Supplementary materials

Antibacterial Activity of Peptide Derivatives of Phosphinothricin against Multidrug-Resistant *Klebsiella pneumoniae*

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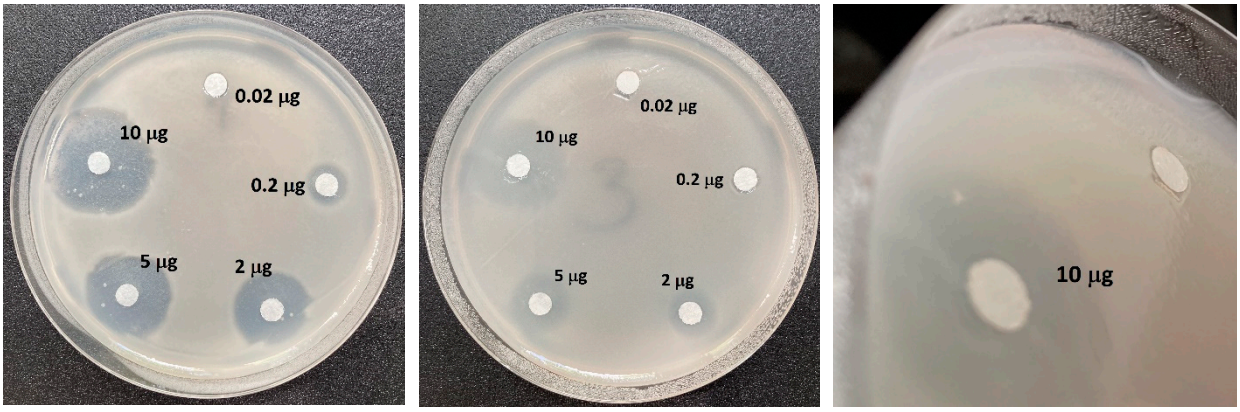
Keywords: multidrug-resistant bacteria; *Klebsiella pneumoniae*; *Escherichia coli*; Glutamine synthetase; phosphinothricin; Bialaphos



(a) **(b)** **(c)**

Figure S1. Inhibition of the growth of reference strain *Klebsiella pneumoniae* ATCC 13883 with Bialaphos and the reverse of the effect with Gln. **(a)** – dose-dependent effect of Bialaphos, μg per disk; **(b) –** same as (a), but with the agar containing 0.5 mM Gln; **(c)** – zoomed (b) taken with angle.

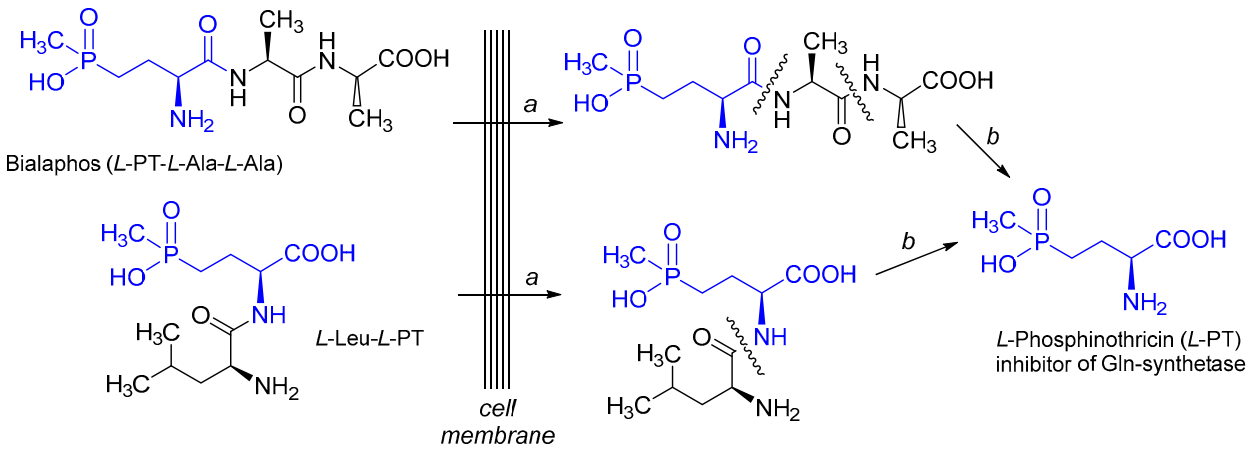


Figure S2. Proposed mechanism of antibacterial activity of Bialaphos and *L*-Leu-*L*-PT**. a**– peptidyl permeases; **b**– peptidases.

