Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

A new chemo-enzymatic route to chiral 2-hydroxy-4-phenylbutyrates by combining lactonase-mediated resolution with hydrogenation over Pd/C

Bing Chen,[a] Hai-Feng Yin,[b] Zhen-Sheng Wang,[b] Jia-Ying Liu,[a] and Jian-He Xu*[a]

1. Materials and Methods

1.1 *General Information*

^{1}H NMR spectra were recorded on a Bruke 300 MHz spectrometer. Chemical shifts were reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (CDCl3, δ = 7.26; d6-DMSO, δ = 2.5). Data are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), integration, and assignment. ^{13}C NMR data were collected on a 75 MHz spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from the tetramethylsilane with the solvent resonance as internal standard (CDCl3, δ = 77.0).

1.2 ***Synthesis of 2-hydroxyl-4-butyrolactones 3*** It was prepared according to procedures reported previously except that the step of separation by column chromatography was unnecessary.

1.3 ***Generation of four reFPL variants*** For reFPL1, the lactonase sequence with the signal peptide was inserted into plasmid pET28a; for reFPL2, the lactonase sequence with the signal peptide was inserted into plasmid pET24a; for reFPL3, the lactonase sequence without signal peptide was inserted into plasmid pET24a; for reFPL4, the lactonase sequence without signal peptide was inserted into plasmid pET28a. The lactonase gene with signal peptide has now been deposited in GenBank with an accession number of EU596535.

1.4 ***Amino acid sequence of FPL***

With signal peptide:

"MPSSISVLAAGILVPVLGAVAAKLPPTAQIIDQKSFNVLKDVPPPAVANDSLVFTWPGVTEE
SLVEKPFHVYDEEFYDVIGKDPSLTLIATSDTDPIFHEAVVWYPPTEEVFFVQNAGAPAAGT
GLNKSSIIQKISLKEADEVRKGKKDEVKVTVVDSNPQVINPNGGTYYKGNIIFAGEGQGDD
VPSALYLMNPLPPYNTTTLLNNYFGRQFNSLNDVGINPRNGDLYFTDTLYGYLQDFRPVP
GLRNQVYRYNFDTGAVTVVADDFTLPNGIGFGPDGKKVYVTDTGIALGFYGRNLSSPASV
YSFDVNQDGTLQNRKTFAYVASFIPDGVHTDSKGRVYAGCGDGVHVWNPSGKLIGKIYT
GTVAANFQFAGKGRMIITGQTKLFYVTLGASGPKLYD"

Without signal peptide:

"MAKLPSTAQIIDQKSFNVLKDVPPPAVANDSLVFTWPGVTEESLVEKPFHVYDEEFYDVIG
KDPSLTHIATSDTDPIFHEAVVWYPPTEEVFFVQNAGAPAAGTGLNKSSIIQKISLKEADEV
RKGKKDEVKVTVVDSNPQVINPNGGTYYKGNIIFAGEGQGDDVPSALYLMNPLPPYNTTT
LLNNYFGRQFNSLNDVGINPRNGDLYFTDTLYGYLQDFRPVPGLRNQVYRYNFDTGAVT
VVADDFTLPNGIGFGPDGKKVYVTDTGIALGFYGRNLSSPASVYSFDVNQDGTLQNRKTF
AYVASFIPDGVHTDSKGRVYAGCGDGVHVWNPSGKLIGKIYTGTVAANFQFAGKGRMIIT
GQTKLFYVTLGASGPKLYD"

1.5 ***Expression of recombinant Fusarium proliferatum (reFPL) lactonase*** FPL gene sequence was ligated to pET28a (or pET24a). The ligation mixture was introduced by transformation into *E. coli* BL21 (DE3) and then screening of clones containing the restriction fragment was performed. The resultant plasmid was designated as pET28a-FPL. The recombinant *E. coli* BL21 (DE3) containing pET28a-FPL was cultured aerobically at 37 °C in 50 ml LB medium until OD_{600} reached 0.7, and then the cultures were immediately shifted to 15 °C. IPTG was added at a final concentration of 0.17 mM to induce the *lac* promoter. After further cultivation, the cells were harvested by centrifugation and washed thoroughly with physiological saline, and then were used for further studies.

