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A Novel, Greener Synthesis and DFT Studies of Stereoisomers of Daclatasvir

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A novel and green method is reported to synthesize the stereoisomers of daclatasvir, an antiviral agent. This work mainly explains the synthesis and characterization of two stereoisomers (*S*,*S*,*R*,*S*) and (*R*,*S*,*R*,*R*) of daclatasvir. The slow addition procedure was designed in order to get high yields in the presence of green solvents like DMSO, 2-methyl tetrahydrofuran and isopropyl alcohol. To gain a better understanding of the stability of intermediates, the DFT calculations were also carried out and presented.

Keywords: Daclatasvir, Stereoisomers, Synthesis, Green solvents.

INTRODUCTION

Around 170 million people globally are infected with hepatitis C virus (HCV), which is a significant quantity compared to other human diseases like HIV. As it is a major human pathogen, it effects the liver to a substantial degree of damage due to this liver cancer and hepatocellular carcinoma diseases were increased in humans around the globe in a rapid manner. HCV therapy has retained efficacy in 40% of patients, however new drugs are needed to tackle this problem, thus developing effective hepatitis C treatments is necessary [1,2].

In HCV infected cell, the polyprotein is cleaved easily and this leads to formation of various proteins including non-structural proteins. To mitigate HCV, substances which inhibit protein particularly NS5A are highly preferable. Based on the extended research daclatasvir, promoted as the name Daklinza in the market, is available as daily oral tablets as hydrochloride salt form to mitigate this problem. However, as an NS5A inhibitor, Daklinza primarily blocks the virus that causes hepatitis C to spread inside the body.

There are few reports are available for the preparation of daclatasvir (Fig. 1), Hui *et al*. [3] reported the condensation reaction of daclatasvir key intermediate (*S*,*S*) isomer hydrochloride and *N*-methyloxycarbonyl-L-valine in presence of base and dichloromethane/ethyl acetate to obtain daclatasvir. However, in this method, the usage of dichloromethane (DCM) is the major setback with the reference to green chemistry. Jingping *et al.* [4] reported the condensation between *N*-(methoxycarbonyl)-L-valine and daclatasvir key intermediate (*S*,*S*) isomer hydrochloride in presence of coupling reagent EDC·HCl and 2-oxime ethyl cyanoacetate as catalyst. But in this method, the yield is low and hazardous chemicals usage was drawback of the process. Qing *et al*. [5] esterified 4,4′-*bis* (2-halogenated acetyl)biphenyl as raw material with *N*-(methloxycarbonyl)-L-valine-L-proline in presence of organic solvents and alkali medium, then carrying out cyclodehydration with ammonium acetate followed by reaction with alkali to obtain daclatasvir. The high cost starting materials and poor overall yield is the major setback of this synthetic method.

The commercial daclatasvir configuration possesses four chiral centers (*S*,*S*,*S*,*S*). Based on the chemical structure two stereoisomers out of all possible stereoisomers were intended to synthesize, which are crucial stereoisomers since these diastereomers having different configuration at middle chiral centers. Even though some patents are reported for the preparation of marketed daclatasvir [3-6], however, no reports are available for daclatasvir isomers. In this perspective, we present the synthesis of daclatasvir impurities by using greener technologies and characterization of stereoisomers.

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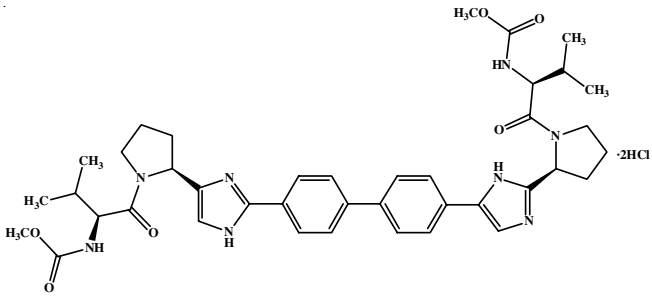


Fig. 1. Chemical structure of daclatasvir dihydrochloride

EXPERIMENTAL

All the chemicals, reagents and solvents were procured from the commercial sources and utilized without additional purification. The IR spectra were recorded on Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan). The progress of reactions was supervised by TLC and HPLC techniques. ^{1}H and ^{13}C NMR spectra of the synthesized compounds were recorded on a Bruker 500 MHz spectrometer in DMSO-d_6 using TMS as internal standard and mass spectra were recorded on a LC/MS/MS Mass Spectrometer (3200 QTRAP, AB Sciex Instruments).

