Supporting Information Appendix

Engineered Ketoreductase-Catalyzed Stereoselective Reduction of Ethyl 2'-Ketopantothenate and Its Analogues: Chemoenzymatic Synthesis of D-Pantothenic Acid

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Table of Contents **S-2**

Table S1. The details of genes used in this study **S-3**

Nucleotide and amino acid sequences of SSCR **S-4**

Nucleotide and amino acid sequences of M3 ..... **S-5**

Figure S1. SDS-PAGE analysis of N-terminal-His$_6$-SSCR and mutants after IMAC purification. **S-6**

Figure S2. SDS-PAGE analysis of cell-free extracts of recombinant E. coli strains coexpressing M3 and GDH. **S-6**

Table S2. Screening of KREDs for the stereoselective reduction of K-PaOEt (1a) **S-7**

Figure S3. Selected amino acid residues within 5 Å of the docked substrate K-PaOEt (1a). **S-8**

Figure S4. Double mutant-catalyzed reduction of K-PaOEt (1a) to (*R*)-PaOEt ((*R*)-2a). **S-9**

Figure S5. Dependence of the activity of SSCR and its mutants on the concentration of K-PaOEt (1a) **S-10**

Table S3. Genetic construction of recombinant *E. coli* strains **S-11**

Figure S6. Reaction condition optimization for the reduction of K-PaOEt (1a) to (*R*)-PaOEt ((*R*)-2a). **S-12**

Table S4. Calculation of E-factor for recombinant strain *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed synthesis of (*R*)-PaOEt at gram-scale **S-13**

Table S5. Primers used for the mutagenesis study **S-14**

Scheme S1. Synthesis of KPL. **S-17**

Scheme S2. Synthesis of compound 1a **S-17**

Scheme S3. General procedure for the synthesis of compounds 1. ..... **S-18**

Scheme S4. *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed stereoselective synthesis of 2. ..... **S-22**

Scheme S5. Synthesis of compounds 2c or 2k using (*R*)-PL. **S-26**

References **S-27**

^{1}H NMR and ^{13}C NMR Spectra **S-28**

Chiral HPLC Spectra **S-51**

Table S1. The details of genes used in this study

Name	Accession No.	Source	aa
KdoADH	CDO95209.1	*Kluyveromyces dobzhanskii*	342
YGL039w	NP_011476	*Saccharomyces cerevisiae*	348
CgKR2	XP_448118.1	*Candida glabrata*	311
LtCR	XP_002554048.1	*Lachancea thermotolerans*	281
YDR541c	AAB64983.1	*Saccharomyces cerevisiae*	344
BYueD	WP_134982026.1	*Bacillus subtilis*	243
RasADH	EU485985	*Ralstonia sp.* DSMZ 6428	250
*Ch*KRED20	AHC30841.1	*Chryseobacterium sp.* CA49	244
YDL124w	NP_010159.1	*Saccharomyces cerevisiae*	312
KmCR2	XP_022675166.1	*Kluyveromyces marxianus* CBS4857	341
PkADH	WP_114811150.1	*Paraburkholderia kururiensis*	251
KRED-SL-10	WP_131435658.1	*Exiguobacterium sp. SL-10*	248
KRED-F42	WP_023468191.1	*Exiguobacterium sp.* MH3	249
SSCR	Q9UUN9.3	*Sporobolomyces salmonicolor* AKU4429	343
KRED-Bt	WP_103592444.1	*Bacillus thuringiensis*	253

Nucleotide sequence of SSCR (synthetic gene, codon optimized for expression in *E. coli*)

ATGGCCAAAATCGATAACGCAGTGCTGCCGGAAGGCTCTTTAGTTCTGGTGACCG
GTGCCAATGGTTTTGTGGCCAGCCATGTGGTTGAGCAGCTGCTGGAGCATGGTTAT
AAAGTGCGCGGTACCGCCCGCAGTGCCAGCAAACTGGCCAACTTACAGAAACGC
TGGGATGCCAAGTATCCGGGTCGCTTCGAAACCGCCGTGGTGGAAGACATGCTGA
AACAAGGTGCCTACGATGAGGTGATTAAAGGTGCCGCCGGTGTGGCCCATATCGC
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CAGCACAAGCGGTTGGATGATGTCTTTATTCAACGGTGAAGTGAGCCCGGCCTTAG
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GTTGTTTAGTGCTGCCGCAGATTGAACGTCGTCGCGTGTATGGCACAGCCGGCACC
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CCCGGCCGATTTTCCGGACCAAGGTCAAGATCTGAGCAAATTCGATACCGCCCCGT
CTTTAGAAATTCTGAAGAGCTTAGGCCGTCCGGGCTGGCGCAGCATTGAAGAAAG
TATTAAAGATCTGGTTGGTAGCGAAACCGCCTAA

Amino acid sequence of SSCR

MAKIDNAVLPEGSLVLVTGANGFVASHVVEQLLEHGYKVRGTARSASKLANLQKRW
DAKYPGRFETAVVEDMLKQGAYDEVIKGAAGVAHIASVVSFSNKYDEVVTPAIGGTL
NALRAAAATPSVKRFVLTSSTVSALIPKPNVEGIYLDEKSWNLESIDKAKTLPESDPQK
SLWVYAASKTEAELAAWKFMDENKPHFTLNAVLPNYTIGTIFDPETQSGSTSGWMMS
LFNGEVSPALALMPPQYYVSAVDIGLLHLGCLVLPQIERRRVYGTAGTFDWNTVLATF
RKLYPSKTFPADFPDQGQDLSKFDTAPSLEILKSLGRPGWRSIEESIKDLVGSETA

Amino acid sequence of SSCR with a N-terminal-His_6-tag

MGSSHHHHHHSSGLVPRGSHMAKIDNAVLPEGSLVLVTGANGFVASHVVEQLLEHGY
KVRGTARSASKLANLQKRWDAKYPGRFETAVVEDMLKQGAYDEVIKGAAGVAHIAS
VVSFSNKYDEVVTPAIGGTLNALRAAAATPSVKRFVLTSSTVSALIPKPNVEGIYLDEK
SWNLESIDKAKTLPESDPQKSLWVYAASKTEAELAAWKFMDENKPHFTLNAVLPNYT
IGTIFDPETQSGSTSGWMMSLFNGEVSPALALMPPQYYVSAVDIGLLHLGCLVLPQIER
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WRSIEESIKDLVGSETA

Nucleotide sequence of M3

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GTGCCAATGGTTTTGTGGCCAGCCATGTGGTTGAGCAGCTGCTGGAGCATGGTTAT
AAAGTGCGCGGTACCGCCCGCAGTGCCAGCAAACTGGCCAACTTACAGAAACGC
TGGGATGCCAAGTATCCGGGTCGCTTCGAAACCGCCGTGGTGGAAGACATGCTGA
AACAAGGTGCCTACGATGAGGTGATTAAAGGTGCCGCCGGTGTGGCCCATATCGC
AAGCGTGGTGAGC**TTG**AGTAATAAATACGATGAAGTTGTGACCCCCGCTATCGGTG
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GCTGACAAGTAGTACCGTGAGCGCCTTAATTCCGAAGCCGAATGTGGAGGGCATC
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CGGAGAGCGATCCGCAGAAA**ATT**TTATGGGTGTACGCCGCCAGTAAAACCGAGGC
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CAGCACAAGCGGTTGGATGATGTCTTTATTCAACGGTGAAGTGAGCCCGGCCTTAG
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GTTGTTTAGTGCTGCCGCAGATTGAACGTCGTCGCGTGTATGGCACAGCCGGCACC
TTTGATTGGAATACCGTGCTGGCCACCTTTCGCAAACTGTATCCGAGCAAAACCTT
CCCGGCCGATTTTCCGGACCAAGGTCAAGATCTGAGCAAATTCGATACCGCCCCGT
CTTTAGAAATTCTGAAGAGCTTAGGCCGTCCGGGCTGGCGCAGCATTGAAGAAAG
TATTAAAGATCTGGTTGGTAGCGAAACCGCCTAA

Amino acid sequence of M3

MAKIDNAVLPEGSLVLVTGANGFVASHVVEQLLEHGYKVRGTARSASKLANLQKRW
DAKYPGRFETAVVEDMLKQGAYDEVIKGAAGVAHIASVVS**L**SNKYDEVVTPAIGGTL
NALRAAAATPSVKRFVLTSSTVSALIPKPNVEGIYLDEKSWNLESIDKAKTLPESDPQK
ILWVYAASKTEAELAAWKFMDENKPHFTLNAVLPNYTIGTIFDPETQSGSTSGWMMS
LFNGEVSPALALM**L**PQYYVSAVDIGLLHLGCLVLPQIERRRVYGTAGTFDWNTVLATF
RKLYPSKTFPADFPDQGQDLSKFDTAPSLEILKSLGRPGWRSIEESIKDLVGSETA

Amino acid sequence of M3 with a N-terminal-His$_6$-tag

MGSSHHHHHHSSGLVPRGSHMAKIDNAVLPEGSLVLVTGANGFVASHVVEQLLEHGY
KVRGTARSASKLANLQKRWDAKYPGRFETAVVEDMLKQGAYDEVIKGAAGVAHIAS
VVS**L**SNKYDEVVTPAIGGTLNALRAAAATPSVKRFVLTSSTVSALIPKPNVEGIYLDEK
SWNLESIDKAKTLPESDPQK**I**LWVYAASKTEAELAAWKFMDENKPHFTLNAVLPNYT
IGTIFDPETQSGSTSGWMMSLFNGEVSPALALM**L**PQYYVSAVDIGLLHLGCLVLPQIER
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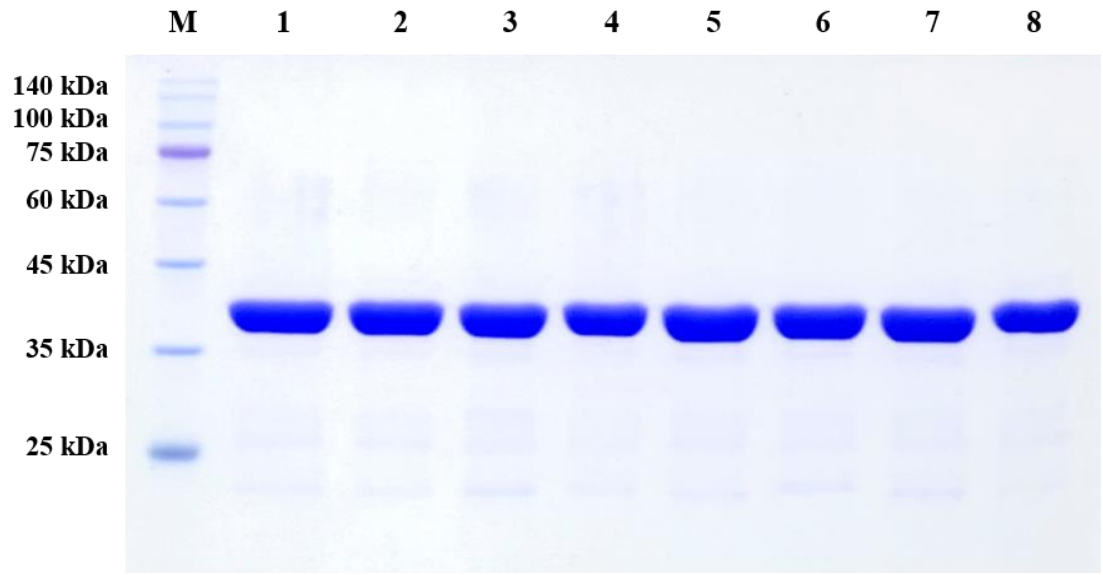


Figure S1. SDS-PAGE analysis of N-terminal-His_6-SSCR and mutants after IMAC purification. Coomassie staining. M: RealBand 3-color Regular Range Protein Marker (Sangon Biotech, China). Lane 1: SSCR. Lane 2: M3. Lane 3: F97L. Lane 4: S173I. Lane 5: P243L. Lane 6: F97L-S173I. Lane 7: F97L-P243L. Lane 8: S173I-P243L.

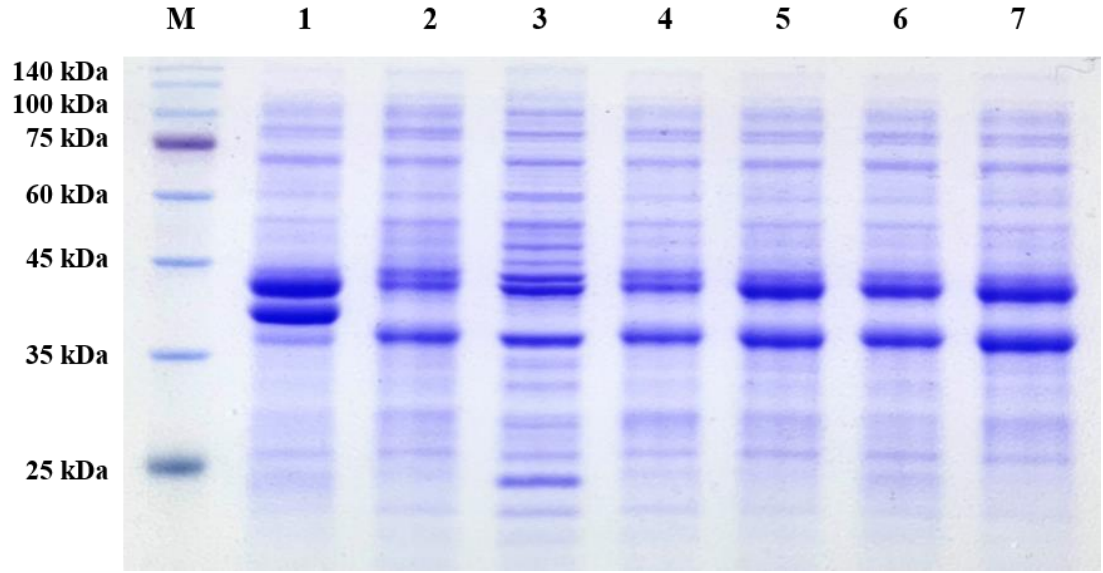
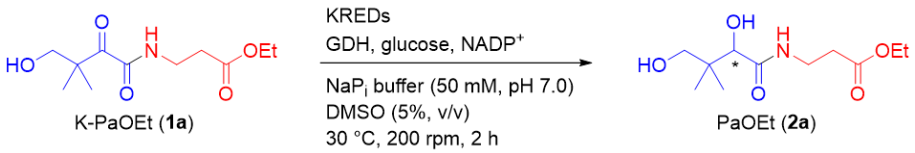


Figure S2. SDS-PAGE analysis of cell-free extracts of recombinant *E. coli* strains coexpressing M3 and GDH. M: RealBand 3-color Regular Range Protein Marker (Sangon Biotech, China). Lane 1: *E. coli* (pET28a-M3/pACYCDuet-1-GDH). Lane 2: *E. coli* (pETDuet-1-M3-GDH). Lane 3: *E. coli* (pACYCDuet-1-M3-GDH). Lane 4: *E. coli* (pRSFDuet-1-M3-GDH). Lane 5: *E. coli* (pETDuet-1-GDH-M3). Lane 6: *E. coli* (pACYCDuet-1-GDH-M3). Lane 7: *E. coli* (pRSFDuet-1-GDH-M3).

Table S2. Screening of KREDs for the stereoselective reduction of K-PaOEt (1a)[a]



Entry	Enzyme	Conv. (%)[b]	Ee (%)[c]
1	KdoADH	45	66 (*S*)
2	YGL039w	8	20 (*R*)
3	CgKR2	<5	n.d.[d]
4	LtCR	<5	n.d.
5	YDR541c	<5	n.d.
6	BYueD	<5	n.d.
7	RasADH	97	96 (*S*)
8	*Ch*KRED20	73	62 (*S*)
9	YDL124w	<5	n.d.
10	KmCR2	89	24 (*R*)
11	PkADH	7	94 (*R*)
12	KRED-SL-10	5	86 (*R*)
13	KRED-F42	<5	n.d.
14	SSCR	>99	>99 (*R*)
15	KRED-Bt	<5	n.d.

[a]Reaction conditions (3 mL): **1a** (50 mM), glucose (100 mM), $NADP^+$ (1 mM), DMSO (5%, v/v), 50 g/L cell-free extract (CFE) (wet cell weight) of KREDs, and 75 g/L CFE (wet cell weight) of GDH in NaP_i buffer (100 mM, pH 7.0). Reaction mixtures in the Eppendorf tubes were shaken in a temperature-controlled orbital shaker at 30 ℃ and 200 rpm for 2 h. [b]The conversion was determined by ^1H NMR analysis. [c]The ee was determined by chiral HPLC analysis upon benzoylation of the product, and the absolute configuration of the product was assigned by comparing the optical rotation data to literature data. [d]n.d.: not determined.

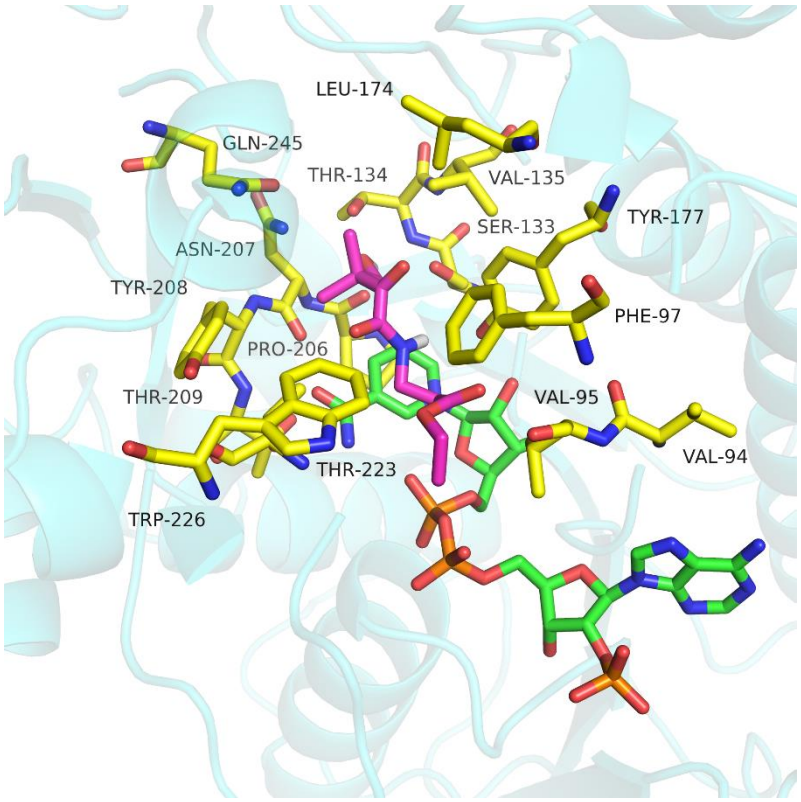


Figure S3. Selected amino acid residues within 5 Å of the docked substrate K-PaOEt (**1a**).

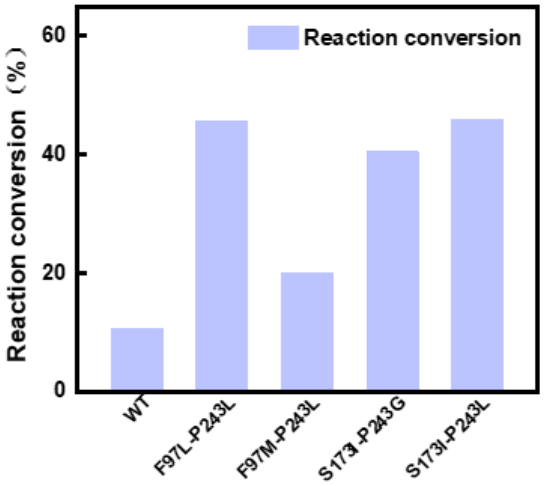


Figure S4. Double mutant-catalyzed reduction of K-PaOEt (**1a**) to (*R*)-PaOEt ((*R*)-**2a**. Reaction conditions (3 mL): **1a** (50 mM), glucose (100 mM), $NADP^+$ (1 mM), DMSO (5%, v/v), 0.17 g/L cell-free extract (CFE) (wet cell weight) of KREDs, and 75 g/L CFE (wet cell weight) of GDH in NaP_i buffer (100 mM, pH 7.0). Reaction mixtures in the Eppendorf tubes were shaken in a temperature-controlled orbital shaker at 30 ℃ and 200 rpm for 1 h.

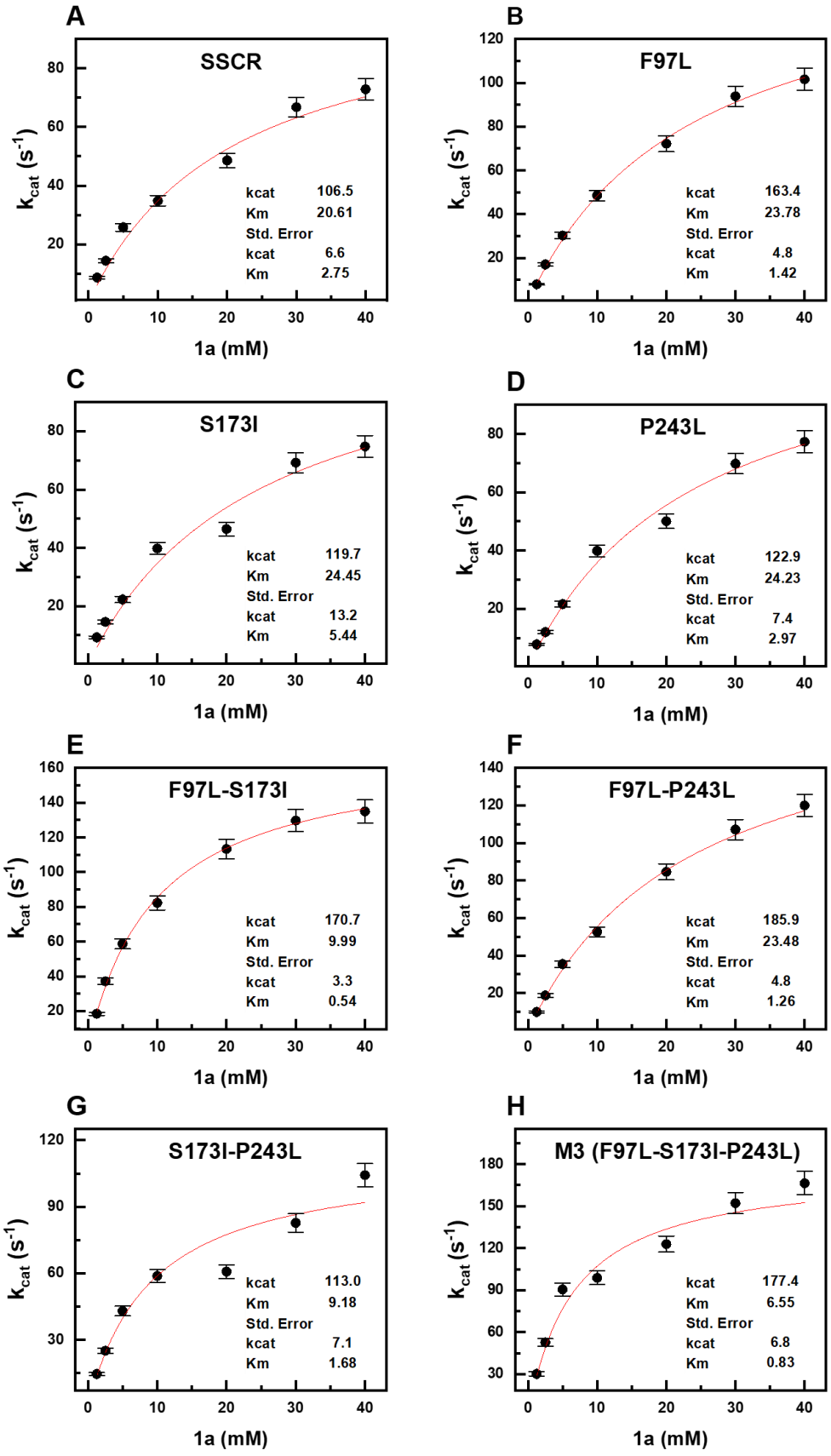
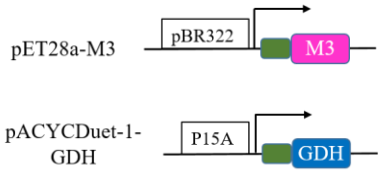
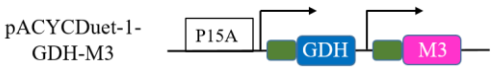
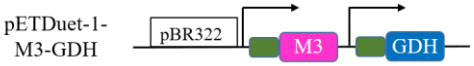
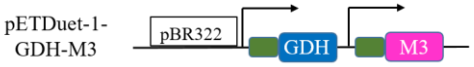


Figure S5. Dependence of the activity of SSCR and its mutants on the concentration of K-PaOEt (**1a**).

Table S3. Genetic construction of recombinant *E. coli* strains

Entry	Recombinant *E. coli* strains	Plasmids
1	*E. coli* (pET28a-M3/ pACYCDuet-1-GDH)	
2	*E. coli* (pACYCDuet-1-M3-GDH)	
3	*E. coli* (pACYCDuet-1-GDH-M3)	
4	*E. coli* (pETDuet-1-M3-GDH)	
5	*E. coli* (pETDuet-1-GDH-M3)	
6	*E. coli* (pRSFDuet-1-M3-GDH)	
7	*E. coli* (pRSFDuet-1-GDH-M3)	

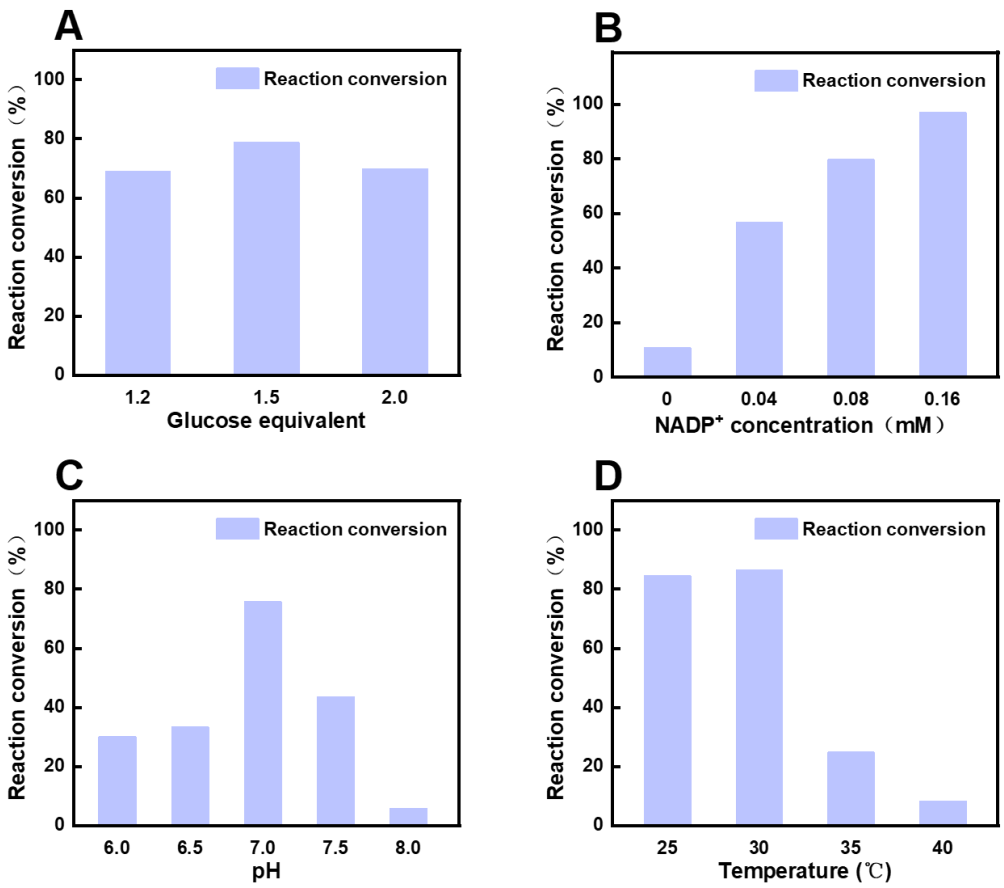


Figure S6. Reaction condition optimization for the reduction of K-PaOEt (**1a**) to (*R*)-PaOEt ((*R*)-**2a**). (A) Glucose concentration. Reaction conditions (5 mL): **1a** (100 g/L), glucose (variable amounts), $NADP^+$ (0.08 mM), toluene (10%, v/v), wet cells of recombinant *E. coli* strains (0.1 g, 20 g/L) in NaP_i buffer (100 mM, pH 7.0). Reaction mixtures in the round-bottom flasks were stirred in a metal heating block at 30 ℃ and 1500 rpm for 3 h. (B) $NADP^+$ concentration. Reaction conditions (5 mL): **1a** (100 g/L), glucose (1.5 equiv.), $NADP^+$ (variable amounts), toluene (10%, v/v), wet cells of recombinant *E. coli* strains (0.1 g, 20 g/L) in NaP_i buffer (100 mM, pH 7.0). Reaction mixtures in the round-bottom flasks were stirred in a metal heating block at 30 ℃ and 1500 rpm for 3 h. (C) pH. Reaction conditions (5 mL): **1a** (100 g/L), glucose (1.5 equiv.), $NADP^+$ (0.08 mM), toluene (10%, v/v), wet cells of recombinant *E. coli* strains (0.1 g, 20 g/L) in 100 mM NaP_i buffers of different pHs (6.0-8.0). Reaction mixtures in the round-bottom flasks were stirred in a metal heating block at 30 ℃ and 1500 rpm for 3 h. (D) Temperature. Reaction conditions (5 mL): **1a** (100 g/L), glucose (1.5 equiv.), $NADP^+$ (0.08 mM), toluene (10%, v/v), wet cells of recombinant *E. coli* strains (0.1 g, 20 g/L) in NaP_i buffer (100 mM, pH 7.0). Reaction mixtures in the round-bottom flasks were stirred in a metal heating block at different temperatures (25-40 ℃) and 1500 rpm for 3 h.

Table S4. Calculation of E-factor for recombinant strain *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed synthesis of (*R*)-PaOEt at gram-scale[a]

	Compound	Weight (g)	
Product	(*R*)-PaOEt	1.3	
Input	substrate	1.5	
	glucose	1.83	
	E. coli cells (corresponding to dry cell weight)	0.11	
	salt in 100 mM PBS (pH 7.0)	13.5 mL, 0.162 g	
	toluene	1.5 mL, 1.308 g	
	$NADP^+$	0.0009 g	
	silica gel	5	
	DCM	53	
	H_2O	13.5	
Waste	Including water	75.11	
	Excluding water	61.61	
E-factor	Including water		57.78
	Excluding water		47.39

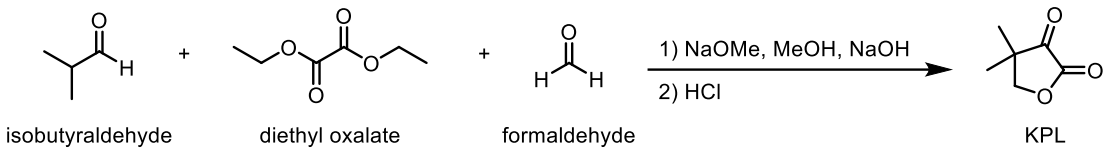
[a]100 g/L substrate, 15 mL reaction scale.

