Preparation of the β^2-Homoselenocysteine Derivatives Fmoc-(*S*)-β^2hSec(PMB)-OH and Boc-(*S*)-β^2hSec(PMB)-OH for Solution and Solid-Phase Peptide Synthesis

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Dedicated to Prof. *Carmen Nájera* on the occasion of her 60th birthday

Fmoc-β^2hSer(^{t}Bu)-OH was converted to Fmoc-β^2hSec(PMB)-OH in five steps. To avoid elimination of HSeR, the selenyl group was introduced in the second last step (Fmoc-β^2hSer(Ts)-OAll → Fmoc-β^2hSec(PMB)-OAll). In a similar way, the *N*-Boc-protected compound was prepared. With the β^2hSe-derivatives, 21 β^2-amino-acid building blocks with proteinogenic side chains are now available for peptide synthesis.

1. Introduction. – 1.1. *Selenocysteine.* Selenium is a trace element for mammals. It is part of selenocysteine [1–3] (**1**; Sec, H-Sec-OH or U), the DNA-encoded 21st proteinogenic amino acid that is found in more than 25 mammalian enzymes (selenoproteins) such as glutathione peroxidase, iodothyronine deiodinase, and thioredoxin reductase. Selenoproteins are important, *e.g.*, for the control of a cell's redox status, for the endocrine system, antioxidant defense, immune response, or sperm maturation. Therefore, there is a correlation between the presence or lack of Se and several human diseases, and Se is believed to be important in cancer prevention [4–6]. The biosynthetic machinery responsible for incorporation of selenocysteine into proteins is well-understood [7][8]. The basis for the incorporation of Sec into proteins has been established by *Böck*'s pioneering work on *Escherichia coli* mutants [9]. Selenocysteine is incorporated into selenoproteins by a process that decodes the UGA codon as selenocysteine [7][8]. For most human selenoproteins, 'homologs' exist, in which selenocysteine is replaced by cysteine. However, these enzymes show typically 10- to 100-fold lower enzymatic efficiencies when compared to the corresponding selenoproteins. One of the reasons might be that the SeH group ($pK_a \approx 5.7$) is more acidic than the SH group ($pK_a \approx 8.5$). Thus, in contrast to cysteine, selenocysteine is ionized at physiological pH (although not believed to exist in free form in cells). Another important property of the Se-atom is its higher polarizability, which makes the SeH group in Sec a 'softer' S_N2-type nucleophile and thus causes higher rates in

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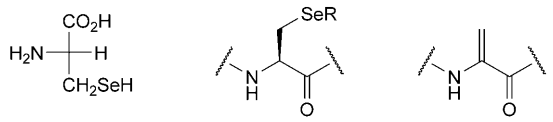
HOMO/LUMO-controlled reactions with electrophiles, which cannot be rivaled by the SH group of Cys.

Moreover, Sec is capable of catalyzing one-electron and two-electron-transfer reactions, much more efficiently than Cys. Diselenides have a lower reduction potential than disulfides, and Sec-containing peptides form diselenides more rapidly than the corresponding Cys-containing peptides form disulfides [10][11]. Additionally, the presence of Se in an amino acid sequence provides a spectroscopic as well as a mechanistic probe for studying peptide properties [12][13].

Finally, the SeH group of selenocysteine can be easily eliminated under mild basic or oxidative conditions[2]) to form dehydroalanines, which are convenient precursors for the preparation of peptide conjugates [14]. For all these reasons, interest in selenocysteine and its derivatives has dramatically increased in recent years.

Many different syntheses of Sec derivatives have been reported [12]. One of the first preparations of Sec was published by *Fredga*, starting from 3-chloroalanine methyl ester and K_2Se_2 in aqueous solution, but the yields were low and poorly reproducible [15]. Later, the common approach to prepare Sec and its derivatives was based on displacement of the activated OH group of serine by various organoselenide anions. In the late 1960s, *Walter* and co-workers introduced this method when they prepared the enantiomerically pure Sec(Bn) derivatives in good yields [16][17]. Recently, this method was optimized by *van der Donk* and co-workers, who described the preparation of the *N*-Fmoc- and *Se-p*-methoxybenzyl (PMB)-protected derivative, Fmoc-Sec(PMB)-OH, on g-scale, ready for solid-phase peptide synthesis (SPPS) [18]. Replacement of benzyl (Bn) by PMB is a great improvement for the syntheses of Sec-containing peptides, since Se(Bn) turned out to be a much better leaving group, which led to low yields of selenopeptides. On the other hand, an even better leaving group, PhSe, is used for the generation of dehydroalanine moieties in the use of convergent ligation strategies for the preparation of peptide conjugates [14]. Today, PMB is the most widely used protecting group for Sec, and it can be removed under various conditions [19][20].

SPP Synthesis with Sec requires special care due to the greater susceptibility to β-elimination of RSe^- *vs.* RS^-. This can lead to enantiomerization during coupling, elimination (**2** → **3**), or formation of β-piperidyl adducts to dehydroalanine during Fmoc deprotection with piperidine. Therefore, the coupling is usually performed with DIC/HOBt[3]) activation or with pre-formed pentafluorophenyl (Pfph) esters rather



H-Sec-OH (**1**) **2** **3**

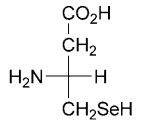
[2]) ... which causes problems for the synthetic chemist working on the preparation of Sec derivatives or analogs.

[3]) For abbreviations, see *Exper. Part.*

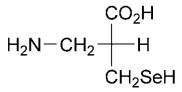
than by methods requiring tertiary base during coupling (*e.g.*, HATU/DIEA). To minimize elimination during Fmoc or final deprotection, the reactions are carried out with much shorter reaction times, lower temperatures, and at lower concentrations of basic or acidic reagents [19].

Although there are examples in the literature for SPPS of selenopeptides containing Sec in the middle of the sequence, taking into account the problem of side reactions mentioned above during repetitive coupling and deprotection, it is advisable to use the so-called native chemical ligation for the synthesis of longer sequences. Optimized and popularized by *Kent* and co-workers [21], native chemical ligation, *i.e.*, coupling of two unprotected peptides, has proved to be a most useful tool for syntheses of large peptides. In this convergent strategy, an N-terminal Cys residue of one peptide (protein) reacts with C-terminal thioesters of another peptide (protein). The thioester-linked intermediate undergoes spontaneous and rapid intramolecular S → N acyl shift, forming the amide bond at the ligation site[4]). This technique has been extended to the coupling of a C-terminal thioester with an N-terminal Sec moiety [22]. Furthermore, expressed protein ligation has been used for preparation of selenoproteins – the thioesters were generated by recombinant DNA technology and coupled with a synthetic Sec-containing segment [22–24].

1.2. *β-Peptides.* β-Peptides built of homologated proteinogenic amino acids represent a biomedically promising class of peptidomimetics [25]. Similar to their α-peptidic counterparts, β-peptides fold to secondary structures such as turns and helices, and they form sheets [26]. Furthermore, they can mimic α-peptidic hormones, as well as peptide–protein, protein–protein, and protein–DNA and protein–RNA interactions [25][27][28]. Due to the different dimensions, geometries, and polarities of the β-peptidic structures, the biological properties of β-peptides differ from those of α-peptides in those cases where exact fitting is mandatory: they do not bind to the active sites of peptidases, and are proteolytically [29a–g] and metabolically [29h,i][30] stable, and they may be orally bioavailable [30]. The β^2- and β^3-amino acid homologs of the 20 common proteinogenic α-amino acids have all been prepared and incorporated in β-peptides, the properties of which have been studied extensively [31]. We have previously also prepared derivatives of H-β^3hSec-OH (**4**; homolog of DNA-encoded amino acid No. 21), and used them for selenoligations [32].



H-β^3hSec-OH (**4**)



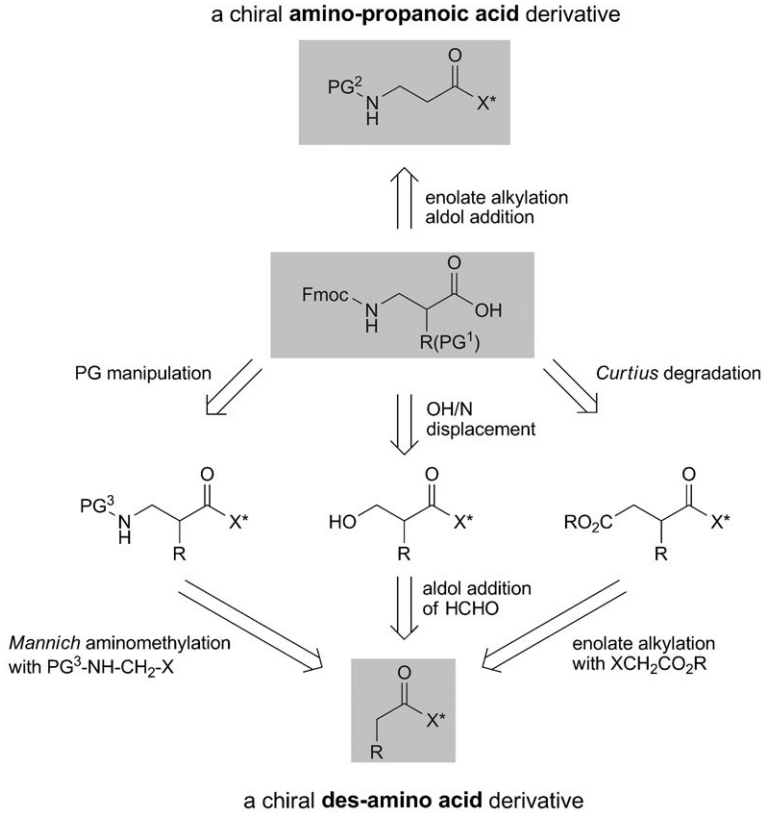
H-β^2hSec-OH (**5**)

