Supporting Information

One-Step Heck Reaction Generates Non-Immunosuppressive FK506 Analogs for Pharmacological BMP Activation

Yuefan Wang[1,2], Brandon Peiffer[1,2], Qi Su[3], Jun O Liu[1,2]

[1]Department of Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA.
[2]The SJ Yan and HJ Mao Laboratory of Chemical Biology, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA.
[3]Department of Chemistry, Johns Hopkins University, 3400N. Charles St., Baltimore, MD 21218, USA
Email: joliu@jhu.edu

Table of Contents

EXPERIMENTAL METHODS AND EQUIPMENT

Normal phase disposable flash columns RediSep®Rf for flash chromatography were purchased from Teledyne Isco, Inc. Solvent was used extra dry over molecular sieve, stabilized, AcroSeal®. Yields refer to chromatographically homogeneous materials. Reactions were monitored by mass spectrometry provided by Agilent 6120 Quadrupole LC/MS. PLC Silica gel 60 F_{254}, 1mm supplied by EMD Millipore using UV light as visualizing agent.

^{1}H-NMR was recorded on Bruker Avance III 500 MHz NMR spectrometer. TMS was used as internal standard for ^{1}H-NMR (0 ppm). High-resolution mass spectra (HRMS) were recorded on Waters Synapt G2-Si mass spectrometer using ESI (electrospray ionization). FK-506 (Tacrolimus) was purchased from Biotang Inc. Zhan-1b catalyst, palladium acetate and tris(o-tolyl)phosphine were purchased from Aldrich.

Cell Culture and Transfections

Jurkat (E6.1, ATCC) cells were cultured in RPMI (buffered to pH=7.5) with 10% FBS and 1.5% PennStrep. Jurkat cells (1 x 106) were transfected with 10 µg of BRE-Luciferase (kindly provided by Martine Roussel & Peter ten Dijke) or NFAT-Luciferase cDNA (Promega) by electroporation (BioRad, square-wave, 250V, 950µF) in 400 µL serum/antibiotic free RPMI with 0.5% DMSO. Thirty minutes after transfection, cells were transferred to complete RPMI and rested overnight. Before plating, cells were re-suspended in fresh media and diluted to 0.5x106 cells/mL.
HUVEC cells were cultured in Lonza Endothelial cell Growth Medium (EGM-2) and used between passages 3 and 7.

All cells were grown and assayed at pH=7.5 (extracellular)*, 37°C, with 5% added CO_2.

*In the pH range between 6.8 and 7.4, lymphocytes maintain a constant internal pH of 7.17 +/- 0.06 pH unit.

Cell Viability Assays

HUVEC cells were plated at 1000 cells/well in 180µL growth media before addition of 20µL of 10X drug/protein stock. After 72-hour treatment, 22uL of a resazurin sodium salt solution (0.1mg/mL stock in water) was added to each well and allowed to incubate at 37°C. The metabolic conversion of resazurin dye was monitored by absorbance at 570nm after 6 hours. After background subtraction (media only + dye), absorbance values were left as arbitrary absorbance units or normalized to those obtained by DMSO.

BMP and NFAT Pathway Reporters

Jurkat cells used for each experiment were transfected at the same time and cultured together overnight until plating and treatment the following day.

Jurkat T cells transfected with BRE-Luc were split into a 96-well plate (80uL/well of 0.5x106 cells/mL)) and treated with previously stated compounds (20µL of 5X stock in RPMI, 0.5% DMSO) for 18 hours. Cells were lysed and measured for luminescence as previously reported (Peiffer et al., 2018). Luminescence values were background subtracted (lysis buffer + substrate) and normalized to DMSO control values.

Jurkat T cells transfected with NFAT-Luc were split into a 96-well plate (80uL/well of 0.5x106 cells/mL) and treated with indicated compounds (20µL of 5X stock in RPMI, 0.5% DMSO) 30 min before activation with PMA/Ionomycin (40nM/1µM). After 6 hours, wells were lysed and measured for luminescence as previously stated (B. Peiffer et al., 2018). FK506 and served as positive control while DMSO and non-activated wells gave negative and background control values, respectively.

Western Blot

Jurkat cells were plated at $1.5x10^6$ cells/well in a 12 well plate before addition of compounds or vehicle control (DMSO). After 2 hours, cells were collected, centrifuged (300g, 5min), and washed with PBS before lysis with 75µL RIPA buffer (including protease/phosphatase inhibitor cocktail, Cell Signaling Technologies). Protein levels were normalized with DC protein assay (BioRad), and boiled with 4X laemmli buffer. 25ug of each cell lysate was run on a 10% PAGE gel before transfer to a nitrocellulose (0.2µm) membrane. Blots were blocked with milk and stained overnight at 4°C with primary antibodies for phosphor-SMAD1/5 (1:500, Cell Signaling Technologies) and HSP90 (Loading control, 1:1000, Santa Cruz). After washing, secondary antibodies (HRP conjugate, 1:7500, Cell Signaling Technologies) were incubated for 2 hours before washing and addition of chemi-luminescent substrate. Developed blots were imaged on a SynGene gel imager.

EC_{50} Calculations

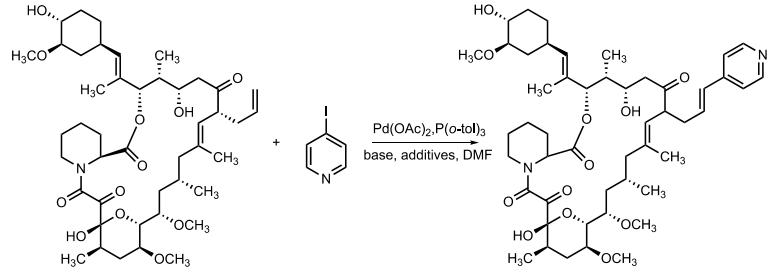
Calculations were performed using GraphPad Prism 6. Curves were fit using non-linear, log(agonist) vs. response (three parameters). 95% confidence intervals of EC50 values are reported below.

EXPERIMENTAL DATA

Optimization of Heck reaction conditions:

To a mixture of FK-506(0.0500 mmol, 40 mg, 1.0 equiv), $Pd(OAc)_2$(0.00500 mmol, 1.1 mg, 0.10 equiv), P(*o*-tol)$_3$(0.0100 mmol, 3.0 mg, 0.20 equiv), base and additives in flame-dried 10 mL-Schlenk tube, dry DMF(1.0 mL) was added under Ar balloon protection, and the mixture was stirred at specific temperature. The reactions were monitored by mass spectrometry.

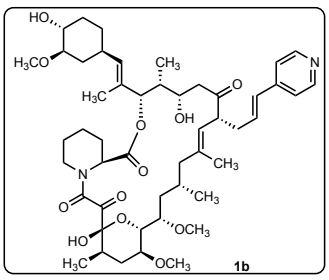
Table S1. Survey of best reaction condition for Heck reaction



Entry	base	additives	temperature	time / h	Yield (%)[b]
1	$NaHCO_3$	$^nBu_4NBr$	100 °C	24	N. D.
2	$NaHCO_3$	none	100 °C	24	decomposed
3	K_2CO_3	$^nBu_4NBr$, H_2O	50 °C	2	decomposed
4	$NaHCO_3$	$^nBu_4NCl$	100 °C	24	decomposed
5	2,6-lutidine	none	100 °C	24	low conversion
6	Et_3N	none	100 °C	24	66

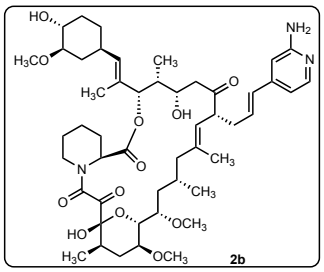
General procedure for the Heck reaction

To a mixture of FK-506 (0.0500 mmol, 40 mg, 1.0 equiv), aryl halides (2.0 equiv) and $Pd(OAc)_2$(0.00500 mmol, 1.1 mg, 0.10 equiv) and P(*o*-tol)$_3$(0.0100 mmol, 3.0 mg, 0.20 equiv) in flame-dried 10 mL-Schlenk tube, dry DMF(1.0 mL) and Et_3N(0.10 mL) dried over K_2CO_3 was added under Ar balloon protection, and the mixture was stirred at 100 °C. The reactions were monitored by mass spectrometry. When the reaction was finished, the reaction mixture cooled to room temperature, and was purified by flash column with gradient solvent (dichloromethane and methanol) to give the corresponding product. If necessary, PLC was used as further purification to separate epimers.

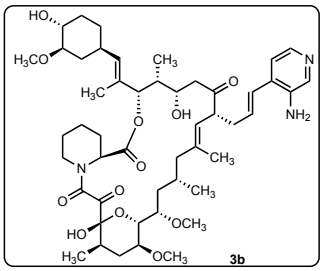


1b: ^{1}H-NMR (500MHz, $CDCl_3$) 8.51 (br s, 2H), 7.18 (br s, 2H), 6.40-6.32

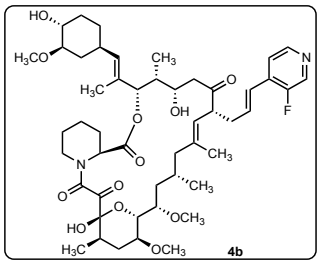
(m, 2H), 5.33 and 5.18 (rotamers, d, *J* = 1.05, 1H), 5.11-5.05 (m, 2H), 4.99 and 4.65 (rotamers, d, *J* = 4.55, 1H), 4.71 and 4.24 (rotamers, s, 1H), 4.43 and 3.72 (rotamers, d, *J* = 12.5, 1H), 3.94-3.84 (m, 1H), 3.72-3.61 (m, 2H), 3.44-3.35 (m, 9H), 3.34-3.29 (m, 3H), 3.06-2.96 (m,2H), 2.84-2.61 (m,3H), 2.33-2.25 (m,2H), 2.21-1.96 (m, 7H), 1.95-1.85 (m, 2H), 1.72-1.44 (m, 18H), 1.03-0.81 (m, 11H); HRMS (ESI): m/z calcd for $C_{49}H_{73}N_2O_{12}$ $[M+H]^+$: 881.5164, found 881.5164.



