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Synthesis, Properties, and Reactivity of Cocaine Benzoylthio Ester Possessing the Cocaine Absolute Configuration

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Abstract: One aspect of immunopharmacotherapy for cocaine abuse involves the use of a catalytic monoclonal antibody (mAb) to degrade cocaine via hydrolysis of the benzoate ester. A cocaine benzoylthio ester analogue provides a means to implement high-throughput selection strategies to potentially isolate mAbs with high activity. The required analogue was synthesized starting from (−)-cocaine hydrochloride and possessed the cocaine absolute configuration. Key points in the preparation were the introduction of the sulfur atom at C-3 via a bromomagnesium thiolate addition to the exo face of anhydroecgonine, separation of C-2 diastereomers, recycling of a C-2 thio ester byproduct, and formation of the necessary C-2 methyl and C-3 benzoylthio esters. Effects resulting from the lower electronegativity and greater hydrophobicity of sulfur compared to oxygen were observed. These characteristics could result in interesting drug properties. Furthermore, the analogue was found to be a substrate for catalytic mAbs that hydrolyze cocaine as monitored by HPLC and also spectrophotometry by coupling cleavage of the benzoylthio ester to the disulfide exchange with Ellman's reagent. Screening antibody libraries with the new cocaine analogue using the spectroscopic assay provides an avenue for the high-throughput identification of catalysts that efficiently breakdown cocaine.

Cocaine abuse continues to be prevalent. Recent surveys for the United States indicate that more than 23 million people have tried cocaine and approximately 2.5 million are considered chronic abusers.[1] Numerous medical problems, including cardiovascular toxicity, brain damage, and death, often accompany cocaine use, and the association of the drug with the spread of AIDS is of concern.[2] Despite intensive efforts, the development of effective therapies for cocaine craving and addiction remain elusive. New strategies are necessary to increase the effectiveness of rehabilitative programs and for the treatment of overdose cases. We, and others, have shown that one aspect of immunopharmacotherapy, the antibody-mediated binding of cocaine to impede passage of the drug into the central nervous system, resulted in a suppression of its characteristic actions.[3,4] Administration of a monoclonal antibody (mAb) endowed with not only binding but also catalytic activity to metabolize cocaine would have enhanced therapeutic effects if the kinetic properties of the mAb were sufficient.

Both spontaneous[5] and esterase-catalyzed[5c,6] hydrolysis of cocaine contribute to the short in vivo half-life of ∼30 min in human blood. Yet, for enzyme or catalytic mAb therapy to be effective, extensive clearance of cocaine must take place within seconds. The injection of purified human plasma cholinesterase reduced cocaine toxicity in mice,[7] but the efficiency would not be suitable to achieve cocaine clearance in the human condition at clinically manageable concentrations of enzyme. Both our laboratory and that of Landry et al. used a transition-state (TS) analogue approach for hapten design and reported the direct isolation of several cocaine-hydrolyzing mAbs.[8,9] In this model, the benzoyl ester at C-3 of the cocaine framework is replaced by a phenylphosphonate that approximates the TS for ester

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hydrolysis. However, similar to the enzymatic esterases, none of the mAbs studied thus far are endowed with the catalytic power required to meet the demands of hydrolyzing cocaine rapidly enough to alter its pharmacokinetic profile and psychoactive effects in humans.

In an effort to isolate more efficient mAbs that degrade cocaine, Cashman and co-workers initiated a novel program for high-throughput screening of hybridoma cultures obtained from immunizations with a TS analogue.[10] The key component in their approach is a benzoylthio ester analogue of cocaine that liberates a thiol-containing product upon hydrolysis, which then reacts with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) to produce a chromophoric analyte.[10,11] This method allows for the rapid and continuous spectrophotometric assay of many reactions without the need for extraction or derivatization. Although the assay itself proved viable, the mAbs selected from large panels of hybridomas were not significantly better catalysts when tested with cocaine than those obtained directly using TS analogues and standard protocols.[8,9] The problem likely resides in the fact that the benzoylthio ester substrate utilized by Cashman does not correspond to the natural isomer of cocaine, but rather to allococaine, which has the opposite configuration of the benzoyl ester at the C-3 position. With the aim of developing a high-throughput selection of efficient cocaine catalytic mAbs from cell cultures and from phage-display libraries, we herein report the synthesis and properties of cocaine benzoylthio ester with the correct stereochemistry at C-3 and in optically pure form, as well as its reactivity with respect to interaction with catalytic mAbs.

Results and Discussion

In recent years, a vast number of cocaine analogues bearing a substituted benzoyl ring, another modified aryl substituent, or an alkyl substitutent at C-3 were prepared for structure−activity studies.[12] However, heteroatoms, other than oxygen, directly attached to the tropane nucleus at C-3 were unknown until our laboratory introduced a nitrogen atom in the preparation of a cocaine diamide hapten **1** (Figure 1).[13] This unique example reflected the difficulties 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 2β,3β isomer. For the installation of a sulfur-bonded functionality, comparable problems might be expected. A concise strategy was used by Carroll et al. in synthesizing 3-(phenylthio) analogues **3** by nucleophilic addition/elimination of sodium thiophenoxide to the enol triflate **2**, followed by samarium iodide reduction, which resulted in the 2β,3β, 2α,3β and 2β,3α isomers.[14] Cashman subsequently employed an amine-catalyzed addition of 4-methoxy-α-toluenethiol to anhydroecgonine methyl ester **4** which gave an endo mode of attack and a mixture of 2β,3α and 2α,3α isomers.[11] The isomers were separable, and the free thiol was unmasked and benzoylated to yield the allo (2β,3α) and allopseudo (2α,3α) thio ester analogues **5**.

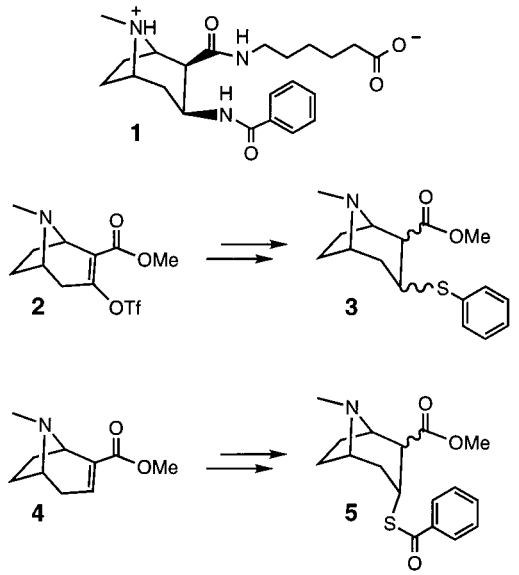

Figure 1. Previously reported cocaine analogues containing a nitrogen or sulfur heteroatom at C-3.

