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# Design and Synthesis of a Cocaine-Diamide Hapten for Vaccine Development

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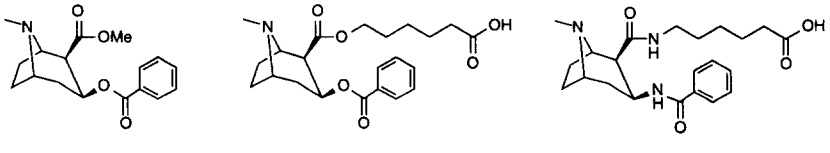
***Abstract***: A cocaine-diamide hapten was designed in an effort to obtain a potent, long-lasting anti-cocaine immune response for the treatment of cocaine abuse. The analogue incorporated an amido linker functionality in place of the carbomethoxy group at C-2 and a benzoylamino replacement of the benzoyloxy group at C-3 of the cocaine framework. Compound **3** was synthesized in 6 chemical steps starting from (+)-2-carbomethoxy-3-tropinone. Copyright © 1996 Elsevier Science Ltd

The abuse of cocaine **1** continues to be prevalent. Recent surveys for the United States indicated that more than 23 million people have tried cocaine, nearly 400,000 use it daily and that 5,000 new users are added each day.[1] The powerful psychotropic effect is strongly reinforcing and usually leads to repeated use of the drug.[2, 3] Studies have established that blockage of the reuptake of dopamine is the most critical factor in mediating the effects of cocaine most likely through inhibition of the dopamine transporter.[4-6]

Despite intensive efforts, there are no proven medications for cocaine craving and addiction.[7] Immunopharmacotherapy offers an alternative for addressing the cocaine problem. Early work demonstrated that antibodies specific for certain drugs were useful in the attenuation of their effects.[8] It had also been shown that catalytic antibodies could degrade cocaine *in vitro*, but rates must be improved to be of practical value.[9] Perhaps the ultimate goal in an immunological approach aimed at the abatement of cocaine abuse is the *de novo* design of a vaccine. Recently, we took steps in this direction and brought together immunochemistry and a well-defined behavioral model and demonstrated the suppression of the psychoactive effects of cocaine.[10]




**1** **2** **3**

**Fig. 1**

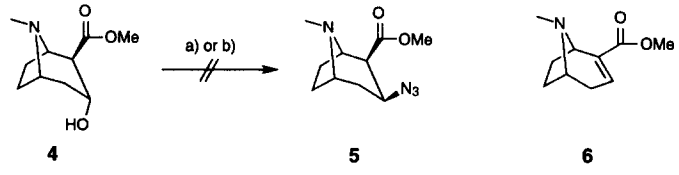
The design and preparation of a cocaine immunogen requires special regard for the stability of free cocaine in solution and particularly as a haptenic determinant to be of therapeutic value.[10] Our initial work employed the hapten **2** that furnished both polyclonal and monoclonal antibodies highly specific for cocaine rather than cocaine

metabolites. We now propose that the diamide hapten **3** (Fig. 1) could lead to a more effective "second generation" vaccine. Our rationalization is founded on 1) the hydrogen-bonding interactions induced by the amide functionalities of **3** at the antibody combining site then available for additional favorable binding to cocaine, and 2) the enhanced stability of immunoconjugates of **3** whereby there would be both a greater sustained antigen concentration together with the absence of trace competitive antigens during immunization.

A number of cocaine analogues have been synthesized as potential cocaine antagonists.[6] However, cocaine analogues bearing nitrogen substituents directly attached to the tropane nucleus at C-3 have not been reported. This might be indicative of difficulties encountered not only in forming the C-N bond, but also in achieving the correct stereochemistries at C-2 and C-3 as well as isolation of the appropriate isomer. We explored several entries into the new cocaine framework embodied in **3** and found one approach to be successful.

