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# Methods of Synthesis and Properties of β-Dimethylaminoethyl and Choline Esters of Aminoacids and Peptides

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The literature data on the synthesis of β-dimethylaminoethyl and choline esters of aminoacids and peptides are surveyed. The N-protecting groups used and methods for their removal as well as the physicochemical and biological properties of the above compounds are considered.
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## I. INTRODUCTION

The choline esters of aminoacids and peptides have a comparatively short history. Their structure resembles that of acetylcholine (AC), which has aroused much interest in recent years in their synthesis and biological activity. After a comparatively short time, research into both active cholinomimetics, isolated from plant and animal raw materials[1-4], and synthetic choline esters of aminoacids and peptides developed on a fairly wide scale. Studies have also been made[5-7] in which dialkylaminoalkyl esters were used as C-protecting groups for peptide synthesis.

This review deals with methods of synthesis of β-dimethylaminoethyl and choline esters of aminoacids and peptides and also with their physicochemical and biological properties. In the development of methods of synthesis of dialkylaminoalkyl aminoacid and peptide esters use is made of the fundamental postulates of peptide chemistry according to which the appropriate protecting groups, methods of synthesis of the ester, and methods for the removal of the protecting group and the isolation of the final product are selected.

## II. PROTECTING GROUPS

In peptide synthesis one of the main tasks is the protection of the amino-group of the aminoacid or peptide. This problem remains just as acute in the synthesis of choline and β-dialkylaminoalkyl esters of aminoacids and peptides.

The majority of investigators[5,8-14] used the benzyloxycarbonyl (Z) group† as the *N*-protecting group in the synthesis of the esters, its removal after the synthesis of the aminoesters being achieved by treatment with hydrogen bromide in glacial acetic acid[5,8-13] and by catalytic hydrogenolysis over palladium[5]; these groups have been removed from the choline esters of certain Z-aminoacids with phosphonium tri-iodide[15] or by hydrogenolysis over $PtO_2$.[16-19] It has been noted[16] that this involves partial cleavage of the ester linkage.

†Apart from the standard abbreviations recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, the following abbreviations have been adopted here: Aba = aminobenzoic acid; ε-Aca = ε-aminocaproic acid; γ-A-β-Hbu = γ-amino-β-hydroxybutyric acid; ω-Ava = ω-aminovaleric acid.

The t-butoxycarbonyl (Boc) group[6,9,10,12,20] has been removed from β-dimethylaminoethyl esters by passing dry gaseous hydrogen chloride through an ethereal solution of the β-dimethylaminoethyl ester of the Boc-aminoacid[6] and also by treatment with trifluoracetic acid[9,12]. The protecting group has been removed from the choline ester of Boc-ε-aminocaproic acid[20] with a dilute solution of hydrogen bromide in acetic acid (10 min, 20 °C) or with a 48% solution of hydrogen bromide (2 min, 0 °C) and a 0.1 *N* solution of hydrogen chloride in formic acid (10 min, 20 °C).

The triphenylmethyl [trityl (Trit)] group has also been used successfully in the synthesis of the choline ester of glutamic acid; it was removed by treatment with hydrogen chloride[21] or acetic acid[22,23].

The possibility of using toluene-*p*-sulphonyl [tosyl (Tos)] and phthalyl (Pht) protecting groups has also been investigated[11]. It has been established that hydrazinolysis or acid hydrolysis under various conditions for the Pht group and reduction with sodium in liquid ammonia for the Tos group lead to partial cleavage of the ester linkage. According to the data of Mndzhoyan and Agadzhanyan[11], these protecting groups are less favourable for the synthesis of dialkylaminoalkyl esters of aminoacids with a free amino-group, in contrast to the Z and Boc groups. In the synthesis of the hydrochlorides of the β-dimethylaminoethyl and choline esters of glycine, the *o*-nitrobenzenesulphenyl (Nbs) group was used as the *N*-protecting group[24] and was removed by treatment with hydrogen chloride.

The majority of investigators using the acid chloride method for the synthesis of β-dimethylaminoethyl or choline esters of aminoacids protected the amino-group by protonation[15,25,26]. The study of Freundenberg and Keller[27], who used the acid chlorides of α-azidoacids, is unique in this respect (see Section III, 1 below). After the synthesis of the choline esters, the azido-group was converted into an amino-group by reduction over Pd/C in the presence of hydrogen chloride.

The dansyl (Dns) group has been used[28] in the synthesis of choline esters of certain aminoacids. In the synthesis of choline esters of aminoacids and peptides Kanaoka and coworkers[8,9] protected other functional groups as well as the amino-group, namely the hydroxy-group of tyrosine was protected by benzylation, the guanidino-group of arginine was protected by nitration, and the β- and γ-carboxy-groups of aspartic and glutamic acid were protected by benzylation. The β-carboxy-group of aspartic acid was protected[9] by t-butylation. The benzyl group has been used[6] to protect

the imidazole ring of histidine and the hydroxy-group of serine.

Table 1 presents data, in relation to glycine, concerning the use and conditions for the removal of various *N*-protecting groups in the synthesis of β-dimethylaminoethyl and choline esters by various methods. Evidently the best *N*-protecting groups which can be used in the synthesis of aminoesters of aminoacids and peptides are the Z, Boc, and Nbs groups: when they are removed, the final product is obtained in high yields.

