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Molecular structures and ab initio molecular orbital calculations of the optically active derivatives of 1-aminocyclopropane-1-carboxylic acid

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Abstract

The novel optically active derivatives of 2,2′-disubstituted-1-aminocyclopropane-1-carboxylic acid (−)-**2** and (+)-**3** were synthesised from the *spiro*-azlactone (+)-**1**. Oxidation of the diol moiety of (+)-**3** gave by ring enlargement the racemic mixture of 2,3-dihydrofuran derivative (±)-**6**. This conversion is explained by stepwise rearrangement of the initially formed tetrasubstituted cyclopropanecarbaldehyde **4** through zwitterionic's reactive intermediate **5**. The formation of (±)-**6** is preferred energetically as established by ab initio calculations of the ground states and possible intermediates for that rearrangement. The crystal structure and absolute configuration of the compounds (+)-**1**, (−)-**2**, (+)-**3** and (−)-**7** were determined by single-crystal X-ray diffraction method. All four compounds possess *Z*-configuration of the cyclopropane ring. The dioxolane ring in the structures (+)-**1** and (−)-**2** adopts *half-chair* conformation, while the cyclopropane ring and geminally substituted groups in the structures (−)-**2**, (+)-**3** and (−)-**7** possess the *anticlinal* conformation. The molecules of the compound (+)-**1** are connected by very weak *inter*molecular hydrogen bond of C-H···O type. In the compounds (−)-**2**, (+)-**3** and (−)-**7** *inter*- and *intra*molecular hydrogen bonds of N-H···O type were observed. The *spiro*-compound (+)-**1** exhibited a more pronounced inhibitory activity against the proliferation of murine leukemia and human T-lymphocytes cells than other type of tumor cell lines and normal human fibroblast cells.

Keywords: 1-Aminocyclopropane-1-carboxylic acid derivatives; Synthesis; Rearrangement; Ab initio MO calculations; Density functional theory, Single crystal X-ray analysis; Cytostatic activities; Antiviral activities

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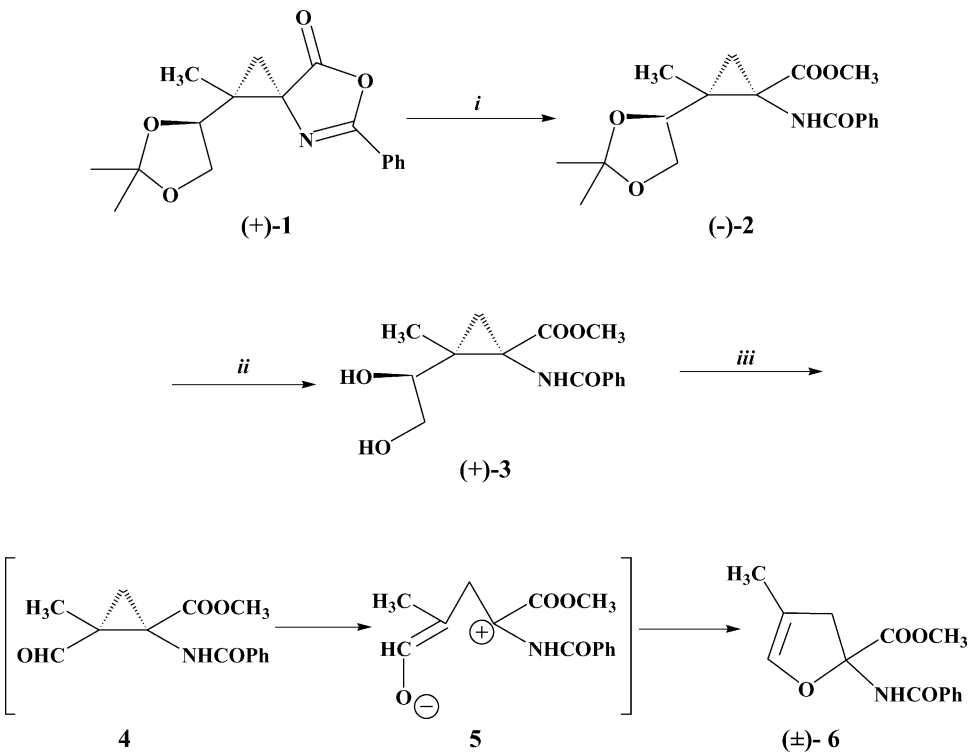
1. Introduction

Cyclopropane derivatives occupy an important role in the synthetic organic chemistry, notably in the synthesis of several natural products [1]. Considerable efforts were directed toward the synthesis of 1-aminocyclopropane-1-carboxylic acid [2] (ACC) and its 2-substituted and 2,2′-disubstituted derivatives [3]. Enantiomerically enriched ACC's have been extensively used in the design and synthesis of conformational constrained peptidomimetics [4,5]. The present study deals with the synthesis of the optically active derivatives of 2,2′-disubstituted 1-aminocyclopropane-1-carboxylic acids (−)-**2** and (+)-**3** and it was undertaken initially to evaluate their cytostatic and antiviral activities. Since exact stereostructure of these molecules were of importance for biological activities and in particular for elucidation of the mechanism of the [1,3]-sigmatropic rearrangement of tetrasubstituted cyclopropane derivative (+)-**3** to **2,3**-dihydrofuran derivative (±)-**6** (Scheme 1), the X-ray crystal structure analyses were performed. In this work we also calculated the energies of the most stable conformers for that rearrangement and compared them with the oxidation of the corresponding trisubstituted cyclopropane derivative (−)-**7**, which under the same reaction condition do not rearrange [6].

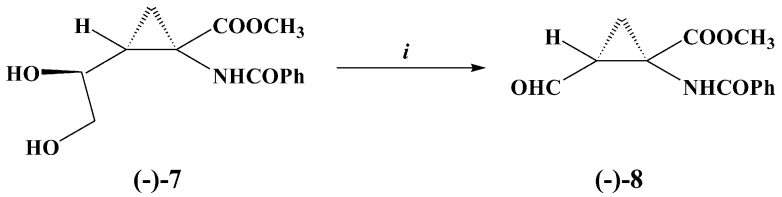
2. Results and discussion

2.1. Synthesis

The synthesis of 2,2′-disubstituted-1-aminocyclopropane-1-carboxylic acid derivatives (−)-**2** and (+)-**3** and the rearrangement of (+)-**3** into 2,3-dihydrofuran derivative (±)-**6** was accomplished as described in Scheme 1. Methanolysis of the azlactone ring of the *spiro*-compound (+)-**1** [7] gave 1-aminocyclopropane-1-carboxylic acid derivative (−)-**2** possessing geminally substituted benzamido and carboxymethyl ester groups. Subsequent acid hydrolysis of the dioxolane moiety of (−)-**2** afforded tetrasubstituted cyclopropane derivative



Scheme 1. Reagents: (*i*) CH_3ONa, CH_3OH; (*ii*) HCl, CH_3OH; (*iii*) $NaIO_4$, THF.



