BIOORGANIC CHEMISTRY 6, 431–441 (1977)

Nucleophilic Substitution at C-2 of *S*-Alkylated 2-Thiocytidines by Cysteine and Lysine

A New Method for Specific Covalent Linking of Peptides to Nucleic Acids

MANFRED KRÖGER[1,2] AND FRIEDRICH CRAMER

Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, Hermann-Rein-Strasse 3, D-3400 Göttingen, West Germany

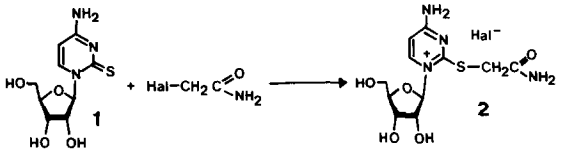
Received April 1, 1977

S-Alkylated 2-thiocytidine can be substituted at C-2 by nucleophilic agents. This reaction has been investigated with model compounds as well as with tRNA using the amino acids cysteine and lysine in order to develop a new affinity label linking covalently tRNA and a protein. Reaction with *N*-protected cysteine gives 2-*S*-alkyl-pyrimidines, while unprotected cysteine yields an *N*-alkyl-pyrimidine, after intramolecular substitution. With the ε-amino group of lysine a fast replacement at C-2 is observed, leading to an unstable 2-*N*-alkyl-pyrimidine. All products have been characterized both chemically and spectroscopically.

INTRODUCTION

For the investigation of protein–nucleic acid interactions by chemical methods, affinity labeling of biological macromolecules has been developed during the last few years (*1*), usually achieved by the modification of enzymatic substrates through the introduction of a side chain bearing a functional group. However, this approach led to ambiguity in the interpretation of results, since although the modified substrate still binds to the active site, a lengthy side chain may enable the functional group to react outside the active site. Therefore, one turns to analogs of the biological substrates which deviate minimally from the natural substrate but are still reactive (*2–6*). Covalent bond formation should then occur only at the active site, and one hopes that this involves one of the amino acids necessary for the binding of the natural substrate.

Since it is possible to incorporate 2-thiocytidine (**1**) into the CpCpA end common to all transfer RNAs (*7*, *8*), we investigated the properties of *S*-alkylated 2-thiocytidine **2** (*9–11*) in order to see whether its electrophilic C-2 site can be attacked by various nucleophiles (*12*, *13*). We were especially interested in the nucleophilic amino acids cysteine and lysine with a view toward future affinity labeling experiments at the transfer RNA–protein level (*14*).



[1] Part of this author's Ph.D. thesis, Technical University, Braunschweig, 1976.

[2] New address: University of California, Department of Molecular Biology, Virus Laboratory, Wendell M. Stanley Hall, Berkeley, California 94720.

ISSN 0045–2068

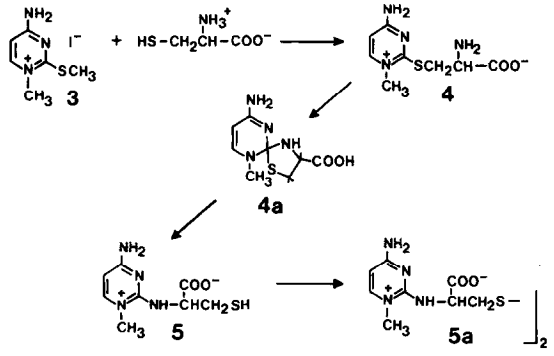
RESULTS AND DISCUSSION

Reactions with Cysteine

In order to avoid side reactions (*10*), cysteine was allowed to react with 4-amino-1-methyl-2-(methylthio)pyrimidinium-iodide (**3**) instead of the nucleoside **2**, under conditions similar to those used for the reaction with ethyl mercaptan (*12*). After 1 hr at 50°C and with a tenfold excess of cysteine, no residual starting material could be observed. We had expected as the product from the reaction the zwitterionic *S*-(4-amino-1-methylpyrimidinium-2-yl)cysteine **4**, which should have an uv spectrum analogous to **2** and **3** and give a positive test with ninhydrin.

When the reaction was followed by thin-layer chromatography (silica gel; ethanol/water, 1 : 1), the formation of a product showing a positive ninhydrin test could be observed. However, before complete reaction of the starting material had occurred, a second product, which did not give a positive ninhydrin test, began to appear. After several days only the second product could be observed.

Although both products could be separated by chromatography techniques, the ninhydrin-positive product could not be isolated in a pure form, since it was converted to the second product. This stable rearrangement product could be obtained in a good yield. Its uv spectrum differed clearly from that of the starting material **3**, but was very similar to the spectrum of 4-amino-1-methyl-2-(methylamino)pyrimidine (*12*).



