

Electronic Supplementary Information

Development of Fructose-1,6-bisphosphate aldolase enzyme peptide mimics as biocatalysts in direct asymmetric aldol reactions

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1. General information and materials

All solvents and reagents were obtained from commercial sources. Solvents for column chromatography (ethyl acetate and hexanes) were purchased from Protea Chemicals (South Africa) and distilled before use to remove non-volatile components. Aldol reactions were monitored using thin layer chromatography (TLC) plates (0.2 mm silica gel 60 with fluorescent indicator UV254) were obtained from Sigma-Aldrich and further visualization was done by staining with potassium permanganate (KMnO₄) solution followed by heating. Purification of aldol products was conducted on Merk normal silica gel (particle size 0.063-0.200 mm) and flash silica gel (particle size 0.040-0.063).

¹H NMR and ¹³C NMR spectra were recorded on either a Bruker AVANCE 300 MHz, Bruker AVANCE 400 MHz or on a Bruker AVANCE III 500 MHz spectrometer. 2D NMR spectra which include COSY, 13C-HSQC, TOCSY, NOESY and ROESY experiments, were recorded at 293, 300, and 308 K on a 500 MHz NMR Bruker III 500 MHz spectrometer. All 2D spectra were recorded at the phase sensitive mode using time proportional phase increment (TPPI). The residual water peak of DMSO-d₆ was suppressed by a presaturation pulse of 2 s duration. The first NOESY experiments were recorded with mixing time of 150, 200 and 250 ms; ROESY spectrum were recorded with mixing times of 100, 150, 200, 250 and 300 ms; while TOCSY was recorded with mixing times of 48, 64, 70, 80 and 100 ms; each increment was the sum of 32 scans with a relaxation delay of 2.0 s; 2048 data point were collected per experiment.

The enantiomeric excess (ee) was determined by chiral high performance liquid chromatography (HPLC) analysis on a Dionex HPLC Ultimate 3000 instrument (CHROMELEON version 6.80 software); coupled to a pump and photodiode array detector. A Lux 5 μ cellulose-2 column was used for the analysis with hexane and isopropyl alcohol (IPA) as the mobile phase. CD spectra were recorded on a JASCO J-18 spectropolarimeter between the range of 190 to 250 nm in the specified solvents (water and phosphate buffer), with 10 scans at 20°C. Analytical LC-MS analysis was carried out on an Ultra High Performance Liquid Chromatography (Thermo Scientific Ultimate 3000, RS diode array detectors)-High Resolution Mass Spectrometer (Bruker Compact quadruple time of-flight) coupled to Diode Array (215 and 254 nm).

2. Synthesis of peptides catalysts.

Peptide were synthesized on an automated Protein Technologies, Inc PS-3TM peptide synthesizer and manually following the general Fmoc solid phase synthesis.

2.1 General procedure for the automated and manual SPPS synthesis

2.1.1-Swelling, activation and coupling of the first amino acid

The Fmoc-rink amide resin, 600 mg, (0, 160 mmol/g) was swelled in DMF (10.0 mL) for 20 minutes in a 70 mL glass reaction vessel with fritted filters. The DMF was removed by suction and a 20% piperidine solution in DMF (5 mL) was added and mixed by bubbling for 5 minutes with inert nitrogen gas (N₂). The piperidine solution was then filtered out and the

resin was washed with DMF (5×5 mL) for 30 sec second per each wash. The first amino acid cysteine (1.20 mmol, 0.2 M), and coupling reagent HBTU (1.14 mmol, 0.19 M) were dissolved in a solution of 1.0 M DIPEA in DMF (6 mL) and added into the reaction vessel. An additional 6 mL of DMF was also added. The reaction mixture was allowed to react for 45 minutes while a gentle flow of N₂ was bubbled into the reaction. For double coupling, the resin was washed with (3×5 mL) DMF, and the coupling was repeated using the same mixture for 60 minutes. The resin was then washed with (3×5 mL) DMF, and (2×5mL) DCM.

After the first coupling, Fmoc protecting group was removed using (2×10 mL) 20% piperidine solution and the resin was washed with DMF (5×5 mL) before the second amino acid was coupled. A solution containing 6 mL of 1.0 M DIPEA, (1.14 mmol, 0.19 M) HBTU, and (1.02 mmol, 0.2 M) arginine was added to the resin. The reaction mixture was mixed gently by bubbling nitrogen gas for 45 minutes. The remaining amino acids in the sequence were also coupled using the same procedure until the full peptide was synthesized.

2.1.2 General procedure for cleaving peptides from the resin

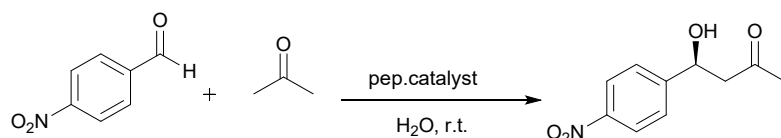
The resin bound peptide was washed with (3 ×5 mL) DMF and then (3×5 mL) DCM and dried by suction. A cleavage cocktail (10 mL) containing 94% TFA: 2.5%EDT:2.5%H₂O:1%TIS was then reacted with the resin bound peptide for 3 hrs. The cleavage solution was filtered off, and the resin was washed with 5 mL of TFA and the filtrated was divided into portions in and poured into 50 ml centrifuged tubes. Cold diethyl ether was added to the filtered solution upon which a white precipitate of the crude peptide was formed. The two samples were centrifuged at 5000 rpm for 10 minutes. The step was repeated 3 times with cold diethyl ether. The obtained white precipitate was then dissolved in 10 mL (60/40; H₂O/MeCN) for further analysis and purification.

2.2 Purification of the peptides

Peptides were purified via an Agilent 1260 Infinity semi-preparative HPLC system with a UV/VIS detector and an automated fraction collector on a Kinetix® 5 μm B-C18, 100 Å (250 ×230 nm) column. A two-buffer system was employed; Buffer A consisted of 0.1% formic acid in H₂O and buffer B consisted of 0.1% formic acid in CH₃CN. A flow rate of 20 ml/min or 15 ml/min and UV wavelengths of 215 and 254 nm were utilized

3. Typical procedures for Aldol reaction

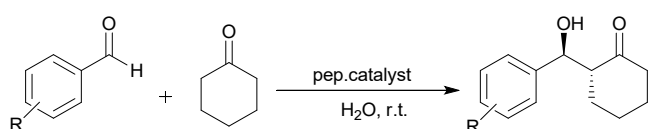
3.1 Aldol reactions in acetone in the presence of water



A peptide catalyst (0.0064 mmol, 4 mol%) was added to a vial containing 0.75 mL of 3:1 acetone/water and the mixture was stirred for 15 min. An acceptor aldehyde (0.157 mmol, 24 mg) was then added, and the resulting reaction mixture was stirred vigorously at room temperature for 24-72 hours. The reaction was stopped upon completion as indicated by

TLC and acetone was evaporated under reduced pressure. The crude product was extracted using (3 ×10 mL) ethyl acetate (EtOAc) and 2 mL water. The combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was then purified by flash silica gel column chromatography using ethyl acetate/hexane (1:3). The pure product was then subjected to chiral-phase HPLC analysis to determine the ee.

3.2 Procedures for the aldol reactions between aromatic aldehydes and cyclohexanone



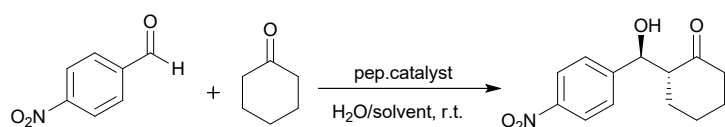
3.2.1 Aldol reaction between cyclohexanone and aromatic aldehydes (homogenous)

A peptide catalyst (0.0064 mmol, 4 mol%) was added to the ketone (0.45 mL) in water (40 μL) and stirred for 15 minutes. An acceptor aldehyde was then added, and the resulting reaction mixture was left to stir for 24-72 hours at room temperature. The reaction was stopped upon completion as indicated by TLC. The reaction was quenched by extraction with (3×10 mL) EtOAc and brine solution (2 mL). The combined organic extract was washed with brine and dried with Na₂SO₄, filtered and concentrated in vacuo. Diastereomeric ratio of the crude product was determined by ¹H NMR analysis. The crude aldol product was purified by flash silica-gel column chromatography using EtOAc/Hexane (1:3) and the desired aldol product was subjected to chiral-phase HPLC analysis to determine the ee.

3.2.2 Aldol reaction between cyclohexanone and aromatic aldehydes (heterogenous)

A peptide catalyst (0.0064 mmol, 4 mol%) was added to a solution of a ketone (0.450 mL) and 60 μL of water and stirred for 15 minutes. An acceptor aldehyde (0.157 mmol, 24 mg) was then added to reaction mixture which was stirred for 24-72 hours. The reaction was monitored by TLC and the reaction was quenched by extraction with (3×10 mL) EtOAc and (1×2 mL) brine solution. The combined organic layers were dried with Na₂SO₄, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel using EtOAc: hexane (1:3).

3.3 General procedure for aldol reaction between cyclohexanone and aromatic aldehydes in organic solvent



A peptide catalyst (0.0064 mmol, 4 mol%) was added to a vial containing a 0.75 mL solvent mixture (0.5173 mL solvent and 0.237 mL water) and cyclohexanone (0.628 mmol, 70 μL) and the mixture was stirred for 15 minutes. An acceptor aldehyde (0.157 mmol, 24 mg) was then added to the reaction mixture which was stirred for 24 -72 hours. The reaction progress was monitored by TLC and was extracted with EtOAc (3×10 mL) and brine solution

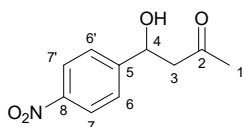
(1×2.0 mL). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. Diastereomeric ratio of the crude product was determined by ¹H NMR analysis. The crude product was purified by flash chromatography on silica-gel with hexane/ethyl acetate (3:1). The pure products were then analyzed by the chiral HPLC and the ee values were determined

3.4 Catalyst recyclability for peptide catalyzed aldol reaction

The filtered aqueous layers obtained after extraction of the crude product, were combined and washed with Et₂O (3 mL) and C₂H₅O (4 mL). The peptide was catalyst obtained was after drying and decantation of the resuspended mixture. The recycled peptide was added to a vial containing a 0.75 mL solvent mixture (0.5173 mL solvent and 0.237 mL water) and

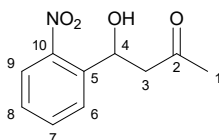
4. Analytical data for Aldol products

4-Hydroxy-4-(4'-nitrophenyl)-butan-2-one (TP_A1N4ACE)



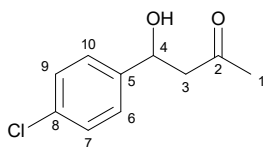
Yellow solid: R_f = 0.27 (30% ethyl acetate/hexane). IR (Vmax/cm⁻¹): 3459 (O-H), 3118 (=C-H), 3068 (C-H), 1574 (C=C), 1706(C=O), 1323(C-O). ¹H NMR (500 MHz, CDCl₃) δ: 8.22 (d, 2H, H-7 & H-7'), 7.54 (d, 2H, H-6 and H-6'), 5.30 – 5.21 (m, 1H, H-4), 3.55 (brs, J = 3.3 Hz, 1H, OH), 2.87 – 2.77 (m, 2H, H-3), 2.23 (s, 3H, H-1). ¹³C NMR (126 MHz, CDCl₃) δ: 208.44(C-2), 126.45(C-6), 126.34(C-6'), 123.80(C-7), 123.72(C-7'), 68.62(C-4), 50.83(C-3), 30.77(C-1). HRMS (ESI) m/z: Calculated for C₁₀H₁₁NO₄: 209.068, found: 232.0910[M+23]⁺

4-Hydroxy-4-(2'-nitrophenyl)-butan-2-one (TP_A2N2ACE)



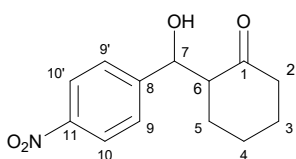
Colourless solid: R_f = 0.32 (30% ethyl acetate/hexane). IR (Vmax/cm⁻¹): 3461 (O-H), 3068(=C-H), 2922 (C-H), 1609 (C=O), 1518 (C=C), 1333(C-O). ¹H NMR (500 MHz, CDCl₃) δ 8.04 – 7.80 (m, 2H, H-9 and H-8), 7.77 – 7.58 (m, 1H, H-6), 7.50 – 7.38 (m, 1H, H-7), 5.68 (dd, 1H, H-4), 3.79 (brs, 1H, OH), 3.11 (d, 1H, H-3), 2.74 (dd, J = 17.8, 9.4 Hz, 1H, H-3), 2.24 (s, 2H H-1). ¹³C NMR (126 MHz, CDCl₃) δ: 208.76 (C-2), 147.12(C-10), 138.47(C-5), 133.82(C-7), 128.28(C-6), 128.19(C-9), 124.43(C-8), 65.60(C-4), 51.12(C-3), 30.43(C-1). HRMS (ESI) m/z: Calculated for C₁₀H₁₁NO₄: 209.07, found: 232.0583 [M+23]⁺

4-Hydroxy-4-(4'-chlorophenyl)-butan-2-one (TP_A3ClACE)



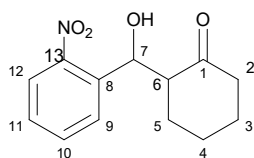
Colourless oil: $R_f = 0.28$ (30% ethyl acetate/hexane). IR (V_{max}/cm^{-1}): 3421 (O-H), 3117(=C-H), 2967 (C-H), 1701 (C=O), 1519 (C=C), 1315(C-O). 1H NMR (500 MHz, $CDCl_3$) δ : 7.33 – 7.26 (m, 4H, Ar-H), 5.12 (d, $J = 9.0, 3.4$ Hz, 1H, H-4), 3.47 (brs, 1H, OH), 2.89 – 2.72 (m, 2H, H-3), 2.19 (s, 3H, H-1). ^{13}C NMR (126 MHz,) δ : 208.86, 141.25, 133.30, 128.65, 127.03, 69.16, 51.82, 30.76. HRMS (ESI) m/z : Calculated for $C_{10}H_{11}ClO_4$:198.0488, found: 221.0334 $[M+23]^+$

2-(Hydroxy(4-nitrophenyl)methyl)cyclohexanone (TP_A4N4CY)



Yellowish solid: $R_f = 0.26$ (30% ethyl acetate/hexane). IR (V_{max}/cm^{-1}): 3426(O-H), 3077(=C-H), 2949 (C-H), 1698 (C=O), 1524 (C=C), 1024(C-O). 1H NMR (500 MHz, $CDCl_3$) δ : 8.21 (d, $J = 8.9, 2.3$ Hz, 2H, H-10 and H-10'), 7.50 (d, $J = 7.5$ Hz, 2H, H-9 and H-9'), 4.90 (d, $J = 8.3, 3.0$ Hz, 1H, H-7), 4.07 (brs, 1H, OH), 2.63 – 2.55 (m, 1H, H-6), 2.54 – 2.45 (m, 1H, H-2a), 2.41 – 2.31 (m, 1H, H-2b), 2.16 – 2.08 (m, 1H-H-3a), 1.72 – 1.64 (m, 1H-H-3b), 1.63 – 1.48 (m, 4H, H-4 and H-3). ^{13}C NMR (126 MHz, $CDCl_3$) δ : 214.73 (C-1), 147.96 (C-11), 127.87 (C-8), 126.59 (C-9 and C-9'), 123.52 (C-10 and C-10'), 74.05 (C-7), 70.14 (C-6), 57.20 (C-2), 42.69 (C-5), 30.77 (27.64 , 24.75 HRMS (ESI) m/z : Calculated for $C_{13}H_{15}NO_4$:249.1001, found 272.0890 $[M+23]^+$.

2-Hydroxy(2-nitrophenyl)methyl)cyclohexanone (TP_A5N2CY)



Yellow oil: $R_f = 0.29$ (30% ethyl acetate/hexane). IR (V_{max}/cm^{-1}): 3454(O-H), 2922 (=C-H), 2853 (C-H), 1693 (C=O), 1512 (C=C), 1199(C-O). 1H NMR (500 MHz, $CDCl_3$) δ : 7.94 – 7.72 (m, 2H), 7.64 (t, $J = 7.7$ Hz, 1H), 7.43 (t, $J = 7.8$ Hz, 1H), 5.45 (d, $J = 7.0$ Hz, 1H), 4.07 (d, $J = 37.1$ Hz, OH), 2.82 – 2.68 (m, 1H), 2.51 – 2.28 (m, 2H), 1.92 – 1.52 (m, 5H), 1.26 (d, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 214.96 (C-1), 148.73 (C-13), 136.63 (C-8), 133.07 (C-10), 129.00 (C-9), 128.40 (C-12), 124.10 (C-11), 69.81 (C-7), 57.31 (C-6), 42.85 (C-2), 31.14 (C-5), 27.77 (C-3), 25.00 (C-4). Calculated for $C_{13}H_{15}NO_4$:249.1001, found 272.0890 $[M+23]^+$.

5. LC-MS spectra for TP_Asp and T_ADlys peptide catalysts

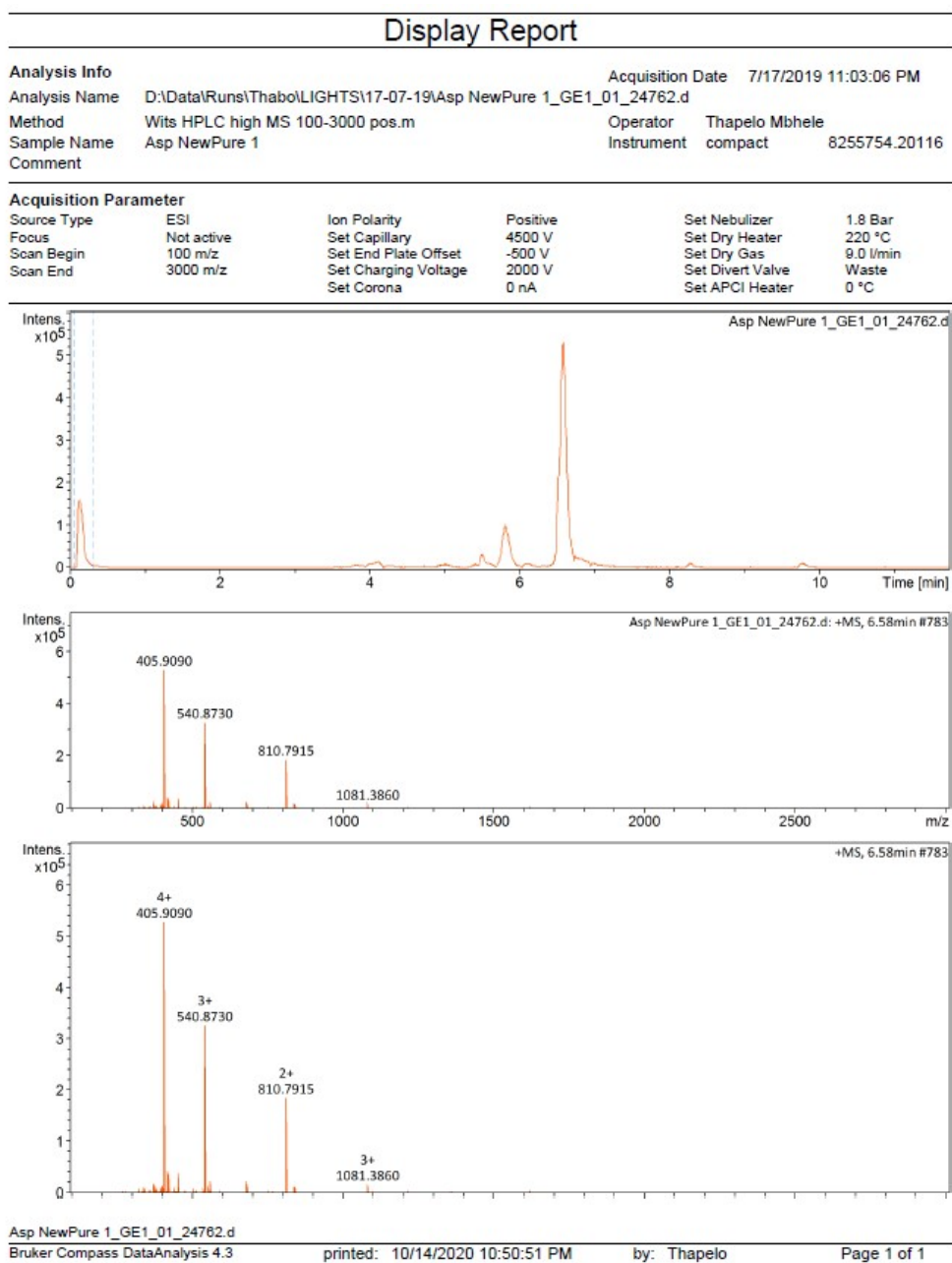


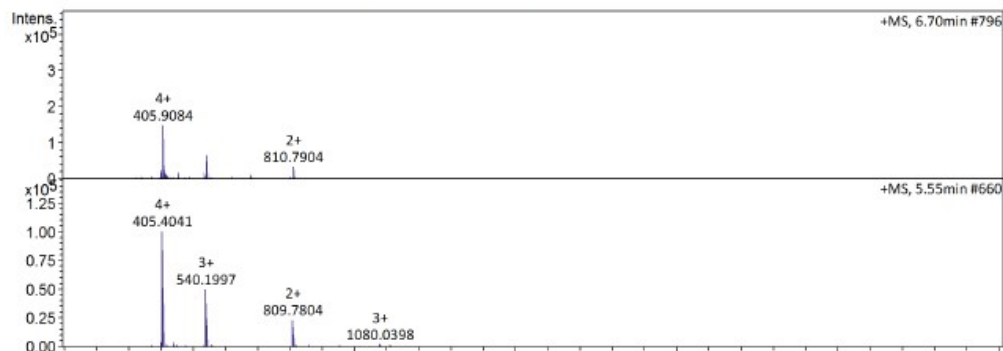
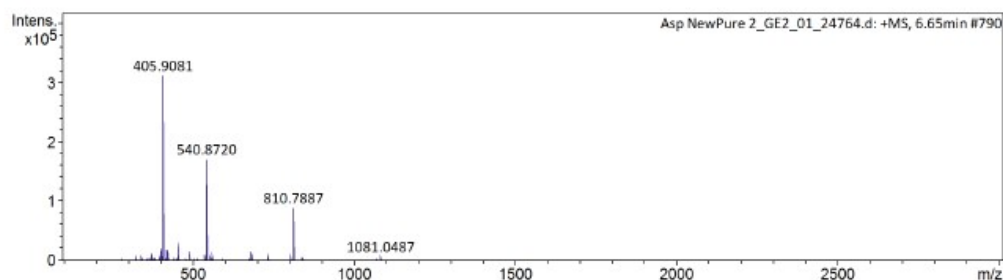
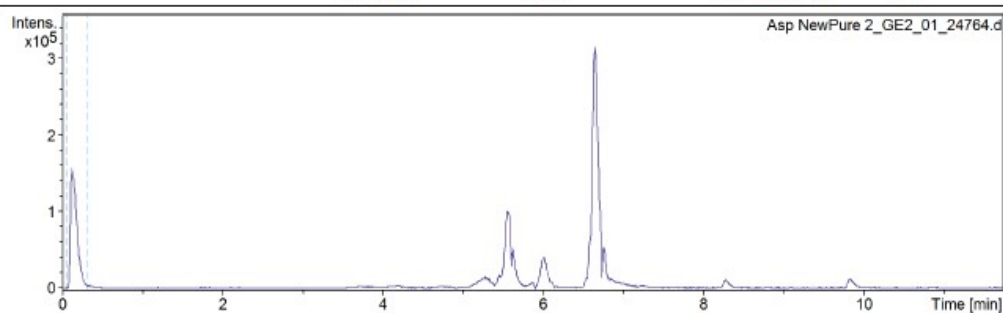
Figure S1: LC-MS spectrum of TP_Asp conformation 1 (peak 1)

Display Report

Analysis Info
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Method Wits HPLC high MS 100-3000 pos.m
Sample Name Asp NewPure 2
Comment
Acquisition Date 7/17/2019 11:30:38 PM
Operator Thapelo Mbhele
Instrument compact 8255754.20116

Acquisition Parameter

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Asp NewPure 2_GE2_01_24764.d

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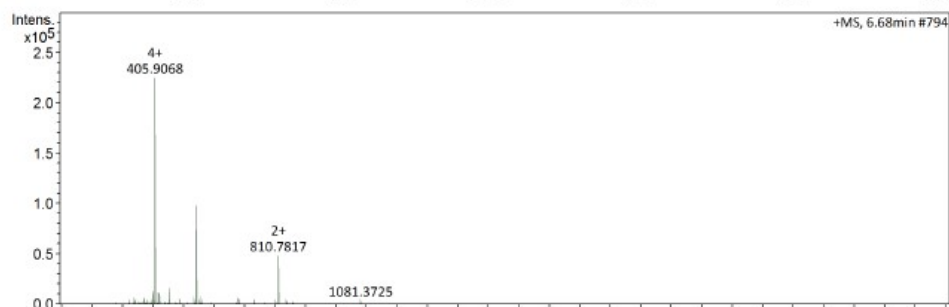
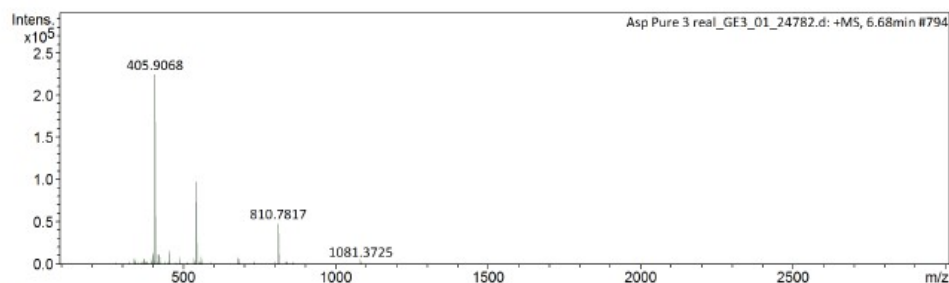
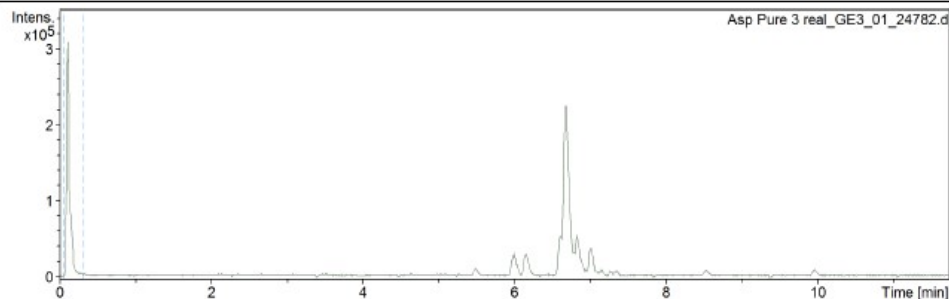
Figure S2: LC-MS spectrum of TP_Asp conformation 2 (peak 2)

Display Report

Analysis Info
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Method Wits HPLC high MS 100-3000 pos.m Operator Thapelo Mbhele
Sample Name Asp Pure 3 real Instrument compact 8255754.20116
Comment

Acquisition Parameter

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Asp Pure 3 real_GE3_01_24782.d
Bruker Compass DataAnalysis 4.3

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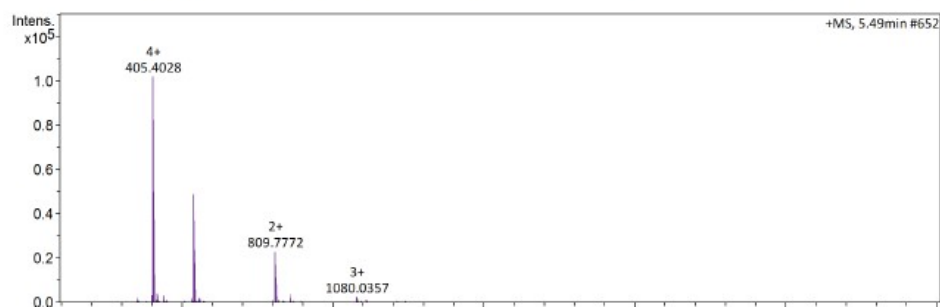
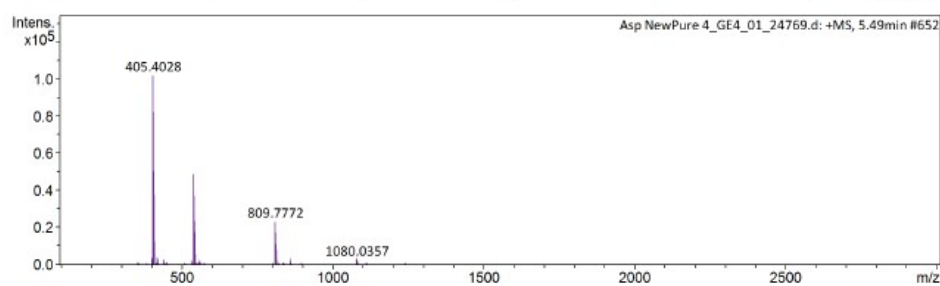
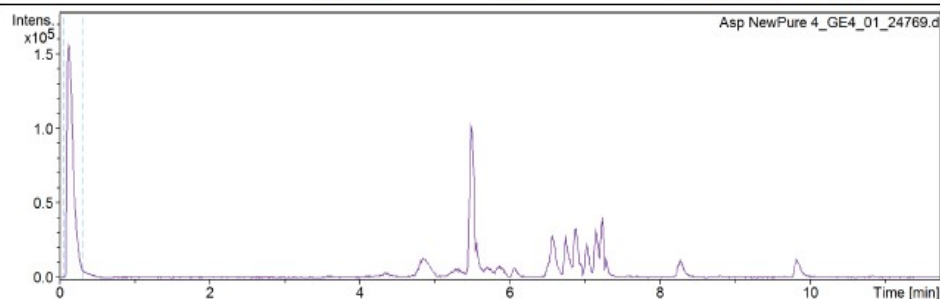
Figure S3: LC-MS spectrum of TP_Asp conformation 3 (peak 3)

Display Report

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Method Wits HPLC high MS 100-3000 pos.m Operator Thapelo Mbhele
Sample Name Asp NewPure 4 Instrument compact 8255754.20116
Comment

Acquisition Parameter

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|-------------|------------|----------------------|----------|------------------|-----------|
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| Scan Begin | 100 m/z | Set End Plate Offset | -500 V | Set Dry Gas | 9.0 l/min |
| Scan End | 3000 m/z | Set Charging Voltage | 2000 V | Set Divert Valve | Waste |
| | | Set Corona | 0 nA | Set APCI Heater | 0 °C |



Asp NewPure 4_GE4_01_24769.d

Bruker Compass DataAnalysis 4.3

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Figure S4: LC-MS spectrum of TP_Asp conformation 4 (peak 4)

Display Report

Analysis Info
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Method: Wits HPLC high MS 100-3000 pos.m
Operator: Thapelo Mbhele
Sample Name: D Lys final Pure 1 re
Instrument: compact 8255754.20116
Comment:

Acquisition Parameter

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| | | Set Corona | 0 nA | Set APCI Heater | 0 °C |

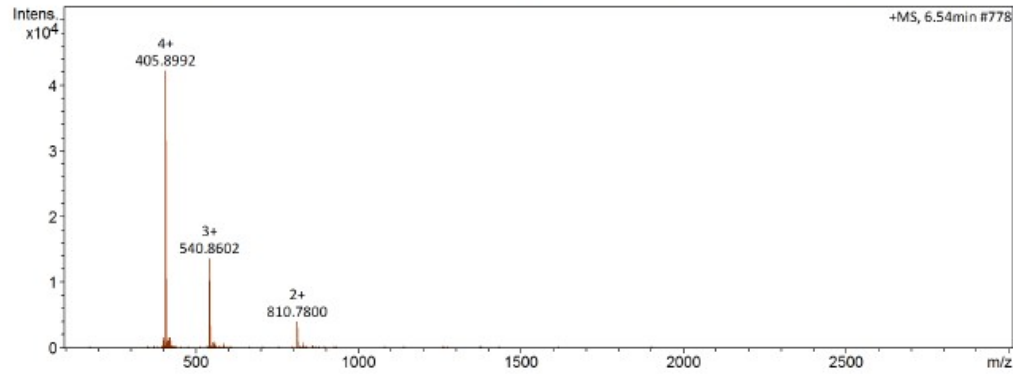
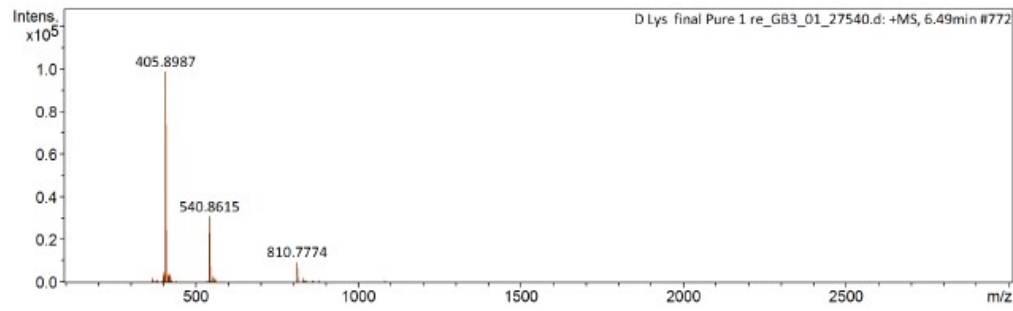
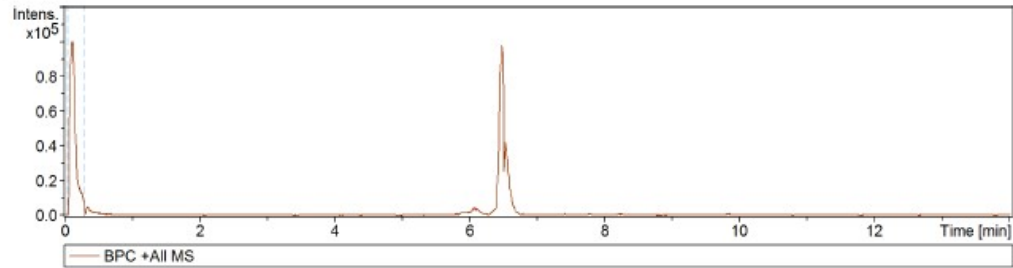


