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Supporting Information

for

Characterization of two new degradation products of atorvastatin calcium formed upon treatment with strong acids

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Materials and methods; stress tests and analytical data of the products obtained thereby; details of characterization of 6 and 7 by X-ray data; crystallographic data for 6 and 7, NMR spectra of artefacts 2, 3, 6, and 7

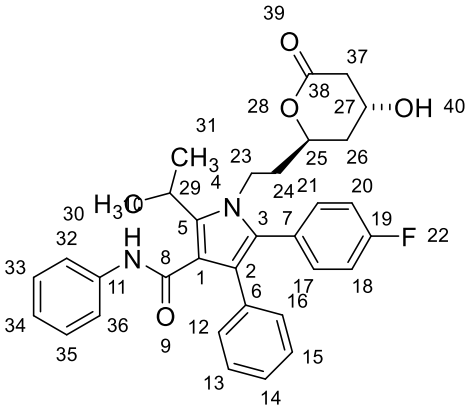
Table of contents:

1. Materials and methods

Atorvastatin calcium trihydrate (Ph. Eur.) was a gift of Prof. Dr. W. Frieß, LMU Munich. All solvents used were of HPLC grade or p.a. grade and/or purified according to standard procedures. Chemical reagents were purchased from Sigma-Aldrich (Schnelldorf, Germany) and Acros (Geel, Belgium). IR-spectra: Jasco FT/IR 4600 series (KBr pellet method); MS: Hewlett Packard MS-Engine, electron ionisation (EI) 70 eV, chemical ionisation (CI) with CH_4 (300 eV); MS spectra: Thermo Q Exactive GC Orbitrap or Finnigan MAT 95 spectrometer, HRESIMS spectra: Thermo Finnigan LTQ FT. NMR: Avance III HD 400 MHz Bruker BioSpin (^{1}H: 400 MHz, ^{13}C: 100 MHz); 500 MHz Avance III HD 500 MHz Bruker BioSpin (^{1}H: 500 MHz, ^{13}C: 125 MHz); melting points: Büchi Melting Point B-540 (not corrected); flash column chromatography (FCC): silica gel 60 (230–400 mesh, E. Merck, Darmstadt); HPLC: Shimadzu LC 10 pump, Shimadzu column oven CTO-10AS, Shimadzu autosampler SIL 10A, UV-detector Shimadzu LC 10 AS, column: Eurospher 100 – C18, 4 mm ID, (Knauer). Polarimeter: Perkin Elmer 241.

2. Stress tests and analytical data of the products obtained thereby

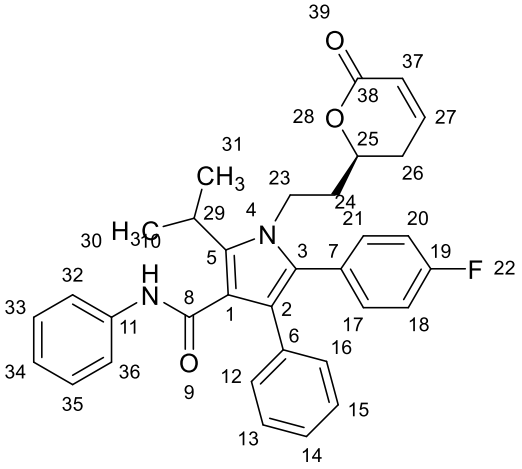
5-(4-Fluorophenyl)-1-(2-((2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-2-isopropyl-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (**2**)



1.025 g (0.848 mmol) of atorvastatin calcium trihydrate (**1**) was dissolved in 50 mL 2 M aqueous hydrochloric acid and stirred for 2 h. Then the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by flash column chromatography (ethyl acetate) to give 503 mg (55%) of **2** as a white solid. The same product could be isolated when **1** was treated with 2 M aqueous hydrochloric acid under reflux for 4 h to give 595 mg (65%) of **2**. M.p. 165 °C (ref. [1] 160 – 162

°C). $[\alpha]_D^{20}$ = + 19.6 (c 0.745, DMF); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 – 7.04 (m, 9 H, 9 arom. CH), 7.02 – 6.89 (m, 5 H, 5 arom. CH), 6.80 (s, 1 H, NH), 4.56-4.48 (m, 1 H, CH, 25-H), 4.34-4.27 (m 1 H, CH, 27-H), 4.22 (ddd, *J* = 14.9, 10.3, 5.0 Hz, 1 H, CH_2, 23-H), 4.03 (ddd, *J* = 14.9, 9.9, 5.7 Hz, 1 H, CH_2, 23-H), 3.62 – 3.45 (m, 1 H, CH, 29-H) , 2.66 (dd, *J* = 17.7, 4.8 Hz, 1 H, CH_2, 37-H), 2.56 (ddd, *J* = 17.8, 3.5, 1.6 Hz, 1 H, CH_2, 37-H), 1.86-1.74 (m, 1 H, CH_2, 24-H), 1.74 – 1.60 (m, 2 H, 2 CH_2, H-24, H-26), 1.56 – 1.49 (m, 1 H, CH_2, H-26), 1.58 – 1.49 (m, 6 H, 2 CH_3, 31-H, 30-H)). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3 (CO, C-38), 164.9 (CO, C-8), 162.4 (d, *J* = 249.6 Hz, quat. C, C-19), 141.4 (quat. C), 138.2 (quat. C), 134.4 (quat. C), 133.1 (d, *J* = 9,0 Hz, 2 arom. CH), 130.4 (2 arom. CH), 128.7 (quat. C), 128.7 (2 arom. CH), 128.4 (2 arom. CH), 128.0 (d, *J* = 3.2 Hz, quat. C), 126.7 (arom. CH), 123.7 (arom. CH), 122.1 (quat. C), 119.7 (2 arom. CH), 115.7 (quat. C), 115.6 (d, *J* = 20.76 Hz, 2 arom. CH), 73.0 (CH, C-25), 62.5 (CH, C-27), 40.8 (CH_2, C-23), 38.5 (CH_2, C-37), 37.2 (CH_2, C-24), 35.7 (CH_2, C-26), 26.2 (CH, C-29), 22.0 (CH_3), 21.7 (CH_3). IR (KBr): ν (cm^{-1}) = 3405, 2963, 2930, 1724, 1653, 1507, 1437, 1314, 1225, 1156, 1110, 1074, 1031, 1011, 915, 885, 808, 754, 694, 617. HR-ESI-MS calcd. for $C_{33}H_{34}FN_2O_4^+$ $[M+H]^+$: 541.2497, found: 541.2498.