The elemental analysis was in agreement with that expected of *S*-pyrimidinylcysteine **4**. It therefore seems probable that **4** is initially formed under kinetic control and this then rearranges to give the thermodynamically more stable aminopyrimidine **5**, which is isolated as its disulfide **5a**. Such a rearrangement of the cysteine residue is well known (*15*). The following data support the structure **5**/**5a** assigned to the product:

1. After reduction with Zn/HCl the compound shows a positive SH test with sodium nitroprusside, i.e., after reduction of a disulfide.

2. The product was stable to sodium hydroxide treatment at room temperature, while heating for 1 hr at 60°C in 0.1 *M* sodium hydroxide led to 1-methylcytosine (**13**) (*12*, *16*).

3. The spectroscopically determined pK_a value 14.1 is very similar to that of comparable 2-*N*-alkylpyrimidines, 14.0 (*12*, *16*).

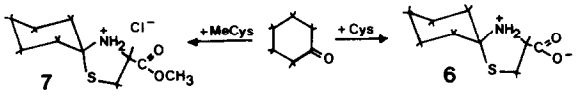
4. The ^{1}H-nmr spectrum in D_2O showed the expected coupling pattern for the aliphatic protons of substituted cystines. The observed data for higher-order spectra (*ABX*-type) were in good agreement with all calculated values.

5. The uv spectrum showed a pH dependence indicating a cationic and a neutral form.

These data were, however, not sufficient to permit a decision as to whether this rearrangement product was the result of a complete (that is, product **5**/**5a**) or of an incomplete Smiles rearrangement, which would have led to the structure **4a**. Such spiro-structures are known, since cysteine is a protecting group for the carbonyl function, forming a thiazolidine (*17–20*). In order to distinguish between the two possible structures **4a** and **5**/**5a** the ^{1}H-nmr and CD spectra of the product were compared to those of model compounds.

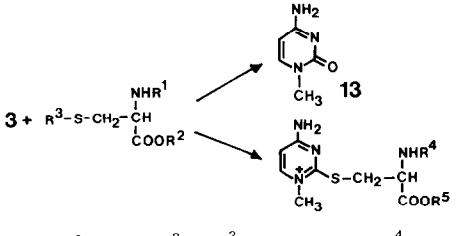
Synthesis of Model Compounds for Structural Proof

As models for the six-ring spiro-structure, 1-thia-4-azaspiro[4.5]decan-3-carboxylic acid (**6**) and its methyl ester (**7**) were prepared from cyclohexanone and cysteine or cysteine methyl ester hydrochloride (*17*).



7 6

The pyrimidinium cation **3** was also allowed to react with various amino- and mercapto-protected cysteine derivatives which cannot form the spiro-structure **4a**. Using *N*-acetyl-cysteine (**8**) under conditions similar to those for the reaction with cysteine, *S*-alkylpyrimidine *S*-(4-amino-1-methylpyrimidinium-2-yl)-*N*-acetyl-cysteine (**9**) is formed and shows the expected uv spectrum. The same result was obtained with the model peptide glutathione (**10**), yielding the *S*-(glutathionyl)pyrimidine **11**. The reaction with the cysteine methyl ester hydrochloride, however, only led to a product identical to that found with cysteine since the alkaline medium of the reaction hydrolyzed the amino acid ester. The 1-day reaction of **3** with *S*-methylcysteine (**12**) at pH 9.0 and at 60°C led to the hydrolysis product 1-methylcytosine (**13**), although during the reaction the formation of another product could be observed by thin-layer chromatography (*12*).



	R^1	R^2	R^3			R^4	R^5
8	acetyl -	H	H	→	**9**	acetyl -	H
10	glutaminyl-	glycyl -	H	→	**11**	glutaminyl-	glycyl -
12	H	H	CH_3	→	**13**	-	-

Spectroscopic Properties of the Products

The spiropyrimidine **4a** contains two chiral carbon atoms, which should produce a marked CD signal. Goodmann *et al.* (*21*) reported for the *N*-acetyl(*S*)-thiazolidine-4-carboxylic acid methyl ester at neutral pH a CD spectrum with a main maximum at