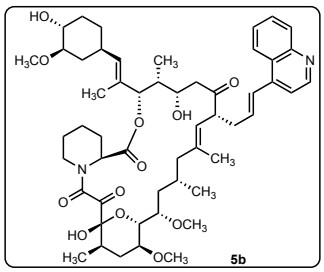
2b: ^{1}H-NMR (500MHz, $CDCl_3$) 7.93 (br s, 1H), 6.60 (br s, 1H) , 6.38 (br s, 1H), 6.32-6.18 (m, 2H), 5.33 and 5.18 (rotamers, d, *J* = 1.05, 1H), 5.12-5.02 (m, 2H), 4.94 and 4.64 (rotamers, d, *J* = 4.50, 1H), 4.69(br s, 2H), 4.43 and 3.72 (rotamers, d, *J* = 14.6, 1H), 3.95-3.84 (m, 1H), 3.74-3.66 (m, 1H), 3.60-3.53 (m, 1H), 3.46-3.23 (m, 12H), 3.04-2.99 (m,2H), 2.82-2.62 (m,2H), 2.40-2.24 (m,4H), 2.17-1.99 (m, 8H), 1.85-1.71 (m, 3H), 1.71-1.31 (m, 16H), 1.03-0.82 (m, 11H); HRMS (ESI): m/z calcd for $C_{49}H_{74}N_3O_{12}$ $[M+H]^+$: 896.5272, found 896.5261.



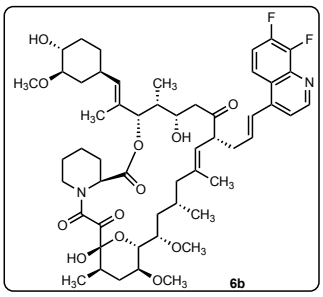
3b: ^{1}H-NMR (500MHz, $CDCl_3$) 8.04 (s, 1H), 7.95 (s, 1H) , 7.05-6.98 (m, 1H), 6.40-6.35 (m, 1H), 6.20-6.10 (m, 1H), 5.34 and 5.20 (rotamers, s, 1H), 5.12-5.04 (m, 2H), 4.94 and 4.64 (rotamers, d, *J* = 4.40, 1H), 4.43 and 3.72 (rotamers, d, *J* = 13.4, 1H), 4.00-3.90 (m, 1H), 3.72 (br s, 2H), 3.61-3.47 (m, 2H), 3.44-3.35 (m, 9H), 3.33-3.28 (m, 3H), 3.04-2.98 (m,2H), 2.83-2.62 (m,3H), 2.42-2.07 (m,8H), 1.98-1.86 (m, 3H), 1.75-1.43 (m, 19H), 0.99-0.82 (m, 11H); HRMS (ESI): m/z calcd for $C_{49}H_{74}N_3O_{12}$ $[M+H]^+$: 896.5272, found 896.5262.



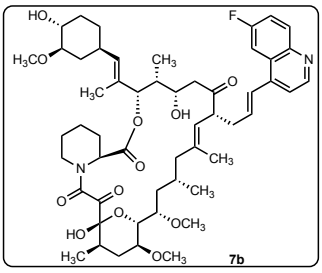
4b: ^{1}H-NMR (500MHz, $CDCl_3$) 8.40 (s, 1H), 8.31 (d, *J* = 4.6, 1H), 6.52-6.41 (m, 2H), 5.33 and 5.18 (rotamers, s, 1H), 5.12-5.02 (m, 2H), 4.99 and 4.65 (rotamers, d, *J* = 4.40, 1H), 4.72 and 4.24 (rotamers, s, 1H), 4.43 and 3.70 (rotamers, d, *J* = 14.6, 1H), 3.94-3.85 (m, 1H), 3.67-3.52 (m, 2H), 3.43-3.35 (m, 9H), 3.34-3.28 (m, 3H), 3.05-2.98 (m,2H), 2.76-2.62 (m,3H), 2.49-2.25 (m,4H), 2.21-1.96 (m, 7H), 1.94-1.69 (m, 5H), 1.68-1.61 (m, 7H), 1.51-1.36 (m, 7H), 1.04-0.85 (m, 11H); HRMS (ESI): m/z calcd for $C_{49}H_{72}N_2O_{12}F$ $[M+H]^+$: 899.5069, found 899.5060.



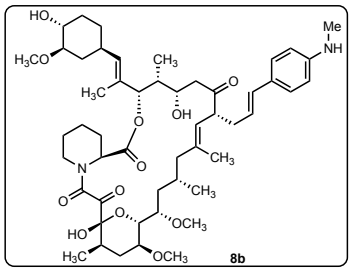
5b: ^{1}H-NMR (500MHz, $CDCl_3$) 8.82 (d, *J* = 4.55, 1H), 8.10-8.05 (m, 2H), 7.70 (t, *J* = 7.42, 1H), 7.55 (t, *J* = 7.52, 1H), 7.36 (dd, *J* = 9.2, 4.55, 1H), 7.10 (d, *J* = 15.6, 1H), 6.36 (dt, *J* = 15.4, 7.53, 1H), 5.34 and 5.20 (rotamers, s, 1H), 5.15-5.00 (m, 2H), 4.95 and 4.65 (rotamers, d, *J* = 4.85, 1H), 4.74 and 4.27 (rotamers, s, 1H), 4.43 and 3.72 (rotamers, d, *J* = 13.5, 1H), 4.14-3.79 (m, 2H), 3.73-3.65 (m, 1H), 3.60-3.51(m, 2H), 3.44-3.35 (m, 9H), 3.34-3.29 (m, 3H), 3.04-2.98 (m,2H), 2.88-2.73 (m,2H), 2.57-2.44 (m,1H), 2.36-2.07 (m,6H), 2.05-1.93 (m, 4H), 1.87-1.66 (m, 9H), 1.61-1.33 (m, 8H), 1.03-0.78 (m, 11H); HRMS (ESI): m/z calcd for $C_{53}H_{75}N_2O_{12}$ $[M+H]^+$: 931.5320, found 931.5322.



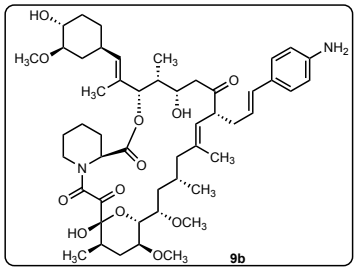
6b: ^{1}H-NMR (500MHz, $CDCl_3$) 8.89-8.86 (m, 1H), 7.85-7.81 (m, 1H), 7.53-7.36 (m, 2H), 7.06-6.99 (m, 1H), 6.45-6.32 (m, 1H), 5.78-5.67 and 5.40-5.34 (rotamers, m, 1H), 5.19-4.98 (m, 2H), 4.94 and 4.65 (rotamers, d, *J* = 5.00, 1H), 4.43 and 3.72 (rotamers, d, *J* = 13.9, 1H), 4.15-3.83 (m, 2H), 3.75-3.50(m, 3H), 3.44-3.35 (m, 9H), 3.34-3.30 (m, 3H), 3.08-2.95 (m,2H), 2.86-2.67 (m,2H), 2.58-2.43 (m,1H), 2.36-1.98 (m,8H), 1.89-1.40 (m, 20H), 1.06-0.79 (m, 11H); HRMS (ESI): m/z calcd for $C_{53}H_{73}N_2O_{12}F_2$ $[M+H]^+$: 967.5132, found 967.5135.



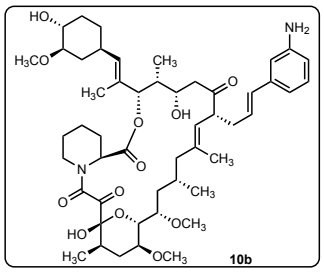
7b: ^{1}H-NMR (500MHz, $CDCl_3$) 8.79 (s, 1H), 8.14-8.06 (m, 1H), 7.66 (d, *J* = 9.50, 1H), 7.52-7.35 (m, 2H), 6.97 (d, *J* = 14.4, 1H), 6.41-6.31 (m, 1H), 5.34 and 5.20 (rotamers, s, 1H), 5.15-4.98 (m, 2H), 4.95 and 4.66 (rotamers, d, *J* = 4.85, 1H), 4.72 and 4.27 (rotamers, s, 1H), 4.43 and 3.70 (rotamers, d, *J* = 13.2, 1H), 4.14-3.85 (m, 2H), 3.74-3.50 (m, 3H), 3.47-3.27 (m, 12H), 3.15-3.10 (m,2H), 2.87-2.64 (m,3H), 2.59-2.43 (m,1H), 2.38-2.19 (m,3H), 2.04-1.73 (m, 6H), 1.70-1.46 (m, 17H), 1.10-0.85 (m, 11H); HRMS (ESI): m/z calcd for $C_{53}H_{74}N_2O_{12}F$ $[M+H]^+$: 949.5226, found 949.5234.



