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Short Note

1-[2,3-Bis(tetradecyloxy)propyl]-3-[2-(piperazin-1-yl)ethyl]urea

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Abstract: Starting from 2,3-bis(tetradecyloxy)propan-1-amine (**1**), the synthesis of the target compound 1-[2,3-bis(tetradecyloxy)propyl]-3-[2-(piperazin-1-yl)ethyl]urea (**2**) is reported. The title compound was characterized by ^{1}H-NMR, ^{13}C-NMR and ESI/MS analysis.

Keywords: amino lipid; cationic lipid; delivery

1. Introduction

Non-viral vectors have emerged in the last decade as a promising approach for the treatment of diseases involving DNA and RNA molecules. [1] There is a good number of examples in which non-viral vectors have been linked with nucleic acids by using covalent approaches or combined with each other by optimized formulations. These two approaches have allowed the synthesis of the anticipated nucleic acid conjugates or complexes, respectively. Cationic lipids [2], dendrimers [3], cell-penetrating peptides [4] or polymers [5] have become the most common units used to improve the nucleic acids efficacy in cellular transfection processes. Despite the existence of an arsenal of non-viral vectors, the use of cationic lipids remains one of the most used transfecting agents due to their simple preparation, biodegradability and their wide use in clinical trials.

The pioneering work carried out by Felgner *et al*. in 1987 [6] demonstrated how a synthetic glycerol-based cationic lipid (DOTMA) was able to interact with DNA and impart cellular uptake,

leading to the expected lipid-DNA complex fusion with the plasma membrane. Since then, a wide number of cationic lipids has been reported and tested as potential non-viral carriers [7].

As a general structure, synthetic glycerol-based cationic lipids are mostly made of a glycerol backbone which contains two points of diversity: a hydrocarbonated alkyl chain and a cationic head group (Figure 1). This has led to the synthesis of novel cationic lipids series in a combinatorial fashion in order to optimize the initial "lead compound." Examples of these combinatorial libraries have been reported [8,9].

Recently, we reported the ability of a synthetic amino lipid (**1**) to interact with plasmid DNA and impart efficiently cellular uptake. These studies were carried out both *in vitro* and *in vivo*, obtaining promising transfection results (Figure 1A) [10,11]. This transfecting agent contained a double-tailed hydrocarbonated alkyl chain with ether linkages, a glycerol backbone and a primary amino group as a cationic head. As part of our ongoing efforts in the synthesis of novel cationic lipids to improve the cellular uptake of genetic materials, we herein report the synthesis of 1-[2,3-bis(tetradecyloxy)propyl]-3-[2-(piperazin-1-yl)ethyl]urea (**2**), a glycerol-based cationic lipid which contains two hydrocarbonated alkyl ether chains of fourteen atoms of carbon and a piperazine moiety that would act as a cationic head. The covalent linkage between the hydrophobic and the hydrophilic part was accomplished by forming the anticipated urea derivative (Figure 1B).

