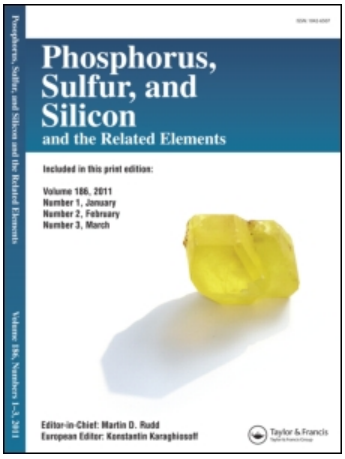


## Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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**To cite this Article** Tacke, R. , Heinrich, T. , Kornek, T. , Merget, M. , Wagner, S. A. , Gross, J. , Keim, C. , Lambrecht, G. , Mutschler, E. , Beckerss, T. , Bernd, M. and Reissmann, T.(1999) 'Bioorganogermanium Chemistry: Studies on C/Si/Ge Bioisosterism', Phosphorus, Sulfur, and Silicon and the Related Elements, 150: 1, 69 — 87

**To link to this Article: DOI:** 10.1080/10426509908546372

**URL:** http://dx.doi.org/10.1080/10426509908546372

*Phosphorus, Sulfur and Silicon*, 1999, Vol. 150-151, pp. 69-87
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# Bioorganogermanium Chemistry: Studies on C/Si/Ge Bioisosterism

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In context with systematic investigations on C/Si/Ge bioisosterism, the following studies were carried out: (a) synthesis and pharmacological characterization of centrochiral enantiomerically pure germanium-based muscarinic antagonists; (b) synthesis and pharmacological characterization of a germanium-containing decapeptide; (c) studies on the metabolism of a germanium-based drug in the rat; (d) synthesis of centrochiral enantiomerically pure germanes using biotransformations with whole microorganisms or isolated enzymes. These investigations demonstrated that there are distinct bioisosteric relationships between the C/Si/Ge analogues studied.

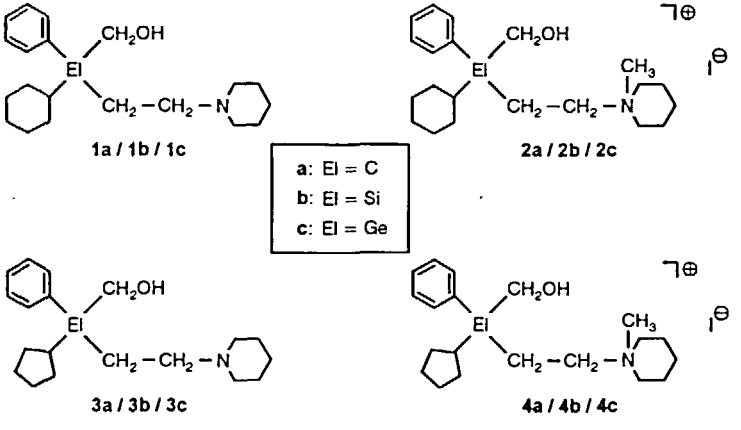
*Keywords:* bioorganogermanium chemistry; C/Si/Ge bioisosterism; sila-and germa-drugs; silicon- and germanium-containing α-amino acids and peptides; metabolism of sila- and germa-drugs; stereoselective biocatalysis

## INTRODUCTION

Bioorganosilicon chemistry represents a fascinating, rapidly expanding branch of organosilicon chemistry (for reviews, see refs. 1–8). In recent years, practical aspects of this research field have become of increasing importance. The development of silicon-based drugs and agrochemicals and the application of biocatalysis in synthetic organosilicon chemistry are examples of this. Compared to the extensive research activities in bioorganosilicon chemistry, bioorganogermanium chemistry is significantly less explored (for reviews, see refs. 9 and 10). In this article we report on some results of our own studies in this particular field. The investigations presented here were carried out with a special emphasis on the aspect C/Si/Ge bioisosterism.

