Synthesis of *C*-Pseudonucleosides Bearing Thiazolidin-4-one as Novel Potential Immunostimulating Agents

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1 Experimental procedure and data:

1.1 General Methods: Melting points were measured on an SGW® X-4 micro melting point apparatus and were uncorrected. Optical rotations were determined on an SGW®-1 automatic polarimeter. The CD spectra were measured on a Bio-Logic MOS-450/AF-CD. ^{1}H NMR, and ^{13}C NMR spectra were measured on a RT-NMR Bruker AVANCE 600M, NMR spectrometer using tetramethylsilane (Me_4Si) as an internal standard. Mass Spectra (MS) and High Resolution Mass Spectra (HRMS) were carried out on a FTICR-MS (Ionspec 7.0T) mass spectrometer with electrospray ionization (ESI). X-ray crystallographic measurements were made on a Bruker SMART CCD diffractometer. The optical densities for examining the activities of immunological activities were measured on a BioRad Model 3550 microplate spectrophotometer. Flow cytometry assay were analyzed on a FACSCalibur (BD Biosciences). Thin-layer chromatography (TLC) was performed on precoated plates (Qingdao GF_{254}) with detection by UV light or with phosphomolybdic acid in EtOH/H_2O followed by heating. Column chromatography was performed using reverse silica gel (C18, 50 μM).

Concanavalin A (type IV) and LPS were purchased from Sigma. Non-Radioactive Cytotoxicity Assay Kit was purchased from Promega. Mouse IL-4, IL-2, and IFN-γ measurement kits were obtained from Jingmei Company, Shenzhen, China. Other reagents were from commercial sources. Cell culture and cytokine assays experiments were carried out under sterile conditions.

1.2 General procedure for the synthesis of compounds 4 and 5:

The unprotected aldehyde **1** (0.162 g, 1 mmol) was dissolved in 3 mL anhydrous EtOH, to the solution, amine **2** (1 mmol) was added and the mixture was stirred in room temperature for 15-30 min. Then, mercaptoacetic acid **3** (0.14 ml, 2 mmol) was added. After continued stirring for 30 min in r. t., the mixture was neutralized with solid K_2CO_3. Solvent was evaporated under reduced pressure to get a crude product, which was purified using reverse silica (C_{18}) gel column chromatography (H_2O-MeOH V/V = 30:70 for **4**, **5(a-c)**, **4e** and **5e**, H_2O-MeOH V/V = 2:98 for **4d** and **5d**) to get the mixture of two diastereomers **4** and **5** (Table 1).

Table 1. The synthesis of *C*-pseudonucleosides **4** and **5**

Entry	R	Yield (%)[a]			
		Total	**4** (*S*)	**5** (*R*)	**4**:**5**
1	**a**	42	22	20	1.1
2	**b**	41	17	24	0.7
3	**c**	39	15	24	0.6
4	**d**	36	14	22	0.6
5	**e**	43	14	29	0.5

a: isolated yield.

(*S*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-methylthiazolidin-4-one **(4a)**: yellow syrup, $[\alpha]_D$ -8.4 (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 3.09 (3H, s, CH_3), 3.52 (1H, d, *J* =15.6 Hz, H-3), 3.66 (1H, dd, *J* =12.0 Hz, 4.8 Hz, H-5'), 3.72- 3.76 (2H, m, H-5, H-5'), 3.98-4.00 (1H, m, H-4'), 4.04 (1H, t, *J* =6.0 Hz, H-1'), 4.15 (1H, t, J =4.8 Hz, H-2'), 4.19 (1H, t, *J* =4.8Hz, H-3'), 4.96 (1H, dd, *J* =6.6 Hz, 2.4Hz, H-2); δ_C (125MHz, CD_3OD): 31.0, 31.3, 61.7 (C-2), 64.6, 77.3, 78.4, 84.8, 85.7, 172.4; HRMS (ESI): Calcd for $C_9H_{15}NO_5SNa$ $(M+Na)^+$, 272.0568, Found: 272.0563.

(*R*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-methylthiazolidin-4-one **(5a)**: white solid, mp 178 ^oC, $[\alpha]_D$ +82.2 (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 2.99 (3H, s, CH_3), 3.40 (1H, d, *J* =15.0 Hz, H-5), 3.62 (1H, dd, *J* =12.6 Hz, 4.8 Hz, H-5'), 3.74 (1H, d, *J* =14.4Hz, H-5'), 3.78 (1H, dd, *J* =12.6 Hz, 4.8 Hz, H-5), 3.91-3.94 (1H, m, H-4'), 4.07-4.10 (2H, m, H-2', H-1'), 4.22 (1H, d, *J* =6.6 Hz, H-3'), 4.87 (1H, s, H-2); δ_C (125MHz, CD_3OD): 29.2, 32.0, 61.4 (C-2), 66.2, 76.1, 77.8, 80.3, 83.8, 172.9 (C-4); HRMS (ESI): Calcd for $C_9H_{15}NO_5SNa$ $(M+Na)^+$, 272.0568, Found: 272.0559.

(*S*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-butylthiazolidin-4-one **(4b)**: yellow syrup, $[\alpha]_D$ -57.4 (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 0.91 (3H, s, *J* =7.2Hz, CH_3), 1.24-1.31 (2H, m, CH_2), 1.52-1.60 (2H, m, CH_2), 3.28 -3.32 (1H, m, CH), 3.42 (1H, d, *J* =15.6Hz, H-5), 3.58 (1H, dd, *J* = 14.0Hz, 5.4Hz, H-5), 3.65 (1H, t, *J* = 5.4Hz, H-1'), 3.76 (1H, dd, *J* =7.8 Hz, 3.6 Hz, CH), 3.71-3.76 (1H, m, H-5'), 3.89-3.91 (1H, m, H-4'), 3.95 (1H, t, *J* = 5.4 Hz, H-5'), 4.05 (1H, t, *J* =4.8Hz, H-2'), 4.08 (1H, t, *J* =4.2Hz, H-3'), 4.94 (1H, dd, *J* = 6.6 Hz, 1.8Hz, H-2); δ_C (125 MHz, CD_3OD): 12.7, 19.6, 28.6, 31.3, 44.0, 61.7 (C-2), 62.1, 77.4, 78.7, 85.1, 86.3, 172.6 (C-4); HRMS (ESI): Calcd for $C_{12}H_{21}NO_5SNa$ $(M+Na)^+$, 314.1038, Found: 314.1033.

(*R*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-butylthiazolidin-4-one **(5b)**: yellow syrup, $[\alpha]_D$ +35.1 (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 0.98 (3H, *J* =7.2 Hz, CH_3), 1.31-1.41 (2H, m, CH_2), 1.56-1.67 (2H, m, CH_2), 3.08-3.12 (1H, m, CH), 3.37 (1H, d, *J* =15.6 Hz, H-5), 3.60 (1H, dd, *J* =12.0 Hz, 4.8 Hz, H-5), 3.72-3.77 (3H, m, CH, H-1', H-5'), 3.89-3.93 (1H, m, H-4'), 4.04-4.08 (2H, m, H-5', H-2'), 4.17 (1H, d, J =6.6Hz, H-3'), 4.91 (1H, s, H-2); δ_C (125MHz, CD_3OD): 12.7, 19.6, 28.8, 32.5, 42.2, 61.4 (C-2), 64.1, 76.0, 77.9, 80.6, 83.8, 172.9 (C-4); HRMS (ESI): Calcd for $C_{12}H_{21}NO_5SNa$ $(M+Na)^+$, 314.1038, Found: 314.1035.

(*S*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-octylthiazolidin-4-one **(4c)**: colorless syrup, $[\alpha]_D$ -20.9 (c 1.0,CH_3OH), δ_H (600 MHz, CD_3OD): 0.94 (3H, t, *J* =6.6Hz, CH_3), 1.33-1.36 (10H, m, $5CH_2$), 1.61-1.69 (2H, m, CH_2), 3.34 -3.35 (1H, m, CH); 3.49 (1H, d, *J* =15.6 Hz, H-5), 3.65 (1H, dd, *J* =13.2 Hz, 7.2Hz, H-5'), 3.72-3.75 (2H, m, CH, H-5), 3.78-3.83 (1H, m, H-4'), 3.98 (1H, q, J =5.4Hz, H-1'), 4.03(1H, t, *J* = 4.8 Hz, H-2'), 4.15(1H, q, *J* =4.8Hz, H-3'), 5.01 (1H, dd, J =7.2 Hz, 4.8 Hz, H-2); δ_C (125MHz, CD_3OD); 13.2, 22.4, 26.5, 29.0, 29.1, 31.4, 31.7, 44.3, 61.9 (C-2), 62.2, 77.5, 78.9, 85.4, 86.6, 172.6 (C-4); HRMS (ESI): Calcd for $C_{16}H_{29}NO_5SNa$ $(M+Na)^+$, 370.1664, Found: 370.1657.

(*R*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-octylthiazolidin-4-one **(5c)**: colorless syrup, $[\alpha]_D$ +38.1, (c 1.0,CH_3OH), δ_H (600 MHz, CD_3OD): 0.92 (3H, t, *J* =6.6Hz, CH_3), 1.32-1.35 (10H, m, $5CH_2$), 1.56-1.69 (2H, m, CH_2), 3.07-3.11(1H, m, CH), 3.37 (1H, d, *J* =15.6 Hz, H-5), 3.65 (1H, dd, J =12.0Hz, 4.8Hz, H-5'), 3.67-3.77 (3H, m, CH, H-5, H-5'), 3.89-3.92 (1H, m, H-4'), 4.03-4.08

(2H, m, H-2', H-1'), 4.16 (1H, d, *J* =6.6Hz, H-3'), 4.90 (1H, s, H-2); δ_C (125 MHz, CD_3OD): 13.0, 22.3, 26.4, 26.6, 28.9, 28.9, 31.5, 32.4, 42.45, 61.5(C-2), 64.1, 76.1, 77.8, 80.6, 83.8, 172.9 (C-4); HRMS (ESI): Calcd for $C_{16}H_{29}NO_5SNa$ $(M+Na)^+$, 370.1664, Found: 370.1665.

