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Synthesis and characterization of some penicillins modified with germanium-containing moieties

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Penicillin derivatives, penicillin G (1), penicillin V (2) and ampicillin (3), were modified with germanium-containing moieties and their structures were confirmed based on NMR spectroscopy and MALDI-TOF. Their antibacterial ability was tested. None of these exhibited activity stronger than the parent penicillins. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: penicillin G; penicillin V; ampicillin; organogermanium compound; ^{1}H NMR spectra; ^{13}C NMR spectra; antibacterial activity

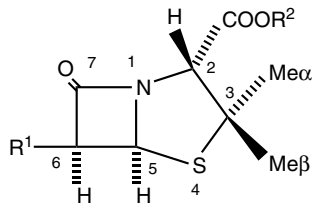
INTRODUCTION

The difficult problem associated with antibiotics in general is the facile emergence of resistant bacteria for which the antibiotics lose their effect. This was particularly the case with penicillin, the first antibiotic. When penicillin became widely available during the World War II it was a medical miracle; but, only 4 years after drug companies began mass-production of penicillin in 1943, microbes began appearing that could resist it.[1]

The first bacterium to battle penicillin was *Staphylococcus aureus*. This bacterium is often a harmless passenger in the human body, but it can cause illness, such as pneumonia or toxic shock syndrome, when it overgrows or produces a toxin. In 1967, another type of penicillin-resistant bacterium, *Streptococcus pneumoniae*, was discovered. The number of bacteria that can resist penicillin was rapidly increasing.

It was found that antibiotic resistance is inevitable, but there are measures that can slow it down. Efforts are under way on several fronts: improving infection control, developing new antibiotics, and using drugs more appropriately. There should be many strategies in order to develop new drugs. We thought that the modification of antibiotics with a germanium-containing moiety would enable such analogs of antibiotic that lost their effects to resistant bacteria to recover their antibiotic ability. There are several reasons for us to think that germanium may have an important role in the development of new drugs. First, germanium is a Group 14 element. Hence, organogermanium compounds resemble their carbon analogues with respect to physical properties such as lipophilicity, which will be an important property as a medicine. Second, organogermanium compounds are, in general, less toxic than their organotin and organolead analogs. Third, it has been established that some organogermanium compounds exhibit biological activity.[2]

To the best of our knowledge, germylation of antibiotics has not been reported. We chose penicillins as the antibiotics to be modified with a germanium-containing moiety because penicillins are readily available and, at the same time, are well known to have become ineffective against resistant bacteria. Among penicillins, we chose those with a carboxy group that can react with germanium-containing alcohols or halides to afford the desired germyl esters of penicillin. Thus, we used 3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (penicillin G) (**1**), 3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (penicillin V) (**2**) and 6-[amino(phenyl)acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (ampicillin) (**3**).



	R^1	R^2
1: $PhCH_2CONH$-		K
2: $PhOCH_2CONH$-		K
3: $PhCH(NH_2)CONH$-		H

Several methods are possible for introducing germanium-containing moieties into organic compounds, and a few

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typical ones are:[3]

(i) hydrogermylation of an alkene:

$$R{-}CH{=}CH_2 + R_3Ge{-}H \longrightarrow R{-}CH_2{-}CH_2GeR_3 \quad (1)$$

(ii) reaction between a lithio derivative of an organogermanium compound and a haloalkane:

$$R{-}X + R_3Ge{-}Li \longrightarrow R{-}GeR_3 + LiX \quad (2)$$

(iii) esterification of an alcohol with a germanium-containing carboxylic acid (or its derivative) or esterification of a carboxylic acid with a germanium-containing alcohol or halide, or by analogous reactions:

$$R{-}OH + R_3Ge{-}COOH \longrightarrow R_3Ge{-}COOR + H_2O \quad (3a)$$

$$R{-}COOH + R_3Ge{-}OH \longrightarrow R_3Ge{-}OOCR + H_2O \quad (3b)$$

$$R{-}COOH + R_3Ge{-}X \longrightarrow R_3Ge{-}OOCR + HX$$

Which method is to be employed will depend on the availability/reactivity of both substrates (alkenes, halides, alcohols or carboxylic acids) and germanium-containing reagents. Since penicillins have a carboxy group, method (iii) will be the method of choice. It must be added that several attempts to prepare new antibiotics have been made by esterification of penicillins.[4,5] Thus, we expected that these penicillins (as a free acid or as a potassium salt) will react with 3-(trimethylgermyl)-1-propyl iodide (**4a**), 3-(triethylgermyl)-1-propyl iodide (**4b**) and 3-(triphenylgermyl)-1-propyl iodide (**4c**) to afford the germyl esters. In one instance, a diiodide, bis(3-iodo-1-propyl)diphenylgermane (**4d**) was used.

RESULTS AND DISCUSSION

Synthesis of germanium-containing reagents

1-Trichlorogermylpropanoic acid (**5**), obtained from ASAI Germanium Research Institute, is the common starting material for **4a**–**4c**. Thus, **5** was reacted with an appropriate Grignard reagent (MeMgI, EtMgI or PhMgBr) to afford organogermylpropanoic acids (**6a**–**6c**).[6]

The carboxylic acids **6a**–**6c** were reduced to the corresponding alcohols (**7a**–**7c**) by lithium aluminum hydride (LAH).[6] The alcohols were converted to the iodides **4a**–**4c** via the corresponding tosylates (**8a**–**8c**), as outlined in Scheme 1.