Scheme 2. Reagent: (*i*) $NaIO_4$, THF.

(+)-**3**. Oxidation of the diol moiety of (+)-**3** gave the racemic mixture of the 2,3-dihydrofuran derivative (±)-**6**. The formation of this product is explained by the rearrangement of the initially formed tetra-substituted cyclopropanecarbaldehyde **4** through zwitterionic's reactive intermediate **5**. The structure of the rearranged product (±)-**6** was deduced from their one- and two-dimensional ^{1}H and ^{13}C NMR spectra (see Experimental part).

On the contrary, oxidation of trisubstituted cyclopropane derivative (−)-**7** led under the same reaction condition to the formation of trisubstituted cyclopropanecarbaldehyde (−)-**8** [6] (Scheme 2).

2.2. X-Ray crystal structure study

Molecular Structures.-In order to elucidate the mechanism of the rearrangement of (+)-**3** to (±)-**6** the structures of the compounds (+)-**1**, (−)-**2**, (+)-**3** and (−)-**7** were determined by X-ray crystallographic analysis. The compounds (+)-**1**, (−)-**2**, (+)-**3** and (−)-**7** (Schemes 1 and 2) crystallized in the monoclinic space group $P2_1$ and their perspective views with the atom numbering scheme are displayed in Figs 1–4. The absolute configuration of the carbon atoms C8, C6 and C5 in all four compounds was determined to be *S*, *R* and *S*.

The skeleton of the *spiro*-compound (+)-**1** consists of the cyclopropane, azlactone, phenyl and dioxolane ring (Fig. 1). The values of the bond distances and angles in the cyclopropane, azlactone and phenyl ring are consistent with those found in structurally related derivatives of 1-spiro-(4′(2′-phenyl-5′(4′H)-oxazolone))cyclopropane [8–10]. The dihedral angle between the planes of the cyclopropane and azlactone ring amounts to 88.0(2)°; that is, those rings are almost perpendicular to each other. On the contrary, the phenyl and azlactone ring are nearly coplanar; the dihedral angle between the l.s. planes of those rings is 6.8(1)°.

The dioxolane ring in the compounds (+)-**1** and (−)-**2** exhibits half-chair conformation twisted around the bonds O1–C3 in (+)-**1** (Fig. 1) and C4–C5 in (−)-**2** (Fig. 2). Both compounds exhibit an almost identical conformation of the moiety consisting of the cyclopropane and dioxolane ring; this is

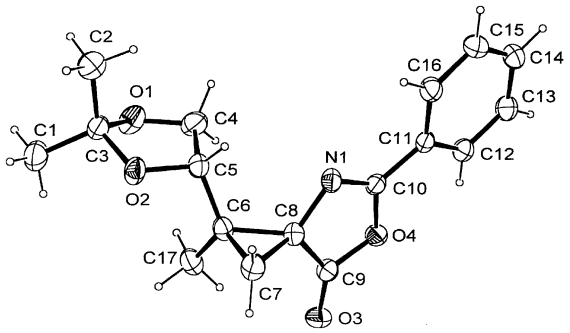


Fig. 1. The molecular structure and labelling of the compound (+)-**1**. Displacement ellipsoids are drawn at the 20% probability level.

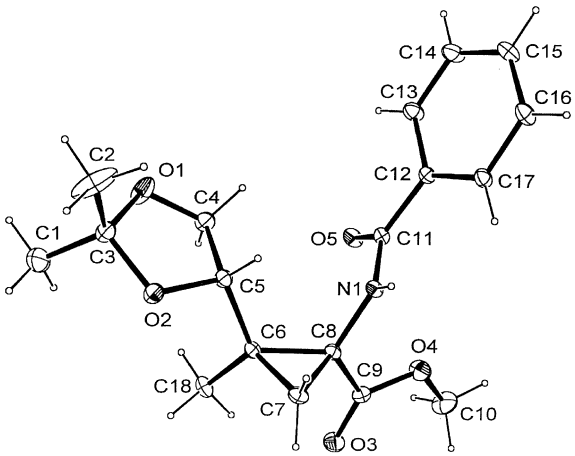


Fig. 2. The molecular structure and labeling of the compound (−)-**2**. Displacement ellipsoids are drawn at the 20% probability level. Disordered atoms C1 and C2 are represented in the Figure in the positions of higher occupancy factor.

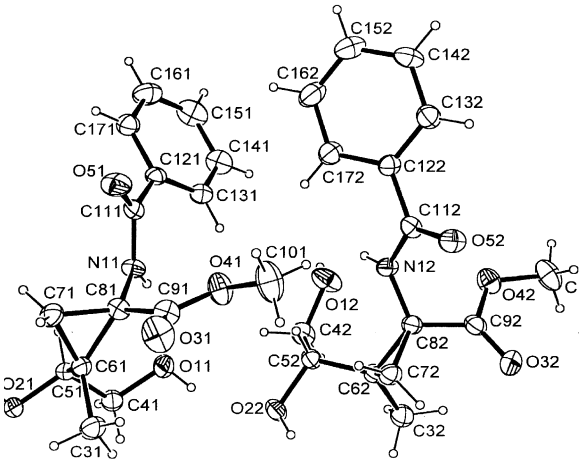


Fig. 3. The molecular structure and labeling of the compound (+)-**3**. Displacement ellipsoids are drawn at the 20% probability level.

shown by the values of the torsion angles C4–C5–C6–C7 and O2–C5–C6–C7 amounting 156.6(2)° and −87.0(3) in (+)-**1** appropriate and 156.0(2)° and −88.8(2)° in (−)-**2**.

The bond distances between the equatorial (C1–C3) and axial (C2–C3) bond of the dioxolane ring in (+)-**1** were found to be equal within standard uncertainties [1.499(4) and 1.504(4) Å], although the difference between those bond distances in the similar dioxolane substituted cyclopropanes were found to be even 0.11 Å [11].

The common structural feature of the compounds (−)-**2** (Fig. 2), (+)-**3** (Fig. 3) and (−)-**7** (Fig. 4) is benzamido and methylcarboxylate group substituted at the atom C8 of the cyclopropane ring. Beside this, the compounds (+)-**3** and (−)-**7** have the common diol-substituent at the atom C6 of the cyclopropane ring as well as two crystallographically independent molecules in the unit cell. All three compounds possess *Z*-configuration of the cyclopropane ring. The values of the torsion angle N1–C8–C6–C5 only slightly deviate from the standard value for *cis*-substituted cyclopropanes (Table 1).

