ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2008, Vol. 34, No. 6, pp. 743–746. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © S.G. Romanova, A.A. Shtil', G.A. Serebrennikova, 2008, published in Bioorganicheskaya Khimiya, 2008, Vol. 34, No. 6, pp. 827–830.

Synthesis and Cytotoxicity of Novel Phosphorusless Analogues of Edelfosine

S. G. Romanova[a, 1], A. A. Shtil'[b], and G. A. Serebrennikova[a]

[a] *Lomonosov State Academy of Fine Chemical Technology, pr. Vernadskogo 86, Moscow, 117571 Russia*

[b] *Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Kashirskoe sh. 24, Moscow, 115478 Russia*

Received October 2, 2007; in final form, October 29, 2007

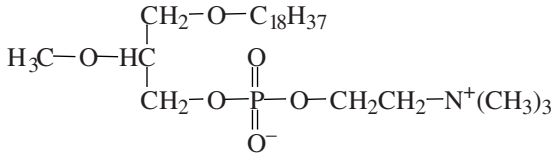
Abstract—Modified series of phosphorusless edelfosine analogues bearing the polar heads of aliphatic bases, *N,N*-dimethylethanolamine and *N,N,N*1,N[1]-tetramethylethylenediamine, were synthesized, with the length of the spacer varying from three to four methylene units. The cytotoxic characteristics of the compounds synthesized were studied.

Key words: antineoplastic activity, edelfosine, edelfosine analogues, ether lipids

DOI: 10.1134/S1068162008060150

INTRODUCTION

In the last few years the structure–activity specialties of the representatives of a new lipid class, cation nonphosphorus ether glycerolipids, are under intensive study[2]. Glycerophospholipid Edelfosine (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) possessing antitumor activity is their prototype.

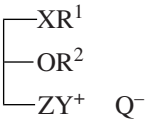


Edelfosine (Et-18-OMe)

Nowadays Edelfosine is used for antitumor therapy. The advantage of Edelfosine and the related compounds over many of antitumor drugs is in the absence of mutagenic effect of these compounds [1, 2].

The practical use of ether glycerolipids requires the solution of problems of the chemical synthesis of this class of compounds. Preparation of Edelfosine and its analogues is a multistage process, which requires long-term purification at every step. This decreases the yield of the target product and increases its cost. Furthermore, these syntheses deal with toxic phosphorus-containing reagents.

The main features of the antiproliferative action of antitumor lipids, the possibility of inhibition of cell proliferation and the selectivity of antiproliferative action, were previously established; the attempt to correlate the results of the study afore-mentioned with the literal data on the parent phosphorus-containing compounds was also undertaken [3, 4]. The most pronounced antitumor activity is observed in for the series of ether glycerolipids. The biological tests [5[a1]] of the nonphosphorus glycerolipids synthesized allowed us to amplify the main requirements to the structure of alkyl glycerolipids reported in literature [6[a2]].



X_2= O, S, OCONH; R^1 = alkyl, alkenyl, acyl (C_{10}–C_{20}), alkyls with aromatic and heterocyclic moieties; R^2 = long-chain (C_{10}–C_{20}) or short-chain (C_1–C_4) alkyl and acyl substituents; Z = spacer moieties of various types, can be absent; Y^+ = ammonium, sulfonium, or heterocyclic moieties with positively charged nitrogen and sulfur atoms; Q^- = counterions Hal^-, AcO^- or $TosO^-$.

It turned out that in most cases the long-chain hydrocarbon residue attached by the ether, thioether, or amine bond should be present at C1 of the glycerol backbone. The short-chain (C_1–C_4) alkoxy group is required at C2 of the glycerol backbone. The spacer region between the polar domain and the glycerol backbone can be absent or to be a connective moiety of alkyl, acyl, or amide type (C_1–C_8). As a rule, the cation

[1] Corresponding author; phone: +7 (495) 936-8903; e-mail: romfill@mail.ru.

[2] Abbreviations: DMAE, *N*,*N*-dimethylethanolamine; TMEDA, *N*,*N*,*N*[1],*N*[1]-tetramethylethylenediamine.

