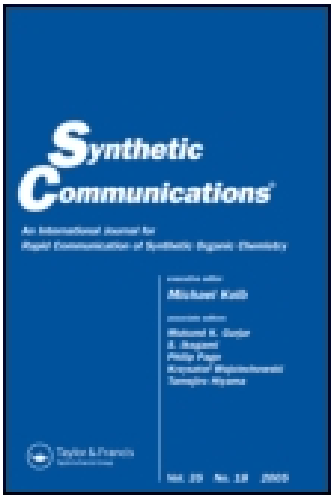

Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

Improved Procedure for the Synthesis of DAMGO

P. Anantha Reddy [a] , Anita H. Lewin [a] & F. Ivy Carroll [a]
[a] Organic and Medicinal Chemistry , Science and Engineering Group, RTI International , Research Triangle Park, North Cardina, USA
Published online: 11 Jul 2007.

To cite this article: P. Anantha Reddy , Anita H. Lewin & F. Ivy Carroll (2007) Improved Procedure for the Synthesis of DAMGO, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 37:14, 2345-2348, DOI: 10.1080/00397910701411044

To link to this article: http://dx.doi.org/10.1080/00397910701411044

Synthetic Communications®, 37: 2345–2348, 2007

ISSN 0039-7911 print/1532-2432 online
DOI: 10.1080/00397910701411044

Improved Procedure for the Synthesis of DAMGO

P. Anantha Reddy, Anita H. Lewin, and F. Ivy Carroll
Organic and Medicinal Chemistry, Science and Engineering Group,
RTI International, Research Triangle Park, North Cardina, USA

Abstract: A short and straightforward synthesis of DAMGO is described.

Keywords: DAMGO trifluoroacetate, μ-opioid receptor ligand, peptide synthesis

As a selective opioid μ-receptor-specific ligand, DAMGO (Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol) is a widely used peptide in investigations of μ-receptor-mediated pharmacology.[1,2] As part of our program on opioid peptides, we had developed a solution methodology suitable to the preparation of gram amounts of highly pure DAMGO. The synthetic scheme consisted of a $(3 + 1 + 1)$ approach in which synthetic Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OH (**1**, R = H) (Chart 1) was prepared starting from Boc-Nα-Me-Phe-OH in three steps to give TFA•DAMGO in 6% overall yield. We now report our new synthetic methodology for introduction of the Gly-ol residue. Specifically, we have determined that heating the tetrapeptide, Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OR (**1**, R = Me), in excess ethanolamine leads to amidation of the Nα-methylpenylalanine methyl ester residue cleanly and quantitatively. Thus, the protected tetrapeptide, Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OR (**1**, R = Me) was prepared in three steps (68% overall yield), following the previously described methodology. Condensation with ethanolamine

Received in the USA December 8, 2006
Address correspondence to P. Anantha Reddy, Organic and Medicinal Chemistry, Science and Engineering Group, RTI International, P.O. Box 12194, Research Triangle Park, North Cardina 27709-2194, USA. E-mail: ananth@rti.org

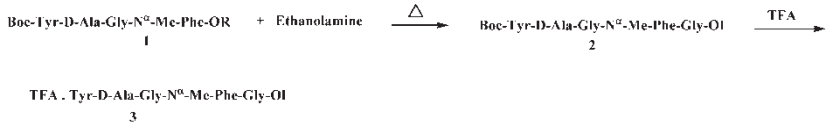

Chart 1.

quantitatively afforded the protected pentapeptide, Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (**2**). Deprotection of the Boc group using 50% TFA in CH_2Cl_2 afforded DAMGO trifluoroacetate (**3**) in 58% overall yield after purification by preparative high performance liquid chromatography (HPLC). The significantly higher yield resulted primarily from the improved yield obtained in the preparation of precursor tetrapeptide Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OR (**1**, R = Me) (68%) vs. the precursor Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OR (**1**, R = Bn) (25%) and in the introduction of the fifth Gly-ol residue (85% vs. 24%).

In summary, we have developed a racemization-free synthetic protocol that is relatively short and affords the product in high overall yield. We have prepared multigram quantities of highly pure DAMGO suitable for biophysical and pharmacological studies utilizing this procedure.

EXPERIMENTAL

^{1}H NMR spectra were determined on a Bruker 300 and 500 spectrometer using tetramethylsilane as an internal standard. Mass spectral data were obtained using a Finnegan LCQ electrospray mass spectrometer in positive ion mode at atmospheric pressure. Optical rotation was measured at the sodium D line using a Rudolph Research Autopol III Polarimeter. Thin-layer chromatography (TLC) was carried out on precoated plates (silica gel 60 F_{254}, 250 μm; Merck Darmstadt, Germany), and the following solvent systems were used (all v/v): EtOAc/EtOH (95:5); $CHCl_3$/MeOH/HOAc (80:18:2); n-BuOH/HOAc/H_2O/pyridine (15:3:12:10). HPLC was performed utilizing two Dynamax solvent-delivery system model SD-300 pumps and a model UV-D II Dynamax absorbance detector. Elemental analysis was carried out by Atlantic Microlabs, Inc.

Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OR (1, R = Me)

The synthesis of this intermediate was analogous to that reported for the preparation of Boc-Tyr-D-Ala-Gly-N$^{\alpha}$ -Me-Phe-OR (**1**, R = Bn).[3] Yield: 68%. TLC single spot, R_f 0.7 [EtOAc/EtOH (95:5)]; ^{1}H NMR (DMSO-d_6): δ 1.22 (3H, d, Ala $\beta C\mathbf{H}_3$), 1.31 [9H,s, $C(C\mathbf{H}_3)_3$], 2.71–2.78 (2H, m, Tyr $\beta C\mathbf{H}_2$), 2.76 (3H, s, Phe N$^{\alpha}C\mathbf{H}_3$), 3.04–3.16 (2H, m, Phe $\beta C\mathbf{H}_2$), 3.63

(3H, s, OC**H**$_3$), 3.68–3.71 (2H, m, Gly αC**H**$_2$), 4.1 (1H, m, Ala αC**H**), 4.3 (1H, m, Tyr αC**H**), 4.95 (1H, m, Phe αC**H**), 6.63 (2H, d, Tyr Ar**H**), 6.83 (1H, d, NH), 7.02 (2H, d, Tyr Ar**H**), 7.19–7.29 (5H, m, Ar**H**), 8.05 (2H, m, 2 × N**H**), and 9.14 (1H, s, Tyr O**H**); ESI MS, m/z 485.4 (M-Boc + H), 607.8 (M + Na).

Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (2)

A mixture of Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-OR (**1**, R = Me) (4.42 g, 7.2 mmol) and ethnolamine (20 mL) was heated at 60°C (oil bath) overnight. TLC [$CHCl_3$/MeOH/HOAc (80:18:2)] analysis showed complete disappearance of the starting material. The excess ethanolamine was evaporated under reduced pressure, and the residue was dissolved in methanol (25 mL). Upon addition of hexanes, a white precipitate formed. The precipitate was collected by filtration and washed with hexanes. This solid was vacuum dried to obtain the title compound as a white solid (4.65 g, 100%): TLC single spot, R_f 0.60 [EtOAc/MeOH/HOAc (80:18:2)]; ^{1}H NMR (MeOH-d_4) δ 1.15 (3H, dd, AlaβC**H**3), 1.3 [9H, s, C(C**H**$_3$)$_3$], 2.8 (3H, s, Phe N$^{\alpha}$C**H**$_3$), 2.85–3.0 (2H, m, PheβC**H**$_2$), 3.43–3.51 (2H, m, GlyαC**H**$_2$), 3.9 (1H, br s, Ala αC**H**), 4.0–4.15 (1H, m, Tyr αC**H**), 4.3–4.45 (1H, m, PheαC**H**), 6.65 (2H, d, Tyr Ar**H**), 6.95 (2H, d, Tyr A**rH**), 7.1–7.2 (5H, m, PheAr**H**); ESI MS m/z 614.7 (M + H), m/z 514.8 [M-Boc + H)], 637.2 (M + Na).

TFA • Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (3)

To a chilled solution of Boc-Tyr-D-Ala-Gly-N$^{\alpha}$-Me-Phe-Gly-ol (**2**) (4.0 g, 6.52 mmol) in CH_2Cl_2 (60 mL), TFA (40 mL). was added. The resultant mixture was stirred 1 h at room temperature. After evaporation of the volatiles, the residue was triturated with Et_2O (100 mL), causing formation of a white precipitate. The solid was collected by filtration, washed with ether (2 × 50 mL) and vacuum dried, affording the title compound as a white powder (3.93 g, 96%). The crude peptide was purified by preparative reversed phase HPLC on a Dynamax C_{18} column (8 m) (41.4 mm × 25 cm) using a gradient 10%B → 60%B over 30 min at 15-mL/min flow rate, with detection at 215 nm, solvent A being 0.1% TFA/H_2O, and solvent B being 0.1% TFA/CH_3CN. The peptide eluting at 10.2 min was collected and lyophilized to obtain a fluffy white sold in 85% yield. TLC, single spot, R_f, 0.75, [n-BuOH/HOAc/H_2O/pyridine (15:3:12:10); HPLC single peak (99%) R_t 10.71 min, on a Dynamax column (5 μ) (4.6 mm × 25 cm) using a gradient 10%B → 60%B over 25 min at 1.0 mL/min, with UV detection at 215 nm, solvent A being 0.10% TFA/H_2O, and solvent B being 0.10%TFA/CH_3CN. ESI MS m/z 514.8 (M + H); $[\alpha]_D^{25}$: +26.85 (c. 0.5 MeOH). Anal.

calcd. for $C_{28}H_{36}F_3N_5O_8 \cdot 0.33\ CF_3CO_2H \cdot 1.1\ H_2O$: C, 50.29; H, 5.62, F, 11.06, N, 10.22. Found: C, 50.26; H, 5.64; F, 10.06; N, 10.18.

ACKNOWLEDGMENT

This work was supported by the National Institute on Drug Abuse, Contract No. NO1DA-3-7736.

REFERENCES

1. Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. *Eur. J. Pharmacol.* **1981**, *70*, 531.
2. Kosterlitz, H. W.; Paterson, S. J. *Br. J. Pharmacol.* **1981**, *73*, 299.
3. Reddy, P. A.; Hayes, J. P.; Lewin, A. H.; Brine, G. A. *Org. Prep. Proc. Int.* **1995**, *27*, 469.