8b: ^{1}H-NMR (500MHz, $CDCl_3$) 7.17-7.13 (m, 2H), 6.54 (d, *J* = 8.30, 2H), 6.29 (d, *J* = 15.0, 1H), 5.90-5.82 (m, 1H), 5.32 and 5.20 (rotamers, s, 1H), 5.14-5.03 (m, 2H), 5.01 and 4.62 (rotamers, d, *J* = 4.10, 1H), 4.80 and 4.27 (rotamers, s, 1H), 4.43 and 3.73 (rotamers, d, *J* = 15.0, 1H), 3.97-3.86 (m, 2H), 3.69-3.64 (m, 1H), 3.59-3.55 (m, 1H), 3.42-3.35 (m, 10H), 3.34-3.29 (m, 3H), 3.04-2.98 (m,2H), 2.83 (s, 3H), 2.75-2.54 (m,3H), 2.35-2.24 (m,3H), 2.16-1.97 (m, 6H), 1.84-1.70 (m, 4H), 1.68-1.59 (m, 11H), 1.50-1.39 (m, 4H), 1.03-0.85 (m, 11H); HRMS (ESI): m/z calcd for $C_{51}H_{77}N_2O_{12}$ $[M+H]^+$: 909.5477, found 909.5473.

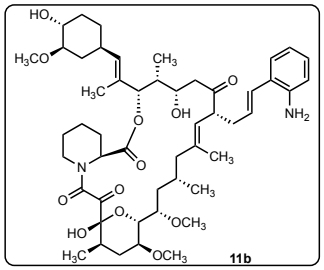


9b: ^{1}H-NMR (500MHz, $CDCl_3$) 7.13-7.09 (m, 2H), 6.61 (d, *J* = 8.15, 2H), 6.28 (d, *J* = 15.7, 1H), 5.92-5.84 (m, 1H), 5.32 and 5.19 (rotamers, s, 1H), 5.10-5.03 (m, 2H), 4.93 and 4.62 (rotamers, d, *J* = 5.05, 1H), 4.80 and 4.27 (rotamers, s, 1H), 4.43 and 3.74 (rotamers, d, *J* = 14.5, 1H), 3.97-3.89 (m, 1H), 3.69-3.64 (m, 1H), 3.61-3.52 (m, 2H), 3.42-3.37 (m, 9H), 3.32-3.28 (m, 3H), 3.03-2.98 (m,2H), 2.75-2.63 (m,2H), 2.33-2.25 (m,3H), 2.20-2.03 (m, 6H), 1.83-1.72 (m, 3H), 1.66-1.48 (m, 19H), 1.00-0.86 (m, 11H); HRMS (ESI): m/z calcd for $C_{50}H_{75}N_2O_{12}$ $[M+H]^+$: 895.5320, found 895.5320.



10b: ^{1}H-NMR (500MHz, $CDCl_3$) 7.07 (t, *J* = 7.75, 1H), 6.72-6.69 (m, 1H), 6.64 (s, 1H), 6.54 (d, *J* = 7.80, 1H), 6.30 (d, *J* = 15.7, 1H), 6.08-6.00 (m, 1H), 5.32 and 5.20 (rotamers, s, 1H), 5.12-5.04 (m, 2H), 4.94 and 4.64 (rotamers, s, 1H), 4.75 and 4.26 (rotamers, s, 1H), 4.43 and 3.69 (rotamers, d, *J* = 13.5, 1H), 3.98-3.89 (m, 1H), 3.72-3.57 (m, 3H), 3.47-

3.36 (m, 9H), 3.34-3.27 (m, 3H), 3.06-2.97 (m,2H), 2.69-2.56 (m,2H), 2.36-2.25 (m,3H), 2.19-1.97 (m, 6H), 1.82-1.71 (m, 3H), 1.69-1.47 (m, 19H), 1.03-0.85 (m, 11H); HRMS (ESI): m/z calcd for $C_{50}H_{75}N_2O_{12}$ $[M+H]^+$: 895.5320, found 895.5320.



11b: ^{1}H-NMR (500MHz, $CDCl_3$) 7.15 (t, *J* = 7.50, 1H), 7.04(t, *J* = 7.65, 1H), 6.75-6.69 (m, 1H), 6.65 (d, *J* = 7.95, 1H), 6.42 (d, *J* = 15.6, 1H), 5.99-5.90 (m, 1H), 5.33 and 5.20 (rotamers, s, 1H), 5.14-5.04 (m, 2H), 4.95 and 4.62 (rotamers, d, *J* = 4.40, 1H), 4.73 and 4.31 (rotamers, s, 1H), 4.43 and 3.72 (rotamers, d, *J* = 13.6, 1H), 3.97-3.83 (m, 2H), 3.76-3.64 (m, 2H), 3.59-3.55 (m, 1H), 3.41-3.37 (m, 9H), 3.32-3.27 (m, 3H), 3.05-2.96 (m,2H), 2.70-2.60 (m,2H), 2.37-2.25 (m,3H), 2.15-1.98 (m, 6H), 1.93-1.72 (m, 6H), 1.70-1.61 (m, 9H), 1.50-1.35 (m, 6H), 1.03-0.85 (m, 11H); HRMS (ESI): m/z calcd for $C_{50}H_{75}N_2O_{12}$ $[M+H]^+$: 895.5320, found 895.5316.

Table S2. EC_{50} values for BMP pathway reporter activation using top performing analogs.

Compound	EC_{50} (nM)
1	7.247 to 21.10

2	19.87 to 46.96
3	16.07 to 34.08
FK506	26.10 to 45.47

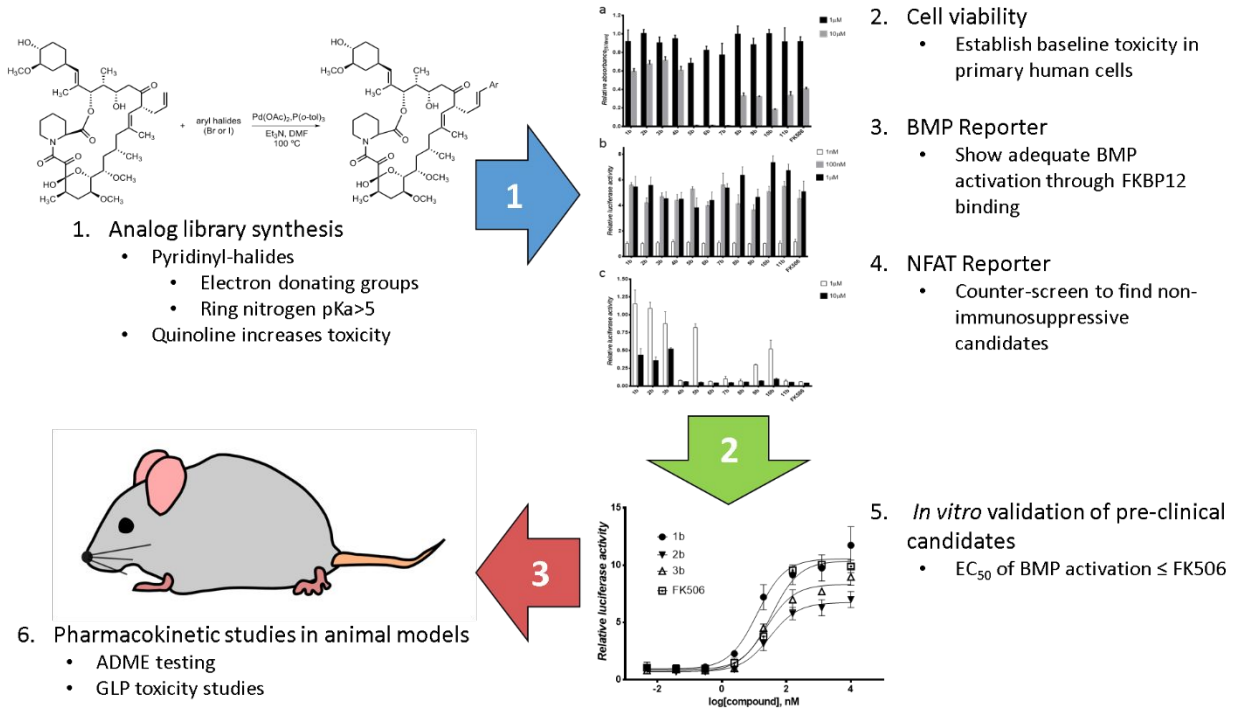
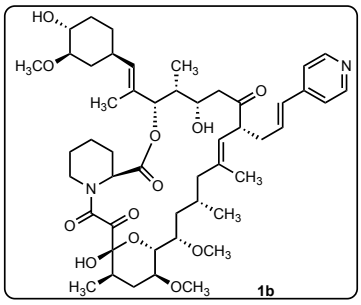
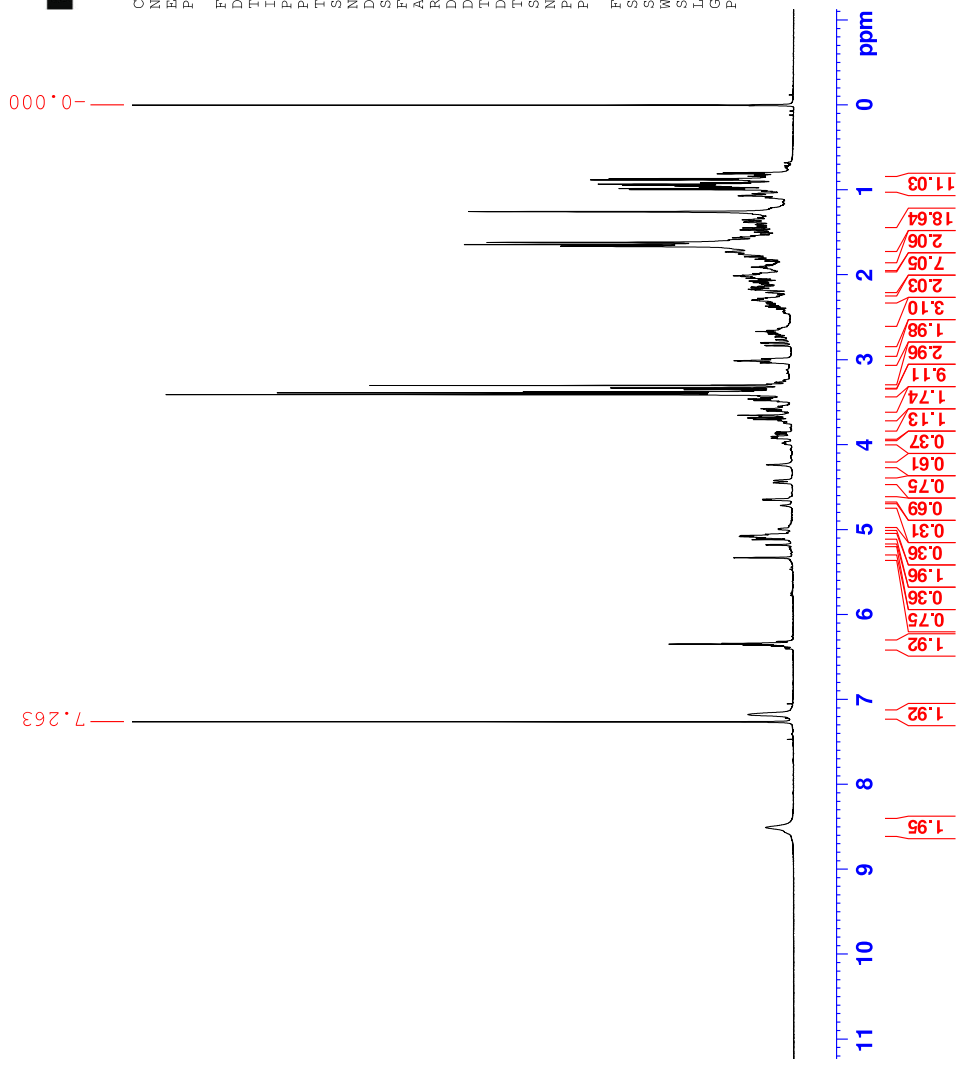


