Supporting Information

L-Rhamnose-containing supramolecular nanofibrils as a potential immunosuppressive material

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1. Materials and General Methods

Chemical reagents and solvents were used as received from commercial sources. All the reagents for peptide solid phase synthesis, including all the amino acids and 2-chlorotrityl chloride resins were purchased from Shanghai GL Biochem. The rest chemicals are from Sigma-Aldrich and Fisher Scientific. HPLC purification was carried out on Waters Delta600 HPLC system equipped with an in-line diode array UV detector using a XTerra C18 RP column with CH_3CN (0.1% of trifluoroacetic acid) and water (0.1% of trifluoroacetic acid) as the eluent. ^{1}H NMR spectra were obtained on Varian Unity Inova 400 (Varian), LC-MS on Waters Acouity UPLC with Waters MICROMASS detector (Waters), TEM on Morgagni 268 transmission electron microscope after stained with 2.0% (w/v) uranyl acetate and rheology test on TA ARES-G2 with the parallel plate (diameter = 25 mm).

2. Synthesis

4: **4** was synthesized by Zemplén deacetylation of **2**, which was obtained from the literature method.[1] For the Zemplén deacetylation, briefly, sodium methoxide (0.8 eq.) in anhydrous methanol was adding into anhydrous methanol containing **2**, and the reaction was quenched by adding dry ice until the pH reached 7. ^{1}H NMR (400 MHz, *d*-DMSO) δ 4.65-4.51 (m, 2H), 3.83-3.59 (m, 6H), 3.51 (m, 2H), 2.04 (m, 2H), 1.64 (m, 2H), 1.06 (d, J = 6.1 Hz, 3H). ESI MS (m/z): $[M-H]^-$ calcd for $C_{10}H_{17}O_7^-$ 249.0980, found 248.8203.

1: Using the same Zemplén condition, **1** was obtained by Zemplén deacetylation of **3**, which was synthesized by following the reported procedure except that N,N'-dicyclohexylcarbodiimide (DCC) was replaced by N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).[2] **2** (188.1 mg, 0.5 mmol), EDC (105.5 mg, 1.1 eq.) and N-hydroxysuccinimide (NHS, 57.5 mg, 1 eq.) were added into 10 mL $CHCl_3$. The reaction was stirred in room temperature for 4 hours. The solvent was removed by rotary evaporation and the solid was dissolved in 10 mL acetone. The acetone solution was added into the aqueous solution of dissolved NapFFK-H TFA salt (361.2 mg, 1 eq.) at pH=8 (10 mL). NapFFK-H TFA salt was synthesized using Fmoc-based peptide solid phase synthesis according to the literature.[3] The reaction was stirred overnight at room temperature and acetone was removed by rotary evaporation. The solution was acidified by 1.0 M HCl solution and the crude product was filtered and collected for purification by column. After obtaining **3**, Zemplén deacetylation yielded **1** quantitatively and purified by RP C18 HPLC (total yield: 79%). ^{1}H NMR (400 MHz, *d*-DMSO) δ 8.47 (m, 3H), 7.85 – 7.64 (m, 4H), 7.58 (s, 1H), 7.52 (s, 1H), 7.47 – 7.36 (m, 2H), 7.29 – 7.03 (m, 10H), 5.29 (m, 2H), 5.04 (s, 1H), 4.44 (m, 3H), 3.75 (s, 1H), 3.65 – 3.38 (m, 3H), 3.24 – 2.61 (m, 7H), 2.06 (m, 2H), 1.73 – 1.49 (m, 4H), 1.34 – 1.12 (m, 4H), 1.06 (d, J = 6.1 Hz, 3H). ESI MS (m/z): $[M-H]^-$ calcd for $C_{46}H_{55}N_4O_{11}^-$ 839.3873, found 839.4681.

