Syntheses and Properties of Silicon- and Germanium-Containing α-Amino Acids and Peptides: A Study on C/Si/Ge Bioisosterism§

Reinhold Tacke,*,† Markus Merget,† Rüdiger Bertermann,† Michael Bernd,‡ Thomas Beckers,‡ and Thomas Reissmann‡

Institut für Anorganische Chemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany, and ASTA Medica AG, Konzernforschung, Weismüllerstrasse 45, D-60315 Frankfurt, Germany

Received February 23, 2000

The unnatural silicon-containing α-amino acids (*R*)- and (*S*)-$H_2NCH(CH_2SiMe_3)COOH$ [(*R*)-**2** and (*S*)-**2**], (*R*)-$H_2NCH(CH_2SiMe_2Ph)COOH$ [(*R*)-**4**], and (*R*)-$H_2NCH(CH_2SiMe_2CH{=}CH_2)$-COOH [(*R*)-**6**] as well as the unnatural germanium-containing α-amino acids (*R*)- and (*S*)-$H_2NCH(CH_2GeMe_3)COOH$ [(*R*)-**3** and (*S*)-**3**] and (*R*)-$H_2NCH(CH_2GeMe_2Ph)COOH$ [(*R*)-**5**] were prepared in three-step syntheses, starting from (*R*)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine [(*R*)-**10**]. All amino acids were isolated as enantiomerically pure (≥99% ee) compounds. The (*R*)- and (*S*)-enantiomers of *β*-(trimethylsilyl)alanine [(*R*)-**2** and (*S*)-**2**] and *β*-(trimethylgermyl)alanine [(*R*)-**3** and (*S*)-**3**] are sila-analogues and germa-analogues, respectively, of the (*S*)- and (*R*)-enantiomers of the nonproteinogenic amino acid *β-tert*-butylalanine [(*S*)- and (*R*)-$H_2NCH(CH_2CMe_3)COOH$; (*S*)-**1** and (*R*)-**1**]. The C/Si/Ge-analogous (L-configurated) amino acids (*S*)-**1**, (*R*)-**2**, and (*R*)-**3** were treated with (fluoren-9-yl)methyl chloroformate to give the corresponding *N*-Fmoc derivatives (*S*)-**26**, (*R*)-**27**, and (*R*)-**28**. These *N*-Fmoc-protected amino acids were used as building blocks for the solid-phase syntheses of the C/Si/Ge-analogous decapeptides **7**−**9** [Ac-D-Nal[1]-4-Cl-D-Phe[2]-D-Pal[3]-Ser[4]-Me_3El-Ala[5]-D-Cit[6]-Leu[7]-Arg[8]-Pro[9]-D-Ala[10]-NH_2 (**7**, El = C; **8**, El = Si; **9**, El = Ge)]. The C/Si/Ge analogues **7**−**9** are derivatives of the GnRH antagonist Cetrorelix[INN], which bears an (*S*)-tyrosine residue [instead of the (*S*)-Me_3C-Ala, (*R*)-Me_3Si-Ala, or (*R*)-Me_3Ge-Ala residue] in position 5 of its decapeptide backbone. The decapeptides **7**−**9** were studied in vitro in receptor binding and functional assays using recombinant cell lines expressing the human GnRH receptor. All compounds behaved as potent GnRH antagonists, the binding affinities and antagonistic potencies of the three C/Si/Ge analogues being quite similar. Compounds **7**−**9** were also studied for their in vivo activities in the male rat after s.c. administration. They produced both a strong testosterone suppression (single-dose treatment, 1.5 mg/kg) and a strong LH suppression (castrated male rat; single-dose treatment, 0.05 mg/kg). For the silicon- and germanium-containing decapeptides **8** and **9** the testosterone and LH suppression lasted for a significantly longer period of time compared with the effects of the carbon analogue **7**.

Introduction

Enantiomerically pure amino acids with unnatural side chains are gaining increasing importance in chemistry and life sciences.[1] Synthetic unnatural amino acids have proven useful for probing the structural requirements for the biological activity of numerous peptides and proteins and have been used as building blocks for the synthesis of novel biologically active peptides. In addition, unnatural amino acids are of interest as precursors of drugs and plant-protective agents and are used as chiral auxiliaries in asymmetric organic synthesis. In the course of our systematic studies on bioorganosilicon and bioorganogermanium chemistry,[2] we became interested in enantiomerically pure silicon- and germanium-containing amino acids of the formula type $H_2NCH(CH_2SiR_3)COOH$ and $H_2NCH(CH_2GeR_3)$-COOH (R = organyl). With their CH_2SiR_3 and CH_2GeR_3 groups as side chains, these *β*-(triorganylsilyl)alanine

* To whom correspondence should be addressed.

§ Dedicated to Professor Horst Kessler on the occasion of his 60th birthday.

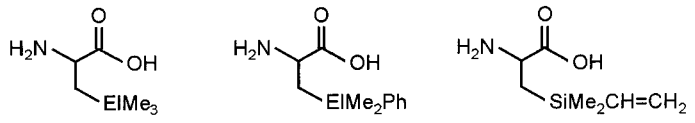
† Universität Würzburg.
‡ ASTA Medica AG.

(1) (a) Giannis, A.; Kolter, T. *Angew. Chem.* **1993**, *105*, 1303−1326; *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267. (b) Gante, J. *Angew. Chem.* **1994**, *106*, 1780−1802; *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720. (c) Kleemann, A.; Leuchtenberger, W.; Hoppe, B.; Tanner, H. In *Ullmann's Encyclopedia of Industrial Chemistry*; Gerhartz, W., Ed.; VCH: Weinheim, Germany, 1985; pp 57−97. (d) Ishida, H.; Inoue, Y. *Rev. Heteroat. Chem.* **1998**, *19*, 79−142.

(2) Reviews dealing with bioorganosilicon and bioorganogermanium chemistry: (a) Tacke, R.; Linoh, H. In *The Chemistry of Organic Silicon Compounds, Part 2*; Patai, S., Rappoport, Z., Eds.; Wiley: Chichester, U.K., 1989; pp 1143−1206. (b) Lukevics, E.; Ignatovich, L. *Appl. Organomet. Chem.* **1992**, *6*, 113−126. (c) Tacke, R.; Wagner, S. A. In *The Chemistry of Organic Silicon Compounds, Vol. 2, Part 3;* Rappoport, Z., Apeloig, Y., Eds.; Wiley: Chichester, U.K., 1998; pp 2363−2400. (d) Tacke, R.; Heinrich, T.; Kornek, T.; Merget, M.; Wagner, S. A.; Gross, J.; Keim, C.; Lambrecht, G.; Mutschler, E.; Beckers, T.; Bernd, M.; Reissmann, T. *Phosphorus, Sulfur, Silicon* **1999**, *150/151*, 69−87.

10.1021/om000169l CCC: $19.00
Publication on Web 08/01/2000

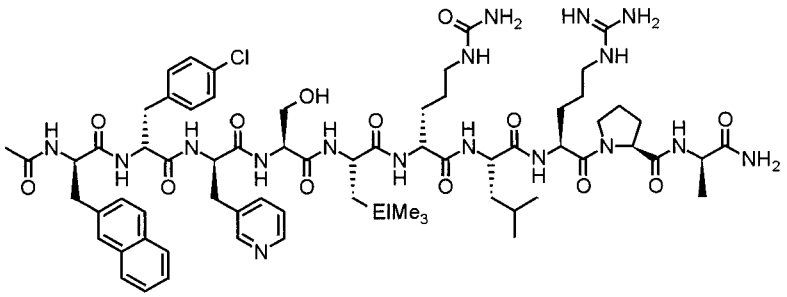
Chart 1



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]—[Me_3C-Ala[5]]—D-Cit[6]—Leu[7]—Arg[8]—Pro[9]—D-Ala[10]—NH_2

8: El = Si, Ac—D-Nal[1]—4-Cl-D-Phe[2]—D-Pal[3]—Ser[4]—[Me_3Si-Ala[5]]—D-Cit[6]—Leu[7]—Arg[8]—Pro[9]—D-Ala[10]—NH_2

9: El = Ge, Ac—D-Nal[1]—4-Cl-D-Phe[2]—D-Pal[3]—Ser[4]—[Me_3Ge-Ala[5]]—D-Cit[6]—Leu[7]—Arg[8]—Pro[9]—D-Ala[10]—NH_2

and *β*-(triorganylgermyl)alanine derivatives are unique amino acids combining hydrophobicity and sterically demanding bulkiness with the common hydrophilic behavior of amino acids. We report here on the syntheses of the enantiomerically pure α-amino acids (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, (*S*)-**3**, (*R*)-**4**, (*R*)-**5**, and (*R*)-**6**. The amino acids *β*-(trimethylsilyl)alanine (**2**) and *β*-(trimethylgermyl)alanine (**3**) are sila- and germa-analogues, respectively, of the nonproteinogenic amino acid *β*-*tert*-butylalanine (**1**) (see Chart 1).

Furthermore, we report on the syntheses and pharmacological characterization of the decapeptides **7**−**9**. These peptides contain the C/Si/Ge-analogous L-configurated amino acid residues Me_3C-Ala, Me_3Si-Ala, or Me_3Ge-Ala in position 5 of their backbone. The C/Si/Ge analogues **7**−**9** are derivatives of the GnRH antagonist Cetrorelix[INN], which bears an (*S*)-tyrosine residue in position 5 [instead of the Me_3El-Ala (El = C, Si, Ge) residues derived from (*S*)-**1**, (*R*)-**2**, or (*R*)-**3**].[3] The pharmacological studies of **7**−**9** presented here were carried out with a special emphasis on C/Si/Ge bioisosterism.

Silicon-containing α-amino acids [*rac*-**2**,[4,5] (*R*)-**2**,[6−9] (*S*)-**2**,[8] *rac*-**4**,[10] (*R*)-**4**,[7] (*R*)-$H_2NCH(SiMePh_2)COOH$,[7] *rac*-$H_2NCH[Si(CH_2)_nMe]COOH$[10] (*n* = 4, 5)] have already been described in the literature; however, alternative strategies have been used for their synthesis. Furthermore, the ethyl ester of (*R*)-**2**,[11] the *N*-Fmoc,[7,11] and *N*-Boc derivatives[11] of (*R*)-**2**, the *N*-Boc derivatives of the α-amino acid esters (*S*)-$H_2NCH(CH_2R)COOMe$ (R = $SiPh_3$, $SiMe_2Ph$, $SiMePh_2$, Si^tBuPh_2),[12] the *N*-Fmoc derivatives of (*R*)-**4** and (*R*)-$H_2NCH(SiMePh_2)COOH$,[7] and the ethyl esters of *rac*- and (*R*)-4,4-dimethyl-4-silaproline[13] have been described. In addition, there are short reports on some tripeptides bearing the silicon-containing amino acid (*R*)-**2**.[7,11] To the best of our knowledge, germanium-containing α-amino acids and peptides have not yet been described in the literature.

The studies presented here were carried out as part of our systematic investigations on biologically active organosilicon and organogermanium compounds (in this context, see refs 2a, 2c, and 2d). Some preliminary results of these studies have already been published elsewhere.[2d,14]

Results and Discussion

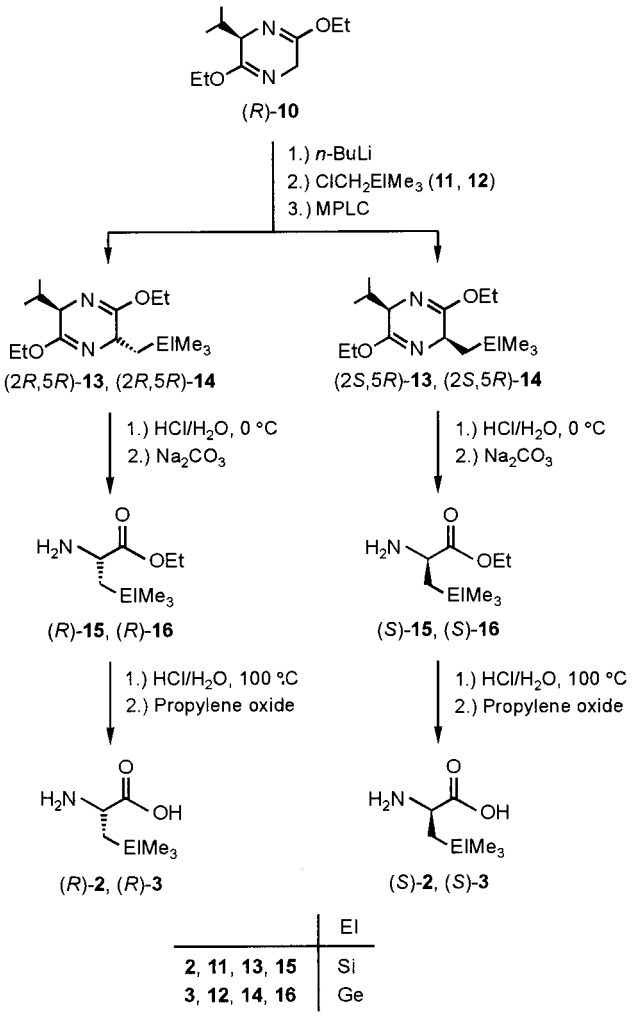
Syntheses of the α-Amino Acids. The silicon- and germanium-containing amino acids (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, and (*S*)-**3** were prepared by three-step syntheses according to the Schöllkopf approach,[15] starting from the dihydropyrazine (*R*)-**10** (Scheme 1).[16]

Metalation of the dihydropyrazine (*R*)-**10** with *n*-butyllithium and subsequent treatment with $ClCH_2$-

(3) Reviews dealing with Cetrorelix[INN]: (a) Kutscher, B.; Bernd, M.; Beckers, T.; Polymeropoulos, E. E.; Engel, J. *Angew. Chem.* **1997**, *109*, 2240−2254; *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2148−2161. (b) Reissmann, T.; Engel, J.; Kutscher, B.; Bernd, M.; Hilgard, P.; Peukert, M.; Szelenyi, I.; Reichert, S.; Gonzales-Barcena, D.; Nieschiag, E.; Comaru-Schally, A. M.; Schally, A. V. *Drugs Future* **1994**, *19*, 228−237.

