Highly Regioselective Protecting Group-Free Synthesis of the Antimalarial Drug MMV693183

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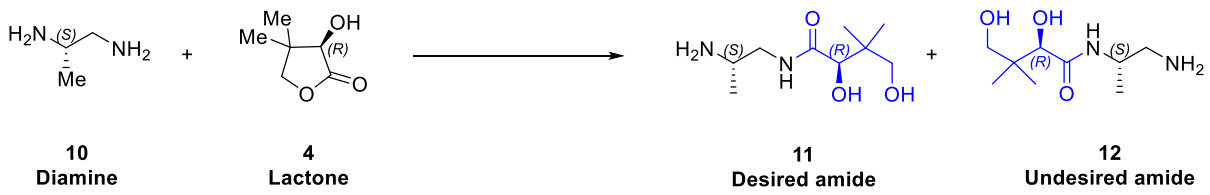
Table S1. Analytical HPLC-HILIC Method for the separation of 1st step regioisomers

Structures & IDs:		
		
Conditions:		
Column: Agilent Poroshell 120 HILIC-Z (3.0 x 100 mm, 2.7 µm)		
Mobile Phase A: Acetonitrile		
Mobile Phase B: 25 mM ammonium formate adjusted to pH 3.0 ± 0.1 with formic acid		
Injection volume: 1 µL	Column temp: 30 °C	Flow rate: 1.0 mL/min
Detector wavelength(s): 210 nm		
LC Gradient Table: Time (min) | %A | %B	Sample preparation: Prepare samples at approximately 1.0 mg/mL in methanol	

0.0	90%	10%
12.0	90%	10%

Post-run equilibration: N/A

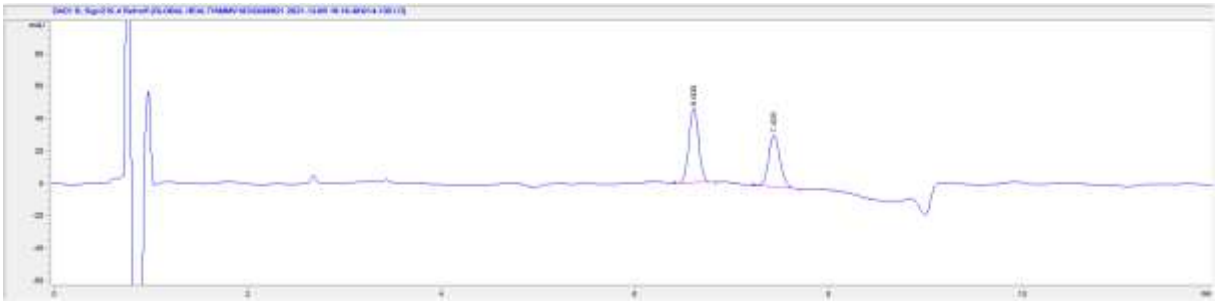
Retention Times

Compound	Time (min)	Relative RF (mg/mL)*	Relative RF (M)*
Desired amide regioisomer **11**	6.6		
Undesired amide regioisomer **12**	7.4		

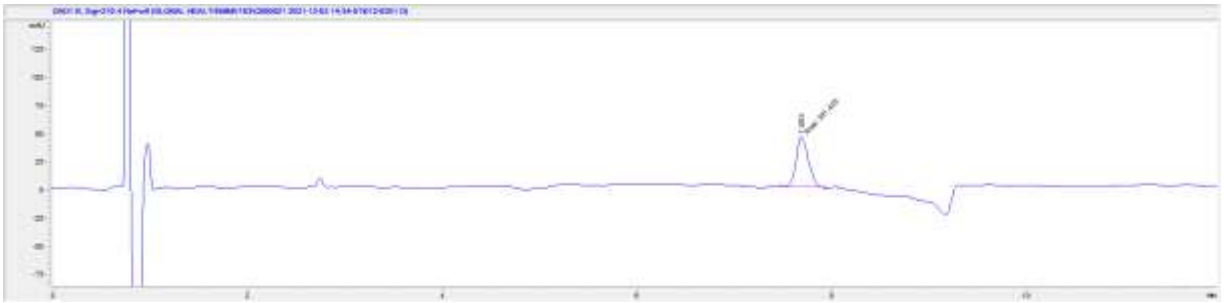
Notes:

- Ensure thorough equilibration (thermal and mobile phase) prior to starting analyses
- $*Relative\ RF = \frac{\left(Analyte\ 2\ Conc./Analyte\ 2\ Peak\ Area\right)}{\left(Analyte\ 1\ Conc./Analyte\ 2\ Peak\ Area\right)}$

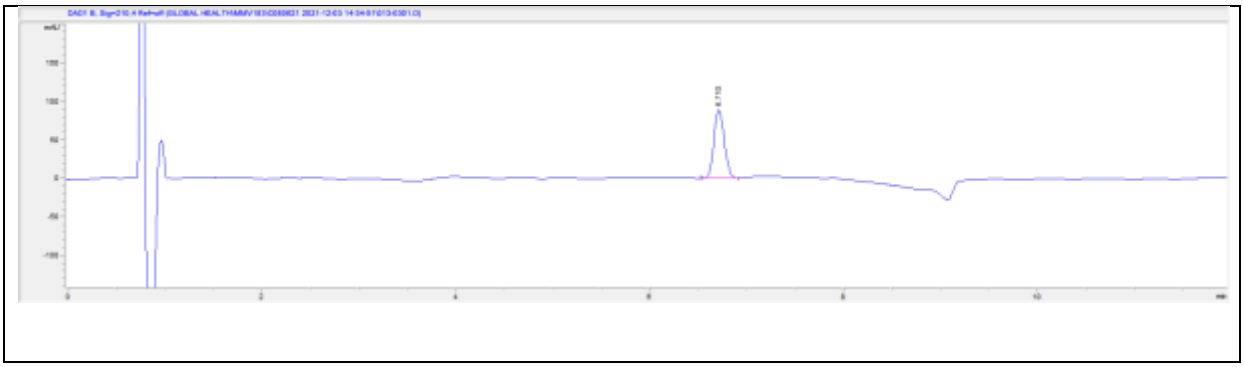
Representative Chromatogram(s) (attach additional chromatograms and spectra as needed)



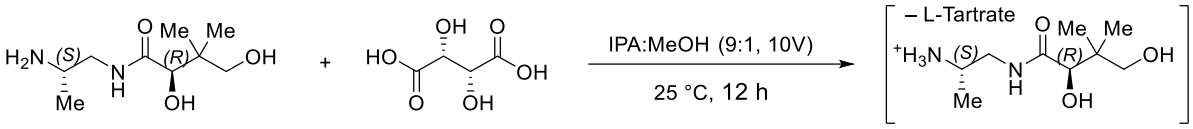
Desired amide 11:



Undesired amide 12:



Scheme S1. Step-II Pictures of Tartrate Salt Formation (18)

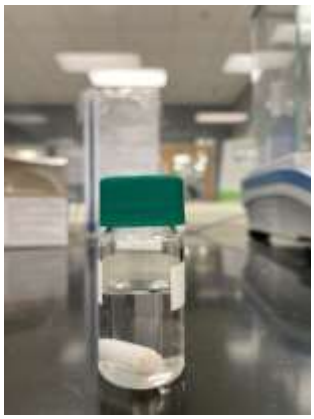


9:1 Mix (**11**:**12**)
1.0 equiv
Column Purified material

L-(+)Tartaric acid
0.9 equiv

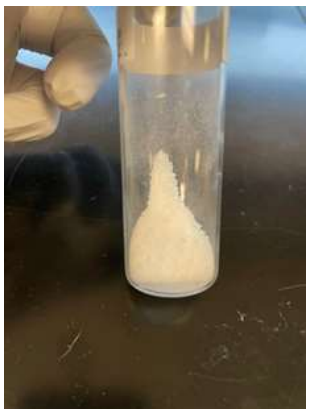
18

9:1 mix of regioisomers in 10 V of IPA:MeOH.



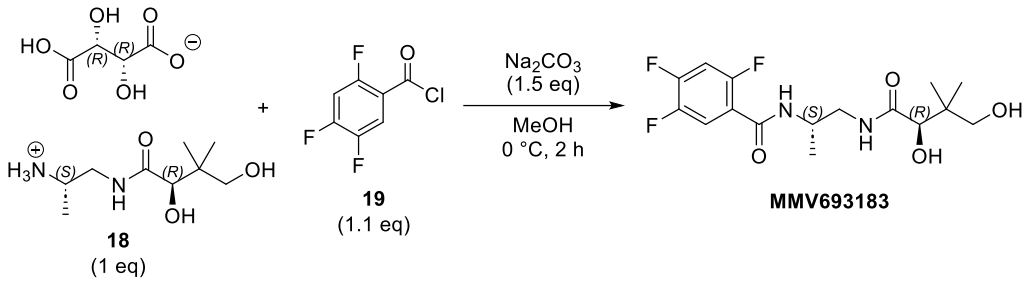
Immediately after addition of 0.9 eq. of *L-(+)*-tartaric acid

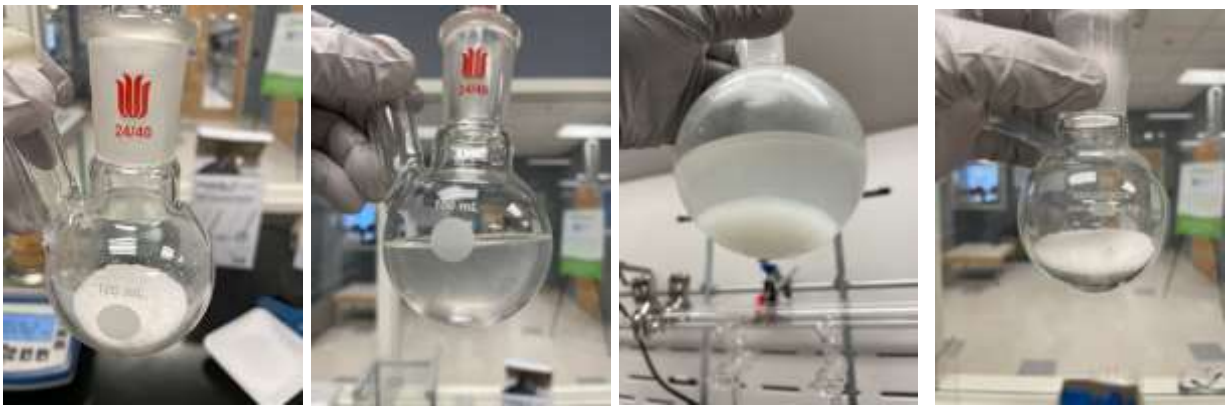
After 12 h, a white solid precipitates out.



Isolated pure desired tartrate salt after filtration and drying.

Scheme S2. Step-III Pictures of *N*-Acylation Reaction

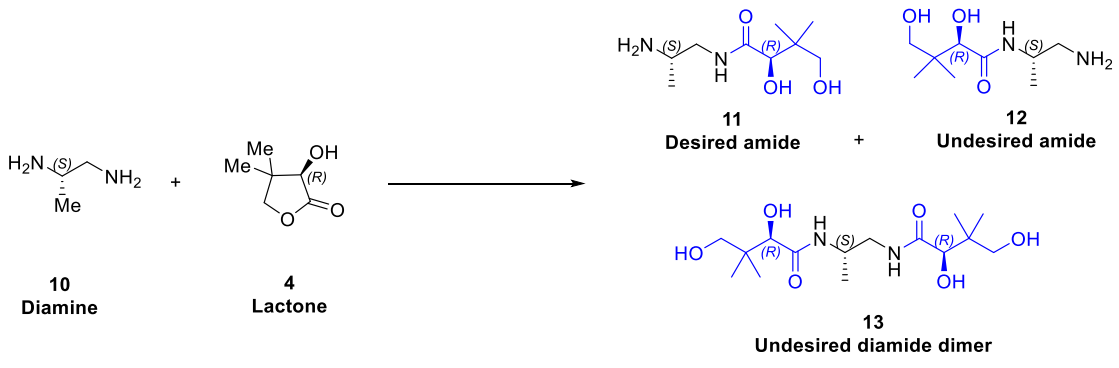


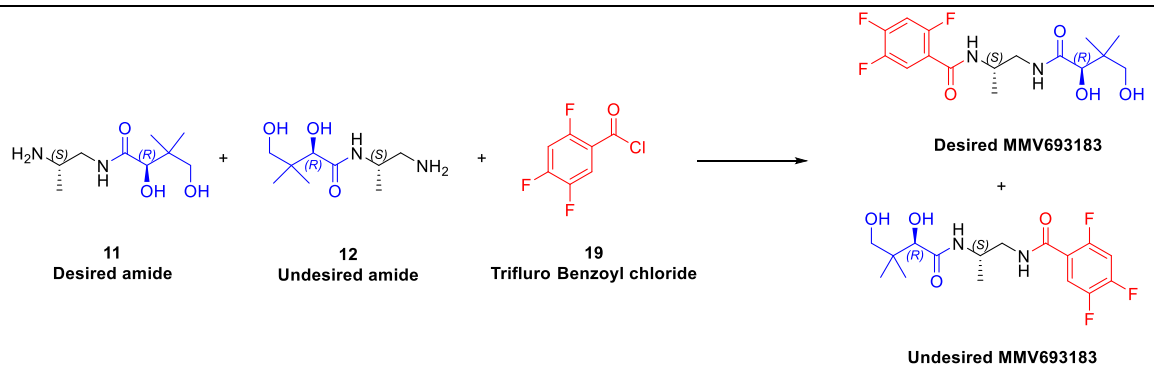


Pure Desired Tartrate salt **18**	Immediately after addition of 10 V of dry MeOH.	After stirring with Na_2CO_3 at 0 °C for 2h, salts precipitate out.	Isolated Desired-MMV693183 after column purification.

Table S2. Analytical HPLC Method for the separation of 3rd step regioisomers

This HPLC method did not resolve the desired and undesired regioisomers of 1st step.

Structures & IDs:




Conditions:

Column: Agilent Eclipse XDB-C18 (4.6 x 250 mm, 5 µm)

Mobile Phase A: 0.1% phosphoric acid in water (1 mL HPLC grade H_3PO_4 in 1000 mL HPLC grade water)

Mobile Phase B: Methanol

Injection volume: 1 µL | Column temp: 40 °C | Flow rate: 1.5 mL/min

Detector wavelength(s): 210 nm

LC Gradient Table:

Time (min)	%A	%B
0.0	90%	10%
0.5	90%	10%
5.0	55%	45%
14.0	55%	45%

Post-run equilibration: 4 min

Sample preparation: Prepare samples at approximately 1.0 mg/mL in ethanol. Step two samples will require sonication to help convert the trifluoro benzoyl chloride to its ethyl ester for monitoring.

