pound was obtained according to the above method as a colorless oil: 1.31 g (98%); R_f (3) 0.81; $[\alpha]^{25}_D$ −12.3° (c 1.4, MeOH).

N-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminocyclooctanecarboxylic Acid Methyl Ester (11e). The compound was obtained according to the above method as a colorless oil: 0.659 (90%); R_f (2) 0.53; $[\alpha]^{25}_D$ −9.9° (c 1.1, MeOH).

Deprotection by Trifluoroacetic Acid. L-Aspartyl-D-alanine Benzyl Ester (1). Ice-cold trifluoroacetic acid (50 mL) was added to *N*-(*tert*-butyloxycarbonyl)-L-aspartyl-*tert*-butyl-D-alanine benzyl ester (**1b**; 5.0 g, 11.1 mmol). The reaction was allowed to proceed for 90 min at room temperature. The solvent was evaporated under reduced pressure. The residue was triturated with isopropyl ether to give a white solid, which was collected by filtration. The trifluoroacetate salt of 1 was dissolved in a mixture of water (30 mL) and methanol (10 mL). The pH of the solution was adjusted to 5 with an aqueous $NaHCO_3$ solution, where 1 precipitated from the solution. It was collected and recrystallized from water: 2.55 g (78%); mp 158–159 °C; R_f (6) 0.60; $[\alpha]^{23}_D$ +26.5° (c 1.0, MeOH). Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

L-Aspartyl-D-α-aminobutyric Acid Benzyl Ester (2). The compound was obtained according to the above method as colorless crystals: 2.90 g (67%); mp 158.5–159 °C; R_f (6) 0.57; $[\alpha]^{25}_D$ +32.3° (c 1.1, MeOH). Anal. ($C_{15}H_{20}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

L-Aspartyl-α-aminoisobutyric Acid Benzyl Ester (3). The compound was obtained according to the above method as a white solid: 0.066 g (43%); mp 173.5–174 °C; R_f (5) 0.39; $[\alpha]^{25}_D$ +20.3° (c 0.5, MeOH). Anal. ($C_{15}H_{20}N_2O_5$) C, H, N.

L-Aspartyl-α-aminocyclopropanecarboxylic Acid Methyl Ester (6). The compound was obtained according to the above method: 0.024 g (79%); mp 110–111 °C dec; R_f (5) 0.18; $[\alpha]^{25}_D$ +35.5° (c 1.0, MeOH). Anal. ($C_9H_{14}N_2O_5$) C, H, N.

Deprotection by Catalytic Hydrogenolysis. L-Aspartyl-α-aminoisobutyric Acid Methyl Ester (4). *N*-(Benzyloxycarbonyl)-β-benzyl-L-aspartyl-α-aminobutyric acid methyl ester (**4b**; 1.73 g, 3.79 mmol) was dissolved in methanol (50 mL). Nitrogen was bubbled into the solution for 10 min. Palladium-black (ca. 5% by weight) was added at once. Nitrogen addition was continued for 5 min and then a slow stream of hydrogen was bubbled through the solution for 4 h. After filtration and evaporation of the solvent, the solid was precipitated from methanol/ether as a white solid: 0.825 g (94%); mp 100–103 °C; R_f (7) 0.33; $[\alpha]^{25}_D$ +24.1° (c 0.8, MeOH); NMR (CD_3OD) δ 1.46 and 1.50 (2 s, 6 H, CH_3), 2.53 and 2.71 (2 dd, ABX, $^1J_{AB}$ = 17 Hz, $^3J_{AX}$ = 9 Hz, $^3J_{BX}$ = 5 Hz, 2 H, Asp C_β H_2), 3.65 (s, 3 H, OCH_3), 4.02 (dd, $^3J_{XA}$ = 9 Hz, $^3J_{XB}$ = 5 Hz, 1 H, Asp C_α H). Anal. ($C_9H_{16}N_2O_5$) C, H, N.

L-Aspartyl-α,α-diethylglycine Methyl Ester (5). The compound was obtained according to the above method as a white solid: 0.055 g (96%); mp 104–105 °C; R_f (7) 0.44; $[\alpha]^{25}_D$ +18.6° (c 1.2, MeOH). Anal. ($C_{11}H_{20}N_2O_5$) C, H, N.

L-Aspartyl-α-aminocyclobutanecarboxylic Acid Methyl Ester (7). The compound was obtained according to the above method as a white solid: 0.048 g (86%); mp 112–113 °C dec; R_f (5) 0.30; $[\alpha]^{25}_D$ +34.2° (c 0.86, MeOH). Anal. ($C_{10}H_{16}N_2O_5$) C, H, N.

