ELECTRONIC SUPPLEMENTARY INFORMATION

Vancomycin dimer formation between analogues of bacterial peptidoglycan surfaces probed by force spectroscopy

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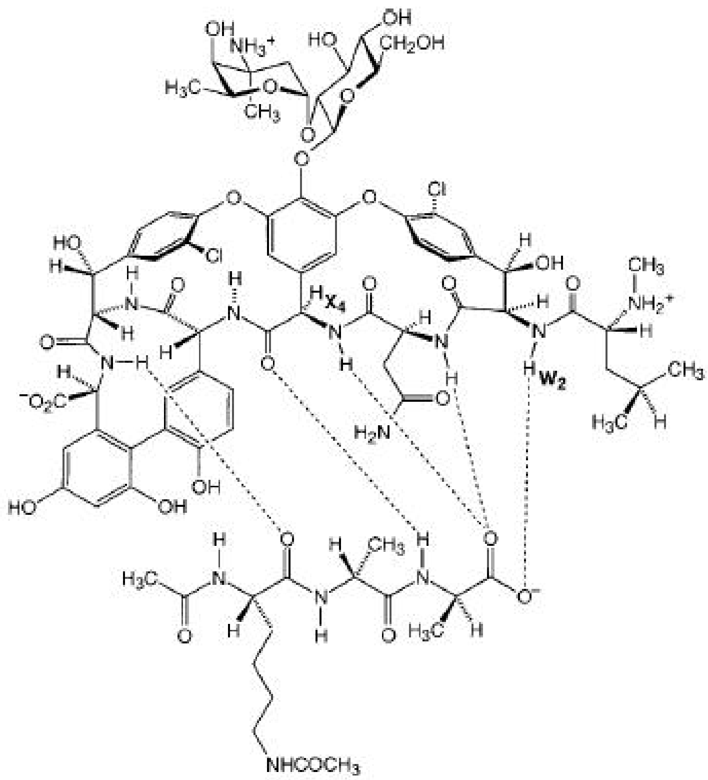


Fig. S1 Illustration of the specific interactions between vancomycin and Ac_2-L-Lysyl-D-Alanyl-D-Alanine (Ac_2-KDADA). The broken lines indicate specific hydrogen bonds. Mutation of the terminal D-Alanine to D-Lactate (as in the case of KDADLac) removes a hydrogen bond (second from the left) which results in the significantly weaker binding (K_d increased by ~ 3 orders of magnitude) associated with bacterial resistance to vancomycin. D. H. Williams and B. Bardsley: The vancomycin group of antibiotics and the fight against resistant bacteria, *Angew. Chem., Int. Ed.*, 1999, **38**, 1173–1193. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

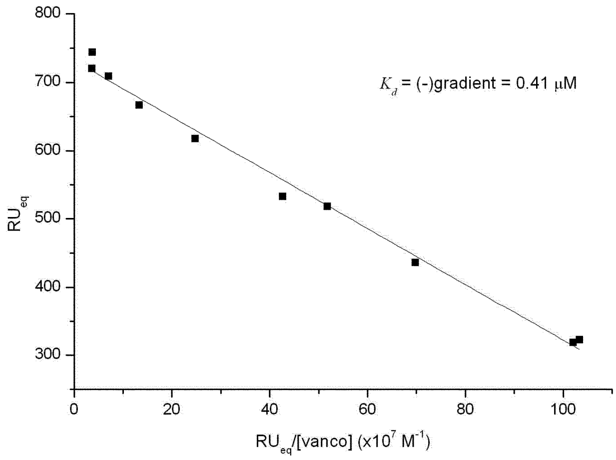


Fig. S2 An example of a Scatchard plot drawn to directly calculate K_d from equilibrium measurements at different analyte concentrations.

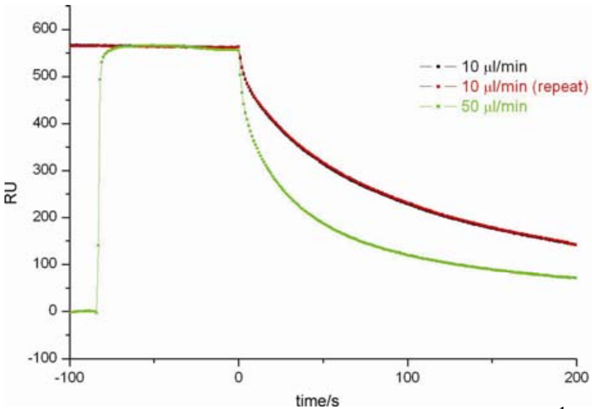


Fig. S3 The effect of increasing the flow rate from 10 to 50 µl min^{-1} on the dissociation rate of vancomycin from a 1 : 9 **1** : **4** surface.

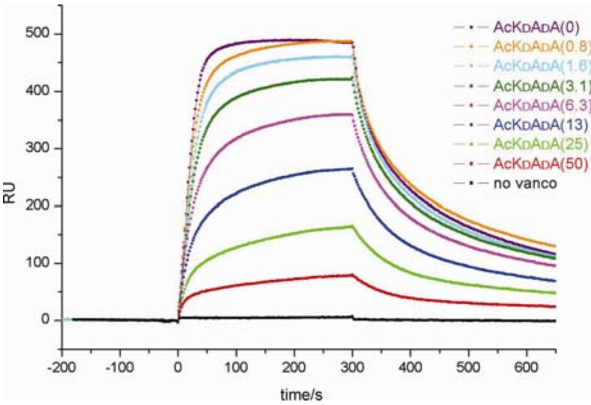


Fig. S4 The effect of introducing AcKDADA (concentrations in μM in brackets) on the binding profile of a 1 μM solution of vancomycin to a 1 : 9 **1** : **4** surface.

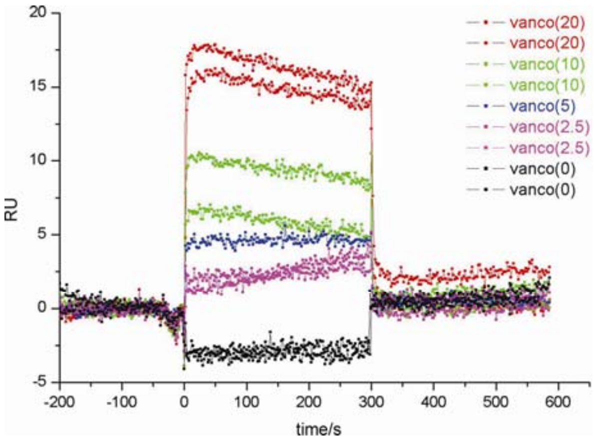


Fig. S5 Overlaid sensorgrams showing typical SPR responses to vancomycin solutions (concentrations in μM in brackets) for a 1 : 9 **2** : **4** (KDALA) surface.

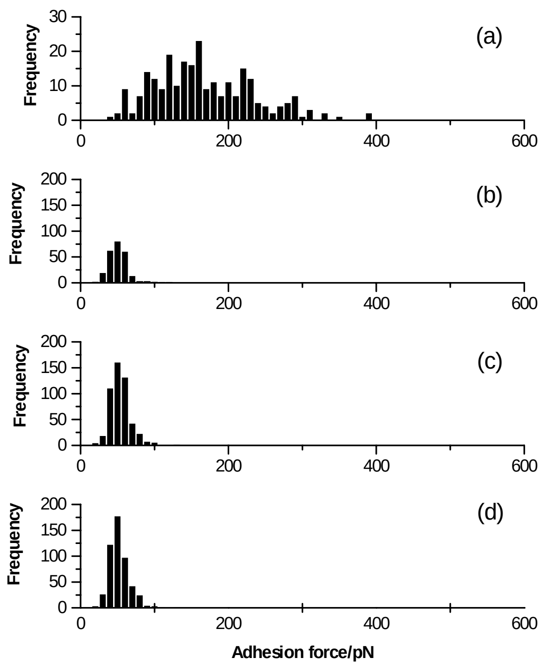


Fig. S6 Histograms of adhesion forces collected using the same KDADA-modified probe in a PBS solution containing 1 μM vancomycin on a: (a) KDADA surface (*n*=249), (b) KDALA surface (*n*=248), (c) KDADLac surface (*n*=500), and (d) PEG-OH surface (*n*=500).

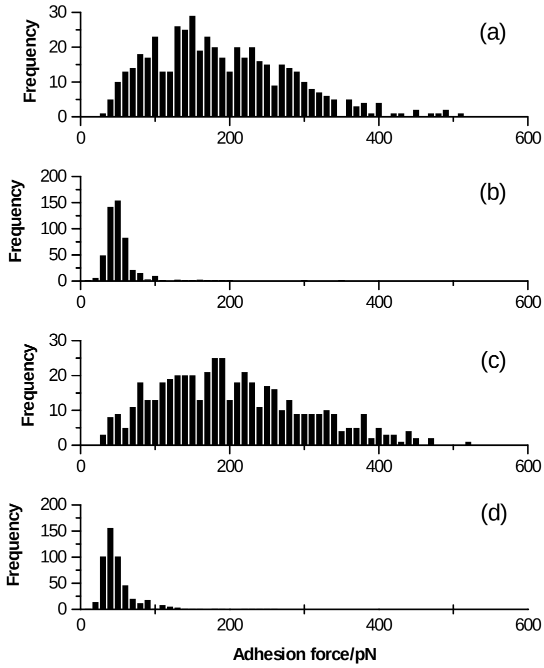


Fig. S7 Histograms of adhesion forces collected using the same KDADA probe-surface combination in PBS solutions containing: (a) 1 μM vancomycin (n=500), (b) 1 μM vancomycin and 50 μM AcKDADA (n=500), (c) 1 μM vancomycin (n=500), and (d) 50 μM AcKDADA (n=500).

