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DESIGN AND SYNTHESIS OF ANALOGS OF VITAMIN E: ANTIPROLIFERATIVE ACTIVITY AGAINST HUMAN BREAST ADENOCARCINOMA CELLS[1]

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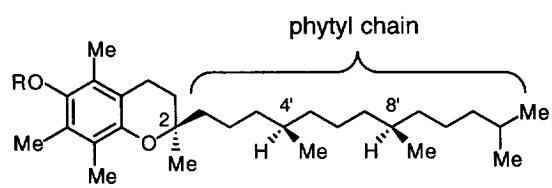
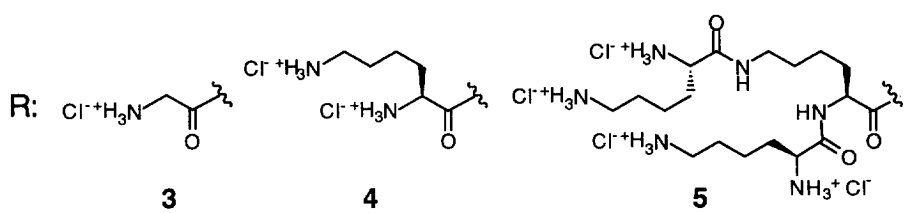
Abstract: Analogs of α-tocopherol (vitamin E; compounds **3–9**) have been synthesized and tested for their antiproliferative activity using the human breast cancer cell line, MCF7. Compounds **6–9** were synthesized from a common synthon, *rac*-Trolox (**14**) and are soluble/miscible at physiological *p*H. Compounds **4**, **8**, and **9** were found to have antiproliferative activity at micromolar concentrations. © 1998 Elsevier Science Ltd. All rights reserved.

It is well known that reactive oxygen-derived free radicals (e.g., •OH, •OOH, O_2-) are able to damage cellular components and can play an important role in initiating biological disorders, leading to diseases such as cancer, cardiovascular disease, and aging.[3] Vitamin E (α-tocopherol, α-TOH, **1**, Figure 1), the major lipid-soluble antioxidant found in mammalian cells plays a vital role in the maintenance of tissue homeostasis and cellular defence against oxidative stress.[4,5] Increasingly, attention is being paid to the role that this natural antioxidant, and its analogs, may play in reducing the incidence of heart disease and cancer.[6]

Figure 1



R: H, Vitamin E (RRR-α-Tocopherol, α-TOH, **1**)
R: Ac, (RRR-α-Tocopheryl acetate, α-TOAc, **2**)



3 **4** **5**

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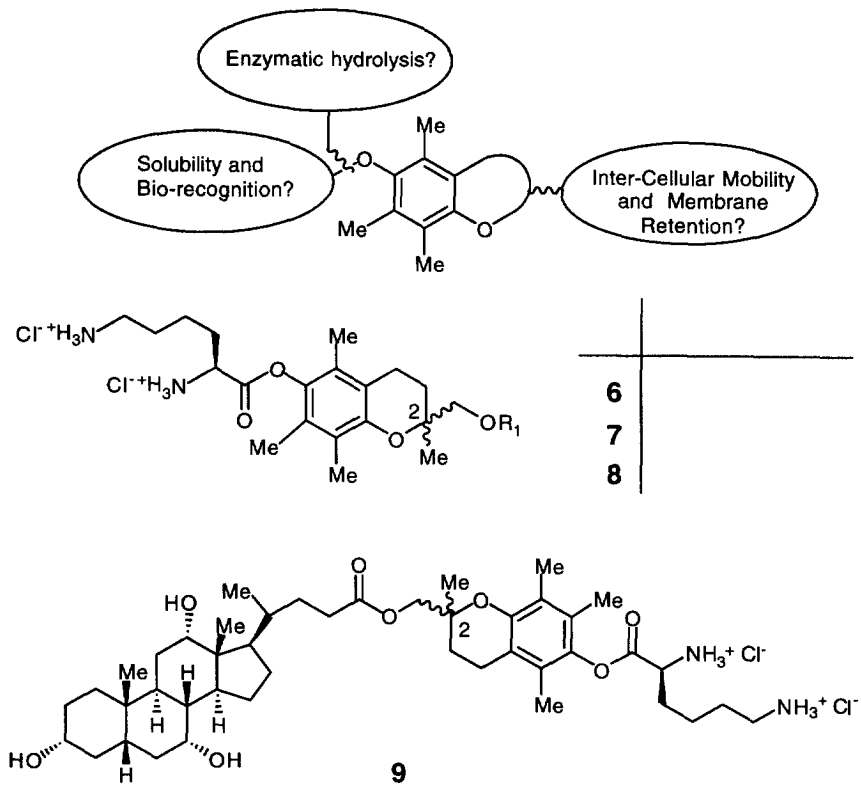
One of the drawbacks of using vitamin E for rapid therapeutic intervention is its extreme insolubility in water. This limitation affects its pharmacokinetics and tissue pharmacodistribution. This communication reports our efforts to "engineer" new analogs of vitamin E for rapid distribution into tissues. As part of this project, we have also begun to explore the antiproliferative properties of the new analogs. Two important factors were considered for our synthetic strategy (Figure 2): (i) the derivatization of the phenolic hydroxyl of vitamin E with

