Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

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1. Materials

Pyruvic acid (**2b**) was obtained from Kanto Chemical Co. (Tokyo, Japan). Trimethylpyruvic acid (**2i**) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Isopropylamine (**3c**) was purchased from Junsei Chemical Co. (Tokyo, Japan). Materials used for preparation of culture media including yeast extract, tryptone and agar were purchased from Difco. (Spark, MD, USA). All other chemicals were purchased from Sigma Aldrich Co. (St. Louis, MO, USA).

2. Cloning of $AR_{mut}TA$ gene

The codon-optimized $AR_{mut}TA$ gene[1] was synthesized and cloned into a pGEM-T vector by gene synthesis service of Bioneer Inc. (Daejon, Korea). Cloning of the $AR_{mut}TA$ gene into a pET28a(+) expression vector (Novagen, WI, USA) was carried out by standard molecular biology techniques.[2] PCR primers for subcloning were purchased from Bioneer Inc. (Daejon, Korea): forward (5´-GATATACCATGGCATTTAGCGCAGAT-3´) and reverse (5´-GTGGTGCTCGAGATACTGAACCGGTGTCAG-3´). The forward and reverse primers carry an NcoI and an XhoI site, respectively, which are underlined in the primer sequences. The PCR-amplified gene fragment after restriction enzyme treatment was ligated with a NcoI/XhoI cut of pET28a(+). Cloning products were confirmed by DNA sequencing.

3. Preparation of enzymes

Escherichia coli BL21(DE3) cells transformed with the expression vector harboring the ω-TA or TD gene were cultivated at 37 ^{o}C in LB medium (3 L) supplemented with kanamycin (50 μg mL^{-1}). Protein expression was induced by IPTG (final concentration = 1 mM) around 0.6 OD_{600} and the cells were cultivated further for 10 hr. Cells were harvested by centrifugation (10,000 × g, 10 min, 4 ^{o}C) and then resuspended in 20 mL buffer (50 mM Tris-HCl, 50 mM NaCl, 1 mM β-mercaptoethanol, 0.1 mM PMSF, 0.5 mM PLP, pH 7.0). The cell suspension was disrupted by an ultrasonic disruptor and centrifuged (17,000 × g, 30 min, 4 ^{o}C). Protein purification was carried out on ÄKTAprime plus (GE Healthcare, Piscataway, NJ, USA). The His_6-tagged proteins were purified by a HisTrap HP column using an elution buffer (20 mM sodium phosphate, 0.5 M sodium chloride, 0.5 mM PLP, pH 7.4) with a linear gradient of imidazole (0 - 0.5 M). Buffer exchange was carried out by a HiTrap desalting column using an elution buffer (50 mM sodium phosphate, 0.15 M sodium chloride and 0.5 mM PLP). When necessary, the purified protein solution was concentrated by an ultrafiltration kit (Ultracel-30) from Millipore Co. (Billerica, MA, USA).

4. HPLC analysis

Analysis of acetophenone was performed on a Waters HPLC system (Milford, MA, USA) using a Symmetry column from Waters Co. with isocratic elution of 60 % methanol/40 % water/0.1 % trifluoroacetic acid at 1 mL min^{-1}. UV detection was done at 254 nm. Retention time of acetophenone was

3.8 min. α-Keto acids (**2a-b**, **2d-e** and **2n**) were analyzed using Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) with isocratic elution of 5 mM H_2SO_4 aqueous solution at 0.5 mL min^{-1}. UV detection was done at 210 nm and column oven temperature was set to 40 ^oC. Retention times of **2a**, **2b**, **2d**, **2e** and **2n** were 12.0, 11.3, 9.9, 9.8 and 37.9, respectively. For determination of amino acid concentration and enantiomeric excess, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) was used for derivatization.[3] In a typical GITC derivatization procedure, 20 μL of 100 mM GITC stock dissolved in acetonitrile, 20 μL of 100 mM triethylamine stock dissolved in acetonitrile and 90 μL TDW were added to 70 μL reaction samples that were previously diluted with acetonitrile to make the final total concentration of amine and amino acid less than 0.3 molar equivalent of GITC. After 90 min incubation at room temperature, aliquots of the mixtures (20 μL) were analyzed by the Symmetry column with UV detection at 254 nm. Elution conditions and retention times of L/D-enantiomers of **1a**, **1b**, **1d**, **1e** and **1n** are shown below.

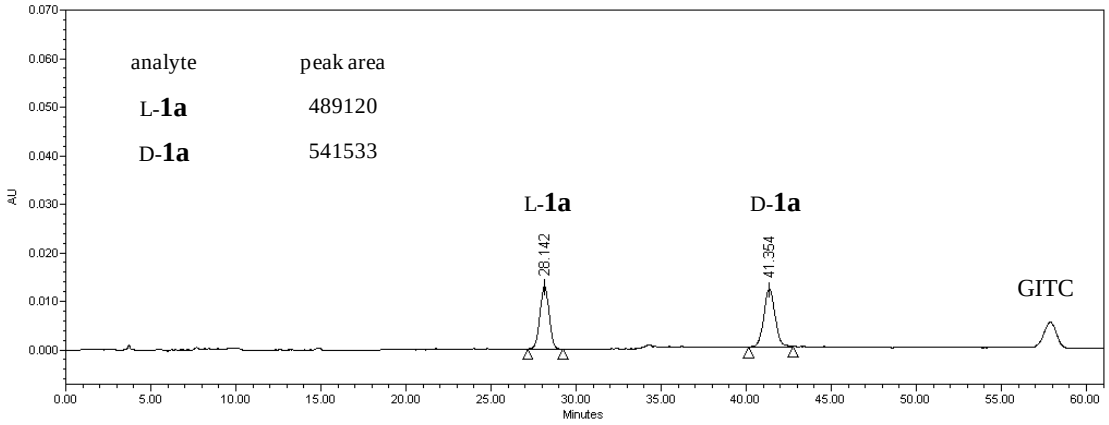
HPLC analysis of the amino acids after GITC derivatization.

