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Total synthesis of a possible specific and effective acid-targeted cancer diagnostic, a camphor derived bis-N-oxide dimer

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ABSTRACT

Possible specific and effective acid-targeted cancer diagnostics and therapeutics, a camphor derived bis-N-oxide dimer was synthesized in 12-steps from commercially available (+)-camphoric acid and seven-steps from a common intermediate, a camphor derived primary amine.

It is well known that most tumors larger than about 1 mm in diameter contain hypoxic (oxygen deficient)/acidic areas and the extracellular pH within such hypoxic areas is significantly lower than the pH in normal tissue under physiological conditions.[1–4] Recently, researchers have begun to exploit the hypoxic/acidic properties of tumors.[5] Ginos et al. have shown that ^{11}C-DMO (carbon 11-labeled 5,5-dimethyl-2,4-oxazolidinedione), containing a single weak-acid moiety (pK_a of 6.3) and a radioisotope could be used to detect acidic areas of tumors.[6,7] As a cytotoxic agent, chlorambucil which contains a weak-acid moiety and can penetrate into acidic area of tumors has been used to treat tumors for a long time.[8,9] However, both ^{11}C-DMO and chlorambucil afford only limited specificity for detecting and treating tumors containing acidic areas.[10] PAP-1, an 18 amino acid pH activated peptide showed potential for exploiting a common property of tumors to achieve highly specific medical intervention. However, biological studies demonstrated that this acid-targeted peptide hangs up in the kidneys and such hang up probably cannot be adequately prevented.[1] Therefore, small-molecule onco-tools, which allow substantially greater versatility in design strategies, need to be developed. According to calculations with the Henderson–Hasselbach equation combined with a binomial expansion, the specificity of a molecule for acidic areas of a tumor should be dramatically increased by incorporating multiple acid moieties in the molecule. However, when typical carboxylic acid moieties having pK_a values around 4.8 are used, the dramatic increases in specificity are counter balanced by corresponding drastic decreases in efficacy factor. Yet an adequate efficacy factor is crucial for cancer treatment because if the efficacy factor is too small then insufficient amounts of the agent will be sequestered in the tumors.[10–12] In order to achieve the increased level of specificity that comes from incorporating multiple acid moieties while avoiding much of the efficacy decrease, adjusting the pK_a of one or more of the acid moieties becomes necessary. Molecules **1**–**4** (Fig. 1) containing a weak-acid moiety with H-bond acceptor (N-oxide or N) and a cargo component (R_1 or R_2) to which a radioisotope can be stably attached were synthesized and have shown increased pK_a's of 5.8, 6.6, 5.5, and 6.0, respectively, compared to a pK_a of 4.8 of a conventional carboxylic acid without an internal H-bond. According to calculations, compound **2** should afford a moderate tumor specificity factor of 4 and an excellent efficacy factor of 61%.[10]

Initial results in hands, we designed a camphor derived bis-N-oxide dimer **5** (Fig. 1) intended to afford greater tumor specificity while still providing an acceptable efficacy factor. The same camphor framework as in compound **2** and the bis-N-oxide moieties of molecule **5** should provide a pK_a between 6.0 and 6.6, and therefore afford an adequate efficacy factor. At the same time, two weak-acid moieties in molecule **5** are calculated to afford a much higher tumor specificity factor.

We envisioned that our target molecule **5** could be accessed by sequential deprotection/oxidation of diamino diester **6**, which in turn, should be available by coupling aziridinium salt **7** with secondary amine **8**. Aziridinium salt **7** and amine **8** should be derivable from a common intermediate, amine **9** obtained from commercially available (+)-camphoric acid (Fig. 2).

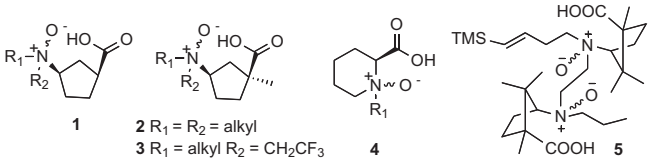

Figure 1. Novel acid-targeted molecules.