L-Aspartyl-α-aminocyclopentanecarboxylic Acid Methyl Ester (8). The compound was obtained according to the above method as a white solid: 0.068 g (86%); mp 114–117 °C dec; R_f (5) 0.33; $[\alpha]^{25}_D$ +26.5° (c 1.1, MeOH). Anal. ($C_{11}H_{18}N_2O_5 \cdot {}^2/_3H_2O$) C, H, N.

L-Aspartyl-α-aminocyclohexanecarboxylic Acid Methyl Ester (9). The compound was obtained according to the above method as a white solid: 0.046 g (90%); mp 114–115 °C dec; R_f (5) 0.47; $[\alpha]^{25}_D$ +27.3° (c 1.0, MeOH). Anal. ($C_{12}H_{20}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N.

L-Aspartyl-α-aminocycloheptanecarboxylic Acid Methyl Ester (10). The compound was obtained according to the above method as a white solid: 0.13 g (80%); mp 125–126 °C dec; R_f (5) 0.49; $[\alpha]^{25}_D$ +26.5° (c 1.0, MeOH). Anal. ($C_{13}H_{22}N_2O_5 \cdot {}^2/_3H_2O$) C, H, N.

L-Aspartyl-α-aminocyclooctanecarboxylic Acid Methyl Ester (11). The compound was obtained according to the above method as a white solid: 0.11 g (82%); mp 129–132 °C dec; R_f (5) 0.23; $[\alpha]^{25}_D$ +23.3° (c 1.0, MeOH). Anal. ($C_{14}H_{24}N_2O_5$) C, H, N.

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Registry No. 1, 92398-37-3; **1a**, 41036-32-2; **1b**, 92398-55-5; 2, 92398-38-4; **2a**, 92470-56-9; **2b**, 92398-56-6; 3, 92398-39-5; **3a**, 60421-20-7; **3b**, 92398-57-7; 4, 92398-40-8; **4a**, 15028-41-8; **4b**, 92398-58-8; 5, 92398-41-9; **5a**, 5455-34-5; **5b**, 92398-53-3; **5c**, 92398-54-4; **5d**, 92398-59-9; 6, 92398-42-0; **6a**, 72784-42-0; **6b**, 92398-60-2; 7, 92398-43-1; **7a**, 89691-88-3; **7b**, 22264-50-2; **7c**, 92398-47-5; **7d**, 92398-61-3; 8, 92420-21-8; **8a**, 699-51-4; **8b**, 52-52-8; **8c**, 92398-48-6; **8d**, 60421-23-0; **8e**, 92398-62-4; 9, 92398-44-2; **9a**, 702-62-5; **9b**, 2756-85-6; **9c**, 39692-17-6; **9d**, 37993-32-1; **9e**, 92398-63-5; 10, 92398-45-3; **10a**, 707-16-4; **10b**, 6949-77-5; **10c**, 92398-49-7; **10d**, 92398-50-0; **10e**, 92398-64-6; 11, 92398-46-4; **11a**, 710-94-1; **11b**, 28248-38-6; **11c**, 92398-51-1; **11d**, 92398-52-2; **11e**, 92398-65-7; benzyl α-aminoisobutyrate tosylate, 79118-16-4; 3-pentanone, 96-22-0; α-aminoisobutyric acid, 62-57-7; *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid-β-*tert*-butyl ester dicyclohexylamine, 1913-12-8; *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid-β-*tert*-butyl ester, 1676-90-0; cyclobutanone, 1191-95-3; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; cycloheptanone, 502-42-1; cyclooctanone, 502-49-8; α-aminocyclopropanecarboxylic acid, 22059-21-8.

Peptide Sweeteners. 7. Taste Relationships of Trifluoroacetyl-L-aspartylanilides

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A series of analogues of trifluoroacetyl-α-L-aspartylanilides substituted at various positions on the aromatic ring was synthesized and tasted. The position of the substitution is essential for the nature of the taste response. The results clearly establish the close relationship between sweet and bitter taste for these compounds. Combined electronic and topochemical contributions are discussed.

The relationship between structure and taste has been extensively studied for L-aspartyl dipeptide analogues.[1-9] Similar studies have been carried out for a related class of trifluoroacetyl-L-aspartylamides.[10-13] In this paper, our efforts focus on taste–structure relationships for compounds with selected substitutions on the aromatic ring

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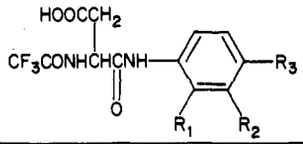
Table I. Relationship of Taste to Aryl Substitution Patterns



no.	R^1	R_2	R_3	taste[a]
1	H	H	H	12[10]
2	H	H	Cl	120[10]
3	H	Cl	H	+/–[b]
4	Cl	H	H	–[c]
5	H	H	CN	3000[10]
6	H	CN	H	+/-
7	CN	H	H	–
8	H	H	NO_2	1500[11]
9	H	NO_2	H	+/---
10	NO_2	H	H	–
11	H	H	CH_3	0[c]
12	H	CH_3	H	+/–
13	CH_3	H	H	0
14	H	H	CF_3	not sweet[10]
15	H	H	I	not sweet[10]