Table S5. Primers used for the mutagenesis study

Name	Sequence (5'→3')
V94A-F	TATCGCAAGC**GCG**GTGAGCTTTAGTAATAAAT
V94A-R	TAAAGCTCAC**CGC**GCTTGCGATATGGGCCACAC
V95A-F	CGCAAGCGTG**GCG**AGCTTTAGTAATAAATACG
V95A-R	TACTAAAGCT**CGC**CACGCTTGCGATATGGGCC
F97A-F	CGTGGTGAGC**GCT**AGTAATAAATACGATGAAGT
F97A-R	ATTTATTACT**AGC**GCTCACCACGCTTGCGATAT
T134A-F	GACAAGTAGT**GCC**GTGAGCGCCTTAATTCCGAAG
T134A-R	AGGCGCTCAC**GGC**ACTACTTGTCAGCACGAAGC
V135A-F	AAGTAGTACC**GCG**AGCGCCTTAATTCCGAAGCC
V135A-R	TTAAGGCGCT**CGC**GGTACTACTTGTCAGCACG
S173A-F	TCCGCAGAAA**GCT**TTATGGGTGTACGCCGCCAG
S173A-R	ACACCCATAA**AGC**TTTCTGCGGATCGCTCTCCG
L174A-F	GCAGAAATCT**GCA**TGGGTGTACGCCGCCAGT
L174A-R	CGTACACCCA**TGC**AGATTTCTGCGGATCGCTCT
S180A-F	GTACGCCGCC**GCT**AAAACCGAGGCAGAACTGGC
S180A-R	CCTCGGTTTT**AGC**GGCGGCGTACACCCATAAAG
P206A-F	CGCCGTGCTG**GCG**AACTACACCATCGGCACC
P206A-R	TGGTGTAGTT**CGC**CAGCACGGCGTTTAAAGT
N207A-MP -F	CGTGCTGCCG**GCC**TACACCATCGGCACCATT
N207A-MP-R	TGGTCCGGAAAATCGGCCGGGAAGGT
Y208A-F	GCTGCCGAAC**GCC**ACCATCGGCACCATTTTCG
Y208A-R	TGCCGATGGT**GGC**GTTCGGCAGCACGGCGTT
T209A-F	GCTGCCGAAC**GCC**ACCATCGGCACCATTTTCG
T209A-R	TGGTGCCGAT**GGC**GTAGTTCGGCAGCACGGCGT
T223A-F	GAGCGGCAGC**GCA**AGCGGTTGGATGATGTCTTT
T223A-R	TCCAACCGCT**TGC**GCTGCCGCTCTGGGTTTCCG
W226A-F	CACAAGCGGT**GCG**ATGATGTCTTTATTCAACGG
W226A-R	AAGACATCAT**CGC**ACCGCTTGTGCTGCCGCTCT
M242A-F	CTTAGCTTTA**GCG**CCTCCGCAGTACTATGTTAG
M242A-R	ACTGCGGAGG**CGC**TAAAGCTAAGGCCGGGCTC
P243A-F	AGCTTTAATG**GCT**CCGCAGTACTATGTTAGCGC
P243A-R	AGTACTGCGG**AGC**CATTAAAGCTAAGGCCGGGC
Q245A-F	AATGCCTCCG**GCG**TACTATGTTAGCGCCGTGG
Q245A-R	TAACATAGTA**CGC**CGGAGGCATTAAAGCTAAGG
Y246A-F	GCCTCCGCAG**GCC**TATGTTAGCGCCGTGGATAT
Y246A-R	CGCTAACATA**GGC**CTGCGGAGGCATTAAAGCT
F97X-F	CGTGGTGAGC**NNK**AGTAATAAATACGATGAAGT
F97X-R	ATTTATTACT**MNN**GCTCACCACGCTTGCGATAT
S173X-F	TCCGCAGAAA**NNK**TTATGGGTGTACGCCGCCAG
S173X-R	ACACCCATAA**MNN**TTTCTGCGGATCGCTCTCCG
M242X-F	CTTAGCTTTA**NNK**CCTCCGCAGTACTATGTTAG

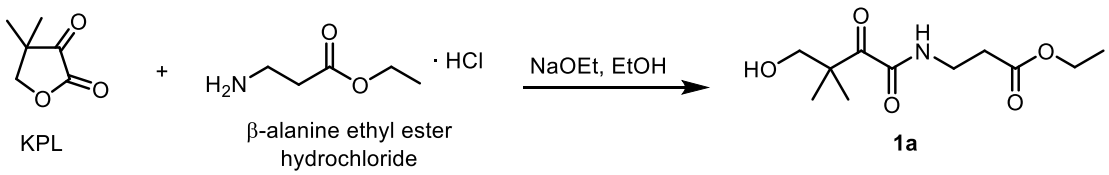
M242X-R	ACTGCGGAGG**MNN**TAAAGCTAAGGCCGGGCTC
P243X-MP-F	AGCTTTAATG**NNK**CCGCAGTACTATGTTAGCGC
P243X-MP-R	AGGCGGTTTCGCTACCAACCAGATC
V135C-F	AAGTAGTACC**TGT**AGCGCCTTAATTCCGAAGCC
V135C-R	TTAAGGCGCT**ACA**GGTACTACTTGTCAGCACG
V135D-F	AAGTAGTACC**GAT**AGCGCCTTAATTCCGAAGCC
V135D-R	TTAAGGCGCT**ATC**GGTACTACTTGTCAGCACG
V135E-F	AAGTAGTACC**GAG**AGCGCCTTAATTCCGAAGCC
V135E-R	TTAAGGCGCT**CTC**GGTACTACTTGTCAGCACG
V135F-F	AAGTAGTACC**TTT**AGCGCCTTAATTCCGAAGCC
V135F-R	TTAAGGCGCT**AAA**GGTACTACTTGTCAGCACG
V135G-F	AAGTAGTACC**GGG**AGCGCCTTAATTCCGAAGCC
V135G-R	TTAAGGCGCT**CCC**GGTACTACTTGTCAGCACG
V135H-F	AAGTAGTACC**CAT**AGCGCCTTAATTCCGAAGCC
V135H-R	TTAAGGCGCT**ATG**GGTACTACTTGTCAGCACG
V135I-F	AAGTAGTACC**ATT**AGCGCCTTAATTCCGAAGCC
V135I-R	TTAAGGCGCT**AAT**GGTACTACTTGTCAGCACG
V135K-F	AAGTAGTACC**AAG**AGCGCCTTAATTCCGAAGCC
V135K-R	TTAAGGCGCT**CTT**GGTACTACTTGTCAGCACG
V135L-F	AAGTAGTACC**CTG**AGCGCCTTAATTCCGAAGCC
V135L-R	TTAAGGCGCT**CAG**GGTACTACTTGTCAGCACG
V135M-F	AAGTAGTACC**ATG**AGCGCCTTAATTCCGAAGCC
V135M-R	TTAAGGCGCT**CAT**GGTACTACTTGTCAGCACG
V135N-F	AAGTAGTACC**AAC**AGCGCCTTAATTCCGAAGCC
V135N-R	TTAAGGCGCT**GTT**GGTACTACTTGTCAGCACG
V135P-F	AAGTAGTACC**CCG**AGCGCCTTAATTCCGAAGCC
V135P-R	TTAAGGCGCT**CGG**GGTACTACTTGTCAGCACG
V135Q-F	AAGTAGTACC**CAG**AGCGCCTTAATTCCGAAGCC
V135Q-R	TTAAGGCGCT**CTG**GGTACTACTTGTCAGCACG
V135R-F	AAGTAGTACC**AGG**AGCGCCTTAATTCCGAAGCC
V135R-R	TTAAGGCGCT**CCT**GGTACTACTTGTCAGCACG
V135S-F	AAGTAGTACC**TCG**AGCGCCTTAATTCCGAAGCC
V135S-R	TTAAGGCGCT**CGA**GGTACTACTTGTCAGCACG
V135T-F	AAGTAGTACC**ACG**AGCGCCTTAATTCCGAAGCC
V135T-R	TTAAGGCGCT**CGT**GGTACTACTTGTCAGCACG
V135W-F	AAGTAGTACC**TGG**AGCGCCTTAATTCCGAAGCC
V135W-R	TTAAGGCGCT**CCA**GGTACTACTTGTCAGCACG
V135Y-F	AAGTAGTACC**TAT**AGCGCCTTAATTCCGAAGCC
V135Y-R	TTAAGGCGCT**ATA**GGTACTACTTGTCAGCACG
P243R-F97M-F	CGTGGTGAGC**ATG**AGTAATAAATACGATGAAGT
P243R-F97M-R	ATTTATTACT**CAT**GCTCACCACGCTTGCGATAT
P243R-F97L-F	CGTGGTGAGC**TTG**AGTAATAAATACGATGAAGT
P243R-F97L-R	ATTTATTACT**CAA**GCTCACCACGCTTGCGATAT
P243R-S173I-F	TCCGCAGAAA**ATT**TTATGGGTGTACGCCGCCAG

P243R-S173I-R	ACACCCATAA**AAT**TTTCTGCGGATCGCTCTCCG
F1	TTTAACTTTAAGAAGGAGATATACCATGGCAACTGAAC AGAAAGCCATTG
R1	CTGCAGGCGCGCCGAGCTCGAATTCTCACTGCCACTTT ATCACCGTCT
F2	TTAAGTATAAGAAGGAGATATACATATGGCCAAAATCGA TAACGCAGTGC
R2	CAGCGGTTTCTTTACCAGACTCGAGTTAGGCGGTTTCG CTACCAACCAG
F3	TTTAACTTTAATAAGGAGATATACCATGGCAACTGAACA GAAAGCCATTGT
R3	CTGCAGGCGCGCCGAGCTCGAATTCTCACTGCCACTTT ATCACCGTCTTTAT
F4	TTAAGTATAAGAAGGAGATATACATATGGCAACTGAAC AGAAAGCCATTG
R4	CAGCGGTTTCTTTACCAGACTCGAGTCACTGCCACTTT ATCACCGTCTT
F5	TTTAACTTTAAGAAGGAGATATACCATGGCCAAAATCG ATAACGCAGTGC
R5	CTGCAGGCGCGCCGAGCTCGAATTCTTAGGCGGTTTCG CTACCAACCAG
F6	TTTAACTTTAATAAGGAGATATACCATGGCCAAAATCGA TAACGCAGTGC
R6	CTGCAGGCGCGCCGAGCTCGAATTCTTAGGCGGTTTCG CTACCAACCAG



Scheme S1. Synthesis of KPL.[1]

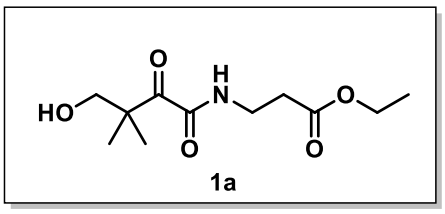
This is a modified literature procedure.[1] To a mixture of 30% methanolic NaOMe (74 g, 0.41 mol, 1.2 equiv.) and diethyl oxalate (50 g, 0.34 mol, 1.0 equiv.) was added isobutyraldehyde (27.1 g, 0.38 mol, 1.1 equiv.) at 0 ℃, and then the mixture was stirred for 1 h at the same temperature. Then 37% HCHO solution (28.1 g, 0.35 mol, 1.02 equiv.) was added, and the mixture was stirred for another hour at 0℃. 40% NaOH solution (51 g, 0.51 mol, 1.5 equiv.) was added at 0 ℃. After stirring for 1 h, conc. HCl (75 mL) was added and the stirring continued for another hour. Upon adjusting the pH to 3 with 40% NaOH solution, NaCl formed was formed and filtered off, and the filtrate was evaporated to remove MeOH. The residual aqueous solution was extracted with EtOAc. The combined organic layer is dried with Na_2SO_4 and evaporated to give the crude product, which was washed with MTBE to afford KPL as white solid in 83% yield. The characterization data of thus synthesized KPL matched well with that reported in literature.



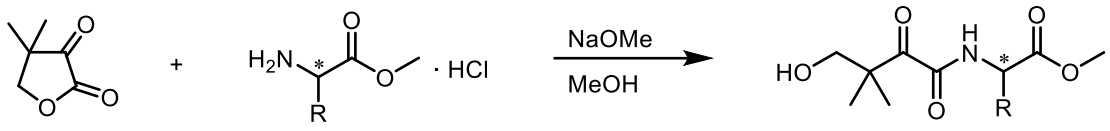
Scheme S2. Synthesis of compound **1a**.

A mixture of NaOEt (4.7 g, 69 mmol, 1.06 equiv.), β-alanine ethyl ester hydrochloride (10.6 g, 69 mmol, 1.06 equiv.), and KPL (8.4 g, 65 mmol) in anhydrous EtOH (190 mL) was stirred overnight at room temperature. Ethanol was removed, and the mixture was dissolved in water, extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (PE/EA = 5:1) to afford **1a** as colorless oil (10 g, 40 mmol, 62%).

Ethyl 3-(4-hydroxy-3,3-dimethyl-2-oxobutanamido)propanoate



Rf = 0.6 (PE/EA = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.76 (s, 2H), 3.57 (q, J = 6.3 Hz, 2H), 2.59 (t, J = 6.3 Hz, 2H), 1.28 (t, J = 7.5 Hz, 3H), 1.27 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3, 172.0, 161.0, 68.9, 61.0, 48.9, 34.7, 33.6, 21.4, 14.2. HRMS (ESI, m/z) calcd for $C_{11}H_{19}NO_5Na$ $[M + Na]^+$ 268.1155, found 268.1156.

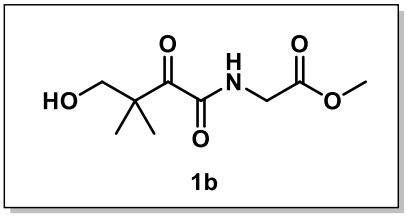


KPL amino methyl ester hydrochloride **1**

Scheme S3. General procedure for the synthesis of compounds **1**.

A mixture of NaOMe (1.1 equiv.), amino methyl ester hydrochloride (1.05 equiv.), and KPL (20 mmol, 1.0 equiv.) in anhydrous MeOH (40 mL) was stirred overnight at room temperature. Methanol was removed, and the mixture was dissolved in water, extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography to afford **1**.

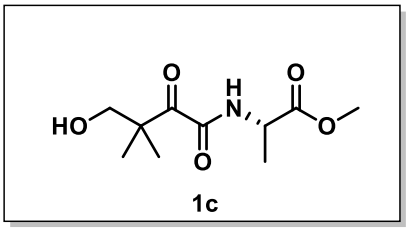
Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl) glycinate



1b

Compound **1b** was prepared and purified in 73% yield (3.2 g, 14.7 mmol) as yellow solid via column chromatography (5/1 PE/EA), starting from 20 mmol of KPL. Rf = 0.5 (PE/EA = 1:1). m.p. = 45.0-47.3 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 4.09 (d, J = 5.7 Hz, 2H), 3.80 (s, 3H), 3.79 (s, 2H), 1.31 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.8, 169.4, 161.0, 69.0, 52.7, 48.9, 40.9, 21.4. HRMS (ESI, m/z) calcd for $C_9H_{15}NO_5Na$ $[M + Na]^+$ 240.0842, found 240.0842.

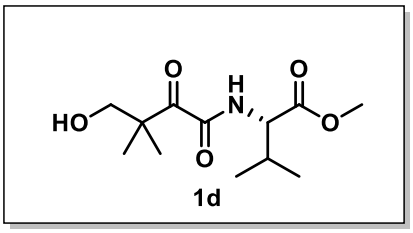
Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-L-alaninate



1c

Compound **1c** was prepared and purified in 65% yield (3 g, 12.99 mmol) as colorless oil via column chromatography (4/1 PE/EA), starting from 20 mmol of KPL. Rf = 0.5 (PE/EA = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 8.0 Hz, 1H), 4.51-4.43 (m, 1H), 3.75-3.65 (m, 5H), 1.40 (d, J = 7.3 Hz, 3H), 1.21 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.2, 172.5, 160.8, 68.9, 52.7, 48.9, 47.9, 21.4, 21.3, 17.7. HRMS (ESI, m/z) calcd for $C_{10}H_{17}NO_5$ $[M + H]^+$ 232.1179, found 232.1177.

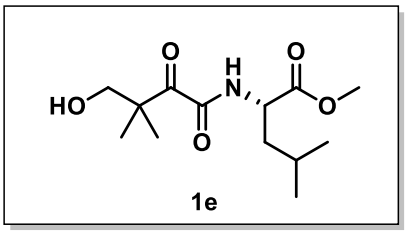
Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-L-valinate



1d

Compound **1d** was prepared and purified in 58% yield (3 g, 11.58 mmol) as colorless oil via column chromatography (5/1 PE/EA), starting from 20 mmol of KPL. Rf = 0.6 (PE/EA = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 9.2 Hz, 1H), 4.45 (dd, J = 9.0, 5.0 Hz, 1H), 3.74 (s, 3H), 3.73 (s, 2H), 2.20 (td, J = 6.9, 5.0 Hz, 1H), 1.26 (s, 6H), 0.93 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.2, 171.4, 160.9, 68.9, 57.1, 52.4, 48.9, 31.2, 21.4, 21.3, 19.0, 17.7. HRMS (ESI, m/z) calcd for $C_{12}H_{21}NO_5$ $[M + H]^+$ 260.1492, found 260.1487.

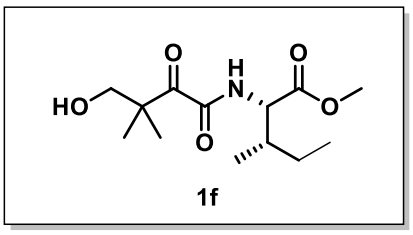
Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-L-leucinate



1e

Compound **1e** was prepared and purified in 73% yield (4.0 g, 14.7 mmol) as colorless oil via column chromatography (5/1 PE/EA), starting from 20 mmol of KPL. Rf = 0.7 (PE/EA = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 8.6 Hz, 1H), 4.56-4.47 (m, 1H), 3.69 (s, 3H), 3.68 (s, 2H), 3.10 (s, 1H), 1.66-1.60 (m, 1H), 1.59-1.53 (m, 2H), 1.22 (s, 6H), 0.88 (d, J = 6.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1, 172.4, 160.7, 69.0, 52.6, 50.7, 48.9, 41.2, 24.9, 22.7, 21.8, 21.43, 21.36. HRMS (ESI, m/z) calcd for $C_{13}H_{23}NO_5$ $[M + H]^+$ 274.1649, found 274.1645.

Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-L-isoleucinate

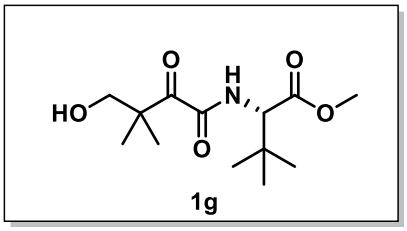


1f

Compound **1f** was prepared and purified in 71% yield (5 g, 18.32 mmol) as colorless oil via column chromatography (5/1 PE/EA), starting from 25.7 mmol of KPL. Rf = 0.7 (PE/EA = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, J = 9.0 Hz, 1H), 4.46 (dd, J = 8.9, 4.9 Hz, 1H), 3.69 (s, 3H), 3.68 (s, 2H) 1.89 (ddt, J = 9.4, 7.0, 4.8 Hz, 1H), 1.41-1.34 (m, 1H), 1.22 (s, 6H), 1.18-1.11 (m, 1H), 0.89-0.84 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1, 171.4, 160.7, 69.0,

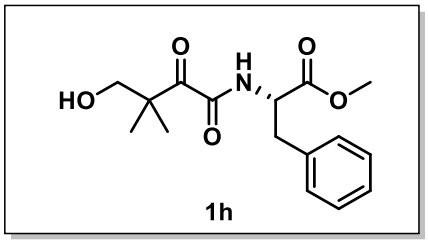
56.4, 52.3, 48.9, 37.8, 25.1, 21.4, 21.3, 15.5, 11.5. HRMS (ESI, m/z) calcd for $C_{13}H_{23}NO_5$ [M + H]$^+$ 274.1649, found 274.1649.

Methyl (S)-2-(4-hydroxy-3,3-dimethyl-2-oxobutanamido)-3,3-dimethylbutanoate



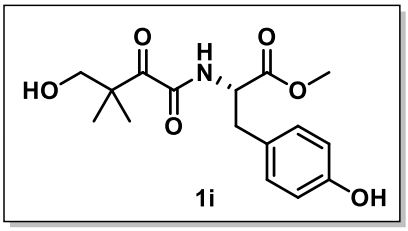
Compound **1g** was prepared and purified in 55% yield (3.0 g, 11.0 mmol) as colorless oil via column chromatography (5/1 PE/EA), starting from 20 mmol of KPL. Rf = 0.7 (PE/EA = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, J = 9.8 Hz, 1H), 4.32 (d, J = 9.7 Hz, 1H), 3.69 (s, 3H), 3.68 (s, 2H), 1.23 (s, 6H), 0.93 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.0, 170.9, 160.4, 69.0, 60.2, 52.1, 48.9, 35.1, 26.5, 21.4. HRMS (ESI, m/z) calcd for $C_{13}H_{23}NO_5$ [M + H]$^+$ 274.1649, found 274.1643.

Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-L-phenylalaninate



Compound **1h** was prepared and purified in 62% yield (2.5 g, 8.14 mmol) as yellow oil via column chromatography (5/1 PE/EA), starting from 13 mmol of KPL. Rf = 0.4 (PE/EA = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 8.3 Hz, 1H), 7.32-7.25 (m, 3H), 7.13 (dd, J = 8.0, 1.6 Hz, 2H), 4.84 (dd, J = 14.0, 6.9 Hz, 1H), 3.75 (s, 3H), 3.72 (s, 2H), 3.21 (dd, J = 13.9, 5.6 Hz, 1H), 3.10 (dd, J = 13.9, 6.9 Hz, 1H), 1.25 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.8, 171.0, 160.4, 135.4, 129.2, 128.7, 127.4, 69.0, 53.1, 52.6, 48.9, 37.9, 21.34, 21.31. HRMS (ESI, m/z) calcd for $C_{16}H_{21}NO_5$ [M + H]$^+$ 308.1492, found 308.1497.

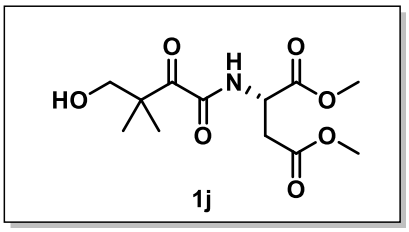
Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-L-tyrosinate



Compound **1i** was prepared and purified in 59% yield (3.8 g, 11.76 mmol) as colorless oil via column chromatography (2/1 PE/EA), starting from 20 mmol of KPL. Rf = 0.4 (PE/EA = 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 6.66 (d, J = 8.5 Hz, 2H), 4.70 (dd, J = 14.0, 6.8 Hz, 1H), 3.68 (s, 3H), 3.65 (s, 2H), 3.05 (dd, J = 14.1, 5.5 Hz, 1H), 2.94 (dd, J = 14.0, 6.8 Hz, 1H), 1.17 (s, 3H), 1.16 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 171.3, 160.6, 155.4, 130.4, 126.7, 115.7, 68.9, 53.3, 52.7, 48.8, 37.0, 21.4, 21.3. HRMS (ESI, m/z) calcd for $C_{16}H_{21}NO_6$ $[M + H]^+$ 324.1442, found 324.1444.

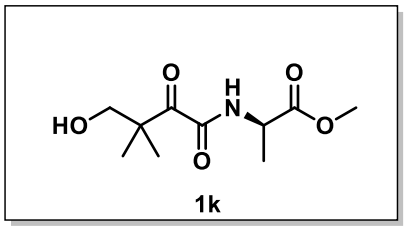
Dimethyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-L-aspartate



1j

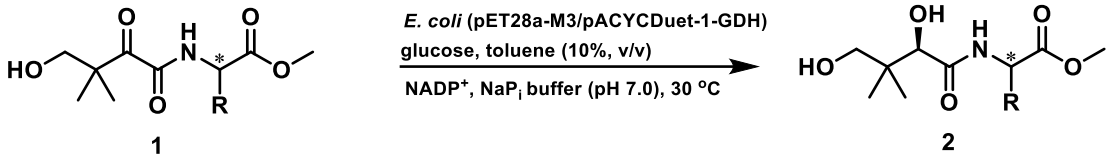
Compound **1j** was prepared and purified in 56% yield (3.2 g, 11.07 mmol) as yellow oil via column chromatography (5/1 PE/EA), starting from 20 mmol of KPL. Rf = 0.5 (PE/EA = 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 8.4 Hz, 1H), 4.85 (dt, J = 9.2, 4.8 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 5H), 3.72 (s, 3H), 3.08 (dd, J = 17.2, 5.0 Hz, 1H), 2.89 (dd, J = 17.2, 4.6 Hz, 1H), 1.30 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.8, 171.0, 170.2, 160.7, 69.1, 53.1, 52.3, 48.9, 48.4, 35.8, 21.4, 21.3. HRMS (ESI, m/z) calcd for $C_{12}H_{19}NO_7$ $[M + H]^+$ 290.1234, found 290.1231.

Methyl (4-hydroxy-3,3-dimethyl-2-oxobutanoyl)-D-alaninate



1k

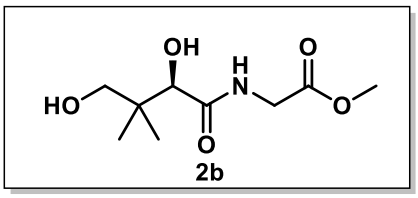
Compound **1k** was prepared and purified in 64% yield (2.5 g, 10.82 mmol) as colorless oil via column chromatography (4/1 PE/EA), starting from 17 mmol of KPL. Rf = 0.5 (PE/EA = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, J = 7.8 Hz, 1H), 4.57-4.44 (m, 1H), 3.73 (s, 3H), 3.72 (s, 2H), 1.43 (d, J = 7.2 Hz, 3H), 1.24 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1, 172.5, 160.7, 68.9, 52.7, 48.9, 48.0, 21.4, 21.3, 17.7. HRMS (ESI, m/z) calcd for $C_{10}H_{17}NO_5Na$ $[M + Na]^+$ 254.0999, found 254.0997.



Scheme S4. *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed stereoselective synthesis of **2**.

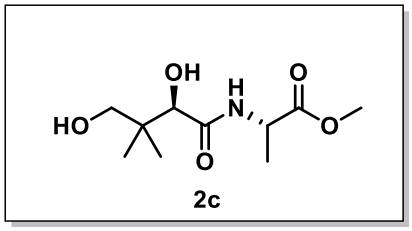
A mixture of **1** (10 mM), glucose (20 mM), $NADP^+$ (0.2 mM), toluene (5 mL, 10%, v/v) and wet cells of *E. coli* (pET28a-M3/pACYCDuet-1-GDH) (2.5 g) in NaP_i buffer (45 mL, 100 mM, pH 7.0) was in a round-bottom flask and stirred in a metal heating block at 30 ℃ and 600 rpm for 12 h. Silica was added and the resulting mixture was stirred for 20 min, and then filtered. DCM (5 x 20 mL) was employed for extraction, and the combined organic layer was dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by preparative TLC (1/2 PE/EA) to afford product **2**.

Methyl (*R*)-(2,4-dihydroxy-3,3-dimethylbutanoyl)glycinate



Compound **2b** was prepared in 50% yield (81 mg, 0.37 mmol) as colorless oil, starting from 0.74 mmol of **1b**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.5 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H), 4.51 (d, J = 5.1 Hz, 1H), 4.10 (s, 1H), 4.07 (d, J = 6.1 Hz, 2H), 3.76 (s, 3H), 3.52 (s, 2H), 2.40 (s, 1H), 1.03 (s, 3H), 0.95 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 170.6, 77.4, 71.0, 52.5, 40.7, 39.3, 21.1, 20.5. HRMS (ESI, m/z) calcd for $C_9H_{17}NO_5$ $[M + H]^+$ 220.1179, found 220.1178. $[\alpha]_D^{20}$ = +44.33 (c = 0.6, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 5.2 min (major), t_2 = 8.1 min; >99% ee (determined upon benzoylation).

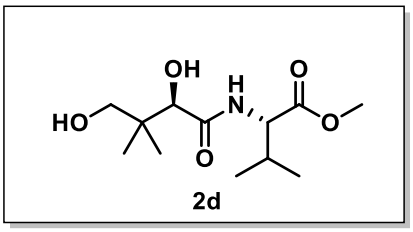
Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-alaninate



Compound **2c** was prepared in 50% yield (130 mg, 0.56 mmol) as colorless oil, starting from 1.13 mmol of **1c**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.5 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 7.8 Hz, 1H), 4.62-4.55 (m, 1H), 4.26 (s, 1H), 4.05 (s, 1H), 3.76 (s, 3H), 3.52 (s, 2H), 1.45 (d, J = 7.3 Hz, 3H),

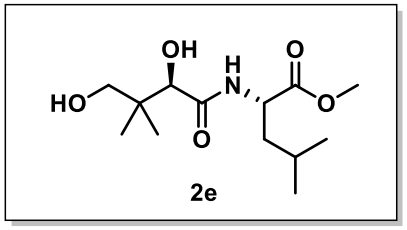
1.05 (s, 3H), 0.97 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 172.9, 77.8, 71.2, 52.5, 47.6, 39.4, 21.1, 20.7, 17.9. HRMS (ESI, m/z) calcd for $C_{10}H_{19}NO_5$ $[M + H]^+$ 234.1336, found 234.1338. $[\alpha]_D^{20}$ = +22.55 (c = 1.1, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 5.4 min (major), t_2 = 6.9 min; >99% de (determined upon benzoylation).

Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-valinate



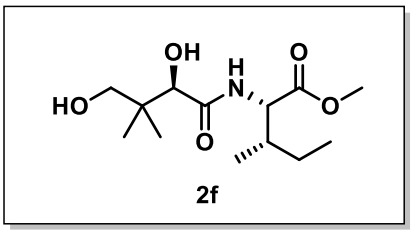
Compound **2d** was prepared in 71% yield (100 mg, 0.38 mmol) as colorless oil, starting from 0.53 mmol of **1d**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.6 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.9 Hz, 1H), 4.75 (s, 1H), 4.41 (dd, *J* = 8.8, 5.0 Hz, 1H), 4.01 (s, 1H), 3.67 (s, 3H), 3.42 (s, 2H), 2.19-2.11 (m, 1H), 0.94 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 172.2, 77.5, 71.0, 56.9, 52.2, 39.4, 30.8, 20.9, 20.6, 19.1, 17.7. HRMS (ESI, m/z) calcd for $C_{12}H_{23}NO_5$ $[M + H]^+$ 262.1649, found 260.1647. $[\alpha]_D^{20}$ = +16.63 (c = 0.4, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 5.2 min (major), t_2 = 14.9 min; >99% de (determined upon benzoylation).

Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-leucinate



Compound **2e** was prepared in 82% yield (290 mg, 1.05 mmol) as colorless oil, starting from 1.28 mmol of **1e**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.7 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.3 Hz, 1H), 4.62-4.55 (m, 1H), 4.05 (s, 1H), 3.74 (s, 3H), 3.50 (s, 2H), 1.71-1.58 (m, 3H), 1.03 (s, 3H), 0.96 (s, 3H), 0.94 (d, *J* = 5.5 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 77.7, 71.1, 52.4, 50.4, 41.0, 39.4, 24.9, 22.8, 21.7, 21.0, 20.7. HRMS (ESI, m/z) calcd for $C_{13}H_{25}NO_5$ $[M + H]^+$ 276.1805, found 276.1808. $[\alpha]_D^{20}$ = +12.13 (c = 2.4, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 4.9 min (major), t_2 = 7.1 min; >99% de (determined upon benzoylation).

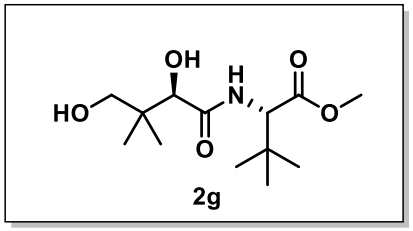
Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-isoleucinate



2f

Compound **2f** was prepared in 71% yield (100 mg, 0.36 mmol) as colorless oil, starting from 0.51 mmol of **1f**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.7 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 8.6 Hz, 1H), 4.45 (dd, J = 8.6, 4.9 Hz, 1H), 4.00 (s, 1H), 3.67 (s, 3H), 3.42 (s, 2H), 1.88 (ddt, J = 9.4, 7.0, 4.7 Hz, 1H), 1.42-1.33 (m, 1H), 1.20-1.10 (m, 1H), 0.94 (s, 3H), 0.89-0.81 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 172.2, 77.4, 71.0, 56.2, 52.2, 39.3, 37.4, 25.1, 20.8, 20.6, 15.6, 11.5. HRMS (ESI, m/z) calcd for $C_{13}H_{25}NO_5$ $[M + H]^+$ 276.1805, found 276.1805. $[\alpha]_D^{20}$ = +31.88 (c = 0.6, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 5.0 min (major), t_2 = 8.9. min; >99% de (determined upon benzoylation).