Germylation of penicillins

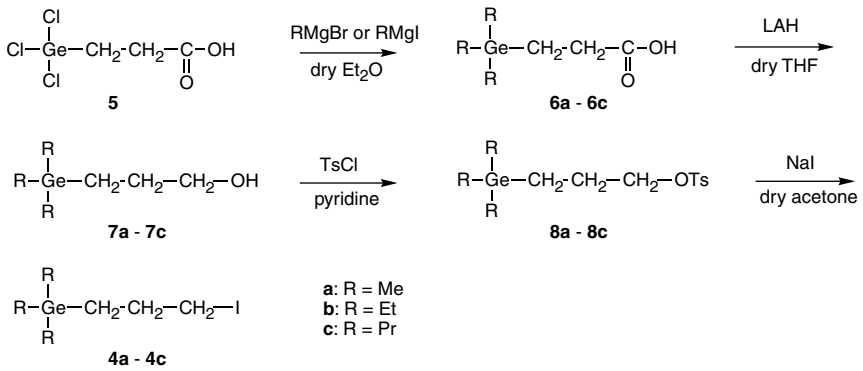
The reaction between a penicillin and a germanium reagent, i.e. germylation of penicillins, was carried out in the following way.

With **1** and **2**, their potassium salts were reacted with iodides **4a**–**4c** in dry dimethylformamide (DMF). The hydrolysis and subsequent usual work-up afforded the desired germyl esters, as indicated in Scheme 2.

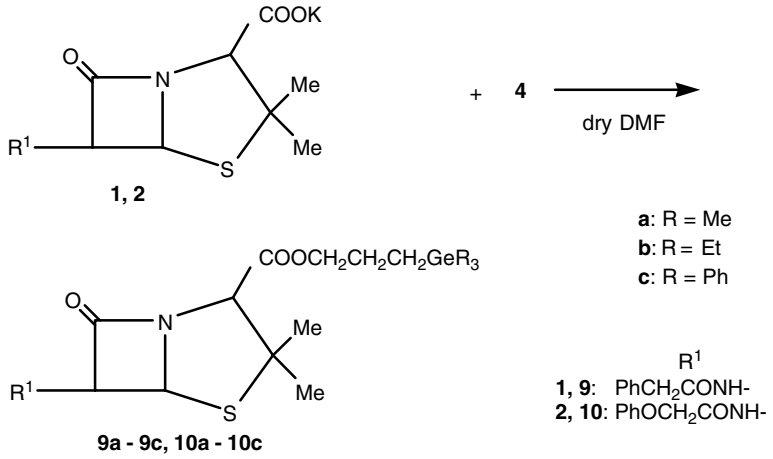
From **1**, 3-(trimethylgermyl)-1-propyl-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (**9a**), 3-(triethylgermyl)-1-propyl-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (**9b**), 3-(triphenylgermyl)-1-propyl-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylate (**9c**) were obtained.

From **2**, 3-(trimethylgermyl)-1-propyl-3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (**10a**), 3-(triethylgermyl)-1-propyl-3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylate (**10b**) and 3-(triphenylgermyl)-1-propyl-3,3-dimethyl-7-oxo-6-[(phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (**10c**) were obtained.

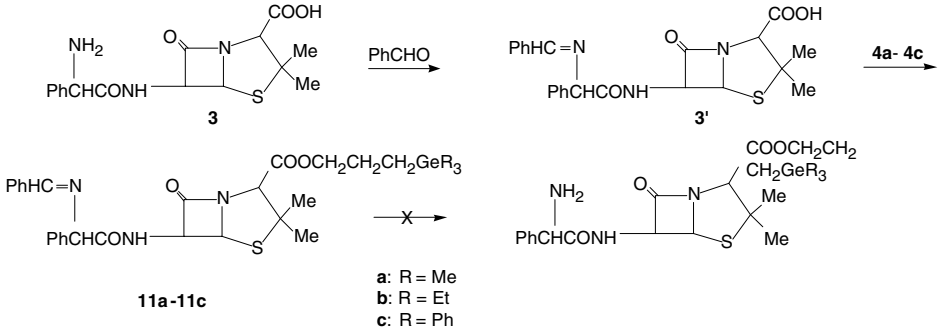
With **3**, it is necessary to protect the amino group before esterification. The method of protection reported by Sakamoto *et al*.[7] was employed. Thus, **3** was treated with benzaldehyde to give a Schiff base **3′**. Some authors assigned an imidazolidinone structure rather than a Schiff base.[5] Without isolation, **3′** was reacted with an appropriate halide in the presence of some base to give the protected ester. It was assumed that the subsequent hydrolysis of this ester with aqueous HCl would give the desired ampicillin ester. Though the germylation of **3′** with germanium-containing iodide gave germylated ampicillin **11a**–**11c** successfully, the subsequent hydrolysis



Scheme 1. Synthesis of germylpropyl iodides.



Scheme 2. Synthesis of germylated penicillins.



Scheme 3. Attempted synthesis of germylated ampicillin.

of **11a**–**11c** for deprotection always gave intractable resinous material under various conditions (Scheme 3). Hence, the pharmaceutical activity was tested for **11a**–**11c**.

We also prepared an organogermanium compound with two penicillin moieties, bis(3-{3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxy}propyl)diphenylgermane (**12**) by the reaction between **1** and bis(2-iodoethyl)diphenylgermane (**4d**) (Scheme 4) to assess the effect of germanium atom per penicillin molecule.