(*S*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-octadecylthiazolidin-4-one **(4d)**: yellow solid, mp 43-45 ^oC, $[\alpha]_D$ -7.0 (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 0.92 (3H, t, *J* = 7.2 Hz, CH_3), 1.31-1.37 (30H, m, $15CH_2$), 1.61-1.70 (2H, m, CH_2), 3.34-3.39 (1H, m, CH), 3.47 (1H, d, *J* =15.6Hz, H-5), 3.64 (1H, dd, J =12.0Hz, 5.4 Hz, H-5), 3.65-3.71 (1H, m, H-1'), 3.73 (1H, t, *J* =3.6 Hz, H-5'), 3.77-3.82 (1H, m, CH), 3.96-3.98 (1H, m, H-4'), 4.02 (1H, t, *J* =5.4 Hz, H-5'), 4.11 (1H, t, *J* =4.2 Hz, H-2'), 4.14 (1H, t, *J* = 4.8Hz, H-3'), 5.00 (1H, dd, J =7.2 Hz, 1.8 Hz, H-2); δ_C (125 MHz, CD_3OD): 13.1, 22.3, 26.4, 28.9, 29.1, 29.3, 29.4, 29.4, 31.3, 31.7, 44.3, 61.8 (C-2), 62.0, 77.4, 78.7, 85.2, 86.5, 172.5 (C-4); HRMS (ESI): Calcd for $C_{26}H_{49}NO_5SNa$ $(M+Na)^+$, 510.3229, Found: 510.3232.

(*R*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-octadecylthiazolidin-4-one **(5d)**: white solid, mp 98 ^oC, $[\alpha]_D$ +10.4 (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 0.93 (3H, t, *J* = 6.6 Hz, CH_3), 1.32-1.36 (30H, m, $15CH_2$), 1.58-1.68 (2H, m, CH_2, H-5), 3.31-3.38 (2H, m, H-1', H-5), 3.59-3.82 (3H, m, CH, H-5', H-1), 3.86-3.94 (1H, m, H-4'), 4.06-4.09 (1H, m, H-2'), 4.17-4.19 (1H, m, H-3'), 4.91 (1H, s, H-2); δ_C (125 MHz, CD_3OD): 13.1, 22.2, 26.6, 28.9, 29.1, 29.4, 29.7, 31.3, 32.5, 42.3, 61.5 (C-2), 64.1, 76.0, 78.8, 83.6, 84.5, 172.5 (C-4); HRMS (ESI): Calcd for $C_{26}H_{49}NO_5SNa$ $(M+Na)^+$, 510.3229, Found: 510.3225.

(*S*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-phenylthiazolidin-4-one **(4e)**: white solid, mp 168-170 ^oC, $[\alpha]_D$ -54.4, (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 3.51 (1H, dd, *J* =12.0Hz, 4.8Hz, H-5), 3.62 (1H, dd, *J* =12.0Hz, 3.6Hz, H-5'), 3.66 (1H, d, *J* =15.6Hz, H-5), 3.74-3.77 (1H, m, H-4'), 3.95-3.99 (2H, m, H-1', H-5'), 4.05 (1H, dd, *J* =7.8 Hz, 4.2 Hz, H-2'), 4.30 (1H, t, *J* = 6.6 Hz, H-3'), 5.55 (1H, dd, *J* =4.8 Hz, 1.8 Hz, H-2), 7.33-7.36 (1H, m, CH), 7.44-7.48 (4H, m, 4CH); δ_C (125 MHz, CD_3OD): 32.0, 61.7 (C-2), 65.3, 77.1, 77.5, , 82.8, 83.6, 126.4, 127.3, 128.8, 138.2, 172.1 (C-4); HRMS (ESI): Calcd for $C_{14}H_{18}NO_5S$ $(M+H)^+$, 312.0905, Found: 312.0908.

(*R*)-2-((2*S*,3*S*,4*S*,5*R*)-tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-3-phenylthiazolidin-4-one **(5e)**: white solid, mp 171-173 °C, $[\alpha]_D$ +93.3 (c 1.0, CH_3OH), δ_H (600 MHz, CD_3OD): 3.59 (1H, d, *J* = 15.6 Hz, H-5), 3.63 (1H, dd, *J* =12.0 Hz, 5.4Hz, H-5'), 3.64 (1H, dd, *J* =12.6 Hz, 2.4Hz, H-5), 3.96- 4.02 (4H, m , H-5', H-4', H-1', H-2'), 4.10 (1H, t, *J* = 7.8 Hz, H-3'), 5.34 (1H, s, H-2), 7.40-7.46 (3H, m, 3CH), 7.51 (2H, t, *J* = 8.4 Hz, 2CH); δ_C (125 MHz, CD_3OD): 32.8, 61.4 (C-2), 67.3, 76.3, 77.7, 80.8, 83.9, 126.9, 127.8, 129.2, 137.2, 172.6 (C-4); HRMS (ESI): Calcd for $C_{14}H_{18}NO_5S$ $(M+H)^+$, 312.0905, Found: 312.0910.

1.3 Immunological activities assay

1.3.1 Preparation and cultivation of splenocytes

The spleens from BALb/c mice were taken out in sterile conditions and soaked in non-serum RPMI-1640 cell culture medium. The spleens were grinded using a wire mesh. Filter the cell suspension with a 200-mesh nylon net. The filtrate of the splenocytes was centrifuged at 2000 g for 10 min and then the supernatant was removed. Dissolving the precipitation in 5 mL of pH 7.2 Tris-NH_4Cl solution and incubating the cells at 37 °C for 6-10 min in order to lyse the red cells. Then the cells were centrifuged at 2000 g for 7 min and the cell pellets were dissolved in RPMI-1640 culture medium with 10% Newborn Calf Serum and 20 μM ConA or 10 μg/mL LPS. Counting cell and adjusting the concentration of cells solution to 5×10^6/ml. Add 4.5×10^5 cells into each well of 96 well plates. Subsequently adding different concentration of each compound into each well, and incubating at 37 °C, 5% CO_2 for 72 hrs. The supernatant was collected and centrifuged at 2000 g for 5 min. The supernatant was collected and stored at -20 °C until for assay.

1.3.2 Measurement of the Proliferation of T cells and B cells

Splencytes from BALb/C mice were aseptically removed and minced, and cell suspensions were incubated at 4.5×10^5 cell/well，90 μL/well in 96-well microtiter plates using an RPMI 1640 medium with 10% FCS. Spleen cells were cultured with 20 μM of Con A or 10 μg/mL Lps for 72 h at 37 °C in 5% CO_2 in the presence or absence of the tested compounds. Wells containing Con A or LPS without tested compounds were used as blanks. All the tests were performed at least three times in quadruplicate ($P<0.01$). Cell proliferation was measured using the MTT assay, testing OD (A) at wavelength 570 nm. Cell proliferation (%)= $(A_{treated}-A_{control}) / A_{control} \times 100\%$.

1.3.3 Measurement of the secretion of IL-2, IL-4, and IFN-γ from splenocytes

Mouse splenocytes were pretreated with Con A at the final concentration of 20 μM at 37 ^{o}C for 72 hours in medium containing 10% newborn bovine serum (NBS) and 5% CO_2. 96-well plates were coated with anti-mouse IFN-γ, IL-2, and IL-4 MAb in advance (commercial products). Different concentrations of compounds and IL-2, IL-4, or IFN-γ standards (500, 250, 125, 62.5, 31.25, 15.63 pg/ml) were added into each well. Incubating at 20-25 ^{o}C for 120 min. The levels of IFN-γ, IL-2, and IL-4 secreted from immunized mice splenocytes were detected using the cytokine-specific ELISA Kits (R & D). Standard curves were determined using known concentration of the IL-2, IL-4, or IFN-γ. According to the standard curve, the amount of the samples was then determined.

1.3.4 Flow cytometry assay

Briefly, 5 μl of FITC conjugated rat anti-mouse CD3, CD4, CD8 and CD19 antibodies (Caltag Laboratories) were incubated with each anti-coagulated whole blood sample of 1×10^6 cells with heparin (25U/ml) in the dark for 20 min at room temperature. Red blood cells were lysed with whole blood lysing reagents (Beckman Coulter). The cells were then analyzed by two-color flow cytometry on a FACSCalibur (BD Biosciences). All experimental protocol were performed according to the manufacturer's instructions strictly. FITC conjugated rat IgG2 isotype controls were used as isotype matched negative controls. The stained cells were analyzed on FACSCalibur flow cytometer and CellQuest software (BD Biosciences).

1.4 Single-crystal X-ray crystallographic analysis of 5a (Figure 1)

A single crystal of compound **5a** was obtained by recrystallization from the solution of CH_3OH. X-ray diffraction data for a crystal were performed with graphite-monochromated Mo-Kα radiation (0.71073Å) on a Bruker APEX II diffractometer and collected by the ω/2θ scan technique at 296(2) K. The crystal structures were solved by direct methods. All non-hydrogen atoms were refined anisotropically by full- matrix least-squares methods on F^2. All calculations were performed using the programs SH ELXS-97 and SHELXL-97.The intensity data were collected with the ω/2θ scan technique at 296(2) K using the h/2x scan technique from a single crystal of $0.55 \times 0.41 \times 0.38$ mm, and a semi empirical absorption correction was applied for all complexes. The crystal system was Orthorhombic, and the space group was P2(1)2(1) 2(1). The structures were solved by direct methods and refined by full-matrix

least-squares on F^2. The absolute structure parameter was 0.00(6).

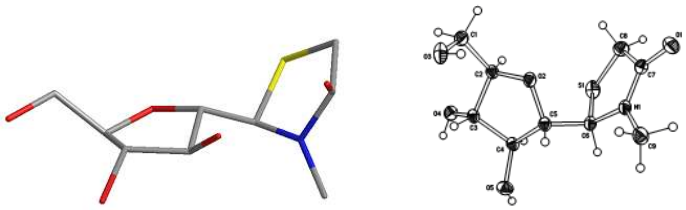
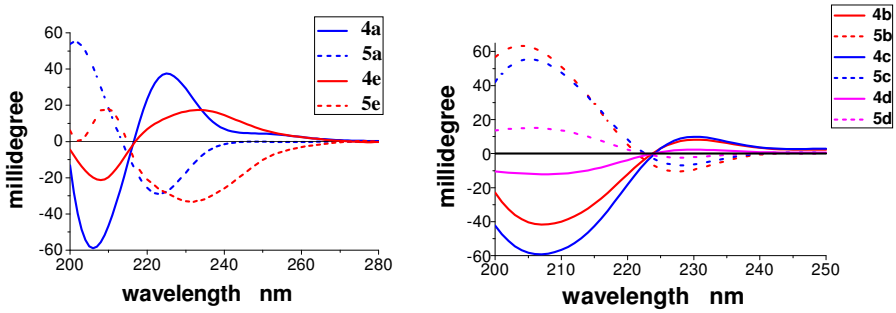


Figure 1. X-ray crystallographic structures of **5a.**

Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC no. 820672. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via: www.ccdc.cam.ac.uk)

1.5 The circular dichroism (CD) spectra of compounds 4 and 5

CD measurements were performed on a a Bio-Logic MOS-450/AF-CD using a quartz cuvettes of 1 cm optical path length and over a wavelength range of 200-280 at 1 nm bandwidth, 1 nm step size, and 1 s time per point. The compounds at a final concentration of 1 mg/mL was resolved in methanol to be tested (**Figure 2**). The CD spectra were obtained by taking the average of at least three scans at room temperature. Final analysis of the data was carried out using Origin 7.0 (OriginLab Corp.).



