

Article

Peptide-Functionalized Gold Nanoparticles as Organocatalysts for Asymmetric Aldol Reactions

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1. Materials and general information

All chemicals were obtained from commercial suppliers and used without further purification unless otherwise noted. Reagents used in the peptide synthesis, such as diisopropylethylamine (DIPEA), piperidine, triisopropylsilane (TIS), formic acid, trifluoroacetic acid (TFA) and 1,2-ethanedithiol (EDT) were purchased from Sigma-Aldrich South Africa, while Fmoc-protected amino acids, (2-(1Hbenzotriazol-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo [4,5-b]pyridinium 3-oxide hexafluorophosphate (HBTU) and Rink amide MBHA were purchased from DLD scientific. Hydrogen tetrachloroaurate trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) and trisodium citrate were obtained from Sigma Aldrich Johannesburg, SA.

2. Experimental section

2.1. General procedure for the synthesis of peptide catalyst and purification

Peptides were synthesized manually using Fmoc solid-phase synthesis. The Fmoc-rink amide resin, 600 mg, (0, 160 mmol/g) was swelled in DMF (10.0 mL) for 20 min 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 min with inert nitrogen gas (N_2). The piperidine solution was then filtered out and the resin was washed with DMF (5×5 mL) for 30 sec 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. The reaction mixture was allowed to react for 45 min while a gentle flow of N_2 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 min. The resin was then washed with (3×5 mL) DMF and (2×5 mL) DCM. The resin was then dried by suction for 30 min. After the first amino acid coupling, the 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, HBTU (1.14 mmol, 0.19 M) and arginine (1.02 mmol, 0.2 M) was added to the resin. The reaction mixture was mixed gently by bubbling nitrogen gas for 45 min. The formation of the peptide bond was confirmed by mass spectrometry or by the Kaiser test. The cycle of coupling an Fmoc-protected amino acid and Fmoc group removal was repeated until an intended sequence was completed. On completion, the resin-supported peptide was washed with (3×5 mL) DMF and (2×5 mL) DCM, then dried under reduced pressure. A cleavage cocktail (10 mL) containing 94% TFA, 2.5% EDT, 2.5% H_2O and 1% TIS was then reacted with the resin-bound peptide for 3 h. The cleavage solution was filtered off, and the resin was washed with 5 mL of TFA. The filtrate was divided into portions and poured into 50 mL centrifuged tubes. Cold diethyl ether was added to the filtered solution, forming a white precipitate of the crude peptide. The two samples were centrifuged at 5000 rpm for 10 min. The step was repeated 3 times with cold diethyl ether. The obtained white precipitate was then dissolved in 10 mL (60/40; $\text{H}_2\text{O}/\text{MeCN}$) for further analysis and purification.

2.2. General procedure for the synthesis of AuNPs

The synthesis of citrate-capped AuNPs was conducted according to the procedures developed by Turkevich et al. All glassware used was thoroughly cleaned with aqua regia (HCl/HNO_3) and rinsed with Millipore-Q water before use. An aqueous solution of tetrachloroauric acid (HAuCl_4) (1 mM, 250 mL) was heated at 100 °C with vigorous mechanical stirring for 20 min. Once the solution started boiling, an aqueous solution of trisodium citrate dihydrate (0.04 M, 10 mL) was then added dropwise while the reaction was stirred vigorously. The solution was allowed to boil for an additional 10 min to ensure a complete reduction of HAuCl_4 . A colour change from colourless to deep ruby-red indicated the

formation of AuNPs. The solution was gradually cooled down to room temperature for 3 h.

To obtain different sizes of AuNPs, various volumes (10, 20, 30 and 50 mL) of aqueous tri-sodium citrate were added to the mixture. After cooling, the colloidal solutions of AuNPs were washed with water by centrifuging at 1500 rpm for 15 min using a Hettich Universal 320R centrifuge (Tuttligen, Germany), in order to eliminate excess unreacted ions and the starting material in the supernatant. The concentrated dispersed AuNP particles at the bottom of the centrifuge tube free of excess salts were diluted back to the original concentration with Millipore-Q water. The AuNP solutions were transferred into glass vials wrapped with aluminium foil to avoid interactions with light which may cause aggregation of the particles. The vials were stored in the fridge and could be used for over 6 months without aggregation.

2.3. General procedure for the functionalization of TP_ADLYs peptide to AuNPs

The centrifuged solution of AuNPs was divided into 3 aliquots of 10 mL. This was further divided into fractions of 1.5, 1.0 and 0.5 mL, which were added to different peptide concentrations of 1.5, 2.8, 4 and 5 mol% and stirred for 3 h at room temperature to allow the complete exchange of citrate with thiols on the particle surface. The ruby-red colour changed to a pale red colour, and the excess non-immobilized peptide was removed by centrifugation at 1500 rpm for 15 min in a Universal 320R centrifuge.

2.4. General procedure for aldol reaction using peptide-capped AuNPs

The corresponding ketone (0.628 mmol, 70 μ L) was added to a 2 mL Eppendorf vial containing 1 mL of the DMSO/AuNP-pep solution (0.5:0.5 mL, v: v) and the mixture was stirred for 15 min. This was followed by the addition of the acceptor aldehyde (0.157 mmol, 24 mg), and the resulting mixture was stirred for 24–72 h. Conversion of the product was monitored by TLC, upon which the reaction mixture was extracted with EtOAc (3 \times 5 mL) and the combined organic phases were dried over Na₂SO₄ and concentrated in vacuo. The diastereomeric ratio of the crude product was determined by ¹H NMR analysis. The crude aldol product was purified using flash silica gel column chromatography and EtOAc/Hexane (1:3), and the desired aldol product was subjected to chiral-phase HPLC analysis to determine the ee.

3. Characterization

Peptide purification was achieved via an Agilent 1260 Infinity semi-preparative HPLC system (Waldbronn, Germany) with a UV/VIS detector and an automated fraction collector on a Kinetix® 5 μ m BC18 (Torrance California, USA, 100 Å (250 \times 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 was utilized.

Analytical LC-MS analysis was carried out on an Ultra High-Performance Liquid Chromatography (Thermo Scientific Ultimate 3000, RS diode array detectors, (Basel, Switzerland) High-Resolution Mass Spectrometer (Bruker Compact quadrupole time of-flight) coupled to Diode Array (215 and 254 nm) Bruker Daltonics, Bremen, Germany. A two-buffer system was employed with a flow rate of 0.3 mL/min on a C18 column (5 μ m, 100 Å, 4.60 mm \times 150 mm). The buffer system utilized formic acid as the ion-pairing agent, with solvents A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid).

Analytical thin layer chromatography (TLC) plates (0.2 mm silica gel 60 with fluorescent indicator UV₂₅₄) were obtained from Sigma-Aldrich. Aldol reactions were monitored using TLC, and further visualization was conducted by staining with potassium permanganate (KMnO₄) solution followed by heating. The compounds were purified using column chromatography on normal silica gel (particle size 0.063–0.200 mm) and flash silica gel

(particle size 0.040–0.063) purchased from Merck. Ethyl acetate and hexane were used as eluting solvents. Solvent ratios are reported as (30/70, volume by volume)

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, (Basel, Switzerland), 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. Detailed solvent ratios are outlined in the experimental section below.

¹H-NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer (Rheinstetten, Germany). The chemical shift (δ) values are reported in parts per million (ppm) relative to deuterated chloroform (CDCl₃, 7.26 ppm) and dimethyl sulfoxide (DMSO-*d*₆, 2.49 ppm), and referenced against the internal standard, tetramethylsilane (TMS, 0.00 ppm). The spectra were analysed as first order, and the values of the coupling constant (J) are reported as Hertz (Hz). Signal multiplicities are expressed as s = singlet, br s = broad singlet, d = doublet, dd = double doublet, t = triplet, q = quartet and m = multiplet.

¹³C-NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. The chemical shift values are reported in (ppm) relative to deuterated chloroform (CDCl₃, 77.2 ppm), (DMSO-*d*₆, 39.52 ppm) and TMS as an internal standard.

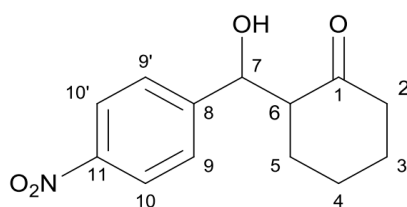
CD spectra were recorded on a JASCO J-18 spectropolarimeter in the range of 190 to 250 nm in the specified solvents (water and phosphate buffer), with 10 scans at 20°C. The quartz cuvette used had a pathlength of 0.2 cm with a resolution of 0.2 nm, a bandwidth of 1.0 nm and a sensitivity of 10–20 mdeg. The peptides (0, 00061 mol L^{−1}) were prepared in water and a 20 mM phosphate buffer (pH 8). The value of absorption was measured as molar ellipticity per residue deg·cm²·dmol^{−1})

Transmission electron microscopy (TEM) analysis was performed using FEI Tecnai-12 microscopy (Hillsboro, USA) at an electron acceleration voltage of 200 KV and beam size of 10–100 nm. Sample analysis was carried out by placing a drop of the gold colloidal on carbon-coated copper TEM grids. The sample was transferred to the grid and allowed to dry in air for a few minutes before analysis.

UV–vis was used to monitor the optical properties of gold colloidal solution. The analysis was conducted using a Varian Cary Eclipse (Cary 50) UV–vis spectrophotometer (Agilent Technologies (Santa Clara, USA), with a Quartz cuvette of 1 cm optical length.

4. Analytical data for Aldol products

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



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, CDCl₃) 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). ¹³C NMR (126 MHz, CDCl₃) 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 $[M+23]^+$.