Retention Times

Compound	Time (min)	Relative RF (mg/mL)*	Rlative RF (M)*
Desired amide regioisomer **11**	2.3		
Undesired amide regioisomer **12**	2.3		
Lactone **4**	4.5		
Diamide dimer **13**	6.6		
Trifluoro benzoyl chloride (as ethyl ester) **19**	10.1		

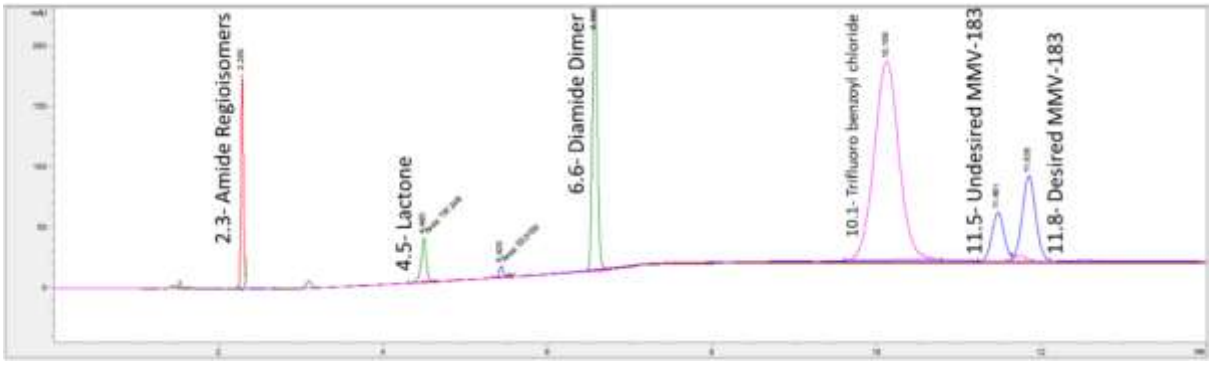
Undesired MMV-183 regioisomer	11.5		
Desired MMV-183 regioisomer	11.8		

Notes:

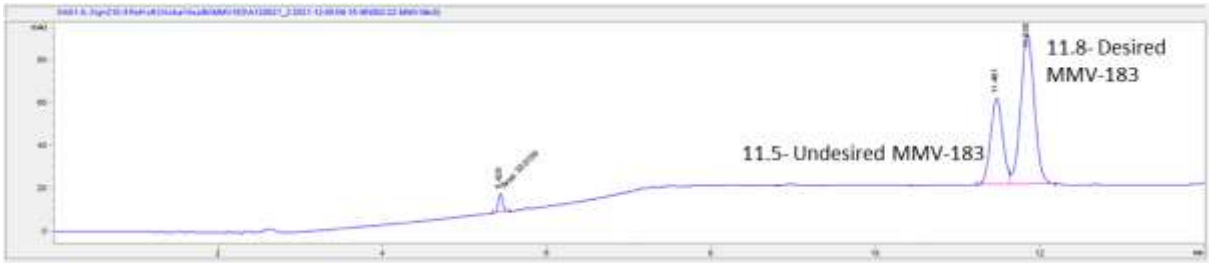
Trifluorobenzoyl chloride and benzoic acid have an RT of 11.7 if not converted to the ester. This would interfere with rection monitoring.

$$*Relative\ RF = \frac{\left({}^{Analyte\ 2\ Conc.}/_{Analyte\ 2\ Peak\ Area}\right)}{\left({}^{Analyte\ 1\ Conc.}/_{Analyte\ 2\ Peak\ Area}\right)}$$

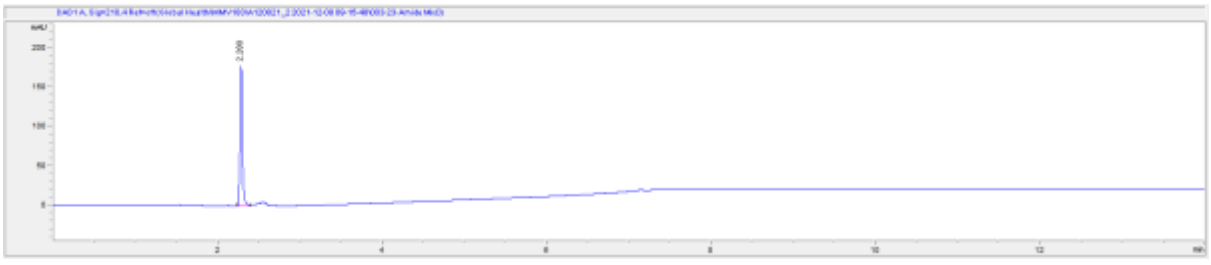
Representative Chromatogram(s) (attach additional chromatograms and spectra as needed)



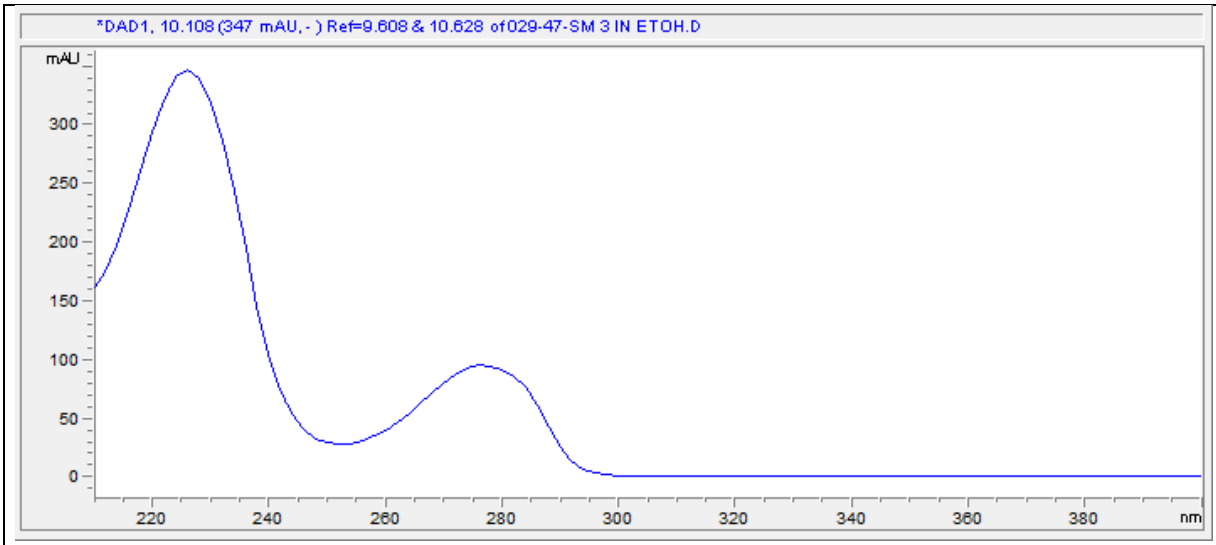
MMV-183 regioisomers:



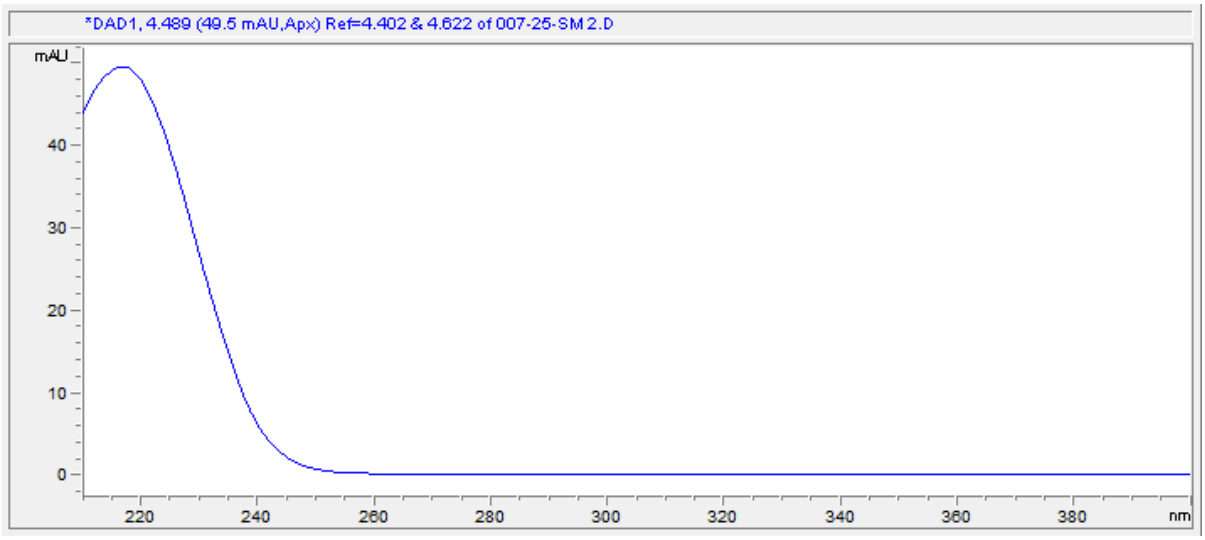
Amide regioisomers 11+12 as a inseparable mixture:



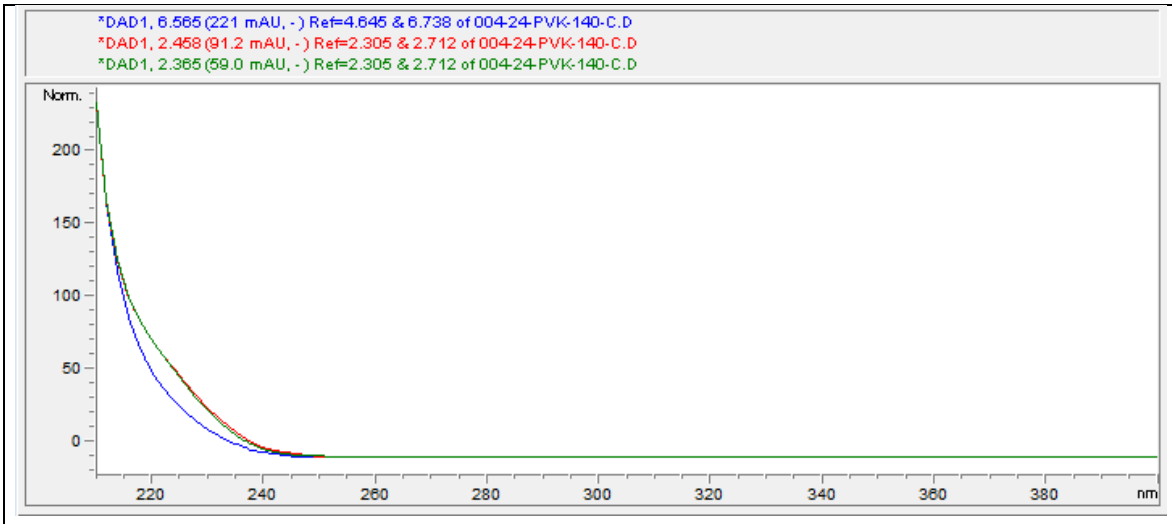
Trifluoro benzoyl chloride UV:



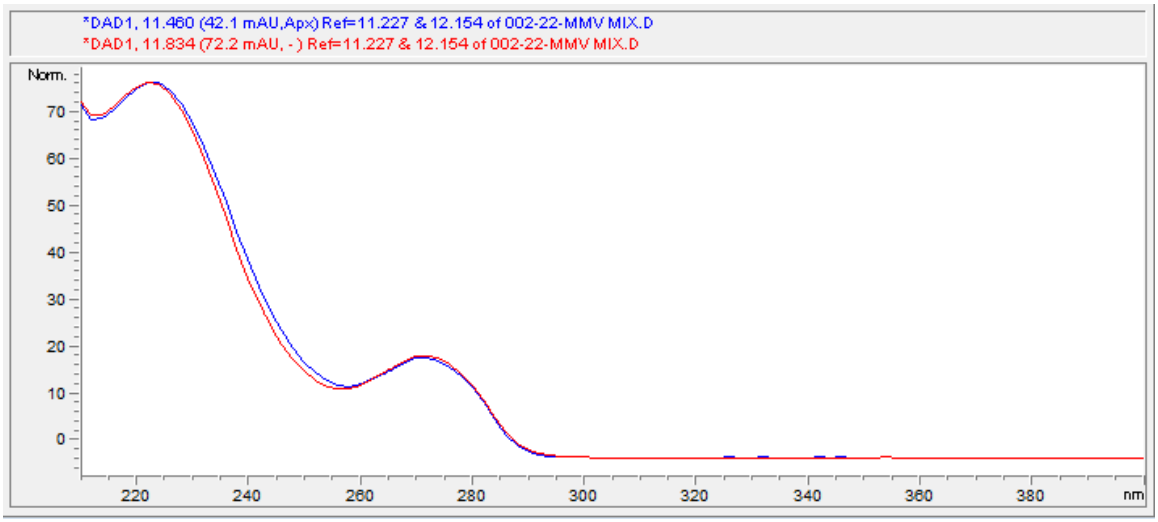
Lactone UV:



Amide regioisomers and dimer UV:

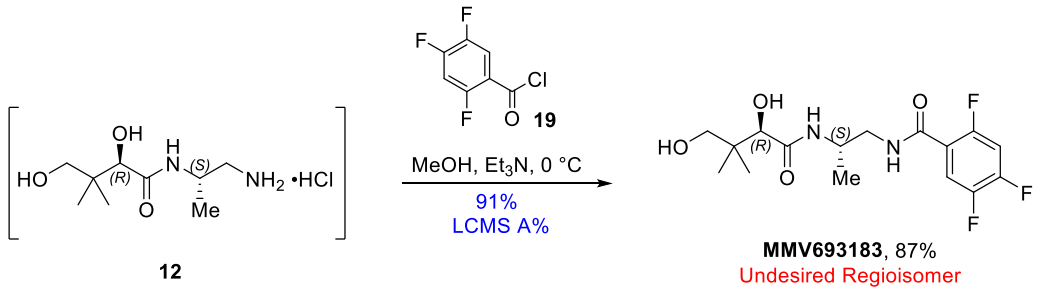


MMV-183 regioisomers UV:



For comparison, the undesired isomer of MMV693183 was prepared from pure compound 12:

Synthesis of N-((S)-2-((R)-2,4-dihydroxy-3,3-dimethylbutanamido)propyl)-2,4,5-trifluorobenzamide (MMV693183-Undesired)



To an oven dried two-necks round bottom flask with magnetic stirrer was added HCl salt **12** (500 mg, 1.0 eq., 2.1 mmol) and dry MeOH (5.0 mL, salt **12** was completely soluble in MeOH) under nitrogen atmosphere. The mixture was cooled to 0 °C with an ice bath and Et_3N (0.43 mL, 1.5 eq., 3.1 mmol) was added. The resulting mixture was allowed to stir for 15 mins at the same temperature. To this mixture was added trifluorobenzoyl chloride **19** (0.29 mL, 1.1 eq., 2.3 mmol) dropwise 0 °C and the resulting mixture was stirred for 1 h. After completion (monitored by TLC) the reaction mixture was allowed to warm to 25 °C. The organic solvent was removed under vacuum and the resulting crude mixture was purified by column chromatography (gradient: hexanes to 1:9 EtOAc:hexanes) to afford the pure undesired regioisomer of **MMV693183-undesired** (0.66 g, 87%).) to afford the pure undesired regioisomer of **MMV693183-undesired** (0.66 g, 87%), which exhibits different ^{1}H, ^{13}C and ^{19}FNMR and melting point.