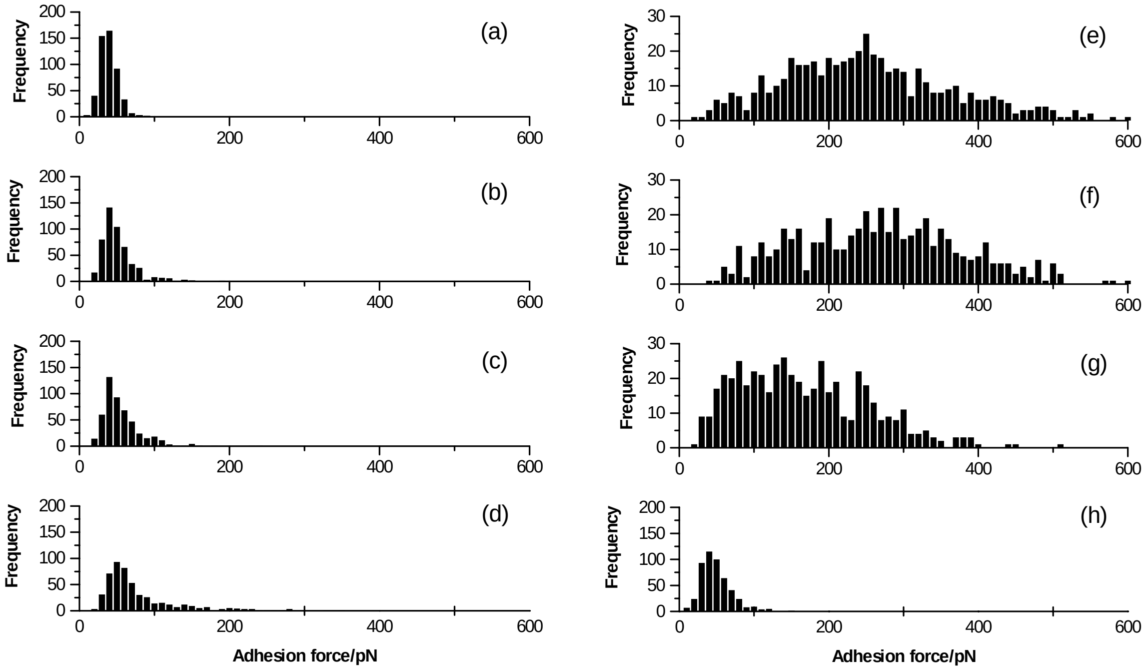
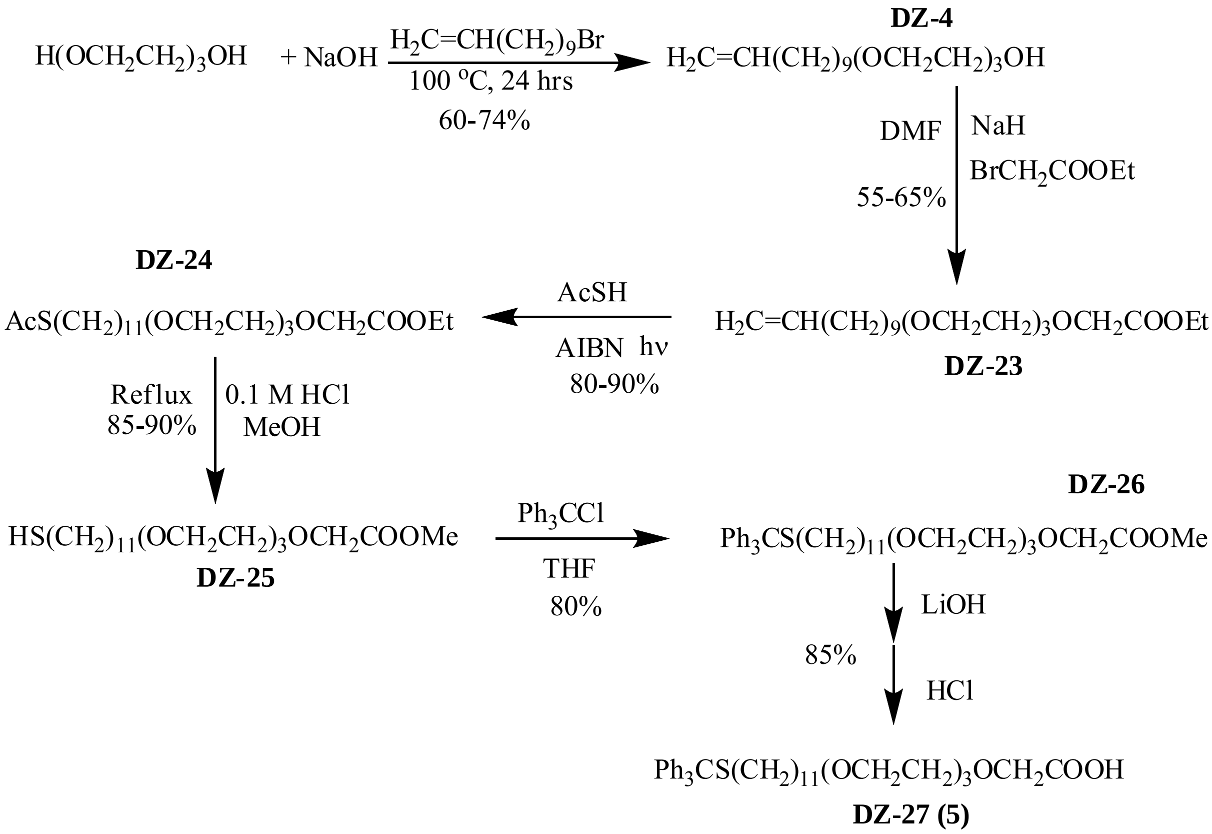


Fig. S8 Histograms of adhesion forces collected using the same KDADA probe-surface combination in PBS solutions containing: (a) 0 nM vancomycin (n=500), (b) 1 nM vancomycin (n=500), (c) 10 nM vancomycin (n=498), (d) 100 nM vancomycin (n=500), (e) 1 μM vancomycin (n=500), (f) 10 μM vancomycin (n=500), (g) 100 μM vancomycin (n=499), and (h) 0 nM vancomycin, after rinsing with buffer (n=499).

Synthesis of compound 5



Scheme S1 Synthesis of compound **5**

DZ-4. A mixture of 13 M NaOH (1.7 mL, 21.5 mmol) and tri(ethylene glycol) (16.11 g, 105 mmol) was stirred in an oil bath at 100 °C under an atmosphere of argon for 30 minutes. 11-Bromoundec-1-ene (5 g, 21.5 mmol) was then added. After 24 hours, the reaction mixture was cooled and extracted with hexane. Concentration of the combined hexane extracts by rotary evaporation at reduced pressure gave a yellow oil containing a mixture of mono and diether, according to analysis by ^{1}H NMR spectroscopy. Purification by column chromatography on silica gel (EtOAc) gave (4.4 g, 68%) of ether **DZ-4**, R_f(EtOAc) 0.51.

IR ν_{max} (film)/cm^{-1} 3464(O-H), 3074(C-H), 1639(C=C), 1121(C-O); ^{1}H NMR δ ppm (400 MHz, $CDCl_3$) 1.16-1.33 (m, 12H, 6CH_2), 1.50 (p, J = 7.0 Hz, 2H, C**H₂**), 1.95 (q, J = 7.0 Hz, 2H, C**H₂**-), 2.94 (s, br, 1H, -OH), 3.37 (t, J = 7.0Hz, 2H, -C**H₂**-PEG), 3.47-3.67 (m, 12H, 3PEG), 4.81-4.93 (m, 2H, C**H₂**=CH-), 5.65-5.77 (m, 1H, CH_2=C**H**-). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 139.46 (-

CH=CH2), 114.44 (-CH=C**H$_2$**), 72.92, 71.86, 70.95, 70.92, 70.69, 70.35 (6 PEG **C**), 61.97 (-**C**H$_2$-PEG), 34.11, 29.91, 29.85, 29.77, 29.75, 29.43, 29.24, 26.39.