5: Synthesis of **5** follows the same procedure as the synthesis of **3** except using butyric acid (44.1 mg, 0.5 mmol) instead of **2**. The HPLC purification gave pure product **5** (total yield: 84%). ^{1}H NMR (400 MHz, *d*-DMSO) δ 8.29 (d, J = 8.7 Hz, 1H), 8.26 (d, J = 7.8 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 7.3 Hz, 1H), 7.76 (m, 3H), 7.58 (s, 1H), 7.46 (m, 2H), 7.30 – 7.10 (m, 10H), 4.58 (m, 1H), 4.51 (m, 1H), 4.18 (m, 1H), 3.52 (dd, J = 39.0, 14.0 Hz, 2H), 3.08 – 2.92 (m, 4H), 2.89 – 2.65 (m, 2H), 2.01 (t, J = 7.1 Hz, 3H), 1.67 (m, 2H), 1.48 (m, 2H), 1.34 (m, 4H), 0.82 (t, J = 7.3 Hz, 3H). ESI MS (m/z): $[M-H]^-$ calcd for $C_{40}H_{45}N_4O_6^-$ 677.3345, found 677.4011.

3. ELISA

All ELISA assays used alkaline phosphatase-coupled secondary antibodies, with p-nitrophenyl phosphate as the substrate. ELISA plates were read on an Emax spectrophotometer from Molecular Devices after 30 minutes of development in the dark.

3.1 ELISA for anti-L-rhamnose natural antibodies

Nunc Covalink NH 96 well plates were purchased from Thermo Scientific and stored at 4°C. 100 μL of solution containing 2.0 mg/mL of **4** and 1.1 mg/mL of NHS in PBS was added into the first row. Each subsequent row was 5 fold diluted. Then 50 μL of EDC solution (3.3 mg/mL) was added into each well. The plates were ready for use after incubation at r.t. for 4 hours, blocking with 2% milk for 2 hours and three washes. Then purified human or mouse IgM/IgG was added to the first column and serial diluted 2-fold. Secondary antibodies conjugated with alkaline phosphatase were added in a 1/1000 dilution and incubated for 1 hour. The plates were washed three times before 100μl of p-nitrophenyl phosphate was added.

3.2 ELISA for anti-PE antibodies

DNA-BIND plates were purchased from Corning and stored at 4°C. 100 μL of PE in PBS (31.25 μg/mL) was added into each well. The plates were ready for use after incubation at 4°C overnight, blocking by milk for 2 hours and three washes. Serum collected from mice was added and serially dilution (2-fold). Secondary antibodies conjugated with alkaline phosphatase were added in a 1/1000 dilution and incubated for 1 hour. The plates were washed three times before 100μl of p-nitrophenyl phosphate was added.

4. Immunizations

As indicated, some mice received intravenous (i.v.) injected of purified human IgM 12 hours before immunization in order to increase the amount of natural IgM. The time point of 12 hours was chosen to guarantee the presence of IgM throughout the mouse. For immunization, i.p. injection for each mouse is 100 μL containing 50 μg PE and 0.4 mg **1**, 0.4 mg **5** or 0.13 mg **4**. The samples were all heated to 40°C for half a minute to obtain either hydrogels or solutions. All the mice received a boost with only PE (5μg) at day 21. Blood was collected from the orbital sinus at day 10 and day 28.

The procedure for i.pl injection is the same as i.p. injection except the injection volume was reduced to 5 μL per injection and each mouse received two injections, one for each hind paw, instead of one per mouse for i.p. injection.

Supporting Figures

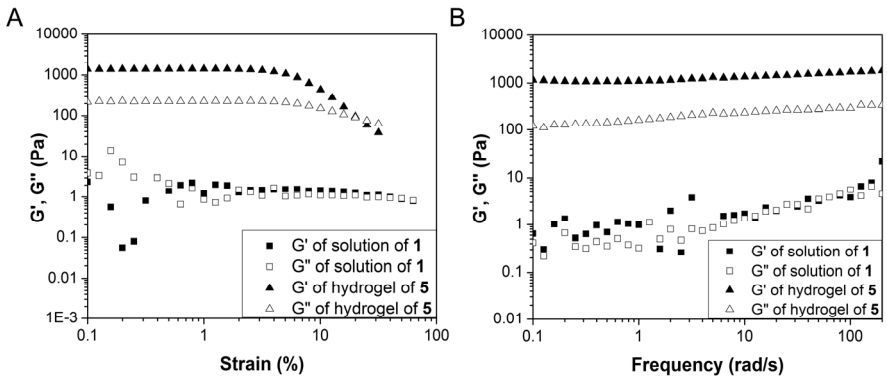


Figure S1. The dynamic strain sweeps (at 6.28 rad/s) and dynamic frequency seeps of weak hydrogel of **1** and hydrogel of **5** at the concentration of 0.4% (w/v).