1.6 ***Enzyme assay*** The standard assay mixture comprised of 2.5% (*w/v*) racemic pantolactone and appropriate amount of the cells with a final volume of 10.0 ml. The reaction was performed at 30 °C, and the pH was automatically controlled at 6.7-6.9 with 0.1 M NaOH. The hydrolysis rate of lactone was calculated based on the rate of NaOH titration. One unit of lactonase (1 IU) was defined as the amount of the enzyme that converted 1.0 μmol of pantolactone into pantoic acid per minute under the above conditions.

1.7 ***Typical procedure for resolution of substrates by recombinant E. coli cells*** Appropriate amount of wet cells were re-suspended in 10 ml potassium phosphate buffer (KPB, 50 mM, pH 6.4) and the

enzyme activity of this aqueous solution was measured as 10.3 IU/100 µl. General procedures were as following: To a 10 ml potassium phosphate buffer (KPB, 50 mM, pH 6.4) was added appropriate volume of this aqueous solution and 0.1 mmol of *cis*-**3** (or *trans*-**3**) in 200 µl methanol. This mixture in 100 ml flask was kept at 30°C and shaken at 180 rpm. The resolution process was monitored by chiral HPLC (AD-H or OJ-H columns) and terminated at appropriate time. (2*S*)-**3** was extracted with ethyl acetate directly, while the solution containing (2*R*)-**3** was worked up by acidification (pH 2.0, 2N HCl) and heating (60°C, 1 hour) after which the product was extracted with ethyl acetate.

1.8 Reaction process of resolution of cis-3 monitored by chiral HPLC

	10 min			20 min			30 min			60 min		
	Ee_s	Ee_p	Conv.	Ee_s	Ee_p	Conv.	Ee_s	Ee_p	Conv.	Ee_s	Ee_p	Conv.
ReFPL1	74.9	>99	42.8	95.4	96.9	49.6	>99	96.9	50.6	>99	90.3	52.5
ReFPL2	49.6	>99	33.2	76.9	98.0	44.0	89.2	95.3	48.3	>99	93.3	51.7
ReFPL3	70.8	97.9	42.0	93.9	96.1	49.4	>99	96.2	51.0	>99	90.1	52.6
ReFPL4	57.0	96.9	37.0	85.2	96.7	46.8	94.5	92.8	50.5	>99	91.6	52.2

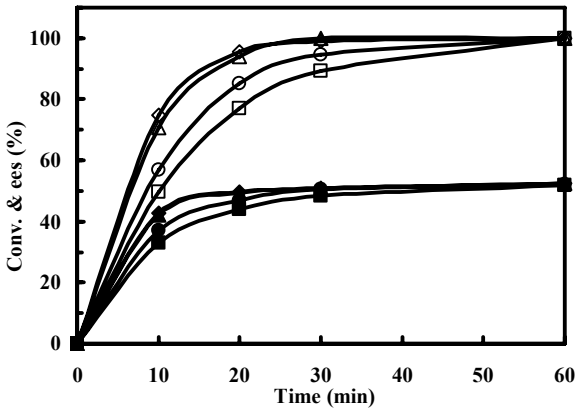


Figure 1. Reaction progress of *cis*-4-phenyl-2-hydroxyl-4-butyrolactone hydrolysis catalyzed by four reFPL variants. Open diamond ee_s of reFPL1; Open square ee_s of reFPL2; Open triangle ee_s of reFPL3; Open circle ee_s of reFPL4; Filled diamond conv. of reFPL1; Filled square conv. of reFPL2; Filled triangle conv. reFPL3; Filled circle conv. of reFPL4. Reaction condition: 10 mM substrate, 10 ml KPB buffer (50 mM, pH 6.4), 200 µl MeCN, 30°C, 180 rpm. The amount of enzymes was controlled at 10 IU towards racemic pantolactone (substrate-to-catalyst ratio > 1/1).