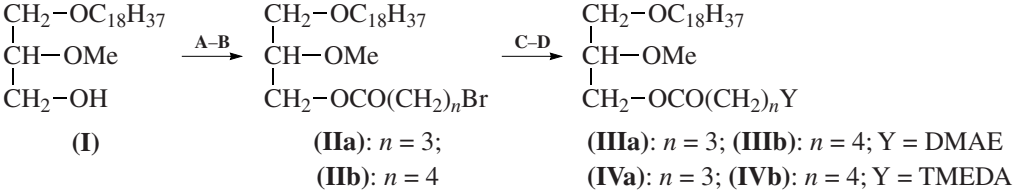
head is of ammonium or sulfonium type with short (C_1–C_3) substituents or a hetero atom involved in heterocyclic systems (e.g., pyridinium or thiasolinium cation heads). A halogen ion can serve as a counterion.

We have developed an approach to the synthesis of novel nonphosphorus ether glycerolipids suited to the requirements afore-mentioned. The glycerolipids under study designated as (**IIIa**), (**IIIb**), (**IVa**), and (**IVb**) were synthesized and characterized for the first time.

RESULTS AND DISCUSSION

To study the structure–function characteristics of nonphosphorus analogues of Edelfosine and to determine their biological activity, new modification series of cationic ether glycerolipids differing in the spacer length represented by the residues of valeric or butyric acids and in the type of the polar head formed by DMAE or TMEDA residues (see scheme) have been synthesized. The influence of an individual structure-forming component on the activity was estimated for *N*,*N*-{4-[(2-methoxy-3-octadecyloxy)propyl]oxycarbonylpropyl}-*N*,*N*-dimethyl-*N*-(2-hydroxyethyl)ammonium iodide (**IIIa**), *rac*-*N*-{4-[(2-methoxy-3-octadecyloxy)propyl]oxycarbonylpropyl}-*N*,*N*-dimethyl-*N*-(2-dimethylaminoethyl) ammonium iodide (**IVa**), *rac*-*N*-{4-[(2-methoxy-3-octadecyloxy)propyl]oxycarbonylbutyl}-*N*,*N*-dimethyl-*N*-(2-hydroxyethyl)ammonium iodide (**IIIb**), and *rac*-*N*-{4-[(2-methoxy-3-octadecyloxy)propyl]oxycarbonylbutyl}-*N*,*N*-dimethyl-*N*-(2-dimethylaminoethyl)ammonium iodide (**IVb**).

The choice of the amine type is dictated by the high antitumor activity of glycerolipids containing the residue of *N*,*N*-dimethylethanolamine as a polar head (the so-called reverse choline type cationic head) [6]. Furthermore, in order to clarify the influence of the hydroxyl group on the cytotoxicity, *N*,*N*,N^1,N^1-tetramethylethylenediamine containing the dimethylamino moiety instead of the hydroxyl group was chosen as a polar head. The possibility of creation of dicationic compounds that widens the area of search for antitumor agents is another specialty of these lipids. The key compounds in the synthesis of lipids (**IIIa**), (**IIIb**), (**IVa**), and (**IVb**), *rac*-1-octadecyl-2-methyl-3-(4-bromobutanoyl)glycerol (**IIa**) and *rac*-1-octadecyl-2-methyl-3-(5-bromopentanoyl)glycerol (**IIb**), were obtained by acylation of the starting diglyceride (**I**) with 4-bromobutyric and 5-bromovaleric acid chlorides, respectively.



Scheme. Synthesis of phosphorusless cationic glycerolipids of alkyl type.
A: (**IIa**), $Br(CH_2)_3COCl$, $Py/CHCl_3$, 20°C, 0.5 h; **B**: (**IIb**), $Br(CH_2)_4COCl$, $Py/CHCl_3$, 20°C, 0.5 h;
C: (**IIIa**) и (**IIIb**): DMAE, NaI/DMSO, 55°C, 4.5 h; **D:** (**IVa**) и (**IVb**): TMEDA, NaI/DMSO, 50°C, 5 h.

The cationic head was introduced to the lipid molecule by quaternization of the corresponding tertiary amine with bromides (**IIa**) and (**IIb**) in the presence of NaI.