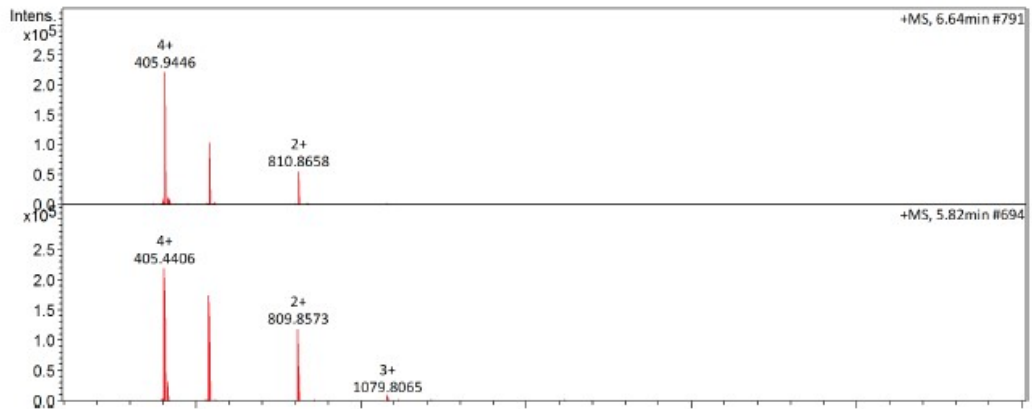
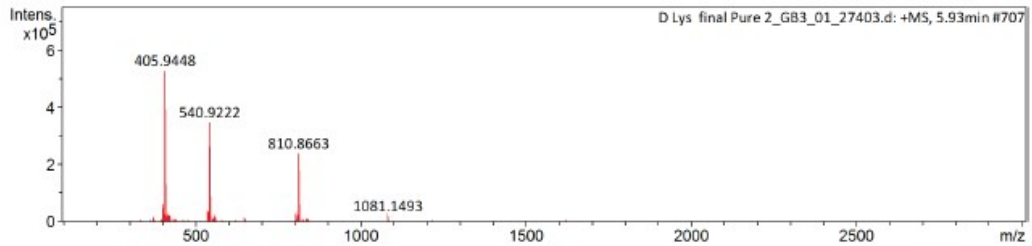
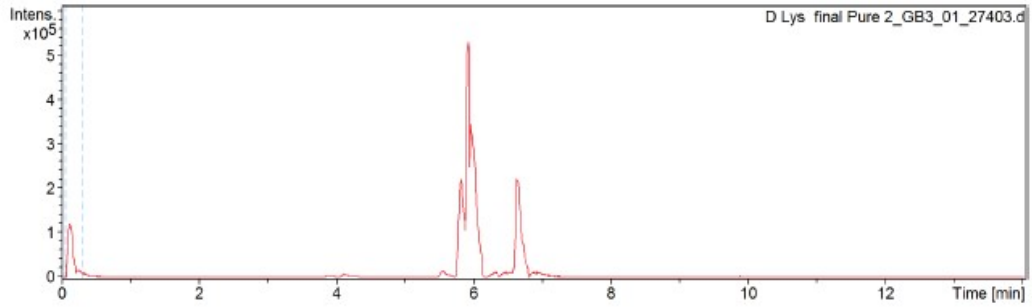
Figure S5: LC-MS spectrum of TP_ADlys conformation 1 (peak 1)

Display Report

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Sample Name: D Lys final Pure 2
Comment:
Acquisition Date: 10/4/2019 6:10:52 PM
Operator: Thapelo Mbhele
Instrument: compact 8255754.20116

Acquisition Parameter

| | | | | | |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 1.8 Bar |
| Focus | Not active | Set Capillary | 4500 V | Set Dry Heater | 220 °C |
| Scan Begin | 100 m/z | Set End Plate Offset | -500 V | Set Dry Gas | 9.0 l/min |
| Scan End | 3000 m/z | Set Charging Voltage | 2000 V | Set Divert Valve | Waste |
| | | Set Corona | 0 nA | Set APCI Heater | 0 °C |



D Lys final Pure 2_GB3_01_27403.d

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by: Thapelo

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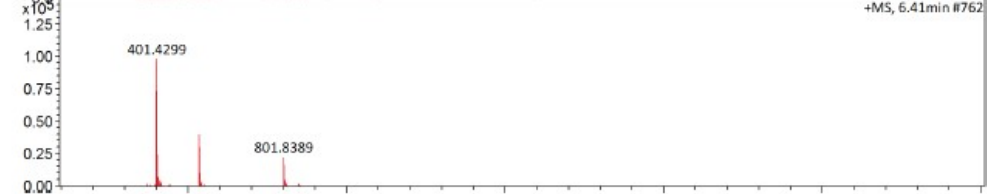
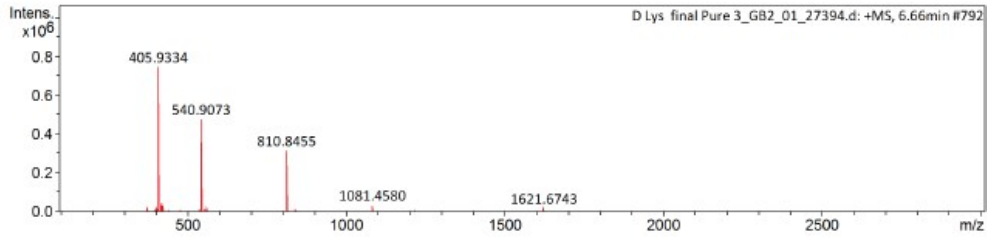
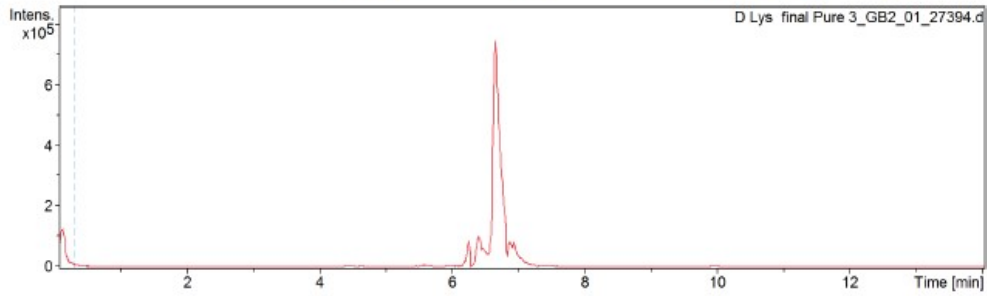
Figure S6: LC-MS spectrum of TP_ADlys conformation 2 (peak 2)

Display Report

Analysis Info
Analysis Name D:\Data\runs\thabo\lights\04-10-19\D Lys final Pure 3_GB2_01_27394.d Acquisition Date 10/4/2019 2:50:04 PM
Method Wits HPLC high MS 100-3000 pos.m Operator Thapelo Mbhele
Sample Name D Lys final Pure 3 Instrument compact 8255754.20116
Comment

Acquisition Parameter

| | | | | | |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 1.8 Bar |
| Focus | Not active | Set Capillary | 4500 V | Set Dry Heater | 220 °C |
| Scan Begin | 100 m/z | Set End Plate Offset | -500 V | Set Dry Gas | 9.0 l/min |
| Scan End | 3000 m/z | Set Charging Voltage | 2000 V | Set Divert Valve | Waste |
| | | Set Corona | 0 nA | Set APCI Heater | 0 °C |



D Lys final Pure 3_GB2_01_27394.d

Bruker Compass DataAnalysis 4.3

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by: Thapelo

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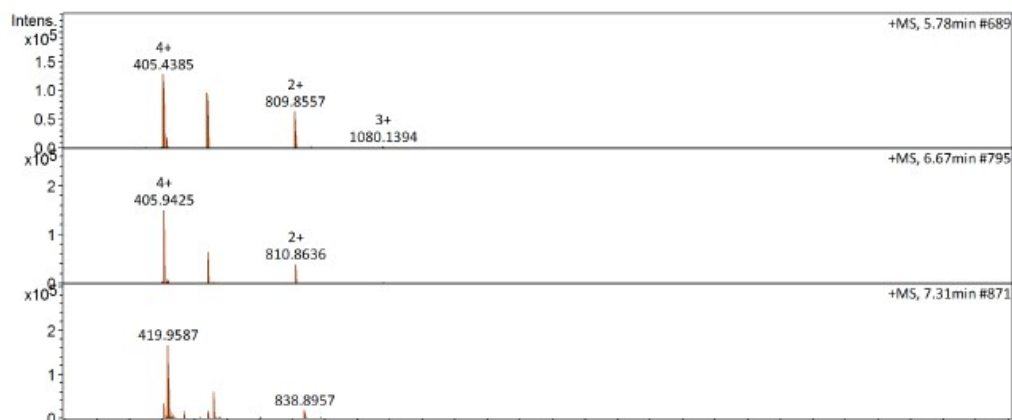
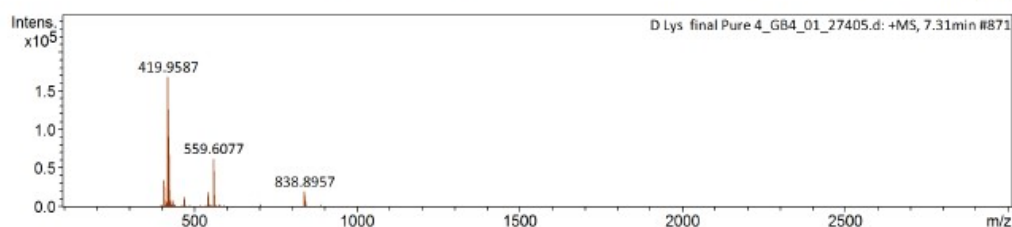
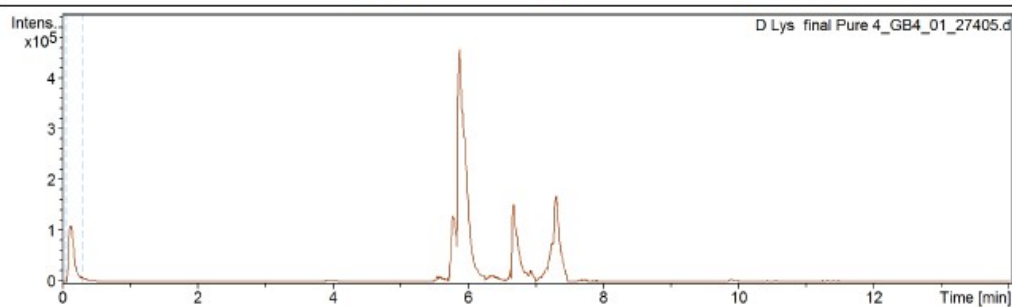
Figure S7: LC-MS spectrum of TP_AD Lys conformation 3 (peak 3)

Display Report

Analysis Info
Analysis Name: D:\Data\runs\thabo\lights\04-10-19\D Lys final Pure 4_GB4_01_27405.d
Acquisition Date: 10/4/2019 6:38:23 PM
Method: Wits HPLC high MS 100-3000 pos.m
Operator: Thapelo Mbhele
Sample Name: D Lys final Pure 4
Instrument: compact 8255754.20116
Comment:

Acquisition Parameter

| | | | | | |
|-------------|------------|----------------------|----------|------------------|-----------|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 1.8 Bar |
| Focus | Not active | Set Capillary | 4500 V | Set Dry Heater | 220 °C |
| Scan Begin | 100 m/z | Set End Plate Offset | -500 V | Set Dry Gas | 9.0 l/min |
| Scan End | 3000 m/z | Set Charging Voltage | 2000 V | Set Divert Valve | Waste |
| | | Set Corona | 0 nA | Set APCI Heater | 0 °C |



D Lys final Pure 4_GB4_01_27405.d

Bruker Compass DataAnalysis 4.3

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Figure S8: LC-MS spectrum of TP_ADlys conformation 3 (peak 3)