A: **4a**, **5a**, **4e**, and **5e** **B**: **4b**, **5b**, **4c**, **5c**, **4d**, and **5d**

Figure 2. The CD spectra of compounds **4** and **5**

1.6 Purity of target compounds (Table 2)

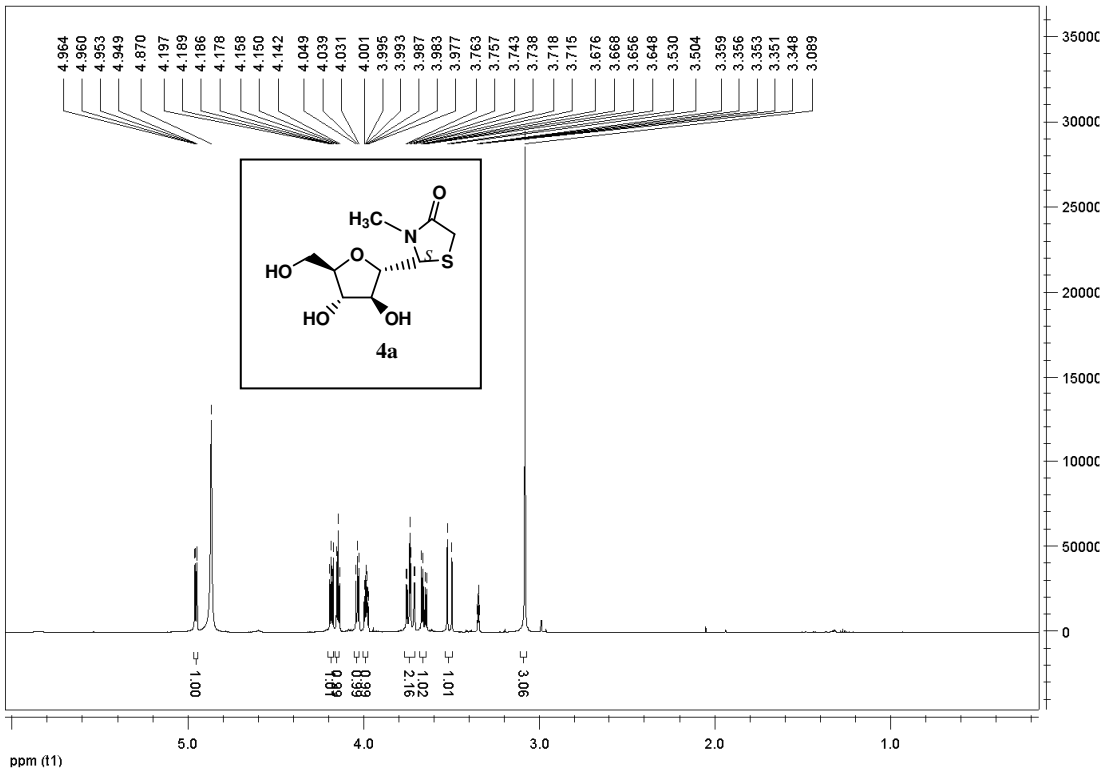
General method for HPLC: Analytical HPLC was performed on an Agilent HP-1100, with UV detection at 220 nm at a flow rate of 0.5 ml/min on BaseLine C18 reverse column (10 μm, 250mm×4.6 mm) column with methanol-water as mobile

phase. Condition A: methanol-water V/V 47:53. Condition B: methanol-water V/V 42:58. Condition C: methanol-water V/V 98:2.

Table 2. HPLC purity

Compds	R_t (min)	Purity	Condition	Compds	R_t (min)	Purity	Condition
4a	5.68	98.5%	A	**5a**	4.74	98.9%	A
4b	12.30	98.1%	A	**5b**	11.38	98.3%	A
4c	21.39	97.5%	B	**5c**	19.92	97.1%	B
4d	18.84	92.4%	C	**5d**	18.34	92.6%	C
4e	8.41	98.6%	A	**5e**	7.67	99.2%	A

