

## Cyclization of Tetra-L-alanine Induced by Complexation of Technetium

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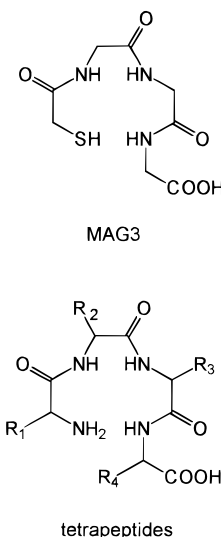
Complexation of technetium with tetraalanine yields an unstable complex that converts to a monooxotechnetium(V) complex of cyclic tetraalanine. The structure of the latter was determined by single-crystal X-ray analysis. In this negatively charged complex, the technetium atom is coordinated to one oxygen and four amide nitrogen atoms. The more polar complex that is initially formed is assumed to be an oxotechnetium(V) complex of tetraalanine, with the oxotechnetium moiety coordinated to three amide nitrogen atoms and the amine nitrogen atom. In this complex, the carboxyl group would be forced in the vicinity of the free amine group, thereby facilitating amide formation and cyclization. Cyclization of tetra-L-alanine occurs on both no carrier added (technetium-99m) and carrier added (technetium-99) scale.

### Introduction

Technetium-99m is the most intensively used radioisotope in diagnostic nuclear medicine. It owes its popularity to its excellent physical characteristics ( $T^{1/2} = 6.02$  h; photon energy of 140 keV; no corpuscular radiation) and chemical properties (transition metal) and to its availability from a  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator. Only very small amounts of Tc (typically about 1–40 ng) are present in radiopharmaceutical preparations, which necessitates the use of milligram amounts of the long-lived  $^{99}\text{Tc}$  ( $T^{1/2} = 2.12 \times 10^5$  years) for the structure determination of  $^{99\text{m}}\text{Tc}$ -labeled agents by X-ray crystallography. The oxotechnetium(V) complex of mercaptoacetyltriglycine ( $^{99\text{m}}\text{Tc}$ -MAG3), in which the technetium atom is coordinated to an oxygen atom, a thiol sulfur atom, and three nitrogen amide atoms,<sup>1</sup> is an established compound for the evaluation of renal function.<sup>2</sup> To assess the involvement of the thiol group for efficient renal extraction of this type of tracer agent, we have synthesized technetium-99m complexes with tetrapeptides that can be considered derivatives of mercaptoacetyltriglycine in which the thiol group has been replaced by an amino group (Figure 1).<sup>3</sup>

In contrast to MAG3, which forms a single complex with technetium-99m in standard conditions, labeling of the tetrapeptide tetra-L-alanine (A4) with technetium-99m yields two different complexes ( $^{99\text{m}}\text{Tc}$ -A4-A and  $^{99\text{m}}\text{Tc}$ -A4-B) that can be separated by HPLC.<sup>3</sup> Variable amounts of the two complexes were obtained depending on the labeling conditions, but the more hydrophilic complex  $^{99\text{m}}\text{Tc}$ -A4-A appears to be less stable as it slowly converts to  $^{99\text{m}}\text{Tc}$ -A4-B. This conversion also takes place after isolation of  $^{99\text{m}}\text{Tc}$ -A4-A with HPLC. This indicates that the original technetium complex ( $^{99\text{m}}\text{Tc}$ -A4-A) is modified and that technetium is not simply transferred to another ligand.

Biological evaluation in mice revealed that  $^{99\text{m}}\text{Tc}$ -A4-B is characterized by an almost exclusive hepatobiliary clearance



**Figure 1.** Comparison of the structures of mercaptoacetyltriglycine (MAG3) and tetrapeptides.

from plasma, whereas  $^{99\text{m}}\text{Tc}$ -A4-A is cleared by both the hepatobiliary and renal systems in almost equal proportions.<sup>3</sup>

Electrophoresis experiments showed that  $^{99\text{m}}\text{Tc}$ -A4-A is negatively charged at alkaline pH, but neutral in acidic conditions, suggesting the presence of an ionizable carboxylic acid group. The behavior of  $^{99\text{m}}\text{Tc}$ -A4-B in electrophoresis clearly is different as migration does not vary as a function of pH.<sup>3</sup>

Prompted by the diverging biological characteristics and the puzzling results of electrophoresis, we have conducted complexation experiments with long-lived technetium-99 to be able to determine the exact structure of the different complexes.

