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Synthesis and spectroscopic characterization of dimethylgermanium derivatives of dipeptides, crystal structure of dimethylgermanium glycylglycinate and *in vivo* effects of dimethylgermanium glycylglycinate against murine leukemia P388

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Dimethylgermanium derivatives of dipeptides, Me_2GeAA (H_2AA = H_2glygly, H_2glyala, H_2glyval, H_2glyleu, H_2glymet) have been obtained by the reaction of Me_2GeBr_2 with H_2AA in the presence of triethylamine. The crystal structure of Me_2Geglygly has been determined by single-crystal X-ray diffraction. The dipeptide is tridentately coordinated to germanium, which has a distorted trigonal bipyramidal environment. From infrared and Raman data, analogous molecular structures are inferred for the other dimethylgermanium derivatives of dipeptides. ^{1}H NMR measurements show that Me_2Geglygly is completely hydrolyzed in aqueous solution to give H_2glygly and $(Me_2GeO)_x$.

***In vivo* tests with Me_2Geglygly showed no toxic or antitumor activity against murine leukemia P388.**

Keywords: Organogermanium, dipeptide, structure, toxicity

INTRODUCTION

Diorganotin derivatives of dipeptides R_2SnAA (H_2AA = dipeptide) are of interest since such compounds were proved to possess activity against leukemia P-388 (AA = glygly, R = Me, n-Bu, n-Oct, Ph),[1–3] and according to recent *in vitro* studies also against human breast cancer cells (e.g. AA = glyala, R = cyclo-C_6H_{11}).[4] The solid-state structures of R_2SnAA are characterized by tridentate bis-chelating AA ligands and a distorted trigonal bipyramidal environment of Sn.[5,6] The same molecular structure with $O_{carboxylate}$ and N_{amino} in axial and C_{methyl} and N_{pept} in equatorial positions has been found in methanol solution. In aqueous solutions hydrolysis occurs quickly via a pentacoordinated species in which the $Sn-N_{pept}$ bond is retained. It seemed worthwhile to extend these investigations to include diorganogermanium derivatives of dipeptides, which had not been studied before. We were interested in preparing the first examples of R_2GeAA compounds, in determining their structure and their possible antitumor activity and, finally, in a comparison of such compounds with the corresponding organotin compounds.

EXPERIMENTAL

Me_2GeBr_2 was prepared according to Ref. 7. The dipeptides were a gift from Degussa. Toluene, methanol (MeOH) and triethylamine (Et_3N) were commercial products and were dried as usual. All reactions were carried out in dry nitrogen atmosphere.

Typically, a solution of 2 mmol (0.52 g) of Me_2GeBr_2 in 5 cm^3 toluene was slowly added to a suspension of 2 mmol of the appropriate dipeptide and 4 mmol Et_3N in 50 cm^3 of toluene at room temperature. Then, after stirring for 10 min the reaction mixture was heated to 80°C. After 4 h the mixture was filtered and the solid residue was washed with

Table 1 Analytical data for dimethylgermanium derivatives of dipeptides Me_2GeAA

Compound	Yield (%)	M.p. (°C)	Mol. wt in MeOH: Found (Calcd)	Analysis (%): Found (Calcd) C	H	N
$Me_2Geglygly$	48	204[a]	253[b]	31.10	5.30	12.10
$C_6H_{12}O_3N_2Ge$			(233)	(30.96)	(5.20)	(12.04)
$Me_2Geglyala$	35	169–171	235	33.90	5.60	11.40
$C_7H_{14}O_3N_2Ge$		188[a]	(247)	(34.07)	(5.72)	(11.35)
$Me_2Geglyval$	67	198[a]	250	40.10	7.10	10.40
$C_9H_{18}O_3N_2Ge$			(275)	(39.33)	(6.60)	(10.19)
$Me_2Geglyleu$	55	194[a]	294	42.10	7.50	9.80
$C_{10}H_{20}O_3N_2Ge$			(289)	(41.58)	(7.00)	(9.70)
$Me_2Geglymet$	59	199[a]	317	35.60	6.50	9.20
$C_9H_{18}O_3N_2SGe$			(307)	(35.22)	(5.91)	(9.13)

Abbreviations: Hgly, NH_2CH_2COOH; Hala, $CH_3CH(NH_2)COOH$; Hval, $(CH_3)_2CHCH(NH_2)COOH$; Hleu, $(CH_3)_2CHCH_2CH(NH_2)COOH$; Hmet, $CH_3SCH_2CH_2CH(NH_2)COOH$.

