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Journal of Organometallic Chemistry 628 (2001) 183–194

Journal of Organometallic Chemistry

www.elsevier.nl/locate/jorganchem

Syntheses of racemic and non-racemic silicon- and germanium-containing α-amino acids of the formula type $H_2NCH(CH_2ElR_3)COOH$ (El = Si, Ge; R = organyl) and incorporation of D-$H_2NCH(CH_2SiMe_3)COOH$ and D-$H_2NCH(CH_2GeMe_3)COOH$ into biologically active decapeptides: a study on C/Si/Ge bioisosterism

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Received 19 December 2000; accepted 7 March 2001

Abstract

Two novel efficient methods for the synthesis of racemic silicon- and germanium-containing α-amino acids of the formula type *rac*-$H_2NCH(CH_2ElR_3)COOH$ (El = Si, Ge; R = organyl), starting from 3,6-diethoxy-2,5-dihydropyrazine, have been developed. Racemic α-amino acids synthesized: *rac*-$H_2NCH(CH_2SiMe_3)COOH$ (*rac*-**2**), *rac*-$H_2NCH(CH_2GeMe_3)COOH$ (*rac*-**3**), *rac*-$H_2NCH(CH_2SiMe_2Ph)COOH$ (*rac*-**4**), *rac*-$H_2NCH(CH_2GeMe_2Ph)COOH$ (*rac*-**5**), and *rac*-$H_2NCH(CH_2SiMe_2CH{=}CH_2)COOH$ (*rac*-**6**). Preparative liquid-chromatographic resolution of *rac*-**2** and *rac*-**3** [CHIROBIOTIC T (glycopeptide Teicoplanin covalently linked to spherical silica gel) as the stationary phase] yielded the α-amino acids (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, and (*S*)-**3**. The (*R*)- and (*S*)-enantiomers of β-(trimethylsilyl)alanine [(*R*)- and (*S*)-**2**] and β-(trimethylgermyl)alanine [(*R*)- and (*S*)-**3**] are sila-analogs and germa-analogs, respectively, of the antipodes of the non-proteinogenic α-amino acid β-*tert*-butylalanine [(*S*)- and (*R*)-$H_2NCH(CH_2CMe_3)COOH$; (*S*)- and (*R*)-**1**]. Starting from the *N*-Fmoc-protected C/Si/Ge-analogous (D-configurated) α-amino acids (*R*)-**1**, (*S*)-**2**, and (*S*)-**3**, the C/Si/Ge-analogous decapeptides **7**–**9** [Ac-D-Nal^1-4-Cl-D-Phe^2-D-Pal^3-Ser^4-*N*-Me-Tyr^5-D-Hci^6-Nle^7-Arg^8-Pro^9-D-Me_3El-Ala^{10}-NH_2 (**7**, El = C; **8**, El = Si; **9**, El = Ge)] were prepared by sequential solid-phase synthesis. The decapeptides **7**–**9** were studied in vitro in a functional assay using a recombinant cell line expressing the human GnRH receptor (agonist Triptorelin). Compounds **7**–**9** behaved as medium-potent GnRH antagonists, the antagonistic potencies of these three C/Si/Ge analogs being very similar.

Keywords: α-Amino acids; C/Si/Ge bioisosterism; Germanium; GnRH antagonists; Peptides; Silicon

1. Introduction

In the course of our systematic studies on bioorganosilicon and bioorganogermanium chemistry [1], we became interested in silicon- and germanium-containing α-amino acids [1d,2–4] and peptides [1d,4] (for further publications dealing with silicon-containing amino acids and their derivatives, see Refs. [5–15]). We have now succeeded in developing efficient methods for the synthesis of racemic silicon- and germanium-containing α-amino acids of the formula type $H_2NCH(CH_2SiR_3)COOH$ and $H_2NCH(CH_2GeR_3)COOH$ (R = organyl) and for the preparative chromatographic resolution of the α-amino acids L- and D-$H_2NCH(CH_2SiMe_3)COOH$ as well as L- and D-$H_2NCH(CH_2GeMe_3)$-

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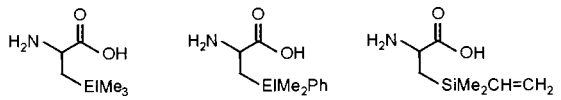
0022-328X/01/$ - see front matter
PII: S0022-328X(01)00783-5

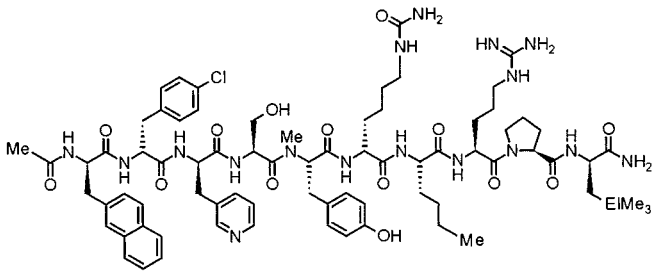
COOH. We report here on the syntheses of the α-amino acids *rac*-**2**–*rac*-**6** and on the chromatographic resolutions of *rac*-**2** and *rac*-**3** to yield the respective α-amino acids (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, and (*S*)-**3**. β-(Trimethylsilyl)alanine (**2**) and β-(trimethylgermyl)alanine (**3**) are sila- and germa-analogs of the non-proteinogenic α-amino acid β-*tert*-butylalanine (**1**).



1: El = C
2: El = Si
3: El = Ge

4: El = Si
5: El = Ge

6



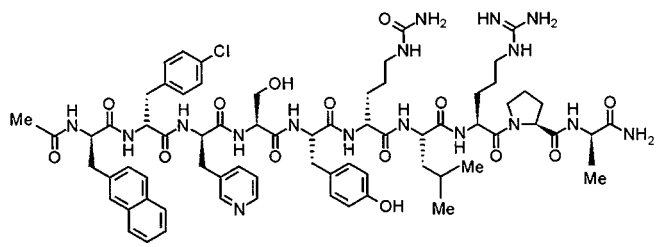
7: El = C, Ac – *D*-Nal[1] – (4-Cl)-*D*-Phe[2] – *D*-Pal[3] – Ser[4] – *N*-Me-Tyr[5] – *D*-Hci[6] – Nle[7] – Arg[8] – Pro[9] – [*D*-Me$_3$C-Ala[10]] – NH_2

8: El = Si, Ac – *D*-Nal[1] – (4-Cl)-*D*-Phe[2] – *D*-Pal[3] – Ser[4] – *N*-Me-Tyr[5] – *D*-Hci[6] – Nle[7] – Arg[8] – Pro[9] – [*D*-Me$_3$Si-Ala[10]] – NH_2

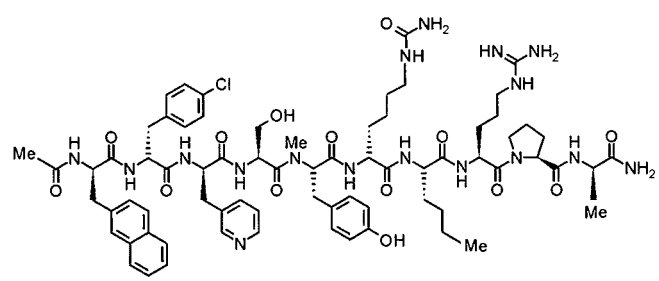
9: El = Ge, Ac – *D*-Nal[1] – (4-Cl)-*D*-Phe[2] – *D*-Pal[3] – Ser[4] – *N*-Me-Tyr[5] – *D*-Hci[6] – Nle[7] – Arg[8] – Pro[9] – [*D*-Me$_3$Ge-Ala[10]] – NH_2

Furthermore, we report on the syntheses of the decapeptides **7**–**9** and their antagonistic potencies at the human GnRH receptor. These peptides contain the C/Si/Ge-analogous D-configurated amino acid residues Me_3C-Ala, Me_3Si-Ala, or Me_3Ge-Ala in position 10 of their backbone. The C/Si/Ge analogs **7**–**9** are structurally related to the GnRH antagonist Cetrorelix[INN] (for reviews dealing with Cetrorelix[INN], see Ref. [16]). This decapeptide bears an (*S*)-tyrosine residue in position 5 [instead of (*S*)-*N*-methyltyrosine], an (*R*)-citrulline residue in position 6 [instead of (*R*)-homocitrulline], an (*S*)-leucine residue in position 7 [instead of (*S*)-neoleucine], and an (*R*)-alanine residue in position 10 [instead of the Me_3El-Ala (El = C, Si, Ge) residues derived from (*R*)-**1**, (*S*)-**2**, and (*S*)-**3**]. The pharmacological studies of **7**–**9** presented here were carried out with special emphasis on C/Si/Ge bioisosterism.

