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STUDIES ON AMINO ACIDS AND PEPTIDES—IV†

1,2,4-TRIAZINES FROM THIOACYLATED AMINO-ACID ESTERS

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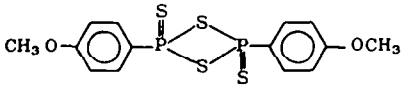
Abstract—Ethyl esters **2** of N-acyl derivatives of glycine, S-alanine, S-phenylalanine, and S-proline, were thionated by use of 2,4 - bis(4 - methoxyphenyl) - 1,3,2,4 - dithiadiphosphetane 2,4-disulfide (Lawesson's Reagent) **1**. The N-thioacyl compounds **3** were reacted with hydrazine hydrate and in most cases a ring-closure reaction took place, giving 1,2,4-triazine derivatives **4**. The structural proofs of **4** were obtained by ^1H, ^{13}C, and ^{15}N NMR spectroscopy.

In connection with our general studies of peptide analog (especially thiopeptides,[1-3] thiotripeptides,[4] and thiopentapeptides[5]) we are also investigating the chemistry of the thiopeptide functional group or more generally thionated amino-acid derivatives. It is thus known that N-acylated amino-acid esters, when reacted with hydrazine hydrate, produce the hydrazides and no ring-closure reaction is observed. However, we assumed that the N-thioacylated amino-acid derivatives would be more reactive and thus more suitable for an internal nucleophilic attack from the hydrazide part of the molecule, making a ring-closure reaction possible and leading to 1,2,4-triazine derivatives. The experiments confirmed this assumption. When the present investigations were about to be finished, a short note appeared[6] about the same problem. This prompted us to publish our results at the present stage of the investigation.

Starting materials

The N-acyl amino acid ethyl esters **2** (Scheme 1) were prepared by standard procedures and the amide function was thionated as described earlier[7] using **1** as a thionation reagent thus obtaining the corresponding N-thioacyl amino-acid ethyl esters **3** (Scheme 1) (see also Experimental). The experimental and physical data for **2** and **3** are presented in Tables 1 and 2.



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The ^1H NMR data for compounds **2** and **3** are presented in Table 3. It is observed that going from >C=O to >C=S causes a downfield shift for the hydrogens attached to nitrogen and to carbon number 2. The latter hydrogens of the glycine derivatives are shifted downfield *ca* 0.4 ppm and the hydrogen of the phenylalanine derivatives is shifted downfield *ca.* 0.5 ppm. The same change of the corresponding alanine and proline derivatives cannot easily be determinated due to overlap with the resonance of $-O\underline{CH_2}CH_3$. The hydrogen attached to N is observed to shift downfield more than 1 ppm.

The methylene hydrogens at C^2 of the glycine derivatives show doublet resonances with a coupling constant of approx. 5 Hz except for **3c** and **3d** which both have a coupling constant of approx. 4 Hz in accordance with earlier results.[8]

The hydrogen of the formyl group resonates as a singlet at 8.29 ppm (**2a**) and at 8.12 ppm (**2f**). The hydrogen of the thioformyl group shows two singlets at 9.40 ppm and 9.50 ppm (**3a**) and at 9.45 ppm and 9.55 (**3f**), due to different conformations.

Changing the acyl to a thioacyl group not only effects the shift value of C^α which is the position where the main change takes place, but it also effects the shift value of C^2 and—to a smaller degree—the shift value of C^β (C^β is the carbonyl carbon of the ester group). C^α is shifted about 30 ppm downfield and C^2 about 5 ppm downfield. Contrary to the shift downfield of C^α and C^2, C^β undergoes an upfield shift of about 1 ppm.

The assignment of the carbonyl signals of compounds **2g, 2h**, and **2l** was made by using a relation between the ^{13}C chemical shifts of the thiocarbonyl and corresponding carbonyl carbons of altogether 11 compounds: $\delta_{C=S} = 1.46\,\delta_{C=O} - 46.8$. This equation was calculated by a least square linear regression analysis and is quite accurate, the correlation coefficient being 0.992. It should also be added that the carbons of the formyl and thioformyl groups give rise to two resonances in the ^{13}C NMR spectra due to different conformations present in the solution (see Table 4).