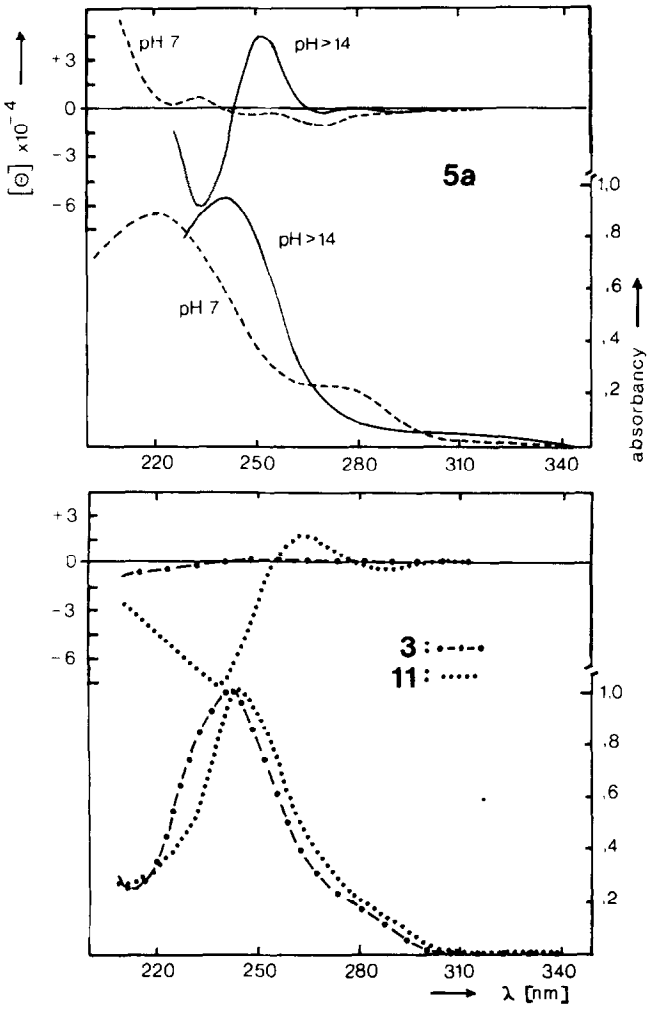


FIG. 1. uv and CD spectra of 1-methylpyrimidines **5a**, **3**, and **11**. The CD spectrometer was not capable of measuring below 210 nm.

198 nm and a small maximum at 230 nm (10:1), similar to those of **5a** (shown in Fig. 1). However, the latter shows a significant Cotton effect at high pH which would not be expected for the spiro structure **4a**, since it should only exist in the same form at both neutral and high pH. The starting pyrimidine **3** did not show any signal at neutral pH as expected, but the cation **11** showed a significant signal caused by the three optically active amino acids (*22*). Neither of the *S*-alkyl pyrimidines **3** and **11** was stable at high pH as mentioned above.

For product **4a/5a** an *ABX*-type ^{1}H-nmr spectrum was recorded [see Fig. 2 and Table 1 (*23*, *24*)]. All other *S*-alkylcysteines also showed nmr spectra of higher order (see Table 1). The coupling constants of the three alkyl protons are similar to those of cysteine in alkaline medium, of the pyrimidinyl-cysteines **9** and **11**, and of the product

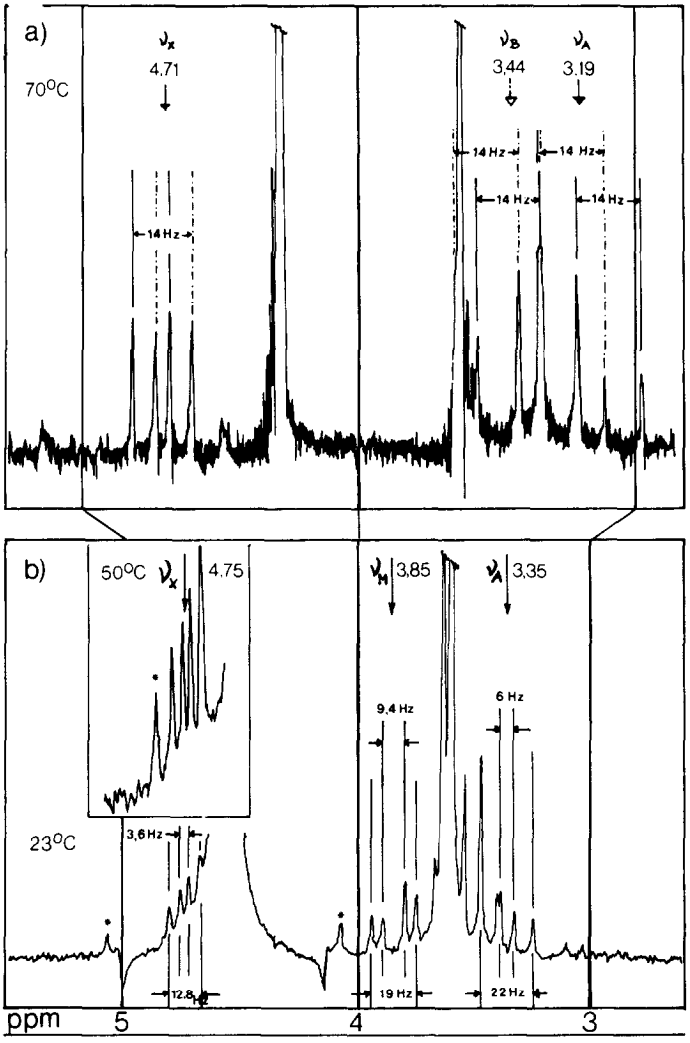


FIG. 2. ^{1}H-nmr spectra of cysteine and glutathione substitution products. (a) After rearrangement to the 2-*N*-alkylpyrimidine **5a** an *ABX*-type spectrum is obtained. (b) The glutathione product **11** gives an *S*-alkylpyrimidine *AMX*-type spectrum. For details see text and Table 1.

