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# CYCLIZATION REACTIONS OF N-(2-CHLOROETHYLCARBAMOYL) AMINO ACIDS

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**Abstract**—Intramolecular cyclization of the N - (2 - chloroethylcarbamoyl) derivatives of glycine, valine and phenylalanine give imidazolidone, oxazoline and hydantoin derivatives. The imidazolidone and oxazoline derivatives possess alkylating activity toward NBP, but the hydantoins do not. N - (2 - Chloroethylcarbamoyl)glycine reacts with the mercapto group of cysteine; thus, this alkylating group may be useful for receptor labelling.

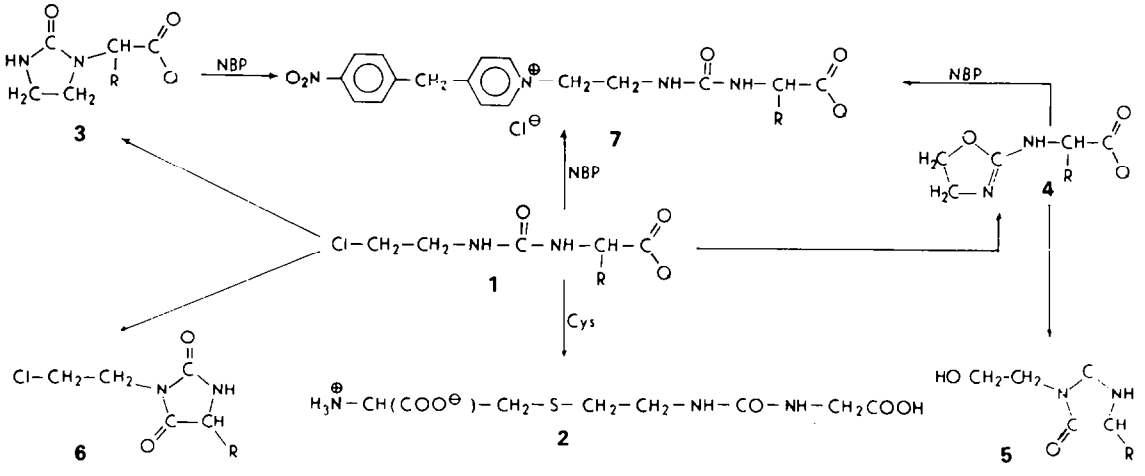
Alkylating derivatives of biologically active peptides can be used for the covalent labelling of specific receptors. The alkylating substituent should not react too soon with the nucleophilic groups but should form covalent bonds only with the sufficiently reactive functional groups of the receptor molecule. We assumed the N - (2 - chloroethylcarbamoyl) group to be a moderately reactive alkylating unit of this kind, which is easily formed by the reaction of 2-chloroethylisocyanate with the amino group of amino acids.[1]

For the investigation of the properties of this group, the N - (2 - chloroethylcarbamoyl) derivatives of glycine, valine and phenylalanine were synthesized and their intramolecular cyclization reactions as well as their reactivities toward cysteine and 4 - (4' - nitrobenzyl) - pyridine (NBP) were studied.

## RESULTS

It is known that alkylating agents react on heating with NBP and bases giving N - alkyl - pyridinium salts then a violet-coloured compound.[2] This reaction proved to be useful also for the chromatographic detection and quantitative determination of N - (2 - chloroethylcarbamoyl) amino acids. With certain considerations, for the modelling of the alkylating reaction in biological systems, the mercapto group was chosen as nucleophile. N - (2 - chloroethylcarbamoyl) glycine reacts slowly with cysteine in $N_2$ at pH 8 and 37°C (15% conversion after 5 hours, measured by the iodometric titration of the SH group of cysteine), but more rapidly at 60°C (63% conversion after 5 hr). From the reaction mixture the S - alkyl - cysteine derivative **2** could be isolated by preparative chromatography in 30% yield (Scheme 1). The structure of the product was determined by its IR spectrum (see Experimental). In the UV region the compound has an absorption at 206 nm (log $\varepsilon$ 3.19) due to the $-CH_2-S-CH_2-CH_2-$ moiety.

