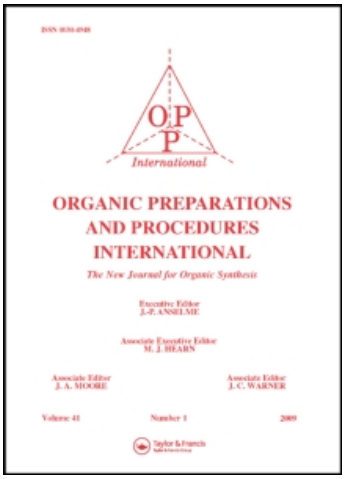

Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

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To cite this Article Rzeszotarska, Barbara and Masiukiewicz, Elzbieta(1990) 'ARGININE, HISTIDINE AND TRYPTOPHAN IN PEPTIDE SYNTHESIS. THE INDOLE FUNCTION OF TRYPTOPHAN', Organic Preparations and Procedures International, 22: 6, 655 — 706
To link to this Article: DOI: 10.1080/00304949009457901
URL: http://dx.doi.org/10.1080/00304949009457901

ORGANIC PREPARATIONS AND PROCEDURES INT., 22 (6), 655-706 (1990)

ARGININE, HISTIDINE AND TRYPTOPHAN IN PEPTIDE SYNTHESIS. THE INDOLE FUNCTION OF TRYPTOPHAN†

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INTRODUCTION

Tryptophan is one of the most reactive amino acids though it seems to occur in proteins rarely.[1] Of the proteinogenic amino acids, it contains the largest aromatic side-chain, and almost every atom in free or bound tryptophan is capable of reacting selectively under appropriate conditions, both *in vivo* as well as *in vitro*.[2] Tryptophan is metabolized by several different pathways leading to compounds of great biological importance.[2,3] Some of them are of interest to synthetic peptide chemistry, e.g. tryptophan sulfur derivatives (Fig. 1) as building components of toxic peptides from the mushroom *Amanita phalloides*[4] and as compounds similar to side-products (Fig. 12 and 13) in some tryptophan peptide syntheses. The cyclic derivative of tryptophan (Fig. 2) found in ilamycin B_1,[5] can be regarded as a counterpart of alkylated tryptophan molecules (Fig. 19) resulting from acidolytic removal of protecting groups *via* S_N1 mechanism. The particular biochemical and chemical reactivity

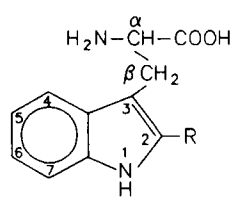


Figure 1.

R = $SCH_2CH(NH_2)COOH$
tryptathionine

$SOCH_2CH(NH_2)COOH$
tryptathionine (*R*)–sulfoxide

$SOCH_3$
2–(methylsulfinyl)tryptophan

SO_2CH_3
2–(methylsulfonyl)tryptophan

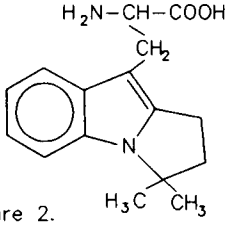


Figure 2.

of tryptophan and its exogenous character account for the fact that the chemistry of tryptophan is more intensively studied than that of other amino acids. Only sulfur amino acids have received comparable attention from chemists and biochemists.[3] Nevertheless problems of the assembly of a peptide chain containing the tryptophan residue(s) were poorly known untill 1974. This is evidenced in Wünsch's monograph by the three pages devoted to tryptophan while nearly four hundred pages are devoted to the systematic coverage of the incorporation of trifunctional amino acids into this chain.[6]

I. REACTIONS OF THE SIDE-CHAIN OF TRYPTOPHAN IN PEPTIDE SYNTHESIS

The mobile π-electron system is involved in activating all seven positions of the indole nucleus for the attack of oxidants,[2] which can dramatically result in the complete lack of tryptophan recovery after the acidic hydrolysis of proteins and peptides.[3] On the other hand, the oxidants which tie down the mobile π-electron system, preferentially by forming a π-complex, often easily recognized by intense color formation, attack the α and β positions of tryptophan to give α,β-dehydrotryptophan.[2] For reviews of the richness of processes and products of the oxidation of both free as well as bound tryptophan, references 2 and 3 should be consulted.

Tryptophan oxidation was not observed during the oxidation of peptide methionine residues to sulfoxide or sulfone[7-9] or during that of the phenylhydrazide to the carboxyl group.[10] However, the oxidation of cysteine to cystine residues may[11-15] or not may[8,11,16-20] correlate with tryptophan oxidation or with other indole modifications such as iodination[12] and sulfenylation[21,22] (Fig. 13). The reduction of Met(O) residues can be accompanied by considerable tryptophan transformation, most probably by the oxindolylalanine residue formation.[7] Since, tryptophan oxidation may also occur during acidolytic deprotections,[23] various measures are used to minimize it. Lower-

ing of the reaction temperature[16,23-27] and work in an inert gas atmosphere[6,16,23,25,28-30] are the simplest of these. The addition of antioxidant minimizes this side-reaction more effectively.[23,31] Among these compounds, one of the best,[31] though not without drawbacks,[32-34] seems to be ethanedithiol which moreover functions as a carbocation scavenger.[28,34,35] It is commonly used in all acidolytic deprotecting reagents acting through both the S_N1 and S_N2 mechanism.[15,34,36-39] A solution of ethanedithiol in trifluoroacetic acid should be freshly prepared, because on standing, the compounds form thioorthoester (Fig. 3) which can acylate the tryptophan position-2 completely within 20 hrs (Fig. 17).[34] Further standing of the reagent affords the precipitated thioorthoester "dimer" (Fig. 3)[32] almost quantitatively within 24 hrs. Moreover, while ethanedithiol polymerizes extensively in mixtures containing hydrogen fluoride at high concentration, it does not polymerize significantly in these containing hydrogen fluoride at low concentration.[33] It is also added to protect the tryptophan residue during the tyrosine sulfation.[40] Another method used to prevent tryptophan oxidation in acidolytic deprotections or in oxidizing procedures is to block the indole nitrogen with an electron-withdrawing acyl group (Section IV).

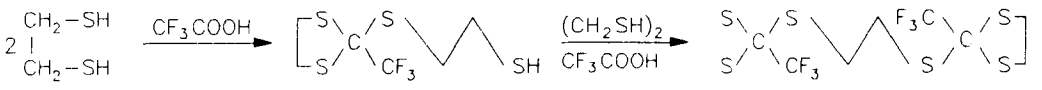


Figure 3.

The tryptophan susceptibility to oxidation is sometimes exploited. The oxidation of tryptophan possessing the free amino and carboxyl groups with one equivalent peracetic acid leads to 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carboxylic acid[41] which serves for the tryptathionine synthesis (Fig. 4).[42] The resulting tryptathionine was used for the synthesis

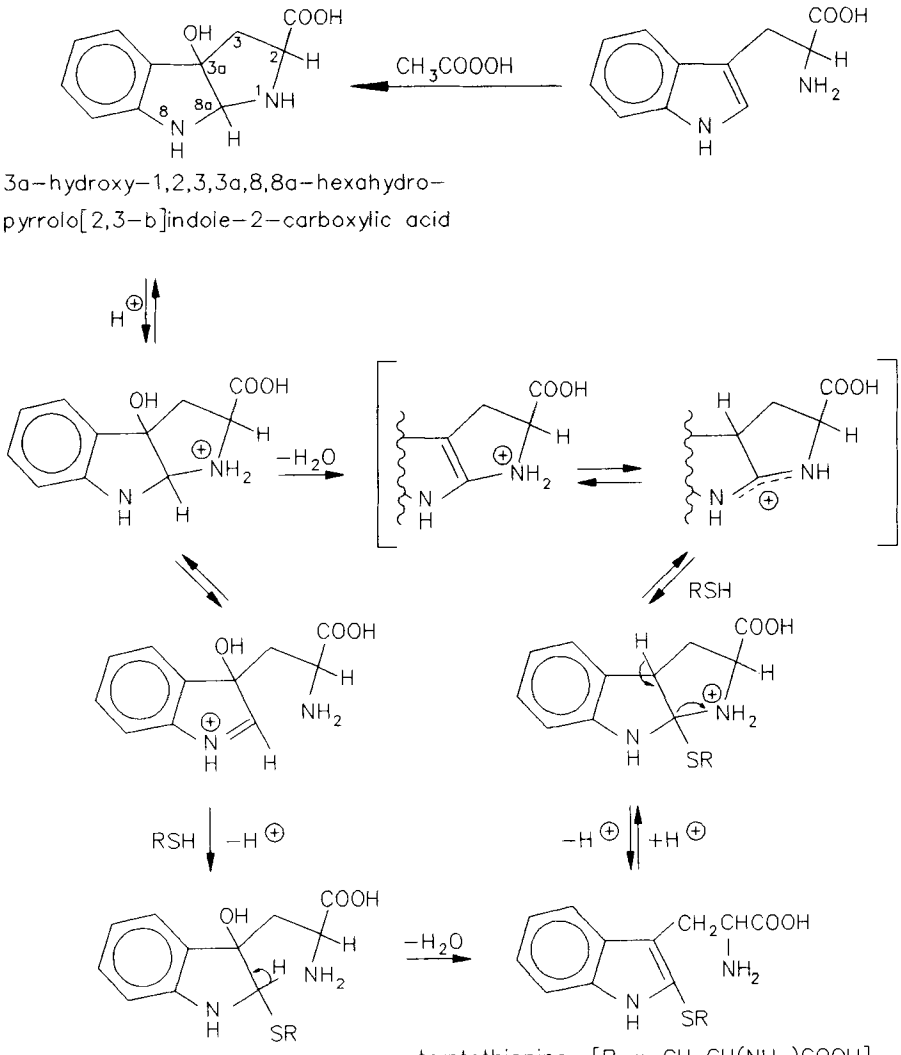


Figure 4.

of the amatoxine analogues in order to investigate structure-toxicity relationships.[4] The *tert*-butyl hypochlorite oxidation of N^{α}-acetyltryptophan with the protected carboxyl function gives 2,3-dihydropyrrolo[2,3-b]indoles, which are efficiently converted to 2-(alkylthio)tryptophan derivatives (Fig. 5).[43]

The tryptophan moiety can be processed selectively in the presence of amino acid residues (other than cysteine and methionine) to the 2-oxindolyl-