[a] Times as sweet as sucrose. [b] (+) sweet, (0) tasteless, (–) bitter, (---) very bitter. [c] Described as "not sweet".[10]

of the trifluoroacetyl-L-aspartylanilides. This represents an extension of our earlier work on side-chain homologues of this series of compounds.[14]

Synthesis. We employed two routes to synthesize our target molecules. Procedure A (a classical approach) was used to synthesize the meta-substituted anilides starting with *N*-(*tert*-butyloxycarbonyl)-β-*tert*-butylaspartate. Although this method gives poor yields, it leads unambiguously to the expected α-substituted aspartylanilides. Because of the reduced nucleophilicity of o-cyano and o-nitroaniline, we could not use the above method. Procedure B was therefore developed on the basis of the addition of trifluoroacetyl-L-aspartic anhydride to a large excess of the appropriate aniline in the molten state (i.e., ~130 °C). This reaction leads to a mixture of the two isomeric α- and β-L-aspartylanilides. We assumed, as previously reported,[14] that the faster moving compound on TLC is the α-isomer. This was verified by synthesizing trifluoroacetyl-α-L-aspartyl-2-methyl-3-nitroanilide (**19**) both by procedures A and B.

The possibility exists that procedure B leads to racemization. We therefore synthesized the *p*-cyanoanilide **5** by this method and showed that the product has an optical rotation $[\alpha]^{25}_D$ –40.8° (*c* 1.1, acetone), while the value obtained in the literature according to Lapidus and Sweeney's method[10] was $[\alpha]^{25}_D$ –40.4° (c 1.1, acetone). We conclude that procedure B does not lead to racemization in the preparation of the anilides discussed in this paper.

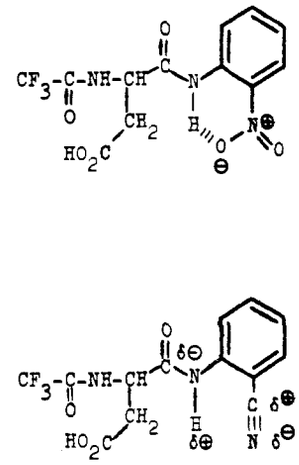


Figure 1. Schematic representation of intramolecular hydrogen bond and dipole–dipole interactions for ortho-substituted trifluoroacetyl-L-aspartylnitro- and -cyanoanilides.

In order to examine the role of the amide bond in the taste of trifluoroacetyl-α-L-aspartylanilides, we have synthesized the N-methylated analogue **16** of trifluoroacetyl-α-L-aspartyl-*p*-nitroaniline, by procedure B. We also attempted to obtain the ester analogue (trifluoroacetyl-α-L-aspartic acid *p*-nitrophenyl ester, **17**) but have been unsuccessful, since the expected compound is highly unstable. We assume that the side-chain carboxylate reacts with the α-ester bond.

We have not been able to obtain the trifluoroacetyl-α-L-aspartyl-2,4-dinitroanilide. Although we observed its formation (method B), its instability did not allow us to purify it. Thus, we synthesized the 4-chloro-2-nitro- and 2-chloro-4-nitroanilides **23** and **24**, which have rather similar steric and electronic features as the 2,4-dinitroanilide.

Results and Discussion

Lapidus and Sweeney[10] reported on the taste of trifluoroacetyl-α-L-aspartylanilide (**1**). The sweet taste is enhanced by the presence in the para position of the aromatic ring of an electron-withdrawing or simply electronegative group (compounds **2**, **5**, **8**, Table I). It is important to note that this group cannot exceed a certain size, since the *p*-trifluoromethyl **14** and the *p*-iodoanilide **15** (Table I) are described as not sweet.

