A Large-Scale Synthesis of [MeTyr^1, MeArg^7, D-Leu^8]Dynorphin A-(1—8)-NHEt (E-2078) by Application of the Trifluoroacetic Acid–Pentamethylbenzene Deprotecting Procedure in the Final Stage

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A large-scale synthesis of [MeTyr^1, MeArg^7, D-Leu^8]dynorphin A-(1—8)-NHEt (E-2078), which is a systemically active dynorphin A-(1—8) analogue, was accomplished by the classical solution method applying the trifluoroacetic acid–pentamethylbenzene deprotecting procedure in the final stage.

Keywords dynorphin A; octapeptide; large-scale synthesis; trifluoroacetic acid–pentamethylbenzene; E-2078

Recently, we demonstrated that a metabolically stable analogue of dynorphin A-(1—8), MeTyr–Gly–Gly–Phe–Leu–Arg–MeArg–D-Leu–NHEt (E-2078; **1**), not only retains opioid receptor selectivity similar to that of dynorphin A but also produces a more potent analgesic effect than morphine even when administered subcutaneously to mice.[1] We also reported the structure–activity relationships of dynorphin A-(1—8) analogues, together with a description of a small-scale synthesis of **1** by the solution method applying the HF deprotecting strategy.[1] In this paper, we describe a large-scale synthesis of **1** by application of a new deprotecting procedure, trifluoroacetic acid–pentamethylbenzene (TFA–PMB),[2] in the final stage.

In general, for the purpose of synthesizing a large amount of peptide, it seems desirable to apply a final acidolytic cleavage using a highly volatile, low-toxicity acid for easy handling. Recently, we found that TFA–PMB rapidly deprotects Tyr(Bzl) without formation of 3-benzyltyrosine (by-product) and that this agent deprotects Arg(Mtr) and Lys(Z) more rapidly than TFA alone.[2] The TFA–PMB method seemed to be superior to the TFA–thioanisole method[3] for the large-scale deprotection of Tyr(Bzl) residue, because 1-benzyl-2,3,4,5,6-pentamethylbenzene formed by the former can be more easily removed than benzylmethylphenylsulfonium ion formed by the latter. Therefore, we planned to synthesize **1** by the TFA–PMB strategy using MeTyr(Bzl), Arg(Mtr),[4] and MeArg(Mtr)[5] as side-chain-protected amino acids. This is the first application of the TFA–PMB deprotecting procedure to the synthesis of a peptide which is longer than kyotorphin (Tyr–Arg).

The synthetic route to the octapeptide is illustrated in Fig. 1. The C-terminal pentapeptide ethylamide, Z–Phe–Leu–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt (**2**), was prepared in a stepwise manner starting with H–D-Leu–NHEt. Each coupling was achieved by the mixed anhydride procedure except for the coupling of Z–Arg(Mtr)–OH with MeArg(Mtr)–D-Leu–NHEt (**3**), where a large amount of by-product was observed on thin-layer chromatography (TLC). Z–Arg(Tos)–OH was reported to have a tendency to form a 2-piperidone derivative when its carboxyl group was activated.[6] Therefore, the lactam seemed to be formed preferentially from the mixed anhydride prepared from

