Electronic Supplementary Material (ESI) for MedChemComm.

Scaffold hopping via ANCHOR.QUERY: β-lactams as potent p53-MDM2 antagonists

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SUPPORTING INFORMATION

Contents

1. General methods

1.1 Virtual Screening. The receptor and small molecule **1** (derived from PDB ID 4MDN) were uploaded into ANCHOR.QUERY (http://anchorquery.csb.pitt.edu/). The pharmacophore character of the benzylic phenyl group was changed in ANCHOR.QUERY from hydrophobe to aromatic. Under the filters tab > hit reduction, the maximum hits per molecules were set to 1. The maximum total hits was set to 200 and ranking was done according to lowest molecular weight. The hit compounds are energy optimized, were further visually inspected and the results were evaluated with the small network analysis software SCORPION.

1.2. Synthesis and analysis. All syntheses were performed using general procedures described below in details. Reagents were obtained from commercial suppliers (Sigma Aldrich, ABCR, Acros and AK Scientific) and used without further purification unless otherwise noted. All microwave irradiation reactions were carried out in a Biotage Initiator™ Microwave Synthesizer. Nuclear magnetic resonance spectra were recorded on a Bruker Avance 500 spectrometer {^{1}H NMR (500 MHz), ^{13}C NMR (126 MHz)}. Chemical shifts for ^{1}H NMR were reported as δ values and coupling constants were in hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = double of doublets, ddd = double doublet of doublets, m = multiplet. Chemical shifts for ^{13}C NMR were reported in δ relative to the solvent peak. Thin layer chromatography was performed on Fluka precoated silica gel plates (0.20 mm thick, particle size 25 µm). Flash chromatography was performed on a Teledyne ISCO Combiflash Rf, using RediSep Rf Normal-phase Silica Flash Columns (Silica Gel 60 Å, 230-400 mesh) and on a Reveleris® X2 Flash Chromatography, using Grace® Reveleris Silica flash cartridges (12 grams). Elemental analysis was performed on a Vario Micro Cube apparatus. Electrospray ionization mass spectra (ESI-MS) were recorded on a Waters Investigator Semi-prep 15 SFC-MS instrument or on Shimadzu LCMS-2020 apparatus.

1.3. Protein expression and purification. *N*-terminal domain of human MDM2 (residues 1-118) was cloned into pET-20 vector (Novagen) and expressed in *Escherichia coli* BL21(DE3) as described previously.[1] In brief, cells were grown at 37 °C and induced with 1 mM IPTG at OD_{600} of 0.8 and grown for additional 5 h at 37 °C. Cells were collected by centrifugation and lysed by sonication. Inclusion bodies were collected by centrifugation, washed with PBS containing 0.05% Triton-X100 and subsequently solubilized in 6 M guanidine hydrochloride in 100 mM Tris-HCl, pH 8.0, containing 1 mM EDTA and 10 mM β-mercaptoethanol. The protein was dialyzed against 4 M guanidine hydrochloride, pH 3.5 supplemented with 10 mM β-mercaptoethanol. Following, the protein was refolded by dropwise addition into 10 mM Tris-HCl, pH 7.0, containing 1 mM EDTA and 10 mM β-mercaptoethanol and incubating overnight at 4 °C. Ammonium sulphate was added to the final concentration of 1.5 M and the refolded protein was recovered on Butyl Sepharose 4 Fast Flow (GE Healthcare). The protein was eluted using 100 mM Tris-HCl, pH 7.2, containing 5 mM β-mercaptoethanol and further purified by gel filtration on HiLoad 16/60 Superdex75 (GE Healthcare) in 50 mM phosphate buffer pH 7.4 containing 150 mM NaCl and 5 mM DTT.

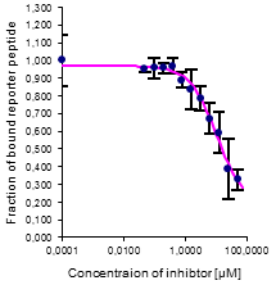
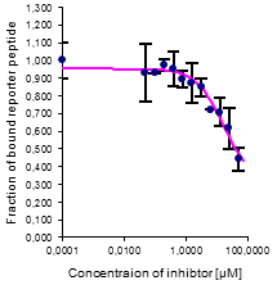
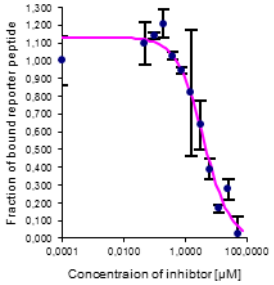
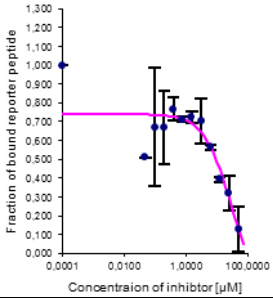
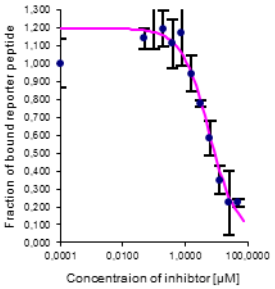
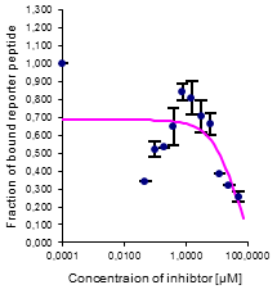
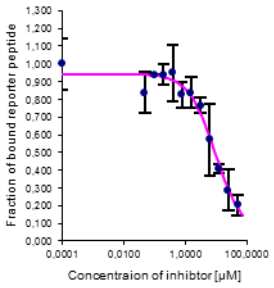
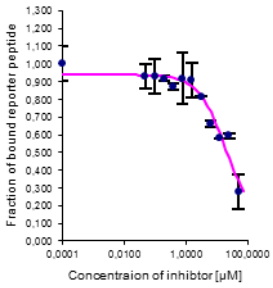
N-terminal domain of human MDMX (residues 1-134) was cloned into pET-46Ek/LIC vector (Novagen). Cells were grown at 37 °C and induced with 0.5 mM IPTG at OD600 nm of 0.6. The recombinant protein expression was carried for 12 h at 20 °C. The protein was purified under native conditions using Ni-NTA Agarose (GE Healthcare). Preparation was polished by gel filtration on HiLoad 16/60 Superdex75 (GE Healthcare).

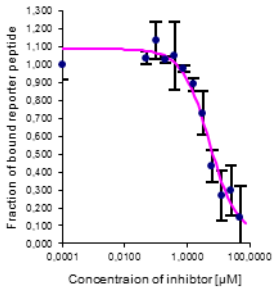
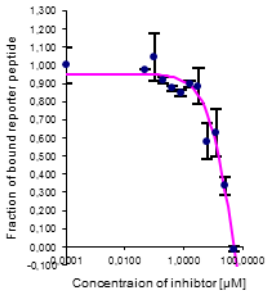
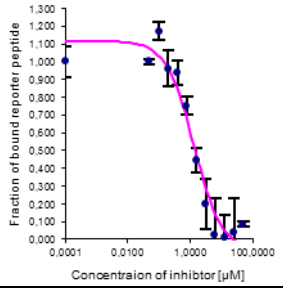
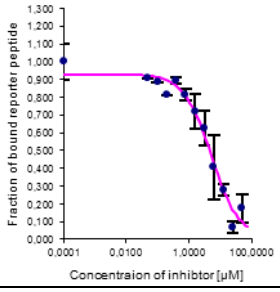
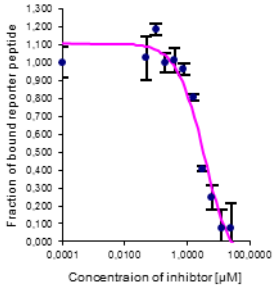
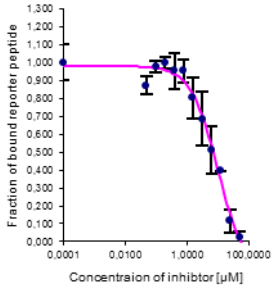
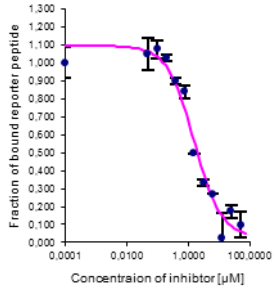
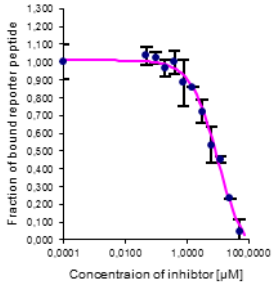
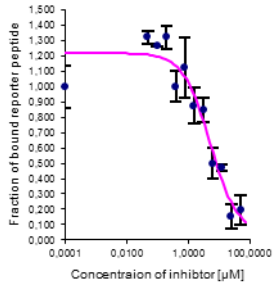
1.4. Fluorescence polarization binding assay. Fluorescence polarization experiments were performed as previously reported by Czarna et al.[1] using Tecan InfinitePro F200 plate reader with the 485 nm excitation and 535 nm emission filters. The fluorescence intensities, parallel and perpendicular to the plane of excitation, were measured in Corning black 96-well NBS assay plates at room temperature. Fluorescence polarization values were expressed in millipolarization units (mP). All the experiments were performed in duplicates and plates were read 15 min after mixing of all assay components. For each assay, new protein stocks were thawed and the protein concentrations were determined using Bradford method. Assay buffer contained 50 mM NaCl, 10 mM Tris pH 8.0, 1 mM EDTA and 5% DMSO. Competition binding assays were performed using 10 nM fluorescent P2 peptide (5'-FAM-LTFEHYWAQLTS) and protein concentration equivalent to f_0 = 0.8. Tested compounds, dissolved in DMSO were evaluated at serial dilutions. Nutlin 3 (Cayman Chemicals) and peptide Z (SQETFSDLWKLLPEN) served as positive controls for MDM2 and MDMX, respectively. Inhibition curves were fitted using Excel program to obtain IC_{50} and K_i values. All calculations were done according to Huang et al.[2]

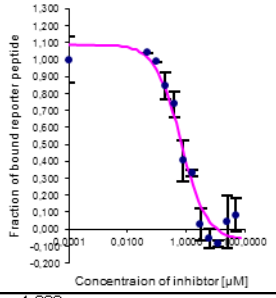
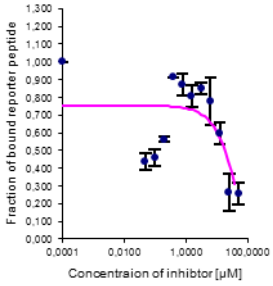
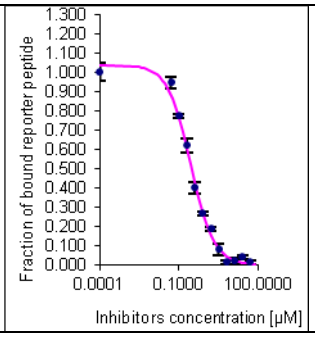
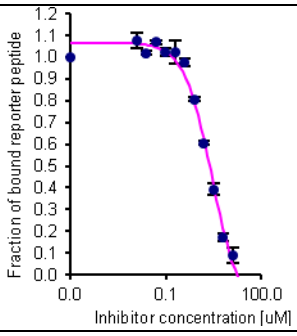
1.5. ^{1}H-^{15}N HSQC binding assay. Uniform ^{15}N isotope labeling was achieved by expression of the protein in the M9 minimal media containing $^{15}NH_4Cl$ as the sole nitrogen source. 10% (v/v) of D2O was added to the samples to provide lock signal. All the spectra were recorded at 300K using a Bruker Avance 600 MHz spectrometer. ^{1}H-^{15}N heteronuclear correlations were obtained using the SOFAST-HMQC pulse sequence. Assignment of the amide groups of MDM2 was obtained as previously reported.[3]

2. Fluorescence polarization binding assays

Table 1. Results of the evaluation of inhibitory activity of compounds towards MDM2/X

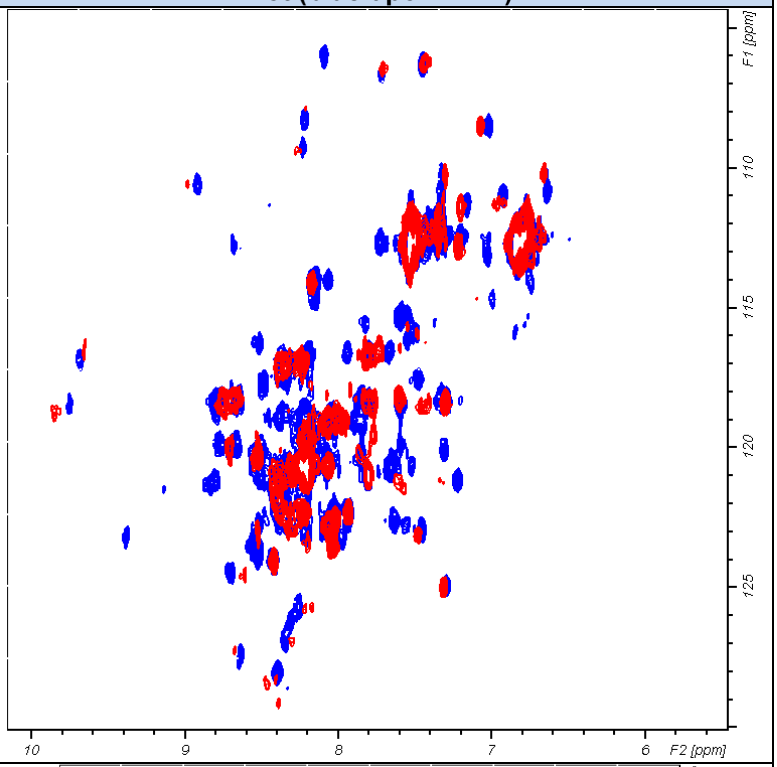
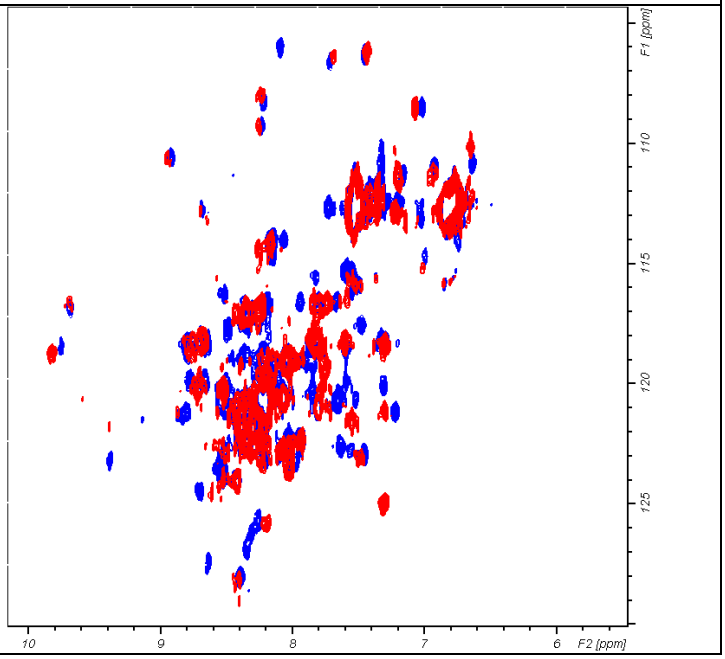
Entry	Compound	K_i MDM2 [μM]	K_i MDMX [μM]	Plot MDM2	Plot MDMX
1	**2a**, diastereoemer A	3,2	12,8		
2	**2a**, diastereoemer B	1,2	2,1		
3	**2b**, diastereoemer A	2,1	2,6		
4	**2b**, diastereoemer B	1,9	6,7		

5	**2c**, diastereoemer A	1,9	4,7		
6	**2c**, diastereoemer B	0,4	1,4		
7	**2d**, diastereoemer A	0,9	2,2		
8	**2d**, diastereoemer B	0,6	2,6		
9	**2e**, diastereomer A	1,99	not active (Ki > 66 µM)		-

10	**2e**, diastereomer B	0,17	4,18		
11	Nutlin-3a (positive control for MDM2)	0,04	-		-
12	Peptide Z (positive control for MDMX)	-	0,49	-	

3. Titration of ^{15}N-labeled MDM2 with inhibitors

Table 2. Results of the evaluation of inhibitory activity of compounds towards MDM2/MDMX (MDM2 conc. = 0.24 mM, inhibitor conc. = 50 mM, ratio cmp/ MDM2 1:1)

Compound	Binding	Plot (blue-apo/MDM2)
2c, diastereoemer B	Yes (tight K_D < 1 μM)	
2d, diastereoemer B	Yes (tight K_D < 1μM)	

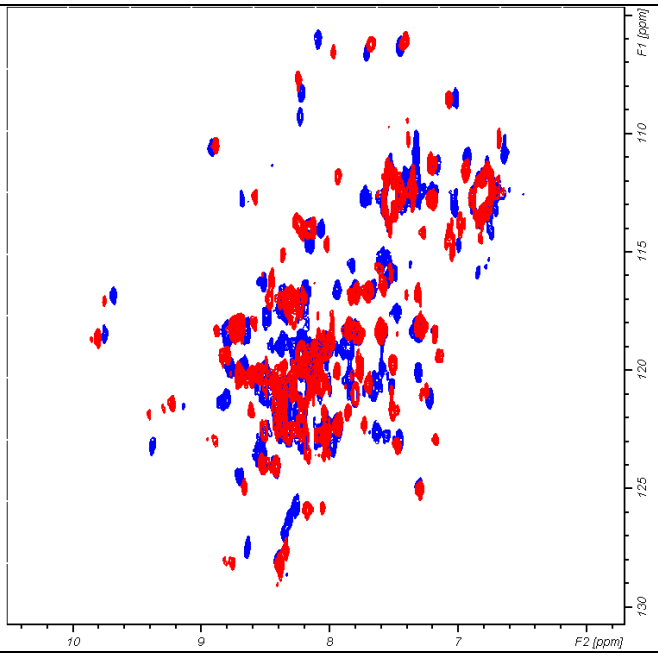
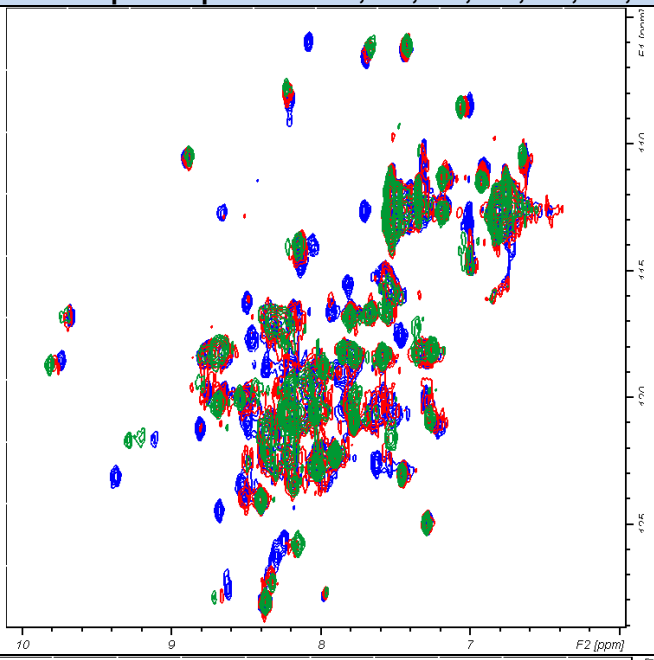
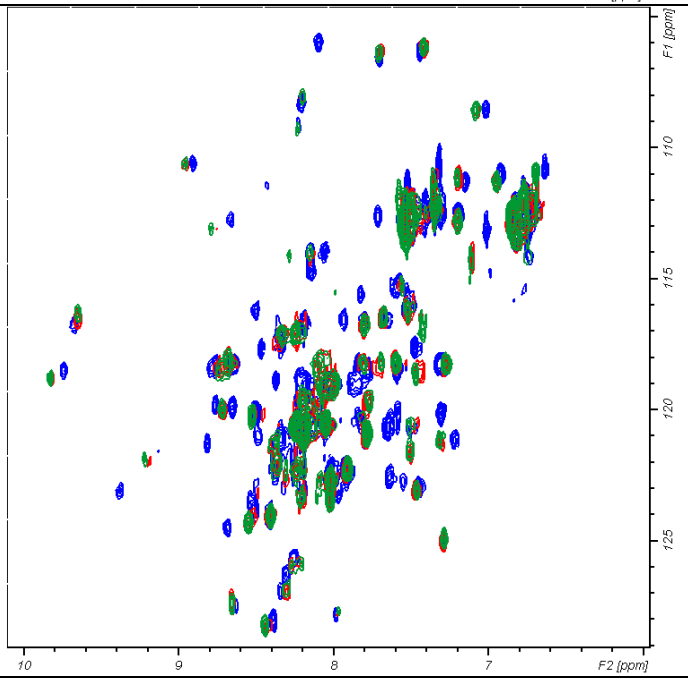
2e, diastereomer B	Yes (tight K_D < 1μM)	

Table 2. Results of the evaluation of inhibitory activity of compounds towards MDM2/MDMX (MDM2 conc. = 0.33 mM, inhibitor conc. = 50 mM)

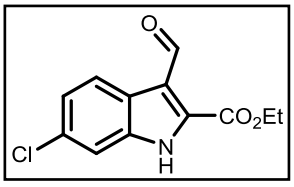
Compound	Binding	Plot (blue-MDM2, red-Cmp/MDM2 1:2, green-Cmp/MDM2 2:1) [Titration steps: Cmp/MDM2: 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 5:1]
2e, diastereomer B	Yes	
2a	Yes	

4. Synthetic procedures and analytical data

4.1 Synthetic procedure and analytical data of compound 4

6-Chloro-1*H*-indole-2-carboxylic acid ethyl ester (5.0 g, 22.3 mmol) and DMF (20 mL) were placed in round-bottom flask equipped with $CaCl_2$ tube. Then, $POCl_3$ (2.49 mL, 26.8 mmol) was added dropwise and reaction mixture was heated overnight (16 h) at 50 ºC. Afterwards, the reaction was cooled to rt, quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate. Organic layer was collected, washed with water and brine, dried over anhydrous $MgSO_4$ and evaporated. The crude product was washed with diethyl ether giving compound **4** as a light yellow solid in 92% (5.15 g) yield.

6-Chloro-3-formyl-1*H*-indole-2-carboxylic acid ethyl ester 4

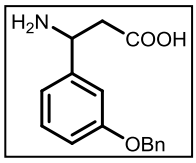


Light yellow solid, 92% yield (5.15 g), mp: 243-244 ºC; NMR: ^{1}H (600 MHz, DMSO-d_6): δ 12.90 (s, 1H), 10.58 (s, 1H), 8.22 (d, J = 8.6 Hz, 1H), 7.56 (d, J = 1.5 Hz, 1H), 7.32 (dd, J = 8.6, 1.9 Hz, 1H), 4.46 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H); ^{13}C (151 MHz, DMSO-d_6): δ 187.5, 159.9, 136.1, 133.5, 130.4, 124.0, 123.9, 123.4, 118.2, 112.6, 62.0, 14.1; IR (ATR) [cm^{-1}]: 3154, 2992, 2908, 1726, 1639, 1571, 1532, 1433, 1300, 1221, 1195, 1100, 1163, 1030, 916, 854, 779, 698; Elemental analysis: Calcd for $C_{12}H_{10}ClNO_3$: C, 57.27; H, 4.01; N, 5.57, found: C, 57.51; H, 4.26; N, 5.50; LC-MS (DAD/ESI): t_R = 2.97 min, Calcd for $C_{12}H_{10}ClNO_3$ (m/z): [M-H]$^-$ 250.03, [M+2-H]$^-$ 250.02, found: [M-H]$^-$ 250.05, [M+2-H]$^-$ 252.05.

4.2 General synthetic procedure and analytical data of compounds 10a-c

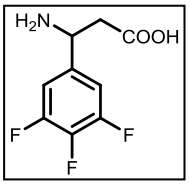
A mixture of malonic acid (1.0 equiv.), substituted benzaldehydes **7a-c** (1.0 equiv.) and ammonium acetate (2.0 equiv.) was refluxed in ethanol overnight. The precipitated amino acid was isolated by filtration, without any further purification.[4]

3-Amino-3-(3-(benzyloxy)phenyl)propanoic acid (10b)



White solid, 50% yield; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.45 – 7.27 (m, 6H), 7.12 (s, 1H), 7.02 – 6.90 (m, 2H), 5.09 (s, 2H), 4.23 (dd, J = 8.6, 6.0 Hz, 1H), 2.41 – 2.27 (m, 2H).

3-Amino-3-(3,4,5-trifluorophenyl)propanoic acid (10c)

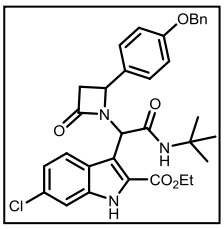


White solid, 50% yield; ^{1}H NMR (500 MHz, D_2O+K_2CO_3) δ: 7.03-6.99 (m, 2H), 4.13-4.09 (m, 1H), 2.67 – 2.21 (m, 2H).

4.3 General synthetic procedure and analytical data of compounds 3

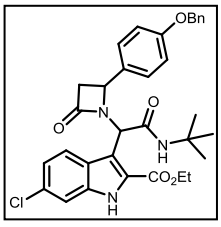
The substituted β-aminoacids (1.0 mmol), the 6-chloro-1*H*-indole-2-carboxylic acid ethyl ester (1.0 mmol) and the corresponding isocyanides (1.0 mmol) were placed in CF_3CH_2OH (1 mL) and the mixture was irradiated in a microwave oven at 130 °C for 90 min. The solvent was removed under reduced pressure followed by purification by flash chromatography on silica gel to afford the diastereomeric products. The diastereomeric products were separated by column chromatography on neutral alumina, using EtOAc/petroleum ether/NEt_3 (1:10:0.1 for the derivatives **3a**, **3b**, 3.5:10:0.1 for the derivative **3c**, 4:10:0.1 for the derivative **3d**) as eluent.

Ethyl 3-(1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(tert-butylamino)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylate (**3a**, *diastereomer A*)



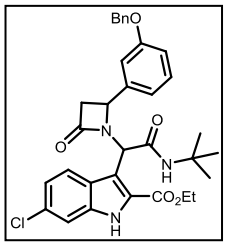
White solid, yield 54% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.83 (s, 1H), 7.50 – 7.35 (m, 6H), 7.13 – 7.11 (m, 2H), 6.90-6.87 (m, 3H), 6.85 (d, *J* = 1.8 Hz, 1H), 6.29 (s, 1H), 6.12 (s, 1H), 5.08 (s, 2H), 4.83 (dd, *J* = 5.7, 2.5 Hz, 1H), 4.35 – 4.10 (m, 2H), 3.43 (dd, *J* = 15.1, 5.7 Hz, 1H), 2.90 (dd, *J* = 15.1, 2.5 Hz, 1H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.11 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 168.8, 166.7, 161.4, 158.8, 136.8, 136.4, 131.3, 130.6, 128.7, 128.3, 128.1, 127.4, 124.8, 124.2, 122.8, 121.3, 116.3, 115.1, 111.8, 70.0, 61.3, 54.9, 54.1, 51.4, 45.6, 28.4, 14.1. MS (ESI) m/z calculated $[M-H]^+$: 586.22; found $[M-H]^+$: 586.35.

Ethyl 3-(1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(tert-butylamino)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylate (**3a**, *diastereomer B*)



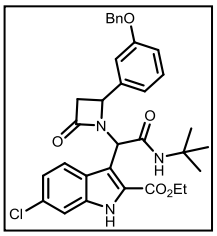
White solid, yield 54% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.79 (s, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.50 – 7.30 (m, 5H), 7.13 – 6.97 (m, 2H), 6.71 – 6.61 (m, 2H), 6.57 – 6.46 (m, 2H), 6.25 (s, 1H), 6.00 (s, 1H), 4.96 (d, J_{AB} = 10 Hz, 1H), 4.93 (d, J_{AB} = 10 Hz, 1H), 4.59 (dd, *J* = 5.3, 2.4 Hz, 1H), 4.42 – 4.23 (m, 2H), 3.38 (dd, *J* = 14.8, 5.3 Hz, 1H), 2.74 (dd, *J* = 14.8, 2.4 Hz, 1H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.32 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.9, 167.4, 160.8, 158.0, 137.0, 135.8, 131.4, 130.5, 128.6, 128.0, 127.4, 126.9, 126.1, 124.8, 122.6, 121.8, 114.2, 113.9, 111.6, 69.7, 61.5, 54.5, 52.4, 51.9, 46.3, 28.6, 14.2. MS (ESI) m/z calculated $[M-H]^+$: 586.22; found $[M-H]^+$: 586.25.

Ethyl 3-(1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(tert-butylamino)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylate (**3b**, *diastereomer A*)



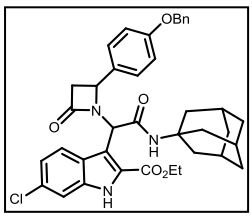
White solid, yield 69% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.03 (br s, 1H), 7.49 – 7.36 (m, 5H), 7.36 – 7.30 (m, 1H), 7.30 – 7.19 (m, 1H), 6.96 – 6.84 (m, 3H), 6.84 – 6.73 (m, 2H), 6.39 (s, 1H), 6.19 (s, 1H), 4.92 (s, 2H), 4.83 (dd, *J* = 5.6, 2.5 Hz, 1H), 4.34 – 4.10 (m, 2H), 3.44 (dd, *J* = 15.1, 5.6 Hz, 1H), 2.92 (dd, *J* = 15.1, 2.5 Hz, 1H), 1.34 (t, *J* = 7.1 Hz, 3H), 1.14 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 168.8, 168.7, 166.8, 161.4, 159.0, 140.2, 136.6, 136.4, 131.2, 129.8, 128.6, 128.0, 127.5, 127.5, 124.9, 124.0, 122.6, 121.2, 119.2, 116.1, 114.7, 113.6, 111.9, 69.9, 61.3, 55.3, 54.2, 51.4, 45.5, 28.3, 14.1. MS (ESI) m/z calculated $[M+H]^+$: 588.22; found $[M+H]^+$: 588.23.

Ethyl 3-(1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(tert-butylamino)-2-oxoethyl)-6-chloro-1H-indole-2-carboxylate (**3b**, *diastereomer B*)



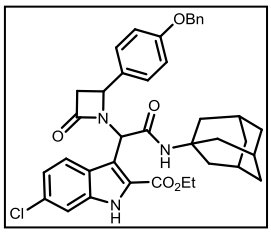
White solid, yield 69% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.82 (br s, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.44 – 7.36 (m, 4H), 7.36 – 7.30 (m, 1H), 7.19 – 7.16 (m, 1H), 7.14 – 7.07 (m, 1H), 6.87 (t, *J* = 7.9 Hz, 1H), 6.64 – 6.58 (m, 1H), 6.42 (dt, *J* = 7.6, 1.2 Hz, 1H), 6.36 (t, *J* = 2.0 Hz, 1H), 6.30 (s, 1H), 5.95 – 5.84 (m, 1H), 4.76 (d, J_{AB} = 10 Hz, 1H), 4.72 (d, J_{AB} = 10 Hz, 1H), 4.65 (dd, *J* = 5.4, 2.3 Hz, 1H), 4.44 – 4.27 (m, 2H), 3.42 (dd, *J* = 14.8, 5.4 Hz, 1H), 2.75 (dd, *J* = 14.8, 2.3 Hz, 1H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.32 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.9, 167.3, 160.8, 158.4, 140.3, 136.8, 135.7, 131.8, 128.9, 128.6, 128.0, 127.5, 126.2, 125.0, 122.8, 122.2, 118.2, 114.4, 114.0, 111.7, 111.5, 69.7, 61.8, 55.0, 52.3, 52.0, 46.6, 28.7, 14.3. MS (ESI) m/z calculated $[M-H]^+$: 586.22; found $[M-H]^+$: 586.28.

Ethyl 3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylate (**3c,** *diastereomer A*)



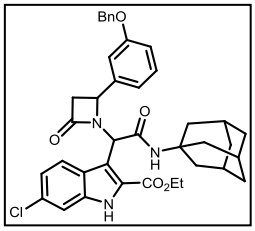
White solid, yield 48% (mix diastereomers);^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.87 (br s, 1H), 7.72 (d, *J* = 8.6 Hz, 1H), 7.49 – 7.32 (m, 5H), 7.13 – 7.00 (m, 2H), 6.70 – 6.59 (m, 2H), 6.50 (d, *J* = 8.3 Hz, 2H), 6.25 (s, 1H), 5.70 (s, 1H), 4.94 (s, 2H), 4.61 (dd, *J* = 5.1, 2.1 Hz, 1H), 4.45 – 4.27 (m, 2H), 3.43 – 3.33 (m, 1H), 2.80 – 2.66 (m, 1H), 2.06 – 2.00 (m, 3H), 1.96 (s, 6H), 1.64 (s, 6H), 1.42 – 1.34 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.8, 167.1, 160.9, 158.0, 137.0, 135.8, 131.4, 130.6, 128.6, 128.0, 127.4, 126.8, 126.1, 124.8, 122.8, 121.8, 114.1, 113.9, 111.6, 69.7, 61.5, 54.4, 52.6, 52.2, 46.4, 41.3, 36.2, 29.3, 14.3.