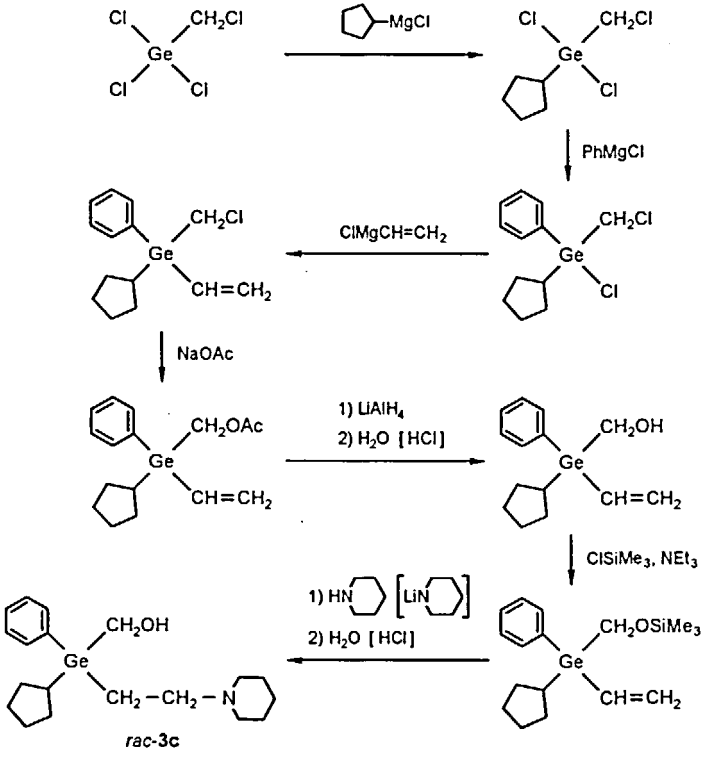
## CHIRAL GERMANIUM-BASED MUSCARINIC ANTAGONISTS

During the past decade, we have developed a variety of highly potent and receptor-selective silicon-based muscarinic antagonists (for selected publications, see refs. 11–15 and references cited therein). Compound



(*R*)-**2b** is an example of this particular type of drug.[11]

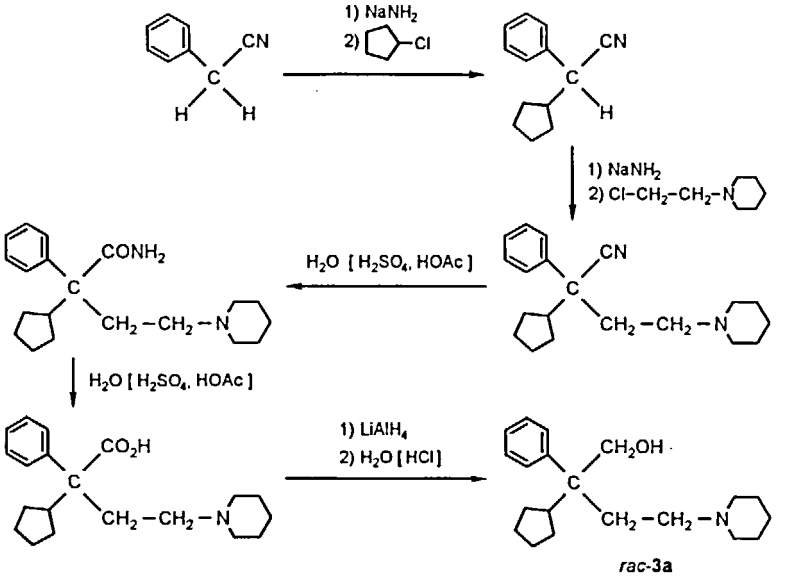
In the course of these studies, we became also interested in the muscarinic receptor binding of related carbon and germanium analogues, such as compounds (*R*)-**2a**[15,16] and (*R*)-**2c**[14]. We report here on the syntheses and antimuscarinic properties of the enantiomerically pure (*R*)- and (*S*)-enantiomers of the centrochiral C/Si/Ge analogues **1a/1b/1c**, **2a/2b/2c**, **3a/3b/3c**, and **4a/4b/4c**.




SCHEME 1

The strategy for the synthesis of the racemic germanes **1c**[14] and **3c**[17] is

outlined in Scheme 1 (example: synthesis of *rac*-**3c**).The corresponding silicon analogues *rac*-**1b**[11] and *rac*-**3b**[17] were prepared analogously [starting from $(MeO)_3SiCH_2Cl$], whereas the carbon compounds *rac*-**1a**[16] and *rac*-**3a**[17] were obtained by a quite different approach as shown in Scheme 2 (example: synthesis of *rac*-**3a**).

