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Integrated Multi-Step Continuous Flow Synthesis of Daclatasvir Without Intermediate Purification and Solvent Exchange

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1. General

Material and method used in experiments

Most of the reagents and chemicals are bought from Spectrochem, AVRA and Sigma-Aldrich, which were used as such without any further purification. Common organic chemicals and salts were purchased from AVRA chemicals, India. Water used was deionized water (18.2 mS conductivity) in the experiments. All work-up and purification procedures were carried out with reagent-grade solvents. Analytical thin-layer chromatography (TLC) was performed using analytical chromatography silica gel 60 F254 pre-coated plates (0.25 mm). The developed chromatogram was analyzed by a UV lamp (254 nm). PTFE (id = 100-1000 μm) tubing, T-junction, and back-pressure regulator (BPR) were procured from Upchurch IDEX HEALTH & SCIENCE. HPLC Pump used was from KNAUER. SS318 capillary bought from the spectrum market, Mumbai, India. The heating reactor used was from the Thales Nano Nanotechnology, Inc.

Measurement method.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 600, 500, 400 or 300 MHz in $CDCl_3$ or DMSO-d_6 solvent. Chemical shifts for ^1H NMR are expressed in parts per million (ppm) relative to tetramethylsilane (δ 0.00 ppm). Chemical shifts for ^{13}C NMR are expressed in ppm relative to $CDCl_3$ (δ 77.0 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, quin = quintet, sext = sextet, m = multiplet), coupling constant (Hz), and integration. For scanning electron microscopy (SEM), gold sputter coating was carried out on desired samples at a pressure ranging in between 1 and 0.1 Pa. The sample was loaded in the chamber of JSM-7610F and recorded by operating at 10-2 to 10-3 Pa with EHT 15.00 kV with 300 V collector bias. LC-MS was conducted on the Shimadzu technology LCMS-8040 instrument equipped with LUNA C8 (250 ´ 4.6 mm, 5.0 u) and in

build triple quadrupole detector. GC/MS analysis was conducted on Shimadzu technology GCMS-QP2010 instrument equipped with an HP-5 column (30 m × 0.25 mm, Hewlett-Packard) and inbuilt MS 5975C VL MSD system with a triple-axis detector. Sonication (Power-Sonic 405) was used for washing the metal surface. ATR analysis was conducted on Portable FTIR spectrometer Bruker ALPHA. High-resolution mass spectra (HRMS) were obtained from a JMS-T100TD instrument (DART) and Thermo Fisher Scientific Exactive (APCI). Datalog model DCS-PS-6401 power supply system was used to supply the constant current. Han's Yueming laser series (model CMA0604-B-A, Carbon dioxide-based, laser power 80W) equipment was used for the fabrication of micro-channel.

Optimization for the continuous flow production of DCV intermediate 3.

Reaction optimization condition. A solution of compound **2** in DCE was charge in one syringe. 2-chloroacetyl chloride, aluminum chloride dissolved in DCE was charged in the capillary microreactor with a T-mixer using separate syringe pumps. The two solutions were introduced into a T-mixer with variable flow rates to maintain the stoichiometric and then passed through PFA tubing for Friedel-Craft acylation reaction. Optimization conditions were varied depending on the nature of the catalyst reagent, retention time, temperature, and pressure, etc. (Table 1).

Optimized condition for the semi-continuous production of DCV intermediate 3. A solution of **2** dissolved in DCE (0.16 M) was charged in one syringe. Another syringe was filled with the solution of chloroacetyl chloride (0.4 M in DCE), and $AlCl_3$ (0.4 M) (Figure.S1). The two solutions were introduced to a T_1-mixer in a flow rate with the ratio of 1: 6.25 (compound 2: chloroacetyl chloride) to maintain the stoichiometry, and then passed through a PFA tubing (id = 1000 µm, l = 3.8 m, vol. = 3 ml) for the Friedel-craft acylation reaction to occurs with the residence time 8.9 min, 100 °C temperature, and 3 bar pressure (Figure S1). The resulting solution was quenched with cold water and extracting through the regular known prior art batch process.

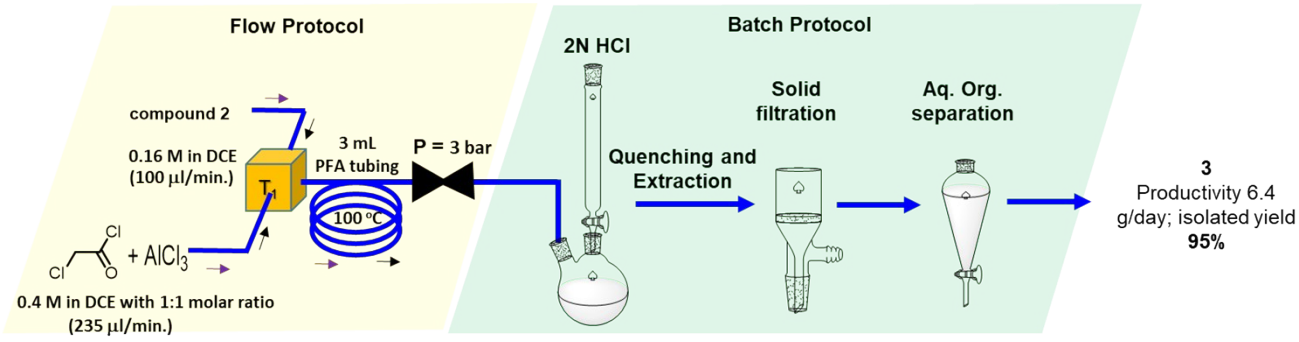
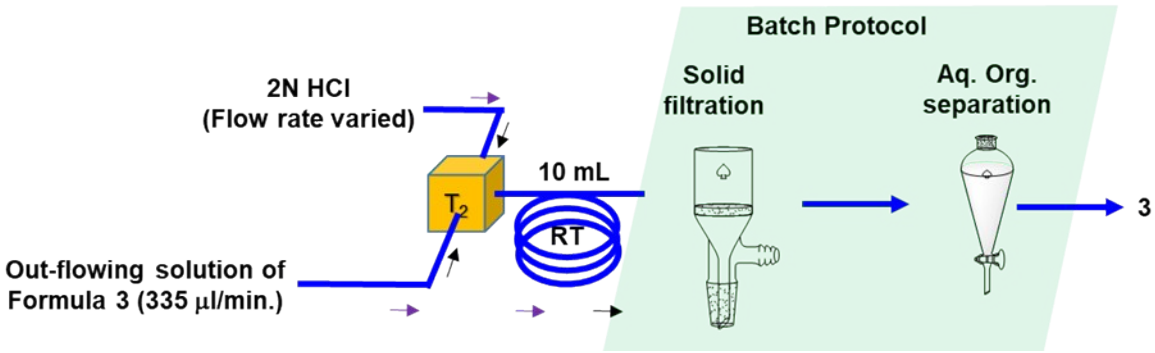


Figure S1. Schematic presentation of semi-continuous flow process to synthesize daclatasvir intermediate **3**.

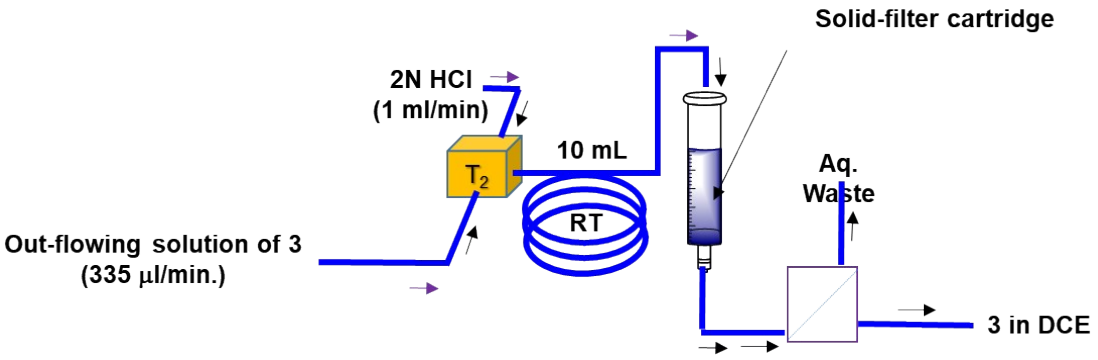
Table S1. Optimization of the quenching reagent for the synthesis of DCV intermediate **3**.



Yields are based on LC-Mass; # represent the tube clogging problem.

Entry	Flow rate (µl/min.) 2N HCl	Temperature (°C)	Residence time (min.)	(%) Yield
1[a]	50	RT	26	#
2[a]	1000	RT	7.5	98
3[a]	3000	RT	2.9	97

Table S2. Continuous-flow solid filter screening.



Entry	Solid filter cartridge	Remark
1	Activated Carbon	Blocking
2[a]	Alumina	Solid cake formation
4[a]	Celite	Blocking
5[a]	Whatmann filter paper	Blocking
6[a]	Silica	Smooth running

*Integrated continuous-flow DCV synthesis of intermediate **3**, and downstream process.* A solution of compound **2** in dichloroethane (DCE; 0.16 M) and a solution of chloro-acetyl chloride in DCE (0.4 M) were introduced into the capillary microreactor with a T-mixer using two separate pumps. The flow rate of the compound **2** solution was kept at same the rate of 2-chloroacetyl chloride, following the stoichiometry of reagent and substrates. The two solutions were introduced to a T-mixer in a flow rate with the ratio of 1: 2.35 (biphenyl: 2-chloroacetyl chloride) to maintain the stoichiometry, and then passed through a PFA tubing (id = 1000 µm, l = 12.8 meters, vol. = 10 ml) for the Friedel-Craft acetylation reaction during 8.9 min of residence time and 100 ^{o}C temperature and 3 bar pressure (Table 1, entry 4). Next, the output of the crude reaction mixture was quenched with aq. 2N HCl with varied flow rate and compound **3** and smoothly passed through perfluoro alkoxy (PFA) tubing (id = 1000 µm, l = 12.8 meters, vol. = 10 ml) for the extraction to occur. A residence time of 7.5 min, 25 ^{o}C was found to be enough for the extraction of the compound **3**. After the successful completion of the extraction next the solution was passed through the silica filled cartridge (id = 60 mm, length 50 mm, silica weight = 80 gm) to remove the solid impurity. A residence time of 2 min, 25 ^{o}C was found to be enough for the solid removal from the compound **3** solution. Further, the aqueous and DCE continuous flow droplet was separated through in house made micro-separator. A residence time of 0.5 min., 0-1 bar pressure was found to be enough for the aqueous waste removal and separation of the crude organic solution of intermediate **3**. The reaction mixture solvent was removed under the vacuum and further triturated by n-pentane, or n-hexane to give the intermediate **3** with a 98% yield. The spectra data matched with values reported in the literature.[1]

