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Syntheses of Potentially Antineoplastic Amides and Esters of *N*-[*N'*-(2-Chloroethyl)-*N'*-nitrosocarbamoyl]amino Acids, II[5]

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Syntheses of *N*-[*N'*-(2-chloroethyl)-*N'*-nitrosocarbamoyl]amino acid amides and esters as potential antineoplastic substances are reported. *N*-[*N'*-(2-chloroethyl)-*N'*-nitrosocarbamoyl]amino acids (with the exception of the glycine derivative) were prepared by reaction of 2-chloroethyl isocyanate with the sodium salt of an amino acid in a heterogenous medium followed by nitrosation with sodium nitrite under acidic conditions. Condensation with amines or alcohols using 1,1-carbonyldiimidazole led to the amides or esters.

Synthese von potentiell antineoplastischen Amiden und Estern von N-[N'-(2-Chlorethyl)-N'-nitroso-carbamoyl]-aminosäuren, 2. Mitt.

Die Synthese von N-[N'-(2-Chlorethyl)-N'-nitroso-carbamoyl]-aminosäureamiden und -estern als potentiell antineoplastischen Substanzen wird beschrieben. N-[N'-(2-Chlorethyl)-N'-nitroso-carbamoyl]aminosäuren (mit Ausnahme der Glycinverbindung) werden durch Umsetzung von 2-Chlorethylisocyanat mit dem Natriumsalz einer Aminosäure in heterogener Phase und anschließender Nitrosierung mit Natriumnitrit in saurem Medium dargestellt. Durch Kondensation mit Aminen bzw. Alkoholen unter Verwendung von 1,1-Carbonyldiimidazol lassen sich die entsprechenden Amide bzw. Ester erhalten.

Nitrosoureas such as N,N'-bis-(2-chloroethyl)-N-nitrosourea (BCNU)[+++)], N-(2-chloro-ethyl)-N'-cyclohexyl-N-nitrosourea (CCNU) and N-(2-chloroethyl)-N'-(4-methylcyclohexyl)-N-nitrosourea (MeCCNU) are important antitumour agents in the treatment of human cancer. In addition, a number of new nitrosoureas with better pharmacokinetic and therapeutic properties have been synthesized and tested for cytostatic activity during recent years[1,2]. Among those with good therapeutic activity against various tumour models were nitrosoureas in which the nitrosocarbamoyl residue was linked to an amino acid or its derivatives (e.g. esters or amides)[1–6]. We have therefore synthesized a series of nitrosoureas of this type with a view to obtaining drugs with an improved therapeutic index.

[+++)] CNU = $Cl\text{-}CH_2\text{-}CH_2\text{-}N(NO)\text{-}CO\text{-}NH$

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Table 1: *Data of N-[N'-(2-Chloroethyl)-N'-nitrosocarbamoyl]amino acid amides* **(4)**