In all mass spectra of **2** and **3** $[M]^+$ is seen, except in the spectra of **2a**, **2f**, **2i**, and **3j** where $[M+1]^+$ is observed. $[M]^+$ is base peak of **3a–d**, **3i**, and **3m**. $[M+1]^+$ is base peak of **3j**. When a benzoyl or thiobenzoyl group is present $[C_6H_5-\overset{\overset{X}{\Vert}}{C}]^+$ (X = O, S) is the base peak. In all mass spectra of the ethyl esters loss of $CH_3CH_2O^\cdot$ from $[M]^+$ is seen followed by loss of CO. $[M-SH^\cdot]^+$ is always observed in the mass spectra of the thio compounds.[21]

In IR **2** shows absorptions in the region 3300–3490 cm^{-1} (N–H stretching) and carbonyl absorptions in the region 1730–1755 cm^{-1} (ester), and 1620–1675 cm^{-1} (amide I). There are absorptions in the region 1185–1210 cm^{-1} due to C–O stretching. **3**

†Part III, see Ref. 3.
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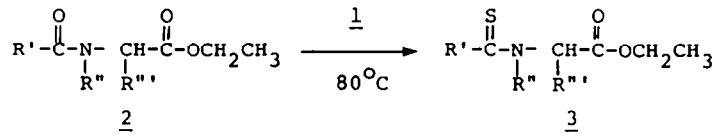


	R'	R"	R"'
a	H	H	H
b	CH_3	H	H
c	$-CH(CH_3)_2$	H	H
d	$-C(CH_3)_3$	H	H
e	Ph	H	H
f	H	H	CH_3
g	CH_3	H	CH_3
h	Ph	H	CH_3
i	$-CH(CH_3)_2$	H	$-CH_2-Ph$
j	$-C(CH_3)_3$	H	$-CH_2-Ph$
k	Ph	H	$-CH_2-Ph$
l	CH_3	$-(CH_2)_3-$	
m	$-CH(CH_3)_2$	$-(CH_2)_3-$	
n	Ph	$-(CH_2)_3-$	

Ph = Phenyl.

Scheme 1.

shows absorptions in the region 3250–3470 cm^{-1} (N–H stretching), 1720–1750 cm^{-1} (ester), 1440–1520 cm^{-1} (thioamide II), and 1170–1350 cm^{-1} (thioamide I or ester).

RESULTS AND DISCUSSION

When **3** was reacted with hydrazine hydrate (80% or 99% solution) in refluxing ethanol, refluxing dioxane, or dioxane at 100°C in a 1:1 or 1:4 molar ratio the 1,2,4-triazine derivatives were formed in most cases. It was impossible to obtain the cyclized products from S-phenylalanine derivatives (**3i–k**), N - (2,2 -dimethyl-1-thioxopropyl) glycine ethyl ester (**3d**), and N-(2-methyl-1-thioxopropyl) S-propline ethyl ester (**3m**) and hydrazine hydrate, probably due to steric hindrance and/or electronic effects. From the reaction of **3d** with hydrazine hydrate a small amount of the corresponding hydrazide $(CH_3)_3C-C(S)NH-CH_2C(O)NHNH_2$ was identified by mass and ^1H NMR spectra.

It should also be noted that the use of hydrazine hydrate is crucial. **3c** and **3e** were both reacted with anhydrous hydrazine in anhydrous dioxane at 100° and no cyclized product from **3c** was isolated even after 9 hr reaction. The yield of **4e** from **3e** was only 57% after 5 hr. With hydrazine hydrate **4c** was isolated in 46% yield and **4e** in 79% yield after the reaction time of $1\frac{1}{2}$ and 1 hr, respectively (Table 5 and experimental section).

As to the mechanism for the formation of **4** there are two routes possible: either that the hydrazine first gives the hydrazide, which subsequently ringclosures under elimination of H_2S (Route 1), or that the hydrazine first attacks the thiocarbonyl function,[22] followed by an internal attack on the ester function to give **4** (Route 2). When R" = H, Route 1 gives the possibility of formation of another product **5**, an isomer of **4**.

The structure **5** was discarded for the following reasons: from the ^1H NMR and the ^{13}C NMR spectra we were not able to distinguish between the two possible isomers, **4** and **5**. The observed coupling constant for the methylene hydrogens of the glycine derivatives (a–e) showed a remarkable decrease from 4 to 5 Hz (**2a–e**, **3a–e**) to 0–1.5 Hz (**4a–c**; Table 6) which could be due to either a small three-bond coupling constant (**4**) or a large four-bond coupling constant (**5**). However, investigations of the compound **4e**/**5e** by natural abundance ^{15}N NMR spectroscopy combined with SINEPT[23] polarization transfer techniques and a new experimental method for correlating of ^1H and ^{15}N NMR spectra[24] proved the structure to be **4e** (only) in solution. Comparing the NMR data from the other compounds with those of **4e** we have concluded that the structure of **4e** is valid for the rest.

The 1,2,4-triazines from S-alanine and S-proline show optical activity and assuming no inversion at the chiral centres during the reaction the assignment S has been used for the optically active 1,2,4-triazines **4**.