In contrast to β^3-homo-amino acids, which are readily available in enantiomerically pure form from the natural proteinogenic amino acids by the *Arndt–Eistert* or *Kolbe* homologation, the β^2-homo-amino acids are much more challenging to prepare. The most common and – in our group – well-established way of preparing enantiomerically pure β^2-homo-amino acids is the *Mannich*-type transformation of des-amino-acid

[4]) See discussion in *Sect. 3* (**A**, **B**, and **C**), below.

derivatives using a chiral auxiliary (*Scheme 1*, *bottom*) [33][34]. Depending on the nature of the side chain, there are three synthetically equivalent, diastereoselective reactions of the corresponding chiral enolates: a 'real' *Mannich* reaction with an electrophilic aminomethylating reagent (X–CH_2–NPG), the (alkoxycarbonyl)methylation (with X–CH_2–CO_2R), and the hydroxymethylation (with HCHO), followed by *Curtius* degradation and O/N substitution, respectively. Otherwise, chiral 3-aminopropanoic acid derivatives can be alkylated (*Scheme 1*, *top*) [34]).

Scheme 1. *Retrosynthetic Analysis of the Most Frequently Used Routes to Fmoc-β²-homo-Amino Acids* (Fmoc-β²hXaa(PG)-OH) *for Solid-Phase Synthesis of β-Peptides.* X* = A chiral auxiliary group; PG^1 = an acid-labile protecting group. For a recent review article on the preparation of β²-amino acid derivatives, see [34].



Herein, we describe the preparation of derivatives of β²-homo-selenocysteine (**5**) (through β²-homoserine as an intermediate) and their incorporation into a peptide.

2. Preparation of Fmoc-β²hSec(PMB)-OH and Boc-β²hSec(PMB)-OH. – The initial efforts to prepare β²-homo-selenocysteine by the method of diastereoselective reactions of a Ti-enolate, derived from our modified *Evans* auxiliary DIOZ [35][36]

with appropriate electrophiles (for aminomethylation or hydroxymethylation) [33–38], did not yield the desired products at all or only in unacceptable yields (*Scheme 2*)[5]). The synthetic difficulties arose from the presence of the PMB-Se group, which is very sensitive to various reaction conditions. In particular, the tendency for elimination is much more pronounced for β^2hSec than for α-Sec derivatives.

These unsatisfactory results forced us to re-design the synthetic route in such a way that the RSe group was introduced in as late a step of the synthesis as possible, in order to avoid exposure of Se-containing synthetic intermediates to unfavorable reaction conditions. To fulfill this requirement, protected β^2-homo-selenocysteine was prepared from Fmoc-β^2hSer(^{t}Bu)-OH (**9**), which, in turn, was obtained from 3-aminopropanoic acid (*Scheme 3*; *cf.* also top part of *Scheme 1*). The conversion of this commercially available starting material to **9** required eight steps[6]), according to our procedures published in [33][37] and somewhat modified by others [39]. The conversion to Fmoc-β^2hSec(PMB)-OH (**14**) took another five steps (*Scheme 4*). The allyl protecting group was introduced (→**10**) by means of a phase-transfer-catalysis reaction [40][41]. The ^{t}Bu protecting group was then removed with Me_3SiI (→**11**) [42], and the resulting OH group was activated for substitution by tosylation (→**12**). Reaction with *in-situ*-generated PMB-selenolate produced the PMB-selenomethyl-substituted allyl ester **13**. Removal of the allyl protecting group was performed with [$(Ph_3P)_4$]Pd/morpholine[7]) in THF to yield Fmoc-β^2hSec(PMB)-OH (**14**) in 34% yield over a total of 13 steps. Due to the lability towards β-elimination, all procedures (reaction conditions, workup, isolation, purification) with the *p*-toluenesulfonate **12** and with the seleno derivatives **13** and **14** had to be performed with special care.

For a thio or seleno ligation, the Cys or Sec residue should be in N-terminal position of one of the two peptides to be coupled. In an SPP synthesis of such a peptide, following the Fmoc strategy, it is convenient to use the *N*-Boc-protected Cys or Sec building block, to allow for simultaneous deprotection of all functional groups and removal of the peptide from the resin under acidic conditions in the final step.

Thus, we have also prepared Boc-β^2hSec(PMB)-OH (**18**) from Fmoc-β^2hSer-OAll (**11**) by Fmoc/Boc interchange [43] (→**15**), tosylation (→**16**), substitution by PMBSe (→**17**), and cleavage of the allyloxy group (→**18**; *Scheme 5*).

3. Preparation of a β^2hSec-Containing β-Tetrapeptide. – Fmoc-β^2hSec(PMB)-OH (**14**) was used for the preparation of the β-peptide **19**, which could possibly be used for a seleno ligation. As mentioned in the *Introduction*, in α-peptide synthesis, incorporation of Sec is usually performed with the corresponding pentafluorophenyl (Pfph) esters

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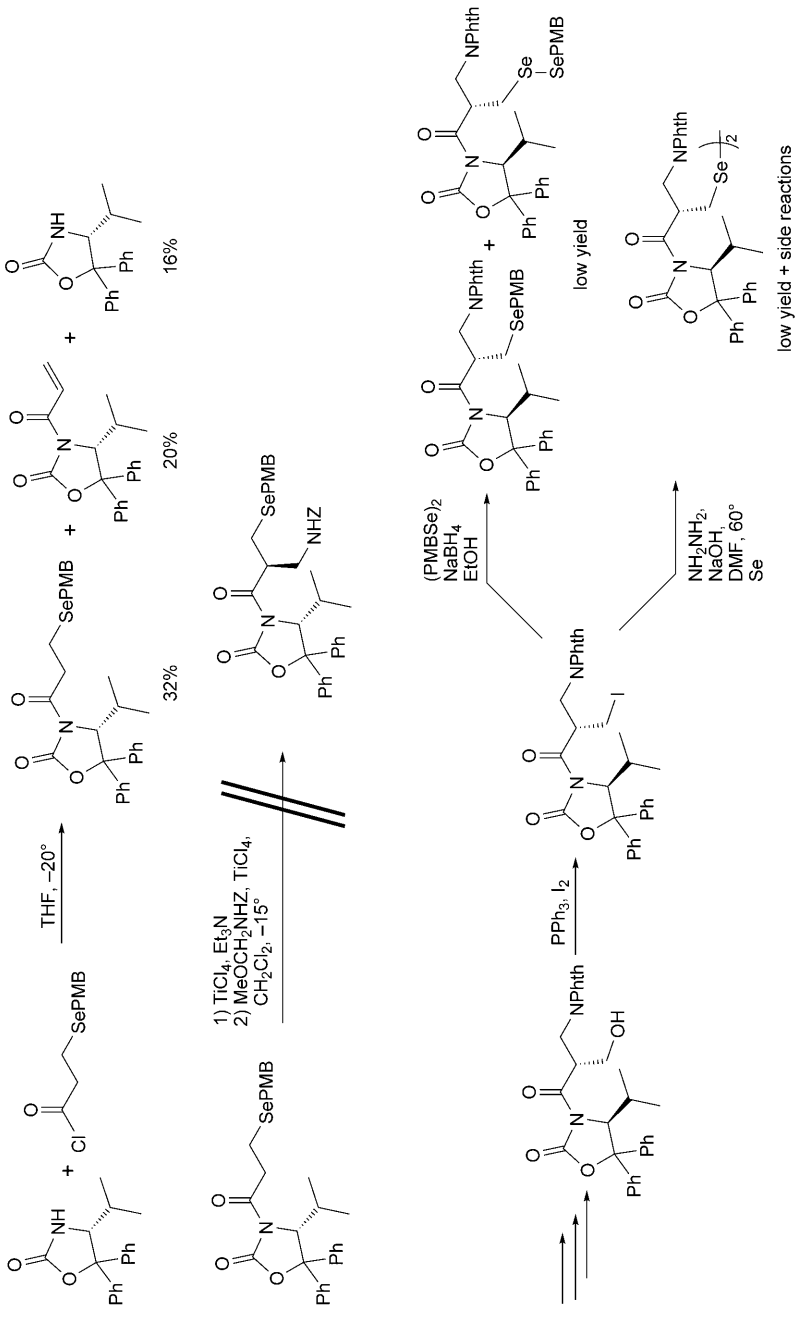
5) These experiments were carried out by Dr. *O. Flögel* as part of his postdoctoral work at ETH Zürich (2004–2006).