RESULTS AND DISCUSSION

Mass spectrometry

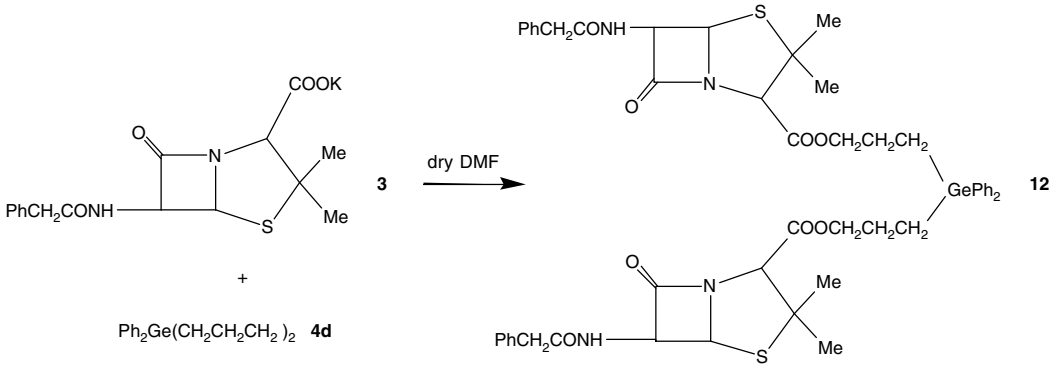
The characterization of germyl esters **9a**–**9c**, **10a**–**10c**, **11a**–**11c** and **12** was carried out by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Shimadzu Biotech; MALDI-TOFMS AXIMA (S/W Version 2.2)) and ^{1}H and ^{13}C NMR spectroscopy. The MALDI-TOF mass spectrum of **9a** is shown in Fig. 1.

The molecular ion signal (observed: 517.1434; calculated for $(M + Na)^+$: 517.1200) was observed. In addition, the characteristic pattern, shown in Fig. 1a, representing the isotopic distribution of species containing one germanium nucleus is clearly demonstrated. The results for other germylated penicillins are given in Table 1.

The MALDI-TOF spectrum of **12** is shown in full range in Fig. 2 for reference. The multiplet characteristic for

Table 1. Molecular weights of germylated penicillins

Compound	Molecular weight	
	Observed (MALDI-TOF)	Calculated $(M + Na)^+$
9a	517.1434	517.1192
9b	559.1409	559.1662
9c	703.4622	703.1700
10a	533.1509	533.1141
10b	575.1863	575.1661
10c	719.5426	719.1611
11a	620.6915	620.1614
11b	662.7203	662.2084
11c	806.8192	806.2084
12	1001.6124	1001.2649



Scheme 4. Synthesis of organogermanium antibiotics with two penicillin moieties.

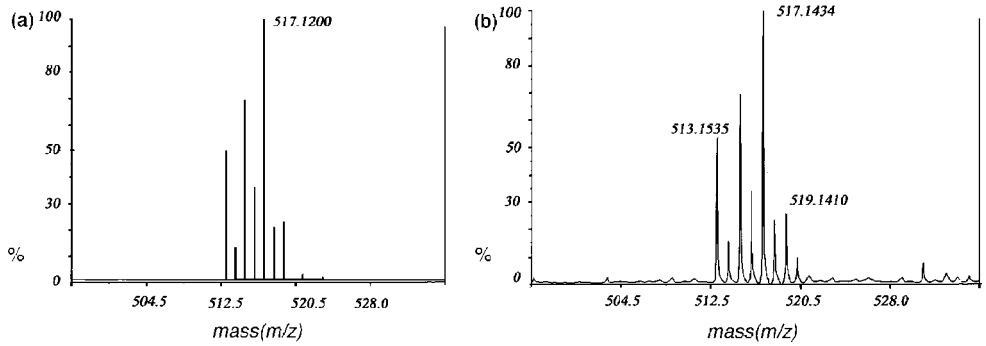


Figure 1. MALDI-TOF mass spectrum of **9a**: (a) calculated; (b) observed.

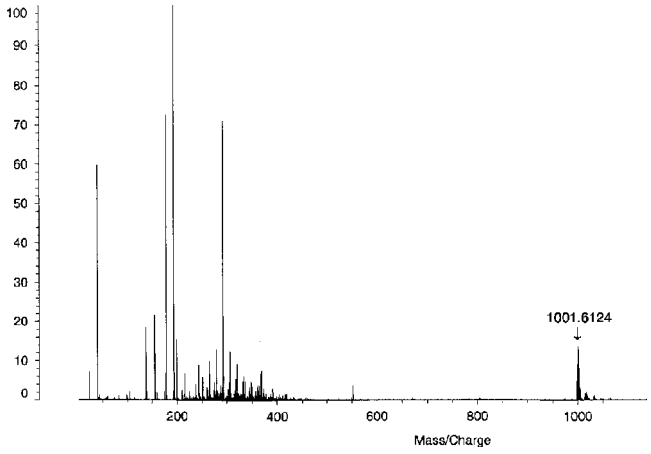


Figure 2. MALDI-TOF mass spectrum of 12.

compounds containing one germanium atom is clearly observable.

NMR spectroscopy

^{1}H NMR spectroscopy of β-lactam antibiotics has been extensively investigated from the early 1960s. Signals common to the spectra of all penicillin are the Me3α and Me3β, the H2 and the H5/H6 signals. The assignment of the H3 signal, which appears as a singlet, is unambiguous. However, the specific assignment of the Me3α/Me3β and H5/H6 signals (both separated only by less than 0.1 ppm) was carried out only for some compounds[8] and the assignments for the other compounds are based on the comparison with such data.

For instance, the evidence of a specific assignment of H5/H6 signals for **1** was obtained from biosynthetic material produced in D_2O in which H6, but not H5, was replaced by deuterium, allowing the assignment of the higher field doublet to H6.[9]

For the assignment of the 3Meα/3Meβ resonances of penicillins, Cooper *et al.*[10] determined the nuclear Overhauser enhancement of the methyl ester of penicillin V and concluded that the Me3β signal is the lower field one.