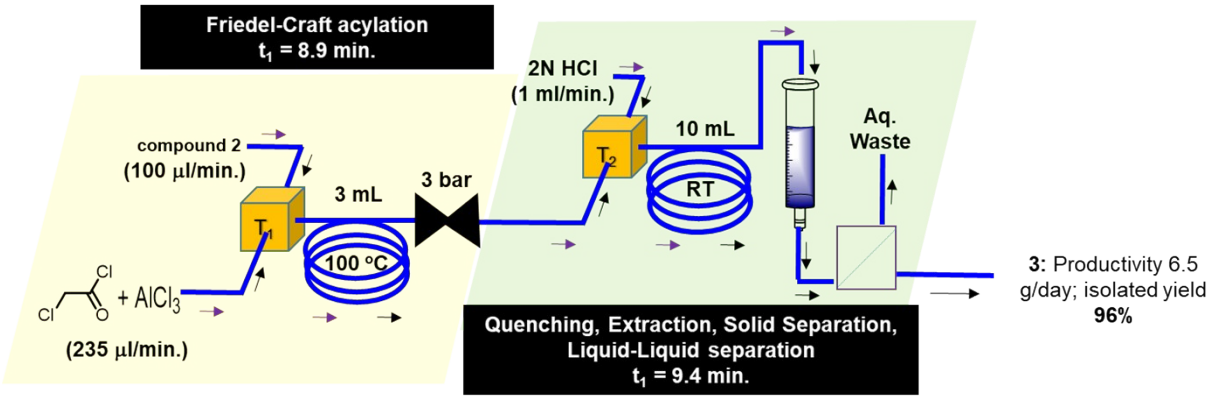


Figure S2: Schematic presentation of the integrated continuous-flow DCV intermediate **3**, and downstream process.

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (d, *J* = 8.37, 4H), 7.76 (d, *J* = 8.37, 4H), 4.74 (s, 4H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 190.81, 145.05, 133.99, 129.50, 127.93, 45.96.

IR (ν_{max}): 3361.48, 2987.28, 2944.15, 1694.05, 1602.82, 1399.29, 1306.11, 1217.05, 997.10, 806.23, 767.09 cm^{-1}

MS (EI): *m/z* 307.17 (M^+).

Batch process peptide (4) synthesis: Reported batch method has been followed for the preparation of compound **4**.[2]

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 – 5.74 (m, 1H), 4.57 – 4.51 (m, 1H), 4.31 – 4.25 (m, 1H), 3.87 – 3.81 (m, 1H), 3.69 – 3.67 (m, 1H), 3.65 (s, 3H), 2.21 – 2.15 (m, 2H), 2.07 – 1.98 (m, 3H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H).

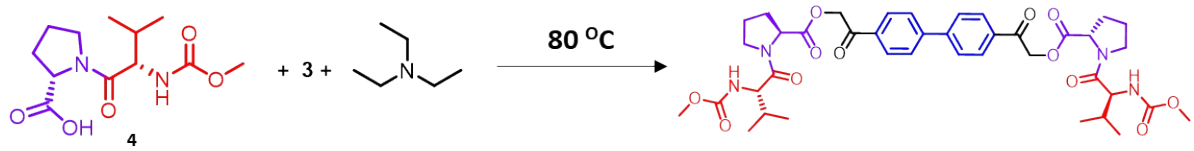
^{13}C NMR (101 MHz, $CDCl_3$): δ 174.76, 172.39, 157.44, 59.21, 57.86, 52.42, 47.69, 31.17, 28.75, 24.95, 19.20, 17.92.

MS (EI): *m/z* 272.14 (M^+).

Individual continuous-flow DCV intermediate (5) formation:

The mixture of solvent system as optimized under the batch process condition: To an oven-dried 4 mL Ace pressure tubes equipped with a Teflon coated magnetic stir bar, ester product **3** (0.14 mmol, 42 mg), peptide (0.28 mmol, 76 mg), and trimethylamine (0.7 mmol, 71 ml) were adding. Then MeCN and DCE (total volume 3.0 mL) were added as per given Table S3 using a syringe. Then the tube was sealed by a Teflon screwcap and placed in an oil bath at 80 °C as pre-heated. After reaction for an 8 h, the regular post-synthetic protocols were following for purification and analysis of the product.

Table S3: Solvent ratio screening.



4

Entry	Solvent Ratio (% v/v)		Reaction time (h)	(%) Yield
	DCE	MeCN		
1	100	0	8	NA
2[a]	66	33	8	10
3[a]	50	50	8	60
4[a]	33	66	8	81
5[a]	20	80	8	81
6[a]	0	100	8	81

Yields are based on the LC-MS.

Figure S4. Precipitation under the capillary micro-reactor.

T

he mixture of solvent optimization under the continuous flow process for esterification reaction: The outflowing crude mixture of **3** in DCE, stock solution of peptide **4** (0.04 M in MeCN), and triethylamine were dissolved in MeCN (0.1 M) and connected as per table 2 design. Compound **3**, compound **4**, and trimethylamine were mix in an X-shaped mixer, and the combined mixture went through stainless steel tubing (id = 1000 μm, length = 12.2 meters, volume = 10 mL). The flow rates for compound **4** and triethylamine have varied to maintain the stoichiometry (table 2). A residence time of 5.4 min, temperature 100 ^{o}C, and 5 bar pressure was found enough for the synthesis of compound **5**. The processed mixture left the flow reactor as a one-phase DCE/MeCN solution. The solvent from the organic phase was removed under reduced pressure, and the regular extraction and purification process provided compound **5** in 89% yield.

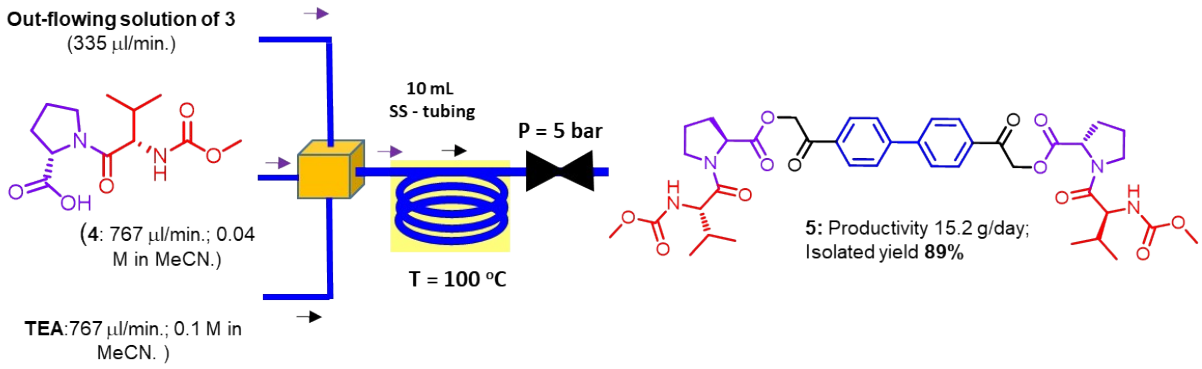


Figure S3. Production of daclatasvir intermediate **5** under the continuous-flow mode.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (d, *J* = 8.00 Hz, 4H), 7.73 (d, *J* = 8.00 Hz, 4H), 5.56 (d, *J* = 16.0 Hz, 2H), 5.39 (d, *J* = 9.0 Hz, 2H), 5.26 (d, *J* = 16.0 Hz, 2H), 4.72 – 4.70 (m, 2H), 4.33 (t, *J* =7.4 Hz, 2H), 3.85 – 3.80 (m, 2H), 3.75 – 3.70 (m, 2H), 3.66 (s, 6H), 2.41 – 2.31 (m, 4H), 2.23 – 2.15 (m, 2H), 2.11 – 2.03 (m, 4H), 1.03 (d, *J* = 6.6 Hz, 6H), 0.94(d, *J*= 6.5 Hz, 6 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 191.51, 171.59, 171.29, 157.28, 145.03, 133.73, 128.60, 127.90, 66.39, 58.87, 57.61, 52.42, 47.46, 31.41, 29.33, 25.09, 19.35,17.66

IR (ν_{max}): 3378.48, 3309.73, 2966.27, 2886.57, 1752.77, 1707.32, 1643.73, 1523.04, 1436.97, 1369.23, 1235.25, 1175.45, 1103.35, 1036.54, 973.38, 820.96, 759.37, 674.01 cm^{-1}.

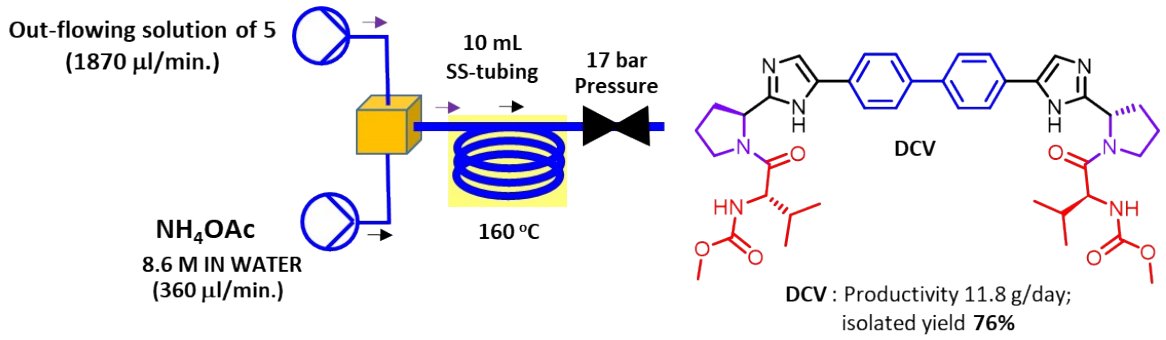
MS (EI): *m/z* 778.34 (M^+).