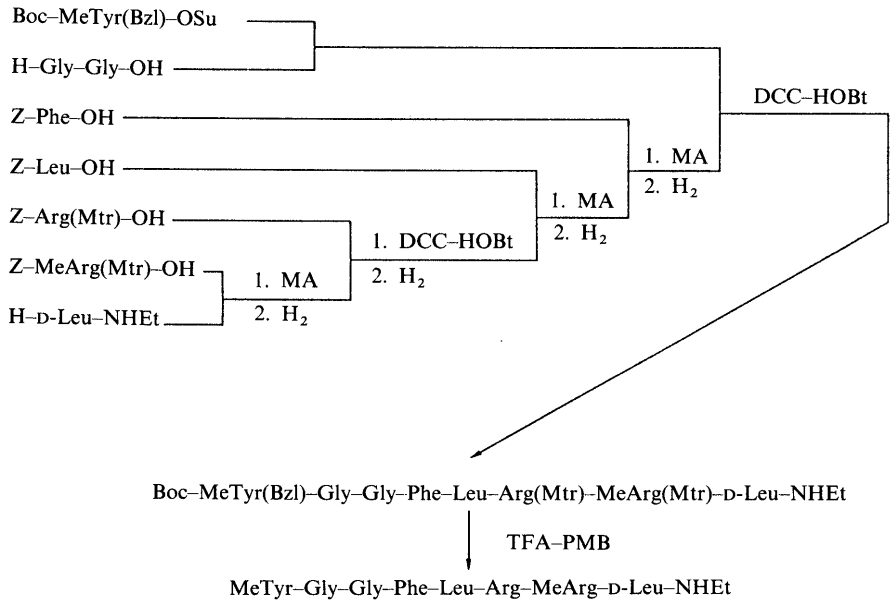

Fig. 1. Synthetic Route to [MeTyr^1, MeArg^7, D-Leu^8]Dynorphin A-(1—8)-NHEt

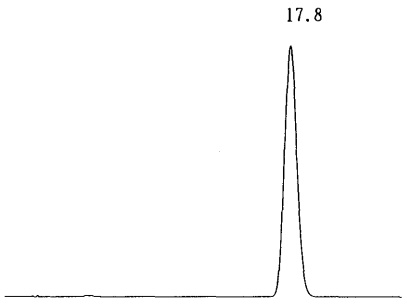

Fig. 2. Result of Analytical HPLC of [MeTyr[1], MeArg[7], D-Leu[8]]Dynorphin A-(1—8)-NHEt (OD at 220 nm)

Z–Arg(Mtr)–OH because of the severe steric hindrance around the amino group of the amine component (**3**). Our next attempt was conducted by changing the coupling method to DCC–HOBt. Furthermore, the concentration of each reagent in the solvent (DMF) was increased as much as possible in order to promote the intermolecular reaction. As a result, Z–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt was obtained in fairly good yield (48%). In this case, changing the solvent from DMF to THF resulted in a dramatically reduced yield, suggesting that the polarity of the solvent has a great influence on the reactivity of the reactants.

The N-terminal tripeptide, Boc–MeTyr(Bzl)–Gly–Gly–OH (**4**), was prepared by condensation of Boc–MeTyr(Bzl)–OSu with H–Gly–Gly–OH. The coupling of **4** with N^{α}-deprotected **2** to yield Boc–MeTyr(Bzl)–Gly–Gly–Phe–Leu–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt (**5**) was achieved by the DCC–HOBt method.

In the final step, the protected octapeptide (**5**) was treated with TFA–PMB overnight at 35 °C to ensure the complete removal of all the protecting groups. The resulting deprotected peptide was precipitated with ether, dissolved in water, and converted to the corresponding acetate with Amberlite IRA-93 (acetate form). The crude product was purified by reverse-phase column chromatography on YMC·GEL (ODS 60A 60/200 mesh) using H_2O–CH_3CN (90 : 10) containing 0.005% HCl as an eluent. A highly purified peptide (**1**) was obtained in 36% overall yield from the protected octapeptide (**5**). The homogeneity of this peptide was confirmed by TLC and analytical high performance liquid chromatography (HPLC) (Fig. 2). The peptide thus obtained possessed the same physicochemical properties and biological activities as those of an authentic sample synthesized by applying the usual HF deprotecting strategy.[1b]

On the basis of the present results it is clear that the TFA–PMB method is useful for the deprotection of the Tyr(Bzl) residue even in the case of large-scale peptide synthesis.

Experimental

General experimental methods employed in this investigation were essentially the same as described in our previous synthesis of dynorphin A-(1—8) analogues.[1] *Rf* values in TLC, performed on precoated silica gel plates (60F254, Merck), refer to the following solvent systems (v/v): Rf_1 MeOH–$CHCl_3$ (1 : 7) and Rf_2 MeOH–$CHCl_3$–AcOH (4 : 12 : 1). Analytical HPLC was conducted with a Shimadzu LC-6A pump and a Shimadzu SPD-6AV detector with a Inertsil ODS-2 (Gasukuro Kogyo Inc.) column (4.6 × 150 mm) using H_2O–CH_3CN (72 : 28) containing 0.5% $HClO_4$ as the eluent.