DZ-23. Sodium hydride (520 mg, 13 mmol, 60% suspension in mineral oil) was added to a solution of undec-1-en-11-yltri(ethylene glycol) **DZ-4** (2.6 g, 8.6 mmol) in dry DMF (3 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 10 minutes, then ethyl-bromoacetate (1.92 mL, 13 mmol) was added under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 hours. An additional amount of ethyl-bromoacetate (1.92 mL, 13 mmol) was added and the reaction heated at 40 °C overnight. On cooling, the reaction mixture was extracted into EtOAc (3 × 20 mL), washed with distilled water (2 × 20 mL) and with brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness. Purification by column chromatography on silica gel (hexane/EtOAc 1:1) gave the ester **DZ-23** (4.5 g, impure, maybe including ethyl-bromoacetate and mineral oil), R_f (silica gel, hexane/EtOAc 1:1) 0.34.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20-1.37 (m, 15H, $(CH_2)_6$ + CH_3), 1.48-1.58 (m, 2H, C**H$_2$**), 1.95-2.03 (m, 2H, C**H$_2$**), 3.40 (t, J = 6.9 Hz, 2H, -C**H$_2$**PEG), 3.51-3.71 (m, 12H, 3PEG), 4.10 (s, 2H, -CH_2COOEt), 4.17 (q, J = 7.1 Hz, 2H, -COOCH_2), 4.85-4.96 (m, 2H, H_2C=), 5.70-5.82 (m, 1H, =CH-).; ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 170.81 (C=O), 139.55 (-CH=), 114.46 (H_2C=), 71.88, 71.26, 71.00, 70.95, 70.42, 69.09 (PEG, C), 61.12 (-O-PEG), 34.15, 29.99, 29.88, 29.81, 29.78, 29.42, 29.28, 26.44, 14.57 (CH_3). **LCMS** (ES^+): 389 (40%, $[M+H]^+$), 407 (100%, $[M+NH_4]^+$.

DZ-24. To 8 Pyrex tubes, each containing undec-1-en-11-yltri(ethylene glycol)-ethyl ester **DZ-23** (0.39 g, 1 mmol) in MeOH (5 mL) was added AIBN (30 mg), and thioacetic acid (0.12 mL, 2.4 mmol). Each tube was bubbled with N_2 for 2 minutes. The tubes were then sealed, put into a photochemical reactor and irradiated with a mercury lamp at 340 nm for 24 hours. On cooling, the tubes were taken out from the reactor. The reaction mixtures were then combined and evaporated to dryness. The solvent was then removed under reduced pressure and the residue purified by chromatography on a silica gel column (hexane/EtOAc 2:1) to obtain the ester **DZ-24** as a slightly yellowish oil (3.36 g, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.18-1.32 (m, 17H, $(CH_2)_7$+CH_3), 1.46-1.56 (m, 2H, C**H$_2$**), 2.27 (s, 3H, CH_3(C=O)S), 2.81 (t, 2H, J = 7.2 Hz, $AcSCH_2$-), 3.40 (t, J = 7.0 Hz, 2H, -C**H$_2$**PEG), 3.50-3.70 (m, 12H, 3PEG), 4.10 (s, 2H, -CH_2COOEt), 4.17 (q, J = 7.1 Hz, 2H, -$COOCH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 201.30 (CH_3(C=O)S-), 175.72 (C=O), 76.78, 76.13, 75.87, 75.29, 73.97, 73.12 (PEG, C), 66.03, 35.90, 29.99, 29.88, 29.81, 29.78, 29.42, 29.28, 26.44, 14.57 (CH_3). **LCMS** (ES^+), 465 (40%, $[M+H]^+$), 482 (100%, $[M+NH_4]^+$).

DZ-25. To 1-thioacetateundec--11-yltri(ethylene glycol)-ethyl ester **DZ-24** (3.36 g, 7.2 mmol) in MeOH (10 mL) was added 0.1 M HCl (8 mL). The reaction mixture was heated at reflex under N_2 for 8 hours. The solvent was then evaporated and the residue purified by column chromatography on silica gel (hexane/EtOAc 1:1) obtained the acid **DZ-25** as a slightly yellowish oil (2.70 g, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.20-1.40 (m, 14H, 7C**H$_2$**), 1.50-1.61 (m, 4H, 2C**H$_2$**), 2.49 (q, 2H, J = 7.2 Hz, -C**H$_2$**SH), 3.41 (t, 2H, J = 6.9 Hz, -C**H$_2$**PEG), 3.51-3.75 (m, 15H, 3**PEG** + C**H$_3$**), 4.15 (s, 2H, -C**H$_2$**COOMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 170.68 (C=O), 71.31, 70.69, 70.40, 70.34, 69.81, 68.40 (PEG, C), 51.57 (**C**H_3), 33.82, 29.39, 29.32, 29.27, 28.83, 28.14, 25.85, 24.43. **LCMS** (ES^+): 409 (45%, $[M+H]^+$), 426 (100%, $[M+NH_4]^+$.

DZ-26. 2-[11-Tritylthiolundec-1-yltri(ethylene glycol)] acetic acid **4.7** was synthesized according to the literature procedure (see Houseman, B. T.; Mrksich, M. *J. Org. Chem.* **1998**, *63*, 7552-7555.), and purified by column chromatography on silica gel (hexane/EtOAc 1:1) (Rf = 0.23 (1:1 EtOAc/hexane) as a slightly yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.09-1.41 (m, 16H, 8C**H$_2$**), 1.51-1.60 (m, 2H, C**H$_2$**), 2.11 (t, 2H, J = 7.2 Hz, Ph_3CSC**H$_2$**), 3.42 (t, 2H, J = 6.9 Hz, -C**H$_2$**PEG), 3.54-3.75 (m, 15H, 3**PEG** + C**H$_3$**), 4.15 (s, 2H, -C**H$_2$**COOMe), 7.15-7.21 (m, 3H, 3Ph-**H** para), 7.22-7.30 (m, 6H, 6 Ph-**H**), 7.37-7.42 (m, 6H, 6Ph-**H**); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 171.53 (**C**=O), 145.70, 130.22, 128.42, 127.11 (Ph-**C**), 72.17, 71.55, 71.26, 71.20, 70.67, 69.26 (PEG-**C**), 52.42 (**C**H_3), 32.64, 30.26, 30.18, 30.11, 30.03, 29.81, 29.64, 29.21, 26.71. **LCMS** (ES^+): 668 (80%, $[M+NH_4]^+$).

DZ-27 (5). The synthesis of DZ-27 (**5**) was prepared following a literature procedure and purified by followed by purified by column chromatography on silica gel (10% MeOH/CH_2Cl_2, Rf = 0.27) as a slightly yell oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.08-1.30 (m, 14H, 7C**H$_2$**), 1.31-1.41 (m, 2H, C**H$_2$**), 1.50-1.60 (m, 2H, C**H$_2$**), 2.11 (s, 2H, J = 7.1 Hz, -C**H$_2$**$SCPh_3$), 3.42 (t, J = 7.0 Hz, 2H, -C**H$_2$**PEG), 3.54-3.72 (m, 12H, 3**PEG**), 4.13 (s, 2H, -C**H$_2$**COOH), 7.15-7.22 (m, 3H, 3Ph-**H**), 7.22-7.30 (m, 6H, 6Ph-**H**), 7.36-7.42 (m, 6H, 6Ph-**H**). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 173.06 (C=O), 145.61, 128.97, 127.16, 125.86, (Ph-**C**), 70.98, 69.73, 69.55, 69.51, 69.36, 69.12 (PEG, C), 31.39, 28.94, 28.88, 28.81, 28.57, 28.40, 27.96, 25.39. HRMS, (TOF MS), found 659.3391, required for $C_{38}H_{52}O_6SNa$ $[M+Na]^+$, 659.3382, dev., 1.27 ppm. R_f (10% MeOH/CH_2Cl_2), 0.27.

