Electronic Supplementary Material (ESI) for RSC Advances

β-Lactam-host defence peptide conjugates as antibiotic prodrug candidates targeting resistant bacteria

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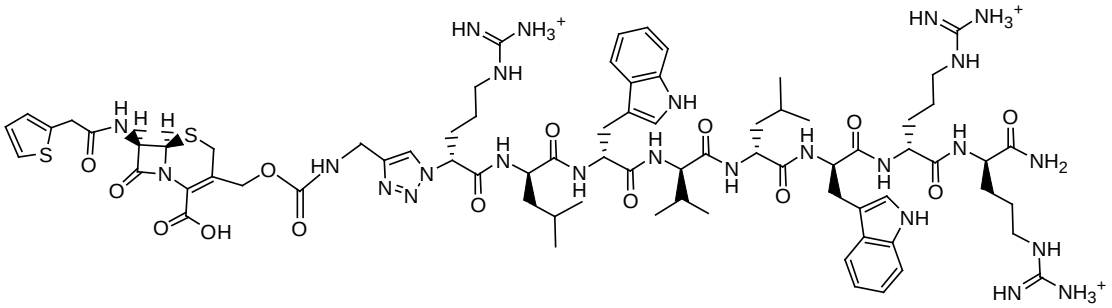
Supporting Information.

1. **cephalothin-D-Bac8c(Leu^{2,5}) (8)**



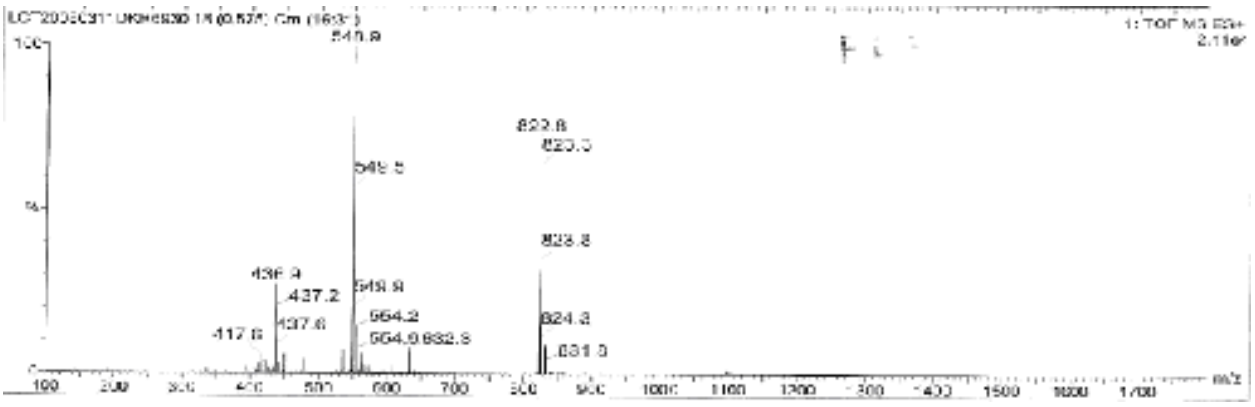


Figure S1. EI-MS recorded at 35eV.
The signal at m/z = 822.8 corresponds to a doubly charged ion, *i.e.* $[M + 2H]^{2+}$; the signal at m/z = 548.9 corresponds to a triply charged ion, *i.e.* $[M + 4H]^{4+}$; the signal at m/z = 436.8 is unidentified.

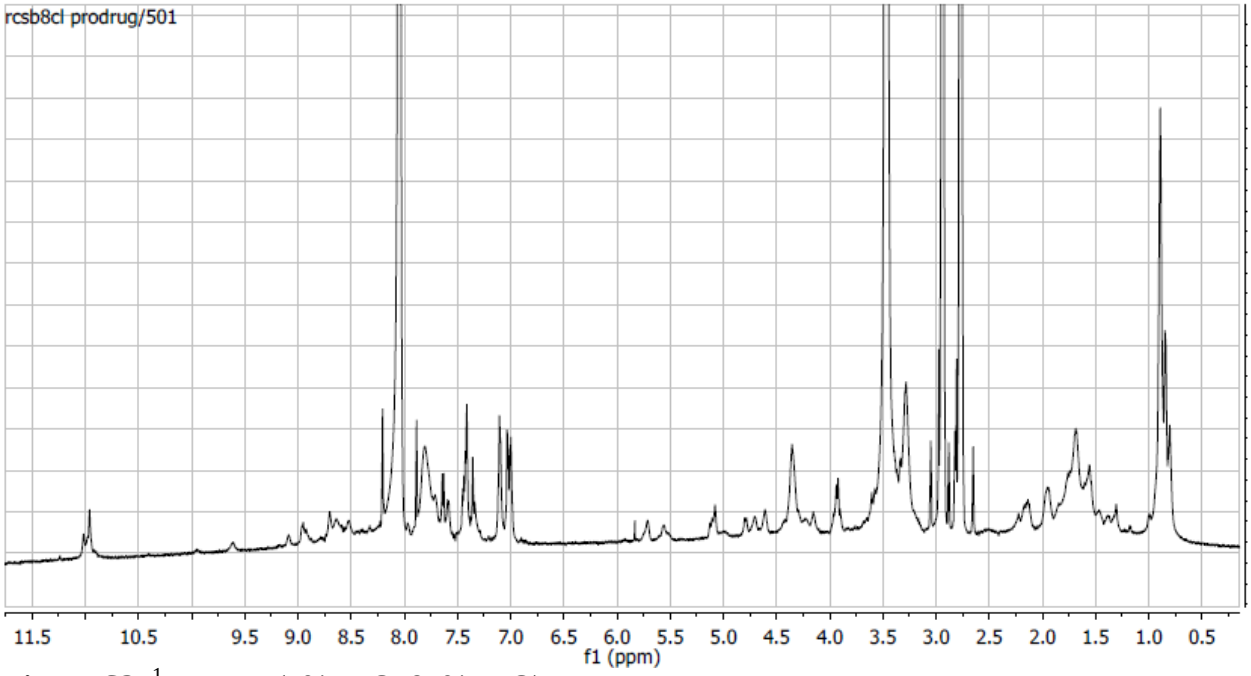


Figure S2. ^{1}H NMR (5% D_2O, 95% H_2O).

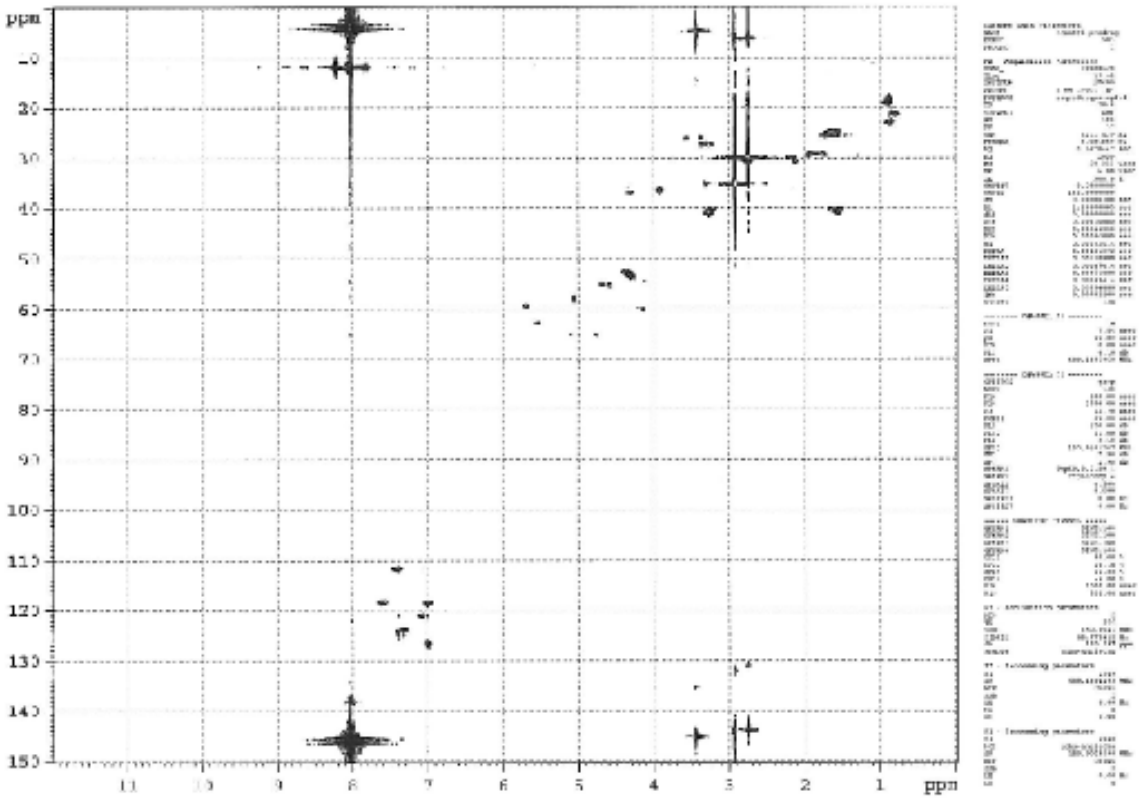
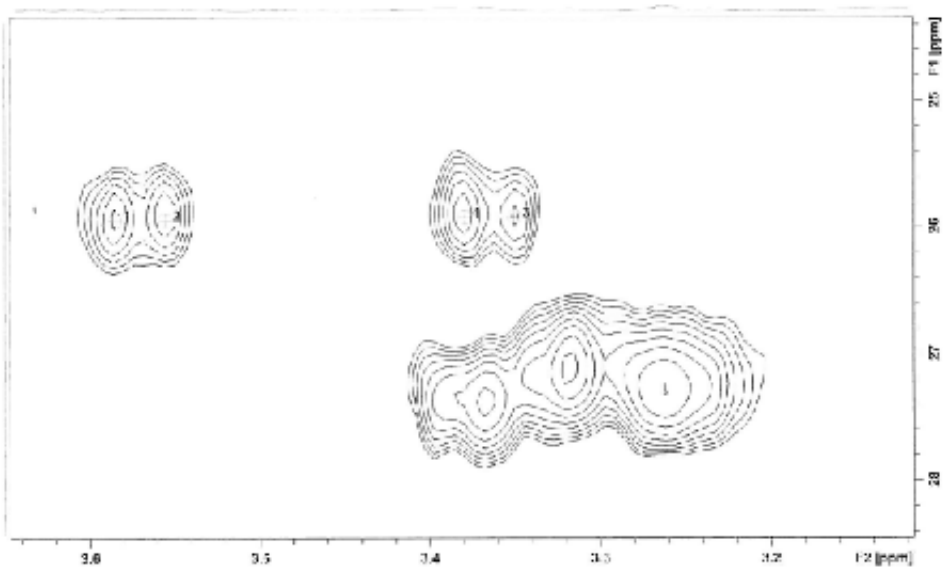


Figure S3a. C-H correlation NMR.



Aug 3, 2009 (1:02:44 PM) "rcsb8c1 prodrug" 503 1 C:\Bruker... Page 1/1

Peak	v(F2) [ppm]	v(F1) [ppm]	Intensity	Annotation
1	3.5849	25.9408	-1083465.97	
2	3.5557	25.9408	-1033846.97	
4	3.3801	25.9187	-1192568.98	
3	3.3507	25.9193	-907340.97	

Figure S3b. Enlargement of the C-H correlation spectrum and H^2 constant coupling table.

2. Activation assays

Chemical and enzymatic hydrolyses of cephalothin-D-Bac8c(Leu^{2,5}) **(8)**: these assays were performed at pH 7.25 in a 10 mM PBS buffer, by monitoring with a UV-spectrophotometer the disappearance of the cephalothin's β-lactam bond at 260 nm. The chemical hydrolysis assay was carried out in a 0.41 M NaOH solution using a 0.21 mM solution of cephalothin (Fig. S4) or a 0.140 mM solution of **(8)** (Fig. S5). β-Lactamase-mediated reactivation assays were performed with a purified P99 enzyme from *Enterobacter cloacae* (0.8 mg, 0.32 μM) using a 9.9 mM solution of cephalothin (Fig. S6) or a 13 mM solution of **(8)** (Fig. S7).

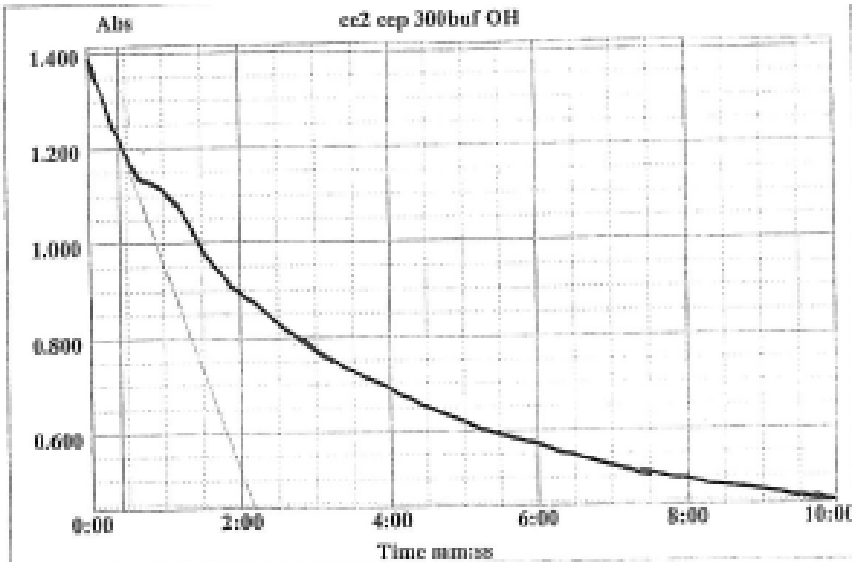


Figure S4. UV spectra for the alkaline hydrolysis of cephalothin (the linear graph is the kinetic derivative).

