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Novel Bioconjugates of Aminolevulinic Acid with Vitamins

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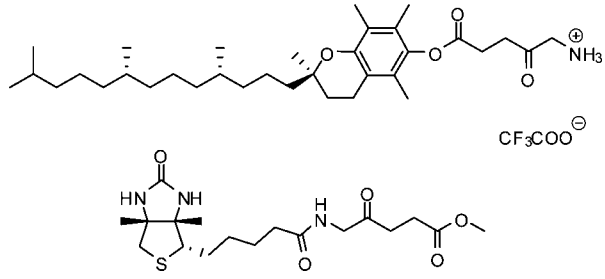
ABSTRACT



5-Aminolevulinic acid (ALA) and derivatives thereof have been successfully used in photodynamic cancer therapy (PDT). The synthesis of novel bioconjugates combining ALA with two lipophilic and one hydrophilic vitamins is reported. The new bioconjugates allow studying the potential synergies between the two components in PDT. The synthetic methodology is robust giving the bioconjugates in good to satisfactory yield.

Photodynamic therapy (PDT) is a minimally invasive treatment for the cure of malignant diseases.[1−3] As clearing of the photosensitizer is often a limiting factor in PDT, the study of 5-aminolevulinic acid (ALA), the natural precursor of the endogenous photosensitizer protoporphyrin IX (PpIX), has been receiving increasing attention.[4,5] ALA is unstable at neutral or slightly basic pH[6] and lacks selectivity toward malignant tissues.[7] Esters of ALA have been successfully applied to circumvent the problem of the insufficient lipophilicity.[8] Bioconjugates of ALA with amino acids and simple peptides have been synthesized and studied to target tumor cells (Figure 1).[9] We recently synthesized a new class of ALA bioconjugates, using monosaccharides.[10] On the

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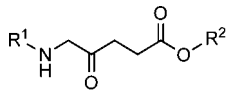


1 R^1 = Ala, R^2 = Me
2 R^1 = Phe, R^2 = Me
3 R^1 = Lys, R^2 = Me
4 R^1 = Asp, R^2 = Me
5 R^1 = Gly-Pro-, R^2 = Et
6 R^1 = Gly-Gly-, R^2 = Et
7 R^1 = Boc-Gly-Pro-, R^2 = Et
8 R^1 = Boc-Gly-Gly-, R^2 = Et

Figure 1. Examples of ALA pseudopeptides synthesized and tested in PDT as reported in refs 9a and b.

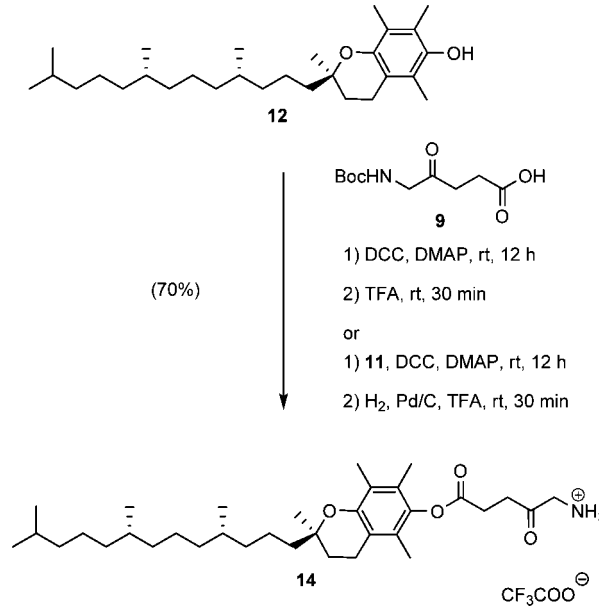
basis of our synthetic methodology, we have undertaken studies investigating a variety of bioconjugates of ALA. We were interested to expand our approach to novel classes of bioconjugates coupling ALA with lipophilic and hydrophilic vitamins. Herein, we report the first successful synthesis of these new bioconjugates. Our approach is robust and can therefore be used for the synthesis of many other derivatives of ALA.

In the past, 5-ALA-hydrochloride (ALA-HCl) has been used as the starting material of choice. ALA-HCl is hygroscopic and easily forms side products. In our hands, the Boc-protected form of ALA **9** (Boc-ALA), the activated oxime ester of Boc-ALA **10**, or the 5-azidolevulinic acid **11** proved to be reliable precursors for the synthesis of novel ALA bioconjugates[11] avoiding most of the problems associated with the use of 5-ALA-HCl.

Bioconjugates between vitamins and ALA were attractive choices to show the applicability of our methodology. We choose vitamin E (**12**) and vitamin D_3 (**16**) as representatives for the lipophilic vitamins and biotin (**19**) as an example of hydrophilic vitamins. α-Tocopherol was chosen because of its ease of incorporation into natural membranes, which might facilitate the transport of ALA into the interior of cancer cells.[12] The lipophilic character of cholecalciferol (**16**) should facilitate the incorporation of the bioconjugate into cell membranes. It has been proposed that the observed anticancer effects of vitamin D are mediated through the vitamin D receptors (VDR).[13] In contrast to the lipophilic vitamins mentioned above, biotin (vitamin H) is water soluble, and no reports on activity related to cancer have been reported. We decided to test the application of our strategy to this vitamin to find out if our methodology is compatible with water-soluble components.

