One-pot Synthesis of *N*-Formyl-*O*-acyl- *threo*- and *erythro*-*DL*-β-phenylserine Ethyl Esters and their Antiviral Properties

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Abstract. A series of *N*-formyl-*O*-acyl-β-phenylserine derivatives **1b**–**7b** were prepared by the interaction of *N*-acyl-β-phenylserine ethyl esters **1a**–**7a** with formic acid in presence of 1.5% HF. One-pot acyl group *N*→*O* migration followed *N*-formylation under elaborated reaction conditions. The kinetics of the reaction was investigated. The carboxylic acid moiety in the structure of β-phenylserine had a strong influence on the reproduction of the used test-viruses. The toxicity and antiviral activity is dependent on the diastereomeric forms of evaluated compounds.

The growing importance of amino acids and their derivatives is associated with their importance as constituents of biologically active products. In recent years great efforts have been made to synthesize receptor-selective amino acids and peptides. Acyl derivatives of amino acids are synthetically useful and widely explored compounds. Moreover, acyl migration reactions attract wide interest since acyl transfer in biological systems, *e.g.* proteins splicing [1], produces active intermediates, and analogous process could control replication of viruses. This observation may be useful employing *N*→*O* acyl migration for the synthesis of potential peptide prodrugs [2, 3]. The search of new selective antiviral agents is connected with several problems, since viruses share many of the metabolic processes of the host cell. The antiviral agents are tested in cell culture measuring the ability of compounds to stop growing of the viruses without affecting the host cells. At present, some virus-specific enzymes are known that are potential targets for drugs [4]. Current search for new chemical agents focuses on finding inhibitors of the viral protease enzyme since many antivirals are nucleoside analogues, however, some other useful classes of synthetic compounds have emerged that suppress virus growth [5, 6].

Earlier, we have synthesized a series of *N*- and *O*-substituted amino acids for evaluation of their antiviral properties [7]. In the course of previous work, it became evident that among different amino acids used, the non-proteinogenic amino acid β-phenylserine is a rather promising structure for chemical modifications in search of new antiviral agents. As an extension of this research, our attention turned to the modification of the key structure by introducing two different acyl groups into the β-phenylserine molecule. A facile synthetic method to prepare *N*-formyl-*O*-acyl-β-phenylserine derivatives was elaborated. *N*→*O* acyl group migration and subsequent *N*-formylation with formic acid in the presence of hydrogen fluoride afforded the target structures. The synthesized compounds were tested for their potential antiviral activity.

Results and Discussion

Synthesis of N-Formyl-O-acyl-β-phenylserine Derivatives

The *N*-acetyl-*threo*-*DL*-β-phenylserine and *N*-acetyl-*erythro*-*DL*-β-phenylserine ethyl esters **1a** and **2a**, respectively, used as starting compounds, were obtained by the method described previously [8]. The ethyl esters of *N*-propionyl-, *N*-(3-bromopropionyl)-, *N*-phenylacetyl-, *N*-(9-fluorenylacetyl)-threo-*DL*-β-phenylserine **3a**–**6a** and *N*-(9-fluorenylacetyl)-*erythro*-*DL*-β-phenylserine **7a** were obtained by condensation of respective acid chlorides with the corresponding ethyl esters of *threo*- and *erythro*-*DL*-β-phenylserine hydrochlorides [9] in the presence of triethylamine. The *N*-acyl derivatives of β-phenylserine **1a**–**7a** by heating in formic acid with 1.5% HF solution were converted to ethyl esters of *N*-formyl-*O*-acyl-*threo*- and *erythro*-*DL*-β-phenylserines **1b**–**7b**, respectively, as shown in Scheme 1.

This conversion was studied by ^{1}H NMR spectroscopy. The *N*→*O* acyl migration in the corresponding *threo*- and *erythro*-*DL*-β-phenylserines has been studied earlier, and the corresponding chemical shift differences and spin-spin couplings permitted to assign signals for H_a at β–C and H_b at α-C (Scheme 1) in the initial, intermediate and final structures [10, 11]. In this work, the reaction of acyl derivatives with formic acid in presence of 1.5% HF was performed directly in the NMR tube. Spectral parameters of the H_a and H_b protons were determined (see, for example compound **1a**, Figure 1) recording spectra over appropriate time intervals. The reaction rate was determined from the integral intensities of the methine protons H_a and H_b which have the higher chemical shift values for *N*-acyl derivatives and lower chemical shift values for the corresponding *N*-formyl-*O*-acyl derivatives, respectively. However, no signals for intermediate cyclic structures were observed (*cf.* [11]).