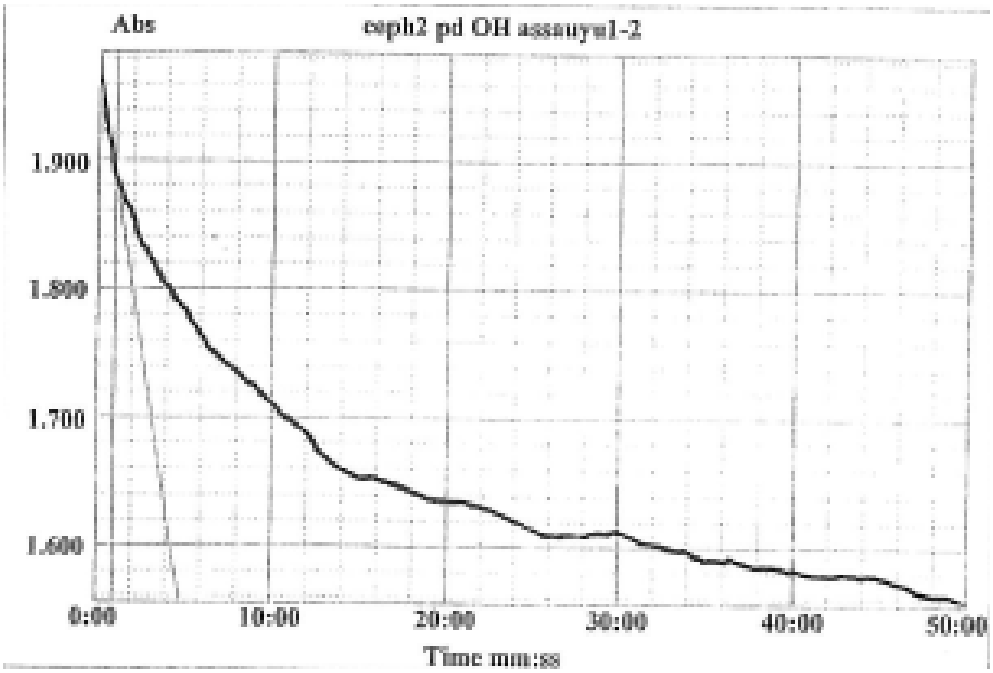


Figure S5. UV spectra for the alkaline hydrolysis of **(8)**.

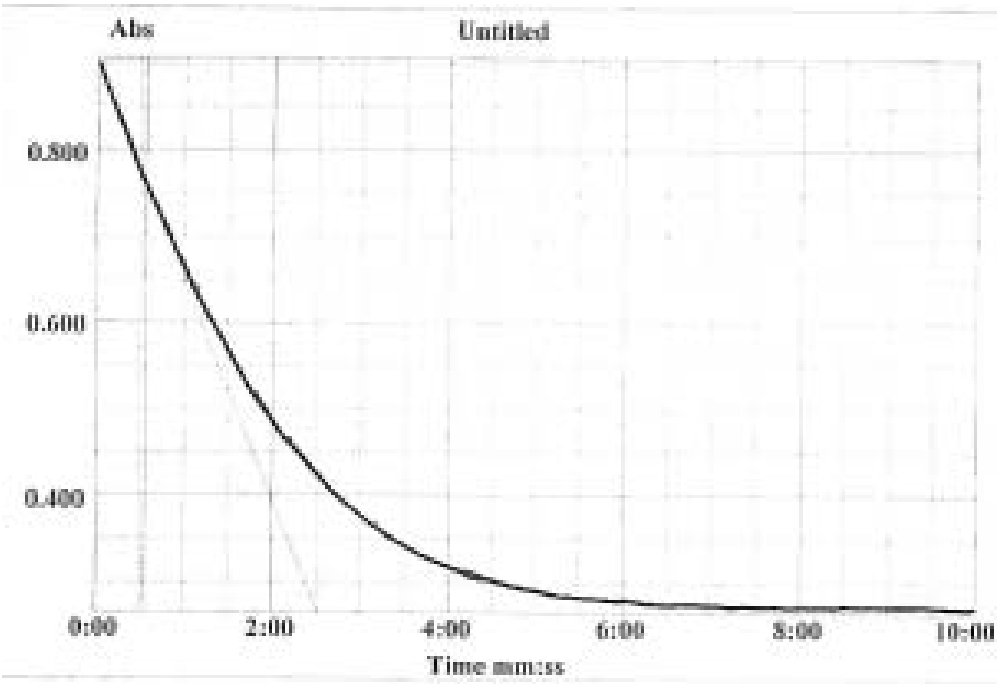


Figure S6. UV spectra for the enzymatic hydrolysis of cephalothin.

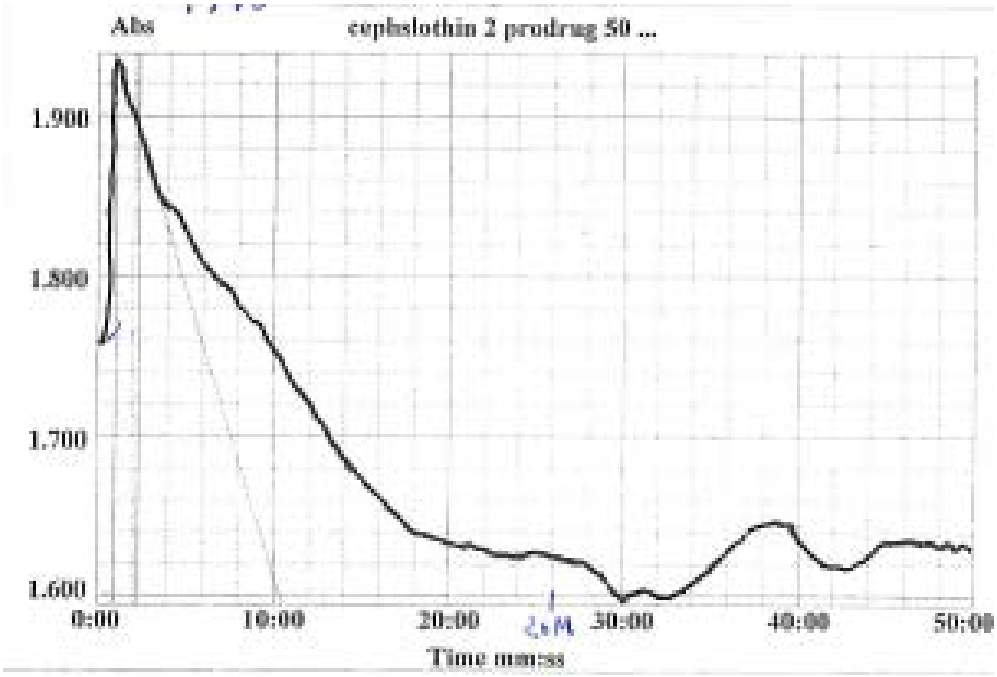


Figure S7. UV spectra for the enzymatic hydrolysis of **(8)**.

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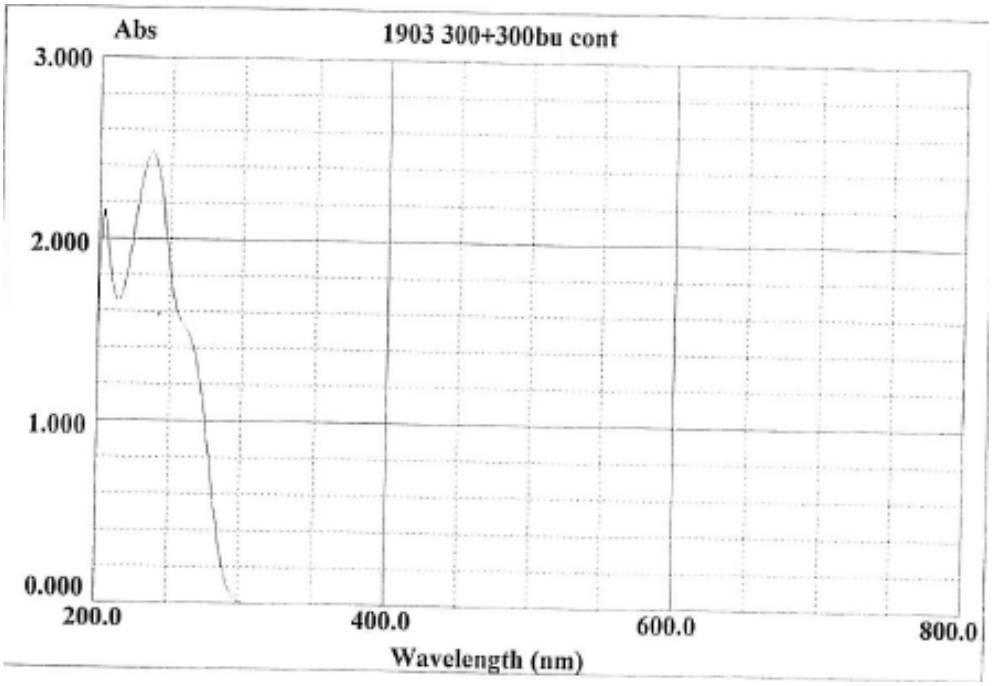


Figure S8. UV wave scans of cephalothin (0.1 mM), before hydrolysis.

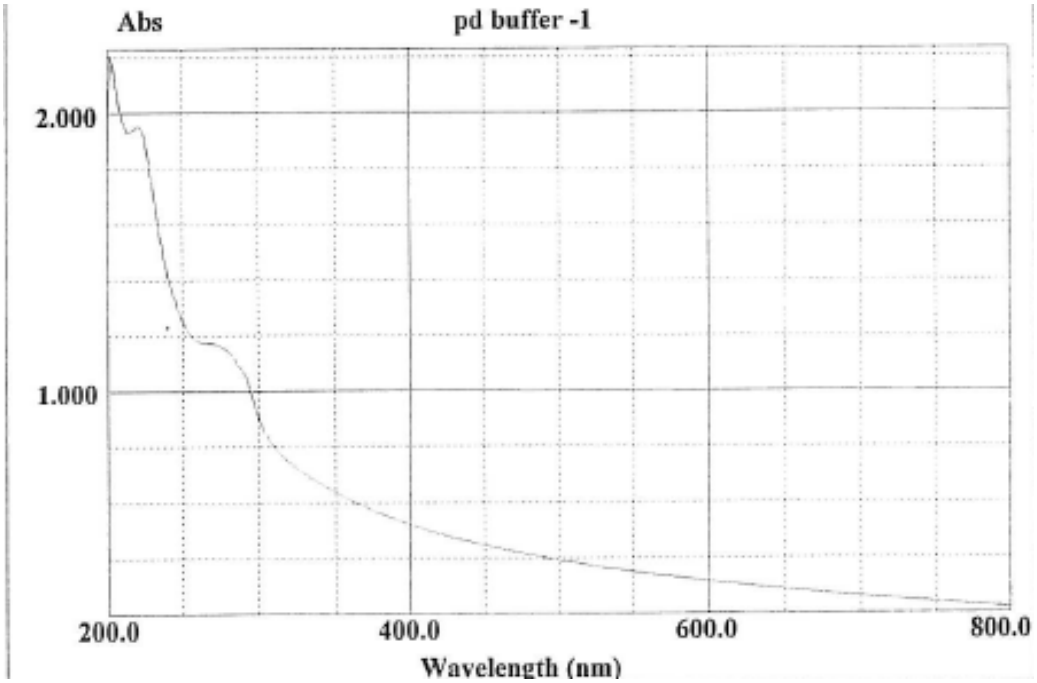


Figure S9. UV wave scans of **(8)** (0.070 mM), before hydrolysis.

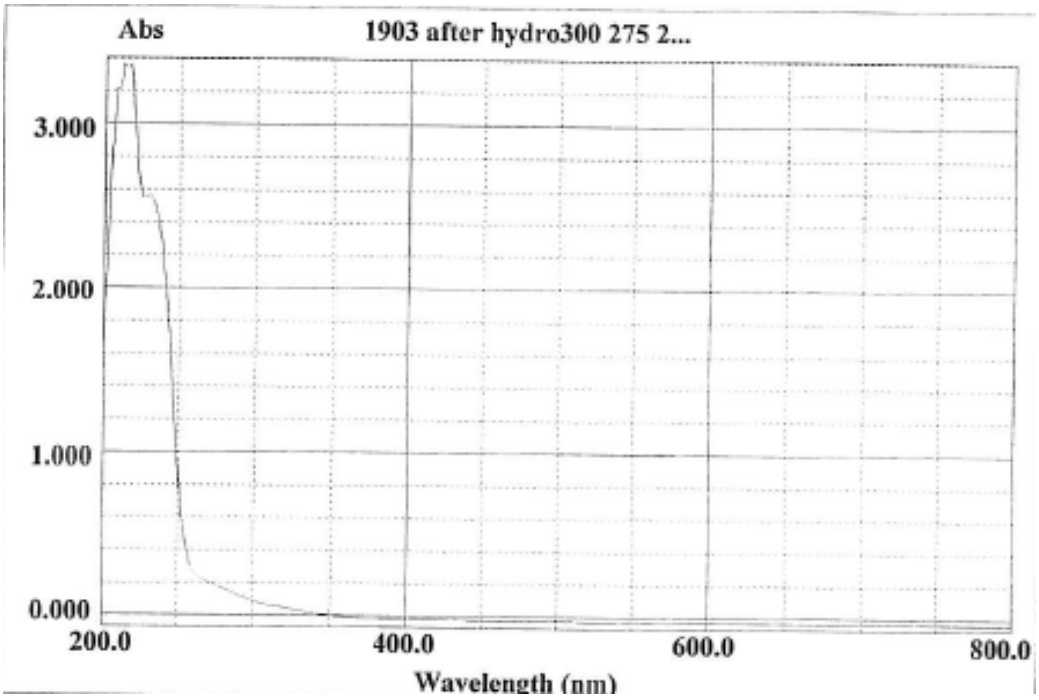


Figure S10. UV wave scans of cephalothin (0.1 mM) after chemical hydrolysis.

