Supplementary data

Substrate-like water soluble lipase inhibitors from *Filipendula kamtschatica*

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General methods.

Commercially available chemicals were purchased from Wako Pure Chem. Ind. Ltd. (Osaka, Japan) and used without further purification unless otherwise noted. Jeol JNM-EX 270 or Bruker AMX500 was used to obtain NMR spectrum and residual solvent peak was used as an internal standard (^{1}H NMR: CD_3OD 3.30 ppm, DMSO-d_6 2.49 ppm; ^{13}C NMR: CD_3OD 49.0 ppm, DMSO-d_6 39.5 ppm). Thermo Scientific Exactive (ESI-MS) was used to obtain mass spectrum. Absorbance was measured by Synergy^{TM} MX (Bio-tech Instruments Inc.,) microplate reader.

Plant Material.

The aerial parts of *Filipendula kamtschatica* were collected in July 2006 at Mt. Piyashiri, Nayoro, Japan and identified by one of the authors (T. S). A voucher specimen (14813-03HK) is deposited in the Hokkaido Division, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Nayoro, Japan.

Lipase inhibitory activity assay.

An emulsified solution of L-α-lecithin (Sigma, P4279, 10 mg), triolein (16 mg), sodium taurocholate (5 mg) in Tris buffer (Tris 13 mM, NaCl 150 mM, $CaCl_2$ 1.3 mM, pH8.0, 9 mL) was used as a substrate. Porcine pancreatic lipase (Sigma, L3126, 4.5 mg) was dissolved in Tris buffer and used as an enzyme solution. Substrate (100 μL) and sample (50 μL, in 50% aq. DMSO) was pre-incubated at 37ºC for 5 min. The reaction was started by the addition of an enzyme solution (50 μL) and reacted for 30 min. at 37ºC. The reaction was stopped by the addition of orlistat (20 μM, 50 μL) and the liberated oleic acids were extracted by hexane (400 μL). The hexane layer (200 μL) was dried and redissolved in DMSO (100 μL) and the oleic acid was quantitated by NEFA C-testwako (Wako Pure Chem. Ind. Ltd). The assay was performed three times for each sample and the mean value was used to evaluate IC_{50}. Orlistat was used as a positive control, which showed 55 % inhibition at 0.4 μM.

Purification of lipase inhibitors from *Filipendula kamtschatica*.

Dried aerial part of *F. kamtschatica* (150 g) was extracted twice by 50% aq. MeOH (2 L). The extract was evaporated to remove methanol and then separated between EtOAc. The water layer was evaporated to remove the remaining EtOAc and then charged to DIAION HP-20 column (Mitsubishi Chem. Co., ϕ7×65 cm) and eluted stepwise by water, 50% aq. MeOH and MeOH. The most active Fraction 2, eluted by 50% aq. MeOH, was evaporated and then loaded to Cosmosil 75C18-OPN column (ϕ7.5×70 cm). The column was washed by water, eluted stepwise by 10%, 20%, 30%, 50% aq. MeOH and the elution was fractionated. Each fraction was tested for the lipase inhibitory activity and the 10% aq. MeOH fraction and the 30% aq. MeOH fraction was selected for further separation.

30% aq. MeOH fraction was purified by HPLC using Inertsil ODS-3 (GL Sciences Inc., ϕ20×250 mm) with gradient elution from 30% to 50% aq. MeOH to obtain **1** (220 mg) as a single peak. 10% aq. MeOH fraction was separated again with Cosmosil 75C18-OPN column chromatography (ϕ3.2×30 cm) and then purified by HPLC using Inertsil ODS-3 (GL Sciences Inc., ϕ20×250 mm) with gradient elution from 30% to 44% aq. MeOH to obtain **2** (110 mg) and **3** (54 mg) as a separated peak.

