BIOORGANIC CHEMISTRY **8,** 323–332 (1979)

The Triphenylphosphine–Sulfur Trioxide Adduct as a Coupling Reagent in Peptide Synthesis[1]

I. J. GALPIN, G. W. KENNER, AND A. MARSTON

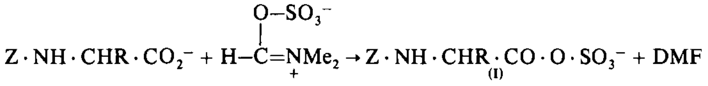
The Robert Robinson Laboratories, University of Liverpool, Liverpool L69 3BX, England

Received February 1, 1979

Several adducts of sulfur trioxide with electron-donor molecules have been prepared. Of these the air-stable triphenylphosphine–SO_3 complex has been shown to be the most useful in the synthesis of peptides. It has been demonstrated that a variety of solvents may be used for couplings, but that in order for reaction to occur the solvent must itself be able to form an adduct. This behaviour is due to the fact that SO_3 is transferred from the triphenylphosphine–SO_3 complex to the solvent, thus forming an intermediate complex which is responsible for activation of the carboxylate unit. The carboxyl component is generally present as its tetramethylguanidinium salt. The method has been used to synthesize several model peptides and to prepare a fully biologically active sample of methionine–enkephalin.

INTRODUCTION

The complex formed between *N*,*N*-dimethylformamide (DMF) and sulfur trioxide was originally investigated as a coupling reagent in peptide synthesis in the early 1950s (*1*, *2*). The reagent was prepared by direct interaction of DMF with sulfur trioxide at low temperature, but when formed was rather hygroscopic and could not be reliably stored for any length of time. In spite of the difficulties of working with this complex it was allowed to react with *N*-protected amino acid salts to form a sulfuric mixed anhydride (**I**) which was used as the activated species in peptide coupling reactions.



$$Z\cdot NH\cdot CHR\cdot CO_2^- + H{-}\overset{\overset{\displaystyle O{-}SO_3^-}{|}}{C}{=}\underset{+}{N}Me_2 \rightarrow \underset{(I)}{Z\cdot NH\cdot CHR\cdot CO\cdot O\cdot SO_3^-} + DMF$$

Good yields of products were obtained, and it was shown by analysis using countercurrent distribution of the products that the coupling of benzyloxycarbonylglycylalanine[2] with phenylalanylglycine ethyl ester was free of racemization (*2*). However, as the compound was very difficult to work with, due to its hygroscopic nature, it was not used widely in peptide synthesis. As the problems encountered when using the reagent were not associated with the activated coupling species but with the source of the sulfur trioxide, it seemed appropriate to reinvestigate the method by looking for a new

---

[1] This manuscript is dedicated to the memory of Professor George Kenner and describes an area of Chemistry which had intrigued him for many years.

[2] All amino acids are of the L-configuration unless specified and nomenclature follows Specialist Periodical Reports, "Amino-acids, Peptides and Proteins" (G. T. Young, Ed.), Vol. 4, Chapter 5, Chemical Society, London, 1972.

0045-2068/79/030323–10$02.00/0

Printed in Great Britain

"electron-donor reagent" with which to form a sulfur trioxide adduct. Ideally such an adduct should be a crystalline, nonhydroscopic solid with the ability to act as a moderated source of sulfur trioxide.

RESULTS AND DISCUSSION

A wide range of sulfur trioxide adducts was prepared in order to study their stability and potential use in peptide-bond formation (Table 1). Two methods of preparation have been used. The first involves distilling sulfur trioxide from oleum, then adding the liquid sulfur trioxide to a solution of the electron-donor reagent in an inert solvent (CH_2Cl_2) at low temperature. In the second method chlorosulfonic acid is added to a cooled solution of the electron-donor reagent in an inert solvent. Both methods employ anhydrous conditions for the formation of the complex.