Analyte	Elution conditions[a]	Retention time (min)	
		L-form	D-form
1a	gradient elution from 30 A /70 B to 40 A / 60 B for 60 min	28.1	41.3
1b	gradient elution from 30 A /70 B to 40 A / 60 B for 60 min	17.6	25.1
1d	gradient elution from 30 A /70 B to 40 A / 60 B for 60 min	18.2	25.6
1e	isocratic elution 20 A /80 B	139.7	145.6
1n	isocratic elution 60 A /40 B	15.1	20.9

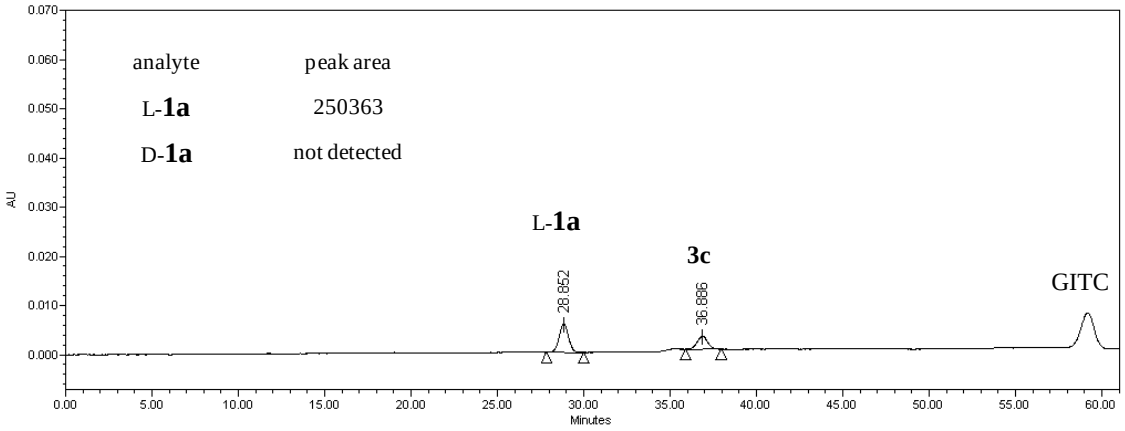
[a] A: MeOH (0.1 % TFA), B: TDW (0.1 % TFA)

HPLC chromatograms for chiral analysis

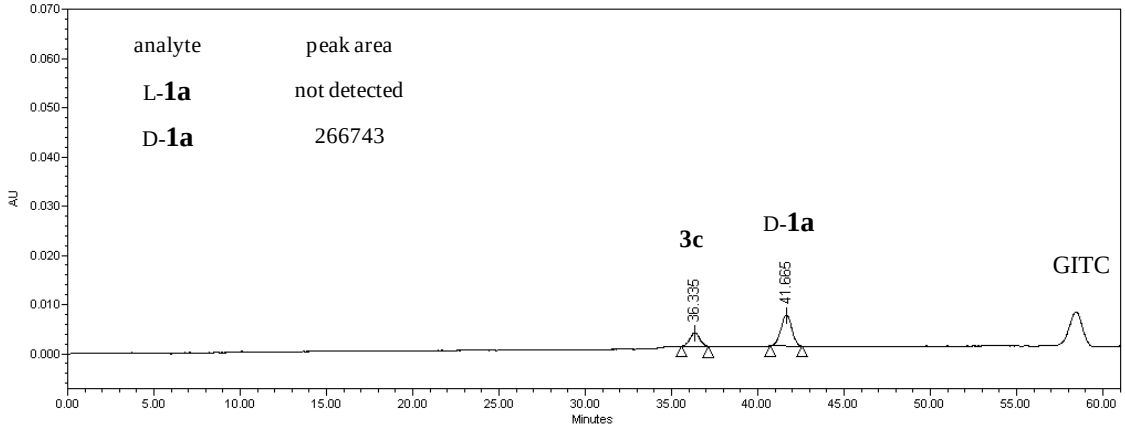
HPLC separation of GITC derivatives of *rac*-**1a** (1 mM)



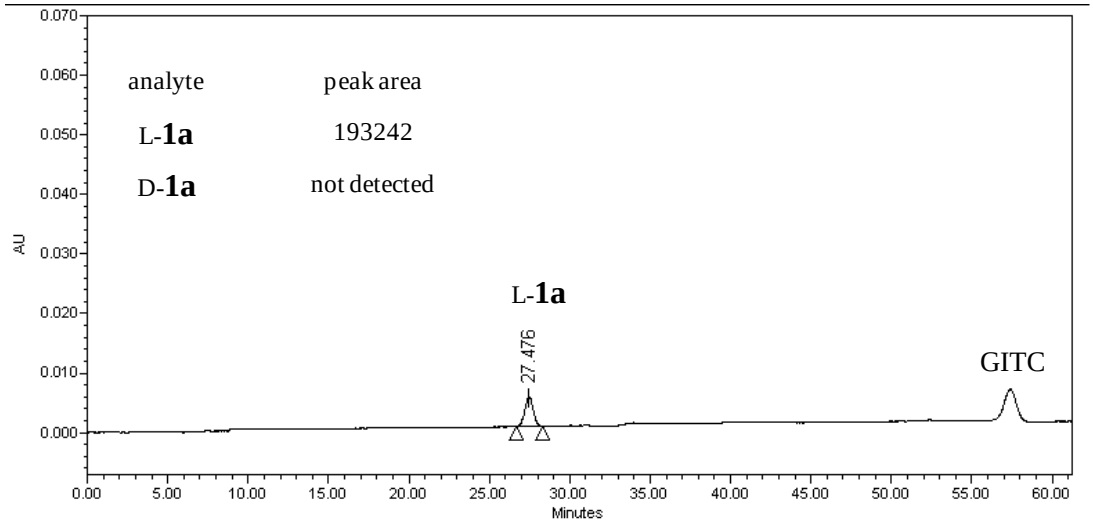
Reaction sample for asymmetric synthesis of L-**1a** from **2a**