*Quercetin 3-O-β-xylopyranosyl-(1→2)-O-β-galactopyranoside (**1**).* ^{1}H NMR (500 MHz, CD_3OD, rt): 7.70 (1H, d, *J* = 2.2 Hz, H-2'), 7.64 (1H, dd, *J* = 2.2, 8.5 Hz, H-6'), 6.86 (1H, d, *J* = 8.5 Hz, H-5'), 6.37 (1H, d, *J* = 2.1 Hz, H-6), 6.18 (1H, d, *J* = 2.1 Hz, H-8), 5.40 (1H, d, *J* = 7.9 Hz, Glc_H-1), 4.75 (1H, d, *J* = 6.6 Hz, Xyl_H-1), 4.02 (1H, dd, *J* = 7.9, 9.5 Hz, Glc_H-2), 3.94 (1H, dd, *J* = 5.0, 11.7 Hz, Xyl_H-5a), 3.84 (1H, br d, *J* = 3.2 Hz, Glc_H-4), 3.71 (1H, dd, *J* = 3.2, 9.5 Hz, Glc_H-3), 3.62 (1H, dd, *J* = 6.0, 11.4 Hz, Glc_H-6a), 3.55 (1H, dd, *J* = 6.3, 11.4 Hz, Glc_H-6b), 