1.9 ***Preparation of (S)-2-hydroxy-4-phenylbutyric acid*** Appropriate amount of wet cells were resuspended in 10 ml potassium phosphate buffer (KPB, 50 mM, pH 6.4). To 500 ml water was added appropriate volume of this aqueous solution and 20.0 g of *cis*-/*trans*-**3** in the mixture in 150 ml MeCN. This mixture in 1 l beaker was kept in room temperature and stirred. The pH was automatically controlled at 6.3~6.5 with 0.1 M NaOH. The resolution was monitored by HPLC (AD-H column) and terminated when *ee* value of *cis*-(2*S*,4*S*)-**3** reached >99% (The rate of hydrolysis of *cis*-lactone was slower than that of *trans*-lactone). The biocatalyst was centrifuged and the supernatant was extracted with ethyl acetate directly. Concentration of this organic layer afforded 6.5 g of (2*S*)-**3** as yellow solid.

The yellow solid was dissolved in 100 ml acetate. To this solution was added 5% Pd/C and submitted to hydrogenation at room temperature and 1 atm for overnight. Then this mixture was concentrated under vacuum to remove acetate. The target product (*S*)-2-hydroxy-4-phenylbutyric acid was then re-crystallized from 1,2-dichloroethane (5.7 g).

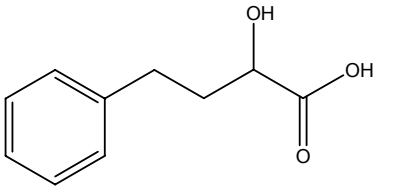
1.10 ***Preparation of (R)-2-hydroxy-4-phenylbutyric acid*** Appropriate amount of wet cells were re-suspended in 10 ml potassium phosphate buffer (KPB, 50 mM, pH 6.4). To 500 ml water was added appropriate volume of this aqueous solution and 20.0 g of *cis*-**3** in 150 ml MeCN. This mixture was kept in room temperature and stirred. The pH was automatically controlled at 6.2~6.4 with 0.1 M $NH_3 \cdot H_2O$. The resolution was terminated when conversion reached around 30%. The biocatalyst was

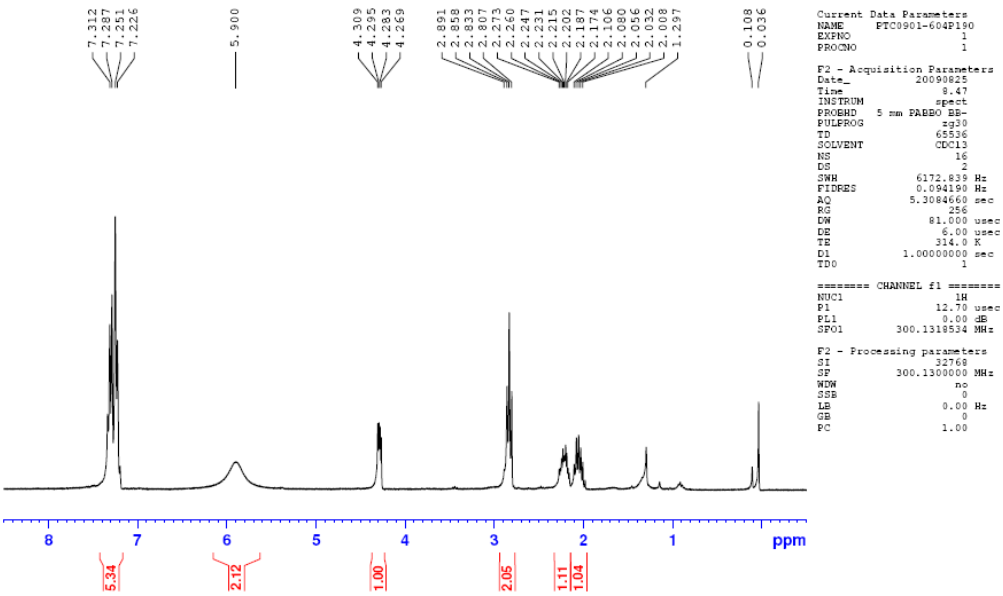
centrifuged and the supernatant was concentrated in vacuum to remove MeCN and then extracted with ethyl acetate to remove the (2*S*)-**3** completely. While (2*R*)-**3** in this aqueous layer was acidified and heated, after which the product was extracted with ethyl acetate to afford 7.1 g of solid.

