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A LABELED GUANIDINE LIGAND FOR STUDYING SWEET TASTE

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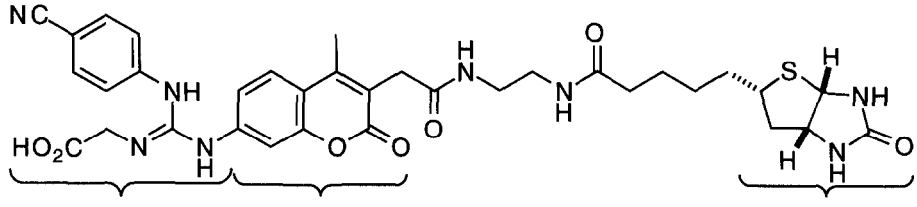
Abstract: A synthesis of a biotinylated-, coumarin-substituted-*N,N'*-diarylguanidine **1** is reported. This ligand has structural features conducive to studying sweet taste including a fluorescent tag to facilitate spectroscopic studies of binding to protein-receptors for sweet ligands and an anchor for affinity purification.

Certain substituted urea and guanidine compounds are extremely potent with respect to initiating the sensation of sweet taste.[1] As a comparison, aspartame (H-Asp-Phe-OMe) and some of its derivatives are of the order of hundreds of times sweeter than sucrose (as determined by human subject taste threshold tests), whereas some substituted guanidines can have sweetness potencies of more than 200,000 times that of sucrose.[2] Consequently, there is a possibility that commercial sweeteners superior to aspartame could be developed.

Unfortunately, the molecular basis for sweet taste is difficult to study and is not completely understood. Sweet taste response to natural ligands appears to be mediated by mediated by 7-transmembrane, G-protein linked receptors specific to taste bud cells.[3] However, several intense sweet tasting non-carbohydrate compounds evoke electrophysiological responses which are distinct from those evoked by sweet carbohydrates implying that there may be at least two different receptors or binding sites involved in the perception of sweetness.[4] For these reasons there is a need for ligands that can be used for receptor isolation and spectroscopic studies[5] of receptor/sweet-ligand interactions. This *Letter* reports a synthesis of ligand **1** that is designed to facilitate both objectives.



