Acylated Prodrugs of $PGF_{2\alpha}$ Analogues

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*The synthesis of triacylated Cloprostenol isopropyl ester **2a**-**2d** were realized for improving the hydrophobic character of the molecule and thus to increase the uptake of these prodrugs into the cell through the cellular membrane. The same was realized for Bimatoprost by acetylation of all 9,11,15-hydroxyl groups. Cloprostenol-1-ethanolamide was esterified to the four hydroxyl groups of the molecule. In the esters synthesized, the chain of the acid was varied from acetyl to oleoyl and stearoyl. The structure of the prodrug compounds was confirmed by ^{1}H- and ^{13}C-NMR spectra.*

Keywords: $PGF_{2\alpha}$ analogues, acylation; $PGF_{2\alpha}$ ethanolamide analogues, Cloprostenol, Bimatoprost

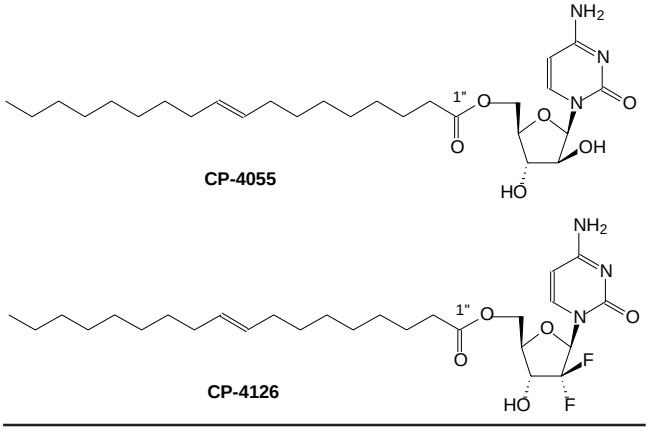
The substances containing hydroxyl or carboxyl functions are well known to penetrate with difficulty the cellular membranes. To overcome this inconvenience, the carboxyl functions are usually esterified or transformed in amides. Sometimes carboxyl groups are reduced to hydroxyl groups. The acylation of hydroxyl groups of drug substances with alkyl, especially fatty alkyl carboxylic acids make the substance, now a prodrug, to become more hydrophobic and to cross easier through the cell membrane. Other useful effects were also observed for acylated compounds. For example, the compound CP-4055, which is the 5'- elaidic ester of cytarabine, "cross cell membranes independently of nucleoside transport proteins and provides prolonged pharmacological activity inside the tumor cell" [1]. The same enhance cellular drug uptake is observed for the elaidic acid ester of gemcitabine (CP-4126) [2] (fig. 1). Antiviral nucleosides were also substituted at the 5'-position as esters with elaidic, oleic, *cis*- and *trans*-eicosaenoic fatty acids [3]. The acylation was also realized at amine group of cytosine, as in Sapacitabine (fig. 1).

In the field of prostaglandins, the esterification of 1-carboxyl function reduced the ocular irritation in topic application, reduced the dose of active substance and increase the uptake of the drug into the cell. But some side effects were observed: induce conjunctival hyperemia of varying duration and severity, foreign body sensation, smarting, etc. and in formulation presents solubility problems in some ophthalmic formulations. Also, amidation or reduction of carboxyl function is known to reduce some secondary effects and to enhance the membrane uptake of prostaglandin analogue [4]. Mono-acylation of 11-hydroxil [5a, 5b] of $PGF_{2\alpha}$ as pivaloate, acetyl, iso-butiryl, valeroyl and iso-valeroyl and of 15-hydroxyl [5c] was presented in patents. A study of the activity of acylated $PGF_{2\alpha}$ esters was also realized [5d]. Diacylation of 9,11-hydroxyls of $PGF_{2\alpha}$ or $PGF_{3\alpha}$, [5e] of 11, 15-, 9,11- (fig. 2) and 9,15-hydroxyls [5f] and mono-acylation of Bimatoprost [5g] keeps the ocular antihypertensive activity of the so protected compounds and reduce their ocular irritation on topic application.

In scheme 1 is presented the synthesis of 9,11-diacylated $PGF_{2\alpha}$ or $PGF_{3\alpha}$.

Generally, the synthesis of diacylated $PGF_{2\alpha}$ suppose the protection of one hydroxyl group, followed by acylation of the remaining two hydroxyl groups (scheme 1).

The enhance of the prostaglandin analogues acylated prodrugs uptake into the cell is expected to take place also by substitution of all hydroxyls with alkyl or fatty acid esters. Some favorable effects were observed for triacylated $PGF_{2\alpha}$ or its C-1 esters [6]. Enzyme deprotection of the acyl function by hydrolases and delivery of the active prostaglandins into the cell is expected to improve the activity of the drug. Also, the acylated prodrugs could be easier formulated (at least in certain cases) as drugs.