On the other hand, compounds **7**–**9** were also synthesized as part of ASTA Medica's search for potent

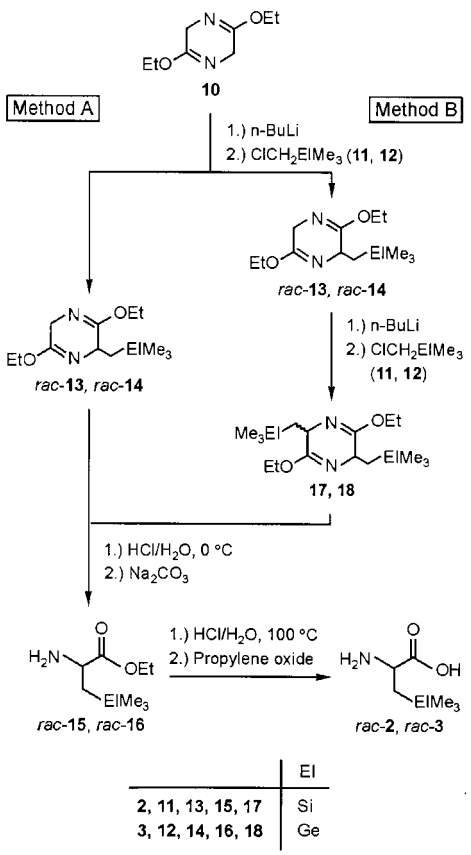


Cetrorelix[INN]: Ac – *D*-Nal[1] – (4-Cl)-*D*-Phe[2] – *D*-Pal[3] – Ser[4] – Tyr[5] – *D*-Cit[6] – Leu[7] – Arg[8] – Pro[9] – *D*-Ala[10] – NH_2



D-63153: Ac – *D*-Nal[1] – (4-Cl)-*D*-Phe[2] – *D*-Pal[3] – Ser[4] – *N*-Me-Tyr[5] – *D*-Hci[6] – Nle[7] – Arg[8] – Pro[9] – *D*-Ala[10] – NH_2

peptide analogs of Cetrorelix[INN] and its derivative D-63153. While Cetrorelix[INN] (Cetrotide®) has already been approved by EU authorities as the first GnRH antagonist for marketing in controlled ovarian stimulation for assisted reproduction (COS/ART), the decapeptide D-63153 is currently in advanced preclinical development due to its favorable pharmacological and physicochemical properties, such as a high water solubility [17]. A high water solubility is of special importance for the development of GnRH antagonist depot formulations for clinical use in hormone-dependent cancer therapy [18]. The decapeptides **7**–**9** are derivatives of D-63153 bearing a Me_3El-Ala residue [El = C, Si, Ge; derived from (*R*)-**1**, (*S*)-**2**, or (*S*)-**3**] in position 10 instead of an (*R*)-alanine residue.



Scheme 1.

Table 1
Yields of the α-amino acid ethyl esters *rac*-**15**, *rac*-**16**, and *rac*-**25**–*rac*-**27** obtained by hydrolysis of the respective dihydropyrazines **10**/*rac*-**13**, **10**/*rac*-**14**, **10**/*rac*-**22**, **10**/*rac*-**24**, *rac*-**13**/**17**, *rac*-**14**/**18**, *rac*-**22**/**28**, and *rac*-**23**/**29**

Product	Yield (educt) (%) [a]	Yield (educt) (%) [b]
rac-**15**	60 (**10**/*rac*-**13**)	66 (*rac*-**13**/**17**)
rac-**16**	42 (**10**/*rac*-**14**)	71 (*rac*-**14**/**18**)
rac-**25**	35 (**10**/*rac*-**22**)	64 (*rac*-**22**/**28**)
rac-**26**		60 (*rac*-**23**/**29**)
rac-**27**	35 (**10**/*rac*-**24**)	

[a] Monoalkylation of the dihydropyrazine **10** (Method A); yields relative to **10**.

[b] Dialkylation of the dihydropyrazine **10** (Method B); yields relative to the two-fold molar amount of **10**.

Table 2
Yields of the α-amino acids *rac*-**2**–*rac*-**6** obtained by hydrolysis of the respective α-amino acid esters *rac*-**15**, *rac*-**16**, and *rac*-**25**–*rac*-**27** as well as overall yields

Product	Yield (educt) (%)	Overall yield (%) [a]	Overall yield (%) [b]
rac-**2**	84 (*rac*-**15**)	42	47
rac-**3**	85 (*rac*-**16**)	30	51
rac-**4**	63 (*rac*-**25**)	21	38
rac-**5**	43 (*rac*-**26**)		22
rac-**6**	42 (*rac*-**27**)	12	

[a] Monoalkylation of the dihydropyrazine **10** (Method A); yields relative to **10**.

[b] Dialkylation of the dihydropyrazine **10** (Method B); yields relative to the two-fold molar amount of **10**.

2. Results and discussion

2.1. Syntheses of the α-amino acids

The silicon- and germanium-containing α-amino acids *rac*-**2** and *rac*-**3** were prepared by three- (Method A) or four-step (Method B) syntheses, starting from the dihydropyrazine **10** (Scheme 1).

Metalation of the dihydropyrazine **10** with *n*-butyllithium and subsequent treatment with $ClCH_2SiMe_3$ (**11**) or $ClCH_2GeMe_3$ (**12**) yielded mixtures of the respective dihydropyrazines *rac*-**13** and *rac*-**14**, along with the non-reacted educt **10** (Method A). After treatment of these mixtures (*rac*-**13** and *rac*-**14** not isolated and characterized) with hydrochloric acid at 0°C and subsequent separation of the byproduct glycine ethyl ester by extraction, the respective α-amino acid ethyl esters *rac*-**15** and *rac*-**16** were obtained (for the yields, see Table 1). Hydrolysis of these esters in boiling hydrochloric acid and subsequent treatment of the resulting α-amino acid hydrochlorides with propylene oxide finally yielded the α-amino acids *rac*-**2** and *rac*-**3** (for the yields, see Table 2).

The overall yields and the atom economy [19] for the syntheses of *rac*-**2** and *rac*-**3** could be improved by two-fold alkylation of the dihydropyrazine **10** (Method B). After metalation of **10** with *n*-butyllithium and subsequent treatment with $ClCH_2SiMe_3$ (**11**) or $ClCH_2GeMe_3$ (**12**), this metalation/alkylation procedure was repeated to yield the dihydropyrazines **17** and **18** (stereochemistry not analyzed), along with the respective dihydropyrazines *rac*-**13** and *rac*-**14**. Treatment

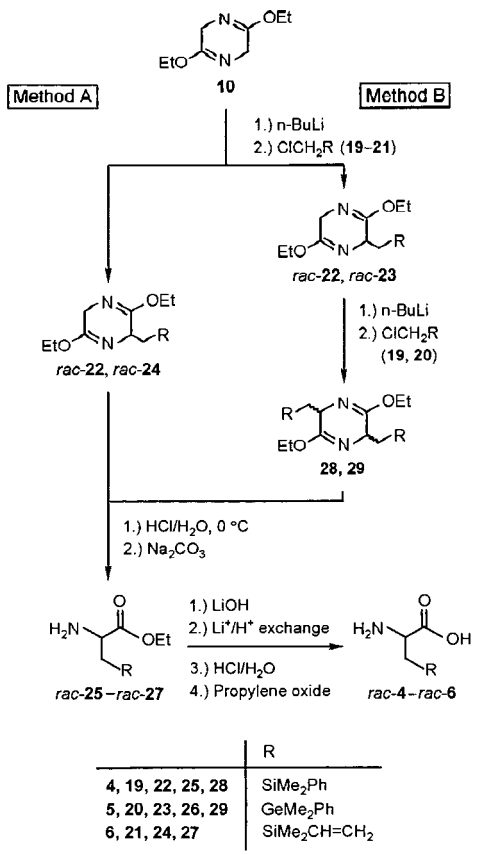
of these mixtures (*rac*-**13**, *rac*-**14**, **17**, and **18** not isolated and characterized) with hydrochloric acid at 0°C and separation of the byproduct glycine ethyl ester by extraction gave the α-amino acid esters *rac*-**15** and *rac*-**16** (for the yields, see Table 1), which were then converted into the α-amino acids *rac*-**2** and *rac*-**3** (for the yields, see Table 3) in analogy to Method A.

The structurally related α-amino acids *rac*-**4**–*rac*-**6** were prepared according to Scheme 2. Thus, metalation of the dihydropyrazine **10** with *n*-butyllithium and subsequent treatment with $ClCH_2SiMe_2Ph$ (**19**) or $ClCH_2SiMe_2CH{=}CH_2$ (**21**) yielded mixtures of the respective dihydropyrazines *rac*-**22** and *rac*-**24**, along with the non-reacted educt **10** (Method A). Treatment

Table 3
Antagonistic potencies (IC_{50} values) of the decapeptides Cetrorelix[INN], D-63153, and **7**–**9** at the human GnRH receptor [a]

Decapeptide	IC_{50} (nM)
Cetrorelix[INN]	0.28
D-63153	0.26
7	14
8	27
9	15

[a] For details, see Section 2.