Figure S1. Proposed workflow for identification of clinic-ready FK506 analogs for pharmacological BMP activation. (**1**) A rationally designed FK506 analog library is screened using a high-throughput compatible, three-assay system. (**2**) Top performing candidates are then validated *in vitro* through EC50 analysis in the BMP-reporter assay. (**3**) Validated compounds are moved into pre-clinical animal studies to determine inter-analog differences in ADME (absorption, distribution, metabolism, excretion) and *in vivo* toxicity profiles.

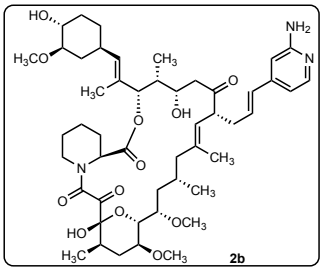
Spectrum of the synthesized compounds

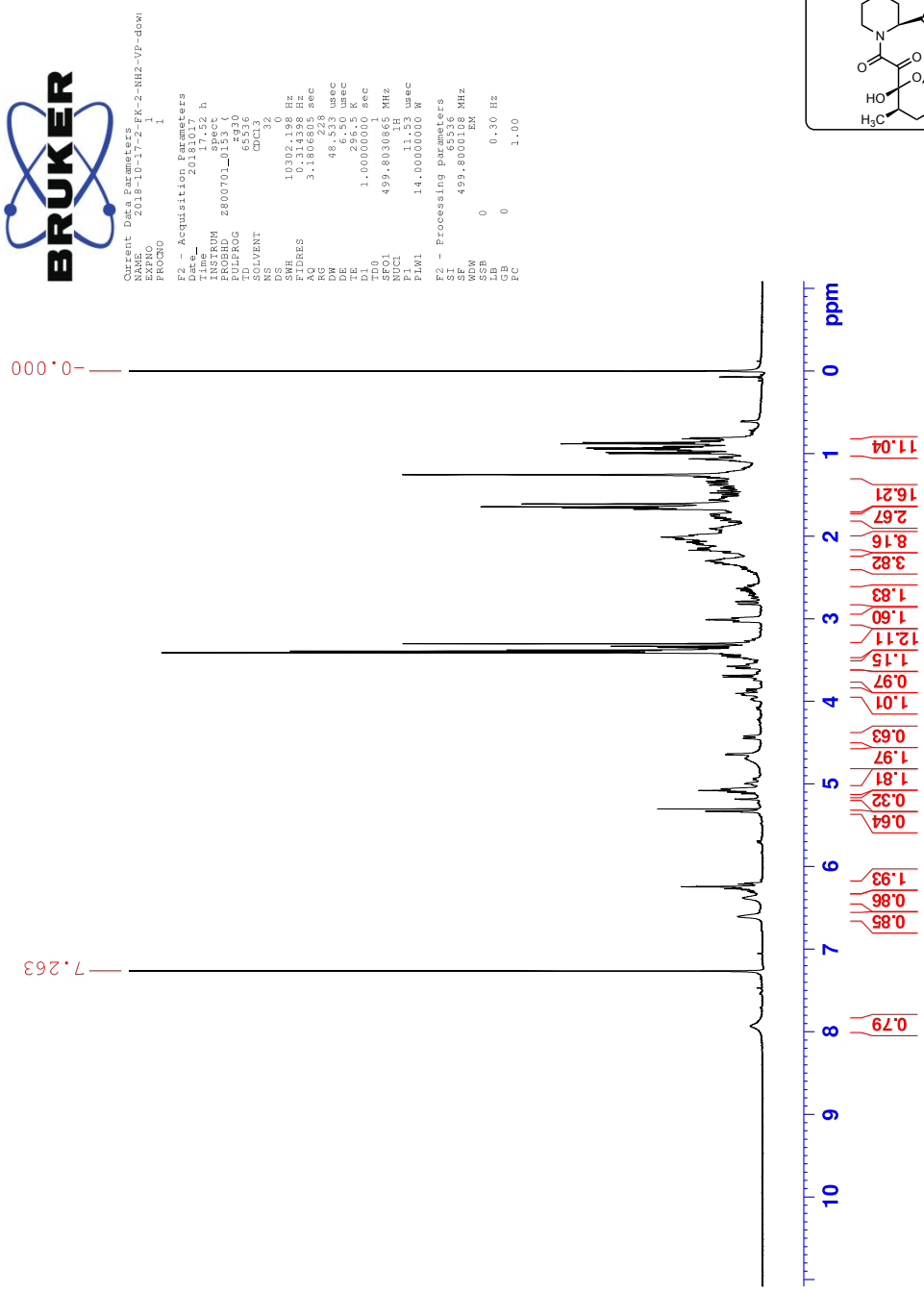
1b: ^{1}H-NMR



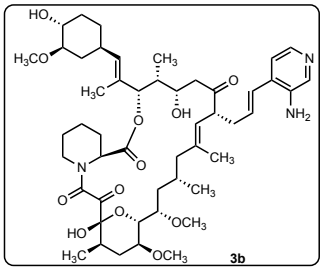


