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Design and synthesis of multifunctional phospholipids

Eugenia Drakopoulou,[a,*] Gerasimos M. Tsivgoulis,[b] Anneke Mukhopadhyay [a] and Alain Brisson [a,*]

[a] *Groningen Biochemistry and Biotechnology Institute, Biophysical Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands*

[b] *Department of Polymer Chemistry and Materials Science Centre, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands*

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Abstract

The synthesis of a bifunctionalized phosholipid capable of binding streptavidin or poly-histidine-tagged proteins is reported for the first time. The head group containing both a biotin and an NTA chelator is synthesized via a new approach using solid phase synthesis.

Keywords: solid phase synthesis; molecular recognition; phospholipids; nickel-chelators; biotin.

Biotin-streptavidin and Ni^{2+}-nitrilotriacetic acid (Ni^{2+}-NTA)-oligohistidine,[1] are the two most popular interactions used for chromatography and molecular recognition based assays. Functionalized lipids, especially with NTA or biotin, have been successfully applied to protein immobilization and crystallization.[2,3] Our group has reported the use of a biotinylated dioctadecylamine (DODA) molecule capable of forming tubular structures supporting the formation of ordered helical arrays of streptavidin.[4] Functionalized lipids of this kind are valuable tools to structural biology since the helical symmetry facilitates the calculation of the three-dimensional protein structure.[5] An extra function such as an NTA will provide the additional possibility to crystallize poly-histidine-tagged proteins. We have recently observed that the tendency of tubular formation was higher for the biotinylated phospholipids compared to the DODA-biotin (Brisson et al. unpublished data). Based on these results, a 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) able to bind both streptavidin and poly-histidine-tagged proteins was prepared. Although it is difficult to predict whether the addition of an NTA-moiety close to the biotin group could interfere with the formation of tubes, it has been shown in the case of galactosylceramide tubes that the addition of NTA-lipids does not prevent their formation.[6] Therefore, we designed a molecule that contains all the structural elements identified as important for the formation of lipid tubes such as chirality of the biotin moiety, saturated nature of the lipid chains, presence of amide bonds and length of the hydrophilic spacer.[7]

* Corresponding authors. E-mail: e.drakopoulou@chem.rug.nl (E. Drakopoulou), a.brisson@chem.rug.nl (A. Brisson)

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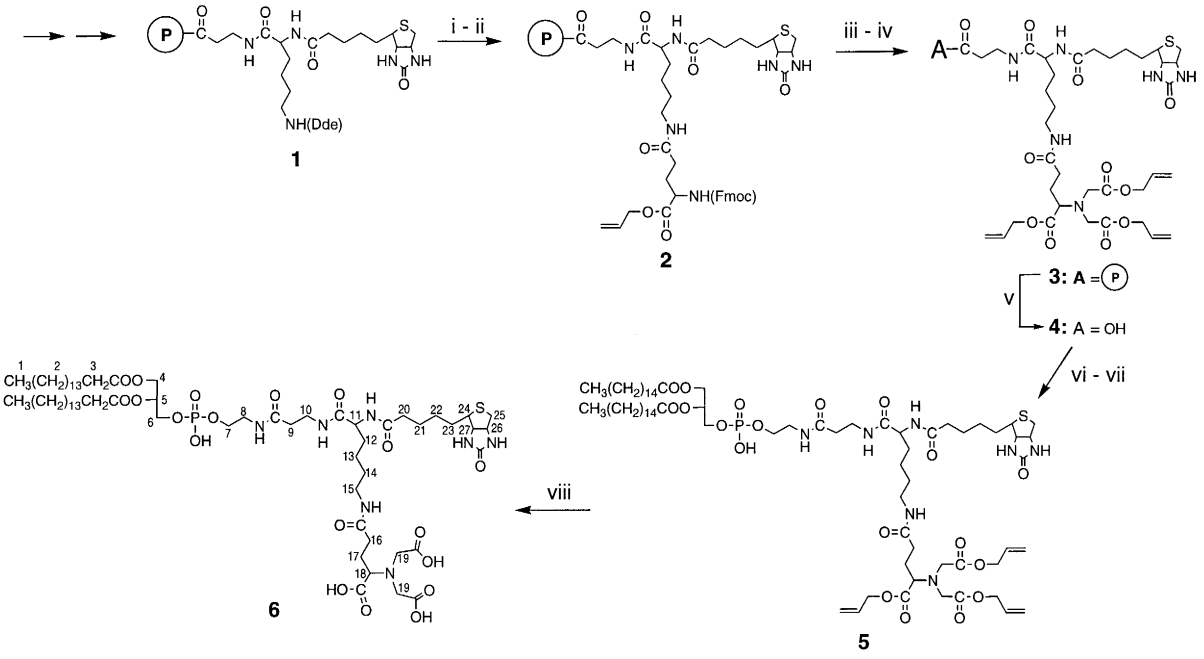
Unfortunately, the limited solubility of phospholipids in a number of solvents and their chemical instability make their functionalization difficult. This certainly explains the low yield of the synthesis of the NTA-DPPE.[8] Therefore, for the synthesis of the proposed bifunctionalized phospholipid a new approach was developed that is presented here. Particularly, a peptide skeleton that bears a free carboxyl group is used for the attachment of the biotin and the NTA chelator to the lipid. The synthesis of this peptidic part (head group) was easily performed on a solid support using solid phase peptide chemistry which is simple and necessitates one single purification step. The NTA function with its carboxyl groups protected as allylesters was also formed on the solid support. In the next step, this head group was bound to the phosholipid using a well known and high yield reaction (coupling between an amino group and NHS activated carboxyl group).

The total synthesis is shown in Scheme 1. β-Alanine, N^{ε}-1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-lysine (Dde-Lys) and biotin were sequentially coupled on a Wang resin using Fmoc chemistry[9] to give molecule **1**.



