2nd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-2), http://www.mdpi.org/ecsoc/, September 1-30, 1998

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Design and Synthesis of a Photoaffinity Label for the Enzyme Tocopherol Cyclase

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Received: 31.08.98

Abstract: A mechanism based, photoaffinity labelled inhibitor of the enzym tocopherol cyclase was synthesized

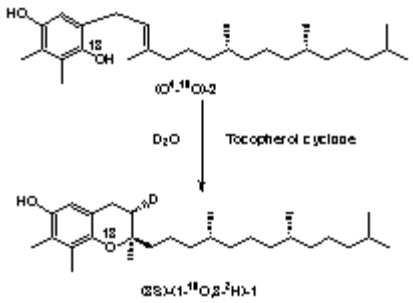
Keywords: enzyme, reaction mechanism, photoaffinity label, tocopherol cyclase, transition state analogue

Introduction

Recently, we have identified a new enzyme in the blue-green algae *Anabaena variabilis* KUETZING (*Cyanobacteria*) which catalyzes the formation of g-tocopherol **1** from the phytyl-hydroquinone **2**, scheme 1.This enzyme, tocopherol cyclase, plays a key role in the biosynthesis of the chromanol substructure of the vitamin E family which is important for animal and human nutrition.

It was shown that the cyclization was stereospecific and could be driven to quantitative substrate turnover under strictly defined conditions such as incubating the 2,6-di-O-methyl-b-cyclodextrin complex of **2** in the presence of ascorbic acid with spheroplasts prepared by lysozyme treatment of intact *Anabaena* cells [1].

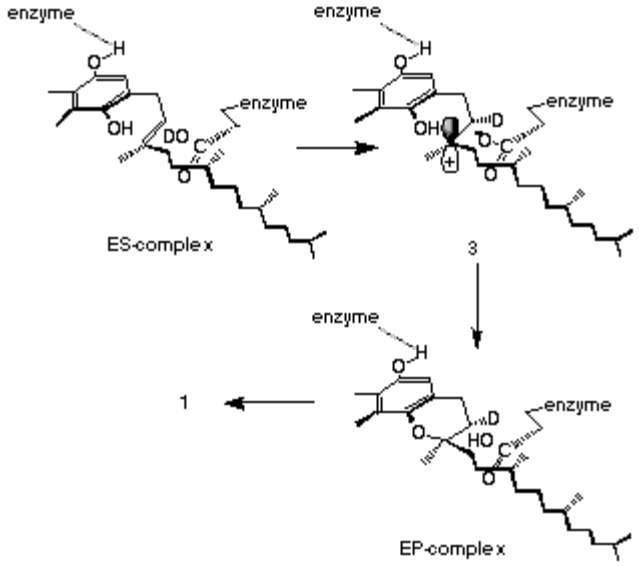
Scheme 1



Subsequent purification of the enzyme proved to be very difficult because the protein is membrane bound and extremely hydrophobic. Using the non-ionic detergent lauryl maltoside the 42kD protein was finally obtained pure by SDS gel electrophoresis (purification factor PF: 13.850) after several chromatographic steps including Mono-Q, Mono-P, and size exclusion chromatography [2]. In order to improve the purification procedure in the presence of a substrate analogue, and furthermore to understand the recognition of the substrate by the protein's active site, we investigated the substrate specificity of the enzyme both in its enriched form and as intact spheroplasts of *Anabaena variabilis* [3]. This study revealed that the the OH group at C(1) of **2**, the (E) double bond as well as the length of the side chain are important for substrate recognition. Several experiments also indicated that the substrate enters the enzyme's active site with the hydrophobic tail [3]. Mechanistic ivestigations with the ^{18}O-labelled substrate in D_2O, as depicted in scheme 1, revealed that the tocopherol cyclase is operating by *si* -protonation of the double bond of **2** and *re*-attack of the phenolic O-atom, Scheme 1 [4]. For the chemical cyclization of **2** to **1**, epimeric at C(1), strong

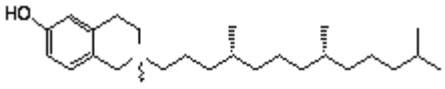
acids or irradiation are required, in contrast the epoxide of **2** cyclyzes spontaneously to the five membered benzodihydrofuran system in agreement with the Baldwin rules. Taken together these experiments favor a non-synchronous enzymatic process and the formation of a carbocation **3** along the reaction coordinate which due to the tight orientation in the active site can cyclize only be by *re*-site-attack of the phenolic OH group, scheme 2.

Scheme 2



It was therefore concluded that a reasonable transition state analogue of the enzymatic cyclization would be the tetrahydroisoquinolinium derivative **4**, scheme 3. **4** was prepared in a straightforward manner by the Picktet-Spengler method and investigated for its binding by incubation with the purified enzyme. In agreement with theory and expectation **4** turned out to be a competitive inhibitor for the cyclization of **2** to **1** at nm concentrations [5]. Accordingly **4** is a good candidate to design haptens suitable for the preparation of monoclonal antibodies catalyzing the cyclization [5]. On the other hand the good IC_{50} value (147nM) of **4** suggested that inhibitors can be developed that could be used to improve the purification of tocopherol cyclase, in particular when covalent attachment of the substrate analogue could be accomplished by means of a photolabile group.