It is known, however, that 2-chloroethylureas are converted to 2 - (alkylamino) - 2 - oxazolines on heating in aqueous solution, and to 1 - alkyl - 2 - oxo - imidazolidines on boiling with alcoholic potassium hydroxide.[1a] Investigation of the cyclization of N - (2 - chloroethylcarbamoyl) glycine **1Aa** on heating in water, followed by estimation of the resulting chloride ion




Aa R = H, Q = OH
Ab R = H, Q = OMe
Ac R = H, Q = OBut
Ad R = H, Q = $NH_2$

B R = iPr
Ba R = iPr, Q = OH

C R = $CH_2Ph$
Ca R = $CH_2Ph$, Q = OH
Cc R = $CH_2Ph$, Q = OMe

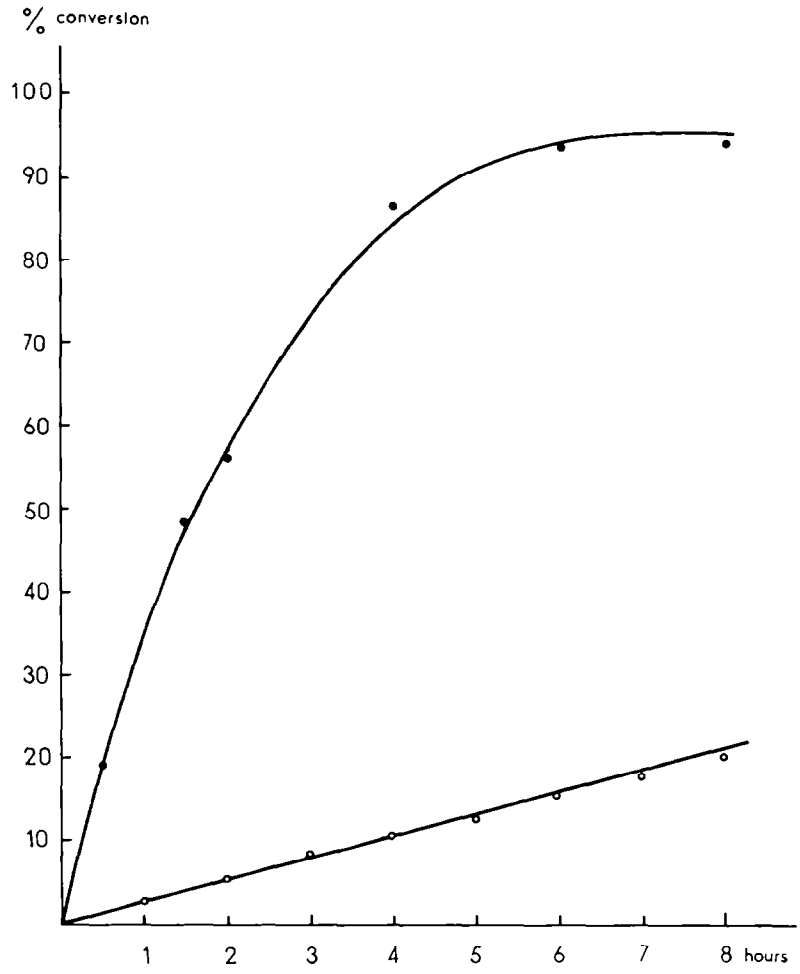



Fig. 1. Formation of $Cl^-$ in the cyclization of **1Aa** (-●-) and the reaction of **1Aa** with NBP (-○-); $(1Aa)_0$ = 7.3 mM/1; $[NBP]_0 = 0.062$ M; $t = 60°$; solvent: ethylene glycol: water = 2:1.

showed that this process is more rapid than the alkylation of the NBP (Fig. 1).

The final product of the cyclization isolated after a two hour reaction with 76% yield in crystalline state proved to be the 1-carboxymethylimidazolid - 2 - one **3Aa**, according to elemental analysis, IR and mass spectroscopic measurements. The same compound formed on boiling the N - (2 - chloroethylcarbamoyl) glycine amide **1Ad** or methyl ester **1Ab**, the ester or amide group having been hydrolysed by the HCl formed during the cyclization. The amide **3Ad** is formed, however, if N - (2 - chloroethylcarbamoyl) glycine amide is boiled with KOH in EtOH. It is remarkable that the isolated imidazolidones react with NBP producing a coloured product with the same absorption maximum (583 nm) as in the case of the N - (2 - chloroethylcarbamoyl) glycine. **3Aa** aslo reacts with the mercapto group of cysteine and even faster than **1Aa** (83% conversion after 5 hr).