Scheme 2.

of these mixtures (*rac*-**22** and *rac*-**24** not isolated and characterized) with hydrochloric acid at 0°C and subsequent separation of the byproduct glycine ethyl ester by extraction gave the respective α-amino acid ethyl esters *rac*-**25** and *rac*-**27** (for the yields, see Table 1). Attempts to prepare the α-amino acids *rac*-**4** and *rac*-**6** by hydrolysis of these esters in boiling hydrochloric acid failed: under the conditions applied to the hydrolysis of *rac*-**15** and *rac*-**16**, Si–C (Si–Ph, Si–$CH{=}CH_2$) bond cleavage was observed. However, this problem was solved by avoiding acidic conditions. Thus, treatment of the α-amino acid esters *rac*-**25** and *rac*-**27** with lithium hydroxide (molar ratio 1:1) in a water–dioxane mixture, followed by a special workup (including an Li^+/H^+ exchange; see Ref. [4]), yielded the respective α-amino acids *rac*-**4** and *rac*-**6**.

Compound *rac*-**4** was prepared alternatively by starting with a two-fold alkylation of the dihydropyrazine **10** (Method B). The same procedure was also used for the synthesis of the α-amino acid *rac*-**5**. Thus, after deprotonation of **10** with *n*-butyllithium and subsequent treatment with $ClCH_2SiMe_2Ph$ (**19**) or $ClCH_2GeMe_2Ph$ (**20**), this metalation/alkylation procedure was repeated to yield the dihydropyrazines **28** and **29** (stereochemistry not analyzed), along with the respective dihydropyrazines *rac*-**22** and *rac*-**23**. Treatment of these mixtures (*rac*-**22**, *rac*-**23**, **28**, and **29** not isolated and characterized) with hydrochloric acid at 0°C and subsequent separation of the byproduct glycine ethyl ester by extraction gave the respective α-amino acid ethyl esters *rac*-**25** and *rac*-**26** (for the yields, see Table 1). To avoid Si–C (Si–Ph) and Ge–C (Ge–Ph) bond cleavage, the conversions of these α-amino acid esters into the respective α-amino acids *rac*-**4** and *rac*-**5** were carried out in analogy to Method A by treatment with lithium hydroxide in a water–dioxane mixture, followed by a special workup.

The α-amino acids *rac*-**2**–*rac*-**6** were isolated as colorless crystalline solids, whereas *rac*-**15**, *rac*-**16**, and *rac*-**25**–*rac*-**27** were obtained as colorless liquids. The identities of all compounds were established by elemental analyses (C, H, N), NMR-spectroscopic studies (^1H, ^{13}C, ^{29}Si), and mass-spectrometric investigations.

2.2. Preparative liquid-chromatographic resolutions

The Si/Ge analogs *rac*-**2** and *rac*-**3** were resolved by preparative liquid chromatography using CHIROBIOTIC T (glycopeptide Teicoplanin covalently linked to spherical silica gel) as the chiral stationary phase and methanol or methanol–water [90:10 (v/v)] as the eluents [yields (relative to the racemic α-amino acid): (*R*)-**2**, 36%; (*S*)-**2**, 23%; (*R*)-**3**, 32%; (*S*)-**3**, 21%]. The enantiomeric purities (see Section 2.3) of the resolved antipodes were as follows: (*R*)-**2**, 99% ee; (*S*)-**2**, 99% ee;

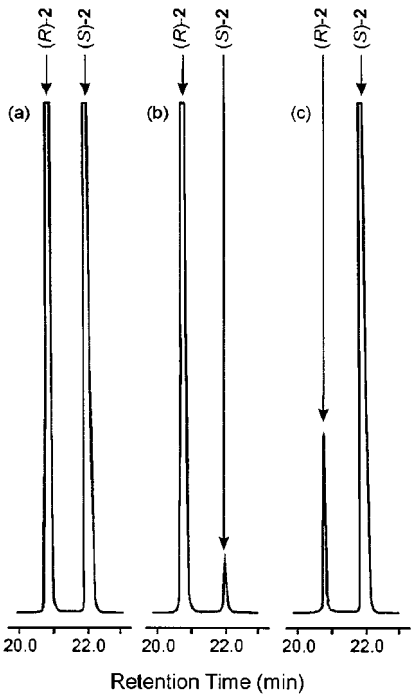


Fig. 1. Quantitative analytical gas-chromatographic determination of the molar ratio of the (*R*)- and (*S*)-enantiomers of the chemically modified α-amino acid **2**. Gas chromatograms: (a) 50:50 mixture of (*R*)-**2**/(*S*)-**2** obtained by hydrolysis of *rac*-**15** (analysis of the crude product); (b) enantiomerically pure (99% ee) isomer (*R*)-**2** obtained by preparative liquid-chromatographic separation; (c) enantiomerically pure (99% ee) isomer (*S*)-**2** obtained by preparative liquid-chromatographic separation. For details, see Section 3.

(*R*)-**3**, 99% ee; (*S*)-**3**, 93% ee. To the best of our knowledge, the resolutions of *rac*-**2** and *rac*-**3** are the first examples of preparative liquid-chromatographic separations of the antipodes of chiral silicon- and germanium-containing α-amino acids. The enantiopure α-amino acids (*R*)-**2** and (*R*)-**3** have already been prepared by asymmetric syntheses, along with the byproducts (*S*)-**2** and (*S*)-**3** [4]. The method described in this paper (synthesis of compounds *rac*-**2** and *rac*-**3** and their chromatographic resolution) represents an efficient alternative approach for the preparation of (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, and (*S*)-**3**.

2.3. Determination of the enantiomeric purities and assignment of absolute configurations

To monitor the preparative liquid-chromatographic resolutions of *rac*-**2** and *rac*-**3** and to determine the enantiomeric purities of the antipodes of **2** and **3**, analytical chromatographic methods were developed. The (*R*)- and (*S*)-enantiomers of **2** were separated, after conversion into the respective *N*-(trifluoroaceto)amino acid propyl esters (see Section 3), by analytical capillary gas chromatography using a Chiralsil-D-Val column (Fig. 1). The retention times of the chemically modified enantiomers are as follows: (*R*)-**2**, 20.8 min; (*S*)-**2**, 22.1 min. The (*R*)- and (*S*)-enantiomers of **3** were separated by analytical liquid chromatography (HPLC) using CHIROBIOTIC T (glycopeptide Teicoplanin covalently linked to spherical silica gel) as the chiral stationary phase (see Fig. 2). The retention times of the enantiomers are as follows: (*R*)-**3**, 9.2 min; (*S*)-**3**, 11.2 min. The assignment of absolute configurations to the antipodes of **2** and **3** was made by comparison with authentic samples of (*R*)-**2** [4] and (*R*)-**3** [4].

2.4. Syntheses of the decapeptides

To incorporate the α-amino acids (*R*)-**1**, (*S*)-**2**, and (*S*)-**3** into the decapeptides **7**–**9**, the *N*-(fluoren-9-yl)methoxycarbonyl (*N*-Fmoc) derivatives of these amino acids were prepared. The *N*-Fmoc derivatives (*R*)-**30**, (*S*)-**31**, and (*S*)-**32** were synthesized according to Scheme 3 the treatment of (*R*)-**1**, (*S*)-**2**, or (*S*)-**3** with (fluoren-9-yl)methyl chloroformate (Fmoc-Cl) and were isolated as colorless solids [yields: (*R*)-**30**, 80%; (*S*)-**31**, 68%; (*S*)-**32**, 59%].

The decapeptides **7**–**9** were prepared by solid-phase syntheses, using standard methods [20] for the sequential synthesis of oligopeptides and starting with the C-terminal attachment of (*R*)-**30**, (*S*)-**31**, or (*S*)-**32**. The decapeptides **7**–**9** were purified by high-performance liquid chromatography, and their identities were established by mass-spectrometric studies (ESI MS and ESI MS/MS).

2.5. Pharmacological characterization of the decapeptides

The decapeptides **7**–**9** were studied in vitro in a functional assay using a recombinant cell line expressing the human GnRH receptor. Triptorelin (D-Trp^6-GnRH) served as the agonist in these studies. Compounds **7**–**9** were found to be medium-potent GnRH antagonists. As can be seen from Table 3, the antagonistic potencies (IC_{50} values) of **7**–**9** are quite similar, but significantly reduced compared with that of Cetrorelix^{INN} and D-63153. The results obtained clearly indicate strongly pronounced bioisosteric relationships between the C/Si/Ge-analogous decapeptides **7**–**9**. This is in line with the findings of a series of further biological studies with other C/Si/Ge-analogous drugs [1d].