**Table 1.** Conditions for the elimination of *N*-protecting groups from the β-dimethylaminoethyl and choline esters of *N*-protected glycines R-Gly-$OCH_2CH_2X$.

| Conditions for elimination of *N*-protecting group | | | References |
|---|---|---|---|
| X=$NMe_2$ | X=$NMe_3$·Hal | yield, % | |
| **Boc-group** | | | |
| $F_3C$—COOH; $t_{room}$, 1 h | — | 94.4 | [12] |
| **Z-group** | | | |
| HBr/AcOH | — | 100 | [5] |
| $H_2$/Pd | — | — | [5] |
| 34% HBr/AcOH soln; $t_{room}$, 2 h | — | 64.9 | [12] |
| sat. HBr/AcOH soln | — | 90 | [10] |
| 28% HBr/AcOH soln; $t_{room}$, 40 min | — | 65 | [13] |
| | 25% HBr/AcOH soln; $t_{room}$, 30-60 min | — | [8] |
| — | 28% HBr/AcOH soln; $t_{room}$ | 73 | [13] |
| **Nbs-group** | | | |
| Methanol solution of HCl | — | 89.5 | [24] |
| — | methanol solution of HCl | 81.5 | [24] |
| **Pht group** | | | |
| 1 M $N_2H_4$ soln in methanol; refluxing for 1 h | — | * | [11] |
| $N_2H_4$ (1 : 1); 50°C, 1 h | — | * | [11] |
| $N_2H_4$ (1 : 2.2); refluxing for 1 h | — | 0 | [11] |
| 5 *N* HCl soln; water bath, 4 h | — | * | [11] |
| 5 *N* HCl soln; refluxing for 2 h | | * | [11] |
| **Tos group** | | | |
| $Na/NH_3$ | | | [11] |

*Mixture of products of the cleavage of the ester linkage and of the *N*-protecting group in the β-dimethylaminoethyl ester of *N*-protected glycine.

## III. METHODS OF SYNTHESIS

### 1. The Acid Chloride Method

The acid chloride method was used mainly in early studies on the synthesis of choline esters of aminoacids and peptides. The hydrochlorides of the choline esters of glycine[15,25], glycylglycine[15], and methionine[26] were obtained by the reaction of the hydrochloride of the aminoacid chloride or peptide with choline on heating. The acid chlorides of *N*-protected aminoacids were used in the synthesis of both β-dimethylaminoethyl esters[11,15,29] and β-halogenoethyl esters[15], which are the starting materials in the synthesis of choline esters of aminoacids. It has been shown that the acid chloride method and the mixed anhydride method using the methyl ester of chlorocarbonic acid are approximately equally effective for the synthesis of the dialkylaminoalkyl esters of Tos- and Pht-glycine[11].

α-Azidoacyl chlorides[27] have also been used as the starting compounds for the synthesis of the choline esters of four aminoacids (glycine, DL-alanine, DL-leucine, and DL-valine):

Scheme 1

$$N_3\text{—}\underset{\displaystyle R}{\underset{|}{CH}}\text{—}CO\text{—}Cl + HOCH_2CH_2NMe_2 \rightarrow N_3\text{—}\underset{\displaystyle R}{\underset{|}{CH}}\text{—}COOCH_2CH_2NMe_2 \xrightarrow{MeI}$$

$$\rightarrow N_3\text{—}\underset{\displaystyle R}{\underset{|}{CH}}\text{—}COOCH_2CH_2\overset{+}{N}Me_3\bar{I} \xrightarrow{AgCl} N_3\text{—}\underset{\displaystyle R}{\underset{|}{CH}}\text{—}COOCH_2CH_2\overset{+}{N}Me_3\bar{Cl} \xrightarrow{Pd/H_2}$$

$$\rightarrow HCl\cdot H_2N\text{—}\underset{\displaystyle R}{\underset{|}{CH}}\text{—}COOCH_2CH_2\overset{+}{N}Me_3\bar{Cl}\ .$$

### 2. The Mixed Anhydride Method

This method has been used by many investigators in the synthesis of β-dimethylaminoethyl esters of *N*-protected aminoacids and peptides[5,10-13,30]. Mixed anhydrides obtained from *N*-acylaminoacids and benzenesulphonyl chloride, tosyl chloride, or methyl chlorocarbonate have been employed for the synthesis. However, in the case of the reaction of Pht-glycine with methyl chlorocarbonate the yield of the β-dimethylaminoethyl ester was insignificant[11]. Best results were obtained using isobutyl chlorocarbonate[12], but one should note that in this case the method involving the azeotropic distillation of water from the mixture of β-dimethylaminoethanol, *N*-acylaminoacid, and xylene proved to be more effective[13] than the mixed anhydride method.

It follows from a comparison of data published in different communications concerning the application of condensing agents in the synthesis of the β-dimethylaminoethyl ester of *N*-benzyloxycarbonylglycine by the mixed anhydride method (Table 2) that the best results are obtained when ethyl and isobutyl chlorocarbonates are used.

**Table 2.** The influence of substituents on the reactivity of mixed anhydrides in the synthesis of the β-dimethylaminoethyl ester of *N*-benzyloxycarbonylglycine via the mechanism Z-Gly-O—R + $HOCH_2CH_2NMe_2$ → Z-Gly-$OCH_2CH_2NMe_2$.

| R | Yield, % | Refs. |
|---|---|---|
| $C_6H_5SO_2$ | 13 | [10] |
| *n*-$CH_3C_6H_4SO_2$ | 26 | [10] |
| MeOOC | 40 | [10] |
| EtOOC | 58 | [5] |
| iso-BuOOC | 61* | [12] |

*According to Shamshin et al.[13], the yield is 23.8%.

It is noteworthy that the yield of the β-dimethylaminoethyl ester of Z-glycine, obtained using isobutyl chlorocarbonate, was only 23.8%.[13] However, in other studies[12,30], the β-dimethylaminoethyl esters of *N*-protected aminoacids and peptides were synthesised using this reagent in high yields, which also confirms the effectiveness of isobutyl chlorocarbonate in the synthesis of the above compounds.