1

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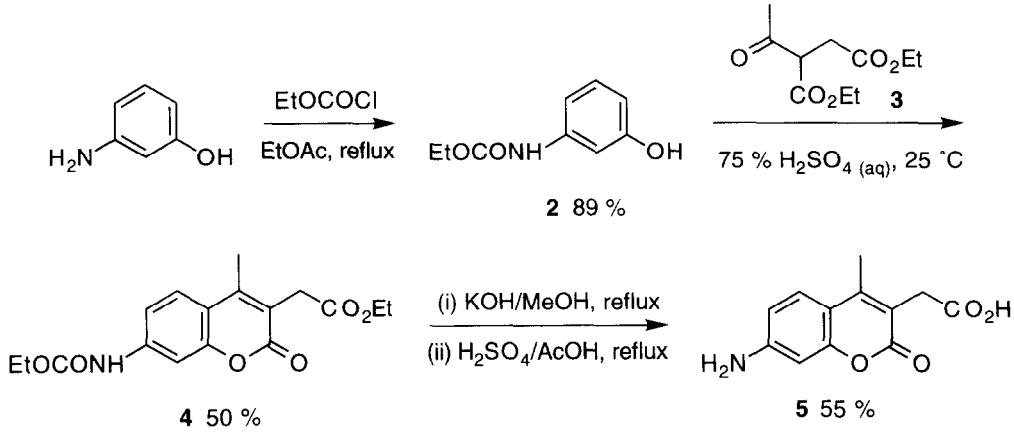
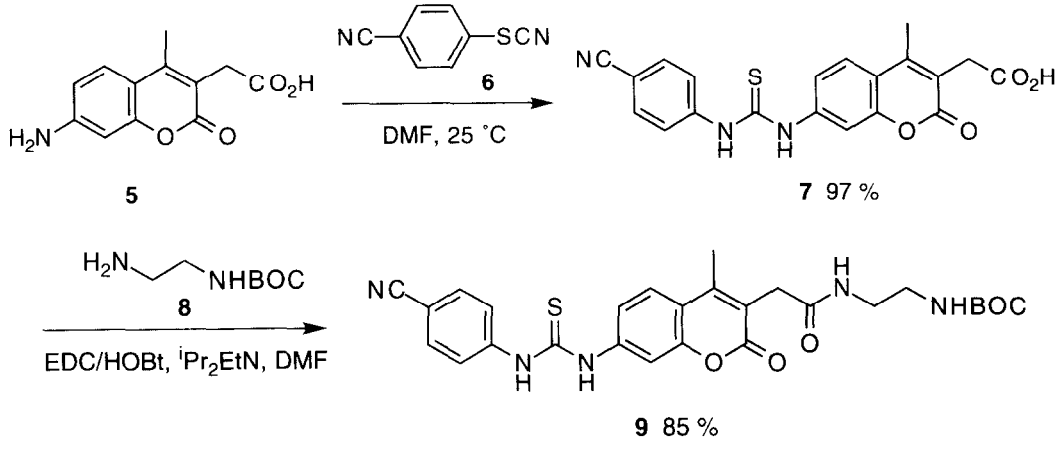
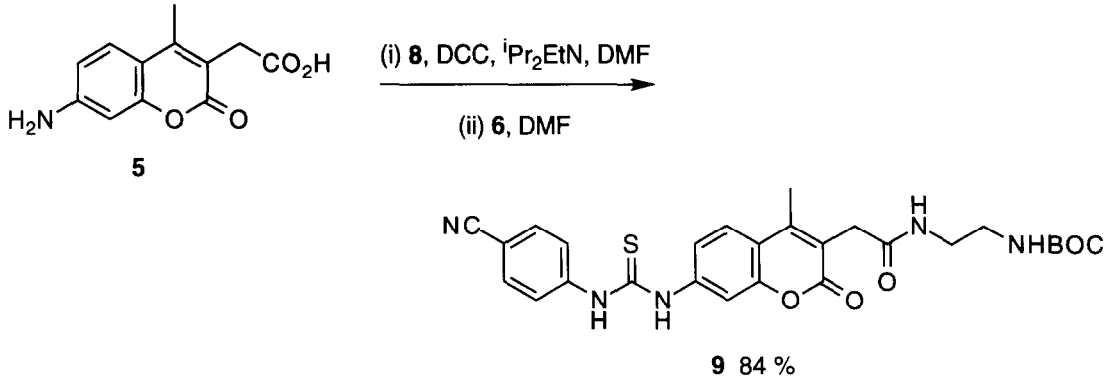
An appropriately substituted coumarin for the preparation of compound **1** was prepared from 3-aminophenol, which was first protected as the ethyl carbamate **2**. A Pechmann condensation of the commercially available succinate derivative **3** with this carbamate gave the coumarin **4**. This material was then subjected to hydrolysis to give the amino acid **5**.



Two routes were developed to prepare the thiourea intermediate **9**. The first involved reaction of amino acid **5** with isothiocyanate **6** then derivatization of the acid with the mono-protected ethylene diamine **8**. This diamine was prepared via a literature procedure, but is now commercially available (Aldrich).



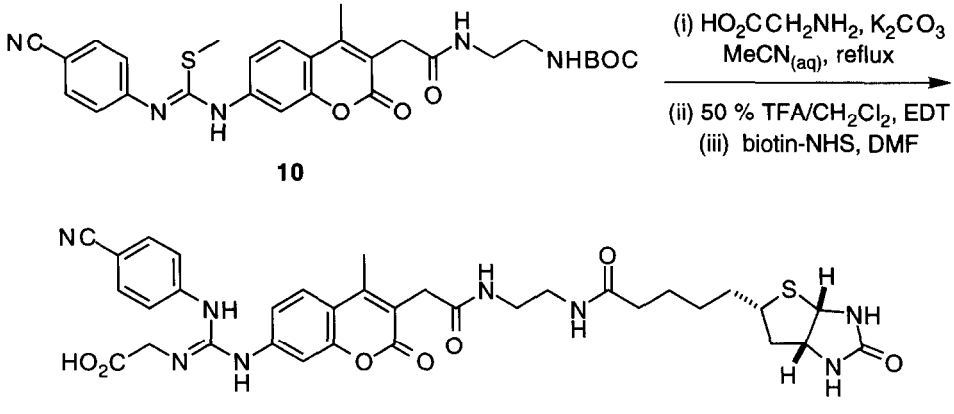
In the second route, these steps were performed in reverse order. It was a concern that amine **5** might couple with itself in this process, but in reality that was not a complication. Both routes were experimentally convenient and the yields obtained were very similar.