3.50 (1H, ddd, *J* = 5.0, 6.9, 9.5 Hz, Xyl_H-4), 3.45 (1H, br dd, *J* = 6.0, 6.3 Hz, Glc_H-5), 3.40 (1H, dd, *J* = 6.6, 8.2 Hz, Xyl_H-2), 3.38 (1H, dd, *J* = 6.9, 8.2 Hz, Xyl_H-3), 3.23 (1H, dd, *J* = 9.5, 11.7 Hz, Xyl_H-5b) ppm. For HRMS, see main document.

*2-O-caffeoyl-4-O-galloyl-L-threonic acid (**2**).* $[\alpha]_D^{28}$ +49.5º (*c* = 1.0, MeOH). For HRMS and ^{1}H/^{13}C NMR data, see main document.

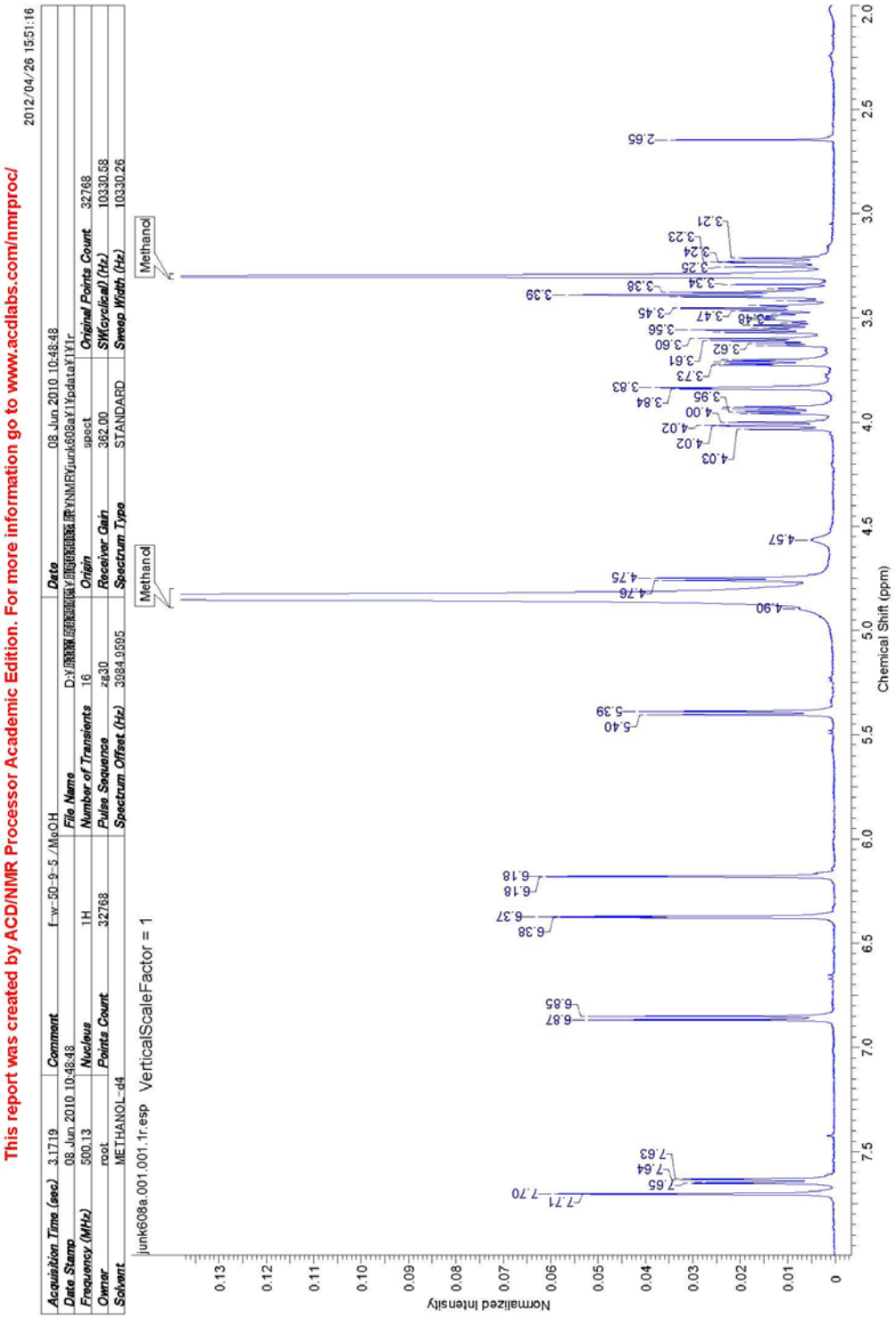
*3-O-caffeoyl-4-O-galloyl-L-threonic acid (**3**).* $[\alpha]_D^{28}$ -70.1º (*c* = 1.0, MeOH). For HRMS and ^{1}H/^{13}C NMR data, see main document.