(4) (a) Birkofer, L.; Ritter, A. *Angew. Chem.* **1956**, *68*, 461−462. (b) Birkofer, L.; Ritter, A. *Liebigs Ann. Chem.* **1958**, *612*, 22−33.
(5) Porter, T. H.; Shive, W. *J. Med. Chem.* **1968**, *11*, 402−403.
(6) Fitzi, R.; Seebach, D. *Tetrahedron* **1988**, *44*, 5277−5292.
(7) Walkup, R. D.; Cole, D. C.; Whittlesey, B. R. *J. Org. Chem.* **1995**, *60*, 2630−2634.
(8) Yamanaka, H.; Fukui, T.; Kawamoto, T.; Tanaka, A. *Appl. Microbiol. Biotechnol.* **1996**, *45*, 51−55.
(9) Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. *J. Am. Chem. Soc.* **1997**, *119*, 656−673.
(10) Smith, R. J.; Bratovanov, S.; Bienz, S. *Tetrahedron* **1997**, *53*, 13695−13702.
(11) Weidmann, B. *Chimia* **1992**, *46*, 312−313.
(12) Sibi, M. P.; Harris, B. J.; Shay, J. J.; Hajra, S. *Tetrahedron* **1998**, *54*, 7221−7228.
(13) Handmann, V. I.; Merget, M.; Tacke, R. *Z. Naturforsch.* **2000**, *55b*, 133−138.
(14) Merget, M.; Bartoschek, S.; Willeke, R.; Tacke, R. In *Organosilicon Chemistry IV−From Molecules to Materials*; Auner, N., Weis, J., Eds.; VCH: Weinheim, Germany, 2000; pp 33−36.
(15) Schöllkopf, U. *Tetrahedron* **1983**, *39*, 2085−2091.
(16) This method has also been used for the synthesis of the ethyl ester of (*R*)-**2** (see ref 11).

Scheme 1



$SiMe_3$ (**11**) or $ClCH_2GeMe_3$ (**12**) yielded mixtures of the respective diastereomeric dihydropyrazines (2*R*,5*R*)-**13**/(2*S*,5*R*)-**13** and (2*R*,5*R*)-**14**/(2*S*,5*R*)-**14** (for the diastereomeric excess, see Table 1), along with the nonreacted educt (*R*)-**10**. These mixtures were separated by medium-pressure liquid chromatography (MPLC) on silica gel to yield the diastereomerically pure (≥99% de) dihydropyrazines (2*R*,5*R*)-**13**, (2*S*,5*R*)-**13**, (2*R*,5*R*)-**14**, and (2*S*,5*R*)-**14** (for the yields, see Table 1). After treatment of the dihydropyrazines (2*R*,5*R*)-**13**, (2*S*,5*R*)-**13**, (2*R*,5*R*)-**14**, and (2*S*,5*R*)-**14** with hydrochloric acid at 0 °C and subsequent liquid-chromatographic separation of the byproduct (*R*)-valine ethyl ester (silica gel as stationary phase), the respective enantiomerically pure (≥99% ee) amino acid ethyl esters (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, and (*S*)-**16** were obtained (for the yields, see Table 2). Hydrolysis of these esters in boiling hydrochloric acid and subsequent treatment of the resulting amino acid hydrochlorides with propylene oxide finally yielded the amino acids (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, and (*S*)-**3** (for the yields, see Table 3).

The structurally related amino acids (*R*)-**4**−(*R*)-**6** were prepared according to Scheme 2. Thus, metalation of the dihydropyrazine (*R*)-**10** with *n*-butyllithium and subsequent treatment with $ClCH_2SiMe_2Ph$ (**17**), $ClCH_2GeMe_2Ph$ (**18**), or $ClCH_2SiMe_2CH{=}CH_2$ (**19**), followed by medium-pressure liquid chromatography on silica gel, yielded the diastereomerically pure (≥99% de) dihydropyrazines (2*R*,5*R*)-**20**, (2*R*,5*R*)-**21**, and (2*R*,5*R*)-**22** [diastereomers (2*S*,5*R*)-**20**, (2*S*,5*R*)-**21**, and (2*S*,5*R*)-**22** not isolated]. Some experimental data for these syntheses are listed in Table 1. Treatment of the dihydropyrazines (2*R*,5*R*)-**20**, (2*R*,5*R*)-**21**, and (2*R*,5*R*)-**22** with hydrochloric acid at 0 °C and subsequent liquid-chromatographic separation of the byproduct (*R*)-valine ethyl ester (silica gel as stationary phase) yielded the respective enantiomerically pure (≥99% ee) amino acid esters (*R*)-**23**−(*R*)-**25** (for the yields, see Table 2). Attemps to prepare the amino acids (*R*)-**4**−(*R*)-**6** by hydrolysis of the respective ethyl esters (*R*)-**23**−(*R*)-**25** in boiling hydrochloric acid failed: Under the conditions applied to the hydrolysis of (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, and (*S*)-**16**, Si−C (Si−Ph, Si−$CH{=}CH_2$) and Ge−C (Ge−Ph) bond cleavage was observed. However, this problem could be solved by avoiding acidic conditions. Thus, treatment of the amino acid esters (*R*)-**23**−(*R*)-**25** with lithium hydroxide (molar ratio 1:1) in a water/dioxane mixture, followed by a special workup (including an Li^+/H^+ exchange; see Experimental Section), yielded the respective amino acids (*R*)-**4**−(*R*)-**6** (for the yields, see Table 3).

The amino acids (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, (*S*)-**3**, and (*R*)-**4**−(*R*)-**6** were isolated as colorless crystalline solids, whereas the dihydropyrazines (2*R*,5*R*)-**13**, (2*S*,5*R*)-**13**, (2*R*,5*R*)-**14**, (2*S*,5*R*)-**14**, and (2*R*,5*R*)-**20**−(2*R*,5*R*)-**22** as well as the amino acid esters (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, (*S*)-**16**, and (*R*)-**23**−(*R*)-**25** were obtained as colorless liquids. The identity of all compounds was established by elemental analyses (C, H, N), NMR-spectroscopic studies (^1H, ^{13}C, ^{29}Si), and mass-spectrometric investigations (EI MS).

Determination of the Absolute Configurations. The assignment of the absolute configurations of the (2*R*,5*R*)- and (2*S*,5*R*)-diastereomers of **13**, **14**, and **20**−**22** is based on the well-established stereochemistry of the Schöllkopf approach[15] and is supported by the characteristic $^5J_{GK}$ coupling constants in the ^1H NMR spectra of the (2*R*,5*R*)-isomers (*trans*-isomers of **13**, **14**, and **20**−**22**: $^5J_{GK}$ = 3.3−3.7 Hz) and (2*S*,5*R*)-isomers (*cis*-isomers of **13** and **14**: $^5J_{GK}$ = 4.1 and 4.2 Hz) (see Figure 1). As the hydrolytic cleavage of the dihydropyrazines **13**, **14**, and **20**−**22** does not affect the absolute configurations of the two centers of chirality, the absolute configurations of the resulting amino acids can be deduced from the absolute configurations of the respective dihydropyrazines (see Schemes 1 and 2).

Determination of the Diastereomeric Purities. The (2*R*,5*R*)- and (2*S*,5*R*)-diastereomers of the dihydropyrazines **13**, **14**, and **20**−**22** could be separated by analytical capillary gas chromatography. This is demonstrated for the diastereomers of **14** in Figure 2. The retention times of the respective stereoisomers of the dihydropyrazines **13**, **14**, and **20**−**22** are listed in Table 4. This gas-chromatographic method was used as an analytical tool to determine the molar ratio of the (2*R*,5*R*)- and (2*S*,5*R*)-diastereomers of **13**, **14**, and **20**−**22**.

Determination of the Enantiomeric Purities. The enantiomeric purities of the amino acid esters (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, (*S*)-**16**, and (*R*)-**23**−(*R*)-**25** were determined by ^1H NMR experiments using the chiral solvating agent (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(*R*)-TFAE]. As shown for **16** in Figure 3, the two

Table 1. Experimental Data for the Syntheses of the Dihydropyrazines 13, 14, and 20−22 Starting from (*R*)-10

product	ratio (2*R*,5*R*)/(2*S*,5*R*)[a]	ratio [(2*R*/5*R*),(2*S*,5*R*)]/(*R*)-**10**[b]	yield (2*R*/5*R*)[c]	yield (2*S*/5*R*)[d]
13	85:15 (70% de)	75:25	52%	8%
14	86:14 (72% de)	85:15	54%	6%
20	80:20 (60% de)	65:35	41%	n.i.[e]
21	83:17 (66% de)	82:18	41%	n.i.[e]
22	87:13 (74% de)	80:20	58%	n.i.[e]

[a] Molar ratio of the (2*R*,5*R*)- and (2*S*,5*R*)-diastereomers (analysis of the crude product by capillary gas chromatography); diastereomeric excess in parentheses. [b] Molar ratio of the dihydropyrazines (mixture of diastereomers) and the educt (*R*)-**10** (analysis of the crude product by capillary gas chromatography). [c] Yield [relative to (*R*)-**10**] of the diastereomerically pure (≥99% de) (2*R*,5*R*)-isomer after liquid-chromatographic separation. [d] Yield [relative to (*R*)-**10**] of the diastereomerically pure (≥99% de) (2*S*,5*R*)-isomer after liquid-chromatographic separation. [e] Not isolated.

Table 2. Yields of the Amino Acid Esters 15, 16, and 23−25 Obtained by Hydrolysis of the Respective Dihydropyrazines 13, 14, and 20−22

product	yield [educt]	product	yield [educt]
(*R*)-**15**	86% [(2*R*,5*R*)-**13**]	(*R*)-**23**	83% [(2*R*,5*R*)-**20**]
(*S*)-**15**	84% [(2*S*,5*R*)-**13**]	(*R*)-**24**	74% [(2*R*,5*R*)-**21**]
(*R*)-**16**	73% [(2*R*,5*R*)-**14**]	(*R*)-**25**	70% [(2*R*,5*R*)-**22**]
(*S*)-**16**	80% [(2*S*,5*R*)-**14**]		

Table 3. Yields of the Amino Acids 2−6 Obtained by Hydrolysis of the Respective Amino Acid Esters 15, 16, and 23−25 as Well as Overall Yields

product	yield [educt]	overall yield[a]	product	yield [educt]	overall yield[a]
(*R*)-**2**	79% [(*R*)-**15**]	35%	(*R*)-**4**	53% [(*R*)-**23**]	18%
(*S*)-**2**	60% [(*S*)-**15**]	4%	(*R*)-**5**	45% [(*R*)-**24**]	14%
(*R*)-**3**	64% [(*R*)-**16**]	25%	(*R*)-**6**	42% [(*R*)-**25**]	15%
(*S*)-**3**	39% [(*S*)-**16**]	2%			

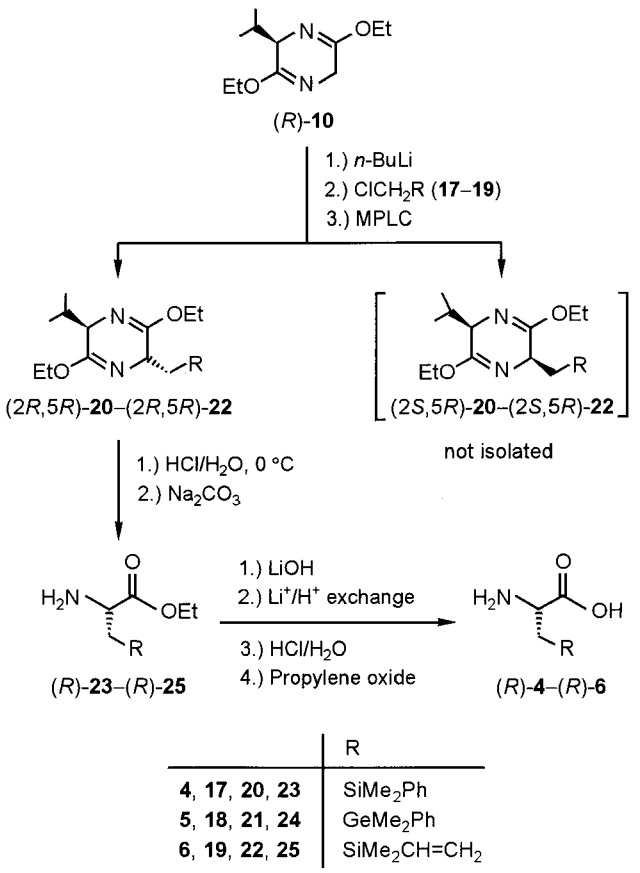
[a] Overall yield relative to (*R*)-**10**.

enantiomers of this amino acid ester can be clearly discriminated by NMR spectroscopy and therefore quantitatively determined by integration of their characteristic resonance signals. According to this method, the enantiomeric purities of (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, (*S*)-**16**, and (*R*)-**23**−(*R*)-**25** were determined to be ≥99% ee. As the hydrolysis of these amino acid esters does not affect the absolute configurations of the centers of chirality, the same enantiomeric purities (≥99% ee) can be assumed for the resulting amino acids (*R*)-**2**, (*S*)-**2**, (*R*)-**3**, (*S*)-**3**, (*R*)-**4**, (*R*)-**5**, and (*R*)-**6**.

Syntheses of the Decapeptides. To incorporate the amino acids (*S*)-**1**, (*R*)-**2**, and (*R*)-**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 (*S*)-**26**, (*R*)-**27**, and (*R*)-**28** were synthesized according to Scheme 3 by treatment of the amino acids (*S*)-**1**, (*R*)-**2**, and (*R*)-**3** with (fluoren-9-yl)-methyl chloroformate (Fmoc-Cl) and were isolated as colorless solids [yields: (*S*)-**26**, 80%; (*R*)-**27**, 69%; (*R*)-**28**, 47%].

The decapeptides **7**−**9** were prepared by solid-phase syntheses, using standard methods[17] for the sequential synthesis of oligopeptides and starting with the C-terminal attachment of the D-alanine residue. 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). The characteristic isotopic ratios of silicon and germanium were very helpful in these studies. As an example of this, the ESI mass spectrum of the germanium-containing decapeptide **9** is shown in Figure 4.