Table 1. Experimental and physical data for N-acylamino-acid esters **2**

	M.p.[°C] Found	M.p.[°C] Reported	$[\alpha]_D^{22}$ Found	$[\alpha]_D^{22}$ Reported	Yield %
a	oil	oil[9]	-	-	53
b	44-46	48[10]	-	-	91
c	34-35	oil[11]	-	-	69
d	23.5-25.5	oil[12]	-	-	93
e	57-58.5	60.5[13a], 67.5[13b] 67[14], 59.5[15]	-	-	94
f	oil	-	-62.0 (c 1.82,EtOH)	-	37
g	oil	oil[16] 34-35[17,18]	-55.3 (c 1.26,EtOH)	-80(c 3,H_2O)[16] -57.3(7%,EtOH)[17]	81
h	95	97-98[18]	-7.78 (c 0.73,EtOH)	-	73
i	63	-	+2.56 (c 1.72,EtOH)	-	84
j	55	-	-2.20 (c 1.73,EtOH)	-	80
k	98-100	100-102[19]	-34.7 (c 2.00,EtOH)	-40.0(c 4.3,MeOH, 25°C)[19]	70
l	30-32	oil[10]	-89.9 (c 11.60,EtOH)	-80.43(c 11.60, EtOH,24°C)[10]	50
m	oil	-	-93.4 (c 1.00, MeOH)	-	75
n	oil	-	-91.0 (c 1.00,MeOH)	-	61

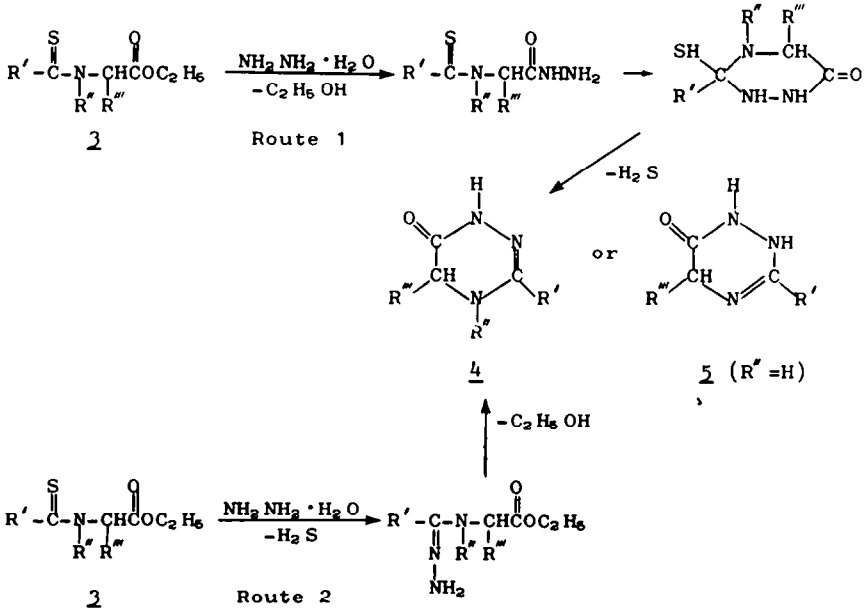


Table 2. Experimental and physical data for N-thioacylamino-acid esters 3

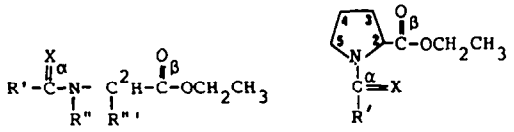
	M.p.[°C] Found	M.p.[°C] Reported	$[\alpha]_D^{22}$ Found	$[\alpha]_D^{22}$ Reported	Yield %
a	oil	-	-	-	89
b	37	-	-	-	89
c	38.5-39.5	-	-	-	74
d	22-23	-	-	-	56
e	49-50	51-52[20]	-	-	86
f	oil	-	-93.9 (c 0.82,EtOH)	-	98
g	oil	-	-99.2 (c 2.06,EtOH)	-	81
h	117-120	-	-29.5 (c 0.47,EtOH)	-	88
i	oil	-	+62.2 (c 2.25,EtOH)	-	86
j	oil	-	+73.1 (c 1.72,EtOH)	-	81
k	oil	-	+91.7 (c 3.03,EtOH)	-	93
l	69-71	-	-191 (c 1.00,MeOH)	-	75
m	oil	-	-119 (c 1.00,MeOH)	-	79
n	88-89.5	-	-141 (c 1.00,MeOH)	-	83

Table 3. ^{1}H NMR data for **2** and **3** ($CDCl_3$)