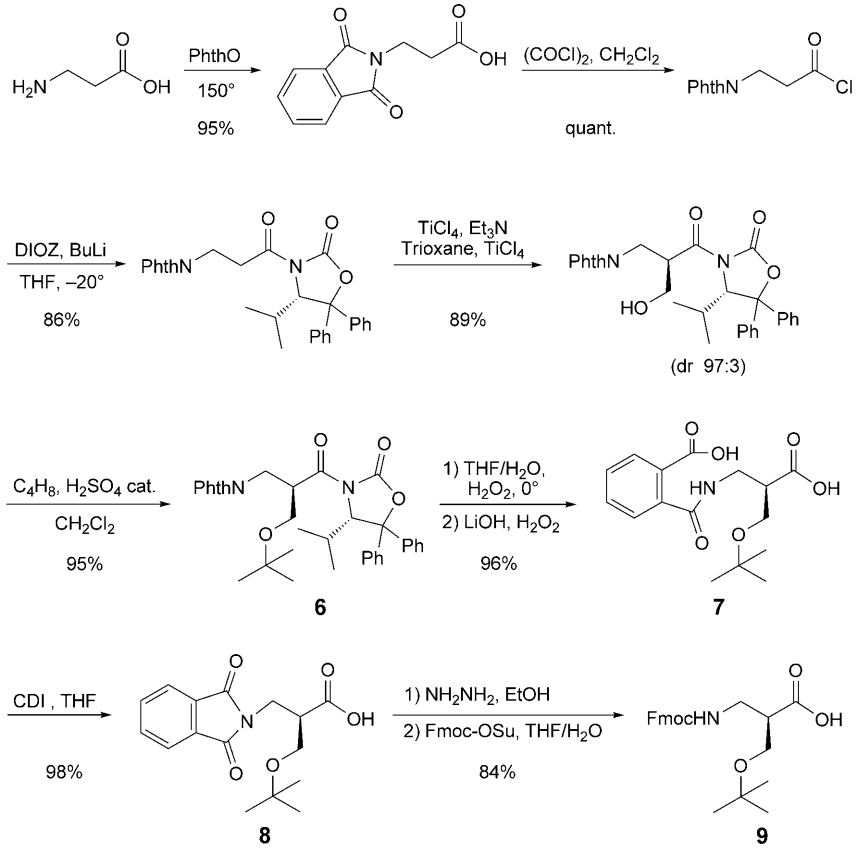
6) Any new general method of preparing such β^2-amino acid derivatives with functionalized side chains, properly protected for Fmoc-solid-phase peptide synthesis, will be most welcome to the community of chemists interested in β-amino acid-containing peptides. As demonstrated in a 2009 review [34], there are no short enantioselective routes to this type of β^2-amino acid derivatives, if we *honestly* count all steps from commercial starting materials.

7) Fortunately, the seleno group did not poison the catalyst; see the high yield of de-allylation. With the Boc derivative, the yield of this deprotection was much lower (*Scheme 5*).

Scheme 2. *Aborted Attempts at the Preparation of β²-homo-Selenocysteine.* PMB = *p*-methoxybenzyl; Z = (benzyloxy)carbonyl; Phth = phthaloyl.



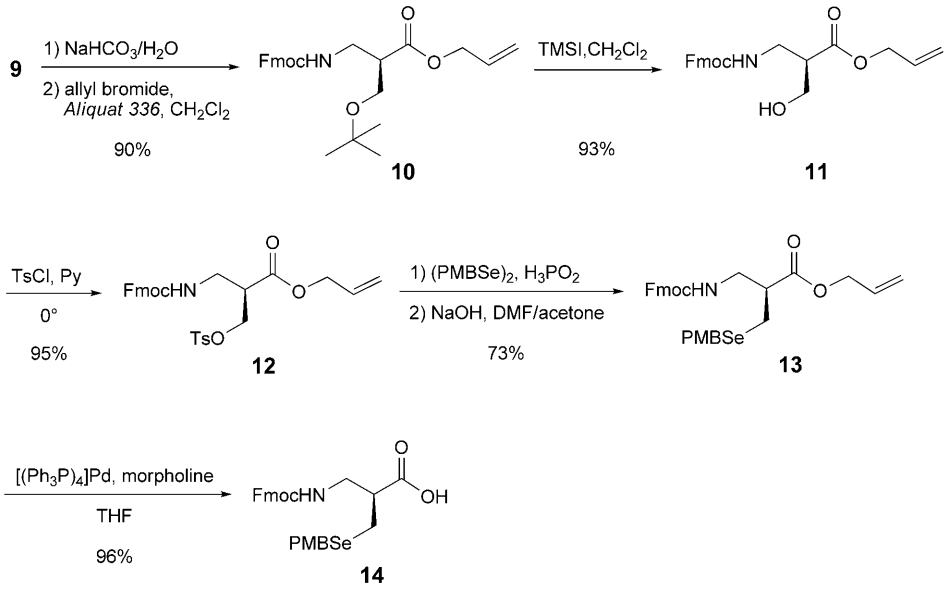
Scheme 3. *Conversion of the Commercially Available 3-Aminopropanoic acid* (βh-glycine or β-alanine) *to Fmoc-β^2hSer(^{t}Bu)-OH* (**9**) *as Described Previously* [33][37][39] *with a Modification of the DIOZ-Cleavage Step* (DIOZ = 4-isopropyl-5,5-diphenyl-1,3-oxazolidin-2-one)



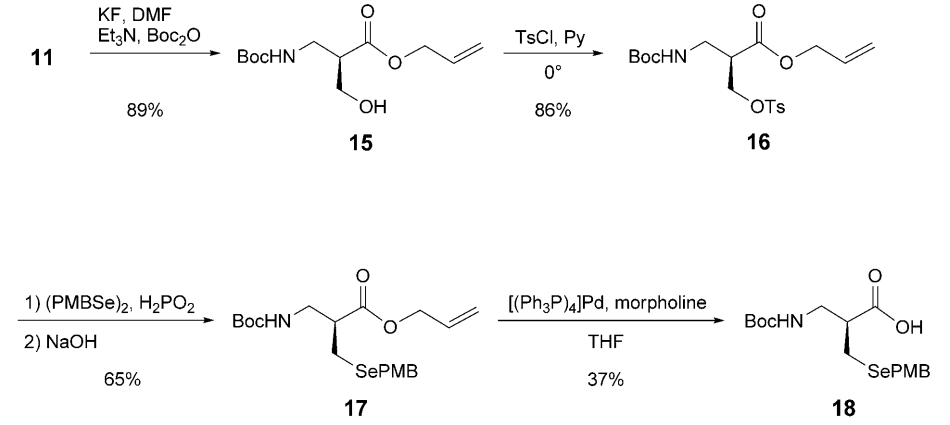
C_4H_8 = Isobutylene = 2-methylprop-1-ene

under HOBt catalysis to avoid racemization and elimination. Since the β-elimination is an especially severe problem with the β^2-homo-selenocysteine moiety, the synthesis of the β-tetrapeptide **19** with an N-terminal β^2hSec residue was carried out in the following way: we first used conventional solid-phase coupling on a 2-chlorotrityl chloride resin. The couplings prior to incorporation of β^2hSec were performed with HATU and HOAt as coupling reagents, using *Hünig*'s base (DMF, 3 h). Activation of Fmoc-β^2hSec(PMB)-OH (**14**) was achieved with DIC and HOBt (CH_2Cl_2, 1 h) and the resulting solution was added to the resin, swollen in DMF and carrying the H-β^3hAla-β^3hLeu-β^3hIle segment. The final terminal Fmoc deprotection was performed with a standard solution of 20% piperidine in DMF using short reaction times (3×3 min). The reaction was followed by LC/MS analysis of analytical amounts of the peptide cleaved from the resin. It turned out that, after two 3-min deprotection periods, there

Scheme 4. *Conversion of Fmoc-β²hSer(ᵗBu)-OH* (**9**) *to Fmoc-β²hSec(PMB)-OH* (**14**) *in Five Steps, Avoiding HX Elimination in the α-Position of the Carbonyl Group* (*Aliquat 336* = *N*-methyl-*N,N*-dioctyloctan-1-ammonium chloride)



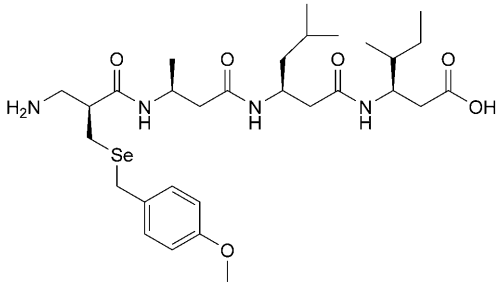
Scheme 5. *Preparation of Boc-β²hSec(PMB)-OH from Fmoc-β²hSer-OAll*



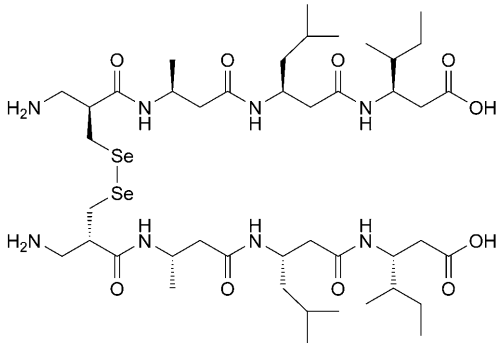
was still *ca.* 10% Fmoc-peptide, while, after the third base treatment, only traces were detectable by LC/MS[8]). No product of elimination could be detected.