^{1}H NMR (600 MHz, CD_3OD) δ/ppm: 7.74-7.66 (m, 1H), 7.34-7.27 (m, 1H), 4.21-4.13 (m, 1H), 3.87 (s, 1H), 3.54-3.32 (m, 4H), 1.21 (d, J = 6.7 Hz, 3H), 0.87 (s, 3H), 0.86 (s, 3H).

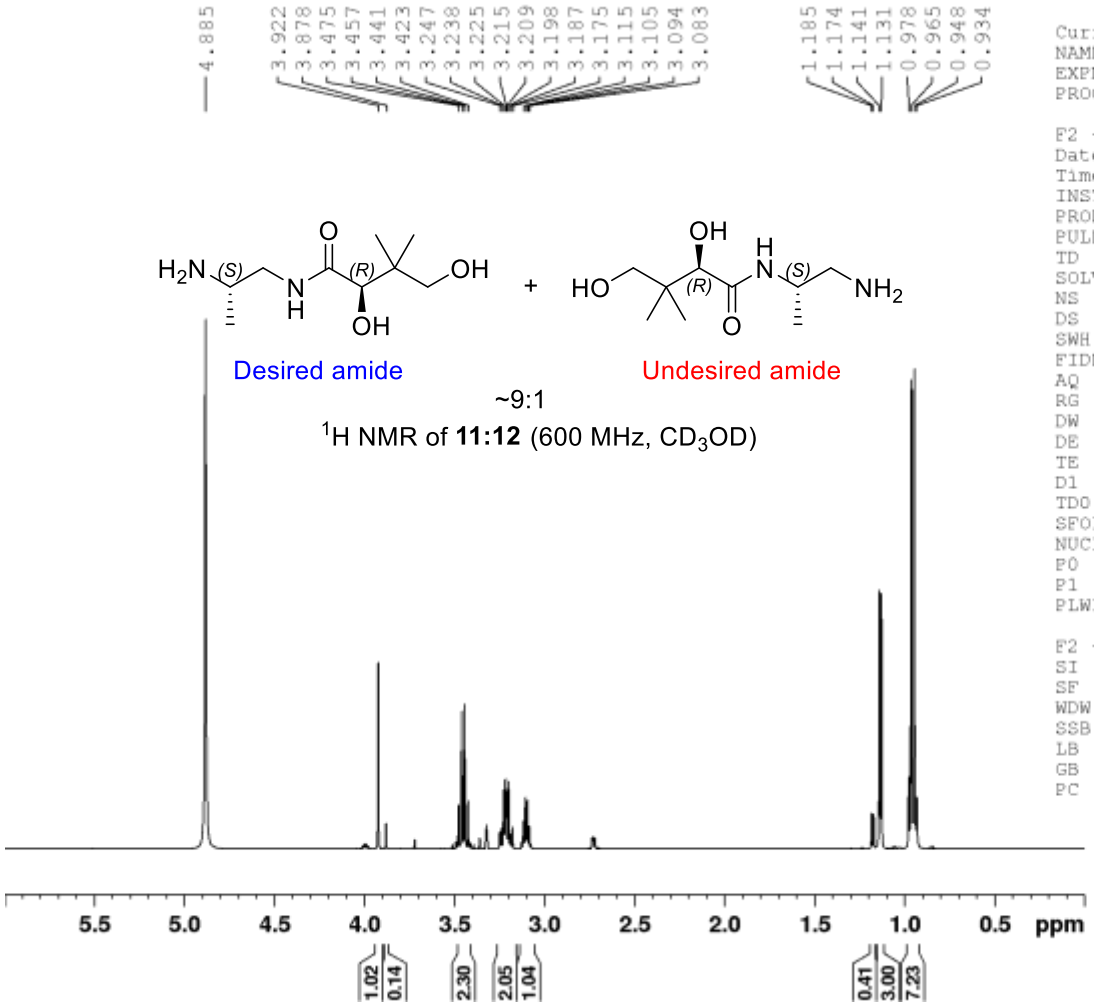
^{13}C NMR (150 MHz, CD_3OD) δ/ppm: 175.9, 164.8, 157.0 (ddd, J = 250.0, 10.0, 2.2 Hz), 153.1 (dt, J = 254.0, 13.8 Hz), 148.1 (ddd, J = 245.0, 13.0, 3.0 Hz), 120.7 (dt, J = 16.5, 4.4 Hz), 119.7 (dd, J = 21.0, 2.5 Hz), 107.6 (dd, J = 23.6, 8.0 Hz), 77.4, 70.2, 46.5, 40.3, 21.5, 20.9, 18.3.

^{19}F NMR (564 MHz, CD_3OD) δ/ppm: -114.3 (dd, J = 17.2, 6.5 Hz, 1F), -130.7 (dd, J = 22.6, 6.3 Hz, 1F), -142.6 (dd, J = 23.1, 6.4 Hz, 1F).

HRMS (ESI) *m/z*: $[M + Na]^+$ Calculated for $C_{16}H_{21}F_3N_2O_4Na$: 385.1453; Found: 385.1462

Melting Point: 106 °C.

II. ^{1}H, ^{19}F and ^{13}C NMR Spectra of all new compounds:

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PROCNO                1

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PULPROG           zg30
TD               65536
SOLVENT           MeOD
NS                  16
DS                   2
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FIDRES         0.366798 Hz
AQ            2.7262976 sec
RG                31.31
DW               41.600 usec
DE               10.33 usec
TE               296.7 K
D1          20.00000000 sec
TD0                  1
SFO1        600.0087050 MHz
NUC1                 1H
P0                5.17 usec
P1               15.50 usec
PLW1        13.23200035 W

F2 - Processing parameters
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SF          600.0050055 MHz
WDW                 EM
SSB                  0
LB                0.30 Hz
GB                   0
PC                1.00
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Fig S1. ^{1}HNMR of compound **11** in CD_3OD.

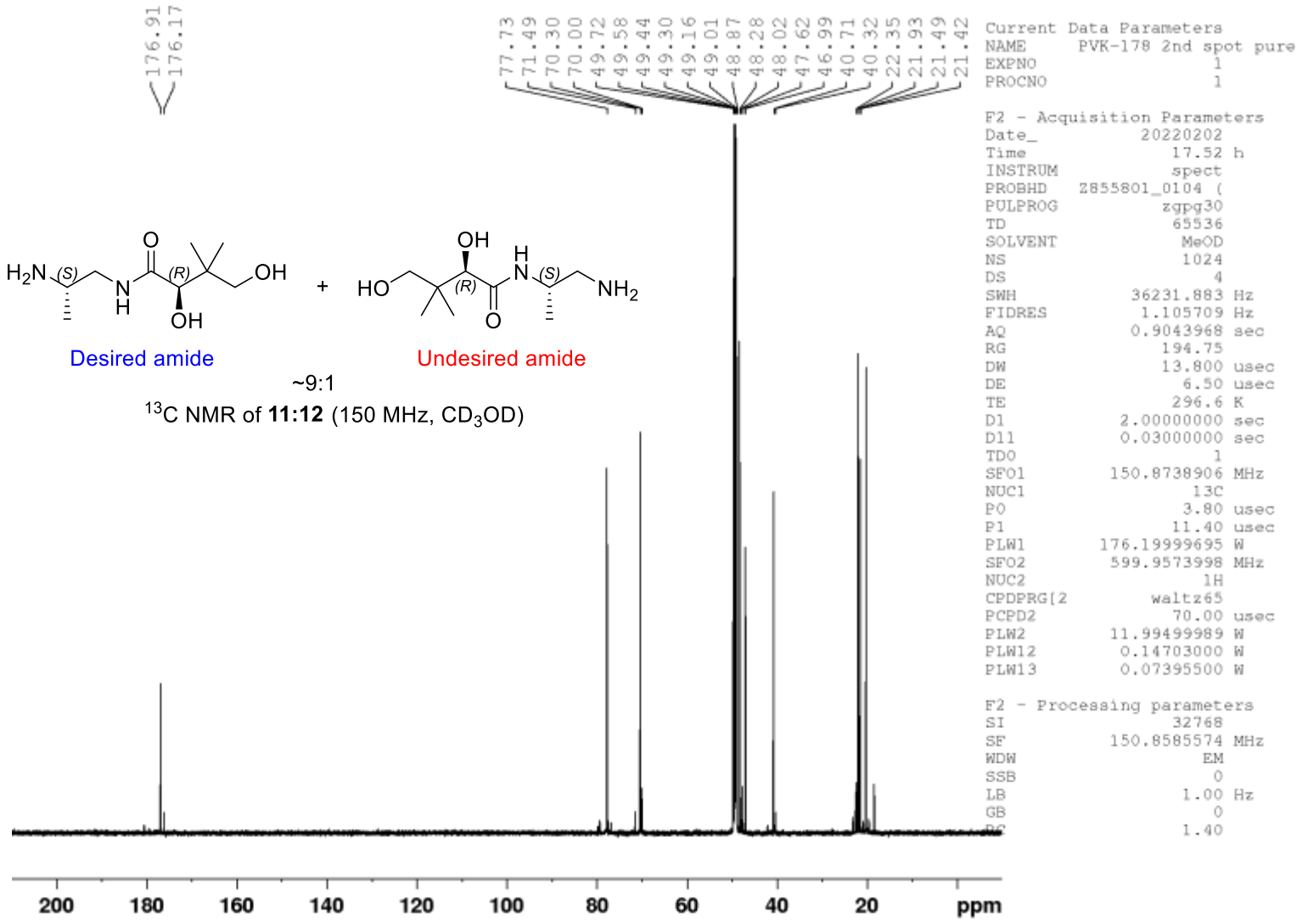
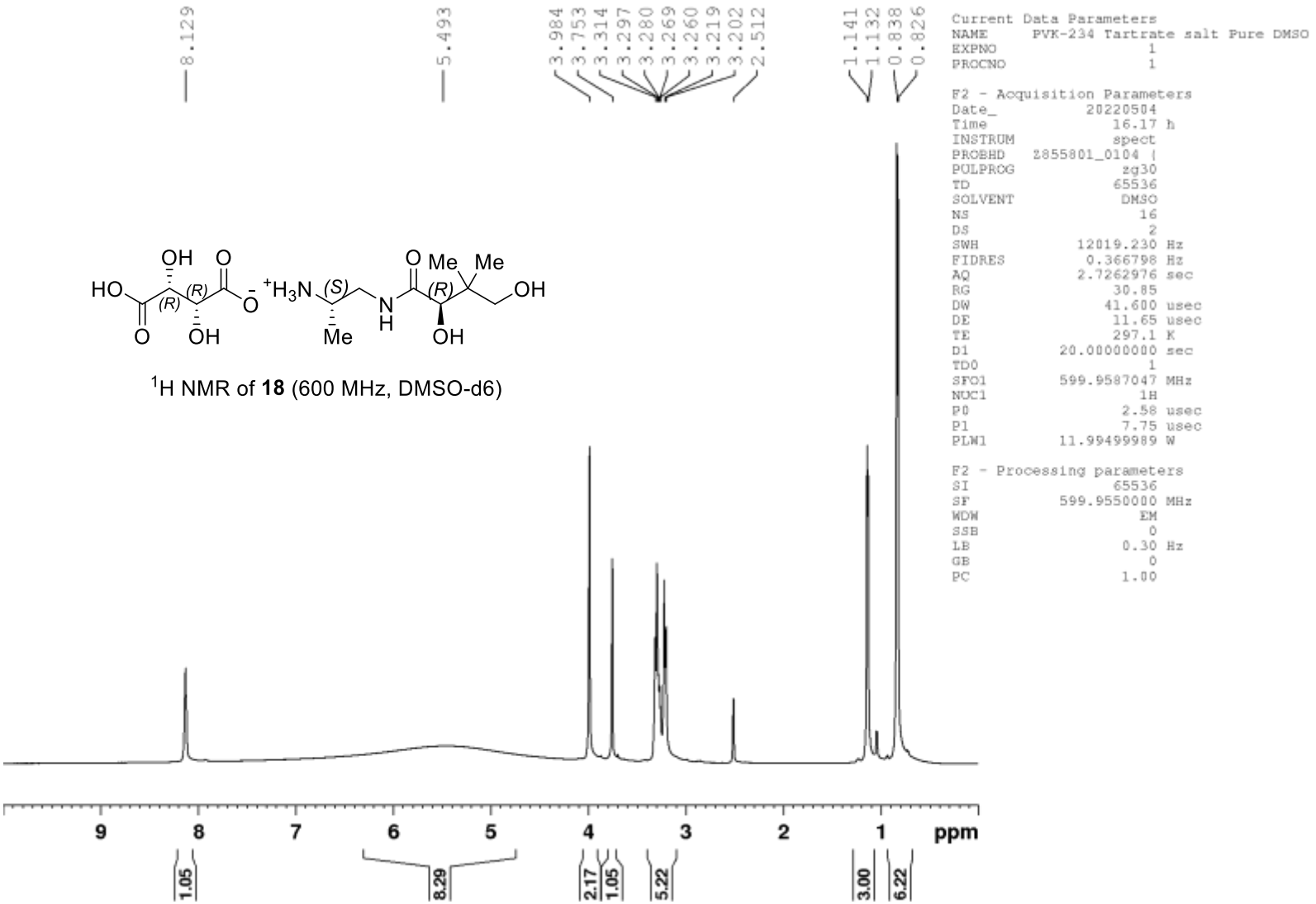
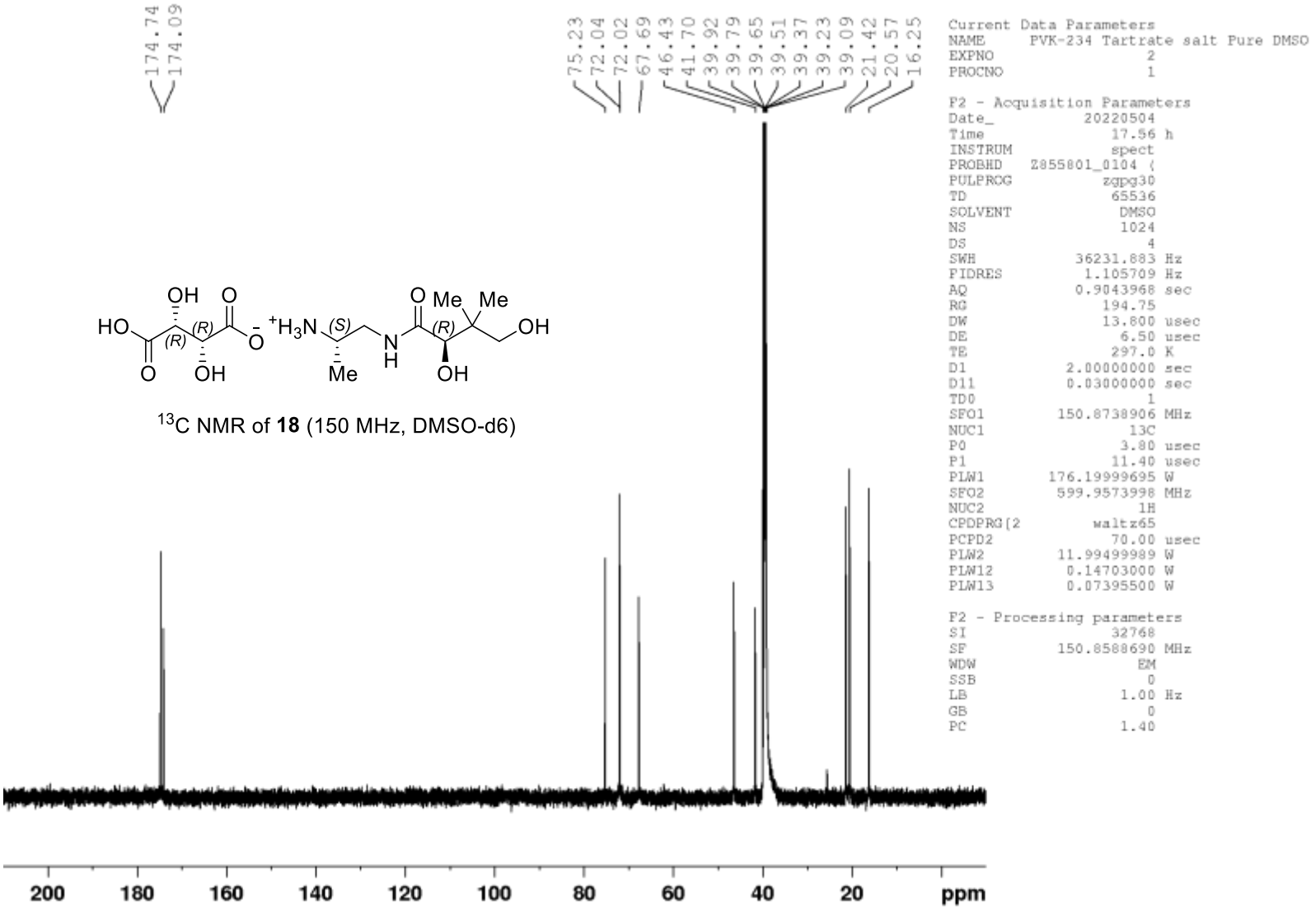