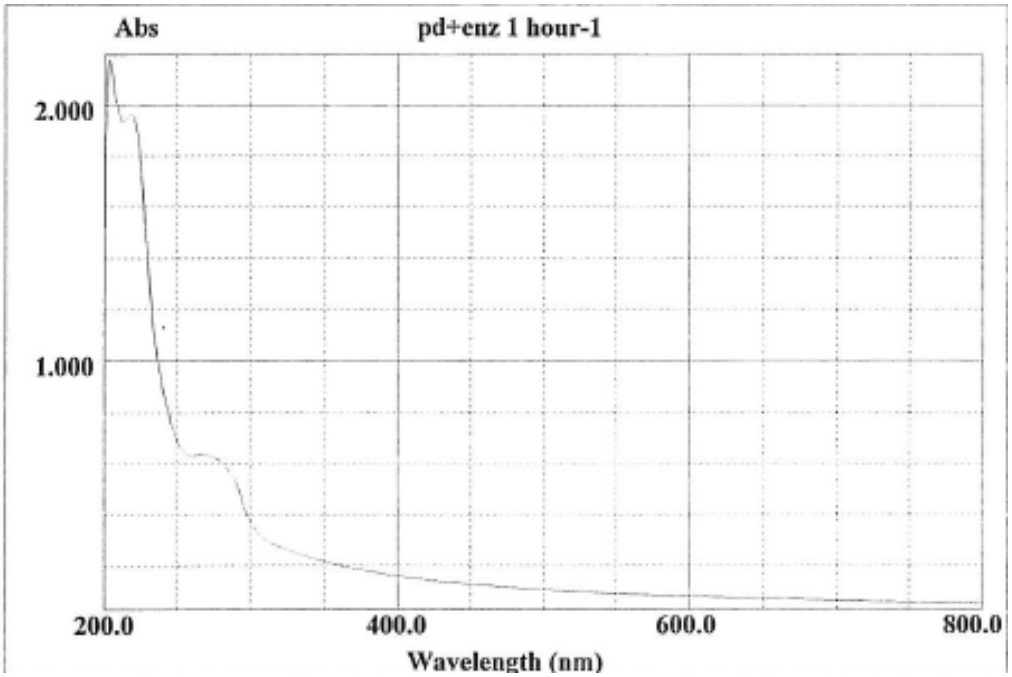


Figure S11. UV wave scans of **(8)** (0.07 mM) after chemical hydrolysis.

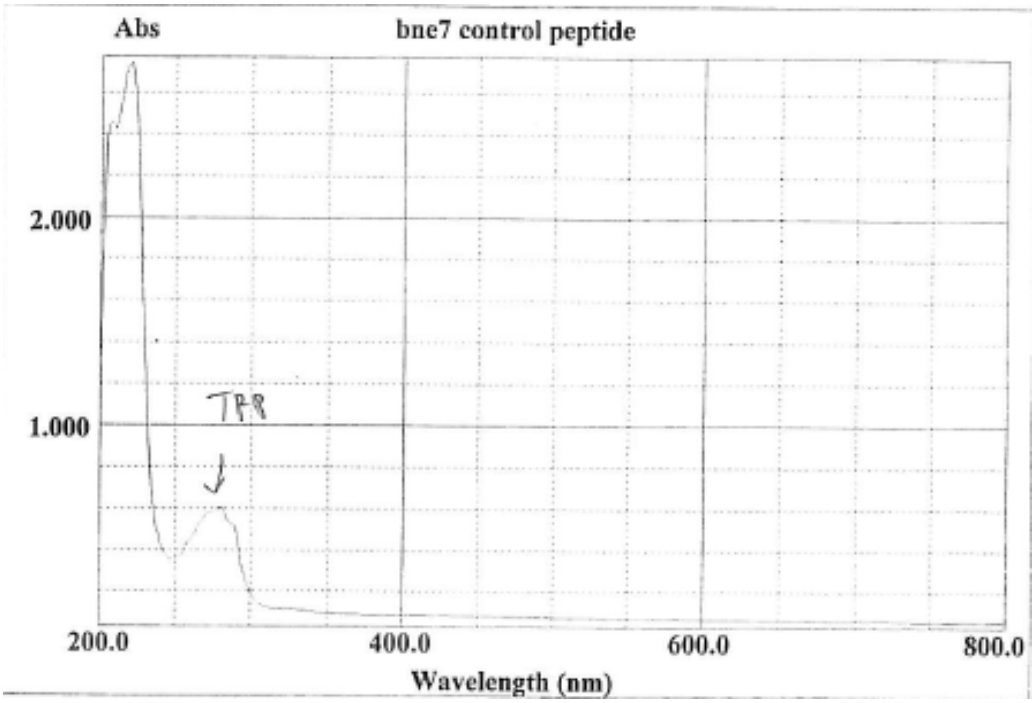
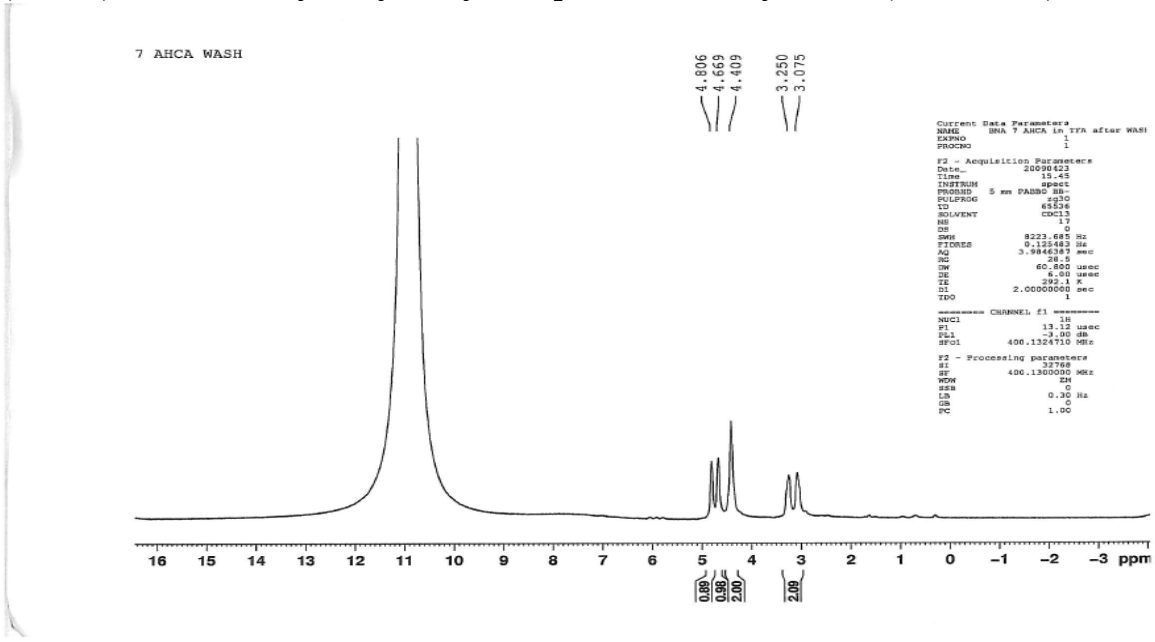


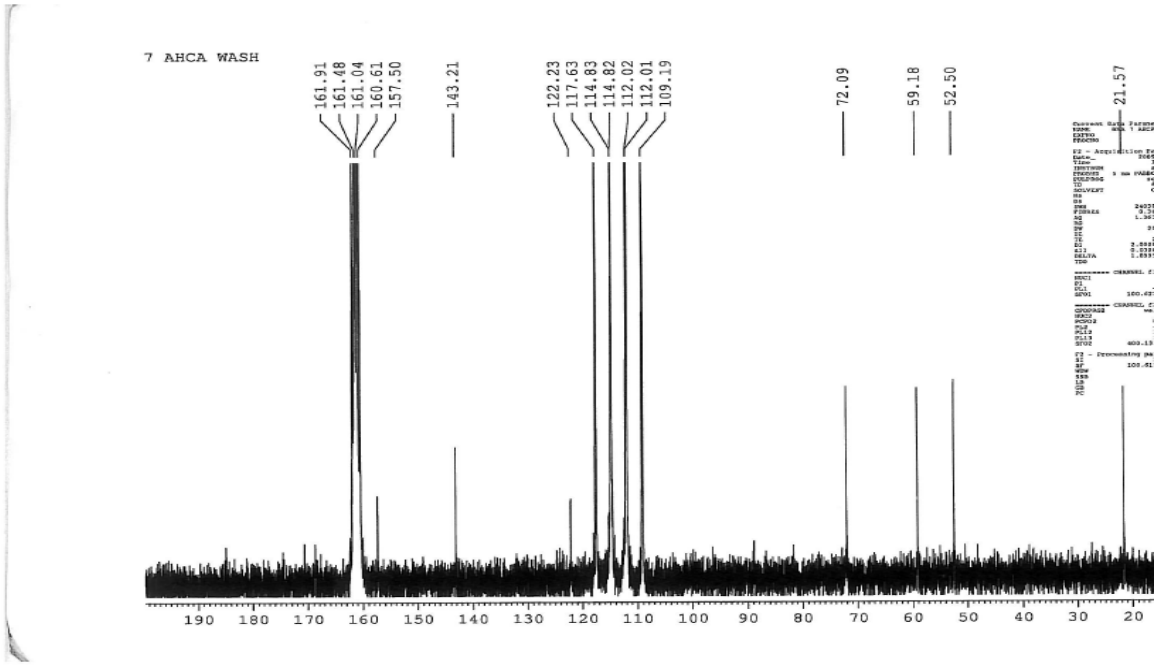
Figure S12. UV wave scans of the triazole peptide **(9)** (0.075 mM).

3. Spectra and chromatograms

(6*R*, 7*R*)-7-Amino-3-hydroxymethyl-3-cephem-4-carboxylic acid (7-AHCA, 3)

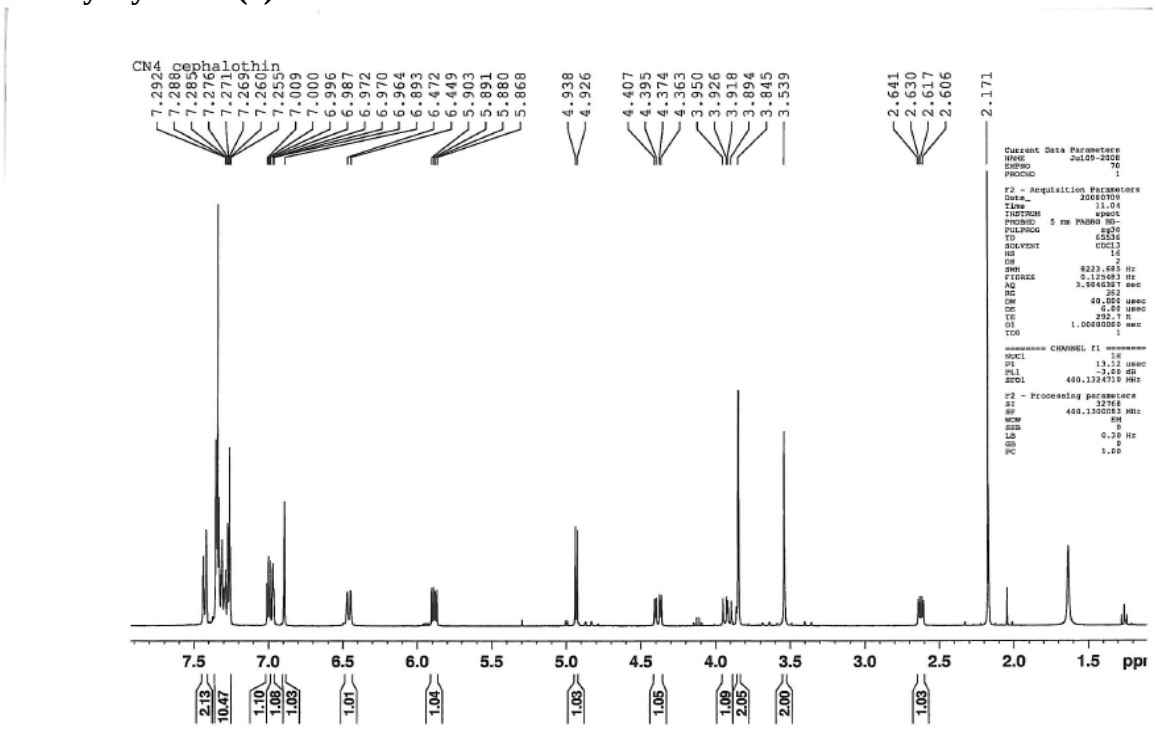


^{1}H NMR (400 MHz, TFA)

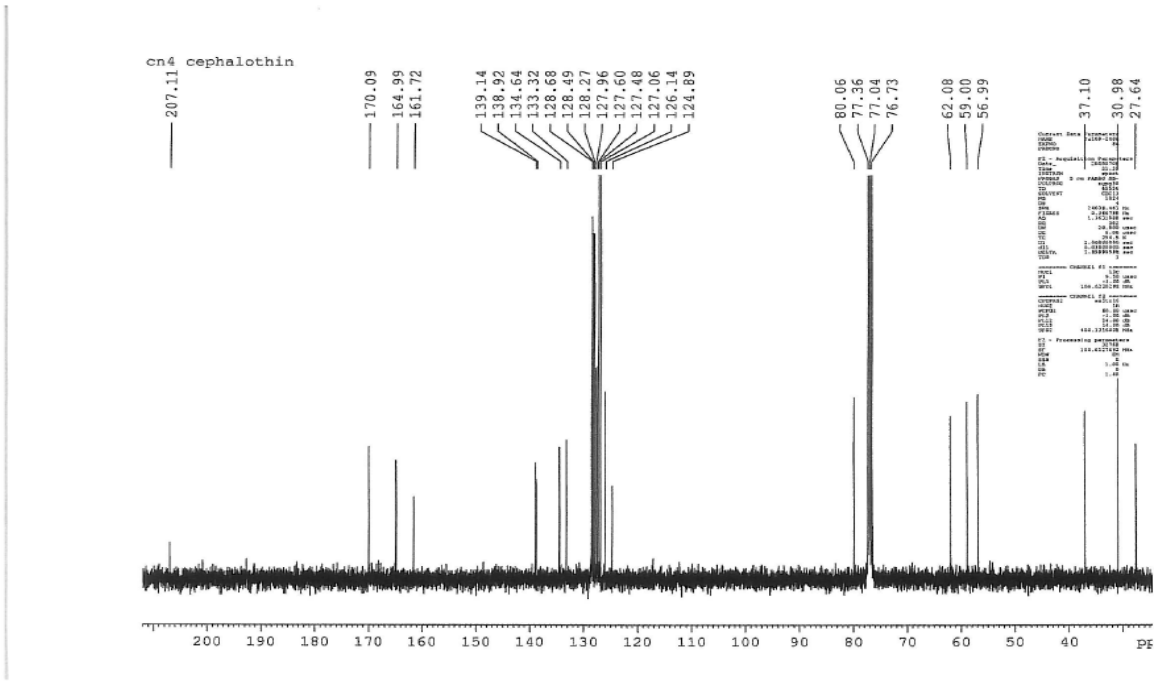


^{13}C NMR (100.6 MHz, TFA)