The substituted analogues based on *p*-chloro- and *p*-fluoroaniline are sweet, while the ortho analogues are not sweet.[19] We synthesized the ortho analogues of the intensely sweet trifluoroacetyl-α-L-aspartyl-*p*-cyano and *p*-nitroanilides; they are slightly bitter (Table I). These structural modifications do not involve drastic changes of the elelctronic character of the aryl ring but lead to an alternation of the steric features of the molecule as a whole. For instance, in the case of the *o*-nitro compound **10**, a strong hydrogen bond can exist between the amide NH and the nitro group (Figure 1). The *o*-cyano compound **7** (the o-chloroanilide **4**, as well) likely involves a dipole–dipole interaction between the amide and the partial negatively charged end of the cyano (or chloro) group (Figure 1). The strong bitter taste (following a weak sweet taste) of the *N*-methyl-*p*-nitroanilide **16** clearly indicates that an unsubstituted amide bond is required for sweet taste, possibly because of its interaction with the receptor. Intramolecular hydrogen bonds or interactions such as

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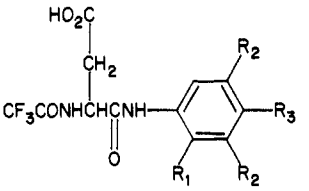
Table II. Taste Relationships for Disubstituted Trifluoroacetyl-α-L-aspartylanilides



	R_1	R_2 (R_2')	R_3	taste[a]
18	CH_3	H	NO_2	+/--
19	CH_3	NO_2	H	--
20	CH_3	H(NO_2)	H	--
21	H	NO_2	NO_2	+/--
22	NO_2	NO_2	H	--
23	NO_2	H	Cl	--
24	Cl	H	NO_2	--
25	H	CH_3	NO_2	+/--

[a] (+) sweet, (--) very bitter.

those described above can interfere with the amide's interaction with the sweet taste receptor. On the contrary, an *o*-methyl substitution (compound **13**, Table I) which does not involve any hydrogen bond or a dipole–dipole interaction with the amide NH does not lead to a bitter taste but rather to a tasteless compound. In addition, these effects likely alter the preferred rotamers around the C_{Ar}–NH bond which can affect receptor–tastant interactions.

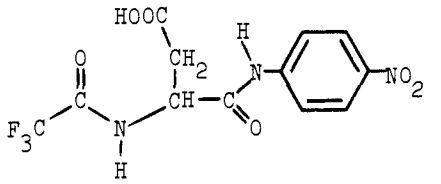
The meta-substituted analogues (**3**, **6**, **9**, and **12**, Table I) all exhibit an initial sweet taste, followed by a long-lasting bitter taste. The intensity of the sweet taste follows the order CN > Cl > NO_2 > CH_3. We suggest that these compounds cannot adapt to the dimensions of the sweet taste receptor as well as their para analogues. If the bitter taste involves interactions of the tastant with the same receptor locale but over a slightly larger retion, we can explain our results. Thus, it is not surprising that the *m*-nitro compound **9** exhibits a much stronger bitter aftertaste than the other meta analogues, since the nitro group is much larger than the cyano, chloro, or methyl groups. Our findings are consistent with Temussi's description of the gross features of the sweet and bitter receptors.[15]

In order to probe the effects of positions of substitutions on taste, we prepared a series of disubstituted anilides (Table II). All these compounds exhibit a strong bitter taste. In addition, the o-methyl-*p*-nitro **18**, *m*,*p*-dinitro **21**, and *m*-methyl-*p*-nitro **25** derivatives are slightly sweet. These results can be explained on the same basis as the compounds in Table I but give more information about the features or both sweet and bitter taste receptors.

The *o*-methyl-*p*-nitroanilide **18** exhibits a slightly sweet taste followed by a srong bitter aftertaste. The *o*-methyl group induces a steric interaction with the adjacent amide group which must affect the rotation around the C_{Ar}–NH bond (Figure 2). Thus, the electronic effects of the *p*-nitro group leading to a sweet taste are overwhelmed by the steric effect of the *o*-methyl substitution.

The same effect leads of a total disappearance of sweet taste in the case of the o-methyl-*m*-nitroanilides **19** and **20**, which only exhibit a bitter taste.

The *m*,*p*-dinitroanilide **21** and the *m*-methyl-*p*-nitroanilide **25** have the same taste. This result clearly shows, as assumed for the meta-substituted compounds **6**, **9**, **12** (Table I), that the effect of a meta substitution is steric



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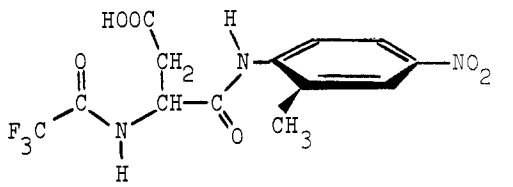

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Figure 2. Rotation about the C_{Ar}–NH bond for the trifluoroacetyl-α-L-aspartyl-*o*-methyl-*p*-nitroanilide.

since the methyl and the nitro group have quite different electronic features.

Compounds **22–24** are substituted in the ortho position by a group involving either a hydrogen bond or a dipole–dipole interaction with the adjacent amide NH. Thus, it is not surprising that these compounds exhibit a strong bitter taste, similar in intensity to the N-methylated analogue **16**.