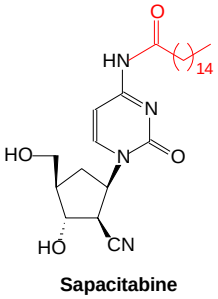


Fig. 1. Elaidic esters of cytarabine and gemcitabine

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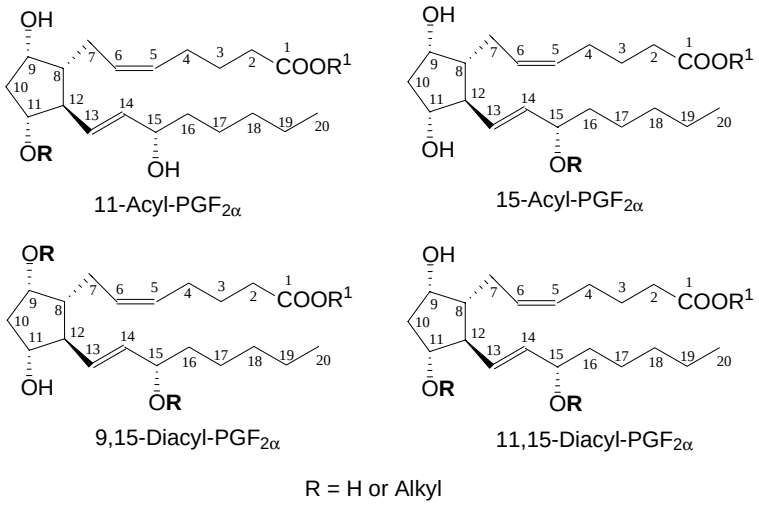
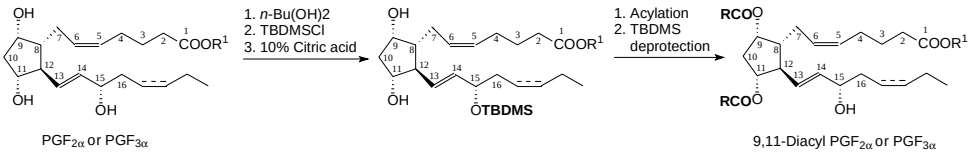


Fig. 2. 11- and 15-Mono-acyl $PGF_{2\alpha}$ and 9,15-and 11,15-diacyl-$PGF_{2\alpha}$



Scheme 1. Synthesis of 9-11-diacyl $PGF_{2\alpha}$ or $PGF_{3\alpha}$

Experimental part

IR spectra were recorded on a FT-IR-100 Perkin Elmer spectrometer, in solid phase by ATR and frequencies are expressed in cm^{-1}, with the following abbreviations: w = weak, m = medium, s = strong, v = very, br = broad. ^{1}H-NMR and ^{13}C-NMR spectra are recorded on Varian Gemini 300 BB spectrometers (300 MHz for ^{1}H and 75 MHz for ^{13}C), chemical shifts are given in ppm relative to TMS as internal standard. Complementary spectra: 2D-NMR and decoupling were done for correct assignment of NMR signals. The numbering of the atoms in compounds is presented in schemes 2-4. Progress of the reaction was monitored by TLC on Merck silica gel 60 or $60F_{254}$ plates (Merck) eluted with the solvent system presented in table 1. Spots were developed with sulfuric acid (15% in ethanol).

General Procedure for synthesis of acylated prosta-glandin analogues compounds [7]

The prostaglandin analogue: (±)- and (+)-Cloprostenol, Bimatoprost (1 mmole) or (+)-Cloprostenol-1-ethanol-amide, was dissolved in 5 mL pyridine and 10 mL anh. toluene, 100 mg DMAP was added, the solution was cooled on an ice-bath and acylating reagent (acetic anhydride or pivaloyl-, oleoyl, stearoyl or lauroyl chloride), 2 equivalents/ hydroxyl group, was dropwise added, and the reaction mixture was stirred overnight at room temperature, monitoring the end of the reaction by TLC in solvent systems presented in table 1.

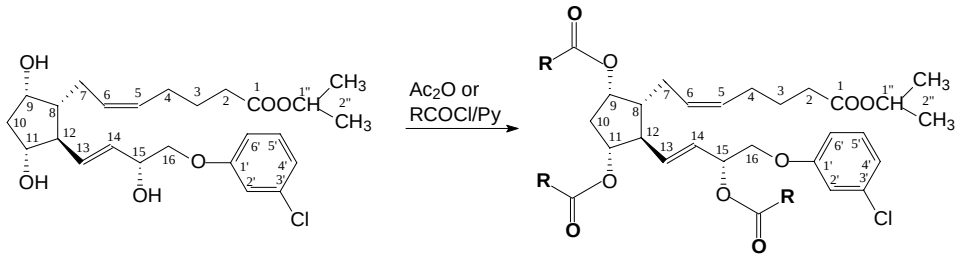
In some cases, more acylating reagent was added. The reaction mixture was poured on ice, stirred >1h, phases were separated, organic phase was washed with sat. soln. $NaHCO_3$ (2×10 mL), dried (Na_2SO_4), filtered and

