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SUPPORTING INFORMATION

Enantioselective Total Synthesis of ROCK-inhibitor (*S*)-Netarsudil (Rhopressa) *via* Asymmetric Organocatalysis.

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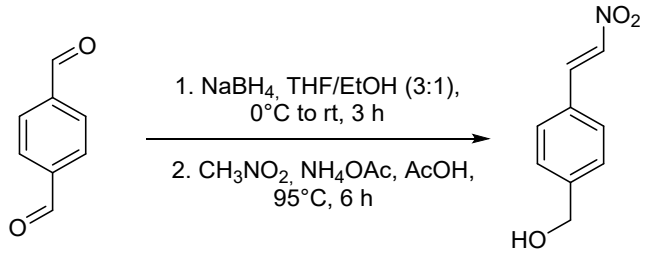
General Information

The ^{1}H, ^{13}C NMR spectra were recorded on a 300 MHz and 600 MHz spectrometers. For ^{1}H NMR, chemical shifts (δ) were given in ppm using residual undeuterated solvent as internal standard ($CDCl_3$ at 7.28 ppm, DMSO-d_6 at 2.51 ppm, CD_3OD at 3.33). For ^{13}C NMR, chemical shifts (δ) were reported in ppm using solvent as internal standard ($CDCl_3$ at 77.0 ppm, DMSO-d_6 at 40.0 ppm, CD_3OD at 47.6). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, tt = triplet of triplets, m = multiplet, brs = broad singlet. Coupling constants are reported as a *J* value in Hertz (Hz). The high-resolution mass spectra (HRMS) were recorded using electrospray ionization (ESI) and a time-of-flight (TOF) mass analyzer. The measurements were taken in the positive ion mode (interface capillary voltage 4500 V) in the mass range from m/z = 50 Da to m/z = 3000 Da; external and internal calibrations were done with the electrospray calibrant solution. For centrifugation was used ultramicrocentrifuge with 21.000 rpm speed. HPLC analyses were performed on an HPLC system equipped with chiral stationary phase columns (AD-H, OD-H, OJ-H, AS-H), detection at 220 nm. Allomaltol **8** are commercially available. Catalysts **I-XII** were synthesized by reported procedures,[1-6] catalysts **XIII**, **XIV** are commercially available. Reagents and solvents were purified according to standard methods.

2. Experimental procedures

2.1. Stage 1. Synthesis of *β*-nitrostyrene 10

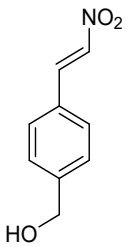


6 81% **10**

Compound **10** was prepared according to a modified literature procedure[7]. $NaBH_4$ (5.6 g, 0.15 mol, 0.25 equiv.) was added to a stirred solution of terephthalic aldehyde (80.0 g, 0.60 mol) in EtOH/THF (1:3, 400 mL) by small portions for 15 min at the 0°C. The reaction mixture was stirred for 3 h. Then HCl (1M) was added to pH 4. The solvents were evaporated, the slurry residue was redissolved in EtOAc (250 mL) and washed with water (3 x 150 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent was evaporated to afford crude 4-(hydroxymethyl)-benzaldehyde which was used in the next step without further purification.

NH_4OAc (137.9 g, 1.79 mol, 3 equiv.) and $MeNO_2$ (160 mL, 2.99 mol, 5 equiv.) were added sequentially to a solution of the crude 4-(hydroxymethyl)-benzaldehyde in AcOH (450 mL). The reaction mixture was gently refluxed at 95 °C for 6 h and cooled down to ambient temperature. The acetic acid was evaporated under reduced pressure, the EtOAc (300 mL) was added to the residue and the organic phase was washed with brine (3 x 150 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. MeOH (150 mL) and AcCl (0.9 mL, 12 mmol, 2 mol.%) were sequentially added to the residue and the mixture was stirred. for 3 h. The solvent was evaporated, the residue was recrystallized from $CHCl_3$ to afford analytically pure *β*-nitrostyrene **10**.

(*E*)-(4-(2-Nitrovinyl)phenyl)methanol (**10**)



10

Physical state yellow powder;

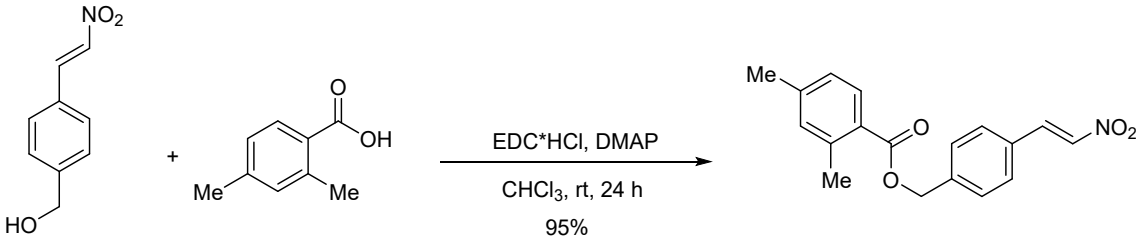
Yield 86.6 g (81 %);

MP 113-114 °C (lit.[8] 114 - 115.5 °C)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 13.7 Hz, 1H), 7.62-7.55 (m, 3H), 7.47 (d, *J* = 8.1 Hz, 2H), 4.78 (s, 2H), 1.97 (brs, 1H).

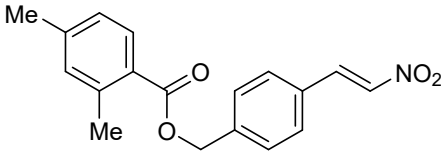
The ^{1}H NMR spectrum corresponds to literature data.[9]

2.2. Stage 2. Synthesis of *β*-nitrostyrene 7



β-Nitrostyrene **10** (35.8 g, 0.20 mol) was added to a solution of 2,4-dimethylbenzoic acid (**11**) (30.0 g, 0.20 mol), EDC*HCl (57.5 g, 0.30 mol, 1.5 equiv.), and DMAP (2.5 g, 20 mmol, 10 mol.%) in chloroform (250 mL). The reaction mixture was stirred for 24 h and washed with aq. $NaHCO_3$ (3 x 150 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from *n*-hexane/EtOAc solvent system to afford analytically pure *β*-nitrostyrene **7**.

(*E*)-4-(2-Nitrovinyl)benzyl 2,4-dimethylbenzoate (**7**)



7

Physical state yellow needles;

MP 79-81°C;

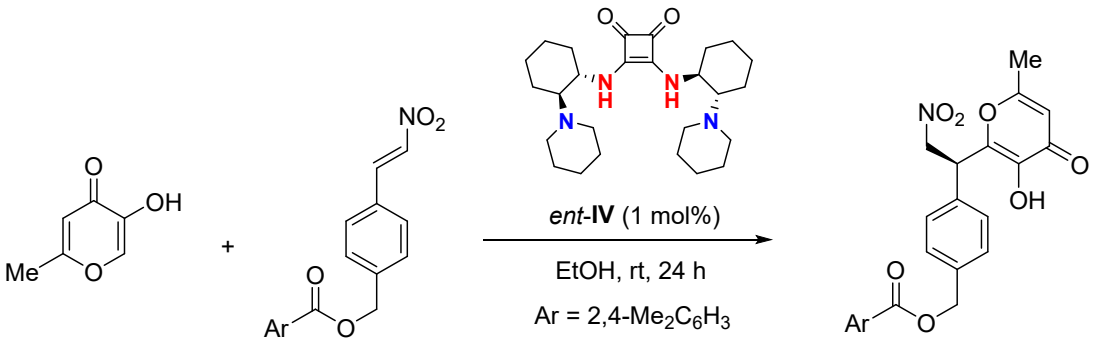
Yield 59.1 g (95 %);

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, *J* = 13.7 Hz, 1H), 7.91 (d, *J* = 7.9 Hz, 1H), 7.64-7.52 (m, 5H), 7.09-7.07 (m, 2H), 5.38 (s, 2H), 2.60 (s, 3H), 2.38 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 191.8, 167.0, 143.0, 140.8, 140.7, 138.5, 137.3, 132.7, 130.9, 130.0, 129.8, 129.4, 128.7, 128.1, 126.6, 126.0, 65.4, 21.9, 21.4;

HRMS (ESI-TOF) m/z calcd. for [$C_{18}H_{18}NO_4^+$] ($M+H^+$) 312.1230, found 312.1235.

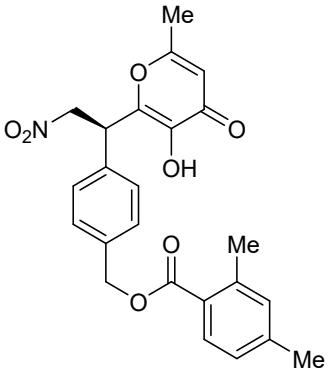
2.3. Stage 3. Asymmetric organocatalytic Michael reaction



8, **6.3 g** **7**, **15.6 g** *ent*-**9**, **19.6 g**, **90%**, **94% *ee***

Catalyst *ent*-**IV** (221 mg, 0.5 mmol, 1 mol.%) was added to a solution of allomaltol **8** (6.3 g, 50.0 mmol) and *β*-nitrostyrene **7** (15.6 g, 50.0 mmol) in EtOH (95%) (120 mL) and the reaction mixture was stirred for 24 h. The solvent was evaporated. The residue was dissolved in Et_2O (100 mL) and filtered through a short plug of SG. Evaporation of the solvent afforded analytically pure adduct *ent*-**9**.

(*S*)-4-(1-(3-Hydroxy-6-methyl-4-oxo-4H-pyran-2-yl)-2-nitroethyl)benzyl 2,4-dimethylbenzoate (*ent*-**9**)



ent-**9**

Physical state brown foam;

Yield 19.6 g (90 %);

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.8 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.07-7.04 (m, 2H), 6.24 (s, 1H), 5.32 (s, 2H), 5.25-5.09 (m, 2H), 4.94 (dd, *J* = 12.6, 6.2 Hz, 1H), 2.59 (s, 3H), 2.36 (s, 3H), 2.32 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 167.1, 165.7, 146.1, 142.8, 141.7, 140.6, 136.9, 135.3, 132.6, 130.9, 128.9, 128.0, 126.5, 126.2, 110.9, 75.3, 65.6, 43.0, 21.9, 21.4, 20.1;

HPLC data: 94% *ee* (CHIRALPAK AD-H column, *n*-hexane/*i*-PrOH 90:10, flow rate 1.00 mL/min, 220 nm; $t_{(R)}$minor = 19.8 min, $t_{(R)}$major = 25.4 min,);

HRMS (ESI-TOF) m/z calcd. for [$C_{24}H_{24}NO_7^+$] ($M+H^+$) 438.1547, found 438.1559.

2.4. Procedure for regeneration of catalyst *ent*-IV

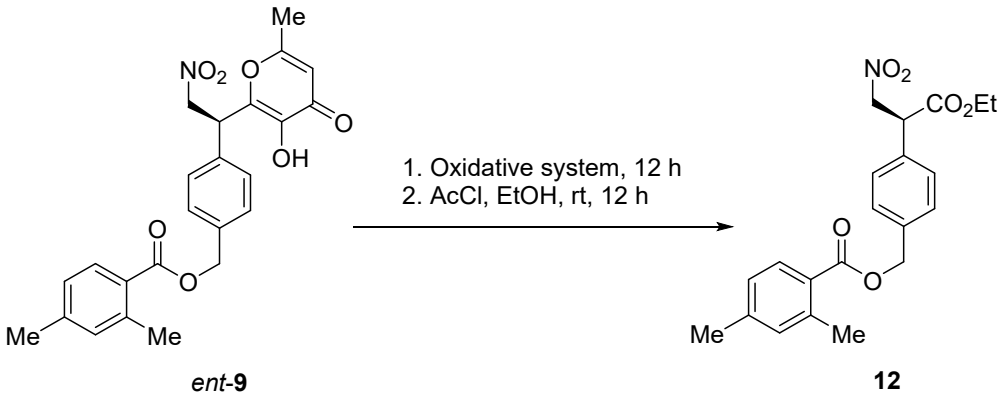
Catalyst *ent*-**IV** (1 mol%, 4 mg) was added to a solution of allomaltol **8** (126 mg, 1 mmol) and *β*-nitrostyrene **7** (311 mg, 1 mmol) in ethanol (5 mL) and the reaction mixture was stirred at ambient temperature for 24 h. The solvent was evaporated and the Michael adduct *ent*-**9** was extracted with Et_2O (3 x 15 mL) with careful decantation of the organic layers. The solid catalyst *ent*-**IV** remaining in the flask was dried under reduced pressure (50 Torr, 60^oC, 1 h). Then, fresh portions of reagents **8**, **7**, and EtOH were added to the flask and the reaction was re-performed as described above. The same catalyst sample could be recovered 4 times while retaining high product yield and very good stereoinduction (see table S1).