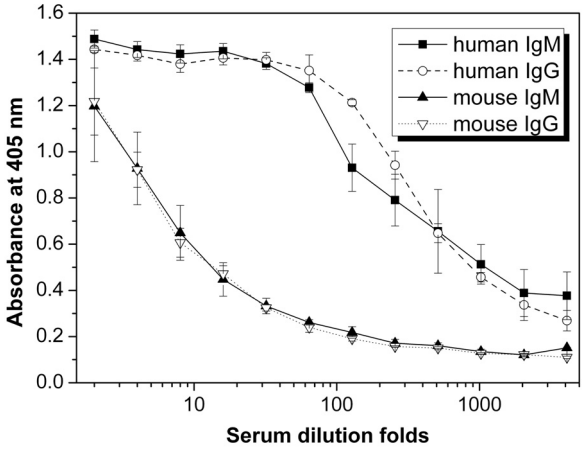


Figure S2. The anti-rhamnose IgG and IgM from normal human serum and wild-type mouse serum with serial dilution.

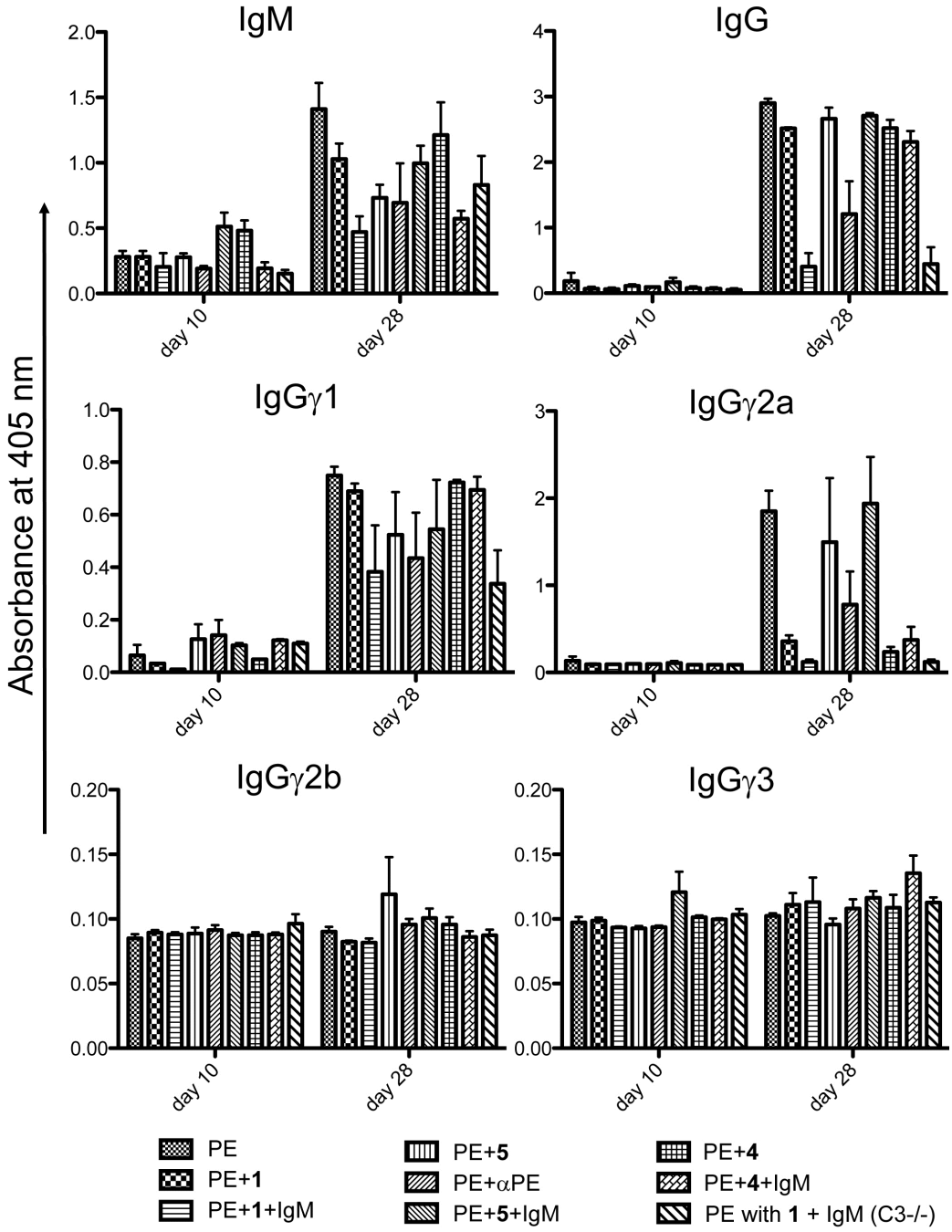


Figure S3. The anti-PE titering of IgM, IgG and isotypes of IgG ($IgG_{\gamma 1}$, $IgG_{\gamma 2a}$, $IgG_{\gamma 2b}$ and $IgG_{\gamma 3}$) from the serum of the wild type mice and complement component 3 knock-out (C3-/-) mice at 10 and 28 days after intraperitoneal immunization respectively with PE, PE with α-PE, PE in **1**, PE in **1** with injected human IgM, PE in **5**, PE in **5** with injected human IgM, PE with **4**, and PE with **4** with injected human IgM. The boosting was followed by injection of PE at day 21. Each data point represents the data from three mice.