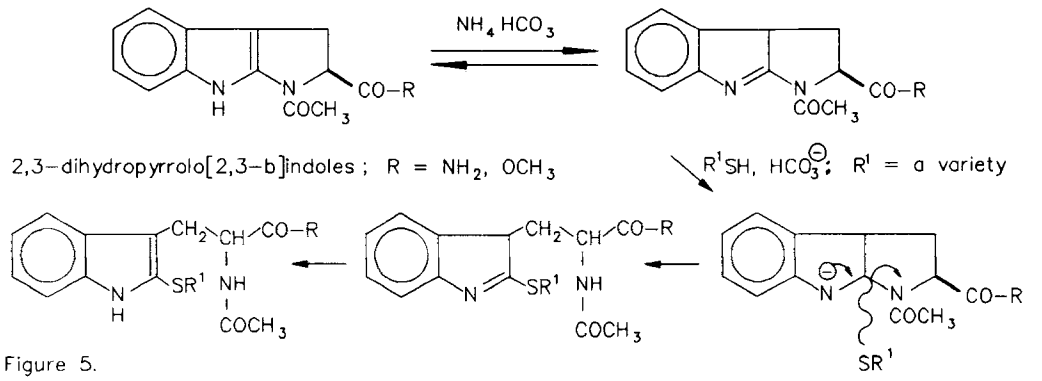


Figure 5.

alanine residue with a mixture of dimethyl sulfoxide and concentrated hydrochloric acid. The postulated mechanism is illustrated in Figure 6.[44] 2-Oxindolylalanine is sometimes formed nearly quantitatively,[44] while in other cases, a mixture of 2-oxindolylalanine and 2-chloroindolylalanine is produced in a *ca* 5:1 to 4:1 ratio.[45] The reaction serves as a synthetic approach to the tryptophan residue modification both to 2-oxo- and to 2-chloroindolylalanine, without cleavage of the peptide chain to exploration of structure-biological activity relationships of tryptophan peptides.[44,45] On the other hand, tryptophan resistance to oxidation in an acidic aqueous medium containing 20% dimethyl sulfoxide during the cyclization of disulfide bridges with this reagent has been described.[19]

The indole system, both free[46-50] and N^i-protected,[51-54] especially the latter according to some authors,[51,52] is reduced in the course of classi-

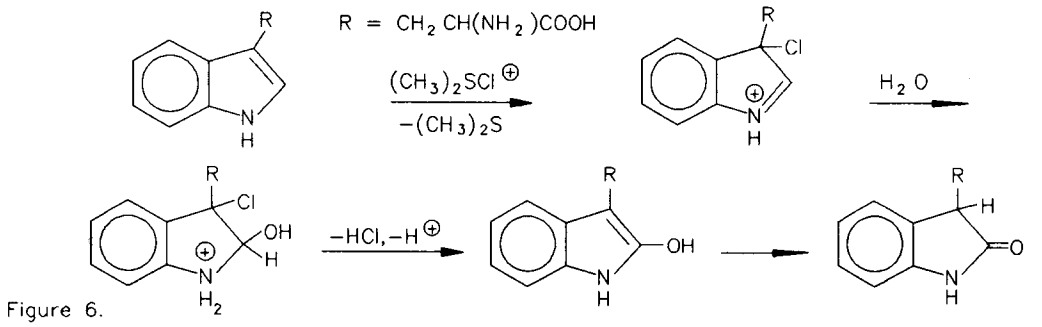


Figure 6.

cal[46,49-54] or catalytic-transfer hydrogenation;[47-50] however, tryptophan bearing the free amino group could not be reduced at all in formic acid as the hydrogen donor.[48] Depending on conditions, especially on the reaction time, either 2,3-dihydro-[48,49,52] or octahydrotryptophan[46,50] results. To diminish the amount of 2,3-dihydrotryptophan, cyclohexene was proposed as the hydrogen donor instead of formic acid or ammonium formate.[49] According to some workers,[55] reductive deprotection of tryptophan peptides in the presence of cyclohexene does not occur. The reduction of tryptophan limits the use of the hydrogenolysis for tryptophan-containing petides.[49,50,56] In spite of these difficulties, cleavage of benzyl-type protective groups from tryptophan peptides by hydrogenolysis including that in liquid ammonia is successful.[30,57-66] This is similar to the reduction of tryptophan-containing peptides by means of sodium in liquid ammonia. Although the reagent attacks tryptophan,[67] this reduction is still currently used.[68]

Although tryptophan undergoes photodegradation under normal light conditions, no change was observed on a methanolic solution of N^{α}-[1-(1-adamantyl)-1-methylethoxycarbonyl]tryptophan.[69] The photolytic deprotection of peptides on irradiation with 350 nm light in neutral media occurs most probably without affecting tryptophan.[12,70,71]

Nitrosation of the indole nitrogen can occur during the generation of azides from hydrazides.[3,72,73] Therefore the azides are not recommended for the tryptophan carboxyl group activation.[17,74-78] N^{α}-Acetyl-N^{i}-nitrosotryptophan contains 65% of *E*- and 35% of *Z*-isomer.[79] The N^{i}-nitroso grouping is removed from tryptophan derivatives by mineral acids, e.g. hydrogen bromide in acetic acid,[72] sulfuric acid[80] or perchloric acid.[81] Some N^{i}-nitrosotryptophan compounds are extremely photolabile.[72]

Simple derivatives or peptides of tryptophan dimerize significantly in hydrogen fluoride or trifluoroacetic acid. The pyrrole ring binds a proton

and the resulting indoline carbocation attacks the second pyrrole ring to furnish a dimer of *trans* and *cis* geometry. When Ac-Trp-OCH_3 was dissolved in trifluoroacetic acid and immediately concentrated under reduced pressure, the dimerized product was isolated in about 9% yield. After storage of a solution of 10% Ac-Trp-OCH_3 in trifluoroacetic acid at 20^0 for 3 hrs, the dimer amounts to 75% including 60% of *trans*-isomer; the *cis* and *trans*-isomers, (Fig. 7)

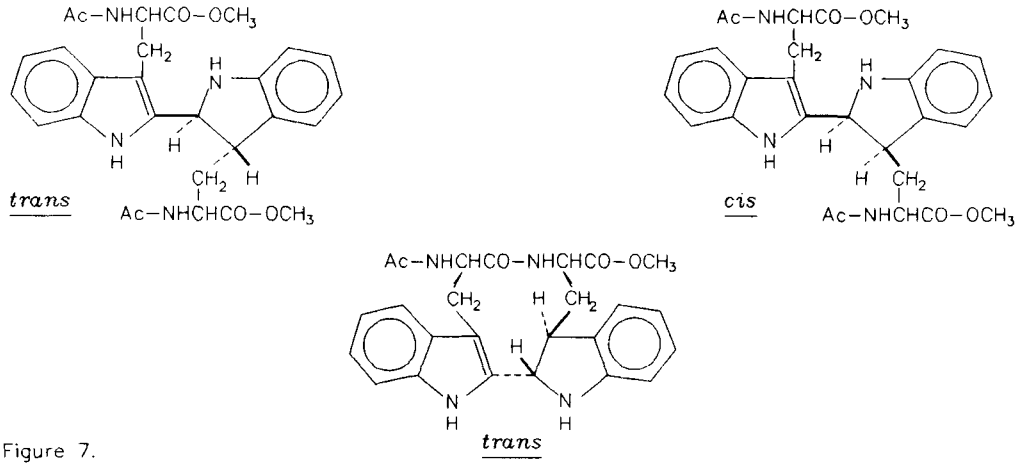


Figure 7.

which are stable in trifluoroacetic acid at room temperature, could be isolated in 5% and 28% yields, respectively. When stored in trifluoroacetic acid, Ac-Trp-Trp-OCH_3 affords the intramolecular *trans*-isomer (Fig. 7).[60,82] (The same system of two sequential tryptophan residues occurs in some neurotoxines,[83,84] lysozyme,[85] epidermal growth factor,[18,77,78] and has been introduced into some biologically active peptide analogues.[86]) The observed facts of dimerization of indole systems suggest[12] that acidic deprotection of tryptophan peptides in the presence of an indole compound as a carbocation scavenger[20,23,25,64,74,82,84,85,87-95] may cause the irreversible tryptophan modification.[96]

The reactions described up to this point result from the specificity of the aromatic system of tryptophan. In addition, this amino acid undergoes the general reactions of all aromatic compounds, among others alkylation, hydroxyalkylation and acylation; the most important of these, alkylation, will be discussed in a separate section.

The intramolecular hydroxyalkylation of tryptamine or N^{α}-acyl derivatives of tryptophan, known as early as 30 years ago, proceeds under the influence of many acids especially by heating to give Bischler-Napieralski's type cyclodehydration.[97,98] Under the influence of light, the resulting 3,4-dihydro-β-carboline derivatives are oxidized and decarboxylated; this is illustrated in Fig. 8 for the derivative, produced from N^{α}-acetyltryptophan.[97] On prolonged

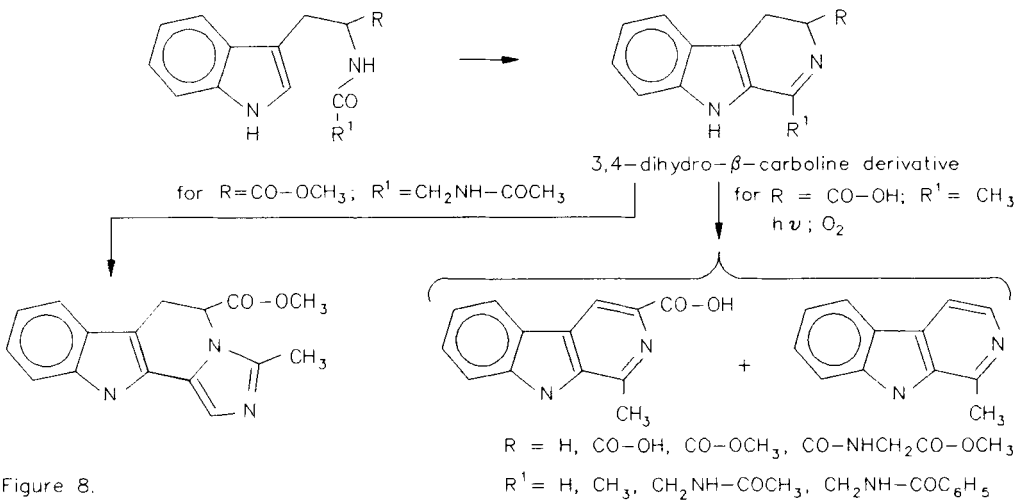


Figure 8.

heating with polyphosphate ester, the derivative, formed from N^{α}-acetylglycyltryptophan methyl ester undergoes further cyclodehydration to yield the tetracyclic compound (Fig. 8).[98] The N^{i}-formyl group deactivates the pyrrole ring to prevent the hydroxyalkylation. However, in the presence of hydrochloric acid N^{α}-formyltryptophan in a formic acid gives not only $N^{\alpha,i}$-diformyltryptophan but also 3,4-dihydro-β-carboline-3-carboxylic acid (Fig. 9).[99]

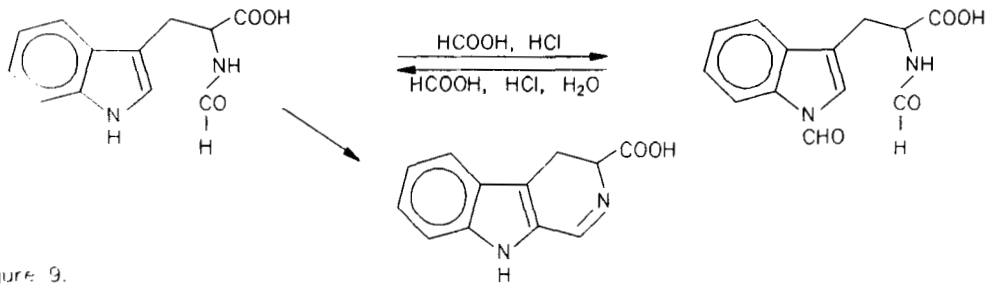


Figure 9.

