Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 23, pp. 2875-2878, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00501-3

LIGANDS FOR THE STUDY OF SWEET TASTE RECEPTORS: SYNTHESIS OF FLUORESCENT N,N'-DISUBSTITUTED GUANIDINEACETIC ACIDS.

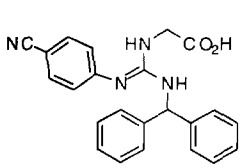
Michelle M. Sulikowski,[a] Kyungjin Kim,[a] Gary A. Sulikowski,[a] Srinivan Nagarajan,[d] and D. Scott Linthicum[b,c]*

[a]*Departments of Chemistry,* [b]*Biochemistry and Biophysics, and* [c]*Veterinary Pathobiology, Texas A&M University, College Station, Texas 77843-4467*

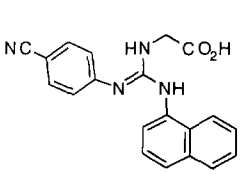
[d]*Monsanto, 700 Chesterfield Parkway BB4I, St. Louis, MO 63198*

Abstract. The synthesis of three different fluorescent N,N'-disubstituted guanidino acetic acids with intense sweet taste properties is reported. The preparation of disubstituted guanidino acetic acids containing an alpha-naphthylamine, beta-naphthylamine or methylcoumarin substitution produced compounds with fluorescent properties which will make them useful for spectroscopic studies of sweetener-receptor binding site interactions.

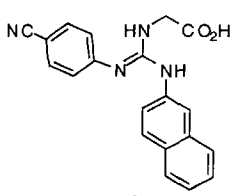
Until recently, it was believed that there was only one receptor or binding site for sweet taste sensation, but the discovery that several intensely sweet plant proteins and superpotent guanidino compounds could evoke electrophysiological responses independently from those evoked by small sweet tasting carbohydrates and related compounds, has led to the notion that different receptors or binding sites may exist.[1] The "receptors" for sweet taste appear to have precise stereochemical requirements or recognition units, as demonstrated by the fact that relatively few compounds elicit a sweet taste sensation. Shallenberger and Acree[2] were the first to propose a model of the receptor based on two recognition units, a hydrogen bond donor (AH) separated by 2.5-4.0 Å from a H-bond acceptor (B). Later, Kier[3] proposed a model that included an additional hydrophobic site which would coordinate with the AH and B units. A recent model proposed by Tinti, et al.,[4] contains eight molecular recognition sites, an observation which lead to the discovery of the superpotent guanidino compounds, some of which have sweetness potencies more than 300,000 times that of sucrose [cf. NC-174 (**1**)], as assessed by a taste threshold test.[5] We report here the synthesis of three fluorescent guanidino derivatives **2-4** as potential probes for sweet taste receptor studies. These ligands have fluorescence properties that make them suitable for a number of spectroscopic techniques involving receptor-ligand interactions.



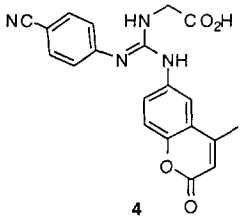
NC-174 (1)