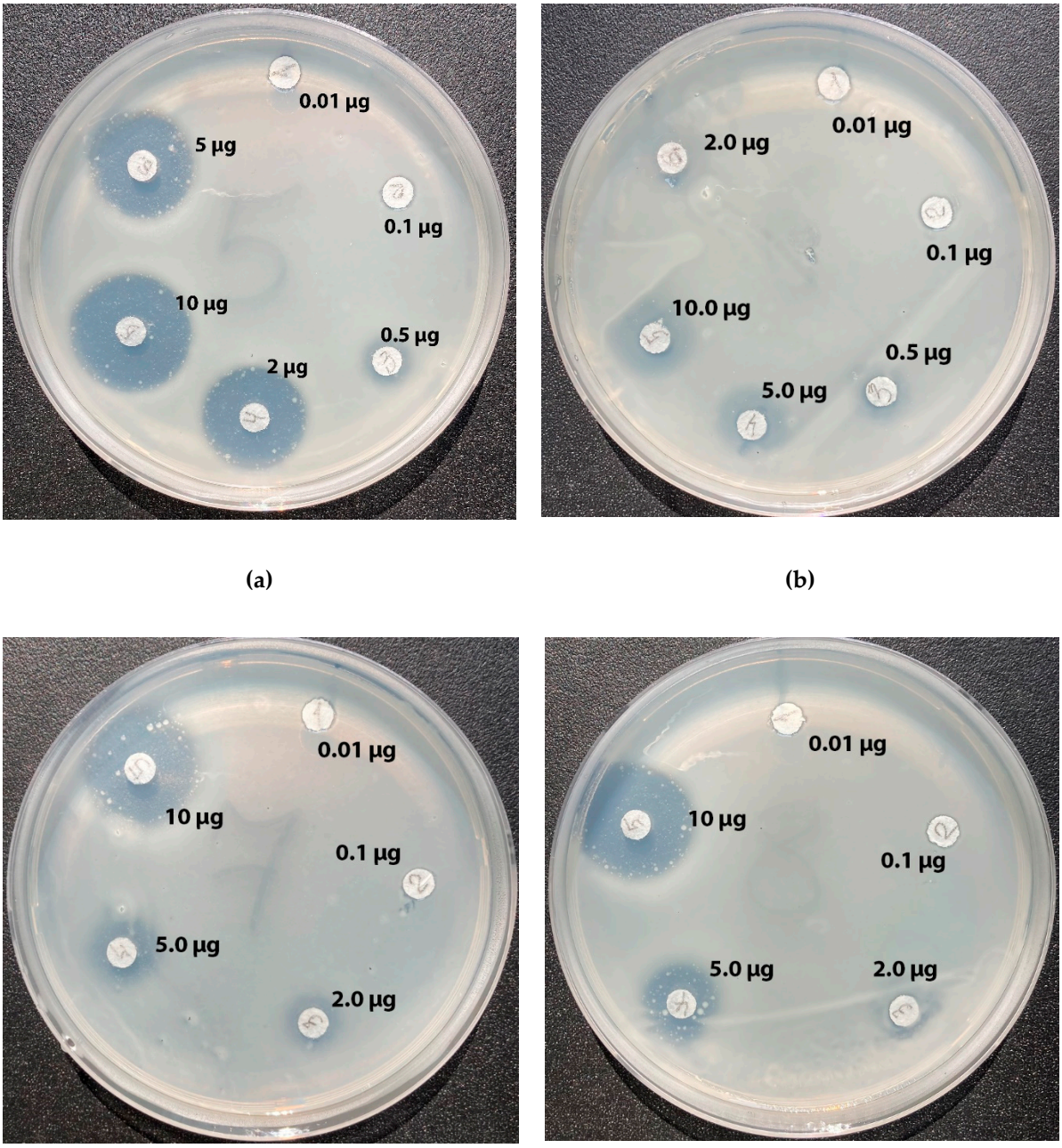
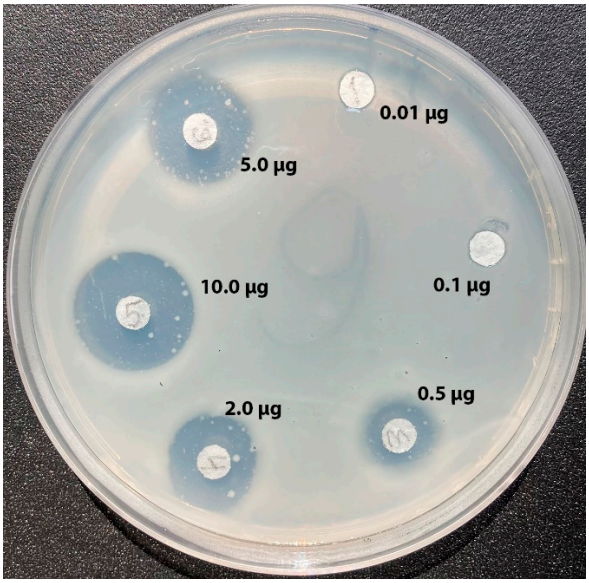
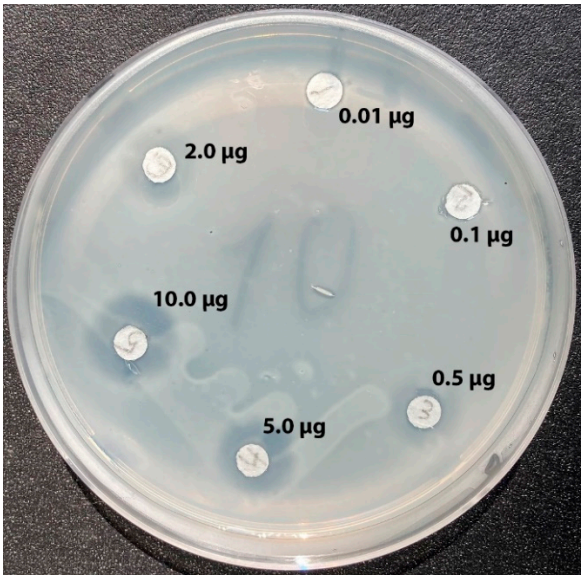


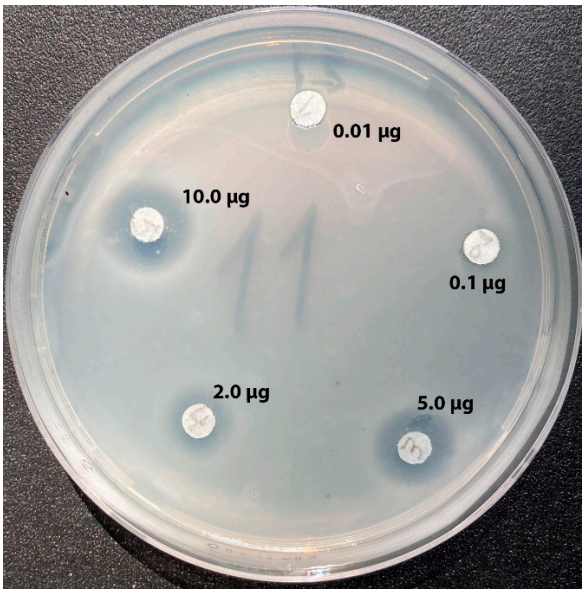
Figure S3. Glutamine reverses Bialaphos-induced inhibition of the growth of reference strain of *Klebsiella pneumoniae* ATCC 13883 doze-dependently. Figures at dishes show the amount of bialaphos applied on the disk. **(A) –** no Gln in agar; **(B) –** agar containing 0.5 mM Gln; **(C)** – agar containing 0.1 mM Gln; **(D)** – agar containing 0.02 mM Gln.