Comp. no.	Physical state, m.p.	$[\alpha]_D$	Yield %	R_f	Analysis		
					IR	NMR	Elem. anal.
2a	Oil	+28.9°(1% in EtOH)	83.9	0.81[1]		+	+
2b	Oil	+19.65°(1% in EtOH)	93.6	0.83[1]		+	+
2c	45-47°C	-	64.7	0.55[2]		+	+
(±)-2d	Oil		89			+	
(+)-2d	Oil	+18.7 °(2% in $CHCl_3$)			+	+	
4	Oil	+35.8°(1% in EtOH)	80	0.72[3]	+	+	
5	Oil	+14.30°(1% in EtOH), +9.95°(1% in $CHCl_3$)			+	+	
7a	Oil	+11.86 °(1% □n EtOH)	93.4	0.67[3]	+	+	
7b	Oil	+19.6 °(1% □n EtOH)	85	0.74[3] 0.80[4]	+	+	
7c	49-50°C	+24.1° (2% □n $CHCl_3$)	88	0.80[3]	+	+	
8	Oil	+9.95° (1% □n $CHCl_3$) +7.6 °(1% □n EtOH)				+	

Table 1
CHARACTERISTIC PROPERTIES FOR ACYLATION OF PROSTAGLANDIN ANALOGUES

[1]benzene-ethyl acetate-methanol-80% formic acid, 20:80:3:2, [2]hexane-ethyl acetate, 10:1, [3]Acetone-dichloromethane-methanol-80% formic acid, 10:80:7:2, [4] hexane-ethyl acetate-acetic acid, 5:3:0.1.

Scheme 4. Synthesis of tetra-acylated derivatives of (+)-Cloprostenol 1-ethanolamide



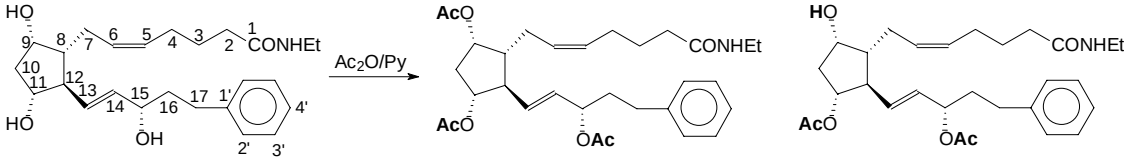
Cloprostenol-1-isopropylester

1

Cloprostenol-1-isopropylester 9,11,15-triacylated

2a, R = CH_3
2b, R = $C(CH_3)_3$
2c, R = $(CH_2)_{16}CH_3$
2c, R = $(CH_2)_7CH=CH(CH_2)_7CH_3$

Scheme 2. Synthesis of 9,11,15-triacyl derivatives of Cloprostenol isopropyl esters.



Bimatoprost

3

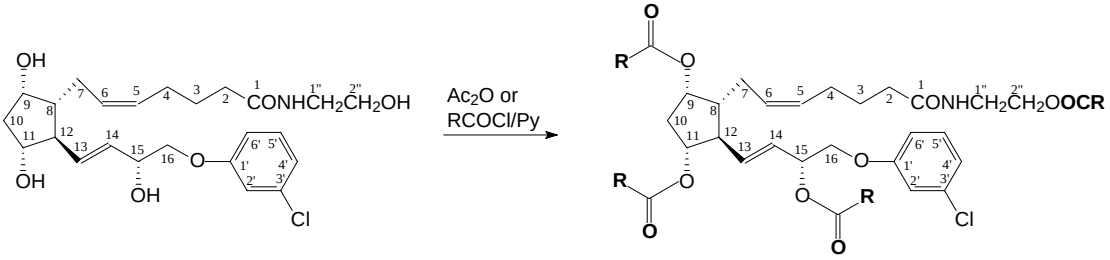
Bimatoprost 9,11,15 triacetate

4

Bimatoprost 11,15 triacetate

5

Scheme 3. Synthesis of Bimatoprost 9,11,15-triacetate



Cloprostenol-1-ethanolamide

6

Cloprostenol-1-ethanolamide tetraacetat

2a, R = CH_3
2b, R = $C(CH_3)_3$
2c, R = $(CH_2)_{10}CH_3$

Scheme 4. Synthesis of tetra-acylated derivatives of (+)-Cloprostenol 1-ethanolamide

concentrated under reduced pressure (Aqueous phases were more extracted with 2×20 mL toluene). The concentrate was co-evaporated with toluene to remove the residual pyridine and the crude product was purified by pressure chromatography on a silica gel column (eluent: hexane-ethyl acetate 5:2 to 2:1), resulting the pure products as oils, with exception of compound **2d** (D-Cloprostenol isopropylester tristearate) and **7c** (tetralauroyl D-Cloprostenol 1-ethanolamide). Analytical data for pure acylated prostaglandin analogues are presented in table 1 and ^{1}H- and ^{13}C-NMR signals for protons and carbon atoms are presented in tables 2 to table 4.

Results and discussions

We have realized a different way for acylation in which we esterified not one or two hydroxyl groups, but all the hydroxyl groups of the prostaglandine $F_{2\alpha}$ analogues present in the molecule; This kind of tri-substitution was realized for $PGF_{2\alpha}$[100b] and esters [6]. Thus, the hydrophobic character of the molecule is expected to be seriously enhanced and the uptake of the prodrug into the cell will be increased. In the same time we suppose that deacylation of the prodrug by hydrolase enzymes will improve the drug delivery within the cell for a longer period of time.

