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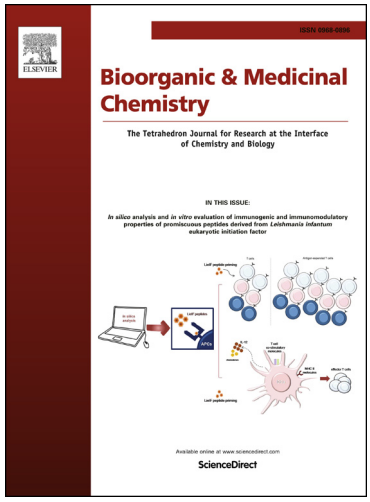

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Synthesis of 1,5-Anhydro-D-fructose derivatives and evaluation of their inflammasome inhibitors

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Abstract

Synthesis of several 1,5-Anhydro-D-fructose (1,5-AF) derivatives to evaluate inhibitory activities of the inflammasome was carried out. Recently, 1,5-AF reported to suppress the inflammasome, although with only low activity. We focused on the hydration of 2-keto form of 1,5-AF and speculated that this hydration was the cause of low activity. Therefore, we synthesized some 1,5-AF derivatives that would not be able to form the dimer conformation and can be expected to have high activity against inflammasome, and then evaluated their inhibitory activities with respect to the NLRP3 inflammasome by using mouse bone marrow–derived macrophages and human THP-1 cells. As a result, some synthesized 2-keto form compounds had much higher inhibitory activities with respect to the NLRP3 inflammasome than did 1,5-AF.

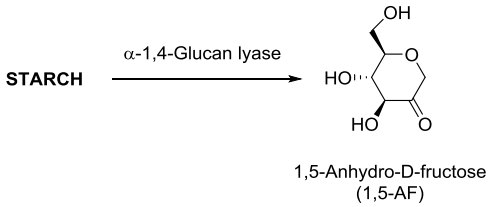
1. Introduction

Natural immunity is a biological defense mechanism present in lower organisms through to higher organisms, and central to the innate immune response are many pattern recognition receptors (PRRs).

Inflammasomes are protein complexes consisting of particular PRRs, ASC, and caspase-1 which are formed intracellularly in response to various stimulating factors such as pathogenic components, endogenous factors, and exogenous factors.[1, 2] The mechanism of inflammasome formation can be explained as follows. First, a PPR such as NLRP3, AIM2, or NLRC4 recognizes a particular stimulating factor (agonist). This recognition triggers structural changes in the PRR and encourages the assembly of complexes with proteins such as ASC and caspase-1. Finally, the PRRs form large multiprotein oligomers called inflammasomes. In inflammasomes, caspase-1 (which is a kind of protease) is activated, and the active caspase-1 then induces inflammation by promoting the maturation and secretion of inflammatory cytokines such as IL-1β and IL-18.[3-6] Inflammatory responses based on the formation of inflammasomes are often effective for defense against infections. However, negative aspects of this inflammasome response are also known. Excessive inflammasome formation induced by certain kinds of stimulator can be inappropriately raised, resulting in persistent inflammation. This mechanism is implicated in the development of various inflammatory responses such as seen in infection, diabetes mellitus, arteriosclerosis, and autoimmune diseases. Therefore, elucidating the mechanisms underlying the control of inflammasome formation or activity will lead to a deeper understanding of the mechanisms of pathogenesis, and is expected to contribute to diagnosis and treatment.

As the mechanisms of inflammasome formation have been gradually revealed, many studies into inflammasome inhibitors have been carried out with the aim of developing diagnostic and therapeutic medicines.[7]

1,5-Anhydro-D-fructose (1,5-AF) is a functional monosaccharide that can be formed directly from starch and glycogen *in vivo* by α-1,4-glucan lyase (**Scheme 1**).[8] It occurs in edible morels, red seaweeds, and certain mammalian tissues, and has been revealed to exhibit strong anti-oxidant activity and antibacterial activity.[9] Therefore, 1,5-AF is expected to find use in various fields such as health foods and medicines.

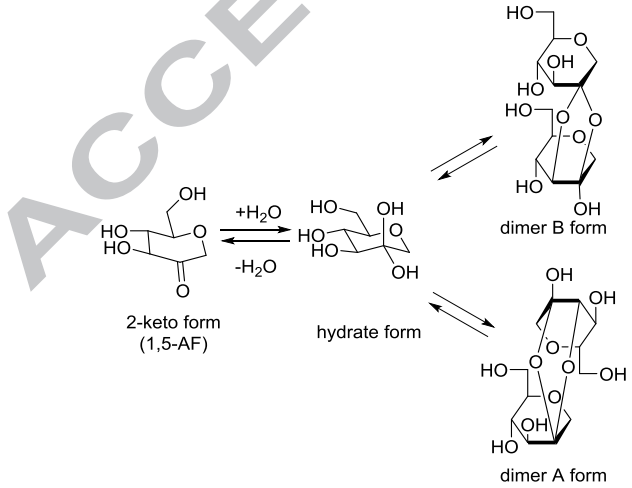


Scheme 1. Synthesis of 1,5-AF from starch.

Recently, 1,5-AF was reported to suppress the inflammasome, although with only low activity.[10] Herein, we show the synthesis and the evaluation of inflammasome inhibitors of which 1,5- AF is a lead compound.

2. Results and Discussion

We focused first on the 2-keto form of 1,5-AF. 1,5-AF has never been observed in purely monomeric form in H_2O. Owing to its presumably hygroscopic nature, it has only been isolated as mixtures of monomeric and dimeric forms or as mixtures of the monomeric (2-keto) and the hydrated forms **(Scheme 2)**. As a result, the abundance ratio of the 2-keto form of 1,5-AF is only a few percent.

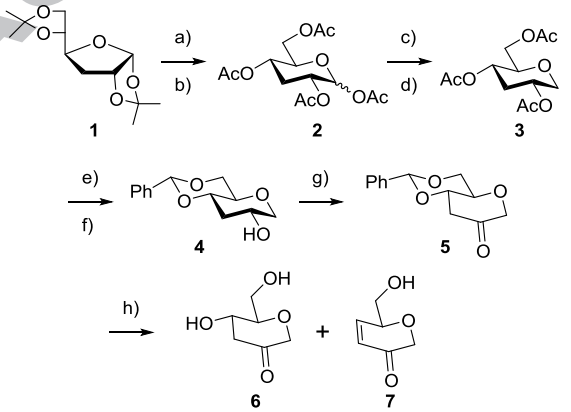


Scheme 2. Conformations of 1,5-AF.

We inferred that this was one of the reasons that 1,5-AF had low inhibitory activity with respect to inflammasomes. We carried out the synthesis of several 1,5-AF derivatives that we expected would not be able to form the dimer conformation, and we evaluated the inhibitory activities of those derivatives with respect to inflammasomes.

2.1. Synthesis and evaluation of 3-deoxy derivative 6 and enone 7

To suppress dimer formation and increase the abundance ratio of the keto form, we first synthesized 3-deoxy-1,5-AF (**6**) (S**cheme 3**). Compound **1**,[11] which had a furanose conformation, was treated with 0.1 M H_2SO_4, and subsequent acetylation of all hydroxyl groups afforded compound **2**, which had a pyranose conformation, in 90%. Bromination of the anomer position of compound **2** was conducted with HBr-AcOH solution; this was followed by removal of the bromine under reductive conditions by using $(TMS)_3SiH$ and Et_3B to give compound **3** in 63%. All acetyl groups of compound **3** were removed with NaOMe, and the subsequent formation of the benzylidene acetal produced alcohol **4** in 79%. Oxidation of the hydroxyl group of **4** was performed using Dess–Martin oxidation[12] to afford compound **5** in 98%. Finally, the removal of the benzylidene acetal was conducted with 70% aq. AcOH to give the 3-deoxy derivative **6** in 85%. In this step, enone derivative **7** was obtained as a byproduct. As expected, the abundance ratio of the 2-keto form in H_2O of compound **6** was improved up to about 20%[13]. We then evaluated the inhibitory activity of compounds **6** and **7** with respect to the inflammasome.



Scheme 3. Synthesis of 3-deoxy derivative **6** and enone **7**. Reagents and conditions: a) 0.1 M H_2SO_4, 60 ^oC; b) Ac_2O, Pyridine, 2 steps 90%; c) HBr-AcOH, CH_2Cl_2; d) $(TMS)_3SiH$, Et_3B, Tol-1,4-dioxane, 2 steps 63%; e) NaOMe, MeOH; f) $PhCH(OMe)_2$, CSA, MeCN, 2 steps 79%; g) Dess-Martin periodinane, CH_2Cl_2, 98%; h) 70% aq. AcOH 70 ^oC, 85%(**6**), 10%(**7**).

2.2. In vitro assay of compounds 6 and 7

We measured the amount of IL-1β produced by bone marrow–derived macrophages (BMDMs) stimulated with lipopolysaccharide (LPS) and the NLRP3 activator Nigericin. The NLRP3 inflammasome–dependent events follow a two-stage process. In the first priming stage, IL-1β expression is induced through NF-κB-mediated signaling, and in the second stage, the NLRP3 inflammasome is assembled and activated by different stimuli. We found that both 1,5-AF and compound **6** inhibited IL-1β secretion.

Compound **6** dose-dependently inhibited the amount of IL-1β in culture supernatants produced by BMDMs (Fig. 1a). The half-maximal inhibitory concentration (IC_{50}) of compound **6** in BMDMs was approximately 2 mM, which was much smaller than that of 1,5-AF (Table 1). Activation of the NLRP3 inflammasome causes pyroptosis (a caspase-1-dependent programmed cell death) in BMDMs. The anti-pyroptotic effects of compound **6** were also determined by measuring the amount of intracellular ATP (Fig. 1b). Without compound **6** pre-incubation, Nigericin treatment caused cell death and resulted in a pronounced decrease in intracellular ATP. Pre-treatment with compound **6** dose-dependently restored the intracellular ATP level, preventing the NLRP3-dependent pyroptosis of BMDMs. As was the case for compound **6**, the enone derivative **7** also dose-dependently inhibited IL-1β secretion and pyroptosis (data not shown). The half-maximal inhibitory concentration (IC_{50}) of compound **7** in BMDMs was approximately 15 μM, which was much smaller than that of compound **6** (Table 1). Inhibition of IL-1β release was also confirmed by western blotting of IL-1β. Although almost equal amounts of pro-IL-1β (37 kDa) were produced in all of the LPS-primed cells

regardless of treatment, compounds **6** and **7** prevented their processing to the mature 17 kDa form caused by Nigericin stimulation (Fig. 2a).

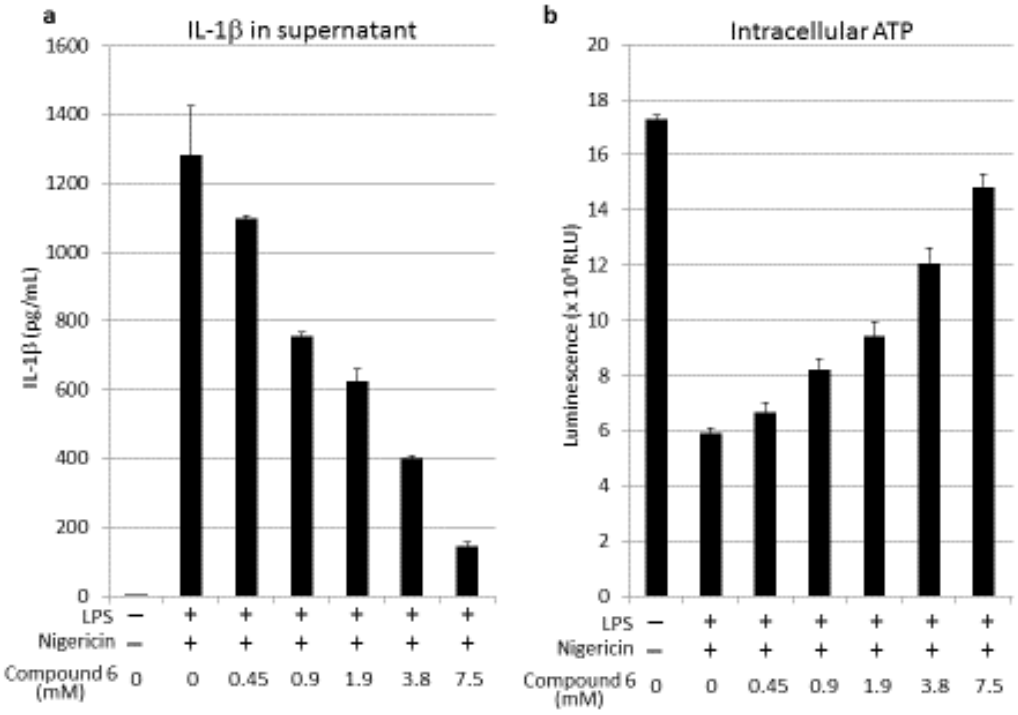


Figure 1. 3-Deoxy-1,5-AF (6) inhibits IL-1β secretion and pyroptosis in response to Nigericin-induced NLRP3 inflammasome activation.

a) Concentration of IL-1β in supernatants from BMDMs stimulated with LPS and Nigericin and treated with compound **6** (0.45–7.5 mM) measured by ELISA.

b) Amount of intracellular ATP in BMDMs stimulated with LPS and Nigericin and treated with compound **6** (measured by CellTiter-Glo reagents). Intracellular ATP was measured after aliquots of supernatants were removed from BMDM cultures. Data are expressed as mean ± standard deviation (n = 3). This data is representative of at least three independent experiments.

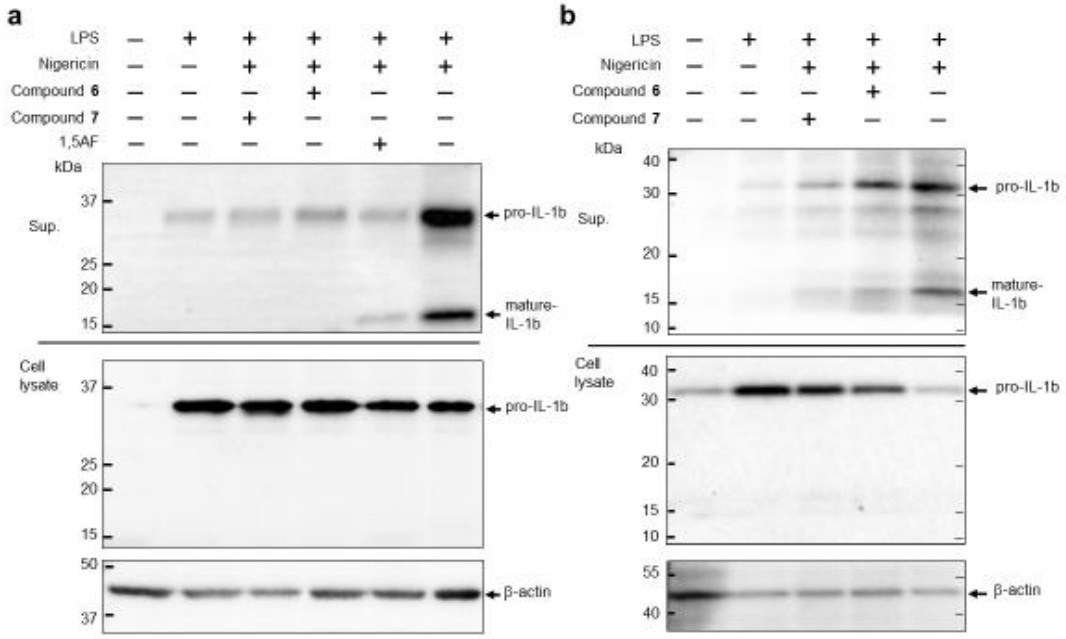


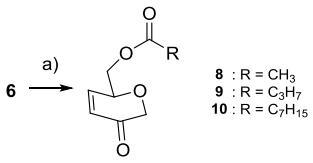
Figure 2. Synthesized compounds inhibit Nigericin-induced NLRP3 inflammasome activation.

a) BMDMs primed with LPS (100 ng/mL) for 4 h were treated with compound **7** (100 μM), compound **6** (6.8 mM), or 1,5-AF (60 mM) for 1 h prior to stimulation with Nigericin (5 μM) for 1 h. The cells were lysed with lysis buffer and the collected supernatants were concentrated with 10 K MWCO centrifugal filters (Amicon Ultra). Immunoblots of pro-IL-1β and mature-IL-1β in culture supernatants (Sup.) and immunoblots of pro-IL-1β, mature-IL-1β, and β-actin in cell lysates are shown.

b) THP-1 cells primed with LPS (100 ng/mL) for 3 h were treated with compound **7** (150 μM) or compound **6** (3.4 mM) for 1 h prior to stimulation with Nigericin (2.5 μM) for 1 h. The cell lysates and collected supernatants were prepared as for BMDMs above. Immunoblots of pro-IL-1β and mature-IL-1β in culture supernatants and immunoblots of pro-IL-1β, mature-IL-1β, and β-actin in cell lysates are shown. All data shown are representative of three independent experiments.

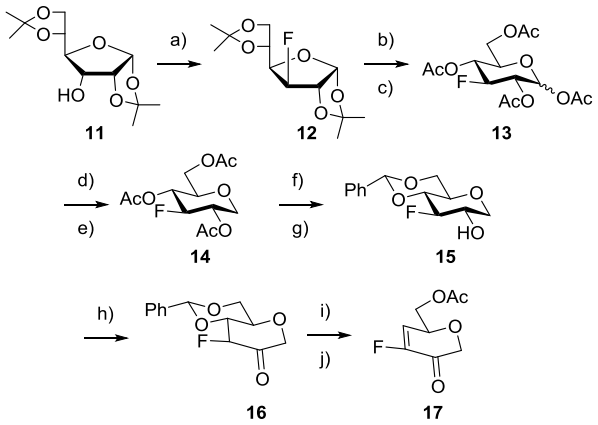
2.3. Synthesis of enone derivatives

Based on this result, we designed and synthesized compound **8**-**10** and fluorinated enones **17** and **23**, assuming that the structure of the enone is important. First, we attempted the synthesis enone derivatives **8**, **9**, and **10** which can be easily converted from the compound **6**. Each enone derivative was obtained by acylation of compound **6**, with good yield of the corresponding anhydride of carboxylic acids (**Scheme 4**).



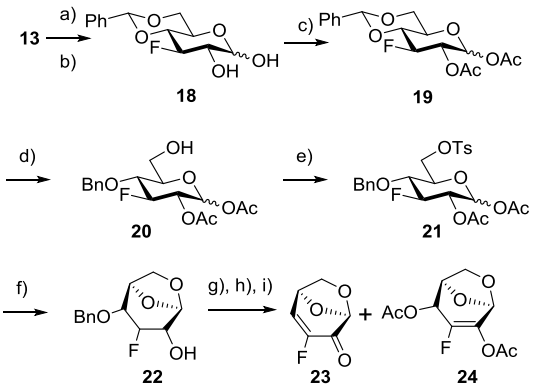
Scheme 4. Synthesis of acyl enones **8**, **9** and **10**. Reagents and conditions: a) $(RC(O))_2O$, Pyridine.

Next, we synthesized the fluorinated enone derivative **17**. Compound **11**[14] was treated with *N*, *N*-diethylaminosulfur trifluoride (DAST) to afford fluoride **12** in 90%. Compound **12** was reacted with 0.1 M H_2SO_4, followed by acetylation to give compound **13** in quant. Compound **13** was brominated by treating with HBr-AcOH solution; subsequent removal of bromine by the radical reaction produced compound **14** in 90%. Removal of the acetyl groups of compound **14** was conducted by using NaOMe. The formation of the benzylidene acetal produced compound **15** in 88%. Compound **15** was oxidized with 1-Me-AZADO[15, 16] in the presence of $PhI(OAc)_2$ to give compound **16** in 75%. The removal of the benzylidene group of compound **16** was conducted by hydrogenation; this was followed by acetylation to give the desired compound **17** in 87% (**Scheme 5**).