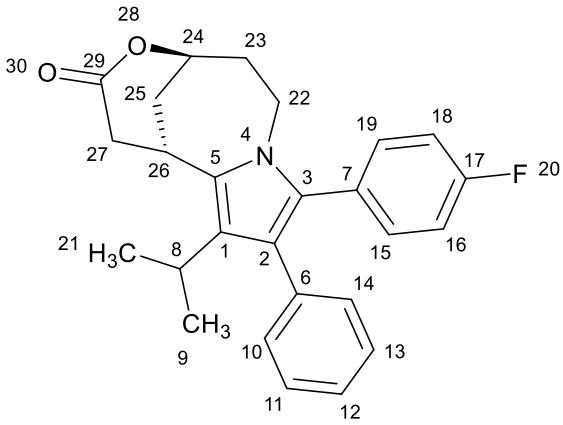
(*S*)-5-(4-Fluorophenyl)-2-isopropyl-1-(2-(6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (3)



1.00 g (0.841 mmol) of atorvastatin calcium trihydrate (**1**) was dissolved in 200 mL toluene, 200 mg (1.07 mmol) *p*-toluenesulfonic acid were added and the suspension was refluxed for 5 h at a water separator. The solvent was evaporated, the residue dissolved in 50 mL aqueous 2 M hydrochloric acid and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, the solvent was evaporated and the residue was purified by flash column chromatography (isohexane/ethyl acetate 1:1) to give 795 mg (95%) of **3** as an amorphous solid with an extremely broad melting interval (168–195 °C). $[\alpha]_D^{20}$ = −49.6 (c 0.74, DMF). The same

product could be isolated in >98% yield after refluxing atorvastatin calcium trihydrate (**1**) for two hours with 20% aqueous sulfuric acid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 – 7.11 (m, 9 H, 9 arom. CH), 7.06 (d, *J* = 7.7 Hz, 2 H, 2 arom. CH), 7.04 – 6.94 (m, 3 H, 3 arom. CH), 6.87 (s, 1 H, NH), 6.78 (ddd, *J* = 8.9, 5.7, 2.6 Hz, 1 H, -CH=, 27-H), 6.00 – 5.94 (m, 1 H, CH=, 37-H), 4.29-4.18 (m, 2 H, CH, CH_2, 23-H, 25-H), 4.10-4.00 (m, 1 H, CH_2, 23-H), 3.63-3.51 (m, 1 H, CH, 29-H), 2.22 - 2.06 (m, 2 H, CH_2,26-H), 2.04 - 1.92 (m, 1 H, CH_2, H-24), 1.84 – 1.73 (m, 1 H, CH_2, H-24), 1.57-1.51 (m 6 H, 2 CH_3, 30-H, 31-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.7 (CO), 163.5 (CO), 162.3 (d, *J* = 247.8 Hz, quat. C), 144.7 (CH=, C-27), 141.3 (quat. C), 138.3 (quat. C), 136.35 (quat. C), 133.1 (d, *J* = 8.1 Hz, 2 arom. CH), 130.4 (2 arom. CH), 128.7 (2 arom. CH), 128.4 (2 arom. CH), 128.1 (d, *J* = 4.2 Hz, quat. C), 127.5 (quat. C), 126.7 (arom. CH), 123.6 (arom. CH), 122.1 (quat. C), 121.3 (CH=, C-37), 119.6 (2 arom. CH), 115.7 (d, *J* = 21.4 Hz, 2 arom CH), 75.2 (CH, C-25), 40.6 (CH_2, C-23), 36.4 (CH_2, C-24), 29.1 (CH_2, C-26), 26.2 (CH, C-29), 22.0 (CH_3), 21.7 (CH_3). IR (KBr): ν (cm^{-1}) = 3402, 2959, 2928, 1722, 1665, 1595, 1526, 1242, 1155, 1043, 816, 753, 693. MS (EI): m/z = 522 (M^+, 10), 430 (100). HR-MS calcd. for $C_{33}H_{31}O_3N_2F^{+\cdot}$ [$M^{+\cdot}$]: 522.2313, found: 522.2313.

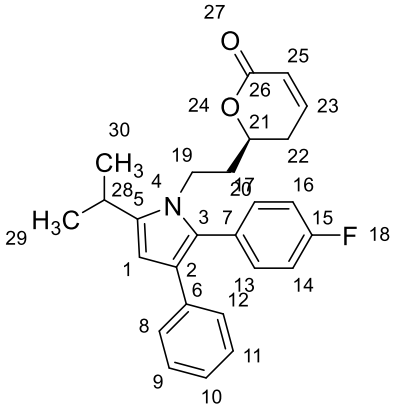
(1*S*,5*R*)-9-(4-Fluorophenyl)-11-isopropyl-10-phenyl-1,2,6,7-tetrahydro-3*H*,5*H*-1,5-methanopyrrolo[1,2-*e*][1,5]oxazonin-3-one (6)



2.41 g (2.00 mmol) of atorvastatin calcium trihydrate (**1**) was dissolved in 50 mL concentrated (37%) aqueous hydrochloric acid and refluxed for 5 h. After addition of 40 mL water the mixture was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by flash column chromatography (isohexane-ethyl acetate 1:1) to give 775 mg (96%) of **6** as a white solid. M.p.: 208 °C. $[\alpha]_D^{20}$ = -68.7 (c 2.07, DMF); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 – 7.03 (m, 3 H, 3 arom. CH), 7.02 – 6.91 (m, 4 H, 4 arom. CH), 6.90 – 6.79 (m, 2 H, 2 arom. CH), 5.09 – 5.03 (m, CH, 24-