The cyclopropane ring has many properties which are found to be more similar to those of the vinyl group; e.g. the hybrid orbitals are closer to sp^2 than sp^3, while a conformation-dependent conjugative ability is exhibited with π-acceptor substituents. Thus, the carbonyl group induces distal bond shortening and adjacent bond lengthening of the cyclopropane ring [12–14]. Such influence of the carbonyl group was observed in the compounds (−)-**2**, (−)-**7** and one crystallographically independent molecule of the compound (+)-**3** where the shortest bond was found to be C6–C7 (Table 1). The values of the endocyclic bond angles in the cyclopropane ring of (−)-**2**, (+)-**3** and (−)-**7** are close to 60° what is in accord with the expected ones. The smallest angle in the cyclopropane ring of (−)-**2**, (−)-**7** and one crystallographically independent molecule of the compound (+)-**3** was observed at the atom C8. The exocyclic bond angle N1–C8–C9 in (−)-**2**, (+)-**3** and (−)-**7** varies in the range from 113.3(2) to 115.4(2)°; that is, it deviates significantly from the tetrahedral angle value (Table 1). This marked angle deviation may be attributed to the steric congestion

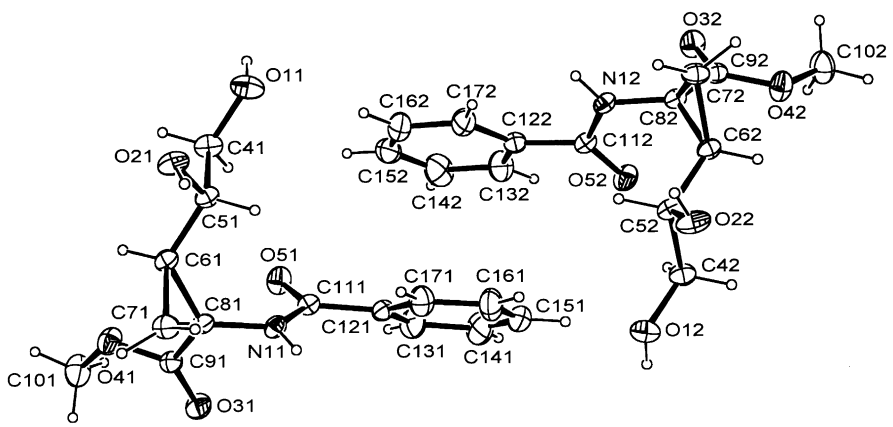


Fig. 4. The molecular structure and labeling of the compound (−)-**7**. Displacement ellipsoids are drawn at the 20% probability level.

Table 1
Selected bond distances (Å), bond and torsion angles (°) for the compounds (−)-**2**, (+)-**3** and (−)-**7**

	(−)-**2**	(+)-**3**	(−)-**7**
C6–C7	1.495(3)	1.498(4), 1.505(4)	1.506(3), 1.516(3)
C6–C8	1.546(3)	1.547(4) 1.528(4)	1.533(3) 1.530(3)
C7–C8	1.516(2)	1.511(4), 1.496(4)	1.521(3), 1.518(3)
C8–N1	1.434(2)	1.426(4), 1.443(4)	1.425(3), 1.431(3)
C8–C9	1.496(3)	1.498(5), 1.489(4)	1.510(3), 1.505(3)
C6–C5	1.517(3)	1.524(4), 1.525(4)	1.515(3), 1.514(3)
C6–C7–C8	61.8(1)	61.9(2), 61.2(2)	60.9(1), 60.6(1)
C6–C8–C7	58.4(1)	58.7(2), 59.7(2)	59.1(1), 59.7(1)
C7–C6–C8	59.8(1)	59.5(2), 59.1(2)	60.1(1), 59.8(1)
N1–C8–C9	115.4(2)	114.7(3), 114.0(2)	113.3(2), 113.3(2)
C5–C6–C18	115.0(2)	–	–
C5–C6–C3	–	115.7(3), 115.5(3)	–
C7–C6–C5–C4	−156.0(2)	−154.9(2), −154.6(3)	−169.3(2), −169.8(2)
N1–C8–C6–C5	−0.3(2)	0.1(4), 4.2(4)	−5.1(3), −3.9(3)
C8–N1–C11–C12	−179.4(2)	173.0(3), 171.1(3)	−179.0(2), −175.6(2)
C8–C9–O4–C10	175.2(2)	−171.2(3), −176.2(3)	−177.5(2), −177.4(2)
C11–N1–C8–C9	65.8(2)	−68.9(4), −56.4(4)	58.0(3), 59.7(3)
N1–C8–C9–O4	3.3(2)	−17.1(4), −32.5(4)	−158.8(2), −162.7(2)
C6–C5–C4–O1	−140.7(2)	63.4(3), 68.9(4)	178.4(2), −179.0(2)

around the cyclopropane ring, which forces two geminal benzamido and methylcarboxylate groups to be remoted from each other. The torsion angles defined by the cyclopropane ring atoms and connected nitrogen atom N1 (C6–C7–C8–N1; C7–C6–C8–N1) as well as the corresponding carbon atom C9 of the carboxylate group (C6–C7–C8–C9; C7–C6–C8–C9) in (−)-**2**, (+)-**3** and (−)-**7**, respectively vary in the range of 103.7(2) − 111.3(2)°. These values corroborate the *anticlinal* conformation of those moieties. The methyl ester group in the compounds (−)-**2** and (+)-**3** adopts *synperiplanar* conformation [the torsion angle O3–C9–O4–C10 amounting −5.7(4)° in (−)-**2** and 6.9(5) and 5.5(5)° in (+)-**3**] with the carbonyl O3 atom eclipsing the cyclopropane ring. The corresponding torsion angle M–C8–C9–O3, where *M* is the midpoint of the distal bond of the cyclopropane ring (C6–C7) is 8.2(3)°. In two crystallographically independent molecules of (+)-**3** the torsion angle M–C8–C9–O3 amounts to −17.4(6)° and −31.2(5)°. Although the *synperiplanar* conformation prevails in derivatives of cyclopropane, the *synclinal* and *anticlinal* conformations have also been observed due to conjugative interactions of the substituents with the cyclopropane ring [12,15]. Two crystallographically independent molecules of (−)-**7** exhibit the *antiperiplanar*, i.e. *anticlinal* conformation with the oxygen atom O4 eclipsing the cyclopropane ring. The values of the torsion angle M–C8–C9–O3 in these two independent molecules are 164.5(3) and 147.1(3)°.

The geometries of the structures (−)-**2**, (+)-**3** and (−)-**7** are not significantly different from the structurally related alkyl 1-benzamido-1-cyclopropanecarboxylic acid derivatives [7,16–20] and 1,2-dihydroxyethyl substituted cylopropane derivatives [21–22]. The methyl ester group and benzamido moiety adopt *antiperiplanar* conformation, defined by the torsion angles C8–N1–C11–C12 and C8–C9–O4–C10 in all three structures (Table 1). The values of the torsion angle N1–C8–C9–O4 in (−)-**7** deviate significantly from the corresponding one of the compounds (−)-**2** and (+)-**3**. This torsion angle depends on the bond angle N1–C8–C9 which is widened in (−)-**2** and (+)-**3** to release close contacts between the main chain atoms of the neighbouring residues. This is in accord with that what has been found for structurally related derivatives of cylopropanes [8,23–25]. The conformation of the 1,2-dihydroxyethyl group in the structure of the compound (−)-**7** defined by the torsion angle C6–C5–C4–O1 is *antiperiplanar*, while the conformation of that group in the compound (+)-**3** is *synclinal*.