Synthesis of intermediate 3: To a stirred mixture of 4,4-*bis*(bromomethyl)biphenyl (92 g, 0.232 mol) in DMSO, (750 mL) Boc protected L-proline (50 g, 0.232 mol) and 65% w/w NaH (9.4 g, 0.254 mol) in DMSO (750 mL) was added dropwise at room temperature for a period of 4 h with continuous stirring. Thereafter, water (4.5 L), EtOAc (1.5 L) were added, after 0.5 h filtered through hyflo filter, then treated with brine solution (1 L) and separated the organic and aqueous layer. Distilled out the organic layer, crude mass was purified with column chromatography (eluent: 35-40% v/v ethyl acetate in hexanes) to afford 85 g, 70% yield intermediate **3**. ^{1}H NMR: δ 8.11-8.06 (m, 2H), 8.05-8.01 (m, 2H), 7.78-7.73 (d = 10 *J*, 4H), 5.62-5.19 (m, 2H), 4.72 (m, 1H), 4.50-4.39 (m, 2H), 3.57-3.42 (m, 2H), 2.40-2.32 (m, 2H), 2.08-2.03 (m, 1H), 1.96-1.92 (m, 1H), 1.43 (s, 9H); MS: *m/z* 531 $[M+1]^+$.

Synthesis of intermediate 4: To compound **3** (30 g, 0.0567 mol) in DMSO (300 mL), Boc protected D-proline (12.2 g, 0.0567 mol) and diisopropyl ethyl amine (8 g, 0.0623 mol) was added at room temperature stirred for 24 h. Thereafter, water (100 mL) and EtOAc (100 mL) were also added. After separation, the organic layer was treated with 10% brine solution and separated organic and aqueous layers. Distilled out the organic layer to afford 35.7 g, 95% yield intermediate **4**. IR (KBr, ν_{max}, cm^{-1}): 3446, 2931, 2713, 2344, 1566, 1316, 1278, 1028, 1004, 923, 861, 768, 745, 724, 695, 663, 595, 560, 544, 477, 464; ^{1} H NMR: δ 8.11 (d *J* = 4.5, 4H), 8.09 (d, *J* = 4, 4H), 5.65-5.49 (m, 4H), 4.40-4.35 (m, 2H), 3.42-3.31 (m, 4H), 2.39-2.23 (m, 2H), 2.19-2.13 (m, 2H), 1.93-1.85 (m, 4H), 1.40 (s, 9H), 1.39 (s, 9H); MS: *m/z* 687 $[M+Na]^+$; HPLC: 91.58%.

Synthesis of intermediate 5: To a compound **4** (35 g, 0.052 mol) in toluene (350 mL) ammonium acetate was added (40 g, 0.052 mol) at ambient temperature thereafter raised the temperature to 95 ºC and stirred for 18 h. After cooling it to 80 ºC, purified water was added (350 mL) followed by filtration and distilled out filtrate to afford 30.7 g, 70% yield intermediate **5**. IR (KBr, ν_{max}, cm^{-1}): 3909, 3786, 3411, 2939, 2886, 1921, 1614, 1544, 1443, 1347, 1326, 1314, 1296, 1249, 1078, 1022, 1003, 968, 945, 921, 902, 857, 717, 590, 558, 535, 509; ^{1}H NMR: δ 11.91 (brs, 1H), 11.85 (brs, 1H), 7.89-7.66 (m, 8H), 7.51 (s, 2H), 4.85-4.79 (m, 2H), 3.55-3.41 (m, 2H), 3.38-3.30 (m, 2H), 2.20-2.15 (m, 2H), 2.01-1.85 (m, 6H), 1.31(S, 9H), 1.21 (s, 9H); MS: *m/z* 625 $[M+1]^+$; HPLC: 83.49%.

