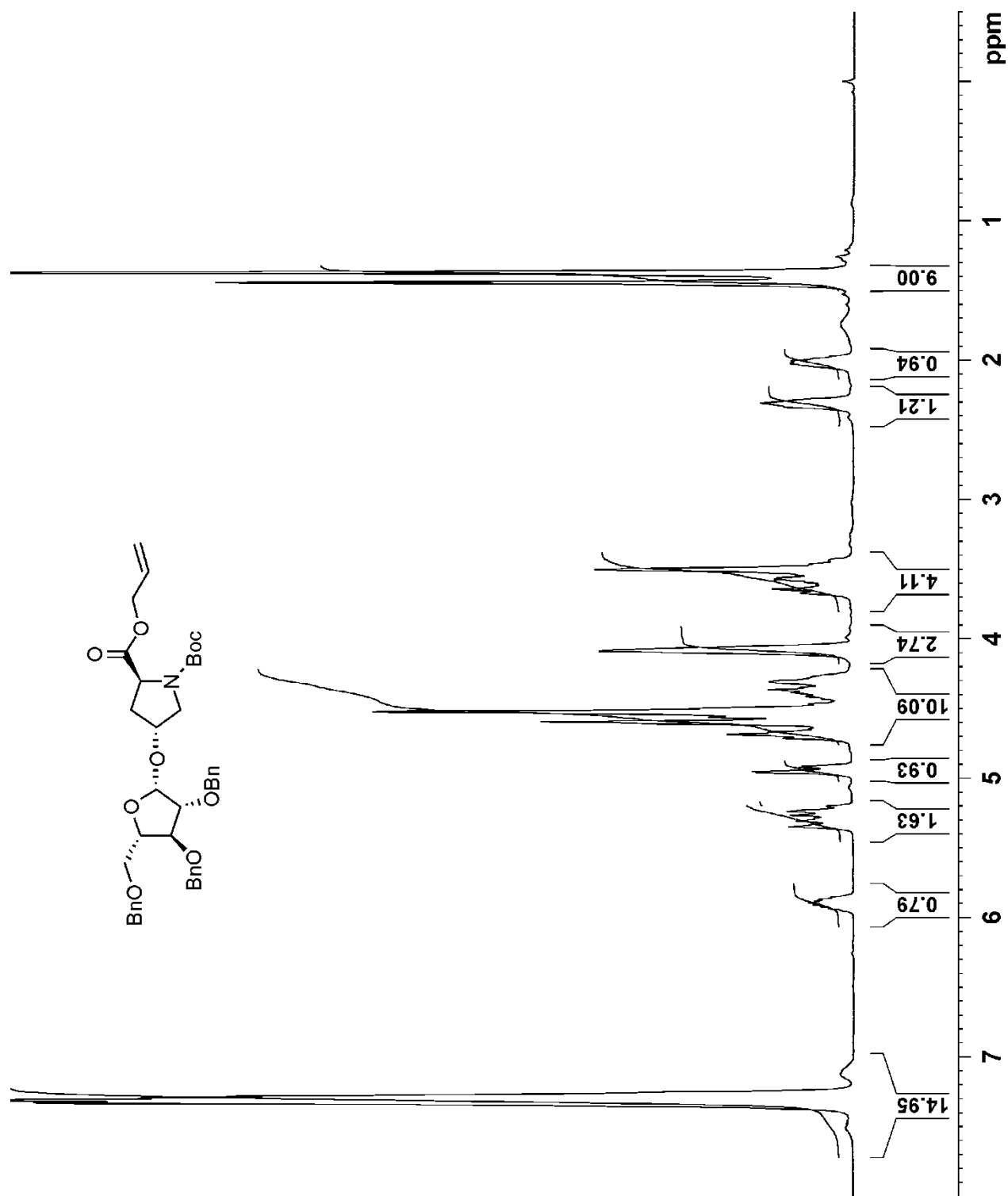
Compound **9** – perbenzylated thioglycoside – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