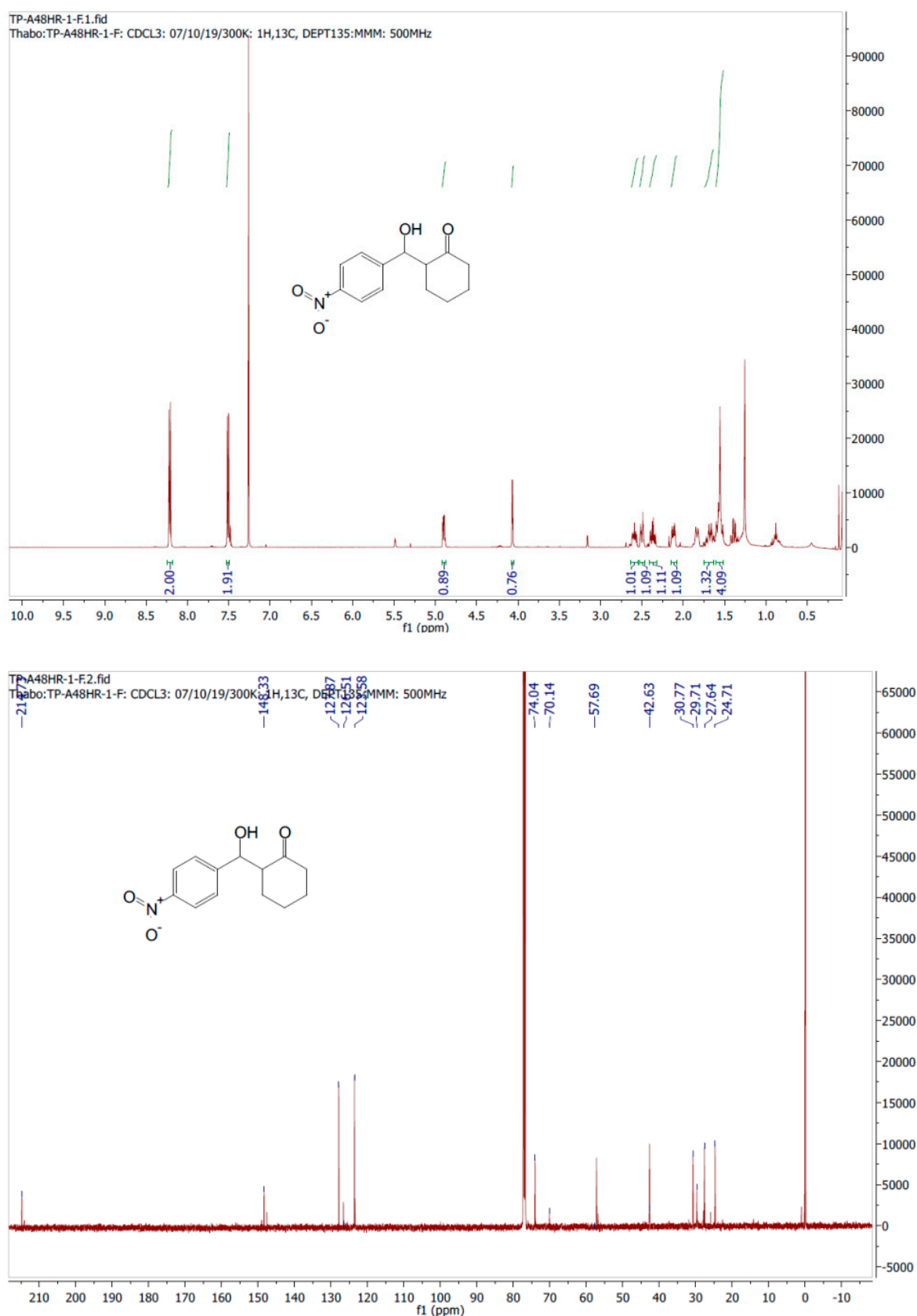


Figure S1: 1H and ^{13}C NMR spectra of the aldol product TP_N4CY

5. Selected chiral HPLC chromatograms for aldol products obtained

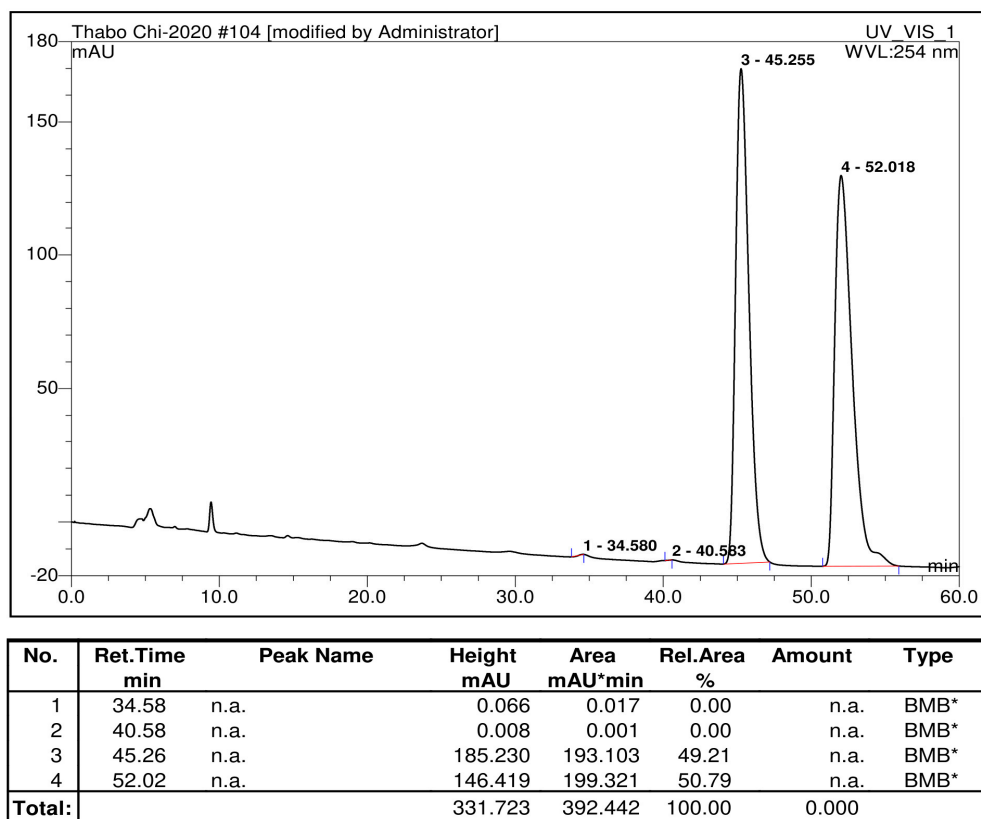


Figure SR2: Chiral HPLC chromatogram of racemate TP_N4CY

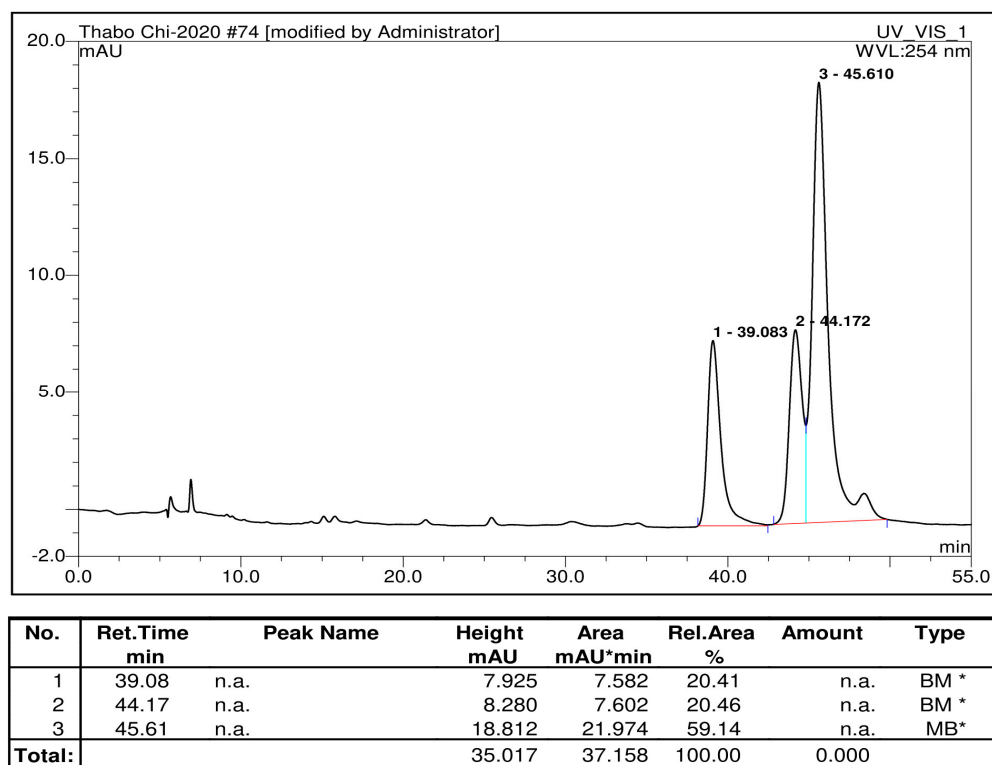


Figure S2: Chiral HPLC chromatogram of TP_N4CY1

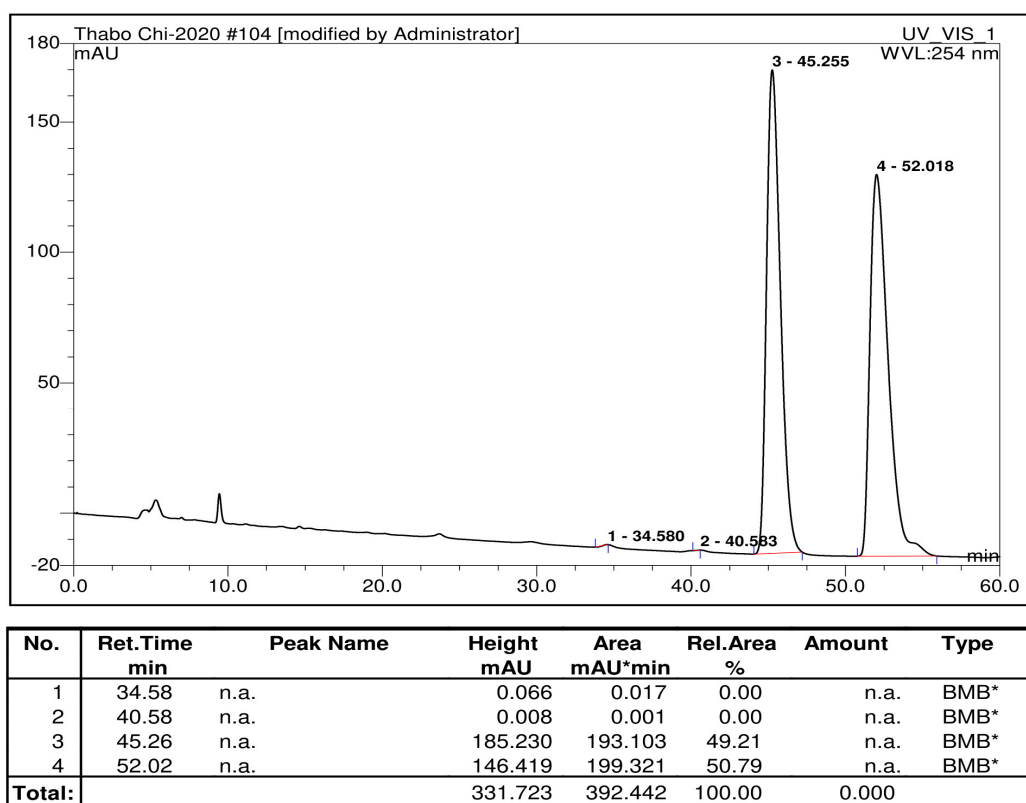


Figure SR3: Chiral HPLC chromatogram of racemate TP_N4CY

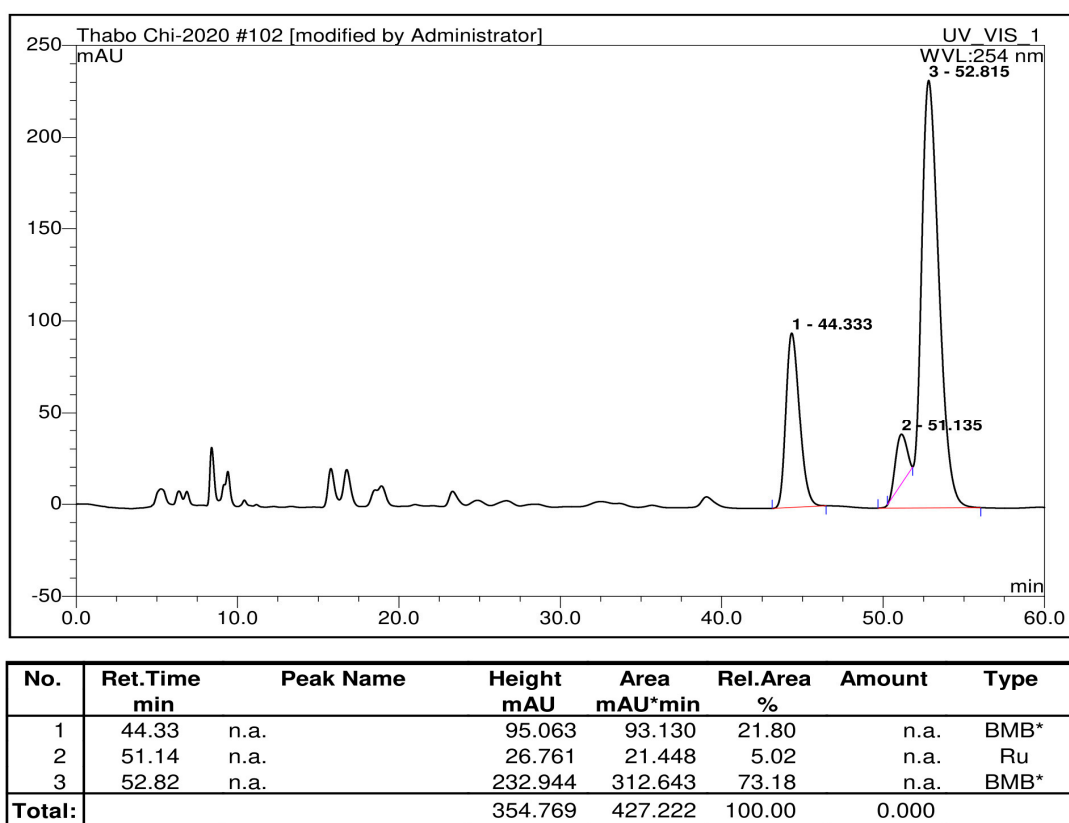


Figure S3: Chiral HPLC chromatogram of TP_N4CY2