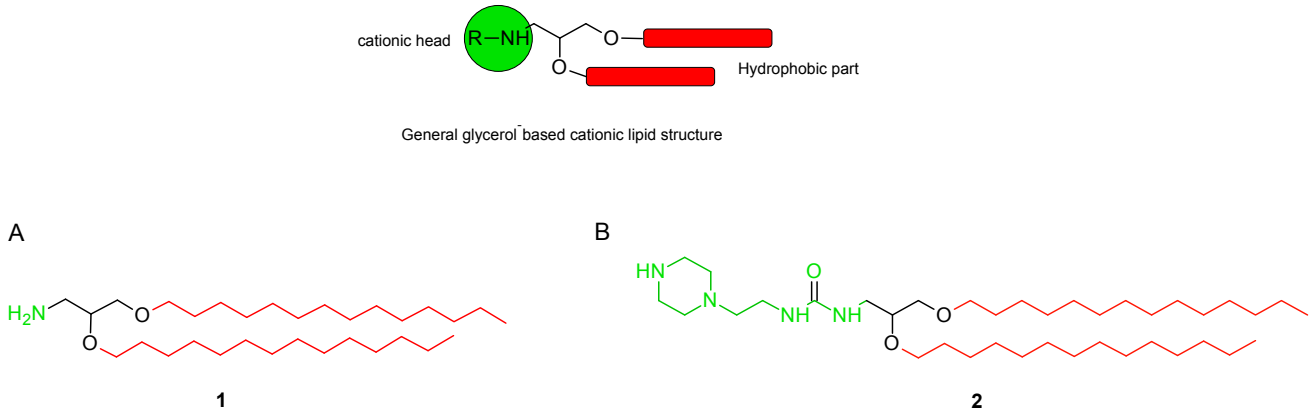


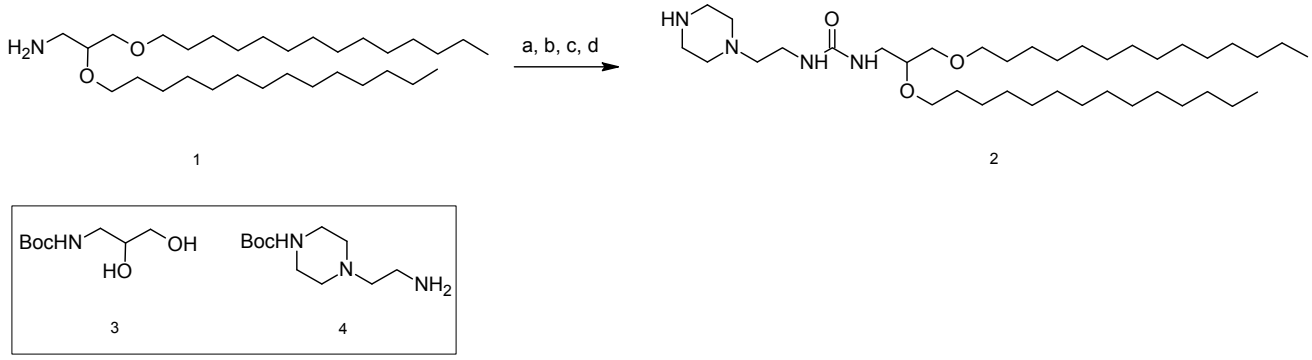
Figure 1. Di(*O*-alkyl)glycerol-based cationic lipids general structure. (**A**) Compound (**1**) was successfully evaluated as a transfecting agent; (**B**) The title compound (**2**) was designed as a second-generation molecule from our "lead compound **1**".

2. Results and Discussion

The synthesis of the title cationic lipid (**2**) is displayed in Scheme 1. As previously reported, *N*-Boc-protected diol (**3**) was used as a starting material for the synthesis of 2,3-bis(tetradecyloxy) propan-1-amine (**1**). Compound (**3**) was subjected to alkylation by phase-transfer catalyst under basic conditions at 60 °C followed by a Boc-deprotection reaction under acidic conditions to achieve the anticipated amino lipid compound (**1**) in quantitative yield [10].

The introduction of the piperazine derivative (**4**) and the synthesis of the title compound (**2**) was carried out following two consecutive reactions: Firstly, the conversion of the corresponding primary amine (**1**) to the intermediate 4-nitrophenyl chloroformate at room temperature; and secondly, the displacement of the 4-nitrophenyl moiety promoted by the protected *N*-Boc-piperazine amino

derivative (**4**). These reactions afforded the anticipated protected urea compound after purifying by flash chromatography (CH_2Cl_2:MeOH 6%) in good yield (81%). Finally, the *N*-Boc protecting group was conveniently removed by treatment with 10% trifluoroacetic acid (TFA) in an organic solvent like dichloromethane (CH_2Cl_2) at room temperature with high yields (85%). Following treatment with TFA, the amine salt was properly liberated with polymer-supported carbonate base (Amberlite IRA 900 $NaCO_3^-$ form) in ethyl acetate at room temperature [12]. This protocol generated the expected compound (**2**) in good yield (85%).



Reagents and conditions: a. 4-nitrophenyl chloroformate (1.5 eq), CH_2Cl_2:THF (1:1), DIEA (1.5 eq), r.t., 5 h; b. **4** (1.0 eq), TEA (1.0 eq), DMF, r.t., overnight; c. 10% TFA:CH_2Cl_2, r.t., 1 h; d. Polymer-supported carbonate (10.0 eq), AcOEt, r.t., 1 h.

Scheme 1. Synthesis of the title compound **2**.