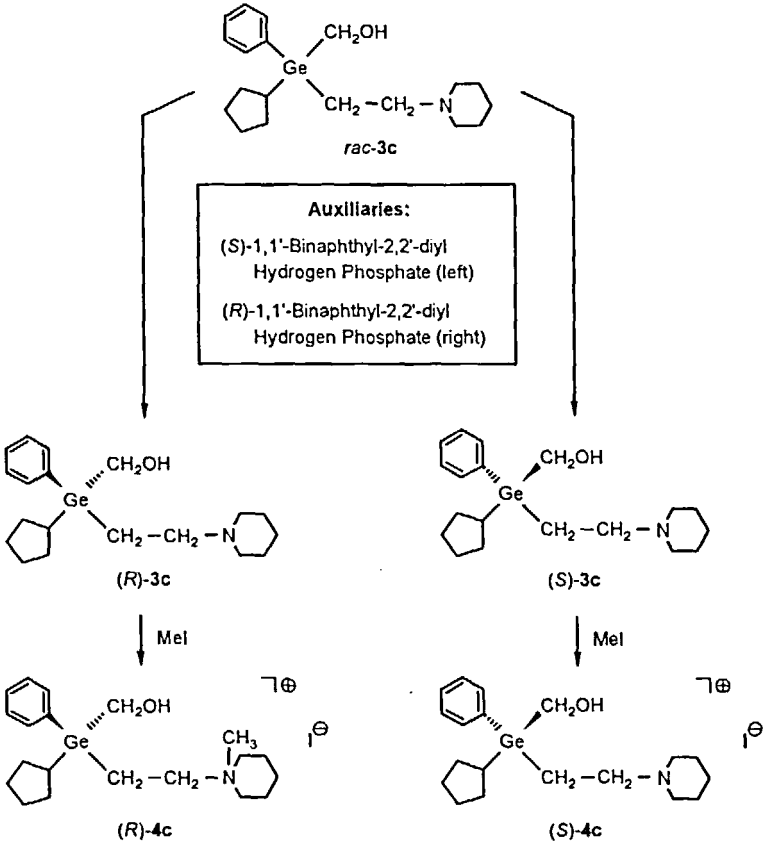



*rac*-**3a**

SCHEME 2

As demonstrated in Scheme 3, the enantiopure compounds (*R*)-**3c** and (*S*)-**3c** were obtained by classical resolution of *rac*-**3c** using the antipodes of 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate as resolving agents.[17] Subsequent quaternization of (*R*)-**3c** and (*S*)-**3c** with methyl iodide gave the enantiopure compounds (*R*)-**4c** and (*S*)-**4c**, respectively.[17] The (*R*)- and (*S*)-enantiomers of **1a**,[16] **1b**,[11] **1c**,[14] **2a**,[16] **2b**,[11] **2c**,[14] **3a**,[17] **3b**,[17] **4a**,[17] and **4b**[17] were prepared analogously. For these syntheses, the

antipodes of 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (for **1a**), 2,3-di-*p*-toluoyltartaric acid (for **1b**, **1c**, and **3b**), and 2,3-dibenzoyltartaric acid (for **3a**) were used as resolving agents.




SCHEME 3

The (*R*)- and (*S*)-enantiomers of the C/Si/Ge analogues **1a/1b/1c** and **2a/2b/2c** were studied in functional pharmacological experiments for their affinities ($pA_2$ values) at muscarinic M1 (rabbit vas deferens), M2 (guinea-pig atria), and M3 receptors (guinea-pig ileum) using 4-F-PyMcN$^+$ (M1)

or arecaidine propargyl ester (M2, M3) as the agonist.[11,14,16] The antipodes of the C/Si/Ge analogues **3a/3b/3c** and **4a/4b/4c** were investigated for their affinities ($pK_i$ values) at recombinant human muscarinic receptor subtypes m1–m5 stably expressed in CHO cells (binding studies with [$^3$H]-*N*-methylscopolamine as the radioligand).[17] Generally, very similar stereoselectivities and pharmacological selectivities were observed in these studies for the respective C/Si/Ge analogues, indicating strongly pronounced bioisosteric relationships between these analogous carbon, silicon, and germanium compounds. This is illustrated for the antipodes of **2a/2b/2c** and **4a/4b/4c** in Figures 1 and 2.



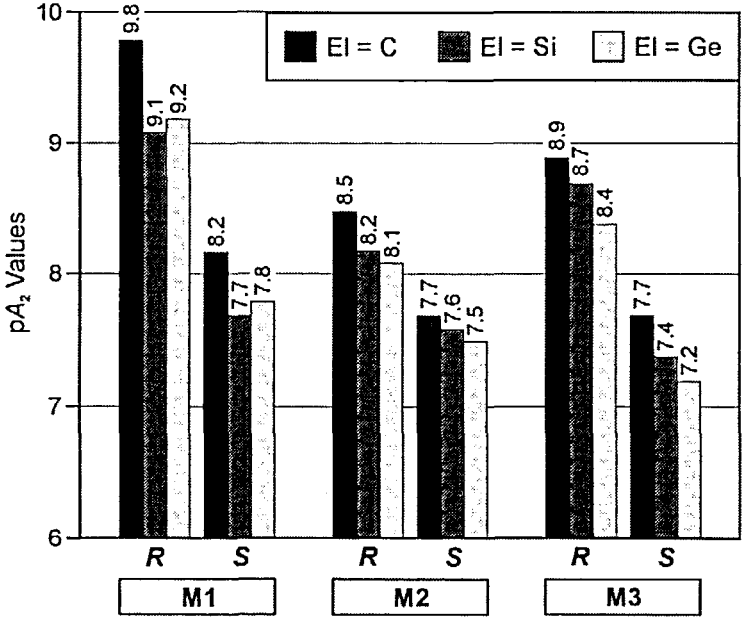



FIGURE 1 Affinity profiles of the antipodes of the C/Si/Ge analogues **2a/2b/2c** at muscarinic M1, M2, and M3 receptors.