	2		3	
	H at N	H at C^2	H at N	H at C^2
a	7.06 (br)	4.06 (d, J=5 Hz)	8.74 (br)	4.43 (d, J=5 Hz)
b	6.03 (br)	4.00 (d, J=5 Hz)	8.00 (br)	4.38 (d, J=5 Hz)
c	6.42 (br)	3.96 (d, J=5 Hz)	7.78 (br)	4.34 (d, J=4 Hz)
d	6.46 (br)	4.01 (d, J=5 Hz)	7.98 (br)	4.36 (d, J=4 Hz)
e	arom.	4.18 (d, J=5 Hz)	8.10 (br)	4.44 (d, J=5 Hz)
f	7.43 (br)	4.40 (m) §§	8.83 (br)	5.20 (m)
g	7.70 (d, J=7 Hz)	4.30 (m) §§	8.50 (br)	5.09 (m)
h	6.80 (br)	4.74 (m)	8.13 (br)	5.23 (m)
i	5.93 (br)	4.83 (m)	7.83 (d, J=8 Hz)	5.38 (m)
j	6.20 (br)	4.83 (m)	7.77 (br)	5.40 (m)
k	6.60 (br)	5.03 (m)	arom.	5.47 (m)
l	-	4.25 (m) §§	-	4.92 (m)
m	-	4.28 (m) §§	-	5.05 (m)
n	-	4.32 (m) §§	-	5.08 (m)

§§ Resonate with $-CH_2-$ from the ester function.

Table 4. ^{13}C NMR data for **2** and **3** ($CDCl_3$)



X = O, 2
X = S, 3

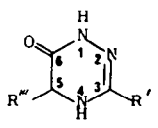
	C^{α}	C^{β}	C^{2}	C^{3}	C^{4}	C^{5}
2a	161.8/164.5[a]	169.0	39.3	-	-	-
2b	170.5	169.7	41.0	-	-	-
2c	177.4	169.6	40.7	-	-	-
2d	178.5	169.8	41.1	-	-	-
2e	167.6	169.7	41.5	-	-	-
2f	161.7/163.7[b]	171.9	46.6	-	-	-
2g	169.8	172.6	47.6	-	-	-
2h	166.7	172.7	48.2	-	-	-
2i	176.2	171.5	52.6	-	-	-
2j	177.3	171.2	52.5	-	-	-
2k	166.6	171.3	53.4	-	-	-
2l	168.7	171.8	58.2	28.9	24.3	47.3
2m	175.1	171.7	58.2	28.5	24.3	46.1
2n	169.2	171.9	59.1	29.1	25.1	49.7
3a	189.5/192.8[c]	168.1	44.4	-	-	-
3b	201.6	168.2	46.9	-	-	-
3c	212.2	168.4	46.5	-	-	-
3d	213.6	168.8	47.5	-	-	-
3e	199.0	168.6	47.6	-	-	-
3f	188.5/190.9[d]	171.5	50.8	-	-	-
3g	200.7	171.8	53.4	-	-	-
3h	198.3	172.1	53.8	-	-	-
3i	211.0	170.6	57.5	-	-	-
3j	212.4	170.7	57.9	-	-	-
3k	198.0	170.3	58.6	-	-	-
3l	197.6	169.8	64.8	29.1	24.5	51.2
3m	209.0	170.1	64.9	28.4	24.3	49.9
3n	199.0	170.0	64.6	29.4	24.8	53.8

[a] Intensity ratio: 9; [b] 1.5; [c] 11; [d] 8.

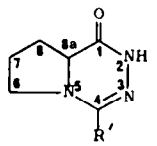
Table 5. Experimental and physical data for **4**

	M.p.[°C]	$[\alpha]_D^{22}$	Yield %
a	183	-	100
b	180	-	97
c	129-132	-	46
e	209-215	-	79
f	136	-3.07 (c 0.26,EtOH)	88
g	186-187	+12.4 (c 0.40,EtOH)	100
h	194	+4.18 (c 0.84,EtOH)	99
l	182-187	-157 (c 1.00,MeOH)	77
n	63-66	-37.9 (c 1.00,MeOH)	82

Table 6. ^{1}H NMR data for **4**. Compounds **4a–c**, **4e–h** in DMSO-d_6. Compounds **4l**, **n** in $CDCl_3$)



4a-c,e-h.



4l,n.

	N^1	C^3	N^4	C^5
a	9.97 (br)	6.73 (d, J=5 Hz)	6.73§	3.67 (s)
b	9.89 (br)	-	6.77 (br)	3.65 (d, J=1.5 Hz)
c	9.79 (br)	-	6.57 (br)	3.61 (d, J=1.3 Hz)
e	10.39 (br)	-	arom.	3.81 (d, J=1.5 Hz)
f	9.98 (br)	6.78 (d, J=5 Hz)	6.93 (br)	3.83 (m)
g	9.83 (br)	-	6.82 (br)	3.76 (m)
h	10.34 (br)	-	arom.	3.93 (m)

	N^2	C^6	C^7	C^8	C^{8a}
l	9.00 (br)	3.50 (m)§§		2.03(m)§§§	3.50(m)§§
n	8.87 (br)	3.45 (m)	2.37(m)/1.85(m)		4.08(m)

§ Hidden under the doublet. §§ in the same multiplet.