Two versions of the synthesis of the β-dimethylaminoethyl ester of *N*-benzyloxycarbonylglycylglycine (I) by the mixed anhydride method were investigated[30]:

$$\left.\begin{array}{l} Z\text{—}Gly\text{—}OH + H\text{—}Gly\text{—}OCH_2CH_2NMe_2 \xrightarrow{a} \\ Z\text{—}Gly\text{—}Gly\text{—}OH + HOCH_2CH_2NMe_2 \xrightarrow{b} \end{array}\right\} \rightarrow Z\text{—}Gly\text{—}Gly\text{—}OCH_2CH_2NMe_2\ . \quad (I)$$

The results obtained[30] show that pathway *a* gives higher yields of compound (I).

### 3. The Carbodiimide Method

The carbodiimide method has been used in the synthesis of choline[8], β-halogenoethyl[20], and β-dimethylaminoethyl[10,12,24] esters of *N*-protected aminoacids and peptides[12,30]. *N,N'*-Dicyclohexylcarbodiimide (Dcc) has been largely used as the condensing agent.

Studies[10,12] in which the β-dimethylaminoethyl esters of Z-glycine and L-valylglycine as well as Boc-glycine were synthesised by both the mixed anhydride and carbodiimide methods have shown that the latter method is less effective.

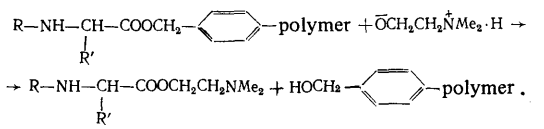
It has been reported[10] that the yield of the β-dimethylaminoethyl ester of Z-glycine obtained by the condensation of Z-glycine and β-dimethylaminoethanol with the aid of Dcc varies with the reactant ratio and reaction time. The authors[10] noted the formation of an appreciable amount (up to 50%) of *N*-benzyloxycarbonylglycyldicyclohexylurea as a side product. They also noted that at a reduced temperature the yield of the β-dimethylaminoethyl ester increases.

Subsequently the carbodiimide method was used successfully[8] in the condensation of certain *N*-acylaminoacids with choline. The carbodiimide method has been employed for the cross-linking of the methobromide of the β-dimethylaminoethyl ether of ε-aminocaproic acid with Sepharose 2B.[20]

### 4. The Activated Ester or Transesterification Method

*p*-Nitrophenyl esters of *N*-protected aminoacids have been largely used in the synthesis of β-dimethylaminoethyl choline esters by the activated ester method. The method has been applied in the synthesis of the choline ester of Z-glycine[24]. The β-dimethylaminoethyl esters of Z-L-leucylglycine and of the γ-methyl ester of L-glutamylglycine have been obtained by the reaction of the β-dimethylaminoethyl ester of glycine with the *p*-nitrophenyl esters of Z-L-leucine and of the γ-methyl ester of L-glutamic acid[5]. The cyanomethyl ester has been employed in the synthesis of the β-dimethylaminoethyl ester of *N*-benzoylsarcosine[31].

After synthesis by the solid phase method, the peptide linked to the polymer in the form of the benzyl ester has been subjected[6] to transesterification by treatment with β-dimethylaminoethanol at room temperature in accordance with the following scheme:



The influence of the solvent on the transesterification process has been tested in relation to Boc-glycine[6]. It was established that treatment with a 1:1 (by volume) mixture of β-dimethylaminoethanol and dimethylformamide (DMF) in the course of 70 h ensures virtually quantitative transesterification. When dioxan or methylene chloride was used, the degree of transesterification of the Boc-Gly-O-polymer with β-dimethylaminoethanol was much lower. The rate of transesterification in DMF has been studied in greater detail[6] in relation to the polymer-bound pentapeptide Boc-O-Bzl-L-Ser-L-Gln-$NO_2$-L-Arg-$N^{im}$-Bzl-L-His-Gly-O-polymer. It was established that the length of the peptide chain does not affect the rate of transesterification. The authors[6] also point out that the reaction is hindered in the case of valine and proline. It has also been found in relation to the transesterification of the compound Boc-L-Leu-L-Ala-O-polymer with β-dimethylaminoethanol that the degree of racemisation is insignificant (approximately 0.3 ± 0.1%).

The β-dimethylaminoethyl esters of Tos-glycine[31], hippuric acid, and hippurylglycine[32] have been obtained by the transesterification method involving the reaction of the methyl or ethyl esters of the *N*-protected aminoacids with β-dimethylaminoethanol in the presence of potassium cyanide as the catalyst.

### 5. Methods Involving the Azeotropic Distillation of Water

The possibilities of the synthesis of dialkylaminoalkyl esters of *N*-protected aminoacids by this method have been studied in detail in relation to the synthesis of the β-dimethylaminoethyl ester of Z-glycine[10,11] using different solvents and reaction times (Table 3). According to Mndzhoyan and Agadzhanyan[11], charring takes place in *o*-dichlorobenzene, while an increase of the duration of distillation of the azeotropic mixture in toluene results in an increase of the yield of the β-dimethylaminoethyl ester of Z-glycine. Subsequently this method was used successfully to synthesise the β-dimethylaminoethyl esters of Z-α- and ω-aminoacids[13].