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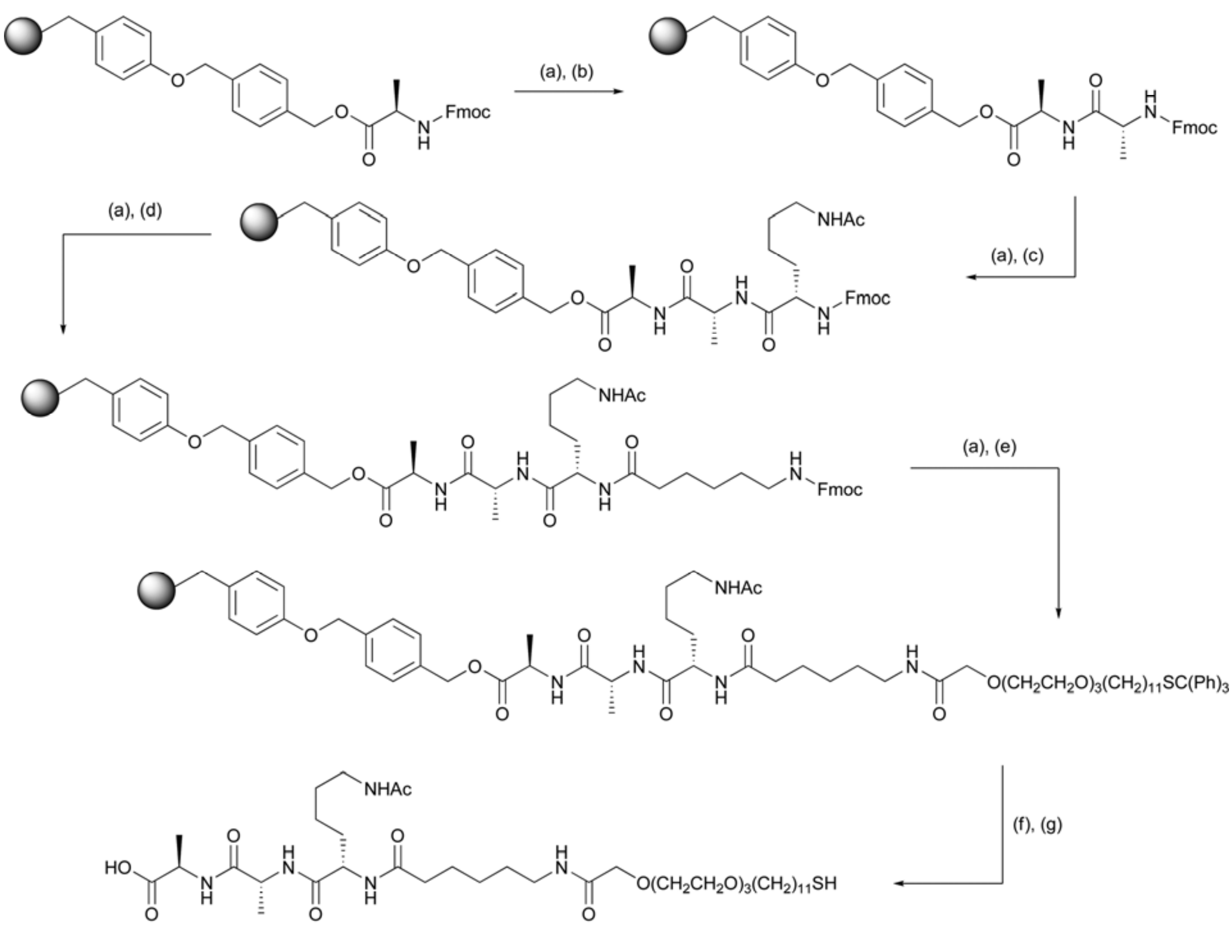
Synthesis of peptide-thiols

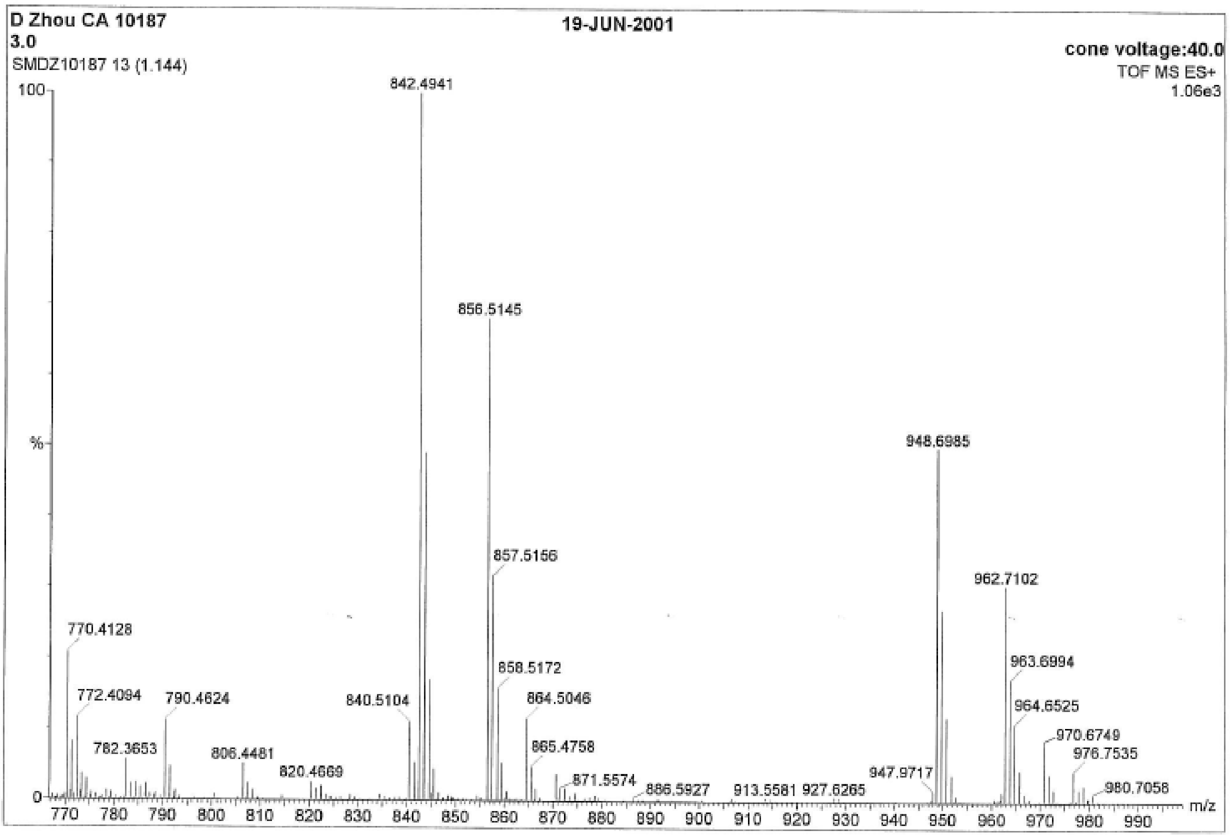


1

Scheme S2 Synthesis of the KDADA-modified thiol (**1**). Conditions: (a) piperidine, DMF; (b) Fmoc-DAla-OH, EDC, HOBt, DMF; (c) Fmoc-LLys-OH, EDC, HOBt, DMF; (d) Fmoc-Ahx-OH, EDC, HOBt, DMF; (e) **5**, EDC, HOBt, DMF; (f) TFA, DCM, Et_3SiH; (g) TFA, $CHCl_3$, BuSH.