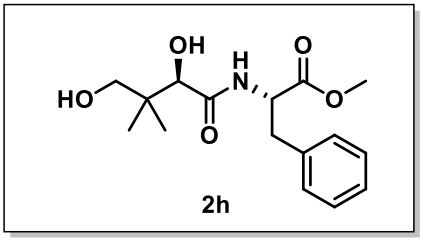
Methyl (S)-2-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-3,3-dimethylbutanoate



2g

Compound **2g** was prepared in 62% yield (90 mg, 0.33 mmol) as colorless oil, starting from 0.53 mmol of **1g**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.7 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (d, J = 8.9 Hz, 1H), 4.28 (d, J = 9.0 Hz, 1H), 4.00 (s, 1H), 3.66 (s, 3H), 3.41 (s, 2H), 0.93 (s, 12H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 171.7, 77.4, 71.1, 60.0, 51.8, 39.4, 34.3, 26.6, 20.8, 20.4. HRMS (ESI, m/z) calcd for $C_{13}H_{25}NO_5$ $[M + H]^+$ 276.1805, found 276.1799. $[\alpha]_D^{20}$ = +58.75 (c = 0.4, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 5.4 min (major), t_2 = 14.9 min; >99% de (determined upon benzoylation).

Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-phenylalaninate

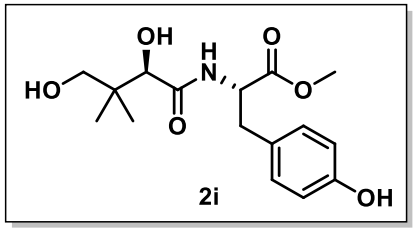


2h

Compound **2h** was prepared in 75% yield (240 mg, 0.78 mmol) as yellow oil, starting from 1.03 mmol of **1h**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid

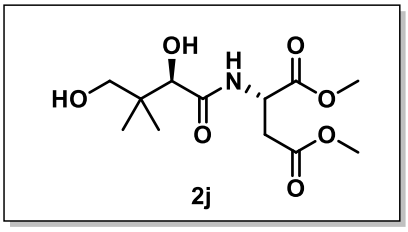
staining. Rf = 0.5 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.17 (m, 3H), 7.14 (d, J = 7.1 Hz, 1H), 7.07 (d, J = 7.1 Hz, 2H), 4.75 (q, J = 7.5 Hz, 1H), 3.90 (s, 1H), 3.62 (s, 3H), 3.32 (q, J = 11.1 Hz, 2H), 3.12-2.95 (m, 2H), 0.86 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 172.0, 135.8, 129.1, 128.7, 127.2, 77.3, 70.9, 52.9, 52.4, 39.2, 37.7, 20.8, 20.6. HRMS (ESI, m/z) calcd for $C_{16}H_{23}NO_5$ $[M + H]^+$ 310.1649, found 310.1650. $[\alpha]_D^{20}$ = +44.25 (c = 0.8, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 6.6 min (major), t_2 = 10.9 min; >99% de (determined upon benzoylation).

Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-tyrosinate



Compound **2i** was prepared in 75% yield (240 mg, 0.74 mmol) as colorless oil, starting from 0.98 mmol of **1i**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.4 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, J = 8.3 Hz, 1H), 6.98 (d, J = 8.1 Hz, 2H), 6.71 (d, J = 8.2 Hz, 2H), 4.86 – 4.78 (m, 1H), 4.27 (d, J = 4.0 Hz, 1H), 3.94 (d, J = 4.7 Hz, 1H), 3.75 (s, 3H), 3.43 (s, 2H), 3.14 (dd, J = 14.1, 5.1 Hz, 1H), 2.95 (dd, J = 14.2, 7.8 Hz, 1H), 0.95 (s, 3H), 0.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 172.2, 155.3, 130.3, 127.3, 115.7, 77.5, 70.9, 52.9, 52.6, 39.3, 36.9, 21.0, 20.6. HRMS (ESI, m/z) calcd for $C_{16}H_{23}NO_6$ $[M + H]^+$ 326.1598, found 326.1594. $[\alpha]_D^{20}$ = +53.50 (c = 0.6, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 10.2 min (major), t_2 = 15.4 min; >99% de (determined upon benzoylation).

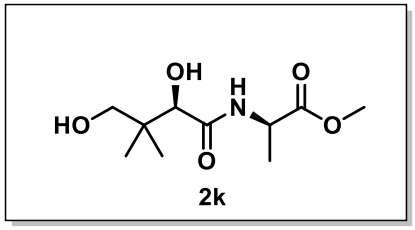
Dimethyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-aspartate



Compound **2j** was prepared in 51% yield (80 mg, 0.27 mmol) as yellow oil, starting from 0.53 mmol of **1j**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.5 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 8.6 Hz, 1H), 4.90 (dt, J = 9.2, 4.8 Hz, 1H), 4.29 (s, 1H), 4.07 (s, 1H), 3.77 (s, 3H), 3.71 (s, 3H), 3.52 (s, 2H), 3.06 (dd, J = 17.1, 5.1 Hz, 1H), 2.87 (dd, J = 17.1, 4.6 Hz, 1H), 1.04 (s, 3H), 0.97 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 171.6, 171.1, 77.8, 71.1, 52.9, 52.2, 48.1, 39.3, 35.9, 21.0, 20.7. HRMS (ESI, m/z) calcd for $C_{12}H_{21}NO_7$ $[M + H]^+$ 292.1391, found 292.1390. $[\alpha]_D^{20}$ = +40.67 (c = 0.6, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow

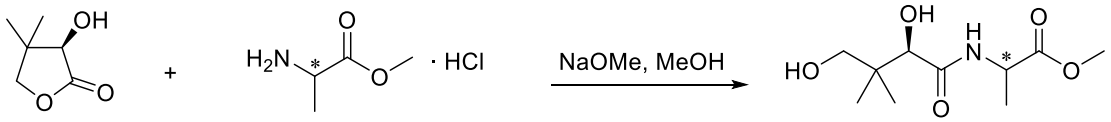
rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 8.1 min (major), t_2 = 13.0 min; >99% de (determined upon benzoylation).

Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-D-alaninate



2k

Compound **2k** was prepared in 61% yield (210 mg, 0.90 mmol) as colorless oil, starting from 1.48 mmol of **1k**, and purified by preparative TLC (1/2 PE/EA) with phosphomolybdic acid staining. Rf = 0.5 (PE/EA = 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 4.2 Hz, 1H), 4.54-4.47 (m, 1H), 3.98 (s, 1H), 3.69 (s, 3H), 3.42 (s, 2H), 1.36 (d, J = 7.3 Hz, 3H), 0.92 (s, 3H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 173.4, 76.7, 70.8, 52.6, 47.7, 39.3, 21.0, 20.2, 18.1. HRMS (ESI, m/z) calcd for $C_{10}H_{19}NO_5Na$ $[M + Na]^+$ 256.1155, found 256.1164. $[\alpha]_D^{20}$ = +63.00 (c = 1.6, EtOH). HPLC Chiracel AD-H, 250 mm × 4.6 mm column, 60/40/ hexane/isopropanol, flow rate of 1.0 mL/min, 254 nm UV lamp, 30 ℃, t_1 = 4.5 min (major), t_2 = 6.0 min; >99% de (determined upon benzoylation).

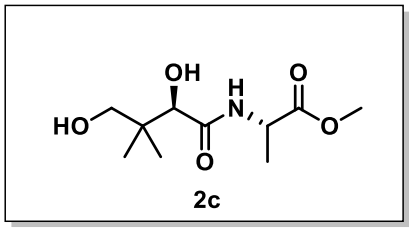


(*R*)-PL L-alanine methyl ester hydrochloride or D-alanine methyl ester hydrochloride **2c** or **2k**

Scheme S5. Synthesis of compounds **2c** or **2k** using (*R*)-PL.

A mixture of NaOMe (1.1 equiv.), L-alanine methyl ester hydrochloride or D-alanine methyl ester hydrochloride (1.05 equiv.), and (*R*)-PL (1.0 equiv.) in anhydrous MeOH was stirred overnight at room temperature. Methanol was removed, and the mixture was dissolved in water, extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated *in vacuo* to give the crude product. A portion of the crude product was purified by preparative TLC to afford **2c** or **2k** for characterization and derivatization purposes.

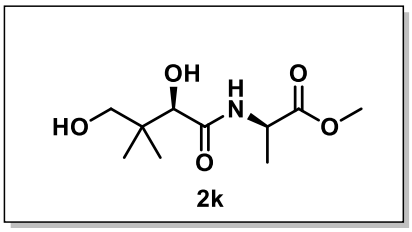
Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-L-alaninate



2c

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J = 7.6 Hz, 1H), 4.58-4.51 (m, 1H), 4.03 (d, J = 4.8 Hz, 1H), 3.97 (s, 1H), 3.74 (s, 3H), 3.53-3.44 (m, 2H), 1.43 (d, J = 7.3 Hz, 3H), 1.01 (s, 3H), 0.94 (s, 3H). $[\alpha]_D^{20}$ = +22.91 (c = 2.3, EtOH).

Methyl ((*R*)-2,4-dihydroxy-3,3-dimethylbutanoyl)-D-alaninate



2k

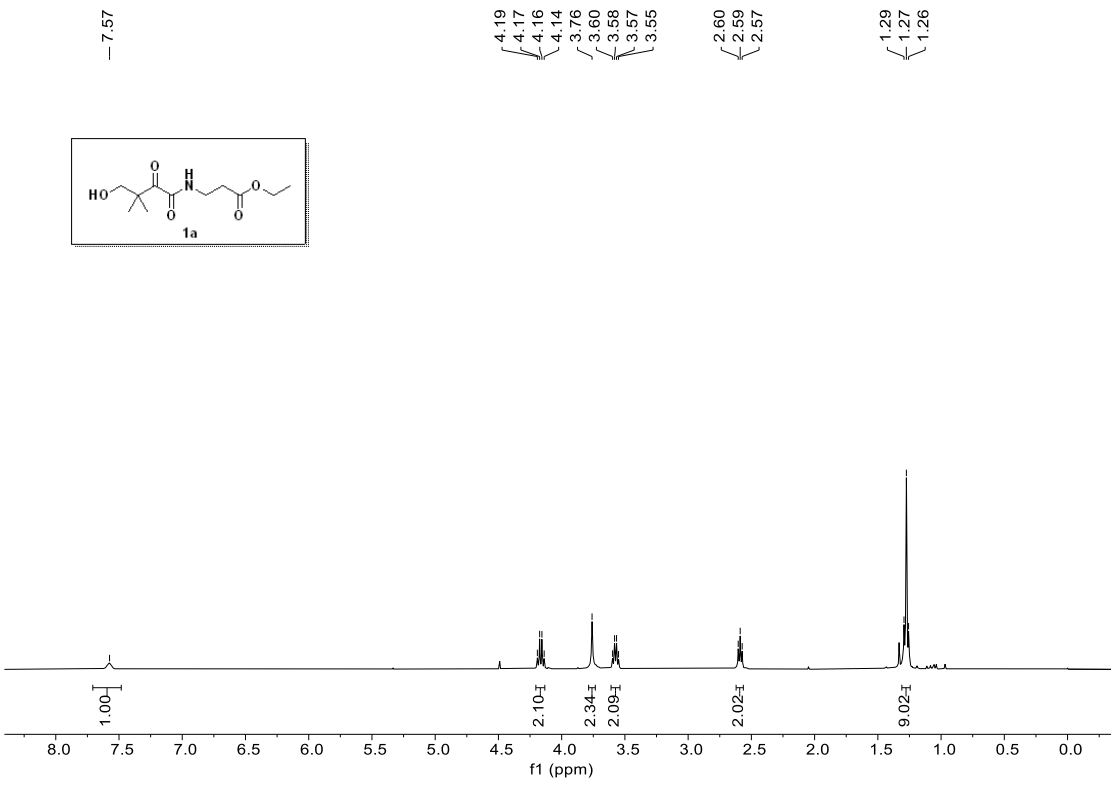
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 7.7 Hz, 1H), 4.53-4.46 (m, 1H), 3.98 (s, 1H), 3.68 (s, 3H), 3.41 (s, 2H), 1.36 (d, J = 7.3 Hz, 3H), 0.90 (s, 3H), 0.85 (s, 3H). $[\alpha]_D^{20}$ = +58.37 (c = 1.6, EtOH).

References

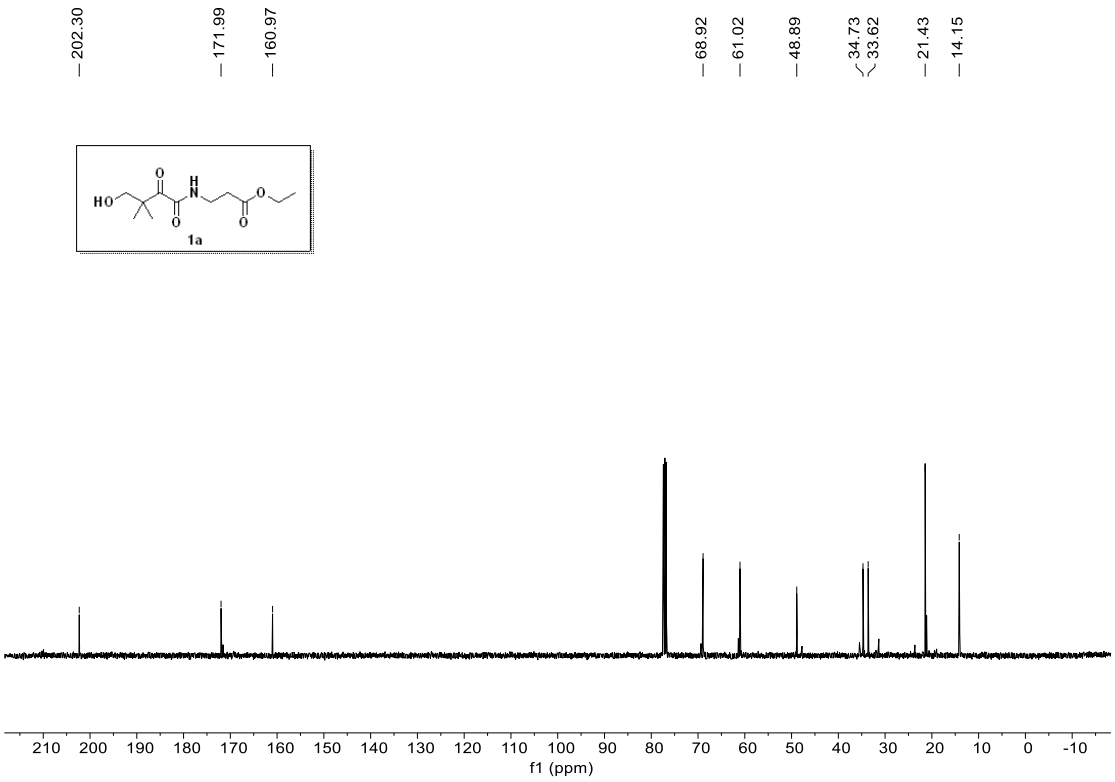
[1] A. Gruessner, H. Hata, T. Morishita, S. Akutsu and M. Kawamura, *Synthesis*, 1991, **4**, 289-291.

^{1}H-NMR and ^{13}C-NMR Spectra

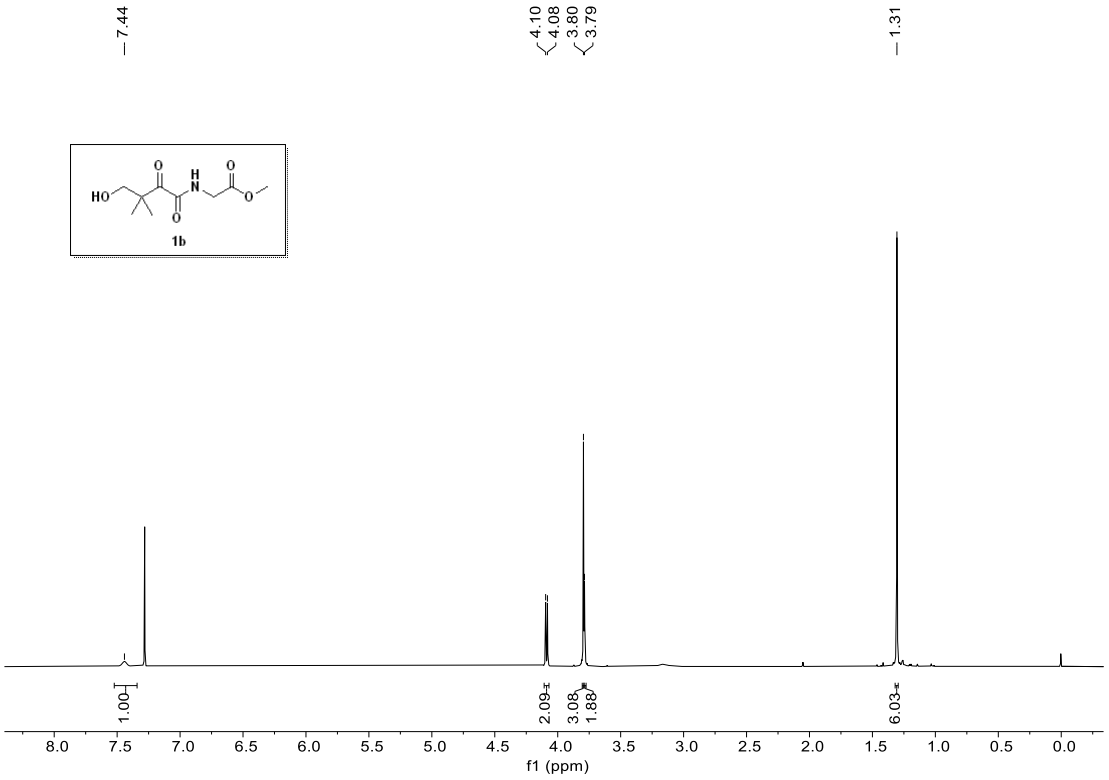
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1a**



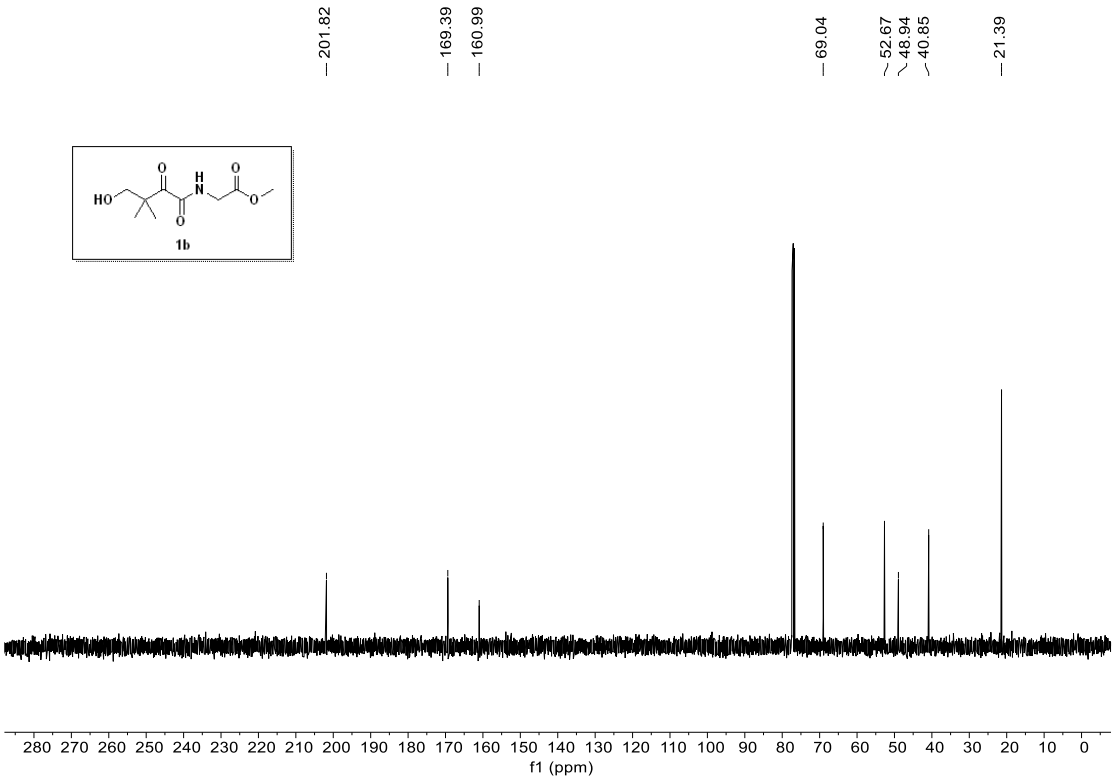
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1a**



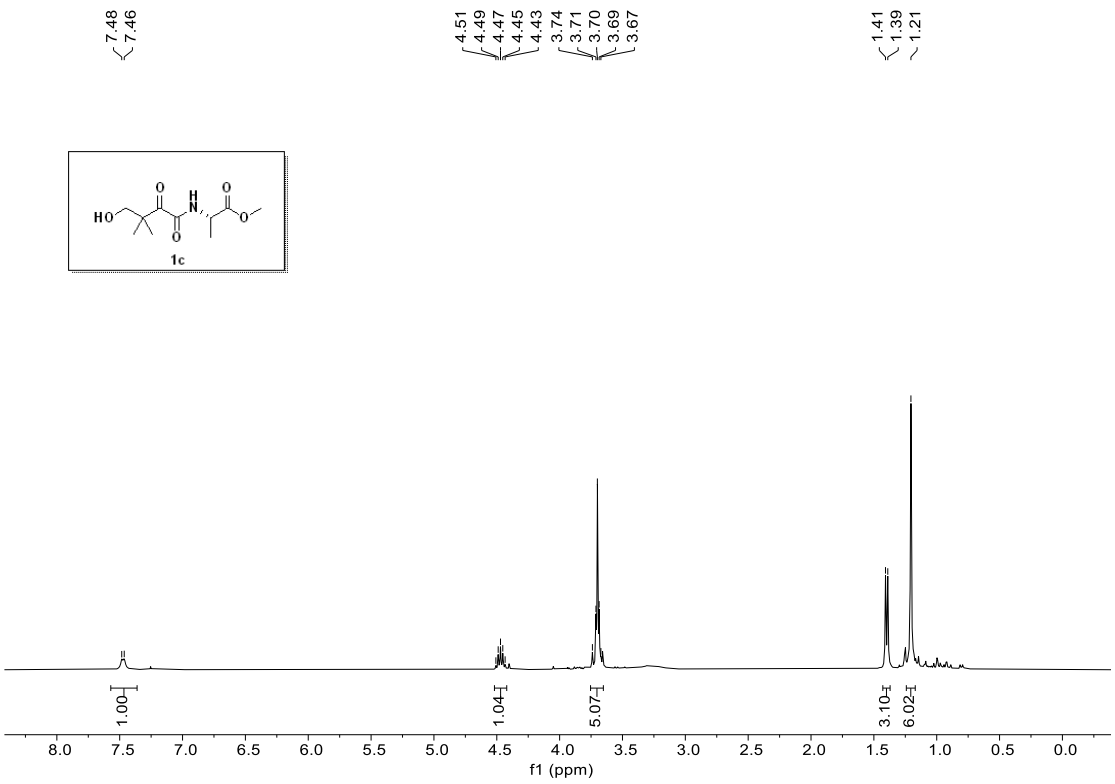
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1b**



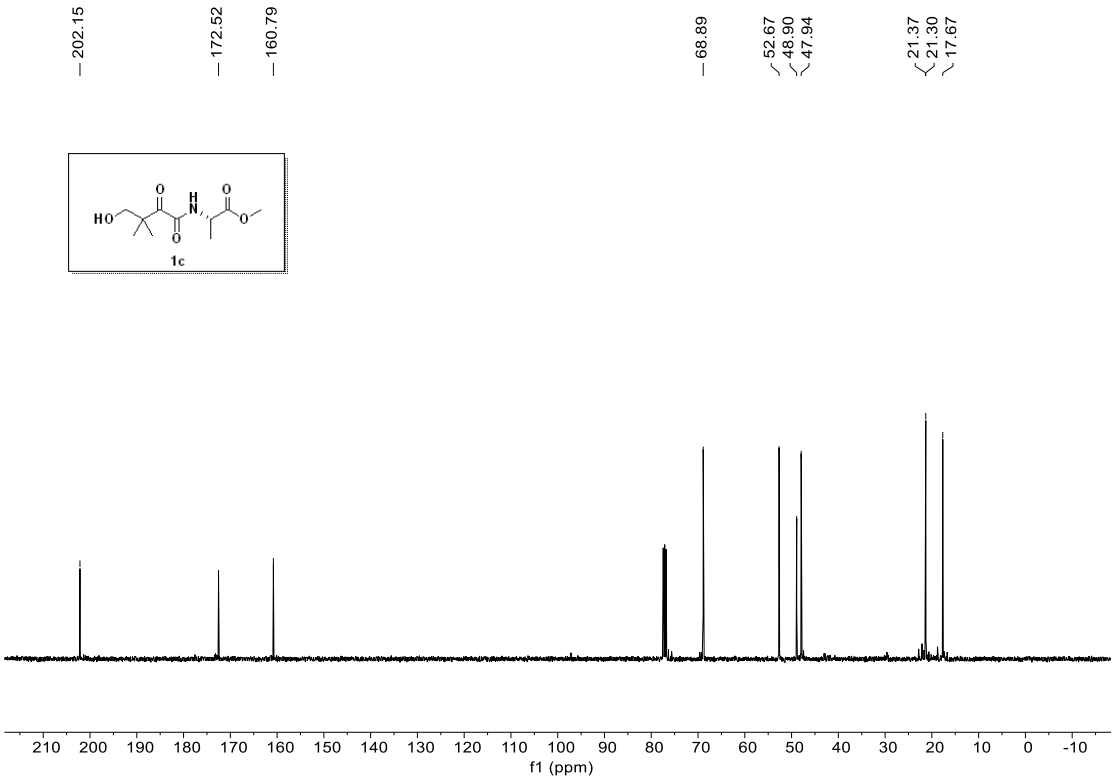
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1b**



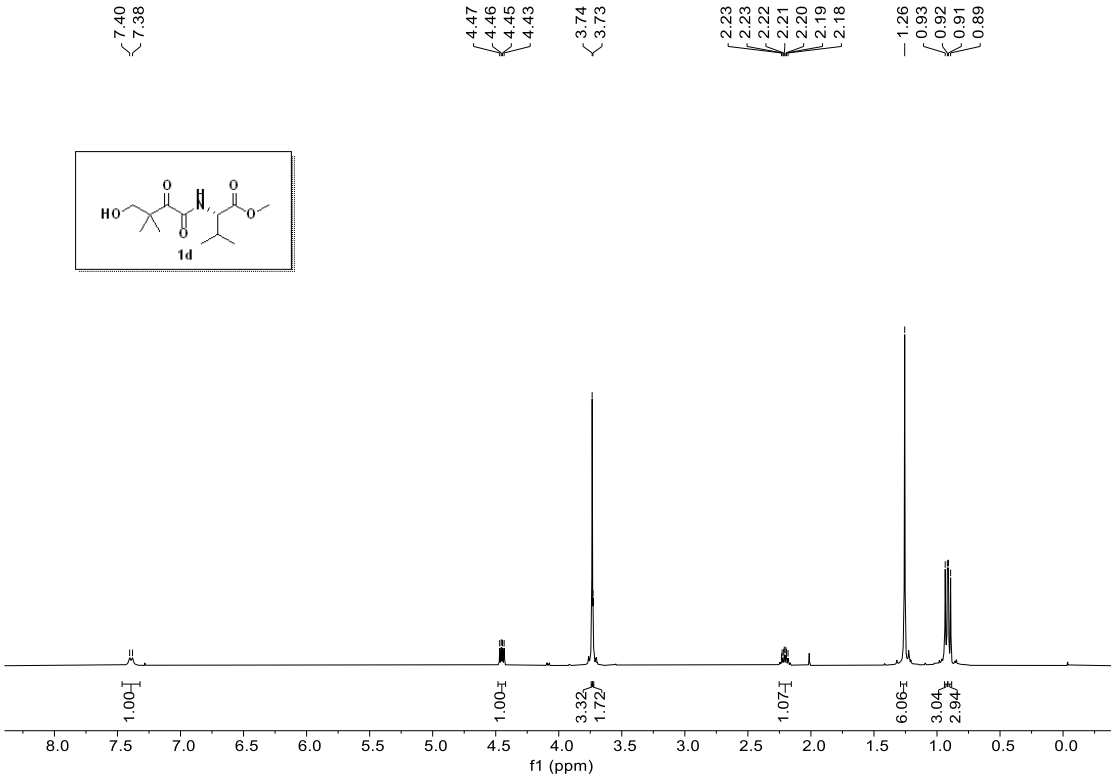
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1c**



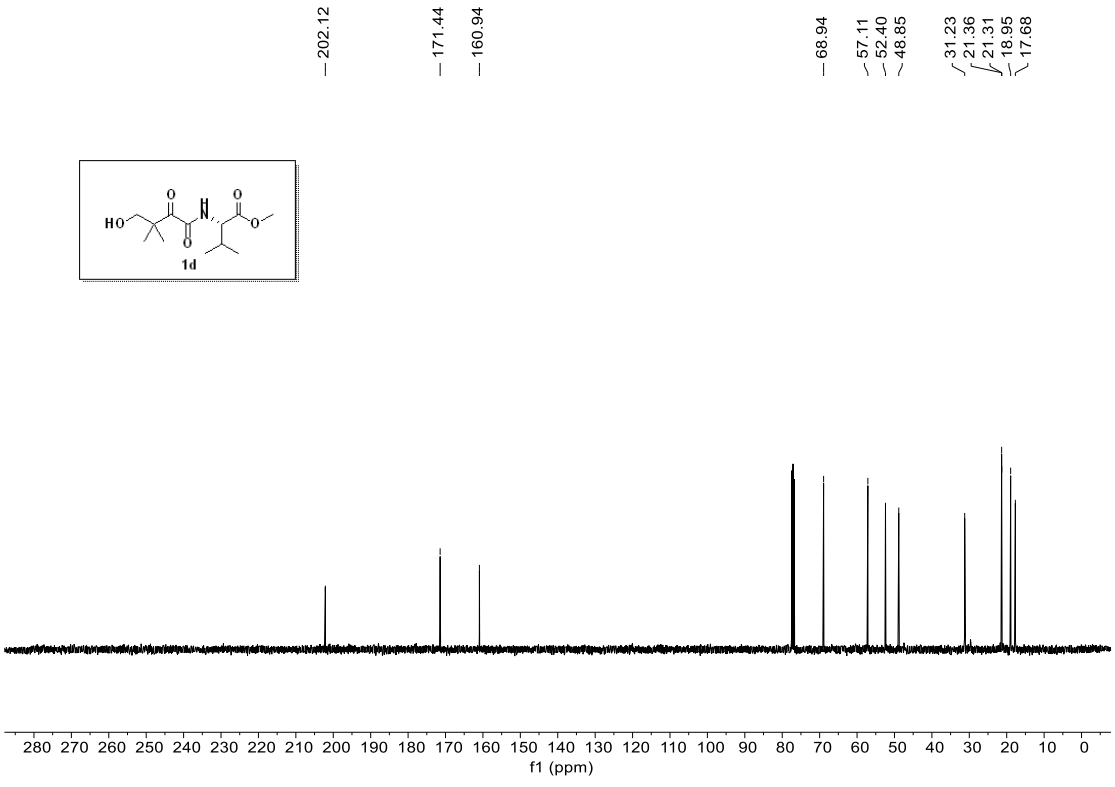
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1c**