Fig S2. ^{13}CNMR of compound **11** in CD_3OD



^{1}H NMR of **18** (600 MHz, DMSO-d6)

Fig S3. ^{1}HNMR of compound **18** in DMSO-d_6



^{13}C NMR of **18** (150 MHz, DMSO-d6)

Fig S4. ^{13}CNMR of compound **18** in DMSO-d$_6$

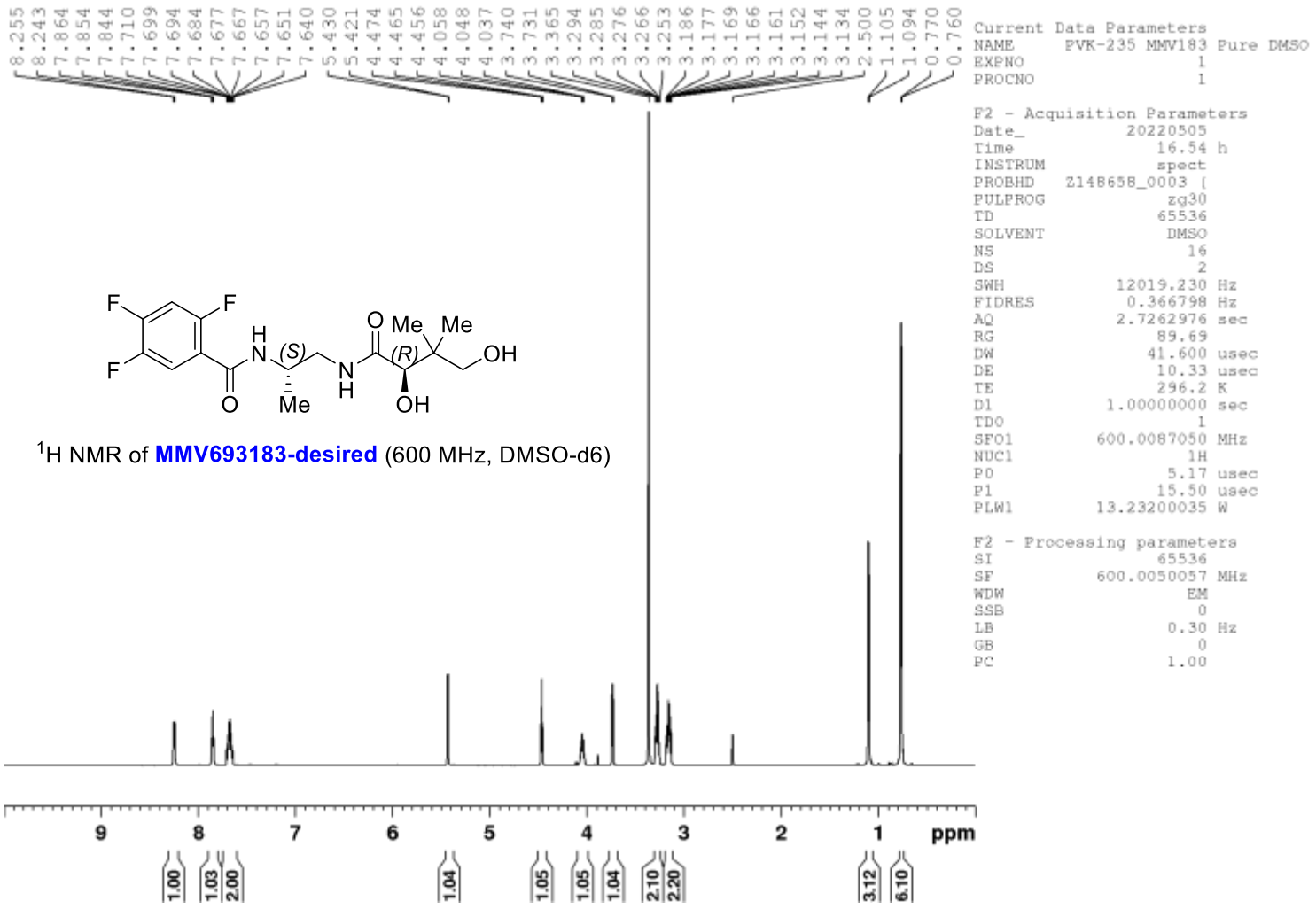


Fig S5. [1]HNMR data of MMV693183 (desired isomer) in DMSO-d_6

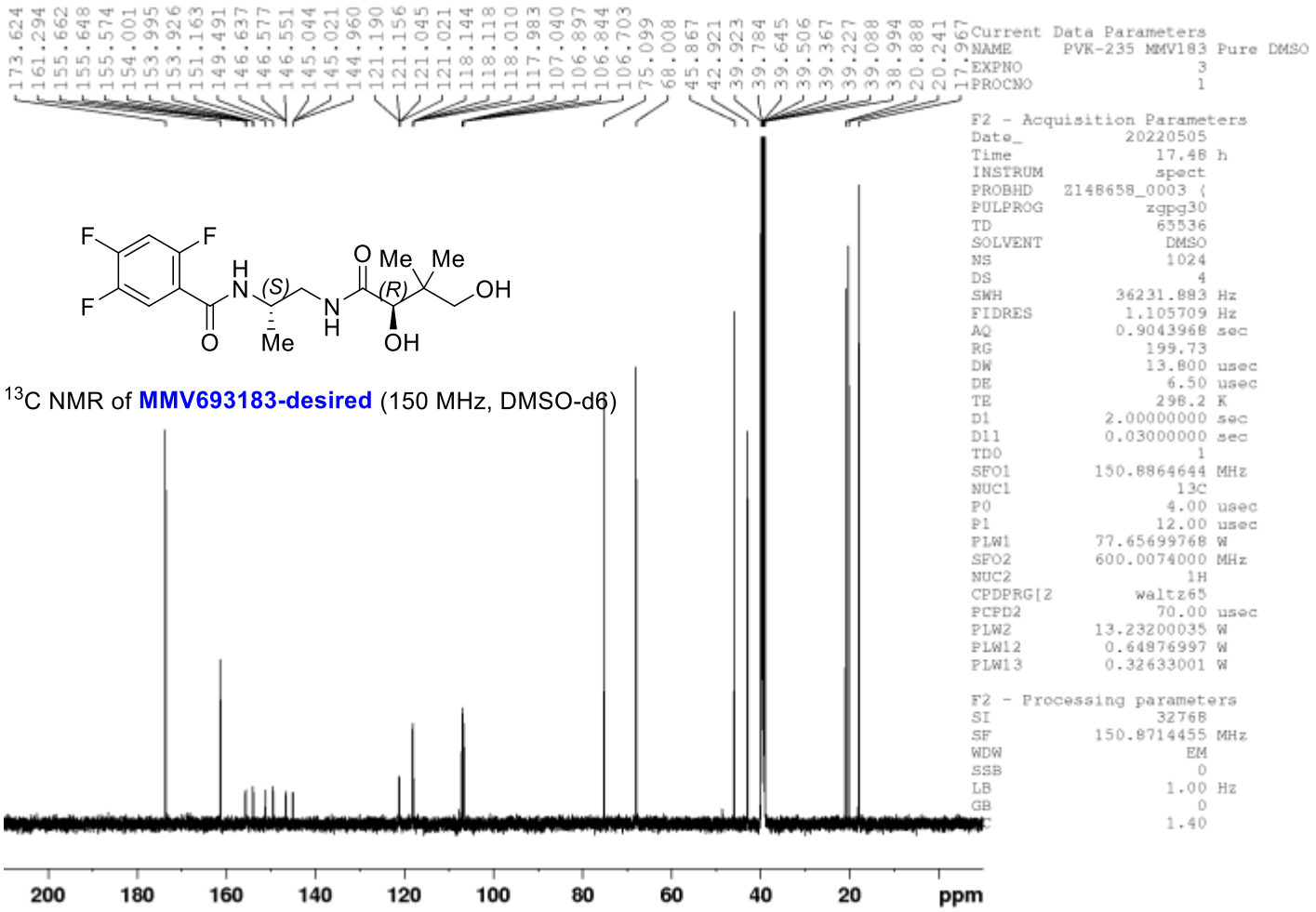
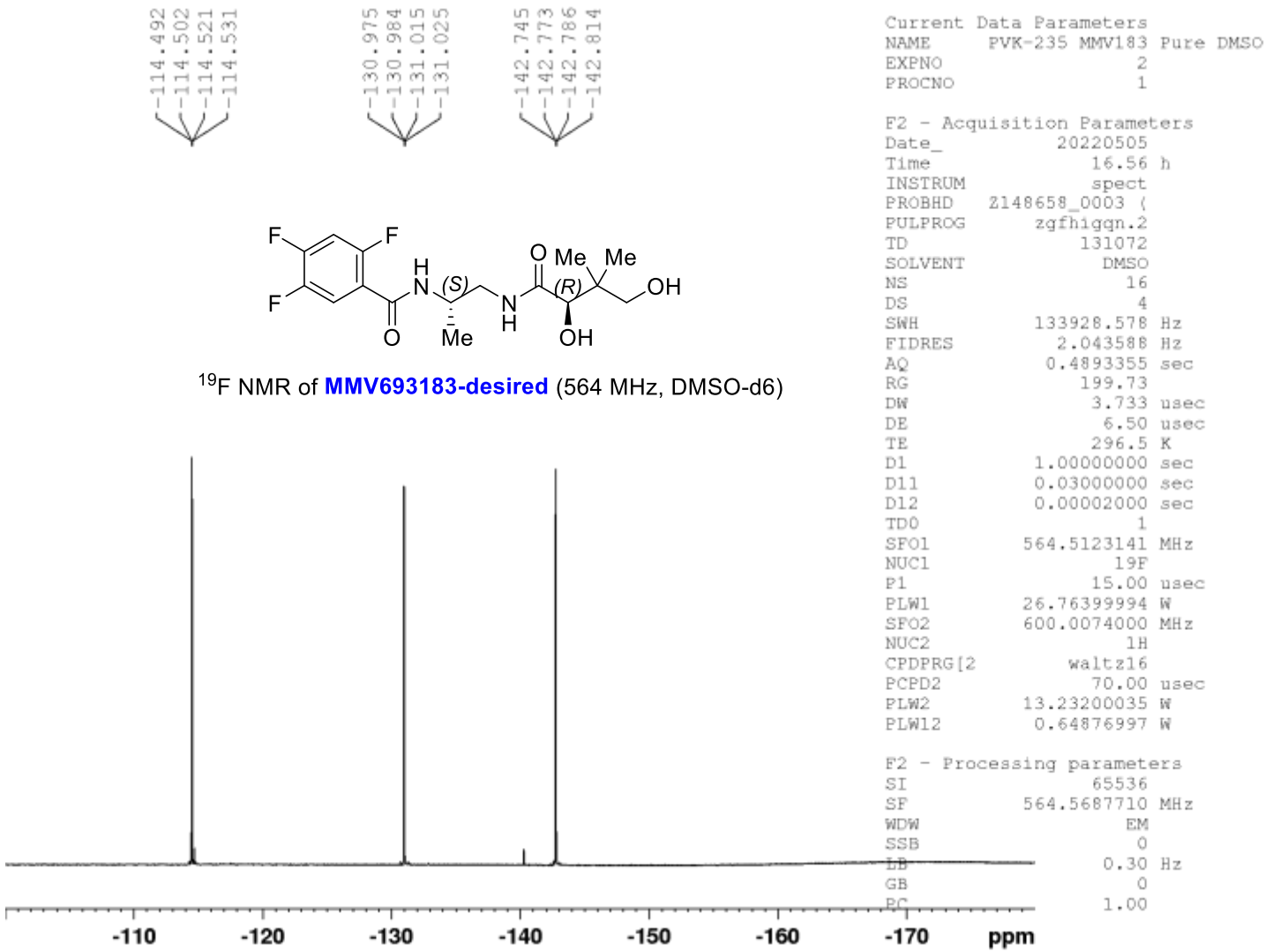