TABLE 1

SULFUR TRIOXIDE ADDUCTS WITH ELECTRON-DONOR MOLECULES

Electron-donor	Method[a]	Stability	Reference
N,*N*-Dimethylformamide	A	Hygroscopic	(*1*, *2*)
N,*N*-Diphenylformamide	A	Hygroscopic	—
N,*N*-Dimethylacetamide	A	Hygroscopic	(*3*)
Tetramethylurea	A	Hygroscopic	(*4*)
N,*N*,2,2-Tetramethylpropionamide (*12*)	A	Hygroscopic	—
N-Methylpyrrolidone	A	Hygroscopic	—
Dimethylsulfoxide	A	Hygroscopic	(*5*)
Pyridine	A, B	Air stable	(*6*, *7*)
Triethylamine	A, B	Air stable	(*8*)
Tri-*n*-butylamine	A	Air stable	(*9*)
1,4-Diazabicyclo-[2.2.2]-octane	A	Hygroscopic	—
Hexamethylphosphoramide	A	Hygroscopic	(*10*)
Triphenylphosphine oxide	A	Hygroscopic	(*11*)
Triphenylphosphine	A, B	Air stable	(*11*)

[a] Method: (A) Direct reaction of SO_3 with electron-donor molecule. (B) Reaction of chlorosulfonic acid with electron-donor molecule.

The adducts are all white, crystalline solids; but many are hygroscopic, and the air-stable adducts with tertiary amines defied all attempts at recrystallization. In contrast, the stable adduct formed with triphenylphosphine could easily be recrystallized from acetonitrile, giving air-stable crystals with a melting point of 196°C.

All the adducts could be used for the formation of peptide bonds providing that moisture was rigorously excluded, for example, the hexamethylphosphoramide–SO_3 adduct gave an 85% yield of the dipeptide Z · Ala · Gly · OMe. As a result of the initial studies the triphenylphosphine–SO_3 adduct was selected for further study, as it was clearly the most easily handled.

The general procedure for peptide bond formation using the reagent may be summarized as follows: The suitably protected carboxyl component is dissolved in dry

TABLE 2

BASES USED IN THE PREPARATION OF Z·ALA·GLY·OME USING $Ph_3\underset{+}{P}-SO_3^-$

Base	Yield of dipeptide (%)
LiOMe	81
Tetramethylguanidine	85
Diisopropylethylamine	67
N-Ethylpiperidine	40
N-Methylmorpholine	5
2,6-Lutidine	0

DMF and base (1 equivalent) added to form the carboxylate salt. After addition of the SO_3 complex, 1 hr is allowed for activation, the amino component is then added, and the reaction is stirred for a further 2 hr. Usually a simple acid/base wash yields the required product after crystallization although occasionally column chromatography has been used to isolate the product.

In the early studies on the DMF–SO_3 complex (*1*, *2*), lithium methoxide was used to generate the carboxylate anion. However, this base was not entirely satisfactory, as the lithium carboxylate salts were not always sufficiently soluble. A range of bases was examined for suitability for use in combination with the triphenylphosphine–SO_3 adduct (Table 2), the suitability of the base being assessed by measuring the yield of dipeptide obtained by coupling benzyloxycarbonylalanine and glycine methyl ester hydrochloride. A second equivalent of the base was needed to liberate glycine methyl ester from its hydrochloride. From the yields in Table 2 it is clear that tetramethylguanidine (TMG) is a good alternative for lithium methoxide, although the high basicity of both of these bases might reasonably give some concern when the possibility of racemization during coupling is considered. Unfortunately the frequently employed base *N*-methylmorpholine gave a very low yield of product and was not considered to be suitable.

The choice of solvent for the coupling reaction is another important factor which has a marked effect on product yield. Table 3 shows a variety of solvents which have been employed, and from the available data it may be seen that solvents which are them-

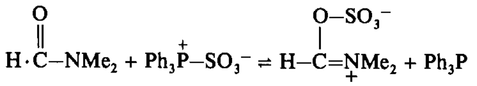
TABLE 3

SOLVENTS USED FOR THE FORMATION OF MIXED ANHYDRIDE

Solvent	Yield of Z · Ala · Gly · OMe (%)
N,*N*-Dimethylformamide	85
Hexamethylphosphoramide	57
N-Methylpyrrolidone	62
Pyridine	30
Dimethylsulfoxide	0
Acetonitrile	0
2,2,2-Trifluorethanol	0
Dichloromethane	0

selves able to form SO_3 adducts give the highest yield when the triphenylphosphine–SO_3 adduct is used for coupling.