NMR and HR-MS Spectra of the peptide-thiols

High resolution mass spectroscopy
KDADA



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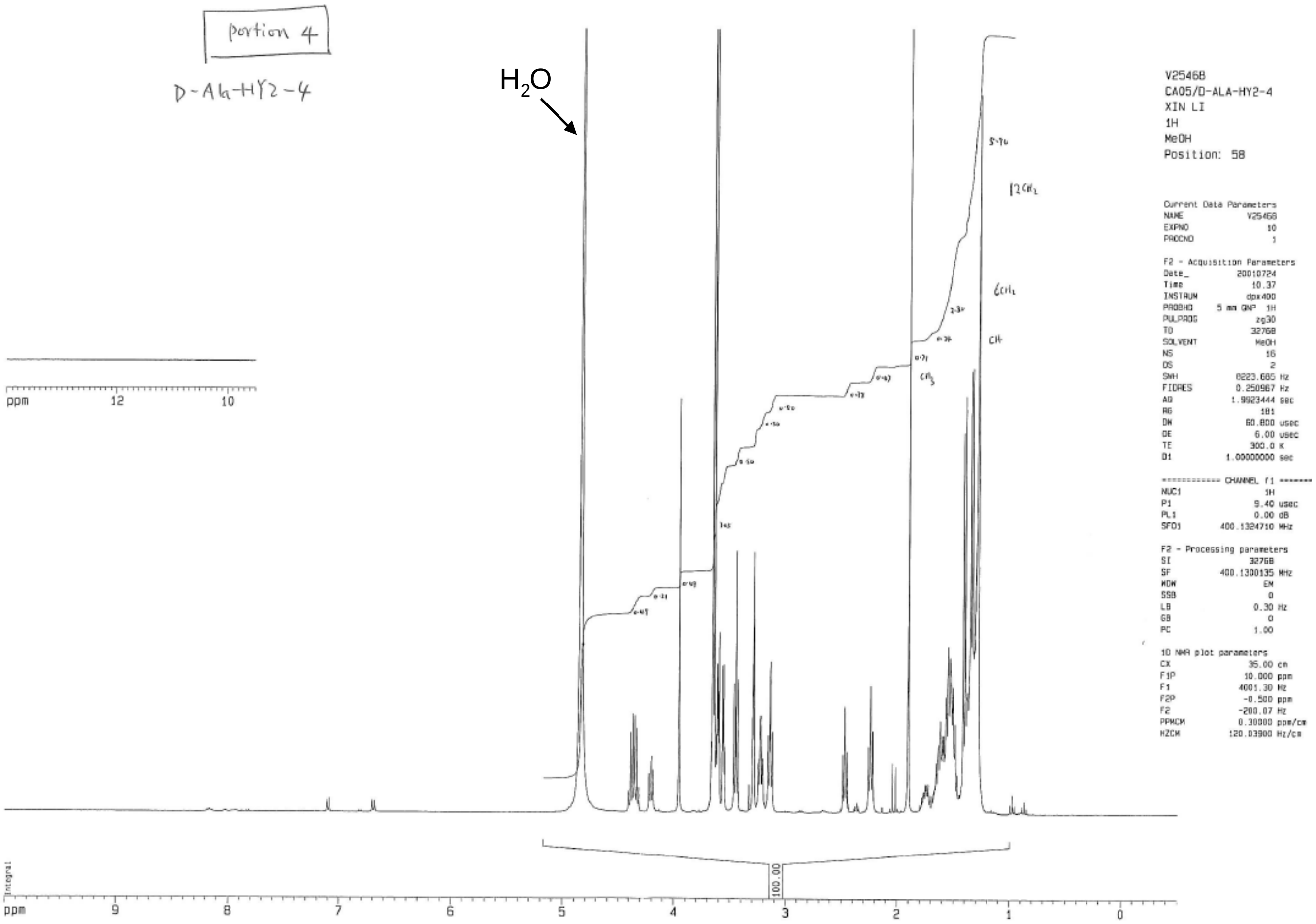
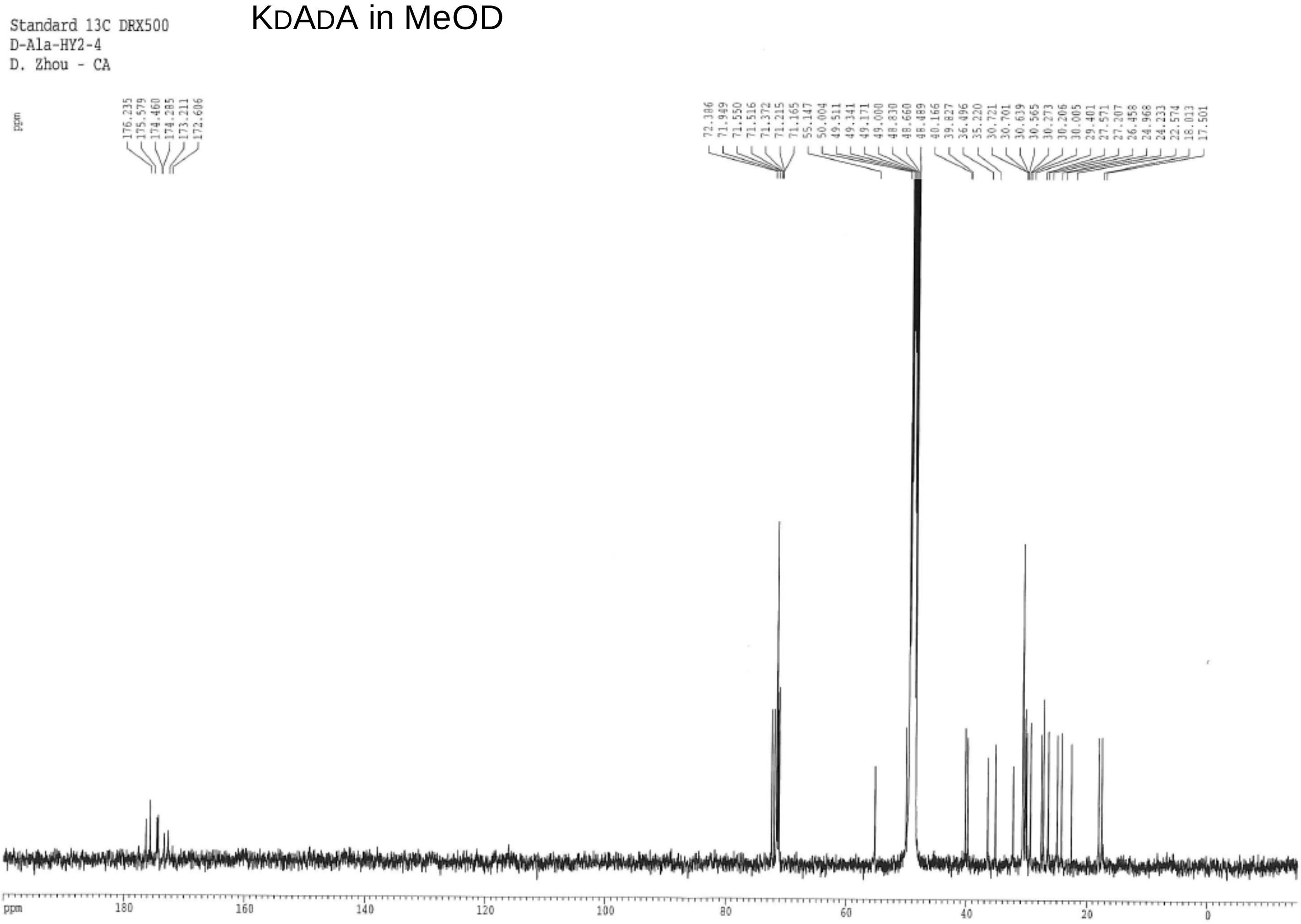
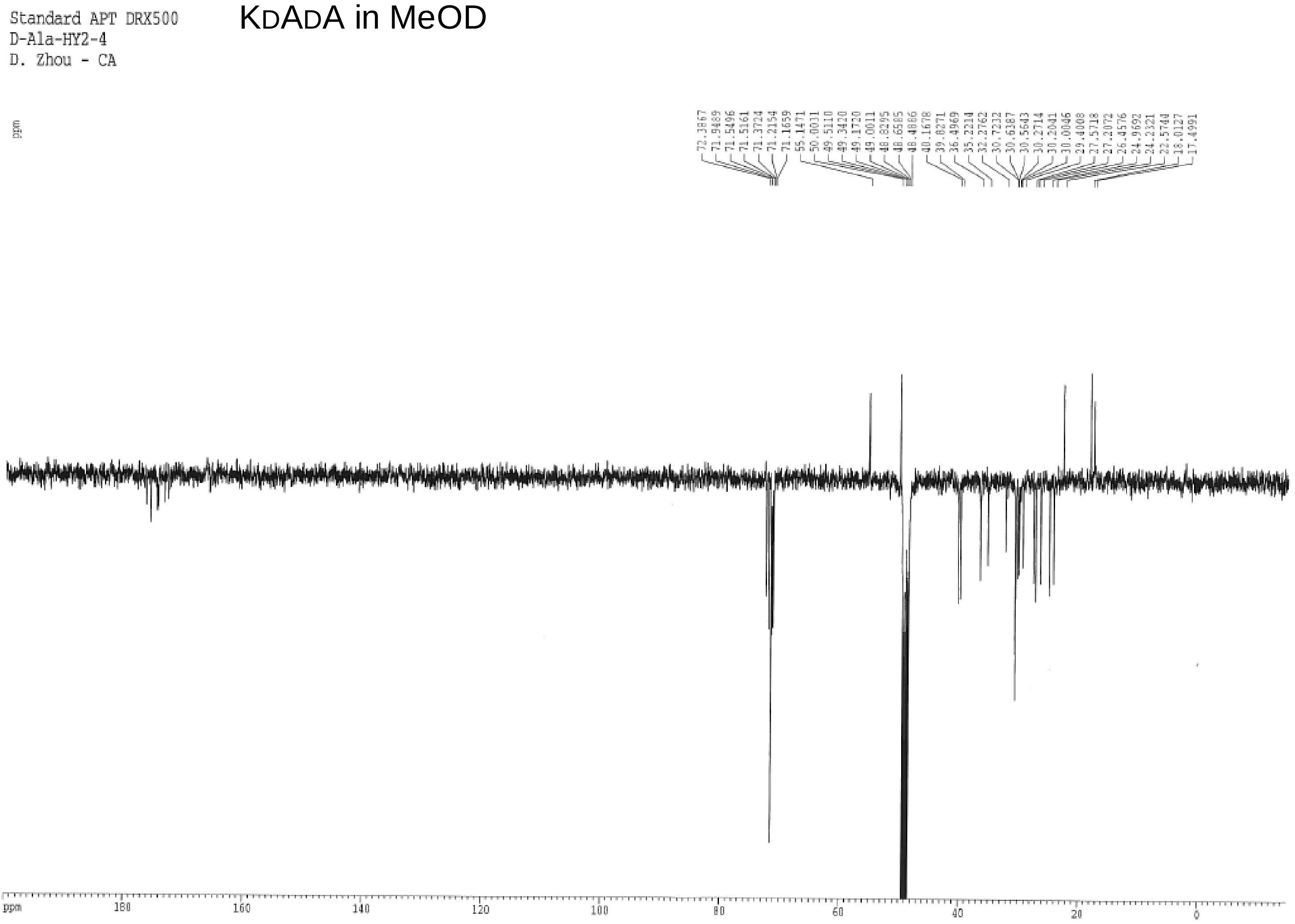
^1H NMR (500 mHz)
KDADA in MeOD