4a/5a, while the spirocarboxylic acid **6** and the ester **7** differ from these. By means of the Karplus equation (*23–27*) a ring structure such as the thiazolidine leads to coupling constants $J_{MX,BX}$ of ~7 Hz, while the *S*-methyl-cysteine shows $J = 2.06$ Hz; so that the observed $J_{MX,BX} = 4.43$ for the product **4a/5a** only can be attributed to an open cysteine structure with a relatively small rotation. Coupling constants of ~4.5 Hz are also shown by other derivatives of cysteine (see Table 1).

TABLE 1

^{1}H-nmr PARAMETERS OF CYSTEINE DERIVATIVES[a]

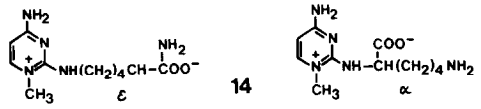
		Chemical shift (ppm)			Coupling constants (Hz)			X-part						
								$2(D_+ + D_-)$		$2(D_+ - D_-)$		$I_{10} : I_{11}$		
	Type[b]	*A*	*B,M*	*C,X*	$J_{AB,AM}$	J_{AX}	$J_{BX,MX}$	Calcd	Found	Calcd	Found	Calcd	Found	Ref.
Cysteine + —	AB_2	3.07	3.99			5								
—	*ABC*	3.77	3.84	4.71	−13.24	8.28	4.22							25
4-Thiazolidine carboxylic acid	*ABX*	3.66	4.11	4.57	−10.2	8.1	6.8							26
Spirocarboxylic acid **6**	*ABX*	3.38	3.52	4.57	12	7.86	7.14	29	—	0.4	0	0.99	1.02	
Spirocarboxylic acid methyl ester **7**	*ABX*	3.48	3.60	5.02	12	9.15	7.11	28	—	1	1	0.99	0.9	
Cysteine methyl ester	AB_2	3.18	4.47			5								
S-Methyl cysteine **12**	*ABX*	3.01	3.08	3.94	12.5	9.44	2.06	27.2	—	2.1	2	0.97	0.94	
					15	9.7	2.3							26
Product **4a/5a**	*ABX*	3.20	3.45	4.57	14	8.57	4.43	41	—	3	3	0.99	1	
Glutathione **10**	AB_2	2.95	4.57			6								
Glutathione oxide	*ABX*	2.98	3.31	4.76	14	9.66	4.56	48.5	—	3.4	3.5	0.99	1	
Glutathione pyrimidine **11**	*AMX*	3.35	3.84	4.75	14.1	8.1	4.7							
N-Acetyl-cysteine pyrimidine **9**	*AMX*	3.48	3.99	4.63	14	8	4.5							

[a] Interpretation according to Refs. (*23*, *24*).
[b] Type or computations schema.

The chemical and spectroscopical data are therefore consistent with structure **5a**, and not **4a**.

Reactions with Lysine

After incubation of **3** for 1 hr at room temperature with a 10-fold excess of lysine, no residual starting material could be observed. In the thin layer electrophoresis at pH 6.5 a new uv-active product with a positive ninhydrin reaction could be observed. The product could not be fully separated from lysine, although only the expected product **14** should be soluble in cold dry alcohol. Attempted purification by silica gel chromatography with ethanol/water, as used for the product **5a**, also failed. Repeated purification only afforded the hydrolysis product 1-methylcytosine (**13**), as was found for the reaction with *S*-methylcysteine (**12**).



The ^{1}H-nmr spectrum of the lysine-contaminated *N*-(4-amino-1-methylpyrimidinium-2-yl)lysine (**14**) showed that the chemical shift of the N–CH_3, H-5 and H-6 protons were rather similar to those of 2-*N*-alkylpyrimidines. The data for product **5a** differ clearly, so that one has to assume that the ε-*N*-alkyl product was formed in a fast, mild reaction.

Reaction at S-Alkylated 2-Thiocytidine in tRNA^{Phe} from Yeast

$tRNA^{Phe}$-C_{74}-s^2C_{75}-A_{76} was alkylated by [^{14}C]iodoacetamide (*9*, *13*) and then incubated with a 10^3-fold excess of unlabeled cysteine or lysine in order to determine the decay of radioactivity in the polynucleotide chain.[3] The reaction of both cysteine and lysine can be seen clearly (Fig. 3). However, cysteine reacts faster than lysine under identical conditions.