The cyclization procedure was also carried out with N - (2 - chloroethylcarbamoyl) phenylalanine. On heating it in water, through the intermediate formation of the corresponding oxazoline **4Ca**, which, when isolated, is reactive toward NBP ($\lambda_{max}$: 588 nm), hydantoin derivatives **5C** and **6C** were formed, from which the 3 - (2 - chloroethyl) 5 - benzyl - hydantoin **6C** could be isolated. It should be noted that N - carbamoyl - glycine ethyl ester is converted to hydantoin in dilute HCl too.[3] In the same experiment from N - (2 - chloroethylcarbamoyl) - phenylalanine methyl ester, through the intermediary oxazoline derivative (TLC), the 3 - (2 - hydroxyethyl) - 5 - benzyl - hydantoin **5C** was formed, though the TLC analysis of the reaction mixture showed the presence of **6C** as well. In KOH/EtOH the cyclization of N - (2 - chloroethylcarbamoyl) - phenylalanine methylester to the hydantoin **6C** proceeded much faster than in aqueous medium. The hydantoin derivative **5C** does not react with NBP.

N - (2 - chloroethylcarbamoyl) - valine **1Ba** gave oxazoline **4Ba** on heating according to its IR spectrum, but, in agreement with the literature, it could not be isolated in crystalline form.[4] This compound also reacts with NBP, probably with the formation of the corresponding N-alkyl compound. It is of interest, that the oily N - (2 - chloroethylcarbamoyl) valine and the N - (2 - chloroethylcarbamoyl) phenylalanine crystallize on standing for several weeks. These crystalline compounds are not the oxazolines, but the hydantoins **6B**, **6C**.

## DISCUSSION

While functioning as direct alkylating agents in the presence of a suitable nucleophile, N - (2 - chloroethylcarbamoyl) amino acids undergo intramolecular cycliza-

Table 1. Products of the cyclization reactions of N - (2 - chloroethylcarbamoyl) amino acids

| Reactant | | | Reaction conditions | | Products | |
|---|---|---|---|---|---|---|
| | R | Q | solvent | time | isolated | detected by TLC |
| 1Aa | H | OH | $H_2O$ | $2^h$ | 3Aa | |
| 1Ab | H | OMe | $H_2O$ | $2^h$ | 3Aa | |
| 1Ad | H | $NH_2$ | $H_2O$ | $2^h$ | 3Aa | |
| 1Ad | H | $NH_2$ | KOH-EtOH | 15' | 3Ad | |
| 1Ba | iPr | OH | $H_2O$ | $1^h$ | 4Ba | 6B |
| 1Ba | iPr | OH | solid-phase | 2 months | 6B | 4Ba |
| 1Ca | $CH_2Ph$ | OH | $H_2O$ | $1^h$ | 4Ca | 5C, 6C |
| 1Ca | $CH_2Ph$ | OH | $H_2O$ | $3^h$ | 6C | 4Ca, 5C |
| 1Cb | $CH_2Ph$ | OMe | $H_2O$ | $3^h$ | 5C | 6C, 4Ca |
| 1Cb | $CH_2Ph$ | OMe | KOH-EtOH | 15' | 6C | |
| 1Ca | $CH_2Ph$ | OH | solid-phase | 2 months | 6C | 4Ca |

tion reactions on heating alone in aqueous solution. The glycine derivative, presumably owing to the steric availability of the amino nitrogen, gives imidazolidone. The valine and phenylalanine derivatives afford hydantoins as final products, through the intermediacy of the corresponding oxazoline which can be isolated in both cases. Both the N - (2 - chloroethylcarbamoyl) amino acids and their oxazoline derivatives, as well as the imidazolidone derivative in the case of glycine, are potent alkylating compounds when using NBP as nucleophile. The alkylation of the cysteine mercapto group is a slow reaction and it may be even slower under physiological conditions. Since ring closure into the unreactive hydantoins occurs only on prolonged heating, this side reaction is highly unlikely in biological systems. N - (2 - Chloroethylcarbamoyl) peptides and their reactive cyclic derivatives are therefore possible candidates for the affinity-labelling of the specific receptor, but only in cases when the latter contains sufficiently reactive nucleophilic groups.

## EXPERIMENTAL

All melting points are uncorrected. TLC was carried out on plates (DC-Plastikfolien Kieselgel 60, E. Merck, Darmstadt). Column chromatography was performed on silica gel open columns (Merck Kieselgel 60, Art. 10832). Solvent systems (volumes): (1) butanol: acetic acid : water = 4 : 1 : 1; (2) ethylacetate : pyridine : acetic acid : water = 30 : 20 : 6 : 11; (3) ethylacetate : pyridine : acetic acid : Water = 60 : 20 : 6 : 11; (4) methanol ; water = 3 : 1; (5) ethylacetate : pyridine : acetic acid : water = 120 : 10 : 3 : 5.5. Detection by $Cl_2$/tolidine and 4-nitrobenzylpyridine/triethylamine. IR and UV spectra were recorded on Specord IR 75 and Specord UV-VIS spectrometers (Karl Zeiss Jena), respectively.