No.	R^1	R^2	R^3	mp. (°C)	formula	yield (%)	elemental analyses calcd. found C	H	Cl	N	spectral data IR (cm^{-1}) ^{1}H-NMR δ (ppm)	
4a	H	H	CH_2-CH_2-S $R^5-CH_2-CH_2-S$	124	$C_{14}H_{24}Cl_2N_8O_6S_2$		31.4 31.6	4.51 4.33	13.2 13.4	20.9 20.7	2.80, 3.20–4.30, 8.22, 8.33	3360, 3310, 1725, 1670, 1540, 1500
4b	CH_3	H	CH_3	92	$C_7H_{13}ClN_4O_3$	87	35.5 35.5	5.54 5.63	15.0 15.2	23.7 24.0	1.53, 2.88, 3.50 4.16, 4.64, 6.61, 7.67	3380, 3310, 1685, 1675, 1530, 1500
4c	CH_3	H	C_2H_5	90	$C_8H_{15}ClN_4O_3$	74	38.3 38.5	6.03 6.14	14.1 14.3	22.4 22.3	1.28, 1.57, 3.38, 4.18, 4.64, 6.75, 7.75	3400, 3300, 1715, 1660, 1530, 1490
4d	CH_3	H	C_8H_{17}	78	$C_{14}H_{27}ClN_4O_3$	45	50.2 50.6	8.13 8.37	10.6 10.8	16.7 16.6	1.17, 3.40, 4.18, 4.62, 6.78, 7.60	3350, 3300, 1720, 1660, 1535, 1490
4e	CH_3	H	$(CH_2)_2OH$	105 (dec.)	$C_8H_{15}ClN_4O_4$	38	36.0 36.1	5.67 5.44	13.3 13.3	21.0 20.8	1.58, 3.50, 3.76, 4.18, 4.62, 6.78, 7.60	3350, 3300, 1698, 1662, 1532, 1495
4f	CH_3	H	CH_2-CH_2-S $R^5-CH_2-CH_2-S$	86	$C_{16}H_{28}Cl_2N_8O_6S_2$	15	34.1 34.4	5.00 4.83	12.6 12.6	19.9 19.6	1.41, 2.80, 3.46, 4.28, 8.25, 8.60	3360, 3310, 1725, 1660, 1525, 1490
4g	CH_3	CH_3	CH_3	/	$C_8H_{15}ClN_4O_3$	63	38.3 38.1	6.03 6.15	14.1 13.9	22.4 22.3	1.49, 3.01, 3.14, 3.47, 4.15, 5.00, 7.92	3390, 3320, 1730, 1650, 1515, 1490
4h	CH_3	C_2H_5	C_2H_5	/	$C_{10}H_{19}ClN_4O_3$	78	43.1 43.3	6.87 6.69	12.7 12.9	20.1 20.2	1.35, 3.41, 4.17, 4.95, 7.88	3390, 3300, 1725, 1645, 1525, 1490

4i	CH_3	$-CH_2-CH_2$ \ CH_2 / $-CH_2-CH_2$		/	$C_{11}H_{19}ClN_4O_3$	62	45.4 45.6	6.59 6.76	12.2 12.3	19.3 19.2	1.64, 3.53, 4.18, 4.99, 8.05	3390, 1725, 1640, 1520, 1490
4k	CH_3	$-CH_2-CH_2$ \ O / $-CH_2-CH_2$		/	$C_{10}H_{17}ClN_4O_4$	59	41.0 41.2	5.86 6.00	12.1 12.2	19.1 18.9	1.51, 3.65, 4.18, 5.00, 8.03	3400, 3320, 1730, 1655, 1525, 1490
4l	CH_3	$-CH_2-CH_2$ \ $N(CH_2)_2OH$ / $-CH_2-CH_2$		/	$C_{12}H_{22}ClN_5O_4$	41	42.9 42.7	6.60 6.77	10.6 10.7	20.9 20.7	1.50, 2.62, 3.64, 4.19, 5.01, 8.04	3390, 1725, 1640, 1520, 1490
4m	$CH-CH_3$ C_2H_5	H	$(CH_2)_2OH$	93 (dec.)	$C_{11}H_{21}ClN_4O_4$	42	42.8 43.0	5.86 6.87	11.5 11.7	18.1 17.9	0.6–2.3, 3.53, 4.19, 4.44	3340, 3300, 1725, 1660, 1540, 1500
4n	CH_3-S CH_2-CH_2	H	$(CH_2)_2OH$	70	$C_{10}H_{19}ClN_4O_4S$	42	36.8 36.9	5.86 5.82	10.9 11.0	17.1 17.2	2.13, 2.60, 3.48, 4.12, 4.67	3440, 3360, 3315, 1725, 1710, 1670. 1640, 1530, 1500