We started this plan with Cloprostenol isopropyl ester, a $PGF_{2\alpha}$ analogue used as veterinary luteolytic drug for the control of reproduction in farm animals [8] and also studied for lowering of intraocular pressure [9]. In the literature we observed that (±)-Cloprostenol methyl ester was triacetylated for elaboration of a method for quantitative gas chromatography-mass spectrometry determination of prostaglandins at picomol level [10a] and ^{13}C-NMR was also realized on $PGF_{2\alpha}$ derivatives [10b]. We also synthesized (±)-Cloprostenol triacetate and tested it for cytotoxic effect on chicken embryo fibroblasts (CEF) and on human cells (HeLa), [11] but its synthesis was not yet published.

As depicted in scheme 2, (±)- or (+)-Cloprostenol isopropyl esters were acylated with acetic anhydride or an acyl chloride (pivaloyl chloride, stearoyl chloride, oleoyl chloride) in the presence of pyridine as organic base and in the presence or absence of an inert solvent (like toluene or dichloromethane), in the usual way. The crude products were purified by pressure chromatography and the pure 9,11,15-tri-acylated compounds **2a-2b** and **2d** were obtained as oils and stearoyl compound **2c** was obtained crystalized. The characteristic properties of compounds **2a-2d** are presented in table 1. ^{1}H- and ^{13}C-NMR spectra are presented in table 2 and confirmed their molecular structure.

The prostaglandin $F_{2\alpha}$ analogue Bimatoprost **3** is used in the treatment of glaucoma and in the reduction of intraocular pressure by increasing the outflow of aqueous liquid from the eyes [12a]. Now it is used also in cosmetics for darkening and lengthening eyelashes [12b]and stimulating hair growth [12c]. It was found that

Table 2

THE SIGNALS OF THE ATOMS IN THE ^{1}H- AND ^{13}C-NMR SPECTRA OF THE TRI-ACYLATED DERIVATIVES OF CLOPROSTENOL ISOPROPYLESTER