5

9 84 %

Conversion of thiourea **9** into the corresponding guanidine was achieved by methylating the sulfur[6] (MeI, CH_2Cl_2/DMF) giving compound **10** (97 %) followed by reaction nucleophillic displacement with glycine to give the desired guanidine functionality. The resulting product was then *N*-deprotected via treatment with TFA in the presence of ethanedithiol (EDT) as a cation scavenger. The amine formed was reacted directly with biotin *N*-hydroxysuccinamide (biotin-NHS) to give the target compound **1**.[7]



10

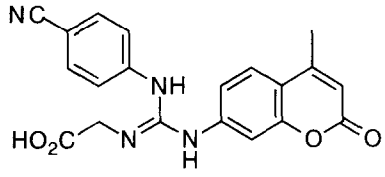
1 16 %

Quantitative measurements of the absorbance of compound **1** were made. Thus a 20 mM solution was prepared in 3:7 DMF:EtOH, diluted to 200 μM in 100% EtOH, and samples ranging in concentration from 8 μM to 100 nM were prepared by dilution in phosphate buffered saline (PBS), 0.1 M NaCl, 0.01 M phosphate, pH 7.4. Absorbance measurements from 400 to 240 nm were taken. A plot of absorbance at λ_{max} (275 nm) versus molar concentration was fit by linear regression and the molar absorbance was calculated to be 20951 M^{-1} cm^{-1}.

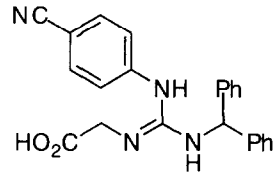
Excitation and emission fluorescence spectra of compound **1** were recorded on 1 μM solutions prepared as indicated above. Compound **11**, a similar, but non-biotinylated, coumarin/guanidine previously prepared[8] had a peak emission at 457 nm. Consequently, emission at that wavelength was monitored first, followed by

determination of the optimal excitation wavelength. Optimal peak excitation and emission was observed at 345 and 460 nm, respectively.

Binding of **1** with monoclonal antibody NC10.14 was determined by competitive radioimmunoassay. NC10.14 is a monoclonal antibody which binds the superpotent sweet tasting guanadino compound **12** with a K_d of 18 ± 6 nM.[9] The K_d value observed for interaction of 1 with this antibody was 184 ± 19 nM. The reduced affinity of **1** relative to **12** is relatively small, and is unimportant for the proposed application of the labeled compound.



11



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Further spectral studies and applications of the fluorescent-, biotinylated-ligand **1** will be reported shortly.

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7. Spectral data for compound **1**: MALDI MS, *m/z*, 701.06 (M-1). ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 10.65 (b, 2H), 9.22 (b, 1H), 8.00 (s, 1H), 7.87 (m, 3H), 7.80 (b, 1H), 7.44 (m, 3H), 7.27 (b, 1H), 6.43 (s, 1H), 6.35 (s, 1H), 4.32 (m, 1H), 4.23 (s, 2H), 4.13 (m, 1H), 3.51 (s, 2H), 3.10 (s, 5H), 2.82 (dd, *J* = 5.0, 12.0 Hz, 1H), 2.58 (d, *J* = 13 Hz, 1H), 2.37 (s, 3H), 2.06 (t, *J* = 7.5 Hz, 2H), 1.70-1.12 (m, 6H). $[\alpha]^{21}_D$ = +90 ° (c = 0.2, DMSO).
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