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# NITROANANDAMIDE, NITROPROSTAMIDES $E_2$ AND $F_{2\alpha}$, AND THEIR ANALOGS

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*Synthetic methods were developed for nitrates of the natural bioactive lipids anandamide and prostamides $E_2$ and $F_{2\alpha}$ in addition to their analogs that contained nitrates of ethanolamine and 3-amino-1,2-propanediol as the NO-generating fragment.*

**Keywords:** anandamide, prostamides, prostaglandins, endocannabinoids, arachidonic acid, amides, nitric oxide, organic nitrates, aminoalcohol nitrates.

Acyl derivatives of ethanolamine that contain fatty acids belong to a recently discovered family of endogenous bioactive lipids with a broad spectrum of activity and are effectors of the endocannabinoid and endovanilloid brain systems [1]. *N*-Arachidonylethanolamine (anandamide or AA-EA) is the most studied member of this family of endocannabinoids [2]. Anandamide is synthesized and released by neurons in response to membrane depolarization and is considered to be a neuronal messenger [3]. It is a substrate of enzymes of the cyclooxygenase oxidative metabolism of fatty acids. The resulting prostaglandin ethanolamides, prostamides, are biologically active compounds with an altered activity profile that apparently constitute a separate group of prostanoids [4]. Anandamide and prostamides exhibit high biological activity and are capable of interacting with several signal transduction systems. They are promising templates for constructing prototypes of polyfunctional drugs targeted primarily for treating neurodegenerative diseases.

A fertile approach to discovering new multi-functional drugs is the introduction into a pharmacologically active compound of a supplemental pharmacophore, e.g., an NO donor [5]. The usefulness of this approach has been demonstrated [6] using as examples 1,3-dinitroglycerin esters of prostaglandins, nitroxylipins. These are nitro derivatives of cyclooxygenase metabolites of the endocannabinoid 2-arachidonylglycerin. Both pharmacophores in nitroxylipin fulfill their function and intensify the activity of each other. The prostaglandin part directs the molecule to specific receptors. The nitro ester interacts with target cells and releases NO.

We synthesized amides of arachidonic and eicosapentenoic acids and the cyclooxygenase metabolites of arachidonic acid prostaglandins $E_2$ and $F_{2\alpha}$ in addition to 11-deoxyprostaglandin $E_1$ with nitrates of ethanolamine (**1a**) and 3-amino-1,2-propanediol (**1b**) as the NO-generating fragments. First, nitrate salts of the nitroxyalkylamines (**1a** and **1b**) were prepared from the aminoalcohols by direct nitration with conc. $HNO_3$ in $CH_2Cl_2$ [7, 8] (Scheme 1). Then, these nitrates of the aminoalcohols were condensed with the acids to produce the target compounds. The optimal synthetic conditions for the nitroamides were found for each class of starting acids.

Amides of prostaglandins were prepared via activation of the carboxyls, converting them into mixed anhydrides with isobutyl carbonate [9] (Method A) or into activated imidazolides [10] (Method B) (Scheme 1). For example, reaction of 11-deoxyprostaglandin $E_1$ (**2**) with isobutylchloroformate or carbonyldiimidazole produced the mixed anhydride (**3**) or imidazolide (**4**), respectively. These were reacted without isolation with the nitroxyalkylamines (**1a** and **1b**) to produce nitroethanolamide (**5a**) or amide (**5b**) (Scheme 1). The nitroethanolamides of prostaglandin $E_2$ (**6a**) and $F_{2\alpha}$ (**7a**)