### Experimental Section

$\text{NH}_4^{99}\text{TcO}_4$  as a black solid was acquired as a gift from Mallinckrodt Medical (Petten, The Netherlands).  $\text{Na}^{99\text{m}}\text{TcO}_4$  was eluted from an Ultratechnekow  $^{99\text{m}}\text{Tc}$  generator (Mallinckrodt Medical). Commercially available reagents and solvents were of pro analysi quality and were used without further purification. Fab mass spectra were recorded by using a *m*-nitrobenzyl alcohol matrix on a VG 30-250 spectrometer (VG Instruments Inc.) at the probe temperature. Xenon was used as the primary beam gas, and the ion gun was operated at 8 keV and 100  $\mu\text{A}$ . Data were collected over the mass range 100–1000 Da at 0.7 s/scan.

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**Warning:** Technetium-99 is a long-lived ( $T^{1/2} = 2.12 \times 10^5$  years) weak  $\beta^-$  emitter ( $E_{\max} = 292$  keV) and requires special radioprotective measurements during handling.<sup>4</sup>

**Preparation of  $\text{TcOCl}_4(\text{Ph})_4\text{As}$ .**<sup>5</sup>  $\text{NH}_4^{99}\text{TcO}_4$  (90 mg 0.5 mmol) was dissolved in 10 mL of 12 N HCl and spiked with 200 MBq of  $\text{Na}^{99\text{m}}\text{TcO}_4$  in 2 mL of generator eluate. Tetraphenylarsonium chloride (420 mg, 1 mmol) was added and the solution was stirred for 30 min at room temperature. The precipitate was filtered off and washed with two portions of 8 mL of 12 N HCl and two portions of a 2-propanol diethyl ether (40:60) mixture. The precipitate was dissolved in 30 mL of methanol.

**Preparation of  $^{99\text{m}}\text{Tc}/^{99}\text{Tc}$ -tetra-L-alanine (Tc-A4).** Sodium (15.84 mg, 0.66 mmol) was dissolved in 20 mL of methanol, and 100 mg (0.33 mmol) of tetra-L-alanine was added to this solution.  $\text{TcOCl}_4(\text{Ph})_4\text{As}$  (225 mg, 0.33 mmol) in 10 mL of methanol was added via a dropping funnel to the latter solution under stirring at room temperature. The solution immediately turned black due to the precipitation of  $\text{TcO}_2$ .  $\text{TcO}_2$  was filtered off and the volume of the filtrate was reduced by evaporation under reduced pressure to a final volume of 4 mL. A fraction of the concentrated solution was stored in an open vial at room temperature to enable a slow evaporation of methanol, yielding bright yellow crystals.

**HPLC Analysis.** The compounds were analyzed on a  $250 \times 4.6$  mm column filled with Hypersil ODS  $5 \mu\text{m}$  (Shandon Scientific, England) eluted at a flow rate of 1 mL/min with a ternary gradient mixture of ethanol (A)—0.025 M phosphate buffer (pH 5.85) (B)—water (C):  $T = 0$  min, B = 100%;  $T = 15$  min, A = 20%, B = 80%;  $T = 20$  min, A = 50%, B = 50%;  $T = 20.1$  min, A = 50%, C = 50%;  $T = 25$  min, A = 90%, C = 10%.

The column effluent was monitored for UV absorbance at 215 nm and for radioactivity using a 2 in.  $\text{NaI}(\text{Tl})$  scintillation detector.

**Preparation of No Carrier Added  $^{99\text{m}}\text{Tc}$ -A4 and Kinetic Analysis of the Conversion Rate.** Tetra-L-alanine (1 mg) was dissolved in 0.1 mL of 0.5 M phosphate buffer (pH 11).  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  (40  $\mu\text{g}$ ) dissolved in 20  $\mu\text{L}$  of 0.05 N HCl was added immediately followed by 2 mL of  $^{99\text{m}}\text{TcO}_4^-$  (740 MBq) solution in saline obtained from a technetium generator (Ultratechnekow, Mallinkrodt Medical).

The solution was neutralized with 0.5 M  $\text{H}_3\text{PO}_4$ , and an aliquot of 500  $\mu\text{L}$  was subjected to reversed-phase HPLC as described earlier. The peak with a retention time corresponding to  $^{99\text{m}}\text{Tc}$ -A4-A was collected and immediately divided into four fractions of 200  $\mu\text{L}$  that were added to 0.5 mL of 0.5 M phosphate buffer with pH's of 4, 6, 8, and 10, respectively.