Scheme 5. Synthesis of 3-F derivative **17**. Reagents and conditions: a) DAST, CH_2Cl_2, 40 °C, 90%; b) 0.1 M H_2SO_4, 60 °C; c) Ac_2O, Pyridine, 2 steps quant.; d) HBr-AcOH, CH_2Cl_2; e) $(TMS)_3SiH$, $E_{t3}B$, Tol-1,4-dioxane, 2 steps 90%; f) NaOMe, MeOH; g) $PhCH(OMe)_2$, CSA, MeCN, 2 steps 88%; h) 1-Me-AZADO CH_2Cl_2, 75%; i) H_2 gas, Pd/C, EtOH-EtOAc; j) Ac_2O, Pyridine, 2 steps 87%.

We also synthesized fluorinated levoglucosenone **23**. Removal of the acetyl groups of compound **13,** followed by formation of the benzylidene acetal provided compound **18** in 56%. Compound **18** was acetylated to prepare compound **19** in 96%. Regioselective ring opening of the benzylidene acetal yielded compound **20** in 49%, which was then tosylated to give **21** in 91%. Compound **21** was then treated with 3.03 M NaOH solution to prepare bicyclic compound **22** in 69%. Compound **22** was reacted with 1-Me-AZADO to afford the corresponding ketone, followed by the hydrogenationin in the presence of Pd/C was conducted to remove benzyl ether. Finally, acetylation could afford the desired compound **23** in 39% with enol acetate **24** in 8% as a byproduct (**Scheme 6**).



Scheme 6. Synthesis of bicyclo derivative **23**. Reagents and conditions: a) NaOMe, MeOH; b) $PhCH(OMe)_2$, CSA, MeCN, 2 steps 56%; c) Ac_2O, Pyridine, 96%; d) Et_3SIH, $PhBCl_2$, CH_2Cl_2, -78 °C, 49%; e) TsCl, Pyridine, 91%; f) 3.03N aq. NaOMe, Pyridine, 69%; g) 1-Me-AZADO, $PhI(OAc)_2$, CH_2Cl_2, 52%; h) H_2 gas, Pd/C, EtOH-EtOAc, 76%; i) Ac_2O, Pyridine, 3 steps 39%.

2.4. In vitro assay of enone derivatives (compounds 8–10, 17, 23, and 24) and levoglucosenone

We measured the inhibitory activity of newly synthesized compounds (**8**–**10**, **17**, **23**, and **24)** and levoglucosenone[17] with respect to Nigericin in the same way as we did for compound **6**. These compounds dose-dependently inhibited IL-1β secretion and pyroptosis as was the case for compound **6**. The half-maximal inhibitory concentration (IC_{50}) of 1,5-AF and the synthesized compounds (**8**–**10**, **17**, **23**, and **24)** and levoglucosenone with respect to NLRP3 stimuli are listed in Table 1. It is clear that the IC_{50}s of compound **7**, levoglucosenone, and their derivatives are much lower than those of 1,5-AF and compound **6**.

Furthermore, fluorination and/or *O*-acylation of compound **7** increased the inhibitory activity by several times that of **6**. It is not known whether macrophages have a specific transport system for 1,5-AF and its derivatives. However, it is expected that fluorination and/or *O*-acylation would elicit hydrophobicity in these compounds, and because it is well known that the membrane permeability of

a compound increases with its hydrophobicity, this may be part of the reason that the inhibitory activity is enhanced.

The effects of various compounds on IL-1β release were also evaluated in human macrophage cells (THP-1 cells). Cells were pretreated with several inhibitors and then cultured with Nigericin; consequent IL-1β production was then estimated by cell-based assay and western blotting. As was shown in BMDMs, all of the tested compounds except for 1,5-AF significantly inhibited IL-1β secretion in a dose-dependent manner (data not shown). These compounds also restored the ATP levels in THP-1 cells in a dose-dependent manner (data not shown), revealing that the synthesized compounds prevented NLRP3-dependent pyroptotic cell death triggered by Nigericin in THP-1 cells.

The IC_{50} values of the tested compounds with respect to Nigericin are summarized in Table 1. Compound **6** exhibited an IC_{50} value of 2.3 ± 0.03 mM, and the modified enone compounds (**8**–**10**, **17**, **23**, **24**, and levoglucosenone) exhibited slightly improved potency, with IC_{50} values in THP-1 cells that ranged from 3.7 ± 1.4 to 6.6 ± 2.8 μM.

We also confirmed the reduction of IL-1β in concentrated supernatants by immunoblots with anti-IL-1β monoclonal antibody in THP-1 cells (Fig. 2b).

Table 1. Inhibitory effect (IC_{50}) of compounds on release of IL-1β from BMDMs and THP-1 cells triggered by Nigericin stimulation.

3. Conclusion

1,5-AF derivatives and related compounds were designed and synthesized, and their inhibitory activities with respect to the NLRP3 inflammasome were evaluated *in vitro* by using mouse BMDMs and human THP-1 cells. Synthesized compounds (**6**–**10**, **17**, **23**, **24)** and levoglucosenone have much higher inhibitory activities with respect to the NLRP3 inflammasome than does 1,5-AF. *In vivo* evaluations of the effects of the newly synthesized compounds and investigation of target molecules are ongoing.

From these results, it is suggested that the enone structure may be an important factor in their inhibitory activities with respect to the NLRP3 inflammasome.

4. Experimental Section

4.1. General methods for chemical synthesis

All reagents were purchased from commercial sources and were used without further purification unless noted. All reactions were carried out under positive pressure of air at room temperature unless specified and were monitored using TLC on silica gel 60-F254 (Merck KGaA, Darmstadt, Germany). Organic solvents were evaporated under reduced pressure and the products were purified using column chromatography on silica gel (silica-gel 60N, spherical, neutral, 40-50 μm, KANTO CHEMICAL CO., INC., Tokyo, Japan). The yields reported are after purification. ^{1}H NMR spectra were recorded at 600 MHz and Chemical shifts are reported in ppm and calibrated using tetramethylsilane (0.00 ppm for ^{1}H in $CDCl_3$ and CD_3OD), HOD (4.76 ppm for 1H in D_2O). ^{13}C NMR spectra were recorded at 150 MHz and chemical shifts were referenced to $CDCl_3$ (77.0 ppm) or CD_3OD (49.0 ppm). ^{19}F NMR were recorded at 600 MHz and chemical shifts were referenced to $PhCF_3$ (-63.2 ppm). Assignments of NMR spectra were based on two-dimensional experiments (^{1}H-^{1}H COSY, HMQC and HMBC). High resolution mass spectra were recorded on an JMS-T100LP (JEOL Ltd., Tokyo, JAAPN), JMS-T100GCv (JEOL Ltd., Tokyo, JAAPN), Exactive (Thermo Scientific, MA, USA) and LTQ Orbitrap XL (Thermo Scientific, MA, USA).

1,2,4,6-Tetra-*O*-acetyl-3-deoxy-D-glucopyranose (2)[18]**:** 0.1 M sulfulic acid (500 mL, 0.05 mmol) was added to 3-deoxy-1,2,5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**; 13.9 g, 56.9 mmol). After stirring at 60 ^{o}C for 20 h, the reaction was cooled to room temperature and neutralized with $NaHCO_3$ (9.24 g, 0.11 mmol). The reaction solution was concentrated under reduced pressure. The resulting residue was dried under high vacuum for 3 hours and used without any further purification. Acetic anhydride (100 mL) and catalytic amount of DMAP were added to a solution of the oil from above in pyridine (200 mL) at 0 ^{o}C. After stirred at room temperature for 14 h, MeOH (150 mL) was added to the reaction mixture at 0 ^{o}C, followed by concentrated under reduced pressure. The residual oil was diluted with AcOEt and washed with H_2O, 1 N HCl, saturated aq. $NaHCO_3$, and saturated aq.

NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 3 : 2) to give compound **2** (17.1 g, 90%, α/β = 33/67, inseparable) as a color less oil. R_f = 0.37 (*n*-Hexane : AcOEt = 1 : 1). Spectrum data were consistent with those of known compound.

2,4,6-Tri-*O*-acetyl-1,5-anhydro-3-deoxy-D-glucitol (3): HBr (33% in acetic acid, 40.8 mL) was added to a mixture of **2** (16.8 g, 50.6 mmol) in dry CH_2Cl_2 (70 mL) at room temperature. After stirring at room temperature for 3 h, ice chips were added to the reaction mixture and very carefully extracted with CH_2Cl_2. The organic layer was washed twice with H_2O, saturated aq. $NaHCO_3$, and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to afford the anomeric bromide (16.4 g) which was used without any further purification.
To a mixture of the bromide from above and TTMSS (46.8 mL, 15.2 mmol) in toluene (160 mL) and 1,4-dioxane (80 mL) was added 1.0 M triethylborane in THF (15.2 mL, 15.2 mmol) at 0 ^oC. After stirring for 16 h at room temperature, the reaction mixture was concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 2 : 1) to give compound **3** (8.72 g, 2steps 63%) as a color less oil. Rf = 0.34 (*n*-Hexane : AcOEt = 2 : 1). ^1H NMR (600 MHz, $CDCl_3$): δ = 4.94-4.86 (m, 1H, H-2), 4.79 (dt, *J* = 9.6, 4.8 Hz, 1H, H-4), 4.20 (dd, *J* = 12.4, 2.8 Hz, 1H, H-6a), 4.16 (dd, *J* = 12.4, 5.5 Hz, 1H, H-6b), 4.14-4.07 (m, 1H, H-1a), 3.49 (ddd, *J* = 9.6, 5.5, 2.8 Hz, 1H, H-5), 3.22 (t, *J* = 10.3 Hz, 1H, H-1b), 2.62-2.55 (m, 1H, H-3a), 2.10 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.56 (q, *J* = 11.0 Hz, 1H, H-3b) ; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.87 (C=O), 169.78 (C=O), 169.57 (C=O), 77.16 (C-5), 68.91 (C-1), 66.08 (C-4), 66.04 (C-2), 62.81 (C-2), 34.80 (C-3), 20.95 (Ac), 20.92 (Ac), 20.84 (Ac); HRMS (ESI-TOF MS.): Calcd for $C_{12}H_{18}O_7Na$ *m/z* $[M+Na]^+$: 297.0945, Found: 297.0939.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (4): To a solution of the compound **3** (7.94 g, 28.9 mmol) in dry MeOH (160 mL) was added sodium methoxide (160 μL, 28% in MeOH solution) at room temperature. After stirring for 5 h at room temperature, the reaction mixture was treated with

Amberlite IR-120 (H^+ form). After filtration, the filtrate was concentrated to afford the corresponding triol (4.19 g) which was used without any further purification.

Benzaldehyde dimethylacetal (12.8 mL, 86.8 mmol) and (±)-10-Camphorsulfonic acid (600 mg, 2.58 mmol) were added to a solution of the triol from above in MeCN (300 mL). After stirred at room temperature for 20 h, the reaction mixture was quenched by Et_3N (3 mL), followed by concentrated under reduced pressure. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 1 : 1) to give compound **3** (5.39 g, 2steps 79%) as a white powder. Rf = 0.30 (*n*-Hexane : AcOEt = 1 : 1). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.51-7.47 (m, 2H, Ph), 7.40-7.33 (m, 3H, Ph), 5.53 (s, 1H, PhC*H*-), 4.32 (dd, *J* = 11.0, 4.8 Hz, 1H, H-6a), 4.08-4.02 (m, 1H, H-2), 3.67 (t, *J* = 10.3 Hz, 1H, H-6b), 3.58-3.51 (m, 1H, H-4), 3.28 (dt, *J* = 10.3, 4.8 Hz, 1H, H-5), 3.21 (t, *J* = 10.3 Hz, 1H, H-1b), 2.56-2.49 (m, 1H, H-3a), 1.65 (q, *J* = 11.7 Hz, 1H, H-3b), 1.56 (d, *J* = 6.9 Hz, 1H, 2-O*H*); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 137.30 (Ph), 129.14 (Ph), 128.37 (Ph), 126.17 (Ph), 101.72 (Ph*C*H-), 76.39 (C-4), 73.15 (C-5), 72.48 (C-1), 69.25 (C-6), 65.63 (C-2), 38.46 (C-3); HRMS (APCI-TOF MS.): Calcd for $C_{13}H_{17}O_4$ *m/z* $[M+H]^+$: 237.1121, Found: 237.1126.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-D-fructose (5): Dess-Martin Periodinane (9.28 g, 21.9 mmol) was added to a solution of **4** (4.62 g, 19.6 mmol) in dry CH_2Cl_2 (200 mL) under Ar atmosphere. After stirring for 2 h at room temperature, saturated aq. $NaHCO_3$ (100 mL) was added to the reaction mixture and stirred for 0.5 h. The resulting mixture was diluted with CH_2Cl_2 and washed with saturated aq. $NaHCO_3$, and saturated aq. NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica-gel (Toluene : AcOEt = 10 : 1) to give compound **5** (4.47 g, 98%) as a white powder. Rf = 0.41 (Toluene : AcOEt = 10 : 1). ^{1}H NMR (600 MHz, CDCl3): δ = ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.53-7.46 (m, 2H, Ph), 7.42-7.34 (m, 3H, Ph), 5.58 (s, 1H, PhC*H*-), 4.45 (dd, *J* = 10.3, 4.8 Hz, 1H, H-6a), 4.17 (dd, *J* = 16.5, 1.4 Hz, 1H, H-1a), 4.04 (d, *J* = 16.5 Hz, 1H, H-1b), 3.97-3.90 (m, 1H, H-4), 3.79 (t, *J* = 10.3 Hz, 1H, H-6b), 3.63 (dt, *J* = 9.6, 4.8 Hz, 1H, H-5), 3.07 (dd, *J* = 16.5, 5.5 Hz, 1H, H-3a), 2.66 (dd, *J* = 16.5, 11.7 Hz, 1H, H-3b); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 204.03 (C-2),

136.89 (Ph), 129.31 (Ph), 128.42 (Ph), 126.14 (Ph), 101.41 (Ph<u>C</u>H-), 75.82 (C-4), 74.78 (C-1), 71.58 (C-5), 69.08 (C-6), 44.77 (C-3) ; HRMS (APCI-TOF MS.): Calcd for $C_{13}H_{15}O_4$ *m/z* $[M+H]^+$: 235.0965, Found: 235.0971.

1,5-Anhydro-3-deoxy-D-fructose (6) and 1,5-Anhydro-3-deoxy-D-*arabino*-hex-3-en-2-ulose (7): 70% aq. acetic acid (50 mL) was added to a solution of **5** (475 mg, 2.03 mmol) in dry CH_2Cl_2 (10 mL). After stirring for 1.5 h at 70 ^oC, the reaction mixture was concentrated. The residue was purified by column chromatography on silica-gel ($CHCl_3$: MeOH : H_2O = 85 : 15 : 1 and *n*-Hexane : AcOEt = 1 : 2) to give the desired compound **6** (251 mg, 85%) and compound **7** (25.1 mg, 10%) as a color less oil respectively.

Data for 1,5-Anhydro-3-deoxy-D-fructose (**6**): Rf = 0.41 ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2). ^1H NMR (600 MHz, $CDCl_3$): δ = 4.24-4.19 (m, 1H, H-4), 4.15 (d, *J* = 16.5 Hz, 1H, H-1a), 3.98-3.91 (m, 1H, H-6a), 3.96 (d, *J* = 16.5 Hz, 1H, H-1b), 3.91-3.85 (m, 1H, H-6b), 3.59-3.54 (m, 1H, H-5), 2.98 (dd, *J* = 16.5, 5.5 Hz, 1H, H-3a), 2.50 (dd, *J* = 16.5, 7.6 Hz, 1H, H-3b), 2.25 (brs, 1H, 4-O<u>H</u>), 1.96 (brs, 1H, 6-O<u>H</u>); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 206.28 (C-2), 80.40 (C-5), 73.40 (C-1), 67.12 (C-4), 63.23 (C-6), 46.21 (C-3) ; HRMS (ESI-TOF MS.): Calcd for $C_6H_{10}O_4Na$ *m/z* $[M+Na]^+$: 169.0471, Found: 169.0472.

Data for 1,5-Anhydro-3-deoxy-D-*arabino*-hex-3-en-2-ulose (**7**): Rf = 0.30 (*n*-Hexane : AcOEt = 1 : 3). ^1H NMR (600 MHz, $CDCl_3$): δ = 7.01 (dd, *J* = 10.3, 2.1 Hz, 1H, H-3), 6.24 (dd, *J* = 10.3, 2.7 Hz, 1H, H-4), 4.49-4.43 (m, 1H, H-5), 4.34 (d, *J* = 15.8 Hz, 1H, H-1a), 4.17 (dd, *J* = 15.8, 2.1 Hz, 1H, H-1b), 3.90-3.83 (m, 1H, H-6a), 3.82-3.76 (m, 1H, H-6b); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 194.43 (C-2), 148.40 (C-3), 128.35 (C-4), 74.47 (C-5), 71.28 (C-1), 64.09 (C-6) ; HRMS (APCI-TOF MS.): Calcd for $C_6H_9O_3$ *m/z* $[M+H]^+$: 129.0546, Found: 129.0550.

6-*O*-Acetyl-1,5-anhydro-3-deoxy-D-*arabino*-hex-3-en-2-ulose (8): Acetic anhydride (1.0 mL) was added to a solution of the compound **6** (47.5 mg, 312 μmol) in pyridine (2.0 mL) at room temperature. After stirred at room temperature for 20 h, the reaction mixture was concentrated. The

residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 2 : 1) to give compound **9** (42.5 mg, 82%) as a white powder. R_f = 0.23 (*n*-Hexane : AcOEt = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.97 (dd, *J* = 11.0, 2.1 Hz, 1H, H-3), 6.24 (dd, *J* = 11.0, 2.1 Hz, 1H, H-4), 4.60-4.56 (m, 1H, H-5), 4.36 (d, *J* = 15.8 Hz, 1H, H-1a), 4.35 (dd, *J* = 11.7, 6.2 Hz, 1H, H-6a), 4.27 (dd, *J* = 11.7, 4.1 Hz, 1H, H-6b), 4.16 (dd, *J* = 16.5, 1.4 Hz, 1H, H-1b), 2.12 (s, 3H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 193.91 (C-2), 170.66 (C=O), 146.88 (C-3), 128.47 (C-4), 71.92 (C-5), 71.13 (C-1), 64.38 (C-6), 20.76 (Ac) ; HRMS (APCI-TOF MS.): Calcd for $C_8H_{11}O_4$ *m/z* $[M+H]^+$: 170.0652, Found: 170.0658.

1,5-Anhydro-6-*O*-butanoyl-3-deoxy-D-*arabino*-hex-3-en-2-ulose (9): Butylic anhydride (335 μL, 2.05 mmol) and catalytic amount of DMAP were added to a solution of the compound **6** (100 mg, 684 μmol) in pyridine (1 mL) at room temperature. After stirred at room temperature for 18 h, MeOH (5 mL) was added to the reaction mixture at 0 ^{o}C, followed by diluted with AcOEt and washed with H_2O, 5% aq. citiric acid, saturated aq. $NaHCO_3$, and saturated aq. NaCl, dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 3 : 1) to give compound **9** (85.6 mg, 63%) as a yellow oil. R_f = 0.36 (*n*-Hexane : AcOEt = 2 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.97 (dd, *J* = 10.3, 2.1 Hz, 1H, H-3), 6.24 (dd, *J* = 10.3, 2.1 Hz, 1H, H-4), 4.59-4.55 (m, 1H, H-5), 4.37 (dd, *J* = 11.7, 6.2 Hz, 1H, H-6a), 4.35 (d, *J* = 16.5 Hz, 1H, H-1a), 4.26 (dd, *J* = 11.7, 4.1 Hz, 1H, H-6b), 4.15 (dd, *J* = 16.5, 2.1 Hz, 1H, H-1b), 2.35 (t, *J* = 7.6 Hz, 2H, -C$\underline{H_2}$CH$_2$CH$_3$), 1.67 (sext, *J* = 7.6 Hz, 2H, -CH$_2$C$\underline{H_2}$CH$_3$), 0.96 (t, *J* =7.6 Hz, 3H, -CH$_2$CH$_2$C$\underline{H_3}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 194.00 (C-2), 173.35 (C=O), 147.07 (C-3), 128.43 (C-4), 72.01 (C-5), 71.16 (C-1), 64.16 (C-6), 35.93 (-$\underline{C}$H$_2$CH$_2$CH$_3$), 18.37 (-CH$_2\underline{C}$H$_2$CH$_3$), 13.63 (-CH$_2$CH$_2\underline{C}$H$_3$) ; HRMS (APCI-TOF MS.): Calcd for $C_{10}H_{15}O_4$ *m/z* $[M+H]^+$: 199.0965, Found: 199.0972.

