Biocatalytic Radical $C(sp^3)$–N Coupling via Active Site Templating

Zayed Alassad[1], Todd K. Hyster[1]*

[1]Princeton University, Princeton, NJ 08544, USA.

Email: thyster@princeton.edu

Table of Contents

1. General information *4*

2. Synthesis of substrates *5*

2.1 Tertiary alkyl bromides (starting materials) **5**

2.1.1 General Procedure A: alkylation of propanoic acid 5

2.1.2 General Procedure B: amidation of 2-methyl-3-phenylpropanoic acid 5

2.1.3 General Procedure C: bromination of amide 6

2.2 Substrate characterization (starting materials) **7**

Overview of the synthesis plan **16**

2.3.1 General Procedure D: Imine formation 16

2.3.2 General Procedure E: Arylation 17

2.3.3 General Procedure F: Deprotection 17

2.3.4 General Procedure G: N-alkylation 18

2.3.5 General Procedure H: Amidation 18

2.4 Product characterization **19**

2.5 Synthesis of the hydroarylation byproduct (overview) **43**

2.5.1 General Procedure K: Amidation 43

2.5.2 General Procedure L: Methylation 44

2.5.3 General Procedure M: Arylation 44

2.5.4 General Procedure N: Reduction 44

2.6 Synthesis of N-Methyl product **46**

2.7 Synthesis of ester product **47**

2.8 Determination of absolute stereoconfiguration **48**

3. Protein sequence and expression *50*

3.1 Morphinone reductase (MorB) **50**

3.2 Other proteins evaluated in this work **51**

3.3 MorB Protein Expression and Purification **54**

3.4 Dialyzed, Cell-Free Lysate Preparation **55**

4. Site Saturation Mutagenesis of MorB for N-alkylation *56*

4.1 Screening procedure for N-alkylation with MorB mutants in 96-well plates **56**

4.2 Plate preparation **56**

4.4 Summary of Iterative Site Saturation Mutagenesis **66**

5. Enzymatic reactions *67*

5.1 Initial screening and reaction discovery (photoredox, ketoreductases and '*ene*'-reductases) **67**

5.1.1 Photoredox catalysts 68

5.1.2 Prozomix ketoreductase (KREDs) library screening ... 70
6.1.2 '*Ene*'-reductases (EREDs) library screening ... 71
5.2 Buffer screening ... 72
5.3 Solvent screening ... 73
5.4 General procedure for the enzymatic reaction ... 74
5.5 Larger scale ... 76
5.6 Dialyzed lysate reaction ... 77
5.7 Enzymatic reaction scope ... 78
5.8 Unreactive substrates ... 104
6. Mechanistic studies ... 105
6.1 Control experiments ... 105
6.2 Reaction progress analysis ... 106
6.3 Radical trap ... 107
6.4 UV-Vis studies ... 108
6.4.1 Photoreduction in tricine buffer ... 108
6.4.2 Photoreduction in phosphate buffer (oxidative diradical mechanism) ... 109
6.5 Catalytic cycle ... 111
6.6 Reaction with dithionite ... 112
6.6.1 Reduction with irradiation ... 112
6.6.2 Reduction without light ... 113
6.7 Reactions with NADPH ... 114
6.8 No Hydroxylation (radical polar crossover) ... 115
6.9 DFT ... 116
6.9.1 C-N coupling step ... 116
6.9.2 Redox potential (no water) ... 117
6.9.3 Redox with water (with water) ... 118
6.10 ProteinX ... 119
7. Modeling ... 120
7.1 Hammett analysis ... 120
7.2 Statistical modeling ... 121
7.3 Aniline xyz structure ... 123
8. NMR spectra ... 135
9. HPLC traces ... 176
References ... 203

1. General information

Unless otherwise noted, all chemicals and reagents for chemical reactions were obtained from commercial suppliers and used as received (Sigma-Aldrich, Oakwood Chemical, Combi-Blocks, TCI, and VWR). Silica gel chromatography purifications were carried out using AMD Silica Gel 60. NMR spectra were recorded on a Bruker UltraShield Plus (500 and 126 MHz, respectively) instrument, and are internally referenced to residual proton signals in $CDCl_3$ (7.26 ppm), CD_3CN (1.94 ppm) or CD_3OD (3.31 ppm). ^1H NMR data are reported as follows: multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift relative to $CDCl_3$ (77.16 ppm), CD_3CN (1.32 ppm and 118.26 ppm) or CD_3OD (49.00 ppm). High resolution mass spectra (HRMS) were obtained on an Agilent 6220 LC/MS with an electrospray ionization time-of-flight (ESI-TOF) detector.

LED Lamps: For Cyan light: Lumidox Gen 2 Cyan LED (30 mA LED Output, 505 nm) (Array Analytical Sales and Services Inc.), Cyan LED chips (490 nm) were used.

Sonications were performed with Sonciator QSonica Q500 Ultra Sonicator. Spin-gravity concentration was performed with Amicon Ultra 15 mL concentrators (30 kDa MW cutoff).

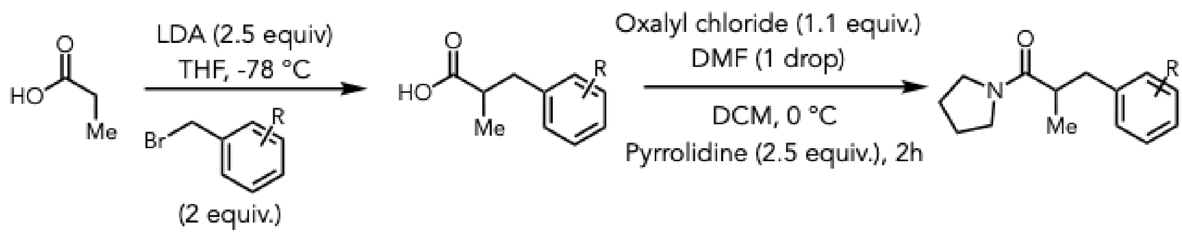
Chromatography. Analytical high performance liquid chromatography (HPLC) and Electron Spray Ionization (ESI) mass spectrometry were carried out using an Agilent 1260 Infinity LCMS System. Yields and conversions were determined on a Poroshell C18 column (4.6 x 50 mm, 2.7 µm) against an internal standard 1,3,5-tribromobenzene (TBB) at 210 nm. Chiral HPLC was conducted using an Agilent 1260 Infinity Chiral HPLC system with isopropanol and hexane as the mobile phases. Chiral OJ-H, OD- H, IA-H, IB-H, IC-H, and AS-H columns were used to separate enantiomers (4.6 x 250 mm, 5 µm).

Cloning. pET22b (+) was used as a cloning and expression vector for all enzymes described in this study. Genes for all 'ene'-reductases were purchased as gBlocks from IDT and cloned using the Gibson cloning method.[1] All C-terminal 6xHis-tagged constructs were cloned directly between the NdeI and XhoI restriction sites. N-terminal 6xHis-tagged constructs were created by the introduction of an N-terminal 6xHis sequence directly after the NdeI site and replacement of the C-terminal 6xHis tag with an XhoI cut site. Cloned plasmids were transformed into *E. coli.* DH5-α cells for storage, and *E. coli.* BL21 (DE3) electrocompetent cells for expression.

2. Synthesis of substrates

2.1 Tertiary alkyl bromides (starting materials)

This synthesis was adapted from Biegasiewicz et. al[2]



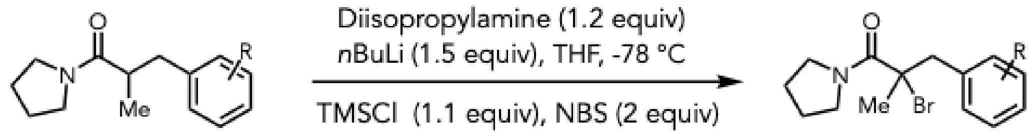
2.1.1 General Procedure A: alkylation of propanoic acid

Propanoic acid (1.0 equiv) was added dropwise to a stirred solution of lithium diisopropylamide (LDA, 2.5 equiv, 2.5 M in THF) at –78 °C. After stirring for 30 minutes at this temperature, the reaction mixture was warmed to room temperature and then heated to 60 °C for an additional hour. It was subsequently recooled to –78 °C, and benzyl bromide (2.0 equiv) in THF was added dropwise. The mixture was then allowed to warm to room temperature and stirred overnight. The reaction was quenched with water, and ethyl acetate was added. The aqueous layer was separated, acidified with 3 M HCl, and extracted with ethyl acetate (three times). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to afford the crude product.

2.1.2 General Procedure B: amidation of 2-methyl-3-phenylpropanoic acid

2-Methyl-3-phenylpropionic acid from the previous step (1.0 equiv) was dissolved in dry dichloromethane (DCM) in a sealed flask equipped with an outlet needle under a nitrogen atmosphere and cooled to 0 °C. After adding two drops of dry DMF at 0 °C, oxalyl chloride (1.1 equiv) was introduced dropwise. The mixture was stirred for 1 hour, allowing it to warm to room temperature. The solvent was then removed *in vacuo*, and the resulting crude acid chloride was dried under vacuum for 1 hour before proceeding to the next step without additional purification.

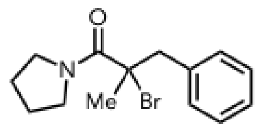
The crude from the last step was dissolved in dry DCM and cooled to 0 °C. Pyrrolidine (2.5 equiv) was added dropwise, and the reaction was stirred for 2 h while warming to room temperature. The reaction was quenched with water and extracted with DCM. The organic extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give the product, which was purified by flash column chromatography.



2.1.3 General Procedure C: bromination of amide

Diisopropylamine (1.2 equiv) was dissolved in dry THF and cooled to -78 °C. *n*BuLi (1.5 equiv) was added dropwise, and the solution was stirred at -78 °C for 30 minutes. The amide from the previous step dissolved in dry THF was added and stirred at -78 °C for 1 hour. TMSCl (1.1 equiv) dissolved in dry THF was added, and the mixture was warmed to room temperature over 3 hours. The mixture was cooled to -78 °C, and NBS (2 equiv) was added and stirred for 1 hour at -78 °C before being warmed to room temperature over 1 hour. The reaction was quenched with water, and the aqueous layer was extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the crude product, which was purified by flash column chromatography.

2.2 Substrate characterization (starting materials)



2-bromo-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**2a**)

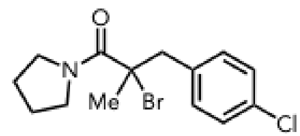
Synthesized according to general procedures A, B, C (16% yield over 3 steps).

^{1}H-NMR (500 MHz; CDCl3): δ 7.32-7.25 (m, 3H), 7.23-7.19 (m, 2H), 3.96 (bs, 1H), 3.75 (bs, 1H), 3.58 (bs, 1H), 3.56 (d, 1H, 20 Hz), 3.40 (d, 1H, 20 Hz), 2.01-1.75 (m, 4H), 1.88 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 168.3, 136.2, 130.6, 128.2, 127.2, 62.5, 48.8, 47.7, 29.4, 27.2, 23.2.

HRMS (ESI-MS): m/z calculated for $C_{14}H_{19}BrNO$ ($M+H^{+}$): 296.0527; found 296.0637

IR (neat, cm^{-1}): 2974, 1753, 1625, 1436, 1407.



2-bromo-3-(4-chlorophenyl)-2-methyl-1-(pyrrolidin-1-yl)propan-1-one (**2b**)

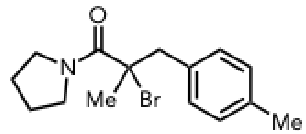
Synthesized according to general procedures A, B, C (15% yield over 3 steps).

^{1}H-NMR (500 MHz; CDCl3): δ 7.27-7.23 (m, 2H), 7.17 (d, 2H, J= 8 Hz), 3.93-3.72 (m, 2H), 3.64-3.46 (m, 3H), 3.39 (d, 1H, J= 15 Hz), 2.02-1.78 (m, 4H), 1.84 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 168.1, 134.8, 133.2, 132.1, 130.6, 128.3, 128.2, 172.2, 62.1, 49.1, 48.8, 47.7, 47.2, 29.4, 28.9, 27.2, 23.2.

HRMS (ESI-MS): m/z calculated for $C_{14}H_{19}BrClNO$ ($M+2H^{+}$): 332.0182; found 332.0256

IR (neat, cm^{-1}): 2976, 1623, 1492, 1407, 1095.



2-bromo-2-methyl-1-(pyrrolidin-1-yl)-3-(*p*-tolyl)propan-1-one (**2c**)

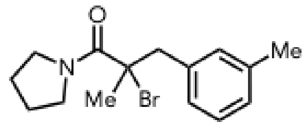
Synthesized according to general procedures A, B, C (8% yield over 3 steps).

^{1}H-NMR (500 MHz; CDCl3): δ 7.09 (s, 4H), 3.98 (s, 1H), 3.75 (s, 1H), 3.55 (s, 2H), 3.53 (d, 1H, J= 15 Hz), 3.37 (d, 1H, J= 15 Hz), 2.31 (s, 3H), 2.02-1.79 (m, 4H), 1.85 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 168.3, 136.8, 133.1, 130.4, 128.9, 62.8, 49.1, 48.8, 47.2, 29.4, 27.2, 23.4, 21.1.

HRMS (ESI-MS): m/z calculated for $C_{15}H_{21}BrNO$ ($M+H^{+}$): 310.0728; found 310.0823

IR (neat, cm^{-1}): 2974, 1627, 1515, 1405, 1054.



2-bromo-2-methyl-1-(pyrrolidin-1-yl)-3-(*m*-tolyl)propan-1-one (**2d**)

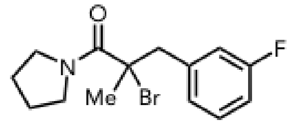
Synthesized according to general procedures A, B, C (20% yield over 3 steps).

^{1}H-NMR (500 MHz; CDCl3): δ 7.17 (t, 1H, 10 Hz), 7.08 (d, 2H, 10 Hz), 7.01 (m, 2H), 3.97 (bs, 1H), 3.75 (bs, 1H), 3.52 (bs, 1H), 3.52 (d, 1H, 20 Hz), 3.39 (d, 1H, 20 Hz), 2.33 (s, 3H), 2.01-1.78 (m, 5H), 1.88 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 168.3, 137.7, 136.1, 131.3, 128.1, 127.5, 62.7, 49.1, 47.6, 29.5, 27.2, 23.2, 21.5.

HRMS (ESI-MS): m/z calculated for $C_{15}H_{21}BrNO$ (M+H^{+}): 310.0728; found 310.0825

IR (neat, cm^{-1}): 2972, 1627, 1449, 1407, 1056.



2-bromo-3-(3-fluorophenyl)-2-methyl-1-(pyrrolidin-1-yl)propan-1-one (**2e**)

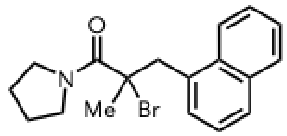
Synthesized according to general procedures A, B, C (17% yield over 3 steps).

^{1}H-NMR (500 MHz; CDCl3): δ 7.28-7.21 (m, 1H), 7.02-6.92 (m, 3H), 3.89 (bs, 1H), 3.79 (bs, 1H), 3.61 (bs, 1H), 3.59 (d, 1H, 20 Hz), 3.44 (d, 1H, 20 Hz), 2.33 (s, 3H), 2.01-1.78 (m, 5H), 1.88 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 168.1, 163.8, 161.3, 138.7, 129.6, 126.4, 117.7, 114.1, 61.9, 49.1, 47.5, 29.1, 27.2, 23.2.

HRMS (ESI-MS): m/z calculated for $C_{14}H_{18}BrFNO$ (M+H^{+}): 314.0478; found 314.0511

IR (neat, cm^{-1}): 2976, 1673, 1487, 1254, 1142.



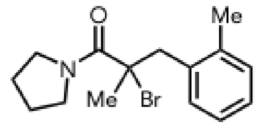
2-bromo-2-methyl-3-(naphthalen-1-yl)-1-(pyrrolidin-1-yl)propan-1-one (**2f**)

Synthesized according to general procedures A, B, C (18% yield over 3 steps).

^{1}H-NMR (500 MHz; CDCl3): δ 7.82-7.74 (m, 3H), 7.69 (s, 1H), 7.48-7.43 (m, 2H), 7.36 (d, 1H, 10 Hz), 3.94 (bs, 1H), 3.80 (bs, 1H), 3.59 (bs, 1H), 3.72 (d, 1H, 20 Hz), 3.58 (d, 1H, 20 Hz), 2.33 (s, 3H), 2.01-1.78 (m, 5H), 1.93 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 166.4, 131.9, 131.3, 130.6, 127.7, 126.8, 125.8, 125.7, 124.1, 123.9, 60.7, 47.2 (d), 45.9, 27.5, 25.3, 21.3.

HRMS (ESI-MS): m/z calculated for $C_{18}H_{21}BrNO$ ($M+H^{+}$): 346.0728; found 346.0785



2-bromo-2-methyl-1-(pyrrolidin-1-yl)-3-(*o*-tolyl)propan-1-one (**2g**)

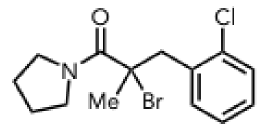
Synthesized according to general procedures A, B, C (7% yield over 3 steps).

^{1}H-NMR (500 MHz; CDCl3): δ 7.21-7.08 (m, 4H), 3.98 (bs, 1H), 3.75 (bs, 1H), 3.64-3.54 (m, 2H), 3.58 (d, 1H, 20 Hz), 3.52 (d, 1H, 20 Hz), 2.35 (s, 3H), 2.01-1.78 (m, 5H), 1.93 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 168.6, 137.2, 134.8, 130.7, 130.4, 127.2, 125.8, 63.6, 48.9, 43.3, 29.8, 27.2, 23.2, 20.4.

HRMS (ESI-MS): m/z calculated for $C_{15}H_{21}BrNO$ ($M+H^+$): 310.0728; found 310.0832

IR (neat, cm^{-1}): 2976, 1627, 1408, 1058.



2-bromo-3-(2-chlorophenyl)-2-methyl-1-(pyrrolidin-1-yl)propan-1-one (**2h**)

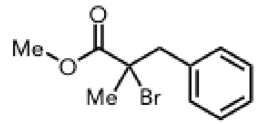
Synthesized according to general procedures A, B, C (12% yield over 3 steps)

^{1}H-NMR (500 MHz; CDCl3): δ 7.21-7.08 (m, 4H), 3.98 (bs, 1H), 3.75 (bs, 1H), 3.64-3.54 (m, 2H), 3.58 (d, 1H, 20 Hz), 3.52 (d, 1H, 20 Hz), 2.35 (s, 3H), 2.01-1.78 (m, 5H), 1.93 (s, 3H).

^{13}C-NMR (126 MHz; CDCl3): δ 168.6, 137.2, 134.8, 130.7, 130.4, 127.2, 125.8, 63.6, 48.9, 43.3, 29.8, 27.2, 23.2, 20.4.

HRMS (ESI-MS): m/z calculated for $C_{14}H_{19}BrClNO$ ($M+2H^{+}$): 332.0182; found 332.0264

IR (neat, cm^{-1}): 2974, 1718, 1623, 1407, 1056.



methyl 2-bromo-2-methyl-3-phenylpropanoate **(2i)**

Synthesized according to general procedures A, B, C (12% yield over 3 steps)

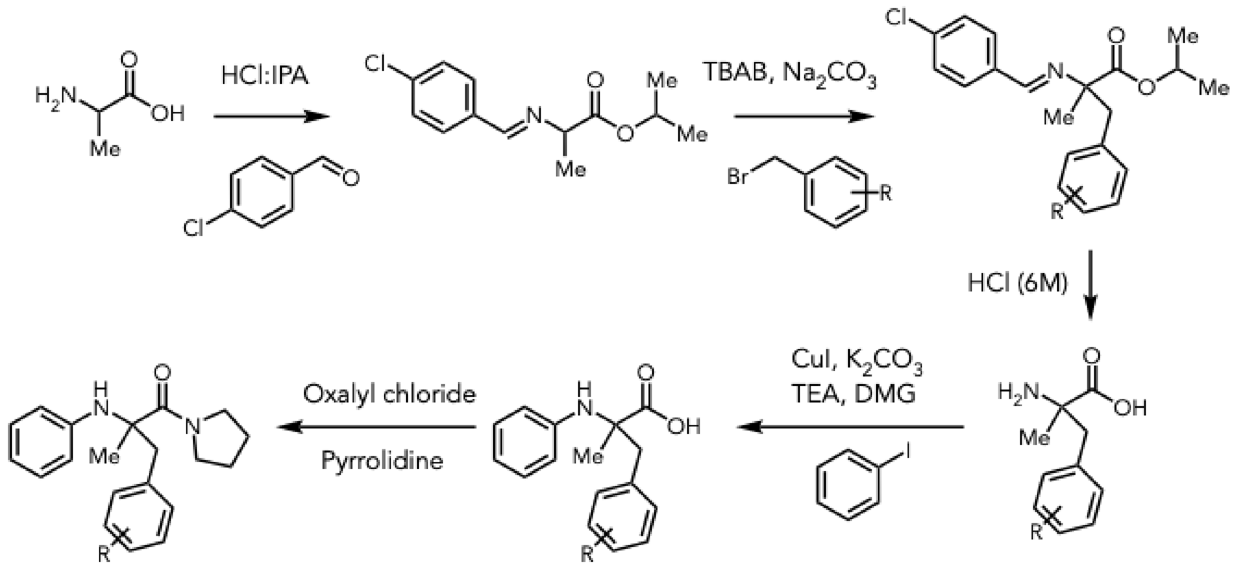
^{1}H-NMR (500 MHz; CDCl3): δ 7.19-7.11 (m, 3H), 7.10-7.05 (m, 2H), 3.67 (s, 3H), 3.49 (d, 1H, J= 15Hz), 3.23 (d, 1H, J= 15 Hz)

^{13}C-NMR (126 MHz; CDCl3): δ 171.5, 135.8, 130.47, 128.3, 127.4, 59.9, 53.0, 48.2, 27.4.

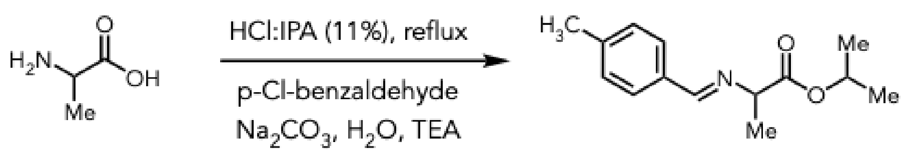
IR (neat, cm^{-1}): 2953, 1736, 1451, 1278, 1208, 1101.

2.3 Product synthesis (general route)

Overview of the synthesis plan

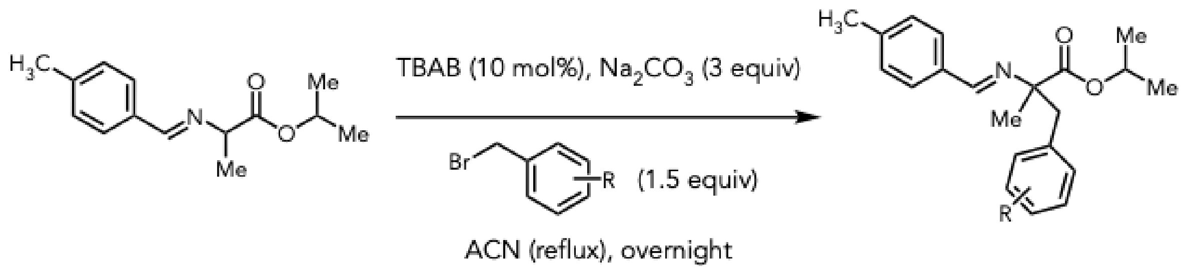


2.3.1 General Procedure D: Imine formation



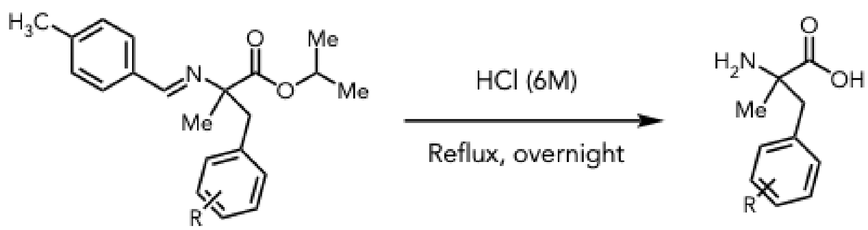
This protocol was adapted from Casas et al.[3,4] DL-alanine was dissolved 11% HCl/isopropanol and heated to reflux for four hours. After completion, the reaction was concentrated *in vacuo*. The residue was resuspended in acetonitrile and concentrated *in vacuo* to ensure complete removal of isopropanol. Water, 4-chlorobenzaldehyde (1 equiv.), and sodium carbonate (1.5 equiv.) were then added to the crude residue, and the mixture was stirred vigorously at room temperature overnight. The reaction mixture was extracted twice with ethyl acetate, and the combined organic layers were dried over sodium sulfate. The dried organic layer was then concentrated *in vacuo* to yield a pure product for the next steps.

2.3.2 General Procedure E: Arylation



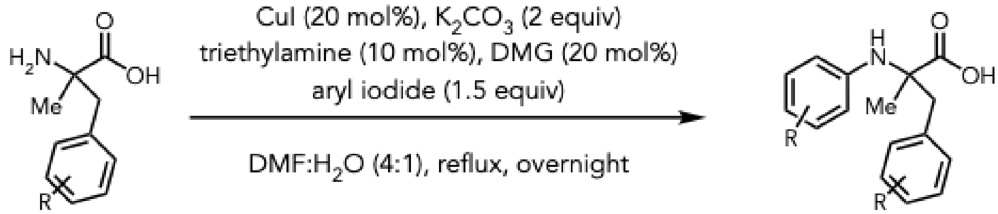
The procedure was adapted from O'Donnell et al.[5] A flame-dried flask was charged with isopropyl 2-[(E)-(4-chlorophenyl)methyleneamino]propanoate (1 equiv), benzyl bromide (1.5 equiv), tetra-n-butylammonium bromide (TBAB, 0.1 equiv), and anhydrous potassium carbonate (3 equiv). The mixture was dissolved in dry acetonitrile and heated under reflux until the reaction was complete, as monitored by LC-MS. After cooling, the mixture was filtered through a pad of Celite. The filtrate was concentrated *in vacuo*, and the resulting residue was carried forward without further purification.

2.3.3 General Procedure F: Deprotection



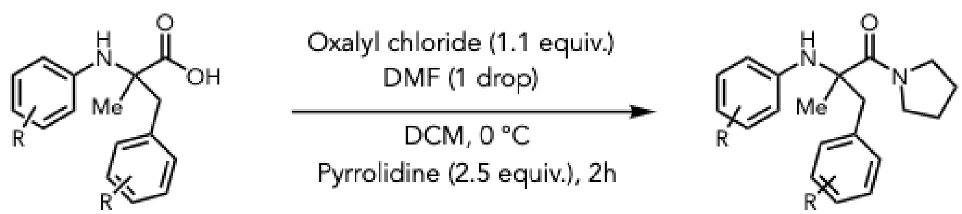
The residue from the previous step was dissolved in 1:1 HCl (6 M) and diethyl ether and stirred vigorously overnight. The aqueous layer was washed three times with diethyl ether, and then the aqueous layer was refluxed overnight. The HCl was removed *in vacuo,* and the residue was recrystallized using a mixture of acetonitrile/diethyl ether.

2.3.4 General Procedure G: N-alkylation



This procecdure was adapted from literature.[6] The acid product from the previous step was transferred to a flame-dried round-bottom flask under an inert atmosphere. To the flask, copper(I) iodide (0.2 equiv), potassium carbonate (2.0 equiv), triethylamine (0.1 equiv), and dimethylglycine (0.2 equiv), and aryl iodide (1.5 equiv) were added. The reaction vessel was subjected to three cycles of vacuum evacuation and nitrogen backfilling to ensure an oxygen-free environment. A pre-degassed mixture of DMF and water (4:1, v/v) was then added to the flask under a nitrogen atmosphere. The resulting reaction mixture was heated to reflux and stirred for 18 hours under nitrogen.

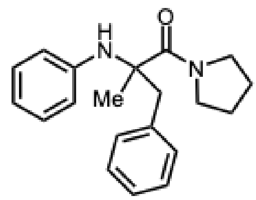
2.3.5 General Procedure H: Amidation



The acid from the previous step (1.0 equiv) was dissolved in dry dichloromethane (DCM) in a sealed flask equipped with an outlet needle under a nitrogen atmosphere and cooled to 0 °C. After adding two drops of dry DMF at 0 °C, oxalyl chloride (1.1 equiv) was introduced dropwise. The mixture was stirred for 1 hour, allowing it to warm to room temperature. The solvent was then removed *in vacuo*, and the resulting crude acid chloride was dried under vacuum for 1 hour before proceeding to the next step without additional purification.

The crude from the last step was dissolved in dry DCM and cooled to 0 °C. Pyrrolidine (2.5 equiv) was added dropwise, and the reaction was stirred for 2 h while warming to room temperature. The reaction was quenched with water and extracted with DCM. The organic extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give the product.

2.4 Product characterization



(*S*)-2-methyl-3-phenyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**3**)

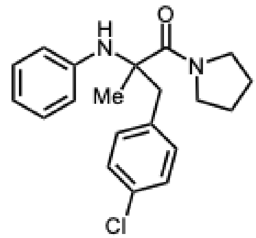
Synthesized according to general procedures D, E, F, G, H (17% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.27-7.19 (m, 3H), 7.14 (t, 2H, 10 Hz), 7.07-7.02 (m, 2H), 6.65 (t, 1H, 10 Hz), 6.53 (d, 2H, 10 Hz), 4.48 (s, 1H), 3.65-3.39 (m, 4H), 3.27 (d, 1H, 20 Hz), 3.19 (d, 1H, 20 Hz), 1.75-1.55 (m, 4H), 1.28 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.7, 146.8, 137.9, 131.5, 129.8, 128.4, 126.9, 117.9, 113.8, 61, 48.5, 47.4, 41.5, 27.5, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated for $C_{15}H_{15}FN$ ($M+H^+$): 309.1961; found 309.1968

IR (neat, cm^{-1}): 3118, 2976, 1671, 1406, 1209, 1046.



(*R*)-3-(4-chlorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**5**)

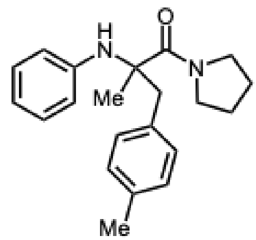
Synthesized according to general procedures D, E, F, G, H (18% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.26 (d, 2H, J=10 Hz), 7.17 (t, 2H, J=10 Hz), 7.03 (d, 2H, J=10 Hz), 6.68 (d, 2H, J=10 Hz), 6.54 (d, 2H, J=10 Hz), 4.53 (s, 1H), 3.70-3.42 (m, 4H), 3.34 (d, 2H, 20 Hz), 3.19 (d, 2H, 20 Hz), 1.80-1.55 (m, 4H), 1.29 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.7, 146.6, 137.1, 133.2, 132.3, 129.8, 128.2, 117.9, 113.8, 60.8, 48.5, 47.3, 40.3, 27.5, 23.3, 22.8,

HRMS (ESI-MS): m/z calculated for $C_{20}H_{24}ClN_2O$ ($M+H^+$): 343.1572; found 343.1560

IR (neat, cm^{-1}): 3002.5, 2252.9, 1442.9, 1375.3, 1038.7, 918.2, 750.3.



(R)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(p-tolyl)propan-1-one (**6**)

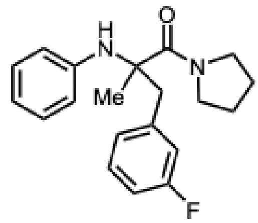
Synthesized according to general procedures D, E, F, G, H (14% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.15 (t, 2H, J= 8 Hz), 7.08 (d, 2H, J=8 Hz), 6.67 (d, 2H, J=8 Hz), 6.54 (d, 2H, J=10 Hz), 4.51 (s, 1H), 3.65-3.50 (m, 4H), 3.45 (t, 2H, J= 5 Hz), 3.19 (dd, 2H, 20 Hz), 2.31 (s, 3H), 1.77-1.59 (m, 4H), 1.30 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 173, 152.3, 140.7, 138, 131.5, 128.4, 126.9, 117.3, 115.1, 61.4, 55.7, 48.5, 47.4, 41.8, 27.5, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O$ ($M+H^+$): 323.2118; found 323.2108

IR (neat, cm^{-1}): 3002.9, 2252.9, 1442.6, 1375.3, 1038.9, 918, 749.5.



(*R*)-3-(3-fluorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**7**)

Synthesized according to general procedures D, E, F, G, H (6% yield over 5 steps).

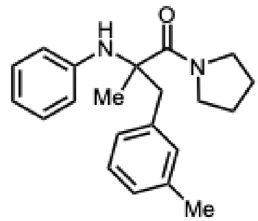
^{1}H-NMR (500 MHz; CD_3CN): δ 7.26 (q, 1H, J=10 Hz), 7.17 (t, 1H, J= 10Hz), 6.97 (t, 1H, j=10 Hz), 6.89 (d, 1H, J=10 Hz), 6.76 (d, 1H, J= 12Hz), 6.68 (t, 1H, J=10 Hz), 6.55 (d, 2H, J= 10 Hz), 4.55 (s, 1H), 3.70-3.42 (m, 4H), 3.37 (d, 1H, J= 15 Hz), 3.20 (d, 1H, J= 15 Hz), 1.80-1.54 (m, 4H), 1.29 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.7, 164.1, 161.7, 146.5, 141.0, 129.9, 129.8, 127.6, 118.2, 117.6, 113.7, 113.4, 60.7, 48.5, 47.4, 27.5, 23.2, 22.9.

^{19}F-NMR (470 MHz, $CDCl_3$): δ -114.31

HRMS (ESI-MS): m/z calculated for $C_{20}H_{24}FN_2O$ ($M+H^+$): 327.1867; found 327.1860

IR (neat, cm^{-1}): 3002.2, 2292.5, 1442.8, 1375.3, 1038.8, 918, 749.3.



(R)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(*m*-tolyl)propan-1-one (**8**)

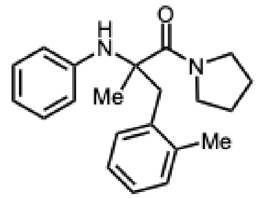
Synthesized according to general procedures D, E, F, G, H (12% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.19-7.11 (m, 3H), 7.07 (t, 1H, J= 12 Hz), 6.89 (m, 2H), 6.68 (t, 1H, J= 12 Hz), 6.55 (d, 2H, J= 12 Hz), 4.50 (s, 1H), 3.66-3.42 (m, 4H), 3.25 (d, 1H, J= 15 Hz), 3.15 (d, 1H, J= 15 Hz), 2.28 (s, 3H), 1.76-1.59 (m, 4H), 1.31 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.8, 146.8, 137.8, 132.3, 129.7, 128.5, 127.5, 123.0, 117.9, 117.5, 113.9, 61.0, 54.8, 48.5, 47.3, 41.6, 27.5, 23.3, 22.9, 21.0.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O$ ($M+H^+$): 323.2118; found 323.2109

IR (neat, cm^{-1}): 3002.5, 2292.5, 1442.4, 1375.3, 1038.9, 918.1, 749.9.



(*R*)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(*o*-tolyl)propan-1-one (**9**)

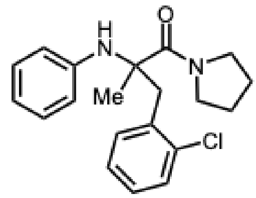
Synthesized according to general procedures D, E, F, G, H (7% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.20 (d, 2H, J= 10 Hz), 7.13-7.05 (m, 3H), 6.96 (d, 2H, J= 10 Hz), 6.62 (t, 1H, J= 12 Hz), 6.48 (d, 2H, J= 10 Hz), 4.46 (s, 1H), 3.62-3.35 (m, 4H), 3.27 (d, 1H, J= 15 Hz), 3.12 (d, 1H, J= 15 Hz), 2.08 (s, 3H), 1.22 (s, 3H)

^{13}C-NMR (126 MHz; CD_3CN): δ 172.6, 146.5, 137.1, 133.2, 129.8, 128.3, 117.9, 113.8, 60.8, 48.5, 47.3, 40.3, 27.5, 23.3, 22.8.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O$ ($M+H^+$): 323.2118; found 323.2102

IR (neat, cm^{-1}): 3001.5, 2292.4, 2252.9, 1442.6, 1375.3, 1038.9, 918, 749.8.



3-(2-chlorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**10**)

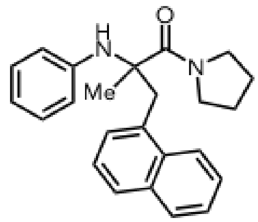
Synthesized according to general procedures D, E, F, G, H (10% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.42 (d, 1H, J= 10 Hz), 7.26-7.15 (m, 4H), 7.03 (d, 1H, J= 10 Hz), 6.96 (t, 1H, J= 7.5 Hz), 6.57 (d, 1H, J= 10 Hz), 4.68 (s, 1H), 3.68 (d, 1H, J= 15 Hz), 3.64-3.57 (m, 1H), 3.52-3.44 (m, 3H), 3.27 (d, 1H, J= 15 Hz), 3.33 (d, 1H, J= 15 Hz), 1.77-1.56 (m, 4H), 1.32 (s, 3H)

^{13}C-NMR (126 MHz; CD_3CN): δ 172.4, 146.4, 136.1, 135.6, 133.7, 129.8, 127.1, 117.9, 117.6, 113.8, 61.7, 48.6, 47.4, 37.1, 23.2, 27.5, 22.2.

HRMS (ESI-MS): m/z calculated for $C_{20}H_{24}ClN_2O$ (M+H$^+$): 343.1572; found 343.1550

IR (neat, cm^{-1}): 3000, 2202, 2158, 1443, 918.



2-methyl-3-(naphthalen-1-yl)-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**11**)

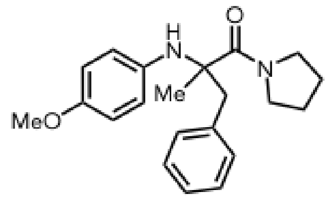
Synthesized according to general procedures D, E, F, G, H (22% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.86 (m, 1H), 7.78-7.73 (m, 2H), 7.54 (s, 1H), 7.48 (quintet, 2H, J= 10 Hz), 7.41-7.15 (m, 7H), 6.71 (t, 1H, J= 10 Hz), 6.58 (d, 2H, J= 12 Hz), 4.58 (s, 1H), 3.69-3.33 (m, 6H), 1.80-1.58 (m, 4H), 1.35 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.8, 146.8, 139.6, 135.8, 135.8, 133.8, 132.8, 130.2, 130.0, 129.1, 128.0, 127.4, 126.5, 126.1, 117.9, 113.9, 61.2, 48.5, 47.4, 41.4, 31.8, 27.5, 26.9, 23.3, 23.0.

HRMS (ESI-MS): m/z calculated for $C_{24}H_{27}N_2O$ ($M+H^+$): 359.2118; found 359.2096.

IR (neat, cm^{-1}): 3002.1, 2292.4, 2253, 1442.9, 1375.3, 1038.7, 918.1, 745.



2-((4-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**12**)

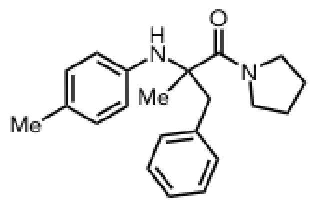
Synthesized according to general procedures D, E, F, G, H (9% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.29-7.21 (m, 3H), 7.08 (d, 2H, J=10 Hz), 6.77 (d, 2H, J=10 Hz), 6.50 (d, 2H, J=10 Hz), 4.21 (s, 1H), 3.71 (s, 3H), 3.69-3.42 (m, 4H), 3.22 (dd, 2H, 20 Hz), 1.80-1.59 (m, 4H), 1.27 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 173, 152.3, 140.7, 138, 131.5, 128.4, 126.9, 117.3, 115.1, 61.4, 55.7, 48.5, 47.4, 41.8, 27.5, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated for $C_{15}H_{15}FN$ ($M+H^{+}$): 339.2067; found 339.2050

IR (neat, cm^{-1}): 3002.9, 2252.9, 1442.8, 1375.3, 1034, 918.2, 749.4.



2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*p*-tolylamino)propan-1-one (**13**)

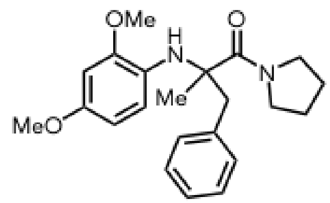
Synthesized according to general procedures D, E, F, G, H (25% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.15 (t, 2H, J= 8 Hz), 7.08 (d, 2H, J=8 Hz), 6.67 (d, 2H, J=8 Hz), 6.54 (d, 2H, J=10 Hz), 4.51 (s, 1H), 3.65-3.50 (m, 4H), 3.45 (t, 2H, J= 5 Hz), 3.19 (dd, 2H, 20 Hz), 2.31 (s, 3H), 1.77-1.59 (m, 4H), 1.30 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.9, 144.3,138.0, 131.5, 130.2, 128.4, 126.9, 117.9, 114.0, 61.1, 48.5, 47.3, 41.5, 27.5, 23.3, 22.9, 19.9.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O$ ($M+H^+$): 323.2118; found 323.2108

IR (neat, cm^{-1}): 3002.9, 2252.9, 1442.6, 1375.3, 1038.9, 918, 749.5.



2-((2,4-dimethoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**14**)

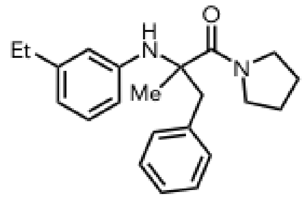
Synthesized according to general procedures D, E, F, G, H (5% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.31-7.21 (m, 3H), 7.03 (d, 2H, J=10 Hz), 6.56 (t, 1H, J=8 Hz), 6.39 (d, 2H, J=8 Hz), 4.15 (s, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.63-3.43 (m, 4H), 3.28 (d, 1H, J= 20 Hz), 3.17 (d, 1H, J= 20 Hz), 1.79-1.60 (m, 4H), 1.29 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 173.1, 152.6, 148.6, 137.8, 131.4, 130.2, 128.4, 127.0, 115.6, 111.8, 104.6, 100.0, 61.0, 55.7, 48.5, 47.4, 41.7, 27.5, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated for $C_{22}H_{29}N_2O_3$ ($M+H^+$): 369.2173; found 369.2173

IR (neat, cm^{-1}): 2937.5, 1619.9, 1515.5, 1405.3, 1033, 705.3.



2-((3-ethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**15**)

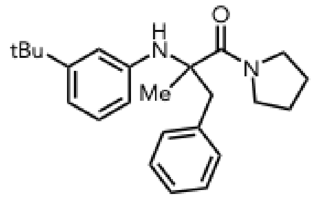
Synthesized according to general procedures D, E, F, G, H (16% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.30-7.21 (m, 3H), 7.10-7.03 (m, 3H), 6.54 (d, 1H, J=8 Hz), 6.41 (m, 1H), 6.38 (d, 1H, J= 8 Hz), 4.44 (s, 1H), 3.66-3.44 (m, 4H), 3.30 (d, 1H, J= 20 Hz), 3.18 (d, 1H, J= 20 Hz), 1.79-1.58 (m, 4H), 1.30 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.9, 146.8, 145.9, 138.0, 131.6, 129.7, 128.4, 126.9, 117.9, 117.3, 113.3,111.5, 61.0, 48.5, 47.4, 41.6, 29.3, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated $C_{22}H_{29}N_2O$ ($M+H^+$): 337.2274; found 337.2279

IR (neat, cm^{-1}): 3349.1, 2964.7, 1605.1, 1410, 701.8.



2-((3-(*tert*-butyl)phenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**16**)

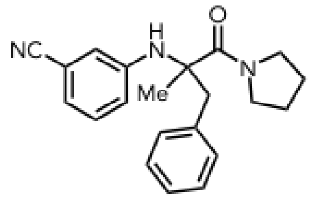
Synthesized according to general procedures D, E, F, G, H (15% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.29-7.20 (m, 3H), 7.10-7.04 (m, 3H), 6.74 (d, 1H, J= 10 Hz), 6.63 (t, 1H, J= 5 Hz), 6.38 (d, 1H, J= 10 Hz), 4.45 (s, 1H), 3.65-3.42 (m, 4H), 3.31 (d, 1H, J= 20 Hz), 3.19 (d, 1H, J= 20 Hz), 1.78-1.58 (m, 4H), 1.31 (s, 3H), 1.27 (s, 9H).

^{13}C-NMR (126 MHz; CD_3CN): δ 173.0, 162.9, 152.7, 146.5, 138.0, 131.5, 129.7, 129.3, 128.8, 126.9, 117.9, 114.6, 111.7, 111.0, 61.1, 48.6, 47.3, 38.5, 34.9, 31.2, 27.8, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated for $C_{24}H_{33}N_2O$ ($M+H^+$): 365.2587; found 365.2642

IR (neat, cm^{-1}): 3350, 2962.8, 1603.2, 1414.2, 779, 702.6.



3-((2-methyl-1-oxo-3-phenyl-1-(pyrrolidin-1-yl)propan-2-yl)amino)benzonitrile (**17**)

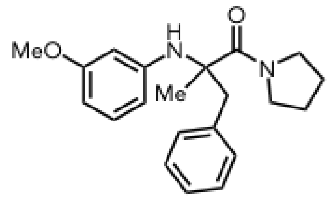
Synthesized according to general procedures D, E, F, G, H (20% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.32-7.23 (m, 4H), 7.08-7.04 (m, 2H), 7.00 (d, 1H, J= 10 Hz), 6.82 (d, 1H, J= 10 Hz), 6.79-6.76 (m, 1H), 5.01 (s, 1H), 3.59-3.42 (m, 4H), 3.29 (d, 1H, J= 20 Hz), 3.21 (d, 1H, J= 20 Hz), 1.78-1.58 (m, 4H), 1.31 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.1, 147.3,137.7, 131.7, 130.9, 128.6, 127.4, 120.9, 119.9, 118.3, 118.1, 116.2, 113.3, 61.2, 48.8, 47.6, 41.4, 27.6, 23.4, 22.7.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{24}N_3O$ (M+H$^+$): 334.1914; found 334.1888

IR (neat, cm^{-1}): 3332, 2972.7, 2226.4, 1600.5, 1536.5, 1421, 1372.4, 703.2.



2-((3-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**18)**

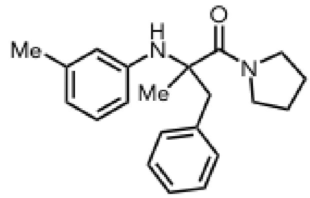
Synthesized according to general procedures D, E, F, G, H (8% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.29-7.21 (m, 3H), 7.09-7.02 (m, 3H), 6.26 (d, 1H, J= 10 Hz), 6.17 (d, 1H, J= 10 Hz), 6.12 (m, 1H), 4.56 (s, 1H), 3.72 (s, 3H), 3.66-3.43 (m, 4H), 3.32 (d, 1H, J= 20 Hz), 3.18 (d, 1H, J= 20 Hz), 1.78-1.58 (m, 4H), 1.29 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.8, 161.4, 148.1, 137.9, 131.6, 130.5, 128.4, 126.9, 117.9, 106.9, 103.0, 99.4, 60.9, 51.1, 48.5, 47.4, 41.4, 27.5, 23.3, 22.8.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O_2$ ($M+H^+$): 339.2067; found 339.2046

IR (neat, cm^{-1}): 3345.6, 2971.1, 1597.5, 1494.8, 1161.9, 703



2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*m*-tolylamino)propan-1-one (**19**)

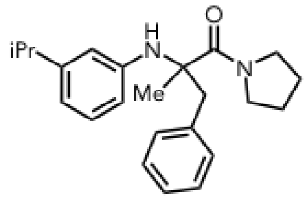
Synthesized according to general procedures D, E, F, G, H (10% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.36-7.24 (m, 4H), 7.09-7.04 (m, 2H), 6.96 (d, 1H, J= 10 Hz), 6.81-6.76 (m, 2H), 4.95 (s, 1H), 3.61-3.42 (m, 4H), 3.29 (d, 1H, J= 20 Hz), 3.22 (d, 1H, J= 20 Hz), 1.78-1.58 (m, 4H), 1.34 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.1, 147.2, 137.6, 131.5, 130.6, 128.4, 127.1,117.9, 117.1, 113.6, 113.5, 109.6, 61.1, 48.5, 47.4, 41.4, 27.5, 23.2, 22.6.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O$ ($M+H^+$): 323.2118; found 323.2105

IR (neat, cm^{-1}): 3347.9, 2971.2, 1603.6, 1492.3, 1182, 770.7, 733.6



2-((3-isopropylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**20**)

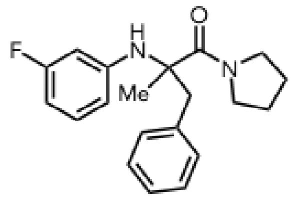
Synthesized according to general procedures D, E, F, G, H (16% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.29-7.22 (m, 3H), 7.10-7.04 (m, 3H), 6.57 (d, 1H, J= 10 Hz), 6.45 (m, 1H), 6.37 (d, 1H, J= 10 Hz), 4.44 (s, 1H), 3.64-3.46 (m, 4H), 3.30 (d, 1H, J= 20 Hz), 3.20 (d, 1H, J= 20 Hz), 2.80 (sep, 1H, J= 7 Hz), 1.78-1.58 (m, 4H), 1.30 (s, 3H), 1.21 (d, 6H, J= 7 Hz).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.9, 150.5, 146.7, 138.0, 131.5, 129.6, 128.3, 126.9, 117.9, 115.9, 111.8, 111.6, 61.1, 48.5, 47.3, 41.6, 34.6, 27.5, 24.0, 23.9, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated for $C_{23}H_{31}N_2O$ ($M+H^+$): 351.2431; found 351.2423.

IR (neat, cm^{-1}): 3350.2, 2962.8, 1603.2, 1414.6, 779, 702.6.



2-((3-fluorophenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**21**)

Synthesized according to general procedures D, E, F, G, H (11% yield over 5 steps).

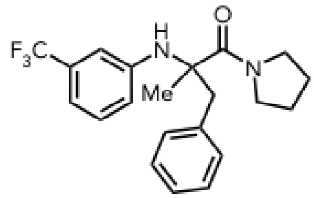
^{1}H-NMR (500 MHz; CD_3CN): δ 7.29-7.22 (m, 3H), 7.14 (q, 1H, J= 8 Hz), 7.09-7.04 (m, 2H), 6.43-6.35 (m, 2H), 6.26 (dt, 1H, J= 1 Hz, 15 Hz), 4.82 (s, 1H), 3.64-3.42 (m, 4H), 3.31 (d, 1H, J= 20 Hz), 3.19 (d, 1H, J= 20 Hz), 1.80-1.60 (m, 4H), 1.32 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.3, 165.4, 163.5, 148.7, 148.6, 137.7, 131.5, 131.1, 131.0, 128.4, 127.0, 117.9, 109.9, 103.6, 103.4, 100.2, 99.9, 61.1, 48.5, 47.4, 41.3, 27.5, 23.3, 22.6.

^{19}F-NMR (470 MHz, $CDCl_3$): δ -114.3.

HRMS (ESI-MS): m/z calculated for $C_{20}H_{24}FN_2O$ ($M+H^+$): 327.1867; found 327.1877

IR (neat, cm^{-1}): 3337.8, 2972.8, 1602.2, 1535, 1495.4, 1338.3, 1179, 735.3



2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-((3-(trifluoromethyl)phenyl)amino)propan-1-one (**22**)

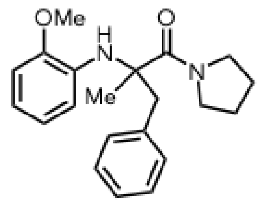
Synthesized according to general procedures D, E, F, G, H (9% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.33 (t, 1H, J= 8 Hz), 7.30-7.22 (m,3H), 7.07 (d, 2H, J = 7.5 Hz), 6.96 (d, 1H, J= 7.5 Hz), 6.81-6.75 (m, 2H), 4.95 (s, 1H), 3.61-3.40 (m, 4H), 3.29 (d, 1H, J= 15 Hz), 3.22 (d, 1H, J= 15 Hz), 1.80-1.56 (m, 4H), 1.34 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.1, 147.1, 137.6, 131.5, 130.6, 128.4, 127.0, 117.9, 117.1, 113.5, 109.6, 61.1, 48.5, 47.4, 41.4, 27.5, 23.2, 22.6.

^{19}F-NMR (470 MHz, $CDCl_3$): δ -63.4

HRMS (ESI-MS): m/z calculated for $C_{21}H_{24}F_3N_2O$ ($M+H^+$): 377.1762; found 377.1792



2-((2-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**23**)

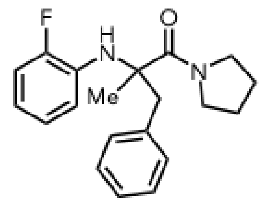
Synthesized according to general procedures D, E, F, G, H (10% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.29-7.22 (m, 3H), 7.04 (d, 2H, J= 8 Hz), 6.90 (d, 1H, J= 8 Hz), 6.81 (t, 1H, J= 8 Hz), 6.69 (t, 1H, J= 8 Hz), 6.48 (d, 1H, J= 8 Hz), 4.51 (s, 1H), 3.80 (s, 3H), 3.60-3.44 (m, 4H), 3.32 (d, 1H, J= 20 Hz), 3.18 (d, 1H, J= 20 Hz), 1.77-1.60 (m, 4H), 1.33 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.8,147.5, 135.9, 131.3, 128.4, 127.0, 121.6, 117.9, 117.5, 111.4, 110.8, 60.7, 56.0, 48.5, 47.2, 41.6, 27.5, 23.3, 22.9.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O_2$ (M+H$^+$): 339.2067; found 339.2035.

IR (neat, cm^{-1}): 2970.24, 1619.7, 1511, 1454.4, 1222, 1028.2, 736.8



2-((2-fluorophenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**24**)

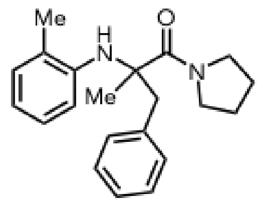
Synthesized according to general procedures D, E, F, G, H (8% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.32-7.25 (m, 3H), 7.09-6.97 (d, 4H), 6.72-6.61 (m, 2H), 4.39 (s, 1H), 3.63-3.45 (m, 4H), 3.32 (d, 1H, J= 20 Hz), 3.22 (d, 1H, J= 20 Hz), 1.79-1.60 (m, 4H), 1.38 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.4, 137.5, 131.3,128.5, 127.2, 125.2, 117.9, 117.6, 117.5, 115.2, 113.7, 61.1, 48.5, 47.4, 27.5, 23.3, 22.8.

HRMS (ESI-MS): m/z calculated for $C_{20}H_{24}FN_2O$ ($M+H^+$): 327.1867; found 327.1848.

IR (neat, cm^{-1}): 3349.5, 2971.7, 1618, 1495.5, 743.4.



2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*o*-tolylamino)propan-1-one (**25**)

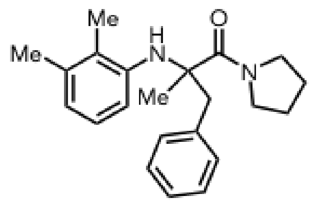
Synthesized according to general procedures D, E, F, G, H (7% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.31-7.22 (m, 3H), 7.09 (d, 1H, J= 7.5Hz), 7.07-7.00 (m, 3H),6.64 (t, 1H, J= 7.5Hz) 6.50 (d, 1H, J= 7.5Hz), 3.81 (s, 1H), 3.63-3.52 (m, 1H), 3.50-3.42 (m, 3H), 3.38 (d, 1H, J= 15Hz), 3.19 (d, 1H, J= 15Hz), 2.02 (s, 3H), 1.77-1.58 (m, 4H), 1.37 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 172.9, 144.1, 137.8, 131.4, 130.9, 128.5, 127.4, 127.1, 123.0, 117.9, 117.6, 111.3, 60.9, 48.5, 47.2, 41.4, 27.5, 23.3, 23.1, 17.5.

HRMS (ESI-MS): m/z calculated for $C_{21}H_{27}N_2O$ ($M+H^+$): 323.2118; found 323.2104.

IR (neat, cm^{-1}): 2970.4, 1605.7, 1495.6, 1406.6, 747.6, 705.



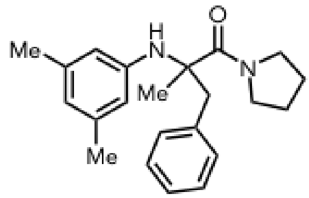
2-((2,3-dimethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**26**)

Synthesized according to general procedures D, E, F, G, H (17% yield over 5 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ 7.31-7.23 (m, 3H), 7.04 (d, 2H, J= 5 Hz), 6.92 (t, 1H, J= 7 Hz), 6.57 (d, 1H, J= 7 Hz), 6.37 (d, 1H, J= 7 Hz), 3.82 (s, 1H), 3.58-3.42 (m, 4H), 3.37 (d, 1H, J= 20 Hz), 3.18 (d, 1H, J= 20 Hz), 2.28 (s, 3H), 1.93 (s, 3H), 1.36 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 173.1, 144.0, 137.8, 137.2, 131.4, 128.5, 127.0, 126.4, 121.2, 119.9, 117.9, 109.6, 61.0, 48.5, 47.2, 41.6, 27.4, 23.3, 23.2, 20.6, 12.5.

HRMS (ESI-MS): m/z calculated for $C_{22}H_{29}N_2O$ ($M+H^+$): 337.2202; found 337.2256



2-((3,5-dimethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**27**)

Synthesized according to general procedures D, E, F, G, H (15% yield over 5 steps).

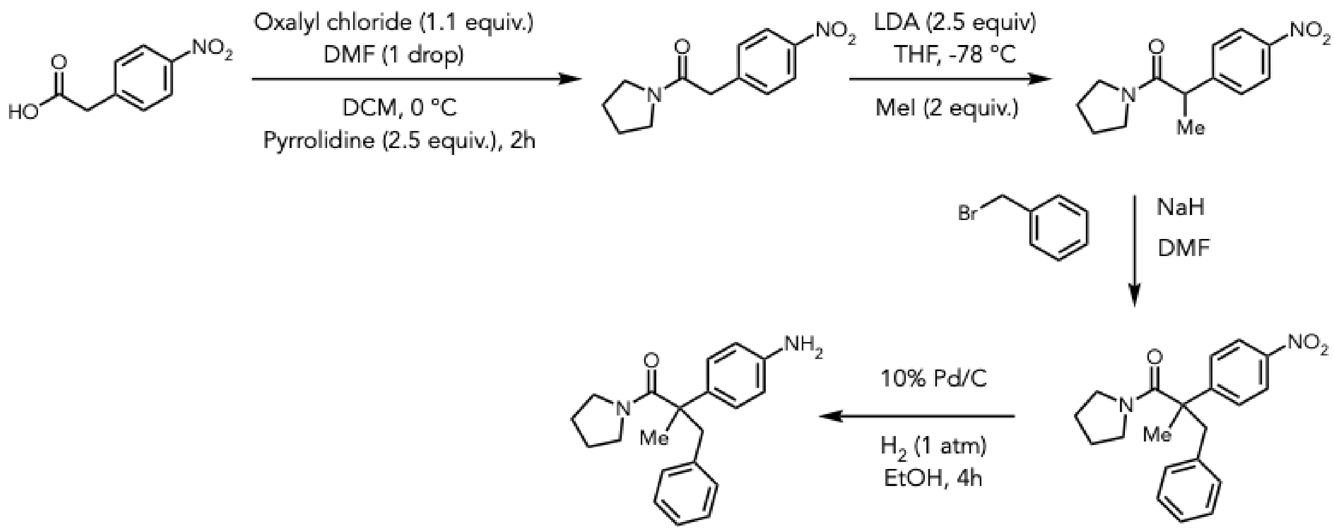
^{1}H-NMR (500 MHz; CD_3CN): δ 7.29-7.21 (m, 3H), 7.08 (d, 2H, J= 5 Hz), 6.35 (s, 1H), 6.18 (s, 2H), 4.35 (s, 1H), 3.64-3.43 (m, 4H), 3.30 (d, 1H, J= 20 Hz), 3.18 (d, 1H, J= 20 Hz), 2.19 (s, 6H), 1.28 (s, 3H).

^{13}C-NMR (126 MHz; CD_3CN): δ 127.8, 146.7, 139.2, 138.0, 131.5, 128.3, 126.9, 119.5, 117.9, 111.8, 61.0, 48.5, 47.3, 41.5, 27.5, 23.3, 22.9, 12.3.

HRMS (ESI-MS): m/z calculated for $C_{22}H_{29}N_2O$ ($M+H^+$): 337.2202; found 337.2278

IR (neat, cm^{-1}): 2974, 1492, 1409, 751, 705.

2.5 Synthesis of the hydroarylation byproduct (overview)



2.5.1 General Procedure K: Amidation

2-(4-nitrophenyl)acetic acid (1.0 equiv) was dissolved in dry dichloromethane (DCM) in a sealed flask equipped with an outlet needle under a nitrogen atmosphere and cooled to 0 °C. After adding two drops of dry DMF at 0 °C, oxalyl chloride (1.1 equiv) was introduced dropwise. The mixture was stirred for 1 hour, allowing it to warm to room temperature. The solvent was then removed *in vacuo*, and the resulting crude acid chloride was dried under vacuum for 1 hour before proceeding to the next step without additional purification.

The crude from the last step was dissolved in dry DCM and cooled to 0 °C. Pyrrolidine (2.5 equiv) was added dropwise, and the reaction was stirred for 2 h while warming to room temperature. The reaction was quenched with water and extracted with DCM. The organic extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give the product.

2.5.2 General Procedure L: Methylation

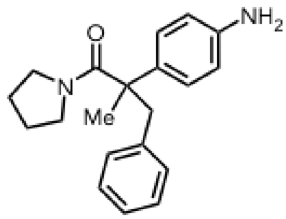
2-(4-nitrophenyl)-1-(pyrrolidin-1-yl)ethan-1-one (1.0 equiv) was added dropwise to a stirred solution of lithium diisopropylamide (LDA, 2.5 equiv, 2.5 M in THF) at –78 °C. After stirring for 30 minutes at this temperature, the reaction mixture was warmed to room temperature and then heated to 60 °C for an additional hour. It was subsequently recooled to –78 °C, and methyl iodide (2.0 equiv) in THF was added dropwise. The mixture was then allowed to warm to room temperature and stirred overnight. The reaction was quenched with water, and ethyl acetate was added. The aqueous layer was separated, acidified with 3 M HCl, and extracted with ethyl acetate (three times). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to afford the crude product.

2.5.3 General Procedure M: Arylation

To a suspension of NaH (60% dispersion in mineral oil, 1.5 equiv.) in anhydrous DMF at 0 °C under N_2 was added a solution of the 2-(4-nitrophenyl)-1-(pyrrolidin-1-yl) propan-1-one (1.0 equiv.) in anhydrous DMF dropwise over 5 min. The reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 30 min. Benzyl bromide (1.5 equiv.) was added dropwise at 0 °C, and the reaction was allowed to warm to room temperature and stirred for 4 h (monitored by TLC). The reaction was quenched with saturated aqueous NH_4Cl at 0 °C and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was purified by flash column chromatography.

2.5.4 General Procedure N: Reduction

In a round-bottom flask, 2-methyl-2-(4-nitrophenyl)-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (1.0 equiv.) was dissolved in EtOH. 10% Pd/C was added, and the reaction vessel was evacuated and backfilled with H_2 (balloon). The suspension was stirred vigorously at room temperature under a H_2 atmosphere for 2 h, until TLC indicated complete consumption of the starting material. The reaction mixture was filtered through a short pad of Celite, washing with EtOAc (3 × 10 mL), and the filtrate was concentrated under reduced pressure. The residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$, and the layers were separated. The aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated.



2-(4-aminophenyl)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**4**)

Synthesized according to general procedures K, L, M, N (28% yield over 4 steps).

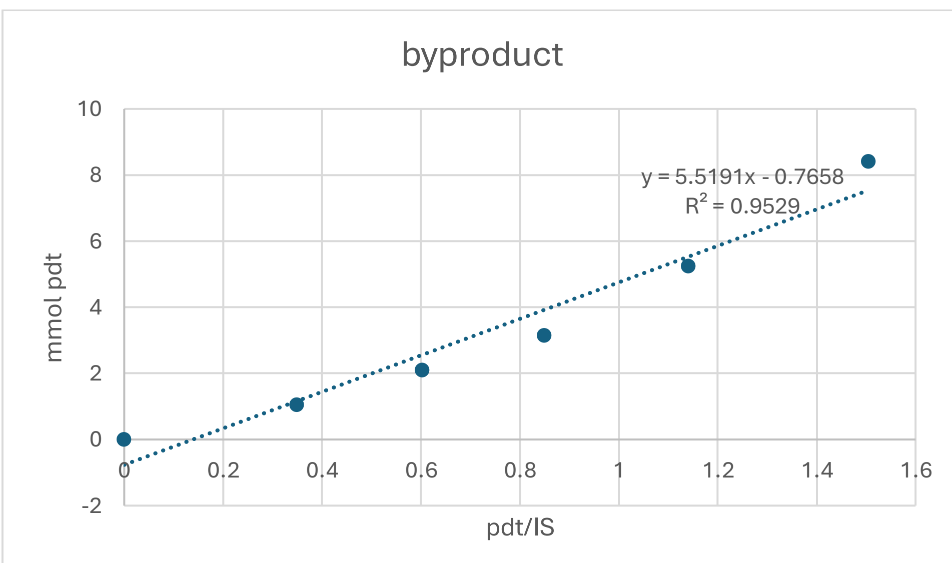
^{1}H-NMR (500 MHz; CD_3CN): δ 7.07-7.03 (m, 3H), 6.77-6.72 (m, 4H), 6.49 (d, 2H, J= 10 Hz), 3.99 (s, 2H), 3.34-3.27 (m, 2H), 3.22 (d, 1H, J= 20 Hz), 3.00 (d, 1H, J= 20 Hz), 3.87-3.69 (m, 2H), 1.66-1.41 (m, 4H), 1.22 (s, 3H)

^{13}C-NMR (126 MHz; CD_3CN): δ 174.4, 146.8, 139.2, 133.2, 131.5, 128.0, 127.7, 126.5, 117.9, 114.8, 51.1, 47.8, 47.4, 45.5, 26.9, 23.5, 23.1.

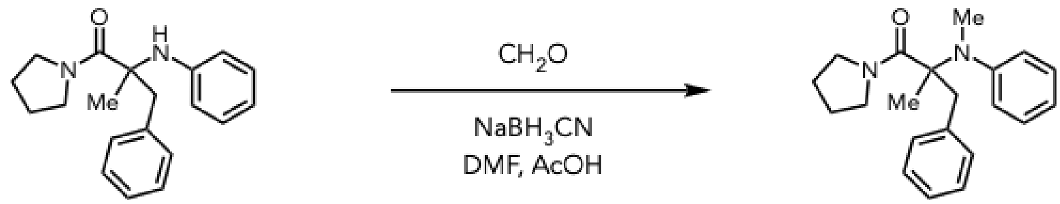
HRMS (ESI-MS): m/z calculated for $C_{20}H_{25}N_2O$ (M+H$^+$): 309.1889; found 309.1805

IR (neat, cm^{-1}): 3345, 2972, 1610, 1517, 1453, 1373, 1287.

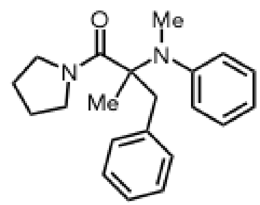
Calibration



2.6 Synthesis of N-Methyl product



This procedure was adapted from literature.[7] In a dry round-bottom flask, the secondary amine (**2a**, 1.0 equiv.) was dissolved in anhydrous DMF. Paraformaldehyde (15.0 mg, 5.0 equiv) and one drop of glacial AcOH were added, and the mixture was stirred at room temperature for 40 min. Sodium cyanoborohydride ($NaBH_3CN$, 1.5 equiv.) was then added dropwise, and the reaction mixture was stirred at room temperature for 16 h. After completion, the reaction mixture was poured into saturated aqueous $NaHCO_3$, and the resulting suspension was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude was purified by flash column chromatography.



2(*S*)-2-methyl-2-(methyl(phenyl)amino)-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**28**)

Synthesized according to general procedure 2.11 (5% yield).

^{1}H-NMR (500 MHz; CDCl3): δ 7.40-7.05 (m, 7H), 6.98 (d, 1H), 6.84 (d, 1H, J= 10 Hz), 6.78 (t, 1H, J= 5Hz), 4.67 (s, 3H), 3.62 (d, 1H, J= 15Hz), 3.52-3.33 (m, 2H), 3.22-2.96 (m, 3H), 1.80-1.56 (m, 4H), 1.45 (s, 3H).

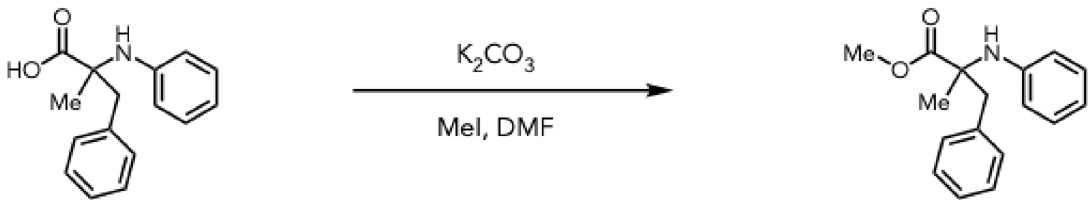
^{13}C-NMR (126 MHz; CDCl3): δ 173.5, 131.6, 131.4, 129.3, 128.7, 128.3, 126.8, 117.9, 47.9, 46.6, 40.5, 38.9, 29.9, 27.4, 23.3, 21.9.

162.4, 160.5, 150.2, 148.8, 141.1, 128.3, 124.7, 115.2, 44.5, 40.5, 21.6.

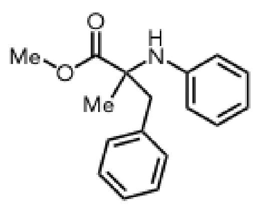
HRMS (ESI-MS): m/z calculated for $C_{22}H_{29}N_2O$ ($M+H^+$): 322.2045; found 322.2008

IR (neat, cm^{-1}): 2981, 1609, 1498, 1405, 747.6.

2.7 Synthesis of ester product



In a dry round-bottom flask, the acid substrate (1.0 equiv.) was dissolved in anhydrous DMF. Powdered K_2CO_3 (3.0 equiv) was added, and the resulting suspension was stirred at room temperature for 20 minutes. Methyl iodide (MeI, 5.0 equiv.) was then added dropwise, and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was cooled to room temperature, diluted with EtOAc, and washed with water and brine. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography.



methyl 2-methyl-3-phenyl-2-(phenylamino)propanoate (**29**)

Synthesized according to general procedure 2.12 (45% yield over 1 steps).

^{1}H-NMR (500 MHz; CD_3CN): δ

7.31-7.23 (m, 3H), 7.19-7.09 (m, 4H), 6.71 (t, 1H, J= 10 Hz), 6.58 (d, 2H, J= 10 Hz), 4.66 (s, 1H), 3.66 (s, 3H), 3.30 (d, 1H, J= 20 Hz), 3.18 (d, 1H, J= 20 Hz), 1.41 (s, 3H).

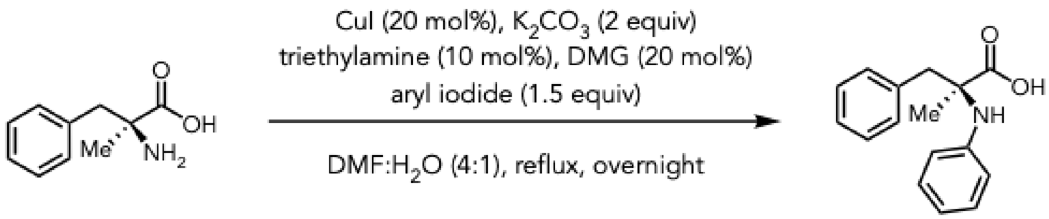
^{13}C-NMR (126 MHz; CD_3CN): δ 176.2, 146.6, 137.0, 131.1, 129.7, 128.5, 127.3, 117.9, 114.9, 67.9, 61.3, 52.4, 43.1, 23.2.

HRMS (ESI-MS): m/z calculated for $C_{17}H_{20}NO_2$ (M+H^{+}): 270.1416; found 270.1512

IR (neat, cm^{-1}): 3408, 2950, 1731, 1603, 1500, 1313.

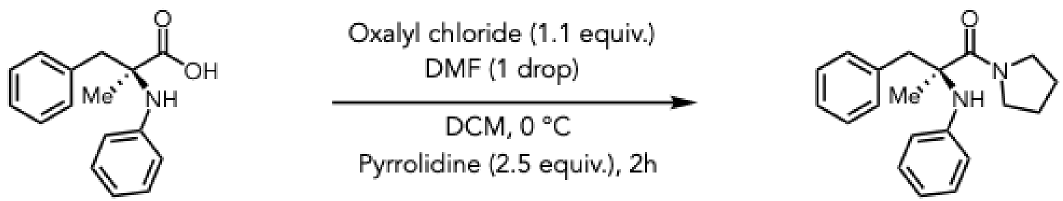
2.8 Determination of absolute stereoconfiguration

The synthesis of the enantioenriched product was performed by copper-catalyzed N-alkylation of enantiopure (*S*)-2-amino-2-methyl-3-phenylpropanoic acid (commercially available), followed by amidation of the crude product.



General Procedure I: N-alkylation

The chiral (*S*)-2-amino-2-methyl-3-phenylpropanoic acid was transferred to a flame-dried round-bottom flask under an inert atmosphere. To the flask, copper(I) iodide (0.2 equiv), potassium carbonate (2.0 equiv), triethylamine (0.1 equiv), and dimethylglycine (0.2 equiv), and aryl iodide (1.5 equiv) were added. The reaction vessel was subjected to three cycles of vacuum evacuation and nitrogen backfilling to ensure an oxygen-free environment. A pre-degassed mixture of DMF and water (4:1, v/v) was then added to the flask under a nitrogen atmosphere. The resulting reaction mixture was heated to reflux and stirred for 18 hours under nitrogen.

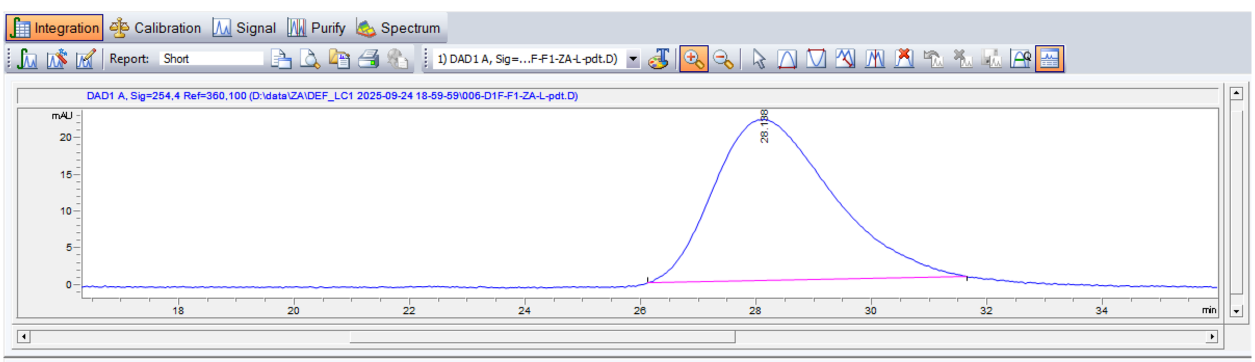


General Procedure J: Amidation

The acid from the previous step (1.0 equiv) was dissolved in dry dichloromethane (DCM) in a sealed flask equipped with an outlet needle under a nitrogen atmosphere and cooled to 0 °C. After adding two drops of dry DMF at 0 °C, oxalyl chloride (1.1 equiv) was introduced dropwise. The mixture was stirred for 1 hour, allowing it to warm to room temperature. The solvent was then removed *in vacuo*, and the resulting crude acid chloride was dried under vacuum for 1 hour before proceeding to the next step without additional purification.

The crude from the last step was dissolved in dry DCM and cooled to 0 °C. Pyrrolidine (2.5 equiv) was added dropwise, and the reaction was stirred for 2 h while warming to room temperature. The reaction was quenched with water and extracted with DCM. The organic extracts were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated to give the product.