DCV free base synthesis:

Optimization reaction condition for the individual continuous-flow DCV free base synthesis. The directly out-flowing crude solution of compound **5** and a solution of ammonium acetate, in water, was taken in the bottle and connected with the pump as described in figure 1. Accordance with the stoichiometry of compound **5** and ammonium acetate, the flow rate was varied, and both solution mixing at T-junction, and the combined mixture went through stainless steel tubing (SS-tubing) inner diameter (id) = 1000 µm, l = 12.2 meters, vol. = 10 mL) to get the ester formed during 4.5 min of residence time and 160 ^{o}C temperature and 17 bar pressure was found enough for the synthesis of DCV free base **6**. The processed mixture comes out of the flow reactor as a two-phase aqueous-MeCN solution. The aqueous phase was removed and discarded, and the solvent from the organic phase was removed under reduced pressure. The crude product was dissolved in ethyl acetate and extracted through the routine process.

Integrated DCV synthesis machine process system. A solution of compound **2** in DCE (0.16 M) and a solution of chloro-acetyl chloride in DCE (0.4 M) were introduced into the capillary microreactor with a T-mixer using two separate pumps. The flow rate of the compound **2** solution was kept at same the rate of 2-chloroacetyl chloride, following the stoichiometry of reagent and substrates. The two solutions were introduced to a T-mixer in a flow rate with the ratio of 1: 2.35 (compound **2**: 2-chloroacetyl chloride) to maintain the stoichiometry, and then passed through a PTFE tubing (id = 1000 µm, l = 12.8 meters, vol. = 10 mL) for Friedel-Craft acetylation to occur over 8.9 min of residence time and 100 ^{o}C temperature and 3 bar pressure (Table 1, entry 4). Next, the output of the crude reaction mixture was quenched with aq. 2N HCl with varied flow rate and compound **3** and smoothly passed through perfluoro alkoxy (PFA) tubing (id = 1000 µm, l = 12.8 meters, vol. = 10 mL) for the extraction to occur. A residence time of 7.5 min, 25 ^{o}C was

found to be enough for the extraction of the compound **3**. After the successful completion of the extraction next the solution was passed through the silica filled cartridge to remove the solid impurity. A residence time of 2 min, 25 ^{o}C was found to be enough for the solid removal from solution **3**. Further, the aqueous and DCE continuous flow droplet was separated through our lab previously reported micro-separator. A residence time of 0.5 min, 0-1 bar pressure was found to be enough for the aqueous waste removal of the crude organic solution of **3**. The outflowing crude solution of product **3** was interconnected with the pump, another pump was connected with the stock solution of trimethylamine in MeCN (0.1 M) and additional one more pump with the stock solution of **4** in MeCN. The outflow of all three solutions was mixed in an X-shaped mixer and the combined mixture went through stainless steel tubing (id = 1000 μm, length = 12.2 meters, volume = 10 mL). A residence time of 5.4 min, temperature 100 ^{o}C, and 5 bar pressure was found enough for the synthesis of compound **5**. Next, the directly out-flowing crude solution of **5** was connected with the HPLC pump to be infused for next reaction. Separately a stock solution of NH_4OAc (8.6 M in water) (Figure 1) connected with high pressure pump. The two solutions were introduced to a T-mixer in a flow rate with the ratio of 1: 236 (**5**: NH_4OAc) to maintain the stoichiometry, and then passed through an SS-tubing (id = 1000 μm, l = 12.2 meters, vol. = 10 mL) for the DCV free base. The DCV free base was observed in 4.5 min of residence time with 160 ^{o}C temperature and 17 bar pressure (Table 3, entry 6). The product was collected in a reservoir containing cold water and extracted and purified through the routine batch process.



Scheme 4. Production of daclatasvir continuous-flow mode.

^{1}H NMR (400 MHz, DMSO-d_6): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 – 7.58 (m, 4H), 7.56 – 7.46 (m, 1H), 7.38 – 7.24 (m, 1H), 5.24 – 5.08 (m, 2H), 4.10 – 4.02 (m, 1H), 3.85 – 3.75 (m, 2H), 3.54 (s, 3H), 2.15 – 2.08 (m, 2H), 2.00 – 1.91 (m, 3H), 0.91 – 0.84 (m, 6H).

^{13}C NMR (101 MHz, DMSO-d_6): ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.38, 156.81, 149.29, 138.87, 137.13, 133.95, 126.20, 124.57, 112.39, 58.01, 54.24, 51.44, 46.83, 30.95, 29.85, 24.25, 18.98, 18.52.

IR (ν_{max}): 3272.37, 2967.00, 1712.36, 1628.75, 1521.98, 1443.10, 1362.04, 1321.99, 1246.05, 1188.32, 1103.53, 1036.86, 830.08, 756.11 cm^{-1}.

MS (EI): *m/z* 738.39 (M^+).

3. Spectra

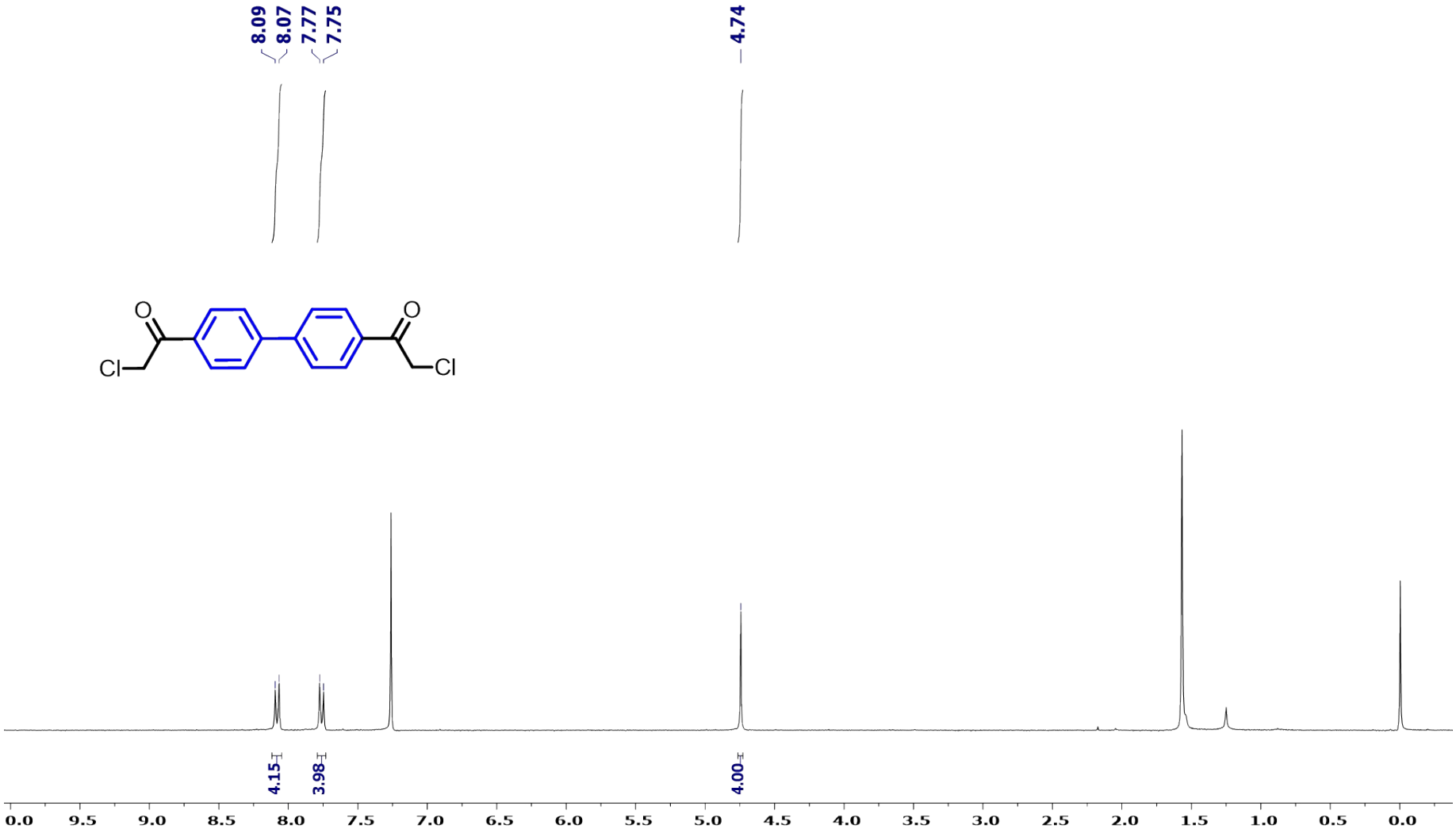


Fig. S5. ^{1}H NMR spectra of 1, 1'-([1, 1'-biphenyl]-4, 4'-diyl)bis(2-chloroethan-1-one) (**3**) in $CDCl_3$.