1.6 Spectra copies of compounds 4 and 5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4.039
4.031
4.001
3.995
3.993
3.987
3.983
3.977
3.763
3.757
3.743
3.738
3.718
3.715
3.676
3.668
3.656
3.648
3.530
3.504
3.359
3.356
3.353
3.351
3.348
3.089
4a
1.00
0.97
0.89
0.89
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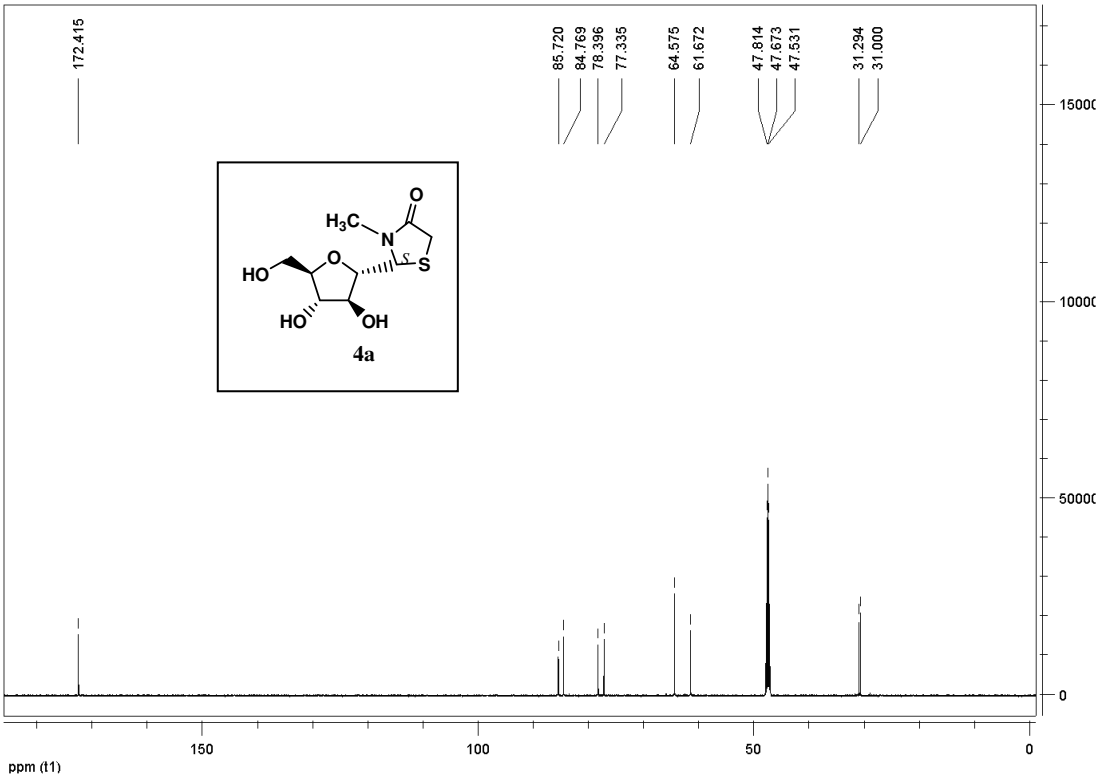
2.16
1.01
3.06
5.0
4.0
3.0
2.0
1.0
ppm (t1)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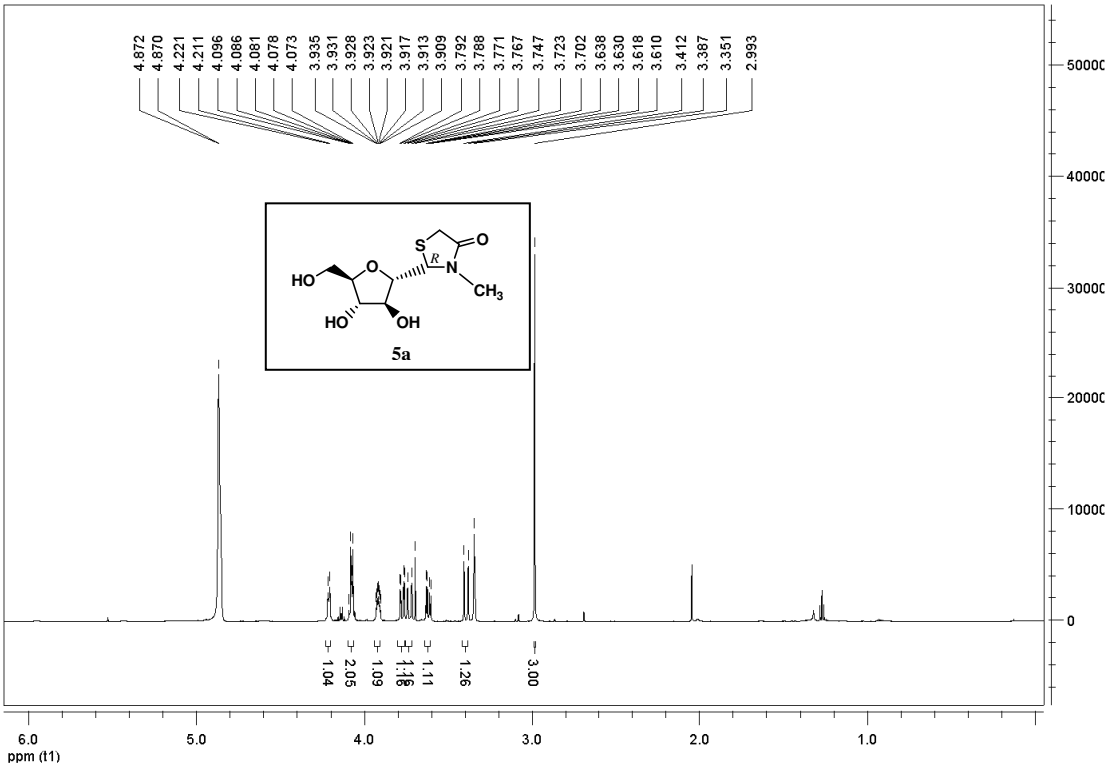
84.769
78.396
77.335
64.575
61.672
47.814
47.673
47.531
31.294
31.000
4a
150
100
50
0
ppm (t1)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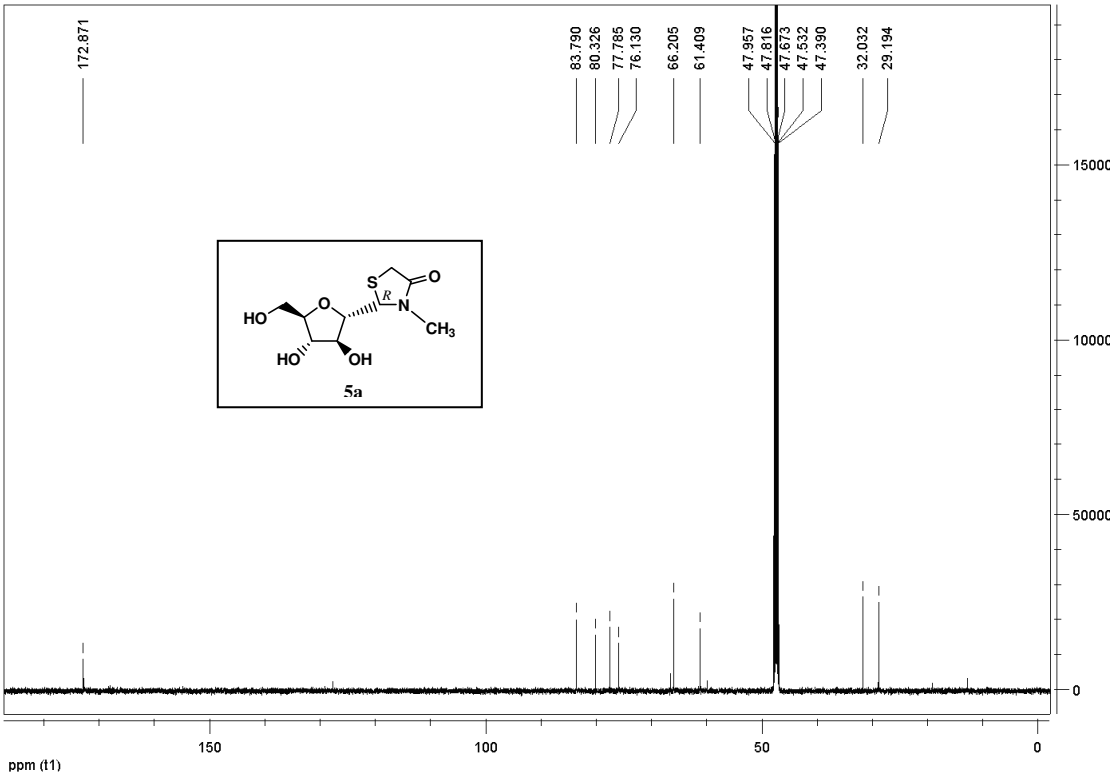
4.870
4.221
4.211
4.096
4.086
4.081
4.078
4.073
3.935
3.931
3.928
3.923
3.921
3.917
3.913
3.909
3.792
3.788
3.771
3.767
3.747
3.723
3.702
3.638
3.630
3.618
3.610
3.412
3.387