Solvolysis of compound 2 and 3.

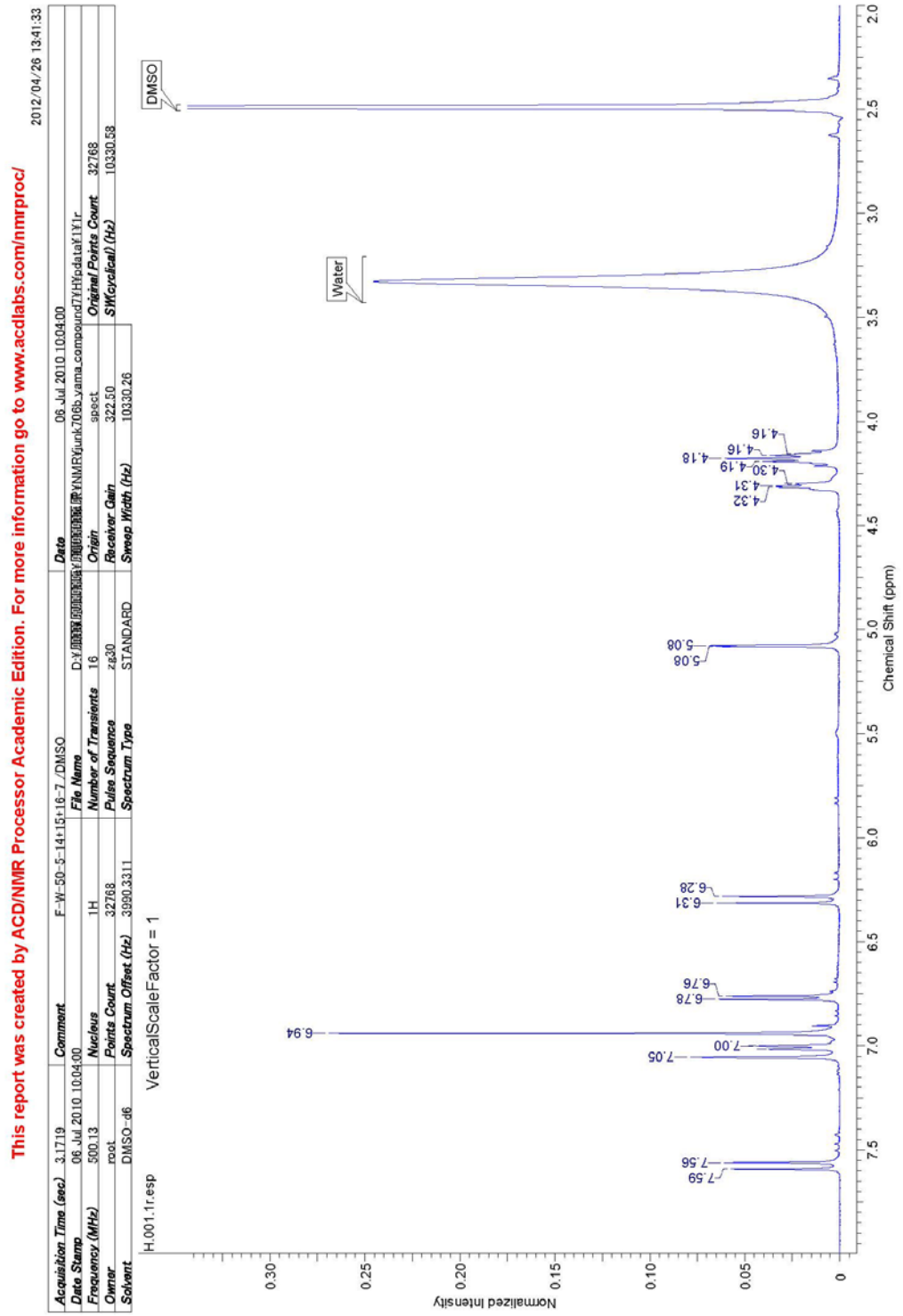
Compound **2** (5.5 mg) was dissolve in 1.25 M hydrogen chloride in methanol (1 mL) and reacted at 37ºC for 5 days. The reaction was monitored by HPLC (InertSustain C18, ϕ4.6×250 mm; gradient elution from 30% to 70% aq. MeOH containing 0.1% trifluoroacetic acid in 20 min., 1 mL/min, 254 nm). Production of methyl gallate and methyl caffeate was confirmed by co-injection with the commercial products. After the reaction finished, the mixture was evaporated to dryness and the residue was partitioned between water and ethyl acetate. The water layer was evaporated and the residue was purified by preparative TLC (chloroform/methanol=3/1) to obtain methyl L-threonate (0.6 mg) and L-threonolactone (0.4 mg). Compound **3** (8.1 mg) was treated as described for **2** and methyl L-threonate (1.2 mg) and L-threonolactone (0.6 mg) was obtained. Synthetic methyl L-threonate was derived from commercial calcium L-threonate by the similar procedure. See page S7 and S8 for ^{1}H NMR comparison of synthetic methyl L-threonate and isolated product derived methyl L-threonate.

Optical rotation of methyl L-threonate. Solvolysis product of **2:** $[\alpha]_D^{28}$ +6.5º (*c* = 0.5, MeOH); Solvolysis product of **3:** $[\alpha]_D^{26}$ +6.9º (*c* = 1.0, MeOH); Synthetic product: $[\alpha]_D^{28}$ +6.6º (*c* = 0.5, MeOH)