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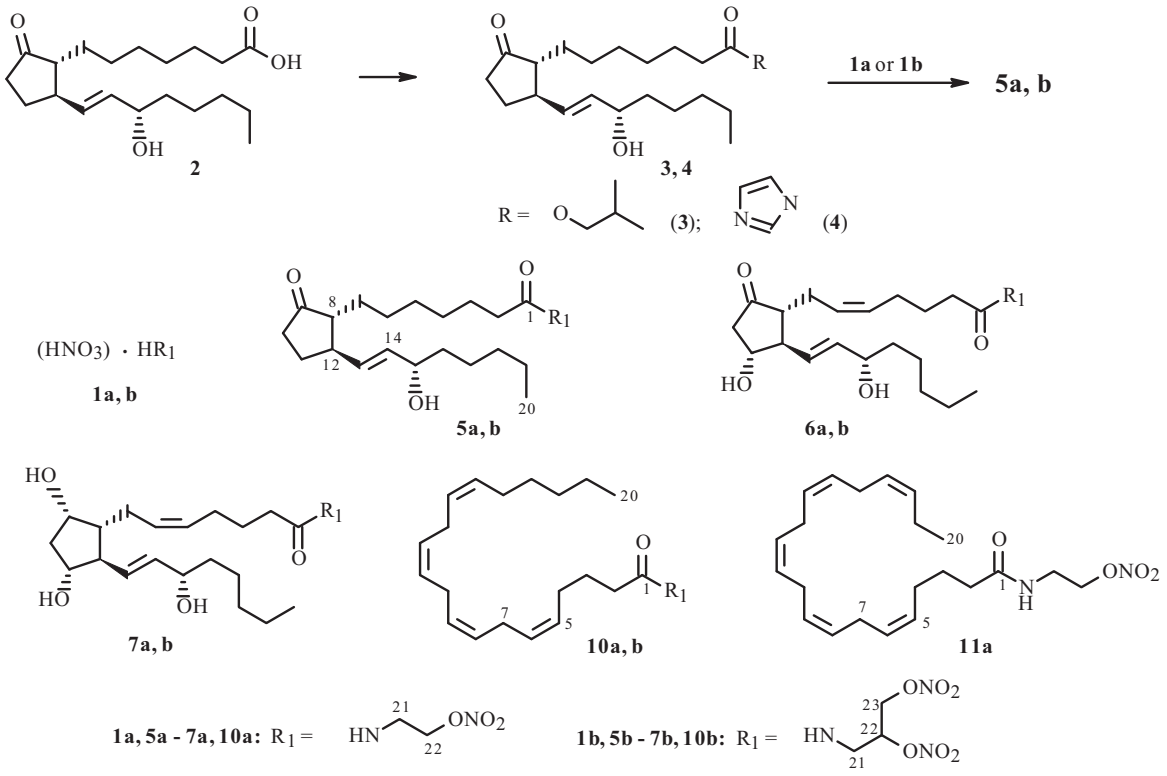
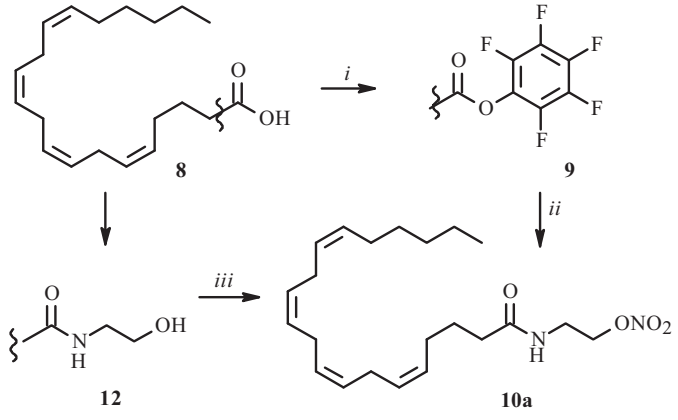
(nitroprostamides) and the amides of these prostaglandins with 3-amino-1,2-propanediol 1,2-dinitrate (**6b**, **7b**) were synthesized analogously (Scheme 1).




Scheme 1

The carboxyl was activated by converting it to the pentafluorophenyl ester (Method C) in the synthesis of the amides of polyunsaturated fatty acids (PUFA). Thus, reaction of arachidonic acid (**8**) with di-pentafluorophenylcarbonate in the presence of *N*-methylmorpholine as the base synthesized the pentafluorophenyl ester of arachidonic acid (**9**), which was then reacted with nitroethanolamine (**1a**) to produce nitroanandamide (**10a**) (Scheme 2). The amide of arachidonic acid with 3-amino-1,2-propanediol 1,2-dinitrate (**10b**) and the nitroethanolamide of eicosapentenoic acid (**11a**) were synthesized analogously (Scheme 1). An advantage of this method for activating the carboxyl is that the intermediate active pentafluorophenyl ester **9** of the fatty acid is a relatively stable compound and can be produced, stored, and used as needed.




*i*: DMF, *N*-methylmorpholine, RT, 15 h; *ii*: DMF, nitroethanolamine, *N*-methylmorpholine, RT, 2 h; *iii*: benzene, $HNO_3$, $Ac_2O$, RT, 30 min.