3. Experimental

3.1. Syntheses

3.1.1. General procedures

Unless otherwise indicated, the reactions were carried out under dry nitrogen. THF was dried and purified

according to standard procedures and stored under nitrogen. The ^{1}H, ^{13}C, and ^{29}Si NMR spectra were recorded at (room temperature) r.t. on a Bruker DRX-300 (^{1}H, 300.1 MHz; ^{13}C, 75.5 MHz; ^{29}Si, 59.6 MHz) or Bruker AMX-400 NMR spectrometer (^{1}H, 400.1 MHz; ^{13}C, 100.6 MHz). Chemical shifts (ppm) were determined relative to internal $CHCl_3$ (^{1}H, δ 7.24, solvent $CDCl_3$), $CDCl_3$ (^{13}C, δ 77.05, solvent $CDCl_3$), HDO (^{1}H, δ 4.82, solvent D_2O), and external Me_4Si (^{13}C, δ 0, solvent D_2O; ^{29}Si, δ 0, solvents $CDCl_3$ and D_2O). The ^{1}H spin systems of the α-amino acids and the α-amino acid esters were analyzed by simulations using the WIN-DAISY software package (version 4.0, Bruker). Assignment of the ^{13}C-NMR data was supported by DEPT 135 experiments. Mass spectra were obtained with a Varian MAT-711 (EI MS, 70 eV), Finnigan MAT-8430 (EI MS, 70 eV; CI MS, NH_3 as reactant gas), Finnigan MAT-8200 (EI MS, 70 eV), or ThermoQuest TRIO 1000 mass spectrometer (EI MS, 70 eV). The selected m/z values given refer to the isotopes ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{28}Si, ^{35}Cl, and ^{74}Ge. IR spectra were obtained with a Bruker Equinox 55 FT-IR spectrometer. The ion exchanger (Aldrich, Art. 21,739-5) was activated by washing with 0.5 M hydrochloric acid (50 ml) and water (3 × 50 ml).

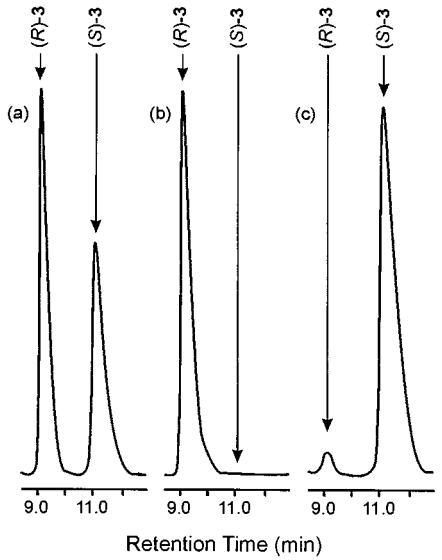
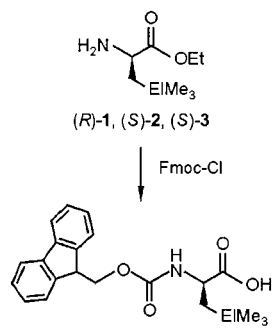


Fig. 2. Quantitative analytical liquid-chromatographic determination of the molar ratio of the (*R*)- and (*S*)-enantiomers of the α-amino acid **3**. Liquid chromatograms: (a) 50:50 mixture of (*R*)-**3**/(*S*)-**3** obtained by hydrolysis of *rac*-**16** (analysis of the crude product); (b) enantiomerically pure (99% ee) isomer (*R*)-**3** obtained by preparative liquid-chromatographic separation; (c) enantiomerically enriched (93% ee) isomer (*S*)-**3** obtained by preparative liquid-chromatographic separation. For details, see Section 3.



	El
1, 30	C
2, 31	Si
3, 32	Ge

Scheme 3.

*3.1.2. (R)-2-Amino-4,4-dimethylpentanoic acid [(R)-β-tert-butylalanine; (R)-**1**]*

This compound was commercially available (Bachem AG).

*3.1.3. rac-2-Amino-3-(trimethylsilyl)propionic acid [rac-β-(trimethylsilyl)alanine; rac-**2**]*

A solution of *rac*-**15** (13.5 g, 71.3 mmol) in hydrochloric acid (6 M, 200 ml) was heated under reflux for 1 h. After the mixture was cooled to r.t., the solvent was removed in vacuo (0.1 mbar, 20°C) and the residue dried at 40°C/0.01 mbar for 1 h and then dissolved in EtOH (250 ml). After addition of propylene oxide (180 ml), the mixture was heated under reflux for 10 min. The resulting precipitate was isolated by centrifugation, washed with EtOH (2 × 25 ml) and Et_2O (2 × 25 ml), and then dried in vacuo (0.01 mbar, 40°C, 36 h) to give *rac*-**2** in 84% yield as a colorless crystalline solid (9.69 g, 60.1 mmol). The NMR and MS data of the product were identical with those described for (*R*)-**2** in Ref. [4]. Anal. Found: C, 44.2; H, 8.9; N, 8.6. Calc. for $C_6H_{15}NO_2Si$: C, 44.68; H, 9.37; N, 8.68%.

*3.1.4. (R)-2-Amino-3-(trimethylsilyl)propionic acid [(R)-β-(trimethylsilyl)alanine; (R)-**2**]*

This compound was obtained by preparative liquid-chromatographic separation (see Section 3.1.27). The IR, NMR, and MS data of the product were identical with those already described for (*R*)-**2** (prepared by an alternative synthesis) in Ref. [4]. Anal. Found: C, 44.8; H, 9.5; N, 8.7. Calc. for $C_6H_{15}NO_2Si$: C, 44.68; H, 9.37; N, 8.68%.

*3.1.5. (S)-2-Amino-3-(trimethylsilyl)propionic acid [(S)-β-(trimethylsilyl)alanine; (S)-**2**]*

This compound was obtained by preparative liquid-chromatographic separation (see Section 3.1.27). The IR, NMR, and MS data of the product were identical with those already described for (*R*)-**2** (prepared by an alternative synthesis) in Ref. [4]. Anal. Found: C, 44.3; H, 9.3; N, 8.5. Calc. for $C_6H_{15}NO_2Si$: C, 44.68; H, 9.37; N, 8.68%.

*3.1.6. rac-2-Amino-3-(trimethylgermyl)propionic acid [rac-β-(trimethylgermyl)alanine; rac-**3**]*

This compound was prepared analogously to the synthesis of *rac*-**2** by heating a solution of *rac*-**16** (3.53 g, 15.1 mmol) in hydrochloric acid (6 M, 70 ml). After treatment of the solution of the residue (*rac*-**3**·HCl) in EtOH (40 ml) with propylene oxide (10 ml), *rac*-**3** was isolated in 85% yield as a colorless crystalline solid (2.65 g, 12.9 mmol). The NMR and MS data of the product were identical with those described for (*R*)-**3** in Ref. [4]. Anal. Found: C, 35.2; H, 7.3; N, 6.7. Calc. for $C_6H_{15}GeNO_2$: C, 35.02; H, 7.35; N, 6.81%.

*3.1.7. (R)-2-Amino-3-(trimethylgermyl)propionic acid [(R)-β-(trimethylgermyl)alanine; (R)-**3**]*

This compound was obtained by preparative liquid-chromatographic separation (see Section 3.1.27). The IR, NMR, and MS data of the product were identical with those already described for (*R*)-**3** (prepared by an alternative synthesis) in Ref. [4]. Anal. Found: C, 35.2; H, 7.5; N, 7.0. Calc. for $C_6H_{15}GeNO_2$: C, 35.02; H, 7.35; N, 6.81%.

*3.1.8. (S)-2-Amino-3-(trimethylgermyl)propionic acid [(S)-β-(trimethylgermyl)alanine; (S)-**3**]*

This compound was obtained by preparative liquid-chromatographic separation (see Section 3.1.27). The IR, NMR, and MS data of the product were identical with those already described for (*R*)-**3** (prepared by an alternative synthesis) in Ref. [4]. Anal. Found: C, 34.9; H, 7.2; N, 6.9. Calc. for $C_6H_{15}GeNO_2$: C, 35.02; H, 7.35; N, 6.81%.