H), 3.95 (ddd, *J* = 15.6, 4.8, 2.8 Hz, 1 H, CH_2 22-H), 3.87 (dd, *J* = 9.3, 5.8 Hz, 1 H, CH, 26-H), 3.63 (dd, *J* = 15.6, 12.2 Hz, 1 H, CH_2, 22-H), 3.06 (dd, *J* = 19.5, 10.2 Hz, 1 H, CH_2), 2.92 – 2.78 (m, 1 H, CH, 8-H), 2.51 – 2.41 (m, 1 H, CH_2, 27-H), 2.35 – 2.20 (m, 1 H, CH_2, 23-H), 2.06 – 1.96 (m, 1 H, CH_2. 27-H), 1.94 - 1.86 (m, 1 H, CH_2), 1.10 (m, , 6 H, 2 CH_3, 21-H, 9-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (CO, C-29), 161.8 (d, *J* = 247 Hz, quat. C, C-17), 136.5 (quat. C), 132.7 (d, *J* = 7.9 Hz, 2 arom. CH), 131.7 (quat. C), 131.1 (2 arom. CH), 129.9 (quat. C), 128.3 (d, *J* = 3.4 Hz, quat. C), 127.6 (2 arom. CH), 125.7 (arom. CH), 125.4 (quat. C), 122.3 (quat. C) 115.1 (d, *J* = 21.24 Hz, 2 arom. CH), 77.3 (CH, C-24), 39.6 (CH_2, C-22), 34.7 (CH_2), 34.3 (CH_2), 30.6 (CH_2, C-27), 26.5 (CH), 25.2 (CH), 24.2 (CH_3), 24.0 (CH_3). IR (KBr): ν (cm^{-1}) = 2961, 2939, 1742, 1719, 1602, 1526, 1508, 1469, 1347, 1241, 1222, 1087, 1057, 846, 704. HR-ESI-MS calcd. for $C_{26}H_{27}FNO_2^+$ $[M+H]^+$: 404.2020, found: 404.2020.

(*S*)-6-(2-(2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-1*H*-pyrrol-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (7)



992 mg (0.821 mmol) of atorvastatin-calcium trihydrate (**1**) was dissolved in 20 mL concentrated sulfuric acid and the mixture was stirred for 2 h at 60 °C. The mixture was carefully diluted with 40 mL ice-water and extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, the solvent was evaporated and the residue purified by flash column chromatography (isohexane-ethyl acetate 1:1) to give 117 mg (18%) of **7** as a grey solid. $[\alpha]_D^{20}$ = -46.7 (c 0.51, DMF); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.24 (m, 2 H, 2 arom. CH), 7.19 – 6.99 (m, 7 H, 7 arom. CH), 6.77 (ddd, *J* = 9.7, 5.9, 2.6 Hz, 1 H, CH=, 23-H), 6.20 (s, 1 H, arom. CH, 1-H), 5.96 (ddd, *J* = 9.7, 2.6, 1.0 Hz, 1 H, CH=, 25-H), 4.21 – 4.06 (m, 2 H, CH, CH_2, 21-H, 19-H), 3.98 (ddd, *J* = 14.9, 9.1, 6.5 Hz, 1 H, CH_2, 19-H), 3.02 (m, 1 H, CH, 28-H), 2.17 – 1.93 (m, 2 H, CH_2), 1.86 (dtd, *J* = 13.9, 8.9, 5.0 Hz, 1 H, CH_2), 1.74 – 1.57 (m, 1 H, CH_2), 1.39 – 1.29 (m, 6 H, 2 CH_3, 29-H, 30-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.7 (CO, C-26), 162.3 (d, *J* = 248.4 Hz, Hz, quat. C, C-15), 144.7 (-CH=, C-23), 140.6 (quat. C), 136.3 (quat. C), 132.9 (d, *J* = 8.2 Hz, 2 arom.

CH), 129.6 (d, *J* = 3.3 Hz, quat. C), 128.0 (2 arom. CH), 127.7 (quat. C), 127.5 (2 arom. CH), 125.0 (arom. CH), 122.6 (quat. C), 121.3 (-CH=, C-25), 115.9 (d, *J* = 16.0 Hz, 2 arom. CH), 103.9 (arom. CH, C-1), 75.1 (CH, C-21), 39.5 (CH_2, C-19), 36.2 (CH_2, C-20), 29.1 (CH_2, C-22), 25.7 (CH, C-28), 23.8 (CH_3), 23.5 (CH_3). MS (EI) m/z = 403 (M^+, 100), 388 (59), 360 (19), 276 (56). IR (KBr): ν (cm^{-1}) = 2962, 2925, 1720, 1599, 1523, 1507, 1348, 1240, 1222, 1087, 851, 695. HR-MS calcd. for $C_{26}H_{26}FNO_2$ [M^+]: 403.1948, found: 403.1951.

3. Details of characterization of 6 and 7 by X-ray data

The data of **6** have been collected at room temperature on a Bruker D8 Quest IμS diffractometer while those of **7** have been collected at 112 K on a Bruker D8 Venture TXS diffractometer. Mo Kα radiation monochromated by multilayer mirror optics was applied in both experiments. The frames were integrated with the Bruker SAINT software package [2] using a narrow-frame algorithm. Data were corrected for absorption effects using the Multi-Scan method of SADABS [3]. The structures were solved and refined using the Bruker SHELXTL Software Package [4]. The hydrogen atoms were calculated in ideal geometry riding on their parent atoms. The data quality of **6** did not allow the determination of the correct absolute structure (the randomly chosen structure solution used to draw Figure 2 in the manuscript shows (*R*)-configuration at C3 and (*S*) configuration at C5 (numbering used in the graphic in Figure 2), as well as the stereocenters of the two independent molecules not displayed herein: C29, C31, C55 and C57). Hence, this structure has been refined as perfect inversion twin. However, the data of **7** led to a Flack parameter of 0.1(2) indicating the correct absolute structure as that with an (*S*)-configured stereocenter (data have been collected up to a resolution of 0.60 Å). The figures were drawn at the 50% ellipsoid probability level (ORTEP [5]).