Scheme 2

The nitroethanolamides of arachidonic (nitroanandamide) and eicosapentenoic acids were also produced by nitration of the corresponding ethanolamides. First the ethanolamides of the acids, e.g., anandamide (**12**), were synthesized from the acids and ethanolamine by either method (using isobutylchloroformate, carbonyldiimidazole, or dipentafluorophenylcarbonate). Then, these were nitrated by a mixture of $HNO_3$ and $Ac_2O$ in benzene to produce the corresponding nitroethanolamides (**10a**, **11a**) (Schemes 1 and 2). An advantage of this scheme is the ability to synthesize the intermediate ethanolamides of PUFA, which can be used as reference compounds in the biological tests.

Thus, we synthesized for the first time nitro derivatives of anandamide, prostamides $E_2$ and $F_{2\alpha}$, and their analogs containing 3-amino-1,2-propanediol dinitrate as the aminoalcohol.

## EXPERIMENTAL

Prostaglandins $E_2$ and $F_{2\alpha}$ were obtained from the pilot plant of the Institute of Chemistry (Tallin, Estonia); 11-deoxyprostaglandin $E_1$, from the Institute of Organic Chemistry (Riga, Latvia). Fast-atom bombardment (FAB) mass spectra were recorded on an MS-50TS instrument (Kratos, Great Britain); PMR spectra, on Bruker WM500 and CXP-200 spectrometers (Bruker, Germany). Proton chemical shifts (δ, ppm) are given relative to $Me_4Si$ for $CDCl_3$ solutions. TLC was performed on Silufol UV 254 plates (Kavalier, Czech Rep.) with detection by phosphomolybdic acid solution (5%) in alcohol; column chromatography, using silica gel L (Chemapol, Czech Rep.). The course of the separation was monitored by TLC. Solutions were evaporated in a rotary evaporator (water aspirator) at a bath temperature <30°C.

**Synthesis of Acid Amides with Aminoalcohol Nitrates. Method A.** A solution of prostaglandin or PUFA (0.2 mM) in acetone (2 mL) under Ar was treated with $Et_3N$ (0.4 mM) and isobutylchloroformate (0.37 mM), stirred at room temperature for 30 min, and evaporated in vacuo. The solid mixed anhydride (**3**) was dissolved in $CHCl_3$ (1 mL), treated with a solution of nitroxyalkylamine nitrate (0.3 mM, **1a**, **1b**) and $Et_3N$ (0.4 mM) in $CHCl_3$ (1 mL), stirred at room temperature for 18 h, diluted with water, acidified with HCl solution (1 M), and extracted with EtOAc (3 × 5 mL). The combined organic extract was washed with water and saturated aqueous NaCl and dried over anhydrous $Na_2SO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The solid was purified by column chromatography over silica gel L (100–250 μm) using a $C_6H_6$:EtOAc gradient. Fractions containing the product (monitored using TLC) were combined. Solvent was evaporated in vacuo.

**Method B.** Prostaglandin or PUFA (0.28 mM) was dissolved in acetonitrile (2 mL) under Ar, treated with carbonyldiimidazole (0.43 mM), stirred at 21°C for 1.5 h until $CO_2$ evolution ceased, treated with a solution of nitroxyalkylamine nitrate (0.4 mM, **1a**, **1b**) and $Et_3N$ (0.5 mM) in anhydrous $CHCl_3$ (1 mL), stirred for 2 h, and diluted with water (4 mL) and EtOAc (20 mL). The organic layer was separated; washed with $NaHSO_4$ solution (2 M), water, and brine; and dried over $Na_2SO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The solid was purified by column chromatography over silica gel L (100-250 μm) using a $C_6H_6$:$Me_2CO$ gradient. Fractions containing the product (monitored using TLC) were combined. Solvent was evaporated in vacuo.

**Method C.** A solution of nitroxyalkylamine nitrate (0.3 mM, **1a**, **1b**) in DMF (0.5 mL) was treated successively with *N*-methylmorpholine (0.6 mM) and a solution of PUFA pentafluorophenyl ester (0.15 mM) in DMF (250 μL), stirred at room temperature for 2 h, and diluted with EtOAc (10 mL) and HCl solution (4 mL, 0.1 M). The organic layer was separated, washed with water and saturated aqueous NaCl solution, and dried over anhydrous $Na_2SO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The solid was purified by column chromatography over silica gel L (100–250 μm) using a $CHCl_3$:$Me_2CO$ gradient. Fractions containing the product (monitored using TLC) were combined. Solvent was evaporated in vacuo.