First, substitution of the hydroxyl group at C-3 of alloecgonine methyl ester[11] **4** by $S_N2$ displacement with azide ion was investigated. This reaction would establish the required equatorial (*S*)-configuration of the nitrogen atom. Activation using trifluoromethanesulfonic anhydride in the presence of pyridine in $CH_2Cl_2$ at 0 °C followed by addition of either $NaN_3$, $NaN_3$-15-Crown-5,[12] or tetra-*n*-butylammonium azide (*n*-$Bu_4NN_3$) did not afford the desired azido derivative **5**, but rather the elimination product **6**, anhydroecgonine methyl ester[13] (Scheme 1). The Mitsunobu reaction with diethyl azodicarboxylate (DEAD), triphenylphosphine and diphenylphosphoryl azide (DPPA)[14] gave similar results. Since the orientation between the hydrogen at C-2 and the hydroxyl group at C-3 in **4** is not diaxial, it seemed evident that rapid epimerization at C-2 occurred even under the weakly basic conditions. This was surprising despite the known lability of the C-2 axial stereochemistry via proton abstraction.[15]

**Scheme 1**

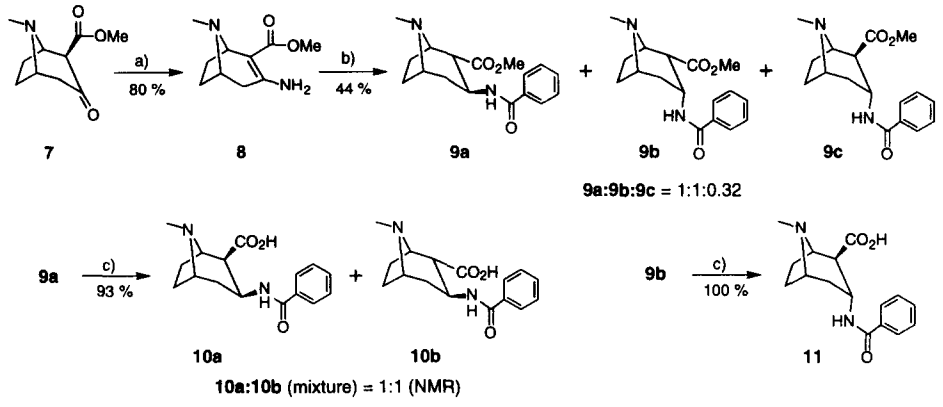



**4** **5** **6**

*Reagents and Conditions*: a) (i) $Tf_2O$, Pyr, $CH_2Cl_2$; (ii) $NaN_3$, or $NaN_3$-15-Crown-5, or *n*-$Bu_4NN_3$, $CH_2Cl_2$ or DMF. or b) DEAD, $PPh_3$, DPPA, THF.

Next, introduction of the requisite stereocenter at C-3 through addition of a hydrogen atom to a trigonal carbon was examined. To this end, (1*R*)-(+)-2-carbomethoxy-3-tropinone[16] **7** and ammonium acetate in refluxing benzene and acetic acid[17] gave enamine **8** in good yield (Scheme 2). While several hydrogenation protocols for the reduction of **8** were completely unsuccessful, sodium cyanoborohydride[18] at pH 4 proved adequate. The highly polar amine intermediates were benzoylated *in situ* and afforded a mixture of diastereomers **9a**, **9b** and **9c**, and unreacted **8**. Even though this transformation was not stereoselective, the three isomers could be separated. The configuration of these compounds was determined using $^1H$ NMR by comparison with the reported spectra for the four C-2, C-3 stereoisomers of cocaine.[11, 16b] The additional amide hydrogens of **9a**, **9b** and **9c** at C-3 complicated the spectra in $CDCl_3$, a problem alleviated upon changing the solvent to $CD_3OD$. The coupling constants between H-2 and H-3, or H-3 and the two H-4 protons are characteristic. For example, in compound **9a**, the coupling pattern of H-3 is a doublet of triplets ($J$ = 6.2, 11.4 Hz) indicative of one axial-equatorial and two axial-axial interactions.