Branch *et al.*[8] pointed out that, with the aid of a high-field instrument, it is possible to observe the three-bond H–N–C–H6 proton–proton coupling, which is unequivocal evidence for the assignment. Thus, the assignment of ^{1}H NMR signals of most penicillins has been completed, and we can depend on the published data of penicillins. Our own measurements and assignments (Table 2) are consistent with the previous data.

The situation is somewhat different for ^{13}C NMR spectroscopy. Most of the available data are based on chemical shift consideration, and modern techniques such as two-dimensional spectroscopy have not been employed so often. With this in mind we determined ^{13}C one-dimensional

Table 2. ^{1}H NMR chemical shifts[a,b] for penicillins **1, 2** and **3**

Assignment	1	2	3
H2	4.23 (4.23)	4.6 (4.52)	4.52 (4.52)
Me3α	1.51 (1.50)	1.55 (1.52)	1.55 (1.52)
Me3β	1.53 (1.53)	1.55 (1.52)	1.55 (1.52)
H5	5.51 (5.58)	5.58 (5.53)	5.53 (5.53)
H6	5.51 (5.57)	5.6 (5.59)	5.59 (5.59)
PhCH_2 or PhC*H*	3.61 (3.60)	4.4 (4.28)	4.28 (4.28)
Aromatic	7.41 (7.29)	6.9–7.3 (6.8–7.6)	6.8–7.6 (6.8–7.6)

[a] In $CDCl_3$: chemical shifts in ppm (δ_H) relative to internal tetramethylsilane (TMS).
[b] Values in parentheses are taken from Ref. 8.

(1D) NMR spectra, distortion enhancement by polarization transfer (DEPT), heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple bond coherence (HMBC) spectra of **1–3**, **9a–9c**, **10a–10c**, **11a–11c** and **12**. As expected, the use of ^{1}H–^{13}C correlations facilitated the assignment of ^{13}C signals to a considerable extent.

As an example, the detailed analysis of the ^{13}C NMR spectra of **1** (as the potassium salt) will be presented. The procedure is applicable to the spectral analyses of all other compounds, including germylated penicillins.

The DEPT spectra of **1** are shown in Fig. 3. Figure 3(a) is the ^{13}C 1D spectrum with broadband decoupling in which each signal is given a letter code (a to k). Altogether, 14 signals are recorded. With the aid of DEPT-135 (Fig. 3b) and DEPT-90 (Fig. 3c), it is possible to differentiate ^{13}C signals based on the number of attached protons (step 1).

Signals e, k, l, m and n disappear in both DEPT-135 and DEPT-90. Hence, these are due to quaternary carbon nuclei. Since the signal k is in the aromatic region (*ipso*-carbon) and signals l, m and n are in the carbonyl region, signal e is unequivocally assigned to C3.

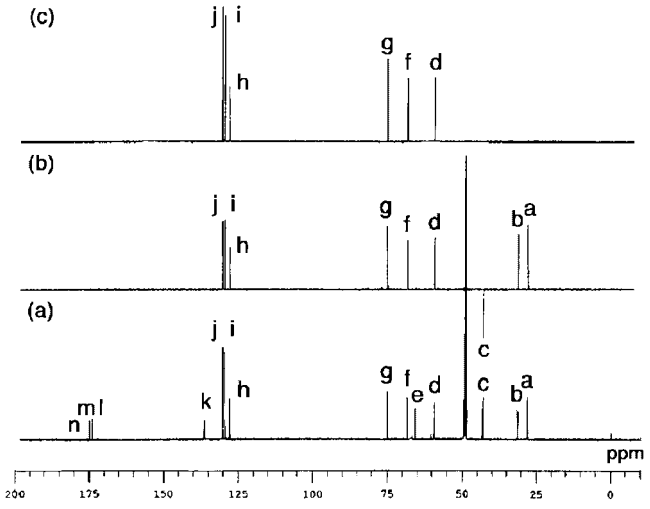


Figure 3. ^{13}C DEPT spectra of **1**: (a) broadband decoupling; (b) DEPT-135; (c) DEPT-90.

The signal e inverts in DEPT-135. Hence, it is due to a CH_2 and the signal c is assigned to $PhCH_2CO$. Signals a and b disappear only in DEPT-90 and are due to methyl groups.

Three signals in the aliphatic region, d, f and g, and three other signals in the aromatic region, i, j, and k, remain in both DEPT-135 and DEPT-90. These, then are due to CH carbon nuclei. Hence, signals d, f and g belong to C2, C5 or C6, and signals h, i and j are the *o*-, *m*- and *p*-carbon nuclei of the benzene ring. From the signal intensity, it is possible to assign peak i to the *p*-carbon. The assignments by step 1, together with the assignments by steps 2 and 3, are shown in Table 3. Step 2 is based on the use of the HMQC spectrum (Fig. 4). Four assignments remain: a/b, d/f/g, i/j and l/m/n signals.

Fig. 4 clearly indicates that signals a and b correlate with proton signals A(Me3α) and B(Me3β), establishing that signals a and b are assigned to Me3α and Me3β respectively. The correlation between signal g and proton signal D established that signal g is assigned to C2.

To complete the remaining assignments, i.e. differentiation between d/f, i/j and l/m/n signals, HMBC is expected to be useful, which is used in step 3. The HMBC spectrum of **1** is shown in Fig. 5.