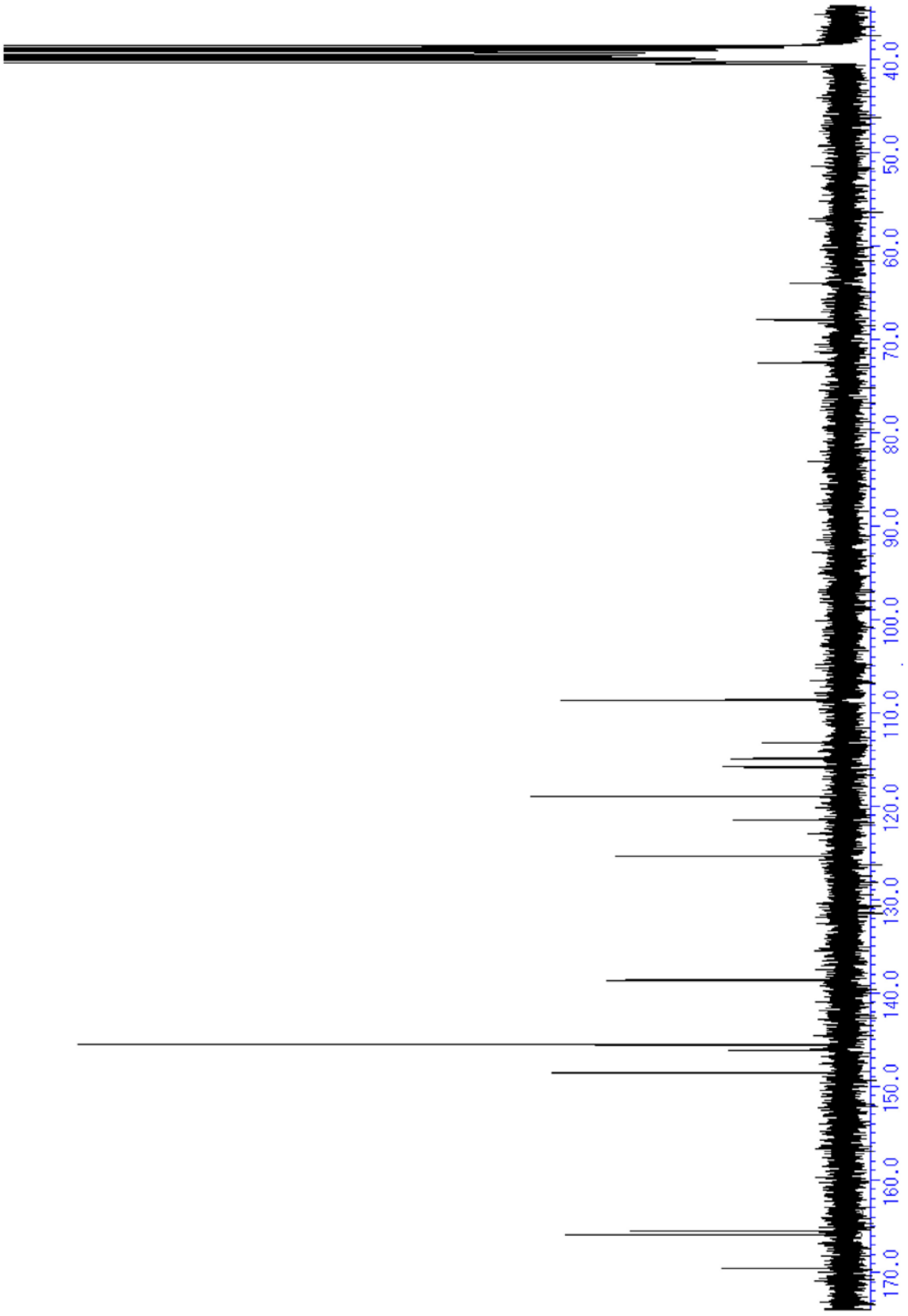
[1]H NMR spectra of 1 (500 MHz, DMSO-d6, rt)



[1]H NMR spectra of 2 (500 MHz, DMSO-d6, rt)

