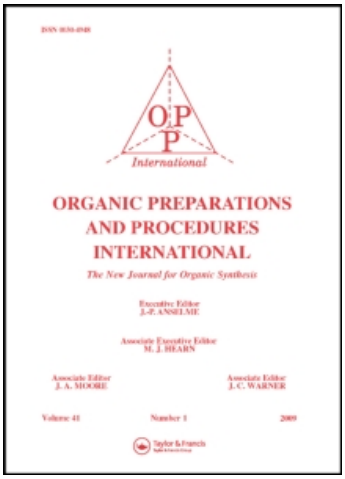

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ORGANIC PREPARATIONS AND PROCEDURES INT. 20(5), 427-464 (1988)

ARGININE, HISTIDINE AND TRYPTOPHAN IN PEPTIDE SYNTHESIS.

THE GUANIDINO FUNCTION OF ARGININE†

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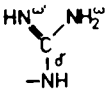
INTRODUCTION

The reactivity of amino acid side-chains which is the underlying reason for the biological specificity of proteins and peptides, is also a major factor in the synthesis of these compounds. Accordingly, for peptide chemists, each of the twenty common constituents of proteins may result in specific problems during the synthesis of its derivatives and/or especially of peptides. The determination of which amino acid presents the greatest difficulty depends on the current state of peptide synthesis methodology and on the possibility of controlling the final synthetic results. For example, in a discussion of the successful synthesis of crystalline bovine ribonuclease A, Yajima remarked that the aspartic residue reactivity was the greatest obstacle during the synthesis.[1]

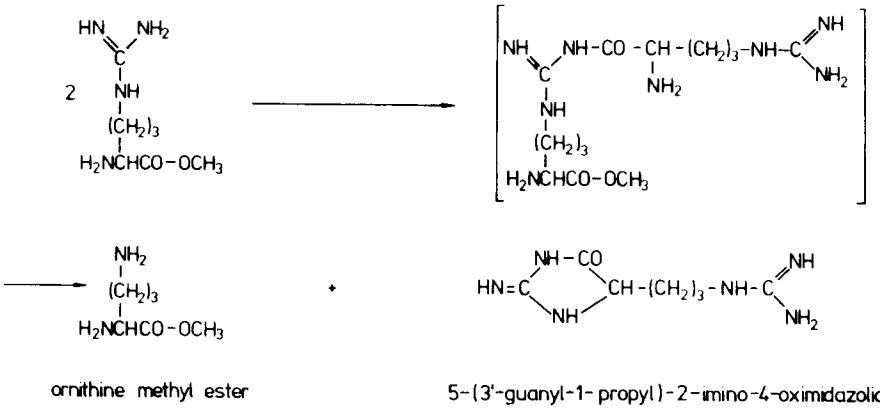
The present series of reviews will deal with three amino acids, arginine, histidine and tryptophan, currently considered to present synthetic difficulties. Each of them involves a characteristic side-chain function: guanidine in arginine, imidazole in histidine and indole in tryptophan. These functional groups are responsible for the unique biochemistry and chemistry of the amino acids as well as the specific problems created by them during peptide synthesis. The individuality of the amino acids in question has attracted the attention of peptide researchers

who have devoted separate comprehensive reviews to them.[2,3] We plan to survey, in three related reviews, the reactivity of the side-chain function of arginine, histidine and tryptophan in peptide synthesis as well as the recent progress in the methodology of protection of these functions. The latter area will, as a rule, be based on papers which appeared since the excellent monograph by Wünsch[4] in 1974 and completed up to 1987 (Chemical Abstracts). Earlier data will be used only to the extent needed to put this review in perspective. A list of abbreviations is given at the end of this review.

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

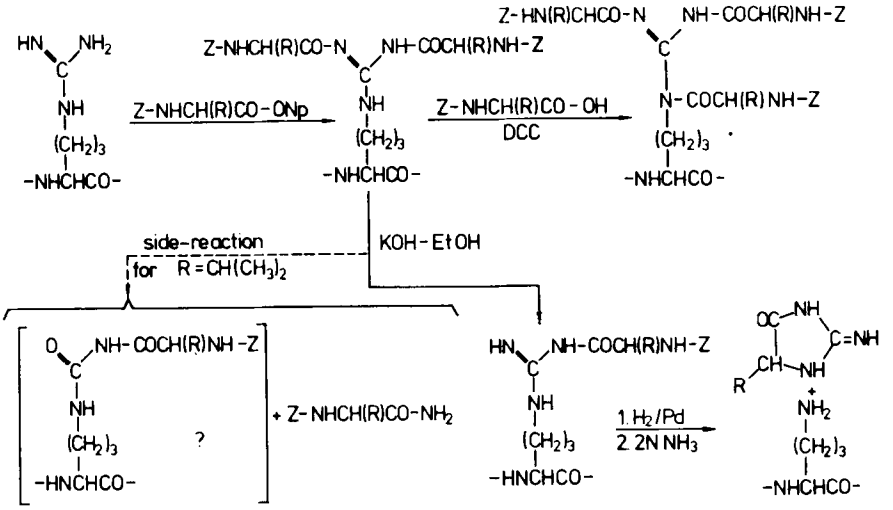


The trifunctional guanidino group of arginine displays a strong nucleophilic character and thus readily undergoes acylation in a short period of time. An example of the ease of acylation is self-condensation of arginine methyl ester resulting in its quantitative disproportionation into ornithine methyl ester and 5-(3'-guanyl-1-propyl)-2-imino-4-oximidazolidine.[5]



More powerful activations than methyl ester, e.g. p-nitrophenyl ester, can result in bisaminoacylation as a major path. From the bisaminoacylated derivatives, one acyl residue is readily cleaved by alkali.

However, steric hindrance in the amino acid side-chain, e.g. with R = $CH(CH_3)_2$, promotes generation of amide as a side-reaction, probably accompanied by citrulline formation (not firmly established). Removal of the N^{α}-blocking group (Z in Figure) from N^{ω}-(N^{α}-blocked aminoacyl) derivative is followed, especially under alkaline conditions, by a reaction similar to disproportionation of ω-arginylarginine, i.e. ornithine methyl ester and 5-alkyl-2-imino-4-oximidazolidine production.[5]



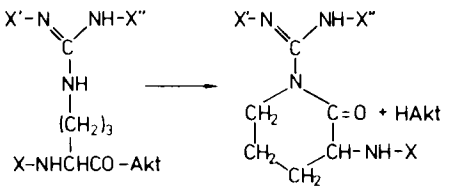
N^{g},$N^{g'}$-bisaminoacylated compounds can be transformed into N^{δ},N^{ω},$N^{\omega'}$-trisaminoacylated derivatives with stronger activation, e.g. DCC. Acylation of the N^{α}-protected arginine with three N^{α}-protected amino acid residues was described only once[5] and therefore this type of derivatives is not well-known. These compounds can be assumed to be acylating agents transferring the aminoacyl residue from the imino group onto a nucleophilic amine in a manner analogous to N^{δ},N^{ω},$N^{\omega'}$-triacyl derivatives of arginine.[4]

Consequently, the guanidino group of arginine has to be protected during peptide synthesis. Therefore, in this section, reactions of the side-chain of N^{g}-blocked arginine, and not of arginine itself, will be reviewed. At present, the following residues are used for the protection of arginine N^{g}-position:

1. the nitro group
2. urethane groups
3. arylsulfonyl groups
4. proton
5. 1,2-dihydroxycyclohex-1,2-ylene

All three nitrogen of the guanidino group should be blocked;[3] it should be kept in mind that the protection is not always secured by the accumulation of any types of blocking groups. Nevertheless, such total protection is not used in practice. We know only one example of $N^{\delta},N^{\omega},N^{\omega'}$-threefold-blocked arginine, *viz.*, $N^{\delta},N^{\omega},N^{\omega'}$-tritritylarginine which however was never utilized in peptide synthesis.[3,4,6] With N^{g}-mono or $N^{g},N^{g'}$-diprotected arginine, side reactions may take place and some of these are characteristic of almost all the types of blocking groups (intramolecular acylation) or of a large number of them (ornithine generation). The two last-mentioned reactions will be discussed below.