We synthesized the direct conjugate between vitamin E (**12**) and ALA using the Boc-protected precursors of ALA **9** (Scheme 1). Standard coupling conditions using DCC in

Scheme 1. Synthesis of Tocopherol-ALA Esters Using the Boc-Protected ALA **9** or the Azido-Protected ALA **11**



the presence of DMAP allowed us to achieve the efficient synthesis of the bioconjugates between **9** and vitamin E. Deprotection of the Boc group under acidic conditions provided the vitamin E ALA bioconjugate **14** in good yield and high purity. The coupling of **9** with vitamin D_3 (**16**) was realized successfully under standard conditions. Trials to deprotect the Boc group of **17** using trifluoroacidic acid led to degradation (Scheme 2), probably due to the sensitivity of the vitamin part.

We tested an alternative approach to these two bioconjugates using the azido-protected ALA **11** as a precursor (see Schemes 1 and 2). We could couple **11** with the two vitamins under standard coupling conditions in good yields. Treating the mixture of **11** with α-tocopherol (**12**) in equimolar amounts with DCC as the coupling reagent and DMAP in catalytic quantities gave an excellent yield of the ester **15** of 83% in high purity. The azido derivative is stable and can be stored without degradation. The conversion of the azido function in the bioconjugate with tocopherol **15** could be achieved by catalytic hydrogenation using palladium on charcoal in the presence of trifluoroacetic acid to trap the amino group. The deprotected bioconjugate **14** could be isolated in 90% yield and in excellent purity. The bioconjugate **18** between azido-protected ALA **11** and cholecalciferol (**16**) could be obtained in moderate yield (Scheme 2). We were not able to transform the azido function into the protonated amine under the conditions mentioned above.

To form the bioconjugate between biotin and the amine of ALA via an amide linkage, we synthesized the pentafluoro phenyl ester of biotin first, which was treated with methyl 5-azidolevulinate (**21**) under catalytic hydrogenation conditions (Scheme 3).

The methyl azidolevulinate (**21**) is reduced under these conditions in situ to methyl aminolevulinate. The newly

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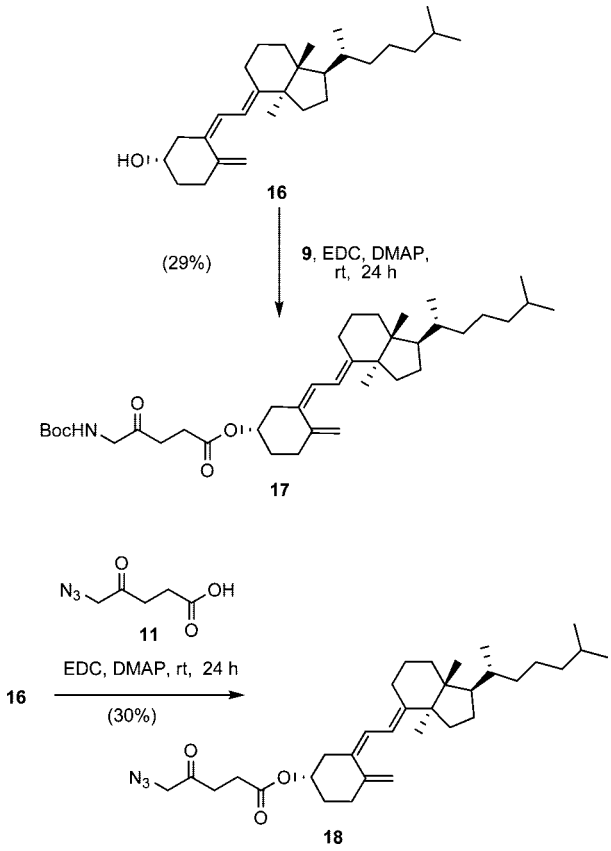
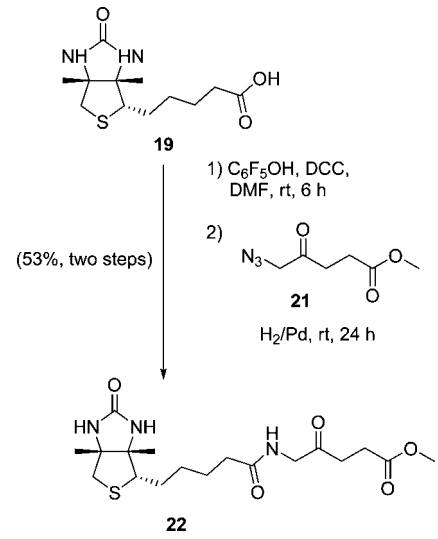
Scheme 2. Synthesis of Cholecalciferol-ALA Esters Using the Boc-Protected ALA **9** and the Azido-Protected ALA **11**



Scheme 3. Synthesis of the Biotin-ALA Amide



formed amine attacks the activated pentafluoro phenyl ester of biotin. The activated pentafluoro phenyl ester of biotin was added in excess to prevent the formation of the pyrazine-type impurity by self-condensation of two methyl aminolevulinate molecules. Under these optimized conditions, a moderate, but reproducible, yield of the bioconjugate **22** could isolated.

We report the synthesis of bioconjugates combining ALA with lipophilic and hydrophilic vitamins. These novel compounds open the opportunity to target the delivery of ALA to tumor cells and to enhance the efficiency of PDT. The concomitant delivery of both partners of the bioconjugate to the cancer cells might create synergies. The synthetic methodologies reported for the synthesis of bioconjugates are robust and general. The most delicate step is the final deprotection, which has not been successful in the case of the bioconjugate with the acid-sensitive cholecalciferol.

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Supporting Information Available: Detailed procedures for the preparation and characterization of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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