It is known that quaternization proceeds rather slowly (20–70 h) at heating in the presence of the amino component [6]. In our case, the reaction time varied from 3 to 5 h. The substitution of the more reactive iodide for bromide in the Finkelstein reaction allowed us to decrease the reaction time and to mollify the temperature conditions of the process in order to avoid the resinification of t he reaction products, and, thus, to increase the yield of the target compounds.

Bromides (**IIa**) and (**IIb**) were put into interaction with DMAE and TMEDA to give lipids (**IIIA**), (**IIIb**), (**IVa**), and (**IVb**) differing in the length of the spacer group and the structure of the cationic head.

The study of the cytotoxic activity of the compounds obtained was carried out using MTT test [7] on K562 (leukemia), HCT-116 (colon cancer), and MCF-7 (breast cancer) cultured cell lines.

In seemed to be appropriate to include cationic lipid (**V**) previously synthesized in our laboratory [6], which contained DMEA in the polar domain and the ethyl substituent instead of the methyl moiety in C2 of the glycerol backbone, to the test program.

Lipids (**IIa**), (**IIIb**), and (**V**) appeared to be the most toxic for K562 cells (IC_{50} 4.0 ± 0.8; 3.9 ± 1.0, and 3.6 ± 1.2 μM, respectively). In the case of Edelfosine, the value of IC_{50} is 4.7 ± 0.9 μM. Compounds (**IVa**) and (**IVb**) appeared to display almost no cytotoxicity towards these cell lines.

Thus, estimation of cytotoxicity of the new compounds showed that the length of the spacer region has no essential effect on the activity of the cationic glycerolipids with an aliphatic base as a polar domain. The structure of the polar domain has the marked influence on the cytotoxicity of glycerolipids: lipids with the reversed choline cationic head are toxic for K562 cells,

whereas the compounds with TMEDA cationic head have no cytotoxic effect on the cell lines under study.

EXPERIMENTAL

Solvents ands reagents used in this study were distilled. The compounds synthesized and the monitoring of the reactions were carried out by TLC on Silica gel 60 (Merck, Germany) precoated plates using (A) chloroform and (B) 4 : 1 chloroform–methanol as developing systems. Spots were visualized by treating with 10% phosphomolybdic acid and subsequent heating.

Column chromatography was carried out on Silica gel 60 (0.040–0.063 μm, Merck, Germany) in (1) chloroform and (2) 6 : 1 chloroform–methanol. Melting points were taken on a Bohetius (Germany) instrument. ^{1}H NMR spectra (δ, ppm; *J*, Hz) were registered on a Bruker MSL-200 (200 MHz) spectrometer in $CDCl_3$ using tetramethylsilane as the internal standard.

***rac*-1-Octadecyl-2-methylglycerol (I)** was obtained according to the standard procedure [6, 8]; ^{1}H NMR: 0.83 (3 H, t, *J* 6.8, $((CH_2)_{15}C\underline{H}_3)$; 1.24 (30 H, br. s, $(C\underline{H}_2)_{15}CH_3$); 1.49–1.56 (2 H, m, $OCH_2C\underline{H}_2$), 3.31 (1 H, m, $C\underline{H}OCH_3$), 3.36 (3 H, c, $OC\underline{H}_3$), 3.48–3.52 (4 H, m, $C\underline{H}_2OCH_2$; $C\underline{H}_2OH$). Found, %: C 73.64; H 12.90; $C_{22}H_{46}O_3$. Calc., %: C 73.68; H 12.93.

5-Bromovaleric and 4-bromobutyric acid chlorides were obtained by treating the corresponding acids with the excess (10 equiv.) of thionyl chloride in anhydrous chloroform (24 h at 20°C). The solvent and the excess of thionyl chloride were removed *in vacuo*. The resulting acid chlorides were put into reaction without additional purification.