(6*R*, 7*R*)-3-Hydroxymethyl-7-(2-thiophen-2-yl-acetylamino) 3-cephem-4-carboxylic acid benzhydryl ester (4)

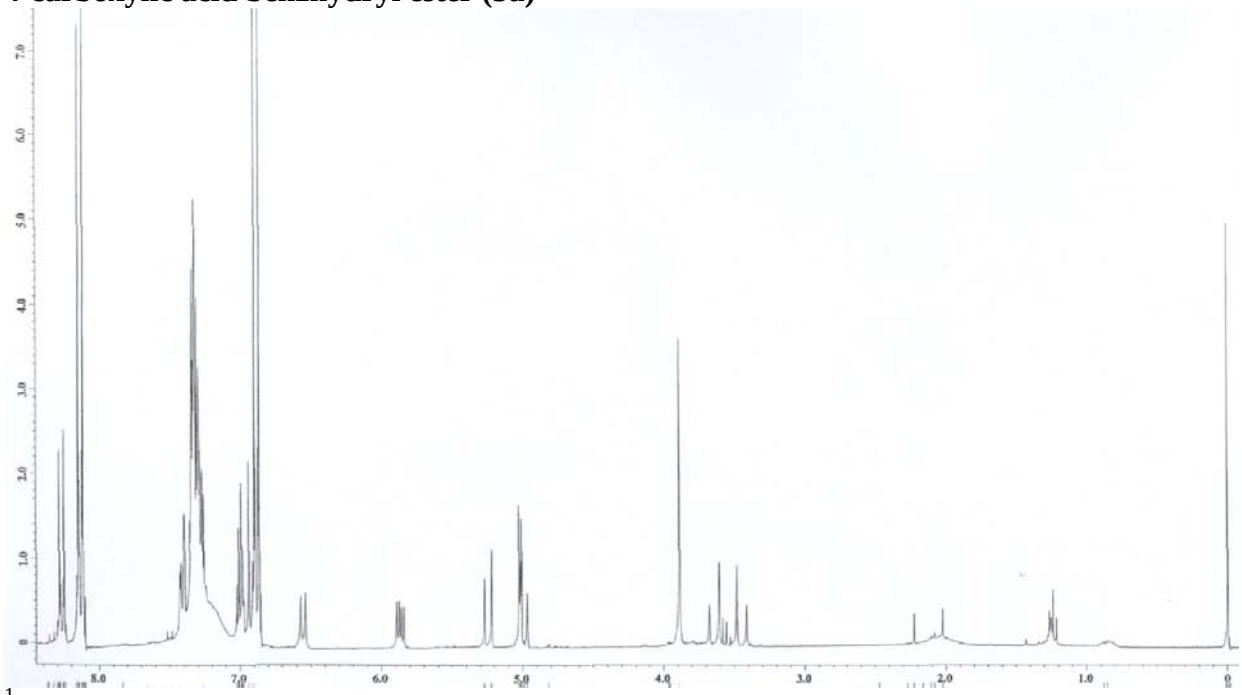


^{1}H NMR (400 MHz, $CDCl_3$)



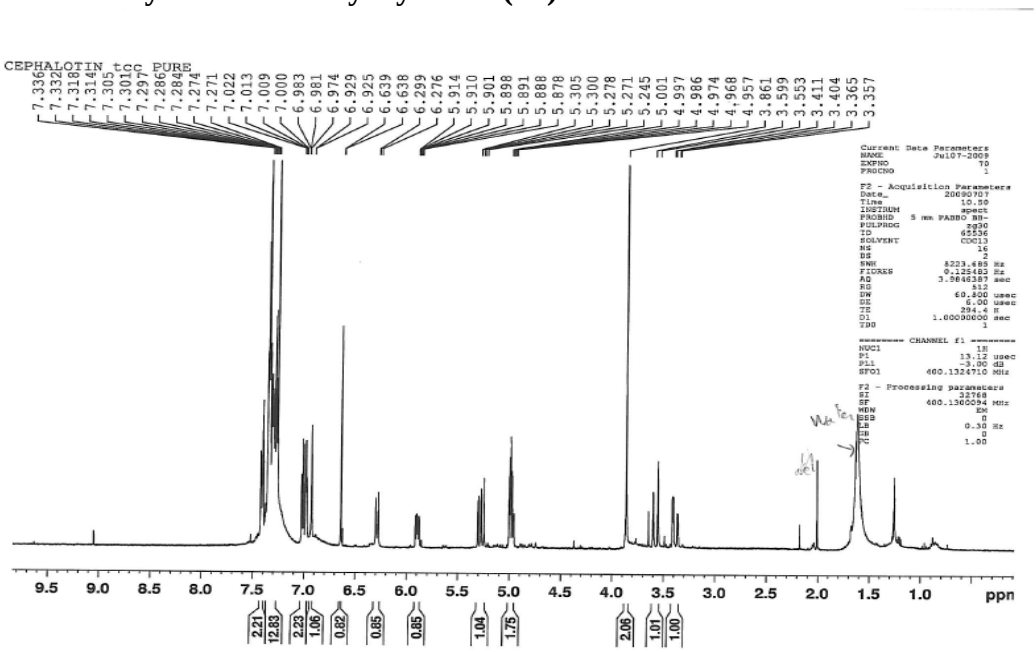
^{13}C NMR (100.6 MHz, $CDCl_3$)

(6*R*, 7*R*)-3-(*p*-nitrophenoxycarbonyloxymethyl)-7-(2-thiophen-2-yl-acetylamino) 3-cephem-4-carboxylic acid benzhydryl ester (5a)

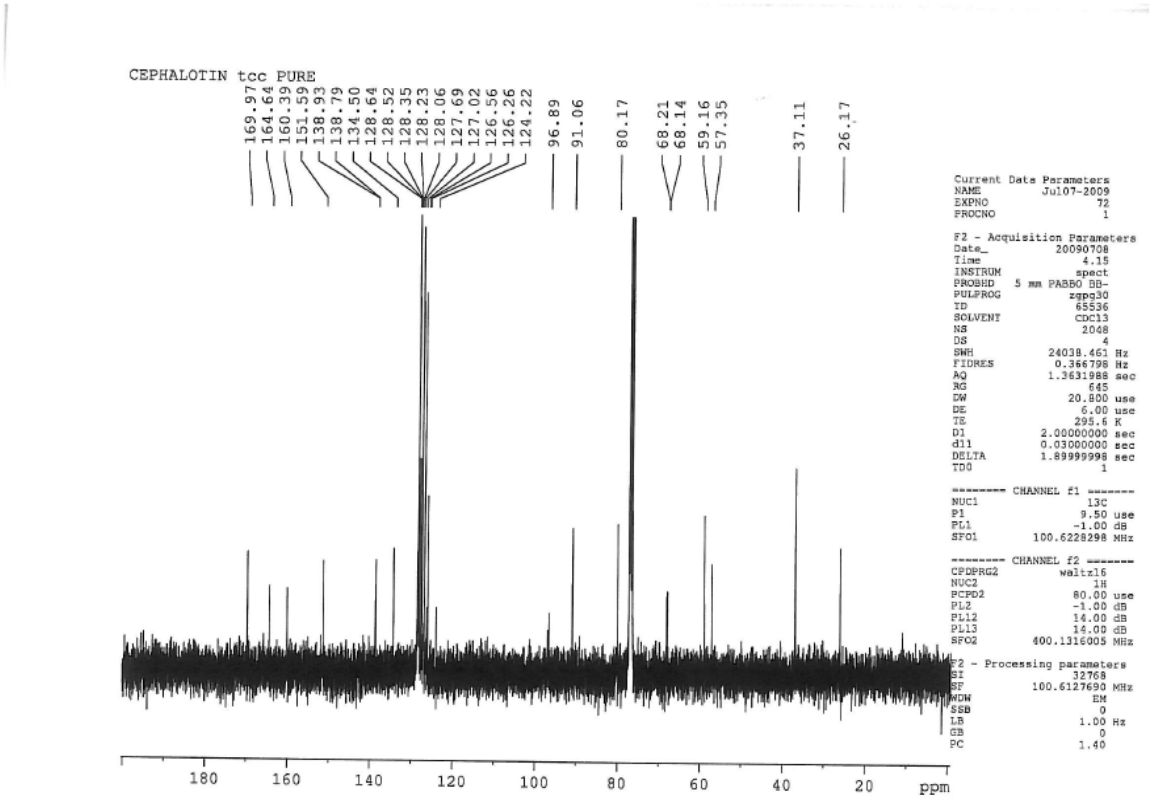


^{1}H NMR (4270 MHz, $CDCl_3$)

(6*R*, 7*R*)-3-(1,2,2,2-Tetrachloro-ethoxycarbonyloxymethyl)-7-(2-thiophen-2-yl-acetylamino) 3-cephem-4-carboxylic acid benzhydryl ester (5b)

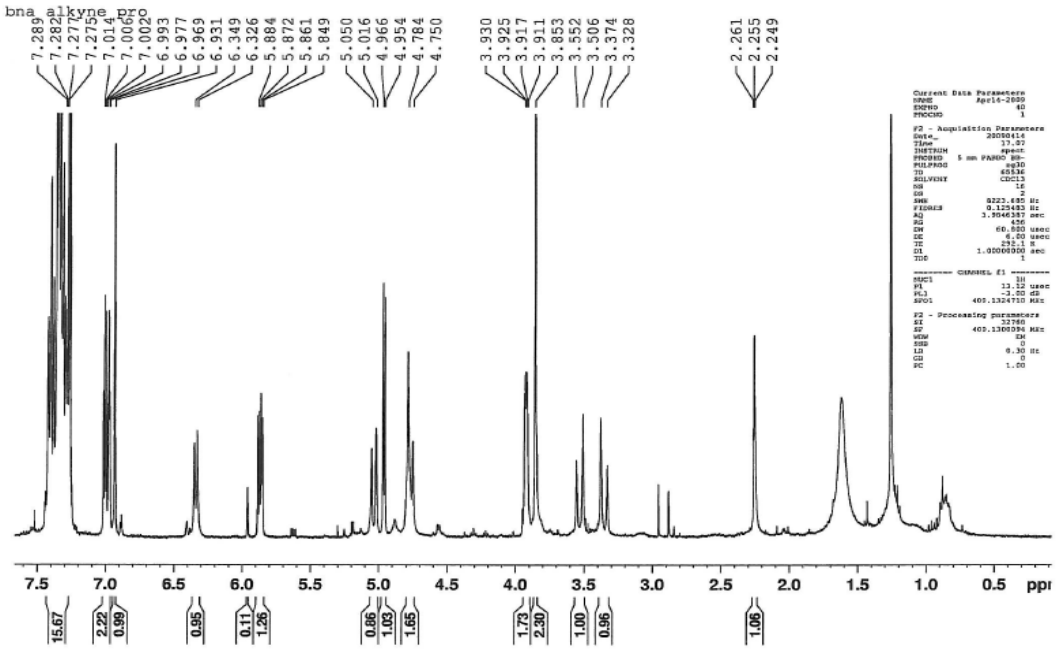


^{1}H NMR (400 MHz, $CDCl_3$)

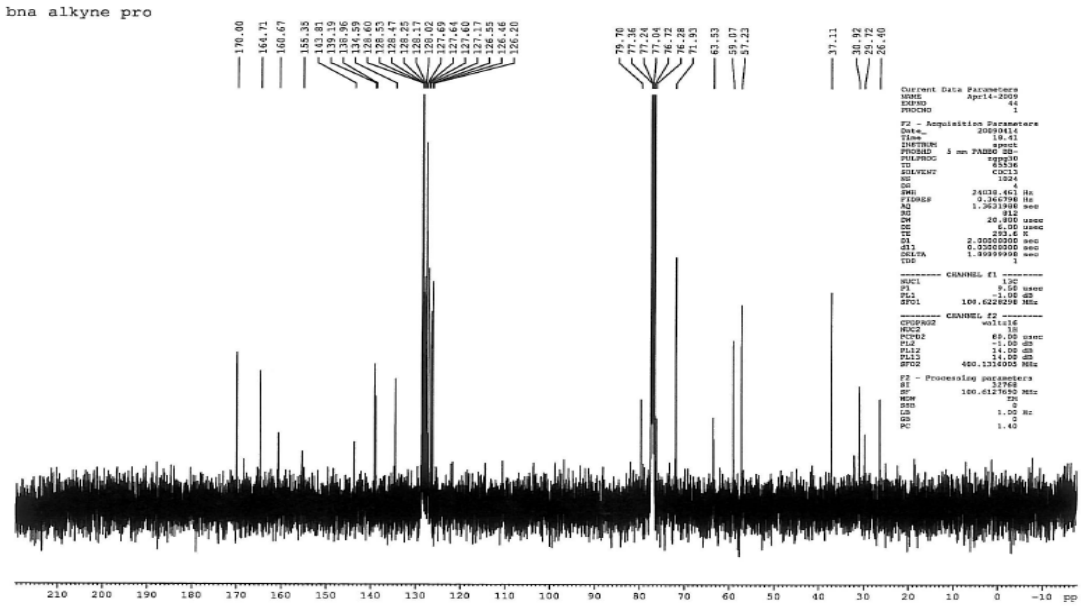


^{13}C NMR (100.6 MHz, $CDCl_3$)