A very common reaction of N^{g}-mono- or of most $N^{\omega},N^{\omega'}$-disubstituted and of activated arginine derivatives is δ-lactam formation.[3] The lactam is assumed to be already produced during the synthesis of N^{α},N^{g}-deriva-



X' = NO_2 X" = H
X' = H X" = Tos
X' = H X" = Z
X' = H X" = Z(NO_2)

X' = H X" = Boc
X' = H X" = Itbz
X' = Trt X" = Trt
X' = Ac X" = Ac
(X' = H X" = H) · HAn

tives of arginine, when the introduction of blocking group reagent may result in the formation of a mixed anhydride with the carboxy group of arginine. Indeed, this side-product was observed in the course of acylation of N^{α}-benzyloxycarbonylarginine with tosyl chloride at pH 8.2.[3,4,7] The presence of δ-lactam in N^{α},N^{g}-derivatives is regarded as one of the reasons for their difficult crystallization and modest yield.[3] δ-Lactam formation also accompanies arginine active ester synthesis.[8,9] Recently, syntheses of new arginine active esters have succeeded and, fortunately,

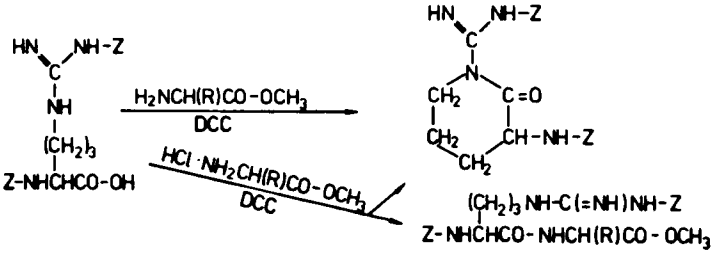
those of known active esters have been improved (Table 1). All the derivatives have been used in peptide synthesis.[10-17]

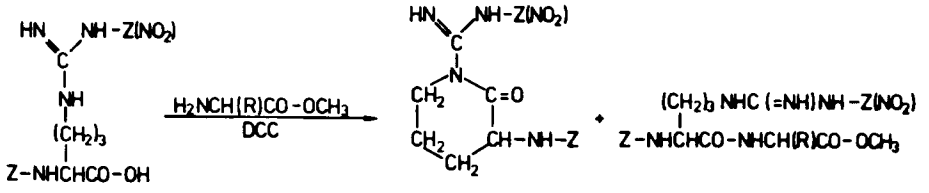
TABLE 1. *Active Esters of X-Arg(X')-OH (1974 - 1986)*

Active Ester	mp. (°C)	$[\alpha]_D^{20-24}$ (°)	lit.
Z-Arg$^{\delta,\omega}$(Z)$_2$-OTcp	124-126	- 8.6 (c 1, THF)	10
Z-Arg-ONp.HNO_3	123-125	- 25.2 (c 2.7, AcOH)	11
Z-Arg-ONp.$C_6H_2OH(NO_2)_3$	-	- 18.6 (c 2.4, DMF)	11
Boc-Arg$^{\omega'}$(NO_2)-OPcp.1/6C_6H_5OH	115-116	- 23.4 (c 1.02, AcOEt)	12
Fmoc-Arg$^{\delta,\omega'}$(Adc)$_2$-ONp	-	-	13
Z-Arg$^{\omega,\omega'}$(DHCH)-OSu.HCl	-	-	14
Boc-Arg-OPfp.HCl	-	-	15
Boc-Arg(Boc)$_2$-ONd	55-60[a]	- 14.3 (c 1, EtOH)	16
Boc-Arg(Boc)$_2$-OSu	64-65	- 17.0 (c 1, EtOH)	16

a. Decomposition.

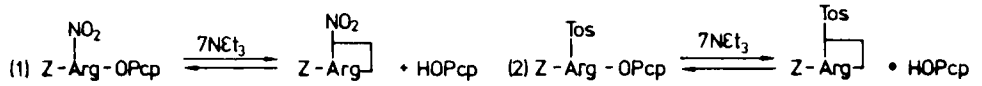
Above all, however, the competition between formation of δ-lactam with peptide bond formation, that is the relative rate of the intra- and





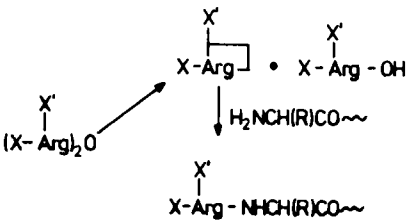
intermolecular aminolysis, depends on the type of N^g-masking group and that of activation. The N^ω-benzyloxycarbonyl derivative which is particularly prone to δ-lactam formation gives the δ-lactam exlusively on coupling with a free amino acid ester in the presence of DCC. Only the use of the hydrochloride as a proton donor for the benzyloxycarbonylguanidino group instead of the free ester makes peptide formation possible as well.[4,18] However, the N^ω-p-nitrobenzyloxycarbonyl derivative gives a mixture of δ-lactam and peptide on reaction with the same free amino acid ester in the presence of DCC.[4] Also very prone to δ-lactam reaction are $N^{\omega'}$-nitro derivatives. For instance, Z-Arg(NO_2)-OPcp under the catalytic influence of triethylamine forms the lactam 20 times faster than Z-Arg(Tos)-OPcp but couples with an amine more slowly.[19] In some cases, this side-reaction is observed during aminolysis of protonated arginine derivatives,[3,6,13,20] especially with active esters of Fmoc-Arg(H^+)[13] but not in other cases.[21-23] Tetraphenylborate, a solubilizing counterion for protonated guanidino group has been suggested to combat this problem.[23] An example of the influence of the type of activation on the relative rate of peptide and δ-lactam formation is the necessity to use DCC with 1-hydroxybenzotriazole instead of the symmetrical anhydride during incorporation of Boc-Arg(Tos) onto peptide chain under solid phase synthesis conditions.[24,25]

δ-Lactam formation can be a reversible reaction if the resulting HAct is a nucleophile. Thus, in the presence of triethylamine, 13% and 9% esters are found in the reaction mixture (1) and (2), respectively. As



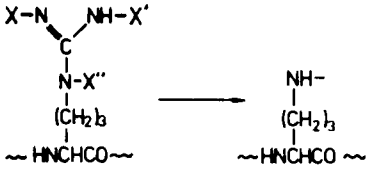
seen, δ-lactams are compounds with the moderately active carboxyl group towards phenoxide ions.[19] Most probably they display also a moderate activity during aminolysis and this property explains the 200-fold slower

coupling of symmetrical anhydrides of N^g-substituted arginine derivatives with amines as compared with the coupling of symmetrical anhydrides of



other amino acids.[24,26] Hydrazinolysis of N^{α}-benzyloxycarbonyl-N^{ω}-p-nitrobenzyloxycarbonylarginine δ-lactam is known.[4] An example of another type of electrophilic reactivity of δ-lactam, namely that its guanidino imido group (and not its carbonyl) undergoes a reaction,[4,26] will be discussed in the section covering properties of $N^{\omega'}$-nitro protection. Removal of the lactam side-products is usually a simple operation[16,20,27] but in some instances, this side-reaction and its consequences force changes of synthesis strategy[27] or tactics.[24]

In reactions of arginine-containing peptides, the ornithine residue is often formed. This side-reaction can occur when nitro, urethane, tosyl or a proton is used as a protecting group.[3,6,20] Conditions and mechanisms for formation of the ornithine are different and will be described in sections dealing with the corresponding N^g-masking groups.