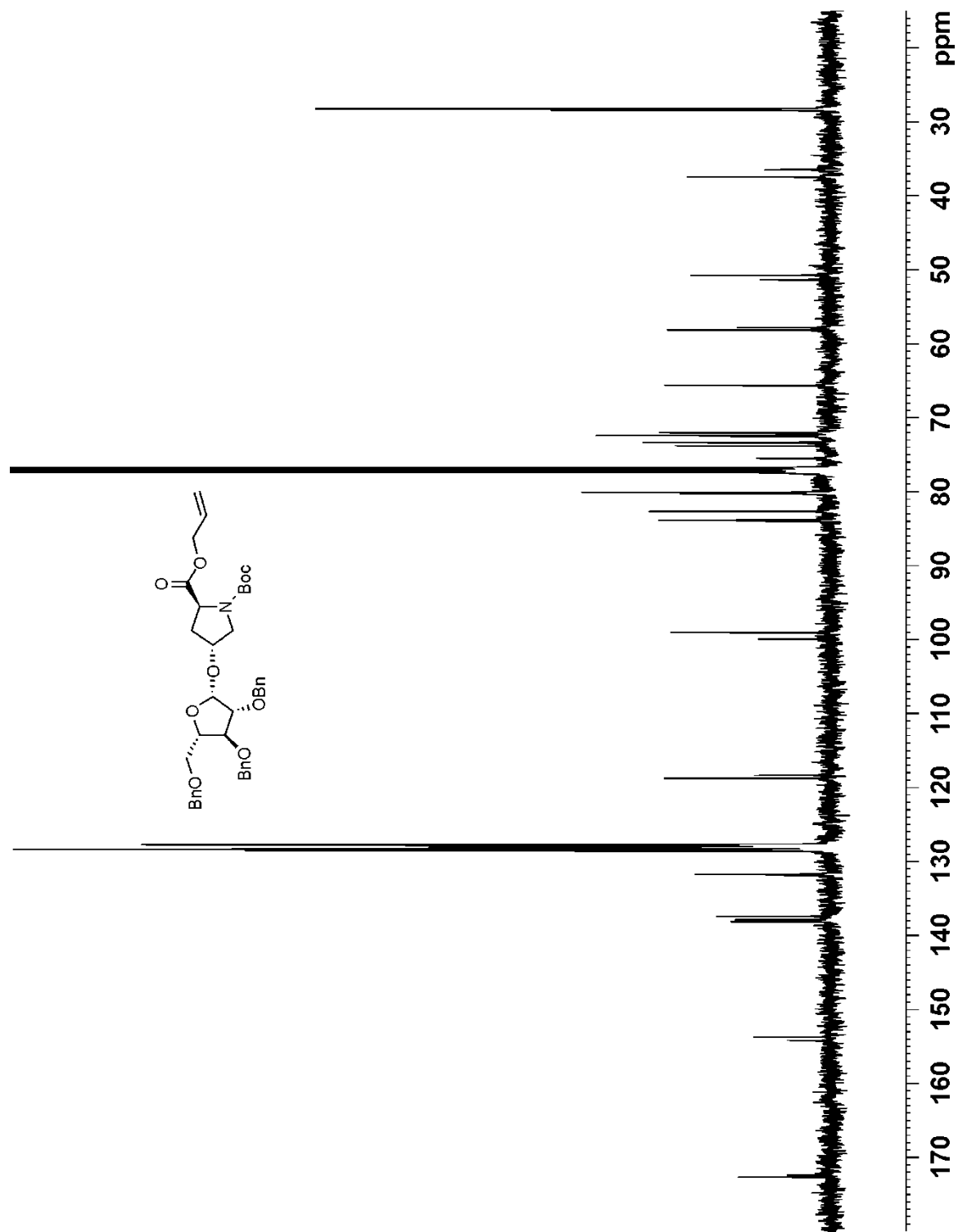
Compound **10** – perbenzylated sulfoxide – ^1H NMR spectrum - CDCl_3 , 400 MHz