This finding prompted us to look in more detail at the mechanism of the reaction in DMF. Both tlc and ^{31}P nmr showed that triphenylphosphine (+5.8 ppm) was produced when the triphenylphosphine–SO_3 complex was dissolved in DMF, although no triphenylphosphine was formed when the complex was dissolved in dichloromethane. Also if the DMF solution was quenched with water after 1 hr 71% of the triphenylphosphine–SO_3 adduct was recovered along with triphenylphosphine (29%). This observation prompted us to speculate that when triphenylphosphine–SO_3 is dissolved in DMF the equilibrium shown below is set up:

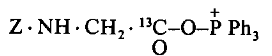


$$\mathrm{H\cdot\overset{\overset{O}{\|}}{C}{-}NMe_2 + Ph_3\overset{+}{P}{-}SO_3^- \rightleftharpoons H{-}\overset{\overset{O-SO_3^-}{|}}{C}{=}\underset{+}{N}Me_2 + Ph_3P}$$

Normally, the position of equilibrium lies largely to the left; but if a carboxylate is present, the equilibrium moves over to the right because the carboxylate reacts with the DMF–SO_3 adduct. We believe that such a transfer of sulfur trioxide by the triphenylphosphine adduct to an intermediate complex (in this case DMF–SO_3) is an essential part of the activation process, as no coupling whatsoever is observed in solvents which are unable to form intermediate sulfur trioxide complexes (e.g., CH_2Cl_2).

In order to study the mechanism from a different viewpoint, the reaction with $Z\cdot NH\cdot CH_2\cdot {}^{13}CO_2^-$ $TMG\cdot H^+$ was examined. The enriched anion showed a single peak at 174.3 ppm in DMF; addition of the triphenylphosphine–SO_3 adduct immediately produced a single peak at 174.9 ppm which was attributed to the sulfuric mixed anhydride (I). Gradually a small peak appeared at 170.5 ppm, ascribable to the symmetrical anhydride, by comparison with authentic material. It appears that this is formed by disproportionation of the sulfuric mixed anhydride, as all the ^{13}C-labeled carboxylate was consumed in the initial reaction, thus excluding the reaction of carboxylate with the sulfuric mixed anhydride as a source of the symmetrical anhydride.

The singlet nature of the signal from the activated intermediate rules out any possibility of attack of carboxylate on the phosphorous atom of the adduct, as this would lead to an acyloxyphosphonium salt as shown:



$$\mathrm{Z\cdot NH\cdot CH_2\cdot {}^{13}\underset{O}{C}{-}O{-}\overset{+}{P}\,Ph_3}$$

Such a system would be expected to exhibit a $^{13}C/^{31}P$ interaction as has been shown for the related compound $Z\cdot NH\cdot CH_2\cdot {}^{13}CO\cdot O\cdot PO\cdot Ph_2$, which has a coupling constant of 9.5 Hz (the signal appears at 169.1 ppm).

When an amine component was added, a new peak rapidly appeared which was due to the corresponding amide. Thus it appears that the majority of the reaction proceeds through the sulfuric mixed anhydride (I), especially when the amine component is added within 1 hr of activation. If a high proportion of the reaction involved the symmetrical anhydride, a signal due to $Z\cdot NH\cdot CH_2\cdot {}^{13}CO_2^-$ would be apparent in the nmr.

As the triphenylphosphine–SO_3 furnishes a highly activated mixed anhydride, we checked the extent of racemization using the Izumiya test (*13*), expressing the extent of racemization according to the Bodanszky formula (*14*) (see Table 4). From these results it may be seen that in the sensitive Izumiya test a small degree of racemization

TABLE 4

EXTENT OF RACEMIZATION BY THE IZUMIYA TEST

Coupling agent	Racemization[a]
$DMF-SO_3$[b]	1.6
$Ph_3\overset{+}{P}-SO_3^-$[c]	32.4
$Ph_3\overset{+}{P}-SO_3^-$[b]	8.9
DCCI[b]	18.2

[a] Percentage racemization = 100 × [D-isomer]{[L-isomer] + [D-isomer]} (*14*).

[b] TMG as base.

[c] LiOMe as base.

was observed using the original DMF–SO$_3$ method, although the extent of racemization is still low for a mixed anhydride method. Use of the triphenylphosphine–SO$_3$ adduct with lithium methoxide as base gave massive racemization, although this dropped dramatically when tetramethylguanidine was used as base.