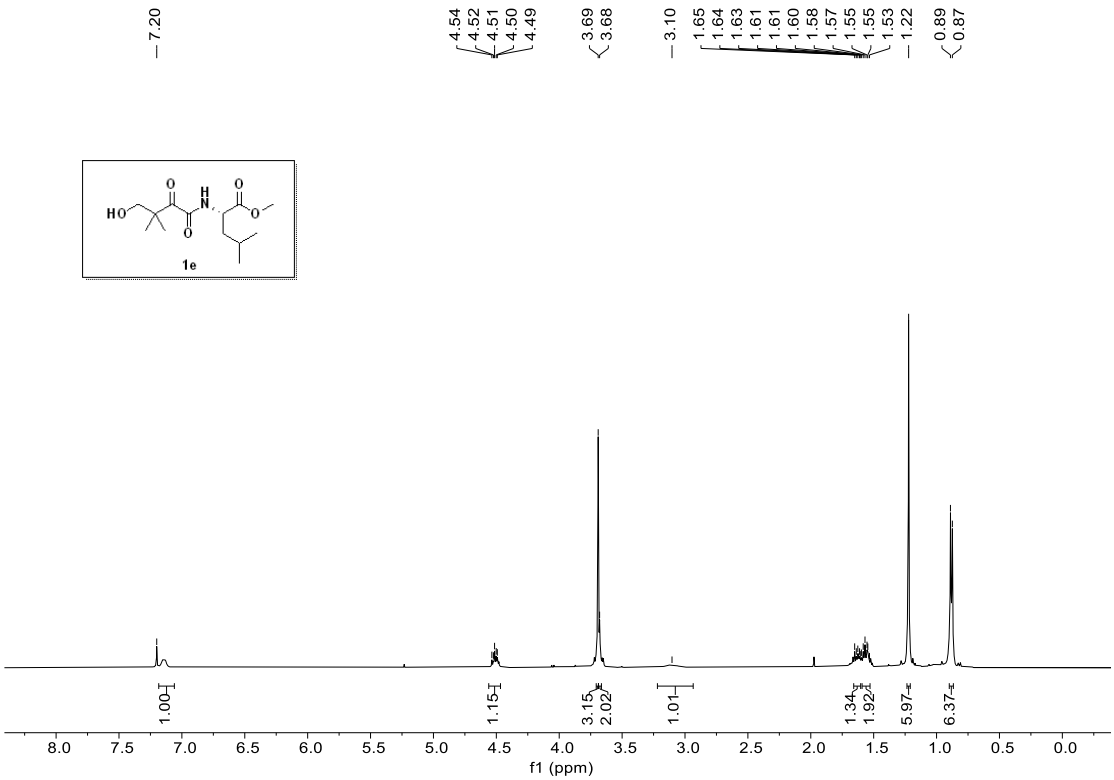
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1d**



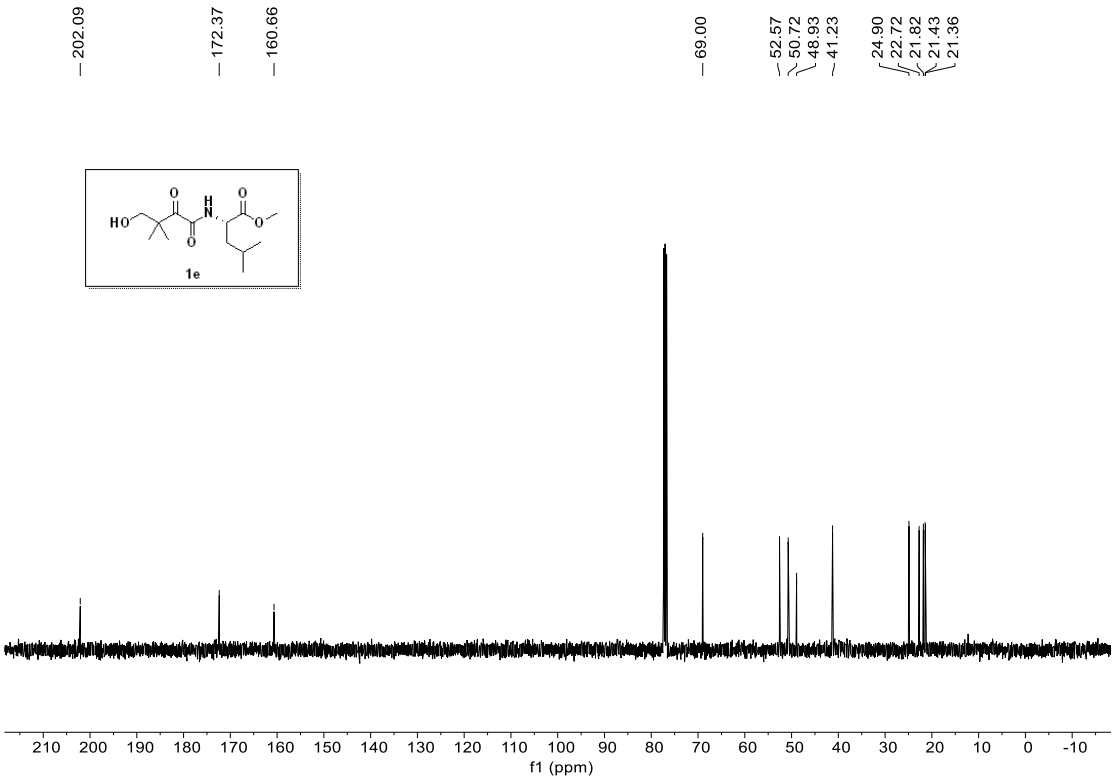
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1d**



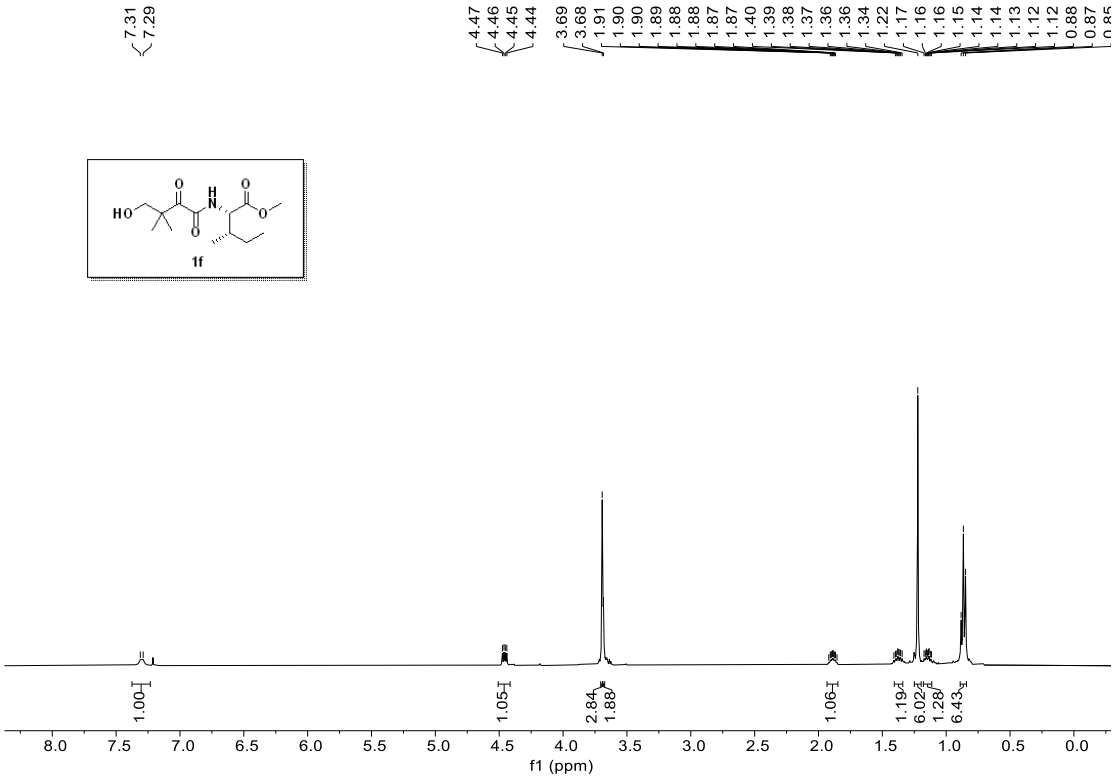
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1e**



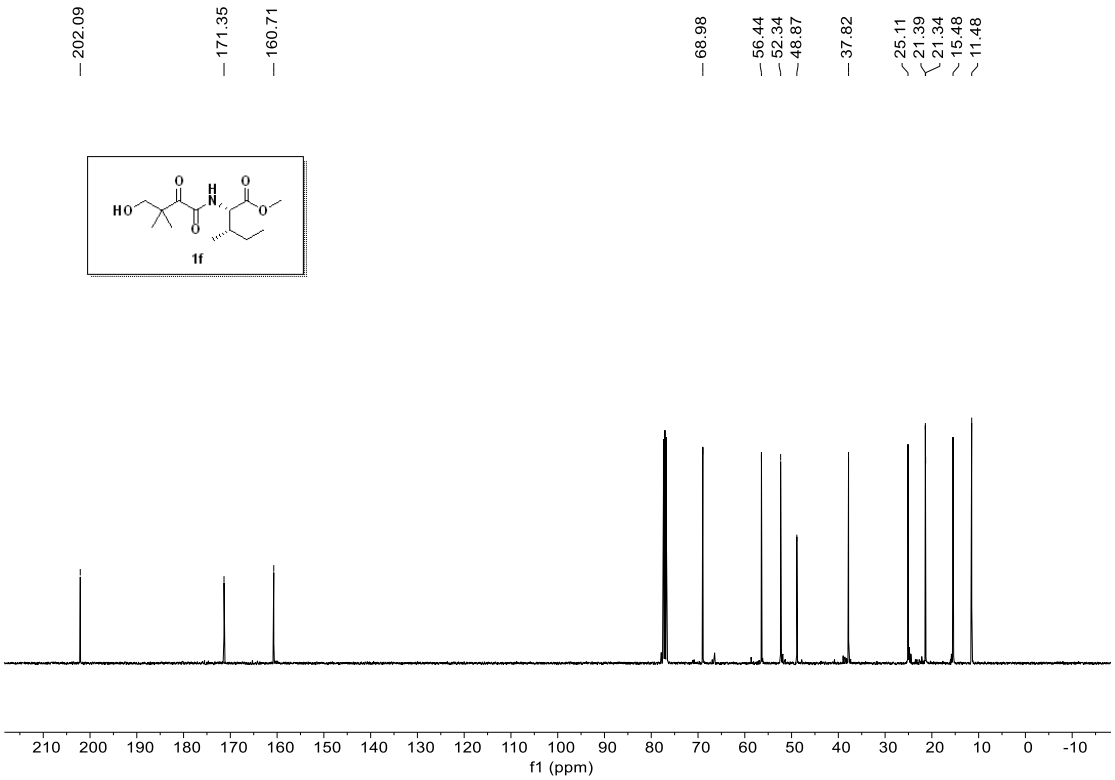
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1e**



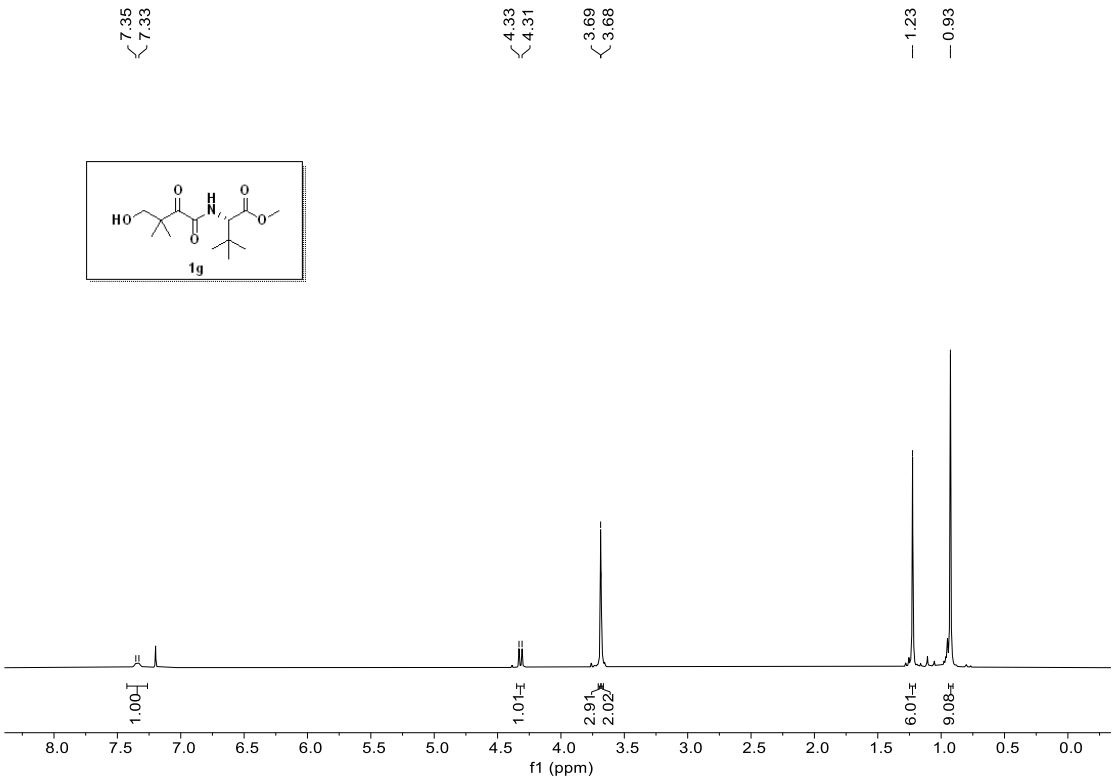
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1f**



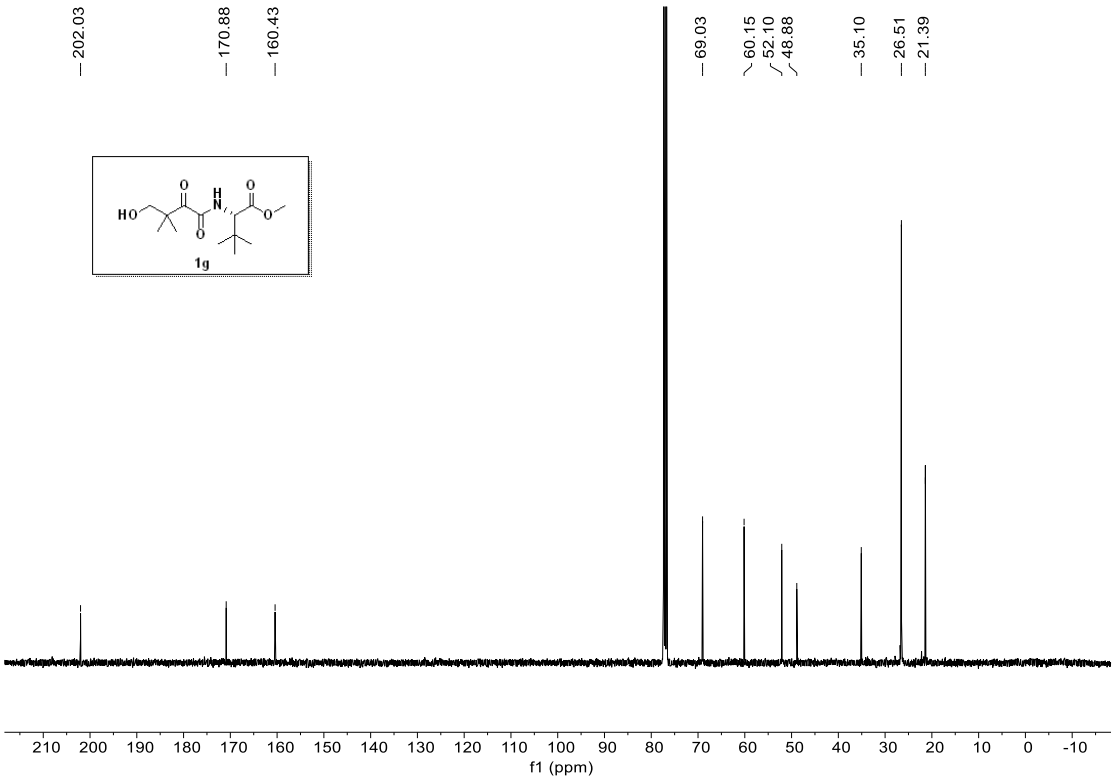
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1f**



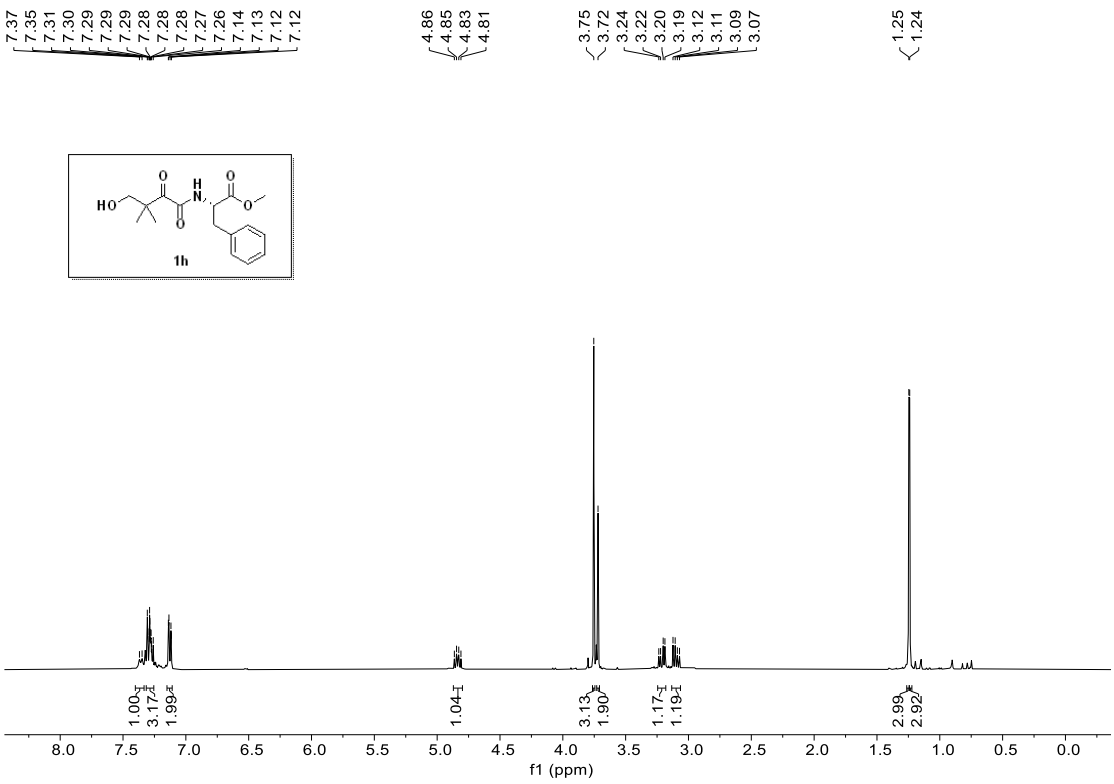
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1g**



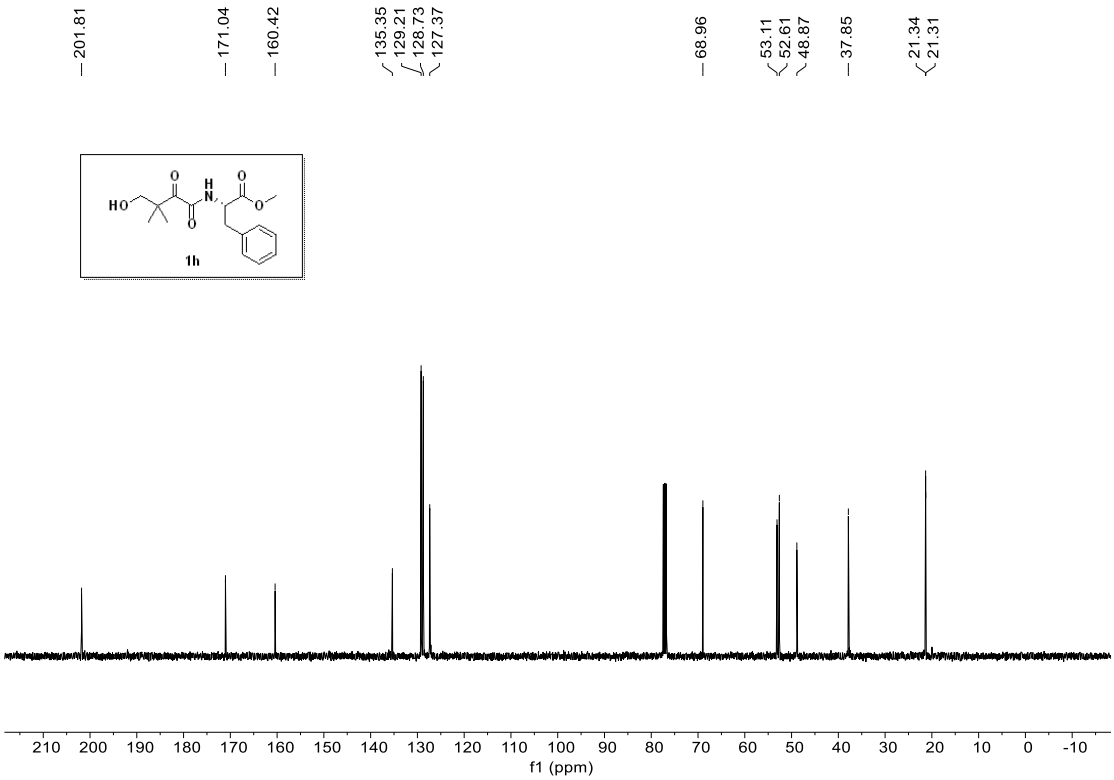
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1g**



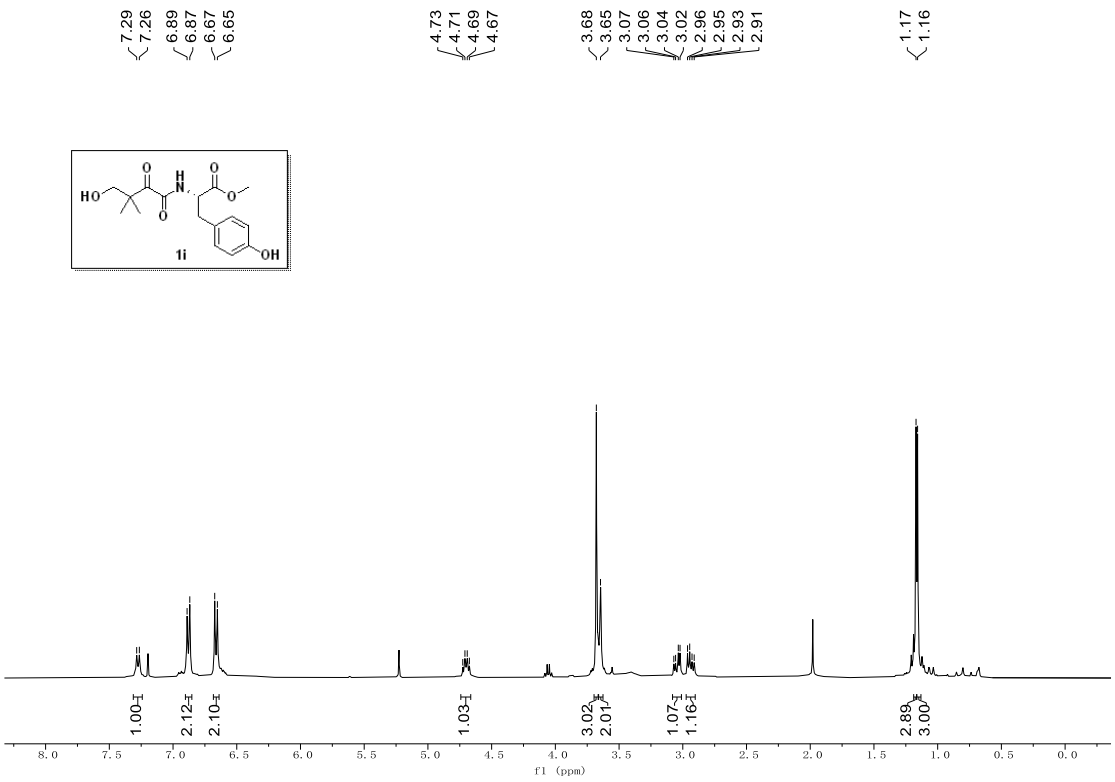
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1h**



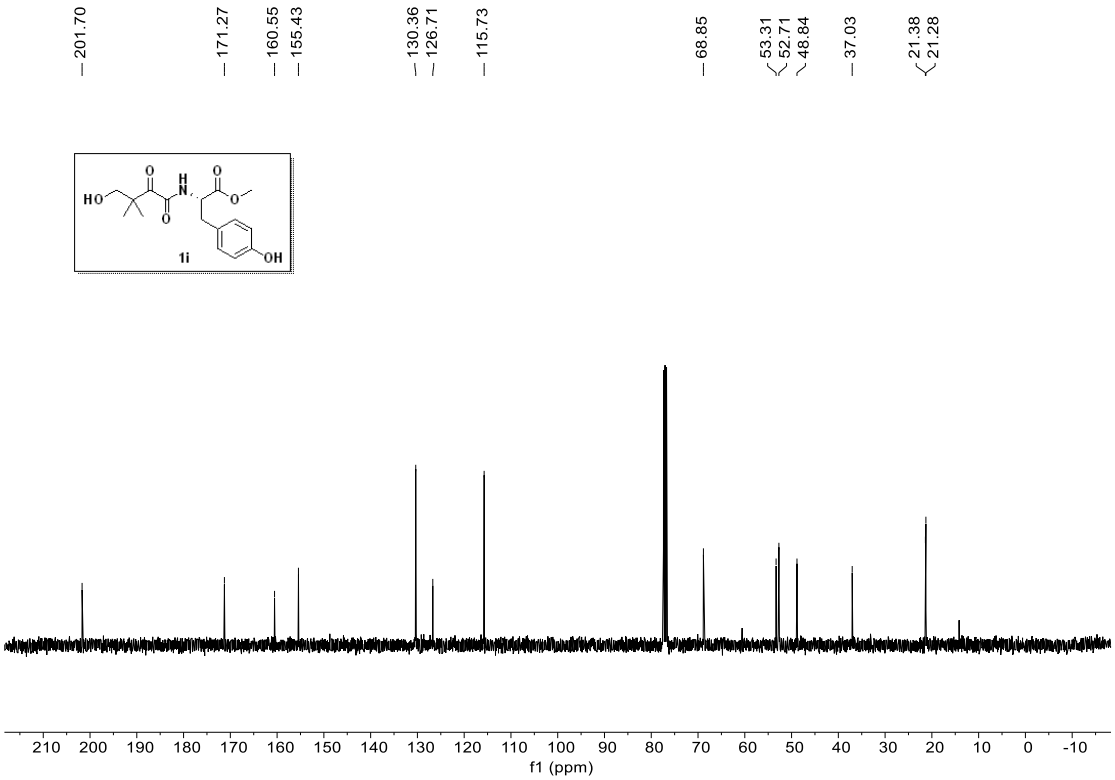
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1h**



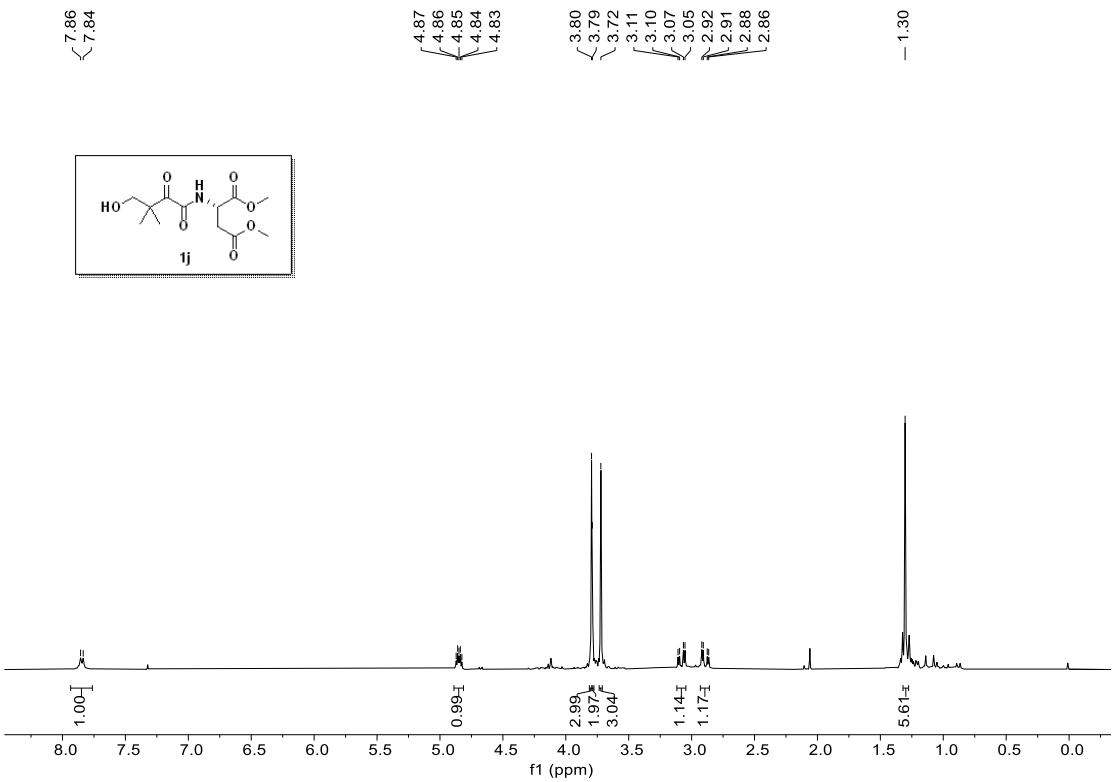
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1i**



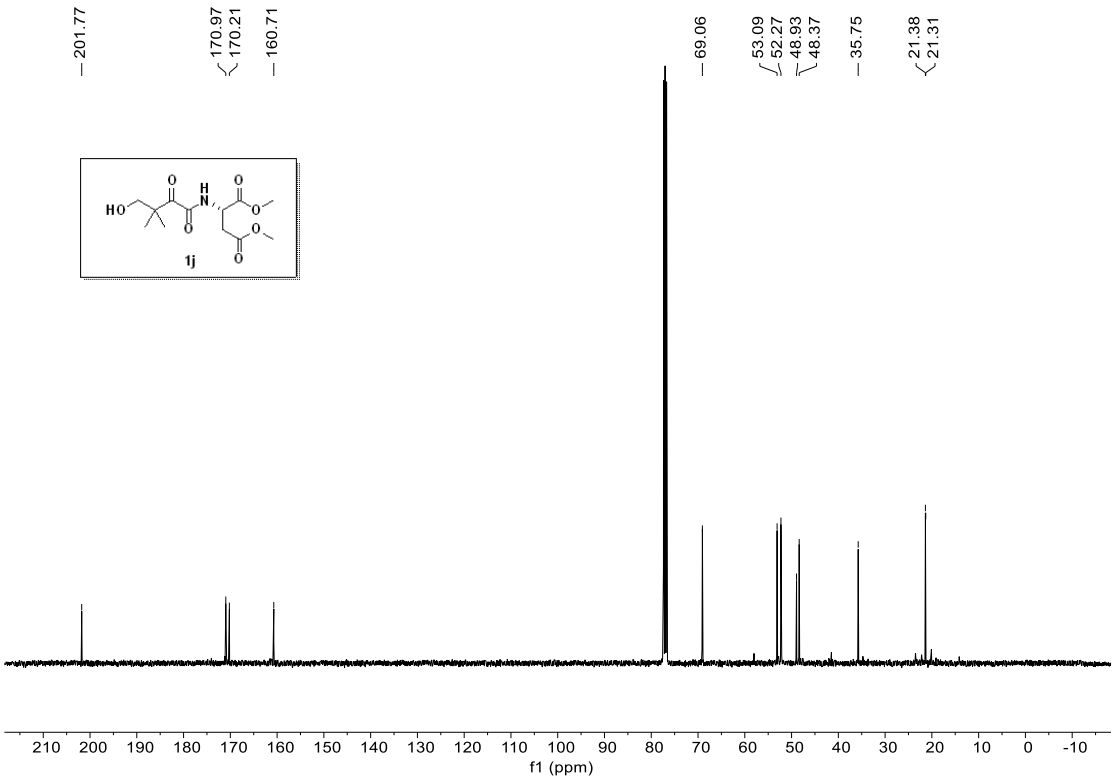
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1i**