2



3

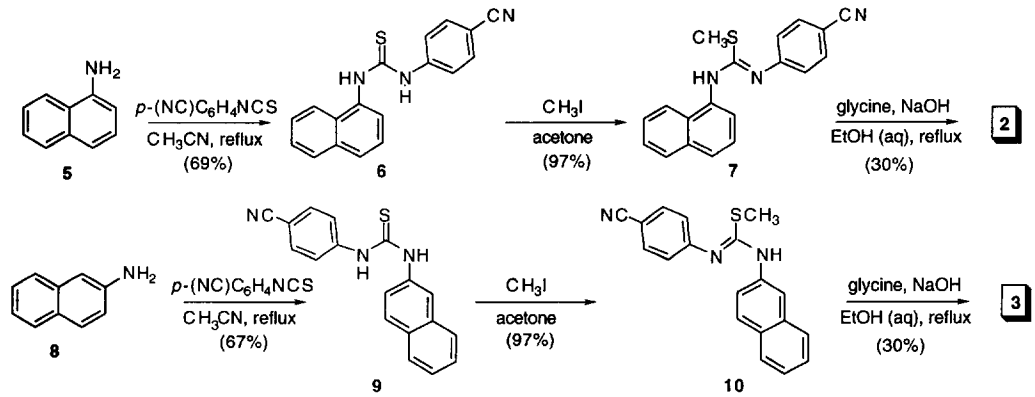


4

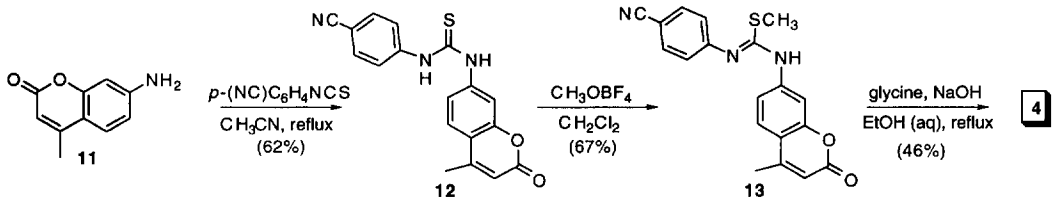
Chemistry. The preparation of N,N'-disubstituted guanidino acetic acids **2** and **3** followed a modification of the procedure of Muller, et al. as outlined in Scheme 1.[5] The preparation of **2** began with heating a mixture of 1-aminonaphthalene (**5**) and *p*-cyanophenyl isothiocyanate in acetonitrile at reflux for 5 h to produce thiourea **6** in 69% yield. Without purification, thiourea **6** was directly methylated to provide S-methylisothiourea

7. Finally, bringing a mixture of **7**, glycine and alkaline aqueous ethanol to reflux provided **2** in 20% overall yield.[6] Following a parallel sequence of reactions guanidinoacetic acid **3** was prepared in 19.5% overall yield starting from 2-aminonaphthalene.[6] Alpha-naphthylamine derivative **2** displays favorable solubility and spectral properties. On the other hand, beta-naphthylamine derivative **3** is sparingly soluble in dimethylsulfoxide and as a result was of limited utility in the binding and fluorescent studies.

Scheme 1



The preparation of coumarin derivative **4** required a minor modification of the synthetic route outlined in Scheme 1. Specifically, we were unable to methylate thiourea **12** using methyl iodide. However, S-methylation proceeded smoothly using trimethyloxonium tetrafluoroborate in methylene chloride. Coupling of the resultant isothiourea (**13**) with glycine under basic conditions followed by careful acidification and subsequent removal of water by lyophilization provided **4** in 93% yield.[6]



Results and Discussion. Several N,N'-disubstituted guanidineacetic acid, such as NC 174 (**1**), analogues possess very intense sweet taste properties. Replacements of the *p*-cyanophenyl and acetic acid portions of the these compounds can reduce sweetness significantly.[5] However, variations of the remaining hydrophobic group, such as diphenylmethyl (cf. **1**), cyclooctyl, cyclohexyl, biphenyl, do not drastically reduce sweet taste. The derivatives in this study (**2**-**4**), consisting of aminonapthalene or coumarin subtitutions, did not abolish sweetness. The naphthalene derivative (**2**) has a potency of 60,000 that of 2% sucrose; the coumarin analogue (**4**) has a similar potency.[5]