These results show the close relationship between sweet and bitter taste for the trifluoroacetyl-α-L-aspartylanilides. Minor changes in the structure of a sweet molecule produce dramatic changes in its taste properties.

We have established some important features of the trifluoro-α-L-aspartylanilides required for a sweet taste.

(1) The anilide NH bond must interact with the sweet taste receptor, since the *N*-methylanilide **16** and the compounds involving an intramolecular hydrogen bond or a dipole–dipole interaction between the ortho substituent and the NH bond (**4**, **7**, **10**, **22**, **23**, **24**) all exhibit a bitter taste.

(2) Any substitution in the meta position induces a steric projection that dramatically reduces the interaction with the hydrophobic zone of the sweet taste receptor. As implied by Temussi,[15] the bitter receptor has larger dimensions. Thus, these projections still allow interactions with the bitter taste receptor.

(3) The bitter taste of the *o*-methyl-substituted nitroanilides leads us to assume that the NH anilide bond and the aromatic ring of a sweet trifluoroacetyl-α-L-aspartylanilide have to be coplanar (see Figure 2). This assumption will be investigated by spectroscopic techniques including ultraviolet, infrared, and nuclear magnetic resonance spectroscopy.

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 with a 10-cm water-jacketed cell. NMR spectra were obtained from either a 60-MHz or a 360-MHz Fourier-transform spectrometer. All chemical shifts are reported in parts per million downfield from Me_4Si. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. The analytical values are within 0.4% of the theoretical values.

Analytical TLC plates were purchased from E. Merck: silica gel 60 F-254, aluminum backed. Preparative TLC plates were

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purchased from Analtech: silica gel GF, 2000 μm, glass backed. Column chromatographies were performed on E. Merck Kieselgel 60 (230–400 mesh ASTM) under pressure of nitrogen (flow rate = 10 mL/min).

The TLC plates were developed with ninhydrin, Cl_2/tolidine reagent, or UV light (254 nm). The following developing systems were used: (A) $CHCl_3$/MeOH/AcOH, 85:10:5; (B) EtOAc/AcOH, 99.5:0.5. The compounds were synthesized according to either method A or B.

The new compounds were qualitatively taste tested by four volunteers from our laboratories as solids (equal amounts of each, including standards) or in solution. Tasting of solutions was carried out with a standard 8% sucrose solution and the new compounds dissolved in doubly distilled water. At least three double-blind tests were performed by the panel on each compound to achieve reproducible results. The anilines were purchased from Aldrich or prepared according to the literature.

Method A. The dicyclohexylamine salt of *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid β-(*tert*-butyl ester (0.468 g, 1 mmol) was dissolved in 1 N $NaHSO_4$ and extracted into ether. The ethereal solution was washed with water and brine and dried over $MgSO_4$, and the solvent was removed under reduced pressure to yield a clean oil of *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid β-*tert*-butyl ester. The oil was dissolved in 10 mL of THF and cooled to −20 °C, triethylamine (0.14 mL, 1 mmol), and isobutyl chloroformate (0.13 mL, 1 mmol) were added. After 15 min, the mixture was warmed to 0 °C and the substituted aniline (1.2 mmol) was added. The mixture was stirred at 0 °C for 1 h and at room temperature for 20 h. The solvent was removed under reduced pressure to leave an oil, which was dissolved in 5 mL of ethyl acetate. The solution was washed with several 50-mL portions of 0.1 N HCl, brine, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the corresponding anilide. The compound was dissolved in 5 mL of trifluoroacetic acid at room temperature. The mixture was stirred for 40 min and trifluoroacetic anhydride (0.14 mL, 1 mmol) was added. Additional trifluoroacetic anhydride (0.14 mL) was added after 30 min.

After an additional 30 min the reaction mixture was concentrated under reduced pressure. The oily residue was dissolved in 10 mL of ethyl acetate and extracted with a cold saturated aqueous solution of $NaHCO_3$ (3 × 15 mL). The combined aqueous layers were washed with ethyl acetate (2 × 10 mL), acidified to pH 3 with 2 N HCl, and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine until neutral pH, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and precipitated in hexane to yield the desired product as a powder.

Method B. The substituted aniline (2 mmol) was melted and heated at 130 °C. *N*-(Trifluoroacetyl)-L-aspartic acid anhydride[10] (0.211 g, 1 mmol) was added. The mixture was stirred for 2 min at this temperature and quickly cooled to room temperature. The residue was dissolved in 10 mL of ethyl acetate and extracted with a cold saturated solution of $NaHCO_3$ (3 × 15 mL). The combined aqueous layers were washed with ethyl acetate (2 × 10 mL), acidified to pH 3 with 2 N HCl, and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine until neutral pH, dried over $MgSO_4$, and concentrated under reduced pressure. A TLC (solvent system A) showed two spots corresonding to the α- and β-substituted compounds.