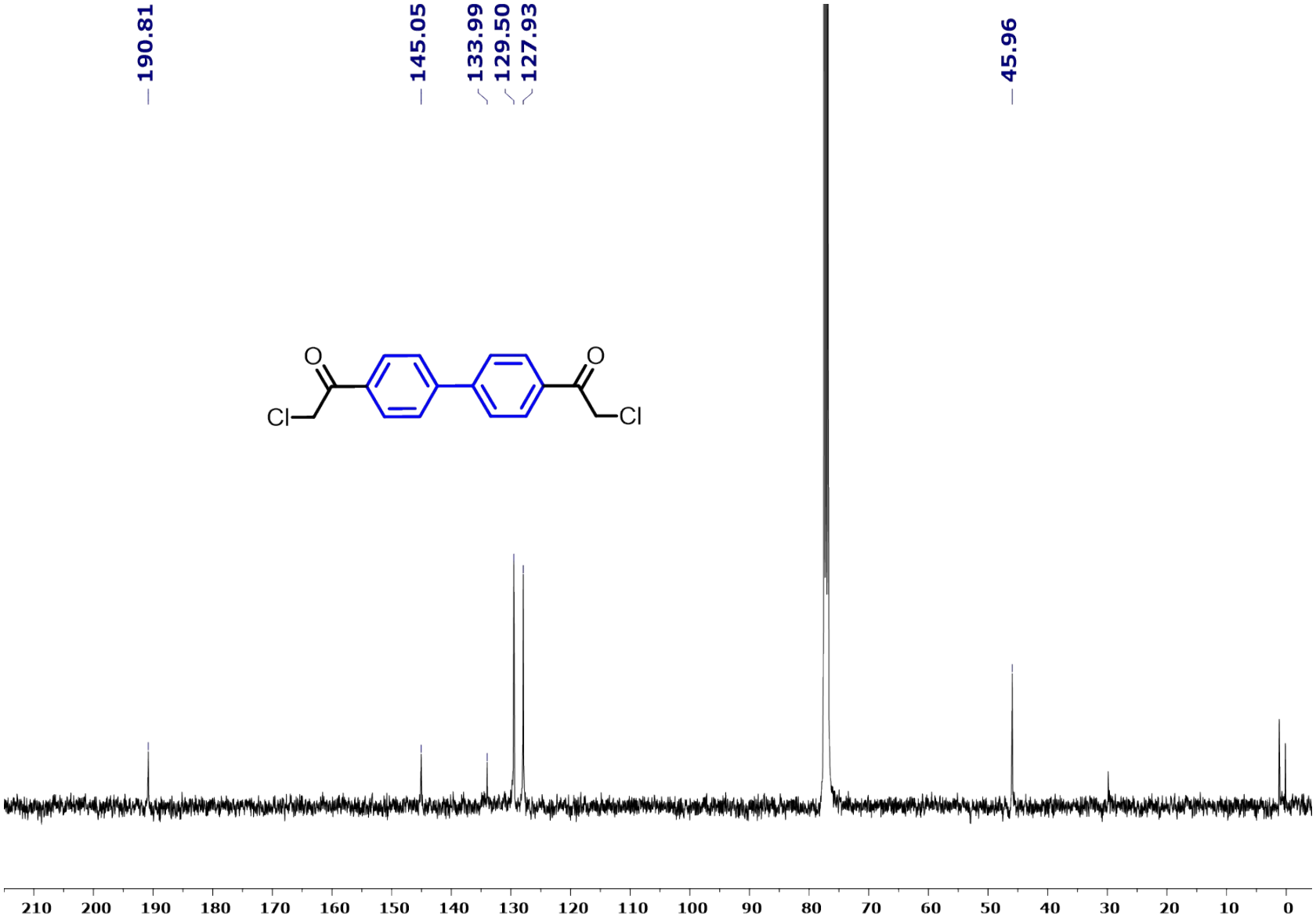


Fig. S6. ^{13}C NMR spectra of 1, 1'-([1, 1'-biphenyl]-4, 4'-diyl)bis(2-chloroethan-1-one) (**3**) in $CDCl_3$.

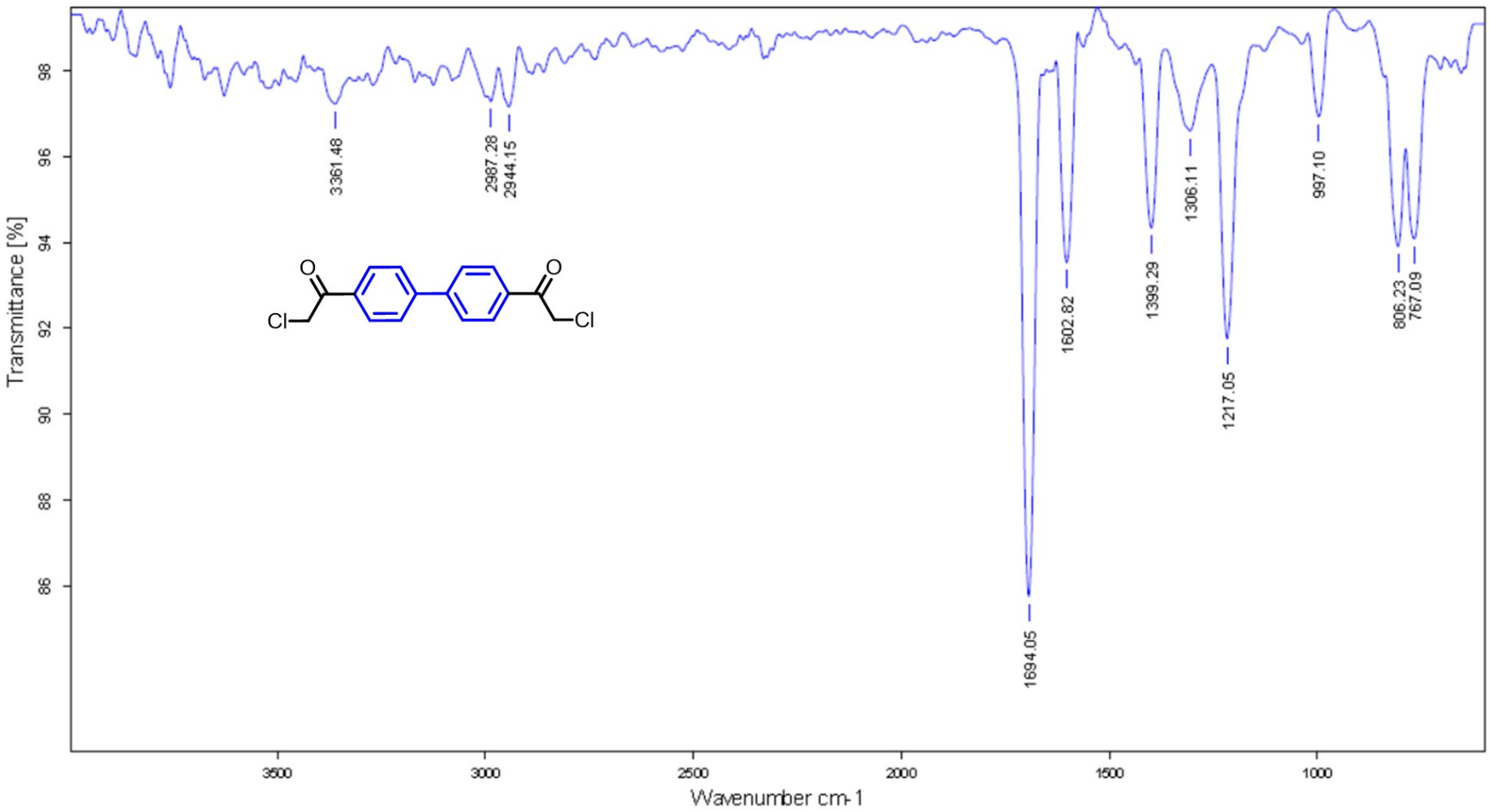


Fig. S7. IR spectra of 1, 1'-([1, 1'-biphenyl]-4, 4'-diyl)bis(2-chloroethan-1-one) **(3)**.

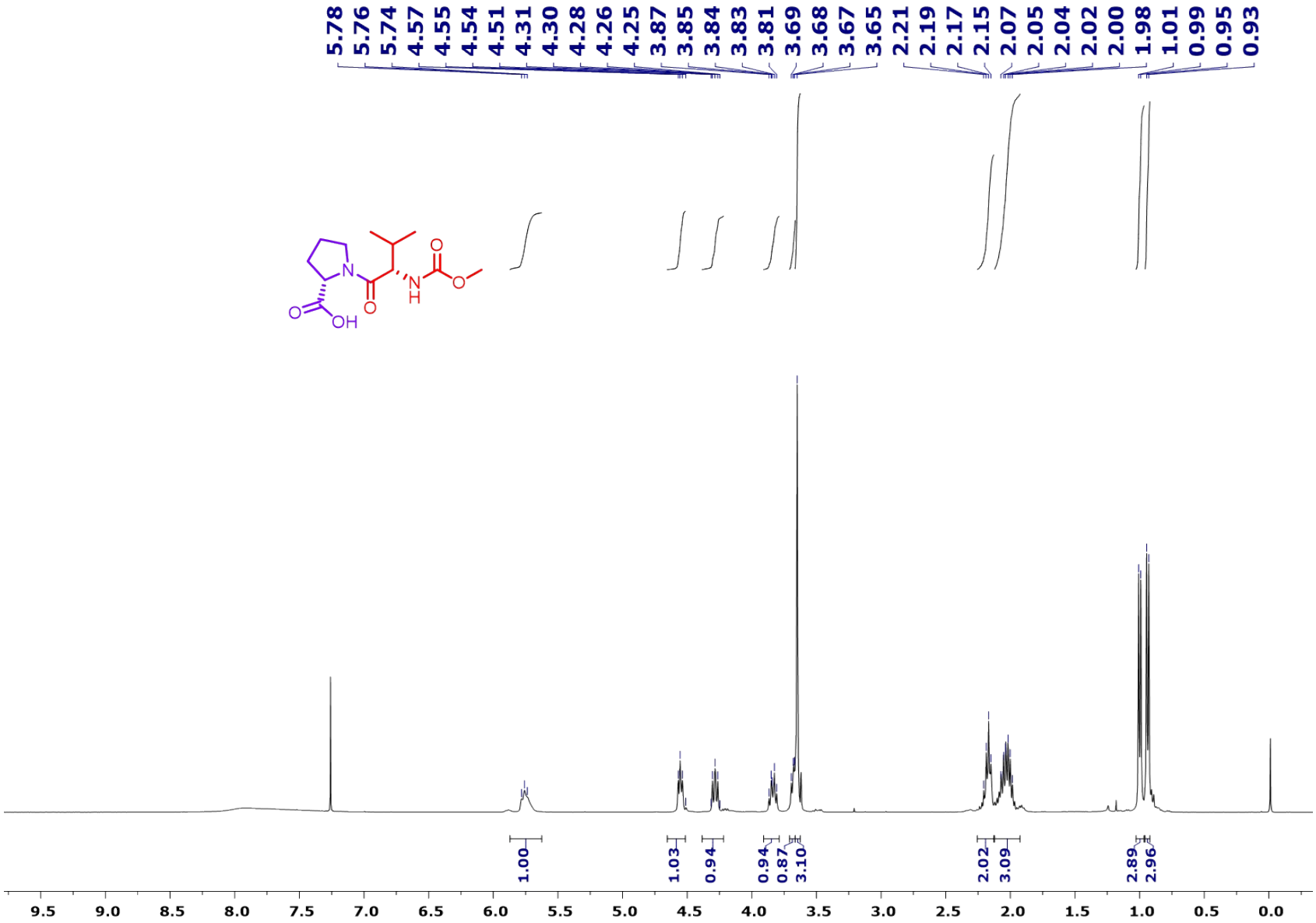


Fig. S8. ^{1}H NMR spectra of (methoxycarbonyl)-L-valyl-L-proline **(4)** in $CDCl_3$.