[a] Decomposition. [b] Molecular weight in water (37°C) = 122.

30 cm^3 of warm $CHCl_3$ and recrystallized from methanol. Analytical data are summarized in Table 1.

Infrared (IR) spectra (KBr) were recorded on a Perkin–Elmer grating spectrometer PE 580 B, ^1H NMR spectra on a Perkin–Elmer R-32 at 37°C. Melting points are uncorrected. Molecular weights were determined osmometrically. Single crystals of $Me_2Geglygly$ were obtained by crystallization from methanol after addition of petroleum ether (40–60°C) and diethyl ether.

For the *in vivo* test of the antitumor activity[8–10] of $Me_2Geglygly$ with the murine leukemia P388, three groups of six female CD_2F_1 mice each were used, one as negative control, one as positive control, and one as test group: 10^6 cells of the P388 leukemia were implanted intraperitoneally into each mouse. On days 1, 5 and 9 the negative controls were treated with a solution of 1.8% aqueous sodium chloride /polyethylene glycol 400 (PEG 400). The negative controls died after 9 days. The positive controls were treated with Cisplatinum (*cis*-$Pt(NH_3)_2Cl_2$) at a concentration of 5 × 10^{-6} mol kg^{-1} weight in 1.8% aqueous NaCl/PEG 400 at days 1, 5 and 9. The positive controls showed a weight difference (day 5 − day 1) of minus 0.77 g; they died at day 18 corresponding to a Test/Control (T/C) value of 200%. The six mice of the test group were treated with a solution of $Me_2Geglygly$ at a concentration of 4 × 10^{-5} mol kg^{-1} weight at days 1, 5 and 9. The weight difference (day 5 − day 1) was plus 3.3 g. The mice died at day 9.

A crystal of dimensions 0.19 mm × 0.16 mm × 0.16 mm mounted on a glass fibre was used to obtain cell data, and subsequently for intensity measurements. Crystal data were as follows: M_r = 232.76, a = 8.023(4), c = 14.156(5) Å, V = 911.3 $Å^3$, Z = 4, D_x = 1.697 mg m^{-3}, space group = $P4_1$.

The intensities of 3193 ($1.5° \leq \theta \leq 30.0°$; $0 \leq h \leq 11$; $0 \leq k \leq 11$; $-19 \leq l \leq 19$) reflexions were measured on a Nonius CAD-4 diffractometer, graphite-monochromated Mo Kα radiation, λ = 0.71069 Å, μ = 3.3 mm^{-1}, T = 291(1) K; $F(000)$ = 472, $\omega/2\theta$ scans, scan speed 1.1–3.3° min^{-1}. Lattice parameters are taken from least-squares fit with 25 reflexions up to 2θ = 31.2° after Lorentz-polarization correction and absorption correction via ψ scans. After averaging equivalent reflexions, 2648 unique reflexions (R_{int} = 0.01) remained from which 1951 reflexions with $F \geq 3.0\,\sigma(F)$ were used for the structure determination via Patterson function, ΔF syntheses and full-matrix least-squares refinements with anisotropic temperature factors for all non-H atoms and a common isotropic temperature factor for H atoms, which were placed in geometrically calculated positions (C–H 0.96 Å). Complex neutral atom scattering factors were taken from the *International Tables*

Table 2 Atomic coordinates and equivalent isotropic thermal parameters ($Å^2 \times 10^3$)