EXPERIMENTAL

Melting points were determined on the Monoskop (Reichert, Austria) and are not corrected. ^{1}H-nmr spectra were taken with a Bruker HX-60 spectrometer and Varian HA 100 spectrometer and are reported in δ values (ppm) relative to tetramethylsilane as internal standard. The uv spectra were measured with the Zeiss PMQ II and III or with the Shimadzu uv 200 spectrometer, respectively, and the CD spectra with the Cary 61 spectrometer. The mass spectrum was recorded on a Varian-MAT CH-4 instrument by the Gesellschaft für Molekularbiologische Forschung (Stöckheim), microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen).

Chromatography

Column chromatography was performed on silica gel 60 (70-230 mesh, Merck, Darmstadt) using LKB fraction collector with Uvicord (LKB, Sweden). Silica gel for

[3] The incorporation of radioactive amino acids could not be followed, since excessive amounts of [^{14}C]amino acid would have been necessary.

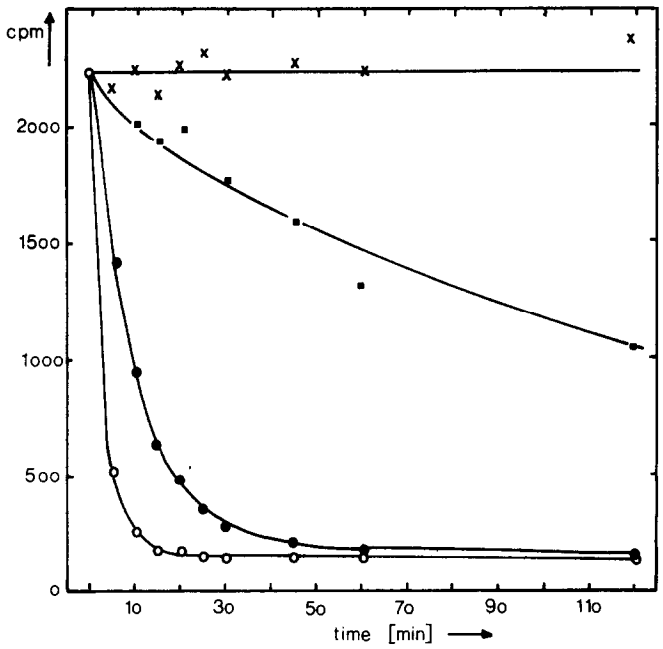


FIG. 3. Decay of ^{14}C-radioactivity during the reaction of $tRNA^{Phe}$-C_{74}-([^{14}C]IAA)s^2C_{75}-A_{76} with lysine at pH 9.0 (■), cysteine at pH 7.0 (●), and pH 9.0 (○), control (×) at 37°C (for details see text).

chromatography with water-containing solvents was suspended four times in 1 liter of water each and then slowly (100 ml/hr) washed with 20 liters of the appropriate solvent to remove water-soluble silica gel. Thin-layer chromatography was performed on silica gel plates (60 F_{254}, Merck, Darmstadt) and on microcards SI F 37340 (Riedel de Haën, Seelze) and compounds detected by uv_{254} absorption or the ninhydrin reaction.

Electrophoresis

Analytic thin-layer electrophoresis was performed on thin-layer plates (ICN-Woelm, Eschwege) in a Desaga TLE-double chamber (Desaga, Heidelberg). The distances are given as R_i value as distance relative to substance *i*. Sodium citrate (0.1 *M*, pH 6.5) was used as buffer system.

Materials

Triethylammonium hydrogencarbonate (1 *M*, TBK buffer, pH 9.0) was made by saturation of a 1 *M* solution of triethylamine with CO_2 at 0°C. 4-Amino-1-methyl-2-(methylthio)pyrimidinium iodide (**3**) was synthesized according to Brown and Jacobsen (*16*). All other chemicals were purchased from Merck (Darmstadt), EGA-Chemie (Steinheim), and Sigma (St. Louis, Mo.). Phenylalanine transfer ribonucleic acid from baker's yeast ($tRNA^{Phe}$) with 2-thiocytidine (**1**, s^2C) instead of cytidine (C) at position 75 was synthesized according to published procedures (*8*, *9*). [1-^{14}C]Iodoacetamide (sp act 56 Ci/mol) was purchased from Amersham/Braunschweig. The filter assay was performed on Whatman 3MM paper discs from Balston (England), and the ^{14}C radioactivity was determined in a Tri-Carb liquid scintillation spectrometer (3385, Packard-Instruments, Frankfurt).