To obtain the cocaine benzoylthio ester corresponding to the natural 2β,3β configuration, we explored several strategies and found one to be successful. The methodology of Carroll was first examined by using the sodium salts of 4-methoxy-α-toluenethiol or thiobenzoic acid for the conjugate addition, as noted above, to **2** (Scheme 1). Both reactions proceeded favorably to give **7** and **8** containing sulfur at an unsaturated C-3 position. We were particularly satisfied with the result in obtaining **7**, since this compound already contained the desired thio ester group. However, in neither case was the SmI_2 reduction successful. The alkylthio enolether functionality in **8** apparently lacked sufficient reactivity, and the acylated counterpart in **7**, on the other hand, was too labile with the evidence suggesting predominant C−S bond cleavage. In an attempt to make further use of **8**, the 4-methoxybenzyl group was cleaved with trifluoroacetic acid, affording the thio analogue **9** of 2-(carbomethoxy)-3-tropinone **6**. It has long been known that **6** could be reduced with sodium amalgam under acidic, equilibrating conditions to provide ~28% of ecgonine methyl ester (2β,3β stereochemistry), together with a similar amount of the 2α,3β pseudoconfigured isomer.[15] The allo isomers, which

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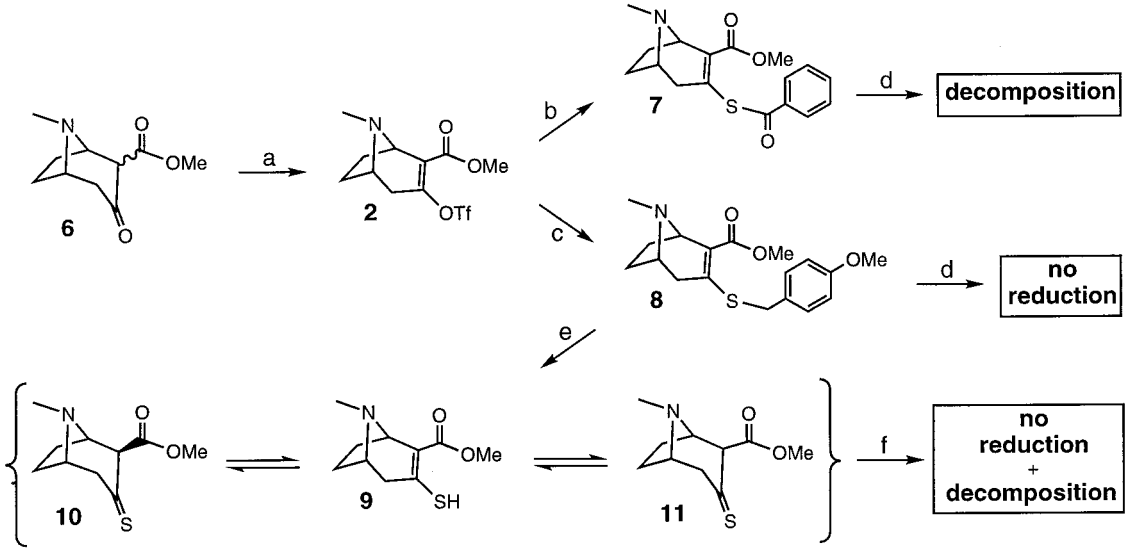
Scheme 1. Unsuccessful Strategies toward the Preparation of Cocaine Benzoylthio Ester[a]



[a] Conditions: (a) sodium bis(trimethylsilylamide), *N*-phenyltrifluoromethanesulfonamide, THF (90% yield); (b) NaH, PhCOSH, DMF (26% yield); (c) sodium bis(trimethylsilylamide), *p*-MeOPhCH$_2$SH (89% yield); (d) SmI$_2$, MeOH, THF; (e) TFA, reflux (95% yield); (f) Na(Hg), H$_2$SO$_4$, pH 3.4, 0 °C.

have the axially disposed C-3 hydroxyl, are not formed. Unfortunately, reduction of **9**, existing as a mixture of tautomers as in the case of **6**, yielded only starting material and an unidentifiable byproduct. A number of other reducing agents were also found to be unsuccessful in reactions with **8** and **9**, resulting in either no reaction, decomposition, the incorrect stereochemistry at C-3, or some combination of these outcomes.

From our earlier work, we were aware that attempted substitution of an activated hydroxyl group at C-3 of alloecgonine methyl ester by S_N2 displacement would result only in elimination to **4** promoted by rapid epimerization at C-2.[13,16] At this point, our only recourse was a thorough reevaluation of what was known in the literature concerning the chemistry in forming tropane derivatives. It occurred to us that although Cashman[11] observed only endo attack of an amine thiolate on **4**, previous investigations utilizing various aryl Grignard reagents afforded exclusively exo addition and a mixture of 2β,3β and 2α,3β isomers.[12h,l] In a related context, tertiary amines were reported to have a favorable effect on the rates of some Grignard reactions and so perhaps interact with this type of organometallic compound.[17] Hence, we speculated that the bridgehead nitrogen atom of **4** might participate in chelation control or, to some degree in just complexation, and direct or bias the magnesium-containing Grignard species to the exo face (Figure 2). Even if the interaction were only weak, it could be enough to overcome the presumably greater steric barrier imposed by the N−Me bridge compared to the endo ethylene bridge, as was apparently the case with the aryl Grignard additions.

With this hypothesis in hand, **4** was prepared[18] from (−)-cocaine hydrochloride (**12**) and reacted with the bromomagnesium salt of 4-methoxy-α-toluenethiol (Scheme 2). A mixture of three compounds was obtained and, to our delight, evidence

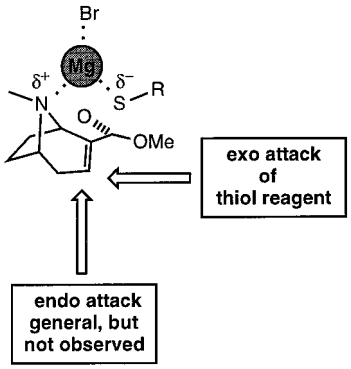


Figure 2. Possible rationale for exo selectivity in the conjugate addition of magnesium-containing nucleophiles to anhydroecgonine methyl ester.