The dihedral angles between the plane of the cyclopropane ring and the plane defined by atoms N1–C8–C9 in (−)-**2**, (+)-**3** and (−)-**7** are 88.6(2), 89.0(4) and 89.0(3)° [the mean values for (+)-**3**

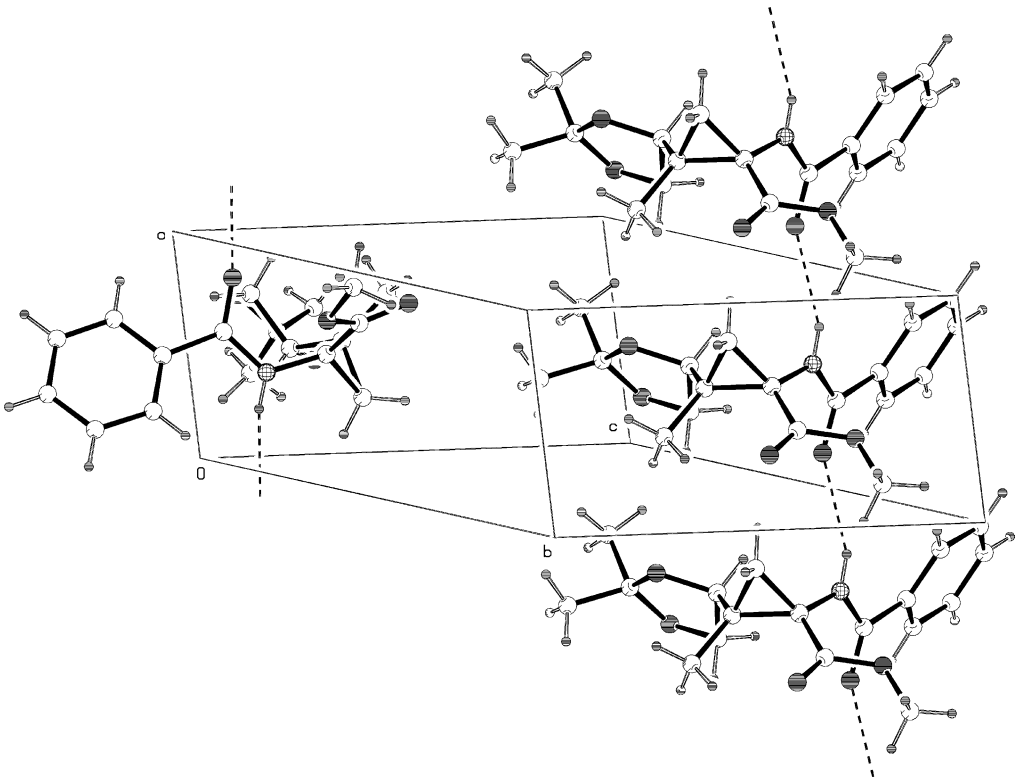


Fig. 5. Crystal packing diagram of the compound (−)-**2**.

and (−)-**7** are given]; that is, those planes are almost perpendicular to each other.

Crystal Structures.-In the crystal structure of the compound (+)-**1** the molecules are connected by very weak *inter*molecular hydrogen bond C14···O3 with D···A distance of 3.378(4) Å and D-H···A angle of 145.5(2)° [symmetry code: x, + y + 1, + z]. The azlactone and phenyl ring of the molecule are oriented parallel to the crystallographic axis *b* with the corresponding dihedral angles of 3.4(1) and 2.9(1)°.

In the 1-aminocyclopropane-1-carboxylic acid derivatives (−)-**2**, (+)-**3** and (−)-**7** *inter*- and *intra*molecular hydrogen bonds of the N H···O type, characteristic for the amide bond were observed. Such very weak *inter*molecular hydrogen bond in the crystal structure of the compound (−)-**2** connecting the molecules along axis *a* forming infinite chains (Fig. 5) with N1···O5 distance of 3.113(2) Å and angle of 156.5(1)° [symmetry code: x + 1, + y, + z].

In both independent molecules of the compound (+)-**3** two *intra*molecular hydrogen bonds of the N-H···O type (Table 2) build seven-membered ring (Fig. 6). Two crystallographically independent molecules are connected in the crystal structure by *inter*molecular hydrogen bond O11···O22. The molecules are additionally connected by three *inter*-molecular hydrogen bonds of the O-H···O type forming three-dimensional network.

The hydrogen bonding pattern of the compound (−)-**7** (Fig. 7) is different to that of an almost similar compound (+)-**3**. Two hydrogen bonds N1···O3 form ten-membered ring of the $R_2^2(10)$ type. Each independent molecule is additionally bonded by hydrogen bonds O1···O2 and O2···O5 with its symmetry related molecules forming two almost identical two-dimensional networks. In one of these networks all hydrogen bonds are slightly longer (Table 2). Furthermore, the hydrogen bond pattern of the compound (−)-**7** has remarkable influence on the density, which is found to be higher than those of the compounds (+)-**1**, (−)-**2** and particularly comparing to the density of its methylated derivative (+)-**3**.

Table 2
Hydrogen-bonding geometry (Å, °) for the compounds (+)-**3** and (−)-**7**

Compound	D–H···A	D···A(Å)	D–H···A(°)	Symmetry code
(+)-**3**	N11–H11N···O11	2.865(4)	143(3)	
	N12–H12N···O12	2.871(4)	145(4)	
	O11–H11···O22	2.829(4)	155(1)	
	O12–H12···O52	2.811(4)	169(5)	x, + y − 1, + z
	O21–H21···O51	2.745(4)	169(3)	− x, + y − 1/2, − z
	O22–H22···O21	2.804(3)	165(1)	− x + 1, + y + 1/2, − z
(−)-**7**	N11–H11N···O31	3.062(3)	173(3)	− x + 2, + y − 1/2, − z
	O11–H11···O21	2.901(3)	163(1)	− x + 1, + y + 1/2, − z
	O21–H21···O51	2.771(3)	170(1)	x, + y − 1, + z
	N12–H12N···O32	3.023(3)	161(3)	− x + 1, + y − 1/2, − z + 1
	O12–H12···O22	2.865(2)	156(1)	− x + 2, + y + 1/2, − z + 1
	O22–H22···O52	2.733(2)	175(1)	x, + y − 1, + z

2.3. Ab initio MO calculations

To clarify the mechanism of the rearrangement of (+)-**3** to (±)-**6** (Scheme 1) the ab initio calculations of the ground states and possible intermediates for that process and the hypothetical one of **8** to **II** were performed (Table 3). All calculations were carried out on DFT [26] pBN/DN** level with the SPARTAN