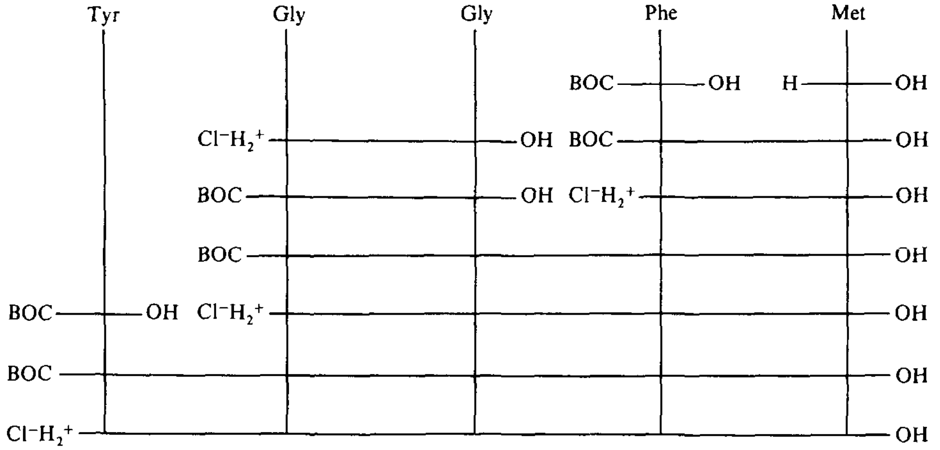
In order to demonstrate the use of the triphenylphosphine–SO$_3$ adduct in the synthesis of small model peptides, the compounds shown in Table 5 were prepared. The carboxyl component was activated in DMF solution for 1 hr at room temperature using tetramethylguanidine as the base. The amine component was then added in either DMF or water depending on whether or not a salt coupling procedure was being employed. The yields were variable in these model systems, but sufficient confidence in the method was obtained to encourage us to apply the technique to the synthesis of a small biologically active test peptide.

Methionine–enkephalin (*15*) was chosen as the test peptide, as the biological activity is well established and may be easily assayed. Before the synthesis was commenced the stability of methionine and tyrosine to the triphenylphosphine–SO$_3$ adduct was checked, showing that protection of the side chains of these residues was not necessary. The salt coupling approach was used throughout as indicated in Scheme 1; *t*-butoxycarbonyl-phenylalanine, -glycylglycine, and -tyrosine were added stepwise. At the intermediate stages the hydrochlorides were obtained by cleavage of the BOC-groups with hydrogen chloride. The final coupling of *t*-butoxycarbonyltyrosine gave a relatively

TABLE 5

REPRESENTATIVE PEPTIDES PREPARED USING $Ph_3\overset{+}{P}-SO_3^-$

Peptide	Solvent (NH_2 component)	Yield (%)
(Tos·Gly-cyclohexylamide)	—	82
Z·Ala·Gly·OMe	DMF	85
Z·Ala·Gly·OMe	H_2O	66
Z·Ala·Gly·OH	H_2O	41
Z·Gly·Ala$_3$·OH (2 + 2 coupling)	H_2O	56
Dpp·Ala·Gly·OMe	DMF	59



SCHEME 1. Synthesis of methionine–enkephalin using Ph$_3\overset{+}{P}$–SO$_3^-$

poor yield (39%); but the other two couplings were achieved in good yield, thus demonstrating the efficiency of the reagent. The final product was deprotected and chromatographed on Sephadex G25, the free peptide obtained after lyophilization showing full biological activity when tested *in vitro* [guinea pig ileum test (*16*)].

In conclusion, an air-stable, easy to prepar sulfur trioxide adduct has been obtained which has some value in peptide synthesis, not least because unreacted carboxyl components can be recovered after coupling. Clearly, racemization is a problem as with most, if not all, mixed anhydride forming reagents. However, by employing a stepwise approach during peptide synthesis the effect of this disadvantage can be minimized. The adducts formed between electron-donor molecules and sulfur trioxide may thus be added to the considerable armory of reagents which may be used to form the peptide bond.

EXPERIMENTAL

General Procedures

Thin layer chromatograms were run on Merck Kieselgel 60$_{F254}$ pre-prepared thin layer plates eluting with: (A) CHCl$_3$/^{i}PrOH/AcOH (45 : 5 : 1), (B) ^{n}BuOH/AcOH/H$_2$O (3 : 1 : 1), (C) CHCl$_3$/MeOH/AcOH (18 : 2 : 1), or (D) EtOAc/Py/AcOH/H$_2$O (60 : 20 : 6 : 11). The compounds were visualized by uv light (254 nm), by treatment with fluorescamine (*17*) or by the chlorine–starch–potassium iodide method (*18*). Amino acid analyses were carried out using a Jeol 6AH analyzer, generally after peptides had been subjected to acid hydrolysis (6 *M* HCl, 105°C, 24 hr). Optical rotations were determined using a Bendix Type 143 automatic polarimeter. The ^{31}P(CW) and ^{13}C(FT) nmr spectra were recorded on a Varian XL100 nmr spectrometer, the infrared spectra on a Pye Unicam SP200 spectrophotometer. All solvents were purified by standard methods except DMF which was subjected to azeotropic distillation using 10% benzene, then fractionally distilled from calcium hydride and stored over 3A molecular sieves.