Fig S6. ^{13}CNMR data of MMV693183 (desired isomer) in DMSO-d_6



^{19}F NMR of **MMV693183-desired** (564 MHz, DMSO-d6)

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PROCNO                1

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PULPROG      zgfhigqn.2
TD               131072
SOLVENT            DMSO
NS                   16
DS                    4
SWH          133928.578 Hz
FIDRES         2.043588 Hz
AQ            0.4893355 sec
RG               199.73
DW                3.733 usec
DE                6.50 usec
TE               296.5 K
D1           1.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
TD0                  1
SFO1         564.5123141 MHz
NUC1                19F
P1                15.00 usec
PLW1        26.76399994 W
SFO2        600.0074000 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2        13.23200035 W
PLW12        0.64876997 W

F2 - Processing parameters
SI                65536
SF          564.5687710 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
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Fig S7. ^{19}FNMR data of MMV693183 (desired isomer) in DMSO-d_6

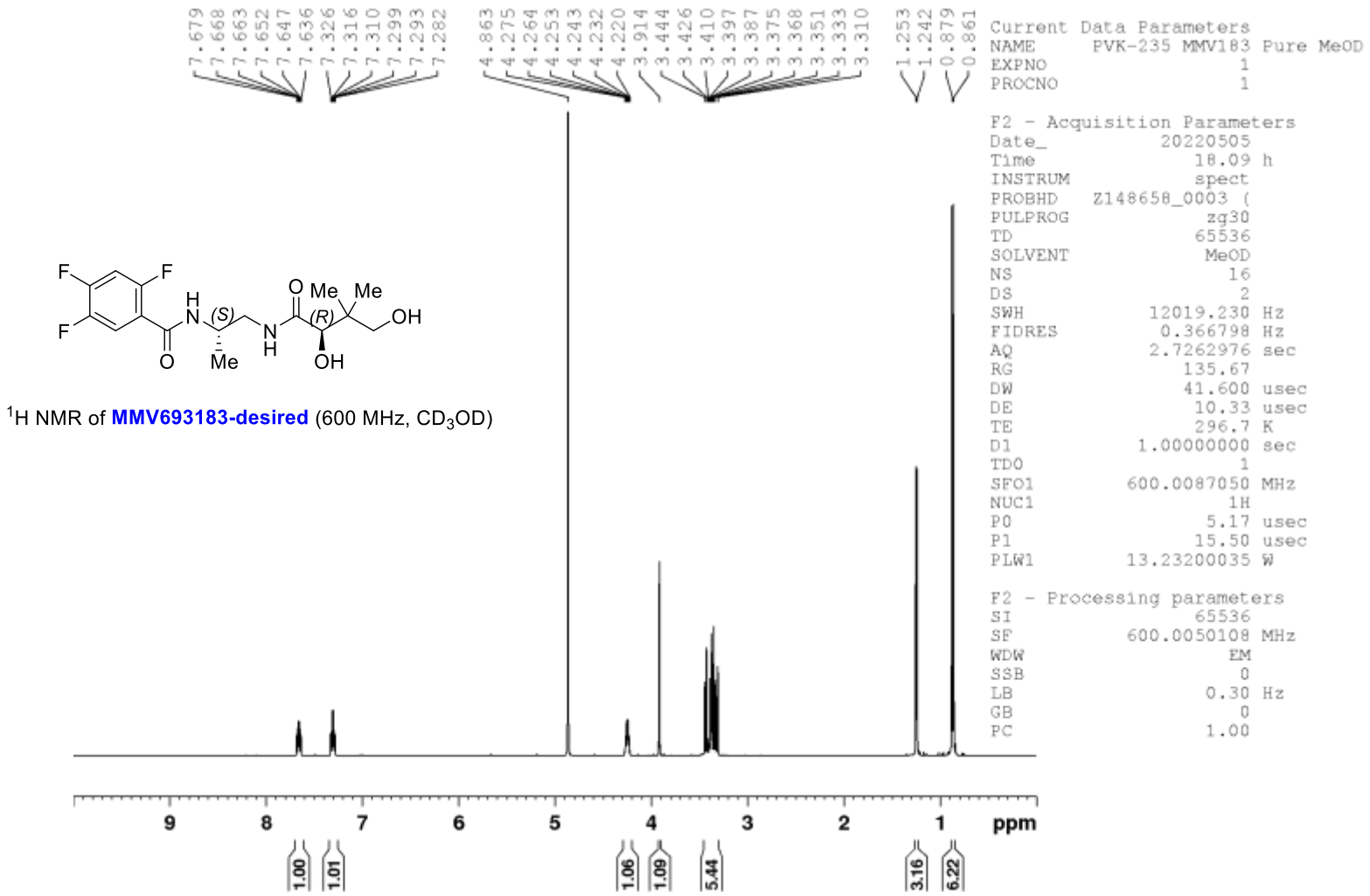


Fig S8. ^{1}HNMR data of MMV693183 (desired isomer) in CD_3OD

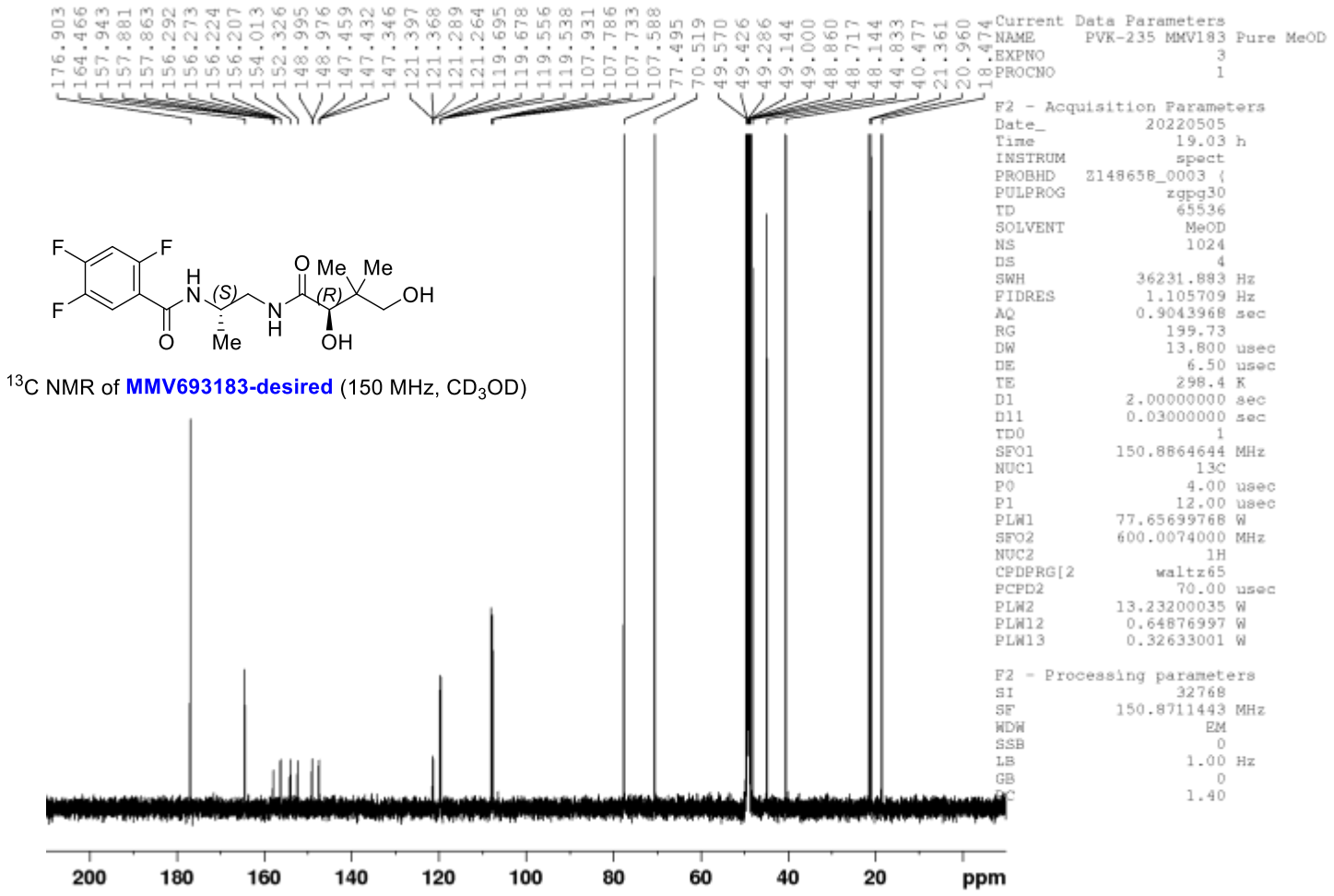
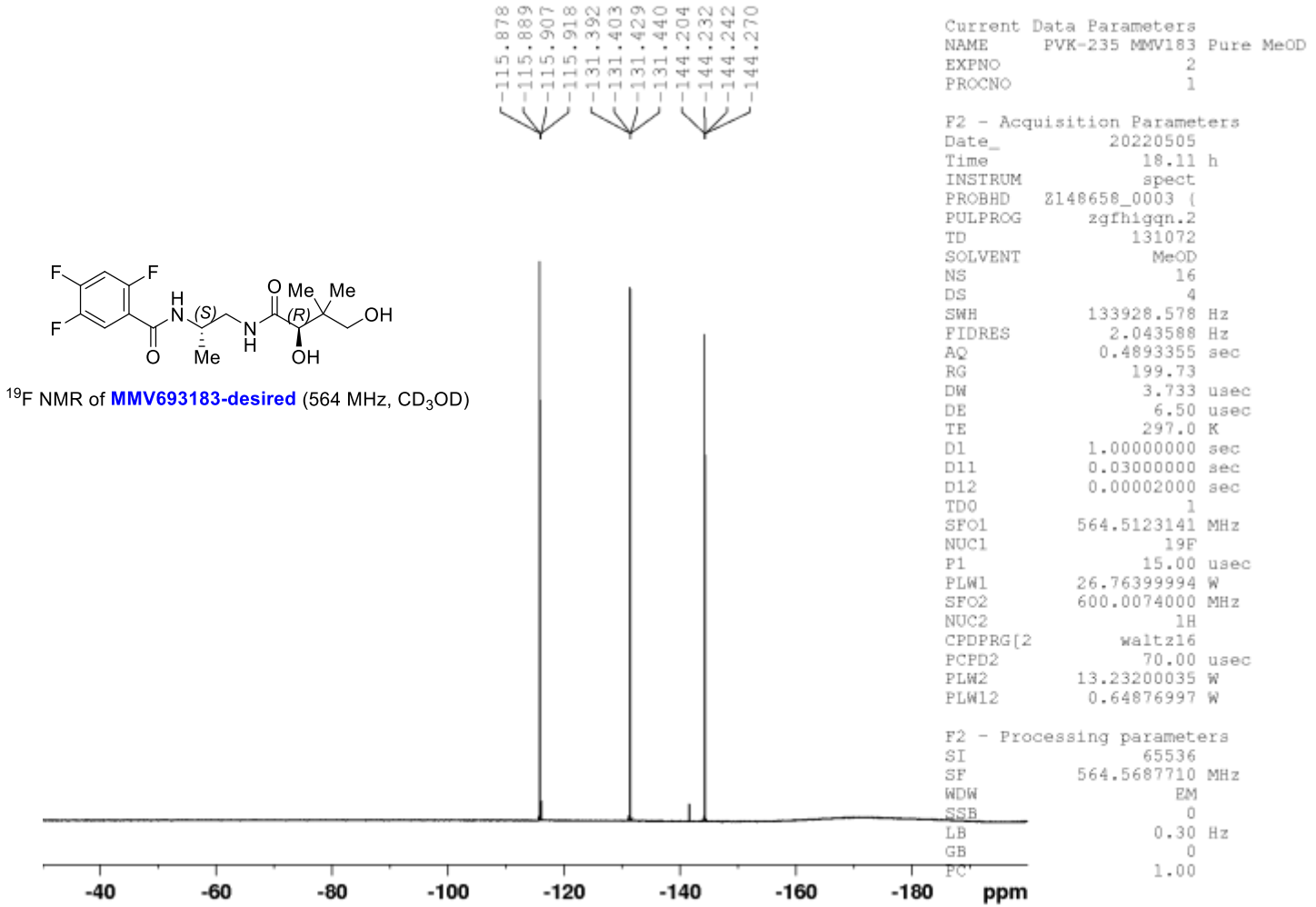


Fig S9. ^{13}CNMR data of MMV693183 (desired isomer) in CD_3OD



^{19}F NMR of **MMV693183-desired** (564 MHz, CD_3OD)

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PROCNO                 1

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PULPROG       zgfhigqn.2
TD                131072
SOLVENT             MeOD
NS                    16
DS                     4
SWH           133928.578 Hz
FIDRES          2.043588 Hz
AQ              0.4893355 sec
RG                199.73
DW                 3.733 usec
DE                 6.50 usec
TE                297.0 K
D1             1.00000000 sec
D11            0.03000000 sec
D12            0.00002000 sec
TD0                    1
SFO1         564.5123141 MHz
NUC1                 19F
P1                15.00 usec
PLW1         26.76399994 W
SFO2         600.0074000 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2             70.00 usec
PLW2         13.23200035 W
PLW12         0.64876997 W

F2 - Processing parameters
SI                 65536
SF           564.5687710 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
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Fig S10. ^{19}FNMR data of MMV693183 (desired isomer) in CD_3OD