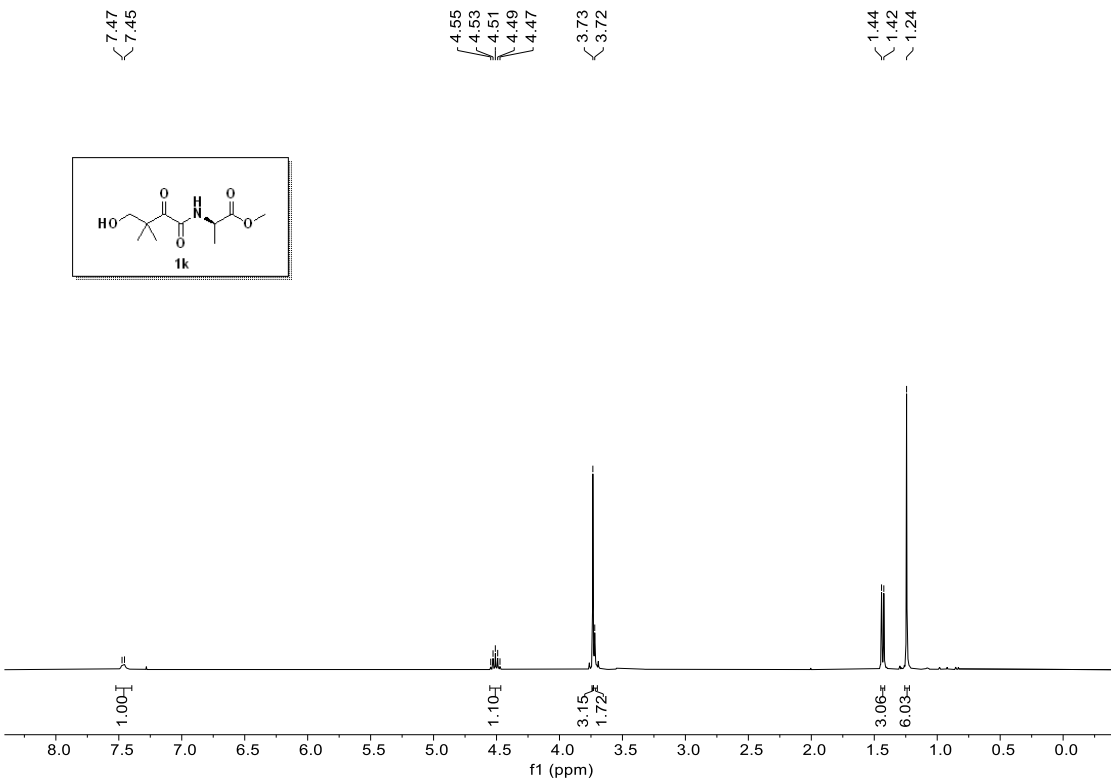
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1j**



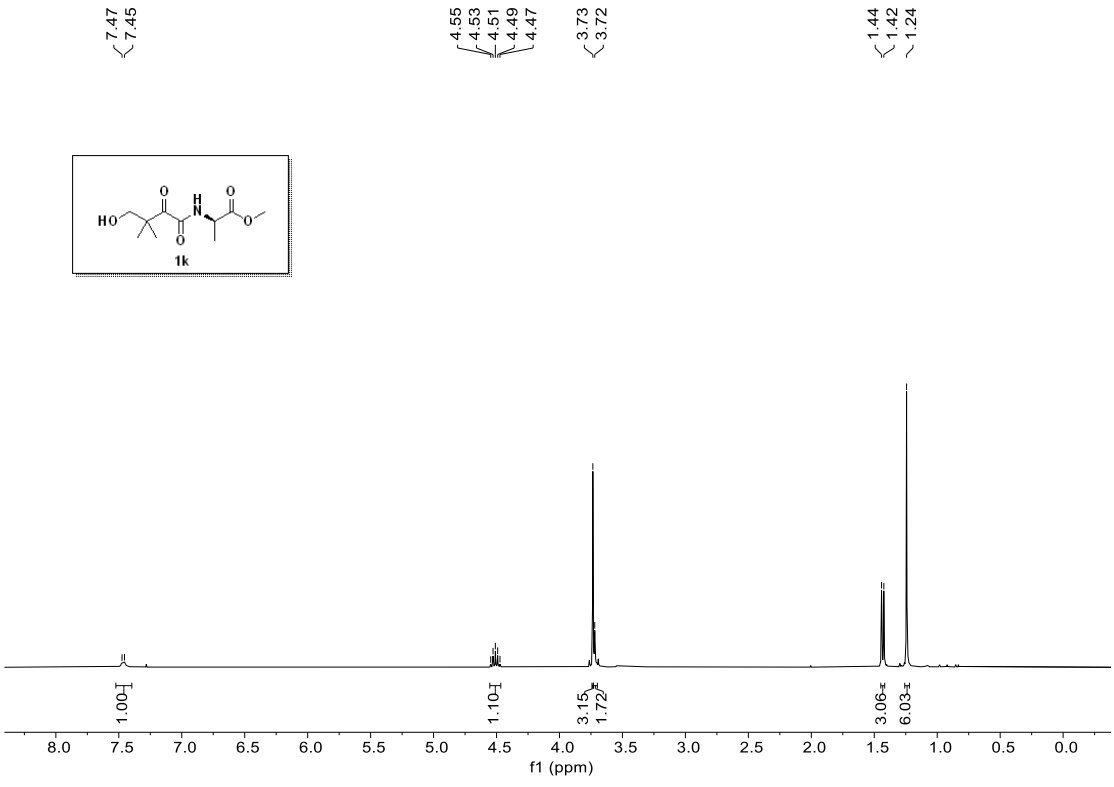
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1j**



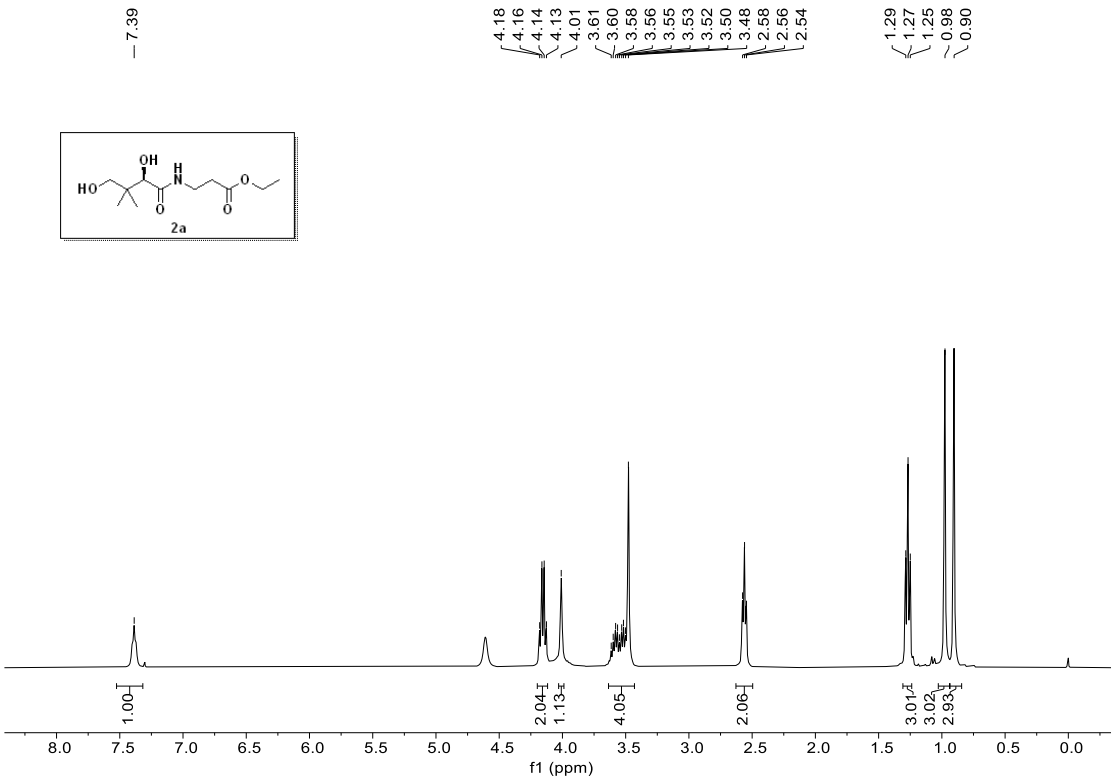
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1k**



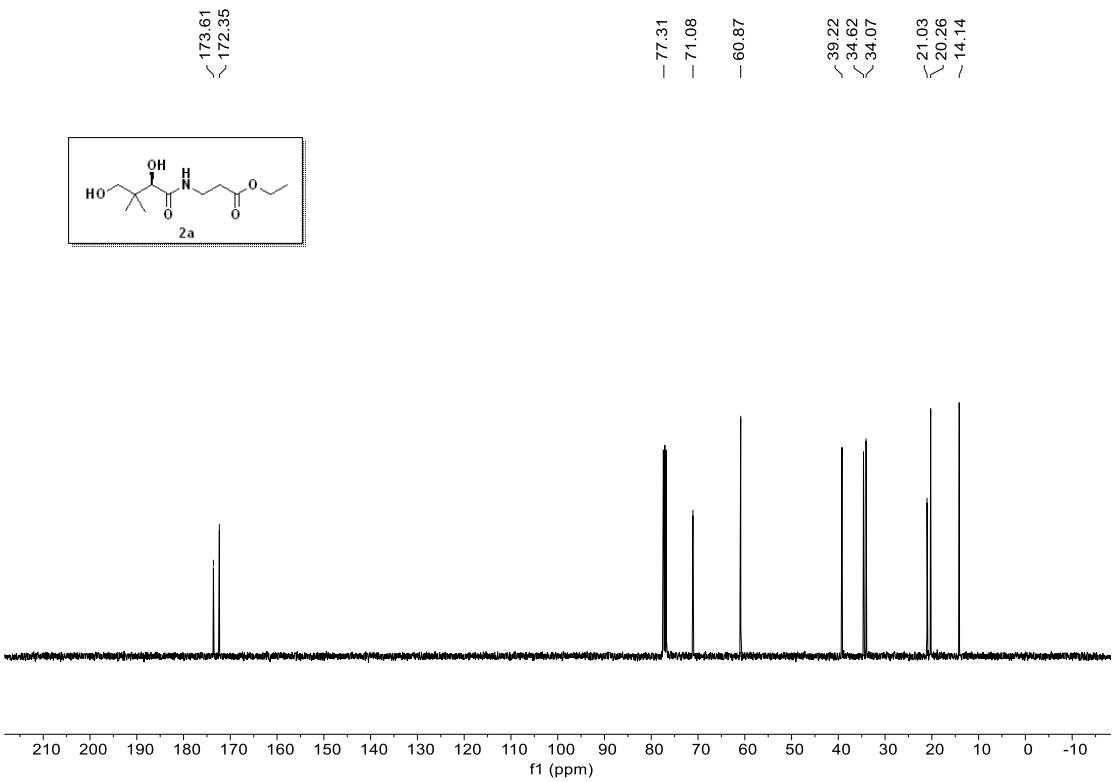
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **1k**



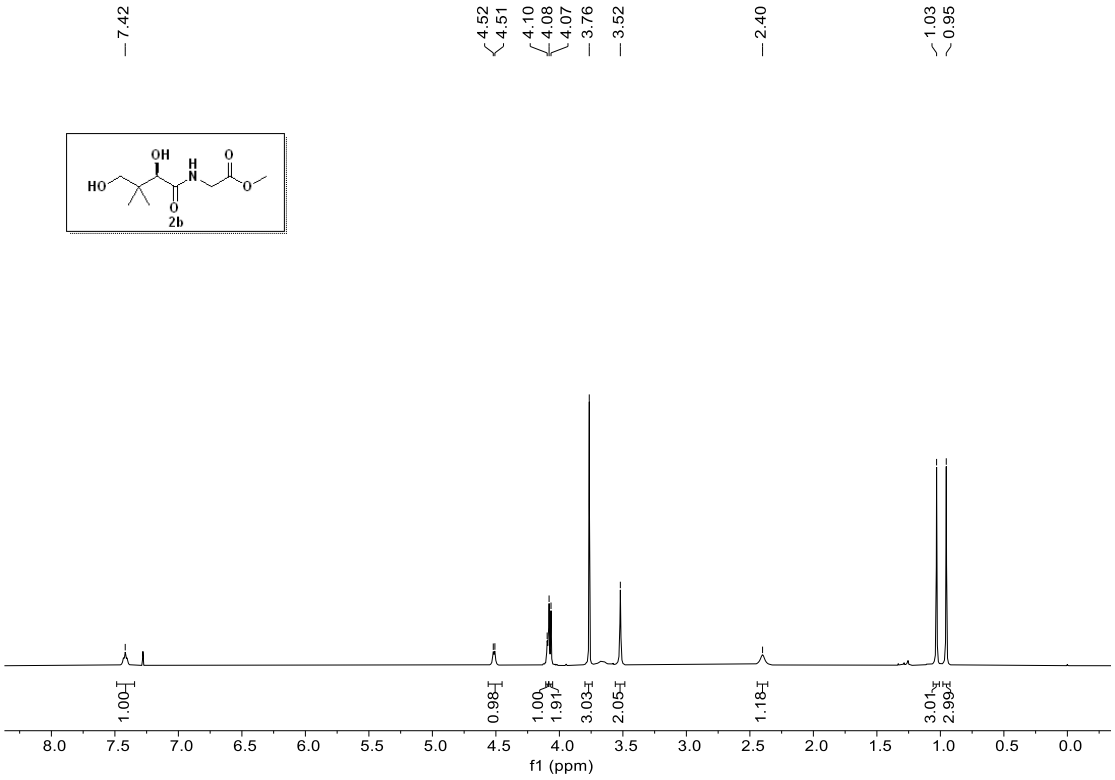
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2a**



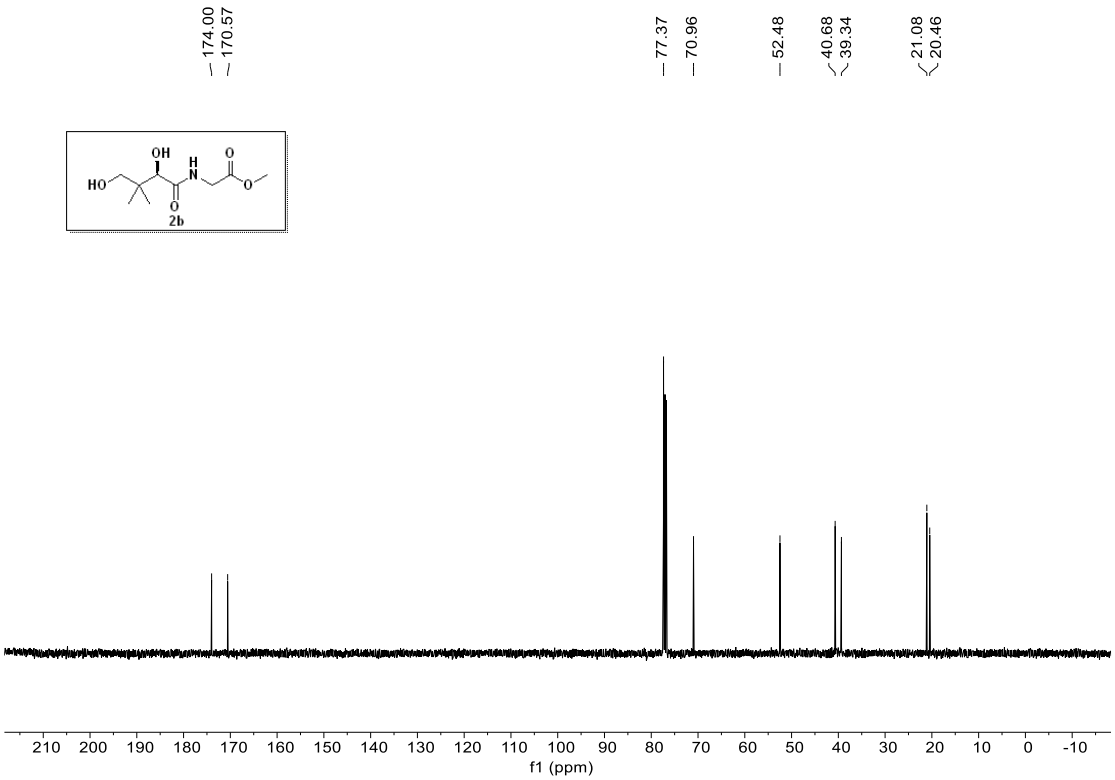
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2a**



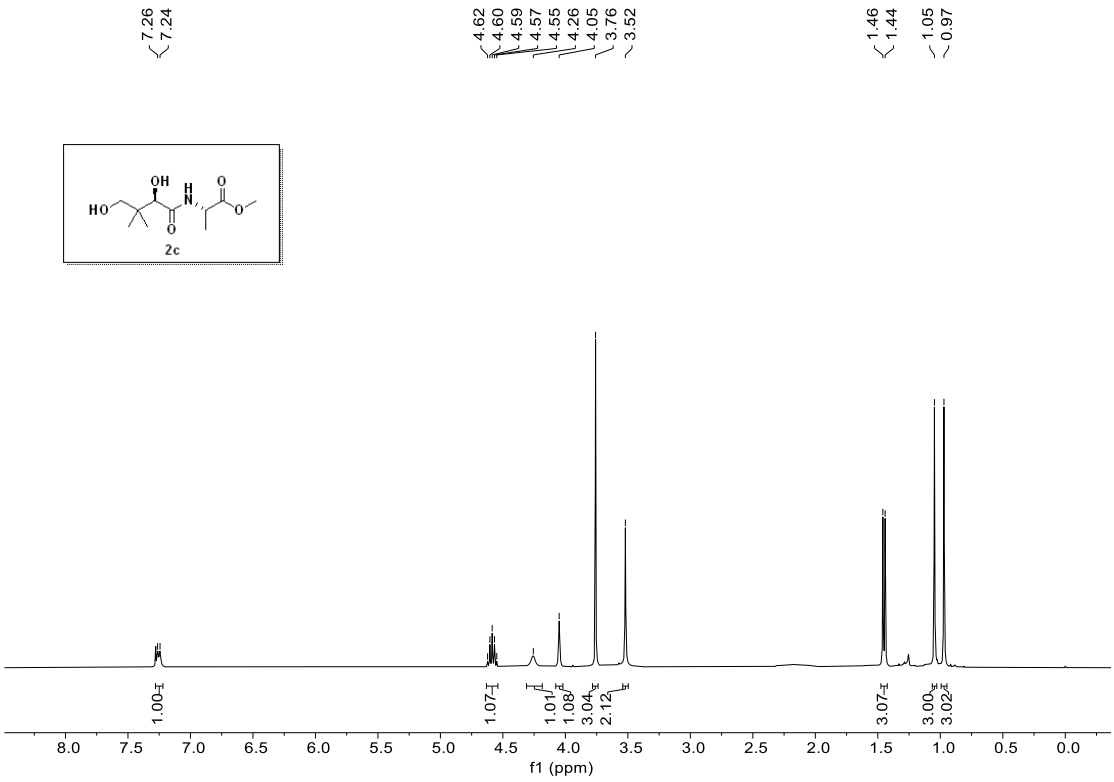
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2b**



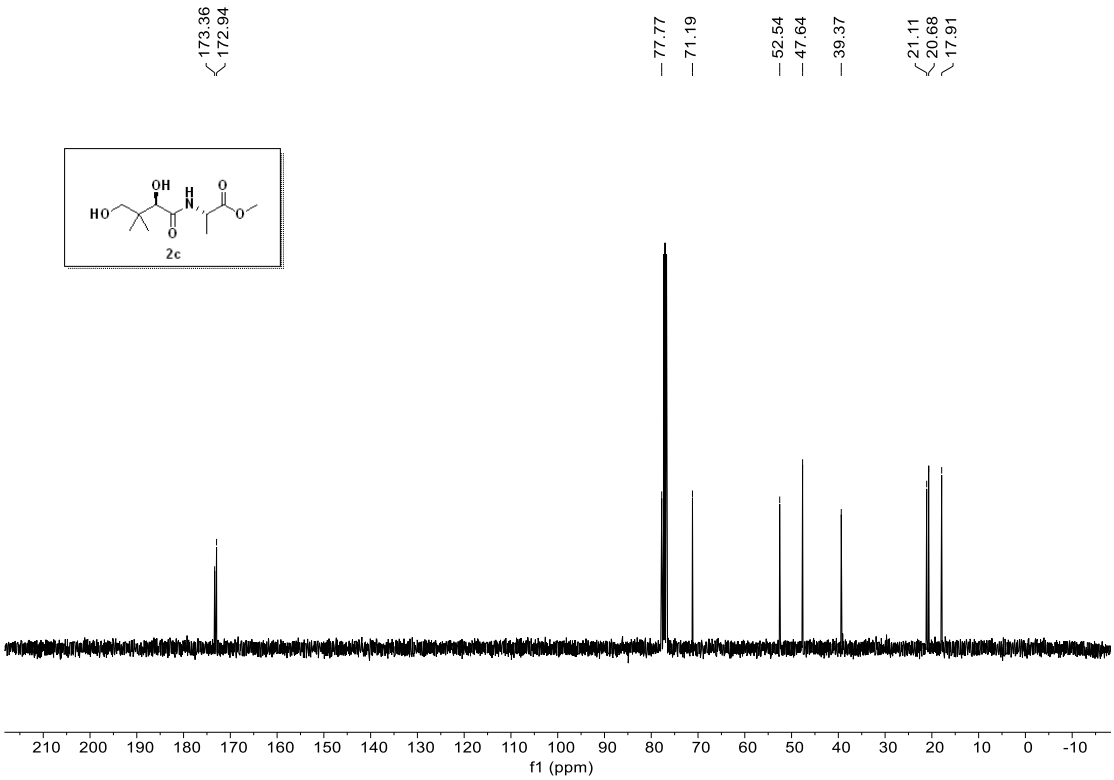
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2b**



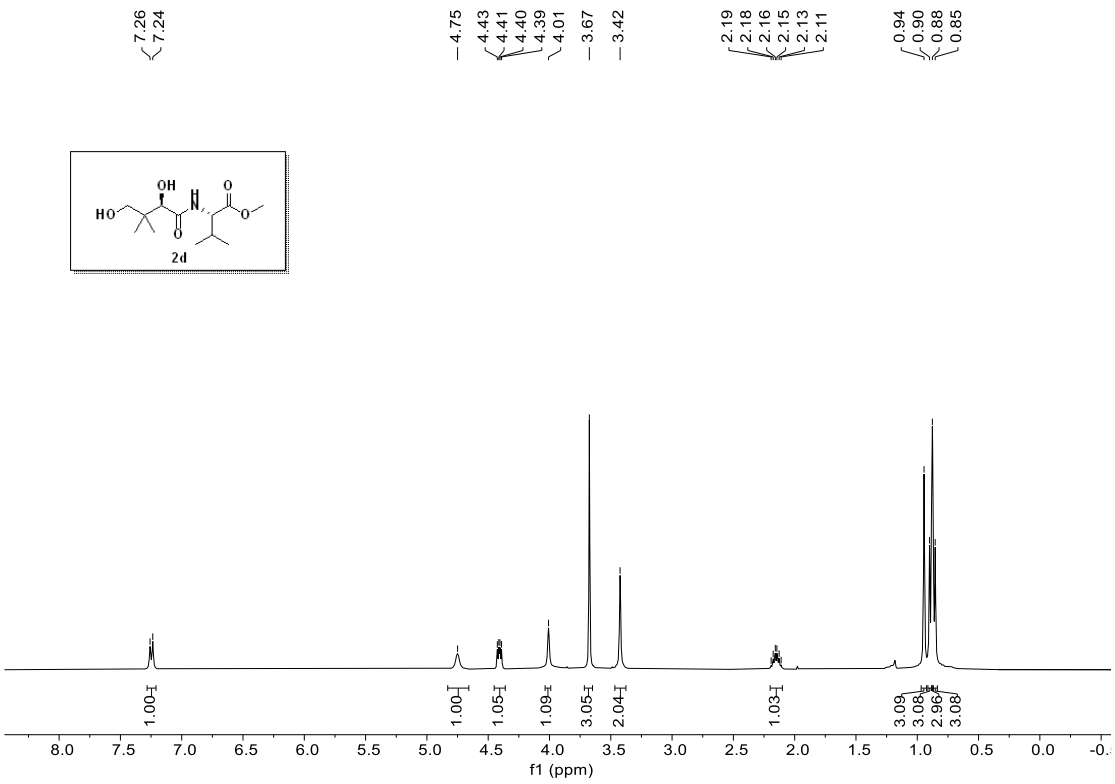
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2c**



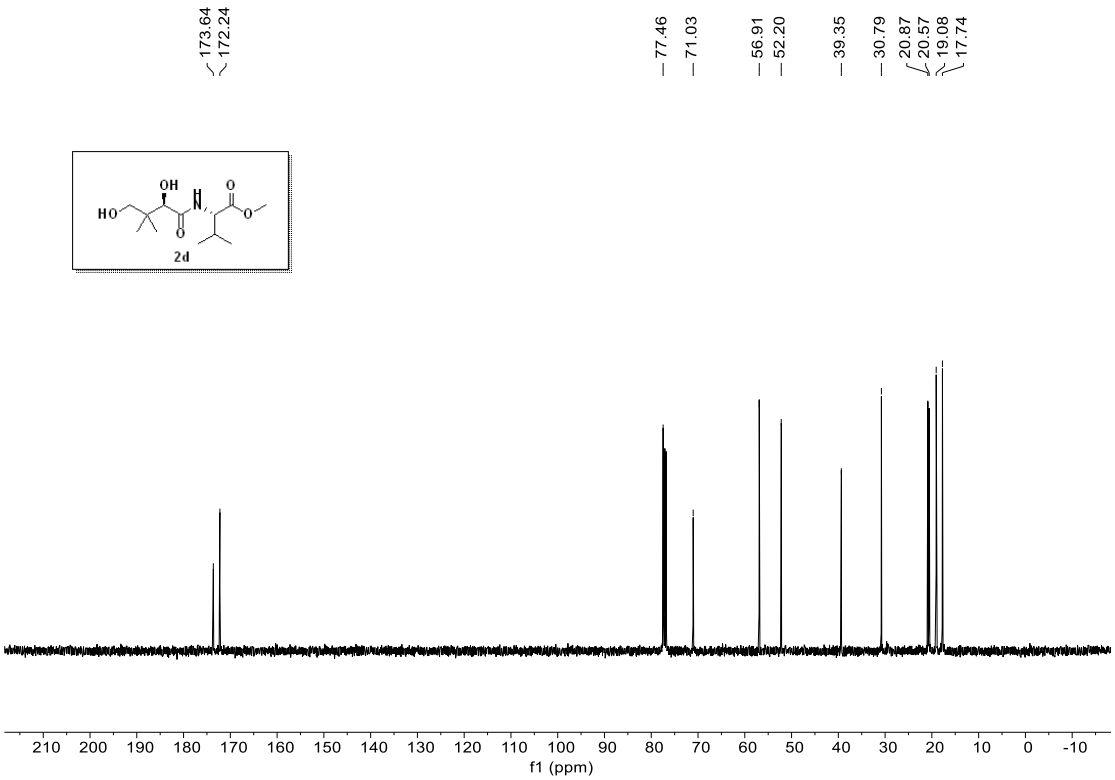
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2c**



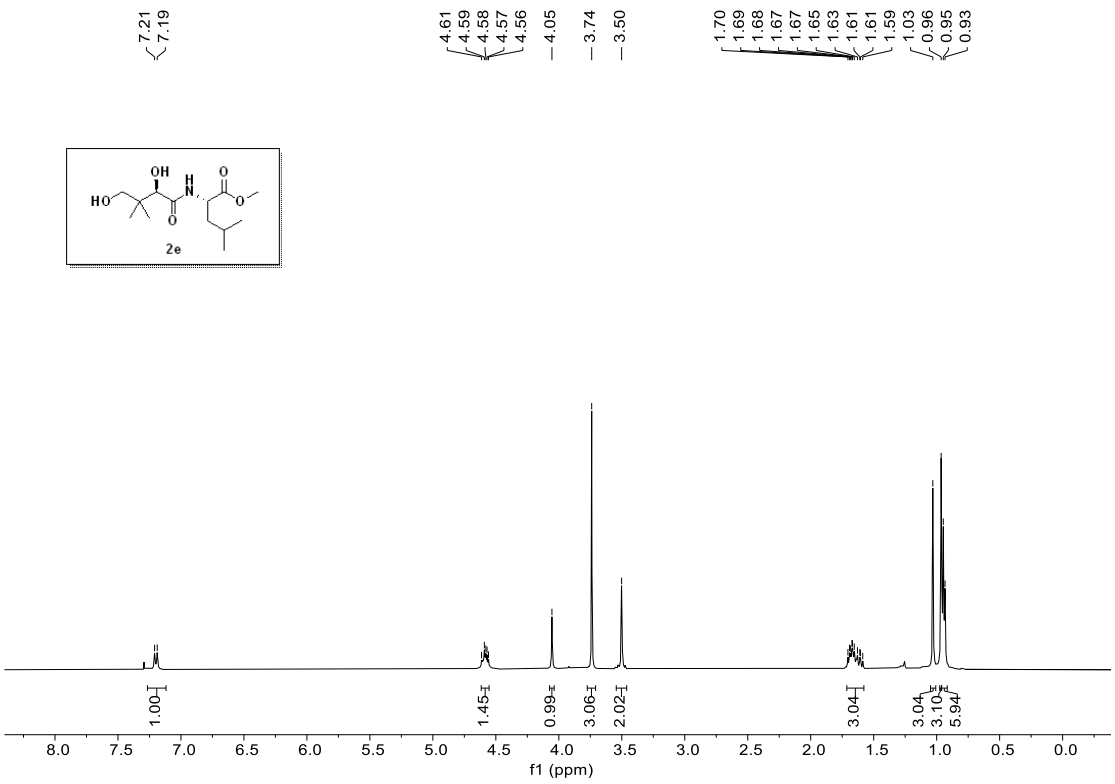
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2d**



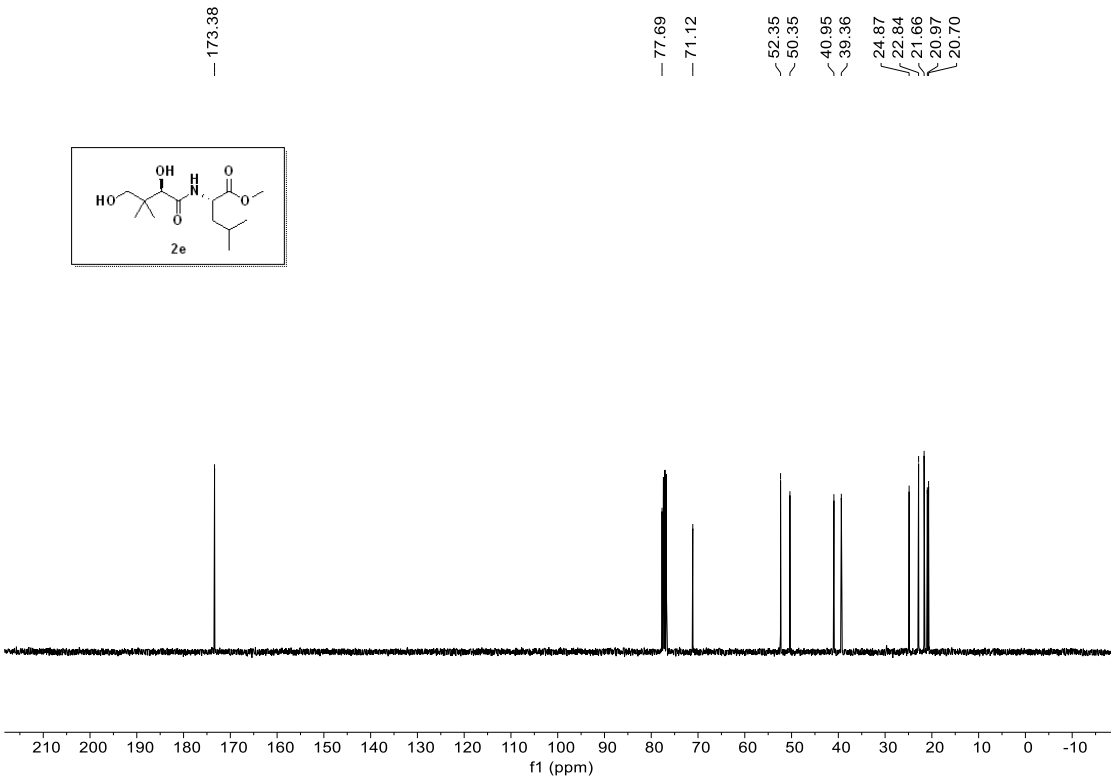
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2d**