1,5-Anhydro-3-deoxy-6-*O*-octanoyl-D-*arabino*-hex-3-en-2-ulose (10): *n*-Octanoic anhydride (628 μL, 2.11 mmol) and catalytic amount of DMAP were added to a solution of the compound **6** (103

mg, 705 μmol) in pyridine (1 mL) at room temperature. After stirred at room temperature for 22 h, MeOH (5 mL) was added to the reaction mixture at 0 ^{o}C, followed by diluted with AcOEt and washed with H_2O, 5% aq. citiric acid, saturated aq. $NaHCO_3$, and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 4 : 1) to give compound **10** (109 mg, 61%) as a color less oil. R_f = 0.29 (*n*-Hexane : AcOEt = 3 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.97 (dd, *J* = 10.3, 2.1 Hz, 1H, H-3), 6.24 (dd, *J* = 10.3, 2.1 Hz, 1H, H-4), 4.60-4.55 (m, 1H, H-5), 4.39-4.32 (m, 2H, H-1a, H-6a), 4.26 (dd, *J* = 11.7, 4.1 Hz, 1H, H-6b), 4.16 (dd, *J* = 16.5, 1.4 Hz, 1H, H-1b), 2.36 (t, *J* = 7.6 Hz, 2H, -$C_7\underline{H_{15}}$), 1.68-1.59 (m, 2H, -$C_7\underline{H_{15}}$), 1.38-1.20 (m, 8H, -$C_7\underline{H_{15}}$), 0.88 (t, *J* =7.6 Hz, 3H, -$C_7\underline{H_{15}}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 193.99 (C-2), 173.56 (C=O), 147.08 (C-3), 128.43 (C-4), 72.01 (C-5), 71.17 (C-1), 64.16 (C-6), 34.07 (-$\underline{C_7}H_{15}$), 31.64 (-$\underline{C_7}H_{15}$), 29.03 (-$\underline{C_7}H_{15}$), 28.89 (-$\underline{C_7}H_{15}$), 24.86 (-$\underline{C_7}H_{15}$), 22.56 (-$\underline{C_7}H_{15}$), 14.07 (-$\underline{C_7}H_{15}$); HRMS (ESI-TOF MS.): Calcd for $C_{14}H_{22}O_4Na$ *m/z* $[M+Na]^+$: 277.1410, Found: 277.1414.

3-Deoxy-3-fluoro-1,2-5,6-di-*O*-isopropylidene-α-D-glucofuranose (12)[19]**:** (Diethylamino)sulfur trifluoride (4.83 mL, 49.5 mmol) was added to a solution of 1,2-5,6-di-*O*-isopropylidene-α-D-allofuranose (**11**; 6.44 g, 24.7 mmol) in THF (60 mL). After stirring at 40 ^{o}C for 15 h, the reaction was cooled to room temperature and quenched by MeOH (10 mL) and Et_3N (5 mL). The reaction mixture was diluted with ethyl acetate and washed with H_2O, saturated aq. $NaHCO_3$, and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 4 : 1) to give compound **12** (5.81 g, 90%) as yellow oil. Rf = 0.50 (*n*-Hexane : AcOEt = 4 : 1). Spectrum data were consistent with those of known compound.

1,2,4,6-Tetra-*O*-acetyl-3-deoxy-3-fluoro-D-glucopyranose (13)[19]**:** 0.1 M sulfulic acid (180 mL, 18.0 mmol) was added to **12** (5.81 g, 22.2 mmol). After stirring at 60 ^{o}C for 17 h, the reaction was cooled to room temperature and neutralized with $NaHCO_3$ (3.33 g, 39.6 mmol). The reaction

solution was concentrated under reduced pressure. The resulting residue was dried under high vacuum for 3 hours and used without any further purification.

Acetic anhydride (30 mL) was added to a solution of the oil from above in pyridine (60 mL) at 0 ^{o}C. After stirred at room temperature for 14 h, MeOH (30 mL) was added to the reaction mixture at 0 ^{o}C, followed by concentrated under reduced pressure. The residual oil was diluted with AcOEt and washed with H_2O, 1 N HCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 3 : 2) to give compound **13** (7.89 g, quant.) as a color less oil. R_f = 0.35 (*n*-Hexane : AcOEt = 3 : 2)

Spectrum data were consistent with those of known compound.

2,4,6-Tri-*O*-acetyl-1,5-Anhydro-3-deoxy-3-fluoro-D-glucitol (14)[19]**:** HBr (33% in acetic acid, 22.0 mL, 65.9 mmol) was added to a mixture of **13** (7.69 g, 22.0 mmol) in dry CH_2Cl_2 (35 mL) at room temperature. After stirring at room temperature for 3 h, ice chips were added to the reaction mixture and very carefully extracted with CH_2Cl_2. The organic layer was washed with twice H_2O, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated to afford the anomeric bromide (8.20 g) which was used without any further purification.

To a mixture of the bromide from above and TTMSS (16.9 mL, 54.9 mmol) in toluene (80 mL) and 1,4-dioxane (40 mL) was added 1.0 M triethylborane in THF (6.59 mL, 6.59 mmol) at room temperature. After stirring for 16 h at room temperature, the reaction mixture was concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 2 : 1) to give compound **14** (5.78 g, 2steps 90%) as a color less oil. Rf = 0.22 (*n*-Hexane : AcOEt = 2 : 1).

Spectrum data were consistent with those of known compound.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-fluoro-D-glucitol (15)[19]**:** To a solution of the compound **14** (4.24 g, 14.5 mmol) in dry MeOH (80 mL) was added sodium methoxide (240 μL,

28% in MeOH solution) at room temperature. After stirring for 22 h at room temperature, the reaction mixture was treated with Amberlite IR-120 (H^+ form). After filtration, the filtrate was concentrated to afford the corresponding triol (2.49 g) which was used without any further purification.

Benzaldehyde dimethylacetal (6.43 mL, 43.5 mmol) and (±)-10-Camphorsulfonic acid (300 mg, 1.29 mmol) were added to a solution of the triol from above in MeCN (150 mL). After stirred at room temperature for 19 h, the reaction mixture was quenched by triethylamine (2 mL), followed by concentrated under reduced pressure. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 3 : 2) to give compound **15** (3.26 g, 2steps 88%) as a white powder. Rf = 0.35 (*n*-Hexane : AcOEt = 3 : 2). Spectrum data were consistent with those of known compound.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-3-fluoro-D-fructose (16): $PhI(OAc)_2$ (770 mg, 2.40 mmol) was added to a mixture of the compound **15** (405 mg, 1.59 mmol) and 1-Me-AZADO (2.65 mg, 15.9 μmol) in CH_2Cl_2 (16 mL) at room temperature. After stirred for 22 h at room temperature, the reaction was quenched by saturated aq. $Na_2S_2O_3$ (4 mL) and saturated aq. $NaHCO_3$ (4 mL). The mixture was stirred for 0.5 h at room temperature, followed by diluted with AcOEt and washed with H_2O, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 1 : 1) to give compound **16** (299 mg, 75%) as a white powder. R_f = 0.28 (*n*-Hexane : AcOEt = 1 : 1). ^{1}H NMR (600 MHz, CDCl3): δ = 7.56-7.49 (m, 2H, Ph), 7.42-7.36 (m, 3H, Ph), 5.61 (s, 1H, PhC*H*-), 5.11 (dd, *J* = 49.5, 10.3 Hz, 1H, H-3), 4.88 (ddd, *J* = 10.3, 4.8, 2.1 Hz, 1H, H-6a), 4.31 (dd, *J* = 15.1, 4.1 Hz, 1H, H-1a), 4.12 (d, *J* = 15.1 Hz, 1H, H-1b), 4.12-4.03 (m, 1H, H-4), 3.84 (t, *J* = 10.3 Hz, 1H, H-6b), 3.79 (dt, *J* = 8.9, 4.8 Hz, 1H, H-5); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 197.48 (d, *J* = 13.0 Hz, C-2), 136.33 (Ph), 129.43 (Ph), 128.40 (Ph), 126.17 (Ph), 101.32 (Ph*C*H-), 91.84 (d, *J* = 202.3 Hz, C-3), 80.65 (d, *J* = 17.3 Hz, C-4), 74.24 (C-1), 70.10 (d, *J* = 7.2 Hz, C-5), 68.47 (C-6); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -202.08 (d, *J* = 43.6 Hz); HRMS

(APCI-TOF MS.): Calcd for $C_{13}H_{14}O_4F$ *m/z* $[M+H]^+$: 253.0871, Found: 253.0878.

1,5-Anhydro-3-deoxy-3-fluoro-D-*arabino*-hex-3-en-2-ulose (17): A solution of the compound **16** (289 mg, 1.15 mmol) in EtOH (1.5 mL)–AcOEt (1.5 mL) was added to a suspension 10% Pd/C (243 mg) in EtOH (8.5 mL)–AcOEt (8.5 mL). After bubbling with hydrogen for 1 h at room temperature, the reaction mixture was filtered on Celite and concentrated in vacuo, and used without any further purification.

Acetic anhydride (2.0 mL) was added to a solution of the oil from above in pyridine (3.0 mL). After stirred at room temperature for 18 h, MeOH (4 mL) was added to the reaction mixture at 0 ^oC, followed by diluted with AcOEt and washed with H_2O, twice 1 N HCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 2 : 1) to give compound **17** (164 mg, 2steps 76%) as a color less oil. R_f = 0.28 (*n*-Hexane : AcOEt = 2 : 1). ^1H NMR (600 MHz, $CDCl_3$): δ = 6.48 (dd, *J* = 12.4, 2.1 Hz, 1H, H-4), 4.81-4.76 (m, 1H, H-5), 4.46 (dd, *J* = 16.5, 3.4 Hz, 1H, H-1a), 4.36 (dd, *J* = 11.7, 6.7 Hz, 1H, H-6a), 4.24 (dd, *J* = 12.4, 4.1 Hz, 1H, H-6b), 4.23 (dd, *J* = 16.5, 1.4 Hz, 1H, H-1b), 2.13 (s, 3H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 187.20 (d, *J* = 17.3 Hz, C-2), 170.55 (C=O), 152.15 (d, *J* = 274.5 Hz, C-3), 122.54 (d, *J* = 8.7 Hz, C-4), 72.36 (d, *J* = 4.3 Hz, C-5), 71.80 (d, *J* = 4.3 Hz, C-1), 64.37 (d, *J* = 2.9 Hz, C-6), 20.73 (Ac) ; ^{19}F NMR (600 MHz, $CDCl_3$): δ = -129.13; HRMS (ESI-TOF MS.): Calcd for $C_8H_9O_4FNa$ *m/z* $[M+Na]^+$: 211.0377, Found: 211.0378.

4,6-*O*-Benzylidene-3-deoxy-3-fluoro-D-glucopyranose (18): To a solution of the compound **13** (8.92 g, 25.5 mmol) in dry MeOH (120 mL) was added sodium methoxide (480 μL, 28% in MeOH solution) at room temperature. After stirring for 24 h at room temperature, the reaction mixture was treated with Amberlite IR-120 (H^+ form). After filtration, the filtrate was concentrated to afford the corresponding triol (4.89 g) which was used without any further purification.

Benzaldehyde dimethylacetal (11.3 mL, 76.4 mmol) and (±)-10-Camphorsulfonic acid (500 mg, 2.15

mmol) were added to a solution of the tetraol from above in MeCN (250 mL). After stirred at room temperature for 2 hours, the reaction mixture was quenched by Et_3N (2 mL), followed by concentrated under reduced pressure. The residual oil was diluted with AcOEt and washed with H_2O, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : AcOEt = 1 : 3) to give compound **18** (3.89 g, 2steps 56%, α/β = 60/40, inseparable) as a white powder. Rf = 0.41 (*n*-Hexane : AcOEt = 1 : 3). ^{1}H NMR (600 MHz, $CDCl_3$): **Data for α isomer**; δ = 7.49-7.43 (m, 2H, Ph), 7.38-7.31 (m, 3H, Ph), 5.59 (s, 1H, PhC*H*-), 5.17 (t, *J* = 3.4 Hz, 1H, H-1), 4.67 (dt, *J* = 55.7, 8.9 Hz, 1H, H-3), 4.21-4.18 (m, 1H, H-6a), 3.99 (dt, *J* = 10.3, 4.8 Hz, 1H, H-5), 3.81-3.69 (m, 3H, H-2, H-4, H-6b); **Data of β isomer**; δ = 7.49-7.43 (m, 2H, Ph), 7.38-7.31 (m, 3H, Ph), 5.58 (s, 1H, PhC*H*-), 4.63 (d, *J* = 7.6 Hz, 1H, H-1), 4.49 (dt, *J* = 53.6, 8.9 Hz, 1H, H-3), 4.30-4.26 (m, 1H, H-6a), 3.81-3.69 (m, 2H, H-4, H-6b), 3.52-3.42 (m, 2H, H-2, H-5); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 138.93 (Ph), 138.85 (Ph), 130.01 (Ph), 129.10 (Ph), 127.42 (Ph), 127.40 (Ph), 102.73 (α-Ph*C*H-), 102.60 (β-Ph*C*H-), 98.46 (d, *J* = 11.6 Hz, β-C-1), 94.97 (d, *J* = 10.1 Hz, α-C-1), 94.32 (d, *J* = 185.0 Hz, β-C-3), 92.68 (d, *J* = 183.5 Hz, α-C-3), 81.08 (d, *J* = 15.9 Hz, α-C-4), 80.39 (d, *J* = 17.3 Hz, β-C-4), 75.60 (d, *J* = 17.3 Hz, β-C-2), 72.76 (d, *J* = 17.3 Hz, α-C-2), 70.06 (α-C-6), 69.61 (β-C-6), 66.29 (d, *J* = 8.7 Hz, β-C-5), 62.84 (d, *J* = 7.2 Hz, α-C-5); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -196.25 (d, *J* = 43.6 Hz), -201.70 (d, *J* = 43.6 Hz) ; HRMS (ESI-TOF MS.): Calcd for $C_{13}H_{15}O_5FNa$ *m/z* $[M+Na]^+$: 293.0796, Found: 293.0803.

1,2-Di-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-fluoro-D-glucopyranose (19): Acetic anhydride (6.0 mL) was added to a solution of the compound **18** (3.83 g, 14.2 mmol) in pyridine (12 mL) at 0 °C. After stirred at room temperature for 16 h, MeOH (10 mL) was added to the reaction mixture at 0 °C, followed by concentrated under reduced pressure. The residual oil was diluted with AcOEt and washed with H_2O, twice 1 N HCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (Toluene : AcOEt = 15 : 1) to give compound **19** (4.84 g, 96%, α/β =

60/40, inseparable) as a white powder. R_f = 0.43 (Toluene : AcOEt = 10 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): **Data for α isomer**; δ = 7.54-7.47 (m, 2H, Ph), 7.41-7.33 (m. 3H, Ph), 6.33 (t, *J* = 3.4 Hz, 1H, H-1), 5.59 (s, 1H, PhC*H*-), 5.23-5.15 (m, 1H, H-2), 4.92 (dt, *J* = 54.3, 8.9 Hz, 1H, H-3), 4.36-4.31 (m, 1H, H-6a), 3.97 (dt, *J* = 10.3, 4.8 Hz, 1H, H-5), 3.92-3.83 (m, 1H, H-4), 3.83-3.75 (m, 1H, H-6b), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac); **Data of β isomer**; δ = 7.54-7.47 (m, 2H, Ph), 7.41-7.33 (m, 3H, Ph), 5.73 (d, *J* = 8.3 Hz, 1H, H-1), 5.57 (s, 1H, PhC*H*-), 5.28 (dt, *J* = 13.7, 8.9 Hz, 1H, H-2), 4.72 (dt, *J* = 52.9, 8.9 Hz, 1H, H-3), 4.44-4.37 (m, 1H, H-6a), 3.92-3.83 (m, 1H, H-4), 3.83-3.75 (m, 1H, H-6b), 3.59 (dt, *J* = 9.6, 4.8 Hz, 1H, H-5), 2.17 (s, 3H, Ac), 2.12 (s, 3H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 169.75 (C=O), 169.16 (C=O), 168.97 (C=O), 168.86 (C=O), 136.46 (Ph), 136.40 (Ph), 129.37 (Ph), 128.37 (Ph), 126.19 (Ph), 101.77 (α-Ph*C*H-), 101.68 (β-Ph*C*H-), 91.84 (d, *J* = 10.1 Hz, β-C-1), 90.40 (d, *J* = 192.2 Hz, β-*C*-3), 89.85 (d, *J* = 10.1 Hz, α-C-1), 87.89 (d, *J* = 190.7 Hz, α-C-3), 78.96 (d, *J* = 17.3 Hz, α-C-4), 78.44 (d, *J* = 17.3 Hz, β-C-4), 71.02 (d, *J* = 18.83 Hz, β-C-2), 70.13 (d, *J* = 17.3 Hz, α-C-2), 68.43 (α-C-6), 68.18 (β-C-6), 65.81 (d, *J* = 8.7 Hz, β-C-5), 64.27 (d, *J* = 7.2 Hz, α-C-5), 20.83 (Ac), 20.76 (Ac), 20.67 (Ac), 20.55 (Ac); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -196.41 (d, *J* = 43.6 Hz), -200.77 (d, *J* = 43.6 Hz); HRMS (ESI-TOF MS.): Calcd for $C_{17}H_{19}O_7FNa$ *m/z* $[M+Na]^+$: 377.1007, Found: 377.1012.

