Bioorganic & Medicinal Chemistry Letters 23 (2013) 3145–3148

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and ionophoric activities of functionalized bis(choloyl) conjugates with a rigid core

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ARTICLE INFO

Article history:
Received 19 February 2013
Revised 7 April 2013
Accepted 9 April 2013
Available online 16 April 2013

Keywords:
Ionophore
Choloyl conjugate
Synthesis
pH Discharge

ABSTRACT

Three bis(choloyl) conjugates bearing a rigid *p*-phenylenediamine/*p*-bis(aminomethyl)benzene linker and amino/acetamido groups were synthesized, and fully characterized on the basis of ^{1}H NMR, ESI-MS and HRMS. Their ionophoric activities were investigated by means of pH discharge assay. The results indicate that these conjugates exhibit potent ionophoric activities across egg-yolk L-α-phosphatidylcholine (EYPC)-based liposomal membranes, via a cation/proton antiport mechanism. They show moderate ion selectivity among alkali metal ions. Of the three conjugates, the ones having amino groups transport alkali metal ions in the order of $Na^+ > Li^+ > K^+ \approx Rb^+ \approx Cs^+$, whereas the one having acetamido groups functions in the order of $Li^+ > Na^+ > K^+ \approx Rb^+ \approx Cs^+$.

The biological significance of ion transport across a membrane has stimulated considerable interest in identifying agents that are capable of mediating ion transport.[1] Such agents, that is, synthetic ion transporters, may find wide applications, for example, in better understanding of the biological functions of natural ion transporters[2] and in new drug discovery.[3] The approaches for creating synthetic ion transporters are essentially aimed at clarifying the structural requirements for large ion flow across a membrane, and at acquiring the skills of regulating ionophoric activities with ion selectivity.

To date, a wealth of nonpeptidic synthetic ion transporters has been reported based on the use of amphiphilic molecules, including cyclodextrins,[4] resorcin[4]arenes,[5] calixarenes[6] and others.[1] Among these building motifs, hydroxylated steroids, in particular cholic acid, appear to be an attractive class of compounds.[7] The flat lipophilic choloyl nucleus, by interacting with the alkyl chains of lipids, provides a rigid framework for the formation of stable pores in a membrane, whereas the inward-directed hydroxyl groups define a relatively hydrophilic pathway for large ion flux. In addition, cholic acid matches lipid monolayer in length.[8] Thus, a dimeric choloyl conjugate that is linked by an appropriate spacer is able to span the whole lipid bilayers in its fully extended conformation. As a consequence, several pore-forming bis(hydroxylated sterol) conjugates have been reported, and found to exhibit promising ionophoric activities.[9,10] These dimers are linked by simple groups, such as terephthaloyl diester and *m*-xylylene dicarbamate. Therefore, except the hydroxyl groups of the hydroxylated sterol framework and the ester/carbamate linking groups, they have no additional functional groups to interact with the ions that are to be transported.[11] Thus, we reason that a transmembrane bis(choloyl)-based conjugate having additional functional groups in between the two choloyl subunits, may exhibit unique ionophoric activity, because the functional groups are always located within the membrane interior and expected to be capable of regulating the ion flow.

With this rationale in mind, herein we describe the synthesis and ionorphoric activities of three bis(choloyl) conjugates **1**–**3** (Chart 1) bearing a rigid linker and amino/acetamido groups that are placed in between the two choloyl subunits. Here, a small, rigid and planar phenyl group is used to connect the two choloyl units so as to enable compounds **1**–**3** to span the entire lipid bilayers. It should be noted that this design principle has been previously used in creating transmembrane artificial ionophores.[9,10,12] To as-

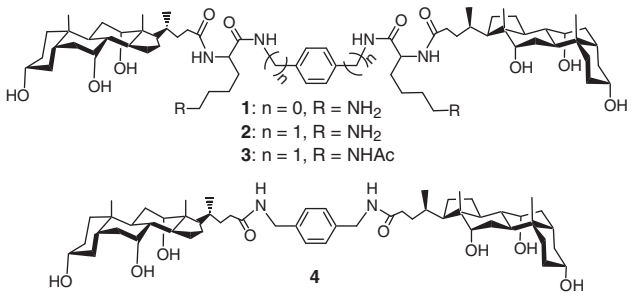

Chart 1. Structures of bis(choloyl) conjugates **1**–**4**.