Synthesis of intermediate 6: To compound **5** (20 g, 0.032 mol) in purified water (24 mL), isopropyl alcohol (56 mL) and conc. HCl (33.4 mL, 0.32 mol) at 27 ºC were added. The reaction mass was heated to 50 ºC and stirred for 4 h. Then the reaction mass was diluted with isopropyl alcohol (250 mL) and stirred again for 30 min. Thereafter cool to 10 ºC and filtered the solid to afford 16.9 g, 90% yield intermediate **6**. IR (KBr, ν_{max}, cm^{-1}): 3852, 3817, 3745, 3709, 3670, 2131, 1333, 1289, 1255, 1177, 1126, 1093, 1078, 1046, 1017, 970, 945, 915, 881, 757, 573, 531, 504; ^{1}H NMR: δ 10.5 (brs, 2H), 9.9 (brs, 2H), 8.17 (S, 2H), 8.05-8.01 (d, 4H), 7.93-7.89 (d, 4H), 5.06-5.02 (m, 2H), 3.78-3.74 (m, 2H), 3.42-3.38 (m, 2H), 2.40 (m,4H), 2.21-2.19 (m, 2H), 2.06-2.02 (m, 2H); MS: *m/z* 425 $[M+1]^+$; HPLC: 93.35%

Synthesis of (*S*,*S*,*R*,*S*)-daclatasvir (7): To a mixture of 1-hydroxybenzotriazole (3.36 g, 0.02192 mol), *N*-(methoxycarbonyl)-L-valine (3.7 g, 0.02105 mol) and EDC·HCl (3.95 g, 0.0206 mol), 2-methyl tetrahydrofuran (50 mL), compound **6** (5 g, 0.00877 mol) and diisopropylethyl amine (4.53 g, 0.03508 mol) was added at 5 ºC. The temperature of the reaction mixture was raised to 15-20 ºC and stirred for 15 h. After completion, the reaction mass was washed with 13% brine solution (30 mL) and then treated with mixture of 1 N NaOH solution. Separate the organic and aqueous layers using separating funnel. The organic layer was dried and then crude mass was purified using column chromatography (eluent: 5% MeOH in CH_2Cl_2) to obtain 4.96 g, 71% yield (*S*,*S*,*R*,*S*)-daclatasvir **7**. IR (KBr, ν_{max}, cm^{-1}): 3276, 3059, 1916, 1003, 945, 921, 895, 857, 828, 732, 639, 608, 559, 523; ^{1}H NMR: δ 12.06 (brs, 1H), 11.77 (brs, 1H), 7.82-7.61 (m, 8H), 7.50-7.43 (m, 2H), 7.29-7.25 (m, 2H), 5.08-5.01 (m, 2H), 4.11-4.01 (m, 2H), 3.86-3.79 (m, 4H), 3.62-3.49 (m, 6H), 2.23-1.83 (m, 10H), 0.91(d, 6H), 0.81 (d, 6H); MS: *m/z* 739.4 $[M+1]^+$; HPLC purity: 92.16%.