Comparison of the chiral HPLC traces of the enantiopure and our enzymatic product led the conclusion that our enzymatic product is (S)-2-methyl-3-phenyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	28.138	BB	3180.1	22.1	1.6862	100.000	0.715

3. Protein sequence and expression

3.1 Morphinone reductase (MorB)

Genebank accession number: AAC43569

Protein Sequence:

MPDTSFSNPGLFTPLQLGSLSLPNRVIMAPLTRSRTPDSVPGRLQQIYYGQRASAGLIISEA
TNISPTARGYVYTPGIWTDAQEAGWKGVVEAVHAKGGRIALQLWHVGRVSHELVQPDG
QQPVAPSALKAEGAECFVEFEDGTAGLHPTSTPRALETDEIPGIVEDYRQAAQRAKRAGF
DMVEVHAANACLPNQFLATGTNRRTDQYGGSIENRARFPLEVVDAVAEVFGPERVGIRL
TPFLELFGLTDDEPEAMAFYLAGELDRRGLAYLHFNEPDWIGGDITYPEGFREQMRQRF
KGGLIYCGNYDAGRAQARLDDNTADAVAFGRPFIANPDLPERFRLGAALNEPDPSTFYG
GAEVGYTDY PFLDNGHDRLGHHHHHH

DNA Sequence:

ATGCCCGACACTTCTTTTTCGAATCCAGGACTTTTTACTCCTCTTCAGTTGGGTAGTCT
GTCTCTTCCAAATCGTGTCATAATGGCACCTTTAACCCGCTCACGCACGCCAGATTCT
GTACCTGGACGCCTTCAACAGATATACTATGGTCAACGCGCCAGCGCCGGGTTAATCA
TCTCCGAAGCGACAAATATCAGTCCCACCGCTCGGGGATACGTATACACGCCAGGCAT
TTGGACTGACGCTCAGGAGGCCGGTTGGAAAGGTGTGGTCGAAGCTGTCCATGCTAA
AGGGGGTCGTATAGCGTTGCAGTTATGGCATGTCGGCCGGGTCTCTCATGAGCTGGTG
CAGCCAGACGGCCAACAACCCGTGGCACCATCCGCCTTAAAAGCCGAAGGGGCCGA
GTGCTTTGTCGAATTCGAGGATGGGACTGCTGGCCTGCACCCTACGTCAACTCCCAG
AGCCCTGGAGACAGATGAGATACCCGGTATTGTTGAAGATTACAGACAGGCCGCGCA
GCGTGCGAAGCGGGCCGGATTCGATATGGTAGAGGTCCACGCGGCAAATGCTTGTCT
TCCTAATCAGTTCTTGGCGACAGGAACCAATCGTCGCACAGACCAGTACGGTGGATC
AATTGAGAACCGGGCTAGATTCCCATTAGAGGTTGTCGATGCTGTAGCCGAGGTATTC
GGGCCCGAAAGAGTGGGGATACGGCTGACTCCTTTCCTGGAGTTATTTGGATTAACG
GATGATGAACCCGAGGCAATGGCTTTTTACCTTGCGGGAGAATTAGACCGGCGTGGT
TTAGCGTATTTACACTTTAATGAACCCGATTGGATAGGTGGGGACATCACGTACCCGG
AAGGGTTTCGTGAGCAAATGCGTCAACGGTTCAAGGGGGGGCTTATATATTGTGGAA
ACTACGACGCAGGTCGGGCCCAAGCCCGGCTTGACGACAATACAGCAGATGCAGTG
GCGTTTGGGCGTCCATTTATTGCCAACCCCGACTTGCCAGAACGTTTCCGCTTAGGAG
CAGCGCTGAACGAACCTGACCCCTCTACTTTTTACGGCGGGGCAGAGGTGGGGTACA
CAGACTACCCGTTCCTGGACAACGGTCATGACCGCCTGGGACTCGAGCACCACCATC
ACCACCACTGA

3.2 Other proteins evaluated in this work

Gluconobacter oxydans enoate reductase (GluER) from Gluconobacter oxydans 621H. Genbank accession number: AAW60280

GluER protein sequence:
MHHHHHHPTLFDPIDFGPIHAKNRIVMSPLTRGRADKEAVPTPIMAEYYAQRASAGLIITE
ATGISREGLGWPFAPGIWSDAQVEAWKPIVAGVHAKGGKIVCQLWHMGRMVHSSVTGT
QPVSSSATTAPGEVHTYEGKKPFEQARAIDAADISRILNDYENAARNAIRAGFDGVQIHA
ANGYLIDEFLRNGTNHRTDEYGGVPENRIRFLKEVTERVIAAIGADRTGVRLSPNGDTQG
CIDSAPETVFVPAAKLLQDLGVAWLELREPGPNGTFGKTDQPKLSPQIRKVFLRPLVLNQ
DYTFEAAQTALAEGKADAIAFGRKFISNPDLPERFARGIALQPDDMKTWYSQGPEGYTD
YPSATSGPN.

GluER DNA sequence:
ATGCACCACCATCACCACCACCCGACCCTTTTCGACCCCATCGATTTCGGACCTATCC
ACGCCAAGAATCGTATCGTCATGTCCCCCCTGACTCGCGGTCGCGCTGACAAAGAGG
CGGTTCCAACCCCCATTATGGCTGAATACTACGCCCAACGCGCTTCGGCGGGTTTAAT
TATCACTGAAGCGACGGGGATTTCACGCGAAGGCTTAGGTTGGCCGTTTGCGCCGGG
AATTTGGTCCGATGCACAGGTTGAGGCGTGGAAACCTATCGTCGCGGGTGTCCATGC
AAAGGGCGGCAAGATCGTATGTCAGCTTTGGCATATGGGCCGTATGGTACATTCTTCA
GTTACAGGGACGCAGCCCGTAAGCAGTTCCGCCACTACTGCTCCAGGTGAGGTTCAC
ACCTATGAGGGCAAGAAGCCCTTCGAACAAGCGCGTGCAATCGATGCTGCAGACATC
TCCCGCATCCTTAACGATTACGAAAATGCAGCACGTAATGCAATCCGCGCGGGTTTCG
ATGGAGTGCAGATCCACGCAGCCAATGGCTACCTTATCGATGAGTTTTTGCGTAACGG
AACCAATCATCGCACCGATGAGTATGGGGGGGTGCCGGAGAACCGTATTCGTTTCTTG
AAAGAGGTAACAGAACGCGTCATCGCGGCGATTGGCGCTGACCGTACGGGTGTGCGT
CTGAGTCCAAACGGTGACACACAGGGTTGTATCGACAGTGCTCCCGAAACCGTTTTT
GTTCCTGCCGCAAAGCTTTTGCAAGATTTAGGGGTAGCGTGGCTTGAGCTGCGTGAA
CCTGGTCCGAATGGTACGTTTGGAAAGACGGATCAACCAAAATTATCTCCACAAATCC
GTAAGGTATTCCTTCGTCCATTGGTCTTAAATCAAGACTATACTTTTGAGGCGGCACAG
ACGGCCCTGGCTGAGGGCAAGGCGGACGCTATTGCGTTTGGCCGTAAGTTCATTTCA
ATCCAGACTTGCCTGAGCGCTTTGCCCGTGGCATCGCACTGCAACCAGACGATATGA
AAACATGGTACTCCCAAGGCCCAGAGGGTTACACAGACTATCCATCCGCAACTTCTG
GGCCGAACT GA

Old yellow enzyme 2 (OYE2) from Saccharomyces cerevisiae.
Genbank accession number: AAA83386.1

OYE2 protein sequence:
MPFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRAQHPGNIPNRDWAVEYYAQ
RAQRPGTLIITEGTFPSPQSGGYDNAPGIWSEEQIKEWTKIFKAIHENKSFAWVQLWVLG
WAAFPDTLARDGLRYDSASDNVYMNAEQEEKAKKANNPQHSITKDEIKQYVKEYVQA
AKNSIAAGADGVEIHSANGYLLNQFLDPHSNNRTDEYGGSIENRARFTLEVVDAVVDAI
GPEKVGLRLSPYGVFNSMSGGAETGIVAQYAYVLGELERRAKAGKRLAFVHLVEPRVTN
PFLTEGEGEYNGGSNKFAYSIWKGPIIRAGNFALHPEVVREEVKDPRTLIGYGRFFISNPDL
VDRLEKGLPLNKYDRDTFYKMS AEGYIDYPTYEEALKLGWDKNHHHHHH.

OYE2 DNA sequence:
ATGCCCTTCGTGAAAGACTTCAAACCTCAAGCCCTGGGCGATACTAATTTATTTAAGC
CAATTAAAATTGGAAACAATGAGTTGTTACACCGCGCTGTAATTCCACCCTTAACCCG
CATGCGCGCCCAACATCCAGGGAACATCCCTAATCGCGATTGGGCAGTCGAGTACTAT
GCTCAGCGTGCTCAGCGTCCGGGTACCCTTATCATCACGGAAGGAACGTTTCCGTCG
CCGCAATCGGGAGGGTATGACAACGCTCCCGGTATCTGGTCGGAAGAACAGATTAAA
GAATGGACCAAAATCTTTAAAGCAATTCATGAGAATAAATCTTTCGCCTGGGTCCAAC
TTTGGGTCCTGGGCTGGGCAGCCTTCCCTGACACATTGGCGCGTGACGGGCTTCGTT
ATGATAGTGCTTCGGATAACGTGTATATGAATGCTGAACAAGAAGAAAAGGCAAAAA
AAGCAAACAATCCACAGCATTCGATTACTAAAGACGAGATTAAGCAGTATGTTAAGG
AATACGTACAAGCAGCAAAGAATTCTATTGCCGCAGGGGCGGACGGGGTAGAAATCC
ACTCTGCTAATGGGTACTTGCTTAACCAGTTCCTGGACCCGCATTCAAACAACCGCAC
TGATGAGTACGGAGGGTCCATCGAAAATCGTGCACGTTTTACTTTAGAGGTCGTAGAT
GCTGTAGTCGACGCGATTGGCCCTGAGAAGGTAGGTTTGCGTTTAAGTCCTTATGGCG
TGTTCAATTCAATGTCAGGGGGCGCTGAAACAGGTATCGTCGCGCAGTACGCATACGT
CTTGGGAGAGCTGGAGCGTCGTGCTAAGGCTGGCAAGCGTTTAGCTTTTGTGCATTTA
GTTGAACCGCGCGTGACAAACCCCTTCTTGACGGAAGGCGAAGGAGAGTATAACGG
AGGATCGAATAAATTTGCGTATTCCATTGGAAGGGCCCGATCATTCGTGCCGGTAACT
TTGCCTTACATCCCGAAGTTGTTCGCGAGGAAGTAAAAGACCCACGTACCTTGATCG
GGTATGGCCGTTTCTTTATTTCAAACCCCGACTTGGTGGATCGCCTTGAAAAAGGTCT
TCCCTTGAATAAGTATGACCGTGATACGTTCTACAAAATGTCAGCCGAAGGTTACATC
GACTACCCCACCTACGAAGAGGCTTTGAAACTTGGTTGGGACAAGAACCACCACCAT
CACCACCACTGA.

Old yellow enzyme 3 (OYE3)

Genbank Accession Number: AAA64522

Amino acid sequence:
MPFVKGFEPISLRDTNLFEPIKIGNTQLAHRAVMPPLTRMRATHPGNIPNKEWAAVYYG
QRAQRPGTMIITEGTFISPQAGGYDNAPGIWSDEQVAEWKNIFLAIHDCQSFAWVQLWS
LGWASFPDVLARDGLRYDCASDRVYMNATLQEKAKDANNLEHSLTKDDIKQYIKDYIH
AAKNSIAAGADGVEIHSANGYLLNQFLDPHSNKRTDEYGGTIENRARFTLEVVDALIETI
GPERVGLRLSPYGTFNSMSGGAEPGIIAQYSYVLGELEKRAKAGKRLAFVHLVEPRVTD
PSLVEGEGEYSEGTNDFAYSIWKGPIIRAGNYALHPEVVREQVKDPRTLIGYGRFFISNPD
LVYRLEEGLPLNKYDRSTFYTMSAEGYTDYPTYEEAVDLGWNKNHHHHHH

DNA sequence:
ATGCCTTTCGTGAAGGGGTTCGAGCCGATCTCTCTGCGCGATACAAACTTGTTCGAA
CCTATCAAGATCGGTAATACCCAATTAGCGCACCGTGCTGTAATGCCCCCATTGACC
CGTATGCGCGCGACGCACCCCGGTAATATTCCCAATAAAGAGTGGGCGGCGGTCT
ACTATGGACAACGTGCGCAACGTCCTGGGACGATGATTATTACTGAAGGTACTTTTA
TTTCACCCCAAGCCGGCGGGTATGATAACGCACCTGGAATCTGGAGTGATGAGCAA
GTGGCTGAGTGGAAAAACATCTTCCTTGCAATCCATGACTGCCAATCTTTTGCTTGG
GTTCAGCTGTGGAGCTTAGGATGGGCATCATTTCCAGATGTATTAGCCCGTGACGG
TCTTCGTTATGATTGTGCTTCAGACCGCGTGTATATGAACGCAACATTACAAGAAAA
AGCCAAAGACGCAAACAACCTTGAGCACTCGCTGACTAAAGACGACATTAAGCAAT
ACATTAAGGACTATATTCATGCAGCGAAGAATAGTATCGCTGCCGGAGCCGATGGC
GTGGAAATTCACAGCGCTAATGGCTACCTGCTGAACCAATTCTTAGACCCCCATTCT
AATAAACGCACTGATGAGTACGGGGGAACGATTGAGAACCGTGCTCGTTTCACATT
AGAGGTAGTCGATGCTTTGATTGAGACGATCGGCCCGGAGCGCGTAGGCCTGCGT
TTGTCCCCCTATGGGACCTTCAACAGTATGAGTGGAGGGGCAGAGCCTGGAATCAT
TGCACAGTATAGCTATGTCCTGGGTGAATTGGAAAAGCGTGCAAAAGCGGGCAAAC
GTCTTGCCTTCGTTCATCTGGTGGAGCCGCGTGTTACCGACCCCTCCTTAGTTGAGG
GAGAGGGAGAGTACAGTGAGGGTACGAATGACTTCGCCTACAGCATCTGGAAGGG
GCCCATCATTCGCGCTGGCAATTACGCCTTGCACCCAGAAGTCGTCCGCGAGCAG
GTAAAGGATCCACGTACACTGATCGGCTATGGGCGCTTCTTCATTTCAAATCCAGAC
TTGGTCTACCGTCTGGAAGAGGGATTACCATTAAATAAATATGACCGCTCCACATTTT
ATACCATGTCGGCTGAGGGGTATACAGACTACCCCACCTATGAGGAAGCAGTGGAT
CTTGGTT GGAACAAGAATCACCACCATCACCACCACTGA

The detailed expression and purification information of other tested ‘ene’-reductases in this study, namely old yellow enzyme 2 (OYE2), and gluconobacter oxydans enoate reductase (GluER), were described previously.[8-11]

3.3 MorB Protein Expression and Purification

Morphinone reductase (MorB) was expressed acoording was produced in *E. coli* BL21 transformed with plasmid encoding MorB. Transformed glycerol stocks were used to initiate a 5 mL overnight culture in Luria-Bertani (LB) media with ampicillin (100 µg/mL at 37 °C and 250 rpm. Expression culture (500 mL in a 2 L baffled shake flask) containing ampicillin (100 µg/mL) and autoinducing mixtures (sterile filtered mixture of 1% glucose, 4% lactose and 15% glycerol, 40 mL/L media) was inoculated with 5 mL of the overnight culture, then grown at 30 oC and 250 rpm for 24 h. The cells were harvested by centrifugation (4000 x g, 20 min, 4 °C) and frozen at -80 °C for further purification.

Frozen cells were thawed and resuspended in buffer A (20 mM KPi pH 7, 300 mM NaCl, 25 mM imidazole) to a final concentration of 2 mL/g of wet cells. The resuspended cells were supplemented with lysozyme (1 mg/mL), FMN (1 mg/mL), DNase I (0.1 mg/mL), phenylmethylsulfonyl fluoride (PMSF, 1 mM) and allowed to shake for 30 min at 37 oC. The cells were further disrupted by sonication (2 x 4 min, output control 5, 35% duty cycle, Sonicator QSonica Q500 Ultra Sonicator). Lysates were centrifuged (20,000 x g, 1 h, 4 °C) to pellet insoluble materials. Proteins were purified using a nickel-NTA column (5 mL HisTrap HP, GE Healthcare, Piscataway, NJ) *via* an AKTAStart purifier FPLC system (GE Healthcare). Enzymes were eluted with buffer B (20 mM KPi pH 7, 300 mM NaCl, 250 mM imidazole) over five column volumes. Yellow fractions containing MorB enzymes were pooled, concentrated through buffer exchange into an imidazole free storage buffer (20 mM KPi, pH 7, 300 mM NaCl). Concentrated enzymes were aliquoted, flash-frozen in liquid N2, and then stored at -80 °C until later use. Protein purity was assessed with SDS-PAGE.

Protein concentrations were determined using the extinction coefficient (12.2 x mM-1 cm-1 at 446 nm) for free FMN released after protein denaturation. Extinction coefficient for MorB: ε = 10.7 x mM-1 cm-1 at 463 nm. Both the WT and the final mutant expressed with an approximate yield of 150 mg protein/L culture.

3.4 Dialyzed, Cell-Free Lysate Preparation

The cell pellet was thawed at room temperature and resuspended in lysis buffer containing kPi (pH 8, 20 mM), FMN (0.5 mg/mL), lysozyme (1 mg/mL), DNAse (0.05 mg/mL), PMSF in ethanol (0.2 mM final concentration). The suspension was incubated at 37 °C for 45 min at 250 rpm, then cooled on ice. The cold suspension was sonicated on ice for 2x 4 min (output control 5, 35% duty cycle) on a Sonicator QSonica Q500 Ultra Sonicator system. The resulting lysed mixture was spun down at 65’000*g* at 4 °C for 1.5 h. The supernatant was transferred into dialysis tubing (10 kDa MW cutoff) and dialyzed into kPi (20 mM, pH 8) at 4 °C (3x 1000 mL) for 12 h each cycle. The resulting wet, cell-free lysate was flash frozen in liquid nitrogen and lyophilized for 48 h. The crude lysate was ground up to a powder, and protein levels were determined by protein gel analysis.

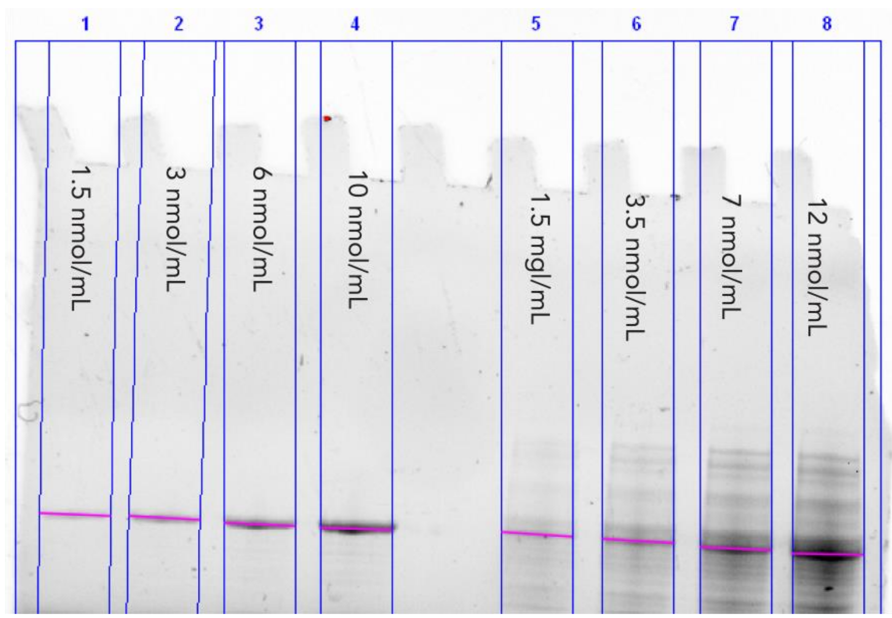


Fig. S1. The calculated amount of final variant protein corresponds to 1.4 nmol/mg of dry lysate.

4. Site Saturation Mutagenesis of MorB for N-alkylation

Site saturation mutagenesis primers were designed in accordance with the 22-codon trick using the PCR protocol.[12] The PCR products were digested with DpnI, and reassembled with NEBuilder® Hi-Fi Assembly, and used to directly transform E. coli. BL21 electrocompetent cells and plated on LB agar plates containing ampicillin (100 µg/mL).

4.1 Screening procedure for N-alkylation with MorB mutants in 96-well plates

Single colonies were picked with sterile toothpicks and used to inoculate 500 µL of LB media containing ampicillin (100 µg/mL) in deep-well 96-well plates, and cultured overnight (30 °C, 250 rpm). Wells A2, B4, C6, D8, E10, F12, and G2 were embedded controls of the parent protein for that round of engineering. Well H12 was used as blank control. A glycerol stock plate of the library was prepared by mixing sterilized glycerol solution (50% *v*/*v*, 50 µL/well) with the overnight cell cultures (50 µL/well). The library was sealed and stored at -80 °C. The expression cultures (950 µL of autoinducing Turbo BrothTM media containing 100 µg/mL of ampicillin) in 96-well plates were inoculated by adding 50 µL of the overnight cultures, and grown for 24 h (30 °C, 250 rpm). After growth and expression, cells were harvested by centrifugation (4000 x g, 20 min, 4 °C) and frozen at -80 °C for later screening. The cell libraries were thawed and resuspended with lysis buffer (100 µL/well) and allowed to shake for 1 h at 37 °C. The crude cell lysates were centrifuged (4000 x g, 20 min, 4 °C) and the supernatants (90 µL/well) were transferred into a new white plastic 96-well plate for desalting.

Desalting: Since NADPH and NADP can halt the reaction progress we aimed to remove these small molecules using desalting plates. We used ZebaTM Spin Desalting Plate from ThermoScientific. 90 µL/well from the supernatants were transferred to the desalting plate, followed by spin at 900 rpm for 1 minute to get the new supernatant lysate for reaction, to which 110 µL tricine pH=9 100 mM, was then then transferred to the glovebox

4.2 Plate preparation

In the glovebox, to the plate containing the lysate supernatant (200 µL/well) substrates (alkyl bromide: 1 mg/well, aniline: 0.1 mg/well in 20 µL DMSO) were added. Then the 96-well plate was sealed with adhesive plate-sealing film and shaken under anaerobic conditions with a fan cooling and irradiated using a Lumidox 96-well Cyan LED array at room temperature for 20 h. Upon completion, the reaction was quenched with acetonitrile (700 µL/well), and the plate was sealed with a reusable silicone mat, shaken for 60 min, and centrifuged (4500 ×g, 20 min). The supernatant (200 µL/well) was filtered through a Millipore 96-well filter plate into a shallow-well plate (1000 x g, 1 min), and the filtrate was retained for mass analysis. Promising hits were chosen and subjected to cultivation in shaking flasks for further confirmation. The DNA plasmids of hits were extracted and submitted for sequencing to identify their mutations.

Recipe for Auto Inducing TB Media. For autoinducing cultures, Turbo BrothTM media was supplemented with a sterile-filtered solution of 1.25% glucose, 5% lactose, and 15% glycerol (40 mL/L media).

Lysis buffer. 100 mM Tricine (pH 9.0) buffer containing lysozyme (1 mg/mL), DNase I (0.1 mg/mL), FMN (1 mg/mL), and PMSF (1 mM).

4.3 PCR for Site Saturation Mutagenesis

Site saturation mutagenesis primers were designed in accordance with the 22-codon trick using the PCR protocol.[12]

The PCR products were digested with DpnI, and reassembled with NEBuilder® Hi-Fi Assembly, and used to directly transform E. coli. BL21 electrocompetent cells and plated on LB agar plates containing ampicillin (100 µg/mL).

Primers for Site Saturation Mutagenesis

Round 2	
Q104-NDT	AAAGGGGGTCGTATAGCGTTGNDTTTATGGCATGTCGGCCGG
Q104-VHG	AAAGGGGGTCGTATAGCGTTGVHGTTATGGCATGTCGGCCGG
Q104-TGG	AAAGGGGGTCGTATAGCGTTGTGGTTATGGCATGTCGGCCGG
Q104-rev	CAACGCTATACGACCCCCTTTAGCATGGACAGC
V73-NDT	TCCCACCGCTCGGGGATACNDTTACACGCCAGGCATTTGG
V73-VHG	TCCCACCGCTCGGGGATACVHGTACACGCCAGGCATTTGG
V73-TGG	TCCCACCGCTCGGGGATACTGGTACACGCCAGGCATTTGG
V73-rev	GTATCCCCGAGCGGTGGGACTGATATTTGTCGC
R110-NDT	CAGTTATGGCATGTCGGCNDTGTCTCTCATGAGCTGGTG
R110-VHG	CAGTTATGGCATGTCGGCVHGGTCTCTCATGAGCTGGTG
R110-TGG	CAGTTATGGCATGTCGGCTGGGTCTCTCATGAGCTGGTG
R110-rev	GCCGACATGCCATAACTGCAACGCTATACGACC
T240-NDT	AGAGTGGGGATACGGCTGNDTCCTTTCCTGGAGTTATTT
T240-VHG	AGAGTGGGGATACGGCTGVHGCCTTTCCTGGAGTTATTT
T240-TGG	AGAGTGGGGATACGGCTGTGGCCTTTCCTGGAGTTATTT
T240-rev	CAGCCGTATCCCCACTCTTTCGGGCCCGAATAC

Round 3	
Y72-NDT	CAGTCCCACCGCTCGGGGANDTAATTACACGCCAGGCATT
Y72-VHG	CAGTCCCACCGCTCGGGGAVHGAATTACACGCCAGGCATT
Y72-TGG	CAGTCCCACCGCTCGGGGATGGAATTACACGCCAGGCATT
Y72-rev	TCCCCGAGCGGTGGGACTGATATTTGTCGCTTC
F246-NDT	ACTCCTTTCCTGGAGTTANDTGGATTAACGGATGATGAA
F246-VHG	ACTCCTTTCCTGGAGTTAVHGGGATTAACGGATGATGAA
F246-TGG	ACTCCTTTCCTGGAGTTATGGGGATTAACGGATGATGAA
F246-rev	TAACTCCAGGAAAGGAGTCAGCCGTATCCCCAC
V111-NDT	TTATGGCATGTCGGCCGGNDTTCTCATGAGCTGGTGCAG
V111-VHG	TTATGGCATGTCGGCCGGVHGTCTCATGAGCTGGTGCAG
V111-TGG	TTATGGCATGTCGGCCGGTGGTCTCATGAGCTGGTGCAG
V111-rev	CCGGCCGACATGCCATAACTGCAACGCTATACG
A190-NDT	GAGGTCCACGCGGCACATNDTTGTCTTCCTAATCAGTTC
A190-VHG	GAGGTCCACGCGGCACATVHGTGTCTTCCTAATCAGTTC
A190-TGG	GAGGTCCACGCGGCACATTGGTGTCTTCCTAATCAGTTC
A190-rev	ATTTGCCGCGTGGACCTCTACCATATCGAATCC
P241-NDT	GTGGGGATACGGCTGACTNDTTTCCTGGAGTTATTTGGA
P241-VHG	GTGGGGATACGGCTGACTVHGTTCCTGGAGTTATTTGGA
P241-TGG	GTGGGGATACGGCTGACTNTGGTCCTGGAGTTATTTGGA
P241-rev	AGTCAGCCGTATCCCCACTCTTTCGGGCCCGAA
Y356-NDT	CCTGACCCCTCTACTTTTNDTGGCGGGGCAGAGGTGGGG
Y356-VHG	CCTGACCCCTCTACTTTTVHGGGCGGGGCAGAGGTGGGG
Y356-TGG	CCTGACCCCTCTACTTTTTGGGGCGGGGCAGAGGTGGGG
Y356-rev	AAAAGTAGAGGGGTCAGGTTCGTTCAGCGCTGC
G281-NDT	AATGAACCCGATTGGATANDTGGGGACATCACGTACCCG
G281-VHG	AATGAACCCGATTGGATAVHGGGGGACATCACGTACCCG
G281-TGG	AATGAACCCGATTGGATATGGGGGGACATCACGTACCCG

G281-rev	TATCCAATCGGGTTCATTAAAGTGTAAATACGC
N275-NDT	TTAGCGTATTTACACTTTNDTGAACCCGATTGGATAGGT
N275-VHG	TTAGCGTATTTACACTTTVHGGAACCCGATTGGATAGGT
N275-TGG	TTAGCGTATTTACACTTTTGGGAACCCGATTGGATAGGT
N275-rev	AAAGTGTAAATACGCTAAACCACGCCGGTCTAAT

Round 4	
I280-NDT	TTTAATGAACCCGATTGGNDTGGTGGGGACATCACGTAC
I280-VHG	TTTAATGAACCCGATTGGVHGGGTGGGGACATCACGTAC
I280-TGG	TTTAATGAACCCGATTGGTGGGGTGGGGACATCACGTAC
I280-rev	CCAATCGGGTTCATTAAAGTGTAAATACGCTAAACC
E135-NDT	TTAAAAGCCGAAGGGGCCNDTTGCTTTGTCGAATTCGAG
E135-VHG	TTAAAAGCCGAAGGGGCCVHGTGCTTTGTCGAATTCGAG
E135-TGG	TTAAAAGCCGAAGGGGCCTGGTGCTTTGTCGAATTCGAG
E135-rev	GGCCCCTTCGGCTTTTAAGGCGGATGGTGCCAC
F137-NDT	GCCGAAGGGGCCGAGTGCNDTGTCGAATTCGAGGATGG GA
F137-VHG	GCCGAAGGGGCCGAGTGCVHGGTCGAATTCGAGGATGG GA
F137-TGG	GCCGAAGGGGCCGAGTGCTGGGTCGAATTCGAGGATGG GA
F137-rev	GCACTCGGCCCCTTCGGCTTTTAAGGCGGATGGT
S34-NDT	ATGGCACCTTTAACCCGCNDTCGCACGCCAGATTCTGTA
S34-VHG	ATGGCACCTTTAACCCGCVHGCGCACGCCAGATTCTGTA
S34-TGG	ATGGCACCTTTAACCCGCTGGCGCACGCCAGATTCTGTA
S34-rev	GCGGGTTAAAGGTGCCATTATGACACGATTTGG
G133-NDT	TCCGCCTTAAAAGCCGAANDTGCCGAGTGCTTTGTCGAA
G133-VHG	TCCGCCTTAAAAGCCGAAVHGGCCGAGTGCTTTGTCGAA
G133-TGG	TCCGCCTTAAAAGCCGAATGGGCCGAGTGCTTTGTCGAA

G133-rev	TTCGGCTTTTAAGGCGGATGGTGCCACGGGTTG
S353-NDT	CTGAACGAACCTGACCCCNDTACTTTTTACGGCGGGGCA
S353-VHG	CTGAACGAACCTGACCCCVHGACTTTTTACGGCGGGGCA
S353-TGG	CTGAACGAACCTGACCCCTGGACTTTTTACGGCGGGGCA
S353-rev	GGGGTCAGGTTCGTTCAGCGCTGCTCCTAAGCG
D283-NDT	ACCCGATTGGATAGGTGGGNDTATCACGTACCCGGAAGGGT
D283-VHG	ACCCGATTGGATAGGTGGGVHGATCACGTACCCGGAAGGGT
D283-TGG	ACCCGATTGGATAGGTGGGTGGATCACGTACCCGGAAGGGT
D283-REV	CCCACCTATCCAATCGGGTTCATTAAAGTGTAA
A62-NDT	GGGTTAATCATCTCCGAANDTACAAATATCAGTCCCACC
A62-VHG	GGGTTAATCATCTCCGAAVHGACAAATATCAGTCCCACC
A62-TGG	GGGTTAATCATCTCCGAATGGACAAATATCAGTCCCACC
A62-REV	TTCGGAGATGATTAACCCGGCGCTGGCGCGTTG

Round 5	
C136-NDT	AAAGCCGAAGGGGCCGAGNDTTTTGTCGAATTCGAGGAT
C136-VHG	AAAGCCGAAGGGGCCGAGNVHGTTGTCGAATTCGAGGAT
C136-TGG	AAAGCCGAAGGGGCCGAGNTGGTTGTCGAATTCGAGGAT
C136-rev	CTCGGCCCCTTCGGCTTTTAAGGCGGATGGTGC
R33-NDT	GTCATAATGGCACCTTTAACCNDTTCACGCACGCCAGATTCT
R33-VHG	GTCATAATGGCACCTTTAACCVHGTCACGCACGCCAGATTCT
R33-TGG	GTCATAATGGCACCTTTAACCTGGTCACGCACGCCAGATTCT
R33-rev	GGTTAAAGGTGCCATTATGACACGATTTGGAAG
W279-NDT	CACTTTAATGAACCCGATNDTATAGGTGGGGACATCACG
W279-VHG	CACTTTAATGAACCCGATVHGATAGGTGGGGACATCACG
W279-TGG	CACTTTAATGAACCCGATTGGATAGGTGGGGACATCACG
W279-rev	ATCGGGTTCATTAAAGTGTAAATACGCTAAACCACGC
L243-NDT	ATACGGCTGACTCCTTTCNDTGAGTTATTTGGATTAACG
L243-VHG	ATACGGCTGACTCCTTTCVHGGAGTTATTTGGATTAACG
L243-TGG	ATACGGCTGACTCCTTTCTGGGAGTTATTTGGATTAACG
L243-rev	GAAAGGAGTCAGCCGTATCCCCACTCTTTCGGG
I59-NDT	GCCAGCGCCGGGTTAATCNDTTCCGAAGCGACAAATATC
I59-VHG	GCCAGCGCCGGGTTAATCVHGTCCGAAGCGACAAATATC
I59-TGG	GCCAGCGCCGGGTTAATCTGGTCCGAAGCGACAAATATC
I59-rev	GATTAACCCGGCGCTGGCGCGTTGACCATAGTAT
E184-NDT	GCCGGATTCGATATGGTANDTGTCCACGCGGCAAATGCT
E184-VHG	GCCGGATTCGATATGGTAVHGGTCCACGCGGCAAATGCT
E184-TGG	GCCGGATTCGATATGGTATGGGTCCACGCGGCAAATGCT
E184-rev	TACCATATCGAATCCGGCCCGCTTCGCACGCTG
P352-NDT	AGCGCTGAACGAACCTGACNDTTCTACTTTTTACGGCGGG

P352-VHG	AGCGCTGAACGAACCTGACVHGTCTACTTTTTACGGCGGG
P352-TGG	AGCGCTGAACGAACCTGACTGGTCTACTTTTTACGGCGGG
P352-rev	GTCAGGTTCGTTCAGCGCTGCTCCTAAGCGGAAACG
V111-NDT	TTATGGCATGTCGGCCGGNDTTCTCATGAGCTGGTGCAG
V111-VHG	TTATGGCATGTCGGCCGGVHGTCTCATGAGCTGGTGCAG
V111-TGG	TTATGGCATGTCGGCCGGTGGTCTCATGAGCTGGTGCAG

Round 6	
T32-NDT	GTCATAATGGCACCTTTANDTCGCTCACGCACGCCAGAT
T32-VHG	GTCATAATGGCACCTTTAVHGCGCTCACGCACGCCAGAT
T32-TGG	GTCATAATGGCACCTTTATGGCGCTCACGCACGCCAGAT
T32-r	TAAAGGTGCCATTATGACACGATTTGGAAGAGACAGACTAC
R238-NDT	GCCCGAAAGAGTGGGGATANDTCTGACTCCTTTCCTGGAG
R238-VHG	GCCCGAAAGAGTGGGGATAVHGCTGACTCCTTTCCTGGAG
R238-TGG	GCCCGAAAGAGTGGGGATATGGCTGACTCCTTTCCTGGAG
R238-rev	TATCCCCACTCTTTCGGGCCCGAATACCTCGGCT
T75-NDT	GCTCGGGGATACGTATACNDTCCAGGCATTTGGACTGAC
T75-VHG	GCTCGGGGATACGTATACVHGCCAGGCATTTGGACTGAC
T75-TGG	GCTCGGGGATACGTATACTGGCCAGGCATTTGGACTGAC
T75-rev	GTATACGTATCCCCGAGCGGTGGGACTGATATTTGTCGC
Y72-NDT	AGTCCCACCGCTCGGGGANDTGTATACACGCCAGGCATT
Y72-VHG	AGTCCCACCGCTCGGGGAVHGGTATACACGCCAGGCATT
Y72-TGG	AGTCCCACCGCTCGGGGATGGGTATACACGCCAGGCATT
Y72-rev	TCCCCGAGCGGTGGGACTGATATTTGTCGCTTC
I237-NDT	CGGGCCCGAAAGAGTGGGGNDTCGGCTGACTCCTTTCCTGG
I237-VHG	CGGGCCCGAAAGAGTGGGGVHGCGGCTGACTCCTTTCCTGG
I237-TGG	CGGGCCCGAAAGAGTGGGGTGGCGGCTGACTCCTTTCCTGG
I237-rev	CCCCACTCTTTCGGGCCCGAATACCTCGGCTAC
G109-NDT	TTGCAGTTATGGCATGTCNDTCGGGTCTCTCATGAGCTG

G109-VHG	TTGCAGTTATGGCATGTCVHGCGGGTCTCTCATGAGCTG
G109-TGG	TTGCAGTTATGGCATGTCTGGCGGGTCTCTCATGAGCTG
G109-rev	GACATGCCATAACTGCAACGCTATACGACCCCCTTTAGCATGG
H186-NDT	TTCGATATGGTAGAGGTCNDTGCGGCACATGCTTGTCTT
H186-VHG	TTCGATATGGTAGAGGTCVHGGCGGCACATGCTTGTCTT
H186-TGG	TTCGATATGGTAGAGGTCTGGGCGGCACATGCTTGTCTT
H186-rev	GACCTCTACCATATCGAATCCGGCCCGCTTCGC

4.4 Summary of Iterative Site Saturation Mutagenesis

Table S1. Evolution campaign

Entry	MorB mutant	Yield	Chemoselectivity	Enantioselectivity e.r
1	WT	25%	66%	52:48
2	N189H	42%	75%	53:47
3	N189H/V73N	58%	80%	54:46
4	N189H/V73N/F246Y	52%	79%	72:28
5	N189H/V73N/F246Y/S353	72%	83%	74:26
6	N189H/V73N/F246Y/S353/I59	78%	85%	81:19
7	N189H/V73N/F246Y/S353/I59/A134V	76%	86%	88:12

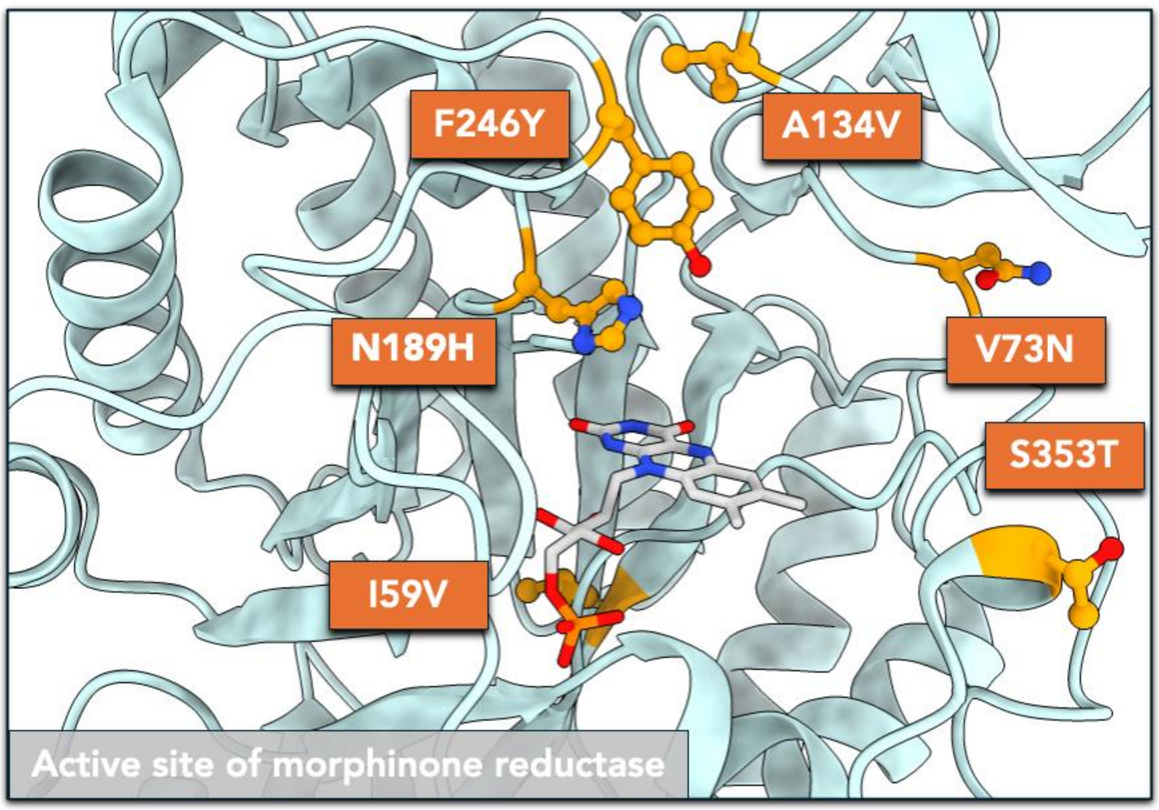
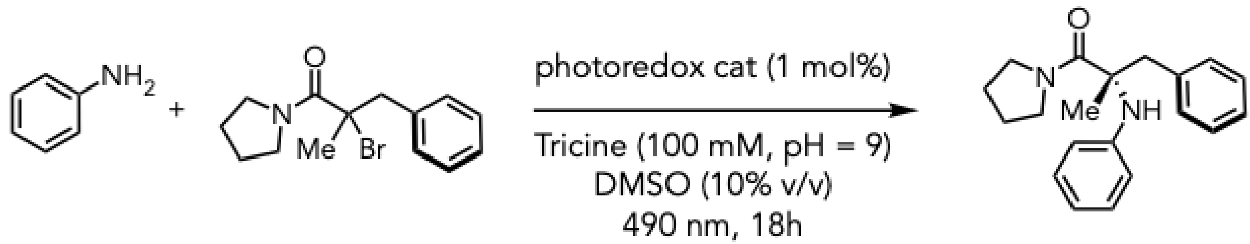


Fig. S2. Site saturation mutagenesis summary

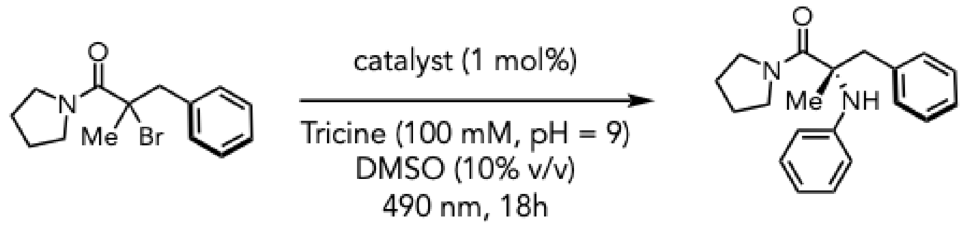
5. Enzymatic reactions

5.1 Initial screening and reaction discovery (photoredox, ketoreductases and '*ene*'-reductases)



Reaction conditions: Vial reactions were set in the MBraun glovebox system (under N_2 atmosphere and oxygen levels of <0.5 ppm). To a 4 mL shell vial sealed with a septum, 450 µL Tris buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL DMSO solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in DMSO). To the mixture, the enzyme was added (50 nmol, 1 mol%), and the total reaction volume is 500 µL with 10% (v/v) DMSO. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

5.1.1 Photoredox catalysts



Reaction conditions: Vial reactions were set in the MBraun glovebox system (under N_2 atmosphere and oxygen levels of <0.5 ppm). To a 4 mL shell vial sealed with a septum, 450 µL Tris buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL DMSO solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in DMSO). To the mixture, the catalyst was added (50 nmol, 1 mol%), a total reaction volume is 500 µL with 10% (v/v) DMSO. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

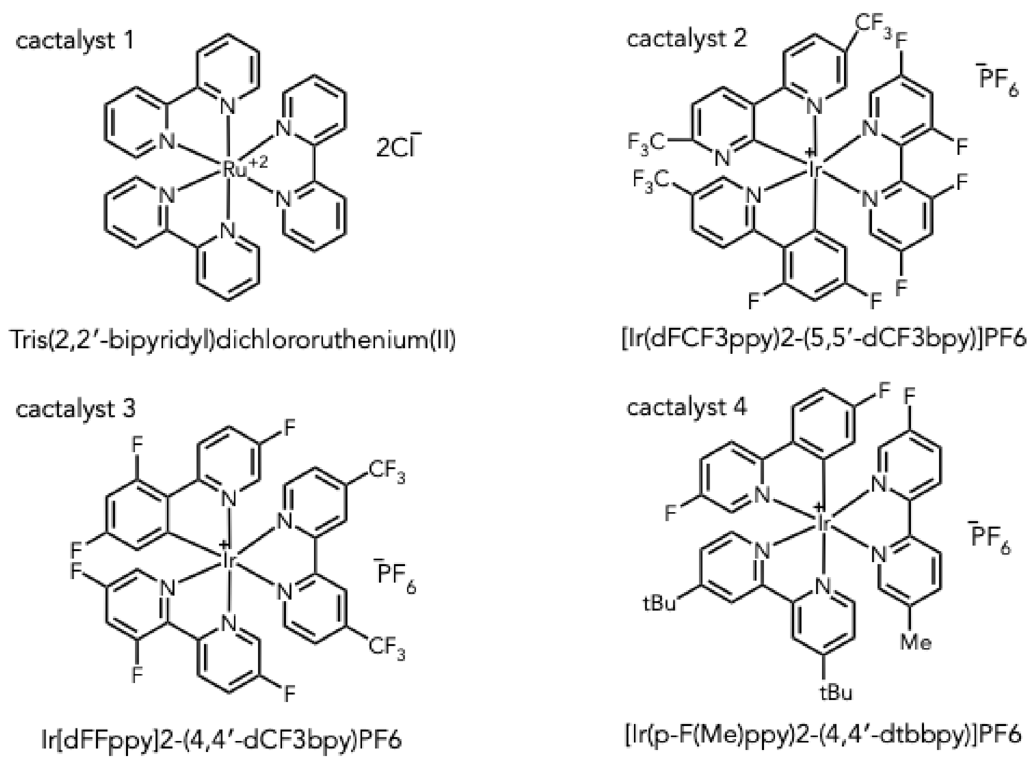
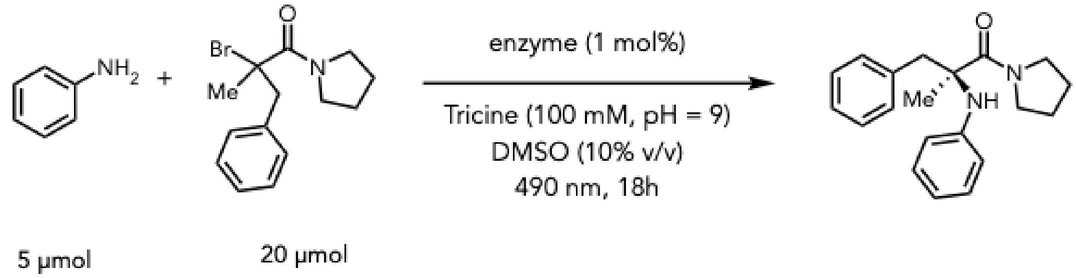


Fig. S3. Photoredox catalyst comparison

Table S2. Photoredox catalyst comparison

Entry	Catalyst	yield	chemoselectivity	enantioselectivity
1	1	~2%	78:22	n.d
2	2	-	n.d	n.d
3	3	-	n.d	n.d
4	4	-	n.d	n.d

5.1.2 Prozomix ketoreductase (KREDs) library screening



Reaction conditions: Vial reactions were set in the MBraun glovebox system (under N_2 atmosphere and oxygen levels of <0.5 ppm). To a 4 mL shell vial sealed with a septum, 450 µL Tris buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL DMSO solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in DMSO). To the mixture, the enzyme was added (50 nmol, 1 mol%), a total reaction volume is 500 µL with 10% (v/v) DMSO. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

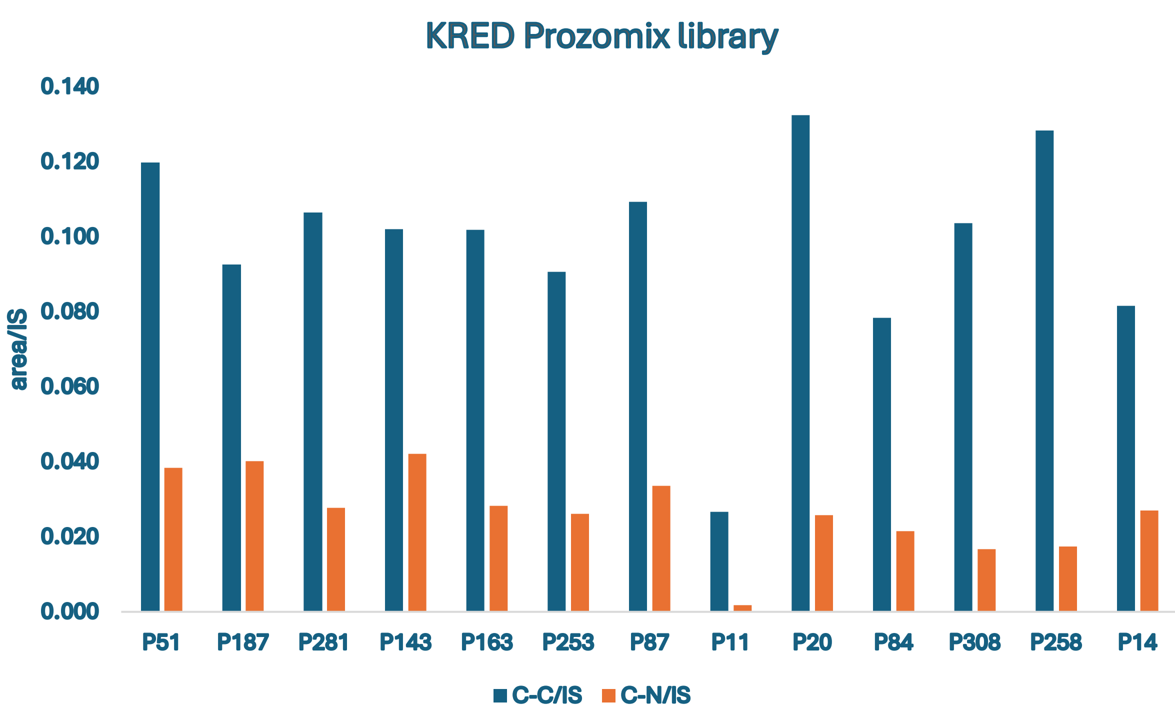
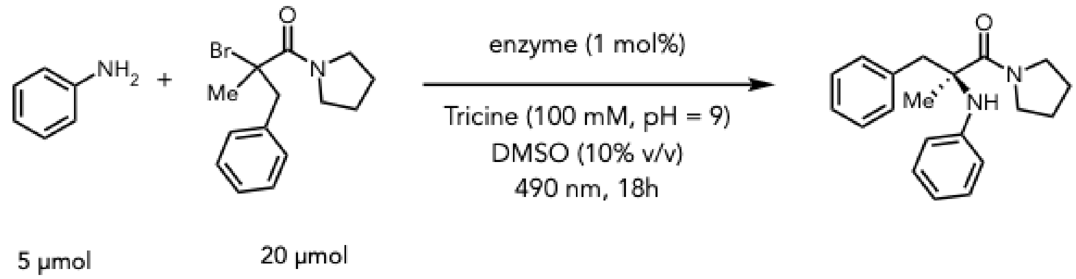


Fig. S4. Prozomix KRED library screening

6.1.2 '*Ene*'-reductases (EREDs) library screening



Reaction conditions: Vial reactions were set in the MBraun glovebox system (under N_2 atmosphere and oxygen levels of <0.5 ppm). To a 4 mL shell vial sealed with a septum, 450 µL Tris buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL DMSO solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in DMSO). To the mixture, the enzyme was added (50 nmol, 1 mol%), a total reaction volume is 500 µL with 10% (v/v) DMSO. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

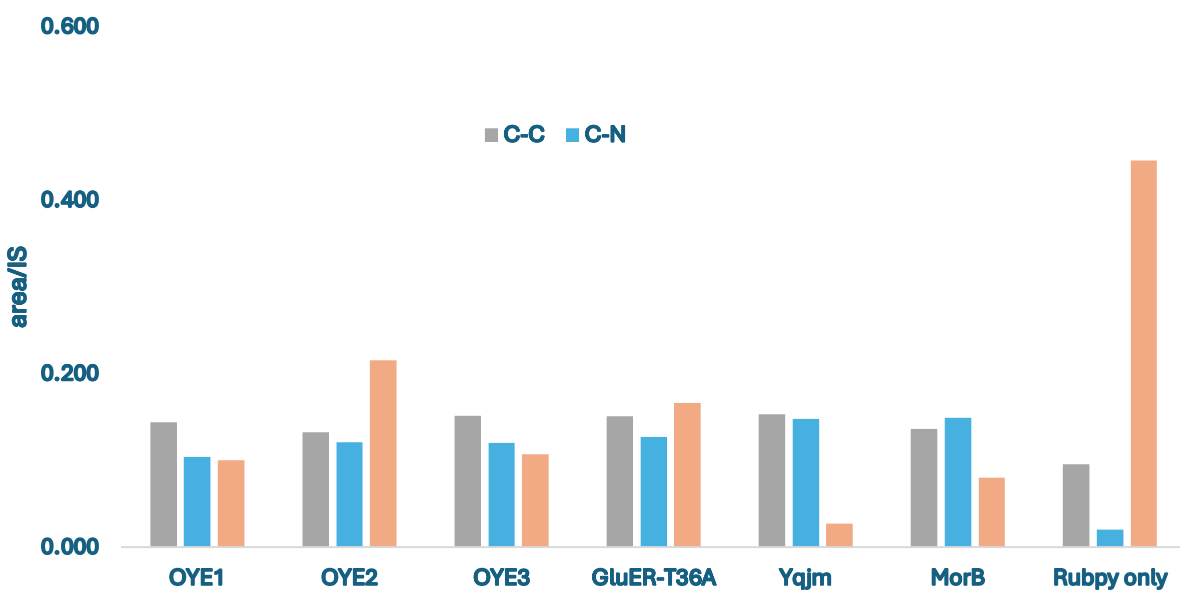
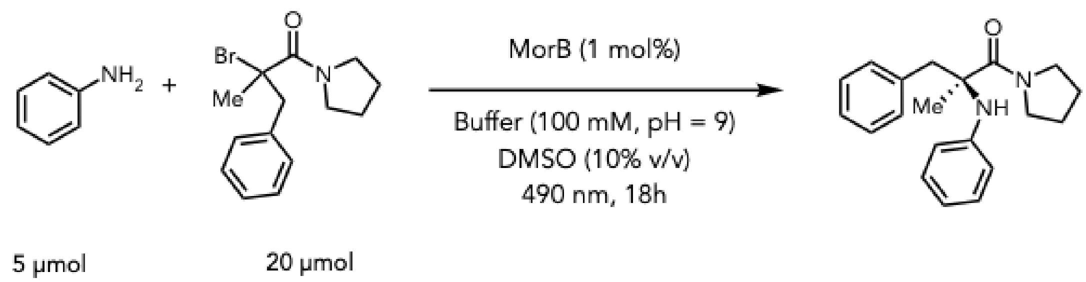


Fig. S5. ERED library screening

5.2 Buffer screening



Reaction conditions: Vial reactions were set in the MBraun glovebox system (under N_2 atmosphere and oxygen levels of <0.5 ppm). To a 4 mL shell vial sealed with a septum, 450 µL buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL DMSO solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in DMSO). To the mixture, the enzyme was added (50 nmol, 1 mol%), a total reaction volume is 500 µL with 10% (v/v) DMSO. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

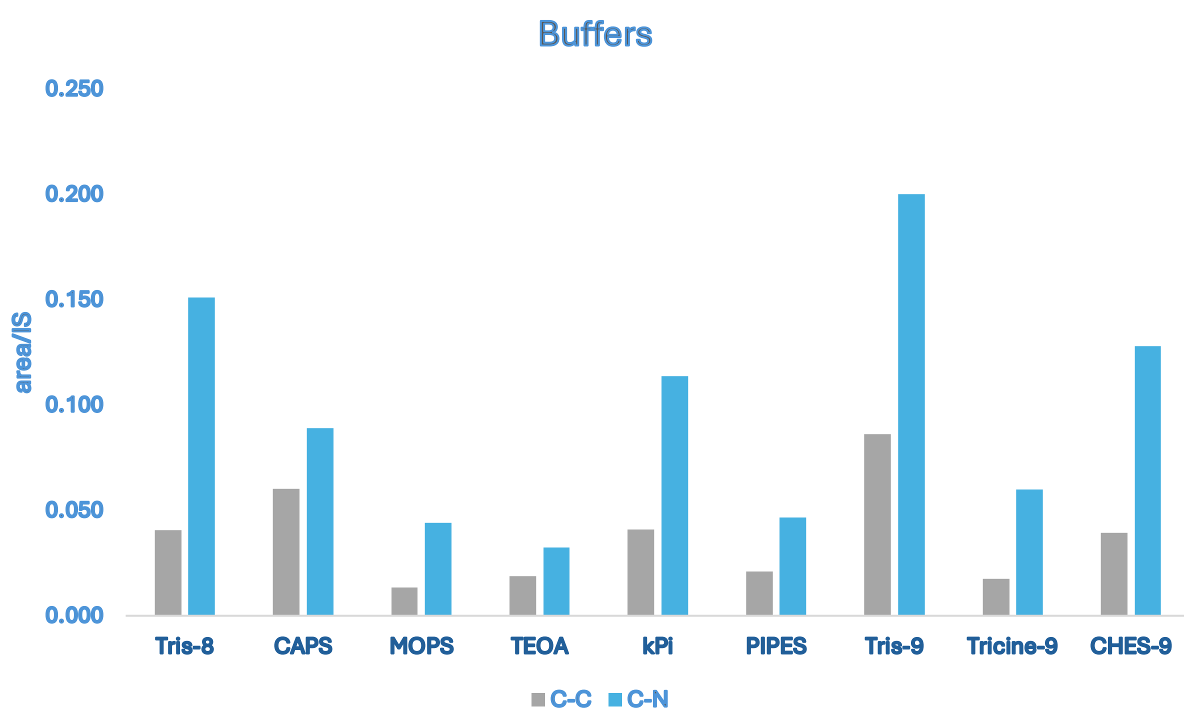
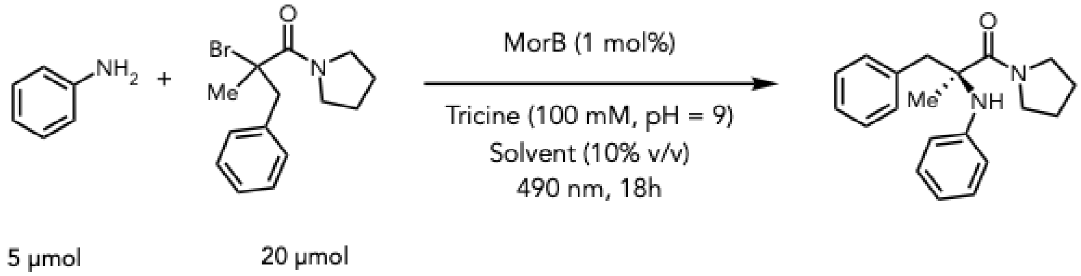


Fig S6. Buffer Screen

5.3 Solvent screening



Reaction conditions: Vial reactions were set in the MBraun glovebox system (under N_2 atmosphere and oxygen levels of <0.5 ppm). To a 4 mL shell vial sealed with a septum, 450 µL tricine buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL solvent solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in solvent). To the mixture, the enzyme was added (50 nmol, 1 mol%), a total reaction volume is 500 µL with 10% (v/v) solvent. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

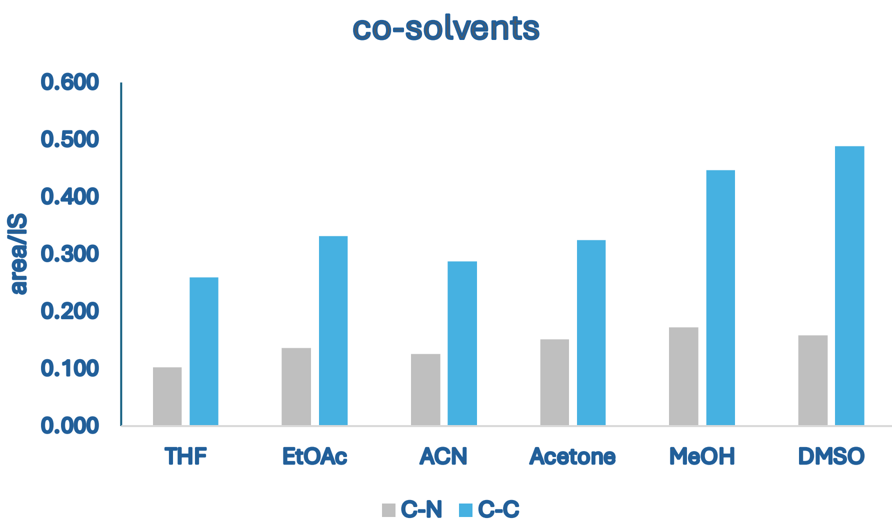
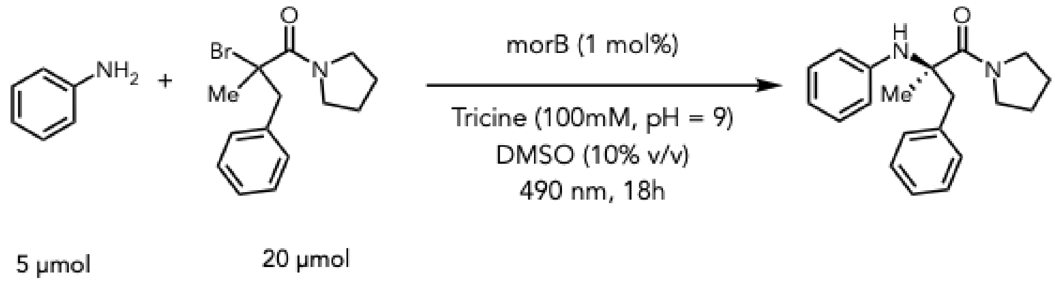


Fig S7. Solvent Screens

5.4 General procedure for the enzymatic reaction



Vial/Schlenk tube reactions were set in the MBraun glovebox system (under N_2 atmosphere and oxygen levels of <0.5 ppm). To a 4 mL shell vial sealed with a septum (or Schlenk tube), 450 µL tricine buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL DMSO solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in DMSO). To the mixture, the enzyme was added (50 nmol, 1 mol%), a total reaction volume is 500 µL with 10% (v/v) DMSO. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation. **Analysis:** After LC-MS analysis, the supernatant was concentrated under reduced pressure, extracted with EtOAc, the combined organic layers were dried over Na_2SO_4, filtered, and passed through a syringe filled with silica. The mixture was then concentrated under reduced pressure. The resulting crude residue was dissolved in 20% isopropanol/hexane (*v*/*v*) for chiral HPLC analysis.

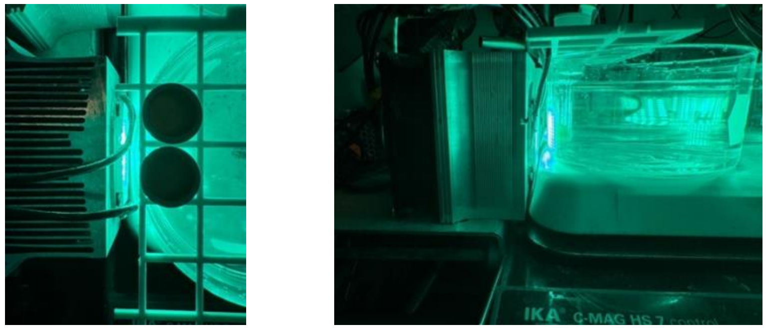


Fig. S8. Vial reaction setup

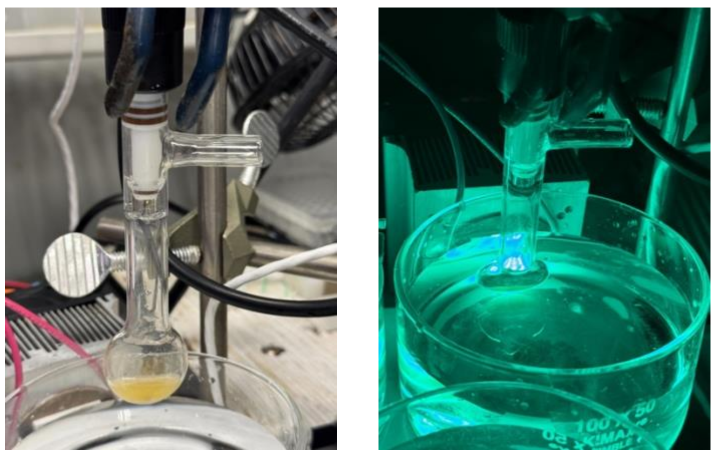
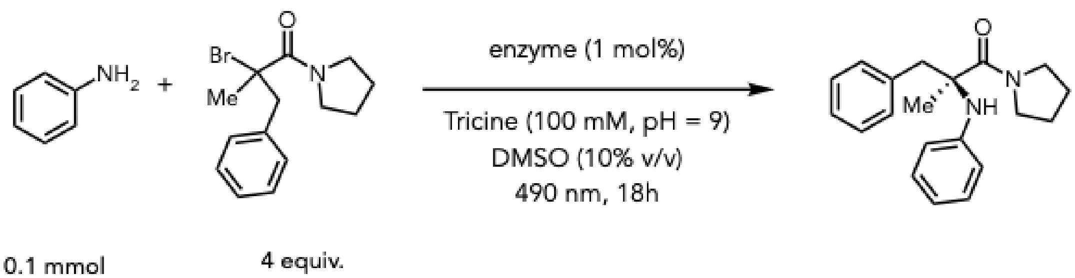


Fig. S9. Schlenk reaction setup

Plate set-up: The site-saturation 96-well plate (stored at -80 °C) was thawed for 30 min at RT and to each well was added lysis buffer (Tricine 100mM, pH=9, 130 µL/well) containing FMN (0.5 mg/mL), lysozyme (1.0 mg/mL), DNAse (0.05 mg/mL) and PMSF (35 µg/mL). The plate was sealed and shaken at 37 °C for 1.5 h. The whole-cell lysates were then 100 µL of the lysate was passed through a desalting plate. The desalted lysate was added to another 100 µL of Tricine buffer, and the plate was brought to the MBraun glovebox. Aniline **1** (0.05 mg, 10 µL from 5mg/1 mL in DMSO) and tertiary bromide **2** (1 mg, 100 mg/1 mL in DMSO) were added. The plate was sealed, and the mixture was stirred under cyan light (490 nm, 55 mW) irradiation for 16 h at RT. To each well was added MeCN (700 µL/well) and the mixtures were stirred for 1 hour at RT. The suspension was centrifuged at 4000 rpm for 10 minutes, and then 200 µL aliquots were filtered for analysis in RapidFire.

5.5 Larger scale



The reaction was performed according to the general procedure 6.4: To a 20 mL vial), 9 mL tricine buffer (100 mM, pH=9), followed by aniline (0.1 mmol), and alkyl bromide (0.5 mmol, in 1 mL DMSO). To the mixture, the enzyme was added (1000 nmol, 1 mol%), and the total reaction volume is 10 mL with 10% (v/v) DMSO. The vial was sealed and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 40 mL of acetonitrile and 2000 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation. **Analysis:** After LC-MS analysis, the supernatant was concentrated under reduced pressure, extracted with EtOAc, the combined organic layers were dried over Na_2SO_4, filtered, and passed through a syringe filled with silica. The mixture was then concentrated under reduced pressure. The resulting crude residue was dissolved in 20% isopropanol/hexane (*v*/*v*) for chiral HPLC analysis.

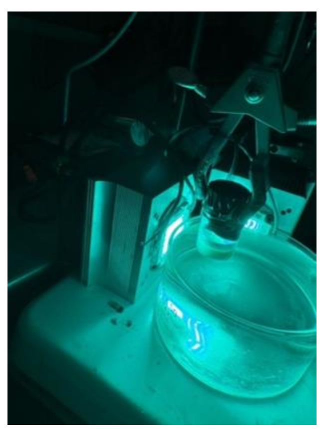
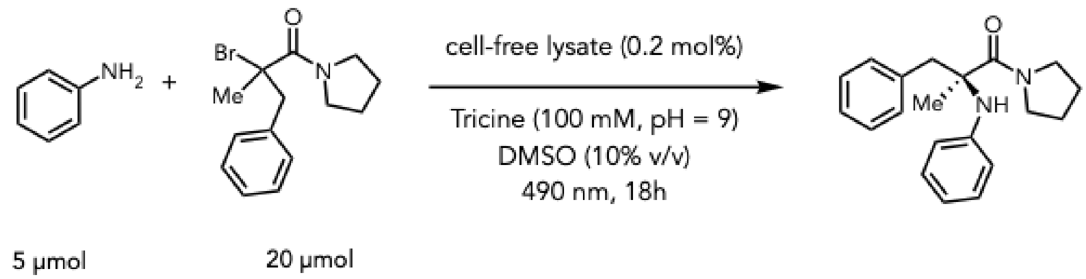


Fig. S10. Large scale reaction

5.6 Dialyzed lysate reaction



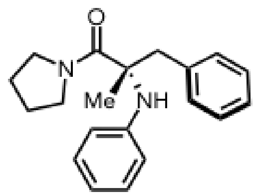
The reaction was performed according to the general procedure 6.4:

To a 4 mL shell vial sealed with a septum, 450 µL tricine buffer (100 mM, pH=9), followed by aniline (5 µmol, 10 µL from a 5 mg/100 µL DMSO solution, prepared in the glovebox), and alkyl bromide (20 µmol in 40 µL in DMSO). To the mixture, the dialized cell-free lysate was added (0.2 mol%, 10 mg), a total reaction volume is 500 µL with 10% (v/v) DMSO. The vial was sealed with a screw cap and stirred under cyan light irradiation and anaerobic conditions at room temperature for 24 h. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation. **Analysis:** After LC-MS analysis, the supernatant was concentrated under reduced pressure, extracted with EtOAc, the combined organic layers were dried over Na_2SO_4, filtered, and passed through a syringe filled with silica. The mixture was then concentrated under reduced pressure. The resulting crude residue was dissolved in 20% isopropanol/hexane (*v*/*v*) for chiral HPLC analysis.

Table S3. Lysate loadings

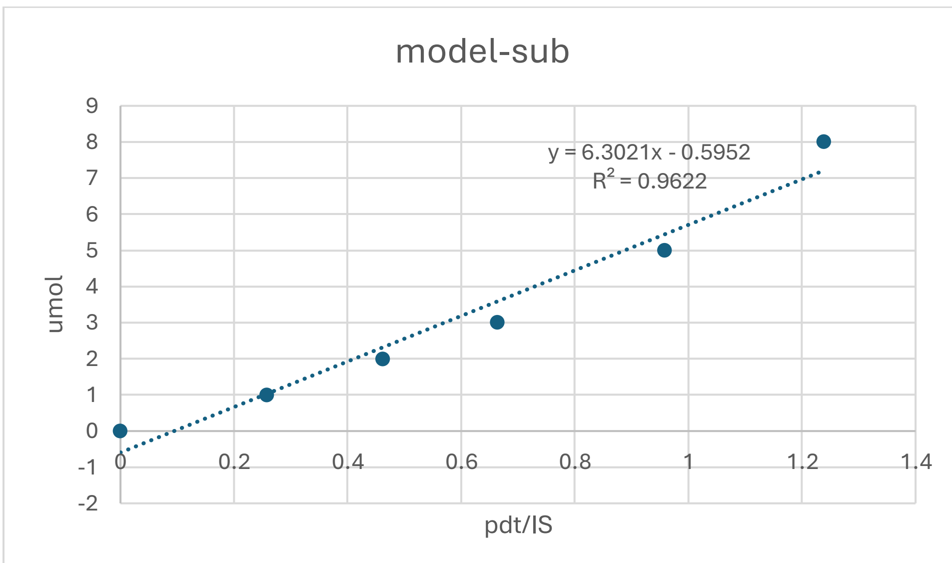
Entry	Catalyst	yield	chemoselectivity	enantioselectivity
1	1 mol%	12%	80:20	n.d
2	0.5 mol%	18%	82:18	85:15
3	0.2 mol%	25%	83:17	86:14
4	0.05 mol%	<5%	n.d	n.d

5.7 Enzymatic reaction scope



(*S*)-2-methyl-3-phenyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**3**)

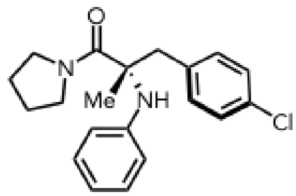
Calibration curve



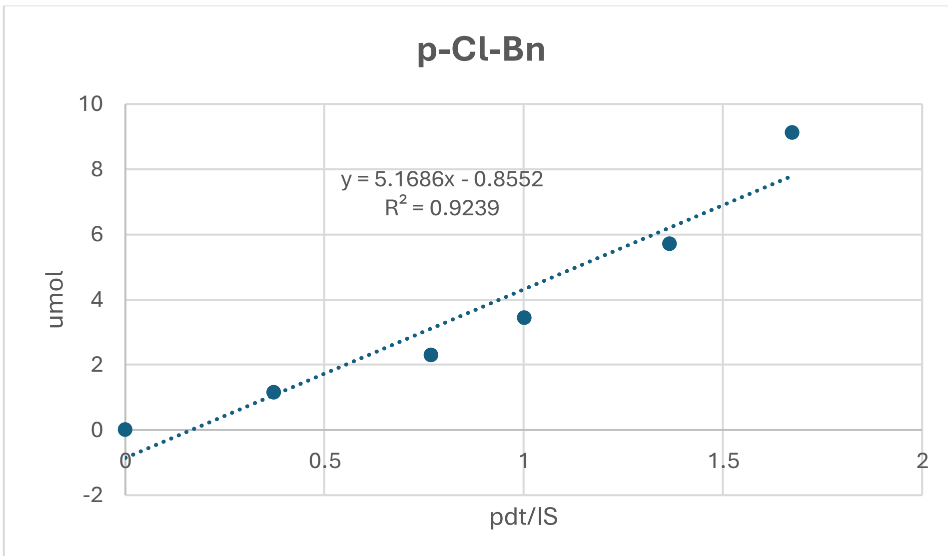
Yield: Schenck: 76%, 74%**.** Vial: 68%, 64%

Chemoselectivity: 85:15, 86:14

Enantioselectivity: 88:12 er. 87:13 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 99.3:0.7, 1.5 mL/min. isocratic.



(S)-3-(4-chlorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**5**)

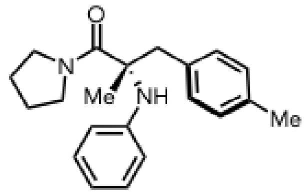


s

Yield: Schenck: 52%, 48%**.** Vial: 42%, 41%

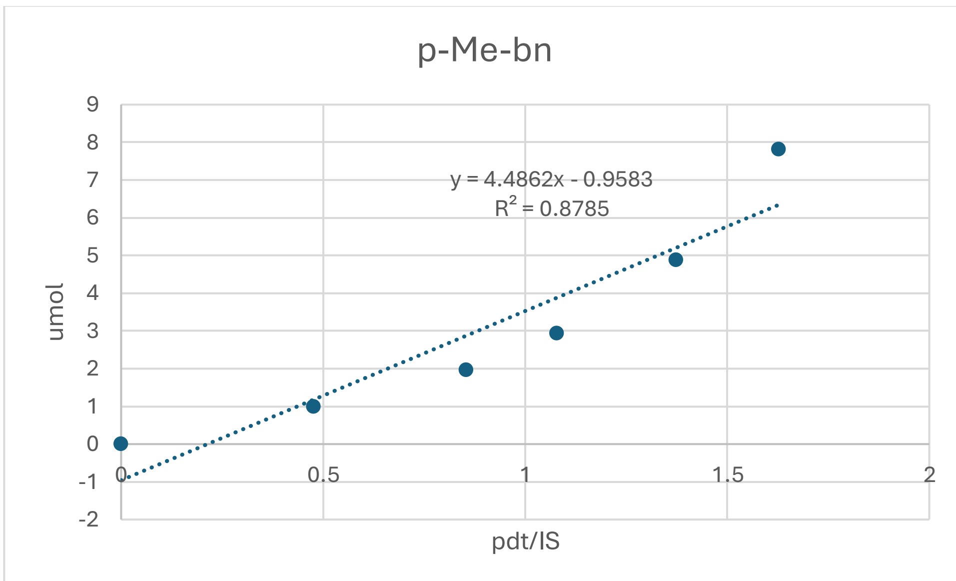
Chemoselectivity: 80:20, 77:23

Enantioselectivity: 80:20 er. 72:28 er. Chiral HPLC: IB column: *n*-hexane/IPA, 96:4, 1 mL/min. isocratic.



(S)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(p-tolyl)propan-1-one (**6**)

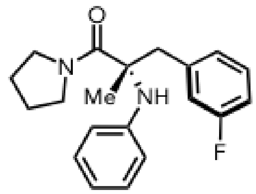
Calibration curve



Yield: Schenck: 78%, 73%**.** Vial: 70%, 61%

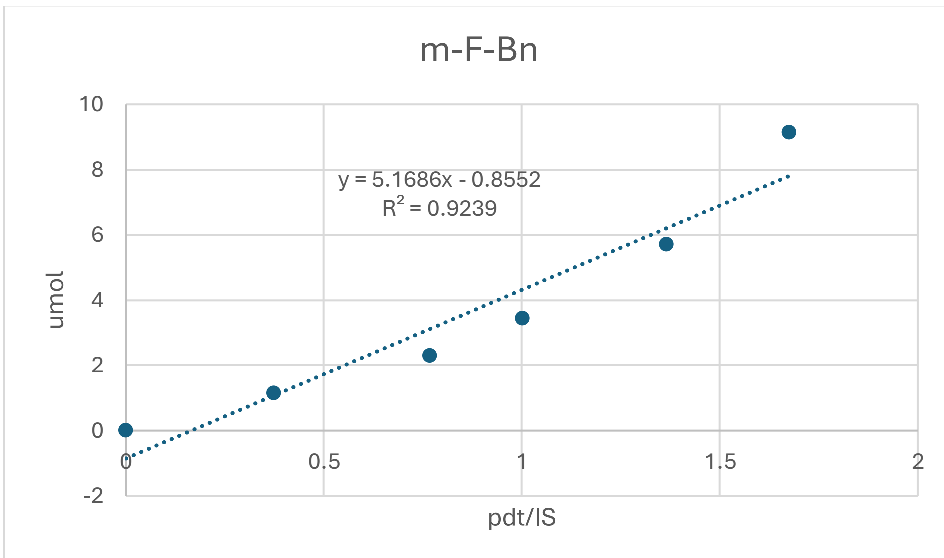
Chemoselectivity: 88:12, 85:15

Enantioselectivity: 72:28 er. 68:32 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



(*S*)-3-(3-fluorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**7**)

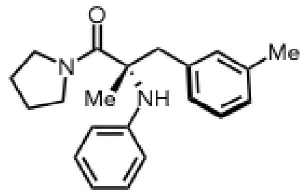
Calibration curve



Yield: Schenck: 18%, 16%**.** Vial: 14%, 10%

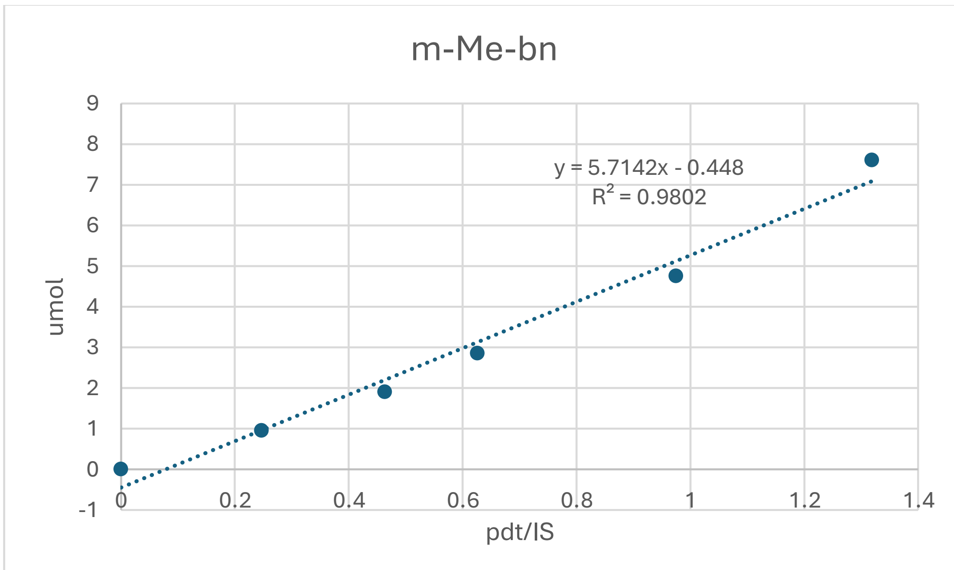
Chemoselectivity: 73:27, 70:30

Enantioselectivity: 73:27 er. 66:34 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.