Ethyl 3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylate (**3c**, *diastereomer B*)



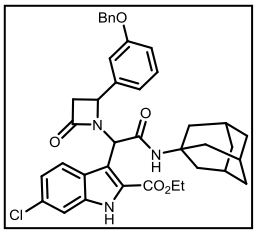
White solid, yield 48% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.08 (s, 1H), 7.51 – 7.32 (m, 6H), 7.17 (s, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.92 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.83 (d, *J* = 8.3 Hz, 2H), 6.14 (s, 1H), 6.10 (s, 1H), 5.06 (s, 2H), 4.57 (dd, *J* = 5.5, 2.5 Hz, 1H), 4.41 – 4.25 (m, 2H), 3.36 (dd, *J* = 15.1, 5.5 Hz, 1H), 2.87 (dd, *J* = 15.1, 2.5 Hz, 1H), 1.84 – 1.79 (m, 3H), 1.61 (s, 6H), 1.56 (s, 6H), 1.38 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 168.8, 166.5, 161.4, 158.9, 136.8, 136.4, 131.1, 130.5, 128.6, 128.3, 128.0, 127.4, 124.8, 124.1, 122.8, 121.2, 116.1, 115.0, 111.8, 70.1, 61.3, 54.8, 54.1, 52.0, 45.4, 41.1, 36.2, 29.2, 14.1. MS (ESI) m/z calculated $[M-H]^+$: 664.27; found $[M-H]^+$: 664.34.

Ethyl 3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylate (**3d**, *diastereomer A*)



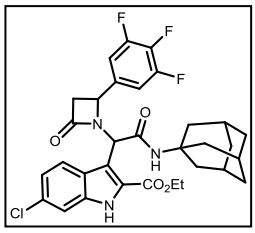
White solid, yield 64% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.07 (s, 1H), 7.77 (d, *J* = 8.7 Hz, 1H), 7.43 – 7.35 (m, 4H), 7.35 – 7.29 (m, 1H), 7.17 (s, 1H), 7.10 (dd, *J* = 8.7, 1.8 Hz, 1H), 6.86 (t, *J* = 7.9 Hz, 1H), 6.59 (dd, *J* = 8.2, 2.5 Hz, 1H), 6.43 (d, *J* = 7.6 Hz, 1H), 6.37 (s, 1H), 6.29 (s, 1H), 5.72 (s, 1H), 4.75 (q, *J* = 15 Hz, 2H), 4.66 (dd, *J* = 5.4, 2.4 Hz, 1H), 4.42 – 4.25 (m, 2H), 3.41 (dd, *J* = 14.8, 5.4 Hz, 1H), 2.74 (dd, *J* = 14.8, 2.4 Hz, 1H), 2.11 – 2.00 (m, 3H), 1.97 (s, 6H), 1.64 (s, 6H), 1.34 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.8, 167.0, 160.9, 158.4, 140.3, 136.7, 135.8, 131.6, 128.8, 128.5, 128.0, 127.5, 126.2, 125.0, 122.8, 122.0, 118.2, 114.3, 113.9, 111.6, 111.6, 69.7, 61.7, 54.9, 52.7, 52.2, 46.6, 41.4, 36.2, 29.4, 14.2. MS (ESI) m/z calculated $[M-H]^+$: 664.27; found $[M-H]^+$: 664.28.

Ethyl 3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylate (**3d**, *diastereomer B*)



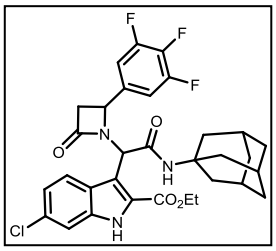
White solid, yield 64% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.94 (s, 1H), 7.48 – 7.39 (m, 5H), 7.39 – 7.33 (m, 1H), 7.31 – 7.24 (m, 1H), 6.99 – 6.90 (m, 2H), 6.90 – 6.83 (m, 2H), 6.80 (s, 1H), 6.22 (s, 1H), 6.19 (s, 1H), 4.95 (s, 2H), 4.85 – 4.78 (m, 1H), 4.39 – 4.18 (m, 2H), 3.46 (dd, *J* = 15.1, 5.6 Hz, 1H), 2.95 (dd, *J* = 15.1, 2.5 Hz, 1H), 1.99 (br s, 3H), 1.85 – 1.73 (m, 6H), 1.60 (s, 6H), 1.38 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 168.7, 166.5, 161.4, 159.1, 140.2, 136.7, 136.4, 131.3, 129.8, 128.6, 128.0, 127.5, 125.0, 124.1, 122.8, 121.3, 119.3, 116.1, 114.7, 113.7, 111.8, 69.9, 61.4, 55.2, 54.3, 52.2, 45.5, 41.1, 36.2, 29.3, 14.1. MS (ESI) m/z calculated $[M-H]^+$: 664.27; found $[M-H]^+$: 664.28.

Ethyl 3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-2-oxo-1-(2-oxo-4-(3,4,5-trifluorophenyl) azetidin-1-yl)ethyl)-6-chloro-1*H*-indole-2-carboxylate (**3e**, *diastereomer A*)



White solid, yield 41% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ:9.26 (s, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.16 (d, *J* = 1.8 Hz, 1H), 7.05 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.91 – 6.86 (m, 2H), 6.06 (s, 1H), 5.85 (s, 1H), 4.82 (dd, *J* = 5.5, 2.4 Hz, 1H), 4.42 – 4.25 (m, 2H), 3.44 (dd, *J* = 15.1, 5.5 Hz, 1H), 2.79 (dd, *J* = 15.1, 2.4 Hz, 1H), 2.04 – 1.96 (m, 3H), 1.85 – 1.75 (m, 3H), 1.63 – 1.55 (m, 9H), 1.39 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.4, 165.9, 161.3, 136.2, 131.9, 124.8, 124.1, 122.9, 121.9, 116.2, 111.9, 111.2, 111.1, 111.0, 61.6, 54.7, 54.0, 52.4, 45.8, 41.2, 36.2, 29.3, 14.2. MS (ESI) m/z calculated $[M-H]^+$: 612.20; found $[M-H]^+$: 612.18.

Ethyl 3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-2-oxo-1-(2-oxo-4-(3,4,5-trifluorophenyl) azetidin-1-yl)ethyl)-6-chloro-1H-indole-2-carboxylate (**3e**, *diastereomer B*)

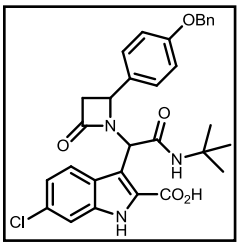


White solid, yield 41% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.33 (s, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 1.8 Hz, 1H), 7.12 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.39 (s, 1H), 6.38 – 6.33 (m, 2H), 5.36 (s, 1H), 4.74 (dd, *J* = 5.4, 2.4 Hz, 1H), 4.58 – 4.35 (m, 2H), 3.49 – 3.45 (m, 1H), 2.67 (dd, *J* = 14.8, 2.4 Hz, 1H), 2.07 – 2.00 (m, 3H), 1.94 (s, 6H), 1.63 (s, 6H), 1.46 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.0, 166.9, 160.7, 135.7, 132.1, 126.1, 124.7, 122.4, 122.4, 113.4, 111.7, 109.7, 109.6, 109.5, 109.5, 62.0, 53.8, 52.8, 51.6, 47.0, 41.4, 36.2, 29.4, 14.3. MS (ESI) m/z calculated $[M-H]^+$: 612.20; found $[M-H]^+$: 612.28.

4.4 General synthetic procedure and analytical data of compounds 2

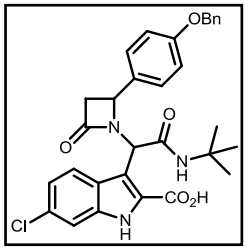
To a stirred solution of the corresponding esters (1.0 mmol) in THF/H_2O (4:1), LiOH (1.5 mmol) was added and the reaction mixture was stirred at rt overnight. Then, THF was removed under reduced pressure. Et_2O and water were then added, and the organic layer was separated and discarded. The aqueous layer was treated with 1 N HCl until it reached pH 2 and then CH_2Cl_2 was added. The organic layer was separated, dried over anhydrous $MgSO_4$ and concentrated to afford the titled product.

3-(1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(*tert*-butylamino)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2a**, *diastereomer A*)



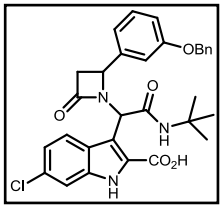
White solid, yield 89% (mix diastereomers); ^{1}H NMR (500 MHz, methanol-*d4*) δ: 7.31-7.43 (m, 6H), 7.25 (d, *J* = 8.8, 1H), 7.14 (s, 1H), 6.97 (m, 2H), 6.71-6.75 (m, 3H), 6.20 (s, 1H), 5.01 (s, 2H), 4.41 (dd, *J* = 5.5, 2.5 Hz, 1H), 3.26 (dd, *J* = 14.9, 5.5 Hz, 1H), 2.82 (dd, *J* = 14.9, 2.6 Hz, 1H), 1.21 (s, 9H). ^{13}C NMR (126 MHz, Methanol-*d4*) δ: 170.8, 169.3, 164.3, 160.2, 138.7, 137.6, 131.7, 131.6, 129.5, 129.4, 128.9, 128.8, 128.5, 127.8, 126.1, 124.2, 121.7, 115.8, 115.1, 112.6, 70.9, 56.1, 55.2, 52.5, 45.6, 28.7. MS (ESI) m/z calculated $[M-H]^+$: 558.19; found $[M-H]^+$: 558.24.

3-(1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(*tert*-butylamino)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2a**, *diastereomer B*)



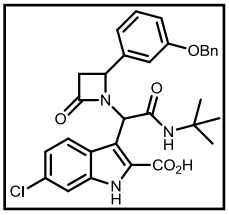
White solid, yield 89% (mix diastereomers); ^{1}H NMR (500 MHz, Methanol-*d4*) δ: 7.71 (d, *J* = 8.8 Hz, 1H), 7.30-7.39 (m, 6H), 7.23 (d, *J* = 1.9 Hz, 1H), 7.04 (dd, *J* = 8.7, 1.9 Hz, 1H), 6.46-6.60 (m, 4H), 6.43 (s, 1H), 4.90 (s, 2H), 4.68 (dd, *J* =5.2, 2.3 Hz, 1H), 3.41 (dd, *J* = 14.8, 5.2 Hz, 1H), 2.67 (dd, *J* = 14.7, 2.3 Hz, 1H), 1.28 (s, 9H). ^{13}C NMR (126 MHz, Methanol-*d4*) δ: 170.5, 170.5, 163.9, 159.6, 138.6, 137.6, 131.8, 131.6, 129.5, 128.8, 128.4, 127.8, 126.3, 123.4, 122.0, 115.0, 113.5, 112.8, 71.0, 56.3, 53.0, 52.6, 46.8, 28.8. MS (ESI) m/z calculated $[M]^+$: 559.19; found $[M]^+$: 559.34.

3-(1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(*tert*-butylamino)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2b**, *diastereomer A*)



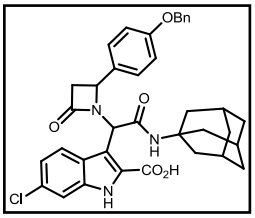
White solid, yield 92% (mix diastereomers); ^{1}H NMR (500 MHz, Methanol-*d4*) δ: 7.40 – 7.22 (m, 9H), 7.10 (d, *J* = 2.7 Hz, 1H), 6.78 – 6.69 (m, 3H), 6.57 (d, *J* = 2.1 Hz, 1H), 6.21 (s, 1H), 4.75 (s, 2H), 4.40 (dd, *J* = 5.5, 2.4 Hz, 1H), 3.25 – 3.20 (m, 1H), 2.75 (dd, *J* = 15.0, 2.6 Hz, 1H), 1.20 (s, 9H). ^{13}C NMR (126 MHz, Methanol-*d4*) δ: 170.6, 169.1, 164.3, 160.2, 141.3, 138.6, 137.6, 131.6, 130.5, 129.4, 128.8, 128.6, 126.0, 124.2, 122.0, 121.8, 120.5, 119.1, 116.1, 115.8, 115.2, 114.0, 112.8, 70.7, 56.6, 55.5, 52.5, 45.8, 28.7. MS (ESI) m/z calculated $[M-H]^+$: 558.19; found $[M-H]^+$: 558.27.

3-(1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(*tert*-butylamino)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid acid (**2b**, *diastereomer B*)



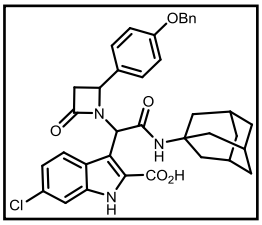
White solid, yield 92% (mix diastereomers); ^{1}H NMR (500 MHz, Methanol-*d4*) δ: 7.75 (d, *J* = 8.8 Hz, 1H), 7.44 – 7.32 (m, 4H), 7.32 – 7.27 (m, 2H), 7.27 – 7.21 (m, 1H), 7.07 (dt, *J* = 8.8, 1.9 Hz, 1H), 6.84 – 6.73 (m, 1H), 6.54 (dt, *J* = 8.2, 1.7 Hz, 1H), 6.48 (s, 1H), 6.34 (d, *J* = 7.5 Hz, 1H), 6.24 (s, 1H), 4.74 – 4.71 (m, 1H), 4.70 (s, 2H), 3.43 (dd, *J* = 14.8, 5.2 Hz, 1H), 2.63 (dd, *J* = 14.7, 1.9 Hz, 1H), 1.28 (s, 9H). ^{13}C NMR (126 MHz, Methanol-*d4*) δ: 170.5, 170.5, 164.0, 159.6, 141.3, 138.6, 137.6, 131.6, 129.6, 129.4, 129.1, 128.8, 128.6, 126.4, 123.3, 122.0, 119.1, 115.8, 113.2, 112.9, 112.1, 70.7, 56.7, 53.0, 52.6, 47.0, 28.8. MS (ESI) m/z calculated $[M-H]^+$: 558.19; found $[M-H]^+$: 558.57.

3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2c**, *diastereomer A*)



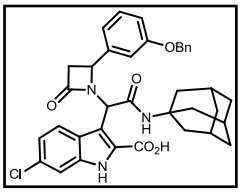
White solid, yield 90% (mix diastereomers); ^{1}H NMR (500 MHz, Methanol-*d4*) δ: 7.90 (s, 1H), 7.49 – 7.42 (m, 2H), 7.41 – 7.35 (m, 3H), 7.34 – 7.27 (m, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 2H), 6.79 – 6.69 (m, 3H), 6.24 (s, 1H), 5.02 (s, 2H), 4.42 (s, 1H), 3.49 (d, *J* = 7.0 Hz, 1H), 2.82 (d, *J* = 14.9 Hz, 1H), 2.00 (s, 4H), 1.88 (s, 5H), 1.66 (s, 6H). ^{13}C NMR (126 MHz, Methanol-*d4*) δ: 169.2, 160.2, 138.7, 137.4, 131.8, 131.2, 129.5, 129.4, 128.9, 128.5, 126.3, 124.2, 121.5, 115.8, 112.5, 102.7, 71.0, 56.0, 55.3, 53.3, 45.6, 42.1, 37.4, 30.8. MS (ESI) m/z calculated $[M-H]^+$: 636.23; found $[M-H]^+$: 636.49.

3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2c**, *diastereomer B*)



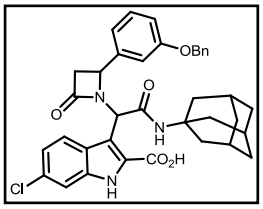
White solid, yield 90% (mix diastereomers); ^{1}H NMR (500 MHz, Methanol-*d4*) δ: 7.71 (d, *J* = 8.8 Hz, 1H), 7.41 – 7.33 (m, 4H), 7.30 (s, 1H), 7.22 (d, *J* = 1.9 Hz, 1H), 7.15 (s, 1H), 7.03 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.60 (d, *J* = 8.3 Hz, 2H), 6.51 – 6.43 (m, 3H), 4.68 (dd, *J* = 5.2, 2.3 Hz, 1H), 3.39 (dd, *J* = 14.7, 5.2 Hz, 1H), 2.67 (d, *J* = 2.4 Hz, 1H), 2.02 (m, 4H), 1.96 (s, 5H), 1.67 (s, 6H). ^{13}C NMR (126 MHz, Methanol-*d4*) δ: 170.5, 170.4, 159.6, 138.7, 137.5, 131.8, 131.3, 129.5, 128.8, 128.5, 127.8, 126.4, 123.4, 121.8, 115.0, 113.0, 112.8, 71.0, 56.3, 53.4, 53.1, 46.8, 42.2, 37.4, 31.0. MS (ESI) m/z calculated $[M-H]^+$: 636.23; found $[M-H]^+$: 636.28.

3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2d**, *diastereomer A*)



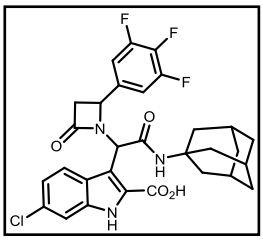
White solid, yield 87% (mix diastereomers); ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 11.50 (s, 1H), 7.75 (d, *J* = 8.8 Hz, 1H), 7.46 – 7.38 (m, 5H), 7.38 – 7.32 (m, 1H), 7.20 (d, *J* = 2.0 Hz, 1H), 7.04 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.77 (t, *J* = 7.8 Hz, 1H), 6.54 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.40 – 6.28 (m, 2H), 6.24 (s, 1H), 4.75 (s, 2H), 4.70 (dd, *J* = 5.4, 2.5 Hz, 1H), 2.56 (dd, *J* = 14.4, 2.3 Hz, 1H), 1.95 (s, 3H), 1.86 (s, 6H), 1.58 (s, 6H). ^{13}C NMR (126 MHz, DMSO-*d6*) δ: 168.1, 166.7, 162.5, 157.6, 141.2, 136.9, 135.7, 128.3, 128.1, 127.8, 127.7, 124.9, 122.4, 119.96, 117.8, 114.0, 112.1, 111.4, 110.6, 68.9, 54.0, 51.4, 51.3, 46.2, 40.8, 35.9, 28.7. MS (ESI) m/z calculated $[M-H]^+$: 636.23; found $[M-H]^+$: 636.21.

3-(2-(((3s,5s,7s)-adamantan-1-yl)amino)-1-(2-(3-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-oxoethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2d**, *diastereomer B*)



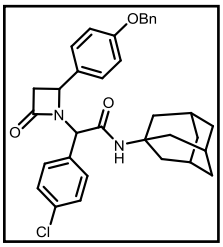
White solid, yield 87% (mix diastereomers); ^{1}H NMR (500 MHz, Methanol-*d4*) δ: 7.43 – 7.32 (m, 6H), 7.33 – 7.22 (m, 2H), 7.06 (t, *J* = 7.8 Hz, 1H), 6.82 – 6.74 (m, 2H), 6.72 (d, *J* = 7.6 Hz, 1H), 6.65 (s, 1H), 6.24 (s, 1H), 4.85 – 4.80 (m, 3H), 4.41 (d, *J* = 3.0 Hz, 1H), 2.81 (dd, *J* = 14.9, 2.3 Hz, 1H), 1.97 (s, 3H), 1.90 (s, 6H), 1.64 (s, 6H). ^{13}C NMR (126 MHz, Methanol-*d4*) δ: 170.7, 168.9, 160.2, 141.4, 138.6, 137.6, 131.6, 130.5, 129.5, 128.8, 128.6, 126.0, 124.2, 121.8, 120.6, 116.0, 114.2, 112.7, 70.8, 56.5, 55.4, 53.4, 49.5, 49.3, 49.2, 49.0, 48.9, 48.8, 48.7, 48.5, 45.8, 42.1, 37.3, 30.8. MS (ESI) m/z calculated $[M-H]^+$: 636.23; found $[M-H]^+$: 636.46.

3-(2-(((3s,5s,7s)-Adamantan-1-yl)amino)-2-oxo-1-(2-oxo-4-(3,4,5-trifluorophenyl)azetidin-1-yl)ethyl)-6-chloro-1*H*-indole-2-carboxylic acid (**2e**, *diastereomer B*)



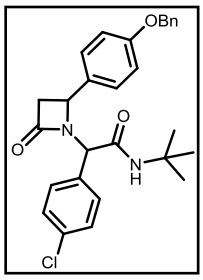
White solid, yield 90% (mix diastereomers); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.98 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.35 (d, *J* = 1.6 Hz, 1H), 7.06 (dd, *J* = 8.7, 1.6 Hz, 1H), 6.45 (s, 1H), 6.43 – 6.37 (m, 2H), 5.58 (s, 1H), 4.72 – 4.66 (m, 1H), 3.51 – 3.40 (m, 1H), 2.63 (dd, *J* = 14.7, 2.1 Hz, 1H), 2.03 (s, 3H), 1.92 (s, 6H), 1.62 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.31, 166.80, 162.63, 136.16, 130.82, 127.25, 124.57, 121.87, 121.49, 112.13, 109.58, 109.54, 109.41, 53.55, 52.39, 51.72, 46.59, 41.18, 36.10, 29.21. MS (ESI) m/z calculated $[M+H]^+$: 586.16; found $[M+H]^+$: 586.20.

_N_-((3s,5s,7s)-Adamantan-1-yl)-2-(2-(4-(benzyloxy)phenyl)-4-oxoazetidin-1-yl)-2-(4-chlorophenyl)acetamide (**2f**, *diastereomer A*)



White solid, yield 82%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 – 7.39 (m, 4H), 7.35-7.34 (m, 1H), 7.13 (s, 4H), 7.05 (d, *J* = 10 Hz, 2H), 6.81 (d, *J* = 10 Hz, 2H), 6.28 (br s, 1H), 5.04 (s, 2H), 4.87 (s, 1H), 4.79 (dd, *J* = 0.5, 5 Hz, 1H), 3.40 (dd, *J* = 15, 5 Hz, 1H), 2.86 (dd, *J* = 15, 0.5 Hz, 1H), 2.07 (br s, 3H), 1.66 (br s, 7H), 1.59 (s, 5H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 167.2, 159.4, 133.7, 129.7, 128.9, 128.1, 127.6, 115.3, 70.3, 62.3, 55.1, 46.4, 41.5, 36.5, 29.6. MS (ESI) m/z calculated $[M+H]^+$: 555.23; found $[M+H]^+$: 555.29.

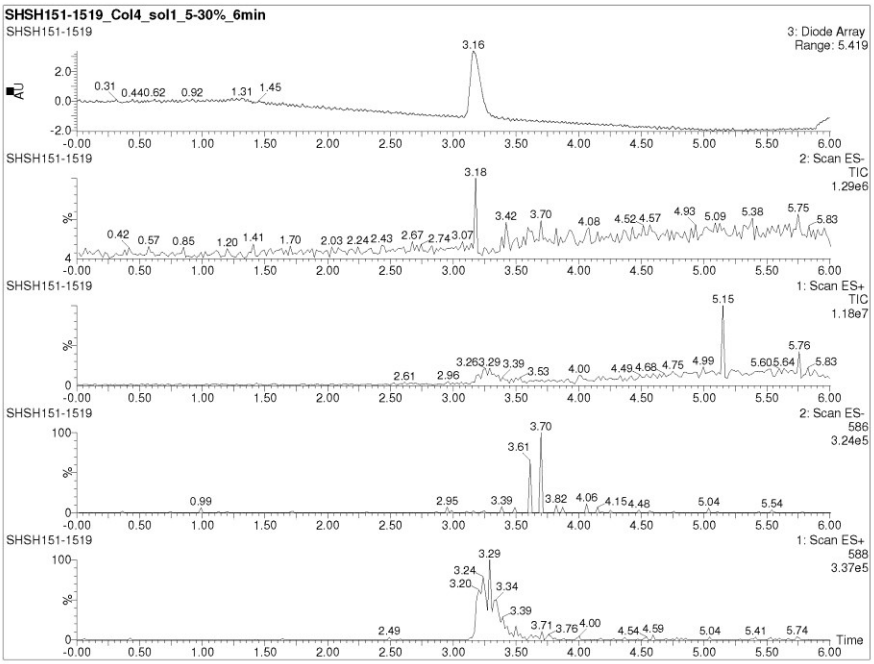
2-(2-(4-(Benzyloxy)phenyl)-4-oxoazetidin-1-yl)-_N_-(tert-butyl)-2-(4-chlorophenyl)acetamide (**2g**, *mixture of diastereomers*)

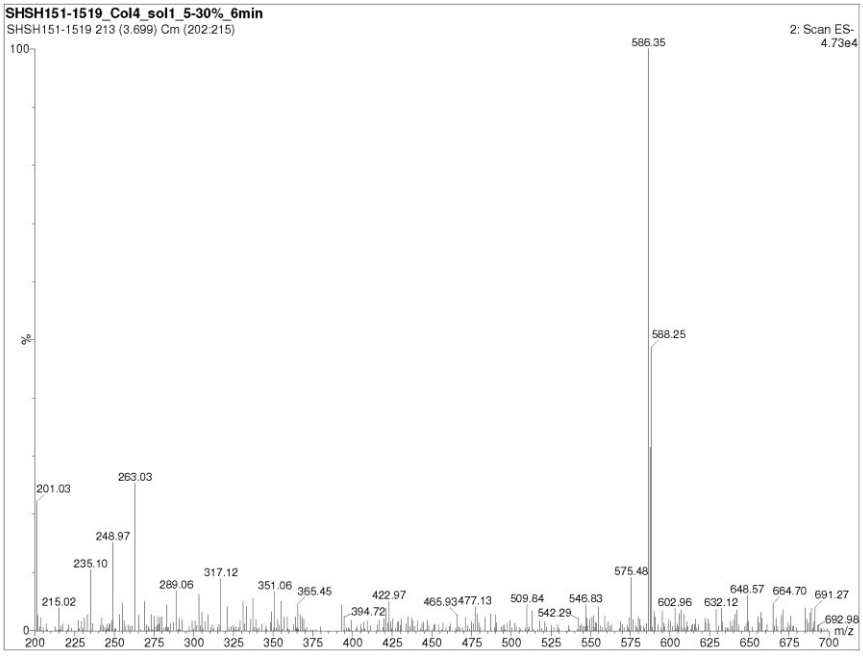


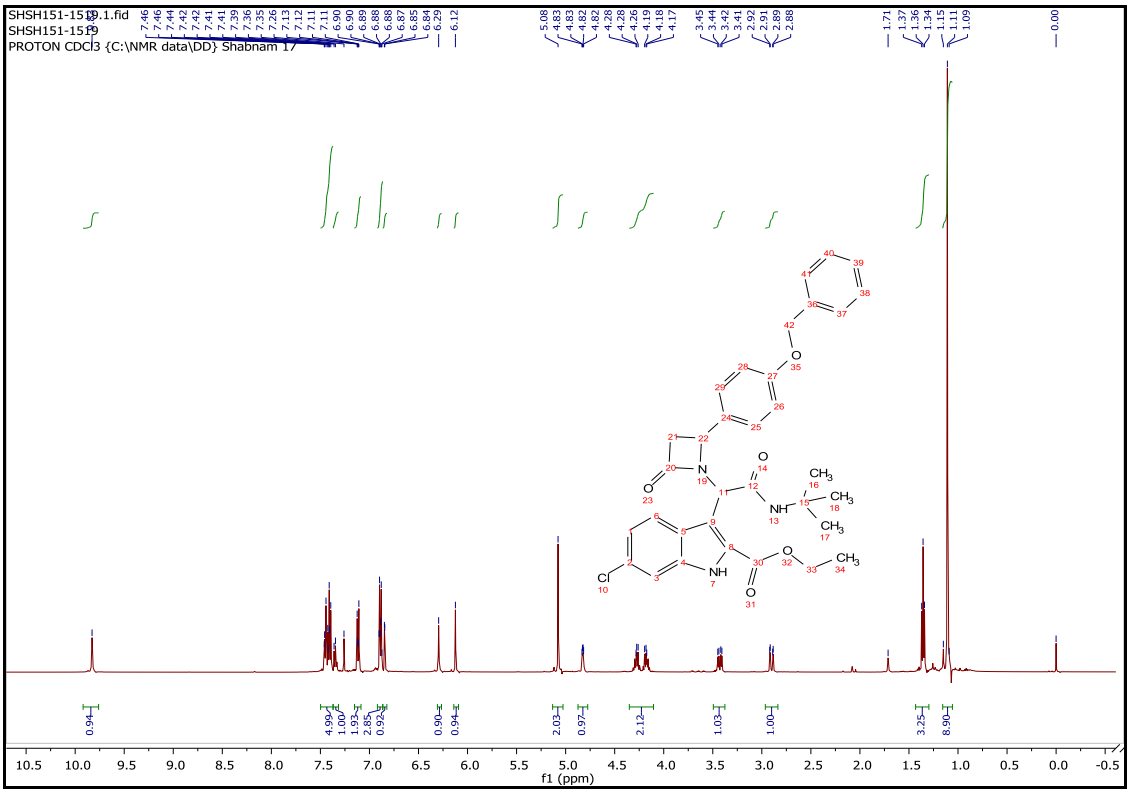
Major isomer reported: White solid, yield 78%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.43 – 7.38 (m, 4H), 7.35 (d, *J* = 6.9 Hz, 1H), 7.13 (s, 4H), 7.05 (d, *J* = 8.7 Hz, 2H), 6.84 – 6.79 (m, 2H), 6.42 (s, 1H), 5.06 (s, 2H), 4.83 (s, 1H), 4.46 (dd, *J* = 5.3, 2.4 Hz, 1H), 3.32 (dd, *J* = 15.0, 5.3 Hz, 1H), 2.97 (dd, *J* = 15.0, 2.4 Hz, 1H), 1.25 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 168.95, 167.62, 159.38, 136.76, 134.41, 133.81, 130.26, 129.62, 129.08, 129.01, 128.86, 128.51, 128.32, 128.25, 128.11, 127.63, 115.53, 115.27, 70.27, 63.75, 62.56, 55.26, 45.76, 28.67. MS (ESI) m/z calculated $[M+Na]^+$: 499.19; found $[M+Na]^+$: 499.26.