Table 3. Assignment of ^{13}C signals of **1**

	Step 1	Step 2	Step 3
C2	d/f/g	g	g
C2-COOH	l/m/n	l/m/n	n
C3	E	e	e
Me3α	a/b	a	a
Me3β	a/b	b	b
C5	d/f/g	d/f	f
C6	d/f/g	d/f	d
C7	l/m/n	l/m/n	l
CONH	l/m/n	l/m/n	m
$PhCH_2$	C	c	c
ipso-Ph	k	k	k
o-Ph	i/j	i/j	j
m-Ph	i/j	i/j	i
p-Ph	h	h	h

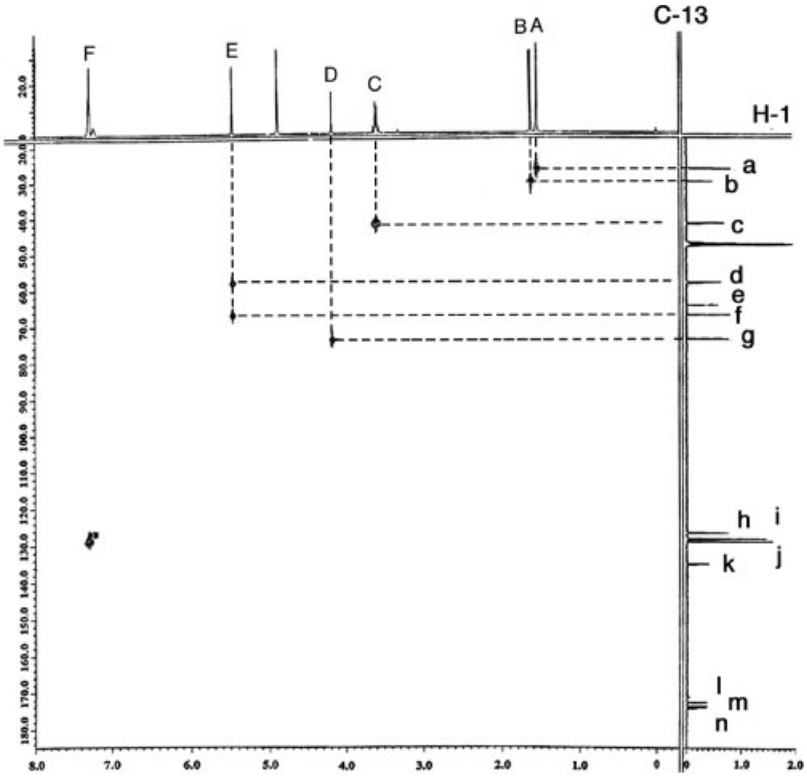


Figure 4. HMQC spectrum of 1.

Figure 5a shows a portion of the HMBC spectrum of **1**, which shows the correlation between ^{13}C nuclei and proton peak D. Figure 5a clearly indicates that signal f is correlated with proton signal D(H2), whereas signal d does not correlate with that proton signal. It is certain that the observed correlation is due to a ^{3}J(C5–H2) coupling, establishing that the signal f is assigned to C5, and hence signal d to C6.

Figure 5b provides another piece of important information obtainable from the HMBC spectrum of **1**, showing the correlation between ^{13}C nuclei and proton peaks C and D. Of carbon signals i and j, only signal j shows a correlation with proton peak C($PhCH_2CO$) due to ^{3}J(C*ortho*–$PhCH_2$), proving that signal j comes from the *o*-carbon (and hence signal i from the *m*-carbon).

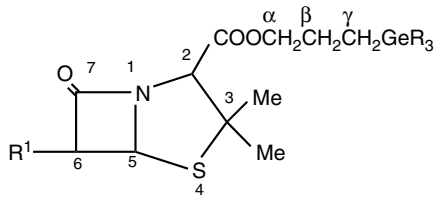
Similarly, of the carbon signals l, m and n, only signal m shows a correlation with proton peak C($PhCH_2CO$). Thus, signal m is assigned to the $PhCH_2CO$ carbon nucleus. The correlation between carbon signal n and proton signal D(H2) indicates that signal n is now assigned to COOR bonded to C2 to complete all the assignments of the ^{13}C spectra of **1**.

Thus, an unequivocal assignment of the ^{13}C NMR spectrum of **1** was completed. The assignment of the ^{13}C NMR spectrum of **2** and **3** was carried out in a similar manner without any ambiguity.

Table 4 lists the data for **1**–**3**, together with the values reported by previous authors.[11] The ^{13}C NMR spectrum of **3** is not reported previously.

The assignment of the signals from the ester parts, based on HMQC and chemical shift considerations, is also straightforward.

The full assignments of the ^{1}H and ^{13}C signals of compounds **9a**–**9c, 10a**–**10b, 11a**–**11c** and **12** are given in Tables 5 and 6 respectively. The numbering of the atoms used in the tables is given below.



R^1	R
1,9: $PhCH_2CONH$	**a**:CH_3
2,10: $PhOCH_2CONH$	**b**:CH_3CH_2
3',11: $PhCH(NH{=}CHC_6H_5)CONH$	**c**:C_6H_5

Pharmacology

Germylated penicillins **9a**–**9c, 10c, 11a**–**11c** and **12** were screened for their *in vitro* antibacterial activity and the results were compared with the activity of **1**. The study was carried out to evaluate the inherent activity of