(*S*)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(*m*-tolyl)propan-1-one (**8**)

Calibration curve

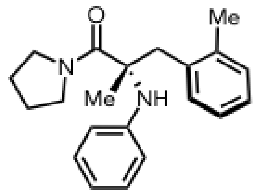


HPLC standard curve prepared with constant amounts of tribromobenzene.

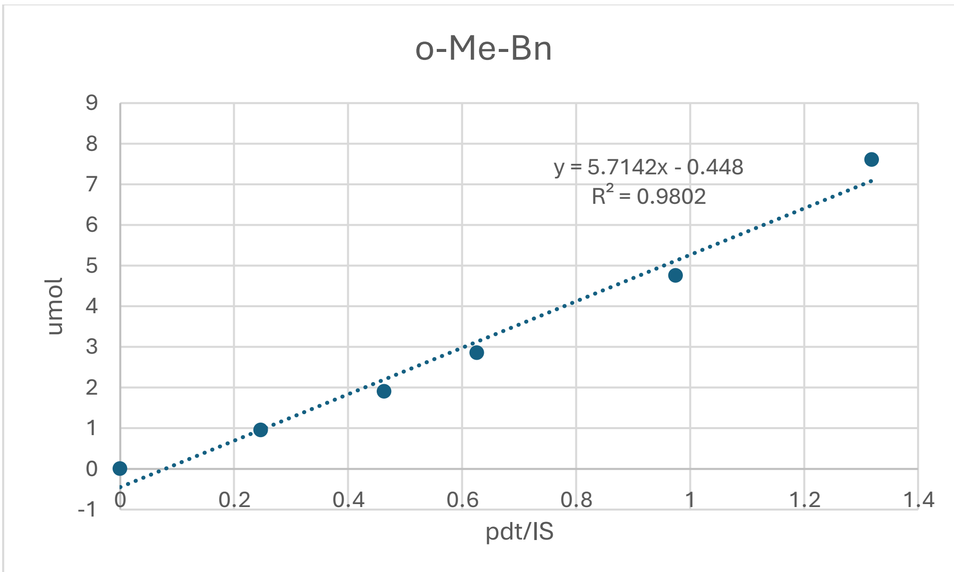
Yield: Schenck: 25%, 22%**.** Vial: 18%, 17%

Chemoselectivity: 70:30, 69:31

Enantioselectivity: 84:16 er. 80:20 er. Chiral HPLC: IB column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.



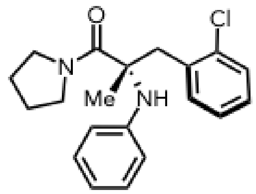
(*S*)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(*o*-tolyl)propan-1-one (**9**)



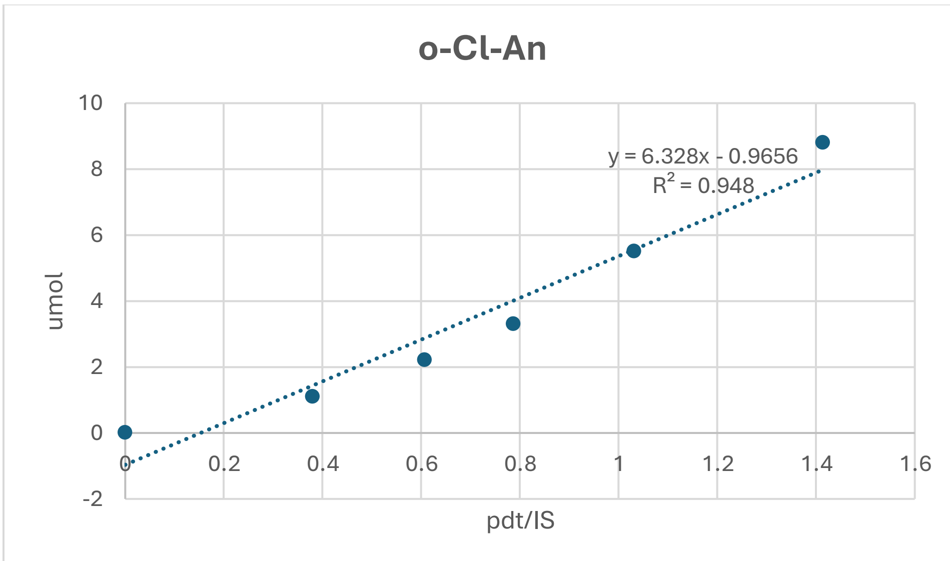
Yield: Schenck: 32%, 25%**.** Vial: 20%, 20%

Chemoselectivity: 66:34, 62:38

Enantioselectivity: 74:26 er. 70:30 er. Chiral HPLC: IB column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.



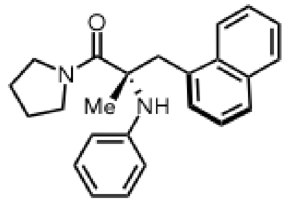
(*S*)-3-(2-chlorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**10**)



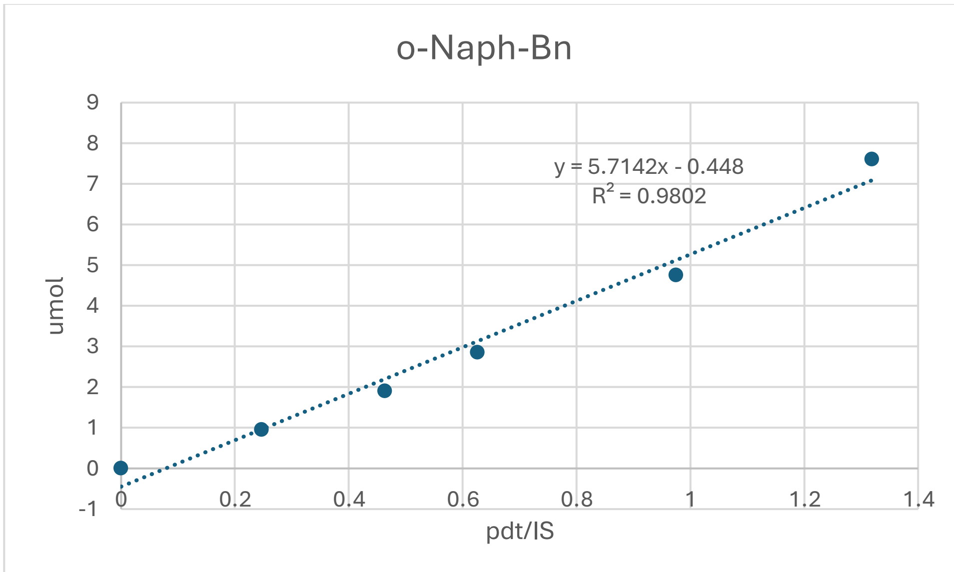
Yield: Schenck: 44%, 40%**.** Vial: 36%, 32%

Chemoselectivity: 60:40, 59:41

Enantioselectivity: 70:30 er. 66:34 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 91:9, 1 mL/min. isocratic



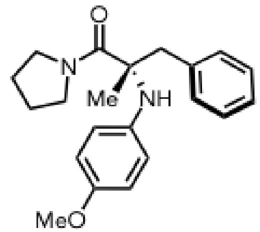
(*S*)-2-methyl-3-(naphthalen-1-yl)-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**11**)



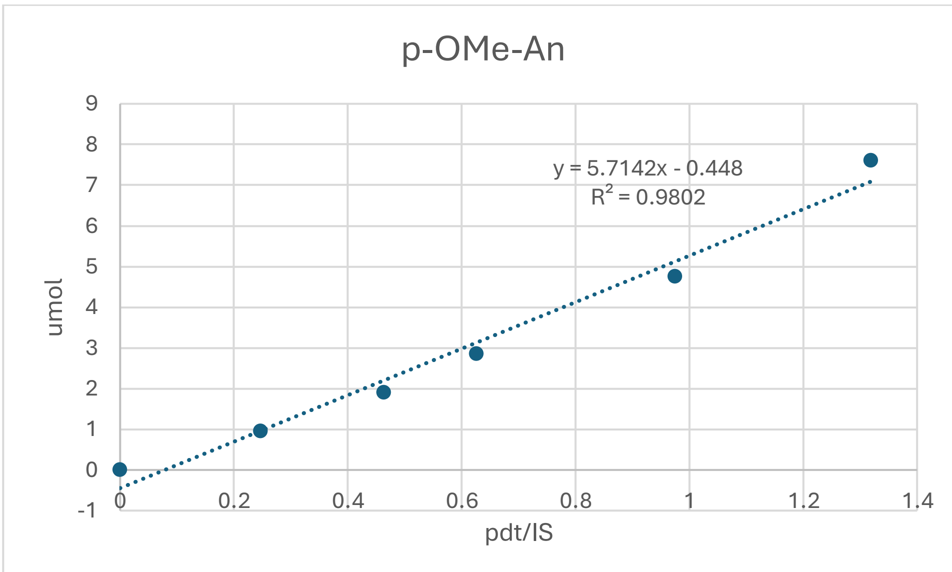
Yield: Schenck: 54%, 53%**.** Vial: 42%, 40%

Chemoselectivity: 73:27, 70:30

Enantioselectivity: 69:31 er. 63:37 er. Chiral HPLC: IB column: *n*-hexane/IPA, 96:4, 1 mL/min. isocratic.



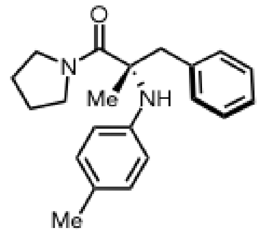
(*S*)-2-((4-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**12**)



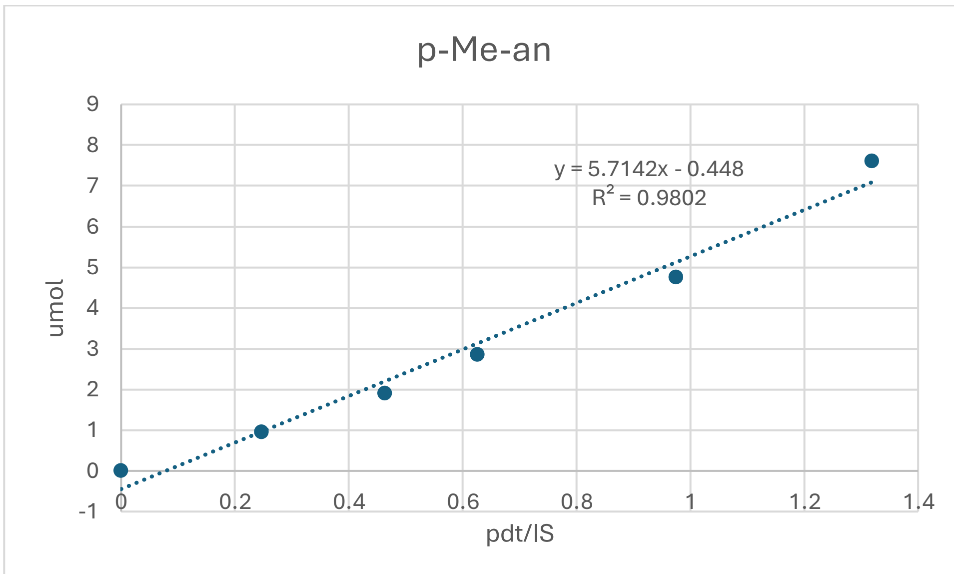
Yield: Schenck: 72%, 67%**.** Vial: 55%, 54%

Chemoselectivity: 100:0, 100:0 *N:C*

Enantioselectivity: 64:36 er. 62:38 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



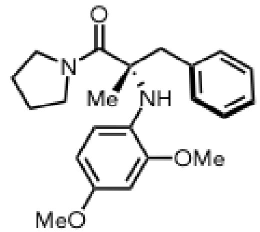
(*S*)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*p*-tolylamino)propan-1-one (**13**)



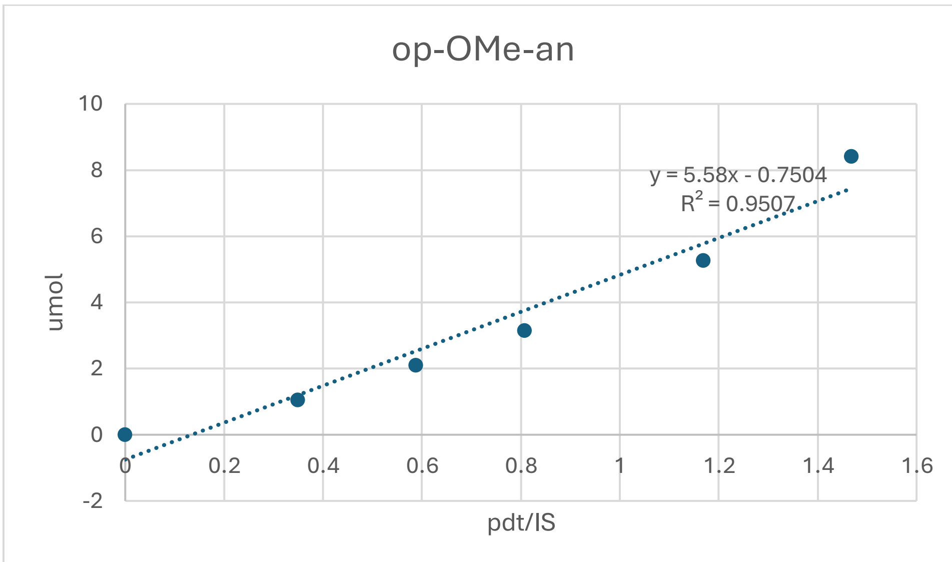
Yield: Schenck: 81%, 74%**.** Vial: 60%, 58%

Chemoselectivity: 100:0, 100:0 *N:C*

Enantioselectivity: 70:30 er. 67:33 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



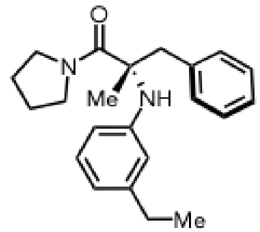
(*S*)-2-((2,4-dimethoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**14**)



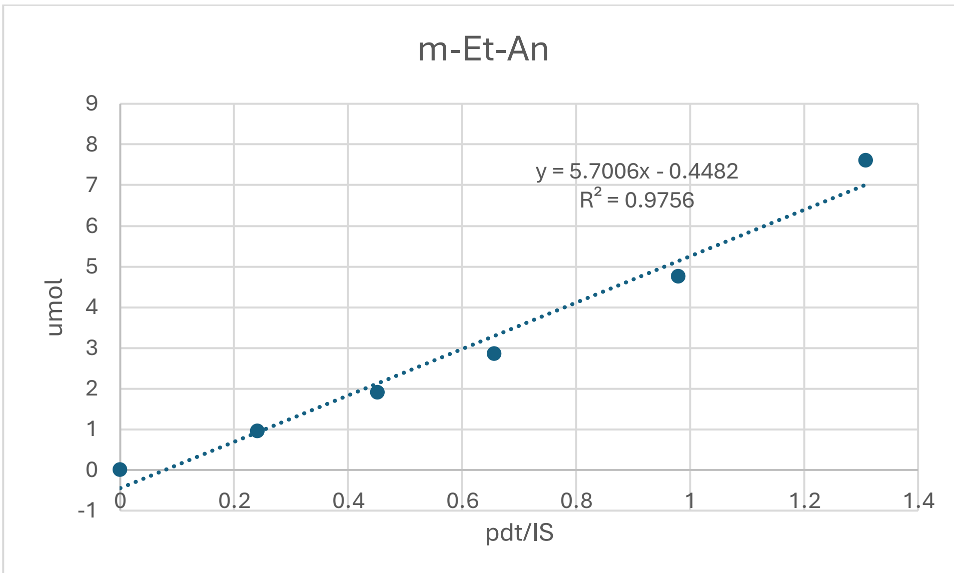
Yield: Schenck: 90%, 84%**.** Vial: 79%, 72%

Chemoselectivity: 100:0, 100:0 *N:C*

Enantioselectivity: 89:11 er. 86:14 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



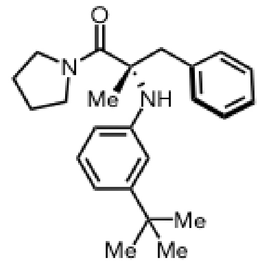
(*S*)-2-((3-ethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**15**)



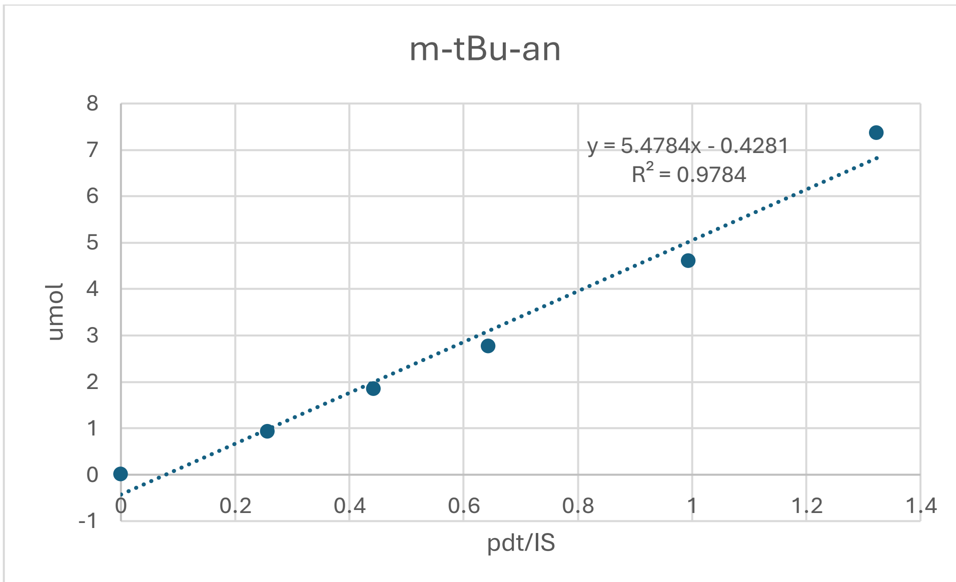
Yield: Schenck: 54%, 52%**.** Vial: 46%, 44%

Chemoselectivity: 83:17, 80:20

Enantioselectivity: 71:29 er. 68:32 er. Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.



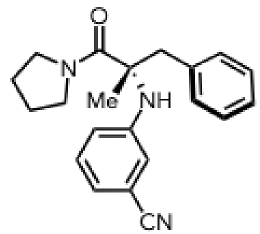
(*S*)-2-((3-(*tert*-butyl)phenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**16**)



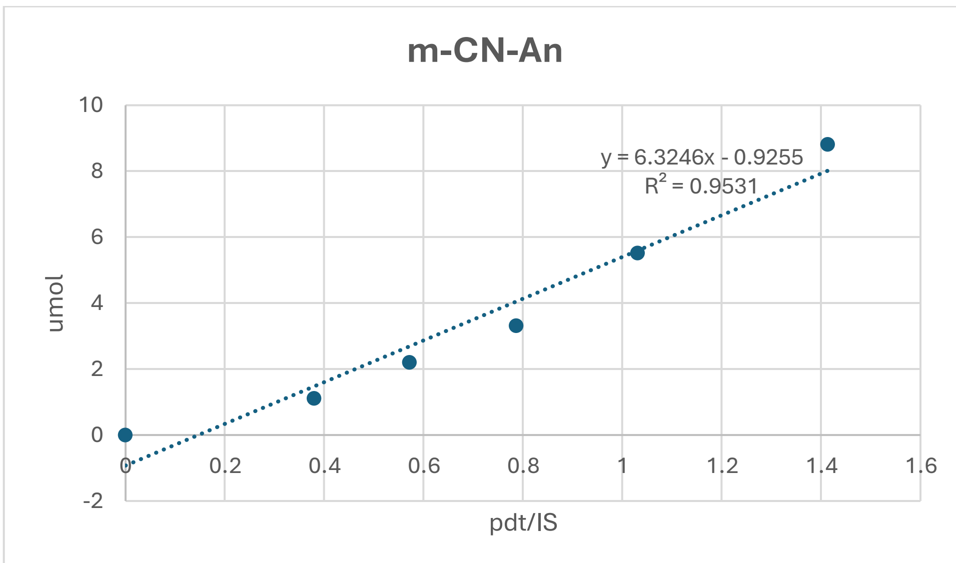
Yield: Schenck: 58%, 56%**.** Vial: 46%, 40%

Chemoselectivity: 93:7, 92:8

Enantioselectivity: 57:43 er. 55:45 er. Chiral HPLC: IC column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.



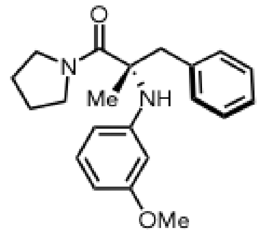
(*S*)-3-((2-methyl-1-oxo-3-phenyl-1-(pyrrolidin-1-yl)propan-2-yl)amino)benzonitrile (**17**)



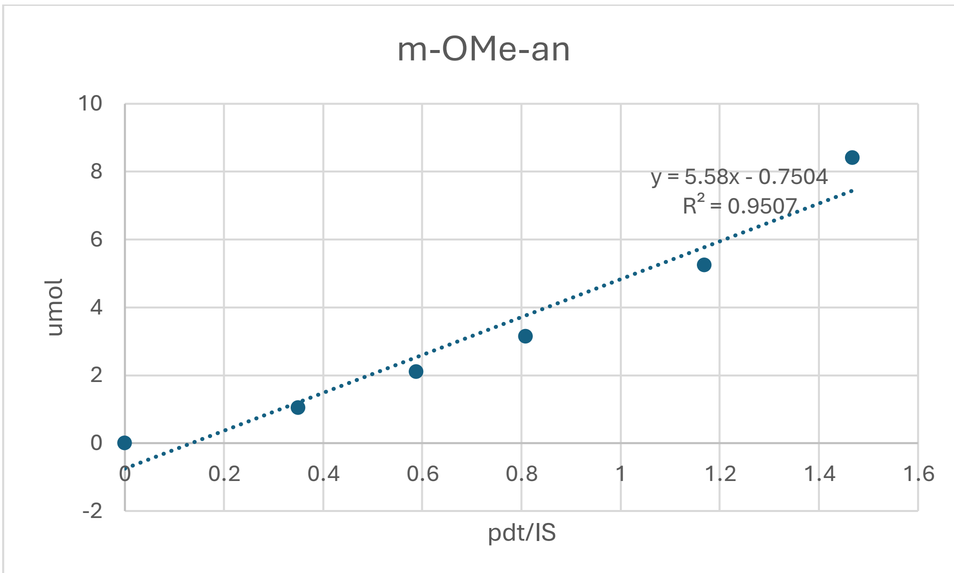
Yield: Schenck: 21%, 19%**.** Vial: 12%, 10%

Chemoselectivity: 52:48, 50:50

Enantioselectivity: 79:21 er. 75:25 er. Chiral HPLC: IA column: *n*-hexane/IPA, 92:8, 1 mL/min. isocratic.



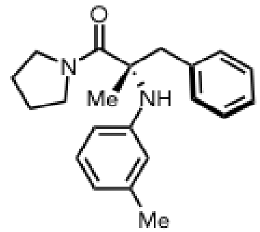
(*S*)-2-((3-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**18)**



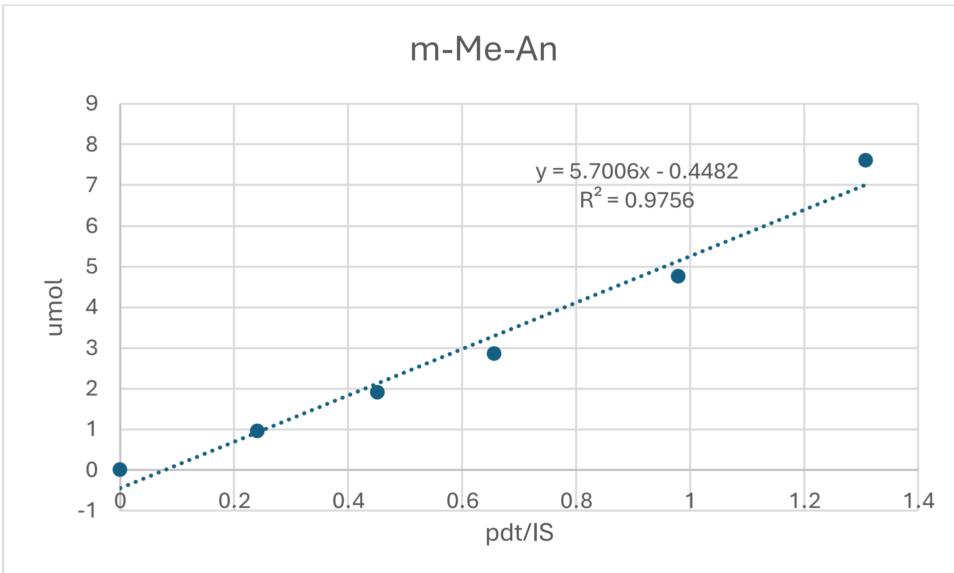
Yield: Schenck: 29%, 27%**.** Vial: 21%, 20%

Chemoselectivity: 65:35, 62:38

Enantioselectivity: 70:30 er. 68:32 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 93:7, 1 mL/min. isocratic.



(*S*)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*m*-tolylamino)propan-1-one (**19**)

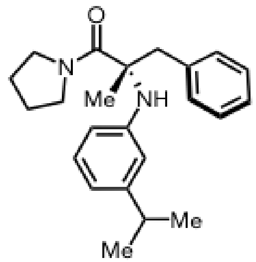


Yield: Schenck: 62%, 59%**.** Vial: 55%, 49%

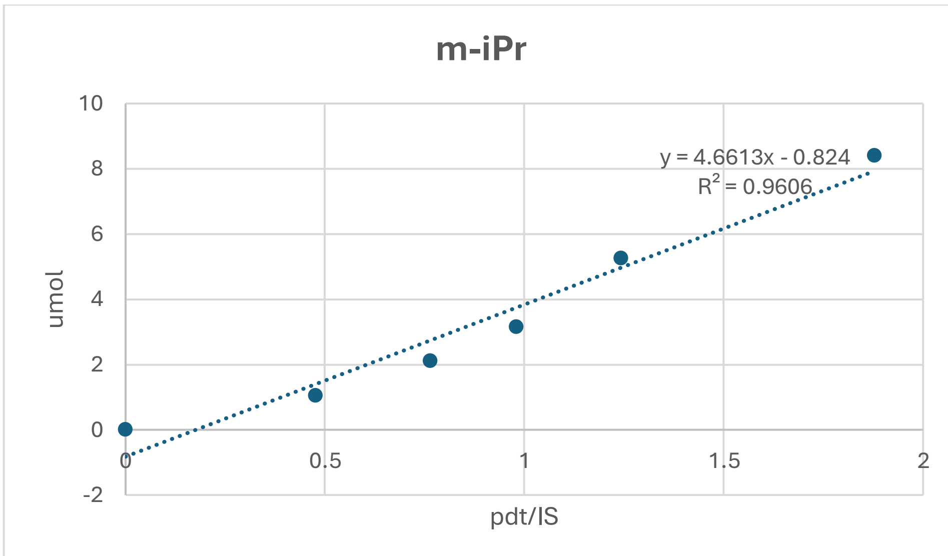
Chemoselectivity: 84:16, 83:17

Enantioselectivity: 62:38, 60:40 er. 80:20 er. Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.

.



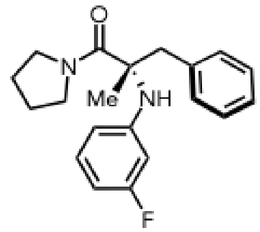
(S)-2-((3-isopropylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**20**)



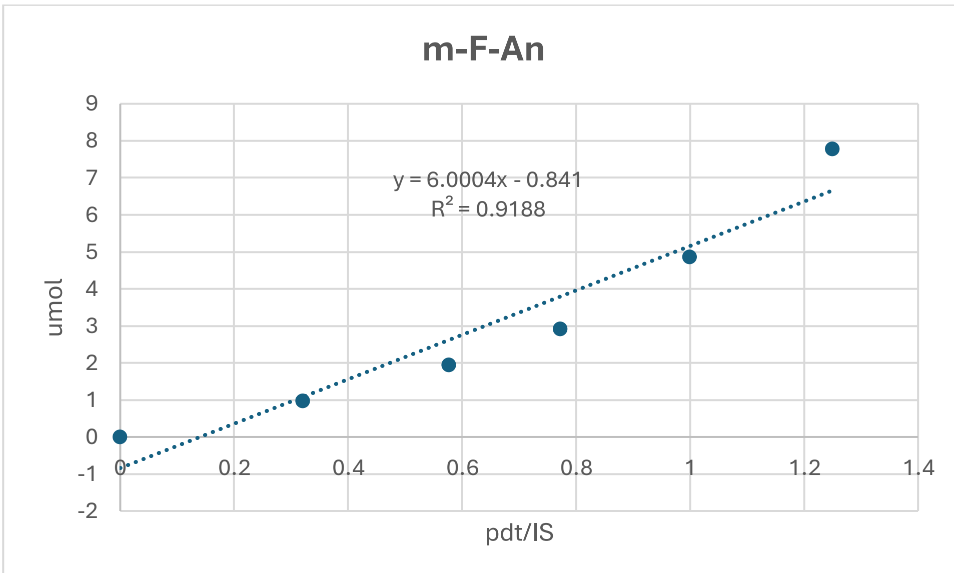
Yield: Schenck: 50%, 48%**.** Vial: 41%, 38%

Chemoselectivity: 91:9, 90:10

Enantioselectivity: 58:42 er. 52:48 er. Chiral HPLC: IC column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



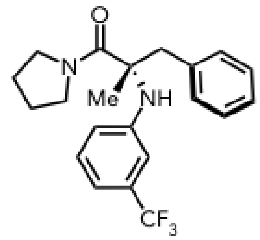
(*S*)-2-((3-fluorophenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**21**)



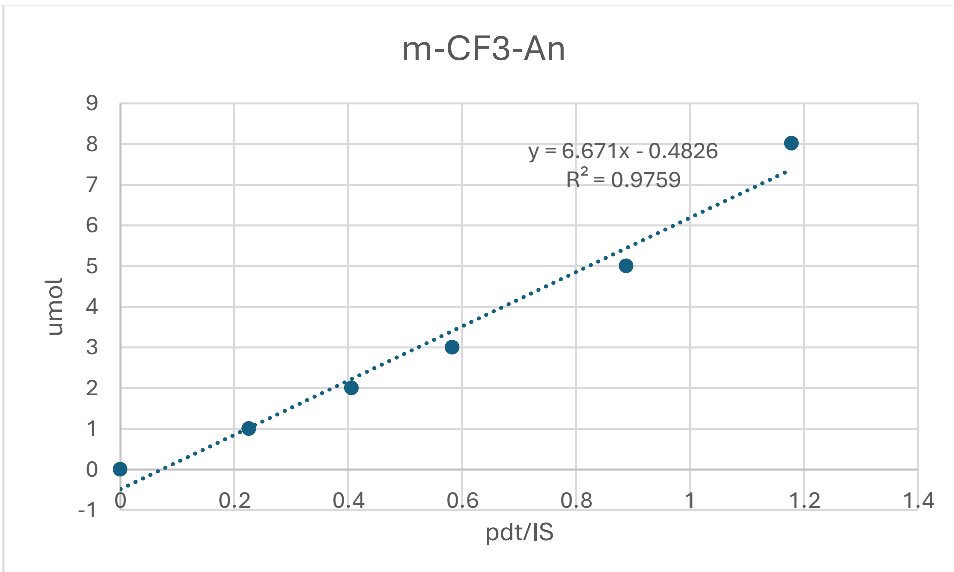
Yield: Schenck: 25%, 22%**.** Vial: 20%, 15%

Chemoselectivity: 66:34, 65:35

Enantioselectivity: 62:38 er. 61;39 er. Chiral HPLC: IA column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



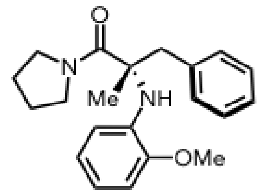
(*S*)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-((3-(trifluoromethyl)phenyl)amino)propan-1-one (**22**)



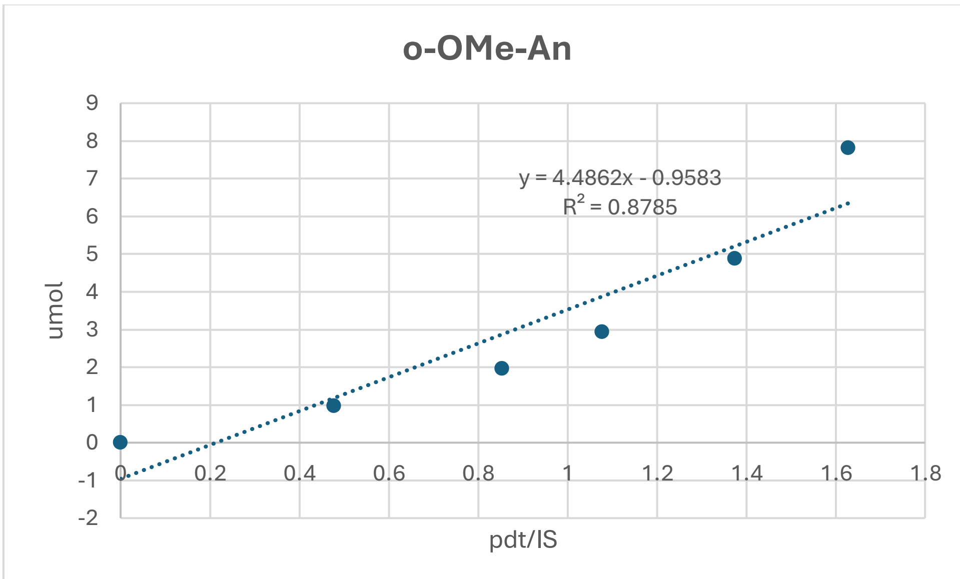
Yield: Schenck: 24%, 22%**.** Vial: 15%, 13%

Chemoselectivity: 70:30, 69:31

Enantioselectivity: 90:10 er. 84:16 er. Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.



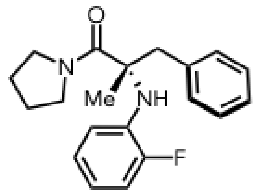
(S)-2-((2-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**23**)



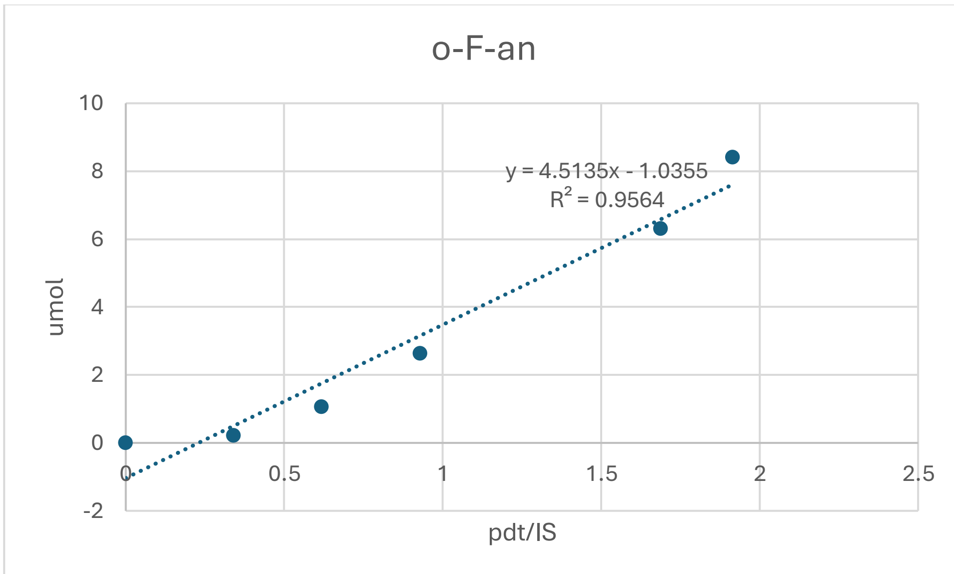
Yield: Schenck: 67%, 62%**.** Vial: 48%, 45%

Chemoselectivity: 70:30, 69:31 *N:C*-alkylation

Enantioselectivity: 70:30 er. 68:32 er. Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



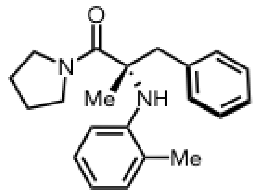
(*S*)-2-((2-fluorophenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**24**)



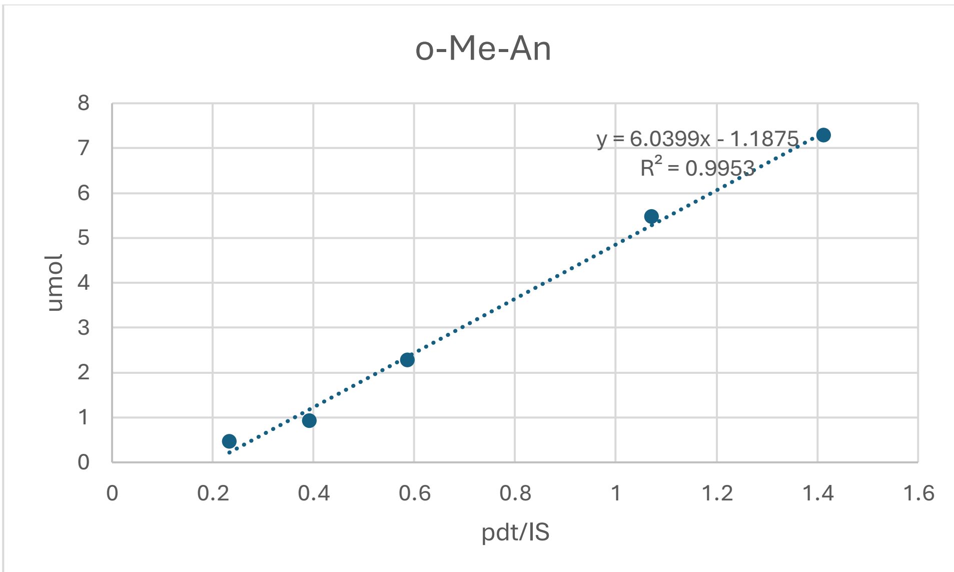
Yield: Schenck: 38%, 35%**.** Vial: 25%, 25%

Chemoselectivity: 72:28, 70:30

Enantioselectivity: 78:22 er. 74:26 er. Chiral HPLC: IB column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.



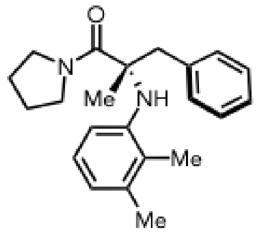
(*S*)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*o*-tolylamino)propan-1-one (**25**)



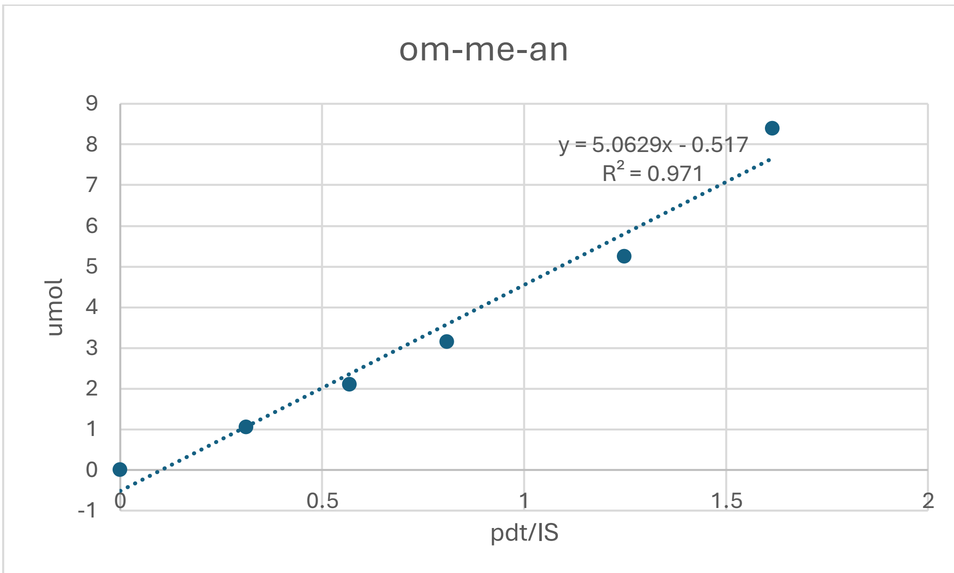
Yield: Schenck: 42%, 38%**.** Vial: 30%, 29%

Chemoselectivity: 75:25, 72:28

Enantioselectivity: 86:14 er. 82:18 er. Chiral HPLC: OJ column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.



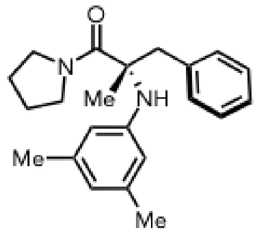
(*S*)-2-((2,3-dimethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**26**)



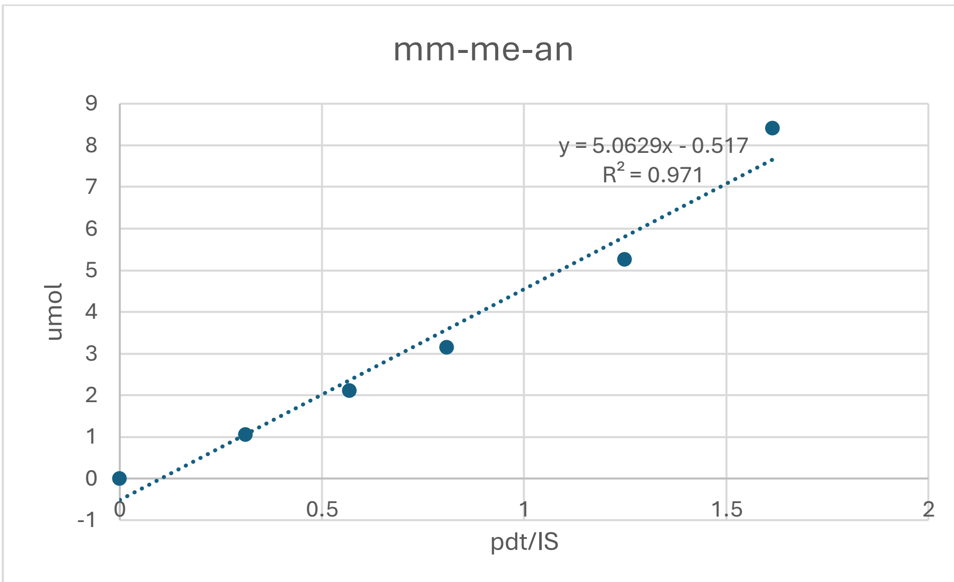
Yield: Schenck: 38%, 35%**.** Vial: 28%, 27%

Chemoselectivity: 70:30, 69:31

Enantioselectivity: 78:22 er. 73:27 er. Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.



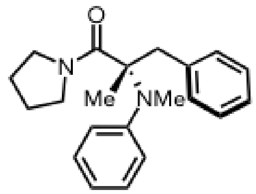
(*S*)-2-((3,5-dimethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**27**)



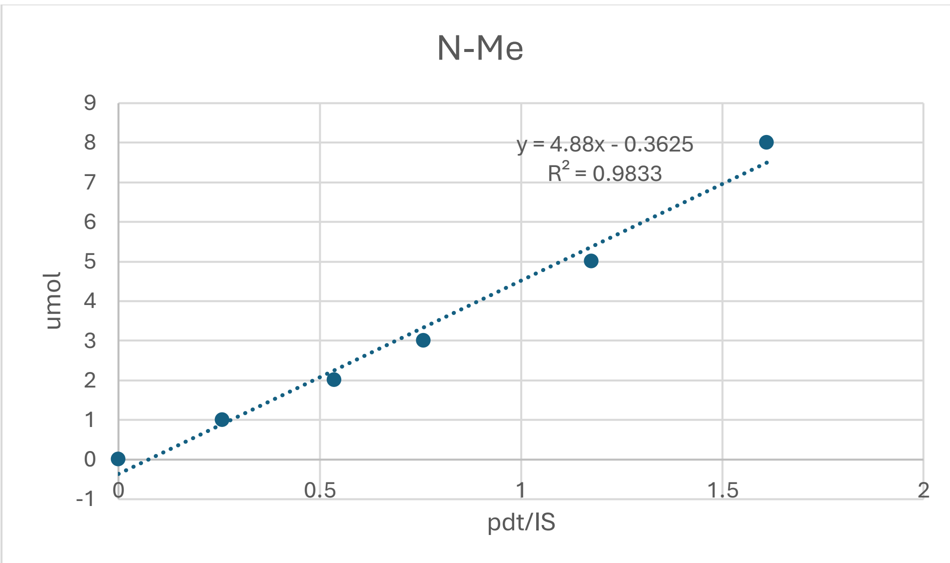
Yield: Schenck: 50%, 47%**.** Vial: 40%, 38%

Chemoselectivity: 62:38, 60:40

Enantioselectivity: 86:14 er. 85:15 er. Chiral HPLC: OJ column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.



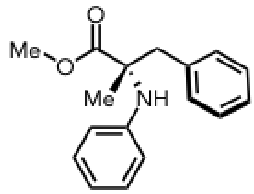
(S)-2-methyl-2-(methyl(phenyl)amino)-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**28**)



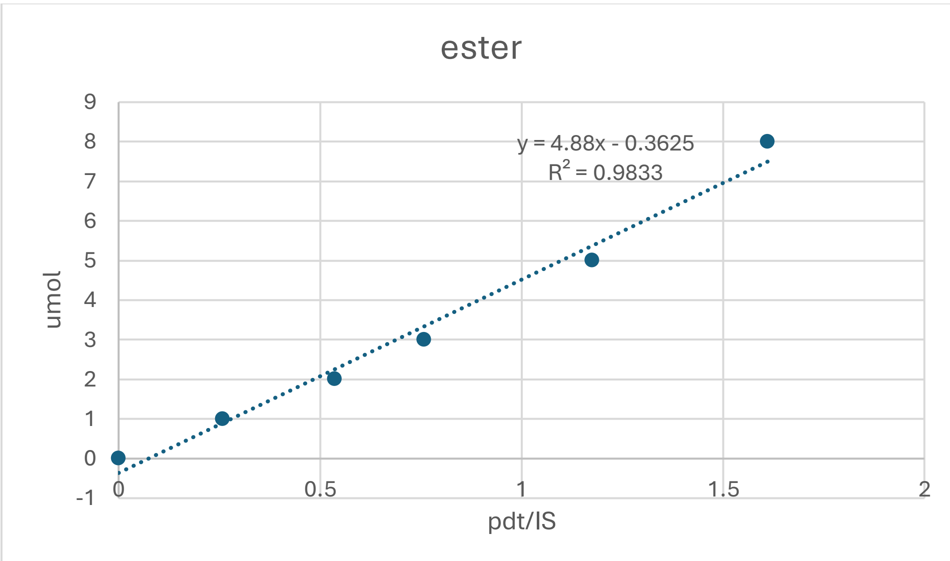
Yield: Schenck: 52%, 50%**.** Vial: 41%, 40%

Chemoselectivity: 72:28, 70:30

Enantioselectivity: 92:8 er. 89:11 er. Chiral HPLC: IA column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.



methyl (*S*)-2-methyl-3-phenyl-2-(phenylamino)propanoate (**29**)

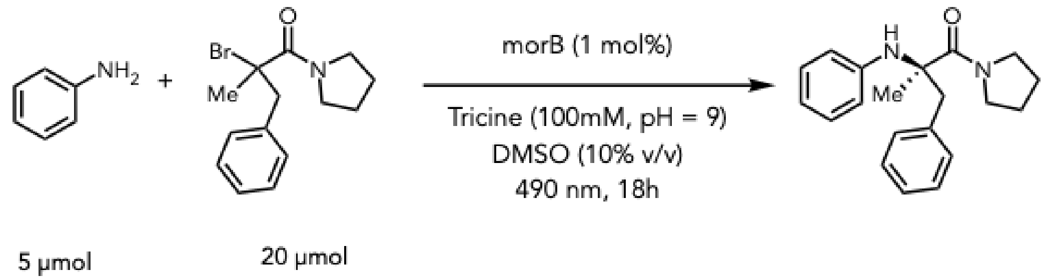


Yield: Schenck: 30%, 27%**.** Vial: 22%, 19%

Chemoselectivity: 72:28, 69:31

Enantioselectivity: 72:28 er. 69:31 er. Chiral HPLC: IA column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

5.8 Unreactive substrates



The following substrates were evaluated under the conditions described in section 5.4 and showed no detectable reactivity.

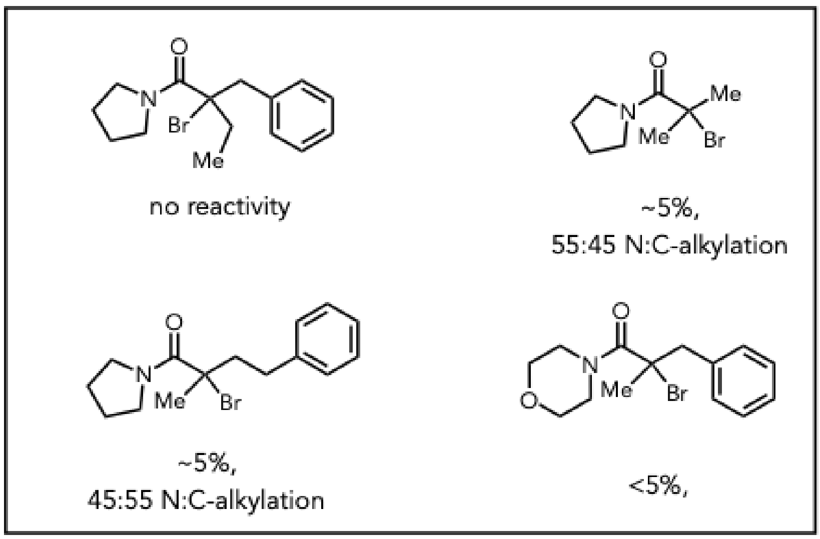


Fig. S10. Unreactive tertiary bromides

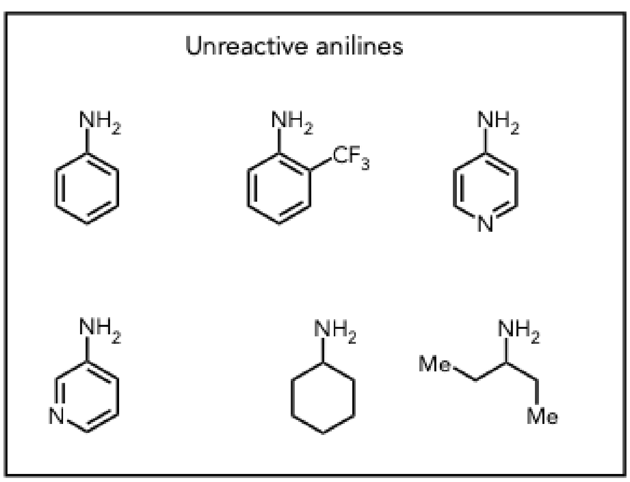


Fig. S11. Unreactive amines

6. **Mechanistic studies**

6.1 Control experiments

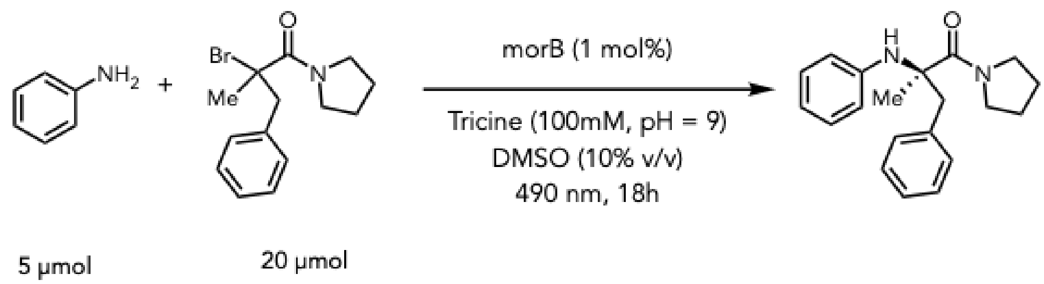
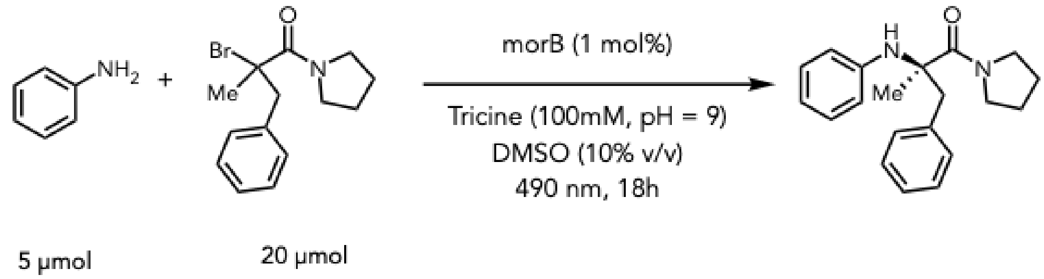


Table. S4. Control experiments

entry	*deviation*	*yield*	*Chemoselectivity (3:4)*	*Enantioselectivity (er.)*
1	none	76%	85:15	88:12
2	No light	0	-	-
3	No enzyme	0	-	-
4	No light and enzyme	0	-	-
5	Just FMN	0	-	-
6	Lysate	25%	83:17	86:14
7	0.1 mmol scale	52%	83:17	86:14

Each reaction was conducted according to the general procedure in section 5.4.

6.2 Reaction progress analysis



Reactions were set up according to the procedure in section 5.4, Each point was performed in duplicates. Upon completion, each reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

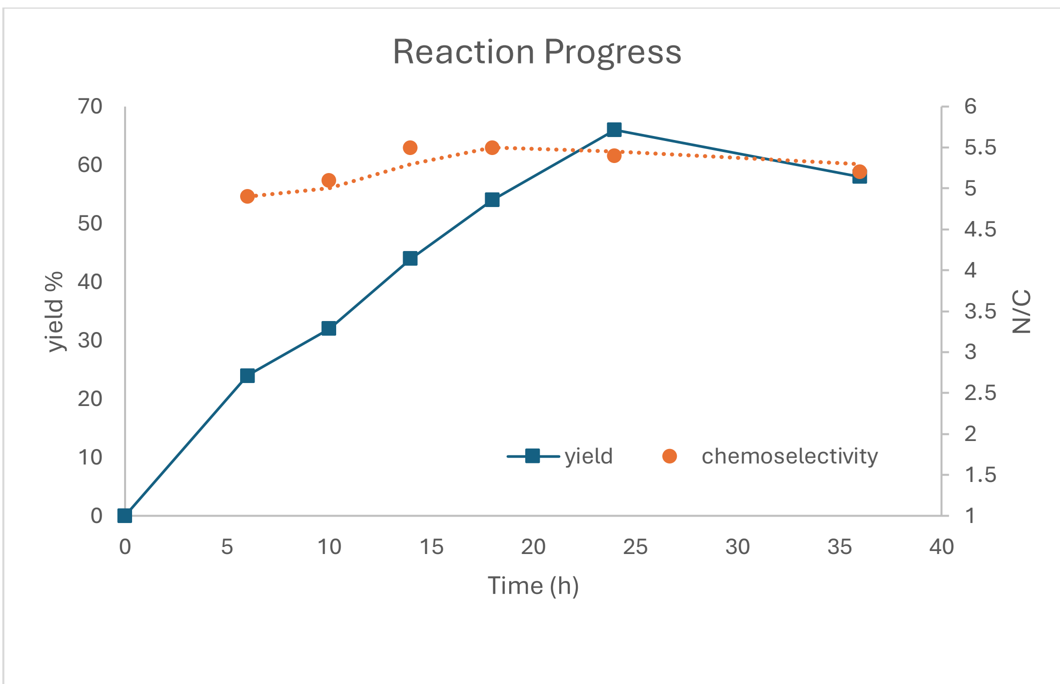
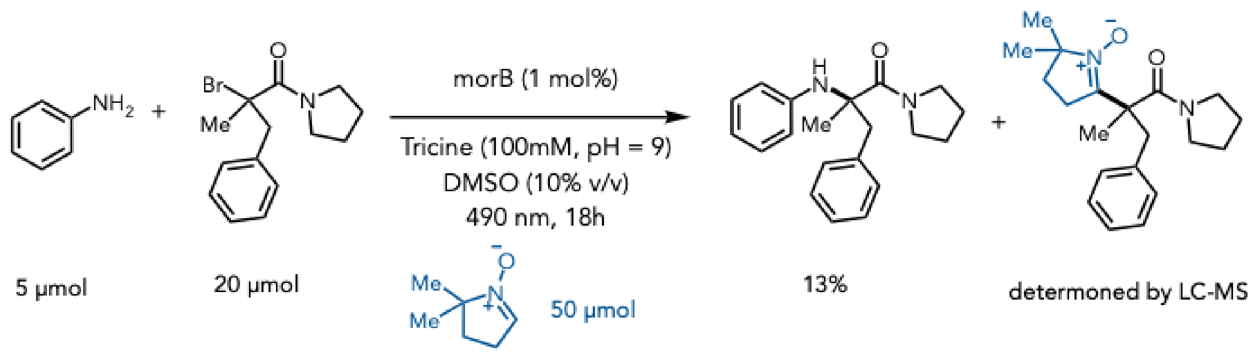


Fig. S12. Time course study

6.3 Radical trap



The reaction was conducted according to general procedure x, with the addition of DMPO (5,5-Dimethyl-1-pyrroline N-oxide, 10 equiv.) to the reaction mixture. Upon completion, the reaction was quenched with 2 mL of acetonitrile and 100 µL of 5 mg/mL 1,3,5-tribromobenzene (TBB) in acetonitrile as the internal standard. The mixture was shaken for 30 min, centrifuged (12000 x g, 5 mins), and the supernatant was filtered and retained for LC-MS analysis for yield calculation.

6.4 UV-Vis studies

6.4.1 Photoreduction in tricine buffer

This procedure is adapted from a previous study in our group, in which irradiating 'ene'-reductases with cyan light in tricine buffer reduces the flavin to its semiquinone state.[8,13]

MorB-mutant (in Tricine pH 9 buffer, 5% DMSO) was irradiated under cyan light (490 nm) for 20 min in a 3 mL quartz cuvette to generate the anionic flavin semiquinone, confirmed by spectroscopy. Addition of substrates 1 and 2 *after* photoreduction, followed by dark incubation, yielded no product (Fig. 5B). Substrate-induced oxidation of the semiquinone thus requires photoexcitation for productive single-electron transfer to the acyl bromide.

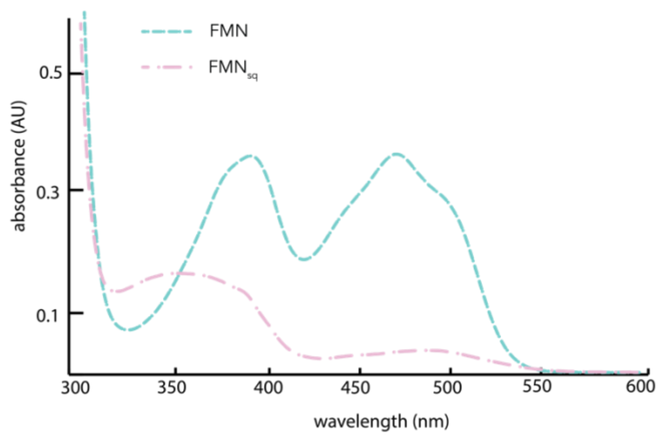


Fig. S13. Photoreduction to flavin semiquinone in tricine buffer

6.4.2 Photoreduction in phosphate buffer (oxidative diradical mechanism)

To rigorously test the diradical mechanism—in which photoexcited FMN oxidizes aniline's lone pair to generate aniline radical cation concurrent with FMN semiquinone formation—we directly compared the kinetics of enzyme-bound FMN photoreduction under cyan light irradiation. If operational, aniline oxidation should accelerate FMN reduction relative to buffer-only controls, detectable via time-dependent loss of the characteristic FMN A_{470} and A_{390} peaks.[13]

In a 3 mL quartz cuvette containing potassium phosphate buffer, purified enzyme was added to a final concentration of 67 nM and irradiated with cyan light. Time-resolved spectra (200-800 nm, 1 nm resolution) were recorded for parallel reactions conducted both in the absence and presence of aniline. FMN photoreduction was monitored by decay of the characteristic A_{450} peak and appearance of the A_{390} characteristic for flavin semiquinone.

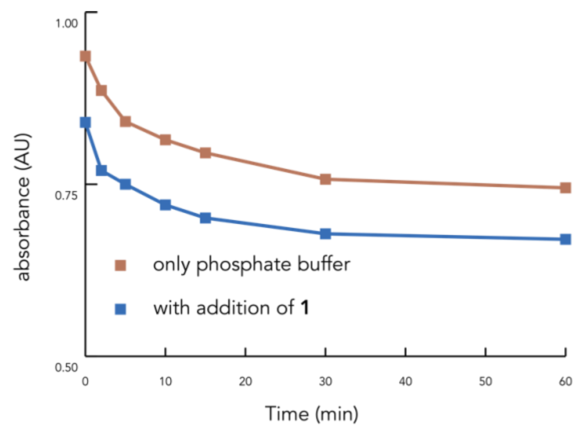


Fig. S14. Kinetics for disappearance at A_{450}

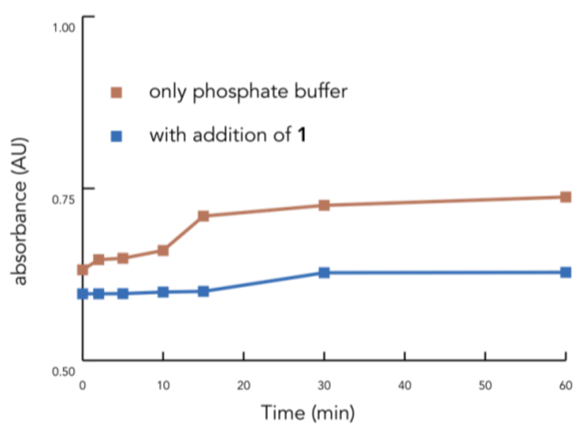


Fig. S15. Kinetics for growth at A_{390}

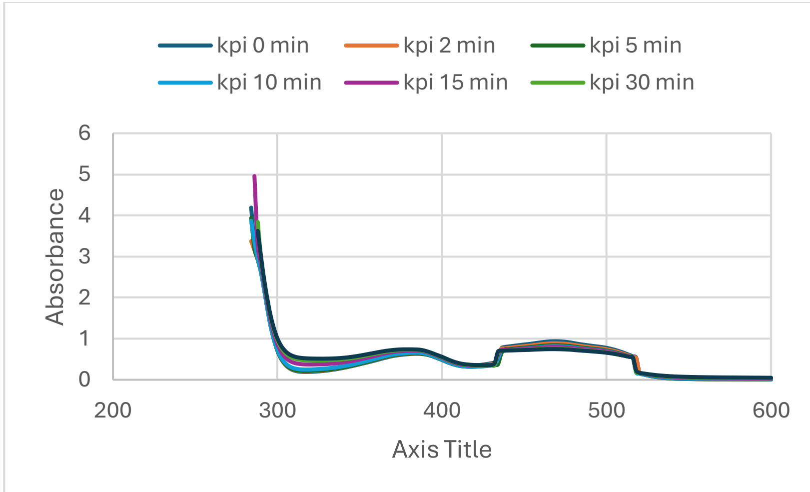


Fig. S16. Full uv-vis data for kPi alone

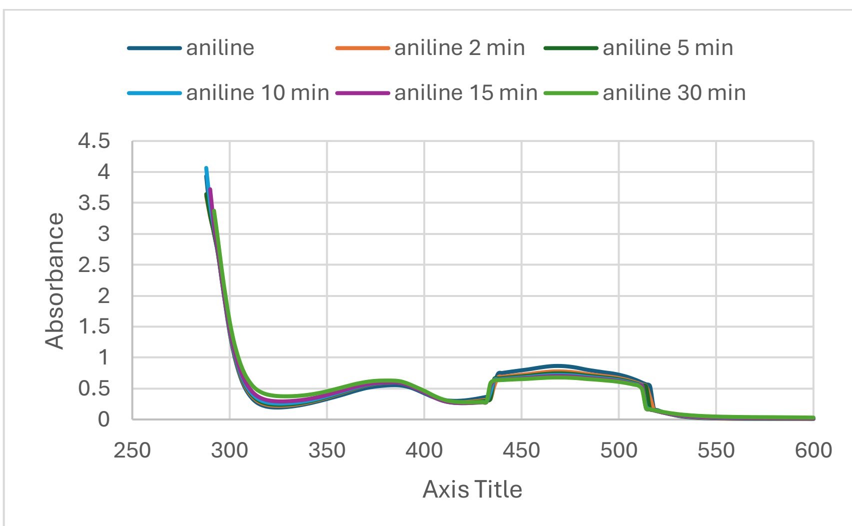


Fig. S17. Full uv-vis data for kPi and aniline

6.5 Catalytic cycle

Photoexcitation of the flavin semiquinone (generated via photoreduction in tricine buffer) drives single-electron reduction of the acyl bromide, generating a tertiary radical. This radical couples selectively with the aniline lone pair through enzyme-templated n→SOMO hyperconjugation, followed by single-electron oxidation and proton transfer to afford the C–N coupled product.[14]

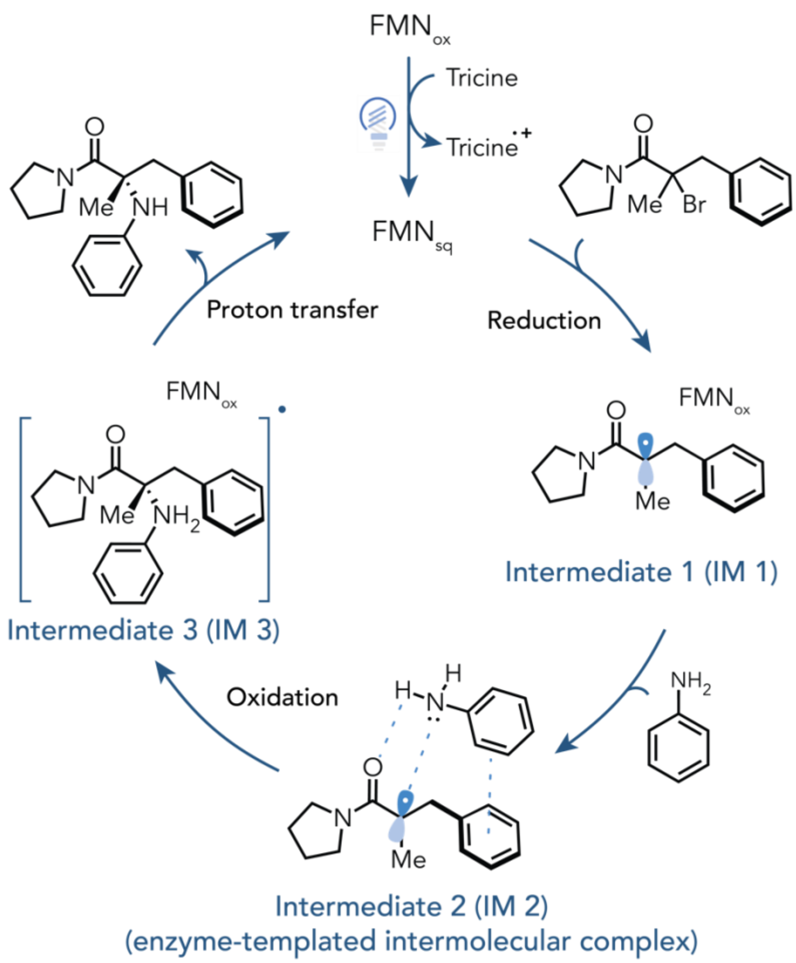


Fig. S18. Catalytic cycle

6.6 Reaction with dithionite

6.6.1 Reduction with irradiation

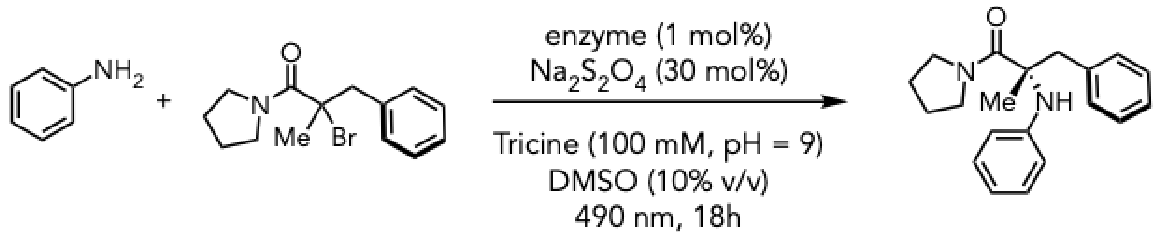


Fig. S19. Dithionite reduction excludes FMNhq Initiation

Reaction was performed as described in section 5.4.

photoirradiation: Standard conditions (section 6.1.1) with $Na_2S_2O_4$ (2.0 μmol, 30 mol%) added *prior to* photoirradiation afforded product in 14% yield (59:41 N:C selectivity). The dramatic drop in yield and selectivity compared to standard conditions demonstrates that excited-state flavin hydroquinone (FMN_{hq}) cannot initiate radical generation. Photoexcitation is thus required to access the reactive flavin species.

6.6.2 Reduction without light

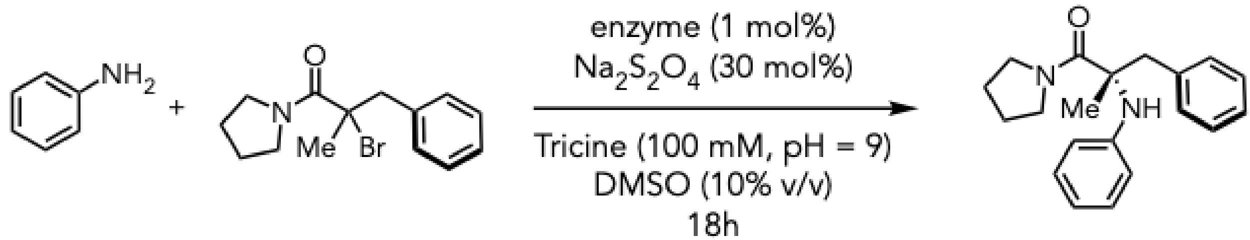


Fig. S20. Dithionite reduction excludes FMNhq Initiation

Dark reaction: Standard conditions (section 5.4) with $Na_2S_2O_4$ (2.0 µmol, 30 mol%) added *without* photoirradiation afforded no product. The result compared to standard conditions demonstrates that ground-state flavin hydroquinone (FMN_{hq}) cannot initiate radical generation. Photoexcitation is thus required to access the reactive flavin species.

6.7 Reactions with NADPH

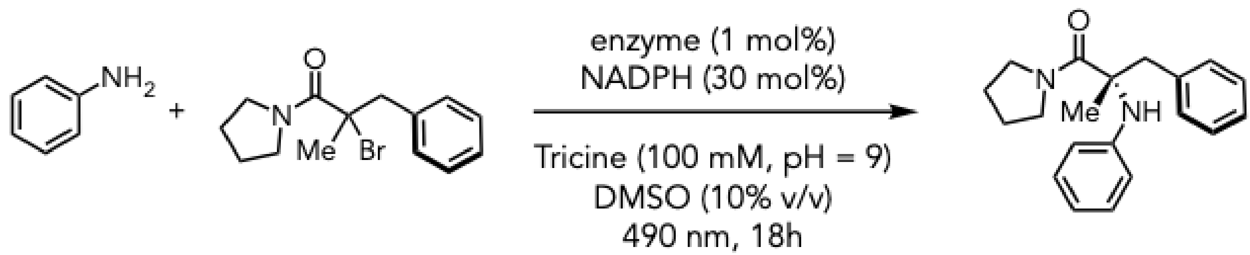


Fig. S21. NADPH reduction excludes FMNhq Initiation

photoirradiation: Standard conditions (section 6.1.1) with NADPH (2.0 µmol, 30 mol%) added *prior to* photoirradiation afforded the product in 29% yield and 69:31 *N:C*-alkylation. The dramatic drop in yield and selectivity compared to standard conditions demonstrates that excited-state flavin hydroquinone (FMN_{hq}) cannot initiate radical generation. Photoexcitation is thus required to access the reactive flavin species.

6.8 No Hydroxylation (radical polar crossover)

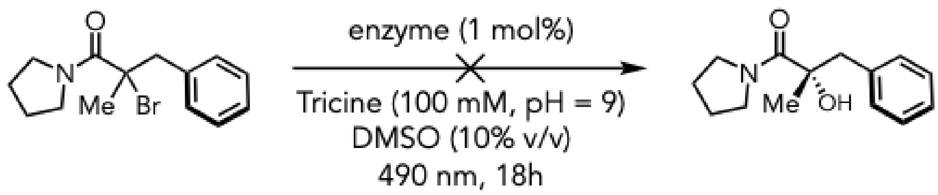


Fig. S22. Radical polar crossover

A radical-polar crossover mechanism, which would generate a carbocation intermediate susceptible to nucleophilic attack by water, is disfavored. Despite water serving as the reaction medium and a competent nucleophile, no hydroxylated products were detected under any standard or control conditions examined. This negative result—combined with high N:C selectivity—provides strong mechanistic evidence that C–N coupling proceeds via a purely radical pathway templated by the enzyme active site, rather than promiscuous ionic intermediates characteristic of crossover mechanisms.

6.9 DFT

DFT calculations were performed using Gaussian 16 on the Princeton Adroit cluster. All input and output files are provided as Supporting Information attachments.

6.9.1 C-N coupling step

A relaxed coordinate scan of the C–N bond dissociation in protonated intermediate IM3 was performed over 15 steps (0.2 Å increments) at the B3LYP/6-31G(d)level.[15] The highest-energy geometry was optimized to a transition state frequency analysis, confirmed by a single imaginary frequency.

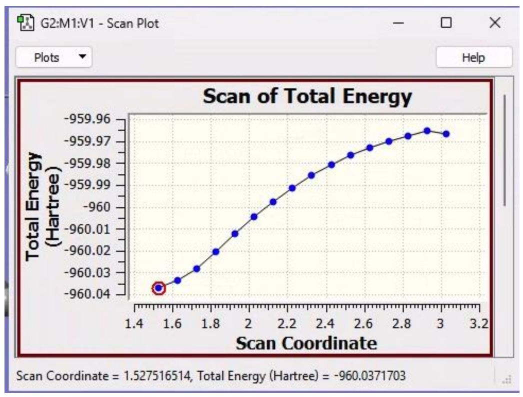


Fig. S23. TS structure

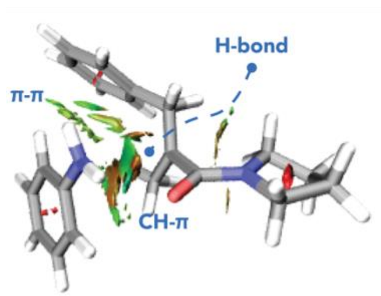


Fig. S24. NCI plot for noncovalent interactions

6.9.2 Redox potential (no water)

To probe whether aniline lone-pair donation stabilizes the transient tertiary radical via n→SOMO hyperconjugation, we computed oxidation potentials (E_{ox}) for the isolated α-acyl radical and its aniline complex using a Born–Haber thermodynamic cycle at the M06-2X/6-311+G(d,p), (MeCN) level in Gaussian 16.[16] The free radical exhibits a high $E_{ox} = -0.02$ V vs SCE, rendering single-electron oxidation unfavorable under reaction conditions. Complexation with aniline lowers E_{ox} dramatically to −0.51 V vs SCE ($\Delta E_{ox} = -0.49$ V), confirming that noncovalent orbital interactions electronically reprogram the radical for selective C–N coupling. Optimized geometries reveal optimal N–C separation (3.1 Å with explicit waters) and frontier orbital overlap consistent with productive hyperconjugation.

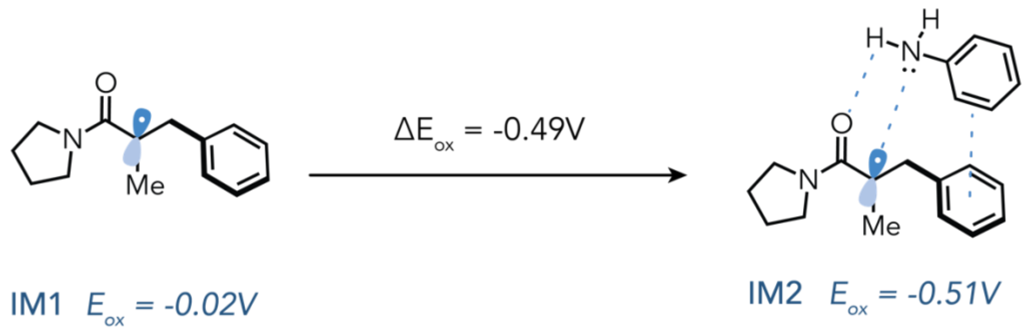


Fig. S25. Oxidation potential calculated at M06-2X/6-311+G(d,p)

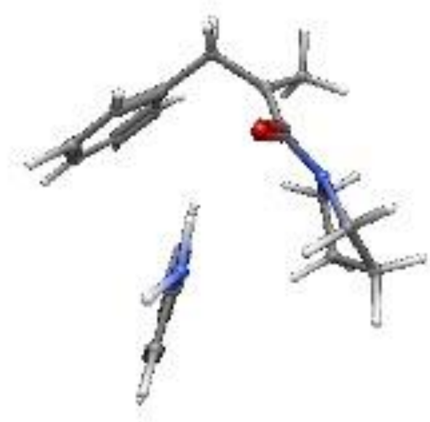


Fig. S26. Structure of the radical intermediate

6.9.3 Redox with water (with water)

Explicit inclusion of two water molecules as H-bond bridges between the aniline nitrogen and α-acyl radical carbonyl dramatically enhances n→SOMO stabilization. The isolated radical exhibits E_{ox} = −0.02 V vs. SCE in MeCN at M06-2X/6-311+G(d,p). The water-bridged aniline complex lowers E_{ox} to −1.21 V vs. SCE (ΔE_{ox} = −1.19 V), compressing the N–C separation to 3.1 Å with optimal frontier orbital overlap. This H_2O-mediated preorganization—not accessible in bulk solution—provides the electronic driving force for selective C–N coupling within the enzyme active site, where analogous water networks and His relays amplify this stabilization.