	$U_{eq} = (1/6\pi^2)\Sigma_i\Sigma_j\beta_{ij}a_ia_j$			
	x	y	z	U_{eq}/U
Ge	0.59957(7)	0.08804(6)	0.14876(7)	40
O(1)	0.6840(5)	0.3199(5)	0.1188(3)	34
O(2)	0.6192(5)	0.5872(5)	0.1277(3)	37
O(3)	0.1887(6)	0.2173(6)	0.2875(4)	53
N(1)	0.4156(5)	0.2108(5)	0.1943(3)	53
N(2)	0.4488(8)	−0.1080(6)	0.1994(4)	76
C(1)	0.5913(9)	0.0184(8)	0.0200(5)	58
C(2)	0.7836(9)	0.0406(9)	0.2295(7)	72
C(3)	0.5862(6)	0.4399(6)	0.1380(4)	38
C(4)	0.4165(7)	0.3897(6)	0.1781(4)	42
C(5)	0.3003(8)	0.1393(8)	0.2480(4)	45
C(6)	0.3117(9)	−0.0441(8)	0.2545(5)	56

Table 3 Bond distances (Å) and angles (degrees)

Bond distances			
O(1)–Ge	2.025(4)		
N(1)–Ge	1.888(4)		
N(2)–Ge	2.110(5)		
C(1)–Ge	1.907(7)		
C(2)–Ge	1.905(7)		
C(3)–O(1)	1.272(6)		
C(3)–O(2)	1.220(6)		
C(5)–O(3)	1.228(7)		
C(4)–N(1)	1.454(7)		
C(5)–N(1)	1.327(7)		
C(6)–N(2)	1.442(9)		
C(4)–C(3)	1.529(7)		
C(6)–C(5)	1.477(8)		
Bond angles			
N(1)–Ge–O(1)	81.6(2)	C(5)–N(1)–Ge	121.0(4)
N(2)–Ge–O(1)	164.4(2)	C(5)–N(1)–C(4)	121.4(5)
N(2)–Ge–N(1)	79.9(2)	C(6)–N(2)–Ge	110.9(4)
C(1)–Ge–O(1)	94.6(2)	O(2)–C(3)–O(1)	125.0(5)
C(1)–Ge–N(1)	116.9(3)	C(4)–C(3)–O(1)	115.4(4)
C(1)–Ge–N(2)	95.0(3)	C(4)–C(3)–O(2)	119.6(5)
C(2)–Ge–O(1)	92.9(3)	C(3)–C(4)–N(1)	108.9(9)
C(2)–Ge–N(1)	120.3(3)	N(1)–C(5)–O(3)	123.3(6)
C(2)–Ge–N(2)	95.2(3)	C(6)–C(5)–O(3)	121.7(6)
C(2)–Ge–C(1)	122.8(3)	C(6)–C(5)–N(1)	115.0(5)
C(3)–O(1)–Ge	116.4(3)	C(5)–C(6)–N(2)	111.6(5)
C(4)–N(1)–Ge	117.2(3)		

for X-ray Crystallography.[11] Refinement on F with 1951 reflexions and 110 refined parameters converged at $R = 0.045$. The largest peak in the final ΔF map was $\pm$ 1.0(4)e $Å^{-3}$. The following programs were used: Enraf–Nonius Structure Determination Package,[12] SHELX76[13] and SCHAKAL.[14]

Final fractional atomic coordinates are listed in Table 2, bond distances and angles in Table 3. Full listings of atomic coordinates and thermal parameters are available upon request from the authors.

RESULTS AND DISCUSSION

The dimethylgermanium derivatives of the dipeptides, Me_2GeAA, listed in Table 1 were prepared by the reaction of Me_2GeBr_2 with the appropriate dipeptide in toluene in the presence of triethylamine according to Eqn [1]:

$$H_2AA + Me_2GeBr_2 + 2\ Et_3N \rightarrow Me_2GeAA + 2[Et_3NH]Br \quad [1]$$

The products are insensitive to dry air but sensitive to moisture. They are not soluble in chloroform and non-polar solvents but they are soluble in methanol and ethanol.