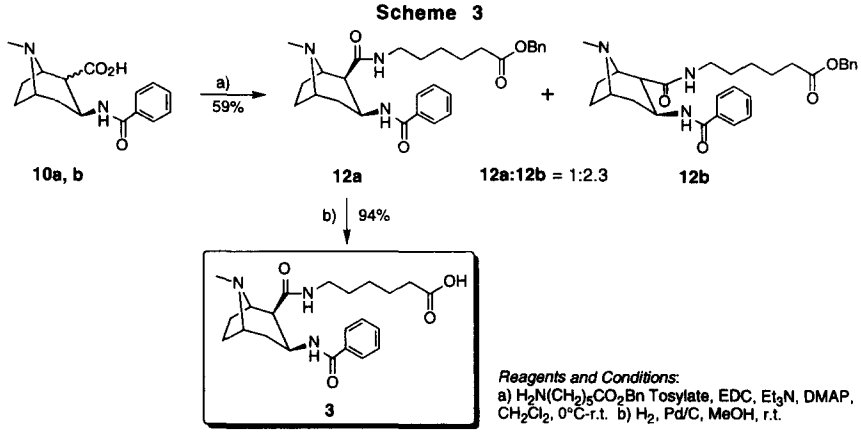
**Scheme 2**




*Reagents and Conditions*: a) $NH_4OAc$, AcOH, benzene, reflux. b) (i) $NaBH_3CN$, pH 4, MeOH, r.t.; (ii) benzoyl chloride, $NaHCO_3$, dioxane-$H_2O$, r.t. c) $H_2O$, reflux.

With the desired C-3 isomer **9a** in hand, it was anticipated that epimerization at C-2 would provide an intermediate compound of the correct configuration at all stereocenters. In cocaine chemistry, it is possible to convert allopseudoecgonine methyl ester to alloecgonine whereby ester hydrolysis accompanies epimerization.[11,16b] Similarly, the allopseudo-analogue **9b** was cleanly converted to **11**. However, the pseudo-isomer **9a** gave a 1:1 equilibrium mixture of the product acid **10a** and unepimerized acid **10b**. This suggests that the stable conformer of tropane-2-carboxylic acid is dependent on C-3 configuration rather than C-3 substituents. The mixture of acids could not be satisfactorily separated at this stage.

**Scheme 3**




*Reagents and Conditions*:
a) $H_2N(CH_2)_5CO_2Bn$ Tosylate, EDC, $Et_3N$, DMAP, $CH_2Cl_2$, 0°C-r.t. b) $H_2$, Pd/C, MeOH, r.t.

Consequently, **10a** and **10b** were coupled with an aminocaproate linker to incorporate the second amide replacement into the cocaine framework (Scheme 3). The diamides **12a** and **12b** could then be cleanly separated using preparative TLC. Finally, **12a** was hydrogenated in the presence of palladium on carbon to afford the diamide hapten **3**.[19]

The hapten formed an immunoconjugate with keyhole limpet hemocyanin (KLH) that was used to immunize mice for the subsequent production of monoclonal antibodies. Preliminary results support the

hypothesis that **3** can elicit a potent anti-cocaine immune response more effective than that of **2**. This tenet will be explored in rat behavioral paradigms in an effort to develop new vaccines for the treatment of cocaine abuse.

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19. 500MHz $^1$H-NMR($CD_3OD$) of **3**: δ 1.20-1.58 (6H, m), 2.03-2.27 (5H, m), 2.38-2.67 (m, 3H), 2.80 (3H, s), 3.13 (1H, dd, $J$ = 3, 6 Hz), 3.15-3.33 (2H, m), 3.92-3.97 (1H, m), 4.05-4.10 (1H, m), 4.60 (1H, dt, $J$ = 6, 13 Hz), 7.48-7.61 (3H, m), 7.81-7.90 (2H, m). The enantiomeric excess (ee) for **3** was estimated to be 93% based on the literature determination[16d] of 99% ee for **(1*R*)-(+)-7** that showed $[\alpha]_D$ = +18.6°. In this regard, we also provide a possible clarification for discrepancies of reported values[16d] of the optical rotations of **(1*R*)-7** and **(1*S*)-7**. In our hands, a value of +26.6° was found for the *R* isomer that could only be obtained for a solution observed within 4 minutes of preparation. We found that aged solutions in methanol had lesser rotations (absolute value) due to epimerization at C-2. For **(1*R*)-7**, an equilibrium value of +17.4° was attained after 30 minutes. Hence, we compared this value to that for material having 99% ee.

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