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TRICARBONYLCHROMIUM TRYPTOPHAN DERIVATIVES AND THEIR USE IN PEPTIDE SYNTHESIS

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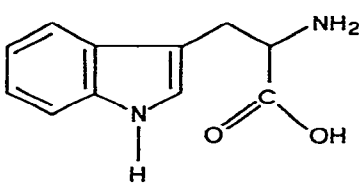
Summary

Incorporation of a $Cr(CO)_3$ ligand into the indole ring of *N*-α-t-butoxycarbonyl-tryptophan methyl ester was achieved in 47% yield. The corresponding *para*-nitrophenyl ester was used in the solid phase synthesis of a peptidic hormone (LHRH) analogue with the aim of decreasing tryptophan alkylation. No improvement was observed.

The side chain of tryptophan **1** (Trp), one of the amino acids found in proteins, involves an indole ring. This heterocyclic system is known to be very sensitive to



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electrophilic agents and this leads to severe problems during incorporation of the Trp residue in peptide synthesis, particularly during the acid cleavage of protecting groups. Among these side reactions, one class consists of oxidative destruction of the Trp residue. This oxidation is generally minimized by the presence of added scavengers such as 2-mercaptoethanol [1] or 1,2-ethanedithiol [2], during the acid treatment (or even throughout the complete syntheses). These scavengers are unfortunately less helpful against the second class of side reactions, which involve electrophilic attack by the carbonium species generated during the deprotection, and lead to alkylated derivatives of Trp.

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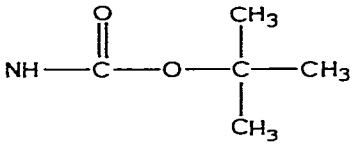
Such derivatives have been well studied during the cleavage of the Boc group **2** (which is the most useful amino protecting group) by trifluoroacetic acid. It has been



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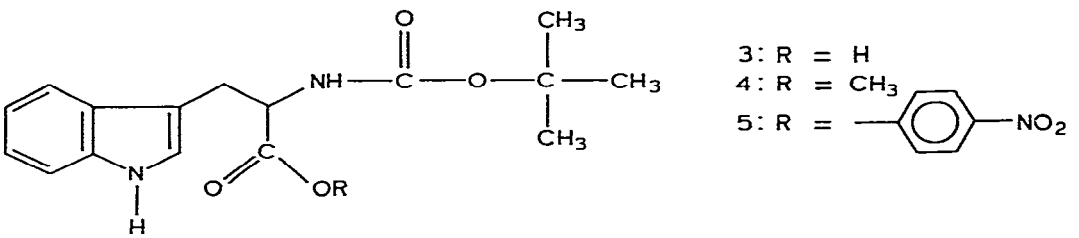
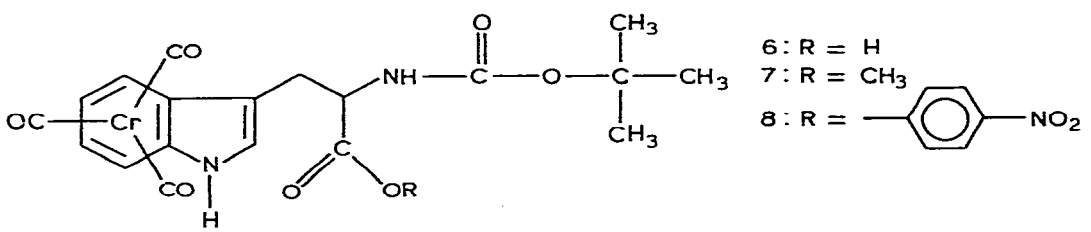
established by mass spectroscopy [3] and NMR spectroscopy [4] that all ring positions but position 4 undergo t-butylation. Nitrogen in position 1 is also alkylated, but in this case protection is afforded by a formyl group.

Complete protection of the indole ring would involve lowering its sensitivity towards electrophilic reagents. Incorporation of tricarbonylchromium in this system could be a useful approach to this problem, since: (i) the effect of a $Cr(CO)_3$ ligand on the reactivity of an aromatic ring towards electrophilic attacks has often been compared to that of a nitro group [5], and (ii) elimination of the $Cr(CO)_3$ ligand at the end of the synthesis can be performed in conditions which are not harmful to peptidic structures.





