Stereoselective Synthesis of *threo*-4, 4, 4-Trichlorothreonine¹⁾Kazuo MATSUMOTO, Yuji URABE, Yasuhiko OZAKI,
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threo-4,4,4-Trichlorothreonine was synthesized completely stereoselectively through *trans*-4-alkoxycarbonyl-5-trichloromethyl-2-oxazoline, which was prepared almost quantitatively by the reaction of 2-isocyano acetate with chloral in the presence of an organic base. Several derivatives of these compounds were also synthesized.

Furthermore, the stereochemistry of the resulting trichloro compounds was chemically elucidated. The trichlorothreonine was easily converted into *threo*-threonine by hydrogenation over Pd/C in the presence of NaHCO₃.

Although the number of compounds containing covalently bound halogens which are produced by living organism is not so many, several chloroamino acids have been found in nature. As an example, 2-amino-4,4-dichlorobutanoic acid (Armentomycin)²⁾ which is well known as an antibiotic was isolated from *Streptomyces armentosus* var. *armentosus*. In another example, 2-amino-5-chloro-4-hexenoic acid³⁾ and 2-amino-4-chloro-4-pentenoic acid⁴⁾ have been recently isolated from *Amanita*.

On the other hand, the halogeno amino acids have become attractive as metabolic inhibitors of biological systems and as intermediates of pharmaceuticals. From this point of view, a number of halogeno amino acids have been synthesized. Of these, most recently, 2-amino-4,4,4-trichlorobutanoic acid and 2-amino-3,4,4,4-tetrachlorobutanoic acid derivatives, which are useful intermediates⁵⁾ for the Armentomycin and the analogs, have been synthesized in our series of the synthetic studies on the halogeno amino acids.^{6,7)} However, the polychloro- β -hydroxyamino acid

has not yet been synthesized.

In the present paper, we wish to report a stereoselective synthesis of *threo*-4,4,4-trichlorothreonine, which is one of the polychloro- β -hydroxyamino acids, using the reaction of 2-isocyano acetates with chloral. With regard to the reaction of the isocyanides with aldehydes, Schöllkopf *et al.*,⁸⁾ Saegusa *et al.*⁹⁾ and Matsumoto *et al.*¹⁰⁾ reported that the oxazoline was formed in the NaCN-EtOH system, copper catalyzed system and NaH-THF system, respectively. However, in the reaction of the isocyano acetate with aldehyde bearing electron-withdrawing group such as chloral, it is expected that the oxazoline would be formed even in the absence of metallic catalysts. Furthermore, it should be anticipated that the *trans*-oxazoline is predominantly formed because of the steric effect of bulky trichloromethyl group. In this way, we carried out the reaction of the isocyano compound (II) with chloral (I) in the presence of several organic bases such as triethylamine, as shown in Scheme I. The resultant oxazoline

SCHEME I.

(III) was purified by the vacuum distillation. The IR spectrum of the compound (*e.g.* R = CH₃) obtained showed the characteristic absorption of CH stretching frequency at 3090 cm⁻¹, ester C=O stretching frequency at 1745 cm⁻¹ and C=N stretching frequency at 1620 cm⁻¹. The NMR spectrum in CDCl₃ indicated the signals at δ 3.89 (s, 3H, OCH₃), 4.84 (d, d, 1H, C4-H, *J*=2.5 Hz, 6.0 Hz), 5.41 (d, 1H, C5-H, *J*=6.0 Hz) and 7.08 (d, 1H, C2-H, *J*=2.5 Hz). The compound was homogeneous by gas liquid chromatography (GLC) using FFAP column. The result should reveal the formation of only one isomer of the diastereoisomers. From these results and the form of "Dreiding Stereo-models," it is suggested that the compound would be the thermodynamically favoured *trans*-4-alkoxycarbonyl-5-trichloromethyl-2-oxazoline (III). The stereochemistry of III was further elucidated by chemical conversion into threonine as shown in Scheme II. The same reaction was also carried out in the presence of metallic bases used in the prior works,^{8,10} as a comparative experiment. These results summarized in Table I show