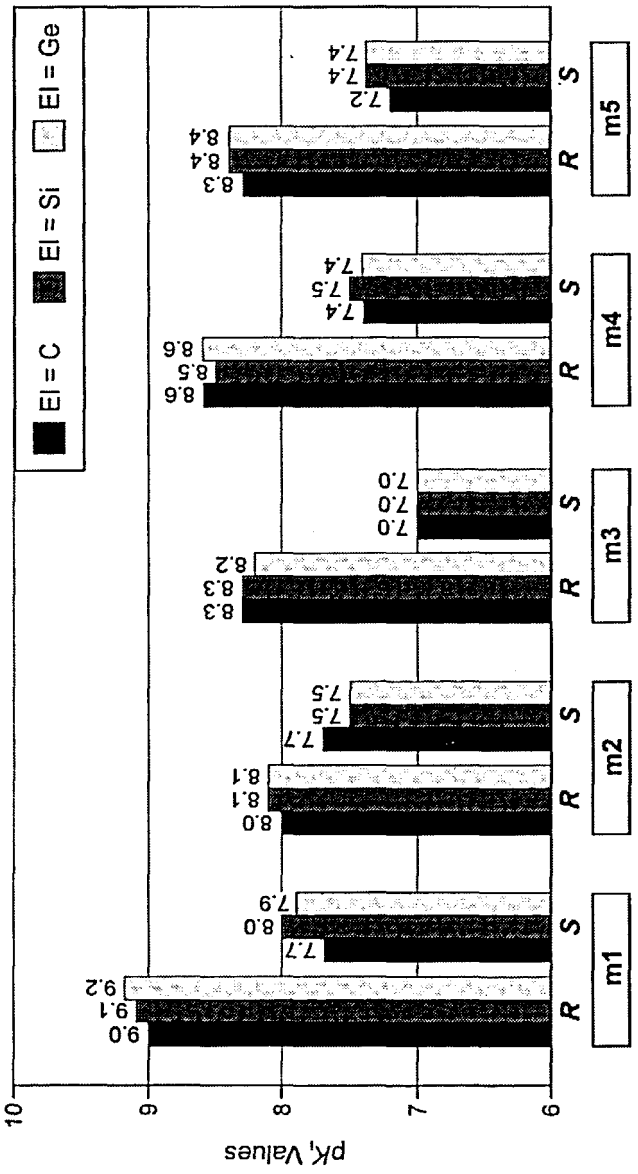
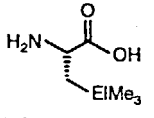



FIGURE 2 Affinity profiles of the C/Si/Ge analogues **4a/4b/4c** at human muscarinic m1, m2, m3, m4, and m5 receptors.

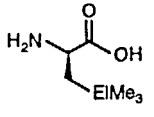
## GERMANIUM-CONTAINING α-AMINO ACIDS AND A GERMANIUM-CONTAINING DECAPEPTIDE

Synthetic amino acids with unnatural side chains have proven useful for probing the structural requirements for the biological activity of numerous peptides and proteins. In addition, unnatural amino acids are of interest as precursors of drugs and plant-protective agents. In context with our studies on silicon-containing α-amino acids, we have succeeded in synthesizing the first germanium-containing α-amino acids, *L*- and *D*-(trimethylgermyl)-alanine [*L*-**5c** and *D*-**5c**].[18]