The rate of conversion of **1a** to **1b** plotted in the semilogarithmic coordinates gave linear relationship (Figure 2).

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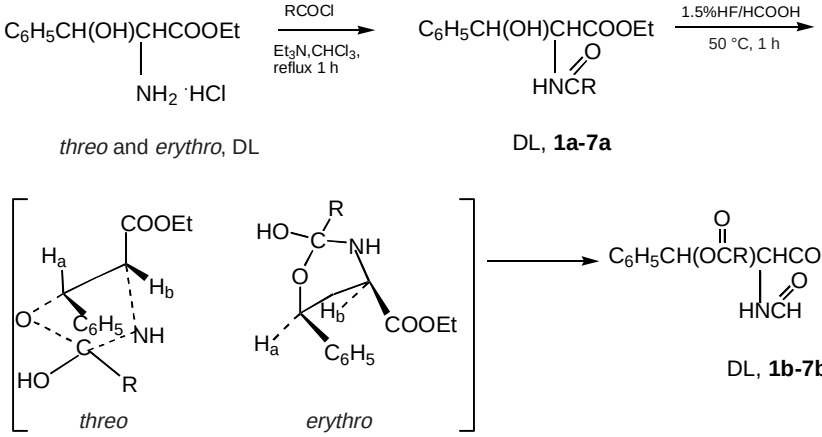

Compound	1a,b	2a,b	3a,b	4a,b	5a,b	6a,b	7a,b
Diastereomer	*threo*	*erythro*	*threo*	*threo*	*threo*	*threo*	*erythro*
R	CH_3	CH_3	CH_3CH_2	$BrCH_2CH_2$	$C_6H_5CH_2$	9-fluorenyl	

Scheme 1 Synthesis of *N*-formyl-*O*-acyl-*threo*-*DL*-*β*-phenylserines **1b**–**7b**

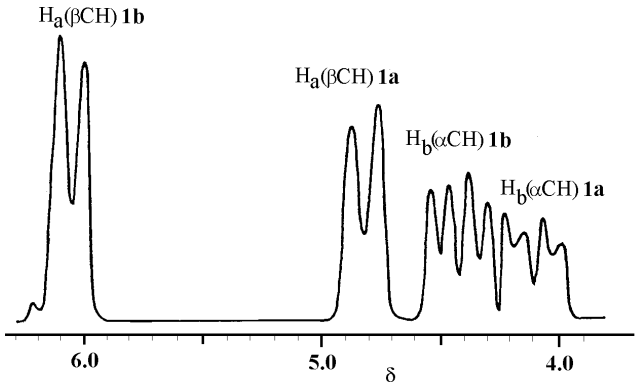

Fig. 1 ^{1}H NMR spectrum of conversion of **1a** to **1b**

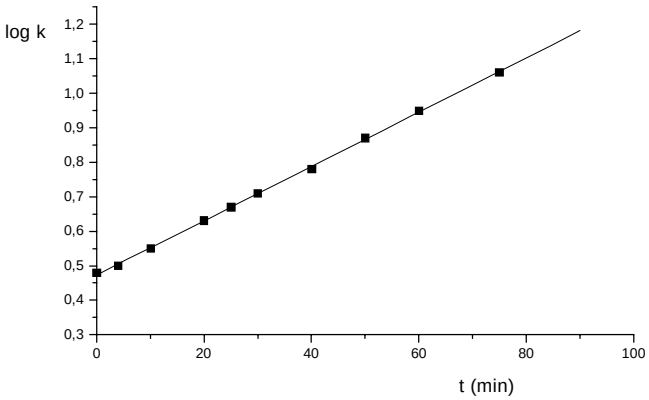

Fig. 2 Rate of conversion of **1a** to **1b** in the semi-logarithmic coordinates

After 60 min almost the entire *N*-acyl derivative was converted into the *O*-acyl product. The reaction rate constant was calculated using the equation for the consecutive first order reactions [12]. A reaction constant for intermediate formation was calculated to be $k_1 = 4.924 \times 10^{-7} \pm 8.690 \times 10^{-11}\,s^{-1}$ and the subsequent conversion of this intermediate $k_2 = 5.400 \times 10^{-6} \pm 1.456 \times 10^{-6}\,s^{-1}$. The kinetic data were used to elaborate a preparative procedure for *N*→*O* migration and synthesis of *NO*-diacyl compounds performing the reaction in less than 1 h.