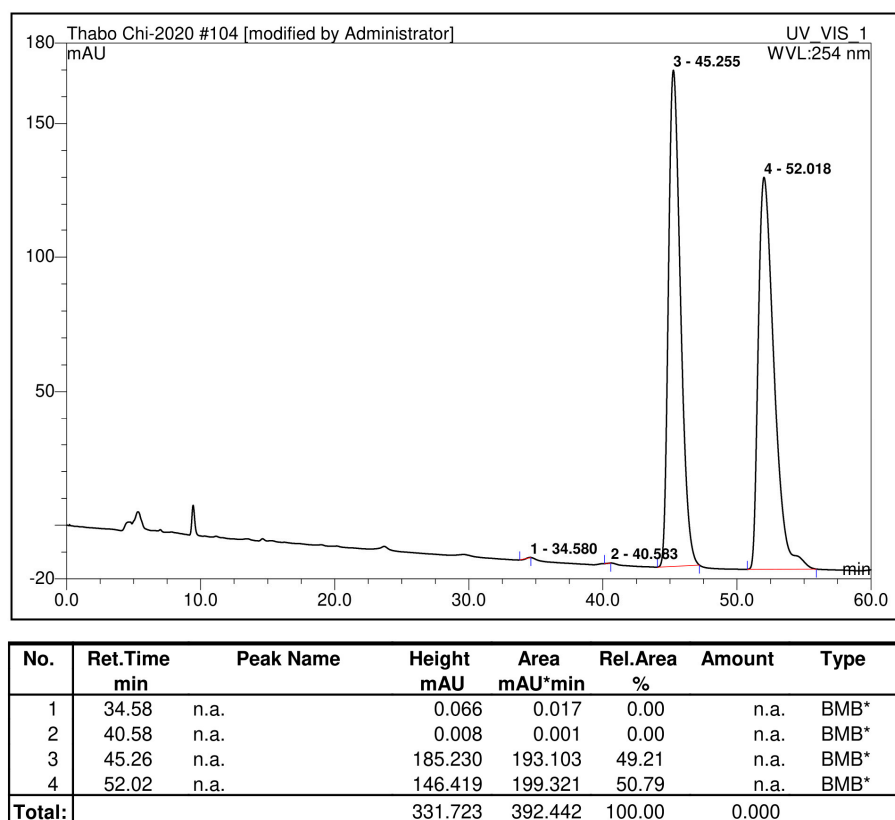


Figure SR4: Chiral HPLC chromatogram of racemate TP_N4CY

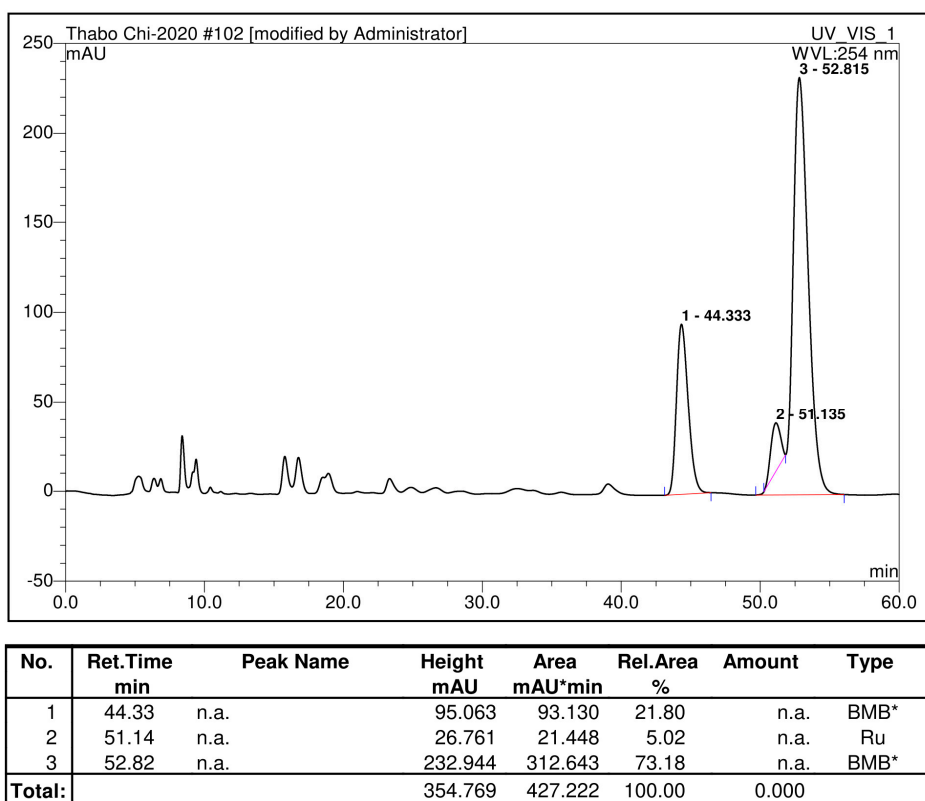


Figure S4: Chiral HPLC chromatogram of TP_N4CY3

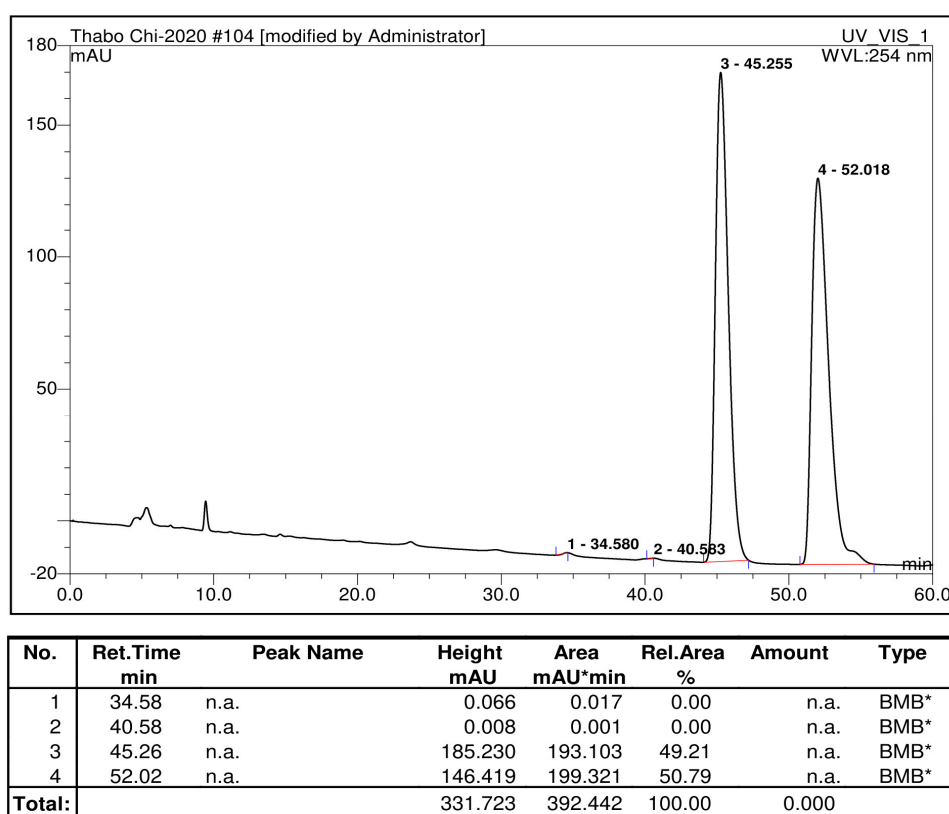


Figure SR5: Chiral HPLC chromatogram of racemate TP_N4CY

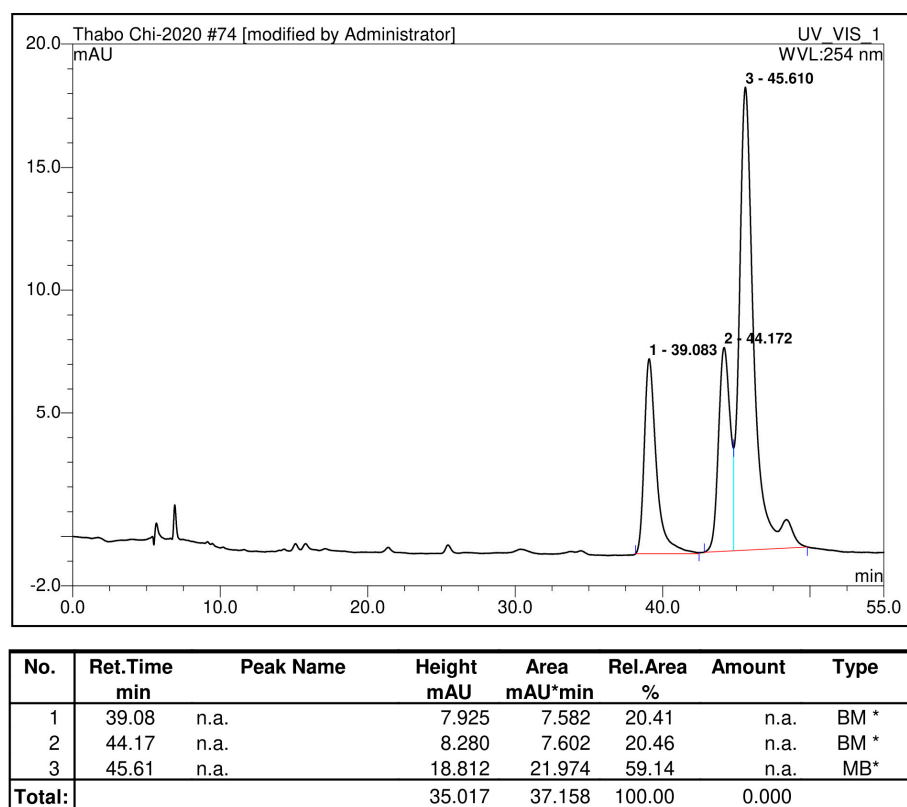


Figure S5: Chiral HPLC chromatogram of TP_N4CY4

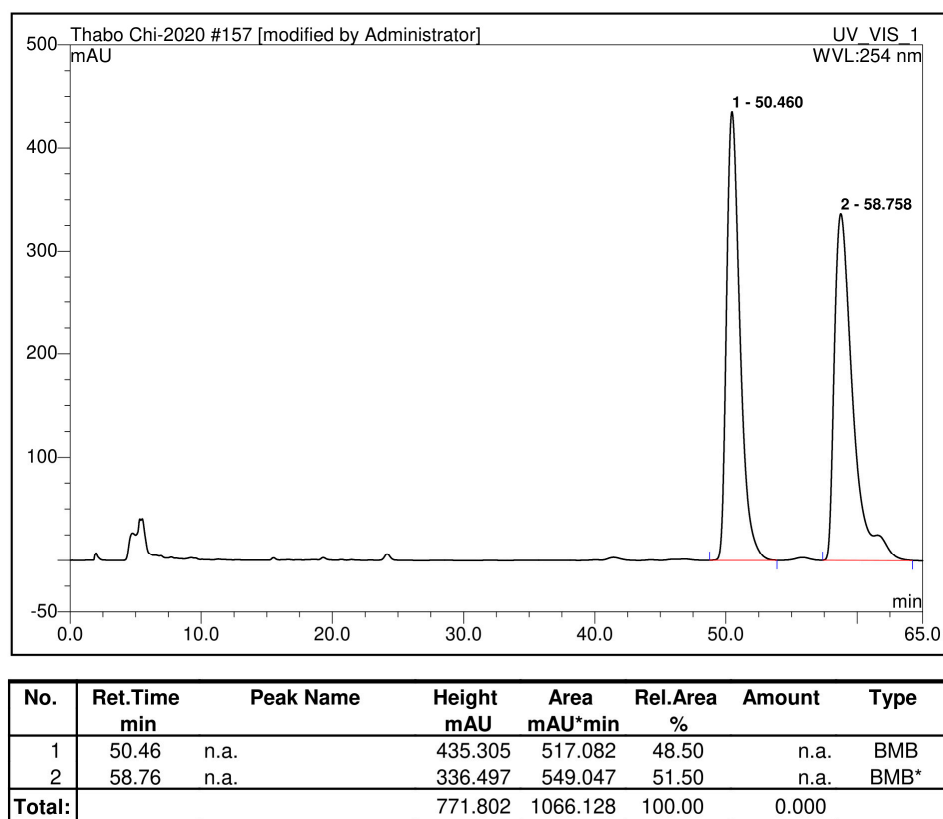


Figure SR6: Chiral HPLC chromatogram of racemate TP_N4CY

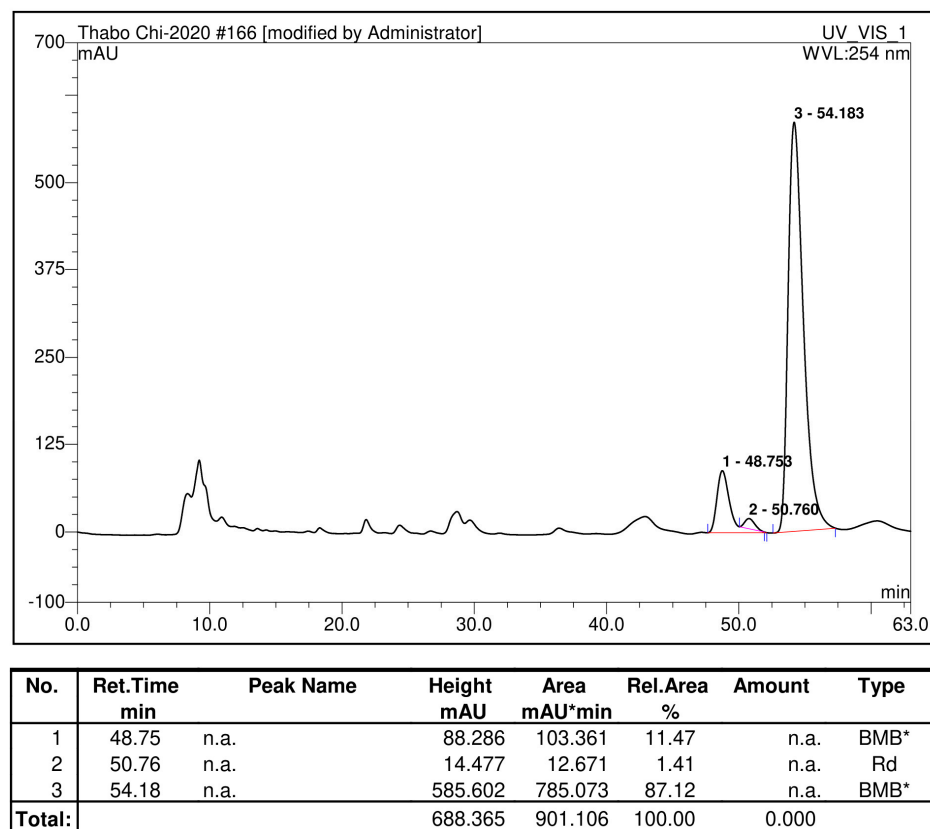


Figure S6: Chiral HPLC chromatogram of TP_N4CY5