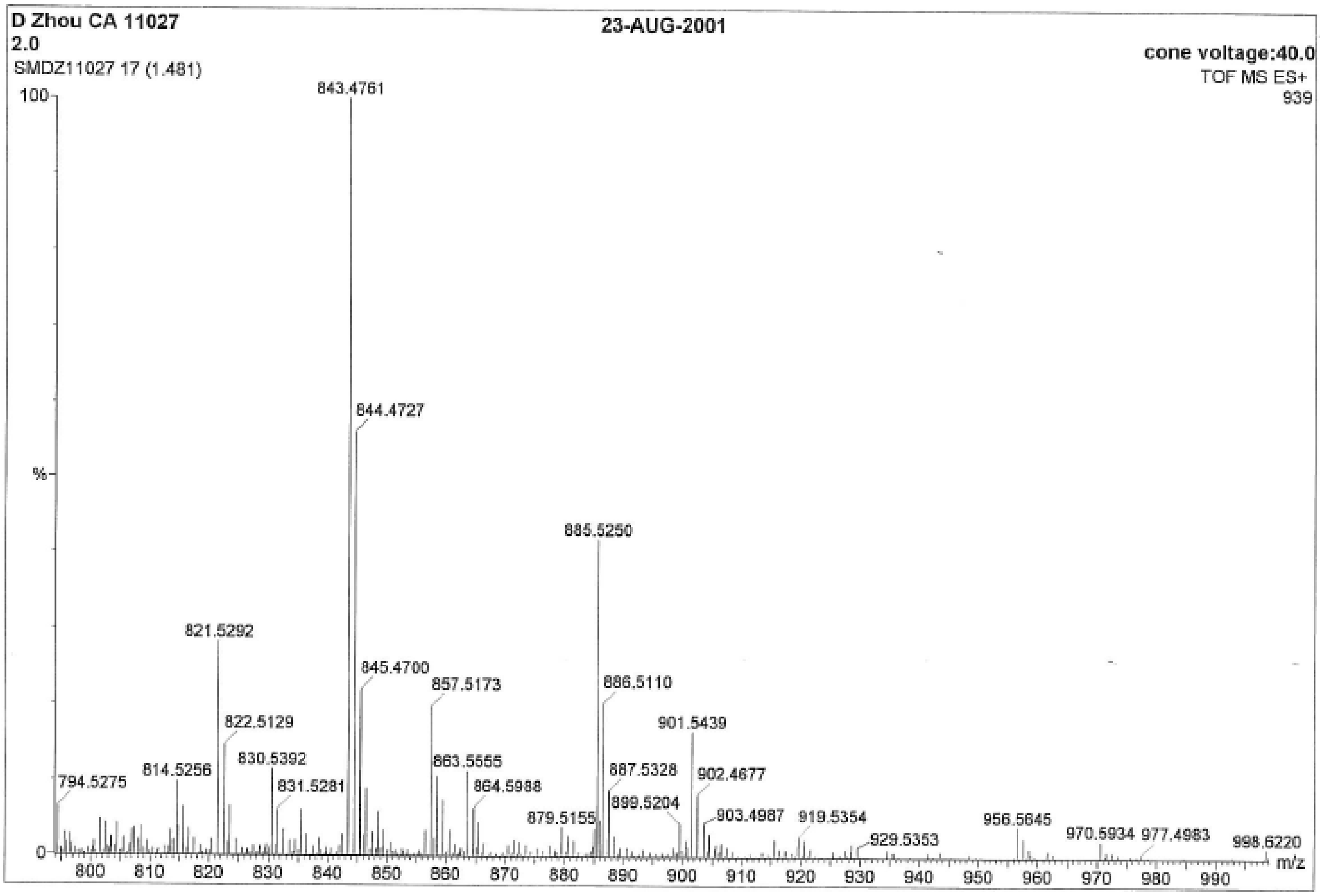
^{13}C NMR (125 MHz)
KDADA in MeOD



^{13}C NMR (APT)
KDADA in MeOD

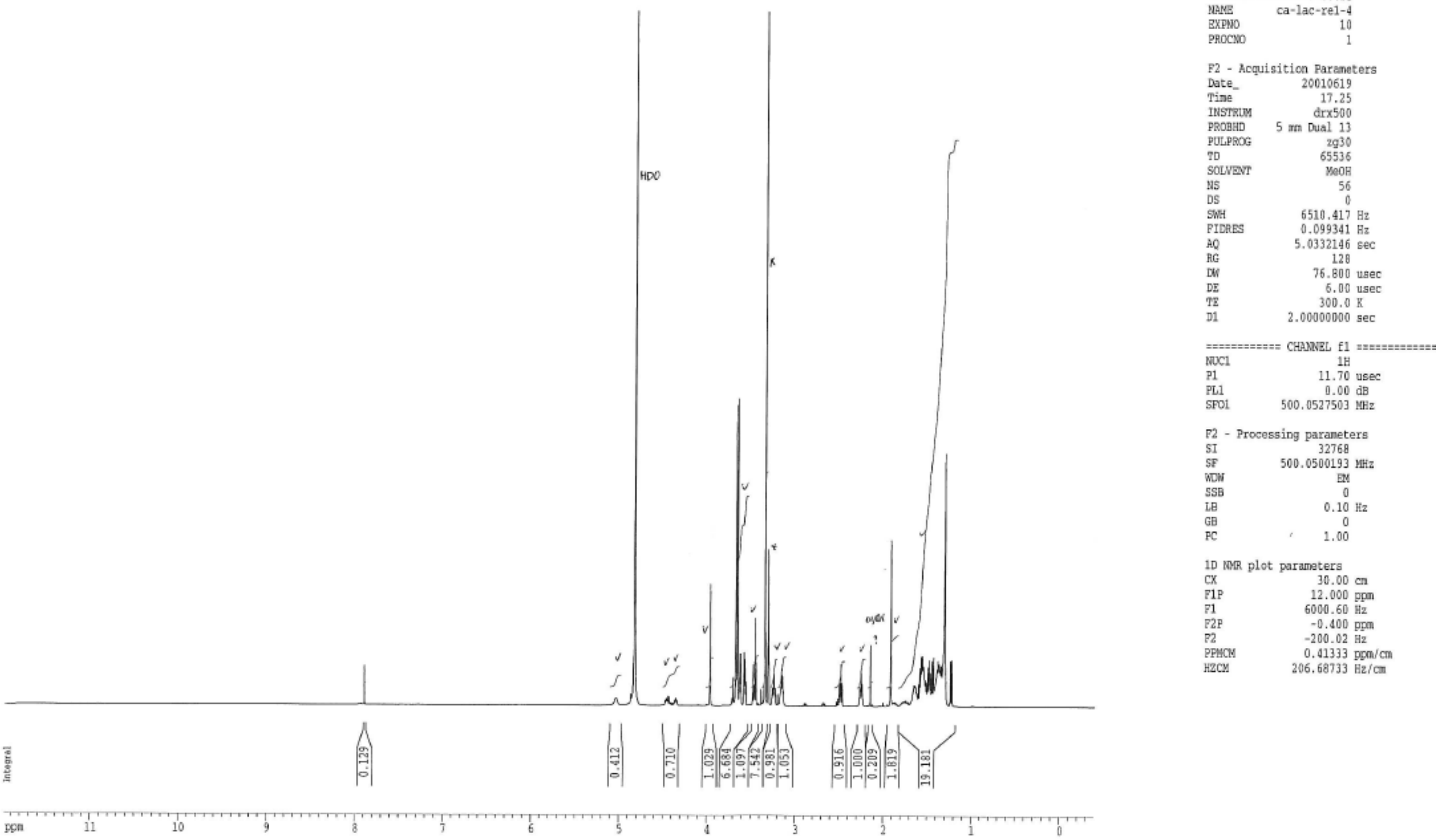


High resolution mass spectroscopy