We report here our synthesis of tricarbonylchromium derivatives of Trp and the first attempt to use them during solid phase synthesis of a peptidic hormone analogue.

1. Synthesis of tricarbonyl chromium (t-butyloxycarbonyl)-L-tryptophan (6) and its para-nitrophenol ester (8)

In order to allow its incorporation during peptide synthesis, a suitably α-amino-protected derivative was necessary, and so the Boc group was chosen. This group is not cleaved off by weak acids such as carboxylic acids at room temperature, but at higher temperatures, such as those required for incorporation of $Cr(CO)_3$, the acidity

of the free α-carboxylic group in **3** is sufficient to lead to removal of the Boc group. We thus decided to use the methyl ester **4**, which was obtained in non-acidic conditions by reaction of the caesium salt of **3** on methyl iodide [6].

Reaction of hexacarbonylchromium (**4**) in dibutyl ether/tetrahydrofuran mixture gave a good yield of **7**, whose structure was characterized by NMR spectroscopy. (A preliminary experiment showed that **6** was fairly stable to the trifluoroacetic treatment which is used during deprotection of the Boc group.) The methyl ester was then hydrolysed in order to prepare **6** with a free carboxylic group, which could then be incorporated into a peptide by classical dicyclohexylcarbodiimide (DCC) activation. We then observed that although **7** was fairly stable, the presence of the free carboxylic group in **6** gave an unstable derivative which reverted to **3** with loss of its $Cr(CO)_3$ ligand.

In order to avoid formation of this free carboxylic group, **6** was not isolated but transformed to **8** by reaction with *para*-nitrophenol and DCC. The activated ester **8** is as stable as **7**, and can be used as acylating reagent in peptide synthesis [7].

2. Comparative use of protected and non protected tryptophan derivatives in solid phase synthesis

An attempt to use $Cr(CO)_3$ modified tryptophan was made during solid phase synthesis of an analogue of luteinizing-hormone releasing hormone (LHRH) having a free carboxy terminus group: pGLu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-OH. The syntheses were performed by the solid phase method, the peptide chain being elongated from the C-terminus amino acid (glycine) which is bound to the resin towards the amino terminus pyroglutamic acid.

The synthetic procedure employed was that we previously used for incorporation of two organometallic amino acid analogues (ferrocenylalanine and cymantrenylalanine) in several peptides [9,10]: this involves use of trifluoroacetic acid (40% in CH_2Cl_2) for deprotection, diisopropylethylamine (5% in CH_2Cl_2) for neutralization, and dicyclohexylcarbodiimide as coupling agent. The trifunctionnal amino acids were protected with tosyl in the case of arginine and histidine and *O*-benzyl in the case of tyrosine and serine.

After incorporation of serine, the peptidyl resin was divided in two parts: (a) with one part (resin 1) the unmodified active ester **5** was incorporated, followed by the next two amino acids. Ethane dithiol (5% was present in the trifluoroacetic acid used for the deprotection steps, (b) with the other part (resin 2) the $Cr(CO)_3$ modified active ester **8** was incorporated. Although all operations were performed in the dark and under nitrogen, a green color appeared on the beads of the resin, probably indicating some change in the organometallic structure. The next amino acids were incorporated as previously.

The two protected peptidyl resins were then treated by anhydrous liquid hydrogen fluoride (1 h, 0°C), with methyl ethyl sulfide and anisole present as scavengers. After evaporation of the hydrogen fluoride, the resins were washed with ether and the peptides extracted with aqueous acetic acid. After lyophilisation, the characteristic bands of the $Cr(CO)_3$ ligand were no longer present in the infrared spectrum of the crude peptide produced from resin 2.

Reversed phase high pressure liquid chromatography was then used to compare the crude peptides formed from resin 1 and 2. As can be seen in Fig. 1, no

