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A Caged Hydrophobic Inhibitor of Carbonic Anhydrase II

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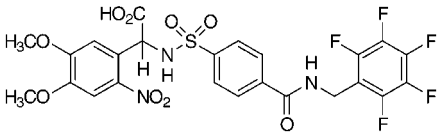
ABSTRACT



A tight-binding, hydrophobic inhibitor of carbonic anhydrase II has been masked with a water-solubilizing, photolabile group derived from *o*-nitrophenylglycine. This caged inhibitor represents our first effort at the site-specific delivery of prodrugs that can be activated by light. Via this approach, we have begun to address the problems of water insolubility and systemic side effects on administration of tight-binding inhibitors of carbonic anhydrase.

The design of effective, useful pharmaceuticals requires consideration of both the means by which a drug will be delivered to its physiological target and the interactions that will enable it to bind tightly to that receptor. The aqueous medium in which many drug targets are found poses a particularly challenging problem. To be delivered directly to a receptor in water, a drug should be water-soluble. Molecular recognition in water, however, is enhanced by hydrophobic contacts between greasy, non-water-soluble inhibitors and nonpolar surfaces of proteins.[1,2] We have modified a hydrophobic inhibitor of carbonic anhydrase II (CA) with a polar caging group, which will (1) increase the water solubility of this drug and (2) be removed by exposure to light of an appropriate wavelength to reveal the active, tight-binding hydrophobic inhibitor in situ. By this approach, we intend to achieve site-specific delivery and activation of a carbonic anhydrase inhibitor (CAI), without having to sacrifice the tight binding that is afforded by hydrophobic drugs.

CA is the target of several successful drugs developed by Merck for the treatment of high intraocular pressure associated with glaucoma.[3,4] The preferred mode of delivery of glaucoma medications is as aqueous drops, but the best drug candidates are water-insoluble.[4] These drugs all contain a primary sulfonamide group, which is essential for binding to CA.[5] By protecting this functional group with a water-soluble, photolabile moiety, one solves the problem of aqueous delivery of a tight-binding, hydrophobic drug *and* addresses the issue of site-specific activation. The caging group that we have prepared should be applicable in any system where a significant component of molecular recognition involves hydrophobic contacts.[1] *o*-Nitrophenylglycine (NPG) derivatives are an ideal choice for this photolabile group, since their photochemistry has been systematically characterized[6] and because these linkers have found applications in several biochemical systems.[6−10] We also find NPG attractive because this group bears a carboxylate moiety,

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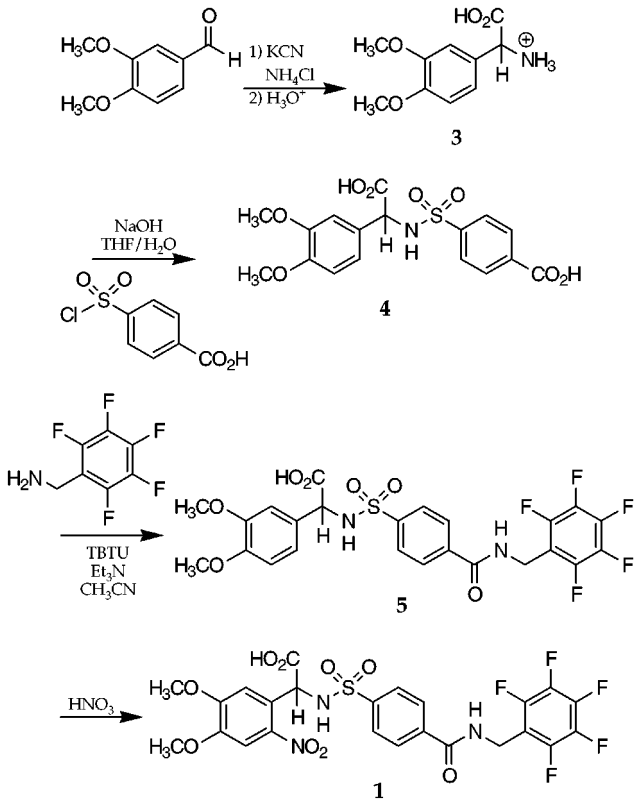
which should significantly increase the water solubility of our caged hydrophobic inhibitors at physiological pH. We chose **1** as the masked inhibitor since, on photolysis, it revealed tightest binding compound from a family of hydrophobic molecules.[11]

Using standard organic transformations, we have completed the synthesis of **1**. This specific analogue was chosen on the basis of calculations using MOPAC/ZINDO employed in CAChe,[12] which predicted that 4,5-dimethoxy-2-nitrophenylglycine should have an absorptivity of about 10^4 M^{-1} cm^{-1} at 360 nm. Holmes has shown that substitution of nitrobenzenes with electron-donating groups increases their rate of photocleavage[5] —for compounds similar to our mask, he measured a $t_{1/2}$ for photocleavage, on irradiation with a source of intensity 10 mW/cm^2, of 2 min. The synthetic plan that we have used for **1** is shown below.



3,4-Dimethoxybenzaldehyde was converted to its cyanoamine by treatment with NaCN and NH_4Cl in concentrated aqueous ammonia.[13] The cyanoamine was hydrolyzed without purification to 3,4-dimethoxyphenylglycine hydrochloride.[14] Our original plan involved nitration at this point, but the product 3,4-dimethoxy-6-nitrophenylglycine decomposed following purification via SGC.[15] Instead, we coupled the amino acid to 4-chlorosulfonylbenzoic acid, via alternate addition of the sulfonyl chloride in THF and 1 N NaOH, to a basic solution of the phenylglycine derivative.[16] The crude yellow precipitate on acidification of the reaction was collected and purified via SGC. Pentafluorobenzylamine[11] was coupled to the less sterically hindered carboxylic acid via treatment of a CH_3CN suspension of the sulfonamide and amine with TBTU and TEA (2 equiv).[17] The crude amide was purified via SGC and nitrated by treatment with HNO_3. The PLM-masked CAI was purified via SGC. All compounds were characterized by ^1H[18] and ^{13}C NMR, using a Bruker DRX-400 spectrometer. Caged inhibitor **1** was also characterized by high-resolution positive ion electrospray mass spectrometry.[19] Yields have not been optimized.

We have determined the kinetics of photolysis of **1** by 366 nm light.[15] We also plan to follow deprotection indirectly, by measuring binding to CA of the deprotected inhibitor via fluorimetry. In the presence of CA saturated with dansylamide (DNSA), a CAI that is fluorescent when bound to the protein,[20] photolysis of **1** will result in a time-dependent decrease in fluorescence as the exposed CAI binds, displacing DNSA.

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Supporting Information Available: ^1H and ^{13}C NMR and mass spectra and detailed descriptions of experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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