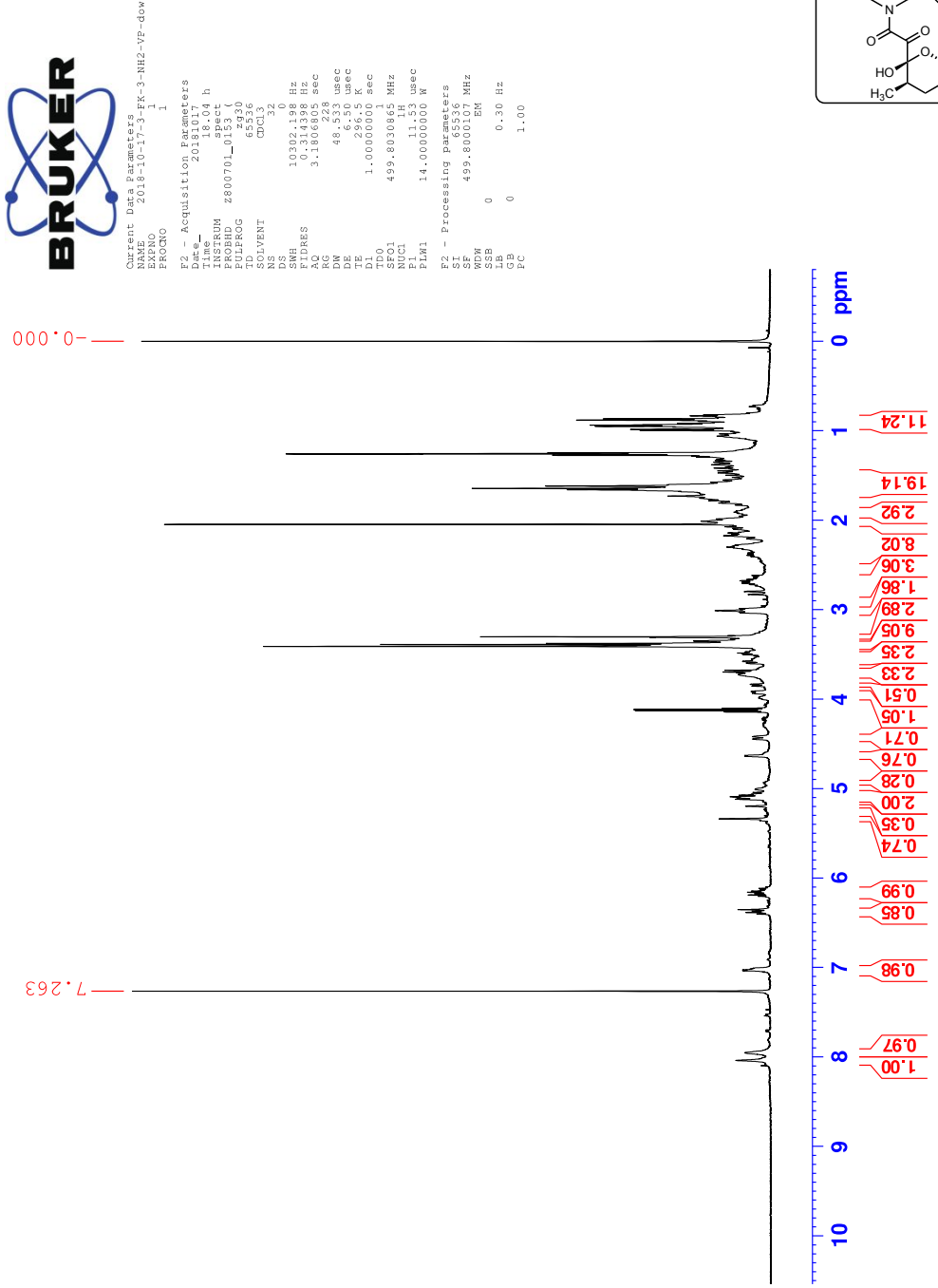
2b: ^{1}H-NMR


HO
H3CO
NH2
CH3
H3C
O
OH
N
CH3
O
N
O
CH3
O
O
HO
OCH3
H3C
OCH3
2b

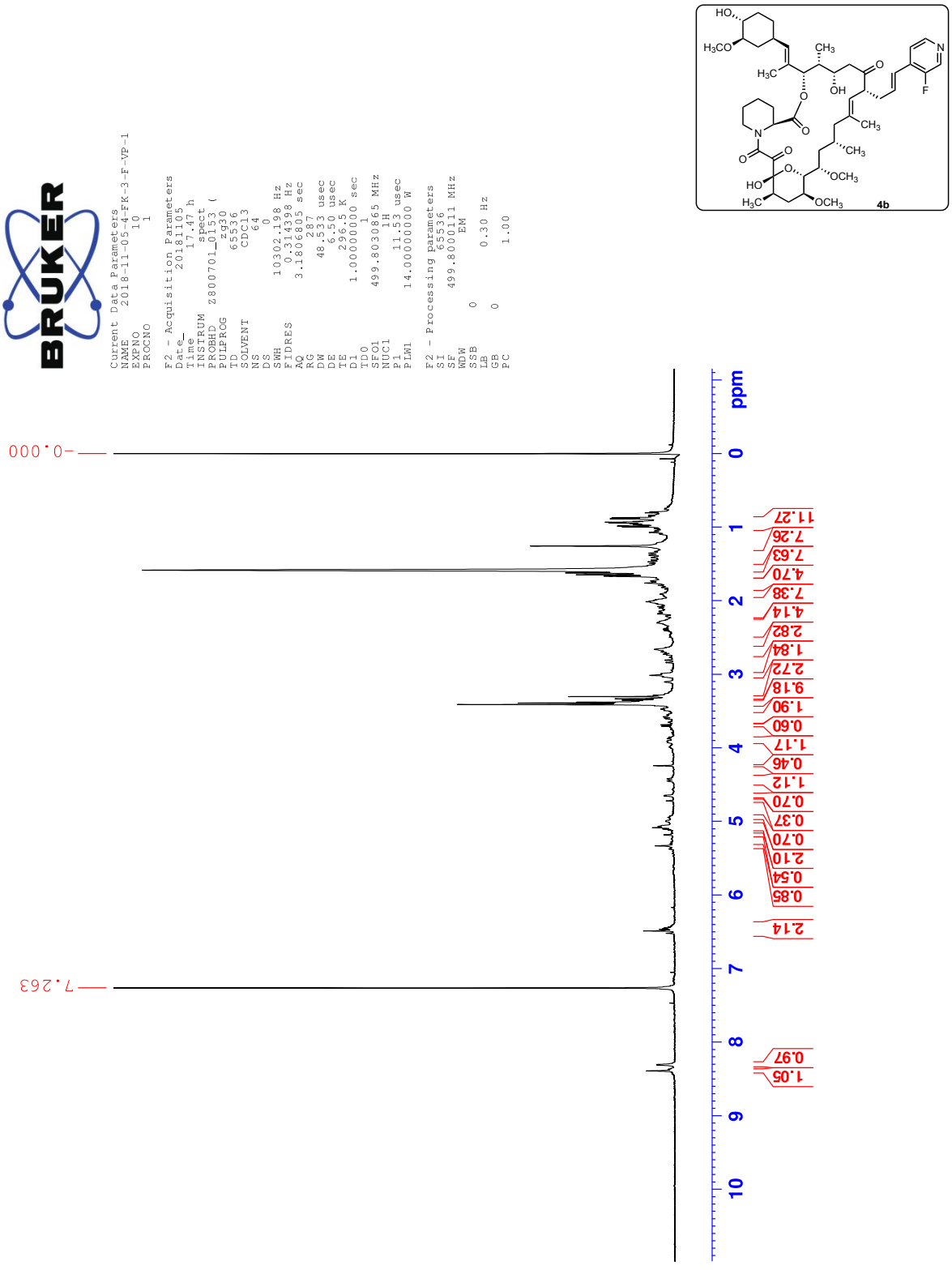

BRUKER
Current Data Parameters
NAME 2018-10-17-2-FK-2-NH2-VP-dcwi
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181017
Time 17.52 h
INSTRUM spect
PROBHD 2800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 32
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 228
DW 48.533 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000108 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
0.000
7.263
ppm
0
1
2
3
4
5
6
7
8
9
10
11.04
16.21
2.67
8.16
3.82
1.83
1.60
12.11
1.15
0.97
1.01
0.63
1.97
1.81
0.32
0.64
1.93
0.86
0.85
0.79