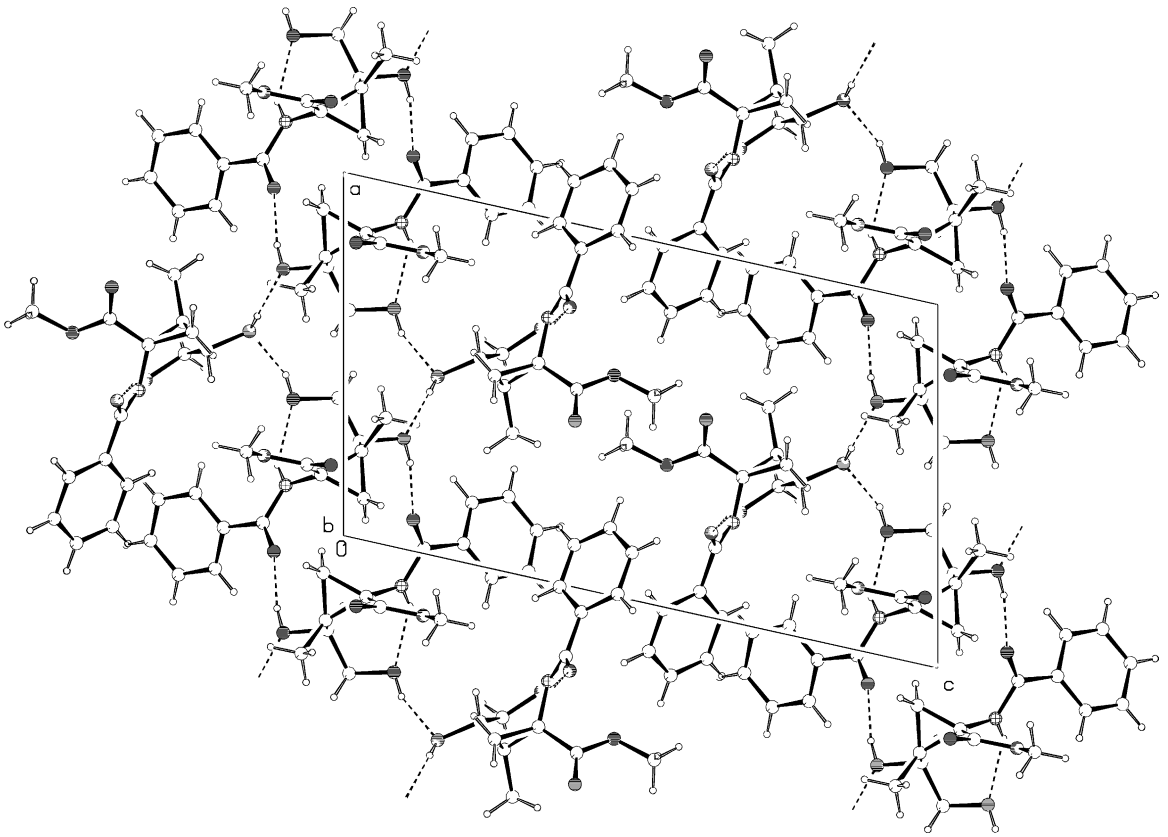


Fig. 6. Crystal packing diagram of the compound (+)-**3** view down the crystallographic axis *b*.

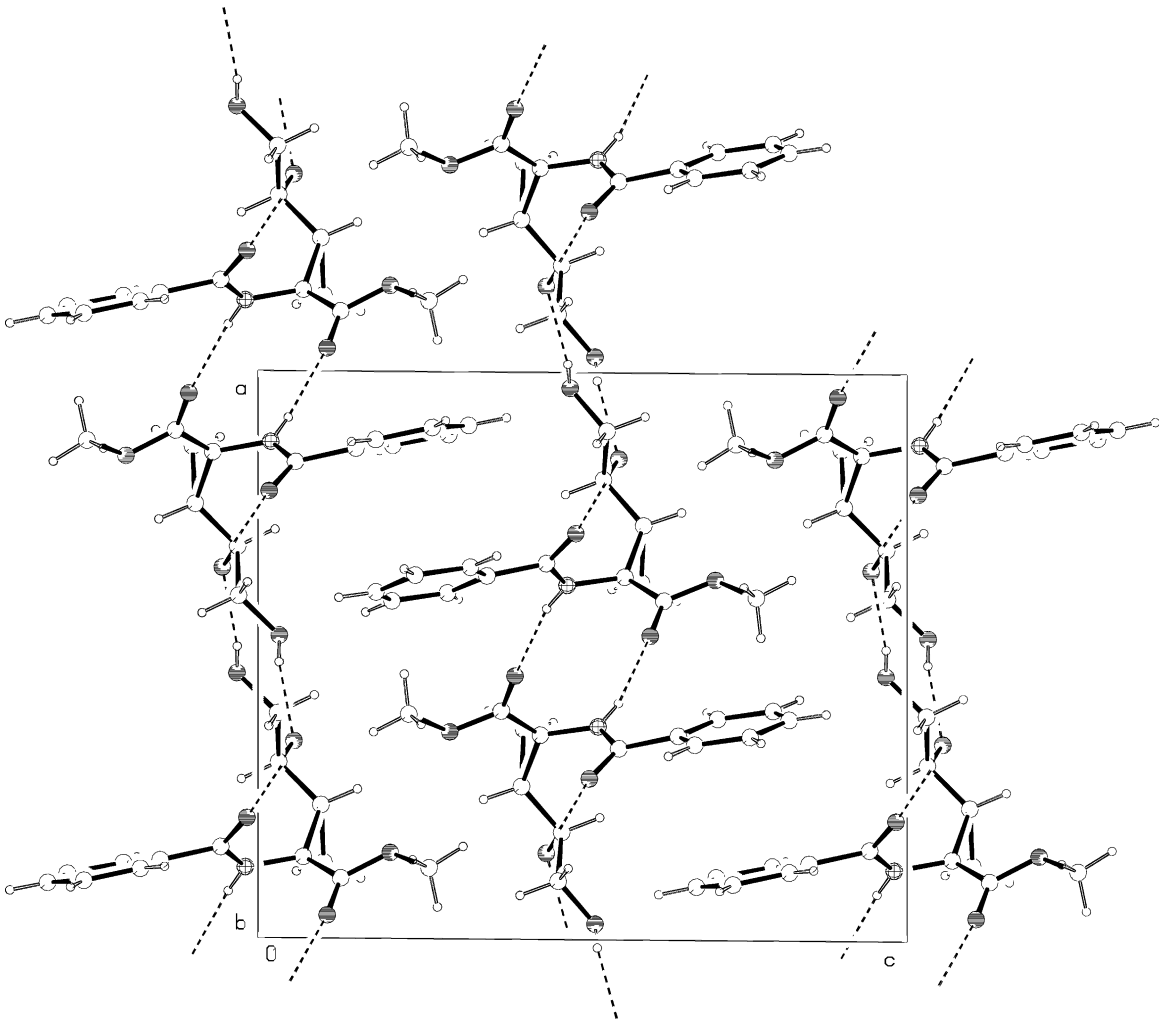
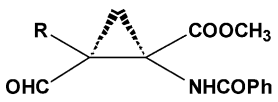


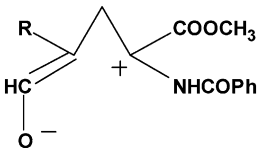
Fig. 7. Crystal packing diagram of the compound (−)-**7** view down the crystallographic axis *b*.