TABLE I. FORMATION OF OXAZOLINE (III)^{a)}

R	Base	Solvent	Yield (%)	bp°C (mmHg)
CH ₃	Et ₃ N	Benzene	85	96(1)
CH(CH ₃) ₂	Et ₃ N	Benzene	90	103(1)
C(CH ₃) ₃	Et ₃ N	Benzene	95	93(0.8)
	(<i>i</i> -Pr) ₂ NH	Benzene	65	
	Pyridine	Benzene	20	
	NaH	THF	40	
	NaCN	EtOH	— ^{b)}	

^{a)} Reaction temp., 22~5°C; reaction time, 4 hr.

^{b)} No reaction.

that the organic bases gave rather high yields. The resulting oxazoline was hydrolyzed with

acetic acid to afford N-formyl-4,4,4-trichloro-threonine (IV) with retention of the configurations at C-4 and C-5. The resultant products were characterized by IR, NMR and elemental analyses. The yields and physico-chemical properties of these compounds are shown in Table II. Compound (IV) was partially hydrolyzed with HCl to form 4,4,4-trichlorothreonate hydrochloride (V) (R = CH₃, CH(CH₃)₂). Compound (V) was further hydrolyzed with HCl to afford *threo*-4,4,4-trichlorothreonine hydrochloride (VI) which was readily converted into the amino acid (VII) by the usual method. The amino acid obtained was homogenous by NMR spectrum in CF₃COOD-D₂O and paper partition chromatography (PPC) using Show-Fox solvent. The result should also show that the product was a single isomer. The physico-chemical properties of these amino acids are summarized in Table III. Moreover, the N-acyl derivatives were also prepared by the usual way, as listed in Table II. The hydrogenation of the trichloroamino acid (VII) over Pd/C in the presence of 3 equivalent moles of NaHCO₃¹¹ was carried out to convert into threonine. The NMR spectrum of the resulting threonine in D₂O showed no signals attributing to *erythro*-threonine. The PPC using Show-Fox solvent showed a single spot, corresponding to *threo*-threonine.

SCHEME II.

Thus, the result manifested that the trichlorothreonine (VII) was *threo*-form and the oxazoline (III) was *trans*-form. This synthetic method must be the first stereoselective synthesis of pure *threo*-threonine which is a biologically important amino acid. It appears

TABLE II. FORMATION OF N-ACYL DERIVATIVES

R ¹	R ²	mp (°C) (Solvent) ^{a)}	Yield (%)	Elemental analysis (%)				
				C	H	N	Cl	
H	CH ₃	164 (dec.)	(A)	63 ^{b)}	27.25 ^{e)}	3.05	5.30	40.21
					27.11 ^{f)}	3.06	5.24	40.55
H	C ₆ H ₅	178~9	(MeOH)	83 ^{b)}	40.46	3.09	4.29	32.57
					40.37	3.23	4.24	32.75
CH ₃	H	96~7	(B)	85 ^{e)}	27.24	3.05	5.30	40.22
					27.02	3.03	5.15	40.68
CH ₃	CH ₃	131~2	(A)	95 ^{d)}	30.19	3.62	5.03	38.19
					30.17	3.83	4.71	38.63
CH ₃	C ₆ H ₅	147~9	(A)	93 ^{d)}	42.32	3.55	4.11	31.23
					41.98	3.49	4.05	30.65
CH ₃	C ₆ H ₅ CH ₂ O	117~8	(A)	96 ^{d)}	42.13	3.81	3.78	28.70
					41.92	3.78	3.79	28.56
CH(CH ₃) ₂	H	165~6	(B)	90 ^{e)}	32.84	4.13	4.79	36.36
					32.97	4.14	4.84	36.73
C(CH ₃) ₃	H	157~9(dec.)	(B)	95 ^{e)}	35.26	4.60	4.57	34.69
					35.22	4.75	4.48	35.03

^{a)} Recrystallization solvent; A: MeOH-(i-Pr)₂O, B: AcOEt-(i-Pr)₂O.