5. ^{1}H, ^{13}C NMR chromatograms and mass spectra of the novel synthesized compounds

Compound **3a**, *diastereomer A*



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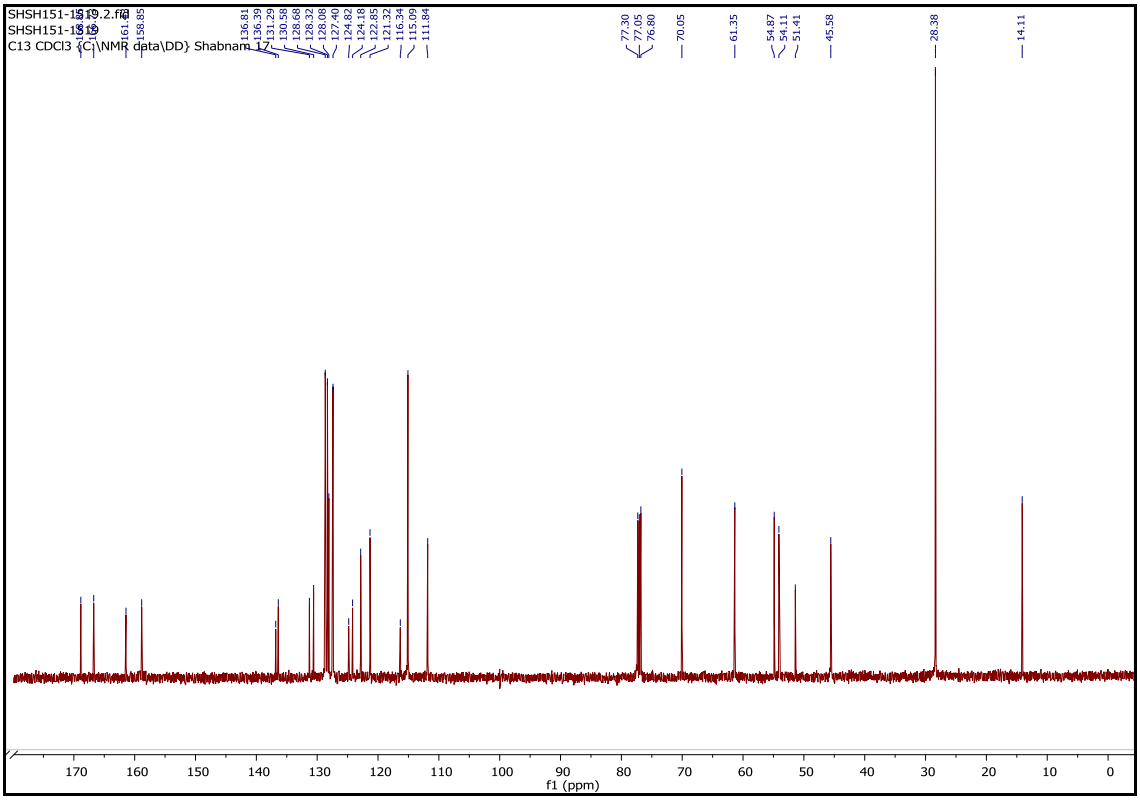
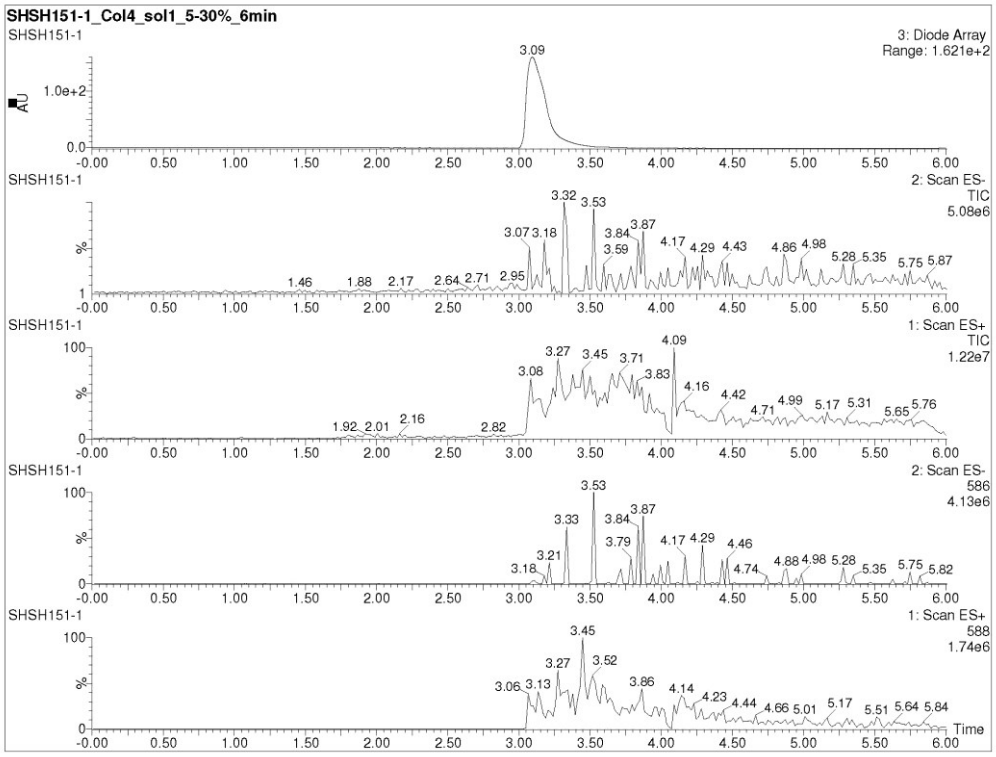
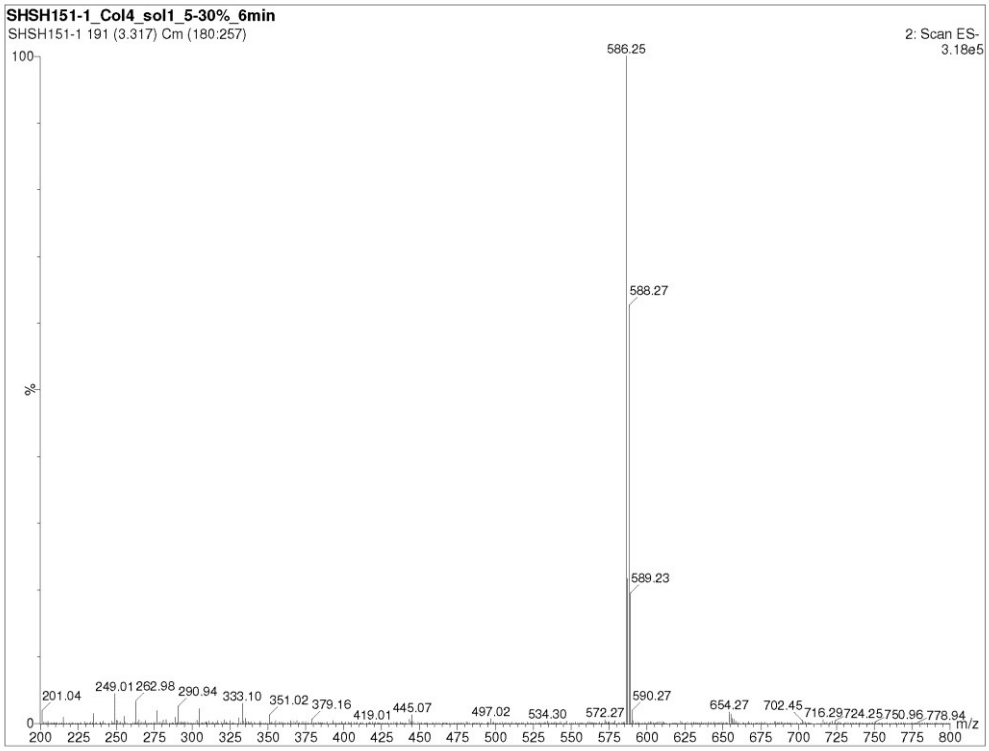
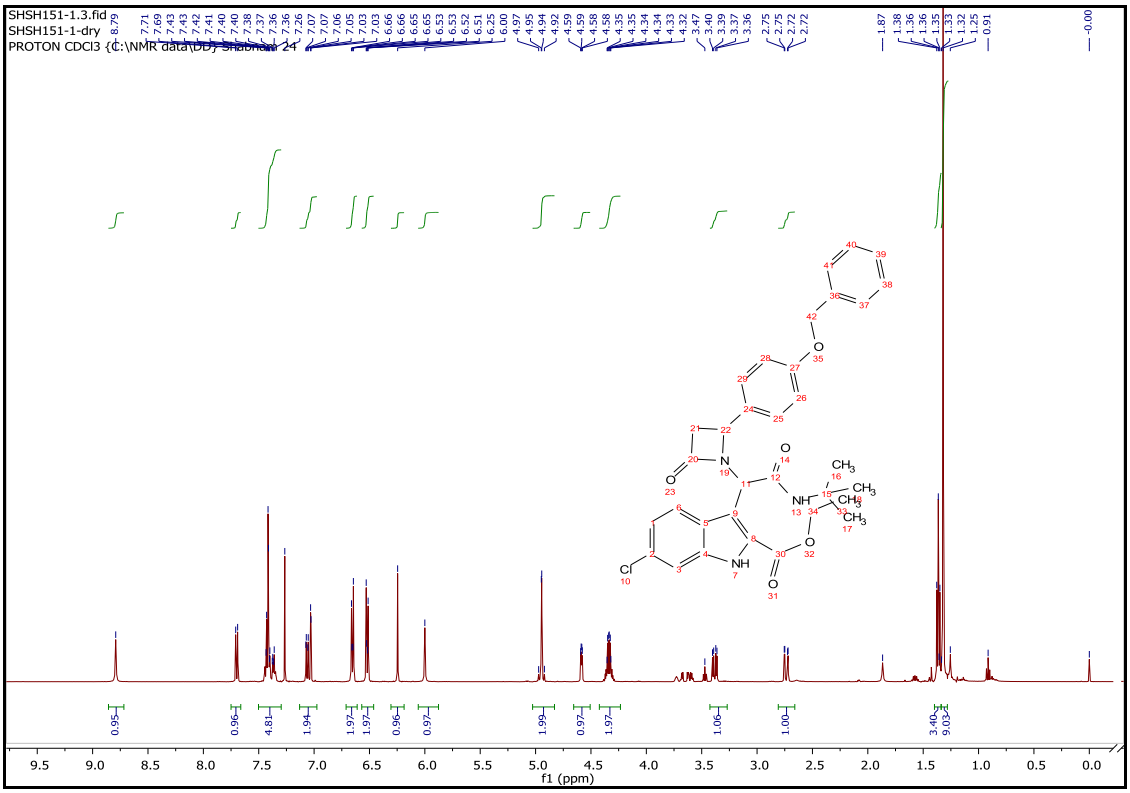
SHSH151-1519
PROTON CDCl3 {C:\NMR data\DD} Shabnam 17
f1 (ppm)

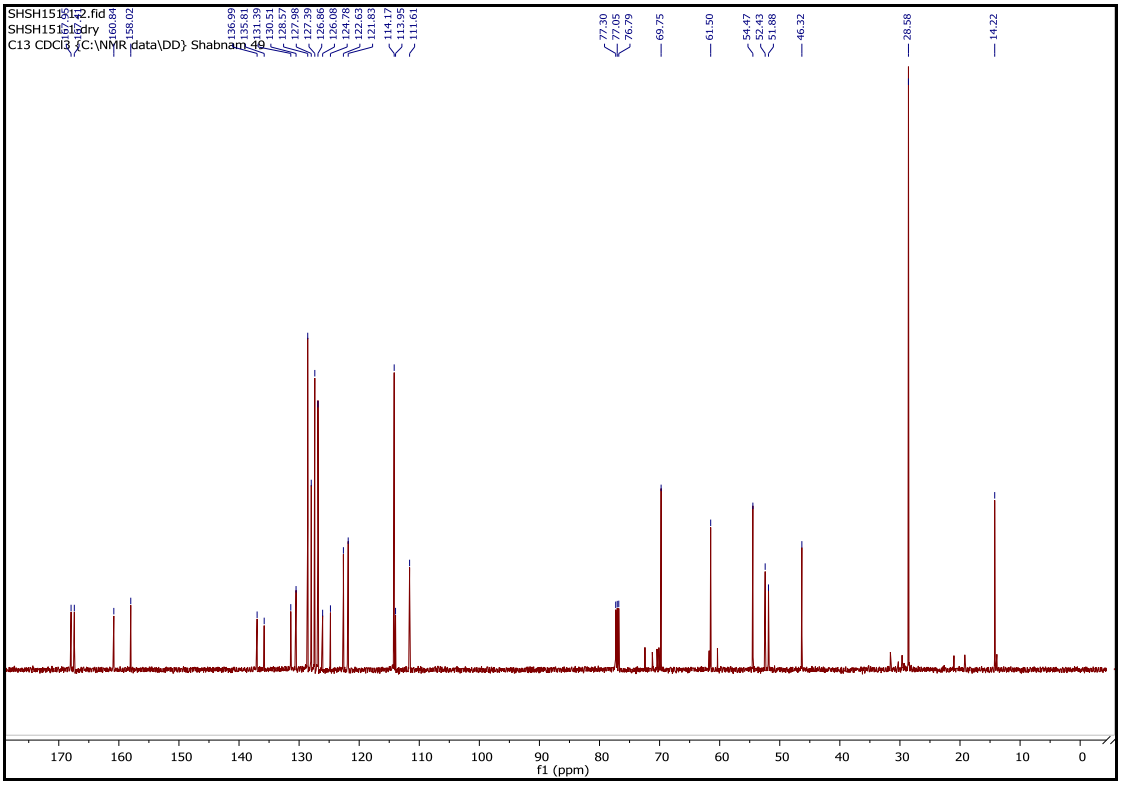

SHSH151-1519.2.fid
SHSH151-1519
C13 CDCl3 {C:\NMR data\DD} Shabnam 17
f1 (ppm)

Compound **3a**, *diastereomer B*

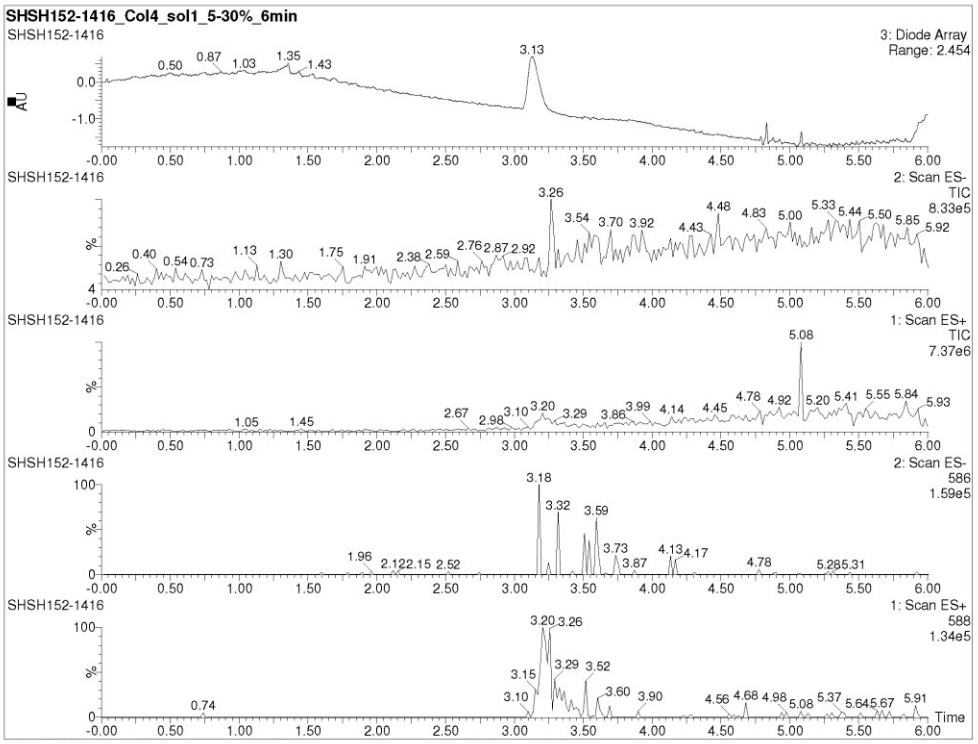


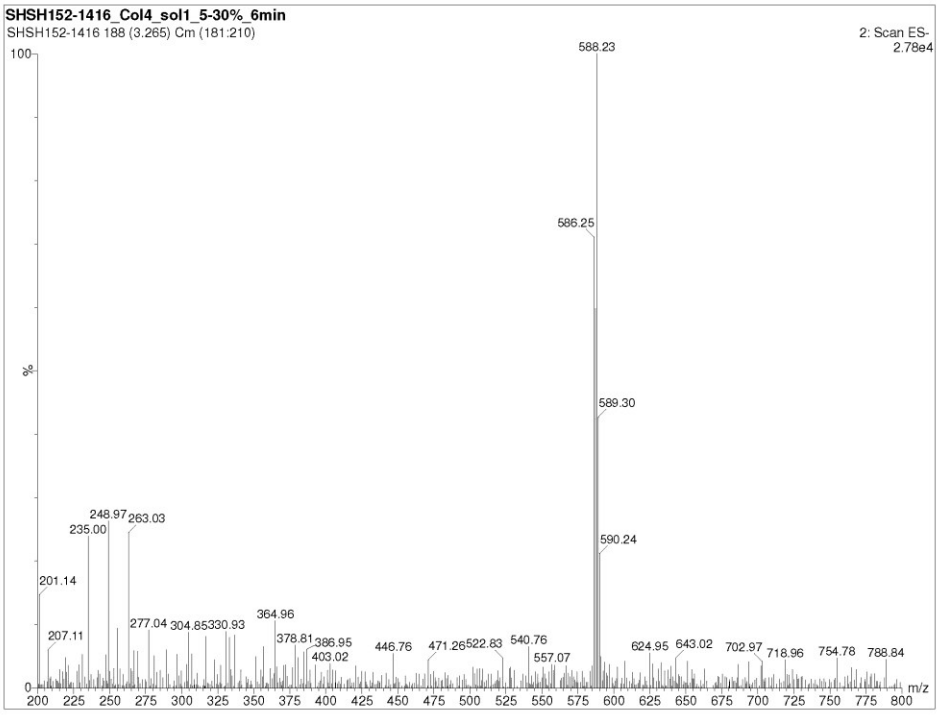


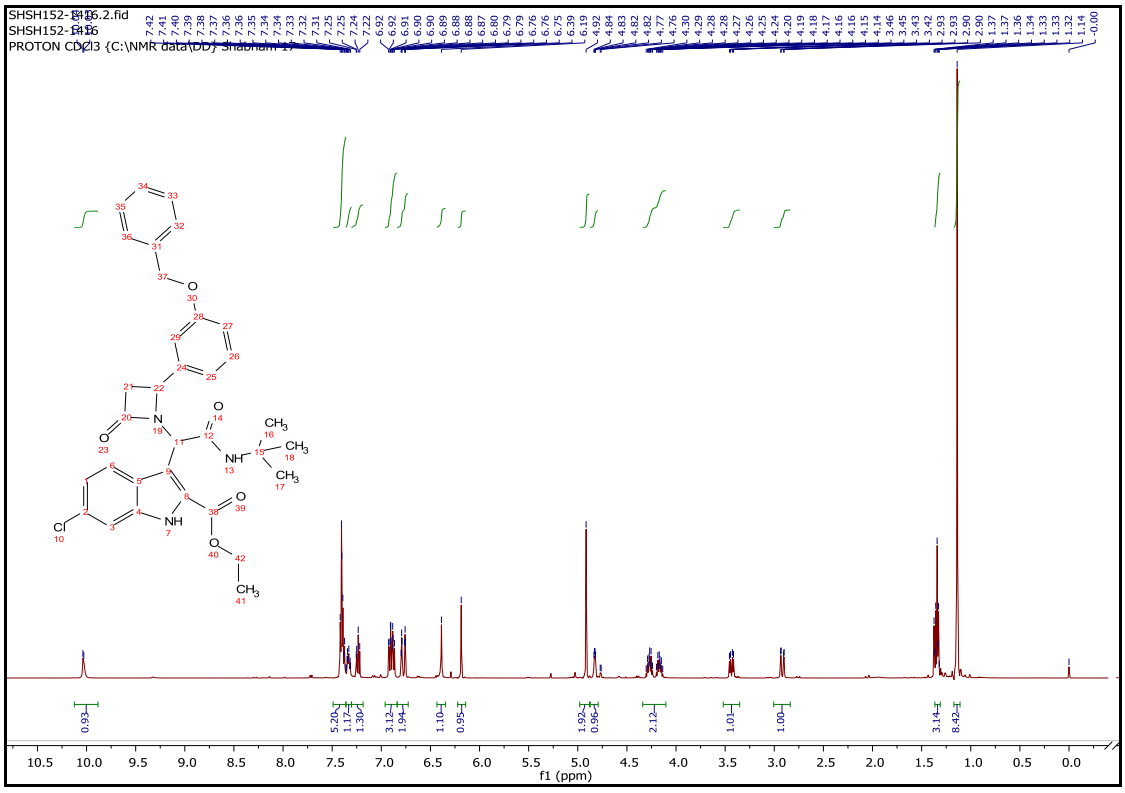

SHSH151-1.3.fid
SHSH151-1-dry
PROTON CDCl3 {C:\NMR data\DD} Shabnam 24
f1 (ppm)

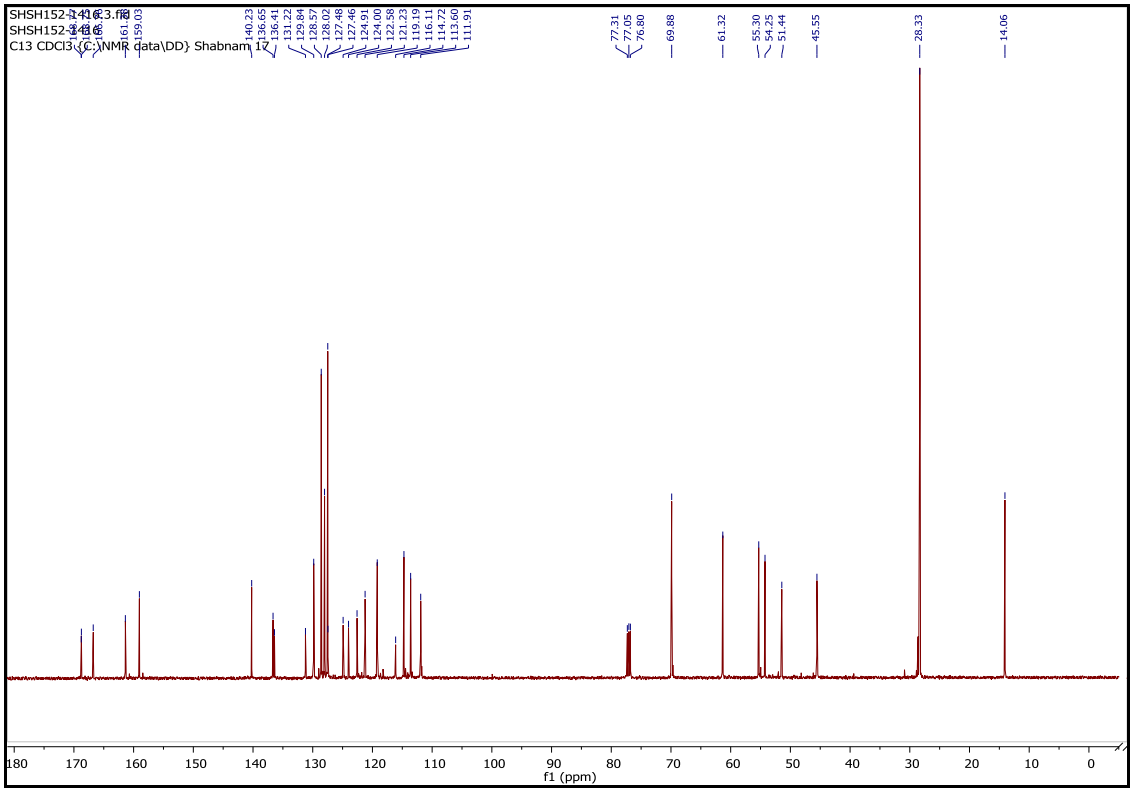

SHSH151-1-2.fid
SHSH151-1-dry
C13 CDCl3 {C:\NMR data\DD} Shabnam 40
f1 (ppm)

Compound **3b**, *diastereomer A*

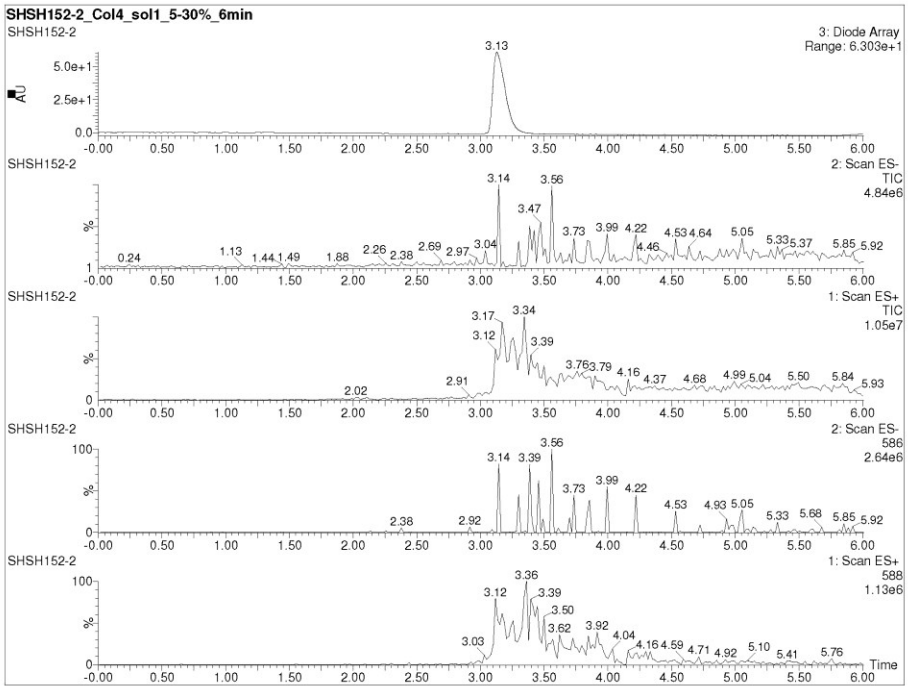


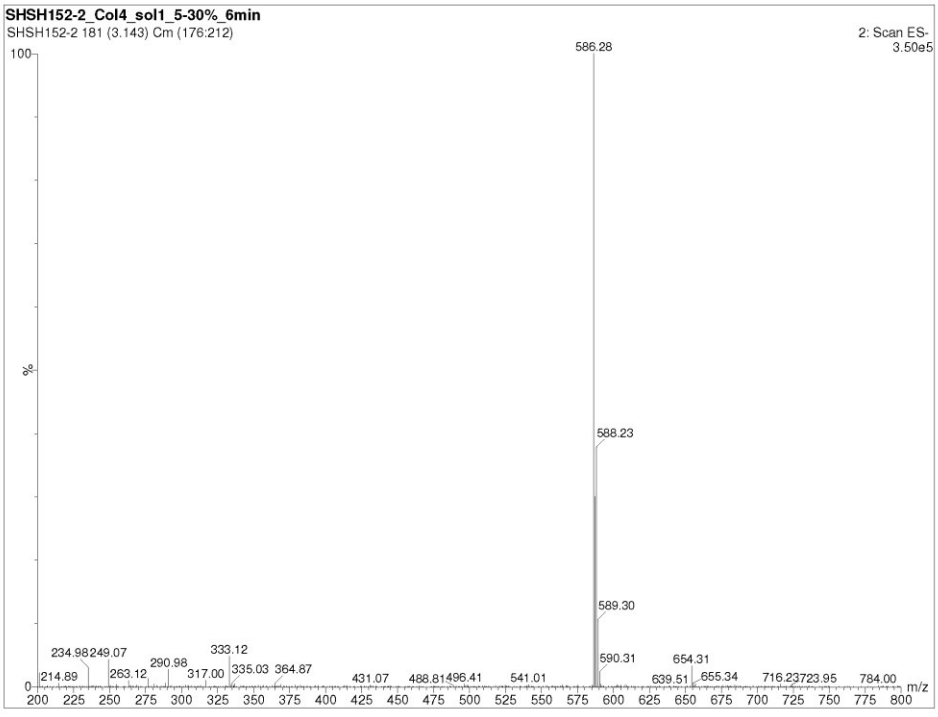


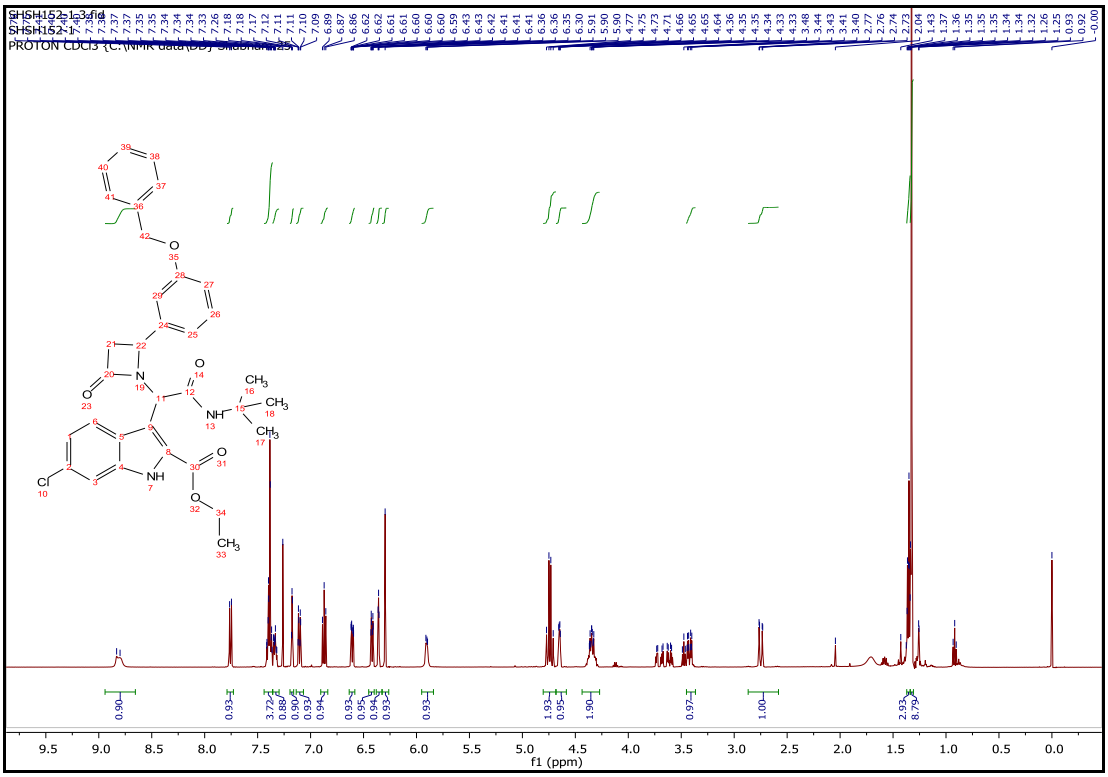

SHSH152-1416.2.fid
SHSH152-1416
PROTON CDCl3 {C:\NMR data\DD} Shabnam 17
f1 (ppm)

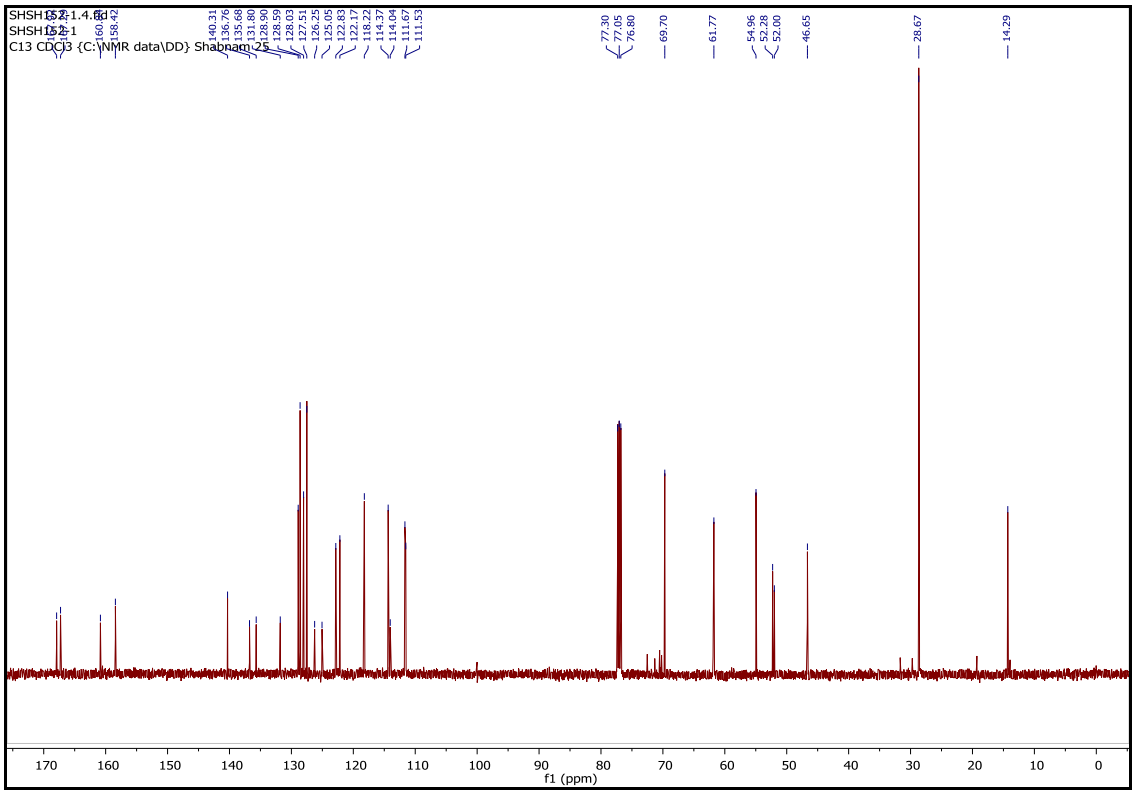

SHSH152-1416C3.fid
SHSH152-1416
C13 CDCl3 {C:\NMR data\DD} Shabnam 12
f1 (ppm)

Compound **3b**, *diastereomer B*

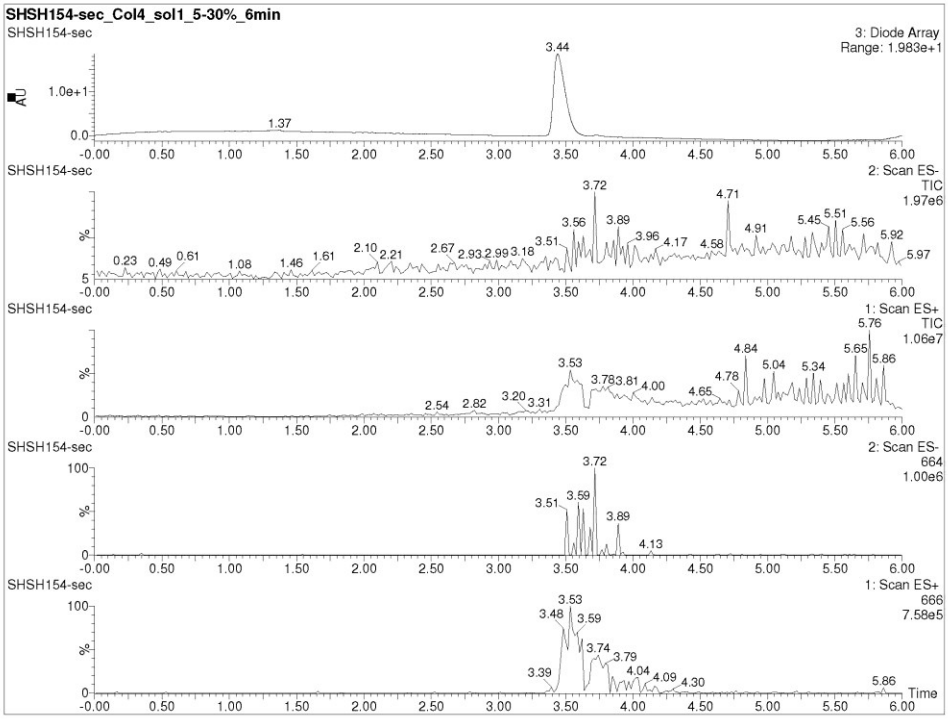


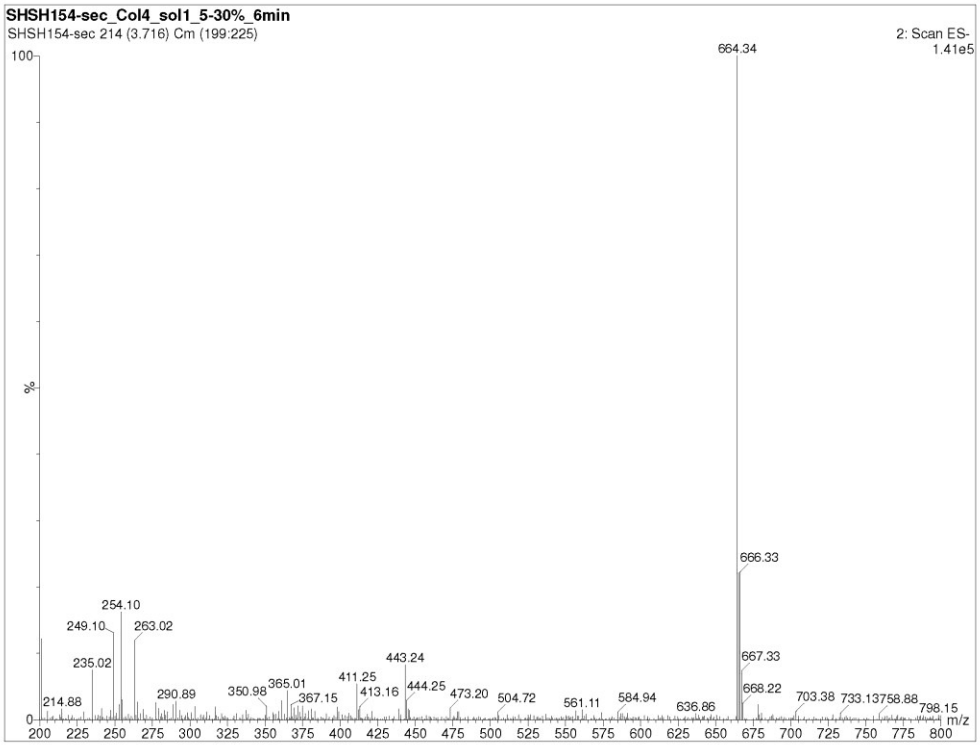


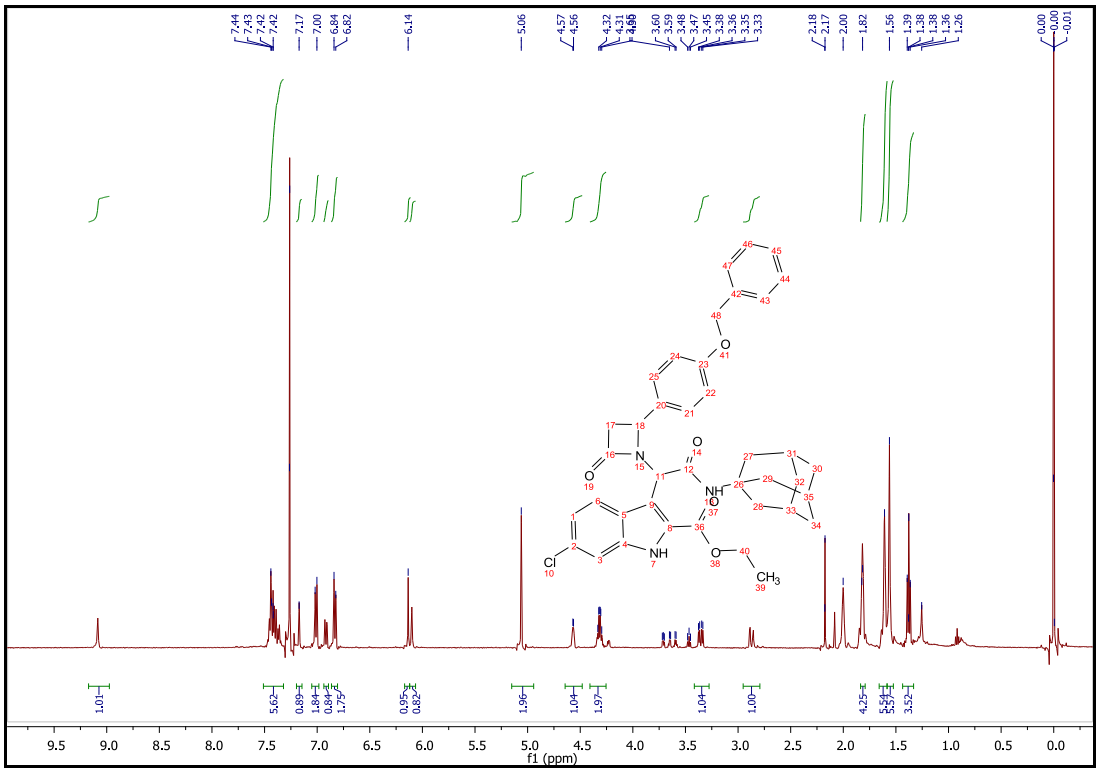

SHSH152-1-3.fid
SHSH152-1
PROTON CDCl3 {C:\NMR data\DD} Shabnam 25
f1 (ppm)