The intermolecular acylation of tryptophan and further reactions were discovered after the hydroxyalkylation. Irradiation of tryptophan with visible light in acetic or formic acid in the presence of a sensitizer gives good conversion to β-carboline (Fig. 10).[100] Tryptophan derivatives react quantita-

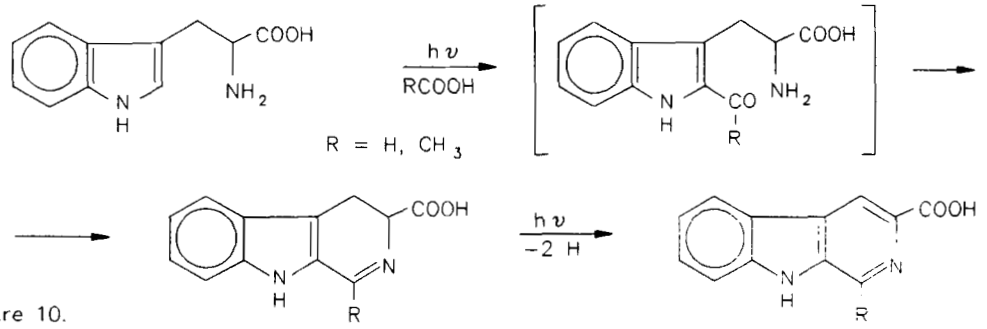


Figure 10.

tively with excess acyl chlorides at room temperature within an hour; only the monoacyl compounds are formed (Fig. 11), in a ratio indicating the following susceptibility of indole positions to acylation: 2 >> 1 > 5; thus, this explains the difficulty in obtaining N^i-acyl derivatives of tryptophan (see Section IV). 2-Acyl compounds (V; in the case of Vb, the α-amino group was previously made free), after 1-2-min heating in water, are transformed quantitatively into 1-alkyl(aryl)-3,4-dihydro-β-carboline-3-carboxylic acids (II); typical yields of isolated products are: 72% (IIa), 17% (IIIa) and 10% (IVa).

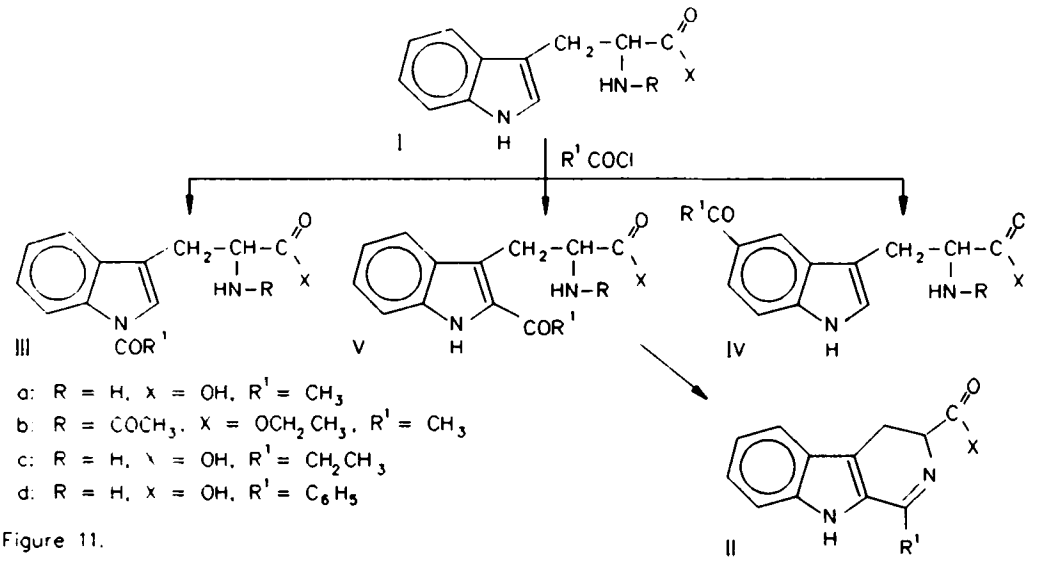


Figure 11.

β-Carbolines were obtained in similar yields, 69-75%, after acylation of proteins.[101]

During the N^{α}-deprotection of N^{α}-2-nitrophenylthiopeptides by means of hydrogen chloride in organic solutions, 2-nitrophenylsulfenyl chloride is

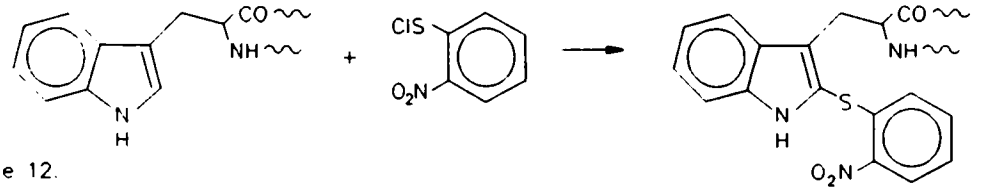


Figure 12.

formed. The nearly quantitative sulfenylation of the tryptophan moiety with this reagent (Fig. 12), discovered more than 20 years ago, may be treated as the first recognized "acylation" of the tryptophan indole in the course of peptide synthesis. The preventive measure relies on adding 10-20 equiv. 1- or 2-methylindole, indole or skatole, listed according to decreasing efficiency, as a substrate to compete with the tryptophan residue;[102] however, they are not always effective in solid phase peptide synthesis.[12]

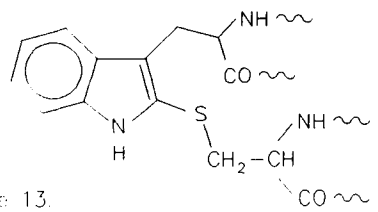


Figure 13.

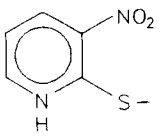


Figure 14. 3-nitro-2-pyridinethio

A similar by-product as that in Figure 12, the thiol derivative of tryptophan indole (Fig. 13) was obtained in the process of oxidation of S-tritylcysteine residues to disulfide with iodine in a peptide containing tryptophan.[22] N^{α}-Desulfenylation with a nucleophile, mostly sulfur one is free from the tryptophan "acylation". A noteworthy proposition is the use of 2-thiopyridone,[103-105] especially as an equimolar mixture with trifluoroacetic acid.[105] Protonated 2-thiopyridone is well-suited for attack on the sulfur atom of the N^{α}-2-nitrophenylthio group and the simultaneous transfer of the proton onto the α-amino group (Fig. 15).[104,105] By the use of this system for

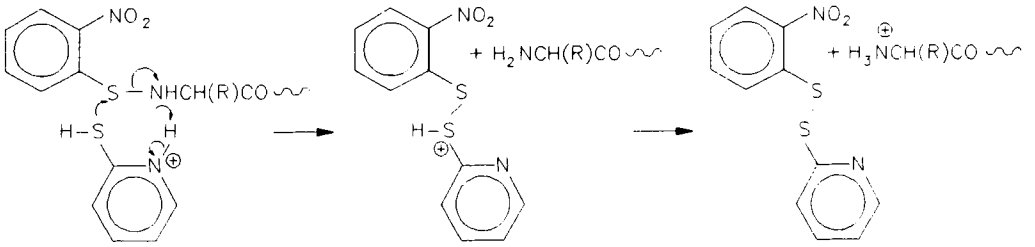


Figure 15.

the deprotection of N^{α}-2-nitrophenylthio groups, human gastrin I, with seventeen residues including ten sensitive to strong acids - among which two tryptophan residues - was synthesized for the first time in solid phase[105] (see also[106,107]). The N^{α}-2-nitrophenylthio and N^{α}-3-nitro-2-pyridinethio groups (Fig. 14) can be removed from peptides, without affecting tryptophan, with triphenylphosphine in the presence of such compounds as N-hydroxy-

succinimide, N-hydroxybenzotriazole or 2-pyridinethiol 1-oxide.[108] The second group is also cleaved off in the presence of *p*-toluenesulfonic acid or pyridine hydrochloride.[109]

2-Nitrophenylsulfenylation can be profitable for the reversible protection of tryptophan, because the protection may be removed by catalytic hydrogenation, even without the destruction of methionine. Hydrogenation with tritium leads simultaneously to the labelling of tryptophan.[3,12] 2-Nitrophenylsulfenylation changes the hydrophobicity and chromatographic mobility of tryptophan, which facilitates HPLC separation of the tryptophan-containing from remaining peptides.[110]

Tryptophan can be sulfonated at the 2-position during sulfation of the tyrosine residues.[111,112] Its acylation with 4-methoxy-2,3,5-trimethylbenzenesulfonyl (Fig. 16) resulting from the N^G-deblockage of arginine with trifluoroacetic acid at 50° has been recently described.[8,34] This side-reaction is most efficiently suppressed by adding a mixture of 15 equiv. ethanedithiol and 10 equiv. 4-(methylmercapto)phenol. However, extended heating in the presence of ethanedithiol leads to another acylation product in the dithioketal form (Fig. 17).[34]

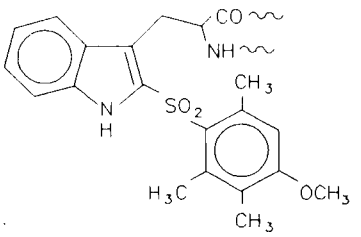


Figure 16.

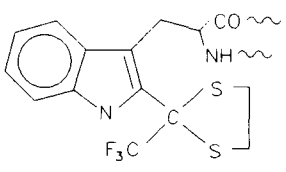


Figure 17.