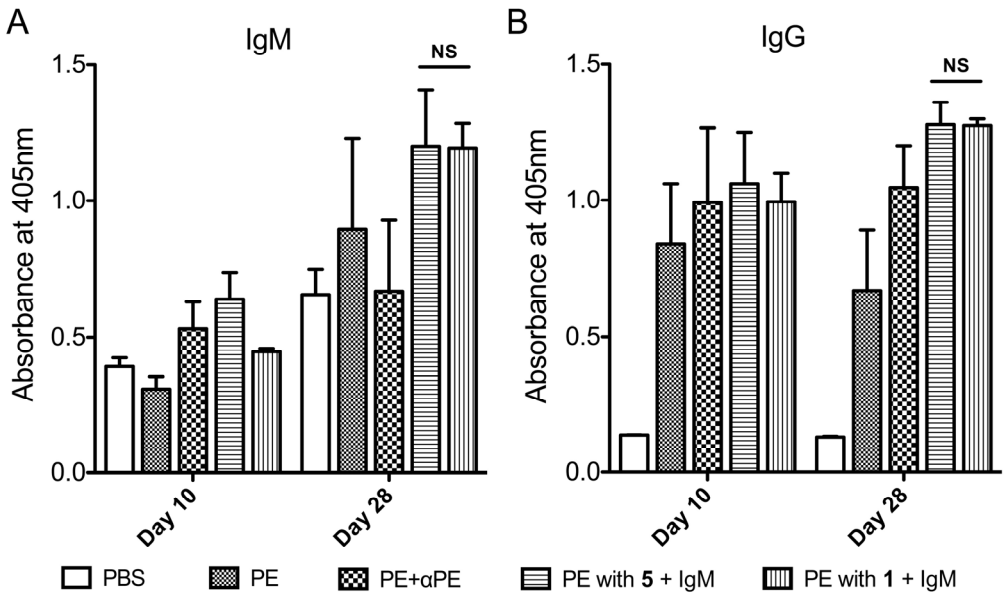
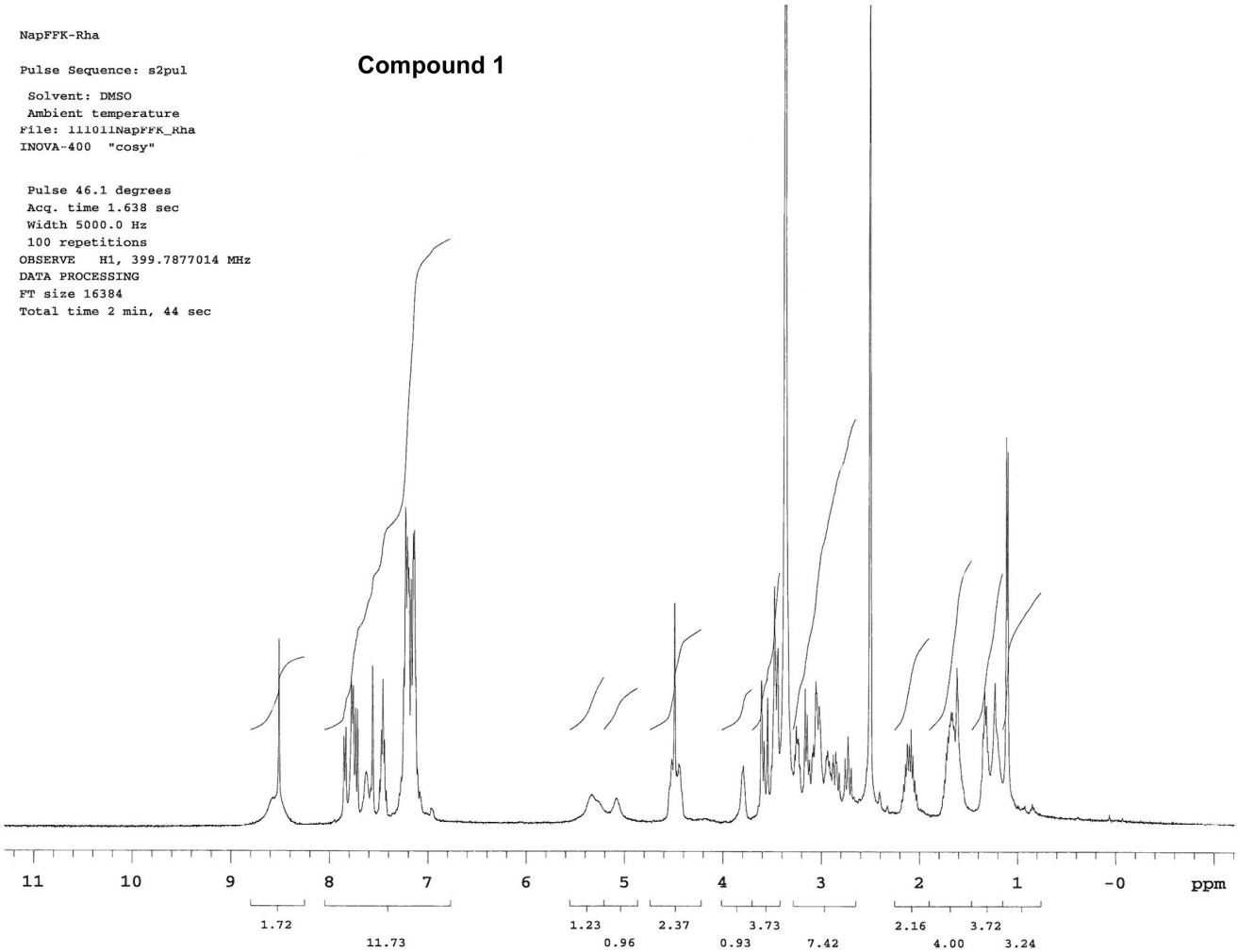


Figure S4. The anti-PE titering IgM and IgG of the serum from the wild type mice at 10 and 29 days after intraplantar (i.pl.) immunization respectively with PE, PE with αPE, PE in **1**, PE in **1** (gel molecule with rhamnose) with injected human IgM. The boosting was followed by injection of PE at day 21. Each data point represents the data from three mice.