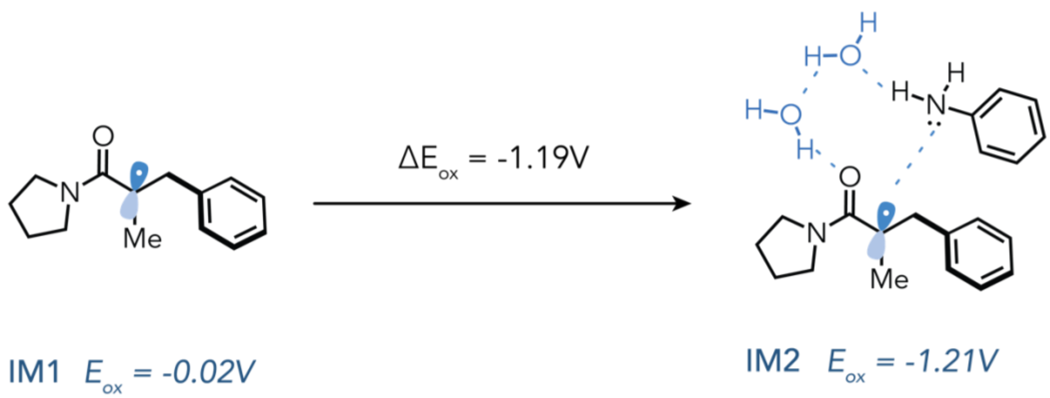


Fig. S27. Oxidation potential for the adduct with water

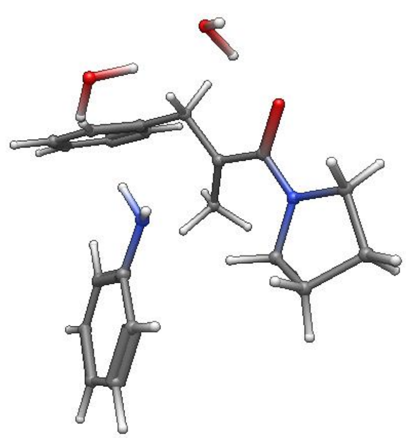


Fig. S28. Structure of encounter complex

6.10 ProteinX

The ProteinX structure was predicted using Protenix-server (https://protenix-server.com), an open-source AlphaFold3 reproduction. The enzyme mutant sequence was submitted via the web interface, generating a high-confidence model (ipTM = 0.97). The refined structure features His186–His189 tandem (N189H from the evolution campaign) forming a water-mediated H-bond relay between aniline N–H and substrate carbonyl.

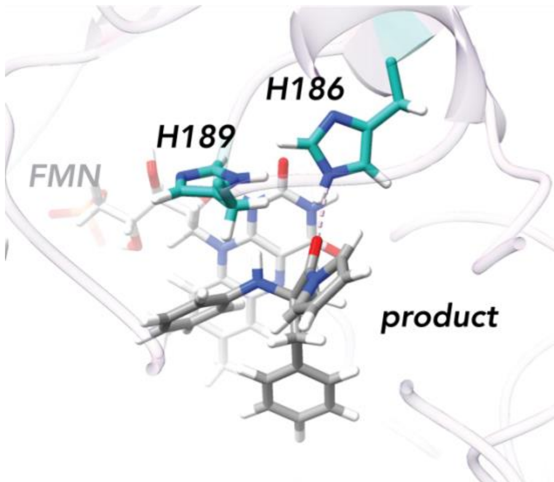


Fig. S29. Mutant structure prediction and substrate docking

7. Modeling

7.1 Hammett analysis

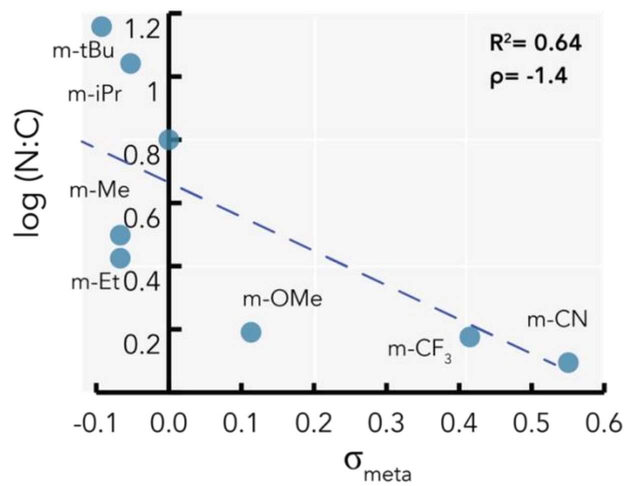
Chemoselectivity values were correlated against Hammett σ constants for meta-substituted aryl derivatives. A poor linear correlation was observed between the product chemoselectivity and σ values for these meta-substituents. The Hammett analysis yielded a negative ρ value, indicating buildup of positive charge character at the selectivity-determining transition state.[17,18] Notably, para substituents completely suppressed arylation byproduct formation, precluding Hammett analysis for that series and highlighting differential electronic effects on competing pathways.

This analysis aligns well with the observation that electron-rich substituents enhance chemoselectivity, consistent with the development of partial positive charge at the postulated selectivity-determining transition state.

Table. S5. Hammett analysis

aniline	σ	**N/C**
H	0	5.5
m-cn	0.56	0.95
m-ethyl	-0.07	5.5
m-ome	0.12	3.9
m-tbu	-0.1	12
m-ipr	-0.04	11
m-me	-0.07	4.8

Fig. S30. Hammett plot



7.2 Statistical modeling

Geometry optimizations and frequency calculations for all aniline structures were carried out using Gaussian 16 software. The functional used for the DFT calculation is M06-2X, which was previously benchmarked for thermodynamic and kinetic accuracy of main-group elements and for non-covalent interactions, and the 6-31g(d) basis-set was chosen.[16]

All regression models were calculated using R (V 4.5.0)9 and R Studio10, and the packages used in the code are moleculaR, caret, car, ggrepel, tidyr, reshape2, scales, tibble, caret, plyr, dplyr, data.table, nnet, ggplot2, knitr, stringr, pracma, and matlib.

Aniline structures were built in ChemDraw and optimized using M06-2X/6-31g(d) method. Sterimol values were calculated for the geometry optimized structures with the use of the Milo group in-house developed Sterimol program (available at https://github.com/Milo-group/SteRimol), based on Verloop's original definitions. [19]

Sterimol values were calculated along the substituent axis such that the principal axis begins on the aniline nitrogen atom. Thus, L is the added length of the substituted ring and the nitrogen–carbon bond distance, B_1 is the minimal width perpendicular to the principal axis L, and B_5 is the maximal width perpendicular to the principal axis L.

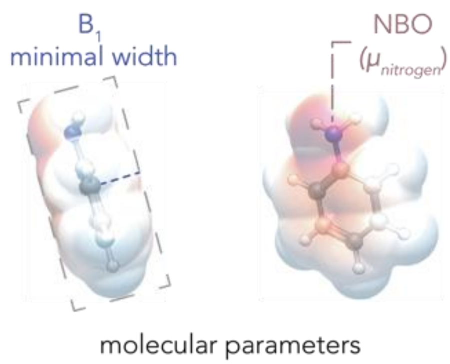


Fig. S31. Parameters

NPA charges were calculated using the NBO 3.1 extension incorporated in Gaussian. Dipole moments for the linear regression model were calculated for the geometry optimized structures with respect to a consistent Cartesian origin, the origin is set at the carbon on the phenyl ring connected to the nitrogen x-axis aligned with the Sterimol L axis, and the y-axis on the plane of the ring towards the *ortho* and *meta* positions (see Figure SX).

Model development involved a selection process that evaluated all possible models with a given range for the number of variables. The maximum number of variables is set to be 2 variables for the overall of 13 observations in this data set. Each parameter was normalized by subtracting its respective mean and dividing by its standard deviation. The resulting models are assessed for their statistical significance and then ranked by a leave- one-out cross-validation. For the most predictive model, a general goodness-of-fit measure, $R^{2,}$ and cross-validation Q^2 are provided.

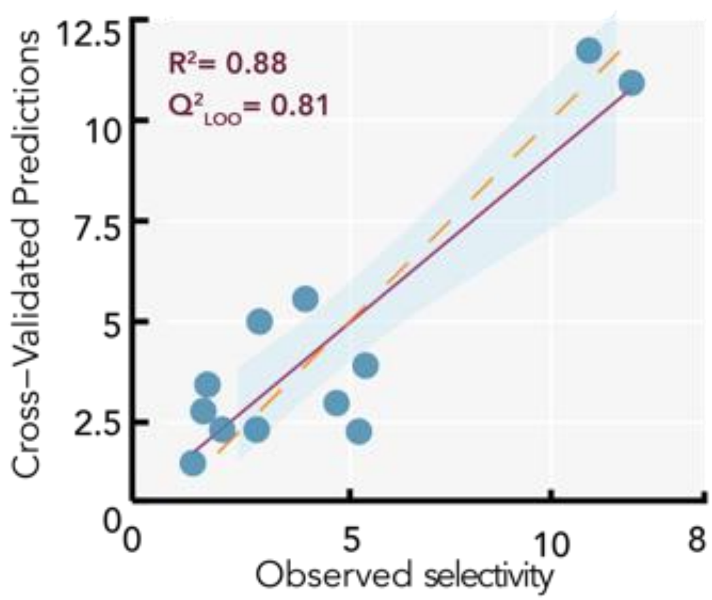


Fig. S32. Multivariate model

7.3 Aniline xyz structure

Aniline-unsubstituted (XYZ)

H -1.700120 -2.142113 0.006738

H 0.760138 -2.144993 -0.015606

C 0.933772 0.000017 -0.008498

C 0.220256 1.202772 -0.005923

C -1.167489 1.197413 0.003494

C -1.874063 -0.000082 0.008832

C -1.166917 -1.197587 0.003689

C 0.220612 -1.202889 -0.006167

H 0.759493 2.144996 -0.016686

H -1.700709 2.141958 0.006998

H -2.957371 -0.000443 0.016377

N 2.327290 0.000333 -0.072497

H 2.774739 0.836028 0.269432

H 2.775771 -0.835628 0.267668

m-Me-aniline (XYZ)

H 0.126119 2.883499 -0.012249

H 2.222354 1.586646 0.000686

C 1.225079 -0.322841 0.008638

C -0.017952 -0.960927 0.018940

C -1.206050 -0.235998 0.011820

C -1.148701 1.157310 0.001413

C 0.083654 1.799610 -0.006594

C 1.265472 1.074355 -0.002807

H -0.055397 -2.047150 0.039868

H -2.485607 -1.894764 0.538382

H -2.065562 1.736861 0.001635

N 2.403149 -1.067707 0.069987

H 2.333003 -2.014396 -0.268205

H 3.226090 -0.601209 -0.277284

H -3.321341 -0.339367 0.423730

C -2.531143 -0.953175 -0.013734

H -2.823862 -1.186175 -1.042531

m-OMe-aniline (XYZ)

H 0.580261 2.881105 -0.008472

H 2.662617 1.567275 0.014311

C 1.642818 -0.336016 0.009221

C 0.401162 -0.963399 0.008507

C -0.772194 -0.211168 0.000922

C -0.721322 1.181545 -0.005880

C 0.528662 1.797738 -0.003822

C 1.701012 1.065479 0.004734

H 0.321106 -2.044821 0.019053

O -1.922290 -0.933460 -0.000540

H -1.618776 1.784187 -0.012564

N 2.813771 -1.088578 0.069354

H 2.736546 -2.038539 -0.257462

H 3.639905 -0.629827 -0.280064

H -3.252666 0.385838 -0.900130

C -3.147785 -0.236204 -0.003953

H -3.926866 -0.996919 -0.002157

H -3.254325 0.391583 0.887956

m-iPr-aniline (XYZ)

H 1.219812 2.894430 -0.012547

H 3.150816 1.361930 0.011605

C 1.937561 -0.416885 0.009844

C 0.626270 -0.904546 0.009381

C -0.468753 -0.048408 -0.000649

C -0.247175 1.330523 -0.009038

C 1.050355 1.822968 -0.006548

C 2.140956 0.964156 0.003585

H 0.462448 -1.979189 0.022743

H -1.787773 -1.705543 0.003859

H -1.084614 2.019469 -0.016912

N 3.017946 -1.297583 0.072963

H 2.836924 -2.226891 -0.272314

H 3.892565 -0.930887 -0.267841

C -2.644304 -0.205948 1.263100

C -1.877116 -0.613272 0.000105

C -2.645072 -0.214457 -1.264984

H -2.105966 -0.519985 -2.165193

H -3.633625 -0.682934 -1.279327

H -2.788091 0.869274 -1.312201

H -2.104901 -0.505976 2.164844

H -2.786339 0.878222 1.302979

H -3.633212 -0.673618 1.280784

m-Et-aniline (XYZ)

H -1.704752 2.738191 0.007987

H -3.094878 0.698842 -0.015017

C -1.405956 -0.635691 -0.009505

C -0.007555 -0.715596 -0.007651

C 0.782619 0.426745 0.001496

C 0.157653 1.677720 0.007659

C -1.225647 1.764952 0.003591

C -2.012434 0.620018 -0.005571

H 0.453792 -1.697698 -0.019251

C 2.297096 0.367635 0.004974

H 0.760890 2.579841 0.015351

N -2.173908 -1.799538 -0.071695

H -1.725053 -2.630394 0.280357

H -3.119048 -1.708985 0.266121

C 2.923241 -1.021863 -0.001828

H 2.658793 0.928403 -0.864222

H 2.655195 0.918619 0.881936

H 2.634069 -1.589717 -0.890596

H 4.013000 -0.944269 0.000452

H 2.631253 -1.599583 0.879753

m-CN-aniline (XYZ)

H -0.884460 2.880050 0.007880

H -2.711125 1.235658 -0.010141

C -1.409252 -0.477570 -0.007234

C -0.077071 -0.895720 -0.008406

C 0.950455 0.045725 -0.000540

C 0.677551 1.413732 0.005750

C -0.649088 1.822143 0.003921

C -1.680500 0.896017 -0.003315

H 0.164327 -1.952599 -0.019551

C 2.312666 -0.408713 0.001688

H 1.487124 2.131667 0.011878

N -2.440140 -1.404410 -0.066284

H -2.219393 -2.338017 0.240255

H -3.340763 -1.088787 0.255386

N 3.405245 -0.772969 0.003872

m-tBu (XYZ)

H -1.764246 2.898764 0.010047

H -3.511120 1.153568 -0.012901

C -2.095283 -0.468777 -0.009638

C -0.735746 -0.803976 -0.009323

C 0.259178 0.167901 -0.000493

C -0.132296 1.512091 0.007025

C -1.475890 1.853023 0.004626

C -2.461955 0.875902 -0.004476

H -0.473679 -1.855739 -0.022626

C 1.751612 -0.187274 0.000949

H 0.612019 2.299806 0.014789

N -3.063061 -1.473241 -0.072274

H -2.772454 -2.372078 0.279240

H -3.974632 -1.212143 0.269256

C 1.996502 -1.700759 -0.006532

C 2.415898 0.411480 -1.252058

C 2.411130 0.398573 1.262438

H 1.955653 0.013929 -2.160910

H 2.323461 1.499671 -1.278083

H 3.482125 0.164219 -1.270220

H 1.576374 -2.175694 -0.897681

H 3.072666 -1.895251 -0.005198

H 1.572654 -2.184877 0.878015

H 1.948086 -0.008682 2.165661

H 3.477431 0.151719 1.281838

H 2.318181 1.486374 1.299575

m-CF_3-aniline (XYZ)

H 1.730191 2.903013 0.001746

H 3.447460 1.143164 0.039078

C 2.034014 -0.479228 0.005754

C 0.676376 -0.807255 -0.017048

C -0.282405 0.196130 -0.036676

C 0.076539 1.538493 -0.027418

C 1.427457 1.862256 -0.002150

C 2.396818 0.872256 0.015672

H 0.371534 -1.847697 -0.023009

F -1.971824 -1.352285 -0.632514

H -0.680204 2.311482 -0.046710

N 2.999805 -1.475250 0.071053

H 2.719346 -2.387253 -0.251464

H 3.922741 -1.218283 -0.239439

F -2.181373 -0.336116 1.260091

C -1.736523 -0.186618 -0.003818

F -2.520510 0.737970 -0.581296

mm-Me-aniline (XYZ)

C 2.490191 1.480079 -0.002201

H 2.170789 -1.205336 0.028410

C 0.032508 -1.417956 0.010431

C -1.188626 -0.735666 -0.002404

C -1.229199 0.652254 -0.013500

C -0.030703 1.368257 -0.015255

C 1.194836 0.709583 -0.000262

C 1.217539 -0.683203 0.013594

H -2.115938 -1.302243 0.000691

H -3.355851 0.752738 -0.382364

H -0.056472 2.453826 -0.029635

N 0.060235 -2.811942 0.073254

H -0.762838 -3.274332 -0.279388

H 0.908011 -3.240388 -0.263390

H -2.815060 1.665263 1.029090

C -2.549570 1.378355 0.006651

H -2.507555 2.291816 -0.591220

H 2.311462 2.556926 -0.012670

H 3.085935 1.245425 0.884600

H 3.094022 1.229690 -0.879230

o-F-aniline (XYZ)

H -2.701355 -1.463539 0.008013

H -0.470462 -2.509118 -0.014895

C 0.598617 -0.651608 -0.008628

C 0.429152 0.732927 -0.004241

C -0.806443 1.341233 0.002016

C -1.949890 0.548359 0.005820

C -1.818497 -0.834896 0.004616

C -0.562573 -1.427914 -0.004236

F 1.549843 1.495478 0.002393

H -0.859797 2.423137 0.006526

H -2.929061 1.010714 0.010615

N 1.870052 -1.205230 -0.072652

H 2.622282 -0.595330 0.206029

H 1.957247 -2.137154 0.298666

o-Me-aniline (XYZ)

H 2.941921 -1.058446 0.004332

H 0.892172 -2.425020 0.017581

C -0.440889 -0.740497 0.004061

C -0.540324 0.661896 -0.000611

C 0.630987 1.412449 -0.010770

C 1.886304 0.815302 -0.009551

C 1.973407 -0.570687 0.001599

C 0.820154 -1.341488 0.006976

C -1.891578 1.323096 0.013547

H 0.553709 2.495576 -0.015569

H 2.781889 1.425062 -0.015420

N -1.594214 -1.525584 0.067627

H -2.422999 -1.125005 -0.342507

H -1.468758 -2.488526 -0.201787

H -2.485420 0.994055 0.872984

H -1.794260 2.408454 0.067116

H -2.467118 1.092513 -0.891637

om-Me-aniline (XYZ)

H 0.137333 3.209546 0.009369

H 2.230007 1.896066 0.013761

C 1.221339 -0.003293 0.002928

C -0.017083 -0.668313 0.005884

C -1.198840 0.084659 -0.006376

C -1.132681 1.477749 -0.005083

C 0.094553 2.125953 0.006256

C 1.268215 1.392367 0.007588

C -0.023182 -2.175079 0.027507

H -2.665263 -1.252086 -0.876550

H -2.050168 2.056144 -0.012542

N 2.409631 -0.738366 0.056401

H 2.385434 -1.646181 -0.380585

H 3.234906 -0.221597 -0.203679

H -2.709385 -1.185714 0.885716

C -2.550619 -0.586570 -0.016116

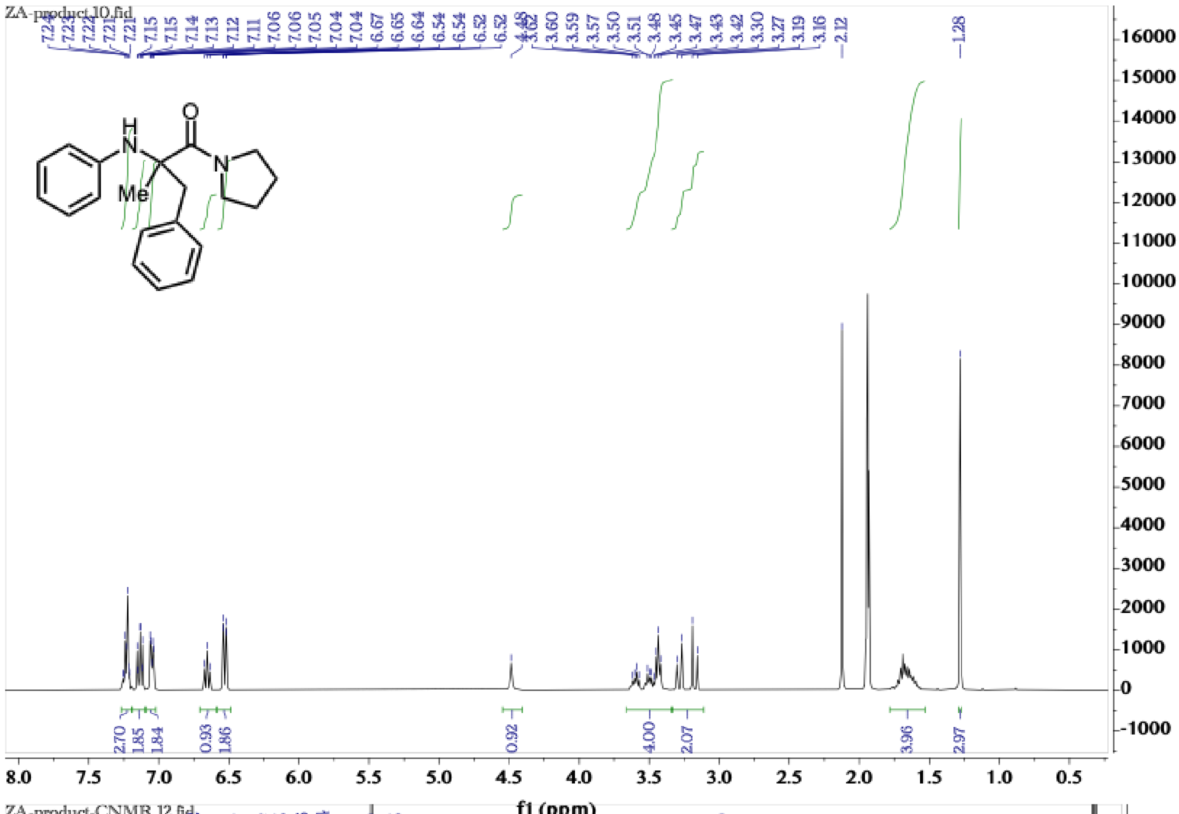
H -3.347597 0.157529 -0.062887

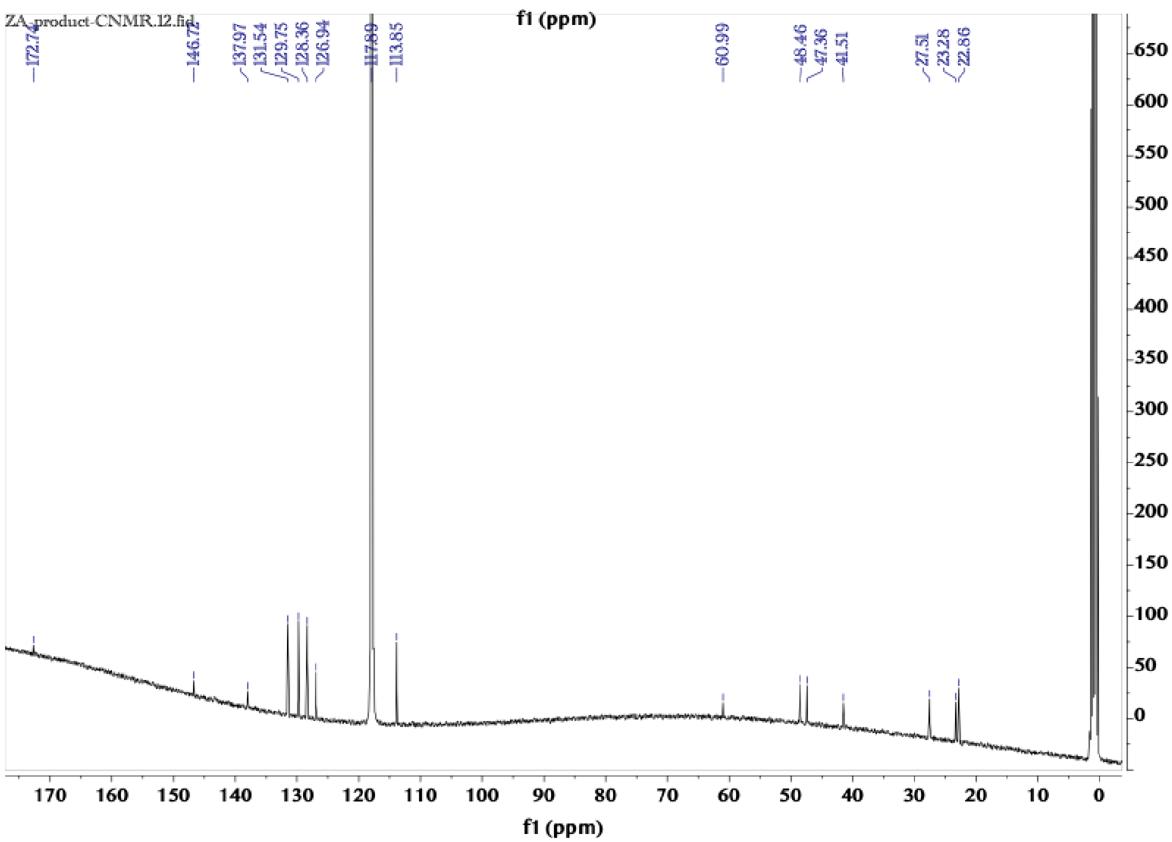
H -1.022787 -2.582117 0.169785

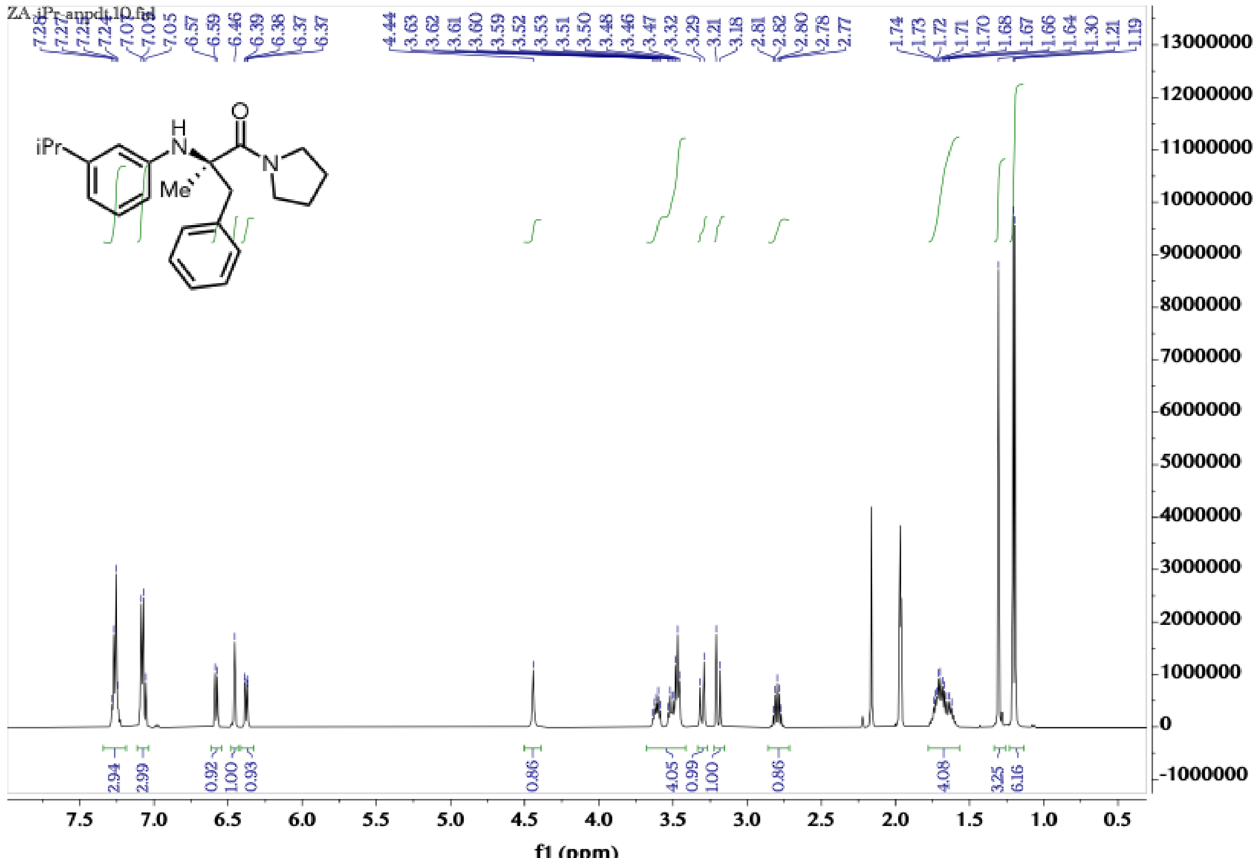
H 0.362485 -2.593555 -0.911221

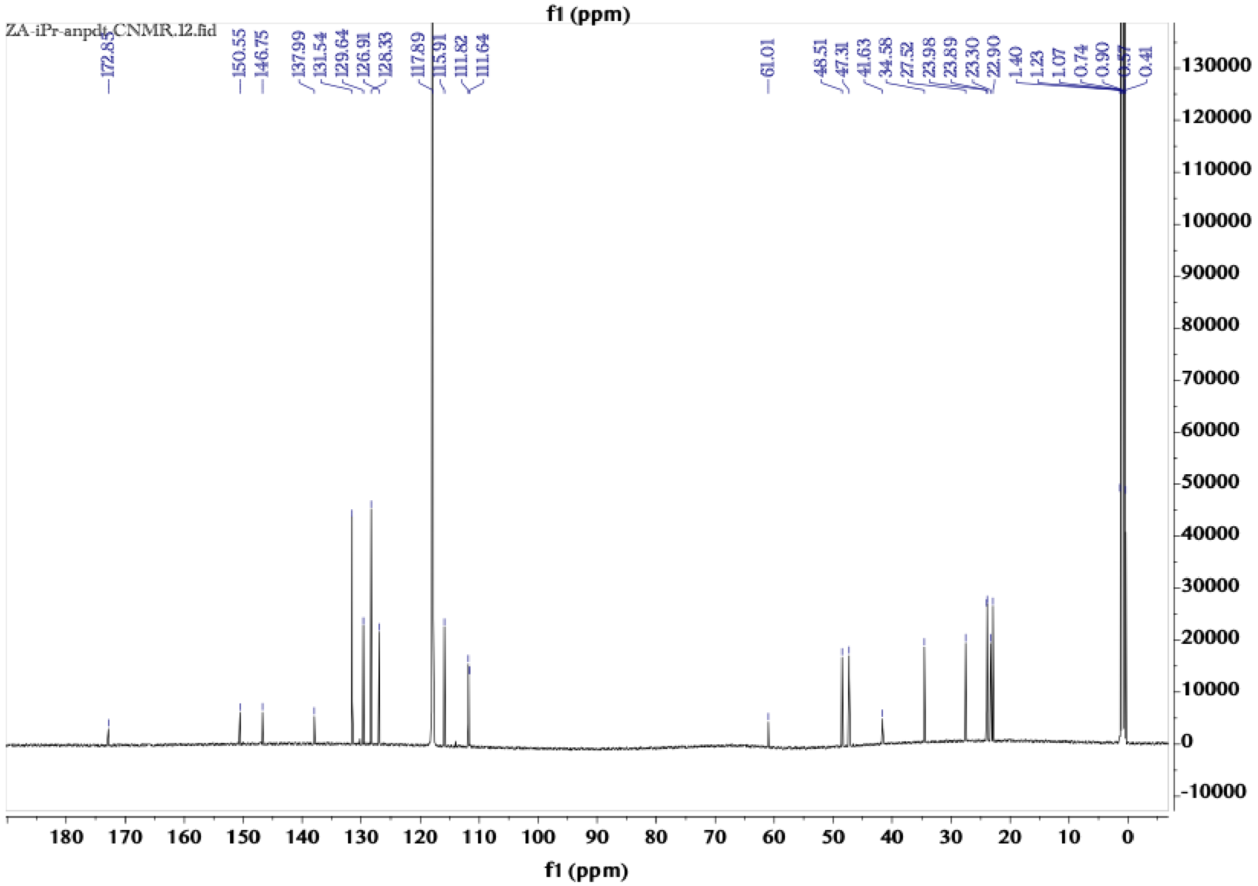
H 0.607409 -2.554303 0.838495

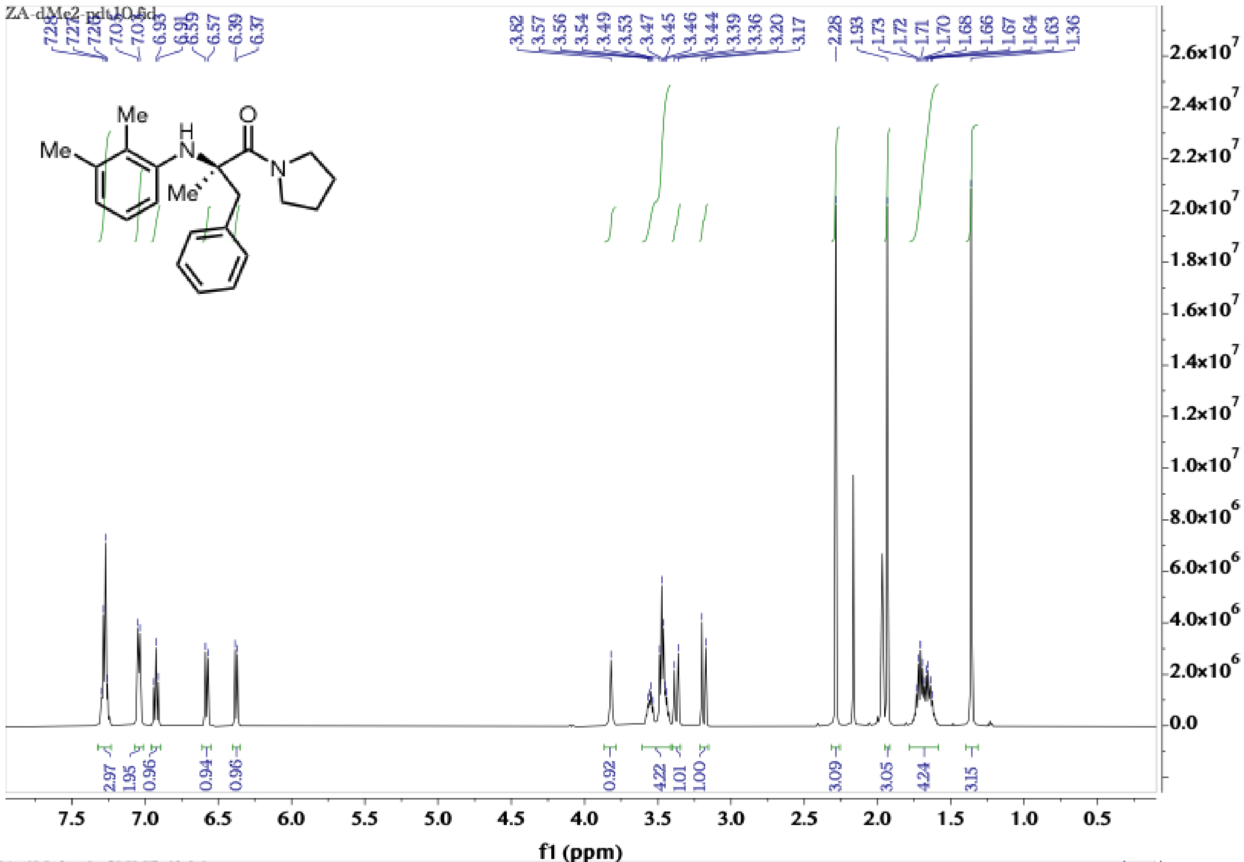
8. NMR spectra

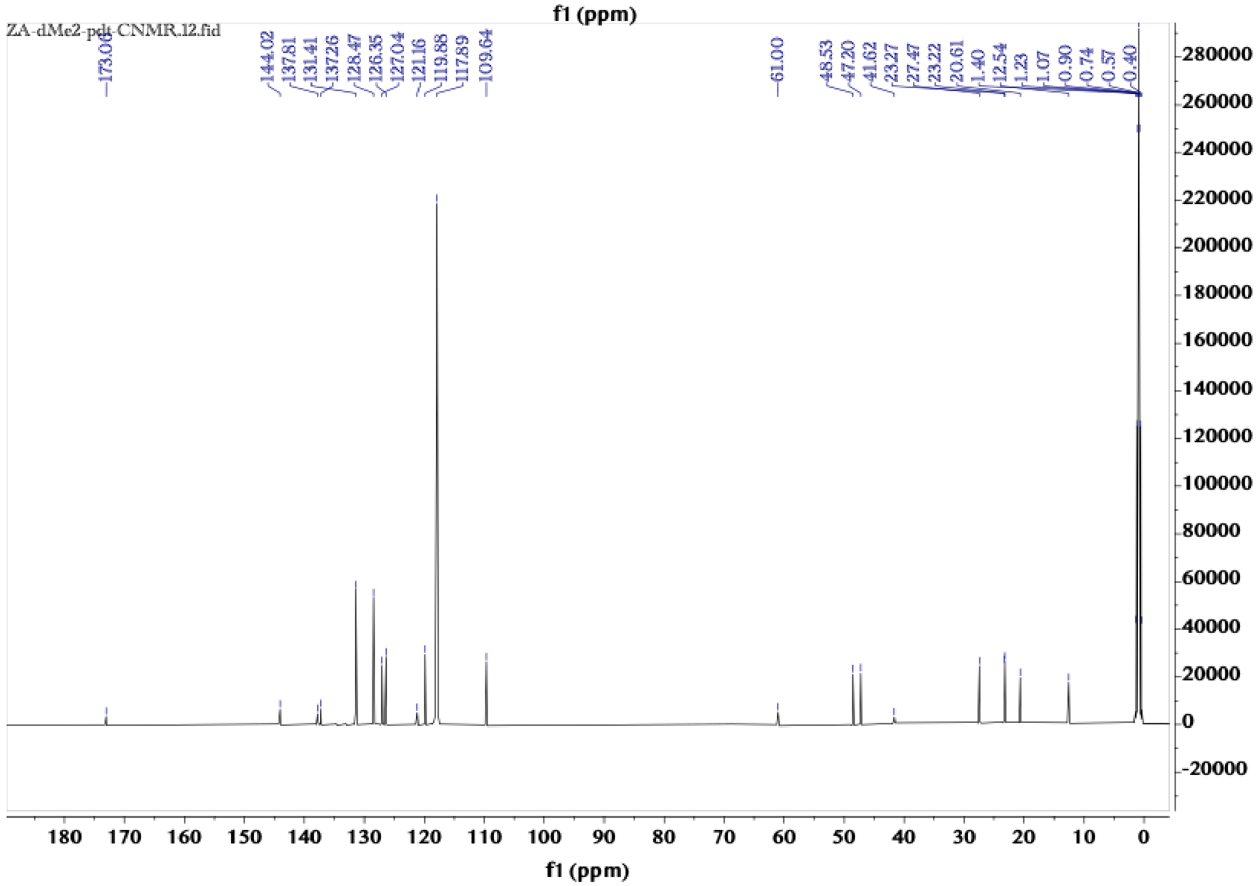


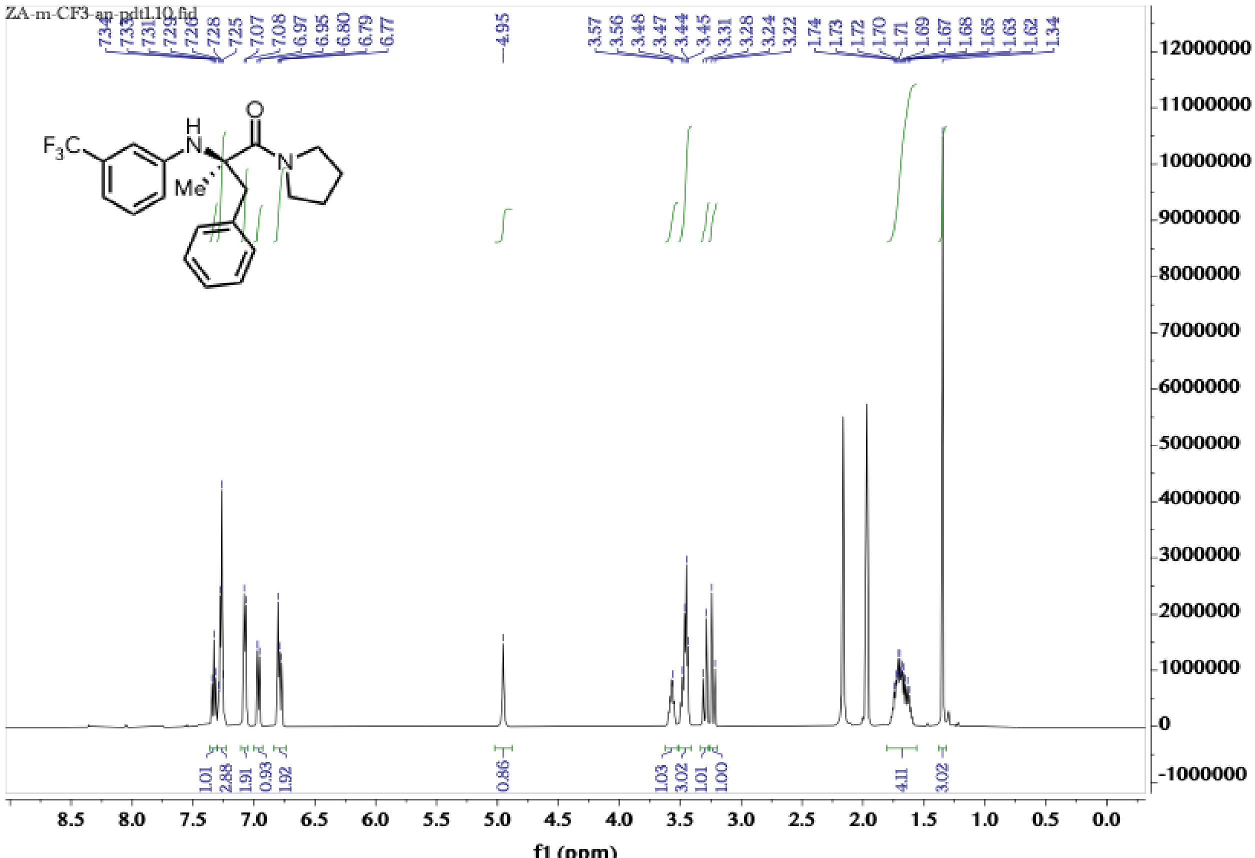


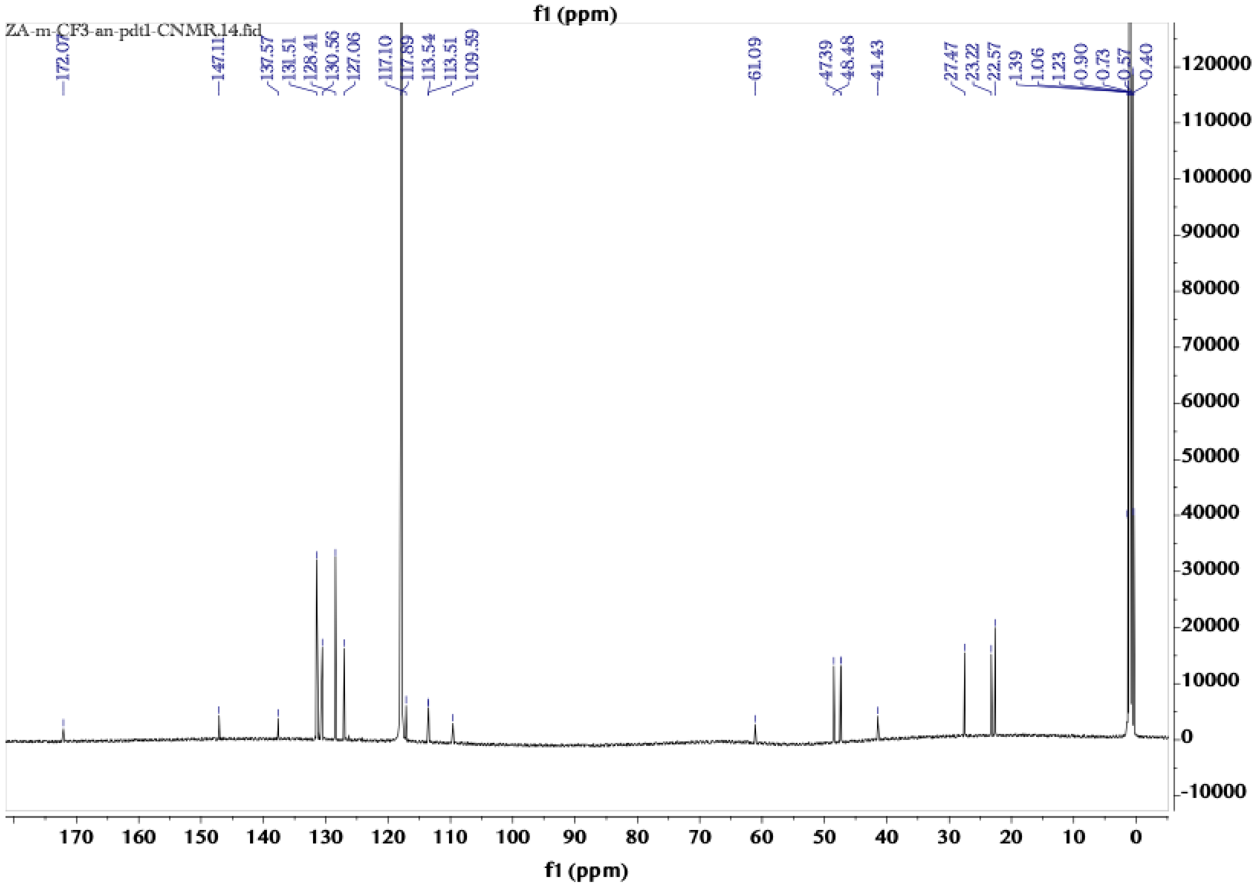

ZA-iPr-anpdt.10.fid
iPr
H
N
O
N
Me
f1 (ppm)

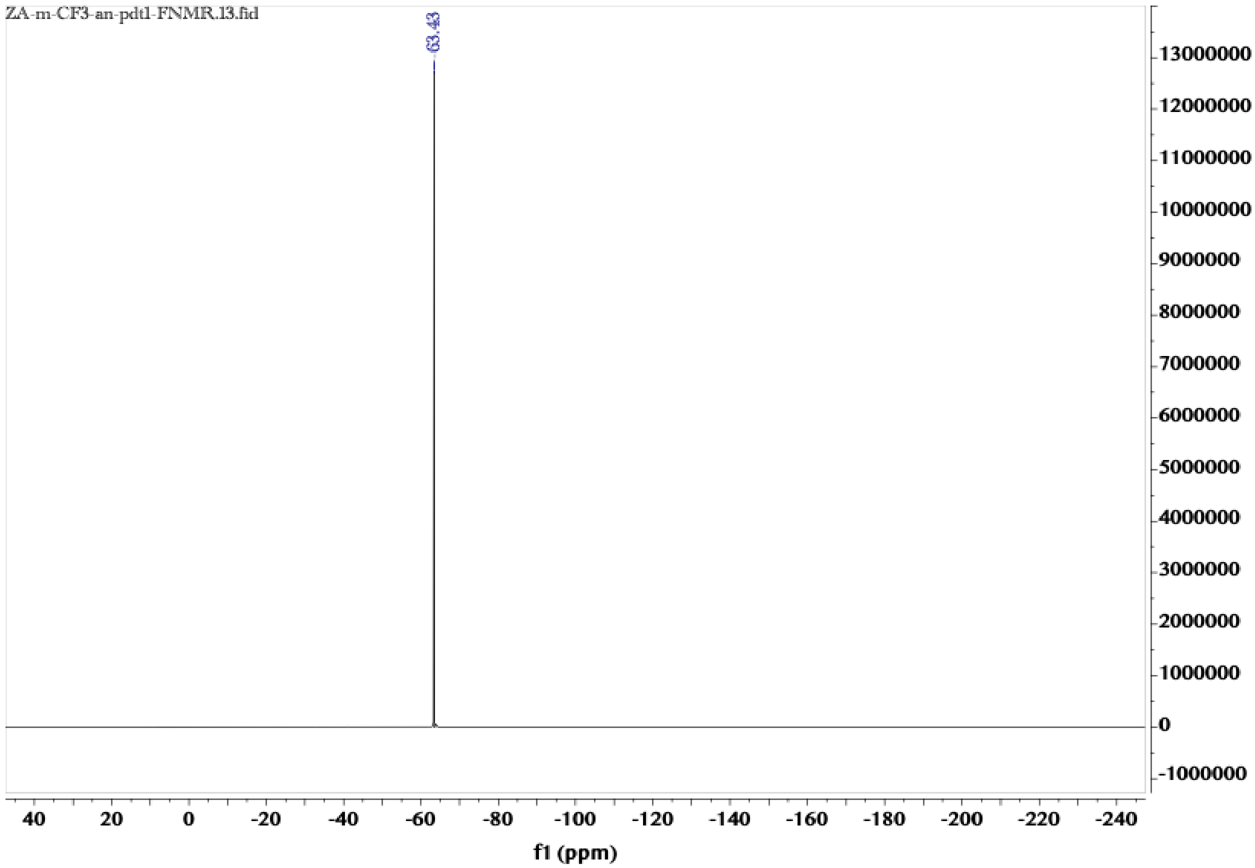

ZA-iPr-anpdt-CNMR.12.fid
f1 (ppm)

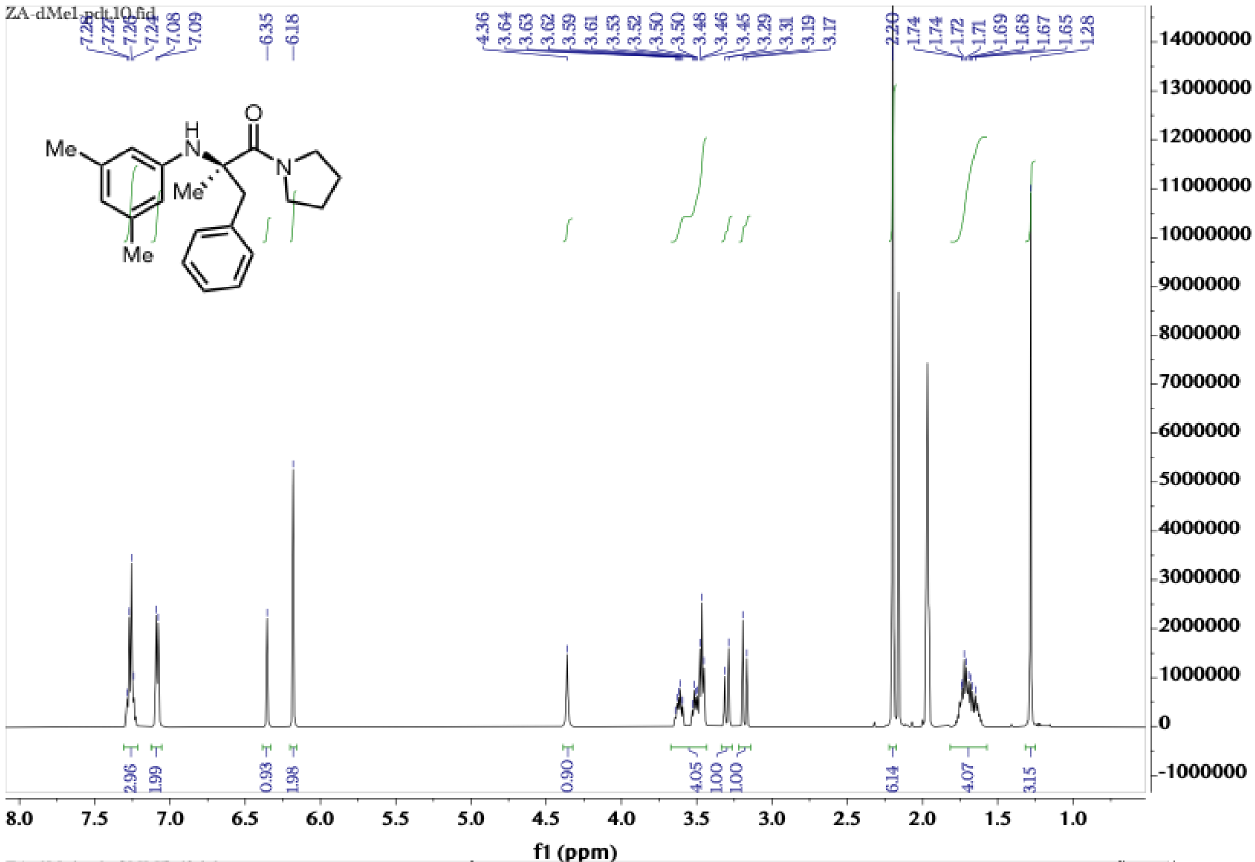

ZA-dMe2-pdt.10.fid
7.28
7.27
7.26
7.04
7.03
6.93
6.91
6.59
6.57
6.39
6.37
3.82
3.57
3.56
3.54
3.49
3.53
3.47
3.45
3.46
3.44
3.39
3.36
3.20
3.17
2.28
1.93
1.73
1.72
1.71
1.70
1.68
1.66
1.67
1.64
1.63
1.36
Me
Me
H
N
O
N
Me
2.97
1.95
0.96
0.94
0.96
0.92
4.22
1.01
1.00
3.09
3.05
4.24
3.15
f1 (ppm)

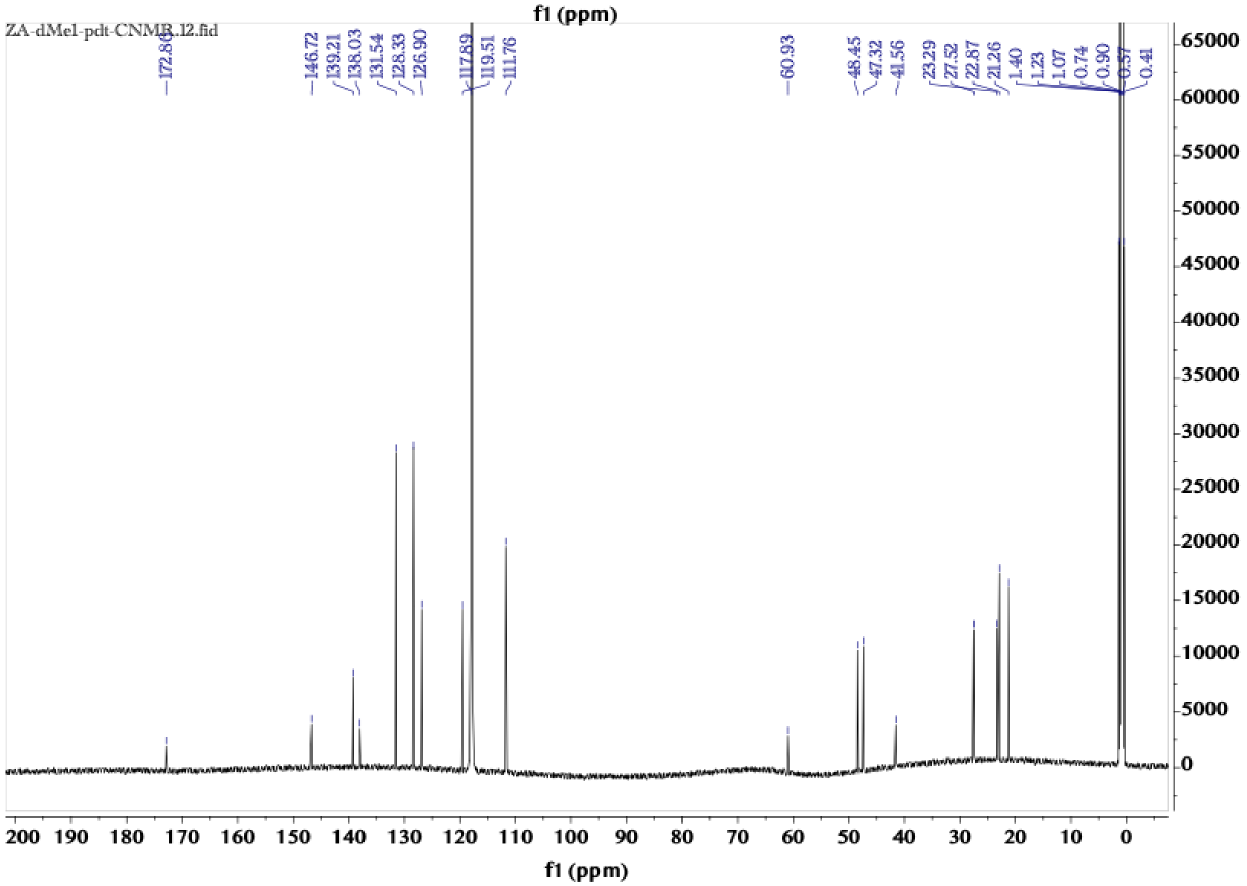

ZA-dMe2-pdt-CNMR.12.fid
173.00
144.02
137.81
131.41
137.26
128.47
126.35
127.04
121.16
119.88
117.89
109.64
61.00
48.53
47.20
41.62
23.27
27.47
23.22
20.61
1.40
12.54
1.23
1.07
0.90
0.74
0.57
0.40
f1 (ppm)

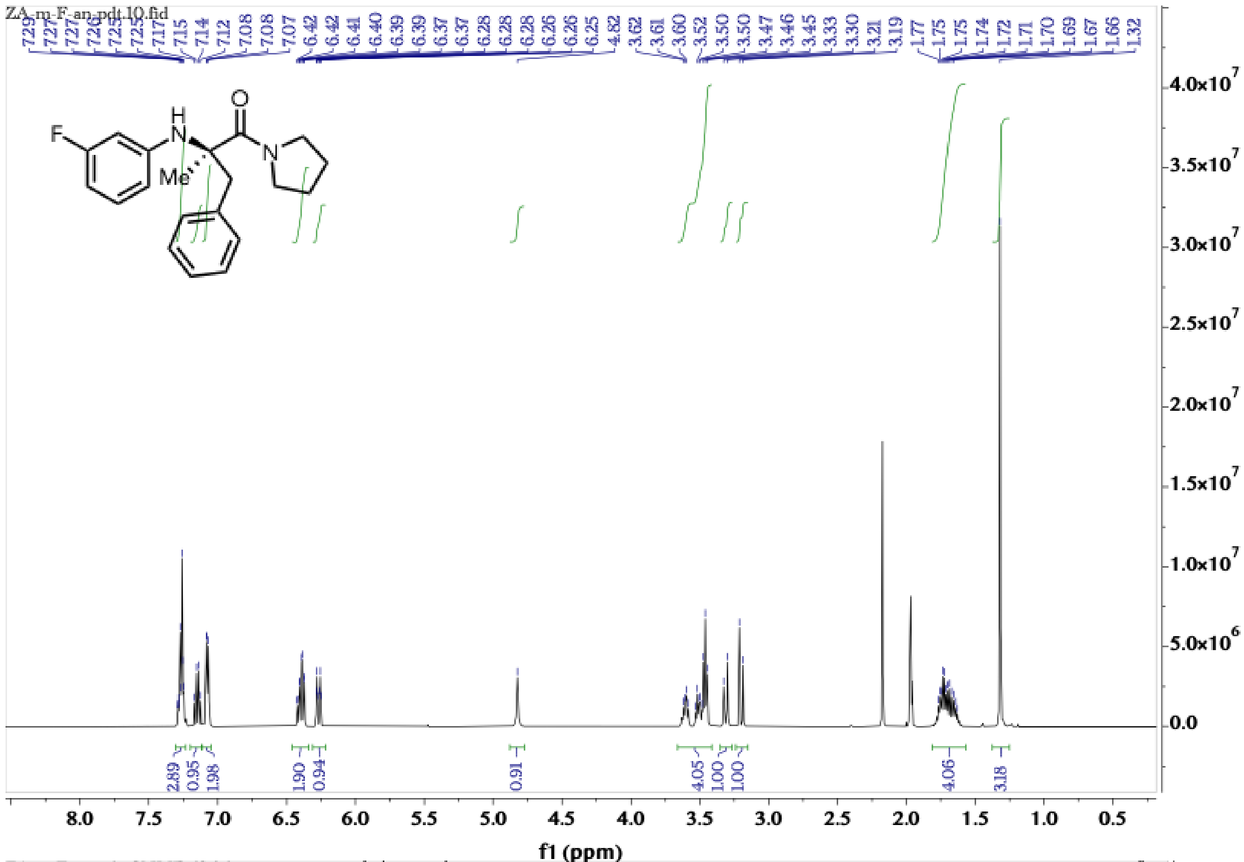

ZA-m-CF3-an-pdt1.10.fid
7.34
7.33
7.31
7.29
7.26
7.28
7.25
7.07
7.08
6.97
6.95
6.80
6.79
6.77
4.95
3.57
3.56
3.48
3.47
3.44
3.45
3.31
3.28
3.24
3.22
1.74
1.73
1.72
1.70
1.71
1.69
1.67
1.68
1.65
1.63
1.62
1.34
F_3C
H
N
O
N
Me
1.01
2.88
1.91
0.93
1.92
0.86
1.03
3.02
1.01
1.00
4.11
3.02
f1 (ppm)

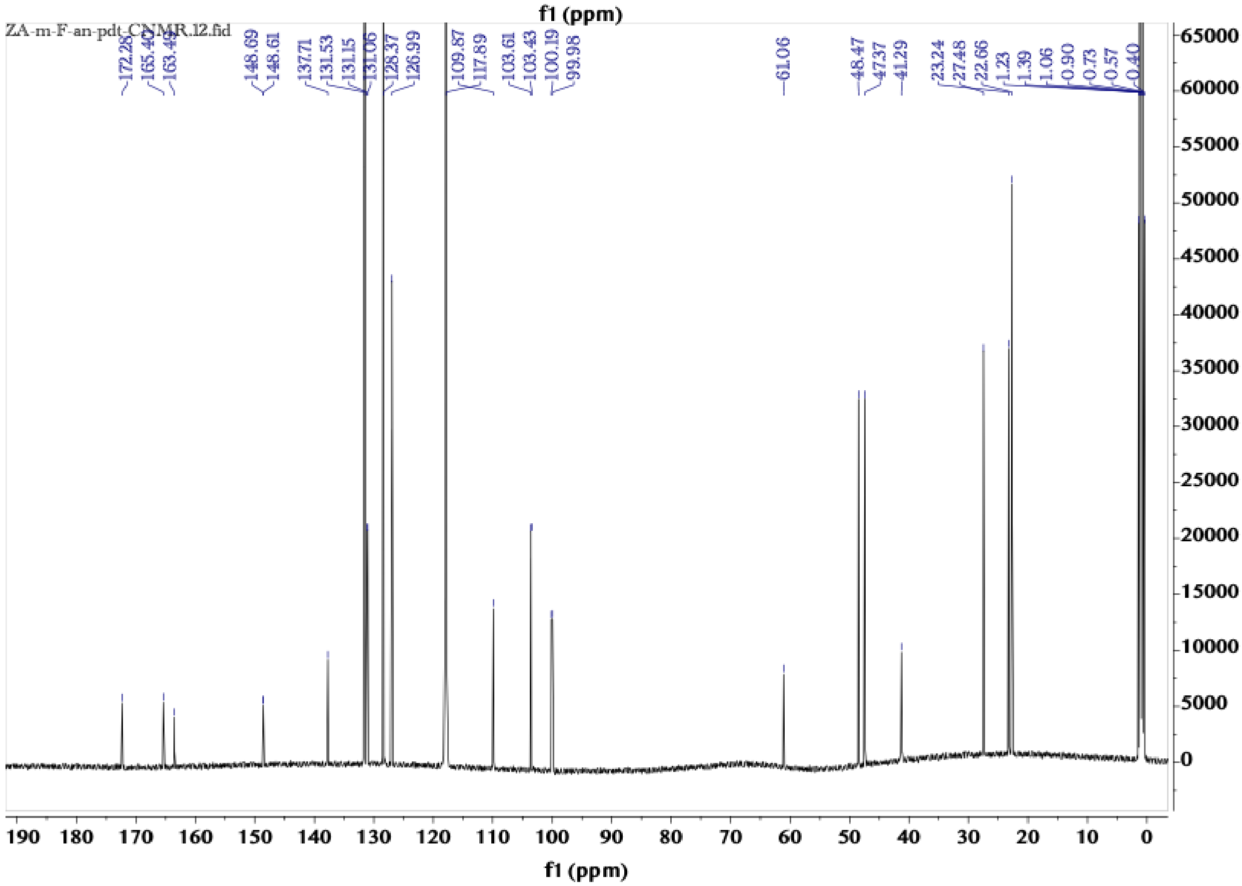

ZA-m-CF3-an-pdt1-CNMR.14.fid
172.07
147.11
137.57
131.51
128.41
130.56
127.06
117.10
117.89
113.54
113.51
109.59
61.09
47.39
48.48
41.43
27.47
23.22
22.57
1.39
1.06
1.23
0.90
0.73
0.57
0.40
f1 (ppm)

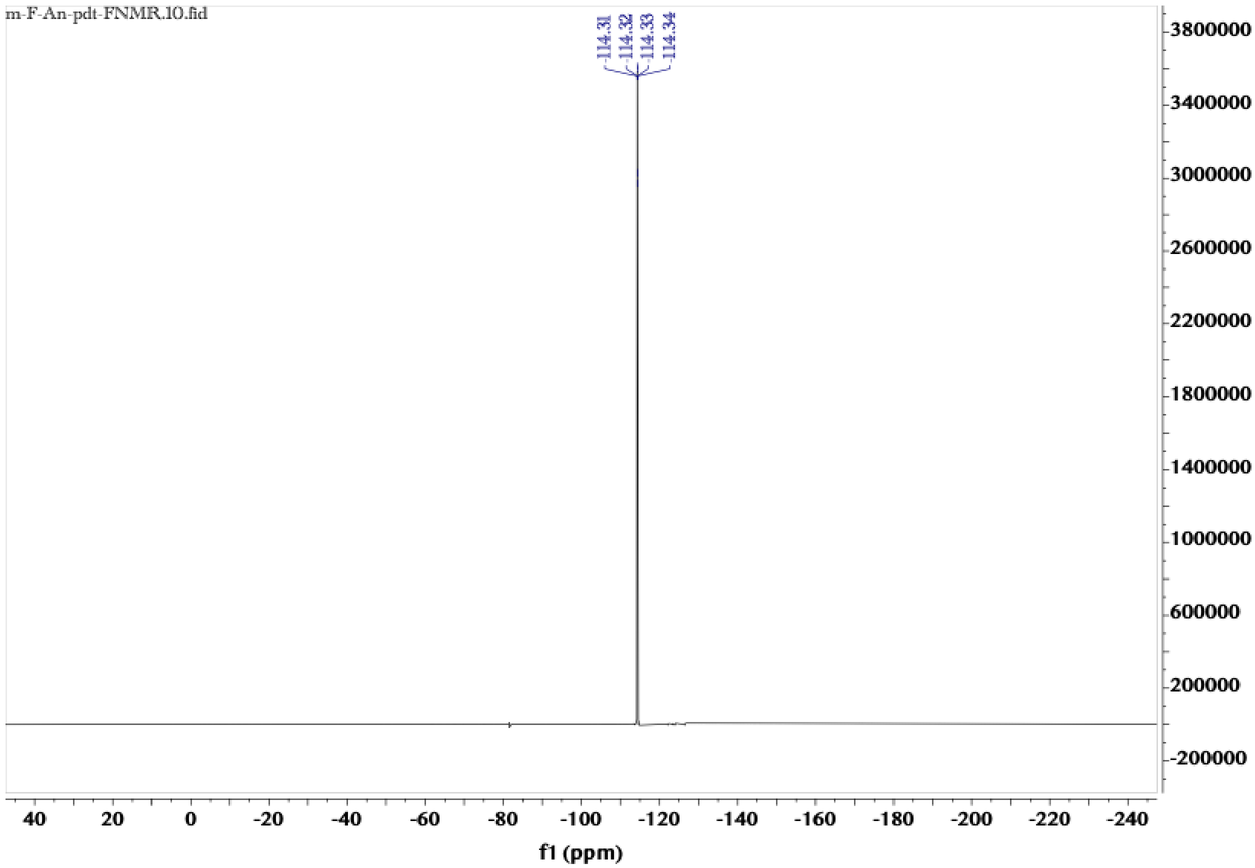

ZA-m-CF3-an-pdt1-FNMR.13.fid
-63.43
13000000
12000000
11000000
10000000
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
-1000000
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
-240
f1 (ppm)


ZA-dMe1-pdt.10.fid
7.26 7.24 7.08 7.09
6.35
6.18
4.36 3.64 3.63 3.62 3.59 3.61 3.53 3.52 3.50 3.50 3.48 3.46 3.45 3.29 3.31 3.19 3.17
2.20 1.74 1.74 1.72 1.71 1.69 1.68 1.67 1.65 1.28
Me
H
N
O
N
Me
Me
2.96 1.99 0.93 1.98 0.90 4.05 1.00 1.00 6.14 4.07 3.15
f1 (ppm)

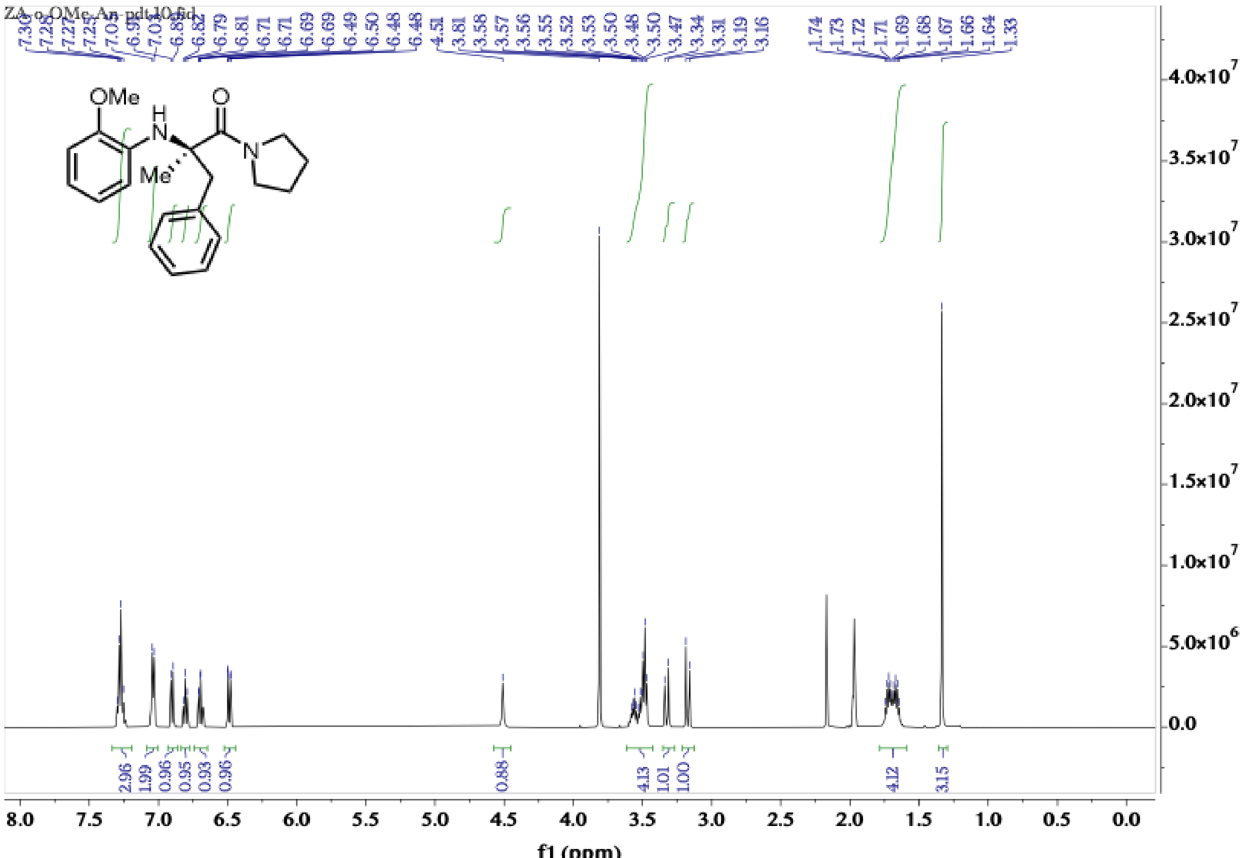

ZA-dMe1-pdt-CNMR.12.fid
172.86
146.72 139.21 138.03 131.54 128.33 126.90
117.89 119.51 111.76
60.93
48.45 47.32 41.56
23.29 27.52 22.87 21.26 1.40 1.23 1.07 0.74 0.90 0.57 0.41
f1 (ppm)

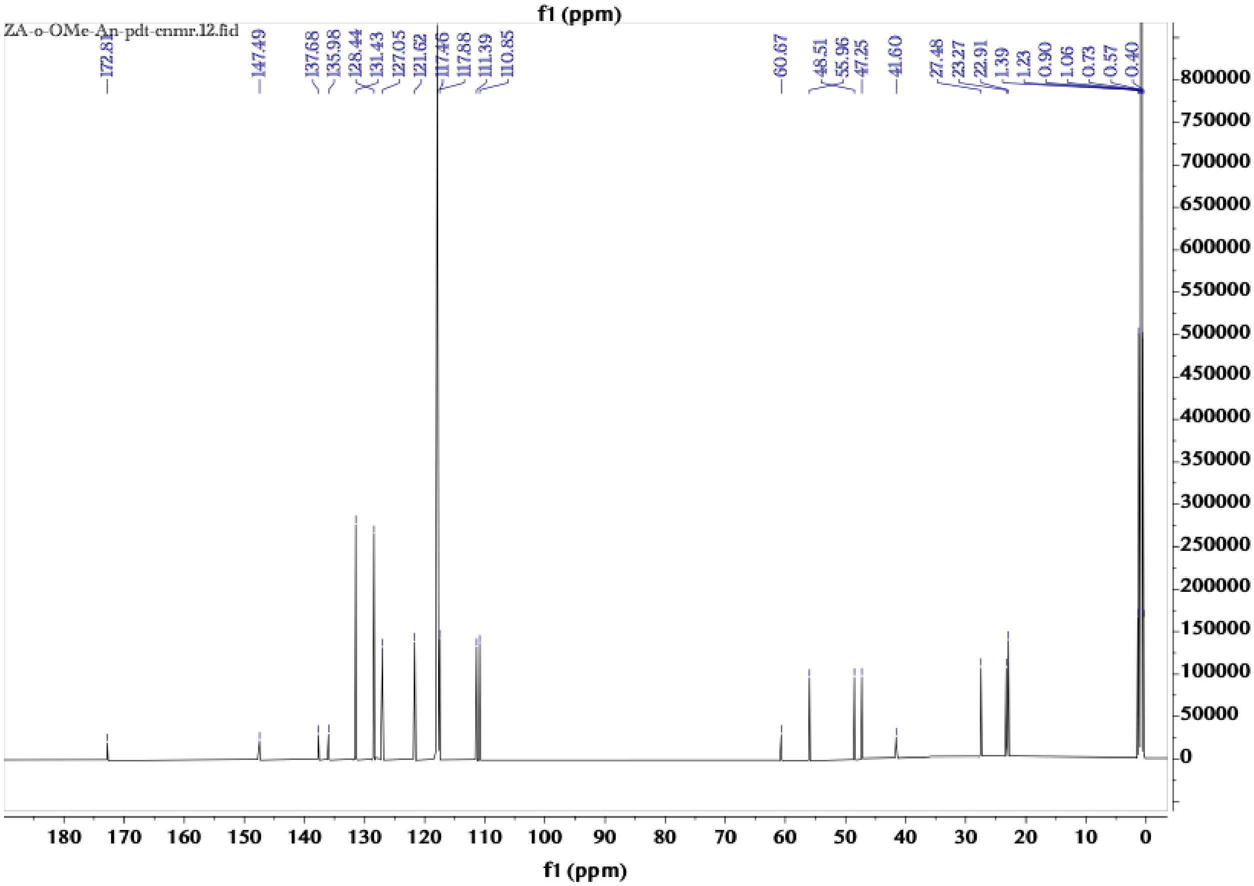

ZA-m-F-an-pdt.10.fid
7.29 7.27 7.27 7.26 7.25 7.25 7.17 7.15 7.14 7.12 7.08 7.08 7.07 6.42 6.42 6.41 6.40 6.39 6.39 6.37 6.37 6.28 6.28 6.28 6.26 6.26 6.25 4.82 3.62 3.61 3.60 3.52 3.50 3.50 3.47 3.46 3.45 3.33 3.30 3.21 3.19 1.77 1.75 1.75 1.74 1.72 1.71 1.70 1.69 1.67 1.66 1.32
F
H
N
O
N
Me
2.89 0.95 1.98 1.90 0.94 0.91 4.05 1.00 1.00 4.06 3.18
f1 (ppm)