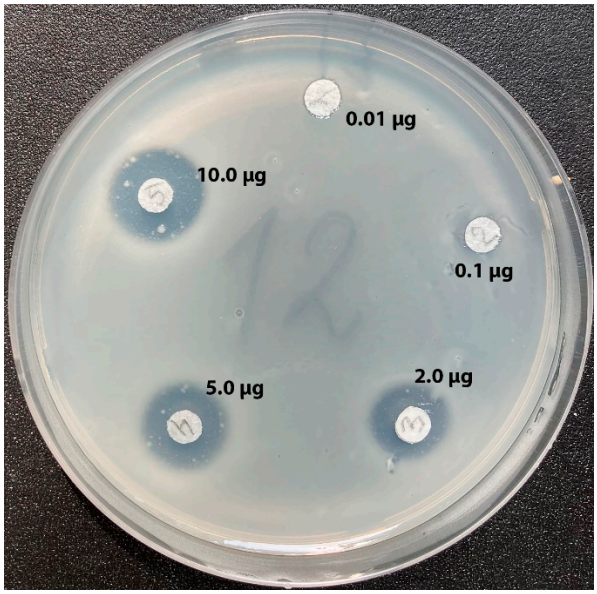
(a)



(b)



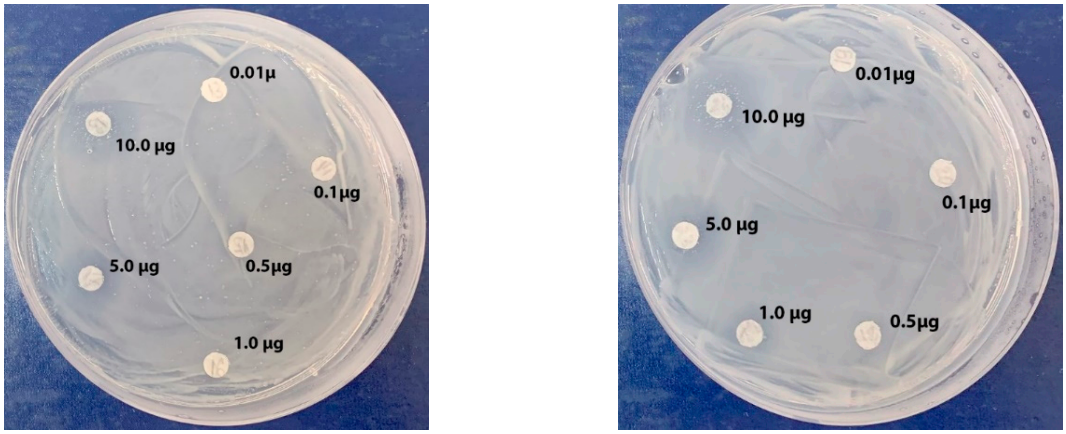
(c)



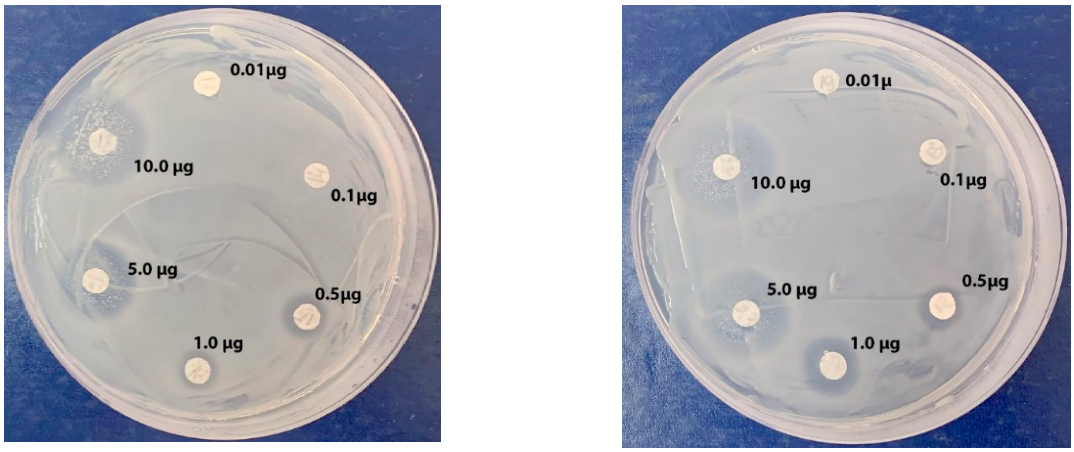
(d)

Figure S4. Glutamine reverses *L*-Leu-*L*-PT-induced inhibition of the growth of reference strain of *Klebsiella pneumoniae* ATCC 13883 doze-dependently. Figures at dishes show the amount of *L*-Leu-*L*-PT applied on the disk. **(A) –** no Gln in agar; **(B) –** agar containing 0.5 mM Gln; **(C)** – agar containing 0.1 mM Gln; **(D)** – agar containing 0.02 mM Gln.

MDR clinical isolate of *Klebsiella pneumoniae* (strain 1161)



MDR clinical isolate of *Klebsiella pneumoniae* (strain 1158)



MDR clinical isolate of *Klebsiella pneumoniae* (strain 1133)

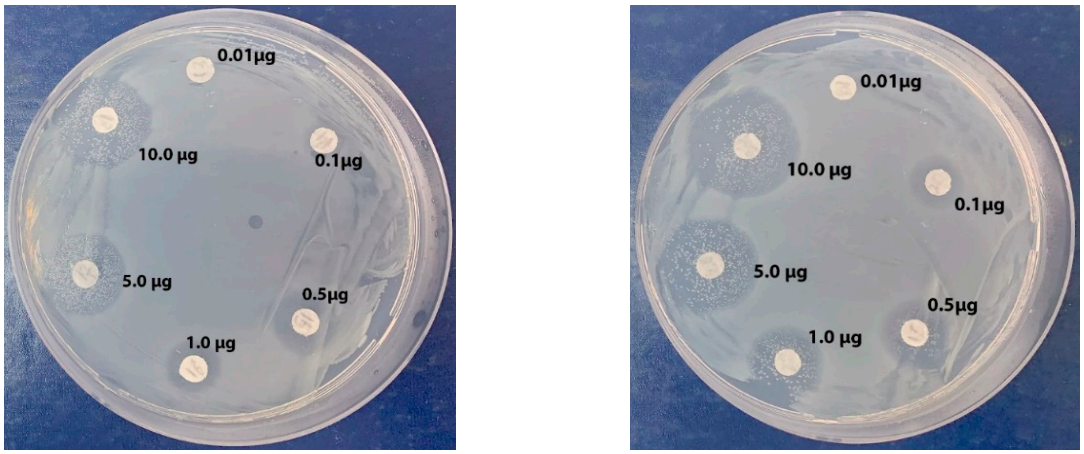


Figure S5. Doze-dependent inhibition of the growth of multi-drug resistant clinical isolates of *Klebsiella pneumoniae* with Bialaphos (left, µg of tripeptide per disk) and *L*-Leu-*L*-PT (right, µg of dipeptide per disk).