[8]) These trace amounts were not visible in the HPLC-UV-analysis mode.

Eventually, the peptide was removed from the 2-chlorotrityl chloride resin under conditions that did not set free the SeH groups (0.8% TFA in CH_2Cl_2, 5×1 min). Purification by preparative HPLC gave the TFA salt of β-tetrapeptide **19** in 92% yield.



19



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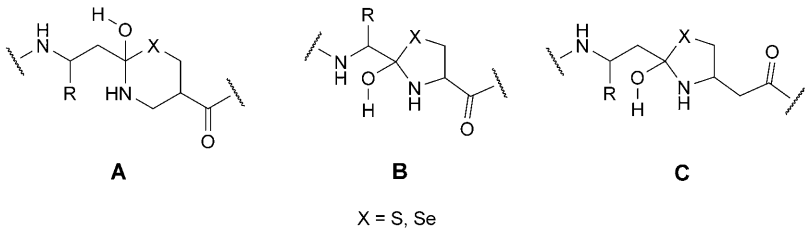
While there are various ways of oxidatively deprotecting the Cys(PMB) or Sec(PMB) groups in peptides, with formation of the corresponding disulfide or diselenide (I_2, $Tl(O_2CCF_3)_3$, DMSO/TFA, DMSO/TMSCl/TFA [19][44], or TMSBr/thioanisole/*m*-cresol/TFA [32][45]), it appeared from the literature that, for Sec(PMB), the use of I_2 is superior to the other methods [44].

Indeed, treatment of the tetrapeptide **19** with 10 equiv. of I_2 in AcOH/H_2O for 1 h, followed by preparative RP-HPLC purification, afforded the diselenide **20** as its TFA salt in 85% yield and, in addition, 10% of the starting material **19** was recovered.

Attempts using the β^2-selenocystein-containing peptide for a ligation with another β-peptide have so far been unsuccessful. This failure must have been caused by the lability of the β^2hSec moiety and not by the fact that the Se/N-shift in the final step of the ligation must occur through a six-ring tetrahedral intermediate **A**, rather than a five-membered heterocyclic intermediate **B** or **C** (α-Cys/Sec and β^3Cys/Sec): the ligation with an N-terminal β^2hCys residue (**A**, X = S) worked perfectly in the ligation of two β^2-decapeptides in a previous investigation [46].



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Experimental Part

1. *General. Abbreviations.* Bn: benzyl, Boc: (*tert*-butoxy)carbonyl, CDI: 1,1′-carbonyldiimidazole, DIC: *N,N′*-Diisopropylcarbodiimide, DIEA: diisopropylethylamine ($EtN(i\text{-}Pr)_2$), ESI: electron spray ionization, FC: flash chromatography, Fmoc: [(9*H*-fluoren-9-yl)methoxy]carbonyl, Fmoc-OSu: *N*-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}oxy)succinimide, GdmCl: guanidinium hydrochloride, HATU: *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HBTU: 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOAt: 1-hydroxy-7-azabenzotriazole, HOBt: 1-hydroxy-1*H*-benzotriazole, HPLC: high-performance liquid chromatography, HR: high resolution, h.v.: high vacuum (0.01 – 0.1 Torr), MALDI: matrix-assisted laser desorption ionization, NMP: *N*-methylpyrrolidin-2-one, Pfph: pentafluorophenyl, PMB: *p*-methoxybenzyl, PyBop: (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, TFA: trifluoroacetic acid, TIPS: $(i\text{-}Pr)_3SiH$, TNBS: 2,4,6-trinitrobenzenesulfonic acid. DTT: dithiothreitol.

2. *Materials and Methods.* All reagents were of synthetic grade and were used without further purification unless stated otherwise. Dry THF was distilled on Na and benzophenone; dry CH_2Cl_2 was distilled on CaH_2. All moisture-sensitive reactions were carried out under a pos. pressure of Ar in oven-dried glassware (140°). Org. extracts were dried over $MgSO_4$. TLC: *Merck* TLC silica gel *60* F_{254} glass plates; visualization by inspection under UV light (254 nm) or by the use of $KMnO_4$ stain, Mo-based stain, bromocresol green stain, or ninhydrin spray. Flash column chromatography (FC): silica gel *60* (0.040 – 0.063 mm; *Fluka*). 2-Chlorotrityl chloride resin was purchased from *Novabiochem*, and amino acids were purchased from *Fluka*. Reversed-phase (RP) HPLC: *Merck*/*Hitachi* HPLC system (*LaChrom*, pump type *L-7150*, UV detector *L-7400*, interface *D-7000*, HPLC Manager *D-7000*). Anal. HPLC: *Macherey-Nagel* C_{18} column (*Nucleosil 100-5* C_{18} (250 × 4 mm)) using a gradient of solvent *A* (0.1% TFA in MeCN) and *B* (0.1% TFA in H_2O) at a flow rate of 1 ml/min. Prep. HPLC: *Macherey-Nagel* C_{18} column (*Nucleosil 100-5* C_{18} (250 × 21 mm)) using a gradient of solvent *A* (0.1% TFA in MeCN) and *B* (0.1% TFA in H_2O) at a flow rate of 10 ml/min. Lyophilization after purification by RP-HPLC was performed using a *Hetosicc* cooling condenser with high-vacuum pump to obtain peptides as their TFA salts. M.p.: *Büchi 510* melting-point apparatus; uncorrected. Optical rotations: *JASCO P-2000* polarimeter (10 cm, 1-ml cell), $[\alpha]_D^{20}$ values are determined at 589 nm (Na-D line). IR Spectra: neat (unless otherwise stated) on a *Perkin-Elmer Spectrum 100* FT-IR spectrometer; selected absorption bands in $\tilde{\nu}$ [cm^{-1}]. NMR Spectra: *Bruker AV-300* (^1H: 300 MHz, ^{13}C: 75 MHz), *AV-400*, *DRX-400* (^1H: 400 MHz, ^{13}C: 100 MHz), *Varian Mercury XL* 300 (^1H: 300 MHz, ^{13}C: 75 MHz); chemical shifts (δ) in ppm rel. to internal standard Me_4Si; coupling constants *J* in Hz; assignments on a routine basis by a combination of 1D and 2D experiments (COSY, HSCQ, HMBC). High-resolution (HR) MS: *IonSpec Ultima* 4.7 T FT Ion Cyclotron Resonance (ICR, HR-MALDI-MS, in 3-HPA matrix) mass spectrometer, in *m*/*z* (% of basis peak). LC/MS: *Thermo Separation Products* (*TSP*) HPLC system connected to a PDA detector (*UV6000LB*, *TSP*) and to an ion-trap mass-spectrometry detector (*LCQdeca*, *Finnigan*).

Elemental analyses were performed by the Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich.

3. *Synthesis of the β-Peptides. General Procedures. Anchoring of* N*-Fmoc-Protected β-Amino Acid on 2-Chlorotrityl Chloride Resin. General Procedure 1* (*GP 1*). The loading of the 2-chlorotrityl chloride resin was performed according to *Barlos et al.* [47][48]. A soln. of the Fmoc-*β*-amino acid (0.2 equiv. rel. to the resin) and DIEA (1 equiv.) in dry CH_2Cl_2 (containing a few drops of DMF to facilitate dissolution of the amino acid) was added to the resin that had been pre-swelled in dry CH_2Cl_2 for 5 min. The suspension was swirled by N_2 bubbling for 2 h at r.t. Subsequently, the resin was filtered off, washed with CH_2Cl_2 and, then, to block unreacted Cl groups, with CH_2Cl_2/MeOH/DIPEA 17:2:1 (3×), DMF (4 ml, 4 × 1 min), CH_2Cl_2 (4 ml, 4 × 1 min), followed by washing with CH_2Cl_2 (3×), DMF (3×), and CH_2Cl_2 (3×), and dried under h.v. overnight. The loading was determined by measuring the absorbance of the benzofulvene–piperidine adduct according to *Gude et al.* [49].

Solid-Phase Peptide Synthesis (SPPS) *on 2-Chlorotrityl Chloride Resin. General Procedure 2* (*GP 2*). The Fmoc group of the first amino acid attached to the resin was removed with 20% piperidine in DMF (4 ml; 2 × 10 min) under N_2 bubbling. After filtration, the resin was washed with DMF (4 ml; 4 × 1 min). SPPS was continued by sequential incorporation of *N*-Fmoc-protected *β*^{3}-homo-amino acids. For each coupling step, the resin was treated with a soln. of Fmoc-*β*^{3}-homo-amino acid (2 equiv.) and DIEA (6 equiv.) in DMF (3 ml), and HATU (1.9 equiv.) and HOAt (1.9 equiv.) in DMF (3 ml). The suspension was mixed by N_2 bubbling for 120 min. Monitoring of the coupling reaction was performed with the TNBS test [50]. The resin was then filtered off and washed with DMF (4 ml; 4 × 1 min) prior to the subsequent Fmoc-deprotection step with 20% piperidine in DMF (4 ml; 4 × 10 min). The resin was then filtered off, washed with DMF (4 ml; 4 × 1 min), CH_2Cl_2 (4 ml; 4 × 1 min), and MeOH (4 ml: 4 × 1 min), dried under h.v. for 24 h, and used for the cleavage step.