of the 2β,3β isomer **13**. Our observation was that the first equivalent of thiolate underwent a rapid transesterification to give **15** but that the 1,4-conjugate addition of the second equivalent was rather sluggish. Although the yield of **13** on moderate scales (10 mmol) was only ~10%, when conducted on a 1-mmol scale ~30% was produced. Significantly, none of the allo isomers were detected. Furthermore, **13**, **14**, and **15** were readily separated by silica gel chromatography and the derivative **15** could be recycled after each reaction by addition of the starting thiolate reagent to eventually obtain a ~60:40 ratio of pure, isolated **13** and **14** in ~50% overall yield. Attempted conversion of these compounds to **16** and **18**, respectively, to provide the required C-2 methyl ester initially proved problematic. The use of sodium methoxide/methanol for ester exchange resulted only in epimerization at C-2 for **13** and no reaction in the case of **14**. Subsequently, we found that a one-pot procedure of aqueous Na_2CO_3/methanol followed by diazomethane effected a smooth transformation. However, it was necessary to conduct the hydrolysis reaction of **13** at room temperature to avoid epimerization, but for **14**, reflux was required to cleave the thio ester and no epimerization occurred. Finally, the 4-methoxybenzyl group of **16** and **18** was cleaved with TFA and the free thiol immediately treated with benzoyl chloride to afford, after purification, the free-base form of (−)-

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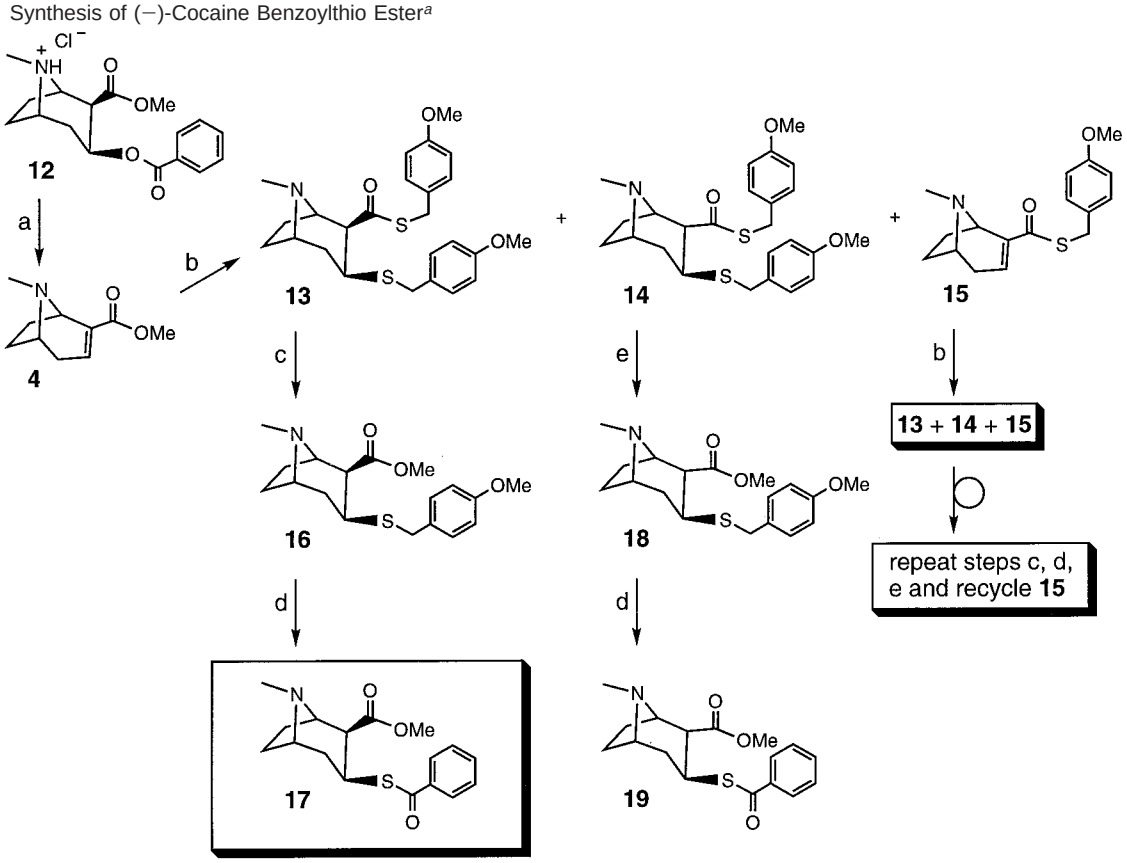
Scheme 2. Synthesis of (−)-Cocaine Benzoylthio Ester[a]



[a](a) Conditions: concentrated HCl, reflux, 20 h; (b) *p*-MeOPhCH$_2$SMgBr (prepared from *p*-MeOPhCH$_2$SH, EtMgBr, THF); (c) (i) 5% Na$_2$CO$_3$, MeOH, room temperature, 72 h; (ii) CH$_2$N$_2$, ether, MeOH; (d) (i) TFA, reflux, 3 h; (ii) benzoyl chloride, NEt$_3$, THF; (e) (i) 5% Na$_2$CO$_3$, MeOH, reflux, 72 h; (ii) CH$_2$N$_2$, ether, MeOH.

cocaine benzoylthio ester **17** corresponding to natural (−)-cocaine, as well as the 2α,3β-isomer **19** analogous to pseudococaine (Scheme 2). Surprisingly, contrary to our previous study of the pseudococaine benzamide analogue,[13] refluxing **19** in water did not result in any epimerization. Notably, the isomer **17** was also stable in this respect. Hence, the C-2 proton in pseudococaine benzoylthio ester **19** is less labile apparently due to the lower electronegativity of sulfur and electron-withdrawing capacity of the benzoylthio group.

Spectral and physical analyses corroborated the structure of **17** and revealed further influences of the sulfur substituent. The coupling constants between H-2 and H-3, and between H-3 and the two H-4 protons, are characteristic for each of the four cocaine structural isomers.[19] In particular, the H-3 coupling pattern is diagnostic and for natural cocaine shows a sharp five-line signal arising from doublet−doublet−doublet couplings (J = 12.0, 6.0, 6.0 Hz) indicative of one axial−axial (H-3−H-4$_{ax}$) and two axial−equatorial (H-2−H-3, H-3−H-4$_{eq}$) interactions. For cocaine benzoylthio ester, the central overlap is slightly spaced due to a small change in coupling values (J = 12.6, 5.6, 5.6 Hz) that suggest an increased puckering in the tropane ring system which brings H-3 and H-4$_{ax}$ somewhat closer to the Karplus optimum 180° dihedral angle. As expected from the lower electronegativity of sulfur compared to oxygen, there is also a marked upfield chemical shift of H-3 from 5.3 ppm in cocaine to 4.16 ppm in the thio analogue. The effect is also dramatically evident from ^{13}C NMR and C−H COSY experiments in which C-3 in the thio compound is found at 34.2 ppm, as opposed to 66.8 ppm in cocaine. However, the lower electronegativity of sulfur influences the carbonyl group differently.[20] The reduced double-bond character of the benzoyl group in **17** and increased partial positive charge on the carbonyl carbon (lower electronic excitation energy of the carbonyl) causes a large downfield shift from 166.0 to 192.4 ppm. With regard to physicochemical properties, the presence of sulfur also imparts enhanced hydrophobicity to the analogue **17**. The log value of the ion-corrected octanol/PBS partition coefficient (P), determined by the shake-flask method,[21] was found to be log P = 3.47 for **17** compared to log P = 2.68 for cocaine. Consistent with experiment, a calculated log P for **17** using 2.68 plus the average difference in π-hydrophobicity constants for acylthio versus acyloxy groups ($\Delta\pi$ = 0.74) affords log P = 3.42.[22] Given the structural similarity between the two compounds, the data suggest that cocaine benzoylthio ester should be more effective in crossing the blood−brain barrier than cocaine itself,[23] and perhaps also in binding to the dopamine transporter, and, therefore, could have interesting agonist/antagonist properties.[24]