Synthesis

Triphenylphosphine–SO_3 adduct. (a) SO_3 method: Triphenylphosphine (6.5 g, 25 m*M*) was dissolved in dry distilled CH_2Cl_2 (40 ml) and the solution cooled to −10°C in a cardice/acetone bath. Sulfur trioxide (2 ml, 25 m*M*) which had been freshly distilled from 60% oleum was slowly added to the stirred solution over 5 min while maintaining the temperature at −10°C. The resulting pale yellow solid was filtered, washed with Et_2O (3 × 20 ml) and air dried, giving the crude product (7.7 g, 90%), mp 188°C. Recrystallization from hot acetonitrile gave a white, highly crystalline solid (6.3 g, 74%) mp 196°C [mp 191°C (*11*)]; infrared (nujol mull) ν 680, 720, 745 cm^{-1} (aromatic C–H str), 1260 cm^{-1} ($-SO_3$ str).

Anal. Calcd for $C_{18}H_{15}O_3PS$: C, 63.25; H, 4.42. Found: C, 63.50; H, 4.66.

(b) $Cl\cdot SO_3H$ method: Triphenylphosphine (6.5 g, 25 m*M*) was dissolved in distilled dioxan (40 ml) and chlorosulfonic acid (2.9 g, 25 m*M*) was added over 10 min to the stirred solution at room temperature. The white precipitate was filtered, washed with Et_2O (3 × 20 ml), and air-dried to give the required adduct (7.4 g, 86%); mp 196°C; infrared (nujol mull) ν 1260 cm^{-1} ($-SO_3$ str), identical to that obtained from method (a).

Anal. Calcd for $C_{18}H_{15}O_3PS$: C, 63.25; H, 4.42. Found: C, 63.11; H, 4.48.

N-Toluene-p-sulfonylglycine cyclohexylamide. N-p-Toluenesulfonylglycine (229 mg, 1 m*M*) was dissolved in DMF (30 ml) and TMG (115 mg, 1 m*M*) was added. Triphenylphosphine–SO_3 (342 mg, 1 m*M*) was added and the solution stirred at room temperature for 1 hr before cyclohexylamine (1 ml, 8 m*M*) in DMF (2 ml) was added. A white precipitate formed immediately but stirring was continued for a further hour before evaporating to dryness. The resulting solid was partitioned between 10% citric acid and EtOAc. After separation, the aqueous phase was further extracted with EtOAc and the combined organic phases were washed with saturated $NaHCO_3$ solution. The EtOAc solution was then washed with brine, dried over Na_2SO_4, and evaporated to give an oil which was crystallized from Et_2O/40–60 petroleum ether. The crude product was crystallized from EtOAc/hexane giving the required amide (255 mg, 82%); mp 114°C [mp 115°C (*2*)]; R_f(A) −0.65.

Anal. Calcd for $C_{15}H_{22}O_3N_2S$: C, 58.04; H, 7.15; N, 9.03. found: C, 57.96; H, 7.23; N, 8.98.

N-benzyloxycarbonylalanylglycine methyl ester. N-Benzyloxycarbonylalanine (223 mg, 1 m*M*) and TMG (115 mg, 1 m*M*) were dissolved in DMF (30 ml), the triphenylphosphine–SO_4 adduct (342 mg, 1 m*M*) was added, and 1 hr allowed for activation at room temperature. A solution of glycine methyl ester hydrochloride (126 mg, 1 m*M*) and TMG (230 mg, 2 m*M*) in DMF (10 ml) was added and the reaction stirred for a further 3 hr. Evaporation of the reaction mixture gave an oil which was dissolved in EtOAc. This solution was washed with 10% citric acid, saturated $NaHCO_3$, and brine then dried over Na_2SO_4 before evaporating to give an oil. This oil was crystallized from $Et_2O/{}^iPr_2O$ (1/1) then recrystallized from EtOAc/hexane giving the pure dipeptide (250 mg, 85%); mp 97–98°C [mp 98–99°C (*19*)]; R_f(A) −0.70; $[\alpha]_D^{20} = -24.9°$ (c = 5, MeOH) $[\alpha]_D^{20} = -25.1°$ [c = 5, MeOH (*19*)].

Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 57.13; H, 6.16; N, 9.52. Found: C, 57.24; H, 6.02; N, 9.69.

N-Benzyloxycarbonylglycylalanylalanylalanine. N-Benzyloxycarbonylglycylalanine (70 mg, 0.25 m*M*) and TMG (29 mg, 0.25 m*M*) were dissolved in DMF (10 ml), triphenylphosphine–SO_3 (86 mg, 0.25 m*M*) was added and the solution stirred for 1 hr. A solution of analylalanine *p*-toluene-sulfonate (83 mg, 0.25 m*M*) in 1 *M* NaOH at pH 7.5 was added over 10 min and the solution maintained at pH 7.5 for 3 hr using a pH stat to control addition of 1 *M* NaOH. The reaction mixture was evaporated to dryness and the residue dissolved in EtOAc. This solution was washed with 10% citric acid, dried over Na_2SO_4, and evaporated to yield an oil. This oil was dissolved in DMF and chromatographed on Sephadex LH20, eluting with DMF. Fractions containing the product were evaporated and the residue crystallized from EtOAc giving the required product (59 mg, 56%); mp 198–200°C; R_f(B) –0.74.

Anal. Calcd for $C_{13}H_{16}N_2O_5 \cdot 1/2H_2O$: C, 52.89; H, 6.31; N, 12.99. Found: C, 53.13; H, 6.16; N, 13.23.

Amino Acid Analysis: $Ala_{3.01}\,Gly_{0.99}$.

N-Diphenylphosphinylalanylglycine methyl ester. N-Diphenylphosphinylalanine (*20*) (289 mg, 1 m*M*) and TMG (115 mg, 1 m*M*) were dissolved in DMF (30 ml), triphenylphosphine–SO_3 (342 mg, 1 m*M*) was added, and the reaction mixture was stirred for 1 hr. A solution of glycine methyl ester hydrochloride (126 mg, 1 m*M*) and TMG (230 mg, 2 m*M*) in DMF (10 ml) was then added and the reaction mixture was stirred for a further 3 hr. The solution was evaporated and the residue dissolved in EtOAc, this solution being worked up by an acid/base washing procedure and the product crystallized from $^{i}Pr_2O$/hexane (211 mg, 59%); mp 130°C; R_f(A) –0.35; $[\alpha]_D^{25} = -35.7°$ ($c = 1$, DMF).

Anal. Calcd for $C_{18}H_{21}N_2O_4P$: C, 59.99; H, 5.88; N, 7.78. Found: C, 60.24; H, 6.06; N, 7.74.

Izumiya racemization test (*13*). *N*-Benzyloxycarbonylglycylalanine (70 mg, 0.25 m*M*) and TMG (28 mg, 0.25 m*M*) were dissolved in DMF (15 ml). Triphenylphosphine–SO_3 (86 mg, 0.25 m*M*) was added and the resulting solution stirred for 1 hr. A solution of leucine benzyl ester *p*-toluene–sulfonate (100 mg, 0.25 m*M*) and TMG (28 mg, 0.25 m*M*) in DMF (10 ml) was then added and the reaction mixture stirred for a further 3 hr.

Evaporation gave an oil which was dissolved in EtOAc, washed with 10% citric acid, saturated $NaHCO_3$ and dried. Evaporation again yielded an oil which was dissolved in 90% AcOH (10 ml). Ten percent Pd/C (300 mg) was added and hydrogen passed through the solution for 48 hr. After removing the catalyst by filtration a sample (1 ml) of the tripeptide solution was mixed with pH 4.25 buffer (5 ml) and introduced to the short column of the Jeol 6AH amino acid analyzer elution: pH 3.20 buffer, 60 ml/hr at 57°C). Glycyl-L-analyl-L-alanine was eluted at 74 min and glycyl-D-alanyl-L-alanine at 93 min and the extent of racemization was calculated to be 8.9% from the integration ratios.