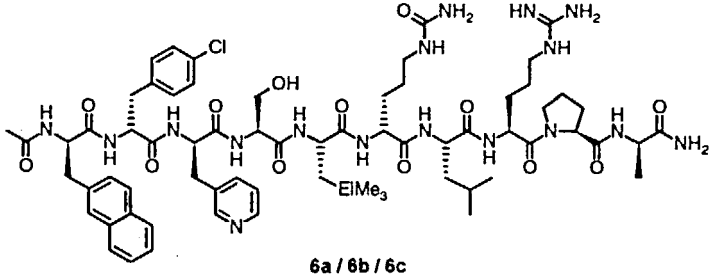
*L*-**5a** / *L*-**5b** / *L*-**5c**

**a**: El = C
**b**: El = Si
**c**: El = Ge



*D*-**5a** / *D*-**5b** / *D*-**5c**

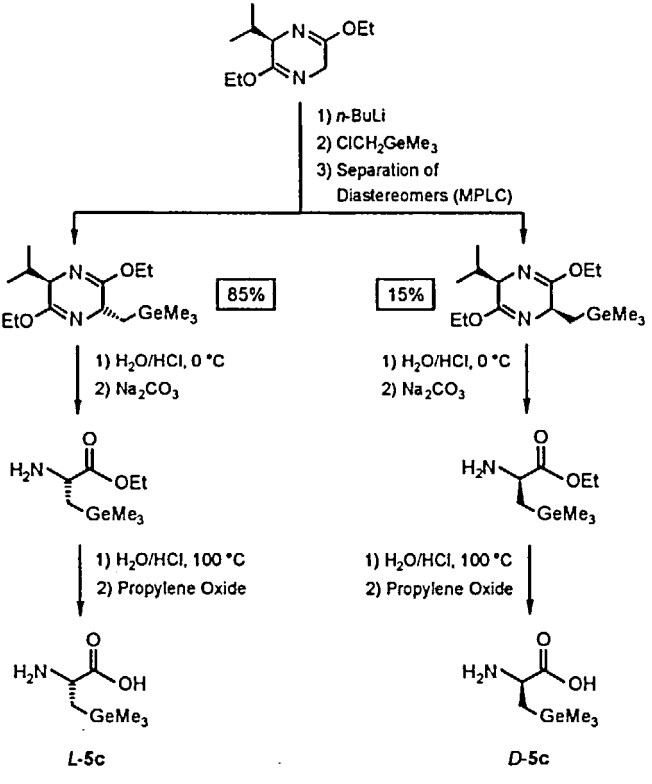
These compounds and their corresponding carbon and silicon analogues, the α-amino acids *L*-**5a**, *D*-**5a**, *L*-**5b**, and *D*-**5b**, are interesting building blocks for new biologically active peptides.



**6a** / **6b** / **6c**

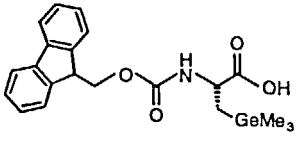
**a**: El = C
**b**: El = Si
**c**: El = Ge

We report here on the first germanium-containing peptide, the decapeptide **6c**, which is a derivative of the GnRH (gonadotropin-releasing hormone) antagonist Cetrorelix[INN] (replacement of *L*-tyrosine by *L*-**5c** in position 5; for recent reviews dealing with Cetrorelix, see refs. 19 and 20). The biological properties of the germanium-containing decapeptide **6c** were compared with those of its corresponding carbon (**6a**) and silicon analogue (**6b**).[21]




SCHEME 4

The $\alpha$-amino acids *L*-**5c** and *D*-**5c** were prepared according to Scheme 4 by three-step syntheses and isolated as almost enantiomerically pure crystalline products.[18] The decapeptide **6c** was prepared by solid-phase synthesis (SPPS) using the Fmoc-protected $\alpha$-amino acid, compound *L*-7.[21]




*L*-7



The decapeptides **6a** and **6b** were synthesized analogously. The identity of **6a**, **6b**, and **6c** was established by NMR studies and mass-spectrometric investigations. As an example of this, an electrospray mass spectrum of the germanium-containing decapeptide **6c** is shown in Figure 3.

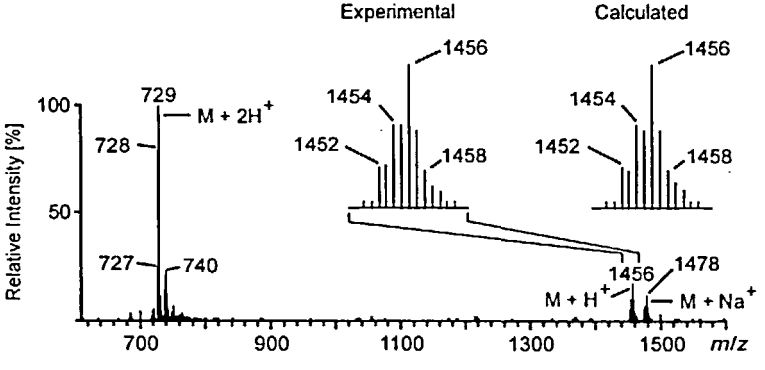



FIGURE 3 Electrospray mass spectrum of the germanium-containing decapeptide **6c**.

The C/Si/Ge analogues **6a/6b/6c** were studied for their antagonistic potencies at the human GnRH receptor using a functional reporter gene assay (tiptorelin as the agonist). The $IC_{50}$ values [nM] determined were as

follows: 0.93 (**6a**), 0.75 (**6b**), 0.50 (**6c**). These preliminary data demonstrate that all decapeptides are potent GnRH antagonists that differ only slightly in their antagonistic potencies, the germanium compound **6c** being somewhat more potent than its carbon and silicon analogue. Thus, there are distinct bioisosteric relationships between the C/Si/Ge analogues **6a/6b/6c**.