The solutions were analyzed at intervals of 40 min over 280 min by RP-HPLC as described earlier, but using isocratic elution at a flow rate of 1 mL/min with 0.025 M phosphate buffer (pH 5.85)—EtOH (70:30) instead of gradient elution.

Linear regression was applied to the plots of the logarithm of the residual fraction of  $^{99\text{m}}\text{Tc}$ -A4-A vs time (seven observations) to obtain the  $k$  values.  $R^2$  values of the regression varied between 0.9981 and 0.9997.

**Esterification and Hydrolysis.** A neutralized solution (500  $\mu\text{L}$ ) of  $^{99\text{m}}\text{Tc}$ -A4-A obtained as described earlier was applied to a Sep-Pak C18 column (Waters, Milford, MA). The column was rinsed twice with 5 mL of  $\text{H}_2\text{O}$  and dried by a stream of nitrogen for 10 min. The Sep-Pak column was eluted with 2 mL of methanol, and 2 mmol of diazomethane<sup>27</sup> in 3.5 mL of ether was added to the eluate, which was then analyzed by HPLC (isocratic elution as described earlier). The previous solution (0.5 mL) was added to 0.5 mL of 1 N NaOH. After 10 min of incubation at room temperature, the solution was neutralized and analyzed by HPLC (isocratic elution as described earlier).

**Crystal Structure.** The crystal structure was determined on a yellow, plate-shaped crystal ( $0.32 \times 0.20 \times 0.04$  mm) isolated from the methanolic mixture. Data were collected at room temperature on a Stoe STADI4 automated four-circle diffractometer (program: DIF4)<sup>6</sup> equipped with graphite-monochromated Mo  $K\alpha$  radiation (see Table

**Table 1.** Crystal Data

chemical formula	$\text{C}_{36}\text{H}_{36}\text{As}_1\text{N}_4\text{O}_5\text{Tc}_1$	$V$ ( $\text{\AA}^3$ )	1637(2)
fw	777.61	$d$ (measd) ( $\text{Mg m}^{-3}$ )	1.5
space group	P1	$d$ (calcd) ( $\text{Mg m}^{-3}$ )	1.577
$a$ ( $\text{\AA}$ )	9.502(6)	$Z$	2
$b$ ( $\text{\AA}$ )	13.154(9)	$\lambda$ ( $\text{\AA}$ )	0.71073
$c$ ( $\text{\AA}$ )	13.889(13)	$\mu$ ( $\text{mm}^{-1}$ )	1.490
$\alpha$ (deg)	89.07(6)	$F(000)$	792
$\beta$ (deg)	88.48(6)	$R, wR(F^2)$	0.042, 0.094
$\gamma$ (deg)	70.64(1)		

**Table 2.** Fractional Atomic Coordinates and Equivalent Isotropic Displacement Parameters ( $\text{\AA}^2$ ) of the (Cyclotetraalanyl)-technetium(V) Anion