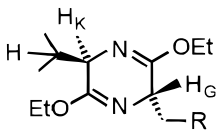
Scheme 2



	R
4, **17**, **20**, **23**	$SiMe_2Ph$
5, **18**, **21**, **24**	$GeMe_2Ph$
6, **19**, **22**, **25**	$SiMe_2CH{=}CH_2$

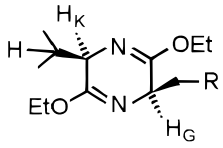
Pharmacological Characterization of the Decapeptides. The decapeptides **7**−**9** were characterized in vitro in receptor binding and functional assays using recombinant cell lines expressing the human GnRH receptor. All compounds behaved as potent GnRH antagonists. As can be seen from Table 5, the binding affinities (K_A values) of **7**−**9** for the human GnRH receptor ([^{125}I]Cetrorelix as the radio ligand) are quite similar, but slightly lower compared with the reference compound Cetrorelix. These findings are in accordance with the results obtained in a reporter gene assay with D-Trp^6-GnRH (Triptorelin) as the agonist: The antagonistic potencies (IC_{50} values) of **7**−**9** are quite similar, but slightly reduced compared with that of Cetrorelix. Thus, incorporation of the (*S*)-Me_3C-Ala, (*R*)-Me_3Si-Ala,

(17) Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*; Pierce Chemical Co: Rockford, 1984.



(2*R*,5*R*)-**13**, (2*R*,5*R*)-**14**,
(2*R*,5*R*)-**20**–(2*R*,5*R*)-**22**

	R
13	$SiMe_3$
14	$GeMe_3$
20	$SiMe_2Ph$
21	$GeMe_2Ph$
22	$SiMe_2CH{=}CH_2$



(2*S*,5*R*)-**13**, (2*S*,5*R*)-**14**

Figure 1. Structure of the *trans*-isomers [(2*R*,5*R*)-configuration] and *cis*-isomers [(2*S*,5*R*)-configuration] of the dihydropyrazines **13**, **14**, and **20**−**22** showing the two coupling protons H_G and H_K.

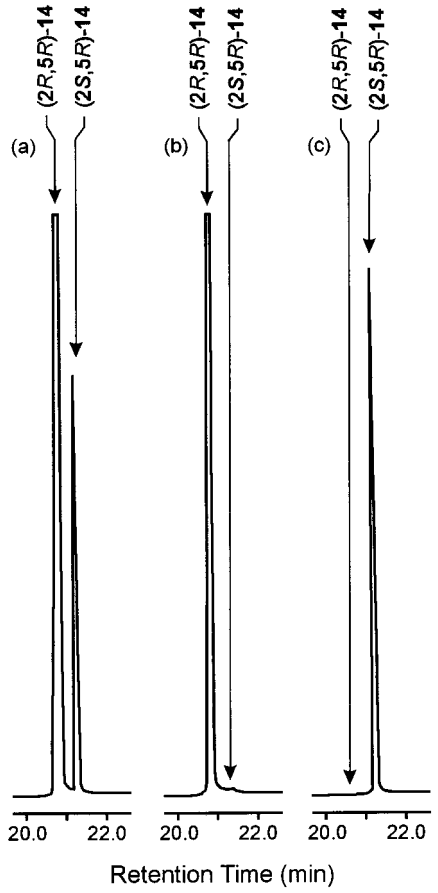


Figure 2. Quantitative gas-chromatographic determination of the molar ratio of the diastereomers of the dihydropyrazine **14**. Gas chromatograms: (a) 86:14 mixture of (2*R*,5*R*)-**14**/(2*S*,5*R*)-**14** obtained by diastereoselective alkylation of (*R*)-**10** (analysis of the crude product); (b) diastereomerically pure (≥99% de) isomer (2*R*,5*R*)-**14** obtained by liquid-chromatographic separation; (c) diastereomerically pure (≥99% de) isomer (2*S*,5*R*)-**14** obtained by liquid-chromatographic separation. For details, see Experimental Section.

or (*R*)-Me_3Ge-Ala residue in position 5 of Cetrorelix causes a marginal reduction in both binding affinity and antagonistic potency, whereas the binding affinities and antagonistic potencies of the C/Si/Ge analogues **7**−**9** are quite similar.

The decapeptides **7**−**9** were also studied for their in vivo activities in the male rat after s.c. administration. With respect to the suppression of testosterone, all three compounds (single-dose treatment, 1.5 mg/kg) produced

Table 4. Retention Times[a] for the Analytical Gas Chromatographic Separations of the (2*R*,5*R*)- and (2*S*,5*R*)-Diastereomers of the Dihydropyrazines 13, 14, and 20−22

compound	retention time (min)	compound	retention time (min)
(2*R*,5*R*)-**13**	18.8	(2*S*,5*R*)-**13**	19.6
(2*R*,5*R*)-**14**	20.8	(2*S*,5*R*)-**14**	21.5
(2*R*,5*R*)-**20**	28.5	(2*S*,5*R*)-**20**	29.1
(2*R*,5*R*)-**21**	30.0	(2*S*,5*R*)-**21**	30.4
(2*R*,5*R*)-**22**	21.9	(2*S*,5*R*)-**22**	22.5

[a] For experimental conditions, see Experimental Section.

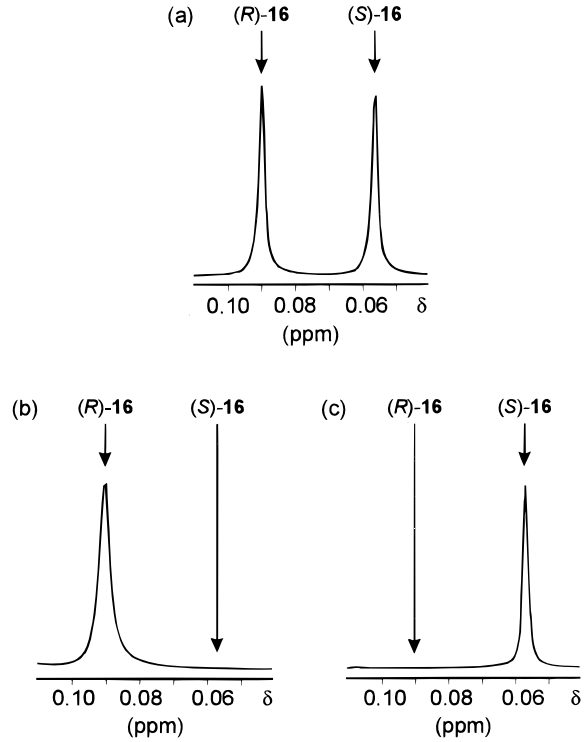


Figure 3. Quantitative NMR-spectroscopic determination of the enantiomeric purities of the antipodes of the amino acid ethyl ester **16**. ^1H NMR partial spectra ($GeCH_3$ groups) of the (*R*)- and (*S*)-enantiomers of **16** in the presence of the chiral solvating agent (*R*)-TFAE: (a) racemic mixture; (b) (*R*)-enantiomer obtained by hydrolysis of (2*R*,5*R*)-**14**; (c) (*S*)-enantiomer obtained by hydrolysis of (2*S*,5*R*)-**14**. For details, see Experimental Section.

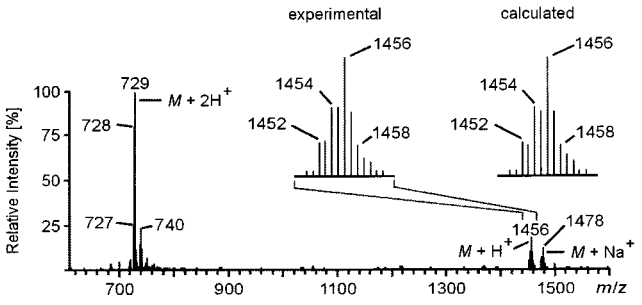
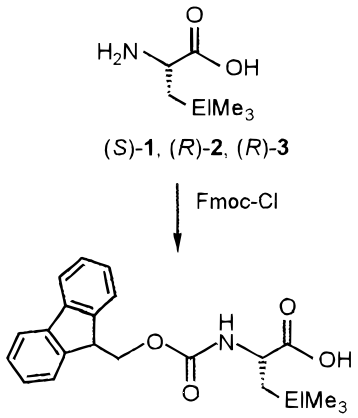


Figure 4. Electrospray mass spectrum of the germanium-containing decapeptide **9**.

The decapeptides **7**−**9** were also studied for their in vivo activities in the male rat after s.c. administration. With respect to the suppression of testosterone, all three compounds (single-dose treatment, 1.5 mg/kg) produced

Scheme 3



(*S*)-**1**, (*R*)-**2**, (*R*)-**3**

Fmoc-Cl

(*S*)-**26**, (*R*)-**27**, (*R*)-**28**

	El
1, **26**	C
2, **27**	Si
3, **28**	Ge

Table 5. Binding Affinities (K_A Values) and Antagonistic Potencies (IC_{50} Values) of the Decapeptides 7−9 and Cetrorelix at the Human GnRH Receptor[a]

decapeptide	K_A [nM^{-1}]	IC_{50} [nM][b]
7	2.20 ± 0.10	0.93 ± 0.30
8	2.82 ± 0.33	0.75 ± 0.17
9	2.01 ± 0.15	0.50 ± 0.21
Cetrorelix	5.94 ± 1.85 (n = 27)	0.25 ± 0.06 (n = 14)

[a] For experimental details, see Experimental Section. [b] Average of three independent experiments.

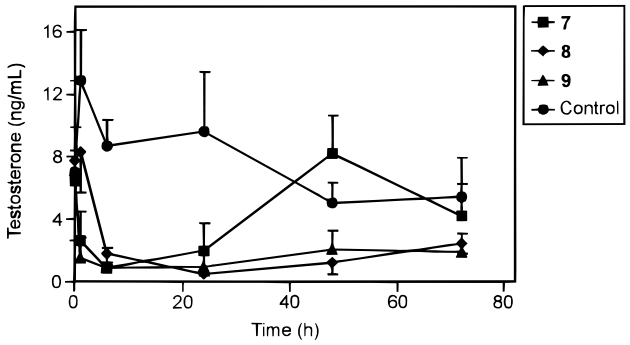


Figure 5. Mean testosterone plasma levels in male rats after single-dose treatment (1.5 mg/kg) with **7**−**9**. For experimental details, see Experimental Section.

an immediate and strong decrease in hormone levels. For the silicon- and germanium-containing decapeptides **8** and **9** this suppression lasted for 48 h, whereas it was 24 h after injection of the carbon analogue **7** until hormone concentrations returned to lower normal levels (Figure 5). To further discriminate the potencies of the C/Si/Ge analogues **7**−**9**, their suppressive effects on the more sensitive parameter of LH were investigated. Again, single-dose treatment (0.05 mg/kg) with **8** and **9** resulted in the most pronounced effects with a strong LH suppression lasting for 24 h, whereas **7** was less effective, with a duration of suppression of only 6 h (Figure 6). Thus, the results obtained for testosterone were also reproduced for the parameter of LH.

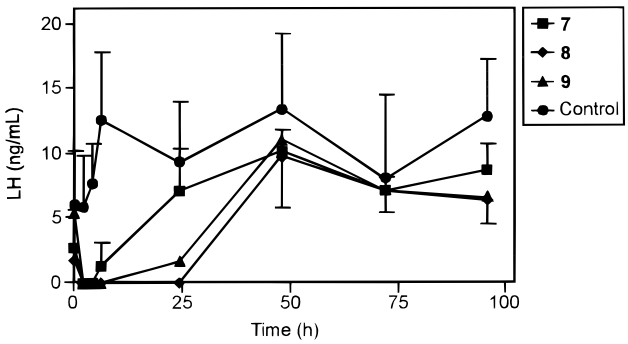


Figure 6. Mean LH serum levels in castrated male rats after single-dose treatment (0.05 mg/kg) with **7**−**9**. For experimental details, see Experimental Section.

In conclusion, the silicon- and germanium-containing decapeptides **8** and **9** showed an advantage over their carbon analogue **7** in vivo, but no significant differences between the Si/Ge analogues **8** and **9** could be detected in both in vivo settings. Although the improved in vivo activities of **8** and **9** cannot yet be explained in terms of differences in the chemical and physicochemical properties of the C/Si/Ge analogues **7**−**9**, the results reported here clearly demonstrate that sila- and germa-substitution of amino acids may be a useful tool to improve the biological properties of peptides. Further chemical and pharmacological studies are in progress to elucidate the potential of this approach systematically.

Experimental Section

General Procedures. Unless otherwise indicated, the reactions were carried out under dry nitrogen. Tetrahydrofuran (THF) and diethyl ether were dried and purified according to standard procedures and stored under nitrogen. ^{1}H NMR spectra were recorded at room temperature on a Bruker DMX-600 (^{1}H, 600.1 MHz), Bruker AMX-400 (^{1}H, 400.1 MHz), or Bruker DRX-300 NMR spectrometer (^{1}H, 300.1 MHz). ^{13}C and ^{29}Si NMR spectra were recorded at room temperature on a Bruker AMX-400 (^{13}C, 100.6 MHz; ^{29}Si, 79.5 MHz) or Bruker DRX-300 NMR spectrometer (^{13}C, 75.5 MHz; ^{29}Si, 59.6 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 $CDCl_3$), and HDO (^{1}H, δ 4.82; solvent D_2O), and external TMS (^{13}C, δ 0; solvent D_2O; ^{29}Si, δ 0, solvents $CDCl_3$ and D_2O). Assignment of the ^{1}H NMR data of **13**, **14**, and **20**−**22** was supported by ^{1}H,^{1}H COSY experiments, and the ^{1}H spin systems of **2**−**6**, **13**−**16**, and **20**−**25** were analyzed by simulations using the Bruker software program WIN-DAISY 4.0.[18] Assignment of the ^{13}C NMR data was supported by DEPT 135 and ^{13}C,^{1}H COSY experiments (**13**, **14**, **20**−**22**). Mass spectra were obtained with a Varian MAT-711 (EI MS, 70 eV), Finnigan MAT-8430 (EI MS, 70 eV; CI MS, ammonia as reactant gas), Finnigan MAT-8200 (EI MS, 70 eV), or ThermoQuest TRIO 1000 mass spectrometer (EI MS, 70 eV). Mass spectra of the decapeptides were measured with a Finnigan LCQ instrument [ESI MS; c(sample), 5 μg/mL; eluent, CH_3CN/H_2O/AcOH (80:20:0.01 (v/v/v)); flow rate, 3 μL/min]. The selected m/z values given refer to the isotopes ^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{23}Na, ^{28}Si, ^{35}Cl, and ^{74}Ge. IR spectra were obtained with a Bruker Equinox 55 FT-IR spectrometer. Preparative liquid chromatography [column: 60 mm i.d. × 350 mm] was performed using silica gel as stationary phase (silica gel 60, 0.063−0.200 mm; Merck, 15111). The ion exchanger (Aldrich, 21,739-5) was activated by washing with 0.5 M hydrochloric acid (50 mL) and water (3 × 50 mL).