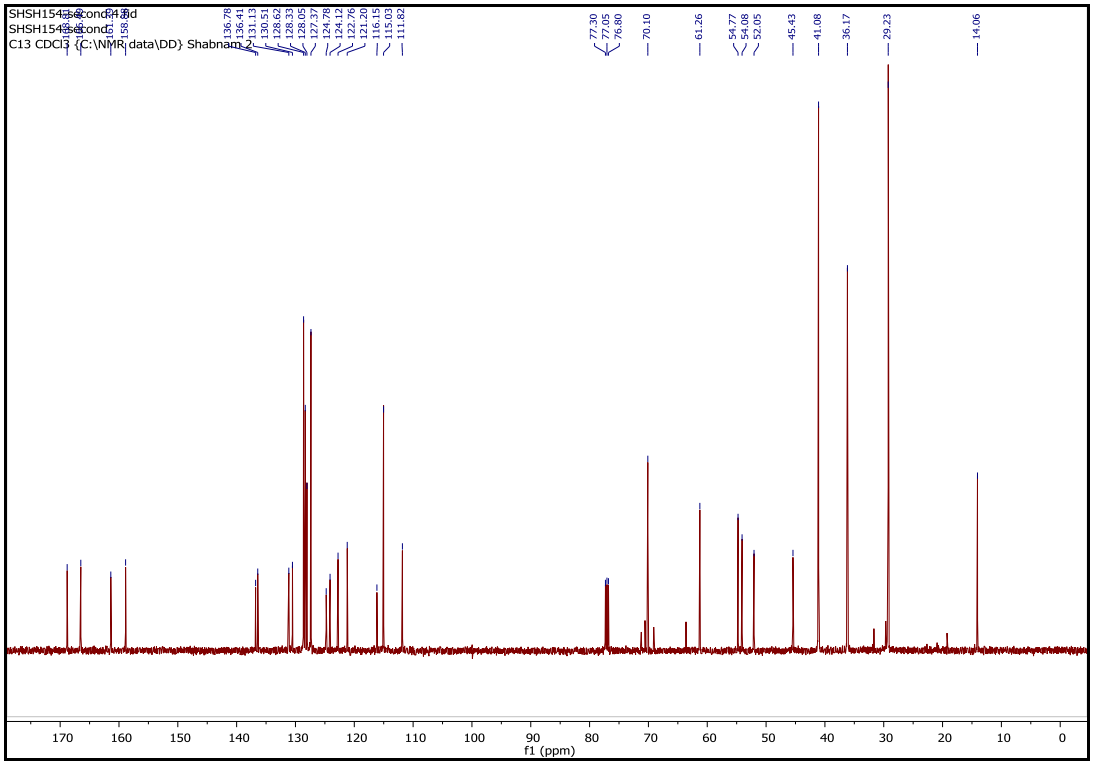

SHSH152-1-4.fid
SHSH152-1
C13 CDCl3 {C:\NMR data\DD} Shabnam 25
f1 (ppm)

Compound **3c**, *diastereomer A*

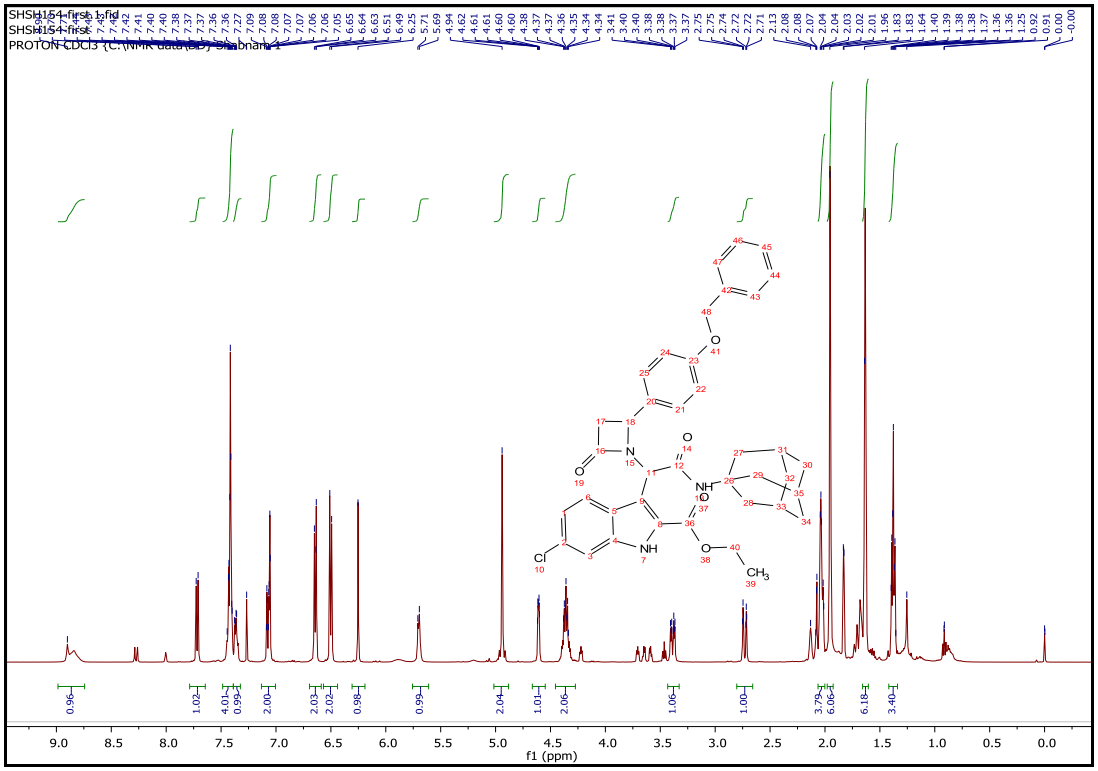


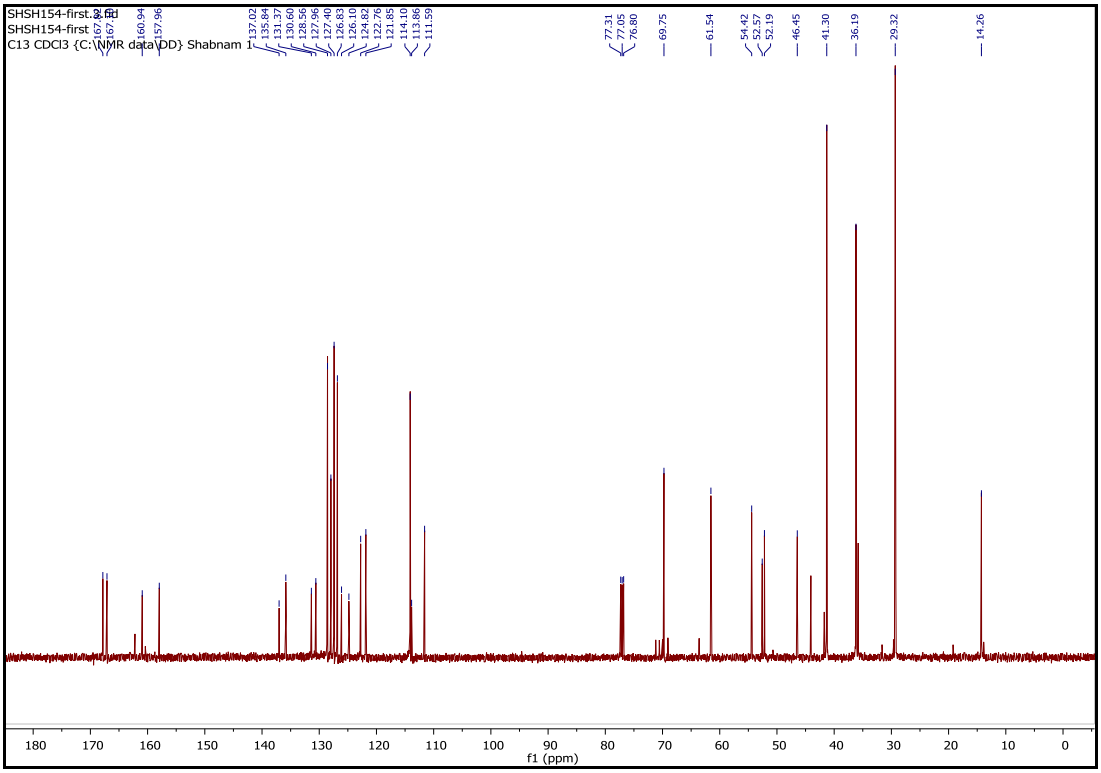



f1 (ppm)

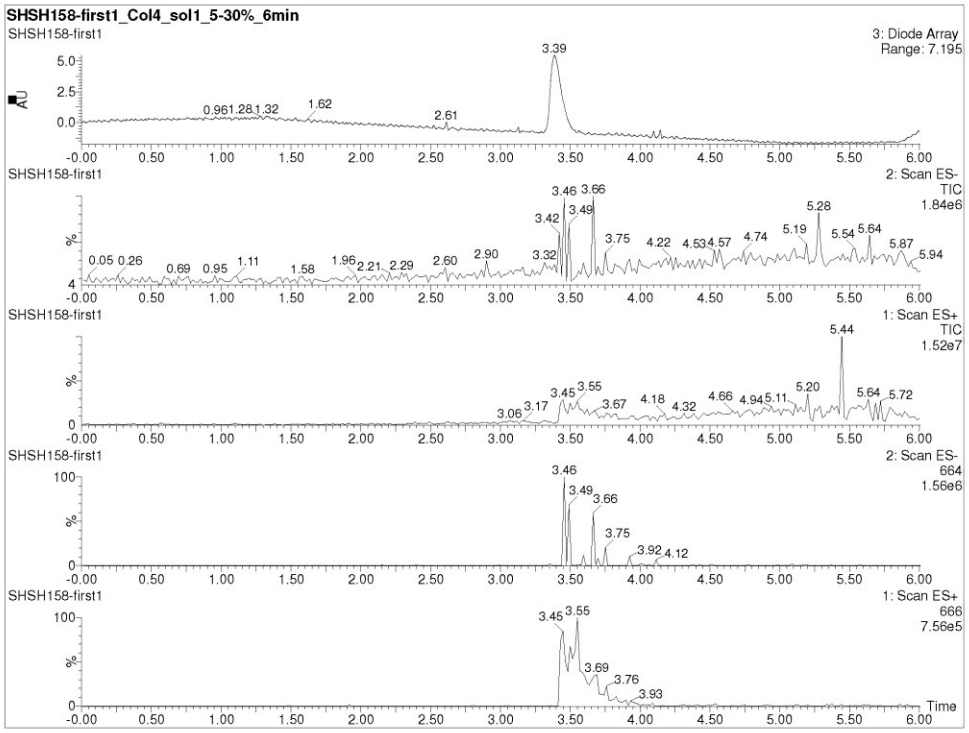

SHSH154sec.cond.4.fid
SHSH154sec.cond.4
C13 CDCl3 {C:\NMR data\DD} Shabnam 2
f1 (ppm)

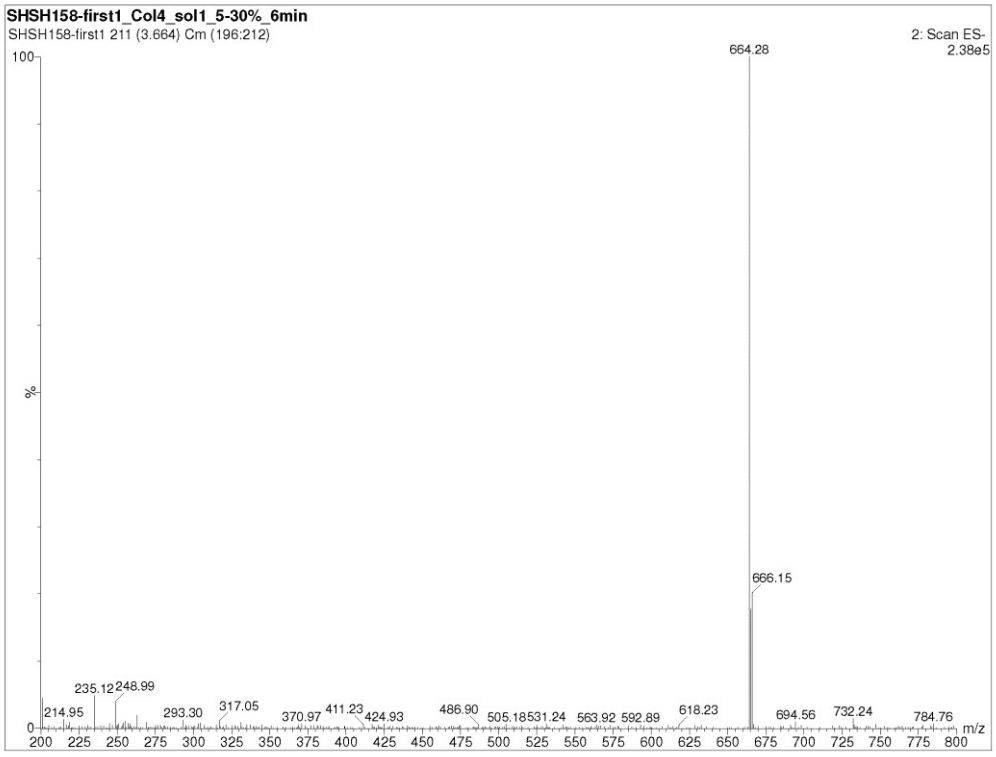
Compound **3c**, *diastereomer B*

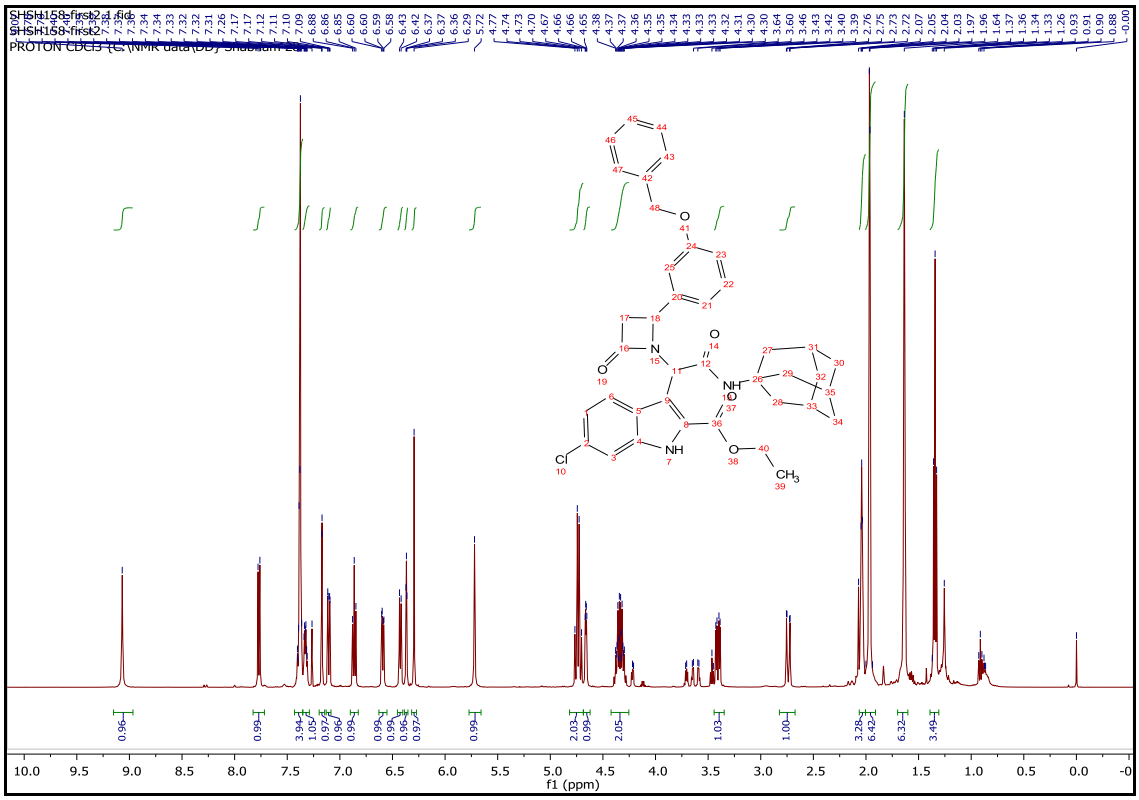


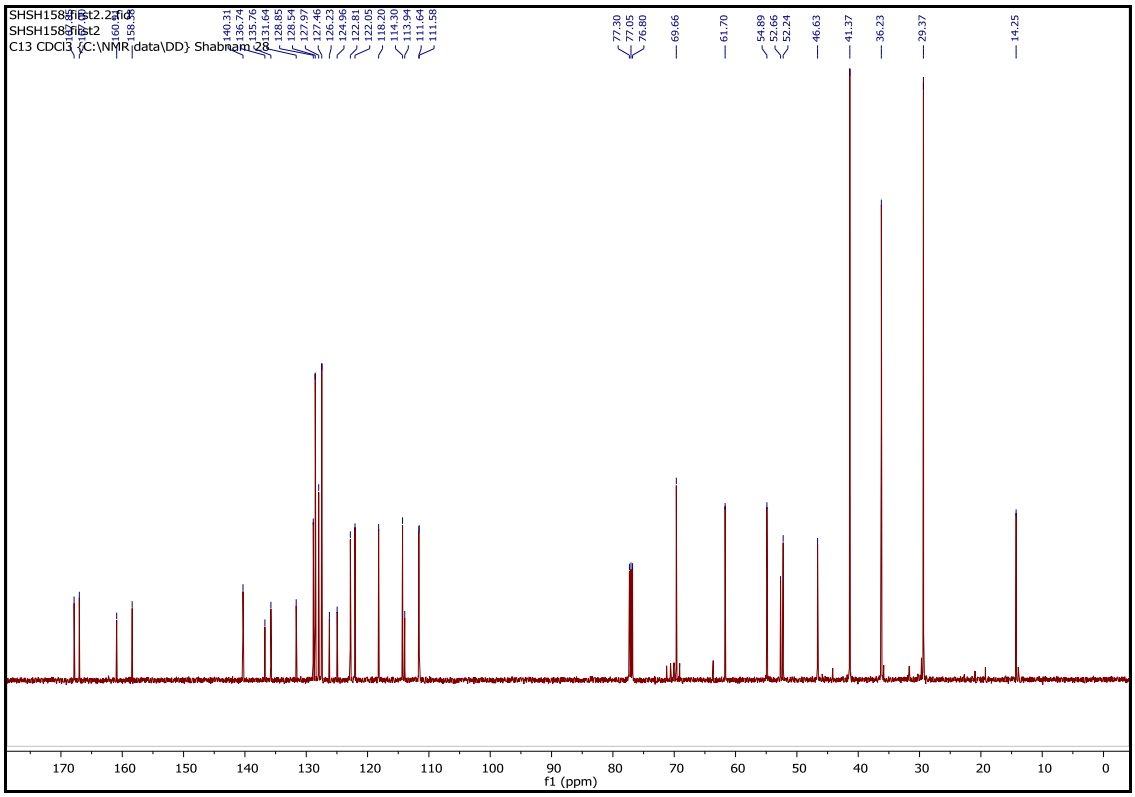


Compound **3d**, *diastereomer A*

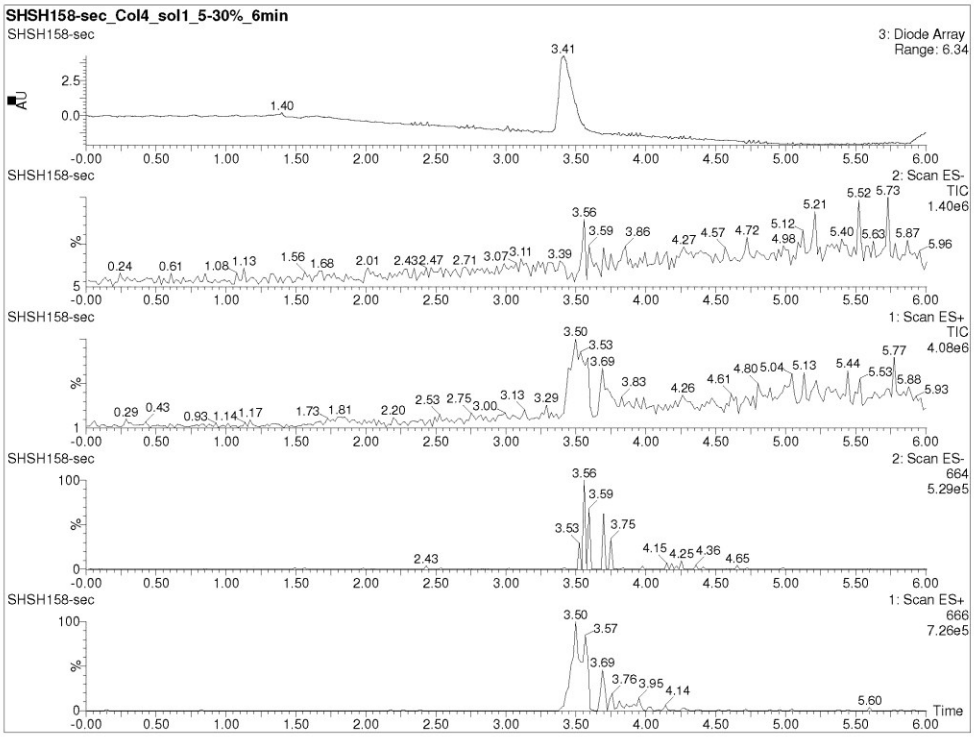


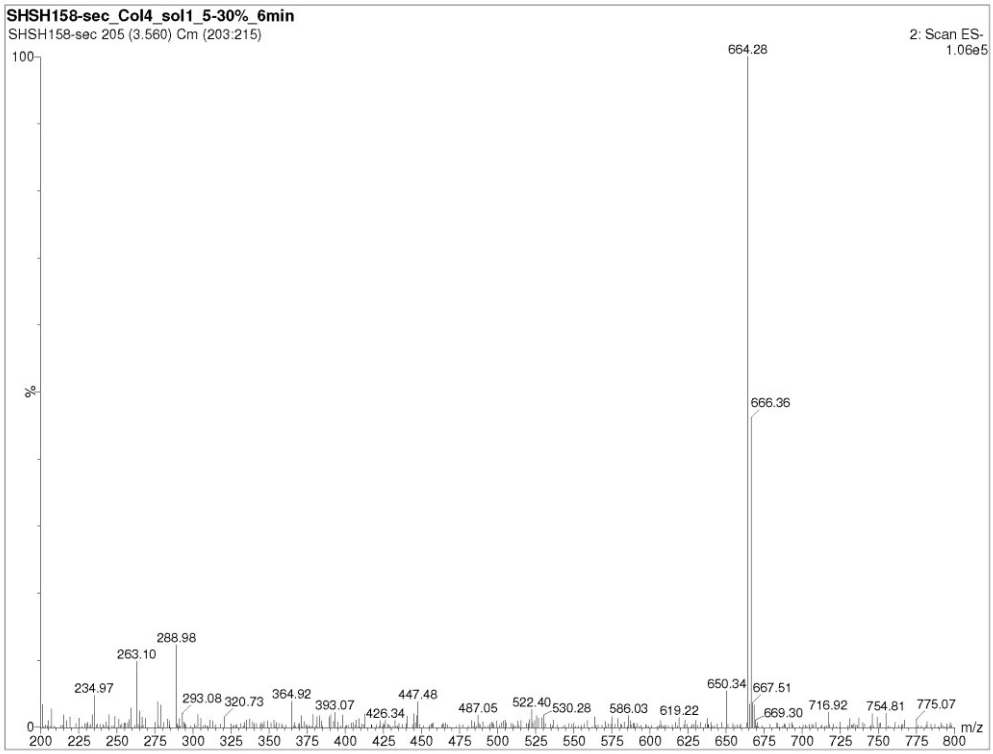


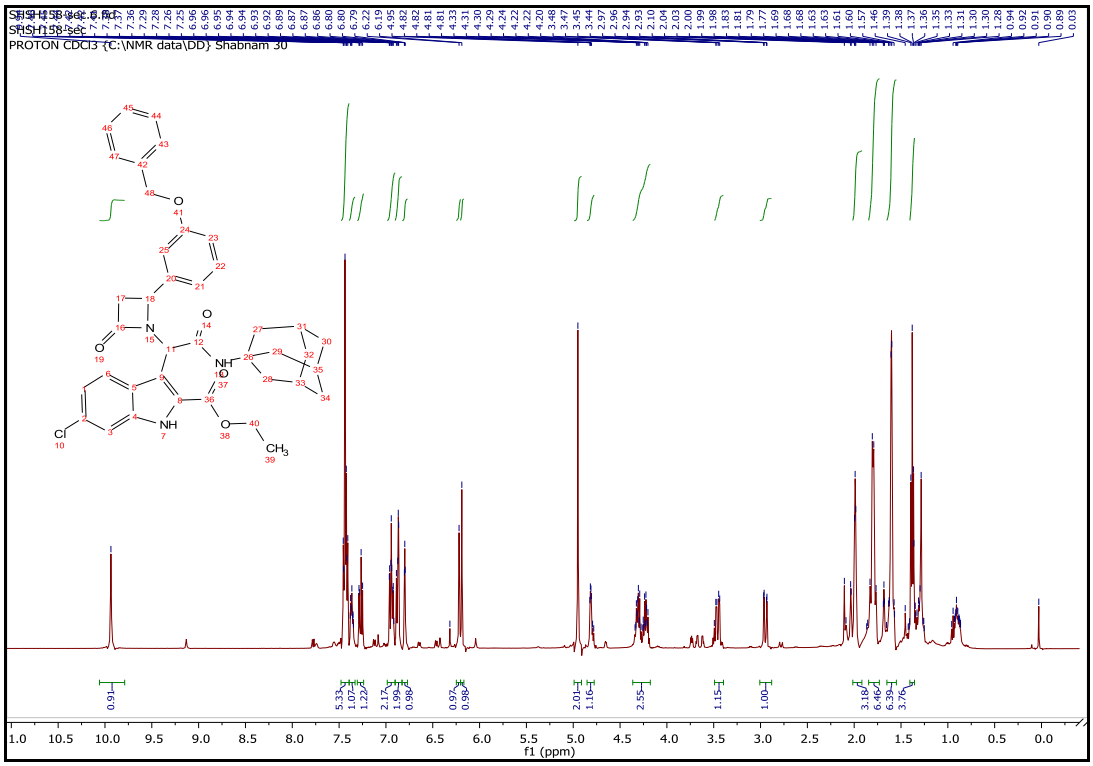

SHSH158-first2.1.fid
SHSH158-first2
PROTON CDCl3 {C:\NMR data\DD} Shabnam 28
f1 (ppm)

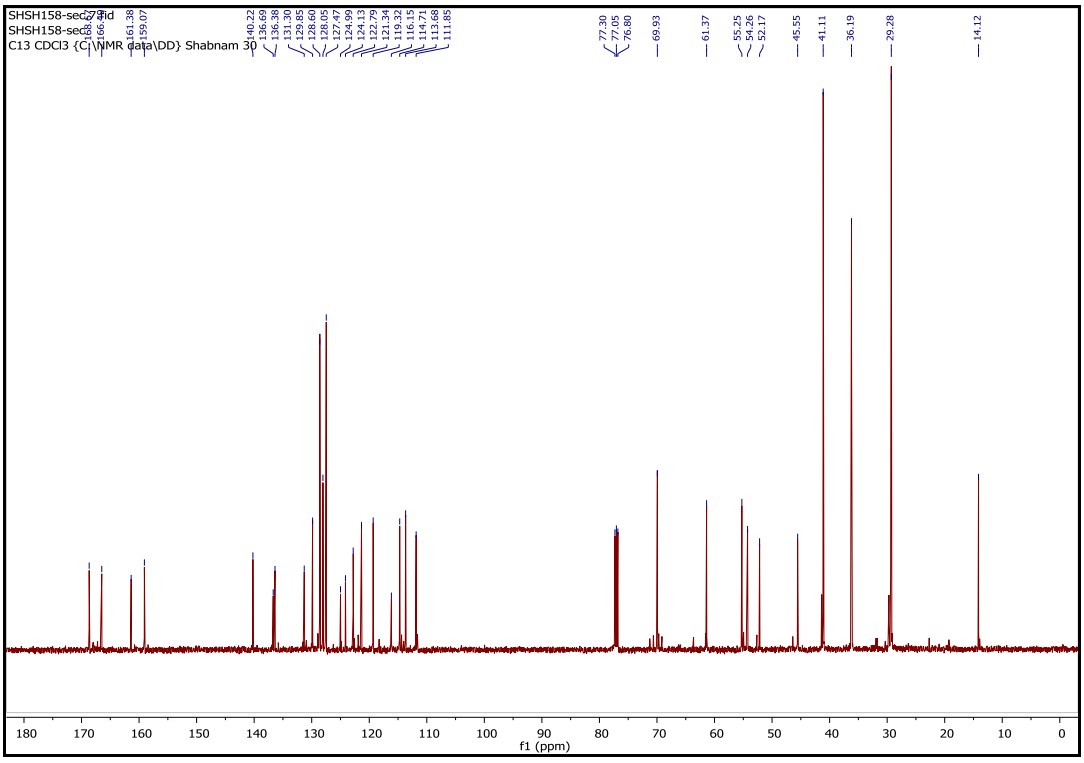

SHSH158-first2.2.fid
SHSH158-first2
C13 CDCl3 {C:\NMR data\DD} Shabnam 28
f1 (ppm)