Comp no.	Atom numbers*																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1'	2'	3'	4'	5'	6'	1"	2"
2a		**2.23** (t, 2H, 7.4)	**1.61** (q_v, 2H, 7.3)	**2.04** (m, 2H)	**5.37** (dd, 6.2, 10.9) ***or 6***	**5.30** (dd, 6.0, 10.9) ***or 5***	**2.11** (m, 2H)	**1.76-1.55** (m)	**5.09** (td, 1.4, 5.8)	**2.53** (ddd, 5.6, 8.9, 15.9) **1.76-1.55** (m)	**4.92** (dt, 4.4, 7.4)	**2.60** (dt, 7.6, 12.1)	**5.73** (dd, 7.6, 15.1)	**5.67** (dd, 5.9, 15.1)	**5.60** (br dt, 4.7, 5.9)	**4.07** (dd, 5.9, 10.2) **4.02** (dd, 4.4, 10.2)		**6.90** (t, 2.1)		**6.98** (ddd, 0.9, 2.0, 8.1)	**7.20** (t, 8.1)	**6.79** (ddd, 0.9, 2.1, 8.1)	**4.99** (sept, 6.2)	**1.22** (d, 6H, 6.2)
	172.9	**33.9**	**24.7**	**26.5**	**127.6**	**130.1**	**24.8**	**47.3**	**74.2**	**38.8**	**77.6**	**51.9**	**134.6**	**127.2**	**71.7**	**69.1**	**159.1**	**115.1**	**134.8**	**121.3**	**130.2**	**113.0**	**67.4**	**21.7**
2b	**Acyl group signals**					**2.10** (s, 3H, CH_3CO), **2.07** (s, 3H, CH_3CO), **2.02** (s, 3H, CH_3CO)											**21.1** (CH_3CO), **21.0** (CH_3CO), **20.96** (CH_3CO)								
		2.22 (t, 2H, 6.9)	**1.63** (q_v, 7.4)	**1.99** (m, 2H)	**5.34** (dt, 5.7, 11.0) ***or 6***	**5.37** (dt, 5.7, 11.0) ***or 5***	**2.13** (m, 2H)	**1.72** (m)	**5.10** (br t, 4.3)	**2.54** (ddd; 5.5, 9.3, 16.0) **1.53** (ddd, 1.1, 4.0, 16.0)	**4.91** (dt, 4.1, 7.7)	**2.62** (dt, 7.4, 12.3)	**5.75** (dd, 7.7, 15.2)	**5.67** (dd, 5.9, 15.2)	**5.57** (br dt, 4.7, 5.9)	**4.06** (dd, 6.3, 10.2) **4.00** (dd, 4.7, 10.2)		**6.88** (br t, 1.9)		**6.94** (ddd, 0.9, 1.9, 8.2)	**7.19** (t, 8.2)	**6.77** (ddd, 0.9, 2.5, 8.2)		**1.21** (d, 6H, 6.2)
	172.9	**34.0**	**24.8**	**26.6**	**127.6** ***or 6***	**130.2** ***or 5***	**25.0**	**47.9**	**74.2**	**39.2**	**77.3**	**52.5**	**134.7**	**127.5**	**71.4**	**69.4**	**159.3**	**115.2**	**134.9**	**121.3**	**130.2**	**113.1**	**67.4**	**21.8**
2c	**Acyl group signals**						**1.22** (s, 9H, *t*-Bu), **1.19** (s, 9H, *t*-Bu), **1.15** (s, 9H, *t*-Bu)									**38.95** (C_q, *t*-Bu), **38.82** (C_q, *t*-Bu), **38.47** (C_q, *t*-Bu), **34.03** (C-2), **27.12** ($3CH_3$, *t*-Bu), **27.07** ($3CH_3$, *t*-Bu), **27.04** ($3CH_3$, *t*-Bu),								
		2.36-2.18 (m, 2H)	**1.75-1.52** (m, 2H)	**2.15-1.92** (m, 2H)	**5.33** (m)	**5.33** (m)	**2.15-1.92** (m, 2H)	**1.75-1.52** (m)	**5.10** (br t, 4.4)	**2.64-2.46** (m) **1.75-1.52** (m)	**4.92** (m)	**2.64-2.46** (m)	**5.79-5.55** (m)	**5.79-5.55** (m)	**5.79-5.55** (m)	**4.03** (m, 2H, 4.3, 5.7, 9.9)		**6.89** (t, 2.2)		**6.93** (br d, 8.2)	**7.18** (t, 8.2)	**6.77** (dd, 2.2, 8.2)	**4.99** (sept, 6.2)	**1.21** (d, 6.2)
	172.9	**34.0**	**24.8**	**26.6**	**127.7** ***or 6***	**130.1** ***or 5***	**25.0**	**47.6** ***or 12***	**74.1**	**39.1**	**77.4**	**52.2** ***or 8***	**134.7**	**127.2**	**71.4**	**69.3**	**159.2**	**115.1**	**134.9**	**121.3**	**130.2**	**113.0**	**67.4**	**21.8**
2d	**Acyl group signals**			**1.75-1.52** (m, 10H, 6H-β-CO-stearoyl), **1.35-1.23** (m, 84H, CH_2-stearoyl), **0.88** (t, 9H, Me-stearoyl, 6.9)													**34.57** (CH_2, C-2'', C3α-stearoyl), **34.41** (CH_2, C-2'', C3α-stearoyl), **34.35** (CH_2, C3α-stearoyl, C-2''), **33.99** (CH_2, C3α-stearoyl), **31.91** (CH_2), **29.70** (CH_2), **29.65** (CH_2), **29.53** (CH_2), **29.50** (CH_2), **29.35** (CH_2), **29.30** (CH_2), **29.21** (CH_2), **29.16** (CH_2), **29.12** (CH_2), **25.01** (CH_2), **24.77** (CH_2), **22.67** (CH_2), **14.10** ($3CH_3$, stearoyl).								
		2.17 (t, 7.6)	**1.66-1.44** (m)	**2.04** (br t, 2H)	**5.32-5.21** (m)	**5.32-5.21** (m)	**1.98-1.87** (m, 2H)	**1.66-1.44** (m)	**5.03** (t, 4.5)	**2.45** (ddd, 5.2, 8.4,	**4.85** (dt, 4.4, 8.8)	**2.52** (dt, 7.4, 12.1)	**5.70-5.50** (m)	**5.70-5.50** (m)	**5.70-5.50** (m)	**3.98** (dd, 6.0, 10.2) **3.93**		**6.82** (tt, 1.4, 2.3)		**6.86** (ddd, 0.8, 2.3,	**7.11** (t, 8.0)	**6.70** (ddd, 0.8, 2.3,	**4.91** (m_{sept}, 6.3)	**1.21** (d, 6.3)
										16.0)						(dd, 4.7, 10.2)								
	172.9	**34.1**	**24.9**	**26.7**	**127.3**	**130**	**25.1**	**47.7** ***or 12***	**74.2** ***8***	**39.1**	**77.4**	**52.3** ***or 8***	**134.8**	**129.6**	**71.5**	**69.4**	**159.3**	**115.3**	**135.0**	**121.4**	**130.3**	**113.2**	**67.4**	**22.0, 21.9**
	Acyl group signals	**2.25** (t, 6H, H-2''-oleoyl, 7.2), **1.34-1.10** (m, nH, CH_2-oleoyl), **0.91** (t, 9H, Me-oleoyl, 7.1).														**34.53** (CH_2, C3α-oleoyl, C-2''), **32.02** (CH_2, C-2'', C3α-oleoyl), **29.78** (nCH_2), **29.64** (nCH_2), **29.43** (CH_2), **27.33** (nCH_2), **25.09** (nCH_2), **22.80** ($3CH_2$), **14.22** ($3CH_3$, oleoyl).								

*The numbering of the atoms is presented in Scheme 2.

bimatoprost also reduce fat by inhibition of adipocyte differentiation and survival [12d, 12e]. Bimatoprost was acetylated similarly with excess acetic anhydride and in the presence of DMAP as catalyst to Bimatoprost triacetate **4** and the crude product was purified by pressure chromatography (scheme 3). The pure product was obtained as oil. In one experiment, a diacetylated compound **5** was isolated in fractions eluted from column chromatography purification, which, from analysis of NMR spectra (table 2), looks to be Bimatoprost 11,15 diacetate. The product was probably formed because of the acetic acid present in the ethyl acetate used in that purification or to an incomplete reaction of esterification (See also its characteristics in table 1). The ^{1}H- and ^{13}C-NMR spectra of both compounds are presented in table 3 and confirmed their molecular structure.