(18) Weber, U.; Thiele, H.; Spiske, R.; Höffken, H.-W.; Hägele, G. *WIN-DAISY 4.0*; Bruker-Franzen GmbH, Bremen, Germany, 1998.

(*S*)-2-Amino-4,4-dimethylpentanoic Acid [(*S*)-β-*tert*-Butylalanine; (*S*)-1]. This compound was commercially available (Bachem AG).

Preparation of (*R*)-2-Amino-3-(trimethylsilyl)propionic Acid [(*R*)-β-(Trimethylsilyl)alanine; (*R*)-2]. A solution of (*R*)-**15** (1.20 g, 6.34 mmol) in 6 M hydrochloric acid (30 mL) was heated under reflux for 1 h. After cooling to room temperature, 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 ethanol (5 mL). After addition of propylene oxide (2 mL), the mixture was heated under reflux for 10 min. The precipitated product was isolated by centrifugation, washed with ethanol (2 × 2 mL) and diethyl ether (2 × 2 mL), and then dried in vacuo (0.01 mbar, 40 °C, 36 h) to give (*R*)-**2** in 79% yield as a colorless crystalline solid (808 mg, 5.01 mmol). IR (KBr, cm^{-1}): ν 1671 (C=O). ^1H NMR (400.1 MHz, D_2O): δ 0.15 (s, 9 H, $SiCH_3$), 1.16 (δ_A), 1.22 (δ_B), and 3.81 (δ_X) ($^2J_{AB}$ = 14.6 Hz, $^3J_{AX}$ = 5.7 Hz, $^3J_{BX}$ = 10.3 Hz, 3 H, Si-CH_AH_B-CH_X); OH and NH_2 not detected (H/D exchange). ^{13}C NMR (100.6 MHz, D_2O): δ −1.6 ($SiCH_3$), 20.5 (SiCH_2CH), 54.3 ($SiCH_2CH$), 176.5 (C=O). ^{29}Si NMR (79.5 MHz, D_2O): δ −0.3. EI MS: 146 [7%, M^+ − Me], 116 [100%, $M^+ - CO_2H$], 73 [90%, $SiMe_3^+$]. Anal. Calcd for $C_6H_{15}NO_2Si$: C, 44.68; H, 9.37; N, 8.68. Found: C, 44.6; H, 9.5; N, 8.6.

Preparation of (*S*)-2-Amino-3-(trimethylsilyl)propionic Acid [(*S*)-β-(Trimethylsilyl)alanine; (*S*)-2]. This compound was prepared analogously to the synthesis of (*R*)-**2** by heating a mixture of (*S*)-**15** (252 mg, 1.33 mmol) in 6 M hydrochloric acid (10 mL). After treatment of the solution of the residue [(*S*)-**2**·HCl] in ethanol (3 mL) with propylene oxide (2 mL), (*S*)-**2** was isolated in 60% yield as a colorless crystalline solid (128 mg, 794 μmol). The IR, NMR, and MS data of the product were identical with those obtained for (*R*)-**2**. Anal. Calcd for $C_6H_{15}NO_2Si$: C, 44.68; H, 9.37; N, 8.68. Found: C, 44.7; H, 9.5; N, 8.6.

Preparation of (*R*)-2-Amino-3-(trimethylgermyl)propionic Acid [(*R*)-β-(Trimethylgermyl)alanine; (*R*)-3]. This compound was prepared analogously to the synthesis of (*R*)-**2** by heating a mixture of (*R*)-**16** (580 mg, 2.48 mmol) in 6 M hydrochloric acid (30 mL). After treatment of the solution of the residue [(*R*)-**3**·HCl] in ethanol (10 mL) with propylene oxide (3 mL), (*R*)-**3** was isolated in 64% yield as a colorless crystalline solid (329 mg, 1.60 mmol). IR (KBr, cm^{-1}): ν 1672 (C=O). ^1H NMR (400.1 MHz, D_2O): δ 0.28 (s, 9 H, $GeCH_3$), 1.29 (δ_A), 1.33 (δ_B), and 3.85 (δ_X) ($^2J_{AB}$ = 13.5 Hz, $^3J_{AX}$ = 5.5 Hz, $^3J_{BX}$ = 10.2 Hz, 3 H, Ge-CH_AH_B-CH_X); OH and NH_2 not detected (H/D exchange). ^{13}C NMR (100.6 MHz, D_2O): δ −0.2 ($GeCH_3$), 21.6 (GeCH_2CH), 56.7 ($GeCH_2CH$), 178.1 (C=O). EI MS: 192 [12%, M^+ − Me], 162 [58%, $M^+ - CO_2H$], 119 [100%, $GeMe_3^+$]. Anal. Calcd for $C_6H_{15}GeNO_2$: C, 35.02; H, 7.35; N, 6.81. Found: C, 35.2; H, 7.3; N, 6.7.

Preparation of (*S*)-2-Amino-3-(trimethylgermyl)propionic Acid [(*S*)-β-(Trimethylgermyl)alanine; (*S*)-3]. This compound was prepared analogously to the synthesis of (*R*)-**2** by heating a mixture of (*S*)-**16** (46.1 mg, 197 μmol) in 6 M hydrochloric acid (5 mL). After treatment of the solution of the residue [(*S*)-**3**·HCl] in ethanol (2 mL) with propylene oxide (2 mL), (*S*)-**3** was isolated in 39% yield as a colorless crystalline solid (15.9 mg, 77.3 μmol). The IR, NMR, and MS data of the product were identical with those obtained for (*R*)-**3**. Anal. Calcd for $C_6H_{15}GeNO_2$: C, 35.02; H, 7.35; N, 6.81. Found: C, 35.1; H, 7.2; N, 6.7.

Preparation of (*R*)-2-Amino-3-[dimethyl(phenyl)silyl]-propionic Acid [(*R*)-β-[Dimethyl(phenyl)silyl]alanine; (*R*)-4]. Water (2.5 mL) and a 1 M aqueous lithium hydroxide solution (1.07 mL, 1.07 mmol LiOH) were added to a solution of (*R*)-**23** (269 mg, 1.07 mmol) in dioxane (7 mL). After stirring the mixture at room temperature 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 (*R*)-**4** in quantitative yield as a colorless solid (245 mg, 1.07 mmol). A mixture of this lithium salt (245 mg, 1.07 mmol) and an activated ion exchanger (11 g) in water (50 mL) was shaken at room temperature for 30 min. After the pH value was adjusted to pH 10−11 by 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 shaking at room temperature 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 room temperature for 5 min. After separation of the ion exchanger by filtration, the solvent of the combined aqueous layers was removed in vacuo (0.1 mbar, 20 °C) and the residue dried at 20 °C/0.01 mbar for 1 h and then dissolved in ethanol (5 mL). After addition of propylene oxide (2 mL), the mixture was heated under reflux for 10 min. The precipitated product was isolated by centrifugation, washed with ethanol (2 × 2 mL) and diethyl ether (2 × 2 mL), and then dried in vacuo (0.01 mbar, 40 °C, 36 h) to give (*R*)-**4** in 53% yield as a colorless crystalline solid (126 mg, 564 μmol). IR (KBr, cm^{-1}): ν 1669 cm^{-1} (C=O). ^1H NMR (300.1 MHz, D_2O): δ 0.40 (s, 3 H, $SiCH_3$), 0.43 (s, 3 H, $SiCH_3$), 1.35 (δ_B), 1.43 (δ_A), and 3.92 (δ_X) ($^2J_{AB}$ = 14.5 Hz, $^3J_{AX}$ = 10.1 Hz, $^3J_{BX}$ = 5.9 Hz, 3 H, Si-CH_AH_B-CH_X), 7.46−7.48 and 7.51−7.54 (m, 5 H, SiC_6H_5); OH and NH_2 not detected (H/D exchange). ^{13}C NMR (75.5 MHz, D_2O): δ −3.7 ($SiCH_3$), −3.2 ($SiCH_3$), 19.3 (SiCH_2-CH), 53.6 ($SiCH_2CH$), 128.6 (C-3/C-5, SiC_6H_5), 130.0 (C-4, SiC_6H_5), 134.1 (C-2/C-6, SiC_6H_5), 137.7 (C-1, SiC_6H_5), 165.2 (C=O). ^{29}Si NMR (59.6 MHz, D_2O): δ −1.2. CI MS (negative ions): 222 [100%, $(M - H)^+$]. Anal. Calcd for $C_{11}H_{17}NO_2Si$: C, 59.16; H, 7.67; N, 6.27. Found: C, 59.2; H, 7.7; N, 6.1.

Preparation of (*R*)-2-Amino-3-[dimethyl(phenyl)germyl]propionic Acid [(*R*)-β-[Dimethyl(phenyl)germyl]-alanine; (*R*)-5]. This compound was prepared analogously to the synthesis of (*R*)-**4** starting from (*R*)-**24** (280 mg, 946 μmol). The product was isolated in 45% yield as a colorless crystalline solid (114 mg, 426 μmol). IR (KBr, cm^{-1}): ν 1674 (C=O). ^1H NMR (300.1 MHz, D_2O): δ 0.52 (s, 3 H, $GeCH_3$), 0.53 (s, 3 H, $GeCH_3$), 1.50 (δ_B), 1.56 (δ_A), and 3.87 (δ_X) ($^2J_{AB}$ = 13.5 Hz, $^3J_{AX}$ = 10.7 Hz, $^3J_{BX}$ = 5.0 Hz, 3 H, Ge-CH_AH_B-CH_X), 7.43−7.52 and 7.62−7.67 (m, 5 H, GeC_6H_5); OH and NH_2 not detected (H/D exchange). ^{13}C NMR (75.5 MHz, D_2O): δ −1.8 ($GeCH_3$), −1.6 ($GeCH_3$), 19.7 (GeCH_2CH), 53.3 ($GeCH_2CH$), 127.9 (C-3/C-5, GeC_6H_5), 128.3 (C-4, GeC_6H_5), 133.2 (C-2/C-6, GeC_6H_5), 141.0 (C-1, GeC_6H_5), 177.5 (C=O). CI MS (negative ions): 268 [5%, $(M - H)^+$], 226 [100%], 224 [80%, $(M - CO_2H)^+$]. Anal. Calcd for $C_{11}H_{17}GeNO_2$: C, 49.32; H, 6.40; N, 5.23. Found: C, 49.2; H, 6.3; N, 5.3.

Preparation of (*R*)-2-Amino-3-[dimethyl(vinyl)silyl]-propionic Acid [(*R*)-β-[Dimethyl(vinyl)silyl]alanine; (*R*)-6]. This compound was prepared analogously to the synthesis of (*R*)-**4** starting from (*R*)-**25** (264 mg, 1.31 mmol). The product was isolated in 42% yield as a colorless crystalline solid (94.4 mg, 545 μmol). IR (KBr, cm^{-1}): ν 1670 (C=O). ^1H NMR (300.1 MHz, D_2O): δ 0.228 (s, 3 H, $SiCH_3$), 0.234 (s, 3 H, $SiCH_3$), 1.24 (δ_B), 1.30 (δ_A), and 3.84 (δ_X) ($^2J_{AB}$ = 14.7 Hz, $^3J_{AX}$ = 9.6 Hz, $^3J_{BX}$ = 6.1 Hz, 3 H, Si-CH_AH_B-CH_X), 5.88 (δ_A), 6.12 (δ_M), and 6.31 (δ_X) ($^2J_{AM}$ = 3.6 Hz, $^3J_{AX}$ = 20.7 Hz, $^3J_{MX}$ = 14.8 Hz, 3 H, Si-CH_X=CH_AH_M); OH and NH_2 not detected (H/D exchange). ^{13}C NMR (75.5 MHz, D_2O): δ −3.8 ($SiCH_3$), −3.6 ($SiCH_3$), 19.0 (SiCH_2CH), 53.5 ($SiCH_2CH$), 131.4 (SiCH=CH_2), 136.1 (SiCH=CH_2), 175.7 (C=O). ^{29}Si NMR (59.6 MHz, D_2O): δ −7.7. CI MS (negative ions): 172 [100%, $(M - H)^+$]. Anal. Calcd for $C_7H_{15}NO_2Si$: C, 48.52; H, 8.72; N, 8.08. Found: C, 48.2; H, 8.5; N, 8.0.

Preparation of the Decapeptides Acetyl-D-2-naphthylalanyl-D-4-chlorophenylalanyl-D-3-pyridylalanyl-seryl-*tert*-butylalanyl-D-citrullyl-leucyl-arginyl-prolyl-D-alaninamide (Ac-D-Nal[1]-4-Cl-D-Phe[2]-D-Pal[3]-Ser[4]-Me_3C-Ala[5]-D-Cit[6]-Leu[7]-Arg[8]-Pro[9]-D-Ala[10]-NH_2; 7), Acetyl-D-2-naphthylalanyl-D-4-chlorophenylalanyl-D-3-pyridylalanyl-seryl-

(trimethylsilyl)alanyl-D-citrullyl-leucyl-arginyl-prolyl-D-alaninamide (Ac-D-Nal[1]-4-Cl-D-Phe[2]-D-Pal[3]-Ser[4]-Me$_3$Si-Ala[5]-D-Cit[6]-Leu[7]-Arg[8]-Pro[9]-D-Ala[10]-NH$_2$; 8), and Acetyl-D-2-naphthylalanyl-D-4-chlorophenylalanyl-D-3-pyridylalanyl-seryl-(trimethylgermyl)alanyl-D-citrullyl-leucyl-arginyl-prolyl-D-alaninamide (Ac-D-Nal[1]-4-Cl-D-Phe[2]-D-Pal[3]-Ser[4]-Me$_3$Ge-Ala[5]-D-Cit[6]-Leu[7]-Arg[8]-Pro[9]-D-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-methoxy-4′-(γ-carboxypropyloxy)benzhydrylamine resin (Fmoc-MBHA resin; Bachem AG, D 1600) was used as polymeric support. The sequential synthesis was performed as follows: *N*-Fmoc-protected D-alanine (C-terminal) was covalently linked to the unprotected MBHA resin by using a 3-fold molar excess of each diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) in a mixture of dichloromethane 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 3-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 acetic acid/2,2,2-trifluoroethanol/dichloromethane [1:1:8 (v/v/v)] at 60 °C for 2.5 h. The solvents were removed under reduced pressure, and the residue was washed with diethyl ether. The precipitate was filtered off and dried at room temperature 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/acetonitrile/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. 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**, 1.64 g; **8**, 1.33 g; **9**, 1.22 g.