Scheme 1. *Reagents and conditions*: (i) 2% hydrazine in DMF; (ii) Fmoc-Glu-Oall/DCC/HOBt; (iii) 30% piperidine in DMF; (iv) allylbromoacetate/diisopropylethylamine; (v) TFA cleavage followed by HPLC purification; (vi) NHS/DCC; (vii) DPPE/Et_3N; (viii) 0.15 equiv. $Pd(PPh_3)_4$ in $CHCl_3$, 19 equiv. morpholine

The ε-amino group of the lysine was deprotected selectively from Dde by 2% hydrazine treatment.[10] Then the $^{\alpha}$allyl ester of N^{α}-Fmoc-glutamic acid was coupled through its γ-carboxyl group to give **2**. Deprotection of the α-amino group of **2** followed by reaction with allylbromoacetate gave the allyl protected NTA **3**. At that stage the whole protected head group **4** was cleaved from the solid support by TFA treatment. The efficiency of the synthesis was high as is shown from the analytical HPLC chromatogram of the crude material (Fig. 1a). The single major peak corresponds to the mass of product **4** as calculated by Electrospray mass spectrometry. Finally, the crude material was purified by preparative reverse phase HPLC giving a total yield of 42%. The allyl protection has been proposed for the successful synthesis of lipopeptides containing sensitive thioesters.[11] In our case it also proved to be crucial since it

remains intact during the TFA cleavage of the peptidic molecule from the solid support while it can be removed under mild conditions that do not affect either the phospholipid or the peptidic unit.

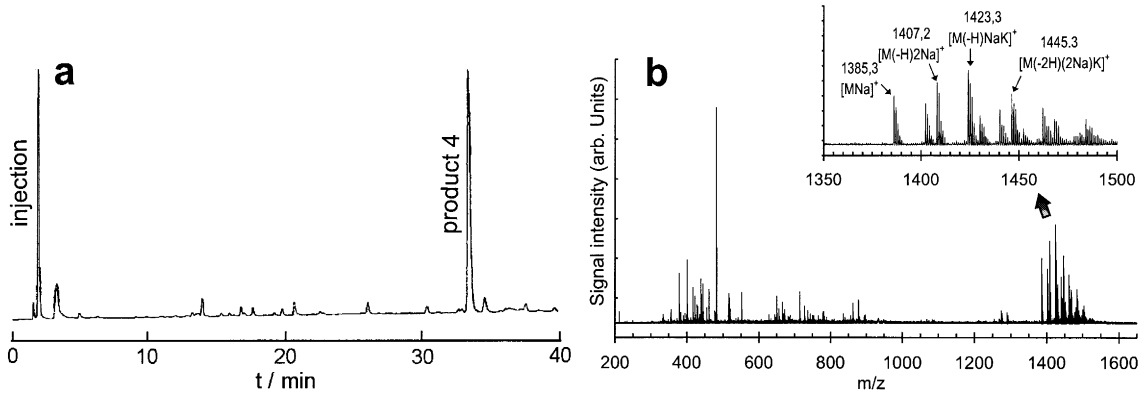


Fig. 1. (a) Analytical HPLC chromatogram of crude **4**. The sample was eluted from a Vydac C_{18} column with a 2–50% acetonitrile in water (0.1% TFA) gradient; (b) MALDI-TOF spectrum in α-cyano-4-hydroxy cinnamic acid matrix of the product **6**

The C-terminus of compound **4** after activation with *N*-hydroxysuccinimide and DCC was reacted with lipid DPPE to give **5**. Purification by preparative TLC followed by diethylether precipitation from a $CHCl_3$ solution gave pure **5** in 60% yield. Finally, the allyl esters were selectively removed by Pd^0-mediated transfer of the allyl group to morpholine giving the final lipopeptide **6**. Products **5** and **6** were characterized by H^1 NMR[12] and MALDI-TOF mass spectrometry (Fig. 1b).

Molecule **6** was designed as a multifunctional reagent. The NTA moiety of **6** was complexed with Ni^{2+} according to Schmitt et al.[8] in order to test its histidine complexing property, while another sample without Ni^{2+} was tested for streptavidin binding ability. These samples were incorporated separately into liposomes and incubated with poly-histidine-tagged proteins and streptavidin, respectively.[13] Indeed, as was detected by SDS-PAGE analysis, both the poly-histidine-tagged proteins and streptavidin bound to liposomes (data not shown), confirming the bifunctional nature of **6**. The morphology of the aggregates formed by this molecule in aqueous solutions is currently under study.

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12. ^{1}H NMR of compound **6** (300 MHz, $CDCl_3$+a few drops CD_3OD), δ ppm: 0.9 (6H, t, H1), 1.2–1.9 (66H, m, H2, H(12–14), H17, H(21–23)), 2.2–2.5 (10H, m, H3, H9, H16, H20), 2.75 (1H, d, H25a), 2.9 (1H, dd, H25b), 3.1–3.2 (3H, m, H15, H24), 3.3–3.7 (9H, m, H8, H10, H18, H19), 3.9 (4H, m, H4, H7), 4.1 (1H, dd, H6a), 4.3 (1H, m, H27), 4.4 (1H, d, H6b), 4.5 (1H, m, H26), 5.2 (1H, m, H5).
13. Product **6** was mixed with dioleoyl-phosphatidyl-choline in 1:4 ratio in $CHCl_3$. After evaporation under vacuum the dry lipid film was rehydrated with a solution containing 25 mM Tris, 100 mM NaCl, pH 7.5 and vortexed. The liposomes obtained were incubated either with streptavidin or a poly-histidine-tagged protein. The material present in both the pellet and supernatant was characterized by SDS-PAGE.