*3.1.9. rac-2-Amino-3-[dimethyl(phenyl)silyl]propionic acid [rac-β-[dimethyl(phenyl)silyl]alanine; rac-**4**]*

Water (2.5 ml) and an aqueous lithium hydroxide solution (1 M, 2.69 ml; 2.69 mmol LiOH) were added to a solution of *rac*-**25** (676 mg, 2.69 mmol) in dioxane (7 ml). After the mixture was stirred at r.t. for 16 h, the solvent was removed in vacuo. The residue was washed with *n*-pentane (10 ml) and then dried in vacuo (0.01 mbar, 20°C, 2 h) to give the lithium salt of *rac*-**4** in quantitative yield as a colorless solid (617 mg, 2.69 mmol). A mixture of this lithium salt (617 mg, 2.69 mmol) and an activated ion exchanger (11 g) in water (35 ml) was shaken at r.t. for 30 min. After the pH value was adjusted to pH 10–11 by the addition of an 0.1 M aqueous diethylamine solution, the mixture was shaken for a further 30 min. The ion exchanger was filtered off, washed with water (20 ml), and then resuspended in 0.5 M hydrochloric acid (35 ml). After the mixture was shaken at r.t. for 5 min, the ion exchanger was filtered off and resuspended in 0.5 M hydrochloric acid (35 ml), and the mixture was shaken once again at r.t. for 5 min. After separation of the ion exchanger by filtration, the solvent of the combined aqueous phases was removed under reduced pressure (0.1 mbar, 20°C) and the residue dried in vacuo (0.01 mbar, 20°C, 1 h) and then dissolved in EtOH (10 ml). After addition of propylene oxide (3 ml), the mixture was heated under reflux for 10 min. The resulting precipitate was isolated by centrifugation, washed with EtOH (2 × 5 ml) and Et_2O (2 × 5 ml), and then dried in vacuo (0.01 mbar, 40°C, 36 h) to give *rac*-**4** in 63% yield as a colorless crystalline solid (377 mg, 1.69 mmol). The NMR and MS data of the product were identical with those described for (*R*)-**4** in Ref. [4]. Anal. Found: C, 59.0; H, 7.5; N, 6.3. Calc. for $C_{11}H_{17}NO_2Si$: C, 59.16; H, 7.67; N, 6.27%.

*3.1.10. rac-2-Amino-3-[dimethyl(phenyl)germyl]-propionic acid [rac-β-[dimethyl(phenyl)germyl]-alanine; rac-**5**]*

This compound was prepared analogously to the synthesis of *rac*-**4** starting from *rac*-**26** (408 mg, 1.38 mmol). The product was isolated in 43% yield as a colorless crystalline solid (159 mg, 594 μmol). The NMR and MS data of the product were identical with those described for *rac*-**5** in Ref. [4]. Anal. Found: C, 48.9; H, 6.2; N, 5.1. Calc. for $C_{11}H_{17}GeNO_2$: C, 49.32; H, 6.40; N, 5.23%.

*3.1.11. rac-2-Amino-3-[dimethyl(vinyl)silyl]propionic acid [rac-β-[dimethyl(vinyl)silyl]alanine; rac-**6**]*

This compound was prepared analogously to the synthesis of *rac*-**4** starting from *rac*-**27** (634 mg, 2.52 mmol). The product was isolated in 42% yield as a colorless crystalline solid (184 mg, 1.06 mmol). The NMR and MS data of the product were identical with those described for *rac*-**6** in Ref. [4]. Anal. Found: C, 48.5; H, 8.6; N, 8.0. Calc. for $C_7H_{15}NO_2Si$: C, 48.52; H, 8.72; N, 8.08%.

*3.1.12. Acetyl-D-2-naphthylalanyl-D-4-chloro-phenylalanyl-D-3-pyridylalanyl-seryl-N-methyltyrosyl-D-homocitrullyl-neoleucyl-arginyl-prolyl-D-tert-butyl-alaninamide (Ac-D-Nal[1]-4-Cl-D-Phe[2]-D-Pal[3]-Ser[4]-N-Me-Tyr[5]-D-Hci[6]-Nle[7]-Arg[8]-Pro[9]-D-Me$_3$C-Ala[10]-NH$_2$; **7**), acetyl-D-2-naphthylalanyl-D-4-chlorophenylalanyl-D-3-pyridylalanyl-seryl-N-methyltyrosyl-D-homocitrul-lyl-neoleucyl-arginyl-prolyl-D-(trimethylsilyl)-alaninamide (Ac-D-Nal[1]-4-Cl-D-Phe[2]-D-Pal[3]-Ser[4]-N-Me-Tyr[5]-D-Hci[6]-Nle[7]-Arg[8]-Pro[9]-D-Me$_3$Si-Ala[10]-NH$_2$; **8**), and acetyl-D-2-naphthylalanyl-D-4-chloro-phenylalanyl-D-3-pyridylalanyl-seryl-N-methyltyrosyl-D-homocitrullyl-neoleucyl-arginyl-prolyl-D-(trime-thylgermyl)alaninamide (Ac-D-Nal[1]-4-Cl-D-Phe[2]-D-Pal[3]-Ser[4]-N-Me-Tyr[5]-D-Hci[6]-Nle[7]-Arg[8]-Pro[9]-D-Me$_3$Ge-Ala[10]-NH$_2$; **9**)*

Compounds **7**–**9** were prepared by solid-phase synthesis using a Labortec SP650 semiautomatic peptide synthesizer. [(Fluoren-9-yl)methoxycarbonyl]-4-meth-

oxy-4′-(γ-carboxypropyloxy)benzhydrylamine resin with a capacity of 0.55 mmol g^{-1} (Fmoc-MBHA resin; Bachem AG, D 1600) was used as polymeric support, starting with 3.5 g of resin for the synthesis of **7** and 2.0 g of resin each for the synthesis of **8** and **9**. The sequential synthesis was performed as follows. The respective *N*-Fmoc-protected α-amino acid (*R*)-**30**, (*S*)-**31**, or (*S*)-**32** [derived from (*R*)-**1**, (*S*)-**2**, and (*S*)-**3**] (C-terminal) was covalently linked to the unprotected MBHA resin by using a two-fold molar excess of both diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) in a mixture of CH_2Cl_2 and DMF [1:1 (v/v)]. Removal of the Fmoc group was performed with a 20% solution of piperidine in DMF as standard routine. Chain elongation according to the respective amino acid sequence was accomplished with a two-molar excess of each *N*-Fmoc-amino acid, DIC, and HOBt within 90 min of coupling time. Completion of acylation was checked via the chloroanil test. Cleavage of the decapeptides from the polymeric support was accomplished by treatment with a mixture of AcOH–2,2,2-trifluoroethanol–CH_2Cl_2 [1:1:8 (v/v/v)] at 40°C for 2.5 h. The solvents were removed under reduced pressure, and the residue was washed with Et_2O. The precipitate was filtered off and dried at r.t. for 12 h. The decapeptides were purified by high-performance liquid chromatography using a Nucleoprep RP-C-18 silica gel column. The experimental conditions were as follows: HPLC pump, Shimadzu LC 8A; detector, Shimadzu SPD 68; column (50 mm i.d. × 250 mm); eluent, water–MeCN–2,2,2-trifluoroacetic acid [A: 97:3:1 (v/v/v); B: 30:70:1 (v/v/v); 45% B → 90% B within 50 min]; injection volume, 20 ml; flow rate, 60.0 ml min^{-1}. After removal of the solvents under reduced pressure, the isolated decapeptides were lyophilized. The yields of the products (white fluffy residues) were as follows: **7**, 1.17 g; **8**, 940 mg; **9**, 1.33 g.

Data for ***7***. ESI MS: 1514 [9%, $(M+H)^+$], 758 [100%, $(M+2\ H)^{++}$]. ESI MS/MS [758; ($C_{76}H_{104}ClN_{17}O_{14}+2\ H)^{++}$]: 1273 [4%, B_8^+], 1117 [3%, B_7^+], 1004 [2%, B_6^+], 859 [10%, $Y_6''^+$], 815 [48%, $(B_5-H_2O)^+$], 736 [100%, $(M+2\ H-HNCO)^{++}$], 682 [42%, $Y_5''^+$], 656 [12%, B_4^+], 398 [3%, $Y_3''^+$].

Data for ***8***. ESI MS: 1530 [28%, $(M+H)^+$], 765 [100%, $(M+2\ H)^{++}$]. ESI MS/MS [765; ($C_{75}H_{104}ClN_{17}O_{14}Si+2\ H)^{++}$]: 1273 [6%, B_8^+], 1117 [4%, B_7^+], 1110 [1%, $Y_8''^+$], 1004 [2%, B_6^+], 962 [2%, $Y_7''^+$], 875 [12%, $Y_6''^+$], 815 [52%, $(B_5-H_2O)^+$], 744 [100%, $(M+2\ H-HNCO)^{++}$], 698 [40%, $Y_5''^+$], 656 [13%, B_4^+], 569 [2%, B_3^+], 527 [1%, $Y_4''^+$], 412 [2%, $Y_3''^+$].

Data for ***9***. ESI MS: 1576 [22%, $(M+H)^+$], 789 [100%, $(M+2\ H)^{++}$]. ESI MS/MS [787; ($C_{75}H_{104}Cl^{70}GeN_{17}O_{14}+2\ H)^{++}$]: 1273 [4%, B_8^+], 1117 [2%, B_7^+], 1152 [1%, $Y_8''^+$], 1004 [3%, B_6^+ or $Y_7''^+$], 915 [12%, $Y_6''^+$], 815 [53%, $(B_5-H_2O)^+$], 765 [100%, $(M+2\ H-HNCO)^{++}$], 740 [42%, $Y_5''^+$], 656 [11%, B_4^+], 569 [8%, B_3^+ or $Y_4''^+$], 456 [2%, $Y_3''^+$].