§§§ Resonate with the methyl group.

Table 7. ^{13}C and ^{15}N NMR data for **4**; solvents as for ^{1}H NMR

	C^3	C^5	C^6	N^1	N^2	N^4
a	137.5	42.1	160.5			
b	144.9	42.5	160.3			
c	152.5	42.8	160.8	-221.5	§	-311.2
e	145.1	42.9	161.2	-219.0	-155.9	-312.9
g	144.9	47.8	163.5			
h	145.1	48.2	164.3			

	C^1	C^4	C^6	C^7	C^8	C^{8a}
l	162.9	146.9	47.0	22.3	27.9	56.1
n	163.7	149.1	51.3	23.7	28.8	56.2

§ Not observed.

From the spectra of **4** (Table 6) is seen that the hydrogen attached to C^5 (proline derivatives C^{8a}) has shifted 0.6–1.4 ppm upfield compared with the data of **3** (hydrogen at C^2). The greatest effect is seen in the alanine derivatives. The same upfield shift is observed at the hydrogen attached to the nitrogen in the amino acid part (N^4 in **4**). This is due to the substitution of a >C=S with a >C=N< and the change of an ester into a hydrazide group both causing a better shielding of the nuclei.

The shift upfield is noted in ^{13}C NMR spectra, too (Table 7). Especially the change of >C=S into >C=N< causes an upfield shift of 50–60 ppm at the substituted carbons (C^{α} in **3**; C^3 respectively C^4 in **4**). The carbonyl carbons of the ester group (C^{β}) are shifted 7–8 ppm upfield compared with the carbons C^6; respectively C^1 of the triazine system.

In IR N–H stretching absorptions are observed in the region 3240–3480 cm^{-1} and carbonyl absorptions in the region 1610–1690 cm^{-1}. In all mass spectra of **4** $[M]^+$ is the base peak.

EXPERIMENTAL

^{1}H NMR spectra were recorded at 60 MHz on a Varian EM-360 spectrometer. ^{13}C NMR were recorded at 20 MHz on a Varian CFT-20 spectrometer. ^{15}N NMR spectra were recorded on a Varian XL-100–15 spectrometer at 10.14 MHz using a homemade[27] 18 mm single coil probe. The NMR solvents were $CDCl_3$ and DMSO-d_6, and the chemical shifts are reported in parts per million on the δ scale, referenced to internal TMS as 0 ppm (for ^{1}H spectra), to the $CDCl_3$ solvent as 77.0 ppm and to the DMSO-d_6 solvent as 39.5 ppm (for ^{13}C spectra) or to external CH_3NO_2 (for ^{15}N spectra). IR spectra were recorded on a Beckmann IR-18 spectrophotometer. Mass spectra were recorded on a Micromass 7070F spectrometer operating at 70 eV using direct inlet. Elemental analyses were carried out by Løvens Kemiske Fabrik, DK-2750 Ballerup (Microanalytical Laboratory). Optical rotations were measured in a 1-dm cell in a Perkin–Elmer 241 polarimeter. Silica gel 60 (Merck) was used for chromatography. M.ps are uncorrected.

Compound **1** (available as Lawesson's reagent from Fluka, Merck-Schuchardt, Aldrich, and Riedel de Haën) was prepared as described earlier.[7]

The ethyl ester hydrochlorides of S-alanine, S-phenylalanine, and S-proline were prepared by standard procedures.[25,26] The ethyl ester hydrochloride of glycine was purchased from Fluka.

Preparation of N-acyl amino acid ethyl esters **2**

2b–d, 2g–n were prepared by the following procedure: In a two-necked flask fitted with a reflux condenser 0.04 mole amino acid ethyl ester hydrochloride was dissolved in 50 ml CH_2Cl_2. 0.08 mole triethylamine was added slowly while stirring. To the stirred solution of the free amino-acid ethyl ester and the remaining triethylamine a solution of 0.04 mole acyl chloride in 15 ml CH_2Cl_2 was added dropwise. The reaction mixture was allowed to stand 1–2 hr after HCl was absorbed in the mixture. Both reactions were run at room temp. The mixture was then evaporated to dryness under reduced pressure and 100 ml anhydrous ether was added. After filtration the residue was washed three times with anhydrous ether. The ether phases were collected and the ether was evaporated under reduced pressure. When possible, the remaining N-acyl amino acid ethyl ester was recrystallized from ether/petrol (**2h** was washed with water only).

Due to contamination of S-proline ethyl ester hydrochloride it was necessary to use a different procedure for the purification of the proline derivatives.