1,2-Di-*O*-acetyl-4-*O*-benzyl-3-deoxy-3-fluoro-6-D-glucopyranose (20): To a mixture of compound **19** (4.81 g, 13.6 mmol) and Et_3SiH (6.50 mL, 40.7 mmol) in dry CH_2Cl_2 (100 mL) was added $PhBCl_2$ (5.89 mL, 44.8 mmol) at -78 °C under Ar atmosphere. After stirring for 30 min at -78 °C, the reaction was quenched by a cocktail of Et_3N (25 mL), MeOH (50 mL) and $CHCl_3$ (50 mL). After stirring for 10 min at room temperature, the reaction mixture was diluted with $CHCl_3$ and washed with saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (*n*-Hexane : EtOH = 10 : 1) to give compound **20** (2.35 g, 49%, α/β = 45/55, inseparable) as a color less oil. R_f = 0.19 (*n*-Hexane : EtOH = 10 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): **Data for α isomer**; δ = 7.40-7.29 (m, 5H, Ph), 6.28 (t, *J* = 3.4 Hz, 1H, H-1), 5.13-5.07 (m, 1H, H-2), 4.94 (dt, *J* = 53.6, 8.9

Hz, 1H, H-3), 4.89 (d, *J* = 11.7 Hz, 1H, PhC*H*$_2$-), 4.67 (d, *J* = 13.1 Hz, 1H, PhC*H*$_2$-), 3.91-3.70 (m, 4H, H-4, H-5, H-6a, H-6b), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.87 (dd, *J* = 7.6, 5.5 Hz, 1H, -O*H*); **Data of β isomer**; δ = 7.40-7.29 (m, 5H, Ph), 5.63 (d, *J* = 8.2 Hz, 1H, H-1), 5.18 (dt, *J* = 13.6, 8.9 Hz, 1H, H-2), 4.86 (d, *J* = 11.0 Hz, 1H, PhC*H*$_2$-),4.70 (dt, *J* = 50.9, 8.2 Hz, 1H, H-3), 4.65 (d, *J* = 11.0 Hz, 1H, PhC*H*$_2$-), 3.91-3.70 (m, 3H, H-4, H-6a, H-6b), 3.52-3.47 (m, 1H, H-5), 2.12 (s, 3H, Ac), 2.10 (s, 3H, Ac), 1.82 (dd, *J* = 7.6, 5.5 Hz, 1H, -O*H*); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 169.93 (C=O), 169.36 (C=O), 169.16 (C=O), 168.99 (C=O), 137.37 (Ph), 137.31 (Ph), 128.55 (Ph), 128.24 (Ph), 128.19 (Ph), 95.43 (d, *J* = 186.4 Hz, β-C-3), 92.83 (d, *J* = 186.5 Hz, α-C-3), 91.27 (d, *J* = 11.6 Hz, β-C-1), 89.51 (d, *J* = 10.2 Hz, α-C-1), 74.93 (d, *J* = 8.7 Hz, β-C-5),74.84 (Ph*C*H$_2$-), 74.82 (d, *J* = 15.9 Hz, α-C-4), 74.67 (d, *J* = 17.3 Hz, β-C-4), 74.56 (PhCH$_2$-), 72.84 (d, *J* = 7.2 Hz, α-C-5), 70.52 (d, *J* = 18.8 Hz, β-C-2), 69.79 (d, *J* = 18.8 Hz, α-C-2), 61.03 (α-C-6), 60.99 (β-C-6), 20.77 (Ac), 20.68 (Ac), 20.58 (Ac); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -192.28 (d, *J* = 43.6 Hz), -196.29 (d, *J* = 43.6 Hz); HRMS (ESI-TOF MS.): Calcd for $C_{17}H_{21}O_7FNa$ *m/z* $[M+Na]^+$: 379.1164, Found: 379.1167.

4-*O*-Benzyl-3-deoxy-3-fluoro-1,2-di-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-D-glucopyranose (21): *p*-toluenesulfonyl chloride (1.84 g, 9.64 mmol) was added to a solution of the compound **20** (2.29 g, 6.63 mmol) in pyridine (25 mL) at room temperature. After stirred for 19 hours, MeOH (10 mL) was added to the reaction mixture at 0 °C, followed by concentrated under reduced pressure. The residual oil was diluted with AcOEt and washed with H_2O, 1 N HCl, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (Toluene : AcOEt = 10 : 1) to give compound **21** (2.97 g, 91%, α/β = 47/53, inseparable) as a white powder. R_f = 0.33 (Toluene : AcOEt = 10 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): **Data for α isomer**; δ = 7.78 (d, *J* = 3.4 Hz, 1H, Ph), 7.39-7.23 (m, 7H, Ph), 6.15 (t, *J* = 3.4 Hz, 1H, H-1), 5.03-4.95 (m, 1H, H-2), 4.88 (dt, *J* = 52.9, 8.9 Hz, 1H, H-3), 4.85 (d, *J* = 11.0 Hz, 1H, PhC*H*$_2$-), 4.59 (d, *J* = 10.3 Hz, 1H, PhC*H*$_2$-), 4.32 (dd, *J* = 11.0 3.4 Hz, 1H, H-6a), 4.20 (dt, *J* = 11.0 Hz, 1H, H-6b), 3.89 (brd, *J* = 10.3 Hz, 1H, H-5), 3.81-3.72 (m, 1H, H-4),

2.44 (s, 3H, PhC$\underline{H_3}$), 2.08 (s, 3H, Ac), 2.07 (s, 3H, Ac); **Data of β isomer**; δ = 7.77 (d, *J* = 3.4 Hz, 1H, Ph), 7.39-7.23 (m, 7H, Ph), 5.54 (d, *J* = 8.3 Hz, 1H, H-1), 5.12 (dt, *J* = 13.7, 8.2 Hz, 1H, H-2), 4.81 (d, *J* = 11.0 Hz, 1H, PhC$\underline{H_2}$-), 4.63 (dt, *J* = 52.2, 8.9 Hz, 1H, H-3), 4.55 (d, *J* = 11.0 Hz, 1H, PhC$\underline{H_2}$-), 4.29 (dd, *J* = 11.0, 3.4 Hz, 1H, H-6a), 4.24 (d, *J* = 11.0 Hz, 1H, H-6b), 3.83-3.72 (m, 1H, H-4), 3.58 (brd, *J* = 9.6 Hz, 1H, H-5), 2.43 (s, 3H, PhC$\underline{H_3}$), 2.09 (s, 3H, Ac), 2.09 (s, 3H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 169.70 (C=O), 169.16 (C=O), 168.90 (C=O), 168.56 (C=O), 145.20 (Ph), 145.08 (Ph), 136.96 (Ph), 136.94 (Ph), 132.58 (Ph), 129.89 (Ph), 129.86 (Ph), 128.57 (Ph), 129.52(Ph), 128.34 (Ph), 128.25 (Ph), 128.23 (Ph), 128.05 (Ph). 128.02 (Ph), 95.26 (d, *J* = 189.3 Hz, β-C-3), 92.63 (d, *J* = 187.8 Hz, α-C-3), 90.94 (d, *J* = 11.6 Hz, β-C-1), 89.16 (d, *J* = 8.7 Hz, α-C-1), 74.90 (d, *J* = 2.9 Hz, α-Ph$\underline{C}$H$_2$-), 74.61 (d, *J* = 2.9 Hz, β-Ph$\underline{C}$H$_2$-), 74.51 (d, *J* = 17.3 Hz, α-C-4), 74.36 (d, *J* = 17.3 Hz, β-C-4), 72.34 (d, *J* = 10.1 Hz, β-C-5), 70.43 (d, *J* = 10.1 Hz, α-C-5), 70.16 (d, *J* = 18.8 Hz, β-C-2), 69.41 (d, *J* = 17.3 Hz, α-C-2), 67.44 (α-C-6), 67.16 (β-C-6), 21.66 (Ph-$\underline{C}$H$_3$), 20.75 (Ac), 20.72 (Ac), 20.63 (Ac), 20.51 (Ac); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -191.93 (d, *J* = 45.6 Hz), -196.21 (d, *J* = 45.6 Hz); HRMS (ESI-TOF MS.): Calcd for $C_{24}H_{27}O_9FNaS$ *m/z* $[M+Na]^+$: 533.1252, Found: 533.1259.

1,6-Anhydro-4-*O*-benzyl-3-deoxy-3-fluoro-β-D-glucopyranoside (22): 3.03 M aq. NaOH (6.27 mL, 19.0 mmol) was added to a solution of the compound **21** (2.94 g, 5.76 mmol) in pyridine (30 mL) at room temperature. After stirred for 17 h, the reaction mixture was diluted with AcOEt and washed with four times 5% aq. citric acid, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (Toluene : AcOEt = 5 : 1) to give compound **22** (1.01 g, 69%) as a white powder. R_f = 0.33 (Toluene : AcOEt = 5 : 1); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.40-7.30 (m, 5H, Ph), 5.45 (s, 1H, H-1), 4.70 (d, *J* = 12.3 Hz, 1H, PhC$\underline{H_2}$-), 4.66 (d, *J* = 12.3 Hz, 1H, PhC$\underline{H_2}$-), 4.64 (d, *J* = 44.7 Hz, 1H, H-3), 4.61 (d, *J* = 6.2 Hz, 1H, H-5), 3.98 (d, *J* = 7.6 Hz, 1H, H-6a), 3.78 (dt, *J* = 6.2, 2.1 Hz, 1H, H-6b), 3.64 (brt, *J* = 10.3 Hz, 1H, H-2), 3.51 (d, *J* = 13.1 Hz, 1H, H-4), 2.65 (d, *J* = 10.3 Hz, 1H, -O$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 136.82 (Ph), 128.68 (Ph),

128.28 (Ph), 127.89 (Ph), 101.26 (C-1), 88.22 (d, *J* = 183.5 Hz, C-3), 74.17 (d, *J* = 27.5 Hz, C-4), 73.58 (C-5), 71.61 (Ph<u>*C*</u>H_2-), 67.37 (d, *J* = 23.1 Hz, C-2), 64.97 (d, *J* = 4.3 Hz, C-6); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -184.94 (d, *J* = 43.6 Hz); HRMS (ESI-TOF MS.): Calcd for $C_{13}H_{15}O_4FNa$ *m/z* $[M+Na]^+$: 277.0847, Found: 277.0850.

1,6-anhydro-3,4-bisdeoxy-3-fluoro-β-D-*glycero*-hex-3-enopyranos-2-ulose (23) and 2,4-di-*O*-acetyl-1,6-anhydro-3-deoxy-3-fluoro-β-D-*glycero*-hex-2-enopyranose (24): $PhI(OAc)_2$ (1.21 g, 3.74 mmol) was added to a mixture of the compound **22** (476 mg, 1.87 mmol) and 1-Me-AZADO (93.4 mg, 562 μmol) in CH_2Cl_2 (20 mL) at room temperature. After stirred for 20 h at room temperature, the reaction was quenched by saturated aq. $Na_2S_2O_3$ (5 mL) and saturated aq. $NaHCO_3$ (5 mL). The mixture was stirred for 0.5 h at room temperature, followed by diluted with AcOEt and washed with H_2O, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel (Toluene : AcOEt = 3 : 2) to give the crude compound **23** (220 mg) which was used without any further purification.

A solution of the oil from above (220) in AcOEt (3 mL) was added to a suspension 10% Pd/C (158 mg) in AcOEt (13 mL). After bubbling with hydrogen for 1.5 h at room temperature, the reaction mixture was filtered on Celite and concentrated in vacuo, and used without any further purification. Acetic anhydride (1.0 mL) was added to a solution of the oil from above in pyridine (1.5 mL). After stirred at room temperature for 2 h, MeOH (2 mL) was added to the reaction mixture at 0 ^{o}C, followed by diluted with AcOEt and washed with three times of 5% aq. citiric acid, saturated aq. $NaHCO_3$ and saturated aq. NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica-gel ($CHCl_3$) to give compound **23** (106 mg, 3 steps 39%) and compound **24** (35.9 mg, 3 steps 8%) as a color less oil respectively.

Data for 1,6-anhydro-3,4-bisdeoxy-3-fluoro-β-D-*glycero*-hex-3-enopyranos-2-ulose (**23**): Rf = 0.42 ($CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.72(dd, *J* = 10.3, 5.5 Hz, 1H, H-4), 5.48 (d, *J* = 6.9 Hz,

1H, H-1), 5.15 (q, *J* = 4.8 Hz, 1H, H-5), 3.95 (t, *J* = 5.5 Hz, 1H, H-6a), 3.85 (d, *J* = 6.9 Hz, 1H, H-6b); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 182.69 (d, *J* = 18.8 Hz, C-2), 152.85 (d, *J* = 280.33 Hz, C-3), 123.06 (d, *J* = 7.2 Hz, C-4), 101.22 (d, *J* = 7.2 Hz, C-1), 71.81 (d, *J* = 7.2 Hz, C-5), 67.16 (d, *J* = 2.9 Hz, C-6); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -131.98; HRMS (APCI-TOF MS.): Calcd for $C_6H_6O_3F$ *m/z* $[M+H]^+$: 145.0296, Found: 145.0300.

Data for 2,4-di-*O*-acetyl-1,6-anhydro-3-deoxy-3-fluoro-β-D-*glycero*-hex-2-enopyranose (**24**): Rf = 0.15 ($CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.68 (d, *J* = 7.6 Hz, 1H, H-1), 5.23 (dd, *J* = 6.9, 1.4 Hz, 1H, H-4), 4.77 (t, *J* = 6.9 Hz, 1H, H-5), 4.01 (t, *J* = 6.9 Hz, 1H, H-6a), 3.66 (dd, *J* = 8.2, 2.1 Hz, 1H, H-6b), 2.25 (s, 3H, Ac), 2.19 (s, 3H, Ac); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.46 (C=O), 166.43 (C=O), 142.94 (d, *J* = 274.6 Hz, C-3), 131.30 (d, *J* = 4.3 Hz, C-2), 97.41 (d, *J* = 4.3 Hz, C-1), 75.31 (d, *J* = 7.2 Hz, C-5), 68.99 (d, *J* = 20.3 Hz, C-4), 63.24 (C-6), 20.85 (Ac), 20.28 (Ac); ^{19}F NMR (600 MHz, $CDCl_3$): δ = -137.93; HRMS (ESI-TOF MS.): Calcd for $C_{10}H_{11}O_6FNa$ *m/z* $[M+Na]^+$: 269.0432, Found: 269.0435.

4.2. Biological studies

Cell culture and reagents

To generate bone marrow–derived macrophages (BMDMs), bone marrow cells were collected from C57BL/6 mouse femurs and cultured for 6–8 days on petri dishes in DMEM/F12 supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA USA), 100 U/mL penicillin, 100 µg/mL streptomycin, and 30% L929 cell-conditioned medium.

The human monocyte cell line THP-1 was cultured in RPMI-1640 medium with 10% FBS, 10 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 50 µM 2-ME (complete RPMI medium) (Wako Pure Chemical Industries, Osaka, Japan).

Anti-murine IL-1β antibody (AF-401-NA) was purchased from R&D Systems (Minneapolis, MN, USA), and anti-human IL-1β monoclonal antibody was purchased from Santa Cruz

Biotechnology, Dallas, TX, USA). Ultrapure LPS from *Escherichia coli* 0111:B4 and Nigericin were purchased from Invivogen (San Diego, CA, USA). 1,5-AF, 3-deoxy-1,5-AF, and enone compounds were dissolved in sterile, endotoxin-free water, and other synthetic compounds were dissolved in sterile, endotoxin-free DMSO; these were stored as stock solutions at −20°C and were diluted with medium just before each experiment. The final DMSO concentration did not exceed 0.1% throughout the study.

Cell stimulation and measurement of IL-1β

BMDMs were seeded onto 96-well plates at 5×10^4 cells per well and were primed with 100 ng/mL LPS in DMEM/F12 supplemented with 10% FBS for 4 h. Cells were washed once with PBS and were incubated with each compound for 1 h prior to stimulation with Nigericin (5 μM, 1 h) in serum-free DMEM/F12 medium. The release of IL-1β in culture supernatants was determined by anti-mouse IL-1β-specific ELISA (eBioscience, San Diego, CA, USA).

THP-1 cells were seeded at 1×10^5 cells per well in a 96-well plate. They were treated with 100 nM PMA (phorbol 12-myristate 13-acetate) for 48 h. LPS (100 ng/mL) was then added to activate the PMA-differentiated THP-1 cells. THP-1 cells primed with LPS for 3 h were washed once with PBS and were incubated with each compound for 1 h prior to stimulation with Nigericin (5 μM, 1 h) in serum-free RPMI medium. At the end of stimulation, supernatants were collected. The levels of IL-1β in supernatants were determined by cell-based assay using HEK-blue-IL-1β cells (Invivogen) following the manufacturer's instructions. In brief, HEK-blue-IL-1β cells were incubated with collected supernatants and assayed for alkaline phosphatase activities by adding QUANTI-Blue substrate (Invivogen) and reading the absorbance at 650 nm.

Intracellular ATP assay

To determine the number of viable cells in culture, we quantified the amount of ATP present, which indicates the presence of metabolically active cells. Equal volumes of CellTiter-Glo reagents (Promega Corporation, Madison, MI, USA) were added directly to the wells. Plates were incubated at room temperature for 10 min on a shaker and luminescence was measured by a luminometer

(TriStar^2 LB 942; Berthold Technology, Bad Wildbad, Germany) according to the manufacturer's instructions.

Western blotting

BMDMs or THP-1 cells (1.5×10^6 cells per 3.5 ⌀ dish) primed with LPS were stimulated with Nigericin (5 μM) in serum-free RPMI or DMEM/F12 medium for 1 h. At the end of stimulation, supernatants were collected and concentrated with 10 K MWCO centrifugal filters (Amicon Ultra, Merck Millipore, Bilerica, MA, USA). The cells were lysed with lysis buffer (20 mM HEPES–KOH, pH 7.5, 150 mM KCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1 mM PMSF, ×100 protease inhibitor mixture (CalbioChem, San Diego, CA, USA). Cell lysates and concentrated supernatants were mixed and boiled with SDS sample buffer for 5 min at 99°C, and were resolved by 12.5% gel SDS-PAGE and were transferred onto PVDF membranes. Generation of IL-1β was determined by immunoblotting with anti-mouse or anti-human IL-1β antibodies followed with peroxidase-conjugated secondary antibodies. Signal detection was achieved using SuperSignal West Dura Substrate (Thermo Fisher Scientific) and blots were imaged using the ChemiDoc XRS Plus system (Bio-Rad, Hercules, CA, USA).

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Keywords

Carbohydrates, 1,5-Anhydro-D-fructose derivatives, enones, inflammasome, NLRP3

References

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13. See supporting information (**Fig. S27.**).
14. Compound **11** is commercially available from Tokyo Chemical Industry Co., Ltd.
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Supporting information

Synthesis of 1,5-Anhydro-D-fructose derivatives and evaluation of their inflammasome inhibitors

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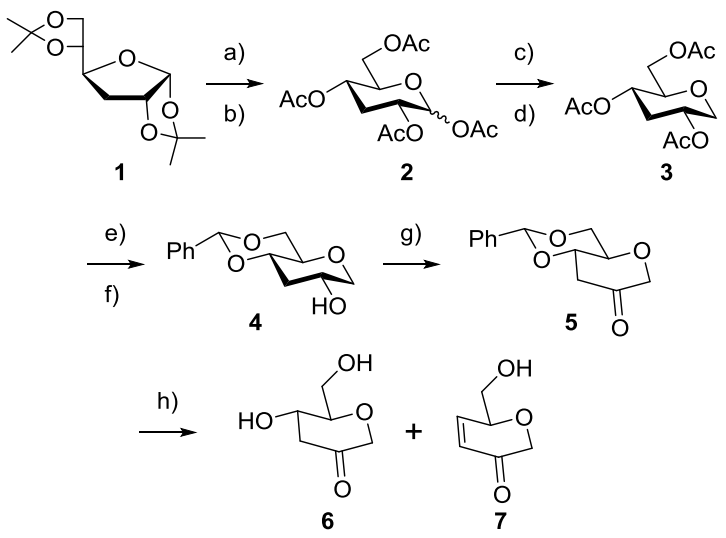
Supporting Information



Scheme S1. Synthesis of 3-deoxy derivative **6** and enone **7**. Reagents and conditions: a) 0.1 M H_2SO_4, 60 ^oC; b) Ac_2O, Pyridine, 2 steps 90%; c) HBr-AcOH, CH_2Cl_2; d) $(TMS)_3SiH$, Et_3B, Tol-1,4-dioxane, 2 steps 63%; e) NaOMe, MeOH; f) $PhCH(OMe)_2$, CSA, MeCN, 2 steps 79%; g) Dess-Martin periodinane, CH_2Cl_2, 98%; h) 70% aq. AcOH 70 ^oC, 85%(**6**) and 10%(**7**).