(6*R*, 7*R*)- 3-Prop-2-ynylcarbamoyloxymethyl -7-(2-thiophen-2-yl-acetylamino) 3-cephem-4-carboxylic acid benzhydryl ester (5c)

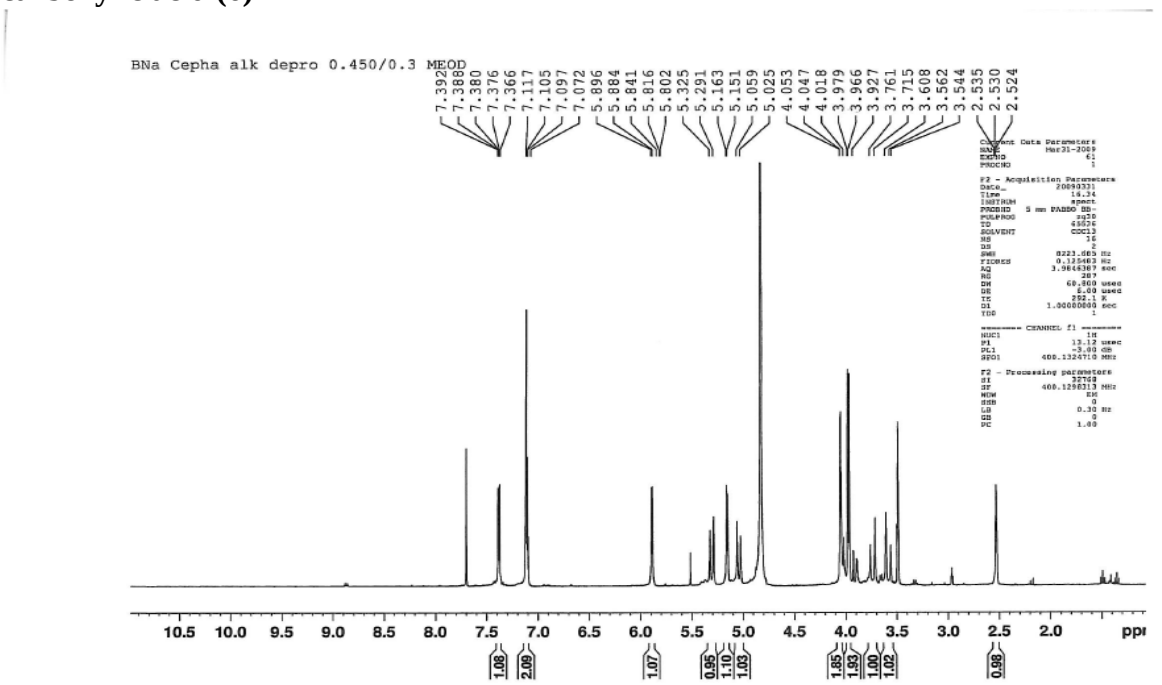


^{1}H NMR (400 MHz, $CDCl_3$)

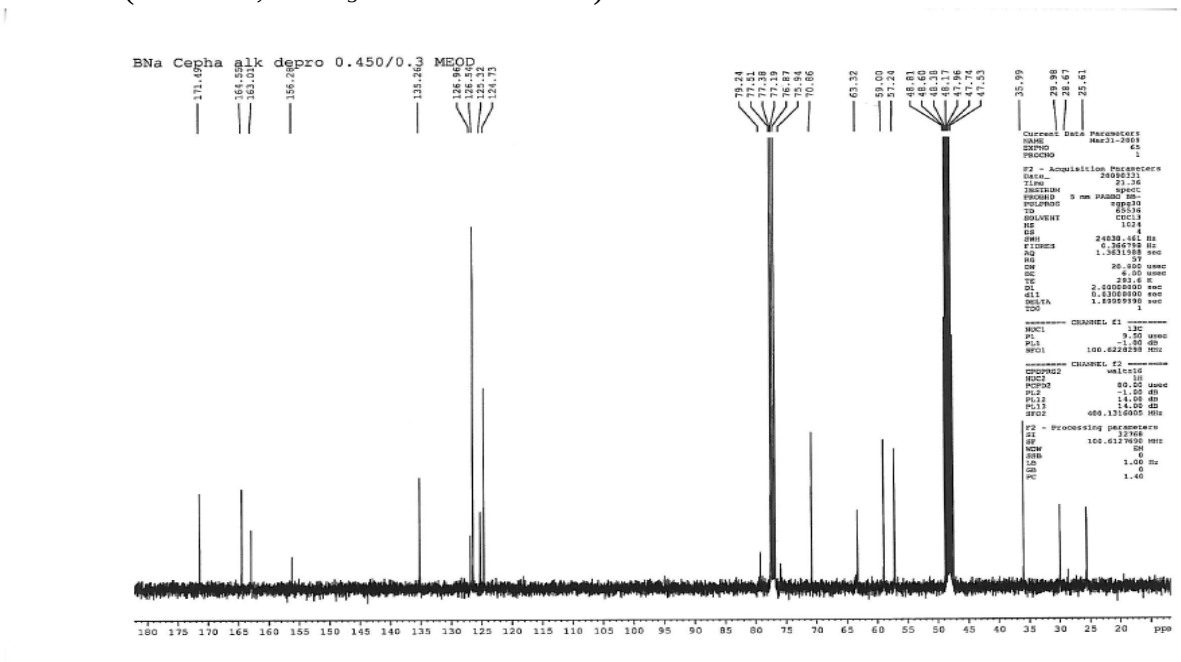


^{13}C NMR (100.6 MHz, $CDCl_3$)

(6*R*, 7*R*)- 3-Prop-2-ynylcarbamoyloxymethyl -7-(2-thiophen-2-yl-acetylamino) 3-cephem-4-carboxylic acid (6)

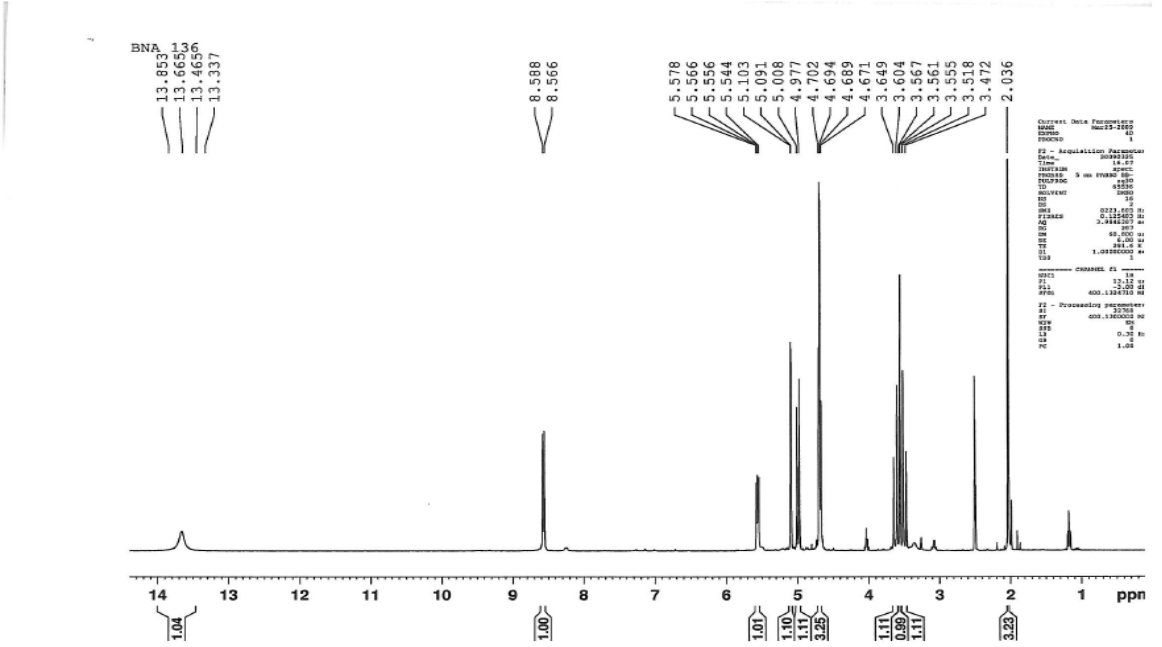


^{1}H NMR (400 MHz, $CDCl_3$/MeOD: 0.450/0.3)

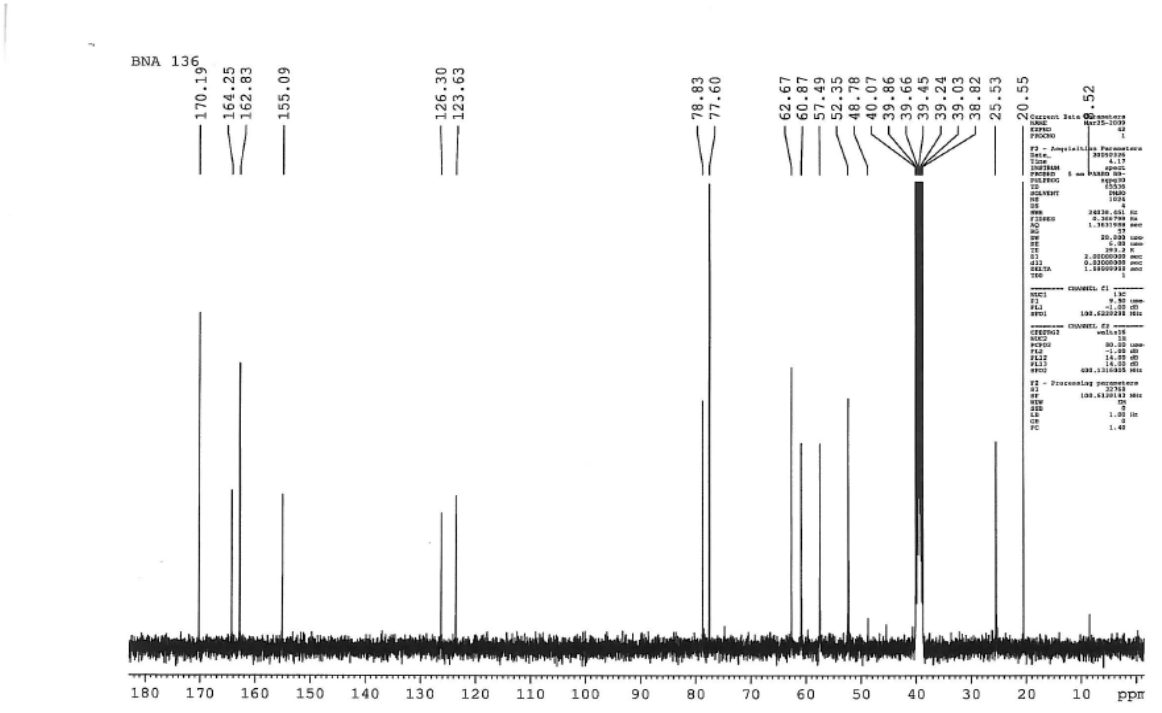


^{13}C NMR (100.6 MHz, $CDCl_3$)

(6*R*, 7*R*)-3-Acetoxymethyl-7-prop-2-ynyloxycarbonylamino 3-cephem-4-carboxylic acid

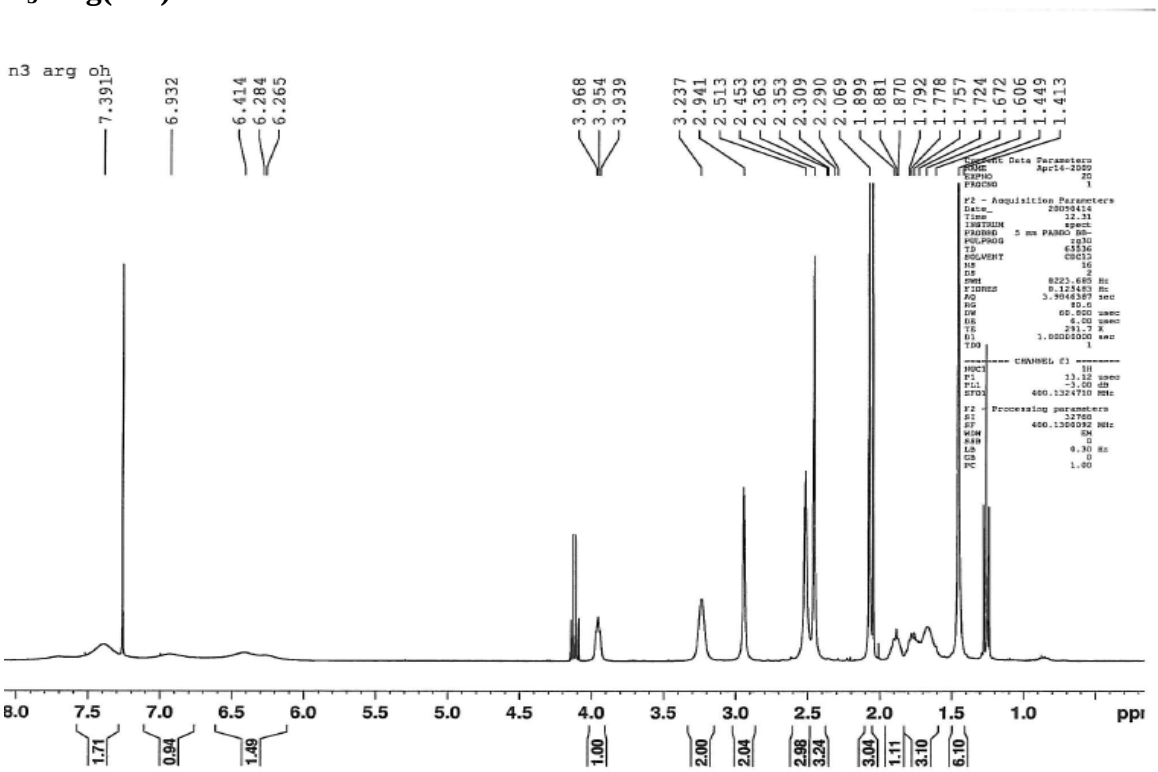


^{1}H NMR (400 MHz, DMSO)