N,N'-bis[4-Amino-1-Methylpyrimidinium-2-yl]-L-cystine (**5a**)

Three hundred milligrams (1.06 mmol) of **3** was stirred at 50°C for 1 hr with 642 mg (5.3 mmol) of L-cysteine in 20 ml of 1 *M* TBK. A single uv product which gave a positive ninhydrin test, could be observed by tlc. The solution was evaporated to dryness; the residue was dissolved in 10 ml of ethanol/water (1:1) and chromatographed on a pretreated silica gel column (42 × 3 cm) with degassed ethanol/water (1:1). Two uv absorbing fractions eluted from the column (fraction 1 ninhydrin positive, fraction 2 negative). These fractions were combined, evaporated to dryness, and the residue was dissolved in 25 ml of ethanol. After several days 222.3 mg (91.8%) of colorless bushy needles had crystallized; mp 229°C (decomp.). These crystals showed no positive ninhydrin test: tlc (ethanol/water, 1:1) $R_f = 0.29$; tlc (isopropylalcohol/NH_3/water, 7:2:1) $R_f = 0.06$; tle (pH 6.5) $R_{cysteine} = 1.12$ (+); uv λ_{max} (water) 221, 272 nm (log ε, 4.55, 3.93); uv λ_{max} (10 *M* NaOH) 237 nm (log ε, 4.45); $pK_a = 14.1$ (determined by uv spectra); MS no molecule peak; CD λ_{max} (1 *M* NaOH) 233,252 nm ($[\theta] \times 10^{-4}$: −5.6, +4.4) see Fig. 1; ^{1}H-nmr (D_2O) 3.19, 3.44, and 4.70 [*ABX*-type, 3*H* (relative to the half-molecule); see Fig. 2 and Table 1], 3.62 (*s*, 3*H*, $-CH_3$), 6.13 (*d*, $J = 7.3$ Hz, 1*H*, H-5), 7.61 ppm (*d*, $J = 7.3$ Hz, 1*H*, H-6); ^{1}H-nmr (5 *M* NaOD) 3.10, 3.38, and 4.43 (*ABX*-type, 3*H*), 3.26 (*s*, 3*H*, $-CH_3$), 5.54 (*d*, $J = 7.5$ Hz, 1*H*, H-5), 7.27 ppm (*d*, $J = 7.5$ Hz, 1*H*, H-6).

Anal. Calcd for $C_{16}H_{22}N_8O_4S_2$ (454.53): C, 42.28; H, 4.88; N, 24.65; S, 14.11. Found: C, 42.25; H, 4.97; N, 24.51; S, 14.06.

1-Thia-4-azaspiro[4,5]decan-3-Carboxylic Acid (**6**)

A suspension of 1.64 ml (1.55 mg, 15.7 mmol) of cyclohexanone, 1.21 g (10 mmol) of cysteine, and 5 ml of absolute ethanol was heated on a water bath for 10 min. A further 10 ml of absolute ethanol was added to the suspension, and heating was continued until a clear solution was obtained. On cooling, 1.43 g (71.3%) of small colorless plates appeared; mp 185°C (decomp. from 135°C); ^{1}H-nmr (D_2O) around 1.65 (*m*, 10*H*), 3.38, 3.52, and 4.57 ppm (*ABX*-type, 3*H*, see Table 1).

Anal. Calcd for $C_9H_{15}NO_2S$ (201.28): C, 53.65; H, 7.48; N, 7.02. Found: C, 53.72; H, 7.51; N, 6.90.

1-Thia-4-Azaspiro[4,5]decan-3-Carboxylic Acid Methylester Hydrochloride (**7**)

A mixture of 1.04 ml (981.5 mg; 10 mmol) of cyclohexanone and 1.72 g (10 mmol) of cysteine methylester hydrochloride was heated on a water bath for 5 min. The solid reaction mixture was recrystallized from 10 ml of absolute ethanol giving 1.36 g (53.9%) of small colorless plates; mp 163–167°C; sublimation (in part) >60°C/1 Torr; ^{1}H-nmr (D_2O) around 1.60 (*m*, 10*H*), 3.88 (*s*, 3*H*, $-CH_3$), 3.48, 3.60, and 5.02 ppm (*ABX*-type, 3*H*; see Table 1).

Anal. Calcd for $C_{10}H_{18}ClNO_2S$ (251.77): C, 47.71; H, 7.21; N, 5.57. Found: C, 47.65; H, 7.23; N, 5.70.