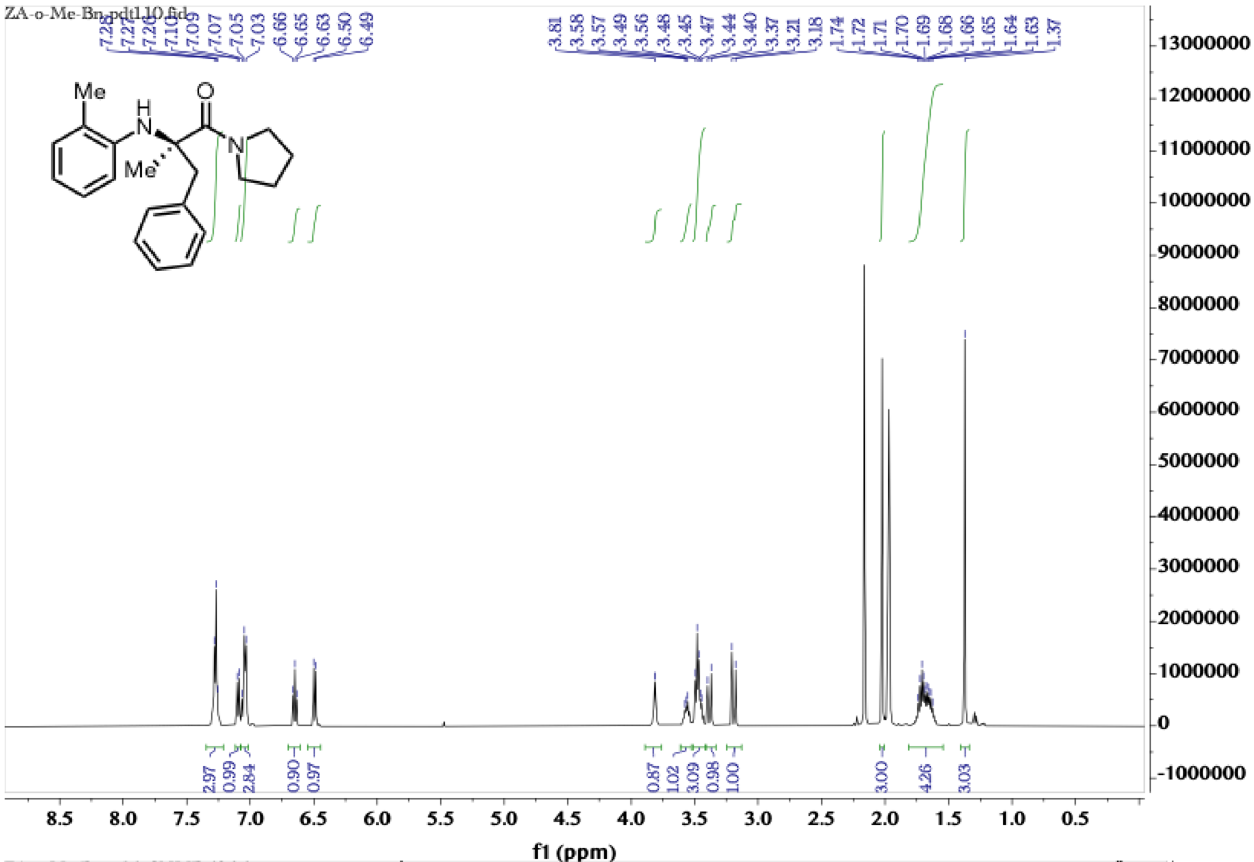

ZA-m-F-an-pdt_CNMR.12.fid
172.28 165.40 163.49 148.69 148.61 137.71 131.53 131.15 131.06 128.37 126.99 109.87 117.89 103.61 103.43 100.19 99.98 61.06 48.47 47.37 41.29 23.24 27.48 22.66 1.23 1.39 1.06 0.90 0.73 0.57 0.40
f1 (ppm)



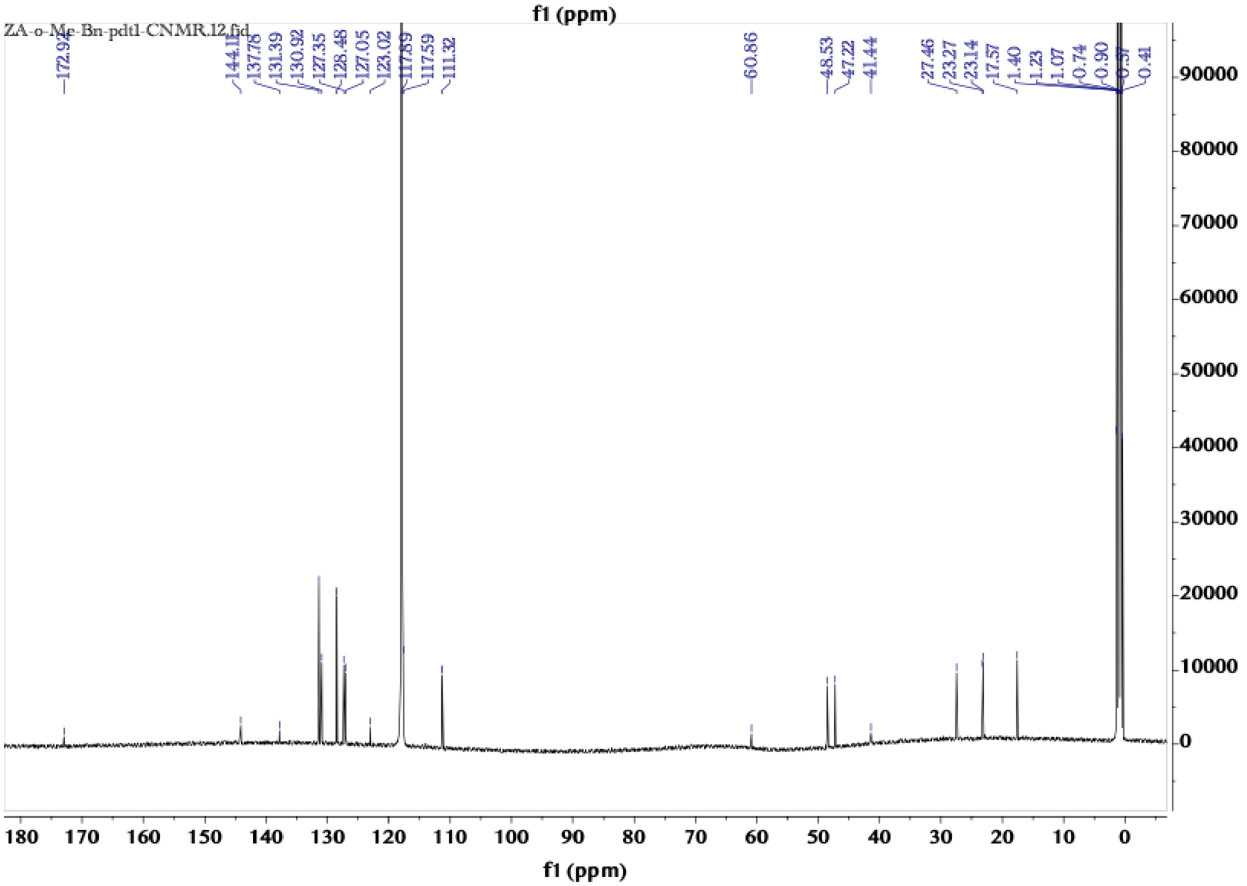
m-F-An-pdt-FNMR.10.fid
-114.31
-114.32
-114.33
-114.34
3800000
3400000
3000000
2600000
2200000
1800000
1400000
1000000
600000
200000
-200000
40 20 0 -20 -40 -60 -80 -100 -120 -140 -160 -180 -200 -220 -240
f1 (ppm)


ZA-o-OMe-An-pdt.10.fid
OMe
H
N
O
N
Me
7.30
7.28
7.27
7.25
7.05
6.91
7.03
6.89
6.82
6.79
6.81
6.71
6.71
6.69
6.69
6.49
6.50
6.48
6.48
4.51
3.81
3.58
3.57
3.56
3.55
3.52
3.53
3.50
3.48
3.50
3.47
3.34
3.31
3.19
3.16
1.74
1.73
1.72
1.71
1.69
1.68
1.67
1.66
1.64
1.33
2.96
1.99
0.96
0.95
0.93
0.96
0.88
4.13
1.01
1.00
4.12
3.15
f1 (ppm)

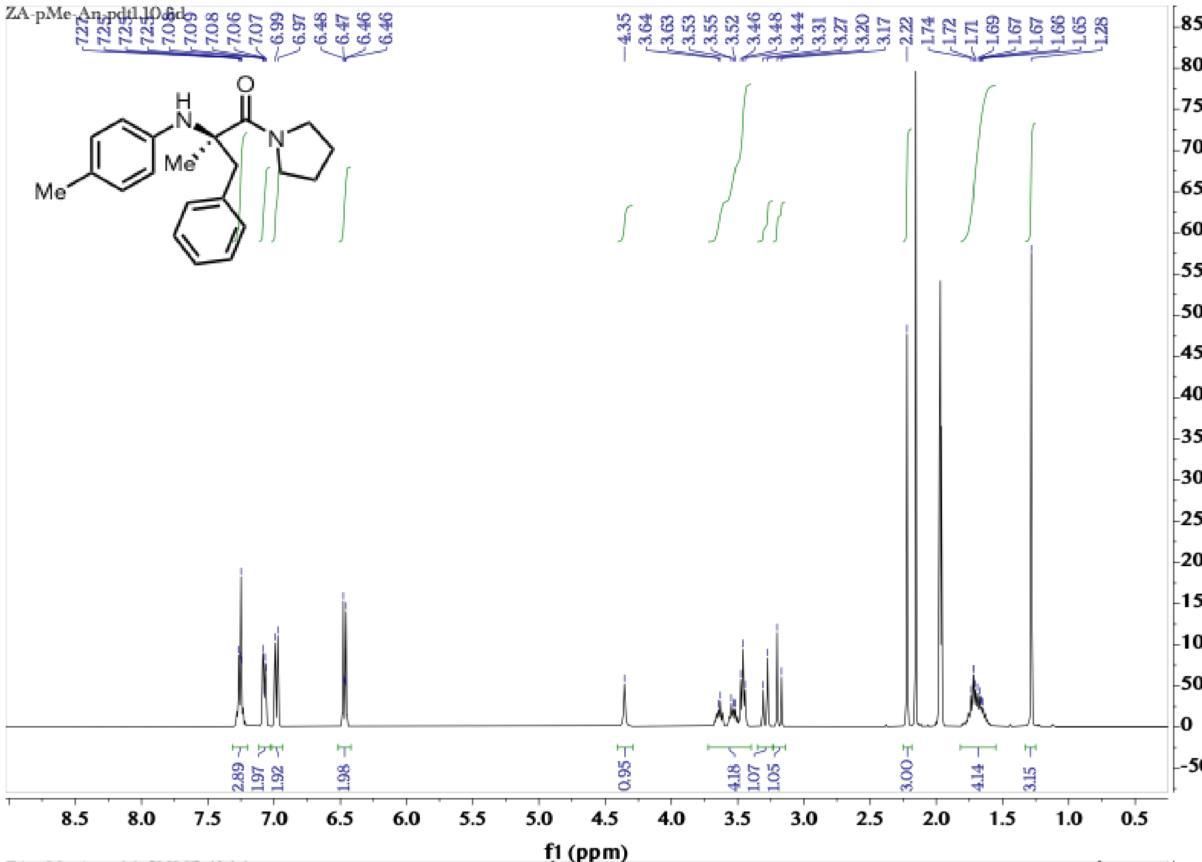
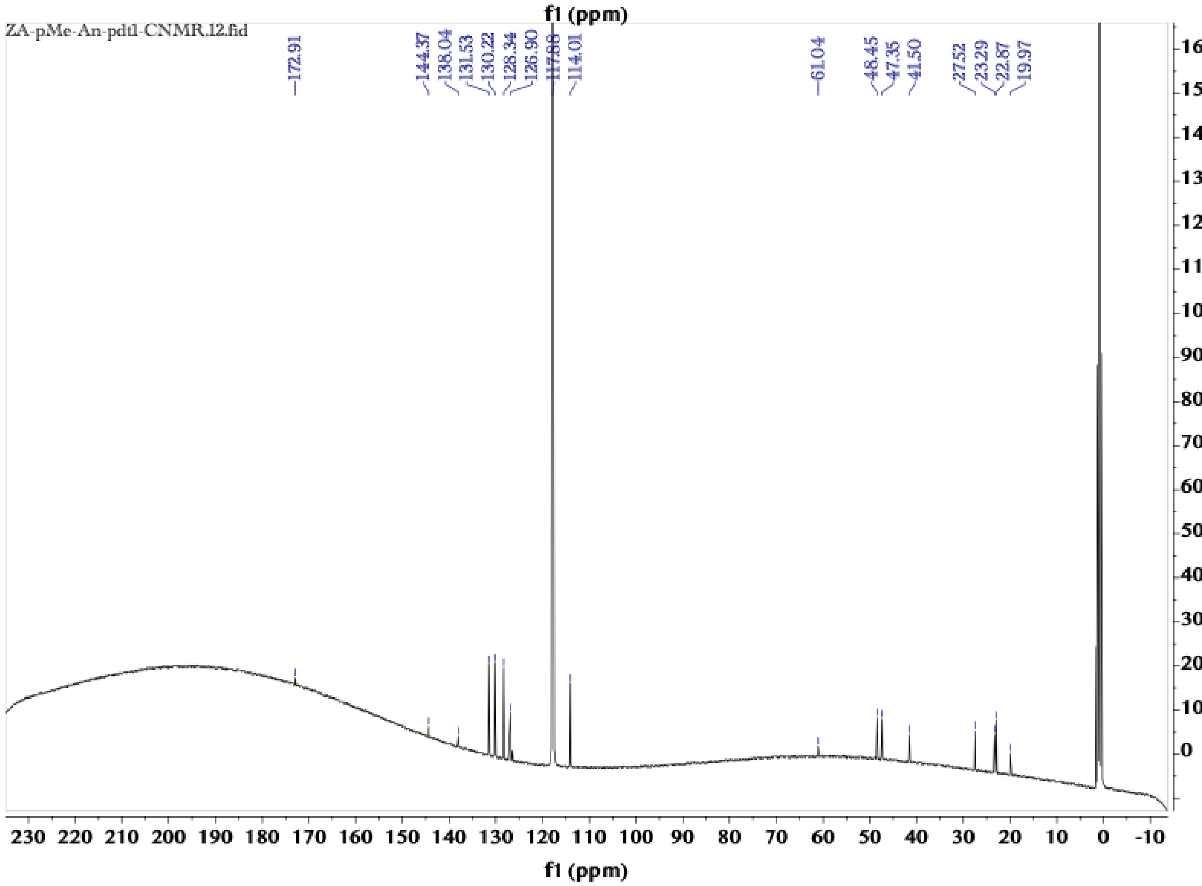

ZA-o-OMe-An-pdt-cnmr.12.fid
172.83
147.49
137.68
135.98
128.44
131.43
127.05
121.62
117.46
117.88
111.39
110.85
60.67
48.51
55.96
47.25
41.60
27.48
23.27
22.91
1.39
1.23
0.90
1.06
0.73
0.57
0.40
f1 (ppm)

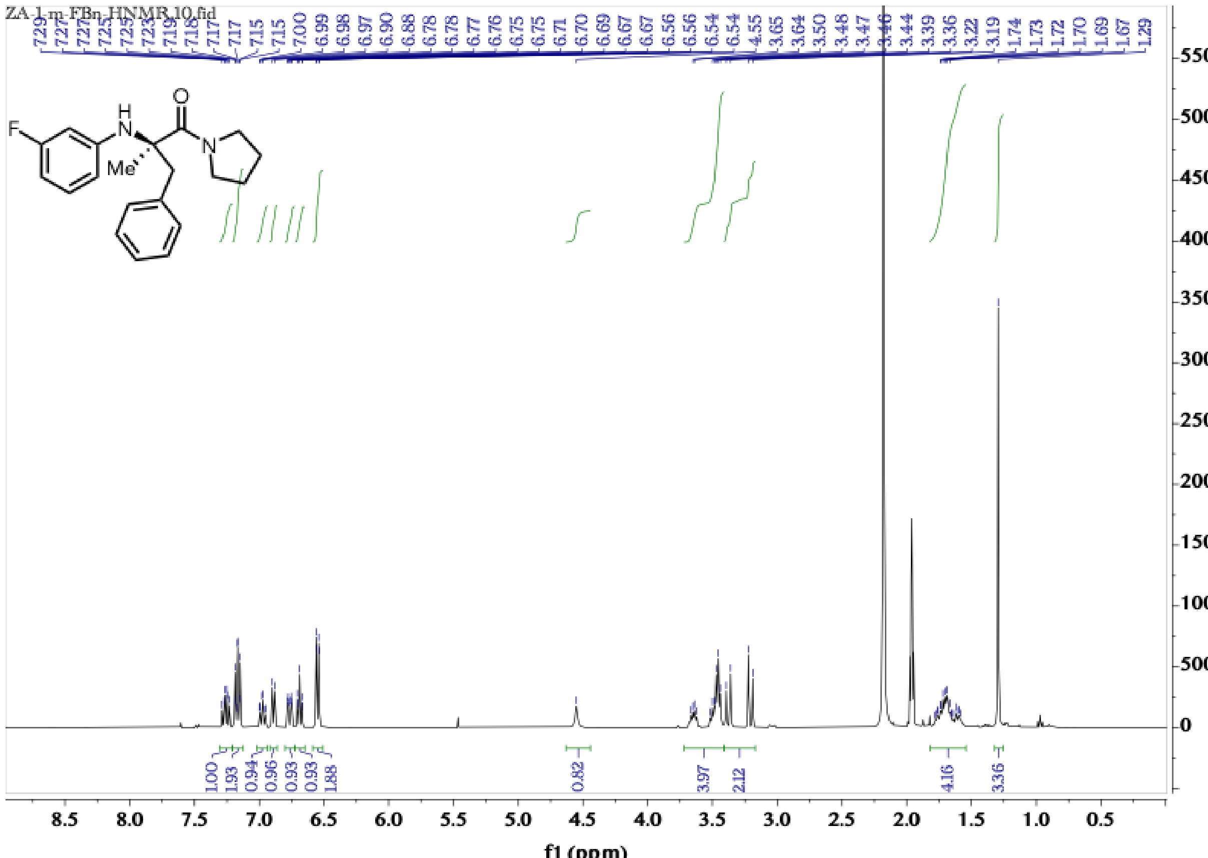


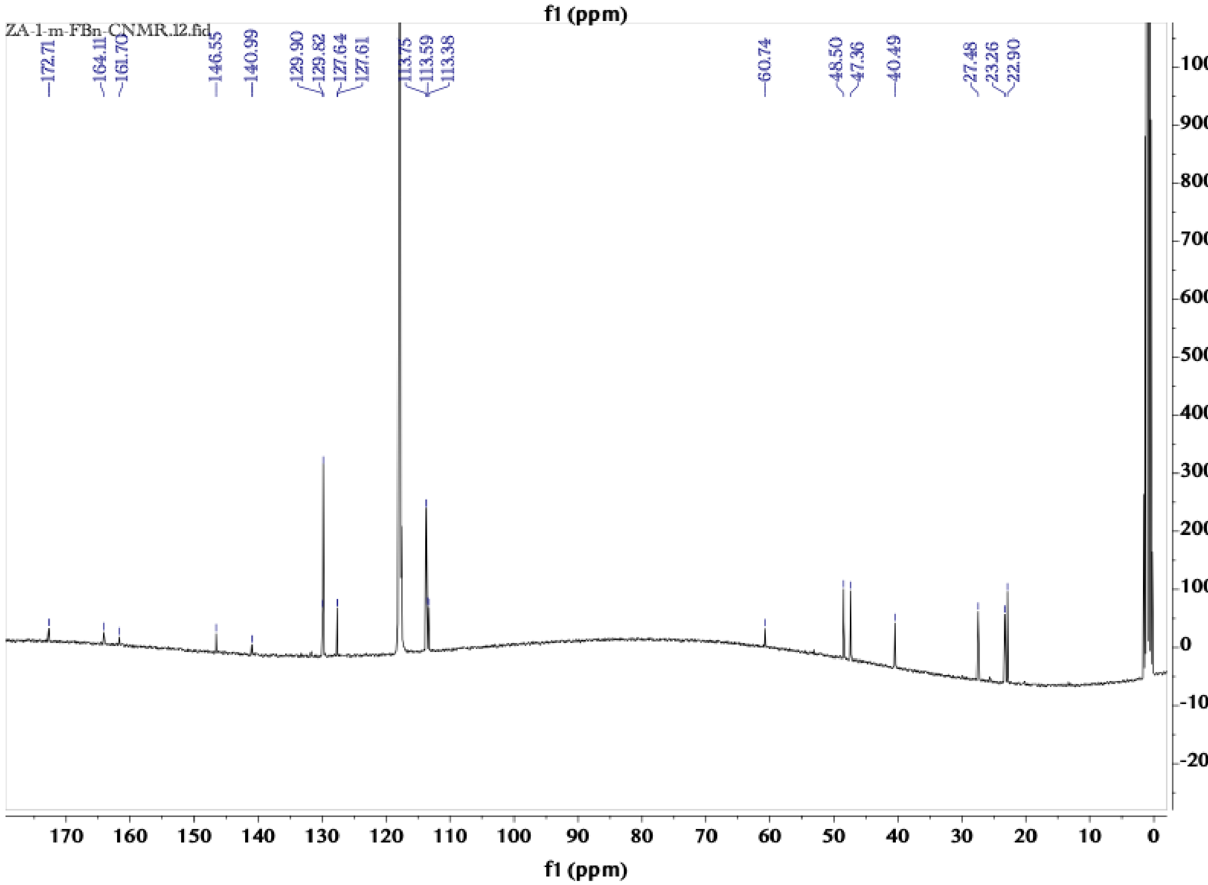
ZA-o-Me-Bn-pdt1.10.fid
Me
H
N
O
N
Me
f1 (ppm)

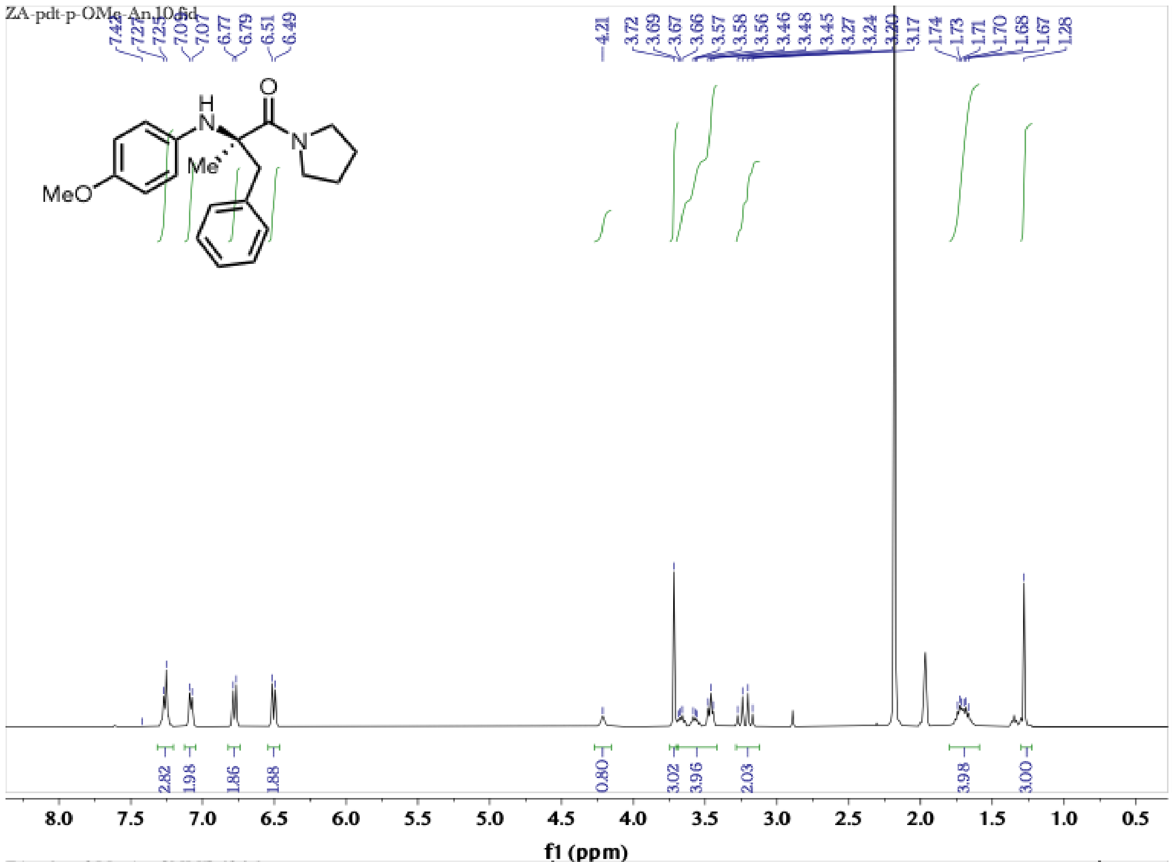


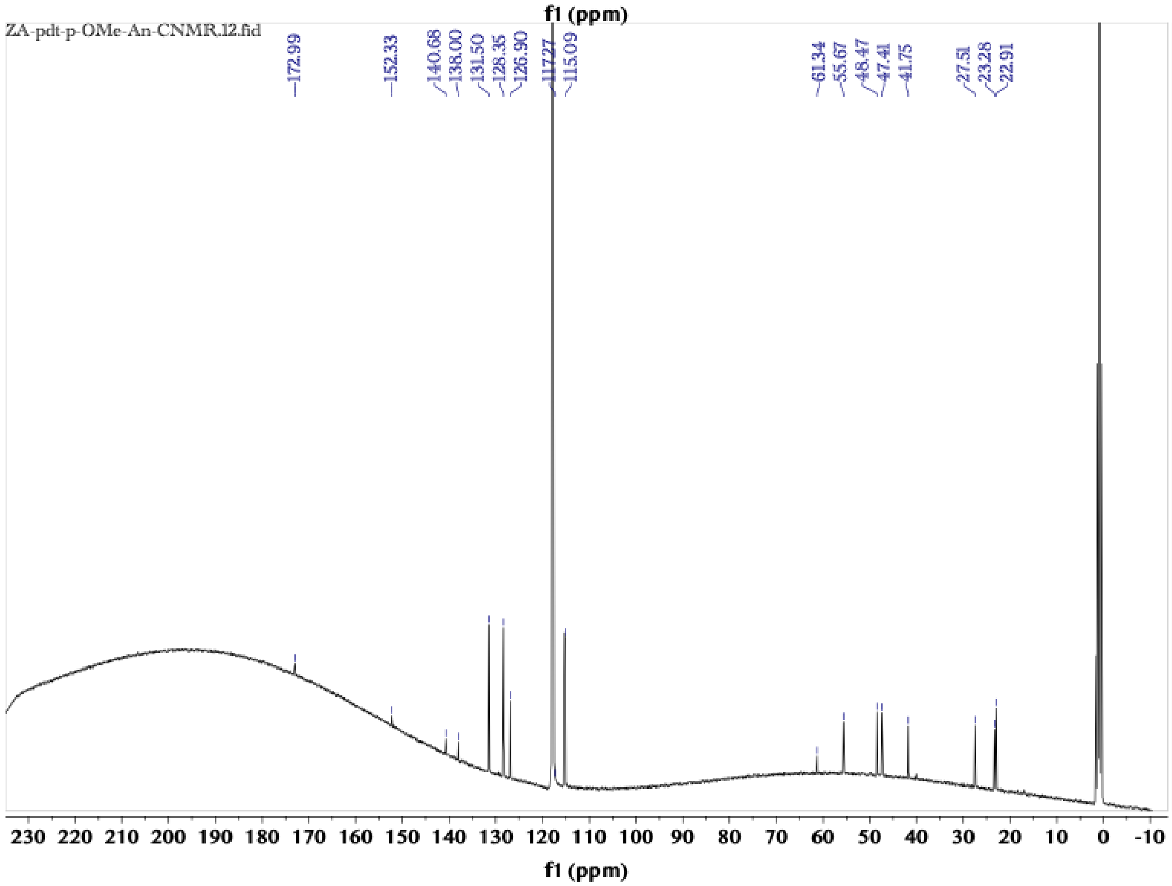
ZA-o-Me-Bn-pdt1-CNMR.12.fid
f1 (ppm)

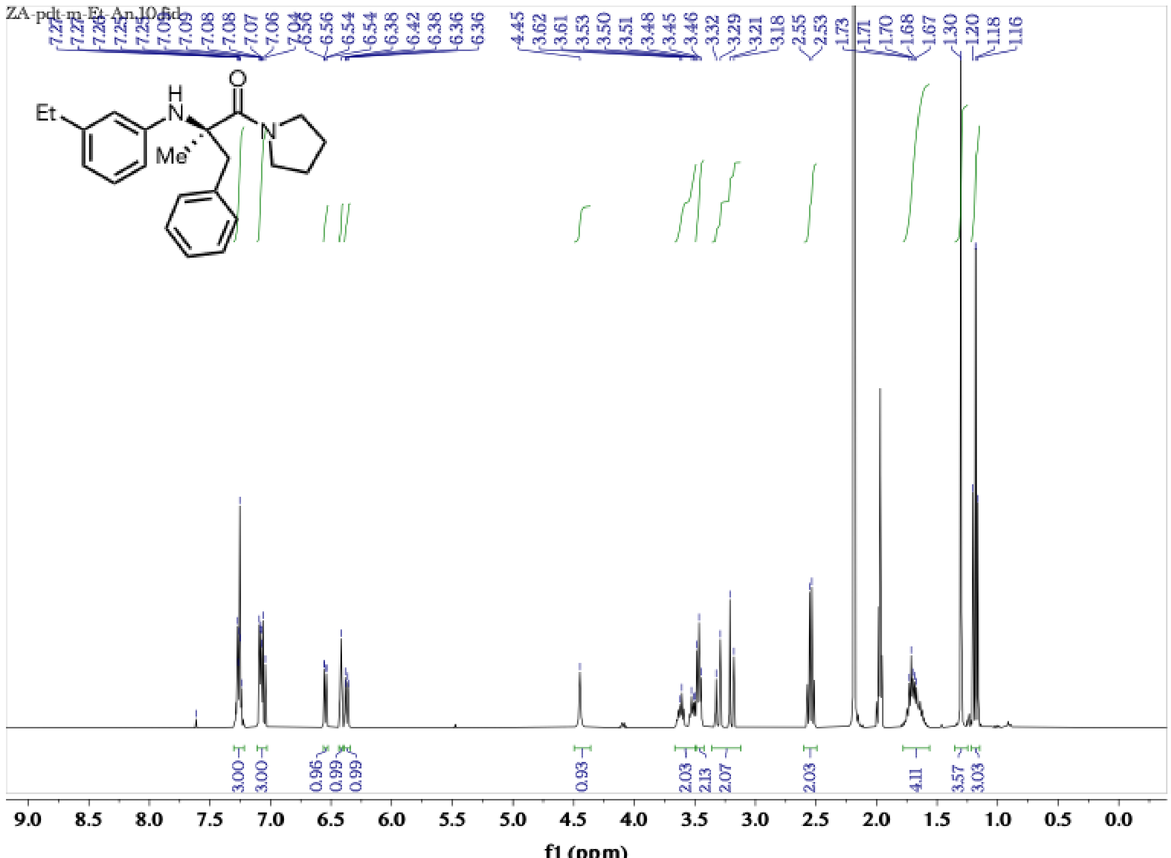

ZA-pMe-An-pdt1.10.fid
7.27 7.25 7.25 7.25 7.08 7.09 7.08 7.06 7.07 6.99 6.97 6.48 6.47 6.46 6.46
4.35 3.64 3.63 3.53 3.55 3.52 3.46 3.48 3.44 3.31 3.27 3.20 3.17 2.22 1.74 1.72 1.71 1.69 1.67 1.67 1.66 1.65 1.28
2.89 1.97 1.92 1.98 0.95 4.18 1.07 1.05 3.00 4.14 3.15
H
N
O
N
Me
Me
f1 (ppm)

ZA-pMe-An-pdt1-CNMR.12.fid
172.91
144.37 138.04 131.53 130.22 128.34 126.90 117.88 114.01
61.04 48.45 47.35 41.50 27.52 23.29 22.87 19.97
f1 (ppm)

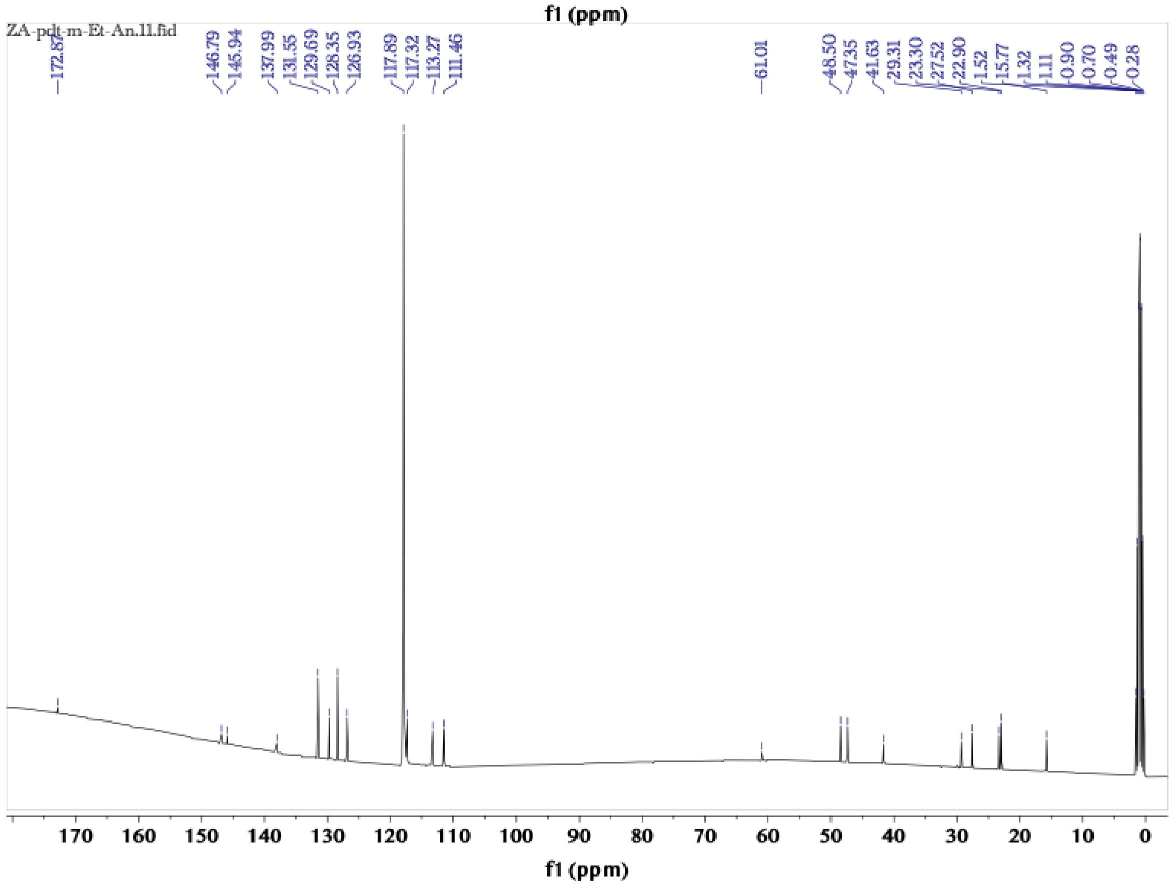

ZA-1-m-FBn-HNMR.10.fid
7.29 7.27 7.27 7.25 7.23 7.23 7.19 7.18 7.17 7.17 7.15 7.15 7.00 6.99 6.98 6.97 6.90 6.88 6.78 6.78 6.77 6.76 6.75 6.75 6.71 6.70 6.69 6.67 6.67 6.56 6.56 6.54 6.54 4.55 3.65 3.64 3.50 3.48 3.47 3.46 3.44 3.39 3.36 3.22 3.19 1.74 1.73 1.72 1.70 1.69 1.67 1.29
F
H
N
O
N
Me
1.00 1.93 0.94 0.96 0.93 0.93 1.88 0.82 3.97 2.12 4.16 3.36
f1 (ppm)

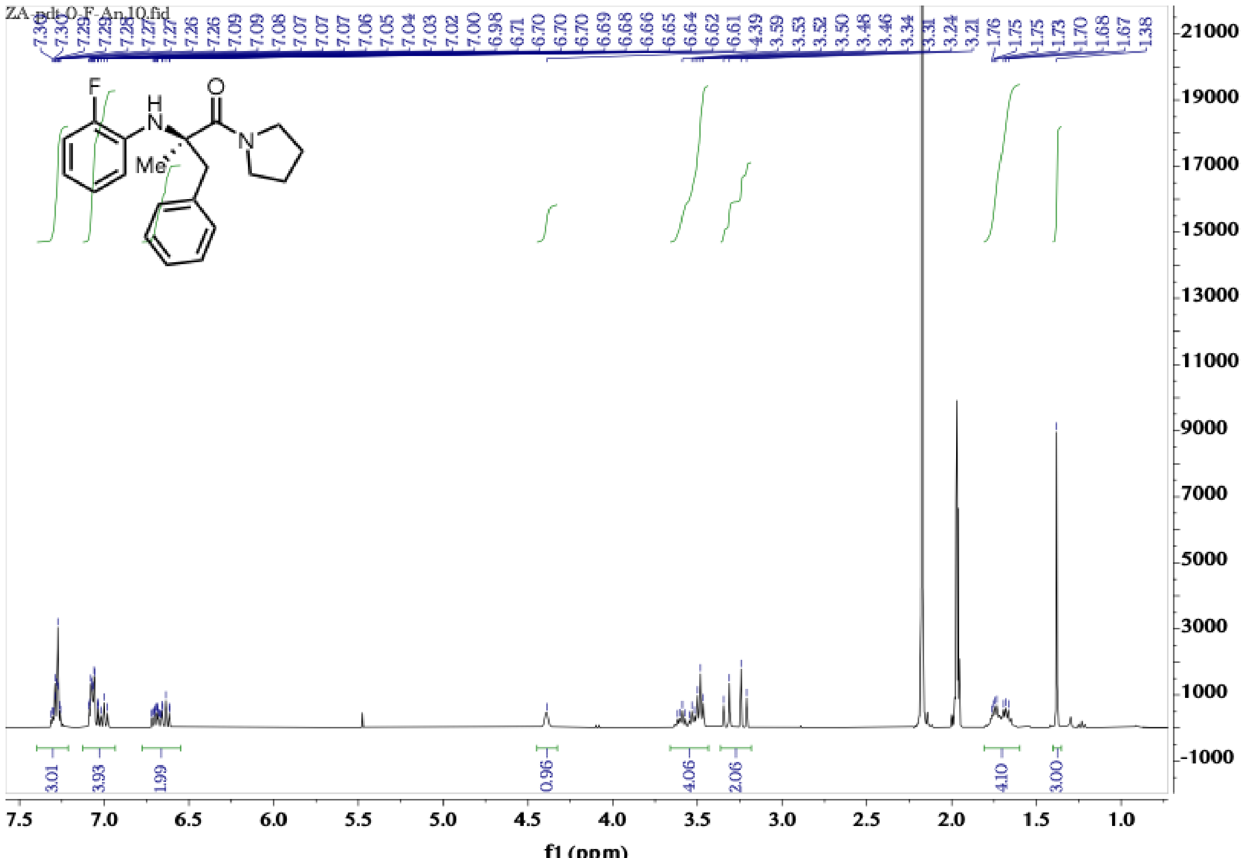

ZA-1-m-FBn-CNMR.12.fid
172.71 164.11 161.70 146.55 140.99 129.90 129.82 127.64 127.61 113.75 113.59 113.38 60.74 48.50 47.36 40.49 27.48 23.26 22.90
f1 (ppm)

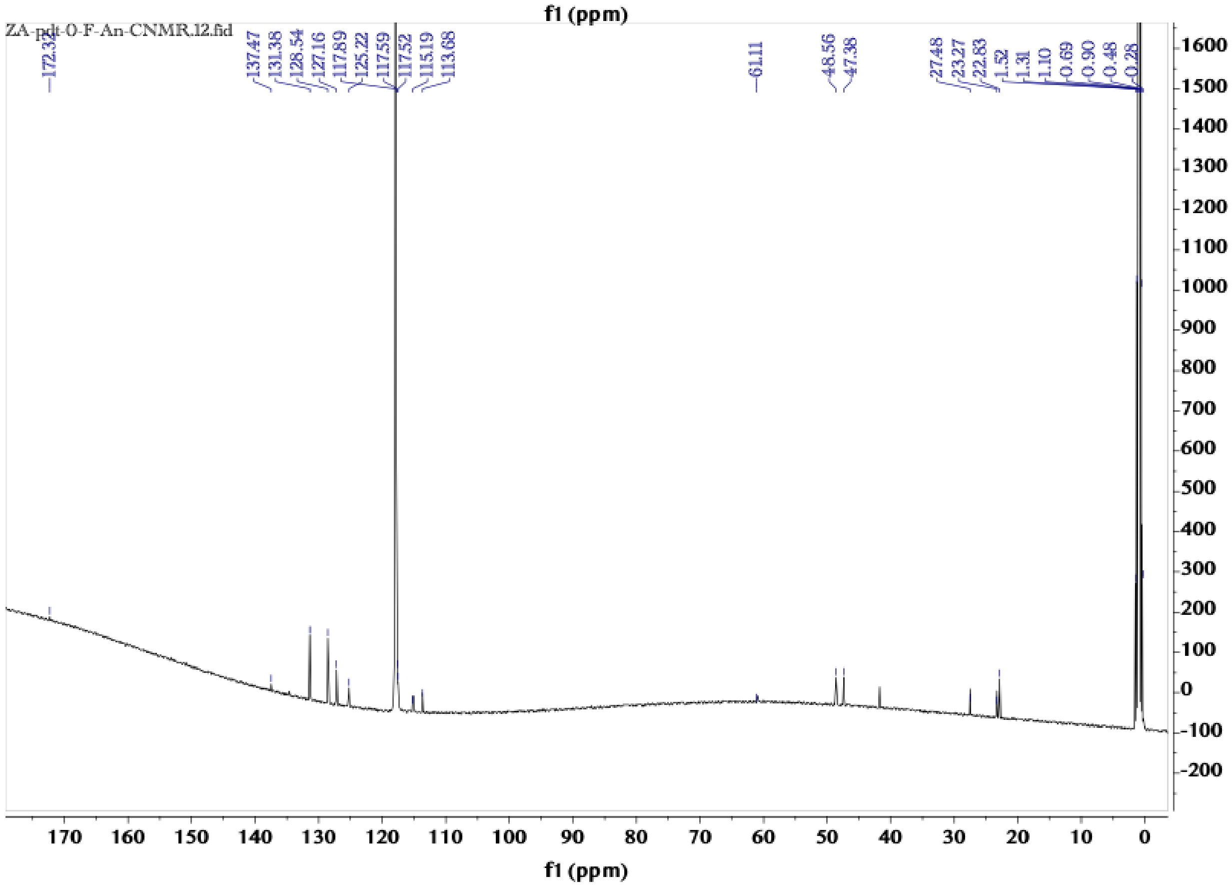

ZA-pdt-p-OMe-An.10.fid
7.42
7.27
7.25
7.09
7.07
6.77
6.79
6.51
6.49
4.21
3.72
3.69
3.67
3.66
3.57
3.58
3.56
3.46
3.48
3.45
3.27
3.24
3.20
3.17
1.74
1.73
1.71
1.70
1.68
1.67
1.28
H
N
O
N
Me
MeO
2.82
1.98
1.86
1.88
0.80
3.02
3.96
2.03
3.98
3.00
f1 (ppm)

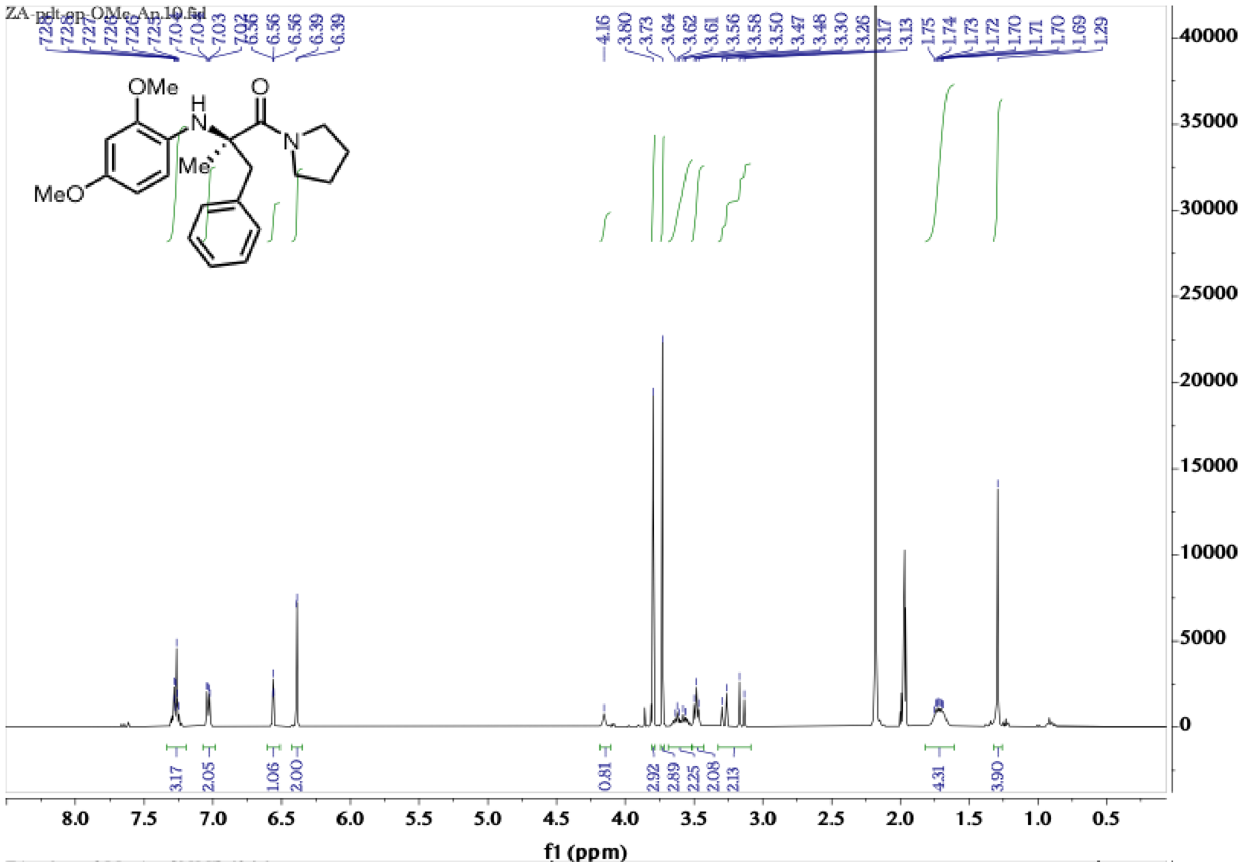
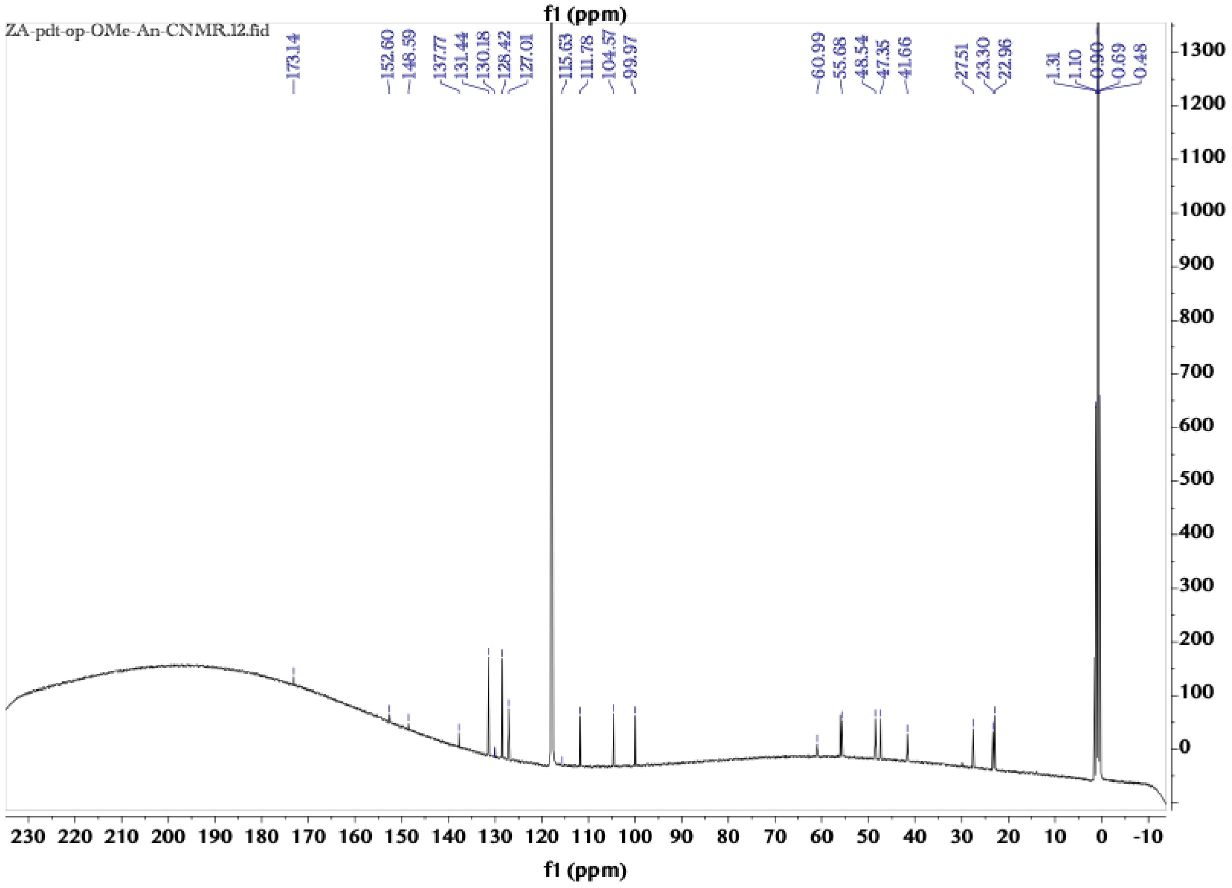

ZA-pdt-p-OMe-An-CNMR.12.fid
172.99
152.33
140.68
138.00
131.50
128.35
126.90
117.27
115.09
61.34
55.67
48.47
47.41
41.75
27.51
23.28
22.91
f1 (ppm)

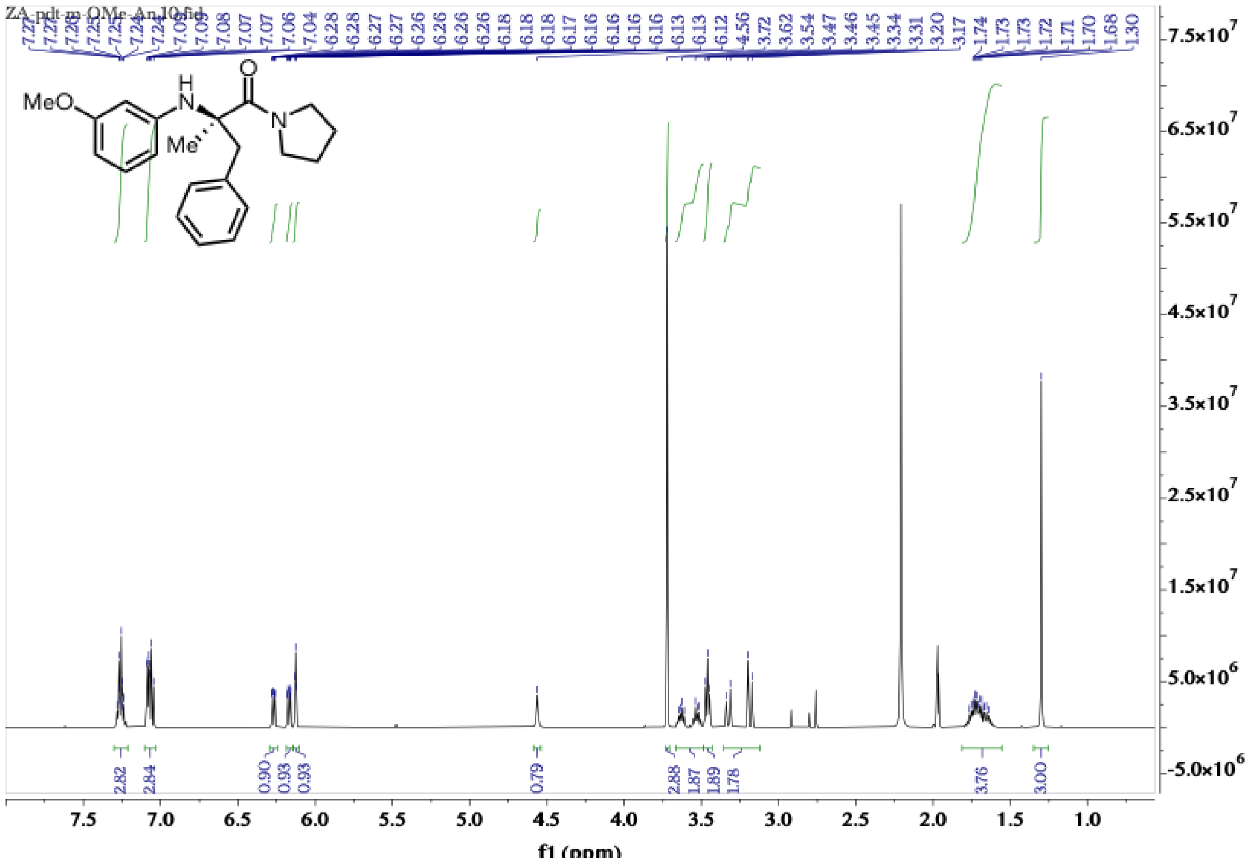

ZA-pdt-m-Et-An.10.fid
Et
H
N
O
N
Me
7.27 7.27 7.26 7.25 7.25 7.09 7.09 7.08 7.08 7.07 7.06 7.04 6.56 6.56 6.54 6.54 6.38 6.42 6.38 6.38 6.36 6.36
4.45 3.62 3.61 3.53 3.50 3.51 3.48 3.45 3.46 3.32 3.29 3.21 3.18 2.55 2.53 1.73 1.71 1.70 1.68 1.67 1.30 1.20 1.18 1.16
3.00 3.00 0.96 0.99 0.99 0.93 2.03 2.13 2.07 2.03 4.11 3.57 3.03
f1 (ppm)

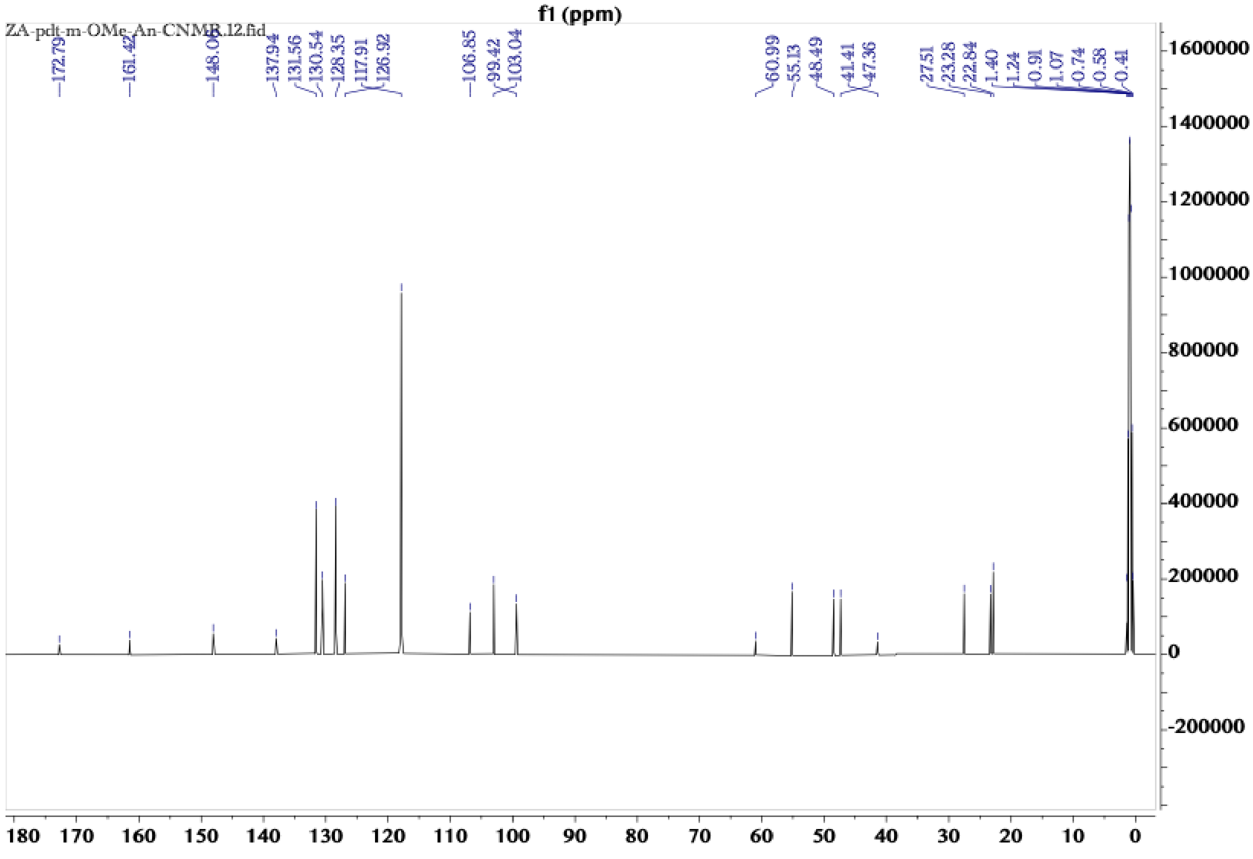

ZA-pdt-m-Et-An.11.fid
172.87 146.79 145.94 137.99 131.55 129.69 128.35 126.93 117.89 117.32 113.27 111.46 61.01 48.50 47.35 41.63 29.31 23.30 27.52 22.90 1.52 15.77 1.32 1.11 0.90 0.70 0.49 0.28
f1 (ppm)

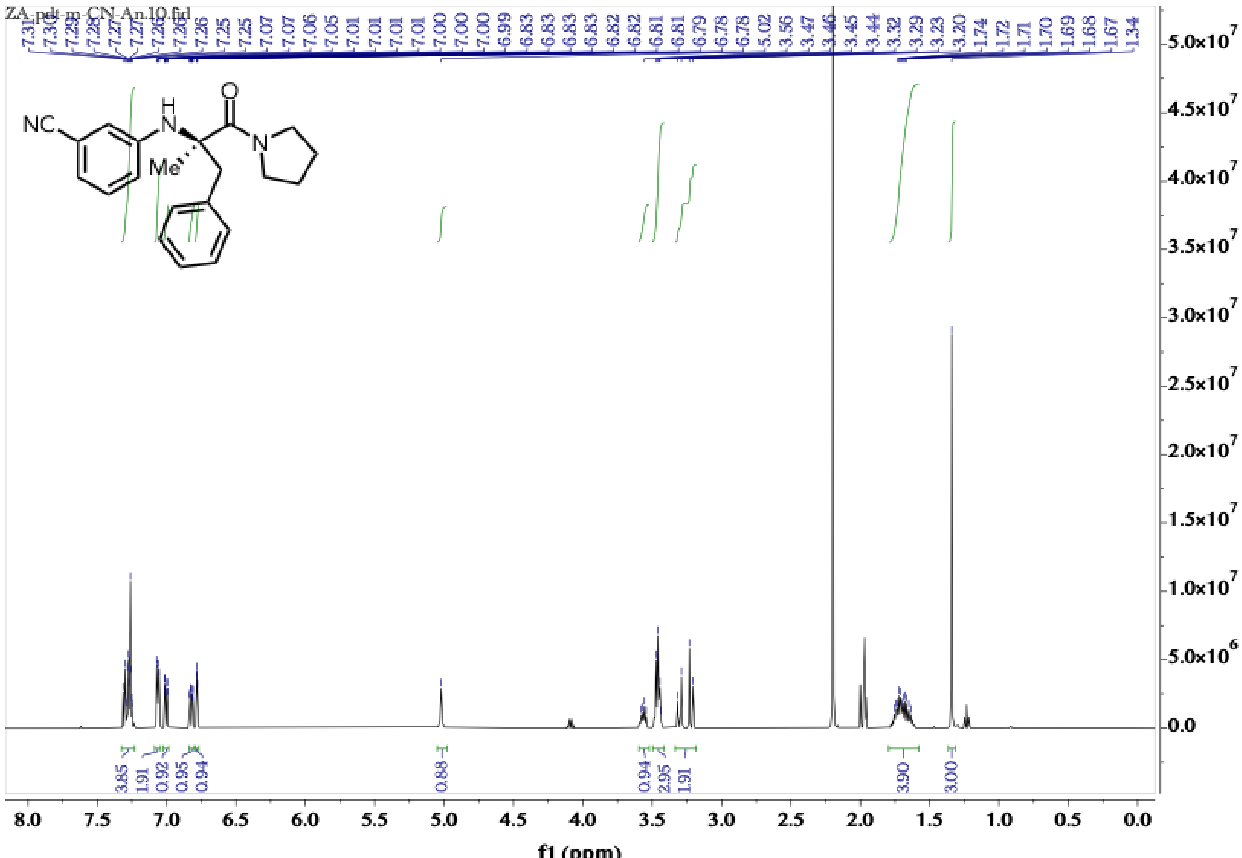

ZA-pdt-O-F-An.10.fid
7.30 7.30 7.29 7.29 7.28 7.27 7.27 7.26 7.09 7.09 7.08 7.07 7.07 7.07 7.06 7.05 7.04 7.03 7.02 7.00 6.98 6.71 6.70 6.70 6.70 6.69 6.68 6.66 6.65 6.64 6.62 6.61 4.39 3.59 3.53 3.52 3.50 3.48 3.46 3.34 3.31 3.24 3.21 1.76 1.75 1.75 1.73 1.70 1.68 1.67 1.38
F
H
N
O
N
Me
3.01
3.93
1.99
0.96
4.06
2.06
4.10
3.00
f1 (ppm)

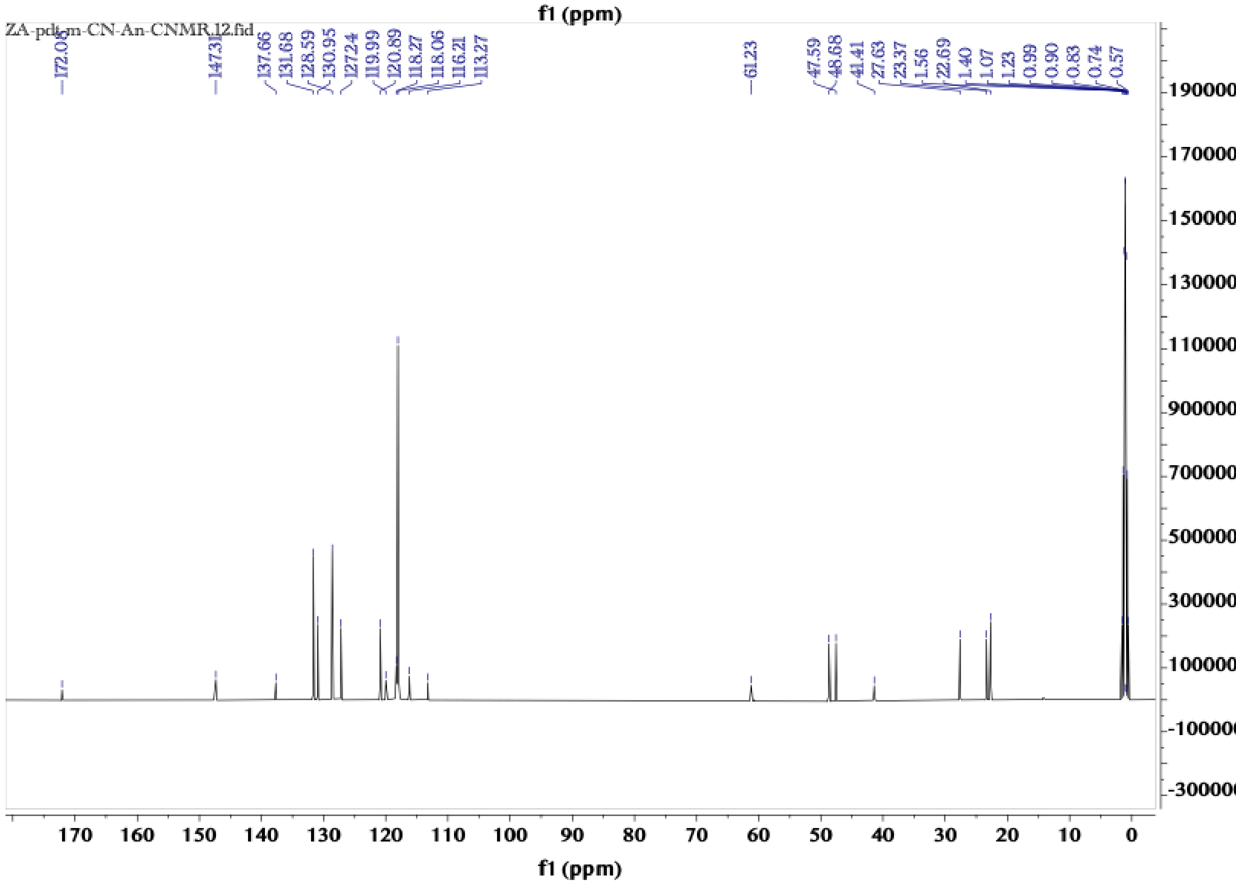

ZA-pdt-O-F-An-CNMR.12.fid
172.32
137.47 131.38 128.54 127.16 117.89 125.22 117.59 117.52 115.19 113.68
61.11
48.56 47.38
27.48 23.27 22.83 1.52 1.31 1.10 0.69 0.90 0.48 0.26
f1 (ppm)

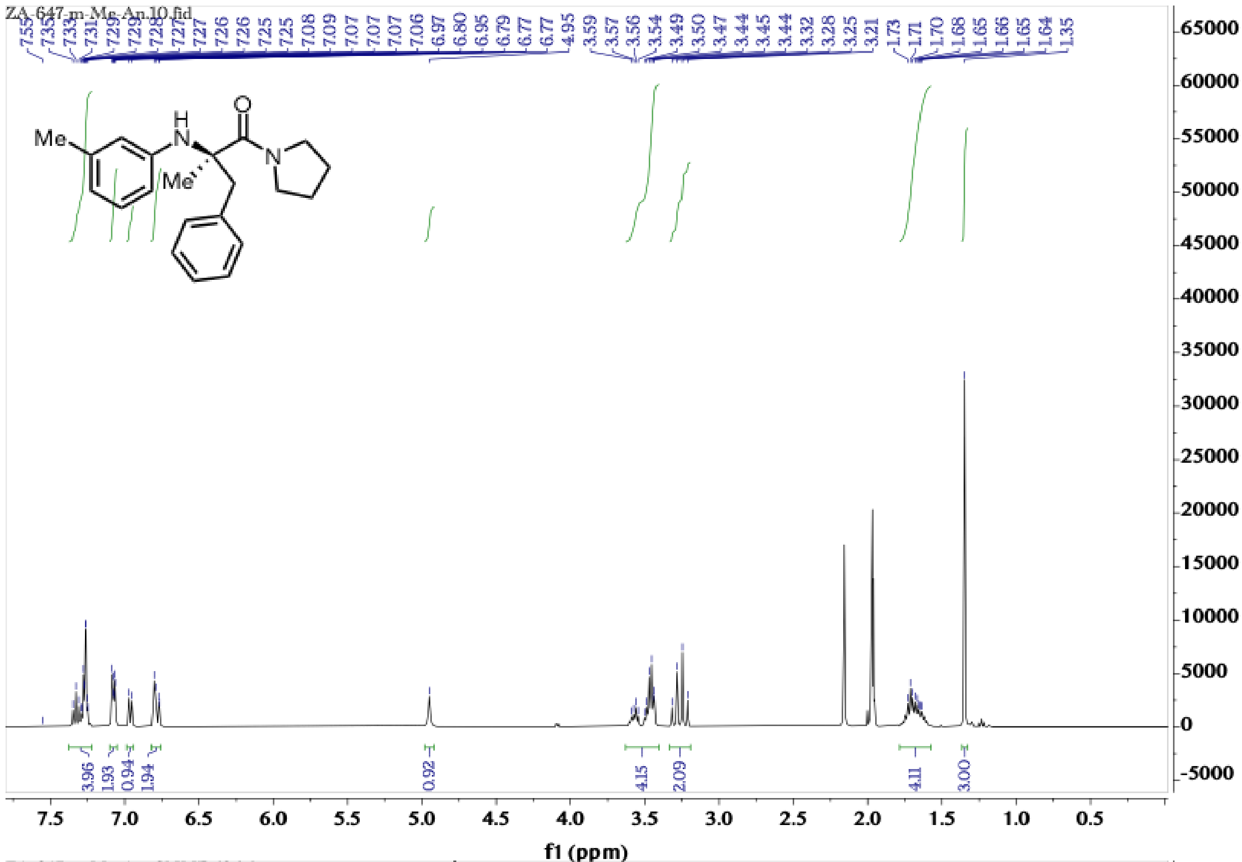

ZA-pdt-op-OMe-An.10.fid
7.28 7.28 7.27 7.26 7.26 7.25 7.04 7.04 7.03 7.02 6.56 6.56 6.56 6.39 6.39
4.16 3.80 3.73 3.64 3.62 3.61 3.56 3.58 3.50 3.47 3.48 3.30 3.26 3.17 3.13 1.75 1.74 1.73 1.72 1.70 1.71 1.70 1.69 1.29
OMe
H
N
O
N
Me
MeO
3.17 2.05 1.06 2.00 0.81 2.92 2.89 2.25 2.08 2.13 4.31 3.90
f1 (ppm)

ZA-pdt-op-OMe-An-CNMR.12.fid
173.14 152.60 148.59 137.77 131.44 130.18 128.42 127.01 115.63 111.78 104.57 99.97 60.99 55.68 48.54 47.35 41.66 27.51 23.30 22.96 1.31 1.10 0.90 0.69 0.48
f1 (ppm)

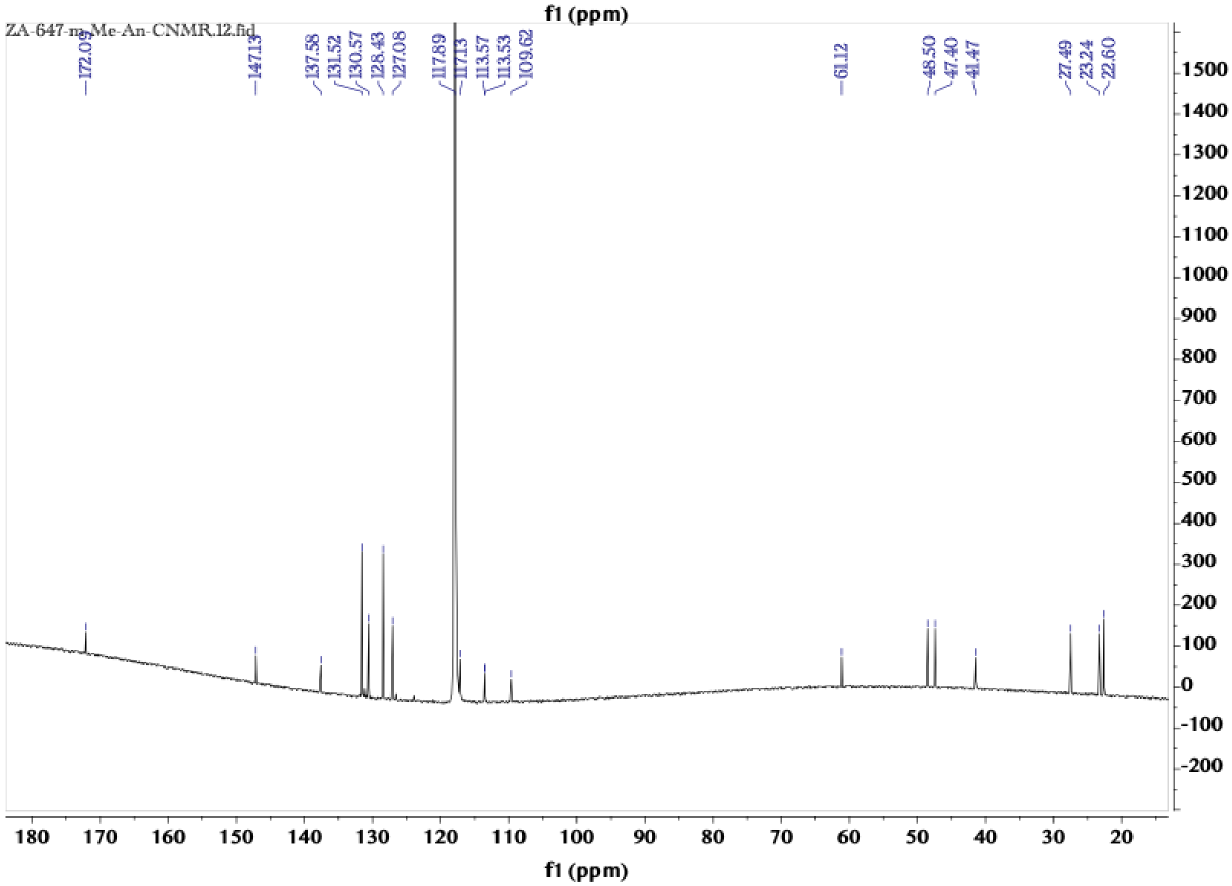

ZA-pdt-m-OMe-An.10.fid
MeO
H
N
O
N
Me
f1 (ppm)

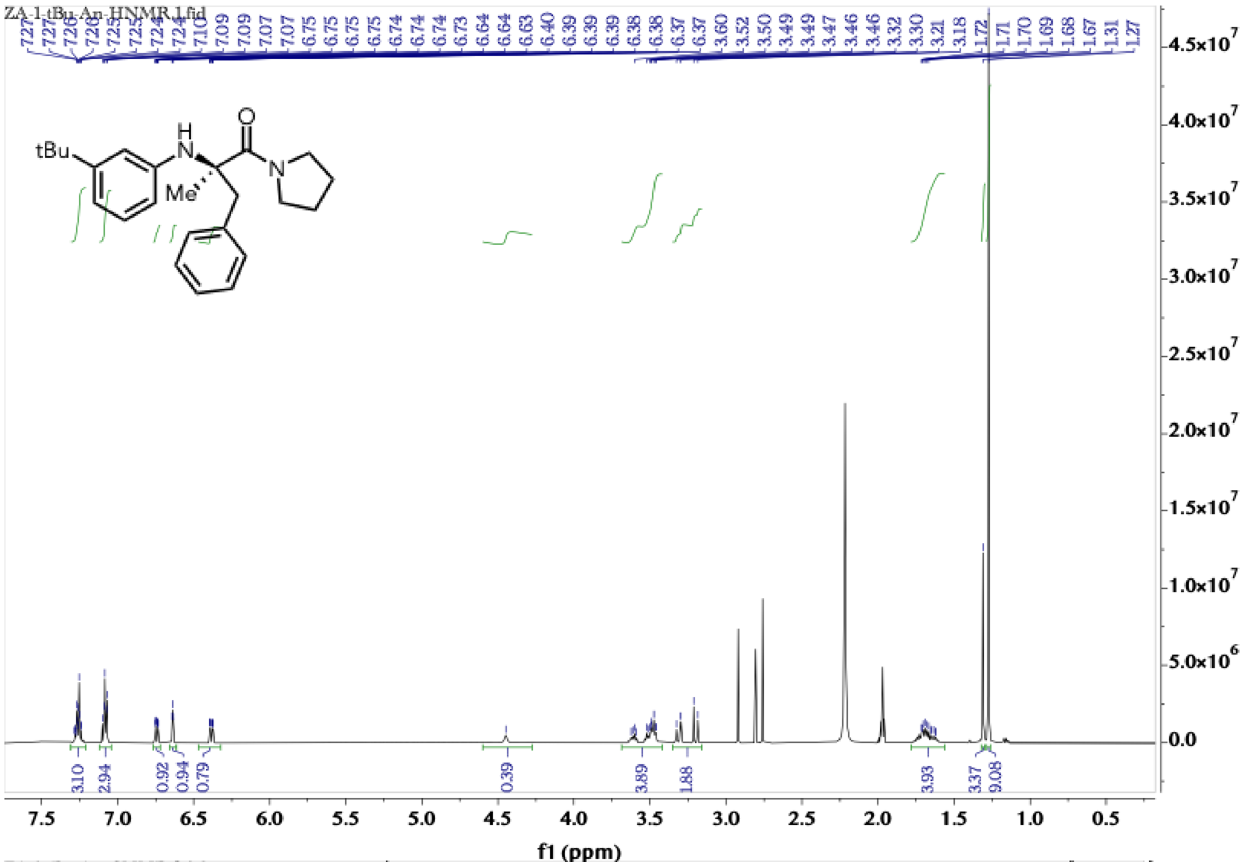

ZA-pdt-m-OMe-An-CNMR.12.fid
f1 (ppm)


ZA-pdt-m-CN-An.10.fid
NC
H
N
O
N
Me
3.85
1.91
0.92
0.95
0.94
0.88
0.94
2.95
1.91
3.90
3.00
f1 (ppm)

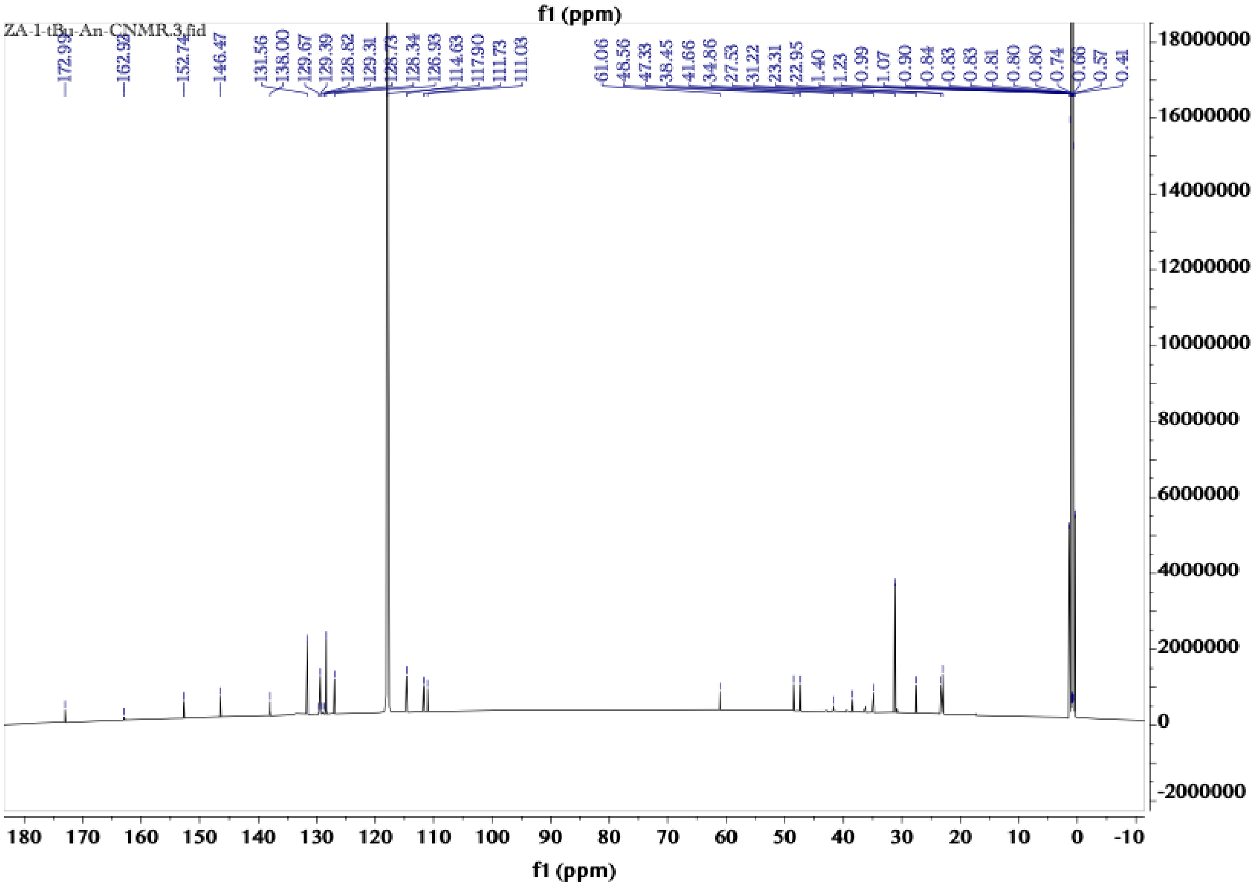

ZA-pdt-m-CN-An-CNMR.12.fid
172.06
147.31
137.66
131.68
128.59
130.95
127.24
119.99
120.89
118.27
118.06
116.21
113.27
61.23
47.59
48.68
41.41
27.63
23.37
22.69
f1 (ppm)