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Table 1. Kinetic Data for GNL Catalytic MAbs That Hydrolyze Cocaine and **17** as Substrates.[a,b]

parameter	GNL3A6	GNL23A6	GNL4D3
k_{cat} (min^{-1})	1.37×10^{-2}	4.91×10^{-3}	1.31×10^{-3}
	4.70×10^{-4}	5.00×10^{-4}	0.750
K_m (μM)	53	430	42
	35	157	81
k_{cat}/K_m (M^{-1} s^{-1})	4.35	0.183	0.517
	2.50×10^{-2}	5.33×10^{-2}	1.54×10^{2}
k_{cat}/k_{uncat}	1.36×10^{3}	4.86×10^{2}	1.30×10^{2}
	36	38	5.70×10^{4}

[a] Determined in 100 mM PB, pH 7.4, 5% DMSO cosolvent, 23 °C. Values in first row for each parameter correspond to reactions with cocaine and values in second row to reactions with **17**. [b] Spontaneous pseudo-first-order k (cocaine) = 1.01×10^{-5} min^{-1}; k (**17**) = 1.32×10^{-5} min^{-1}

Since cleavage of the benzoate ester of cocaine produces the nonpsychoactive metabolite ecgonine methyl ester,[25] the reaction is an excellent target for an immunopharmacological strategy. The outstanding features of **17** reside in its cocaine-like stereochemistry and the ability to use catalysis, rather than merely binding of a TS analogue, as a selectable trait from large libraries of antibodies. To provide support for the premise that catalytic mAbs derived from a selection process using **17** must then also match with cocaine as a substrate, the converse pairing was examined. Hence, three of our cocaine-hydrolyzing mAbs elicited using the TS analogue approach[8] were tested for activity with **17**, assaying the formation of benzoic acid by HPLC (Table 1). Although catalysis was not entirely unexpected, the proficiency of one pairing, GNL4D3 and **17**, was quite surprising. The mAb afforded a 57 000-fold initial rate enhancement over background with an apparent second-order rate constant $k_{cat}/K_m > 10^2$ M^{-1} s^{-1}. Operationally, 5 mol % of GNL4D3 completely degraded an offered sample of **17** in 40 min. The activity was far greater than that of both GNL3A6 and GNL23A6.

Spectrophotometric monitoring of the benzoylthio ester cleavage of **17**, when coupled to the disulfide exchange with Ellman's reagent,[26] was consistent with the differences in activities observed by HPLC. However, the GNL4D3-catalyzed reaction revealed "burst kinetics", whereas GNL3A6 and GNL23A6 showed only typical, linear initial rates (Figure 3, Scheme 3). In light of the unique behavior of GNL4D3, we initially surmised that the burst was indicative of the formation of an acyl−antibody intermediate, as previously observed for a catalytic mAb in our laboratory.[27] Indeed, the experiments demonstrate that there is an intial rapid, exponential phase to liberate 2 equiv of **20** (the mAb has two active sites), which subsequently react with DTNB and form the equivalent concentration of TNB, followed by a much slower steady-state phase turnover. Yet, closer inspection showed that the apparent steady-state rates were very similar and not proportional to the mAb concentration as required for progress curves invoking a burst-derived covalent complex. Also, curvature of this phase of the reaction continued with time and approached a shutdown

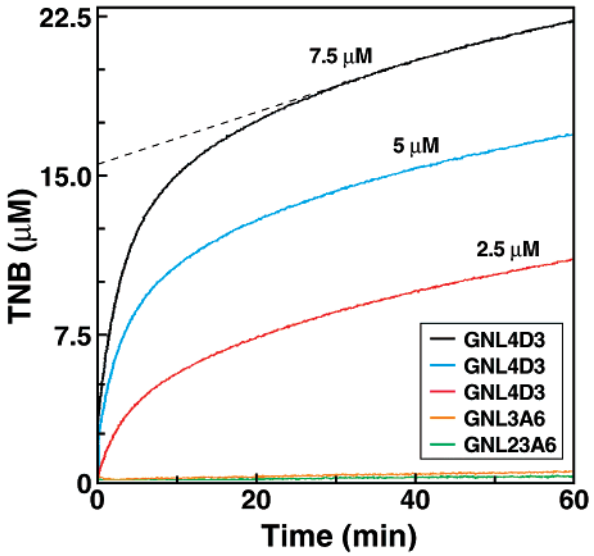

Figure 3. Visible spectrophotometry of the liberation of 2-nitro-5-thiobenzoic acid (TNB) during the mAb-catalyzed hydrolysis of **17** in the presence of Ellman's reagent.

of the velocity. Furthermore, a subsequent radiolabeling experiment of GNL4D3 using [7-^{14}C-*benzoyl*]-**17** did not provide evidence for the existence of a labeled mAb. No radioactivity remained with the antibody after dialysis at low pH designed to trap the benzoyl intermediate. The data suggested that an alternative phenomenon, namely, rapid onset of product inhibition, was responsible for the burst kinetics. In fact, for GNL4D3, single-turnover inhibition would be the cause of the effect. Since HPLC analysis showed complete hydrolysis, the degradation products of **17** were ruled out as inhibitors. On the other hand, HPLC analysis of the reaction used for spectrophotometry, which is GNL4D3 and **17** in the presence of DTNB, correlated with the spectrophotometric result showing the formation of only a stoichiometric amount of benzoic acid followed by a halt in reaction progress. Consequently, the disulfide interchange product **21** must act as a potent inhibitor of the reaction (Scheme 3).