Compounds **6a**, **6b**, and **6c** were also studied for their in vivo properties in the castrated male rat after s.c. administration (0.05 mg/kg). As illustrated for the germanium-containing decapeptide **6c** in Figure 4, a serum LH (luteinizing hormone) suppression was observed after administration of the C/Si/Ge analogues **6a/6b/6c**. Again, distinct bioisosteric relationships were observed for these compounds. Interestingly, the decapeptides **6b** and **6c** were found to act ca. 6 times longer than their carbon analogue **6a**.



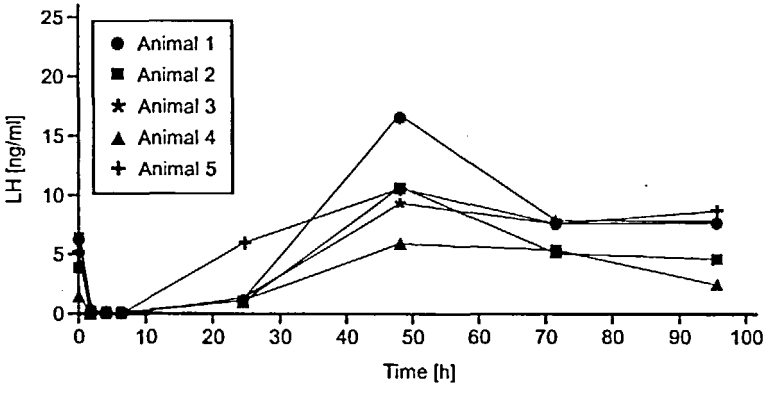
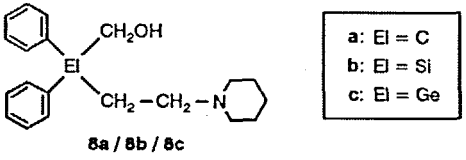



FIGURE 4 Serum LH suppression in the castrated male rat after s.c. administration of the germanium-containing decapeptide **6c** (0.05 mg/kg).

## METABOLISM OF A GERMANIUM-BASED MUSCARINIC ANTAGONIST IN THE RAT

The C/Si/Ge analogues **8a/8b/8c** were studied for their metabolic fate in the rat.[22]




**8a / 8b / 8c**



The phase-I metabolism of these muscarinic antagonists was investigated after p.o. administration of 80 mg/kg. For this purpose, urine samples (collected for a period of 24 hours after administration) were studied with

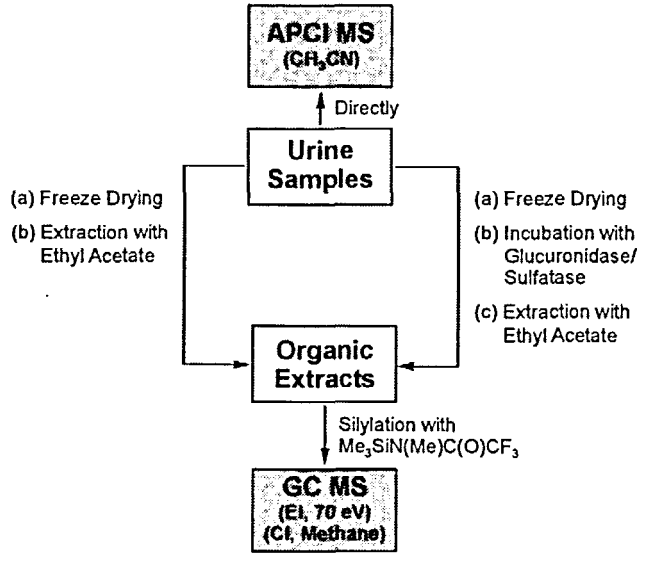



FIGURE 5 Experimental design used for the investigation of the phase-I metabolism of the C/Si/Ge analogues **8a/8b/8c** in the rat after p.o. administration.

mass-spectrometric techniques (for the experimental design, see Figure 5). As shown in Figure 6, APCI MS studies of the untreated urine samples demonstrated the presence of the unchanged parent drugs **8a/8b/8c** (peaks *A*) and three types of metabolites (peaks *B*, *C*, and *D*).