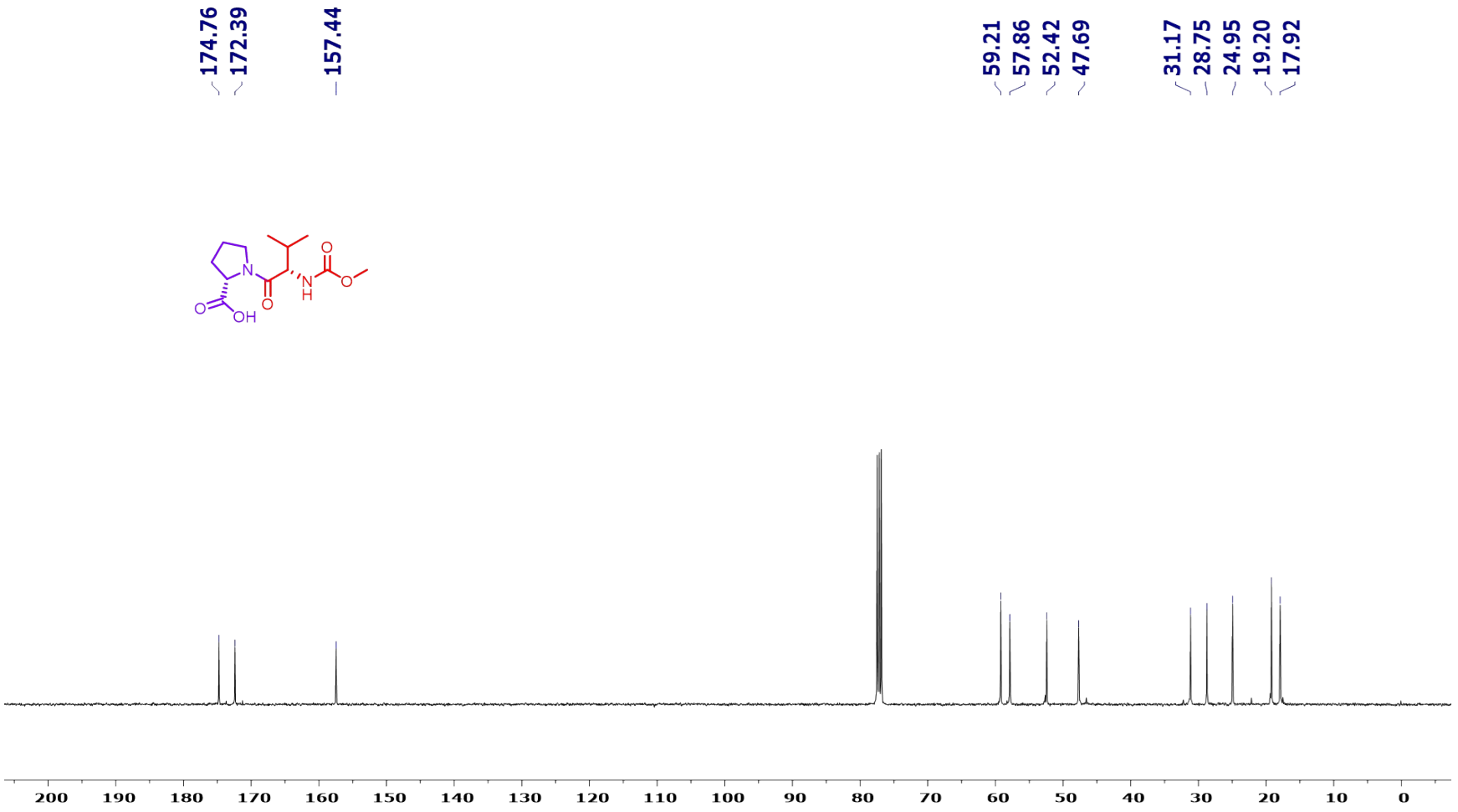


Fig. S9. ^{13}C NMR spectra of (methoxycarbonyl)-L-valyl-L-proline **(4)** in $CDCl_3$.