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1907390 (**6**), 1907391 (**7**)). These supplementary crystallographic data can be obtained free of charge from The Cambridge Crystallographic Data Centre via https://www.ccdc.cam.ac.uk/structures/.

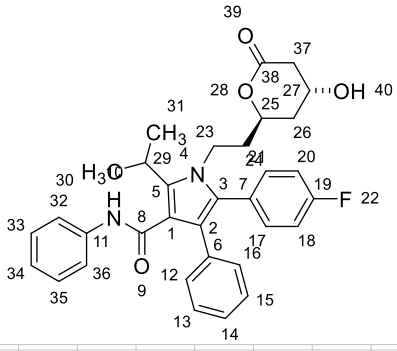
4. Crystallographic data for 6 and 7

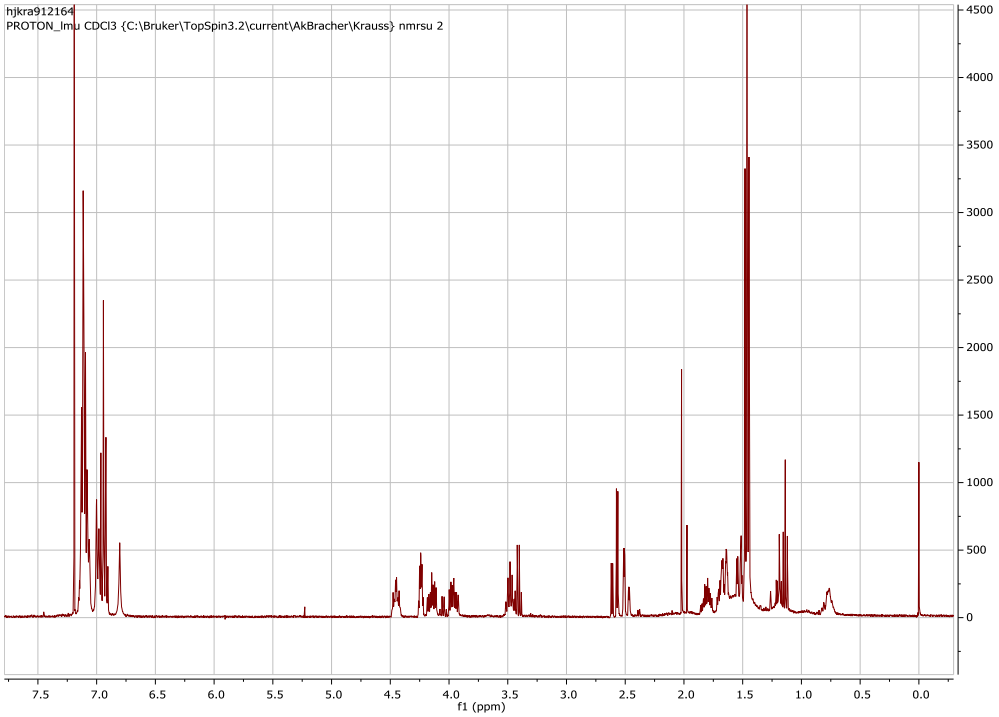
Table S1

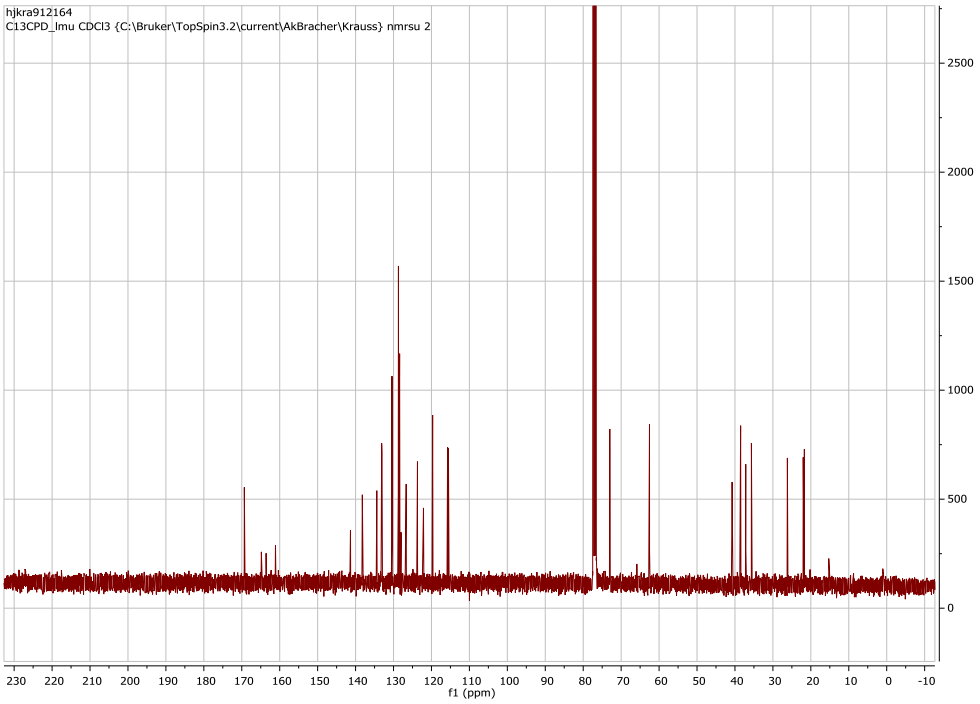
	6	**7**
CCDC	1907390	1907391
net formula	$C_{26}H_{26}FNO_2$	$C_{26}H_{26}FNO_2$
M_r/g mol^{-1}	403.48	403.48
crystal size/mm	0.100 × 0.030 × 0.020	0.100 × 0.070 × 0.060
crystal system	orthorhombic	orthorhombic
space group	'P 21 21 21'	'P 21 21 21'
a/Å	9.6001(8)	9.7398(4)
b/Å	17.4122(16)	10.4787(5)
c/Å	38.547(4)	20.4221(9)
α/°	90	90
β/°	90	90
γ/°	90	90
V/Å^3	6443.5(10)	2084.29(16)
Z	12	4
calc. density/g cm^{-3}	1.248	1.286
μ/mm^{-1}	0.084	0.087
transmission factor range	0.96–1.00	0.96–0.99
refls. measured	77036	48775
R_{int}	0.1383	0.0545
mean σ(*I*)/*I*	0.0889	0.0418
θ range	2.339–25.350	3.484–36.313
observed refls.	7567	8541
x, y (weighting scheme)	0.0342, 2.1173	0.0756, 0.0638
Flack parameter	0.5	0.1(2)
refls in refinement	11793	10078
parameters	818	273
restraints	0	0
$R(F_{obs})$	0.0667	0.0524
$R_w(F^2)$	0.1283	0.1307
S	1.038	1.082
shift/error$_{max}$	0.001	0.001
max electron density/e Å^{-3}	0.172	0.490
min electron density/e Å^{-3}	−0.190	−0.287