The solid was dissolved in 100 ml acetate. To this solution was added 5% Pd/C and submitted to hydrogenation at room temperature and 1 atm for overnight. Then this mixture was concentrated under vacuum to remove acetate. The target product (*R*)-2-hydroxy-4-phenylbutyric acid was then recrystallized from 1,2-dichloroethane (4.7 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.009-2.106 (m, 1H), 2.174-2.273 (m, 1H), 2.807-2.891 (m, 2H), 4.269-4.309 (dd, *J* = 4.2 Hz, 7.8 Hz, 1H), 5.9 (br, 2H), 7.226-7.312 (m, 5H).

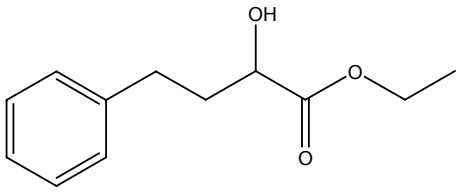
1.11 ***Typical procedure for ethyl 2-hydroxy-4-phenylbutyric acid*** To a stirred 250 ml round-bottom flask, crude compound **4** (100 mg) in 50 ml EtOH was added, followed by Me_3SiCl (10 ml) in one portion at room temperature. The mixture was stirred for 24 h and then concentrated under vacuum to give yellow oil. The separation of the target compounds was done by column chromatography on silica gel (petroleum ether:ethyl acetate = 5:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.257-1.304 (t, *J* = 7.2 Hz, 3H), 1.884-2.007 (m, 1H), 2.063-2.177 (m, 1H), 2.646 (br, 1H), 2.707-2.796 (m, 2H), 4.157-4.244 (m, 3H), 7.165-7.371 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.21, 31.05, 36.00, 61.76, 69.70, 126.04, 128.43, 128.58, 141.21, 175.22.