Even though cocaine and **17** have similar spontaneous rates of hydrolysis (Table 1), the TS mechanism invoked by GNL3A6 and GNL23A6 is better at accommodating cocaine−benzoate cleavage by hydroxide. Apparently, hapten programming of the cocaine hydrolytic TS enforces stereoelectronic effects which are less congruent to the TS for hydrolysis of **17**. However, for GNL4D3, **17** is utilized better than cocaine and very efficiently, even though this mAb was similarly derived from selection using a TS analogue. This suggests that possible additional mechanisms, such as general-acid/base catalysis, are invoked at the mAb active site which favor **17** in the formation or breakdown of the tetrahedral intermediate.[28] In other studies, we observed that a catalytic mAb designed solely on the basis of TS stabilization also fortuitously combined more elaborate chemical mechanisms to provide large rate enhancements.[27] The use of **17** as a selection reagent to directly screen antibody libraries for catalysis will prove invaluable in identifying subsets of mAbs that operate via TS stabilization or other chemical mechanisms,

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(25) Misra, A. L.; Nayak, P. K.; Bloch, R.; Mule, S. J. *J. Pharm. Pharmacol.* **1975**, *27*, 784−786.
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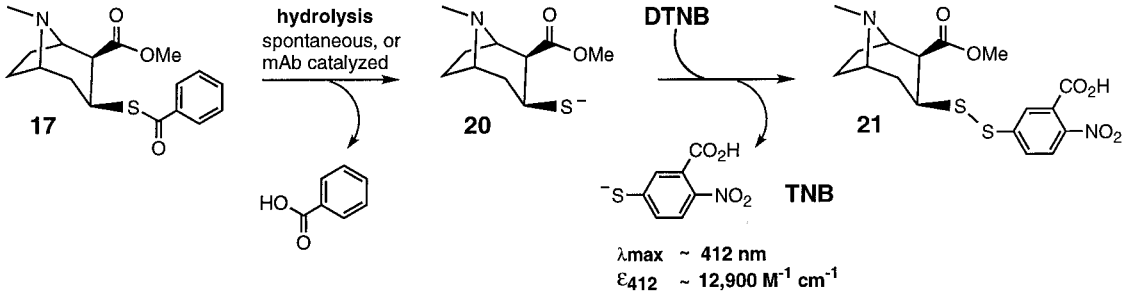
Scheme 3. Pathway for the Formation of the C-3 Thiolate Hydrolysis Product and Trapping Using Ellman's Reagent



including covalent catalysis, some of which should be highly active for cocaine hydrolysis. Spectrophotometry of large panels of hybridoma cell cultures and visual colorimetric assessment of phage-display libraries on agar plates will allow high-throughput analysis and selection of catalysts. Finally, antibody engineering using X-ray structure-based methods and mutagenesis techniques provides the means to modify critical amino acid residues with the aim of obtaining a value of $k_{cat}/K_m \sim 10^4\ M^{-1}\ s^{-1}$ necessary for therapy.[8]

A catalytic mAb that metabolizes cocaine with a sufficient rate would find use in human immunopharmacotherapy both for rehabilitative purposes and in paramedic or emergency room situations for drug overdose cases. In addition, cocaine benzoylthio ester might serve as a novel pharmacotherapeutic in itself by blocking the psychoactive effects of cocaine or as a nonaddictive cocaine surrogate. A drug that interacts at the dopamine transporter to prevent cocaine binding, yet does not effect dopamine reuptake, or has less reinforcing cocaine-like properties would be beneficial for the treatment of cocaine addiction. Studies in these directions are underway in our laboratory.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were measured at 400 MHz on a Brucker AMX-400 spectrometer. Chemical sifts (ppm) are reported relative to internal $CDCl_3$ (^{1}H, 7.26 ppm and ^{13}C, 77.0 ppm). HRMS spectra were measured using MALDI techniques. Infrared spectra were measured using neat samples on a Nicolet Avatar 360 FT-IR equipped with a Nicolet Smart Golden Gate (ZnSe) by the ATR technique. Glassware and solvents were dried by standard methods. Flash chromatography was performed on silica gel 60 (230−400 mesh) and thin-layer chromatography (TLC) on glass plates coated with a 0.25-mm layer of silica gel 60 F-254. The (−)-cocaine hydrochloride was supplied by the National Institute on Drug Abuse (NIDA) and other chemical reagents and solvents were from Aldrich Chem. Co. and used without further purification. Nomenclature is in accord with the Chemical Abstracts Registry Service (CAS).

Introduction of Sulfur onto the Cocaine Framework Using a Thiolate Reagent: 13, 14, and 15. A pear-shaped flask was charged with THF (11.3 mL) and 4-methoxytoluene-α-thiol (1.65 mL, 11.9 mmol) and then the Grignard reagent EtMgBr (12.5 mL, 1.0 M solution in THF, 12.5 mmol) was added at room temperature. After bubbling subsided, the resulting solution of bromomagnesium thiolate (0.5 M in THF) was added dropwise via cannula over a period of ∼15 min to a solution of **4** (1.79 g, 9.89 mmol) in THF (30 mL) at 0 °C with stirring, and then the mixture was allowed to stir at room temperature for 14 h. After this time, the mixture was cooled to −78 °C and TFA (1.16 mL, 15 mmol) was added. The mixture was allowed to warm to room temperature, poured into 1 M HCl, and extracted with Et_2O to remove unreacted thiol. The aqueous layer was brought to pH ∼9 with NH_4OH and extracted several times with CH_2Cl_2; the CH_2Cl_2 layers were washed with brine, dried over Na_2SO_4, and evaporated to give crude thioester **15**. To a solution of the crude reaction product in THF (30 mL) was added the bromomagnesium thiolate [prepared as described above using THF (14 mL), 4-methoxytoluene-α-thiol (2.07 mL, 14.8 mmol), and EtMgBr (15.6 mL, 1.0 M solution in THF, 15.6 mmol)] at 0 °C with stirring, and then the mixture was allowed to stir at room temperature for 40 h. After this time, the mixture was cooled to −78 °C and TFA (1.44 mL, 18.7 mmol) was added. The mixture was allowed to warm to room temperature, poured into 1 M HCl, and extracted with Et_2O to remove unreacted thiol. The aqueous layer was brought to pH ∼9 with NH_4OH and extracted several times with CH_2Cl_2; the CH_2Cl_2 layers were washed with brine, dried over Na_2SO_4, and evaporated. Analysis by TLC [$CHCl_3$/EtOAc/NH_4OH (50:50:1)] showed three major compounds of R_f = 0.85, 0.60, 0.40. The residue was purified by chromatography on silica gel, eluting with $CHCl_3$/EtOAc/NH_4OH (60:40:1), to afford, in order of elution, the desired compound **13** (334 mg, 7.40% yield), the pseudoisomer **14** (205 mg, 4.50% yield), and the thioester **15** (2.39 g, 79.8% yield) as pale yellow oils. The thioester was recycled four times until 1.25 g of **13** (27.6% based on **4**) and 0.940 g of **14** (20.7% based on **4**) were obtained.