After evaporation under reduced pressure of the reaction mixture the N-acyl proline ethyl ester was extracted with ethyl acetate. After filtration the residue was washed with ethyl acetate. The fractions were collected and after evaporation of the solvent, the residue was chromatographed on a silica gel column, using 5% MeOH/CH_2Cl_2 as eluent. The fractions were collected and the solvent evaporated under reduced pressure. **2l** was recrystallized from ether/petrol.

2a and **2f** were prepared the following way. A mixture of 0.05 mole amino acid ethyl ester hydrochloride, 0.05 mole sodium formate, and 0.5 mole formic acid was placed in a flask fitted with a reflux condenser and then heated at 100° for 1 hr. Sodium chloride was filtered off after cooling and the formic acid was evaporated under reduced pressure. In order to remove the formic acid completely the N-formyl amino acid ethyl ester was dissolved in CH_2Cl_2 and $NaHCO_3$ was added. After filtration CH_2Cl_2 was evaporated under reduced pressure.

2e was prepared in the following way. 0.15 mole (26.9 g) hippuric acid (available from EGA-Chemie), 0.30 mole (18 ml) absolute ethanol, and 6 drops of concentrated sulfuric acid were mixed in 75 ml dry benzene where after the azeotropic distillation was started and continued until all the starting material was consumed (as monitored by TLC in 25% MeOH/ether). After cooling, the mixture was poured into 60 ml water. The two phases were separated and the aqueous phase was extracted with 2 × 20 ml ether. The organic phases were collected and dried with $MgSO_4$. After filtration the solvents were evaporated under reduced pressure. The oily residue was freezed to a solid and recrystallized from ether/petrol.

N-(2-Methyl-1-oxopropyl) S-alanine ethyl ester, **2i**. Found: C, 68.46; H, 8.03; N, 5.32. Calc for $C_{15}H_{21}NO_3$: C, 68.44; H, 7.98; N, 5.32%. N - (2 - Methyl - 1 - oxopropyl) S-proline ethyl ester, **2m**. Found: C, 61.71; H, 8.98; N, 6.67. Calc for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57%. N-Benzoyl S-proline ethyl ester, **2n**. Found: C, 67.27; H, 6.93; N, 5.44. Calc for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66%.

3 was prepared from **2** using $\frac{1}{2}$ equiv of **1** in anhydrous benzene or toluene. The reaction mixture was placed in a two-necked flask fitted with a reflux condenser. The flask was placed in an oil-bath heated to 80° and the thionation of the amide function was completed in $\frac{1}{2}$–3 hr depending on the substrates. When possible, the consumption of **2** was monitored by TLC during the reaction course.

After evaporation of the solvent the residue was chromatographed on a silica gel column with a suitable eluent. The fractions were collected and the solvent evaporated under reduced pressure. When possible **3** was recrystallized (see individual compounds for further details).

N-Thioformyl glycine ethyl ester, **3a**

From 0.02 mole **2a** and 0.01 mole **1** in benzene for $1\frac{1}{2}$ hr. Starting eluent CH_2Cl_2 until all 2,4,6-tris (4-methoxyphenyl)-1,3,5,3,4,6-trioxatriphosphorinane-2,4,6-trisulfide,[7] **6**, a product formed from **1** during the reaction, was separated from the other compounds. Elution was continued with 5–10% AcOEt/CH_2Cl_2. Found: C, 40.81; H, 6.20; N, 8.95p S, 20.99. Calc for $C_3H_9NO_2S$: C, 40.82; H, 6.12; N, 9.52; S, 21.77%.

N-Thioacetyl glycine ethyl ester, **3b**

From 0.02 mole **2b** and 0.01 mole **1** in benzene for 1 hr. Eluation as in **3a**. Found: C, 44.90; H, 7.00; N, 8.42; S, 19.51. Calc for $C_6H_{11}NO_2S$: C, 44.72; H, 6.83; N, 8.70; S, 19.88%. The compound is hygroscopic.

N-(2-Methyl-1-thioxopropyl) glycine ethyl ester, **3c**.

From 0.02 mole **2c** and 0.01 mole **1** in 40 ml toluene for 2 hr. Two columns were used to purify **3c**. The first with CH_2Cl_2 as eluent, the second with ether as eluent. The compound was recrystallized from ether/petrol. Found: C, 50.91; H, 7.99; N, 7.44; S, 16.82. Calc for $C_8H_{15}NO_2S$: C, 50.77; H, 7.99; N, 7.40; S, 16.94%.

N-(2,2-Dimethyl-1-thioxopropyl) glycine ethyl ester, **3d**.
From 0.03 mole **2d** and 0.015 mole **1** in 50 ml toluene for 2 hr. **3d** was purified by using two columns both with CH_2Cl_2 as eluent. The oil was frozen and recrystallized from ether/petrol. Anal.: Found: C, 53.23; H, 8.45; N, 6.87; S, 15.58. Calc for $C_9H_{17}NO_2S$: C, 53.17; H, 8.43; N, 6.89; S, 15.77%

N-Thiobenzoyl glycine ethyl ester, **3e**
From 0.02 mole **2e** and 0.01 mole **1** in 30 ml toluene for 1 hr. The reaction was monitored by TLC with ether as eluent. **3e** was purified by using two columns both with CH_2Cl_2 as eluent. The compound was recrystallized from ether/petrol.