Data for 7. ESI MS: 1416 [2%, $(M + Na)^+$], 1394 [10%, $(M + H)^+$], 698 [100%, $(M + 2\ H)^{2+}$]. ESI MS/MS [698; ($C_{68}H_{96}ClN_{17}O_{13} + 2\ H)^{2+}$]: 1209 [20%, B_8^+], 1053 [14%, B_7^+], 1010 [7%, $(B_7 - HNCO)^+$], 974 [2%, $Y_8''^+$], 940 [7%, B_6^+], 897 [9%, $(B_6 - HNCO)^+$], 826 [3%, $Y_7''^+$], 783 [5%, B_5^+], 739 [3%, $Y_6''^+$], 676 [100%, $(M + 2\ H - HNCO)^{2+}$], 612 [5%, $Y_5''^+$], 605 [12%, $B_8'^{2+}$], 569 [2%, B_3^+], 455 [10%, $Y_4''^+$], 342 [12%, $Y_3''^+$].

Data for 8. ESI MS: 1432 [10%, $(M + Na)^+$], 1410 [20%, $(M + H)^+$], 706 [100%, $(M + 2\ H)^{2+}$]. ESI MS/MS [706; ($C_{67}H_{96}ClN_{17}O_{13}Si + 2\ H)^{2+}$]: 1225 [19%, B_8^+], 1069 [13%, B_7^+], 1026 [13%, $(B_7 - HNCO)^+$], 990 [2%, $Y_8''^+$], 956 [8%, B_6^+], 913 [11%, $(B_6 - HNCO)^+$], 842 [3%, $Y_7''^+$], 799 [5%, B_5^+], 755 [4%, $Y_6''^+$], 684 [100%, $(M + 2\ H - HNCO)^{2+}$], 656 [6%, B_4^+], 613 [13%, $B_8'^{2+}$], 612 [5%, $Y_5''^+$], 569 [2%, B_3^+], 455 [12%, $Y_4''^+$], 342 [11%, $Y_3''^+$].

Data for 9. ESI MS: 1478 [10%, $(M + Na)^+$], 1456 [14%, $(M + H)^+$], 729 [100%, $(M + 2\ H)^{2+}$]. ESI MS/MS [727; ($C_{67}H_{96}Cl^{70}GeN_{17}O_{13} + 2\ H)^{2+}$]: 1267 [30%, B_8^+], 1111 [90%, B_7^+], 1068 [35%, $(B_7 - HNCO)^+$], 1032 [2%, $Y_8''^+$], 998 [40%, B_6^+], 955 [42%, $(B_6 - HNCO)^+$], 884 [14%, $Y_7''^+$], 841 [20%, B_5^+], 797 [14%, $Y_6''^+$], 705 [100%, $(M + 2\ H - HNCO)^{2+}$], 656 [15%, B_4^+], 634 [48%, $B_8'^{2+}$], 670 [23%, $Y_5''^+$], 569 [8%, B_3^+], 455 [48%, $Y_4''^+$], 342 [52%, $Y_3''^+$].

Preparation of (*R*)-3,6-Diethoxy-2-isopropyl-2,5-dihydropyrazine [(*R*)-10]. This compound was synthesized in analogy to ref 13. In this context, see also refs 19 and 20.

(Chloromethyl)trimethylsilane (11). This compound was commercially available (Aldrich).

Preparation of (Chloromethyl)trimethylgermane (12). This compound was synthesized from trichloro(chloromethyl)-germane[21] according to ref 22.

Preparation of (2*R*,5*R*)- and (2*S*,5*R*)-3,6-Diethoxy-5-isopropyl-2-[(trimethylsilyl)methyl]-2,5-dihydropyrazine [(2*R*,5*R*)-13 and (2*S*,5*R*)-13]. A 1.6 M solution of *n*-butyllithium in *n*-hexane (5.81 mL, 9.30 mmol *n*-BuLi) was added dropwise at −70 °C within 30 min to a stirred solution of (*R*)-**10** (1.97 g, 9.28 mmol) in THF (40 mL). After the mixture was stirred at −70 °C for 15 min, a solution of **11** (1.14 g, 9.29 mmol) in THF (10 mL) was added dropwise at −70 °C within 30 min, and the reaction mixture was stirred at this temperature for 2 h. The mixture was allowed to warm to room temperature within 12 h, followed by addition of diethyl ether (50 mL) and water (50 mL). The organic phase was separated and the aqueous layer extracted with diethyl ether (3 × 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 150 °C, 0.01 mbar) to give a 75:25 mixture of (2*R*,5*R*)-**13**/(2*S*,5*R*)-**13** (molar ratio 85:15, GC analysis) and (*R*)-**10**. The diastereomerically pure compounds (2*R*,5*R*)-**13** and (2*S*,5*R*)-**13** were obtained by liquid-chromatographic separation (for the general procedure, see below).

Data for (2*R*,5*R*)-13. Yield: 52% (1.43 g, 4.79 mmol), relative to (*R*)-**10**; diastereomeric purity ≥99% de. IR (film, cm^{-1}): ν 1688 (C═N). ^{1}H NMR (600.1 MHz, $CDCl_3$): δ 0.02 (s, 9 H, $SiCH_3$), 0.69 (δ_Q), 0.84 (δ_A), 1.01 (δ_T), 1.19 (δ_B), 2.24 (δ_N), 3.87 (δ_K), and 4.00 (δ_G) [$^2J_{AB}$ = 14.5 Hz, $^3J_{AG}$ = 9.8 Hz, $^3J_{BG}$ = 4.9 Hz, $^5J_{GK}$ = 3.6 Hz, $^3J_{KN}$ = 3.3 Hz, $^3J_{NQ}$ = 6.8 Hz, $^3J_{NT}$ = 6.9 Hz, 11 H, Si-CH_AH_B-CH_G-N═C-CH_K-CH_N(CH_{Q3})-(CH_{T3})], 1.24 (δ_X), 4.03 (δ_A), and 4.11 (δ_B) [$^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.0 Hz, $^3J_{BX}$ = 7.2 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.26 (δ_X), 4.03 (δ_A), and 4.16 (δ_B) [$^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.0 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −0.5 ($SiCH_3$), 14.40 (OCH_2CH_3), 14.43 (OCH_2CH_3), 16.8 ($CHCH_3$), 19.1 ($CHCH_3$), 23.2 ($CHCH_2Si$), 31.9 [$CHCH(CH_3)_2$], 53.3 ($CHCH_2Si$), 60.46 (OCH_2CH_3), 60.50 (OCH_2CH_3), 60.6 [$CHCH(CH_3)_2$], 162.2 (C═N), 165.1 (C═N). ^{29}Si NMR (79.5 MHz, $CDCl_3$): δ 1.2. EI MS: 298 [87%, M^+], 283 [43%, M^+ − Me], 269 [13%, M^+ − Et], 255 [44%, $M^+ - CHMe_2$], 211 [100%, $M^+ - CH_2SiMe_3$], 73 [50%, $SiMe_3^+$]. Anal. Calcd for $C_{15}H_{30}N_2O_2Si$: C, 60.36; H, 10.13; N, 9.38. Found: C, 60.3; H, 10.2; N, 9.3.

Data for (2*S*,5*R*)-13. Yield: 8% (208 mg, 697 μmol), relative to (*R*)-**10**; diastereomeric purity ≥99% de. IR (film, cm^{-1}): ν 1686 (C═N). ^{1}H NMR (600.1 MHz, $CDCl_3$): δ 0.06 (s, 9 H, $SiCH_3$), 0.67 (δ_A), 0.75 (δ_Q), 1.02 (δ_T), 1.21 (δ_B), 2.14 (δ_N), 3.84 (δ_K), and 3.97 (δ_G) [$^2J_{AB}$ = 14.5 Hz, $^3J_{AG}$ = 11.5 Hz, $^3J_{BG}$ = 4.8 Hz, $^5J_{GK}$ = 4.2 Hz, $^3J_{KN}$ = 4.1 Hz, $^3J_{NQ}$ = 6.8 Hz, $^3J_{NT}$ = 6.9 Hz, 11 H, Si-CH_AH_B-CH_G-N═C-CH_K-CH_N(CH_{Q3})(CH_{T3})], 1.23 (δ_X), 4.03 (δ_A), and 4.12 (δ_B) [$^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 6.9 Hz, $^3J_{BX}$ = 7.2 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.25 (δ_X), 4.05 (δ_A), and 4.08 (δ_B) [$^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.2 Hz, $^3J_{BX}$ = 6.9 Hz, 5 H, O-CH_AH_B-CH_{X3}). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −0.5 ($SiCH_3$), 14.1 (OCH_2CH_3), 14.4 (OCH_2CH_3), 17.9 ($CHCH_3$), 19.6 ($CHCH_3$), 24.4 ($CHCH_2Si$), 31.6 [$CHCH(CH_3)_2$], 53.4 ($CHCH_2Si$), 60.3 (OCH_2CH_3), 60.4 (OCH_2CH_3), 61.0 [$CHCH(CH_3)_2$], 162.1 (C═N), 165.1 (C═N). ^{29}Si NMR (79.5 MHz, $CDCl_3$): δ 1.4. EI MS: 298 [76%, M^+], 283 [46%, M^+ − Me], 269 [16%, M^+ − Et], 255 [62%, $M^+ - CHMe_2$], 211 [100%, $M^+ - CH_2SiMe_3$], 73 [52%, $SiMe_3^+$]. Anal. Calcd for $C_{15}H_{30}N_2O_2Si$: C, 60.36; H, 10.13; N, 9.38. Found: C, 60.4; H, 10.2; N, 9.3.

Preparation of (2*R*,5*R*)- and (2*S*,5*R*)-3,6-Diethoxy-5-isopropyl-2-[(trimethylgermyl)methyl]-2,5-dihydropyra-

(19) Schöllkopf, U.; Groth, U.; Deng, C. *Angew. Chem.* **1981**, *93*, 793−795; *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 798−799.
(20) Groth, U.; Schmeck, C.; Schöllkopf, U. *Liebigs Ann. Chem.* **1993**, 321−323.
(21) Tacke, R.; Becker, B. *J. Organomet. Chem.* **1988**, *354*, 147−153, and references therein.
(22) Seyferth, D.; Rochow, E. G. *J. Am. Chem. Soc.* **1955**, *77*, 907−910.

zine [(2*R*,5*R*)-14 and (2*S*,5*R*)-14]. These compounds were prepared analogously to the synthesis of (2*R*,5*R*)-**13**/(2*S*,5*R*)-**13** by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (5.01 mL, 8.02 mmol *n*-BuLi) to a solution of (*R*)-**10** (1.70 g, 8.01 mmol) in THF (40 mL), followed by treatment with a solution of **12** (1.35 g, 8.07 mmol) in THF (10 mL). The crude product was purified by Kugelrohr distillation (oven temperature 160 °C, 0.01 mbar) to give a 85:15 mixture of (2*R*,5*R*)-**14**/(2*S*,5*R*)-**14** (molar ratio 86:14) and (*R*)-**10**. The diastereomerically pure compounds (2*R*,5*R*)-**14** and (2*S*,5*R*)-**14** were obtained by liquid-chromatographic separation (for the general procedure, see below).

Data for (2*R*,5*R*)-14. Yield: 54% (1.47 g, 4.29 mmol), relative to (*R*)-**10**; diastereomeric purity ≥99% de. IR (film, cm^{-1}): ν 1688 (C═N). ^1H NMR (400.1 MHz, $CDCl_3$): δ 0.15 (s, 9 H, $GeCH_3$), 0.69 (δ_Q), 1.00 (δ_T), 1.03 (δ_A), 1.34 (δ_B), 2.24 (δ_N), 3.88 (δ_K), and 4.03 (δ_G) [$^2J_{AB}$ = 13.5 Hz, $^3J_{AG}$ = 9.4 Hz, $^3J_{BG}$ = 5.2 Hz, $^5J_{GK}$ = 3.4 Hz, $^3J_{KN}$ = 3.5 Hz, $^3J_{NQ}$ = 6.8 Hz, $^3J_{NT}$ = 6.9 Hz, 11 H, Ge-CH_AH_B-CH_G-N═C-CH_K-$CH_N(CH_{Q3})$-(CH_{T3})], 1.24 (δ_X), 4.03 (δ_A), and 4.10 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.26 (δ_X), 4.04 (δ_A), and 4.16 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −1.0 ($GeCH_3$), 14.40 (OCH_2CH_3), 14.42 (OCH_2CH_3), 16.8 ($CHCH_3$), 19.1 ($CHCH_3$), 23.6 ($CHCH_2Ge$), 31.9 [$CHCH(CH_3)_2$], 53.8 ($CHCH_2Ge$), 60.45 (OCH_2CH_3), 60.54 (OCH_2CH_3), 60.8 [$CHCH(CH_3)_2$], 162.4 (C═N), 164.9 (C═N). EI MS: 344 [8%, M^+], 329 [40%, $M^+ - Me$], 301 [15%, $M^+ - CHMe_2$], 119 [100%, $GeMe_3^+$]. Anal. Calcd for $C_{15}H_{30}GeN_2O_2$: C, 52.52; H, 8.82; N, 8.17. Found: C, 52.4; H, 9.0; N, 8.3.

Data for (2*S*,5*R*)-14. Yield: 6% (175 mg, 510 μmol), relative to (*R*)-**10**; diastereomeric purity ≥99% de. IR (film, cm^{-1}): ν 1691 (C═N). ^1H NMR (400.1 MHz, $CDCl_3$): δ 0.18 (s, 9 H, $GeCH_3$), 0.76 (δ_Q), 0.85 (δ_A), 1.03 (δ_T), 1.35 (δ_B), 2.16 (δ_N), 3.85 (δ_K), and 3.98 (δ_G) [$^2J_{AB}$ = 13.3 Hz, $^3J_{AG}$ = 11.4 Hz, $^3J_{BG}$ = 5.2 Hz, $^5J_{GK}$ = 4.1 Hz, $^3J_{KN}$ = 4.2 Hz, $^3J_{NQ}$ = 7.0 Hz, $^3J_{NT}$ = 6.8 Hz, 11 H, Ge-CH_AH_B-CH_G-N═C-CH_K-$CH_N(CH_{Q3})(CH_{T3})$], 1.23 (δ_X), 4.02 (δ_A), and 4.09 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.0 Hz, $^3J_{BX}$ = 7.0 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.24 (δ_X), 4.04 (δ_A), and 4.15 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.0 Hz, $^3J_{BX}$ = 7.0 Hz, 5 H, O-CH_AH_B-CH_{X3}). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −1.1 ($GeCH_3$), 14.4 (OCH_2CH_3), 14.5 (OCH_2CH_3), 17.9 ($CHCH_3$), 19.6 ($CHCH_3$), 24.6 ($CHCH_2Ge$), 31.7 [$CHCH(CH_3)_2$], 53.9 ($CHCH_2Ge$), 60.9 (OCH_2CH_3), 61.1 (OCH_2CH_3), 61.0 [$CHCH(CH_3)_2$], 162.2 (C═N), 165.3 (C═N). EI MS: 344 [1%, M^+], 329 [29%, $M^+ - Me$], 301 [18%, $M^+ - CHMe_2$], 119 [100%, $GeMe_3^+$]. Anal. Calcd for $C_{15}H_{30}GeN_2O_2$: C, 52.52; H, 8.82; N, 8.17. Found: C, 52.8; H, 8.8; N, 8.4.