(1*R*,2*R*,3*S*,5*S*)-3-[[(4-Methoxyphenyl)methyl]thio]-8-methyl-8-azabicyclo[3.2.1]octane-2-carbothioic acid *S*-[(4-methoxyphenyl)-methyl] ester 13: ^{1}H NMR ($CDCl_3$) δ 7.24−7.18 (m, 4H), 6.85−6.79 (m, 4H), 4.13 (d, J = 13.5 Hz, 1H), 4.12 (d, J = 13.5 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.66 (d, J = 13.5 Hz, 1H), 3.64 (d, J = 13.5 Hz, 1H), 3.40 (dd, J = 2.1, 7.1 Hz, 1H), 3.15 (m, 1H), 2.72−2.63 (m, 2H), 2.19 (ddd, J = 12.3, 2.6, 2.6 Hz, 1H), 2.12 (s, 3H), 2.05−1.94 (m, 2H), 1.55−1.50 (m, 1H), 1.40−1.29 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 196.8, 158.52, 158.49, 130.3, 129.90, 129.87, 129.0, 113.8, 113.7, 65.8, 62.3, 60.4, 55.2, 55.1, 41.6, 38.4, 35.9, 35.8, 32.5, 25.6, 25.0; HRMS (MALDI-FTMS) calcd for $C_{25}H_{32}NO_3S_2$ (MH^+) 458.1818, found 458.1809; $[\alpha]^{25}_D = -91.0°$ (c = 1.042, $CHCl_3$).

(1*R*,2*S*,3*S*,5*S*)-3-[[(4-Methoxyphenyl)methyl]thio]-8-methyl-8-azabicyclo[3.2.1]octane-2-carbothioic acid *S*-[(4-methoxyphenyl)-methyl] ester 14: ^{1}H NMR ($CDCl_3$) δ 7.23−7.17 (m, 4H), 6.84−6.79 (m, 4H), 4.14 (d, J = 13.8 Hz, 1H), 4.09 (d, J = 13.8 Hz, 1H), 3.79 (s, 3H), 3.76 (s, 3H), 3.66 (d, J = 12.6 Hz, 1H), 3.62 (d, J = 12.6 Hz, 1H), 3.28 (d, J = 6.5 Hz, 1H), 3.15−3.08 (m, 3H), 2.31 (s, 3H), 1.99−1.90 (m, 2H), 1.82−1.72 (m, 2H), 1.57−1.52 (m, 1H), 1.45−1.38 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 199.3, 158.7, 158.5, 130.2, 130.0, 129.9, 129.4, 114.0, 113.7, 64.5, 60.7, 59.3, 55.2 (2), 38.3, 37.1, 36.1, 35.1, 32.8, 26.5, 22.9; HRMS (MALDI-FTMS) calcd for $C_{25}H_{32}NO_3S_2$ (MH^+) 458.1818, found 458.1811.

8-Methyl-8-azabicyclo[3.2.1]oct-2-ene-2-carbothioic acid *S*-[(4-methoxyphenyl)methyl] ester 15: ^{1}H NMR ($CDCl_3$) δ 7.25−7.21 (m, 2H), 6.85−6.81 (m, 2H), 6.79 (t, J = 3.2 Hz, 1H), 4.11 (s, 2H), 3.84 (d, J = 5.3 Hz, 1H), 3.78 (s, 3H), 3.24 (t, J = 5.3 Hz, 1H), 2.62 (br d, J = 19.4 Hz, 1H), 2.33 (s, 3H), 2.17−2.14 (m, 2H), 1.86−1.80 (m, 2H), 1.52−1.48 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 190.9, 158.7, 141.6, 134.4, 130.0, 129.6, 113.9, 58.4, 57.0, 55.2, 36.0, 34.4, 32.2, 31.4, 30.1; HRMS (MALDI-FTMS) calcd for $C_{17}H_{22}NO_2S$ (MH^+) 304.1366, found 304.1362.

On small scales, the protocol used was as follows. A stock solution of the bromomagesium thiolate was prepared as described above using 4-methoxytoluene-α-thiol (1.46 mL, 10.5 mmol), THF (8.54 mL), and EtMgBr (10.0 mL, 1.0 M solution in THF, 10.0 mmol), but the addition was carried out at 0 °C and the mixture stirred at room temperature for 2 h. The bromomagesium thiolate (4.51 mL, 2.25 mmol) was added dropwise via syringe over several minutes to a solution of **4** (136 mg, 0.751 mmol) in THF (1.0 mL) at room temperature and the resultant mixture stirred for 75 h. After this time, the mixture was cooled to −78 °C and TFA (208 μL, 2.70 mmol) was added. The mixture was allowed to warm to room temperature, poured into 1 M HCl, and extracted with Et_2O to remove unreacted thiol. The aqueous layer was brought to pH ∼9 with NH_4OH and extracted several times with CH_2Cl_2; the CH_2Cl_2 layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel, eluting with $CHCl_3$/EtOAc/NH_4OH (60:40:1), to afford, in order of elution, the desired compound **13** (109 mg, 31.8% yield), the pseudoisomer **14** (11.1 mg, 3.2% yield), and the thioester **15** (87.0 mg, 38.2%) as pale yellow oils.

(1*R*,2*S*,3*S*,5*S*)-3-[[(4-Methoxyphenyl)methyl]thio]-8-methyl-8-azabicyclo[3.2.1]octane-2-carbothioic Acid Methyl Ester 16. A mixture of **13** (853 mg, 1.87 mmol), MeOH (20 mL), and 5% Na_2CO_3 (5.76 mL, 2.72 mmol) was stirred at room temperature for 24 h. Then, additional MeOH (5 mL) and 5% Na_2CO_3 (2.18 mL, 1.03 mmol) were added, and the mixture stirred for 48 h. After this time, the mixture was acidified to pH ∼3 with 1 M HCl and then treated with CH_2N_2. The excess CH_2N_2 was quenched with AcOH, and the mixture was evaporated until about half the volume remained and was poured into 1 M HCl and washed with Et_2O. The aqueous layer was brought to pH ∼9 with NH_4OH and extracted several times with CH_2Cl_2; the combined CH_2Cl_2 layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel, eluting with $CHCl_3$/EtOAc/NH_4OH (60:40:1), to afford **16** as a pale yellow oil (235 mg, 37.6% yield): ^{1}H NMR ($CDCl_3$) δ 7.24−7.20 (m, 2H), 6.86−6.82 (m, 2H), 3.80 (s, 3H), 3.70 (s, 3H), 3.64 (d, J = 13.5 Hz, 1H), 3.63 (d, J = 13.5 Hz, 1H), 3.42 (m, 1H), 3.10 (m, 1H), 2.62 (ddd, J = 12.6, 5.3, 5.3 Hz, 1H), 2.47 (t, J = 4.1 Hz, 1H), 2.19 (ddd, J = 12.6, 2.9, 2.9 Hz, 1H), 2.11 (s, 3H), 2.03−1.93 (m, 2H), 1.49−1.44 (m, 1H), 1.37−1.29 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 172.1, 158.5, 130.7, 129.9, 113.8, 65.4, 62.7, 55.2, 53.2, 51.4, 41.6, 38.3, 36.4, 35.7, 25.4, 25.0; HRMS (MALDI-FTMS) calcd for $C_{18}H_{26}NO_3S$ (MH^+) 336.1628, found 336.1627; $[\alpha]^{25}_D$ = −38.2° (c = 0.654, $CHCl_3$).