Compound **3d**, *diastereomer B*

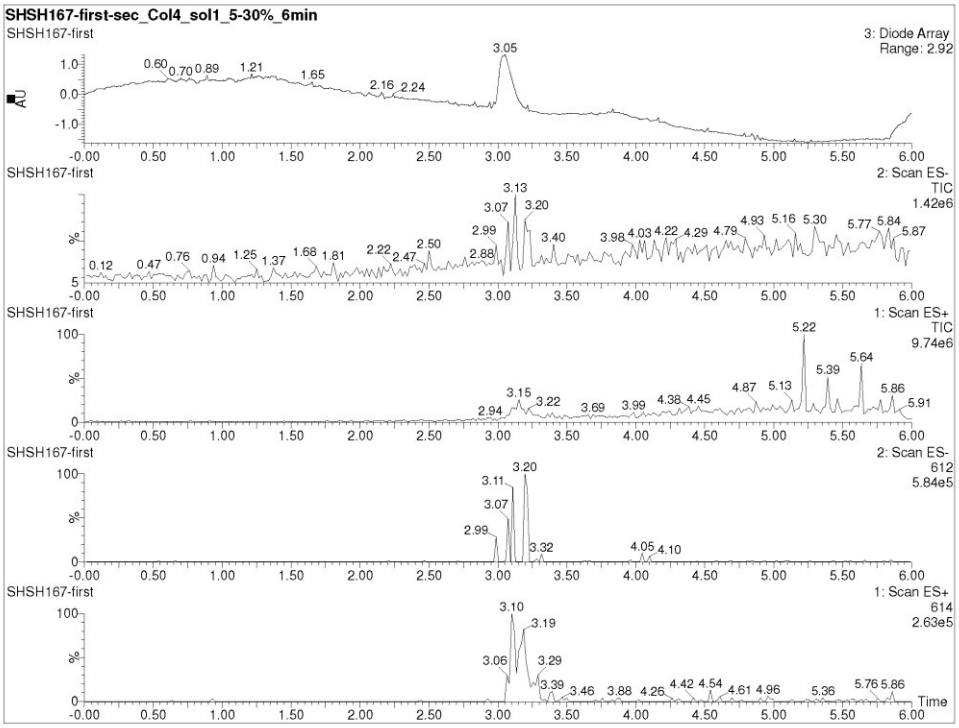


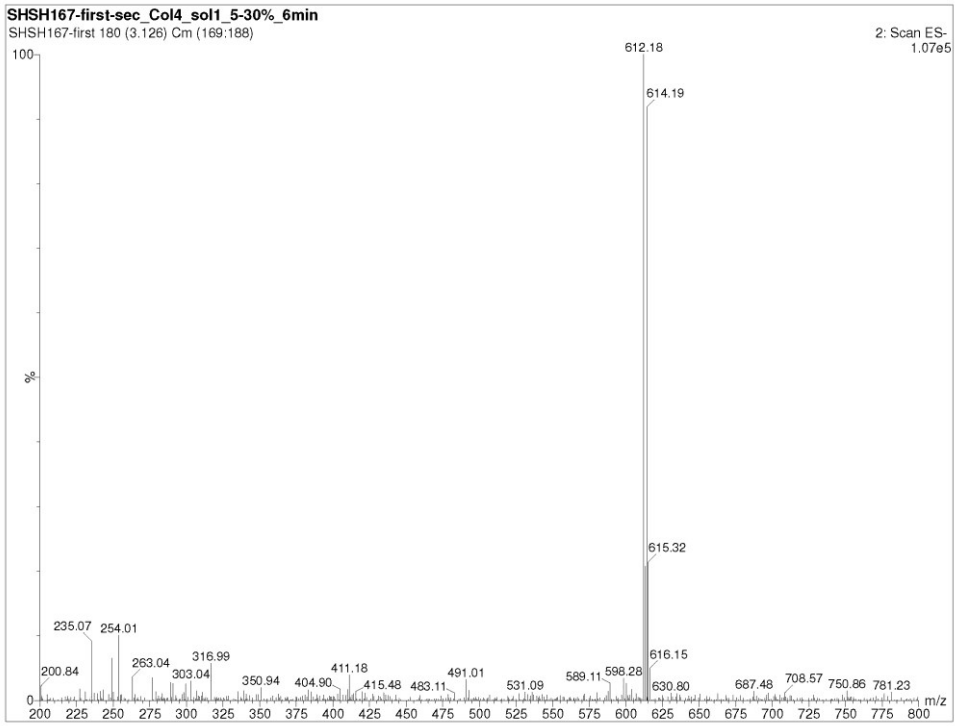


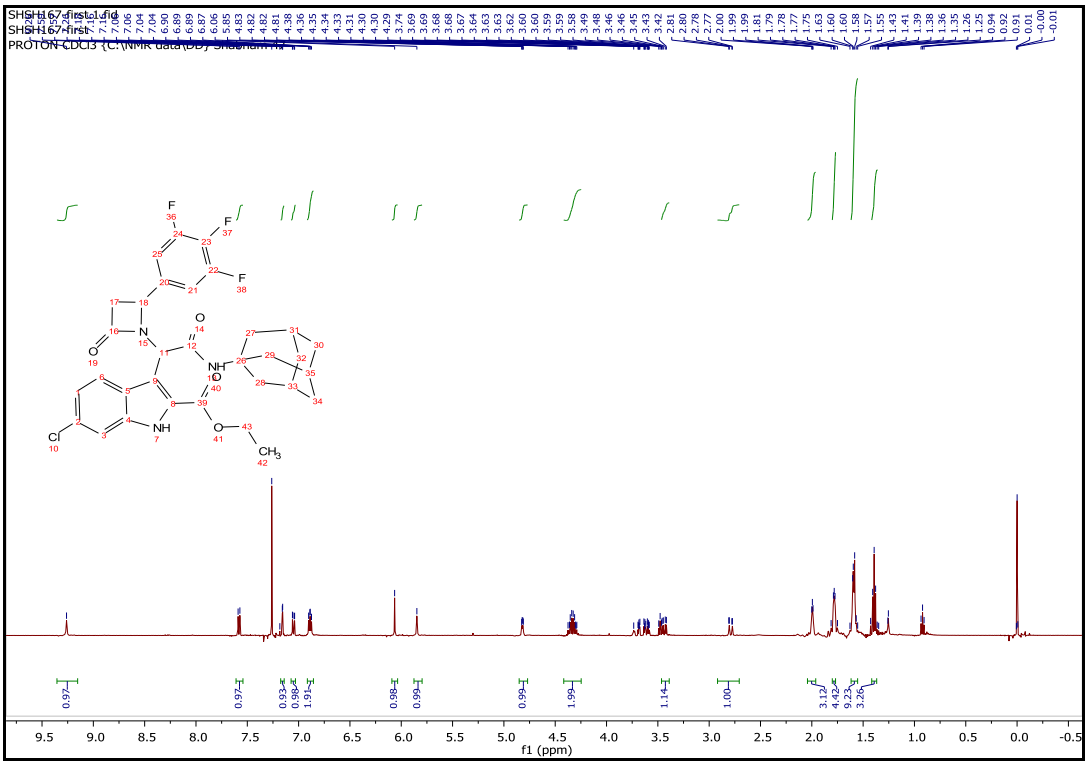

SHSH158-sec.fid
SHSH158-sec
PROTON CDCl3 {C:\NMR data\DD} Shabnam 30
f1 (ppm)

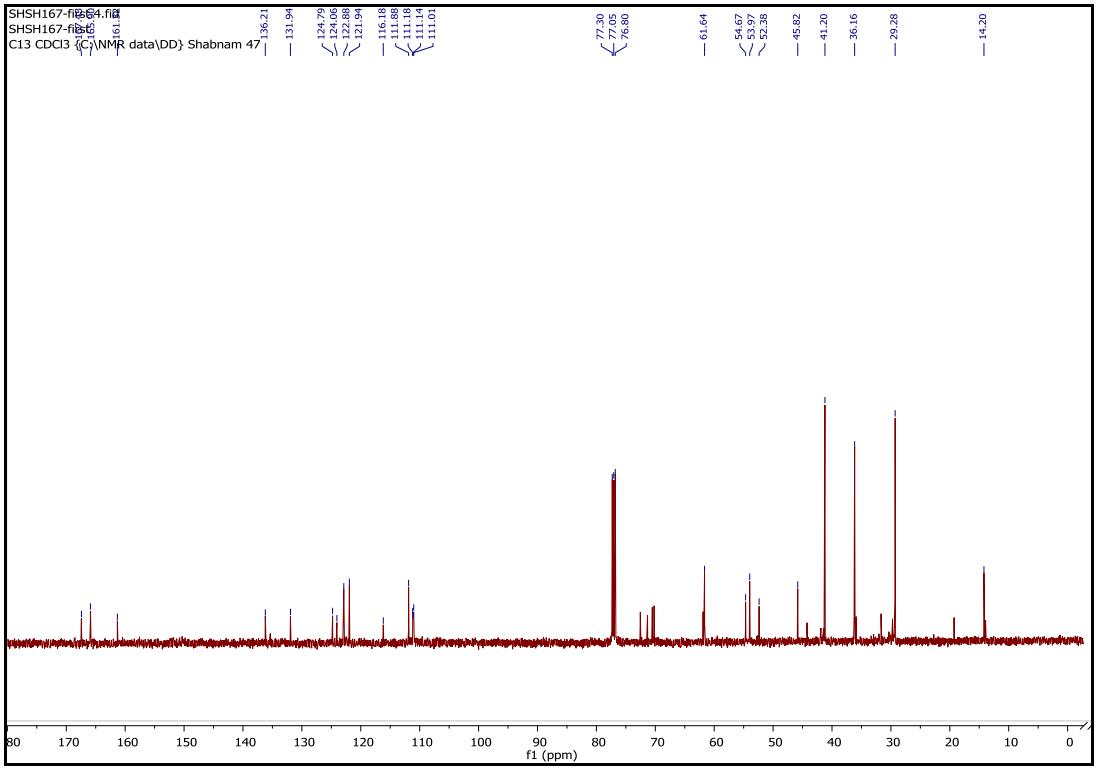

SHSH158-sec.fid
SHSH158-sec
C13 CDCl3 {C:\NMR data\DD} Shabnam 30
f1 (ppm)

Compound **3e**, *diastereomer A*

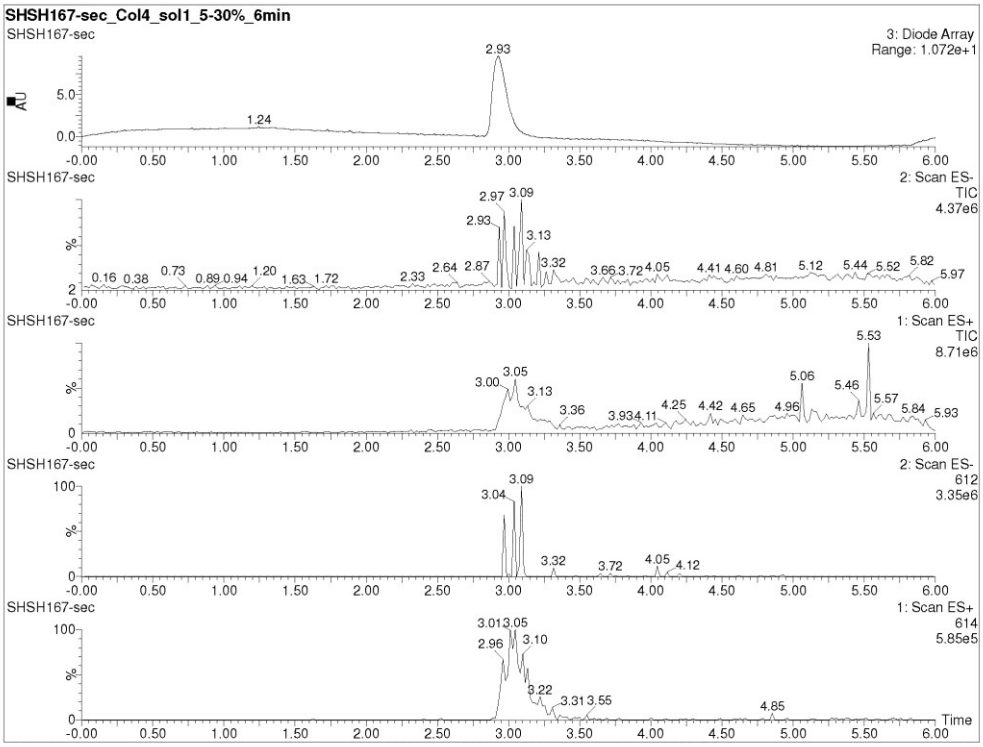


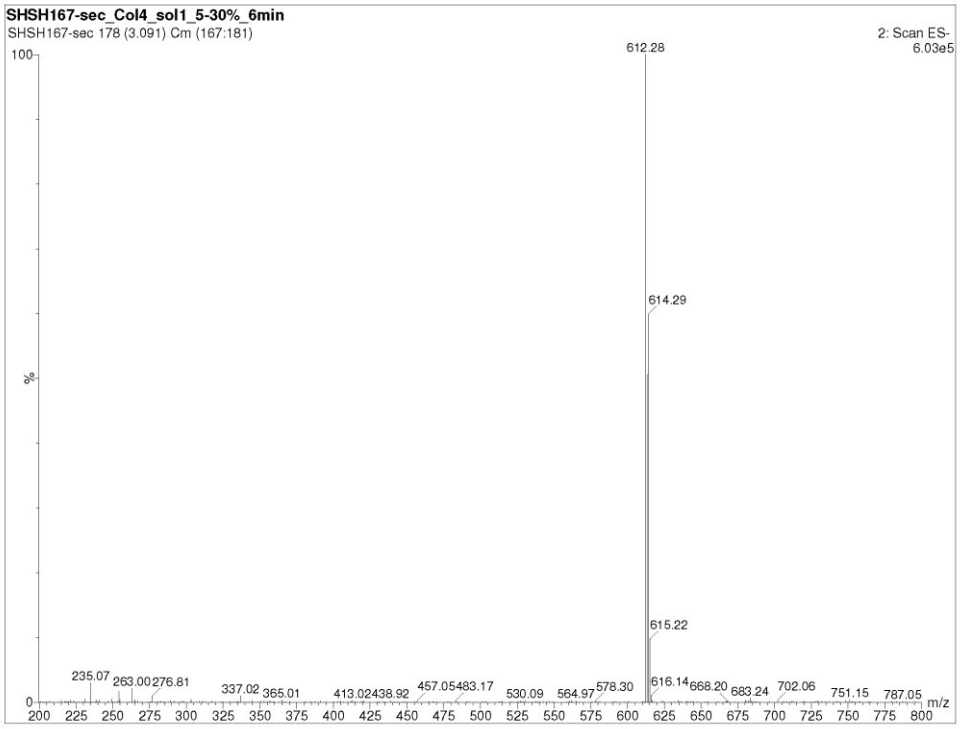


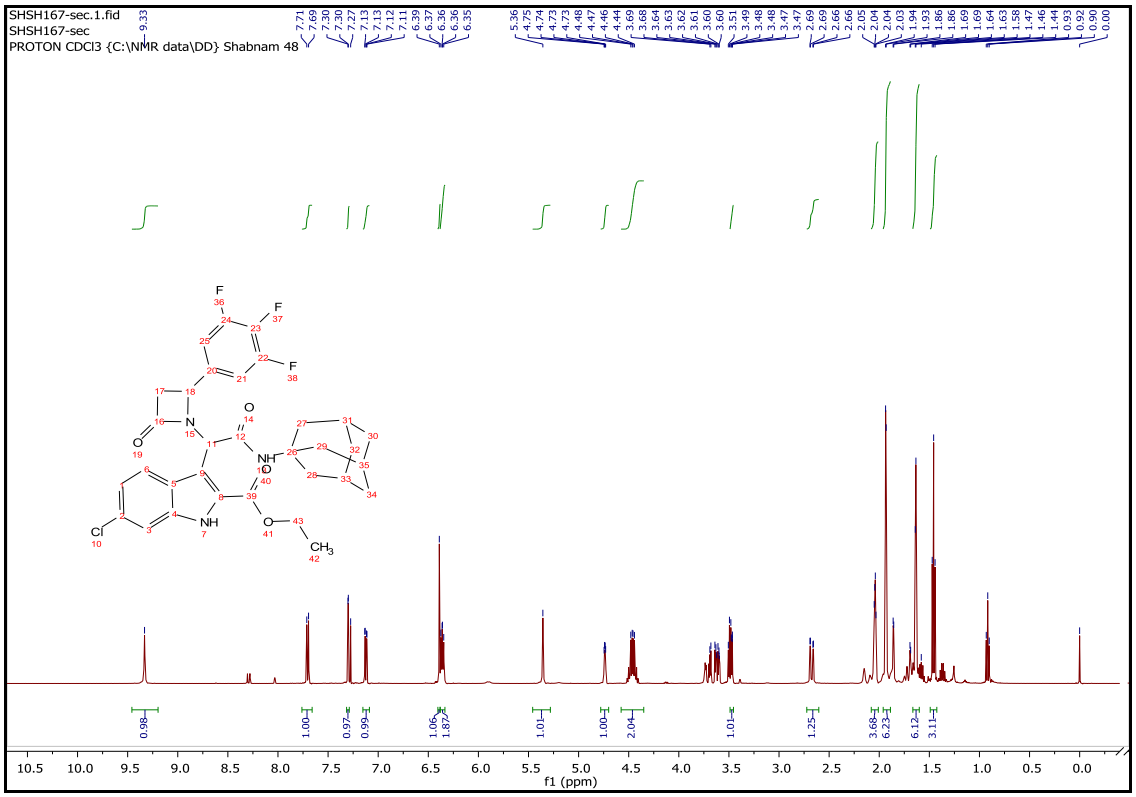

SHSH167-first-1.fid
SHSH167-first
PROTON CDCl3 {C:\NMR data\DD} Shabnam 47
f1 (ppm)

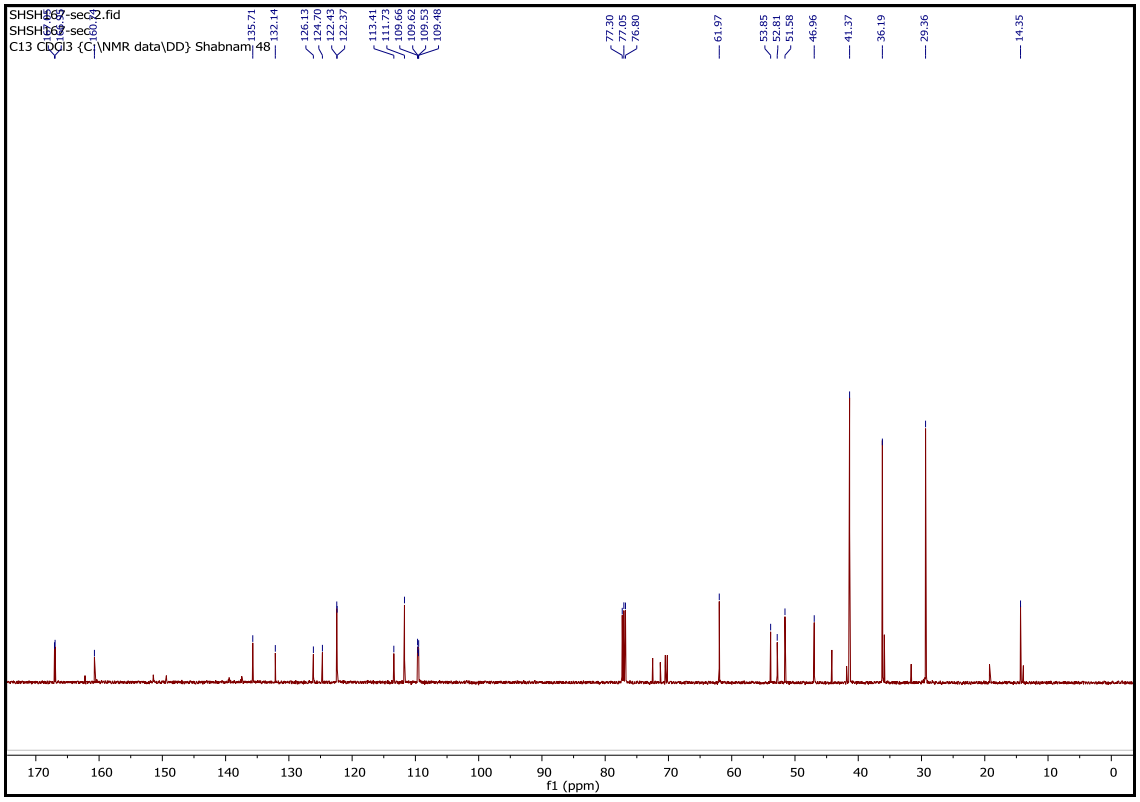

SHSH167-first-C13.fid
SHSH167-first
C13 CDCl3 {C:\NMR data\DD} Shabnam 47
f1 (ppm)

Compound **3e**, *diastereomer B*

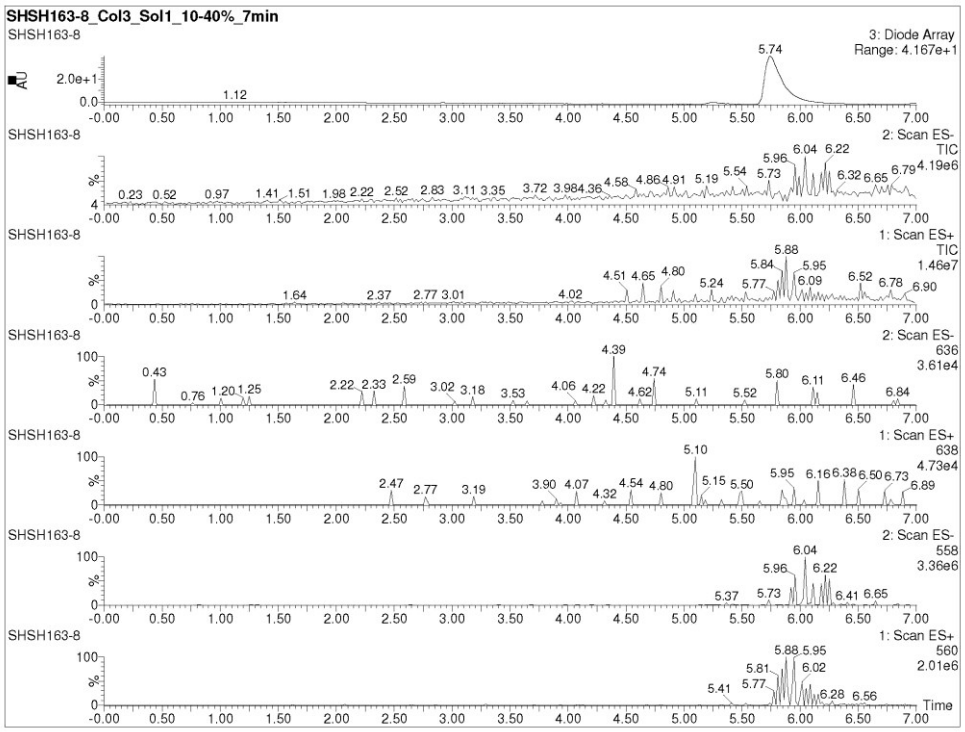


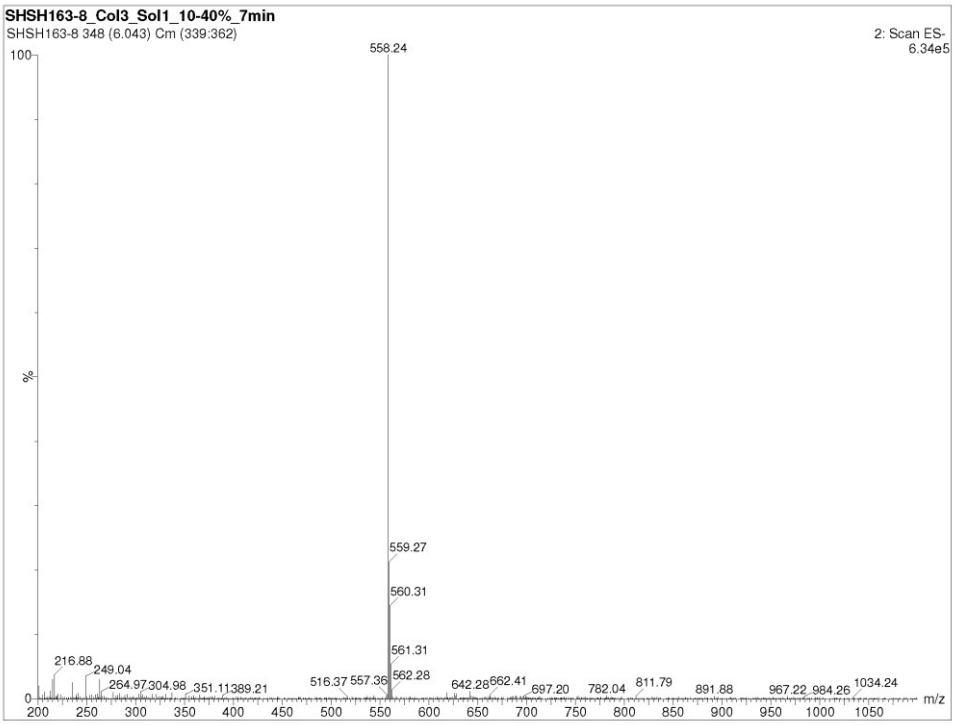


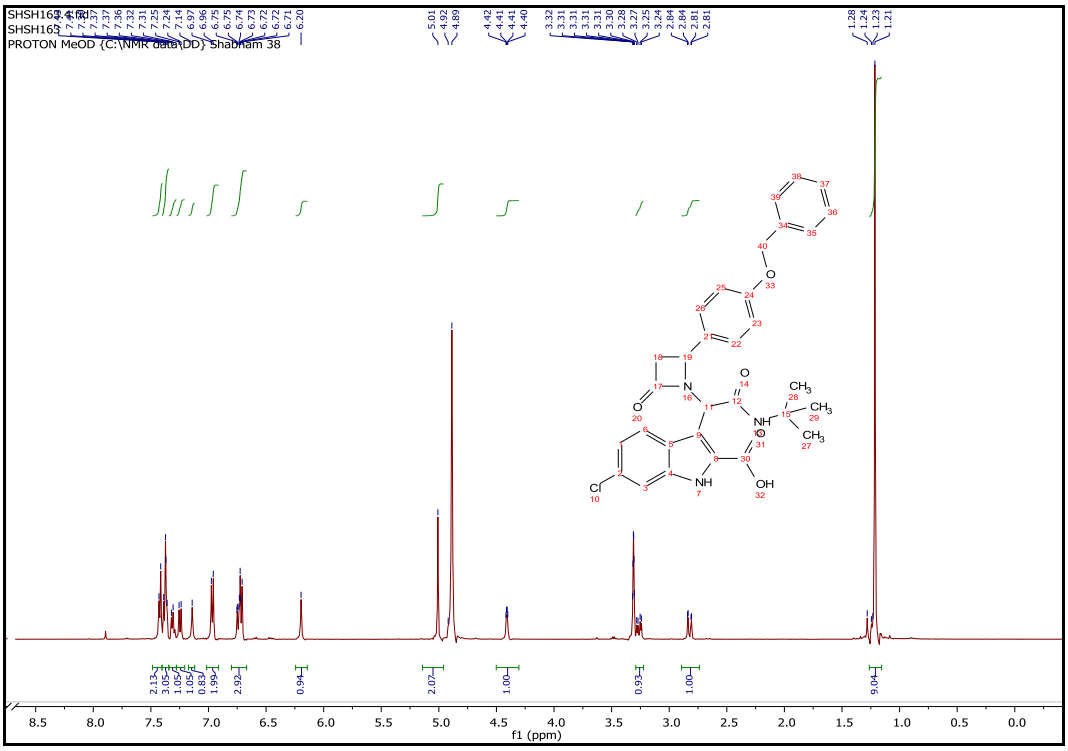

SHSH167-sec.1.fid
SHSH167-sec
PROTON CDCl3 {C:\NMR data\DD} Shabnam 48
f1 (ppm)

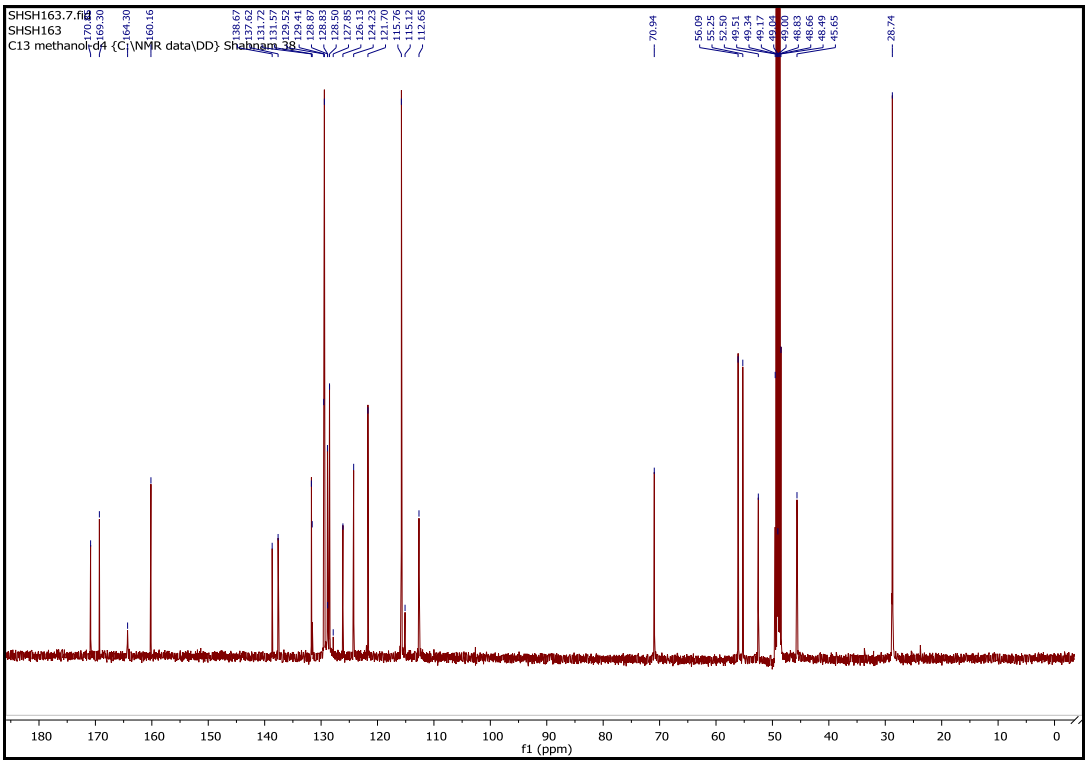

SHSH167-sec2.fid
SHSH167-sec
C13 CDCl3 {C:\NMR data\DD} Shabnam 48
f1 (ppm)

Compound **2a**, *diastereomer A*

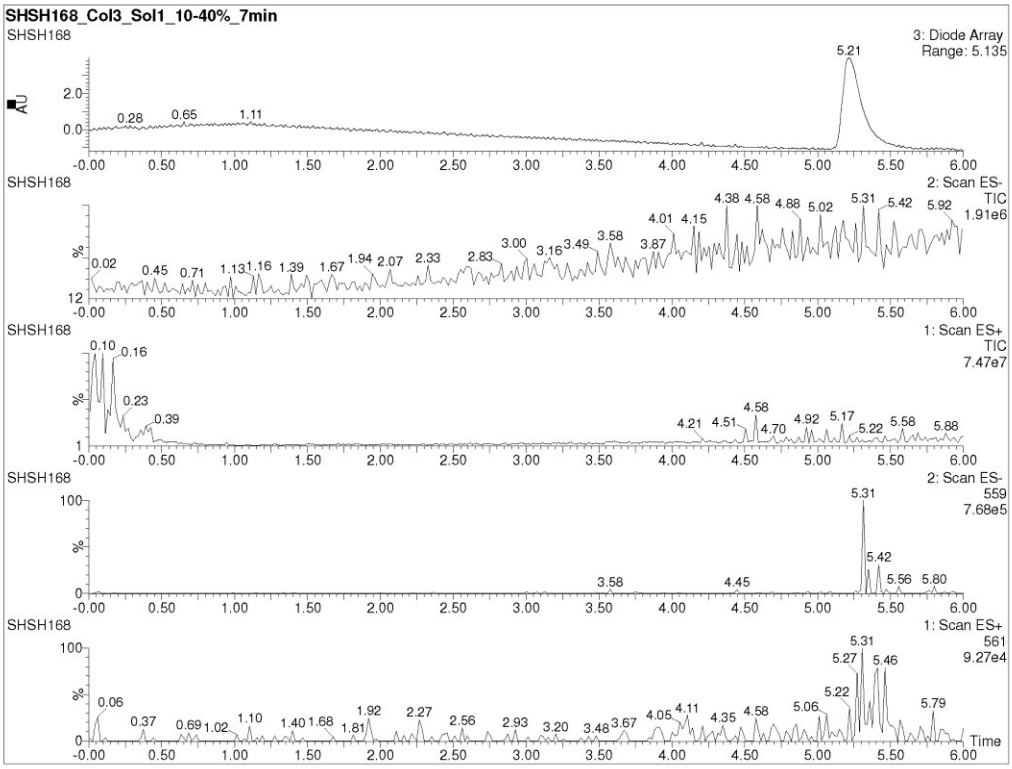


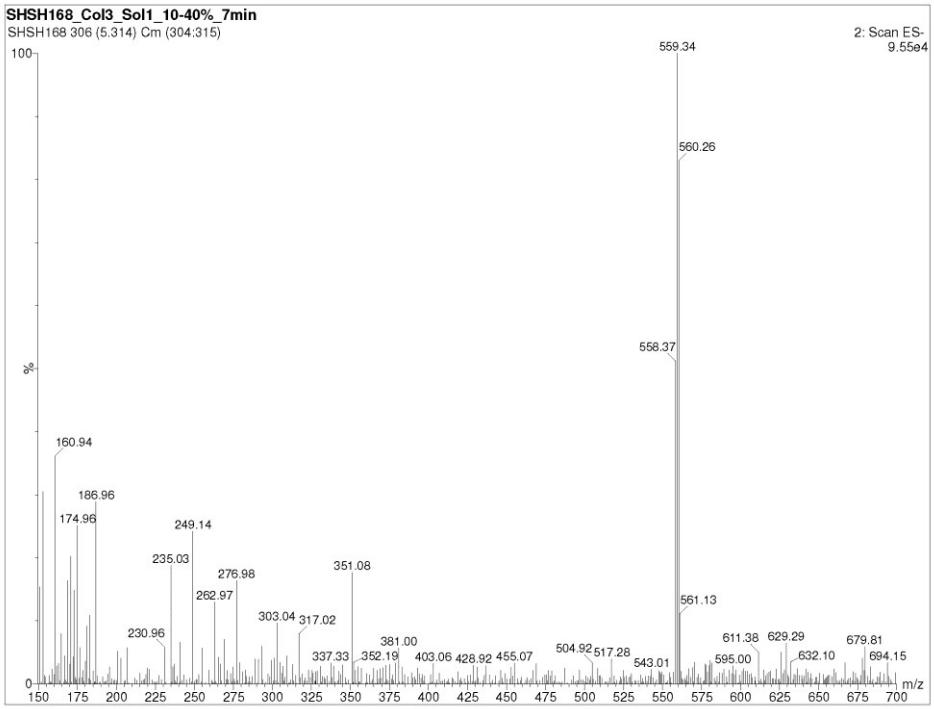


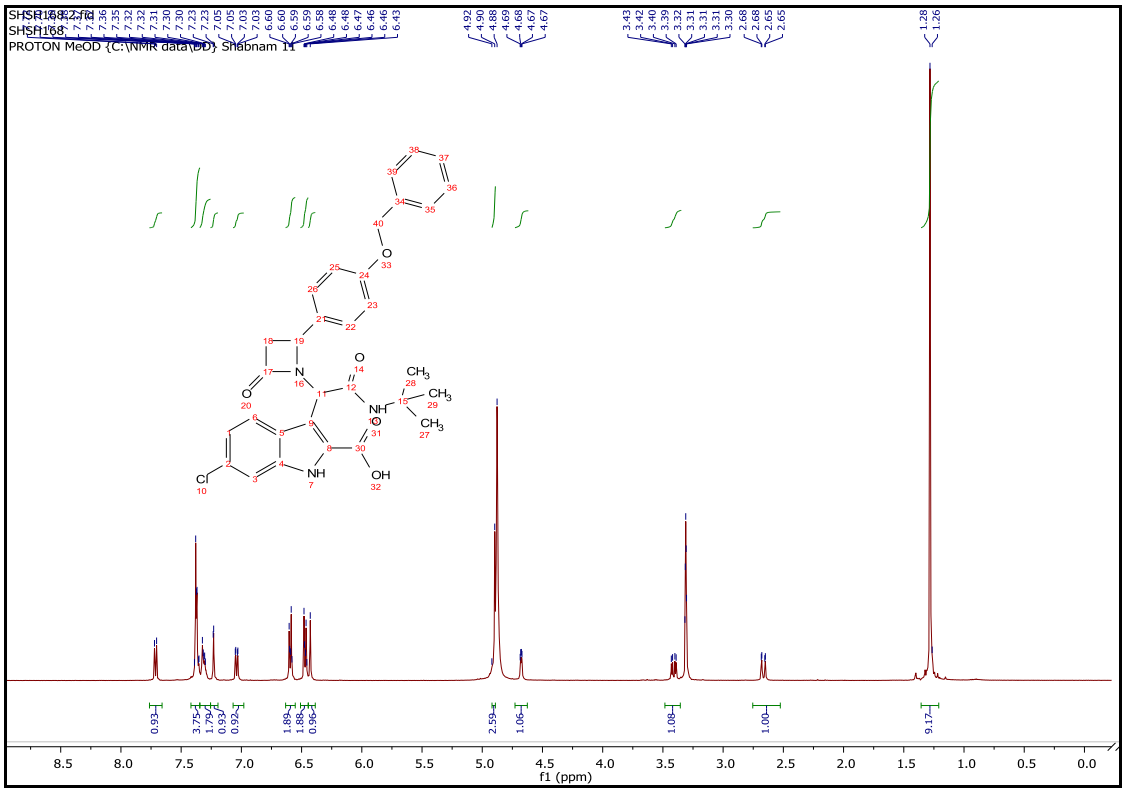

SHSH163.4.fid
SHSH163
PROTON MeOD {C:\NMR data\DD} Shabnam 38
f1 (ppm)