X = NO_2, X', X'' = H
X = X'' = H, X' = ROCO
X = H, X' X'' = ROCO

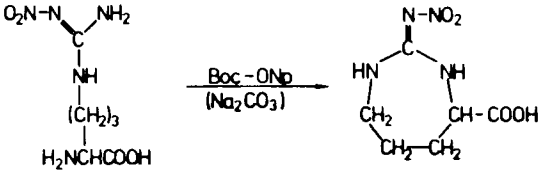
X = X'' = H, X' = Tos
(X = X' = X'') · HAn
R = alkyl or aralkyl

As has already been shown in the example of N^{ω}-benzyloxycarbonyl derivative, some of the N^g-protected arginine derivatives are basic. Thus, after activation of their carboxyl group, racemization may be expected in the absence of other bases in solution. Indeed, Z-Arg(Tos)-OPcp self-racemizes and the rate of this process is concentration-dependent.[19]

II. PROTECTING GROUPS FOR THE GUANIDINO FUNCTION OF ARGININE

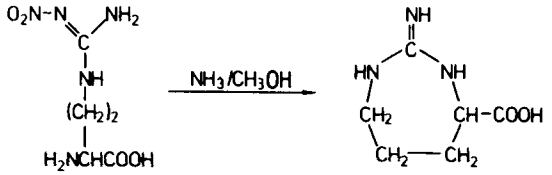
1. The Nitro Group

Protection by the nitro group has been known for over fifty years[4] and there are numerous examples of its successful application in classical peptide synthesis in solution and on polymeric supports[3,4,12,17,20,26-37] although failures have also been noted resulting from difficulties encountered in its reductive removal even from a dipeptide.[38,39] The advantage of the nitro group is the possibility to introduce it without the necessity of protecting for arginine amino and carboxyl functions. For this purpose, a nitrating mixture (HNO_3, H_2SO_4 and 20% oleum[4]) or more conveniently NH_4NO_3 in H_2SO_4 under mild conditions[40] is used. Arginine peptides with the nitro group, in contrast to those with some other N^g-blocking groups, dissolve well in organic solvents.[20] However, the accumulation of nitroarginine residues in homooligoarginyl peptides (e.g. Z-[Arg(NO_2)]$_4$-OCH_3) lowers solubility.[41] In spite of a long period of exploitation of nitroarginine derivatives, the position of the masking group was established as late as 1979, by means of ^1H and ^{15}N NMR. From the four possible isomers, the nitrimine structure exists in an aqueous solution.[42]

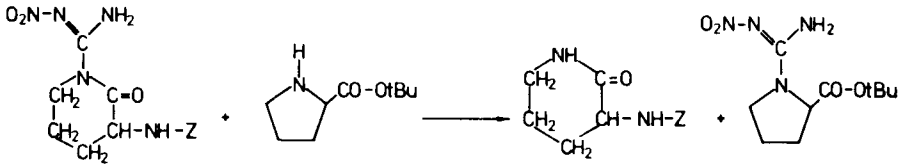


Nitroarginine itself as well as its derivatives are very prone to side-reactions both with nucleophilic and with electrophilic agents. Two such reactions were described as early as 27 years ago. The first was the formation of a 1,3-diazacycloheptane during an attempted synthesis of Boc-Arg(NO_2).[4] The presence of the side-product seems likely to be responsible for the varying quality (different melting points and incorporation of

various amounts of crystallization solvents) of Boc-Arg(NO_2) obtained according to the early preparative procedures.[43-46] Presently, there exist two high-yield methods of synthesis of Boc-Arg(NO_2), free from crystallization solvent and characterized by a high melting point. In one of them, Boc-N_3 and tetramethylguanidine in dimethylformamide solution are used.[47] In the other, t-butyl 4,6-dimethylpyrimidyl-2-thiol carbonate and triethylamine in an aqueous-organic medium are used.[48] A similar arginine cyclization product accompanied by the loss of the nitro group was formed in the presence of ammonia.[49] However, this type of cyclic compound was not observed in peptide-bond-forming synthesis with nitroarginine α-amino group.[50]



Nitroamidination of the proline amino group was the second side-reaction described as early as 27 years ago.[4] According to Merrifield,



during peptide bond formation, the lactam is also capable of transferring the protected amidino moiety onto amines thus resulting in premature termination of peptide chain.[26]

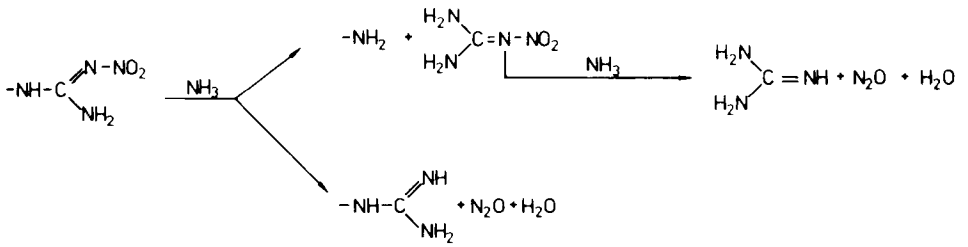
N^{α},N^{g}-Ditosyl-$N^{\omega'}$-nitroarginine was obtained in 1983 by reaction of $N^{\omega'}$-nitroarginine with tosyl chloride in the presence of $NaHCO_3$.[51] This indicates that the nitroarginine group can be acylated not only intramolecularly (δ-lactam formation; previous section) but intermolecularly as well. Peptides bearing nitroarginine residues are also very prone to side-

reactions both during peptide chain elongation and especially during final deprotecting procedures.

Hydrazinolysis of esters of nitroarginine-containing peptides fails to yield the expected hydrazides but leads to a series of side-products,[52] among which ornithine is assumed to be the main one.[20] The side-reactions can be suppressed by treatment of esters for 2 minutes with 100-fold excess hydrazine.[53]

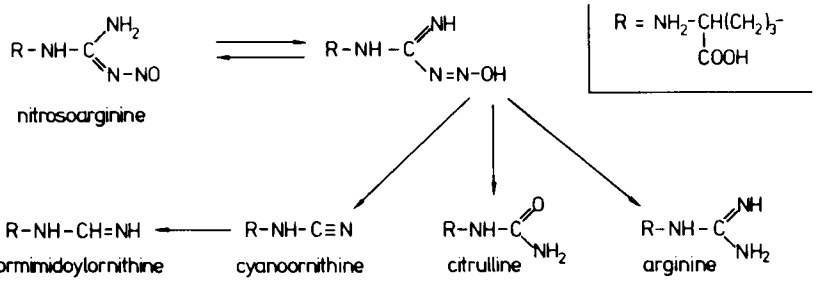
Side-products, unidentified as yet, were found from the action of HBr in acetic acid on peptides with Z-Arg(NO_2) residue.[50,54] The problem can be avoided by removing the Z group by means of liquid HBr at -75°.[54] After acidic removal of Z- or Boc-protecting groups, peptides incorporating -Arg(NO_2)- retain a higher number of acid molecules than one equivalent in relation to the number of their nominal basic groups. For example, for each nitroguanidino group, 0.20-1.25 excess HBr [27,55,56] or 0.17 excess trifluoroacetic acid equivalent[44] were found.