(1*R*,2*S*,3*S*,5*S*)-3-(Benzoylthio)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (Cocaine Benzoylthio Ester) 17. A solution of **16** (269 mg, 0.803 mmol) in TFA (8 mL) was refluxed and stirred for 3 h. After cooling, the mixture was coevaporated with toluene (three times). The residue was dissolved in THF (8 mL) and cooled to 0 °C, and Et_3N (447 μL, 3.21 mmol) and benzoyl chloride (237 μL, 2.01 mmol) were added with stirring. After stirring at room temperature for 2.5 h, MeOH was added to quench the excess benzoyl chloride and the mixture was evaporated. The residue was purified twice by chromatography on silica gel, each time eluting with $CHCl_3$/EtOAc/NH_4OH (60:40:1), to afford **17** as a white solid (154 mg, 60.1% yield): ^{1}H NMR ($CDCl_3$) δ 7.97−7.94 (m, 2H), 7.55−7.51 (m, 1H), 7.43−7.38 (m, 2H), 4.16 (ddd, J = 12.6, 5.6, 5.6 Hz, 1H) (H-3), 3.74 (s, 3H) (OMe), 3.59 (m, 1H) (H-1), 3.22 (m, 1H) (H-5), 2.75 (t, J = 4.1 Hz, 1H) (H-2), 2.32 (dt, J = 12.6, 2.9 Hz, 1H) (H-4_{ax}), 2.18 (s, 3H) (NMe), 2.21−2.08, 1.88−1.78 (m, 4H) (H-6, H-7), 1.68−1.63 (m, 1H) (H-4_{eq}); ^{13}C NMR ($CDCl_3$) δ 192.4 (SCOPh), 172.6 (MeOCO), 136.9 (1′-Ph), 133.3 (4′-Ph), 127.2 (2′,6′-Ph), 128.5 (3′,5′-Ph), 65.7 (C-1), 62.9 (C-5), 52.2 (C-2), 51.7 (OMe), 41.7 (NMe), 37.6 (C-4), 34.2 (C-3), 25.3 (C-6), 25.1 (C-7); HRMS (MALDI-FTMS) calcd for $C_{17}H_{22}NO_3S$ (MH^+) 320.1315, found 320.1312; $[\alpha]^{25}_D$ = −42.8° (c = 0.694, $CHCl_3$); IR (cm^{-1}) 1717 (MeOCO), 1647 (SCOPh); mp 111−113 °C.

(1*R*,2*R*,3*S*,5*S*)-3-[[(4-Methoxyphenyl)methyl]thio]-8-methyl-8-azabicyclo[3.2.1]octane-2-carbothioic Acid Methyl Ester 18. A mixture of **14** (202 mg, 0.442 mmol), MeOH (4 mL), and Na_2CO_3 (70 mg, 0.656 mmol) was refluxed and stirred for 24 h. Then, H_2O (1.5 mL) was added and the mixture was refluxed and stirred for 48 h. After cooling to room temperature, the mixture was acidified to pH ∼3 with 1 M HCl and then treated with CH_2N_2. The excess CH_2N_2 was quenched with AcOH, and then the mixture was evaporated until about half the volume remained and was poured into 1 M HCl and washed with Et_2O. The aqueous layer was brought to pH ∼9 with NH_4OH and extracted several times with CH_2Cl_2; the combined CH_2Cl_2 layers were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel, eluting with $CHCl_3$/EtOAc/NH_4OH (60:40:1) to afford **18** as a colorless oil (71.1 mg, 48.0% yield): ^{1}H NMR ($CDCl_3$) δ 7.26−7.22 (m, 2H), 6.84−6.81 (m, 2H), 3.78 (s, 3H), 3.76 (d, J = 12.9 Hz, 1H), 3.69 (d, J = 12.9 Hz, 1H), 3.69 (s, 3H), 3.25 (dd, J = 6.5, 2.6 Hz, 1H), 3.08 (m, 1H), 3.01 (dt, J = 12.0, 5.9 Hz, 1H), 2.88 (dd, J = 12.0, 2.6 Hz, 1H), 2.31 (s, 3H), 1.99−1.74 (m, 4H), 1.50 (ddd, J = 13.2, 5.9, 2.9 Hz, 1H), 1.43−1.37 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 137.5, 158.5, 130.4, 130.0, 113.7, 63.4, 60.5, 55.2, 51.7, 50.6, 38.1, 36.8, 36.3, 35.0, 26.6, 23.1; HRMS (MALDI-FTMS) calcd for $C_{18}H_{26}NO_3S$ (MH^+) 336.1628, found 336.1624; $[\alpha]^{25}_D$ = −34.2° (c = 0.556, $CHCl_3$).

(1*R*,2*R*,3*S*,5*S*)-3-(benzoylthio)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (Pseudococaine Benzoylthio Ester) 19. A solution of **18** (66.3 mg, 0.198 mmol) in TFA (3.5 mL) was refluxed and stirred for 1 h. After cooling, the mixture was coevaporated with toluene (three times). The residue was dissolved in THF (2 mL) and Et_3N (44.1 μL, 0.317 mmol) and benzoyl chloride (29.9 μL, 0.257 mmol) were added at room temperature and stirred for 2 h. Although TLC showed the reaction was incomplete, additional Et_3N (44.1 μL, 0.317 mmol), benzoyl chloride (29.9 μL, 0.257 mmol), and 1.5 h of stirring did not provide an improvement. Excess benzoyl chloride was quenched with MeOH and the mixture evaporated. The residue was purified twice by chromatography on silica gel, each time eluting with $CHCl_3$/EtOAc/NH_4OH (60:40:1), to afford **19** as a pale yellow oil that crystallized to an off-white solid upon standing in a cold room at 4 °C (48.9 mg, 77.5% yield): ^{1}H NMR ($CDCl_3$) δ 7.91−7.88 (m, 2H), 7.54−7.50 (m, 1H), 7.42−7.38 (m, 2H), 4.12 (dt, J = 12.0, 7.0 Hz, 1H), 3.63 (s, 3H), 3.39 (dd, J = 6.2, 2.9 Hz, 1H), 3.18 (m, 1H), 3.08 (dd, J = 12.3, 2.9 Hz, 1H), 2.39 (s, 3H), 2.10−1.88 (m, 5H), 1.85−1.77 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 190.6, 172.4, 136.9, 133.3, 128.5, 127.2, 63.7, 60.7, 51.8, 48.2, 38.3, 36.4, 35.8, 26.4, 23.2; HRMS (MALDI-FTMS) calcd for $C_{17}H_{22}NO_3S$ (MH^+) 320.1315, found 320.1310; $[\alpha]^{25}_D$ = +27.8° (c = 0.964, $CHCl_3$); mp 61−68 °C.