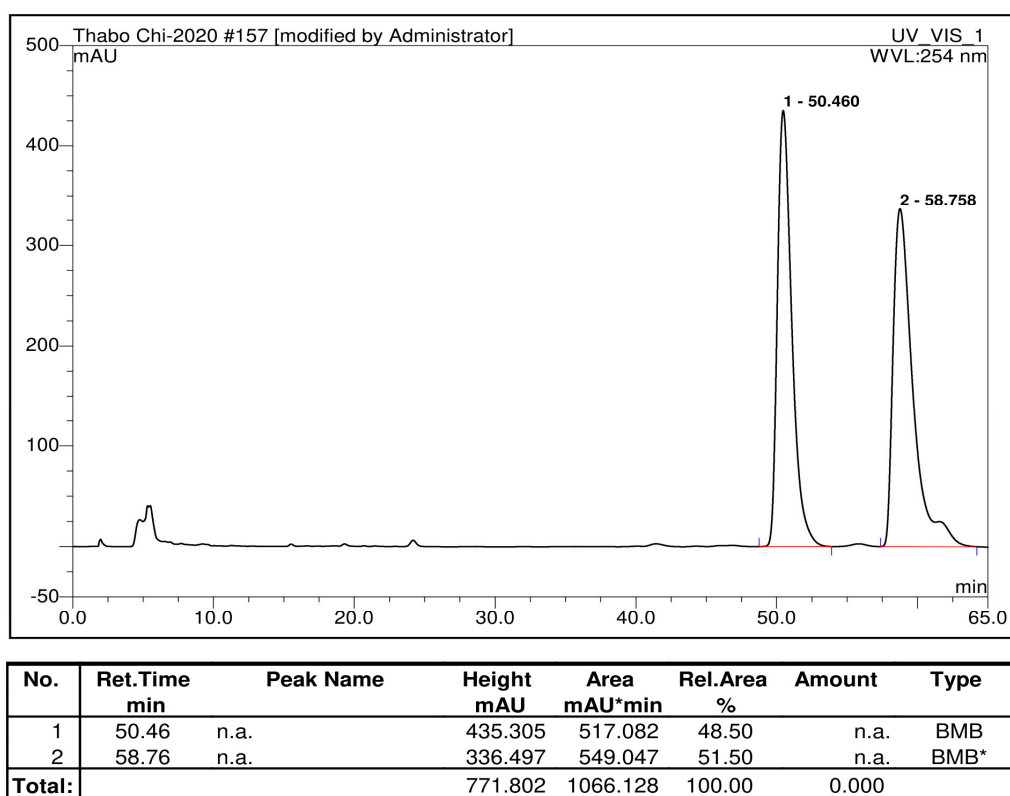


Figure SR7: Chiral HPLC chromatogram of racemate TP_N4CY

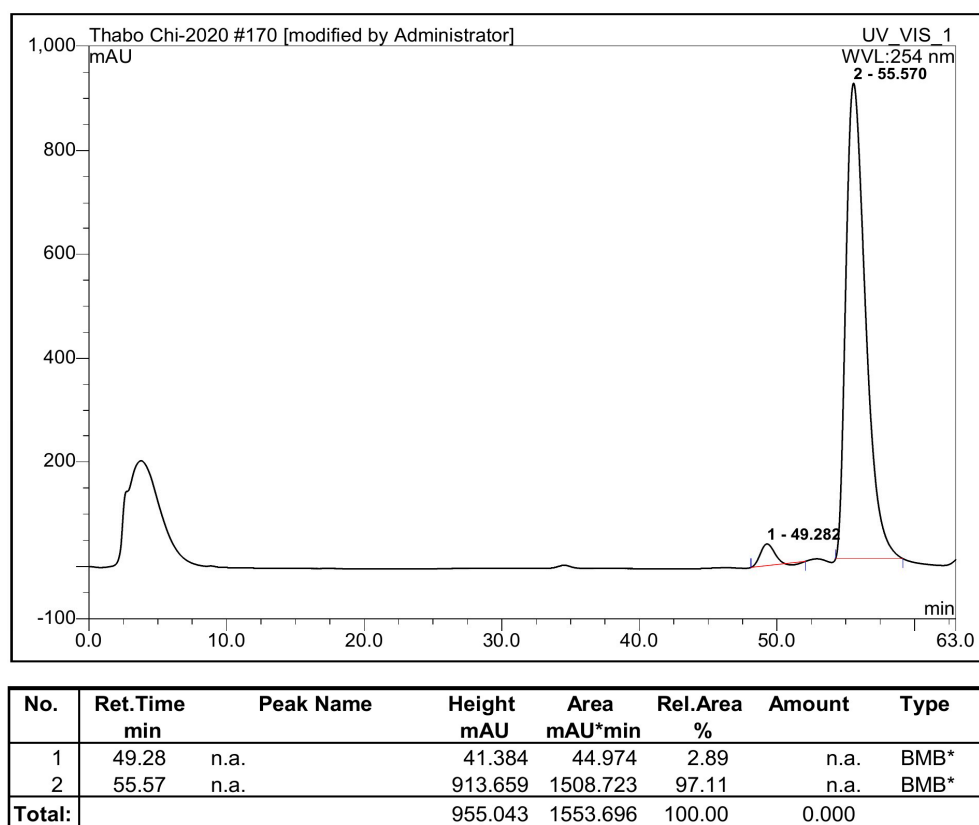


Figure S7: Chiral HPLC chromatogram of TP_N4CY6

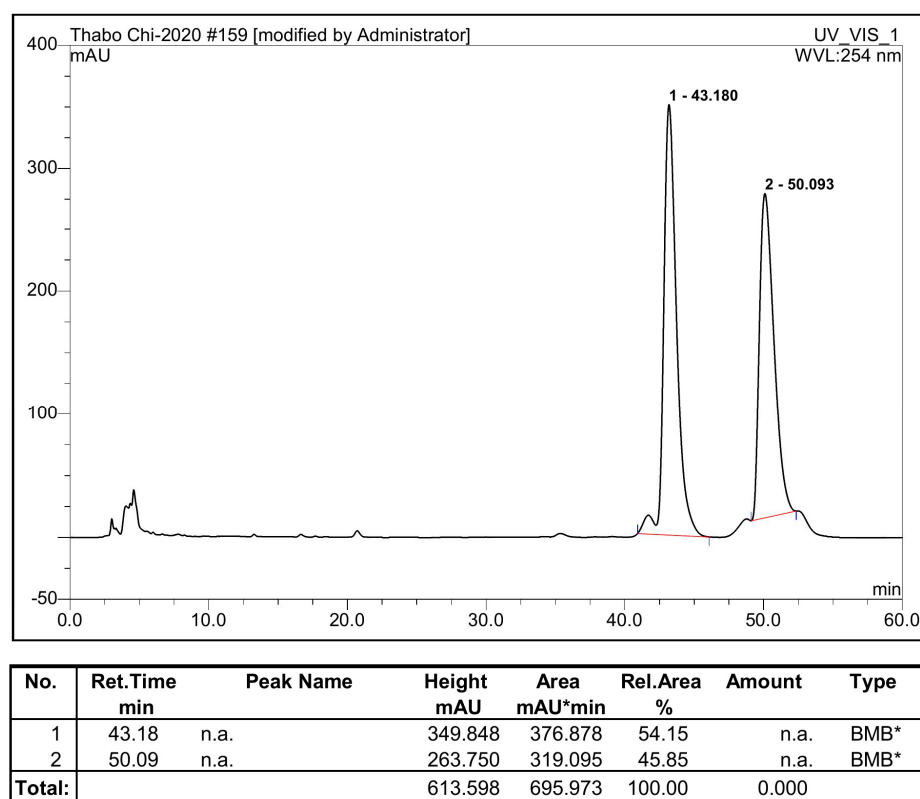


Figure SR8: Chiral HPLC chromatogram of racemate TP_N4CY

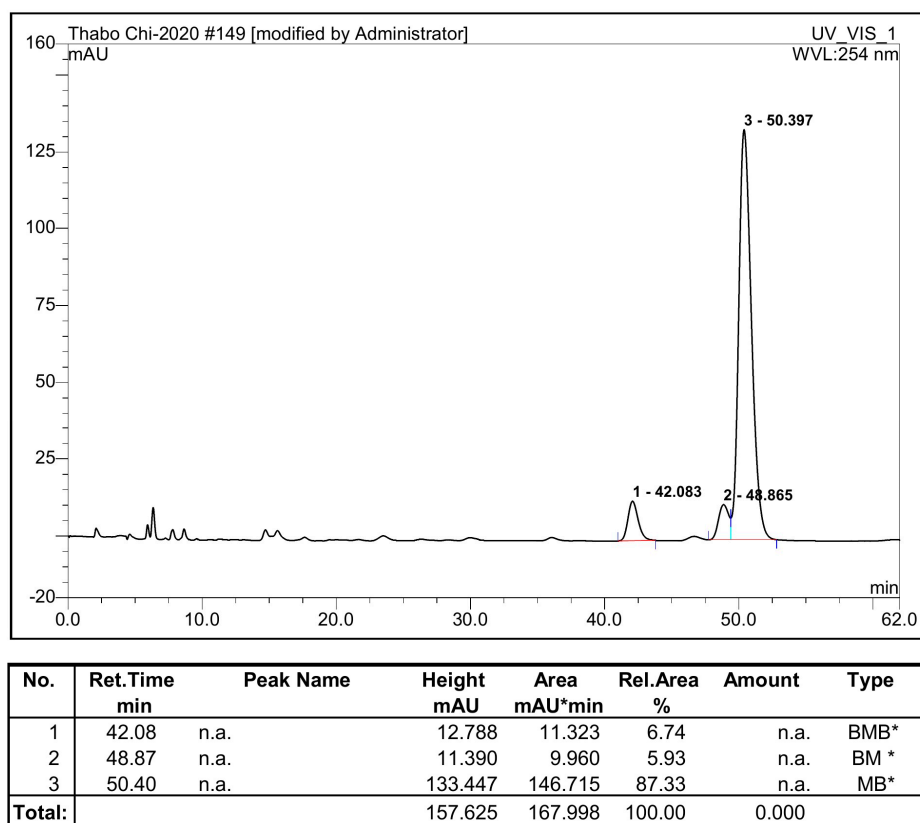


Figure S8: Chiral HPLC chromatogram of TP_N4CY7

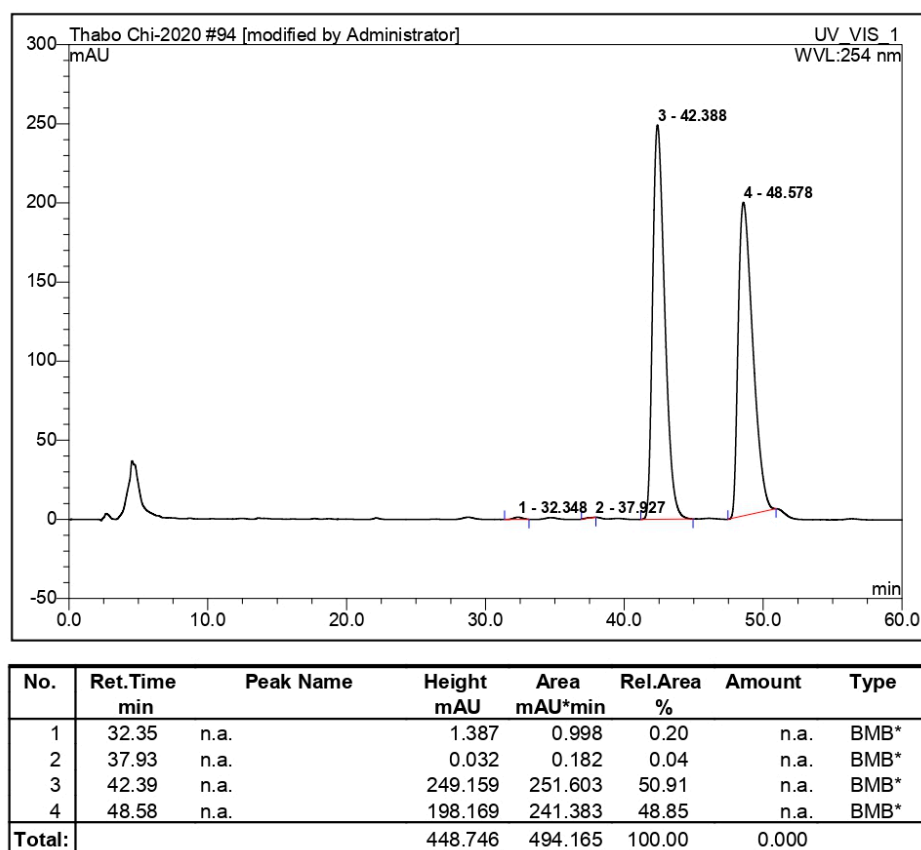


Figure SR9: Chiral HPLC chromatogram of TP_N4CY

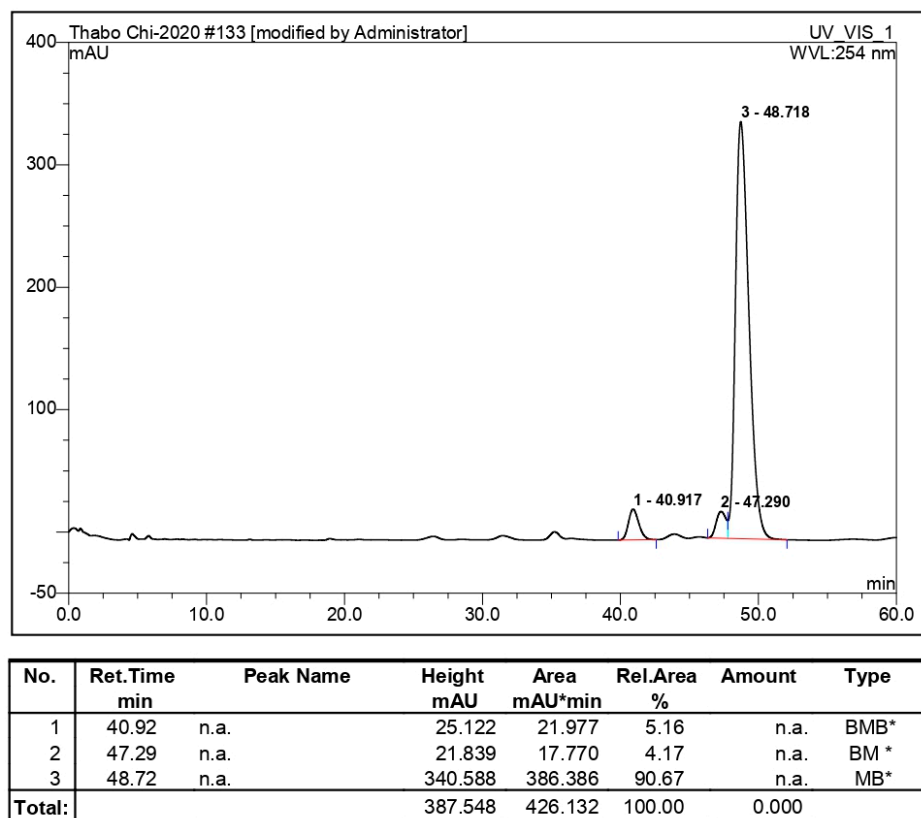


Figure S9: Chiral HPLC chromatogram of TP_N4CY8