Preparation of (*R*)-2-Amino-3-(trimethylsilyl)propionic Acid Ethyl Ester [(*R*)-15]. Hydrochloric acid (3 M, 10 mL) was added dropwise at 0 °C within 10 min to a stirred solution of (2*R*,5*R*)-**13** (1.02 g, 3.42 mmol) in ethanol (25 mL). After stirring the mixture at 0 °C for 2 h, the solvent was removed in vacuo (0.1 mbar, 20 °C), the residue dissolved in dichloromethane, and the resulting solution extracted with a 2 M aqueous Na_2CO_3 solution. After drying of the organic layer over anhydrous Na_2SO_4, the solvent was removed under reduced pressure (rotary evaporator) and the product isolated and purified by liquid chromatography on silica gel [eluent diethyl ether/*n*-hexane (2:1, v/v; before using the eluent, it was washed with concentrated aqueous ammonia solution); R_f[(*R*)-**15**] > R_f[(*R*)-valine]] to give (*R*)-**15** in a 86% yield as a colorless liquid (555 mg, 2.93 mmol). IR (film, cm^{-1}): ν 1734 (C═O). ^1H NMR (300.1 MHz, $CDCl_3$): δ 0.00 (s, 9 H, $SiCH_3$), 0.79 (δ_A), 1.00 (δ_B), and 3.42 (δ_X) ($^2J_{AB}$ = 14.6 Hz, $^3J_{AX}$ = 8.9 Hz, $^3J_{BX}$ = 6.1 Hz, 3 H, Si-CH_AH_B-CH_X), 1.22 (δ_X), 4.07 (δ_A), and 4.10 (δ_B) ($^2J_{AB}$ = 10.9 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.48 (s, 2 H, NH_2). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −1.0 ($SiCH_3$), 14.1 (OCH_2CH_3), 23.4 ($SiCH_2CH$), 52.0 ($SiCH_2CH$), 60.7 (OCH_2CH_3), 177.4 (C═O). ^{29}Si NMR (79.5 MHz, $CDCl_3$): δ 0.9. EI MS: 174 [6%, $M^+ - Me$], 116 [100%, $M^+ - SiMe_3$], 73 [79%, $SiMe_3^+$]. Anal. Calcd for $C_8H_{19}NO_2Si$: C, 50.75; H, 10.12; N, 7.40. Found: C, 50.7; H, 10.1; N, 7.5.

Preparation of (*S*)-2-Amino-3-(trimethylsilyl)propionic Acid Ethyl Ester [(*S*)-15]. This compound was prepared analogously to the synthesis of (*R*)-**15** by treatment of a solution of (2*S*,5*R*)-**13** (150 mg, 503 μmol) in ethanol (10 mL) with 3 M hydrochloric acid (5 mL). After liquid-chromatographic separation on silica gel [see preparation of (*R*)-**15**; R_f[(*S*)-**15**] > R_f[(*R*)-valine]], (*S*)-**15** was isolated in 84% yield as a colorless liquid (80.3 mg, 424 μmol). The IR, NMR, and MS data of the product were identical with those obtained for (*R*)-**15**. Anal. Calcd for $C_8H_{19}NO_2Si$: C, 50.75; H, 10.12; N, 7.40. Found: C, 50.6; H, 10.4; N, 7.3.

Preparation of (*R*)-2-Amino-3-(trimethylgermyl)propionic Acid Ethyl Ester [(*R*)-16]. This compound was prepared analogously to the synthesis of (*R*)-**15** by treatment of a solution of (2*R*,5*R*)-**14** (1.01 g, 2.94 mmol) in ethanol (20 mL) with 3 M hydrochloric acid (5 mL). After liquid-chromatographic separation on silica gel [see preparation of (*R*)-**15**; R_f[(*R*)-**16**] > R_f[(*R*)-valine]], (*R*)-**16** was isolated in 73% yield as a colorless liquid (500 mg, 2.14 mmol). IR (film, cm^{-1}): ν 1734 (C═O). ^1H NMR (400.1 MHz, $CDCl_3$): δ 0.12 (s, 9 H, $GeCH_3$), 0.94 (δ_A), 1.13 (δ_B), and 3.42 (δ_X) ($^2J_{AB}$ = 13.6 Hz, $^3J_{AX}$ = 9.0 Hz, $^3J_{BX}$ = 6.1 Hz, 3 H, Ge-CH_AH_B-CH_X), 1.20 (δ_X), 4.06 (δ_A), and 4.09 (δ_B) ($^2J_{AB}$ = 10.8 Hz, $^3J_{AX}$ = 7.2 Hz, $^3J_{BX}$ = 7.0 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.40 (s, 2 H, NH_2). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −1.5 ($GeCH_3$), 14.1 (OCH_2CH_3), 23.4 ($GeCH_2$-CH), 52.6 ($GeCH_2CH$), 60.6 (OCH_2CH_3), 177.2 (C═O). EI MS: 220 [18%, $M^+ - Me$], 162 [90%, $M^+ - CO_2Et$], 119 [100%, $GeMe_3^+$]. Anal. Calcd for $C_8H_{19}GeNO_2$: C, 41.09; H, 8.19; N, 5.99. Found: C, 41.2; H, 8.3; N, 6.1.

Preparation of (*S*)-2-Amino-3-(trimethylgermyl)propionic Acid Ethyl Ester [(*S*)-16]. This compound was prepared analogously to the synthesis of (*R*)-**15** by treatment of a solution of (2*S*,5*R*)-**14** (114 mg, 332 μmol) in ethanol (10 mL) with 3 M hydrochloric acid (5 mL). After liquid-chromatographic separation on silica gel [see preparation of (*R*)-**15**; R_f[(*S*)-**16**] > R_f[(*R*)-valine]], (*S*)-**16** was isolated in 80% yield as a colorless liquid (62.4 mg, 267 μmol). The IR, NMR, and MS data of the product were identical with those obtained for (*R*)-**16**. Anal. Calcd for $C_8H_{19}GeNO_2$: C, 41.09; H, 8.19; N, 5.99. Found: C, 40.7; H, 8.1; N, 5.6.

(Chloromethyl)dimethyl(phenyl)silane (17). This compound was commercially available (Aldrich).

Preparation of (Chloromethyl)dimethyl(phenyl)germane (18). A Grignard reagent was prepared from bromobenzene (27.2 g, 173 mmol) and magnesium turnings (4.20 g, 173 mmol) in diethyl ether (250 mL) and then added dropwise at room temperature within 2 h to a stirred solution of trichloro(chloromethyl)germane[21] (39.5 g, 173 mmol) in diethyl ether (200 mL). After stirring at room temperature for 14 h and heating under reflux for 1 h, *n*-pentane (100 mL) was added and the precipitate was filtered off. The solvent of the filtrate was removed under reduced pressure and the residue distilled in vacuo (Vigreux column) to give 53.2 g of a mixture consisting of dichloro(chloromethyl)phenylgermane, bromochloro(chloromethyl)phenylgermane, and dibromo(chloromethyl)phenylgermane (molar ratio 17:47:36, ^1H NMR analysis; 130 °C/17 mbar to 85 °C/0.1 mbar). A stirred solution of this mixture in diethyl ether (500 mL) was treated dropwise at −70 °C within 2 h with a 1.6 M solution of methyllithium in diethyl ether (136 mL, 218 mmol MeLi). After stirring for 4 h at this temperature, the mixture was allowed to warm to room temperature within 6 h, followed by addition of water (300 mL). The organic phase was separated and the aqueous layer extracted with diethyl ether (4 × 150 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue distilled in vacuo (Vigreux column) to give **18** in 51% yield as a colorless liquid (20.4 g, 89.0 mmol); bp 124 °C/29 mbar. ^1H NMR (300.1 MHz, $CDCl_3$): δ 0.63 (s, 6 H, $GeCH_3$), 3.19 (s, 2

H, $GeCH_2Cl$), 7.44−7.49 and 7.52−7.57 (m, 5 H, GeC_6H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −4.8 ($GeCH_3$), 30.6 ($GeCH_2Cl$), 128.2 (C-3/C-5, GeC_6H_5), 129.0 (C-4, GeC_6H_5), 133.3 (C-2/C-6, GeC_6H_5), 138.7 (C-1, GeC_6H_5). EI MS: 230 [1%, M^+], 215 [4%, $M^+ - Me$], 181 [100%, $M^+ - CH_2Cl$]. Anal. Calcd for $C_9H_{13}ClGe$: C, 47.15; H, 5.72. Found: C, 47.3; H, 5.7.

Preparation of (Chloromethyl)dimethyl(vinyl)silane (19). This compound was synthesized according to ref 23.

Preparation of (2*R*,5*R*)-3,6-Diethoxy-5-isopropyl-2-{[dimethyl(phenyl)silyl]methyl}-2,5-dihydropyrazine[(2*R*,5*R*)-20]. This compound was prepared analogously to the synthesis of (2*R*,5*R*)-**13**/(2*S*,5*R*)-**13** by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (7.00 mL, 11.2 mmol *n*-BuLi) to a solution of (*R*)-**10** (2.36 g, 11.1 mmol) in THF (40 mL), followed by treatment with a solution of **17** (2.07 g, 11.2 mmol) in THF (10 mL). The crude product was purified by Kugelrohr distillation (oven temperature 170 °C, 0.01 mbar) to give a 65:35 mixture of (2*R*,5*R*)-**20**/(2*S*,5*R*)-**20** (molar ratio 80:20) and (*R*)-**10**. The diastereomerically pure compound (2*R*,5*R*)-**20** was isolated by liquid-chromatographic separation (for the general procedure, see below) as a colorless liquid in 41% yield [relative to (*R*)-**10**; 1.65 g, 4.58 mmol; ≥99% de]. IR (film, cm^{-1}): ν 1691 (C═N). ^1H NMR (600.1 MHz, $CDCl_3$): δ 0.33 (s, 3 H, $SiCH_3$), 0.34 (s, 3 H, $SiCH_3$), 0.67 (δ_Q), 1.00 (δ_T), 1.10 (δ_A), 1.48 (δ_B), 2.22 (δ_N), 3.85 (δ_K), and 4.03 (δ_G) [$^2J_{AB}$ = 14.6 Hz, $^3J_{AG}$ = 10.0 Hz, $^3J_{BG}$ = 4.7 Hz, $^5J_{GK}$ = 3.7 Hz, $^3J_{KN}$ = 3.2 Hz, $^3J_{NQ}$ = 6.8 Hz, $^3J_{NT}$ = 6.9 Hz, 11 H, Si-CH_AH_B-CH_G-N═C-CH_K-CH_N-(CH_{Q3})(CH_{T3})], 1.19 (δ_X), 3.97 (δ_A), and 4.13 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 6.9 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.24 (δ_X), 3.81 (δ_A), and 4.00 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 6.9 Hz, 5 H, O-CH_AH_B-CH_{X3}), 7.31−7.42 and 7.53−7.62 (m, 5 H, SiC_6H_5). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ −2.0 ($SiCH_3$), −1.7 ($SiCH_3$), 14.37 (OCH_2CH_3), 14.38 (OCH_2CH_3), 16.8 ($CHCH_3$), 19.1 ($CHCH_3$), 22.3 ($SiCH_2CH$), 31.9 [$CHCH$-$(CH_3)_2$], 53.1 ($SiCH_2CH$), 60.46 (OCH_2CH_3), 60.51 (OCH_2CH_3), 60.7 [$CHCH(CH_3)_2$], 127.6 (C-3/C-5, SiC_6H_5), 128.6 (C-4, SiC_6H_5), 133.7 (C-2/C-6, SiC_6H_5), 140.1 (C-1, SiC_6H_5), 162.3 (C═N), 164.8 (C═N). ^{29}Si NMR (59.6 MHz, $CDCl_3$): δ 3.3. EI MS: 360 [42%, M^+], 345 [45%, $M^+ - Me$], 331 [11%, $M^+ - Et$], 317 [38%, $M^+ - CHMe_2$], 283 [36%, $M^+ - Ph$], 211 [88%, $M^+ - CH_2SiMe_2Ph$], 135 [100%, $SiMe_2Ph^+$]. Anal. Calcd for $C_{20}H_{32}N_2O_2Si$: C, 66.62; H, 8.95; N, 7.77. Found: C, 66.6; H, 8.8; N, 7.9.