In general, the incorporation of N^i-unmasked tryptopan into a peptide chain proceeds smoothly. Indole is assumed to be too poor a nucleophile to be acylated under the unusually mild conditions of coupling.[12,25] However, the inappropriateness of some methods for the tryptophan carboxyl activation,

e.g., the mixed anhydride[113] or the azide method,[17,74-78] is sometimes mentioned. The synthesis of (Boc-Trp)[20] using dicyclohexylcarbodiimide is accompanied by a side-reaction not observed during that of [Boc-Trp(CHO)]$_2$O.[12] The intramolecular acylation of indole which may occur under some conditions (Fig. 18) can be prevented by N^i-acylation which deactivates the indole.[101]

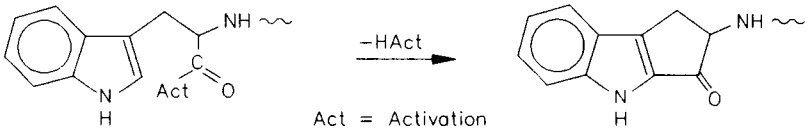


Figure 18.

At the turn of the sixties, the *t*-butylation of tryptophan was discovered during acidolytic N^{α}-Boc deprotections.[23] However, several reasons delayed the systematic studies on the *t*-butylation started for ten years.[114] First, the addition of an antioxidant or the N^i-formylation of tryptophan severely represses *t*-butylation. Second, the total acidic hydrolysis of a peptide removes *t*-butyl groups from the tryptophan residue[115] and no alkylation is observed in the determination of this amino acid after the hydrolysis. Third, as was the case with ACTH fragments, analogues containing *t*-butylated tryptophan can also exhibit biological activity, as much as 40-60% of that of the parent compound.[116,117] All the facts not only failed to suggest this side-reaction, but seem to rule it out. Model experiments, in which a dozen or so of the indole *t*-butylation products were found, showed the tryptophan *t*-butylation problem in the course of peptide synthesis.[87,118-120]

II. TRYPTOPHAN ALKYLATION IN PEPTIDE SYNTHESIS

1. *t*-Butylation of Tryptophan

Thirteen products were detected after 2 hrs storage of Z-Trp-OBzl in a large volume of *t*-butyl acetate and trifluoroacetic acid. Nine of them were

isolated in pure form, mostly N^i- and C^i-mono or di(*t*-butyl) derivatives.[118,119] Eleven products including two tri(*t*-butyl)ated and one tetra(*t*-butyl)ated have been reported after 48 hrs storage of tryptophan in a large volume of *t*-butanol and trifluoroacetic acid[87] (Fig. 19). The effect of the

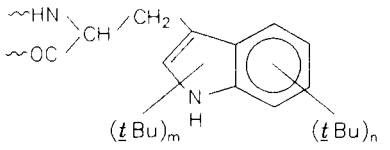


Figure 19.

reagent on *t*-butylation of tryptophan during Boc removal from Boc-Trp-Gly-OCH_3 is presented in Table 1. As can be seen, the yield of *t*-butyl derivative and the position of substitution depend on the type of the acid and the concentration of the peptide. The *t*-butylation is very extensive using boron tristrifluoroacetate or hydrogen fluoride, much less using trifluoroacetic acid (especially at the lower peptide concentration) and ever less in a solution of an organic sulfonic acid in acetic acid. This side-reaction proceeds neither in solutions of hydrogen chloride in methanol, acetic and formic acid nor in the latter alone or in hydrochloric acid.[120] *t*-Butyl cations are the main *t*-butylating species which *t*-butylates tryptophan. Moreover, with trifluoroacetic acid they form stable *t*-butyl trifluoroacetate which also possesses alkylating properties albeit lower than those of *t*-butyl cations.[32,35] The alkylation of tryptophan amounts to 10-30% in trifluoroacetic acid[120,121] and 75% in hydrogen fluoride[120] (Tab. 1). The addition of water to trifluoroacetic acid decreases the *t*-butylation of tryptophan considerably. Water is apparently acting as an effective carbonium ion scavenger.[122,123] Solvents in hydrogen chloride solutions are assumed to take over as water the role of the oxygen scavengers of *t*-butyl cations. Solutions of hydrogen chloride in methanol,[124,125] in ethyl acetate at low temperature[26]

TABLE 1. *Effect of Acidic Deprotecting Agent on the Modification of the Trp Residue within Boc-Gly-Trp-OCH_3 (1.33 mM) at 20° [120]*

Deprotecting agent	ml (time-hrs)	Products *t*-butylated [%] in the position 1	3	5	2+7	TOTAL
TFA	5 (1)	10	-	10	10	30
TFA	50 (1)	3	-	<5	<5	10
TFA-anisole-water (8:1:1)	5 (1)	5	-	<5	<5	10
2 N HCl in EtOAc[a]	5 (1)	-	-	5	-	5
2 N HCl in dioxane	5 (1)	-	-	5	-	5
2 N HCl in methanol	5 (1)	-	-	-	-	0
12 N hydrochloric acid[b]	2.5 (10′)	-	-	-	-	0
2 N HCl in HOAc	5 (1)	-	-	-	-	0
1 N CH_3SO_3H in HOAc	5 (1)	8	-	-	-	8
1 N $C_2H_5SO_3H$ in HOAc	5 (1)	8	-	-	-	8
1 N $HSCH_2CH_2SO_3H$ in HOAc	5 (1)	<5	-	-	-	<5
1 N $BF_3 \cdot O(C_2H_5)_2$ in HOAc	5 (0.5)	5	-	-	-	5
HCOOH	5 (2.5)	<1	-	-	-	<1
0.1 N HCl in HCOOH	80 (0.5)	-	-	-	-	0
1 N HCl in HCOOH	5 (1)	2	-	-	-	2
0.8 M $(TFA\text{-}O)_3B$ in TFA	5 (1)[c]	10	5	80		95
HF	5 (0.5)[c]	5	-	60	10	75
HF-anisole (9:1)	5 (0.5)[c]	-	-	25	5	30

a) Et = ethyl. b) Boc-Trp-Gly (1.39 mM). c) 0°.

and in acetic acid in the presence of ethanethiol[126] are utilised in the large-scale conventional syntheses of gonadoliberin,[124,125] a cyclic hexapeptide analogue of somatostatin[26] and pentagastrin.[126] In formic acid, N^i-formylation of tryptophan occurs most probably, thus preventing *t*-butylation.[6] The above-mentioned reagents, which do not result in *t*-butylation, can lead to other side-reactions;[127,128] therefore, they are not always efficient in deblocking large peptides[127] and their use in solid phase peptide synthesis meets with varing degrees of success.[123] Then, they do not deliver the univer-

sal means for Boc removal, but their efficiency in preventing *t*-butylation prompted a search for cation-scavengers as additives to universal acidolytic reagents.

Nitrogen atom is the most susceptible site to *t*-butylation in the indole system.[88,115] The resulting N^i-*t*-butyl derivative is moderately stable in trifluoroacetic acid and unstable in hydrogen fluoride (Tab. 2). In trifluoroacetic acid, the scavenger must compete against the rapid N^i-alkylation.

TABLE 2. *Stability of Trp(N-tBu) (N) and Trp(C-tBu) (C) in Acidic Media [115]*

Conditions	Substrate	Products [%] Trp	N + C	C
1.0 ml HF, 0.1 ml EDT 0.1 ml anisole, 1 hr, 0°	0.1 mM N 0.1 mM C	92 5	trace	 90
1.0 ml TFA, 0.1 ml EDT 10′, 0°; 50′, 20°	0.1 mM N 0.1 mM C	50 0	40 + 10	 100
6 M HCl + 2% $HSCH_2COOH$ 22 hrs, 108° in an evacuated, sealed tube	0.01 mM N 0.01 mM C	99 84	0	 trace

Therefore in this medium, even the best scavengers known so far are not fully effective (Tab. 3). However, in hydrogen fluoride, due to the reversibility of the N^i-alkylation, the scavenger competes against the slow C^i-*t*-butylation. Hence, in the presence of a rapidly reacting sulfur compound, the discussed reaction may be avoided (Tab. 3).[115] Two groups carried out systematic studies on the efficiency of additives in minimizing the tryptophan *t*-butylation.

Markussen *et al*. examined the reaction rate of carbocation scavengers and antioxidants with *t*-butyl trifluoroacetate (in a 1:1 ratio) in trifluoroacetic acid. Thioanisole and thiophenol react one order of magnitute more rapidly

than tryptophan, mercaptoethanol, ethanedithiol and ethyl methyl sulfide (in this order) - only from six to three times more rapidly. Methionine reacts at a comparable rate. However, anisole and phenol are *t*-butylated more slowly than tryptophan. Since the butylation of thioanisole and thiophenol is reversible and mercaptoethanol is quickly esterified at the hydroxy group, and more slowly, at the thio group, ethanedithiol seems to be the scavenger of choice for general purposes.[32,35] Sakakibara *et al*. investigated possible additives against tryptophan *t*-butylation in the course of N^{α}-Boc deprotection with trifluoroacetic acid or hydrogen fluoride (Tab. 3). A 1:1 mixture of trifluoroacetic acid and ethanedithiol gives *t*-butylated tryptophan in 4.2%

TABLE 3. *Effect of Additives on the Formation of Trp(tBu) from Boc-Trp (1 mM) in Acidic Media [115]*

Conditions	Scavenger	[ml (mM)]	EDT ml (mM)	% Trp(*t*Bu)	Trp
	-		-	14.2	67
	-		3 (36)	5.1	91
	-		10 (120)	4.2	93
	Dimethyl sulfide	10 (137)	1 (12)	1.1	99
		5 (68)	1 (12)	2.6	97
A		3 (41)	1 (12)	4.3	96
	Ethyl methyl sulfide	10 (111)	1 (12)	2.6	97
	Thioanisole	10 (85)	1 (12)	3.2	96
	Ethyl isopropyl sulfide	10	1 (12)	4.9	94
	Diisopropyl sulfide	10 (69)	1 (12)	6.4	92
	Anisole	10 (92)	1 (12)	6.3	91
	-		-	15.6	57
	Anisole	1 (10)	-	2.4	94
B		1 (10)	(10)	-	99
	-		(10)	-	99
	Dimethyl sulfide	0.7 (10)	(10)	-	99

A. 10 ml TFA, 1 hr, 20°. B. 10 ml HF, 1 hr, 0°.

yield. A 1:1:0.1 mixture of this acid, dimethyl sulfide and ethanedithiol (needed as an antioxidant) leads to the *t*-butylation in only 1.1% extent. Other sulfides are less effective and anisole even less so. But in hydrogen fluoride, the addition of 10 equiv. of ethanedithiol alone is sufficient to prevent the alkylation. After acidolysis with hydrogen fluoride in the presence of anisole only, the substituted tryptophan contents amounts to 2.4%.[115]