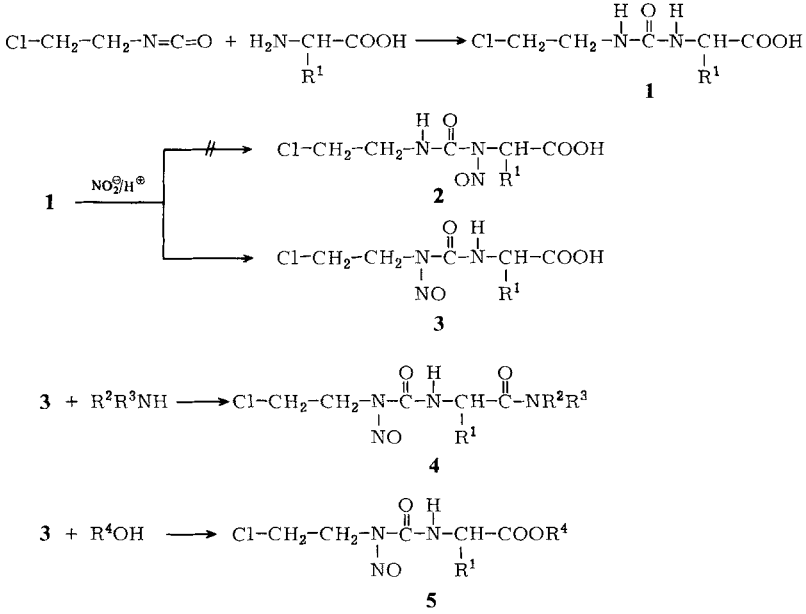
1) *MP*: not corrected; 2) ^{1}H-NMR: Bruker HX 90; IR: Perkin Elmer 580 B; 3) R^5 = Cl-CH_2-CH_2-N(NO)-CO-NH

4a: R^5=Cl–CH_2–CH_2–N(NO)–C(=O)–NH–CH_2–C(=O)–NH–; **4f**: R^5=Cl–CH_2–CH_2–N(NO)–C(=O)–NH–CH(CH_3)–C(=O)–NH–

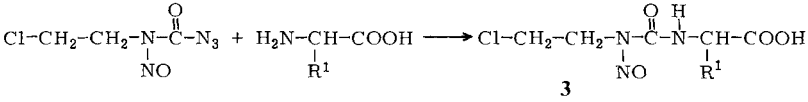
N-[N′-(2-chloroethyl)-N′-nitroso-carbamoyl]-amino acid esters and amides can be synthesized in different ways. Reaction of N-(2-chloroethyl)-N-nitroso-carbamoyl azide with amino acids, as shown in pathway B, gave the corresponding ureas **3** in a yield of about 70 %[5,7]. With exception of the glycine compound **3a** the same ureas can also be prepared by a direct linkage of the sodium salt of an amino acid with 2-chloroethylisocyanate in a water/toluene mixture followed by nitrosation with sodium nitrite under acidic conditions (90 % yield; pathway A). The formation of the isomeric nitrosoureas **2** could not be detected and the compounds so obtained were proved to be identical (by IR and mixed m.p.) with the products of pathway B.

Scheme 1

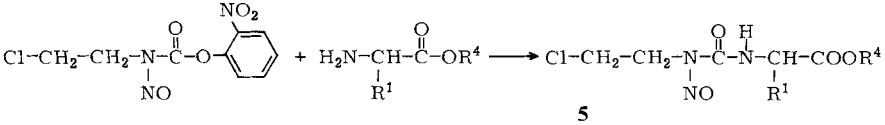
Pathway A



Pathway B



Scheme 2



1, 2 and **3:**
a: $R^1 = H$
b: $R^1 = CH_3$
c: $R^1 = CH(CH_3)(C_2H_5)$

1, 2 and **3:**
d: $R^1 = CH_2-CH_2-SCH_3$
e: $R^1 = CH_2-C_6H_5$
4 and **5:** R^1, R^2, R^3 and R^4 see tables 1 and 2

Condensation of the resulting N-carbamoylated amino acids **3** with amines or alcohols using 1,1-carbonyldiimidazole as condensating agent[8] led to the corresponding amides **4** or esters **5**. The glycine compound **5a** and the phenylalanine compound **5f** were prepared by reaction of the N-(2-chloroethyl)-N-nitroso-carbamate of ortho-nitro-phenol[9] with the ethyl ester of glycine and phenylalanine in methanol (scheme 2).