3b: ^{1}H-NMR

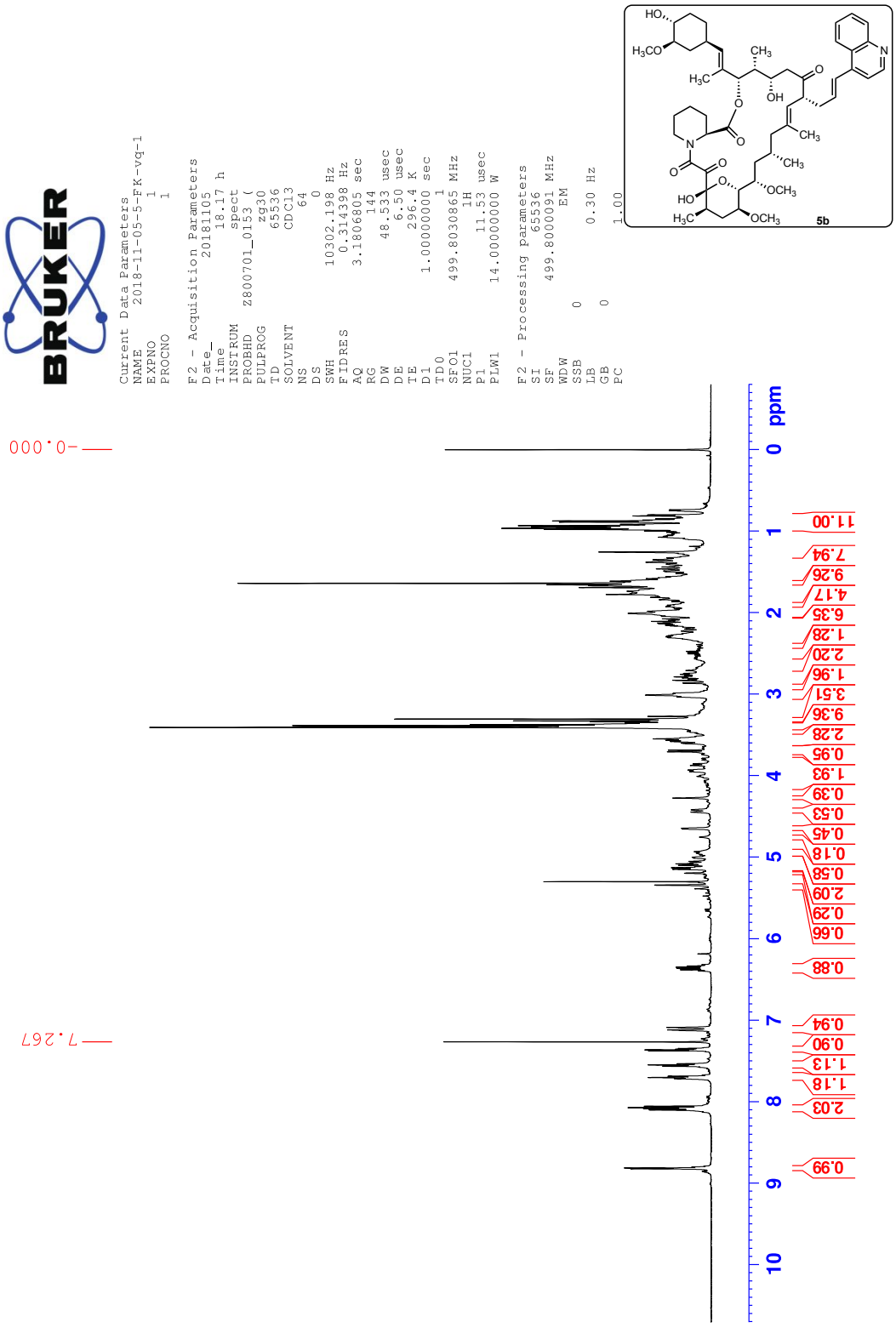




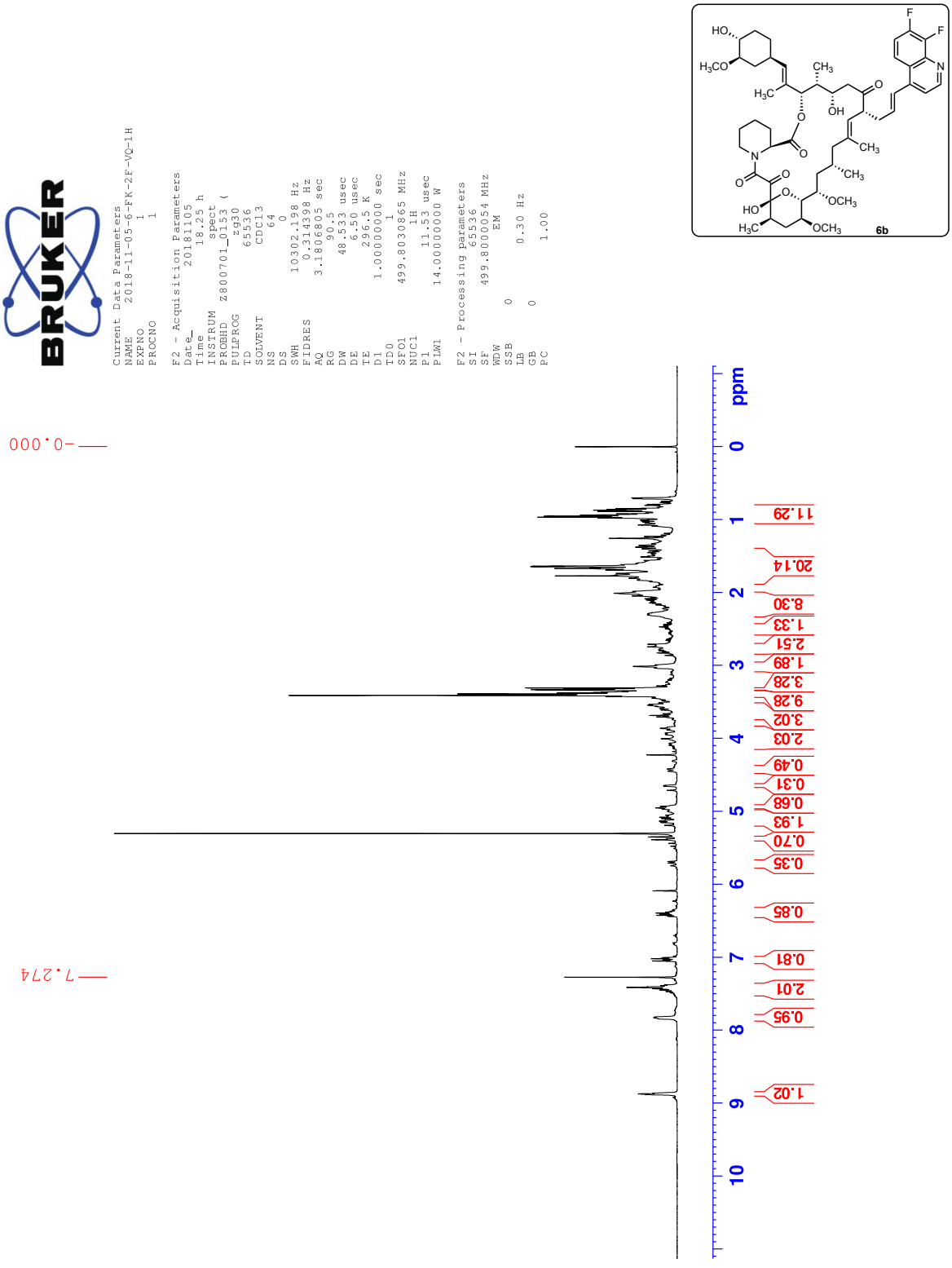
4b: ^{1}H-NMR


Current Data Parameters
NAME 2018-11-05-4-FK-3-F-VP-1
EXPNO 10
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181105
Time_ 17.47 h
INSTRUM spect
PROBHD Z800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 64
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 287
DW 48.533 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000111 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
HO
H3CO
CH3
H3C
O
OH
N
F
CH3
CH3
OCH3
OCH3
HO
H3C
4b
-0.000
7.263
ppm
11.27
7.26
7.63
4.70
7.38
4.14
2.82
1.84
2.72
9.18
1.90
0.60
1.17
0.46
1.12
0.70
0.37
0.70
2.10
0.54
0.85
2.14
1.05
0.97

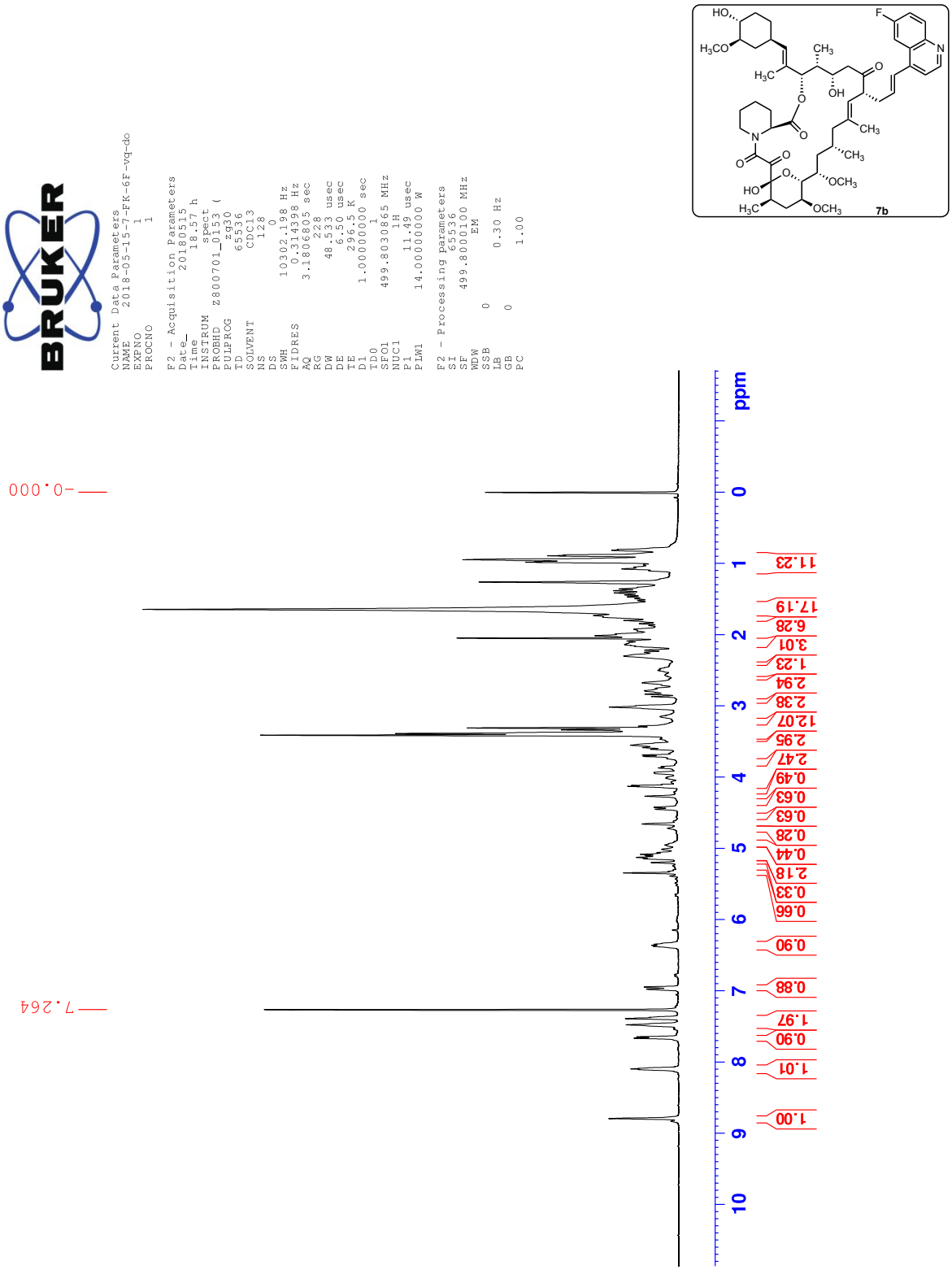
5b: ^{1}H-NMR


Current Data Parameters
NAME 2018-11-05-5-FK-vq-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181105
Time 18.17 h
INSTRUM spect
PROBHD Z800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 64
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 144
DW 48.533 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000091 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
5b
-0.000
7.267
ppm
11.00
7.94
9.26
4.17
6.35
1.28
2.20
1.96
3.51
9.36
2.28
0.95
1.93
0.39
0.53
0.45
0.18
0.58
2.09
0.29
0.66
0.88
0.94
0.90
1.13
1.18
2.03
0.99