3.351
2.993
HO
O
S
R
N
CH3
HO
OH
5a
1.04
2.05
1.09
1.16
1.11
1.26
3.00
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)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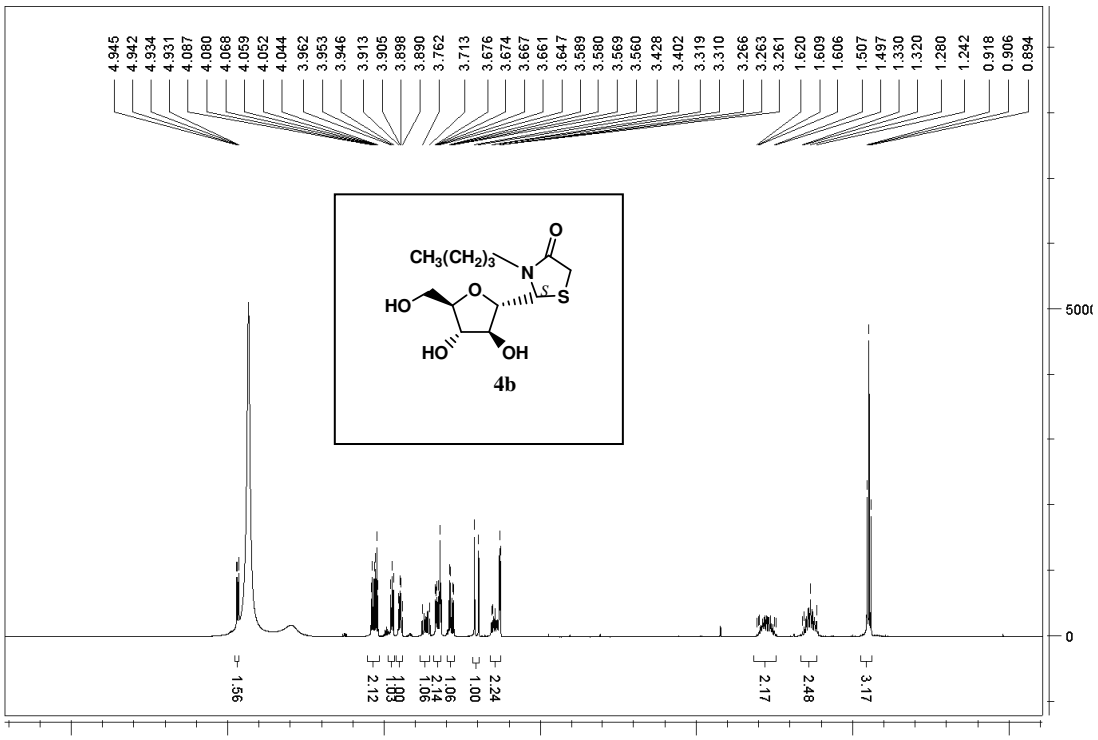
83.790
80.326
77.785
76.130
66.205
61.409
47.957
47.816
47.673
47.532
47.390
32.032
29.194
HO
O
S
R
N
CH3
HO
OH
5a
150
100
50
0
ppm (t1)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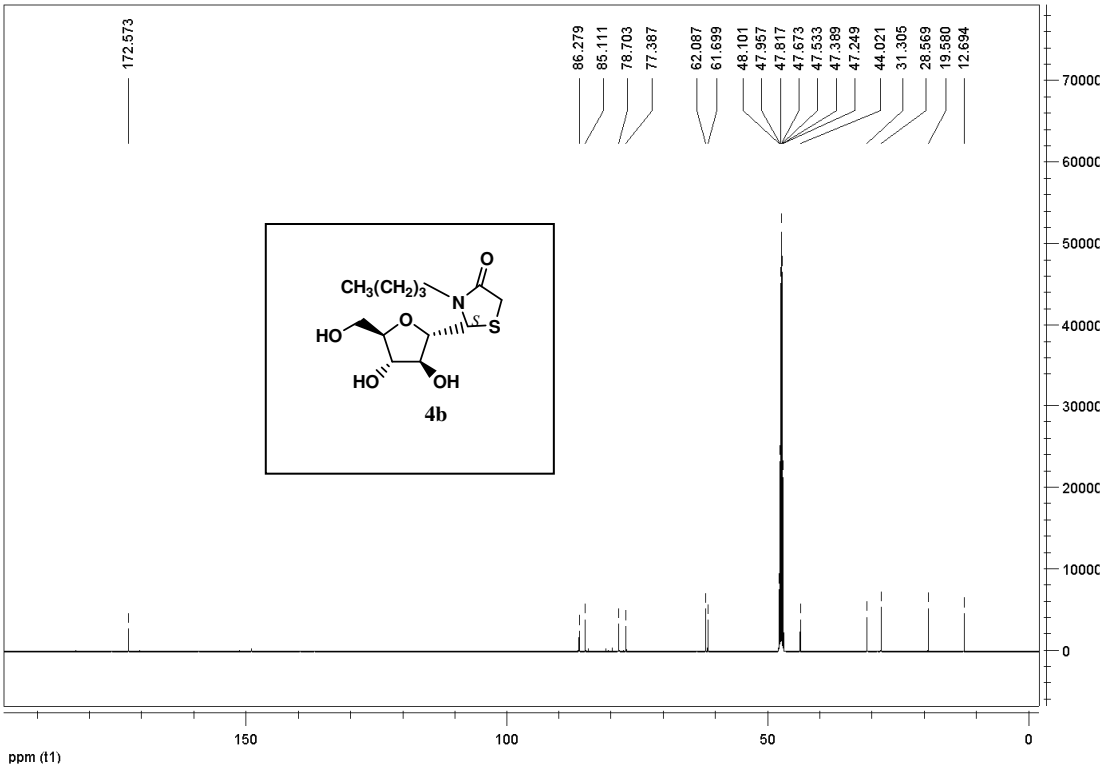
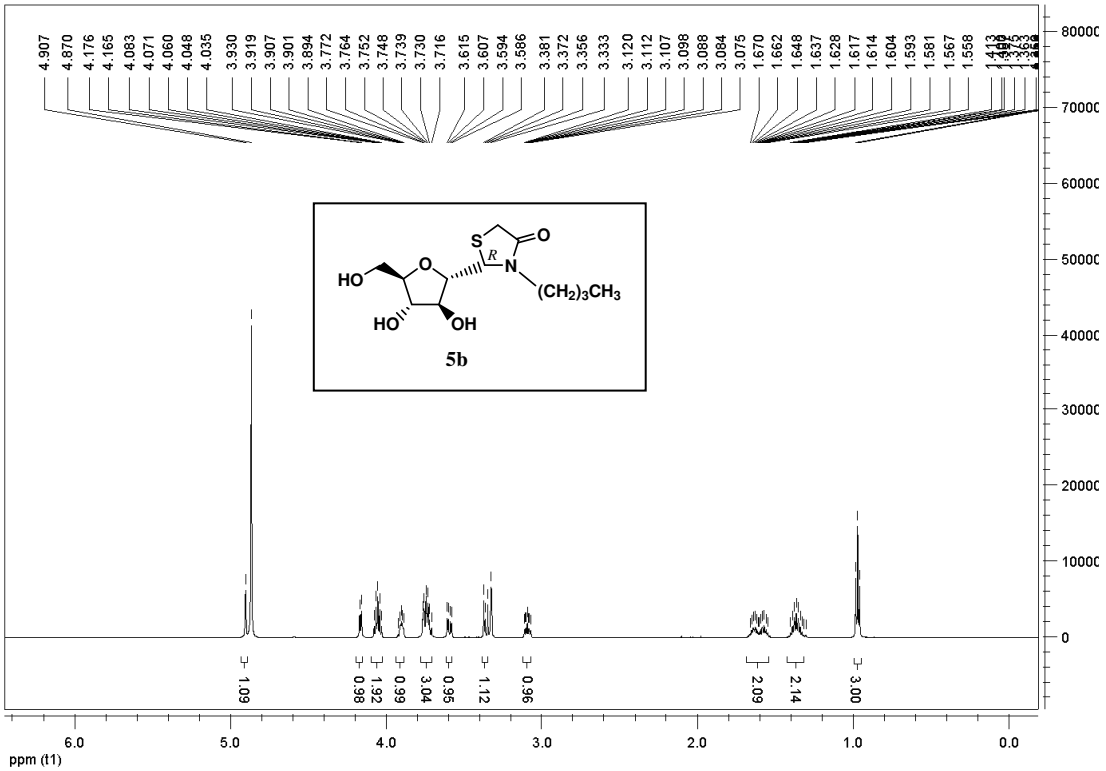
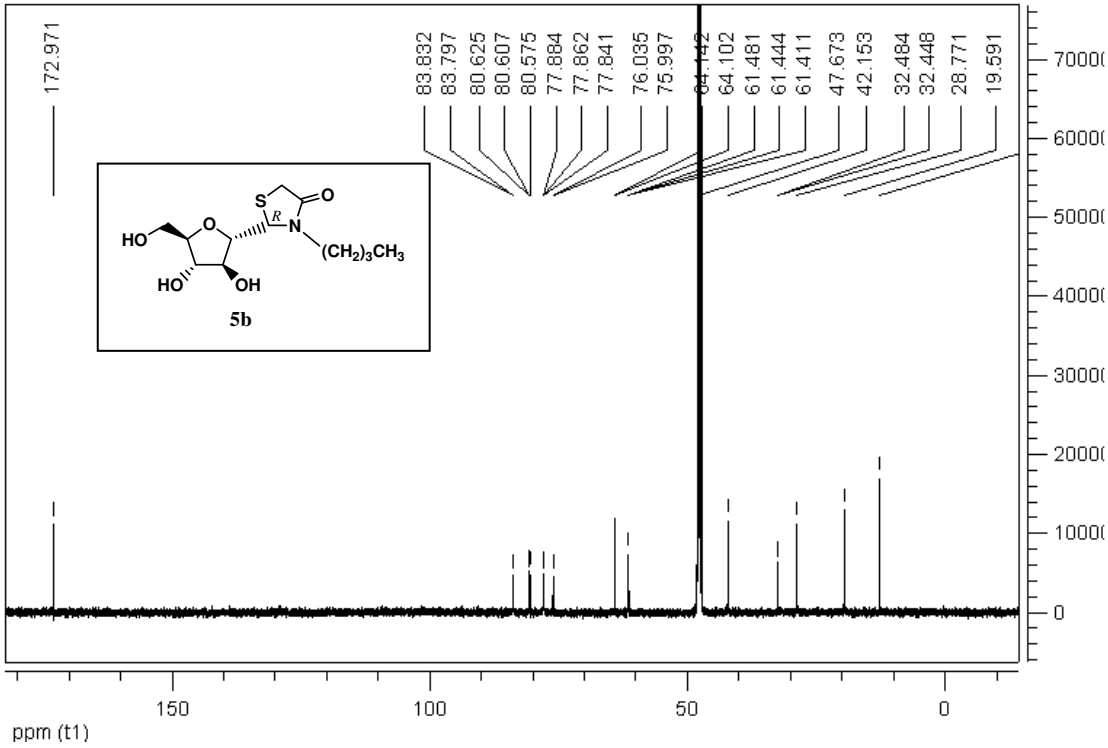
4.942
4.934
4.931
4.087
4.080
4.068
4.059
4.052
4.044
3.962
3.953
3.946
3.913
3.905
3.898
3.890
3.762
3.713
3.676
3.674
3.667
3.661
3.647
3.589
3.580
3.569
3.560
3.428
3.402
3.319
3.310
3.266
3.263
3.261
1.620
1.609
1.606
1.507
1.497
1.330
1.320
1.280
1.242
0.918
0.906
0.894
CH3(CH2)3
N
O
S
HO
HO
OH
4b
1.56
2.12
2.17
2.48
3.17