6. NMR spectra for peptide structures

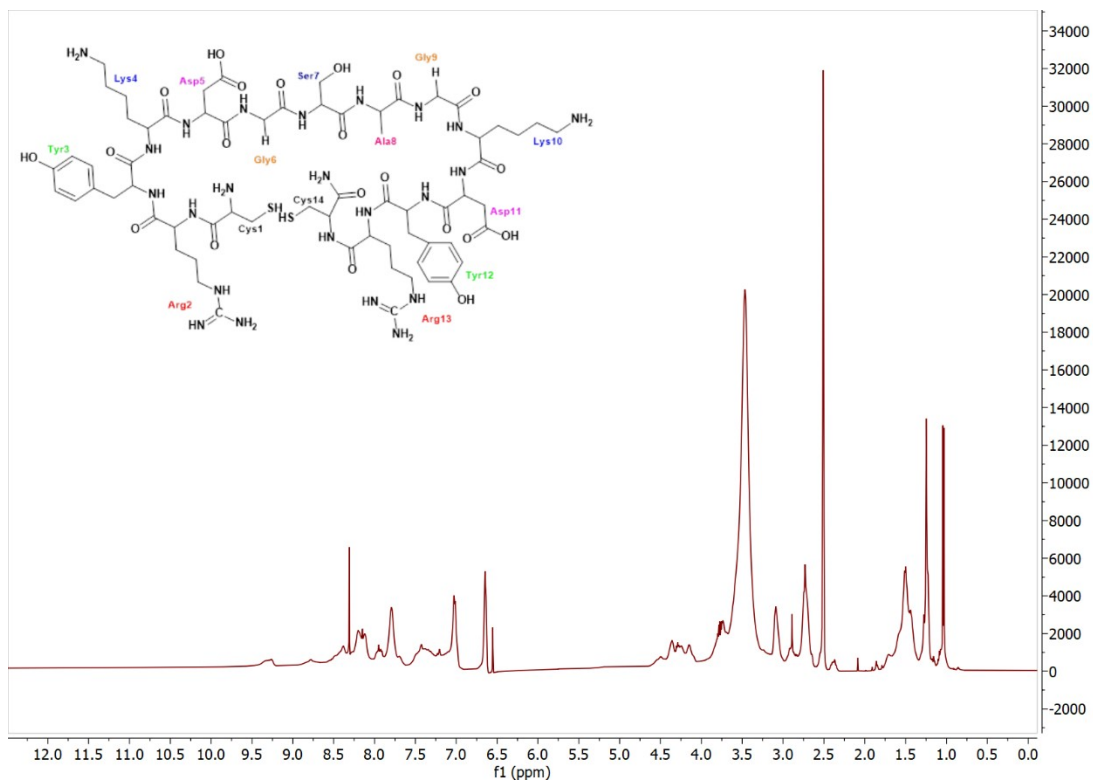


Figure S9: ¹H NMR spectrum of TP_Asp peptide

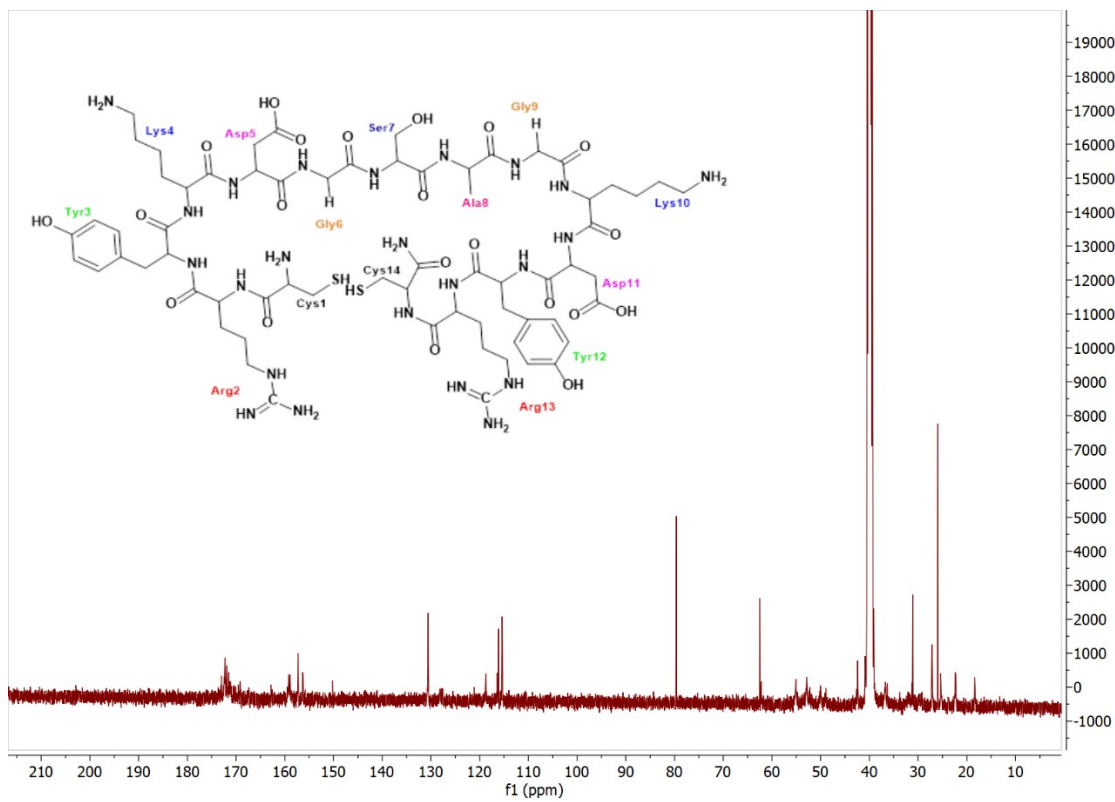


Figure S.2-9: ¹³C NMR spectrum of TP_Asp peptide

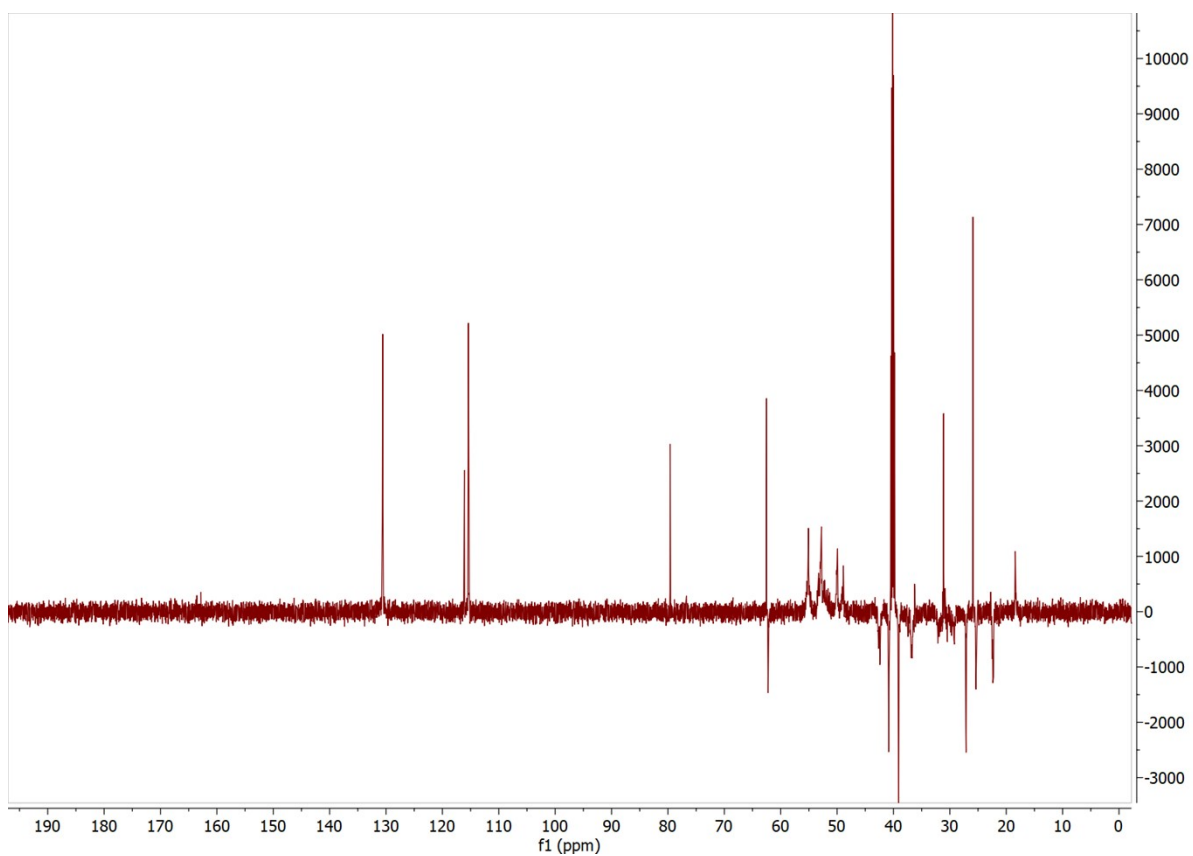


Figure S10: DEPT 135 NMR spectrum of TP_Asp peptide

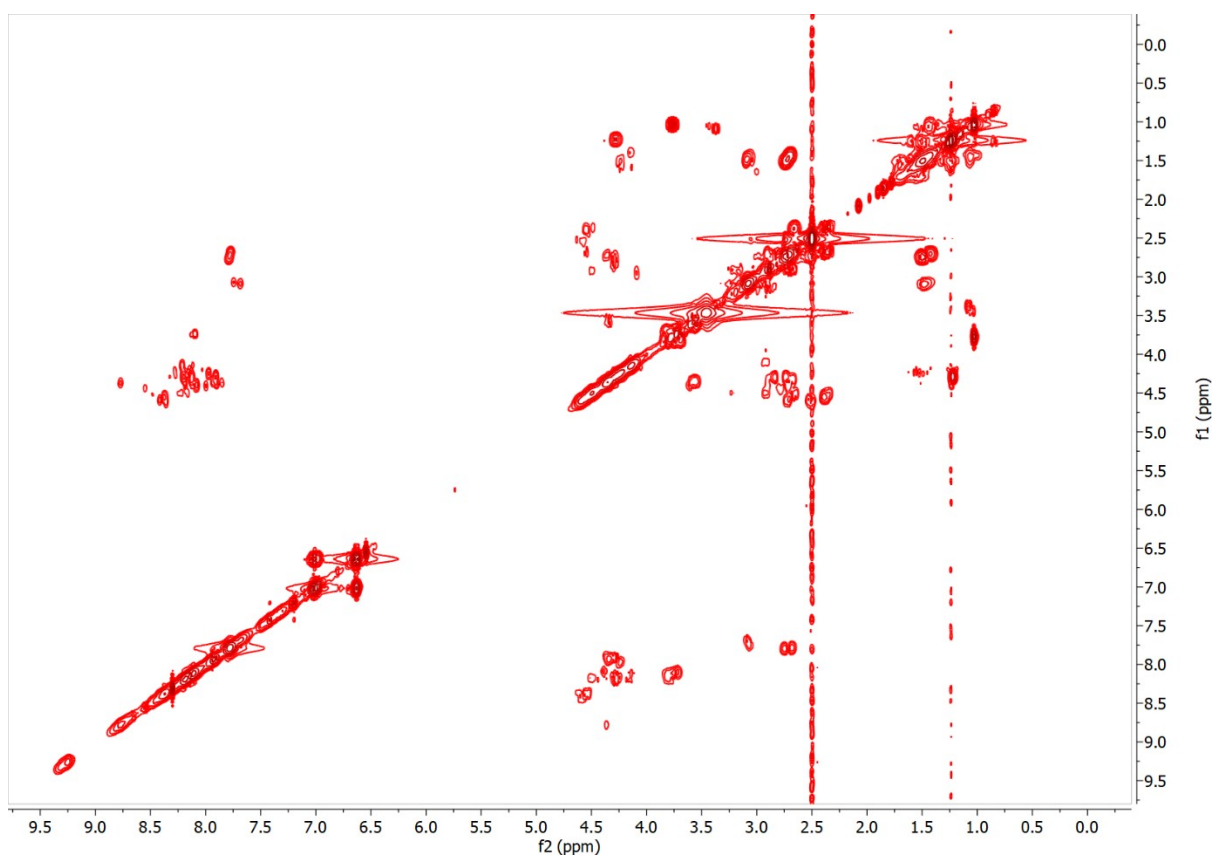


Figure S11: 2D ^1H COSY NMR spectrum of TP_Asp peptide

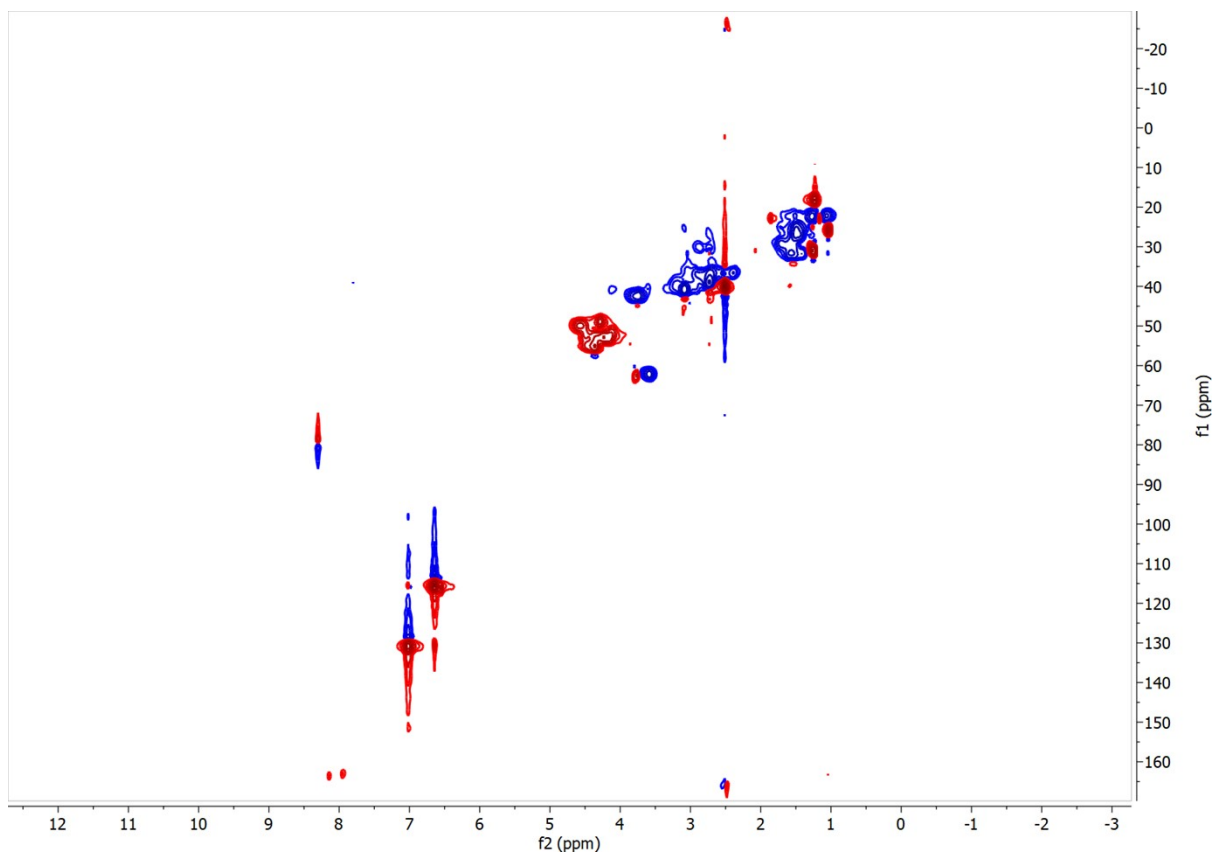


Figure S12: HSQC NMR spectrum of TP_Asp peptide

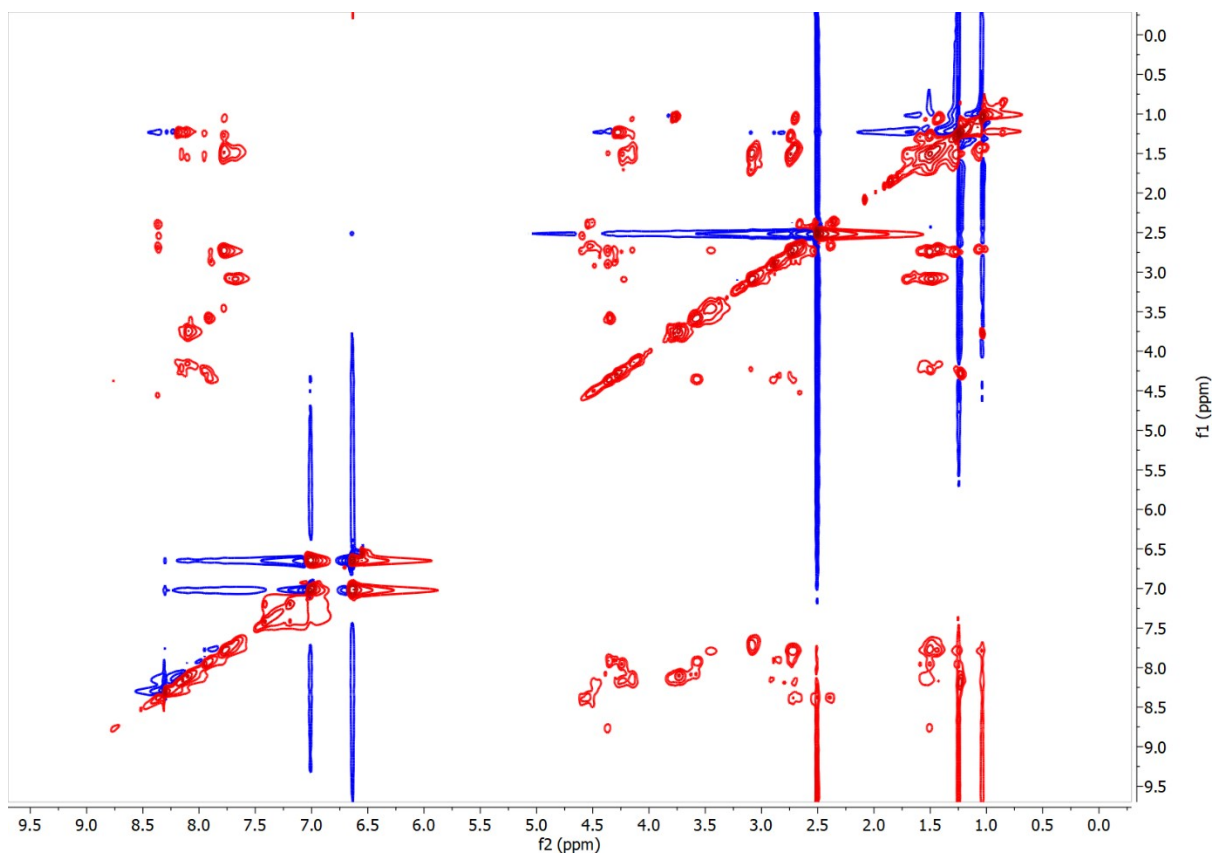


Figure S13: 2D ^1H TOCSY NMR spectrum of TP_Asp peptide

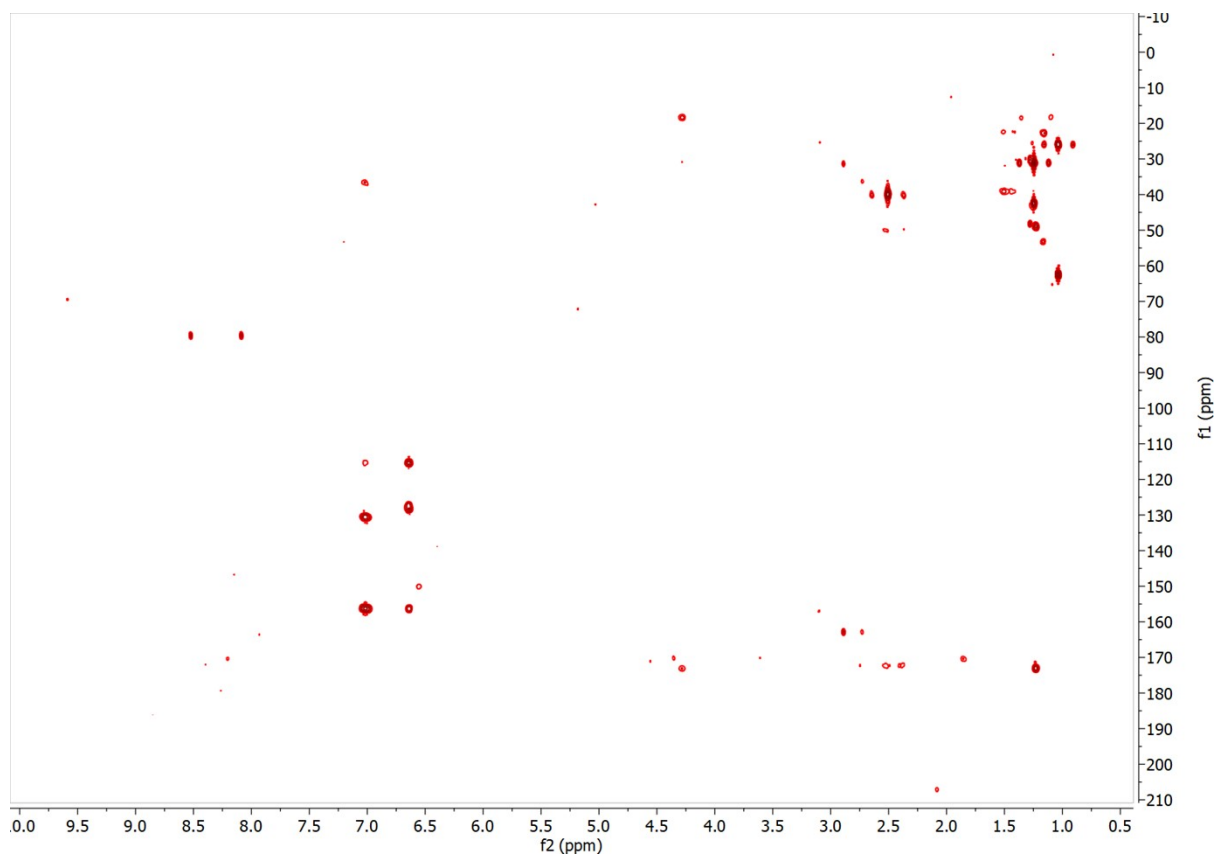


Figure S14: HMBC NMR spectrum of TP_Asp peptide

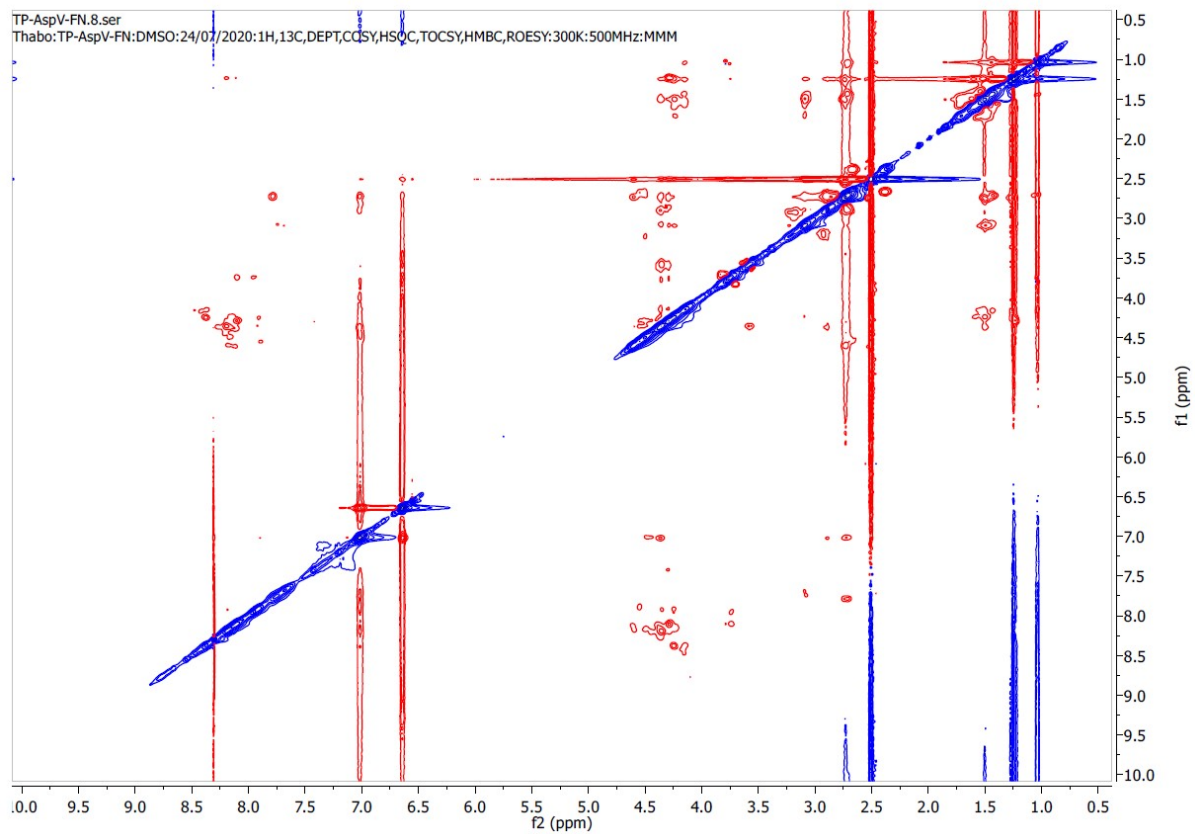


Figure S15: 2D ¹H ROESY NMR spectrum of TP_Asp peptide

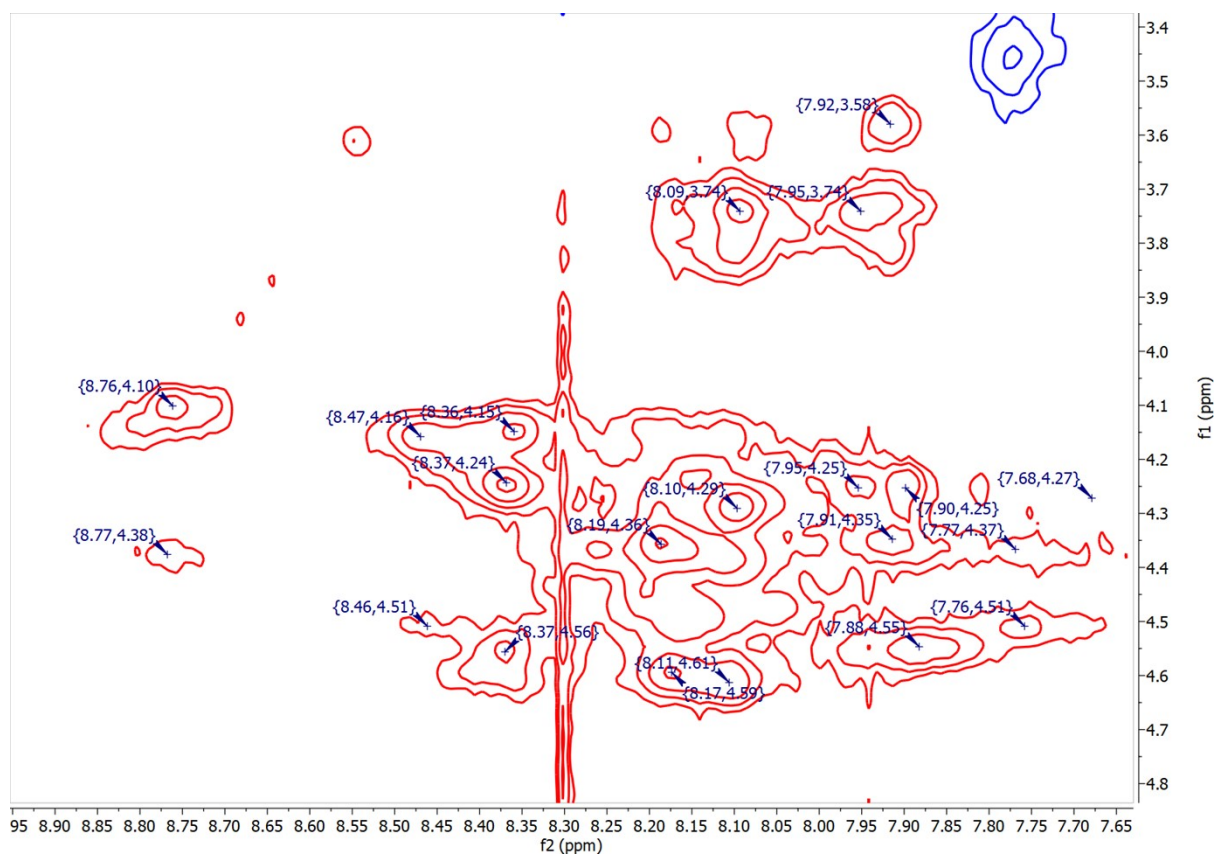


Figure S16: 2D ¹H ROESY NMR spectrum of TP_ADlys peptide, ¹HN region

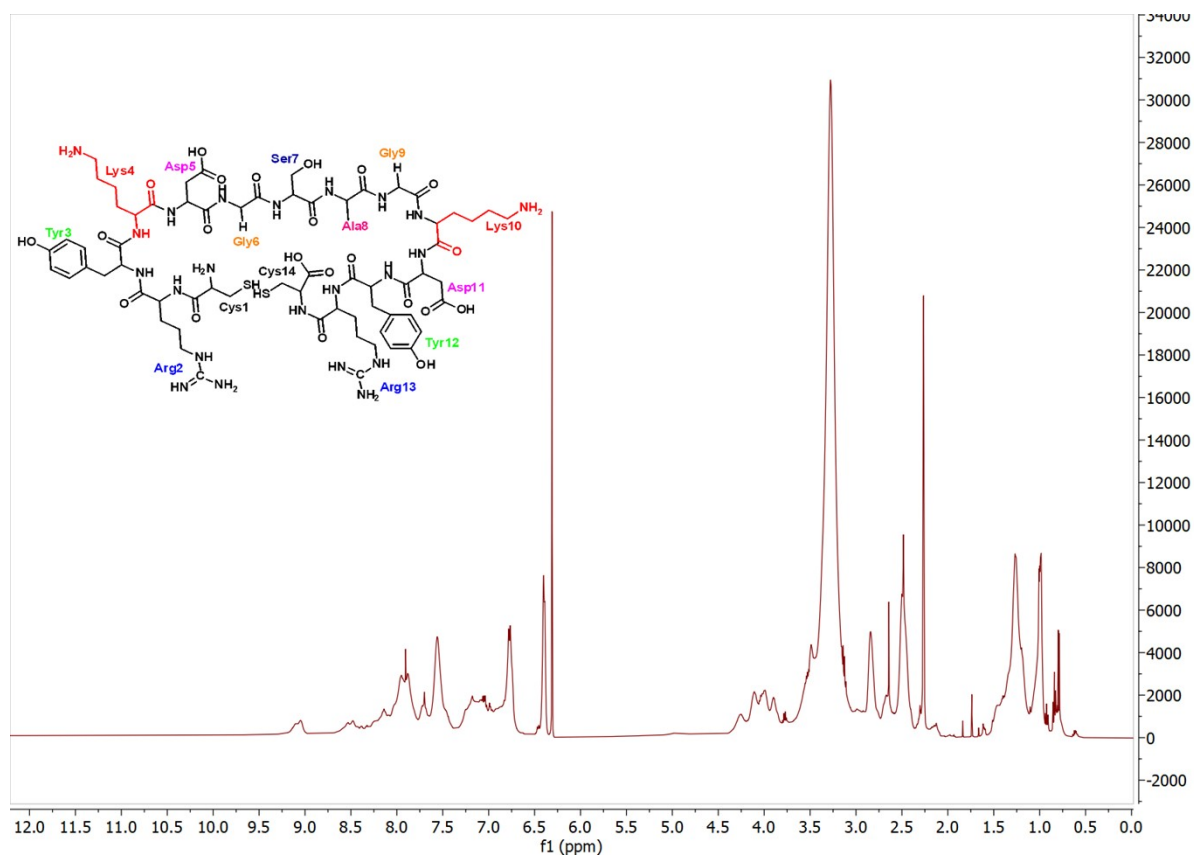


Figure S17: ¹H NMR spectrum of TP_ADlys peptide

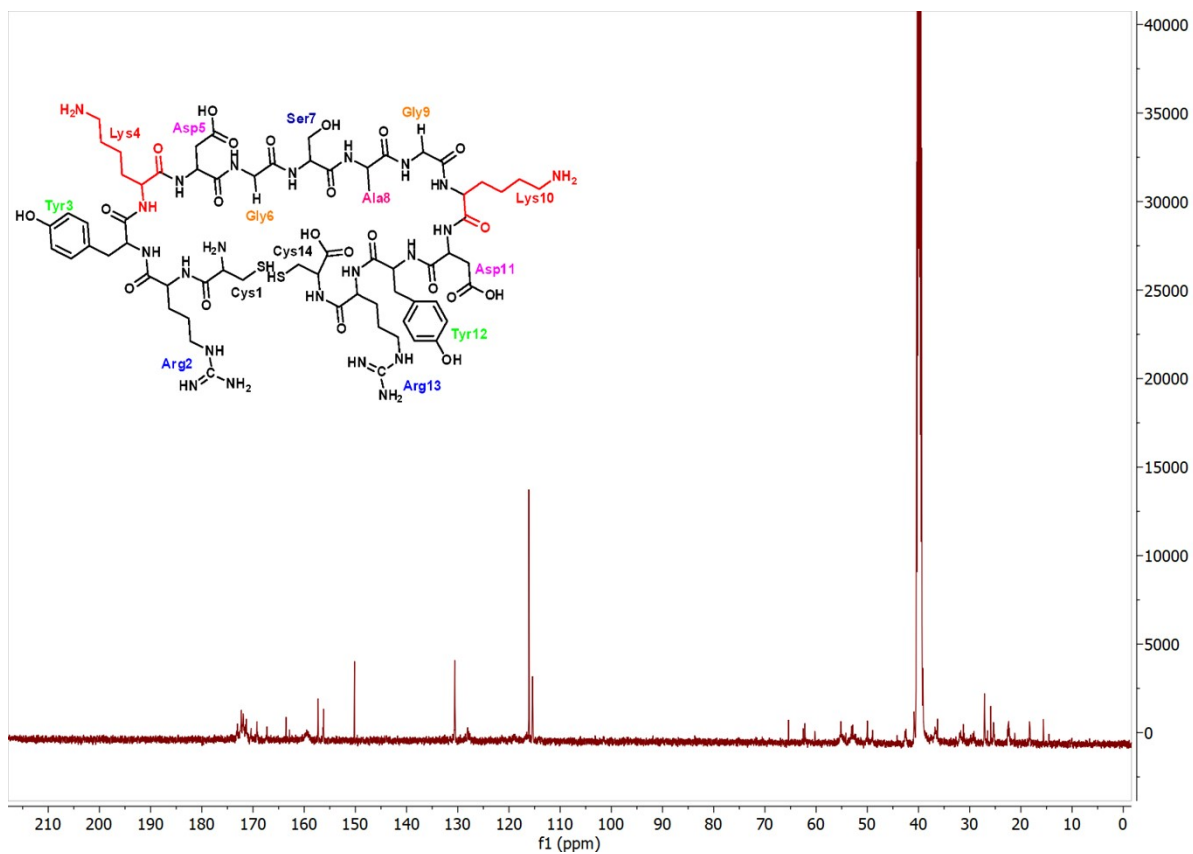


Figure S18: ^{13}C NMR spectrum of TP_ADlys peptide

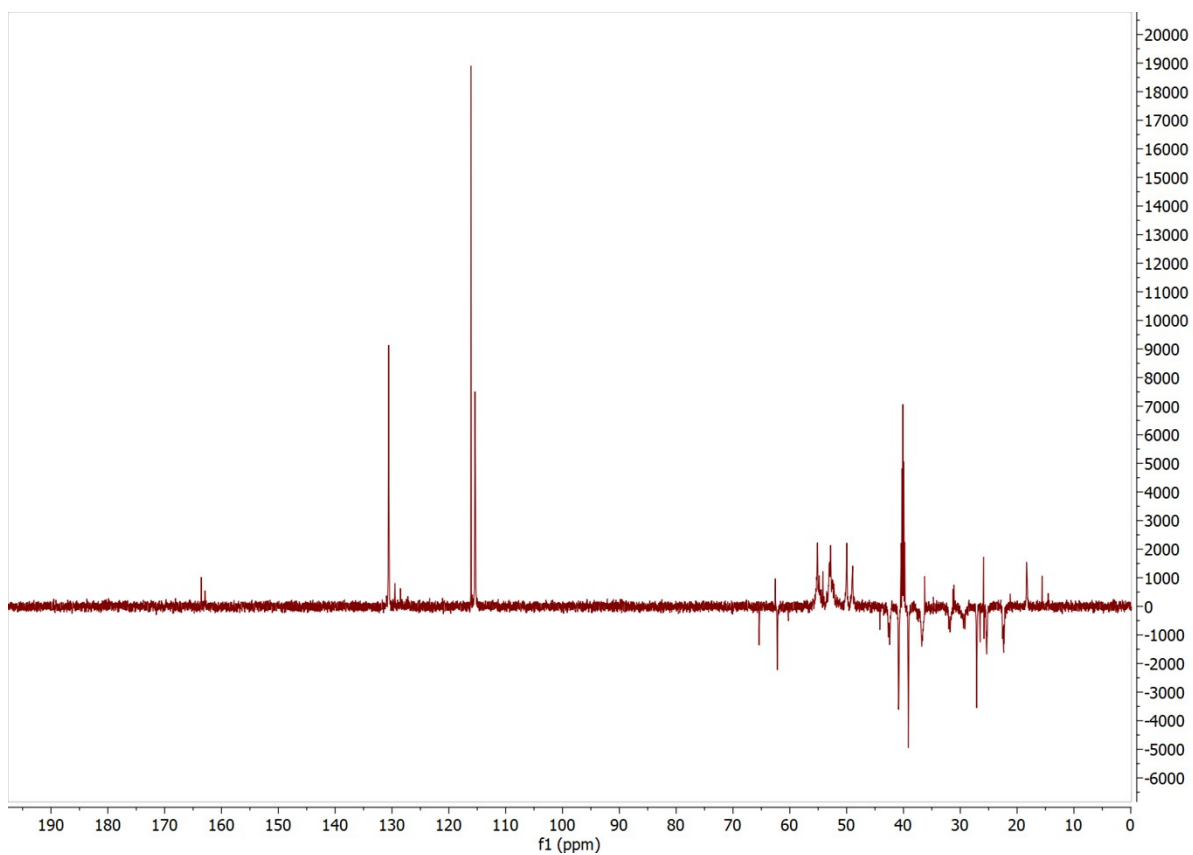


Figure S19: DEPT 135 NMR spectrum of TP_ADlys peptide

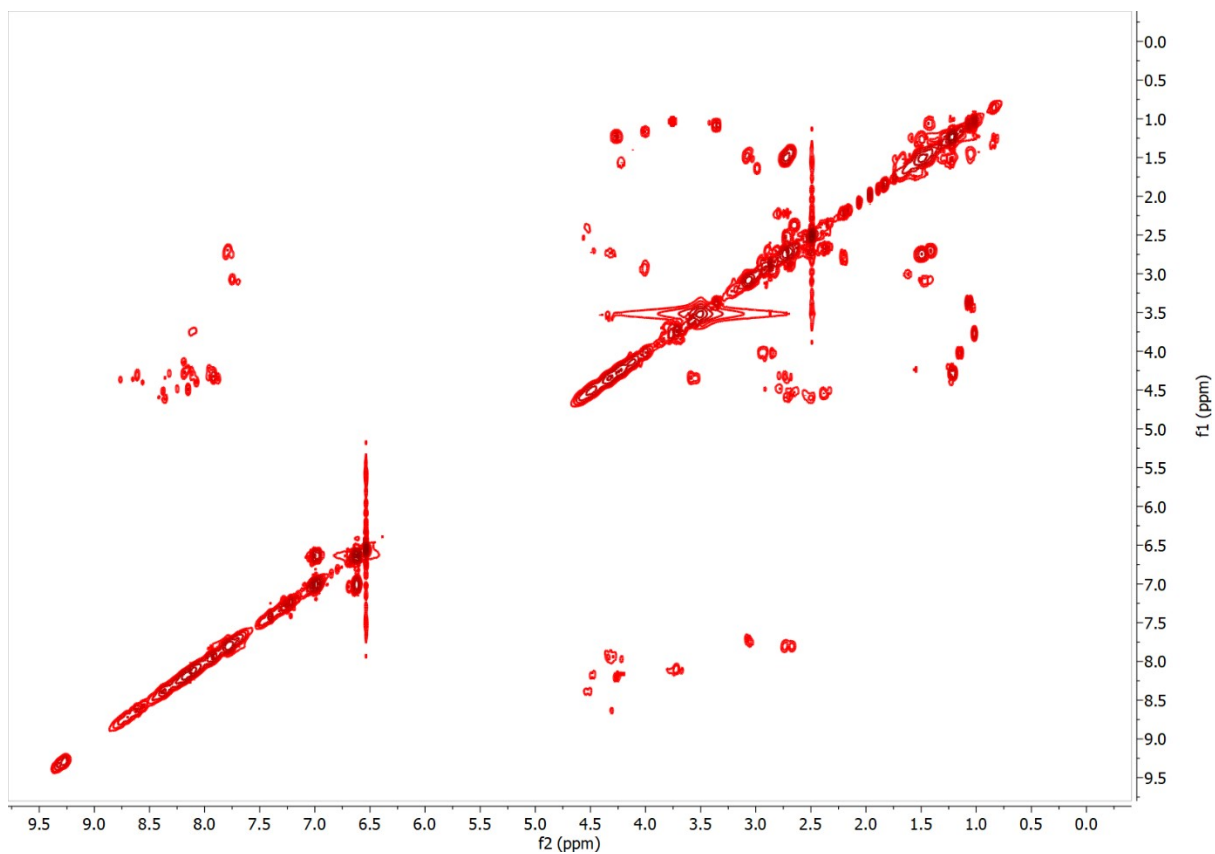


Figure S20: 2D ^1H COSY NMR spectrum of TP_ADlys peptide

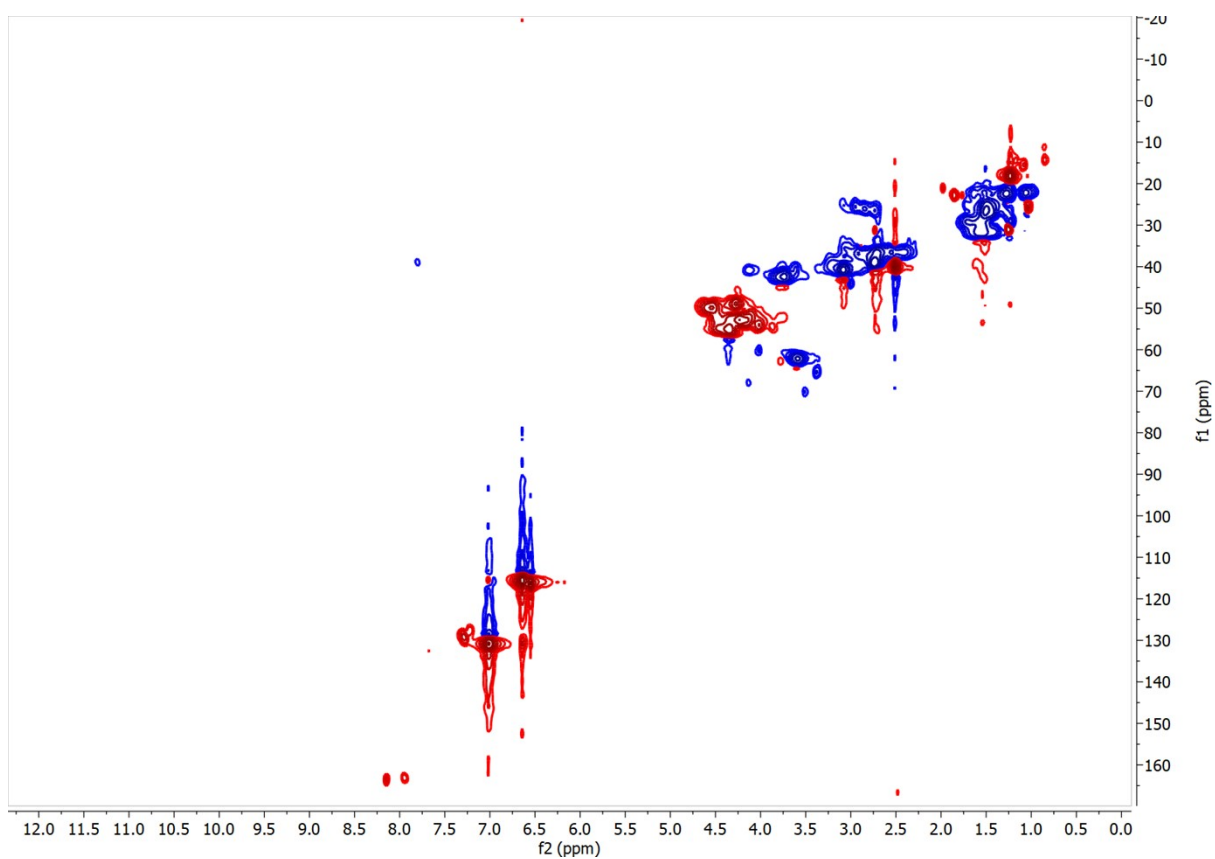


Figure S21: HSQC NMR spectrum of TP_ADlys peptide

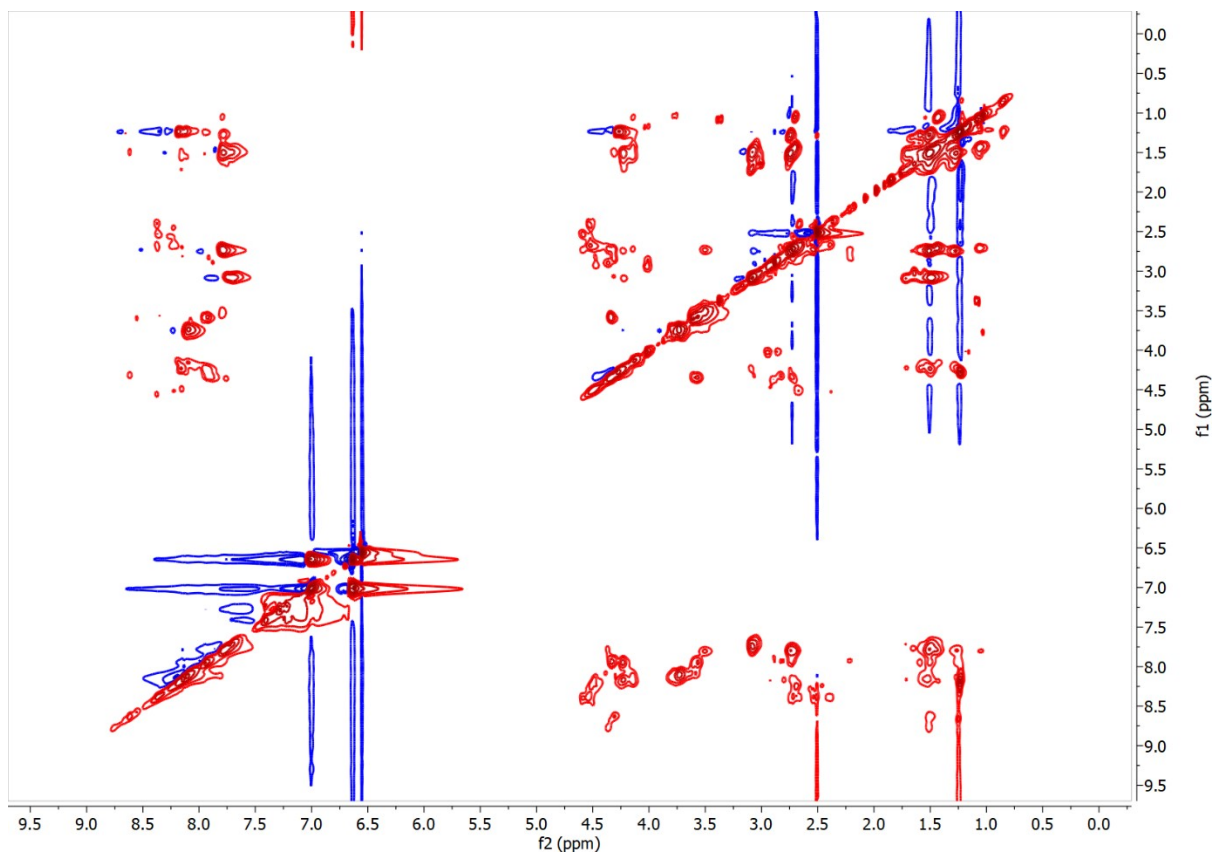


Figure S22: 2D ^1H TOCSY spectrum of TP_ADlys peptide

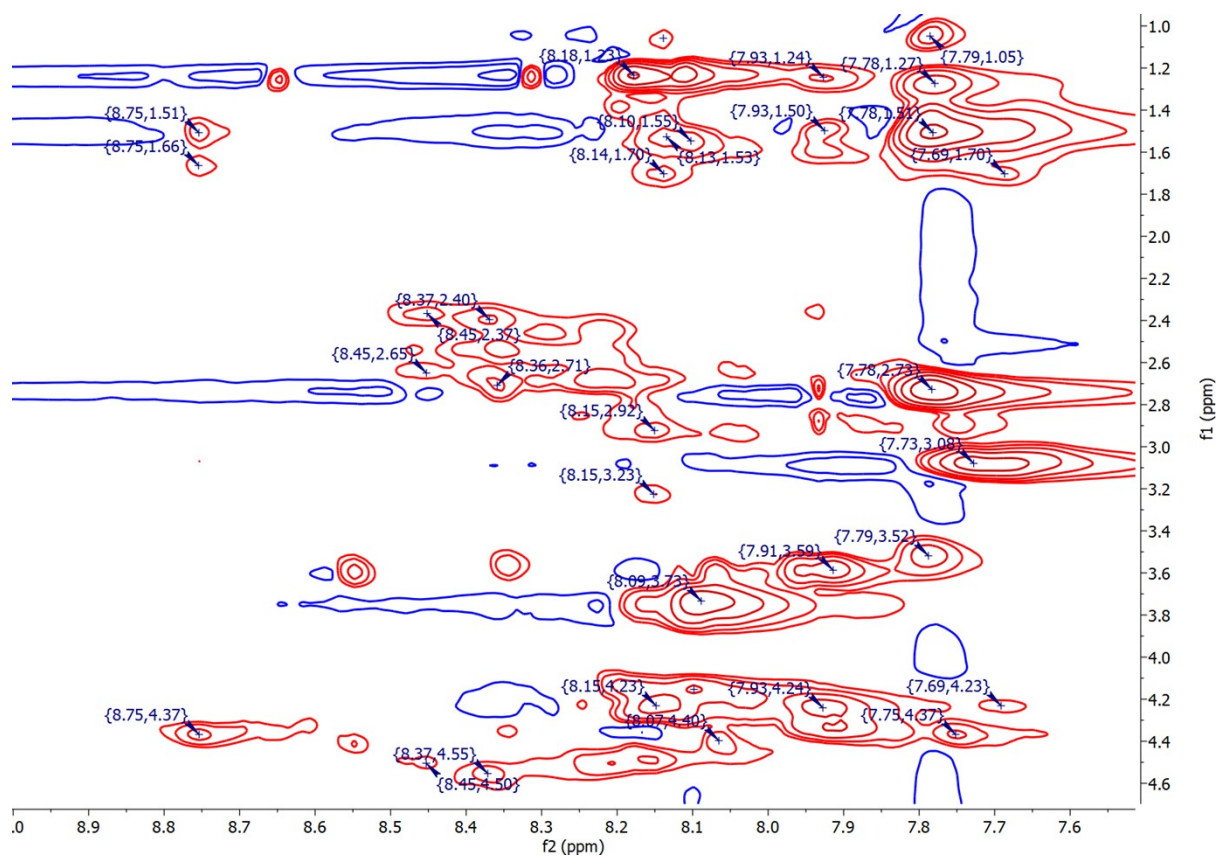


Figure S23: 2D ^1H TOCSY spectrum of TP_ADlys peptide, ^1HN fingerprint region

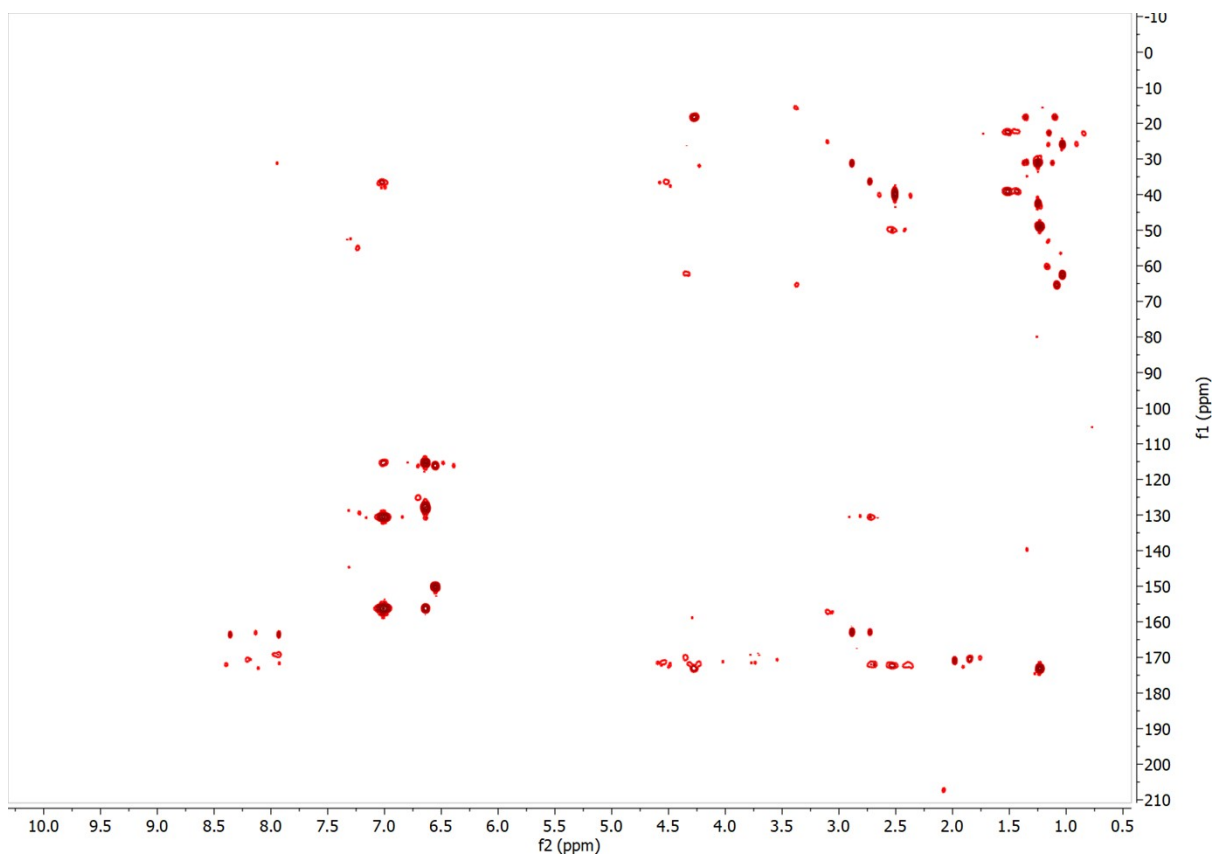


Figure S24: HMBC NMR spectrum of TP_ADlys peptide



Figure S25: 2D ^1H ROESY NMR spectrum of TP_ADlys peptide

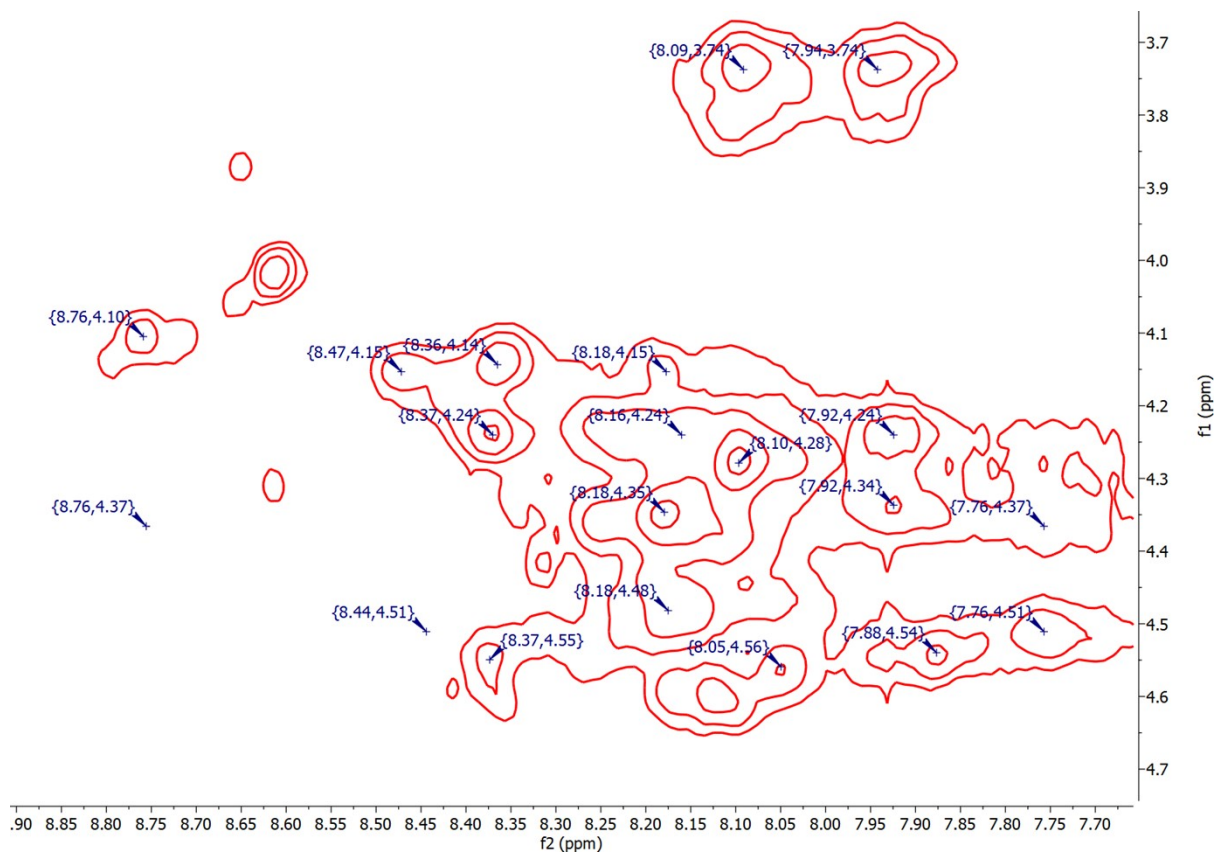


Figure S26: 2D ^1H ROESY NMR spectrum of TP_ADlys peptide, ^1HN region

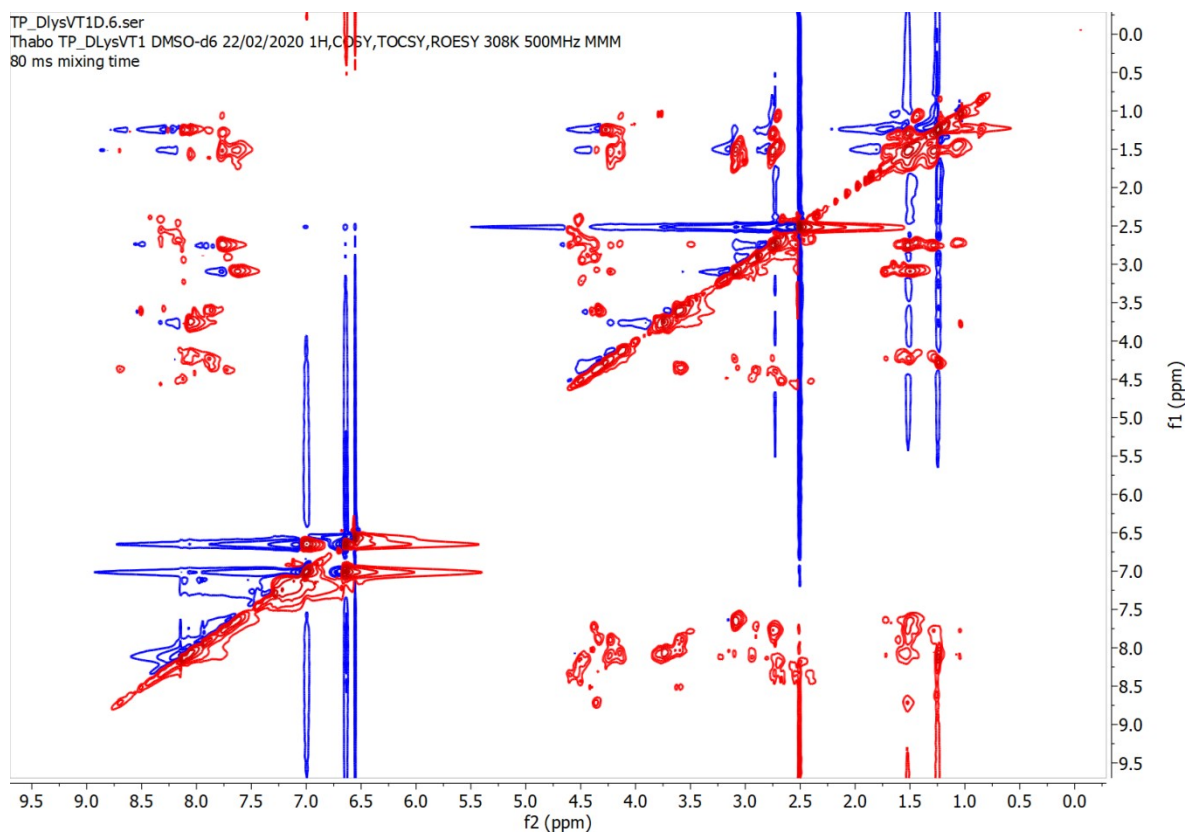


Figure S27: 2D ^1H TOCSY spectrum of TP_ADlys peptide at **308K**

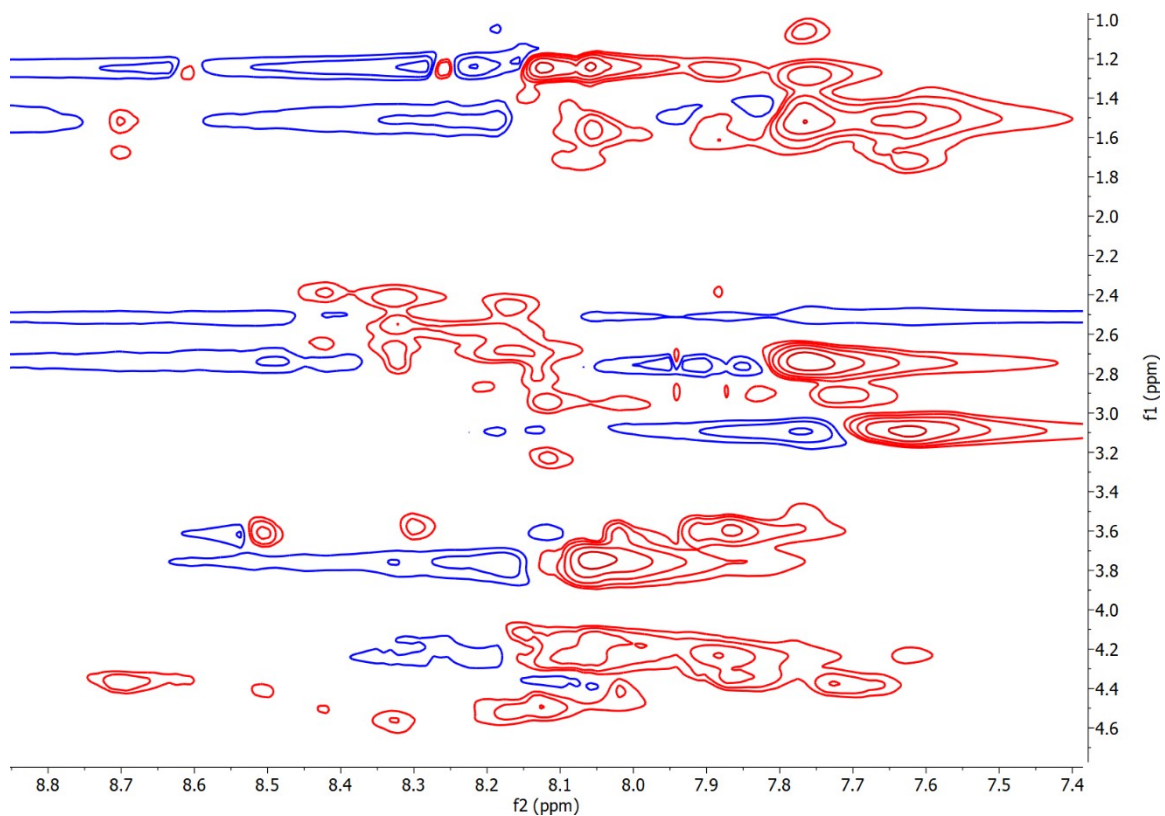


Figure S28: 2D ^1H TOCSY spectrum of TP_ADLYs peptide, ^1HN fingerprint region at 308K