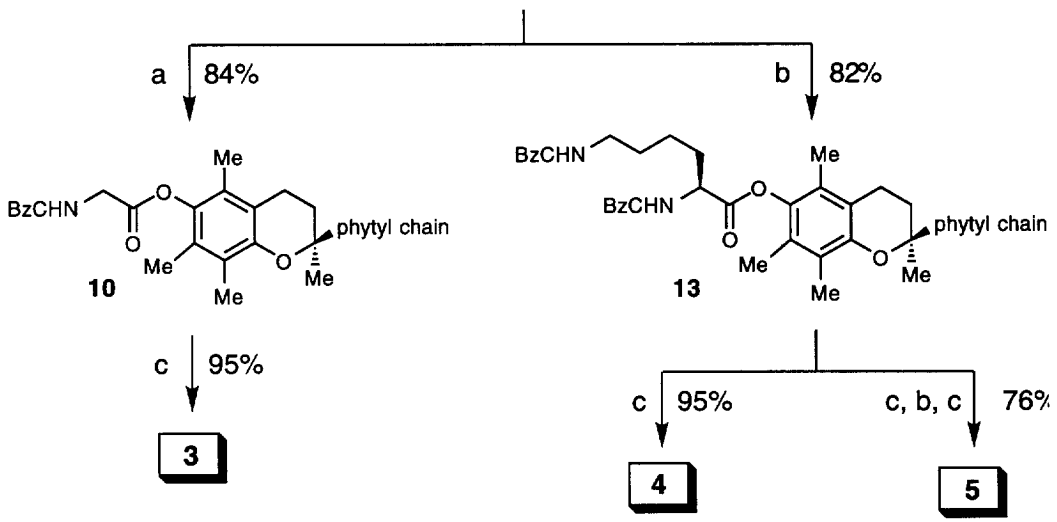
Figure 2



amino acid derivatives via an ester bond, and (ii) modulation of the nature and length of the phytyl chain. Derivatization of the phenolic hydroxyl of vitamin E as an amino acid ester conjugate introduces a positively charged group that is expected to be cleaved by in vitro or in vivo enzymatic hydrolysis (e.g., by cholesterol esterases). Modulation of the chain length is expected to reduce the membrane-philicity of vitamin E and to increase its solubility in aqueous media. The chroman ring was not altered because changing in the ring size (i.e., from six to five members) causes only a modest increase in antioxidant activity.[4] Analogs of vitamin E (**3**–**9**) were evaluated by testing for antiproliferative activity using a human breast cancer cell line, MCF7, and comparing the results with the commercially available vitamin E derivatives [e.g., α-tocopherol (**1**), α-tocopheryl acetate (**2**), α-tocopheryl succinate and *rac*-Trolox (**14**)].

Synthesis of glycine and lysine conjugates of vitamin E (Scheme 1, **3–5**): The CBz-glycine and di–CBz lysine ester of vitamin E (**10** and **13**) were prepared in 82–84% isolated yield, after purification over silica gel, by coupling vitamin E (**1**) to CBz–glycine (**11**) or di–CBz–lysine (**12**) using DCC/DMAP reaction conditions.[7] Compounds **10** and **13** on hydrogenation conditions (H2, 10% Pd/C in 95% EtOH) gave the corresponding free amine derivatives, which were isolated as hydrochloride salts (**3** and **4**) after acidification with dil HCl. Compound **5** was obtained from **13** in 76% isolated yield in four steps: (i) hydrogenation of **13** to obtain the free amine derivative, (ii) coupling with the di–CBz–lysine (**12**) using DCC/DMAP, (iii) hydrogenation to obtain the free amine derivative, and (iv) hydrochloride salt formation.