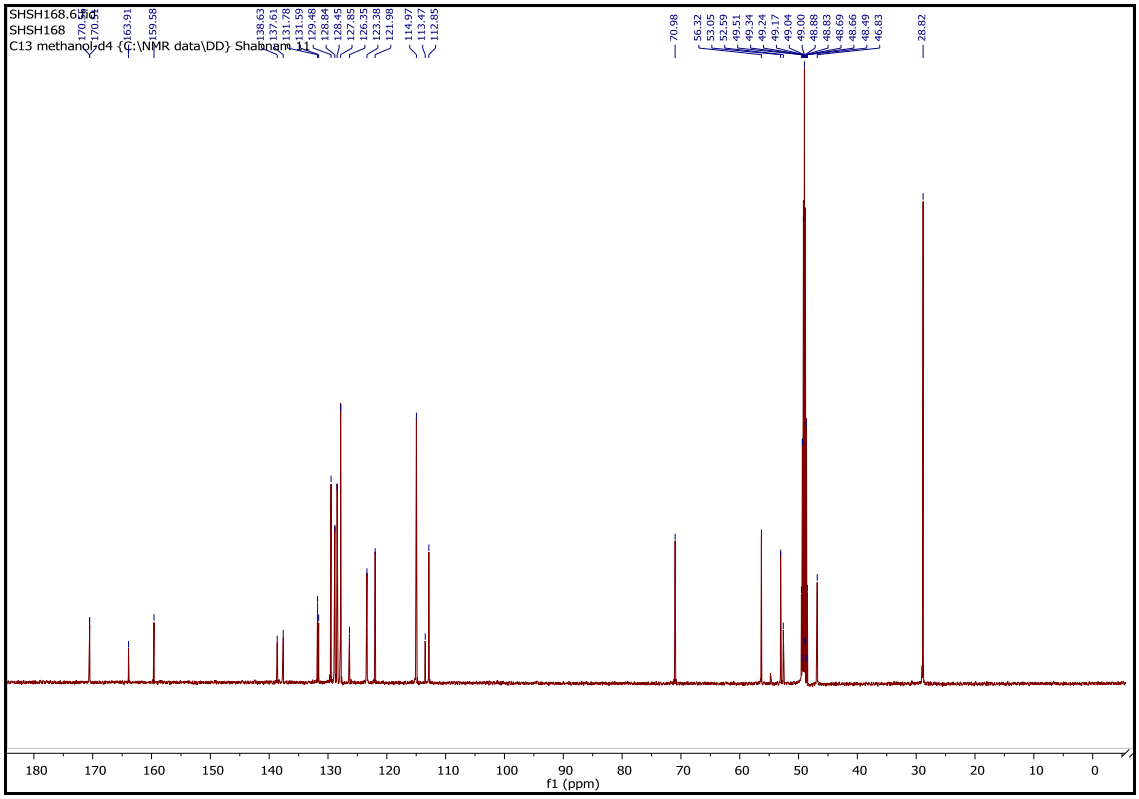

SHSH163.7.fid
SHSH163
C13 methanol-d4 {C:\NMR data\DD} Shabnam 38
f1 (ppm)

Compound **2a**, *diastereomer B*

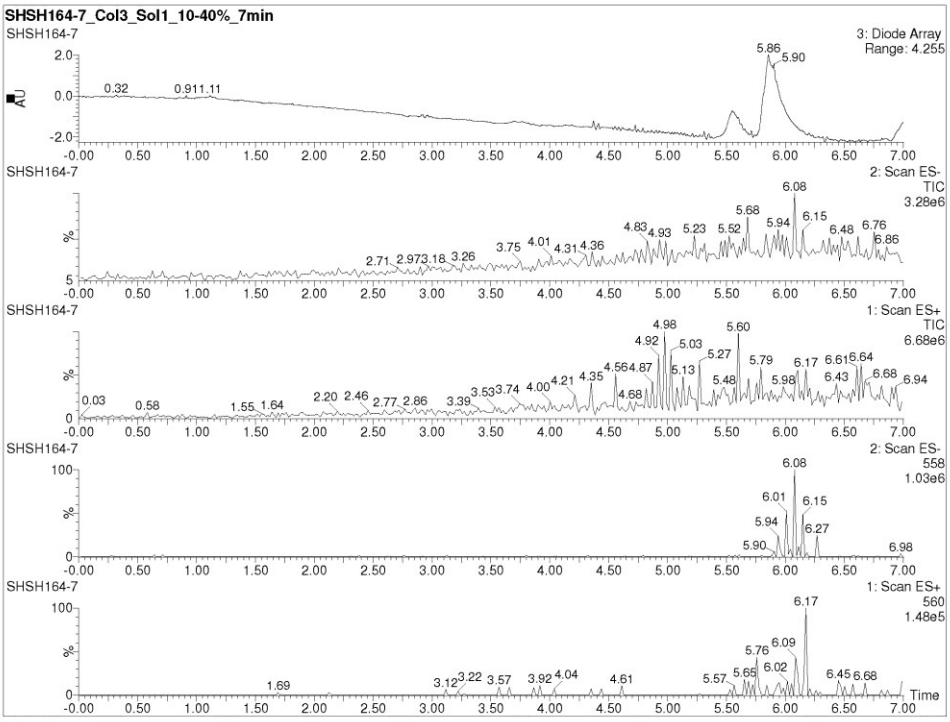


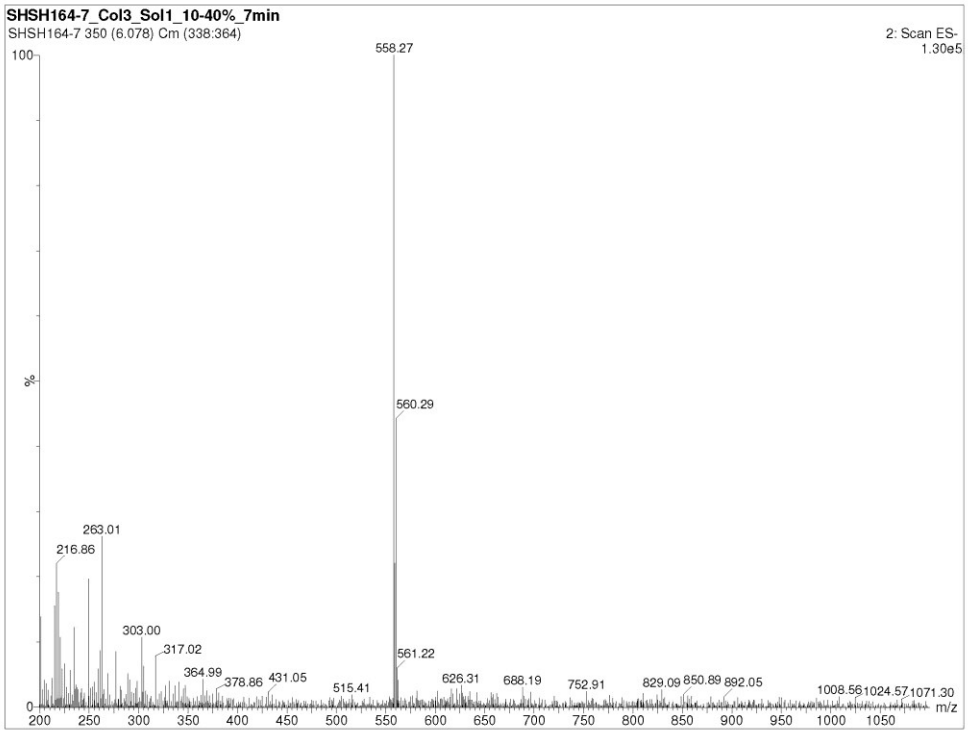


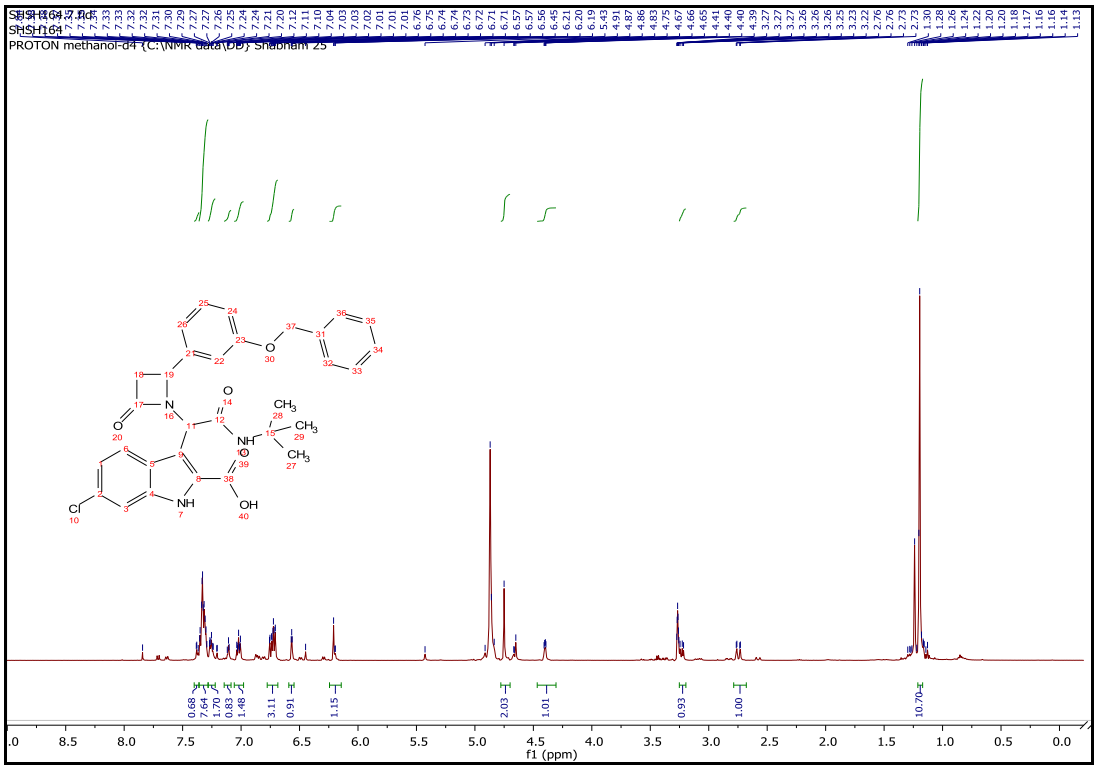

SHSH168
PROTON MeOD {C:\NMR data\DD} Shabnam 11
f1 (ppm)

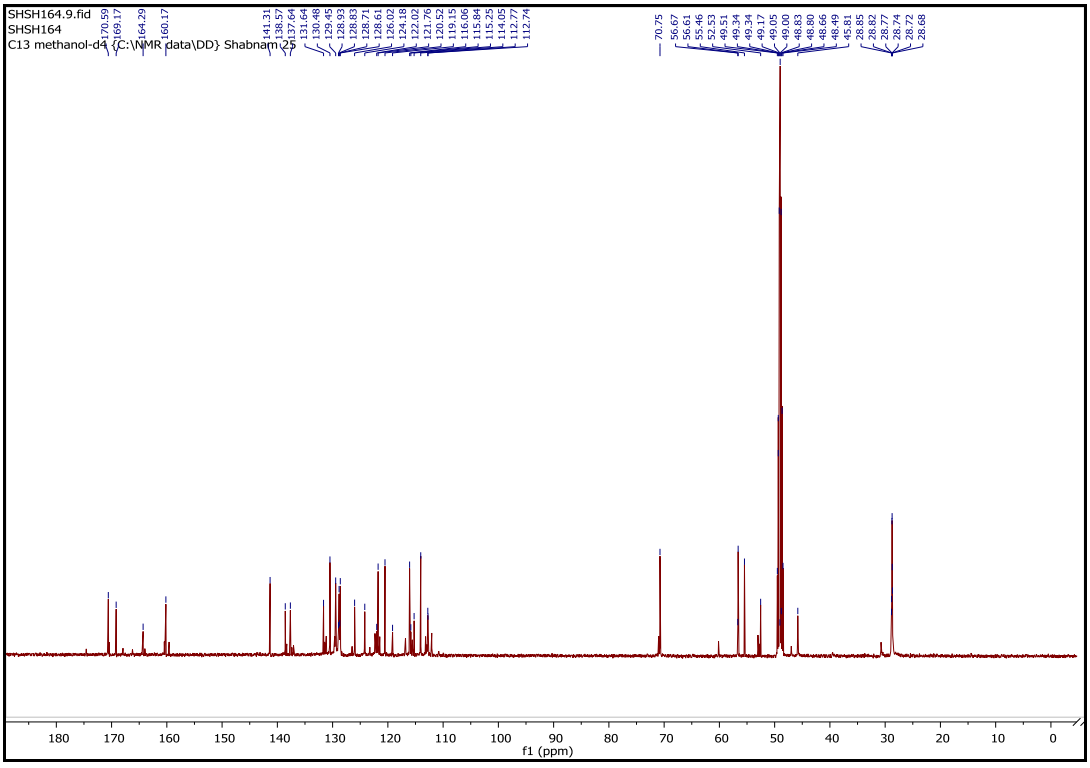

SHSH168
C13 methanol-d4 {C:\NMR data\DD} Shabnam 11
f1 (ppm)

Compound **2b**, *diastereomer A*

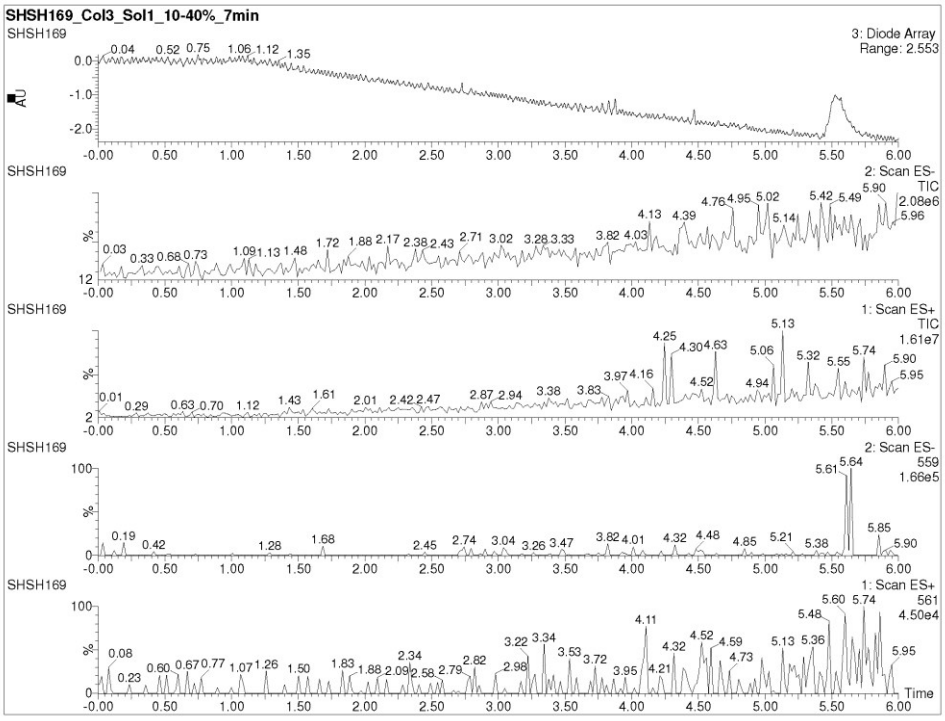


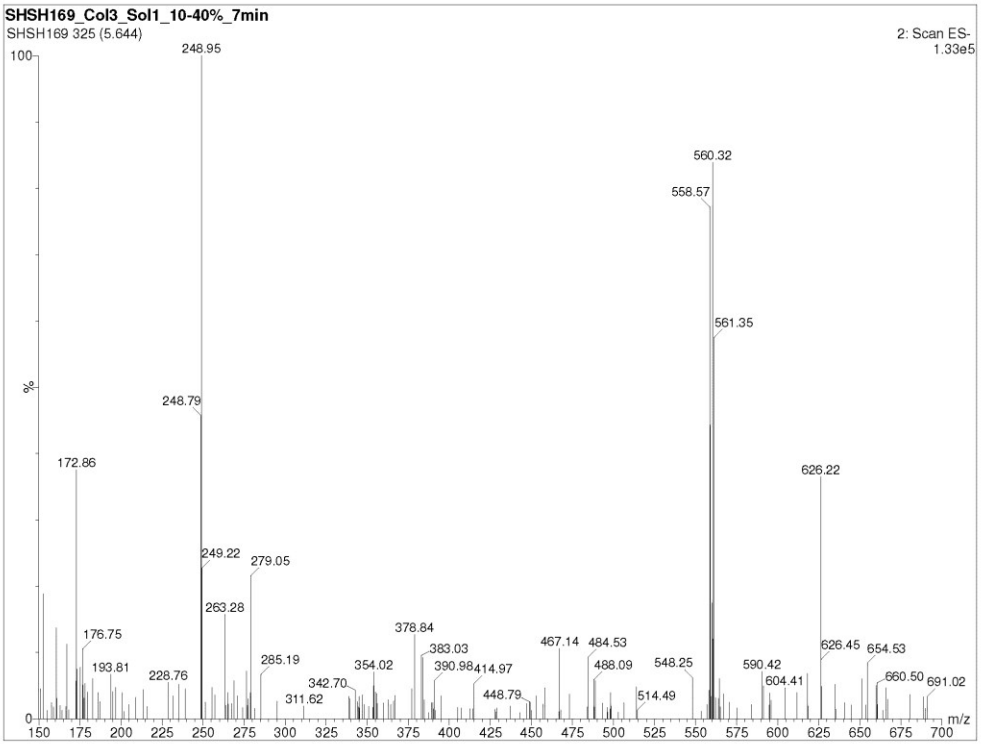


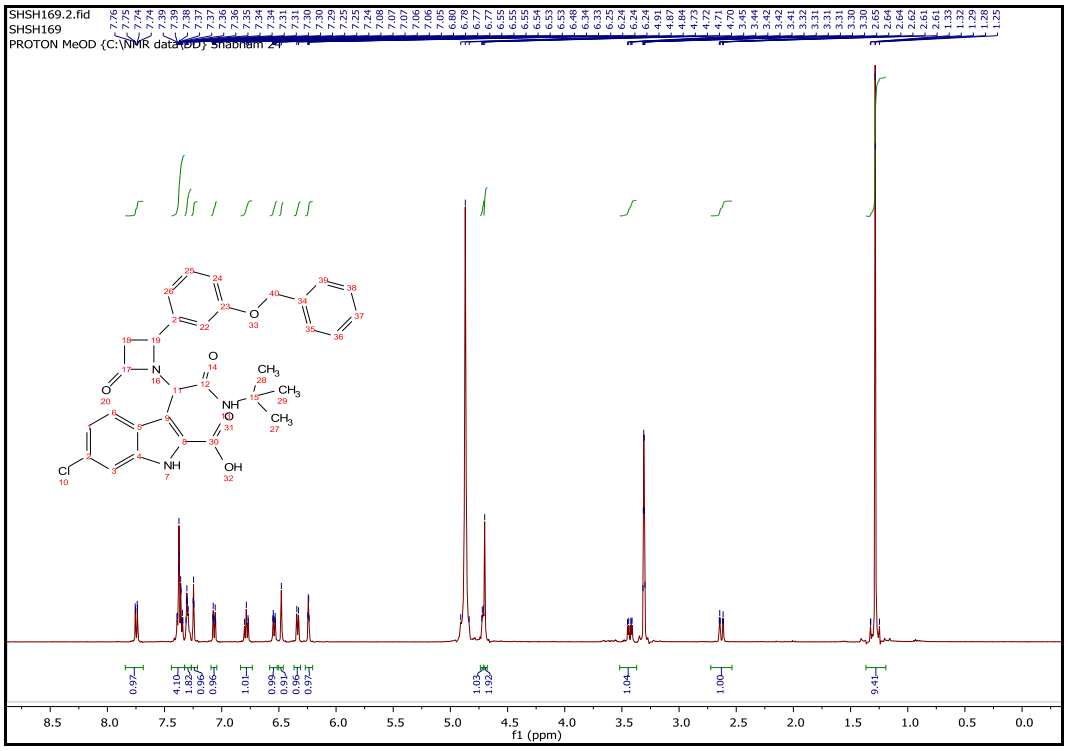

SHSH164.7.fid
SHSH164
PROTON methanol-d4 {C:\NMR data\DD} Shabnam 25
f1 (ppm)

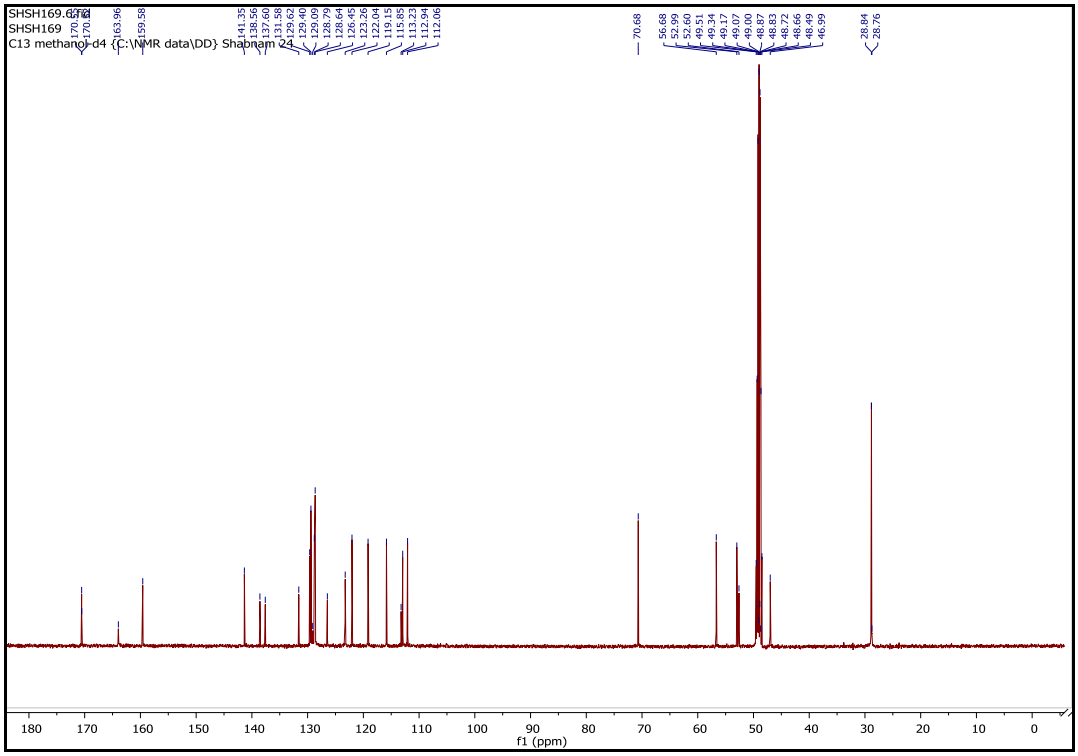

SHSH164.9.fid
SHSH164
C13 methanol-d4 {C:\NMR data\DD} Shabnam 25
f1 (ppm)

Compound **2b**, *diastereomer B*

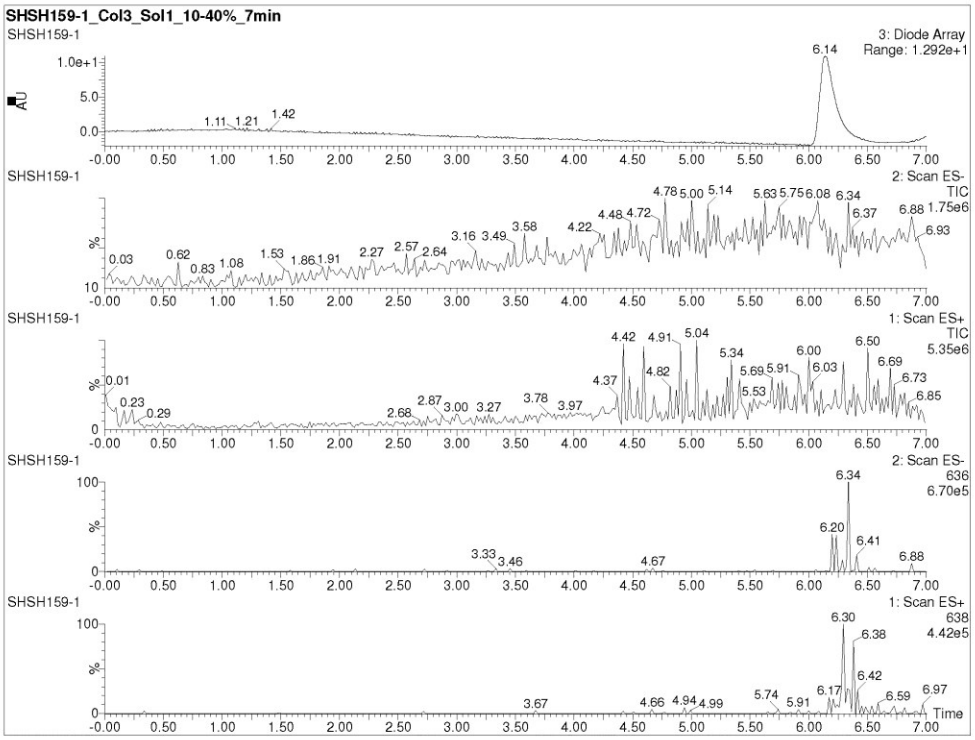


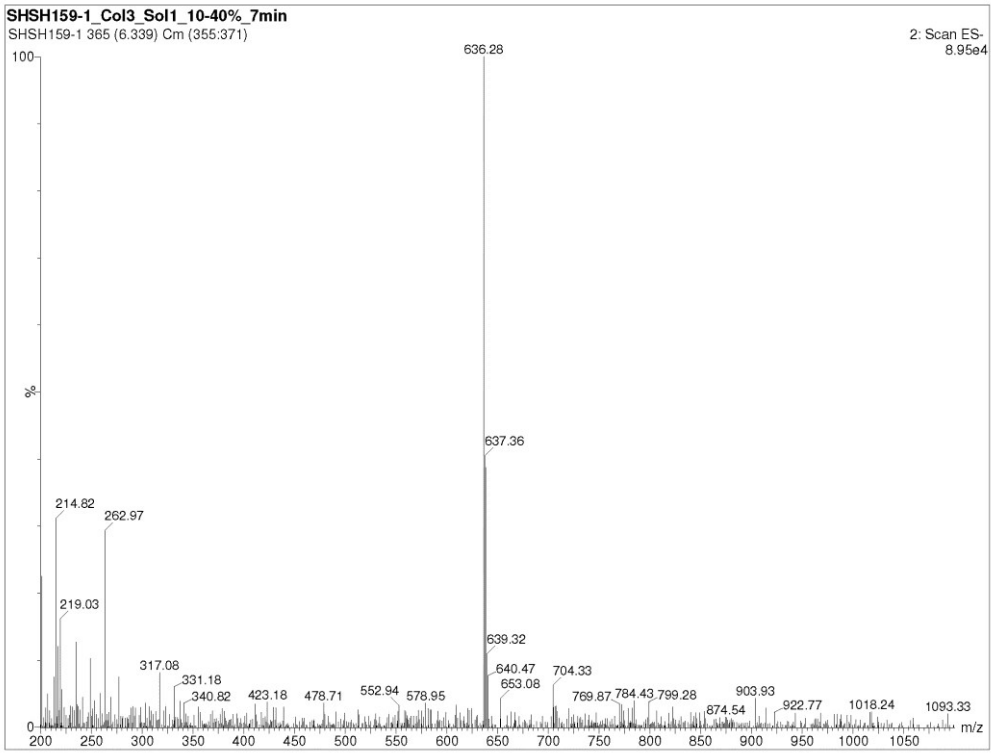


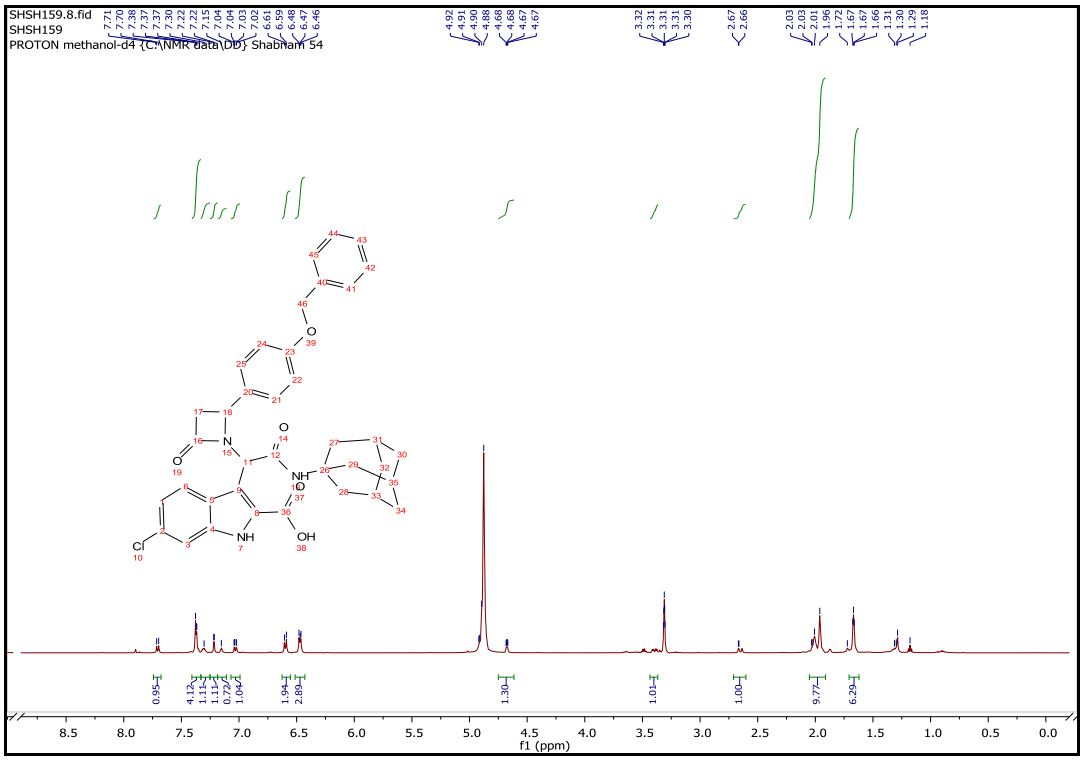

SHSH169.2.fid
SHSH169
PROTON MeOD {C:\NMR data\DD} Shabnam 24
f1 (ppm)