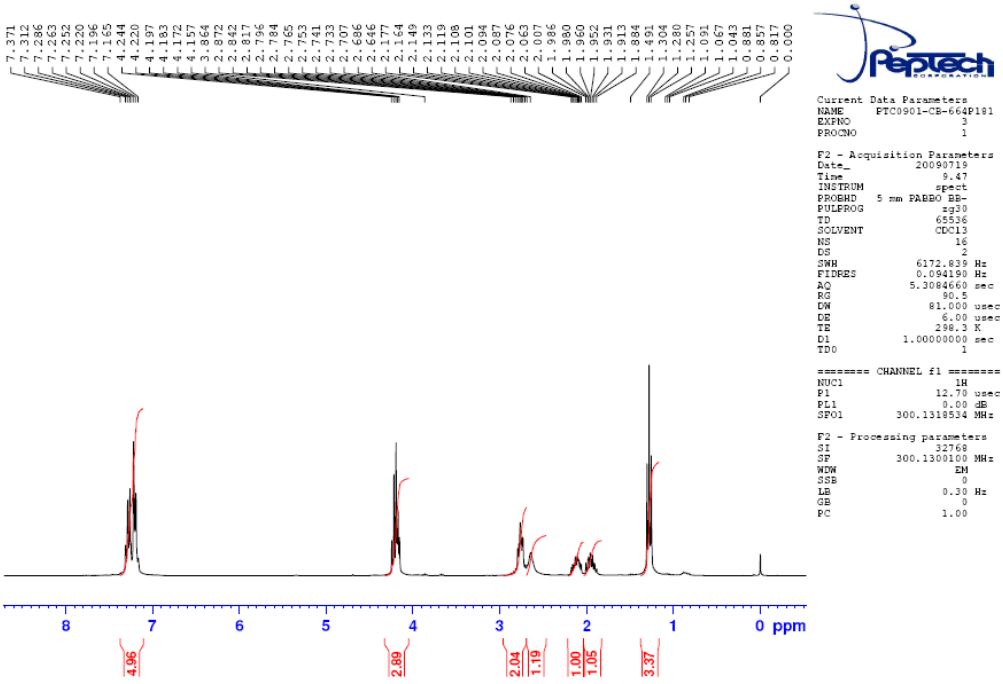
2.1 NMR Spectra for Relative Compounds



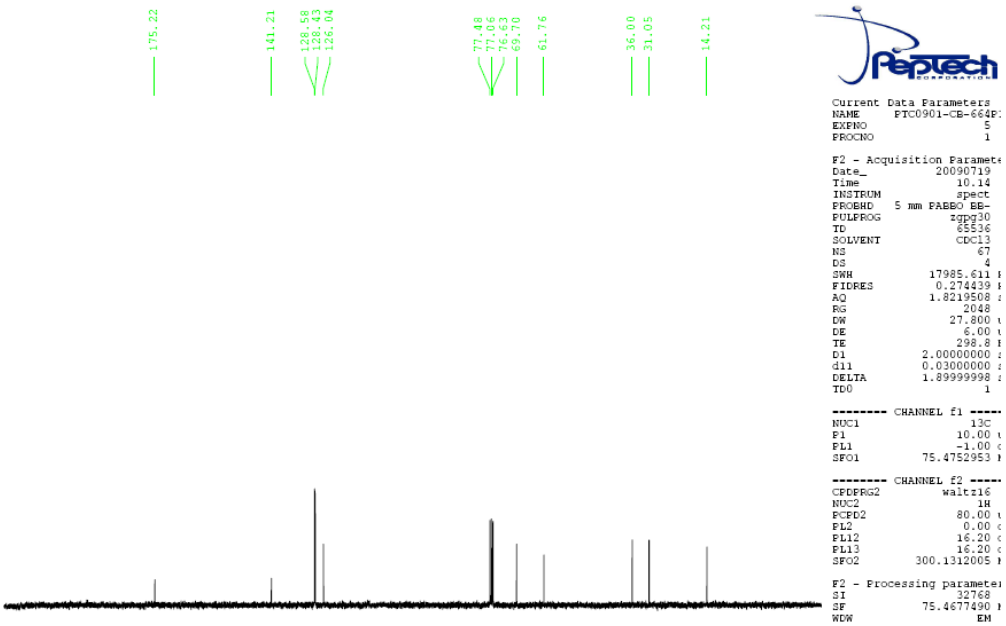


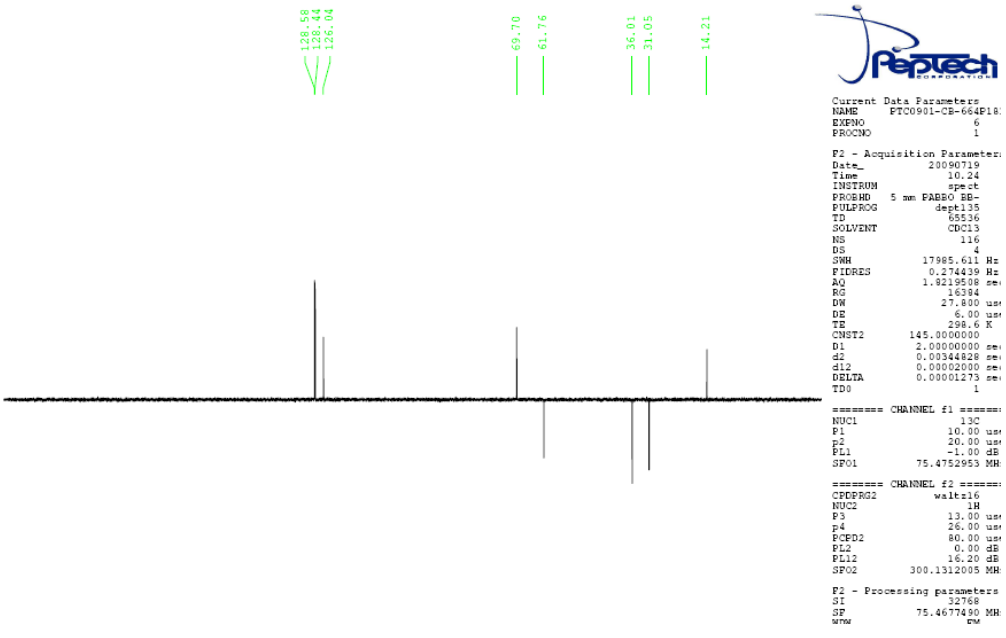

```
Current Data Parameters
NAME       PTC0901-604P190
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20090825
Time                 8.47
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             6172.839 Hz
FIDRES          0.094190 Hz
AQ              5.3084660 sec
RG                   256
DW                81.000 usec
DE                 6.00 usec
TE                314.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 12.70 usec
PL1                0.00 dB
SFO1         300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF           300.1300000 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00
```