***rac*-1-Octadecyl-2-methyl-3-(5-bromopenthanoyl) glycerol (IIb).** Pyridine (0.5 ml) and then a solution of 5-bromopenthanoyl chloride (0.22 ml, 1.2 mmol) in anhydrous chloroform (1.5 ml) were added dropwise at stirring at 0°C to a solution of *rac*-1-octadecyl-2-methylglycerol (**I**) (0.290 g, 2 mmol) in anhydrous chloroform. The mixture was stirred for 30 min at 20°C, diluted with chloroform (20 ml), washed with 1% HCl (3 × 25 ml) and water (2 × 25 ml), and dried with Na_2SO_4. The residue was chromatographed in system (1[a3]) to give 0.379 g (91.2%) of the target product; R_f 0.66 (**A**). ^{1}H NMR: 0.86 (3 H, t, *J* 6.8, $((CH_2)_{15}C\underline{H}_3)$; 1.23 (30 H, br. s, $(C\underline{H}_2)_{15}CH_3$); 1.47–1.59 (2 H, m, $OCH_2C\underline{H}_2$), 1.71–1.91 (4 H, m, $((C\underline{H}_2)_2CH_2CH_2Br)$; 2.35 (2 H, t, *J* 7.0, $OCOC\underline{H}_2$), 3.35–3.46 (5 H, m, $C\underline{H}_2OCH_2CH_2$; $C\underline{H}OCH_3$; $C\underline{H}_2Br$); 3.60 (3 H, s, $OC\underline{H}_3$), 4.07 (1 H, dd, *J* 5.8, 10.6, $CH\underline{H}_aOCO$); 4.25 (1 H, dd, *J* 3.9, 10.6, $CH\underline{H}_bOCO$).

***rac*-1-Octadecyl-2-methyl-3-(4-bromobutanoyl) glycerol (IIa)** was prepared by the procedure described for (**IIb**) starting from *rac*-1-octadecyl-2-methylglycerol (**I**) (0.285 g, 0.796 mmol) in anhydrous chloroform (0.5 ml), pyridine (0.5 ml), and a solution of 4-bromobutyric acid chloride (0.12 ml, 0.20 g, 1.2 mmol) in chloroform (1.5 ml); yield 0.365 g (90.6%); R_f 0.64 (**A**). ^{1}H NMR: 0.84 (3 H, t, *J* 6.8, $((CH_2)_{15}C\underline{H}_3)$; 1.20 (30 H, br. s, $(C\underline{H}_2)_{15}CH_3$); 1.45–1.58 (2 H, m, $OCH_2C\underline{H}_2$), 1.75–1.89 (2 H, q, $C\underline{H}_2CH_2Br$); 2.36 (2 H, t, *J* 7.0, $OCOC\underline{H}_2$), 3.33–3.46 $C\underline{H}_2OCH_2CH_2$; $C\underline{H}OCH_3$; $C\underline{H}_2Br$); 3.58 (3 H, s, $OC\underline{H}_3$), 4.08 (1 H, dd, *J* = 5.8 and 10.6, $CH\underline{H}_aOCO$), 4.23 (1 H, dd, *J* = 3.9 and 10.6, $CH\underline{H}_bOCO$).

***rac*-*N*-{4-[(2-Methoxy-3-octadecyloxy)propyl] oxycarbonylbutyl}-*N*,*N*-dimethyl-*N*-(2-hydroxyethyl) ammonium iodide (IIIb).** A mixture of *rac*-1-octadecyl-2-methyl-3-(5-penthanoyl)glycerol (0.379 g, 0.73 mmol), DMSO (1.6 ml), NaI (0.33 g, 2.2 mmol), and DMEA (0.09 ml, 0.08 g, 0.87 mmol) was kept for 4.5 h at 55°C at occasional stirring. The mixture was cooled, diluted with chloroform (20 ml), and washed with 1% HCl (2 × 25 ml) to pH < 7 and water (5 × 30 ml). The aqueous layer was extracted with chloroform (15 ml). The organic layer was dried with Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica using system (2); yield 0.300 g (82.7%); R_f = 0.56 (B); ^{1}H NMR: 0.83 (3 H, t, *J* 6.8, $((CH_2)_{15}C\underline{H}_3)$; 1.24 (30 H, br. s, $(C\underline{H}_2)_{15}CH_3$); 1.49–1.56 (2 H, m, $OCH_2C\underline{H}_2$), 1.78–1.84 (4 H, m, $(C\underline{H}_2)_2CH_2N^+$); 2.44 (2 H, t, *J* 6.9, $OCOC\underline{H}_2$); 3.38 (6 H, s, $N^+(C\underline{H}_2)_3$), 3.43–3.47 (4 H, m, $C\underline{H}_2OC\underline{H}_2CH_2$); 3.58–3.69 (3 H, m, $N^+CH_2C\underline{H}_2OH$, $C\underline{H}OCH_3$), 3.75–3.81 (2 H, m, $(CH_2)_3C\underline{H}_2N^+$), 4.09 (1 H, dd, *J* = 5.9 and 11.8, $CH\underline{H}_aOCO$), 4.13–4.22 (2 H, m, $N^+C\underline{H}_2OH$), 4.26 (1 H, dd, *J* = 4.2 and 11.8, $CH\underline{H}_bOCO$). Found, %: C 53.75; H 8.76; N 2.26; $C_{31}H_{64}O_5NI$. Calc., %: C 53.71; H 8.73; N 2.21.