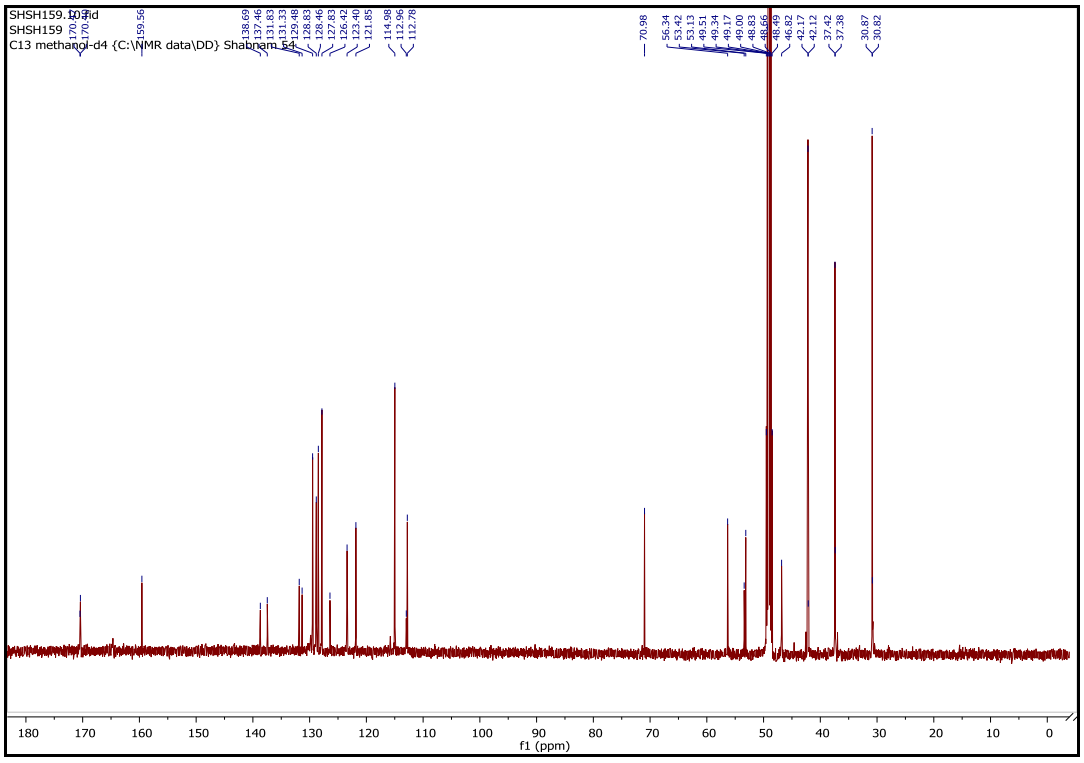

SHSH169.6.fid
SHSH169
C13 methanol-d4 {C:\NMR data\DD} Shabnam 24
f1 (ppm)

Compound **2c**, *diastereomer A*

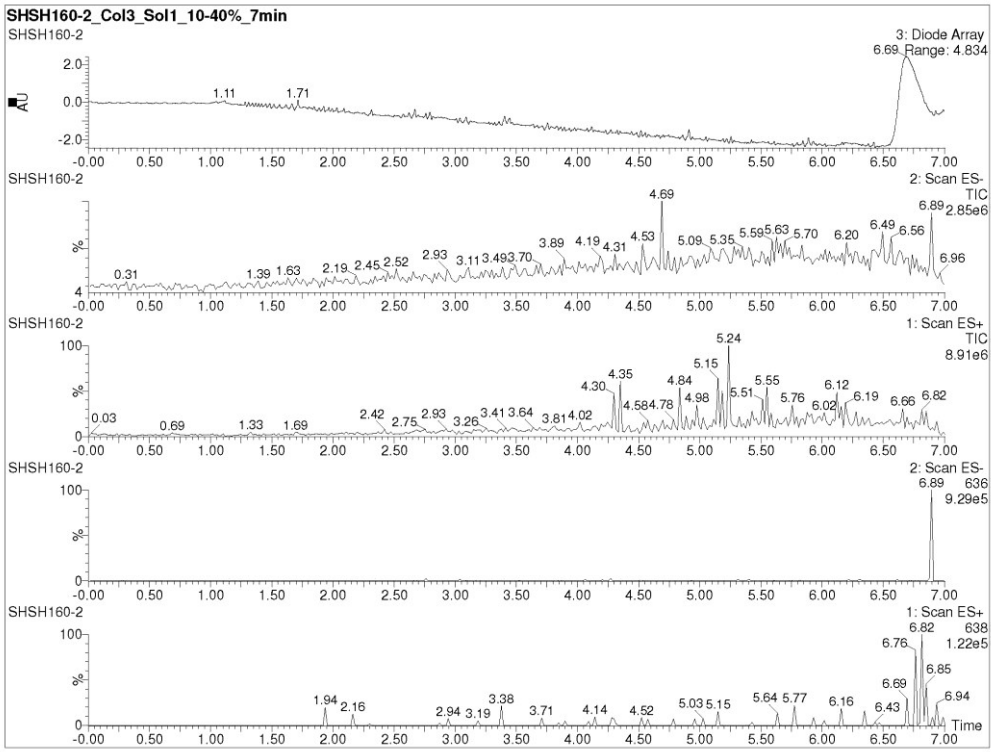


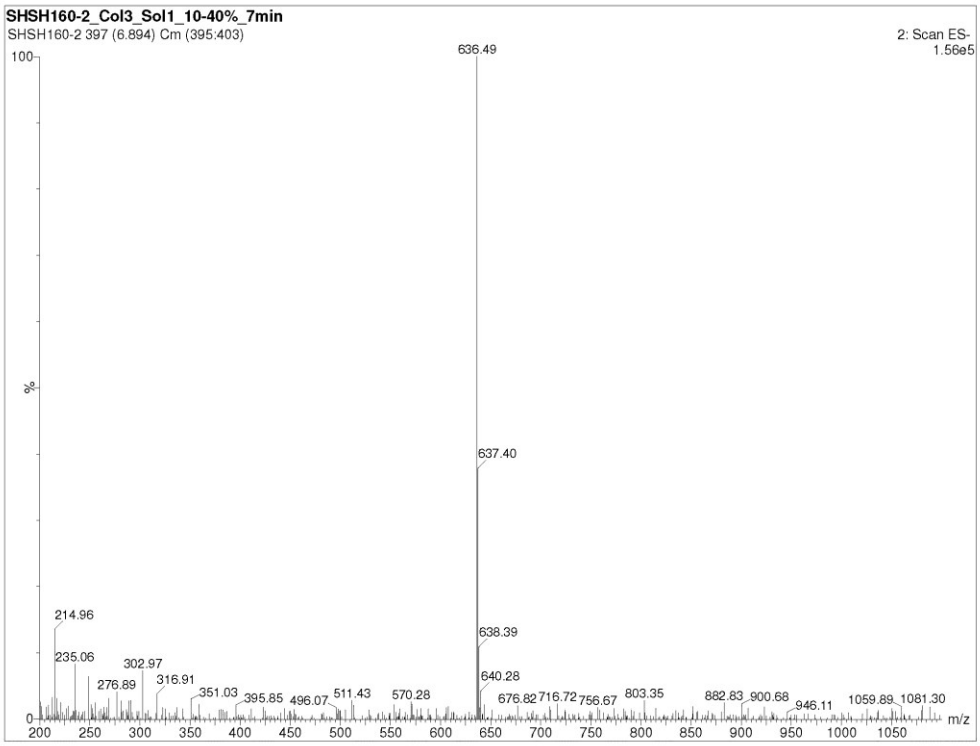


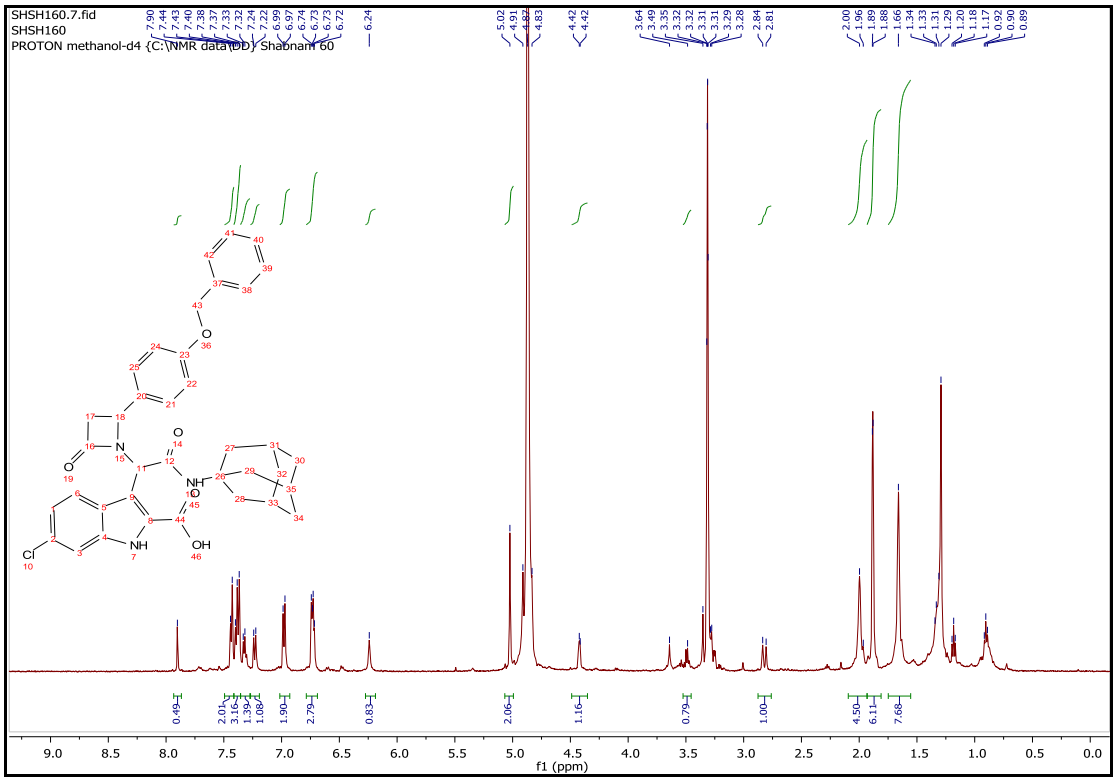

SHSH159.8.fid
SHSH159
PROTON methanol-d4 {C:\NMR data\DD} Shabnam 54
f1 (ppm)

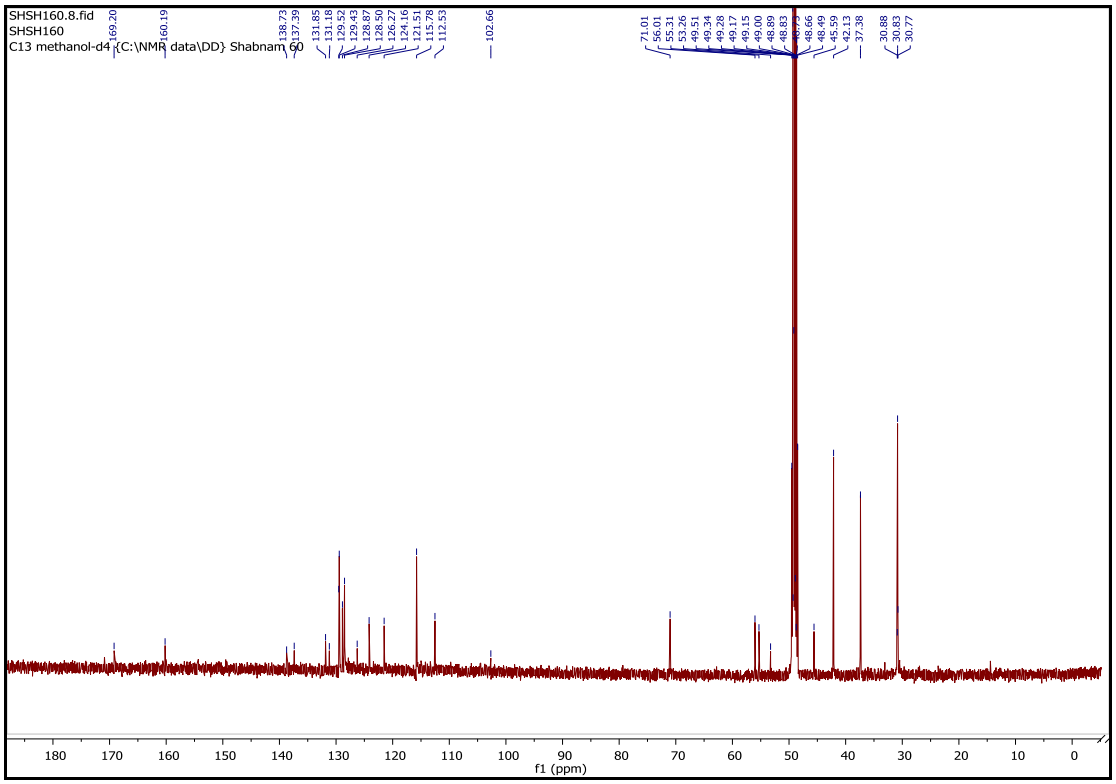

SHSH159.10.fid
SHSH159
C13 methanol-d4 {C:\NMR data\DD} Shabnam 54
f1 (ppm)

Compound **2c**, *diastereomer B*

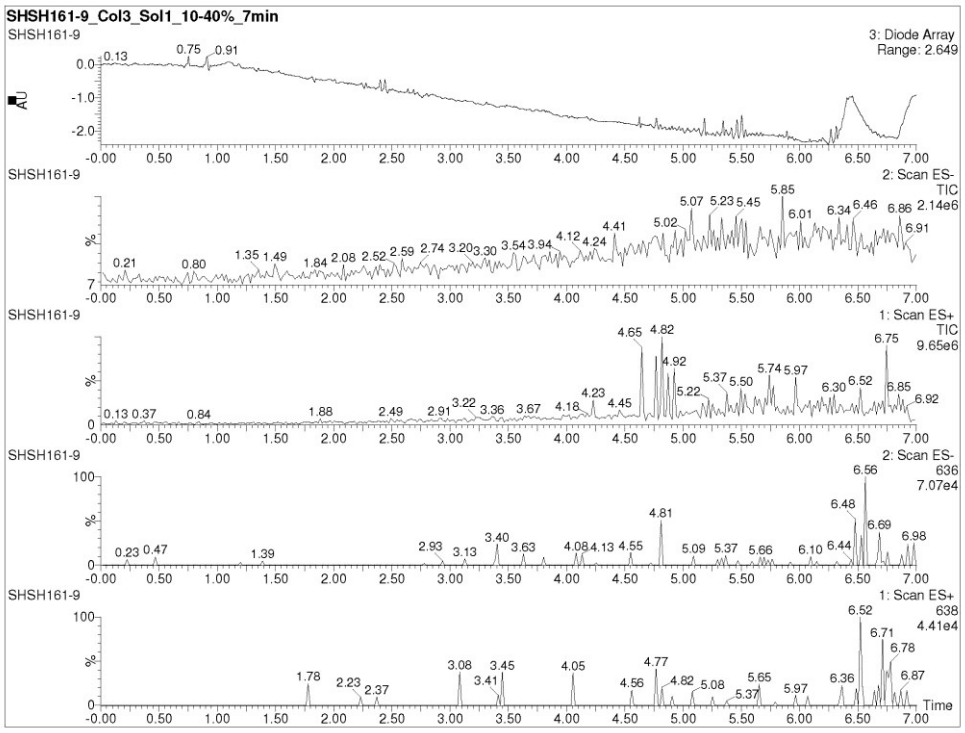


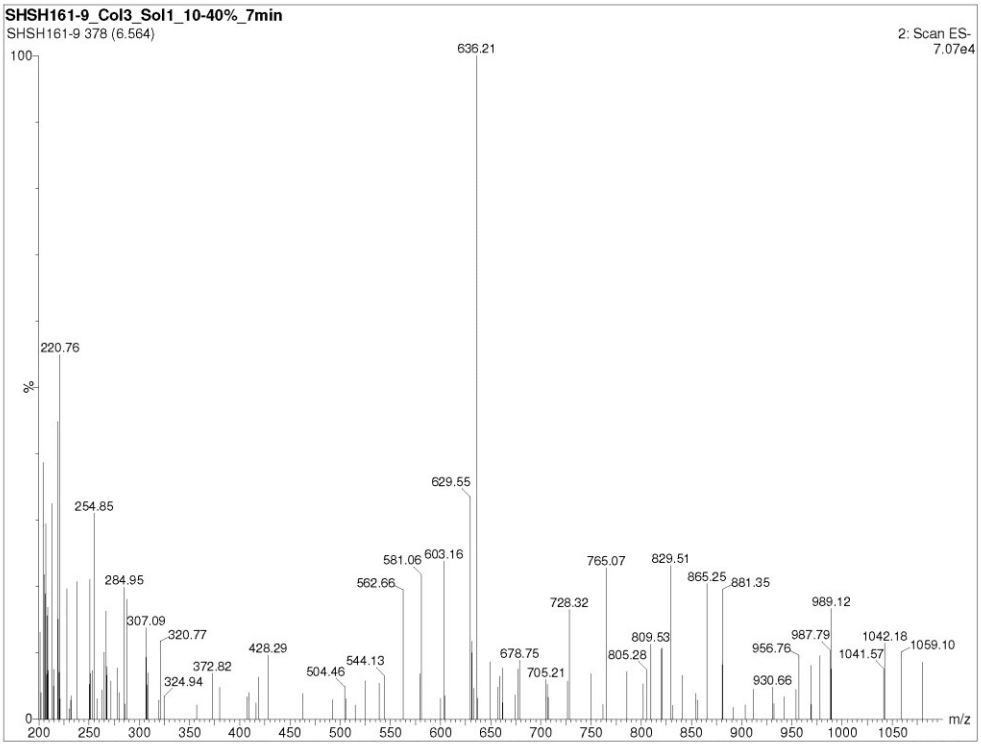


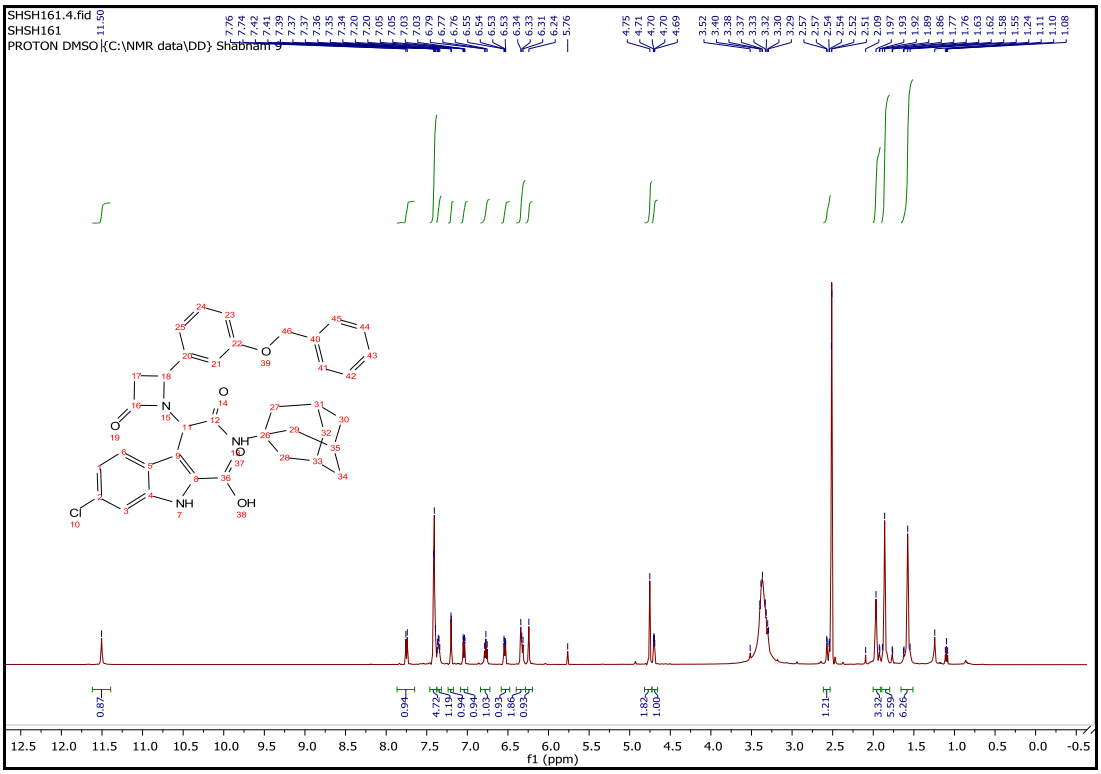

SHSH160.7.fid
SHSH160
PROTON methanol-d4 {C:\NMR data\DD} Shabnam 60
f1 (ppm)

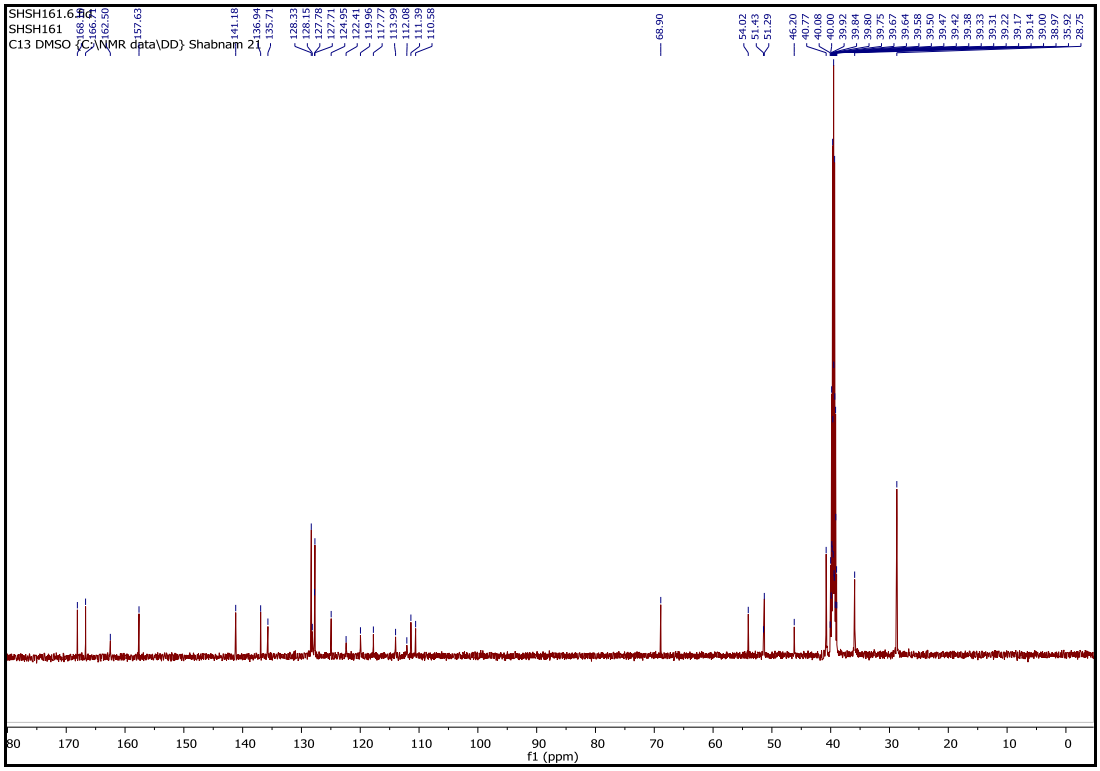

SHSH160.8.fid
SHSH160
C13 methanol-d4 {C:\NMR data\DD} Shabnam 60
f1 (ppm)

Compound **2d**, *diastereomer A*

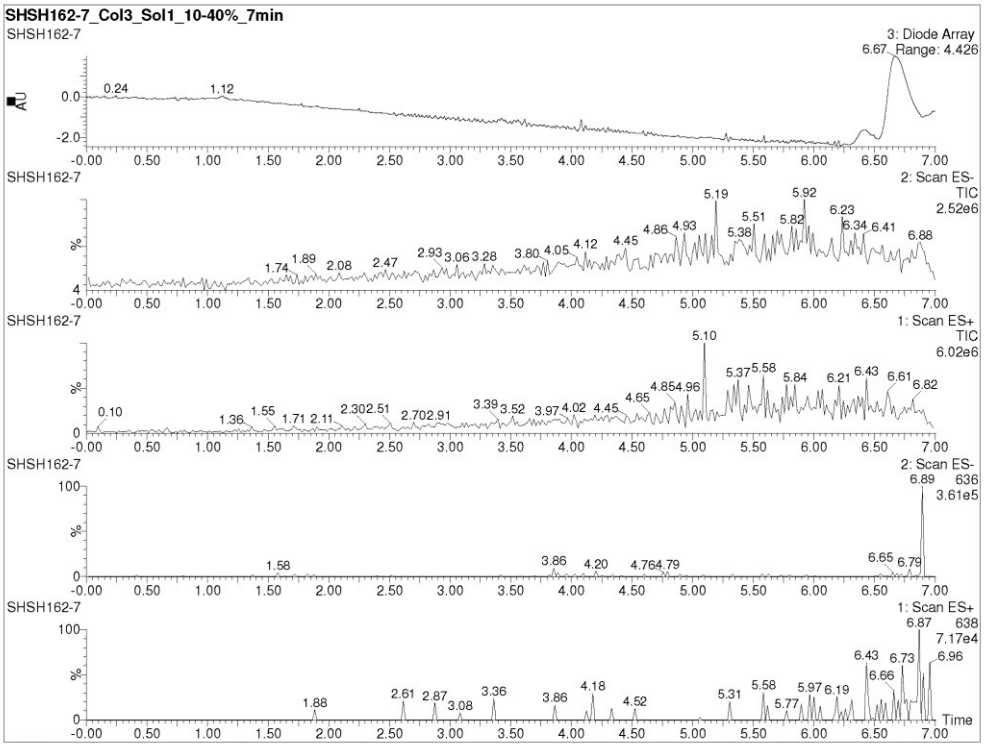


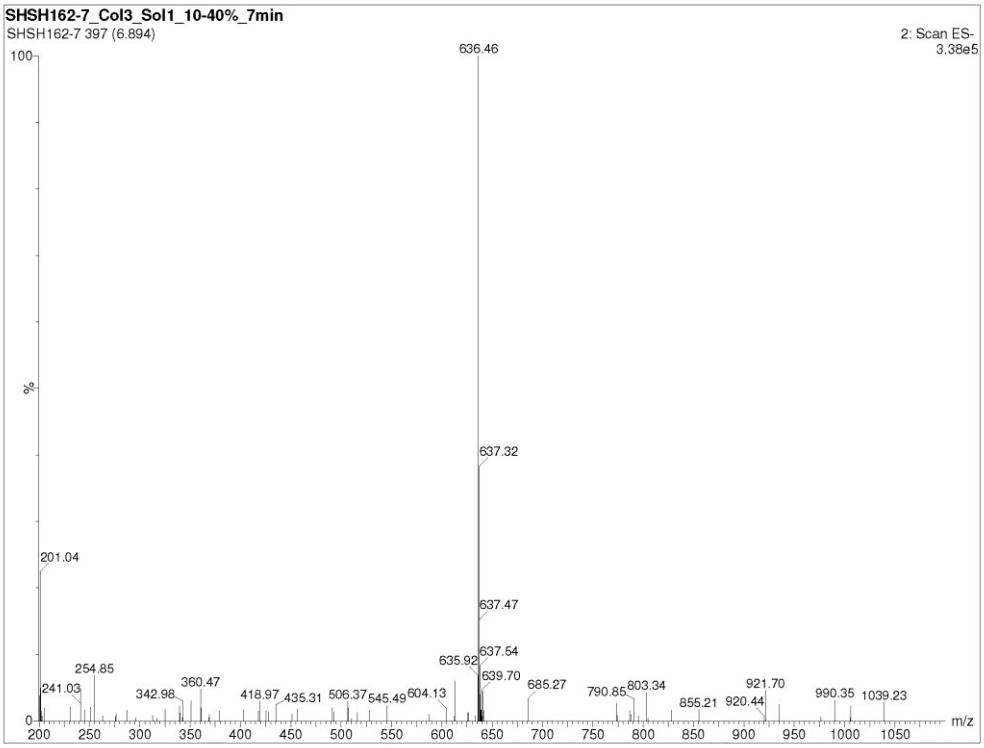


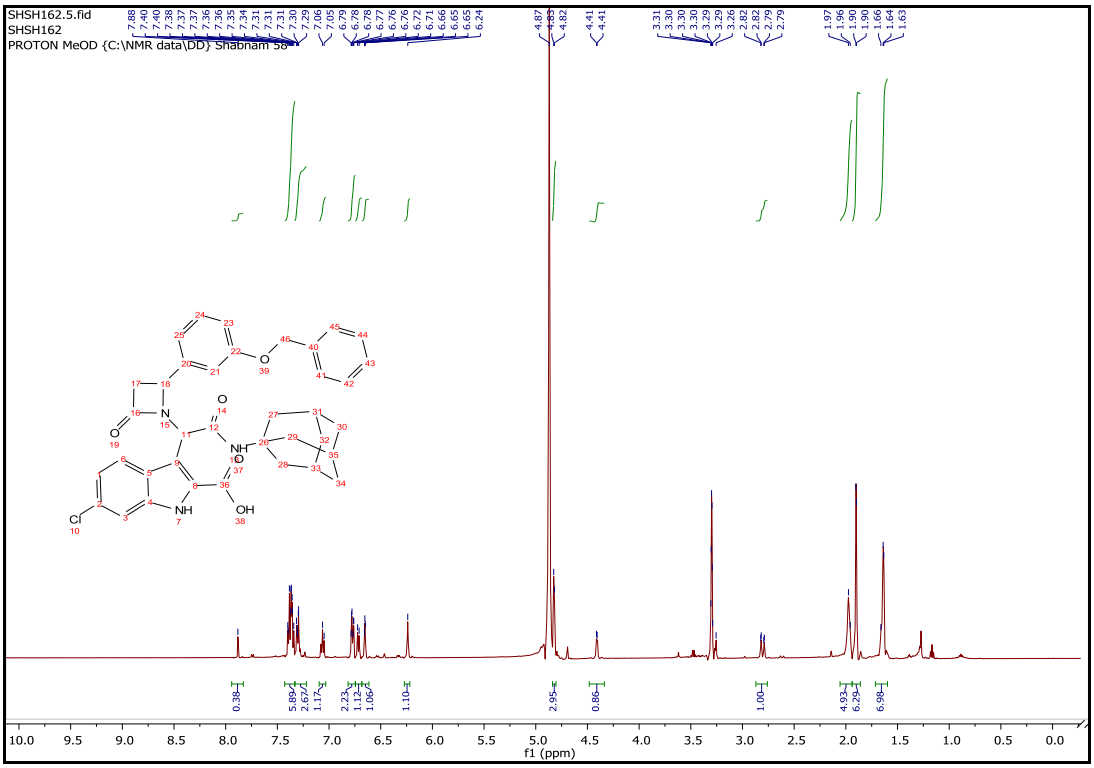

SHSH161.4.fid
SHSH161
PROTON DMSO {C:\NMR data\DD} Shabnam 9
f1 (ppm)

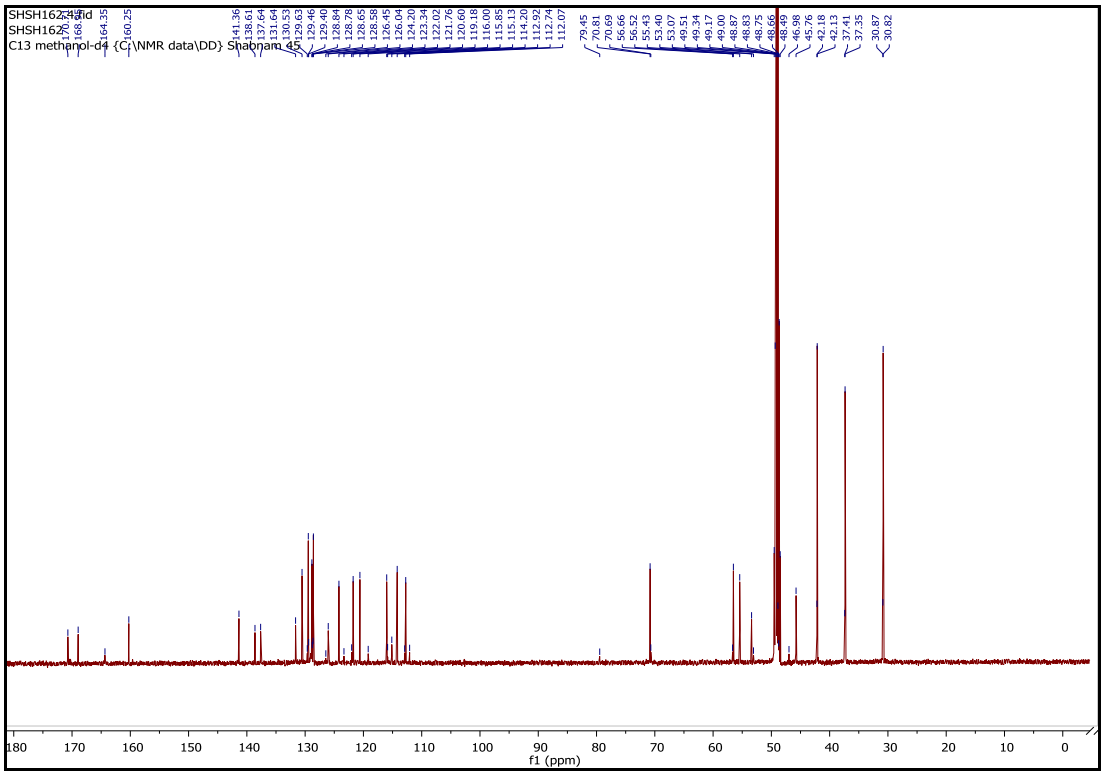

SHSH161.63.fid
SHSH161
C13 DMSO {C:\NMR data\DD} Shabnam 21
f1 (ppm)