**PUFA Pentafluorophenyl Ester.** A solution of PUFA (0.6 mM) in DMF (1 mL) under Ar was stirred, treated with *N*-methylmorpholine (0.7 mM), cooled to 0°C, treated in one portion with di-pentafluorophenylcarbonate (0.6 mM), and stirred with self heating to room temperature until the reaction completed (monitored by TLC, $CHCl_3$ eluent, ~1.5 h). The resulting finely crystalline precipitate was filtered off to afford a transparent slightly yellowish solution of PUFA pentafluorophenyl ester, the concentration of which was taken as 270 mg/mL. This solution was used in reactions without further characterization.

**Nitration of Anandamide.** A solution of arachidonic acid ethanolamide (35 mg, 0.12 mM) in $C_6H_6$ (3 mL) at 0°C was treated dropwise with acetic anhydride (1.5 mL) and $HNO_3$ (67%, 500 μL), stirred at this temperature for 30 min, diluted with water, and extracted with EtOAc (3 × 5 mL). The combined organic extract was washed with water and saturated aqueous

NaCl solution, and dried over anhydrous $Na_2SO_4$. The desiccant was filtered off. The filtrate was evaporated in vacuo. The solid was purified by column chromatography over silica gel L (100–250 μm) using a $C_6H_6$:EtOAc gradient. Fractions containing the product (monitored using TLC) were combined. The solvent was evaporated in vacuo to afford **10a** (32.4 mg, 82%).

**11-Deoxyprostaglandin $E_1$ Nitroethanolamide (5a).** Yield 58%, colorless viscous oil, $R_f$ 0.34 ($C_6H_6$:EtOAc, 1:1).

PMR spectrum (200 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.49 (2H, m, H-13, H-14), 4.47 (2H, t, J = 4.8, H-22), 3.98 (1H, m, H-15), 3.49 (2H, m, H-21), 0.86 (3H, t, J = 6, H-20).

**11-Deoxyprostaglandin $E_1$ Amide of 3-Amino-1,2-propanediol-1,2-dinitrate (5b).** Yield 75%, colorless viscous oil, $R_f$ 0.45 ($CHCl_3$:$Me_2CO$, 4:1). Mass spectrum (*m*/*z*, *I*, %): 486 (24) $[M + H – OH]^+$, 485 (100) $[M + H – H_2O]^+$, 457 (15) $[M + H – H_2O – CO]^+$, 439 (9) $[M – HNO_3]^+$, 438 (18) $[M – H_2O – NO_2]^+$, 394 (7) $[M + H – HNO_3 – H_2O – CO]^+$, 393 (8) $[M – HNO_3 – NO_2]^+$, 392 (24) $[M – HNO_3 – HNO_2]^+$, 377 (8) $[M – HNO_3 – ONO_2]^+$, 376 (33) $[M – 2HNO_3]^+$.

PMR spectrum (200 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.54 (1H, t, J = 6, NH), 5.56 (2H, m, H-13, H-14), 5.40 (1H, m, H-22), 4.84 (1H, dd, J = 2.8, J = 13, H-23a), 4.54 (1H, dd, J = 6, J = 13, H-23b), 4.09 (1H, m, H-15), 3.69 (1H, m, H-21a), 3.50 (1H, m, H-21b), 0.89 (3H, t, J = 6.4, H-20).

**Prostaglandin $E_2$ Nitroethanolamide (6a).** Yield 75%, colorless viscous oil, $R_f$ 0.39 ($C_6H_6$:$Me_2CO$, 1:1).

PMR spectrum (200 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.68 (1H, t, J = 5.4, NH), 5.45 (4H, m, H-5, H-6, H-13, H-14), 4.53 (2H, t, J = 5, H-22), 4.05 (2H, m, H-11, H-15), 3.58 (2H, m, H-21), 0.89 (3H, t, J = 6, H-20).

**Prostaglandin $E_2$ Amide of 3-Amino-1,2-propanediol-1,2-dinitrate (6b).** Yield 82%, colorless viscous oil, $R_f$ 0.42 ($CHCl_3$:$Me_2CO$, 2:1).