ZA-647-m-Me-An.10.fid
Me
N
H
O
N
Me
f1 (ppm)

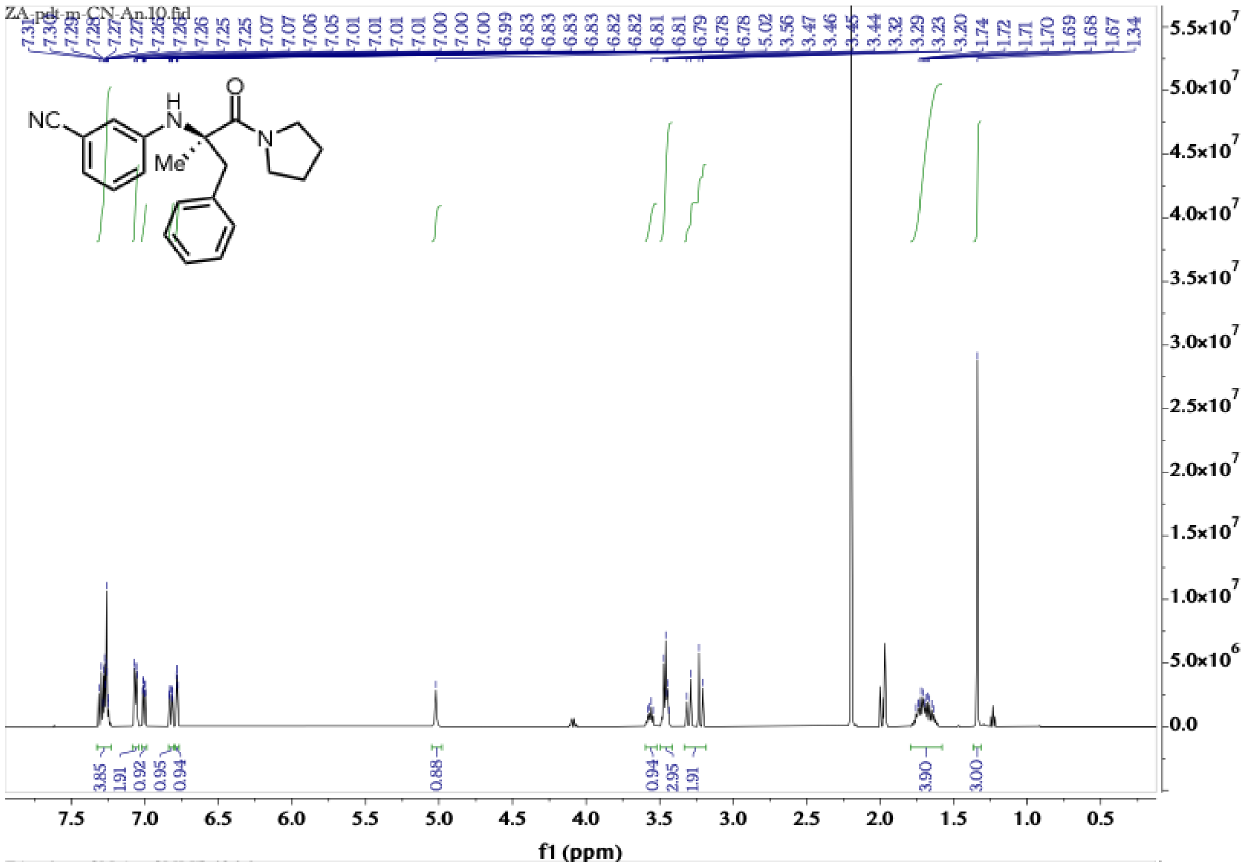

ZA-647-m-Me-An-CNMR.12.fid
f1 (ppm)



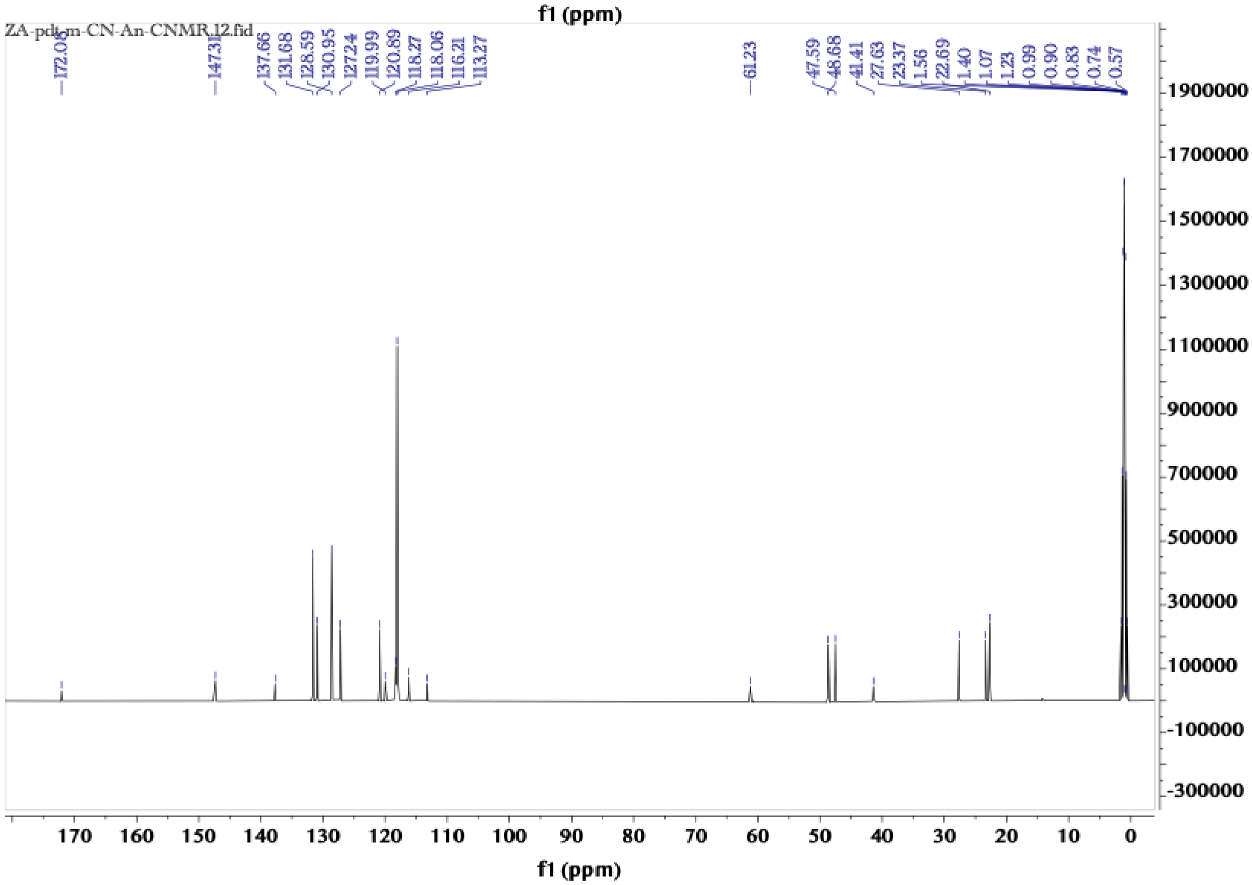
ZA-1-tBu-An-HNMR.1.fid
tBu
H
N
O
N
Me
3.10
2.94
0.92
0.94
0.79
0.39
3.89
1.88
3.93
3.37
9.08
f1 (ppm)



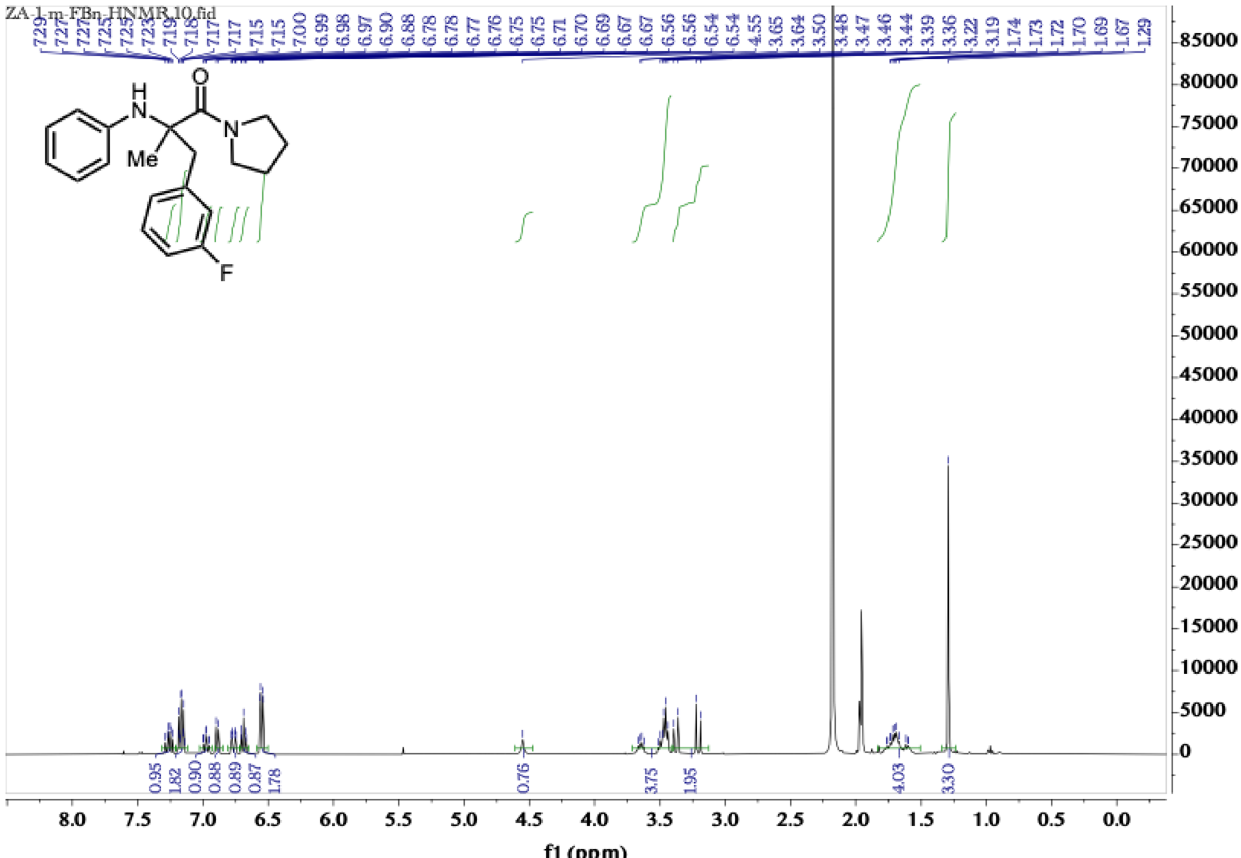
ZA-1-tBu-An-CNMR.3.fid
172.99
162.93
152.74
146.47
131.56
138.00
129.67
129.39
128.82
129.31
128.73
128.34
126.93
114.63
117.90
111.73
111.03
61.06
48.56
47.33
38.45
41.66
34.86
27.53
31.22
23.31
22.95
f1 (ppm)

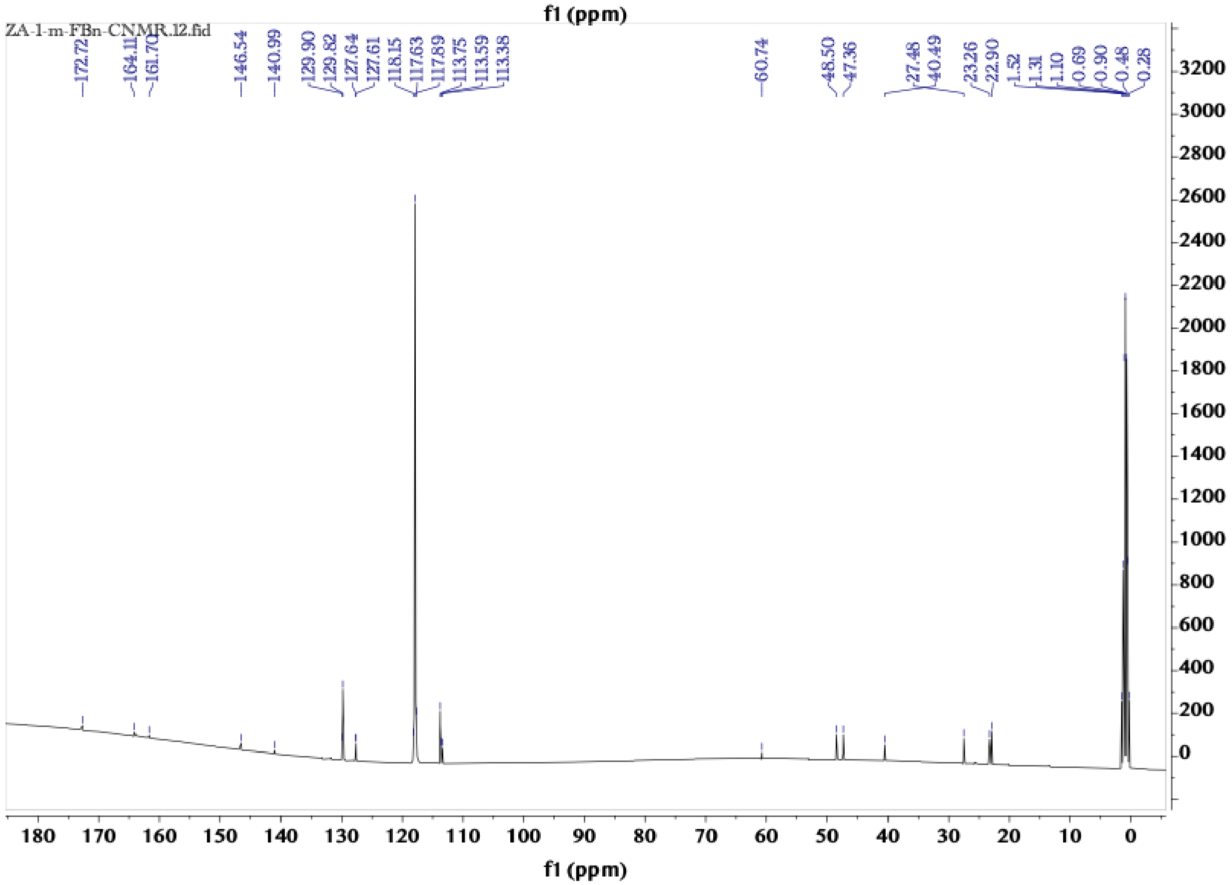


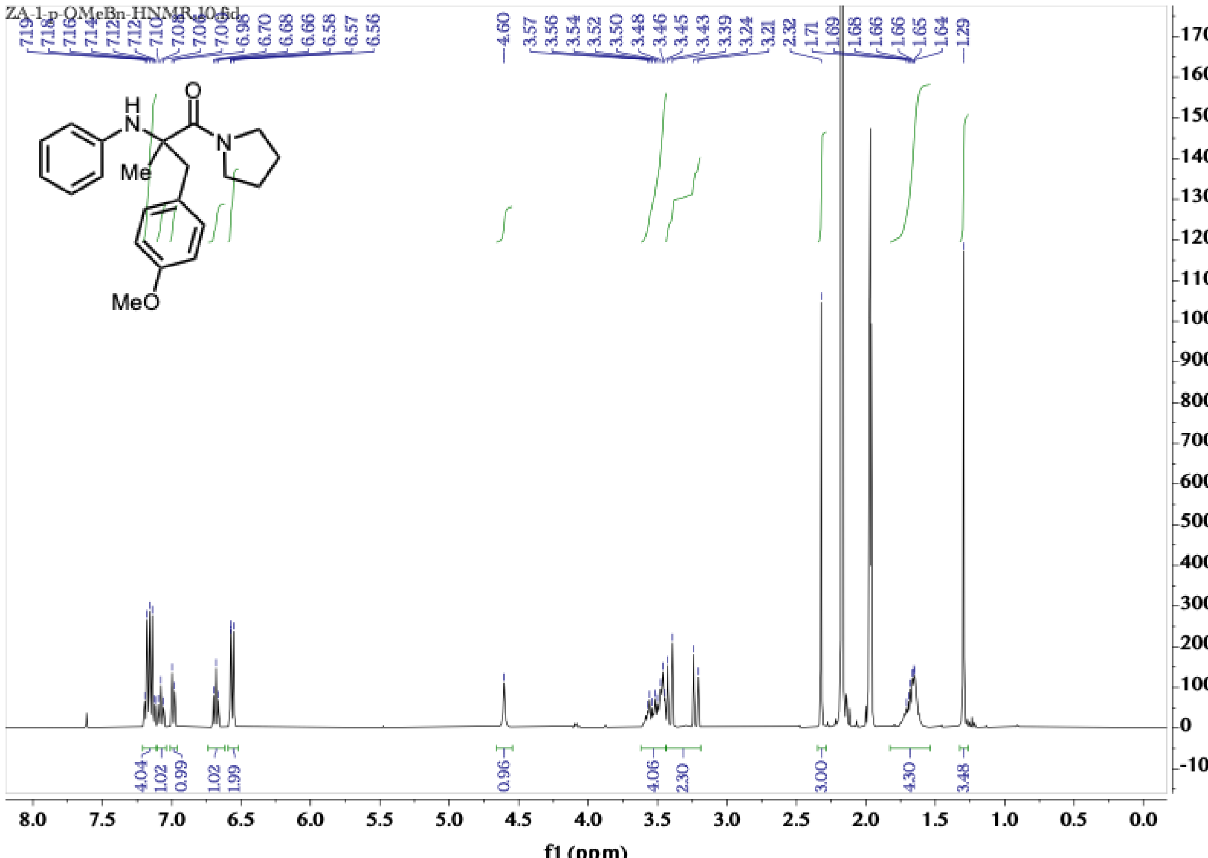
ZA-pdt-m-CN-An.10.fid
f1 (ppm)

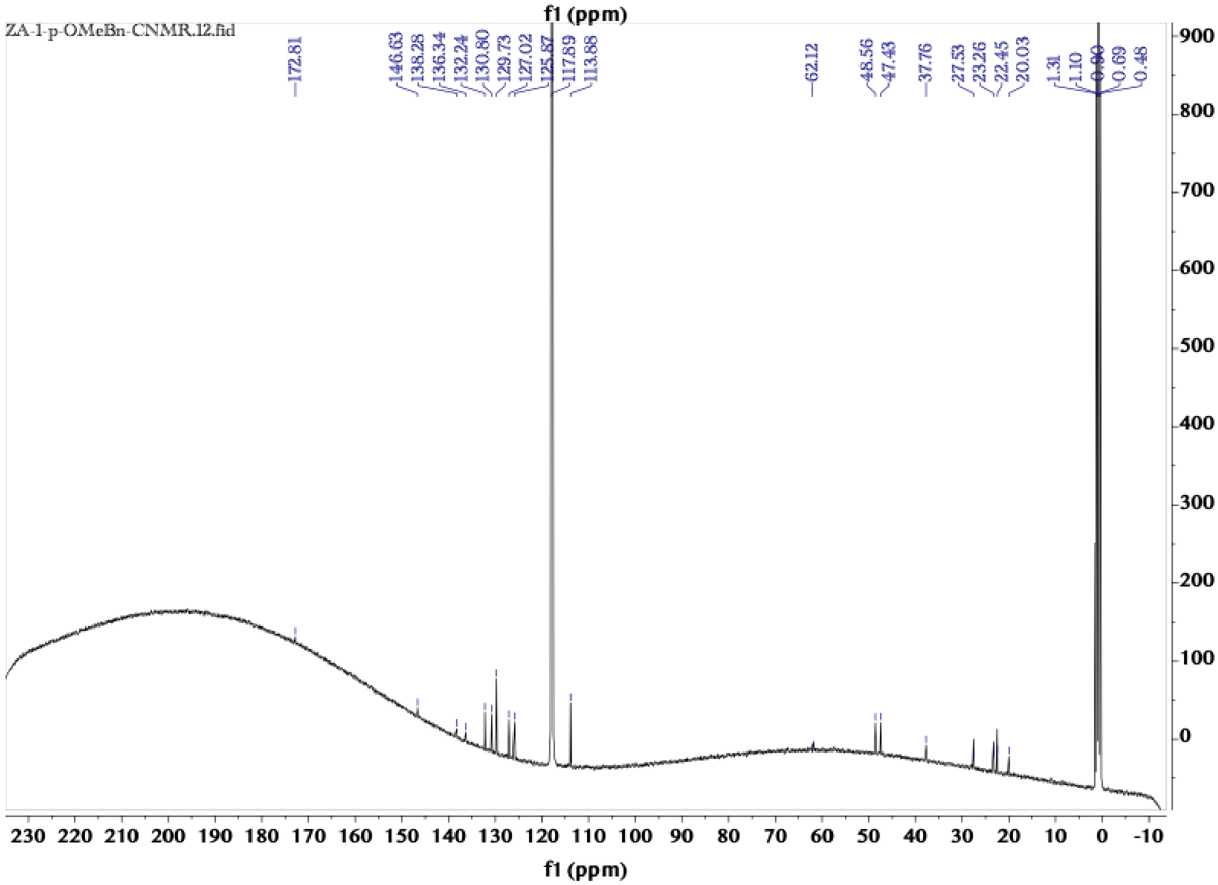


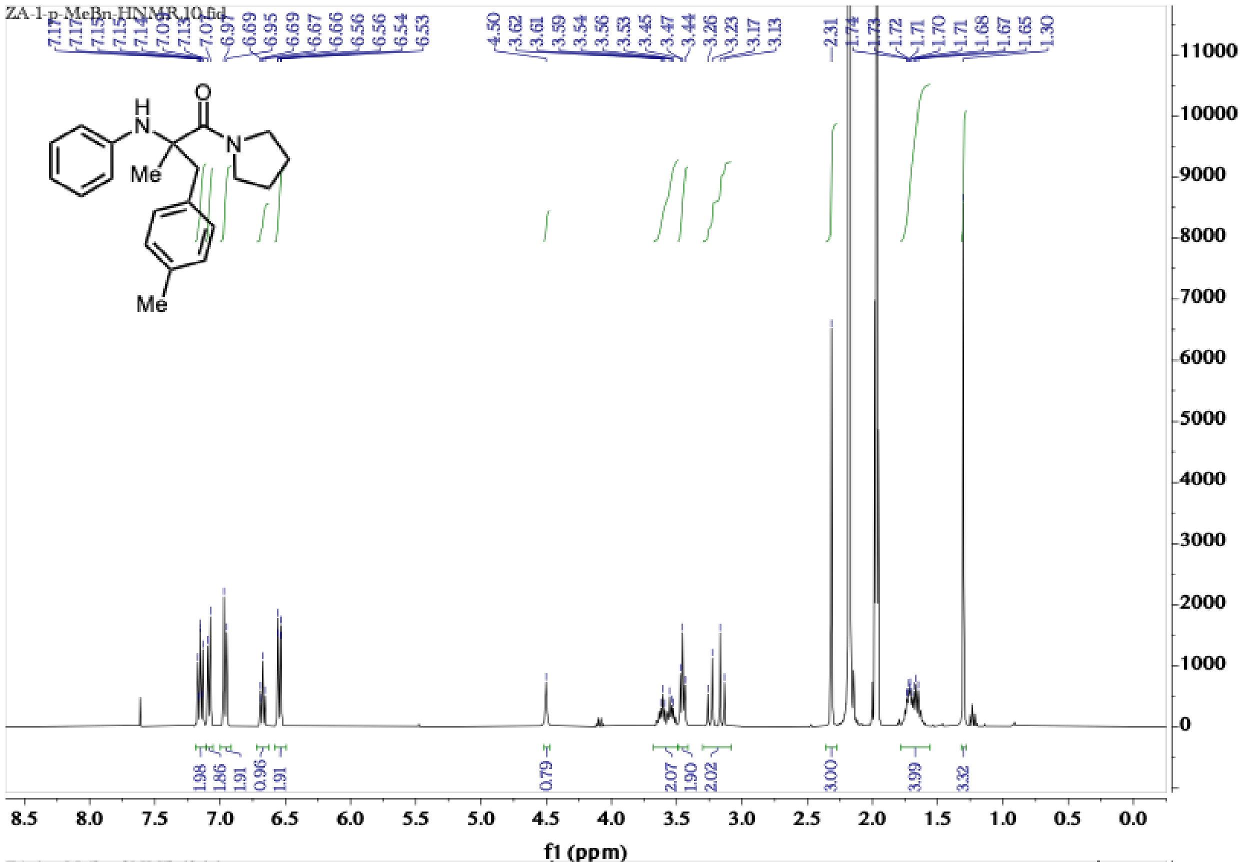
ZA-pdt-m-CN-An-CNMR.12.fid
f1 (ppm)

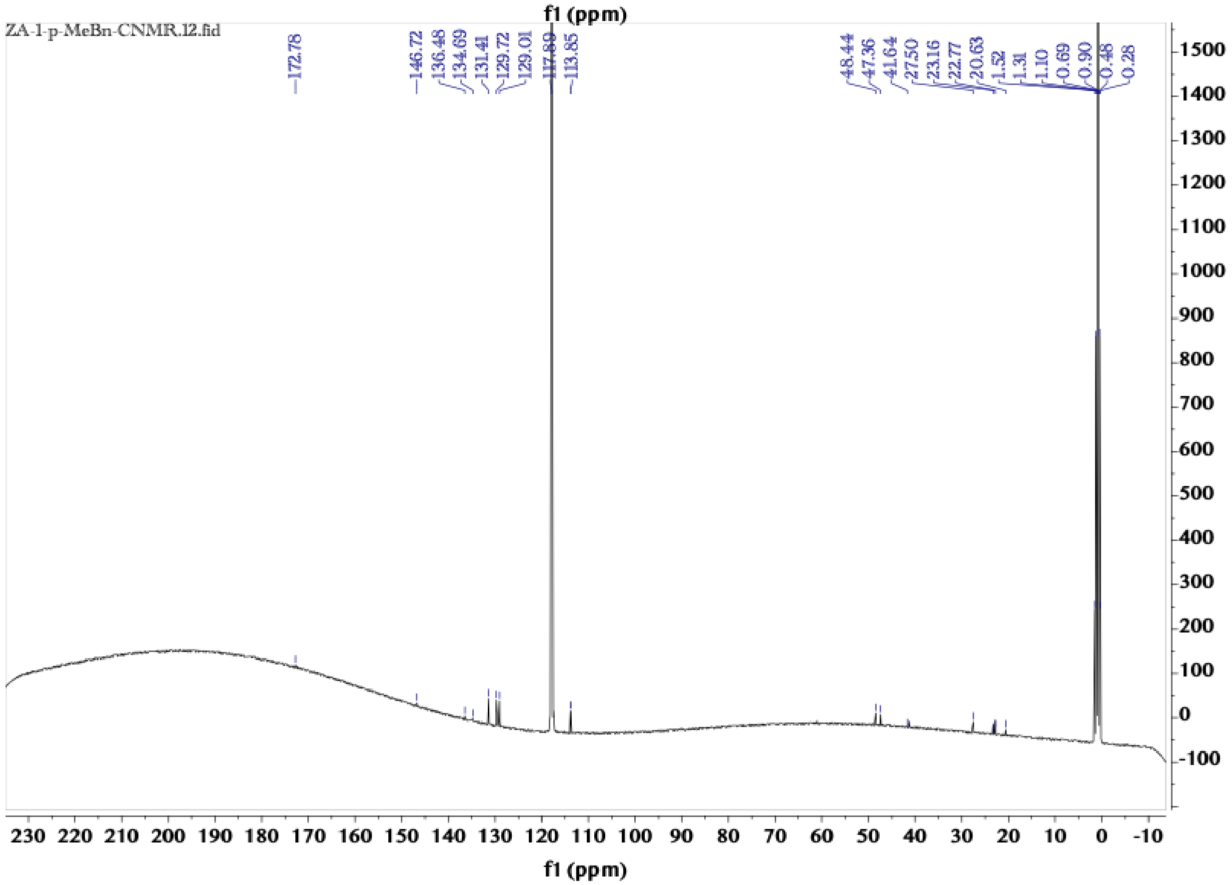

ZA-1-m-FBn-HNMR.10.fid
7.29 7.27 7.27 7.25 7.23 7.23 7.19 7.18 7.17 7.17 7.15 7.15 7.00 6.99 6.98 6.97 6.90 6.88 6.78 6.78 6.77 6.76 6.75 6.75 6.71 6.70 6.69 6.67 6.67 6.56 6.56 6.54 6.54 4.55 3.65 3.64 3.50 3.48 3.47 3.46 3.44 3.39 3.36 3.22 3.19 1.74 1.73 1.72 1.70 1.69 1.67 1.29
0.95 1.82 0.90 0.88 0.89 0.87 1.78 0.76 3.75 1.95 4.03 3.30
H
N
O
N
Me
F
f1 (ppm)

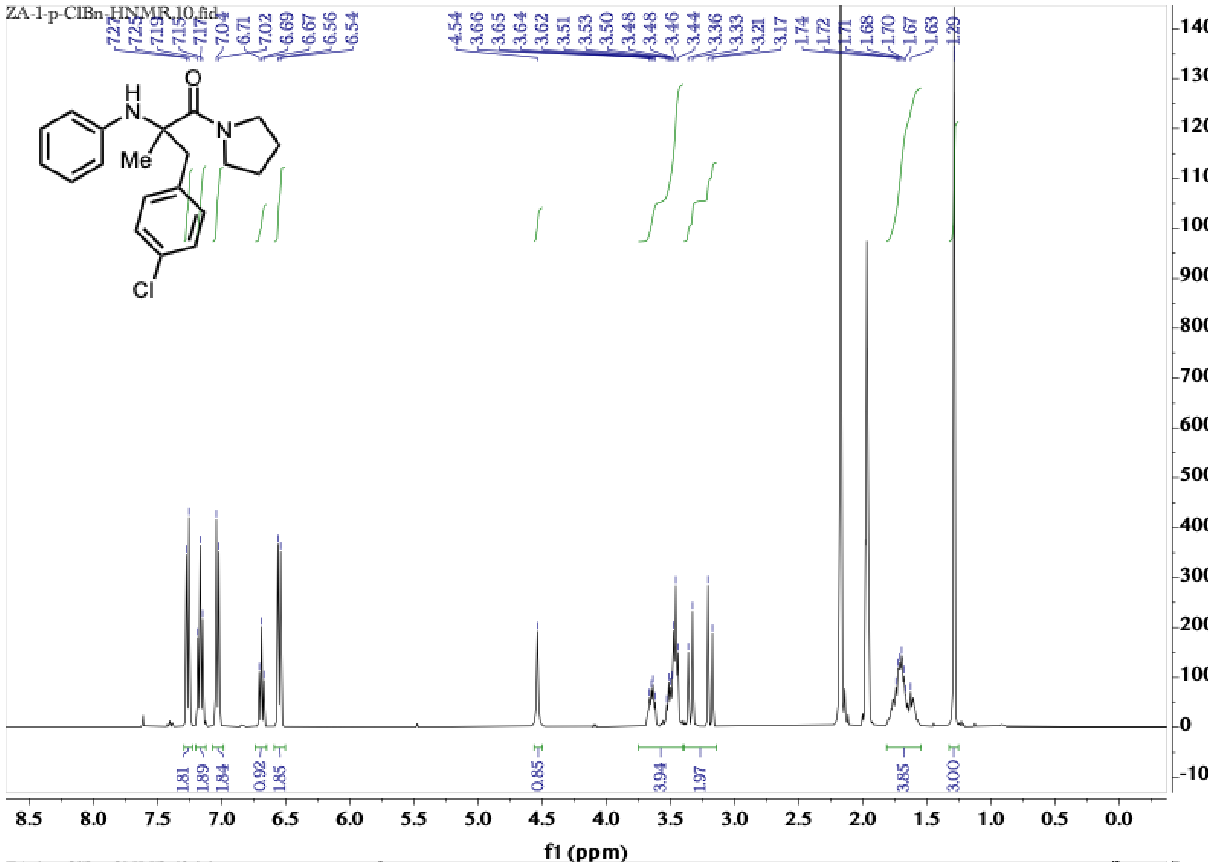
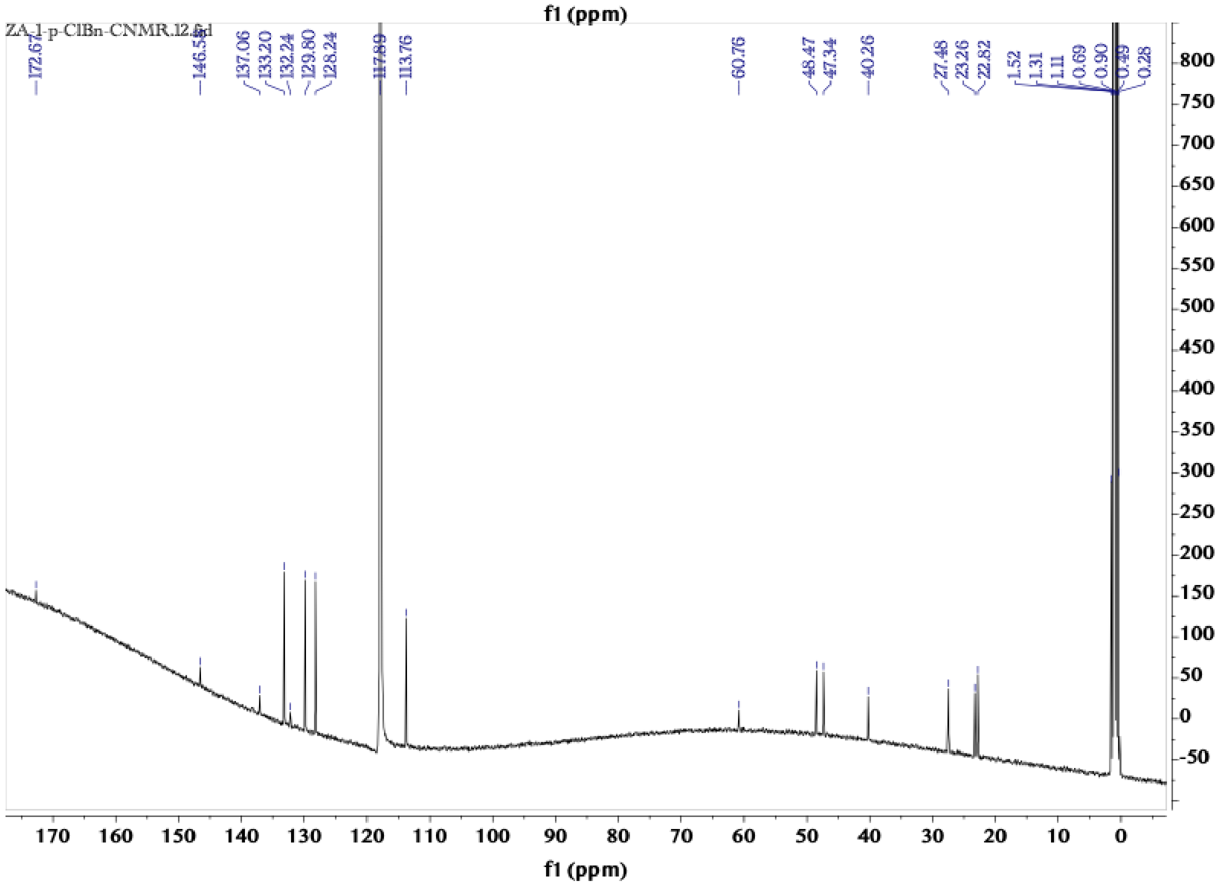

ZA-1-m-FBn-CNMR.12.fid
172.72 164.11 161.70 146.54 140.99 129.90 129.82 127.64 127.61 118.15 117.63 117.89 113.75 113.59 113.38 60.74 48.50 47.36 27.48 40.49 23.26 22.90 1.52 1.31 1.10 0.69 0.90 0.48 0.28
f1 (ppm)

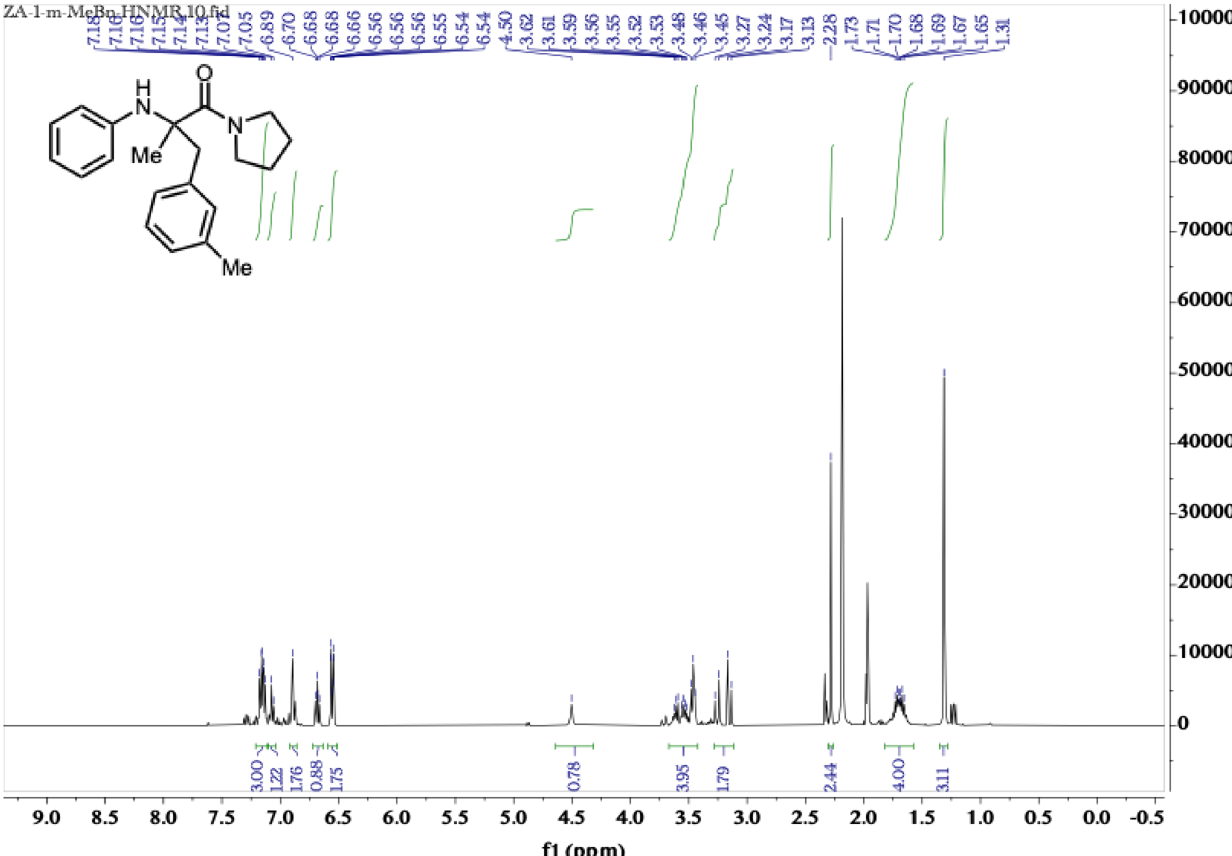

ZA-1-p-OMeBn-HNMR.10.fid
7.19 7.18 7.16 7.14 7.12 7.12 7.10 7.08 7.06 7.04 6.98 6.70 6.68 6.66 6.58 6.57 6.56
4.60
3.57 3.56 3.54 3.52 3.50 3.48 3.46 3.45 3.43 3.39 3.24 3.21
2.32
1.71 1.69 1.68 1.66 1.66 1.65 1.64
1.29
H
N
O
N
Me
MeO
4.04 1.02 0.99 1.02 1.99 0.96 4.06 2.30 3.00 4.30 3.48
f1 (ppm)

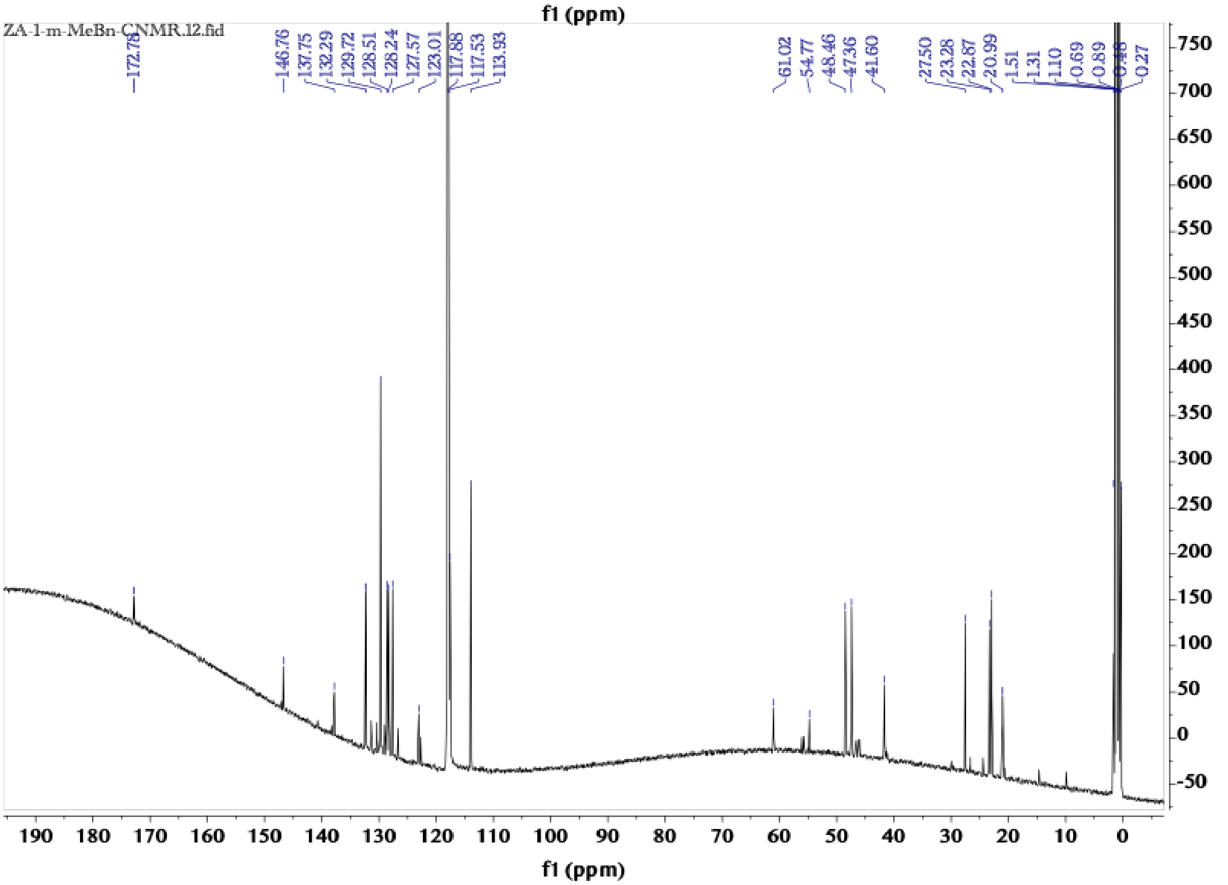

ZA-1-p-OMeBn-CNMR.12.fid
172.81
146.63 138.28 136.34 132.24 130.80 129.73 127.02 125.87 117.89 113.88
62.12
48.56 47.43
37.76
27.53 23.26 22.45 20.03
1.31 1.10 0.90 0.69 0.48
f1 (ppm)

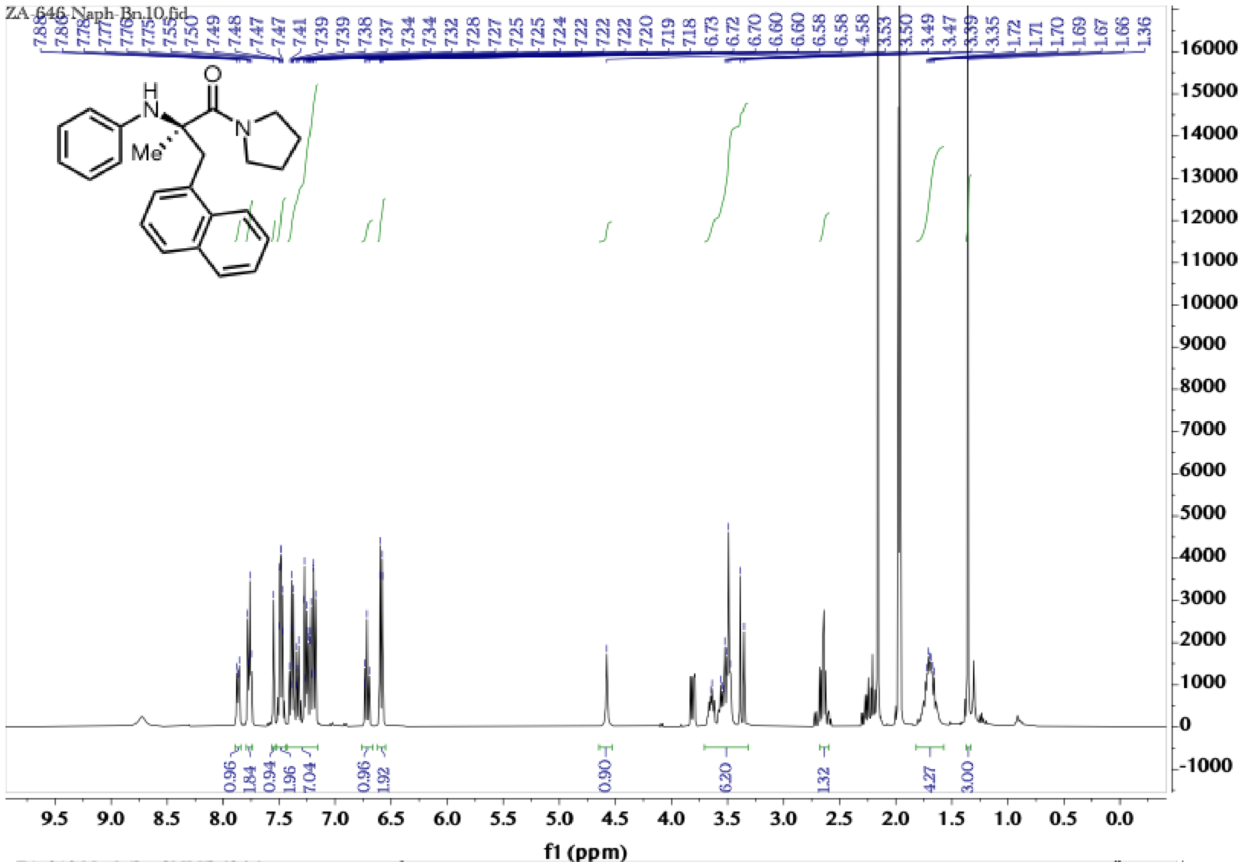

ZA-1-p-MeBn-HNMR.10.fid
f1 (ppm)

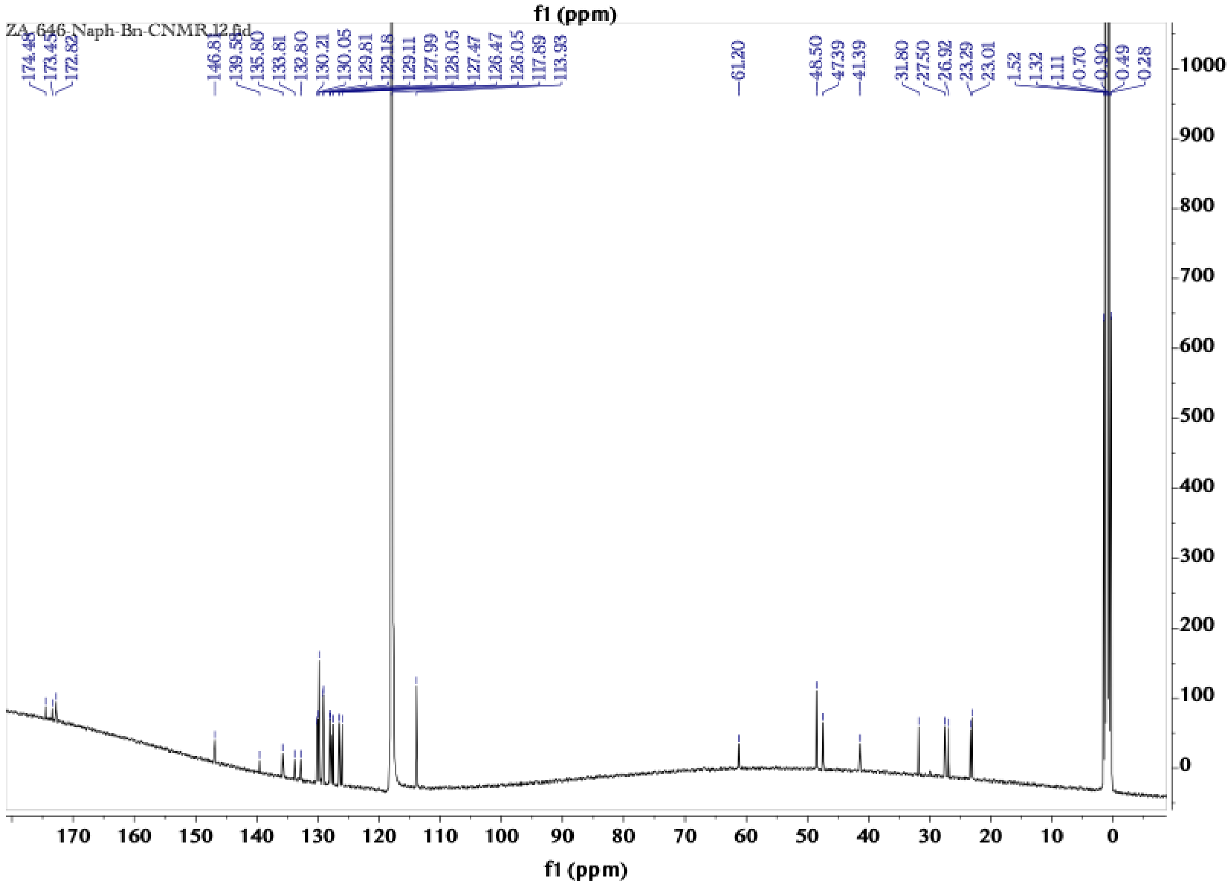

ZA-1-p-MeBn-CNMR.12.fid
172.78
146.72
136.48
134.69
131.41
129.72
129.01
117.80
113.85
48.44
47.36
41.64
27.50
23.16
22.77
20.63
f1 (ppm)

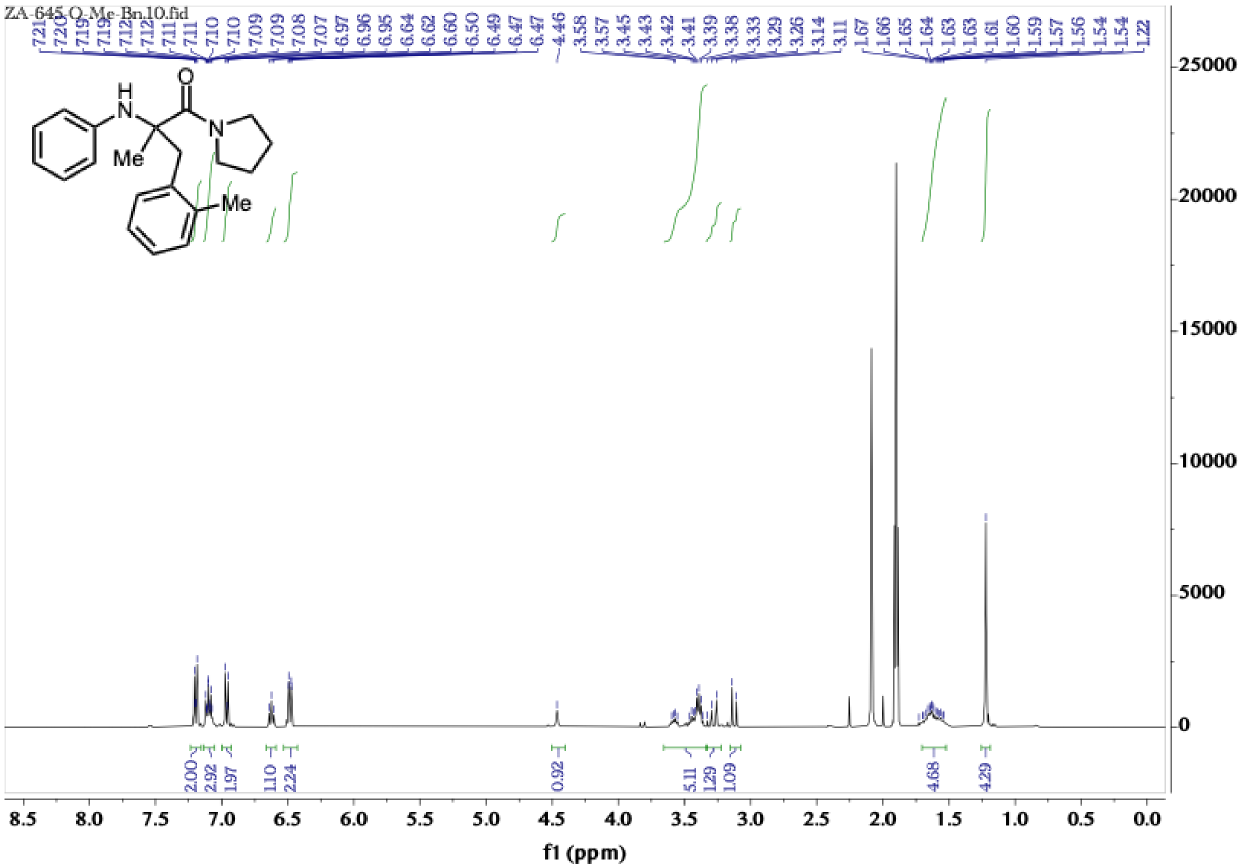

ZA-1-p-ClBn-HNMR.10.fid
7.27 7.25 7.19 7.17 7.15 7.04 7.02 6.71 6.69 6.67 6.56 6.54
4.54 3.66 3.65 3.64 3.62 3.53 3.51 3.50 3.48 3.48 3.46 3.44 3.36 3.33 3.21 3.17 1.74 1.72 1.71 1.70 1.68 1.67 1.63 1.29
H
N
O
N
Me
Cl
1.81 1.89 1.84 0.92 1.85 0.85 3.94 1.97 3.85 3.00
f1 (ppm)

ZA-1-p-ClBn-CNMR.12.fid
172.67 146.58 137.06 133.20 132.24 129.80 128.24 117.89 113.76 60.76 48.47 47.34 40.26 27.48 23.26 22.82 1.52 1.31 1.11 0.90 0.69 0.49 0.28
f1 (ppm)

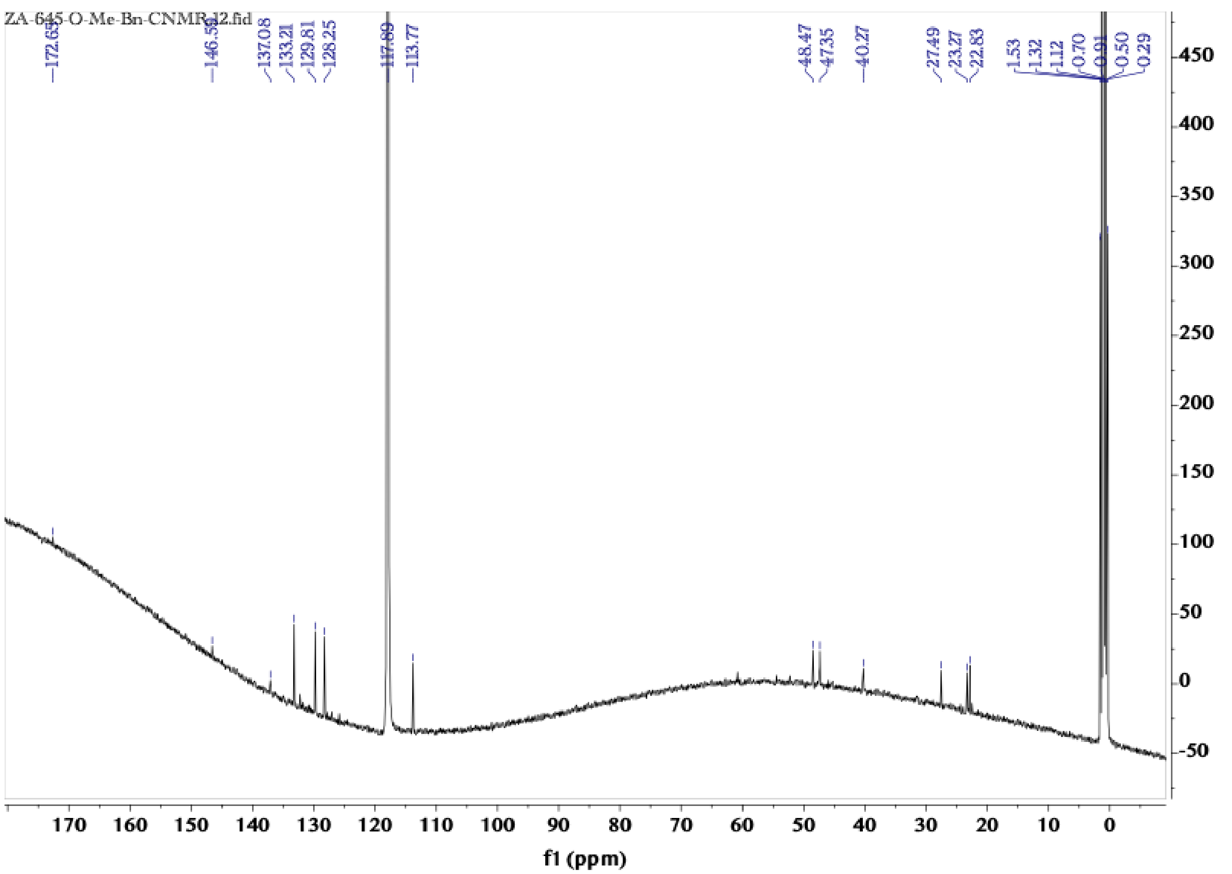

ZA-1-m-MeBn-HNMR.10.fid
7.18
7.16
7.16
7.15
7.14
7.13
7.13
7.05
6.89
6.70
6.68
6.68
6.66
6.56
6.56
6.56
6.55
6.54
6.54
4.50
3.62
3.61
3.59
3.56
3.55
3.52
3.53
3.48
3.46
3.45
3.27
3.24
3.17
3.13
2.28
1.73
1.71
1.70
1.68
1.69
1.67
1.65
1.31
3.00
1.22
1.76
0.88
1.75
0.78
3.95
1.79
2.44
4.00
3.11
f1 (ppm)

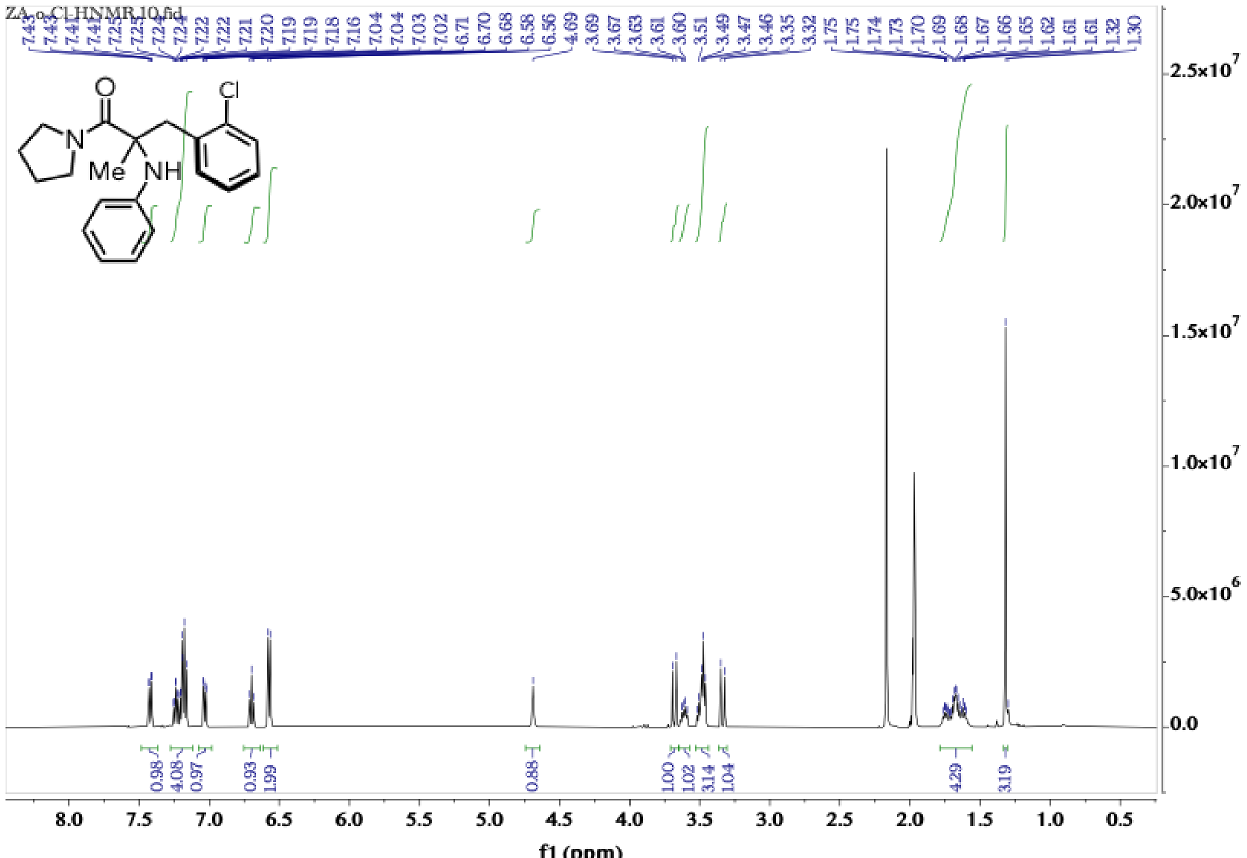

ZA-1-m-MeBn-CNMR.12.fid
172.78
146.76
137.75
132.29
129.72
128.51
128.24
127.57
123.01
117.88
117.53
113.93
61.02
54.77
48.46
47.36
41.60
27.50
23.28
22.87
20.99
1.51
1.31
1.10
0.69
0.89
0.48
0.27
f1 (ppm)

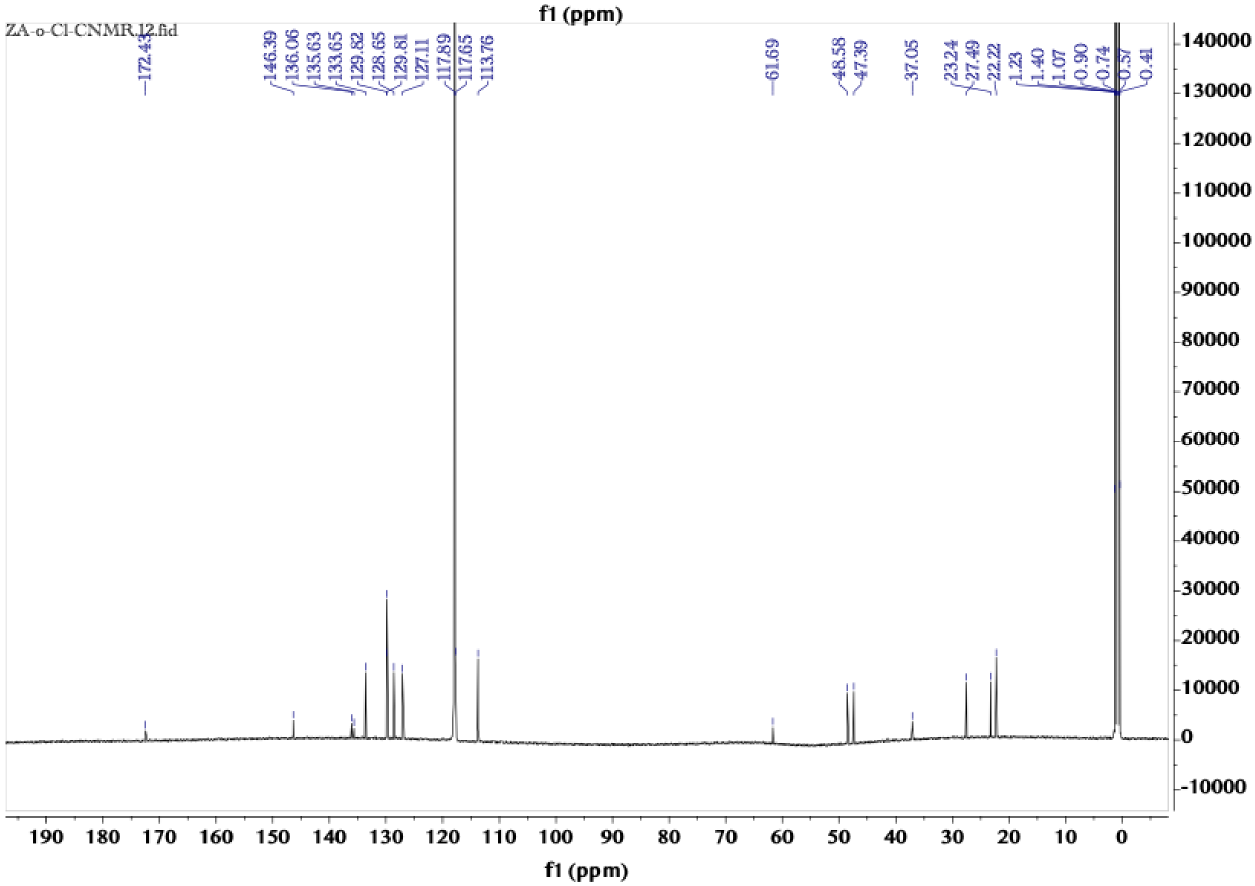

ZA-646-Naph-Bn.10.fid
f1 (ppm)

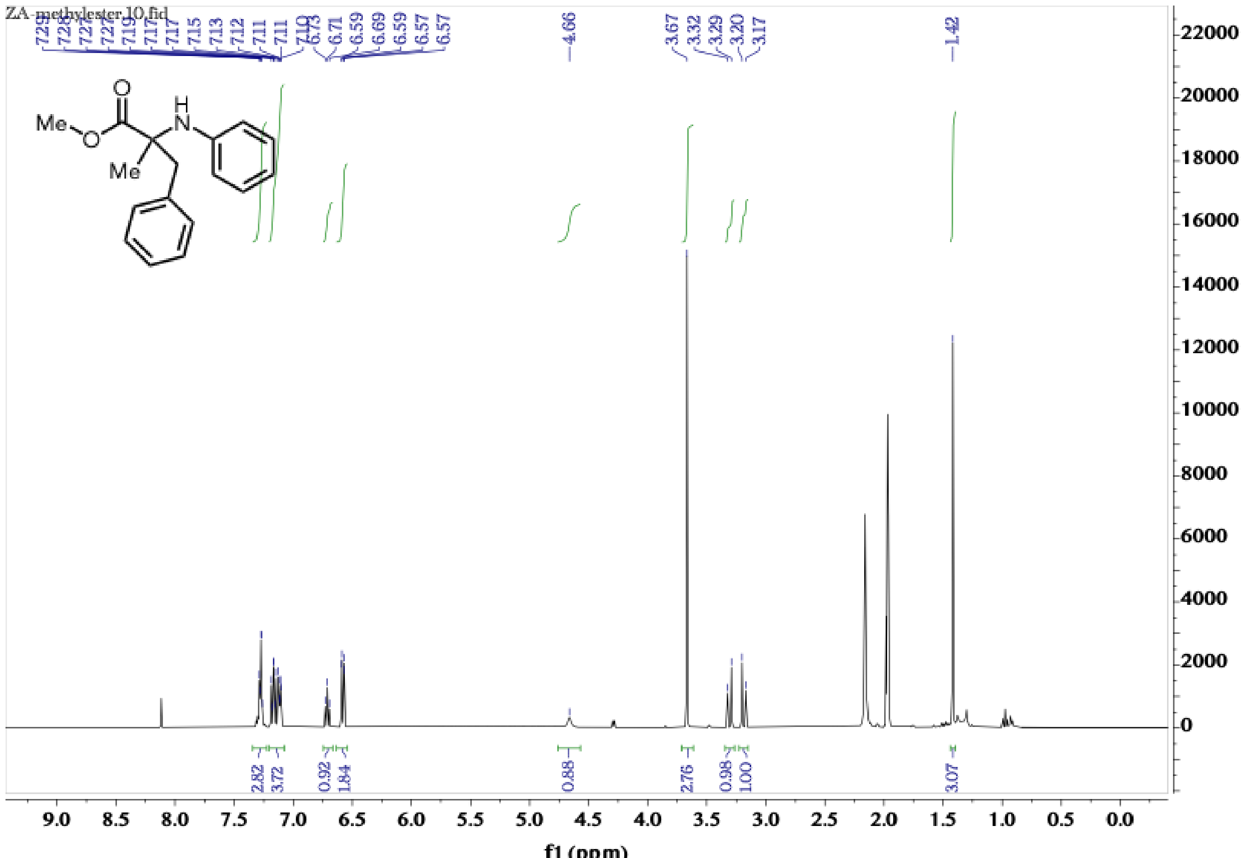

ZA-646-Naph-Bn-CNMR.12.fid
f1 (ppm)

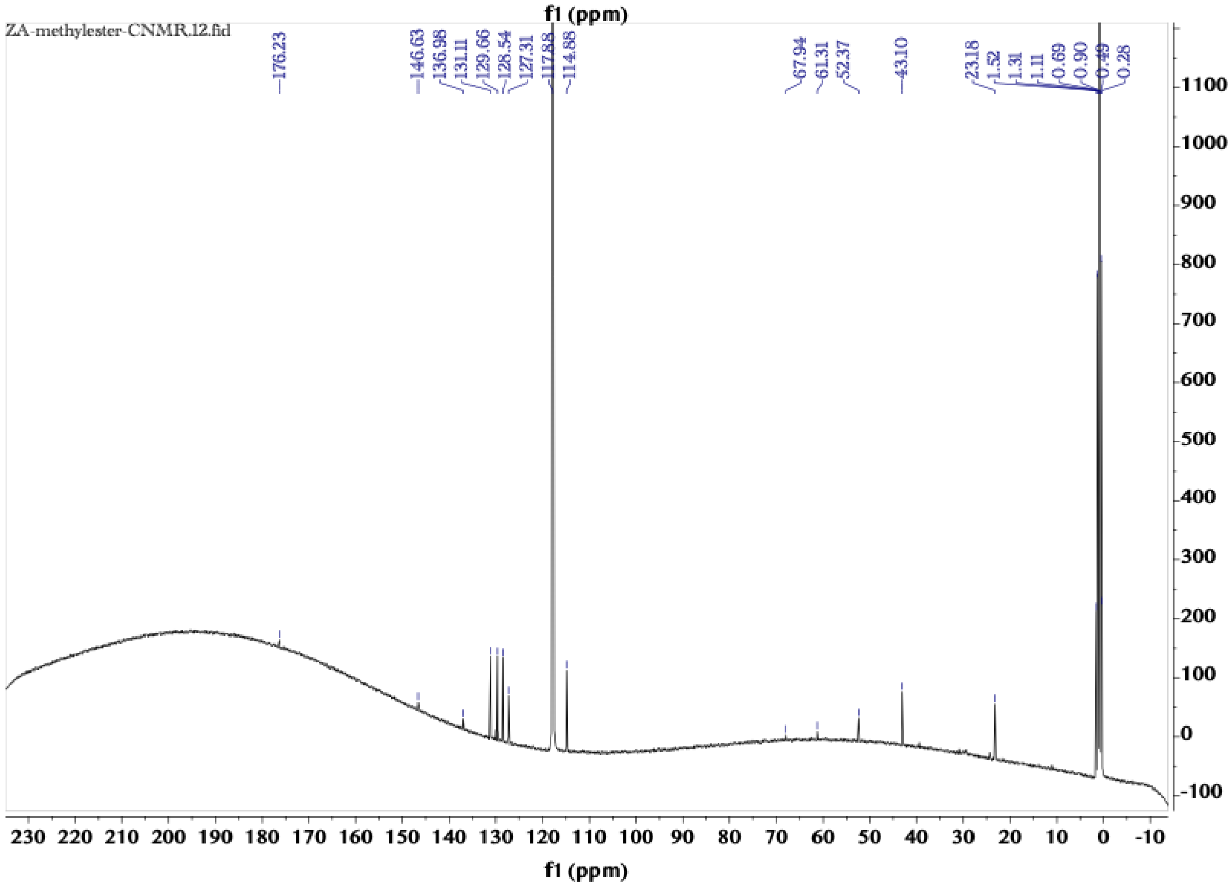

ZA-645-O-Me-Bn.10.fid
Me
Me
f1 (ppm)

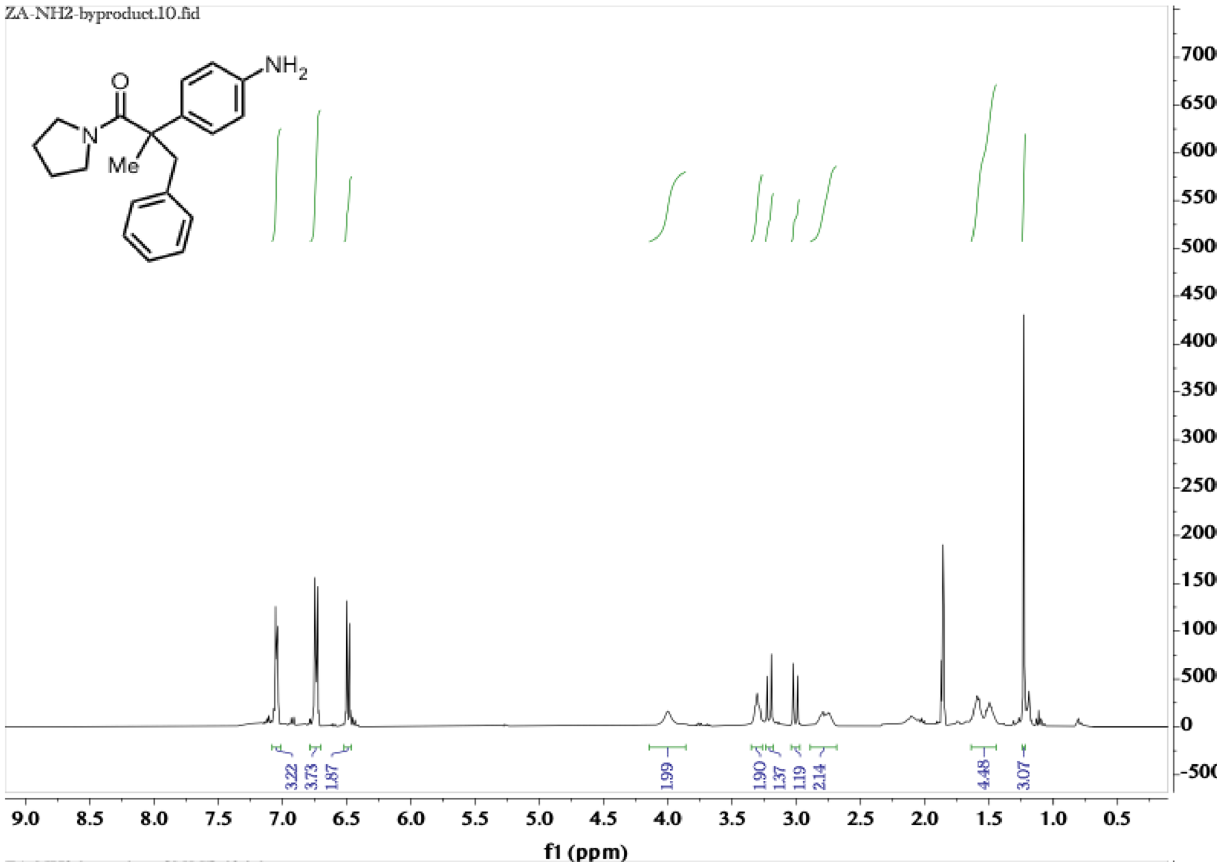

ZA-645-O-Me-Bn-CNMR.12.fid
f1 (ppm)