In order to gain more insight into the acyl migration, the process was subjected to semiempirical calculations by AM1 method using SPARTAN Plus program package [13]. The energies of initial *N*-acyl and final *O*-acyl compounds **1a** and **1b**, respectively, were estimated. Comparison of the ground state energies of these structures showed no considerable difference, namely it is less than 1 kcal/mol, and this indicates that the migration process is controlled kinetically.

Biological Testing

The toxicity and viral inhibition of the studied compounds were determined by methods described in [7, 14]. For investigation of viral inhibition of *N*-formyl-*O*-acyl-*β*-phenylserine derivatives **2b**–**7b**, vesicular stomatitis virus (VSV) (strain Indiana), coxsackievirus CVA-13 (strain Flores), echovirus ECHO-11 (strain Uppsala), and herpes simplex HSV-1 virus (strain L-2) were involved. The cytotoxicity of compounds **2b**–**7b** was evaluated in the culture of transplantable cell line L-41. The most toxic one was *N*-formyl-*O*-(3-bromo-propionyl)-*β*-phenylserine derivative **4b** (maximal tolerated dose MTD = 125 mcg/ml). The rest of the tested compounds were moderate (MTD = 500 mcg/ml for compounds **5b** and **6b**) or slightly toxic (MTD = 5000 mcg/ml for compounds **2b** and **3b** and MTD = 10000 mcg/ml for **7b**) for L-41.

The nature of moieties of carboxylic acids introduced into the structure of *β*-phenylserine had strong influence on the

reproduction of the used test-viruses. Thus, *β*-phenylserine derivative **3b** in its minimum effective dose MED = 1000 mcg/ml (chemotherapeutic index CTI = MTD/MED = 5) completely stops the reproduction of vesicular stomatitis virus, and *β*-phenylserine derivative **5b** in MED = 250 mcg/ml (CTI = 2) completely inhibits the reproduction of herpes simplex virus 1. It is important to point out that the diastereomeric forms of evaluated compounds are decisive for toxicity and antiviral activity. For example, the toxicity of *threo* **6b** analog is twenty times larger compared to the toxicity of *erythro* analog **7b** (data given above). The *threo* **6b** analog completely stops the reproduction of VSV and CVA-13 viruses in doses 250 mcg/ml (CTI = 2), meanwhile the *erythro* diastereomer **7b** did not show any activity against VSV, in contrast against CVA-13 (MED = 1000 mcg/ml, CTI = 10). Compounds **2b** and **4b** had no influence on the reproduction of the used test-viruses and no compound possessed antiviral activity against ECHO-11 at MTD doses.

Thus, four of six investigated *β*-phenylserine derivatives exhibited antiviral activity against three different test-viruses. The obtained results may serve as a basis for further search of novel antiviral compounds among *β*-phenylserine derivatives.

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Experimental

Melting points were determined on a Kofler apparatus and are not corrected. IR spectra were obtained on a Perkin Elmer Spectrum BX FT-IR spectrophotometer in nujol and were consistent with the structures (Tables 2, 3). ^{1}H NMR spectra were recorded on a Hitachi R-22 90 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to hexamethyldisiloxane and signal multiplicities in Tables 4, 5 as follows: s-singlet, d-doublet, dd- doublet of doublets, t-triplet, q-quadruplet, m-multiplet.

Synthesis of *N*-Acyl-*β*-phenylserine Ethyl Esters 3a–7a (General Procedure)

To a mixture of 2.46 g (10 mmol) of *β*-phenylserine ethyl ester hydrochloride and 20 mL of chloroform at 0 °C temperature were added 3 mL (22 mmol) of triethylamine under stirring and then 10 mmol of the corresponding carboxylic acid chloride. The reaction mixture was refluxed for 1h. After cooling to room temperature, the reaction mixture was washed with water, 1N hydrochloric acid, 5% aqueous $NaHCO_3$, and finally once again with water. The organic layer was dried over anhydrous Na_2SO_4. The chloroform was evaporated in vacuo, and the residue purified by recrystallization from corresponding solvents (see Table 1).

