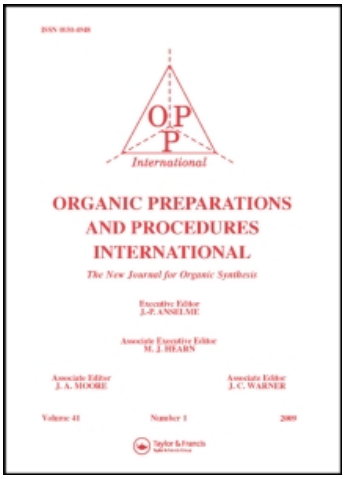

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AN EFFICIENT SYNTHESIS OF DAGO

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AN EFFICIENT SYNTHESIS OF DAGO

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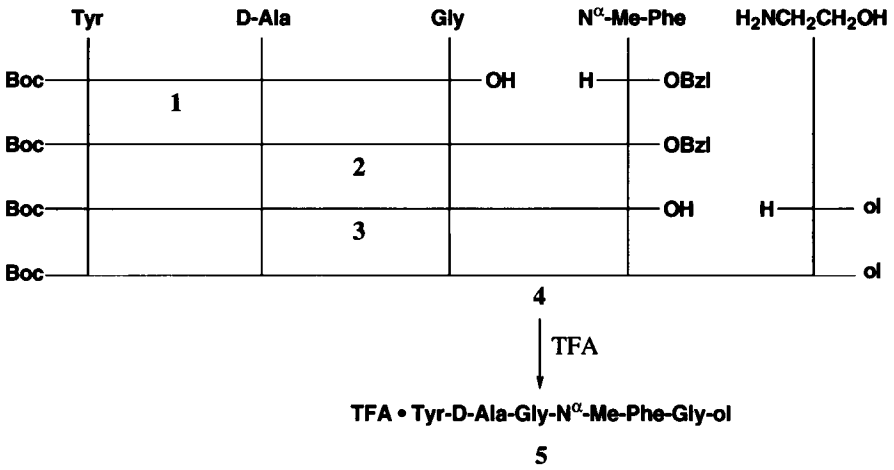
The enkephalin analog DAGO (Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol) is a selective ligand for the opioid μ receptor[1,2] and is used widely in the characterization of μ-receptor mediated pharmacology.[3] As part of a program on opioid peptides, we needed gram amounts of highly pure DAGO for structural and pharmacological studies. We describe here an efficient solution phase synthesis of DAGO. Pharmacological assays on our synthetic material confirmed that it had the expected properties.



Our synthetic route involved a 3 + 1 + 1 construction of DAGO. Synthetic Boc-N$^{\alpha}$-Me-Phe-OH[4] was coupled to benzyl alcohol to give Boc-N$^{\alpha}$-Me-Phe-OBzl (49%) using DCC in the presence of a catalytic amount of DMAP.[5] Subsequent treatment with trifluoroacetic acid:CH_2Cl_2 (1:1) (30 min, room temperature) gave TFA • N$^{\alpha}$-Me-Phe-OBzl in high yield. The fragment coupling of Boc-Tyr-D-Ala-Gly-OH (**1**)[6] to TFA • N$^{\alpha}$-Me-Phe-OBzl was accomplished in moderate yield utilizing DCC/HOBt in the presence of DIEA. The resultant tetrapeptide, Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OBzl (**2**), was purified by flash chromatography on silica gel using EtOAc:hexane (4:1) to afford a powdery white solid, mp. 150-151°. Hydrogenolysis of the benzyl ester (10% Pd/C catalyst, 20 min) gave Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OH (**3**) in quantitative yield. Trituration of the oily hydrogenol-

ysis product with hexane produced a white solid, mp. 165-170°. This material was then coupled to ethanolamine (two-fold excess) using the DCC/HOBt protocol to produce Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (**4**). Chromatographic purification as described for tetrapeptide **2** afforded intermediate **4** in 60% yield. Brief treatment of this intermediate with trifluoroacetic acid:CH_2Cl_2 (1:1) at room temperature removed the protecting group, providing TFA • Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (**5**) (DAGO trifluoroacetate) as a white powder. Gel filtration on Sephadex G15 yielded the pure peptide as a fluffy white solid. In a representative run, we produced a 1.10 g batch of DAGO trifluoroacetate in 24% overall yield from Boc-Tyr-D-Ala-Gly-OH (**1**).