The transformation of nitroarginine peptide into its amide with an excess of ammonia leads to a peptide containing ornithine, sometimes in amounts higher than 50%. In model experiments with N^{α}-acetyl-$N^{\omega'}$-nitroarginine, nitroguanidine, guanidine, N^{α}-acetylarginine and N^{α}-acetylornithine were isolated. Rationalization of these results is as follows.[49]



Reduction of the nitro group, historically the first approach to its removal, is still used in spite of many side reactions and hence is still being improved. Though in recent times no efforts have been made to improve reduction of the nitro group by means of sodium in liquid ammonia

or electrolytically, repeated attempts have been made to find better conditions for catalytic reductions. Catalytic reduction of the nitroarginine residue proceeds very slowly, requiring several days sometimes for larger peptides[3,57] and there are a number of examples which testify to failures depending on peptide sequence.[6,38,39] The course of hydrogenation and the composition of the crude product are also influenced by the type of catalyst employed, the reaction medium and its acidity.[6,57] According to some authors,[57] the generation of side-products may be a source of concern after formation of nitrosoarginine, the initial reduction product of



nitroarginine. In some cases, the formation of aminoguanidine[6] and in other instances that of ornithine[58] has been ascertained. Long periods of hydrogenation may result in the reduction of tyrosine, phenylalanine, tryptophan and histidine as well.[4,6] In summary, the general consensus on the elimination of the nitro group by the classical Pd-catalysed reduction is that 80-90% aqueous acetic acid is recommended as "the lesser evil".[6] The nitro group can also be removed in the presence of palladium black using liquid ammonia as a solvent. However, this procedure requires a long time as well[59] and so far has not been examined in greater detail.

The unsatisfactory results of catalytic reduction of the nitro group induced searches for optimal conditions for removal of the nitro group by means of Zn and various acids. A medium of 2N aqueous trifluoroacetic acid turned out to be the best one. However, in this case, the crude product composition also depends on the model tested and the results were no

better than for classical hydrogenation on Pd in 85% acetic acid.[58] Application of the one-electron reducing agent, $TiCl_3$ is a more recent method. Yet, ornithine formation (1-90% yields) is the serious side reaction which can become the main reaction depending upon the conditions and model investigated.[60]

Because of the difficulties in the removal of the nitro group from larger peptides and a number of side-reactions accompanying this process, the nitro group sometimes serves as a temporary protection. That is, as soon as possible after incorporation of $Arg(NO_2)$ onto peptide chain, the nitro group is cleaved and a peptide segment with the protonated arginyl residue used in the later stages of the synthesis.[17,56,60,61]

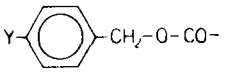
Recently, catalytic transfer hydrogenation with compounds being proton donors[62] has been recommended for reductive final deblocking.[63] It can also be applied for the removal of the $N^{\omega'}$-nitro protection from arginine peptides.[64,65] Regardless of certain details, e.g. palladium black as catalyst and formic acid as hydrogen donor[66] or Pd/C as catalyst and ammonium formate as hydrogen donor[64], the reactions proceed very rapidly unless sulfur-containing amino acids are present in the peptides.[34] If a common precursor is assumed for arginine and for the side-products in the reduction of nitroarginine (Scheme on the previous page), a more unequivocal removal of the nitro group than in classical hydrogenation might be possible and this assumption seems to be true in reality.[27]

The nitro group may also be cleaved by means of liquid hydrogen fluoride.[3,6,20,26,67] 4-Nitrophenylalanine[67] and in one case ornithine (as much as 20%) were observed as side-products, the latter within a peptide containing the arginine residue adjacent to histidine.[68] For elimination of the nitro protection, sulfonic acids are not recommended. Neither methanesulfonic acid nor trifluoromethanesulfonic acid removes this group

completely.[67,69] Fluorosulfonic acid is efficient for this purpose but under these conditions hydroxy amino acids are esterified.[67,69].

2. Urethane Groups

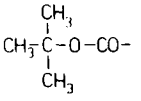
To this type belong:



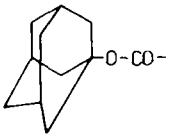
Y = H, benzyloxycarbonyl

Y = O_2N, p-nitrobenzyloxycarbonyl Z(NO_2)

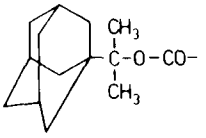
Y = MeO, p-methoxybenzyloxycarbonyl Z(OMe)



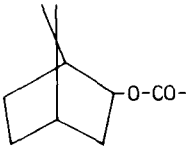
t-butoxycarbonyl (Boc)



adamantyloxycarbonyl (Adc)



1-(1-adamantyl)-1-methyl-ethoxycarbonyl (Adpoc)



isobornyloxycarbonyl (Ibc)

As arginine guanidino function is prone to oligoacylation, an excess acylating agent easily gives rise to bisurethanes or to a mixture of two

TABLE 2. *Known Mono and Bisurethane Protecting Groups for Arginine Side-chain*

Protection(s)	ω Position	Evidence of structure	δ,ω Positions	Evidence of structure	ω,ω' Positions	Evidence of structure
Z	+	-	+[4]	chemical[4]	+[4]	chemical[4]
Z(NO_2)	+[4]	-				
Z(OMe)			+[70]	-		
Boc	+[71]	a	+[71]	a	+[71]	a
Adc			+[72]	^1H NMR[73]		
Ibc			+[74]	-		
Adpoc			+[75]	-		

a. By ^1H and ^{13}C NMR[71]

isomeric ones (Table 2). These bisurethanes differ in their stability. Derivatives: $(Z)_2$,[4] $(Adc)_2$,[72] $(Idc)_2$[74] and most probably $[Z(OMe)]_2$[70] are more stable in the form of N^{δ},N^{ω}-isomers. An exception is the $(Boc)_2$ derivative which is more stable as the $N^{\omega},N^{\omega'}$-form.[71] On the other hand, the less stable $N^{\omega},N^{\omega'}$-$(Z)_2$[4] and N^{δ},N^{ω}-$(Boc)_2$[71] isomer undergo degradation in solutions as shown for Z-Arg$(Boc)_2$ in the following reaction and in Table 3.[71]

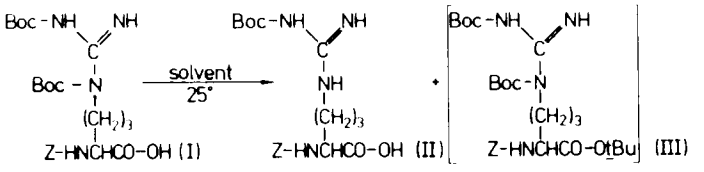
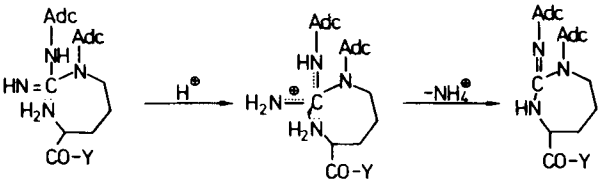