	x	y	z	$U_{\text{eq}}$
Tc1	-0.16686(4)	0.03170(3)	0.49051(3)	0.0276(2)
O1	0.0129(5)	-0.0284(3)	0.4817(3)	0.037(1)
N111	-0.2833(5)	0.0126(3)	0.3824(4)	0.034(1)
C112	-0.3497(6)	-0.0743(4)	0.3916(5)	0.033(2)
C113	-0.2583(9)	-0.1811(5)	0.3396(5)	0.058(3)
C114	-0.3603(6)	-0.0953(5)	0.5002(5)	0.037(2)
O115	-0.4406(4)	-0.1452(3)	0.5297(3)	0.046(2)
N121	-0.2788(5)	-0.0526(4)	0.5526(4)	0.034(1)
C122	-0.3052(7)	-0.0383(5)	0.6590(5)	0.044(2)
C123	-0.1937(9)	-0.1266(5)	0.7161(5)	0.068(3)
C124	-0.2924(6)	0.0689(5)	0.6820(5)	0.037(2)
O125	-0.3300(5)	0.1085(4)	0.7599(3)	0.048(2)
N131	-0.2347(5)	0.1115(4)	0.6088(4)	0.035(1)
C132	-0.2557(7)	0.2279(5)	0.6087(5)	0.042(2)
C133	-0.1400(7)	0.2624(5)	0.6528(5)	0.046(2)
C134	-0.2714(6)	0.2649(5)	0.4986(5)	0.038(2)
O135	-0.3124(5)	0.3554(3)	0.4747(3)	0.037(1)
N141	-0.2402(5)	0.1782(4)	0.4375(3)	0.034(1)
C142	-0.2897(7)	0.1944(4)	0.3417(4)	0.037(2)
C143	-0.1941(7)	0.2099(5)	0.2668(5)	0.046(2)
C144	-0.3305(7)	0.0919(5)	0.3179(5)	0.039(2)
O145	-0.4092(5)	0.0888(3)	0.2494(3)	0.055(2)
Tc2	0.24952(4)	0.45826(3)	-0.02245(3)	0.0256(1)
O2	0.0657(5)	0.5193(4)	-0.0143(4)	0.047(2)
N211	0.3488(5)	0.4031(4)	0.0971(4)	0.034(1)
C212	0.3705(7)	0.2923(5)	0.1221(5)	0.038(2)
C213	0.2429(7)	0.2828(6)	0.1966(5)	0.041(2)
C214	0.3594(6)	0.2353(5)	0.0251(4)	0.032(2)
O215	0.3920(6)	0.1388(4)	0.0234(4)	0.033(2)
N221	0.3120(4)	0.3045(3)	-0.0454(3)	0.045(1)
C222	0.3381(7)	0.2709(5)	-0.1443(5)	0.036(2)
C223	0.2052(8)	0.2464(6)	-0.1861(6)	0.047(2)
C224	0.3627(6)	0.3630(5)	-0.2047(5)	0.037(2)
O225	0.4045(6)	0.3554(4)	-0.2875(3)	0.051(2)
N231	0.3335(5)	0.4538(4)	-0.1512(3)	0.031(1)
C232	0.3885(7)	0.5408(5)	-0.1775(5)	0.035(2)
C233	0.285(1)	0.6297(6)	-0.2313(5)	0.061(3)
C234	0.4288(7)	0.5800(5)	-0.0854(5)	0.034(2)
O235	0.5182(5)	0.6311(4)	-0.0852(4)	0.043(2)
N241	0.3710(5)	0.5508(4)	-0.0069(3)	0.028(1)
C242	0.4238(8)	0.5535(5)	0.0868(5)	0.037(2)
C243	0.3452(8)	0.6493(5)	0.1421(5)	0.044(2)
C244	0.4293(7)	0.4534(5)	0.1431(4)	0.034(2)
O245	0.4885(6)	0.4295(4)	0.2175(3)	0.049(2)

1). Accurate lattice parameters were derived by automatic centering and least-squares analysis of 25 reflections in the range  $16 \leq 2\theta \leq 24^\circ$ . The  $\omega$ - $2\theta$  scan technique (scan width =  $1.20^\circ$  plus  $\alpha_1$ - $\alpha_2$  divergence) was used to collect 6028 intensities with indices  $-11 \leq h \leq 11$ ,  $-15 \leq k \leq 15$ ,  $0 \leq l \leq 16$ . Of these measured reflections, 6028 were unique and 3595 were considered observed ( $I \geq 2\sigma_I$ ). Three reflections (002, 020, 232) monitored periodically (120 min) showed an average decay of  $<2\%$ . The intensities were corrected for Lp and linear decay (program: REDU4).<sup>7</sup> Empirical absorption corrections<sup>8</sup>

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**Table 3.** Selected Bond Lengths and Bond Angles

Bond Lengths (Å)			
Tc1—O1	1.629(4)	Tc2—O2	1.665(4)
Tc1—N111	1.957(5)	Tc2—N211	1.940(5)
Tc1—N121	1.951(5)	Tc2—N221	1.939(5)
Tc1—N131	1.941(5)	Tc2—N231	1.932(5)
Tc1—N141	1.958(5)	Tc2—N241	1.953(5)
Bond Angles (deg)			
N131—Tc1—N141	80.4(3)	N231—Tc2—N241	79.7(3)
N121—Tc1—N141	129.1(4)	N221—Tc2—N241	129.3(4)
N121—Tc1—N131	78.9(3)	N221—Tc2—N231	80.2(3)
N111—Tc1—N141	78.7(3)	N211—Tc2—N241	78.5(3)
N111—Tc1—N131	129.3(4)	N211—Tc2—N231	129.3(4)
N111—Tc1—N121	79.5(3)	N211—Tc2—N221	79.4(3)
O1—Tc1—N141	115.6(4)	O2—Tc2—N241	115.8(4)
O1—Tc1—N131	114.6(4)	O2—Tc2—N231	114.5(4)
O1—Tc1—N121	115.3(4)	O2—Tc2—N221	114.9(4)
O1—Tc1—N111	116.1(4)	O2—Tc2—N211	116.2(4)