**Table 3.** The influence of the solvent and reaction time on the yield of the β-dimethylaminoethyl ester of *N*-benzyloxycarbonylglycine in the azeotropic distillation of water.

| Solvent | Time, h | Yield, % | Refs. |
|---|---|---|---|
| Toluene | 23 | 25 | [10] |
| Toluene | 56 | 60 | [11] |
| *o*-Xylene | 23 | 50 | [11] |
| *o*-Dichlorobenzene | 23 | 20 | [11] |
| Xylene | 15—18 | 76.4 | [13] |

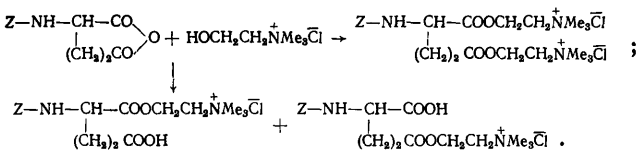
Comparison of the yields of the β-dimethylaminoethyl ester of *N*-benzyloxycarbonylglycine, synthesised by the method involving the azeotropic distillation of water from a mixture of the Z-aminoacid and the corresponding aminoalcohol in different solvents, makes it evident that xylene constitutes the best reaction medium.

### 6. Other Methods of Synthesis

One of the common methods of synthesis of β-dimethylaminoethyl esters of aminoacids and peptides involves the interaction of the *N*-protected aminoacids and peptides with dialkylaminoalkyl halides ($XCH_2CH_2NR_2$; X = Cl or Br) in the presence of bases such as triethylamine[8-10] or potassium carbonate[7,20,24]. The β-dimethylaminoethyl esters of di-, tri-, and tetra-peptides have been synthesised successfully by this method[9]. Good results have been obtained using potassium carbonate in the synthesis of the β-dimethylaminoethyl ester of Boc-ε-aminocaproic acid[20]. The β-dimethylaminoethyl ester of Nbs-glycine has been obtained analogously[24].

In a study of the degree of racemisation of the *C*-terminal aminoacid in the synthesis of choline esters, the authors[9] established in relation to Ac-L-Ile-$OCH_2CH_2\overset{+}{N}Me_3$.$Tos\bar{O}$ that the degree of racemisation does not exceed 1.2% for X = Br and 1.4% for X = Cl.

The interaction of one mole of the cyclic anhydride of Z-glutamic acid with two moles of choline yielded the corresponding dicholine ester[17,33]:



When equimolar amounts of the starting materials are used, a mixture of the α- and γ-choline ester is obtained[16,19,22].

**Table 4.** The β-dialkylaminoethyl and choline esters of *N*-protected aminoacids and peptides R-Am-$OCH_2CH_2NR'_2$.Y, where R' = Me.

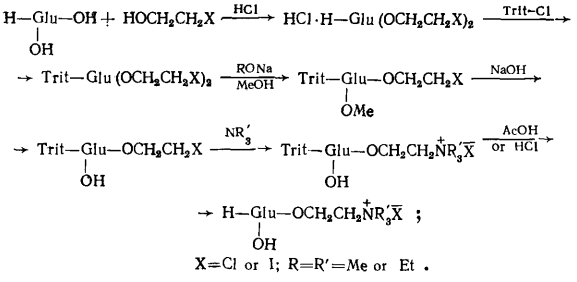
| R | Am | Y | Yield, % | M.p. | Refs. |
|---|---|---|---|---|---|
| Boc | ε-Aca | — | 100 | liq. | [20] |
| Boc | *L*-Ala | — | 65.0 | liq. | [10] |
| Boc | Gly | — | 60.2 | liq. | [6, 12] |
| Boc | Gly-Gly | — | 94.0 | liq. | [6] |
| Boc | *L*-Leu-*L*-Ala | — | — | — | [6] |
| Boc | *L*-Ser(Bzl)-*L*-Gln-*L*-·Arg($NO_2$)- *L* - His (Bzl)-Gly | — | 85.0 | liq. | [6] |
| Bz | Gly * | — | — | liq. | [7] |
| Z | γ-Abu | — | 70.0 | liq. | [13] |
| Z | ε-Aca | — | 74.0 | liq. | [13] |
| Z | *L*-Ala | — | 78.0 | liq. | [13, 30] |
| Z | β-Ala | — | 70.0 | liq. | [13] |
| Z | Gly | — | 76.4 | liq. | [8, 10—13] |
| Z | Gly ** | — | 50.0 | 65—67 | [10, 11] |
| Z | *L*-Ile | — | 57.2 | liq. | [30] |
| Z | *L*-Leu | — | 64.2 | liq. | [30] |
| Z | *D*-Met | — | 65.0 | liq. | [30] |
| Z | *D*-Nval | — | 83.0 | liq. | [13] |
| Z | *D*-Phe | — | 72.0 | liq. | [30] |
| Z | *L*-Val | — | 81.3 | liq. | [13, 30] |
| Z | *m*-Aba-Gly | — | 53.3 | liq. | [30] |
| Z | *p*-Aba-Gly | — | 58.6 | liq. | [30] |
| Z | *L*-Ala-*L*-Phe | — | 93.0 | liq. | [6] |
| Z | *L*-Glu(OMe)-Gly | — | 69.0 | 78—80 | [5] |
| Z | Gly-Gly | — | 62.2 | liq. | [30] |
| Z | *L*-Leu-Gly | — | 61.0 | 72—74 | [5] |
| Z | *L*-Val-Gly | — | 61.0 | liq. | [12] |
| H | Gly | — | — | liq. | [12] |
| Nps | Gly | — | 80.0 | liq. | [24] |
| Pht | Gly | — | 37.0 | 46—48 | [11] |
| Pht | Gly * | — | 30.0 | 88—90 | [11] |
| Tos | Gly | — | 42.0 | 55—57 | [11] |
| Tos | Gly * | — | 36.0 | 56 | [11] |
| Ac | *L*-Ile | TosOMe | 88.0 | 184—186 | [9] |
| Boc | ε-Aca | MeI | 86.0 | 94—95 | [20] |
| Boc | *L*-Trp -*L*-Met-*L*-Asp(OBu-t- )-*L*-Phe | MeBr | 72.0 | 114—116 | [9] |
| Bz | Gly | MeBr | — | 180—181 | [31] |
| Bz | Gly | MeCl | 25.0 | 91 (decomp.) | [32, 34] |
| Bz | Gly | MeCl·$AuCl_4$ | — | 173 (decomp.) | [34] |
| Bz | Gly | MeI | 70.0 | 160—164 | [32] |
| Bz | Gly | $Cr(NH_3)_2(SCN)_4Me$ | — | 135 (decomp.) | [34] |
| Bz | Gly * | MeBr | 74.0 | liq. | [7] |
| Bz | Sar | MeI | — | 125—127 | [31] |
| *p*-$NO_2$Bz | Gly | MeI | — | — | [31] |
| *p*-$NO_2$Bz | Gly | MeCl | 64.0 | 189—192 | [31] |
| | Gly-Gly | MeCl | 81.3 | 222—225 | [32] |
| Z | γ-Abu | MeI | 50.0 | 123—125 | [13] |
| Z | *L*-Ala | TosOMe | — | 88—89 | [8] |
| Z | *L*-Ala | $H_2PtCl_6$ | 34.0 | 195—197 | [8] |
| Z | *L*-Arg($NO_2$) | TosOMe | 85.0 | 85—88 | [8] |
| Z | *L*-Asp(OBzl) | TosOMe | — | 131—133 | [8] |
| Z | *L*-Asp(OBzl) | $H_2PtCl_6$ | — | 172—174 | [8] |
| Z | *L*-$(Cys)_2$*** | MeI | — | 140—142 | [15] |
| Z | *L*-$(Cys)_2$* | MeI | 75.0 | 67—77 | [15] |
| Z | *L*-Glu(OBzl) | $H_2PtCl_6$ | — | 154 | [8] |
| Z | Glu **** | MeCl | 90.6 | — | [17—19, 41] |
| Z | Gly | HCl | — | 110—112 | [5, 7, 11, 12] |
| Z | Gly | $H_2PtCl_6$ | — | 156—161 | [8] |