*N-(2-Chloroethylcarbamoyl)-glycine methyl ester* **1Ab**

To 2.5 g (0.02 M) glycine methyl ester hydrochloride in 15 ml abs dimethylformamide 2.88 ml (0.02 M) triethylamine and 2 ml (2.3 mM) 2 - chloroethylisocyanate was added under stirring and ice-cooling. Stirring was continued for 2 hr and then it was let to stand overnight. The solvent was evaporated *in vacuo*, and the residue was triturated with water and filtered (3.05 g, 78%). The crude product was recrystallized from ethyl acetate (2.4 g, 61%). M.p. 115–116°, $R_f^1$: 0.66, $R_f^3$: 0.88. Found C, 37.45; H, 6.01; N, 14.08; O, 24.25; Cl, 18.34. $C_6H_{11}N_2O_3Cl$ requires C, 37.02; H, 5.69; N, 14.39; O. 24.66; Cl, 18.21%.

*N - (2 - Chloroethylcarbamoyl)glycine t-butyl ester* **1Ac**, *N - (2 - chloroethylcarbamoyl)glycine amide* **1Ad**, *N - (2 - chloroethyl carbamoyl)phenylalanine methyl ester* **1Cb**

These were prepared in similar manner as **1Ab**. **1Ac**: m.p. 116–117°, crystallized from ethylacetate - n - hexane (74%), $R_f^1$: 0.74, $R_f^3$: 0.44. **1Ad**: m.p. 164–165°, crystallized from ethanol (51%), $R_f^3$: 0.65. **1Cb**: m.p. 100–101°, crystallized from ethylacetate - n - hexane (58%), $R_f^3$: 0.92.

*N - (2 - Chloroethylcarbamoyl)glycine* **1Aa**

3.1 g (13.5 mM) 1Ac was dissolved in 20 ml trifluoroacetic acid. After half an hour the soln was evaporated and the residue was triturated with ether, filtered off (2.34 g, 100%) and crystallized from ethanol (1.9 g, 81%). M.p. 145–146°, $R_f^1$: 0.55, $R_f^3$: 0.65. Found C, 33.87; H, 5.34; N, 15.75; Cl, 19.50%. $C_5H_9N_2Cl$ requires C, 33.25; H, 5.02; N, 15.51; Cl, 19.63%.

1 - *Carboxymethyl - imidazolid - 2 - one* **3Aa**

900 mg (5 mM) 1Aa was boiled for 2 hr in 20 ml water containing 0.02 ml Agepon[5] (Agfa Gevaert AG). After cooling the solution was neutralized with dil $NH_4OH$ and evaporated. The residue was triturated with abs ethanol and filtered (550 mg, 76%). A sample was twice recrystallized from 90% ethanol with 50% yield. M.p. 170–172°, $R_f^1$: 0.15, $R_f^3$: 0.25. Found C, 41.69; H, 6.04; N, 19.04; O, 33.20. $C_5H_8N_2O_3$ requires C, 41.67; H, 5.59; N, 19.43; O, 33.30%. IR (KBr): 3300–3200 $cm^{-1}$ (OH), 3010 $cm^{-1}$ (NH), 1710 $cm^{-1}$ (C = O, carboxyl), 1590 $cm^{-1}$ (C=O, imidazolidone); MS: *m/z* 144 ($M^+$ 29%), 100 (41%), 99 (45%) and 30 (100%).

**1Ab** and **1Ad** could be converted in to **3Aa** by the same procedure.

1 - *Carboxamido - imidazolid - 2 - one* **3Ad**

900 mg (5 mM) 1Ad was boiled for 15 min in 20 ml abs. ethanol containing 282 mg (5 mM) KOH. After cooling, the reaction mixture was neutralized with HCl/methanol and KCl was filtered off. After evaporation of the filtrate, the crude product was purified on silica gel column in solvent system 2 (350 mg, 43%). M.p. 160–161°, $R_f^2$: 0.65, $R_f^3$: 0.27. (Found O, 22.40. $C_5H_9O_2N_3$ requires O, 22.35%). IR (KBr): 3375, 3270, 3140 $cm^{-1}$ ($NH_2$, NH), 1675 $cm^{-1}$ (C = O), 1615 $cm^{-1}$ ($NH_2$).