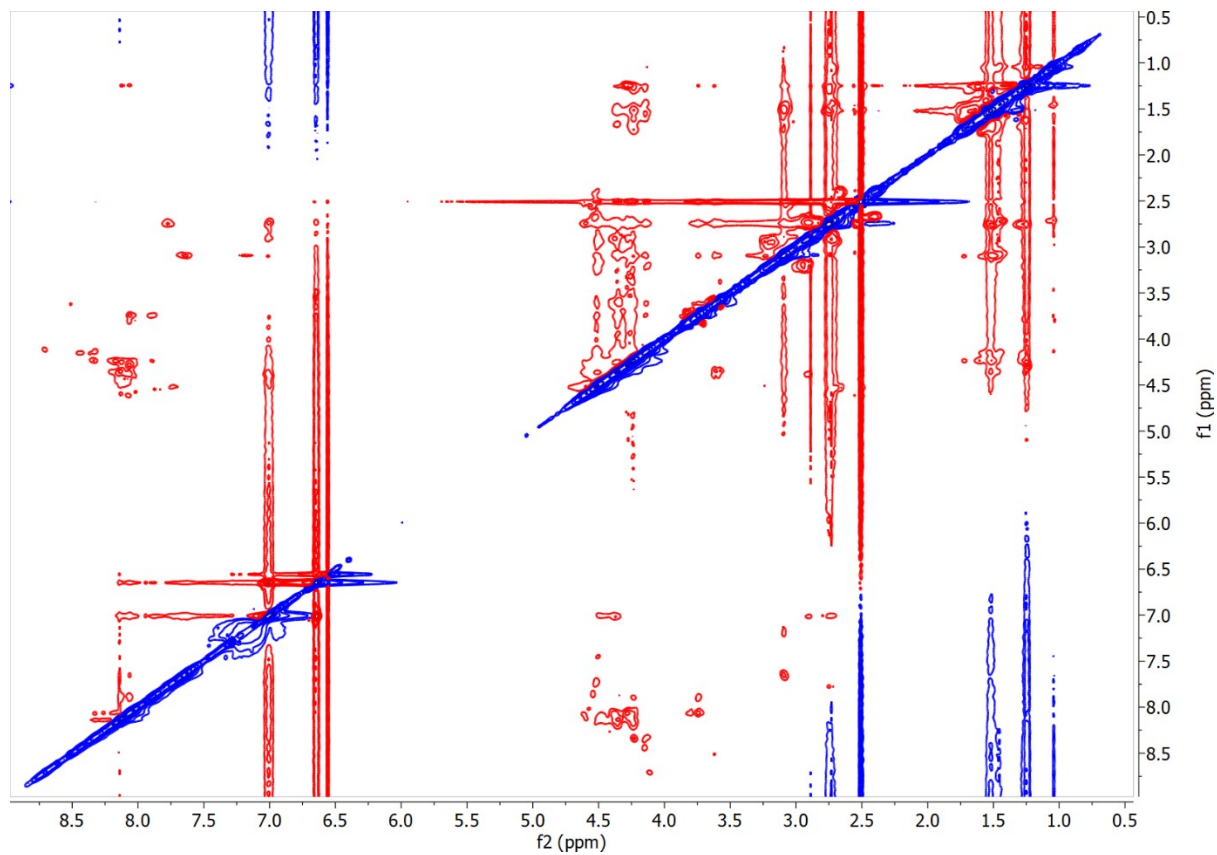


Figure-S29: 2D ^1H ROESY spectrum of TP_ADLYs peptide at 308K

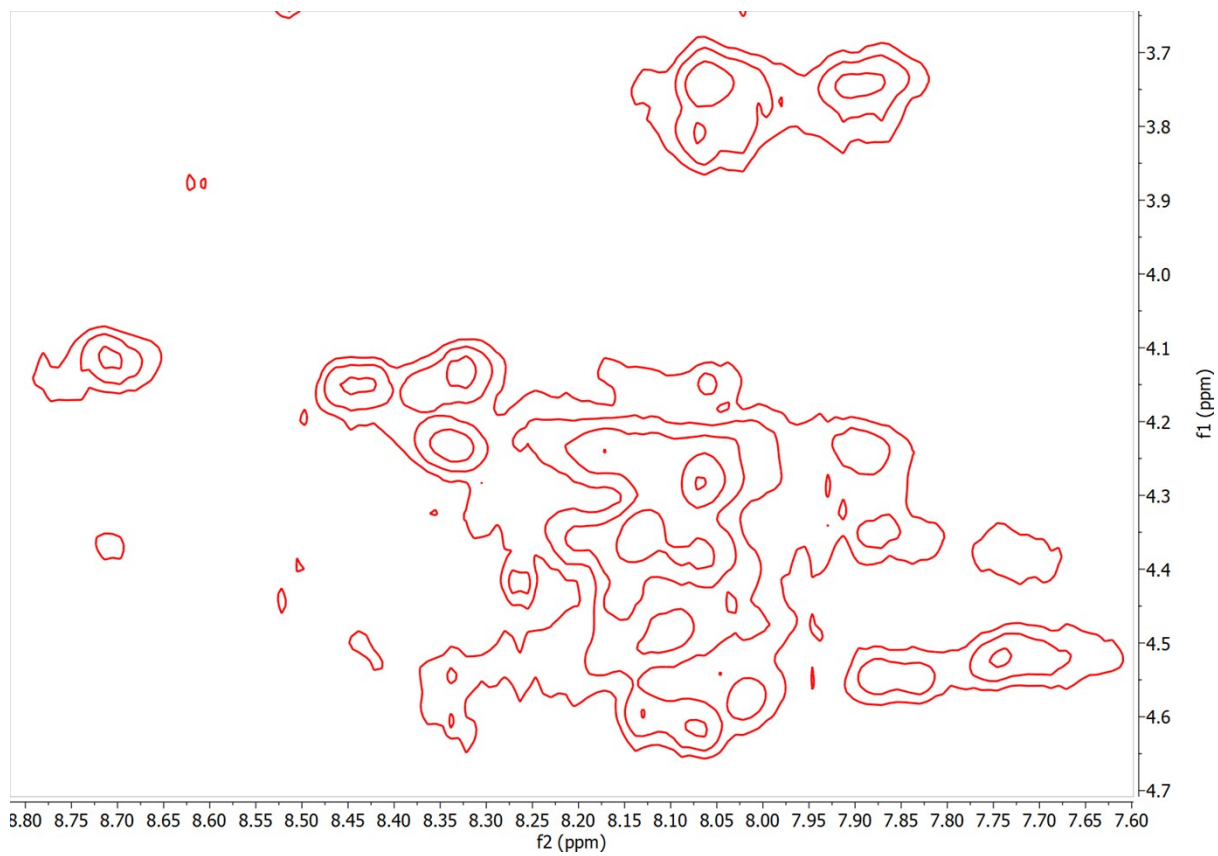


Figure S30: 2D ^1H ROESY spectrum of TP_ADlys peptide, ^1HN fingerprint region at **308K**.

Selected NMR spectra for aldol products

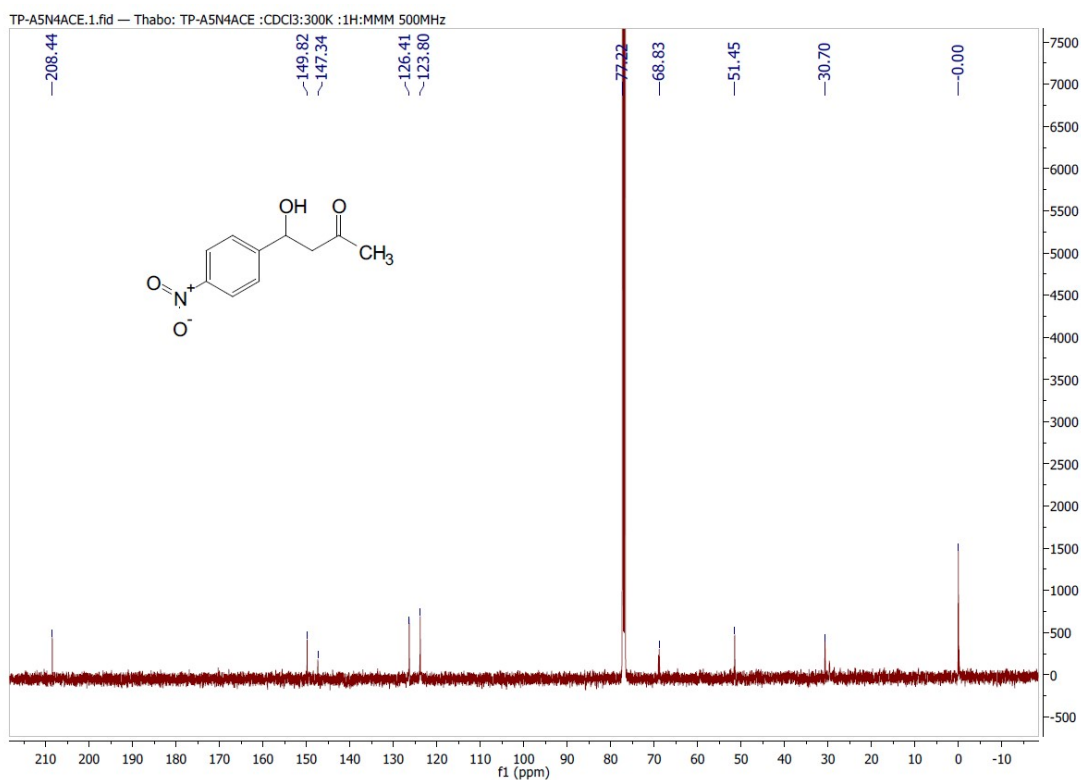
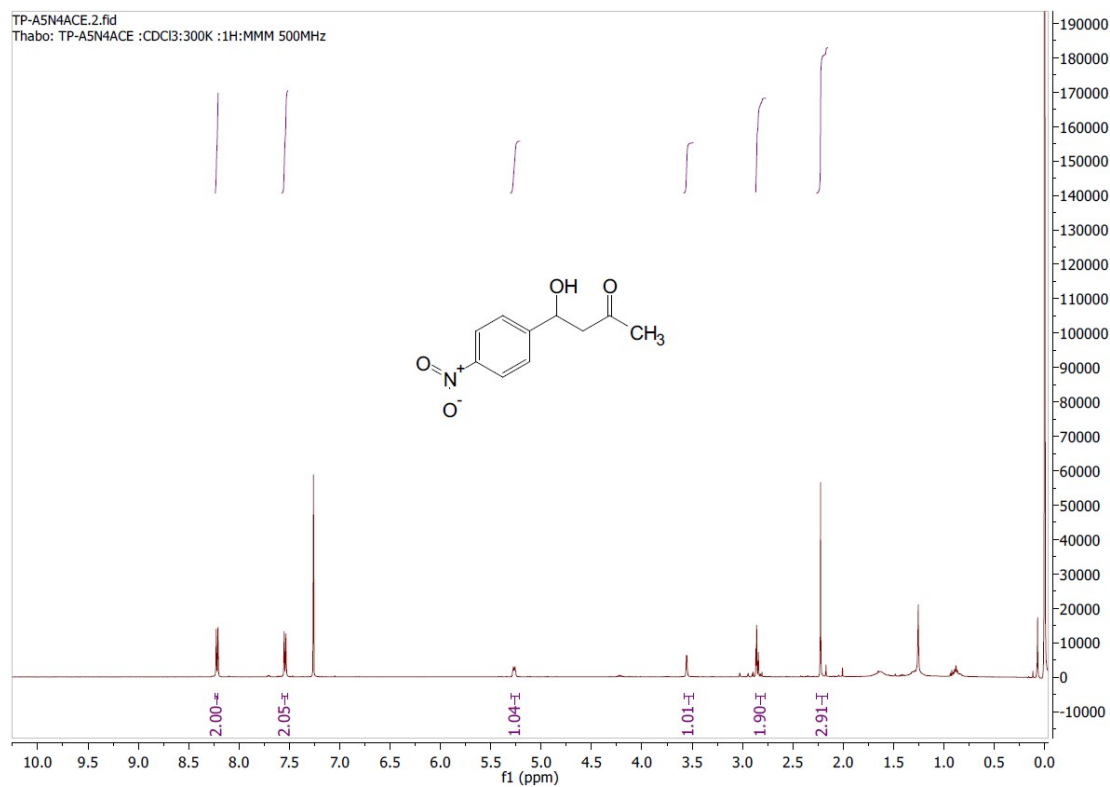


Figure S31: ^1H and ^{13}C NMR spectra of the aldol product TP_A1N4ACE

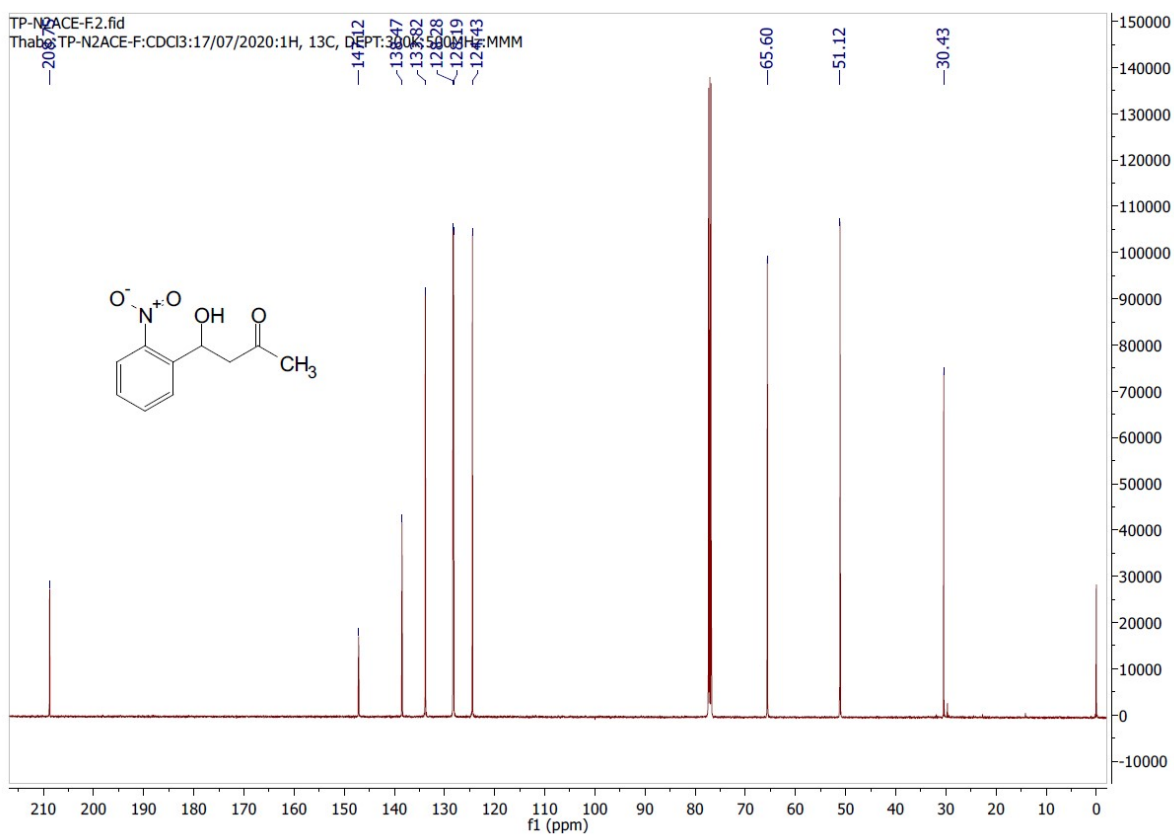
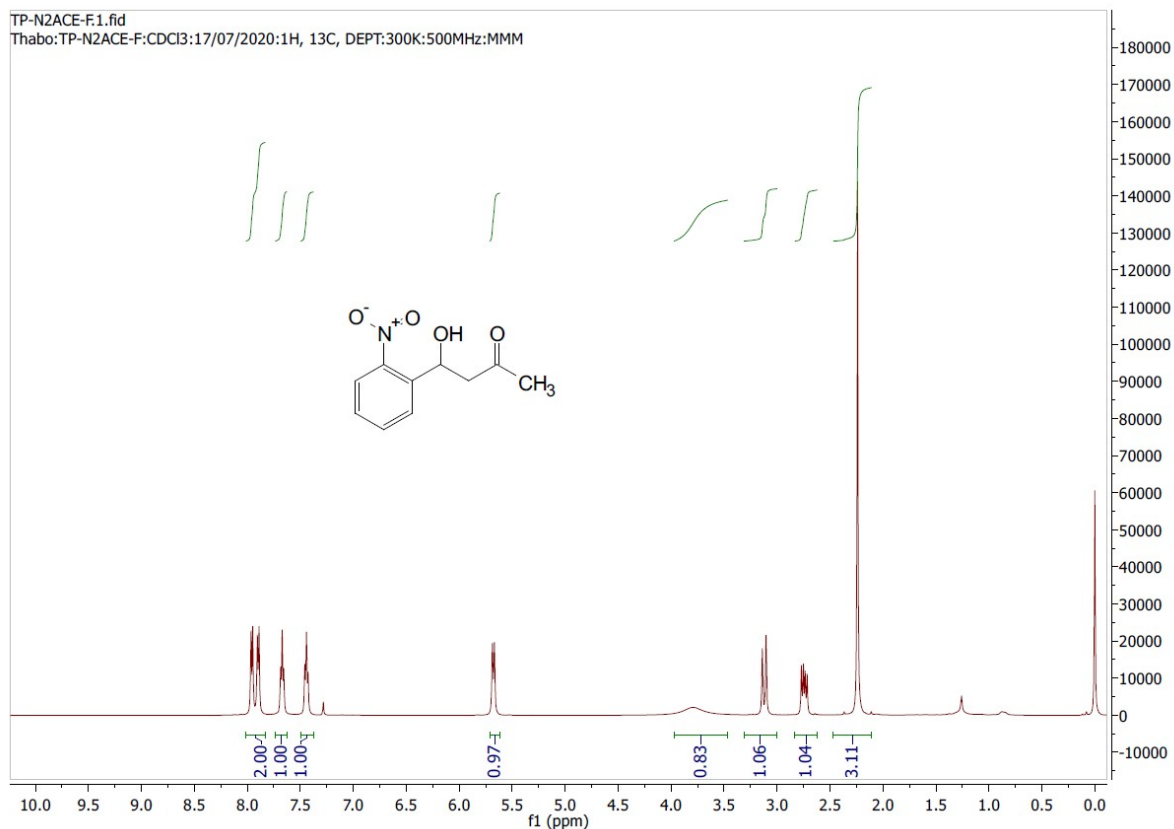


Figure S32: ^1H and ^{13}C NMR spectra of the aldol product TP_A2N2ACE

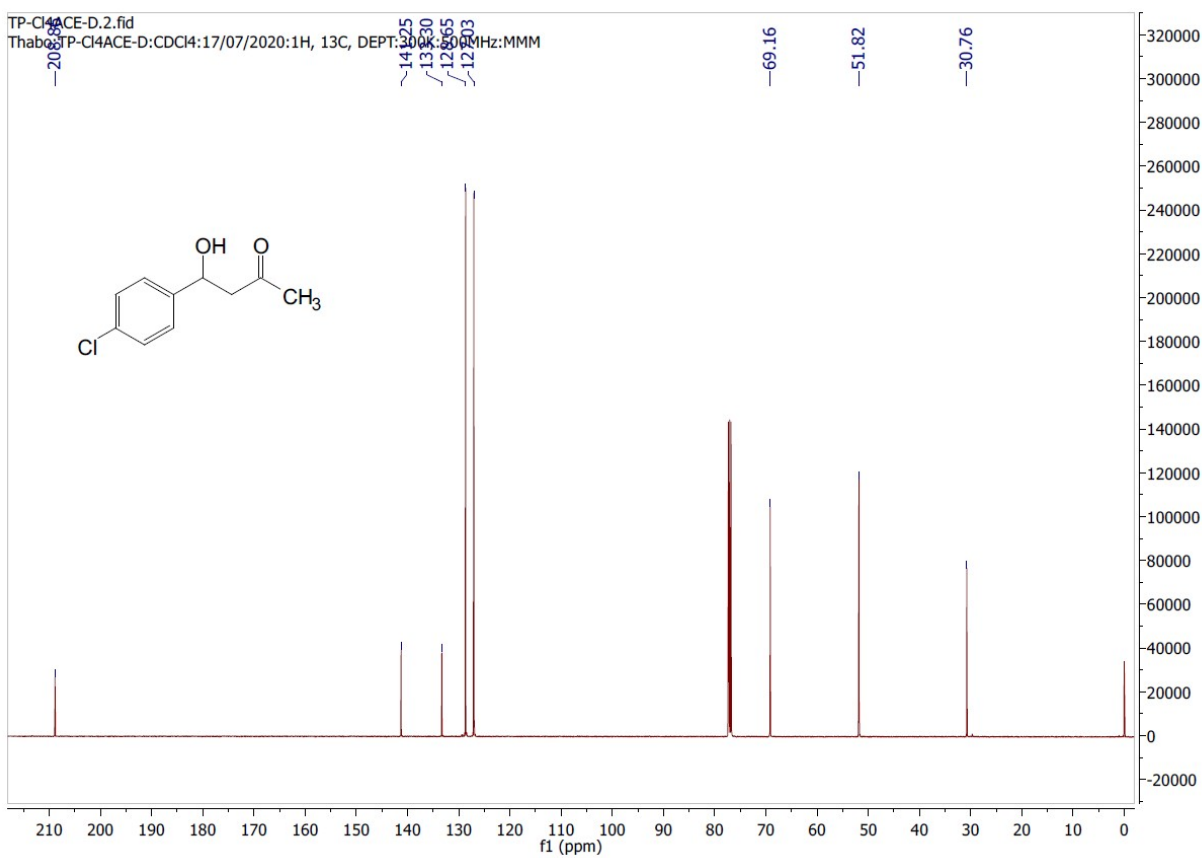
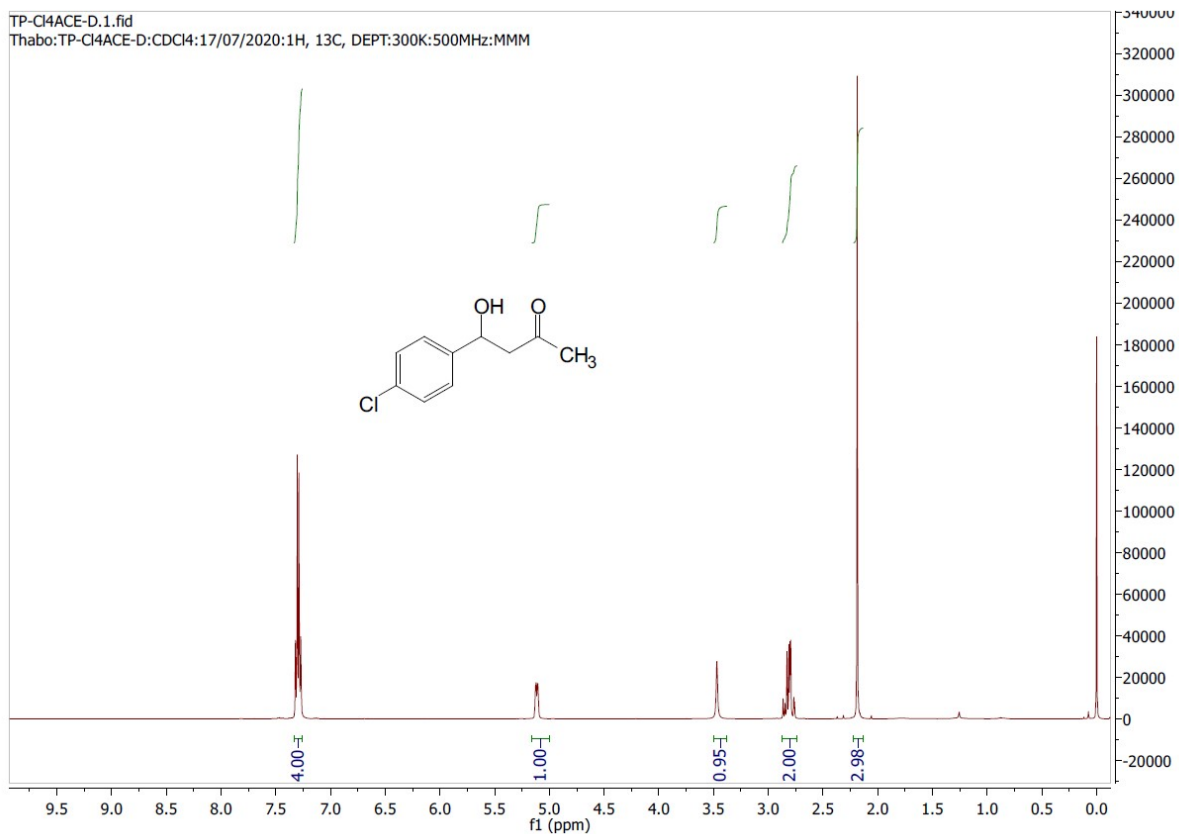


Figure S33: ^1H and ^{13}C NMR spectra of the aldol product TP_A3Cl4ACE

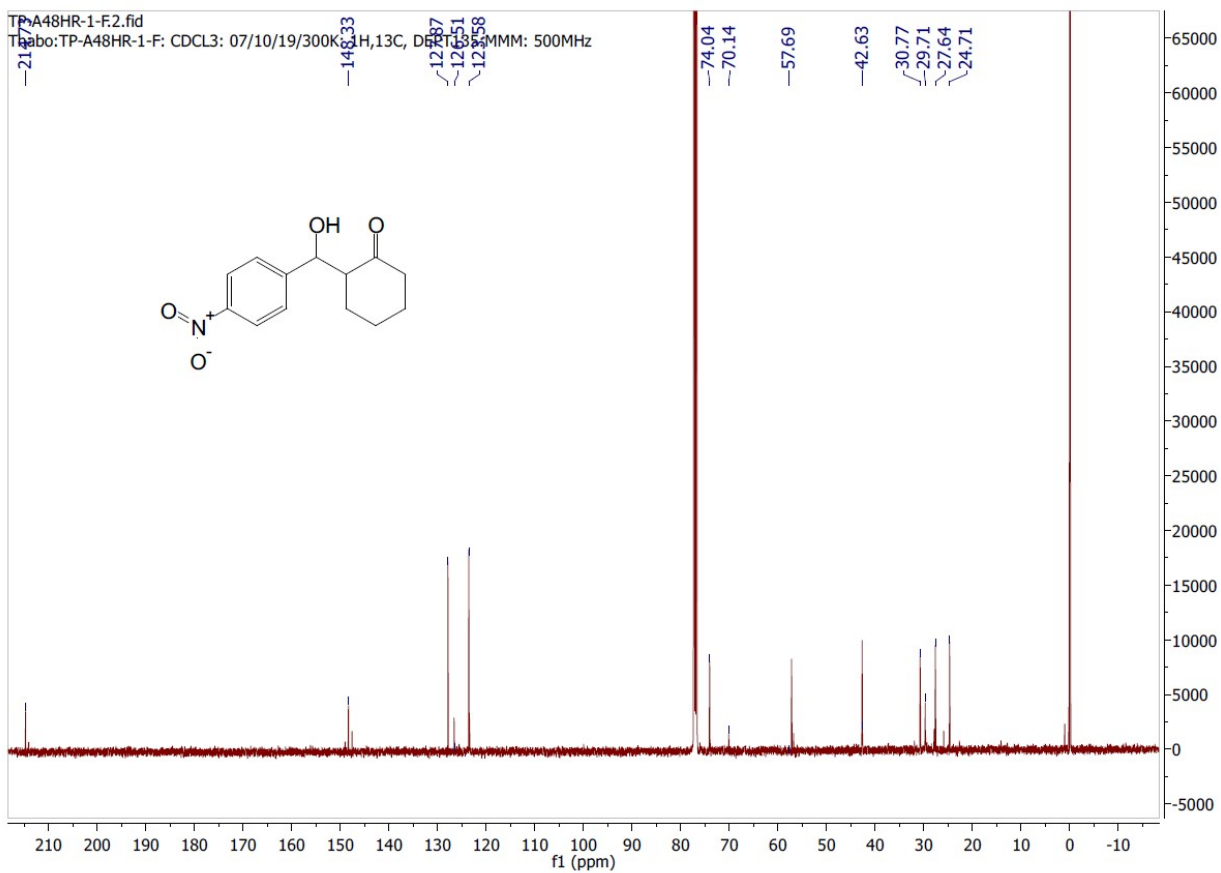
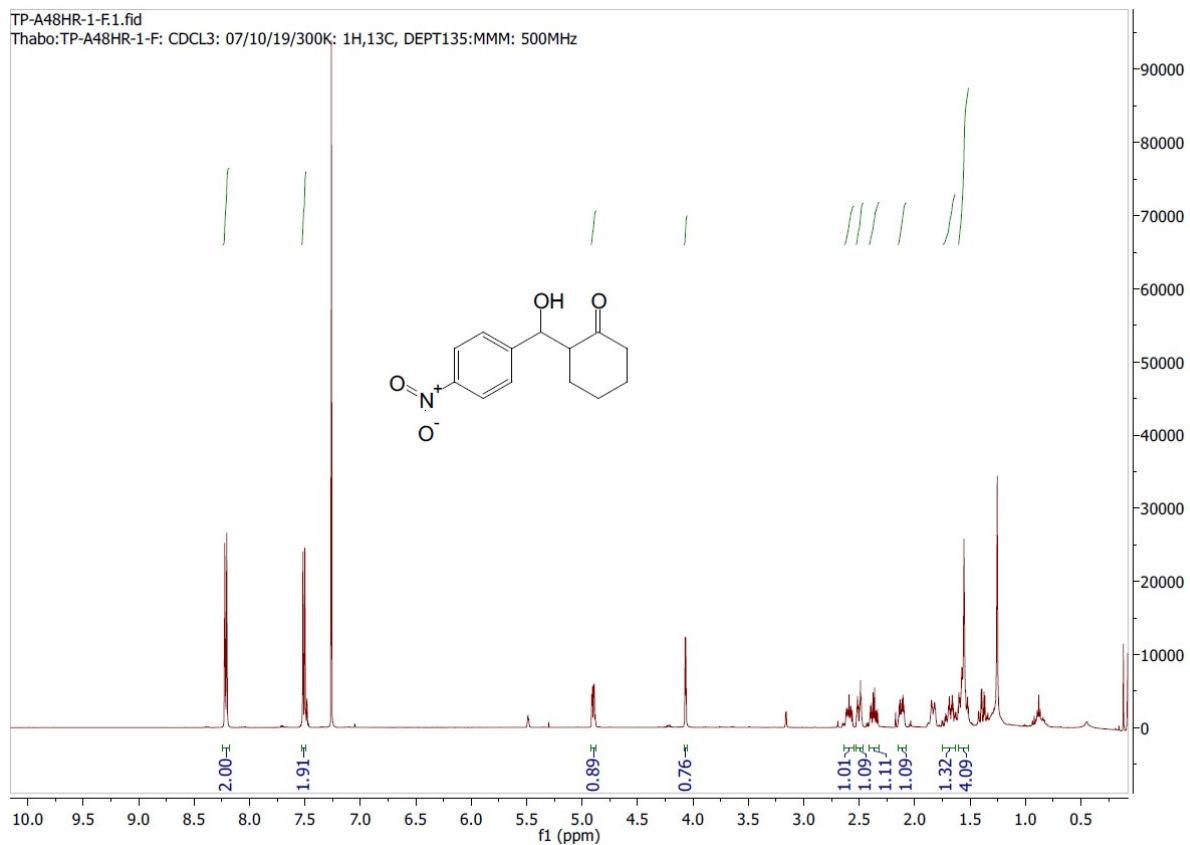