N-Thioformyl S-alanine ethyl ester, **3f**
From 0.03 mole **2f** and 0.015 mole **1** in 20 ml benzene for 1 hr. **3f** was purified by using CH_2Cl_2 as eluent. Precise mass measurement: found: 161.0510. Calc for $C_6H_{11}NO_2S$: 161.0510.

N-Thioacetyl S-alanine ethyl ester, **3g**
From 0.05 mole **2g** and 0.025 mole **1** in benzene for $\frac{1}{2}$ hr.. Purification as **3f**. Found: C, 47.92; H, 7.43; N, 7.74; S, 17.92. Calc for $C_7H_{13}NO_2S$: C, 48.00; H, 7.43; N, 8.00; S, 18.29%.

N-Thiobenzoyl S-alanine ethyl ester, **3h**
From 0.04 mole **2h** and 0.02 mole **1** in 25 ml benzene for 1 hr. **3h** was purified by using 50% ether/petrol. The compound was recrystallized from ether/petrol. Found: C, 60.73; H, 6.28; N, 5.73; S, 13.43. Calc for $C_{12}H_{15}NO_2S$: C, 60.76; H, 6.33; N, 5.91; S, 13.50%.

N-(2-Methyl-1-thioxopropyl) S-phenylalanine ethyl ester, **3i**
From 0.01 mole **2i** and 0.005 mole **1** in 15 ml benzene for $\frac{1}{2}$ hr. **3i** was purified by using 50% ether/petrol as eluent. Found: C, 64.73; H, 7.63; N, 5.00; S, 11.32. Calc for $C_{15}H_{21}NO_2S$: C, 64.52; H, 7.53; N, 5.02; S, 11.47%.

N-(2,2-Dimethyl-1-thioxopropyl) S-phenylalanine ethyl ester, **3j**
As **3i**. Found: C, 66.15; H, 7.90; N, 4.74; S, 10.40. Calc for $C_{16}H_{23}NO_2S$: C, 65.53; H, 7.85; N, 4.78; S, 10.92%.

N-Thiobenzoyl S-phenylalanine ethyl ester, **3k**
As **3i**. Found: C, 69.08; H, 6.18; N, 4.53; S, 10.20. Calc for $C_{18}H_{19}NO_2S$: C, 69.01; H, 6.07; N, 4.47; S, 10.22%.

N-Thioacetyl S-proline ethyl ester, **3l**
From 0.02 mole **2l** and 0.01 mole **1** in 50 ml toluene for 3 hr. **3l** was purified by using two columns. The first column starting with CH_2Cl_2 as eluent until all **6** was separated. Eluation was continued with 2% MeOH/CH_2Cl_2. The second column with 80% ether/petrol as eluent. The compound was recrystallized from ether/petrol. Found: C, 53.59; H, 7.45; N, 6.83; S, 15.89. Calc for $C_9H_{15}NO_2S$: c, 53.70; H, 7.51; N, 6.96; S, 15.93%.

N-(2-Methyl-1-thioxopropyl) S-proline ethyl ester, **3m**
From 0.05 mole **2m** and 0.025 mole **1** in 110 ml toluene for 2 hr. The reaction was monitored by TLC using 5% MeOH/CH_2Cl_2 as eluent. Purification by using two columns both with CH_2Cl_2 as eluent. Found: C, 57.14; H, 8.32; N, 6.04; S, 13.76. Calc for $C_{11}H_{19}NO_2S$: C, 57.61; H, 8.35; N, 6.11; S, 13.98%.

N-Thiobenzoyl S-proline ethyl ester, **3n**
From 0.03 mole **2n** and 0.015 mole **1** in 75 ml toluene for $1\frac{1}{2}$ hr. Monitored by TLC using 5% MeOH/CH_2Cl_2 as eluent. **3n** was purified by using CH_2Cl_2 as eluent. Recrystallized from ether/petrol. Found: C, 63.82; H, 6.53; N, 5.33; S, 12.30. Calc for $C_{14}H_{17}NO_2S$: C, 63.85; H, 6.51; N, 5.32; S, 12.17%.

Preparation of 1,2,4-triazin-6(2H)-ones
The following reactions were monitored by the development of H_2S-gas using a blue gel tube which is coloured black by the presence of H_2S.