Table S1. Inhibition of the growth of clinical isolates of multi-drug resistant Klebsiella pneumoniae (marked light ocher) and reference strain ATCC 13883 (marked Mauve) with Bialaphos, *L*-Leu-*L*-PT and antibiotics of different classes.

	Antibiotics and PT derivatives	**Strain 1161**	**Strain 1158**	**Strain 1133**	**ATCC 13883**
		Diameter of growth zones, mm*)			
1.	Amikacin, 30 µg/disk	0/0	0/0	11/9	23/23
2.	Ampicillin, 10 µg/disk	0/0	0/0	0/0	0/0
3.	Gentamicin, 10 µg/disk	0/0	0/0	19/16	23/24
4.	Ciprofloxacin, 5 µg/disk	0/0	0/0	0/0	33/35
5.	Cefazolin, 30 µg/disk	0/0	0/0	0/0	22/24
6.	Tetracycline, 30 µg/disk	22/22	0/0	0/0	19/20
13.	Bialaphos, 5 µg/disk	16/15	16/16	17/16	18/22
14.	*L*-Leu-*L*-PT, 5 µg/disk	18/17	18/17	18/20	18/18
7.	Cefotaxime, 30 µg/disk	0/0	0/0	0/0	40/42
8.	Levofloxacin, 5 µg/disk	0/0	0/0	0/0	32/34
9.	Polymyxin B, 300 IU/disk	12/10	13/14	14/14	16/18
10.	Tobramycin, 10 µg/disk	0/0	0/0	0/0	21/20
11.	Trimethoprim, 5 µg/disk	0/0	0/0	0/0	18/21
12.	Ampicillin/Sulbactam, 10/10 µg/disk	0/0	0/0	0/0	13/15
13.	Bialaphos, 5 µg/disk	15/14	17/19	17/18	21/23
14.	*L*-Leu-*L*-PT, 5 µg/disk	17/17	16/19	21/21	19/22

*) Dish 1/dish 2 (one of two dishes is depicted at Figure 3, for ATCC 13882, and at Figure 4 for multi-drug resistant strains 1161, 1158 and 1133).
*) Light ocher section of the Table is illustrated by Figure 4; mauve section – by Figure 3.

Table S2. Multi-drug resistant clinical isolates of *Klebsiella pneumoniae*. R – resistant; **S** – sensitive; Antibiotics verified in this study are in the left column and are in blue.

Antibiotic	***K.pneumoniae* strain**			**Antibiotic**	***K.pneumoniae* strain**		
	1161	**1158**	**1133**		**1161**	**1158**	**1133**
Amikacin	R	R	R	Amoxicillin/clavulanic acid	R	R	R
Ampicillin/Sulbactam	R	R	R	Aztreonam	R	R	R
Ampicillin	R	R	R	Cefepime	R	R	R
Cefazolin	R	R	R	Cefoxitin	R	R	R
Cefotaxime	R	R	R	Ceftazidime	R	R	R
Ciprofloxacin	R	R	R	Ceftriaxone	R	R	R
Gentamicin	R	R	**S**	Cefuroxime	R	R	R
Levofloxacin	R	R	R	Ertapenem	R	R	R
Tetracycline	**S**	R	R	Imipenem	R	R	R
Tobramycin	R	R	R	Meropenem	R	R	R
Trimethoprim/sulfamethoxazole	R	R	R	Piperacillin	R	R	R
Polymyxin B	**S**	**S**	**S**	Piperacillin/tazobactam	R	R	R
				Tigecycline	S	**S**	R