KDADLac



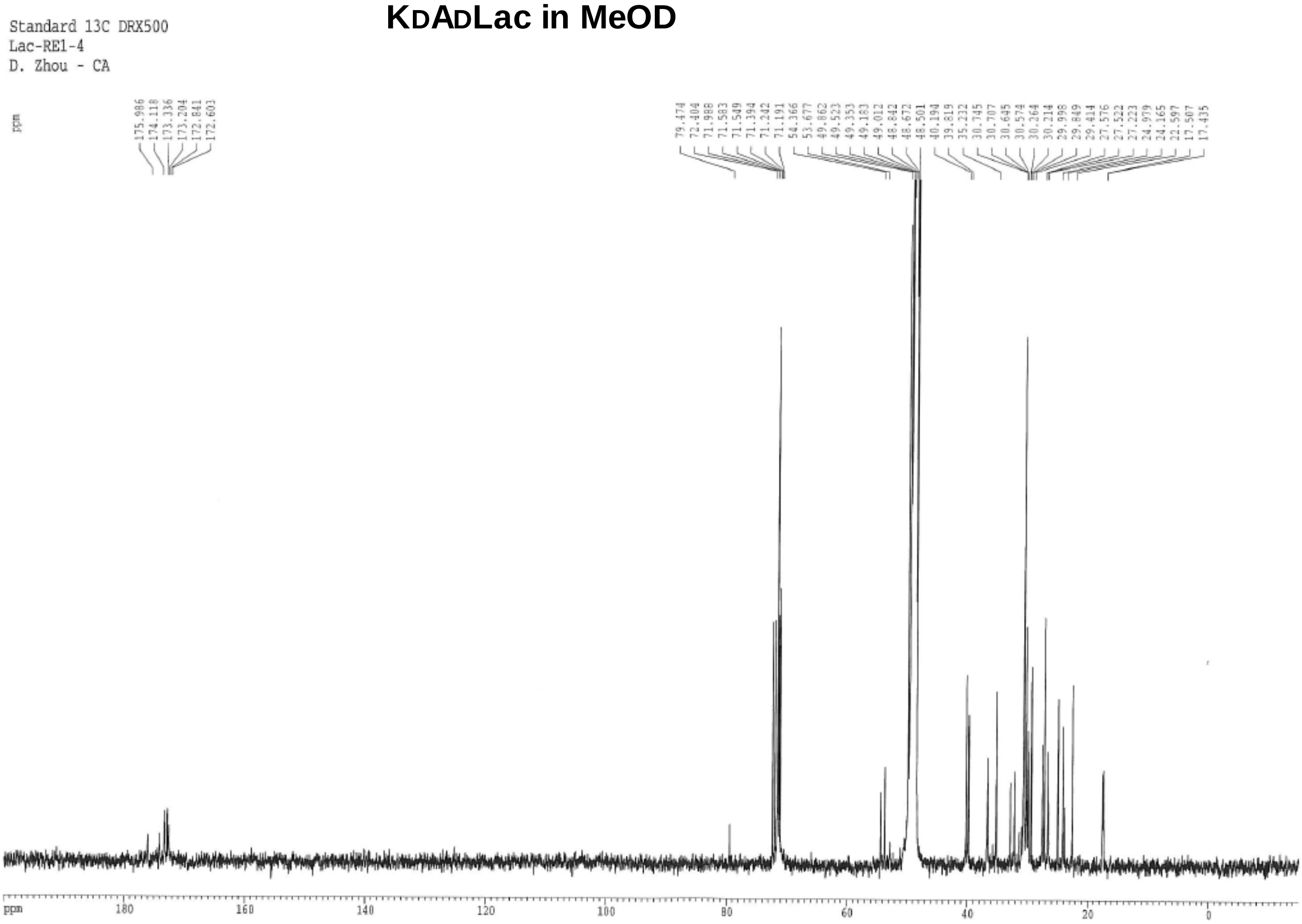
1H-NMR (500 MHz)
KDADLac in MeOD

Standard 1H DRX500
Lac-RE1-4
D. Zhou - CA



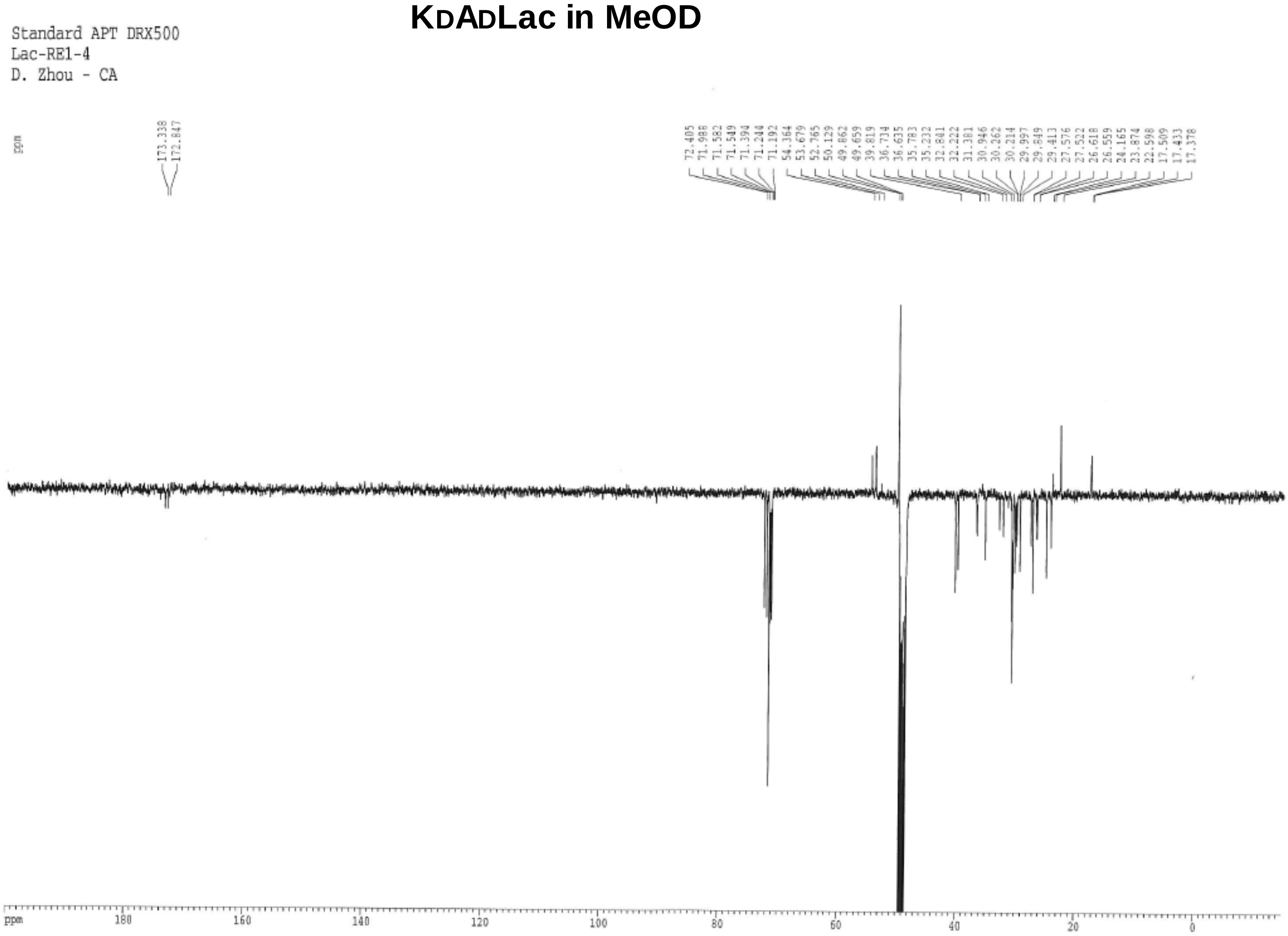
^{13}C-NMR (125 MHz)