Cleavage of the Peptide from 2-Chlorotrityl Chloride Resin. General Procedure 3 (*GP 3*). The cleavage from the resin was performed according to the procedure of *Barlos et al.* [47][48]. To the pre-swelled resin with fully assembled peptide, a soln. of 0.8% TFA in CH_2Cl_2 was added. The suspension was mixed by N_2 bubbling for 1 min, and the filtrate was collected. The procedure was repeated several times (the beads become deeply red), and then the resin was washed with CH_2Cl_2. The collected filtrates were evaporated under reduced pressure to yield an oily residue that was dissolved in H_2O/MeCN and directly purified on prep. HPLC and lyophilized.

*2-[(2*R*)-2-[(1,1-Dimethylethoxy)methyl]-3-[(4*S*)-4-(1-methylethyl)-2-oxo-5,5-diphenyloxazolidin-3-yl]-3-oxopropyl]-1*H*-isoindole-1,3(2*H*)-dione* (**6**). Following our published procedure [33], **6** was obtained on a 18.44-mmol scale (10.5 g, 95% yield). Colorless solid. The anal. data are in agreement with those reported in [33].

*2-({[(2*R*)-2-Carboxy-3-(1,1-dimethylethoxy)propyl]amino}carbonyl)benzoic Acid* (**7**). To a soln. of **6** (6.2 g, 10.9 mmol) in THF/H_2O 2.4:1 (125 ml) was added H_2O_2 (0.17 equiv., 30% aq., 4.5 ml, 1.88 mmol) at 0°. After stirring for 5 min, LiOH/H_2O (2 equiv., 0.928 g, 21.8 mmol) was added. The mixture was stirred at 0° for 3 h. Na_2SO_3 (1.16 g) was added at 0°, and the mixture was stirred for 30 min. THF was evaporated and 16 ml of Et_2O were added. The suspension was stirred for *ca.* 15 min, and the solid was filtered off and washed successively with 1M NaOH (16 ml), H_2O (16 ml), Et_2O (10 ml), and pentane (10 ml) to give 2.7 g of recovered DIOZ (88%). The filtrate was diluted with AcOEt, and the aq. layer was acidified to pH 2 with 1M HCl, which was extracted with AcOEt. The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated to give **7** (3.2 g, 96%). Colorless solid. M.p. 189–192°. R_f (CH_2Cl_2/MeOH 15:1) 0.115. $[\alpha]_D^{20} = +5.05$ ($c = 1.87$, MeOH). IR ($CHCl_3$): 2951.0*w* (br.), 1773.0*w*, 1709.5*s*, 1579.8*m*, 1467.5*m*, 1396.6*m*, 1214.4*m*, 1127.8*m*, 1072.4*m*, 1039.4*m*, 749.1*s*, 721.8*s*, 666.5*m*. ^{1}H-NMR (400 MHz, CD_3OD): 1.20 (*s*, 9 H); 2.96 (*m*, 1 H); 3.62 (*dd*, $J = 5.2, 6.8, 2$ H); 3.72 (*dd*, $J = 4.8, 5.2, 2$ H); 7.44 (*ddd*, $J = 0.4, 1.6, 7.2, 1$ H); 7.53 (*dt*, $J = 1.6, 7.6, 1$ H); 7.60 (*dt*, $J = 1.6, 7.6, 1$ H); 7.96 (*ddd*, $J = 0.4, 1.6, 7.2, 1$ H). ^{13}C-NMR (100 MHz, CD_3OD): 27.76 (Me); 40.05 (CH_2); 47.08 (CH); 62.21 (CH_2); 74.27 (C); 128.84; 130.57 (CH); 130.89 (C); 131.31, 133.02 (CH); 139.90, 169.29, 172.99, 176.38 (C). ESI/LC-MS: 321.9 ($[M-H]^+$, $C_{16}H_{21}NO_6^+$; calc. 323.34).

*(α*R*)-α-[(1,1-Dimethylethoxy)methyl]-1,3-dihydro-1,3-dioxo-2*H*-isoindole-2-propanoic Acid* (**8**). To a soln. of **7** (1 equiv., 3.6 g, 11.8 mmol) in dry THF (100 ml), CDI (3 equiv., 5.74 g, 35.4 mmol) was added. The mixture was stirred at r.t. for 48 h and then concentrated. The residue was re-dissolved in 10%

$NaHCO_3$ (15 ml), and the aq. soln. was acidified to pH 1 with 1M HCl. The resulting precipitate was extracted with AcOEt, and the combined org. layers were dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by FC (CH_2Cl_2/MeOH 15:1, containing 0.1% AcOH) to yield **8** (3.4 g, 98%). Colorless solid. M.p. 116–118°. R_f (CH_2Cl_2/MeOH 15:1) 0.32. $[\alpha]_D^{20} = -1.1$ ($c = 1.19$, $CHCl_3$). IR (CH_2Cl_2): 2974.6*m*, 1774.0*m*, 1713.7*s*, 1468.2*w*, 1438.1*w*, 1394.8*m*, 1364.5*m*, 1192.3*m*, 1087.9*m*, 1022.1*w*, 976.2*w*, 906.7*w*, 794.7*w*, 721.3*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.95 (*s*, 1 H); 3.02 (*m*, 1 H); 3.48 (*dd*, $J = 5.2, 9.2$, 1 H); 3.58 (*dd*, $J = 5.2, 9.2$, 1 H); 3.89 (*dd*, $J = 6.0, 14.0$, 1 H); 3.97 (*dd*, $J = 6.0, 14.0$, 1 H); 7.59 (*dd*, $J = 3.2, 5.2$, 2 H); 7.71 (*dd*, $J = 2.8, 5.2$, 2 H); 8.22 (br. *s*, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 27.22 (Me); 38.10 (CH_2); 46.26 (CH); 61.70 (CH_2); 73.12 (C); 123.12, 133.81 (CH); 132.21, 168.42, 177.60 (C). LC/EI-MS: 304.1 ($[M-H]^+$, $C_{16}H_{19}NO_5^+$; calc. 305.33). Anal. calc. for $C_{16}H_{19}NO_5$ (304.1): C 62.94, H 6.27, N 4.59; found: C 62.68, H 6.33, N 4.59.

*(2*R*)-3-(1,1-Dimethylethoxy)-2-[({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)methyl]propanoic Acid* (Fmoc-β^2hSer(^{t}Bu)-OH; **9**). A soln. of **8** (1 equiv., 2.3 g, 7.53 mmol) and NH_2NH_2 (1 ml) in EtOH (50 ml) was heated under reflux for 30 min (a white precipitate is formed). After cooling to r.t., the solvent was evaporated to give a colorless solid which was washed with CH_2Cl_2 and used directly in the next step without further purification. The residue was dissolved in THF/H_2O 1:1 (4 ml) and cooled to 0°, and Fmoc-OSu (1.2 equiv., 9.036 mmol, 3.08 g) was added, followed by $NaHCO_3$ (10 equiv., 75.3 mmol, 6.26 g). The mixture was stirred overnight at r.t. and concentrated by evaporating THF. The resulting aq. phase was washed with Et_2O, acidified to pH 1 with 1M HCl, and extracted with AcOEt. The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. The crude residue was purified by FC (CH_2Cl_2/MeOH 30:1) to give **9** (2.7 g, 84%). Colorless solid. The anal. data are in agreement with those reported in [33].

*Prop-2-en-1-yl (2*R*)-3-(1,1-Dimethylethoxy)-2-[({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)methyl]propanoate* (Fmoc-β^2hSer(^{t}Bu)-OAll; **10**). To a soln. of $NaHCO_3$ (1 equiv., 0.57 g, 6.8 mmol) and **9** (2.7 g, 6.8 mmol) in 20 ml of H_2O, a soln. of allyl bromide (1.1 equiv., 0.647 ml, 7.48 mmol) and *Aliquat 336* (1 equiv., 3.12 ml, 6.8 mmol) in CH_2Cl_2 was added. The mixture was stirred vigorously at r.t. for 72 h, and then extracted with CH_2Cl_2 (3 × 30 ml); the combined layers were dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by FC (hexane/AcOEt 4:1) to give **10** (2.842 g, 90%). Oil. R_f (hexane/AcOEt 4:1) 0.31. $[\alpha]_D^{20} = +2.8$ ($c = 4.15$, $CHCl_3$). IR ($CHCl_3$): 3330.7*w*, 2973.0*w*, 1724.8*s*, 1648.2*w*, 1589.3*w*, 1515.5*m*, 1477.7*w*, 1449.6*m*, 1392.7*w*, 1363.4*m*, 1329.4*w*, 1246.0*s*, 1181.6*s*, 1091.2*s*, 994.1*m*, 933.7*m*, 875.8*w*, 758.9*s*, 740.7*s*, 621.4*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.16 (*s*, 3 H); 2.83 (*m*, 1 H); 3.47–3.59 (*m*, 2 H); 3.64 (*m*, 2 H); 4.21 (*t*, $J = 7.2$, 1 H); 4.47 (*d*, $J = 7.2$, 1 H); 4.62 (br. *t*, $J = 5.2$, 2 H); 5.23 (*dd*, $J = 1.2, 10.4$, 1 H); 5.33 (*ddd*, $J = 1.2, 2.8, 10.4$, 1 H); 5.40 (br. *t*, NH); 5.91 (*ddt*, $J = 5.6, 10.4, 17.1$, 1 H); 7.3 (*dt*, $J = 0.8, 7.6$, 2 H); 7.39 (br. *t*, $J = 7.6$, 2 H); 7.58 (*d*, $J = 7.6$, 2 H); 7.75 (*d*, $J = 7.2$, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 27.34 (Me); 40.52 (CH_2); 46.10, 47.31 (CH); 61.11, 65.34, 66.72, 118.32 (CH_2); 119.94, 125.08, 127.08, 127.65, 131.98 (CH); 141.33, 144.02, 156.34, 172.41 (C). LC/ESI-MS: 438.1 ($[M+H]^+$, $C_{26}H_{31}NO_5^+$; calc. 437.53). Anal. calc. for $C_{26}H_{31}NO_5$ (437.53): C 71.37, H 7.14, N 3.20; found: C 71.11, H 7.07, N 3.82.