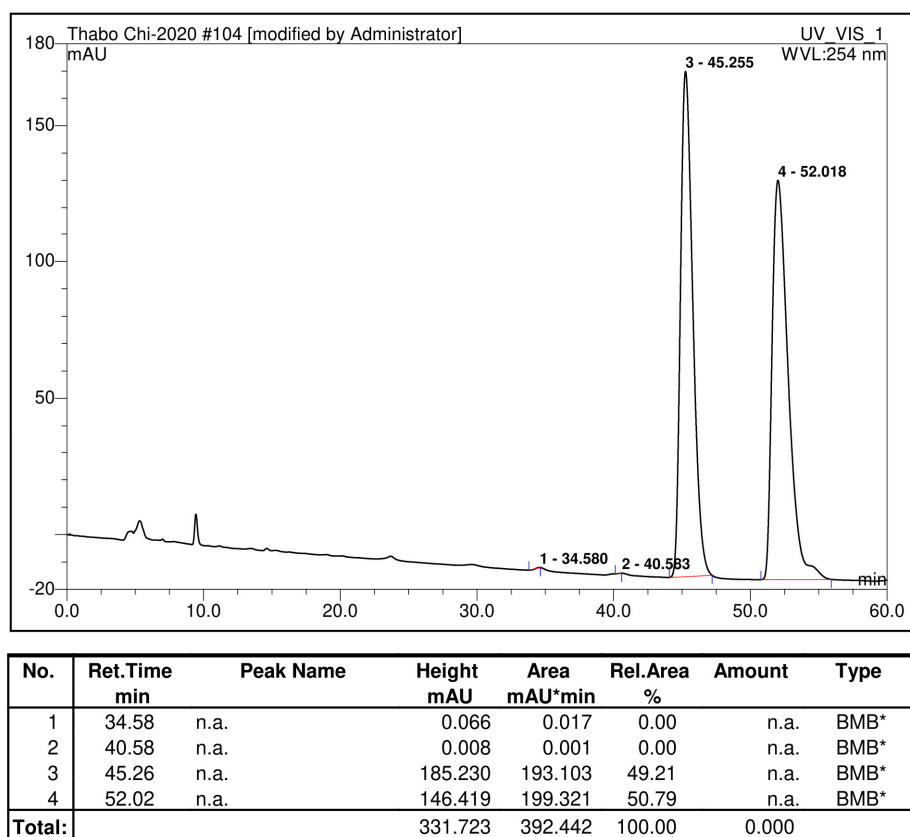


Figure SR10: Chiral HPLC chromatogram of racemate TP_N4CY

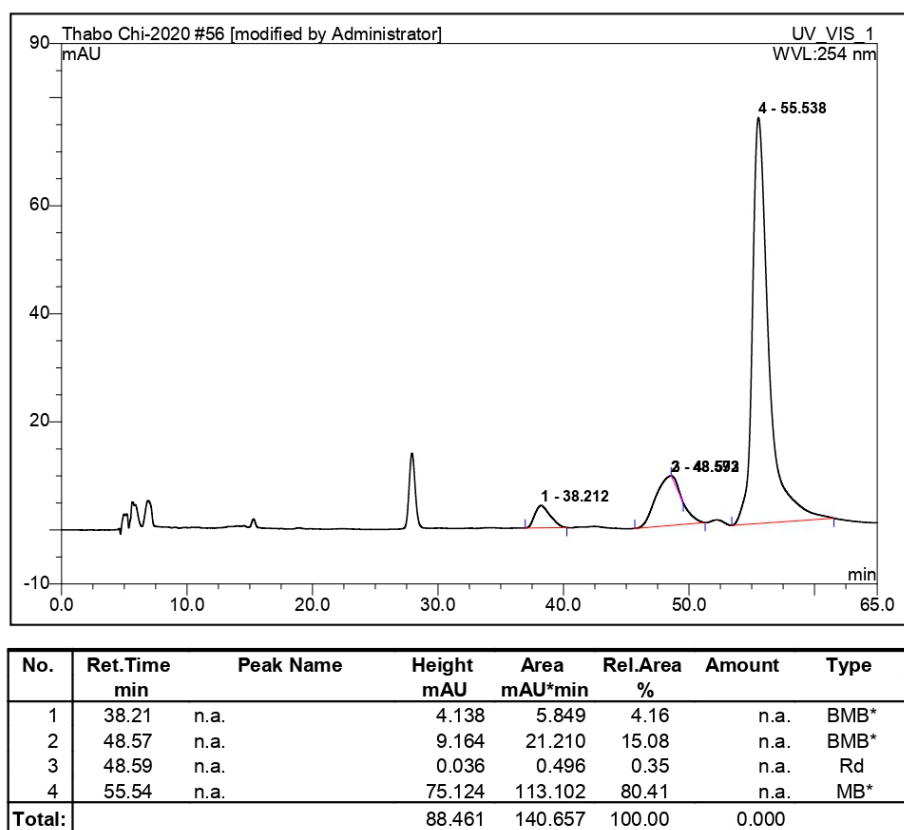


Figure S10: Chiral HPLC chromatogram of TP_N4CY9

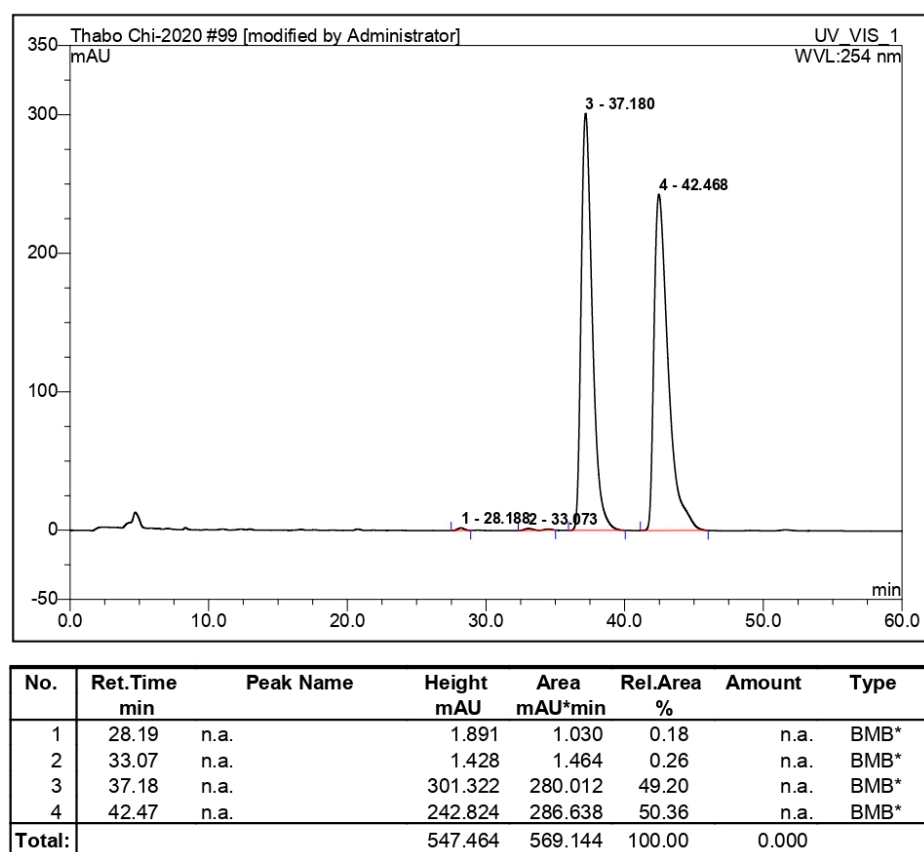


Figure SR11: Chiral HPLC chromatogram of TP_N4CY

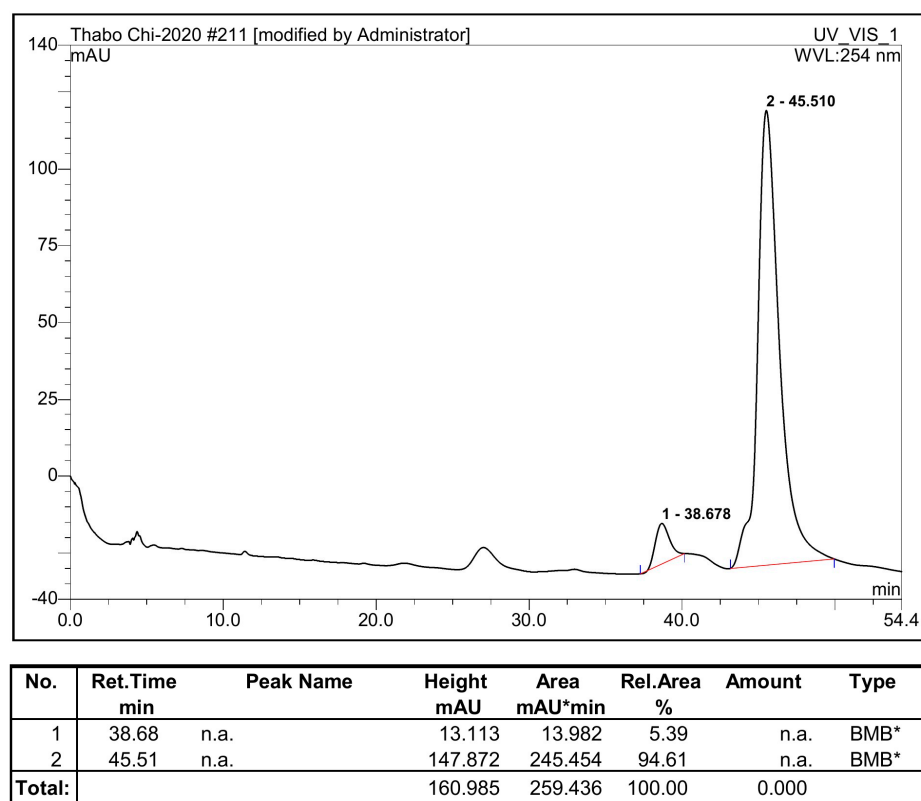


Figure S11: Chiral HPLC chromatogram of TP_N4CY10

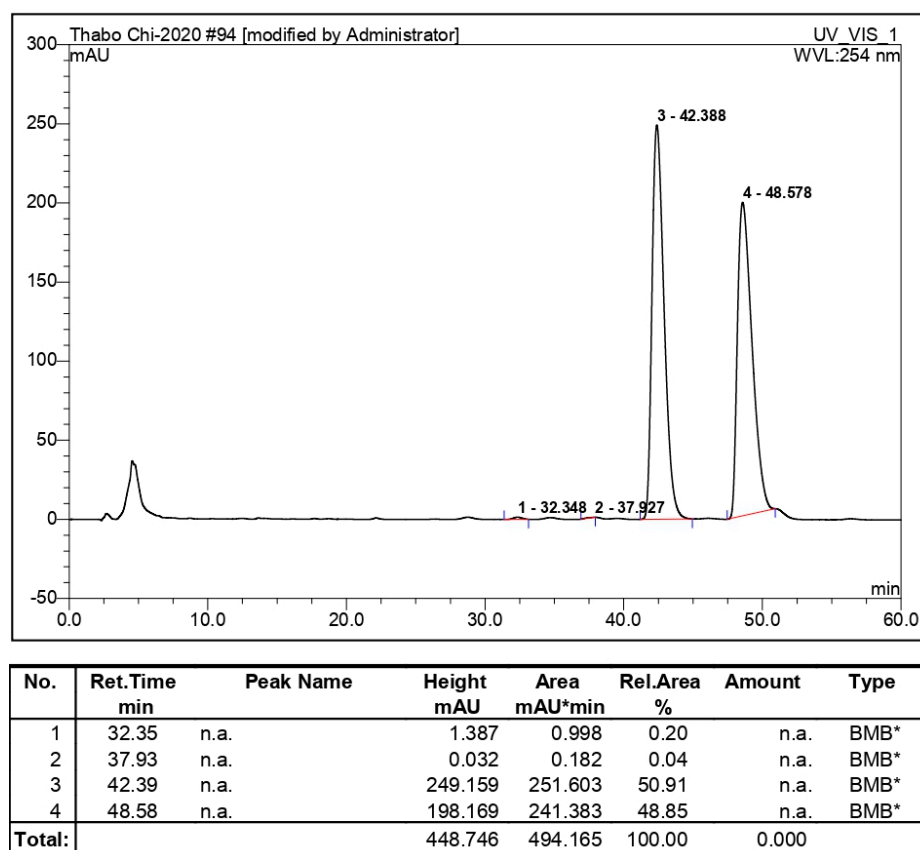


Figure SR12: Chiral HPLC chromatogram of TP_N4CY

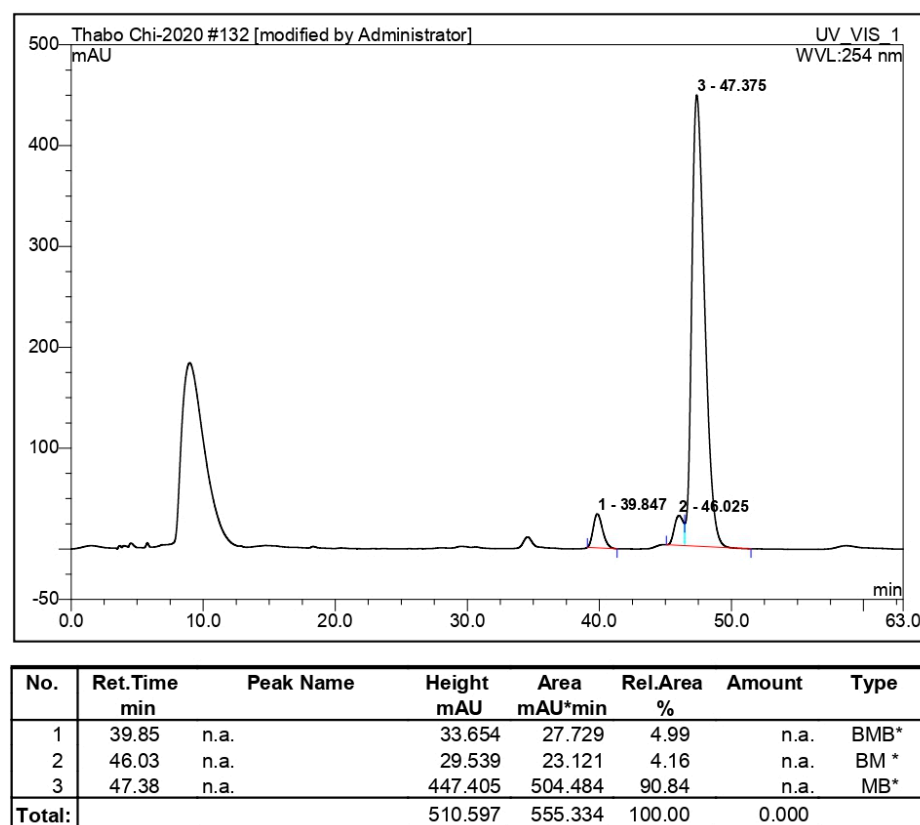


Figure S12: Chiral HPLC chromatogram of TP_N4CY11