A mixed anhydride was prepared as follows: ethyl chloroformate (1 eq) was added at about −20 °C to a solution of a carboxy component (1 eq) and *N*-methylmorpholine (1 eq) in DMF. The mixture was stirred for 5 min and a solution of an amine component was added.

The following abbreviations are used: MeTyr, *N*-methyltyrosine; MeArg, N^{α}-methylarginine; Boc, *tert*-butoxycarbonyl; Z, benzyloxycarbonyl; Bzl, benzyl; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; TFA, trifluoroacetic acid; DCC, dicyclohexylcarbodiimide; HOSu, *N*-hydroxysuccinimide; HOBt, *N*-hydroxybenzotriazole; THF, tetrahydrofuran; DMF, dimethylformamide; IPE, diisopropyl ether.

Z–MeArg(Mtr)–D-Leu–NHEt A solution of H–D-Leu–NHEt [prepared by catalytic hydrogenation (10% Pd/C) of Z-D-Leu–NHEt (789.2 g, 2.7 mol)] in DMF (300 ml) was added to a mixed anhydride prepared from Z–MeArg(Mtr)–OH (1201 g, 2.25 mol) in DMF (2 l). The mixture was stirred at 0 °C for 4 h, then the solvent was evaporated off. The residue was dissolved in AcOEt and the solution was washed with 5% $NaHCO_3$, 5% citric acid, and water followed by concentration to dryness (1503 g, 99%): $[\alpha]_D^{20}$ −5.0° (c = 1.0, DMF), Rf_1 0.61. *Anal.* Calcd for $C_{33}H_{50}N_6O_7S$: C, 58.73; H, 7.47; N, 12.45. Found: C, 58.60; H, 7.51; N, 12.09.

Z–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt Z–Arg(Mtr)–OH (1172 g, 2.25 mol) and MeArg(Mtr)–D-Leu–NHEt (**3**) [prepared by catalytic hydrogenation (10% Pd/C) of the previously mentioned protected dipeptide (1503 g, 2.228 mol)] were dissolved in DMF (1.75 l). To this solution, HOBt·H_2O (413 g, 2.7 mol) and DCC (510 g, 2.48 mol) were added at 0 °C. The mixture was stirred at 4 °C overnight and at room temperature for 22 h, then the precipitate was filtered off and the solvent was removed *in vacuo*. The residue was dissolved in AcOEt and the solution was washed with 5% $NaHCO_3$, 5% citric acid, and water. After removal of the solvent, the residue was purified by column chromatography on silica gel (MeOH–$CHCl_3$, 1 : 15) (1109 g, 48%): $[\alpha]_D^{20}$ −15.0° (c = 1.0, DMF), Rf_1 0.58. *Anal.* Calcd for $C_{49}H_{74}N_{10}O_{11}S_2$: C, 56.41; H, 7.15; N, 13.34. Found: C, 56.32; H, 7.22; N, 13.46.

Z–Leu–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt A solution of H–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt [prepared by catalytic hydrogenation of the previously mentioned protected tripeptide (1148 g, 1.1 mol)] in DMF (2 l) was added to a mixed anhydride prepared from Z–Leu–OH (321 g, 1.21 mol) in DMF (3 l). The mixture was stirred at 0 °C for 4 h, then the solvent was evaporated off and the residue was dissolved in AcOEt. The solution was washed with 5% $NaHCO_3$, 5% citric acid, and water. The AcOEt was removed *in vacuo* and the residue was solidified with CH_2Cl_2–IPE (1215 g, 95%): $[\alpha]_D^{20}$ −21.0° (c = 1.0, DMF), Rf_1 0.54. *Anal.* Calcd for $C_{55}H_{85}N_{11}O_{12}S_2$: C, 57.12; H, 7.41; N, 13.32. Found: C, 56.93; H, 7.41; N, 12.93.