TABLE 3. *Stability of Compound I at 25°*

Days	Degradation product	CH_2Cl_2 %	CH_2Cl_2 1 eqv.Et_3N %	DMF %	DMF 1 eqv.Et_3N %	CH_3OH %	CH_3OH 1 eqv.Et_3N %
3	II	6	3	7	3	21	3
	III	1	0.3	0.4	0.3	0.7	0.3
12	II	11	3	7	3	63	11
	III	1.4	0.3	0.7	0.3	1.1	0.3

In some cases, stable δ,ω-bisurethane derivatives are labilized after deprotection of the α-amino group. For instance, Z(OMe)-[76] and Boc-$Arg^{\delta,\omega}(Z)_2$[77] are successfully employed in peptide synthesis with the $(Z)_2$ blocking group kept until the final deprotection. Although it is possible to crystallize $Arg^{\delta,\omega}(Z)_2$ from aqueous methanol[76], the same $Arg^{\delta,\omega}(Z)_2$ left in a methanol solution decomposes into several fragments whose structures have not yet been identified.[76] Therefore, $Arg^{\delta,\omega}(Z)_2$ is usually utilized as Z-$Arg^{\delta,\omega}(Z)_2$ either as the NH_2-terminal component in a peptide chain or to secure only temporary guanidino function protection

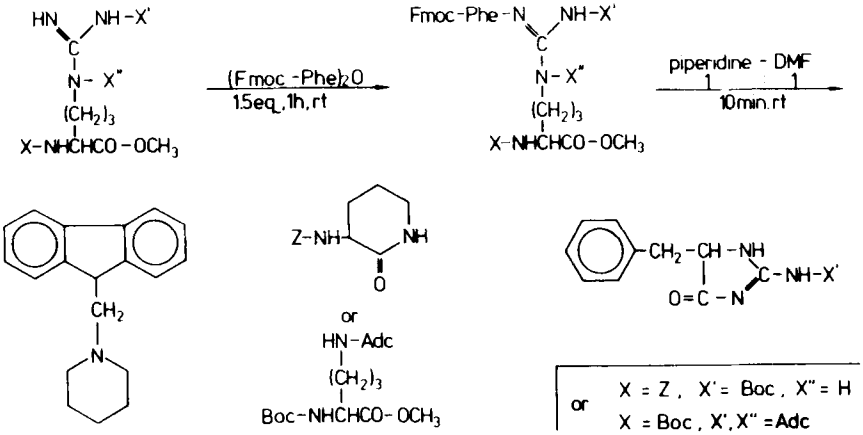
for including the arginine residue onto the peptide chain, followed by acidic removal of all the three benzyloxycarbonyl groups.[78,79] Another type of instability is characteristic of compounds with an NH_2-terminal



Y = OH; $N(CH_3)_2$; $NHCH_2CONH_2$

$Arg^{\delta,\omega}(Adc)_2$ residue. They undergo intramolecular condensation to 1,3-diazacycloheptane derivatives. The condensation in classical peptide synthesis in solution is a source of concern following N^{α}-deprotection, e.g. hydrogenolytic removal of N^{α}-Z group, or peptide bond formation.[72] For instance, this cyclization can be suppressed using formic acid and palladium black for the reduction.[80] While $(Adc)_2$ protection functions mainly in solid-phase peptide synthesis[81,82] as the Fmoc-$Arg^{\delta,\omega}(Adc)_2$ derivative,[80] the significance of 1,3-diazacycloheptane generation under solid-phase conditions is unclear.[26]

In 1984 in a set of model experiments, it was shown that both N^{δ},N^{ω}-bisadamantyloxycarbonyl- and N^{ω}-t-butoxycarbonyl-arginine undergo intermolecular acylation during peptide bond formation mediated by the sym-



metrical anhydride of N-protected amino acid resulting in the formation of ornithine residues. The latter residues were identified, by fast-atom-bombardment mass spectrometry within the pentadecapeptide C-terminus of human leucocyte interferon αF synthesized on a polymeric support by means of symmetrical anhydrides. Amino acid analysis of the pentadecapeptide

TABLE 4. *N^{ω},N^{δ} or $^{\omega'}$-Bisurethane Derivatives of Arginine (1973-1986)*

Compound	mp. (°C)	$[\alpha]_D^{20-25}$ (°)	lit.
Z-Arg(Z)$_2$-OLi	156-157	+ 10.6 (c 1.5, CH_3OH)	70
Z-Arg(Z)$_2$	138-139	+ 15.1 (c 1.5, CH_3OH)	70
Z(OMe)-Arg(Z)$_2$-OLi	209-210	+ 9.9 (c 1.5, CH_3OH)	70
Z(OMe)-Arg(Z)$_2$	139-141	+ 16.6 (c 1.5, $CHCl_3$)	70
Z(OMe)-Arg[Z(OMe)]$_2$-OLi	146-148	+ 17.5 (c 1, DMF)	70
Z(OMe)-Arg[Z(OMe)]$_2$	125-128	+ 1.9 (c 1, DMF)	70
Z-Arg(Ibc)$_2$	124[a]	+ 10.8 (c 1, $CHCl_3$)	74
Z-Arg$^{\delta,\omega}$(Boc)$_2$	-	-	71
Z-Arg$^{\omega,\omega'}$(Boc)$_2$	-	-	71
Z-Arg(Adpoc)$_2$	158-160	+ 15.2 (c 1.2, $CHCl_3$)	75
Z-Arg(Adc)$_2$	118-120	+ 17.7 (c 1, $CHCl_3$)	80
Arg(Adc)$_2$	174-177	- 30.3 (c 1, $CHCl_3$)	80
Fmoc-Arg(Adc)$_2$	154-156	+ 19.2 (c 1, $CHCl_3$)	80
Boc-Arg(Boc)$_2$	123-124	- 5.0 (c 1, DMF), + 8.0 (c 1, EtOH)	16
Boc-D-Arg(Boc)$_2$	118-121	+ 5.0 (c 1, DMF), - 7.5 (c 1, EtOH)	16
Boc-DL-Arg(Boc)$_2$	120-123		16
Boc-Arg(Boc)$_2$-OCh	53-55[a]	- 24.6 (c 1, EtOH)	16

a. Decomposition.

revealed a 50% content of ornithine residues in relation to the expected number of arginine. The data demonstrate clearly that Adc or Boc - and probably all urethane type protecting groups - do not effectively prevent acylation of guanidine under the conditions of peptide synthesis and the subsequent partial conversion of arginine to ornithine occurs when strongly basic conditions have to be used to remove the α-amino protecting groups.[73] On the other hand, however, satisfactory yield have been reported for solid-phase synthesis of fragment 166-174 of human chorionic somatomammotropin by the symmetrical anhydride procedure including $[Fmoc\text{-}Arg^{\delta,\omega}(Adc)_2]_2O$.[82]

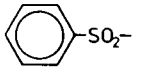
N^g-urethane blocking groups can be similarly removed to the corresponding N^α-urethane protections and in general no difficulties are encountered in these reactions.[3,4,6] However, the romoval of $N^g\text{-}(Boc)_2$ groups by hydrogen chloride in acetic acid is incomplete.[16]

Table 4 lists N^g-urethane derivatives of arginine, both new compound and those previously known, that were synthesized during 1973-1986.