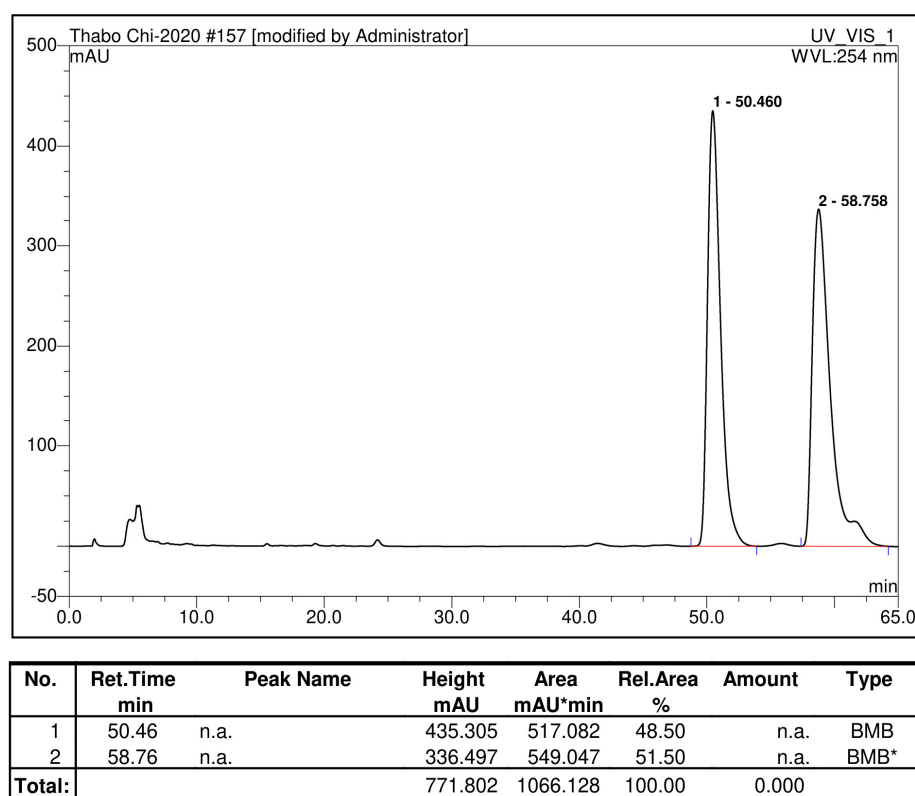


Figure SR13: Chiral HPLC chromatogram of TP_N4CY

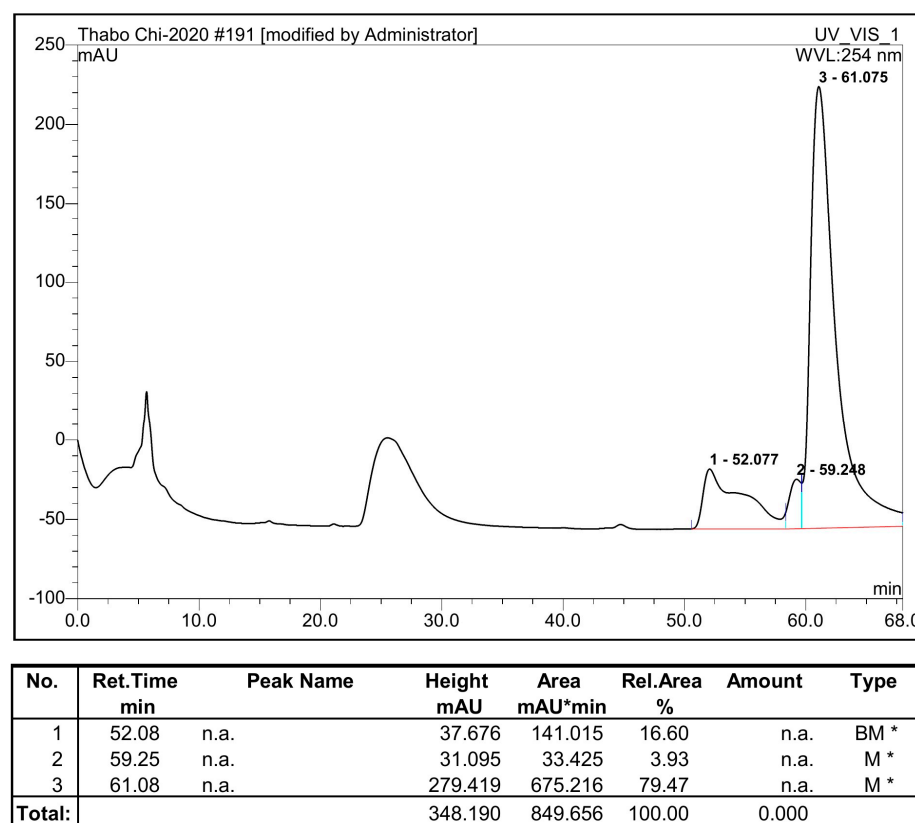


Figure S13: Chiral HPLC chromatogram of TP_N4CY12

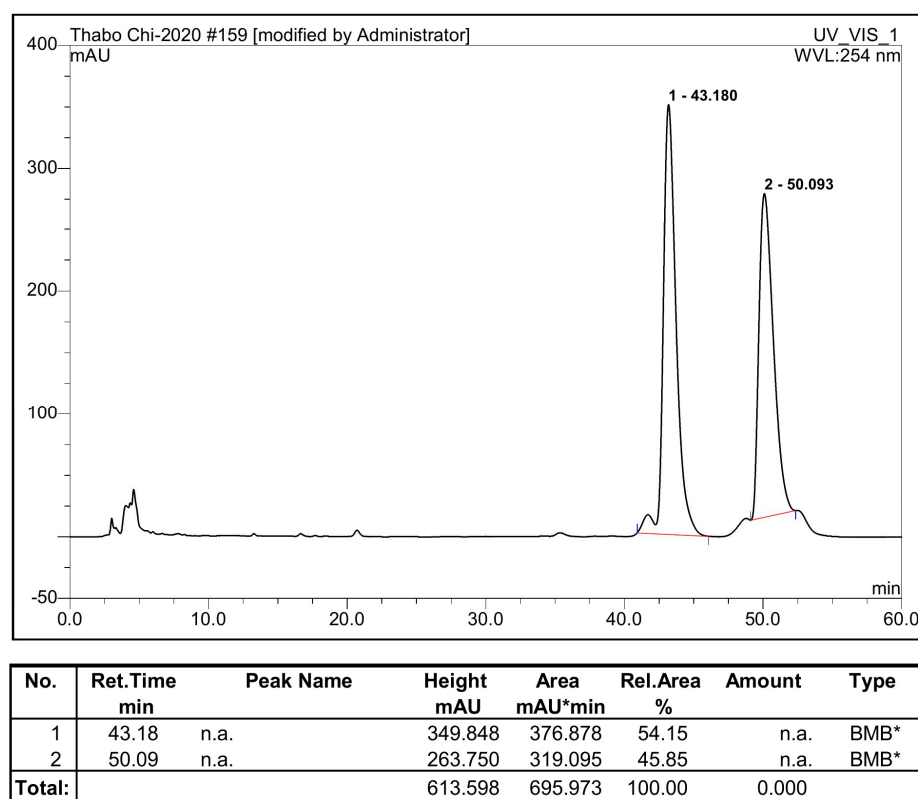


Figure SR14: Chiral HPLC chromatogram of TP_N4CY

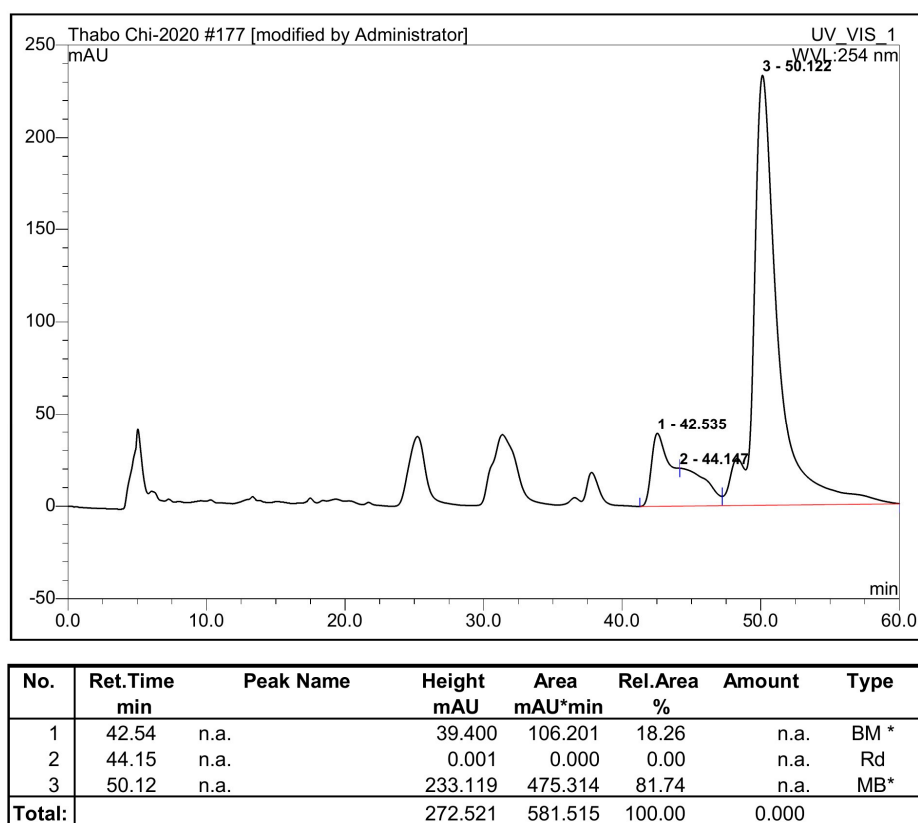


Figure S14: Chiral HPLC chromatogram of TP_N4CY13

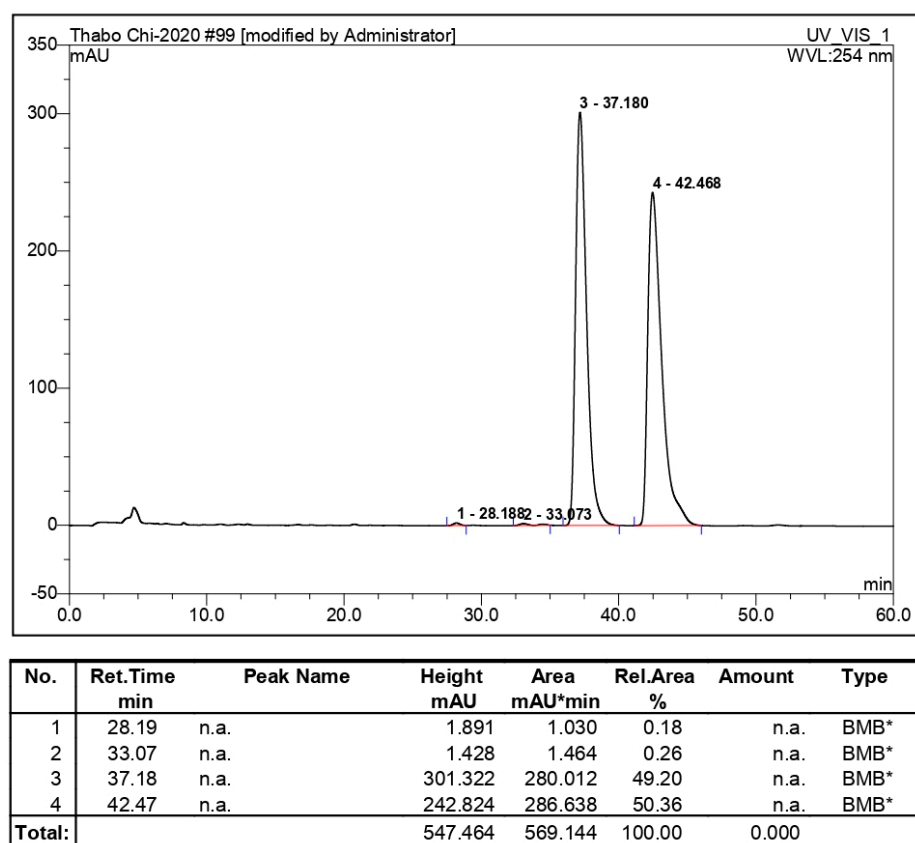


Figure SR15: Chiral HPLC chromatogram of TP_N4CY

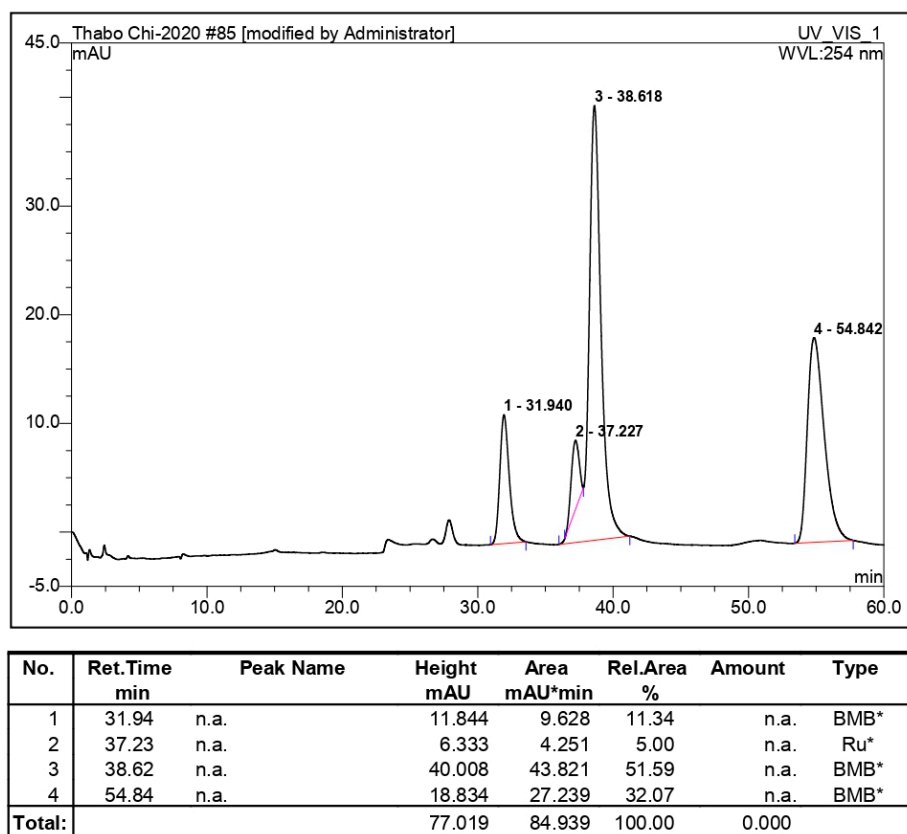
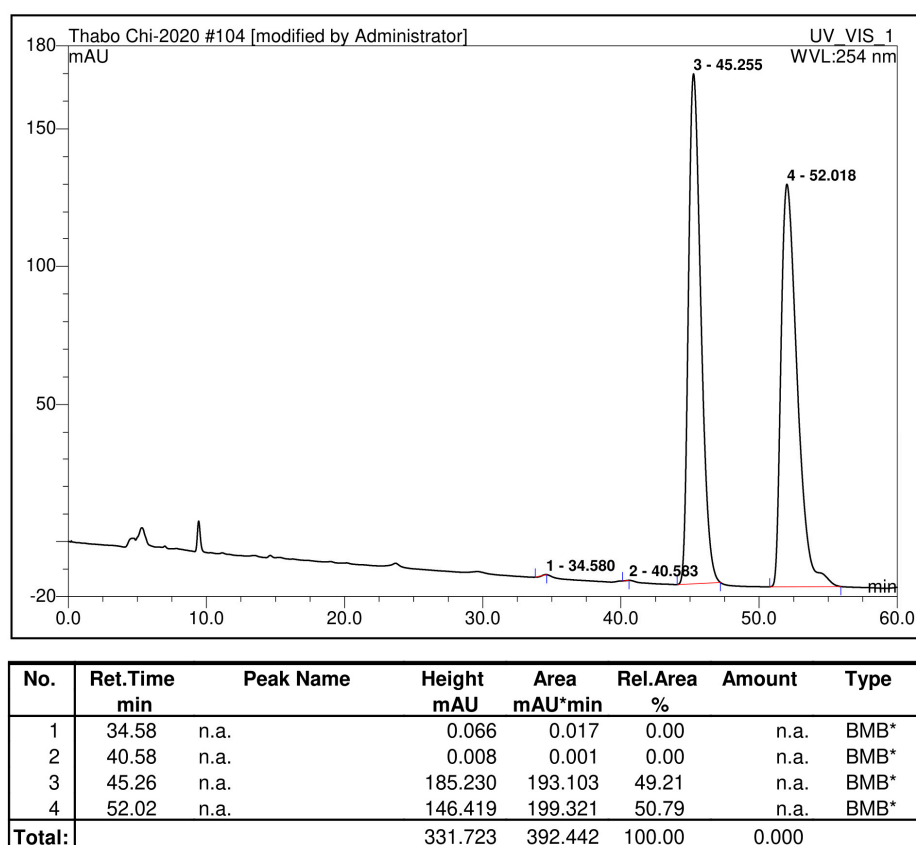