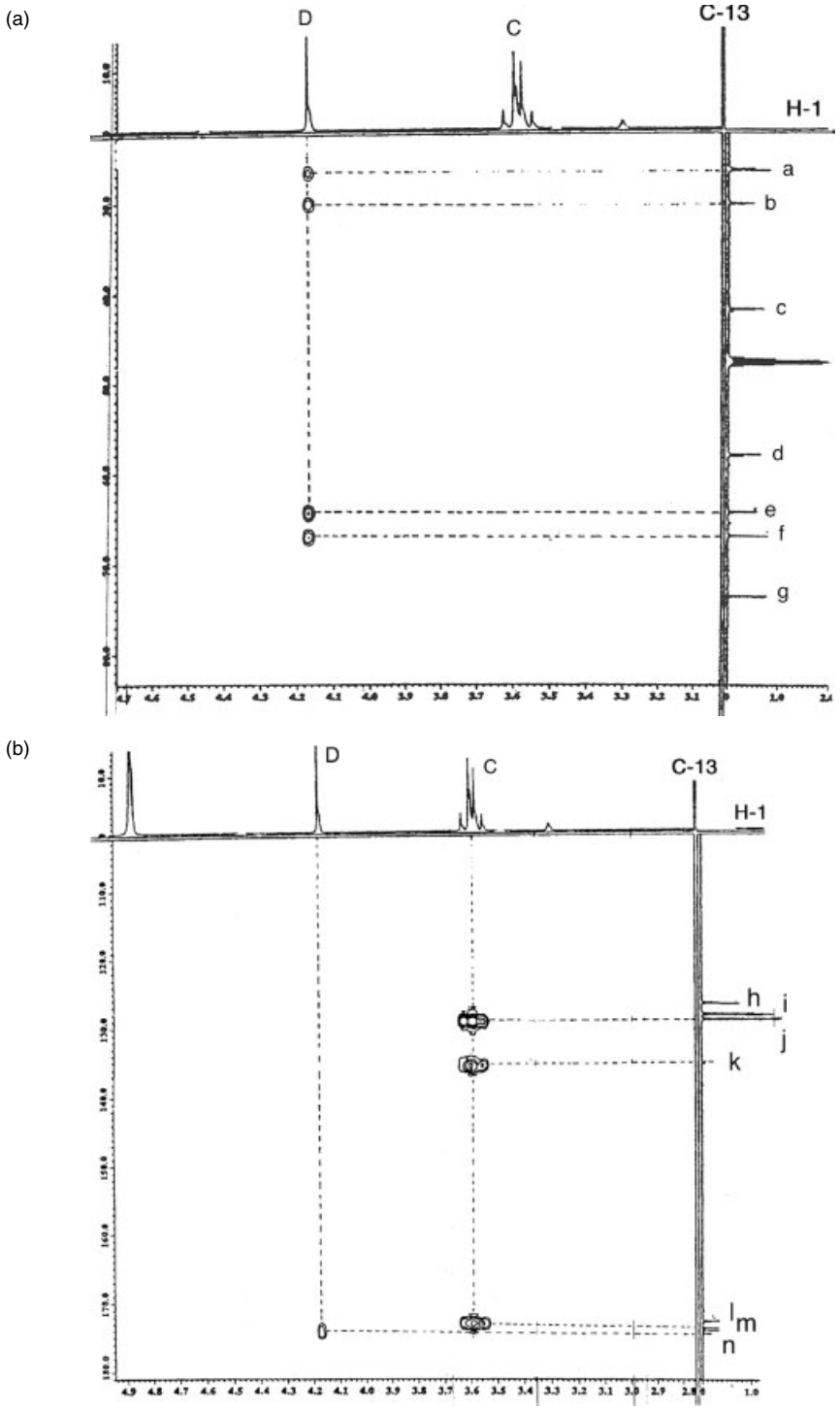


Figure 5. HMBC spectrum of **1**: (a) high-field part of ^{13}C signals; (b) low-field part of ^{13}C signals.

germylated penicillins against Gram-positive and Gram-negative microorganisms. The test was carried out by Shionogi Research Laboratories.

The antibacterial activity was determined after the incubation of bacterial strains at 37 °C for 18 h on Mueller–Hinton agar by using the standard agar-dilution method.

The activity was determined in terms of minimum inhibitory concentrations (mg ml^{-1}). A larger value indicates that the relevant germylated penicillin is less effective than **1**. Unfortunately, all germylated penicillins show larger values than the corresponding value for **1** for all microorganisms investigated.

The activity of germylated penicillins was tested for the following microorganisms: *S. aureus* Smith, *S. aureus* SR3637(H-MRSA), *Saphylococcus epidermidis* ATCC14990, *S. epidermidis* SR25009(MRSE), *S. pneumoniae* type I, *S. pneumoniae* SR16675(PRSP), *Enterococcus faecalis* ATTC49757(Bla+), *E. faecium* SR7917(van A), *E. faecium* SR23546, *Escherichia coli* NIHJ JC-2, *E. coli* SR21003(Toho2), *Klebsiella pneumoniae* SR1, *K. pneumoniae* ATTC700603(SHV), *Enterobacter cloacae* ATCC13047, *E. cloacae* SR4321(bla++), *Serratia marcescens* ATCC13880, *Pseudomonas aeruginosa* ATCC25619, *P. aeruginosa* SR6554(IPM-R), *Haemophilus influenzae* ATCC49766, *H. influenzae* SR11435(BLNAR), *Branhamella catarrhalis* ATCC43617(bla+).

Table 4. ^{13}C chemical shifts of **1**, **2** and **3**

	1		**2**		
Assignment	δ_c	Lit. value	δ_c	Lit. value	**3** δ_c
C2	64.32	64.6	64.76	64.8	64.43
C2–COOH	173.85	174.1	171.03	173.8	174.57
C3	73.69	73.5	70.49	73.4	64.43
3Meβ	26.26	26.8	26.96	26.8	26.48
3Meα	30.29	31.6	31.6	31.6	30.09
C5	66.94	66.9	67.58	66.8	66.63
C6	58.07	58.1	58.08	64.8	58.13
C7	173.51	174.7	173.06	174.0	173.93
CONH	172.60	173.1	168.86	169.6	168.76
$PhCH_2$	42.82	42.3	67.10	66.8	56.60
ipso-Ph	135.38	134.7	157.67	156.9	131.74
o-Ph	128.99	129.5[a]	129.43	114.9	128.39
m-Ph	128.29	128.9[a]	114.57	130.1	129.77
p-Ph	126.71	127.3	121.71	122.2	130.63

[a] Literature values are taken from Ref. 12.