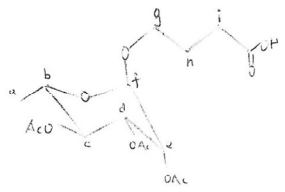


Rha_acid

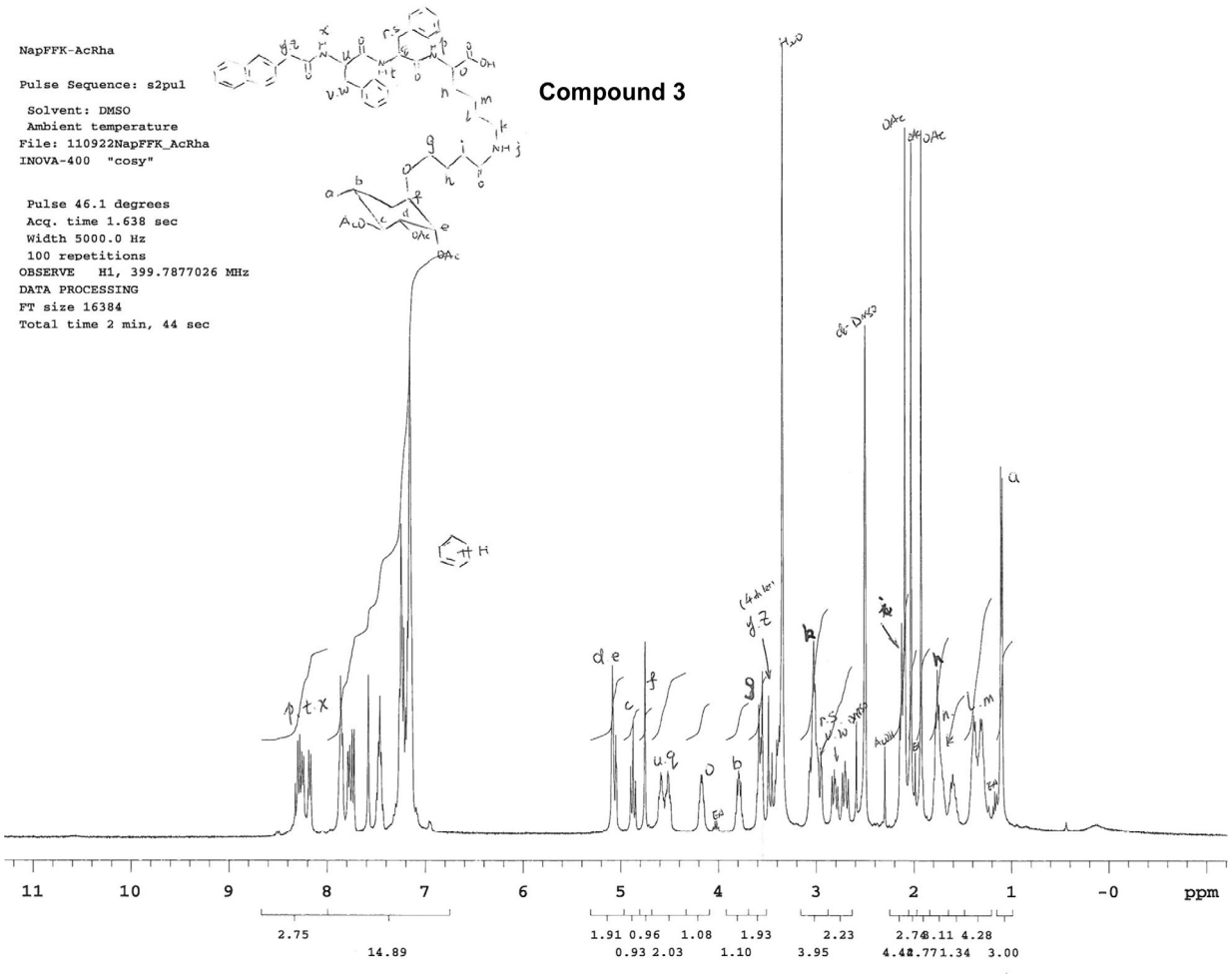
Pulse Sequence: s2pul

Solvent: CDCl3
Ambient temperature
File: 110914Rha_butacid
INOVA-400 "cosy"

Pulse 46.1 degrees
Acq. time 1.638 sec
Width 5000.0 Hz
90 repetitions
OBSERVE H1, 399.7858094 MHz
DATA PROCESSING
FT size 16384
Total time 2 min, 44 sec