Table S1 (Detalization of Figure 2 in the manuscript)

Cycle	Yield of *ent*-9, %	*ee*, %
1	90	94
2	91	93
3	88	94
4	90	93
5	75	91

2.5.1. Optimization of oxidative fragmentation process

Table 2. Optimization of oxidative fragmentation system.[a]

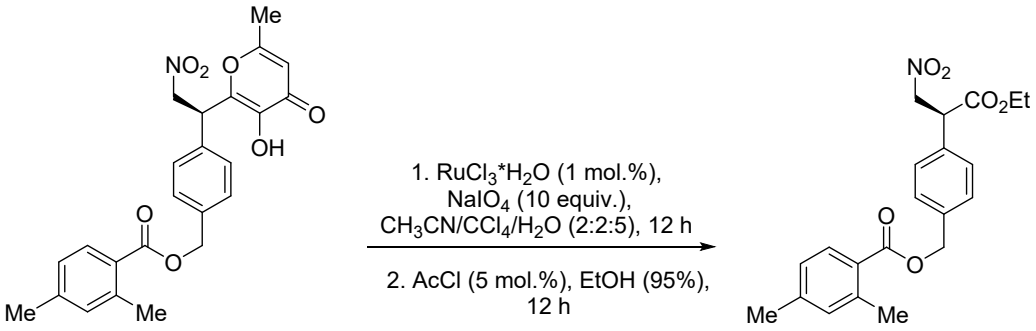


ent-**9** **12**

Entry	Oxidative system	$Ru^{III}/NaIO_4$ (mol.%/ equiv.)	Solvent system (vol.%/vol.%/vol.%)	Yield, **12** (%)[b]
1	$RuCl_3*3H_2O$, $NaIO_4$	1:10	$CH_3CN/CCl_4/H_2O$ (2:2:5)	92%
2	$RuCl_3*3H_2O$, $NaIO_4$	2:10	$CH_3CN/CCl_4/H_2O$(2:2:5)	85%
3	$RuCl_3*3H_2O$, $NaIO_4$	1:20	$CH_3CN/CCl_4/H_2O$(2:2:5)	93%
4	$RuCl_3*3H_2O$, $NaIO_4$	0.5:10	$CH_3CN/CCl_4/H_2O$(2:2:5)	43%
5	$RuCl_3*3H_2O$, $NaIO_4$	1:5	$CH_3CN/CCl_4/H_2O$(2:2:5)	65%
6	$RuCl_3*3H_2O$, $NaIO_4$	0:10	$CH_3CN/CCl_4/H_2O$(2:2:5)	nr
7	$RuCl_3*3H_2O$, $NaIO_4$	1:0	$CH_3CN/CCl_4/H_2O$(2:2:5)	nr
8	$RuCl_3*3H_2O$, $NaIO_4$	1:10	$CH_3CN/EtOAc/H_2O$(2:2:5)	61%
9	$RuCl_3*3H_2O$, $NaIO_4$	1:10	$CH_3CN/DCM/H_2O$(2:2:5)	76%

[a]Unless otherwise specified, the reactions were carried out with corresponding oxidative system, *ent*-**9** (43.7 mg, 0.1 mmol) in the corresponding solvent system (1.0 mL) at ambient temperature for 24 h. [b]Yield obtained after extraction, filtration through SG and evaporation of solvent.

2.5.2. Stage 4. Oxidative fragmentation of Michael adduct *ent*-9



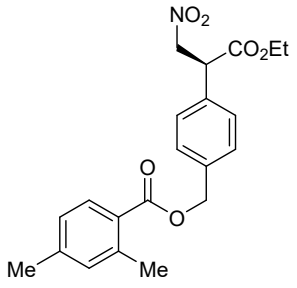
ent-**9**, **9.5 g**, **94% *ee***

12, **7.4 g**, **90%**, **93% *ee***

Water (150 mL) and $NaIO_4$ (46.5 g, 21.7 mmol, 10 equiv.) were added sequentially to a solution of *ent*-**9** (9.5 g, 21.7 mmol) in CH_3CN/CCl_4 (1 : 1) solvent system (120 mL) and the mixture was stirred for 5 min. Then, the $RuCl_3*3H_2O$ (56.8 mg, 0.22 mmol, 1 mol.%) was added and the suspension was stirred at ambient temperature for 12 h. Inorganic components were filtered off and the filtrate was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent was evaporated. The residue was dissolved in Et_2O (70 mL) and the solution was passed through a short plug of SG, the solvent was evaporated to afford crude β-nitrocarboxylic acid.

The crude acid was dissolved in EtOH (95%) (30 mL) and AcCl (1.5 mL) was carefully added to the solution. The reaction mixture was stirred for 12 h. The volatile materials were evaporated. The residue was purified by CC on SG (EtOAc/*n*-hexane = 1:3 eluent system) to afford analytically pure β-nitroester **12**.

(*S*)-4-(1-Ethoxy-3-nitro-1-oxopropan-2-yl)benzyl 2,4-dimethylbenzoate (**12**)



12

Physical state light-yellow oil;

Yield 7.4 g (90 %);

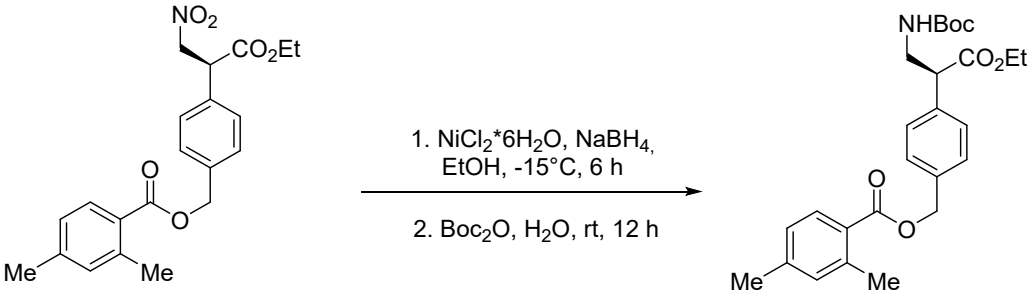
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 5.33 (s, 2H), 5.12 (dd, *J* = 14.5, 9.8 Hz, 1H), 4.57 (dd, *J* = 14.5, 5.2 Hz, 1H), 4.46 (dd, *J* = 9.8, 5.2 Hz, 1H), 4.32-4.22 (m, 1H), 4.22-4.12 (m, 1H), 2.60 (s, 3H), 2.37 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 167.1, 142.8, 140.6, 137.0, 133.2, 132.6, 130.9, 128.9, 128.1, 126.5, 126.2, 65.5, 62.0, 48.5, 21.8, 21.4, 14.0;

HPLC data: 93% *ee* (CHIRALPAK AS-H column, *n*-hexane/*i*-PrOH 90:10, flow rate 1.00 mL/min, 220 nm; $t_{(R)}$major = 13.8 min, $t_{(R)}$minor = 16.1 min);

HRMS (ESI-TOF) m/z calcd. for $[C_{21}H_{27}N_2O_6^+]$ $(M+NH_4^+)$ 403.1864, found 403.1848.

2.6. Stage 5. Reduction of *β*-nitroester 12



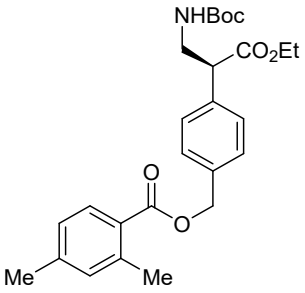
12, 5.1 g, 93% *ee*

13, 5.2 g, 86%, 94% *ee*

Nitroester **12** (5.1 g, 13.2 mmol) and $NaBH_4$ (2.5 g, 66.0 mmol, 5 equiv.) were added sequentially to a stirred solution of $NiCl_2$*$6H_2O$ (3.5 g, 14.5 mmol, 1.1 equiv.) in EtOH (95%) (80 mL) at -15°C (ice/salt bath) and the resulting suspension was stirred for 15 min at 0°C. The reaction mixture was adjusted to pH 3 by HCl (2M) and then adjusted to pH 9 by aq. $NaHCO_3$ The black precipitate was filtered off and the filtrate was extracted with DCM (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent was evaporated to afford crude β-amino ester.

The crude amino ester was dissolved in H_2O (20 mL) and Boc_2O (3.2 g, 14.5 mmol, 1.1 equiv.) was added to the solution in one portion. The reaction mixture was stirred for 12 h, extracted with CH_2Cl_2 (3 x 100 mL) and washed with brine (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, the solvent was evaporated. The residue was purified by CC on SG (EtOAc/*n*-hexane = 1:6 eluent system) to afford analytically pure *N*-Boc-protected β-amino ester **13**.

(*S*)-4-(3-((*tert*-Butoxycarbonyl)amino)-1-ethoxy-1-oxopropan-2-yl)benzyl 2,4-dimethylbenzoate (**13**)



13

Physical state colorless oil;

Yield 5.2 g (86 %);

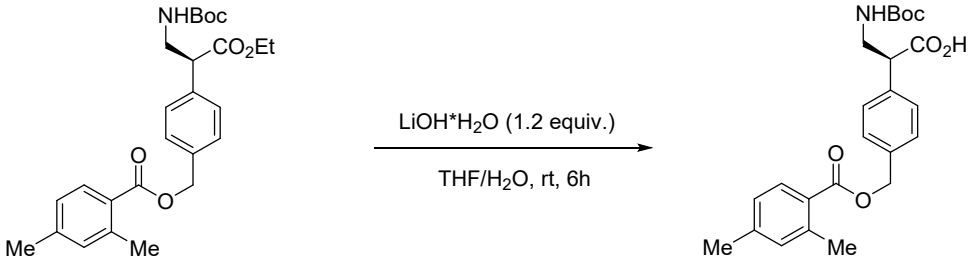
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.07-7.05 (m, 2H), 5.32 (s, 2H), 4.89 (brs, 1H), 4.24-4.11 (m, 2H), 3.93-3.89 (m, 1H), 3.68-3.47 (m, 1H), 2.60 (s, 3H), 2.37 (s, 3H), 1.44 (s, 9H), 1.23 (t, *J* = 7.2 Hz, 3H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 178.7, 167.2, 157.7, 142.7, 140.6, 136.4, 135.8, 132.5, 130.9, 128.5, 128.2, 126.5, 126.4, 79.7, 65.8, 61.1, 51.3, 43.4, 28.3, 21.8, 21.4, 14.1

HPLC data: 94% *ee* (CHIRALPAK OJ-H column, *n*-hexane/*i*-PrOH 90:10, flow rate 1.00 mL/min, 220 nm; $t_{(R)}$major = 6.3 min, $t_{(R)}$minor = 7.5 min);

HRMS (ESI-TOF) m/z calcd. for $[C_{26}H_{37}N_2O_6^+]$ $(M+NH_4^+)$ 473.2646, found 473.2657.

2.7. Stage 6. Hydrolysis of *N*-Boc-protected β-amino ester 13

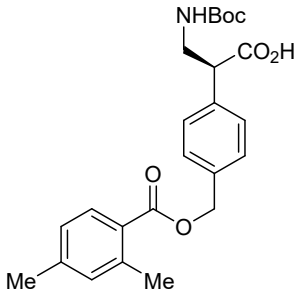


13, **4.2 g**, **94%*ee***

14, **3.8 g**, **96%**, **95% *ee***

A solution of LiOH*H_2O (465 mg, 11.1 mmol, 1.2 equiv.) in H_2O (5 mL) was added in one portion to a stirred solution of ester **13** (4.2 g, 9.2 mmol) in THF (25 mL). The reaction mixture was stirred for 6 h at ambient temperature. Then it was adjusted to pH 5 with HCl (2M), and extracted with EtOAc (3 x 70 mL). The combined organic layer was washed with brine (3 x 70 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated. The residue was recrystallized from *n*-hexane/EtOAc to afford analytically pure *N*-Boc-protected aminoacid **14**.

(*S*)-3-((*tert*-Butoxycarbonyl)amino)-2-(4-(((2,4-dimethylbenzoyl)oxy)methyl)phenyl)propanoic acid (**14**)



14

Physical state colorless crystals;

MP 99-100°C (lit.[11]98-100 °C)

Yield 3.8 g (96 %);

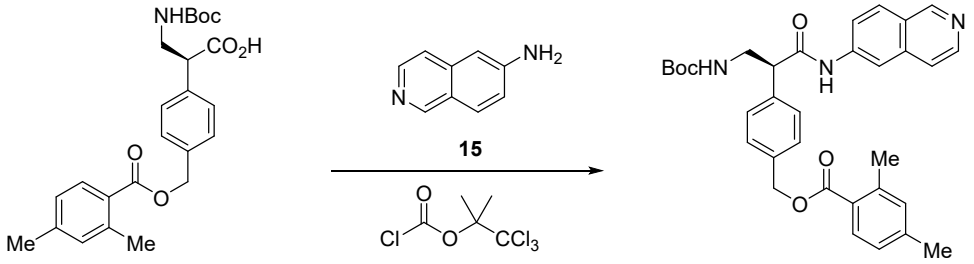
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.9 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.07 (s, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 5.32 (s, 2H), 5.02 (br s, 1H), 3.95–3.85 (m, 1H), 3.62–3.54 (m, 2H), 2.60 (s, 3H), 2.37 (s, 3H), 1.44 (s, 9H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 167.2, 157.9, 142.7, 140.6, 136.0, 135.9, 132.5, 130.9, 128.7, 128.3, 126.5, 126.3, 81.5, 65.8, 52.3, 44.6, 28.3, 21.9, 21.4;

HPLC data: 95% *ee* (CHIRALPAK AD-H column, *n*-hexane/*i*-PrOH 70:30, flow rate 1.00 mL/min, 220 nm; $t_{(R)}$major = 5.5 min, $t_{(R)}$minor = 7.7 min);

HRMS (ESI-TOF) m/z calcd. for [$C_{24}H_{30}NO_6^+$] ($M+H^+$) 428.2068, found 428.2060.

2.8. Stage 7. Amidation reaction between 14 and 15

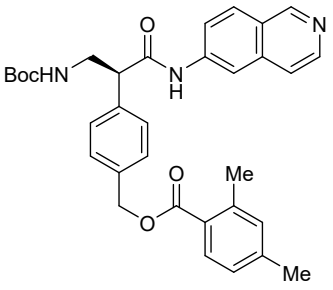


14, **3.8 g**, **95% *ee***

16, **2.9 g**, **60%**, **95% *ee***

6-Aminoisoquinoline (**15**) (1.28 g, 8.9 mmol) and 2,4,6-collidine (1.40 g, 11.6 mmol) were added to a stirred solution of *N*-Boc-protected aminoacid **14** (3.8 g, 8.9 mmol) in DMF (15 mL) at 0 °C. After 10 min, a solution of 2,2,2-trichloro-1,1-dimethylethyl chloroformate (2.78 g, 11.6 mmol) in DMF (10 mL) was added to the reaction mixture. After stirring at 0 °C for 12 h, the mixture was poured into aq. $NaHCO_3$ /EtOAc (1:1, 100 mL) and extracted with EtOAc (3 x 50 mL). The organic layers were washed brine (3 x 70 mL), dried over anhydrous Na_2SO_4. The solvent was evaporated. The residue was purified by CC on SG (EtOAc/*n*-hexane = 4:1 eluent system) to afford analytically pure Boc-amide **16**.