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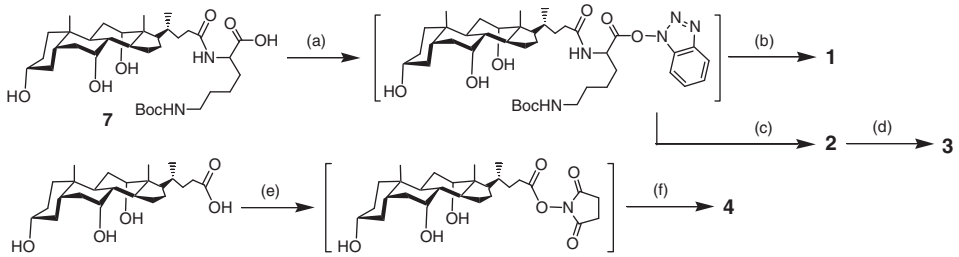
http://dx.doi.org/10.1016/j.bmcl.2013.04.022



Scheme 1. Synthesis of compounds **1**–**4**. Reagents and conditions: (a) HOBt, DCC, THF; (b) (i) *p*-phenylenediamine **5**, THF; (ii) TFA, CH_2Cl_2; (c) (i) *p*-bis(aminomethyl)benzene **6**, THF; (ii) TFA, CH_2Cl_2; (d) Ac_2O, THF–H_2O; (e) NHS, DCC, $CHCl_3$; (f) *p*-bis(aminomethyl)benzene **6**, $CHCl_3$.

sess the effect of the amino/acetamido groups in compounds **1**–**3** on the ionophoric activity, compound **4** (Chart 1) having no additional functional groups was also synthesized. In addition, as compound **2** is acetylated to give compound **3**, comparison of compound **2** with compound **3** will provides a means for evaluating the effect of the amino groups on the ionophoric activity.

Compounds **1**–**4** were synthesized according to the approaches shown in Scheme 1. Thus, acylation of *p*-phenylenediamine **5** and *p*-bis(aminomethyl)benzene **6** with *N*-α-choloyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-lysine **7**[13] that was activated with 1-hydroxybenzotriazole (HOBt), and subsequent Boc-deprotection by TFA, afforded compounds **1** and **2**, respectively. Acylation of compound **2** with acetic anhydride in aqueous THF gave compound **3**. Compound **4** was prepared from the reaction of *p*-bis(aminomethyl)benzene **6** with cholic acid that was activated with *N*-hydroxysuccinimide (NHS). The structures of compounds **1**–**4** were confirmed by ^{1}H NMR, ESI-MS and HRMS.[14]

To determine whether compounds **1**–**4** are capable of mediating the transport of ions across lipid bilayers, we firstly examined their ability to eliminate a pH differential across liposomal membranes derived from egg-yolk L-α-phosphatidylcholine (EYPC).[15] For this purpose, a pH-sensitive dye, 8-hydroxypyrene-1,3,6-trisulfonate (HPTS, or pyranine, pK_a 7.2) was loaded within large unilamellar vesicles (100 nm diameter, extrusion) and used as a fluorescence-responsive reporter of pH changes within the vesicle interior. If there is proton or hydroxide transport across the EYPC bilayers, this leads to a change in the internal pH of the vesicles and is detected by a change in the fluorescence intensity (FI) of pyranine. As a result, addition of compounds **1**–**4** to EYPC liposomal dispersions containing an internal pH of 7.0 and an external aqueous phase of pH of 8.0, led to an increase in the fluorescence intensity of pyranine, indicating that these compounds were capable of inducing pH discharge across the membrane. In each case, apparent first-order kinetics was observed.