S-(4-Amino-1-Methylpyrimidinium-2-yl)-N-Acetylcysteine (**9**)

A solution of 201.2 mg (0.71 mmol) of **3** in 15 ml of TBK-buffer was stirred for 3 hr at 50°C together with 581.5 mg (3.56 mmol) of *N*-acetylcysteine (**8**). The resulting solution was evaporated and the residue was dissolved in 10 ml of ethanol/water (1:1)

and chromatographed on a pretreated silica gel column (42 × 3 cm) with degassed ethanol/water (1 : 1). The uv-active fraction was evaporated and rechromatographed under identical conditions. The uv absorbing fraction was evaporated and the residue was dissolved in methanol. An amorphous colorless material (44.6 mg, 26.2%) was precipitated with ether: tlc (ethanol/water, 1 : 1) $R_f = 0.35$; tle (pH 6.5) $R_{cysteine} = 1.18$ (+); uv λ_{max} (H_2O) 242 nm (log ε, 4.34); CD λ_{max} (H_2O) 290, 265, 241 nm ($[\theta] \times 10^{-4}$: −2.96, +8.55, −38.8); ^{1}H-nmr (D_2O) 2.02 (*s*, 3*H*, see Table 1), 6.57 (*d*, $J = 7.5$ Hz, 1*H*, H-5), 7.92 ppm (*d*, $J = 7.5$ Hz, 1*H*, H-6).

Anal. Calcd for $C_{10}H_{14}N_4O_3S$ (270.31): C, 44.43; H, 5.22; N, 20.73; S, 11.86. Found: C, 44.49; H, 5.33; N, 20.52; s, 11.88.

S-(4-Amino-1-Methylpyrimidinium-2-yl)-Glutathione (**11**)

A solution of 300 mg (1.06 mmol) of **3** in 30 ml of TBK-buffer was stirred for 4 hr at 40°C together with 1.63 g (5.3 mmol) of reduced glutathione (**10**). The solution was evaporated and the residue was dissolved in 20 ml of ethanol/water (1 : 1) and chromatographed on a pretreated silica gel column (42 × 3 cm) with degassed ethanol/water (1 : 1). The uv absorbing fraction was evaporated. The colorless transparent residue could be transformed to 273.8 mg (62.3%) of an amorphous powder by addition of ethanol. The product showed a positive ninhydrin test: tlc (ethanol/water, 1 : 1) $R_f = 0.38$; uv λ_{max} (H_2O) 242 nm (log ε, 4.17); CD λ_{max} (H_2O) 239, 269, 288 nm ($[\theta] \times 10^{-4}$: −7.7, +1.2, −0.4); ^{1}H-nmr (D_2O) 2.30 (*m*, 4*H*, $-CH_2-$), 3.75 (*s*, 3*H*, $-NCH_3$), 3.76 (*t*, $J = 6$ Hz, 1*H*, −CH), 3.78 (*s*, 2*H*, $-CH_2-$), 3.35, 3.84, and 4.75 (*AMX*-type, 3*H*; see Table 1), 6.58 (*d*, $J = 7.5$ Hz, 1*H*, H-5), 7.92 ppm (*d*, $J = 7.5$ Hz, 1*H*, H-6).

Anal. Calcd for $C_{15}H_{22}N_6O_6S$ (414.45): C, 43.47; H, 5.35; N, 20.28; S, 7.74. Found: C, 43.42; H, 5.47; N, 20.15; S, 7.60.

Reaction of **3** *with Lysine to* N^{ε}*-(4-Amino-1-Methylpyrimidinium-2-yl)*-L-*Lysine* (**14**)

The chloride form of **3** (300 mg, 1.57 mmol) was dissolved in 30 ml of water containing 2.00 g (10.95 mmol) of lysine hydrochloride adjusted to pH 9.0. The clear solution was stirred at room temperature for 2 hr. No more starting material could be observed by tlc after that time. After evaporation of the solvent, the residue was stirred several times with absolute ethanol at 35°C; the resulting solution was filtered after cooling and treated with ether. In this way 180 mg of a fine amorphous hydroscopic powder melting higher than 181°C and showing a positive ninhydrin test could be obtained: tlc ($CHCl_3/CH_3OH$, 1 : 1) $R_f = 0.58$; tle (pH 6.5) $R_{13} = 2.22$ (−); uv λ_{max} (H_2O) 218, 274 nm (ε-ratio 1 : 0.27); ^{1}H-nmr (D_2O) around 1.60 (*m*, $-CH_2-$), around 3.00 (*m*, $-CH_2NH$, −CH−), 3.52 (*s*, 3*H*, $-CH_3$) 6.13 (*d*, $J = 7.5$ Hz, 1*H*, H-5), 7.58 ppm (*d*, $J = 7.5$ Hz, 1*H*, H-6).

Reaction of $tRNA^{Phe}$*-*C_{74}*-([*^{14}*C]IAA)*s^2C_{75}*-*A_{76} *with Cysteine and Lysine*

Four different reaction mixtures 100 μl in volume containing 1.99 nmol of $tRNA^{Phe}$-C_{74}-([^{14}C]IAA)s^2C_{75}-A_{76} and 20 μl of standard buffer solution (*28*) with 50 m*M* $MgSO_4$ and 25 m*M* amino acid were prepared: (1) cysteine, potassium phosphate, pH 7.0; (2) cysteine, Theorell–Stenhagen buffer, pH 9.0; (3) lysine, Theorell–Stenhagen buffer, pH 9.0; (4) control: no amino acid, Theorell–Stenhagen buffer pH 9.0. After given times at 37°C, 10-μl probes were pipetted into a trichloroacetic acid assay to determine the amount of residual radioactivity (*8*, *9*). See Fig. 3 for the results.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of B. Seeger and Mrs. G. Daenecke, as well as various discussions, including help in interpreting the nmr data, with Drs. F. Seela, M. Günther, and R. Perraud.