Table 2: *[Data of N-N'-(2-Chloroethyl)-N'-nitrosocarbamoyl]amino acid esters* **(5)**

No	R^1	R^4	formula	yield (%)	elemental analyses calcd. found				spectral data ^{1}H-NMR δ (ppm)	IR (cm^{-1})
					C	H	Cl	N		
5a	H	C_2H_5	$C_7H_{12}ClN_3O_4$	39	35.4 35.7	5.09 5.35	14.9 14.6	17.7 17.4	2.33, 3.50, 4.22, 7.42	3410, 1735, 1535 1495
5b	CH_3	C_2H_5	$C_8H_{14}ClN_3O_4$	42	38.2 38.3	5.61 5.71	14.1 14.4	16.7 16.8	1.32, 1.62, 3.50, 4.21, 4.70, 7.51	3410, 3360, 1725, 1530, 1495
5c	CH_3	$n\text{-}C_4H_9$	$C_{10}H_{18}ClN_3O_4$	68	42.9 43.0	6.49 6.25	12.7 12.7	15.0 15.4	0.95, 1.52, 3.50, 4.13, 4.70, 7.51	3420, 3380, 1730, 1535, 1500
5d	CH_3	$i\text{-}C_4H_9$	$C_{10}H_{18}ClN_3O_4$	59	42.9 43.0	6.49 6.19	12.7 12.5	15.0 15.3	0.98, 1.59, 2.00, 3.48, 4.13, 4.72	3420, 3380, 1735, 1535, 1500
5e	$CH(CH_3)$ $\vert$ C_2H_5	C_2H_5	$C_{11}H_{20}ClN_3O_4$	68	45.0 44.9	6.86 6.73	12.1 12.3	14.3 14.2	1.34, 2.10, 3.53 4.28, 4.70, 7.42	3410, 3360, 1725, 1525, 1495
5f	$CH_2C_6H_5$	C_2H_5	$C_{14}H_{18}ClN_3O_4$	37	51.3 51.0	5.53 5.63	10.8 11.0	12.8 12.7	1.27, 3.24, 4.20, 4.95, 7.25	3410, 1725, 1525, 1495

Results and Discussion

All of the monosubstituted amino acid amides could be obtained in a crystalline form, whereas the disubstituted amides as well as the amino acid esters were yellow oils, sometimes of high viscosity. In all cases the assigned structures were verified by spectroscopy and elementary analyses.

Both described pathways proved to be valuable methods for the selective synthesis of N-nitrosoureas with the nitroso group adjacent to the 2-chloroethyl residue. Pathway B is a general method for the preparation of all CNU-compounds; pathway A, however, is preferable for obtaining CNU-amino acids, because it comprises only two reaction steps beginning with the isocyanate and results in high yields. The CNU-amino acids so obtained can be further modified at their carboxyl groups.

It is also possible to use "active carbamates" as a transport form of the CNU moiety (see scheme 2). In contrast to pathway A, the separation of by-products generated during the reaction (e.g. phenols) is difficult, and in our hands only moderate yields of the desired products were obtained.

Spectral Data

Structural confirmation of the derivatives **4** and **5** could be obtained from ^{1}H-NMR-spectra in combination with IR-spectra. All compounds showed two characteristic triplets at δ 3.4–3.6 ppm and at δ 4.1–4.2 ppm, both with a coupling constant of about 7 Hz caused by the methylene protons of the 2-chloroethyl group. These signals are typical for nitrosated 2-chloroethyl-carbamoyl compounds[10]. The proton at the asymmetric center of the amino acid could be detected as a multiplet centered at δ 4.6 ppm. As exemplified by the alanine compounds it was replaced by a quartet after treatment with D_2O. The N-bound ureido proton, the signal of which could be seen at δ 7.8–8.1 ppm was only slowly exchanged against deuterium. All other remaining protons gave signals with expected intensities and δ-values.