Figure S34: ^1H and ^{13}C NMR spectra of the aldol product TP_A4N4CY

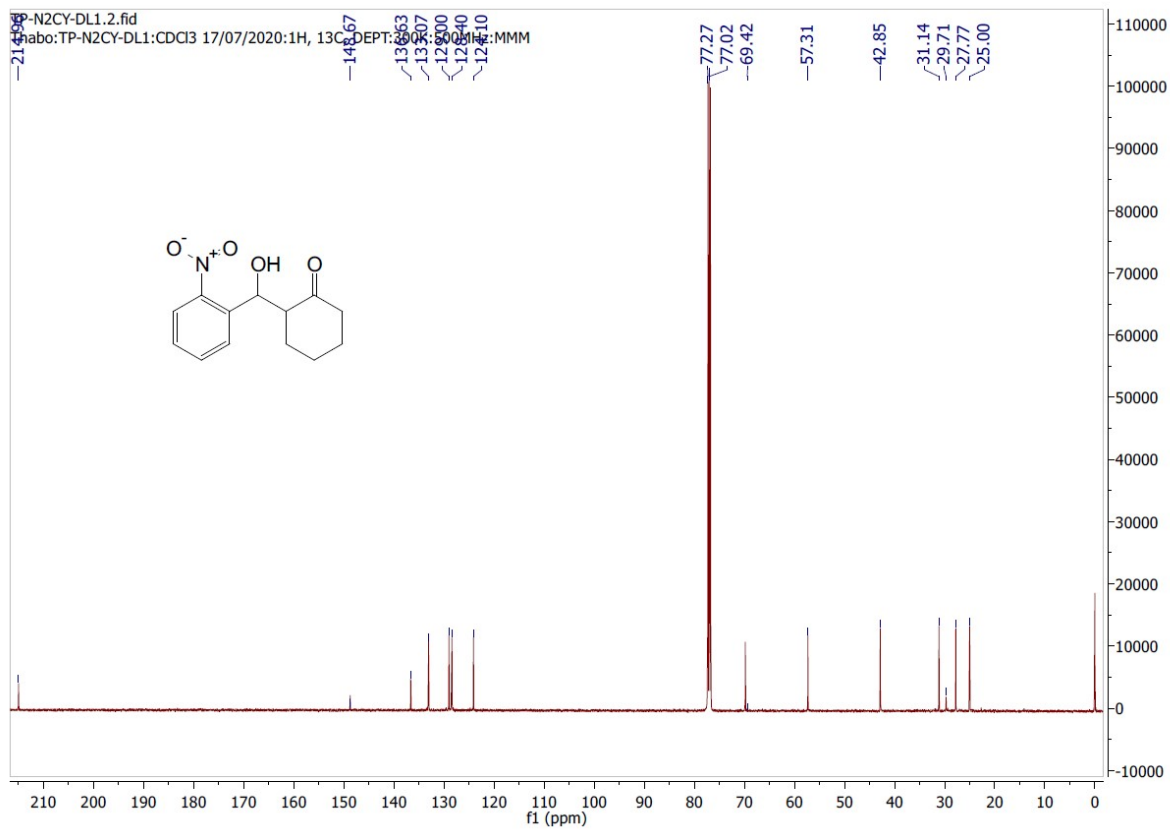
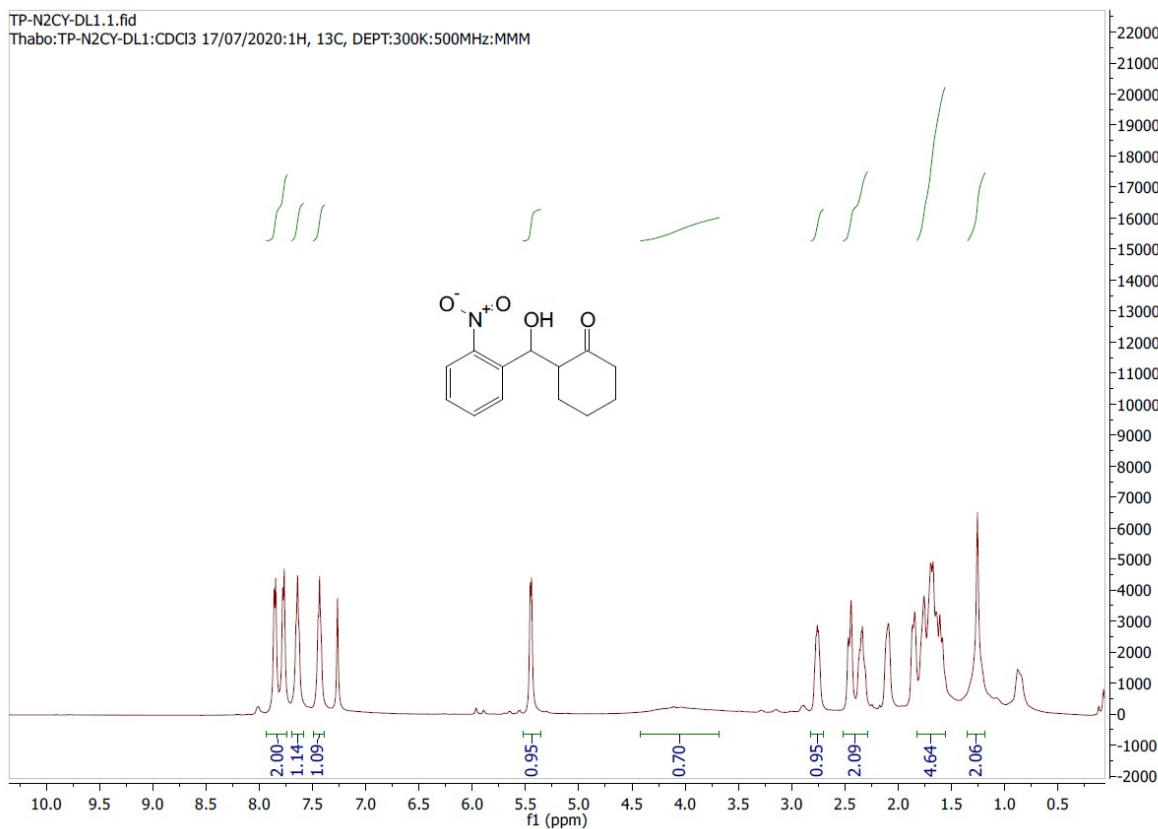


Figure S35: ^1H and ^{13}C NMR spectra of the aldol product TP_A5N2CY

Selected chiral HPLC chromatograms for aldol products

Using Acetone as a solvent and substrate (**Table 2**)

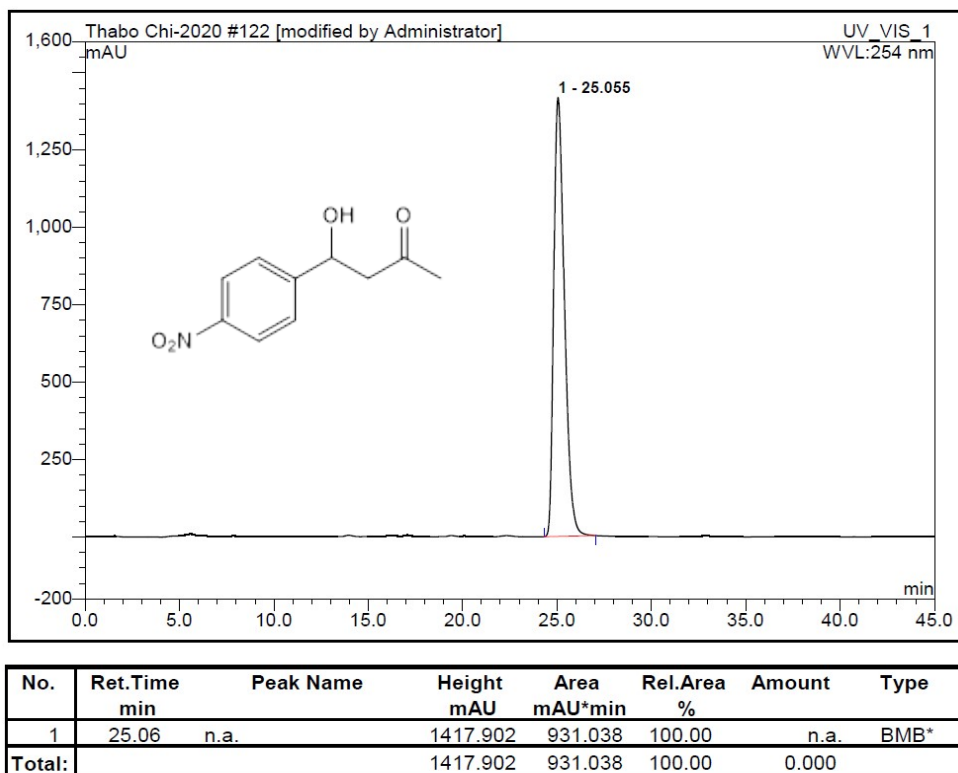


Figure SR: Chiral HPLC chromatogram of TP_A1N4ACE –standard

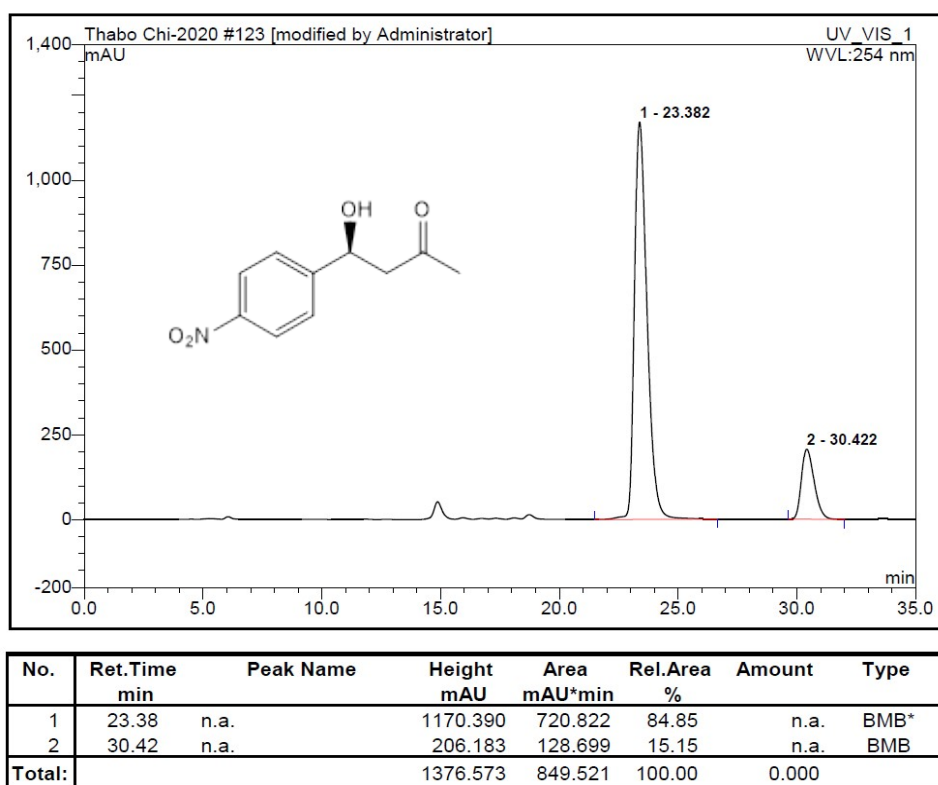
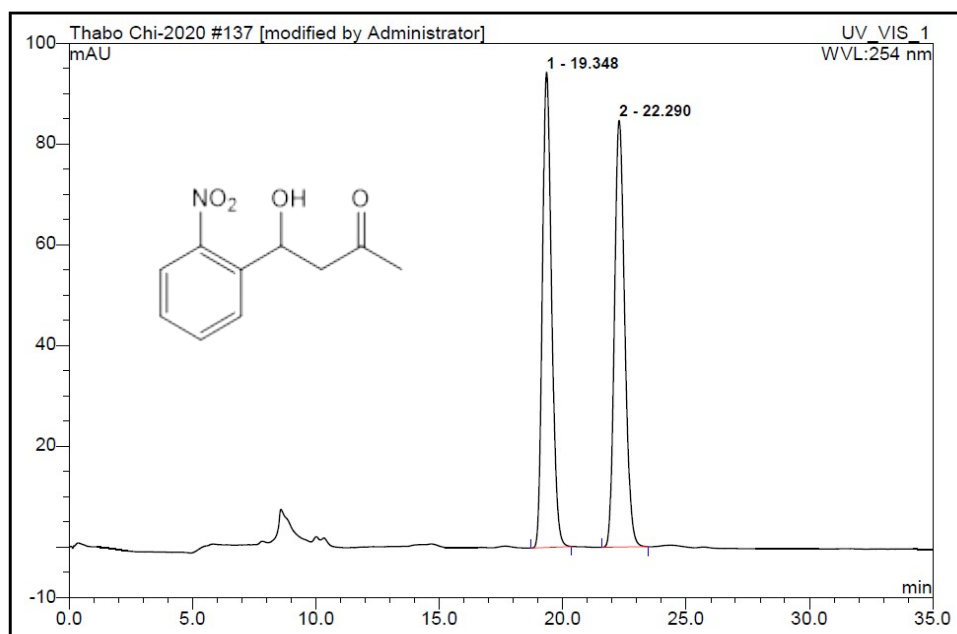
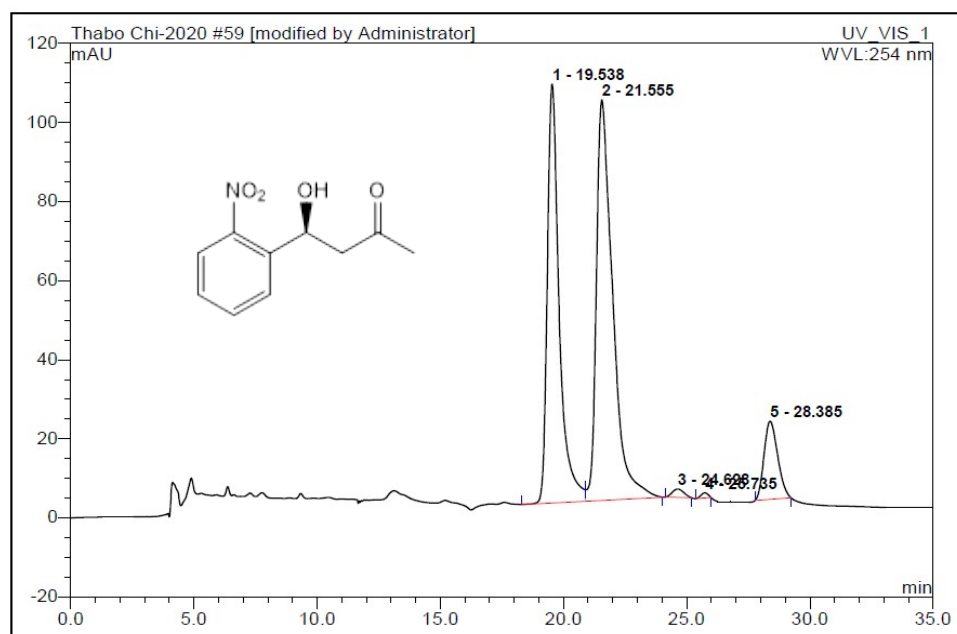


Figure S36: Chiral HPLC chromatogram of TP_A1.1N4ACE (entry1)



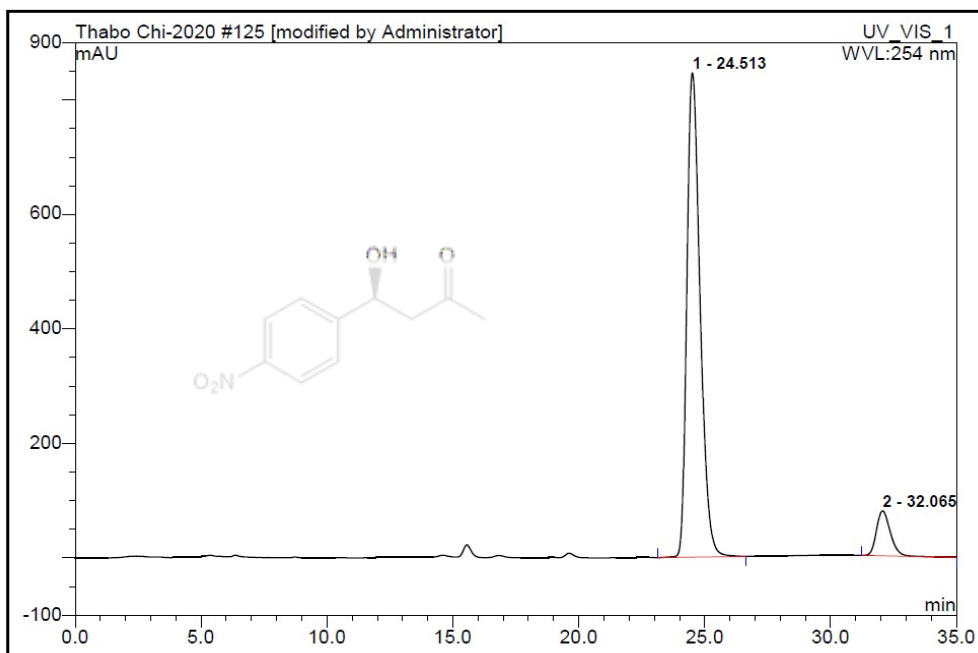
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|--------------|-----------|------------|--------------|------------|--------|------|
| 1 | 19.35 | n.a. | 94.329 | 40.831 | 49.99 | n.a. | BMB* |
| 2 | 22.29 | n.a. | 84.722 | 40.849 | 50.01 | n.a. | BMB* |
| Total: | | | 179.051 | 81.680 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A2N2ACE-S2



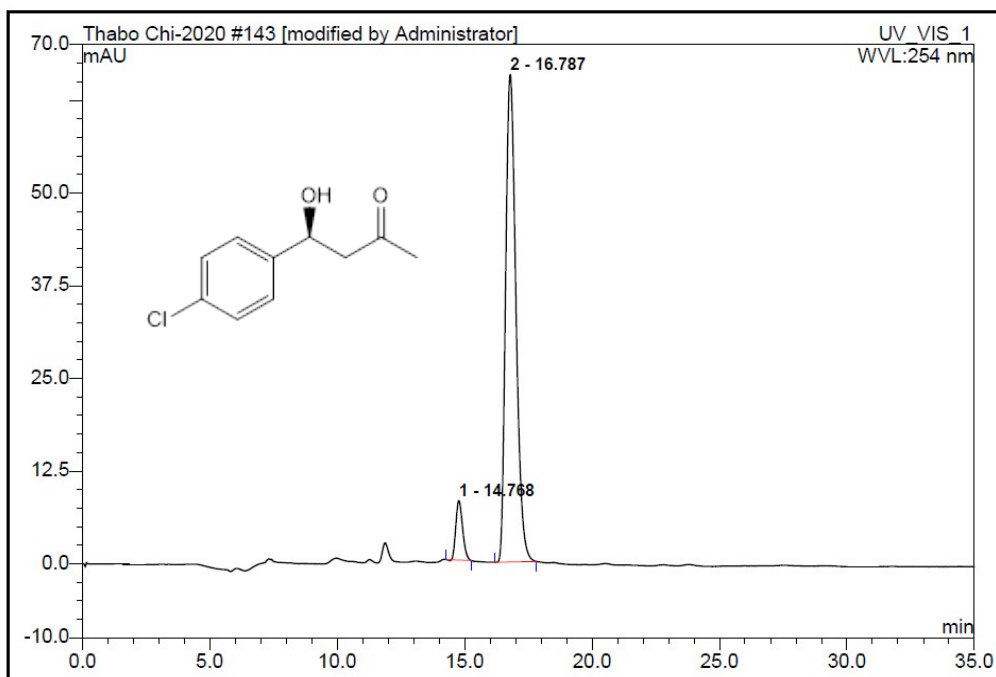
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|--------------|-----------|------------|--------------|------------|--------|------|
| 1 | 19.54 | n.a. | 105.908 | 59.370 | 39.25 | n.a. | BM * |
| 2 | 21.56 | n.a. | 101.309 | 77.648 | 51.34 | n.a. | MB* |
| 3 | 24.63 | n.a. | 2.125 | 1.156 | 0.76 | n.a. | BMB* |
| 4 | 25.74 | n.a. | 1.280 | 0.417 | 0.28 | n.a. | BMB* |
| 5 | 28.39 | n.a. | 19.739 | 12.653 | 8.37 | n.a. | BMB* |
| Total: | | | 230.360 | 151.244 | 100.00 | 0.000 | |

Figure S37: Chiral HPLC chromatogram of TP_A2.1N2ACE (entry 2)



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 24.51 | n.a. | 846.014 | 515.043 | 91.14 | n.a. | BMB* |
| 2 | 32.07 | n.a. | 78.442 | 50.053 | 8.86 | n.a. | BMB* |
| Total: | | | 924.456 | 565.095 | 100.00 | 0.000 | |

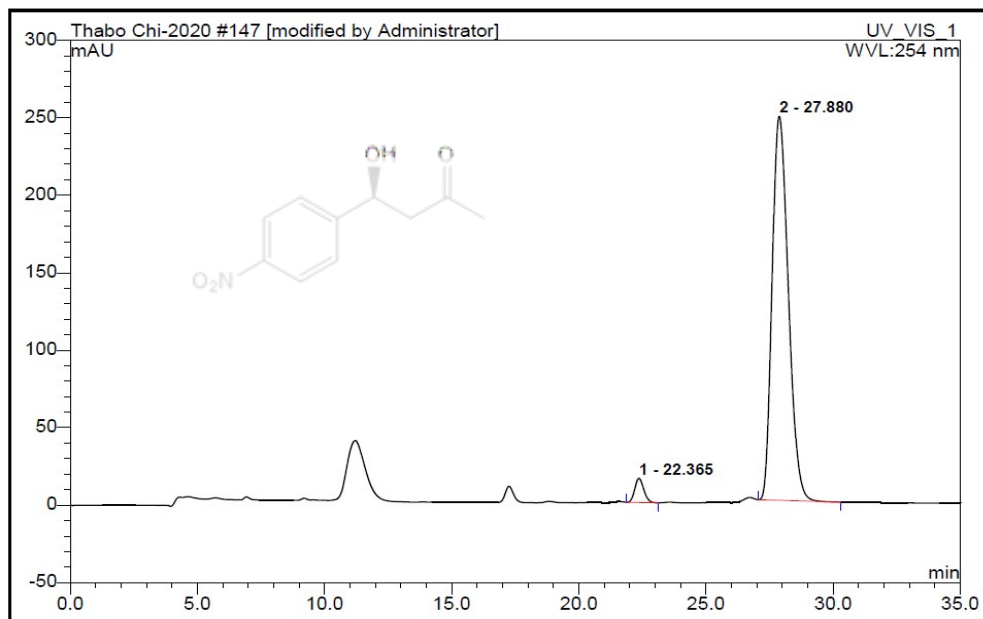
Figure SR: Chiral HPLC chromatogram of TP_A1.2N4ACE (entry 4)



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 14.77 | n.a. | 8.036 | 2.462 | 7.43 | n.a. | BMB* |
| 2 | 16.79 | n.a. | 65.744 | 30.679 | 92.57 | n.a. | BMB* |
| Total: | | | 73.780 | 33.142 | 100.00 | 0.000 | |

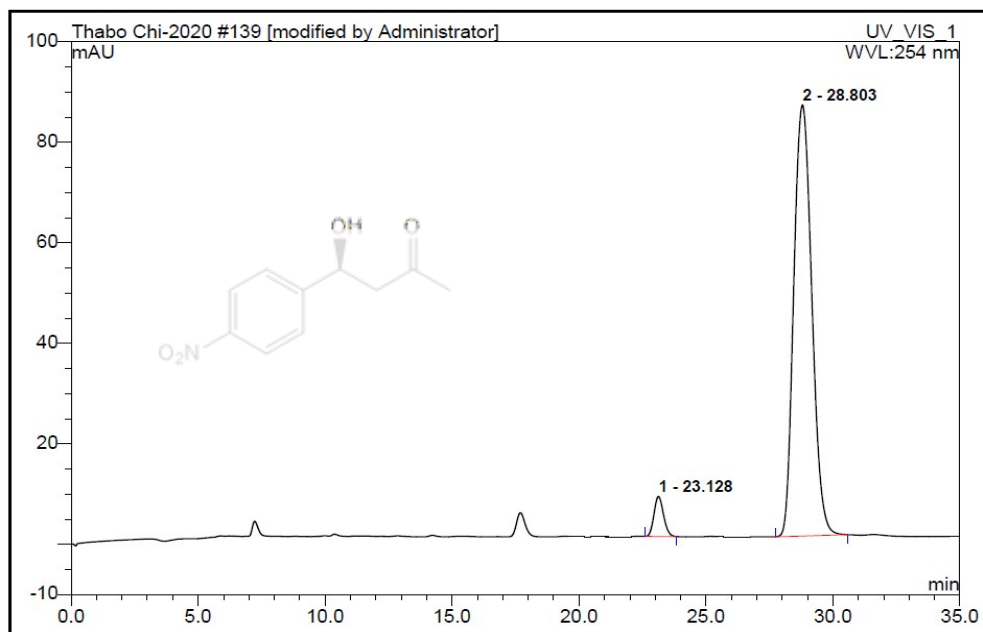
Figure S38: Chiral HPLC chromatogram of TP_A3Cl4ACE (entry 6)

Using 8 mol% for reaction between Acetone and *p*-nitrobenzaldehyde (Table 5)



| No. | Ret. Time min | Peak Name | Height mAU | Area mAU*min | Rel. Area % | Amount | Type |
|---------------|---------------|-----------|------------|--------------|-------------|--------|------|
| 1 | 22.37 | n.a. | 15.513 | 6.386 | 3.37 | n.a. | BMB* |
| 2 | 27.88 | n.a. | 247.796 | 182.974 | 96.63 | n.a. | BMB* |
| Total: | | | 263.309 | 189.360 | 100.00 | 0.000 | |

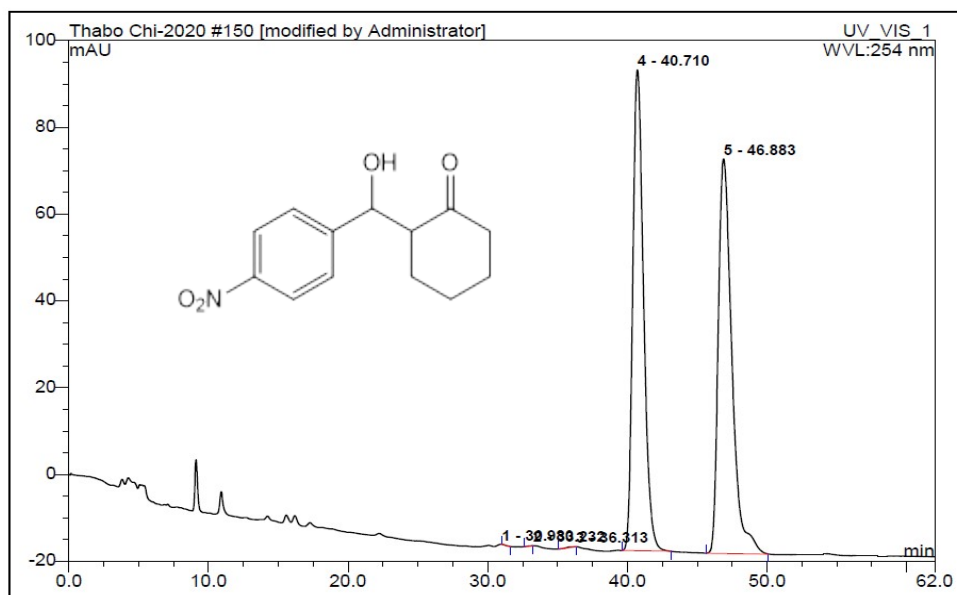
Figure SR: Chiral HPLC chromatogram of TP_A1.3N4ACE (entry 11)



| No. | Ret. Time min | Peak Name | Height mAU | Area mAU*min | Rel. Area % | Amount | Type |
|---------------|---------------|-----------|------------|--------------|-------------|--------|------|
| 1 | 23.13 | n.a. | 7.962 | 3.480 | 4.71 | n.a. | BMB* |
| 2 | 28.80 | n.a. | 85.803 | 70.346 | 95.29 | n.a. | BMB* |
| Total: | | | 93.765 | 73.825 | 100.00 | 0.000 | |

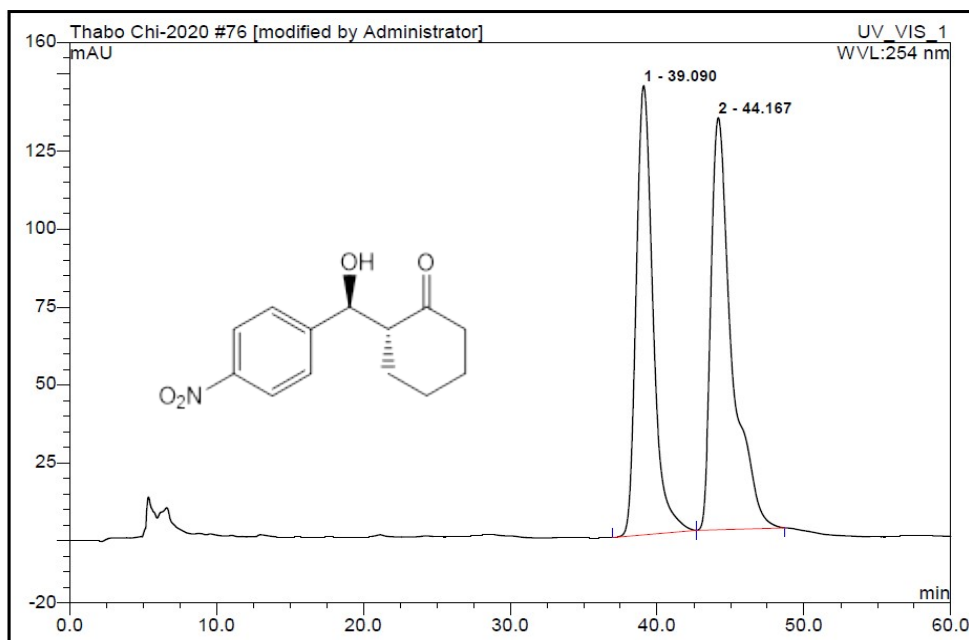
Figure S.39: Chiral HPLC chromatogram of TP-A1.4N4ACE (entry 12)

Effect of substrate: Reaction between cyclohexanone and aromatic aldehydes (Table 3)



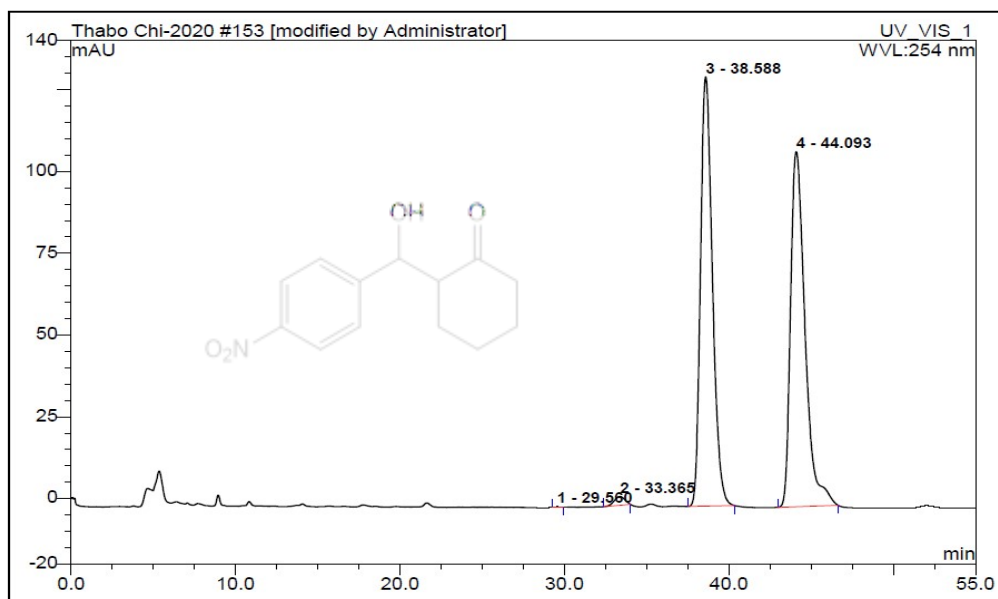
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|--------------|-----------|------------|--------------|------------|--------|------|
| 1 | 30.98 | n.a. | 0.011 | 0.002 | 0.00 | n.a. | BMB* |
| 2 | 33.23 | n.a. | 0.010 | 0.011 | 0.01 | n.a. | BMB* |
| 3 | 36.31 | n.a. | 0.008 | 0.046 | 0.02 | n.a. | BMB* |
| 4 | 40.71 | n.a. | 110.867 | 99.050 | 49.14 | n.a. | BMB* |
| 5 | 46.88 | n.a. | 90.970 | 102.454 | 50.83 | n.a. | BMB |
| Total: | | | 201.866 | 201.564 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R1



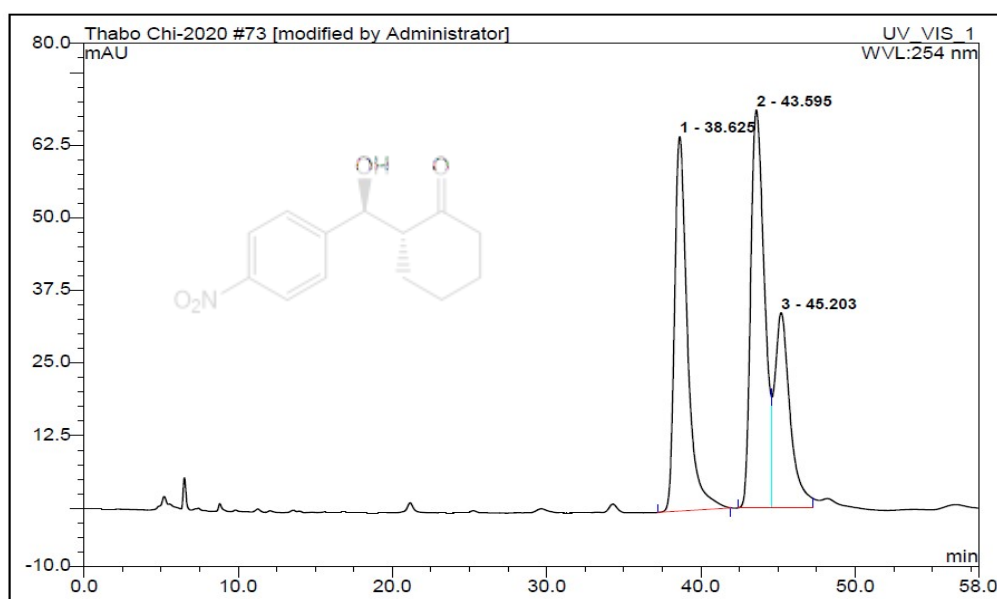
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|--------------|-----------|------------|--------------|------------|--------|------|
| 1 | 39.09 | n.a. | 144.164 | 191.099 | 46.64 | n.a. | BMB* |
| 2 | 44.17 | n.a. | 132.215 | 218.650 | 53.36 | n.a. | BMB* |
| Total: | | | 276.380 | 409.750 | 100.00 | 0.000 | |

Figure S40: Chiral HPLC chromatogram of TP_A4.1N4CY (entry 4)