(*S*)-4-(3-((*tert*-Butoxycarbonyl)amino)-1-(isoquinolin-6-ylamino)-1-oxopropan-2-yl)benzyl 2,4-dimethylbenzoate (**16**)



16

Physical state light-yellow solid;

MP 153-154°C (lit.[11] 155 - 156°C)

Yield 2.9 g (60 %);

^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 10.60 (brs, 1H), 9.15 (brs, 1H), 8.40 (s, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.78-7.70 (m, 3H), 7.44 (brs, 4H), 7.12-7.03 (m, 3H), 5.27 (m, 2H), 4.13 (brs, 1H), 3.57 (brs, 1H), 2.48 (s, 3H), 2.30 (s, 3H), 1.34 (s, 9H);

^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 171.5, 166.9, 156.2, 152.0, 143.7, 142.9, 140.9, 140.0, 136.6, 135.7, 132.8, 130.9, 129.0, 128.7, 128.5, 127.1, 121.5, 120.5, 113.6, 78.2, 66.1, 52.2, 43.5, 28.7, 21.7, 21.3;

HPLC data: 95% *ee* (CHIRALPAK AD-H column, *n*-hexane/*i*-PrOH 70:30, flow rate 1.00 mL/min, 220 nm; $t_{(R)}$minor= 11.7 min, $t_{(R)}$major = 15.3 min);

HRMS (ESI-TOF) m/z calcd. for [$C_{33}H_{35}N_3O_5^+$] ($M+H^+$) 554.2649, found 554.2647.

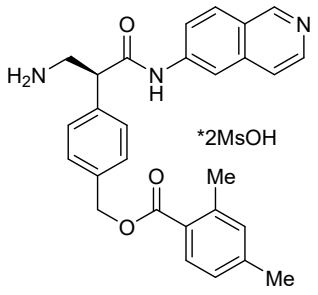
2.9. Stage 8. Boc-deprotection of amide 16: Synthesis of Netarsudil dimesylate (1)

16, **2.9 g**, **95% *ee***

(*S*)-Netarsudil dimesylate (**1**)
3.1 g, **90%**, **>98% ee**

Methanesulfonic acid (0.85 mL, 13.1 mmol, 2.5 equiv.) was added to a solution of *N*-Boc-amide **16** (2.9 g, 5.2 mmol,) in CH_2Cl_2 (15 mL) and the solution was stirred for 24 h at ambient temperature. The solvent was evaporated and the residue was recrystallized from isopropanol to afford analytically pure (*S*)-Netarsudil dimesylate (**1**).

(*S*)-4-(3-Amino-1-(isoquinolin-6-ylamino)-1-oxopropan-2-yl)benzyl 2,4-dimethylbenzoate dimesylate (**1**)



1

Physical state white solid;

MP 123 - 125°C (lit.[11] 122 - 133°C)

Yield 3.1 g (90 %);

^{1}H NMR (600 MHz, MeOH-*d*$_4$) δ 9.51 (s, 1H), 8.73 (d, *J* = 1.6 Hz, 1H), 8.42 (d, *J* = 6.7 Hz, 1H), 8.34 (d, *J* = 9.1 Hz, 1H), 8.21 (d, *J* = 6.6 Hz, 1H), 8.02 (dd, *J* = 9.1, 1.8 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.02 (s, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 5.30 (s, 2H), 4.40 (dd, *J* = 8.8, 5.6 Hz, 1H), 3.75 (dd, *J* = 12.9, 8.9 Hz, 1H), 3.37-3.33 (m, 2H), 2.77 (s, 6H), 2.46 (s, 3H), 2.29 (s, 3H);

^{13}C NMR (150 MHz, MeOH-*d*$_4$) δ 170.77, 167.07, 146.08, 145.32, 142.79, 140.53, 140.07, 137.18, 135.21, 132.06, 131.56, 131.03, 130.37, 128.92, 128.23, 126.14, 124.26, 124.09, 123.83, 113.54, 65.38, 50.13, 41.44, 38.30, 23.88, 20.54, 19.99.

HRMS (ESI-TOF) m/z calcd. for [$C_{28}H_{27}N_3O_3^+$] (M-2CH_3SO_3H+H^+) 454.2125, found 454.2123.

*ee was determined by simple Boc-derivatization to afford amide **16**.*

HPLC data: >98% *ee* (CHIRALPAK AD-H column, *n*-hexane/*i*-PrOH 70:30, flow rate 1.00 mL/min, 220 nm; $t_{(R)}$minor= 11.7 min, $t_{(R)}$major = 15.3 min);

HRMS of (*S*)-Netarsudil dimesylate (1)

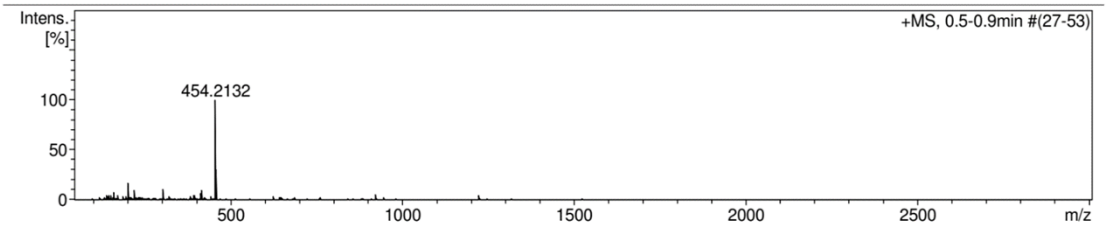
Display Report

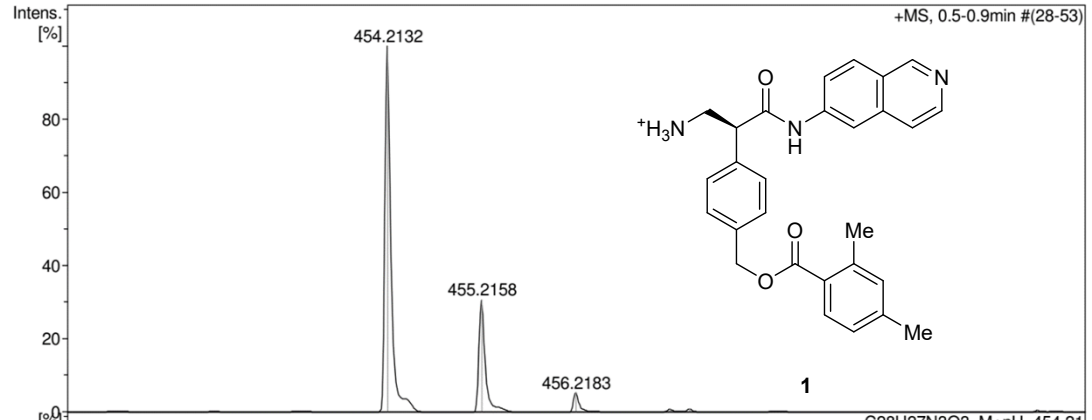
Analysis Info

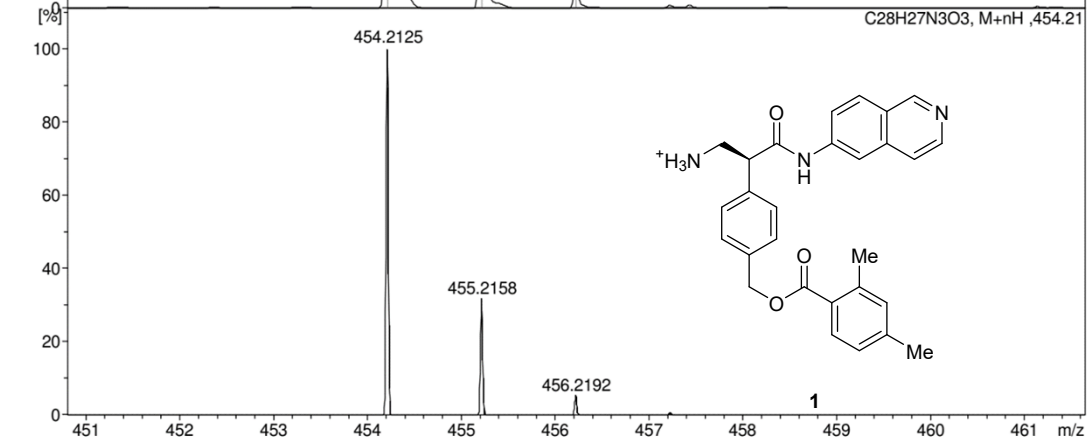
Analysis Name	D:\Data\Kolotyrkina\2025\Kovalevskii\0911013.d		
Method	tune_low.m	Operator	BDAL@DE
Sample Name	/ZSGN RA-767.01	Instrument / Ser#	micrOTOF 10248
Comment	C28H29N3O3 clb added CH3OH		

Acquisition Parameter

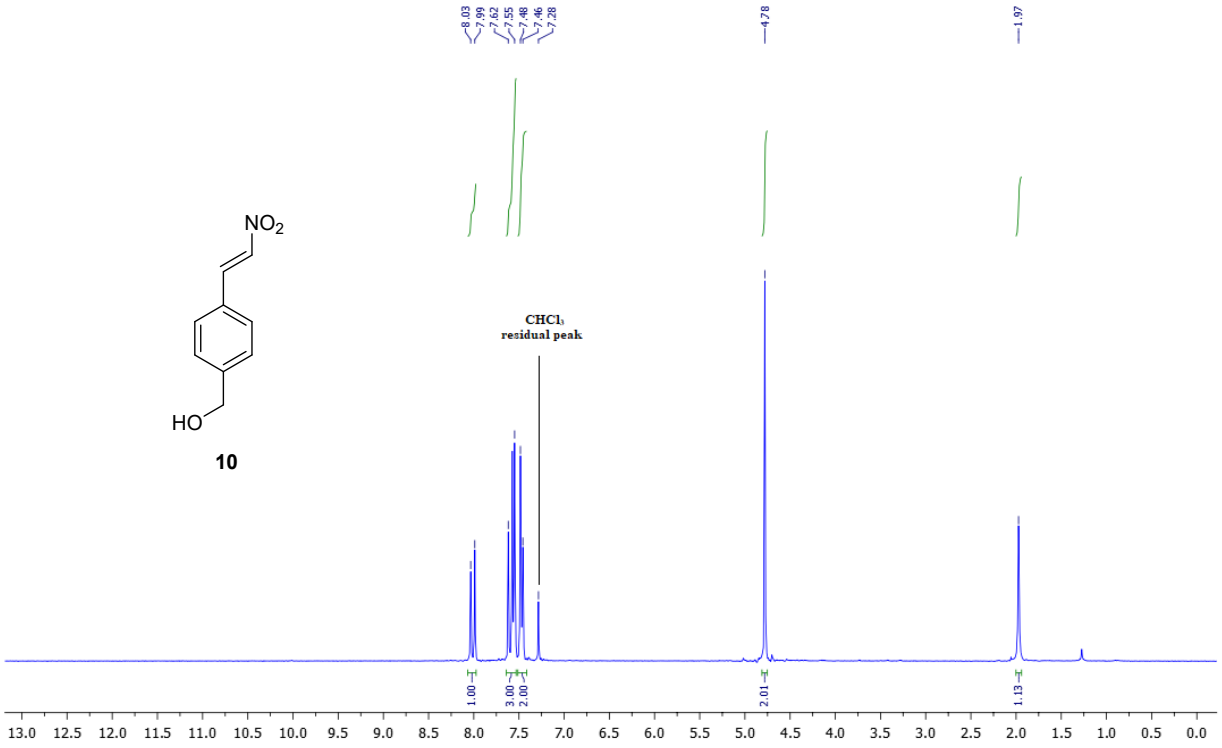
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