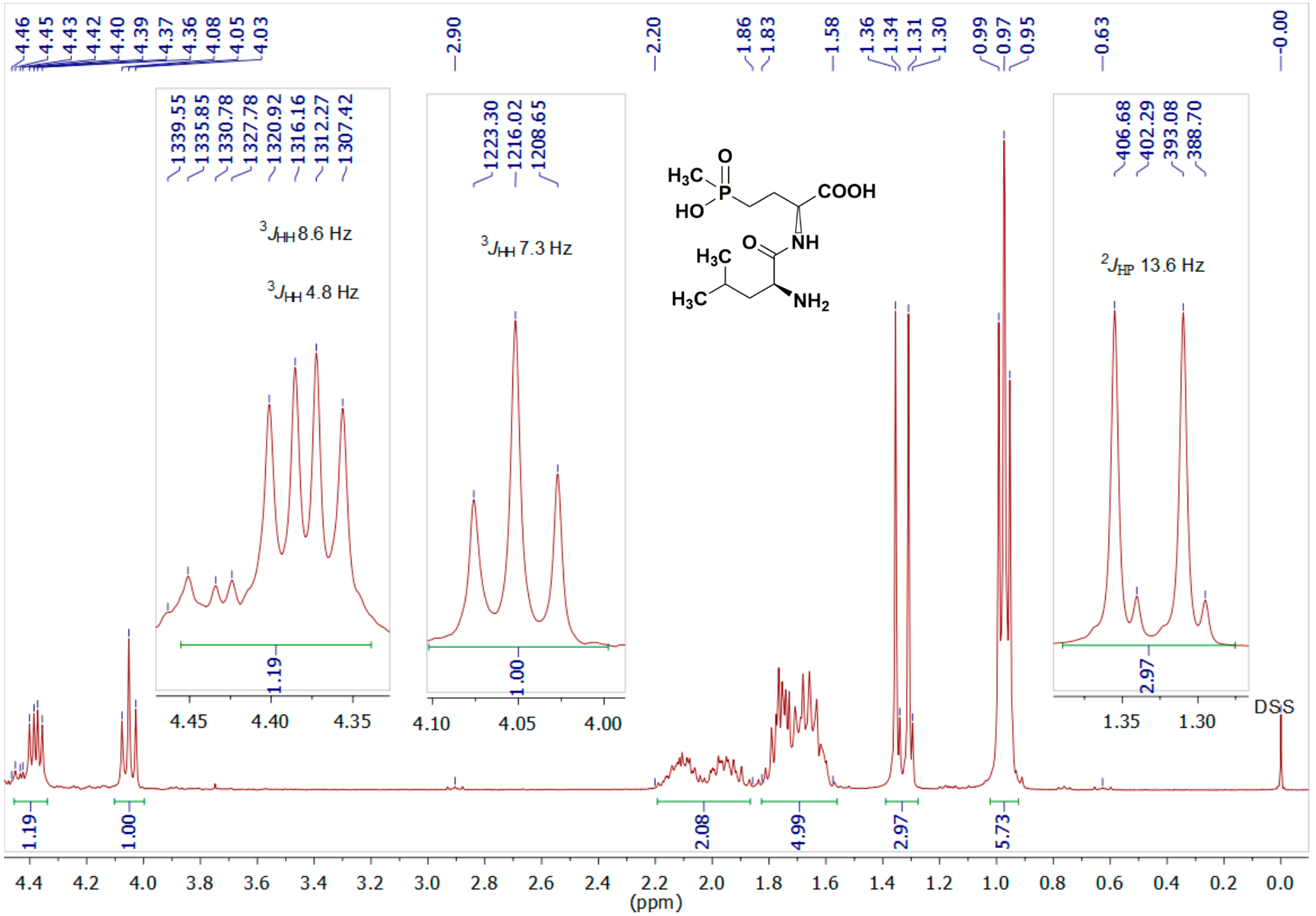


Figure S6. ^{1}H-NMR spectrum of *L*-Leu-*D*-PT with about 10% of *L,L*-diastereomer (fraction n. 7).

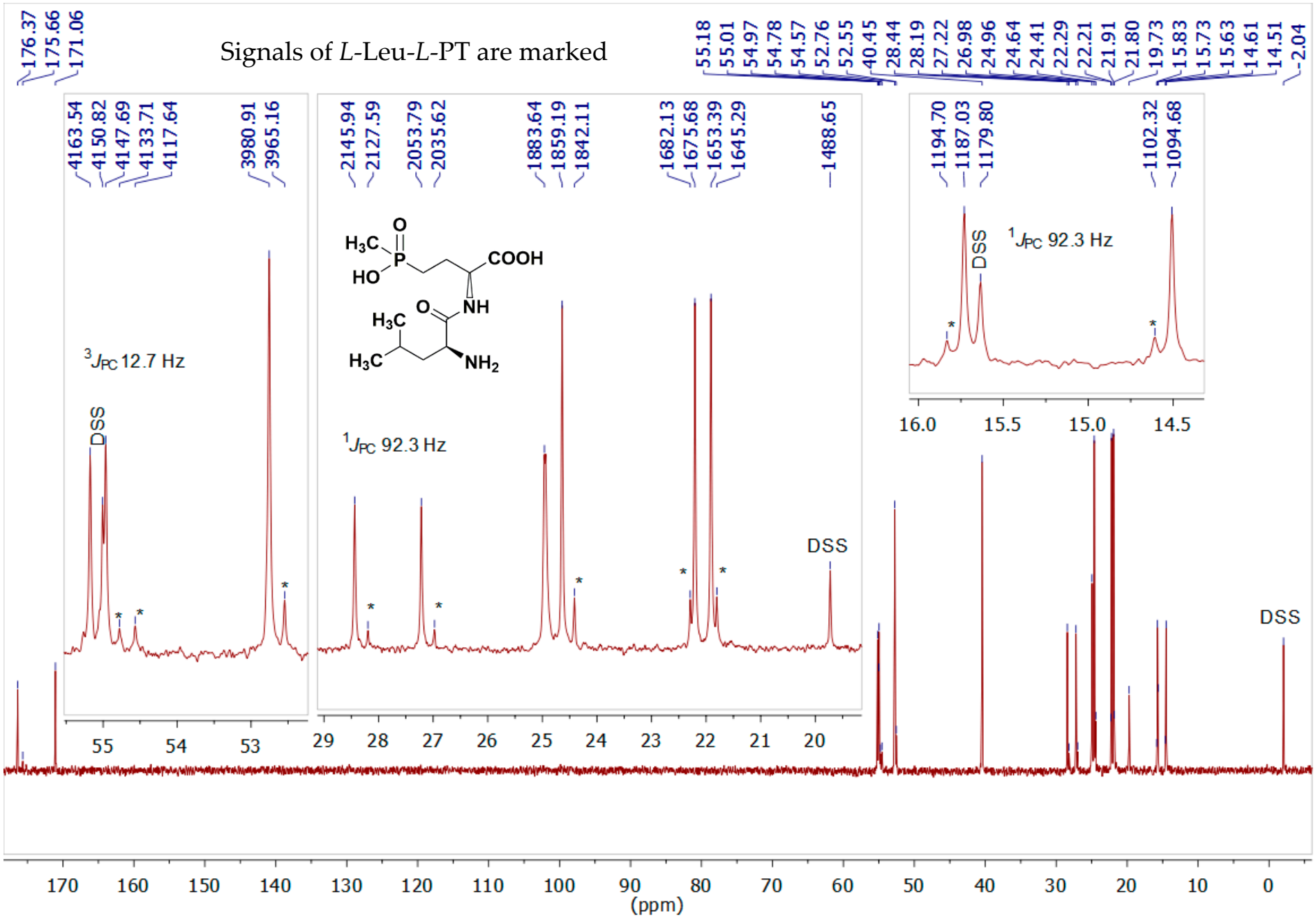


Figure S7. ^{13}C-NMR spectrum of *L*-Leu-*D*-PT with about 10% of *L,L*-diastereomer (fraction n. 7).