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 29.56 | n.a. | 0.181 | 0.069 | 0.03 | n.a. | BMB* |
| 2 | 33.37 | n.a. | 2.682 | 1.999 | 0.90 | n.a. | BMB* |
| 3 | 38.59 | n.a. | 131.271 | 109.853 | 49.38 | n.a. | BMB* |
| 4 | 44.09 | n.a. | 108.542 | 110.528 | 49.69 | n.a. | BMB* |
| Total: | | | 242.676 | 222.450 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R4



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 38.63 | n.a. | 64.448 | 62.770 | 36.38 | n.a. | BMB* |
| 2 | 43.60 | n.a. | 68.545 | 70.791 | 41.03 | n.a. | BM * |
| 3 | 45.20 | n.a. | 33.626 | 38.975 | 22.59 | n.a. | M * |
| Total: | | | 166.619 | 172.535 | 100.00 | 0.000 | |

Figure S41: Chiral HPLC chromatogram of TP_A4.2N4CY (entry 9)

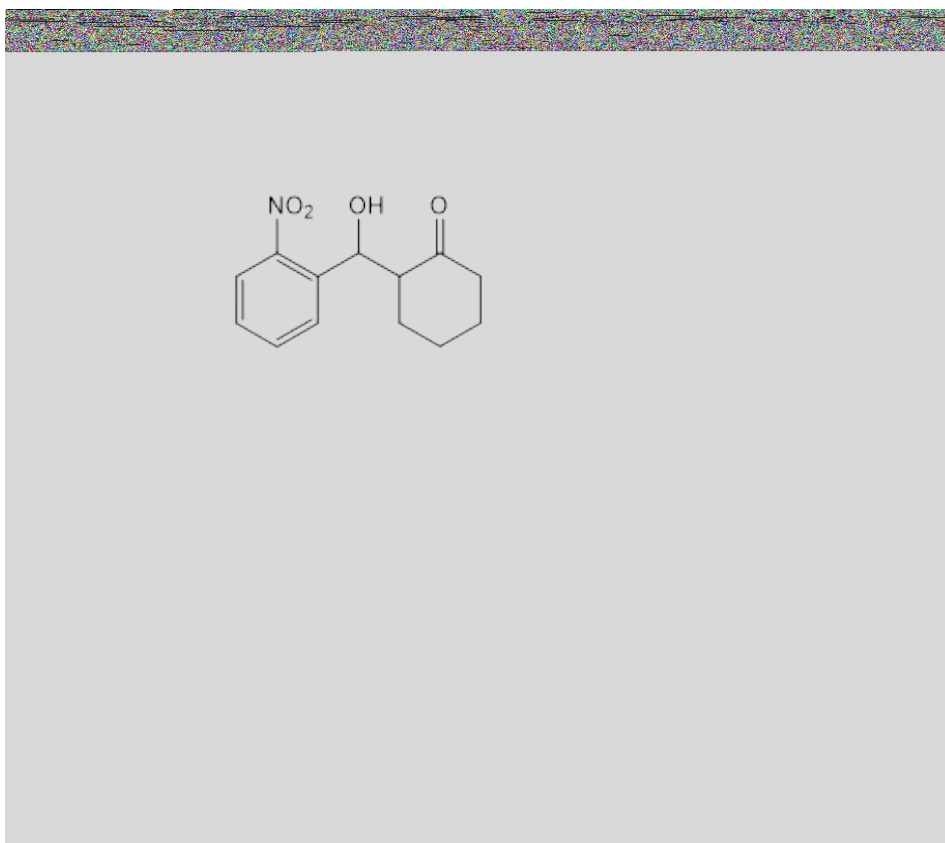
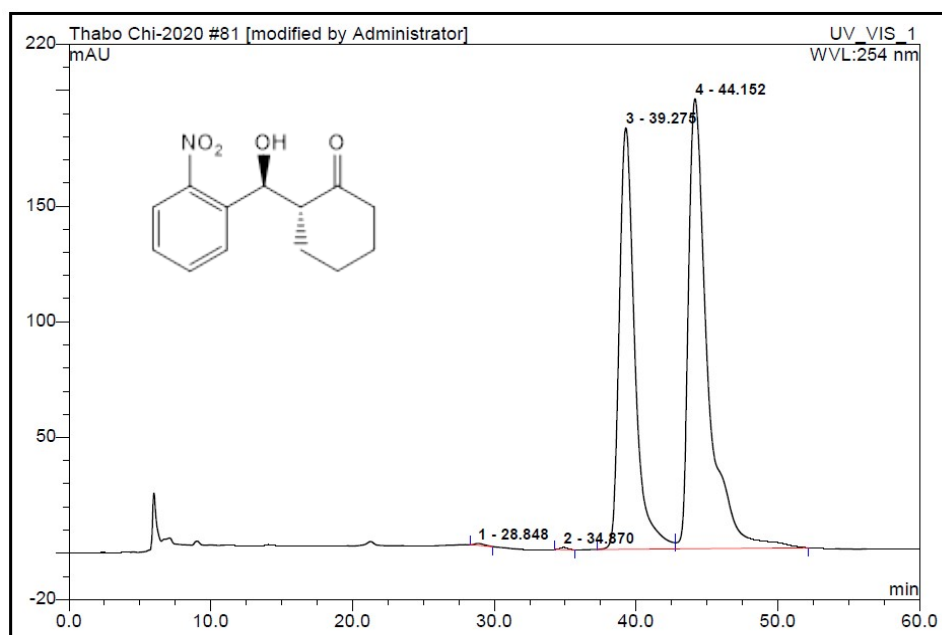


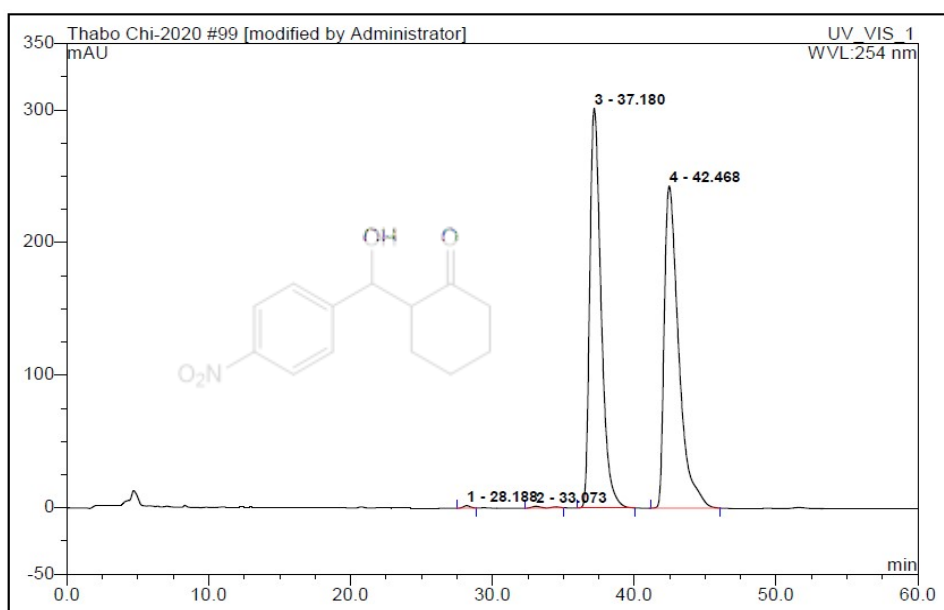
Figure SR: Chiral HPLC chromatogram of racemate TP_A5N2CY-RA5



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 28.85 | n.a. | 0.923 | 0.717 | 0.13 | n.a. | BMB |
| 2 | 34.87 | n.a. | 0.962 | 0.630 | 0.12 | n.a. | BMB* |
| 3 | 39.28 | n.a. | 182.083 | 232.316 | 43.02 | n.a. | BM * |
| 4 | 44.15 | n.a. | 194.427 | 306.341 | 56.73 | n.a. | MB* |
| Total: | | | 378.395 | 540.004 | 100.00 | 0.000 | |

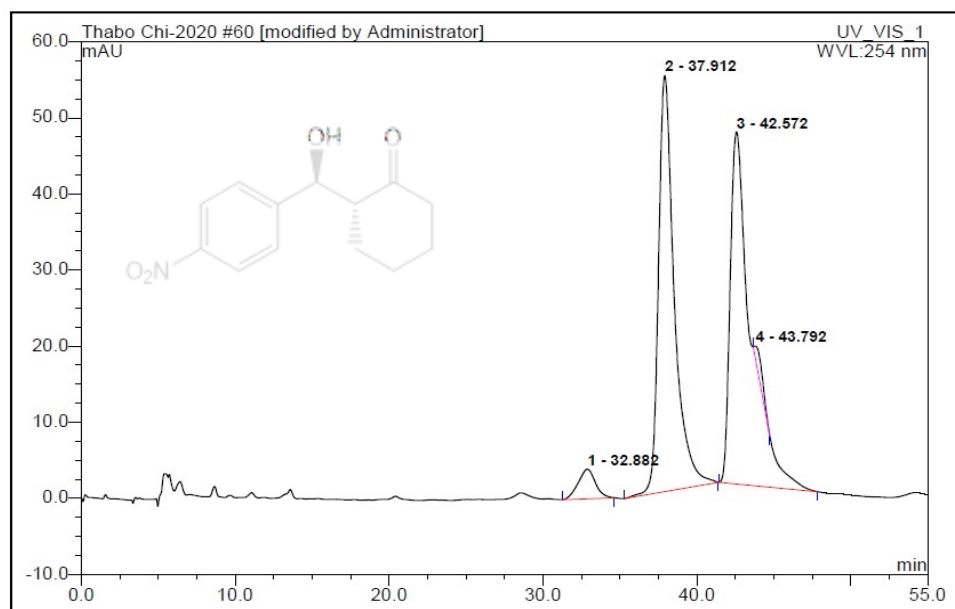
Figure S42: Chiral HPLC chromatogram of TP_A5.1N2CY (entry 10)

Effect of solvents -Reaction between cyclohexanone and *p*-nitrobenzaldehyde (Table 4)



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|--------------|-----------|------------|--------------|------------|--------|------|
| 1 | 28.19 | n.a. | 1.891 | 1.030 | 0.18 | n.a. | BMB* |
| 2 | 33.07 | n.a. | 1.428 | 1.464 | 0.26 | n.a. | BMB* |
| 3 | 37.18 | n.a. | 301.322 | 280.012 | 49.20 | n.a. | BMB* |
| 4 | 42.47 | n.a. | 242.824 | 286.638 | 50.36 | n.a. | BMB* |
| Total: | | | 547.464 | 569.144 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R5

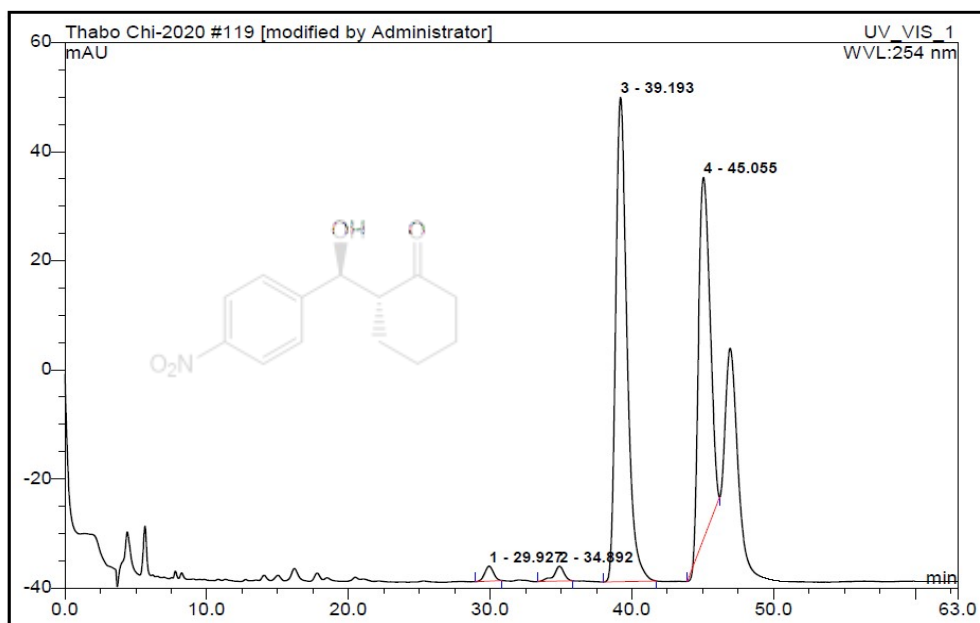


| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|--------------|-----------|------------|--------------|------------|--------|------|
| 1 | 32.88 | n.a. | 3.885 | 5.043 | 3.48 | n.a. | BMB* |
| 2 | 37.91 | n.a. | 54.689 | 65.290 | 45.11 | n.a. | BMB* |
| 3 | 42.57 | n.a. | 46.283 | 72.571 | 50.14 | n.a. | BMB* |
| 4 | 43.79 | n.a. | 1.556 | 1.823 | 1.26 | n.a. | Rd |
| Total: | | | 106.413 | 144.727 | 100.00 | 0.000 | |

Figure S43: Chiral HPLC chromatogram of TP_A4.3N4CY (entry 4)



Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R6

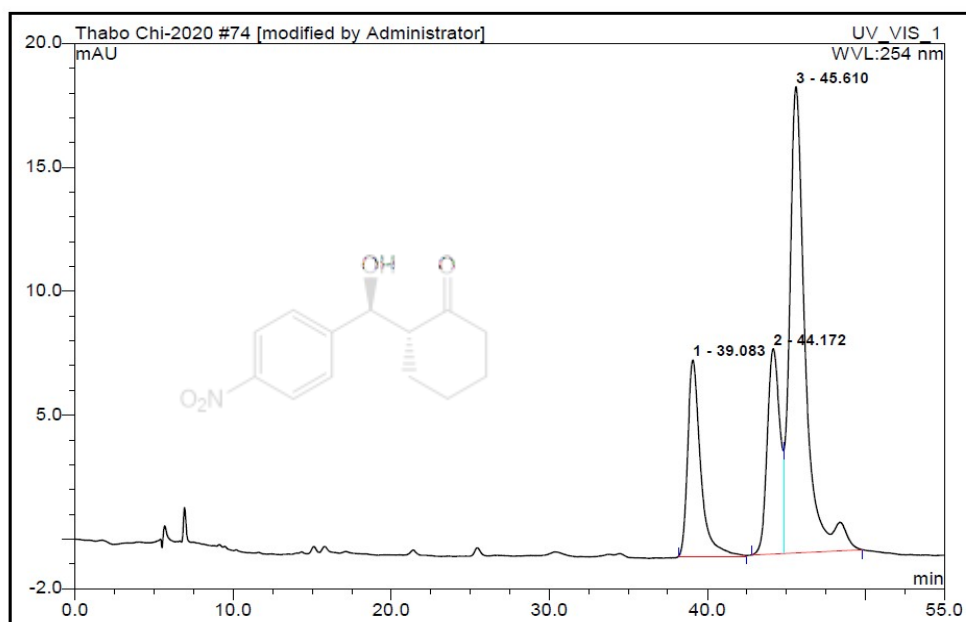


| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 29.93 | n.a. | 2.726 | 1.960 | 1.32 | n.a. | BMB* |
| 2 | 34.89 | n.a. | 2.718 | 2.275 | 1.54 | n.a. | BMB* |
| 3 | 39.19 | n.a. | 88.765 | 82.427 | 55.70 | n.a. | BMB* |
| 4 | 45.06 | n.a. | 66.283 | 61.318 | 41.44 | n.a. | BMB* |
| Total: | | | 160.492 | 147.979 | 100.00 | 0.000 | |

Figure S44: Chiral HPLC chromatogram of TP_A4.4N4CY (entry 6)

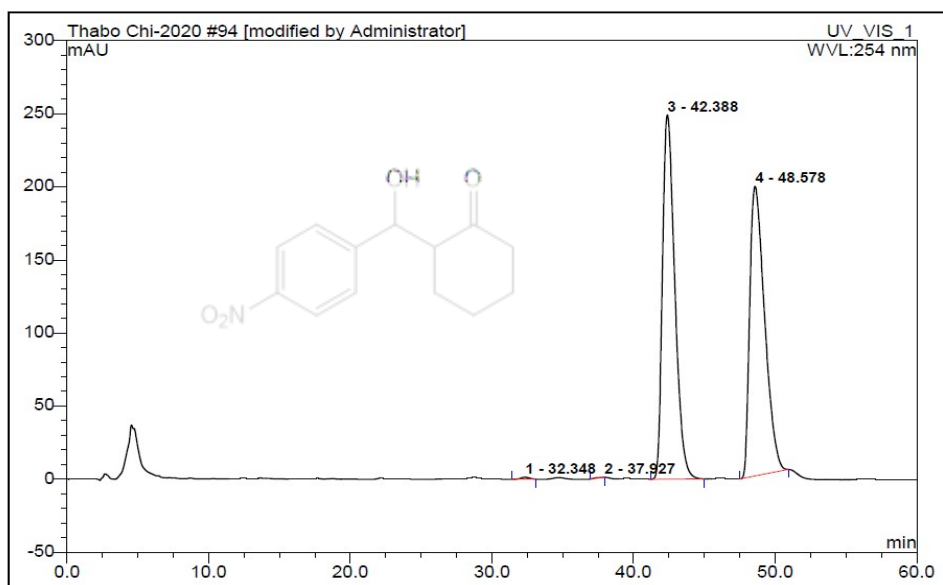


Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R7



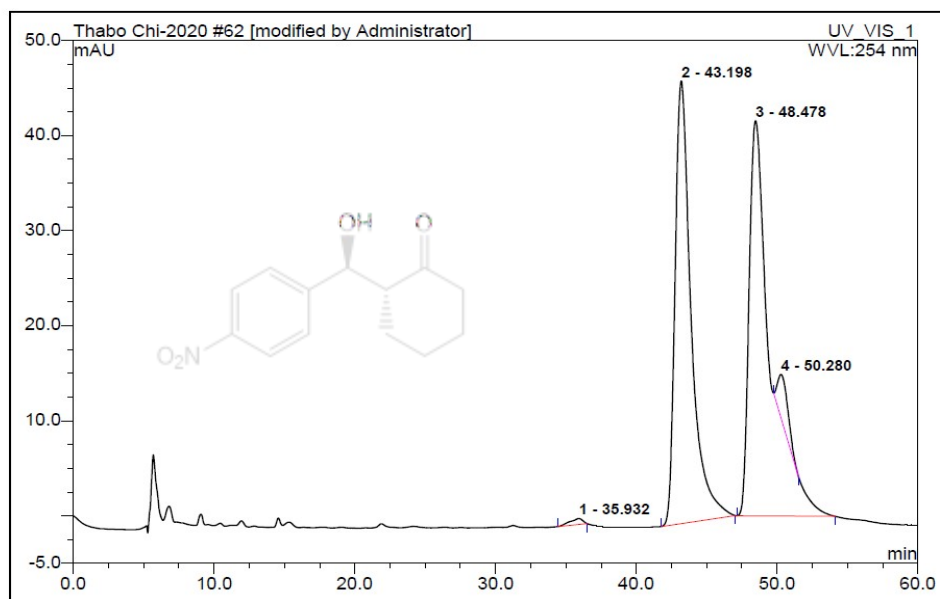
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 39.08 | n.a. | 7.925 | 7.582 | 20.41 | n.a. | BM * |
| 2 | 44.17 | n.a. | 8.280 | 7.602 | 20.46 | n.a. | BM * |
| 3 | 45.61 | n.a. | 18.812 | 21.974 | 59.14 | n.a. | MB* |
| Total: | | | 35.017 | 37.158 | 100.00 | 0.000 | |

Figure S45: Chiral HPLC chromatogram of TP_A4.5N4CY (entry 9)



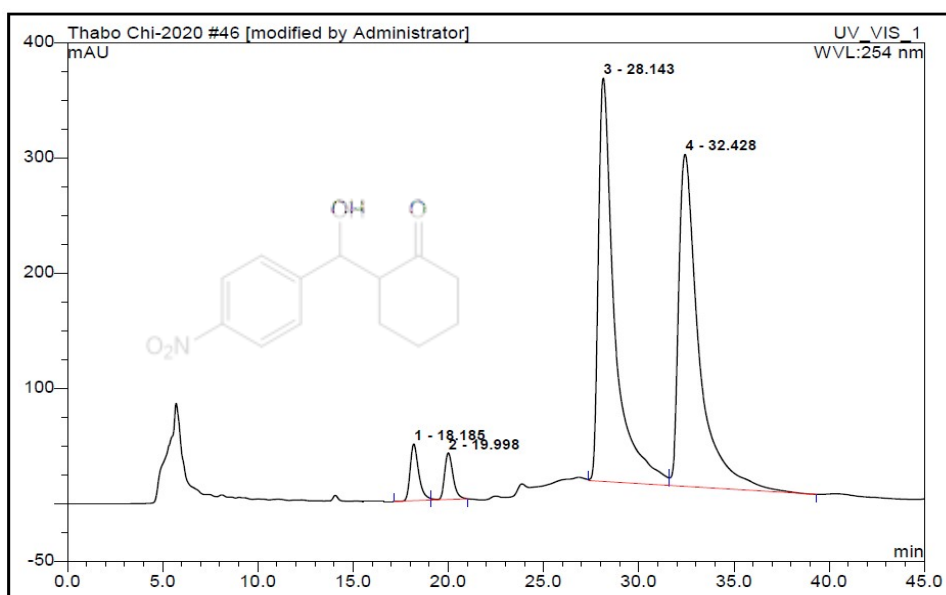
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 32.35 | n.a. | 1.387 | 0.998 | 0.20 | n.a. | BMB* |
| 2 | 37.93 | n.a. | 0.032 | 0.182 | 0.04 | n.a. | BMB* |
| 3 | 42.39 | n.a. | 249.159 | 251.603 | 50.91 | n.a. | BMB* |
| 4 | 48.58 | n.a. | 198.169 | 241.383 | 48.85 | n.a. | BMB* |
| Total: | | | 448.746 | 494.165 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R8



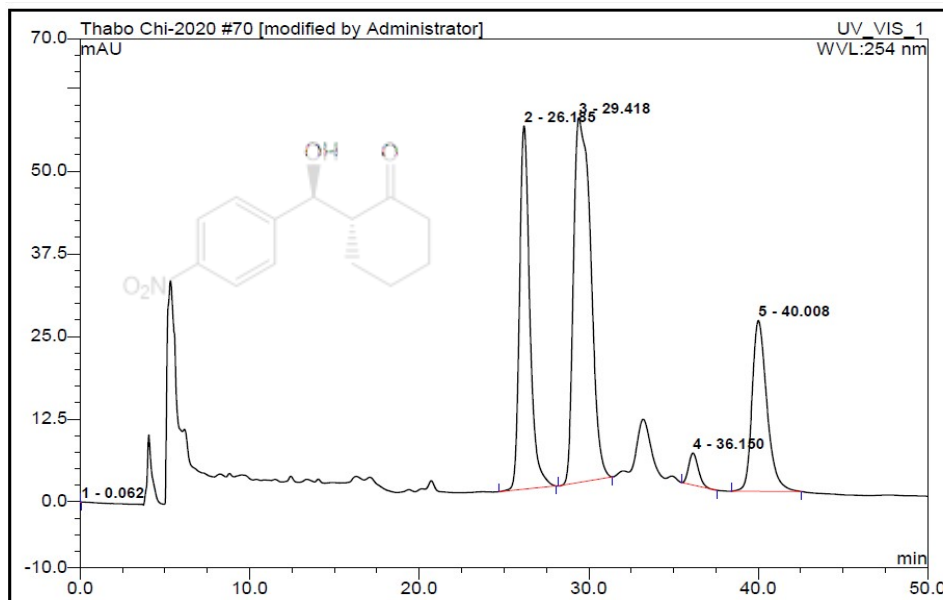
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 35.93 | n.a. | 0.625 | 0.676 | 0.48 | n.a. | BMB* |
| 2 | 43.20 | n.a. | 46.566 | 61.847 | 44.33 | n.a. | BMB* |
| 3 | 48.48 | n.a. | 41.579 | 72.831 | 52.20 | n.a. | BMB* |
| 4 | 50.28 | n.a. | 4.533 | 4.162 | 2.98 | n.a. | Rd |
| Total: | | | 93.304 | 139.516 | 100.00 | 0.000 | |

Figure S46: Chiral HPLC chromatogram of TP_A4.6N4CY (entry 13)



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|----------------|-----------------|---------------|--------------|------|
| 1 | 18.19 | n.a. | 49.255 | 25.866 | 3.44 | n.a. | BM * |
| 2 | 20.00 | n.a. | 40.366 | 20.758 | 2.76 | n.a. | MB* |
| 3 | 28.14 | n.a. | 350.081 | 350.136 | 46.53 | n.a. | M * |
| 4 | 32.43 | n.a. | 288.095 | 355.814 | 47.28 | n.a. | MB* |
| Total: | | | 727.798 | 752.574 | 100.00 | 0.000 | |

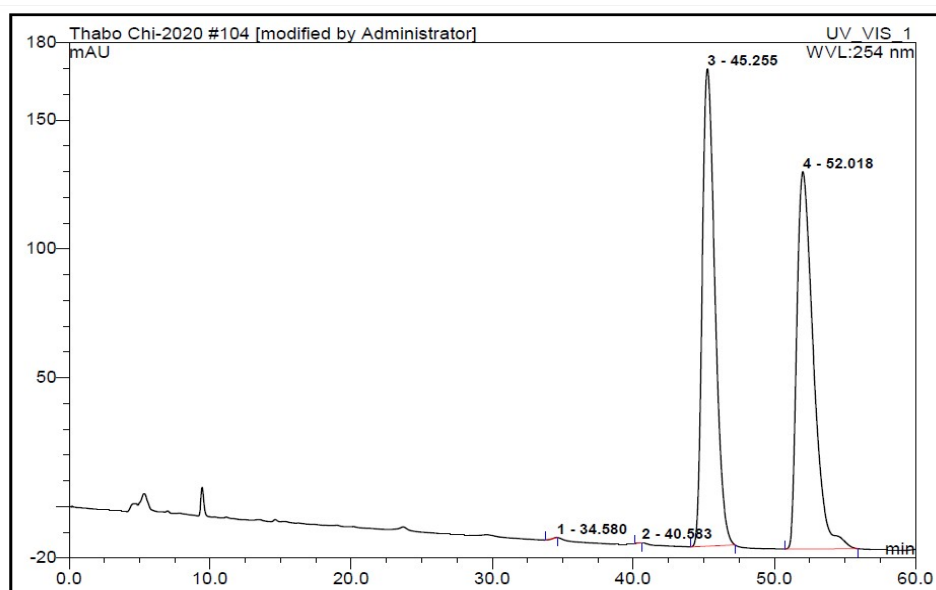
Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R10



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|----------------|-----------------|---------------|--------------|------|
| 1 | 0.06 | n.a. | 0.005 | 0.000 | 0.00 | n.a. | BMB |
| 2 | 26.19 | n.a. | 54.929 | 38.662 | 29.15 | n.a. | BMB* |
| 3 | 29.42 | n.a. | 55.175 | 64.604 | 48.70 | n.a. | BMB* |
| 4 | 36.15 | n.a. | 4.826 | 3.142 | 2.37 | n.a. | BMB* |
| 5 | 40.01 | n.a. | 25.817 | 26.244 | 19.78 | n.a. | BMB* |
| Total: | | | 140.752 | 132.652 | 100.00 | 0.000 | |

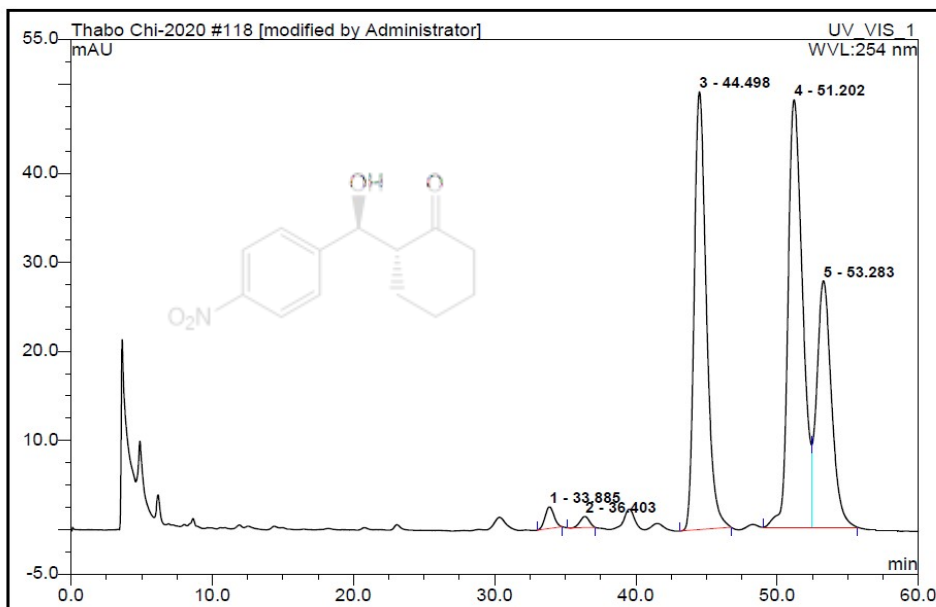
Figure S47: Chiral HPLC chromatogram of TP_A4.7N4CY (entry 15)

Aldol products using 8 mol% between cyclohexanone and *p*-nitrobenzaldehyde (Table 5)



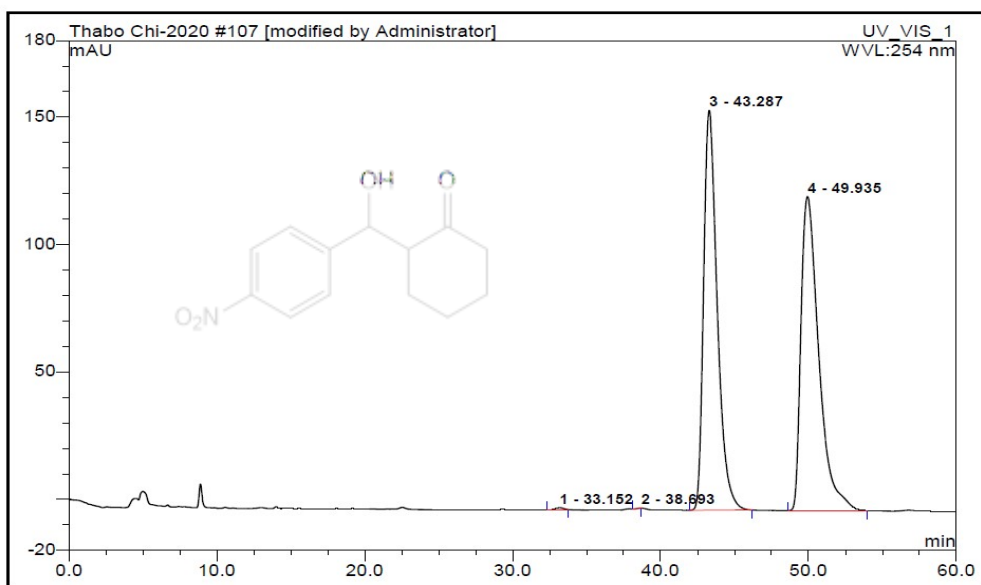
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 34.58 | n.a. | 0.066 | 0.017 | 0.00 | n.a. | BMB* |
| 2 | 40.58 | n.a. | 0.008 | 0.001 | 0.00 | n.a. | BMB* |
| 3 | 45.26 | n.a. | 185.230 | 193.103 | 49.21 | n.a. | BMB* |
| 4 | 52.02 | n.a. | 146.419 | 199.321 | 50.79 | n.a. | BMB* |
| Total: | | | 331.723 | 392.442 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R13



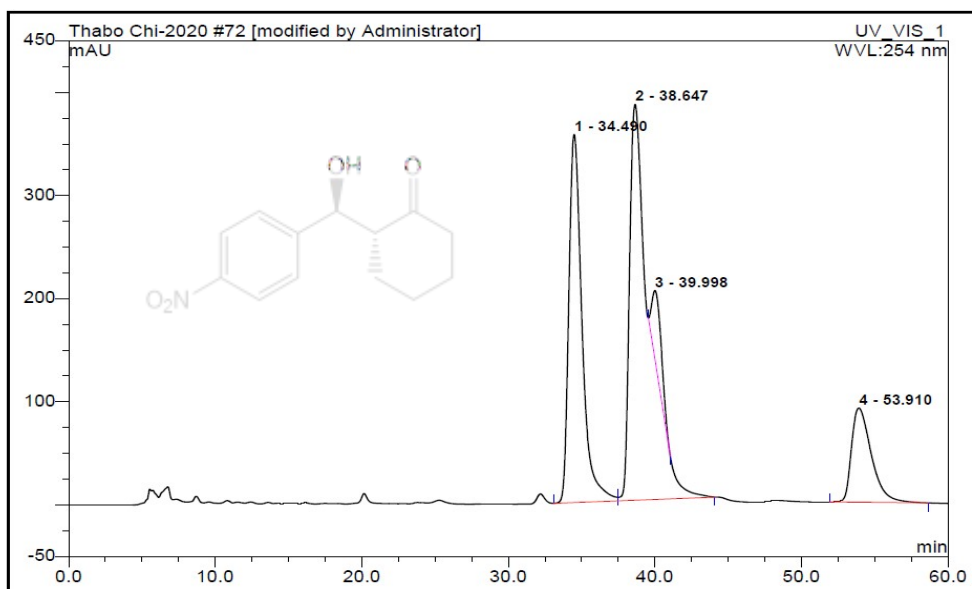
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 33.89 | n.a. | 2.422 | 1.802 | 1.26 | n.a. | BMB* |
| 2 | 36.40 | n.a. | 1.243 | 0.933 | 0.65 | n.a. | BMB* |
| 3 | 44.50 | n.a. | 49.146 | 49.059 | 34.41 | n.a. | BMB* |
| 4 | 51.20 | n.a. | 47.979 | 56.946 | 39.94 | n.a. | BM |
| 5 | 53.28 | n.a. | 27.664 | 33.849 | 23.74 | n.a. | MB* |
| Total: | | | 128.454 | 142.589 | 100.00 | 0.000 | |

Figure S48: Chiral HPLC chromatogram of TP_A4.10N4CY (entry 5)



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 33.15 | n.a. | 0.855 | 0.598 | 0.18 | n.a. | BM * |
| 2 | 38.69 | n.a. | 0.010 | 0.003 | 0.00 | n.a. | BMB* |
| 3 | 43.29 | n.a. | 156.876 | 167.356 | 49.27 | n.a. | BMB* |
| 4 | 49.94 | n.a. | 123.206 | 171.693 | 50.55 | n.a. | BMB |
| Total: | | | 280.947 | 339.650 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R12

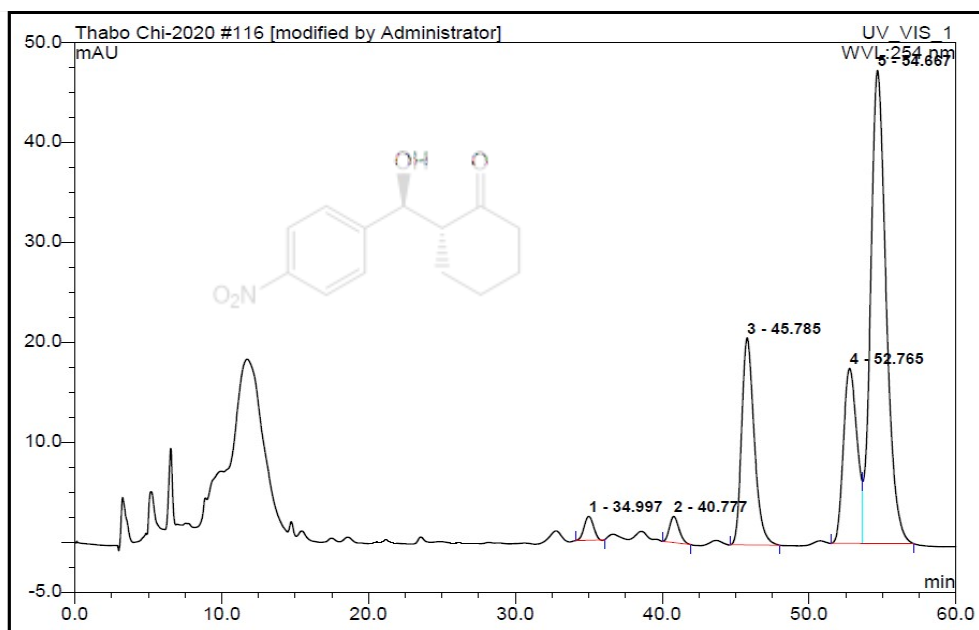


| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 34.49 | n.a. | 356.697 | 372.865 | 31.78 | n.a. | BM * |
| 2 | 38.65 | n.a. | 383.506 | 603.075 | 51.40 | n.a. | MB* |
| 3 | 40.00 | n.a. | 66.234 | 53.515 | 4.56 | n.a. | Rd |
| 4 | 53.91 | n.a. | 91.311 | 143.847 | 12.26 | n.a. | BMB* |
| Total: | | | 897.748 | 1173.302 | 100.00 | 0.000 | |