1,3,4,5-Tetrahydro [1,2,4]triazin 6 (2H)-one, **4a**. From 0.01 mole **3a** and 0.01 mole 99% hydrazine hydrate in 20 ml EtOH. Reflux for 1 hr. After evaporation under reduced pressure of the solvent the residue was washed with cold acetone and recrystallized in hot acetone. Precise mass measurement: Found: 99.0432. Calc for $C_3H_5N_3O$: 99.0432.

1,4,5-trihydro-3-methyl[1,2,4]triazin-6 (2H)-one, **4b**. From 0.005 mole **3b** and 0.005 mole 99% hydrazine hydrate in 20 ml EtOH. Reflux for $\frac{1}{2}$ hr. Purification as **4a**. Found: C, 42.59; H, 6.27; N, 36.85. Calc for $C_4H_7N_3O$: C, 42.48; H, 6.19; N, 37.17%.

1,4,5 - Trihydro - 3 - (1 - methylethyl)[1,2,4]triazin- 6 (2H)-one, **4c**. From 0.015 mole **3c** and 0.06 mole 80% hydrazine hydrate in 35 ml dioxane for $1\frac{1}{2}$ hr at 100°. After evaporation of the solvent and the remaining hydrazine hydrate under reduced pressure (10 mmHg) and using a waterbath (90°) the residue was chromatographed on a silica gel column with 15% MeOH/CH_2Cl_2 as eluent. The fractions were collected and the solvent evaporated under reduced pressure. The residue was recrystallized from MeOH/ether. Found: C, 51.04; H, 7.93; N, 29.19. Calc for $C_6H_{11}N_3O$: C, 51.05; H, 7.85; N, 29.76%.

1,4,5 - Trihydro - 3 - phenyl[1,2,4]triazin - 6(2H) - one, **4e**. From 0.01 mole **3e** and 0.04 mole 80% hydrazine hydrate in 30 ml dioxane for 1 hr by 100°. After evaporation of the solvent and the remaining hydrazine hydrate the residue was washed in cold acetone. Precise mass measurement: Found: 175.0747. Calc for $C_9H_9N_3O$: 175.0746.

5(S) - 1, 3, 4 - Trihydro - 5 - methyl[1,2,4]triazin - 6(2H) - one, **4f**. From 0.005 mole **3f** and 0.005 mole 99% hydrazine hydrate in 10 ml refluxing dioxane for 2 hr. Purification as **4a**. Precise mass measurement: Found: 113.0599. Calc for $C_4H_7N_3O$: 113.0589.

5(S) - 1,4 - Dihydro - 3,5 - dimethyl[1,2,4]triazin - 6(2H) - one, **4g**. From 0.005 mole **3g** and 0.005 mole 99% hydrazine hydrate in 15 ml refluxing EtOH for 2 hr. Purification as **4a**. Precise mass measurement: Found: 127.0745. Calc for $C_5H_9N_3O$: 127.0745.

5(S)-1,4-Dihydro-5-methyl-3-phenyl[1,2,4]triazin-6(2H)-one, **4h**. From 0.005 mole **3h** and 0.005 mole 99% hydrazine hydrate in 10 ml refluxing dioxane for 3 hr. Purification as **4a**. Anal.: Found: C, 63.29; H, 5.93; N, 22.18. Calc for $C_{10}H_{11}N_3O$: C, 63.49; H, 5.82; N, 22.22%.

8a(S)-6,7,8,8a-tetrahydro-4-methylpyrrolo[1,2-d][1,2,4]-triazin-1(2H)-one, **4i**. From 0.01 mole **3i** and 0.04 mole 80% hydrazine hydrate in 30 ml dioxane for $4\frac{1}{2}$ hr at 100°. After evaporation under reduced pressure of the solvent and the remaining hydrazine hydrate the residue was chromatographed in a silica gel column using 10% MeOH/CH_2Cl_2 as eluent. The fractions were collected and the solvent evaporated under reduced pressure. The compound was recrystallized from MeOH/ether. Found: C, 55.04; H, 7.36; N, 27.37. Calc for $C_7H_{11}N_3O$: C, 54.89; H, 7.24; N, 27.43%.

8-(S)-6,7,8,8a-tetrahydro-4-phenylpyrrolo[1,2-d][1,2,4]triazin-1(2H)-one, **4i**. From 0.015 mole **3n** and 0.060 mole 80% hydrazine hydrate in 45 ml dioxane for 3 hr by 100°C. After evaporation under reduced pressure of the solvent and the remaining hydrazine hydrate the residue was chromatographed on a silica gel column using 2% MeOH/CH_2Cl_2 as eluent which yielded a yellow impurity. Eluation was continued with 5% MeOH/CH_2Cl_2. The fractions from the later eluation were collected and the solvent evaporated under reduced pressure. The crystalline solid was without success tried to get recrystallized from several solvent-combinations. Precise mass measurement: Found: 215.1058. Calc for $C_{12}H_{13}N_3O$: 215.1059.

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