Experimental Determination of log *P*. The log P values for cocaine and cocaine benzoylthio ester were derived using the same conditions. Briefly, the extinction coefficients at 254 nm (ϵ_{254}) for both compounds in free-base form were measured in octanol [ϵ_{254} (cocaine) = 783 M^{-1} cm^{-1}; ϵ_{254} (**17**) = 5960 M^{-1} cm^{-1}]. A precisely weighed amount of each compound (∼10 mg proved convenient) was partitioned between octanol and 10 mM phosphate/150 mM NaCl, pH 7.4 (PBS) using accurately dispensed volumes of the two solvents in a ratio such that ∼50−75% of the compound was extracted into the upper octanol phase. Experiments were conducted in screw-capped glass vials or polypropylene tubes and manually inverted for 10 min (∼300 inversions). An aliquot of the octanol phase was then diluted to 1 mL of octanol in a quartz cuvette and the absorbance measured at 254 nm. Back calculations afforded the concentration of compound in the octanol phase, and subtraction from the known total amount of compound afforded the concentration in the PBS phase. The empirical P = [cmpd in octanol]/[cmpd in PBS] resulted in log P (cocaine) = 1.49 and log P (**17**) = 2.27. These values were corrected to account for only free-base partitioning at pH 7.4 by using the known pK_a = 8.6 for cocaine,[29]

(29) Bailey, D. N.; Bessler, J. B.; Sawrey, B. A. *J. Anal. Toxicol* **1997**, *21*, 41−43.

and the assumption of the same value for **17**, and are noted in the text above. An uncorrected log *P* (cocaine) = 1.05 in octanol/water at pH 7.4, which did not specify conditions, was previously reported.[30]

Kinetic Experiments. The kinetic parameters for the spontaneous and mAb-catalyzed hydrolysis of cocaine and **17** were determined in 100 mM sodium phosphate (PB), pH 7.4, 5% DMSO cosolvent at 23 °C. Stock solutions of free-base cocaine and free-base **17** were prepared in DMSO. The initial rates were determined by following the total formation of benzoic acid by analytical reversed-phase HPLC (C-18 column, VYDAC 201TP54; isocratic mobile phase of 17% CH_3CN, 83% H_2O, 0.1% TFA; flow rate, 1.5 mL/min; detector setting, 254 nm; retention times: benzoic acid 8.00 min, benzoylecgonine 5.90 min, cocaine 11.70 min, thiobenzoylecgonine 12.70 min, **17** 27.0 min). Based on our previous measurements with no cosolvent,[8] the mAb catalysis of cocaine hydrolysis was adversely effected by the presence of DMSO, particularly for GNL23A6 and GNL4D3. However, to achieve sufficient solubility of **17**, DMSO was required and so all reactions were performed under identical conditions to allow comparisons. The spontaneous and mAb-catalyzed hydrolysis of **17** and trapping of **20** was observed using spectrophotometry. The reactions were performed in 100 mM PB, pH 7.4, 5% DMSO cosolvent at 23 °C in 1-mL (1 cm) quartz cuvettes. The rates were monitored by the increase in absorbance at 412 nm due to the formation of 2-nitro-5-thiobenzoic acid (TNB) liberated upon reaction of **20** with Ellman's reagent. Reactions were done singly in a Shimadzu UV2100U spectrophotometer equipped with an automatic cell changer, Peltier temperature control, and computer interface. The absorption maximum (412 nm) and extinction coefficient (ϵ_{412} = 12 900 M^{-1} cm^{-1}) of TNB under the reaction conditions was determined by reduction of a known amount of Ellman's reagent with a 20-fold excess of tris(2-carboxyethyl)phosphine (TCEP).[31] Reactions contained a final concentration of 100 μM **17** and 500 μM Ellman's reagent aliquoted from stock solutions in DMSO. The mAb-catalyzed reactions contained various concentrations of GNL4D3 or 5 μM of either GNL3A6 or GNL23A6. After temperature equilibration of buffer containing the Ellman's reagent and in the presence or absence of mAb, a solution was first auto-zeroed and the reaction initiated by addition of **17** and then mixing (Mini-Mix; Precision Cells, Inc.). The total time from addition to the start of data accumulation was ~7 s. Decomposition of Ellman's reagent was ~0.03%/h within the time frame of the experiments. The initial rate for the observed spontaneous hydrolysis of **17** (~0.7%/h) was subtracted from the mAb-catalyzed reactions.

Preparation of Radiolabeled 17. The procedure was analogous to the preparation of unlabeled **17**. The compound **16** (31 mg, 0.0925 mmol) was deprotected and the residue dissolved in THF (0.25 mL) and Et_3N (51.5 μL, 0.370 mmol). A solution of radiolabeled benzoyl chloride [prepared in situ from 1 mCi of [7-^{14}C]-benzoic acid at 55 mCi/mmol (American Radiolabeled Chemicals), unlabeled benzoic acid (24 mg, 0.197 mmol), oxalyl chloride (20 μL, 0.225 mmol), and a microdrop of DMF in THF (1.5 mL) for 1 h] was then added and the reaction stirred until TLC showed completion. The crude material was purified using column chromatography. A stock solution was prepared in DMSO and scintillation counting used to determine the specific activity (4.69 mCi/mmol).

Radiolabeling Experiments with mAb GNL4D3. A 500-μL solution of GNL4D3 (67 μL of a 59.8 μM stock solution to give 4 μM) was prepared in 100 mM PB, pH 7.4 (408 μL), and DMSO (20 μL). An aliquot of radiolabeled **17** (5 μL of a 10 mM stock solution to give 100 μM) was then added and the solution left at 23 °C for 30 min. After this time, the solution was transferred to a Slide-A-Lyzer dialysis cassette (10 000 MWCO) (Pierce) and dialyzed against seven portions of 200 mL of 100 mM ammonium acetate, pH 5.5 at 4 °C, with changing of the buffer every hour. The radioactivity of the mAb solution was measured using scintillation counting. Stoichiometric incorporation would have yielded ~83 000 counts/min, but <1000 counts/min were observed.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra for all prepared compounds. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

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