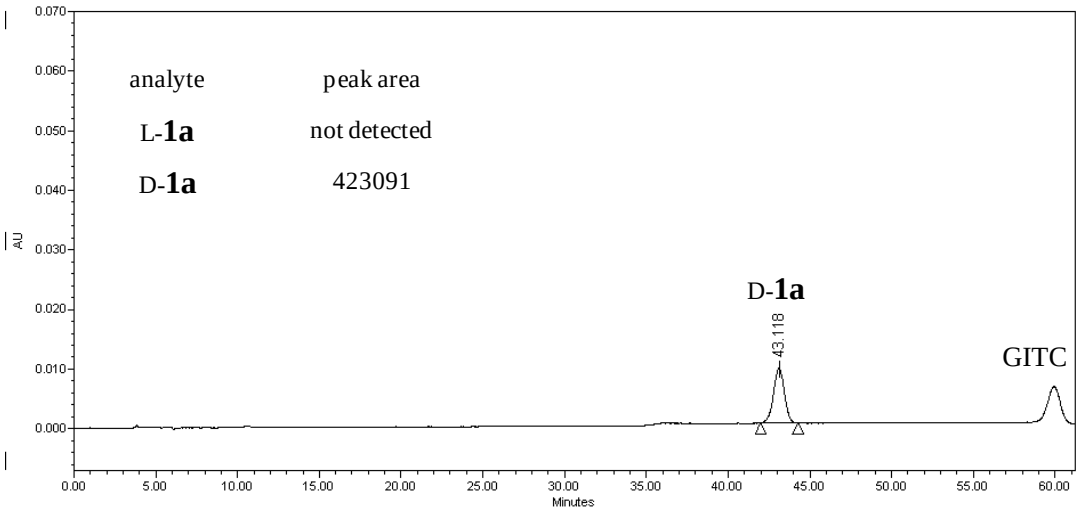
Reaction sample for asymmetric synthesis of D-**1a** from **2a**



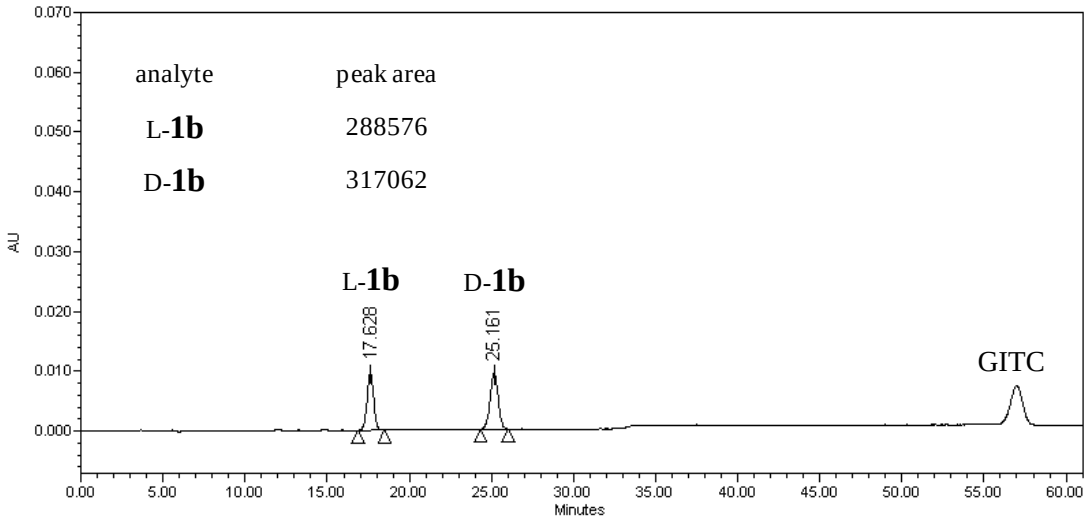
Isolated L-**1a** from the preparative-scale TD/OATA reaction (0.2 mM)



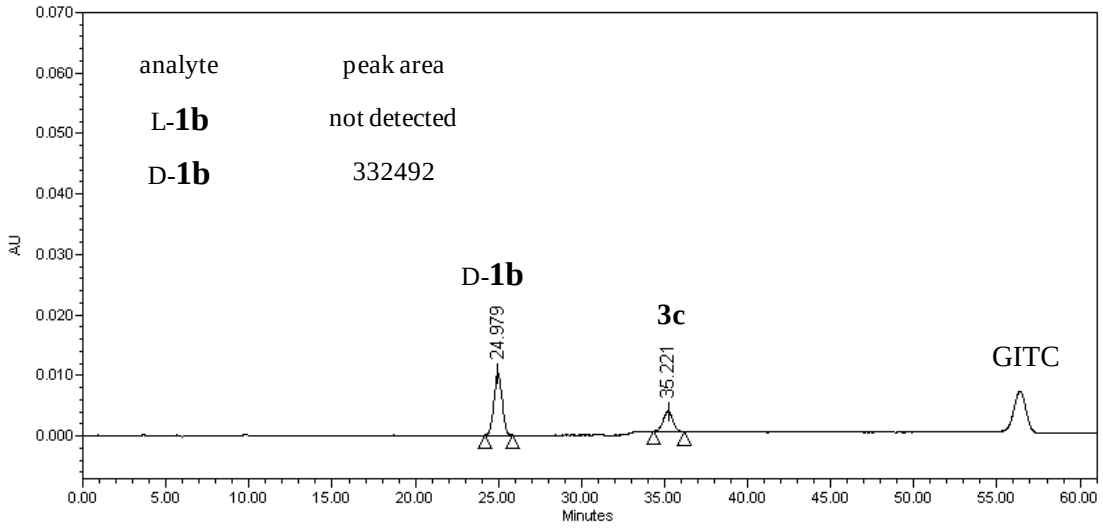
Isolated D-**1a** from the preparative-scale TD/AR_{mut}TA reaction (0.4 mM)



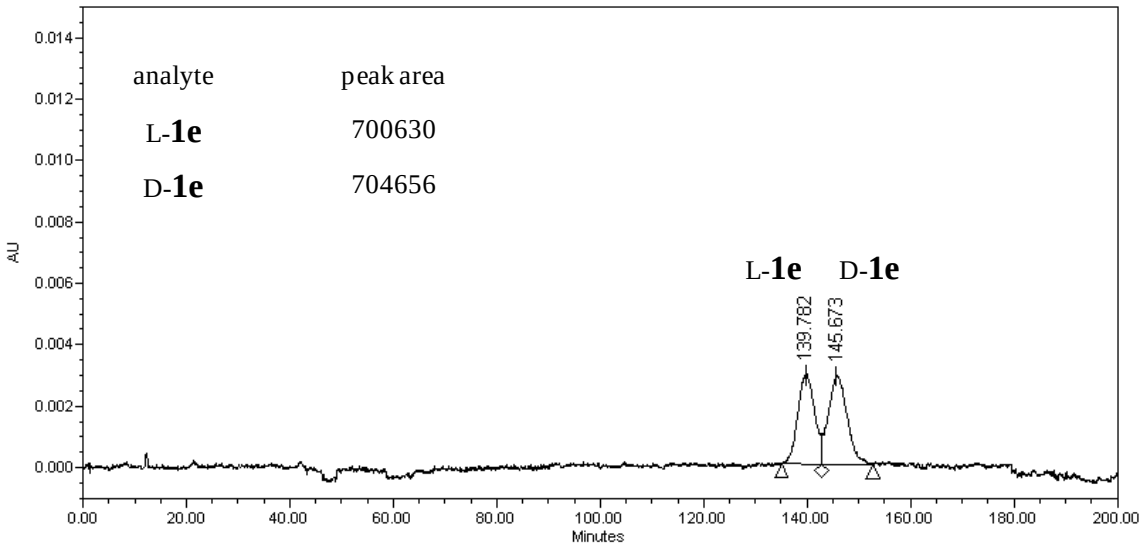
HPLC separation of GITC derivatives of *rac*-**1b** (0.5 mM)