According to molecular weight measurements, the compounds are monomeric in methanol (see Table 1) and we assume that in analogy to the appropriate dimethyltin (Me_2Sn) derivatives of dipeptides[5] they retain in methanol solution the molecular structure as determined in the solid state (*vide infra*). In aqueous solution of $Me_2Geglygly$ only half of the calculated molecular weight was found. Evaporation of this solution to dryness and extraction of the residue with petroleum ether (40–60°C) leads to the separation of free acid and dimethylgermoxane. These products were identified by their characteristic IR frequencies and it is inferred that they result from hydrolysis according to Eqn [2]:

$$Me_2Geglygly + H_2O \rightarrow H_2glygly + \frac{1}{x}(Me_2GeO)_x \quad [2]$$

This proposal would also be consistent with preliminary results of conductivity measurements. The molar conductivity (Λ_M) of 2.32×10^{-3} molar aqueous solutions at 20°C: 28 Ω^{-1} cm^2 mol^{-1}, is comparable with that of $Me_2Snglygly$ solutions of similar concentrations for which the presence of undissociated species in aqueous solutions was inferred.[2]

^1H NMR spectroscopy was used as a diagnostic tool to follow the rate of hydrolysis by measuring the

decrease of the height of the methyl signal of $Me_2Geglygly$ and the increase of that of $(Me_2GeO)_x$ (Fig. 1). Graphical calculations based on ln C/C_0 vs t (Fig. 2) demonstrate that the hydrolysis of $Me_2Geglygly$ is a pseudo-first-order reaction, and

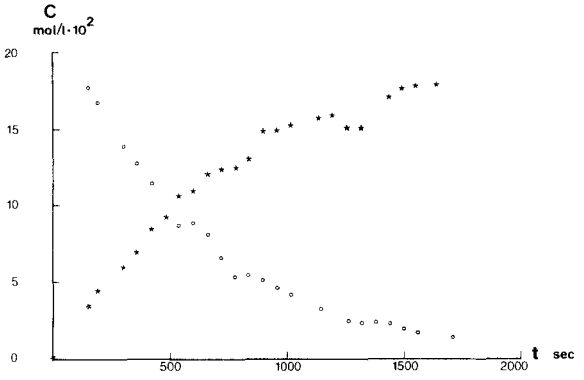

Figure 1 Graph showing the rate of hydrolysis of $Me_2Geglygly$: o, concentration of $Me_2Geglygly$; *, concentration of Me_2GeO, calculated from the height of the appropriate ^1H NMR methyl singlet.

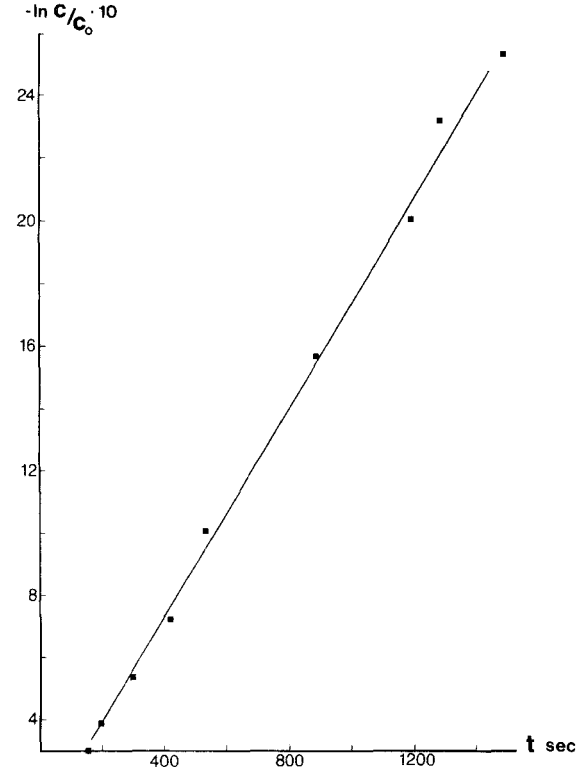

Figure 2 Hydrolysis of $Me_2Geglygly$: ln C/C_0 vs t plot demonstrating pseudo-first-order reaction type (selected values of $Me_2Geglygly$ concentration taken from Fig. 1).

lead to a rate constant of 1.64×10^{-3} s^{-1} and a half-life value of *ca* 6.5 min.