**Table 4** (contd.).

| R | Am | Y | Yield, % | M.p. | Refs. |
|---|---|---|---|---|---|
| Z | Gly | MeBr | 80.0 | 113—114 | [8, 13] |
| Z | Gly | MeI | 68.7 | 195—198 | [11, 13, 24] |
| Z | *L*-Ile | TosOMe | 60.0 | 104—105 | [8] |
| Z | *L*-Ile | $H_2PtCl_6$ | — | 149—150 | [8] |
| Z | *L*-Leu | TosOMe | 80.0 | 150 | [8] |
| Z | *L*-Leu | $H_2PtCl_6$ | — | 190—192 | [8] |
| Z | *L*-Lys | $H_2PtCl_6$ | 80.0 | 126—128 | [8] |
| Z | *L*-Met | TosOMe | 70.0 | 119—121 | [8] |
| Z | *L*-Met | $H_2PtCl_6$ | — | 126, 128 | [8] |
| Z | *D*-Nval | MeI | 41.2 | 95—97 | [13] |
| Z | *L*-Phe | MeBr | 68.0 | 117—119 | [8] |
| Z | *L*-Phe | MeI | — | 59—62 | [15] |
| Z | *L*-Phe | TosOMe | 80.0 | 184—187 | [8] |
| Z | *L*-Pro | TosOMe | 65.0 | 81 | [8] |
| Z | *L*-Pro | $H_2PtCl_6$ | — | 192—194 | [8] |
| Z | *L*-Trp | TosOMe | 35.5 | 83—85 | [8] |
| Z | *L*-Trp | $H_2PtCl_6$ | — | 121—124 | [8] |
| Z | *L*-Tyr(Bzl) | MeBr | 60.0 | 58—61 | [8] |
| Z | *L*-Tyr(Bzl) | $H_2PtCl_6$ | 80.0 | 148—150 | [8] |
| Z | *L*-Val | MeBr | 70.0 | 116—117 | [8] |
| Z | *L*-Val | $H_2PtCl_6$ | 80.0 | 158—162 | [8] |
| Z | *L*-Asp(OBu-t- )-*L*-Phe | MeBr | 75.0 | 81 | [9] |
| Z | *L*-Asp-*L*-Phe | MeBr | 87.0 | 84—85 | [9] |
| Z | *L*-Met-*L*-Asp(OBu)-*L*-Phe | MeBr | 77.0 | 74—76 | [9] |
| Z | *L*-Met-*L*-Asp-*L*-Phe | MeBr | 84.0 | 85—87 | [9] |
| Z | *L*-Trp-*L*-Met-*L*-Asp (OBu- t- *L*-Phe) | MeBr | 73.0 | 89—91 | [9] |
| Z | *L*-Trp-*L*-Met-*L*-Asp-*L*-Phe | MeBr | 82.0 | 118—120 | [9] |
| Z | *L*-Val-Gly | HCl | 86.5 | 102—102 | [12] |
| Dns | γ-Abu | MeBr | — | — | [28] |
| Dns | ω-Ava | MeBr | — | — | [28] |
| Dns | ε-Aca | MeBr | — | — | [28] |
| Dns | β-Ala | MeBr | — | — | [28] |
| Dns | Gly | MeBr | — | — | [28] |
| H | Gly | MeI | 50.3 | 120—130 | [12] |
| Nps | Gly | MeI | 79.2 | 191—192 | [24] |
| Pht | Gly | HCl | 70.0 | 184—185 | [11] |
| Pht | Gly | MeI | 23.0 | 226—229 | [11] |
| Pht | Gly | EtI | 26.01 | 162—174 | [11] |
| Tos | Gly | HCl | 60.0 | 176—178 | [11] |
| Tos | Gly | MeI | 74.0 | 156—159 | [11, 31] |
| Tos | Sar | MeI | 86.0 | 142—144 | [31] |
| Trit | *L*-Glu | MeI | 54.0 | — | [21—23] |
| Trit | *L*-Glu | MeCl | 75.0 | 203 | [33] |

*R' = Et.
**M.p. 35—36 °C.
***Dicholine ester.
****Monocholine ester.