*3.1.13. 3,6-Diethoxy-2,5-dihydropyrazine (**10**)*

Synthesis as described in Ref. [21].

*3.1.14. (Chloromethyl)trimethylsilane (**11**)*

This compound was commercially available (Aldrich).

*3.1.15. (Chloromethyl)trimethylgermane (**12**)*

This compound was synthesized from trichloro(chloromethyl)germane [22] according to Ref. [23].

*3.1.16. rac-2-Amino-3-(trimethylsilyl)propionic acid ethyl ester (rac-**15**)*

Method A. A 1.6 M solution of *n*-butyllithium in *n*-hexane (3.00 ml, 4.80 mmol *n*-BuLi) was added dropwise at −10°C within 10 min to a stirred solution of **10** (6.79 g, 39.9 mmol) in THF (50 ml). After the reaction mixture was cooled to −70°C, a 1.6 M solution of *n*-butyllithium in *n*-hexane (22.0 ml, 35.2 mmol *n*-BuLi) was added dropwise over a period of 20 min. The resulting mixture was stirred at −70°C for 15 min and a solution of **11** (4.89 g, 39.9 mmol) in THF (10 ml) was added dropwise at this temperature within 30 min. After the mixture was stirred at −70°C for 2 h, it was warmed to r.t. within 12 h, followed by addition of Et_2O (50 ml) and water (50 ml). The organic phase was separated and the aqueous layer extracted with Et_2O (2 × 25 ml), and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the oily residue distilled in a Kugelrohr apparatus (oven temperature 140°C, 0.01 mbar) to give a 23:77 mixture (8.70 g) of **10** and *rac*-**13**. Hydrochloric acid (3 M, 10 ml) was added dropwise at 0°C within 15 min to a stirred solution of **10**/*rac*-**13** in EtOH (25 ml). After the mixture was stirred at 0°C for 2 h, the solvent was removed in vacuo (0.1 mbar, 20°C), the residue dissolved in CH_2Cl_2 (25 ml), and the resulting solution extracted with a saturated aqueous Na_2CO_3 solution (2 × 25 ml). After the organic layer was dried over anhydrous Na_2SO_4, the solvent was removed under reduced pressure (rotary evaporator) and the residue purified by distillation in a Kugelrohr apparatus (oven temperature 80°C/0.01 mbar) to give *rac*-**15** in 60% yield as a colorless liquid (4.52 g, 23.9 mmol). The IR, NMR, and MS data of the product were identical with those described for (*R*)-**15** in Ref. [4]. Anal. Found: C, 50.5; H, 10.1; N, 7.4. Calc. for $C_8H_{19}NO_2Si$: C, 50.75; H, 10.12; N, 7.40%.

Method B. A 1.6 M solution of *n*-butyllithium in *n*-hexane (5.00 ml, 8.00 mmol *n*-BuLi) was added dropwise at −10°C within 10 min to a stirred solution of **10** (10.2 g, 59.9 mmol) in THF (250 ml). After the reaction mixture was cooled to −70°C, a 1.6 M solution of

n-butyllithium in *n*-hexane (32.5 ml, 52.0 mmol *n*-BuLi) was added dropwise over a period of 20 min. The resulting mixture was stirred at −70°C for 15 min and a solution of **11** (7.35 g, 59.9 mmol) in THF (15 ml) was added dropwise at this temperature within 30 min. After the mixture was stirred at −70°C for 2 h, it was warmed to r.t. within 12 h. The mixture was cooled again to −70°C and then treated dropwise with a 1.6 M solution of *n*-butyllithium in *n*-hexane (37.5 ml, 60.0 mmol *n*-BuLi). After the mixture was stirred at −70°C for 30 min, a solution of **11** (7.35 g, 59.9 mmol) in THF (15 ml) was added dropwise at this temperature within 30 min. The resulting mixture was stirred at −70°C for 2 h and then warmed to r.t. within 12 h, followed by the addition of Et_2O (500 ml) and water (500 ml). The organic phase was separated and the aqueous layer extracted with Et_2O (2 × 250 ml), and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the oily residue distilled in a Kugelrohr apparatus (oven temperature 180°C, 0.01 mbar) to give a 16:84 mixture (16.6 g) of *rac*-**13** and **17**. Hydrochloric acid (3 M, 150 ml) was added dropwise at 0°C within 15 min to a stirred solution of *rac*-**13**/**17** in EtOH (100 ml). After the mixture was stirred for 2 h at 0°C, the solvent was removed in vacuo (0.1 mbar, 20°C), the residue dissolved in CH_2Cl_2 (150 ml), and the resulting solution extracted with a saturated aqueous Na_2CO_3 solution (75 ml). After the organic layer was dried over anhydrous Na_2SO_4, the solvent was removed under reduced pressure (rotary evaporator) and the residue purified by distillation in a Kugelrohr apparatus (oven temperature 80°C/0.01 mbar) to give *rac*-**15** in 66% yield (relative to the two-fold molar amount of **10**) as a colorless liquid (14.9 g, 78.7 mmol). The IR, NMR, and MS data of the product were identical with those described for *rac*-**15** in Ref. [4]. Anal. Found: C, 50.6; H, 10.0; N, 7.2. Calc. for $C_8H_{19}NO_2Si$: C, 50.75; H, 10.12; N, 7.40%.

*3.1.17. rac-2-Amino-3-(trimethylgermyl)propionic acid ethyl ester (rac-**16**)*

Method A. This compound was prepared analogously to the synthesis of *rac*-**15** (Method A) by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (13.3 ml, 21.3 mmol *n*-BuLi) to a solution of **10** (3.61 g, 21.2 mmol) in THF (25 ml), followed by treatment with a solution of **12** (3.54 g, 21.2 mmol) in THF (10 ml). The crude product was purified by Kugelrohr distillation (oven temperature 150°C, 0.01 mbar) to give a 14:86 mixture (5.89 g) of **10** and *rac*-**14**. Hydrolysis of this mixture was carried out by treatment of a solution of **10**/*rac*-**14** in EtOH (25 ml) with hydrochloric acid (3 M, 10 ml). After extractive separation of glycine ethyl ester, *rac*-**16** was isolated in 42% yield as a colorless liquid (2.06 g, 8.81 mmol). The IR, NMR, and MS data of the product were identical with those described for (*R*)-**16** in Ref. [4]. Anal. Found: C, 41.1; H, 8.2; N, 6.0. Calc. for $C_8H_{19}GeNO_2$: C, 41.09; H, 8.19; N, 5.99%.

Method B. This compound was prepared analogously to the synthesis of *rac*-**15** (Method B) by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (7.38 ml, 11.8 mmol *n*-BuLi) to a solution of **10** (2.01 g, 11.8 mmol) in THF (50 ml), followed by treatment with a solution of **12** (1.97 g, 11.8 mmol) in THF (15 ml). After the metalation/alkylation procedure was repeated [metalation with a 1.6 M solution of *n*-butyllithium in *n*-hexane (7.38 ml, 11.8 mmol *n*-BuLi); alkylation with a solution of **12** (1.97 g, 11.8 mmol) in THF (15 ml)], the crude product was purified by Kugelrohr distillation (oven temperature 190°C, 0.01 mbar) to give a 11:89 mixture (4.38 g) of *rac*-**14** and **18**. Hydrolysis of this mixture was carried out by treatment of a solution of *rac*-**14**/**18** in EtOH (30 ml) with hydrochloric acid (3 M, 50 ml). After extractive separation of glycine ethyl ester, *rac*-**16** was isolated in 71% yield (relative to the two-fold molar amount of **10**) as a colorless liquid (3.91 g, 16.7 mmol). The IR, NMR, and MS data of the product were identical with those described for (*R*)-**16** in Ref. [4]. Anal. Found: C, 41.1; H, 8.2; N, 6.0. Calc. for $C_8H_{19}GeNO_2$: C, 41.09; H, 8.19; N, 5.99%.

*3.1.18. (Chloromethyl)dimethyl(phenyl)silane (**19**)*

This compound was commercially available (Aldrich).

*3.1.19. (Chloromethyl)dimethyl(phenyl)germane (**20**)*

Synthesis as described in Ref. [4].

*3.1.20. (Chloromethyl)dimethyl(vinyl)silane (**21**)*

Synthesis as described in Ref. [24].