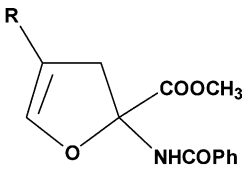
[27] program. The results in Table 3 show that the zwitterionic's intermediate **5** is more stable than the ground state of the molecule **4**. Furthermore, the methyl-substituted dihydrofuran derivative **6** has lower energy than the reactive intermediate **5** by 0.00493 atomic units. The zwitterionic's reactive intermediate **I** is less stable than the ground state of the molecule **8** by 0.00100 atomic units and **II** is less stable than **8** by 0.00325 atomic units. This means that formation of **6** is energetically preferred what is in agreement with our experimental finding. The mechanism of this rearrangement is also in accord with the reported one for 1,2-aminocyclopropane alcohol [28], trialkylsiloxy substituted vinylcyclopropanecarbaldehydes [29] and Cram's work on *cis/trans* isomerizations of substituted cyclopropane derivatives *via* 1,3-zwitterions. [30–31].

Comparison of the calculated ab initio HF/6-31G* and DFT pBN/DN** selected geometrical parameters for (+)-**3** and (−)-**7** with the experimental X-ray data are displayed in Table 4. The average absolute error of the differences between the experimental bond lengths and those obtained by HF/6-31G* basis set are 0.013 and 0.014 Å for two independent molecules of (+)-**3** and 0.013, i.e. 0.012 Å for two independent molecules of (−)-**7**. DFT geometry is also in a very

Table 3
DFT calculated energies of the most stable conformers of the tetra- and trisubstituted cyclopropanecarbaldehydes **4** and **8**, zwitterionic's intermediates **5** and **I** and 2,3-dihydrofurane derivatives **6** and **II** (in atomic units)







4 (R = CH3)	−898.50236	**5** (R = CH3)	−898.50665	**6** (R = CH3)	−898.51158
8 (R = H)	−859.18432	**I** (R = H)	−859.18332	**II** (R = H)	−859.18107

Table 4
Selected bond distances (Å) of (+)-(**3**) and (−)-**7** derived from X-ray crystallography and ab initio (HF/6-31G* and DFT pBN/DN**) calculations. For numbering of the atoms see Fig. 3 and Fig. 4

Bond	(+) − (**3**)			(−) − (**7**)		
	Experimental[a]	HF/6-31G*	DFT	Experimental[a]	HF/6-31G*	DFT
O11–C41	1.427(4)	1.409	1.442	1.417(3)	1.409	1.445
O12–C42	1.419(4)			1.417(3)		
O21–C51	1.438(3)	1.407	1.438	1.433(3)	1.405	1.439
O22–C52	1.436(3)			1.431(3)		
O31–C91	1.203(4)	1.190	1.221	1.200(3)	1.192	1.221
O32–C92	1.209(4)			1.209(3)		
O41–C91	1.339(4)	1.321	1.359	1.329(3)	1.319	1.356
O42–C92	1.331(4)			1.329(3)		
O41–C101	1.453(5)	1.416	1.449	1.449(3)	1.417	1.451
O42–C102	1.447(4)			1.447(3)		
O51–C111	1.233(4)	1.201	1.236	1.235(3)	1.200	1.235
O52–C112	1.244(3)			1.233(3)		
N11–C111	1.347(4)	1.361	1.378	1.352(3)	1.361	1.378
N12–C112	1.342(4)			1.354(3)		
N11–C81	1.426(4)	1.426	1.438	1.425(3)	1.421	1.433
N12–C82	1.443(4)			1.431(3)		
C31–C61	1.508(4)	1.521	1.520	–	–	–
C32–C62	1.514(4)			–		
C41–C51	1.523(4)	1.521	1.531	1.514(3)	1.521	1.532
C42–C52	1.512(4)			1.513(3)		
C51–C61	1.524(4)	1.530	1.536	1.515(3)	1.514	1.518
C52–C62	1.525(4)			1.514(3)		
C61–C71	1.498(4)	1.497	1.512	1.506(3)	1.491	1.507
C62–C72	1.505(4)			1.516(3)		
C61–C81	1.547(4)	1.525	1.550	1.533(3)	1.513	1.532
C62–C82	1.528(4)			1.530(3)		
C71–C81	1.511(4)	1.498	1.515	1.521(3)	1.500	1.513
C72–C82	1.496(4)			1.518(3)		
C81–C91	1.498(5)	1.507	1.514	1.510(3)	1.502	1.514
C82–C92	1.489(4)			1.505(3)		
C111–C121	1.493(4)	1.502	1.509	1.490(3)	1.502	1.510
C112–C122	1.483(4)			1.497(3)		

[a] X-ray crystallograhic determination.

good agreement with the X-ray data; average absolute errors for pBN/DN** calculated bond distances are 0.015 Å, i.e. 0.018 Å for (+)-**3** and 0.015, i.e. 0.014 Å for (−)-**7**. The results of ab initio calculations show that both, HF/6-31G* and DFT pBN/DN** agree quite well with the X-ray structure data.

2.4. Cytostatic and antiviral activity

Compounds (+)-**1**, (−)-**2**, (+)-**3** and (±)-**6** were evaluated for their cytostatic activity against malignant tumor cell lines: murine leukemia (L1210/0), human T-lymphocyte (Molt4/C8 and CEM/0), cervical carcinoma (HeLa), breast carcinoma (MCF7), colon carcinoma (CaCo-2), pancreatic carcinoma (MIAPaCa-2) and laryngeal carcinoma (Hep-2), as well as human fibroblast cells (WI-38). The *spiro*-azlactone (+)-**1** exhibited the most pronounced antiproliferative activity against murine leukemia (L1210/0; $IC_{50=}$41 μM) and human T-lymphocyte cells (Molt4/C8; IC_{50} = 42 μM; CEM/0; $IC_{50=}$40 μM).

Compounds (+)-**1**, (−)-**2**, (+)-**3** and (±)-**6** were also evaluated against varicella-zoster virus (VZV) and cytomegalovirus (CMV) in human embryonic lung (HEL) cells and against human immunodeficiency virus type 1 (HIV-1) and HIV-2 in human limphocyte CEM cells. None of the compounds showed appreciable antiviral activity at subtoxic concentrations, except for some very slight activity of (+)-**1** against CMV.