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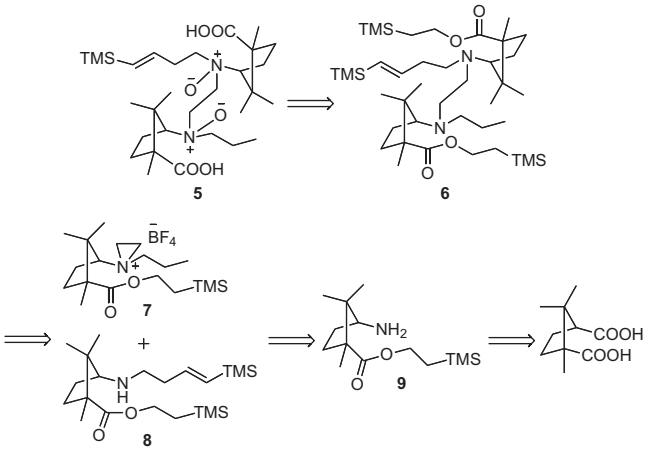
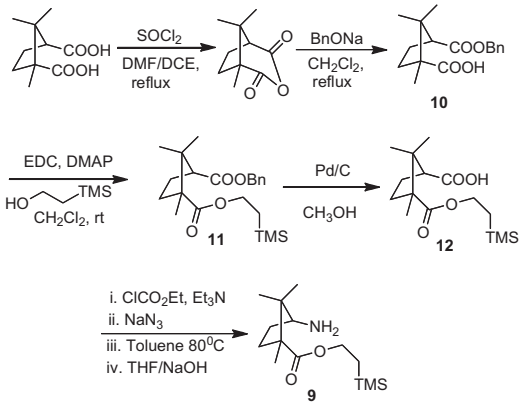


Figure 2. Retrosynthetic strategy for preparation of **5**.

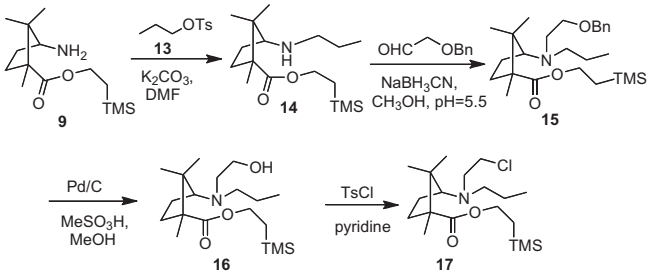
Amine **9** was synthesized in four-steps from known (+)-camphoric anhydride, which was obtained from (+)-camphoric acid in 95% yield (Scheme 1). Ring opening of (+)-camphoric anhydride with sodium salt of benzyl alcohol proceeded smoothly affording benzyl ester **10** in 95% yield,[13] which then underwent esterification to give diester **11** in 75% yield.[14] Hydrogenation of **11** generated acid **12** quantitatively, which was converted into the primary amine **9** by a one-pot Curtius rearrangement and decarboxylation.[15]

N-alkylation of **9** with tosylate **13** generated secondary amine **14** in 75% yield.[16] Amine **14** was then subjected to reductive amination with benzyloxyacetaldehyde to afford amino ester **15** in 85% yield.[17] Cleavage of the benzyl ether was then effected by hydrogenation of amino ester **15** in the presence of 1.0 equiv of CH_3SO_3H to prevent the amino moiety in **15** from poisoning the Pd catalyst afforded primary hydroxy amino ester **16** quantitatively.[15] Conversion of **16** into the chloride **17** then proceeded smoothly in 80% yield upon treatment of **16** with TsCl in pyridine(Scheme 2).[18] At first glance, direct formation of the chloride **17** might seem surprising. However, this conversion is presumed to occur via spontaneous formation of the reactive intermediate aziridinium ion **7** via anchimeric assistance of departure of the tosylate by nitrogen. Ensuing collapse of the aziridinium ion pair to the more stable chloride then occurs upon exchange of the tosylate counterion with chloride (Scheme 2).

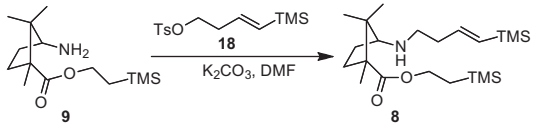
Independently, the carrier for the diagnostic or therapeutic radioisotope (potentially ^{131}I or others) was installed in the form of a vinyl silane. Monoalkylation of primary amine **9** with (*E*)-TMS-vinyl tosylate **18**[19] afforded the desired secondary amine **8** in 75% yield (Scheme 3).[16]



Scheme 1. Synthesis of amine **9**.