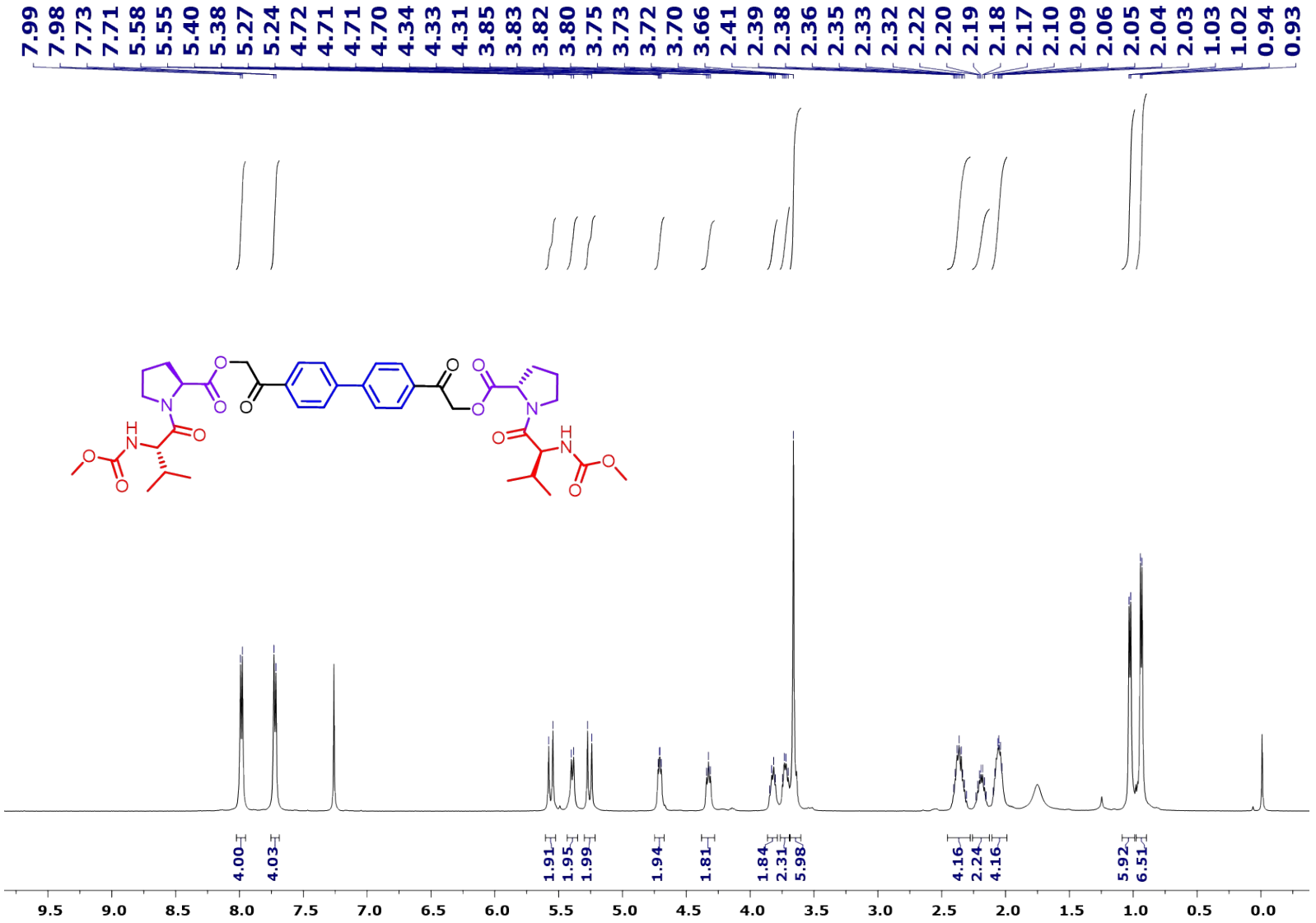


Fig. S10. ^{1}H NMR spectra of DCV intermediate **5.**

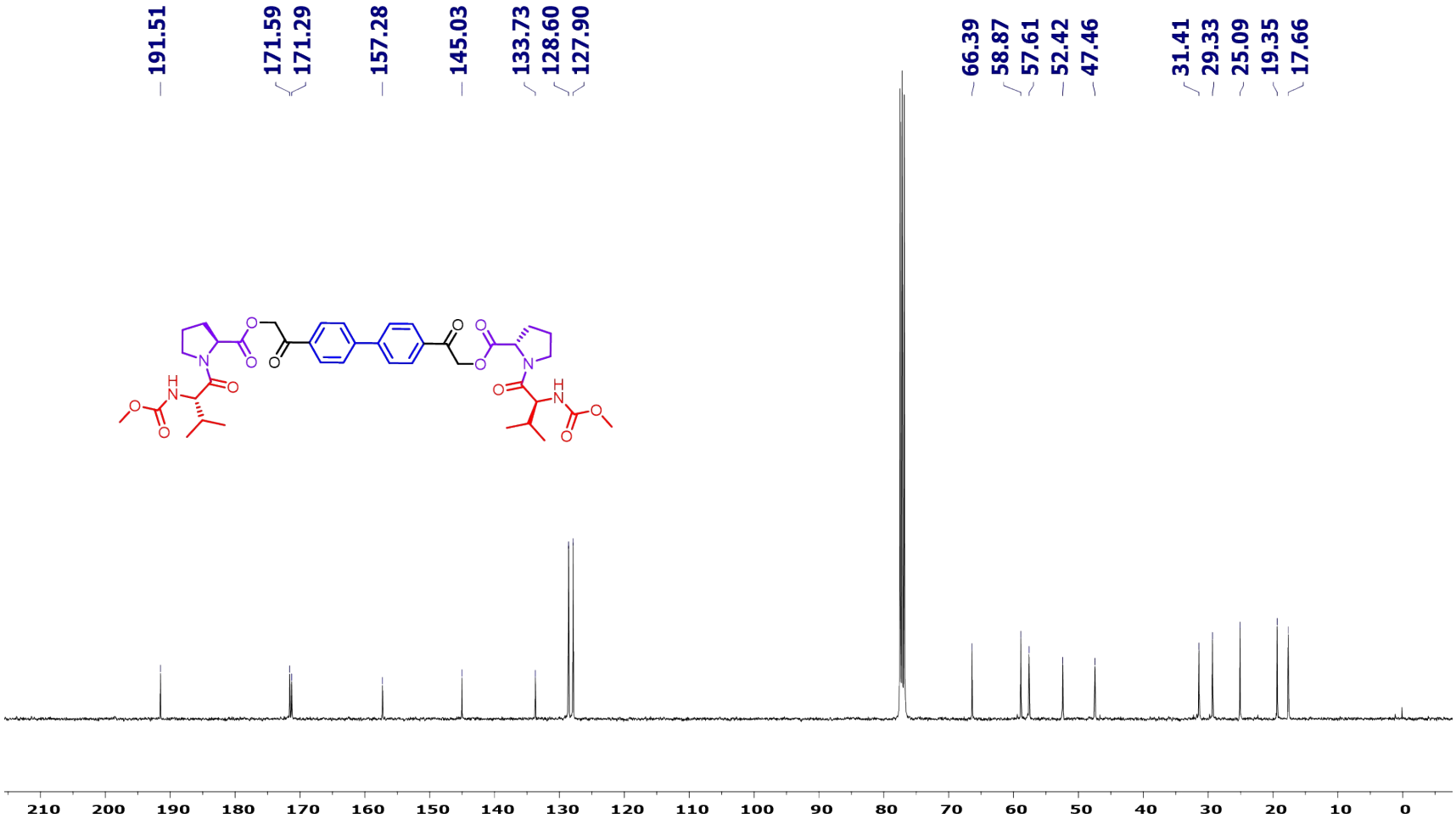
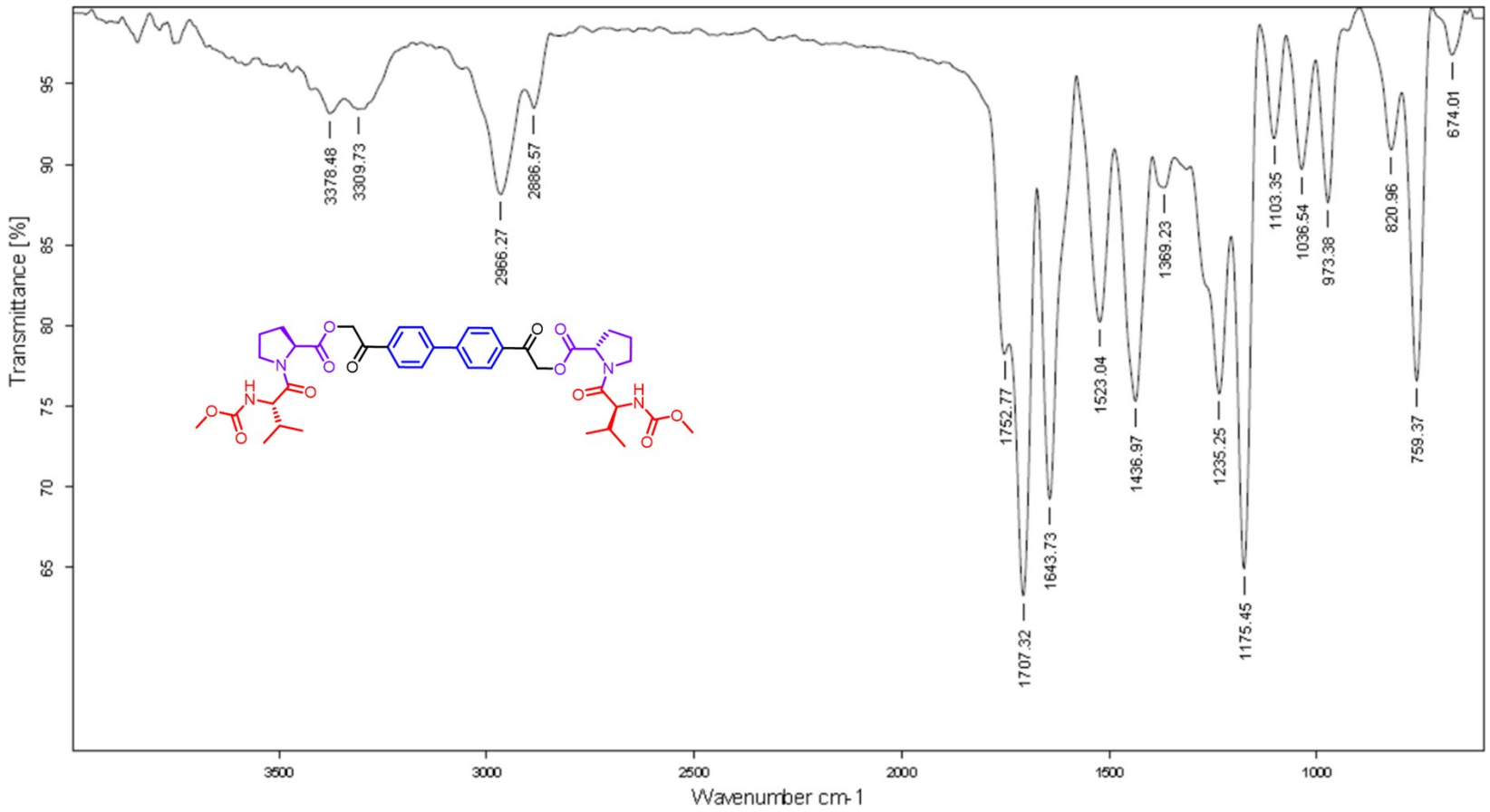


Fig. S11. ^{13}C NMR spectra of DCV intermediate **5.**



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ig. S12. IR spectra of DCV intermediate **5.**

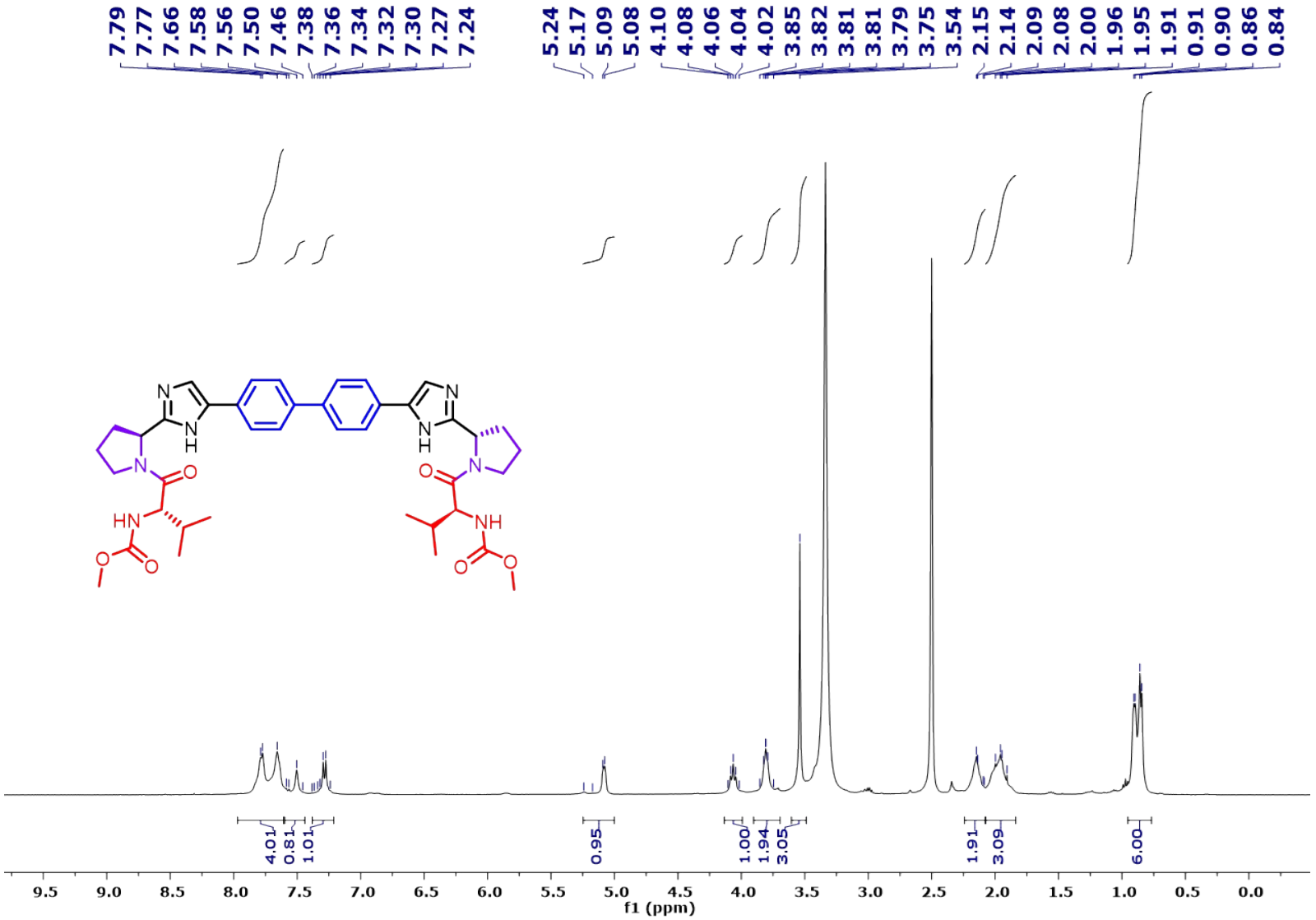


Fig. S13. ^{1}H NMR spectra of DCV free base (**6**) in DMSO-d_6.