3. Conclusions

The aims of the presented work were to evaluate the novel 2,2′-disubstituted-1-aminocyclopropane-1-carboxylic acid derivatives (−)-**2** and (+)-**3**, the *spiro*-azlactone (+)-**1** and 2,3-dihydrofuran derivative (±)-**6** on cytostatic and antiviral activities and to elucidate the mechanism of the rearrangement of (+)-**3** to (±)-**6**. For these reasons the exact stereostructures, particularly the absolute configuration of (+)-**1**, (−)-**2**, (+)-**3** and the related trisubstituted cyclopropane derivative (−)-**7** were determined by X-ray crystal structure analysis. Additionally, the ab initio HF/6-31G* and DFT pBN/DN** calculations were performed to clarify the mechanism of that process. The result of these calculations support the predicted mechanism of the rearrangement of (+)-**3** to (±)-**6** *via* the zwitterionic's reactive intermediate. The DFT method is also shown to be a valuable tool for predicting structure-reactivity relationship. The evaluated compounds didn't show significant cytotoxic and antiviral activities of the examined cell lines; the exception being the *spiro*-compound (+)-**1** which showed a more pronounced inhibitory activity against the proliferation of murine leukemia and human T-lymphocytes cells than other type of tumor cell lines and normal human fibroblast cells.

4. Experimental

Melting points were determined on a Kofler micro hot-stage apparatus (Reichert, Wien) and are uncorrected. Precoated Merck silica gel 60F-254 plates were used for thin-layer chromatography (TLC) and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (0.05–0.2 mm, Merck); glass column was slurry-packed under gravity. The electron impact mass spectra were recorded with an *EXTREL FT MS 2001* instrument with ionizing energy of 70 eV. The IR spectra were recorded on a *Nicolet-Magna IR 760* spectrometer and UV spectra on a *Hewlett-Packard 8452* spectrometer. Optical rotations were measured with an *Optical Activity AA-10 Automatic Polarimeter* in a 1 dm cell; *c* in g/100 ml, at r.t. (*ca.*20°). Elemental analyses were performed by the Central Analytical Service, Ruđer Bošković Institute, Zagreb. The ^{1}H- and ^{13}C- NMR spectra were recorded on a Bruker AMX-300 (^{1}H 300.13; ^{13}C 75 MHz). Chemical shifts in NMR spectra are given in ppm relative to internal TMS (δ scale), *J*in Hz; the chemical shifts assignments of (±)-**6** (*c.f.* Scheme 1) are based on two-dimensional (^{1}H,^{1}H(- DQF-COSY, and long range (^{1}H,^{13}C(-HSQC and HMBC experiments at 500 MHZ (^{1}H) frequency.

4.1. Compounds preparation

Methyl(−)-(1S, 2R)-1-benzamido-2-methyl-2-((S)-2,2-dimethyl-1,3-dioxolan-4yl(cyclopropanecarboxylate **(2).** A suspension of (+)-**1** [7] (0.86 g, 2.85 mmol)

in a solution of sodium metoxide (0.02 g) in absolute methanol (50 ml) was stirred at room temperature for 30 min. The solvent was then evaporated, the residue dissolved in dichloromethane, washed with water and the organic layer dried over $MgSO_4$. After removal of the solvent a colourless solid of (−)-**2** was obtained in a quantitative yield. mp 133–134 °C (from MeOH); $[\alpha]_D$ − 24.0 ° (c 0.25 in MeOH); ν_{max} (KBr)/cm^{-1} 3350, 1733, 1644; λ_{max} (MeOH) 204 (log ϵ 4.13) and 228 (log ϵ 4.14); δ_H ($CDCl_3$) 1.25 (s, 3H), 1.34 (s, 3H) and 1.41 (s, 3H) (2 × CH_3), 1.40 (d, *J* 5.67, 1H, $CH_{2cyclopro}$), 1.81 (d, *J* 5.67, 1H, $CH_{2cyclopro}$), 3.70 (s, 3H, OCH_3), 3.89 (dd, *J* 7.12 and 8.54, 1H, CH_2), 4.10 (dd, *J*6.50 and 8.57, 1H, CH_2), 4.23 (t, *J* 6.78, 1H, CH), 7.19 (s, 1H, NH), 7.39–7.75 (m, 5H, C_6H_5); δ_C 14.16 (C2–*C*H_3), 24.18 (C3), 25.16 and 26.19 (2 × C2′–*C*H_3), 31.4 (C2′), 42.93 (C1), 52.48 (O*C*H_3), 67.68 (C5′), 78.15 (C4′), 109.37 (C2′), 126.95, 128.48, 131.72 and 133.71 (C–Ph), 168.17 (CO–amide), 170.48 (CO–ester); *m/z* (EI) 333.4 (M^+); Anal. calcd for $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20. Found: C, 64.92; H, 7.02; N, 4.12.

Methyl(+)-(1S, 2R)-1-benzamido-2-methyl-2-((S)-1,2-dihydroxyethyl(cyclopropanecarboxylate **(3).** 3 M Hydrochloric acid (1.2 ml) was added to a solution of (−)-**2** (0.6 g, 1.80 mmol) in methanol (50 ml) and the mixture was stirred at room temperature for 24 h. The solvent was evaporated to afford (+)-**3** as a white solid in quantitative yield. Mp 143–146 °C (from water); $[\alpha]_D$ + 26.7° (c 0.34 in MeOH); ν (KBr)/cm^{-1} 3444, 3352, 3315, 1736, 1651; λ_{max} (MeOH) 204 (log ϵ 4.00) and 230 (log ϵ 4.00); δ_H (DMSO-d_6) 1.09 (d, *J* 5.27, 1H, $CH_{2cyclopro}$), 1.70 (d, *J* 5.25, 1H, $CH_{2cyclopro}$), 3.47 (t, 1H, CH, superimposed with CH_2OH), 3.48 (1H) and 3.59 (1H) (2 × dd, CH_2, superimposed with OCH_3 and CH_2OH), 3.58 (s, 3H, OCH_3), 5.07 (d, *J* 4.37, 1H, CHOH), 5.61 (t, *J* 3.65, 1H, CH_2OH), 7.48–7.74 (m, 5H, C_6H_5), 8.86 (s, 1H, NH); δ_C (DMSO-d_6) 10.89 (C2–*C*H_3), 28.36 (C3), 32.18 (C2), 42.39 (C1), 52.07 (OCH_3), 61.93 (C5′), 73.49 (C4′), 126.72, 128.09, 131.74 and 133.69 (C–Ph), 166.33 (CO–amide), 171 (CO–ester); *m/z* (EI) 294.3 (MH^+); Anal. calcd for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.34; H, 6.59; N, 4.68.