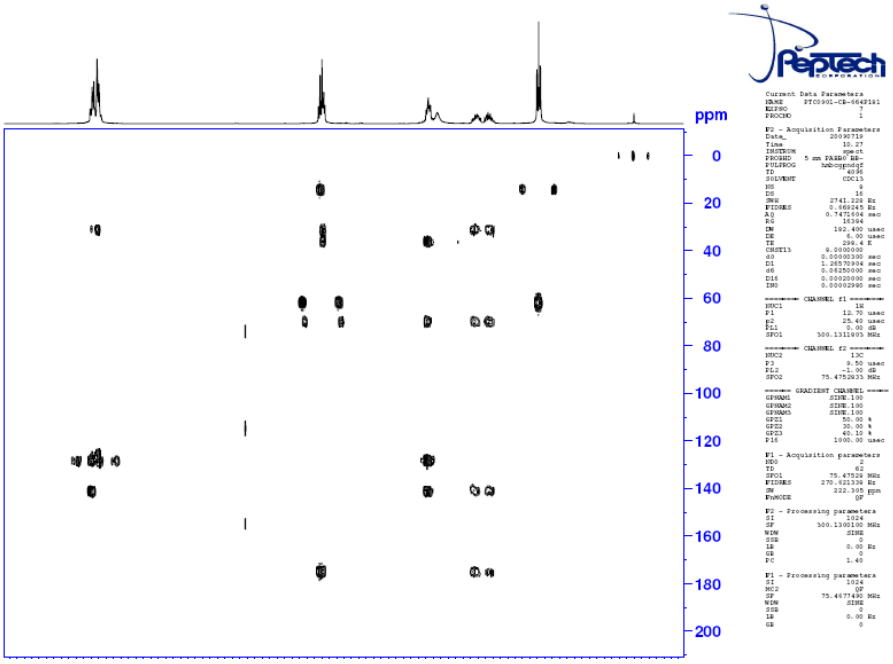


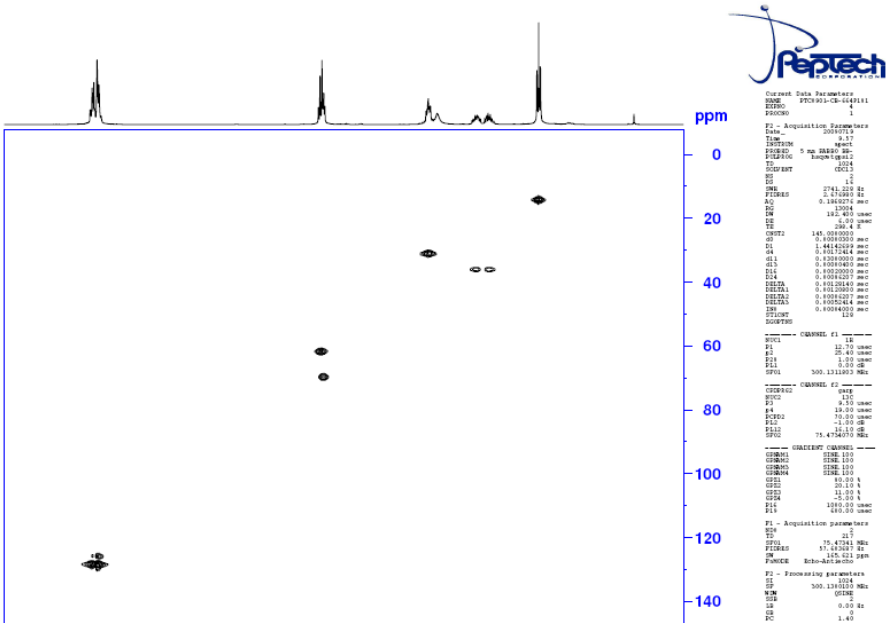



```
Current Data Parameters
NAME       PTC0901-CB-664P181
EXPNO                    3
PROCNO                   1

F2 - Acquisition Parameters
Date_             20090719
Time                 9.47
INSTRUM             spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             6172.839 Hz
FIDRES          0.094190 Hz
AQ              5.3084660 sec
RG                  90.5
DW                81.000 usec
DE                 6.00 usec
TE                298.3 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 12.70 usec
PL1                0.00 dB
SFO1         300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF           300.1300100 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

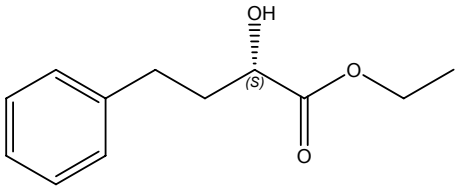

175.22
141.21
128.58
128.43
126.04
77.48
77.05
76.63
69.70
61.76
36.00
31.05