^{b)} From (VI). ^{c)} From (II). ^{d)} From (V). ^{e)} Calculated. ^{f)} Found.

TABLE III. PHYSICOCHEMICAL PROPERTIES OF (V), (VI) AND (VII)

Compound	mp (°C)	C	Elemental analysis (%)		Cl
			H	N	
(V, R=CH ₃)	174~6 (dec.)	22.00 ^{a)}	3.32	5.13	51.96
		21.83 ^{b)}	3.49	5.01	52.36
(V, R=CH(CH ₃) ₂)	144~6	27.93	4.35	4.65	47.12
		28.03	4.33	4.74	47.05
(VI)	193~5 (dec.)	18.55	2.72	5.41	54.77
		18.60	2.69	5.44	54.52
(VII)	148 (dec.)	21.59	2.72	6.29	47.81
		21.82	2.76	6.30	48.21

^{a)} Calculated. ^{b)} Found.

that this route will be practically useful for the preparation of *threo*-trichlorothreonine and *threo*-threonine.

EXPERIMENTAL¹²⁾

General procedure for the preparation of 4-methoxycarbonyl-5-trichloromethyl-2-oxazoline (III)

To a mixture of methyl 2-isocyano acetate (II) (5.0 g, 50 mmole) and Et₃N (5.0 g, 50 mmole) in benzene (40 ml) was added dropwise, chloral (I) (7.4 g, 50 mmole) at 0°C with stirring. After stirring was continued for 4 hr at room temperature, the reaction mixture was neutralized with AcOH and the solvent

was removed under reduced pressure. The residue was dissolved in AcOEt (50 ml) and the solution was washed with water and then dried over MgSO₄. The solvent was removed under reduced pressure and the residual oil was distilled to give oxazoline (III) (R=CH₃) which was homogeneous by GLC using FFAP column (retention time, 15 min.); bp 95°C (1 mmHg), yield 10.6 g (85%). Similarly, 4-isopropoxycarbonyl and 4-*tert*-butoxycarbonyl oxazolines were also prepared from isopropyl and *tert*-butyl 2-isocyano acetates, respectively. The boiling points and yields are shown in Table I.

Methyl *N*-formyl-4,4,4-trichlorothreonate (IV)

Oxazoline (III) (R=CH₃) (6.2 g, 25 mmole) was

dissolved in a mixture of AcOH (10 ml) and THF (50 ml) at room temperature. After the mixture was allowed to stand for 2 hr, the solvent was removed under reduced pressure. The residue was extracted with AcOEt (50 ml) and the extract was washed with water and then dried over MgSO_4 . After the solution was evaporated to dryness under reduced pressure, the resulting crystals were collected by filtration and washed with $(i\text{-Pr})_2\text{O}$. Recrystallization from AcOEt- $(i\text{-Pr})_2\text{O}$ gave (IV) ($\text{R}=\text{CH}_3$), yield 6.3 g (95%). IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1} = 3355, 3170, 1735, 1660$. NMR (CDCl_3) δ : 3.72 (s, 3H, OCH_3), 4.65 (d, d, 1H, C3-H, $J=0.75$ Hz, 8.7 Hz), 5.29 (d, d, 1H, C2-H, $J=0.75$ Hz, 9.8 Hz), 7.60 (d, 1H, OH, $J=8.7$ Hz), 8.14 (br, 1H, CHO), 8.39 (d, 1H, NH, $J=9.8$ Hz). Analogs of isopropyl and *tert*-butyl esters were also prepared in a similar way. The melting points, elemental analyses and yields are summarized in Table II.

Methyl 4,4,4-trichlorothreonate hydrochloride (V)

Compound (IV) ($\text{R}=\text{CH}_3$) (6.61 g, 25 mmole) was dissolved in a mixture of conc. HCl (15 ml) and MeOH (75 ml), and the solution was heated at 50°C for 2 hr with stirring. Subsequently, the solution was evaporated to dryness under reduced pressure and the resulting crystals were collected by filtration. Recrystallization from MeOH gave (V) ($\text{R}=\text{CH}_3$), yield 5.8 g (85%). IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1} = 3150, 2700\sim 2500, 1745$. NMR ($d_6\text{-DMSO}$) δ : 3.79 (s, 3H, OCH_3), 4.32 (d, 1H, C2-H, $J=4$ Hz), 4.63 (d, 1H, C3-H, $J=4$ Hz), 8.68 (br, 4H). Analog of isopropyl ester was prepared in a similar way, yield 90%. The melting points and elemental analyses are shown in Table III. N-Acyl derivatives were prepared by the usual method and the physicochemical properties and yields are listed in Table II.