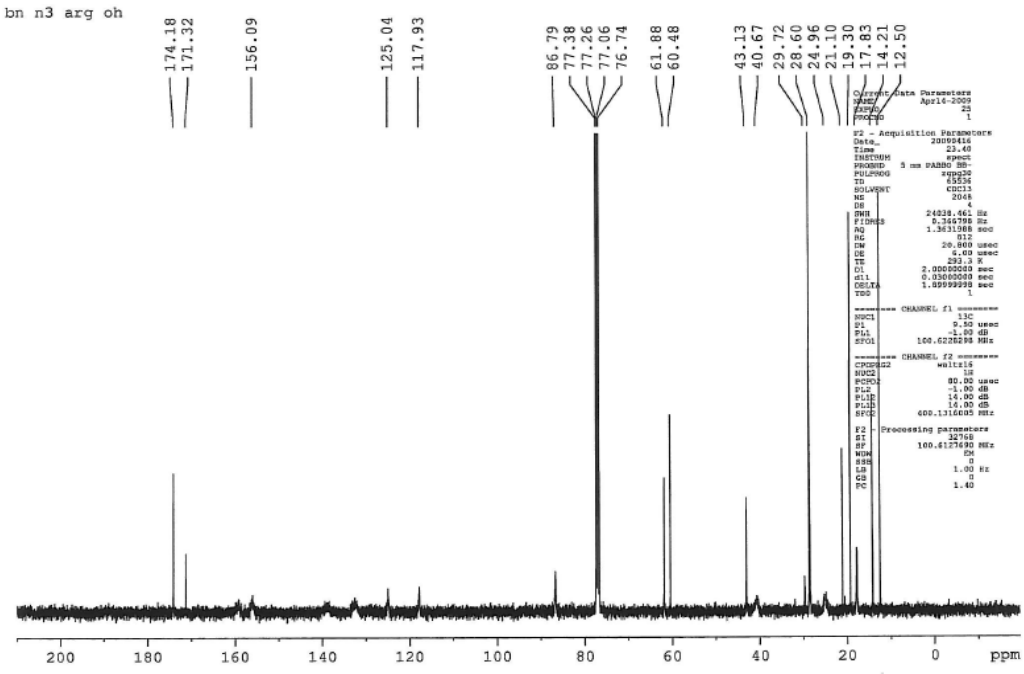


^{13}C NMR (100.6 MHz, $CDCl_3$)

N_3-Arg(Pbf)-OH

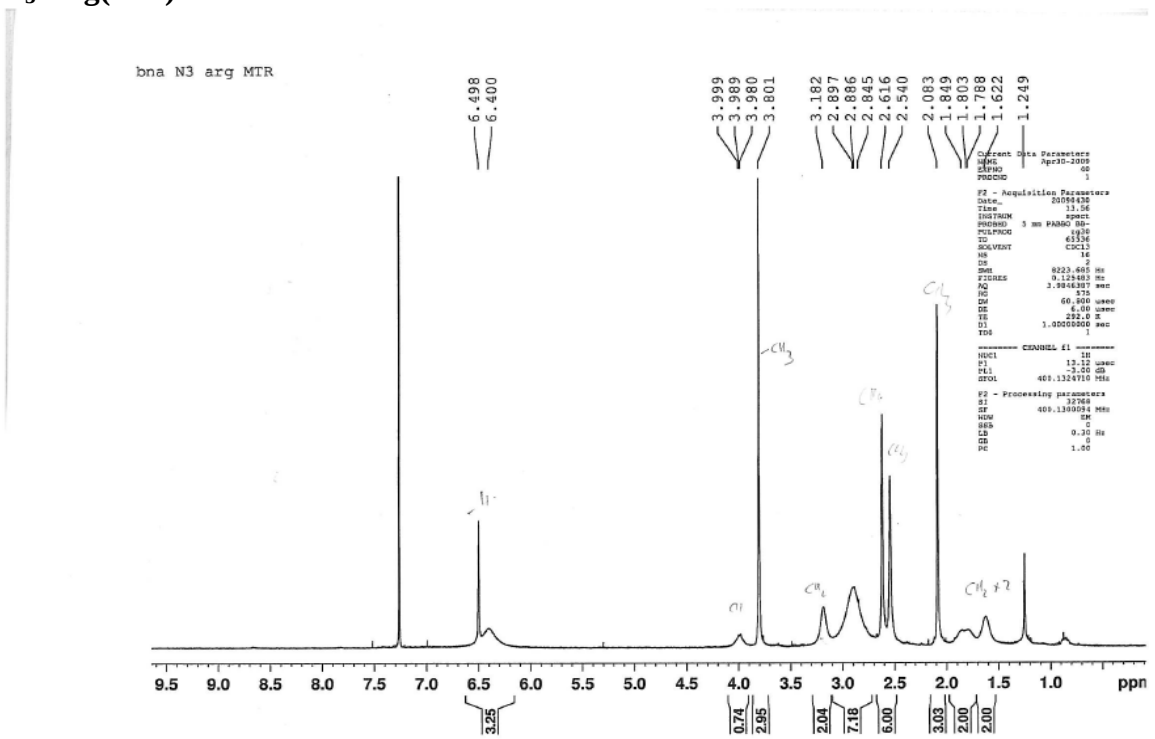


^{1}H NMR (400 MHz, $CDCl_3$)

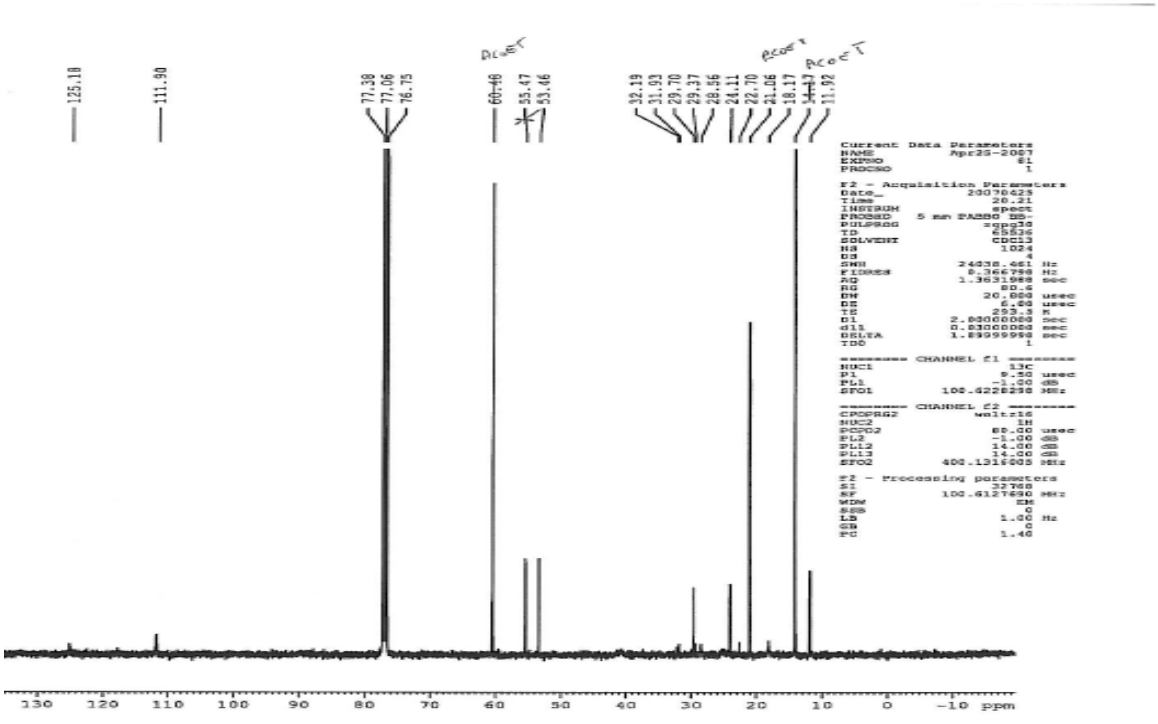


^{13}C NMR (100.6 MHz, $CDCl_3$)

N_3-Arg(Mtr)-OH

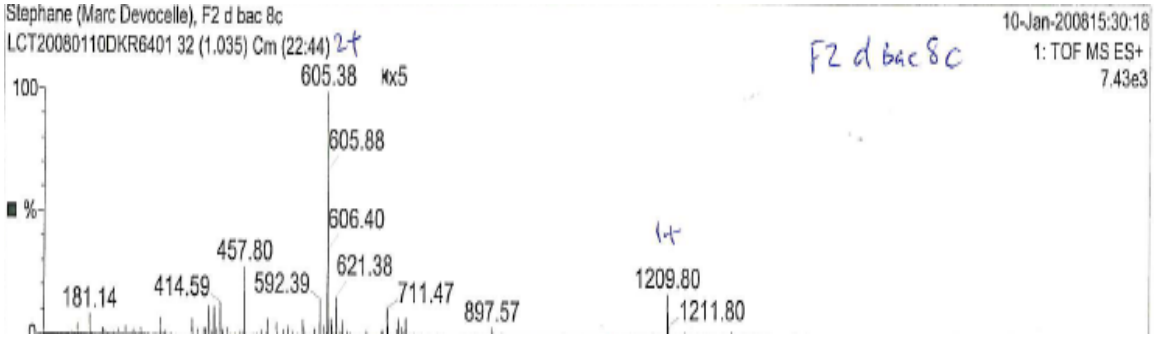


^{1}H NMR (400 MHz, D_2O)



^{13}C NMR (100.6 MHz, $CDCl_3$)

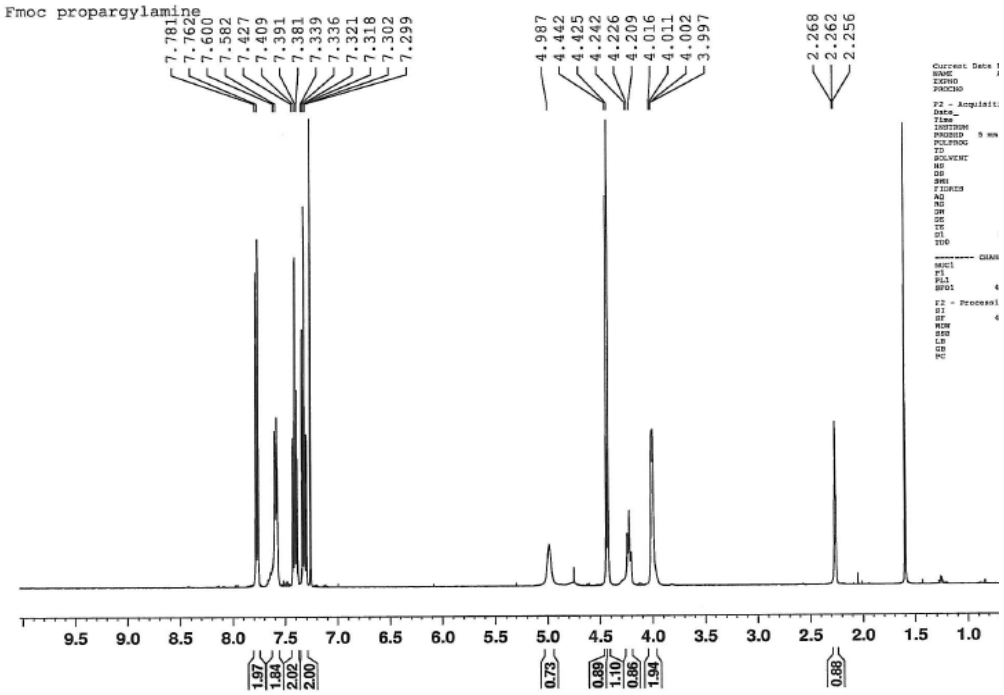
N_3-arg-leu-trp-val-leu-trp-arg-arg-NH_2 (azido- D-Bac8c(Leu^{2,5})-NH_2), 7)



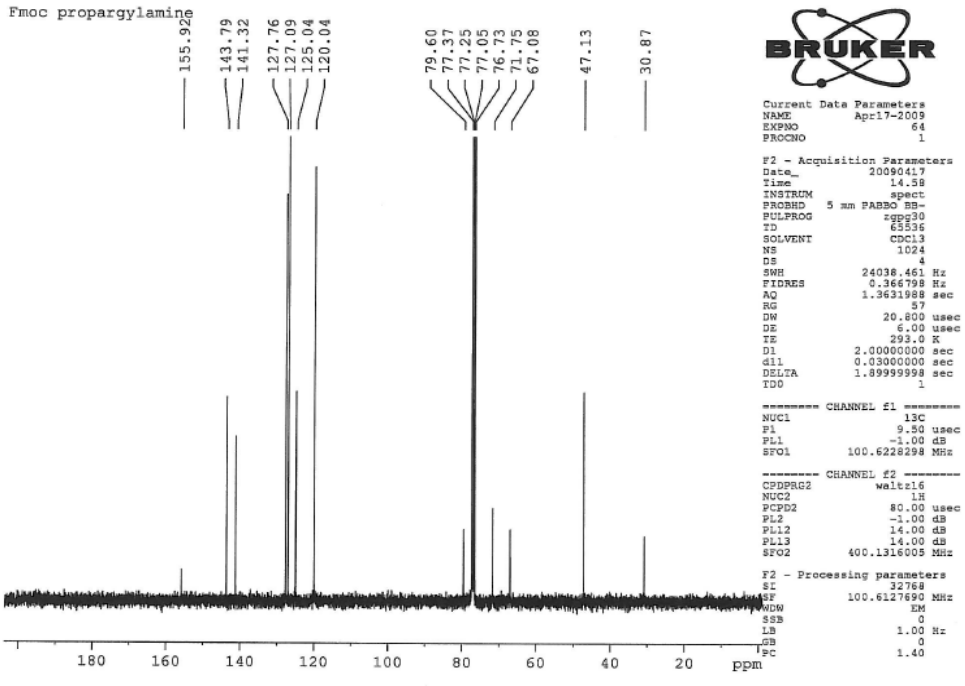
EI-MS

The signal at m/z = 1209.80 corresponds to the molecular ion, *i.e.* $[M + H]^+$; the signal at m/z = 605.38 corresponds to a doubly charged ion, *i.e.* $[M + 2H]^{2+}$.