Figure S49: Chiral HPLC chromatogram of TP_A4.9N4CY (entry 1)



Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R15

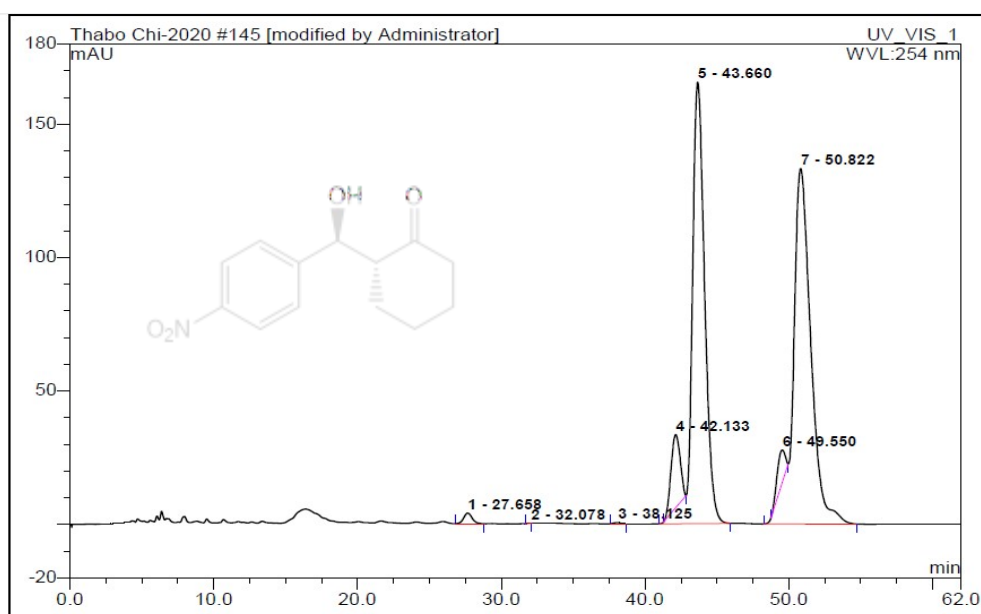


| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 35.00 | n.a. | 2.391 | 1.759 | 1.73 | n.a. | BMB* |
| 2 | 40.78 | n.a. | 2.615 | 1.975 | 1.95 | n.a. | BMB* |
| 3 | 45.79 | n.a. | 20.731 | 20.635 | 20.34 | n.a. | BMB* |
| 4 | 52.77 | n.a. | 17.529 | 19.034 | 18.77 | n.a. | BM * |
| 5 | 54.67 | n.a. | 47.354 | 58.025 | 57.21 | n.a. | MB* |
| Total: | | | 90.620 | 101.428 | 100.00 | 0.000 | |

Figure S50: Chiral HPLC chromatogram of TP_A4.13N4CY (entry 4)



Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R14



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|--------------|-----------|------------|--------------|------------|--------|------|
| 1 | 27.66 | n.a. | 3.954 | 2.686 | 0.69 | n.a. | BMB* |
| 2 | 32.08 | n.a. | 0.008 | 0.003 | 0.00 | n.a. | BMB* |
| 3 | 38.13 | n.a. | 0.484 | 0.284 | 0.07 | n.a. | BMB* |
| 4 | 42.13 | n.a. | 27.400 | 20.285 | 5.18 | n.a. | Ru |
| 5 | 43.66 | n.a. | 165.417 | 168.513 | 43.02 | n.a. | BMB* |
| 6 | 49.55 | n.a. | 12.085 | 8.313 | 2.12 | n.a. | Ru |
| 7 | 50.82 | n.a. | 133.210 | 191.609 | 48.92 | n.a. | BMB* |
| Total: | | | 342.558 | 391.692 | 100.00 | 0.000 | |

Figure S51: Chiral HPLC chromatogram of TP_A4.14N4CY (entry 6)

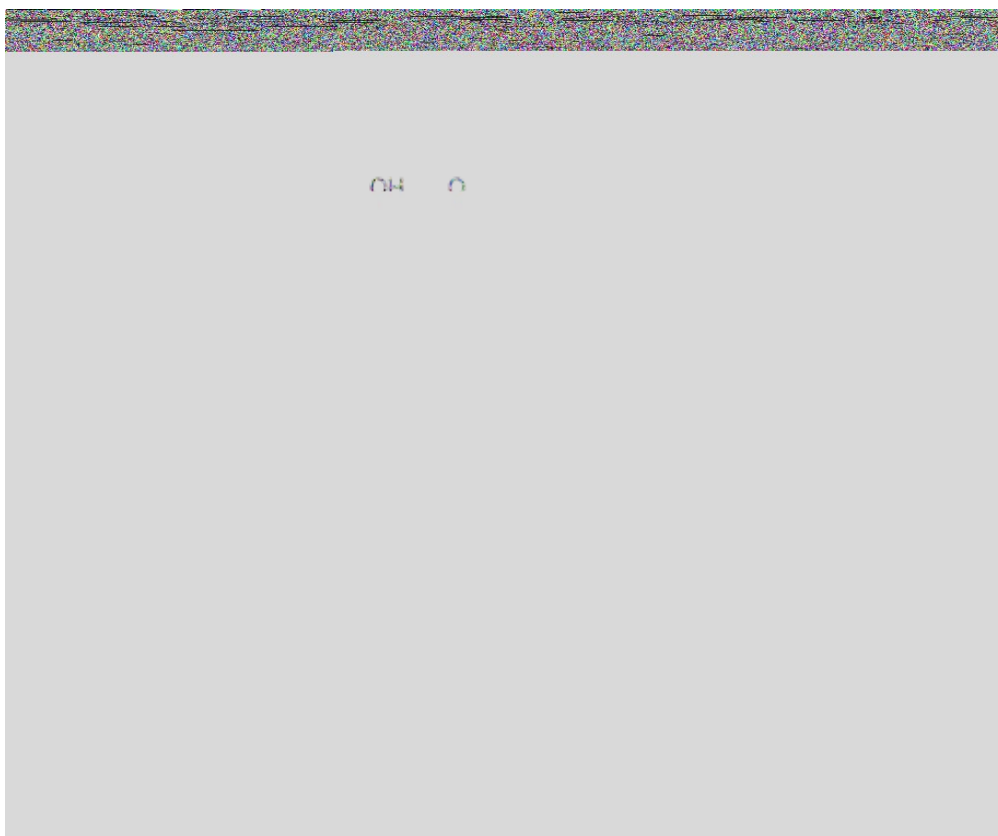
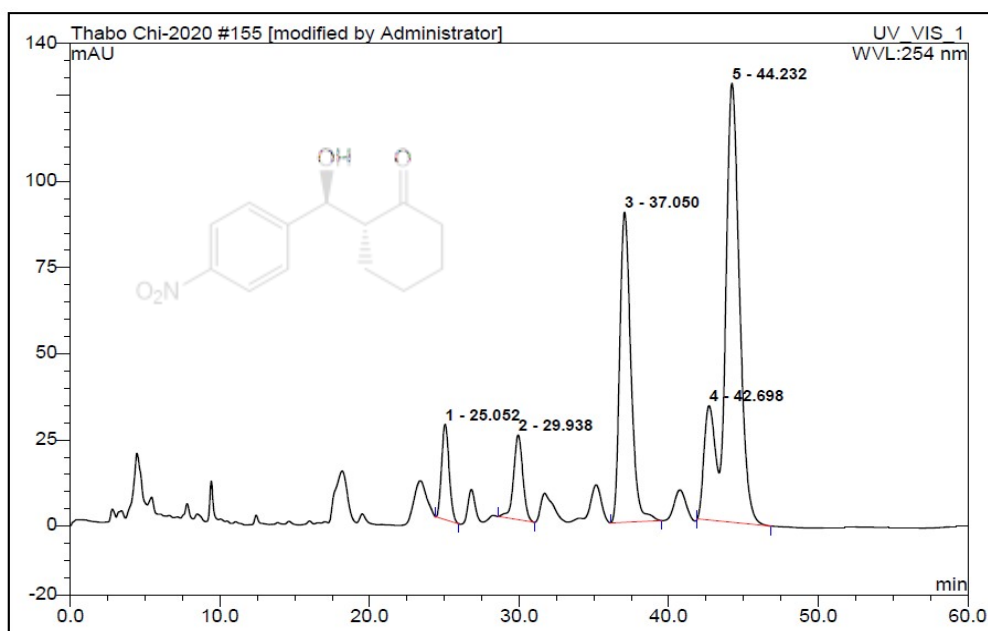
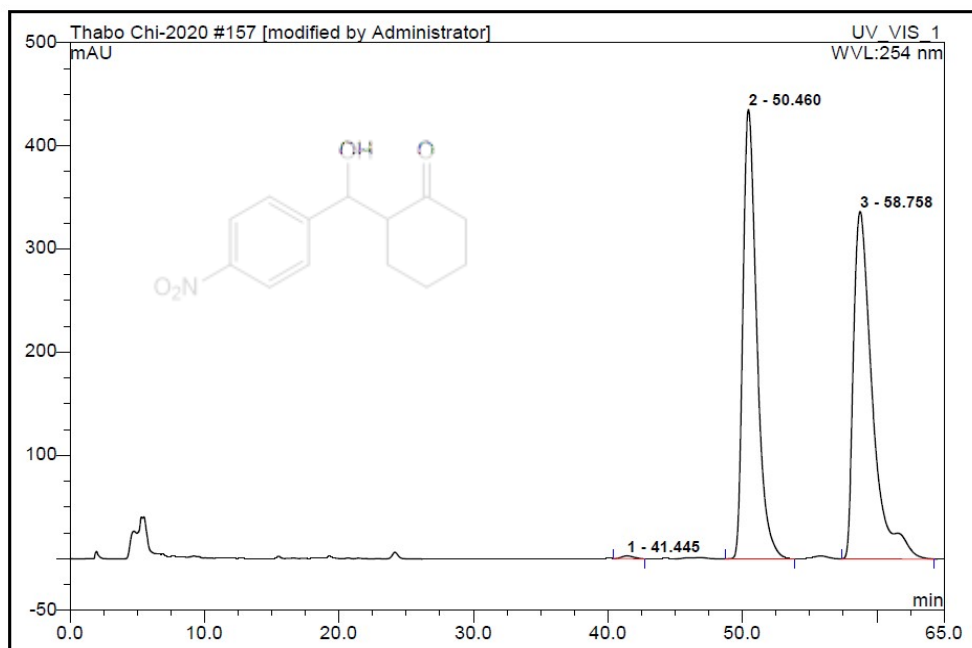


Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-15



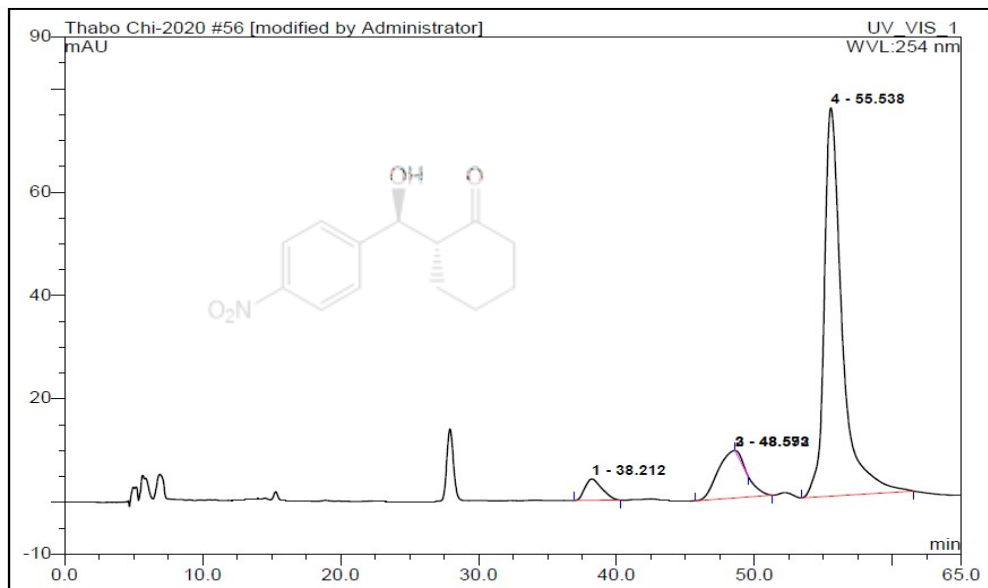
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 25.05 | n.a. | 27.625 | 16.077 | 5.92 | n.a. | BMB* |
| 2 | 29.94 | n.a. | 24.568 | 17.622 | 6.48 | n.a. | BMB* |
| 3 | 37.05 | n.a. | 89.999 | 77.484 | 28.51 | n.a. | BMB* |
| 4 | 42.70 | n.a. | 33.210 | 0.000 | 0.00 | n.a. | BMB* |
| 5 | 44.23 | n.a. | 127.454 | 160.564 | 59.09 | n.a. | bMB* |
| Total: | | | 302.857 | 271.747 | 100.00 | 0.000 | |

Figure S52: Chiral HPLC chromatogram of TP_A4.15N4CY (entry 7)



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 41.45 | n.a. | 2.604 | 2.643 | 0.25 | n.a. | BMB* |
| 2 | 50.46 | n.a. | 435.305 | 517.082 | 48.38 | n.a. | BMB |
| 3 | 58.76 | n.a. | 336.497 | 549.047 | 51.37 | n.a. | BMB* |
| Total: | | | 774.406 | 1068.772 | 100.00 | 0.000 | |

Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R16

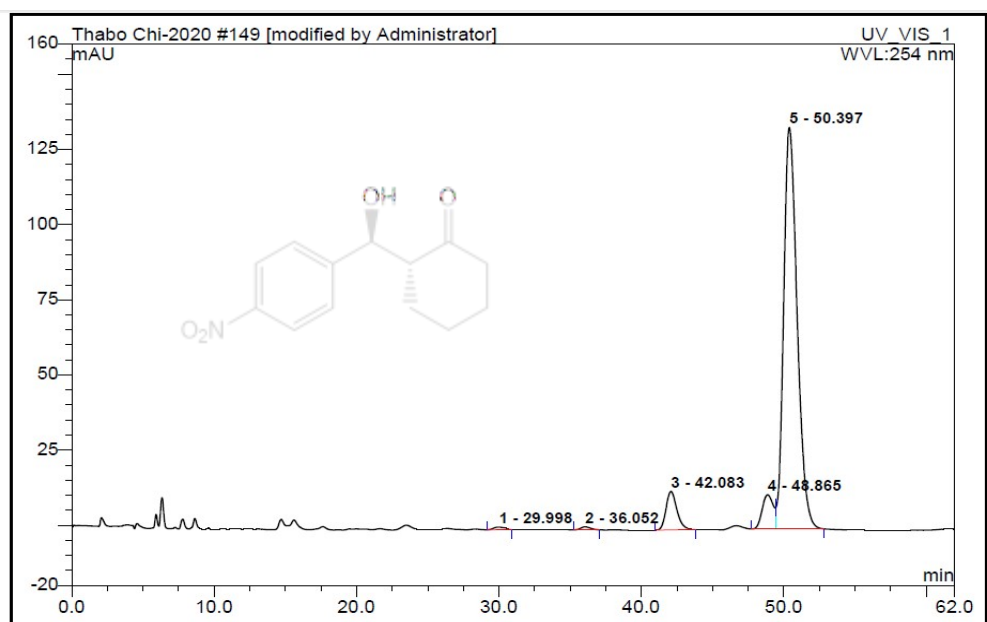


| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 38.21 | n.a. | 4.138 | 5.849 | 4.16 | n.a. | BMB* |
| 2 | 48.57 | n.a. | 9.164 | 21.210 | 15.08 | n.a. | BMB* |
| 3 | 48.59 | n.a. | 0.036 | 0.496 | 0.35 | n.a. | Rd |
| 4 | 55.54 | n.a. | 75.124 | 113.102 | 80.41 | n.a. | MB* |
| Total: | | | 88.461 | 140.657 | 100.00 | 0.000 | |

Figure S53: Chiral HPLC chromatogram of TP_A4.16N4CY (entry 8)



Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R17



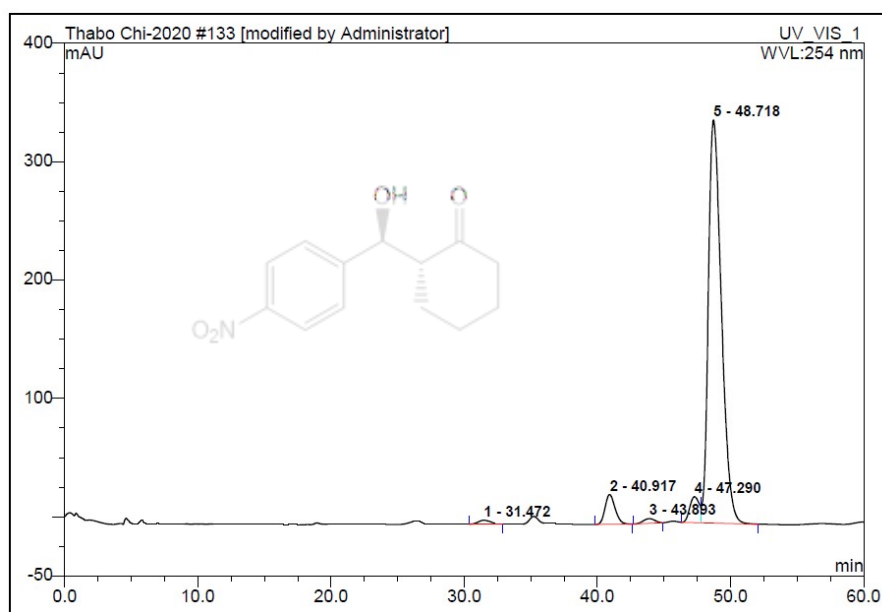
| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 30.00 | n.a. | 0.908 | 0.784 | 0.46 | n.a. | BMB* |
| 2 | 36.05 | n.a. | 1.048 | 0.807 | 0.48 | n.a. | BMB* |
| 3 | 42.08 | n.a. | 12.788 | 11.323 | 6.68 | n.a. | BMB* |
| 4 | 48.87 | n.a. | 11.390 | 9.960 | 5.87 | n.a. | BM * |
| 5 | 50.40 | n.a. | 133.447 | 146.715 | 86.51 | n.a. | MB* |
| Total: | | | 159.581 | 169.589 | 100.00 | 0.000 | |

Figure S54: Chiral HPLC chromatogram of TP_A4.17N4CY (entry 9)

Catalyst Recycling: Reaction between cyclohexanone and *p*-nitrobenzaldehyde (Table 6)



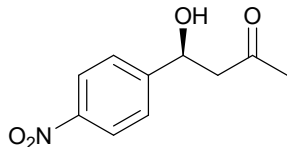
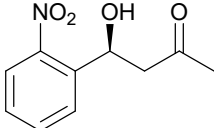
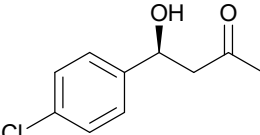
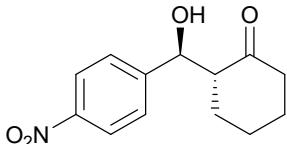
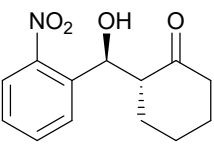
Figure SR: Chiral HPLC chromatogram of racemate TP_A4N4CY-R18

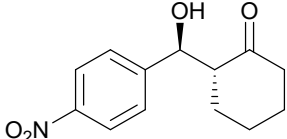
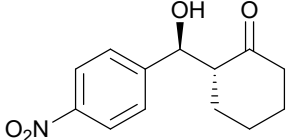


| No. | Ret. Time min | Peak Name | Height mAU | Area mAU*min | Rel. Area % | Amount | Type |
|---------------|------------------|-----------|---------------|-----------------|----------------|--------|------|
| 1 | 31.47 | n.a. | 3.412 | 3.731 | 0.86 | n.a. | BMB* |
| 2 | 40.92 | n.a. | 25.122 | 21.977 | 5.07 | n.a. | BMB* |
| 3 | 43.89 | n.a. | 3.977 | 3.971 | 0.92 | n.a. | BMB* |
| 4 | 47.29 | n.a. | 21.839 | 17.770 | 4.10 | n.a. | BM * |
| 5 | 48.72 | n.a. | 340.588 | 386.386 | 89.06 | n.a. | MB* |
| Total: | | | 394.937 | 433.835 | 100.00 | 0.000 | |

Figure S55: Chiral HPLC chromatogram of TP_A4.18N4CY (entry 5)

Table A: Analytical data and Chiral-phase HPLC spectra analysis for selected aldol products using Lux 5 μ m Cellulose-1 chiral column

| Structure | Aldol product | Eluent; %ratio | Flow rate; Wavelength (nm) | Retention time (min) |
|---|---------------|--------------------------|-------------------------------|---|
|  | TP_A1.1N4ACE | Hexane/IPA; 85/15 | 0.6 mL; 254 | $t_{Rmajor} = 23.38$; $t_{Rminor} = 30.42$ |
| | TP_A1.2N4ACE | Hexane/IPA; 85/15 | 0.6 mL; 254 | $t_{Rmajor} = 24.52$; $t_{Rminor} = 32.07$ |
| | TP_A1.3N4ACE | Hexane/IPA; 85/15 | 0.6 mL; 254 | $t_{Rminor} = 22.37$; $t_{Rmajor} = 27.88$ |
| | TP_A1.4N4ACE | Hexane/IPA; 85/15 | 0.6 mL; 254 | $t_{Rminor} = 23.13$; $t_{Rmajor} = 28.80$ |
|  | TP_A2.1N2ACE | Hexane/IPA; 90/10 | 0.6 mL; 254 | $t_{Rmajor} = 22.75$; $t_{Rminor} = 26.31$ |
| | TP_A2.2N2ACE | Hexane/IPA; 90/10 | 0.6 mL; 254 | $t_{Rmajor} = 19.31$; $t_{Rminor} = 22.29$ |
|  | TP_A3Cl4ACE | Hexane/IPA; 85/15 | 0.7 mL; 254 | $t_{Rminor} = 14.77$; $t_{Rmajor} = 16.79$ |
| | | | | |
| Using cyclohexanone as solvent for reaction between cyclohexanone and aromatic aldehydes (Table 4-2) | | | | |
|  | TP_A4.1N4CY | Hexane/IPA; 94/6 | 0.6 mL; 254 | $t_{Rminor} = 39.09$; $t_{Rmajor} = 44.17$ |
| | TP_A4.2N4CY | Hexane/IPA; 94/6 | 0.6 mL; 254 | $t_{Rmajor} = 38.63$; $t_{Rminor} = 43.60$ |
|  | TP_A5.1N4CY | Hexane/IPA; 95/5 | 0.5 mL; 254 | $t_{Rmajor} = 39.28$; $t_{Rminor} = 44.15$ |
| | TP-A5.2N4CY | Hexane/IPA; 95/5 | 0.5 mL; 254 | $t_{Rmajor} = 38.63$; $t_{Rminor} = 43.60$ |

| | Selected Aldol products using organic solvents (Table 4-3) | | | |
|---|---|-------------------------|-------------------|---|
| | | | | |
|  | TP_A4.3N4CY | Hexane/IPA; 95/5 | 0.7mL; 254 | $t_{Rmajor} = 37.91$; $t_{Rminor} = 42.57$ |
| | TP_A4.4N4CY | Hexane/IPA; 95/5 | 0.6mL; 254 | $t_{Rmajor} = 39.19$; $t_{Rminor} = 45.06$ |
| | TP_A4.5N4CY | Hexane/IPA; 94/6 | 0.7mL; 254 | $t_{Rminor} = 39.08$; $t_{Rmajor} = 45.61$ |
| | TP_A4.6N4CY | Hexane/IPA; 95/5 | 0.6mL; 254 | $t_{Rminor} = 43.19$; $t_{Rmajor} = 48.48$ |
| | TP_A4.7N4CY | Hexane/IPA; 96/4 | 0.8mL; 254 | $t_{Rmajor} = 29.42$; $t_{Rminor} = 26.19$ |
| | Using 8 mol% catalyst loading (Table 4-5) | | | |
|  | TP_A4.10N4CY | Hexane/IPA; 94/6 | 0.6mL; 254 | $t_{Rmajor} = 44.50$; $t_{Rminor} = 51.20$ |
| | TP_A4.9N4CY | Hexane/IPA; 94/6 | 0.7mL; 254 | $t_{Rminor} = 34.49$; $t_{Rmajor} = 38.65$ |
| | TP_A4.13N4CY | Hexane/IPA; 95/5 | 0.6mL; 254 | $t_{Rminor} = 45.79$; $t_{Rmajor} = 54.67$ |
| | TP_A4.14N4CY | Hexane/IPA; 95/5 | 0.6mL; 254 | $t_{Rminor} = 43.66$; $t_{Rmajor} = 50.82$ |
| | TP_A4.15N4CY | Hexane/IPA; 94/6 | 0.6mL; 254 | $t_{Rmajor} = 37.05$; $t_{Rmajor} = 44.23$ |
| | TP_A4.16N4CY | Hexane/IPA; 95/5 | 0.7mL; 254 | $t_{Rminor} = 48.57$; $t_{Rmajor} = 55.54$ |
| | TP_A4.17N4CY | Hexane/IPA; 95/5 | 0.8mL; 254 | $t_{Rminor} = 42.08$; $t_{Rmajor} = 50.40$ |
| | TP_A4.18N4CY | Hexane/IPA; 95/5 | 0.8mL; 254 | $t_{Rminor} = 40.92$; $t_{Rmajor} = 48.72$ |

Selected NMR spectra for determination of the syn/anti (dr) for aldol products by ^1H NMR

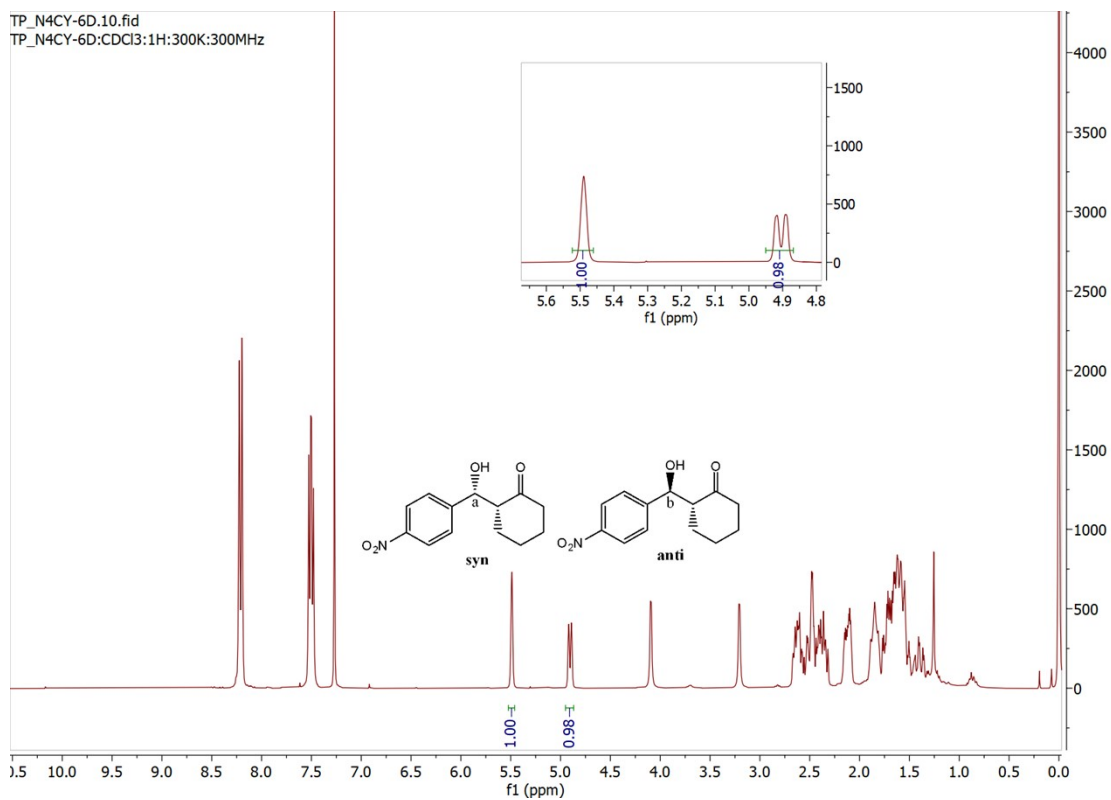
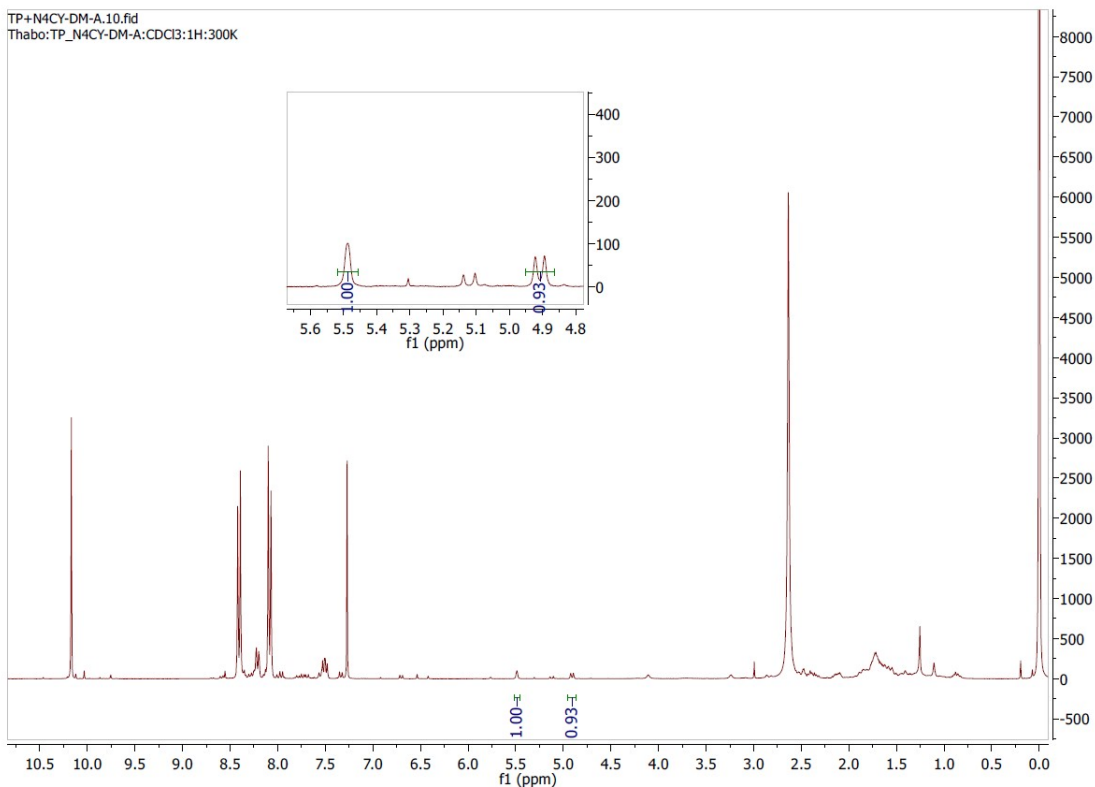