Compound 2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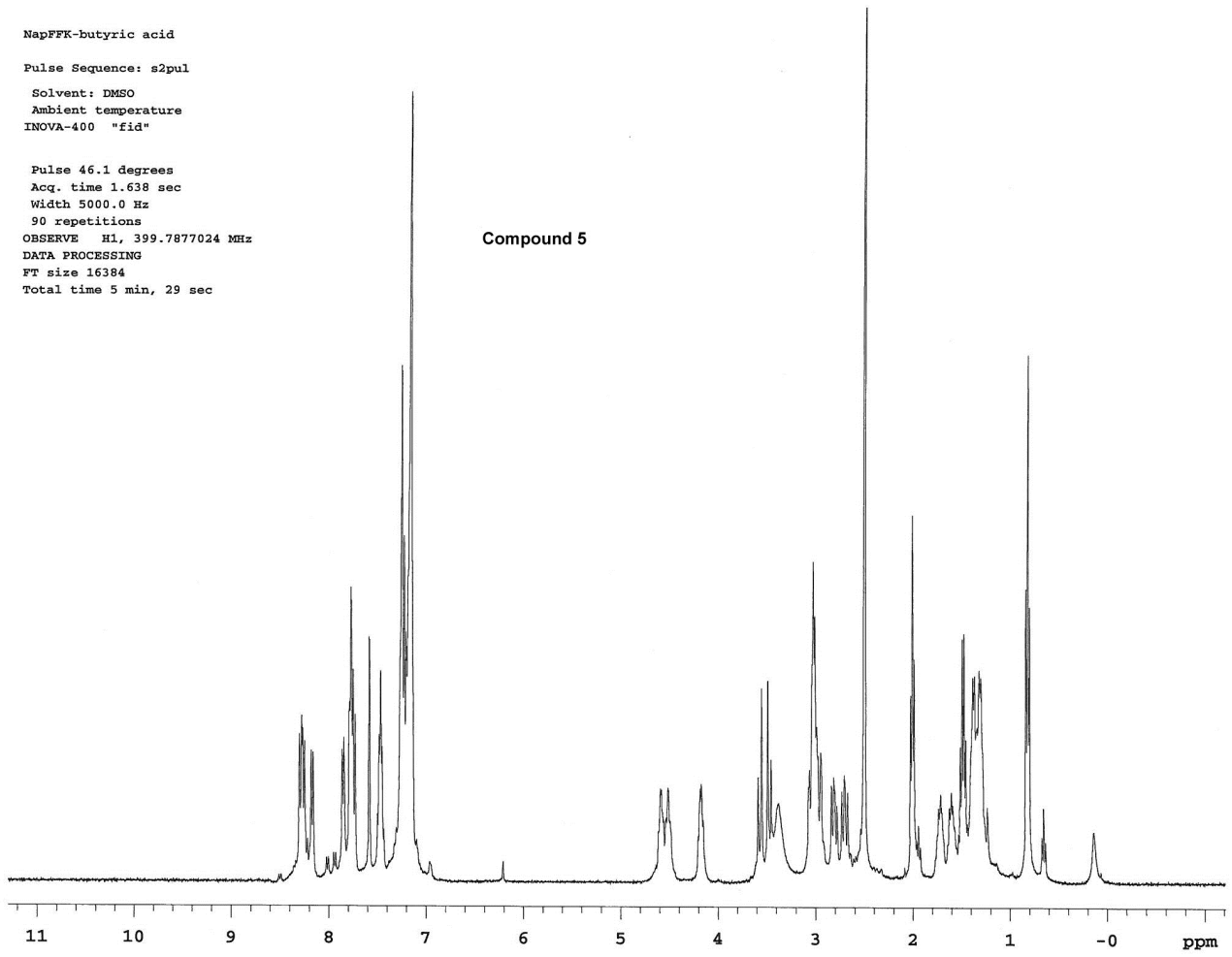
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
File: 110922NapFFK_AcRha
INOVA-400 "cosy"
Pulse 46.1 degrees
Acq. time 1.638 sec
Width 5000.0 Hz
100 repetitions
OBSERVE H1, 399.7877026 MHz
DATA PROCESSING
FT size 16384
Total time 2 min, 44 sec
Compound 3
11 10 9 8 7 6 5 4 3 2 1 -0 ppm
2.75 14.89
1.91 0.96 1.08 1.93 2.23 2.74 8.11 4.28
0.93 2.03 1.10 3.95 4.44 2.77 1.34 3.00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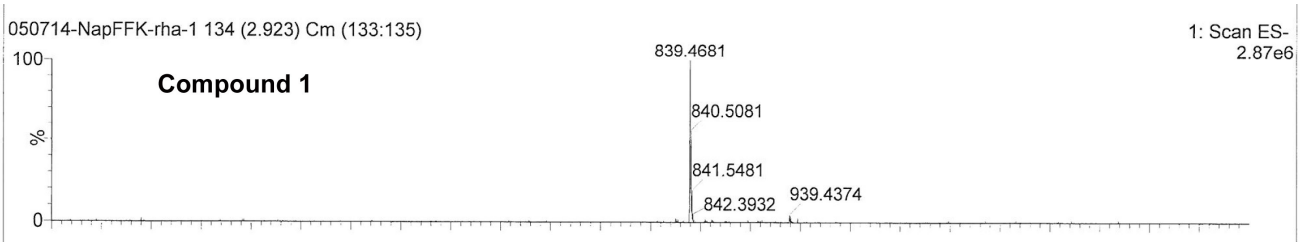