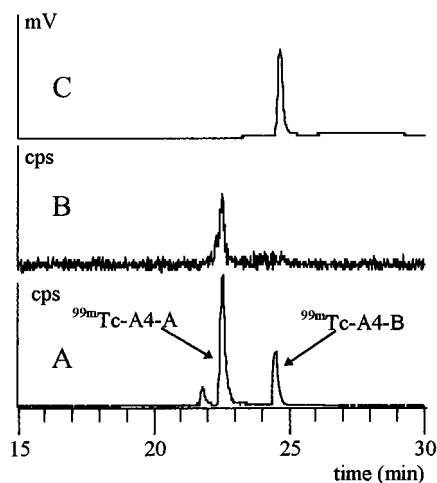
based on  $\psi$  scans were applied (program: EMPIR).<sup>9</sup> Reflections were measured in  $10^\circ$  intervals. The minimum and maximum transmission values ranged from 0.601 to 0.732. The structure was solved by using a combination of the Patterson method and direct methods on the difference structure (program: DIRDIF).<sup>10</sup> The model was refined on  $F^2$  by full-matrix least-squares (program: SHELXL-93).<sup>11</sup> Displacement parameters were anisotropic for all non-hydrogen atoms. H-atoms were located on ideal molecular geometries and refined by using a riding model. Analysis of the final difference map revealed  $-0.68 \text{ e } \text{Å}^{-3}$  as the deepest hole and  $0.70 \text{ e } \text{Å}^{-3}$  as the highest peak. General calculations were performed using PARST.<sup>12</sup> The absolute configuration was determined according to Flack.<sup>13</sup>

Final fractional coordinates and equivalent isotropic displacement parameters with estimated standard deviations (esd) of the (cyclotetra-L-alanyl)oxotechnetium(V) anions are listed in Table 3. Table 3 contains selected bond lengths and bond angles. A complete report is included in the supporting information.

## Results and Discussion

<sup>99m</sup>Tc pertechnetate was spiked with <sup>99m</sup>Tc to facilitate the detection of any contaminations caused during handling and synthesis and to enable radiometric detection with a NaI(Tl) detector during HPLC analysis.

The technetium complex was synthesized in two steps with an initial reduction of technetium(VII) pertechnetate to oxotechnetium(V) tetrachloride in concentrated hydrochloric acid.<sup>5</sup> Oxotechnetium(V) tetrachloride was isolated by precipitation with tetraphenylarsonium chloride, and the salt was added to a solution of the ligand tetra-L-alanine in sodium methanolate/methanol. Previous experiments on the technetium-99m scale indicated that complexation of technetium by tetrapeptides proceeds with a higher yield in an alkaline environment.<sup>3</sup> Accordingly, we used an alkaline solution to facilitate the deprotonation of the amide groups, which is necessary for chelation of technetium by tetra-L-alanine. The colloidal precipitate of TcO<sub>2</sub>, which was also formed during this reaction, was easily removed by filtration to yield a clear yellow solution, the color typical for Tc(V) complexes.<sup>1,14–21</sup> HPLC analysis



**Figure 2.** HPLC chromatograms: (A) analysis of the labeling reaction mixture of <sup>99m</sup>Tc-A4 with the presence of <sup>99m</sup>Tc-A4-A and <sup>99m</sup>Tc-A4-B (radioactivity detection); (B) analysis immediately after the preparation of <sup>99m</sup>Tc-A4 showing the presence of <sup>99m</sup>Tc-A4-A (radioactivity detection); (C) analysis of a sample of the crystals obtained from the <sup>99m</sup>Tc-A4 preparation [(cyclotetra-L-alanyl)oxotechnetium(V)] exhibiting a retention time identical to that of <sup>99m</sup>Tc-A4-B (UV detection 215 nm).