*Prop-2-en-1-yl (2*R*)-3-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-2-(hydroxymethyl)propanoate* (Fmoc-β^2hSer-OAll; **11**). To a soln. of **10** (1 equiv., 2.25 g, 5.15 mmol) in dry CH_2Cl_2 (20 ml), TMSI (1.3 equiv., 0.95 ml, 6.7 mmol) was added *via* a syringe under Ar. The mixture was stirred at r.t., and reaction progress was monitored by TLC. After completion (1 h), the reaction was quenched by addition of MeOH. The residue obtained after evaporation was dissolved in Et_2O (100 ml), the resulting soln. was washed with 1M $NaHSO_3$ (10 ml) and sat. $NaHCO_3$, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by FC (hexane/AcOEt 4:1 → 2:1) to give **11** (1.67 g, 93%). Colorless solid. M.p. 79–81°. R_f (hexane/AcOEt 1:1) 0.31. $[\alpha]_D^{20} = -5.69$ ($c = 1.16$, $CHCl_3$). IR (CH_2Cl_2): 3355.9*w*, 2931.1*w*, 1701.5*s*, 1522.1*m*, 1449.6*m*, 1251.7*s*, 1180.3*m*, 1104.6*m*, 1080.1*m*, 1033.4*m*, 997.2*m*, 934.9*m*, 759.2*s*, 741.2*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.79 (*m*, 1 H); 3.47–3.57 (*m*, 2 H); 3.62–3.88 (*m*, 3 H); 4.21 (*t*, $J = 6.9$, 1 H); 4.44 (*d*, $J = 6.9$, 1 H); 4.63 (*m*, 2 H); 5.33 (*dd*, $J = 1.2, 10.5$, 1 H); 5.44 (br. *t*, 1 H); 5.90 (*ddt*, $J = 5.7, 10.5, 17.1$, 1 H); 7.32 (*dt*, $J = 1.2, 7.5$, 2 H); 7.41 (br. *t*, $J = 7.5$, 2 H); 7.58 (*d*, $J = 7.5$, 2 H); 7.77 (*d*, $J = 7.5$, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 39.22 (CH_2); 47.97, 48.34 (CH); 60.71, 66.22, 67.65, 119.31 (CH_2); 120.69, 125.66, 127.77, 128.43 (C); 131.45 (CH); 132.45, 142.04, 144.49, 158.20, 173.03 (C). LC/ESI-MS: 382.1 ($[M+H]^+$, $C_{22}H_{23}NO_5^+$; calc. 381.16).

*Prop-2-en-1-yl (2*R*)-3-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-2-({[(4-methylphenyl)sulfonyl]oxy}methyl)propanoate* (Fmoc-β^2hSer(Ts)-OAll; **12**). Compound **11** (1 equiv., 450 mg, 1.3 mmol) and TsCl (5 equiv., 1.25 g, 6.5 mmol) were dissolved in pyridine (2 ml), and the soln. was immediately cooled to 0°. The mixture was stirred at 0° under Ar for 12 h. Et_2O (100 ml) was added, and the soln. was washed with H_2O (20 ml), 10% $KHSO_4$ (4 × 20 ml), sat. $NaHCO_3$ (20 ml), brine (20 ml), and again H_2O (2 × 20 ml). After drying ($MgSO_4$), filtering, and evaporating the solvent, the crude product was purified by FC (hexane/AcOEt 2:1) to give **12** (0.855 g, 95%). Yellow oil. R_f (hexane/AcOEt 4:1) 0.11. $[\alpha]_D^{20} = +2.87$ ($c = 5.16$, $CHCl_3$). IR ($CHCl_3$): 3389.7*w*, 2961.7*w*, 1730.1*s*, 1522.1*m*, 1450.4*m*, 1363.9*m*, 1257.5*m*, 1189.9*s*, 1177.1*s*, 1097.0*w*, 977.3*m*, 815.0*w*, 759.9*m*, 742.4*m*, 667.4*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.42 (*s*, 3 H); 2.97 (*m*, $J = 5.6$, 1 H); 3.37–3.59 (*m*, 2 H); 4.18 (*t*, $J = 6.8$, 1 H); 4.21–4.32 (*m*, 2 H); 4.37 (*d*, $J = 6.4$, 1 H); 4.58 (br. *d*, $J = 4.4$, 2 H); 5.09 (br. *t*, 1 H); 5.23 (*dd*, $J = 0.8$, 10.4, 1 H); 5.29 (*dd*, $J = 0.8$, 17.2, 1 H); 5.85 (*ddt*, $J = 6.0$, 11.2, 16.8, 1 H); 7.27–7.34 (*m*, 4 H); 7.40 (*t*, $J = 7.6$, 2 H); 7.56 (*dd*, $J = 2.4$, 7.2, 2 H); 7.77 (*d*, $J = 7.2$, 4 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.64 (Me); 38.95, 44.73 (CH_2); 47.25 (CH); 66.02, 66.84, 67.44, 118.92 (CH_2); 119.99, 125.03, 127.06, 127.75, 127.98, 129.95 (C); 131.45 (CH); 132.49, 141.33, 143.77, 145.18, 156.24, 170.02 (C). LC/ESI-MS: 535.8 ($[M+H]^+$, $C_{29}H_{29}NO_7S^+$; calc. 535.17). Anal. calc. for $C_{29}H_{29}NO_7S$ (535.17): C 65.03, H 5.46, N 2.62; found: C 64.95, H 5.58, N 2.58.

*Prop-2-en-1-yl (2*S*)-3-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-2-({[(4-methoxyphenyl)methyl]selanyl}methyl)propanoate* (Fmoc-β^2hSec(PMB)-OAll; **13**). $(PMBSe)_2$ (1.6 equiv., 0.965 g, 2.4 mmol; prepared from Se powder and *p*-methoxybenzyl chloride according to the procedure of *Gieselman et al.* [18]) was dissolved in a mixture of 20 ml THF/50% H_3PO_2 (1:1.5), and the soln. was heated under reflux and Ar for 1 h. The soln. was allowed to cool to r.t. and extracted under Ar with Et_2O (2 × 20 ml). The combined org. layers were transferred *via* syringe to an Ar-flushed flask containing $MgSO_4$. After drying, the soln. was rapidly filtered, and the solvent was removed under reduced pressure. The selenol thus obtained was dissolved in degassed DMF in a round-bottom flask under Ar, and a degassed aq. 1M soln. of NaOH (1.1 equiv., 1.65 mmol) was added, immediately followed by a soln. of **12** (1 equiv., 0.8 g, 1.5 mmol) in degassed acetone (10 ml). The mixture was stirred under Ar at 0° for 4 h, diluted with AcOEt (50 ml), and washed with sat. NH_4Cl (3 × 10 ml) and brine (3 × 10 ml). The org. layer was dried ($MgSO_4$), filtered, and the solvents were evaporated. The residue was subjected to FC (hexane/AcOEt 9:1 → 5:1) to give **13** (0.63 g, 73%). Colorless solid. M.p. 114–116°. R_f (hexane/AcOEt 3:1) 0.34. $[\alpha]_D^{20} = -1.4$ ($c = 1.56$, $CHCl_3$). IR ($CHCl_3$): 3345.8*w*, 2935.2*w*, 1737.0*m*, 1689.8*s*, 1549.3*m*, 1510.7*m*, 1447.4*w*, 1270.6*m*, 1249.4*m*, 1184.2*w*, 1149.4*w*, 983.4*w*, 824.8*w*, 759.2*m*, 734.6.1*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.65 (*dd*, $J = 6.8$, 12.8, 1 H); 2.74 (*dd*, $J = 6.8$, 12.8, 1 H); 2.88 (*m*, $J = 6.8$, 1 H); 3.47 (*m*, $J = 7.2$, 2 H); 3.78 (*s*, 5 H); 4.23 (*t*, $J = 6.8$, 1 H); 4.42 (*ddd*, $J = 6.8$, 7.6, 17.6, 1 H); 4.64 (*d*, $J = 5.6$, 2 H); 5.04 (br. *t*, 1 H); 5.27 (br. *t*, $J = 6.0$, NH); 5.28 (*dd*, $J = 0.8$, 10.4, 1 H); 5.35 (*ddd*, $J = 1.2$, 2.8, 17.2, 1 H); 5.94 (*ddt*, $J = 5.6$, 11.2, 16.4, 1 H); 6.79 (*d*, $J = 8.4$, 2 H); 7.19 (br. *d*, $J = 8.8$, 2 H); 7.34 (*t*, $J = 7.6$, 2 H); 7.43 (*t*, $J = 7.6$, 2 H); 7.60 (*d*, $J = 7.6$, 2 H); 7.79 (*d*, $J = 7.6$, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.92, 27.38, 42.20 (CH_2); 46.05 (CH); 47.28 (CH); 55.28 (Me); 65.69, 66.75 (CH_2); 114.00, 118.71, 120.00, 125.03, 127.06, 127.72, 130.03 (CH); 130.70 (C); 131.80 (CH); 141.33, 143.88, 156.28, 158.54, 172.90 (C). HR-ESI-MS: 566.144 ($[M+H]^+$, $C_{30}H_{31}NO_5Se^+$; calc. 565.144). Anal. calc. for $C_{30}H_{31}NO_5Se$ (565.144): C 63.83, H 5.53, N 2.48; found: C 63.56, H 5.56, N 2.48.