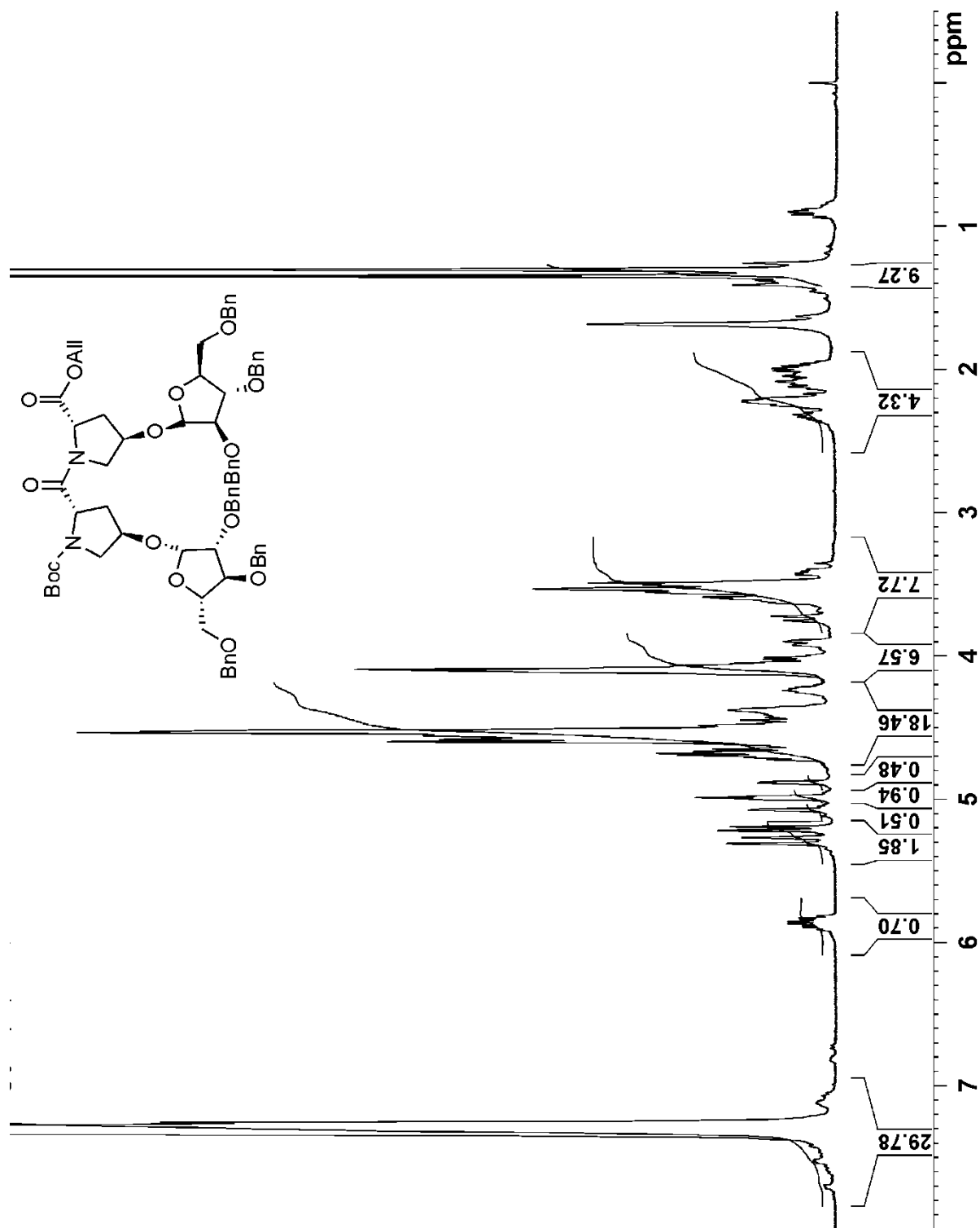


Compound **10** – perbenzylated sulfoxide – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

Compound **11β** – beta-glycoside – ^1H NMR spectrum – CDCl_3 , 400 MHz



Compound **11 β** – beta-glycoside – ^{13}C NMR spectrum - CDCl_3 , 100 MHz

Compound **1** – Boc-[(β -L-Araf)Hyp]₂-OAll – ¹H NMR spectrum - CDCl₃, 400 MHz

Compound **1** – Boc-[(β -L-Araf)Hyp]₂-OAll – ¹³C NMR spectrum - CDCl₃, 100 MHz