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
INOVA-400 "fid"
Pulse 46.1 degrees
Acq. time 1.638 sec
Width 5000.0 Hz
90 repetitions
OBSERVE H1, 399.7877024 MHz
DATA PROCESSING
FT size 16384
Total time 5 min, 29 sec
Compound 5
11
10
9
8
7
6
5
4
3
2
1
-0
ppm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Compound 1
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%
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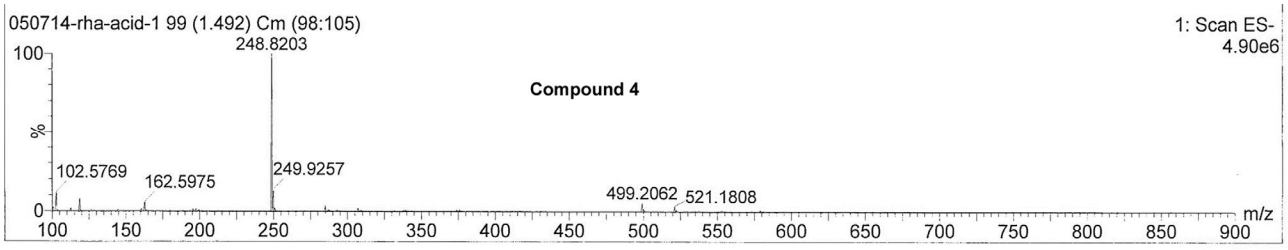
840.5081
841.5481
842.3932
939.4374