Figure S15: Chiral HPLC chromatogram of TP_N4CY14



FigureSR16: Chiral HPLC chromatogram of TP_N4CY

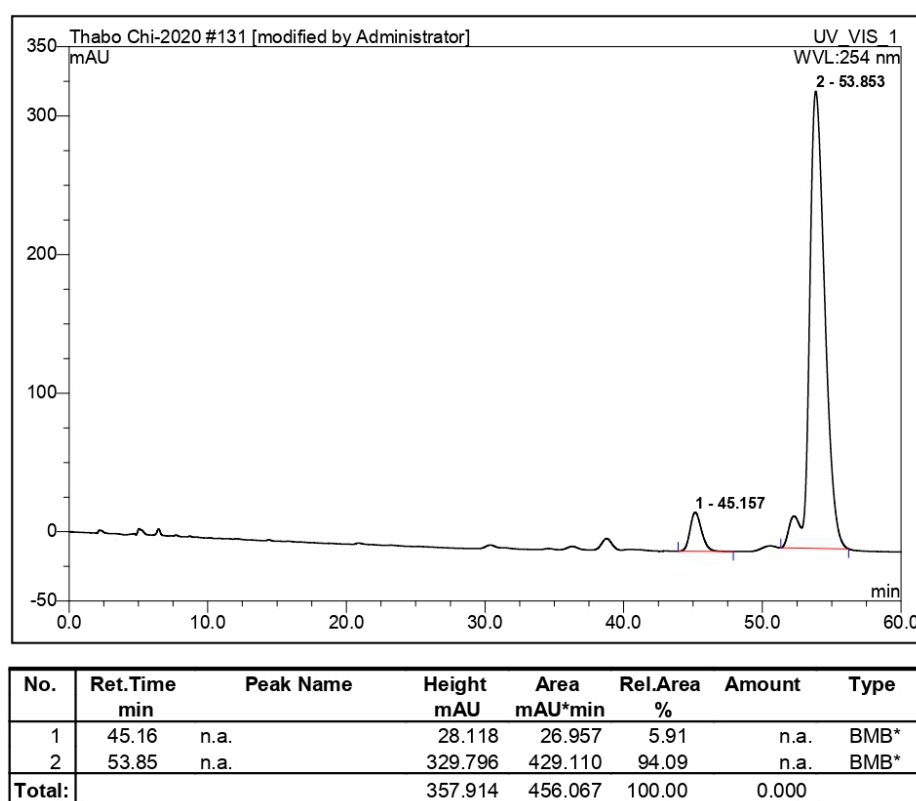


Figure S16: Chiral HPLC chromatogram of TP_N4CY15

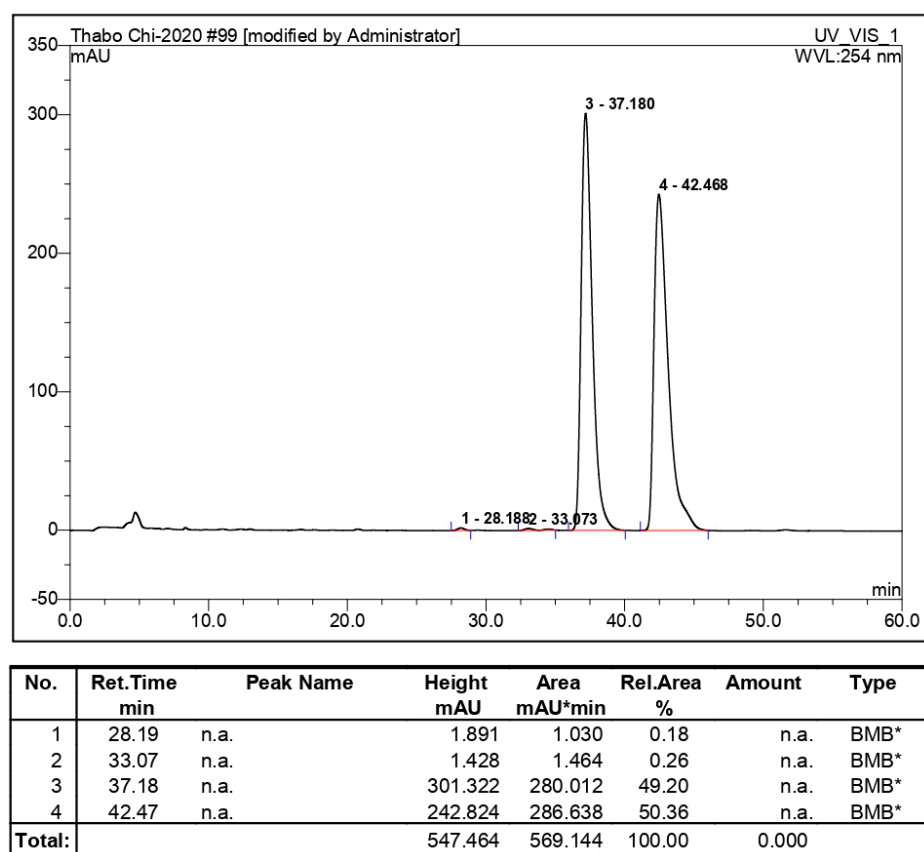


Figure SR17: Chiral HPLC chromatogram of TP_N4CY

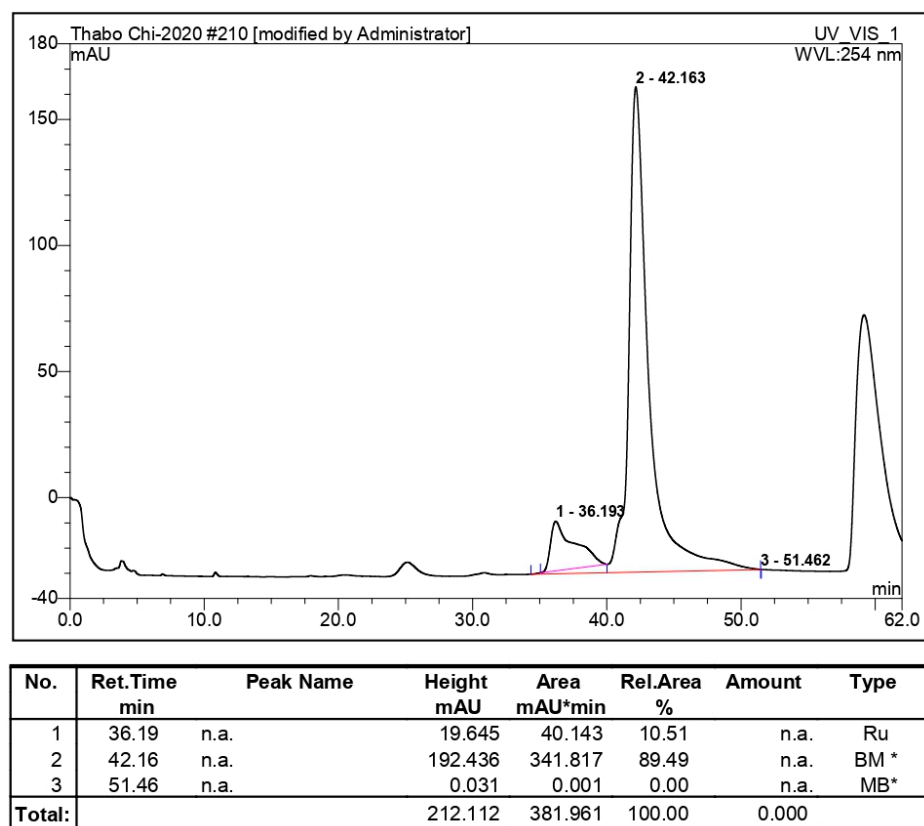


Figure S17: Chiral HPLC chromatogram of TP_N4CY16

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

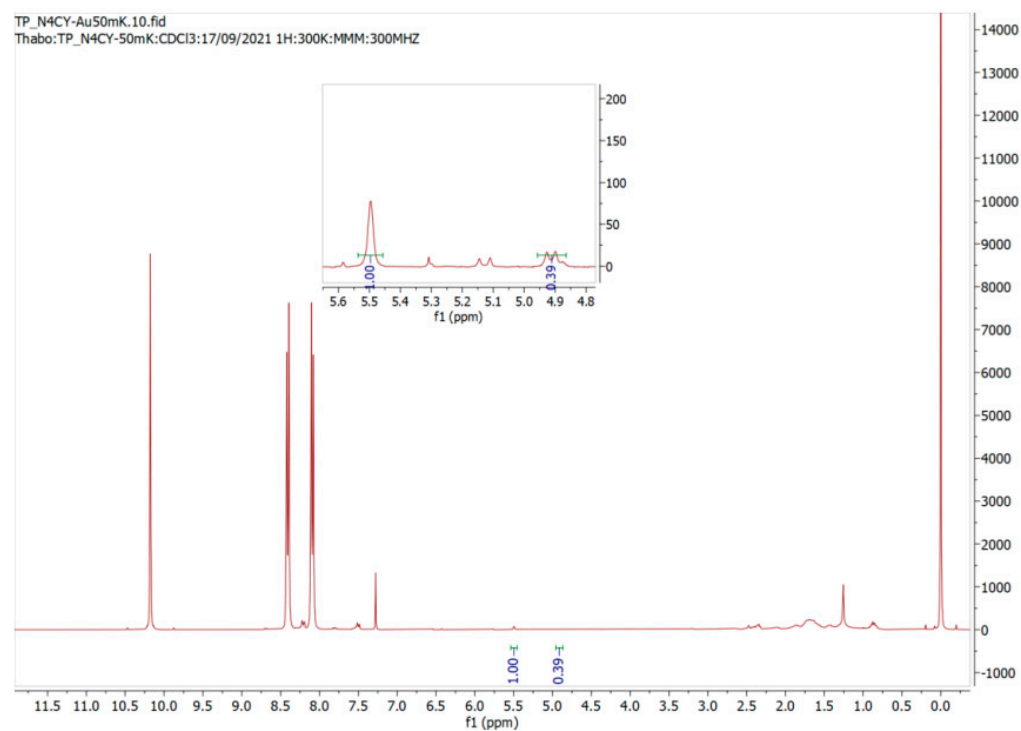


Figure S18: ^1H NMR spectrum of the crude product TP_N4CYR1

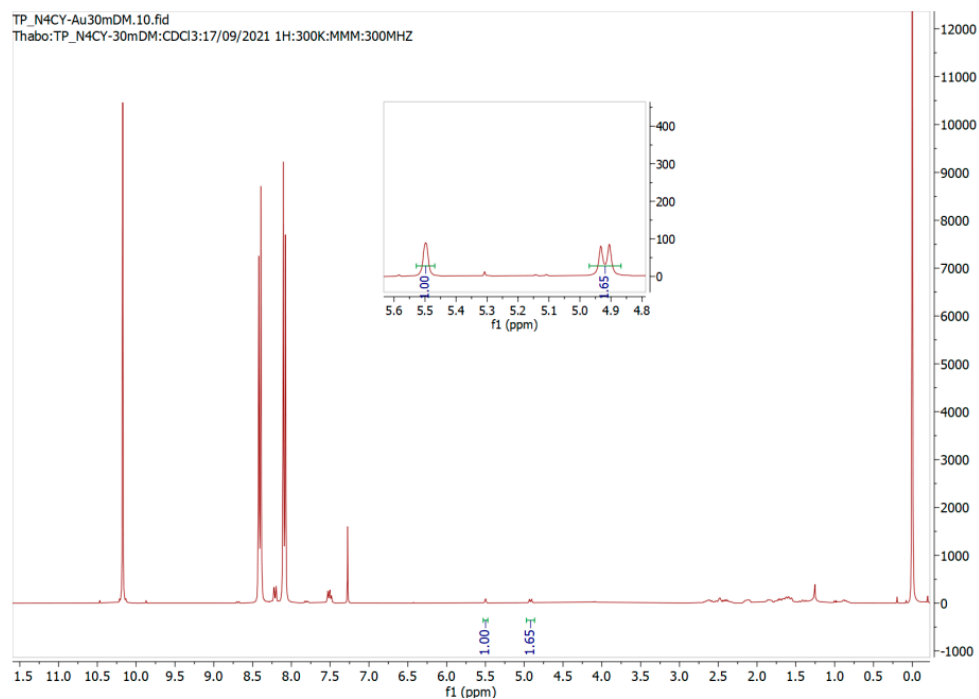
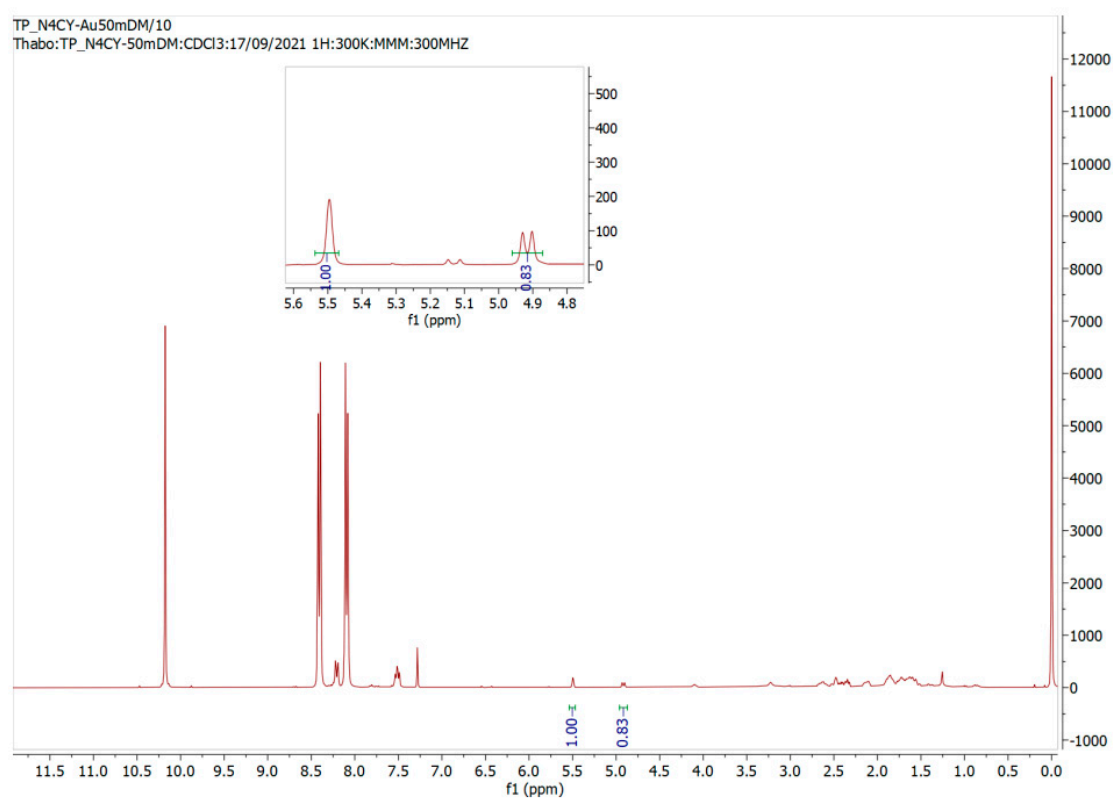
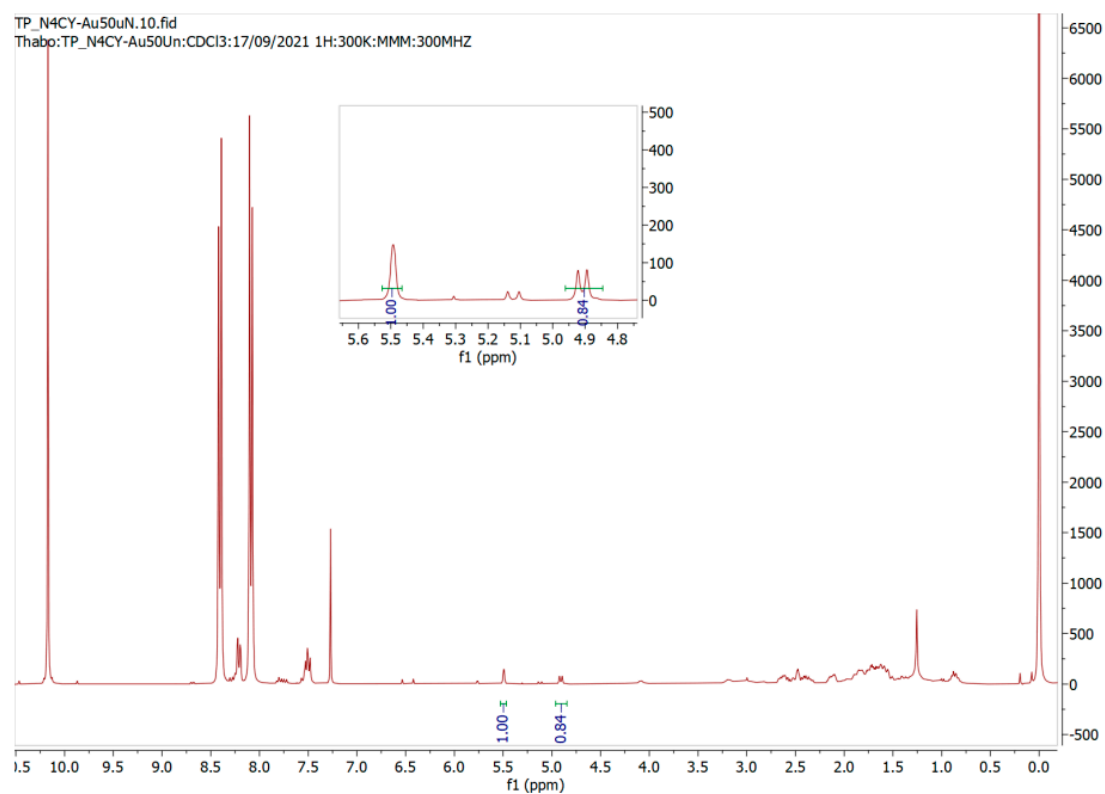


Figure S19: ^1H NMR spectrum of the crude product TP_N4CYR2

Figure S20: ¹H NMR spectrum of the crude product TP_N4CYR3Figure S21: ¹H NMR spectrum of the crude product TP_N4CYR4