Scheme 1

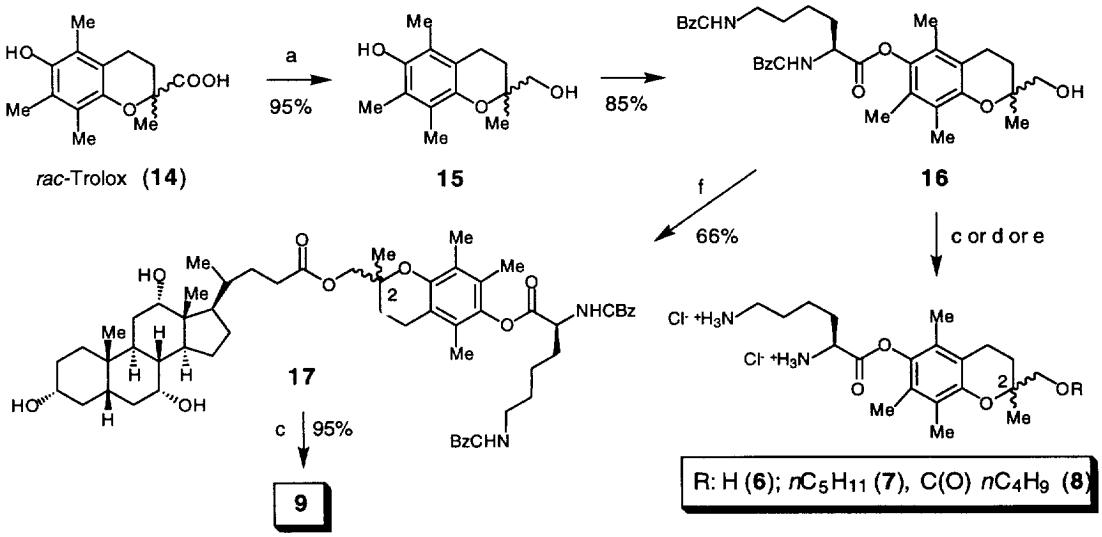


(a) CBz–Gly (**11**), DCC, DMAP (10 mol%), CH_2Cl_2; (b) di–CBz–Lys (**12**), DCC, DMAP (10 mol%), CH_2Cl_2; (c) (i) H_2, 10% Pd–C, 95% EtOH; (ii) dil HCl.

Synthesis of analogs of vitamin E (Scheme 2, **6–9**): Compound **15** was prepared from the *rac*-Trolox (**14**) in 95% isolated yield in two steps, employing esterification followed by LAH reduction. The di-CBz lysine ester derivative (**16**) was obtained in 85% isolated yield, after purification over silica gel, by the coupling of compound **14** with di–CBz–lysine (**12**) using DCC/DMAP reaction conditions.7 Hydrogenation (H_2, 10% Pd/C in 95% EtOH) of **16** gave the corresponding free amine derivative, and was isolated as the hydrochloride salt (**6**) after acidification with dil HCl. Compounds **7** and **8** were obtained by alkylation ($nC_5H_{11}Br$, Et_3N, RT) and acylation (nC_4H_9COCl, Et_3N, RT) of **16**, respectively. The corresponding products were purified over silica gel, hydrogenated and then subjected to hydrochloride salt formation to give compounds **7** and **8**. Compound **16** was also coupled with cholic acid using DIC/DMAP reaction conditions in order to introduce an amphiphilic auxiliary as a substitute for the tail of vitamin E. The cholic acid ester derivative **17** was obtained in 66%

isolated yield after purification over silica gel by column chromatography. As in previous cases, the CBz–groups were removed by hydrogenation to obtain the free amine derivative, which was isolated as the hydrochloride salt (**9**).

Scheme 2



(a) (i) *p*TSA, EtOH, reflux; (ii) LAH, Et_2O; (b) di–CBz–Lys (**12**), DCC, DMAP (10 mol%), CH_2Cl_2; (c) (i) H_2, 10% Pd–C, 95% EtOH; (ii) dil HCl →(**6**); (d) (i) $nC_5H_{11}Br$, Et_3N, THF; (ii) (c) → (**7**); (e) (i) nC_4H_9COCl, Et_3N, THF; (ii) (c) → (**8**); (f) cholic acid, DIC, DMAP (10 mol%), THF.