5. NMR spectra

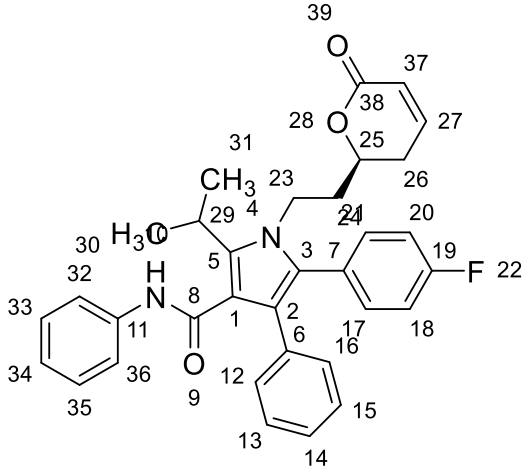
5-(4-Fluorophenyl)-1-(2-((2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl)ethyl)-2-isopropyl-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (**2**)

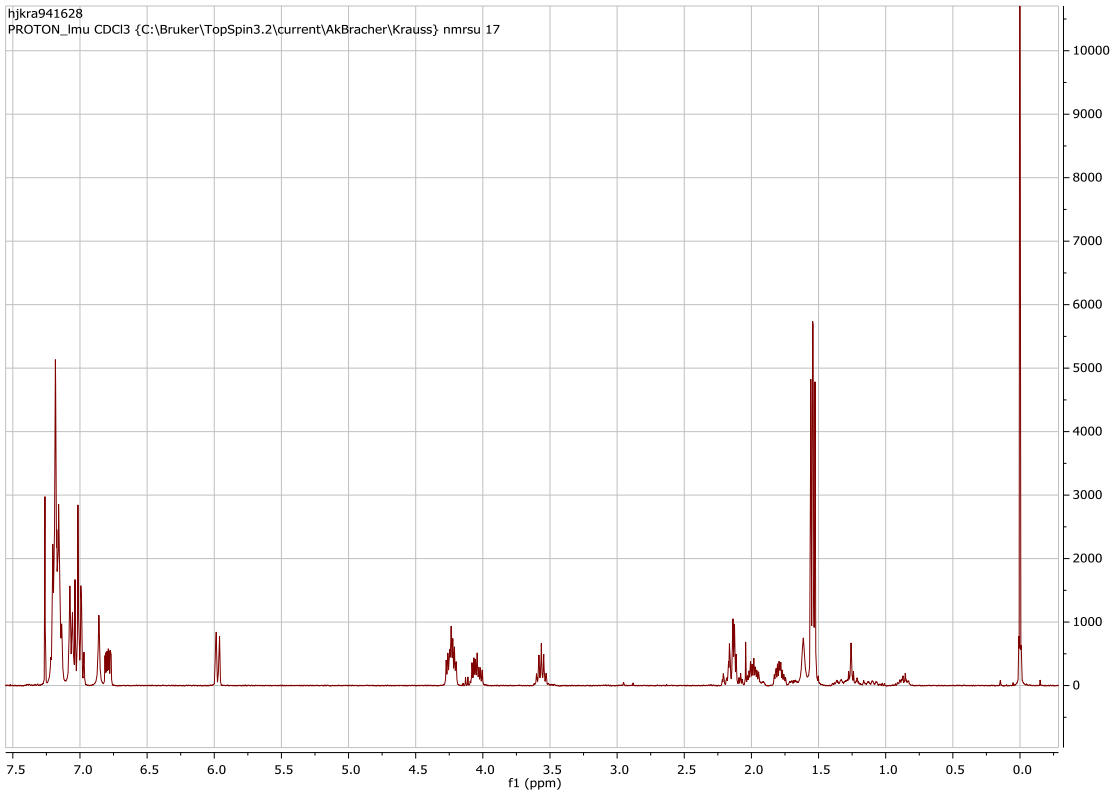






(*S*)-5-(4-Fluorophenyl)-2-isopropyl-1-(2-(6-oxo-3,6-dihydro-2*H*-pyran-2-yl)ethyl)-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide (3)