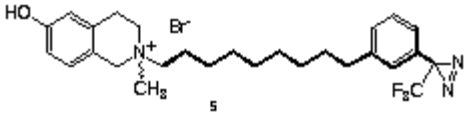
Scheme 3



Results and Discussion

Substructures of **4**, which were believed to be essential for good binding to the enzmye, such as the 5-hydroxy tetrahydroisoquinolinium part and the lipohilic side chain should be maintained to accomplish an inhibitor carrying a photoaffinity label. Accordingly the structure **5** was considered because the hydrophilic part is preserved, the lipophilic side chain, however, is devoid of methyl groups, and the photoactive group replaces the terminal isoprene unit, scheme 4. The 3-trifluoromethyl-3-phenyl-diaziridine group was chosen as the photolaaffinity label because irradiation (358 nm) is distinct from the absorption maximum of the enzyme. Further on irradiation carbenes are generated which have been reported to be more useful than corresponding nitrenes [6], and due to the reactivity of carbenes regarding H. abstraction (from solvents), we should not encounter severe problems with pseudo-photoaffinity labelling.

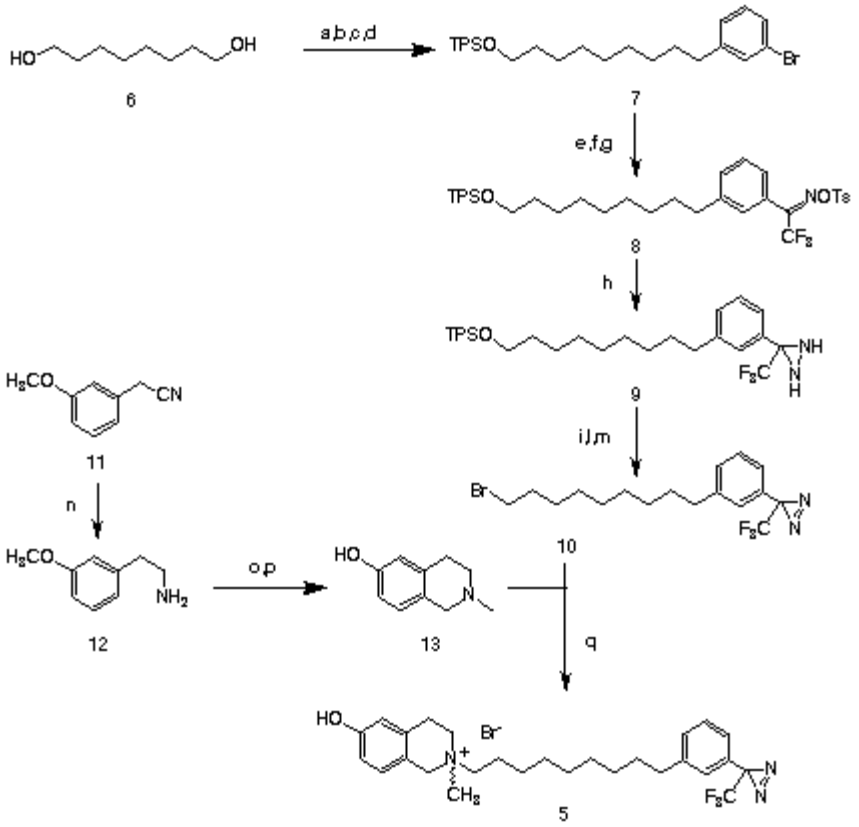
Scheme 4



The side chain of **5** was prepared from 1,8 octanediol **6** via **7**, the precursor for attachment of the required diazirine. Lithiation of **7** followed by addition of trfluoroacetyl-piperidine gave the trifluoroacteyl ketone which was treated with NH_2OH and subsequently with TosCl to yield the tosyl oxime **8**. On reaction of **8** with liquid NH_3 in an autoclave the diaziridine **9** was formed which was oxidized with AgO, and brominated after deprotection to furnish the w-bromodiazrine **10**. The commercially avaiable nitrile **11** was reduced to **12** which was subjected to Pictet-Spengler conditions [7] and deprotected to yield the tetrahydroisoquinoline **12**. Alkylation of the latter with the bromide **10** gave the target compound **5**, scheme 5. Since most of the steps proceed with over 90% yield the preparation of **5** is quite efficient and could be pursued also with radiolabelled **13**.

The HPLC-pure photoaffinity label **5** was investigated for its competitive bindung to the enzyme tocopherol cyclase using the same conditions as for **4**; an IC_{50} = 133nM was determined for **5**. Accordingly replacing the terminal isoprene unit of the side chain of **4** by a trifluoromethylphenyldiazirine unit does not significantly changes the binding of the substrate analogue. The photochemistry of E.5 **complex will be described elsewhere [8].**

Scheme 5



a) 1equ. HBr (48%)toluene, reflux 8h, 77%; b) PPh_3, EtOH, reflux, 48h, 84%; c) 2equ. PhLi, 1 equ. 3-bromobenzaldehyde, THF, -40°, 30 min, 92.5%; d) H_2, Pd/C, EtOH, r.t., 2h, TPSCl, imidazole, DMF, r.t.., 1h, 95%; e)BuLi, $CF_3CONpip$, Et_2O, -50°, 67%; f) NH_2OH/AcONa, EtOH, reflux, 18h, 97%; g) TsCl, py, 120°, 3h, 99%; h) NH_3, Et_2O, -78° to r.t., 18h, 93%; i) AgO, Et_2O, r.t., 1.5h, 91%; l) TBAF, THF, r.t., 1h, 99%; m) Ph_3P, NBS, CH_2Cl_2, O°, 45 min, 91%; n) $LiAlH_4/AlCl_3$, Et_2O, 5°, 2h, 76%; o) HCHO, HCOOH, 95°, 5h, 88%; p) BBr_3, CH_2Cl_2, -78°, 3h, 80%; q) CH_2Cl_2, MeOH, 50°, 18h, 95%.

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Back to Introduction

Comments

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