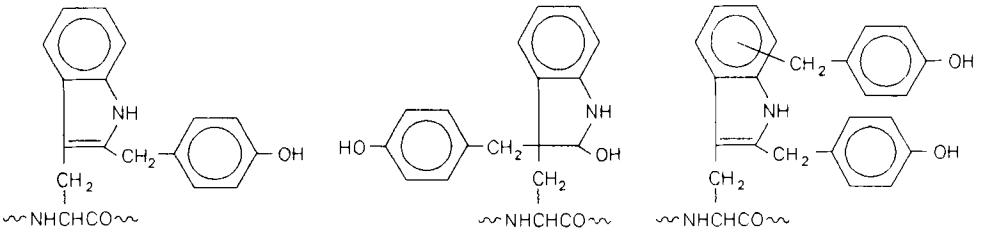
As the practical result from the exploration of carbocation scavengers, N^{α}-Boc removal in solid phase peptide synthesis is sometimes modified by adding dimethyl[129-132] or diethyl[86] sulfide to trifluoroacetic acid. However, the standard mixture of 10:1:0.5 trifluoroacetic acid-anisole-ethanedithiol is proposed for N^{α}-Boc cleavage in conventional laboratory-scale syntheses of polypeptides containing tryptophan; no *t*-butylated products were found in those peptides.[28] Many compounds were studied as potential carbocation scavengers in various acidolytic reagents including ethanesulfonic acid with little satisfactory results.[85,88,133] Yet, 10% sulfuric acid (H_2SO_4 refers to reagent grade concentrated H_2SO_4, *viz.*, 96% H_2SO_4/4% H_2O) in dioxane seems to be the universal reagent deblocking N^{α}-Boc peptides linked to a polymeric support, instead of 50% trifluoroacetic acid in dichloromethane. This reagent is devoid of the disadvantages of the latter acid and found to prevent *t*-butylation. It presents an especially attractive alternative to trifluoroacetic acid for the large-scale synthesis of specific peptides.[123]

2. *p*-Alkoxybenzylation and *p*-Hydroxybenzylation of Tryptophan

Yajima *et al*. prefer the Z(OMe) rather than the Boc for the temporary protection of the α-amino function in classical peptide synthesis in solution.[15,40,134-139] The action of trifluoroacetic acid in the presence of anisole on Z(OMe)-Trp and Boc-Trp led to the introduction of *p*-methoxybenzyl and the *t*-butyl groups in the indole moiety in amounts of 100% and 40%, respectively. In the first case, the 2-(*p*-methoxybenzyl) derivative is the

main product although 2,5- and 1,5-di(*p*-methoxybenzyl) compounds were found in non-negligible quantities. In the second case, N^{i}-*t*-butyltryptophan was the major product along with minor monoalkylated derivatives. Carbocation scavengers such as thioanisole, dimethyl sulfide, skatole or combinations thereof either in trifluoroacetic acid or in 4 N ethanesulfonic acid in trifluoroethanol were less effective towards the *p*-methoxybenzyl than *t*-butyl cations. Therefore, the Z(OMe) protection is not recommended for the synthesis of peptides containing tryptophan.[77,78,88]

Significant quantities (50-65%) of side-products including two monohydroxybenzylated and one bishydroxybenzylated were isolated from a tryptophan-containing peptide removed from the resin of the benzyloxybenzylalcohol anchor (Fig. 20) by 1:1 trifluoroacetic acid in dichloroethane at 20°. In addition,



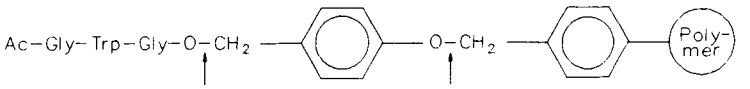


Figure 20.

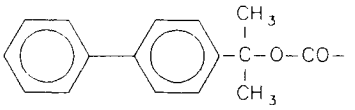
this cleavage is accompanied by irreversible binding of a part of the peptide to the support. This binding can be explained by the alkylation of the indole moiety with the benzyl cations formed from the polymer by cleavage at (a) or (b) (see Fig. 20). The presence of 5% of ethanedithiol suppresses, but does not completely eliminate both side-reactions.[140] A similar stable binding of

tryptophan-containing peptides to a similar matrix after trifluoroacetic acid treatment has been also described by others.[8,141]

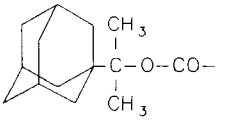
3. Benzylation, 2,4-Dichlorobenzylation and *p*-Bromobenzylation of Tryptophan

Benzyl-type cations are formed in the course of the final removal of *ω*-benzyl and related groups with hydrogen fluoride, trifluoromethanesulfonic or methanesulfonic acid. In hydrogen fluoride, the effectiveness of a scavenger can be quantitatively predicted by its pK_a value. In this acid, *m*-cresol and *p*-cresol appear to be the best scavengers of the 2,4-dichlorobenzyl and *p*-bromobenzyl cations, respectively.[142] In methanesulfonic acid, the best scavenger of the benzyl, *p*-methoxybenzyl, *t*-butyl and *p*-methoxybenzenesulfonylium cations (from the Mbs protection) is *m*-cresol. Its use instead of anisole in the final deprotection of bovine ribonuclease A (which does not contain tryptophan) and peptides containing tryptophan by means of any of the above-mentioned acids gave the crystalline enzyme and those peptides of the correct tryptophan contents.[75,134,135] The cleavage of the Z protection with boron tribromide[143] or trimethylsilyl triflate in dichloromethane (in order "to dissolve" a peptide, it can be pretreated with trimethylcyanosilane)[56] does not bring about the tryptophan alkylation even in the absence of a scavenger. Boron tristrifluoroacetate and trimethyliodosilane lead to equivocal results.[56,144]

4. Tryptophan Alkylation with Carbocations under Mild Acidic Conditions



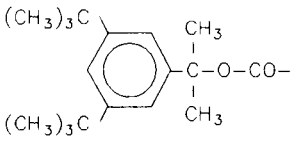
2-(4-biphenylyl)-2-propoxycarbonyl (Bpoc)



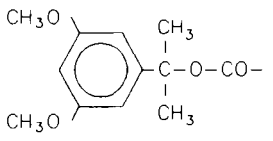
1-(1-adamantyl)-1-methylethoxycarbonyl (Adpoc)

Figure 21.

Another approach to incorporate tryptophan depends on changing the N^{α}-Boc group to urethanes such as the 2-(4-biphenylyl)-2-propoxycarbonyl (Bpoc, Fig. 21),[12,70] the 1-(1-adamantyl)-1-methylethoxycarbonyl (Adpoc, Fig. 21),[69,145] the 1-[3',5'-di(*t*-butyl)phenyl]-1-methylethoxycarbonyl (Bumeoc, Fig. 22),[93,145,146] and the α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl (Ddz, Fig. 22),[70,121] removable by 0.5-5% trifluoroacetic acid in dichloromethane or chloroform.



1-[3',5'-di(*t*-butyl)phenyl]-1-methylethoxycarbonyl (Bumeoc)



α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl (Ddz)

Figure 22.

Among these moieties, the N^{α}-Bpoc is the most commonly used. Successful syntheses of tryptophan-containing peptides both in solid[12,70,147] and in liquid[148] phase have been carried out using N^{α}-Bpoc-amino acids. Treatment of N^{α}-Bpoc amino acid with 0.5% trifluoroacetic acid in dichloromethane leads to a rapid equilibration between the monomeric alkene and the dimer with a carbocation intermediate (Fig. 23). Fmoc-Trp, exposed to the monomeric alkene in 0.5% trifluoroacetic acid in dichloromethane at 20°, reveals no change over a period of 90 min. After 21 hrs, 86% of the tryptophan derivative remains. Assuming that all the loss of tryptophan is the result of alkylation, one can calculate *ca*. 0.5% alkylation of tryptophan during a typical 30-min deblocking cycle. The following approximate reactivity order of various compounds,

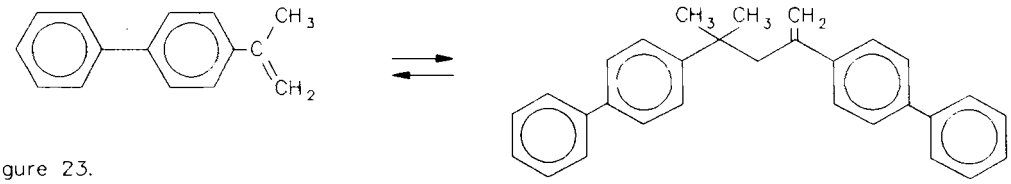


Figure 23.

examined as scavengers was established: benzylmercaptan ≈ thiophenol > indole >> 1,3-dimethoxybenzene ≈ resorcinol > 1,3,5-trimethoxybenzene ≈ dimethyl sulfide ≈ thioanisole. As can be seen, thioethers were found to be inefficient carbocation scavengers for the deblocking reaction. But even benzyl mercaptan, added in ten-fold excess does not totally eliminate the monomeric alkene, since the pure benzyl biphenylyldimethylcarbinyl thioether in 0.5% trifluoroacetic acid in dichloromethane reequilibrates with the thiol and the alkene.[149] Biphenylyldimethylcarbinyl trifluoroethyl ether is formed as the most abundant co-product in N^{α}-Bpoc removal with 1-hydroxybenzotriazole in trifluoroethanol.[150]

2-(1-Adamantyl)-2-propanol causes much less substitution at the indole moiety than *t*-butanol.[145] A somatostatin analogue containing tryptophan was synthesized by the use of the N^{α}-Bumeoc masking.[93] [Trp^4]Met-enkephalin was obtained in liquid phase by means of the N^{α}-Ddz.[121] And finally, the case of the irreversible transfer of the xanthenyl group from the glutamine residue to the adjacent tryptophan residue in 0.4 N HCl in tetrahydrofuran is worthy of mention (Fig. 24).[12]

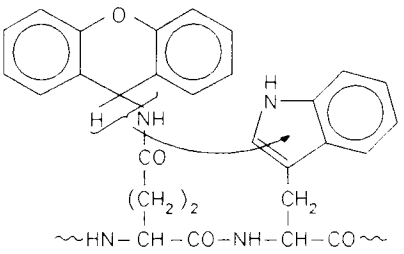


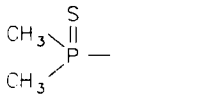
Figure 24.

III. N^{α}-PROTECTIVE GROUPS AND DEPROTECTION METHODS WHICH DO NOT GENERATE CARBOCATIONS

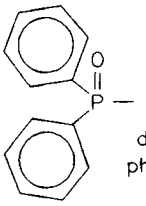
The use of the temporary N^{α}-protective groups which do not generate carbocations avoids the alkylation of tryptophan during the assembly of a

peptide chain. Two types of such groups have been developed: acid-labile and base-labile.