*(2*S*)-3-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-2-({[(4-methoxyphenyl)methyl]selanyl}methyl)propanoic Acid* (Fmoc-β^2hSec(PMB)-OH; **14**). To a soln. of **13** (1 equiv., 150 mg, 0.265 mmol) and $[(Ph_3P)_4]Pd$ (0.1 equiv., 30 mg, 0.026 mmol) in anh. THF, a soln. of morpholine (1.05 equiv., 24.3 µl, 0.278 mmol) in 10 ml of THF was added dropwise over a period of 45 min. The mixture was stirred under Ar at r.t. for an additional 30 min. Then, the mixture was diluted with 50 ml of AcOEt and washed with 1M HCl (3 × 10 ml). The combined org. layers were dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by FC (first CH_2Cl_2/MeOH 50:1, then CH_2Cl_2/0.1% AcOH in MeOH 40:1) to yield **14** (135 mg, 96%). Colorless solid. M.p. 156–158°. R_f (CH_2Cl_2/MeOH 15:1) 0.28. $[\alpha]_D^{20} = -8.5$ ($c = 0.90$, DMF). IR ($CHCl_3$): 3337.4*w*, 1691.5*s*, 1551.6*m*, 1510.3*s*, 1449.8*w*, 1270.9*m*, 1247.9*m*, 1033.3*w*, 759.5*m*, 741.1*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 2.56 (*m*, 2 H); 2.83 (*m*, 1 H); 3.44 (*m*, 2 H); 3.74 (*s*, 3 H); 3.76 (*s*, 2 H); 4.21 (*t*, $J = 6.6$, 1 H); 4.33 (*m*, 2 H); 5.04 (br. *t*, 1 H); 6.79 (*d*, $J = 8.7$, 2 H); 7.19 (*d*, $J = 8.7$, 2 H); 7.31 (*t*, $J = 7.5$, 2 H); 7.39 (*t*, $J = 7.5$, 2 H); 7.58 (*d*, $J = 7.5$, 2 H); 7.76 (*d*, $J = 7.5$, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.63 (CH_2); 27.51 (CH_2); 41.92 (CH_2); 45.81 (CH); 47.26 (CH); 55.28

(CH_3); 66.83 (CH_2); 114.04, 120.01, 125.05, 127.08, 127.7, 130.02 (CH); 130.60, 134.7, 141.34, 143.81, 158.57, 174.3 (C). HR-ESI-MS: 526.1127 ($[M+H]^+$, $C_{27}H_{27}NO_5Se^+$; calc. 525.11). Anal. calc. for $C_{27}H_{27}NO_5Se$ (525.11): C 61.83, H 5.19, N 2.67; found: C 61.55, H 5.25, N 2.68.

*Prop-2-en-1-yl (2*R*)-3-{[(1,1-Dimethylethoxy)carbonyl]amino}-2-(hydroxymethyl)propanoate* (Boc-β^2hSer-OAll; **15**). To a soln. of **11** (1 equiv., 381 mg, 1 mmol) in DMF (3 ml) under Ar, KF (7 equiv., 0.4 g, 7 mmol) was added, followed by Et_3N (2 equiv., 270 µl, 2 mmol). To the mixture stirred at r.t., Boc_2O (1.3 equiv., 284 mg, 1.3 mmol) was added. After stirring overnight (reaction completed), the mixture was diluted with AcOEt, and washed with H_2O, sat. NH_4Cl, sat. $NaHCO_3$, and brine. The org. layer was dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by FC (hexane/AcOEt 4:1 → 2:1) to give **15** (230 mg, 89%). Oil. $[\alpha]_D^{20} = -18.62$ ($c = 1.08$, $CHCl_3$). IR ($CHCl_3$): 3371.9*w*, 2976.2*w*, 1714.2*s*, 1517.9*m*, 1450.0*m*, 1391.7*m*, 1366.7*m*, 1251.6*s*, 1170.7*m*, 1037.6*m*, 997.2*m*, 940.7*m*, 760.1*m*, 733.6*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.41 (*s*, 9 H); 2.73 (*quint.*, $J = 5.2$, 1 H); 3.42 (*dt*, $J = 5.6$, 14.4, 1 H); 3.50–3.60 (*m*, 1 H); 3.73 (*dd*, $J = 7.2$, 11.6, 2 H); 3.85 (*dd*, $J = 4.8$, 11.6, 1 H); 4.58 (*d*, $J = 5.6$, 2 H); 5.18 (br. *t*, NH); 5.22 (*d*, $J = 10.4$, 1 H); 5.29 (*d*, $J = 17.2$, 1 H); 5.88 (*ddt*, $J = 5.6$, 10.4, 17.2, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 28.29 (Me); 37.79 (CH_2); 47.7 (CH); 59.76, 65.40 (CH_2); 80.01 (C); 118.43 (CH_2); 131.80 (CH); 157.25, 172.39 (C). HR-ESI-MS: 282.13 ($[M+Na]^+$, $C_{12}H_{21}NNaO_5^+$; calc. 282.131).

*Prop-2-en-1-yl (2*R*)-3-{[(1,1-Dimethylethoxy)carbonyl]amino}-2-({[(4-methylphenyl)sulfonyl]oxy}-methyl)propanoate* (Boc-β^2hSer(Ts)-OAll; **16**). Compound **15** (1 equiv., 210 mg, 0.81 mmol) and TsCl (5 equiv., 840 mg, 4.05 mmol) were dissolved in pyridine (1.5 ml), and the soln. was immediately cooled to 0°. The mixture was stirred at 0° under Ar for 12 h. Et_2O (50 ml) was added, and the soln. was washed with H_2O (20 ml), 10% $KHSO_4$ (4 × 20 ml), sat. $NaHCO_3$ (20 ml), brine (20 ml) and again H_2O (2 × 20 ml). After drying ($MgSO_4$), filtering, and evaporating the solvent, the crude product was purified by FC (hexane/AcOEt 2:1) to give **16** (288 mg, 86%). Yellow oil. R_f (hexane/AcOEt 2:1) 0.48. $[\alpha]_D^{20} = +1.07$ ($c = 2.8$, $CHCl_3$). IR ($CHCl_3$): 3387.5*w*, 2978.1*w*, 1713.1*s*, 1597.9*w*, 1512.5*m*, 1454.7*m*, 1365.5*s*, 1251.2*m*, 1189.2*s*, 1175.4*s*, 1097.0*w*, 974.5*m*, 939.3*m*, 814.9*m*, 755.2*w*, 665.9*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.41 (*s*, 9 H); 2.45 (*s*, 3 H); 2.96 (*quint.*, $J = 5.6$, 1 H); 3.30–3.50 (*m*, 2 H); 4.26 (*dd*, $J = 2.0$, 5.2, 2 H); 4.53–4.57 (*m*, 2 H); 4.89 (br. *t*, NH); 5.22 (*ddd*, $J = 1.2$, 2.8, 10.4, 1 H); 5.28 (*ddd*, $J = 1.6$, 2.8, 17.2, 1 H); 5.85 (*ddt*, $J = 5.6$, 10.4, 17.2, 1 H); 7.35 (*dd*, $J = 0.8$, 8.8, 2 H); 7.77 (*d*, $J = 8.4$, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.67 (CH_2); 28.30 (Me); 38.61 (CH_2); 44.99 (CH); 65.90 (CH_2); 67.67 (CH_2); 79.70 (C); 118.72 (CH_2); 127.04, 127.99, 129.96 (CH); 130.25 (C); 131.53 (CH); 132.43, 145.14, 155.75, 170.27 (C). HR-ESI-MS: 414.15 ($[M+H]^+$), 436.14 ($[M+Na]^+$, $C_{19}H_{27}NO_7S$; calc. 413.1508).