***rac*-*N*-{4-[(2-Methoxy-3-octadecyloxy)propyl] oxycarbonylpropyl}-*N*,*N*-dimethyl-*N*-(2-hydroxyethyl) ammonium iodide (IIIa)** was obtained as described for (**IIIb**) starting from a solution of *rac*-1-octadecyl-2-methyl-3-(4-bromobutanoyl)glycerol (0.365 g, 0.72 mmol) in DMSO (1.5 m), NaI (0.32 g, 2.16 mmol), and DMEA (0.09 ml, 0.08 g, 0.86 mmol); yield 0.382 g (62.9%); R_f = 0.58 (B); ^{1}H NMR: 0.85 (3 H, t, *J* 6.8, $((CH_2)_{15}C\underline{H}_3)$; 1.26 (30 H, br. s, $(C\underline{H}_2)_{15}CH_3$); 1.51–1.55 (2 H, m, $OCH_2C\underline{H}_2$), 1.81 (2 H, m, $(C\underline{H}_2CH_2N^+)$; 2.41 (2 H, t, *J* 6.9, $OCOC\underline{H}_2$); 3.36 (6 H, s, $N^+(C\underline{H}_2)_3$), 3.43–3.47 (4 H, m, $C\underline{H}_2OC\underline{H}_2CH_2$); 3.56–3.62 (3 H, m, $N^+CH_2C\underline{H}_2OH$, $C\underline{H}OCH_3$), 3.78–3.82 (2 H, m, $(CH_2C\underline{H}_2N^+)$, 4.11 (1 H, dd, *J* = 5.9 and 11.8, $CH\underline{H}_aOCO$), 4.12–4.23 (2 H, m, $N^+C\underline{H}_2OH$), 4.26 (1 H, dd, *J* = 4.2 and 11.8, $CH\underline{H}_bOCO$). Found, %: C 55.91; H 9.75; N 2.17; $C_{30}H_{62}O_5NI$. Calc., %: C 55.97; H 9.70; N 2.24.

***rac*-*N*-{4-[(2-Methoxy-3-octadecyloxy)propyl] oxycarbonylbutyl}-*N*,*N*-dimethyl-*N*-(2-dimethylaminoethyl)ammonium iodide (IVb)** was prepared as described for (**IIIb**) starting from a solution of *rac*-1-O-octadecyl-2-O-methyl-3-O-(5-bromopenthanoyl) glycerol (0.230 g, 0.44 mmol) in DMSO (0.6 ml), NaI (0.20 g, 1.32 mmol), and TMEDA (0.08 ml, 0.06 g, 0.53 mmol); yield 0.142 g (80.1%); R_f = 0.56 (B); ^{1}H NMR: 0.89 (3 H, t, *J* 6.8, $((CH_2)_{15}C\underline{H}_3)$; 1.22 (30 H, br.