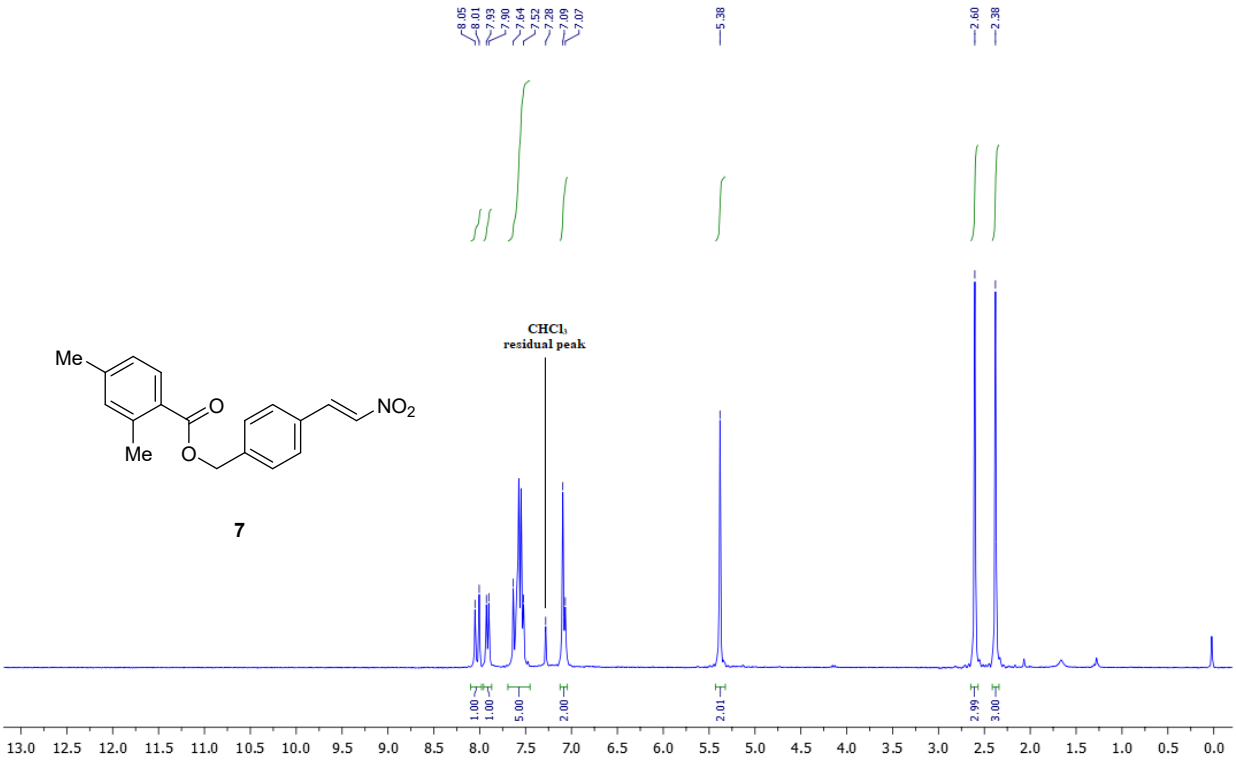


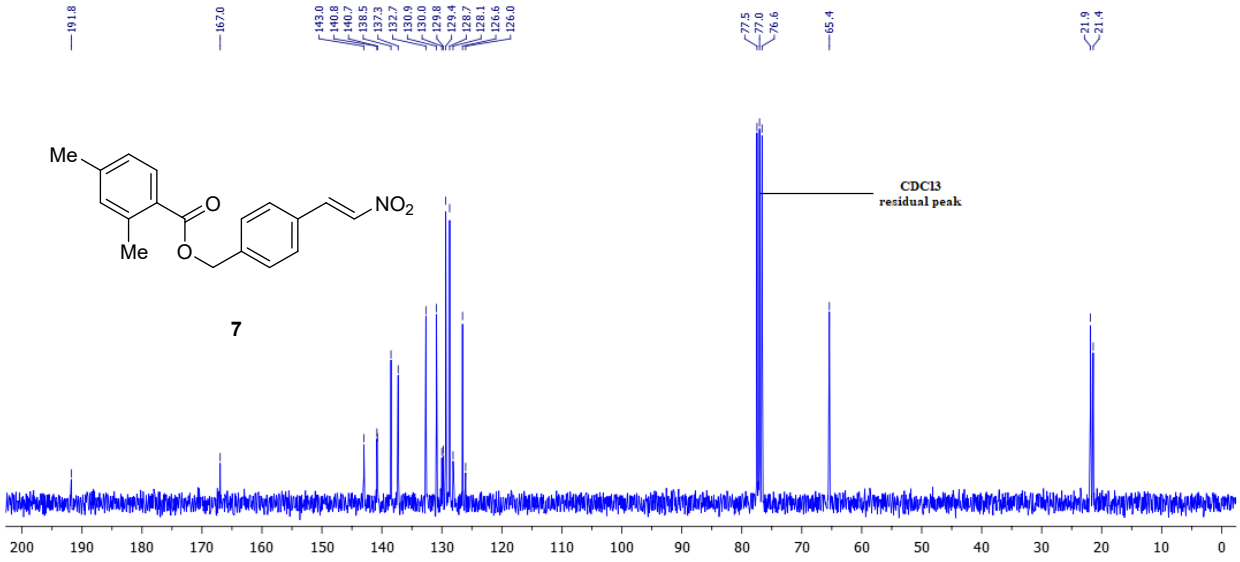


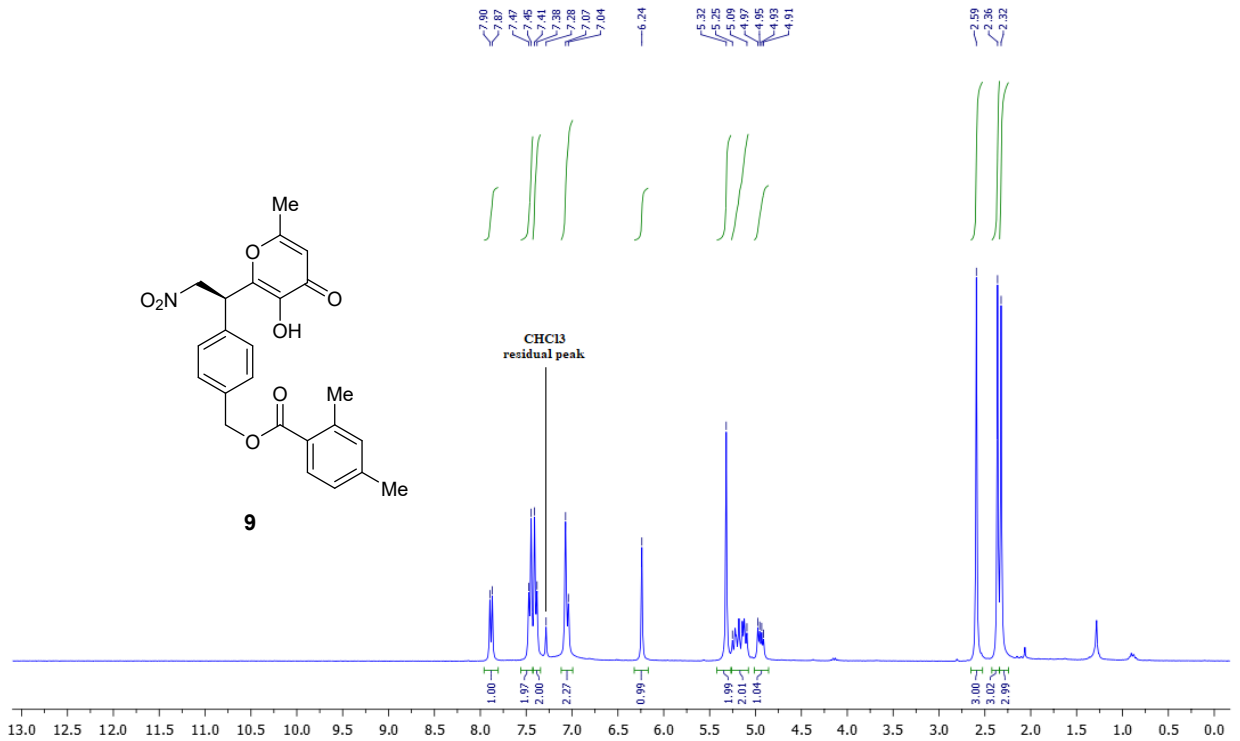


3. NMR pictures for all compounds






191.8
167.0
143.0
140.8
140.7
138.5
137.3
132.7
130.9
130.0
129.8
129.4
128.7
128.1
126.6
126.0
77.5
77.0
76.6
65.4
21.9
21.4
Me
O
NO2
Me
O
CDCl3
residual peak
7
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0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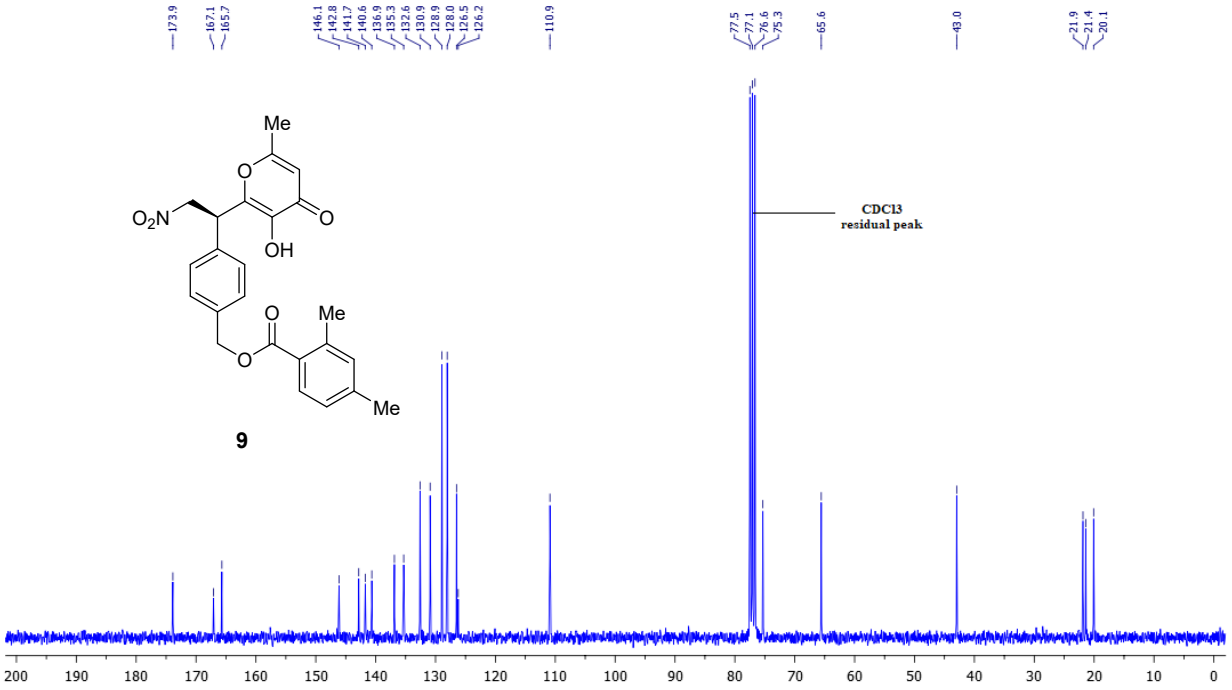
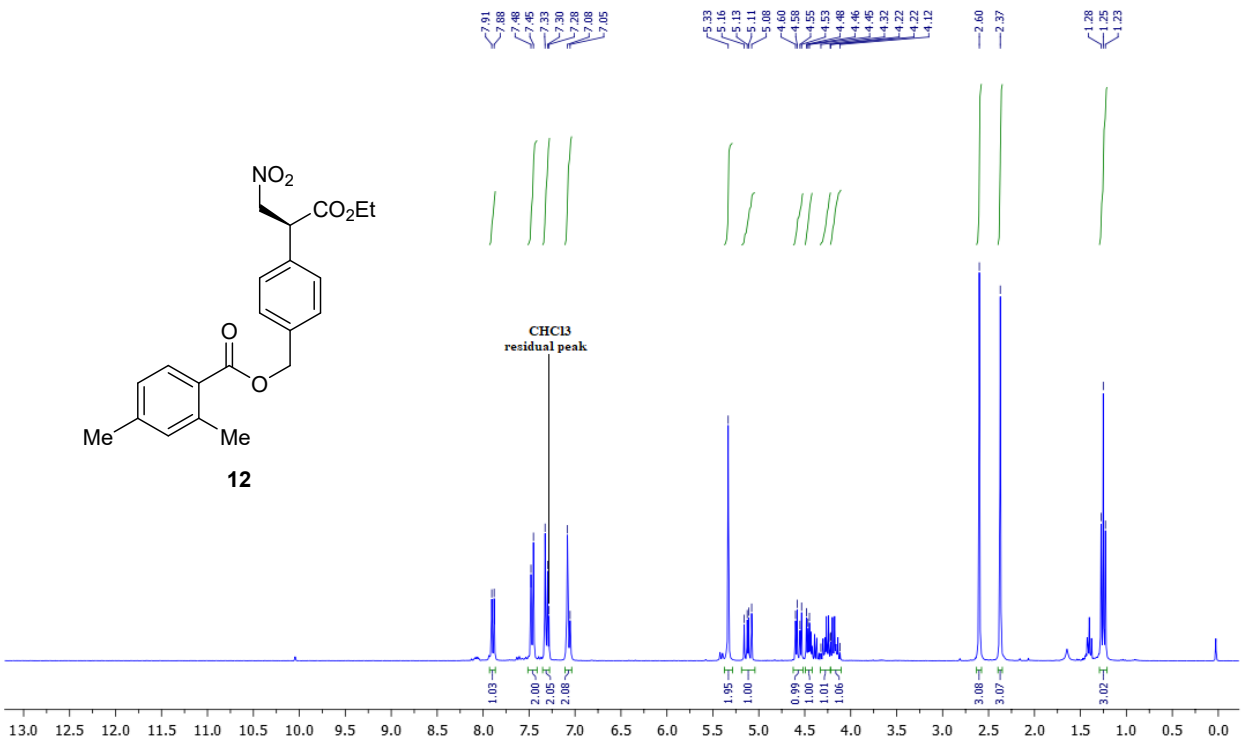
7.87
7.47
7.45
7.41
7.38
7.28
7.07
7.04
6.24
5.32
5.25
5.09
4.97
4.95
4.93
4.91
2.59
2.36
2.32
Me
O
O2N
O
OH
O
Me
O
Me
9
CHCl3
residual peak
1.00
1.97
2.00
2.27
0.99
1.99
2.01
1.04
3.00
3.02
2.99
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


173.9
167.1
165.7
146.1
142.8
141.7
140.6
136.9
135.3
132.6
130.9
128.9
128.0
126.5
126.2
110.9
77.5
77.1
76.6
75.3
65.6
43.0
21.9
21.4
20.1
Me
O
O2N
O
OH
O
Me
O
Me
CDCl3
residual peak
9
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