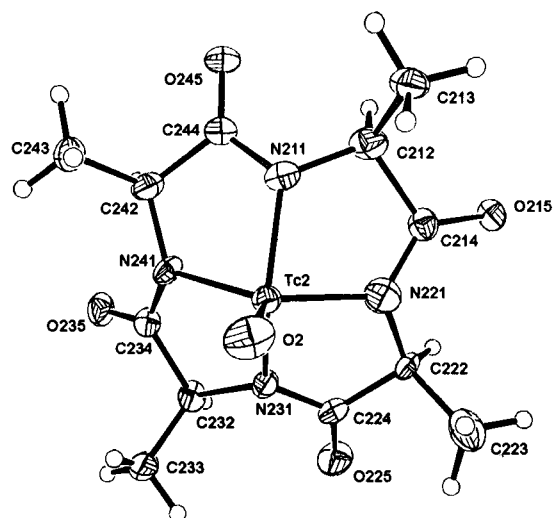
of the reaction mixture immediately after preparation showed the presence of only one compound with a retention time identical to that of <sup>99m</sup>Tc-A4-A, obtained by labeling tetra-L-alanine with technetium-99m in alkaline conditions (Figure 2). HPLC analysis of the mixture was repeated 24 h after synthesis and yielded a chromatogram that shows the presence of two peaks, the retention times of which correspond to <sup>99m</sup>Tc-A4-A and <sup>99m</sup>Tc-A4-B, respectively. The conversion of Tc-A4-A to Tc-A4-B, which was observed on the technetium-99m scale, also appeared to proceed for the carrier-added technetium-99 complex that was initially obtained. Subsequent HPLC analyses showed that this conversion to Tc-A4-B proceeded further as a function of time and was complete after 14 days.

Yellow crystals started to form about 20 days after synthesis, and, as could be expected, the retention time on HPLC of the crystallized compound was identical to that of <sup>99m</sup>Tc-A4-B (Figure 2). Negative ion Fab mass spectroscopy of the crystal surprisingly showed a molecular ion with a mass of 385 Da, corresponding to a Tc complex in which an oxotechnetium core is bound to the four amide nitrogen atoms of cyclic tetra-L-alanine, of which four protons have been withdrawn [(cyclotetra-L-alanyl)oxotechnetium(V)]. This structure was unexpected as we were unsuccessful in previous attempts to chelate technetium-99m with cyclic tetrapeptides.

The structure derived from the results of mass spectroscopy was confirmed by single-crystal X-ray analysis. An ORTEX<sup>22</sup> plot is shown in Figure 3. The asymmetric unit consists of two tetraphenylarsonium cations and two (cyclotetra-L-alanyl)oxotechnetium(V) anions. Apart from the peptide bond atoms, the structure is pseudocentrosymmetric. The (cyclotetra-L-alanyl)oxotechnetium(V) anions have a square pyramidal ar-

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**Figure 3.** ORTEX drawing of the anion (cyclotetra-L-alanyl)oxotechnetium(V).

**Table 4.**  $k$ -Values ( $\text{min}^{-1}$ ) of the Conversion of  $^{99\text{m}}\text{Tc-A4-A}$  to  $^{99\text{m}}\text{Tc-A4-B}$

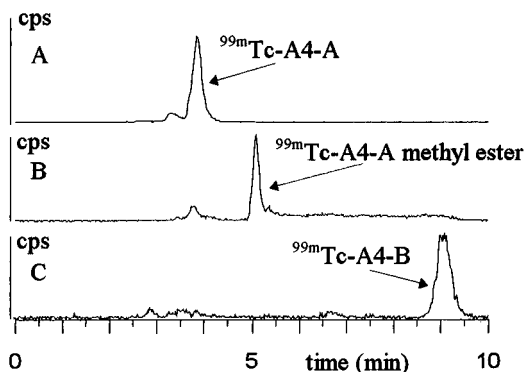
pH	$k$
4	$4.91 \times 10^{-3}$
6	$10.01 \times 10^{-3}$
8	$3.03 \times 10^{-3}$
10	no conversion observed

rangment around the Tc atom with the oxo group occupying the apical position. The maximum deviation from the least-squares plane through the four nitrogen atoms is 0.0026 and 0.0020 Å, respectively. All alanyl methyl substituents adopt the syn configuration with respect to the oxotechnetium group. Technetium bond lengths are given in Table 3 and are very similar to those reported in the literature for other technetium(V) oxo complexes.<sup>23–25</sup>

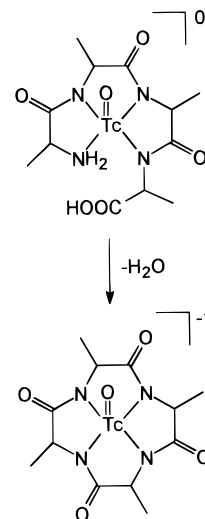
The conversion of  $^{99\text{m}}\text{Tc-A4-A}$  to  $^{99\text{m}}\text{Tc-A4-B}$  follows first-order kinetics, and the rate is largely dependent on the pH. The conversion proceeds with the highest rate at pH 6, whereas at pH 10 no conversion was observed (Table 4).