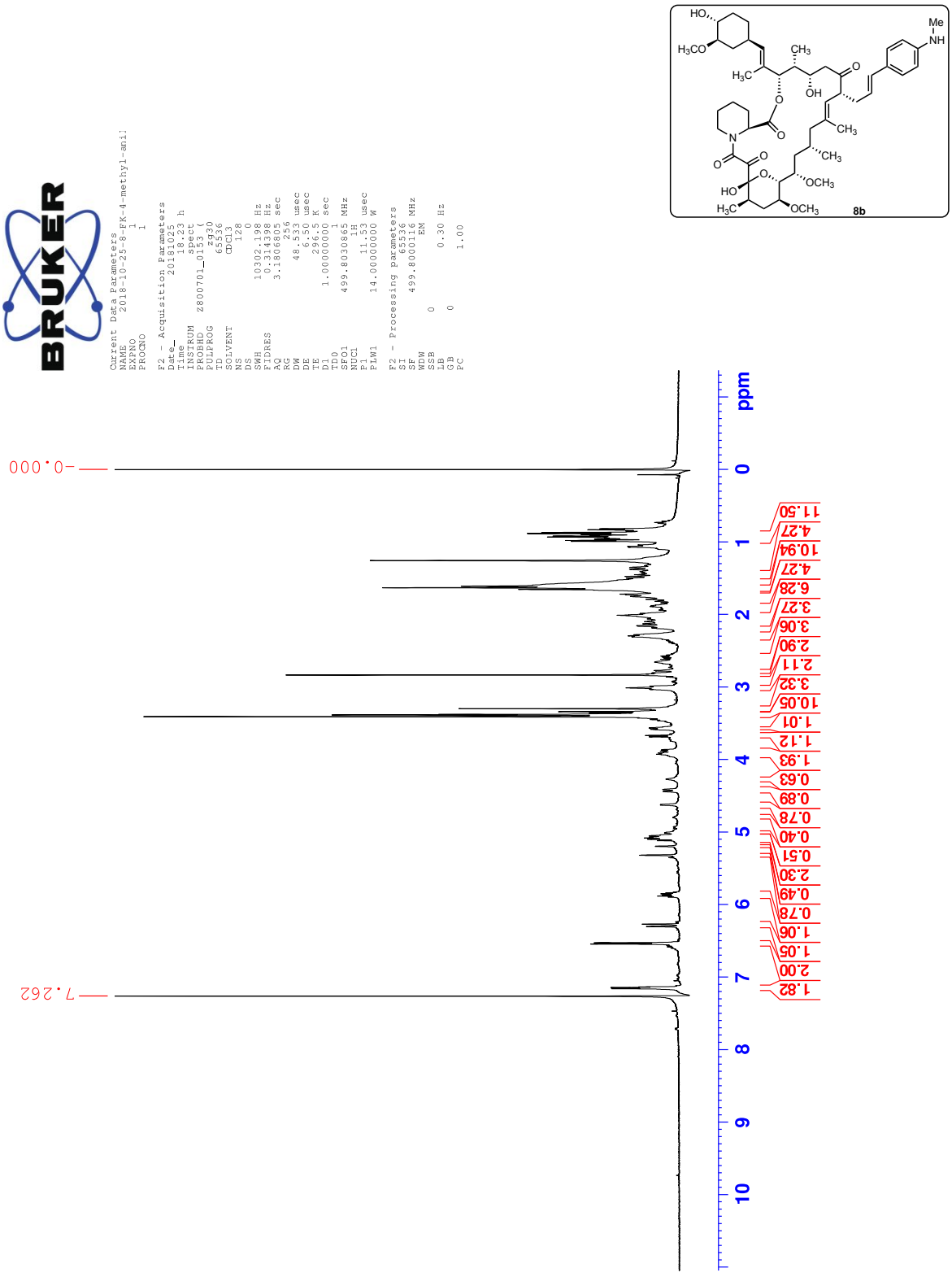
6b: ^{1}H-NMR


Current Data Parameters
NAME 2018-11-05-6-FK-2F-VQ-1H
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181105
Time 18.25 h
INSTRUM spect
PROBHD Z800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 64
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 90.5
DW 48.533 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000054 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
HO
H3CO
CH3
H3C
O
OH
N
F
F
N
OCH3
6b
ppm
11.29
20.14
8.30
1.33
2.51
1.89
3.28
9.28
3.02
2.03
0.49
0.31
0.68
1.93
0.70
0.35
0.85
0.81
2.01
0.95
1.02
7.274
0.000

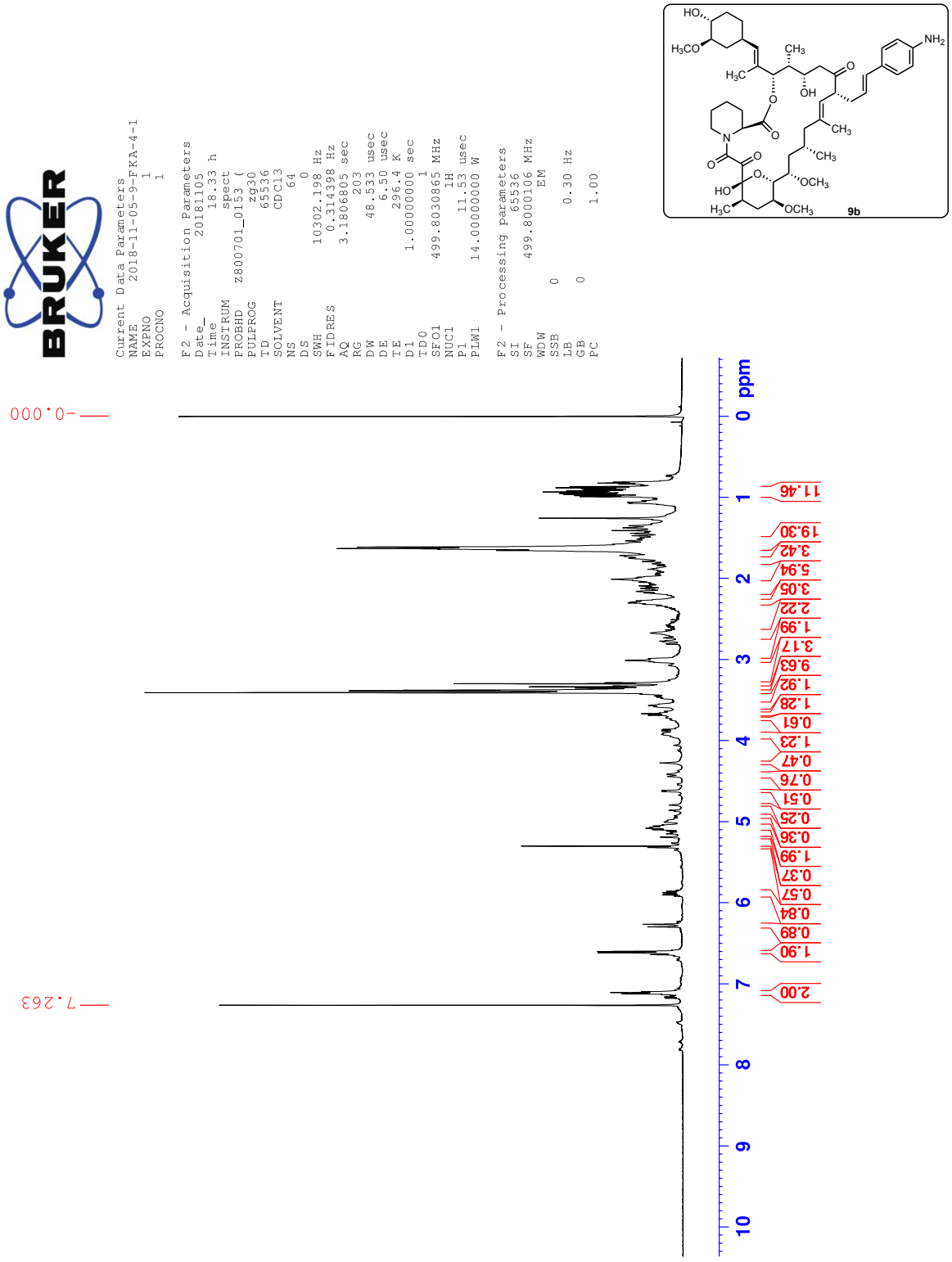
7b: ^{1}H-NMR


Current Data Parameters
NAME 2018-05-15-7-FK-6F-vq-do
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20180515
Time 18.57 h
INSTRUM spect
PROBHD z800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 228
DW 48.533 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.49 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000100 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
ppm
11.23
17.19
6.28
3.01
1.23
2.94
2.38
12.07
2.95
2.47
0.49
0.63
0.63
0.28
0.44
2.18
0.33
0.66
0.90
0.88
1.97
0.90
1.01
1.00
7.264
0.000
7b