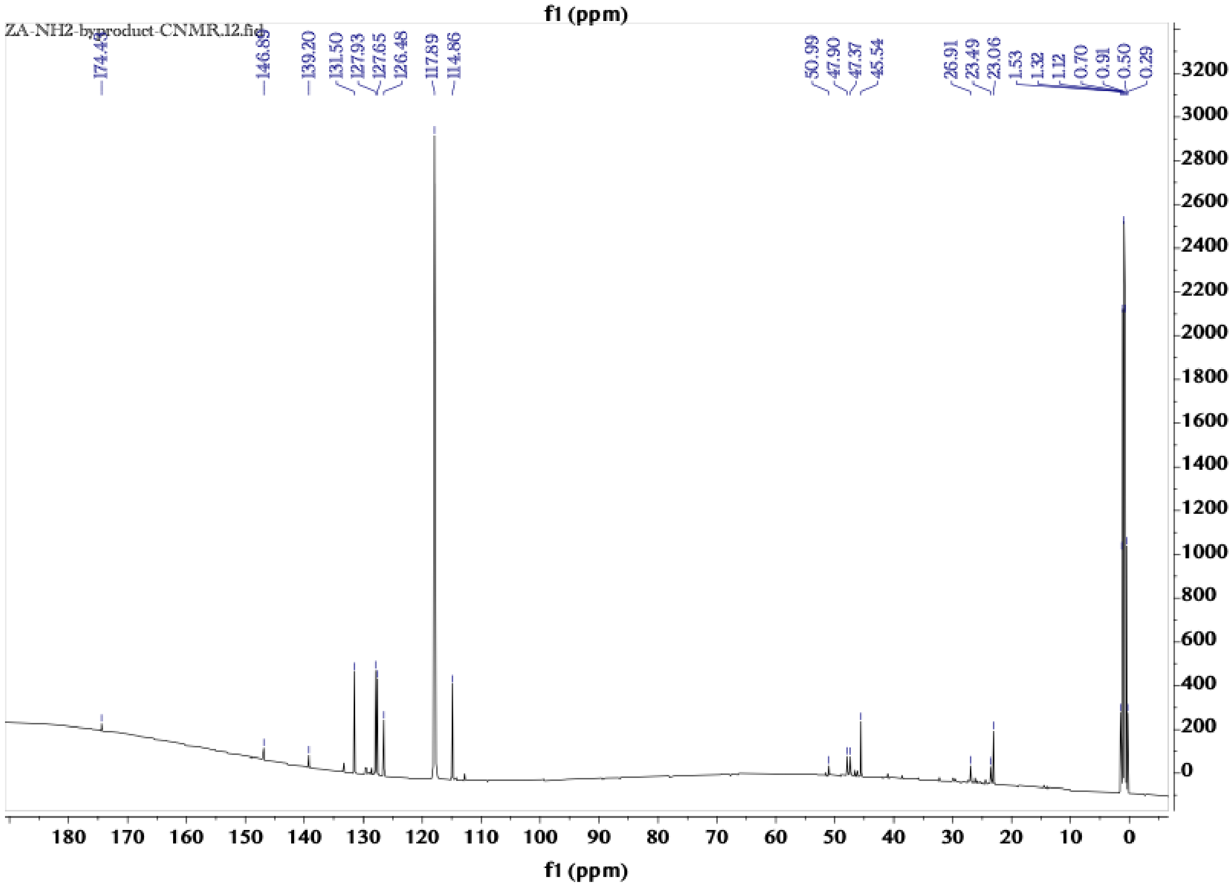

ZA-o-Cl-HNMR.10.fid
7.43 7.43 7.41 7.41 7.25 7.25 7.24 7.24 7.22 7.22 7.21 7.20 7.19 7.19 7.18 7.16 7.04 7.04 7.03 7.02 6.71 6.70 6.68 6.58 6.56 4.69 3.69 3.67 3.63 3.61 3.60 3.51 3.49 3.47 3.46 3.35 3.32 1.75 1.75 1.74 1.73 1.70 1.69 1.68 1.67 1.66 1.65 1.62 1.61 1.61 1.32 1.30
Cl
O
N
Me
NH
0.98 4.08 0.97 0.93 1.99 0.88 1.00 1.02 3.14 1.04 4.29 3.19
f1 (ppm)


ZA-o-Cl-CNMR.12.fid
172.43 146.39 136.06 135.63 133.65 129.82 128.65 129.81 127.11 117.89 117.65 113.76 61.69 48.58 47.39 37.05 23.24 27.49 22.22 1.23 1.40 1.07 0.90 0.74 0.57 0.41
f1 (ppm)

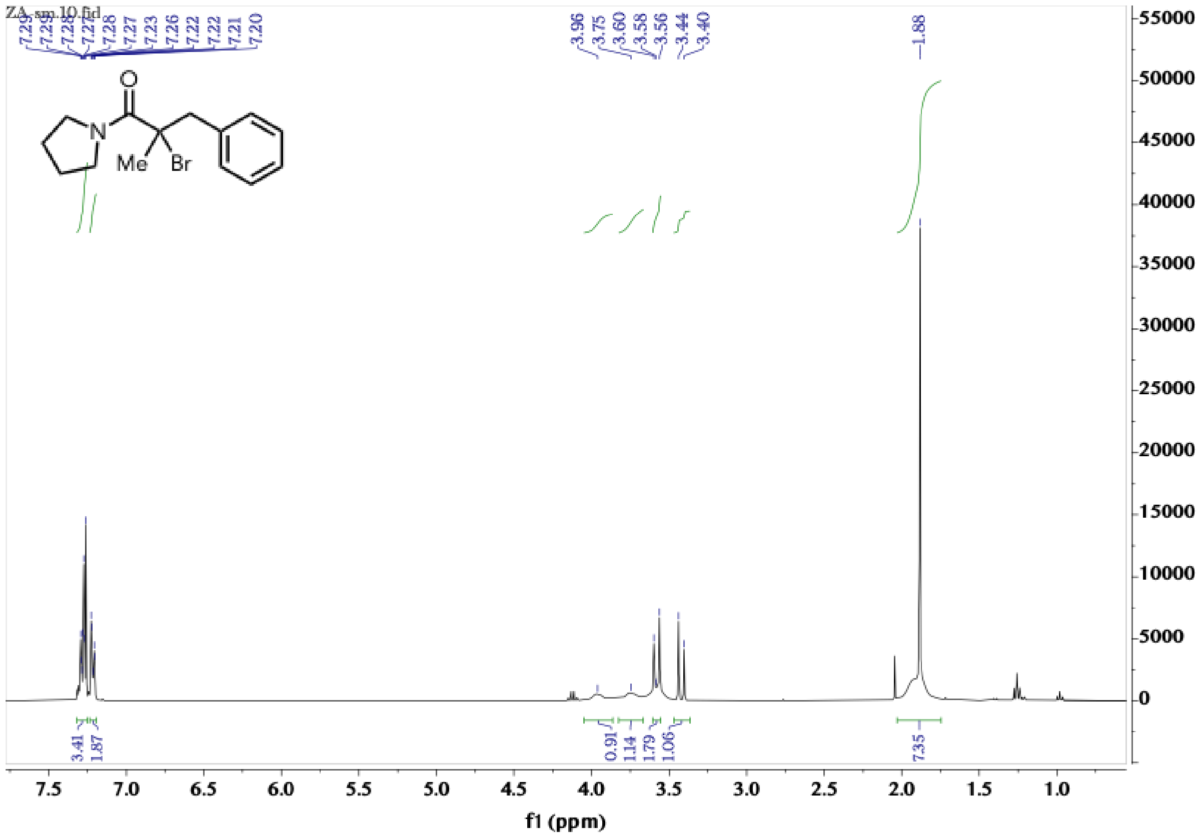

ZA-methylester.10.fid
Me
O
O
H
N
Me
f1 (ppm)

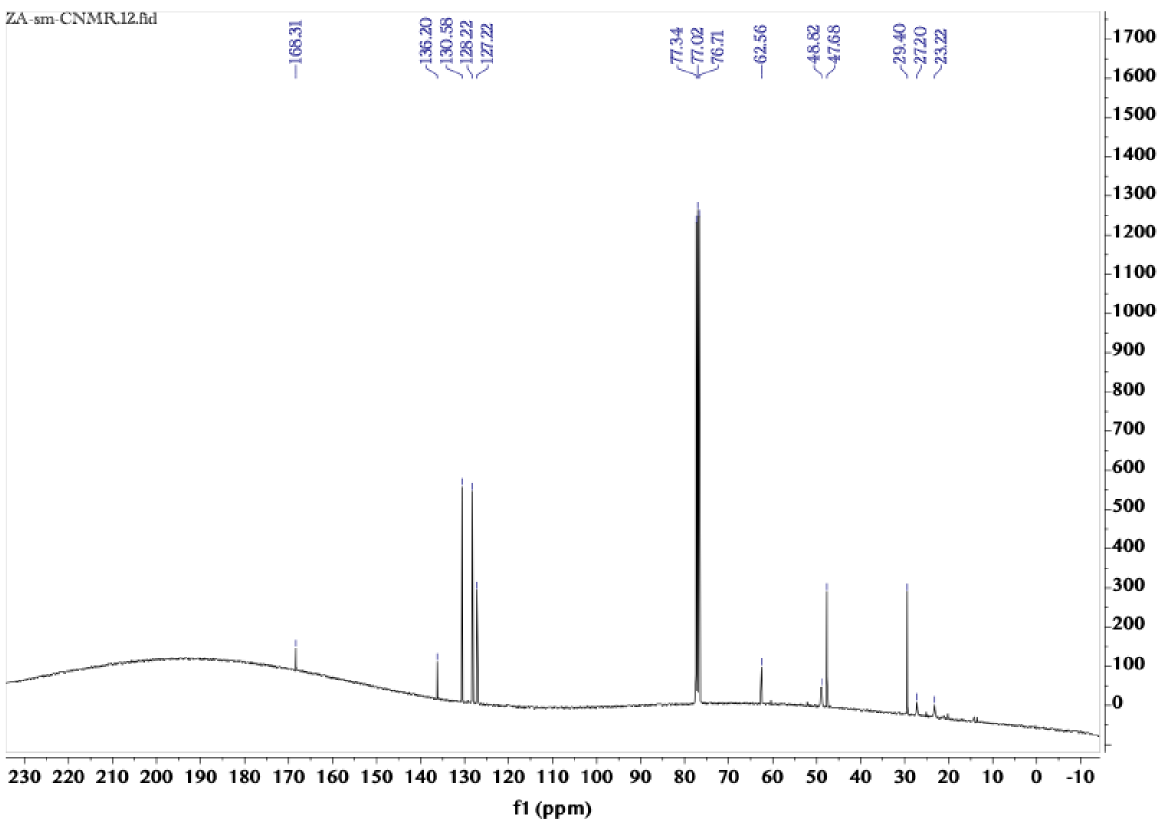

ZA-methylester-CNMR.12.fid
f1 (ppm)

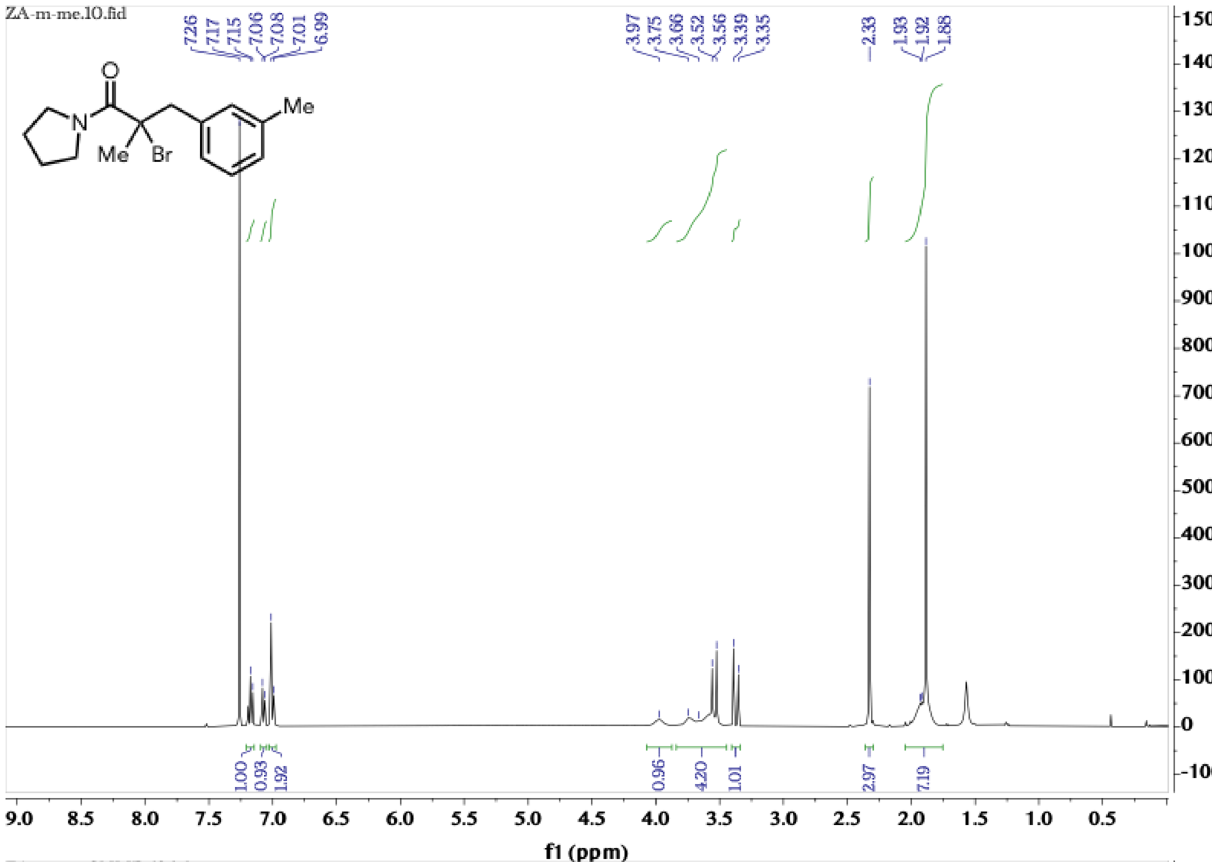

ZA-NH2-byproduct.10.fid
NH2
O
N
Me
3.22
3.73
1.87
1.99
1.90
1.37
1.19
2.14
4.48
3.07
f1 (ppm)

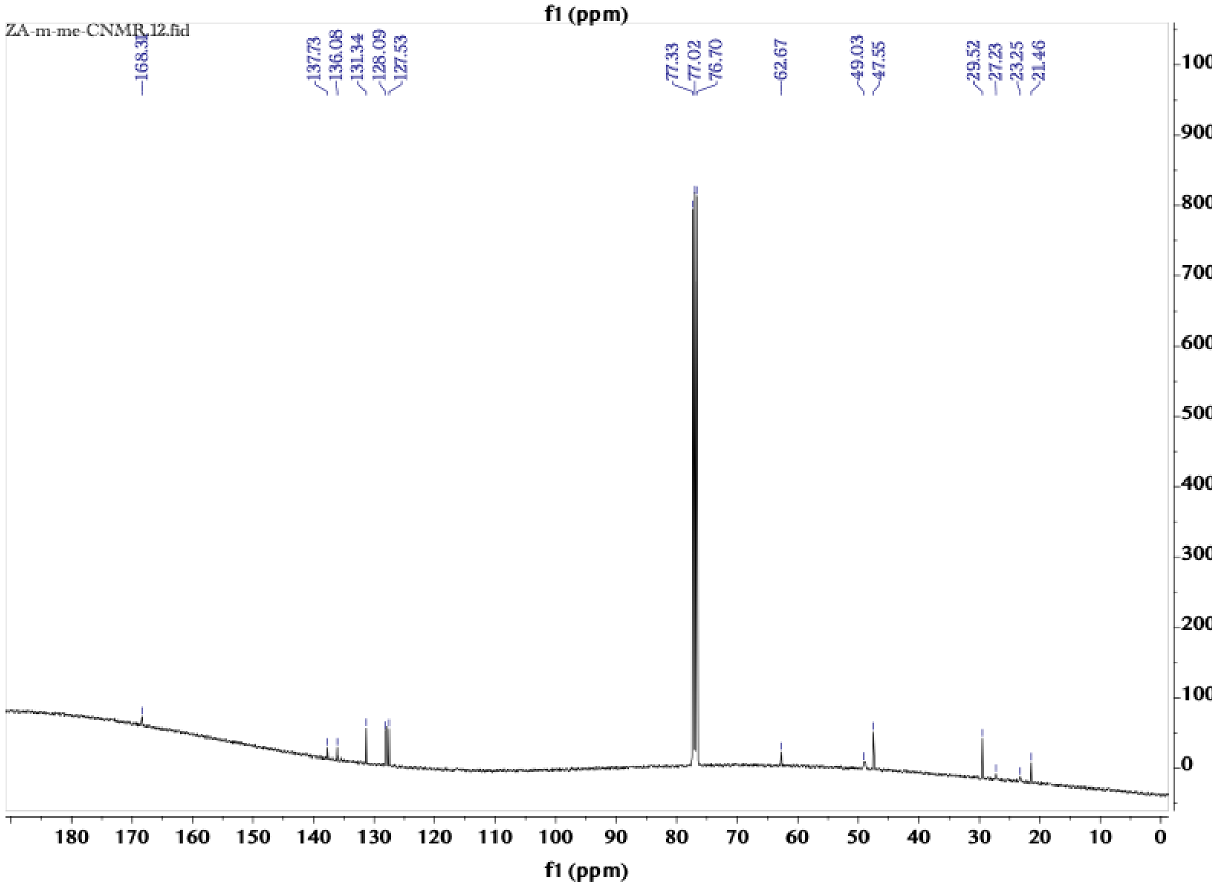

ZA-NH2-byproduct-CNMR.12.fid
174.40
146.89
139.20
131.50
127.93
127.65
126.48
117.89
114.86
50.99
47.90
47.37
45.54
26.91
23.49
23.06
1.53
1.32
1.12
0.70
0.91
0.50
0.29
f1 (ppm)

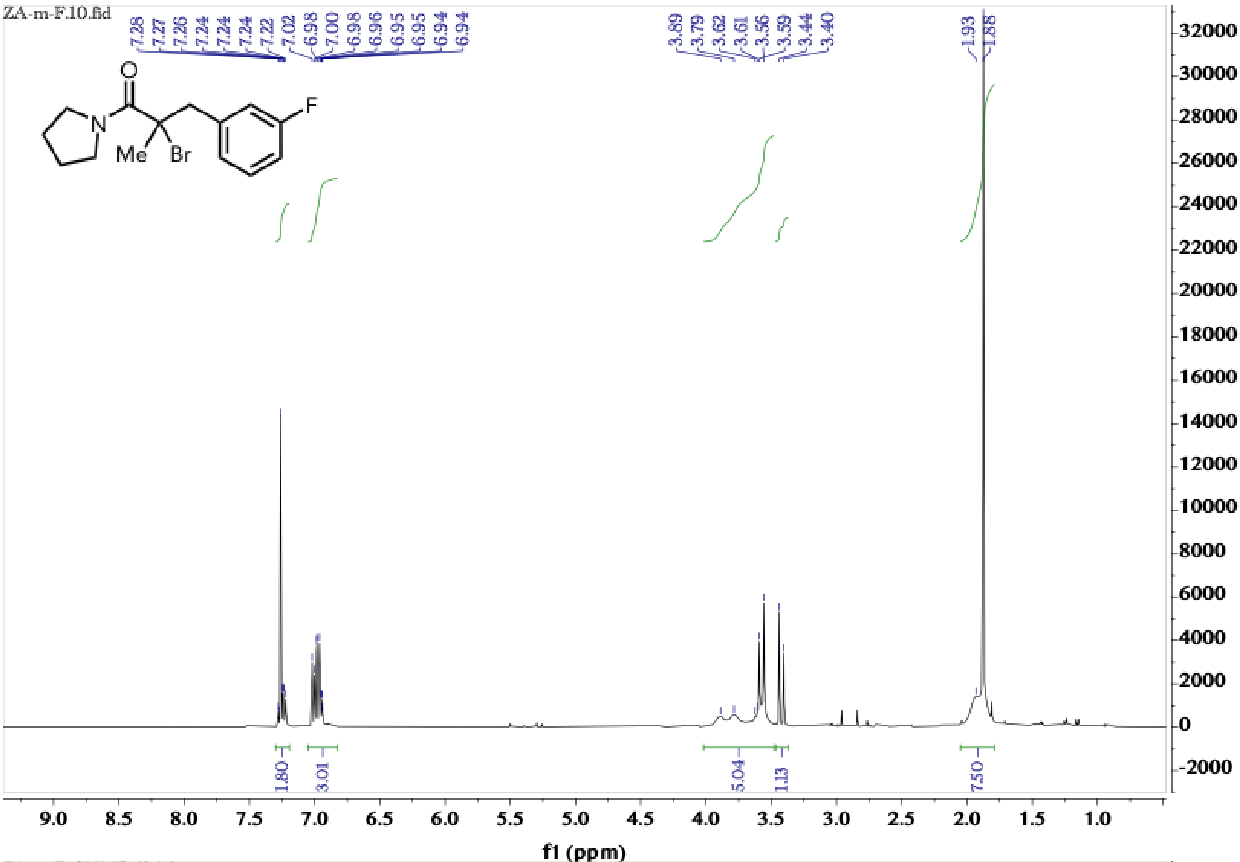
Starting materials

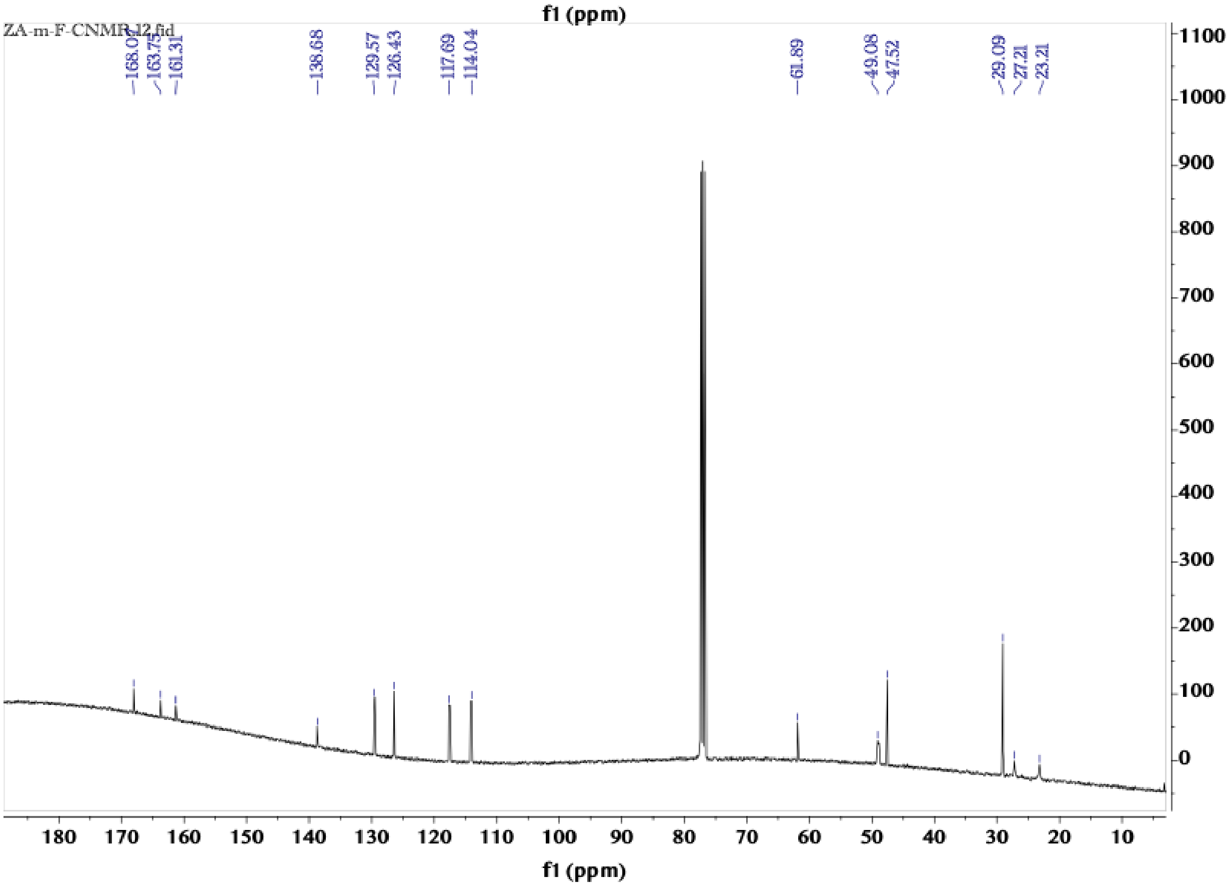


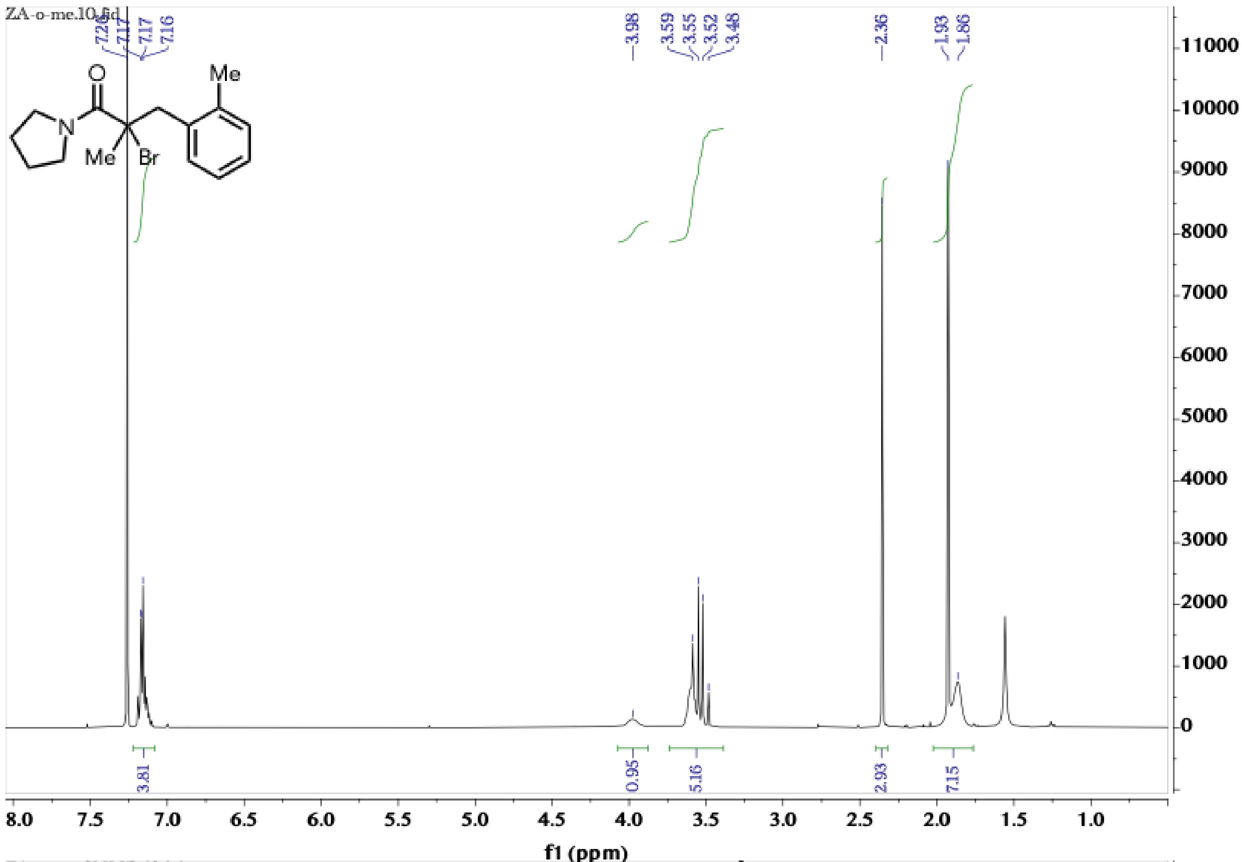
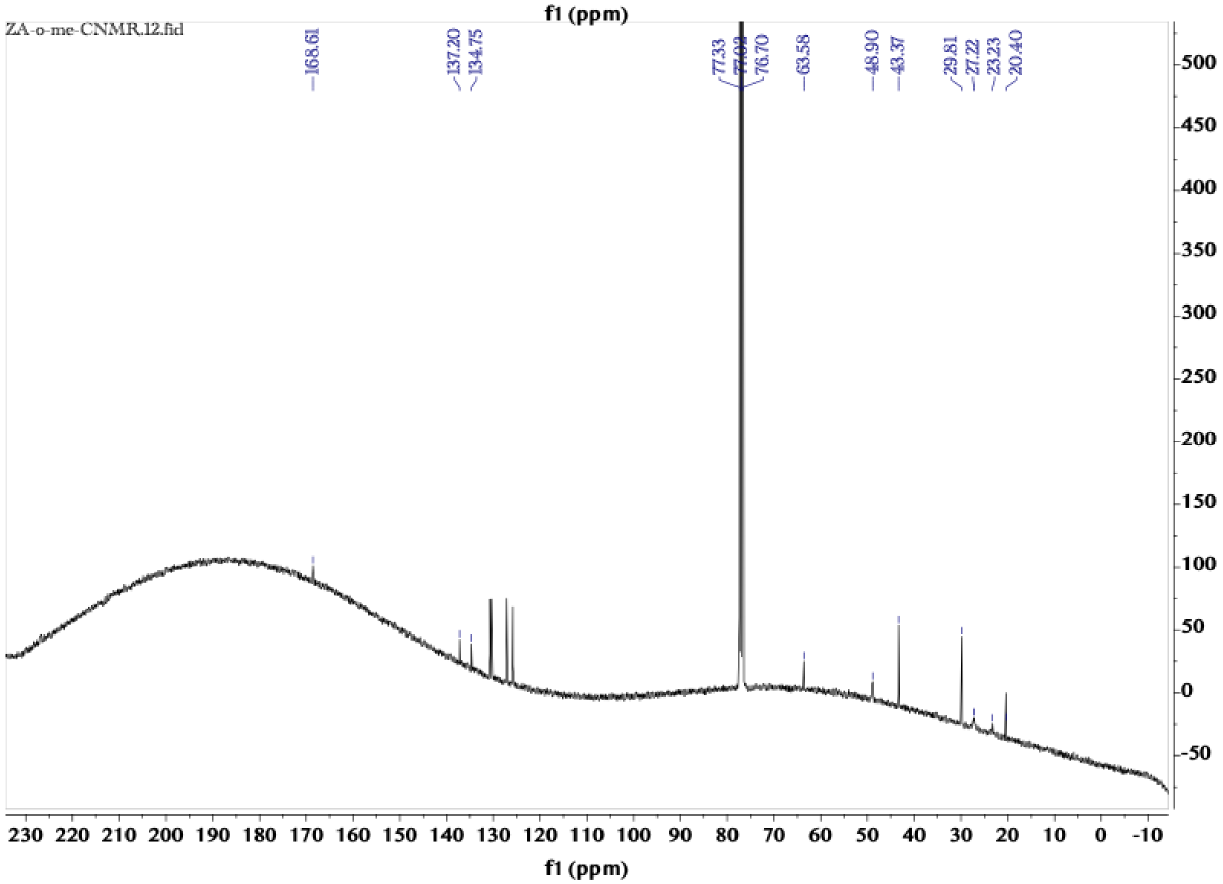


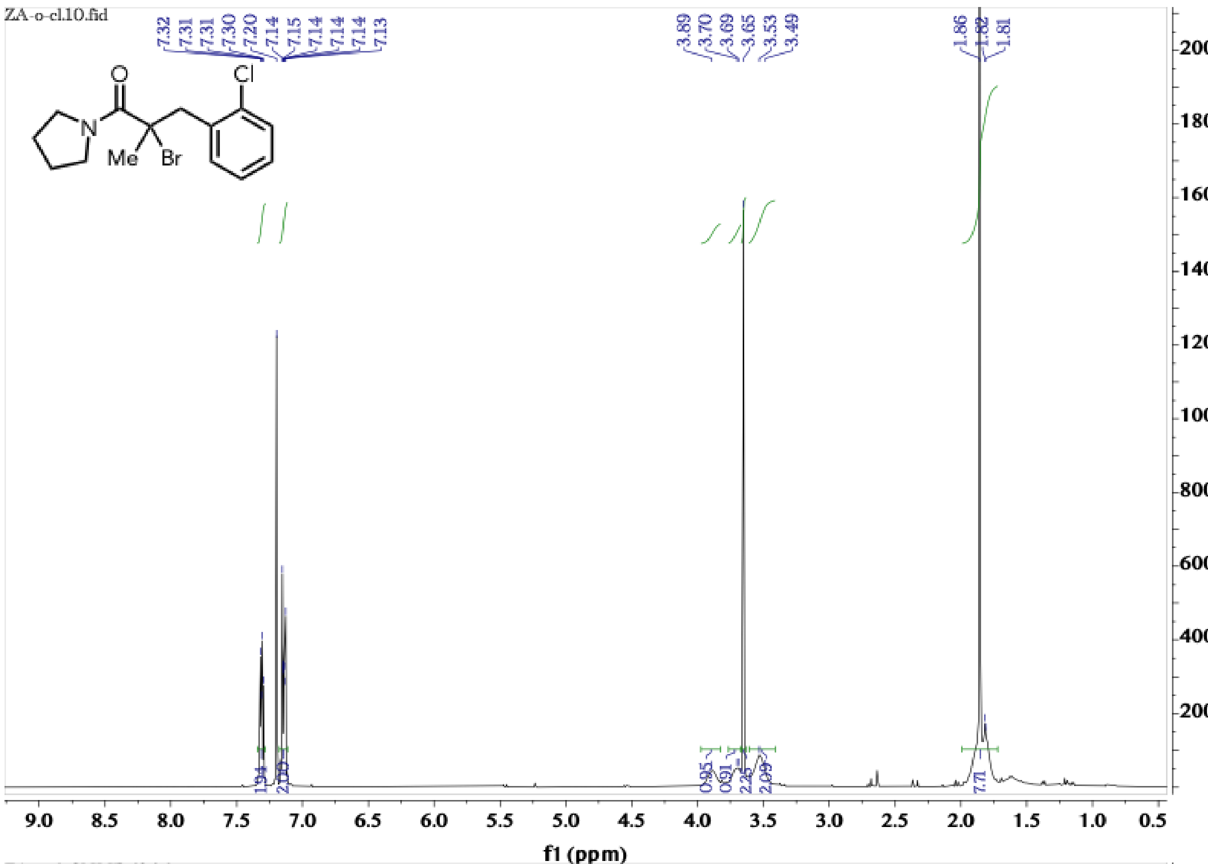

ZA-m-me.10.fid
7.26
7.17
7.15
7.06
7.08
7.01
6.99
3.97
3.75
3.66
3.52
3.56
3.39
3.35
2.33
1.93
1.92
1.88
O
N
Me
Br
Me
1.00
0.93
1.92
0.96
4.20
1.01
2.97
7.19
f1 (ppm)

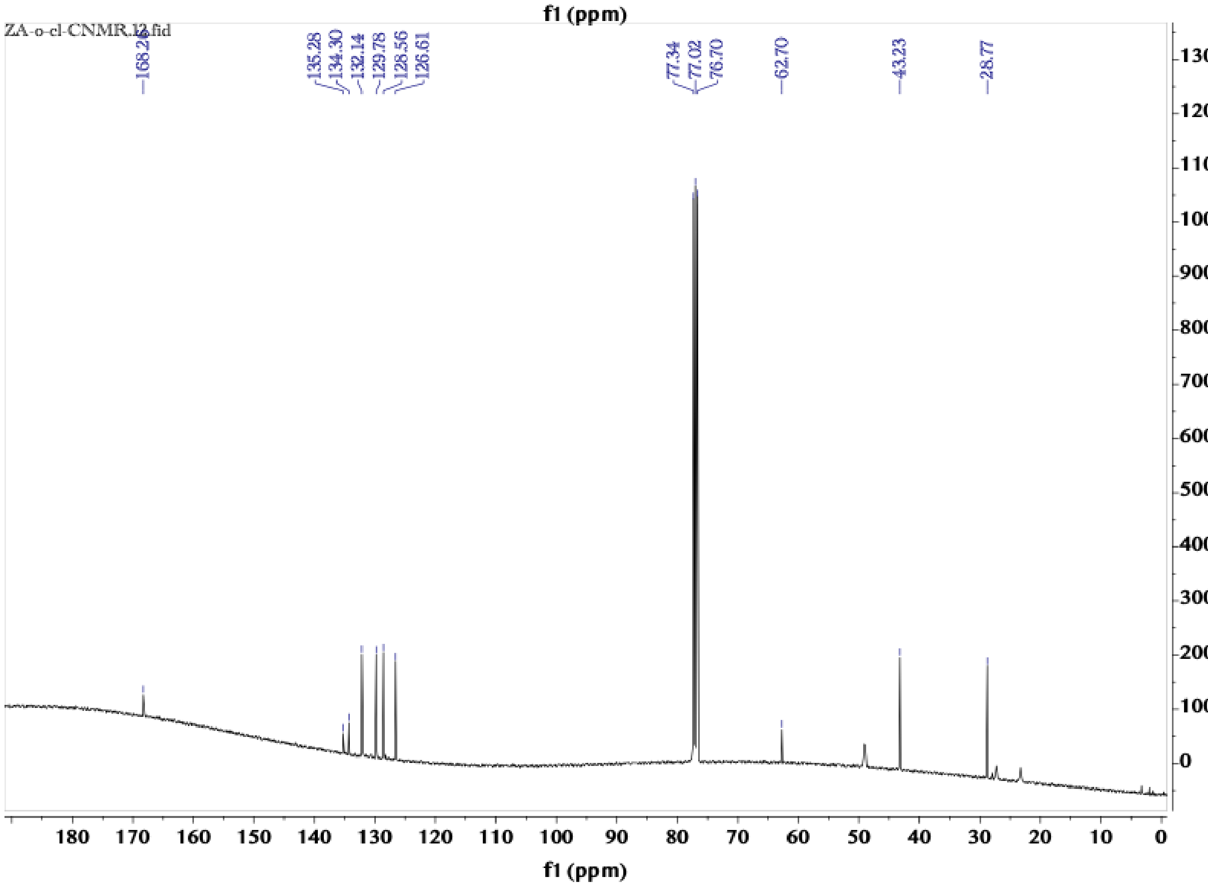

ZA-m-me-CNMR.12.fid
168.31
137.73
136.08
131.34
128.09
127.53
77.33
77.02
76.70
62.67
49.03
47.55
29.52
27.23
23.25
21.46
f1 (ppm)

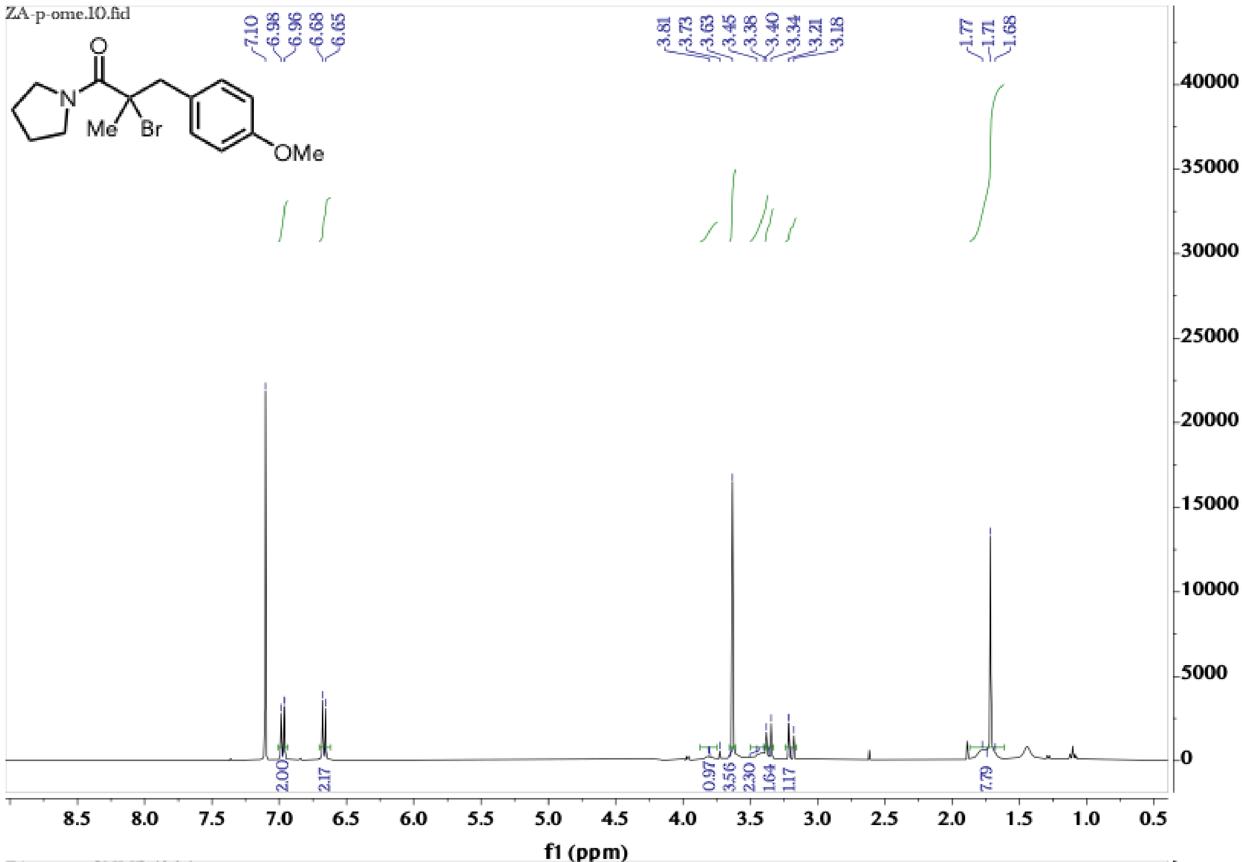

ZA-m-F.10.fid
7.28 7.27 7.26 7.24 7.24 7.24 7.22 7.02 6.98 7.00 6.98 6.96 6.95 6.95 6.94 6.94
3.89 3.79 3.62 3.61 3.56 3.59 3.44 3.40
1.93 1.88
O
N
F
Me
Br
1.80
3.01
5.04
1.13
7.50
f1 (ppm)

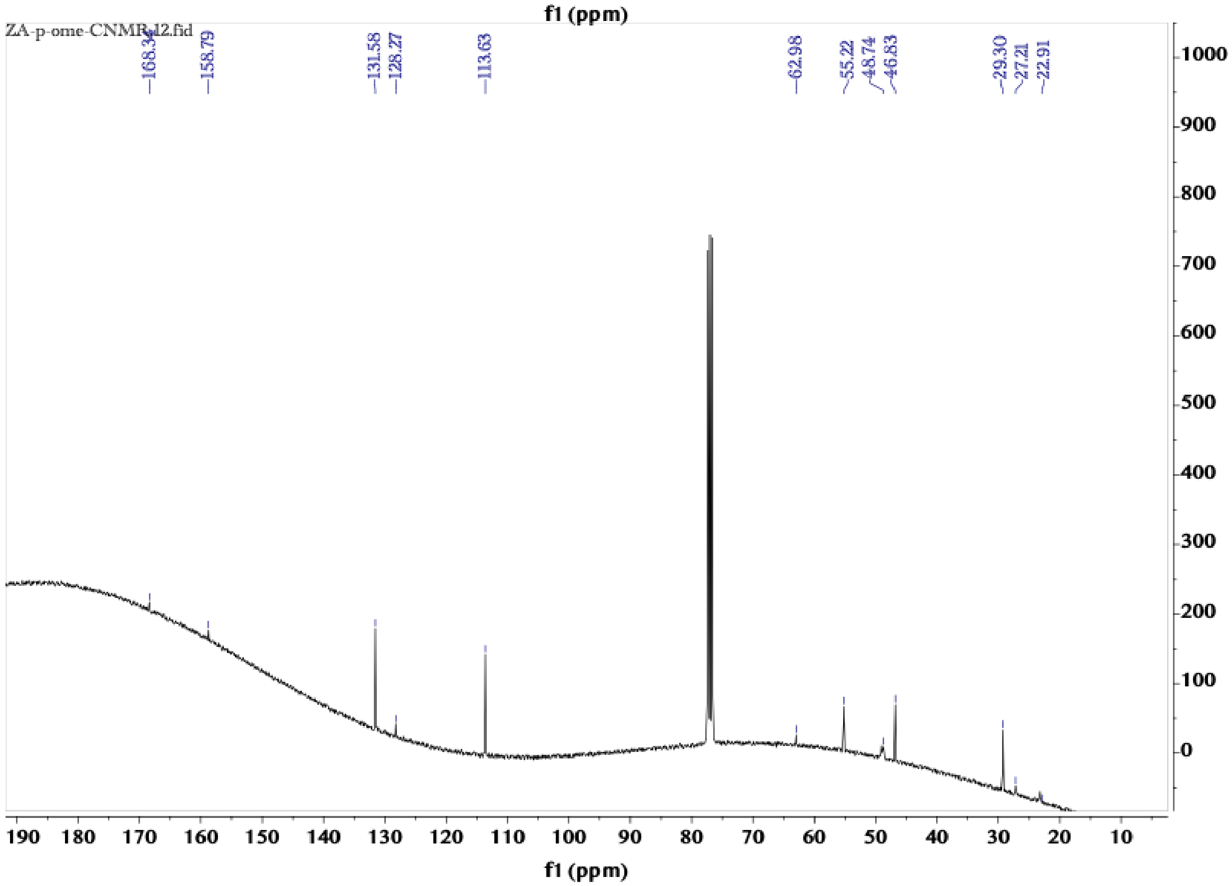

ZA-m-F-CNMR-12.fid
168.02 163.75 161.31
138.68
129.57 126.43
117.69 114.04
61.89
49.08 47.52
29.09 27.21 23.21
f1 (ppm)

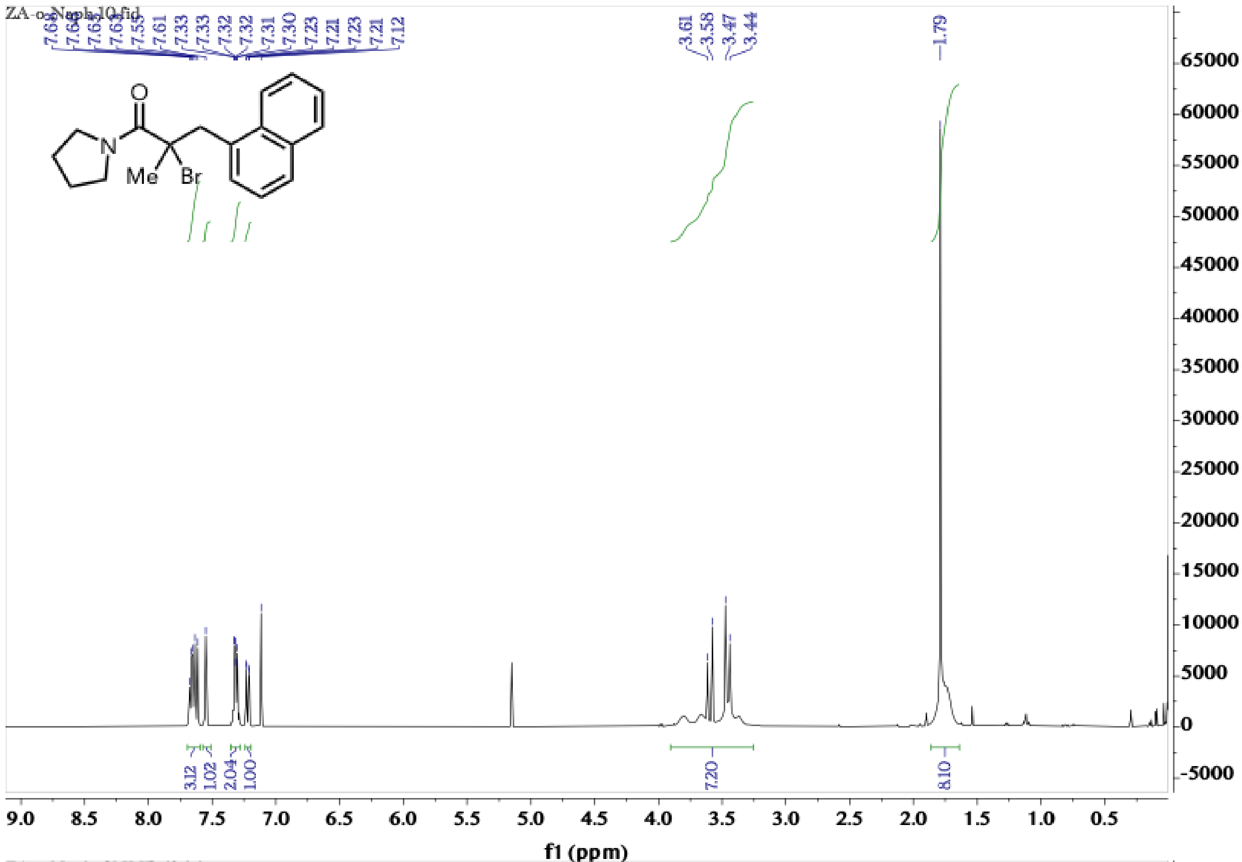

ZA-o-me.10.fid
7.26
7.17
7.17
7.16
3.98
3.59
3.55
3.52
3.48
2.36
1.93
1.86
Me
O
N
Me
Br
3.81
0.95
5.16
2.93
7.15
f1 (ppm)

ZA-o-me-CNMR.12.fid
168.61
137.20
134.75
77.33
76.70
63.58
48.90
43.37
29.81
27.22
23.23
20.40
f1 (ppm)

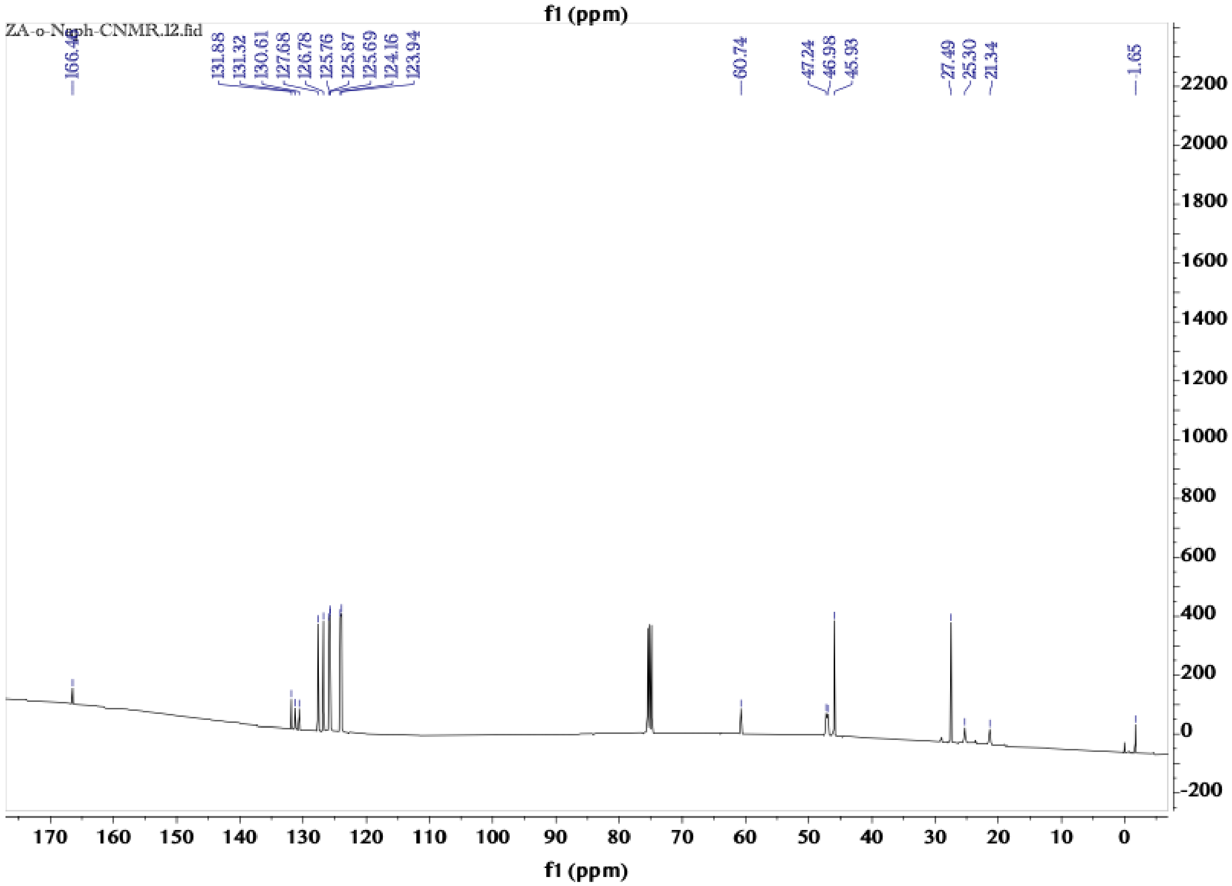

ZA-o-cl.10.fid
7.32
7.31
7.31
7.30
7.20
7.14
7.15
7.14
7.14
7.14
7.13
3.89
3.70
3.69
3.65
3.53
3.49
1.86
1.82
1.81
Cl
O
N
Me
Br
1.34
2.00
0.95
0.91
2.25
2.09
7.71
f1 (ppm)

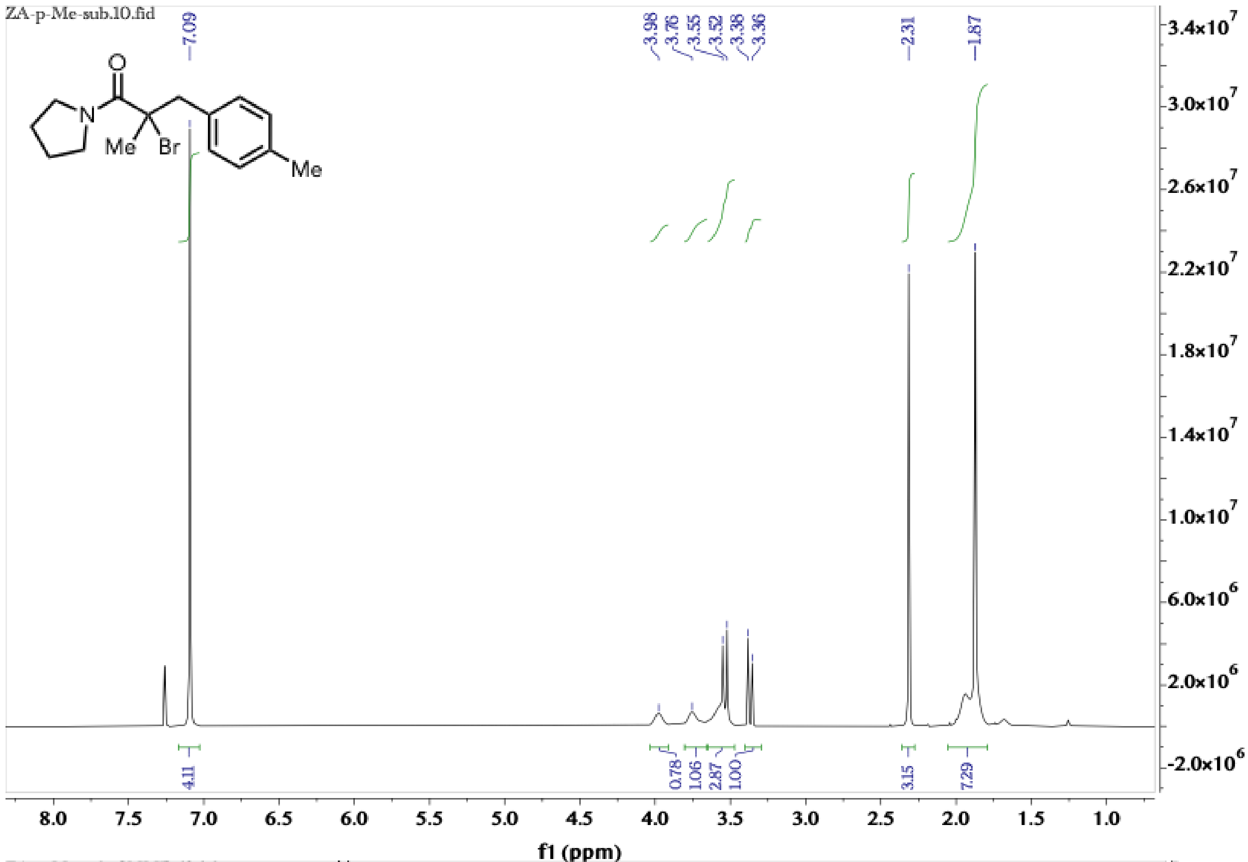

ZA-o-cl-CNMR.12.fid
168.26
135.28
134.30
132.14
129.78
128.56
126.61
77.34
77.02
76.70
62.70
43.23
28.77
f1 (ppm)

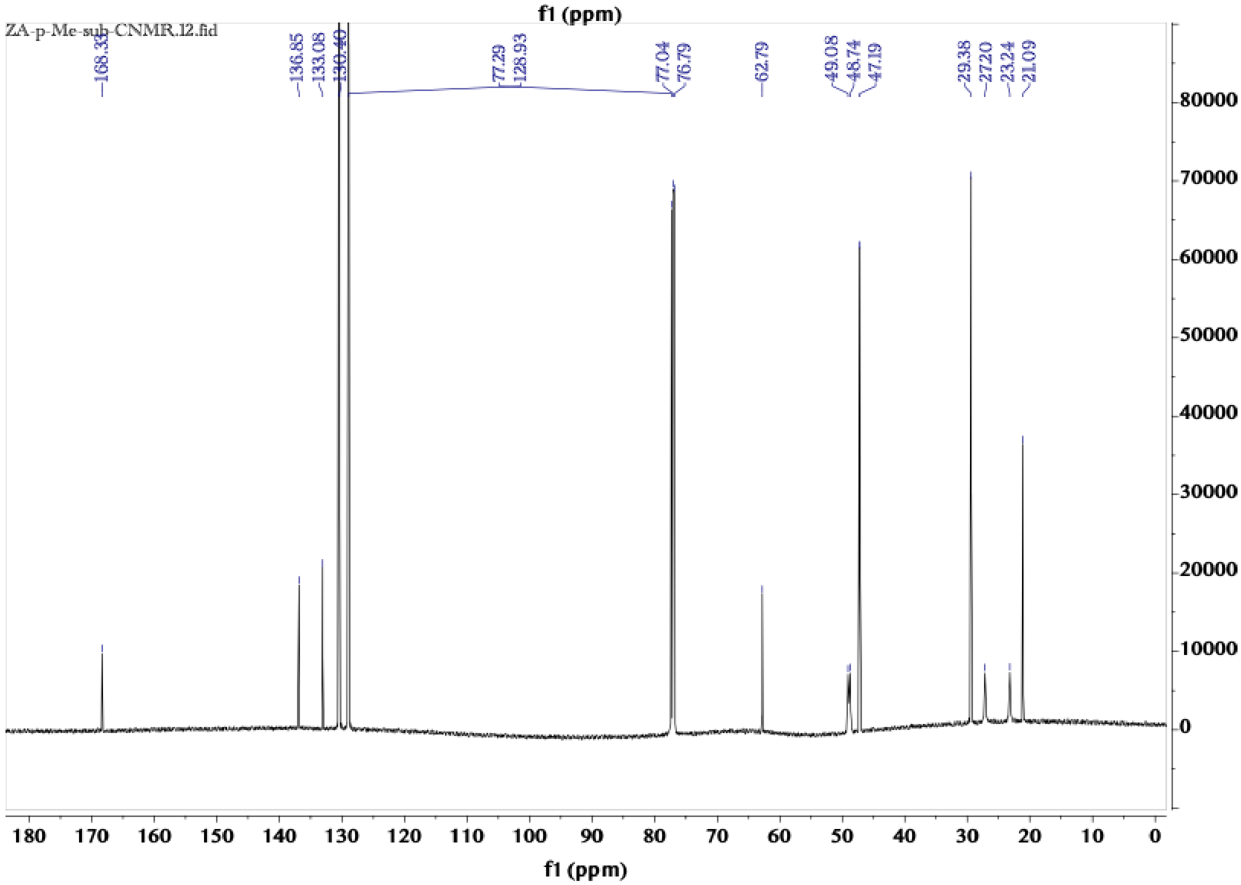

ZA-p-ome.10.fid
7.10
6.98
6.96
6.68
6.65
3.81
3.73
3.63
3.45
3.38
3.40
3.34
3.21
3.18
1.77
1.71
1.68
Me
Br
OMe
2.00
2.17
0.97
3.56
2.30
1.64
1.17
7.79
f1 (ppm)

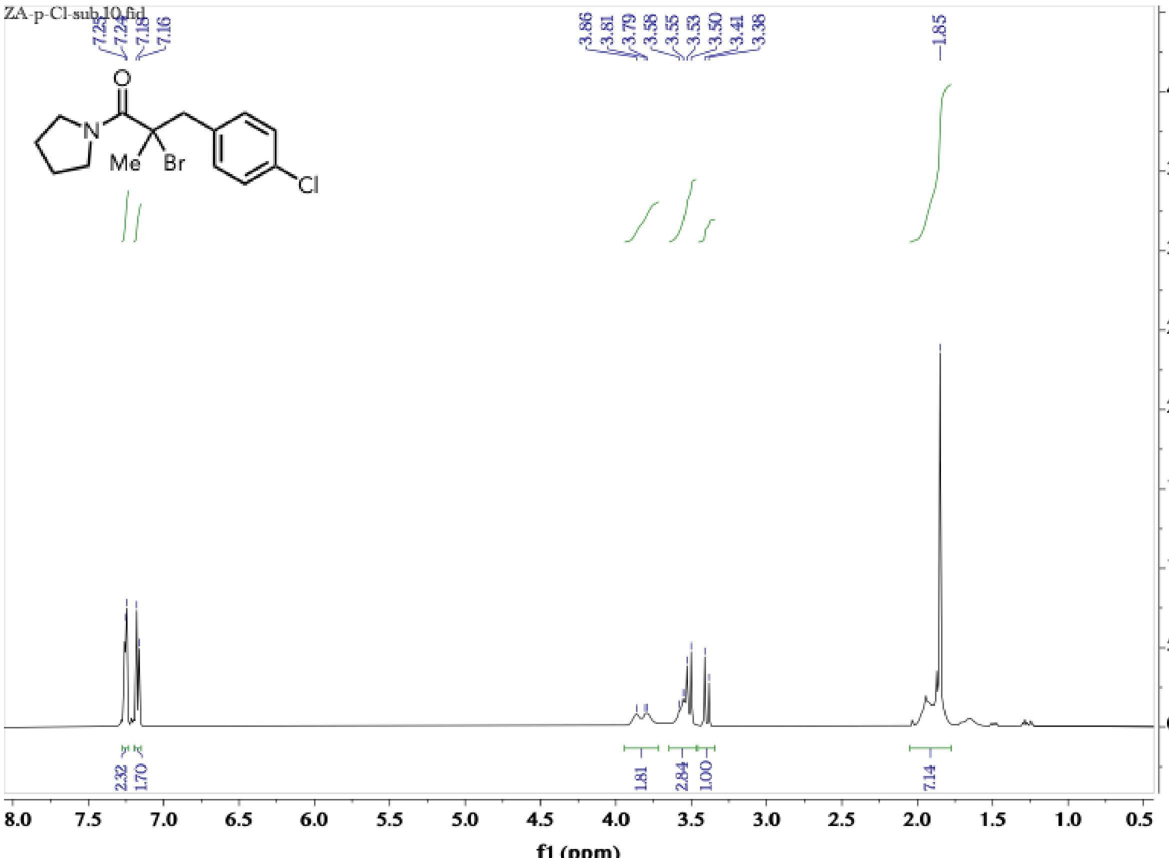

ZA-p-ome-CNMR.12.fid
168.34
158.79
131.58
128.27
113.63
62.98
55.22
48.74
46.83
29.30
27.21
22.91
f1 (ppm)

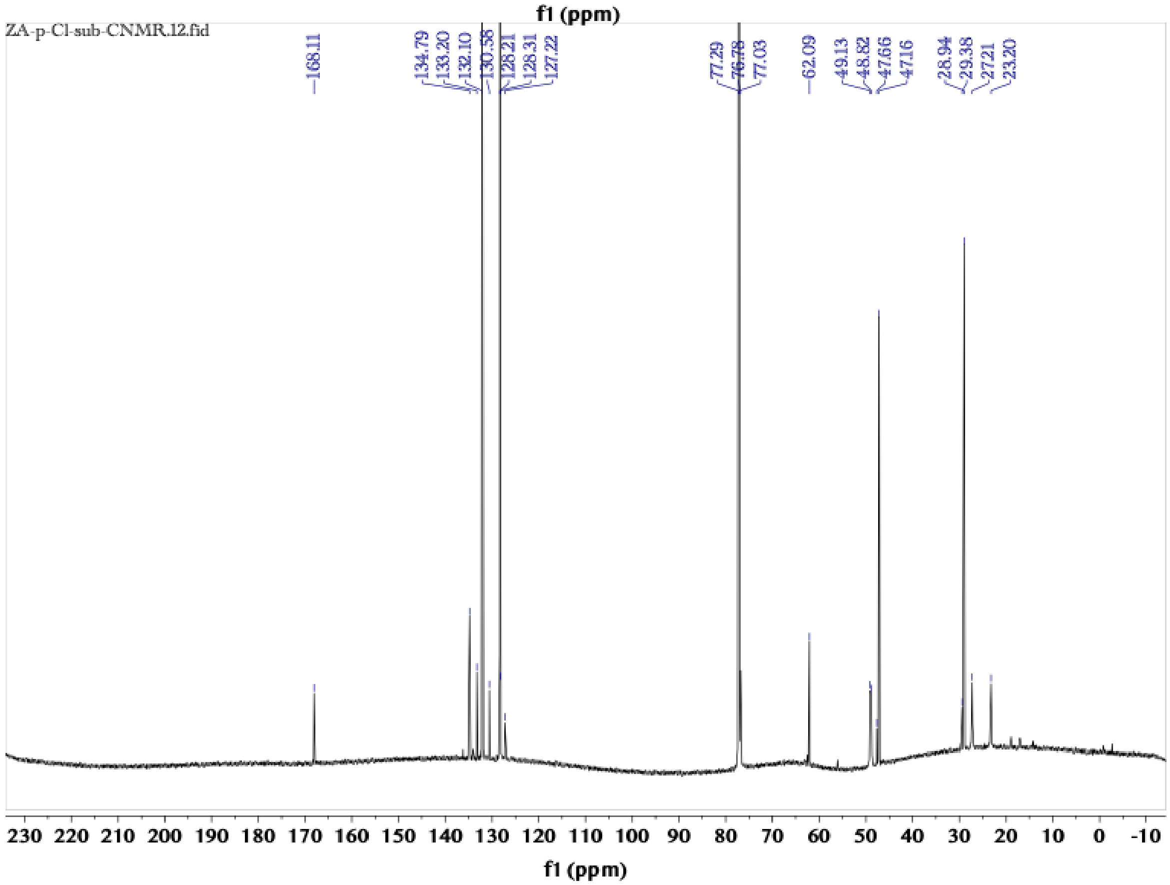

ZA-o-Naph-10.fid
7.66
7.64
7.63
7.63
7.61
7.55
7.33
7.33
7.32
7.32
7.31
7.30
7.23
7.21
7.23
7.21
7.12
3.61
3.58
3.47
3.44
1.79
O
N
Me
Br
3.12
1.02
2.04
1.00
7.20
8.10
f1 (ppm)

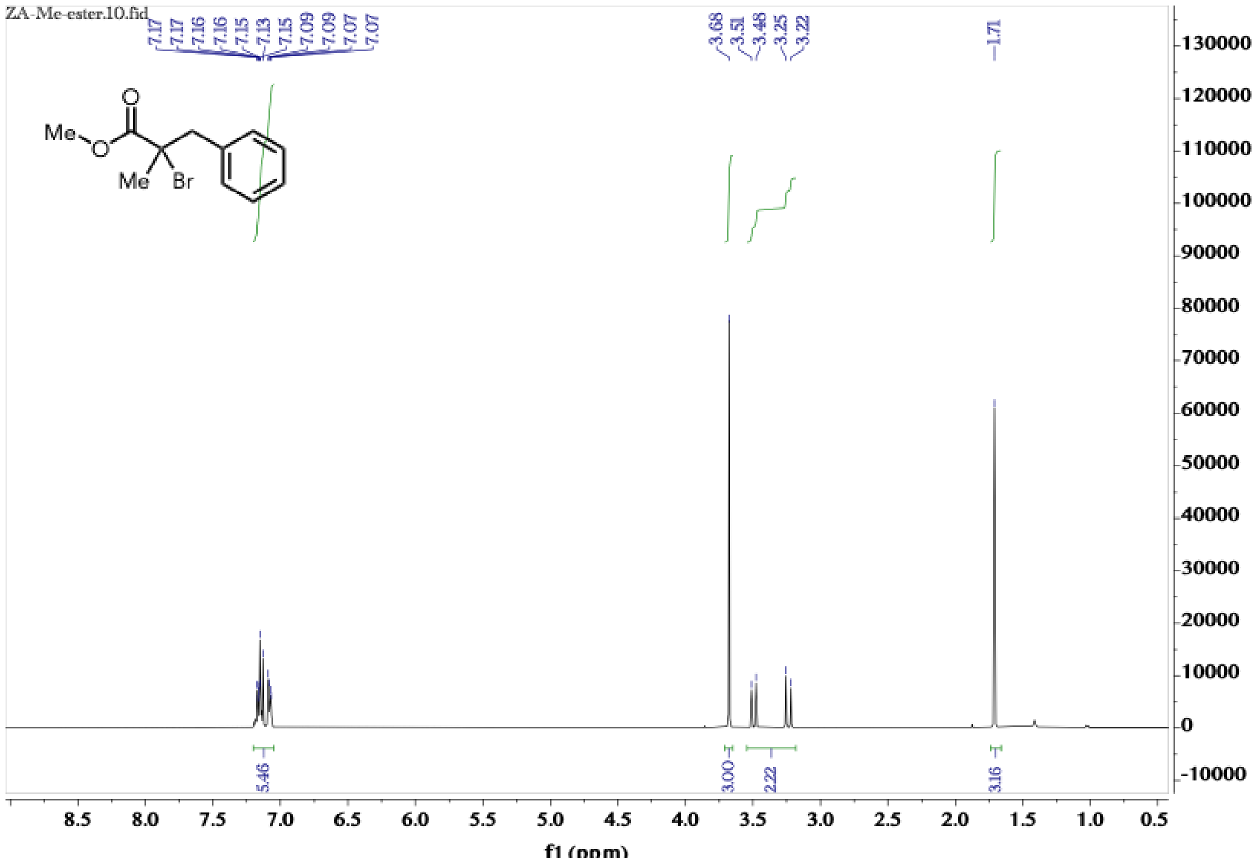

ZA-o-Naph-CNMR.12.fid
166.46
131.88
131.32
130.61
127.68
126.78
125.76
125.87
125.69
124.16
123.94
60.74
47.24
46.98
45.93
27.49
25.30
21.34
1.65
f1 (ppm)

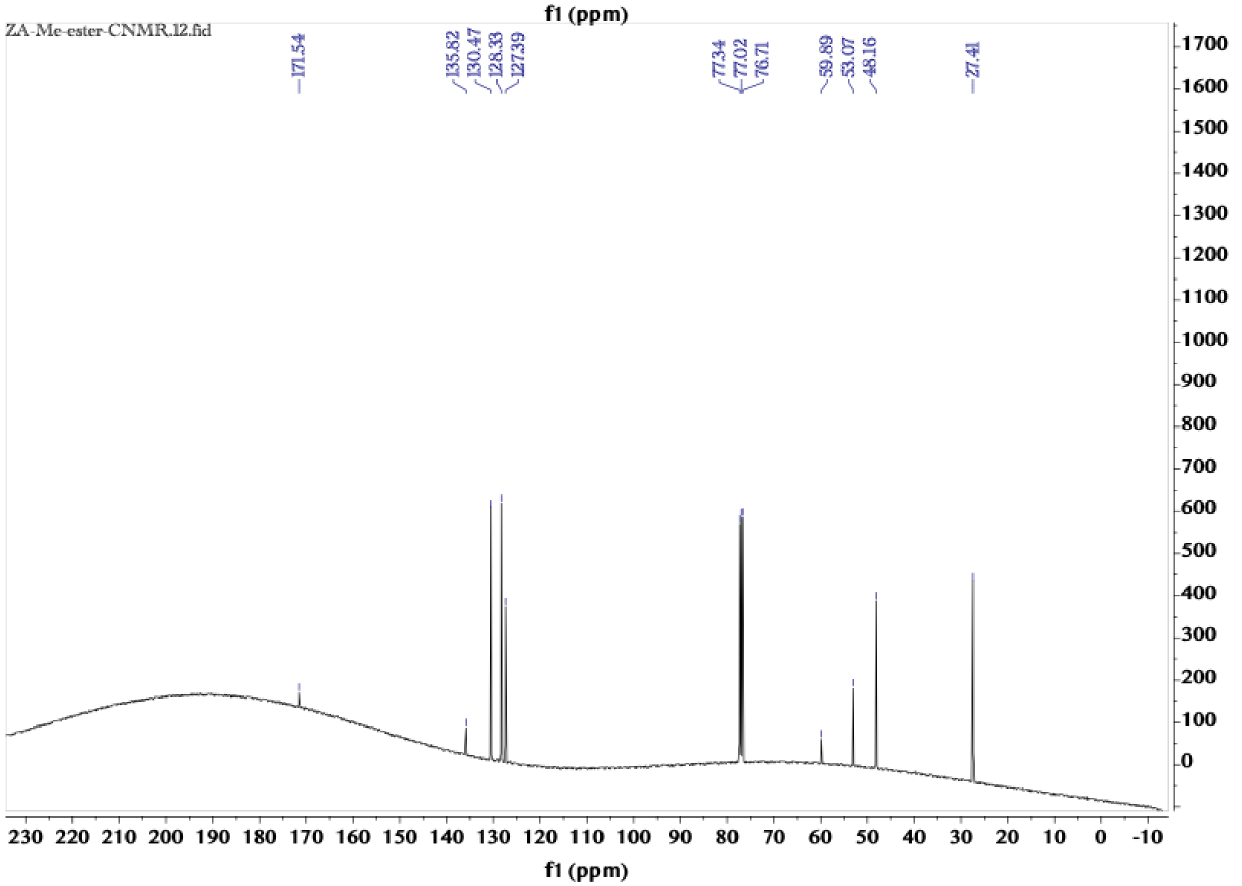

ZA-p-Me-sub.10.fid
7.09
3.98
3.76
3.55
3.52
3.38
3.36
2.31
1.87
Me
Br
Me
4.11
0.78
1.06
2.87
1.00
3.15
7.29
f1 (ppm)


ZA-p-Me-sub-CNMR.12.fid
168.33
136.85
133.08
130.40
77.29
128.93
77.04
76.79
62.79
49.08
48.74
47.19
29.38
27.20
23.24
21.09
f1 (ppm)

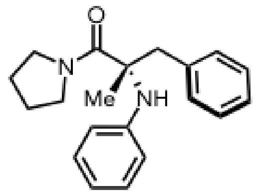

ZA-p-Cl-sub.10.fid
7.25
7.24
7.18
7.16
3.86
3.81
3.79
3.58
3.55
3.53
3.50
3.41
3.38
1.85
O
N
Me
Br
Cl
2.32
1.70
1.81
2.84
1.00
7.14
f1 (ppm)


ZA-p-Cl-sub-CNMR.12.fid
168.11
134.79
133.20
132.10
130.58
128.21
128.31
127.22
77.29
76.78
77.03
62.09
49.13
48.82
47.66
47.16
28.94
29.38
27.21
23.20
f1 (ppm)


ZA-Me-ester.10.fid
7.17
7.17
7.16
7.16
7.15
7.13
7.15
7.09
7.09
7.07
7.07
3.68
3.51
3.48
3.25
3.22
1.71
Me
O
O
Me
Br
5.46
3.00
2.22
3.16
f1 (ppm)


ZA-Me-ester-CNMR.12.fid
171.54
135.82
130.47
128.33
127.39
77.34
77.02
76.71
59.89
53.07
48.16
27.41
f1 (ppm)

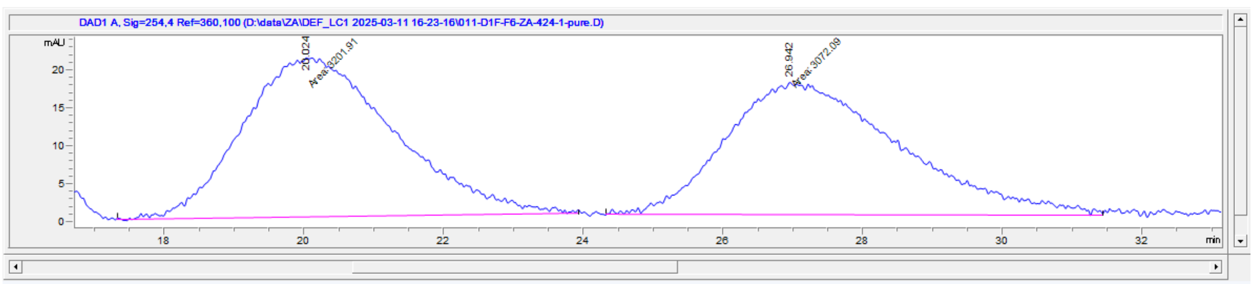
9. HPLC traces



(*S*)-2-methyl-3-phenyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**3**)

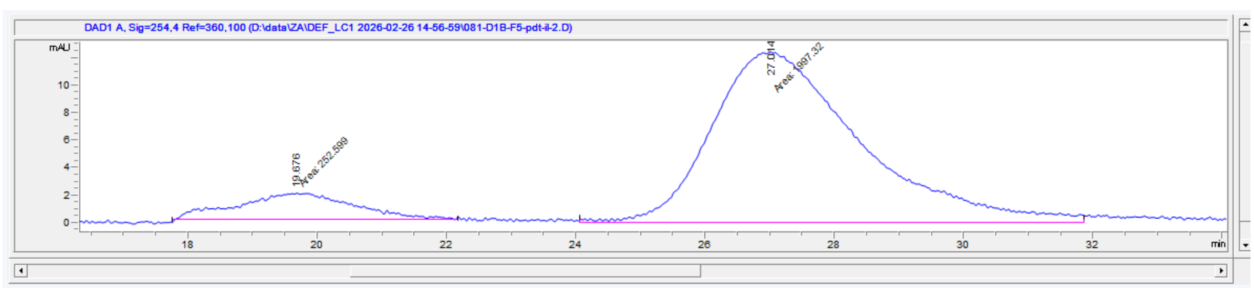
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 99.3:0.7, 1.5 mL/min. isocratic.