Compound **2d**, *diastereomer B*

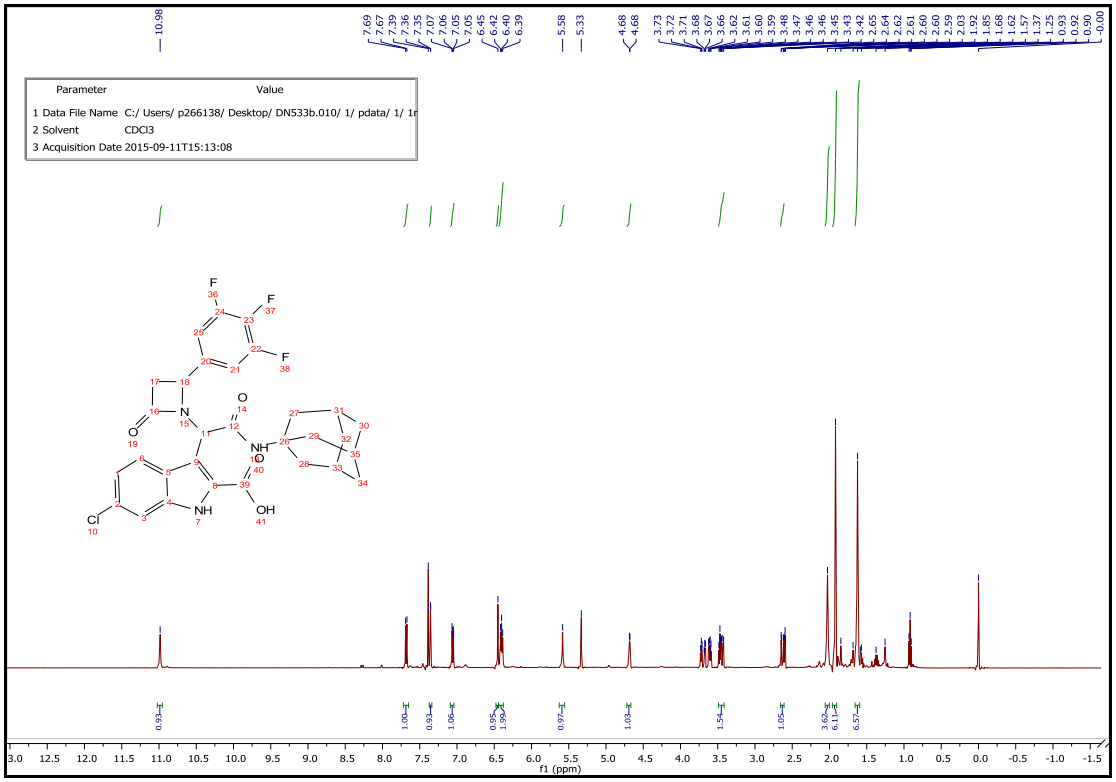


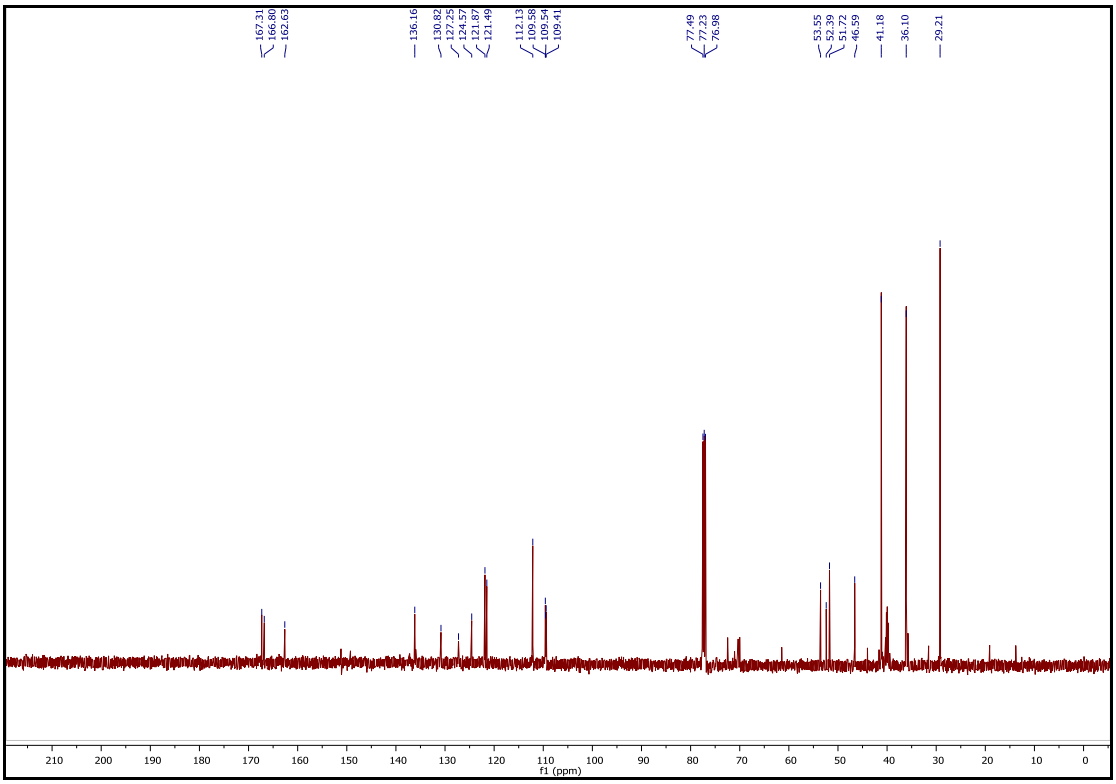



SHSH162.5.fid
SHSH162
PROTON MeOD {C:\NMR data\DD} Shabnam 58
f1 (ppm)

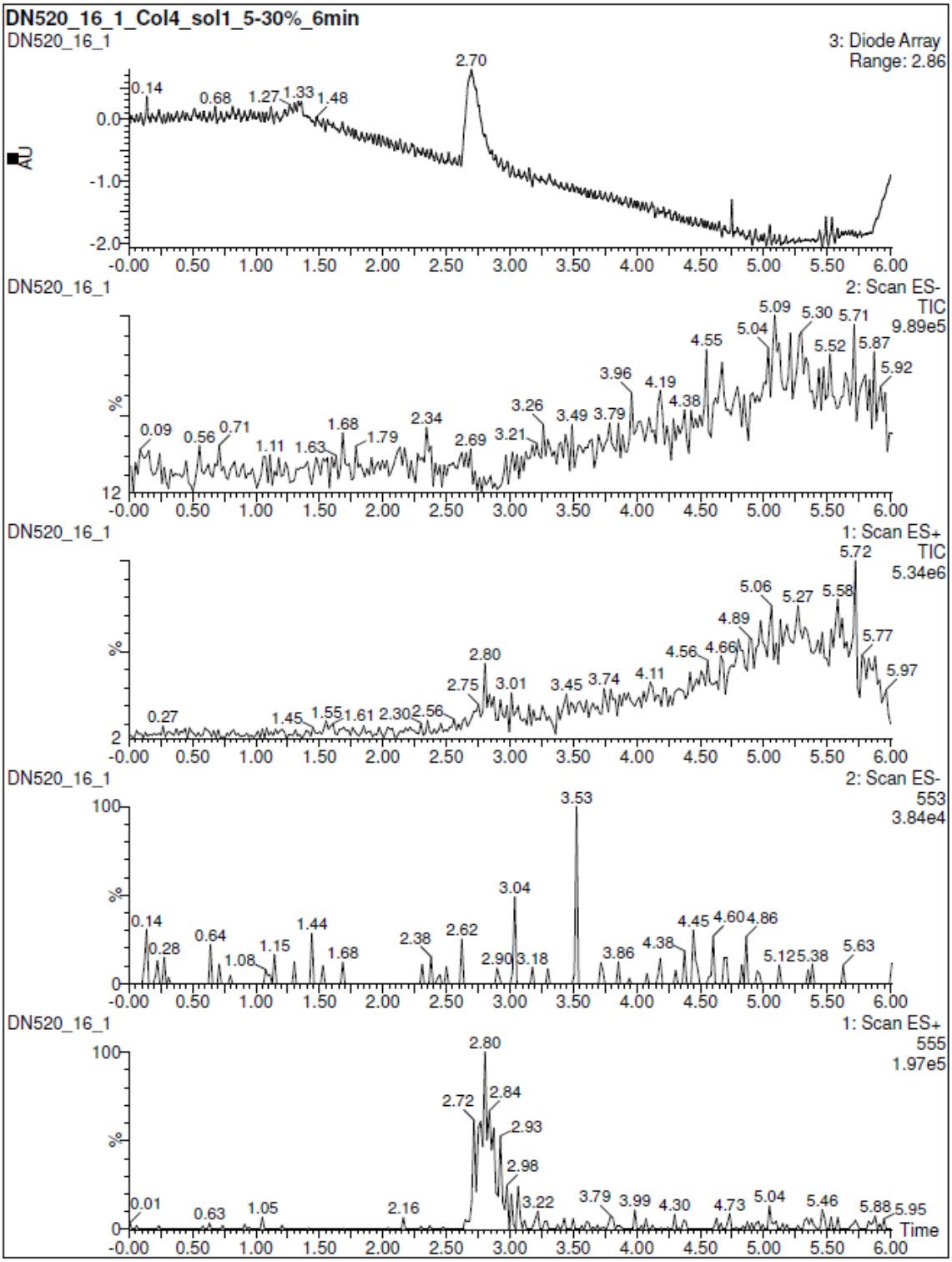

SHSH162.11.fid
SHSH162
C13 methanol-d4 {C:\NMR data\DD} Shabnam 45
f1 (ppm)

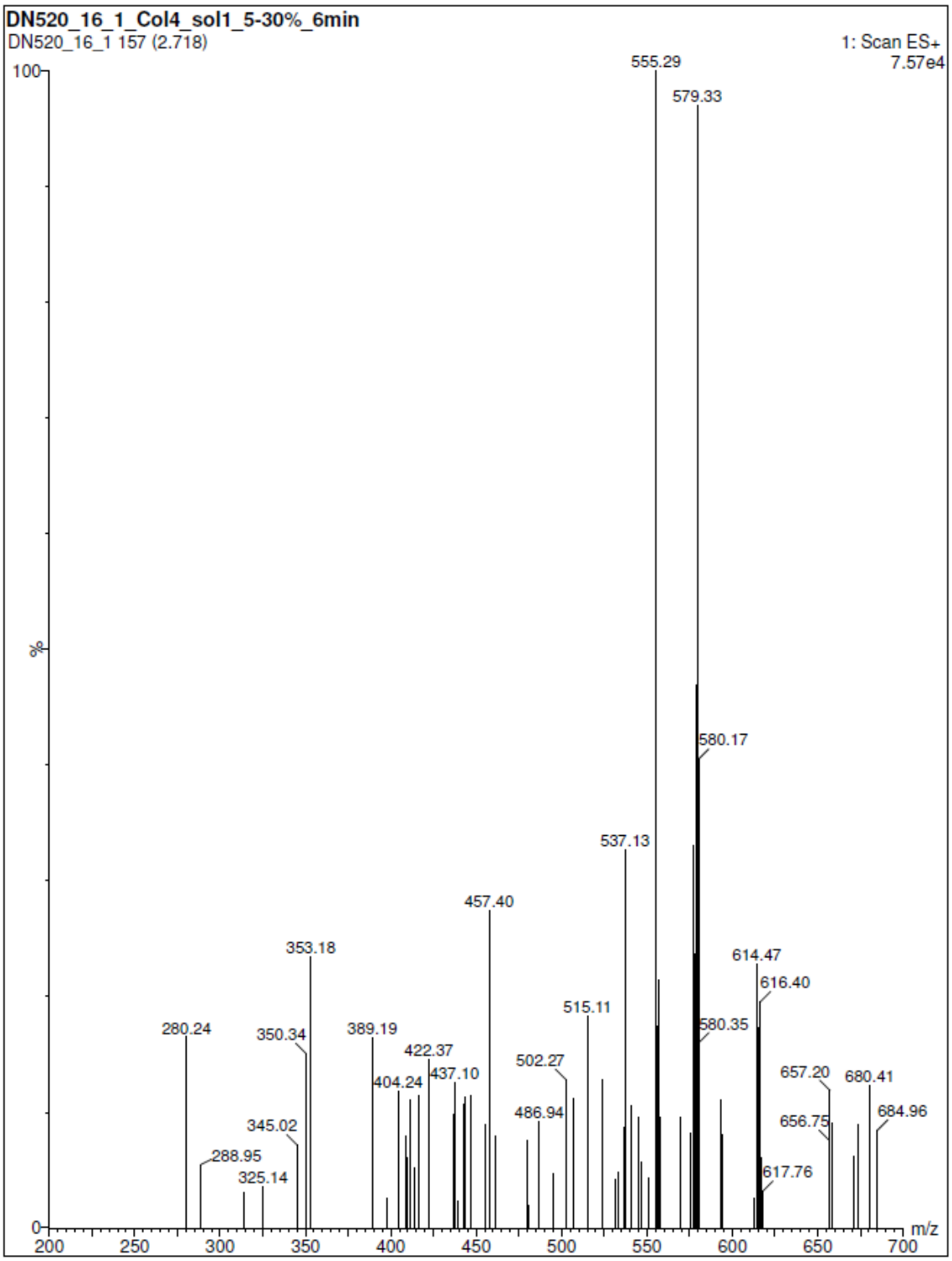
Compound **2e**, *diastereomer A*

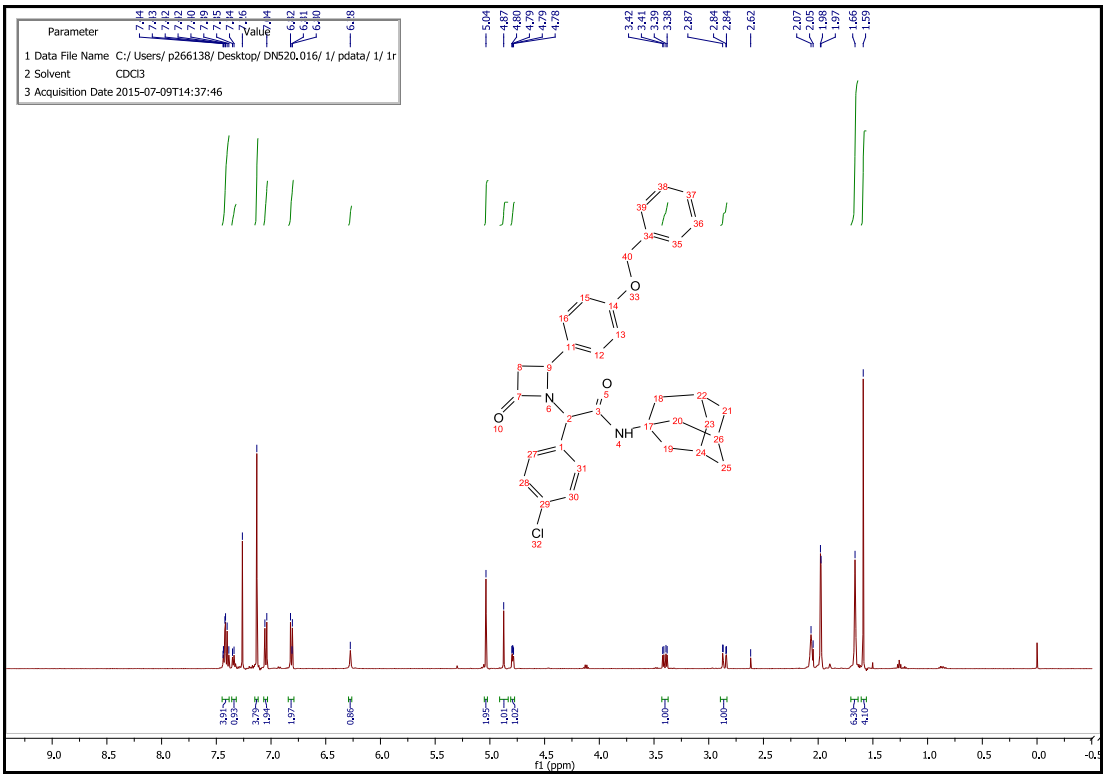


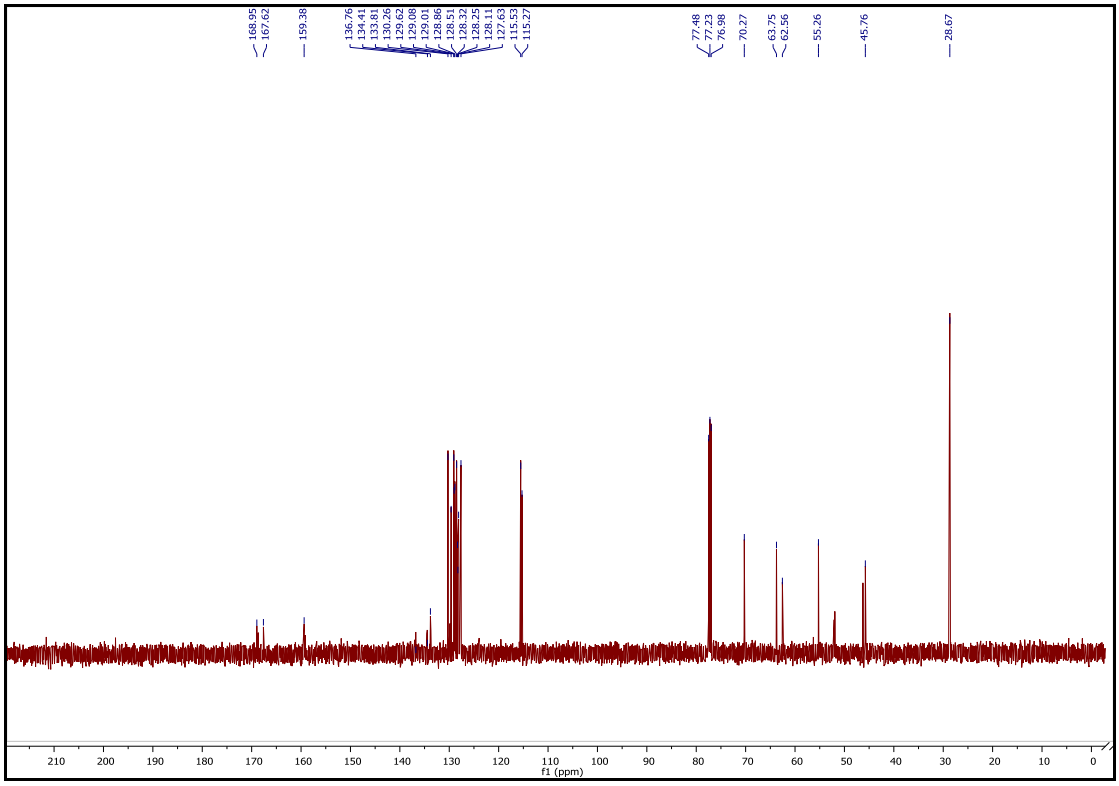


Compound **2f**, *diastereomer A*

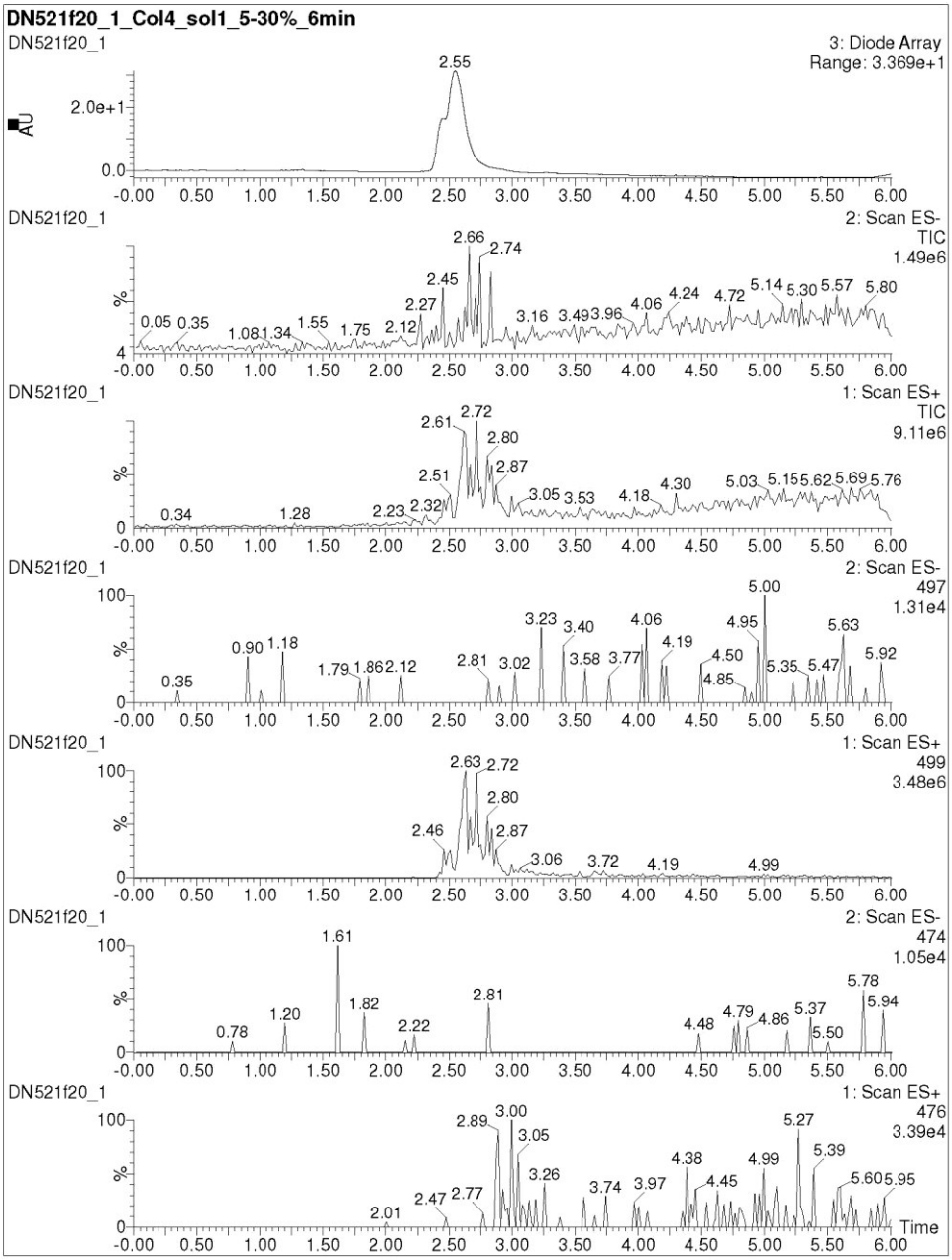


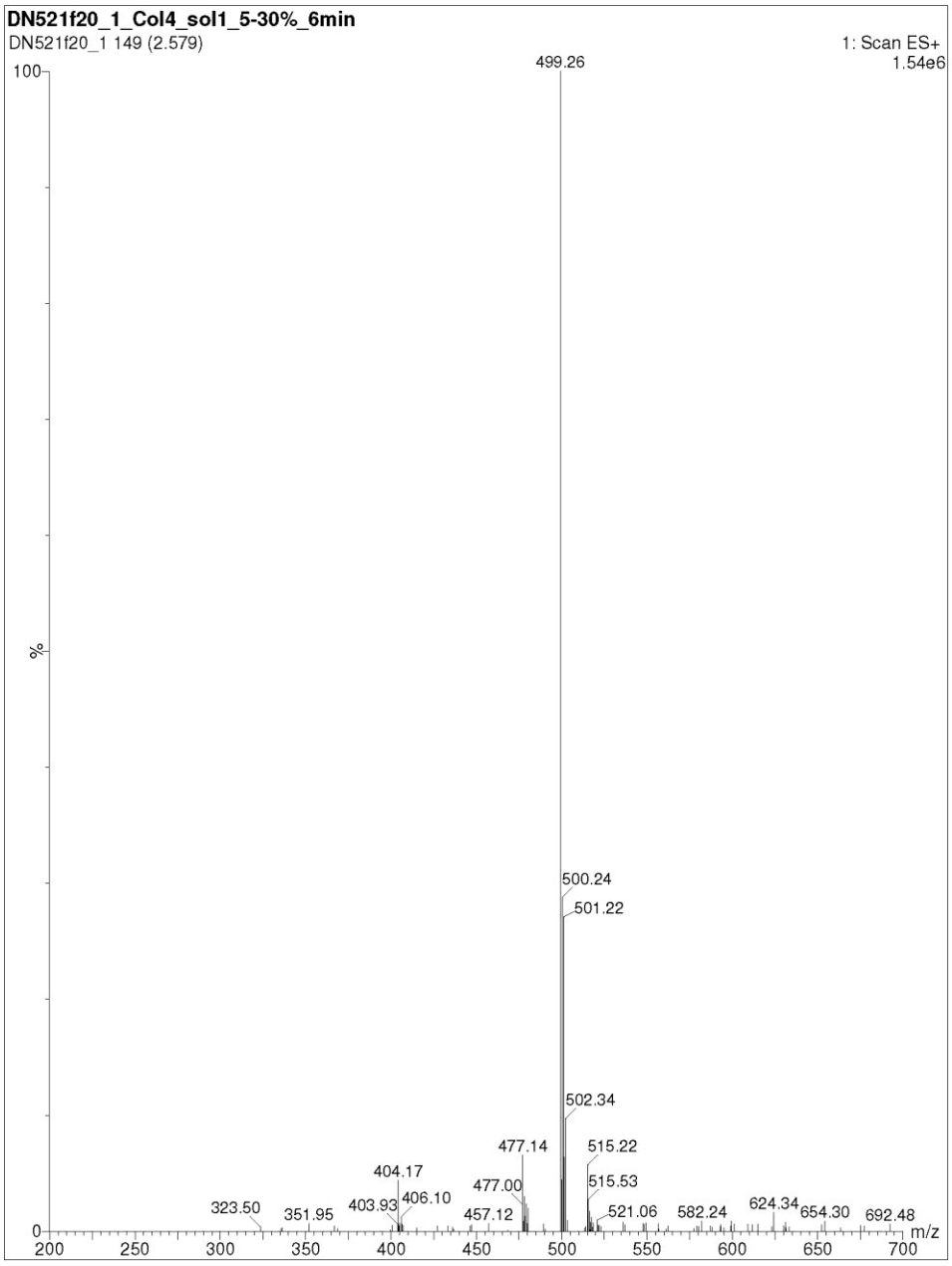

DN520_16_1_Col4_sol1_5-30%_6min
DN520_16_1 157 (2.718)
1: Scan ES+
7.57e4
100
%
0
555.29
579.33
580.17
537.13
457.40
353.18
614.47
616.40
515.11
280.24
350.34
389.19
422.37
437.10
502.27
580.35
404.24
657.20
680.41
345.02
486.94
656.75
684.96
288.95
325.14
617.76
200
250
300
350
400
450
500
550
600
650
700
m/z

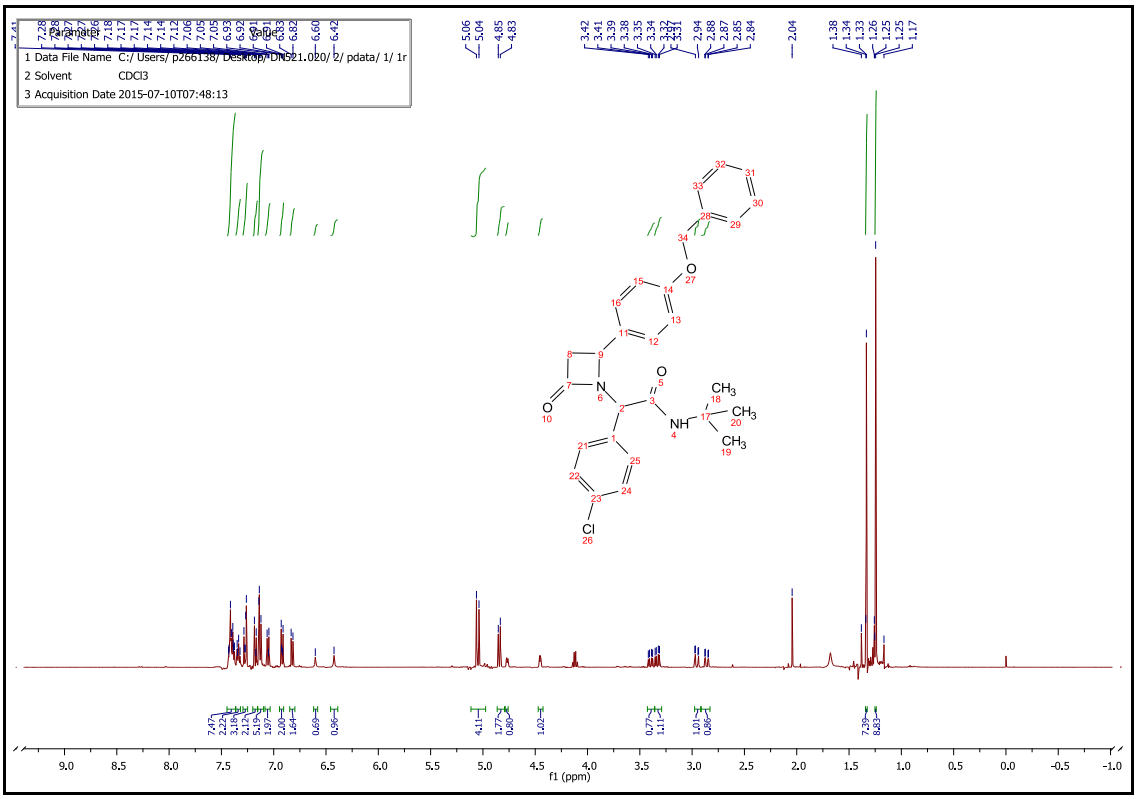

Parameter Value
1 Data File Name C:/ Users/ p266138/ Desktop/ DN520.016/ 1/ pdata/ 1/ 1r
2 Solvent CDCl3
3 Acquisition Date 2015-07-09T14:37:46
f1 (ppm)


f1 (ppm)

Compound **2g**, *mixture of diastereomers*




DN521f20_1_Col4_sol1_5-30%_6min
DN521f20_1 149 (2.579)
1: Scan ES+
1.54e6
100
%
0
499.26
500.24
501.22
502.34
477.14
515.22
404.17
477.00
515.53
406.10
403.93
323.50
351.95
457.12
521.06
582.24
624.34
654.30
692.48
200
250
300
350
400
450
500
550
600
650
700
m/z


Parameter Value
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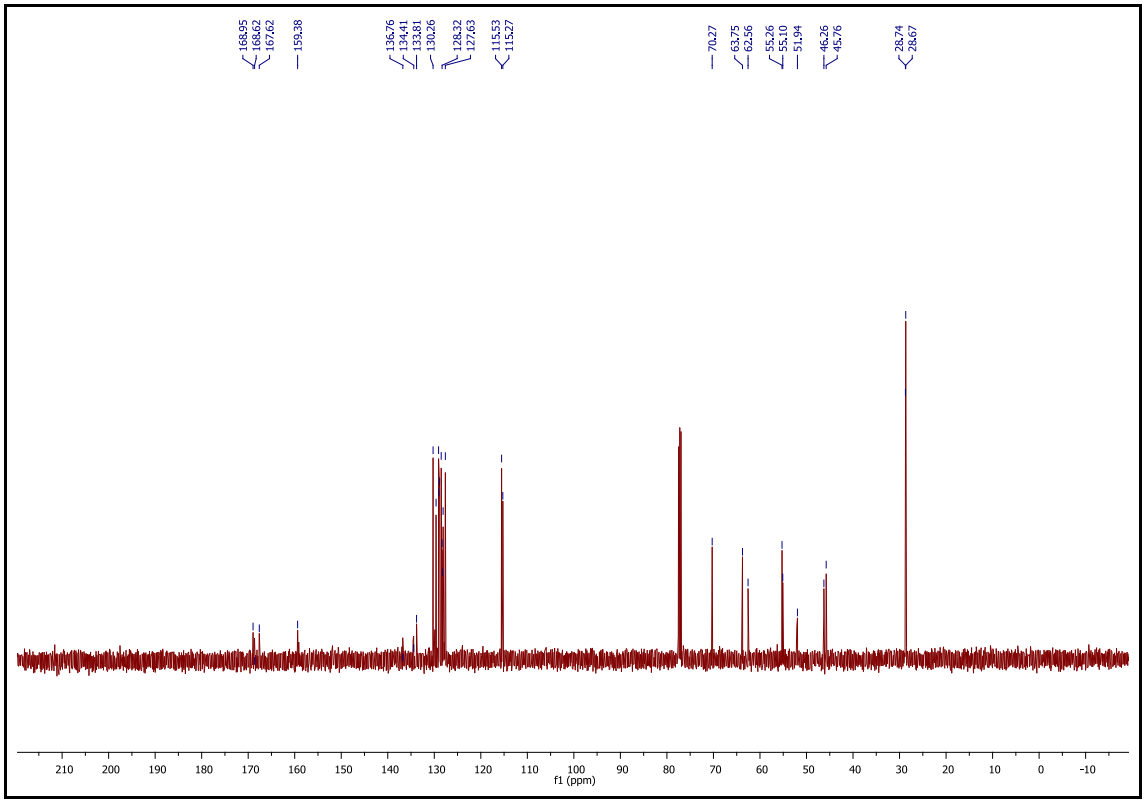
f1 (ppm)


f1 (ppm)

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