It is well known that prostaglandin-ethanolamides, called also prostamides, are found in almost all the tissues of the human body, in which are formed by biosynthesis of anandamine [13]. These substances are neutral lipid mediators and act as local hormones with important biological roles such as regulators of the intraocular pressure, hair growth, adipose tissue methabolism and on central nervous system [14]. Several synthetic derivatives were developed in order to obtain drugs with similar structures, but slightly different, to the natural compounds with prolonged pharmaceutical effects and reduced side-effects. Significant pharmacological effects, like: reducing

Table 3

THE SIGNALS OF THE ATOMS IN THE ^{1}H- AND ^{13}C-NMR SPECTRA OF BIMATOPROST TRI-ACETATE **4** AND OF BIMATOPROST DIACETATE 5

Comp no.	Atom numbers																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	1'	2'	3'	4'	1"	2"
4		**2.20-1.87** (m, 2H)	**2.20-1.87** (m, 2H)	**2.20-1.87** (m, 2H)	**5.35** (dd, 4.7, 10.7)	**5.32** (dd, 4.4, 10.7)	**2.20-1.87** (m, 2H)	**1.70-1.60** (m)	**5.09** (td, 1.4, 5.5)	**2.52** (ddd, 5.8, 9.1, 16.0) **1.70-1.60** (m)	**4.89** (ddd, 1H, 4.4, 7.4, 8.8)	**2.57** (dt, 7.2, 11.2)	**5.62-5.49** (m)	**5.62-5.49** (m)	**5.26** (ddt, 2.7, 5.5, 7.4)	**1.70-1.60** (m, 2H)	**2.65** (dd, 2.8, 6.6) **2.61** (dd, 2.5, 6.6)		**7.18** (m, 2H)	**7.29** (t_t, 2H, 6.8)	**7.18** (m)	**3.25** (br q, 7.1)	**1.10** (t, 3H, 7.1)
	172.8	**36.2**	**25.0**	**26.9**	**130.4** *or 6*	**131.2** *or 5*	**25.7**	**47.7**	**74.3**	**39.1**	**77.9**	**52.0**	**132.5**	**127.8**	**73.5**	**31.7**	**36.2**	**141.4**	**128.5**	**128.6**	**126.2**	**34.4**	**15.0**
	Acyl group signals			**2.06** (3H, C*H*$_3$CO), **2.05** (3H, C*H*$_3$CO), **2.01** (3H, C*H*$_3$CO)												**170.8** (COO), **170.6** (COO), **170.5** (COO), **21.4** (3CH$_3$CO)								
5		**2.28** (m) **2.23-1.86** (m)	**2.23-1.86** (m, 2H)	**2.23-1.86** (m, 2H)	**5.38** (br dt, 2H, H-5, H-6, 5.6)	**5.38** (br dt, 2H, H-5, H-6, 5.6)	**2.23-1.86** (m, 2H)	**1.50** (m)	**4.16** (t_t, 4.3)	**2.40** (ddd, 5.2, 9.1, 15.4) **1.70** (ddd, 4.7, 9.1, 15.4)	**4.89** (ddd, 3.8, 7.1, 8.8)	**2.58** (dt, 7.4, 11.5)	**5.60-5.49** (m)	**5.60-5.49** (m)	**5.27** (dt, 5.5, 7.1)	**1.66** (m, 2H)	**2.65** (dd, 2.8, 6.6) **2.62** (dd, 2.5, 6.6)		**7.15-7.30** (m, 2H)	**7.15-7.30** (m, 2H)	**7.15-7.30** (m)	**3.27** (qd, 2H, 5.5, 7.1)	1.12 (t, 3H, 7.1)
	173.2	**36.0**	**25.0**	**26.8**	**130.4** *or 6*	**130.8** *or 5*	**25.7**	**49.7**	**71.7**	**39.1**	**78.0**	**51.2**	**133.4**	**127.8**	**73.8**	**31.7**	**36.2**	**141.5**	**128.5**	**128.6**	**126.1**	**34.5**	**15.0**
	Acyl group signals			**2.05** (s, 3H, C*H*$_3$CO), **2.03** (s, 3H, C*H*$_3$CO)												**171.0** (COO), **170.5** (COO), **21.4** (3CH$_3$CO)								

Table 4

THE SIGNALS OF THE ATOMS IN THE ^{1}H- AND ^{13}C-NMR SPECTRA OF THE TETRA-ACYLATED DERIVATIVES OF CLOPROSTENOL 1-ETHANOLAMIDE 7**A-7C** AND OF ITS TRI-PIVALOYL DERIVATIVE **8**