*Oxazolinylvaline* **4Ba**

1.1 g (5 mM) **1Ba**[4] was boiled for 1 h in 20 ml water containing 0.02 ml Agepon. After cooling and neutralizing, the solution was evaporated *in vacuo*. The residue was purified on a silica gel column in solvent system 4 (360 mg, 39%). $R_f^4$: 0.52. IR (KBr): 3280 $cm^{-1}$ (NH), 3200–2200 $cm^{-1}$ (OH), 1718 $cm^{-1}$ (C=O), 1700 $cm^{-1}$ (C=N).

3 - (2 - *Chloroethyl*) - 5 - *isopropylhydantoin* **6B**

**1Ba** crystallized on standing for several weeks. Recrystallization from water (75%). M.p. 87–88°, $R_f^4$: 0.91. (Found C, 47.44; H, 6.40; N, 13.79; O, 15.40; Cl, 17.68. $C_8H_{13}N_2O_2Cl$ requires C, 46.94; H, 6.40; N, 13.69; O, 15.63; Cl, 17.32%). IR (KBr): 3290 $cm^{-1}$ (NH), 1755, 1700 $cm^{-1}$ (C=O).

*Oxazolinylphenylalanine* **4Ca**

812 mg (3 mM) 1Ca[4] was boiled for 1 h in 20 ml water containing 0.02 ml Agepon. The solution was cooled, neutralized and evaporated *in vacuo*. The residue was purified on a silica gel column in solvent system 4 (240 mg, 34%). $R_f$[4]: 0.62. IR (KBr): 2920 $cm^{-1}$ (NH), 3400–2200 $cm^{-1}$ (OH), 1715 $cm^{-1}$ (C=O), 1675 $cm^{-1}$ (C=N), 755, 695 $cm^{-1}$ (monosubstituted aromatic ring).

3 - (2 - *Chloroethyl*) - 5 - *benzylhydantoin* **6C**

(a) 812 mg (3 mM) **1Ca** was boiled for 3 h in 20 ml water containing 0.02 ml Agepon. After cooling the solution, the precipitate was filtered off and crystallized from ethylacetate - n - hexane (248 mg, 35%). M.p. 137–138°, $R_f$[4]: 0.96, $R_f$[5]: 0.8 (Found Cl, 13.70. $C_{12}H_{13}N_2OCl$ requires Cl, 14.03%). IR (KBr): 3260 $^{-1}$ (NH), 1772, 1713 $cm^{-1}$ (C=O), 759, 698 $cm^{-1}$ (monosubstituted aromatic ring). (b) 854 mg (3 mM) **1Cb** was boiled for 15 min in 15 ml abs ethanol containing 168 mg (3 mM) KOH. The solvent was evaporated and the residue was dissolved in the mixture of ethyl acetate-water. After separation, the ethyl acetate phase was dried on dry $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from ethylacetate - n - hexane (320 mg, 42%). The physical data are the same as above.

3 - (2 - *Hydroxyethyl*) - 5 - *benzylhydantoin* **5C**

1.42 g (5 mM) **1Cb** was boiled for 3 h in 20 ml water containing 0.02 ml Agepon. After cooling and neutralization with diluted $NH_4OH$ solution, the water was distilled *in vacuo* and the residue was dried over $P_2O_5$ in a desiccator. The dry material was dissolved in abs ethanol and the salt was filtered off. After evaporation the residue was crystallized from ethylacetate (400 mg, 34%). M.p. 115–117°, $R_f$[5]: 0.6. Found C, 61.28, H, 6.02; N, 11.88; O, 20.29. $C_{12}H_{14}N_2O_3$ requires C, 61.52; H, 6.02; N, 11.95; O, 20.49%). IR (KBr): 3280 $cm^{-1}$ (NH, OH), 1769, 1719 $cm^{-1}$ (C=O), 1065 $cm^{-1}$ (C–O), 762, 694 $cm^{-1}$ (monosubstituted aromatic ring).

*Reaction of cysteine with N - (2 - chloroethylcarbanoyl) glycine*

432 mg (2.4 mM) **1Aa** and 313 mg (2 mM) cysteine hydrochloride were dissolved in 25 ml 0.4 M pH: 8.5 phosphate buffer. The reaction mixture was incubated under nitrogen atmosphere at 60° for 24 hr. After concentration *in vacuo* the residue was purified in solvent system 4 on a silica gel column. The crude material was crystallized from water (190 mg, 30%). M.p. 203–204°, $R_f$[4]: 0.7. IR (KBr): 3393, 3330 $cm^{-1}$ (NH), 3250–2200 $cm^{-1}$ ($NH_3^+$, OH), 1695 $cm^{-1}$ (C=O, acid), 1628 $cm^{-1}$ (C=O, amide), 1570, 1400 $cm^{-1}$ ($CO_2^-$).

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