Figure S56: ^1H NMR spectrum of the crude product of TP_A4.2N4CY



e S57: ^1H NMR spectrum of the crude product of TP_A4.3N4CY

Figur

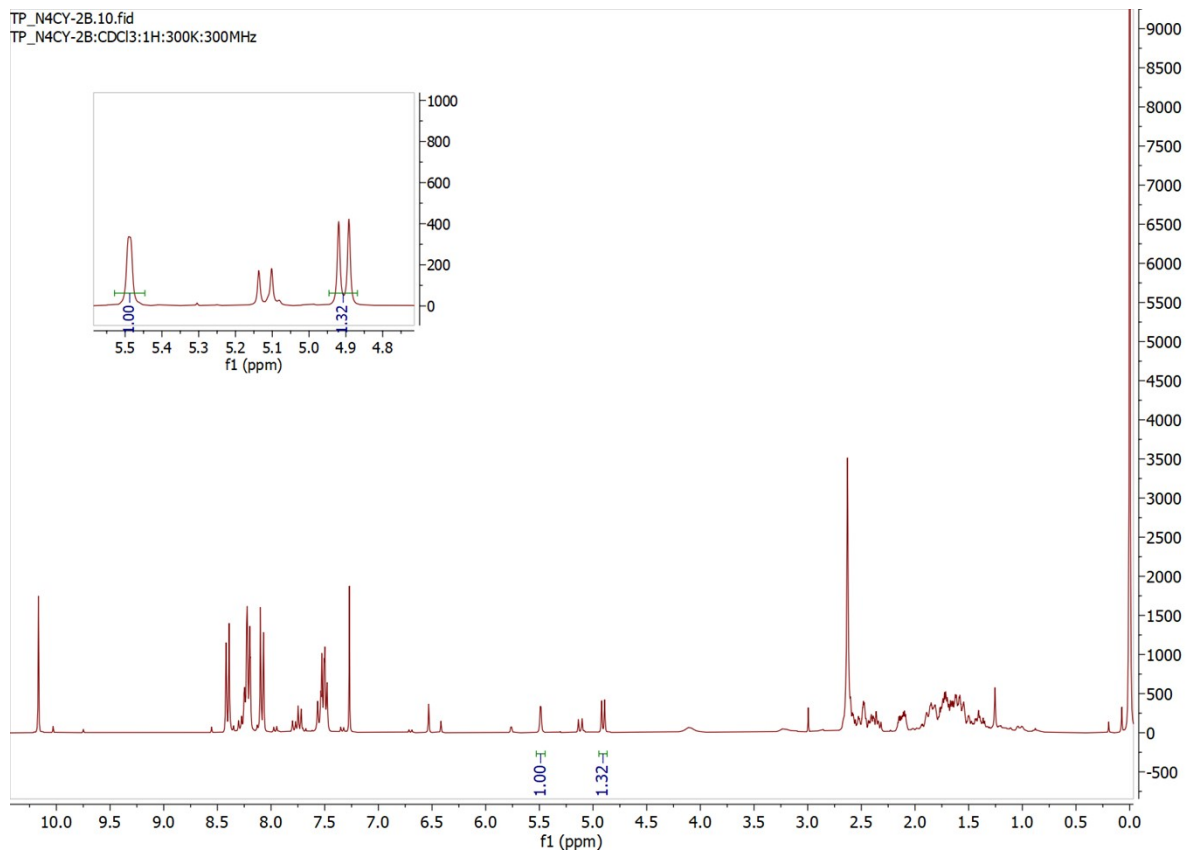


Figure S58: ^1H NMR spectrum of the crude product of TP_A4.4N4CY

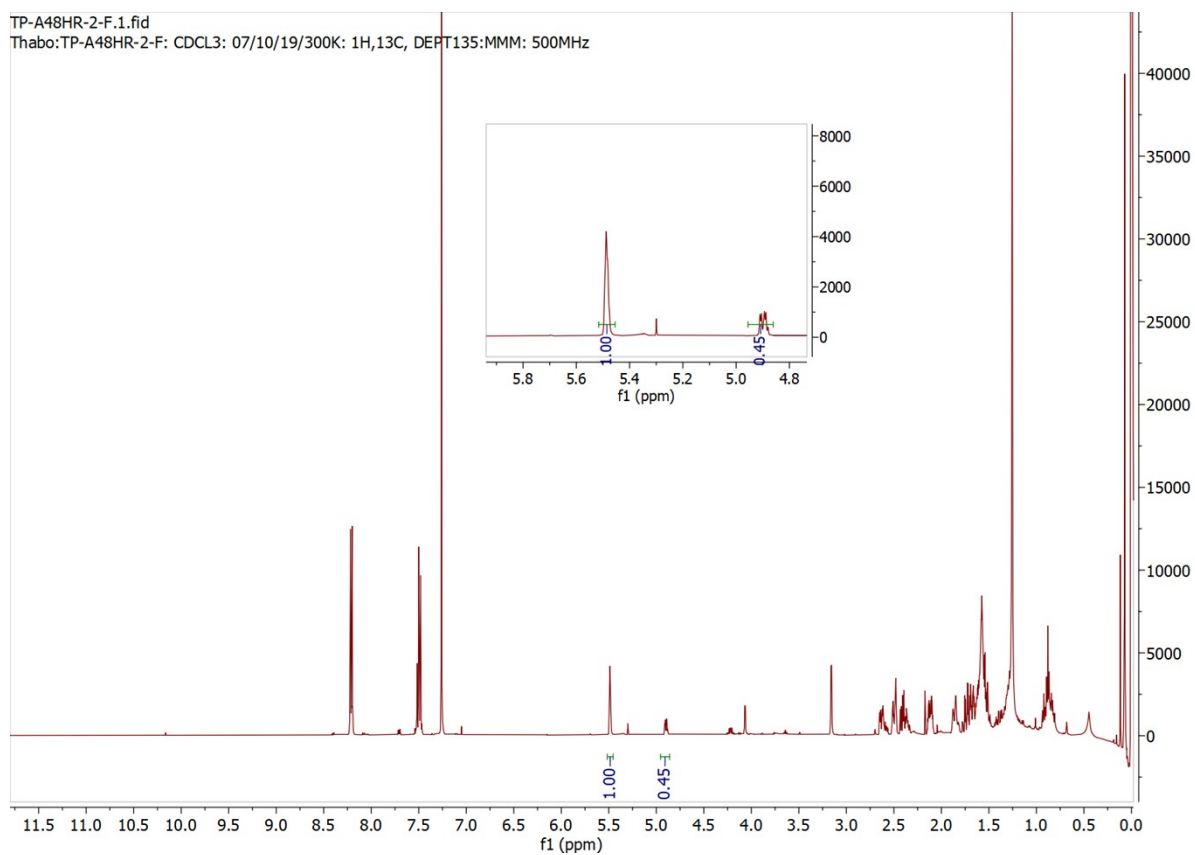


Figure S59: ^1H NMR spectrum of the crude product TP_A4.5N4CY

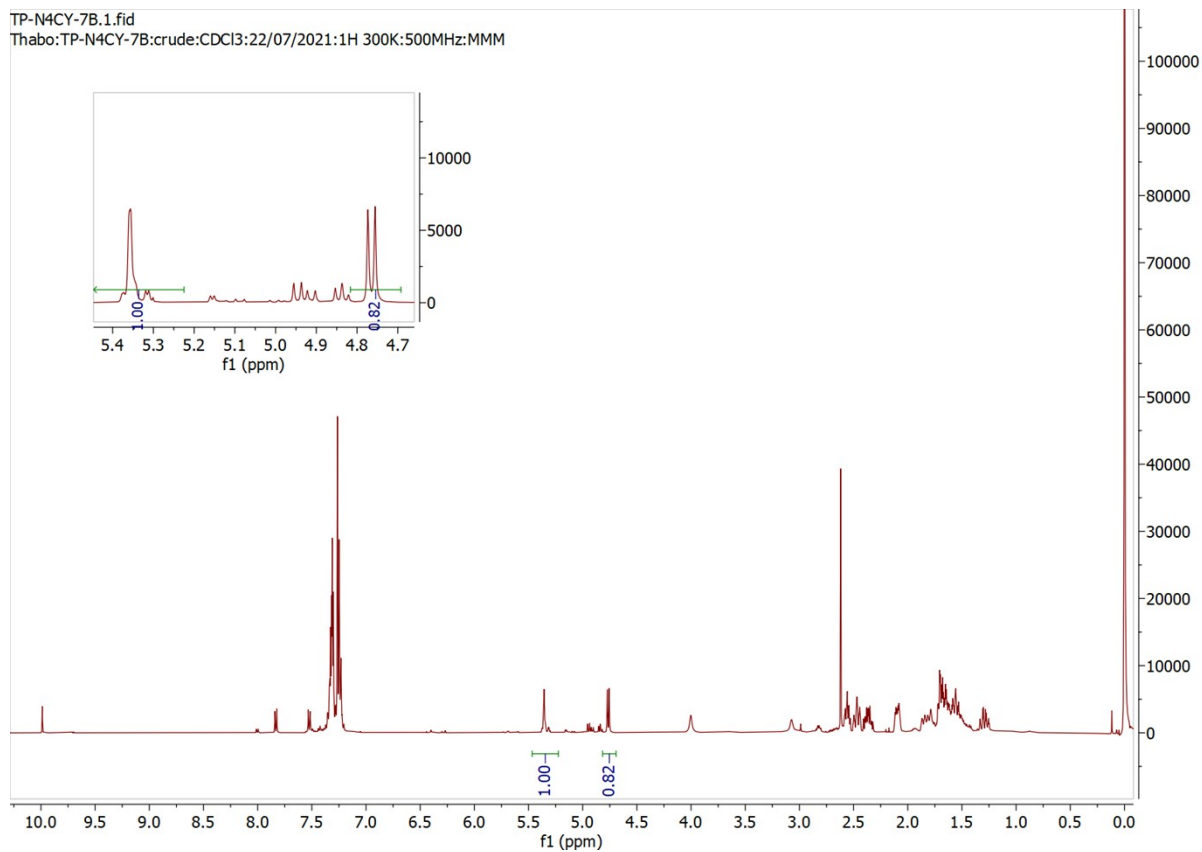


Figure S60: ^1H NMR spectrum of the crude product of TP_A4.6N4CY

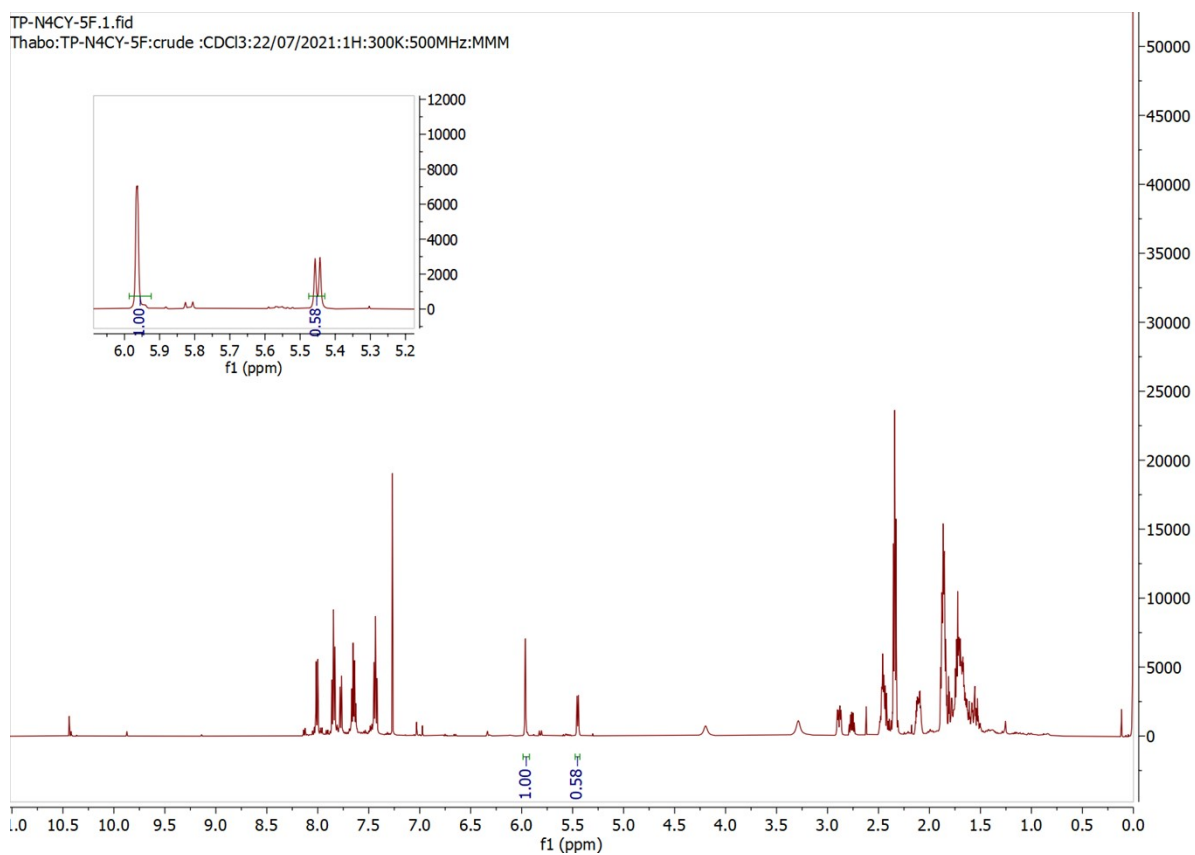


Figure S61: ^1H NMR spectrum of the crude product of TP_A4.8N4CY

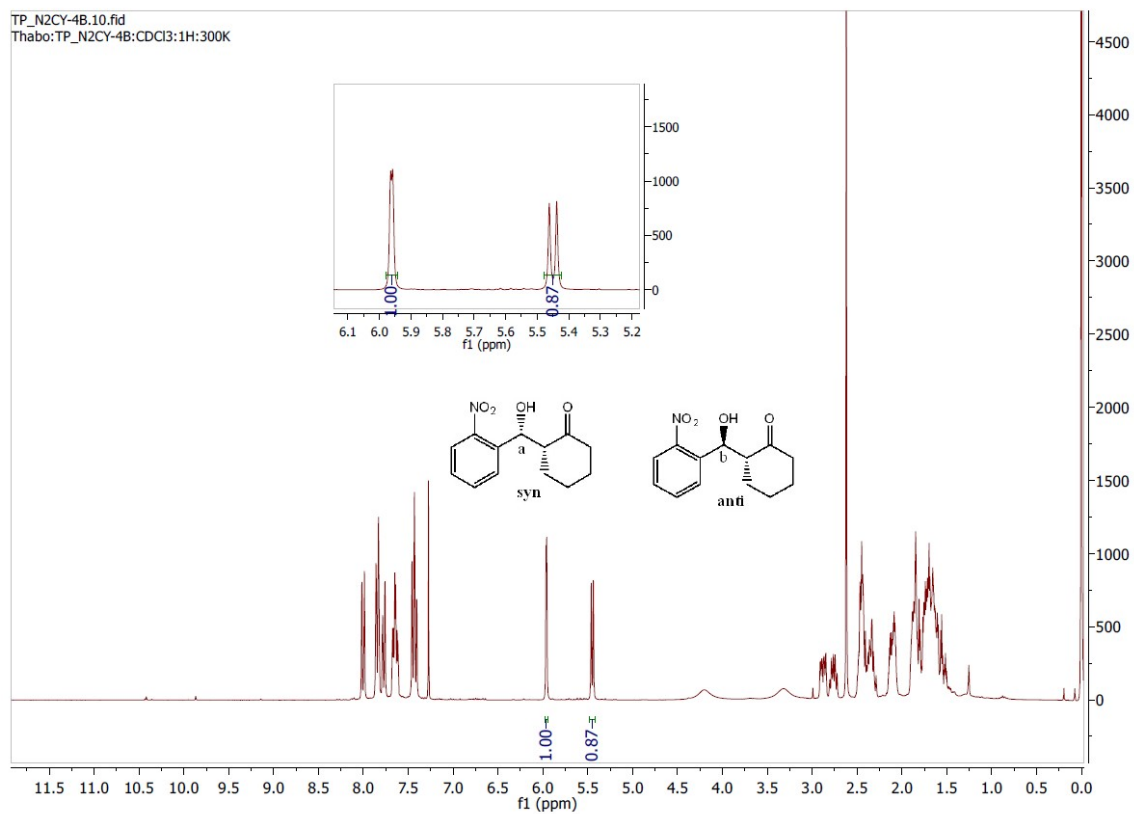


Figure S62: ^1H NMR spectrum of the crude product of TP_A5.1N2CY

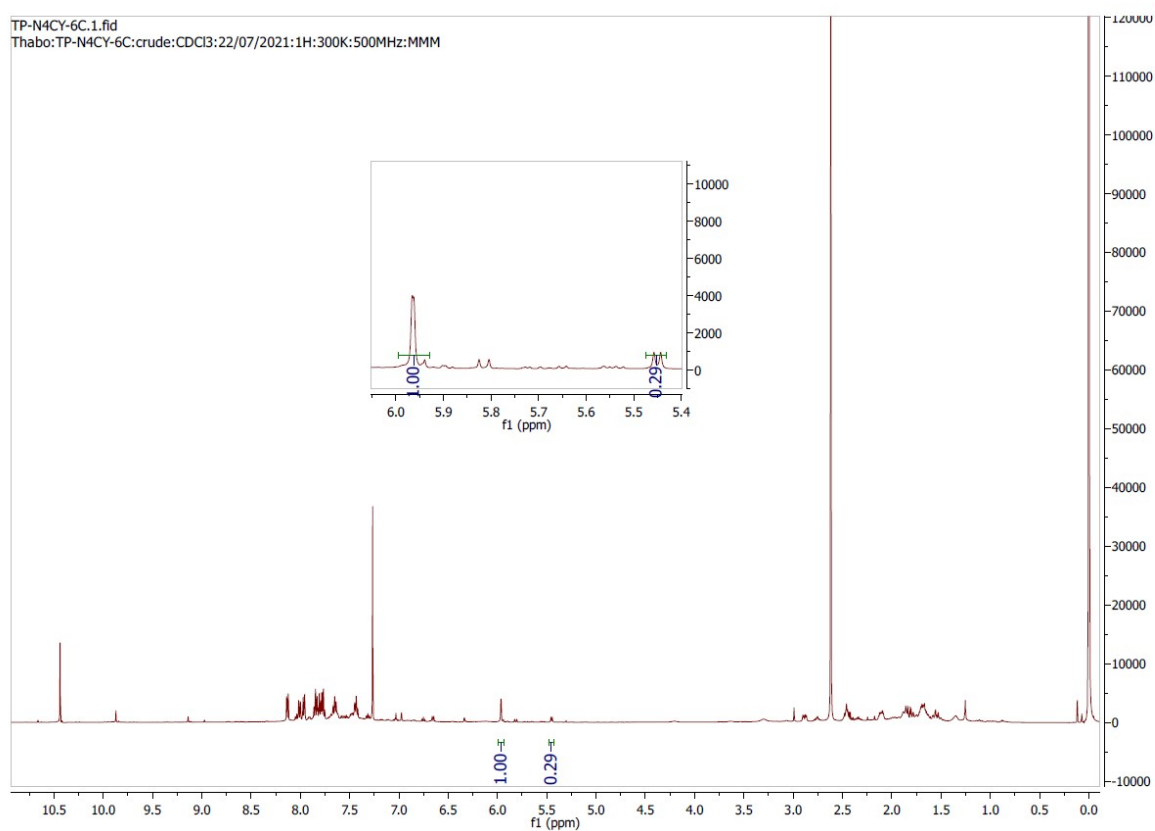


Figure S63: ^1H NMR spectrum of the crude product of TP_A5.2N2CY

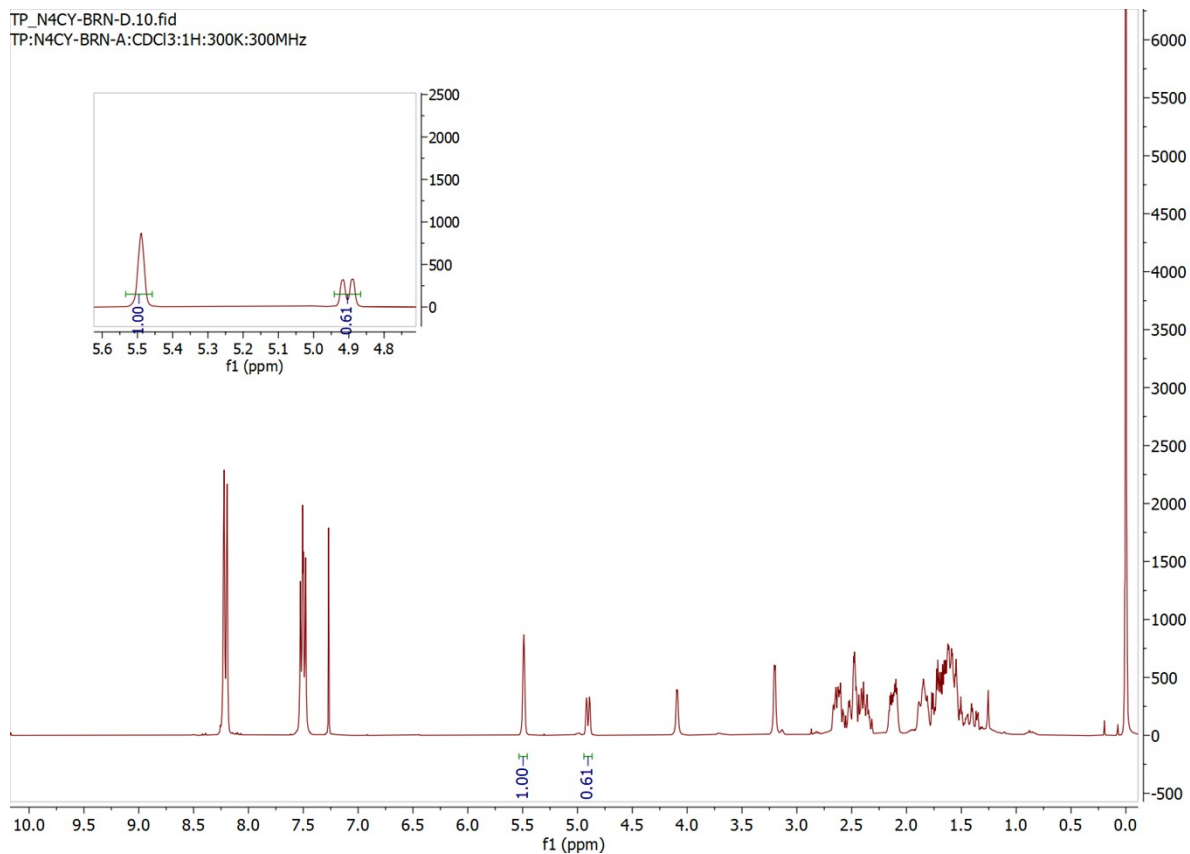


Figure S64: ^1H NMR spectrum of the crude product TP_A4.9N4CY

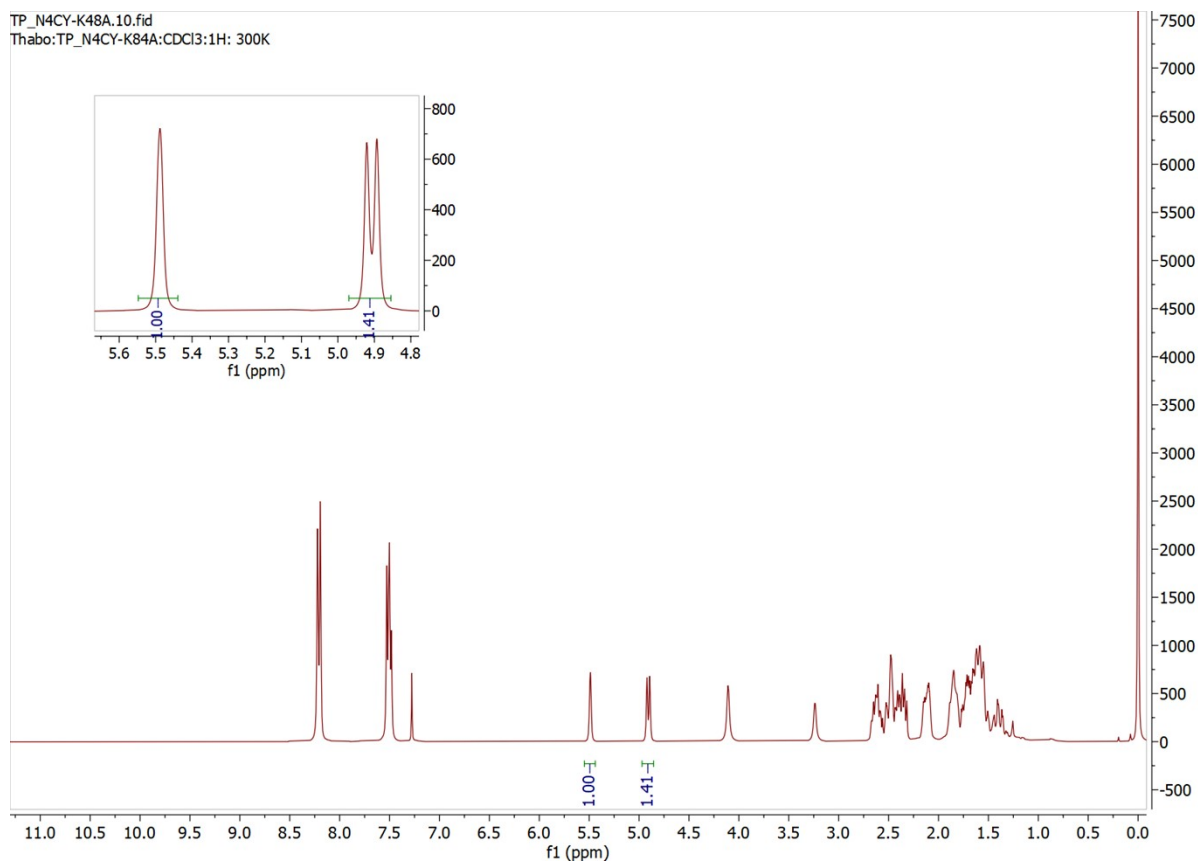


Figure S65: ^1H NMR spectrum of the crude product TP_A4.10N4CY

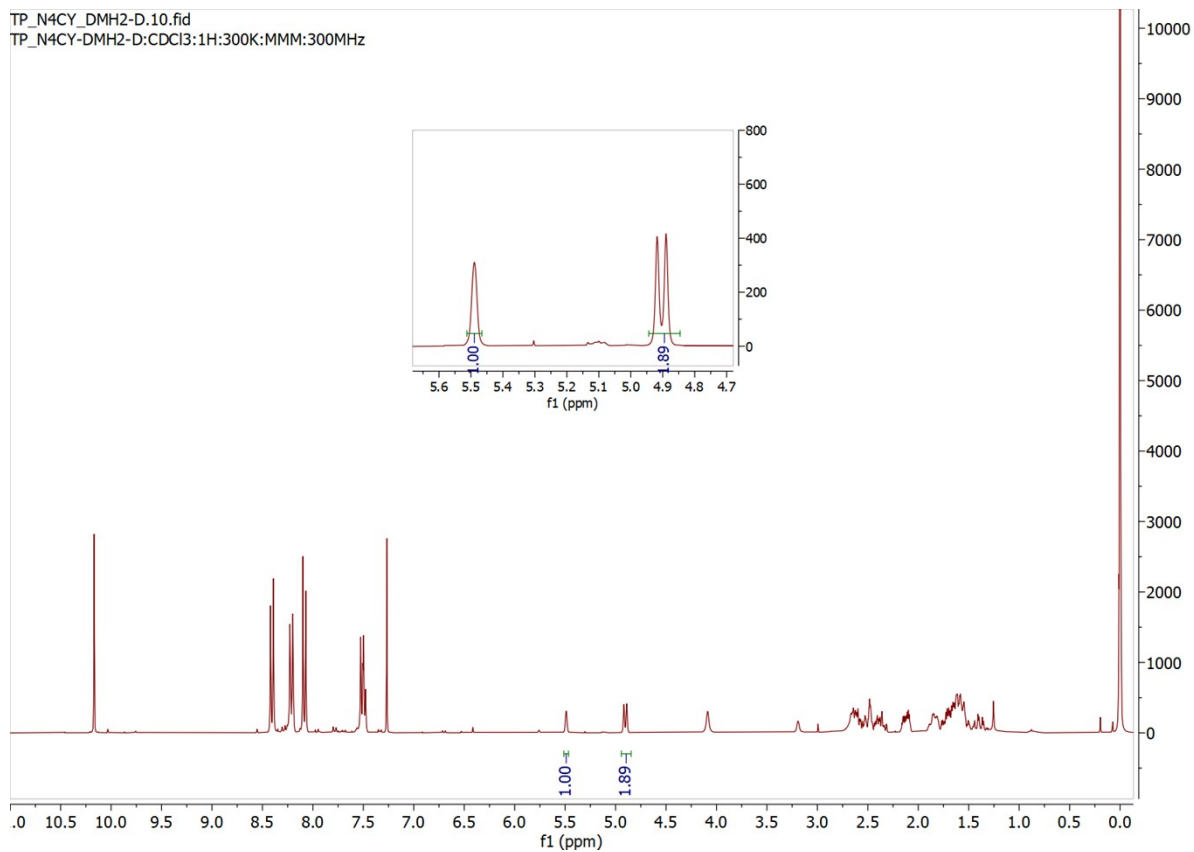


Figure S66: ¹H NMR spectrum of the crude product TP_A4.12N4CY

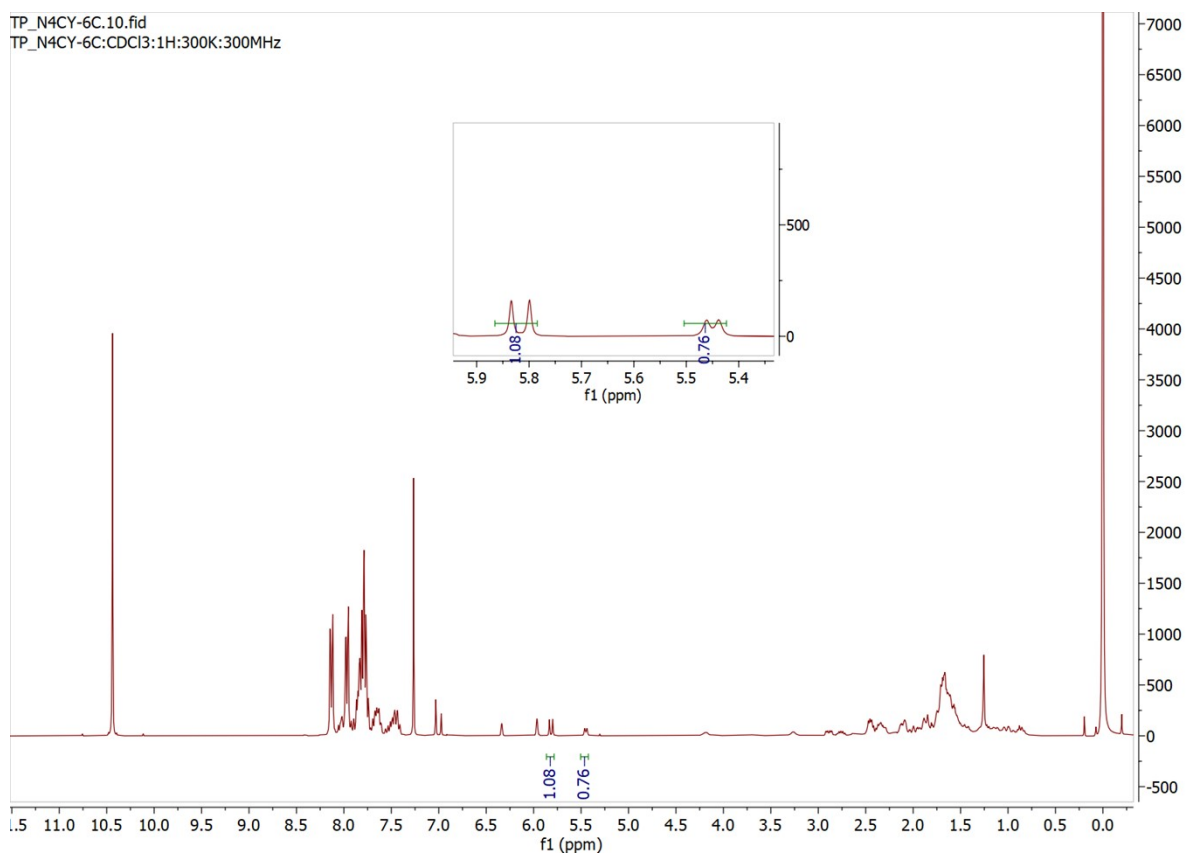


Figure S67: ¹H NMR spectrum of the crude product of TP_A4.14N4CY

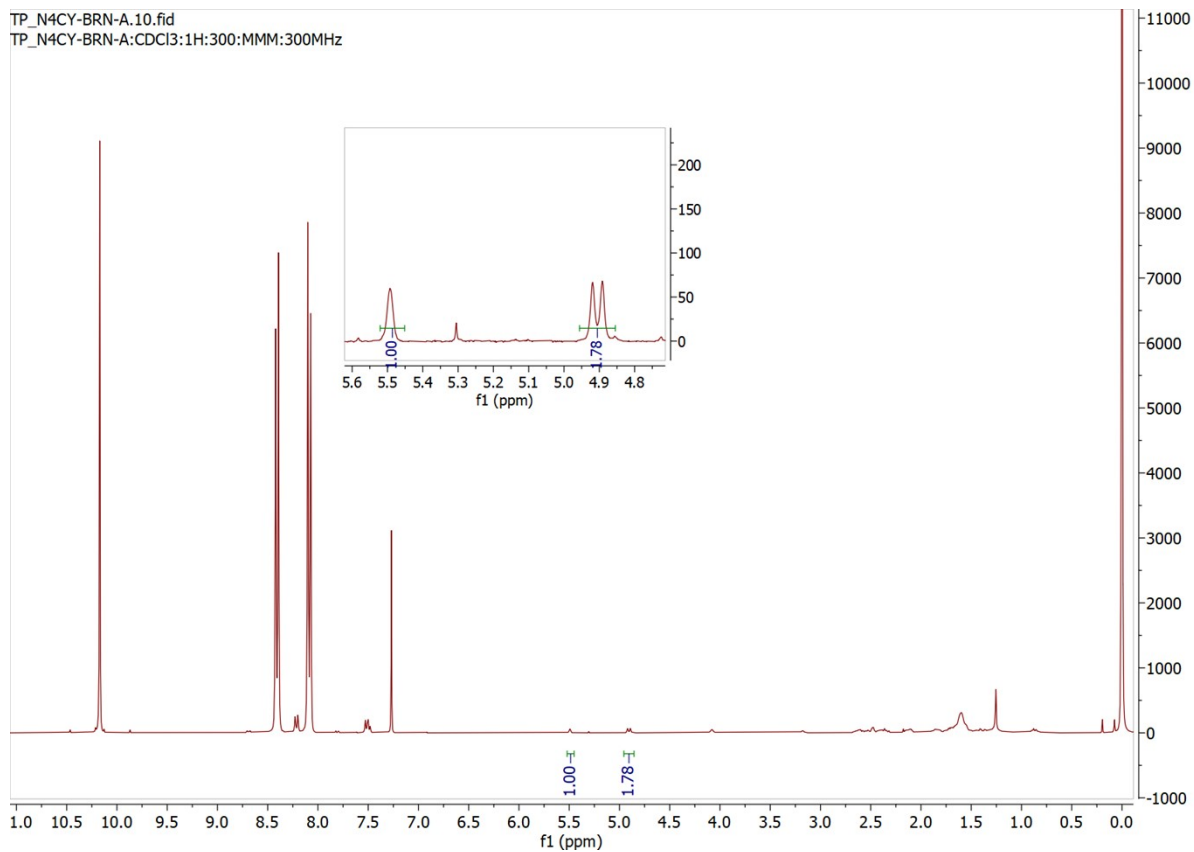


Figure S68: ^1H NMR spectrum of the crude product of TP_A4.16N4CY

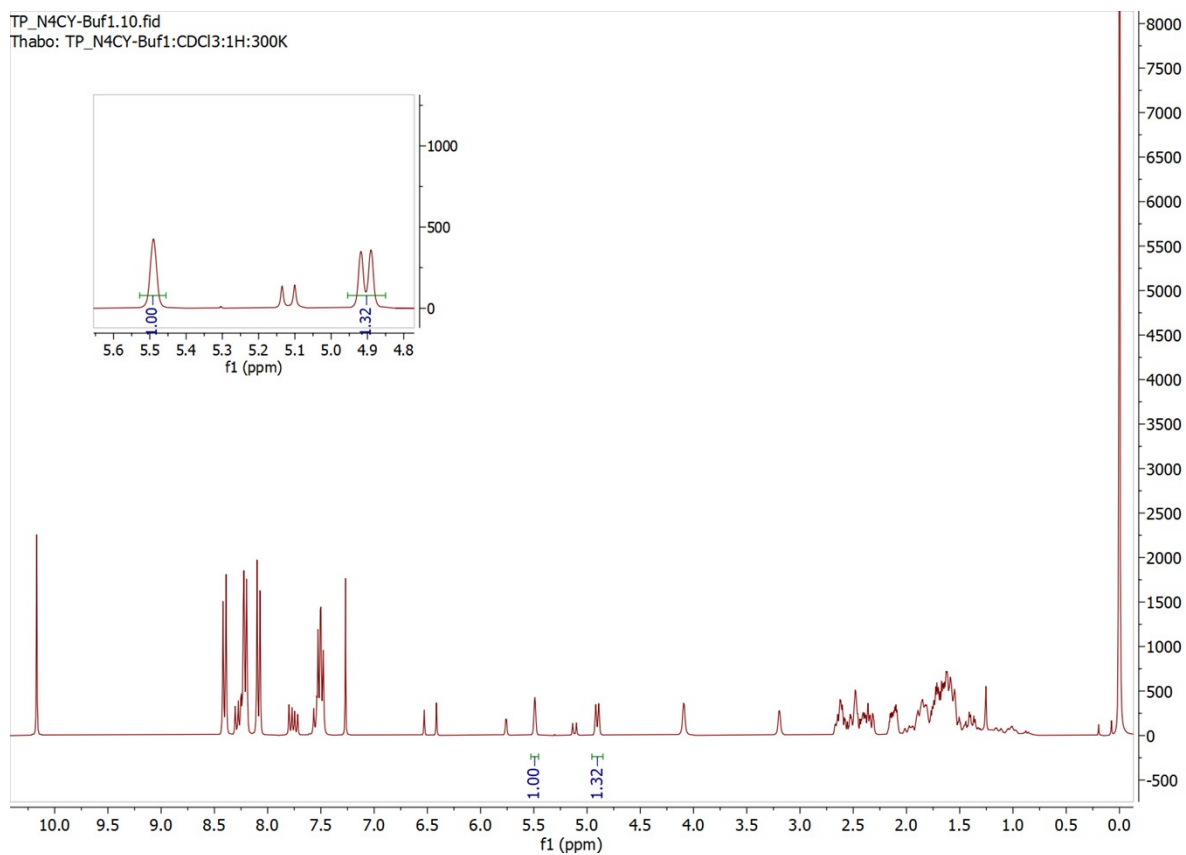


Figure S69: ^1H NMR spectrum of the crude product TP_A4.17N4CY