As the initially formed complex (Tc-A4-A) is not stable, it is difficult to determine its exact structure, but different observations indicate that it is the oxotechnetium(V) complex of tetraalanine in which the oxotechnetium group is bound to the amine nitrogen atom and the three amide nitrogen atoms.

To confirm whether the carboxylic acid group indeed is not involved in the complexation of technetium, we have reacted  $^{99\text{m}}\text{Tc-A4-A}$  with diazomethane, a reagent known to convert carboxylic acids rapidly to their methyl esters. Analysis of the reaction mixture showed the presence of a new radiolabeled compound (probably  $^{99\text{m}}\text{Tc-A4-A}$ -methyl ester) with a retention time intermediate between those of  $^{99\text{m}}\text{Tc-A4-A}$  and  $^{99\text{m}}\text{Tc-A4-B}$  (Figure 4). This conversion supports the theory that the carboxylic acid is not involved in the chelation of the peptide as in a Tc-tetraalanine complex only the carboxylic acid group can be expected to react with diazomethane. Attempts to hydrolyze the supposed methyl ester in alkaline conditions to again obtain  $^{99\text{m}}\text{Tc-A4-A}$  always resulted in the formation of



**Figure 4.** HPLC analysis of  $^{99\text{m}}\text{Tc-A4}$  (A) prior to and (B) after reaction with diazomethane and (C) after attempted alkaline hydrolysis yielding  $^{99\text{m}}\text{Tc-A4-B}$ .



**Figure 5.** Proposed structure of Tc-A4-A and its conversion to Tc-A4-B.

$^{99\text{m}}\text{Tc-A4-B}$ , probably due to the reactivity of the free primary amino group toward the methyl ester (Figure 4).

Electrophoresis of  $^{99\text{m}}\text{Tc-A4-A}$  further showed that the complex has no charge at pH < 3, but migration distance to the anode increases as a function of pH with a steep rise between pH 4 and 6. This observation also confirms the assumption that the carboxylic acid group is not involved in the complexation of tetraalanine.<sup>3</sup>

The yellow color of the Tc-A4-A solution is strongly indicative for an oxotechnetium(V) core as such a yellow color is typical for oxotechnetium(V) complexes. The migration distances of  $^{99\text{m}}\text{Tc-MAG3}$  and  $^{99\text{m}}\text{Tc-A4-A}$  in size exclusion chromatography are identical (results not shown), which indicates that the structure of  $^{99\text{m}}\text{Tc-A4-A}$  could be a 1:1 technetium:ligand complex, as is the case for  $^{99\text{m}}\text{Tc-MAG3}$ .<sup>1</sup>

All of the former observations are compatible only with a structure of Tc-A4-A in which the oxotechnetium(V) core is coordinated by three amide nitrogen atoms and the lone pair of the amine nitrogen atom (Figure 5). In this structure, the carboxylic acid group can be forced in the vicinity of the amine group, facilitating amide formation and subsequent cyclization of the tetraalanine ligand, resulting in the formation of (cyclotetra-L-alanyl)oxotechnetium(V) ( $^{99\text{m}}\text{Tc-A4-B}$ ). To our knowledge, a similar conversion of a ligand induced by complexation of technetium has not been reported before.

Cyclization of tetrapeptides normally requires specialized conditions that provide only low yields.<sup>26</sup> It therefore will be interesting to investigate whether complexation of a stable

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transition metal (e.g., Re) with tetraalanine also induces a similar cyclization of the tetrapeptide. On the condition that the metal can be removed in an appropriate way, this could provide a potential new synthesis procedure for cyclic tetrapeptides.

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**Supporting Information Available:** Tables of positional parameters, displacement parameters, bond distances, bond angles, and torsion angles (17 pages). Ordering information is given on any current masthead page.

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