050714-rha-acid-1 99 (1.492) Cm (98:105)
1: Scan ES-
4.90e6
Compound 4
100
%
0
248.8203
102.5769
162.5975
249.9257
499.2062
521.1808
m/z
100
150
200
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300
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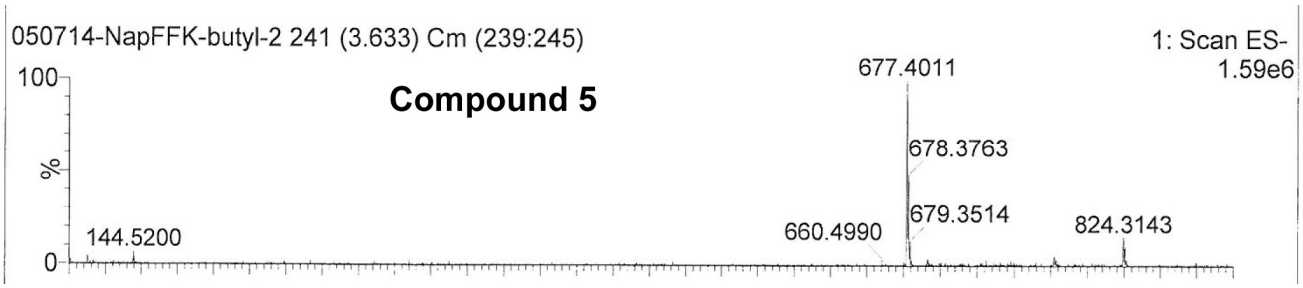

1 S. Sarkar, S. A. Lombardo, D. N. Herner, R. S. Talan, K. A. Wall and S. J. Sucheck, *J. Am. Chem. Soc.*, 2010, **132**, 17236.
2 F. Zhao, Y. Gao, J. Shi, H. M. Browdy and B. Xu, *Langmuir*, 2010, **27**, 1510.
3 Y. Gao, J. Shi, D. Yuan and B. Xu, *Nat Commun*, 2012, **3**, 1033.