We have recently generated a library of monoclonal antibodies to several disubstituted guanidineacetic acid ligands in an effort to mimic the sweet taste receptor ligand binding specificity.[7,8] Some of these antibodies possess ligand binding profiles which correlate with ligand sweetness potencies.[9] The fluorescent ligands synthesized herein should prove useful as spectroscopic probes for the study of the receptor and monoclonal

antibody binding sites[10] due to their overlaps in excitation/emission spectra with protein flurophores. Stryer and Haugland[11] suggested that fluorescence energy transfer could be used as a "spectroscopic ruler" in the 10-60 Å range to reveal proximity relationships in biological macromolecules. Fluorescence energy transfer involves the transfer of electronic excitation between a fluorescent energy donor and a suitable energy acceptor, and such transfer occurs without the appearance of a photon and is primarily a result of dipole-dipole interactions between the donor and acceptor. Forster[12] proposed a theory for this dipole-dipole energy transfer process which postulated that the rate of transfer depends on the inverse sixth power of the distance between the donor and acceptor. This predicted distance dependence was verified by fluorescence studies of donor-acceptor pairs separated by a known distances in well defined model systems.[13] The different fluorescent ligands synthesized in this study possess spectral properties that should permit examination of their energy excitations from receptor binding site tryptophan emissions (335 nm). It has been proposed that the *p*-cyanophenyl moiety of these superpotent sweeteners interacts with an aromatic binding site residue, such as tryptophan. Calculations of the distances between these ligands and the nearest receptor binding site tryptophan residues may be helpful in understanding the geometry and molecular basis for superpotency among the guanidinium family of sweeteners.

Absorption and Fluorescence Spectra Millimolar solutions of compounds **2**, **3** and **4** were made by dissolving a known amount in N,N-dimethylformamide; solutions were diluted in 0.01 M phosphate buffered (pH 7.4) saline (0.1 M NaCl) for spectral measurements. The absorption spectra were recorded on a Beckman DU650 UV-vis instrument. The emission spectra were recorded a SLM8100 spectrofluorimeter (SLM-Aminco, Rochester, NY). All spectral measurements were carried out at rt. The excitation and emission slit widths were maintained at 8 and 16 nm, respectively. The relative quantum yields of the compounds were determined using the known quantum efficiency of 1,N6 ethenoadenosine as described by Brisstee et al.[14] Compound **2** was found to have a maximum absoprtion at 330 nm and an emission maximum at 442 nm, with a quantum yield of 0.01. Compound **3** was found to have a maximum absorption and emission maximum nearly identical to compound **2**. Compound **4** was found to have a maximum absoprtion peak at 324 nm and an emission maximum at 457 nm, with a quantum yield of 0.18; compound **4** would be suitable for energy transfer studies involving protein typtophan emission (335 nm). We have used compound **4** in fluorescence polarization studies with monoclonal antibodies that bind superpotent sweeteners and have established this probe to be highly suitable for affinity constant and kinetic rate determinations (Tetin and Linthicum, unpublished observations).

Acknowledgments. This work was supported by grants from the National Institutes of Health GM46535 to D.S.L. and the Welch Foundation (A-1230) to G.A.S.

References

1. DuBois, G. E.; Walters, D. E.; Kellogg, M. S. In *Flavor measurement* , Ho, C.-T.; Manley, C. H., Eds; M. Dekker: New York, 1993; pp 239-266.
2. Shallenberger, R. S.; Acree, T. E. *Nature* **1967**, *216*, 480.
3. Kier, L. B. *J. Pharm. Sci.* **1972**, *9*, 1394.
4. Tinti, J. M.; Nofre, C; Durozard, D.*Naturwissenshaften* **1981**, *68*, 143.
5. Muller, G. W.; Walters, D. E.; DuBois, G. E. *J. Med. Chem* **1992**, *35*, 740.