*Prop-2-en-1-yl (2*S*)-3-{[(1,1-Dimethylethoxy)carbonyl]amino}-2-({[(4-methoxyphenyl)methyl]selanyl}methyl)propanoate* (Boc-β^2hSec(PMB)-OAll; **17**). $(PMBSe)_2$ (1.6 equiv., 0.44 g, 1.1 mmol; prepared as described above) was dissolved in a mixture of 10 ml THF/50% H_3PO_2 (1:1.5), and the soln. was heated under reflux and Ar for 1 h. The soln. was allowed to cool to r.t. and extracted under Ar with Et_2O (2 × 20 ml). The combined org. layers were transferred *via* syringe to an Ar-flushed flask containing $MgSO_4$. After drying, the soln. was rapidly filtered, and the solvent was removed under reduced pressure. The selenol thus obtained was dissolved in degassed DMF in a round-bottom flask under Ar, and a degassed 1M aq. soln. of NaOH (1.1 equiv., 0.76 ml, 0.76 mmol) was added, immediately followed by a soln. of **16** (1 equiv., 0.28 g, 0.69 mmol) in degassed acetone (5 ml). The mixture was stirred under Ar at 0° for 4 h, diluted with AcOEt (30 ml), and washed with sat. NH_4Cl (3 × 10 ml) and brine (3 × 10 ml). The combined org. layers were dried ($MgSO_4$), filtered, and the solvents were evaporated. The residue was subjected to FC (hexane/AcOEt 9:1 → 5:1) to give, besides the elimination product (50 mg, 0.21 mmol), **17** (crystallizes upon standing, 194 mg, 65%). Yellow solid. M.p. 118–121°. R_f (hexane/AcOEt 5:1) of **17** 0.25, of elimination product 0.37. $[\alpha]_D^{20} = -4.58$ ($c = 3.2$, $CHCl_3$). IR ($CHCl_3$): 3375.0*w*, 2976.6*w*, 2932.6*w*, 1713.6*s*, 1609.1*w*, 1583.1*w*, 1510.5*s*, 1454.5*w*, 1390.0*w*, 1365.9*m*, 1300.6*m*, 1246.8*s*, 1173.1*s*, 1098.1*w*, 1034.0*m*, 936.6*w*, 831.1*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.41 (*s*, 9 H); 2.61 (*dd*, $J = 6.4$, 11.6, 1 H); 2.72 (*dd*, $J = 6.8$, 12.8, 1 H); 2.85 (*m*, 1 H); 3.29–3.44 (*m*, 2 H); 3.75 (*s*, 2 H); 3.78 (*s*, 3 H); 4.61 (br. *d*, $J = 5.6$, 1 H); 4.67 (*d*, $J = 5.6$, 1 H); 4.88 (br. *t*, NH); 5.25 (br. *dd*, $J = 1.4$, 10.4, 1 H); 5.33 (*ddd*, $J = 0.4$, 1.6, 7.2, 1 H); 5.86–6.00 (*m*, 1 H); 6.82 (*d*, $J = 8.4$, 2 H); 7.20 (*d*, $J = 8.8$, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.98 (CH_2); 27.30 (CH_2); 28.38 (Me); 31.60 (CH_2); 41.97 (CH_2); 46.37 (CH); 55.25 (CH_3); 65.55

(CH_2); 79.43 (C); 113.96 (CH); 118.48 (CH_2); 130.00 (CH); 130.14, 130.29, 130.81, 131.92 (CH); 155.75 (C); 158.49 (C); 173.02 (C). HR-ESI-MS: 444.1286 ($[M+H]^+$, $C_{20}H_{29}NO_5Se^+$; calc: 443.1211).

(2S)-3-{[(1,1-Dimethylethoxy)carbonyl]amino}-2-({[(4-methoxyphenyl)methyl]selanyl}methyl)propanoic Acid (Boc-β^2hSec(PMB)-OH; **18**). To a soln. of **17** (1 equiv., 150 mg, 0.338 mmol) and $[(Ph_3P)_4]Pd$ (0.1 equiv., 33.8 mg, 0.034 mmol) in anh. THF, a soln. of morpholine (1.1 equiv., 32.5 µl, 0.37 mmol) in 10 ml THF was added dropwise over a period of 45 min. The mixture was stirred under Ar at r.t. for an additional 30 min. Then, the mixture was diluted with 50 ml of AcOEt and washed with sat. NH_4Cl (3×10 ml). The combined org. layers were dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by FC (first CH_2Cl_2/MeOH 50:1, then CH_2Cl_2/MeOH/AcOH 50:1:0.1) to yield **18** (50 mg, 37%). Colorless solid. M.p. 99–101°. $[\alpha]_D^{20}=-5.71$ ($c=0.49$, $CHCl_3$). R_f (CH_2Cl_2/MeOH, 15:1) 0.4. IR ($CHCl_3$): 2925.4*m*, 2854.2*w*, 1706.0*s*, 1609.3*m*, 1583.2*w*, 1510.3*s*, 1456.3*m*, 1392.7*w*, 1366.6*m*, 1299.7*w*, 1246.5*s*, 1171.1*s*, 1098.3*w*, 1034.3*m*, 949.3*m*, 829.1*m*, 780.2*w*, 740.0*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.46 (*s*, 3 H); 2.65 (*m*, 1 H); 2.78 (*m*, 1 H); 2.83 (*m*, 1 H); 3.44 (*m*, 2 H); 3.79 (*s*, 2 H); 3.81 (*s*, 3 H); 4.91 (br. *t*, NH); 6.85 (*d*, $J=8.4$, 2 H); 7.23 (*d*, $J=8.4$, 2 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.72 (CH_2); 27.48 (CH_2); 28.38 (Me); 41.78 (CH_2); 46.24 (CH); 55.29 (CH_3); 79.82 (C); 114.03 (CH); 130.00 (CH); 130.75 (C); 155.94 (C); 158.53 (C); 178.13 (C). HR-MALDI-MS: 404.1 ($[M+H]^+$), 402.08 ($[M-H]^+$), 426.1 ($[M+Na]^+$, $C_{17}H_{25}NO_5Se^+$, calc. 403.1).

H-β^2hSec(PMB)-β^3hAla-β^3hLeu-β^3hIle-OH (**19**). The 2-chlorotrityl chloride resin (1 g, 1.1 mmol) was derivatized with Fmoc-β^3hIle-OH (55.05 mg, 0.15 mmol) according to *GP 1* to give a loading of 0.13 mmol/g (87%). The unreacted Cl groups were capped with a soln. of CH_2Cl_2/MeOH/DIEA 17:2:1.3; 10 ml). Then, the resin was divided into two portions. SPPS using 600 mg of the resin (0.078 mmol) was performed according to *GP 2* by sequential incorporation of Fmoc-β^3hLeu-OH and Fmoc-β^3hAla-OH. Fmoc-β^2hSec(PMB)-OH (**14**; 2 equiv., 81.7 mg, 0.156 mmol), pre-activated with CDI (2 equiv., 24.2 µl, 0.156 mmol) and HOBt (2 equiv., 21.08 mg, 0.156 mmol) in CH_2Cl_2 for 1 h in an ice bath, was then added to the resin pre-swelled in DMF. The reaction was agitated for 4 h at r.t. The resin was then filtered and washed with DMF and CH_2Cl_2 (4 ml, 4×1 min), and dried under h.v. overnight. Final Fmoc deprotection was performed with 20% piperidine in DMF (3×3 min). Cleavage of the fully assembled peptide from the resin was achieved according to *GP 3*. Purification by RP-HPLC (5–95% B in 50 min: 40 min 60% *B*; 50 min 95% *B*; t_R 40.2 min) yielded **19** (46 mg, 92%) as a colorless fluffy solid. Anal. RP-HPLC (3–97% *B* in 30 min; *C18*): t_R 18.95. HR-MALDI-MS: 643.3 ($[M+H]^+$, $C_{30}H_{51}N_4O_6Se^+$; calc. 642.7012).

H-β^2hSec(H-β^2hSec-β^3hAla-β^3hLeu-β^3hIle-OH)-β^3hAla-β^3hLeu-β^3hIle-OH (**20**). Compound **19** (13 mg, 0.02 mmol) was dissolved in 5 ml of AcOH/H_2O 8:1, and I_2 (10 equiv., 51.7 mg, 0.2 mmol, dissolved in 0.5 ml MeOH) was added. After stirring for 1 h, 0.1M soln. of ascorbic acid was added to quench excess I_2 (the brownish color disappeared), and the mixture was lyophilized to reduce the volume. The crude product thus obtained was dried under h.v. Purification by RP-HPLC (5–95% *B* in 50 min: 40 min 60% *B*; 50 min 95% *B*; t_R 36.8 min) yielded the TFA salt of diselenide **20** (10.2 mg, 85%). Anal. RP-HPLC (3–97% B in 20 min): t_R 14.22. HR-MALDI-MS: 523.4 ($[0.5\,M+H]^+$), 1041.3 ($[M+H]^+$, $C_{44}H_{82}N_8O_{10}Se_2^+$; calc.1041.0895).

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