Comp no.	Atom numbers																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1'	2'	3'	4'	5'	6'	2"	1"
7a		**2.11-1.89** (m, 2H)	**1.65-1.56** (m, 2H)	**2.11-1.89** (m, 2H)	**5.30** (dd, 5.8, 11.0) *or 6*	**5.26** (dd, 6.0, 11.0) *or 5*	**2.11-1.89** (m, 2H)	**1.65-1.56** (m)	**5.03** (td, 1.4, 5.8)	**2.46** (ddd, 5.5, 8.8, 15.9) **1.65-1.56** (m)	**4.86** (ddd, 4.2, 7.4, 8.5)	**2.55** (br dt, dt, 4.7, 7.4)	**5.68-5.55** (m)	**5.68-5.55** (m)	**5.68-5.55** (m)	**4.00** (dd, 5.8, 10.2) **3.95** (dd, 4.4, 10.2)	**171.2** (C$_0$), **170.8** (C$_0$), **170.6** (C$_0$), **170.3** (C$_0$), **21.4** (CH$_3$CO), **21.3** (CH$_3$CO), **21.2** (CH$_3$CO), **21.0** (CH$_3$CO).							
	173.0	**36.1**	**24.9**	**26.8**	**127.8**	**130.4**	**25.5**	**47.7**	**74.4**	**38.8**	**77.7**	**52.1**	**134.6**	**127.4**	**71.9**	**69.3**	**159.3**	**115.3**	**135.0**	**121.6**	**130.5**	**113.2**	**63.5**	**39.1**
	Acyl group signals			**5.85** (br t, 1H, NH), **2.04** (s, 3H, C*H*$_3$CO), **2.00** (s, 6H, C*H*$_3$CO), **1.95** (s, 3H, C*H*$_3$CO).																				
7b		**2.36** (t, 2H, 7.4)	**1.61** (qv, 2H, 7.4)	**1.94** (m_p, 2H, 7.1)	**5.30** (dd, 4.7, 10.6) *or 6*	**5.25** (dd, 5.7, 10.6	**2.05** (m, 2H)	**1.26-0.80** (m)	**5.03** (br t, 4.7)	**2.48** (ddd, 5.5, 9.3, 15.9) **1.47** (dd, 3.8, 15.9)	**4.84** (br dt, 3.8, 7.4)	**2.56** (br dt, 7.7, 12.4)	**5.68** (dd, 7.1, 15.4)	**5.60** (dd, 5.8, 15.4)	**5.50** (q, 5.5)	**3.98** (dd, 6.6, 10.2) **3.93** (dd, 4.7, 10.2)	**178.4** (COO-Piv), **178.1** (COO-Piv), **177.7** (COO-Piv), **177.5** (COO-Piv), **39.1** (C_q-^{t}Bu), **38.9** (C_q-^{t}Bu), **28.5** (CH$_3$-^{t}Bu), **27.2** (CH$_3$-^{t}Bu)							
	175.5	**35.4**	**25.0**	**26.7**	**127.9**	**130.2**	**25.1**	**47.9**	**74.3**	**38.9**	**77.6**	**52.7**	**134.8**	**127.6**	**71.4**	**69.4**	**159.4**	**115.3**	**135.0**	**121.4**	**130.4**	**113.2**	**62.5**	**39.2**
	Acyl group signals			**1.23** (s, 9H, CH$_3$-^{t}Bu), **1.15** (s, 9H, CH$_3$-^{t}Bu), **1.11** (s, 9H, CH$_3$-^{t}Bu), **1.10** (s, 9H, CH$_3$-^{t}Bu), **1.08** (s, 9H, CH$_3$-^{t}Bu).																				
8		**2.34** (t, 2H, H-2, 7.4)	**1.70-1.60** (m, 2H)	**2.03-1.96** (m) **2.11** (m)	**5.34** (br t, 2H, H-5, H-6, 5.5)	**5.34** (br t, 2H, H-5, H-6, 5.5)	**2.12** (t, 2H, 7.4)	**1.70-1.60** (m)	**5.10** (br t, 4.7)	**2.54** (ddd, 5.5, 9.3, 15.4) **1.54** (bdd, 4.1., 15.4)	**4.91** (ddd, 3.8, 8.8, 9.3)	**2.62** (br dt, 6.6, 12.4)	**5.74** (dd, 4.3, 15.1)	**5.67** (dd, 3.0, 15.1)	**5.57** (dt, 4.7, 6.3)	**4.05** (dd, 6.6, 10.4) **4.00** (dd, 4.7, 10.4)	**178.2** (COO-Piv), **177.8** (2COO-Piv), **39.3** (C_q-^{t}Bu), **39.0** ($2C_q$-^{t}Bu), **25.0** (C_q-CH$_3$), **28.5** (CH$_3$-^{t}Bu), **27.2** (CH$_3$-^{t}Bu), **25.6** (C_q-CH$_3$).							
	173.2	**36.1**	**25.0**	**26.7**	**127.9**	**130.4**	**24.9**	**48.1**	**74.3**	**39.3**	**77.4**	**52.7**	**134.6**	**127.7**	**71.5**	**69.5**	**159.4**	**115.3**	**135.0**	**121.5**	**130.5**	**113.3**	**63.2**	**38.6**
	Acyl group signals			**1.26** (s, 3H, CH$_3$-^{t}Bu), **1.23** (s, 6H, CH$_3$-^{t}Bu), **1.22** (s, 6H, CH$_3$-^{t}Bu), **1.19** (s, 6H, CH$_3$-^{t}Bu), **1.15** (s, 6H, CH$_3$-^{t}Bu), **1.15** (s, 6H, CH$_3$-^{t}Bu).																				
7c			**1.62-1.50** (m, 2H)	**2.08-1.90** (m, 2H)	**5.28-5.24** (m)	**5.28-5.24** (m)	**2.08-1.90** (m, 2H)	**1.62-1.50** (m)	**5.04** (br t, 5.2)	**2.43** (m, 5.8) **1.62-1.50** (m)	**4.87** (ddd, 3.8, 7.7, 8.5)	**2.52** (dt, 8.5, 12.1)	**5.68-5.55** (m)	**5.68-5.55** (m)	**5.68-5.55** (m)	**3.98** (dd, 5.2, 10.2) **3.93** (dd, 4.7, 10.2)	**178.2** (COO-Piv), **177.8** (2COO-Piv), **39.3** (C_q-^{t}Bu), **39.0** ($2C_q$-^{t}Bu), **25.0** (C_q-CH$_3$), **28.5** (CH$_3$-^{t}Bu), **27.2** (CH$_3$-^{t}Bu), **25.6** (C_q-CH$_3$).							
	173.2	**36.1**	**25.0**	**26.9**	**127.9**	**130.4**	**25.5**	**47.9**	**74.2**	**38.9**	**77.5**	**52.3**	**134.6**	**127.3**	**71.6**	**69.4**	**159.3**	**115.3**	**135.0**	**113.2**	**130.4**	**121.5**	**63.2**	**39.3**
	Acyl group signals			**2.29-2.15** (m, 11H, 8H-α-CO-lauroyl, 2H-, H-), **1.62-1.50** (m, 12H, 8H-β-CO-lauroyl), **1.26-1.14** (m, 54H, CH$_2$-lauroyl), **0.81** (t, 12H, Me-lauroyl, 6.8)														**36.06** (CH$_2$), **34.76** (2CH$_2$, C-2'', C-3α-lauroyl), **34.59** (CH$_2$, C-2''', C-3α-lauroyl), **34.49** (2CH$_2$, C-3α-lauroyl, C-2''), **34.28** (CH$_2$, C-2'''', C-3α-lauroyl), **32.04** (4CH$_2$), **29.75** (nCH$_2$), **29.64** (nCH$_2$), **29.47** (nCH$_2$), **29.29** (nCH$_2$), **25.14** (3CH$_2$), **25.03** (1CH$_2$), **22.81** (4CH$_2$), **14.24** (4CH$_3$, lauroyl)							