Racemate



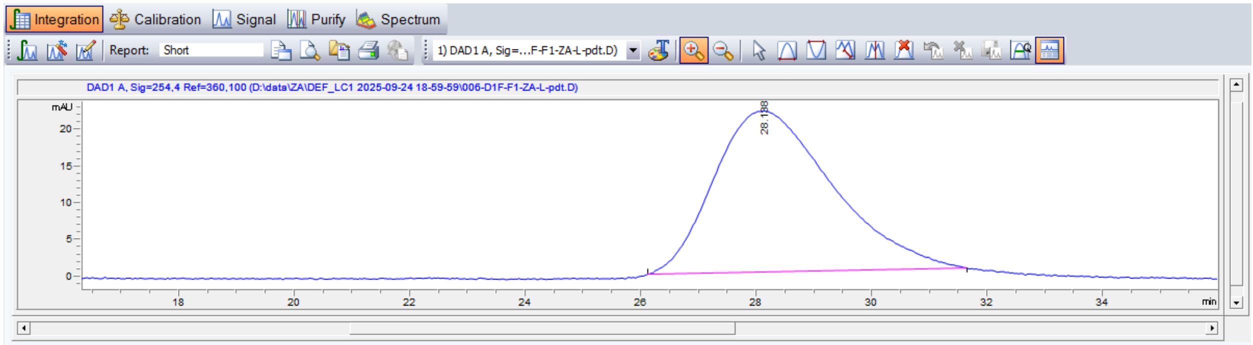
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	20.024	MM	3201.9	21.1	2.5276	51.035	0.718
2	26.942	MM	3072.1	17.6	2.9156	48.965	0.577

Final mutant

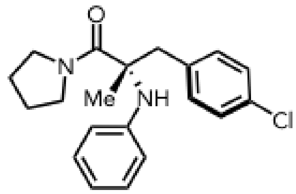


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	19.676	MM	252.6	1.9	2.161	11.227	0.994
2	27.014	MM	1997.3	12.5	2.6647	88.773	0.648

S-product



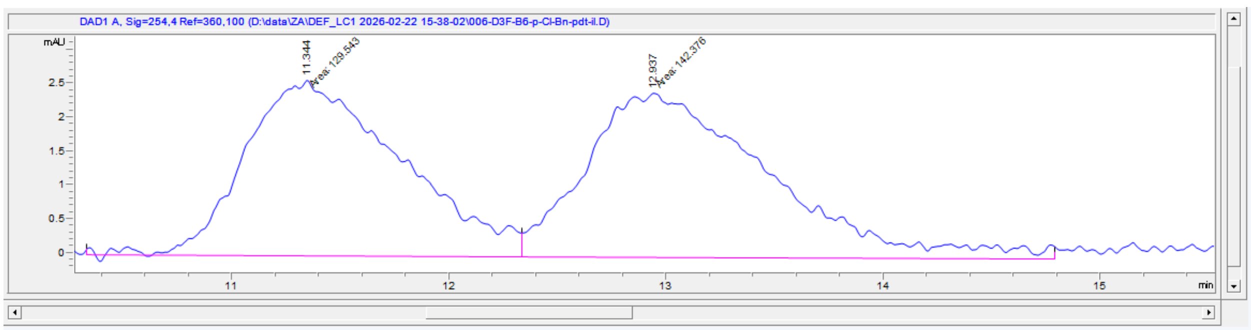
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	28.138	BB	3180.1	22.1	1.6862	100.000	0.715



(*S*)-3-(4-chlorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**5**)

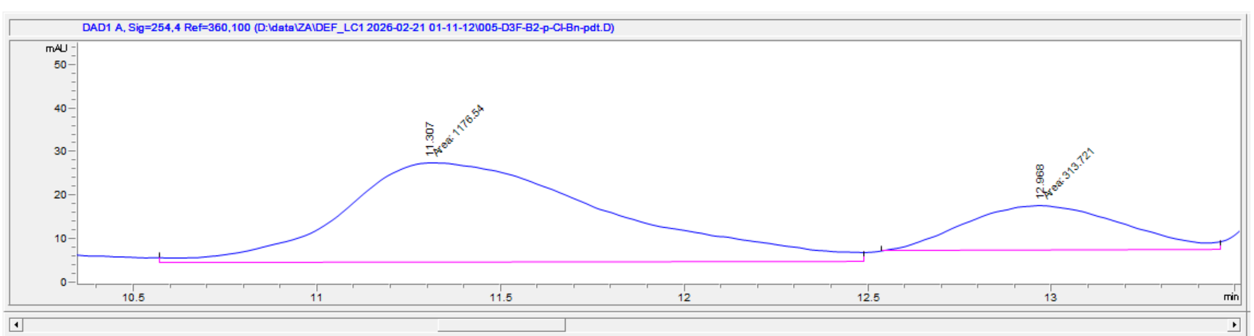
Chiral HPLC: IB column: *n*-hexane/IPA, 96:4, 1 mL/min. isocratic.

Racemate

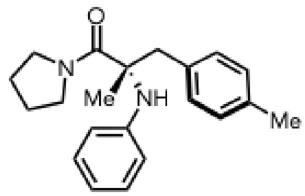


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.344	MF	129.5	2.6	0.8331	47.640	0.657
2	12.937	FM	142.4	2.4	0.9786	52.360	0.569

Final mutant



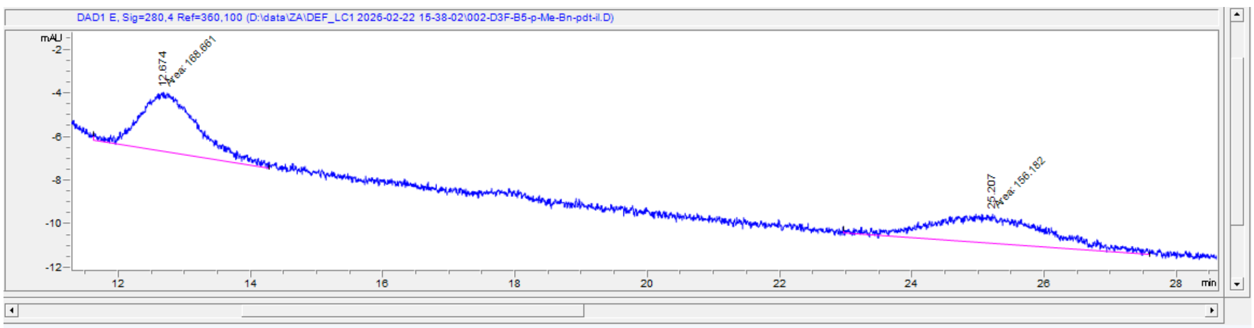
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.307	MM	1176.5	23	0.8512	78.949	0.497
2	12.968	MM	313.7	10.3	0.5054	21.051	0.869



(*S*)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(*p*-tolyl)propan-1-one (**6**)

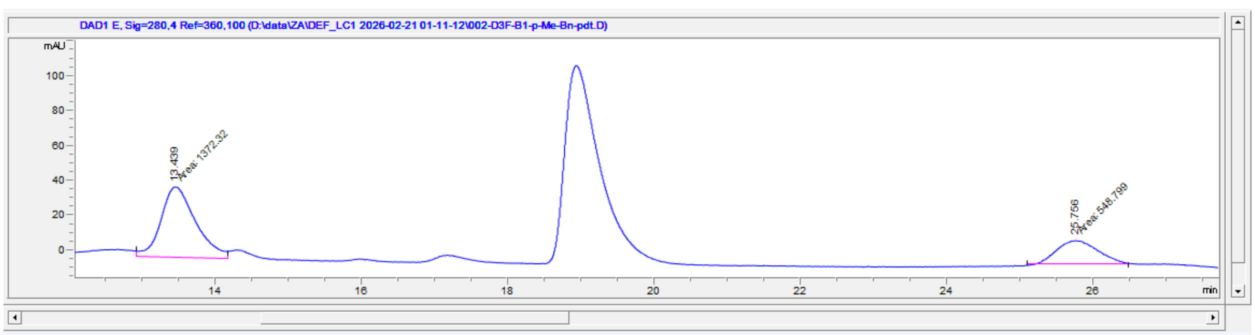
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

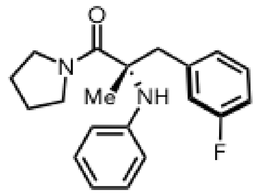


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	12.674	MM	168.7	2.7	1.0258	51.921	0.644
2	25.207	MM	156.2	1.4	1.9169	48.079	0.956

Final mutant



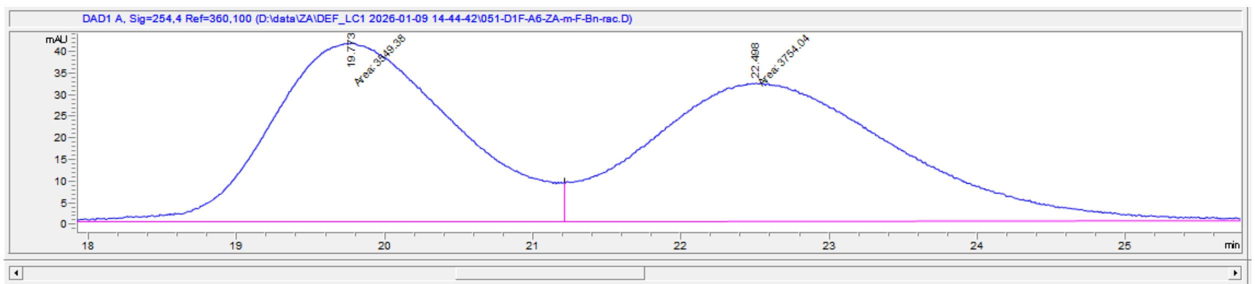
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	13.439	MM	1372.3	41	0.5578	71.433	0.648
2	25.756	MM	548.8	13.9	0.6562	28.567	0.815



(*S*)-3-(3-fluorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**7**)

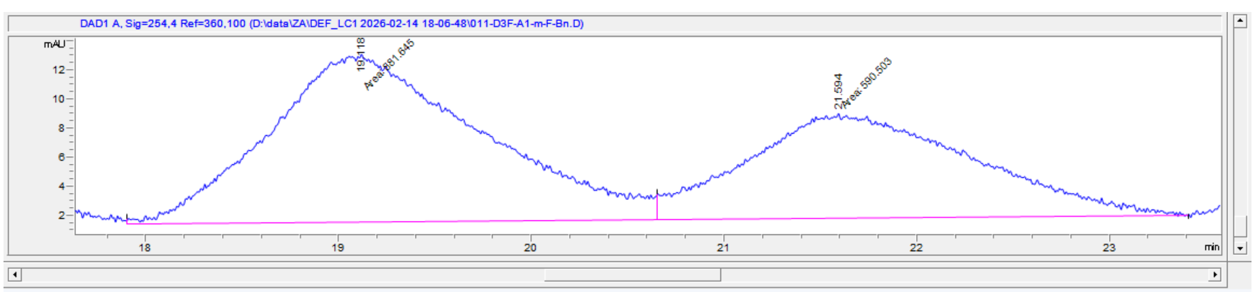
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.

Racemate

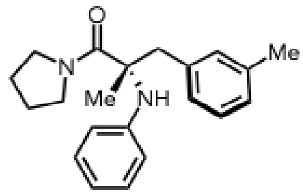


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	19.773	MF	3549.4	41.3	1.432	48.599	0.772
2	22.498	FM	3754	32	1.9563	51.401	0.723

Final mutant



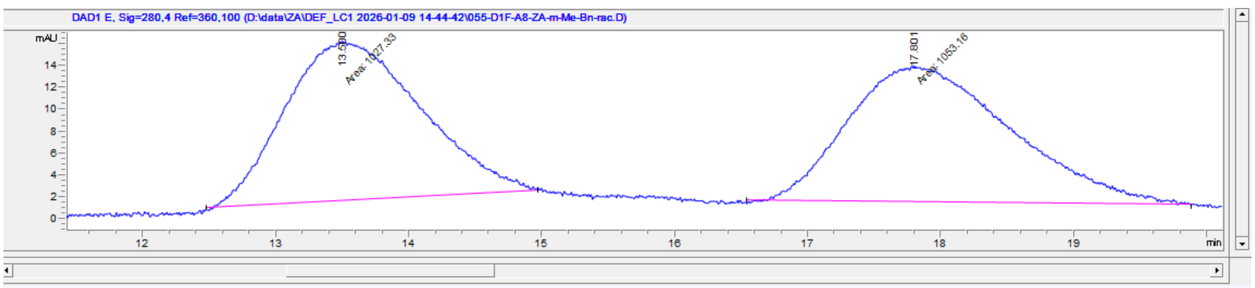
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	19.118	MF	881.6	11.5	1.2797	59.888	0
2	21.594	FM	590.5	7.2	1.3684	40.112	0.671



(*S*)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(*m*-tolyl)propan-1-one (**8**)

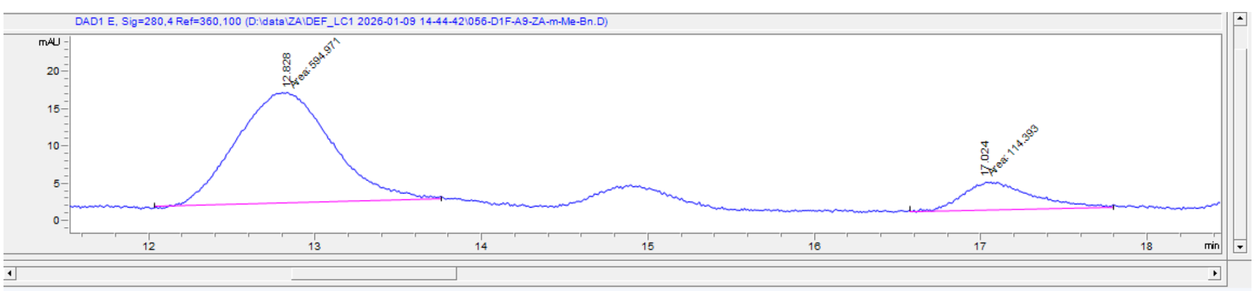
Chiral HPLC: IB column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.

Racemate

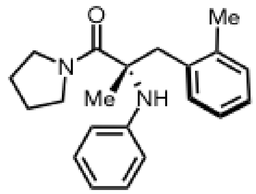


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	13.5	MM	1027.3	14.6	1.1739	49.379	0.741
2	17.801	MM	1053.2	12.4	1.4211	50.621	0.635

Final mutant



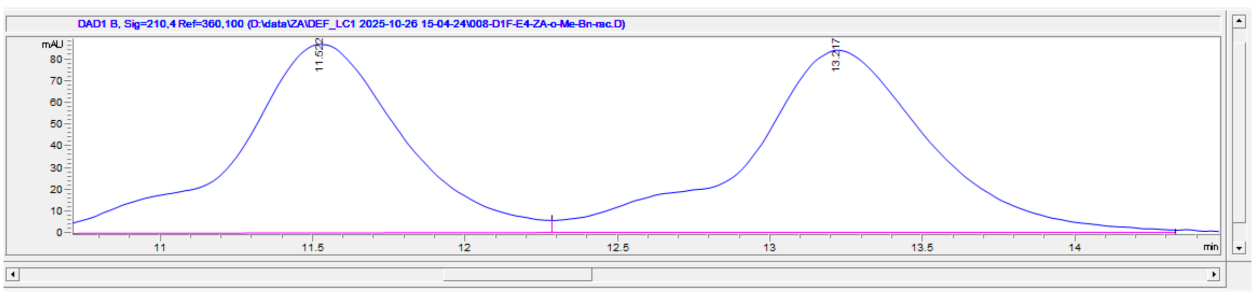
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	12.828	MM	595	14.8	0.6684	83.874	1.067
2	17.024	MM	114.4	3.8	0.4982	16.126	0.497



(*S*)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)-3-(*o*-tolyl)propan-1-one (**10**)

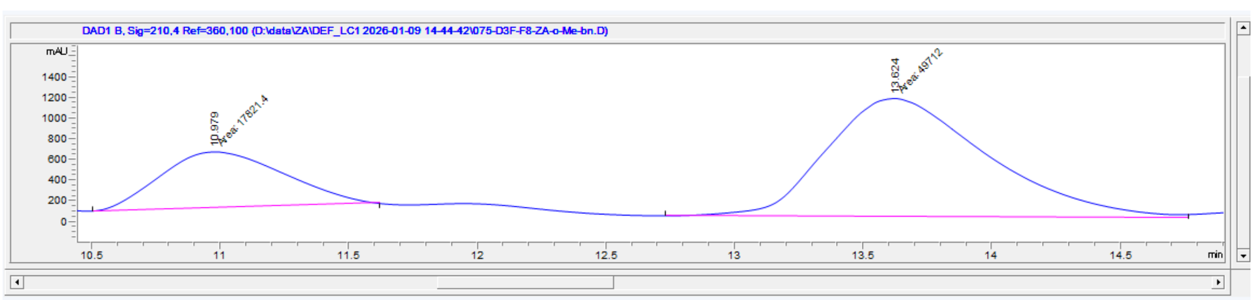
Chiral HPLC: IB column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.

Racemate

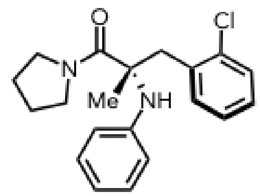


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.522	VV	3329.8	87.3	0.5429	48.660	1.014
2	13.217	VV	3513.2	84.5	0.5731	51.340	0.995

Final mutant



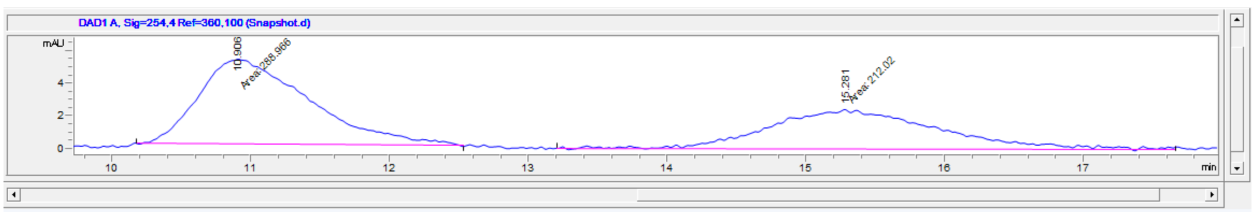
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	10.979	MM	17821.4	537.2	0.5529	26.389	0.804
2	13.624	MM	49712	1139	0.7274	73.611	0.709



(S)-3-(2-chlorophenyl)-2-methyl-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**10**)

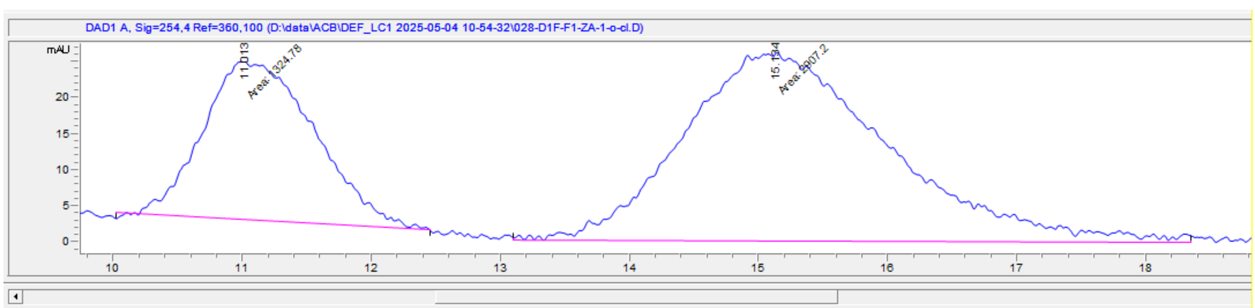
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 91:9, 1 mL/min. isocratic.

Racemate

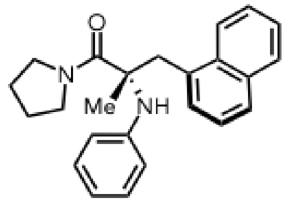


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	10.906	MM	289	5.2	0.9231	57.679	0.545
2	15.281	MM	212	2.5	1.4385	42.321	0.811

Final mutant

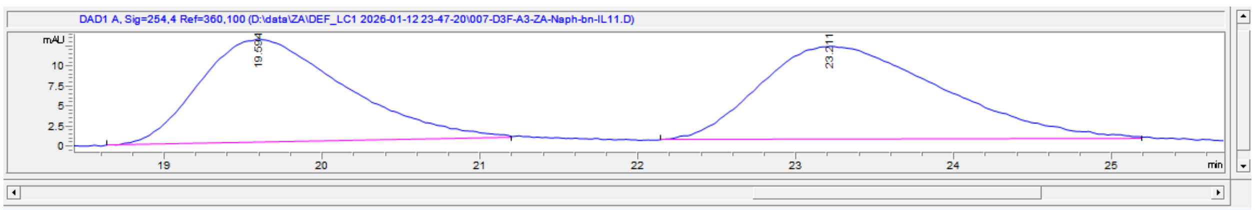


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.013	MM	1324.8	21.8	1.0106	31.304	0.542
2	15.134	MM	2907.2	26.1	1.8555	68.696	0.814

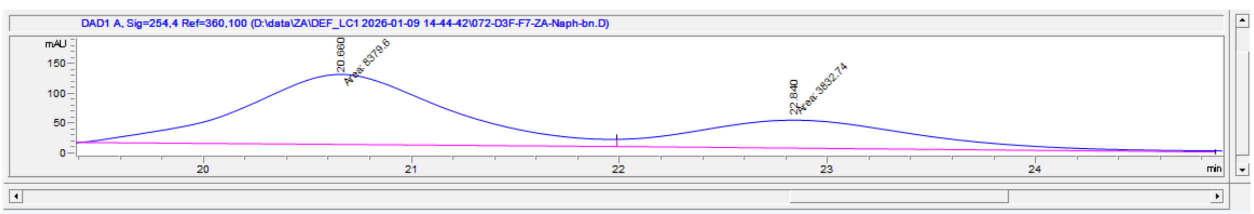


2-methyl-3-(naphthalen-1-yl)-2-(phenylamino)-1-(pyrrolidin-1-yl)propan-1-one (**11**)

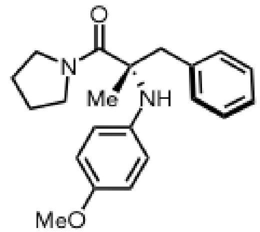
Chiral HPLC: IB column: *n*-hexane/IPA, 96:4, 1 mL/min. isocratic.



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	19.594	BB	821.8	12.8	0.7585	47.025	0.664
2	23.211	BB	925.8	11.6	0.9405	52.975	0.617



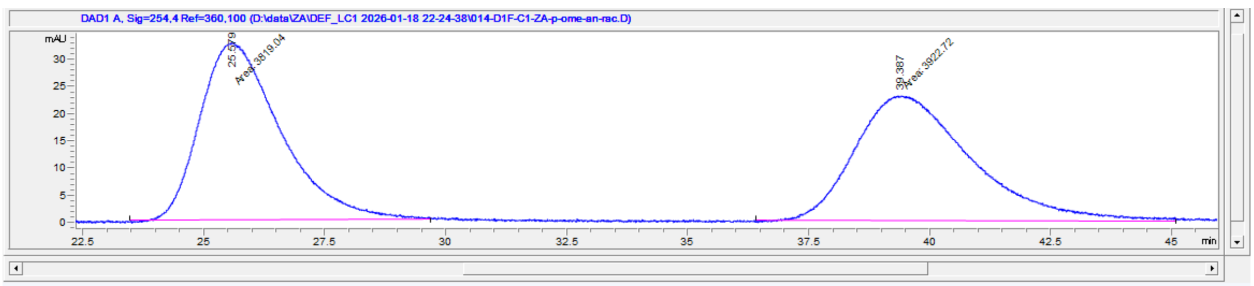
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	20.66	MF	8379.6	120.6	1.158	68.616	0
2	22.84	FM	3832.7	49.2	1.2978	31.384	0.726



(S)-2-((4-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**12**)

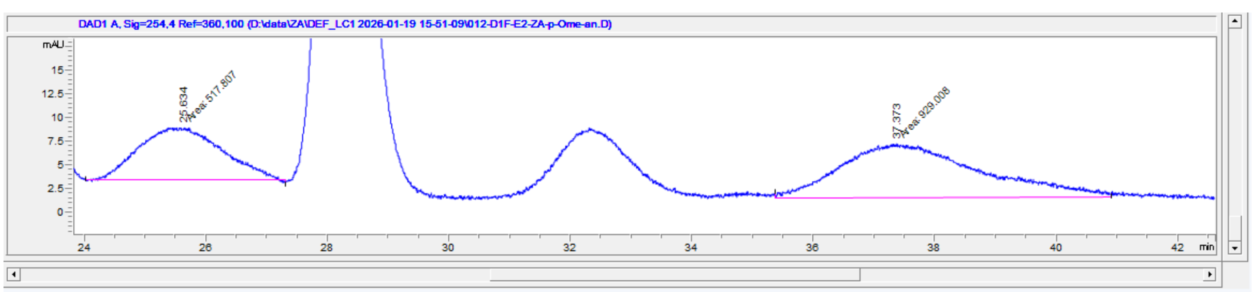
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

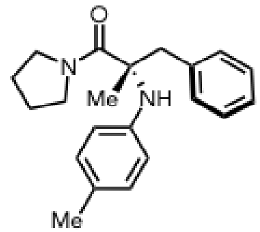


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	25.579	MM	3819	32.8	1.9429	49.330	0.631
2	39.387	MM	3922.7	23.1	2.8244	50.670	0.646

Final mutant



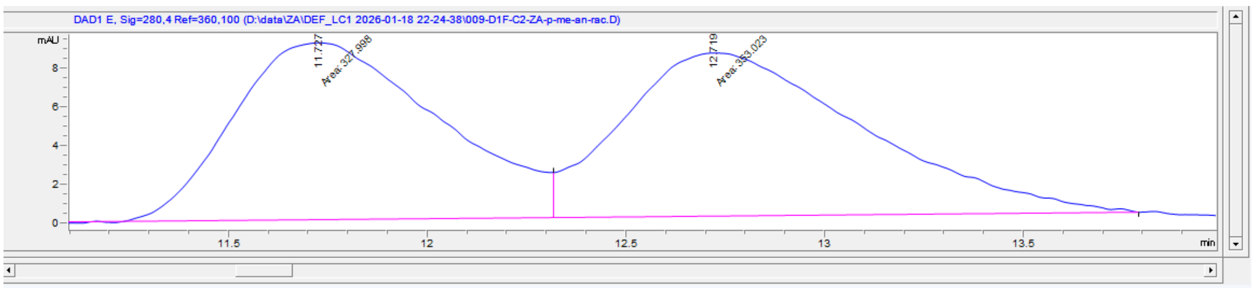
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	25.634	MM	517.8	5.5	1.5778	35.789	1.115
2	37.373	MM	929	5.8	2.6774	64.211	0.665



(S)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*p*-tolylamino)propan-1-one (**13**)

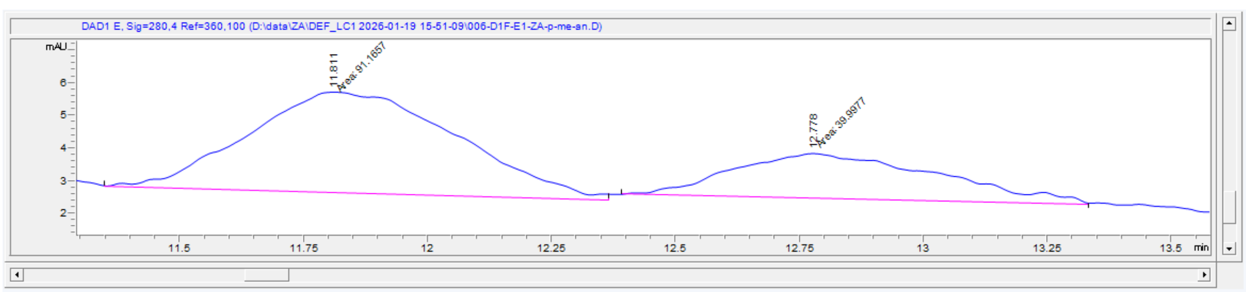
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

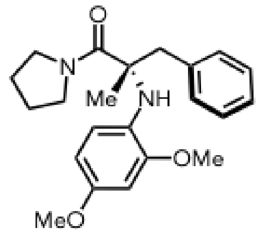


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.727	MF	328	9.1	0.6019	48.163	0.701
2	12.719	FM	353	8.4	0.704	51.837	0.603

Final mutant



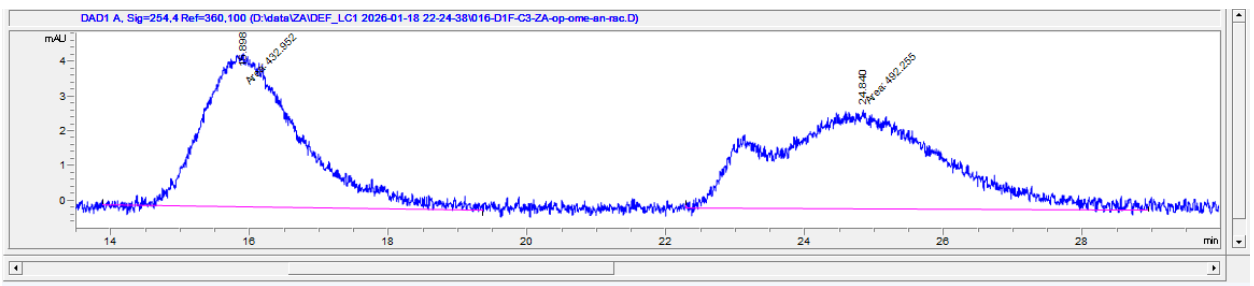
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.811	MM	91.2	3.1	0.4941	69.505	0.671
2	12.778	MM	40	1.4	0.486	30.495	0.625



(S)-2-((2,4-dimethoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**14**)

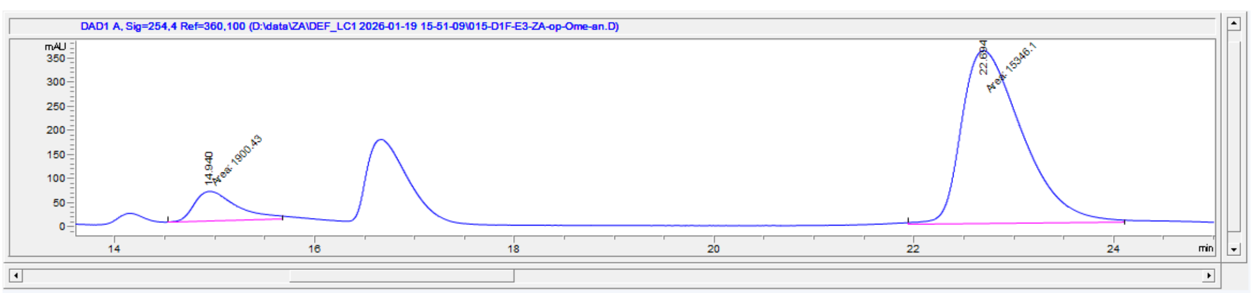
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

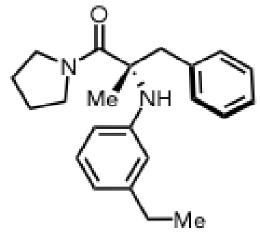


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.898	MM	433	4.5	1.6211	46.795	0.677
2	24.84	MM	492.3	2.9	2.8557	53.205	1.069

Final mutant



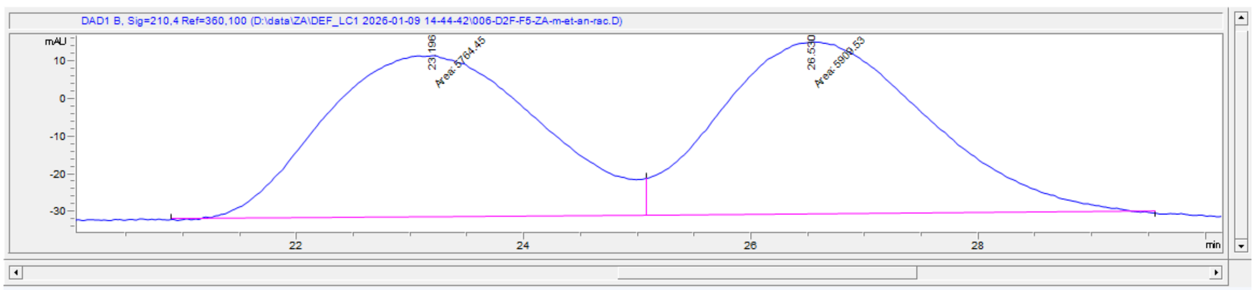
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.94	MM	1900.4	62.7	0.505	11.019	0.526
2	22.694	MM	15346.1	361.3	0.708	88.981	0.539



(S)-2-((3-ethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**15**)

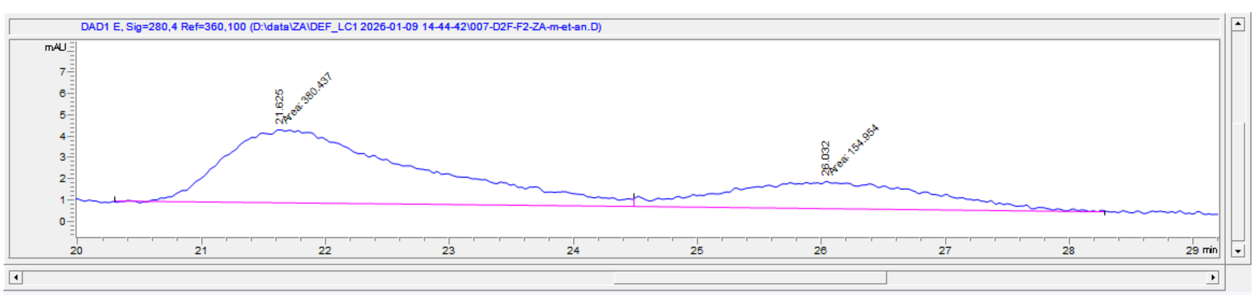
Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.

Racemate

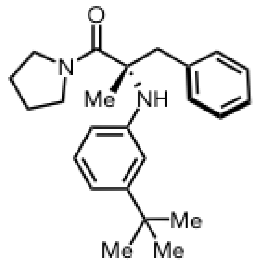


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	23.196	MF	5764.4	43.4	2.2153	49.379	0
2	26.53	FM	5909.5	46.3	2.128	50.621	0.776

Final mutant



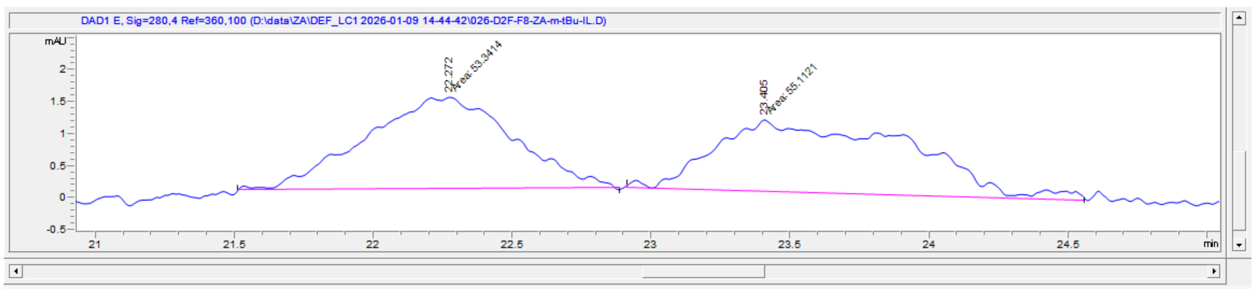
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	21.625	MF	380.4	3.5	1.8326	71.058	0
2	26.032	FM	155	1.3	1.9798	28.942	0.924



(S)-2-((3-(*tert*-butyl)phenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**16**)

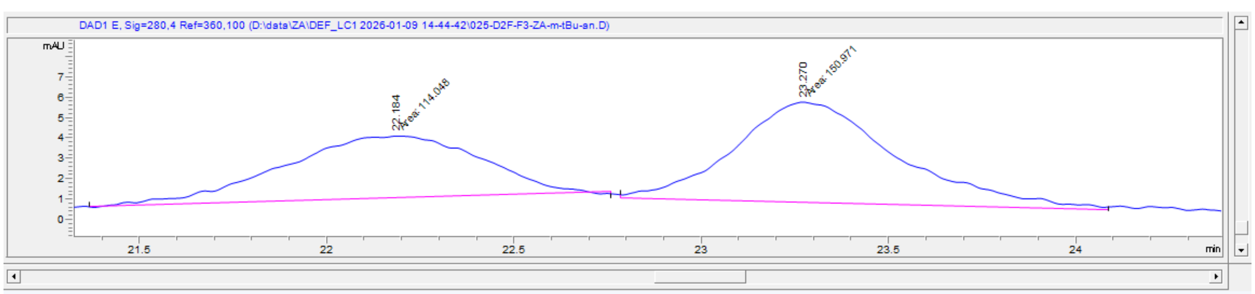
Chiral HPLC: IC column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.

Racemate

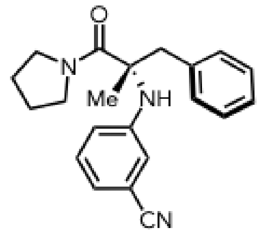


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	22.272	MM	53.3	1.4	0.6204	49.184	1.274
2	23.405	MM	55.1	1.1	0.818	50.816	0.351

Final mutant



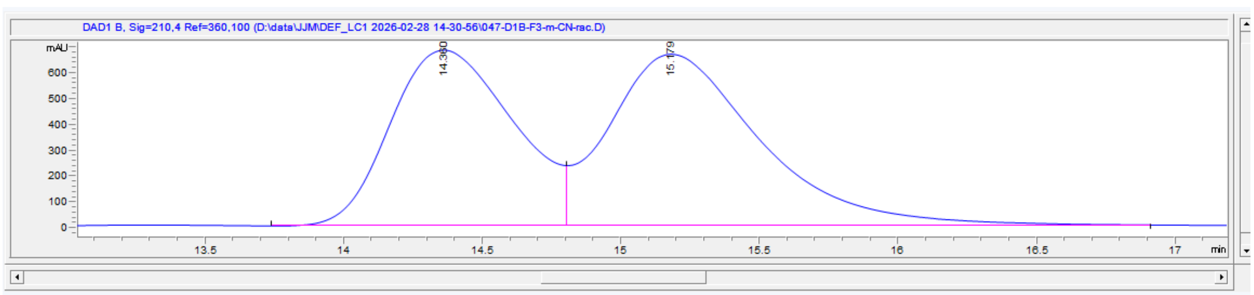
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	22.184	MM	114	3.1	0.6227	43.034	1.364
2	23.27	MM	151	4.9	0.5107	56.966	0.699



(S)-3-((2-methyl-1-oxo-3-phenyl-1-(pyrrolidin-1-yl)propan-2-yl)amino)benzonitrile (**17**)

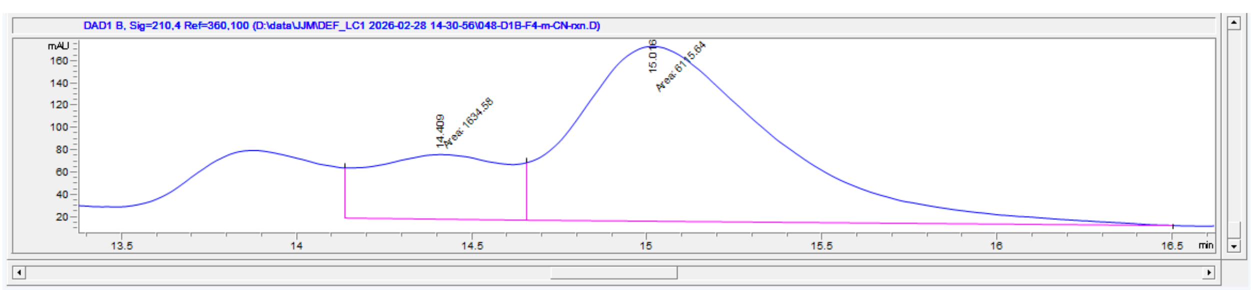
Chiral HPLC: IA column: *n*-hexane/IPA, 92:8, 1 mL/min. isocratic.

Racemate

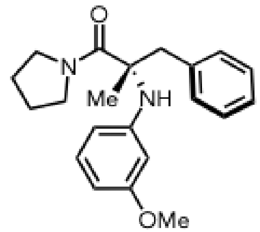


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.36	VV	20955.1	676.3	0.4835	45.567	0.748
2	15.179	VB	25032	660.2	0.5544	54.433	0.653

Final mutant



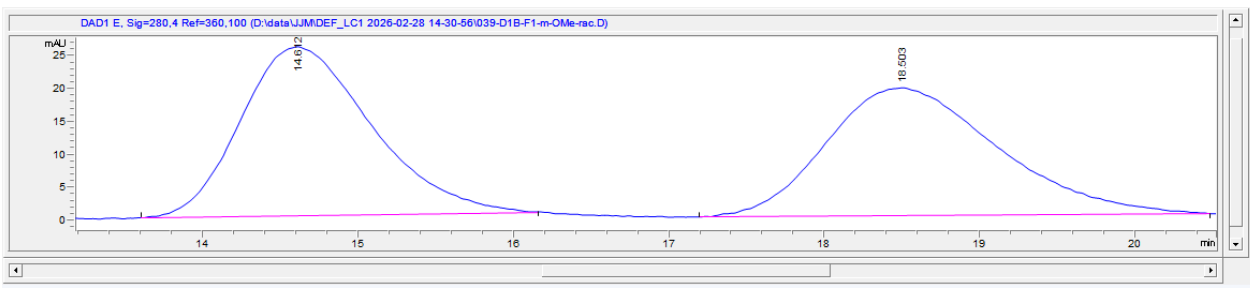
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.409	FM	1634.6	58.5	0.4654	21.091	1.014
2	15.016	FM	6115.6	157.3	0.6481	78.909	0.598



(S)-2-((3-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**18)**

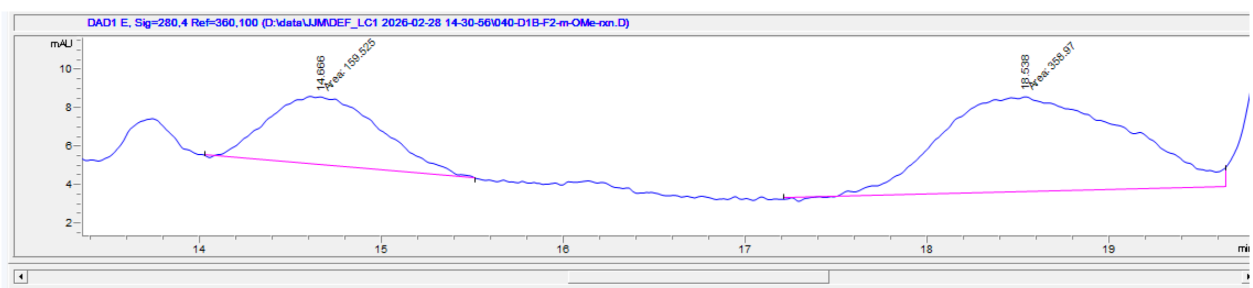
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 93:7, 1 mL/min. isocratic.

Racemate

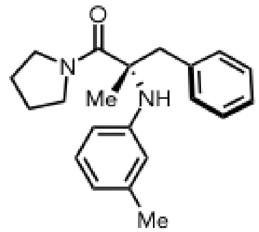


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.612	BB	1513.9	25.8	0.7015	50.354	0.749
2	18.503	BB	1492.6	19.4	0.9142	49.646	0.791

Final mutant



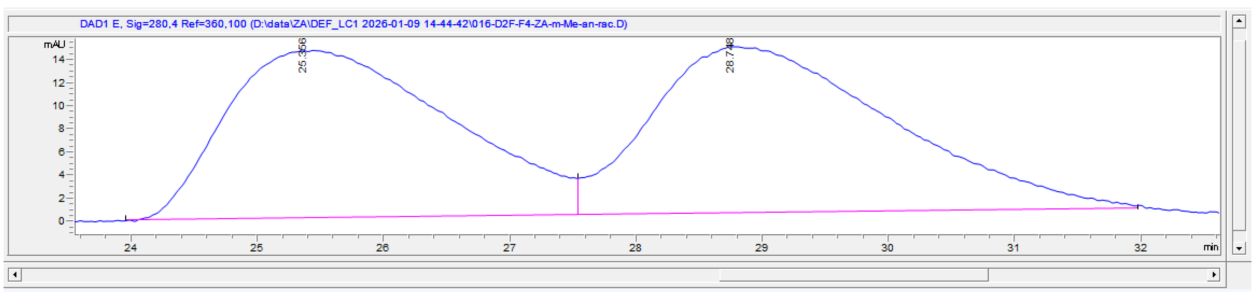
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.666	MM	159.5	3.6	0.7384	30.767	0.812
2	18.538	MM	359	5	1.2011	69.233	0.837



(S)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*m*-tolylamino)propan-1-one (**19**)

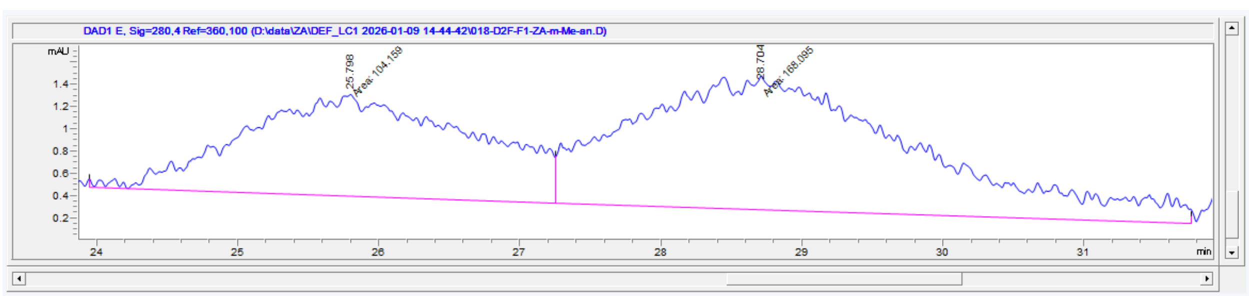
Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.

Racemate

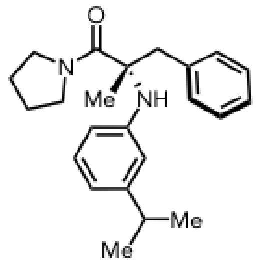


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	25.356	BV	1800.6	14.6	1.4537	48.397	0.516
2	28.748	VB	1919.9	14.4	1.5671	51.603	0.498

Final mutant



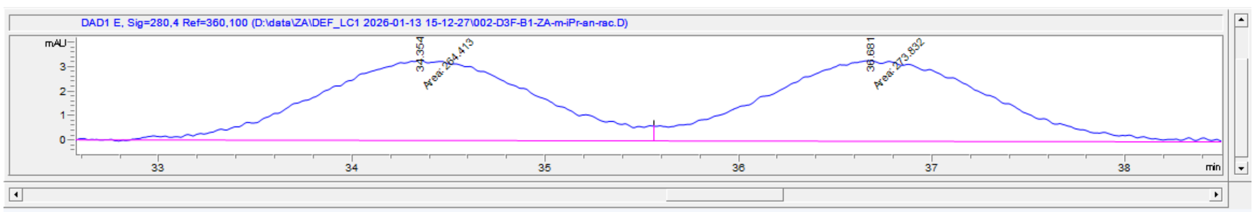
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	25.798	MF	104.2	9.2E-1	1.8874	38.258	0.845
2	28.704	FM	168.1	1.2	2.319	61.742	0.778



(S)-2-((3-isopropylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**20**)

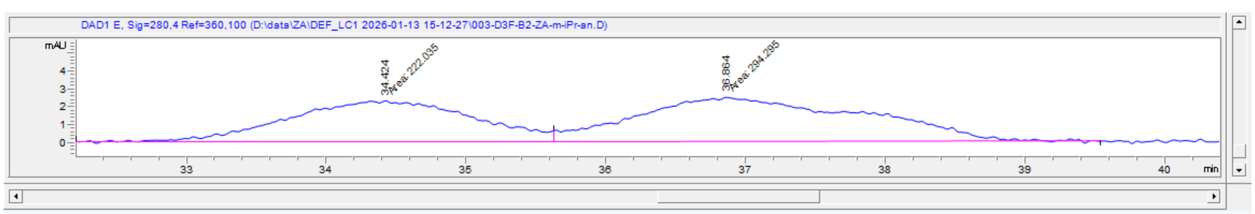
Chiral HPLC: IC column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

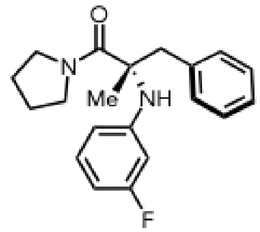


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	34.354	MF	264.4	3.3	1.3201	49.125	0.902
2	36.681	FM	273.8	3.4	1.3595	50.875	0.855

Final mutant



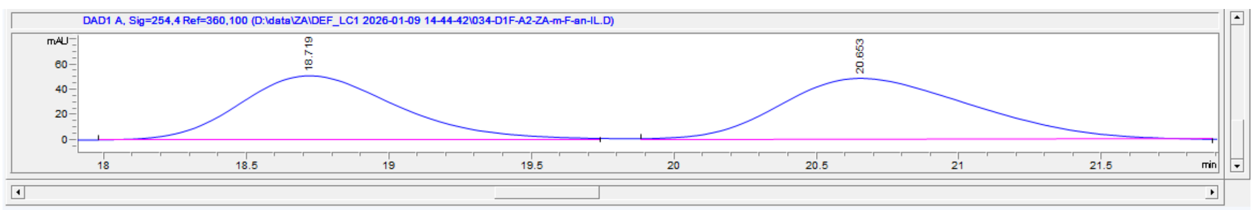
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	34.424	MF	222	2.4	1.5712	43.003	0
2	36.864	FM	294.3	2.6	1.9227	56.997	0.641



(S)-2-((3-fluorophenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**21**)

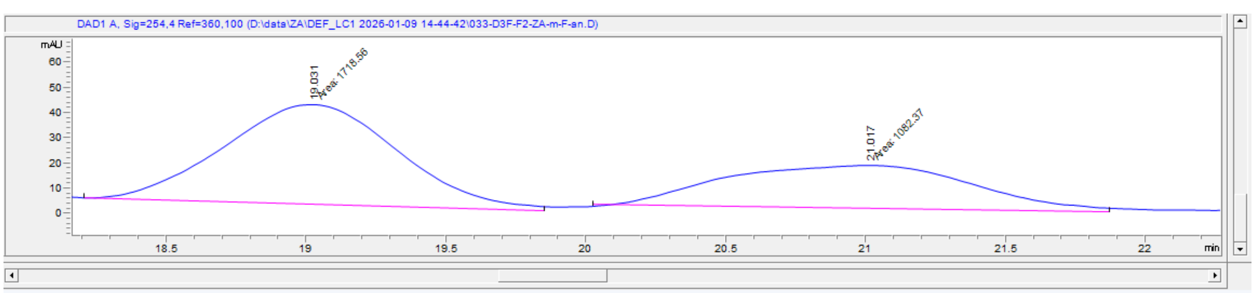
Chiral HPLC: IA column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

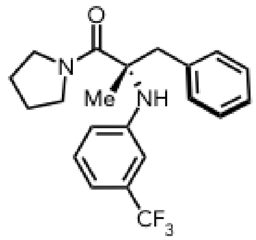


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	18.719	BB	1948	50.4	0.5613	46.082	0.75
2	20.653	BB	2279.3	48.2	0.6513	53.918	0.684

Final mutant



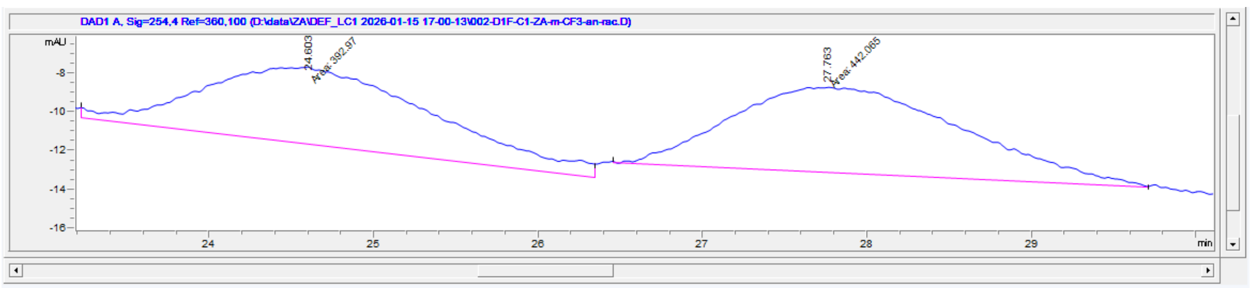
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	19.031	MM	1718.6	39.7	0.7222	61.357	1.003
2	21.017	MM	1082.4	17.3	1.0398	38.643	1.266



(S)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-((3-(trifluoromethyl)phenyl)amino)propan-1-one (**22**)

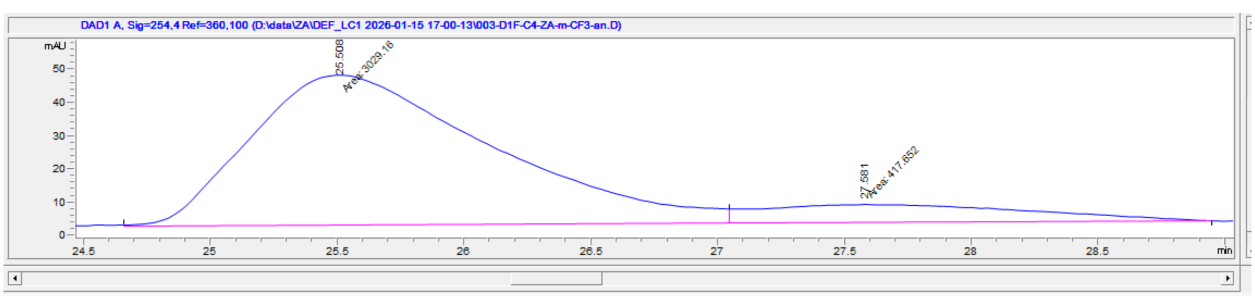
Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.

Racemate

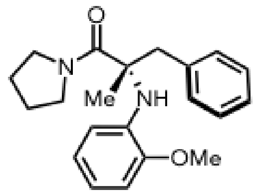


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	24.603	MM	393	4	1.6449	47.060	0.758
2	27.763	MM	442.1	4.4	1.6634	52.940	0.696

Final mutant



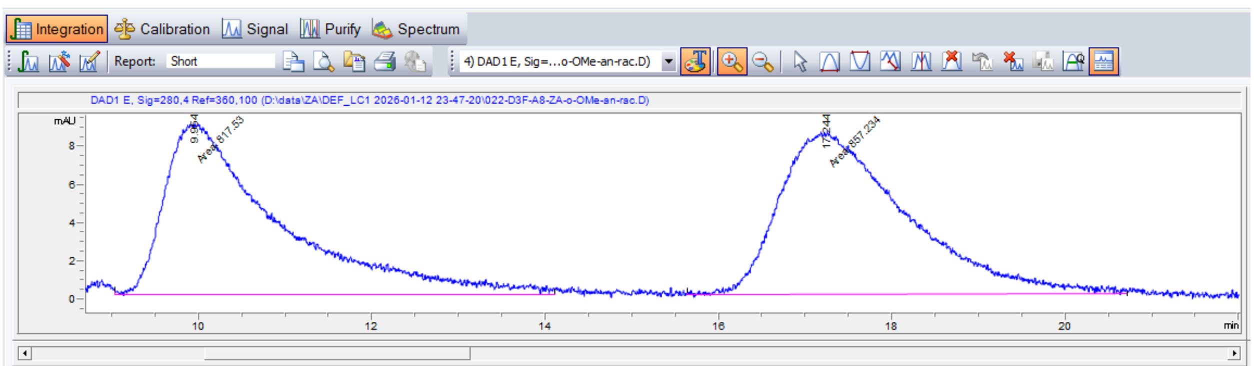
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	25.508	MF	3029.2	45.6	1.1062	87.883	0.571
2	27.581	FM	417.7	5.6	1.2322	12.117	0.62



(S)-2-((2-methoxyphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**23)**

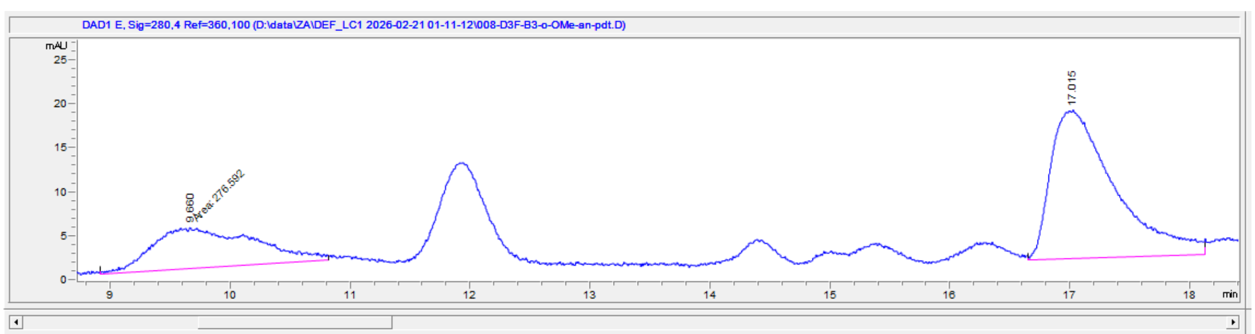
Chiral HPLC: OJ-H column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

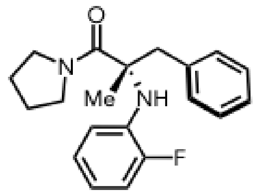


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	9.954	MM	817.5	8.9	1.5273	48.815	0.355
2	17.244	MM	857.2	8.5	1.6742	51.185	0.581

Final mutant



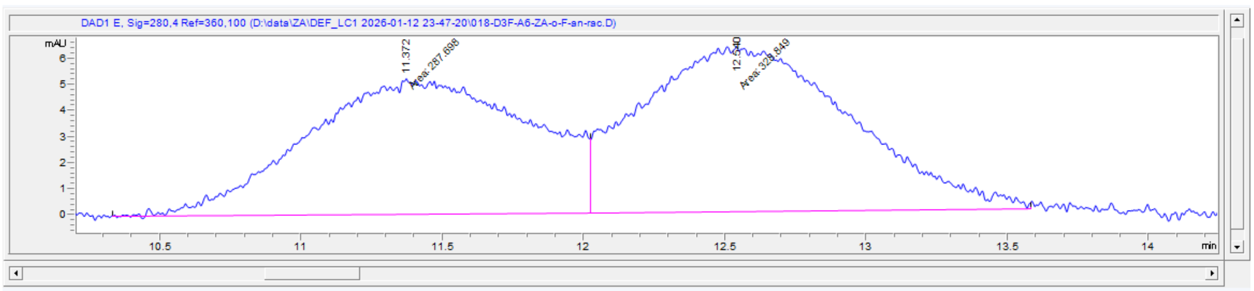
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	9.66	MM	276.6	4.7	0.9839	30.577	0.631
2	17.015	VB	628	16.9	0.4397	69.423	0.512



(S)-2-((2-fluorophenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**24**)

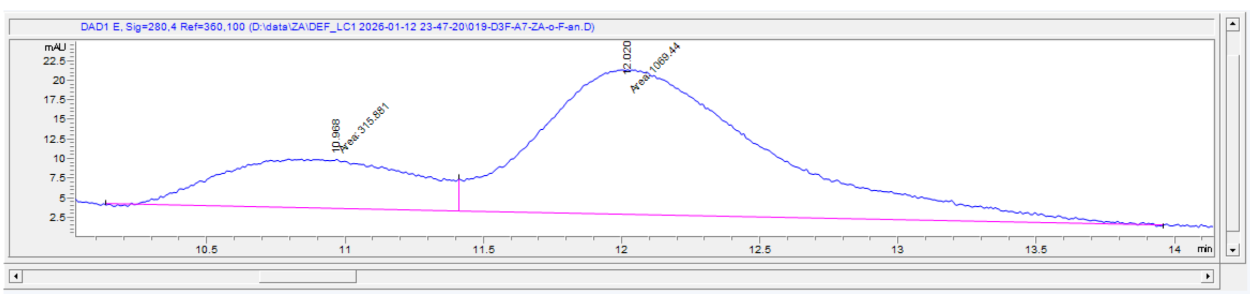
Chiral HPLC: IB column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.

Racemate

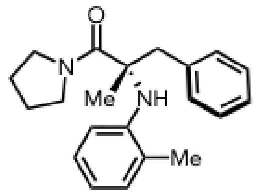


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	11.372	MF	287.7	5.2	0.9199	46.663	0.824
2	12.54	FM	328.8	6.3	0.8632	53.337	0.8

Final mutant



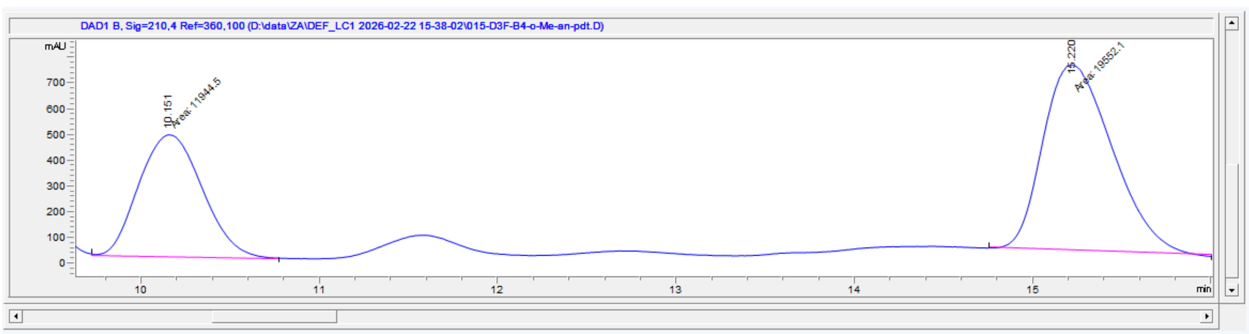
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	10.968	MF	315.9	6.4	0.8276	22.802	0
2	12.02	FM	1069.4	18.6	0.9584	77.198	0.615



(S)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)-2-(*o*-tolylamino)propan-1-one (**25**)

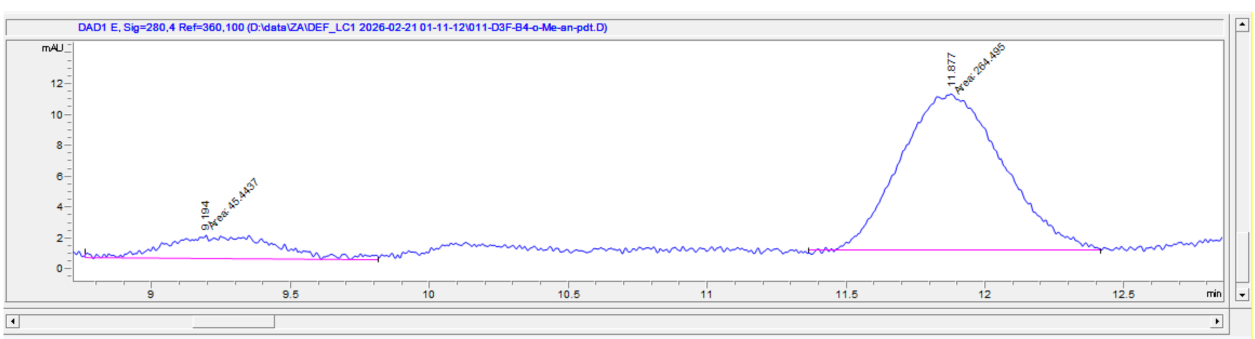
Chiral HPLC: OJ column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate

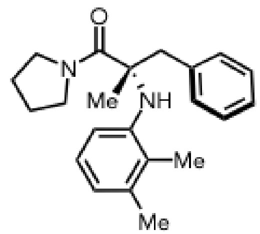


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	10.151	MM	11944.5	478.7	0.4159	37.923	0.85
2	15.22	MM	19552.1	724.1	0.45	62.077	0.706

Final mutant



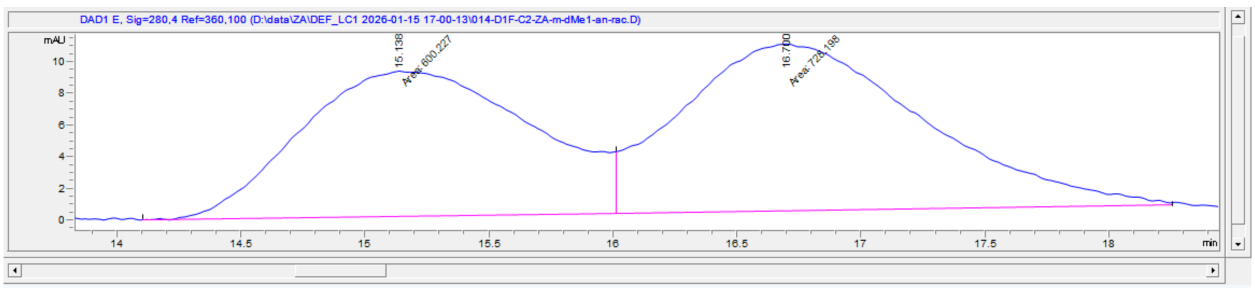
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	9.194	MM	45.4	1.6	0.4831	14.662	0.603
2	11.877	MM	264.5	10.2	0.4323	85.338	0.94



(S)-2-((2,3-dimethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**26**)

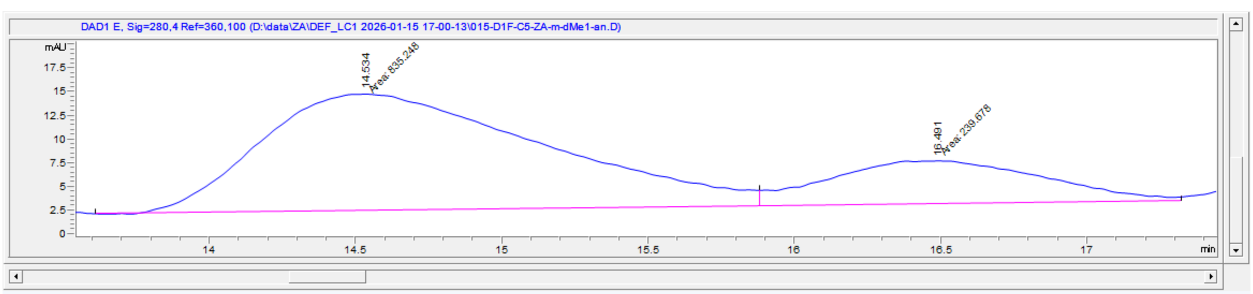
Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.

Racemate

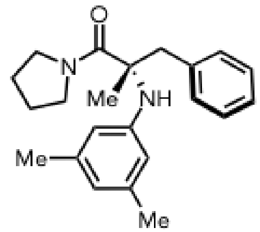


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.138	MF	600.2	9.2	1.0817	45.183	0
2	16.7	FM	728.2	10.6	1.1398	54.817	0.742

Final mutant



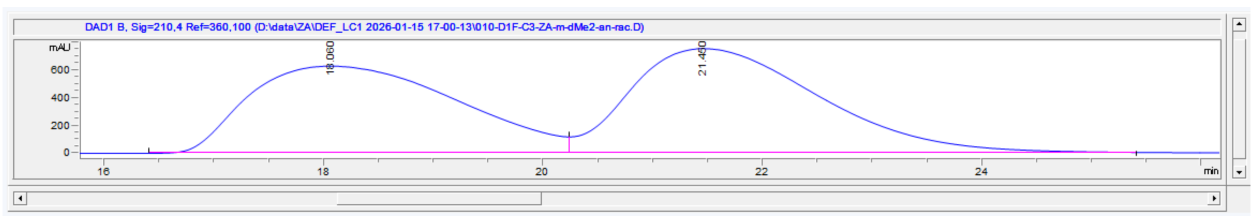
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	14.534	MF	835.2	12.4	1.1251	77.703	0.577
2	16.491	FM	239.7	4.6	0.8701	22.297	0.992



(S)-2-((3,5-dimethylphenyl)amino)-2-methyl-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**27)**

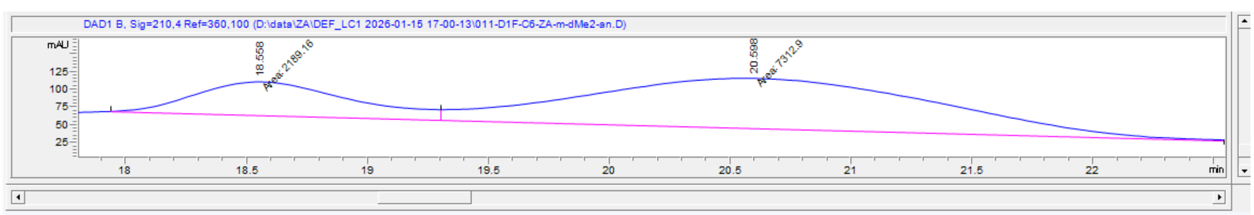
Chiral HPLC: IB column: *n*-hexane/IPA, 99:1, 1 mL/min. isocratic.

Racemate

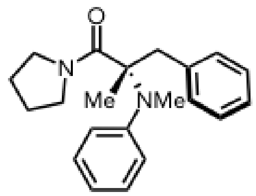


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	18.06	BV	84105.4	634.8	1.5558	48.123	0.626
2	21.45	VB	90665.1	760.8	1.4163	51.877	0.589

Final mutant



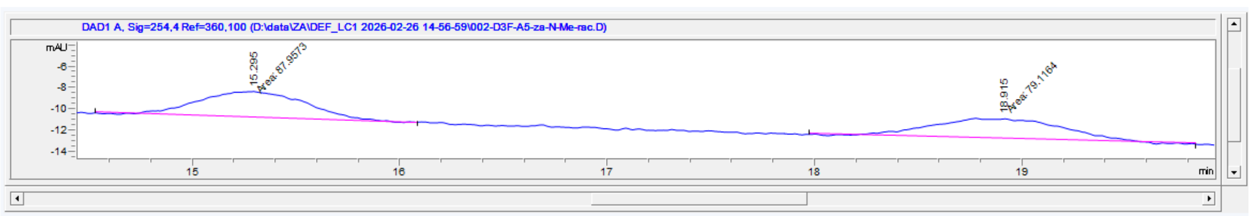
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	18.558	MF	2189.2	48	0.7607	23.039	0.653
2	20.598	FM	7312.9	71.4	1.7076	76.961	0.858



(*S*)-2-methyl-2-(methyl(phenyl)amino)-3-phenyl-1-(pyrrolidin-1-yl)propan-1-one (**28**)

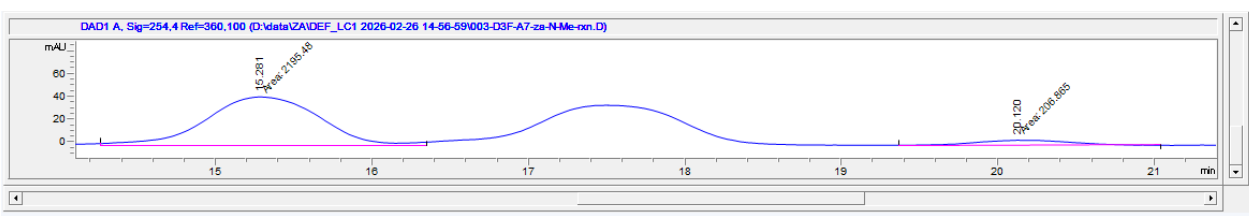
Chiral HPLC: IA column: *n*-hexane/IPA, 98:2, 1 mL/min. isocratic.

Racemate

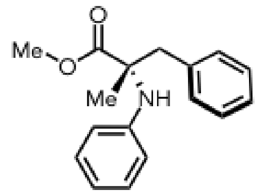


#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.295	MM	88	2.4	0.607	52.646	0.93
2	18.915	MM	79.1	1.8	0.7271	47.354	0.535

Final mutant



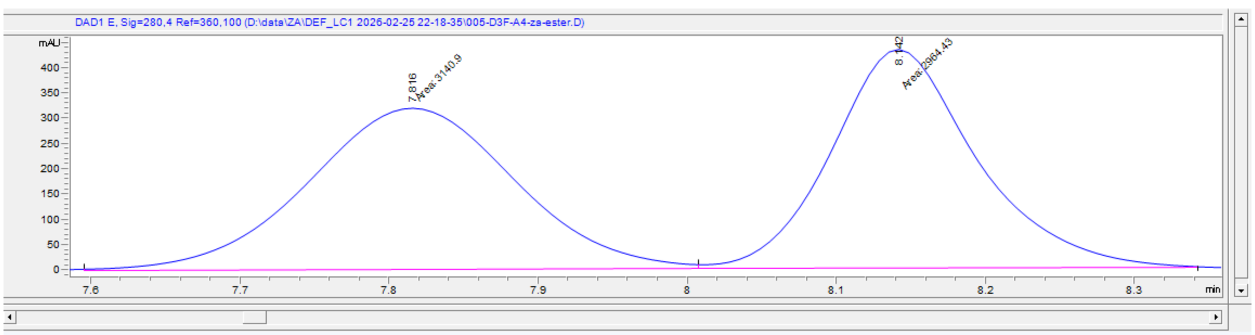
#	Time	Type	Area	Height	Width	Area%	Symmetry
1	15.281	MM	2195.5	43	0.8511	91.389	0.907
2	20.12	MM	206.9	4.7	0.7391	8.611	0.901



methyl (*S*)-2-methyl-3-phenyl-2-(phenylamino)propanoate (**30**)

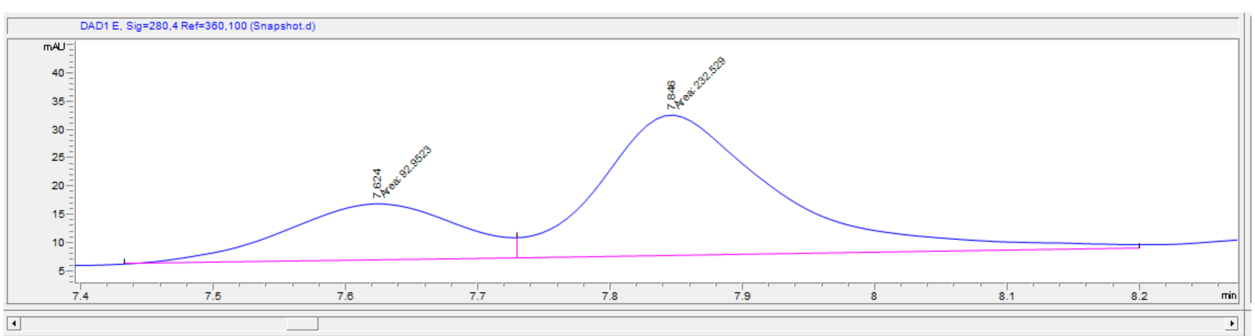
Chiral HPLC: IA column: *n*-hexane/IPA, 97:3, 1 mL/min. isocratic.

Racemate



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	7.816	MF	3140.9	322.4	0.1624	51.445	1.007
2	8.142	FM	2964.4	436.1	0.1133	48.555	0.837

Final mutant



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	7.624	MF	93	10	0.1548	28.558	1.118
2	7.846	FM	232.5	24.9	0.1558	71.442	0.636

10. References

1 Gibson, D. G. *et al.* Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature Methods* **6**, 343–345 (2009). https://doi.org/10.1038/nmeth.1318

2 Biegasiewicz, K. F., Cooper, S. J., Emmanuel, M. A., Miller, D. C. & Hyster, T. K. Catalytic promiscuity enabled by photoredox catalysis in nicotinamide-dependent oxidoreductases. *Nature Chemistry* **10**, 770–775 (2018). https://doi.org/10.1038/s41557-018-0059-y

3 Casas, J., Grigg, R., Nájera, C. & Sansano, José M. The Effect of Phase-Transfer Catalysis in the 1,3-Dipolar Cycloaddition Reactions of Azomethine Ylides − Synthesis of Substituted Prolines Using AgOAc and Inorganic Base in Substoichiometric Amounts. *European Journal of Organic Chemistry* **2001**, 1971–1982 (2001). https://doi.org/https://doi.org/10.1002/1099-0690(200105)2001:10<1971::AID-EJOC1971>3.0.CO;2-U

4 Ouyang, Y., Page, C. G., Bilodeau, C. & Hyster, T. K. Synergistic Photoenzymatic Catalysis Enables Synthesis of a-Tertiary Amino Acids Using Threonine Aldolases. *Journal of the American Chemical Society* **146**, 13754–13759 (2024). https://doi.org/10.1021/jacs.4c04661

5 O'Donnell, M. J. *et al.* Acidities of glycine Schiff bases and alkylation of their conjugate bases. *Journal of the American Chemical Society* **110**, 8520–8525 (1988). https://doi.org/10.1021/ja00233a031

6 Bhunia, S., Pawar, G. G., Kumar, S. V., Jiang, Y. & Ma, D. Selected Copper-Based Reactions for C−N, C−O, C−S, and C−C Bond Formation. *Angewandte Chemie International Edition* **56**, 16136–16179 (2017). https://doi.org/https://doi.org/10.1002/anie.201701690

7 Borch, R. F., Bernstein, M. D. & Durst, H. D. Cyanohydridoborate anion as a selective reducing agent. *Journal of the American Chemical Society* **93**, 2897–2904 (1971). https://doi.org/10.1021/ja00741a013

8 Black, M. J. *et al.* Asymmetric redox-neutral radical cyclization catalysed by flavin-dependent 'ene'-reductases. *Nature Chemistry* **12**, 71–75 (2020). https://doi.org/10.1038/s41557-019-0370-2

9 Biegasiewicz, K. F. *et al.* Photoexcitation of flavoenzymes enables a stereoselective radical cyclization. *Science* **364**, 1166–1169 (2019). https://doi.org/doi:10.1126/science.aaw1143

10 Fu, H. *et al.* Ground-State Electron Transfer as an Initiation Mechanism for Biocatalytic C–C Bond Forming Reactions. *Journal of the American Chemical Society* **143**, 9622–9629 (2021). https://doi.org/10.1021/jacs.1c04334

11 Ouyang, Y., Turek-Herman, J., Qiao, T. & Hyster, T. K. Asymmetric Carbohydroxylation of Alkenes Using Photoenzymatic Catalysis. *Journal of the American Chemical Society* **145**, 17018–17022 (2023). https://doi.org/10.1021/jacs.3c06618

12 Kille, S. *et al.* Reducing Codon Redundancy and Screening Effort of Combinatorial Protein Libraries Created by Saturation Mutagenesis. *ACS Synthetic Biology* **2**, 83–92 (2013). https://doi.org/10.1021/sb300037w

13 Müller, F. & Massey, V. Flavin-Sulfite Complexes and Their Structures. *Journal of Biological Chemistry* **244**, 4007–4016 (1969). https://doi.org/https://doi.org/10.1016/S0021-9258(17)36377-9
14 Raps, F. C., Rivas-Souchet, A., Jones, C. M. & Hyster, T. K. Emergence of a distinct mechanism of C–N bond formation in photoenzymes *Nature* **637**, 362–368 (2025). https://doi.org/10.1038/s41586-024-08138-w
15 Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *The Journal of Chemical Physics* **98**, 5648–5652 (1993). https://doi.org/10.1063/1.464913
16 Zhao, Y. & Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theoretical Chemistry Accounts* **120**, 215–241 (2008). https://doi.org/10.1007/s00214-007-0310-x
17 Alassad, Z., AboRaed, A., Mizrachi, M. S., Pérez-Temprano, M. H. & Milo, A. Metal-Free Multicomponent Strategy for Amidine Synthesis. *Journal of the American Chemical Society* **144**, 20672–20679 (2022). https://doi.org/10.1021/jacs.2c07918
18 Jaffé, H. H. Theoretical Considerations Concerning Hammett's Equation. IV. Calculation of ρ-Values. *The Journal of Chemical Physics* **21**, 415–419 (1953). https://doi.org/10.1063/1.1698922
19 Verloop, A. & Tipker, J. Use of linear free energy related and other parameters in the study of fungicidal selectivity. *Pesticide Science* **7**, 379–390 (1976). https://doi.org/https://doi.org/10.1002/ps.2780070410