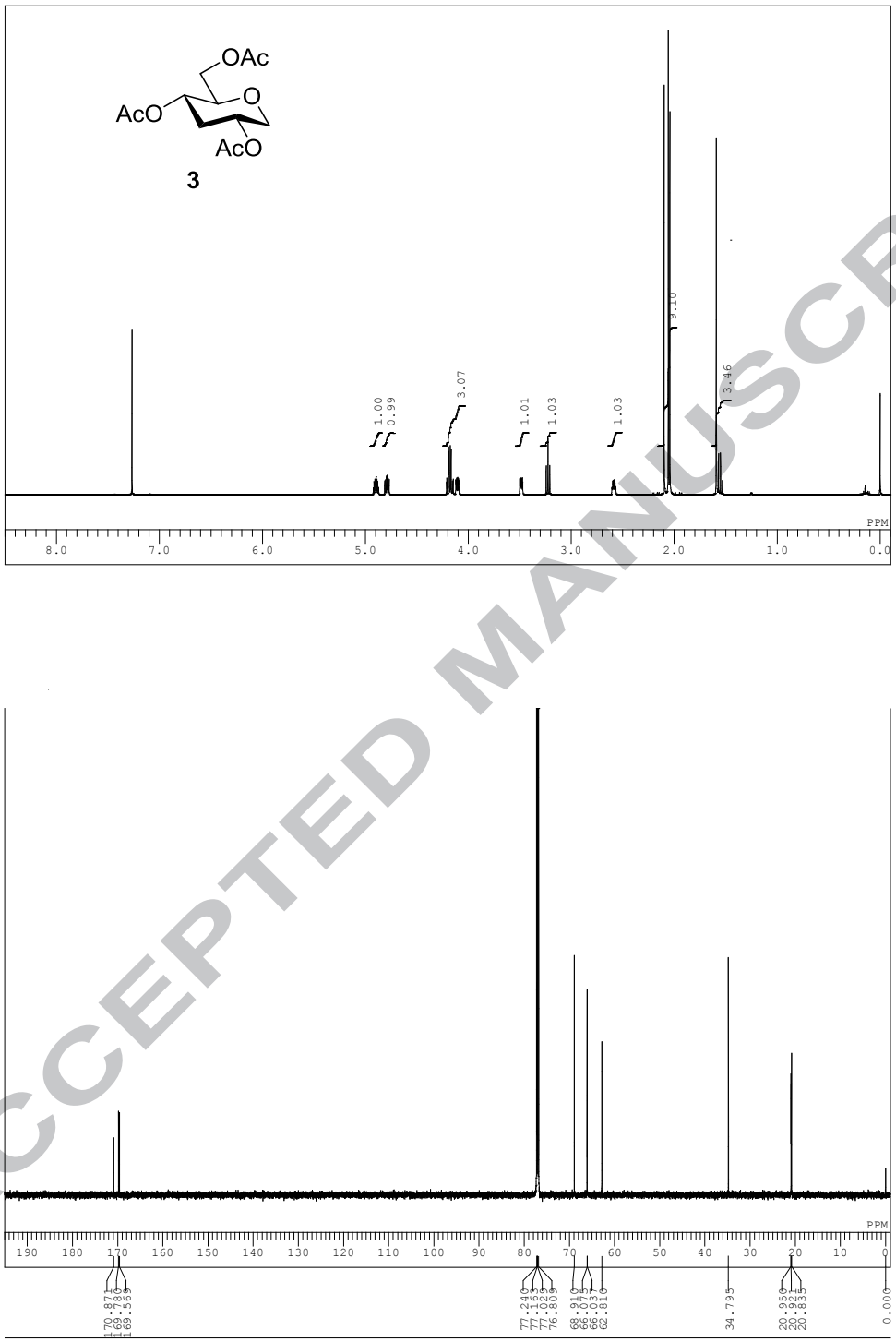


Fig. S1. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **3**.

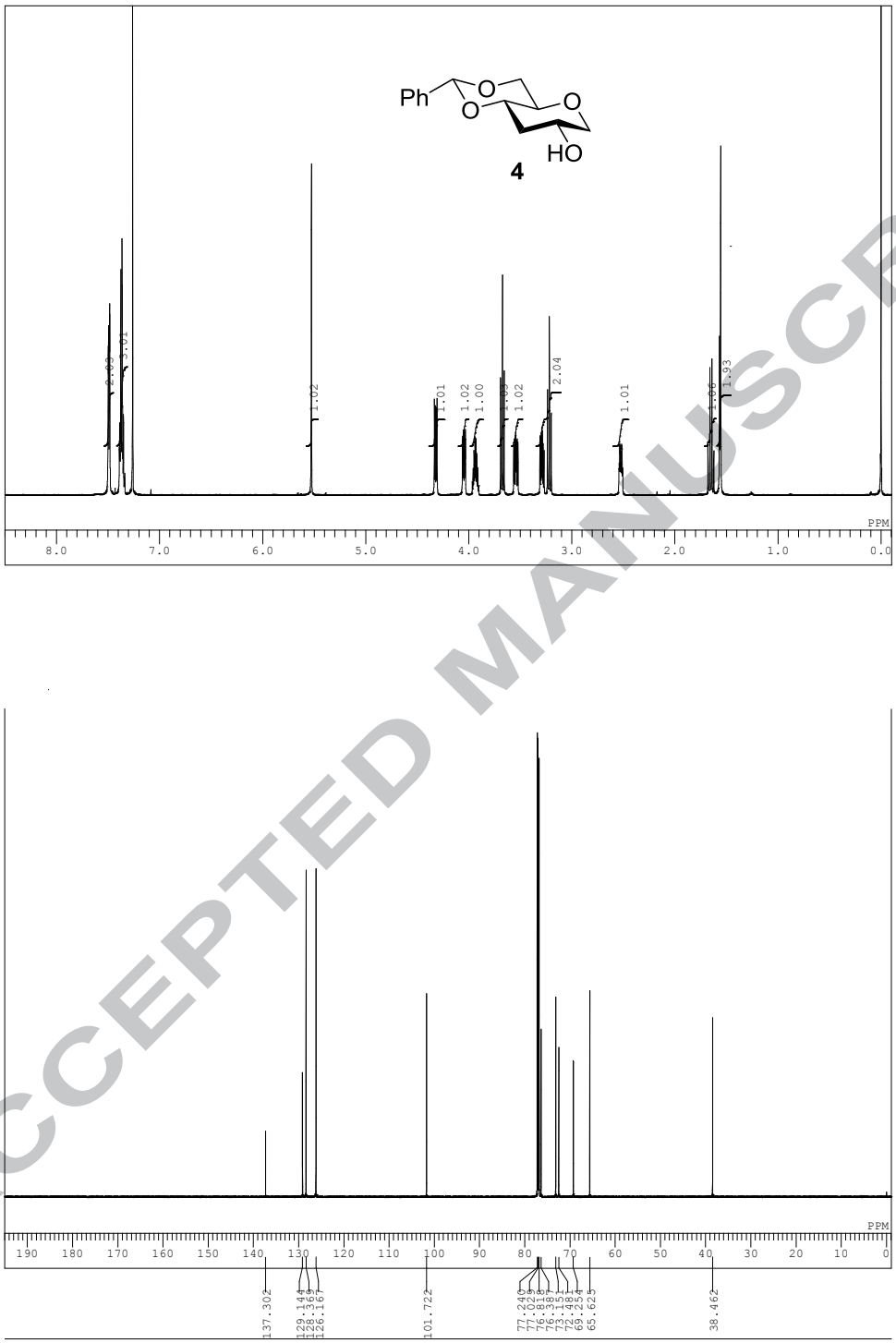


Fig. S2. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **4**.

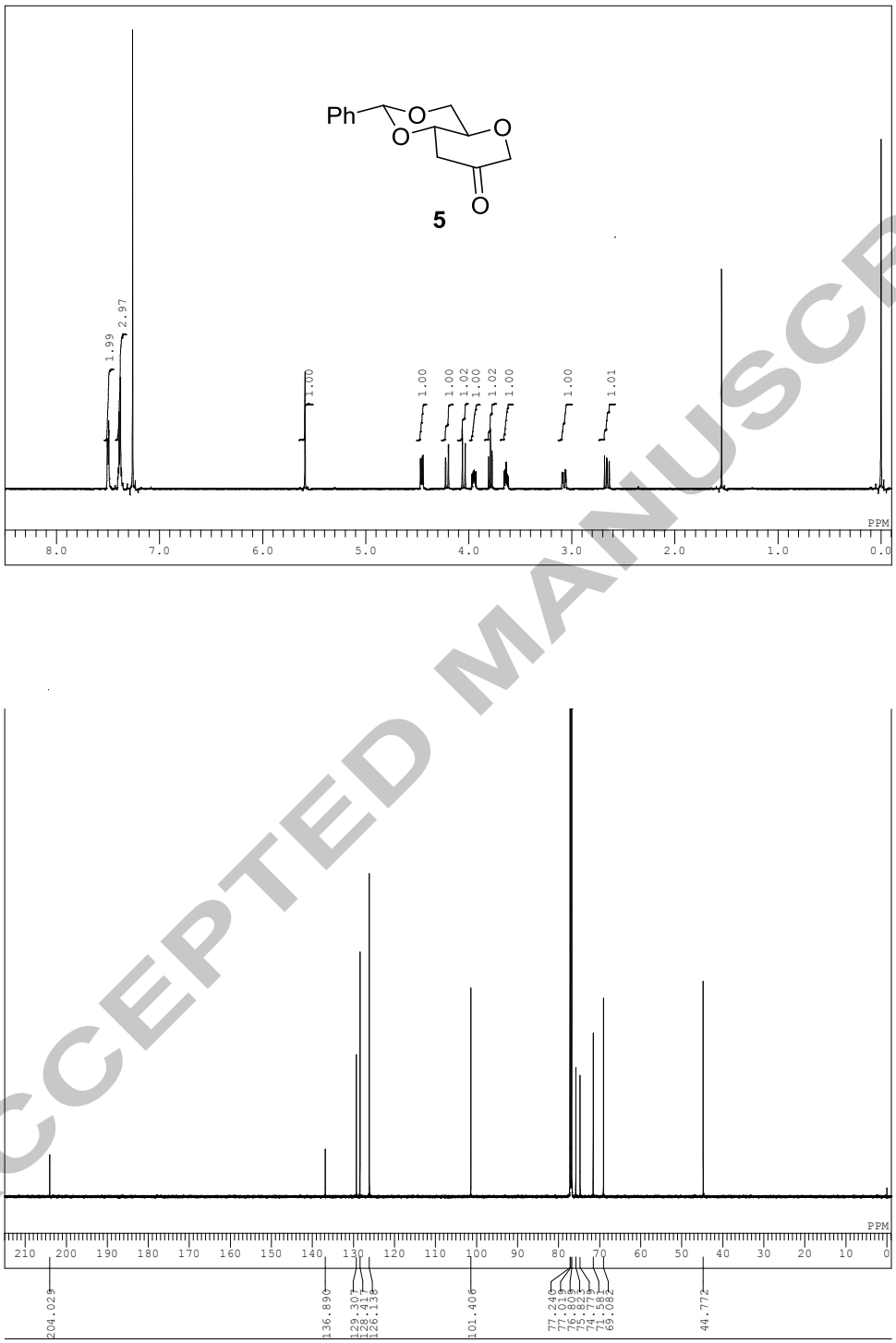


Fig. S3. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **5**.

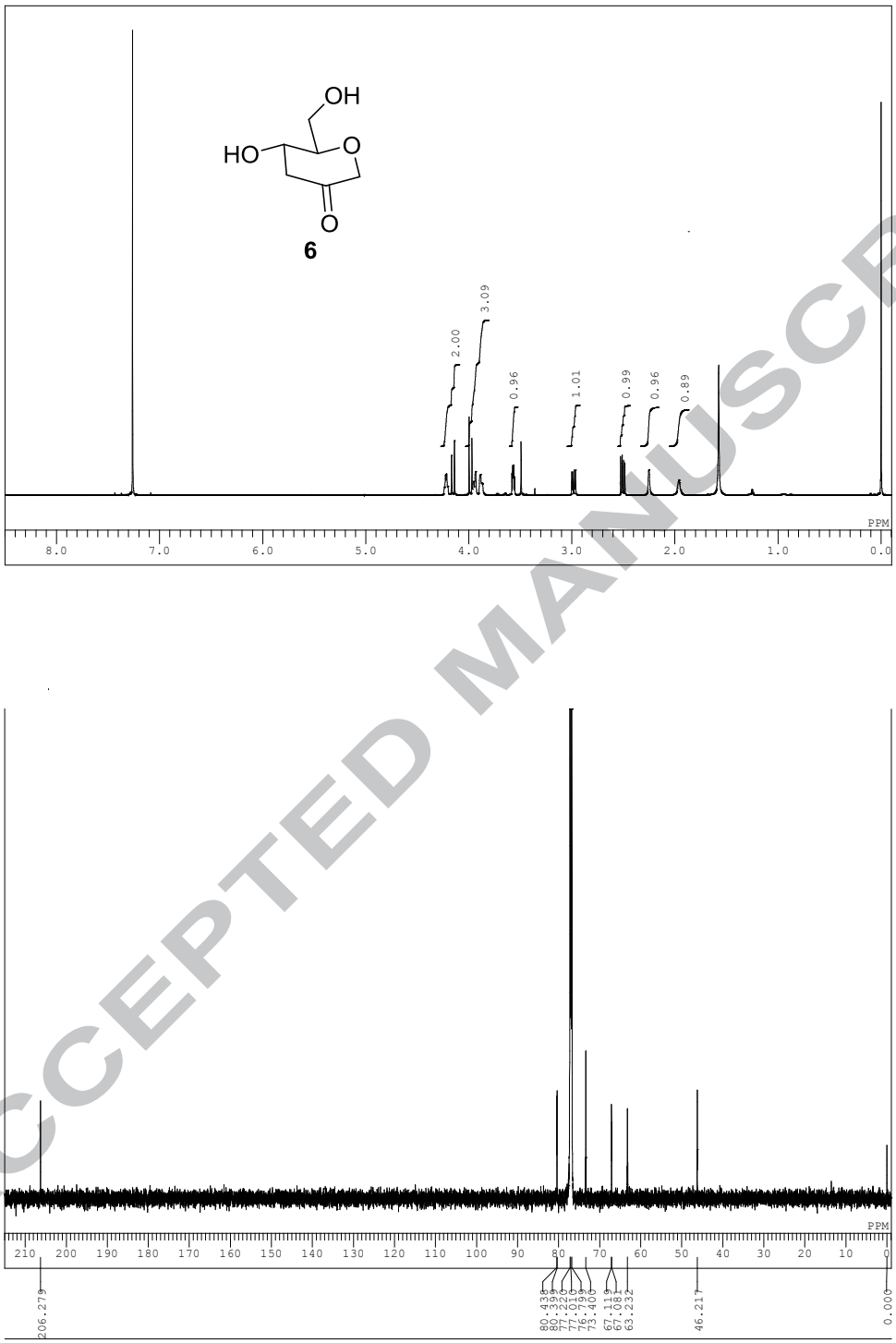


Fig. S4. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **6**.

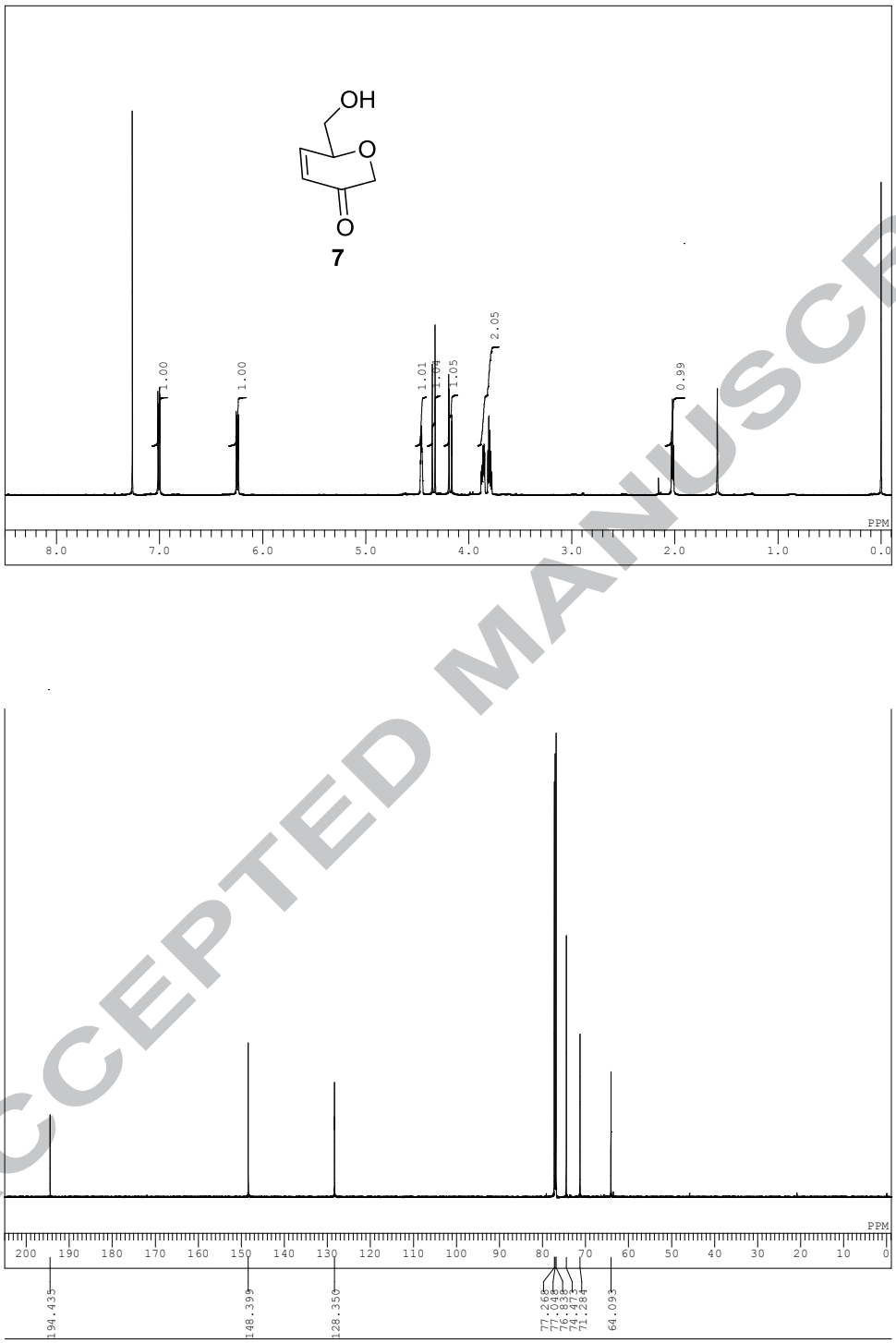
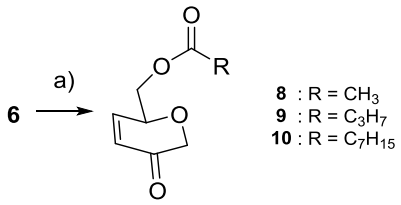


Fig. S5. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **7**.



Scheme S2. Synthesis of acyl enones **8**, **9** and **10**. Reagents and conditions: a) $(RC(O))_2O$, Pyridine.