The two isomers were separated by preparative TLC or column chromatography (solvent system B). The faster moving compound (α-substituted isomer) was dissolved in ethyl acetate and precipitated with hexanes.

Trifluoroacetyl-α-L-aspartyl-*m*-chloroanilide (3). This compound was prepared according to method A and recrystallized in a mixture of ethyl acetate and light petroleum ether: yield 30%; TLC R_f (A) 0.42; mp 154–156 °C; $[\alpha]^{25}_D$ −1.9° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.45 (s, 1 H, NH Ar), 9.87 (m, 1 H, NH_α), 7.90–7.14 (m, 4 H, Ar), 4.78 (m, 1 H, CH), 2.81 (m, 2 H, CH_2). Anal. ($C_{12}H_{10}N_2O_4ClF_3 \cdot 1.5H_2O$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-*o*-chloroanilide (4). This compound was prepared according to method B: yield 39%; TLC R_f (A) 0.42; mp 165–166 °C; $[\alpha]^{25}_D$ −67.5° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 9.80 (s, 1 H, NH Ar), 7.72–7.02 (m, 5 H, NH_α, Ar), 4.85 (m, 1 H, CH), 2.84 (m, 2 H, CH_2). Anal. ($C_{12}H_{10}N_2O_4ClF_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-*m*-cyanoanilide (6). The compound was prepared according to method A and recrystallized in a mixture of ethyl acetate and hexane: yield 25%; TLC R_f (A) 0.26; mp 170–172 °C; $[\alpha]^{25}_D$ −2.0° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.64 (s, 1 H, NH Ar), 9.94 (d, 1 H, NH_α), 8.10–7.57 (m, 4 H, Ar), 4.80 (m, 1 H, CH), 2.84 (m, 2 H, CH_2). Anal. ($C_{13}H_{10}N_3O_4F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-*o*-cyanoanilide (7). This compound was prepared according to method B: yield 37%; TLC R_f (A) 0.49; mp 183 °C; $[\alpha]^{25}_D$ −75.8 (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.32 (s, 1 H, NH Ar), 9.70 (m, 1 H, NH_α), 7.77–7.13 (m, 4 H, Ar), 4.82 (m, 1 H, CH), 2.83 (m, 2 H, CH_2). Anal. ($C_{13}H_{10}N_3O_4F_3$) C, H, N.

Trifluoroacetyl-α-aspartyl-*m*-nitroanilide (9). This compound was prepared according to method A and recrystallized in a mixture of ethyl acetate and light petroleum ether: yield 30%; TLC R_f (A) 0.31; mp 175–177 °C; $[\alpha]^{25}_D$ −1.1° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.75 (s, 1 H, NH Ar), 9.92 (m, 1 H, NH_α), 8.63–7.64 (m, 4 H, Ar), 4.85 (m, 1 H, CH), 2.85 (m, 2 H, CH_2). Anal. ($C_{12}H_{10}N_3O_6F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-*o*-nitroanilide (10). This compound was prepared according to method B: yield 60%; TLC R_f (A) 0.43; mp 167–169 °C; $[\alpha]^{25}_D$ −102.1° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.67 (s, 1 H, NH Ar), 9.95 (m, 1 H, NH_α), 8.00–7.42 (m, 4 H, Ar), 4.90 (m, 1 H, CH), 2.87 (m, 2 H, CH_2). Anal. ($C_{12}H_{10}N_3O_6F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-*p*-toluidide (11). This compound was prepared according to method B: yield 39%; TLC R_f (A) 0.52; mp 182–184 °C; $[\alpha]^{25}_D$ −53.8 (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.33 (s, 1 H, NH Ar), 9.87 (m, 1 H, NH_α), 7.27 (m, 4 H, Ar), 4.83 (m, 1 H, CH), 2.82 (m, 2 H, CH_2), 2.23 (s, 3 H, CH_3). Anal. ($C_{13}H_{13}N_2O_4F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-*m*-toluidide (12). This compound was prepared according to method B: yield 41%; TLC R_f (A) 0.46; mp 173 °C; $[\alpha]^{25}_D$ −50.3 (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.00 (s, 1 H, NH Ar), 9.67 (m, 1 H, NH_α), 7.42–6.77 (m, 4 H, Ar), 4.80 (m, 1 H, CH), 2.80 (m, 2 H, CH_2), 2.27 (s, 3 H, CH_3). Anal. ($C_{13}H_{13}N_2O_4F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-*o*-toluidide (13). This compound was prepared according to method B: yield 50%; TLC R_f (A) 0.51; mp 185–186 °C; $[\alpha]^{25}_D$ −72.9 (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 9.75 (m, 1 H, NH_α), 9.58 (s, 1 H, NH Ar), 7.15 (m, 4 H, Ar), 4.85 (m, 1 H, CH), 2.83 (m, 2 H, CH_2), 2.18 (s, 3 H, CH_3). Anal. ($C_{13}H_{13}N_2O_4F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-4-nitro-*N*-methylanilide (16). The 4-nitro-*N*-methylaniline was prepared according to Johnstone et al.[16]