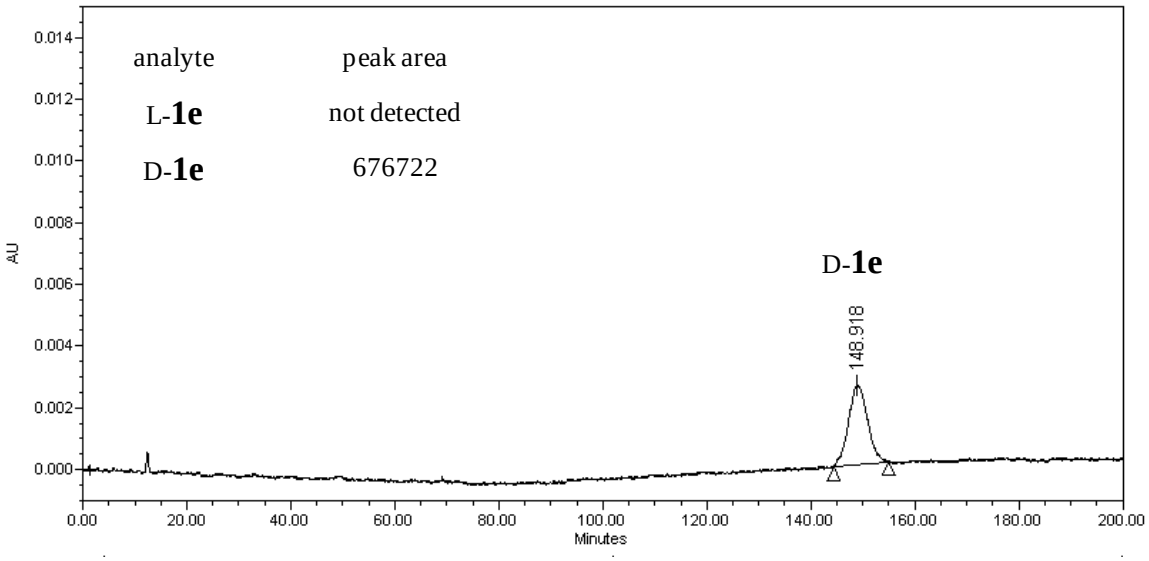
Reaction sample for asymmetric synthesis of D-**1b** from **2b**



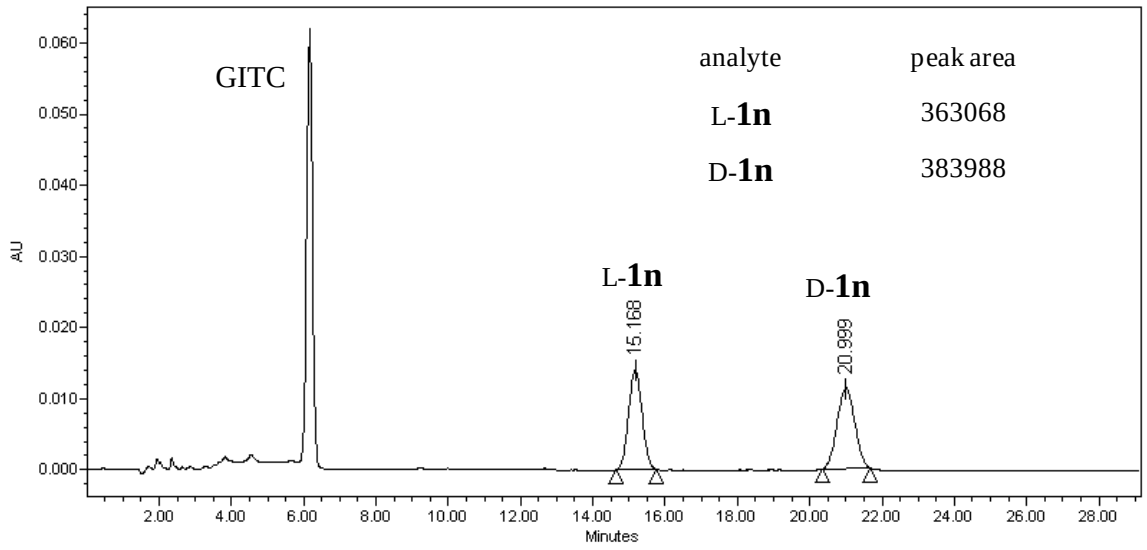
HPLC separation of GITC derivatives of *rac*-**1e** (0.5 mM)