3. Experimental Section

3.1. General Methods

All reagents, dry solvents and chemicals were purchased from Sigma-Aldrich (Tres Cantos, Madrid, Spain) and were used as received without further purification. All reactions were carried out in oven-dried glassware under inert atmosphere of argon. Analytical thin layer chromatography (TLC) was done on E. Merck silica gel 60 F254 plates (Merck, Darmstadt, Germany), visualized by UV and stained with phosphomolybdic acid. Flash chromatography was carried out on silica gel SDS 0.063–0.2 mm/70–230 mesh (Solvent Documentation Syntheses, Peypin, France). ^{1}H- and ^{13}C-NMR spectra were recorded at 25 °C on a Varian Mercury 400 MHz spectrometer (Varian Inc., Palo Alto, CA, USA). Tetramethylsilane (TMS) was used as an internal reference (0 ppm) for ^{1}H spectra recorded in $CDCl_3$ and the residual signal of the solvent (77.16 ppm) for ^{13}C spectra. Chemical shifts were reported in part per million (ppm) in the δ scale, coupling constants (*J*) in Hz and multiplicity as follows: bs (broad singlet), m (multiplet), t (triplet). Electrospray ionization mass spectra (ESI-MS) were recorded on a Micromass ZQ instrument (Waters Corporation, Milford, MA, USA) with single quadrupole detector coupled to an HPLC, and high-resolution (HR) ESI-MS on an Agilent 1100 LC/MS-TOF instrument (Agilent Technologies, Santa Clara, CA, USA).

3.2. 1-[2,3-Bis(tetradecyloxy)propyl]-3-[2-(piperazin-1-yl)ethyl]urea (**2**)

(a) 2,3-bis(tetradecyloxy)propan-1-amine (**1**) (25 mg; 0.052 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 and THF (1:1) (total volume: 1.0 mL). Then, DIEA (1.5 eq) and *p*-nitrophenyl chloroformate (15.7 mg; 0.078 mmol) were carefully added at room temperature. The mixture was stirred at room temperature for 5 h. The solvent was reduced in vacuo obtaining a yellow oil. The crude was used without further purification in the next reaction step.

(b) The resulting product (30 mg; 0.046 mmol) was dissolved in anhydrous DMF (1.0 mL) and the *N*-Boc-protected piperazine amine derivative (**4**) was added dropwise together with TEA (1.0 eq). The resulting mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and purified by flash chromatography (CH_2Cl_2:MeOH 6%). A yellowish oil was obtained (81%).

(c) Finally, the resulting compound (27.5 mg; 0.037 mmol) was dissolved in a mixture of 10% TFA in CH_2Cl_2 (1.0 mL). The reaction was stirred at room temperature for 1 h. The solvent was reduced in vacuo and the anticipated trifluoroacetate salt was used without further purification. The dried crude was dissolved in AcOEt (2.0 mL) and polymer-supported carbonate base (Amberlite IRA 900 $NaCO_3^-$ form) (10.0 eq) was added. The mixture was stirred for 1 h at room temperature (until pH of the organic layer was basic). Finally, the resin was filtered off and the filtrate was removed under vacuum obtaining the expected cationic lipid derivative (**2**) (20.1 mg; 0.031 mmol) with good yields (85%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 5.54 (bs, N<u>H</u>-CO), 5.26 (bs, N<u>H</u>-CO), 3.52 (m, 1H), 3.44 (m, 8H), 3.26 (m, 2H; -N-CH_2-C<u>H</u>$_2$-NH-), 3.18 (m, 4H; piperazine ring), 2.78 (m, 4H; piperazine ring), 2.60 (m, 2H; -N-C<u>H</u>$_2$-CH_2-NH-), 1.51 (m, 4H; 2 C<u>H</u>$_2$ alkyl chain), 1.23 (m, 44H; alkyl chain), 0.85 (t, J = 6.6 Hz; 6H; 2 C<u>H</u>$_3$-CH_2).

^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm) 161.8 (<u>C</u>O), 161.4, 77.6 (<u>C</u>H-O), 71.8 (<u>C</u>H$_2$-O), 70.4 (<u>C</u>H$_2$-O), 58.1 (<u>C</u>H$_2$-O), 48.6 (<u>C</u>H$_2$-N), 40.8 (<u>C</u>H$_2$-N), 40.7 (HO-CH-<u>C</u>H$_2$-N), 34.8 (CH_2-<u>C</u>H$_2$-Pip), 31.9 (CH_2-<u>C</u>H$_2$-N), 29.9, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 28.1, 26.0, 25.9, 22.6 (alkyl chain), 14.0 (<u>C</u>H$_3$-C).

HRMS (ESI$^+$) *m*/*z* calcd for $C_{38}H_{79}N_4O_3$ $[M + H]^+$ 639.6147 found 639.6147.

^{1}H-NMR, ^{13}C-NMR and ESI-MS spectra for the title compound **2** are available in the Supplementary Information.

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Author Contributions

This work is part of a project that has been going on in the RE research group in the field of DNA and RNA delivery. The experimental work was carried out by SG and SN The manuscript was written and corrected by all authors.

Conflicts of Interest

The authors declare no conflict of interest.

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