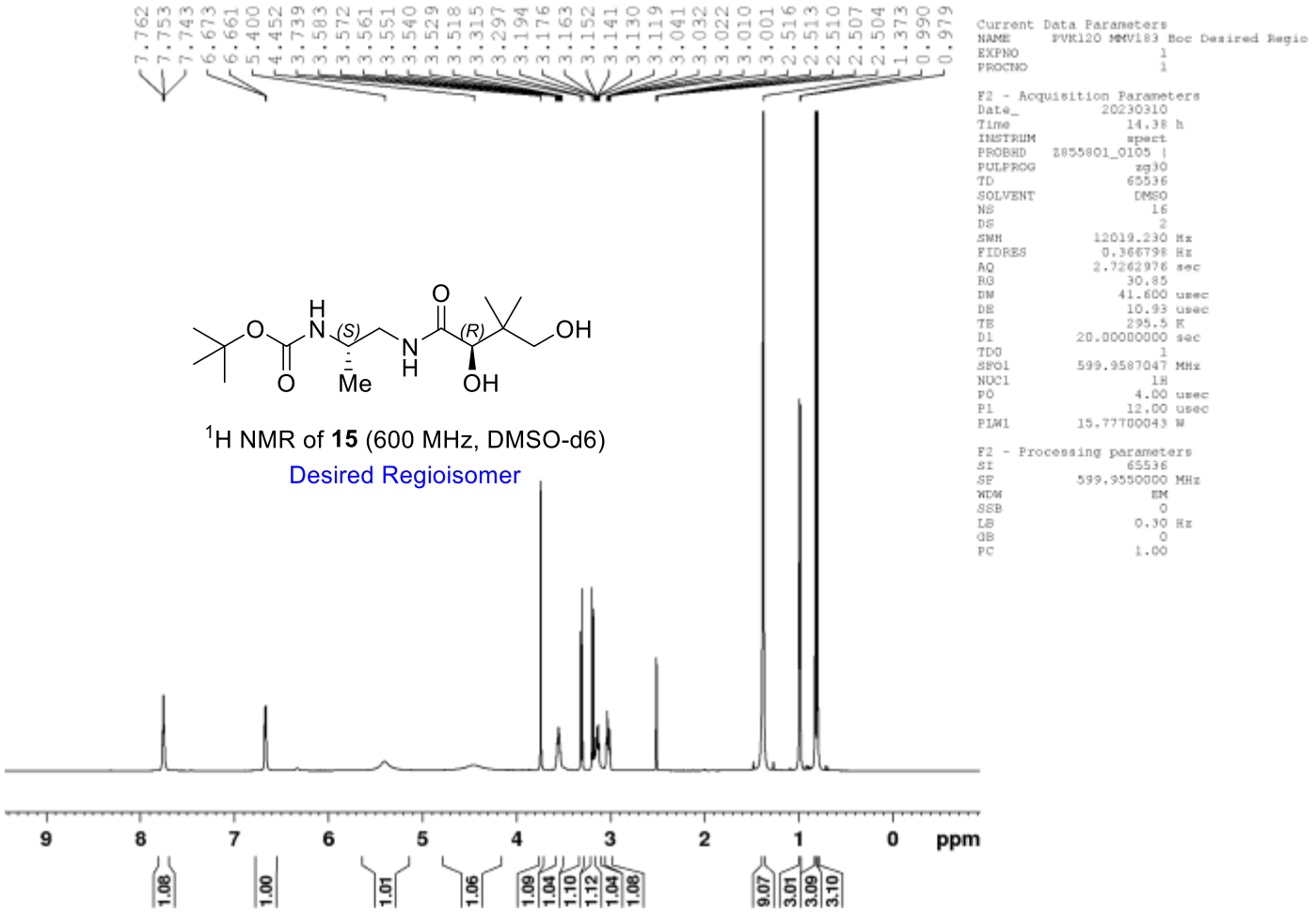


Fig S11. ^{1}HNMR data of compound **15** in DMSO-d$_6$

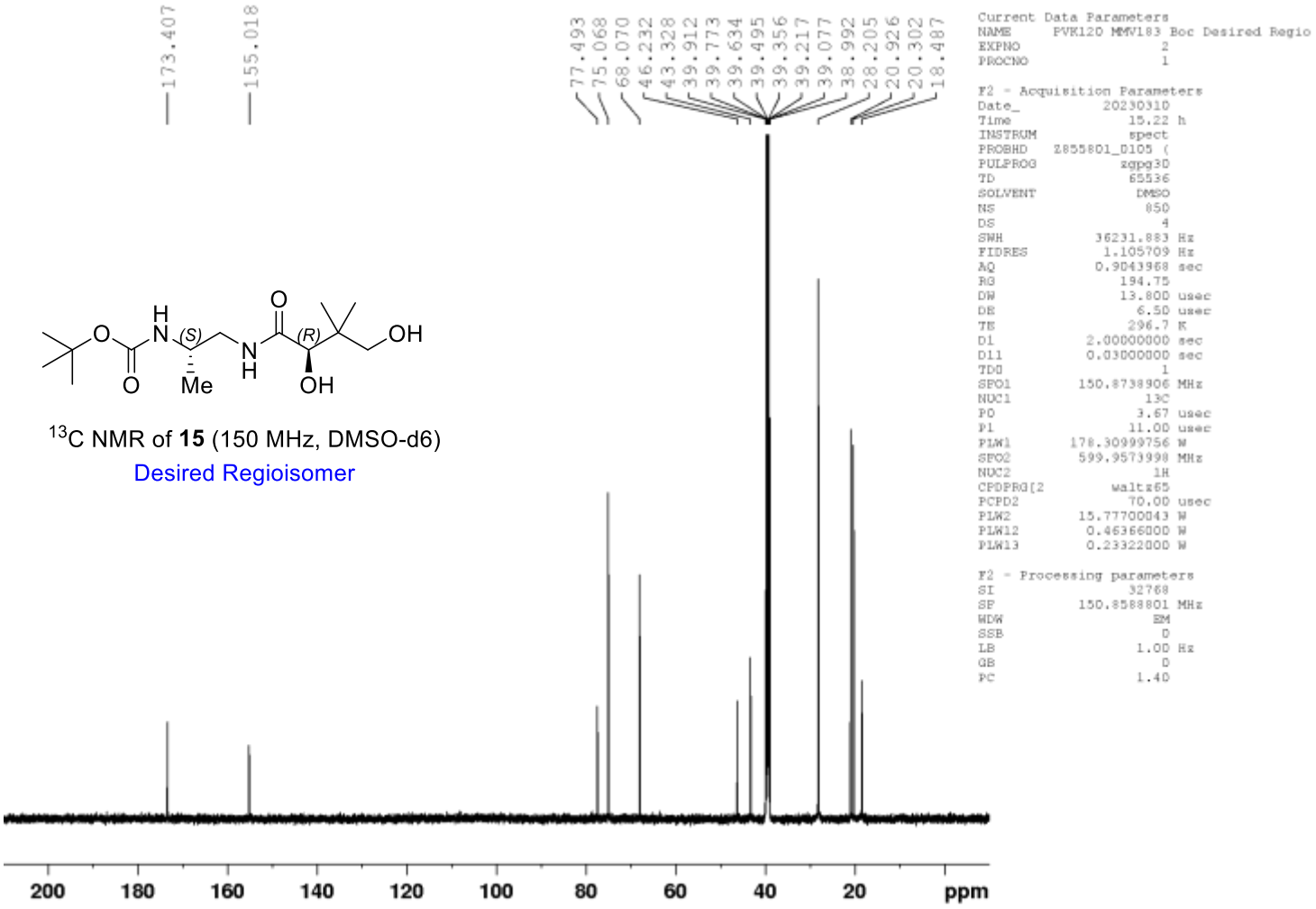
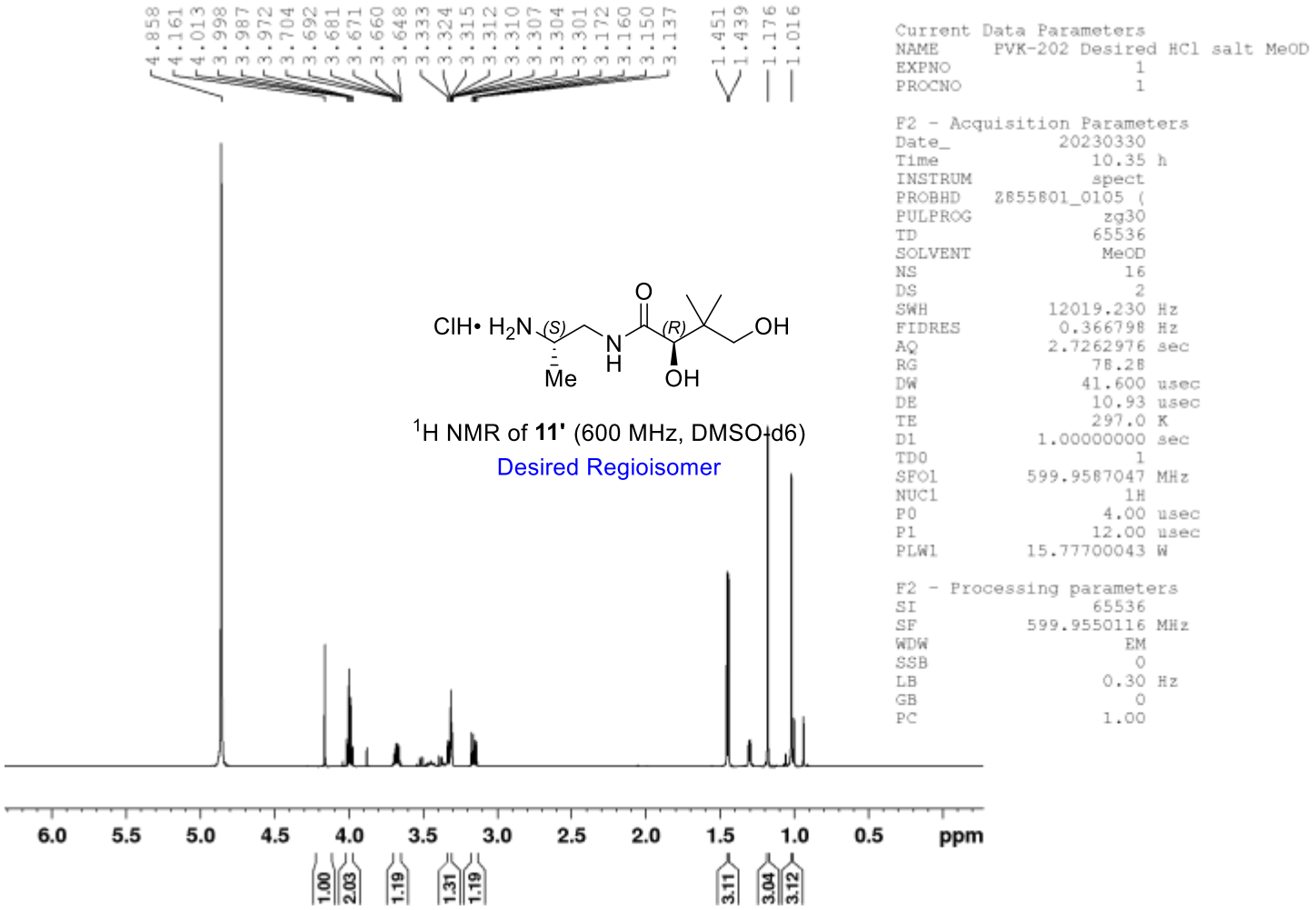


Fig S12. ^{13}CNMR data of compound **15** in DMSO-d_6

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PROCNO                1

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TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           12019.230 Hz
FIDRES         0.366798 Hz
AQ            2.7262976 sec
RG                 78.28
DW                41.600 usec
DE                10.93 usec
TE                297.0 K
D1           1.00000000 sec
TD0                    1
SFO1         599.9587047 MHz
NUC1                  1H
P0                 4.00 usec
P1                12.00 usec
PLW1         15.77700043 W

F2 - Processing parameters
SI                 65536
SF          599.9550116 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