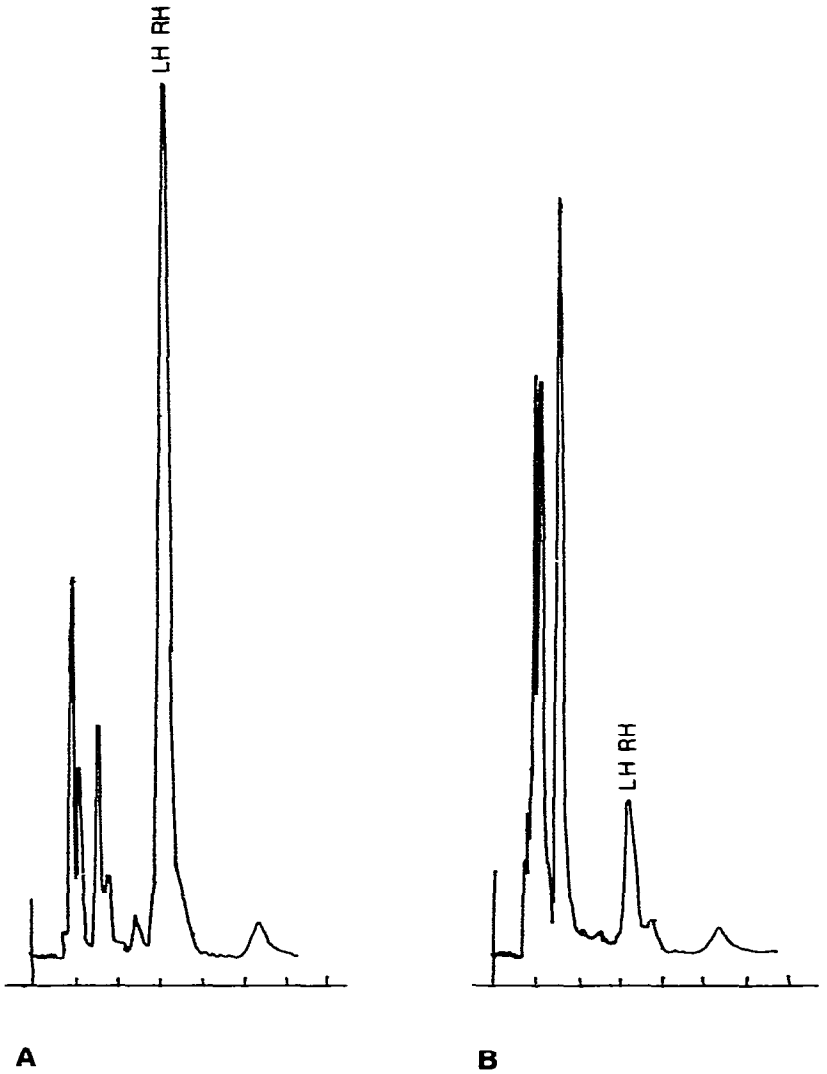


Fig. 1. Reversed phase chromatography of crude peptides (1 mg/0.5 ml) from resin 1 (A) and resin 2 (B) Solvent: CH_3OH (350)/H_2O (600)/PO_4^{3-} (50); flow rate: 2 ml/min; detection: 210 nm/sample size: 5µl; column: µ bondapack C_{18} (Waters).

improvement was observed, in fact, the overall yield of pure LHRH is actually decreased and important additional side products were obtained with resin 2 (B).

Conclusion

From these experiments, it can be concluded that stable tricarbonylchromium tryptophan derivatives can be isolated but that these derivatives are not suitable for protection of a tryptophan side chain under the peptide synthesis conditions we used.

Experimental

Boc amino acids were purchased from UCB or Bachem. The Merrifield resin (chloromethylated copolystyrene-divinyl benzene 1%, 0.7 mequiv. Cl/g) was obtained from Bio Rad Laboratories. Methylene chloride (Prolabo) was distilled from sodium carbonate. TFA, DCC, HOBt (Aldrich) were used without further purification.

HPLC was performed with two model 6000 A pumps, a model U6K injector, a model 660 solvent programmer, and a model 450 variable wavelength detector.

Synthesis of N-t-butoxycarbonyl-L-tryptophan methyl ester (4)

Boc-L-tryptophan (15.2 g, 50 mmol) was dissolved in ethanol (200 ml) and the pH was adjusted to 7 with an aqueous solution of CsOH. The solution was evaporated to dryness and the residue dissolved in DMF. After addition of methyl iodide (3.4 ml, 55 mmol) the mixture was stirred for 20 h at room temperature. DMF was then evaporated under reduced pressure. After addition of water to the residue, the mixture was extracted with ethyl acetate. The organic layer was washed with $NaHCO_3$, dried and evaporated. Recrystallisation from cyclohexane/C_6H_6 gave **4**, 11.8 g (75% yield), m.p. 147°C, α_D (CH_3OH, *c* 0.2) = −3.6. ^1H NMR (δ, ppm, CD_3COCD_3, TMS): t-butyl (1), CH_3 (2.8), $CH_{2\beta}$ (2.5), CH_α (3.3), CH_{indole} (5.5), NH_{indole} (4.5), NH_{amide} (7.7).