*3.1.21. rac-2-Amino-3-[dimethyl(phenyl)silyl]propionic acid ethyl ester (rac-**25**)*

Method A. This compound was prepared analogously to the synthesis of *rac*-**15** (Method A) by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (31.3 ml, 50.1 mmol *n*-BuLi) to a solution of **10** (8.51 g, 50.0 mmol) in THF (25 ml), followed by treatment with a solution of **19** (9.24 g, 50.0 mmol) in THF (10 ml). The crude product was purified by Kugelrohr distillation (oven temperature 160°C, 0.01 mbar) to give a 45:55 mixture of **10** and *rac*-**22**. Hydrolysis of this mixture was carried out by treatment of a solution of **10**/*rac*-**22** in EtOH (25 ml) with hydrochloric acid (3 M, 10 ml). After extractive separation of glycine ethyl ester, *rac*-**25** was isolated in 35% yield as a colorless liquid (4.35 g, 17.3 mmol). The IR, NMR, and MS data of the product were identical with those described for (*R*)-**25** in Ref. [4]. Anal. Found: C, 62.0; H, 8.4; N, 5.6. Calc. for $C_{13}H_{21}NO_2Si$: C, 62.11; H, 8.42; N, 5.57%.

Method B. This compound was prepared analogously to the synthesis of *rac*-**15** (Method B) by addition of a

1.6 M solution of *n*-butyllithium in *n*-hexane (22.1 ml, 35.4 mmol *n*-BuLi) to a solution of **10** (6.01 g, 35.3 mmol) in THF (150 ml), followed by treatment with a solution of **19** (6.52 g, 35.3 mmol) in THF (15 ml). After the metalation/alkylation procedure was repeated [metalation with a 1.6 M solution of *n*-butyllithium in *n*-hexane (22.1 ml, 35.4 mmol *n*-BuLi); alkylation with a solution of **19** (6.52 g, 35.3 mmol) in THF (15 ml)], the crude product was purified by Kugelrohr distillation (oven temperature 210°C, 0.01 mbar) to give a 33:67 mixture of *rac*-**22** and **28**. Hydrolysis of this mixture was carried out by treatment of a solution of *rac*-**22**/**28** in EtOH (100 ml) with hydrochloric acid (3 M, 150 ml). After extractive separation of glycine ethyl ester, *rac*-**25** was isolated in 64% yield (relative to the two-fold molar amount of **10**) as a colorless liquid (11.4 g, 45.3 mmol). The IR, NMR, and MS data of the product were identical with those described for (*R*)-**25** in Ref. [4]. Anal. Found: C, 61.8; H, 8.2; N, 5.5. Calc. for $C_{13}H_{21}NO_2Si$: C, 62.11; H, 8.42; N, 5.57%.

*3.1.22. rac-2-Amino-3-[dimethyl(phenyl)germyl]-propionic acid ethyl ester (rac-**26**)*

This compound was prepared analogously to the synthesis of *rac*-**15** (Method B) by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (2.34 ml, 3.74 mmol *n*-BuLi) to a solution of **10** (637 mg, 3.74 mmol) in THF (25 ml), followed by treatment with a solution of **20** (857 mg, 3.74 mmol) in THF (15 ml). After the metalation/alkylation procedure was repeated [metalation with a 1.6 M solution of *n*-butyllithium in *n*-hexane (2.34 ml, 3.74 mmol *n*-BuLi); alkylation with a solution of **20** (857 mg, 3.74 mmol) in THF (15 ml)], the crude product was purified by Kugelrohr distillation (oven temperature 250°C, 0.01 mbar) to give a 25:75 mixture (1.62 g) of *rac*-**23** and **29**. Hydrolysis of this mixture was carried out by treatment of a solution of *rac*-**23**/**29** in EtOH (10 ml) with hydrochloric acid (3 M, 15 ml). After extractive separation of glycine ethyl ester, *rac*-**26** was isolated in 60% yield (relative to the two-fold molar amount of **10**) as a colorless liquid (1.32 g, 4.46 mmol). The IR, NMR, and MS data of the product were identical with those described for (*R*)-**26** in Ref. [4]. Anal. Found: C, 52.8; H, 7.1; N, 4.4. Calc. for $C_{13}H_{21}GeNO_2$: C, 52.76; H, 7.15; N, 4.73%.

*3.1.23. rac-2-Amino-3-[dimethyl(vinyl)silyl]propionic acid ethyl ester (rac-**27**)*

This compound was prepared analogously to the synthesis of *rac*-**15** (Method A) by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (4.92 ml, 7.87 mmol *n*-BuLi) to a solution of **10** (1.34 g, 7.87 mmol) in THF (25 ml), followed by treatment with a solution of **21** (1.06 g, 7.87 mmol) in THF (10 ml). The crude product was purified by Kugelrohr distillation (oven temperature 150°C, 0.01 mbar) to give a 20:80 mixture (1.51 g) of **10** and *rac*-**24**. Hydrolysis of this mixture was carried out by treatment of a solution of **10**/*rac*-**24** in EtOH (25 ml) with HCl (3 M, 10 ml). After extractive separation of glycine ethyl ester, *rac*-**27** was isolated in 35% yield as a colorless liquid (548 mg, 2.72 mmol). The IR, NMR, and MS data of the product were identical with those described for (*R*)-**27** in Ref. [4]. Anal. Found: C, 53.0; H, 9.3; N, 7.1. Calc. for $C_9H_{19}NO_2Si$: C, 53.69; H, 9.51; N, 6.96%.

*3.1.24. (R)-N-[(Fluoren-9-yl)methoxycarbonyl]-2-amino-4,4-dimethylpentanoic acid [(R)-**30**]*

A solution of (fluoren-9-yl)methyl chloroformate (3.93 g, 15.2 mmol) in dioxane (40 ml) was added dropwise at 0°C within 30 min to a stirred solution of (*R*)-**1** (2.00 g, 13.8 mmol) and Na_2CO_3 (2.93 g, 27.6 mmol) in water–dioxane [3:5 (v/v)] (80 ml). After the mixture was stirred at 10°C for 1 h, it was warmed to r.t. within 12 h. The solvents were removed under reduced pressure (0.1 mbar, 20°C) and the residue acidified with hydrochloric acid (6 M, pH 2). The aqueous solution was extracted with EtOAc (3×5 ml), and the combined organic extracts were dried over anhydrous Na_2SO_4. After the solution was concentrated under reduced pressure, the resulting precipitate was isolated by filtration, washed with petroleum ether (40–60°C) (2×10 ml) and then dried in vacuo (0.1 mbar, 20°C, 2 h) to give (*R*)-**30** in 80% yield as a colorless solid (4.04 g, 11.0 mmol). The IR, NMR, and MS data of the product were identical with those described for (*S*)-**30** in Ref. [4]. Anal. Found: C, 71.3; H, 6.7; N, 3.8. Calc. for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81%.

*3.1.25. (S)-N-[(Fluoren-9-yl)methoxycarbonyl]-2-amino-3-(trimethylsilyl)propionic acid [(S)-**31**]*

This compound was prepared analogously to the synthesis of (*R*)-**30** starting from (*S*)-**2** (1.04 g, 6.45 mmol). The product (*S*)-**31** was isolated in 68% yield as a colorless solid (1.68 g, 4.38 mmol). The IR, NMR, and MS data of (*S*)-**31** were identical with those described for (*R*)-**31** in Ref. [4]. Anal. Found: C, 65.5; H, 6.6; N, 3.6. Calc. for $C_{21}H_{25}NO_4Si$: C, 65.77; H, 6.57; N, 3.65%.

*3.1.26. (S)-N-[(Fluoren-9-yl)methoxycarbonyl]-2-amino-3-(trimethylgermyl)propionic acid [(S)-**32**]*

This compound was prepared analogously to the synthesis of (*R*)-**30** starting from (*S*)-**3** (973 mg, 4.73 mmol). The product (*S*)-**32** was isolated in 59% yield as a colorless solid (1.20 g, 2.80 mmol). The IR, NMR, and MS data of (*S*)-**32** were identical with those described for (*R*)-**32** in Ref. [4]. Anal. Found: C, 58.8; H, 5.8; N, 3.3. Calc. for $C_{21}H_{25}GeNO_4$: C, 58.93; H, 5.89; N, 3.27%.