A comparison of the IR spectra of the free acids H_2AA and of the appropriate solid compounds Me_2GeAA (Table 4) shows that $\nu(NH_{peptide})$ (e.g. 3295 cm^{-1} in $H_2glygly$) disappeared in the spectra of the products, indicating that the organogermanium moiety is bonded to the dipeptide via the amide group. Further structural information ensues from the following spectral observations: (i) the $\nu(NH_{amino})$ values (3280–3120 cm^{-1}, Table 1) are lowered compared with those in alkali metal salts (e.g. 3380 cm^{-1} in Nagly)[15] or with matrix-isolated amino acids (e.g. 3414 cm^{-1});[15] this can be correlated with coordination of the amino group to the central germanium atom, and this would be fully consistent with the situation in the analogous triorganotin derivatives of amino-acids and dipeptides;[16] (ii) from the values of $\Delta\nu$ = 220–260 cm^{-1} [$\Delta\nu = \nu_{as}(COO^-) - \nu_s(COO^-)$], unidentate bonding of the carboxylate group to germanium can be inferred;[17] and (iii) from the appearance of both the $\nu_s(Ge-C)$ and the $\nu_{as}(Ge-C)$ vibration in the IR and Raman spectra, it can be safely assumed that the molecules do not contain a linear C–Ge–C skeleton. In summary it can be postulated that the peptide ligand acts as a tridentate ONN-ligand coordinating to the bent Me_2Ge moiety in analogy to the molecular structure of the corresponding diorganotin derivatives.[1,2]

The result of an X-ray structure determination of $Me_2Geglygly$ is consistent with this proposal. A molecule of $Me_2Geglygly$ is shown in Fig. 3 and a stereoscopic view of the unit cell in Fig. 4. Atomic parameters are given in Table 2 and bond lengths and angles in Table 3. $Me_2Geglygly$ crystallizes in the space group $P4_1$. The atoms bound to germanium form a distorted trigonal bipyramid with peptide-N and the methyl-C atoms occupying the equatorial positions whereas carboxylate-O and amino-N are in axial positions. The molecular structure therefore is in principle identical with that of diorganotin dipeptides.[5,6] Bond lengths and angles are not unreservedly comparable with those found in other germanium compounds, since structures of the same type are not known. The Ge–$N_{peptide}$ bond length of 1.888(4) Å is not significantly shorter than the Ge–N bond in dimethylphenylgermanium-μ-isocyanido-(pentacarbonyl)molybdenum [1.897(6) Å with tetrahedral germanium],[18] but shorter than that found in *trans*-bis(3,3-dimethyl-1-butynyl)hemiporphyrazineger-

Table 4 Characteristic IR and Raman vibrations of Me_2GeAA

Compound	$\nu(NH_{amino})$	$\nu(CO)$	$\nu_{as}(COO^-)$	$\nu_s(COO^-)$	$\Delta\nu^a$	IR		Raman	
						$\nu_{as}(Ge-C)$	$\nu_s(Ge-C)$	$\nu_{as}(Ge-C)$	$\nu_s(Ge-C)$
Me_2Geglygly	3280 s 3220 s 3140 s	1667 s	1648 vs	1420vs	248	640 s	593 s	640	591
Me_2Geglyala	3210 s 3132 vs	1670 sh	1635 s	1410 vs	225	642 s	591 s	641	591
Me_2Geglyval	3210 s,br 3118 s,br	1675 s,br	1650 s,br	1390 s	260	670 vs	592 vs	668	594
Me_2Geglymet	3260 s,br 3165 s,br 3070 s,br	1665 s,br	1622 s,br	1400 vs	222	637 vs	598 vs	635	598
Me_2Geglyleu	3240 s 3128 s	1637 sh	1648 vs	1390 vs	258	632 vs	590 vs	632	595

[a] $\Delta\nu = \nu_{as}(COO^-) - \nu_s(COO^-)$.