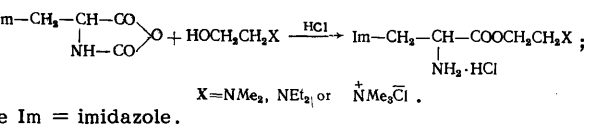
Another method of synthesis of the α-choline ester of glutamic acid has also been developed[21—33]:



Choline esters of hippuric acid[34] and certain Dns-aminoacids[28] have been synthesised analogously via β-halogenoethyl esters.

The synthesis of the hydrochlorides of the choline esters of γ-aminobutyric and β-hydroxy-γ-aminobutyric acids by the reaction of the corresponding acid and choline in the presence of gaseous hydrogen chloride in anhydrous dioxan has been described[35—37]. Choline esters of aminoacids can be obtained via their ammonium salts[26,33].

*N*-Carboxyanhydrides have been used[38] to synthesise the choline ester of L-histidine:



Here Im = imidazole.

Tables 4 and 5 present data on the synthesis of β-dialkylaminoethyl and choline esters of aminoacids and peptides.

Table 5. The hydrohalides of β-dialkylaminoalkyl and choline esters of aminoacids and peptides $HX.H\text{-}Am\text{-}OCH_2.CH_2NR'_2.Y$, where R′ = Me.

| X | Am | Y | Yield, % | M.p. | References |
|---|---|---|---|---|---|
| Br | γ-Abu | HBr | 82.0 | 173,5—175 | [13] |
| Br | γ-Abu | MeBr | 65.0 | 158—160 | [13] |
| Cl | γ-Abu | MeCl | — | 193—194 | [1, 35, 36] |
| Cl | γ-Abu | MeCl·$PtCl_4$ | — | 153—154 (decomp.) | [36] |
| Cl | γ-A-β-Hbu | MeCl | — | 160 (decomp.) | [37] |
| Br | ε-Aca | HBr | 72.0 | 169—172 | [13] |
| Br | ε-Aca | MeBr | 93.0 | 128 | [13, 20] |
| Cl | ε-Aca | MeCl | 75.0 | — | [20] |
| Br | *L*-Ala | HBr | 82.0 | 185—190 | [10, 13, 30] |
| Br | *L*-Ala | MeBr | 17.3 | 213—216 | [8, 13] |
| Cl | *DL*-Ala | MeCl·$PtCl_4$ | — | 237—239 | [27] |
| Cl | *DL*-Ala | MeCl·$2AuCl_3$ | — | — | [27] |
| Br | β-Ala | MeBr | 33.3 | 185—187 | [13] |
| F | *L*-Arg | MeF | — | 56—57 | [8] |
| Br | *L*-Asp | MeBr·$H_2PtCl_6$ | — | 138—140 | [8] |
| I | *L*-$(Cys)_2$* | MeI | — | 83—95 | [15] |
| Br | Glu ** | MeBr·$H_2PtCl_6$ | — | 185—188 | [8] |
| Cl | Glu * | MeCl | 60 | 146 | [33, 41] |
| $CH_3COO$ | *L*-Glu ** | MeI | 77 | 170—180 | [17, 19, 21—23] |
| Cl | *L*-Glu ** | MeCl | — | — | [18] |
| Br | Gly | HBr | 100 | 186—187 | [5, 10, 12, 13] |
| Br | Gly | MeBr | 73.0 | 257—259 | [8, 13] |
| Cl | Gly | MeCl | — | 241 | [15, 20] |
| Cl | Gly | MeCl·$PtCl_4$ | — | 236—238 | [25, 27] |
| Cl | Gly | MeCl·$3AuCl_3$ | — | 180—181 (decomp.) | [25] |
| Cl | Gly | MeCl·$2HgCl_2$ | — | 150—156 (decomp.) | [25] |
| $C_{14}H_7O_7S$ | Gly *** | $C_{14}H_7O_7S$ | — | 259—260 | [15] |
| $2C_6H_3O_7N_3$ | *L*-His | $C_6H_3O_7N_3$ | — | 125—128 | [38] |
| $2C_6H_3O_7N_3$· | *L*-His *** | $C_6H_3O_7N_3$ | — | 142—145 | [38] |
| Cl | *L*-His | MeCl | 88.0 | 230 (decomp.) | [38] |
| Br | *L*-Ile | HBr | 55.7 | liq. | [30] |
| Br | *L*-Ile | MeBr | — | 223—225 | [8] |
| Br | *L*-Leu | HBr | 53.6 | liq. | [30] |
| Br | *L*-Leu | MeBr | — | 188—190 | [8] |
| Cl | *DL*-Leu | MeCl·$PtCl_4$ | — | 203 | [27] |
| Cl | *DL*-Leu | MeCl·$2AuCl_3$ | — | — | [27] |
| Br | *L*-Lys | MeBr | — | 63—65 | [8] |
| Br | *L*-Met | MeBr·$H_2PtCl_6$ | — | 138—140 | [8] |
| Cl | *L*-Met | MeCl | — | 151 | [26] |
| Cl | *L*-Met | MeCl·$PtCl_4$ | — | 195—197 | [26] |
| Cl | *DL*-Met | MeCl | — | 180 | [26] |
| Br | *D*-Nval | MeBr | 66.0 | 120—123 | [13] |
| Br | *D*-Phe | HBr | 61.6 | — | [30] |
| Cl | Phe | MeCl | — | — | [15] |
| I | Phe | MeI | — | 80—83 | [15] |
| Br | *L*-Phe | MeBr | — | 172—176 | [8] |
| Br | *L*-Pro | MeBr | — | 169—171 | [8] |
| Br | *L*-Trp | MeBr | — | 98—101 | [8] |
| Br | *L*-Tyr | MeBr | — | 68—72 | [8] |
| Br | *L*-Val | HBr | 57.6 | — | [30] |
| Br | *L*-Val | MeBr | 20.0 | 229—232 | [8, 13] |
| Cl | *DL*-Val | MeCl·$PtCl_4$ | — | 205—208 | [27] |
| Cl | *DL*-Val | MeCl·$2AuCl_3$ | — | 217—218 | [27] |
| Br | *m*-Aba-Gly | HBr | 50.3 | liq. | [30] |
| Br | *p*-Aba-Gly | HBr | 51.3 | liq. | [30] |
| Br | *L*-Asp-*L*-Phe | MeBr | 75.0 | 126—127 | [9] |
| Br | Gly-Gly | HBr | 62.1 | liq. | [30] |
| Cl | Gly-Gly | MeCl | — | 128—130 | [15] |
| Br | *L*-Met-*L*-Asp-*L*-Phe | MeBr | 75.0 | 144—146 | [9] |
| Br | *L*-Trp-*L*-Met-*L*-Asp-*L*-Phe | MeBr | 77.0 | 126—128 | [9] |
| Br | *L*-Val-Gly | HBr | 93.6 | liq. | [12] |