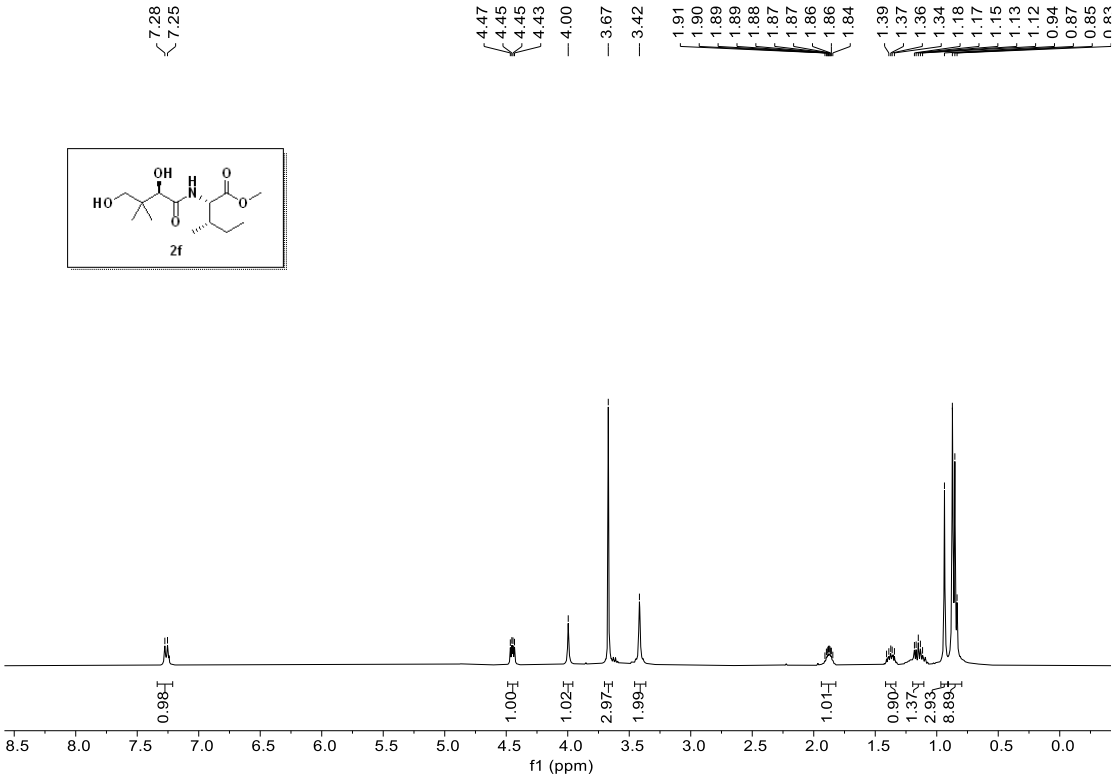
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2e**



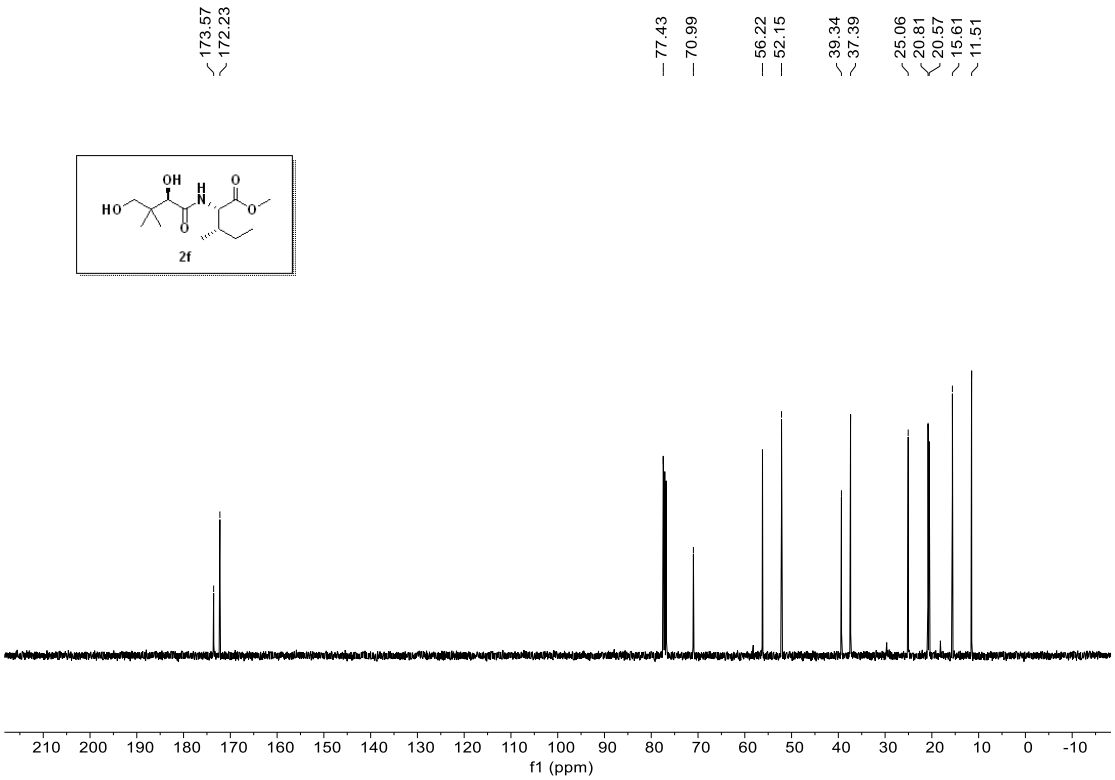
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2e**



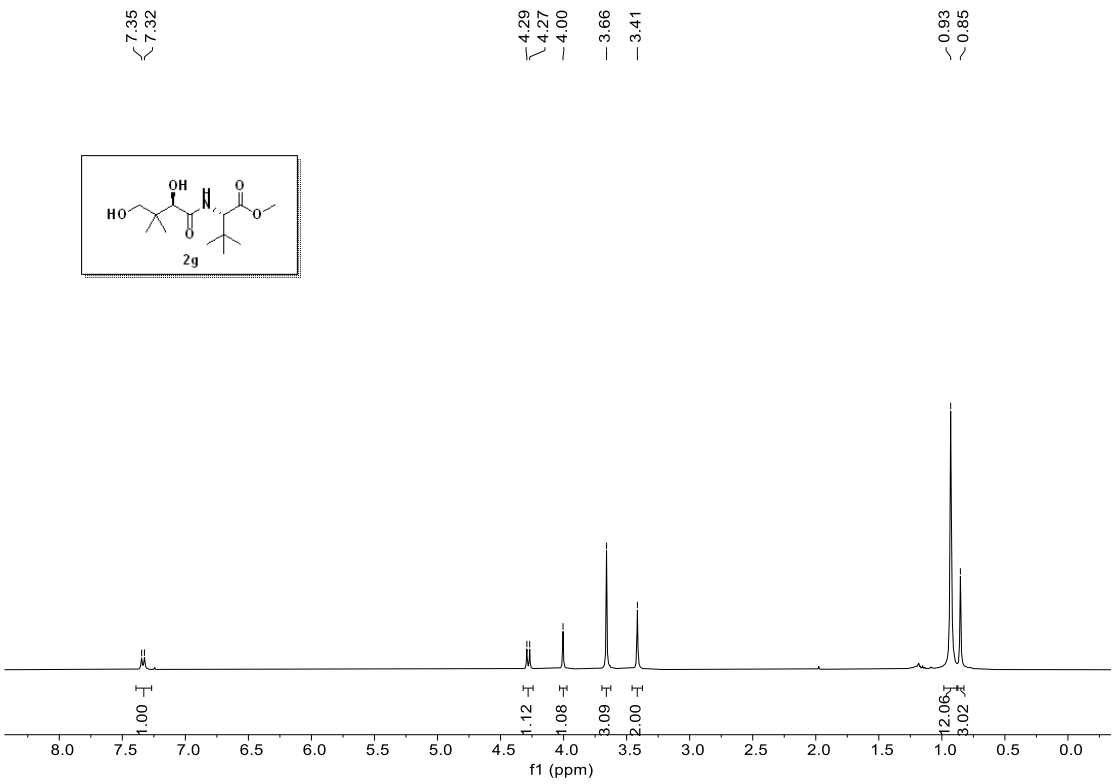
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2f**



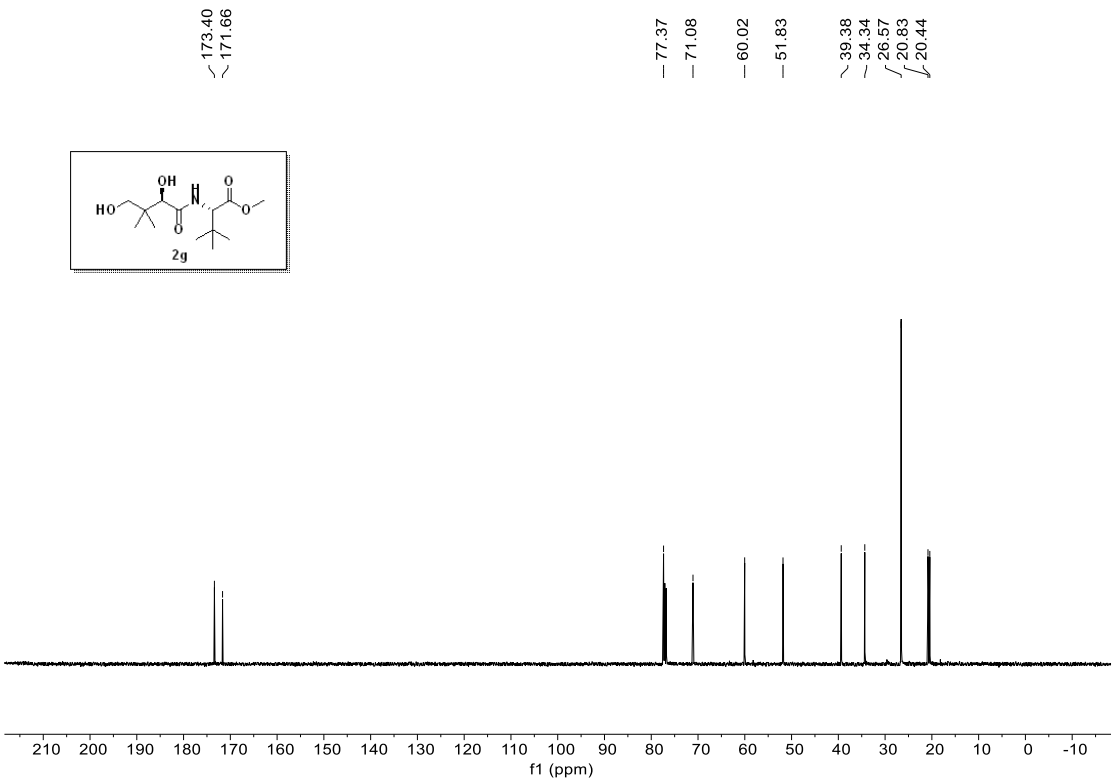
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2f**



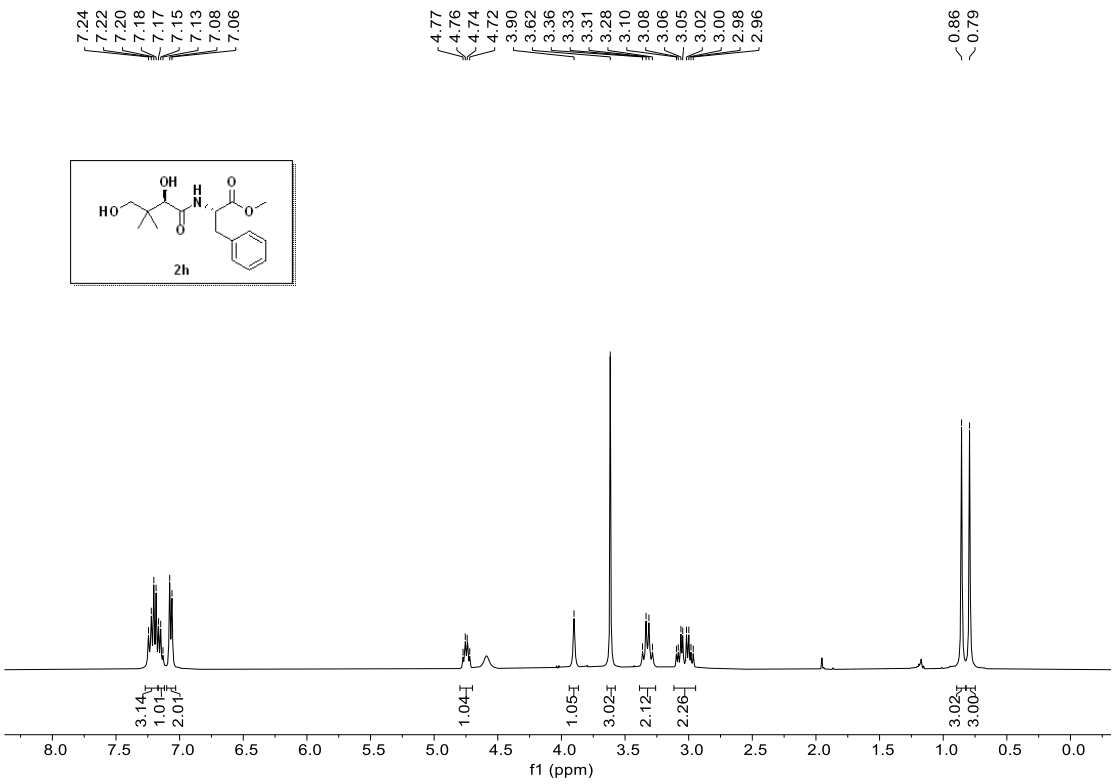
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2g**



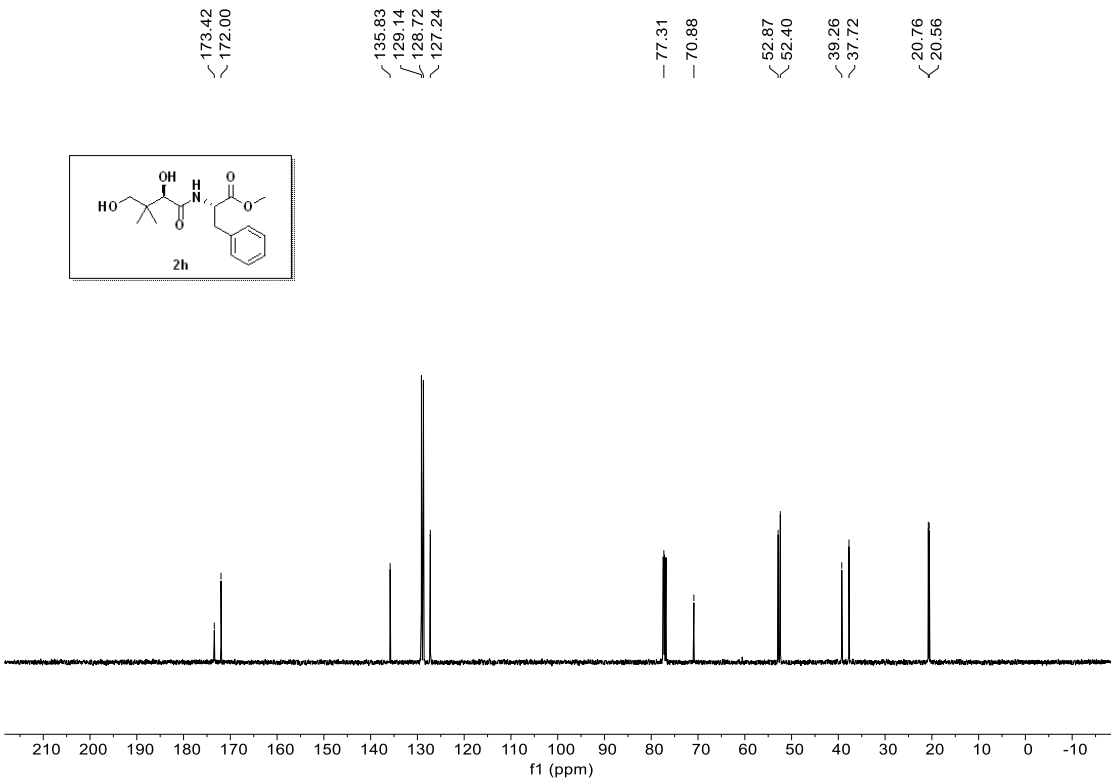
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2g**



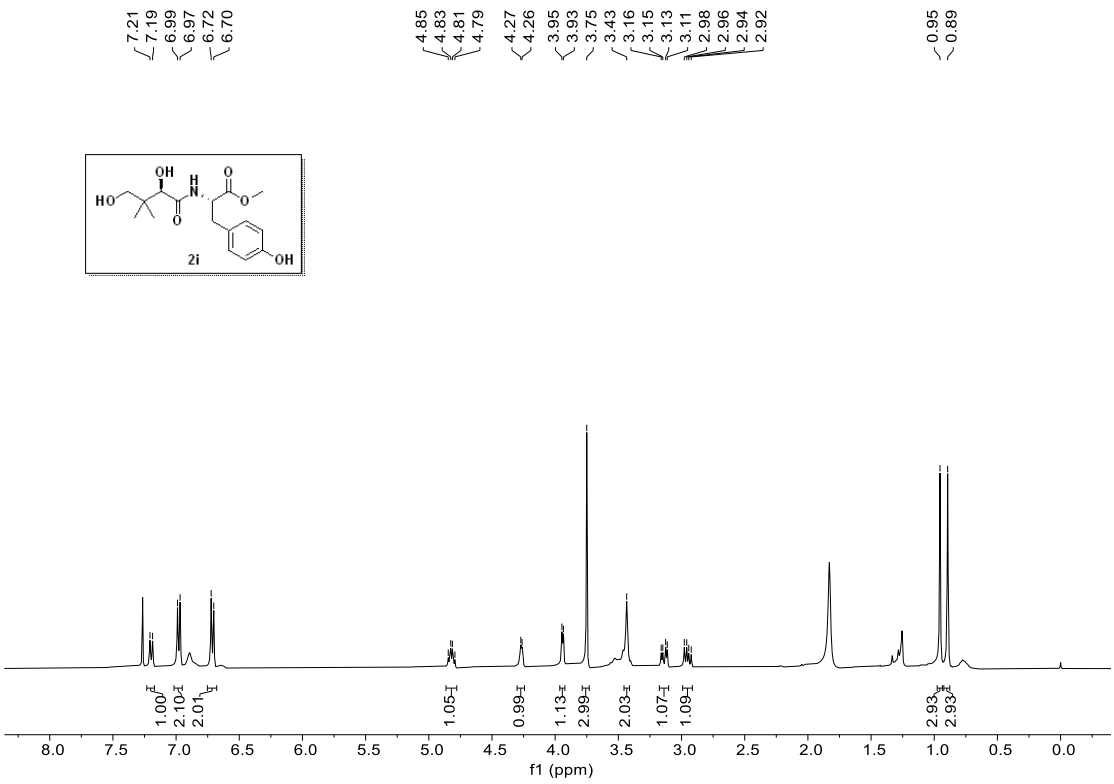
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2h**



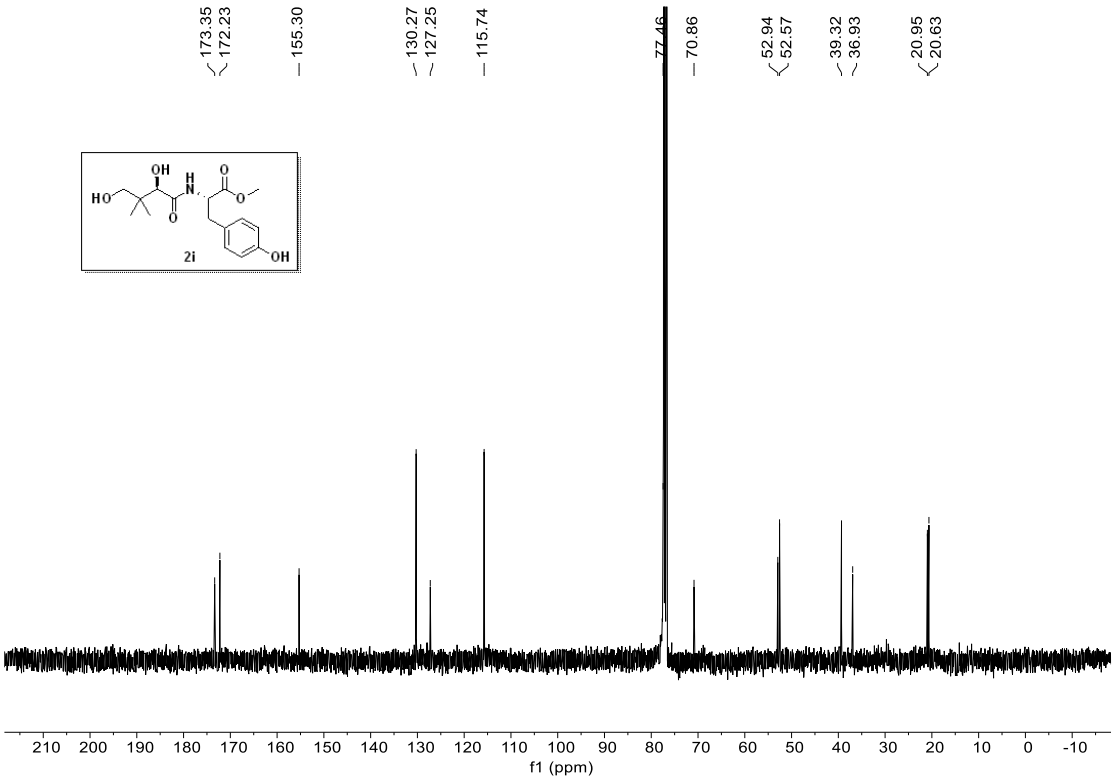
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2h**



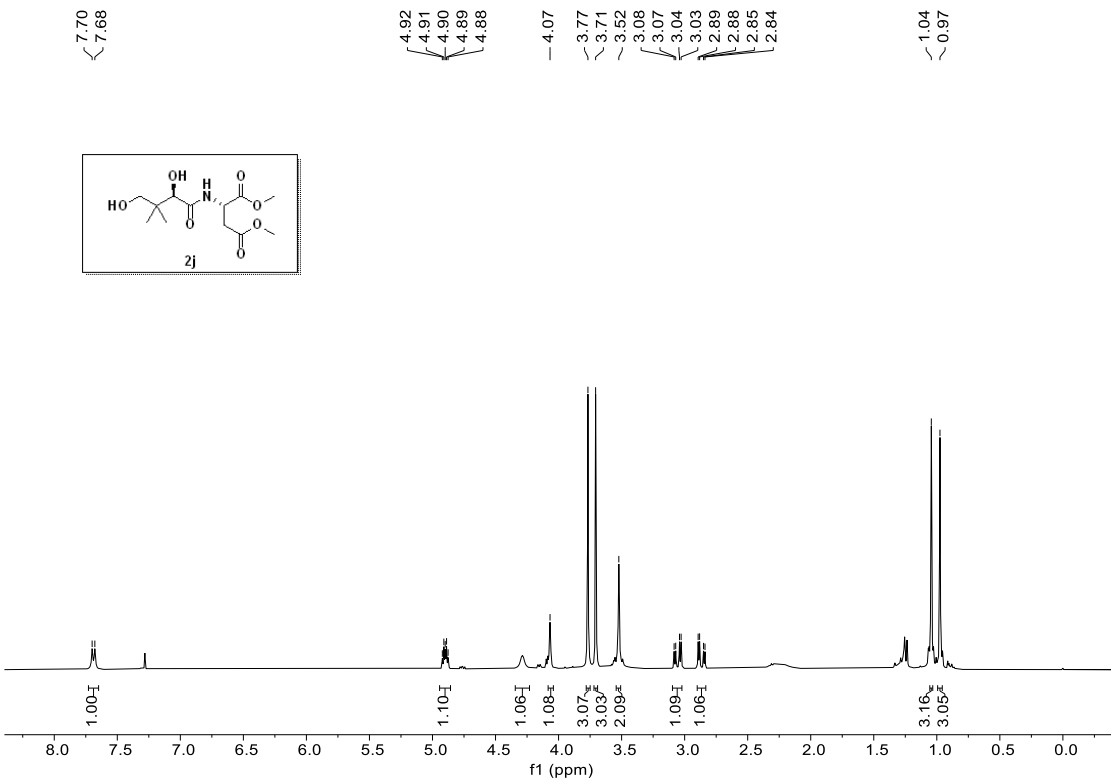
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2i**



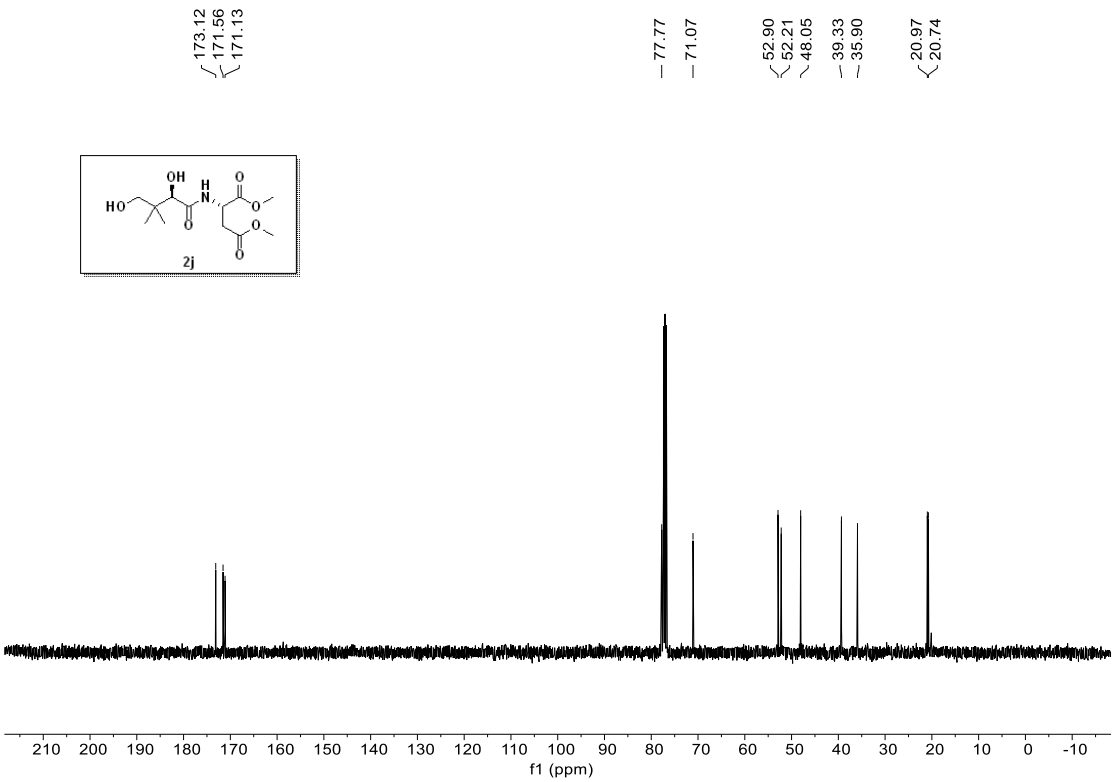
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2i**



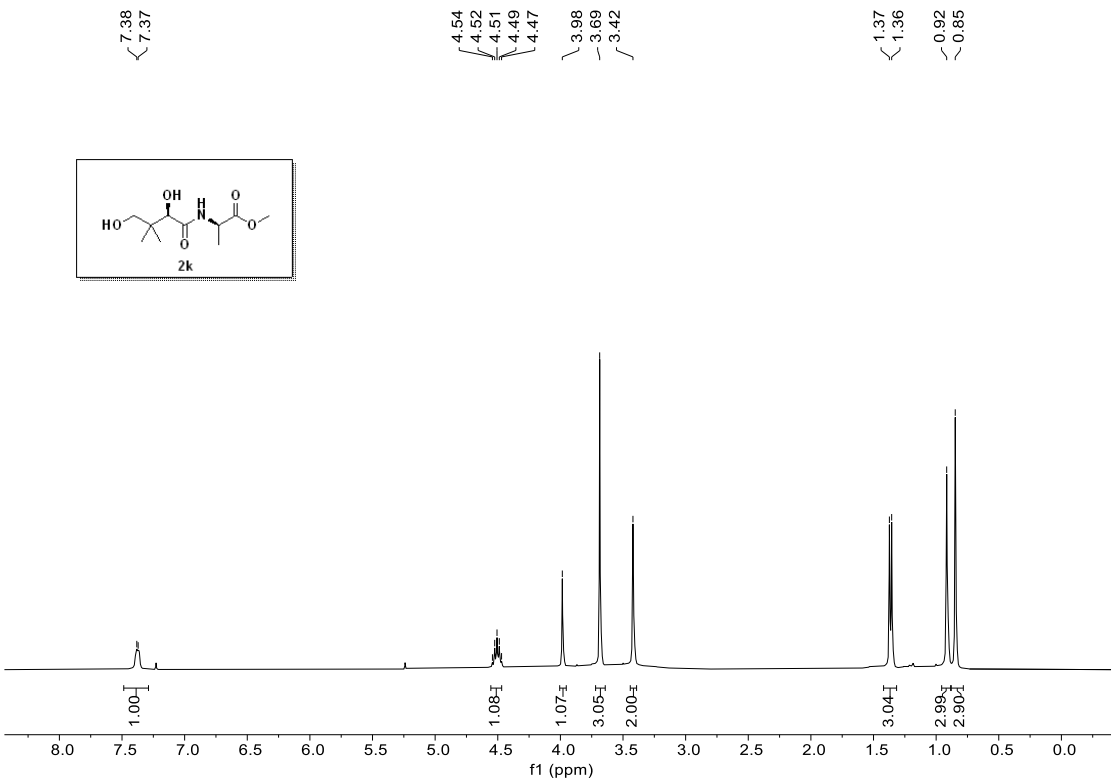
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2j**



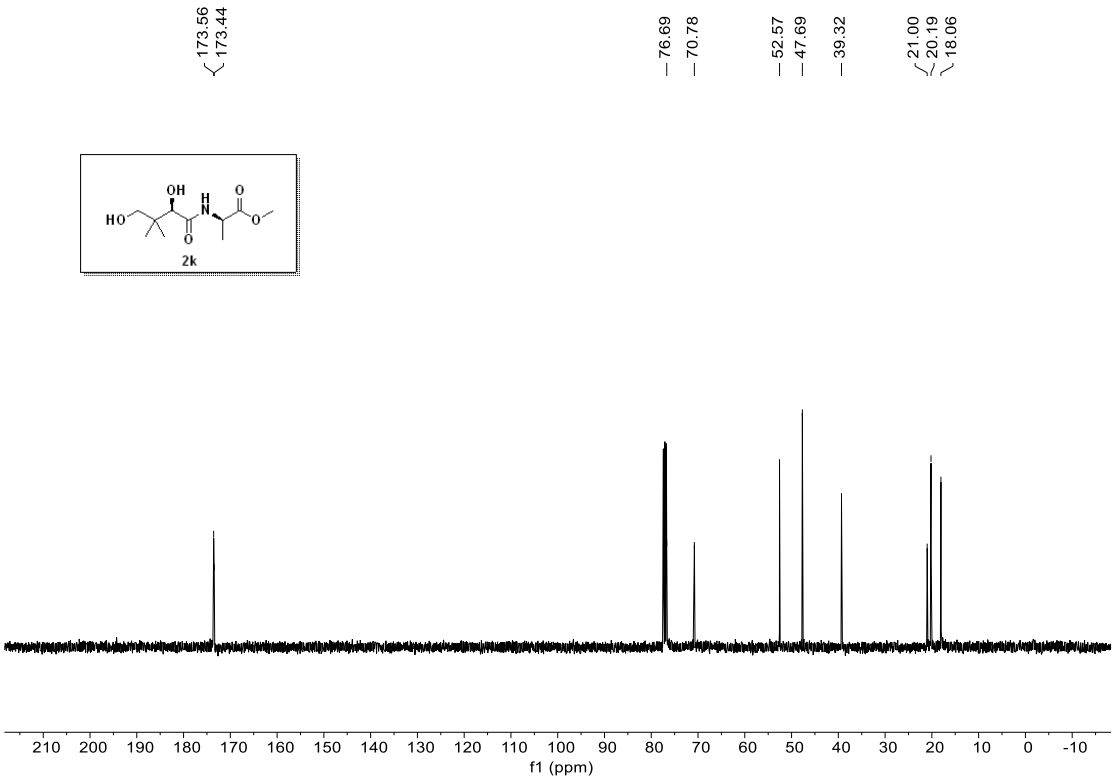
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2j**