intraocular pressure, a protective role against the oxidative stress in different tissues and very low toxicity, were observed in the case of synthetic ethanolamide analogues of Cloprostenol [15]. Disadvantages of Cloprostenol ethanolamides reside from the fact that, like Cloprostenol, are highly lipophilic compounds and only a few derivatives could be used in the studies due to the difficulties of drug formulations [16].

In the case of Cloprostenol 1-ethanolamide, another hydroxyl (of ethanolamide group) was acylated together with the three hydroxyls of prostaglandin molecules. In all cases an excess of acylating reagent was used and all reactions were realized in the presence of DMAP as catalyst. Tetra-acetate **7a** and tetra-pivaloate **7b** compounds were obtained as oils and tetra-lauroyl compound **7c** was obtained crystallized (scheme 5); some characteristic properties are presented also in table 1. The signals of the protons and carbon atoms were presented in table 4 and are in full agreement with molecular structure of the compounds. In all cases, complementary 2D spectra: COSY and HETCOR, were registered for the correct attribution of the signals presented in NMR spectra.

As in the case of Bimatoprost triacetate, when (+)-Cloprostenol-1-ethanolamide tetra-pivaloate was purified by pressure column chromatography on silica gel with the solvent system: hexane-ethyl acetate, a triacetate derivative **8** was obtained, in which, according with NMR data, the 9-hydroxyl group looks to be unprotected (free). The signal of the H-9 proton appeared at an increased field at δ 4.16 ppm as triplet (J = 4.3 Hz) by comparison with the signal of the H-9 proton in Bimatoprost triacetate, **4**, [δ5.09 ppm as triplet of doublets (J = 1.4, 5.5 Hz)]. The same thing happened for ^{13}C: C-9 appeared at δ 71.7 ppm, while in compound **4** it appeared at δ 74.3 ppm.

A patent pending for the poly-acylated compounds was deposited [6].

Conclusions

We have realized the synthesis of triacylated Cloprostenol isopropyl ester **2a-2d** and of tri-acetate ester of Bimatoprost **4** and tetra-acylated derivatives of Cloprostenol-1-ethanolamide **7a**-**7c** as highly hydrophobic prodrugs of the parent nucleoside compounds. These chemical forms are supposed to improve the uptake of the prodrugs into the cells where, hydrolase enzymes deacylate the prodrug and deliver slowly the prostaglandin analogue. In the same time these prodrugs are easier included at least in certain pharmaceutical formulations.

The prodrugs were obtained mainly as optically active compounds and a few as racemic. The structure of the compounds were confirmed mainly by ^{1}H- and ^{13}C-NMR spectroscopy, inclusive complementary 2D-spectra for correct attribution of the signals to proton and carbon atoms presented in each molecule. The use of these prodrugs in pharmaceutical formulation and in the evaluation of their activity will be presented separately in a future paper.

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