Synthesis of (*R*,*S*,*R*,*R*)-daclatasvir (8): A mixture of 1-hydroxybenzotriazole (3.36 g, 0.02192 mol), *N*-(methoxycarbonyl)-D-valine (3.70 g, 0.02105 mol) and EDC·HCl (3.95 g, 0.0206 mol) in 2-methyl tetrahydrofuran (50 mL) at 20-30 ºC and stirred for 1 h. Thereafter cool to 0-5 ºC, added compound **6** (5 g, 0.00877 mol) and diisopropylethyl amine (4.53 g, 0.03508 mol) to the above solution. Then raised the temperature up to 15-20 ºC and stirred again for 15 h. After completion of maintenance, treated reaction mass with 13% NaCl solution (30 mL) followed by treated with mixture of 1molar sodium hydroxide solution and 13% NaCl solution (30 mL). Separated both organic and aqueous layers, the organic layer was dried and distilled out 2-methyl tetrahydrofuran solvent. The crude mass was purified with column chromatography (eluent: 5% v/v methanol in CH_2Cl_2) to afford 4.96 g, 71% yield (*R*,*R*,*S*,*R*)-daclatasvir (**8**). IR (KBr, ν_{max}, cm^{-1}): 3278, 2191, 1915, 970,

945, 921, 895, 857, 728, 639, 608, 560, 522; ^{1}H NMR: δ 12.05 (brs, 1H), 11.61 (brs, 1H), 7.93-7.79 (m, 4H), 7.79-7.61 (m, 5H), 7.60-7.25 (m, 3H), 5.71-5.59 (m, 1H), 5.19-5.09 (m, 1H), 4.19-4.01 (m, 2H), 3.99-3.81 (m, 1H), 3.73-3.51 (m, 9H), 2.39-1.85 (m, 9H), 1.80-1.70 (m,1H), 0.95 (d, 6H), 0.75 (d, 3H), 0.35 (d, 3H); MS: *m/z* 739.3 $[M+1]^+$; HPLC purity: 91.45%.

RESULTS AND DISCUSSION

During the synthesis of daclatasvir dihydrochloride, there is possibility to form chiral and achiral impurities. As per ICH Q3A guidelines any known related substance need to control to less than 0.15% and unknown related substance control to less than 0.10% in drug substance. In the reported literature chiral isomer impurities are separated through HPLC methods but preparation is not available. The Qin Ye-feng [6] reported the HPLC to separate the chiral impurities in daclatasvir hydrochloride, *i.e.* (a*S*, b*S*, c*S*, d*S*), (a*R*, b*S*, c*S*, d*S*), (a*S*, b*S*, c*R*, d*S*), (a*R*, b*S*, c*S*, d*R*), (a*S*, b*R*, c*R*, d*S*) and the self-control of (a*R*, b*R*, c*R*, d*R*).

As per literature survey, the preparation procedures are not reported for the stereoisomes, hence present approach focused on the preparation of two crucial stereoisomers of daclatasvir. This study investigated the novel approaches to green synthesis of daclatasvir isomers. The 3rd and 5th principles of green chemistry demand for the use of safer solvents and the implementation of less hazardous chemical synthesis [7]. Accordingly, the initial reaction in the synthetic process was investigated with various polar aprotic (class 3 solvents) such as anisole, MeOAc, EtOAc, BuOAc, ^{i}BuOAc, ^{i}PrOAc, MTBE and DMSO in different combinations. The reaction conditions with different bases and solvents are shown in Table-1.