7.91
7.88
7.48
7.45
7.33
7.30
7.28
7.08
7.05
5.33
5.16
5.13
5.11
5.08
4.60
4.58
4.55
4.53
4.48
4.46
4.45
4.32
4.22
4.12
2.60
2.37
1.28
1.25
1.23
NO2
CO2Et
O
O
Me
Me
CHCl3
residual peak
12
1.03
2.00
2.05
2.08
1.95
1.00
0.99
1.00
1.01
1.06
3.08
3.07
3.02
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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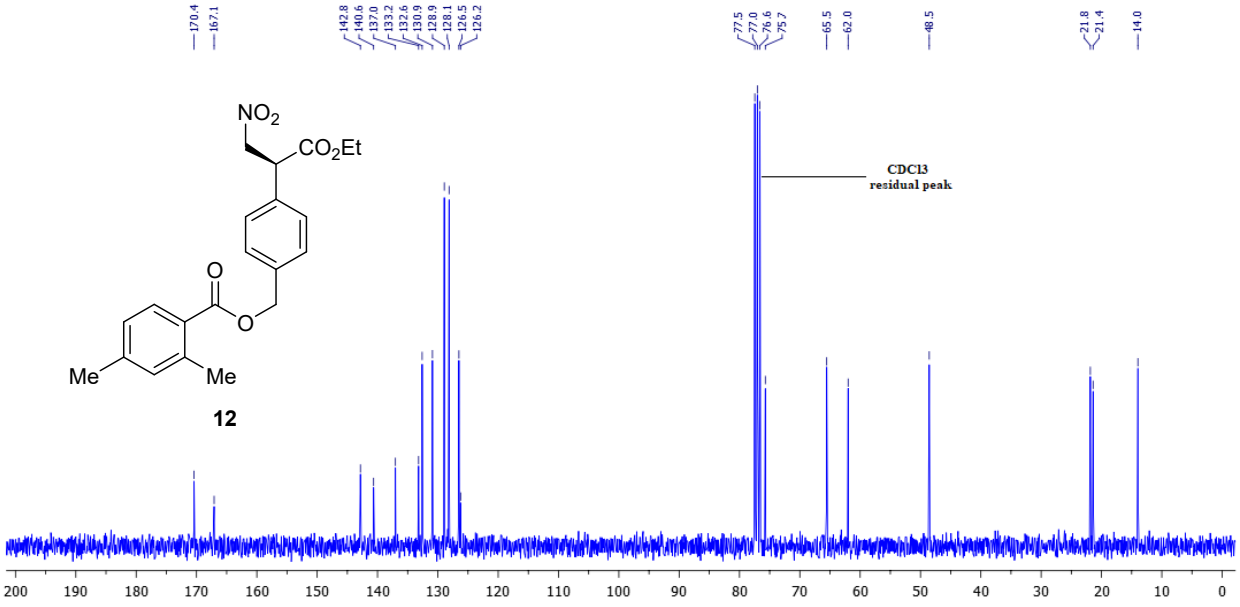
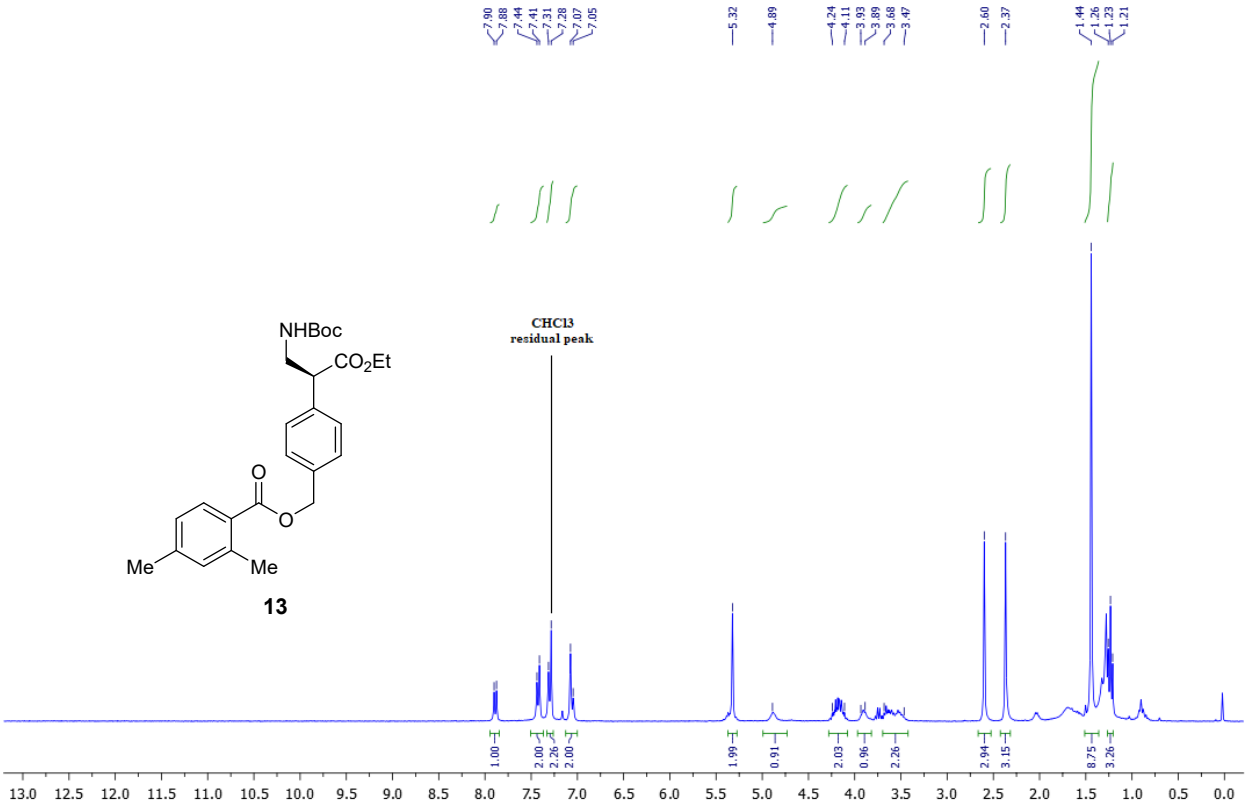
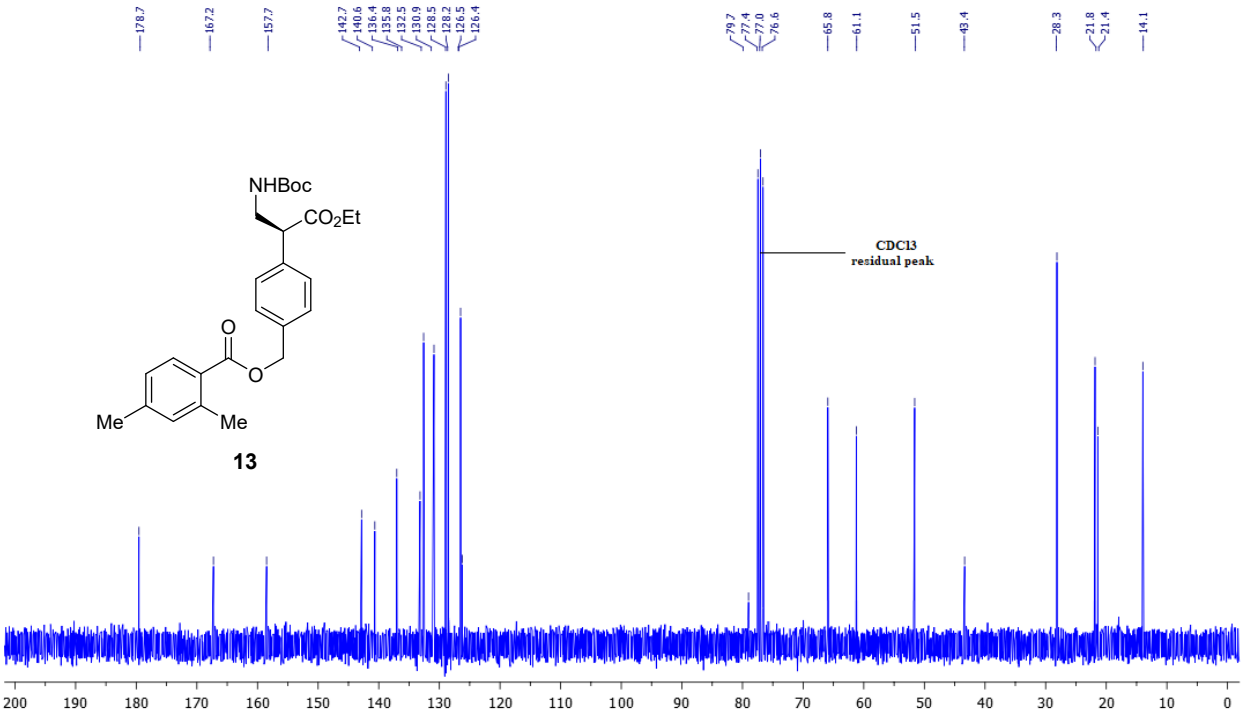
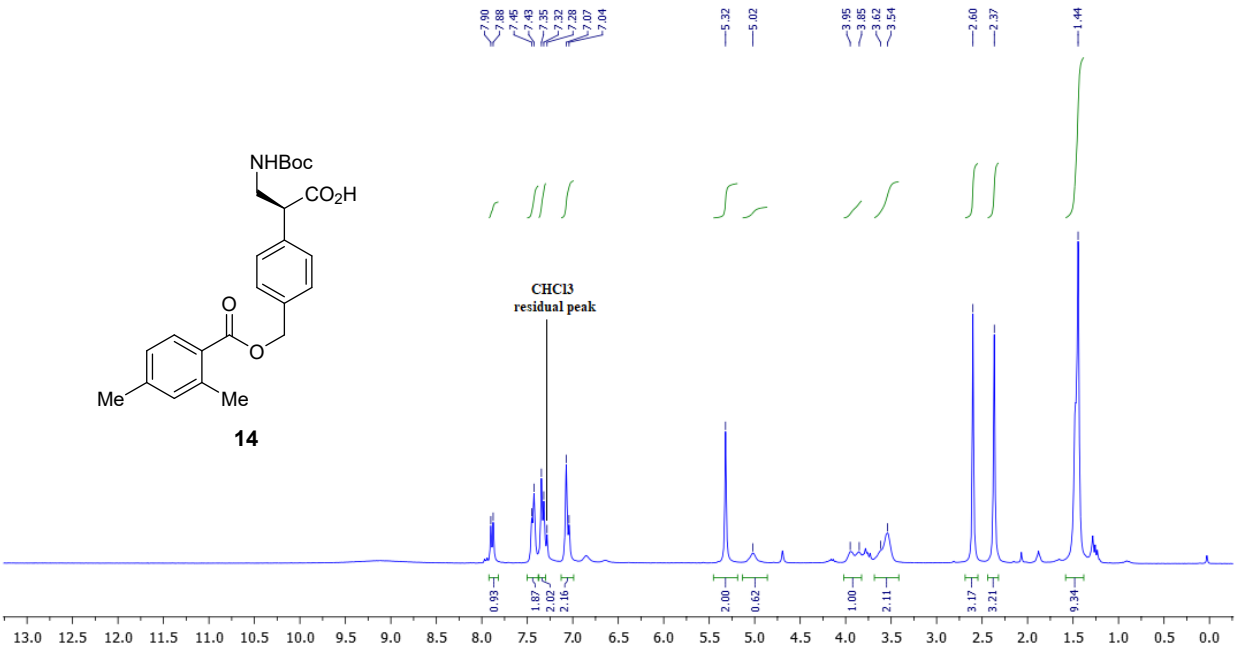
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


170.4
167.1
142.8
140.6
137.0
133.2
132.6
130.9
128.9
128.1
126.5
126.2
77.5
77.0
76.6
75.7
65.5
62.0
48.5
21.8
21.4
14.0
NO2
CO2Et
O
O
Me
Me
12
CDCl3
residual peak
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


7.90
7.88
7.44
7.41
7.31
7.28
7.07
7.05
5.32
4.89
4.24
4.11
3.93
3.89
3.68
3.47
2.60
2.37
1.44
1.26
1.23
1.21
NHBoc
CO2Et
O
O
Me
Me
13
CHCl3
residual peak
1.00
2.00
2.26
2.00
1.99
0.91
2.03
0.96
2.26
2.94
3.15
8.75
3.26
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


178.7
167.2
157.7
142.7
140.6
136.4
135.8
132.5
130.9
128.5
128.2
126.5
126.4
79.7
77.4
77.0
76.6
65.8
61.1
51.5
43.4
28.3
21.8
21.4
14.1
NHBoc
CO2Et
O
O
Me
Me
13
CDCl3
residual peak
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