N-Fmoc-propargylamine

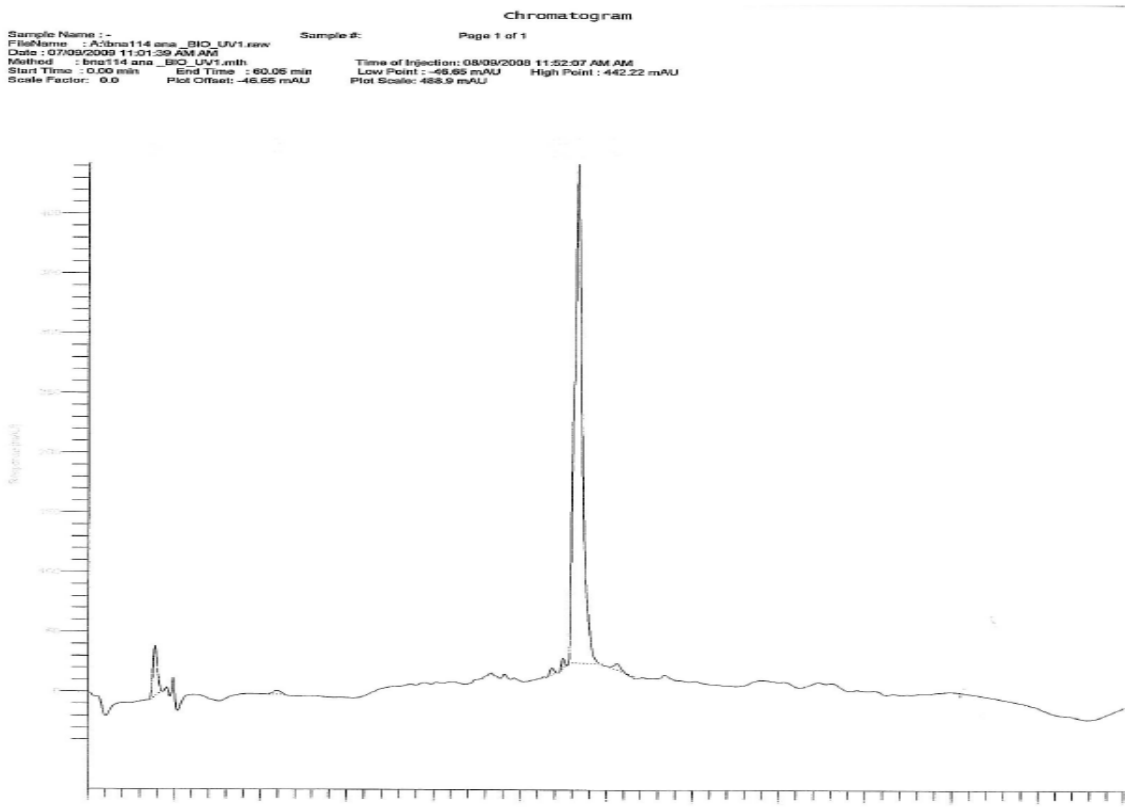


^{1}H NMR (400 MHz, $CDCl_3$)

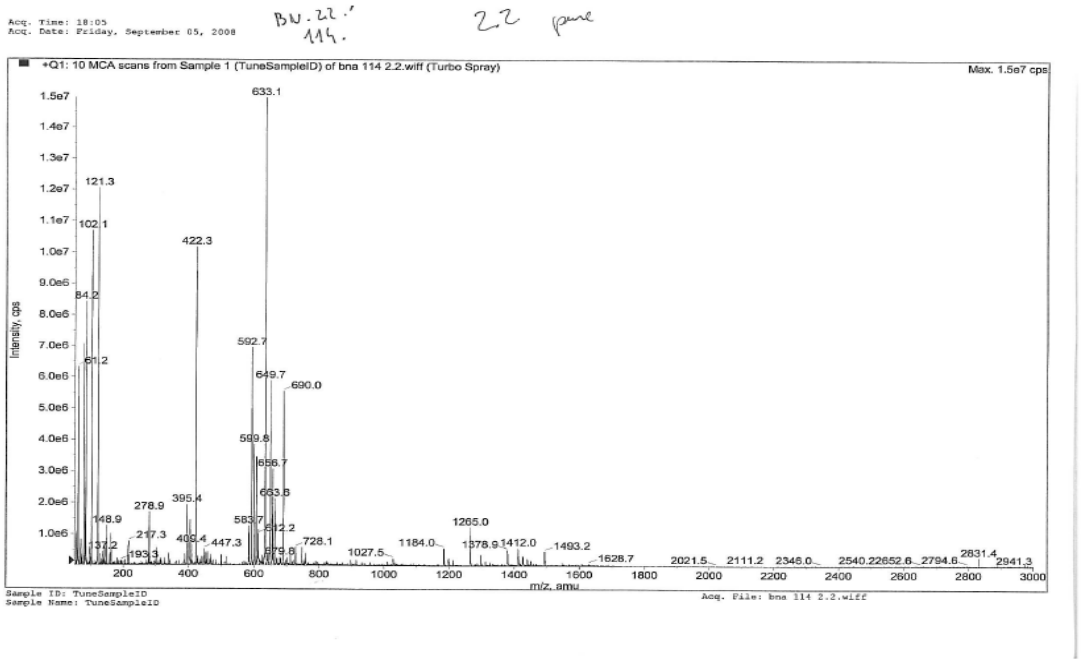


^{1}3C NMR (100.6 MHz, $CDCl_3$)

D-Arg[1]-triazole-ε2- D-Bac8c(Leu[2,5]) (9)



RP-HPLC



EI-MS

The signal at m/z = 1265.0 corresponds to the molecular ion, *i.e.* $[M + H]^+$; the signal at m/z = 633.1 corresponds to a doubly charged ion, *i.e.* $[M +2 H]^{2+}$; the signal at m/z = 422.3 corresponds to a triply charged ion, *i.e.* $[M + 3 H]^{3+}$.

Cephalothin-D-Bac8c(Leu^{2,5}) (8)

Chromatogram : bna22 prodrug pure1_channel1

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User : JVA

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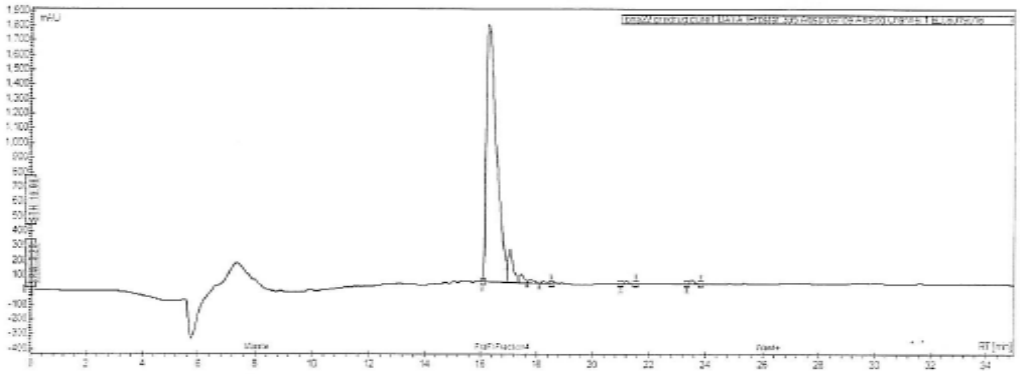
Processed : 29/06/2009 14:53:19
Printed : 29/06/2009 14:53:45



Peak results :

bna22 prodrug pure1_not_saved.DATA [Prostar 335 Absorbance Analog Channel 1 EL08019018]

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	16.27	91.08	1750.3	748.5	91.076
2	UNKNOWN	17.05	5.74	221.0	47.2	5.745
3	UNKNOWN	17.47	1.37	54.9	11.2	1.367
4	UNKNOWN	17.77	0.64	21.6	5.3	0.640
5	UNKNOWN	18.27	0.25	13.3	2.0	0.247
6	UNKNOWN	21.20	0.38	18.8	3.1	0.378
7	UNKNOWN	23.52	0.55	27.7	4.5	0.547
Total			100.00	2107.8	821.9	100.000

RP-HPLC

Control 10

Chromatogram : bn137 pure26_channel1

System : System_1
Method : ana 0.2 70 mn
User : Celine

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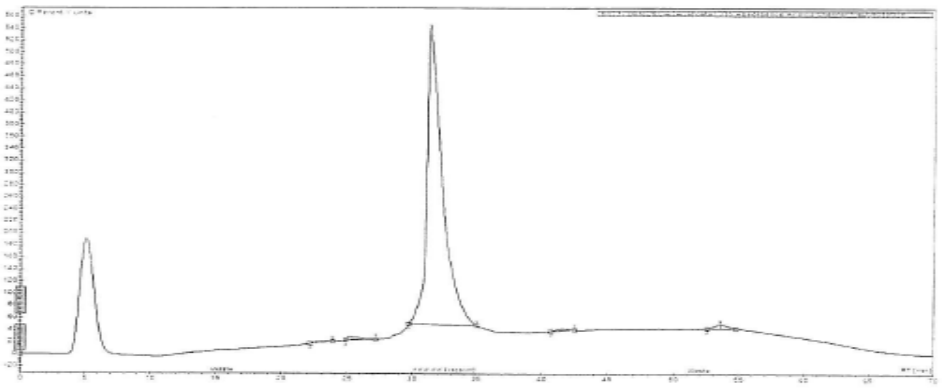
Processed : 18/04/2009 15:29:51
Printed : 29/06/2009 14:48:30



Peak results :

bn137 pure26.DATA [Prostar 335 Absorbance Analog Channel 1 EL08019018]

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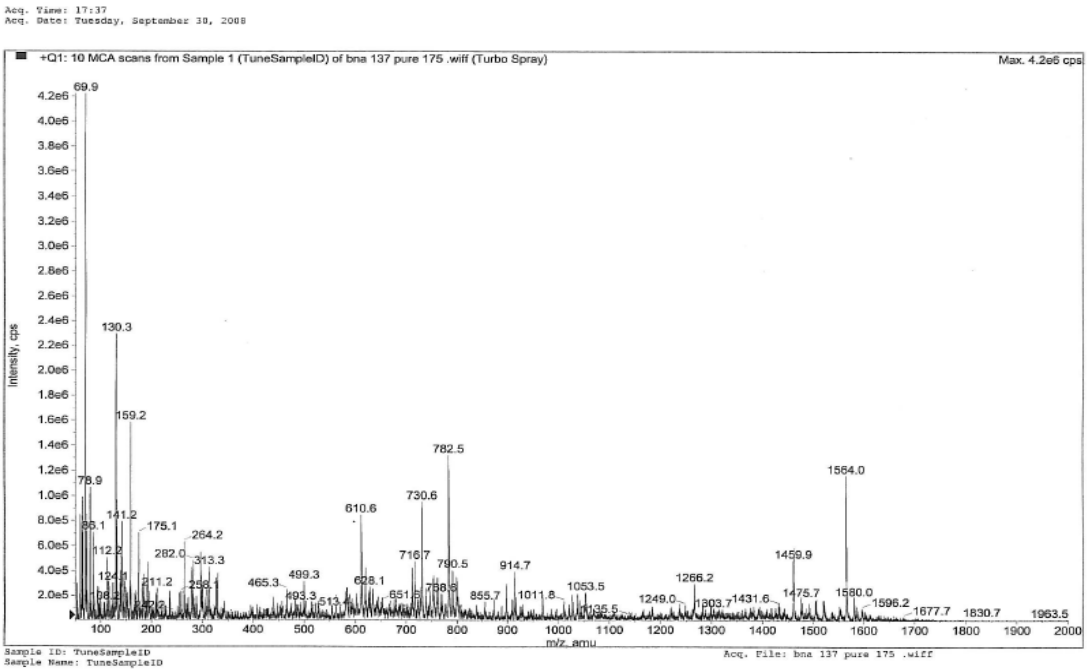
2	UNKNOWN	25.15	0.80	5.3	5.6	0.805
3	UNKNOWN	31.31	97.93	497.1	685.5	97.932
4	UNKNOWN	41.49	0.49	3.5	3.4	0.488
5	UNKNOWN	53.55	0.44	5.4	3.1	0.437
Total			100.00	513.6	700.0	100.000