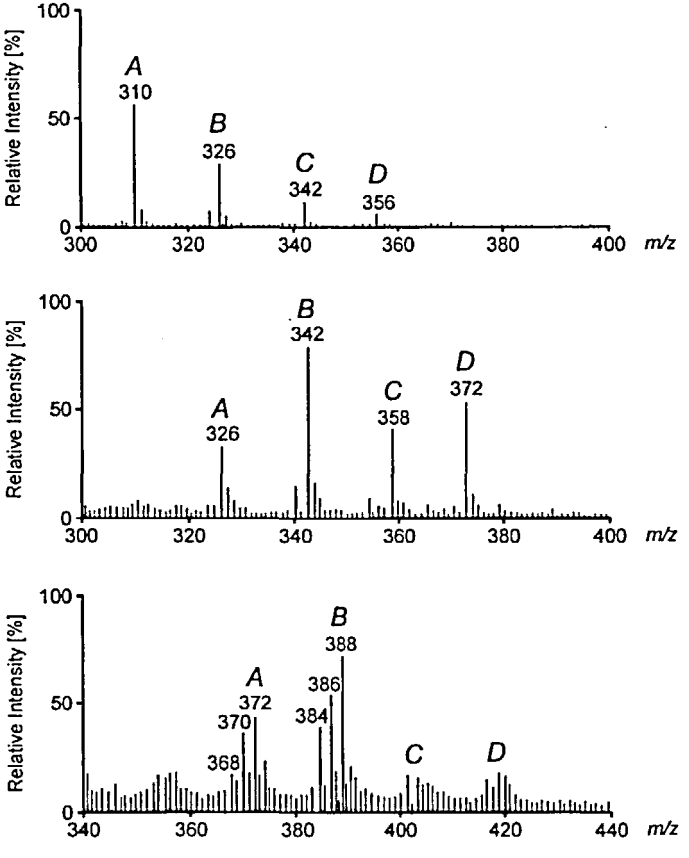
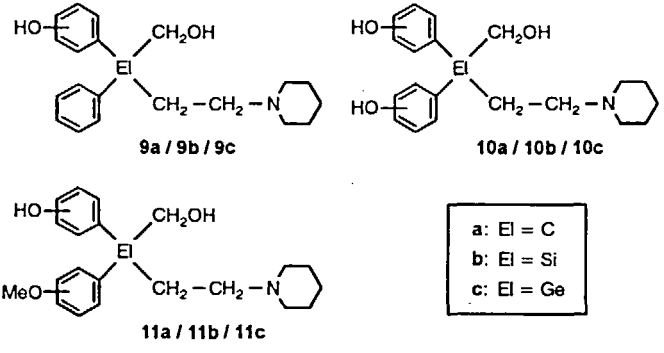



FIGURE 6 APCI MS spectra of urine samples collected after p.o. administration of 80 mg/kg of **8a** (above), **8b** (middle), and **8c** (below).

To get information about the structure of these metabolites, organic extracts of the freeze-dried urine samples were silylated with $Me_3SiN(Me)C(O)CF_3$ and subsequently studied by GC MS experiments (EI, 70 eV; CI, methane). These investigations included urine samples which were treated with glucuronidase/sulfatase (see Figure 5) in order to cleave potential conjugates. The mass-spectrometric studies clearly demonstrated the presence of the metabolites **9a/9b/9c**, **10a/10b/10c**, and **11a/11b/11c**. These preliminary results indicate a similar phase-I metabolism of the C/Si/Ge analogues **8a/8b/8c**.

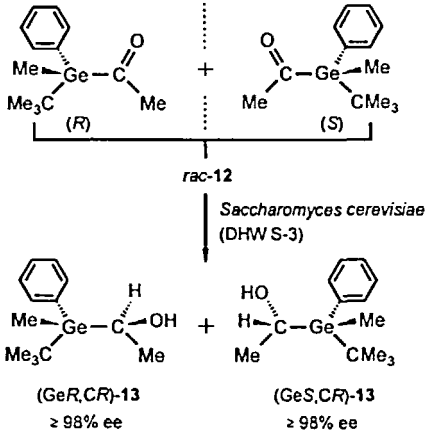





## BIOCATALYSIS AS A PREPARATIVE METHOD FOR THE SYNTHESIS OF OPTICALLY ACTIVE CENTROCHIRAL GERMANES

By analogy with many organic ketones of the general formula type $R_3C–C(O)–CR_3$, related silaketones (acylsilanes) $R_3Si–C(O)–CR_3$ were found to be accepted as substrates by a variety of ketone-reducing microorganisms and to be converted stereoselectively into the corresponding optically active reduction products $R_3Si–C(OH)H–CR_3$ (see ref.

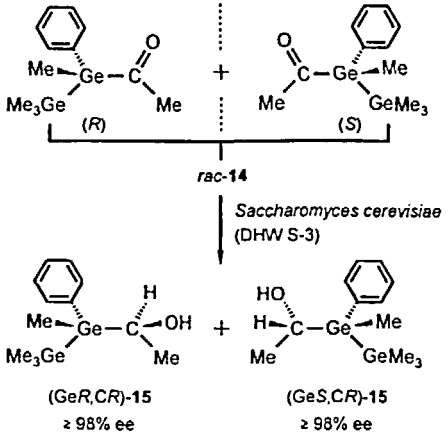
8 and literature cited therein). Related germaketones (acylgermanes) $R_3Ge–C(O)–CR_3$ were also found to undergo stereoselective microbial reductions, indicating again bioisosteric relationships between analogous carbon, silicon, and germanium compounds. The enantioselective microbial reductions shown in Schemes 5–7 are examples of this.[23]