*Dicholine ester.
**Monocholine ester.
***R′ = Et.

## IV. PHYSICOCHEMICAL PROPERTIES

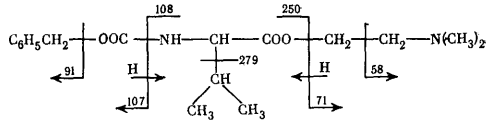
### 1. Physical Properties

The β-dimethylaminoethyl esters of Z- and Boc-aminoacids and peptides are mainly oily substances. Their hydrochlorides and hydrobromides are crystalline substances. The hydrohalides of choline esters of aminoacids and the dihydrohalides of dialkylaminoalkyl esters of aminoacids are as a rule crystalline; however, we should note that some of them are very hygroscopic. The choline esters of *N*-protected aminoacids and peptides are also crystalline.

Infrared spectroscopy has been widely used to identify β-dimethylaminoethyl esters of *N*-protected and unprotected aminoacids and peptides. The infrared absorption spectra of the β-dimethylaminoethyl esters of acylaminoacids have been investigated in detail[12,13] and it has been observed that characteristic peaks occur in the ranges 1680—1760 and 1625—1725 $cm^{-1}$ and refer to the amide and ester carbonyl groups respectively. The presence of the ester bond in the β-dimethylaminoethyl esters of aminoacids and peptides independently of the *N*-protecting group has been confirmed[12, 30] by observing an absorption peak at 1265 $cm^{-1}$.

NMR has also been used to identify β-dimethylaminoethyl and choline esters of aminoacids and their derivatives[6,8,13,20,28].

A mass-spectrometric study of the β-dimethylaminoethyl esters of Z-α- and Z-ω-aminoacids[13] showed that the molecular ion peaks for these compounds have a low intensity or in many instances are not observed at all. This phenomenon has been explained[13] in relation to the β-dimethylaminoethyl ester of Z-valine by the ease of its decomposition with formation of stable radicals of the type $CH_2{=}N(CH_3)_2^+$ (*m/e* 58), $CH_2{=}CH{-}N(CH_3)_2^+$ (*m/e* 71), $C_6H_5CH_2^+$ (*m/e* 91), and $C_6H_5CH_2O^+$ (*m/e* 108):




The β-dimethylaminoethyl esters of p-alkoxybenzoylglycylglycines have also been studied by mass spectrometry[39].

Thin-layer and paper chromatography constitute some of the widely used physical methods for the identification of the β-dimethylaminoethyl and choline esters of aminoacids and peptides[5,6,8,9,11—13,16,20,26,30,33,34,38].

### 2. Chemical Properties

A study of the hydrolysis of the β-dimethylaminoethyl ester of Z-glycine by a 2—4% solution of sodium bicarbonate[5] established that the complete cleavage of the ester linkage takes place in the course of 40—70 min at room temperature. The β-dimethylaminoethyl ester of Boc-glycine in DMF has been hydrolysed[6] in the presence of water. It was observed that the time of complete hydrolysis of the ester diminishes with increase of the amount of water; in the presence of imidazole (0.1 equiv.), the rate of hydrolysis increases.

The ester group hydrolyses on refluxing an acid solution of the β-dimethylaminoethyl ester of Pht-glycine and also on removal of the Tos group by treatment with sodium in liquid ammonia[11].

It has been found that the spontaneous hydrolysis of hippurylcholine in distilled water proceeds at the same rate as the hydrolysis of acetylcholine[34]. The hydrolysis of the choline esters of Tos-glycine[31], hippuric acid[31,32], and hippurylglycine[32] has been investigated. γ-Aminobutyrylcholine (GABC) is unstable at pH 5.5, and is almost completely hydrolysed by 1 *N* HCL at 100 °C in the course of 30 min.[40]

The hydrazinolysis of the γ-choline ester of glutamic acid yielded the corresponding hydrazide[41]. The aminoacid hydrazide is obtained when the Pht group is removed from the aminoester of *N*-phthalylglycine[11].

The aminolysis of the ester linkage in the choline esters of the aminoacids has been investigated. Treatment of the α- or γ-choline esters of *N*-protected glutamic acid with a methanol solution of ammonia[41] yielded the corresponding amides.