Synthesis of N-t-butyloxycarbonyltricarbonylchromium)-L-tryptophan methyl ester (7)

A mixture of **4** (3 h, 9.5 mmol) and $Cr(CO)_6$ (5 g, 23 mmol) was heated in a mixture of dibutyl ether (30 ml) and tetrahydrofuran (6 ml) under N_2 at 135°C for 30 h. Reversed phase HPLC analysis of the reaction medium showed that it consisted of approximatively 80% **7** and 20% **4**. Chromatography on silica gel (eluent: ether/petroleum ether 80/20) yielded about 2 g of yellow crystals (47% yield), m.p. 152–153°C, α_D (CH_3OH, *c* 0.2) +51. ^1H NMR (δ, ppm, CD_3COCD_3, TMS) t-butyl (1), CH_3 (2.8), $CH_{2\beta}$ (2.5), CH (3.3), CH_{indole} (5.5; 4.92; 4.12), N_{indole} (4.5), NH_{amide} (7.7). Found: C, 52.79; H, 4.95; N, 6.02; Cr, 10.67. $C_{20}H_{22}CrN_2O_7$ calcd.: C, 52.86; H, 4.88; N, 6.16; Cr, 11.44%.

Synthesis of N-t-butyloxycarbonyl(tricarbonylchromium)-L-tryptophan p-nitrophenyl ester (8)

7 (2 g, 4.4 mmoles) was treated under N_2 with a solution of 0.5 g KOH in CH_3OH/H_2O mixture (15 ml). The reaction mixture was monitored by TLC. After 1 h, CH_3OH was evaporated under reduced pressure, degassed water (10 ml) was added, and the pH adjusted to 2 with $KHSO_4$. The mixture was extracted with CH_2Cl_2. Evaporation of the organic layer yielded yellow crystals of **6**, which were unstable. The CH_2Cl_2 solution was dried over Na_2SO_4 and some *p*-nitrophenol (0.68 g, 4.8 mmol) was added. The solution was cooled to 0°C and dicyclohexyl carbodiimide (1.8 g, 4.8 mmol) was added. After 2 h stirring, the precipitated dicyclohexylurea was filtered off and the organic solution was washed twice with 1*M* NaOH, then dried and evaporated. The residue was chromatographed on SiO_2 (eluent: ether/petroleum ether 80/20) to give yellow orange crystals of **8**.

Peptide synthesis

Peptide synthesis was carried out under nitrogen in a Beckman Peptide Synthesized mode 990 B according a previously described protocol [9,10].

Boc glycine was attached to a chloromethylated copolymer of styrene and divinylbenzene (1%) using the caesium salt procedure. The degree of substitution as evaluated by residual chlorine determination was 0.34 meq/g. Two grams of resin were used. Trifunctional amino acids were protected with tosyl groups for histidine and arginine, and benzyl groups for tyrosine and serine. Coupling of Boc amino acids were performed with DCC, the completeness of the coupling being determined by the Kaiser test.

After incorporation of serine the protected peptidyl resin was divided into two portions. With one **5** was incorporated, and with the other one **8** was coupled. After this step, ethane dithiol (5%) was added to trifluoroacetic acid during deprotection. The next two amino acids were then incorporated as previously on the two resins.

At the end of the synthesis, both peptidyl resins were treated with anhydrous hydrogen fluoride (10 ml/g resin) in the presence of anisole (1 ml/g resin) and ethyl methyl sulfide (1 ml/g resin) at 0°C for 60 min. After evaporation of HF, the mixture was washed with ether and the peptide extracted from the resin with 5% acetic acid. The extracts were lyophilized. The crude peptides were checked by reversed phase high pressure liquid chromatography on a μ Bondapack C18 column, the elution being with the mixture CH_3OH (350)/H_2O (600)/PO_4^{3-} (50). Each peptide (1 mg) was dissolved in 0.5 ml of eluting buffer; 10 μl injections were made, and the absorbance of the eluate was measured at 210 nm and 280 nm.

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