4,4,4-Trichlorothreonine (VII) and the hydrochloride (VI)

Compound (IV) ($\text{R}=\text{CH}_3$) (5.3 g, 20 mmole) was dissolved in 6 N HCl (50 ml) and the solution was refluxed for 3 hr. Then the solution was evaporated to dryness under reduced pressure. On treatment with acetone of the residue, crystals of VI were obtained. Recrystallization from EtOH-Et₂O gave pure (VI), yield 4.7 g (92%). IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1} = 3320, 3100, 2570, 2450, 1730, 1575$. NMR ($d_6\text{-DMSO}$) δ : 4.19 (d, 1H, C2-H, $J=2$ Hz), 4.68 (d, 1H, C3-H, $J=2$ Hz), 8.75 (br, 5H).

Compound (VI) (4.7 g, 18 mmole) was dissolved in water (20 ml) and the solution was treated with Amberlite IR 120 (H^+ form). The amino acid was eluted with 5% NH_4OH and the eluate was evaporated to dryness under reduced pressure. The residue was crystallized from MeOH to afford VII, yield 3.8 g (95%). This amino acid was homogeneous on PPC [Show-Fox solvent (*n*-BuOH: H_2O : NH_4OH : Acetone = 200: 150: 25: 25), *Rf* 0.55; *n*-BuOH: AcOH: H_2O

= 4: 4: 1, *Rf* 0.53]. IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1} = 3320, 2080, 1620$. NMR ($\text{CF}_3\text{COOD-D}_2\text{O}$) δ : 4.89 (s, 1H, C2-H), 5.08 (s, 1H, C3-H).¹³ The physicochemical properties of VI and VII are shown in Table III.

DL-Threonine (VIII)

A mixture of compound (VII) (3.3 g, 15 mmole), NaHCO_3 (3.8 g, 45 mmole) and water (100 ml) was hydrogenated over 10% Pd/C (0.5 g) for 3 hr at room temperature under 50 psi (initial pressure). After the hydrogenation was over, the catalyst was removed by filtration. The filtrate was treated with Amberlite IR 120 (H^+ form) and the amino acid was eluted with 5% NH_4OH . The eluate was evaporated to dryness under reduced pressure. The resulting crystals were recrystallized from MeOH- H_2O to afford DL-threonine (VIII), yield 1.68 g (95%), mp $235\sim 8^\circ\text{C}$ (dec.). It was found that no *erythro*-threonine was observed in the resulting threonine by PPC using Show-Fox solvent and NMR in D_2O . Anal. Calcd. for $\text{C}_4\text{H}_9\text{O}_3\text{N}$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.28; H, 7.59; N, 11.73.

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- 11) The hydrogenation did not smoothly proceed in the absence of NaHCO_3 .
- 12) All melting points and boiling points are uncorrected. Melting points were measured by the use of a Yamato melting point apparatus. IR spectra were recorded on a Shimadzu IR-27G infrared spectrophotometer. NMR spectra were obtained using a Hitachi Perkin-Elmer R-20A High Resolution NMR spectrometer with TMS in the case of organic solvent and with DSS in

the case of D_2O as internal standard. GLC was carried out using a Shimadzu GC-4B instrument with nitrogen as carrier gas and using a 1 m column with 15% FFAP on Neosorb NS. The temperature was raised from 100°C to 220°C at rate $5^\circ\text{C}/\text{min}$. R_f value on PPC was determined using Toyo filter paper No. 51.

- 13) Coupling constant between C2-H and C3-H was so small as to be observed seemingly as singlets.
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