Preparation of (2*R*,5*R*)-Diethoxy-5-isopropyl-2-{[dimethyl(phenyl)germyl]methyl}-2,5-dihydropyrazine[(2*R*,5*R*)-21]. This compound was prepared analogously to the synthesis of (2*R*,5*R*)-**13**/(2*S*,5*R*)-**13** by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (5.11 mL, 8.18 mmol *n*-BuLi) to a solution of (*R*)-**10** (1.72 g, 8.10 mmol) in THF (40 mL), followed by treatment with a solution of **18** (1.87 g, 8.16 mmol) in THF (10 mL). The crude product was purified by Kugelrohr distillation (oven temperature 200 °C, 0.01 mbar) to give a 82:18 mixture of (2*R*,5*R*)-**21**/(2*S*,5*R*)-**21** (molar ratio 83:17) and (*R*)-**10**. The diastereomerically pure compound (2*R*,5*R*)-**21** was isolated by liquid-chromatographic separation (for the general procedure, see below) as a colorless liquid in 41% yield [relative to (*R*)-**10**; 1.35 g, 3.33 mmol; ≥99% de]. IR (film, cm^{-1}): ν 1691 (C═N). ^1H NMR (600.1 MHz, $CDCl_3$): δ 0.410 (s, 3 H, $GeCH_3$), 0.414 (s, 3 H, $GeCH_3$), 0.67 (δ_Q), 0.99 (δ_T), 1.28 (δ_A), 1.62 (δ_B), 2.21 (δ_N), 3.84 (δ_K), and 4.06 (δ_G) [$^2J_{AB}$ = 13.6 Hz, $^3J_{AG}$ = 9.5 Hz, $^3J_{BG}$ = 4.8 Hz, $^5J_{GK}$ = 3.4 Hz, $^3J_{KN}$ = 3.4 Hz, $^3J_{NQ}$ = 6.8 Hz, $^3J_{NT}$ = 6.9 Hz, 11 H, Ge-CH_AH_B-CH_G-N═C-CH_K-CH_N-(CH_{Q3})(CH_{T3})], 1.19 (δ_X), 3.83 (δ_A), and 4.01 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.23 (δ_X), 3.96 (δ_A), and 4.13 (δ_B) ($^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 7.25−7.32 and 7.45−7.48 (m, 5 H, GeC_6H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −2.5 ($GeCH_3$), −2.3 ($GeCH_3$), 14.36 (OCH_2CH_3), 14.39 (OCH_2CH_3), 16.9 ($CHCH_3$), 19.1 ($CHCH_3$), 22.8 ($GeCH_2CH$), 32.0 [$CHCH$-$(CH_3)_2$], 53.6 ($GeCH_2CH$), 60.5 (OCH_2CH_3), 60.6 (OCH_2CH_3), 60.9 [$CHCH(CH_3)_2$], 127.8 (C-3/C-5, GeC_6H_5), 128.1 (C-4, GeC_6H_5), 133.3 (C-2/C-6, GeC_6H_5), 142.4 (C-1, GeC_6H_5), 162.5 (C═N), 164.6 (C═N). EI MS: 406 [4%, M^+], 391 [14%, $M^+ - Me$], 363 [8%, $M^+ - CHMe_2$], 329 [14%, $M^+ - Ph$], 211 [5%, $M^+ - CH_2GeMe_2Ph$], 181 [100%, $GeMe_2Ph^+$]. Anal. Calcd for $C_{20}H_{32}GeN_2O_2$: C, 59.30; H, 7.96; N, 6.92. Found: C, 59.3; H, 7.7; N, 6.9.

Preparation of (2*R*,5*R*)-Diethoxy-5-isopropyl-2-{[dimethyl(vinyl)silyl]methyl}-2,5-dihydropyrazine [(2*R*,5*R*)-22]. This compound was prepared analogously to the synthesis of (2*R*,5*R*)-**13**/(2*S*,5*R*)-**13** by addition of a 1.6 M solution of *n*-butyllithium in *n*-hexane (5.11 mL, 8.18 mmol *n*-BuLi) to a solution of (*R*)-**10** (1.72 g, 8.10 mmol) in THF (40 mL), followed by treatment with a solution of **19** (1.10 g, 8.17 mmol) in THF (10 mL). The crude product was purified by Kugelrohr distillation (oven temperature 150 °C, 0.01 mbar) to give a 80:20 mixture of (2*R*,5*R*)-**22**/(2*S*,5*R*)-**22** (molar ratio 87:13) and (*R*)-**10**. The diastereomerically pure compound (2*R*,5*R*)-**22** was isolated by liquid-chromatographic separation (for the general procedure, see below) as a colorless liquid in 58% yield [relative to (*R*)-**10**; 1.47 g, 4.73 mmol; ≥99% de]. IR (film, cm^{-1}): ν 1698 (C═N). ^1H NMR (400.1 MHz, $CDCl_3$): δ 0.10 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.68 (δ_Q), 0.90 (δ_A), 1.00 (δ_T), 1.27 (δ_B), 2.23 (δ_N), 3.86 (δ_K), and 4.01 (δ_G) [$^2J_{AB}$ = 14.5 Hz, $^3J_{AG}$ = 9.9 Hz, $^3J_{BG}$ = 4.8 Hz, $^5J_{GK}$ = 3.3 Hz, $^3J_{KN}$ = 3.5 Hz, $^3J_{NQ}$ = 6.8 Hz, $^3J_{NT}$ = 6.9 Hz, 11 H, Si-CH_AH_B-CH_G-N═C-CH_K-CH_N-(CH_{Q3})(CH_{T3})], 1.23 (δ_X), 4.02 (δ_A), and 4.11 (δ_B) ($^2J_{AB}$ = 10.5 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.25 (δ_X), 4.03 (δ_A), and 4.15 (δ_B) ($^2J_{AB}$ = 10.6 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 5.64 (δ_A), 5.89 (δ_M), and 6.17 (δ_X) ($^2J_{AM}$ = 3.8 Hz, $^3J_{AX}$ = 20.3 Hz, $^3J_{MX}$ = 14.7 Hz, 3 H, Si-CH_X═CH_AH_M). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −2.4 ($SiCH_3$), −2.2 ($SiCH_3$), 14.40 (OCH_2CH_3), 14.43 (OCH_2CH_3), 16.8 ($CHCH_3$), 19.1 ($CHCH_3$), 22.2 ($SiCH_2CH$), 31.9 [$CHCH(CH_3)_2$], 53.1 ($SiCH_2CH$), 60.5 (OCH_2CH_3), 60.71 (OCH_2CH_3), 60.76 [$CHCH(CH_3)_2$], 130.9 ($SiCH$═CH_2), 139.9 ($SiCH$═CH_2), 162.3 (C═N), 164.9 (C═N). ^{29}Si NMR (59.6 MHz, $CDCl_3$): δ −6.1. EI MS: 310 [1%, M^+], 295 [5%, $M^+ - Me$], 283 [5%, $M^+ - CH$═CH_2], 267 [24%, $M^+ - CHMe_2$], 211 [49%, $M^+ - CH_2$-$SiMe_2CH$═CH_2], 85 [100%, $SiMe_2CH$═CH_2^+]. Anal. Calcd for $C_{16}H_{30}N_2O_2Si$: C, 61.89; H, 9.74; N, 9.02. Found: C, 61.7; H, 9.5; N, 9.2.

Preparation of (*R*)-2-Amino-3-[dimethyl(phenyl)silyl]-propionic Acid Ethyl Ester [(*R*)-23]. This compound was prepared analogously to the synthesis of (*R*)-**15** by treatment of a solution of (2*R*,5*R*)-**20** (1.13 g, 3.13 mmol) in ethanol (20 mL) with 3 M hydrochloric acid (10 mL). After liquid-chromatographic separation on silica gel [see preparation of (*R*)-**15**; R_f[(*R*)-**23**] > R_f[(*R*)-valine]], (*R*)-**23** was isolated in 83% yield as a colorless liquid (654 mg, 2.60 mmol). IR (film, cm^{-1}): ν 1733 (C═O). ^1H NMR (300.1 MHz, $CDCl_3$): δ 0.33 (s, 3 H, $SiCH_3$), 0.36 (s, 3 H, $SiCH_3$), 1.08 (δ_A), 1.30 (δ_B), and 3.45 (δ_X) ($^2J_{AB}$ = 14.7 Hz, $^3J_{AX}$ = 9.1 Hz, $^3J_{BX}$ = 5.9 Hz, 3 H, Si-CH_AH_B-CH_X), 1.19 (δ_X), 3.97 (δ_A), and 4.05 (δ_B) ($^2J_{AB}$ = 11.0 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.45 (s, 2 H, NH_2), 7.35−7.43 and 7.53−7.62 (m, 5 H, SiC_6H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −2.6 ($SiCH_3$), −2.1 ($SiCH_3$), 14.0 (OCH_2CH_3), 22.5 ($SiCH_2CH$), 51.9 ($SiCH_2CH$), 60.6 (OCH_2-CH_3), 127.7 (C-3/C-5, SiC_6H_5), 128.9 (C-4, SiC_6H_5), 133.5 (C-2/C-6, SiC_6H_5), 138.7 (C-1, SiC_6H_5), 177.1 (C═O). ^{29}Si NMR (59.6 MHz, $CDCl_3$): δ −3.7. EI MS: 236 [1%, $M^+ - Me$], 135 [100%, $SiMe_2Ph^+$]. Anal. Calcd for $C_{13}H_{21}NO_2Si$: C, 62.11; H, 8.42; N, 5.57. Found: C, 61.9; H, 8.6; N, 5.7.

Preparation of (*R*)-2-Amino-3-[dimethyl(phenyl)germyl]propionic Acid Ethyl Ester [(*R*)-24]. This compound was prepared analogously to the synthesis of (*R*)-**15** by treatment of a solution of (2*R*,5*R*)-**21** (608 mg, 1.50 mmol) in ethanol (20 mL) with 3 M hydrochloric acid (10 mL). After liquid-chromatographic separation on silica gel [see prepara-

(23) Altamura, M.; Giammaruco, M.; Taddei, M.; Ulivi, P. *J. Org. Chem.* **1995**, *60*, 8403−8406.

tion of (*R*)-**15**; R_f[(*R*)-**24**] > R_f[(*R*)-valine]], (*R*)-**24** was isolated in 74% yield as a colorless liquid (328 mg, 1.11 mmol). IR (film, cm^{-1}): ν 1735 cm^{-1} (C=O). ^1H NMR (300.1 MHz, $CDCl_3$): δ 0.45 (s, 3 H, $GeCH_3$), 0.46 (s, 3 H, $GeCH_3$), 1.20 (δ_X), 3.49 (δ_A), and 3.52 (δ_B) ($^2J_{AB}$ = 11.0 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.1 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.23 (δ_A), 1.44 (δ_B), and 3.46 (δ_X) ($^2J_{AB}$ = 14.6 Hz, $^3J_{AX}$ = 9.1 Hz, $^3J_{BX}$ = 5.7 Hz, 3 H, Ge-CH_AH_B-CH_X), 1.84 (s, 2 H, NH_2), 7.31−7.38 and 7.42−7.50 (m, 5 H, GeC_6H_5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −3.0 ($GeCH_3$), −2.6 ($GeCH_3$), 14.1 (OCH_2CH_3), 22.7 ($GeCH_2CH$), 52.5 ($GeCH_2CH$), 60.8 (OCH_2CH_3), 128.0 (C-3/C-5, GeC_6H_5), 128.4 (C-4, GeC_6H_5), 133.2 (C-2/C-6, GeC_6H_5), 144.1 (C-1, GeC_6H_5), 177.0 (C=O). EI MS: 282 [17%, M^+ − Me], 224 [18%, M^+ − CO_2Et], 181 [100%, $GeMe_2Ph^+$]. Anal. Calcd for $C_{13}H_{21}GeNO_2$: C, 52.76; H, 7.15; N, 4.73. Found: C, 52.7; H, 7.0; N, 4.6.

Preparation of (*R*)-2-Amino-3-[dimethyl(vinyl)silyl]-propionic Acid Ethyl Ester [(*R*)-25]. This compound was prepared analogously to the synthesis of (*R*)-**15** by treatment of a solution of (2*R*,5*R*)-**22** (991 mg, 3.19 mmol) in ethanol (20 mL) with 3 M hydrochloric acid (10 mL). After liquid-chromatographic separation on silica gel [see preparation of (*R*)-**15**; R_f[(*R*)-**25**] > R_f[(*R*)-valine]], (*R*)-**25** was isolated in 70% yield as a colorless liquid (449 mg, 2.23 mmol). IR (film, cm^{-1}): ν 1732 (C=O). ^1H NMR (300.1 MHz, $CDCl_3$): δ 0.12 (s, 6 H, $SiCH_3$), 0.90 (δ_A), 1.11 (δ_B), and 3.47 (δ_X) ($^2J_{AB}$ = 14.7 Hz, $^3J_{AX}$ = 9.1 Hz, $^3J_{BX}$ = 5.9 Hz, 3 H, Si-CH_AH_B-CH_X), 1.24 (δ_X), 4.10 (δ_A), and 4.13 (δ_B) ($^2J_{AB}$ = 10.8 Hz, $^3J_{AX}$ = 7.1 Hz, $^3J_{BX}$ = 7.2 Hz, 5 H, O-CH_AH_B-CH_{X3}), 1.60 (s, 2 H, NH_2), 5.68 (δ_A), 5.95 (δ_M), and 6.15 (δ_X) ($^2J_{AM}$ = 3.4 Hz, $^3J_{AX}$ = 20.3 Hz, $^3J_{MX}$ = 14.7 Hz, 3 H, Si-CH_X=CH_AH_M). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −2.8 ($SiCH_3$), −2.7 ($SiCH_3$), 14.2 (OCH_2CH_3), 22.3 ($SiCH_2CH$), 51.9 ($SiCH_2CH$), 60.8 (OCH_2CH_3), 132.0 ($SiCH$=CH_2), 138.8 ($SiCH$=CH_2), 177.0 (C=O). ^{29}Si NMR (59.6 MHz, $CDCl_3$): δ −6.4. EI MS: 186 [5%, M^+ − Me], 128 [83%, M^+ − CO_2Et], 85 [100%, $SiMe_2CH$=CH_2^+]. Anal. Calcd for $C_9H_{19}NO_2Si$: C, 53.69; H, 9.51; N, 6.96. Found: C, 53.0; H; 9.3; N, 7.1.

Preparation of (*S*)-*N*-[(Fluoren-9-yl)methoxycarbonyl]-2-amino-4,4-dimethylpentanoic Acid [(*S*)-26]. A solution of (fluoren-9-yl)methyl chloroformate (3.93 g, 15.2 mmol) in dioxane (40 mL) was added dropwise at 0 °C over 30 min to a stirred solution of (*S*)-**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 stirring at 10 °C for 1 h, the reaction mixture was allowed to warm to room temperature within 12 h. The solvents were removed under reduced pressure (0.1 mbar, 20 °C), and the residue was acidified with 6 M hydrochloric acid (→ pH 2). The aqueous solution was extracted with ethyl acetate (3 × 5 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4. After concentrating the solution 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 (*S*)-**26** in 80% yield as a colorless solid (4.04 g, 11.0 mmol). IR (KBr, cm^{-1}): ν 1719 (C=O). ^1H NMR ($CDCl_3$, 300.1 MHz): δ 0.89 (s, 9 H, CCH_3), 1.19 (δ_A), 1.24 (δ_B), and 4.20 (δ_X) ($^2J_{AB}$ = 13.9 Hz, $^3J_{AX}$ = 5.5 Hz, $^3J_{BX}$ = 10.1 Hz, 3 H, C-CH_AH_B-CH_X), 4.35−4.41 (m, 3 H, $CHCH_2O$), 7.27−7.74 (m, 8 H, aromat. H), OH and NH not localized. ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 30.3 (CCH_3), 31.5 (CCH_3), 46.6 (CCH_2CH), 47.9 ($CHCH_2O$), 52.4 (CCH_2CH), 67.0 ($CHCH_2O$), 120.8, 125.7, 125.8, 127.8, 128.4, 142.0, 144.4, and 144.6 (fluorenyl), 156.7 (C=O), 178.4 (C=O). CI MS (negative ions): 733 [100%, 2 M − H^+], 366 [15%, M − H^+]. Anal. Calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.3; H, 6.7; N, 3.8.