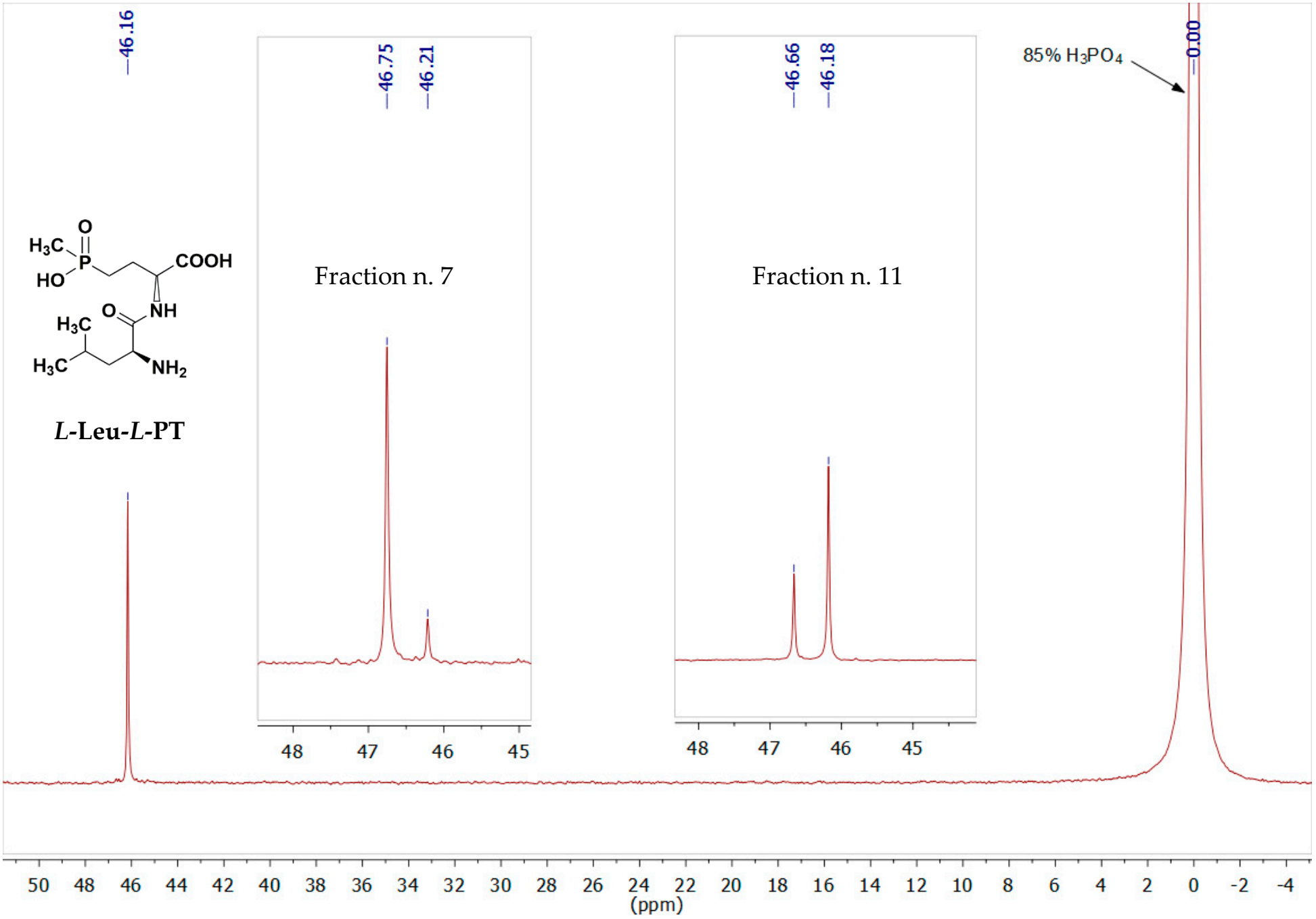


Figure S8. Combined ^{31}P-NMR spectra of *L*-Leu-*L*-PT, *L*-Leu-*D*-PT with about 10% of *L,L*-diastereomer (fraction n. 7) and *L*-Leu-*rac*-PT (fraction 11).

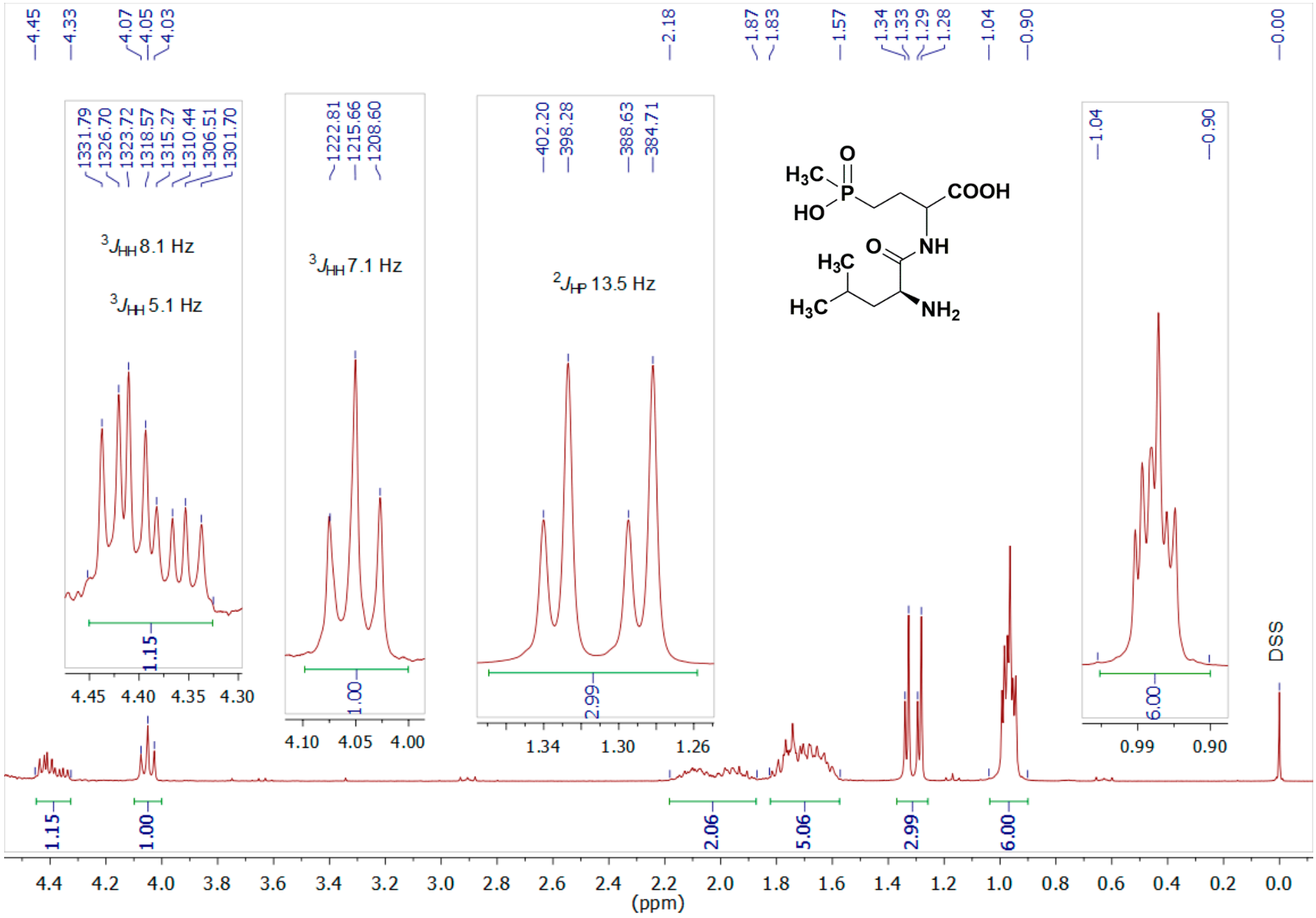


Figure S9. [1]H-NMR spectrum of *L*-Leu-*rac*-PT (fraction n. 11).