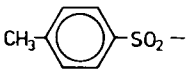
3. Arylsulfonyl Groups

So far, ten types of arylsulfonyl protection of the guanidino function of arginine have been investigated. They are arbitrarily assumed to be on the ω-nitrogen of arginine.[83]

Although the initially proposed benzenesulfonyl group has not yet been used in peptide chemistry,[4,6] the tosyl is widely utilized as the



benzenesulfonyl



tosyl (Tos)

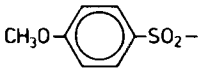
most important N^g-blocking residue of arginine in solid-phase synthesis.[84-97] Side-products, $N^\omega,N^{\omega'}$-ditosyl compound[7] and the above-mentioned δ-lactam,[3,4,7] accompany tosylation of N^α-protected arginine. Therefore, the yield of Boc-Arg(Tos), the arginine derivative employed in solid-phase

synthesis, amounts to only 39%.[68] The N^{ω}-tosyl group is very stable under conditions of peptide chain formation.[4] This advantage becomes a detriment since very drastic methods are necessary for final deprotection.[4,98] One procedure, is the action of sodium in liquid ammonia, which converts a part of arginine residues into ornithine.[3,4,6] Therefore, it is presently applied only rarely.[3,4,99,100] The tosyl moiety is also cleaved by liquid hydrogen fluoride at 0° for 1-2 hrs.[3,68,85,87,89-92] This is a troublesome operation[67] and side-reactions with some amino acid residues are unavoidable.[67,101] Attempts were made to remove the N^{ω}-tosyl group using organic sulfonic acids like methanesulfonic acid and trifluoromethanesulfonic acid.[67] A large excess of trifluoromethanesulfonic acid in the presence of anisole at 40° often for >1.5 hr is required.[67,102,103] A solution of the trifluoromethanesulfonic acid in trifluoroacetic acid in the presence of anisole[104] or m-cresol[105] cleaves the tosyl group at 0° in 30 minutes[104] or at room temperature within one hour.[105] So far however, the solution has been applied only for deprotection of Arg(Tos)[105] or oligopeptides[104] but not for deprotection of polypeptides. The N^{ω}-tosyl group can also be removed using pyridinium polyhydrogen fluoride (70% HF-pyridine)[106], fluorosulfonic acid[69] or electrolytically.[107,108] Yet, these methods have been employed only sporadically for the deprotection of larger peptides.[108] Side-reactions have been noted with fluorosulfonic acid[69] or during electrolytic reduction.[107] Recently, photoinduced donor-acceptor ion pairs with electron-donating aromatics have been proposed for the selective removal of electron-accepting tosyl protecting group for amino function.[109] A similar procedure may be possible to effect cleavage of the tosyl group from the guanidino function.

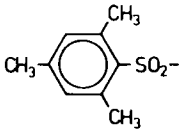
Due to the shortcomings of final deprotecting procedures required for removal of the N^{ω}-tosyl group, investigations started in two directions. One of them covers modifications of blocking groups which would be

more readily removable under acidic conditions. The second involves modifications of acidic agents with the goal of changing the mechanism of deprotecting reactions. In 1981, these two trends became convergent.[1,110]

All modifications of the tosyl group consist of the introduction of electron-donating substituent(s) into the phenyl ring. The first two protecting groups in this series are the Mbs and the Mts groups which are both removable with methanesulfonic acid or with a mixture of trifluoromethanesulfonic acid and trifluoroacetic acid (1:1) at 25°; the second



4-methoxybenzene-sulfonyl (Mbs)



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

group is the more labile one.[111-113] During the deprotection by means of methanesulfonic acid in the presence of anisole, significant esterification of the tyrosine residue[67,112] and, to a lesser extent, of the serine and threonine moieties[67] occurs as a result of acylation with p-methoxybenzenesulfonylium or the mesitylenesulfonylium cation. The same is true with other arylsulfonylium cations and different acidic agents.[67] m-Cresol has been reported to be a rather efficient scavenger of the cations in question.[1,67,110,114]

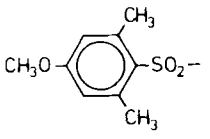
The search for other final deprotecting procedures of general usefulness led to the finding that the presence of sulfur nucleophiles accelerates acidolysis of the blocked amino acid derivatives and peptides,[115] including the N^{ω}-arylsulfonylarginine residues. The tosyl group can be removed with trifluoromethanesulfonic acid in trifluoroacetic acid in the presence of thioanisole at 25°.[116] For comparison, trifluoromethanesulfonic acid alone in trifluoroacetic acid solution requires a temperature of 40° for effective action.[67] Although the Mbs protecting group requires the action of 1 M trifluoromethanesulfonic acid-thio-

anisole in trifluoroacetic acid at 0° for 1 h to achieve cleavage,[1,67] Mts is removed by a solution of thioanisole (2.5 mmol) in trifluoroacetic acid (1 ml) at 25° (within 72 hours).[116]

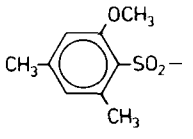
The use of the N^{ω}-Mbs blocking group rendered the synthesis of crystalline bovine ribonuclease A possible.[1,110] Deprotection of the blocked enzyme was effected, for comparison, by the three reagents: a) methanesulfonic acid, b) liquid hydrogen fluoride and c) 1M trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid, in each case in the presence of m-cresol. Procedure (c) appears to be prefered.[67] N^{ω}-Mbs protection has been used in liquid-phase synthesis of the C-terminal nonapeptide of bovine insulin B-chain[117] and in solid-phase synthesis of neurotensin and related peptides.[118] However, at present, N^{ω}-Mts blocking group serves commonly in classical synthesis in solution of both small and large biologically active peptides as well as their analogues.[119-121]

All these successes encouraged the search for similarly modified still more labile N^{ω}-protecting groups which could be cleaved in a shorter period of time with sulfur nucleophiles in trifluoroacetic acid. In comparison to other acidic deprotecting reagents, trifluoroacetic acid minimizes the serious side-reaction of aspartimide formation.[122]

It was suggested that the N^{ω}-Mds group could be removed by a mixture of trifluoroacetic acid and thioanisole (9.5:0.5) at 50° within 1-2 hrs.[123,124] Yet, the preparation of pure of N^{ω}-Mds arginine derivatives presents difficulties. Sulfonation of Z-Arg by means of MdsCl yielded four



4-methoxy-2,6-dimethyl-benzenesulfonyl (Mds)

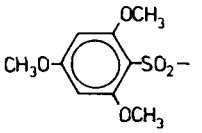


2-methoxy-4,6-dimethyl-benzenesulfonyl (iMds)

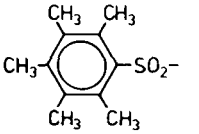
products: Z-Arg(Mds), Z-Arg(iMds), Z-Arg(5-chloro-2-methoxy-4,6-dimethyl-

benzenesulfonyl) and, presumably, Z-Arg(Mds)$_2$. Generation of the second and third products is a source of concern connected with inseparable contaminations of MdsCl. The authors were able to obtain Z-Arg(Mds) in 95% purity by high performance reversed-phase chromatography.[125] According to Fujino et al.,[122] N^{ω}-iMds is removed much more slowly with a mixture of trifluoroacetic acid and anisole (9:1) than N^{ω}-Mds. On the other hand, according to Smyth et al.,[126] since all the protecting groups introduced into the guanidino function of arginine in both the main reaction and the side-reaction are of equal lability, the purity of final products is unaffected. Yet, characterization of intermediate protected peptides is extremely difficult.