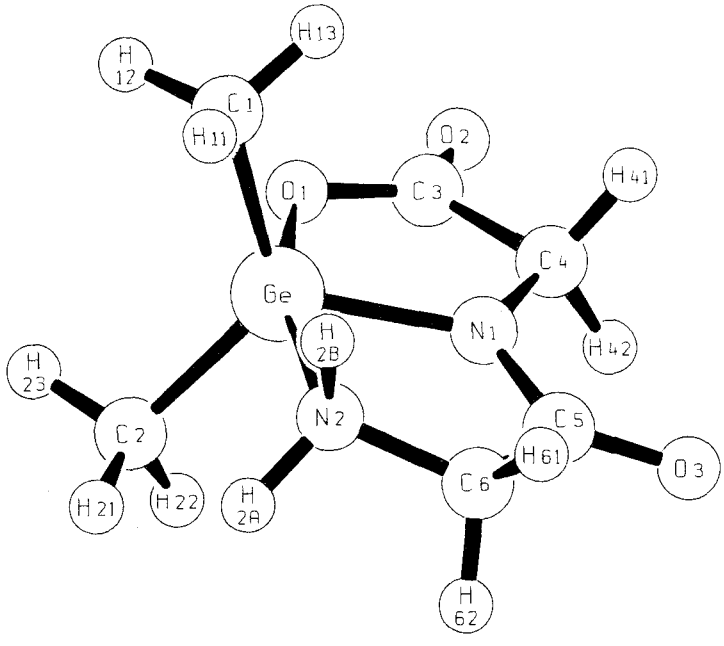

Figure 3 Structure of Me_2Geglygly: view of molecule showing atom numbering scheme.

manium [1.956(2) Å][19] in which Ge is six-coordinated. The $Ge-N_{amino}$ bonds in the latter compound [2.172(3) Å][19] and in organogermatranes[20–22] containing pentacoordinated germanium [2.19(3) to 2.24(2) Å], are distinctly longer than the $Ge-N_{amino}$ bond in Me_2Geglygly with 2.110(5) Å. The Ge–O distance of 2.025(4) Å is appreciably longer than that in $Ph_3GeOCOCF_3$[23] and that in $[(PhCH_2)_3Ge]_2O$,[24] which have been found to be 1.86 and 1.730(1) Å, respectively. The observed intermolecular distances clearly exclude the presence of hydrogen bonds.

The antitumor activity of Me_2Geglygly was tested *in vivo* against the murine P388 leukemia of the CD_2F_1 mouse. In this test, Me_2Geglygly showed neither toxicity nor antitumor activity. The mice died

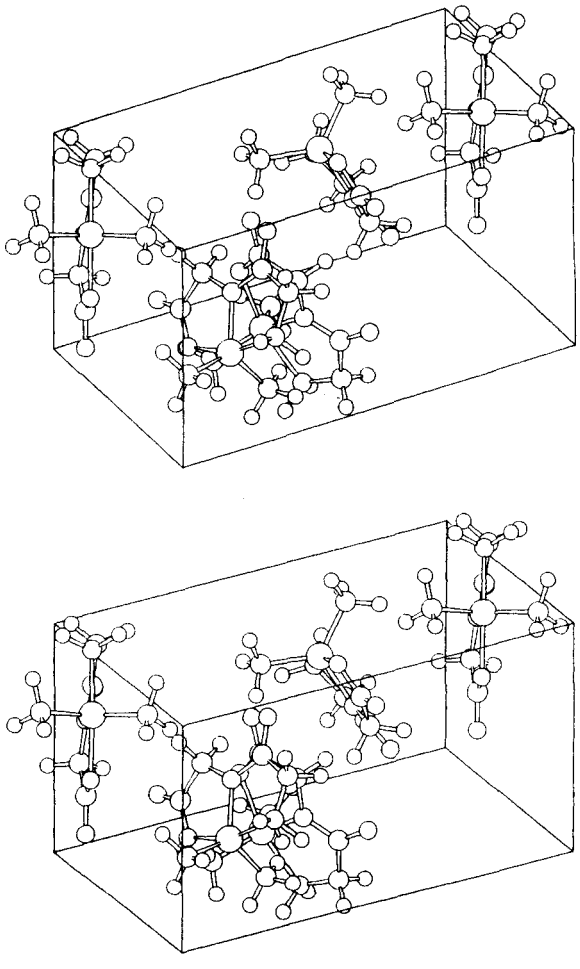

Figure 4 Structure of Me_2Geglygly: stereoscopic view of the unit cell (*a* vertical; *c* horizontal).

at the same time as the negative controls which were treated only with the solvent mixture (1.8% aqueous sodium chloride/polyethylene glycol 400) used to dissolve Me_2Geglygly. There was no increase of lifespan compared with the negative controls and the increase of weight was similar to the negative controls.

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