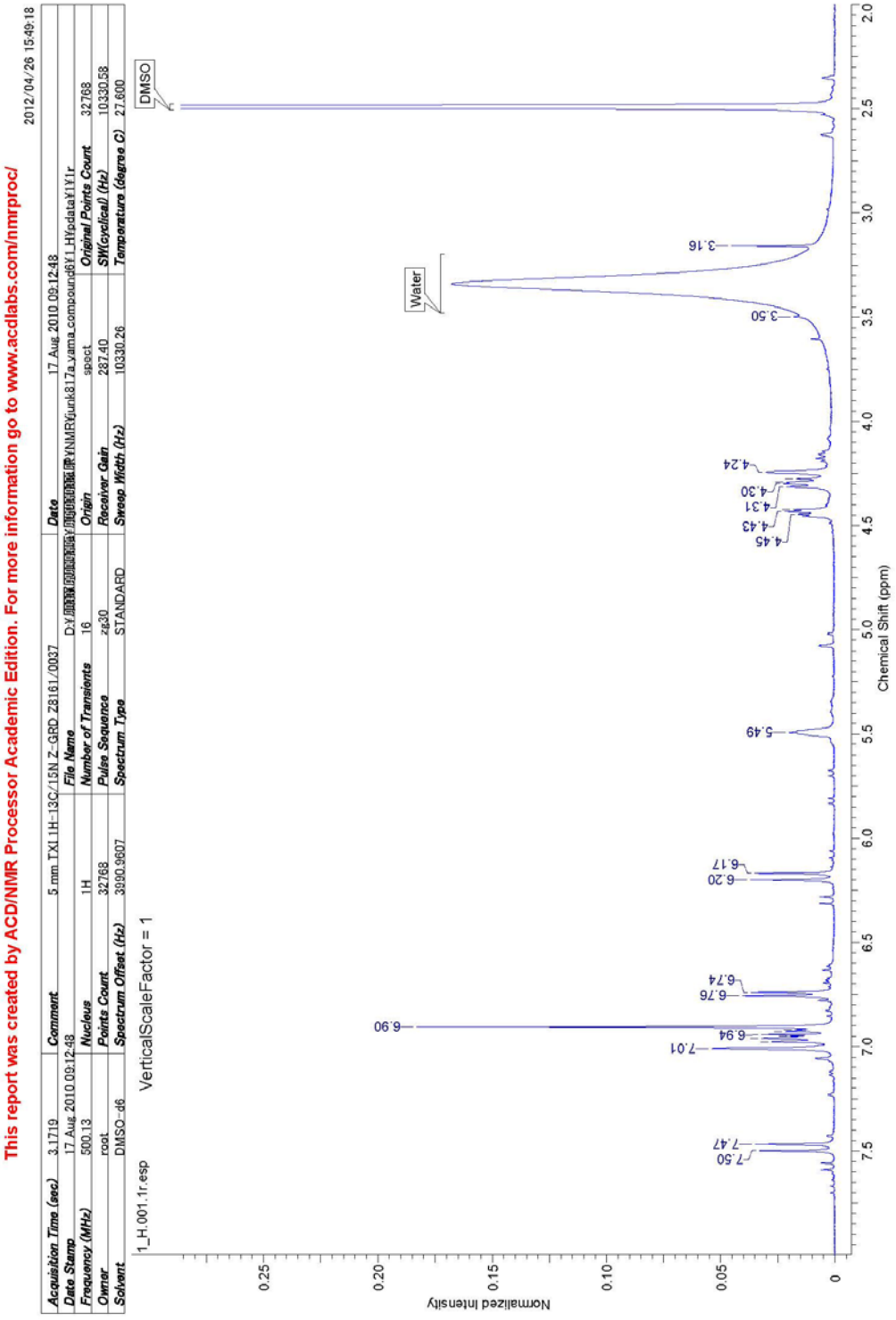


^{13}C NMR spectra of 2 (67.5 MHz, DMSO-d6, rt)