Synthesis of *N*-Formyl-O-acyl-*β*-phenylserine Ethyl Esters 1b–7b (General Procedure)

A mixture of 1.0 g of *N*-acyl-*β*-phenylserine ethyl ester (**1a**–

Table 1 Physico-chemical data of compounds **3a–7a** and **1b–7b**

Compound	Yield [a] (%)	*m.p.* (°C)	Recryst. solvent	Empirical formula (molecular weight)	Calcd./Found: C	H	N
3a	59	129–131	Et_2O/hexane	$C_{14}H_{19}NO_4$	63.38	7.22	5.28
				(265.31)	63.51	7.18	5.34
4a	68	118.5–119	EtOH/H_2O	$C_{14}H_{18}BrNO_4$	48.85	5.27	4.07
				(344.20)	48.08	5.31	3.91
5a	79	151–153	MeOH	$C_{19}H_{21}NO_4$	69.71	6.47	4.28
				(327.38)	70.00	6.31	4.36
6a	84	162–163	EtOH	$C_{26}H_{25}NO_4$	75.16	6.07	3.37
				(415.49)	75.03	5.93	3.39
7a	53	132–133	MeOH	$C_{26}H_{25}NO_4$	75.16	6.07	3.37
				(415.49)	75.40	6.16	3.21
1b	74	103–105	Et_2O	$C_{14}H_{17}NO_5$	60.20	6.13	5.02
				(279.29)	60.47	6.33	5.30
2b	52	152–154	EtOH/H_2O	$C_{14}H_{17}NO_5$	60.20	6.13	5.02
				(279.29)	60.21	6.11	5.09
3b	90	92– 94	Et_2O	$C_{15}H_{19}NO_5$	61.42	6.52	4.77
				(293.32)	61.51	6.46	4.51
4b	63	91.5– 92	EtOH/H_2O	$C_{15}H_{18}BrNO_5$	48.40	4.87	3.76
				(372.22)	48.40	4.99	3.55
5b	68	121–122	EtOH/H_2O	$C_{20}H_{21}NO_5$	67.59	5.95	3.94
				(355.39)	67.60	5.89	4.10
6b	80	129–130	EtOH/H_2O	$C_{27}H_{25}NO_5$	73.12	5.68	3.16
				(443.50)	73.44	5.64	3.18
7b	73	143–145	EtOH/H_2O	$C_{27}H_{25}NO_5$	73.12	5.68	3.16
				(443.50)	73.35	5.55	3.04

[a]) Yields after crystallization from a corresponding solvent

Table 2 IR spectra (ν in cm^{-1}, nujol) of compounds **1a**–**7a**

Comp.	C=O($COOC_2H_5$)	C=O(HNCOR)	NH, OH
1a	1742	1662	3224, 3269, 3353
2a	1711, 1726	1665	3306
3a	1737	1649	3258, 3356
4a	1736	1646	3249, 3329
5a	1729	1651	3230, 3355
6a	1743	1653	3348
7a	1740	1647	3289

Table 3 IR spectra (ν in cm^{-1}, nujol) of compounds **1b**–**7b**

Comp.	C=O($COOC_2H_5$)	C=O(OCOR)	C=O(HNCOH)	NH
1b	1732	1746	1659	3198
2b	1724	1745	1657	3265
3b	1732	1756	1647	3268
4b	1730	1744	1658	3292
5b	1731	1731	1647, 1664	3247
6b	1727	1740	1665	3345
7b	1727	1727	1680	3390

Table 4 ^{1}H NMR spectra (chemical shifts δ in ppm, multiplicity, coupling constants J in Hz) of compounds **3a**–**7a**

Comp.	Solvent	C_6H_5	CH	OH	CH	(NH-	CO R [a])	CO_2CH_2	CH_3
3a	DMSO-d_6	7.02–7.42 m	4.92 dd, J = 4.0, 5.0	5.67 d, J = 5.0	4.43 dd, J = 4.0, 9.0	7.86 d, J = 9.0	1.96(CH_2,q, J = 8.0) 0.98(CH_3, t, J = 7.0)	3.97 q, J = 7.0	0.73 t, J = 7.0
3a	$CDCl_3$	7.20 s	5.09 d, J = 3.0	[b])	4.71 dd, J = 3.0, 9.0	6.29 d, J = 8.0	2.02(CH_2, q, J = 6.5) 1.11(CH_3, t, J = 7.0)	4.04 q, J = 7.0	0.91 t, J = 7.3
4a	$(CD_3)CO$	7.06–7.42 m	5.20 d, J = 3.0	6.18 d, J = 5.0	4.67 dd, J = 3.0, 10.0	7.69 d, J = 10.0	2.09(CH_2, m) 3.58(CH_2Br, m)	3.97 q, J = 7.0	0.8 t, J = 7.0
5a	$(CD_3)_2CO$	7.03–7.24 m	5.15 d, J = 3.2	[b])	4.58 dd, J = 3.2; 9.0	[b])	3.39(CH_2, s) 7.03–7.24(C_6H_5, m)	3.99 q, J = 7.1	1.06 t, J = 7.1
6a	DMSO-d_6	7.09–7.74 m	5.0 m	[b])	4.71 dd, J = 5.0, 9.0	5.27 d, J = 8.0	2.53(CH_2, d, J = 7.0) 4.20(CH ,t, J = 7.0) 7.09–7.74 [$(C_6H_4)_2$C,m]	3.98 q, J = 7.0	1.1 t, J = 7.0
6a	$CDCl_3$	7.09–7.83 m	5.22 d, J = 3.0	[b])	4.98 dd, J = 3.0, 9.0	6.27 d, J = 8.8	2.56(CH_2, q, J = 8,0) 4.37(CH, t, J = 8.0) 7.09–7.83 [$(C_6H_4)_2$C, m]	4.14 q, J = 7.0	1.18 t, J = 7.0
7a	$CDCl_3$	7.11–7.71 m	5.04 dd, J = 3.0, 7.0	[b])	4.94 dd J = 3.0, 7.0	6.11 d, J = 7.0	2.56(CH_2, d, J = 7.0) 4.39(CH, t, J = 7.0) 7.11–7.71 [$(C_6H_4)_2$C, m]	3.98 q, J = 7.0	1.04 t, J = 7.0