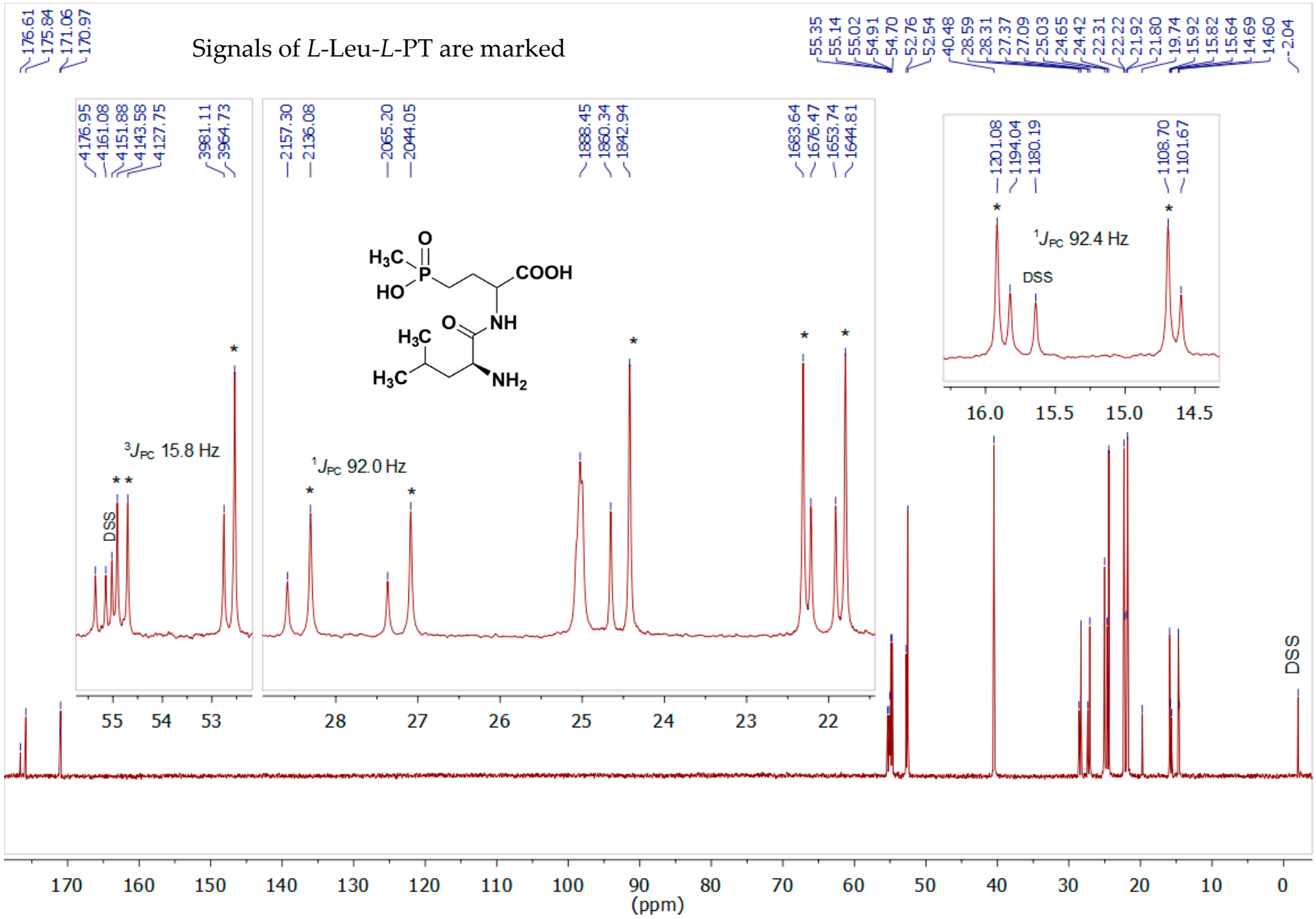


Figure S10. ^{13}C-NMR spectrum of *L*-Leu-*rac*-PT (fraction n. 11).