In the IR-spectra, the following peaks were prominent: Amido peaks of the urea at 1700–1730 cm^{-1}; amide I at 1640–1675 cm^{-1}; amide II at 1515–1535 cm^{-1} and NNO at 1490–1500 cm^{-1}.

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Experimental Part

N-[N'-(2-Chloroethyl)-N'-nitroso-carbamoyl]amino acids **(3)**
N-[N'-(2-Chloroethyl)-N'-nitroso-carbamoyl]-L-alanine **(3b)**

To a solution of 123 mmol L-alanine and 123 mmol NaOH in 130 ml of water, 123 mmol 2-chloroethyl-isocyanate in 85 ml toluene were added dropwise with stirring and ice-cooling. After 1 h both phases were separated. To the aqueous phase 250 mmol sodium nitrite was added followed by 90 ml hydrochloric acid which was added in parts so that the temp. did not rise above 5 °C. Stirring for 30 min was followed by extraction with diethyl ether. The pooled etherial phases were washed with water, dried and the solvent removed by rotary evaporation. The remaining residue was recrystallized from dichloromethane/n-hexane after which light orange crystals, mp. 93 °C, were obtained in a yield of 90 %. The IR-spectrum of this compound was identical with that of the same compound synthesized by pathway B and a mixture of both compounds showed no depression in melting-point.

N-[N'-(2-Chloroethyl)-N'-nitroso-carbamoyl]-L-isoleucine **(3c)**

The procedure was the same as described above, with the exception that 50 ml of diethyl ether per 100 mmol isoleucine were added before nitrosation. A brown oil of high viscosity, yield 90 %, was obtained. The IR-spectrum was identical with that of the same compound synthesized by pathway B.

N-[N'(2-Chloroethyl)-N'-nitroso-carbamoyl]-D,L-methionine **(3d)**

To the aqueous phase obtained after reaction of the amino acid with the isocyanate sodium nitrite was added and the resulting solution added dropwise to a mixture of hydrochloric acid/diethyl ether. A crystalline product, mp. 92°C; yield 82 %, was obtained. IR-spectroscopy and m.p. showed identity with the product of pathway B.

N-[N'-(2-Chloroethyl)-N'-nitrosocarbamoyl]amino acid amides **(4)**

General Procedure: To a solution of 10 mmol of the N-carbamoylated amino acid **3** in 30 ml dichloromethane 10 mmoles of 1,1-carbonyldiimidazole were added with stirring at room temp. When

the liberation of carbon dioxide was finished 10 mmoles of the corresponding amine dissolved in 10 ml of dichloromethane were added and the reaction mixture kept in the dark for about 1–2 h. The solvent was then removed by rotary evaporation and the residual yellow oil dissolved in a small amount of dichloromethane. The solution was purified by silica gel chromatography (eluant: dichloromethane or dichloromethane/methanol 10:1). For the preparation of the 2-hydroxyethylamides **4e**, **4m** and **4n** acetonitrile instead of dichloromethane was used as the solvent.

N-[N'-(2-Chloroethyl)-N-nitrosocarbamoyl]amino acid esters **(5)**

General Procedure: The procedure was the same as described above. A 3-fold molar excess of the corresponding alcohol was used instead of the amine.

The glycine ethylester **5a** *and the phenylalanine ethylester* **5f**

To a solution of 9 mmol o-nitrophenyl N-(2-chloroethyl)-N-nitrosocarbamate (prepared according to *Imbach*[9]) in 40 ml methanol a solution of 9 mmol of the amino acid ethylester in 40 ml methanol was added dropwise within 30 min. After stirring for 2 h the solvent was removed by rotary evaporation. The residual yellow oil was dissolved in a small amount of dichloromethane and separated on a silica gel column with dichloromethane as eluant. The esters were obtained in form of yellow oils.

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[Ph 754]