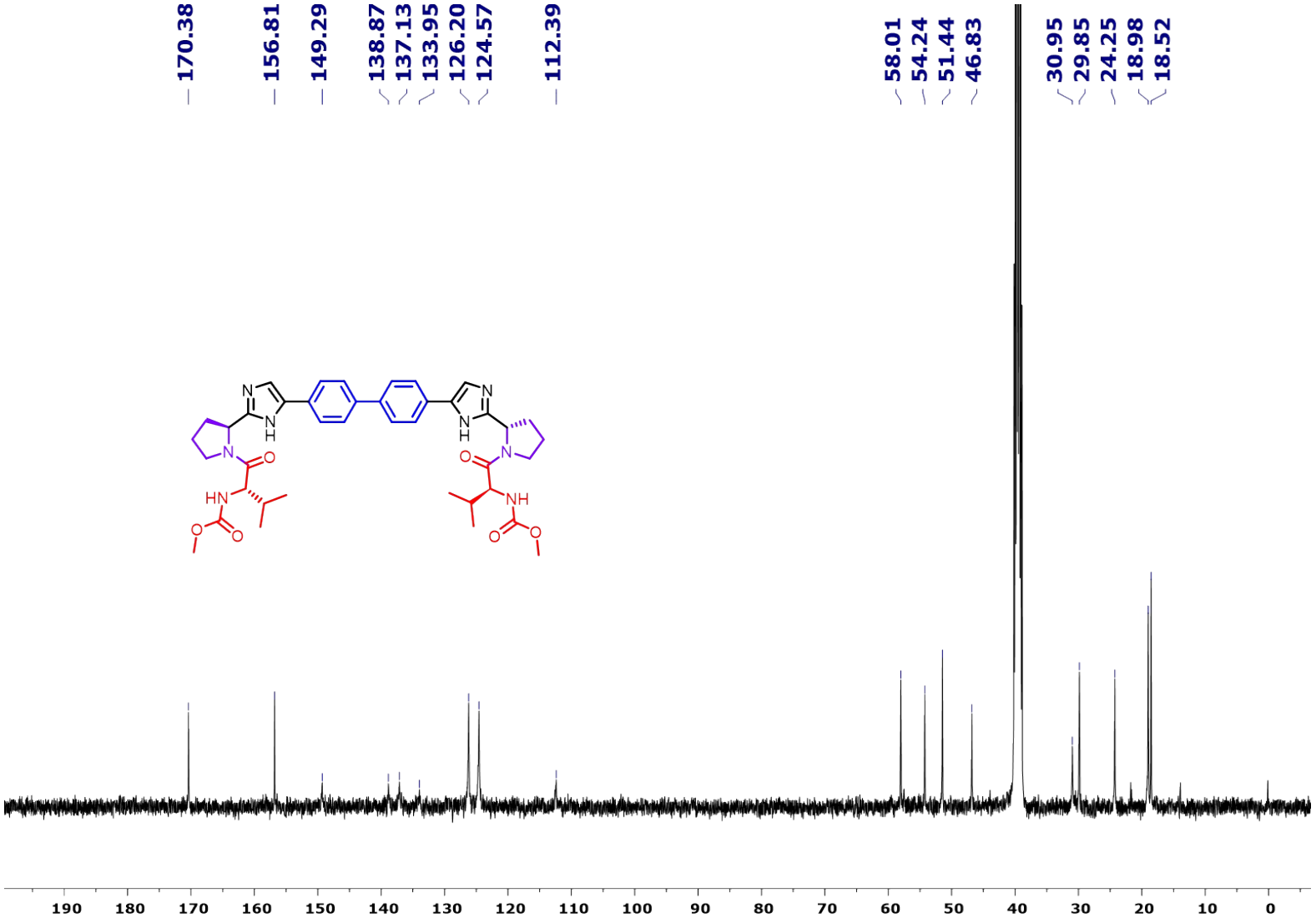


Fig. S14. ^{13}C NMR spectra of DCV free base (**6**) in DMSO-d_6.

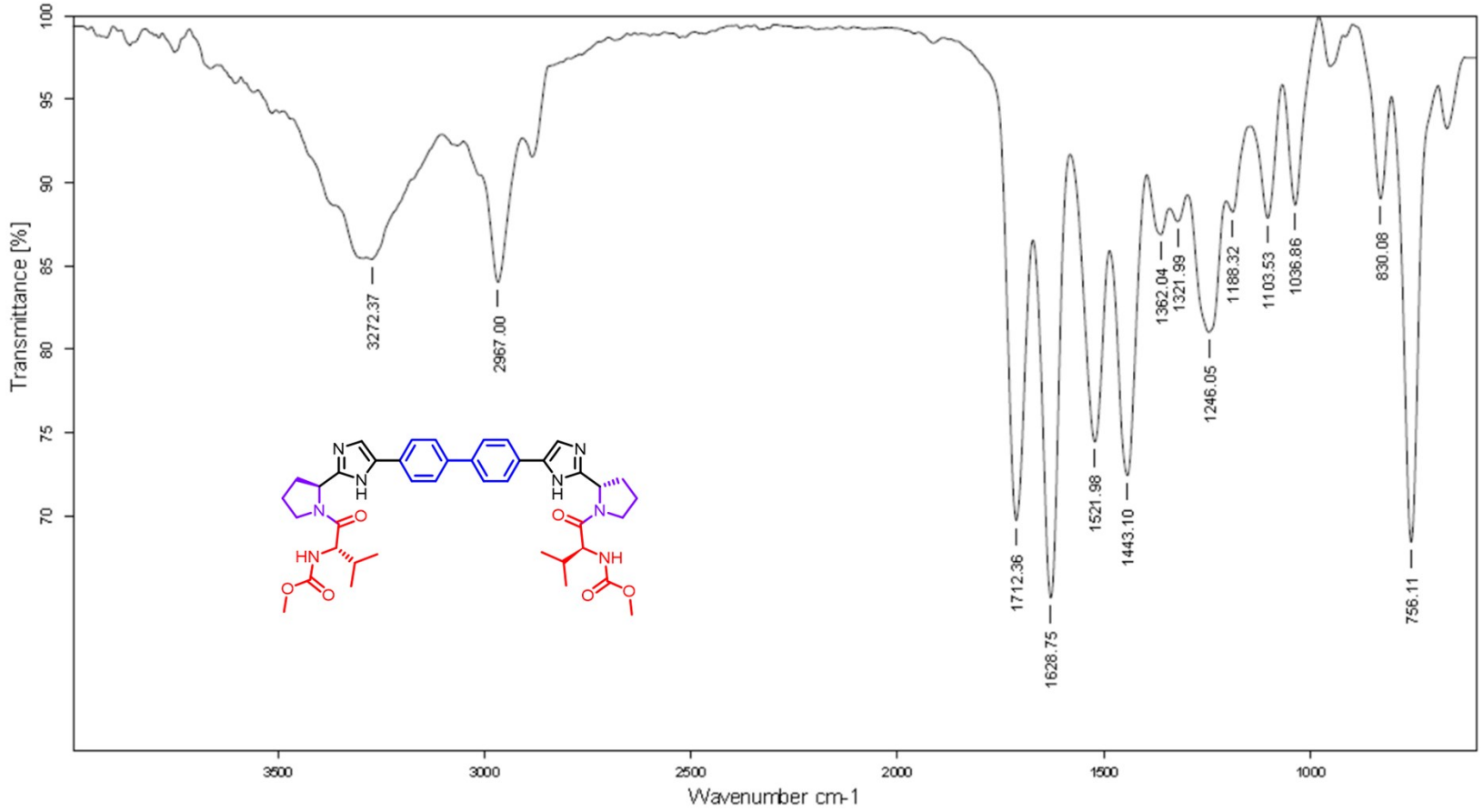


Fig. S15. IR spectra of DCV (**6**).

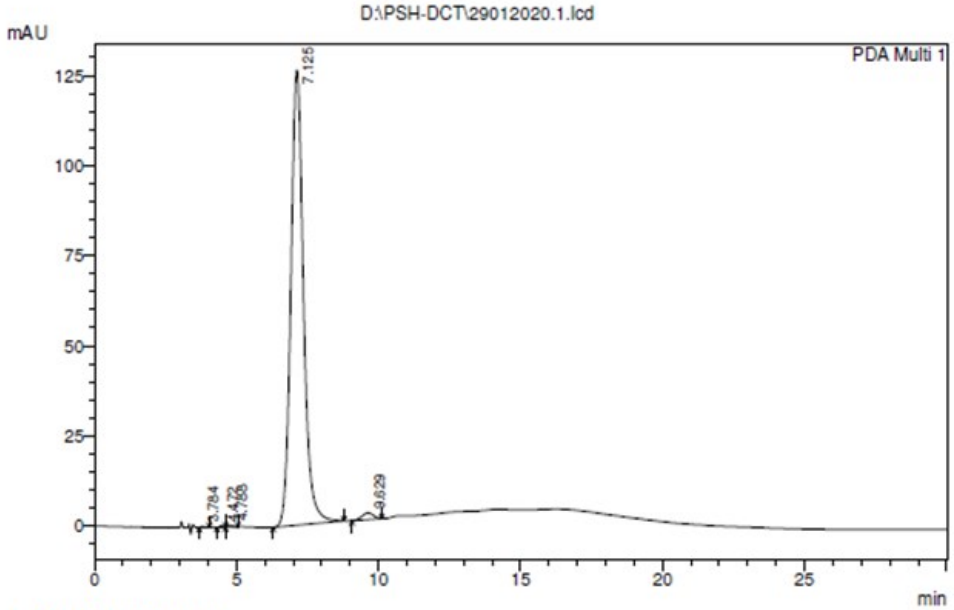
==== Shimadzu LCsolution Analysis Report ====

D:\PSH-DCT\29012020.1.lcd

Acquired by	: Manjula
Sample Name	: PSH-AB-738-A
Sample ID	: PSH-AB-738-A
Tray#	: 1
Vail #	: 1
Injection Volume	: 10 uL
Data File Name	: 29012020.1.lcd
Method File Name	: 29012020.lcm
Batch File Name	:
Report File Name	: Default.lcr
Data Acquired	: 1/29/2020 10:27:14 AM
Data Processed	: 1/29/2020 11:34:52 AM

COLUMN: CHIRAL PAK ID 250 X 4.6mm 5u
MOBILE PHASE : GRADIENT (MEOH (0.1% DEA) IN ACN (0.1% DEA)
FLOW RATE: 1ml/min

<Chromatogram>



1 PDA Multi 1/320nm 4nm

PeakTable

PDA Ch1 320nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.784	2928	339	0.074	0.262
2	4.472	4376	421	0.110	0.325
3	4.788	6664	531	0.168	0.410
4	7.125	3894735	126310	98.291	97.545
5	9.629	53737	1888	1.356	1.458
Total		3962440	129489	100.000	100.000

Fig. S16. HPLC data of the DCV free base.