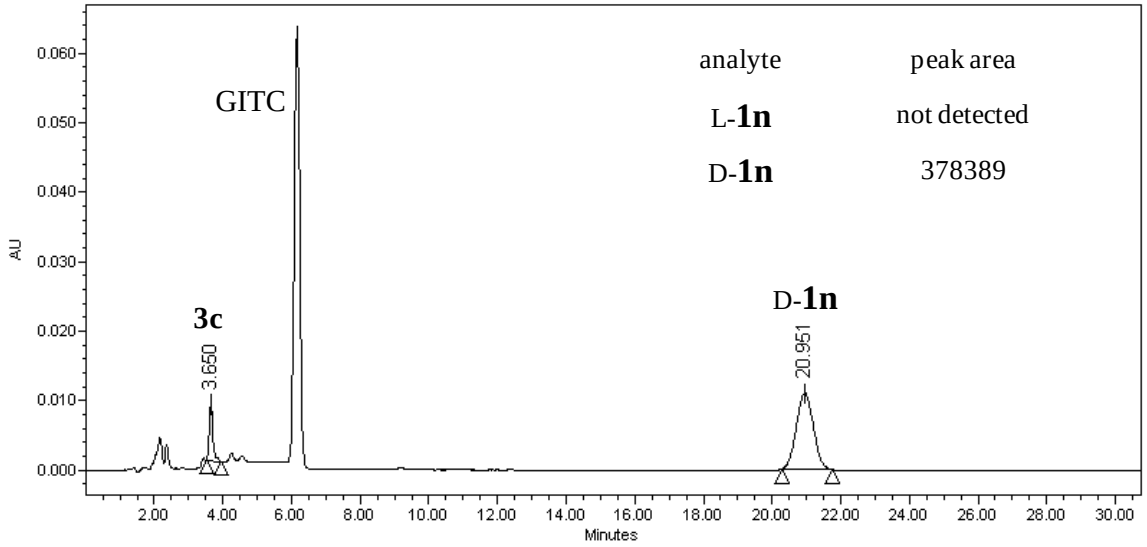
Reaction sample for asymmetric synthesis of D-**1e** from **2e**



HPLC separation of GITC derivatives of **1n** (0.3 mM)



Reaction sample for asymmetric synthesis of D-**1n** from **2n**



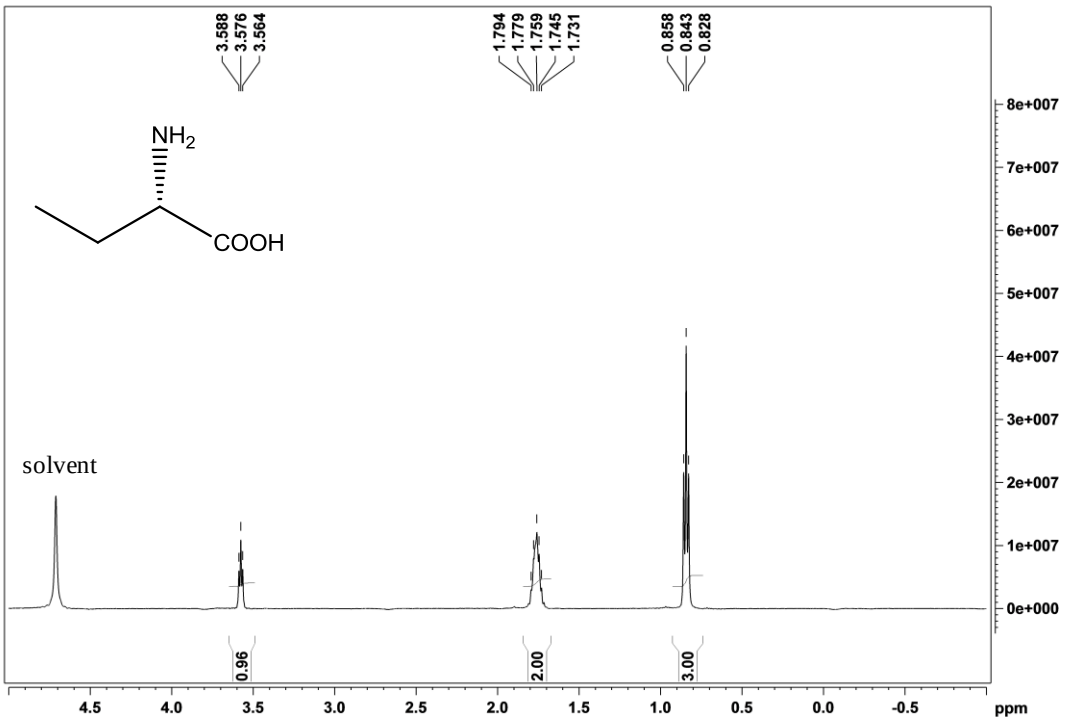
5. Structural characterization of L-1a and D-1a

The white solids of L-**1a** and D-**1a** isolated from the preparative-scale TD/ω-TA coupled reactions were characterized by ^{1}H NMR, ^{13}C NMR, IR, elemental analysis and MS. ^{1}H NMR and ^{13}C NMR spectra were recorded on an FT-NMR spectrometer DRX-500 (500 MHz, Bruker Co.) using tetramethylsilane as a standard. IR spectra were obtained by a Vertex70 FTIR spectrometer (Bruker Co.). Elemental analyses were performed using an Elemental Analyzer 2400 Series II CHNS/O (Perkin-Elmer Inc.). Mass spectral data were obtained with a TSQ Quantum Access MAX Triple Stage Quadrupole Mass Spectrometer (electronspray ionization, positive ion mode, Thermo Fisher Scientific Inc.).

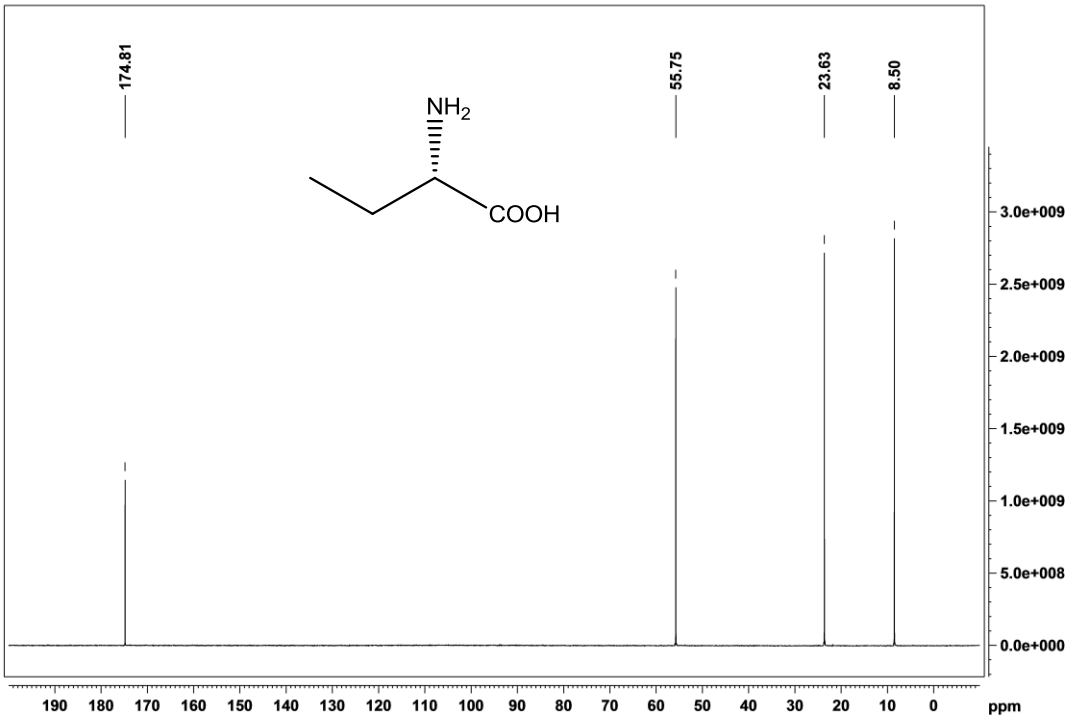
L-**1a** : ^{1}H NMR (500 MHz, D_2O, ppm): δ 0.83-0.86 (t, 3H, CH3), 1.73-1.79 (m, 2H, CH2), 3.56-3.59 (t, 1H, CH). ^{13}C MR (125 MHz, D_2O, ppm): δ 8.5, 23.6, 55.7, 174.8. IR (neat film): 3439, 2971, 2939, 2882, 1584, 1256, 1205, 1178, 1113, 1045, 1034 cm^{-1}. MS (ESI): *m*/*z* 104.17 $[M+H]^+$. Anal. Calcd. for $C_6H_{13}NO_2$: C 46.59, H 8.80, N 13.58; found C 46.69, H 8.79, N 13.56.