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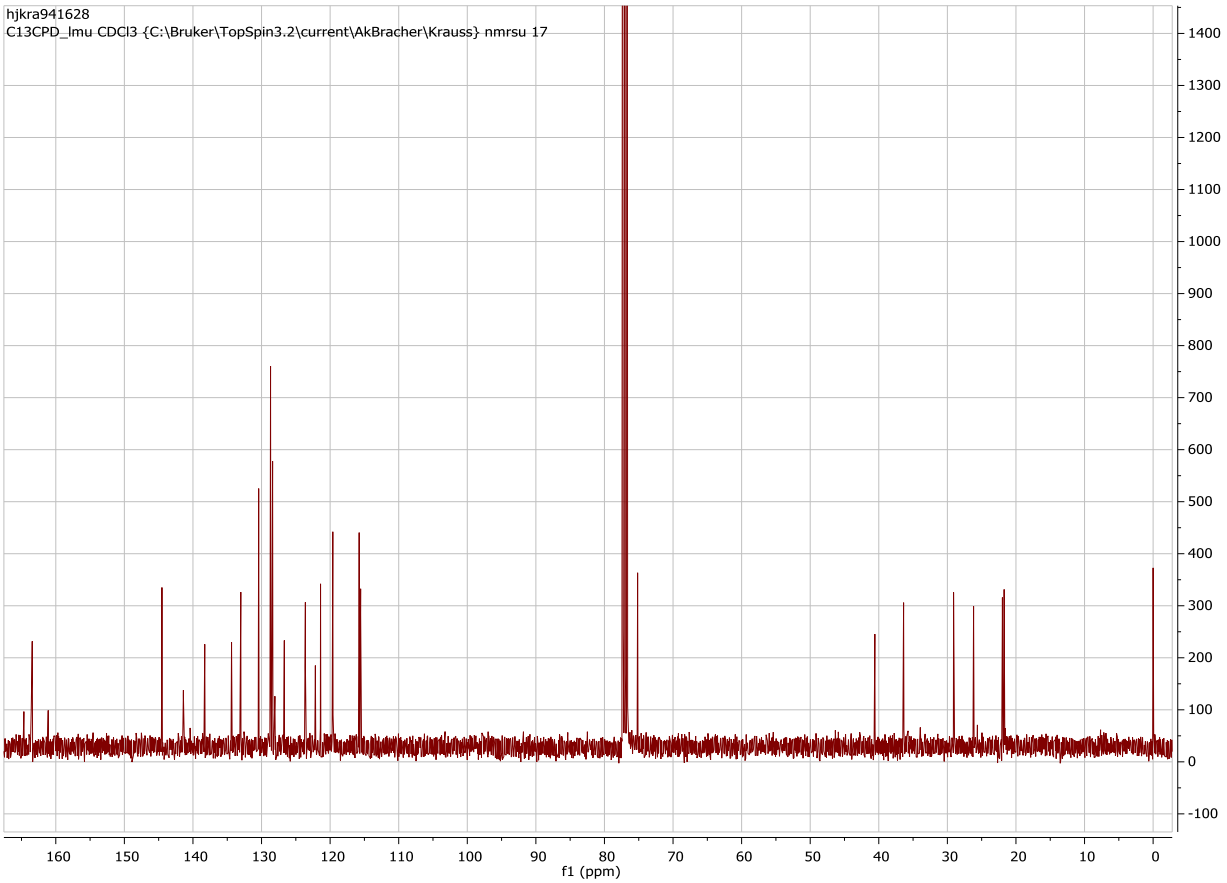
f1 (ppm)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500
400
300
200
100
0
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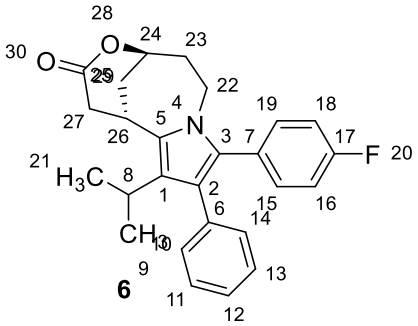
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

(1*S*,5*R*)-9-(4-Fluorophenyl)-11-isopropyl-10-phenyl-1,2,6,7-tetrahydro-3*H*,5*H*-1,5-methanopyrrolo[1,2-*e*][1,5]oxazonin-3-one (6)