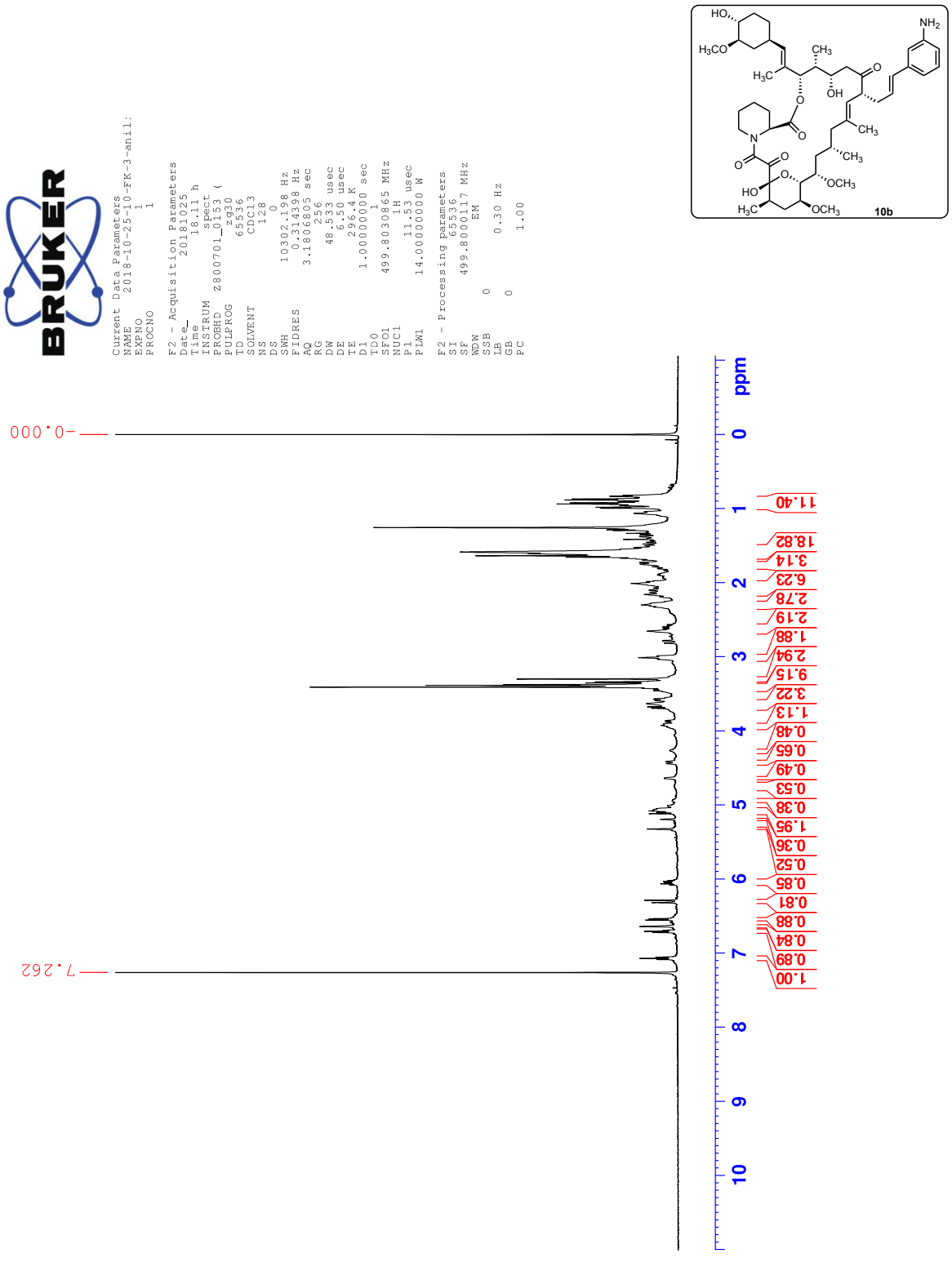
8b: ^{1}H-NMR


Current Data Parameters
NAME 2018-10-25-8-FK-4-methyl-ani
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181025
Time 18.23 h
INSTRUM spect
PROBHD 280070_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 203
DW 48.533 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000116 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
HO
H3CO
CH3
H3C
O
OH
Me
NH
N
OCH3
8b
ppm
7.262
0.000
11.50
4.27
10.94
4.27
6.28
3.27
3.06
2.90
2.11
3.32
10.05
1.01
1.12
1.93
0.63
0.89
0.78
0.40
0.51
2.30
0.49
0.78
1.06
1.05
2.00
1.82

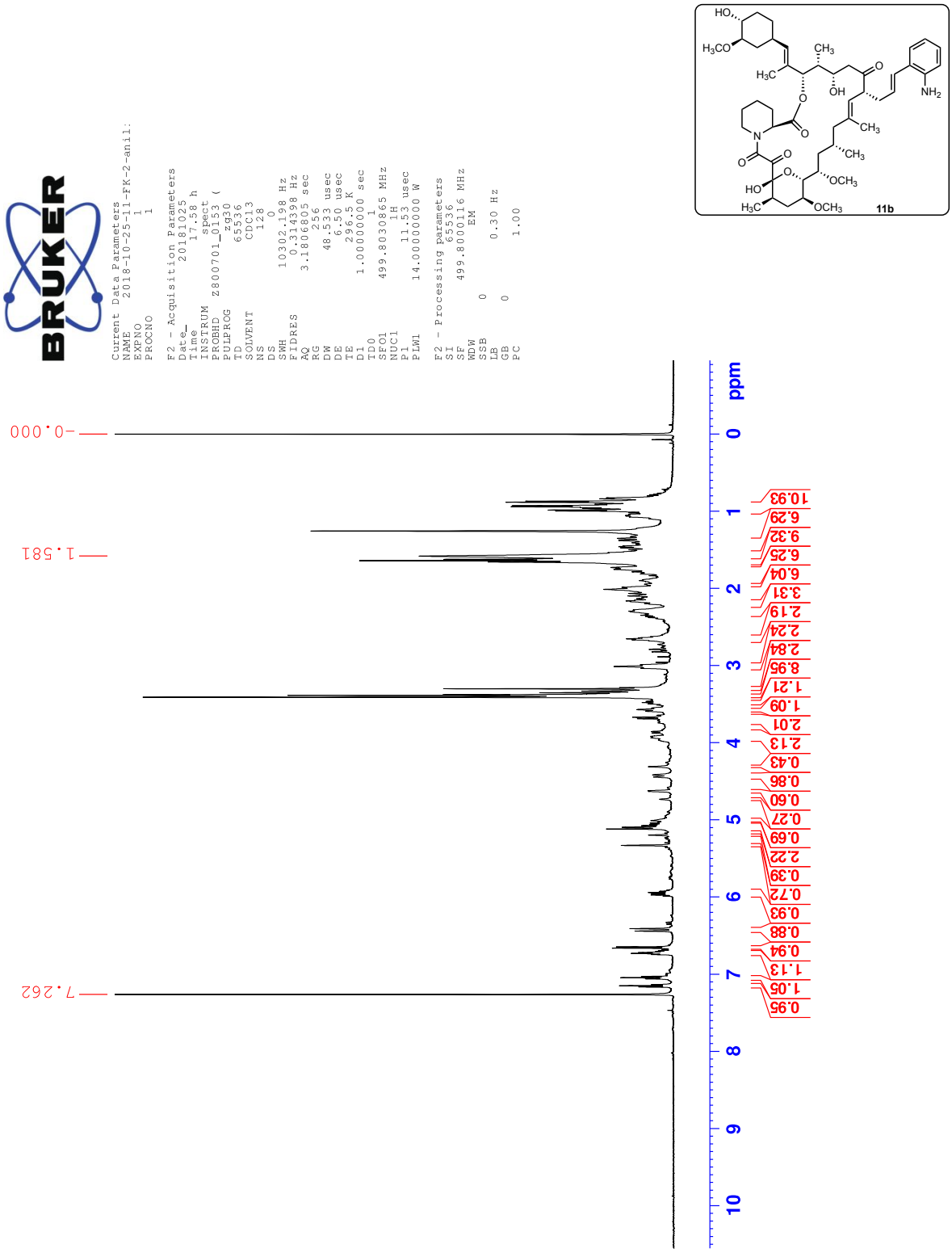
9b: ^{1}H-NMR


Current Data Parameters
NAME 2018-11-05-9-FKA-4-1
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181105
Time 18.33 h
INSTRUM spect
PROBHD Z800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 64
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 203
DW 48.533 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000106 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
9b
0.000
7.263
ppm
11.46
19.30
3.42
5.94
3.05
2.22
1.99
3.17
9.63
1.92
1.28
0.61
1.23
0.47
0.76
0.51
0.25
0.36
1.99
0.37
0.57
0.84
0.89
1.90
2.00

10b: ^{1}H-NMR


Current Data Parameters
NAME 2018-10-25-10-FK-3-anil
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181025
Time 18.11 h
INSTRUM spect
PROBHD Z800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 256
DW 48.533 usec
DE 6.50 usec
TE 296.4 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000117 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
HO
H3CO
CH3
H3C
O
OH
NH2
N
HO
H3C
OCH3
OCH3
10b
-0.000
7.262
ppm
0
1
2
3
4
5
6
7
8
9
10
11.40
18.82
3.14
6.23
2.78
2.19
1.88
2.94
9.15
3.22
1.13
0.48
0.65
0.49
0.53
0.38
1.95
0.36
0.52
0.85
0.81
0.88
0.84
0.89
1.00

11b: ^{1}H-NMR


Current Data Parameters
NAME 2018-10-25-11-FK-2-anil
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20181025
Time 17.58 h
INSTRUM spect
PROBHD z800701_0153 (
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 128
DS 0
SWH 10302.198 Hz
FIDRES 0.314398 Hz
AQ 3.1806805 sec
RG 256
DW 48.533 usec
DE 6.50 usec
TE 296.5 K
D1 1.00000000 sec
TD0 1
SFO1 499.8030865 MHz
NUC1 1H
P1 11.53 usec
PLW1 14.00000000 W
F2 - Processing parameters
SI 65536
SF 499.8000116 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
HO
H3CO
CH3
H3C
OH
O
NH2
N
OCH3
11b
ppm
0
1
2
3
4
5
6
7
8
9
10
7.262
1.581
0.000
10.93
6.29
9.32
6.25
6.04
3.31
2.19
2.24
2.84
8.95
1.21
1.09
2.01
2.13
0.43
0.86
0.60
0.27
0.69
2.22
0.39
0.72
0.93
0.88
0.94
1.13
1.05
0.95