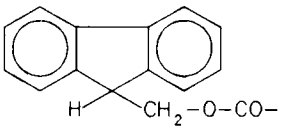
To the first type belong the N^{α}-phosphinothioyl and N^{α}-phosphinoyl groups and among the N^{α}-phosphinothioyl type, the dimethylphosphinothioyl (Fig. 25)



dimethylphosphinothioyl (Mpt)



diphenylphosphinoyl (Dpp)



9-fluorenylmethoxycarbonyl (Fmoc)

Figure 25.

was selected as especially suitable for solid phase synthesis. The residue can be removed by 0.2 M hydrogen chloride-triphenylphosphine in dichloromethane. Employing this blocking group, a series of tryptophan oligohomomeric peptides was synthesized and their homogeneity was established by spectral and HPLC methods.[151] Among the N^{α}-phosphinoyl groups, the diphenylphosphinoyl (Fig. 25) was chosen and it was first checked that this group allows preserving the chirality of amino acids during peptide synthesis. Using this protection and deprotection with 6 equiv. methanolic hydrogen chloride, a series of tryptophan-containing analogues of the C-terminal hexapeptide of substance P was obtained. It was shown by ^{31}P NMR that the diphenylphosphinoyl group was not incorporated in the peptide chain by attack of the indole nitrogen of tryptophan.[152]

To the base-labile N^{α}-protections belong first of all urethane groups capable of undergoing β-elimination. Commonly used is the Fmoc blocking group (Fig. 25)[8,70,107,109,141,153-158] which is cleaved by a variety of bases.[154,158] Recently, the 86-residue protein, coded by Tat gen of human immunodeficiency virus has been synthesized applying Fmoc-amino acids.[157] Other protections were only used in model experiments. Among the more recent ones

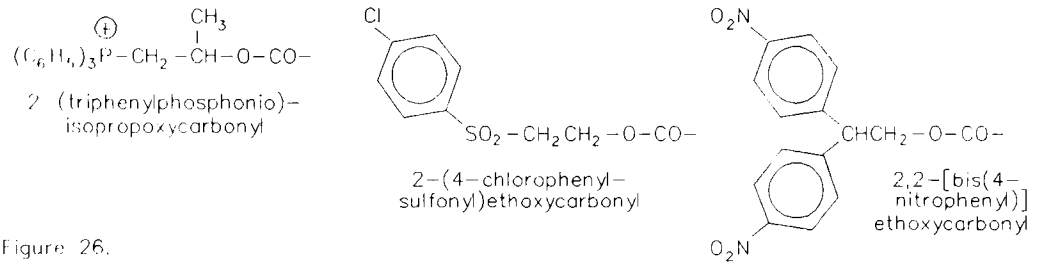


Figure 26.

are the 2-(triphenylphosphonio)isopropoxycarbonyl (Fig. 26) removed by sodium bicarbonate in methanolic-aqueous solution,[159] the 2-(4-chlorophenylsulfonyl)-ethoxycarbonyl (Fig. 26) eliminated with 1,8-diazabicyclo[5.4.0]undec-7-ene,[160] the 2,2-[bis(4-nitrophenyl)]ethoxycarbonyl (Fig.26) cleaved by 1,5-diazabicyclo[4.3.0]nona-5-ene or 1,8-diazabicyclo[5.4.0]undec-7-ene[161] and the 2-[4-(methylsulfonyl)phenylsulfonyl]ethoxycarbonyl (Fig. 27) removed by dimethylamine.[162] The 2-(trimethylsilyl)ethanesulfonyl (Fig. 27), non-urethane N^{α}-protective group, cleaved with fluoride ion[163] is worthy of mention.

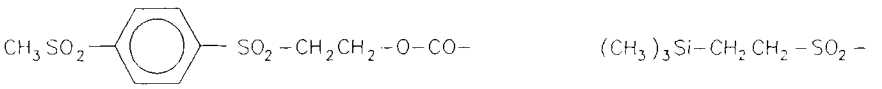


Figure 27.

The use of non carbocation-generating N^{α}-protective groups prevents alkylation only at the stage of the temporary-group removal. The complete repression of this side-reaction requires also that no carbocations be generated during the final acidolytic deprotection. To this end, many methods of total acidolytic deprotection by S_N2 mechanism have been developed. The finding that cleavage with trifluoromethanesulfonic acid was accelerated by the addition of a sulfur compound[164,165] has become the origin of this approach. These deprotecting procedures are presumably based on the push-pull mechanism exemplified in Figure 28.[135] In this approach, various acids are

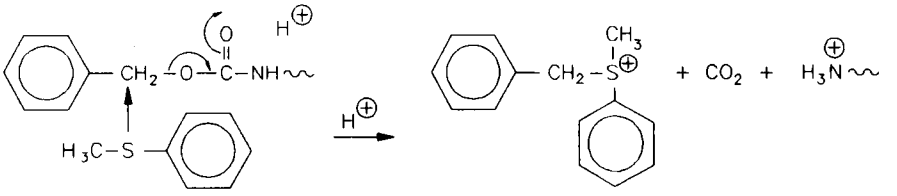


Figure 28.

used, e.g. trifluoroacetic, methanesulfonic, trifluoromethanesulfonic and sulfuric acids and hydrogen fluoride as well as Lewis acids such as trialkyl triflates and trimethylbromosilane. The second component of these systems may be one of the following sulfur or selenium nucleophiles: thioanisole, dimethyl sulfide, diphenyl sulfide, dimethyl selenide. Since alkylated nucleophiles may exhibit alkylating properties, an additional carbocation scavenger such as *m*- or *p*-cresol or sometimes anisole is used.[15,33,106,135,136,139,166-174] Silicone derivatives such as trichloromethylsilane and chlorotrimethylsilane promote the cleavage reaction in trifluoroacetic acid-dimethyl selenide system.[171-174] Many peptides containing tryptophan with its indole either free or N^i-blocked have been efficiently deprotected by means of these final deprotecting procedures by the S_N2 mechanism. On the other hand, no significant improvement has been observed changing hydrogen fluoride alone to the hydrogen fluoride-dimethyl sulfide system in deblocking a peptide containing tryptophan.[130]

IV. PROTECTIVE GROUPS FOR THE INDOLE FUNCTION OF TRYPTOPHAN

The indole nitrogen blocking with an electron-withdrawing substituent which diminishes susceptibility of the aromatic system to carbocation attack was the first approach at preventing tryptophan alkylation. So far, only acyl protections have been tested, even though save for the formyl group, it is difficult to introduce them directly on the indole nitrogen. At present, the following residues are used for the protection of the N^i-position of tryptophan:

1. the formyl group
2. urethane groups
3. arylsulfonyl groups
4. the diphenylphosphinothioyl group

1. The Formyl Group

The formyl is introduced into the indole nitrogen by formic acid in the presence of hydrogen chloride.[6,175] The facile introduction of the N^i-formyl group may, however, result in undesired formylation in the course of N^α-Boc removal with hydrogen chloride in formic acid[175] or of N^α-Z removal with hydrogen catalytically transferred from formic acid.[47] The formyl is the first N^i-blocking group which was used to protect tryptophan against oxidation.[29] It fulfilled this function and in addition another goal, *viz*. mainly as a protection against the *t*-butylation of tryptophan in solid phase peptide synthesis using Boc-amino acids.[176] The N^i-formyltryptophyl residue is not alkylated either during N^α-Boc cleavage with trifluoroacetic acid or boron tristrifluoroacetate or during the *t*-butyl ester synthesis with isobutene. However, 5% of the *t*-butylation did occur in the hydrogen fluoride-mediated N^α-Boc removal.[52,177] The N^i-formyl remains intact in trifluoroacetic acid or hydrogen fluoride.[12,33,178] The group does not protect tryptophan from hydrogenation.[52]

The N^i-masking in question is removed by bases[179,180] and/or nucleophiles. Hydrolysis can cause the formation of several side-products.[179] First of all, the hydrolysis in the presence of a nucleophile as the formyl scavenger is exploited; for example, hydrazine,[181] hydroxylamine,[106,182] piperidine[56,179,183] or a mixture of hydroxylamine and ammonia[184] may be used. Piperidine (0.1 M) in dimethylformamide cleaves the N^i-formyl group, sometimes incompletely.[179] Scavengers do not fully exclude the formyl transfer to the peptide N-terminus.[182] The N^i-formyl group is also cleaved during detachment of a peptide from a polymeric support with ammonia.[185] In acidic-nucleophilic reagents, the N^i-formyl is removed *via* thiolyse by *p*-thiocresol in hydrogen fluoride-dimethyl sulfide (low HF) (Fig. 29),[33] by *p*-thiocresol or ethane-

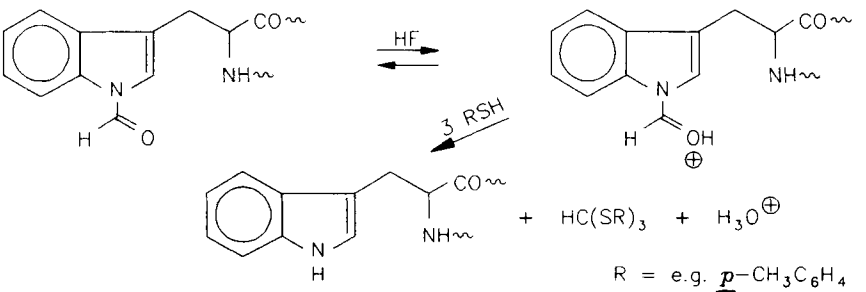


Figure 29.

dithiol in sulfuric acid-dimethyl sulfide in trifluoroacetic acid,[168,186] and by ethanedithiol in trifluoromethanesulfonic acid-dimethyl sulfide,[166] in trifluoromethanesulfonic acid-thioanisole,[187] trimethylsilyl triflate-thioanisole,[38] or trimethylsilyl triflate-dimethyl sulfide,[169] in each case in trifluoroacetic acid. In the low-HF procedure, N^i-formyl cleavage can be far from complete (50%) and increasing the amount of *p*-thiocresol does not help.[1-88] The addition of ethanedithiol is proposed as a means of forcing this reaction to completeness.[189] In the high-HF procedure in the presence of *p*-thiocresol, side-products, most probably addition products of *p*-thiocresol to the N^i-formylindole moiety were observed. They are not formed from tryptophan in which the indole is free.[33] Changing from *p*-thiocresol to 1,4-butanedithiol has been recently suggested.[189] As for N^i-formyl removal by ethanedithiol in the high hydrogen fluoride, data are contradictory.[33,178,190] The N^i-formyl is cleaved very easily by tetra(*n*-butyl)ammonium fluoride trihydrate.[191]