14.21
Current Data Parameters
NAME PTC0901-CB-664P
EXPNO 5
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090719
Time 10.14
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 67
DS 4
SWH 17985.611
FIDRES 0.274439
AQ 1.8219508
RG 2048
DW 27.800
DE 6.00
TE 298.8
D1 2.00000000
d11 0.03000000
DELTA 1.89999998
TD0 1
-------- CHANNEL f1 -----
NUC1 13C
P1 10.00
PL1 -1.00
SFO1 75.4752953
-------- CHANNEL f2 -----
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00
PL2 0.00
PL12 16.20
PL13 16.20
SFO2 300.1312005
F2 - Processing parameters
SI 32768
SF 75.4677490
WDW EM

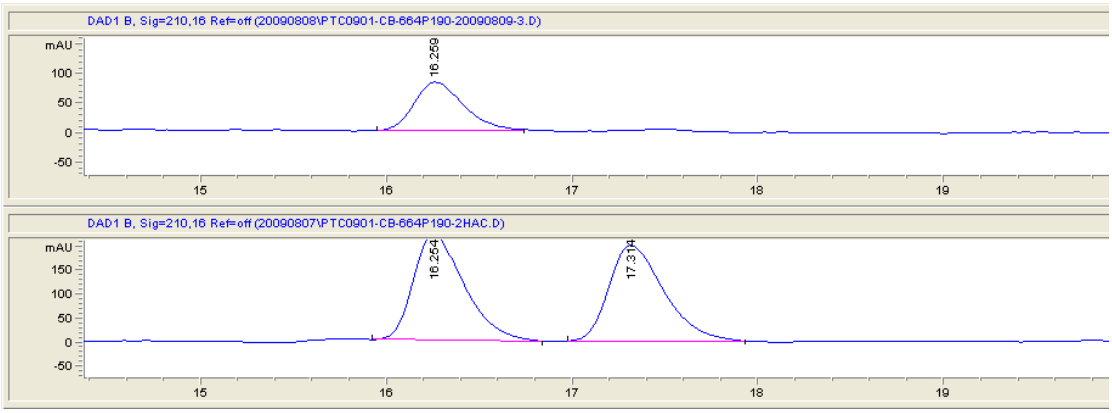

128.58
128.44
126.04
69.70
61.76
36.01
31.05
14.21
Current Data Parameters
NAME PTC0901-CB-664P18
EXPNO 6
PROCNO 1
F2 - Acquisition Parameters
Date_ 20090719
Time 10.24
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG dept135
TD 65536
SOLVENT CDCl3
NS 116