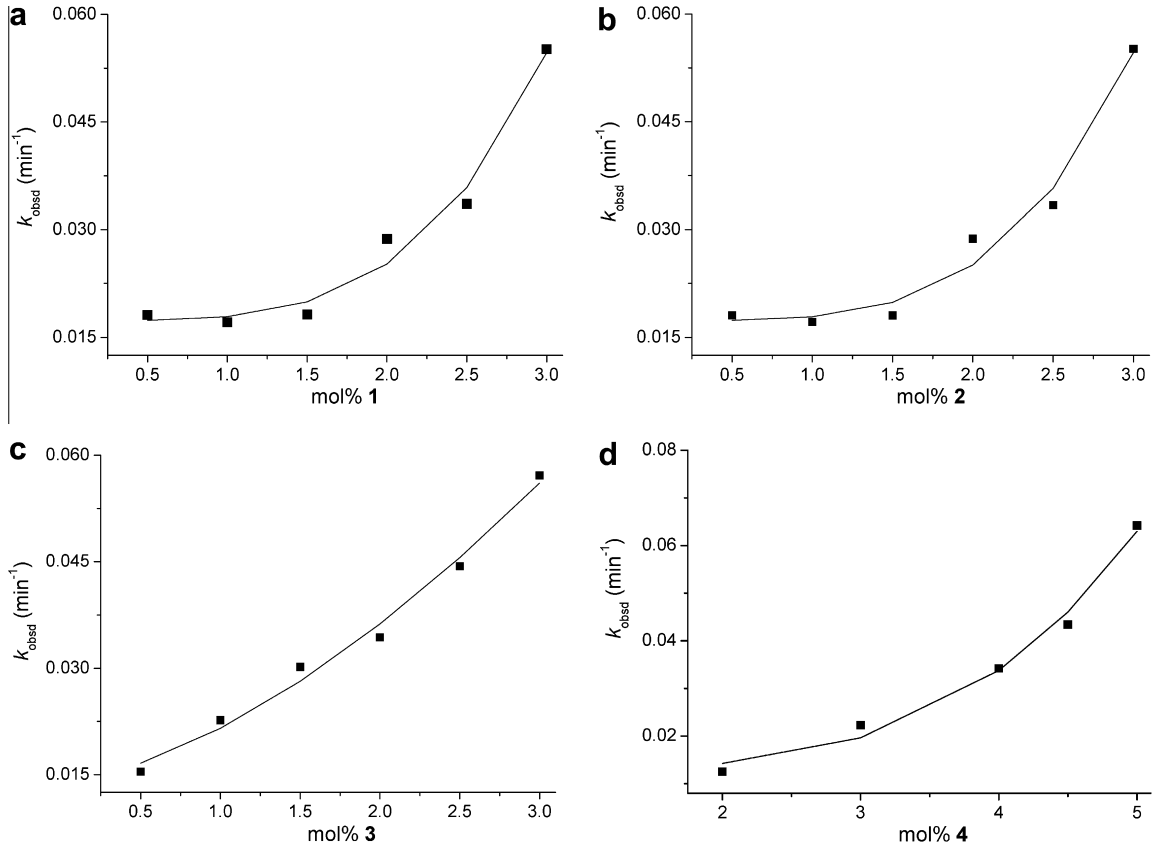


Figure 1. Plots of k_{obsd} versus the mol % concentrations of (a) compound **1**, (b) compound **2**, (c) compound **3** and (d) compound **4** across EYPC-based liposomal membranes at room temperature, under the conditions for internal vesicles: 0.1 mM pyranine in 25 mM HEPES (pH 7.0, 50 mM NaCl) and external vesicles: 25 mM HEPES (pH 8.0, 50 mM NaCl). Ex 460 nm; em 509 nm. The solid lines are nonlinear least-squares fit of the data according to the Eq. $k_{obsd} = k_0 + k_2[\text{monomer}]^n/K$, where n = 3.84, 3.88, 1.54 and 3.92, respectively.