The title compound was prepared according to method B: yield 13%; TLC R_f (A) 0.33; mp 94–96 °C; $[\alpha]^{25}_D$ −67.1° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 9.94 (m, 1 H, NH), 8.33–7.67 (m, 4 H, Ar), 4.81 (m, 1 H, CH), 3.27 (s, 3 H, CH_3), 2.74 (m, 2 H, CH_2). Anal. ($C_{13}H_{12}N_3O_6F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-4-nitro-2-methylanilide (18). This compound was prepared according to method B: yield 48%; TLC R_f (A) 0.47; mp 179–180 °C; $[\alpha]^{25}_D$ −63.6° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 9.88 (m, 2 H, NH), 8.17–7.40 (m, 3 H, Ar), 4.92 (m, 1 H, CH), 2.82 (m, 2 H, CH_2), 2.37 (s, 1 H, CH_3). Anal. ($C_{13}H_{12}N_3O_6F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-3-nitro-2-methylanilide (19). This compound was prepared according to method B: yield 40%; TLC R_f (A) 0.43; mp 200–204 °C; $[\alpha]^{25}_D$ −54.6° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 10.07 (s, 1 H, NH Ar), 9.85 (d, 1 H, NH_α), 7.83–7.37 (m, 3 H, Ar), 4.85 (m, 1 H, CH), 2.86 (m, 2 H, CH_2), 2.25 (s, 3 H, CH_3). Anal. ($C_{13}H_{12}N_3O_6F_3$) C, H, N.

A sample was prepared according to method A and showed the same R_f in either solvent system A or B.

Trifluoroacetyl-α-L-aspartyl-5-nitro-2-methylanilide (20). This compound was prepared according to method B: yield 40%; R_f (A) 0.39; mp 166–168 °C; $[\alpha]^{25}_D$ −69.1° (*c* 1, MeOH); NMR (Me_2SO-d_6) δ 9.92 (s, 1 H, NH Ar), 9.80 (m, 1 H, NH_α), 8.32–7.37 (m, 3 H, Ar), 4.90 (m, 1 H, CH), 2.85 (m, 2 H, CH_2), 2.36 (s, 3 H, CH_3). Anal. ($C_{13}H_{12}N_3O_6F_3$) C, H, N.

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Trifluoroacetyl-α-L-aspartyl-3,4-dinitroanilide (21). The 3,4-dinitroaniline has been obtained according to Nielsen et al.[17]

The title compound was prepared according to method B: yield 27%; TLC R_f (A) 0.47; mp 180–182 °C; $[\alpha]^{25}_D$ −58.5° (c 1, MeOH); NMR (Me_2SO-d_6) δ 11.25 (s, 1 H, NH Ar), 10.00 (m, 1 H, NH_α), 8.40–7.87 (m, 3 H, Ar), 4.83 (m, 1 H, CH), 2.90 (m, 2 H, CH_2). Anal. ($C_{12}H_9N_4O_3F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-2,3-dinitroanilide (22). The 2,3-dinitroaniline was prepared according to Nielsen et al.[17]

The title compound was prepared according to method B: yield 15%; TLC R_f (A) 0.37; mp 182–185 °C; $[\alpha]^{25}_D$ −88.8 °C (c 1, MeOH); NMR (Me_2SO-d_6) δ 10.03 (m, 2 H, NH_α, NH Ar), 8.15–7.90 (m, 3 H, Ar), 4.93 (m, 1 H, CH), 2.90 (m, 2 H, CH_2). Anal. ($C_{12}H_9N_4O_3F_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-2-nitro-4-chloroanilide (23). This compound was prepared according to method B: yield 24%; TLC R_f (A) 0.39; mp 159–161 °C; $[\alpha]^{25}_D$ −95.9° (c 1, MeOH); NMR (Me_2SO-d_6) δ 10.67 (s, 1 H, NH Ar), 9.97 (m, 1 H, NH_α), 8.04–7.77 (m, 3 H, Ar), 4.85 (m, 1 H, CH), 2.83 (m, 2 H, CH_2). Anal. ($C_{12}H_9N_3O_6ClF_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-2-chloro-4-nitroanilide (24). This compound was prepared according to method B: yield 23%; TLC R_f (A) 0.38; mp 162–164 °C; $[\alpha]^{25}_D$ −69.5° (c 1, MeOH); NMR (Me_2SO-d_6) δ 10.10 (m, 2 H, NH_α, NH Ar), 8.33–8.20 (m, 3 H, Ar), 5.03 (m, 1 H, CH), 2.85 (m, 2 H, CH_2). Anal. ($C_{12}H_9N_3O_6ClF_3$) C, H, N.