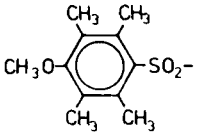
Because of these difficulties with the Mds blocking group, the action of a mixture of trifluoroacetic acid and thioanisole (9:1) at 50° on



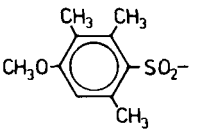
2,4,6 - trimethoxy - benzenesulfonyl (Mtb)



pentamethylbenzene - sulfonyl (Pme)



4 - methoxy - 2,3,5,6 - tetra - methylbenzenesulfonyl (Mte)



4 - methoxy - 2,3,6 - trimethyl - benzenesulfonyl (Mtr)

simple models of Z-Arg(X) with the arylsulfonyl residues X given in the above Figure was investigated and compared with that on Z-Arg(Mds). The following order of lability was determined:

Mtr > Mds > Mtb > Pme > Mte

The most labile Mtr group is removed during 30 minutes (or 2-2.5 hrs at 25°). Mds and Mtb are cleaved within 2 hrs while Pme requires 4 hrs and

Mte is removed in this time only to the extent of 81%. All the arylsulfonyl groups discussed resist catalytic reduction. Consequently, they can be used along with N^{α}-benzyloxycarbonyl protection. For practical peptide synthesis, masking groups removable by means of trifluoroacetic acid and thioanisole should resist the action of trifluoroacetic acid at room temperature. The N^{ω}-Mtr protecting group, after a one hour treatment with this reagent, is cleaved in about 50% yield; N^{ω}-Mds in about 20%; N^{ω}-Pme and N^{ω}-Mte in about 2%. As seen, the Mtr blocking group cannot be employed in syntheses in which trifluoroacetic acid is applied for permanent deprotection.[122] On the other hand, this group was found to be useful in solid-phase peptide synthesis together with α-Fmoc-ω-tBu protecting system due to complete stability under conditions of repeated deprotection of α-amino group and prevention of N^{g}-acylation of arginine, i.e., of its transformation into ornithine. However, conditions for removal of the Mtr moiety cause the destruction of extremely sensitive peptides. Therefore, for splitting the peptide from polymeric support and removal of the Mtr group, the acidic reagent was modified, *viz.*, a mixture of trifluoroacetic acid, phenol and ethylmethylsulfide was used. Although the complete removal of the Mtr protection required 6 hrs at room temperature under these conditions, it led to a much purer product than the treatment with the mixture of trifluoroacetic acid and thioanisole (9:1) for 2-2.5 hrs at the same temperature.[83]

As a result of the investigations of modifications of arginine N^{ω}-arylsulfonyl protecting groups, peptide chemistry was enriched with new derivatives listed in Table 5.

4. Proton

The strongly basic guanidino function of arginine (pK = 12.5) can be selectively protected, in relation to the α-amino group, by protonation.[4,6] Most often hydrogen chloride and hydrogen bromide[4] are used,

TABLE 5. *N^{ω}-Arylsulfonyl Derivatives of Arginine (1976-1983)*

Compound	mp. (°C)	$[\alpha]_D^{21-26}$ (in CH_3OH) (°)	lit.
Z-Arg(Mbs).DCHA.CH_3CN	110-112	+ 5.1 (c 1.35)	111
Arg(Mbs).0.5H_2O	144-146	- 6.1 (c 0.71)	111
Z-Arg(Mbs)-ONp	90-110[a]	- 3.4 (c 0.59, DMF)	111
Z(OMe)-Arg(Mbs).CHA	110-114	+ 25.1 (c 0.6)	126
Z(OMe)-Arg(Mbs)-OCH_3	-	-	126
Z-Arg(Mds).CHA	140-141	+ 5.7 (c 0.5)	123
Arg(Mds)	120-122[a]	- 7.8 (c 0.7)	123
Boc-Arg(Mds)	175-176[a]	+ 3.5 (c 0.5)	123
Fmoc-Arg(Mds)	127-129	-	83
Z(OMe)-Arg(Mts).CHA	125-128	+ 5.5 (c 0.7)	112
Boc-Arg(Mts).CHA	122-125	+ 15.1 (c 0.5)	112
Z-Arg(Mts).CHA	113-114	+ 5.5 (c 1.1)	112
Arg(Mts).H_2O	158-161	- 5.2 (c 0.4)	112
Z(OMe)-Arg(Mts)-NHNHTcc.0.5H_2O	99-101	+ 7.1 (c 1.0, DMF)	127
Z-Arg(Mtb).H_2O	89-93	+ 0.8 (c 0.5)	122
Arg(Mtb).CH_3OH	115-120	- 8.9 (c 0.6)	122
Z-Arg(Pme).CHA	173-175	+ 5.8 (c 1.3)	122
Arg(Pme).0.5H_2O	153-156	- 5.5 (c 0.9)	122
Fmoc-Arg(Pme)	123-125	-	83
Z-Arg(Mte).CHA	127-129	+ 5.9 (c 0.8)	122
Arg(Mte).0.5H_2O	150-152	- 4.5 (c 0.9)	122
Z-Arg(Mtr).CHA	195-197	+ 6.5 (c 1.2)	122
Arg(Mtr).0.5H_2O	100-103	- 4.8 (c 1.3)	122
Fmoc-Arg(Mtr)	68-72	-	83

a. Decomposition

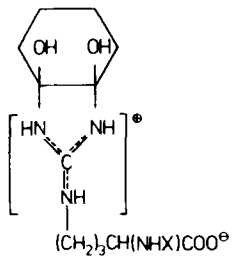
sometimes acetic acid[3] and in scattered instances arginine's own carboxyl group,[128] nitric acid,[11] picric acid[11] or tetraphenylborate.[22] The advantages of these derivatives lie in their ease of preparation and the possibility to synthesize the corresponding peptides with minimum side-function protection, a fact that is particularly attractive in the final deprotection step. On the other hand, the poor solubility of peptides with arginine as a guanidinium salt in organic solvents causes difficulties. The above-mentioned proposition of tetraphenylborate derivatives soluble in dimethylformamide or tetrahydrofuran is an attempt to overcome this problem.[22]

A more serious defect of N^g-salts of arginine is their substantial deprotection at pH>9. Then, the deprotected guanidino function undergoes acylation whereupon the N^g-(N^α-deblocked aminoacyl)arginine residue is converted into ornithine. This phenomenon has been noted in solid-phase peptide synthesis with both the N^α-Boc protection (the cause was the action of triethylamine)[129] and N^α-Fmoc (the cause was the action of piperidine).[130] In the first case, the side-reaction was overcome using a calculated amount of N-methylmorpholine in place of triethylamine. In the second instance, it was suppressed to a great extent by the addition of 1-hydroxybenzotriazole. After several cycles of cleaving α-Fmoc protecting groups as much as 22% of ornithine was observed in the peptide. The addition of 1-hydroxybenzotriazole during the activation step minimized the acylation times and afer 11 cycles of synthesis, the ornithine content of the crude product was only 9%, i.e., at a level of about 1% per cycle.[130] It was remarkable that the ornithine-containing impurities were largely separated from the desired peptide by chromatographic purifications. Therefore in the opinion of the authors of the last cited paper, the masking of arginine by protonation deserves well of recommendation from the practical point of view in peptide synthesis.[130] Accordingly, many exam-

ples of successful applications of protonated arginine derivatives for the syntheses of oligo- and polypeptides in solution have been reported.[78,79,131-139] There are also examples of their use in solid-phase synthesis.[140,141] This type of arginine protection seems to have aquired increased acceptance in the last few years.