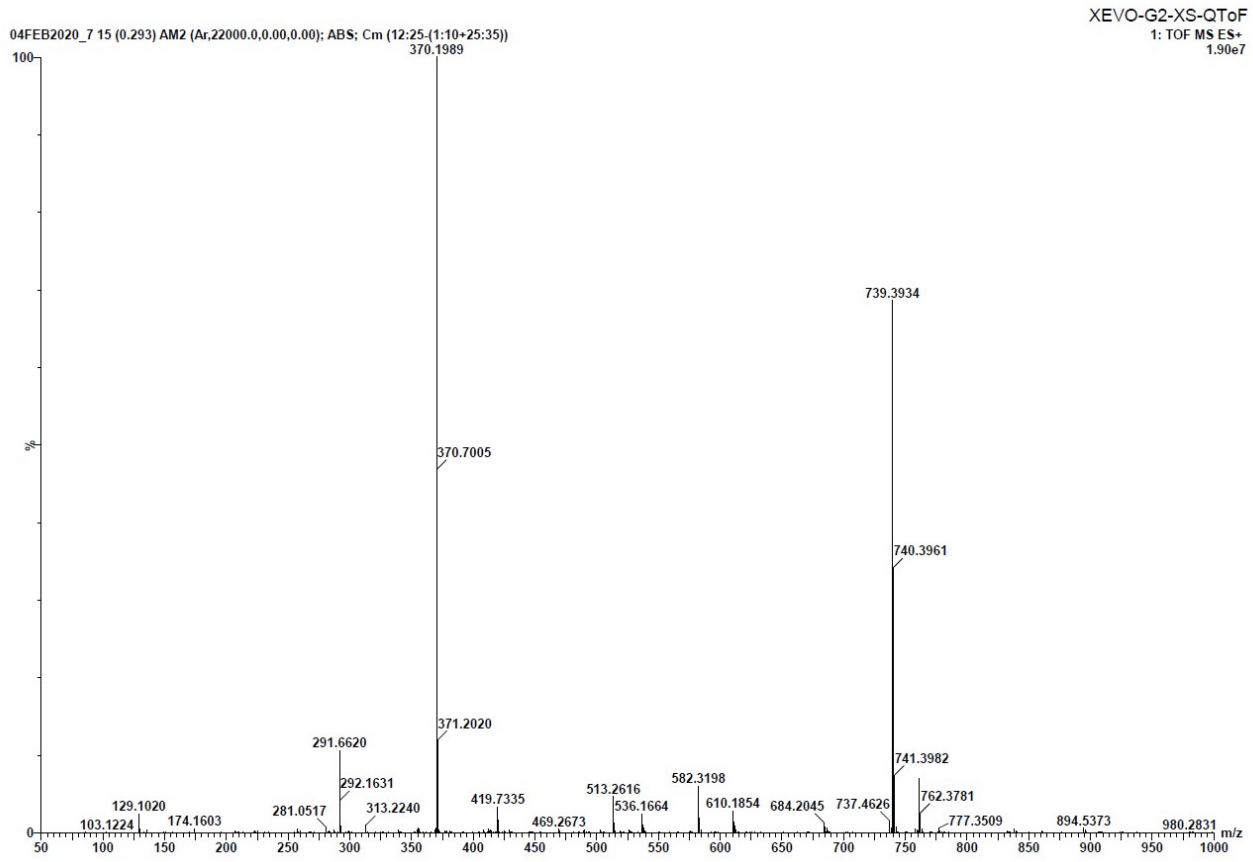


Fig. S17. HRMS data of the DCV free base.

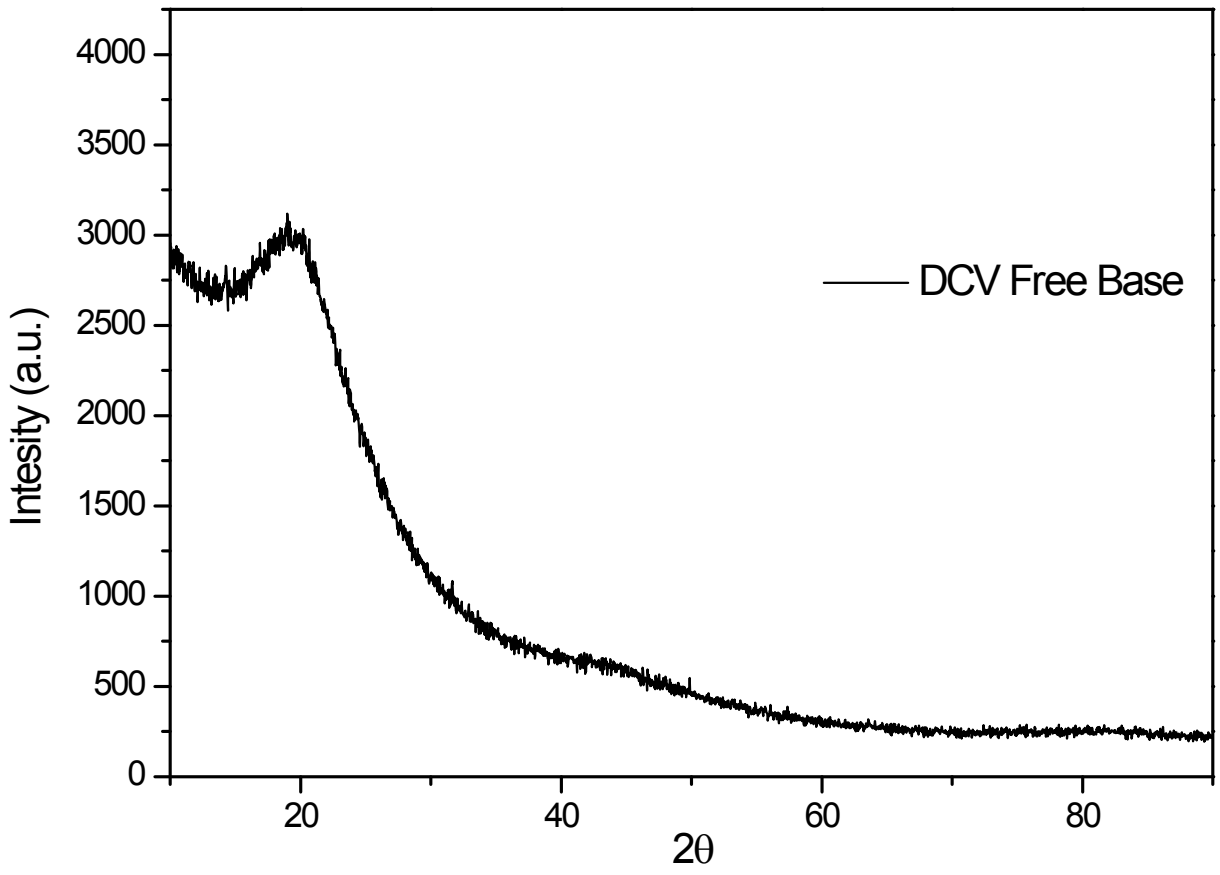


Fig. S18. Powder XRD of DCV free base.

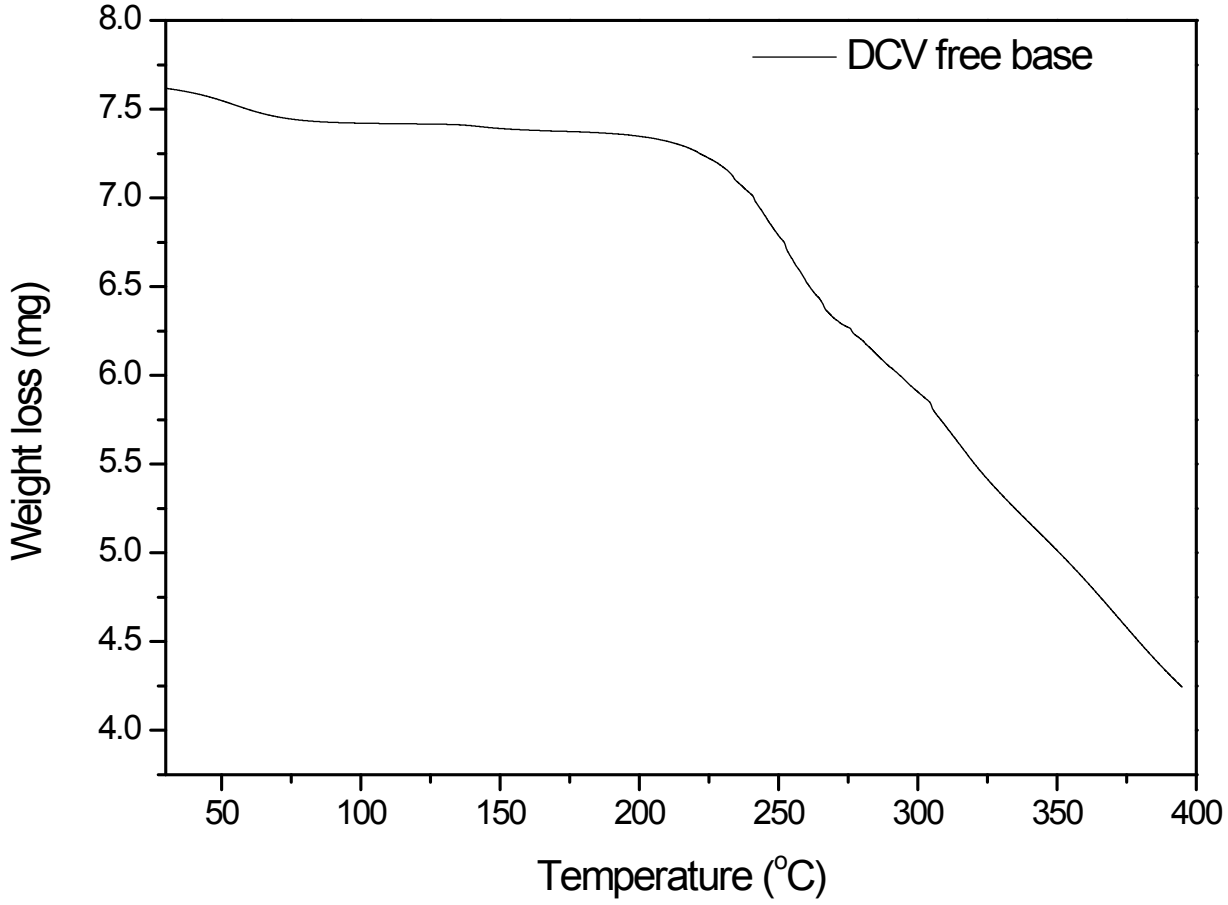


Fig. S19. Thermo-gravimetrical analysis data of the DCV free base.

4. Supporting references.

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