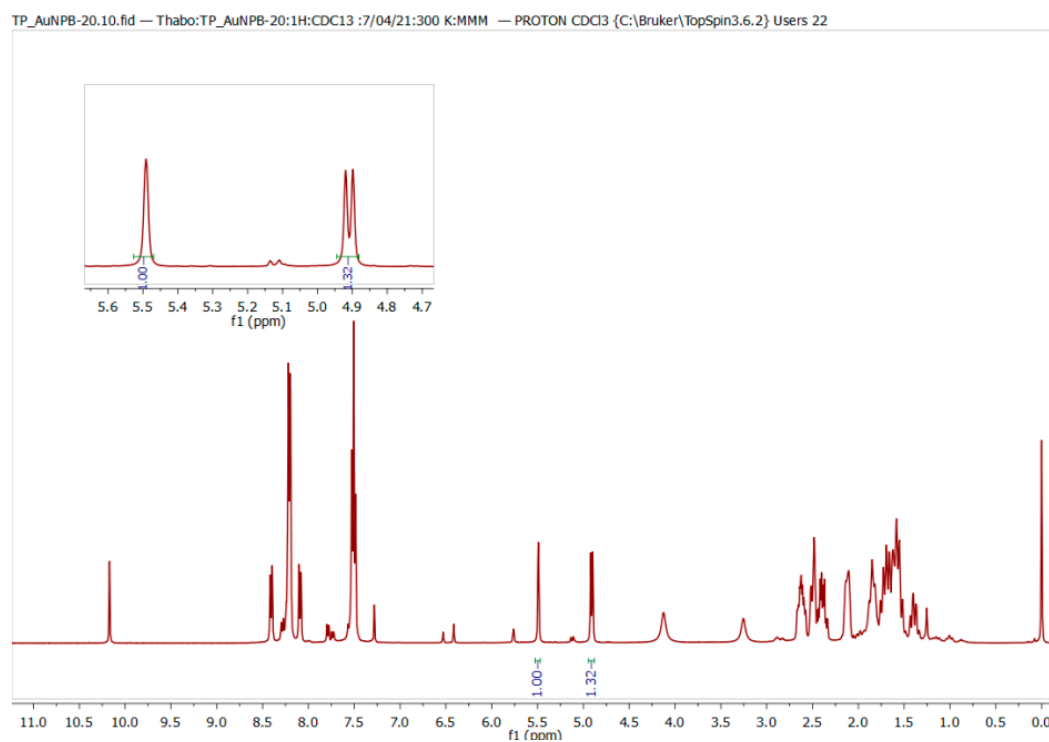


Figure S22: ¹H NMR spectrum of the crude product TP_N4CYR5

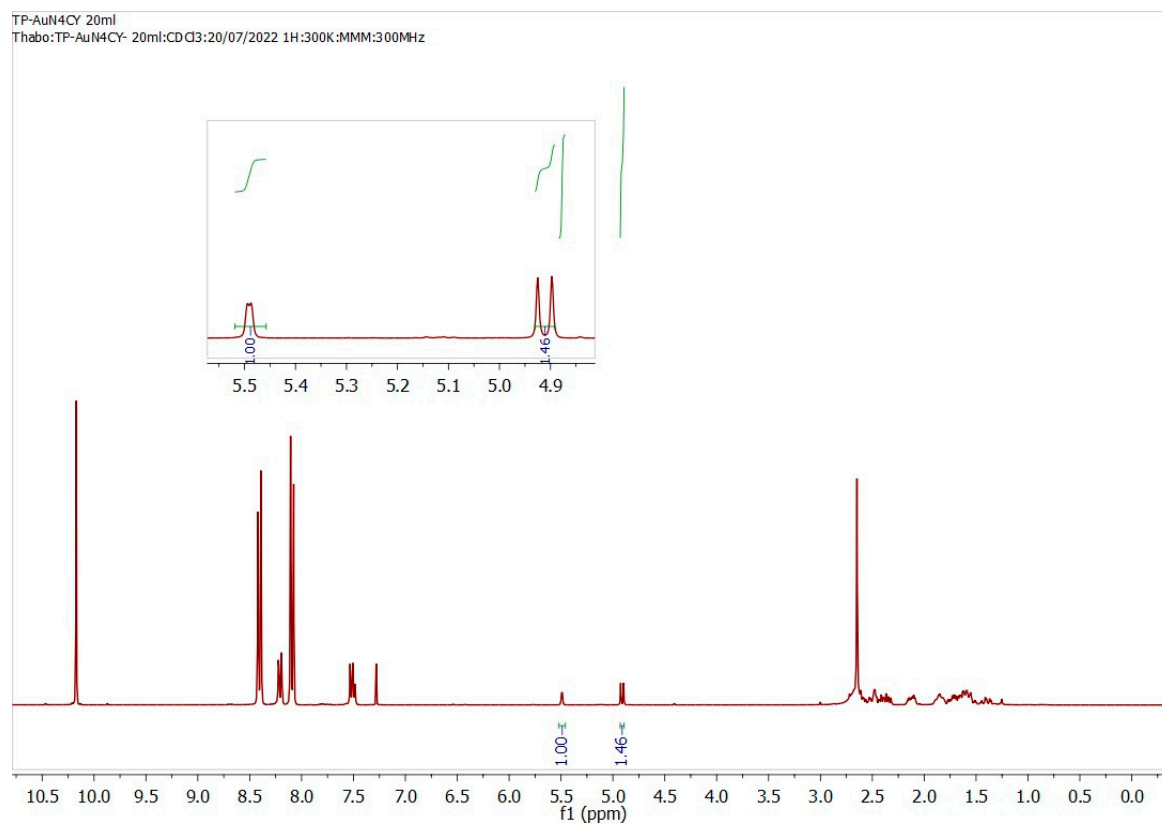


Figure S23: ¹H NMR spectrum of the crude product TP_N4CYR6

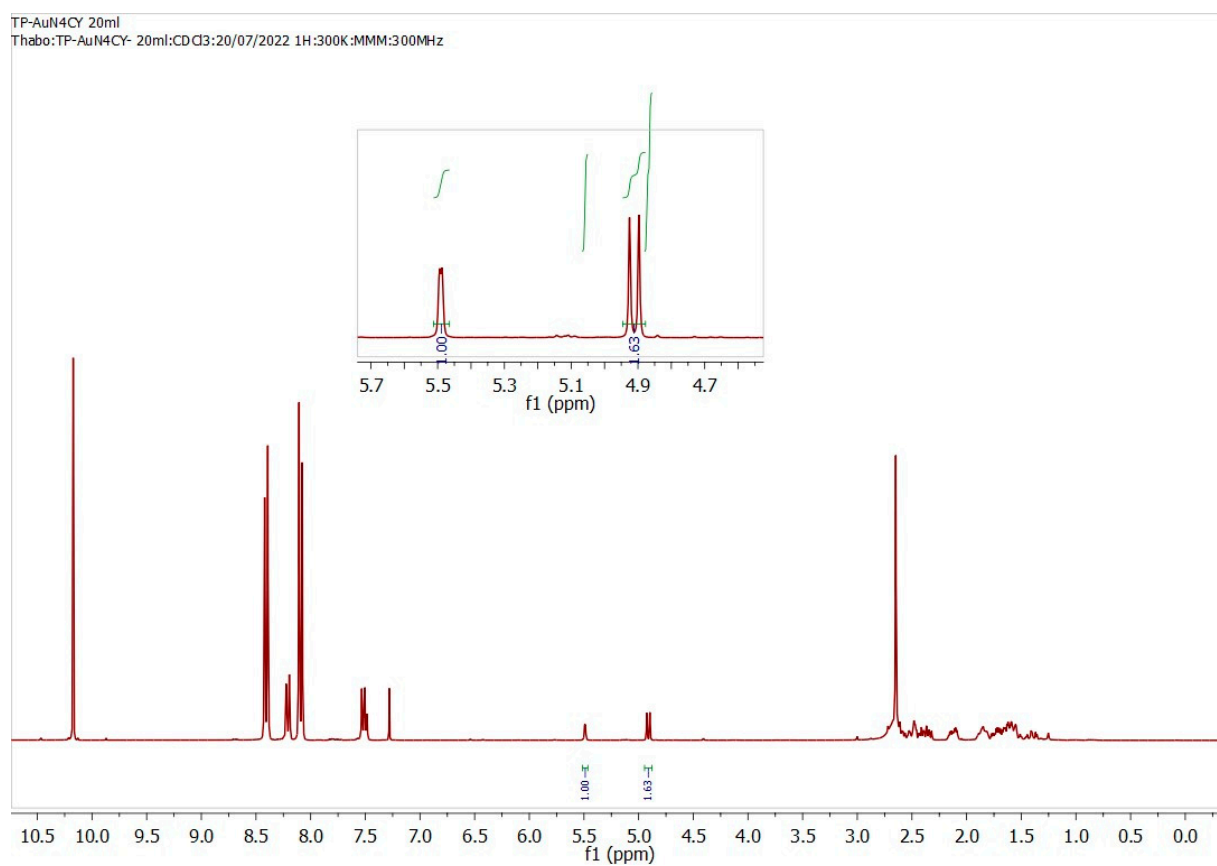


Figure S24: ^1H NMR spectrum of the crude product TP_N4CYR7

7. Selected TEM images, XPS and Raman Spectra

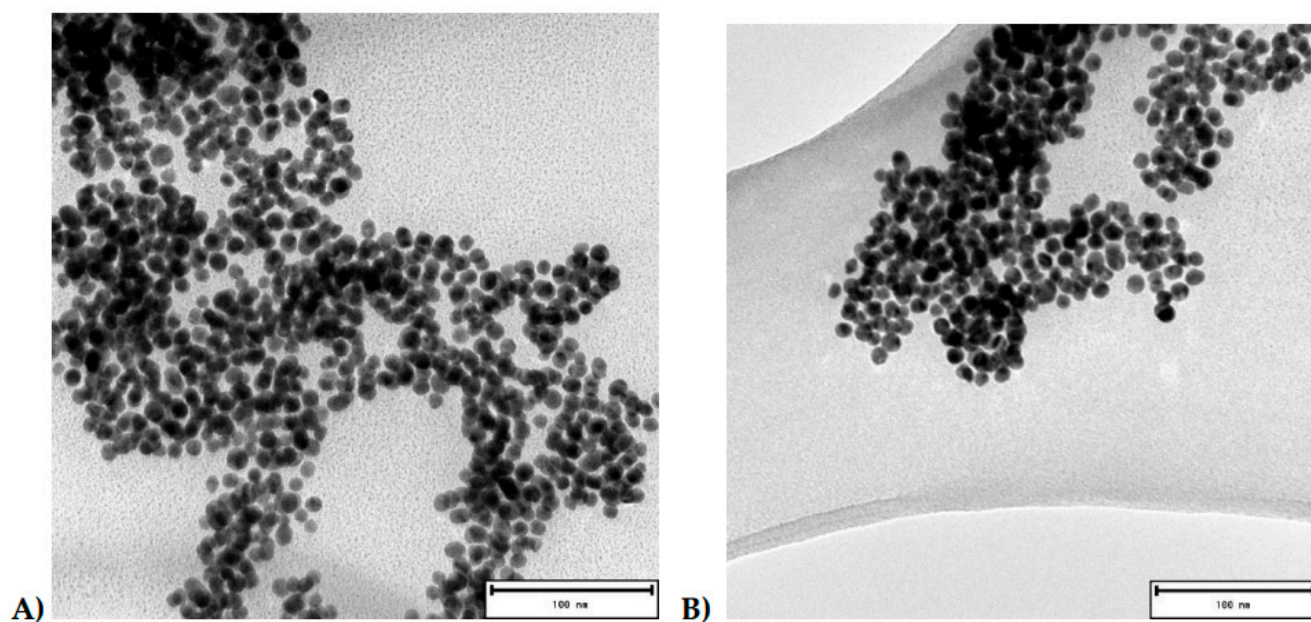


Figure S25: TEM image for the stability study using 12 nm particles size A) at day 1 and B) after 20 days

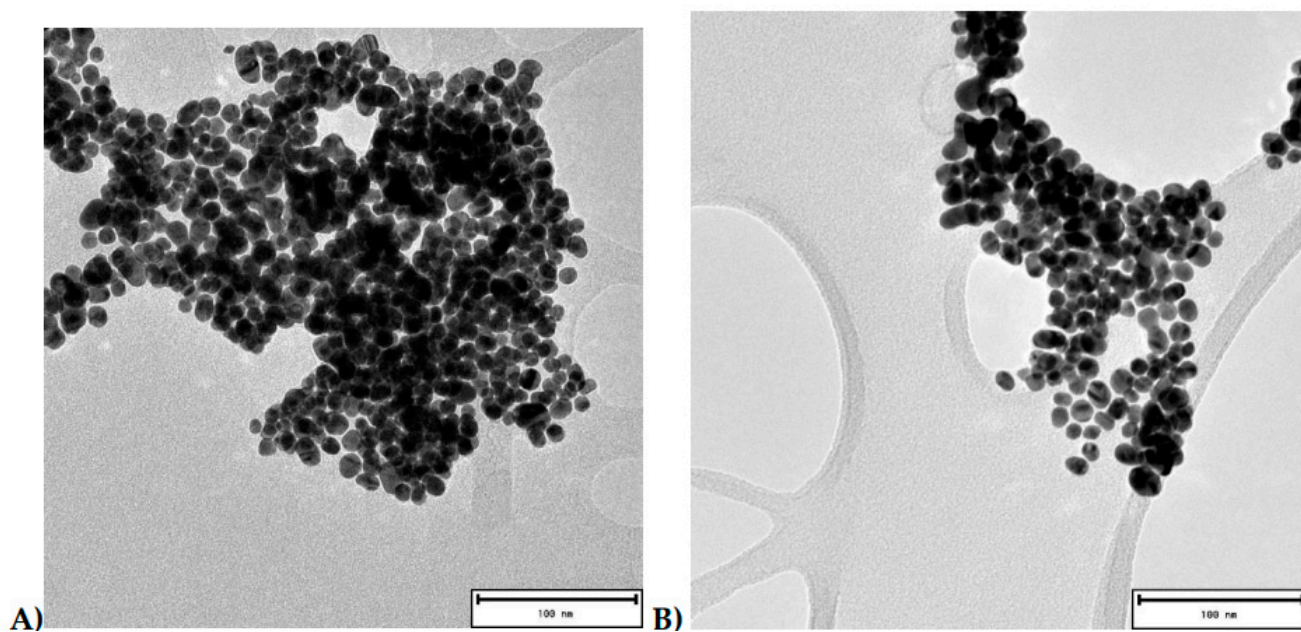
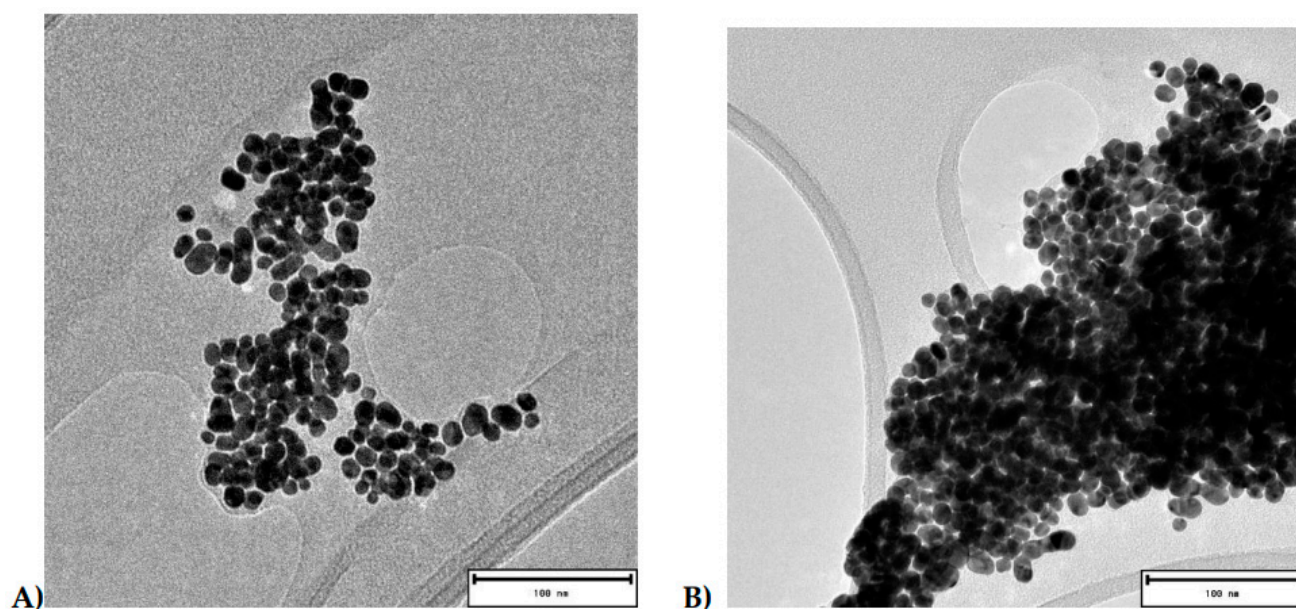


Figure S26: TEM image for the stability study using 15 nm particles size A) at day 1 and B) after 20 days



FigureS27: TEM image for the stability study using 16 nm particles size A) at day 1 and B) after 20 days

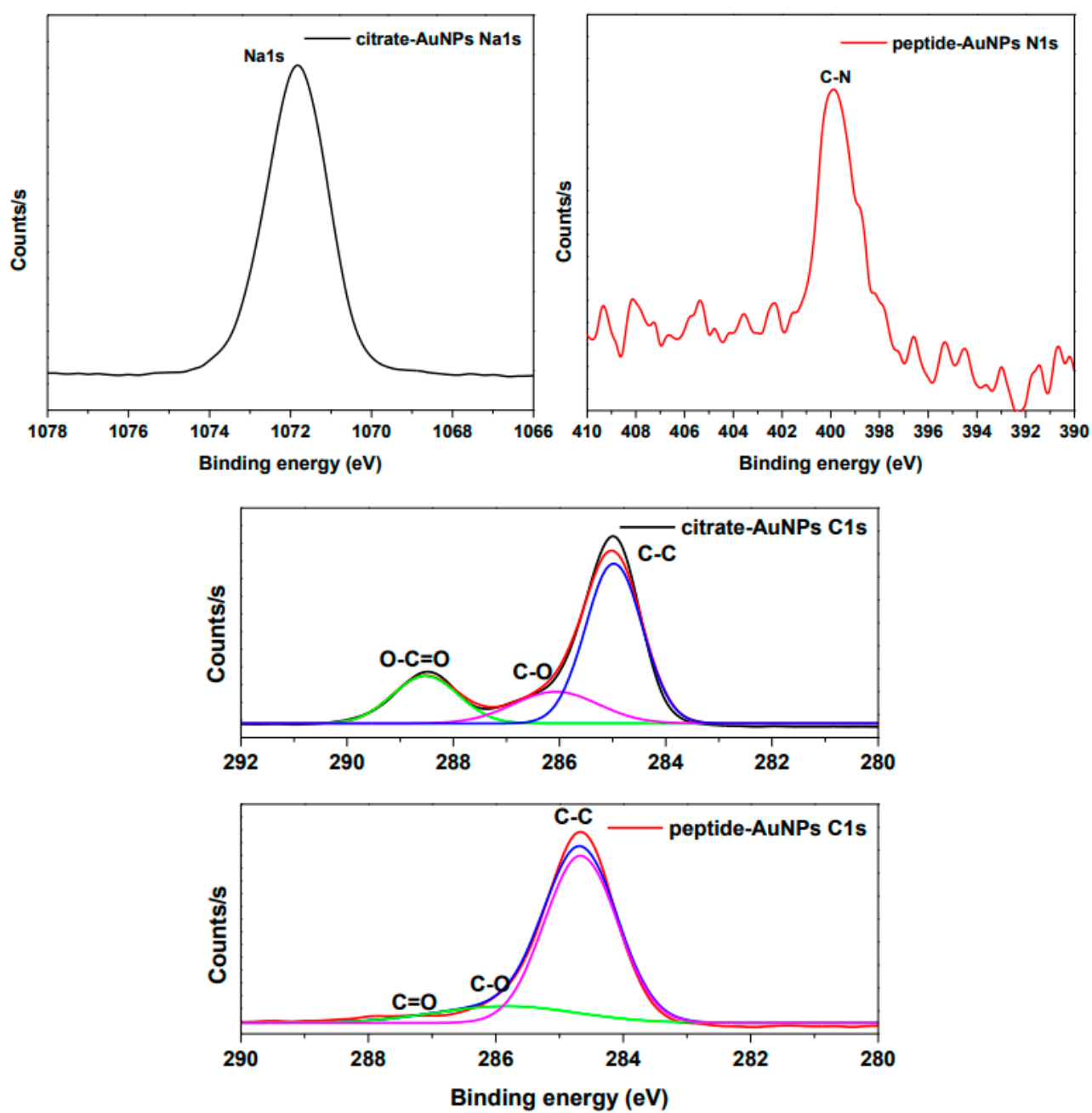


Figure S28: X-ray photoelectron high resolution spectra (Na1s, N1s and C1s) of citrate-capped AuNPs (black line) and peptide-AuNPs (red line)

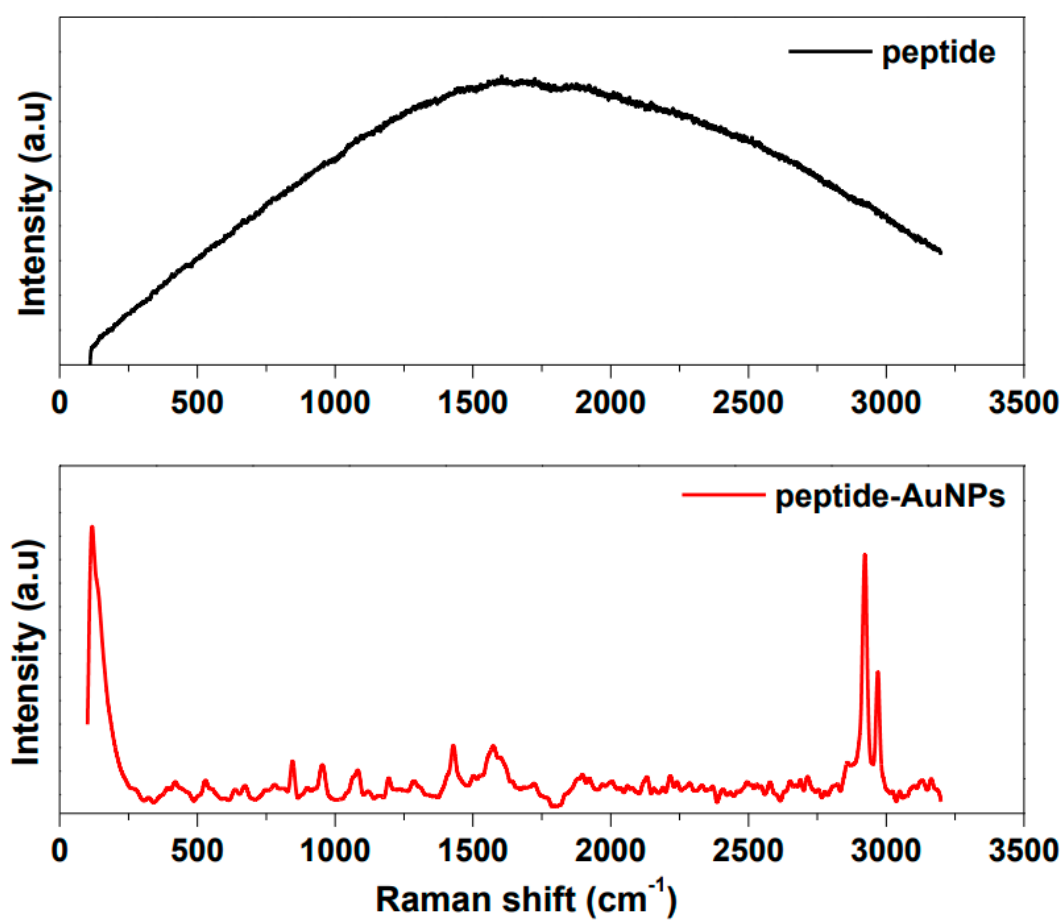


Figure S29: Raman spectra of the peptide (black line) and peptide-AuNPs (red line).

References

Turkevich, J.; Stevenson, P. C.; Hillier, J. A Study of the Nucleation and Growth Processes in the Synthesis of Colloidal Gold. *Discuss. Faraday Soc.* **1951**, *11*, 55–75. <https://doi.org/10.1039/DF9511100055>.