172.573
86.279
85.111
78.703
77.387
62.087
61.699
48.101
47.957
47.817
47.673
47.533
47.389
47.249
44.021
31.305
28.569
19.580
12.694
CH3(CH2)3
N
O
S
HO
HO
OH
4b
150
100
50
0
ppm (t1)


4.907
4.870
4.176
4.165
4.083
4.071
4.060
4.048
4.035
3.930
3.919
3.907
3.901
3.894
3.772
3.764
3.752
3.748
3.739
3.730
3.716
3.615
3.607
3.594
3.586
3.381
3.372
3.356
3.333
3.120
3.112
3.107
3.098
3.088
3.084
3.075
1.670
1.662
1.648
1.637
1.628
1.617
1.614
1.604
1.593
1.581
1.567
1.558
HO
HO
OH
O
S
R
N
(CH2)3CH3
5b
1.09
0.98
1.92
0.69
3.04
0.95
1.12
0.96
2.09
2.14
3.00
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)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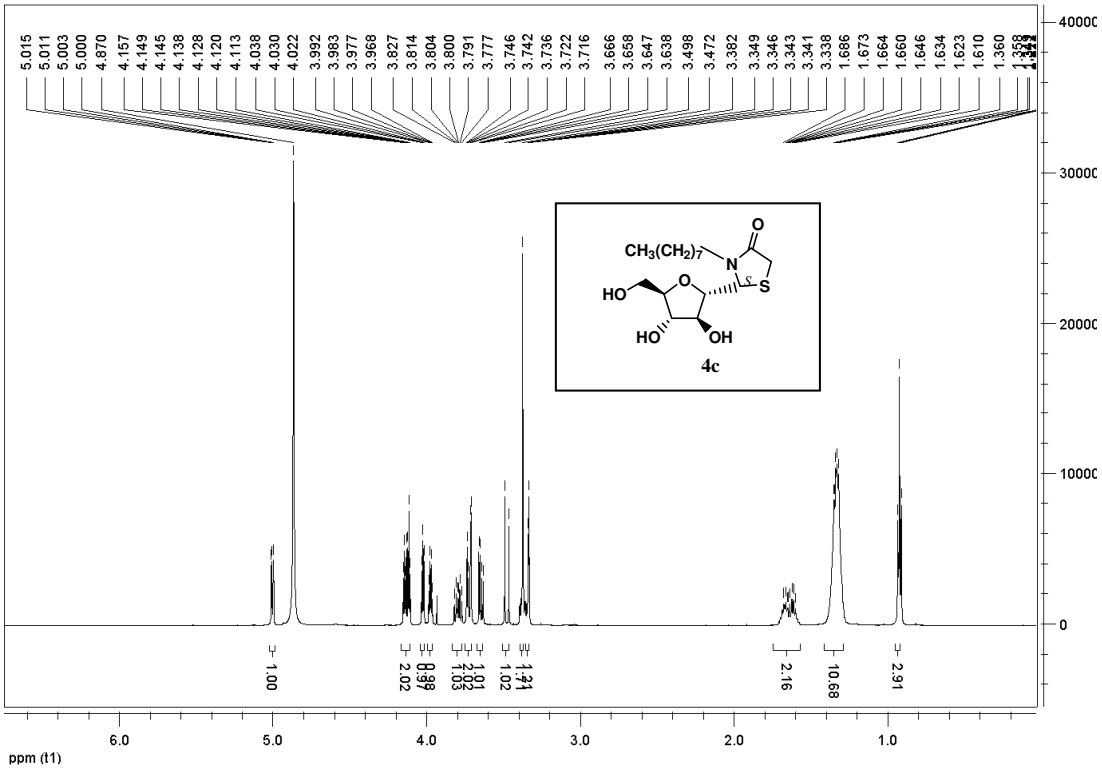
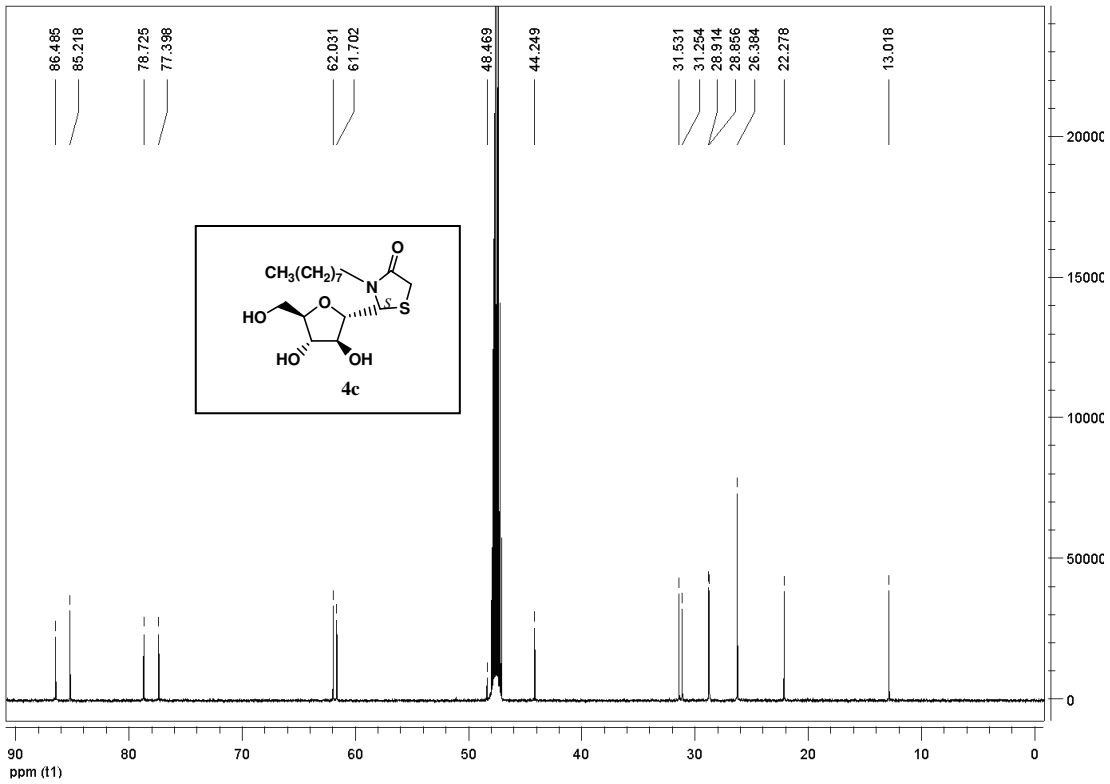
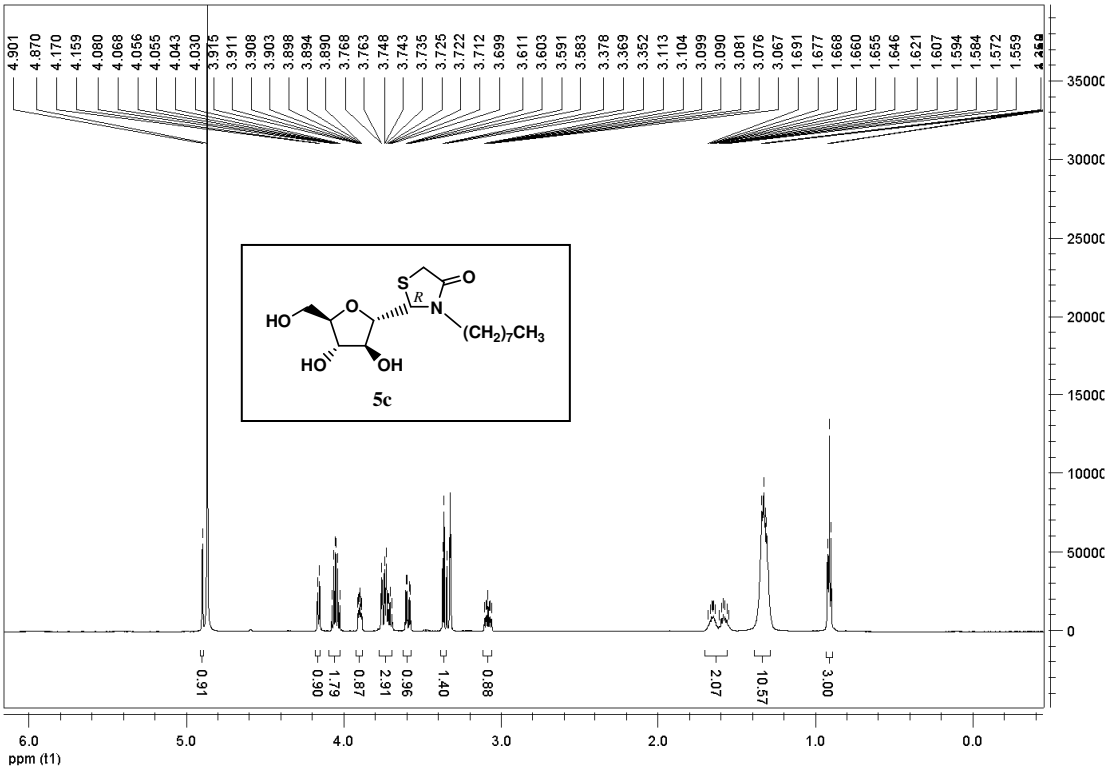
83.832
83.797
80.625
80.607
80.575
77.884
77.862
77.841
76.035
75.997
64.142
64.102
61.481
61.444
61.411
47.673
42.153
32.484
32.448
28.771
19.591
HO
HO
OH
O
S
R
N
(CH2)3CH3
5b
150
100
50
0
ppm (t1)