CONCLUSIONS

Initially we anticipated that selective germylation would be difficult. To our surprise, the reaction proceeded smoothly, and the germylated penicillins were synthesized in a reasonable yield. NMR spectra indicate that all germylated penicillins are pure enough to be characterized by MALDI-TOF mass spectrometry and NMR.

Unfortunately, the antibacterial activity of these germylated penicillins is lower than that of the starting antibiotics. This might indicate that the presence of carboxy moieties in the structure will be one of the essential factors for their biological activity.

Attempts to synthesize novel antibiotics germylated at a site different from the carboxy function are in progress in our laboratory.

EXPERIMENTAL

General

^{1}H NMR spectra were determined with a JEOL ECP 500 spectrometer operating at 500 MHz, and the chemical shifts were reported in δ (ppm) with respect to TMS as the internal standard. ^{13}C NMR spectra were determined on the same spectrometer operating at 125 MHz and the chemical shifts were reported in δ (ppm), also with respect to TMS. Mass spectra were recorded on a PerSeptive Biosystems DE MALDI-TOF mass spectrometer, Voyager Elite XL.

Synthesis of organogermanium propyl tosylates (8a–8c) and organogermanium iodides (4a–4c)

General procedure

3-Substitutedgermyl-1-propanol (**7a**–**7c**) was prepared by the LAH reduction of the corresponding 3-substitutedgermyl-1-propanoic acid (**6a**–**6c**),[6] which in turn was prepared by the

Table 5. ^{1}H chemical shifts (δ_H) for **9a**–**9c**, **10a**–**10b**, **11a**–**11c** and **12**

	9a	**9b**	**9c**	**10a**	**10b**	**11a**	**11b**	**11c**	**12**
2H	4.29	4.339	4.38	4.44	4.44	4.49	4.49	4.47	4.33
3Meα	1.36	1.39	1.43	1.58	1.57	1.61	1.62	1.61	1.41
3Meβ	1.38	1.41	1.42	1.48	1.48	1.41	1.47	1.43	1.37
5H	5.41	5.46	5.47	5.57	5.56	5.55	5.55	5.51	5.43
6H	5.51	5.58	5.64	5.72	5.71	5.71	5.72	5.73	5.62
$PhCH_2$ or PhCH	3.51	3.58	3.64	4.54	4.52	5.01	5.02	5.04	3.62
Aromatic	7.16–7.25	7.2–7.3	7.1–7.7	6.9–7.4	6.9–7.4	7.2–8.3	7.22–8.25	7.25–8.31	7.1–7.5
α	4.01	4.04	4.15	4.11	4.11	4.13	4.11	4.13	4.06
β	1.61	1.64	1.88	1.69	1.69	1.73	1.73	1.88	1.73
γ	0.59	0.64	1.37	0.68		0.67	0.68	1.55	1.23
$GeCH_3$	0.04	–	–	0.12	0.72	0.13	–	–	
$GeCH_2CH_3$	–	0.69	–		0.99	–	0.71	–	
$GeCH_2CH_3$	–	0.97	–			–	1.03	–	
GeC_6H_5	–	–	7.1–7.7			–	–	7.25–8.31	7.1–7.5

Table 6. ^{13}C NMR chemical shifts (δ_C) for **9a–9c, 10a–10c** and **11a–11c** and **12**

	9a	9b	9c	10a	10b	10c	11a	11b	11c	12
C2	70.38	70.44	70.42	70.58	70.59	70.58	70.5	70.5	70.41	70.49
C2–COOR	167.7	167.7	167.9	167.9	167.9	168.1	167.9	168.0	167.9	167.9
C3	64.42	64.48	64.50	64.79	64.77	64.39	64.5	64.3	64.65	64.38
Me3α	26.80	26.84	26.50	26.95	26.94	26.89	26.53	26.52	26.53	26.92
Me3β	31.96	32.06	32.03	32.14	32.09	32.10	32.32	32.33	32.31	32.16
C5	68.15	68.19	68.10	67.91	67.89	67.97	68.39	68.40	68.46	68.12
C6	59.03	58.96	59.00	59.11	58.18	58.19	58.92	58.79	58.69	58.82
$PhCH_2$ or PhCH	43.00	43.29	43.92	67.22	67.21	67.30	76.12	76.20	76.10	43.91
Aromatic-*ipso*	134.23	134.08	126.2–138.1	157.12	157.02	114.2–137.5	127.5–139.1	127.5–139.5	127.0–139.1	127.0–137.3
Aromatic-*o*	129.48	129.57	126.2–138.1	130.01	129.89	114.2–137.5	127.5–139.1	127.5–139.5	127.0–139.1	127.0–137.3
Aromatic-*m*	128.94	129.08	126.2–138.1	114.31	114.83	114.2–137.5	127.5–139.1	127.5–139.5	127.0–139.1	127.0–137.3
Aromatic-*p*	127.41	127.57	126.2–138.1	122.12	122.42	114.2–137.5	127.5–139.1	127.5–139.5	127.0–139.1	127.0–137.3
α	68.11	68.45	67.91	68.24	68.46	68.20	68.15	68.38	68.10	68.95
β	24.24	24.41	24.32	24.33	24.44	24.23	24.31	24.25	24.23	24.18
γ	12.41	7.24	9.55	12.48	7.27	10.08	12.51	7.59	10.11	9.57
$GeCH_3$	−2.39	—	—	−2.36	—	—	−2.35	—	—	—
$GeCH_2CH_3$	—	3.86	—	—	3.89	—	—	3.92		—
$GeCH_2CH_3$	—	9.00	—		9.01		—	9.02		
Ge*Ph*			126–138			114.2–137.5			127.0–139.1	127.0–137.3
N=CPh							127.5–139.1	127.5–139.5	127.0–139.1	

Grignard reaction between 3-trichlorogermylpropanoic acid (**5**) and appropriate Grignard reagents.[6]

The alcohol was converted to the corresponding tosylate (**7a–7c**) which was reacted with NaI to afford 3-substitutedgermyl-1-propyl iodide (**4a–4c**).