6. **1**: mp 160-161°C; ^{1}H NMR (DMSO, 200MHz) δ 8.09 (dd, *J*= 6.4, 5.6 Hz, 1H); 7.81 (dd, *J*= 6.9, 5.6 Hz, 1H); 7.50- 7.20 (m, 8H); 6.97 (d, *J*= 12.3 Hz, 1H); 3.85 (s, 2H); ^{13}C NMR (DMSO, 50 MHz) δ 172.0; 149.2; 147.4; 134.0; 132.9; 128.5; 127.7; 126.0; 125.8; 125.1; 123.7; 122.4; 119.6; 119.4; 117.9; 117.8; 101.9; 44.3; IR (KBr) 3504, 3200-2780 (broad), 2220, 1637, 1592, 1393, 1299, 1169, 828, 728, 620 cm^{-1}; UV-vis l_{max} (CH_3OH) 222 (53 132), 277 (16 795), 330 (4 440); HRMS (FAB) m/z 345.1349 [(M+H)$^+$; calc. for $C_{20}H_{16}N_4O_2$: 345.1351.
2: mp 189°C (decomp); ^{1}H NMR (DMSO-CD_3CO_2D, 200 MHz) δ 7.83-7.63 (m, 4H), 7.60 (d, *J*=8.6 Hz, 2H), 7.43-7.35 (m, 5H); 4.05 (br s, 2H); ^{13}C NMR (DMSO-CD_3CO_2D , 50 MHz) δ 172.2; 155.0; 142.5; 134.7; 134.6; 132.6; 130.7; 128.8; 128.2; 128.1; 127.4; 124.1; 123.5; 123.0; 122.5; 119.6; 109.0; 46.4; IR (KBr) 3720, 3400-3040, 2921, 2225, 1643, 1594, 1378, 1340, 1279, 1011 cm^{-1}; HRMS (FAB) m/z 345.1335 [(M+H)$^+$; calc. for $C_{20}H_{16}N_4O_2$: 345.1351.
3 : mp 190-193 °C (decomp); ^{1}H NMR (DMSO, 200 MHz) δ 7.63 (d, *J* =8.5 Hz, 2H), 7.60 (d, *J* =6.9 Hz, 1H), 7.23 (d, *J* =8.5 Hz, 2H), 7.06 (m, 2H), 6.18 (d, *J* =1.2 Hz, 1H), 4.09 (s, 2H), 2.35 (s, 3H); ; ^{13}C NMR ($CDCl_3$, 50 MHz) δ 177.2; 160.0; 153.9; 153.3; 150.3; 133.2; 125.9; 121.0; 119.4; 118.2; 117.4; 114.2; 111.5; 107.4; 103.4; 44.0; 18.0; IR (KBr) 3600-2800, 1710, 1612, 1390 cm^{-1}; UV-vis l_{max} (DMSO) 294 (0.803), 344 (1.132); HRMS (FAB) m/z 377.1280 [(M+H)$^+$; calc. for $C_{20}H_{16}N_4O_4$: 377.1250].
7. Anchin, J. M.; Droupadi, P. R.; DuBois, G. E.; Kellogg, M. S.; Nagarajan, S.; Carter, J. S; Linthicum, D. S. *J. Immunol.* **1994**, *153*, 3059.
8. Anchin, J. M.; Mandal, C.; Culberson, C.; Subramaniam, S.; Linthicum, D. S. *J. Molec. Graphics* **1994**, *12*, 257.
9. Droupadi, P. R.; Linthicum, D. S. *Int. J. Biochem. Cell Biol.* **1995**, *27*, 351.
10. Droupadi, P. R.; Anchin, J. M.; Meyers, E. A.; Linthicum, D. S. *J. Molec. Recog.* **1992**, *5*, 173.
11. Stryer, L.; Haugland, R. P.*Proc. Nat. Acad. Sci. USA* **1967**, *58*, 719.
12. Forster, T. *Ann. Phys. Leipzig* **1948**, *2*, 55.
13. Wu, P.; Ludwig, B. *Biochemistry* **1992**, *31*, 7939.
14. Brissette, P.; D. P. Ballou, D. P. ; Massey, V. *Anal. Biochem.* **1989**, *181*, 234.

(*Received in USA* 20 *September* 1995; *accepted* 27 *October* 1995)