*3.1.27. Preparative liquid-chromatographic resolution of rac-**2** and rac-**3***

The (*R*)- and (*S*)-enantiomers of **2** and **3** were separated by liquid-chromatographic resolution of *rac*-**2** and *rac*-**3**, respectively, using CHIROBIOTIC T (glycopeptide Teicoplanin covalently linked to spherical silica gel, 40 μm; Advanced Separation Technologies, Inc.) as the chiral stationary phase. The experimental conditions were as follows: HPLC pump, Shimadzu LC8-A; detector, Knauer VW monitor; integrator, Shimadzu C-R3A; column (50 mm i.d. × 500 mm for *rac*-**2**; 50 mm i.d. × 250 mm for *rac*-**3**), Merck Superformance; eluent, MeOH for *rac*-**2** and MeOH–water [90:10 (v/v)] for *rac*-**3**; detection, 215 nm; temperature, 21–23°C; injection volume, 150 ml (2 mg of the sample material in 1 ml of the eluent) for *rac*-**2** and 30 ml (2 mg of the sample material in 1 ml of the eluent) for *rac*-**3**. The solvents of the respective fractions obtained [(*R*)-**2**, first fraction, (*S*)-**2**, second fraction; (*R*)-**3**, first fraction, (*S*)-**3**, second fraction] were removed immediately after the chromatographic separation (rotary evaporator, 35°C, 100 mbar), and the respective residues were combined and then stored at r.t. The yields and enantiomeric purities (for the analytic methods, see below) of the separated enantiomers (colorless solids) were as follows: (*R*)-**2**, 36%, 99% ee; (*S*)-**2**, 23%, 99% ee; (*R*)-**3**, 32%, 99% ee; (*S*)-**3**, 21%, 93% ee.

*3.1.28. Determination of the enantiomeric purities of (R)-**2** and (S)-**2** by capillary gas chromatography*

The (*R*)- and (*S*)-enantiomers of **2** were separated, after chemical transformation (see Section 3.1.29), by capillary gas chromatography. The experimental conditions were as follows: gas chromatograph, HP 6890; column (0.25 mm i.d. × 25 m, film thickness 0.12 μm), Chrompack 7496; carrier gas, hydrogen; pressure, 0.55 bar; temperature program, 50°C (1 min) to 105°C (0 min) with 3°C min^{-1} to 200°C (10 min) with 4°C min^{-1}; injector temperature, 250°C; split, 1:100; detector, FID; detector temperature, 250°C. The retention times of the derivatives of (*R*)-**2** and (*S*)-**2** were as follows: (*R*)-**2**, 20.8 min; (*S*)-**2**, 22.1 min.

*3.1.29. Derivatization of **2** for the GC studies [formation of the N-(trifluoroaceto)amino acid propyl ester]*

A 3.0 M solution of HCl in *n*-propanol (500 μl) was added to a 2.5-mg sample of **2**, and the mixture was heated at 110°C for 45 min. After the excess HCl and *n*-propanol were blown off with a stream of nitrogen gas, the residue was dissolved in CH_2Cl_2 (200 μl) and treated with trifluoroacetic anhydride (200 μl) for 10 min at 110°C. The excess CH_2Cl_2 and trifluoroacetic anhydride were blown off with a stream of nitrogen gas, and the residue was dissolved in toluene (150 μl). A 0.2 μl-sample of this solution was injected into the gas chromatograph.

*3.1.30. Determination of the enantiomeric purities of (R)-**3** and (S)-**3** by liquid chromatography*

The (*R*)- and (*S*)-enantiomers of **3** were separated by liquid chromatography using CHIROBIOTIC T (glycopeptide Teicoplanin covalently linked to spherical silica gel, 5 μm; Advanced Separation Technologies, Inc.) as the chiral stationary phase. The experimental conditions were as follows: HPLC pump, Shimadzu LC6-A; detector, Soma S-3702 UV–vis; integrator, Shimadzu C-R3A; column, 4.6 mm i.d. × 250 mm, Merck Superformance; eluent, MeOH–water [90:10 (v/v)]; flow rate, 600 μl min^{-1}; pressure, 58 bar; detection, 215 nm; temperature, 20°C; injection volume, 20 μl (2 mg of the sample material dissolved in 1 ml of the eluent). The retention times were as follows: (*R*)-**3,** 9.2 min; (*S*)-**3**, 11.2 min.

*3.1.31. Determination of the antagonistic potencies of the decapeptides **7**–**9** at the human GnRH receptor*

The antagonistic potencies of the decapeptides **7**–**9** at the human GnRH receptor were studied in vitro in a functional assay as described in Ref. [4].

Acknowledgements

Financial support of this work by Degussa AG and the Fonds der Chemischen Industrie is gratefully acknowledged. M.M. thanks the Fond der Chemischen Industrie for a postgraduate fellowship.

References

[1] Reviews dealing with bioorganosilicon and bioorganogermanium chemistry: (a) R. Tacke, H. Linoh, in: S. Patai, Z. Rappoport (Eds.), The Chemistry of Organic Silicon Compounds, Part 2, Wiley, Chichester, 1989, pp. 1143–1206. (b) E. Lukevics, L. Ignatovich, Appl. Organomet. Chem. 6 (1992) 113. (c) R. Tacke, S.A. Wagner, in: Z. Rappoport, Y. Apeloig (Eds.), The Chemistry of Organic Silicon Compounds, vol. 2, Part 3, Wiley, Chichester, 1998, pp. 2363–2400. (d) R. Tacke, T. Heinrich, T. Kornek, M. Merget, S.A. Wagner, J. Gross, C. Keim, G. Lambrecht, E. Mutschler, T. Beckers, M. Bernd, T. Reissmann, Phosphorus, Sulfur, and Silicon 150/151 (1999) 69.
[2] M. Merget, S. Bartoschek, R. Willeke, R. Tacke, in: N. Auner, J. Weis (Eds.), Organosilicon Chemistry IV — From Molecules to Materials, Wiley–VCH, Weinheim, 2000, pp. 33–36.
[3] V.I. Handmann, M. Merget, R. Tacke, Z. Naturforsch. B 55 (2000) 133.
[4] R. Tacke, M. Merget, R. Bertermann, M. Bernd, T. Beckers, T. Reissmann, Organometallics 19 (2000) 3486.
[5] (a) L. Birkofer, A. Ritter, Angew. Chem. 68 (1956) 461. (b) L. Birkofer, A. Ritter, Liebigs Ann. Chem. 612 (1958) 22.
[6] T.H. Porter, W. Shive, J. Med. Chem. 11 (1968) 402.
[7] R. Fitzi, D. Seebach, Tetrahedron 44 (1988) 5277.
[8] B. Weidmann, Chimia 46 (1992) 312.

[9] R.D. Walkup, D.C. Cole, B.R. Whittlesey, J. Org. Chem. 60 (1995) 2630.
[10] H. Yamanaka, T. Fukui, T. Kawamoto, A. Tanaka, Appl. Microbiol. Biotechnol. 45 (1996) 51.
[11] A.G. Myers, J.L. Gleason, T. Yoon, D.W. Kung, J. Am. Chem. Soc. 119 (1997) 656.
[12] R.J. Smith, S. Bratovanov, S. Bienz, Tetrahedron 53 (1997) 13695.
[13] M.P. Sibi, B.J. Harris, J.J. Shay, S. Hajra, Tetrahedron 54 (1998) 7221.
[14] H. Ishikawa, H. Yamanaka, T. Kawamoto, A. Tanaka, Appl. Microbiol. Biotechnol. 53 (1999) 19.
[15] C. Bolm, A. Kasyan, K. Drauz, K. Günther, G. Raabe, Angew. Chem. 112 (2000) 2374; Angew. Chem. Int. Ed. 39 (2000) 2288.
[16] (a) B. Kutscher, M. Bernd, T. Beckers, E.E. Polymeropoulos, J. Engel, Angew. Chem. 109 (1997) 2240; Angew. Chem. Int. Ed. 36 (1997) 2148. (b) T. Reissmann, J. Engel, B. Kutscher, M. Bernd, P. Hilgard, M. Peukert, I. Szelenyi, S. Reichert, D. Gonzales-Barcena, E. Nieschiag, A.M. Comaru-Schally, A.V. Schally, Drugs of the Future 19 (1994) 228.
[17] M. Bernd, B. Kutscher, E. Günther, P. Romeis, T. Reissmann, T. Beckers, DE 199 11 771.3, PCT/EP 2000/02165.
[18] F. Haviv, T.D. Fitzpatrick, C.J. Nichols, R.E. Swenson, N.A. Mort, E.N. Bush, G. Diaz, A.T. Nguyen, M.R. Holst, V.A. Cybulski, J.A. Leal, G. Bammert, N.S. Rhutasel, P.W. Dodge, E.S. Johnson, J.B. Cannon, J. Knittle, J. Greer, J. Med. Chem. 36 (1993) 928.
[19] B.M. Trost, Science 254 (1991) 1471.
[20] J.M. Stewart, J.D. Young, Solid-Phase Peptide Synthesis, Pierce Chemical Co, Rockford, USA, 1984.
[21] U. Groth, T. Huhn, B. Porsch, C. Schmeck. U. Schöllkopf, Liebigs Ann. Chem. (1993) 715.
[22] R. Tacke, B. Becker, J. Organomet. Chem. 354 (1988) 147.
[23] D. Seyferth, E.G. Rochow, J. Am. Chem. Soc. 77 (1955) 907.
[24] M. Altamura, M Giammaruco, M. Taddei, P. Ulivi, J. Org. Chem. 60 (1995) 8403.