A typical example

To a pyridine (12 ml) solution of 3-trimethylgermyl-1-propanol (**7a**; 3.54 g, 0.02 mol) was added *p*-toluensulfonyl chloride (TsCl; 2.7 g, 0.029 mol) with stirring under ice cooling. The mixture was stirred for an additional 5 h at room temperature and was acidified by 1 mol dm^{-3} HCl. The solution was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, concentrated to give 3-trimethylgermyl-1-propyl tosylate (**8a**; 4.61 g, 0.014 mol) as a colorless liquid in 70% yield.

To an anhydrous acetone (50 ml) solution of NaI (3.0 g, 0.020 mol) was added dropwise a solution of **8a** (4.61 g, 0.014 mol) in anhydrous acetone (20 ml) under reflux with stirring. After the mixture was stirred for an additional 5 h under reflux, it was cooled to room temperature and concentrated. Water was added to the residue and the organic layer was extracted by CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to give 3-trimethylgermyl-1-propyl iodide (**4a**; 2.88 g, 0.01 mol) as a yellow liquid in 72% yield.

The other iodides **4b** and **4c** were prepared in a similar manner. The iodide **4d** was prepared from bis(3-hydroxy-1-propyl)diphenylgermane (**7d**).[12] The amounts of reagents used and the yields are given in Table 7.

Table 7. Preparation of germylated iodides (**4a–4c**)

Alcohol [mmol]	Tosyl chloride (mmol)	Tosylate [yield (%)]	Tosylate (mmol)	NaI (mmol)	Iodide [yield (%)]
7a [20]	29	**8a** [70]	14	20	**4a** [72]
7b [10]	22	**8b** [76]	7.6	13	**4b** [43]
7c [1.7]	2.6	**8c** [36]	0.94	1.9	**4c** [84]
7d [16.8]	17.2	**8d** [70]	0.94	1.9	**13** [84]

Characterization of 8a–8c and 4a–4c

The tosylates **8a–8c** and the iodides **4a–4c** were characterized by ^1H and ^{13}C NMR spectroscopy. The results are summarized in Table 8. The same numbering as in Tables 5 and 6 is used.

Reaction of penicillins 1 and 2 with germyl iodides (4a–4d)

Typically, to an anhydrous DMF (50 ml) solution of **1** (1.86 g, 0.005 mol) was added dropwise an anhydrous DMF (20 ml) solution of **4a** (1.435 g, 0.005 mol) at room temperature with stirring for 24 h. The mixture was added to ice water and the organic layer was separated, washed with 2% $NaHCO_3$ solution and water, and finally dried over $MgSO_4$. The organic layer was concentrated and purified by high-performance liquid chromatography (gel permeation chromatography column) to give 3-(trimethylgermyl)-1-propyl-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-

Table 8. NMR data for tosylates (**8a**–**8c**) and iodides (**4a**–**4c**)

X	8a	8b	8c	4a	4b	4c
^{1}H NMR parameters						
α	3.85 t	3.85 t	4.00 t	3.10 m	3.09 m	4.92 m
β	1.57 m	1.52 m	1.83 m	1.79 m	1.85 m	2.15 m
γ	0.51 m	0.45 m	1.44 m	0.69 m	0.74 m	1.52 m
$GeCH_3$	0.13 s	—	—	0.13 s	—	—
$GeCH_2CH_3$	—	0.51 m	—	—	0.68	—
$GeCH_2CH_3$	—	0.85 m	—	—	0.99 s	—
GeC_6H_5	—	—	7.20–7.80 m	—	—	7.2–7.8 m
$C_6H_4CH_3$	2.30 s	2.30 s	2.43 s	—	—	—
$C_6H_4CH_3$	7.20–7.80 m	7.20–7.80 m	7.20–7.80 m	—	—	—
^{13}C NMR parameters						
α	71.16	71.16	71.16	30.10	30.50	63.0
β	22.51	22.51	25.90	19.21	13.62	30.1
γ	11.15	6.10	9.5	11.12	11.15	9.50
$GeCH_3$	−2.34	—	—	−2.34	—	—
$GeCH_2CH_3$	—	3.25	—	—	4.21	—
$GeCH_2CH_3$	—	8.52	—	—	9.10	—
GeC_6H_5	—	—	125–148	—	—	125–147
$C_6H_4CH_3$	21.33	21.15	21.73	—	—	—
$C_6H_4CH_3$	125–149	125–149	125–148 m	—	—	—

Table 9. Synthesis of germylated penicillins

Penicillin [mmol]	Iodide [mmol]	Product [yield (%)]
1 [5]	**4a** [5]	**9a** [49]
1 [5]	**4b** [5]	**9b** [52]
1 [5]	**4c** [5]	**9c** [41]
2 [4]	**4a** [5]	**10a** [49]
2 [5]	**4b** [5]	**10b** [55]
2 [4]	**4c** [5]	**10c** [39]
3′ [5]	**4a** [5]	**11a** [29]
3′ [5]	**4b** [5]	**11b** [25]
3′ [5]	**4c** [5]	**11c** [25]
1 [2.7]	**4d** [0.88]	**12** [32]

carboxylate (**9a**) as a colorless viscous liquid in 49% yield (1.24 g, 0.002 mol).

The other reactions were carried out similarly. The results of these reactions are summarized in Table 9.

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