KDADLac in MeOD

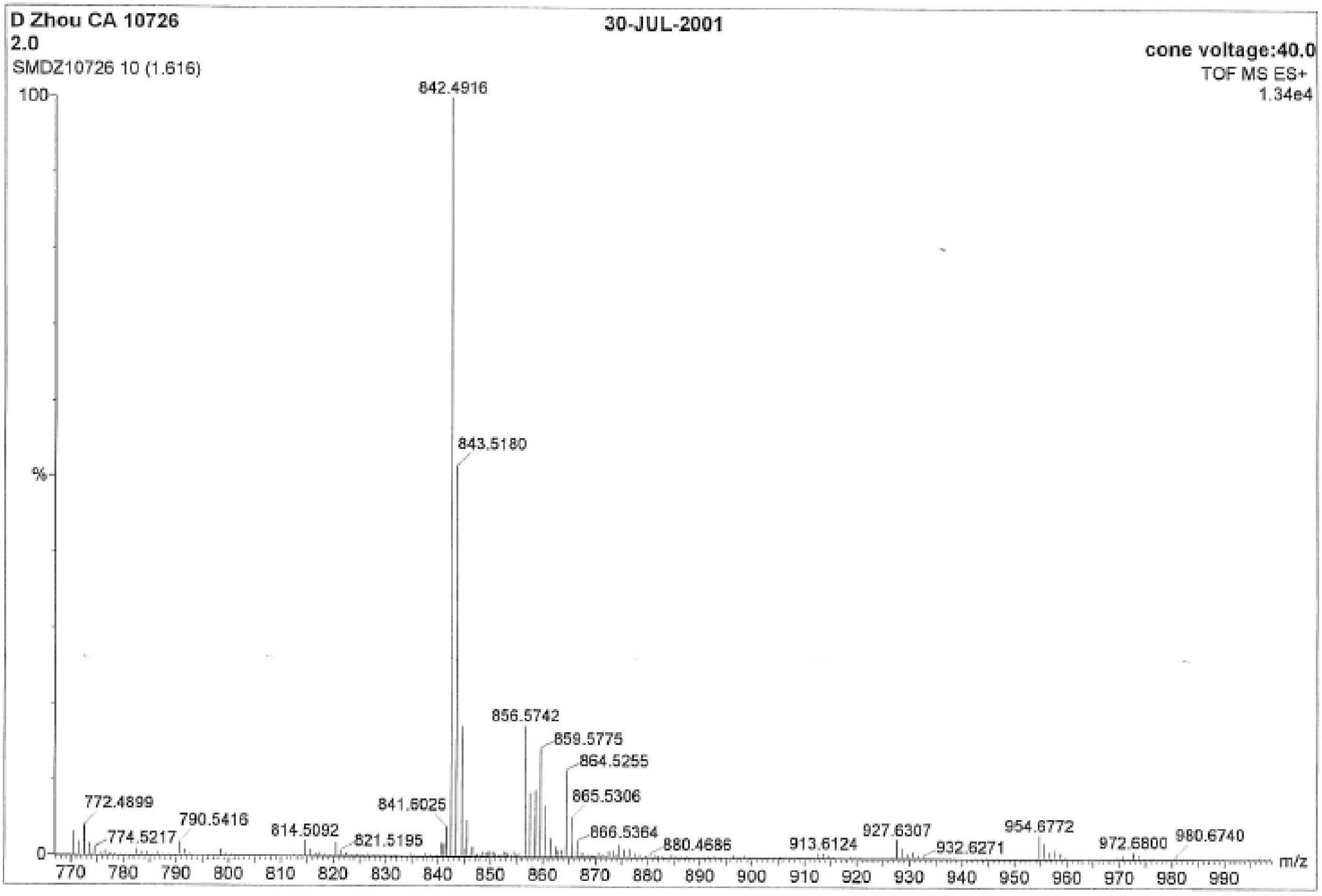


^{13}C-NMR APT

KDADLac in MeOD



High resolution mass spectroscopy
KDALA



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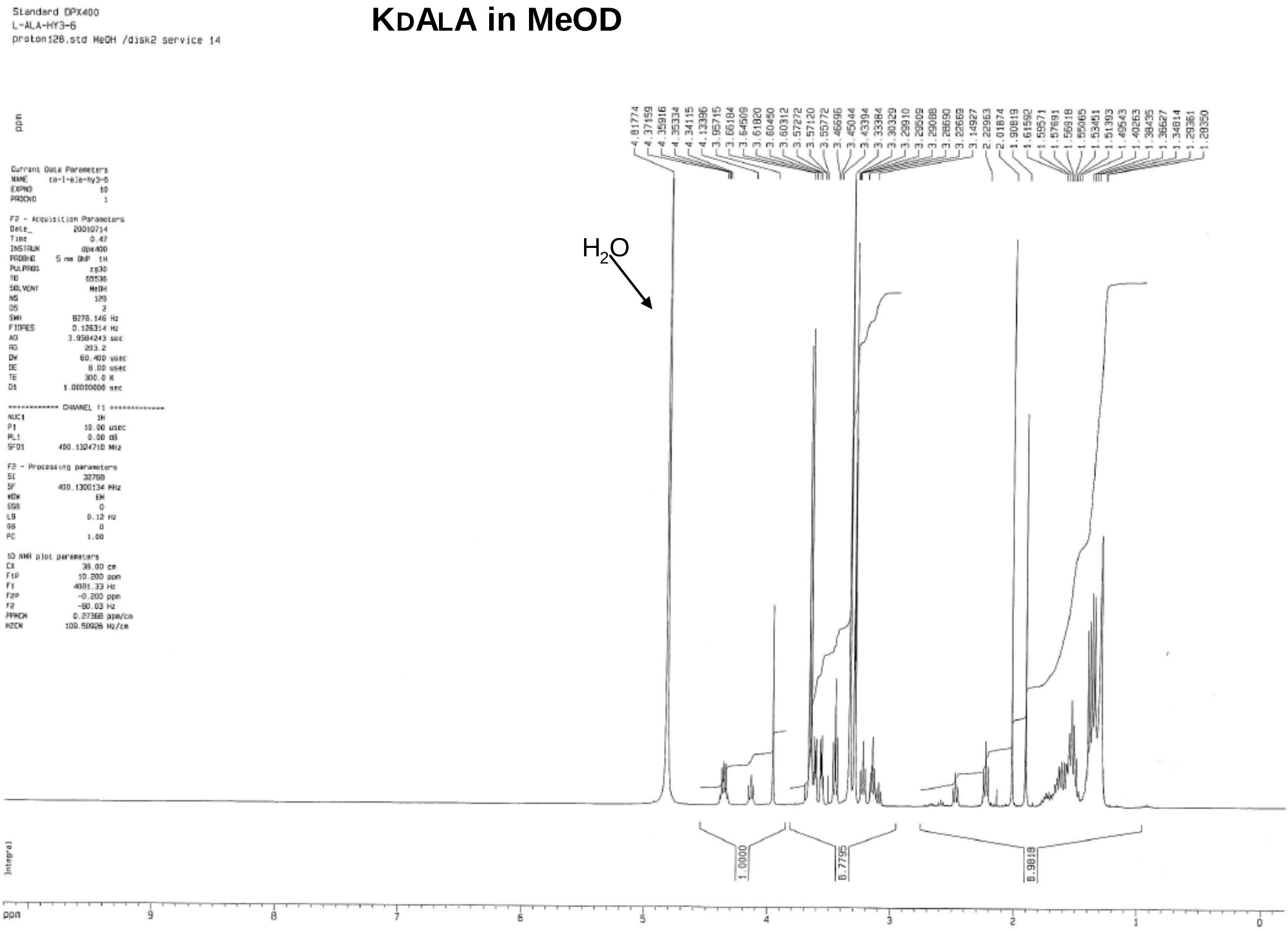
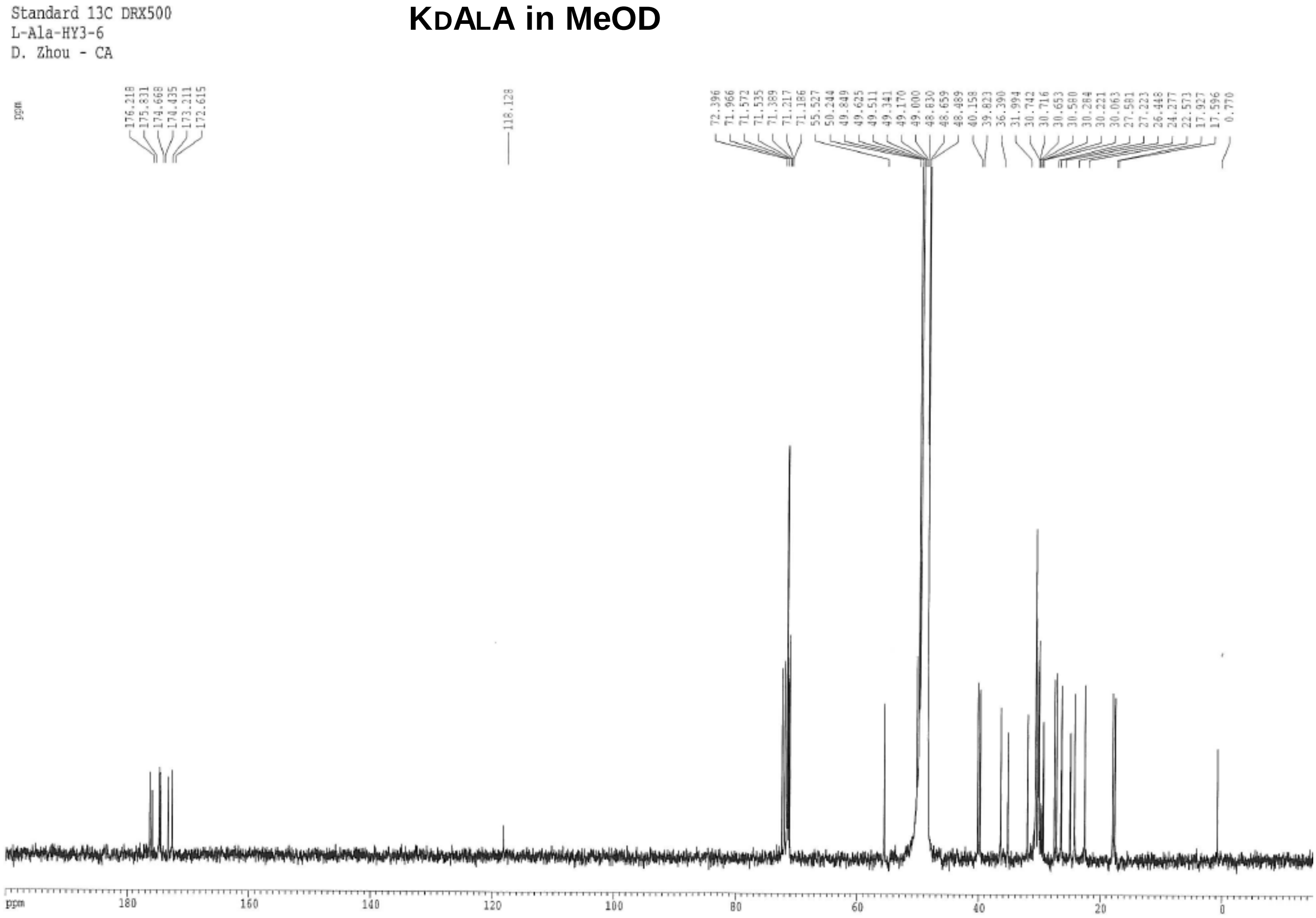
1H-NMR (500 MHz)

KDALA in MeOD



^{13}C-NMR (125 MHz)

KDALA in MeOD



Standard APT DRX500
L-Ala-HY3-6
D. Zhou - CA

^{13}C-NMR APT
KDALA in MeOD