Methionine–Enkephalin

N-t-Butoxycarbonylphenylalanylmethionine. N-t-Butoxycarbonylphenylalanine (2.39 g, 9 m*M*) and TMG (1.04 g, 9 m*M*) were dissolved in DMF (200 ml) and triphenylphosphine–SO_3 (3.08 g, 9 m*M*) was added. After stirring for 1 hr, a solution of methionine (1.34 g, 9 m*M*) and TMG (2.0 g, 18 m*M*) in DMF (80 ml) and water (20 ml) was added and the reaction mixture stirred overnight. Evaporation gave an oil which was dissolved in EtOAc. This solution was partitioned with 10% citric acid and the product extracted from the organic phase with saturated $NaHCO_3$. The alkaline solution was acidified and partitioned with EtOAc and after drying the EtOAc over Na_2SO_4 the product was crystallized by the addition of 40–60 petroleum ether giving (2.3 g, 65%); mp 134–137°C, [mp 138–140°C (*21*)]; R_f(A) –0.49, R_f(B) –0.71; $[\alpha]_D^{20} = -11.2°$ ($c = 1$, DMF), $[\alpha]_D^{20} = -9.8°$ ($c = 1$, DMF) (*21*).

Anal. Calcd for $C_{19}H_{28}N_2O_5S$: C, 57.55; H, 7.12; N, 7.07. Found: C, 57.44; H, 7.10; N, 6.82.

N-t-Butoxycarbonylglycylglycylphenylalanylmethionine. *N-t*-Butoxycarbonylphenylalanylmethionine (1.00 g, 2.5 m*M*) was dissolved in EtOAc (20 ml) containing dimethylsulfide (7 ml, 100 m*M*) and a 27% w/v solution of HCl in EtOAc (20 ml) was added. After 1.5 hr the solution was evaporated to give the white homogenous solid hydrochloride [R_f(B) –0.65]. A solution of *N-t*-butoxycarbonylglycylglycine (0.46 g, 2 m*M*) and TMG (0.23 g, 2 m*M*) in DMF (50 ml) was reacted with triphenylphosphine–SO_3 (0.68 g, 2 m*M*) for 1 hr. A solution of the above hydrochloride (0.8 g, 2 ml) in DMF (20 ml) was added with TMG (0.46 g, 4 m*M*) and 3 hr allowed for completion of the coupling reaction. The solution was evaporated and the resulting oil partitioned between 10% citric acid and EtOAc. The product was extracted from the EtOAc solution with saturated $NaHCO_3$ and the alkaline solution was then acidified with 10% acid and reextracted into EtOAc. After washing with brine, the organic phase was dried (Na_2SO_4) and evaporated to give the product as a white solid. Recrystallization from ^{i}PrOH/Et_2O gave the pure protected tetrapeptide acid (0.8 g, 78%); mp 118–122°C; R_f(B) –0.80; $[\alpha]_D^{20} = -14.5°$ ($c = 1$, DMF).

Anal. Calcd for $C_{23}H_{34}N_4O_7S$: C, 54.10; H, 6.71; N, 10.98. Found: C, 53.89; H, 6.83; N, 10.85.

Glycylglycylphenylalanylmethionine hydrochloride. *N-t*-Butoxycarbonylglycylglycylphenylalanylmethionine (510 mg, 1 m*M*) was dissolved in peroxide-free dioxan (20 ml) containing dimethylsulfide (3.5 ml, 50 m*M*). A 27% w/v solution of HCl in EtOAc (10 ml) was added and the required tetrapeptide hydrochloride slowly precipitated from the solution. After 1.5 hr the reaction mixture was filtered giving the product (350 mg, 78%); mp 198°C; R_f(B) –0.47 (homogeneous).

Anal. Calcd for $C_{18}H_{27}N_4O_5SCl$: C, 48.37; H, 6.39; N, 12.54. Found: C, 47.90; H, 6.39; N, 12.32.

N-t-Butoxycarbonyltyrosylglycylglycylphenylalanylmethionine. Triphenylphosphine–SO_3 (171 mg, 0.5 m*M*) was added to a solution of *N-t*-butoxycarbonyltyrosine (70 mg, 0.5 m*M*) and TMG (57 mg, 0.5 m*M*) in DMF (10 ml). After 1 hr a solution of glycylglycylphenylalanylmethionine hydrochloride (224 mg, 0.5 m*M*) and TMG (115 mg, 1 m*M*) in DMF (10 ml) was added and the reaction mixture stirred for 3 hr. The solution was evaporated to give a yellow oil which was partitioned between EtOAc and 10% citric acid. The organic phase was extracted with saturated $NaHCO_3$ and the alkaline extract acidified with 10% citric acid. This acidic solution was then extracted with EtOAc (3×) and the combined extracts were washed with brine and dried over Na_2SO_4. Evaporation of the solvent gave an oil which was dissolved in a small volume of EtOAc, the required product precipitating as a white solid by the addition of Et_2O (130 mg, 39%); mp 138°C; R_f(B) –0.78, R_f(D) –0.50; $[\alpha]_D^{20} = -17.4°$ ($c = 1$, DMF).