Scheme 2. Synthesis of chloride **17**.



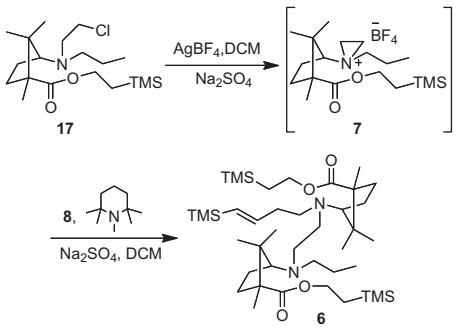
Scheme 3. Synthesis of amine **8**.

Somewhat surprisingly, given the ease of formation of the aziridinium ion from the tosylate above, attempts to couple chloride **17** and amine **8** at reflux resulted in recovery of **17** and **8** with no dimer formation. This suggested that chloride **17** was too poor a leaving group to permit generation an adequate concentration of the required aziridinium salt intermediate thermally.

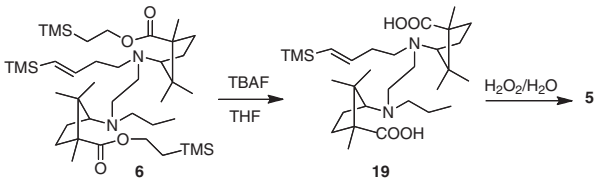
Fortunately, we were able to obtain the highly reactive aziridinium salt intermediate **7** (Scheme 2) by activation of chloride **17** with $AgBF_4$ driven by formation of insoluble AgCl.[20] Owing to its high reactivity and moisture sensitivity, aziridinium salt **7** was prepared and used immediately in the subsequent coupling chemistry (Scheme 4).

Treatment of chloride **17** with $AgBF_4$ in CH_2Cl_2 at rt in the presence of Na_2SO_4 to scavenge any moisture, removal of the precipitated AgCl and concentration afforded aziridinium tetrafluoroborate **7** as a brown oil which was used immediately. Successive addition at rt of anhyd Na_2SO_4 and 1,2,2,6,6-pentamethylpiperidine, a hindered base, and amine **8** to a solution of **7** in CH_2Cl_2 and stirring overnight at rt afforded dimer **6** in 65% yield.[18]

With the desired dimer **6** in hand, we then examined the final deprotection and oxidation sequence. Since bis-N-oxides can be difficult to manipulate owing to ready to Cope elimination, we decided to attempt the desilylation first and construct the bis-N-oxide group during the final transformation.[21] Further the chromatographic properties of the target suggested that we had to find methods which would effect the desired transformations with minimal need for further purification. To this end, diacid **19** was obtained smoothly from dimer **6** by exposure to TBAF in THF in 75% yield (Scheme 5).[22]



Scheme 4. Synthesis of dimer **6**.



Scheme 5. Synthesis of bis N-oxides **5**.

Literature precedent suggested that N-oxides can be formed with *m*CPBA/K_2CO_3 at −78 °C under conditions where epoxidation of the vinyl silane double bond was expected to be slow.[23] To our disappointment, **19** was either recovered unchanged at −78 °C or resulted in formation of an uncharacterizable mixture of products at −50 °C. Remarkably diacid **19** also proved inert to oxidation by H_2O_2/CH_3OH.[24] However, we were pleased to find that treatment with 50% H_2O_2/H_2O finally afforded our target molecule **5** as a mixture of diastereomers.[25]

Unfortunately, upon biological evaluation, the modification of the pK_as provided by the second acid/N-oxide moiety proved insufficient to render bis N-oxide **5** a viable diagnostic or treatment. Nevertheless, the dimer **5** played a valuable role in helping to move forward on the long quest toward possible specific and effective acid-targeted cancer diagnostics and therapeutics by permitting recognition of the importance of other cellular mechanisms such as competing efflux which must be overcome to permit sufficient sequestration and retention in the tumor cells. The delicacy with which the molecular properties must be manipulated continues to delineate the synthesis and design challenges facing the development of such high-specificity acid-targeted agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.01.028.

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