REFERENCES

1. D. S. SIGMAN AND G. MOOSER, *Annu. Rev. Biochem.* **44**, 889 (1975); CH. R. CANTOR, M. PELLEGRINI, AND H. OEN, *in* "Ribosomes" (M. Nomura, Y. Tissières, and Z. Lengyel, Eds.), pp. 573–585, Cold Spring Harbor Monograph Series, 1974.
2. H. SOMMER AND D. V. SANTI, *Biochem. Biophys. Res. Commun.* **57**, 689 (1974).
3. A. L. POGOLOTTI, K. M. IVANETICH, H. SOMMER, AND D. V. SANTI, *Biochem. Biophys. Res. Commun.* **70**, 972 (1976).
4. I. FISER, K. H. SCHEIT, G. STÖFFLER, AND E. KUECHLER, *Biochem. Biophys. Res. Commun.* **60**, 1112 (1974).
5. F. SEELA AND F. CRAMER, *Bioorg. Chem.* **5**, 25 (1976).
6. V. W. ARMSTRONG, H. STERNBACH, AND F. ECKSTEIN, *Biochemistry* **15**, 2086 (1976).
7. M. SPRINZL, K. H. SCHEIT, AND F. CRAMER, *Eur. J. Biochem.* **34**, 306 (1973).
8. M. KRÖGER AND F. CRAMER, *Eur. J. Biochem.*, submitted for publication.
9. M. SPRINZL, E. KRÄMER, AND D. STEHLIK, *Eur. J. Biochem.* **49**, 595 (1974).
10. M. KRÖGER AND F. CRAMER, *Chem. Ber.* **110**, 361 (1977).
11. J. K. DATTAGUPTA, M. KRÖGER, AND W. SAENGER, *Chem. Ber.* **110**, 353 (1977).
12. M. KRÖGER AND F. CRAMER, *Justus Liebigs Ann. Chem.*, to appear.
13. M. KRÖGER, M. SPRINZL, AND F. CRAMER, *Justus Liebigs Ann. Chem.* **1976**, 1395 (1976).
14. M. KRÖGER, H. STERNBACH, M. SPRINZL, AND F. CRAMER, *Eur. J. Biochem.*, submitted for publication.
15. H. P. BURCHFIELD, *Nature (London)* **181**, 49 (1958); A. HOLÝ, I. VOTRUBA, AND K. JOST, *Coll. Czech. Chem. Commun.* **39**, 634 (1974).
16. D. J. BROWN AND N.W. JACOBSEN, *J. Chem. Soc.* **1962**, 3172 (1962).
17. S. LIEBERMAN, P. BRAZEAU, AND L. B. HARITON, *J. Amer. Chem. Soc.* **70**, 3094 (1948).
18. U. SCHULZ AND D. R. MCCALLA, *Canad. J. Chem.* **47**, 2021 (1969).
19. R. BOGNAR, L. SOMORYI, AND Z. GYÖRGYDIÁK, *Justus Liebigs Ann. Chem.* **738**, 68 (1970).
20. J. J. PESEK AND J. H. FROST, *Tetrahedron* **31**, 907 (1975).
21. M. GOODMAN, K. SU, AND C.-C. NIN, *J. Amer. Chem. Soc.* **92**, 5220 (1970).
22. M. OTTNAD, C. OTTNAD, P. HARTTER, AND G. JUNG, *Tetrahedron* **31**, 1155 (1975).
23. H. GÜNTHER, "NMR-Spektroskopie", pp. 172–183, Georg-Thieme-Verlag, Stuttgart, 1973.
24. H. SUHR, *in* "Organische Chemie in Einzeldarstellungen" (Bredereck and Müller, Eds.), Vol. 8, pp. 54–69, Springer Verlag, Berlin, 1965.
25. J. A. GLASEL, *J. Amer. Chem. Soc.* **87**, 5472 (1965).
26. R. B. MARTIN AND R. MATHUR, *J. Amer. Chem. Soc.* **87**, 1065 (1965).
27. J. L. LARICE AND J. ROGGERO, *Bull. Soc. Chem. France* 1971, 2053 (1971).
28. H. A. SOBER (Ed.), "CRC Handbook of Biochemistry, Selected Data for Molecular Biology," pp. 195–198, The Chemical Rubber Company, Cleveland, OH, 1968.