SCHEME 5

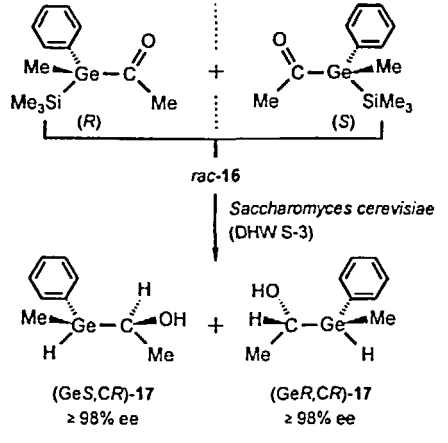
The acetylgermane *rac*-**12** was found to be reduced (*R*)-selectively by resting free cells of *Saccharomyces cerevisiae* (DHW S-3) to give a mixture of the diastereomeric (1-hydroxyethyl)germanes (Ge*R*,C*R*)-**13** and (Ge*S*,C*R*)-**13** (molar ratio 1:1) (Scheme 5). These compounds could be separated by column chromatography on silica gel as almost diastereomerically and enantiomerically pure products.

As shown in Scheme 6, the related acetyldigermane *rac*-**14** could also be reduced (*R*)-selectively with resting free cells of *Saccharomyces cerevisiae* (DHW S-3) to give the diastereomeric (1-hydroxyethyl)digermanes (Ge*R*,C*R*)-**15** and (Ge*S*,C*R*)-**15** (molar ratio 1:1).




SCHEME 6

The enantiomeric purities of these products were >98% ee. Interestingly, the Ge–Ge bonds of the substrate and the products are rather stable against hydrolytic cleavage under the bioconversion conditions used.

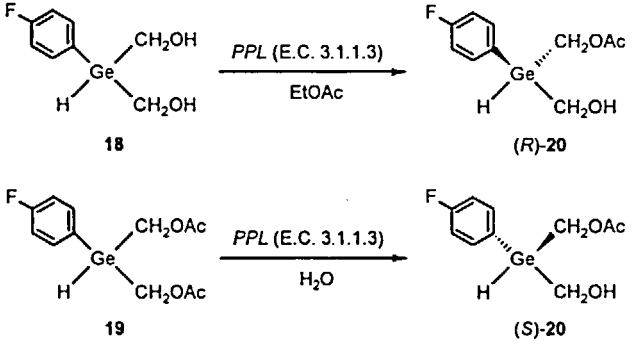



SCHEME 7

A quite different result was obtained when replacing the $Me_3Ge$ group of *rac*-**14** by a $Me_3Si$ moiety (→ *rac*-**16**): Incubation of the acetyl(trimethylsilyl)germane *rac*-**16** with resting free cells of *Saccharomyces cerevisiae* (DHW S-3) gave a 1:1 mixture of the diastereomeric (1-hydroxyethyl)hydridogermanes (Ge*S*,C*R*)-**17** (≥98% ee) and (Ge*R*,C*R*)-**17** (≥98% ee); i.e. a (probably chemically induced) Ge–Si cleavage was observed.

The biotransformations outlined in Schemes 5–7 were performed on a preparative scale indicating that biocatalysis is an efficient preparative method for the synthesis of optically active germanes and digermanes.






SCHEME 8

As illustrated in Scheme 8, bioconversions with isolated enzymes have also a high potential for synthetic germanium chemistry.[24] The optically active hydridogermane (*R*)-**20** (86% ee) was prepared on a preparative scale by an enantioselective transesterification of the prochiral diol **18** with ethyl acetate (acyl donor and reaction medium) using *porcine pancreas lipase* (*PPL*, E.C. 3.1.1.3) as the biocatalyst. The corresponding antipode (*S*)-**20** (94% ee) was obtained by a *PPL*-catalyzed hydrolysis of the prochiral diacetate **19** (phosphate buffer/tetrahydrofuran as reaction medium).

Analogous enzymatic conversions of related diols $R^1R^2El(CH_2OH)_2$ (El = C, Si) and diacetates $R^1R^2El(CH_2OAc)_2$ (El = C, Si) with lipases are also known (see ref. 24 and literature cited therein).

## CONCLUDING REMARKS

The respective C/Si/Ge analogues described in this article were found to undergo quite similar interactions with biological systems, indicating that there are distinct bioisosteric relationships between analogous carbon, silicon, and germanium compounds.



**Acknowledgements**

R. T. wishes to express his sincere thanks to his coworkers and colleagues without whose contributions this article could not have been written; their names are cited in the references. In addition, financial support of our work by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie* and support by the ASTA Medica AG (Frankfurt, Germany), the *Bayer AG* (Leverkusen and Wuppertal-Elberfeld, Germany), and the Merck *KGaA* (Darmstadt, Germany) is gratefully acknowledged.

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