Fig S13. ^{1}HNMR data of compound **11′** in DMSO-d_6

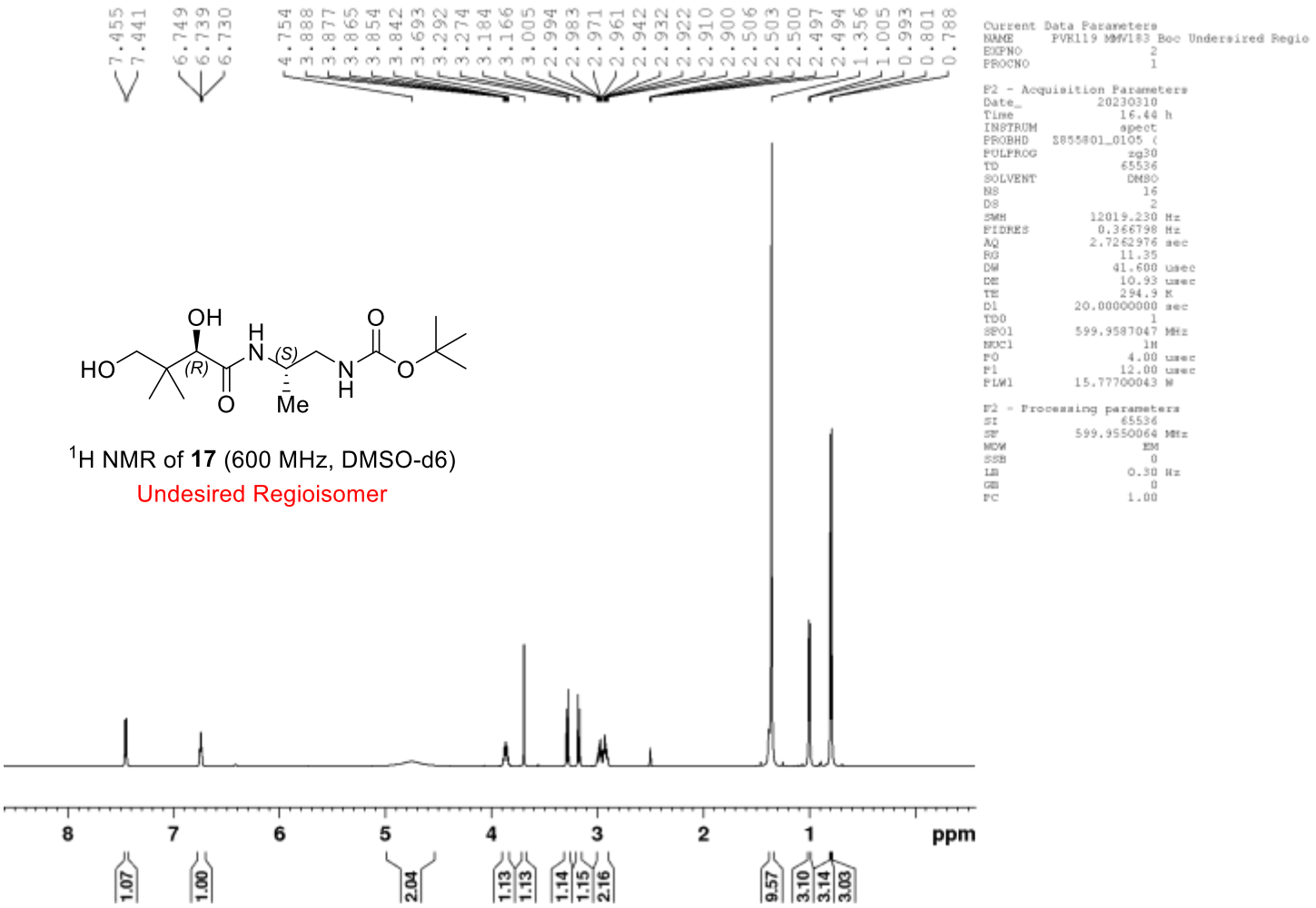


Fig S14. ^{1}HNMR data of compound **17** in DMSO-d_6

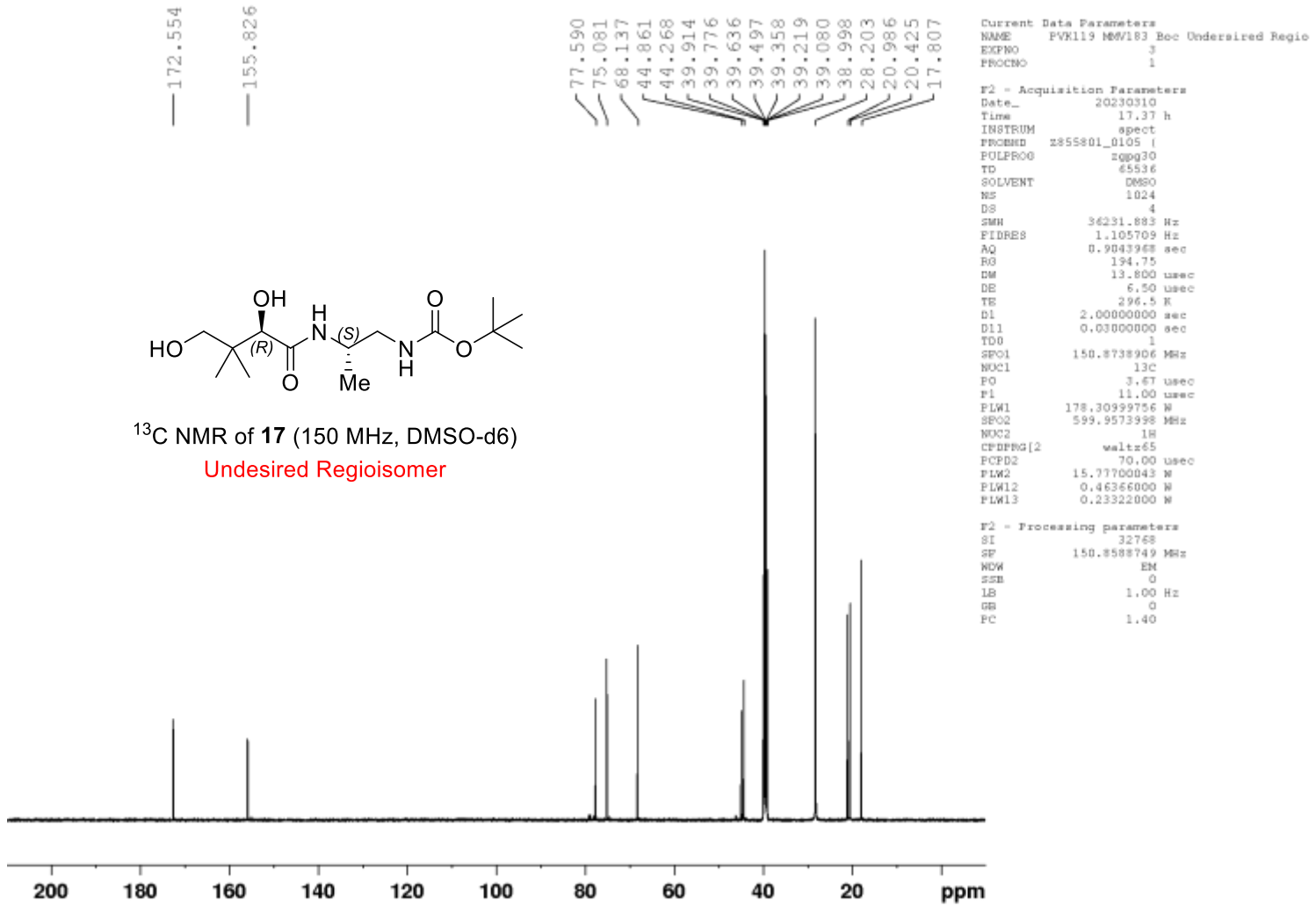
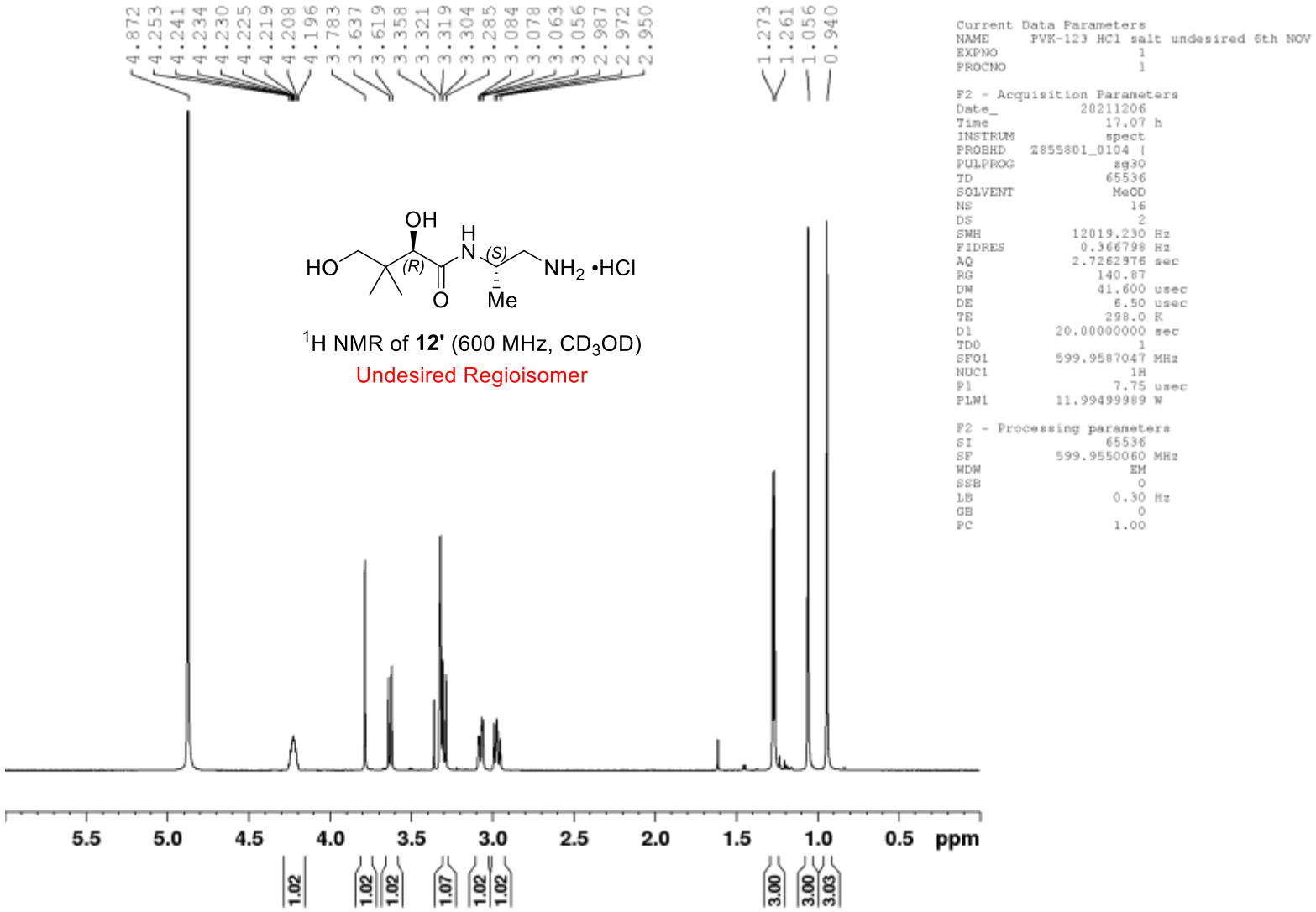