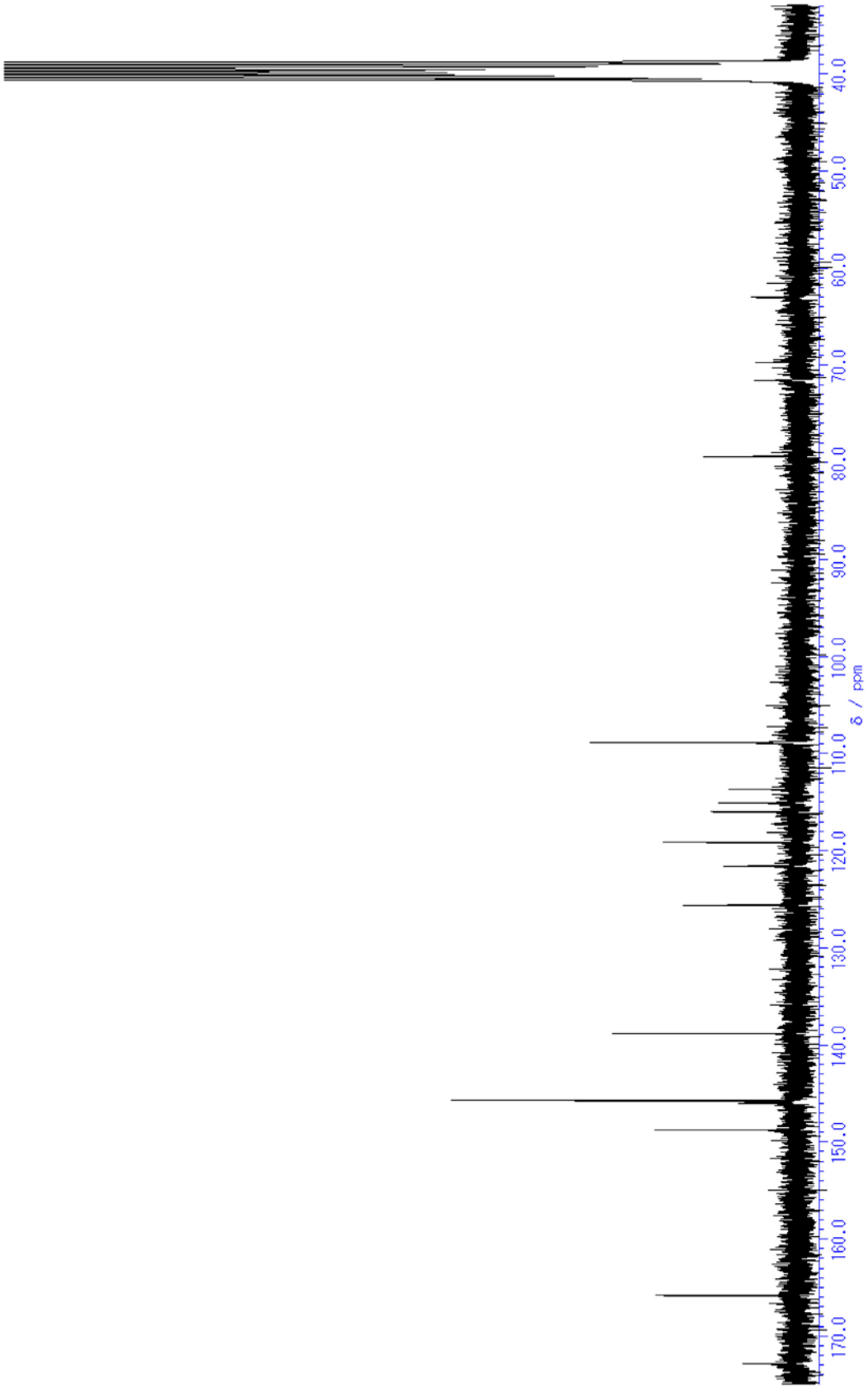


[1]H NMR spectra of 3 (500 MHz, DMSO-d6, rt)