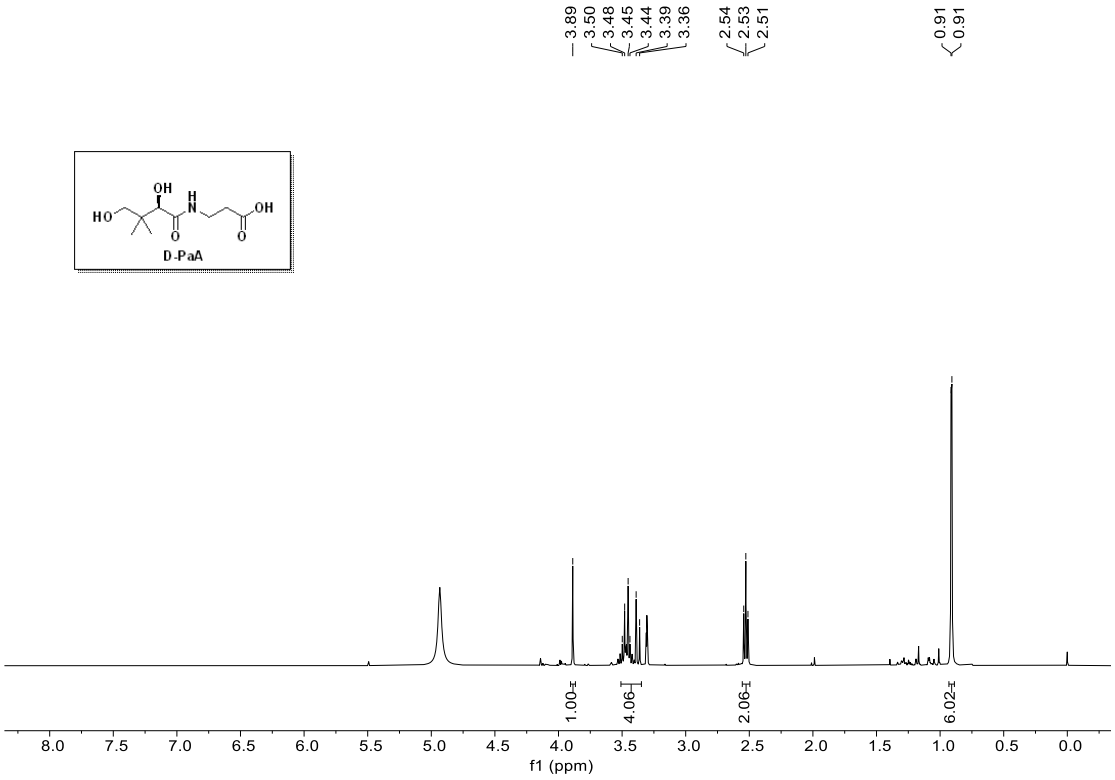
The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2k**



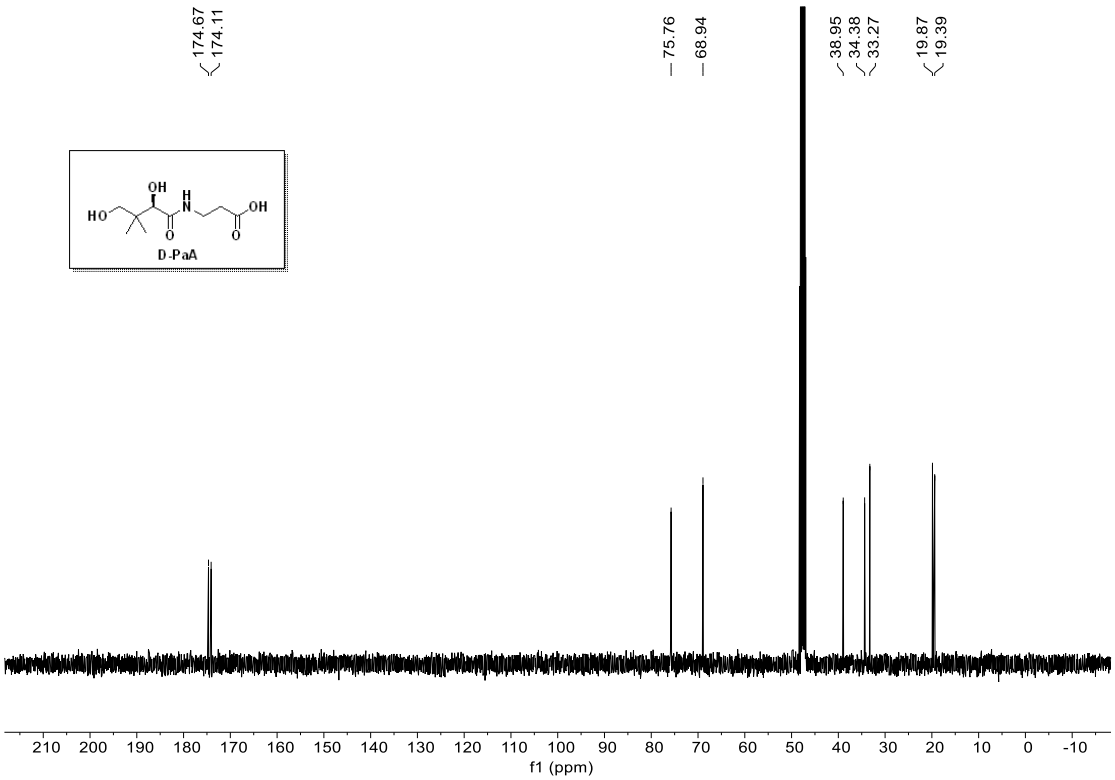
The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **2k**

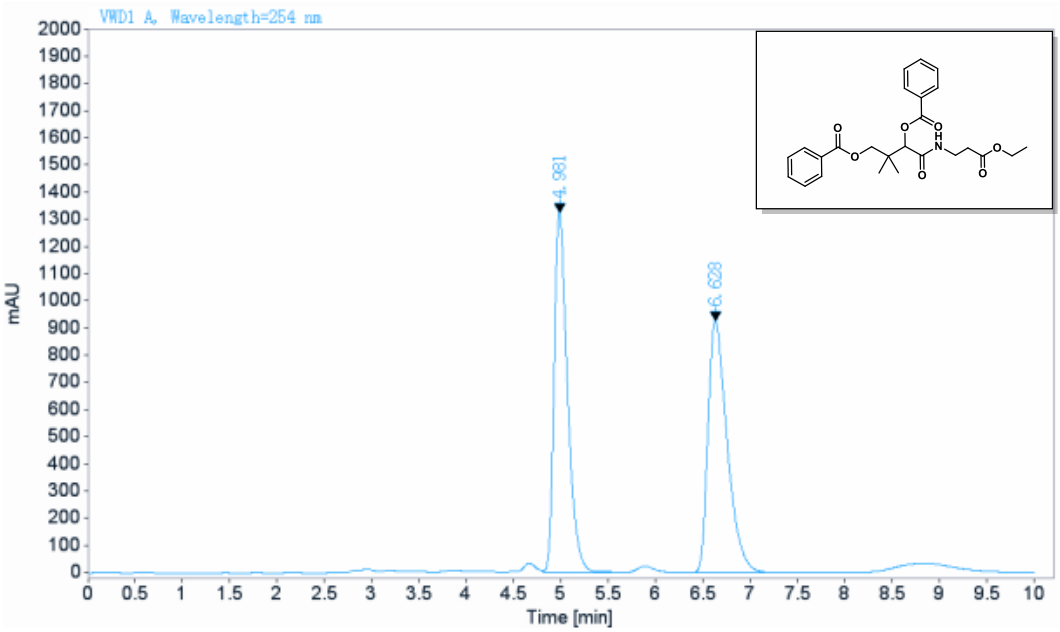


The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **D-PaA**



The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **D-PaA**





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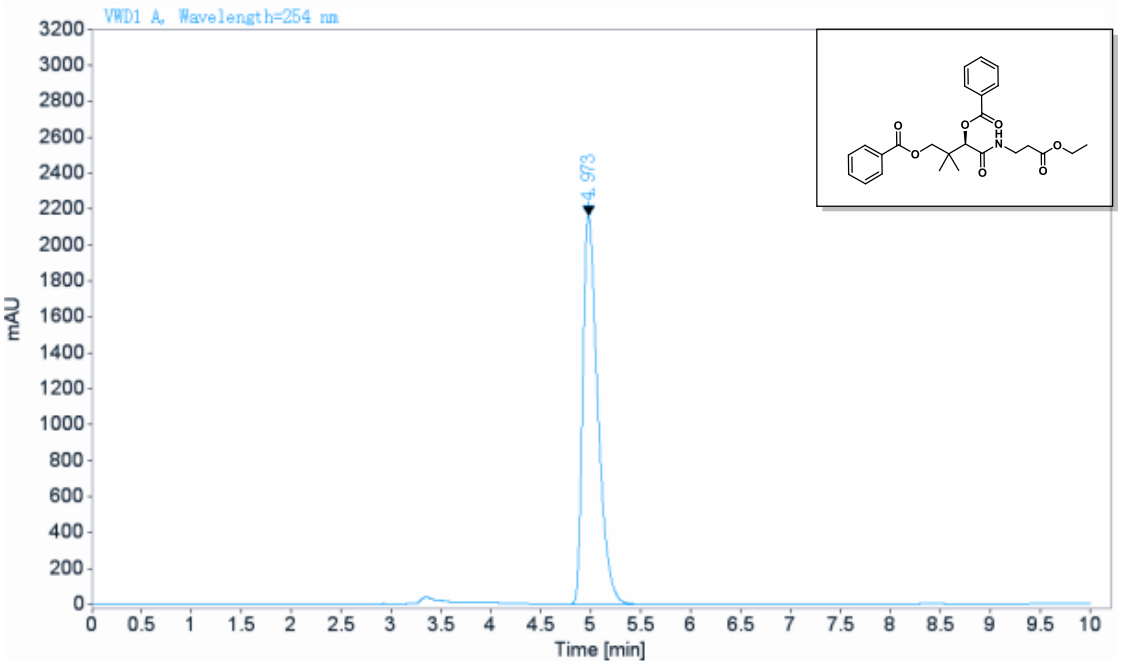
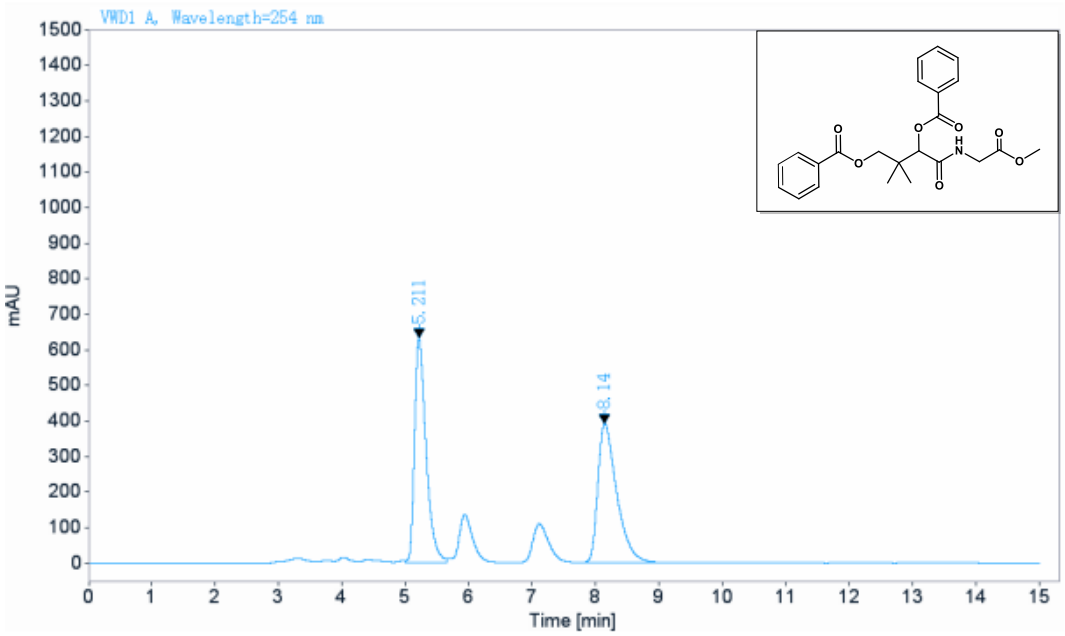
6.628	0.2159	13081.2148	925.6587	50.21

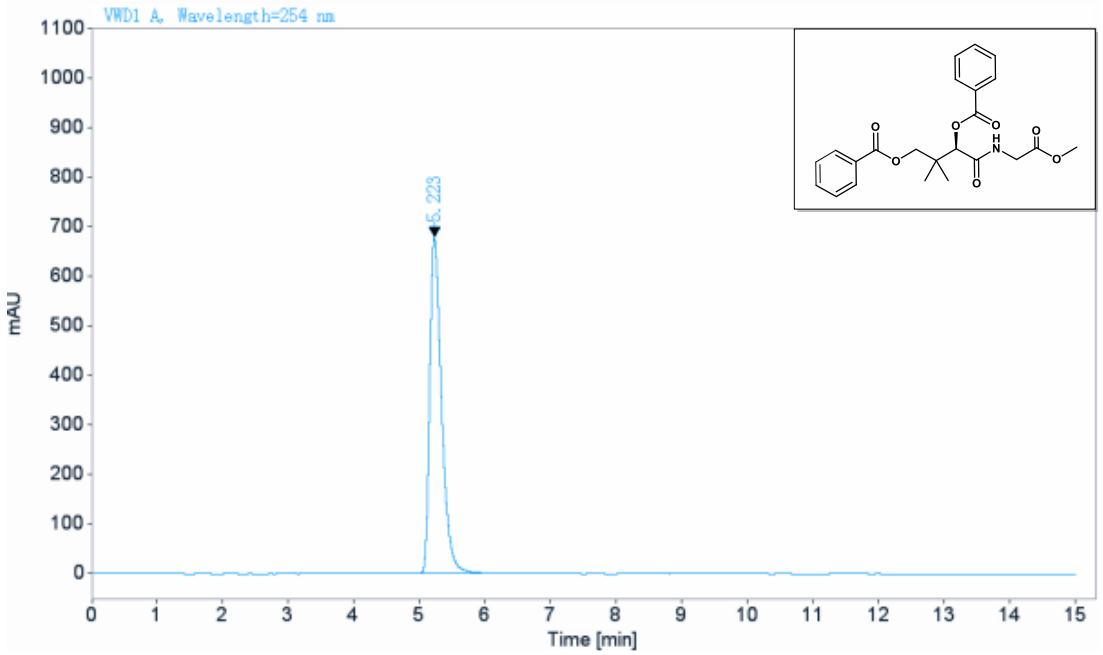


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
4.973	0.1590	22587.6582	2160.0181	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2a**, synthesized through $NaBH_4$-mediated reduction of **1a** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2a**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1a** and benzoylation.

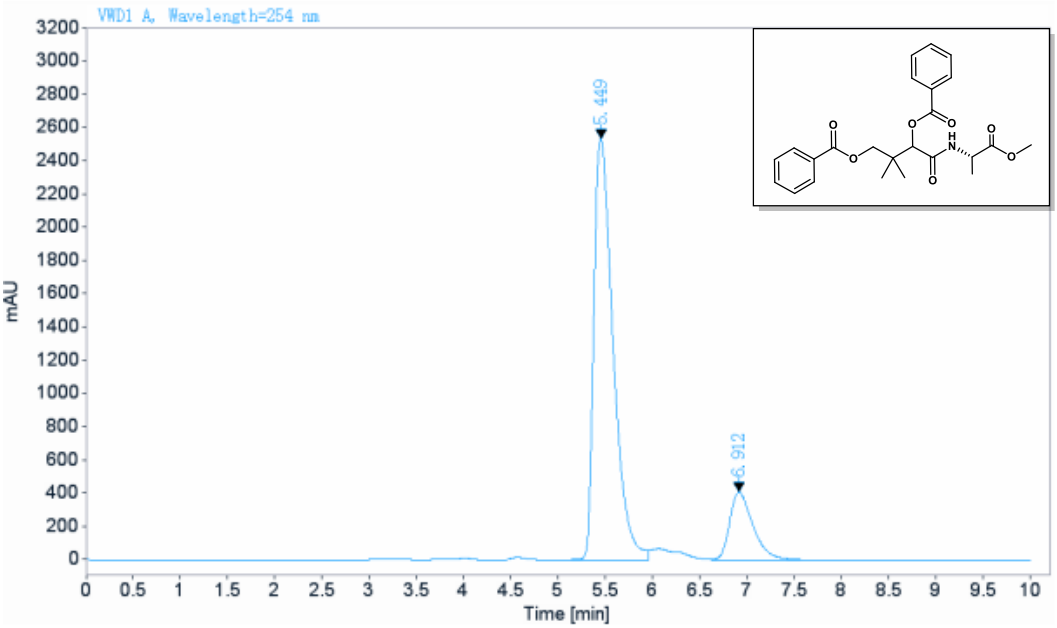


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
5.211	0.1908	7922.2495	629.5263	49.62
8.140	0.3107	8045.0220	390.0132	50.38



RetTime[min]	Width[min]	Area	Height[mAu]	Area%
5.223	0.1905	8525.7988	679.0826	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2b**, synthesized through $NaBH_4$-mediated reduction of **1b** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2b**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1b** and benzoylation.



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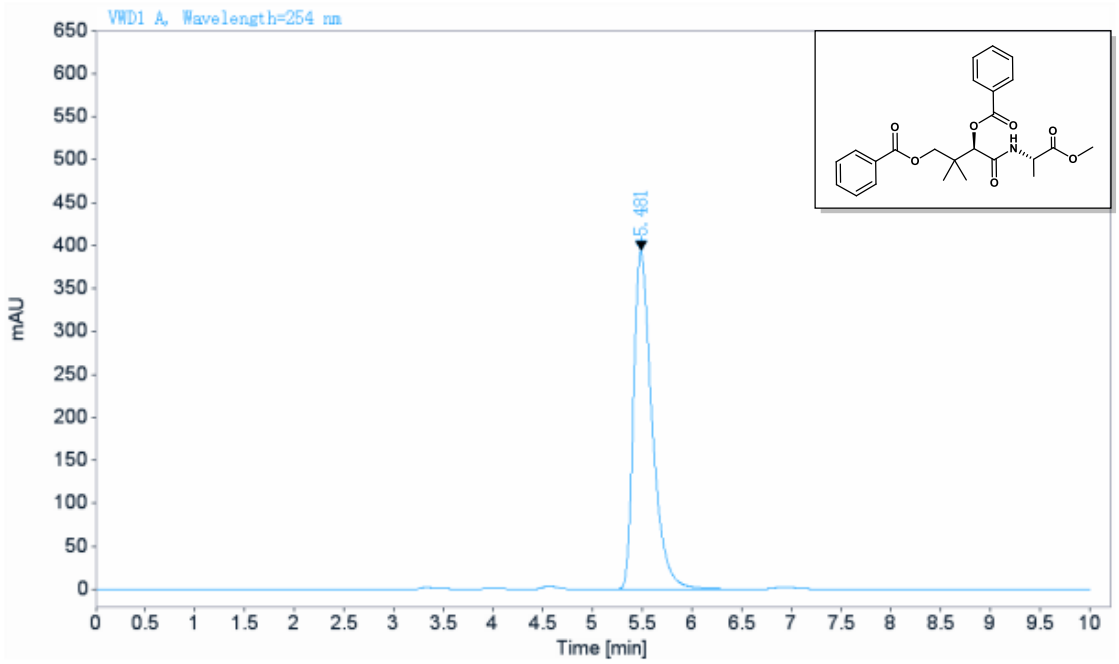
5.449	0.2178	36012.5000	2533.8787	83.62
6.912	0.2631	7052.3975	407.1066	16.38



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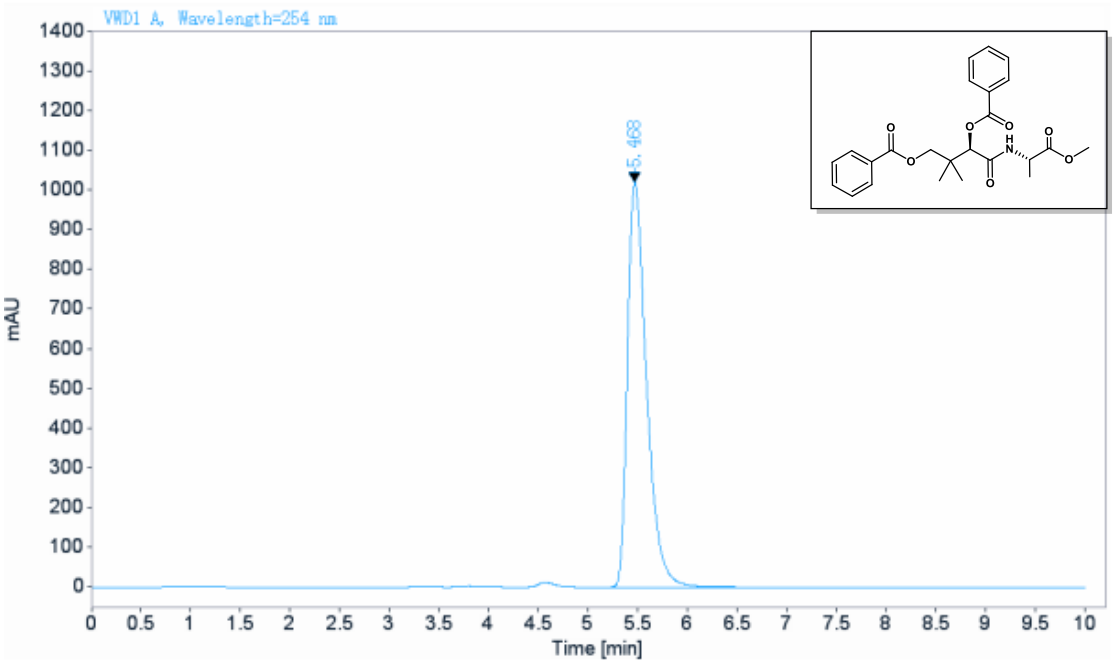
5.481	0.1954	5102.7310	393.2765	100.00



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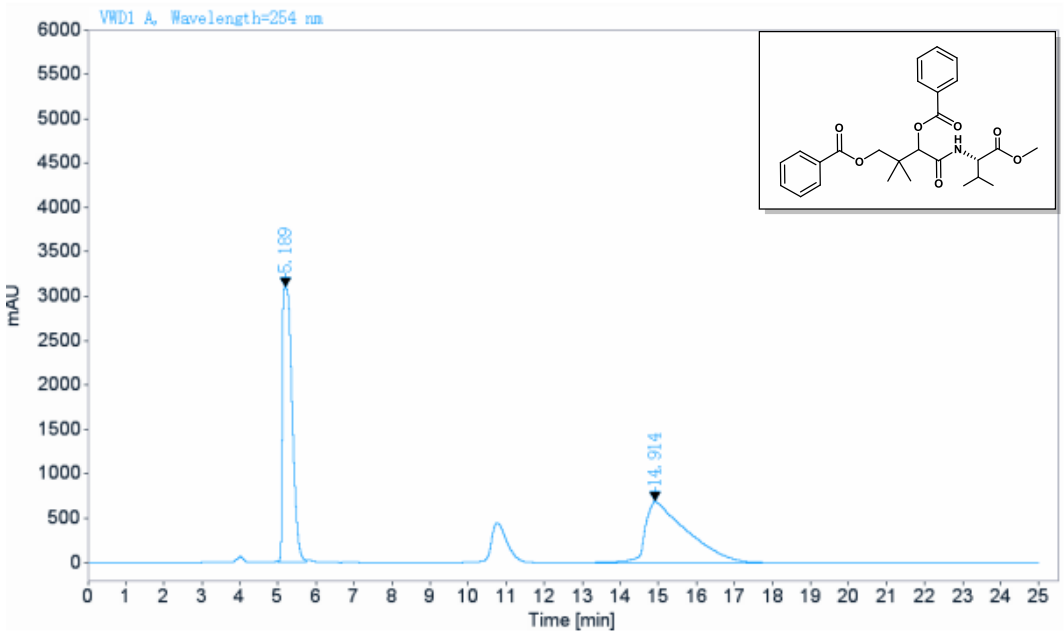
5.468	0.2038	13609.5215	1019.2852	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2c**, synthesized through $NaBH_4$-mediated reduction of **1c** and benzoylation. The middle spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2c**, synthesized through the condensation between (*R*)-pantolactone with L-alanine methyl ester, followed by benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2c**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1c** and benzoylation.



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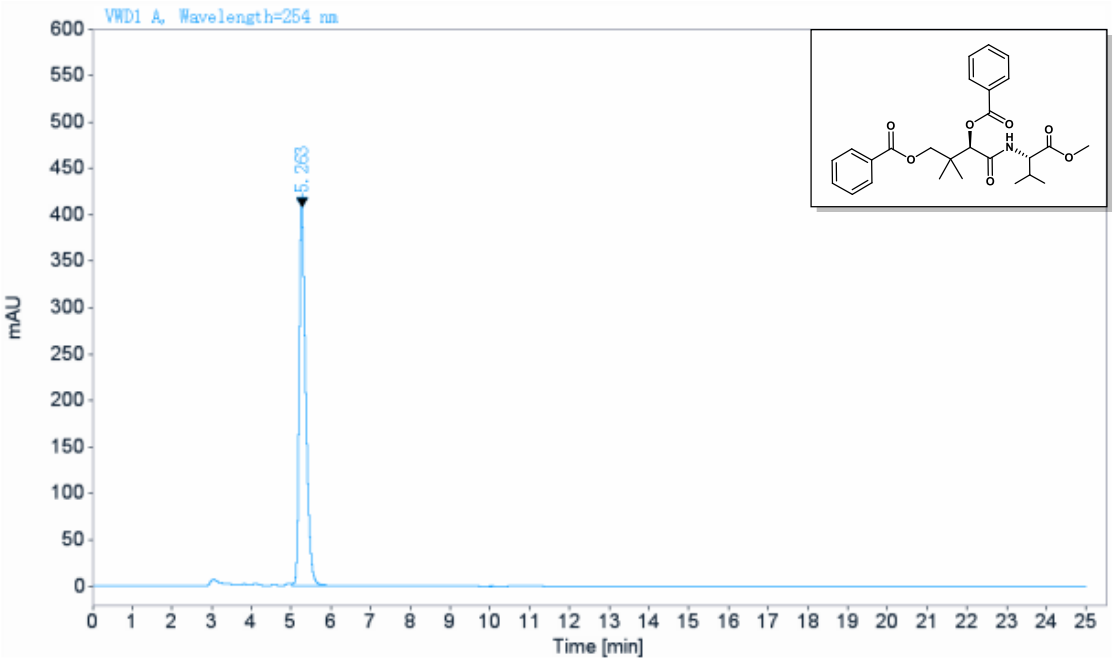
5.189	0.2117	54260.4688	3092.5872	50.84
14.914	1.0366	52461.9531	687.5958	49.16



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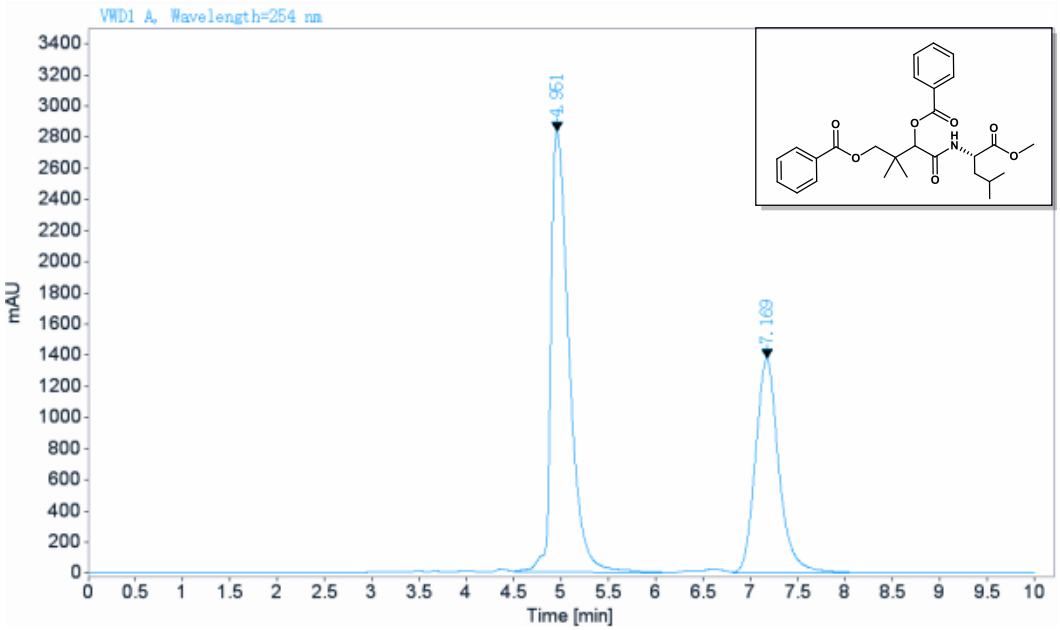
5.263	0.1759	4721.3413	406.9378	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2d**, synthesized through $NaBH_4$-mediated reduction of **1d** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2d**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1d** and benzoylation.