D-**1a** : ^{1}H NMR (500 MHz, D_2O, ppm): δ 0.83-0.86 (t, 3H, CH3), 1.74-1.78 (m, 2H, CH2), 3.56-3.58 (t, 1H, CH). ^{13}C MR (125 MHz, D_2O, ppm): δ 8.5, 23.6, 55.7, 174.8. IR (neat film): 3440, 2971, 2939, 2881, 1585, 1256, 1205, 1178, 1113, 1046, 1035 cm^{-1}. MS (ESI): *m*/*z* 104.16 $[M+H]^+$. Anal. Calcd. for $C_6H_{13}NO_2$: C 46.59, H 8.80, N 13.58; found C 46.66, H 8.78, N 13.54.

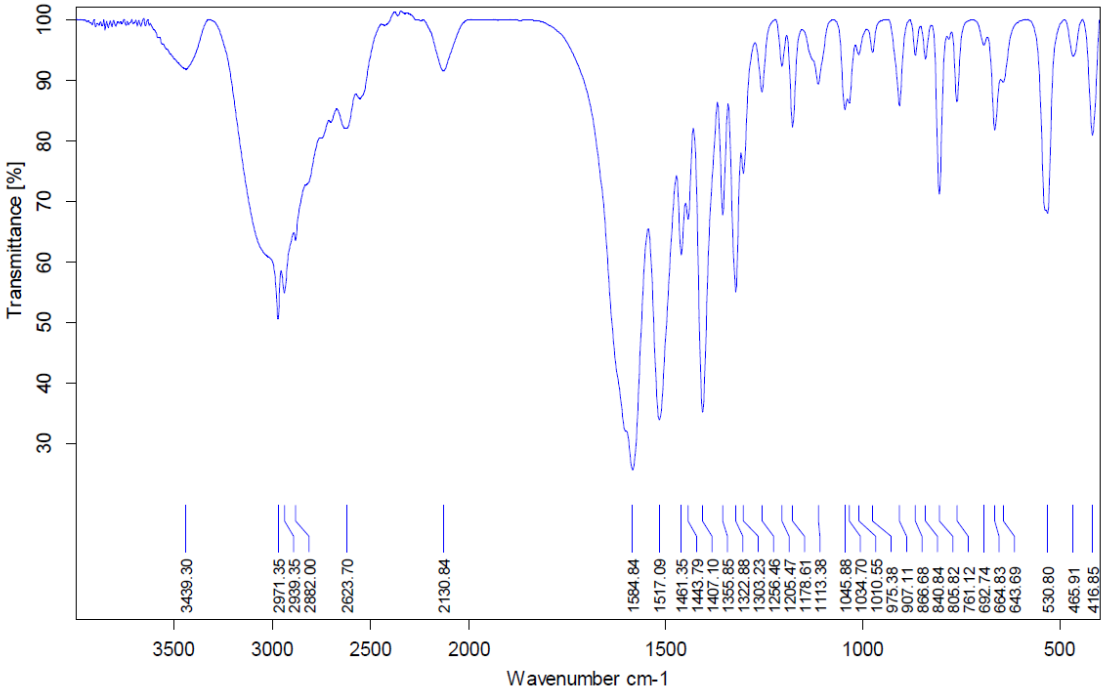
^{1}H NMR (500 MHz, D_2O): L-**1a**



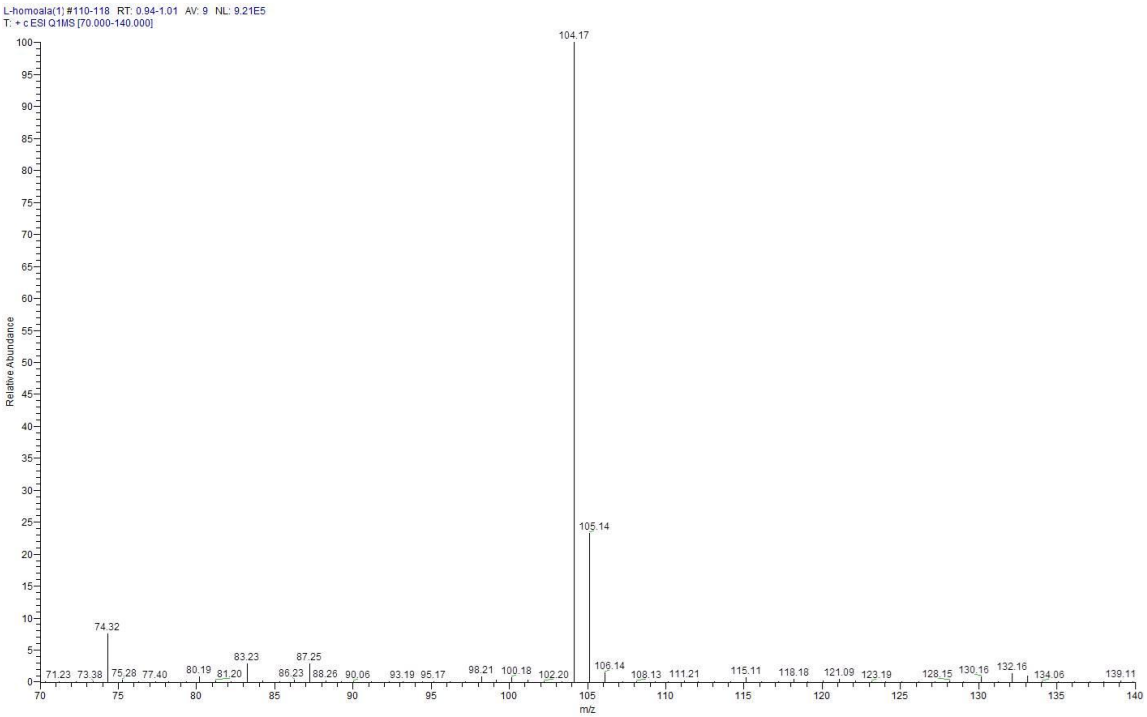
^{13}C NMR (125 MHz, D_2O): L-**1a**



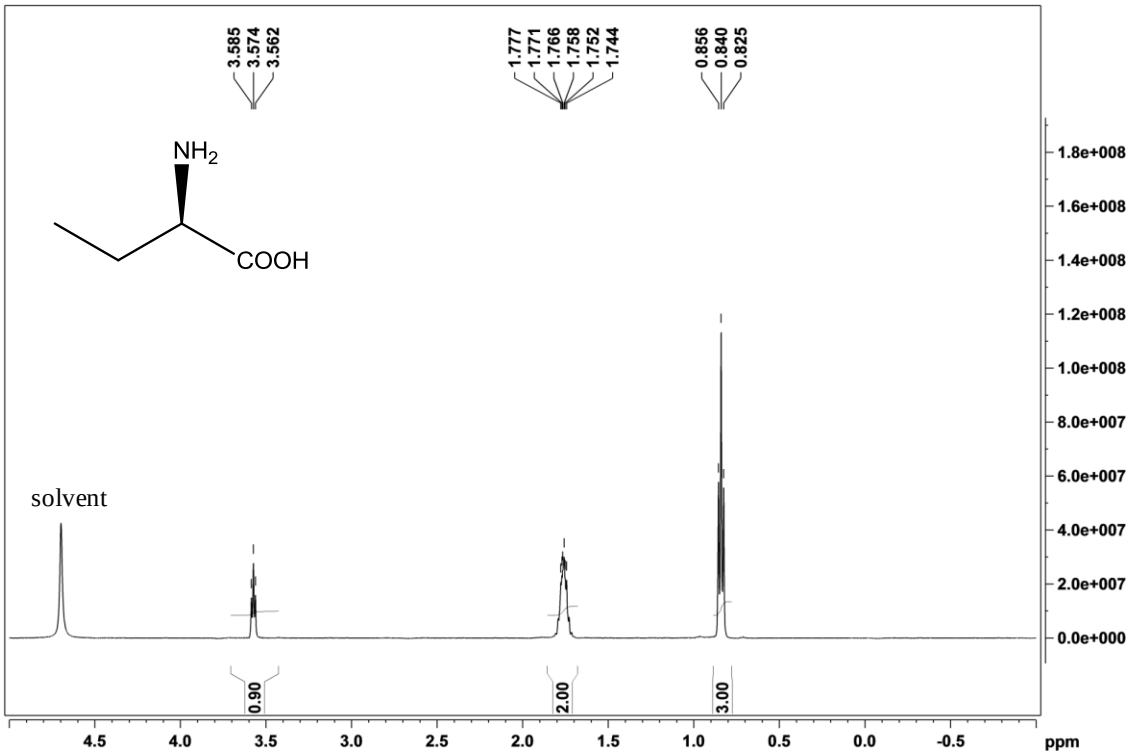
IR: L-**1a**



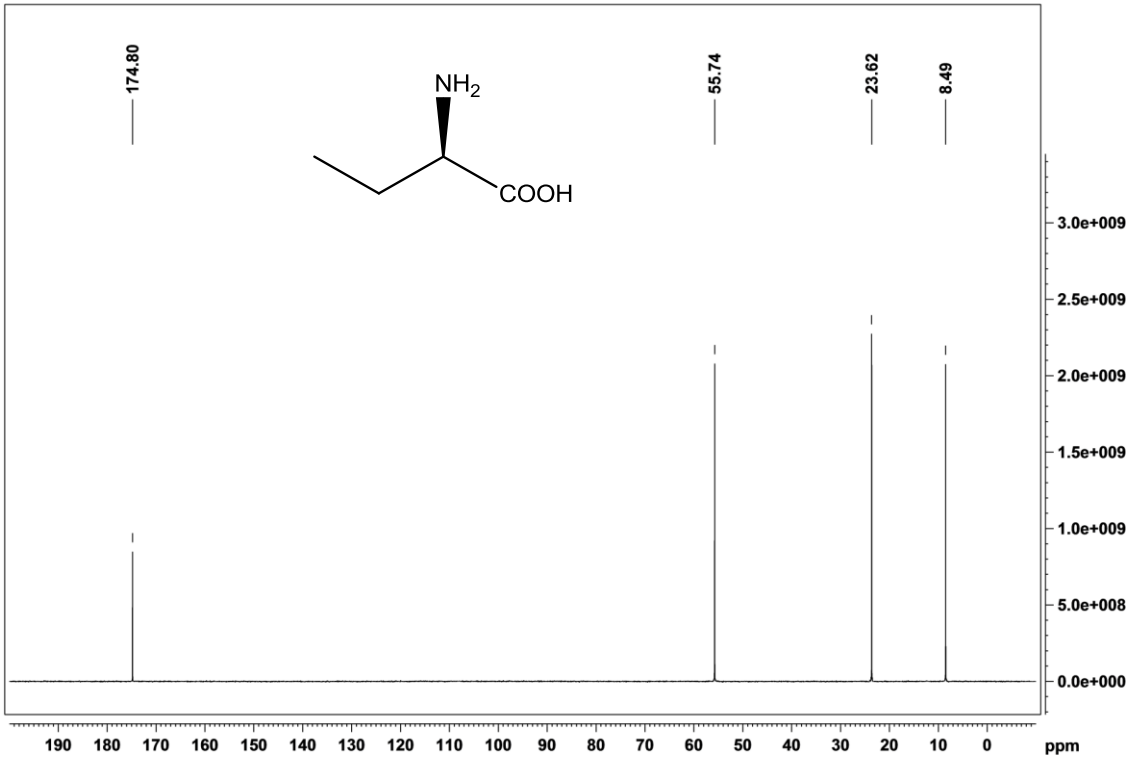
LC/MS: L-**1a**



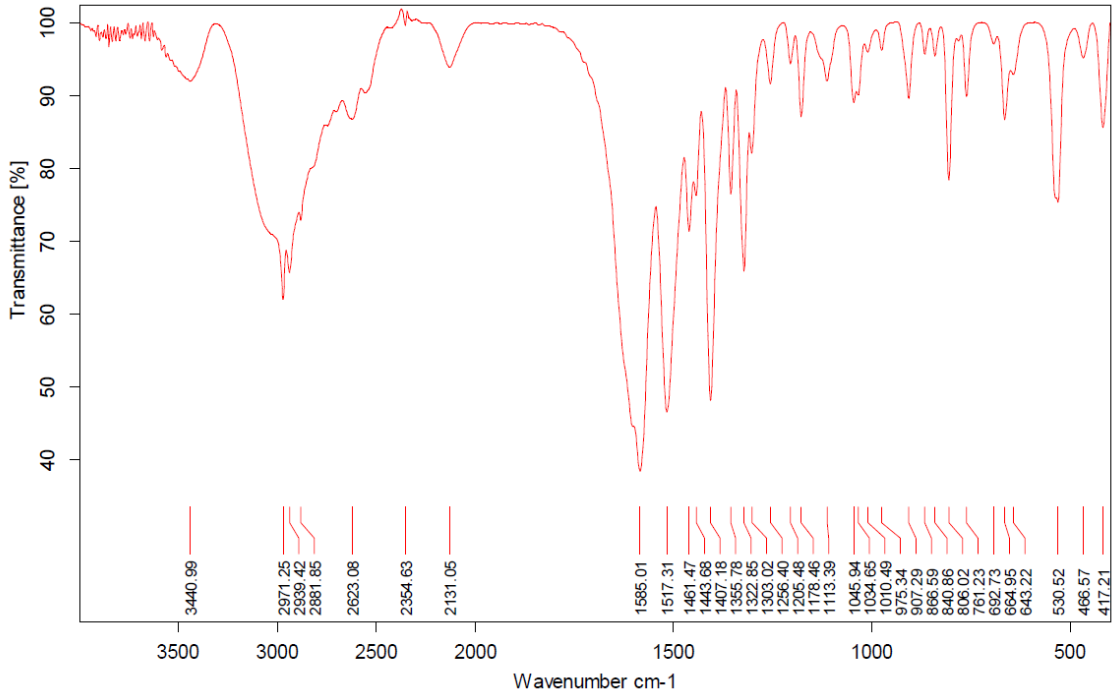
^{1}H NMR (500 MHz, D_2O): D-**1a**



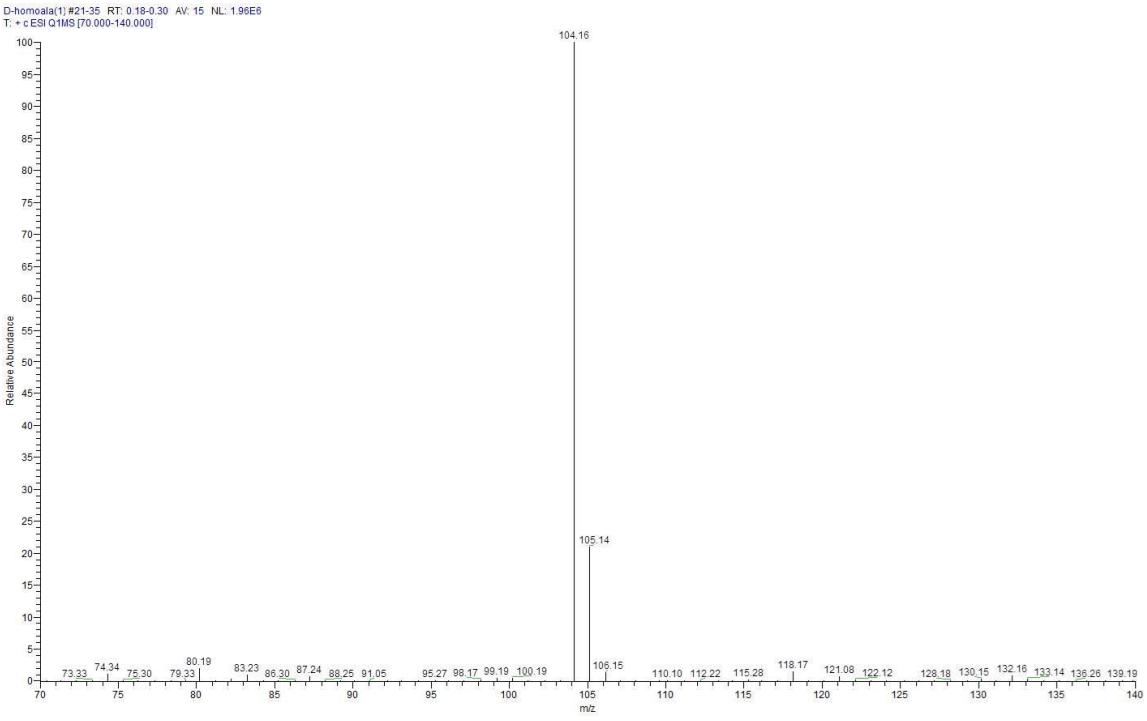
^{13}C NMR (125 MHz, D_2O): D-**1a**



IR: D-**1a**



LC/MS: D-**1a**



6. References

1. F. G. Mutti, C. S. Fuchs, D. Pressnitz, J. H. Sattler and W. Kroutil, *Adv. Synth. Catal.*, 2011, **353**, 3227-3233.
2. J. Sambrook and D. W. Russell, *Molecular cloning : a laboratory manual*, 3rd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001.
3. S. Ito, A. Ota, K. Yamamoto, Y. Kawashima, *J. Chromatogr.* 1992, **626**, 187-196.