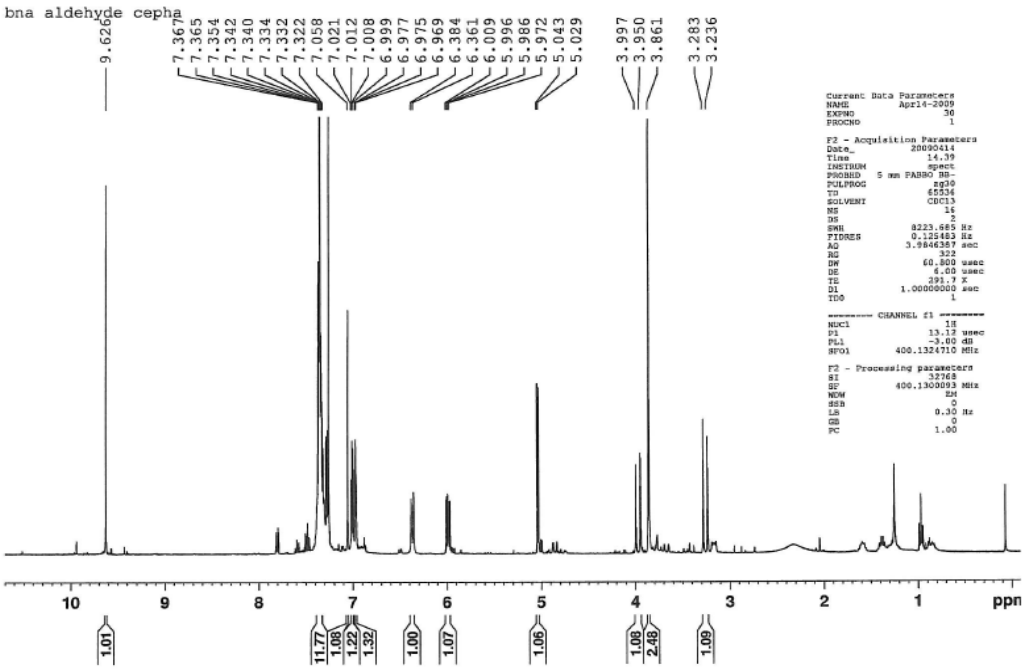
RP-HPLC



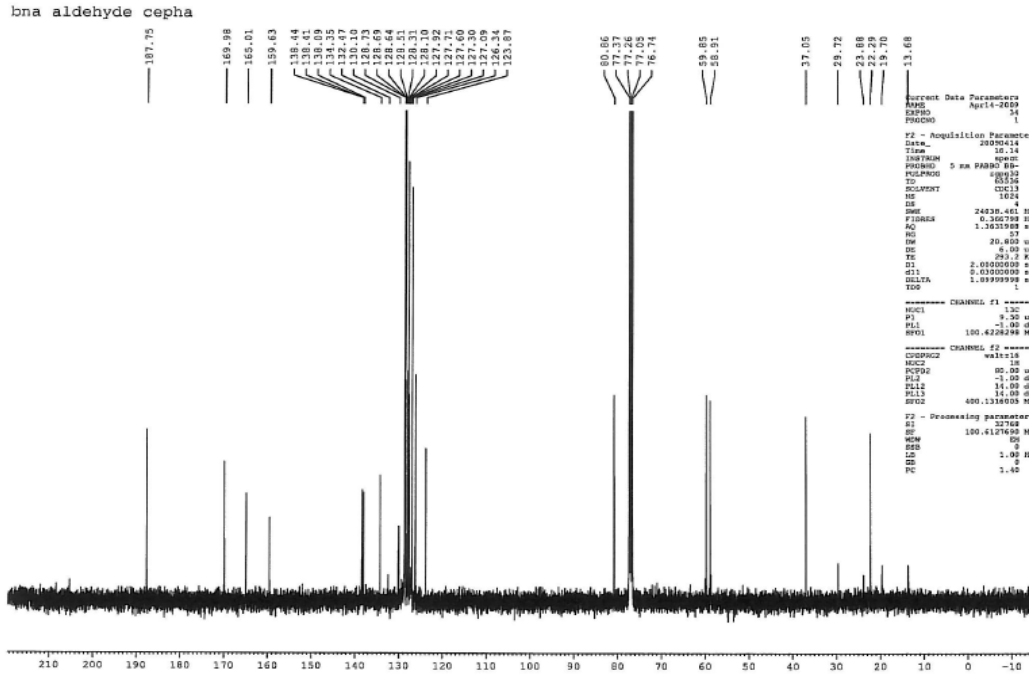
EI-MS

The signal at m/z = 1564.0 corresponds to the molecular ion, *i.e.* $[M + H]^+$; the signal at m/z = 782.5 corresponds to a doubly charged ion, *i.e.* $[M +2 H]^{2+}$.

(6*R*, 7*R*)- 7-(2-thiophen-2-yl-acetylamino)-3-formyl-3-cephem-4-carboxylic acid benzhydryl ester

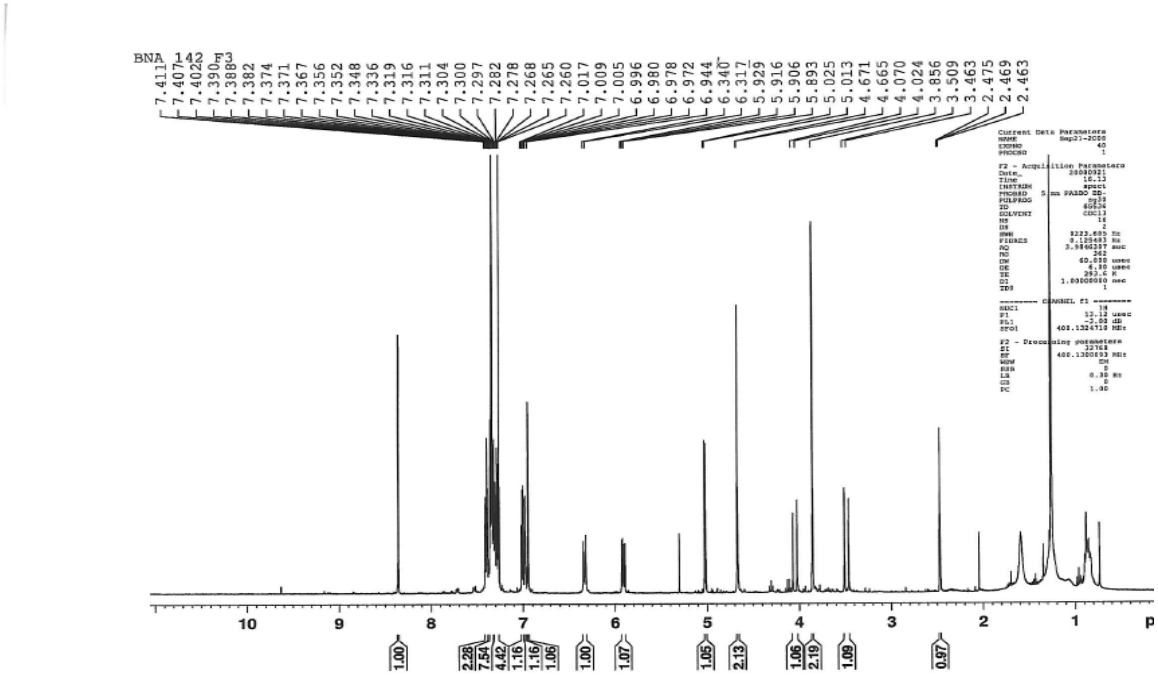


^{1}H NMR (400 MHz, $CDCl_3$)

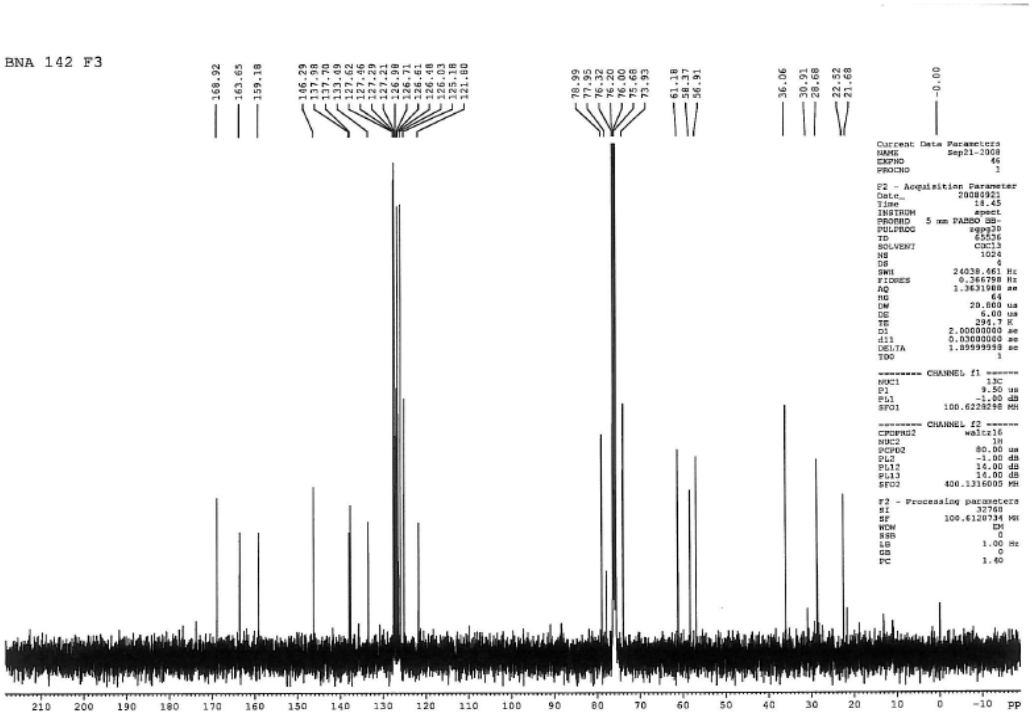


^{13}C NMR (100.6 MHz, $CDCl_3$)

(6*R*, 7*R*)- 7-(2-thiophen-2-yl-acetylamino)-3-(prop-2-ynyloxyimino-methyl)-3-cephem-4-carboxylic acid benzhydryl ester



^{1}H NMR (400 MHz, $CDCl_3$)



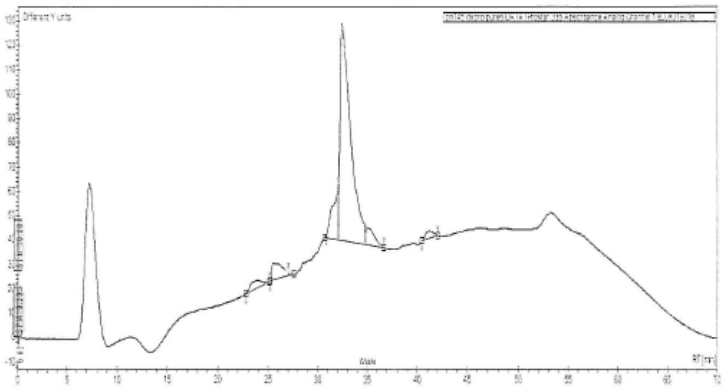
^{13}C NMR (100.6 MHz, $CDCl_3$)

Control 11

Chromatogram : bn145 depro pure6_channel1

System : System_1
Method : ana 0.2 70 mn
User : JVA

Acquired : 30/09/2008 16:07:58
Processed : 01/07/2009 18:12:35
Printed : 01/07/2009 18:13:01

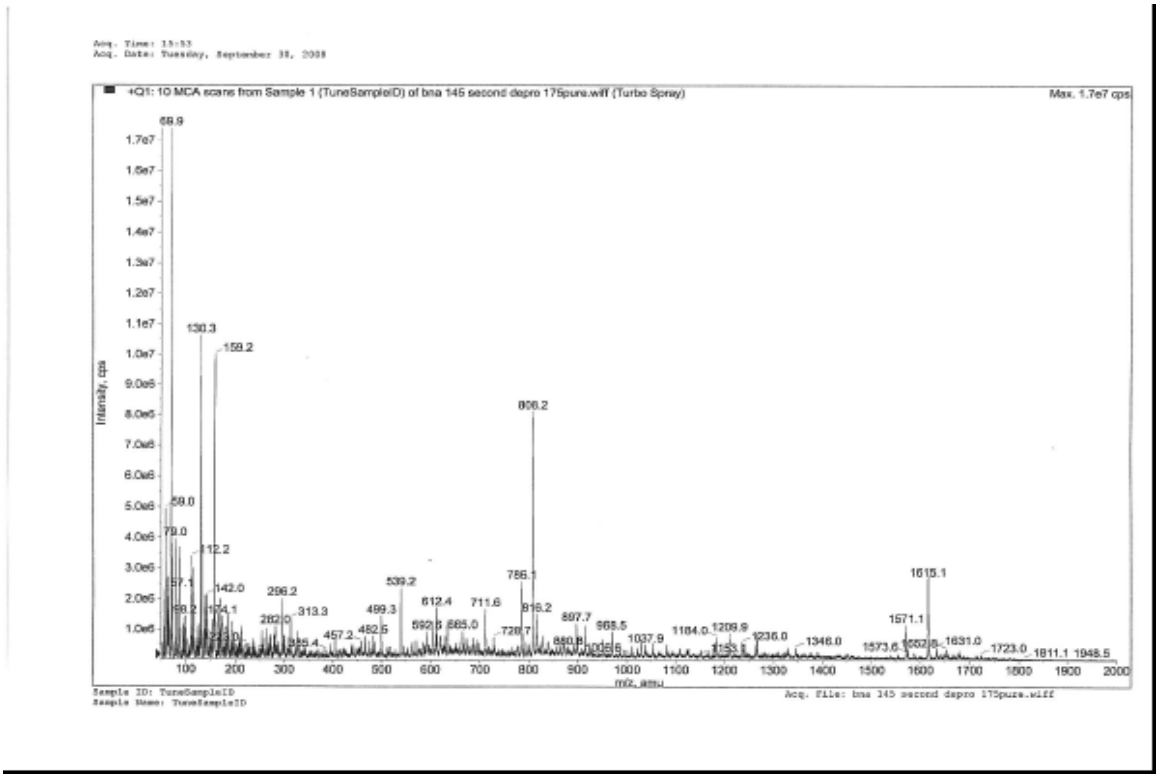


Peak results :

bn145 depro pure6_not_saved.DATA [Prostar 335 Absorbance Analog Channel 1 EL06019018]

Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	23.64	2.81	3.4	4.2	2.811
2	UNKNOWN	25.53	5.46	6.9	8.1	5.461
3	UNKNOWN	32.07	9.56	22.0	14.1	9.556
4	UNKNOWN	32.40	75.23	89.1	111.3	75.232
5	UNKNOWN	34.77	5.01	7.6	7.4	5.007
6	UNKNOWN	41.15	1.91	3.1	2.8	1.913
Total			100.00	132.4	147.9	100.000

RP-HPLC



EI-MS

The signal at m/z = 1615.1 corresponds to the molecular ion, *i.e.* $[M + H]^+$; the signal at m/z = 806.2 corresponds to a doubly charged ion, *i.e.* $[M +2 H]^{2+}$.