Trifluoroacetyl-α-L-aspartyl-3-methyl-4-nitroanilide (25). The 3-methyl-4-nitroaniline was prepared according to Wibaut.[18]

The title compound was prepared according to method B: yield 24%; TLC R_f (A) 0.44; mp 167–169 °C; $[\alpha]^{25}_D$ −55.9 (c 1, MeOH); NMR (Me_2SO-d_6) δ 10.71 (s, 1 H, NH Ar), 9.88 (d, 1 H, NH_α), 8.10–7.55 (m, 3 H, Ar), 4.83 (m, 1 H, CH), 2.85 (m, 2 H, CH_2), 2.53 (s, 3 H, CH_3). Anal. ($C_{13}H_{12}N_3O_6F_3$) C, H, N.

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Registry No. 1, 41696-59-7; 2, 41566-94-3; 3, 92398-66-8; 4, 48193-99-3; 5, 39219-30-2; 6, 92398-67-9; 7, 92398-68-0; 8, 61980-46-9; 9, 92398-69-1; 10, 92398-70-4; 11, 41567-05-9; 12, 92398-71-5; 13, 92398-72-6; 14, 41567-06-0; 15, 41567-00-4; 16, 92398-73-7; 18, 92398-74-8; 19, 92398-75-9; 20, 92398-76-0; 21, 92398-77-1; 22, 92398-78-2; 23, 92398-79-3; 24, 92398-80-6; 25, 92398-81-7; *N*-(*tert*-butyloxycarbonyl)-L-aspartic acid β-*tert*-butyl ester, 1676-90-0; trifluoroacetic anhydride, 407-25-0; 4-nitro-*N*-methylaniline, 100-15-2; 3,4-dinitroaniline, 610-41-3; 2,3-dinitroaniline, 602-03-9; 3-methyl-4-nitroaniline, 611-05-2; *m*-chloroaniline, 108-42-9; *o*-chloroaniline, 95-51-2; *m*-cyanoaniline, 2237-30-1; *o*-cyanoaniline, 1885-29-6; *m*-nitroaniline, 99-09-2; *o*-nitroaniline, 88-74-4; *p*-toluidine, 106-49-0; *m*-toluidine, 108-44-1; *o*-toluidine, 95-53-4; 2-methyl-4-nitroaniline, 99-52-5; 2-methyl-3-nitroaniline, 603-83-8; 2-methyl-5-nitroaniline, 99-55-8; 4-chloro-2-nitroaniline, 89-63-4; 2-chloro-4-nitroaniline, 121-87-9.

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(19) We repeated the synthesis of the o-chloroanilide **4**, which Lapidus and Sweeney described as "not sweet", and found this compound to be slightly bitter.

A ^{1}H NMR Study of the Interactions and Conformations of Rationally Designed Brodimoprim Analogues in Complexes with *Lactobacillus casei* Dihydrofolate Reductase

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A consideration of the detailed structural information available from X-ray crystallographic and NMR studies on complexes of dihydrofolate reductase with inhibitors has led to the design of trimethoprim analogues with improved binding properties. Computer graphic techniques have been used to predict which substituent groups were required at the 3′-O position of brodimoprim (2,4-diamino-5-(3,5-dimethoxy-4-bromobenzyl)pyrimidine) to make additional interactions with the enzyme. NMR spectroscopy provided a convenient method of assessing if the analogues were binding in the predicted manner. On the basis of this approach, the C4,C6-dicarboxylic acid analogue IX was designed to interact with Arg-57 and His-28 in the enzyme, and this analogue was found to bind 3 orders of magnitude more tightly than the parent brodimoprim.

The antibacterial drug trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine) acts by selectively inhibiting dihydrofolate reductase in bacterial cells. In the past, many trimethoprim analogues have been investigated in attempts to find inhibitors that are either more selective or more active against resistant strains. With the recent availability of detailed structural information on complexes of dihydrofolate reductase with inhibitors from both X-ray crystallography[1-4] and NMR spectroscopy,[5,6] we now have a framework for designing trimethoprim analogues with modified binding characteristics. These techniques also provide methods of monitoring the complexes formed with new inhibitors to assess whether or not they are binding in the predicted manner. Kuyper and co-workers[7] have recently used this structural approach to design a series of trimethoprim analogues with aliphatic ω-carboxylic acid substituents arranged to interact favorably with the con-

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