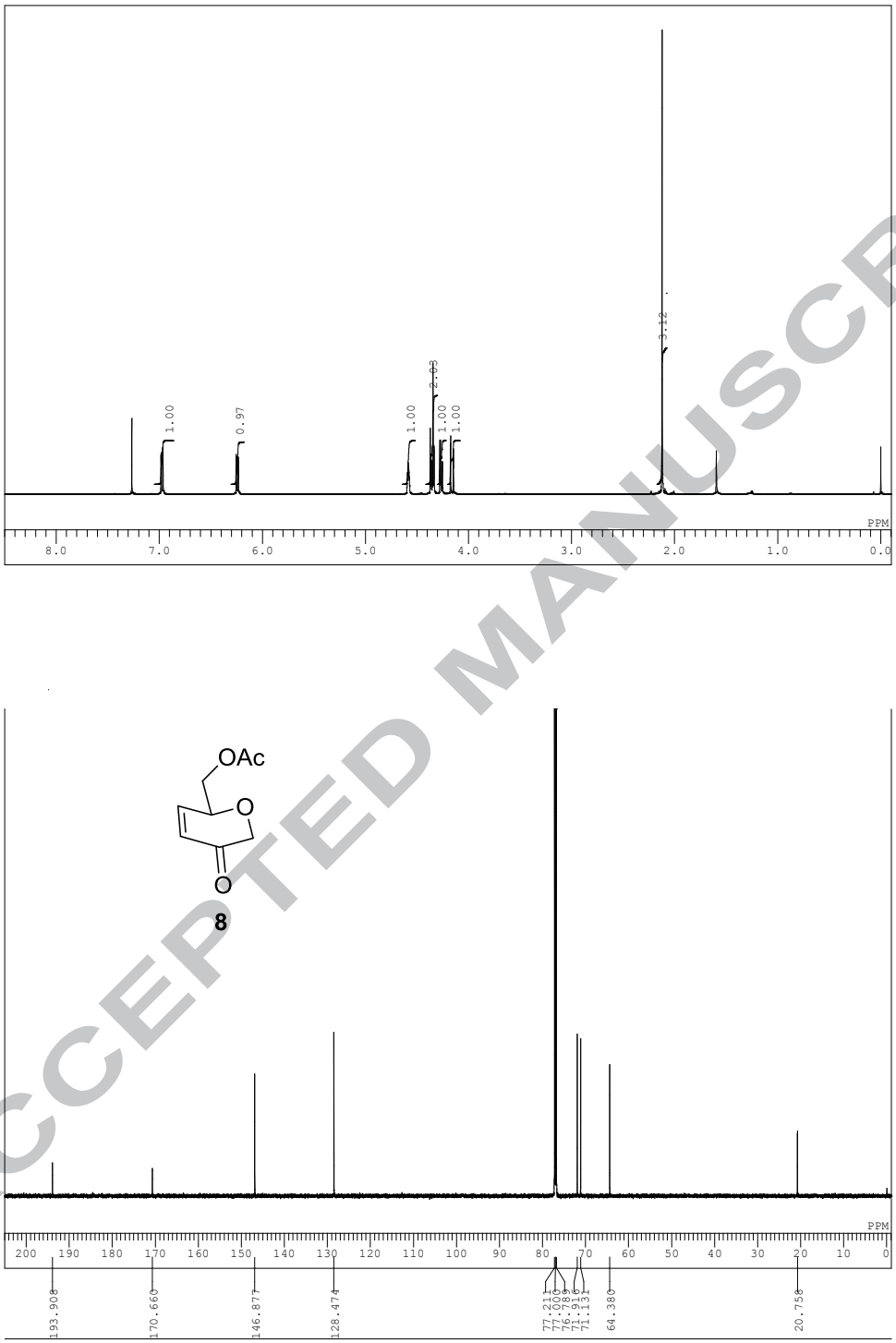


Fig. S6. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of eneone **8**.

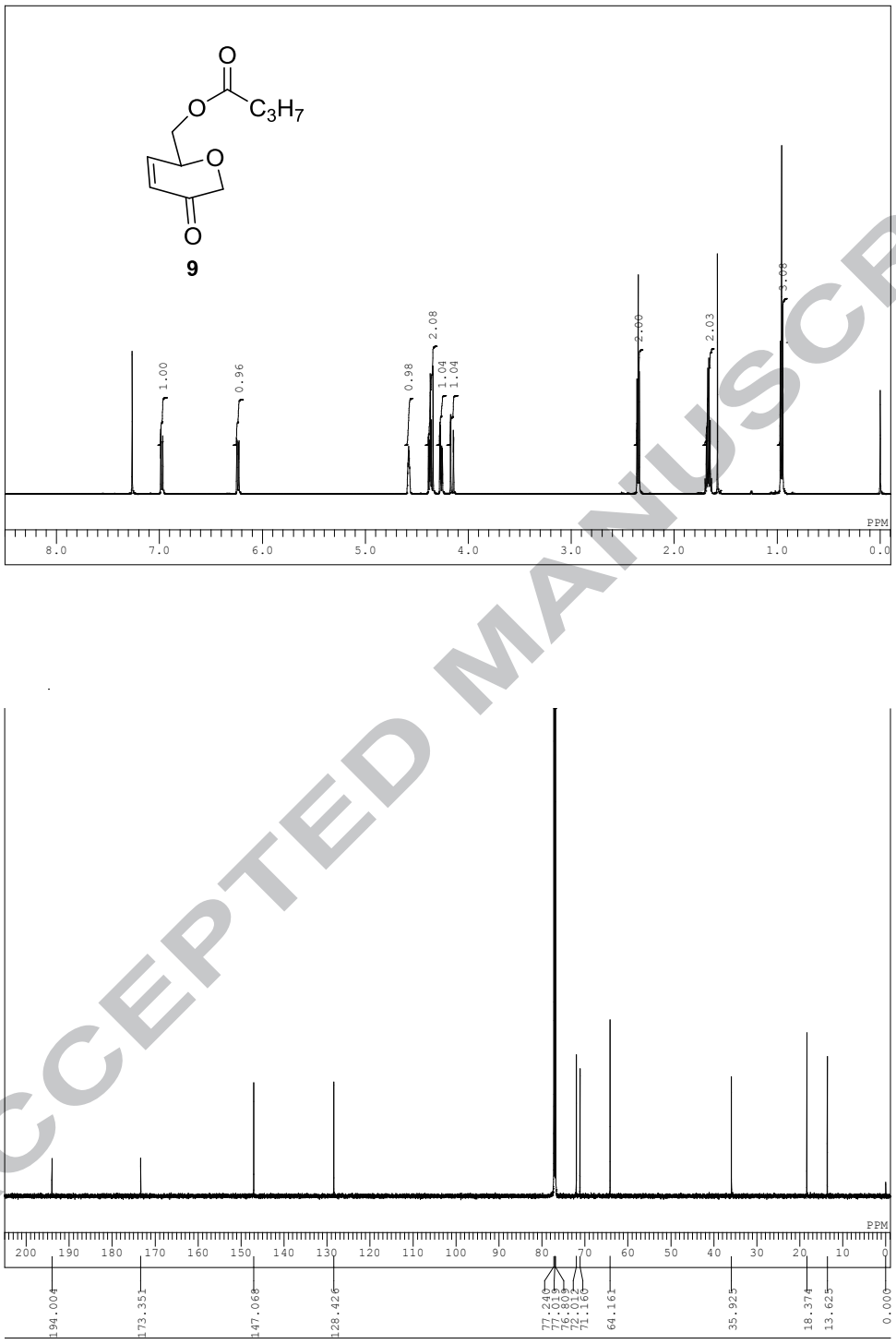


Fig. S7. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of eneone **9**.

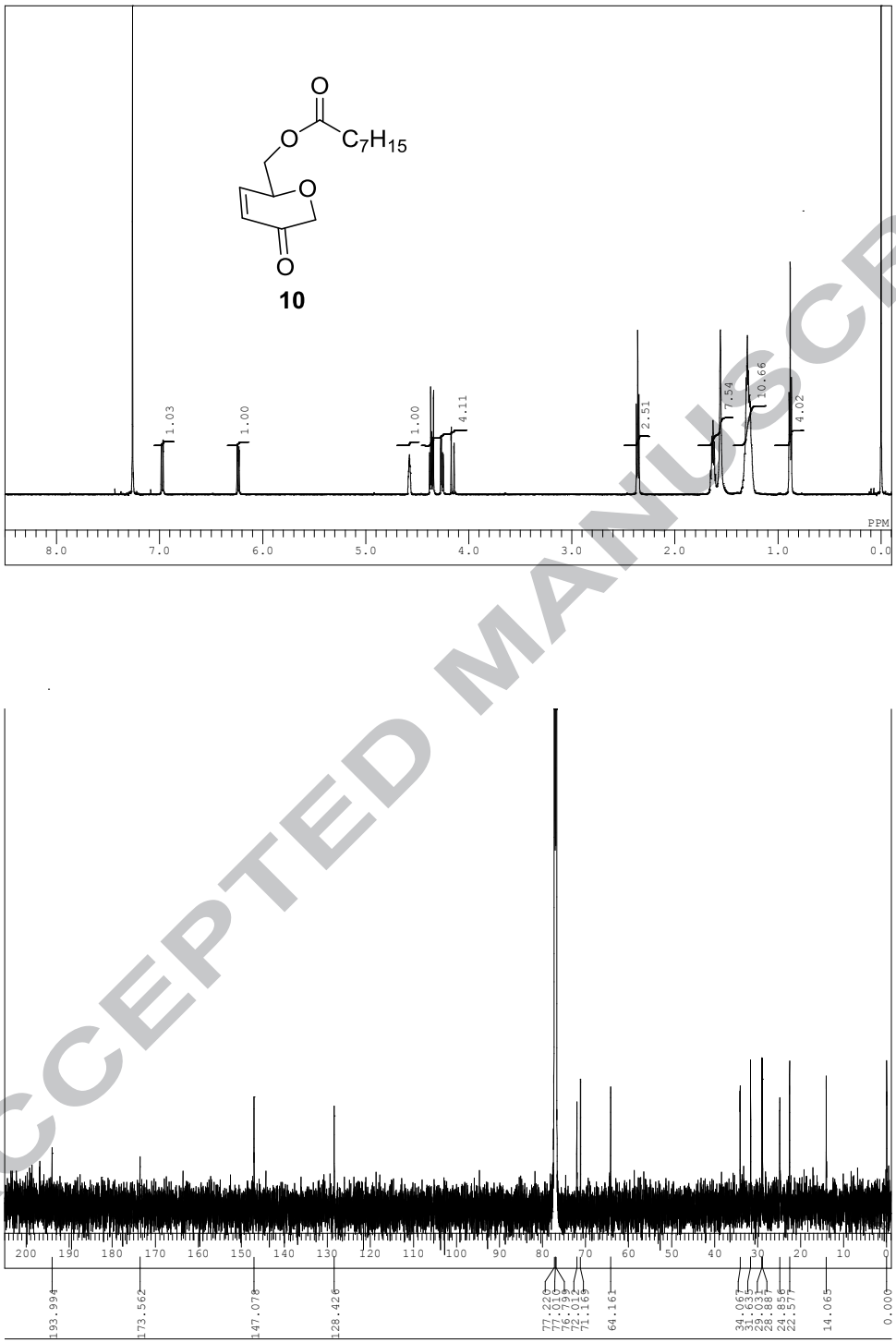
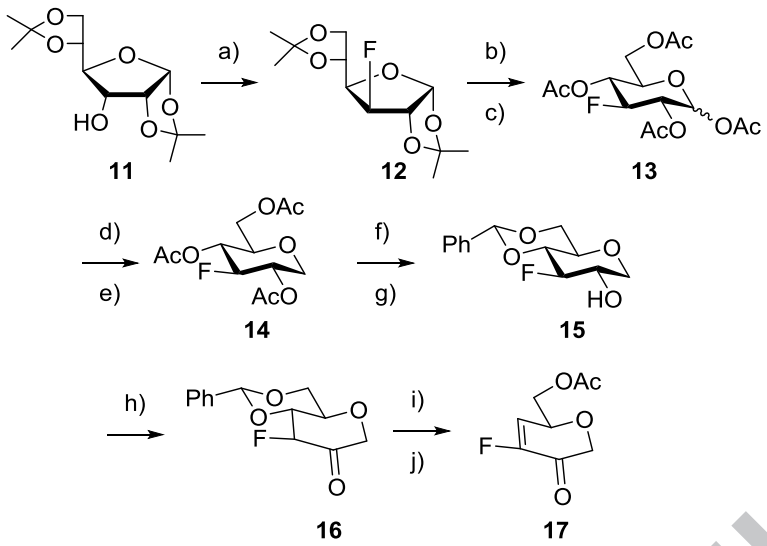


Fig. S8. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of eneone **10**.



Scheme S3. Synthesis of 3-F derivative **17**. Reagents and conditions: a) DAST, CH_2Cl_2, 40 °C, 90%; b) 0.1 M H_2SO_4, 60 °C; c) Ac_2O, Pyridine, 2 steps quant.; d) HBr-AcOH, CH_2Cl_2; e) $(TMS)_3SiH$, $E_{t3}B$, Tol-1,4-dioxane, 2 steps 90%; f) NaOMe, MeOH; g) $PhCH(OMe)_2$, CSA, MeCN, 2 steps 88%; h) 1-Me-AZADO CH_2Cl_2, 75%; i) H_2 gas, Pd/C, EtOH-EtOAc; j) Ac_2O, Pyridine, 2 steps 87%.

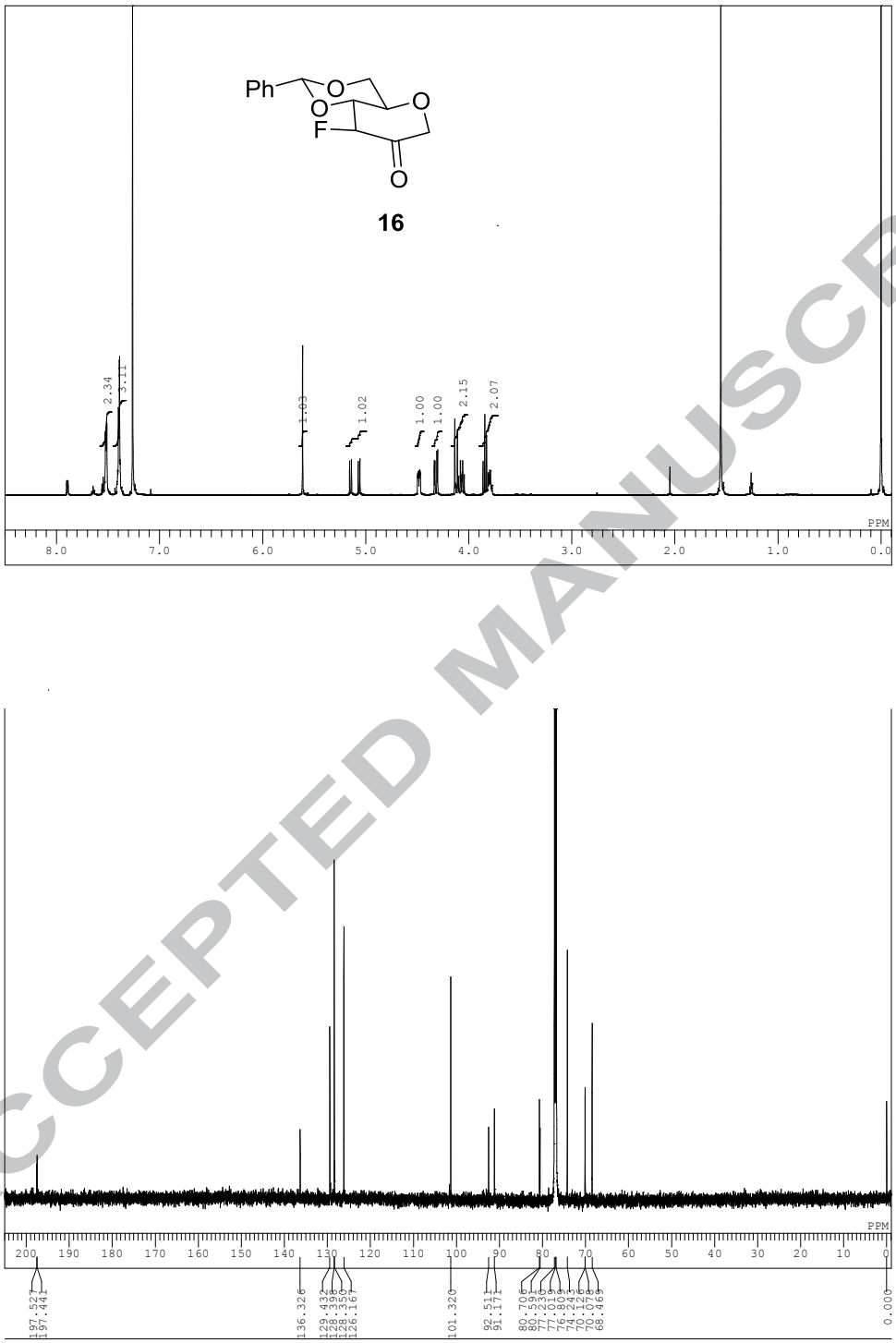


Fig. S9. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **16**.

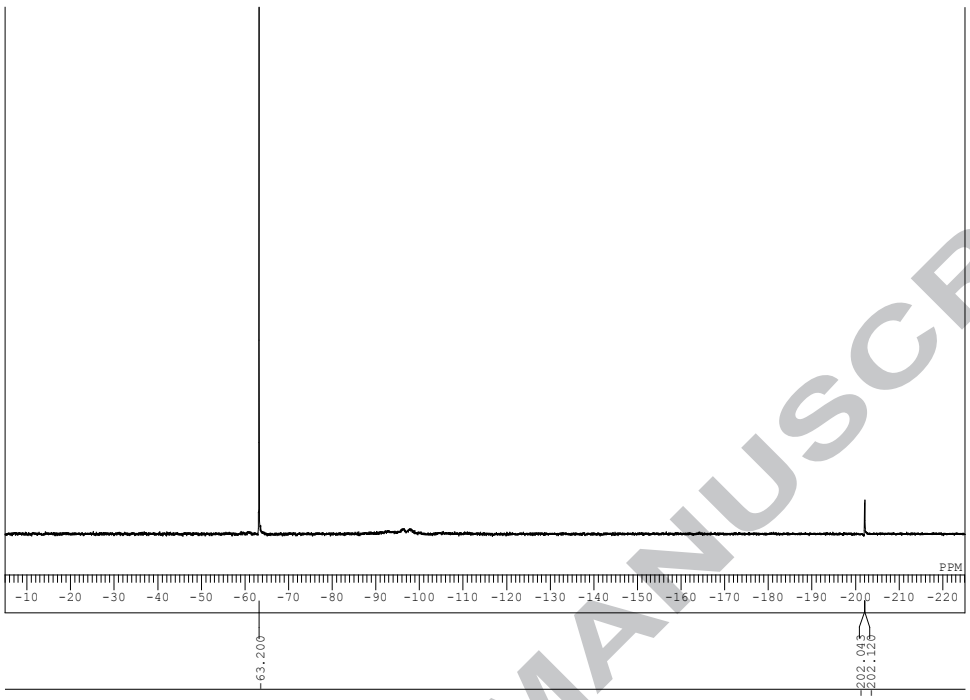


Fig. S10. ^{19}F ($CDCl_3$) spectrum of compound **16**.

Fig. S11. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of fluorinated enone **17**.