Anal. Calcd for $C_{32}H_{43}N_5O_9S$: C, 57.04; H, 6.43; N, 10.40. Found: C, 57.05; H, 6.55; N, 10.25.

Tyrosylglycylglycylphenylalanylmethionine hydrochloride (methionine-enkephalin hydrochloride). *N-t*-Butoxycarbonyltyrosylglycylglycylphenylalanylmethionine (120 mg, 0.18 m*M*) was dissolved in a mixture of EtOAc (20 ml) and peroxide-free dioxan (15 ml) containing dimethylsulfide (1 ml, 14 m*M*). A 27% w/v solution of HCl in EtOAc (10 ml) was added and the solution stirred for 2 hr during which time some precipitate slowly formed. Evaporation gave a viscous oil which was dissolved in water (2 ml) and subjected to gel filtration on Sephadex G25, eluting with water. The fractions containing the product were combined and lyophilized, to give methionine–enkephalin hydrochloride as a white, slightly hygroscopic powder (77 mg, 70%); mp 164°C; R_f(B) 0.59; $[\alpha]_D^{20} = -21.4$ ($c = 1$, DMF).

Anal. Calcd for $C_{27}H_{36}N_5O_7S\ Cl \cdot H_2O$: C, 51.62; H, 6.10; N, 11.15. Found: C, 51.83; H, 6.39; N, 11.24.

Amino acid analysis: Gly 1.94, Met 1.05, Tyr 0.98, Phe 1.03. A sample of this material was found to have an activity of 98% when compared with a standard sample in the guinea pig ileum test.

ACKNOWLEDGMENTS

Our thanks are due to The Royal Society and the SRC for supporting funds and to Dr. B. A. Morgan of Reckitt and Coleman Ltd. for arranging biological testing.

REFERENCES

1. G. W. Kenner and R. J. Stedman, *J. Chem. Soc.* 2069 (1952).
2. D. W. Clayton, J. A. Farrington, G. W. Kenner, and J. M. Turner, *J. Chem. Soc.* 1398 (1957).
3. K. K. Kelly and J. S. Matthews, *J. Org. Chem.* **36**, 2159 (1971).
4. E. E. Gilbert, "Sulphonation and Related Reactions." Wiley Interscience, New York, 1965.
5. R. L. Whistler, A. H. King, G. Ruffini, and F. A. Lucas, *Arch. Biochem. Biophys.* **121**, 358 (1967).
6. P. Baumgarten, *Berichte* **59B**, 1166 (1926).
7. H. H. Sisler and L. F. Ardreith, "Inorganic Syntheses" (W. C. Fernelius, Ed), Vol. 2, p. 173. McGraw-Hill, New York, 1946.
8. W. B. Hardy and R. M. Hardy, *Chem. Abstr.* **48**, 1014 (1954).
9. J. A. Moede and C. Curran, *J. Amer. Chem. Soc.* **71**, 852 (1949).
10. R. Pautrat, R. Cheritat, and J. Marteau, *Chem. Abstr.* **76**, 155286 (1972).
11. M. Becke-Goehring and H. Thielemann, *Z. Anorg. Allgem. Chem.* **308**, 33 (1961).
12. E. Hayon, T. Ibata, N. N. Lichtin, and M. Simić, *J. Amer. Chem. Soc.* **93**, 5388 (1971).
13. N. Izumiya, M. Muraoka, and H. Aoyagi, *Bull. Chem. Soc. Japan* **44**, 3391 (1971).
14. M. Bodanszky and L. E. Conklin, *J. Chem. Soc. Chem. Commun.* 773 (1967).
15. J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature (London)* **258**, 577 (1975).
16. H. W. Kosterlitz and A. J. Watt, *Brit. J. Pharmacol.* **33**, 266 (1968).
17. S. Udenfriend, S. Stein, P. Bohlen, and W. Dairman, *Science* **178**, 871 (1972).
18. H. N. Rydon and P. G. W. Smith, *Nature (London)* **169**, 922 (1952).
19. L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.* **85**, 3660 (1963).
20. G. W. Kenner, G. A. Moore, and R. Ramage, *Tetrahedron Lett.* 3623 (1976).
21. J. D. Bower, K. P. Guest, and B. A. Morgan, *J. Chem. Soc. Perkin I* 2488 (1976).