(±)-*Methyl-2-benzamido-2,3-dihydro-4-methyl-2-furancarboxylate*.**(6)** A suspension of $NaIO_4$ (0.133 g, 0.621 mmol) in water (3 ml) was added dropwise to a stirred solution of (+)-**3** (0.174 g, 0.594 mmol) in THF (50 ml). The mixture was then stirred at room temperature for 6 h and the resultant suspension was filtered. The solvent was removed and the resulting residue was dissolved in dichloromethane, washed with water, dried over $MgSO_4$, filtered and concentrated in vacuo. Purification of the residue by silica gel column chromatography (ethyl acetate: dichloromethane 3: 2) afforded (±)-**6** (0.112 g, 72.2%) as a white solid. mp 162–163 °C (from EtOH); ν (KBr)/cm^{-1} 3321, 1739, 1650, 1530 cm^{-1}; λ_{max} (MeOH) 204 (log ϵ 4.06) and 228 (log ϵ 4.02); δ_H ($CDCl_3$) 1.71 (s, 3H, CH_3), 2.97 (d, *J* 16.4, 1H, CH_2), 3.24 (d, *J* 16.4, 1H, CH_2), 3.86 (s, 3H, OCH_3), 6.12 (s, 1H, CH), 7.27 (s 1H, NH), 7.43–7.83 (m, 5H, C_6H_5); δ_C 10.45 (C4–*C*H_3), 43.65 (C4), 53.26 (OCH_3), 91.60 (C5), 109.47 (C3), 127.20, 128.57, 132.14 and 133.11 (C–Ph), 138.09 (C2), 166.81 (CO–amide), 169.49 (CO–ester); (^1H,^1H(DQF-COSY ($CDCl_3$), cross peaks between: C4–CH_3 and CH_AH_B (CH_2); C4–CH_3 and CH; CH and CH_AH_B (CH_2); (^1H,^{13}C(-HSQC ($CDCl_3$), cross peaks between: C*H*$_A$*H*$_B$ (CH_2) and *C*4; C*H* and *C*2; (^1H,^{13}C(-HMBC ($CDCl_3$), cross peaks between: C*H*$_A$ (CH_2) and C4–*C*H_3; C*H* and *C*4; N*H* and *C*4; C*H*$_A$ (CH_2) and *C*5; N*H* and *C*5; C*H* and *C*3; N*H* and C_6H_5; C4–C*H*$_3$ and *C*2; C*H*$_A$*H*$_B$ (CH_2) and *C*2; N*H* and *C*O–amide; C_6H_5 and *C*O–amide; N*H* and *C*O–ester; O*CH*$_3$ and *C*O-ester; C*H*$_A$*H*$_B$ (CH_2) and *C*O-ester; *m/z* (EI) 261.3 (M^+); Anal. calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.30; H, 5.89; N, 5.45.

Methyl (-)-(1S, 2R)-1-benzamido-2-((S)-1,2-dihydroxyethyl(cyclopropanecarboxylate **(7)** and *methyl (−)-(1S, 2R)-1-benzamido-2-formylcyclopropanecarboxylate* **(8)** were prepared following the procedure given in the literature [6]. The spectroscopic data were in agreement with the reported ones.

4.2. X-ray structure determination

Single crystals suitable for X-ray structure analysis were prepared by growth under slow evaporation at room temperature of a very dilute ethanol solution for the compounds (+)-**1** and (−)-**2** and water solution for the compounds (+)-**3** and (−)-**7**. The intensities were collected at room

temperature on a Philips PW1100 diffractometer updated by Stoe and Cie [32,33] using Mo-K_α radiation. The crystal structures of all compounds were solved by direct methods using SHELXS86 [34] program. All non-hydrogen atoms were refined anisotropically by full-matrix least-squares calculations based on F^2 by application of SHELXL97 [35] program. The coordinates of hydrogen atoms were included in structure factor calculations. The carbon atoms C1 and C2 of the compound (−)-**2** are disordered. The site occupancy factors are 57(4) and 43(4)% for the atom C1 and 53(4) and 47(4)% for the atom C2. The distances between occupation sites of the disordered atoms C1 and C1′ and atoms C2 and C2′ are 0.72(4) and 0.77(4) Å, respectively. The molecular and crystal structure drawings were prepared by ORTEP-3 [36] and PLUTON93 [37] programs. Crystal data, data collection and refinement for the compounds (+)-**1**, (−)-**2**, (+)-**3** and (−)-**7** are summarized in Table 5. Crystallographic data excluding structure factors for the structures (+)-**1**, (−)-**2**, (+)-**3** and (−)-**7** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-167403, CCDC-167404, CCDC-167405 and CCDC-167406. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Table 5
The crystal data, data collection and refinement for the compounds (+)-**1**, (−)-**2**, (+)-**3** and (−)-**7**

Compound	(+)-**1**	(−)-**2**	(+)-**3**	(−)-**7**
Formula	$C_{17}H_{19}NO_4$	$C_{18}H_{23}NO_5$	$C_{15}H_{19}NO_5$	$C_{14}H_{17}NO_5$
Formula weight	301.33	333.37	293.31	279.29
Temperature [*K*]	295	295	293	295
Crystal size [mm]	0.50 × 0.45 × 0.40	0.70 × 0.50 × 0.45	0.89 × 0.42 × 0.15	0.50 × 0.40 × 0.35
Crystal colour	colourless	colourless	colourless	colourless
Crystal system	monoclinic	monoclinic	monoclinic	monoclinic
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
a[Å]	5.725(1)	5.239(1)	11.078(6)	13.530(3)
b[Å]	10.609(2)	14.998(3)	7.570(4)	6.479(1)
c[Å]	13.186(3)	11.377(2)	18.544(12)	15.489(3)
β[°]	93.15(1)	100.56(1)	102.57(6)	90.43(1)
V [Å^3]	799.7(3)	878.8(3)	1517.8(15)	1357.7(5)
Z	2	2	4	4
$D_{calc.}$[gcm^{-3}]	1.251	1.260	1.284	1.366
μ[mm^{-1}]	0.089	0.092	0.097	0.104
F(000)	320	356	624	592
Scan-mode	ω	ω–θ	ω	ω
θ range for data collection [°]	3.09 to 28.98	2.27 to 27.91	3.23 to 28.04	3.01 to 29.01
Index ranges	$-7 \le h \le 7$ $-14 \le k \le 14$ $-17 \le l \le 17$	$-6 \le h \le 6$ $-19 \le k \le 19$ $-14 \le l \le 14$	$-14 \le h \le 14$ $-9 \le k \le 9$ $-21 \le l \le 24$	$-18 \le h \le 18$ $-8 \le k \le 8$ $-21 \le l \le 21$
Collected reflections	4410	4302	7727	7785
Independent reflections/$R_{int.}$	4197/0.0739	4147/0.0833	7226/0.0535	7167/0.0761
Reflection number $I \geqq 2\sigma(I)$	2183	3407	2917	4658
Refinement method	Full-matrix least-squares on F^2			
Data/restrains/parameters	2183/1/200	3407/1/239	2917/1/400	4658/1/372
Weighting parameters *a*,*b*[a]	0.1000, 0	0.0803, 0.1136	0.0697, 0	0.1000, 0
Goodness-of-fit on F^2	0.935	1.018	0.824	1.108
R [$I \geqq 2\sigma(I)$]	0.0581	0.0449	0.0437	0.0630
WR	0.1350	0.1205	0.1020	0.1518
Max.min. elect. dens. [eÅ^{-3}]	0.315/ − 0.268	0.220/ − 0.176	0.219/ − 0.177	0.479/ − 0.444

[a] $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$, where $P = (F_o^2 + 2F_c^2)/3$.

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