[a]) Residues R are the same as in Scheme 1 [b]) Overlapped with other signals

Tab. 5 ^{1}H NMR spectra (chemical shifts δ in ppm, multiplicity, coupling constants J in Hz) of compounds **1b**–**7b**

Comp. [a])	C_6H_5	CH	(OCO - R [b]) -	CH	(NH-	OCH)CO_2	CH_2-	CH_3
1b	7.18 s	6.00 d, J = 5.0	1.62 s	4.77 dd, J = 5.0, 9.0	8.42 d, J = 9.0	8.2 s	3.91 q, J = 7.0	0.93 t, J = 7.0
2b	7.20 s	6.25 d J = 5.0	1.64 s	5.11 dd, J = 5.0, 9.0	7.63 d, J = 9.0	8.11 s	4.01 q, J = 7,0	0.91 t J = 7.0
3b	7.16–7.49 m	6.27 d, J = 5.0	2.02 (CH_2, q, J = 7.0) 1.07(CH_3, t, J = 7.0)	4.98 dd, J = 5.0, 9.0	c)	8.09 s	4.02 q, J = 7.0	0.87 t, J = 7.0
4b	7.14–7.44 m	6.24 d, J = 5.0	2.73(CH_2, t, J = 6.5) 3.42(CH_2Br, t, J = 6.5)	5.02 dd, J = 5.0, 8.0	7.68 d, J = 8.0	8.13 s	4.04 q, J = 7.0	0.90 t, J = 7.0
5b	6.98–7.31 m	6.27 d, J = 4.4	3.42(CH_2, s) 6.98–7.31(C_6H_5, m)	4.99 dd, J = 4.4, 10.0	7.51 d, J = 10.0	8.10 s	3.98 q, J = 7.0	0.91 t, J = 7.0
6b	7.12–7.48 m	6.23 d, J = 4.5	2.56(CH_2, k, J = 8.0) 4.35(CH, t, J = 8.0) 7.12–7.,48[$(C_6H_4)_2$C, m]	4.98 d, J = 4.5, 9.0	7.60 d, J = 9,0	8,13 s	4.05 q, J = 7.0	0.95 t, J = 7.0
7b	7.04–7.73 m	6.21 d, J = 7.0	2.56(CH_2, t, J = 8.0) 4.33(CH, t, J = 8.0) 7.04–7.73[$(C_6H_4)_2$C, m]	5.23 dd J = 4.5, 9.0	c)	8.15 s	4.09 q, J = 7.0	1.14 t, J = 7.0

[a]) The spectra were recorded in $(CD_3)_2CO$ except for **1b** in DMSO-d_6; [b]) The radicals R are the same as in Scheme 1
[c]) Overlapped with other signals

7a) and 10 mL solution of 1.5% HF in formic acid (prepared from 3mL of 48% aqueous HF and 100 mL of 99.7% HCOOH) was heated at 50 °C for 1h. After cooling to room temperature, the reaction mixture was poured on ice. Compounds **1b**–**5b** separated as oily substances and were extracted with chloroform, the organic layer was dried over anhydrous Na_2SO_4, evaporated to dryness *in vacuo* and the residue was purified by recrystallization from corresponding solvents (see Table 1). The precipitated compounds **6b** and **7b** were filtered off, washed with water and recrystallized from an ethanol-water mixture.

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