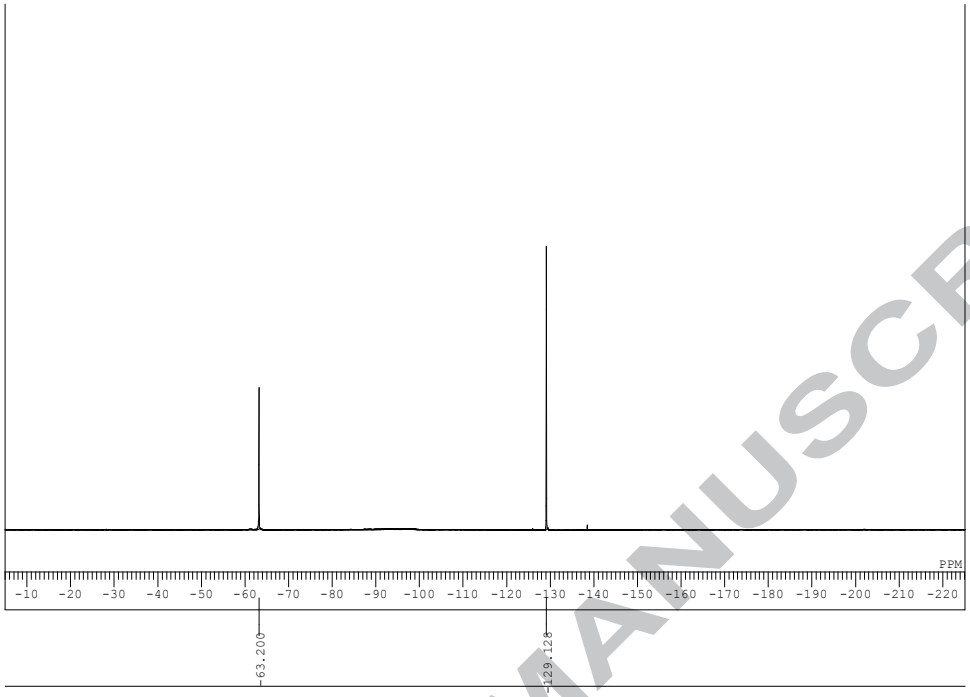
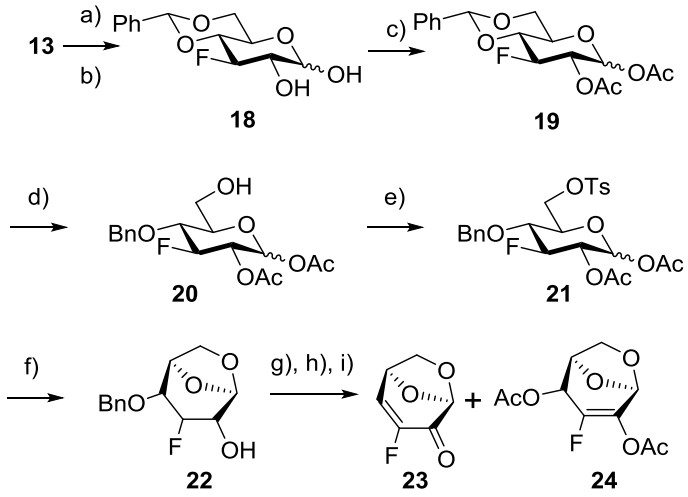


Fig. S12. ^{19}F ($CDCl_3$) spectrum of fluorinated enone **17**.



Scheme S4. Synthesis of bicyclo derivative **23**. Reagents and conditions: a) NaOMe, MeOH; b) $PhCH(OMe)_2$, CSA, MeCN, 2 steps 56%; c) Ac_2O, Pyridine, 96%; d) Et_3SIH, $PhBCl_2$, CH2Cl2, -78 °C, 49%; e) TsCl, Pyridine, 91%; f) 3.03N aq. NaOMe, Pyridine, 69%; g) 1-Me-AZADO, $PhI(OAc)_2$, CH_2Cl_2, 52%; h) H_2 gas, Pd/C, EtOH-EtOAc, 76%; i) Ac_2O, Pyridine, 3 steps 39%.

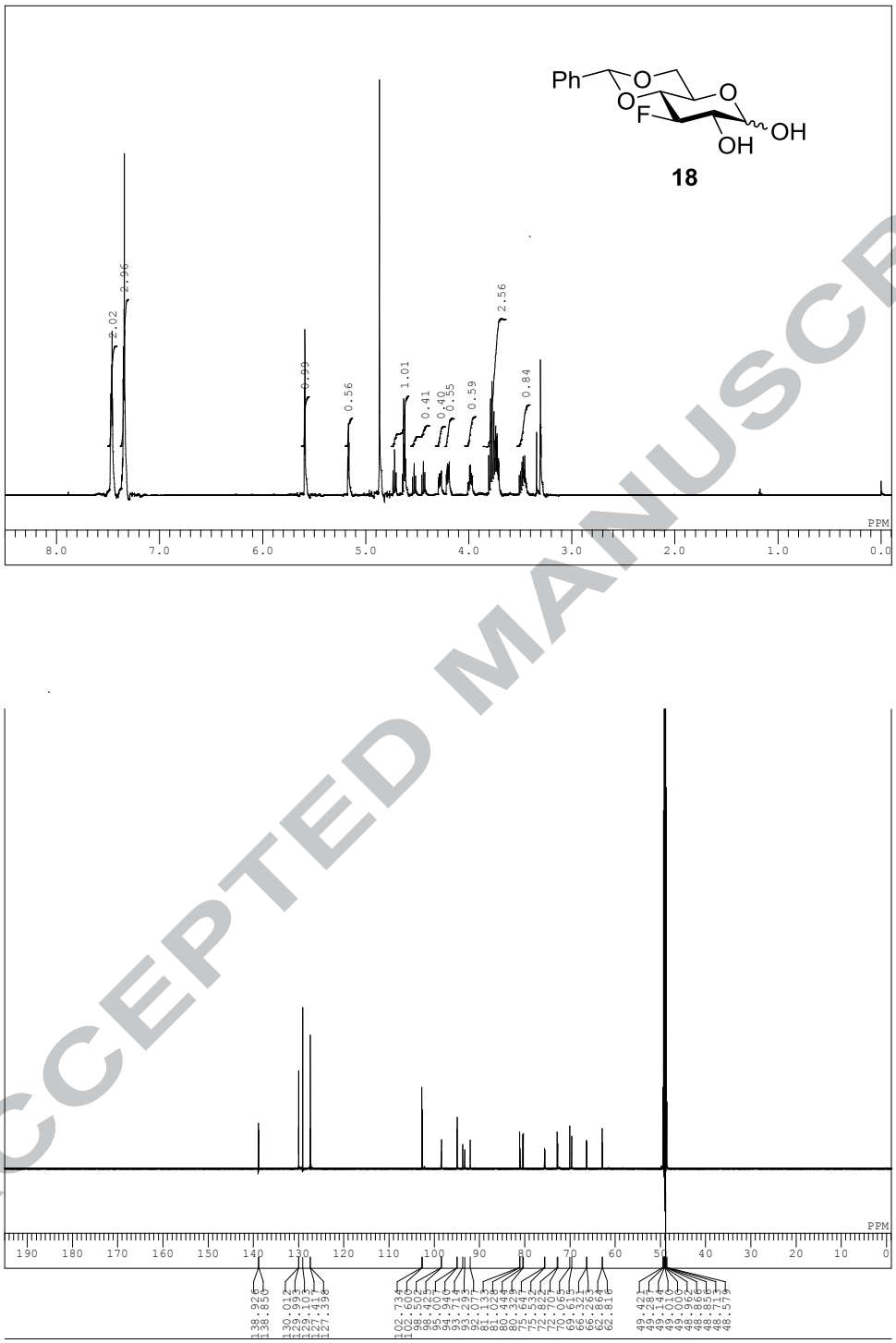


Fig. S13. ^{1}H and ^{13}C NMR (CD_3OD) spectrum of compound **18**.

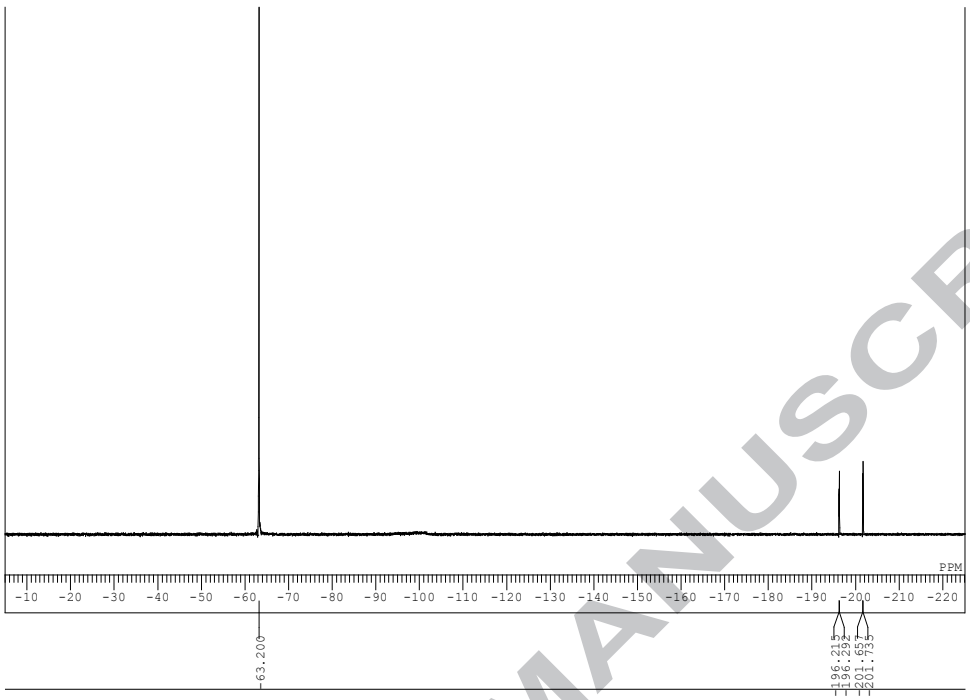


Fig. S14. ^{19}F (CD_3OD) spectrum of compound **18**.

Fig. S15. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **19**.

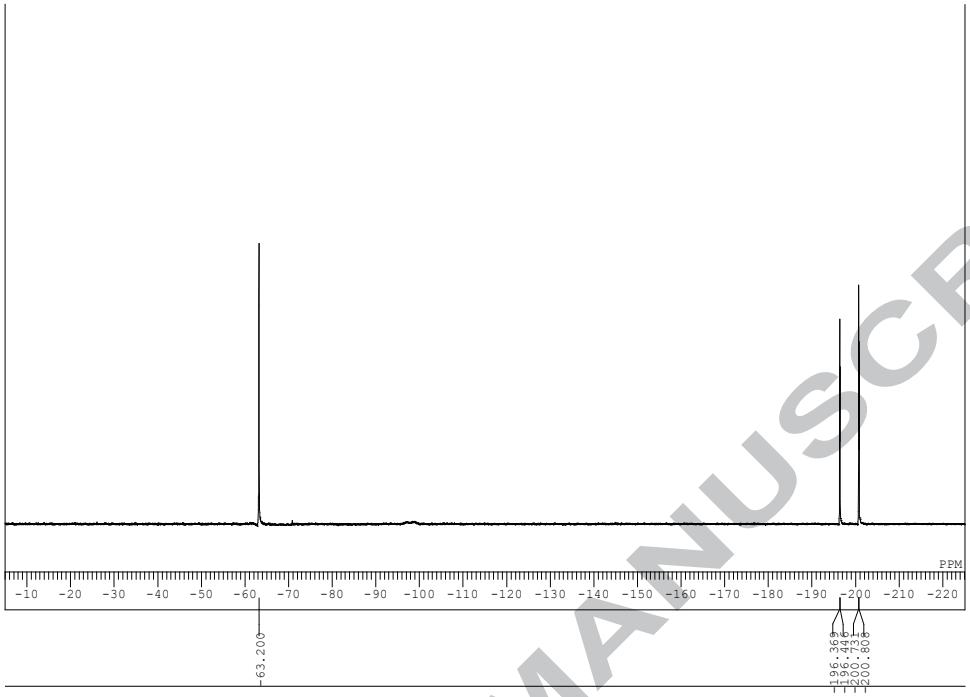


Fig. S16. ^{19}F ($CDCl_3$) spectrum of compound **19**.

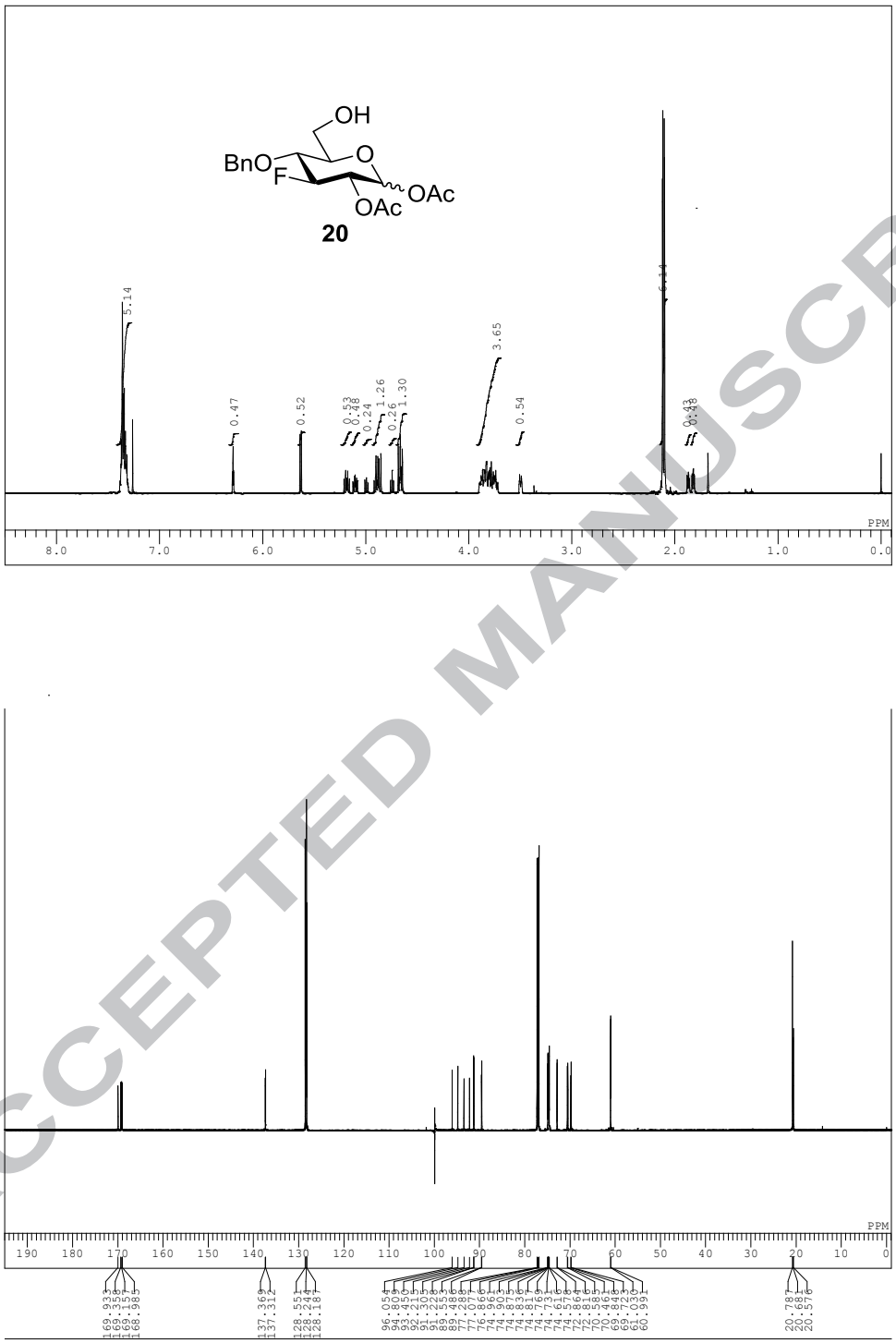


Fig. S17. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **20**.

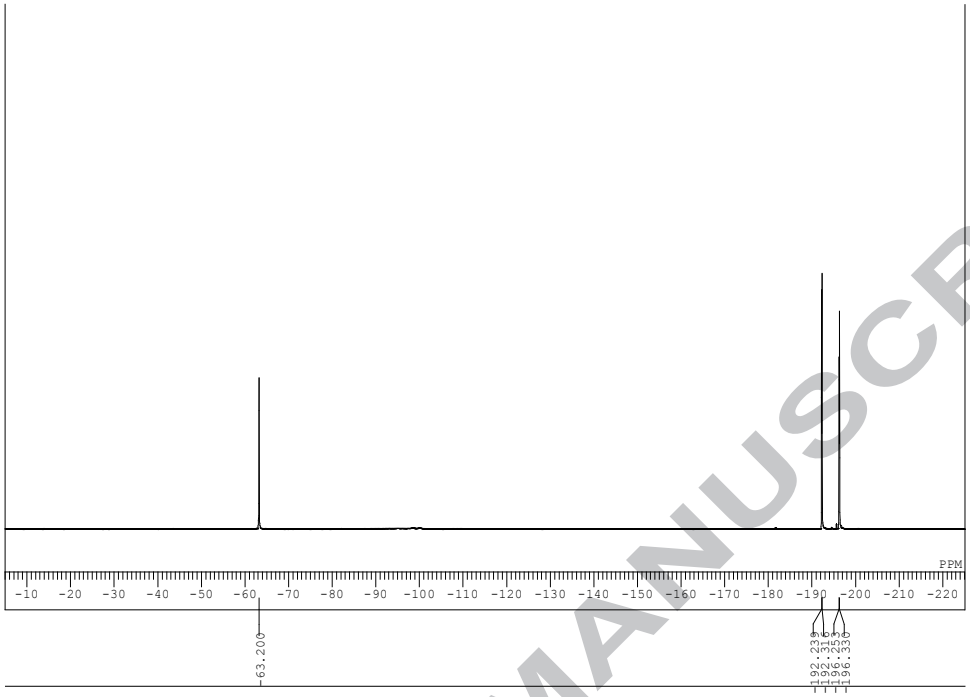


Fig. S18. ^{19}F ($CDCl_3$) spectrum of compound **20**.

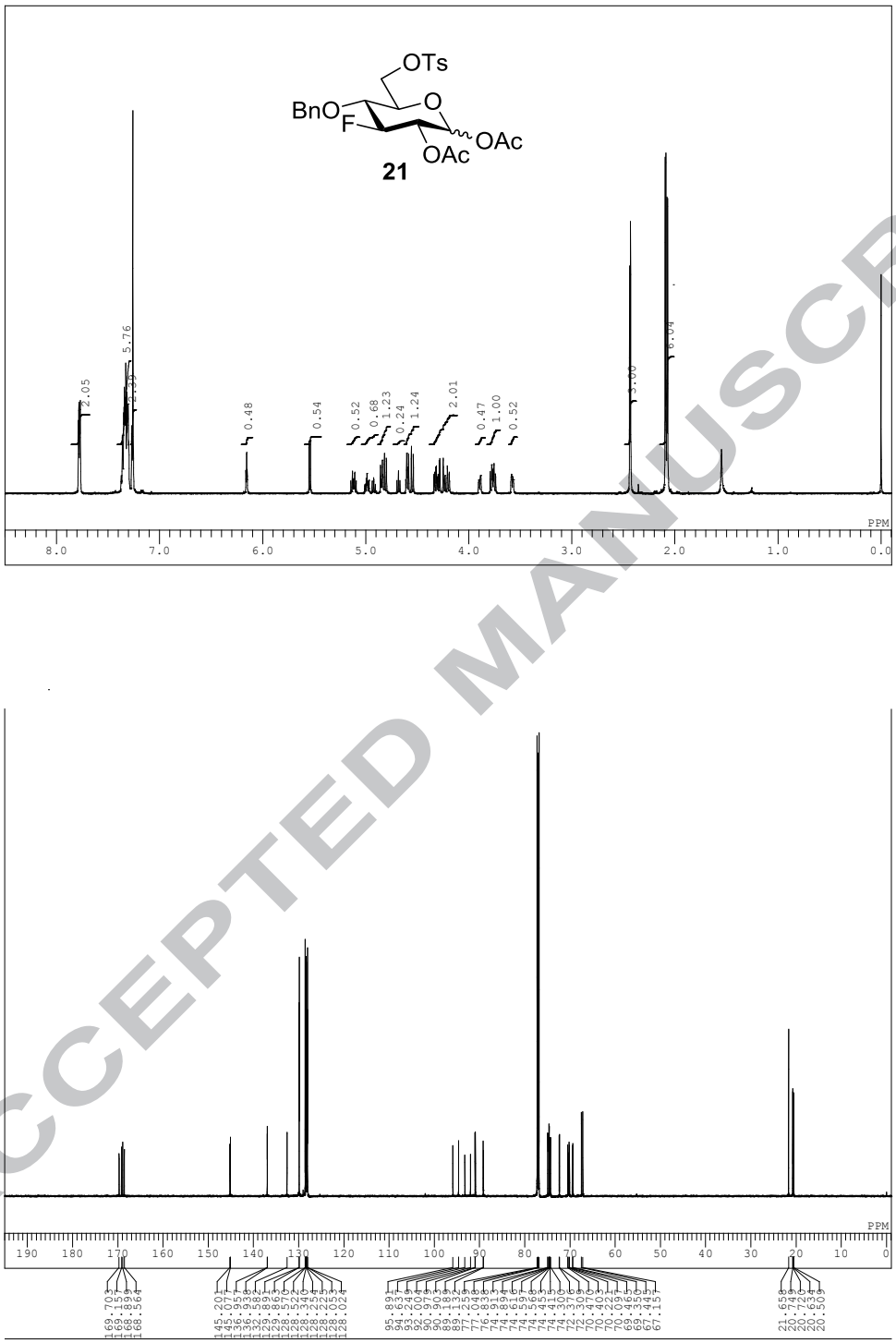


Fig. S19. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **21**.