Fig S15. ^{13}CNMR data of compound **17** in DMSO-d_6




```
Current Data Parameters
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EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_              20211206
Time                 17.07 h
INSTRUM              spect
PROBHD    Z855801_0104 (
PULPROG              zg30
TD                  65536
SOLVENT              MeOD
NS                     16
DS                      2
SWH             12019.230 Hz
FIDRES              0.366798 Hz
AQ                2.7262976 sec
RG                   140.87
DW                  41.600 usec
DE                   6.50 usec
TE                  298.0 K
D1             20.00000000 sec
TD0                     1
SFO1            599.9587047 MHz
NUC1                   1H
P1                   7.75 usec
PLW1            11.99499989 W

F2 - Processing parameters
SI                  65536
SF            599.9550060 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

Fig S16. ^{1}HNMR data of compound **12′** in CD_3OD

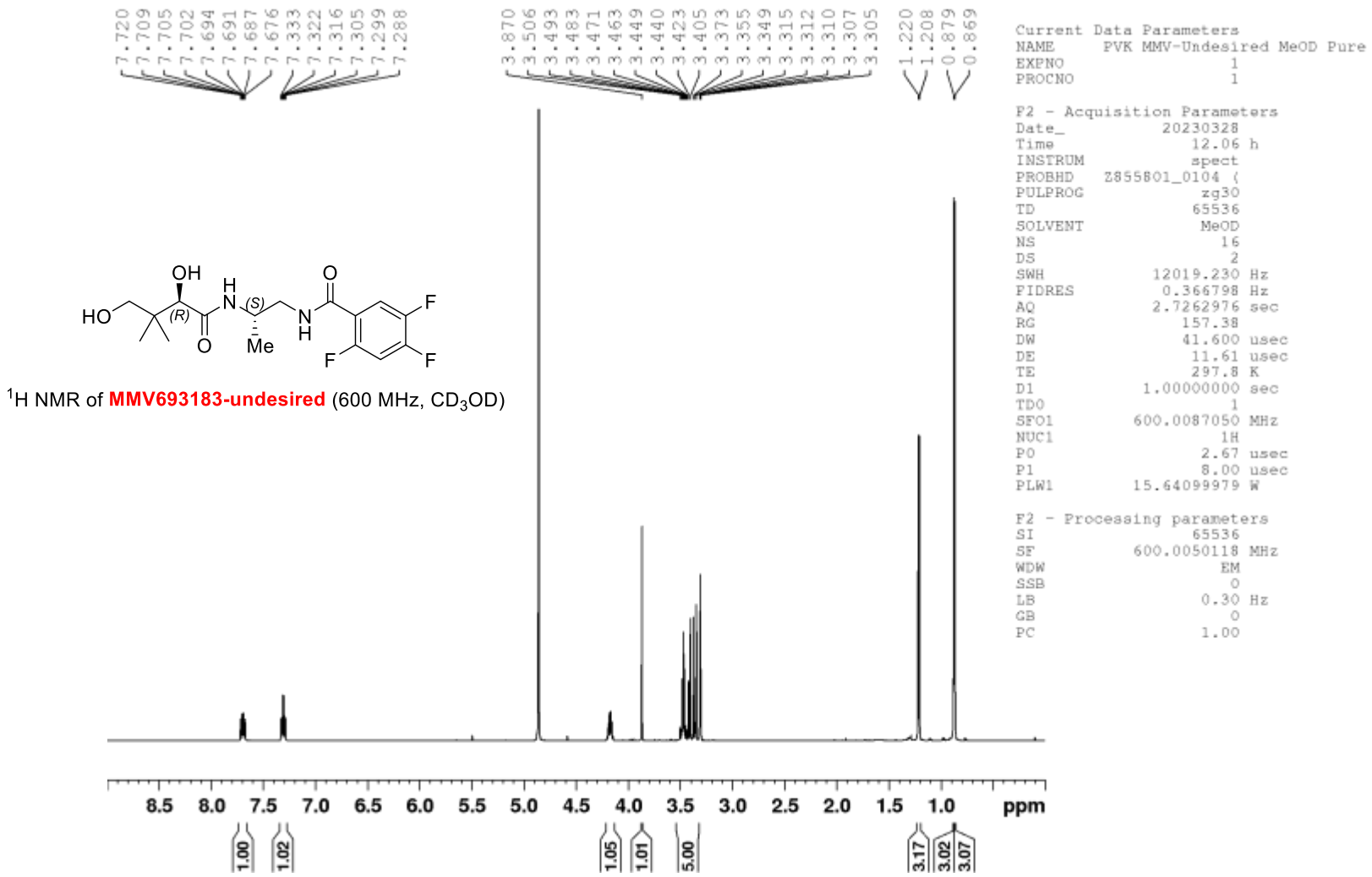
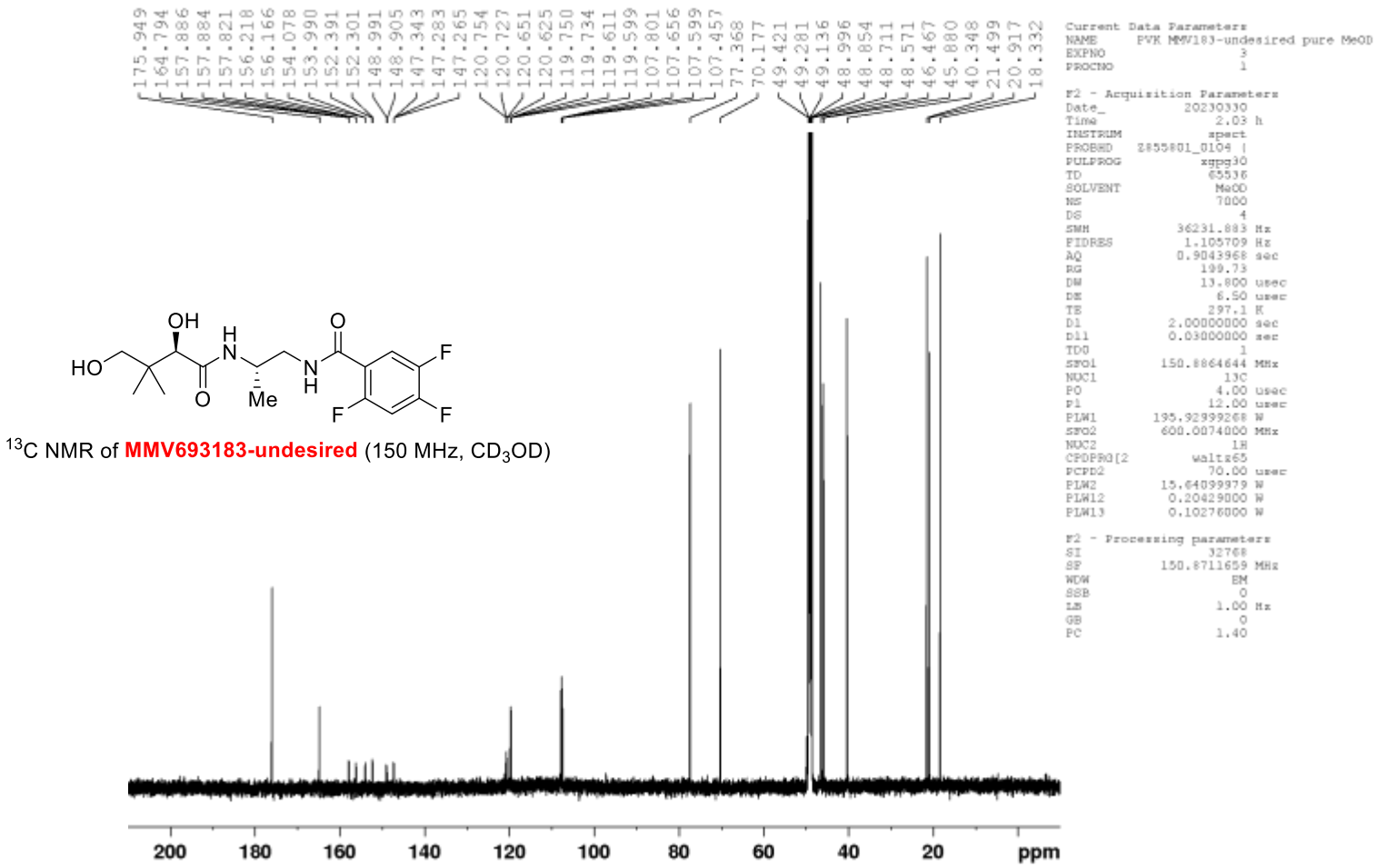


Fig S17. [1]HNMR data of MMV693183-undesired isomer in CD_3OD



^{13}C NMR of **MMV693183-undesired** (150 MHz, CD_3OD)

Fig S18. ^{13}CNMR data of MMV693183-undesired isomer in CD_3OD

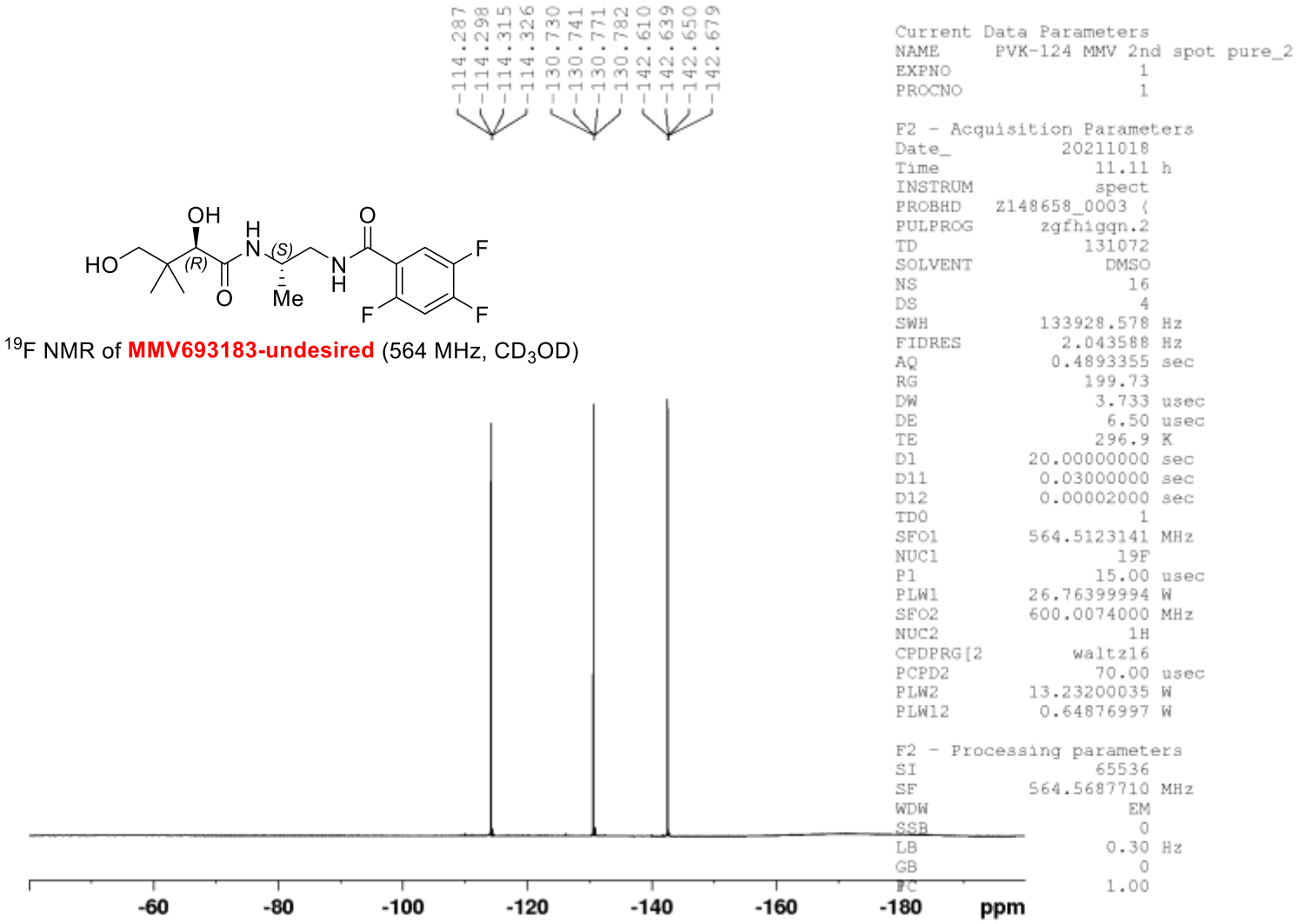


Fig S19. ^{19}FNMR data of MMV693183-undesired isomer in CD_3OD

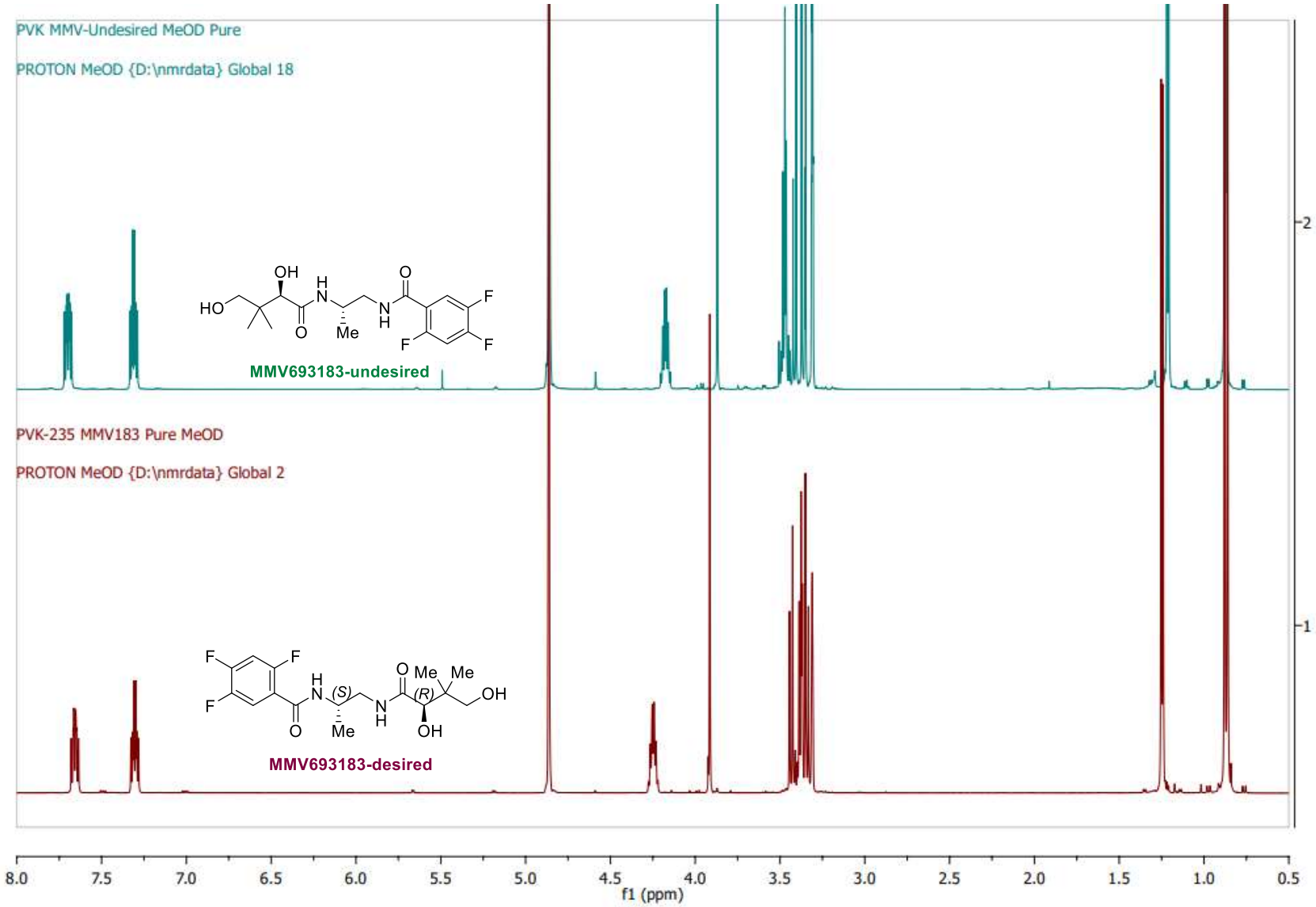


Fig S20. [1]HNMR data comparison of MMV693183 (desired and undesired regisomers) in CD_3OD

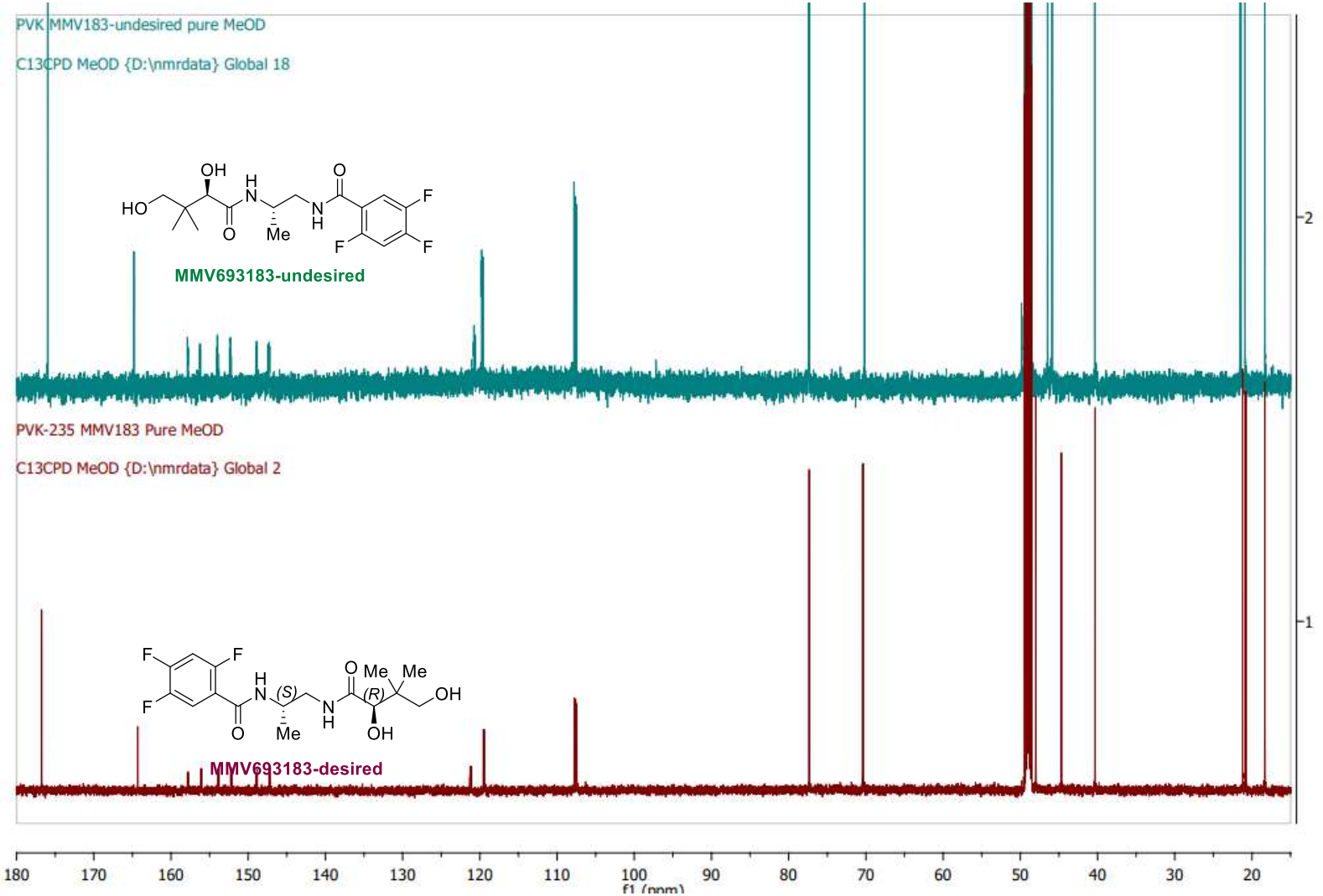


Fig S21. ^{13}CNMR data comparison of MMV693183 (desired and undesired regisomers) in CD_3OD