7.90
7.88
7.45
7.43
7.35
7.32
7.28
7.07
7.04
5.32
5.02
3.95
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3.62
3.54
2.60
2.37
1.44
NHBoc
CO2H
O
O
Me
Me
14
CHCl3
residual peak
0.93
1.87
2.02
2.16
2.00
0.62
1.00
2.11
3.17
3.21
9.34
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.0
3.5
3.0
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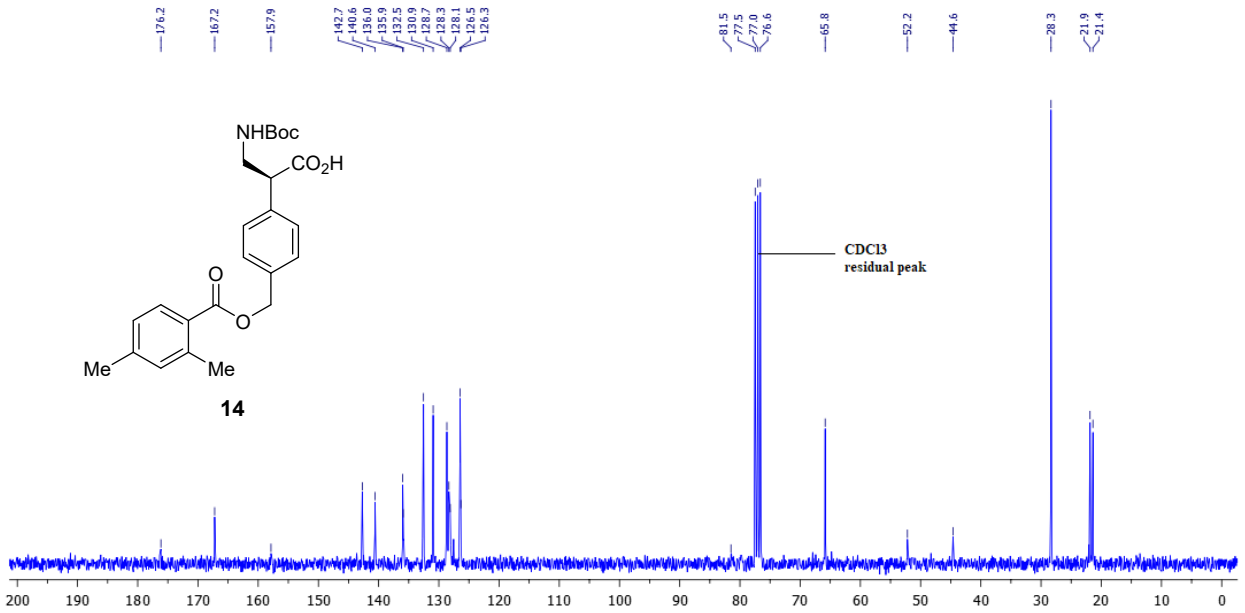
2.0
1.5
1.0
0.5
0.0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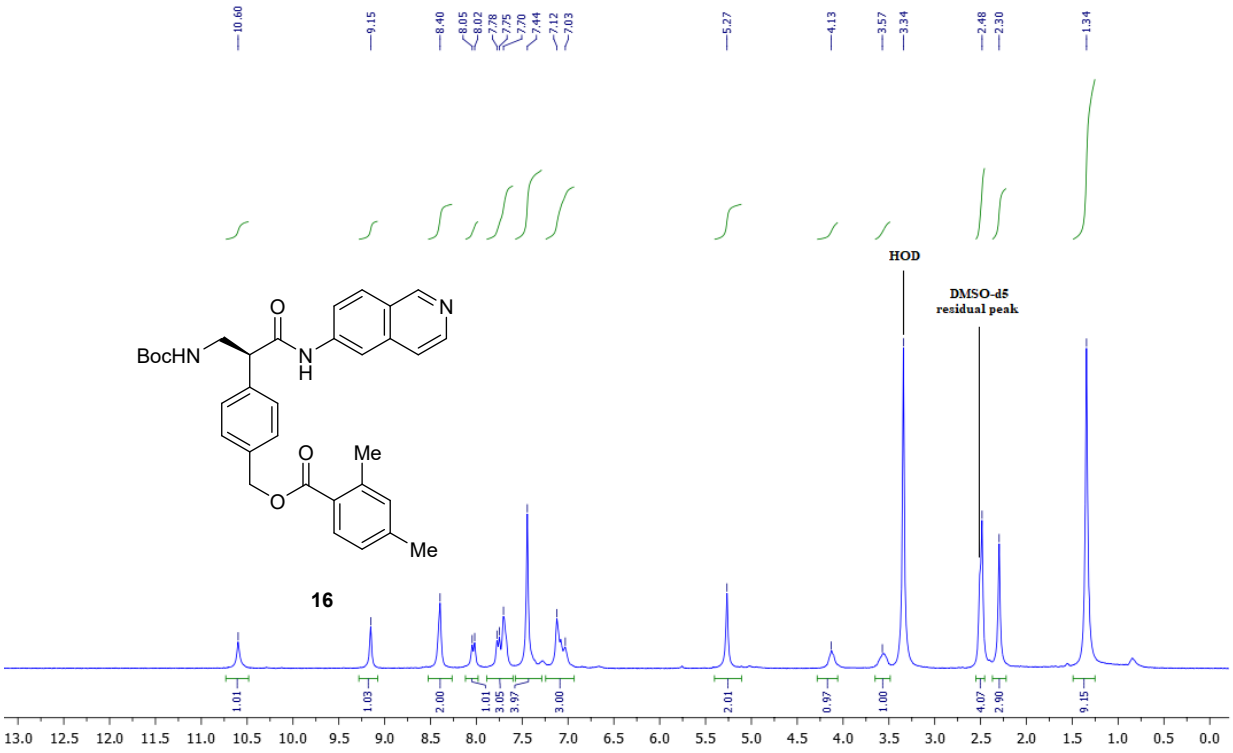
167.2
157.9
142.7
140.6
136.0
135.9
132.5
130.9
128.7
128.3
128.1
126.5
126.3
81.5
77.5
77.0
76.6
65.8
52.2
44.6
28.3
21.9
21.4
NHBoc
CO2H
O
O
Me
Me
14
CDCl3
residual peak
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0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9.15
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8.05
8.02
7.78
7.75
7.70
7.44
7.12
7.03
5.27
4.13
3.57
3.34
2.48
2.30
1.34
HOD
DMSO-d5
residual peak
BocHN
O
N
H
N
O
O
Me
Me
16
1.01
1.03
2.00
1.01
3.05
3.97
3.00
2.01
0.97
1.00
4.07
2.90
9.15
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
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6.5
6.0
5.5
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2.0
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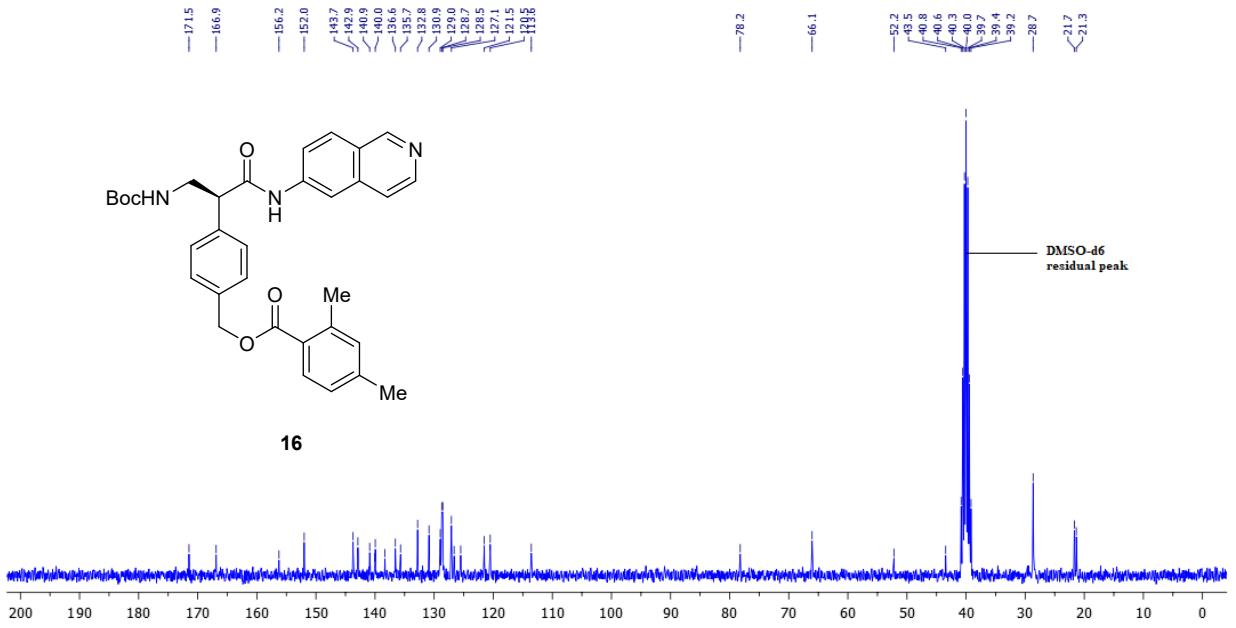
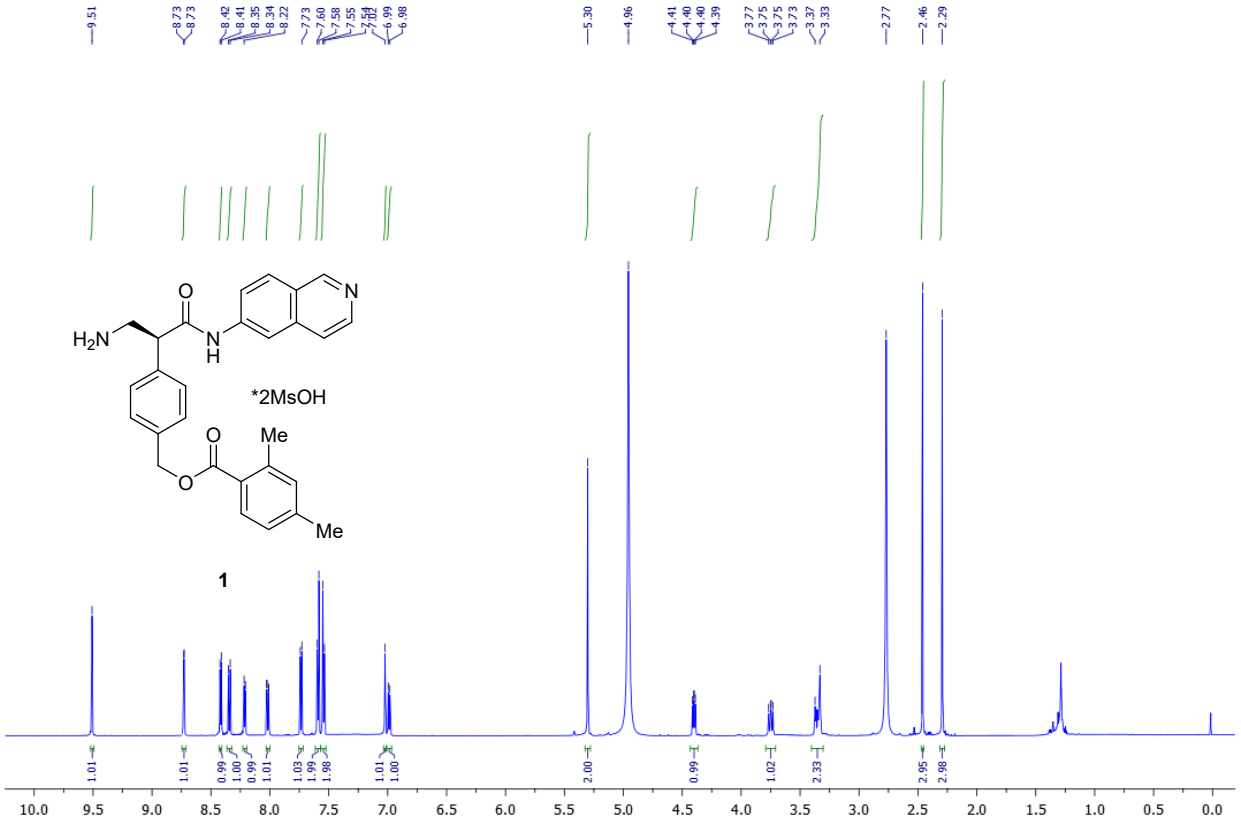
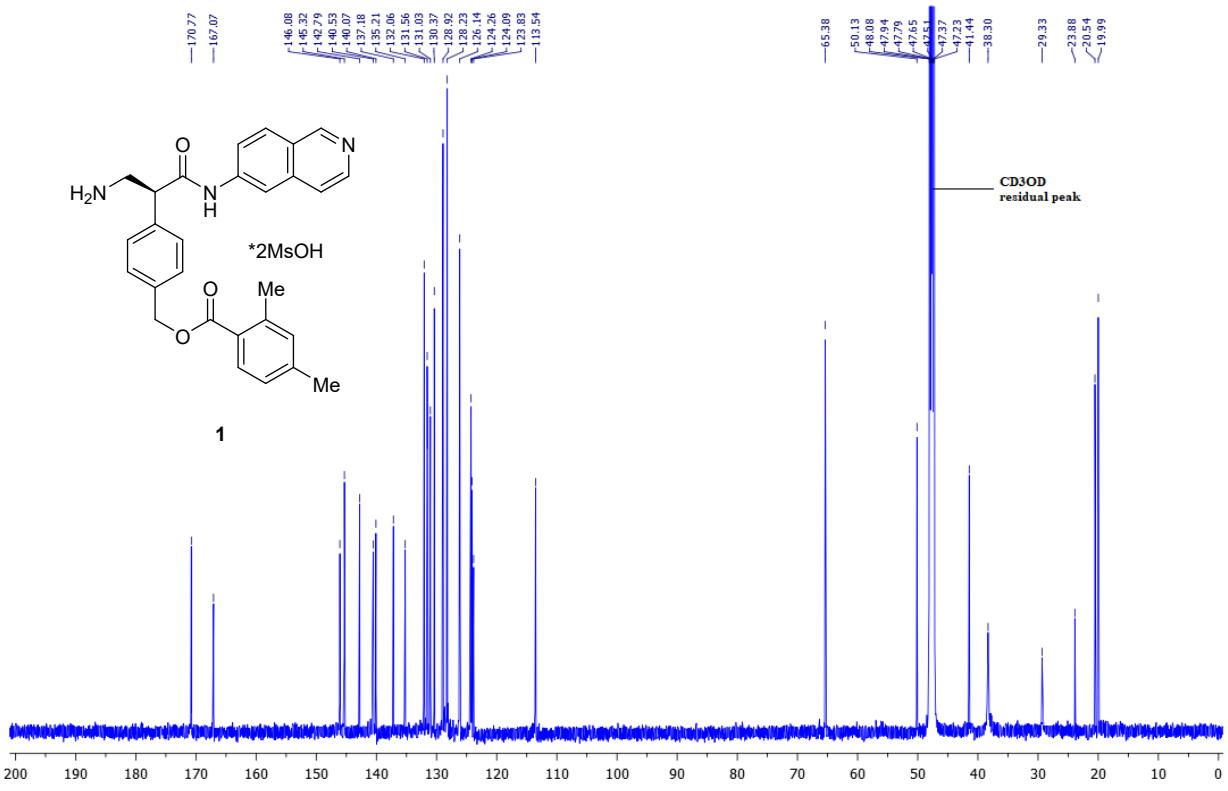