PMR spectrum (200 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.89 (1H, m, NH), 5.58 (2H, m, H-13, H-14), 5.33 (3H, m, H-5, H-6, H-22), 4.83 (1H, dd, J = 2.8, J = 13, H-23a), 4.53 (1H, dd, J = 6.2, J = 13, H-23b), 4.08 (2H, m, H-11, H-15), 3.59 (2H, m, H-21), 2.69 (1H, dd, J = 7.2, J = 18.2, H-10), 0.89 (3H, t, J = 6.2, H-20).

**Prostaglandin $F_{2\alpha}$ Nitroethanolamide (7a).** Yield 84%, colorless viscous oil, $R_f$ 0.39 ($CHCl_3$:$Me_2CO$, 1:1).

PMR spectrum (200 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.70 (1H, t, J = 4.8, NH), 5.42 (4H, m, H-5, H-6, H-13, H-14), 4.53 (2H, t, J = 5, H-22), 3.91–4.15 (3H, m, H-9, H-11, H-15), 3.55 (2H, q, J = 5, H-21), 0.88 (3H, t, J = 6.6, H-20).

**Prostaglandin $F_{2\alpha}$ Amide of 3-Amino-1,2-propanediol-1,2-dinitrate (7b).** Yield 81%, slightly yellow viscous oil, $R_f$ 0.43 ($CHCl_3$:$Me_2CO$, 1:1).

PMR spectrum (200 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.38 (1H, m, NH), 5.53 (2H, m, H-13, H-14), 5.38 (3H, m, H-5, H-6, H-22), 4.85 (1H, dd, J = 2.8, J = 13.5, H-23a), 4.51 (1H, dd, J = 6.7, J = 13.5, H-23b), 4.07 (3H, m, H-9, H-11, H-15), 3.59 (2H, m, H-21), 0.88 (3H, t, J = 6.2, H-20).

**Arachidonic Acid Nitroethanolamide (Nitroanandamide) (10a).** (Synthesis from arachidonic acid and nitroethanolamine). Yield 72%, slightly yellow viscous oil, $R_f$ 0.61 ($C_6H_6$:EtOAc, 2:1).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.84 (1H, m, NH), 5.30 (8H, m, H-5, H-6, H-8, H-9, H-11, H-12, H-14, H-15), 4.49 (2H, t, J = 4.8, H-22), 3.53 (2H, m, H-21), 2.77 (6H, m, H-7, H-10, H-13), 2.09 (6H, m, H-2, H-4, H-16), 1.68 (2H, m, H-3), 1.31 (6H, m, H-17, H-18, H-19), 0.91 (3H, t, J = 6.4, H-20).

**Arachidonic Acid Amide of 3-Amino-1,2-propanediol-1,2-dinitrate (10b).** Yield 75%, colorless viscous oil, $R_f$ 0.66 ($C_6H_6$:$Me_2CO$, 2:1).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.98 (1H, m, NH), 5.31 (8H, m, H-5, H-6, H-8, H-9, H-11, H-12, H-14, H-15), 4.84 (1H, dd, J = 2.8, J = 13.5, H-23a), 4.50 (1H, dd, J = 6.7, J = 13.5, H-23b), 3.60 (2H, m, H-21), 2.78 (6H, m, H-7, H-10, H-13), 2.08 (6H, m, H-2, H-4, H-16), 1.71 (2H, m, H-3), 1.33 (6H, m, H-17, H-18, H-19), 0.91 (3H, t, J = 6.4, H-20).

**Eicosapentenoic Acid Nitroethanolamide (11a).** Yield 69%, colorless viscous oil, $R_f$ 0.61 ($C_6H_6$:EtOAc, 2:1).

PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.91 (1H, m, NH), 5.29 (10H, m, H-5, H-6, H-8, H-9, H-11, H-12, H-14, H-15, H-17, H-18), 4.47 (2H, t, J = 4.8, H-22), 3.52 (2H, m, H-21), 2.79 (8H, m, H-7, H-10, H-13, H-16), 2.11 (2H, t, J = 7.4, H-2), 2.14 (4H, m, H-4, H-19), 1.74 (2H, quin, J = 7.3, H-3), 1.02 (3H, t, J = 7.5, H-20).

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