*some isomerization observed

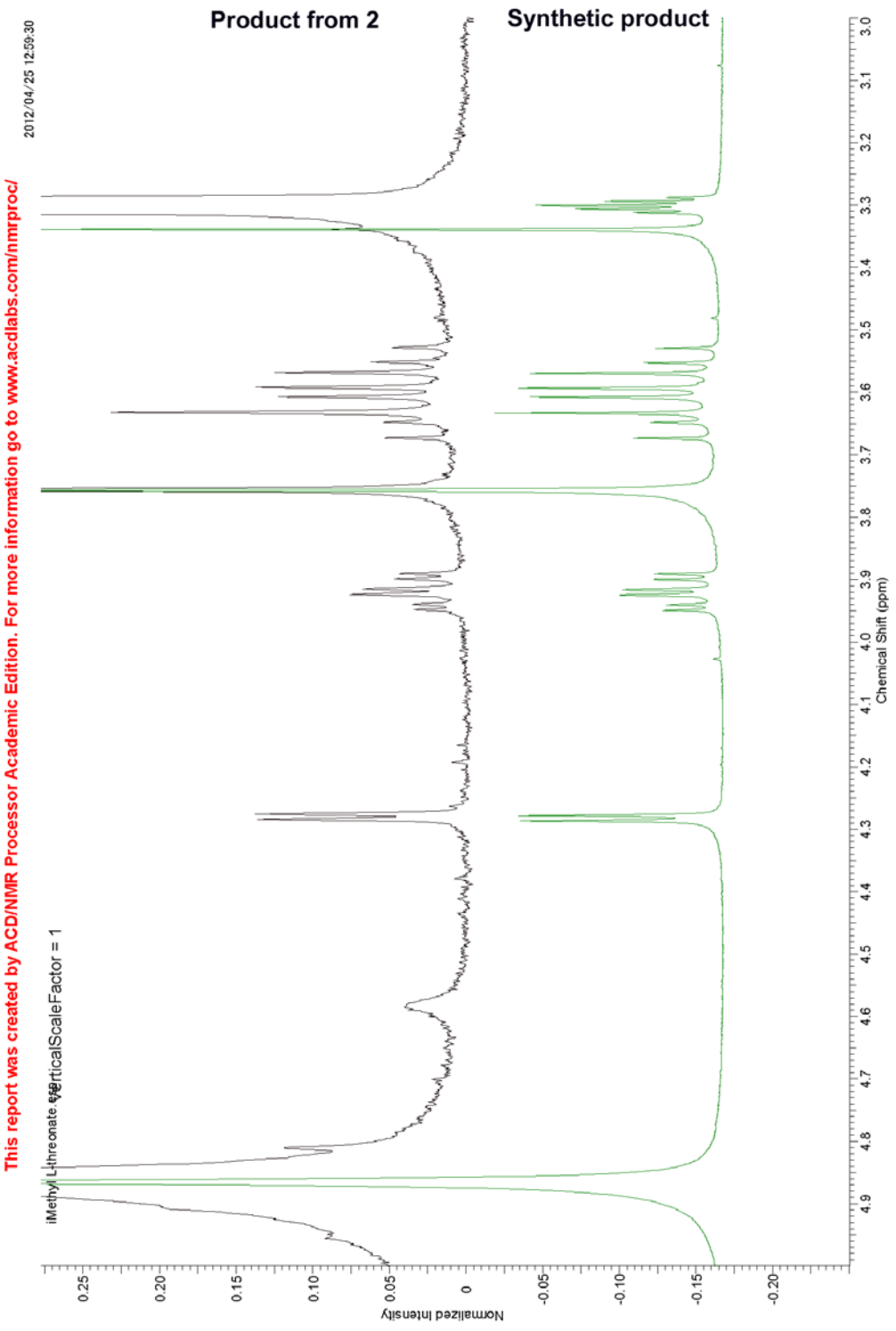


^{13}C NMR spectra of 3 (67.5 MHz, DMSO-d6, rt)



[1]H NMR spectra of methyl L-threonate derived from 2 and synthetic product

(270 MHz, CD_3OD, rt)



[1]H NMR spectra of methyl L-threonate derived from 3 and synthetic product

(500 MHz, CD_3OD, rt)