5.015
5.011
5.003
5.000
4.870
4.157
4.149
4.145
4.138
4.128
4.120
4.113
4.038
4.030
4.022
3.992
3.983
3.977
3.968
3.827
3.814
3.804
3.800
3.791
3.777
3.746
3.742
3.736
3.722
3.716
3.666
3.658
3.647
3.638
3.498
3.472
3.382
3.349
3.346
3.343
3.341
3.338
1.686
1.673
1.664
1.660
1.646
1.634
1.623
1.610
1.360
1.00
2.02
1.03
1.02
2.16
10.68
2.91
4c
ppm (t1)


86.485
85.218
78.725
77.398
62.031
61.702
48.469
44.249
31.531
31.254
28.914
28.856
26.384
22.278
13.018
4c
ppm (t1)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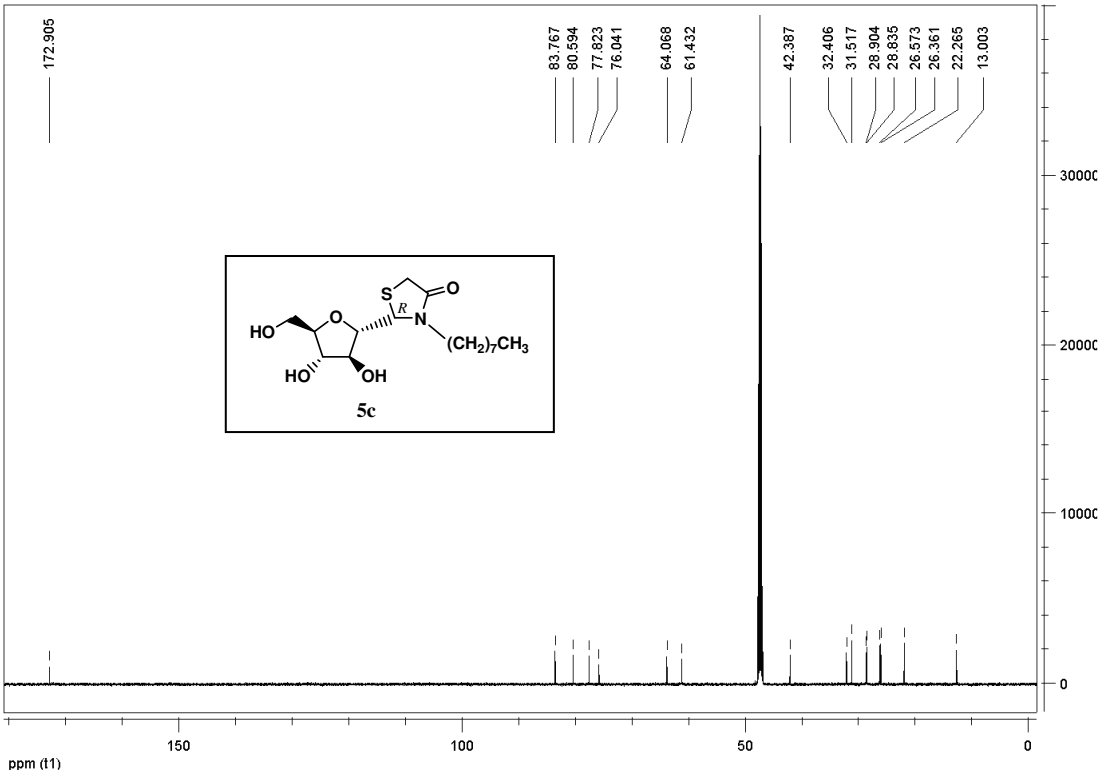
4.870
4.170
4.159
4.080
4.068
4.056
4.055
4.043
4.030
3.915
3.911
3.908
3.903
3.898
3.894
3.890
3.768
3.763
3.748
3.743
3.735
3.725
3.722
3.712
3.699
3.611
3.603
3.591
3.583
3.378
3.369
3.352
3.113
3.104
3.099
3.090
3.081
3.076
3.067
1.691
1.677
1.668
1.660
1.655
1.646
1.621
1.607
1.594
1.584
1.572
1.559
HO
HO
OH
(CH2)7CH3
5c
0.91
0.96
1.79
0.87
2.91
0.96
1.40
0.88
2.07
10.57
3.00
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)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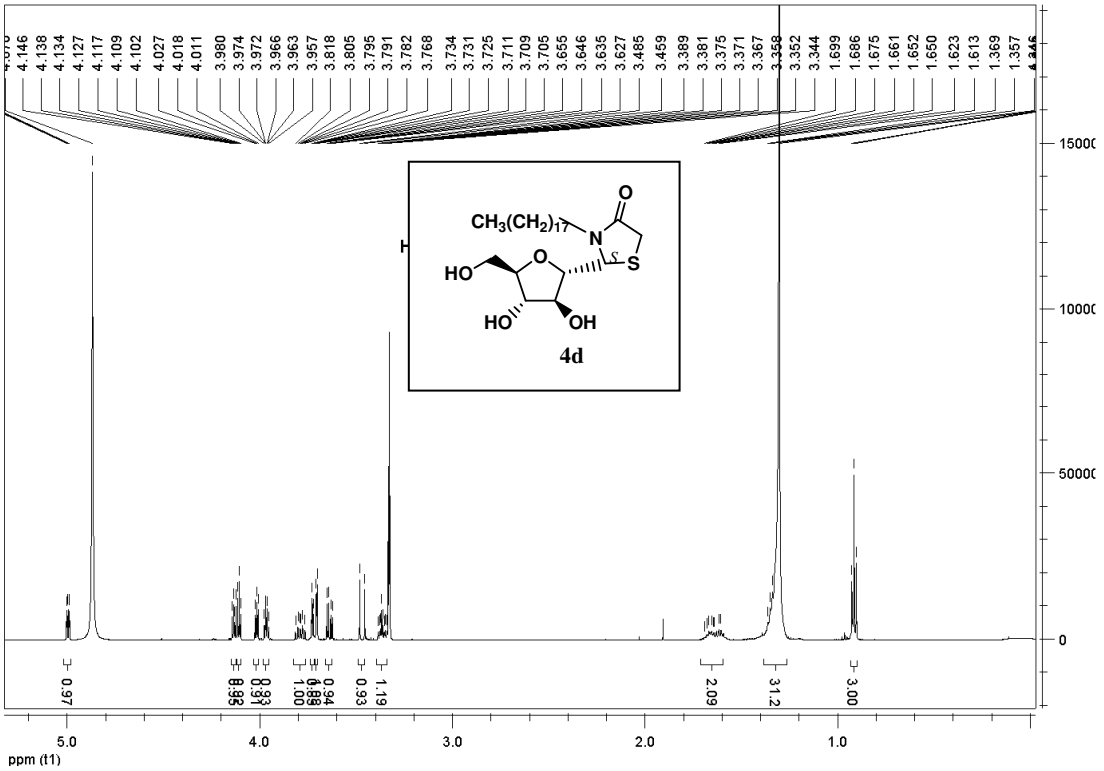
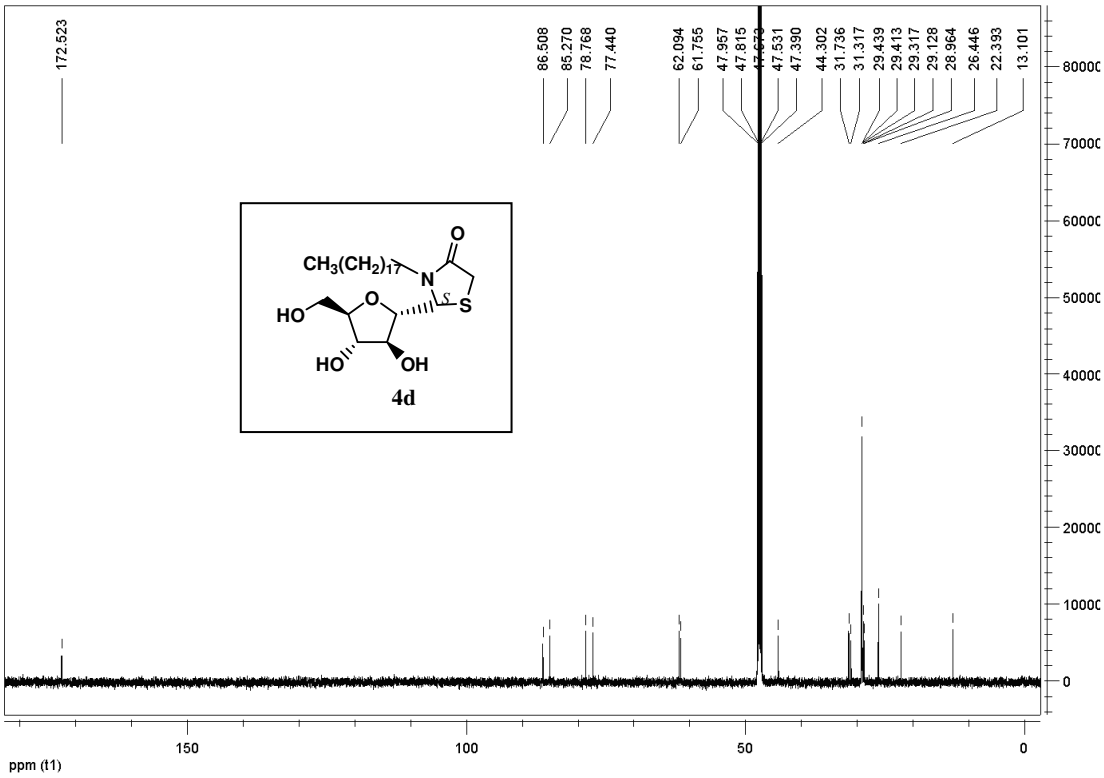
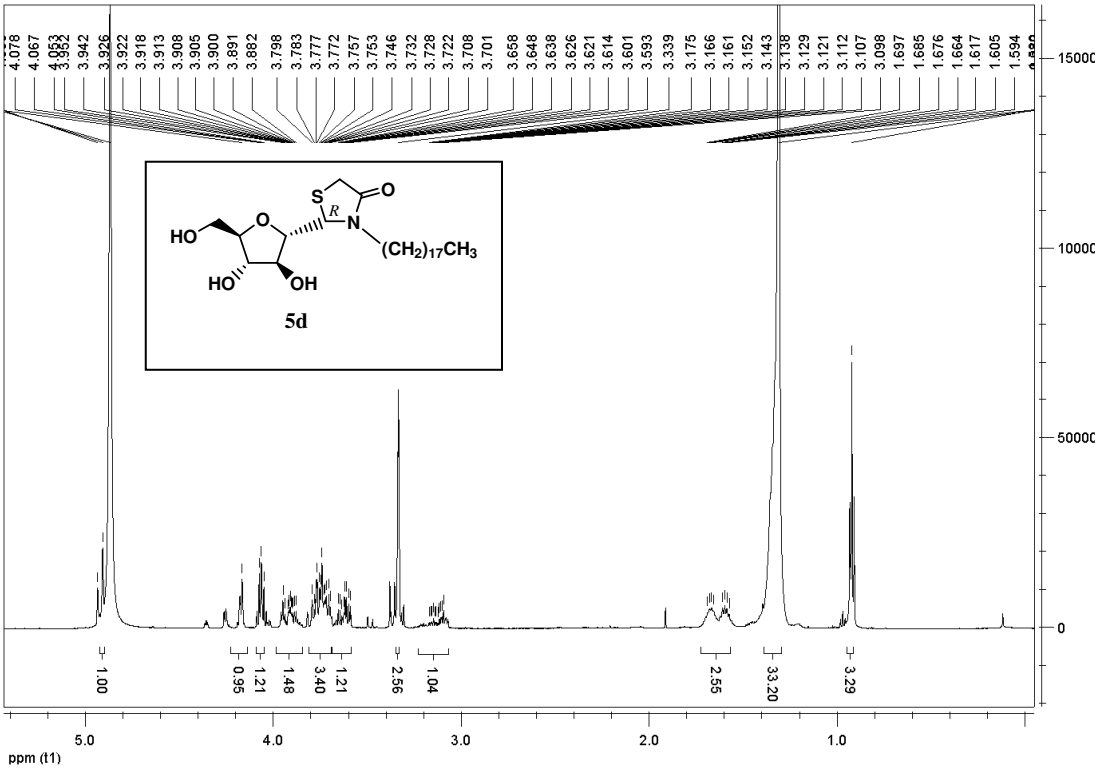
83.767
80.594
77.823
76.041
64.068
61.432
42.387
32.406
31.517
28.904
28.835
26.573
26.361
22.265
13.003
HO
HO
OH
(CH2)7CH3
5c
150
100
50
0
ppm (t1)