In the search for activated esters suitable for the synthesis of peptides, a study has been made[7] of the possibility of using the choline esters. The reaction of the methobromide of the β-dimethylaminoethyl ester of hippuric acid with benzyl-amine gave rise to the expected benzylamide of hippuric acid in a yield up to 34%.

## V. BIOLOGICAL PROPERTIES

The biological properties of the choline esters of aminoacids and peptides have been investigated fairly widely. Frank and coworkers synthesised diazoacetylcholine[42-44] and ε-aminocaproylcholine[20,45] in order to isolate acetylcholinesterase (ACE) and other enzymes specific to acetylcholine from the electric organs of the *electrophorus electricus*. It was established that ε-aminocaproylcholine is a full cholinergic (nicotinic) agonist. Its activity varies between those of acetylcholine and carbocholine; it is not hydrolysed by ACE and is not its inhibitor, but bound to Sepharose 2B it is an effective agent for the isolation of acetylcholine-binding receptors. The choline ester of *N*-dansyl-ε-aminocaproic acid was synthesised for the same purpose[28,46].

The biological action of GABC has been studied in detail. According to the results[1], GABC in a dose of $5 \times 10^{-2}$ M suppresses the contracting action of acetylcholine, in a dose of $5 \times 10^{-3}$ M it suppresses the action of histamine, and in a dose of $1 \times 10^{-4}$ M it suppresses the secretion of gastrin. Eserine enhances and *d*-tubocurarine and flaxedil weaken the inhibiting influence of GABC on the transmission of neuromuscular impulses[47]. At a concentration of $5 \times 10^{-4}$ g ml$^{-1}$, GABC induces the contractions of the small intestine in the guinea-pig and rabbit[48], which are not suppressed by atropine, hexamethonium, phenazoline, or a high concentration of serotonin; morphine appreciably weakens the antiserotonin effect of GABC.

Apart from its inhibiting effect on respiration, GABC also influences the cardiovascular system. GABC and glycylcholine reduce the tonus and the [contraction] amplitude of the isolated heart of the molusc *Mepetrix lusoria*[49]. According to Asano et al.[49], the inhibiting effect of GABC is due to its anticholinergic activity. The neurotropic activity of GABC [50] and its influence on the electric activity of the brain cortex[51] and the central nervous system[52,53] have been investigated. It has been established that GABC reduces the blood supply to the brain[54] but increases the permeability of the membranes[55].

The anticonvulsant properties of GABC [56] have been studied on mice, rabbits, and dogs. Glutamylcholine and GABC have an inhibiting effect on the primary sensory potential when the contralateral peraneal nerve is stimulated[57]. According to Takahashi et al.[57], the absence of an effect by intravenously administered glutamylcholine and GABC on the discharges induced by a solution of corazole is associated with the decomposition of these substances in the blood. It has been shown[40] that GABC is not hydrolysed by the ACE of the blood erythrocytes or the enzyme from the electrical organ of the eel, but is partly susceptible to the action of butyryl cholinesterase of the plasma and tissues, not being, however, a physiological substrate of this enzyme. The enzymatic hydrolysis of GABC [40] is inhibited by inhibitors of cholinesterase—eserine and di-isopropyl phosphorofluoridate. In the presence of α-oxoglutaric acid the action of the guinea-pig brain transaminase on GABC is not accompanied by transamination[40]. It has been established[13] that the choline esters of α- and ω-aminoacids exhibit a weak anticonvulsant activity; some of them possess hypothermic properties.

The results of tests on the influence of the hydrobromides of the choline esters of α-aminoacids on the contraction of smooth muscles[8] have demonstrated a cholinomimetic activity intermediate between those of acetylcholine and choline. It has also been established that the choline esters of cysteine and cystine exhibit an oxytocin activity[15]. Hippurylcholine suppresses the activity of blood serum cholinesterase by 90%.[34]

The choline ester of Nbs-glycine possesses a cholinomimetic activity[24] and also exhibits a pronounced antibacterial activity *in vitro* against *Staphylococcus aureus*. The dihydrochloride of the choline ester of L-histidine in a dose of 2 mg litre$^{-1}$ intensifies the contraction of the isolated rabbit intestine and, when the dose is increased (to 5, 10, and 20 mg litre$^{-1}$), its contraction is inhibited. The picrates of β-dimethyl- and β-diethyl-aminoethyl esters of L-histidine enhance the contraction of the rabbit intestine in doses of 1 and 5 mg litre$^{-1}$ respectively[38]. The methiodides of the β-dimethylaminoethyl esters of certain aminoacids and peptides are inferior in their cholinomimetic activity to carbocholine, but are much less toxic than the latter[58].

The mutagenic activity of the hydrochlorides of the β-dimethylaminoethyl esters of *p*-alkoxybenzoylglycylglycines has also been studied[59]. It has been established that some of them exhibit a pronounced mutagenic activity and that the latter depends on the structure of the alkyl moiety of the alkoxy-group.

The hydrolysis of the choline esters of certain *N*-protected aminoacids by chymotrypsin has been investigated[32,60]. A detailed study has been made of the hydrolysis of the choline esters of *N*-substituted aminoacids by ACE and butyrylcholinesterase[61] and also of their influence on the hydrolysis of acetylcholine. The dimethiodide of the β-dimethylaminoethyl ester of *N,N*-dimethylaminopropionic acid has been used as a model compound in the study of the hydrolysis of the dicholine esters of dicarboxylic acids by butyrylcholinesterase[62]. It has been shown that Z-glutamylcholine increases the activity of transmethylase and choline oxidase, prevents the decrease in the amount of phospholipids, and prevents the accumulation of fats in the liver[63-69].

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