5. 1,2-Dihydroxycyclohex-1,2-ylene

For over 20 years, bifunctional aldehydes and ketones have been used for modification of arginine residues within proteins. Among them 1,2-cyclohexanedione occupies a special position. In neutral or slightly alkaline borate buffer, this compound reacts with arginine to form a single amino acid derivative, $N^{\omega},N^{\omega'}$-(1,2-dihydroxycyclohex-1,2-ylene)-arginine, which is stable in acetic acid solutions and in borate buffers at pH 8-9. This masking group can be removed with hydroxylamine at pH 7 with quantitative recovery of arginine. Because of these properties, 1,2-cyclohexanedione was successfully applied for reversible protection of arginine in a semi-synthesis of human insulin B-chain.[6]



X-Arg$(H^+)^{\omega,\omega'}$(DHCH)-$O^{\ominus}$

The potential of 1,2-cyclohexanedione for the formation of arginine derivatives of use in peptide synthesis was investigated in 1982. Among others, Z-Arg(DHCH,H^+)O^- and Boc-Arg(DHCH,H^+)O^-, two stable compounds, as well as Z-Arg(DHCH,HCl)-OSu were readily obtained. They give blocked di- and tripeptides in good yields and during the reaction neither δ-lactam formation nor acylation of vicinal hydroxyls were observed. Nevertheless, removal of N^{α}-protection is feasible, without side-reactions, by means of hydrogenation exclusively. Unfortunately, acydolysis of N^{α}-masked $N^{\omega,\omega'}$-(DHCH) peptides or cleavage of the DHCH blocking group with nucleophiles lead to a lot of side-products.[14]

III. CONCLUSION

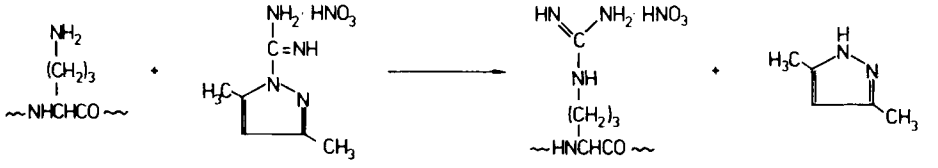
In peptide synthesis, arginine cannot be employed without protection of its guanidino function. The eight types of groups masking this function are known but some of them never left the stage of model investigations. The same is also true for some individual proposals within applied types of protection. Yet, even with arginine having the blocked guanidino function, the several serious side-reactions observed result for two reasons. Firstly, guanidine is trifunctional and the introduction, as used in practice, of one masking group or at most two, does not constitute a sufficient protection of its reactivity. Secondly, guanidine is ambivalent, i.e. it can operate as a nucleophile (its three nitrogen atoms) and as an electrophile (its C = NH group). The protection of guanidine suppressing the reactivity of one type, by necessity, increases in some measure the reactivity of the second type. In connection with this reasoning, it should be borne in mind that protonation of the guanidino group of arginine gives a fairly symmetric resonance-stabilized system which can withstand a brief treatment of supernucleophilic hydrazine.[20] This fact seems to provide a rational basis for the revival of interest in the use of protonation as a means of arginine N^g-protection in recent years.[23,78,79,131-141]

The past decade brought about an explosion of new N^{ω}-protecting groups of oligosubstituted benzenesulfonyl derivatives. Three of them deserve particular attention. The N^{ω}-p-methoxybenzenesulfonyl masking group (Mbs) enabled the synthesis of crystalline bovine ribonuclease A.[1,110] Nowadays, the N^{ω}-mesitylenesulfonyl group is commonly applied by Yajima for peptide synthesis in solution.[119-121] For its removal, the standard reagent for final deprotection, 1 M tifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid in the presence of m-cresol, is used.[1,67,110] The N^{ω}-4-methoxy-2,3,6-trimethylbenzenesulfonyl group has

been tested for possible application in solid phase synthesis along with α-Fmoc-ω-tBu protection system.[83] N^{ω}-arylsulfonyl protecting groups of arginine were investigated with particular emphasis on the ease of their removal in final deprotecting procedures. However, no special attention has been given to intra- and intermolecular acylation of N^{ω}-arylsulfonyl-arginine residues in peptide bond forming steps. There is only one mention of δ-lactam generation from Z-Arg(Mds)[125] and some information regarding the fact that the Arg(Mtr) residue does not undergo intramolecular acylation and does not form the ornithine residue.[83,130] Whether the recently developed oligosubstituted benzenesulfonyl groups will provide an adequate protection of the guanidino function remains to be shown.

The oldest covalent N^{g}-protection, the $N^{\omega'}$-nitro group has had an amazingly long life in peptide synthesis in spite of a large number of side-reactions and difficulties in its removal. A special example is its use for arginine side-chain masking in an orthogonal-scheme synthesis of bradykinin along with the N^{α}-dithiasuccinoyl amino blocking group. The nitro residue was removed by hydrogenation over $Pd/BaSO_4$.[32]

A particular problem is synthesis of protamines containing from 25 to 70 amino acid residues. They have repeated homo-oligoarginyl sequences which include as many as 2-7 residues. In this case, ornithyl precursors are synthesized and subsequently converted into arginine peptides by amidination. 1-Amidine-3,5-dimethylpyrazole nitrate has been reported to be a good amidination agent.[142]



In this series of reviews, comparison of the problems of the side-chain function of arginine, histidine and tryptophan suggests that those

of arginine seem to be the most prevalent. One can venture to say that, at present, the difficulties in blocking the reactivity of the side-chain of the two remaining amino acids are of a more technical nature. On the other hand, in the case of arginine, one still has to take into account the ambivalency of its guanidino function.

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ABBREVIATIONS

Ac	= acetyl	Mtr	= 4-methoxy-2,3,6-trimethyl-benzenesulfonyl
Adc	= adamantyloxycabonyl	Mts	= 2,4,6-trimethylbenzenesul-fonyl = mesitylenesulfonyl
Adpoc	= 1-(1-adamantyl)-1-methylethoxycarbonyl	OCh	= cholesteroxyl
An	= anion	ONd	= 5-borneno-2,3-dicarboxy-imido-oxyl
Boc	= t-butoxycarbonyl	ONp	= 4-nitrophenoxyl
CHA	= cyclohexylamine	OPcp	= pentachlorophenoxyl
DCC	= dicyclohexylcarbodiimide	OPfp	= pentafluorophenoxyl
DCHA	= dicyclohexylamine	OSu	= succinimido-oxyl
DHCH	= 1,2-dihydroxycyclohex-1,2-ylene	OTcp	= 2,4,5-trichlorophenoxyl
DMF	= dimethylformamide	Pme	= pentamethylbenzene-sulfonyl
Et	= ethyl	R	= amino acid side-chain
Fmoc	= fluoren-9-ylmethyloxy-carbonyl	tBu	= t-butyl
Ibc	= isobornyloxycarbonyl	THF	= tetrahydrofuran
iMds	= 2-methoxy-4,6-dimethyl-benzenesulfonyl	Tos	= tosyl
Itbz	= 2-isopropoxycarbonyl-3,4,5,6-tetrachloro-benzoyl	Tcc	= 2,2,2-trichloroethoxy-carbonyl
Mbs	= 4-methoxybenzenesulfonyl	Tri	= trityl
Mds	= 4-methoxy-2,6-dimethyl-benzenesulfonyl	X,X',X''	= protections of functional groups
Mtb	= 2,4,6-trimethoxybenzene-sulfonyl	Z	= benzyloxycarbonyl
		Z(OMe)	= p-methoxybenzyloxycarbonyl
		$Z(NO_2)$	= p-nitrobenzyloxycarbonyl

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