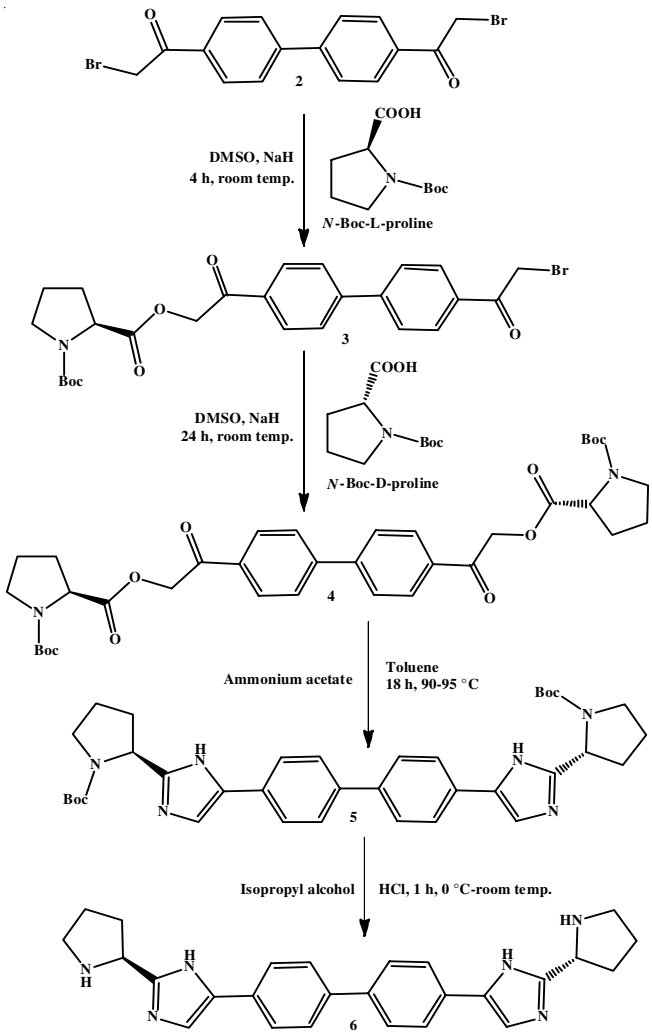
TABLE-1
OPTIMIZATION OF SOLVENT[a,b]

Base	Solvent	% of Product **3** (fast addition)	% of Product **3** (slow addition up to 3 h)
DIPEA	Anisole	31	46
	MeOAc	33	47
	EtOAc	30	46
	BuOAc	29	44
	^{i}BuOAc	30	42
	^{i}PrOAc	34	43
	MTBE	39	54
	DMSO	40	63
Imidazole	Anisole	31	46
	MeOAc	33	47
	EtOAc	30	46
	BuOAc	29	44
	^{i}BuOAc	30	42
	^{i}PrOAc	34	43
	MTBE	39	54
	DMSO	40	63
NaH	Anisole	31	46
	MeOAc	33	47
	EtOAc	30	46
	BuOAc	29	44
	^{i}BuOAc	30	42
	^{i}PrOAc	34	43
	MTBE	39	54
	DMSO	40	75

[a]1 mmol compound **2** and 1.1 mmol base and solvent (10 mL); and [b]Isolated yields.

Ester functional group containing solvents were resulted moderate yields, better yield was observed with DMSO and MTBE solvents. Out of different solvents, DMSO was found to be a better option in two different ways (i) in terms of generating relatively better yields and (ii) to employ class 3 solvent (less harmful solvent in terms of green chemistry) based on solvent selection guidelines from GSK, AstraZeneca and the ACS GCI.

Initially, sodium hydride and BOC protected L-proline in DMSO was added to a predissolved solution of dibromo compound **2** in DMSO at room temperature only 40% of desired product along with disubstituted compound produced only moderate yield after 4 h stirring. When sodium hydride and BOC protected L-proline in DMSO was added dropwise to a predissolved solution of dibromo compound **2** in DMSO at room temperature (implementation of 6th principle of green chemistry in terms of energy efficiency and 12th principle of green chemistry in terms of accident prevention) up to 3 h, after additional 1 h stirring at same temperature remarkable yields were observed. Yield of the process was always increased with the slow addition process.



Scheme-I: Preparation of key intermediate **6**

Later compound **3** was reacted with BOC protected D-proline in DMSO, which resulted in the formation of compound **4** with excellent yield. Cyclization of two ester functionalities in compound **4** with 2 eq. of ammonium acetate afforded (*S*,*R*)-*bis* Boc cyclized compound **5** with 70% yield. The two BOC groups were deprotected in compound **5** with HCl in isopropyl alcohol. During the preparation of crucial stereoisomers of daclatasvir, a common intermediate **6** was synthesized with good yield **Scheme-I**.