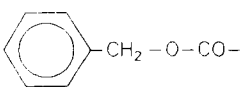
The reactivity of the N^i-formyl group described above predisposes it to solid-phase peptide synthesis with Boc-amino acids. In 1975, Li *et al.* reported overall yields of 10% or 25%, from syntheses of the 18-peptide β-MSH in which tryptophan was free or protected, respectively;[192] since then only a few peptides have been obtained by Merrifield's method without masking the indole nitrogen by the formyl group[7,86,131,179] and if they have, results are not always satisfactory.[86] It has to be stated, however, that a case was

reported in which only a slightly improved result was achieved with Boc-Trp(CHO) in comparison with that with Boc-Trp.[193] Stable $[Boc\text{-}Trp(CHO)]_2O$ {mp. 126-129°; $[\alpha]_D^{25}$ = + 15.1 ° (c 1.2, CH_2Cl_2)} has been prepared and found to be very useful for solid phase synthesis.[194] On the other hand, N^i-formyltryptophan is not recommended for peptide synthesis in solution,[52] but recentlly it has been sometimes employed for this purpose.[180,184]

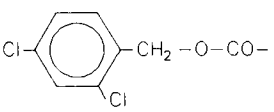
2. Urethane Groups

Seven of this type of protecting groups are known (Fig. 30-32). Treatment of indole with chloroformates leads to a mixture of 1- and 3-isomers.[195] In order to promote 1-substitution, indole is either converted to its anion with alkali or the acylating reagent is activated. In the first case, KF in the presence of crown ethers and the corresponding 4-nitrophenyl carbonates have been used (no racemization was observed)[196,197] or pulverized NaOH in the presence of tetra(*n*-butyl)ammonium bisulfate and the corresponding chloroformates have been more simply and effectively employed.[54,198] In the second case, 4-dimethylaminopyridine served as a catalyst of the acylation.[199,200] The urethane groupings are introduced only into the N^{α}- and C-blocked tryptophan. Therefore, the N^i-urethane derivatives of tryptophan alone were sometimes not obtained, but the tryptophan indole was acylated in protected peptides.[196,197]

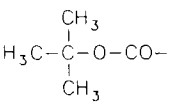
Boc-Trp(Z)-OCH_3 can be converted into TFA.Trp(Z)-OCH_3 by the trifluoroacetic acid-anisole system at 0° for 1 hr, and the decarbobenzoxylation of this compound was incomplete even after 24 hrs at room temperature in a trifluoroacetic acid-thioanisole mixture. This result indicates the N^i-Z group



benzyloxycarbonyl (Z)



2,4-dichlorobenzyloxycarbonyl



t-butoxycarbonyl (Boc)

Figure 30.

is more stable to acidic conditions (see also [196]) than the N^{α}-Z or N^{ω}-Z groups.[54,198] The N^{i}-Z deblocking requires 0.25 M trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid.[198] The N^{i}-2,4-dichlorobenzyloxycabonyl group is *ca*. 80 times more resistant to acids than the N^{i}-Z group.[197] Both N^{i}-blocking groups are cleaved by catalytic hydrogenation, hydrogen fluoride or hydrazine.[196,197]

The N^{i}-*t*-butoxycarbonyl (Fig. 30) is the most promising urethane group readily removable by trifluoroacetic acid without *t*-butylation, most likely because of the formation of the unstable N^{i}-COOH derivative which deactivates the indole nucleus. Boc-Trp(Boc)-OCH_3 can be converted into hydrazide in high yield. The selective deprotection of the N^{α}-Boc group which can be accomplished with 2.7 M hydrogen chloride in dioxane within 3 hrs at room temperature, allows the syntheses of oligopeptides. Prolonged rection time and/or a higher concentration of the acid increases N^{i}-deprotection.[199] Then, the N^{α}-2-phenylisopropoxycarbonyl[200] in conjunction with indole-Boc has been proposed for the synthesis of polypeptides. In 1.5% trifluoroacetic acid in dichloromethane, the N^{α}-blocking group is cleaved while indole-Boc is sufficiently stable to be removed only at the end of synthesis by trifluoroacetic acid-thioanisole.[68]

Figure 31 presents the reactivity of the N^{i}-2,2,2-trichloroethoxycarbonyl group. It resists strong acids, even trifluoromethanesulfonic acid-dimethyl sulfide, and catalytic hydrogenation, although longer exposure to

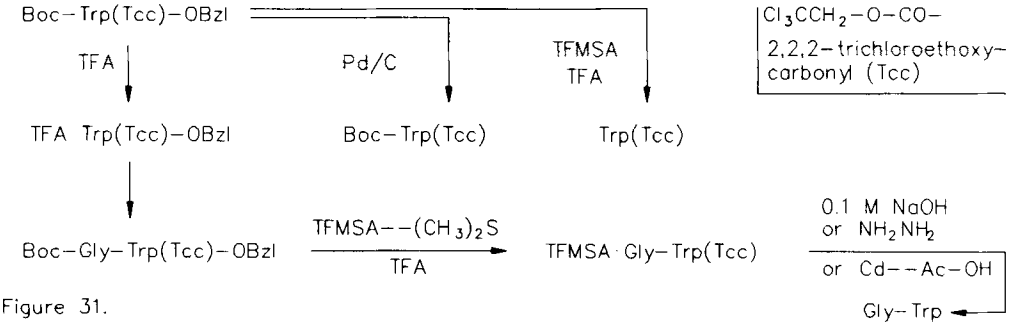


Figure 31.

hydrogen leads to very small amounts of side-products. This group can be quantitatively removed either under basic conditions, e.g., hydrazine, NaOH or by cadmium dust in acetic acid. However, the deprotection of Trp(Tcc) was incomplete in Zn-acetic acid in contrast to the Tcc group attached at the α-amino and hydrazide functions.[54]

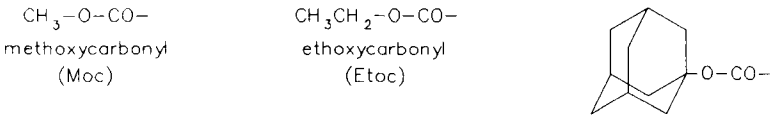


Figure 32.

The N^i-protections displayed in Figure 32 have been little investigated. The N^i-methoxycarbonyl and ethoxycarbonyl groups are removed by hydrazine or alkaline hydrolysis.[198] The N^i-Adc can be selectively removed in the presence of the N^{α}-Z by trifluoroacetic acid or hydrogen chloride in inert solvents.[201] Table 4 lists the N^i-urethane derivatives of tryptophan obtained in 1971-1988.

3. Arylsulfonyl Groups

Seven N^i-protections of this type (Fig. 33-35) have been tested. For the preparation of derivatives (Table 5), the corresponding arylsulfonyl chloride and either sodium hydride or pulverized NaOH in the presence of tetraalkylammonium salt were used;[202-204] NaOH was found not to racemize tryptophan.[202]

The N^i-arylsulfonyl groups, collected in Figure 33 and 34 resist trifluoroacetic acid, bases and catalytic hydrogenation. The only exception was

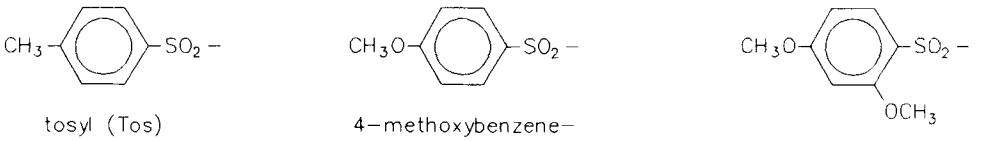


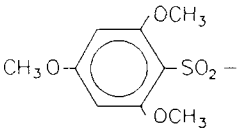
Figure 33.

TABLE 4. *N^i-Urethane Derivatives of Tryptophan (1971-1988)*

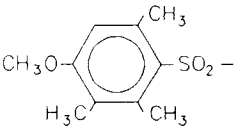
Compound	mp. (°C)	$[\alpha]_D^{25}$ (°)	lit.
Z-Trp(Adc)	-	-	201
Boc-Trp(Tcc)-OBzl	122-125	-	54,198
Boc-Trp(Tcc)	186-188	-	54
TFA·Trp(Tcc)-OBzl	-	-	54
Trp(Tcc)	222-225	-	54
Boc-Trp(Z)-OCH_3	118-120	-	54,198
TFA·Trp(Z)-OCH_3	-	-	54
Boc-Trp(Boc)-OCH_3	-	-	199
Boc-Trp(Boc)-$NHNH_2$	-	-	199
Z-Trp(Boc)-OCH_3	-	-	199
Trp(Boc)-OCH_3	-	-	199
Ppoc-D-Trp(Boc)-OCH_3	white foam	+ 12.1 (c 1.0, DMF)	68
D-Trp(Boc)-OCH_3	-	-	68
Boc-Trp(Moc)-OBzl	114-116	-	198
Boc-Trp(Etoc)-OBzl	102-104	-	198

the formation of a very small amount of an unknown compound when Boc-Trp(Mtr)-OBzl was exposed to trifluoroacetic acid at room temperature for 15 hrs. The protections depicted in Figure 33 are too stable to be exploited in practice under conditions of the final deprotection with hydrogen fluoride-anisole-ethanedithiol at 0° or with methanesulfonic acid-thioanisole-ethanedithiol at 20°. But, the groups shown in Figure 34 are removed smoothly, although the N^i-Mtb in methanesulfonic acid generated an unknown by-product together with tryptophan; thus this acid should then not be used for the N^i-Mtb cleavage.[202]

The Trp(Mtr) residue is even deprotected under the influence of 0.15 M methanesulfonic acid-thioanisole in trifluoroacetic acid.[205] Syntheses of the 17-peptide bombesin (in solution) and of the 17-peptide dynorphin (in solid phase) were proof of the usefullness of N^i-Mtb group.[202] The N^i-Mtr was successfully employed in solution to obtain the 9-peptide gonadoliberin analogue, leuprolide,[202] and the 27-peptide chicken gastrin releasing hormone.[205] During those syntheses, both the N^i-Mtb and the N^i-Mtr resisted the action of 1-hydroxybenzotriazole.[202,205]



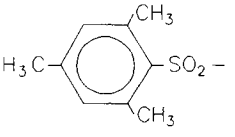
2,4,6-trimethoxybenzenesulfonyl (Mtb)



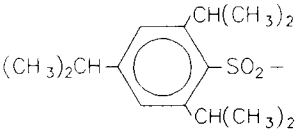
4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr)

Figure 34.

The N^i-Mts and N^i-Tip groups (Fig. 35) were found to be stable to various treatments required for peptide synthesis, *viz*. acid treatment with trifluoroacetic acid, trifluoroacetic acid-thioanisole, 25% hydrogen bromide in acetic acid or saponification with 1 N NaOH and hydrazinolysis.[203,204] The N^i-Mts protection withstands 4 N HCl in dioxane and catalytic hydrogenation.[203] Both groupings are split by 1 M trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid at 0^o, the first rapidly[203] and the second more slowly.[204] The N^i-Mts is also removed by methanesulfonic acid or a 1:1 mixture



2,4,6-trimethylbenzenesulfonyl (Mts)=mesitylenesulfonyl



2,4,6-triisopropylbenzenesulfonyl (Tip)

Figure 35.