BocHN
O
N
H
N
O
Me
O
Me
16
DMSO-d6
residual peak


H2N
O
N
H
N
*2MsOH
O
Me
O
Me
1


170.77
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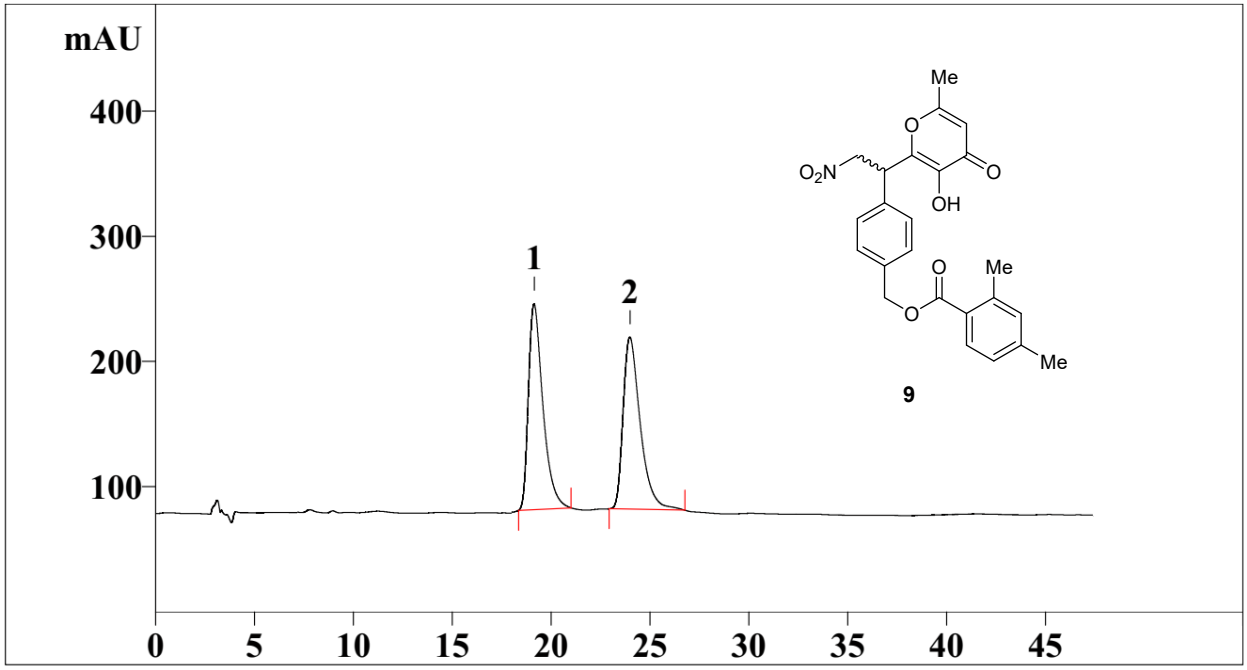
142.79
140.53
140.07
137.18
135.21
132.06
131.56
131.03
130.37
128.92
128.23
126.14
124.26
124.09
123.83
113.54
65.38
50.13
48.08
47.94
47.79
47.65
47.51
47.37
47.23
41.44
38.30
29.33
23.88
20.54
19.99
CD3OD
residual peak
O
N
H2N
N
H
*2MsOH
O
Me
O
Me
1
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

4. HPLC data for all compounds



No	Retention	Area %
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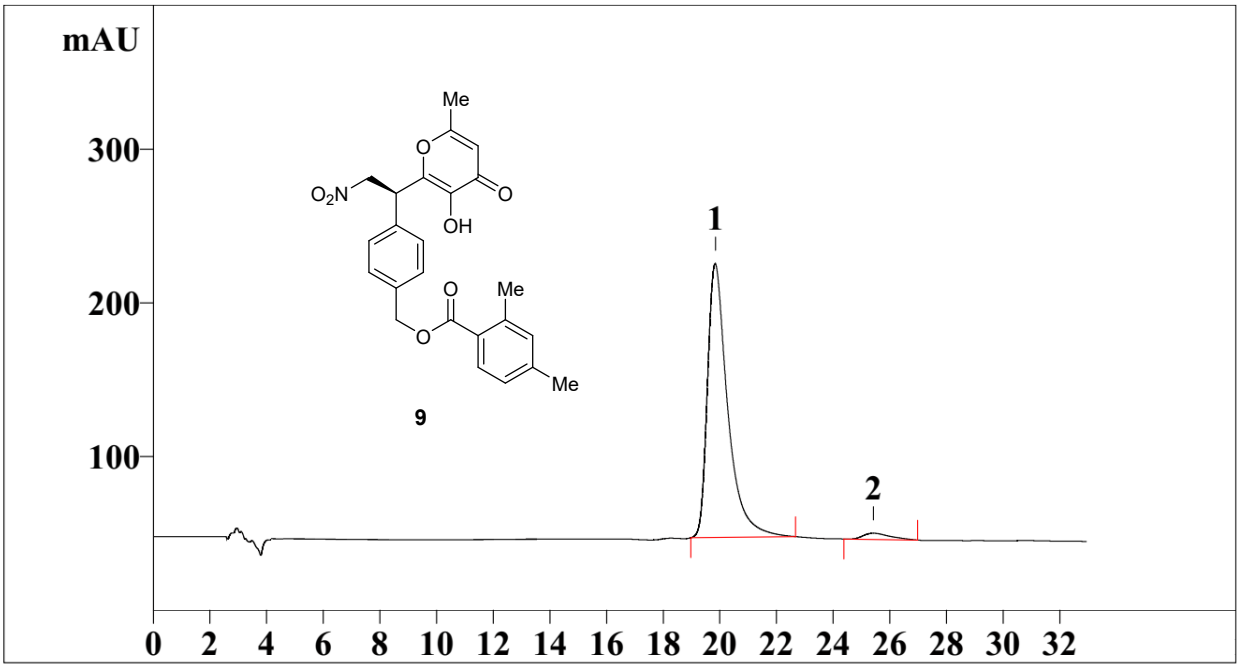
2	23.99	49.742
2	47.4	



No	Retention	Area %
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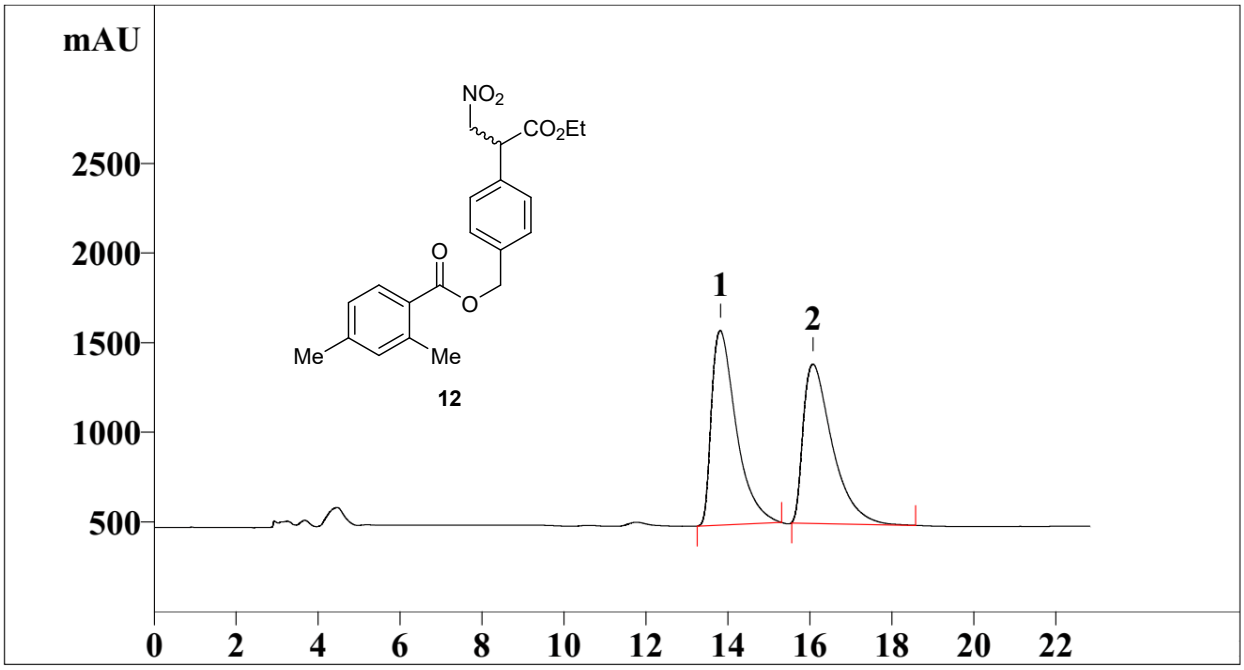
2	25.44	2.92
2	32.94	100.00



No	Retention	Area %
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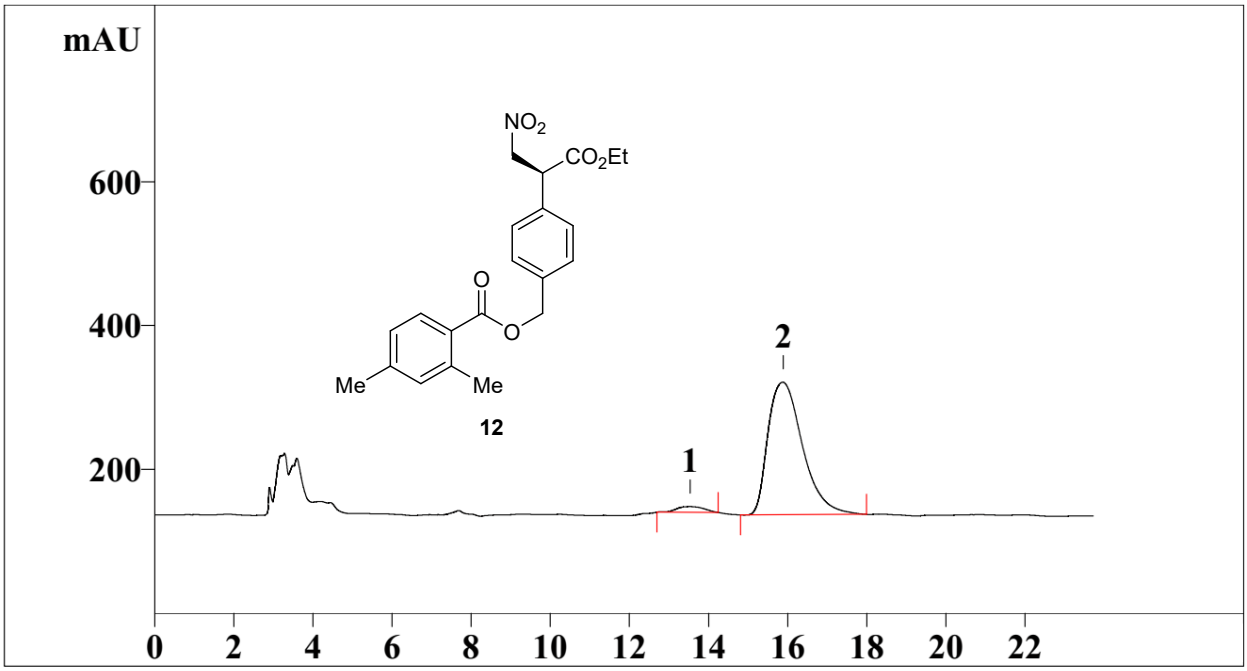
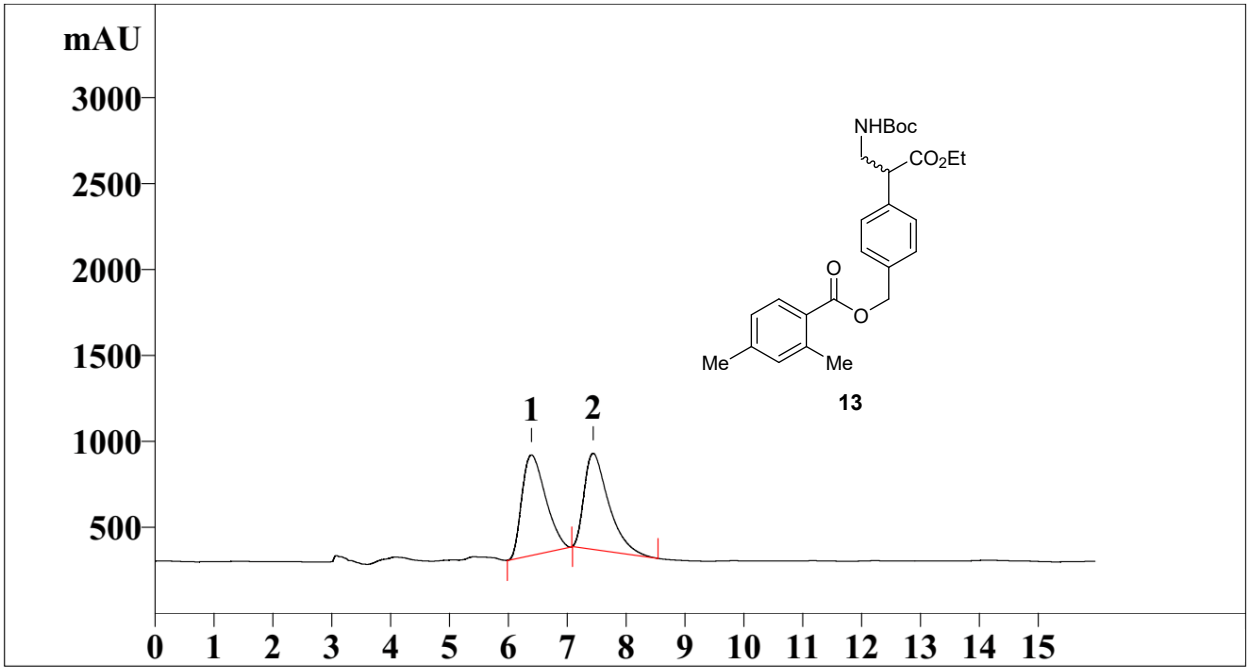
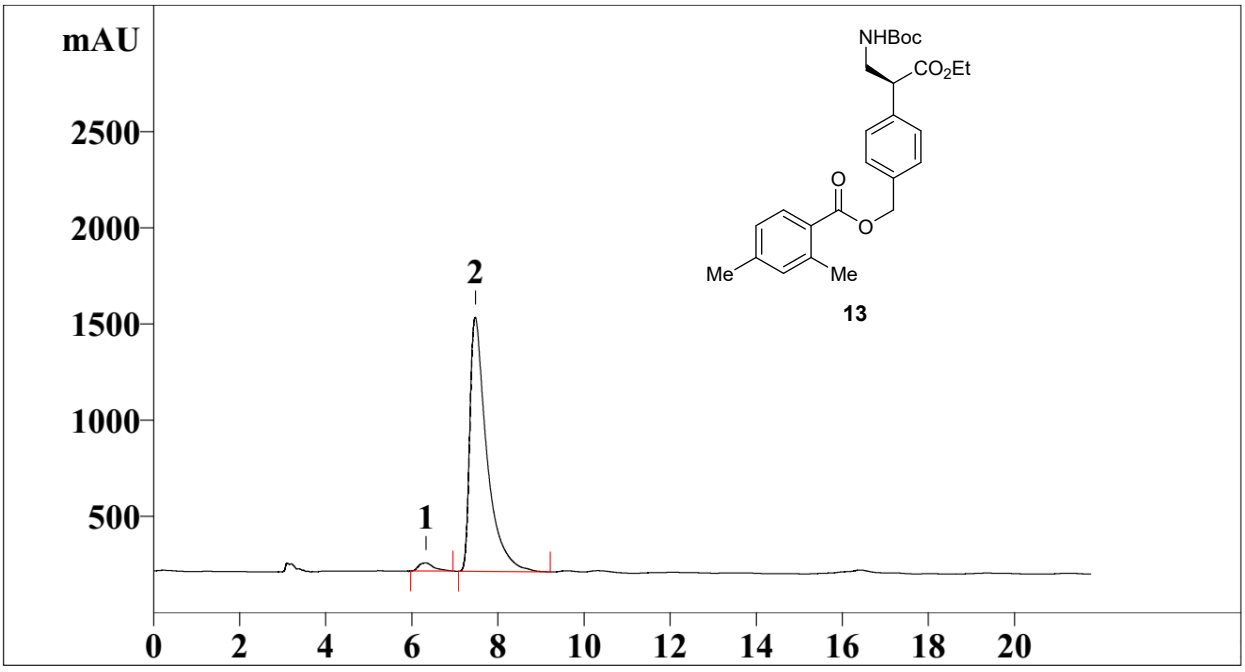
2	16.08	49.038
2	22.84	