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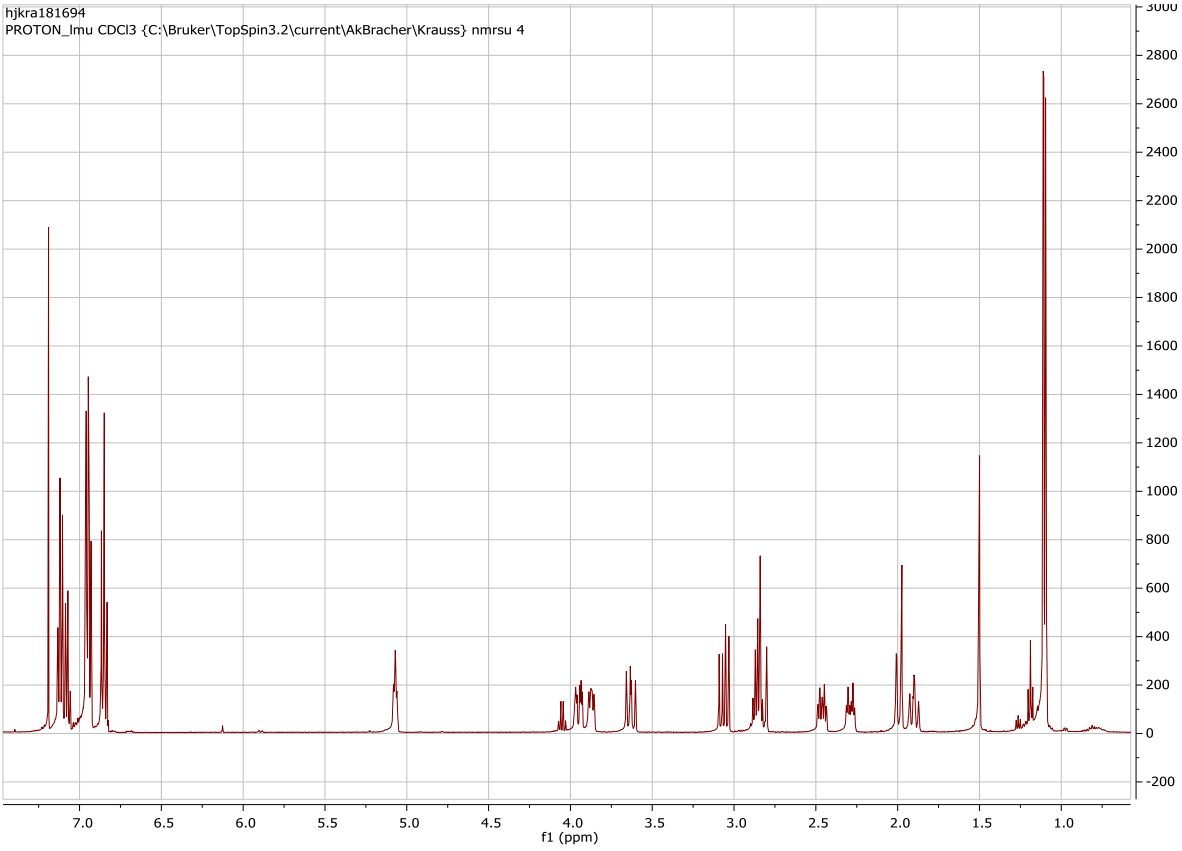
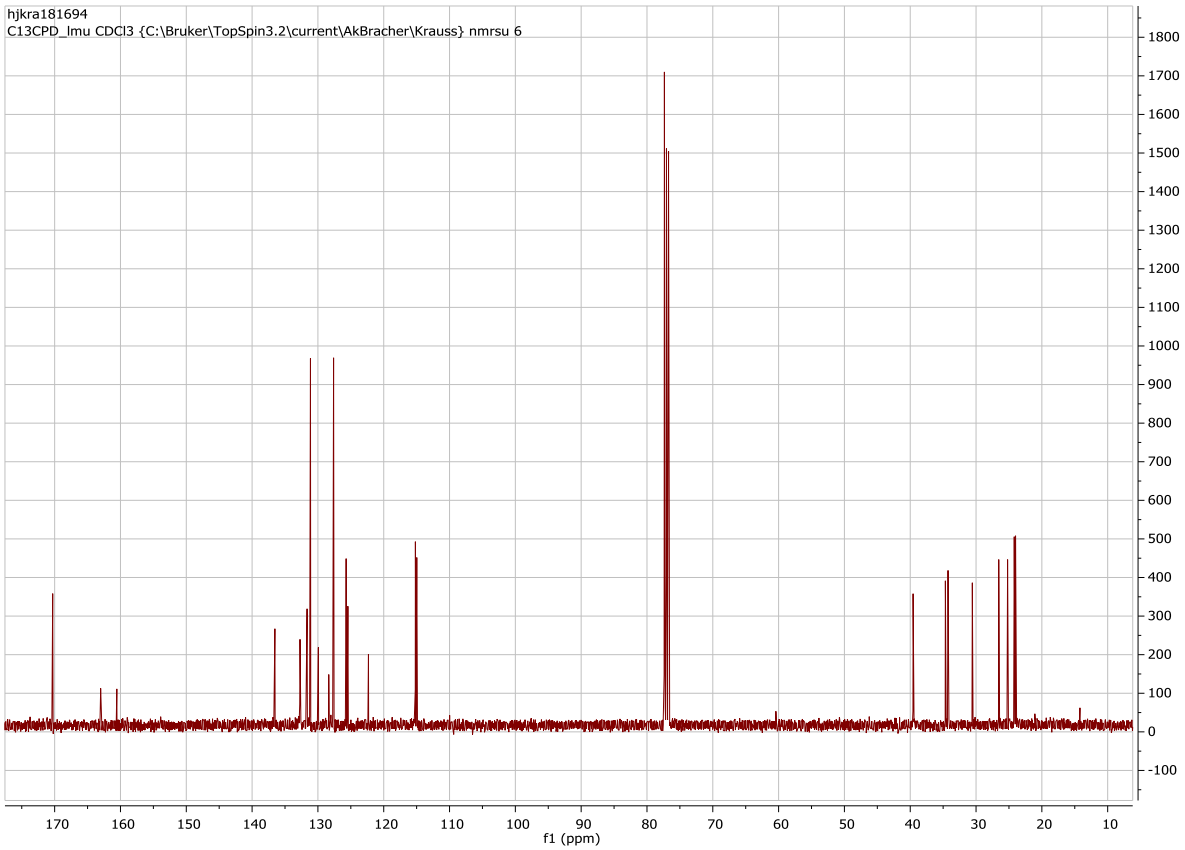
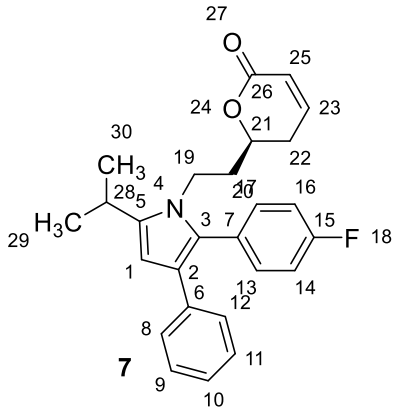
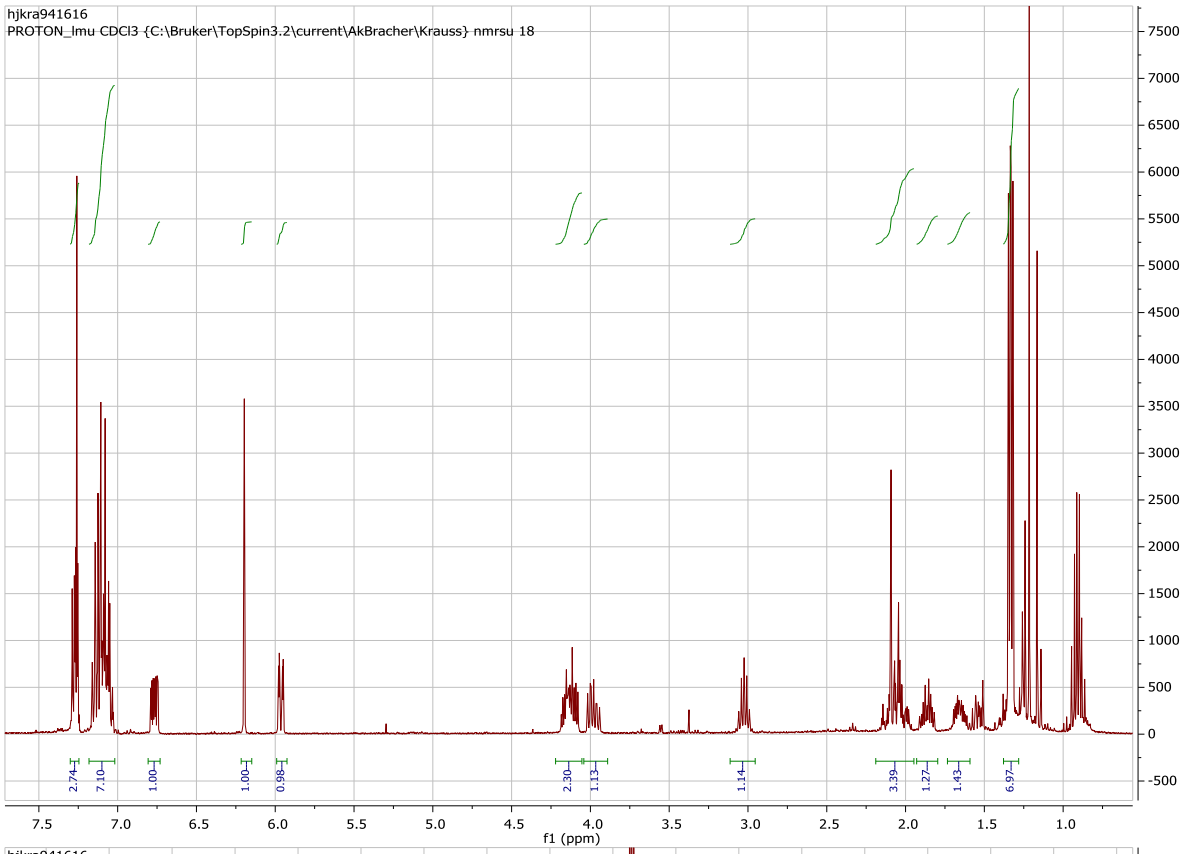