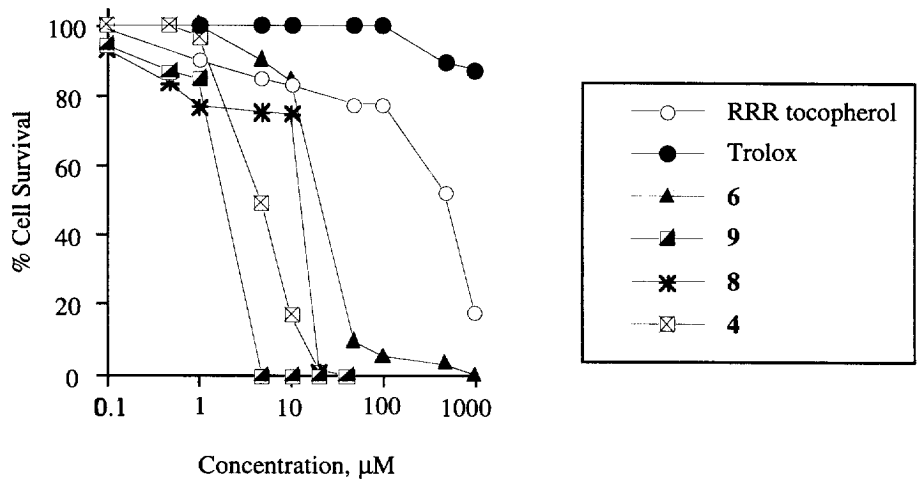
Results and Discussion: The analogs of vitamin E (**4, 6–9**) were tested for antiproliferative activity using a human breast cancer cell line MCF7 (Table 1). Cells were grown in RPMI medium, supplemented with 10% fetal bovine serum and antibiotics under 5% CO_2.[8] MCF7 cells were seeded at a density of 1×10^3 cells/100μL/well in 96-well plates. After 18 h of culture, the cells were treated with various concentrations of the vitamin E analogs **4**, **6–9** for 96 h. The antiproliferative effect was evaluated using the 3(4-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described earlier.[9] The MTT assay distinguishes between viable and nonviable cells on the basis of the requirement of physiologically active mitochondria to metabolize MTT only in viable cells. The maximum absorption of the metabolized MTT occurs at 570 nm. In brief, the culture media was replaced with a solution composed of 20 μL complete media and 20 μL of a solution containing 2.5 mg/mL of MTT in phosphate buffer (pH 7.4). After 4 h of incubation at 37 °C, 100 μL of DMSO was added to dissolve the precipitate of reduced MTT. Absorbance was determined at 570 nm with a microplate reader (BIORAD-450). The IC_{50} was calculated as the dose of each analog causing a 50% reduction in absorbance, in comparison to untreated cells or cells treated with the solvent alone.

In comparison to α–TOH (**1**), α–TOAc (**2**), α–TO–succinate (purchased from Sigma) and *rac*–Trolox (**14**), the new analogs **4**, **7–9** showed anti proliferative activity against the human breast cancer cell line MCF 7

(Table 1). Simple replacement of the acetate of α–TOAc with a lysine moiety (**4**) resulted in a dramatic increase

Table 1: Antiproliferative Effects of Vitamin E Derivatives and Analogs

Compound	Human Breast Cancer Cell Line MCF 7 IC_{50} (μM)
α–TOH (**1**)	329 ± 12
α–TOAc (**2**)	>500
α–TO–succinate	>368
Trolox (**14**)	1461 ± 246
4	12 ± 2
6	194 ± 62
7 (water soluble)	22 ± 6
8 (water soluble)	15 ± 1
9 (water soluble)	4 ± 1



in antiproliferative activity. Furthermore, replacement of the phytyl group of the lysine conjugate, **4**, by a short hydrocarbon chain attached by an ether or an ester bond (**7** or **8**) or by an amphiphilic auxiliary (**9**) enhanced the solubility at physiological *p*H without significantly affecting the antiproliferative activity. *Rac*-Trolox (**14**), in which the phytyl chain of vitamin E has been replaced by the –COOH group, was not active. It is not known at this stage if the different biological responses exhibited by the various analogs result from differences in their ability to penetrate the cells. Further work is required to determine why these analogs exert antiproliferative effects on this transformed cell line.

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References and Notes

1. NRCC publication no. 40879.
2. Post doctoral fellow supported by NSERC grant-STR 0181484.
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