In vitro radioligand inhibition assays[7] on the purified DAGO trifluoroacetate afforded the following K_i values (nM): μ, 0.8; δ, 126.5; κ, 529. The observed μ and δ values agreed well with the literature values (1.29 and 120 nM, respectively) reported for similar assays using rat brain membranes.[1] In the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations our sample had IC_{50} values (nM) of 11.29±1.00 and 94.42±17.34, respectively, also in excellent agreement with reported values.[8] The μ antagonist CTAP[9] produced a six-fold IC_{50} shift while the δ antagonist NTI[10] and the κ antagonist norBNI[11] had little effect on the IC_{50}, providing further evidence for the μ selectivity of the sample.[12]

In summary, we have developed an efficient 3 + 1 + 1 solution synthesis of the μ selective peptide DAGO. The synthesis, which is amenable to scale-up, affords highly pure material suitable for pharmacological studies.

EXPERIMENTAL SECTION

Melting points (uncorrected) were determined on a Thomas Hoover capillary tube apparatus. Proton NMR spectra were recorded on either a Bruker Am-250 MHz or a Bruker AMX-500 MHz supercon spectrometer using tetramethylsilane as the internal standard. Mass spectral determinations were performed on a VG ZAB-E instrument. Optical rotations were measured at the sodium D line using a Rudolph Research Autopol III Polarimeter. Thin layer chromatography (TLC) was carried out on Whatman analytical SiO_2 60 plates. HPLC was performed utilizing two Waters model 510 pumps, a model 680 automated gradient controller, a model 481 Lamba-Max spectrophotometer and a model 745 data module. Elemental analyses were carried out by Atlantic Microlabs, Inc.

TFA • N$^{\alpha}$-Me-Phe-OBzl.- To an ice-cold mixture of Boc-N$^{\alpha}$-Me-Phe-OH[4] (2.80 g, 10 mmol), benzyl alcohol (1.30 g, 12 mmol) and DMAP (0.10 g, 0.8 mmol) in CH_2Cl_2 (100 mL) and DMF (5 mL) was added a solution of DCC (2.27 g, 11 mmol) in CH_2Cl_2 (15 mL). The resultant mixture was stirred 15 hrs at room temperature. The precipitated urea was removed by filtration, the filtrate concentrated in vacuo, and the residue dissolved in EtOAc (50 mL). The solution was washed with 5% citric acid (25 mL), 5% $NaHCO_3$ (25 mL) and saturated NaCl (25 mL), then dried ($MgSO_4$) and evaporated to provide Boc-N$^{\alpha}$-Me-Phe-OBzl (1.80 g, 49%) as a highly viscous oil: TLC single spot, R_f 0.49 [EtOAc:hexane (9:1)]. The ^1H NMR ($CDCl_3$) spectrum was consistent with the structure and with the presence of two rotamers (3:2 ratio). To a solution of the oil (1.50 g, 4.1 mmol) in CH_2Cl_2 (20 mL) was added dropwise trifluoroacetic acid (20 mL), and the resultant mixture was stirred 30 min at room

temperature. Afterwards, the volatiles were evaporated to a viscous white residue. Subsequent trituration with hexane yielded 1.56 g (100%) of the title compound as a powdery white solid, mp. 78-80°; ^{1}H NMR ($CDCl_3$): δ 2.7 (3H, s, NC**H**$_3$), 3.3 (2H, doubled ABq, βC**H**$_2$), 4.15 (1H, overlapping dd, αC**H**), 5.20 (2H, ABq, ArC**H**$_2$O), 7.0-7.4 (10H, m, Ar**H**), 9.7 (1H, br s, N**H**).