After the successful formation of key intermediate **6**, it was planned to synthesize two crucial stereoisomers of daclatasvir by utilizing the environmental less harmful biorenewable solvent 2-methyl tetrahydrofuran, a class 3 solvent. The (*S*,*R*) intermediate **6** was allow to react with N-(methoxycarbonyl)-L-valine in presence of 1-hydroxybenzotriazole and EDC·HCl in 2-methyl tetrahydrofuran to obtain compound (*S*,*S*,*R*,*S*) stereoisomer **7**. Similarly, when (*S*,*R*) intermediate **6** was treated with *N*-(methoxycarbonyl)-L-valine in the presence of 1-hydroxybenzotriazole and EDC·HCl dissolved in 2-methyl tetrahydrofuran get compound (*R*,*S*,*R*,*R*) stereoisomer **8** (**Scheme-II**). It was found that both processes yield 71% of the desired compounds. All the intermediates and final compounds were characterized by using ^{1}H NMR, IR, mass and HPLC spectral techniques.

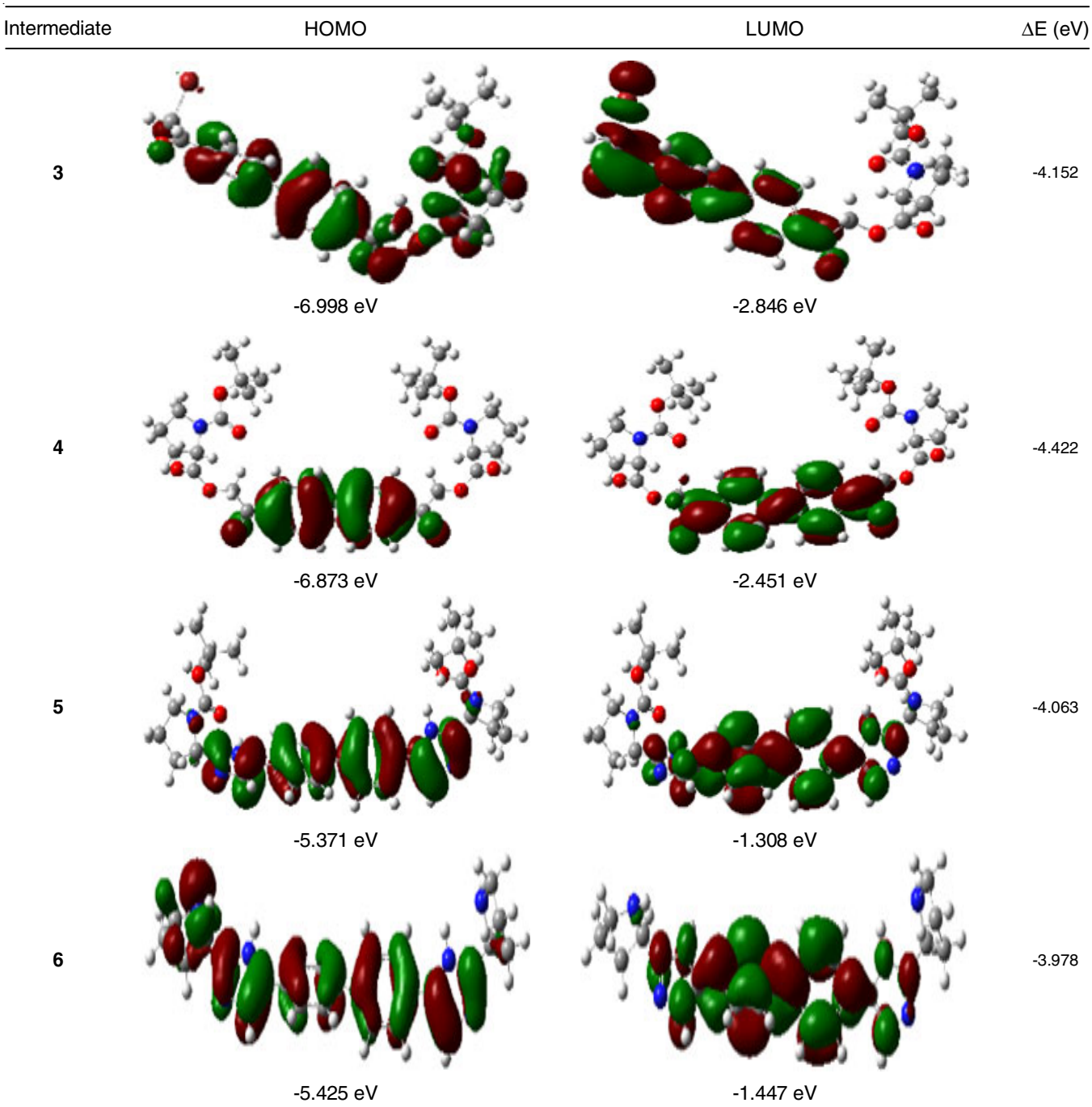
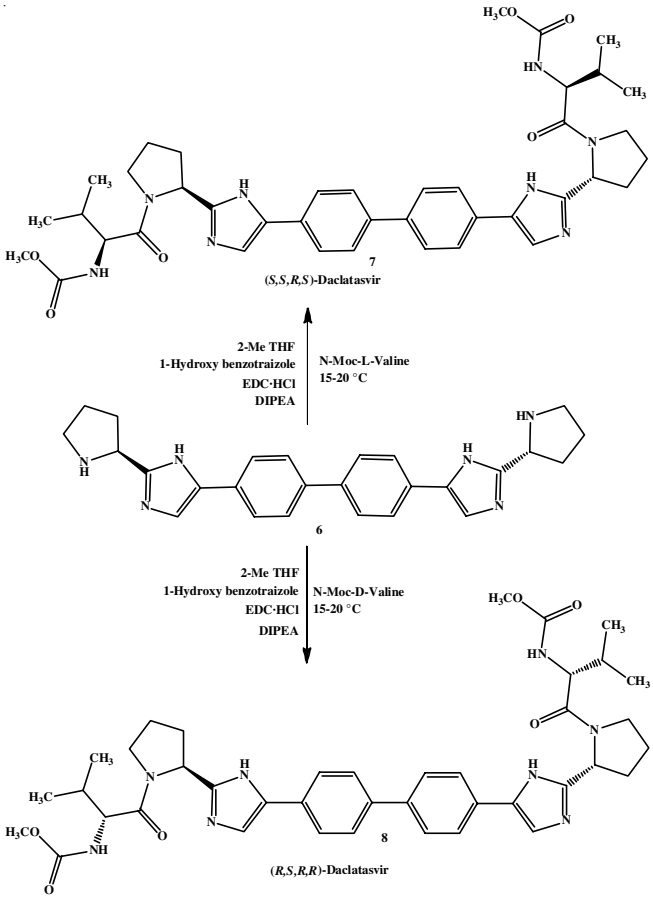