Preparation of (*R*)-*N*-[(Fluoren-9-yl)methoxycarbonyl]-2-amino-3-(trimethylsilyl)propionic Acid [(*R*)-27]. This compound was prepared analogously to the synthesis of (*S*)-**26** starting from (*R*)-**2** (1.20 g, 7.44 mmol). The product (*R*)-**27** was isolated in 69% yield as a colorless solid (1.96 g, 5.11 mmol). IR (KBr, cm^{-1}): ν 1720 (C=O). ^1H NMR ($CDCl_3$, 300.1 MHz): δ 0.01 (s, 9 H, $SiCH_3$), 1.18 (δ_A), 1.24 (δ_B), and 4.20 (δ_X) ($^2J_{AB}$ = 14.5 Hz, $^3J_{AX}$ = 5.8 Hz, $^3J_{BX}$ = 10.2 Hz, 3 H, Si-CH_AH_B-CH_X), 4.35−4.41 (m, 3 H, $CHCH_2O$), 7.27−7.73 (m, 8 H, aromat. H), OH and NH not localized. ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ −1.0 ($SiCH_3$), 20.2 ($SiCH_2CH$), 46.9 ($CHCH_2O$), 51.3 ($SiCH_2CH$), 65.9 ($CHCH_2O$), 120.3, 125.4, 125.5, 127.6, 127.9, 141.8, 144.9, and 143.8 (fluorenyl), 155.6 (C=O), 175.2 (C=O). ^{29}Si NMR ($CDCl_3$, 59.6 MHz): δ 1.8. CI MS (negative ions): 765 [82%, (2 M − $H)^+$], 382 [100%, (M − $H)^+$], 180 [80%]. Anal. Calcd for $C_{21}H_{25}NO_4Si$: C, 65.77; H, 6.57; N, 3.65. Found: C, 65.5; H, 6.6; N, 3.6.

Preparation of (*R*)-*N*-[(Fluoren-9-yl)methoxycarbonyl]-2-amino-3-(trimethylgermyl)propionic Acid [(*R*)-28]. This compound was prepared analogously to the synthesis of (*S*)-**26** starting from (*R*)-**3** (1.16 g, 5.64 mmol). The product (*R*)-**28** was isolated in 47% yield as a colorless solid (1.13 g, 2.64 mmol). IR (KBr, cm^{-1}): ν 1717 (C=O). ^1H NMR ($CDCl_3$, 300.1 MHz): δ 0.18 (s, 9 H, $GeCH_3$), 1.26 (δ_A), 1.30 (δ_B), and 4.19 (δ_X) ($^2J_{AB}$ = 13.5 Hz, $^3J_{AX}$ = 5.4 Hz, $^3J_{BX}$ = 10.1 Hz, 3 H, Ge-CH_AH_B-CH_X), 4.33−4.43 (m, 3 H, $CHCH_2O$), 7.25−7.74 (m, 8 H, aromat. H), OH and NH not localized. ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ −1.6 ($GeCH_3$), 21.1 ($GeCH_2CH$), 47.1 ($CHCH_2O$), 51.8 ($GeCH_2CH$), 67.0 ($CHCH_2O$), 120.0, 125.0, 125.1, 127.1, 127.7, 141.3, 143.7, and 143.8 (fluorenyl), 155.8 (C=O), 178.2 (C=O). CI MS (negative ions): 857 [94%, (2 M − $H)^+$], 428 [100%, (M − $H)^+$]. Anal. Calcd for $C_{21}H_{25}GeNO_4$: C, 58.93; H, 5.89; N, 3.27. Found: C, 58.8; H, 5.8; N, 3.3.

Preparative Liquid-Chromatographic Separation of the Mixtures (2*R*,5*R*)-13/(2*S*,5*R*)-13/(*R*)-10, (2*R*,5*R*)-14/(2*S*,5*R*)-14/(*R*)-10, (2*R*,5*R*)-20/(2*S*,5*R*)-20/(*R*)-10, (2*R*,5*R*)-21/(2*S*,5*R*)-21/(*R*)-10, and (2*R*,5*R*)-22/(2*S*,5*R*)-22/(*R*)-10. The (2*R*,5*R*)- and (2*S*,5*R*)-isomers of **13**, **14**, **20**, **21**, and **22** were separated by liquid chromatography (MPLC) on silica gel (30−60 μm; Baker, 7024-01) starting from the respective mixtures (2*R*,5*R*)-**13**/(2*S*,5*R*)-**13**/(*R*)-**10**, (2*R*,5*R*)-**14**/(2*S*,5*R*)-**14**/(*R*)-**10**, (2*R*,5*R*)-**20**/(2*S*,5*R*)-**20**/(*R*)-**10**, (2*R*,5*R*)-**21**/(2*S*,5*R*)-**21**/(*R*)-**10**, and (2*R*,5*R*)-**22**/(2*S*,5*R*)-**22**/(*R*)-**10**. The experimental conditions were as follows: LC pump, Büchi Pump 688; detector, differential refractometer Knauer K-2300; column, 20 mm i.d. × 250 mm; pressure, 5 bar; eluent, *n*-hexane/diethyl ether (60:1, v/v); injection volume, 1.15 mL (150 mg of the sample material dissolved in 1 mL of diethyl ether); flow rate, 8.0 mL/min. The solvent of the respective fractions obtained [(2*R*,5*R*)-isomers, first fraction; (2*S*,5*R*)-isomers, second fraction; (*R*)-**10**, third fraction] was removed (rotary evaporator), and the diastereomerically products (2*R*,5*R*)-**13**, (2*S*,5*R*)-**13**, (2*R*,5*R*)-**14**, (2*S*,5*R*)-**14**, and (2*R*,5*R*)-**20**−(2*R*,5*R*)-**22** [(2*S*,5*R*)-**20**−(2*S*,5*R*)-**22** not isolated] were purified by Kugelrohr distillation [(2*R*,5*R*)-**13** and (2*S*,5*R*)-**13**, oven temperature 150 °C, 0.01 mbar; (2*R*,5*R*)-**14** and (2*S*,5*R*)-**14**, oven temperature 160 °C, 0.01 mbar; (2*R*,5*R*)-**20**, oven temperature 170 °C, 0.01 mbar; (2*R*,5*R*)-**21**, oven temperature 200 °C, 0.01 mbar; (2*R*,5*R*)-**22**, oven temperature 150 °C, 0.01 mbar].

Separation of the (2*R*,5*R*)- and (2*S*,5*R*)-Diastereomers of the 2,5-Dihydropyrazines 13, 14, and 20−22 by Capillary Gas Chromatography: Determination of Diastereomeric Purities. The (2*R*,5*R*)- and (2*S*,5*R*)-isomers of **13**, **14**, and **20**−**22** were separated by capillary gas chromatography [gas chromatograph, Shimadzu GC-14A; SE-30 column (0.32 i.d. × 10 m), Macherey-Nagel; carrier gas, nitrogen; temperature program, 100 °C (15 min) to 280 °C (10 min) with 7 °C/min; injector temperature, 200 °C; split 1:45; detector, FID; detector temperature, 320 °C]. The retention times of the diastereomers of **13**, **14**, and **20**−**22** are listed in Table 4.

Determination of the Enantiomeric Purities of the Amino Acid Esters (*R*)-15, (*S*)-15, (*R*)-16, (*S*)-16, and (*R*)-23−(*R*)-25 by ^1H NMR Spectroscopy. The enantiomeric purities of (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, (*S*)-**16**, and (*R*)-**23**−(*R*)-**25** were determined by ^1H NMR experiments using the chiral solvating agent (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol [(*R*)-TFAE; Aldrich]. The NMR spectra were recorded at 22 °C on

a Bruker DRX-300 NMR spectrometer operating at 300.1 MHz. The composition of the samples used for the ^{1}H NMR experiments was as follows: (*R*)-**15**, (*S*)-**15**, (*R*)-**16**, (*S*)-**16**, (*R*)-**23**−(*R*)-**25**, 53 μmol; (*R*)-TFAE, 159 μmol; $CDCl_3$, 1.0 mL.

Receptor Binding Assay for Determination of Antagonistic Potency of the Decapeptides 7−9.[24] For receptor binding studies, Cetrorelix was iodinated with ^{125}I (Amersham; specific activity 80.5 Bq/fmol) by using the Iodo-Gen reagent (Pierce). The reaction mixture was purified by reversed-phase high-performance liquid chromatography yielding mono-iodinated Cetrorelix without unlabeled peptide. About 80% of [^{125}I]-Cetrorelix was capable of specific receptor association. The receptor binding assay was performed with intact cells under physiological conditions as follows. Subconfluent cultures of stably transfected LTK$^-$ cells expressing the human GnRH receptor were detached by incubation in NaCl/P_i (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 11.5 mM KH_2PO_4)/1 mM EDTA and collected by centrifugation. The cell pellet was resuspended in binding buffer (Dulbecco's modified Eagle's medium DMEM) without H_2CO_3, with 4.5 g/L glucose, 10 mM Hepes pH 7.5, 0.5% (m/v) BSA, 1 g/L bacitracin, 0.1 g/L soy bean trypsin inhibitor (SBTI), 0.1% (m/v) NaN_3. For displacement assays, 0.25×10^6 cells/100 μL were incubated with approximately 225 pM [^{125}I]Cetrorelix (specific activity $(5-10) \times 10^5$ dpm/pmol) and different concentrations of unlabeled peptide as competitor. The cell suspension in 100 μL binding medium was layered on top of 200 μL 84% (by vol) silicone oil (Merck type 550)/16% (by vol) paraffin oil in 400 μL assay tubes. After incubation for 1 h at 37 °C under slow continuous agitation, the cells were separated from the incubation medium by centrifugation for 2 min at 9000 rpm (rotor type HTA13.8; Heraeus Sepatec). The tips of the tubes containing the cell pellet were cut off. Cell pellet and supernatant were subsequently analyzed by γ-radiation counting. The amount of unspecific binding was determined by including unlabeled Cetrorelix at 1 μM final concentration and was typically ≤10% of total binding. The analysis of binding data was accomplished with the EBDA/Ligand analysis program (Biosoft V3.0).

Functional Assay for Determination of Antagonistic Potency of the Decapeptides 7−9. The assay was performed as described in ref 25 with some modifications. Briefly, 10 000 cells/well expressing the human GnRH receptor and a luciferase reporter gene were cultivated for 24 h in microtiter plates using DMEM with supplements and 1% (v/v) FCS_i. Cells were subsequently stimulated for 6 h with 1 nM D-Trp^6-GnRH. Antagonistic GnRH analogues were added prior to the stimulation, and cells were finally lysed for quantification of cellular Luc (*Photinus pyralis* luciferase) activity. Calculation of the IC_{50} values from dose−response curves was done by nonlinear regression analysis using the Hill model (program EDX 2.0 by C. Grunwald, unpublished). Quantification of Luc activity was carried out essentially as described (Promega Technical Bulletins 101/161) by using the respective luciferase assay system (Promega E4030). By inclusion of coenzyme A (CoA), oxidation occurs from luciferyl-CoA with favorable kinetics (enhanced flash). Briefly, after removal of culture medium from the microtiter plate, cells were lysed by adding 100 μL of lysis buffer (25 mM tris-phosphate pH 7.8, 2 mM dithiothreitol, 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA), 10% (v/v) glycerol, 1% (v/v) Triton X-100). After incubation for 15 min at room temperature, 10 μL of cell lysate was transferred into a white microplate suitable for luminometric detection (Dynatech). The enzymatic reaction was initiated by adding 50 μL of assay buffer (20 mM Tricine pH 7.8, 1.07 mM $4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM dithiothreitol, 270 μM coenzyme A, 470 μM firefly luciferin, 530 μM rATPNa_2). Luminescence with a signal half-life of 5 min was quantified after 1 min for a total time of 1 s by using the EG&G Berthold MicroLumat LB96P.

In Vivo Experiments for Determination of Antagonistic Potency of the Decapeptides 7−9. Male Sprague-Dawley rats weighing 250−290 g were caged under "specific pathogen-free" conditions (SPF) and injected subcutaneously into the right flank with single doses of 1.5 mg/kg of **7**−**9**. The treatment groups consisted of 5 rats each, and the injection volume was 0.5 mL/kg. The test compounds were prepared in 5% mannitol just before used. The solution was then applied to the animal according to the individual body weight. Blood samples were taken under CO_2 short narcosis from *V. sublingualis* at 0, 1, 6, 24, 48, and 72 h after injection for the measurement of testosterone. Analysis of testosterone was performed by using an EIA kit provided by DRG Instruments GmbH and runs on microtiter 96-well plates. Evaluation of the microtiter plates was performed in a Mikrotrak EIA System (Syva) and evaluated via a software based on Excel. The specificity of the test is >98%. A significant suppression is evident with testosterone values below 2 ng/mL since this concentration represents the lower normal values that can be seen sporadically in untreated controls.[26]

To evaluate the effects of the test compounds on luteinizing hormone (LH) serum levels, male rats were castrated one week before the start of treatment in order to ensure high LH levels due to the positive feed back induced by ablation of the testes. Treatment was then performed as described for testosterone, but since the LH in castrated rats is very sensitive to suppression, the dose applied was reduced to 0.05 mg/kg. Blood samples were taken under CO_2 short narcosis from *V. sublingualis* at 0, 2, 4, 6, 24, 48, 72, and 96 h after injection for the LH measurements. Determination of LH in rat serum was performed in triplicate by using a specific rat-LH RIA provided by the NIADDK program (Bethesda, MD).

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

OM000169L

(24) Beckers, T.; Marheineke, K.; Reiländer, H.; Hilgard, P. *Eur. J. Biochem.* **1995**, *231*, 535−543.
(25) Beckers, T.; Reiländer, H.; Hilgard, P. *Anal. Biochem.* **1997**, *251*, 17−23.
(26) Reissmann, T.; Klenner, T.; Deger, W.; Hilgard, P.; McGregor, G. P.; Voigt, K.; Engel, J. *Eur. J. Cancer* **1996**, *32A*, 1574−1579.