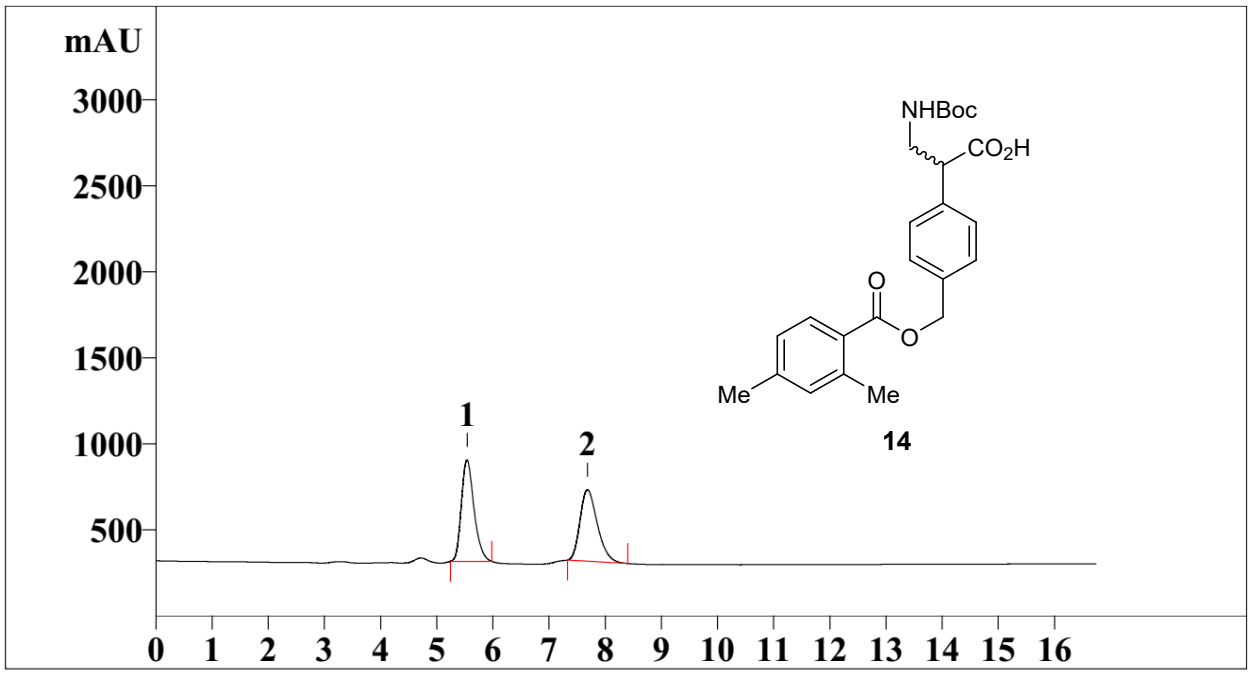
No	Retention	Area %
1	13.53	3.3188
2	15.88	96.681
2	23.73	



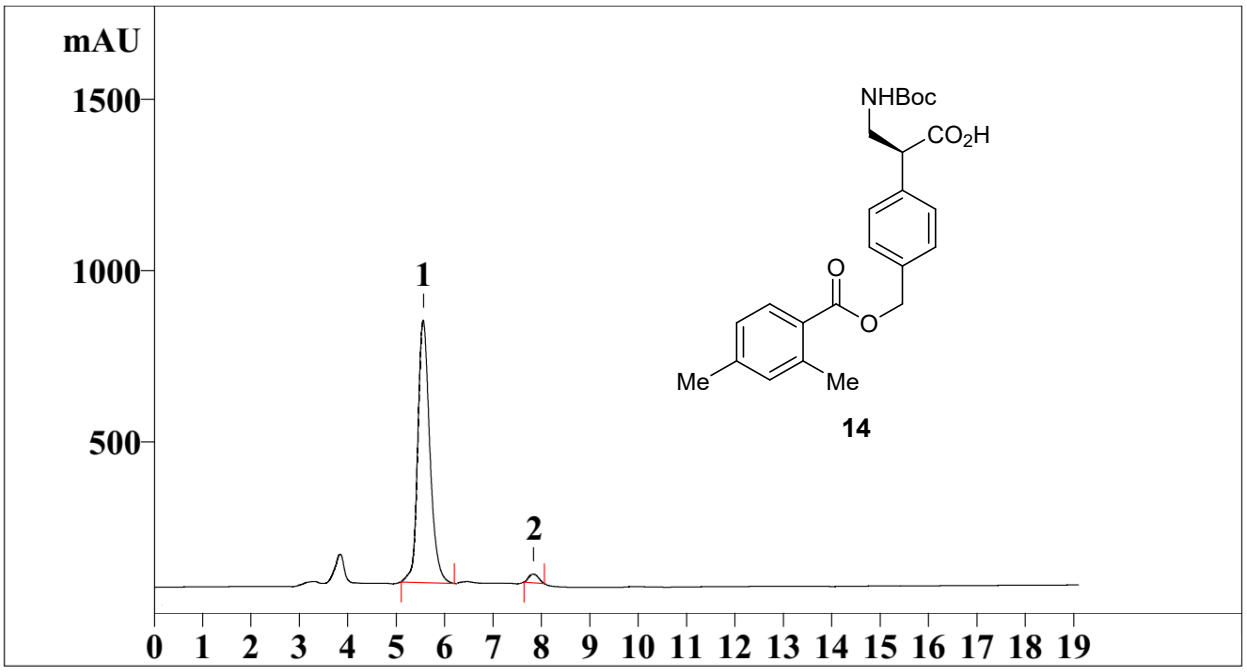
No	Retention	Area %
1	6.388	50.134
2	7.445	49.866
2	15.97	



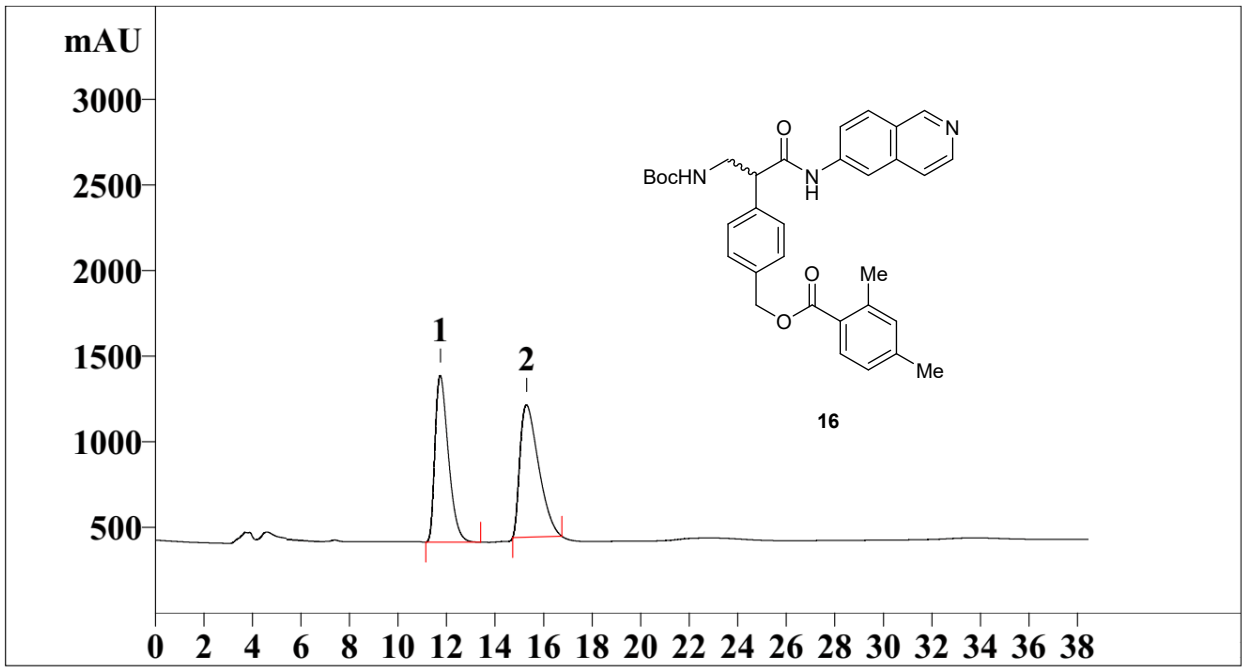
No	Retention	Area %
1	6.254	2.7615
2	7.475	97.239
2	21.78	



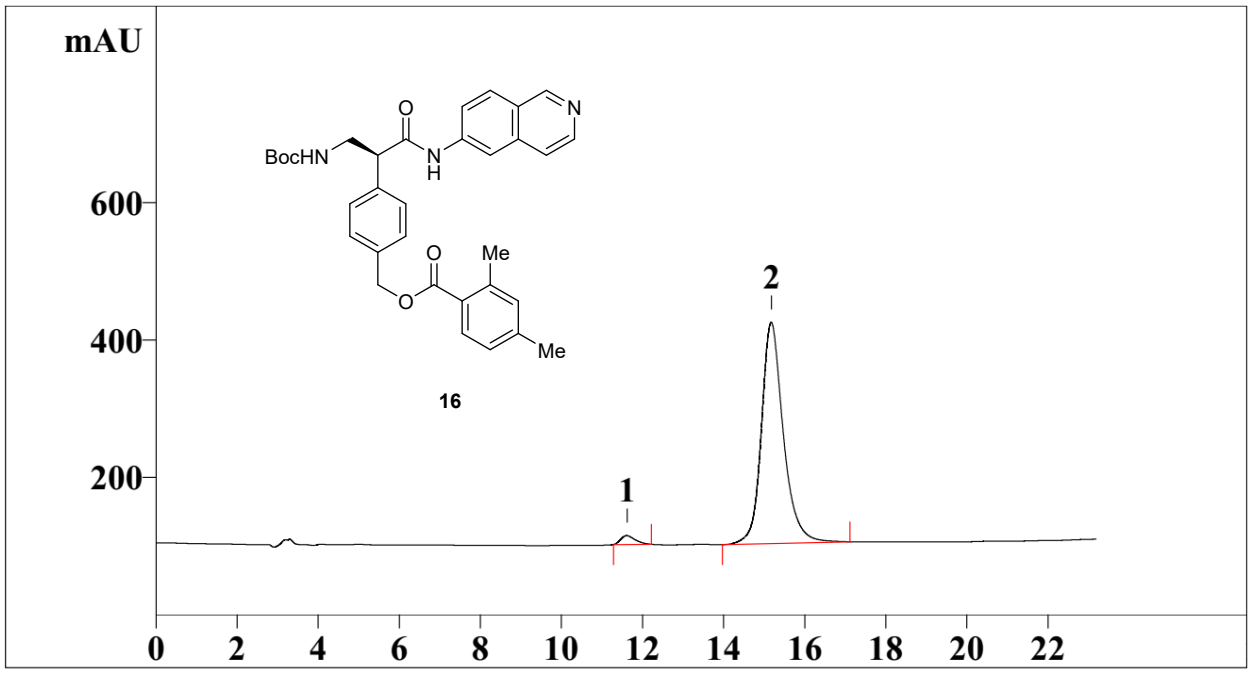
No	Retention	Area %
1	5.541	50.819
2	7.687	49.181
2	16.74	



No	Retention	Area %
1	5.556	97.411
2	7.823	2.5887
2	19.38	



No	Retention	Area %
1	11.74	47.41
2	15.29	52.59
2	38.57	



No	Retention	Area %
1	11.61	2.5309
2	15.34	97.469
2	23.19	

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