Anal. Calcd for $C_{19}H_{20}F_3NO_4$•0.25 CF_3CO_2H: C, 56.87; H, 4.96; N, 3.40
Found: C, 56.75; H, 5.04; N, 3.33

Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OBzl (2).- To an ice-cold solution of Boc-Tyr-D-Ala-Gly-OH[6] (3.20 g, 7.8 mmol) and HOBt hydrate (1.20 g, 7.8 mmol) in CH_2Cl_2 (70 mL) and DMF (5 mL) was added a solution of DCC (1.68 g, 8.1 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred 20 min, then a solution of TFA • N$^{\alpha}$-Me-Phe-OBzl (2.76 g, 8.4 mmol) and DIEA (1.90 g, 15 mmol) in CH_2Cl_2 (45 mL) was added. The latter mixture was stirred 12 hrs at room temperature, then filtered to remove the precipitated urea. The filtrate was evaporated. The oily residue was dissolved in EtOAc (100 mL) with the insolubles being removed by filtration. The filtrate was then washed successively with 5% citric acid (2 x 50 mL), 5% $NaHCO_3$ (2 x 50 mL) and saturated NaCl (2 x 50 mL). The organic layer was dried ($MgSO_4$) and evaporated to afford 2.60 g (50%) of the crude product as a white solid. Chromatography on SiO_2 utilizing EtOAc:hexane (4:1) yielded the title compound as a white powder, mp. 150-151° (dec); TLC single spot, R_f 0.49 [EtOAc:hexane (3:1)]; ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, d, Ala C**H**$_3$), 1.39 [9H, s, C(C**H**$_3$)$_3$], 2.75 (3H, s, NC**H**$_3$), 2.85-3.35 (5H, overlapping m, βC**H**$_2$ and Phe αC**H**), 3.8-4.1 (2H, doubled ABq, Gly αC**H**$_2$), 4.25 (1H, m, Tyr αC**H**), 4.45 (1H, m, Ala αC**H**), 5.20 (2H, br s, ArC**H**$_2$O), 6.20 (2H, d, Tyr Ar**H**), 7.0 (2H, m, N**H**), 7.1 (2H, d, Tyr Ar**H**), 7.2-7.3 (10H, m, Ar**H**).

Anal. Calcd for $C_{36}H_{44}N_4O_8$•0.5 H_2O: C, 64.56; H, 6.77; N, 8.37. Found: C, 64.43; H, 6.73; N, 8.33

Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OH (3).- A mixture of Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OBzl (2.50 g, 3.8 mmol) and 10% Pd/C (0.40 g) in absolute EtOH (100 mL) was hydrogenated 20 min, after which time the reaction was complete (TLC). The catalyst was removed by filtration and the filtrate evaporated to obtain an oily residue. Trituration with hexane afforded the title compound in quantitative yield as a white solid, mp. 165-170°; ^{1}H NMR (CD_3OD): δ 1.10 (3H, br s, Ala C**H**$_3$), 1.37 [9H, s, C(C**H**$_3$)$_3$], 2.80 (3H, s, NC**H**$_3$), 2.85-4.40 (9H, overlapping m, four βC**H**$_2$, three αC**H**, and Gly αC**H**$_2$), 6.72 (2H, d, Tyr Ar**H**), 7.10 (2H, d, Tyr Ar**H**), 7.23 (5H, m, Phe Ar**H**).