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 se
RG 16384
DW 27.800 us
DE 6.00 us
TE 298.6 K
CNST2 145.0000000
D1 2.00000000 se
d2 0.00344828 se
d12 0.00002000 se
DELTA 0.00001273 se
TD0 1
======== CHANNEL f1 ======
NUC1 13C
P1 10.00 us
p2 20.00 us
PL1 -1.00 dB
SFO1 75.4752953 MH
======== CHANNEL f2 ======
CPDPRG2 waltz16
NUC2 1H
P3 13.00 us
p4 26.00 us
PCPD2 80.00 us
PL2 0.00 dB
PL12 16.20 dB
SFO2 300.1312005 MH
F2 - Processing parameters
SI 32768
SF 75.4677490 MH
WDW EM

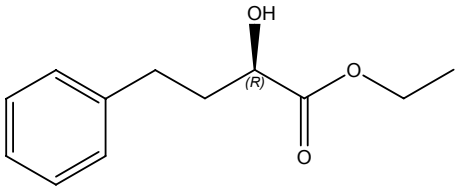

ppm
0
20
40
60
80
100
120
140
160
180
200

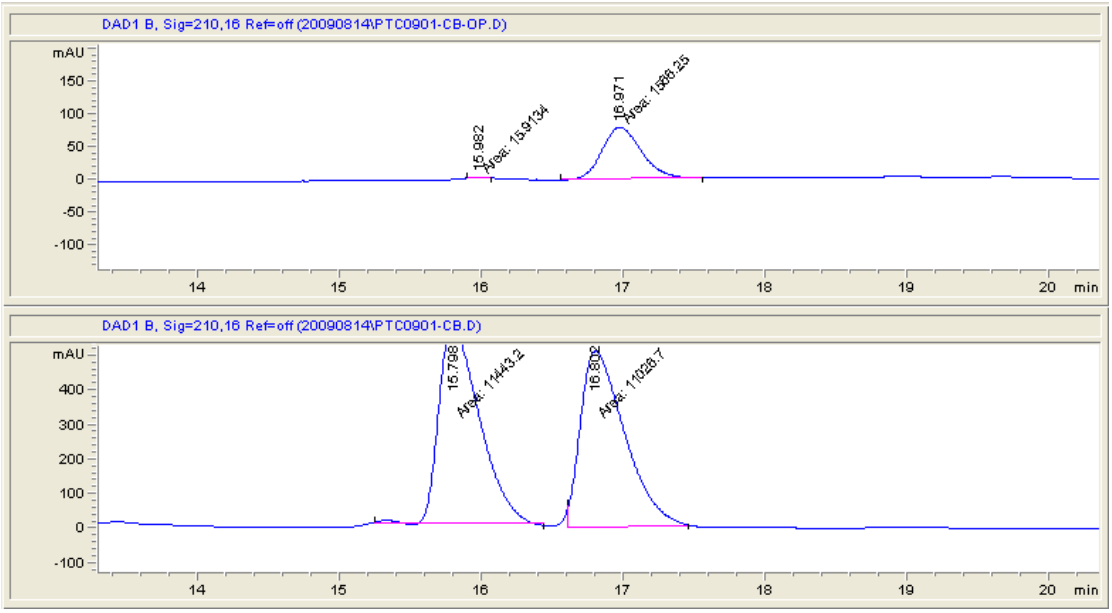

ppm
0
20
40
60
80
100
120
140

2.2 HPLC Spectra for Relative Compounds









#	Time	Area	Height	Width	Area%	Symmetry
1	15.798	11712.3	580.7	0.3362	50.362	0.52
2	16.802	11543.9	513.3	0.3404	49.638	0.49

#	Time	Area	Height	Width	Area%	Symmetry
1	15.982	15.9	1.9	0.1415	1.006	0.89
2	16.971	1566.2	79.4	0.3286	98.994	0.778