(*S*)-6-(2-(2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-1*H*-pyrrol-1-yl)ethyl)-5,6-dihydro-2*H*-pyran-2-one (7)

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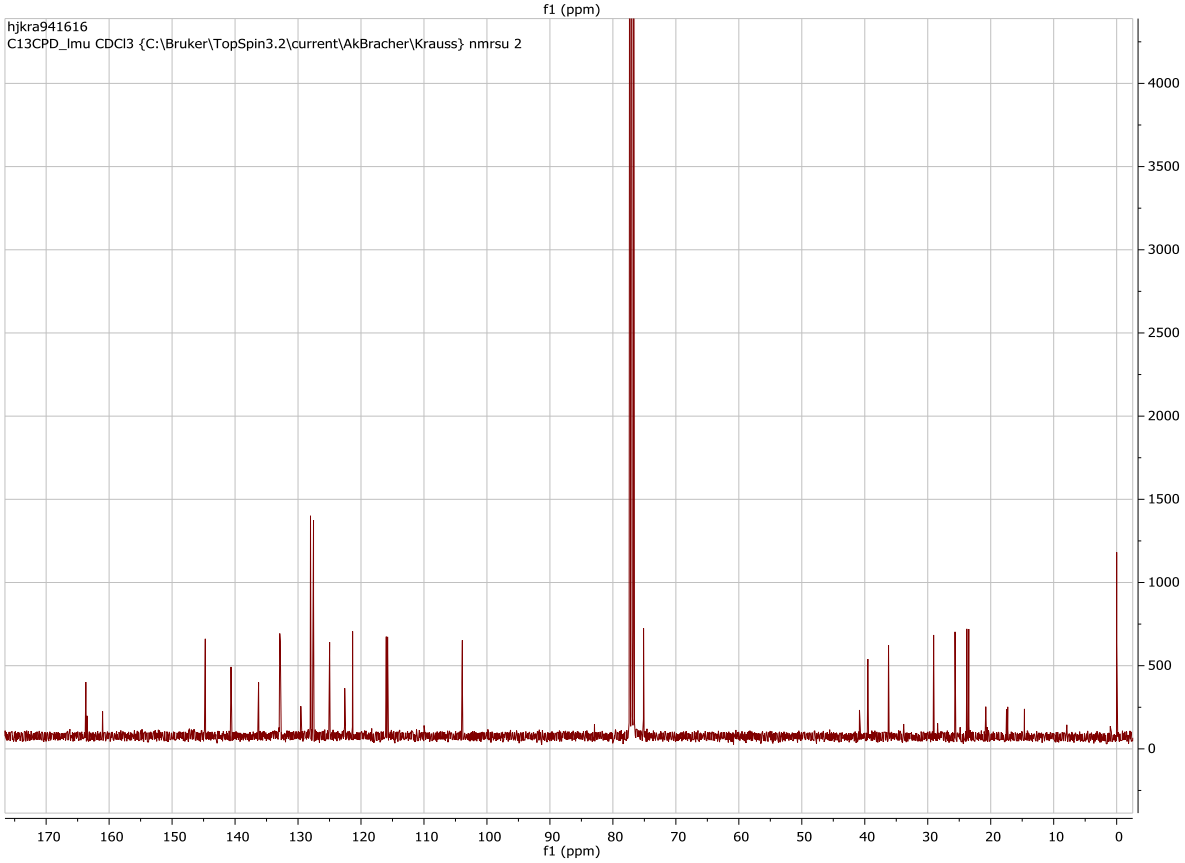
2.30
1.13
1.14
3.39
1.27
1.43
6.97
f1 (ppm)


hjkra941616
C13CPD_lmu CDCl3 {C:\Bruker\TopSpin3.2\current\AkBracher\Krauss} nmrsu 2
f1 (ppm)

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