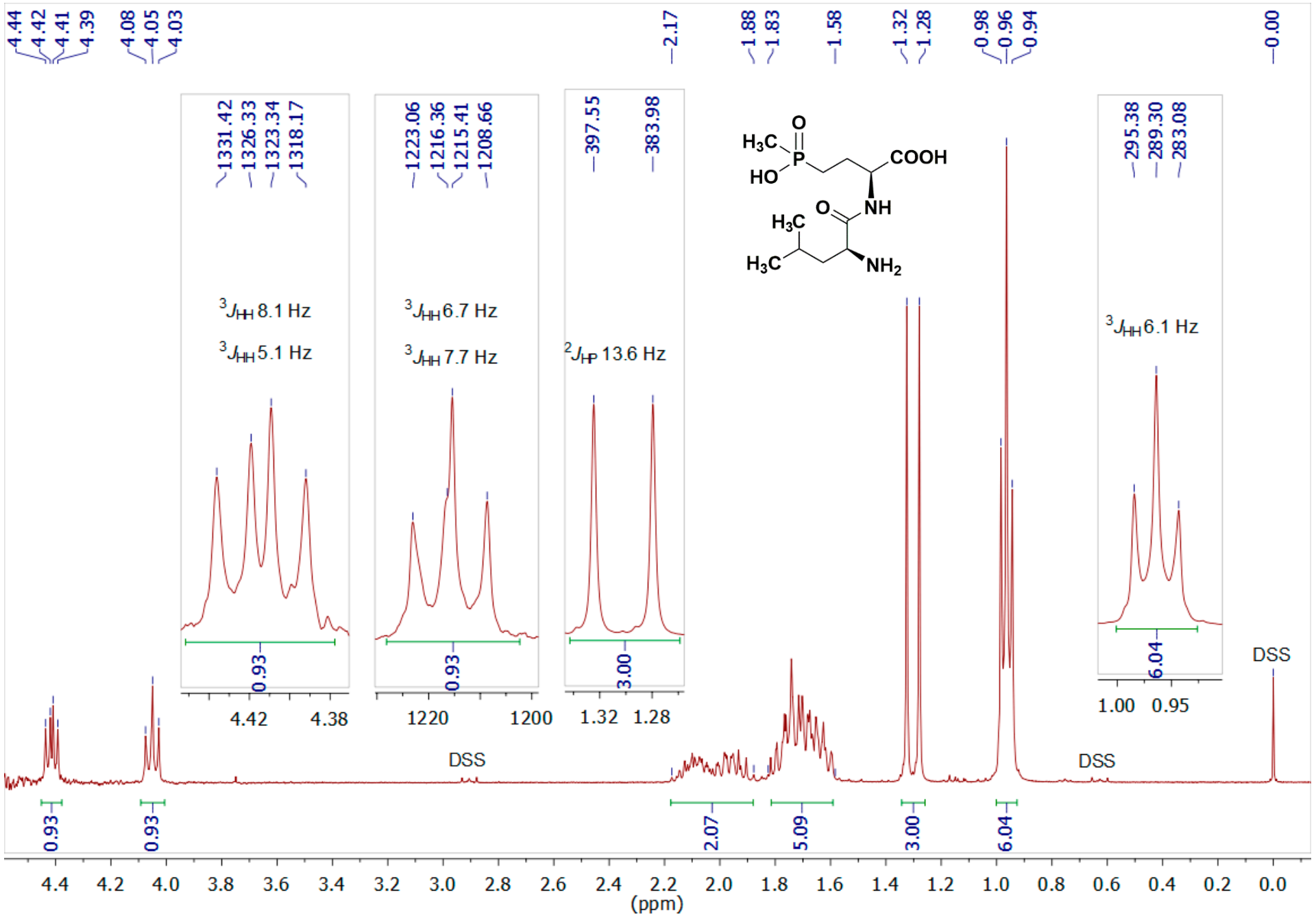


Figure S11. ^{1}H-NMR spectrum of *L*-Leu-*L*-PT.

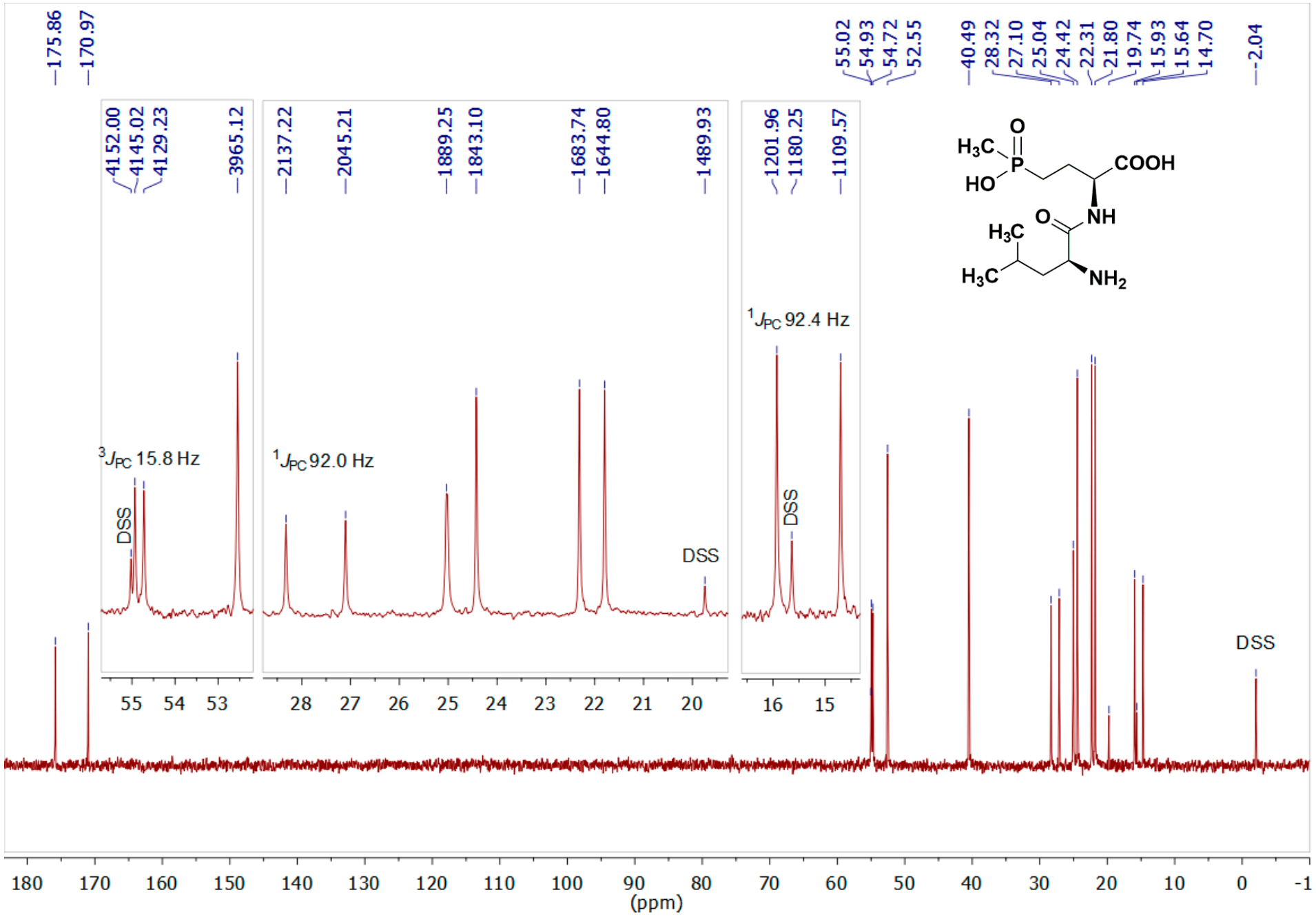


Figure S12. ^{13}C-NMR spectrum of *L*-Leu-*L*-PT.