TABLE 5. *N^i-Arylsulfonyl and N^i-Diphenylphosphinothioyl Derivatives of Tryptophan (1984-1988)*

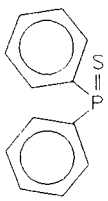
Compound	mp. (°C)	$[\alpha]_D^{18-31}$ (°)	lit.
Trt-Trp(Tos)-OBzl	101-103	+ 47.6 (c 0.5, DMF)	202
Trp(Tos)·0.5H_2O	227[a]	- 29.2 (c 0.5, AcOH)	202
Trt-Trp(Mbs)-OBzl	96	+ 43.2 (c 0.5, DMF)	202
Boc-Trp(Mds)	83-84	- 28.2 (c 0.5, DMF)	202
Trp(Mds)·0.5H_2O	218-220	- 34.8 (c 0.5, DMF)	202
Boc-Trp(Dmb)	100[a]	- 9.43 (c 0.5, DMF)	202
Boc-Trp(Mtb)	82-84	- 15.4 (c 0.5, DMF)	202
Boc-Trp(Mtr)	88-90	- 24.8 (c 0.5, DMF)	202
Z(OMe)-Trp(Mts)-Bzl	94-95	- 16.1 (c 0.6, DMF)	203
Z(OMe)-Trp(Mts)	77-79	- 10.1 (c 1.8, DMF)	203
Z(OMe)-Trp(Mts)-$NHNH_2$	121-123	- 41.3 (c 0.8, DMF)	203
Z(OMe)-Trp(Mts)-OSu	-	-	203
Boc-Trp(Mts)-OBzl	-	-	203
Boc-Trp(Mts)·DCHA	154-155	+ 19.9 (c 1.0, CH_3OH)	203
Boc-Trp(Mts)-OSu	-	-	14
Trp(Mts)	211-213	- 30.0 (c 0.6, DMF)	203
Z(OMe)-Trp(Tip)-OBzl	-	-	204
Z(OMe)-Trp(Tip)	-	-	204
Z(OMe)-Trp(Tip)·DCHA	-	-	204
Trp(Tip)	-	-	204
Boc-Trp(Ppt)-OCH_3	125-126.5	- 8.0 (c 0.6, DMF)	210
Boc-Trp(Ppt)	127-129	+ 17.4 (c 1.0, CH_3OH)	210

a) Decomposition

of this acid and trifluoroacetic acid possibly in the presence of thioanisole.[203] The N^i-Tip is less susceptible to these reagents.[204] Both protections are not completely cleaved by hydrogen fluoride even in the presence of thioanisole.[203,204] 1 M Trialkylsilyl triflates-thioanisole or -diphenyl sulfide[14,15,38,40,139,206] and 1 M trimethylbromosilane-thioanisole,[37] in each case in trifluoroacetic acid at 0° demask the Trp(Mts) residue more effectively than 1 M trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid. Yields are higher or at least equal.[14,15,37,38,139] Since its introduction,[203] the N^i-Mts has been commonly used by Yajima *et al*. for the synthesis of a number of polypeptides, mainly in solution,[9,15,37,38,40,137-139,203,207] but also in solid phase.[208] For example, in the synthesis of urotensin II, Boc-Trp(Mts) was preferred to tryptophan with the free indole moiety.[14,209] The N^i-Tip group was utilized in the synthesis of the 9-residue δ-sleep inducing peptide.[204]

4. The diphenylphosphinothioyl Group

This group (Fig. 36) is introduced into indole by Ppt-Cl in the presence of pulverized NaOH and catalytic amounts of tetra(*n*-butyl)ammonium bisulfate. The N^i-Ppt group is stable to trifluoroacetic acid-thioanisole, tetrachlorosilane-thioanisole (1 hr), NaOH, hydrazine and Zn-acetic acid, but removable by either 0.5 M methanesulfonic acid-thioanisole or 0.3 M trifluoromethanesulfonic acid-thioanisole, in each case in trifluoroacetic acid at 0°. The N^i-Ppt has been found to be smoothly deprotected at 20° by fluorides such as tetra(*n*-butyl)ammonium fluoride trihydrate or KF in the presence of 18-crown-6. Reaction rates depend on the solvent. For the first fluoride, the best solvents are dimethylformamide and dimethyl sulfoxide; for KF, acetonitrile appears the most suitable. To demonstrate the usefulness of Boc-Trp(Ppt), bradykinin potentiating peptide was



diphenyl-phosphino-thioyl (Ppt)

Figure 36.

synthesized by the conventional solution method and deblocked for comparison by the three reagents: a) methanesulfonic acid-thioanisole, b) trifluoromethanesulfonic acid-thioanisole and c) tetra(*n*-butyl)ammonium fluoride trihydrate in dimethylformamide. The latter procedure seems to be preferred.[198,210,211] 1 M Tetrafluoroboric acid-thioanisole in trifluoroacetic acid in the presence of *m*-cresol and of ethanedithiol has been recently found to remove effectively the N^iPpt group in final deprotections.[212] Table 5 lists the N^i-diphenylphosphinothioyl derivatives of tryptophan obtained.

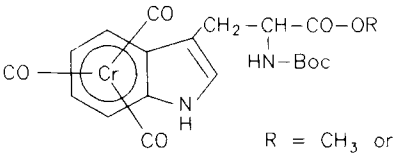
CONCLUSION

The particularly abundant bioreactivity of tryptophan, recognized a relatively long time ago, has some counterparts in peptide synthesis in which tryptophan with its free indole moiety can suffer a number of side-reactions. The oxidation and alkylation of tryptophan are two major side-reactions, frequently observed in the course of acidolytic deprotections. The first one is prevented by the addition of an antioxidant, among which ethanedithiol[31] is commonly used though it is not without shortcomings.[32-34] Attempts to exclude the alkylation of tryptophan during the peptide chain assembly follow two directions, *viz*. (i) the search for temporary N^{α}-groups which do not generate carbocations during deprotections and (ii) the development of N^i-blocking groups for tryptophan which discourage alkylation. Among the first group, the α-Fmoc-protection[154] (Fig. 25), eliminated by bases has become the most important in the synthesis of tryptophan-containing peptides especially in solid phase.[213-215] Using Fmoc-amino acids, there is no need for N^i-protection.[153,155] The Fmoc-based solid-phase peptide synthesis beeing predominantly developed in Europe,[155,214,215] would require a separate review and is beyond the scope of our survey.

Sixteen N^i-protective groups for tryptophan are currently known, all of the acyl type. All the groups are most often believed to prevent the alkyla-

tion and the oxidation of tryptophan. As many as twelve of these groups were devised in the 1980s, after the finding in 1979 of an efficient method for the indole nitrogen acylation.[195] Among the sixteen N^i-masking groups only two are commonly used in complementary fashion: the N^i-formyl mainly in Merrifield's method[153,155] and seldom in solution peptide synthesis,[180,184] and the N^i-Mts (Fig. 35) preferentially in the latter synthesis[15] and rarely in Merrifield's method.[187,208] A peptide chain having been assembled, each of the two latter protections can be removed, fortunately, by an acidic-nucleophilic mixture which does not generate carbocations; however, the N^i-formyl group requires the addition of a thiol, *p*-thiocresol and/or ethanedithiol.[14,15,33,37,38,40,138,139,166,168,169,186,187,203,206] It is worth noting that the N^i-formyl group[191] and the N^i-diphenylphosphinothioyl[210,211] are cleaved by fluoride ion which smoothly splits the N^α-Fmoc[157] and detaches a peptide from the resin of *p*-(carbamoylmethyl)benzyl ester linkage as well.[210] This makes it possible to use Boc-Trp(CHO) and Boc-Trp(Ppt) in two tactics of the peptide chain building: in the N^α-Boc-ω-Z protecting group system with the final acidic-nucleophilic deprotection and in the N^α-Boc-ω-Fmoc protecting group system with the final fluoride, non-acidic deprotection. In this respect, the first experiment with Boc-Trp(Ppt) has been already provided.[210] The development of the N^i-protections is mostly due to Japanese chemists.[29,54,175,198,202-204,210-212]

Looking for efficient N^i-protections, Sergheraert and Tartar[216] took an unusual approach. They synthesized Boc-Trp derivatives with tricarbonylchromium with the view that the strong electron-withdrawing effect of this ligand



R = CH_3 or *p*-$O_2NC_6H_4$

Figure 37.

is comparable to that of the nitro group. These derivatives (Fig. 37) were stable, but the yield and purity of the peptide obtained, after Merrifield's synthesis with the hydrogen fluoride-mediated deprotection were not satisfactory.

The great advance in the methodology of the synthesis of peptides containing tryptophan, accomplished in 1974-1989 (the interval covered by this review) allows us to conclude that peptide chemistry has evolved from the belief of no need for the protection of the indole nitrogen[6] through Hamlet's dilemma "to protect or not to protect"[12,217] and to the current strong acceptance of the advantages resulting from the incorporation into a peptide chain of this amino acid with its indole moiety blocked.

Acknowledgement.- The authors acknowledge financial support of the present study by grant CPBP 05.06.1.5.3.

ABBREVIATIONS

Ac = acetyl
Adc = 1-adamantyloxycarbonyl
Adpoc = 1-(1-adamantyl)-1-methylethoxycarbonyl
Boc = *t*-butoxycarbonyl
Bpoc = 2-(4-biphenylyl)-2-propoxycarbonyl
*t*Bu = *t*-butyl
Bumeoc = 1-[3',5'-di(*t*-butyl)-phenyl]-1-methyl-ethoxycarbonyl
Bzl = benzyl
DCHA = dicyclohexylamine
Ddz = α,α-dimethyl-3,5-di-methoxybenzyloxy-carbonyl
Dmb = 2,4-dimethoxybenzene-sulfonyl
DMF = dimethylformamide
EDT = ethanedithiol
Etoc = ethoxycarbonyl
Fmoc = 9-fluorenylmethoxy-carbonyl
Mbs = 4-methoxybenzenesulfonyl
Moc = methoxycarbonyl
Mtb = 2,4,6-trimethoxybenzene-sulfonyl
Mtr = 4-methoxy-2,3,6-trimethyl-benzenesulfonyl
Mts = 2,4,6-trimethylbenzenesul-fonyl (mesitylenesulfonyl)
OSu = succinimido-oxyl
Ppoc = 2-phenylisopropoxycarbonyl
Ppt = diphenylphosphinothioyl
Tcc = 2,2,2-trichloroethoxy-carbonyl
TFA = trifluoroacetic acid or trifluoroacetyl
TFMSA = trifluoromethanesulfonic acid
Tip = 2,4,6-triisopropylbenzene-sulfonyl
Tos = tosyl
Trt = trityl
Z = benzyloxycarbonyl
Z(OMe) = 4-methoxybenzyloxycarbonyl

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(Received January 3, 1990; in revised form September 10, 1990)