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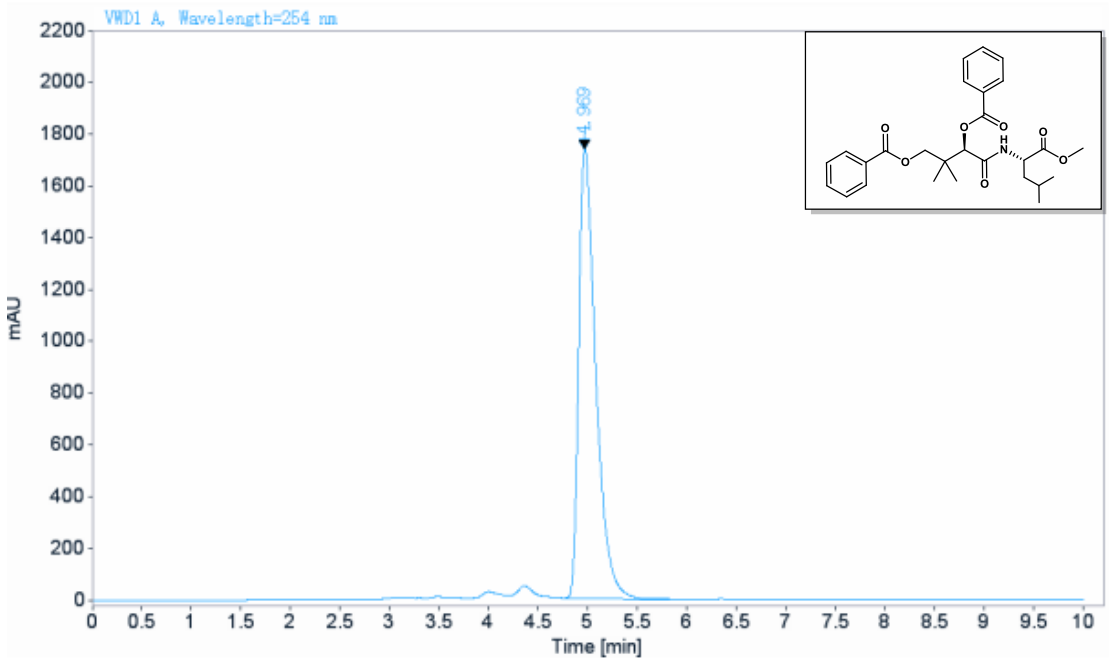
4.951	0.2041	37862.7383	2829.5146	61.61
7.169	0.2612	23593.2578	1374.6018	38.39



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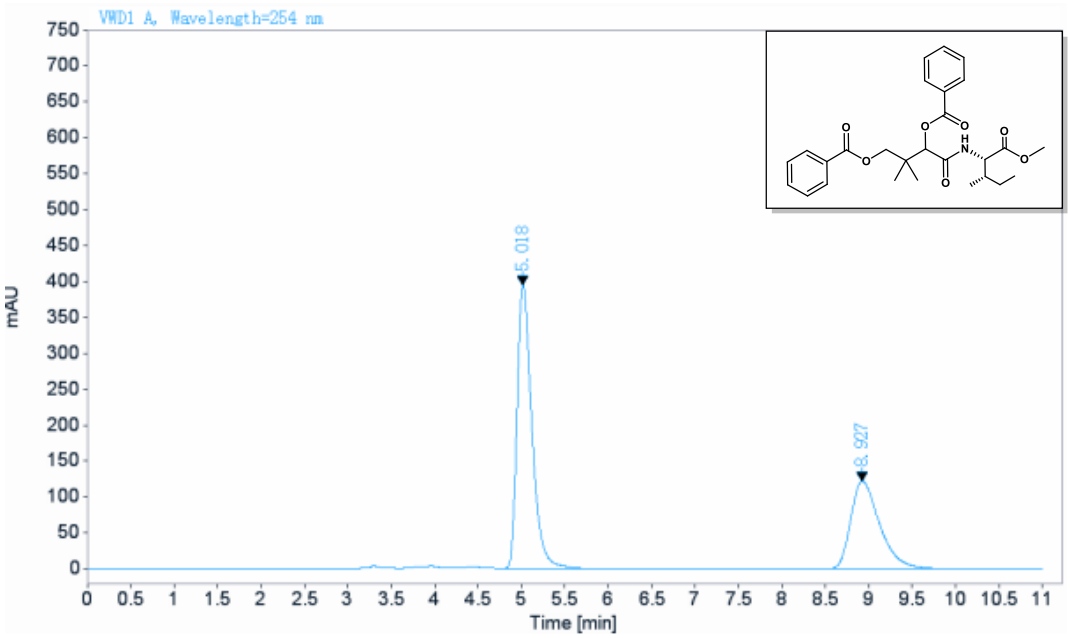
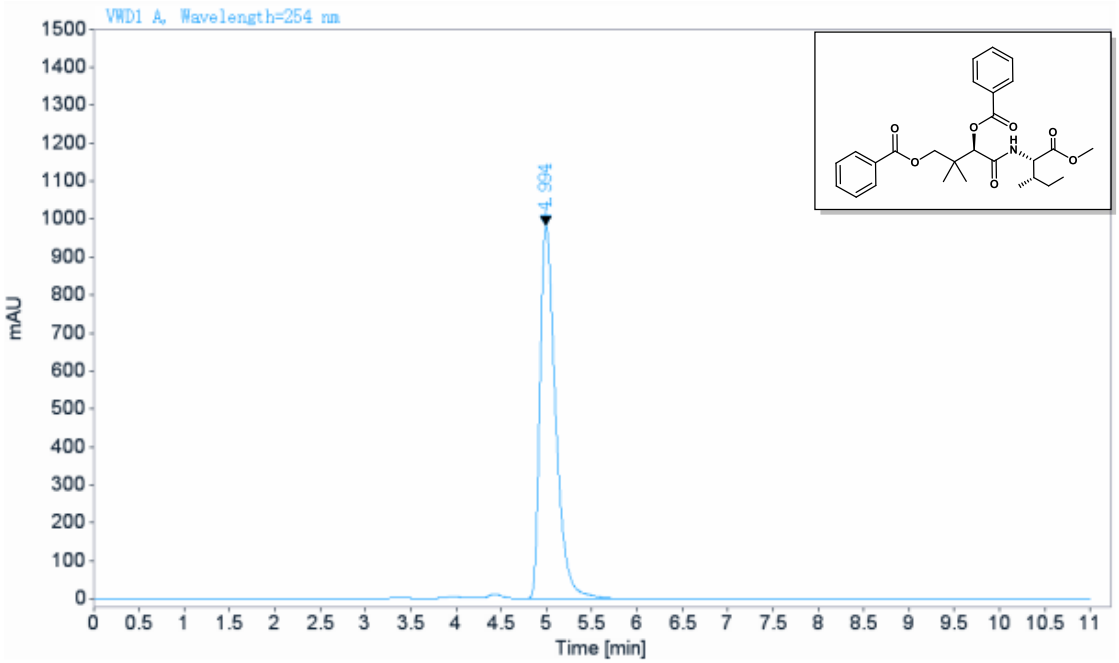
4.969	0.1823	20894.7109	1737.3409	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2e**, synthesized through $NaBH_4$-mediated reduction of **1e** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2e**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1e** and benzoylation.

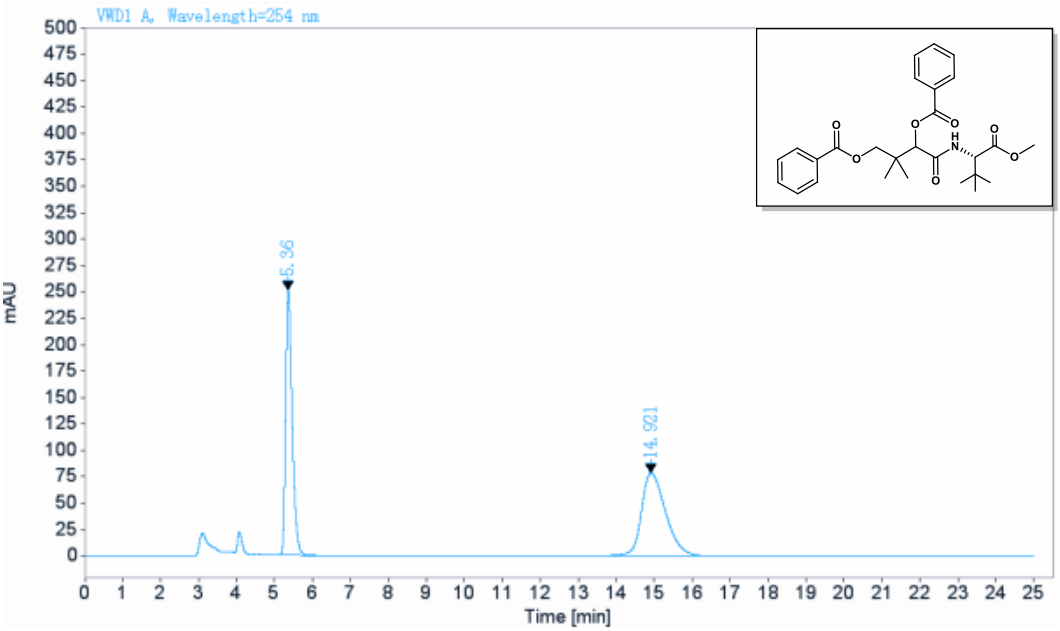


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
5.018	0.1808	4654.1616	393.9989	62.18
8.927	0.3550	2831.1252	121.8952	37.82

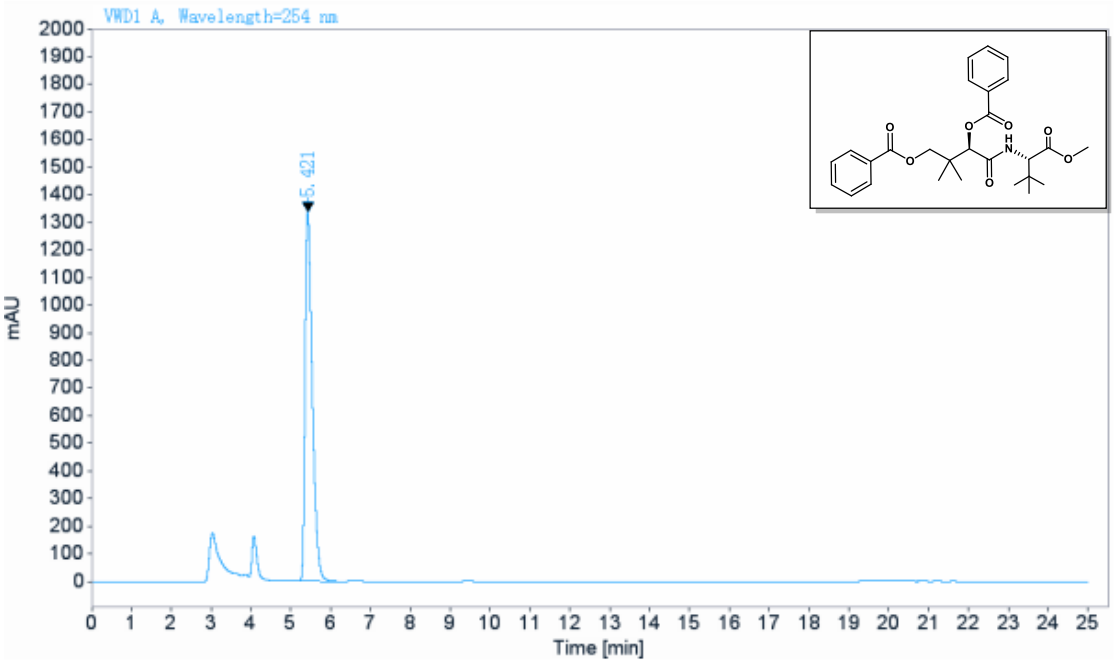


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
4.994	0.1830	11775.8584	981.4606	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2f**, synthesized through $NaBH_4$-mediated reduction of **1f** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2f**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1f** and benzoylation.

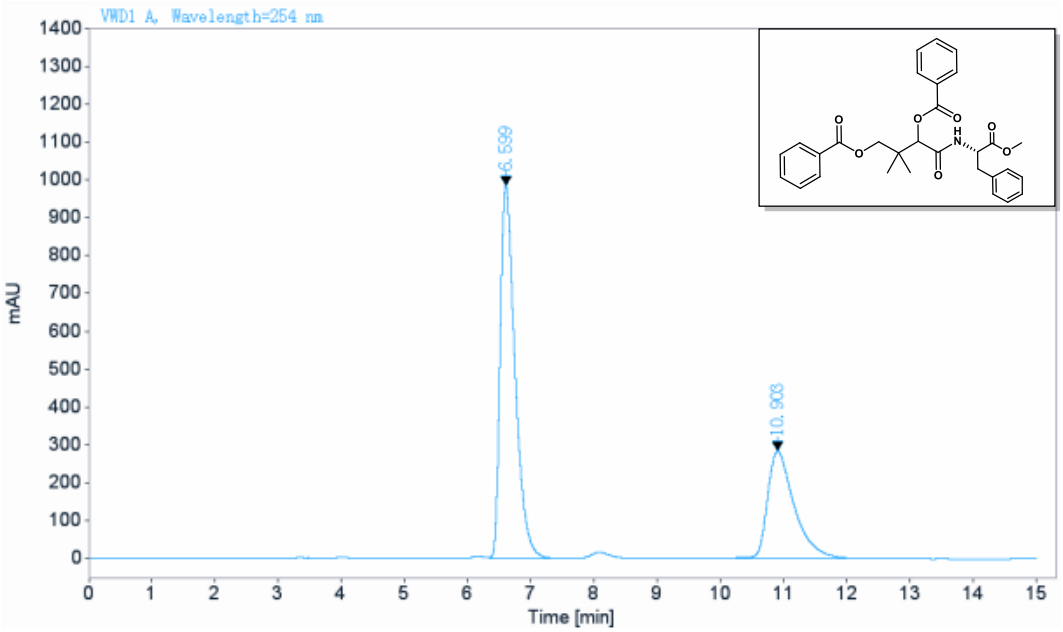


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
5.360	0.1804	2972.1311	250.6213	46.09
14.921	0.6591	3475.9749	78.6492	53.91

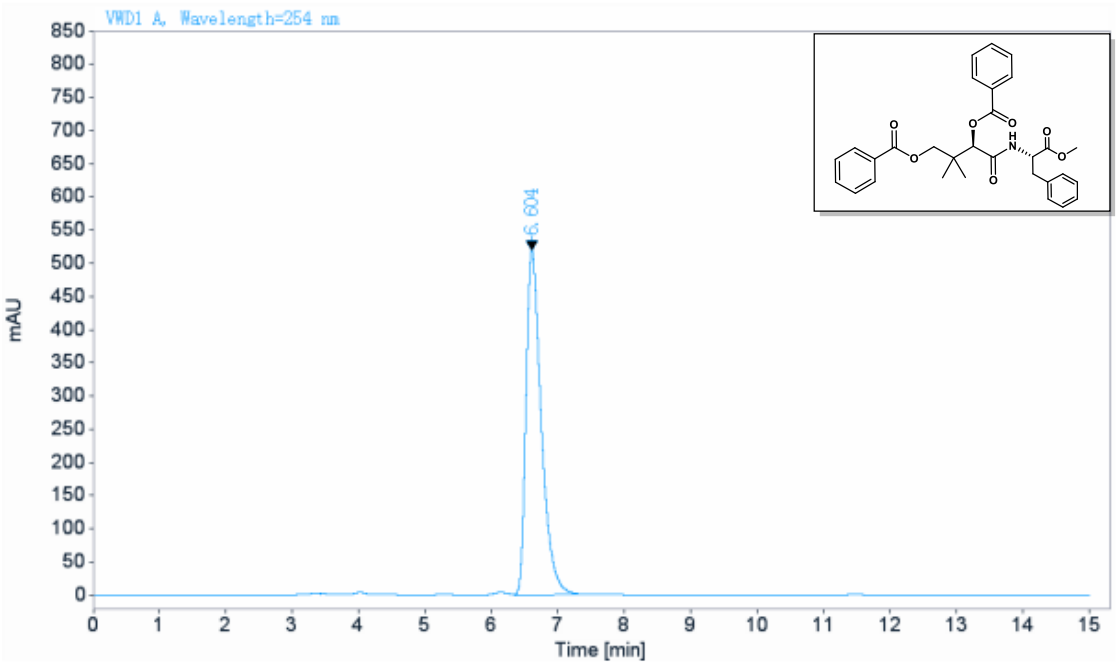


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
5.421	0.1930	16873.8809	1334.9287	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2g**, synthesized through $NaBH_4$-mediated reduction of **1g** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2g**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1g** and benzoylation.

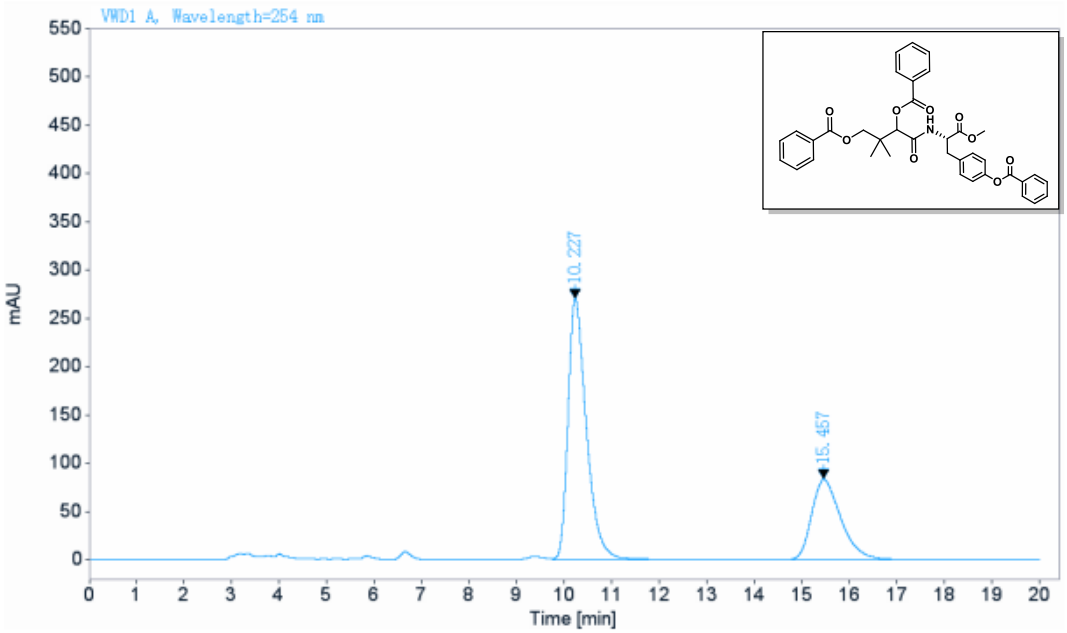


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
6.599	0.2491	16052.2236	985.3956	65.59
10.903	0.4459	8422.7959	283.2592	34.41

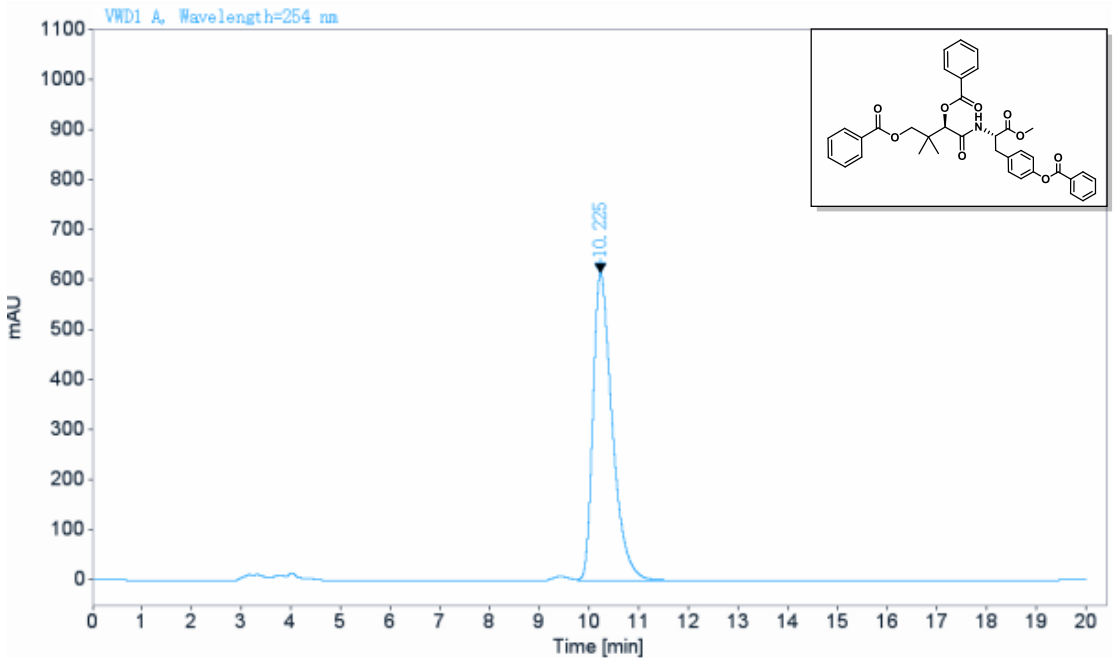


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
6.604	0.2435	8287.2832	518.4904	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2h**, synthesized through $NaBH_4$-mediated reduction of **1h** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2h**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1h** and benzoylation.

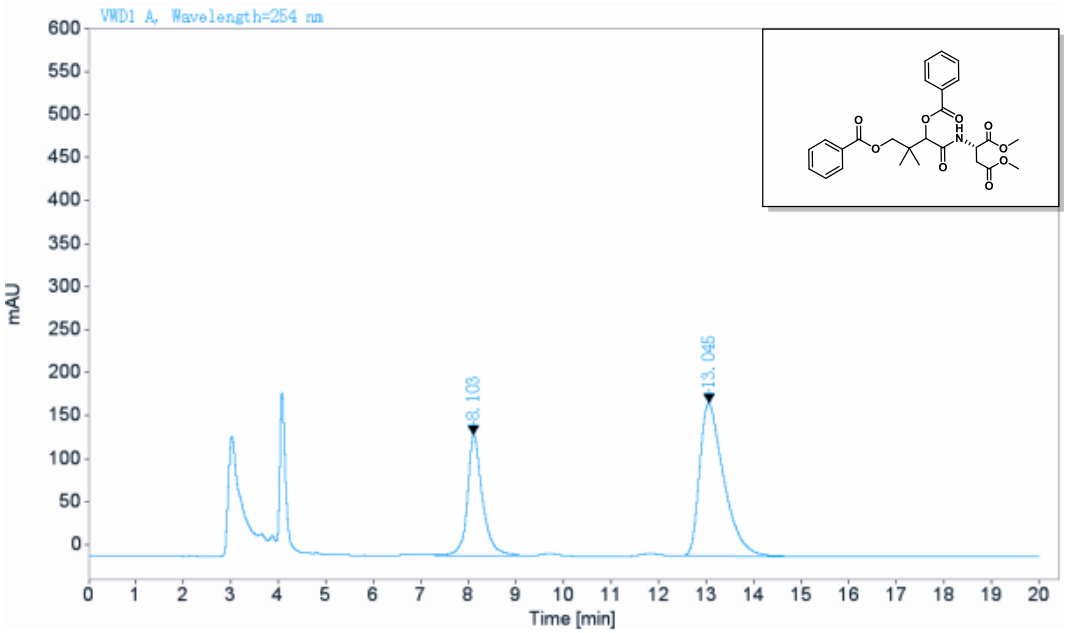


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
10.227	0.4044	7255.1436	270.0489	67.08
15.457	0.6692	3561.3159	83.5603	32.92

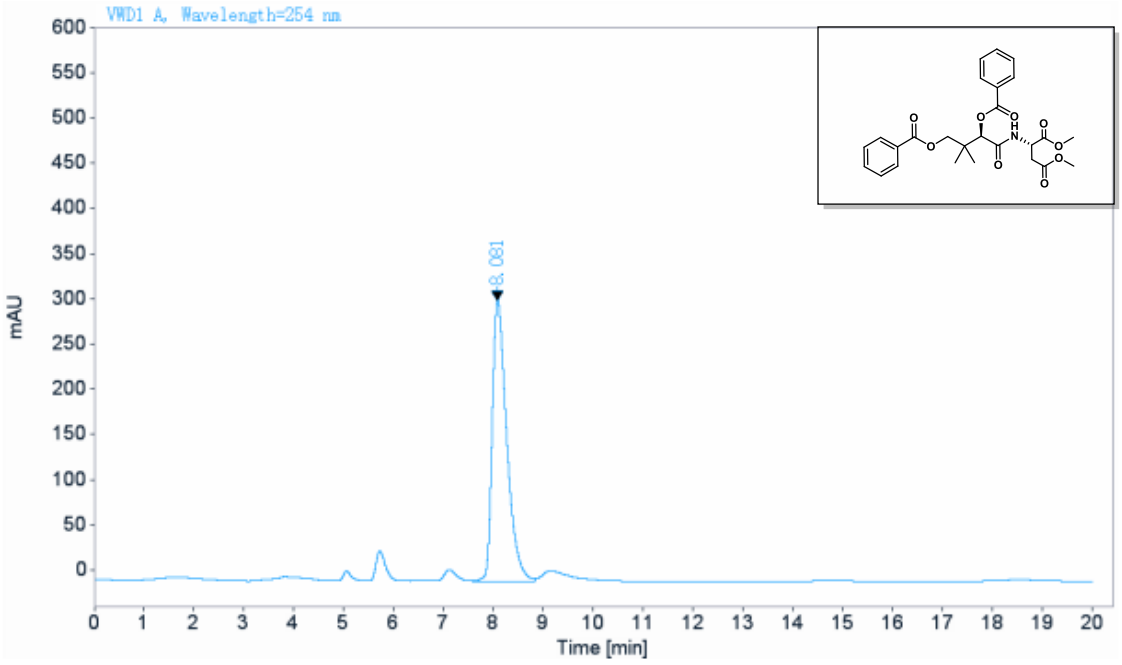


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
10.225	0.4080	16628.6211	613.9702	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2i**, synthesized through $NaBH_4$-mediated reduction of **1i** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2i**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1i** and benzoylation.

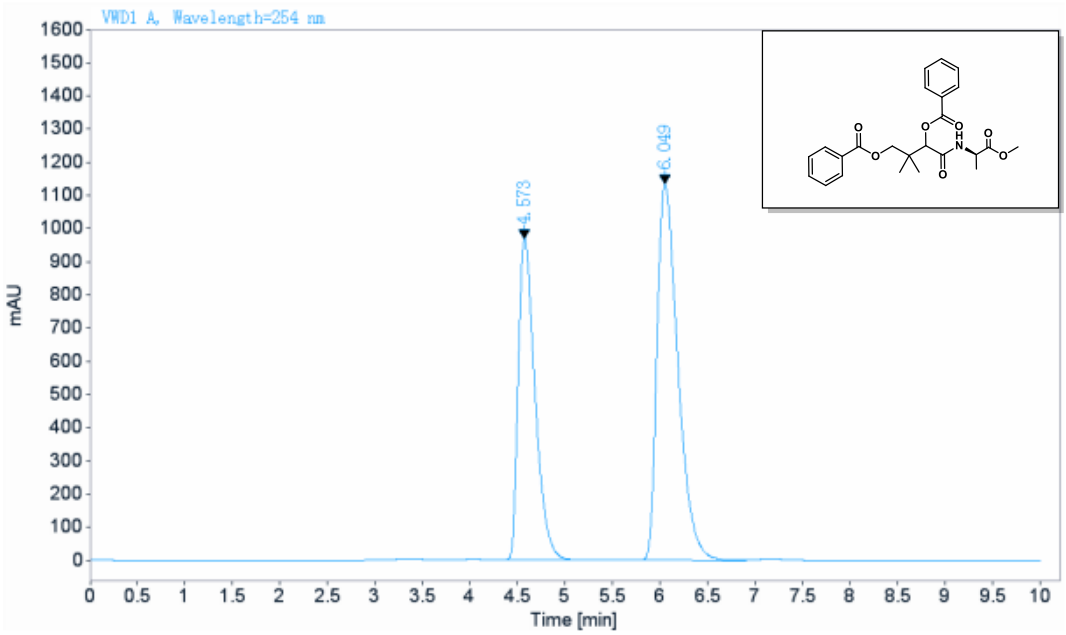


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
8.103	0.3238	3099.2813	140.2567	33.18
13.045	0.5256	6241.7256	177.1197	66.82

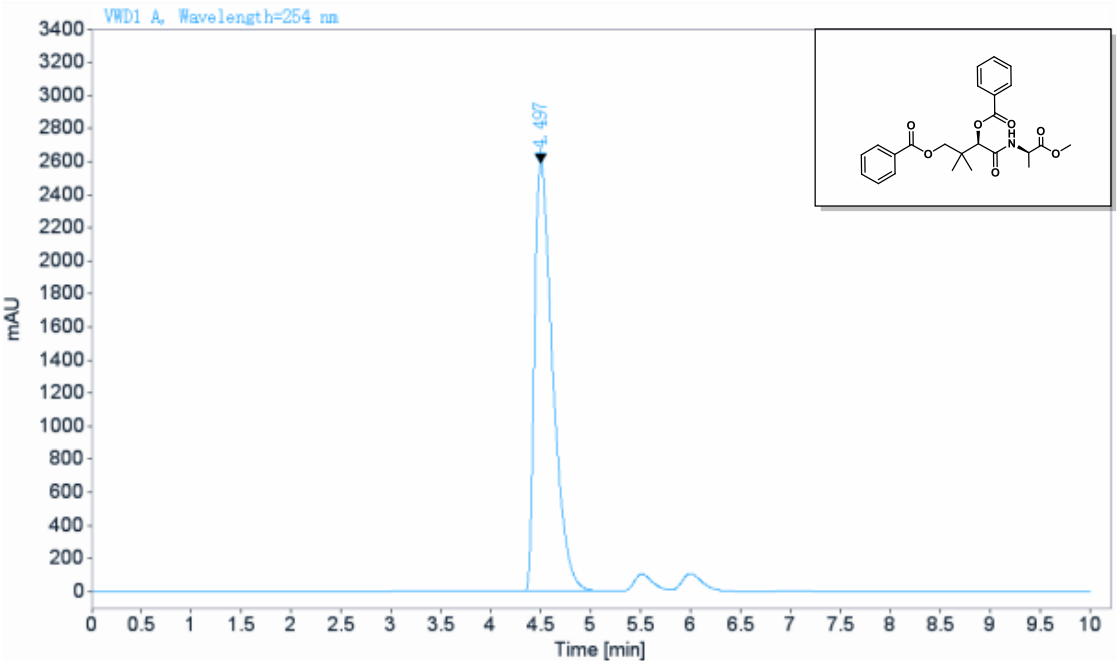


RetTime[min]	Width[min]	Area	Height[mAu]	Area%
8.081	0.3073	6268.2510	310.1697	100.00

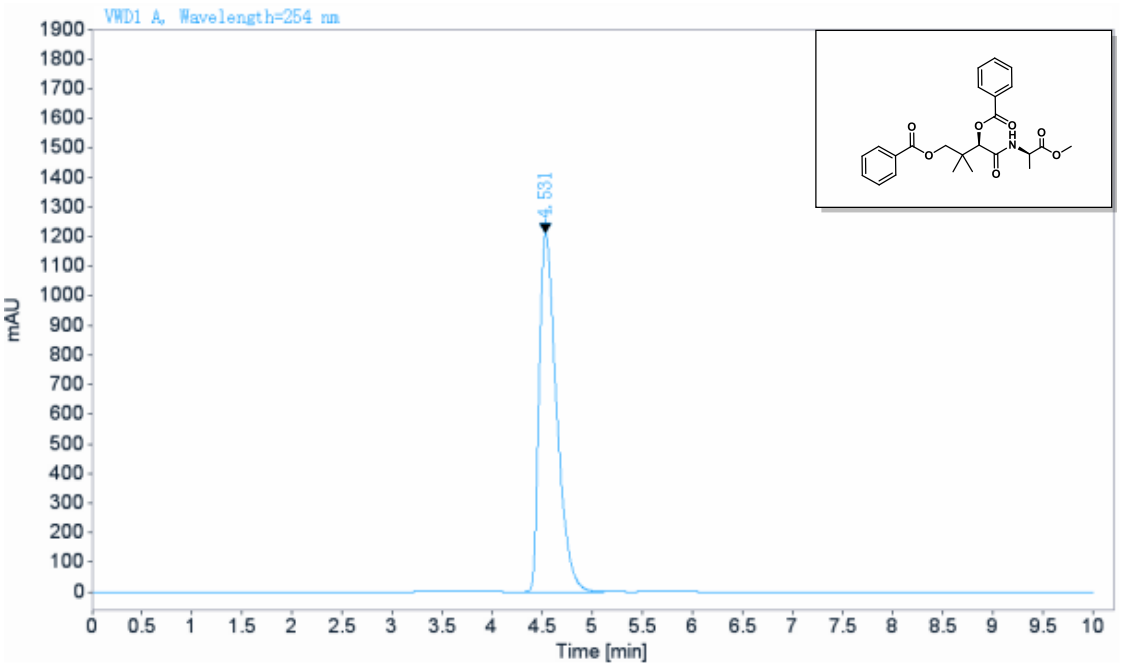
The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2j**, synthesized through $NaBH_4$-mediated reduction of **1j** and benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2j**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1j** and benzoylation.



RetTime[min]	Width[min]	Area	Height[mAu]	Area%
4.573	0.1907	11910.0791	966.9980	40.75
6.049	0.2363	17320.4727	1133.4956	59.25



RetTime[min]	Width[min]	Area	Height[mAu]	Area%
4.497	0.1951	33050.8438	2585.8528	100.00



RetTime[min]	Width[min]	Area	Height[mAu]	Area%
4.531	0.1849	14618.1289	1210.1051	100.00

The top spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2k**, synthesized through $NaBH_4$-mediated reduction of **1k** and benzoylation. The middle spectrum is the chiral HPLC analysis of the benzoylated authentic standard **2k**, synthesized through the condensation between (*R*)-pantolactone with D-alanine methyl ester, followed by benzoylation. The bottom spectrum is the chiral HPLC analysis of the benzoylated **2k**, synthesized through *E. coli* (pET28a-M3/pACYCDuet-1-GDH)-catalyzed reduction of **1k** and benzoylation.