Z–Phe–Leu–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt (2) A solution of H–Leu–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt [prepared by catalytic hydrogenation of the previously mentioned protected tetrapeptide (1215 g, 1.05 mol)] in DMF (2 l) was added to a mixed anhydride prepared from Z–Phe–OH (346 g, 1.155 mol) in DMF (3.5 l). The mixture was stirred at 0 °C for 4 h, then the solvent was evaporated off and the residue was dissolved in AcOEt. The solution was washed with 5% $NaHCO_3$, 5% citric acid, and water. The AcOEt was removed *in vacuo* and the residue was solidified with CH_2Cl_2–IPE (1198 g, 88%): $[\alpha]_D^{20}$ −22.6° (c = 1.0, DMF), Rf_1 0.58. *Anal.* Calcd for $C_{64}H_{94}N_{12}O_{13}S_2$: C, 58.97; H, 7.27; N, 12.89. Found: C, 59.06; H, 7.28; N, 12.44.

Boc–MeTyr(Bzl)–Gly–Gly–OH (4) Boc–MeTyr(Bzl)–OH (325 g, 0.843 mol) and HOSu (107 g, 0.926 mol) were dissolved in THF (4.2 l) and DCC (174 g, 0.843 mol) was added at 0 °C. The mixture was stirred at 4 °C overnight, then the precipitate was removed by filtration and a solution of H–Gly–Gly–OH (123 g, 0.926 mol) and $NaHCO_3$ (78 g, 0.926 mol) in water (1.7 l) was added. The mixture was stirred at 4 °C overnight, then the solvent was evaporated off and 10% citric acid and AcOEt were added to the residue. The AcOEt layer was separated and washed with water. The solvent was removed *in vacuo* and the residue was solidified with *n*-hexane (302 g, 72%): $[\alpha]_D^{20}$ −39.6° (c = 1.0, DMF), Rf_2 0.64. *Anal.* Calcd for $C_{26}H_{33}N_3O_7$: C, 62.50; H, 6.66; N, 8.41. Found: C, 62.67; H, 6.75; N, 8.43.

Boc–MeTyr(Bzl)–Gly–Gly–Phe–Leu–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt (5) Boc–MeTyr(Bzl)–Gly–Gly–OH (**4**) (516 g, 1.03 mol), HOBt·H_2O (184 g, 1.20 mol), and H–Phe–Leu–Arg(Mtr)–MeArg(Mtr)–D-Leu–NHEt [prepared by catalytic hydrogenation of the previously mentioned protected pentapeptide (**2**) (1122 g, 0.86 mol)] were dissolved in DMF (3 l) and DCC (213 g, 1.03 mol) was added at 0 °C. The mixture was stirred at 4 °C overnight and at room temperature for 8 h, then the precipitate was filtered off. The solvent was removed *in vacuo* and the residue was dissolved in AcOEt. The solution was washed with 5% $NaHCO_3$ and water. The AcOEt was removed *in vacuo* and the residue was solidified

with CH_2Cl_2–IPE (1369 g, 96%): $[\alpha]_D^{20}$ −26.1° (c=1.0, DMF), Rf_1 0.53. *Anal.* Calcd for $C_{82}H_{119}N_{15}O_{17}S_2 \cdot H_2O$: C, 59.01; H, 7.31; N, 12.59. Found: C, 58.92; H, 7.21; N, 12.53.

MeTyr–Gly–Gly–Phe–Leu–Arg–MeArg–D-Leu–NHEt (1) TFA (5 l) was added to a solution of the previously mentioned protected octapeptide (**5**) (300 g, 0.182 mol), anisole (450 ml, 4.14 mol), and pentamethylbenzene (900 g, 6.07 mol) in CH_2Cl_2 (2 l). The mixture was stirred overnight at 35 °C, then TFA and CH_2Cl_2 were removed by evaporation *in vacuo* and ether was added. The resulting powder was collected by filtration and dissolved in water. The solution was treated with Amberlite IRA-93 (acetate form) and filtered by suction. The filtrate was applied to a column of YMC·GEL (ODS 60A 60/200 mesh, 10 × 120 cm), which was eluted first with 0.1% HCl (15 l) and then with H_2O–CH_3CN (90 : 10) containing 0.005% HCl (50 l). The main fractions (monitored by UV measurement at 218 nm) were collected, the solvent was removed by evaporation *in vacuo*, and the residue was lyophilized to give a powder (85 g, 36%). The product obtained here was identical with the authentic sample previously prepared.[1b]

References and Notes

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