4.146
4.138
4.134
4.127
4.117
4.109
4.102
4.027
4.018
4.011
3.980
3.974
3.972
3.966
3.963
3.957
3.818
3.805
3.795
3.791
3.782
3.768
3.734
3.731
3.725
3.711
3.709
3.705
3.655
3.646
3.635
3.627
3.485
3.459
3.389
3.381
3.375
3.371
3.367
3.358
3.352
3.344
1.699
1.686
1.675
1.661
1.652
1.650
1.623
1.613
1.369
1.357
15000
10000
5000
0
0.97
0.93
0.91
1.00
0.94
0.93
1.19
2.09
3.12
3.00
5.0
4.0
3.0
2.0
1.0
ppm (t1)
HO
O
N
S
HO
OH
4d


172.523
86.508
85.270
78.768
77.440
62.094
61.755
47.957
47.815
47.673
47.531
47.390
44.302
31.736
31.317
29.439
29.413
29.317
29.128
28.964
26.446
22.393
13.101
80000
70000
60000
50000
40000
30000
20000
10000
0
150
100
50
0
ppm (t1)
HO
O
N
S
HO
OH
4d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4.067
4.053
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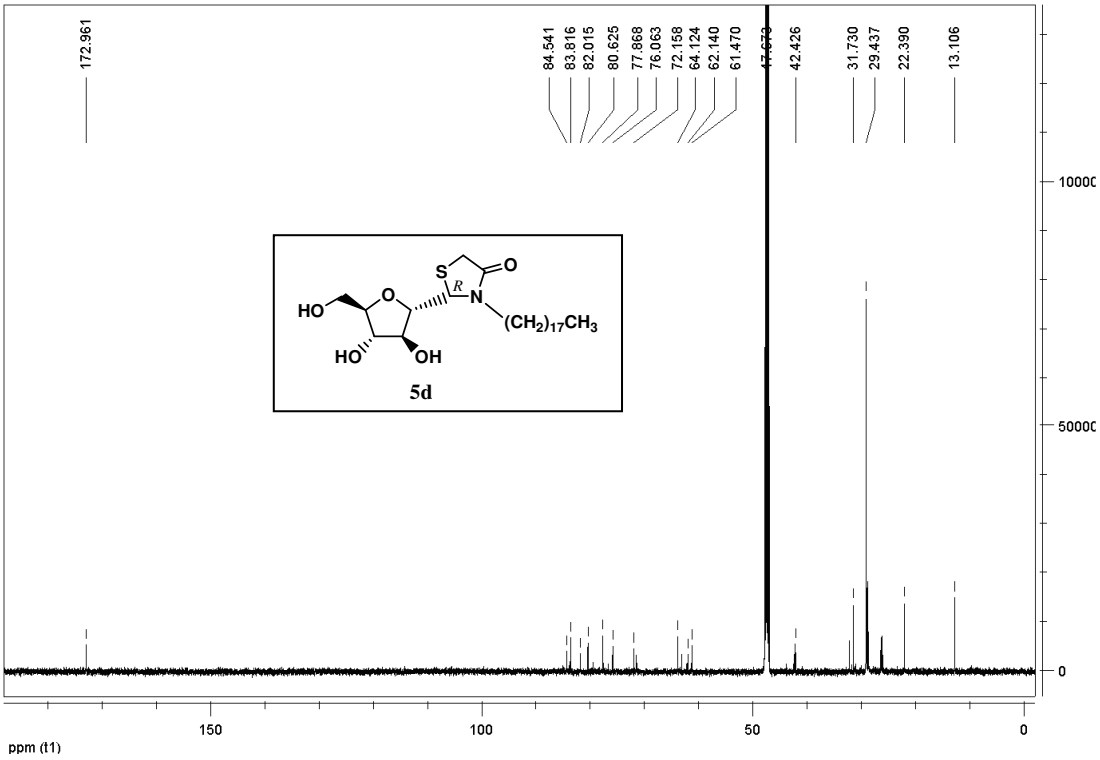
3.926
3.922
3.918
3.913
3.908
3.905
3.900
3.891
3.882
3.798
3.783
3.777
3.772
3.757
3.753
3.746
3.732
3.728
3.722
3.708
3.701
3.658
3.648
3.638
3.626
3.621
3.614
3.601
3.593
3.339
3.175
3.166
3.161
3.152
3.143
3.138
3.129
3.121
3.112
3.107
3.098
1.697
1.685
1.676
1.664
1.617
1.605
1.594
15000
10000
50000
0
1.00
0.95
1.21
1.48
3.40
1.21
2.56
1.04
2.55
33.20
3.29
5.0
4.0
3.0
2.0
1.0
ppm (t1)
HO
O
S
R
N
O
(CH2)17CH3
HO
OH
5d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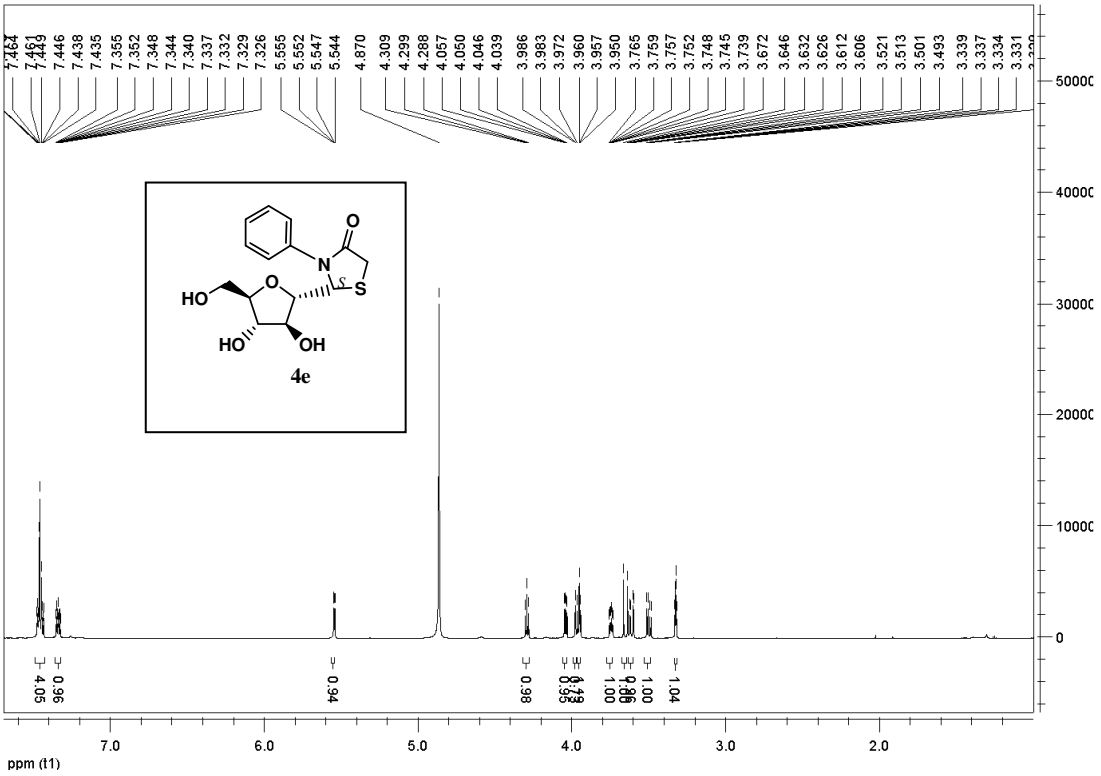
83.816
82.015
80.625
77.868
76.063
72.158
64.124
62.140
61.470
42.426
31.730
29.437
22.390
13.106
10000
50000
0
150
100
50
0
ppm (t1)
HO
O
S
R
N
O
(CH2)17CH3
HO
OH
5d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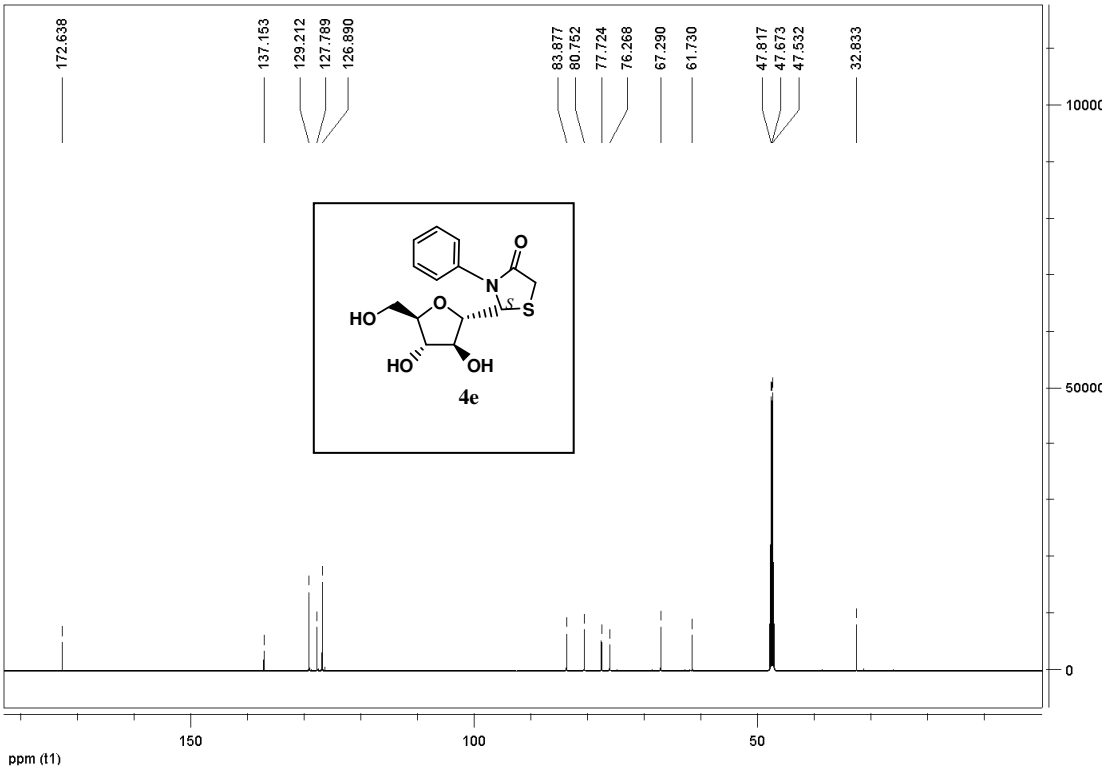
7.435
7.355
7.352
7.348
7.344
7.340
7.337
7.332
7.329
7.326
5.555
5.552
5.547
5.544
4.870
4.309
4.299
4.288
4.057
4.050
4.046
4.039
3.986
3.983
3.972
3.960
3.957
3.950
3.765
3.759
3.757
3.752
3.748
3.745
3.739
3.672
3.646
3.632
3.626
3.612
3.606
3.521
3.513
3.501
3.493
3.339
3.337
3.334
3.331
50000
40000
30000
20000
10000
0
HO
HO
OH
O
N
S
O
4e
4.05
0.96
0.94
0.98
1.00
1.04
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)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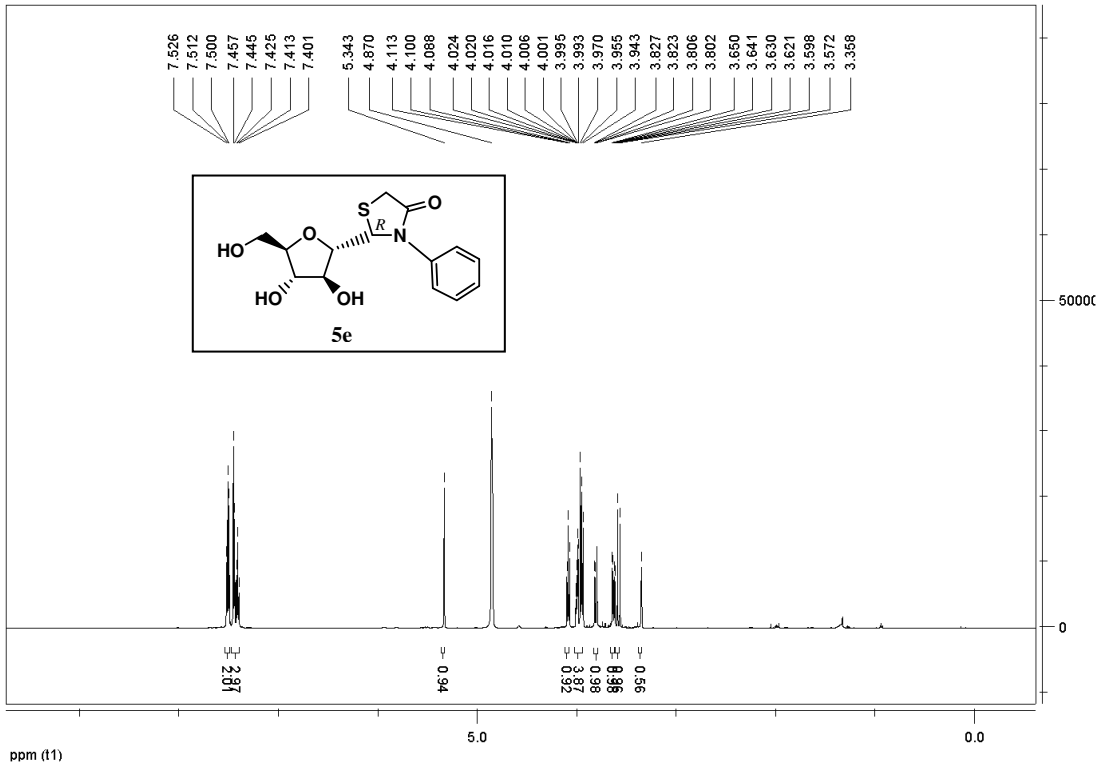
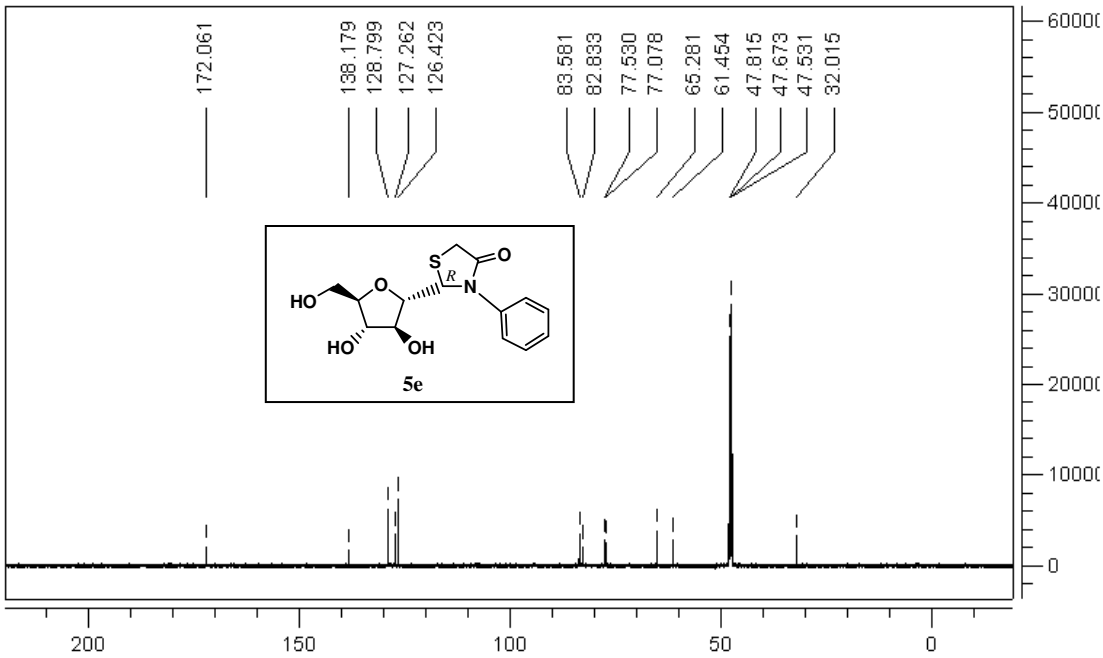
137.153
129.212
127.789
126.890
83.877
80.752
77.724
76.268
67.290
61.730
47.817
47.673
47.532
32.833
10000
50000
0
HO
HO
OH
O
N
S
O
4e
150
100
50
ppm (t1)


7.526
7.512
7.500
7.457
7.445
7.425
7.413
7.401
5.343
4.870
4.113
4.100
4.088
4.024
4.020
4.016
4.010
4.006
4.001
3.995
3.993
3.970
3.955
3.943
3.827
3.823
3.806
3.802
3.650
3.641
3.630
3.621
3.598
3.572
3.358
HO
HO
OH
O
S
N
R
O
5e
50000
0
2.87
0.94
3.87
0.92
0.98
0.96
0.56
5.0
0.0
ppm (t1)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138.179
128.799
127.262
126.423
83.581
82.833
77.530
77.078
65.281
61.454
47.815
47.673
47.531
32.015
HO
HO
OH
O
S
N
R
O
5e
60000
50000
40000
30000
20000
10000
0
200
150
100
50
0