Fig. 2. Frontier molecular orbitals of **3**, **4**, **5** and **6**



Scheme-II: Preparation of (*S*, *S*, *R*, *S*) and (*R*, *S*, *R*, *R*) stereo isomers of daclatasvir

DFT studies: DFT studies were computed using DFT/B3LYP level {6-311++G (d, p) basis set} for compounds **3**, **4**, **5** and **6**. Compounds **3**, **4**, **5** and **6** were shown to have the HOMOs values ranging from -6.998 eV to -5.371 eV and on the other hand LUMOs from -2.846 eV to -1.308 eV (Fig. 2). Therefore, the energy gap values of compounds **3**, **4**, **5** and **6** were found to be between 4.422 eV and -3.978 eV, suggesting that these intermediates were stable enough to be used in a variety of processes.

To predict the kinetics of these intermediates during an interaction with a nucleophiles or an electrophile, electrostatic potential maps were computed. The neutral electrostatic potential region is shown by a light blue shade, partial positive electrostatic potential area is shown by a blue shade, while the negative ve electrostatic potential area is shown by a red shade (Fig. 3).

Conclusion

A straightforward and cost-effective method for separating two stereoisomers of daclatasvir in 5 steps with outstanding yields is reported. Few green chemistry protocols were intended to execute for sustainable environment. The current process is sufficiently robust to produce the required quality of stereoisomers of daclatasvir by controlling the formation of unknown impurities.

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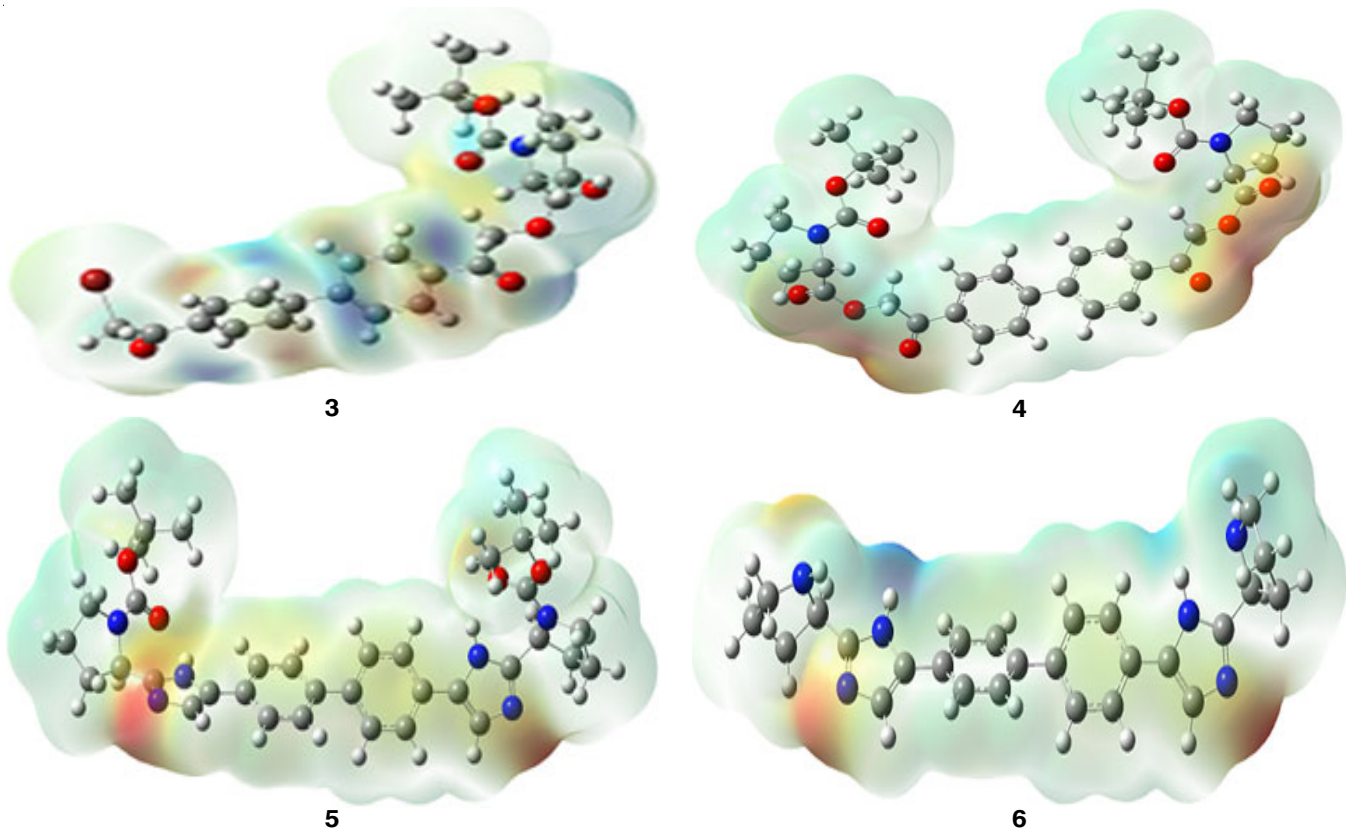

Fig. 3. Electrostatic maps of compounds **3**, **4**, **5** and **6**

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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