Anal. Calcd for $C_{29}H_{38}N_4O_8$•1.8 H_2O: C, 57.76; H, 6.95; N, 9.29. Found: C, 57.50; H, 6.91; N, 9.59

Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (4).- To an ice-cold solution of Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OH (2.00 g, 3.5 mmol) and HOBt hydrate (0.53 g, 3.5 mmol) in CH_2Cl_2 (38 mL) and DMF (2 mL) was added DCC (0.74 g, 3.6 mmol) with exclusion of moisture. To this mixture was added a solution of $H_2NCH_2CH_2OH$ (0.42 g, 6.9 mmol) in CH_2Cl_2 (15 mL). The resultant mixture was stirred 15 hrs at room temperature, then filtered to remove the urea. The filtrate was evaporated. The oily residue was worked up as described above for intermediate **2** to provide 1.30 g (60%) of the title compound as a white solid, mp. 128-131°; TLC single spot, R_f 0.60 [EtOAc:EtOH (95:5)]; ^{1}H NMR (DMSO-d_6): δ 1.1 (3H, d, Ala C**H**$_3$), 1.3 [9H, s, C(C**H**$_3$)$_3$], 2.8 (3H, s, NC**H**$_3$), 2.6-4.6 (13H, overlap-

ping m, other aliphatic C**H** and C**H**$_2$), 5.1 (1H, m, N**H**), 6.6 (2H, d, Tyr Ar**H**), 6.8 (1H, d, N**H**), 7.0 (2H, d, Tyr Ar**H**), 7.2 (5H, m, Phe Ar**H**), 7.9 (2H, overlapping m, N**H**), 9.2 (1H, br s, ArO**H**).

Anal. Calcd for $C_{31}H_{43}N_5O_8 \cdot 0.7\ H_2O$: C, 59.45; H, 7.15; N, 11.18. Found: C, 59.77; H, 7.48; N, 11.08

TFA • Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (5).- Trifluoroacetic acid (25 mL) was added dropwise to an ice-cold suspension of Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (1.28 g, 2.1 mmol) in CH_2Cl_2 (30 mL) with exclusion of moisture. The mixture was stirred 30 min at room temperature, then the volatiles were evaporated. The resultant residue was triturated with Et_2O to obtain 1.20 g (92%) of the crude title compound as an off-white powder. The crude peptide was purified by gel filtration on Sephadex G15 using 5% HOAc to yield 1.10 g of the title compound as a fluffy white solid, mp. 104-106° dec; TLC single spot, R_f 0.37 [n-BuOH:HOAc:H_2O (4:1:5) (organic phase)]; TLC single spot, R_f 0.78 [n-BuOH:H_2O:HOAc:pyridine (15:8:3:10); HPLC single peak (>99%), R_t 5.46 min, on a Vydac C_{18} column (10 μ) (10 mm x 25 cm) using 8:2 A:B at 4.5 mL/min with solvent A being 0.1% TFA/H_2O and solvent B being 0.1% TFA/CH_3CN and with UV detection at 220 nm; $[\alpha]^{25}$: +15.25° (c 0.8, MeOH); ^{1}H NMR (D_2O): δ 1.1-1.2 (3H, two d, Ala C**H**$_3$), 1.9 (1H, m, βC**H**$_2$), 2.9 (3H, s, NC**H**$_3$), 2.9-5.2 (12H, overlapping m, other aliphatic C**H** and C**H**$_2$), 6.9 (2H, d, Tyr Ar**H**), 7.1 (2H, d, Tyr Ar**H**), 7.2-7.4 (5H, m, Phe Ar**H**); FAB MS: m/z 514 (M+1) (base); high resolution FAB MS: 514.2672 [calculated for $C_{26}H_{36}N_5O_6$ (M+H): 514.2666].

Anal. Calcd for $C_{28}H_{36}F_3N_5O_8 \cdot 1.4\ H_2O$: C, 51.51; H, 5.99; F, 8.73; N, 10.73
Found: C, 51.52; H, 5.70; F, 8.28; N, 10.36

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5. Abbreviations: Boc, t-butyloxycarbonyl; Bzl, benzyl; DCC, 1,3-dicyclohexylcarbodiimide; DIEA, N,N-diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; HOBt, 1-hydroxybenzotriazole hydrate; Pd/C, palladium on carbon; TFA, trifluoroacetic acid.

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12. The antagonists CTAP (100 nM) and norBNI (20 nM) were examined in the GPI assay; NTI (1 nM) in the MVD assay.

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