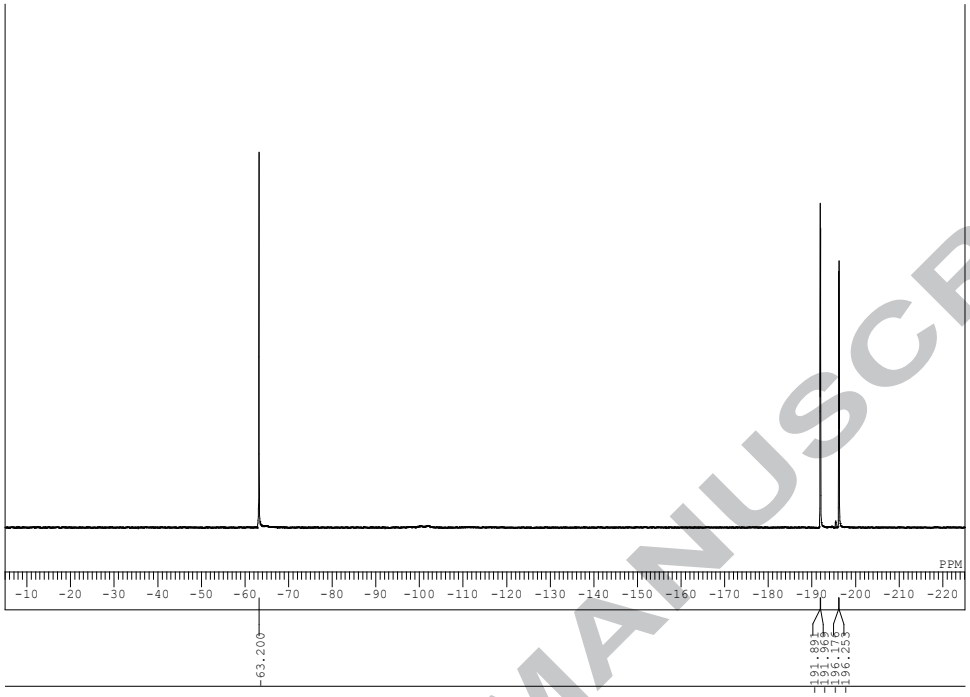


Fig. S20. ^{19}F ($CDCl_3$) spectrum of compound **21**.

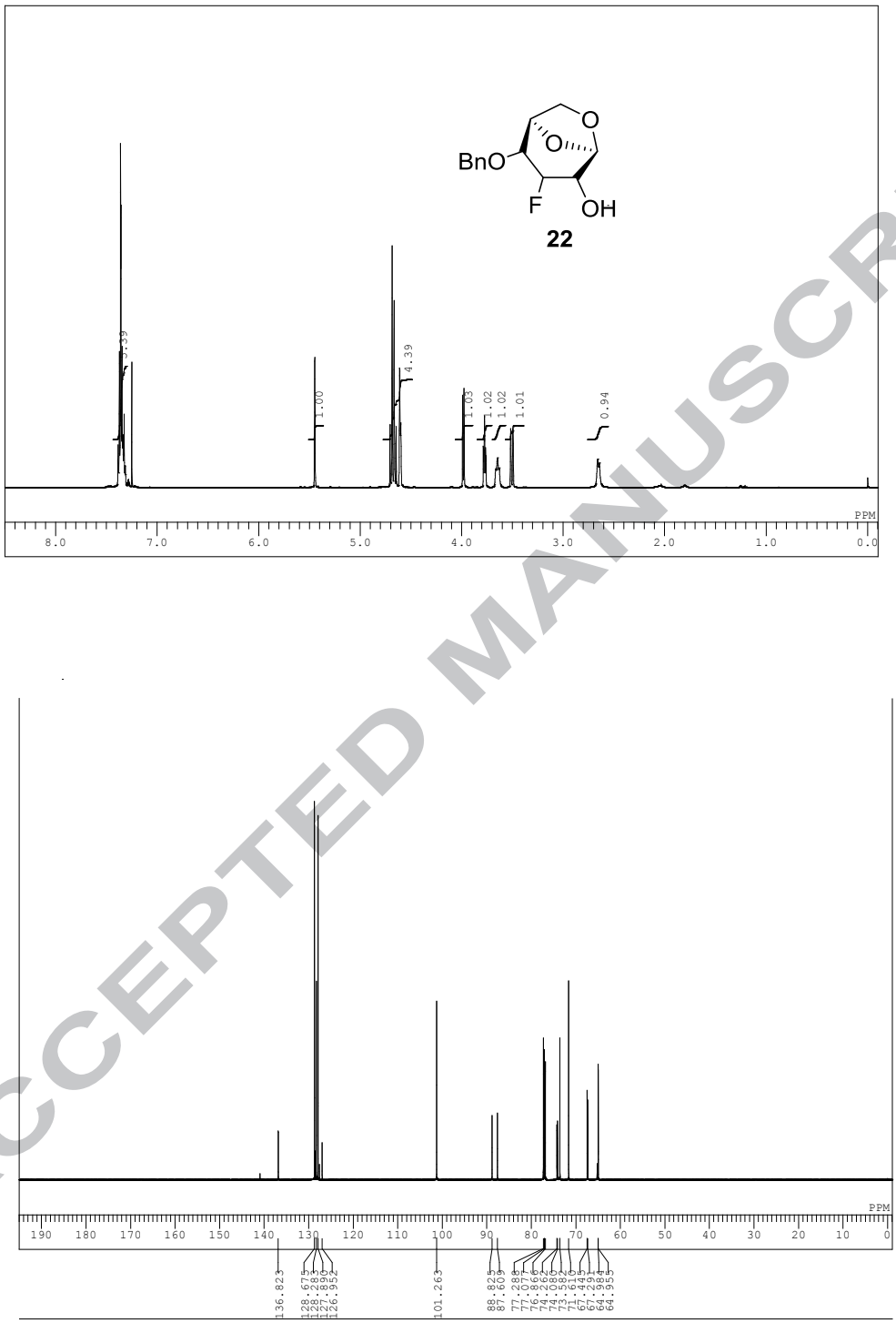


Fig. S21. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of compound **22**.

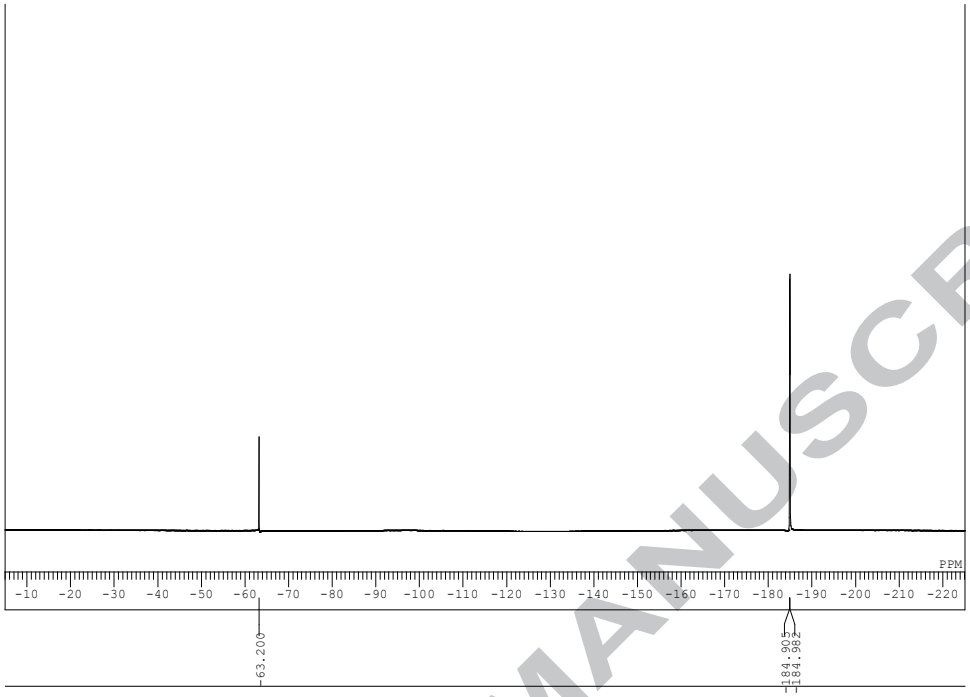


Fig. S22. ^{19}F ($CDCl_3$) spectrum of compound **22**.

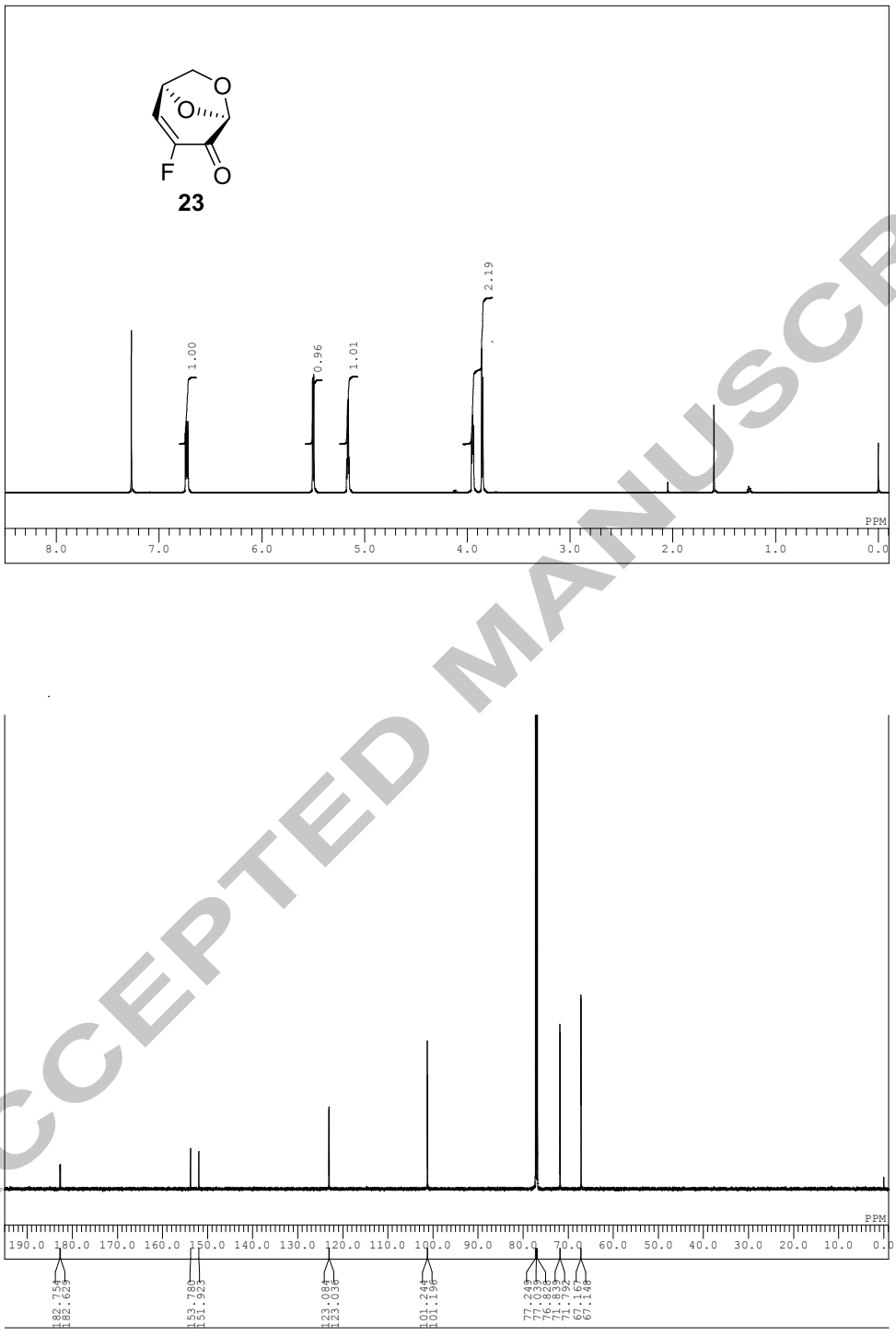


Fig. S23. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of fluorinated levoglucosenone **23**.

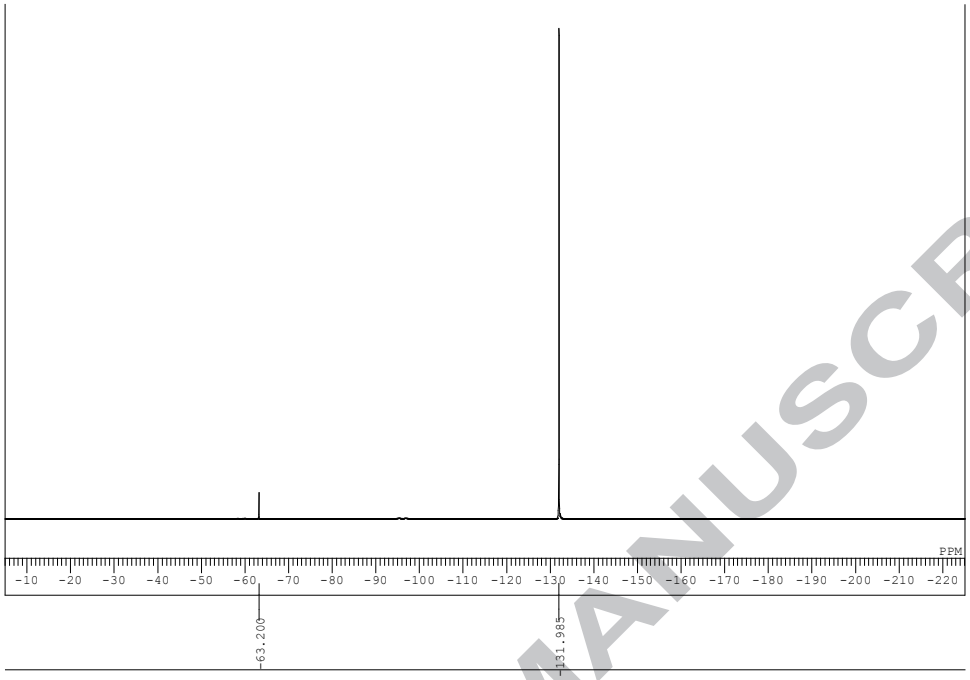


Fig. S24. ^{19}F ($CDCl_3$) spectrum of fluorinated levoglucosenone **23**.

Fig. S25. ^{1}H and ^{13}C NMR ($CDCl_3$) spectrum of enol acetate **24**.

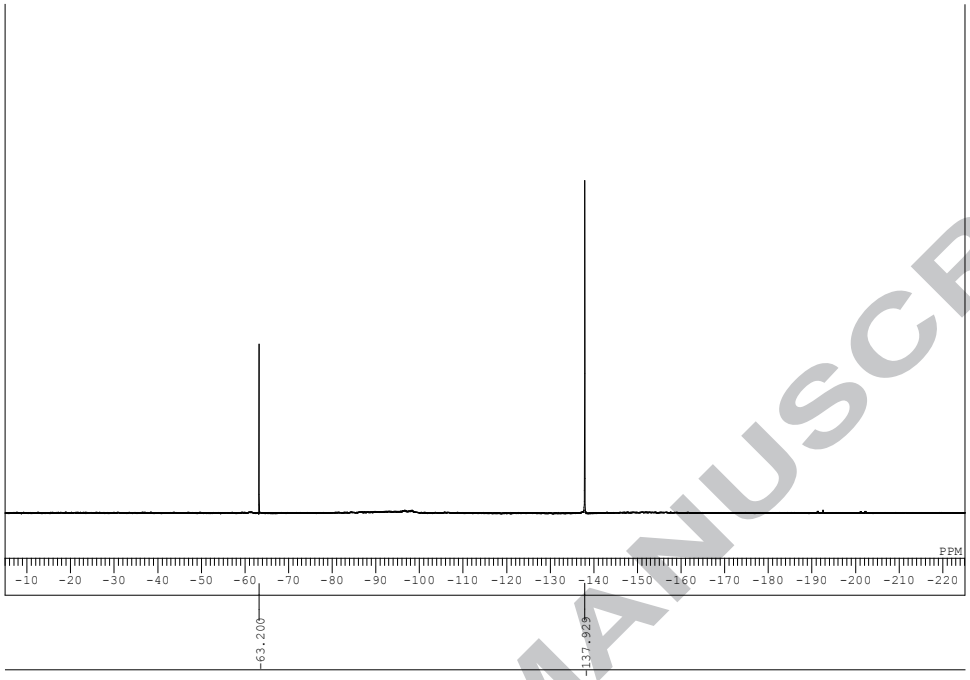


Fig. S26. ^{19}F ($CDCl_3$) spectrum of enol acetate **24**.

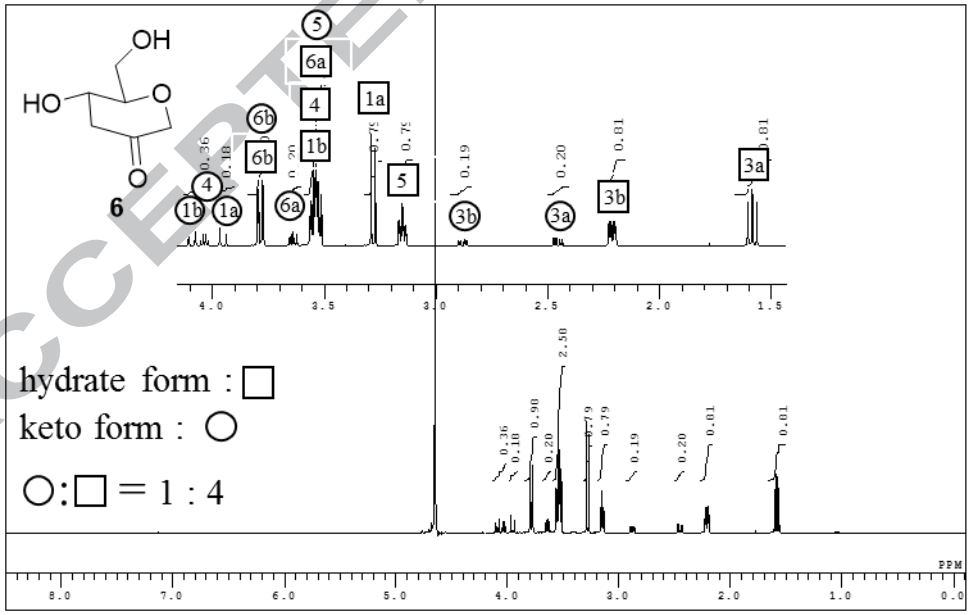


Fig. S27. ^{1}H NMR (D_2O) spectrum of compound **6** (Ref 13).

Highlight

・1,5-Anhydro-D-fructose (1,5-AF) derivatives and related compounds were synthesized.

・Evaluation of synthetic derivatives with respect to inflammasomes was conducted.

・Some synthesized compounds have much higher inhibitory activities than does 1,5-AF.

Graphical Abstract