s, ($C\underline{H}_2)_{15}CH_3$); 1.52–1.56 (2 H, m, $OCH_2C\underline{H}_2$), 2.24 (4 H, m, $((C\underline{H}_2)_2CH_2N^+$); 2.29 (6 H, s, $N(C\underline{H}_3)_2$), 2.36–2.38 (2 H, m, $OCOC\underline{H}_2$); 2.73 (2 H, m, $(CH_2C\underline{H}_2N^+)$); 3.21 (6 H, s, $N^+(C\underline{H}_3)_2$), 3.32 (3 H, s, $CH_2OC\underline{H}_3$), 3.91 (1 H, dd, J = 5.9 and 11.8, $CH\underline{H}_aOCO$), 4.15–4.25 (6 H, m, $C\underline{H}_2OC\underline{H}_2$, $CH_2N^+(CH_2)_3C\underline{H}_2$), 4.28 (1 H, dd, J = 4.2 and 11.8, $CH\underline{H}_bOCO$). Found, %: C 59.44; H 10.39; N 2.01; $C_{33}H_{69}O_4N_2I$. Calc., %: C 59.37; H 10.34; N 2.10.

***rac*-*N*-{3-[(2-Methoxy-3-octadecyloxy)propyl] oxycarbonylpropyl}-*N*,*N*-dimethyl-*N*-(2-dimethylaminoethyl)ammonium iodide (IVa)** was obtained as described for (*IIIb*) starting from a solution of *rac*-1-octadecyl-2-methyl-3-(4-bromobutanoyl)glycerol (0.186 g, 0.36 mmol) in DMSO (0.4 ml), NaI (0.16 g, 1.07 mmol), and TMEDA (0.10 ml, 0.049 g, 0.42 mmol); yield 0.104 g (69.4%); R_f = 0.59 (B); ^{1}H NMR: 0.87 (3 H, t, J 6.8, $((CH_2)_{15}C\underline{H}_3)$; 1.25 (30 H, br. s, $(C\underline{H}_2)_{15}CH_3$); 1.52–1.56 (2 H, m, $OCH_2C\underline{H}_2$), 2.26 (2 H, m, $(C\underline{H}_2CH_2N^+)$; 2.31 (6 H, s, $N(C\underline{H}_3)_2$), 2.35–2.39 (2 H, m, $OCOC\underline{H}_2$); 2.71 (2 H, m, $(CH_2C\underline{H}_2N^+)$), 3.23 (6 H, s, $N^+(C\underline{H}_3)_2$), 3.34 ($CH_2OC\underline{H}_3$), 3.93 (1 H, dd, J = 5.9 and 11.8, $CH\underline{H}_aOCO$), 4.12–4.23 (6 H, m, $C\underline{H}_2OC\underline{H}_2$, $CH_2N^+(CH_2)_3C\underline{H}_2$); 4.26 (1 H, dd, J = 4.2 and 11.8, $CH\underline{H}_bOCO$). Found, %: C 58.82; H 10.24; N 2.17; $C_{32}H_{67}O_4N_2I$. Calc., %: C 58.90; H 10.28; N 2.15.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (project no. 04–3-32452).

REFERENCES

1. Kibera, J., Lienas, V., and Pelekk, Z., *Highlights of Modern Biochemistry*, 1999, vol. 1, pp. 1459–1474.
2. Mollinedo, F., Fernandez-luna, J.L., Gajate, C., Marti-Martin, B., Benito, A., and Martinez-Dalmau, R., *Cancer Res.*, 1997, vol. 57, p. 1320.
3. Gajate, C. and Mollinedo, F., *Curr. Drug Metab.*, 2002, vol. 3, pp. 491–525.
4. Gajate, C., Onteriz, R.I., Cabaner, C., Alvarez-Noves, G., Modollel, M., and Mollinedo, F., *Int. J. Cancer*, 2000, vol. 85, pp. 674–682.
5. Botzler, C., Ellwart, J., Gunther, W., Eissner, G., and Multhoff, G., *Exp. Hematol.*, 1999, vol. 27, pp. 470–478.
6. Plyavnik, N.V., Maslov, M.A., and Serebrennikova, G.A., *Bioorg. Khim.,* 2004, vol. 30, pp. 507–511; *Rus. J. Bioorg. Chem.*, 2004. vol. 30, pp. 454–458.
7. Mossman, T., *J. Immunol. Methods*, 1983, vol. 89, p. 271.
8. The Merck Index, Reagenzien, Diagnostica, Chemikalien, 1987/88.