Synthesis of New Photoactivatable Phenylalanine Analogues and Their Incorporation into a Model Peptide − Phenylseleno Derivatives as Precursors of α,β-Unsaturated Ketones in Peptide Synthesis

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The asymmetric synthesis of (*S*)-Boc-*p*-(propanoyl)phenylalanine (**10**) was performed by alkylation of sultam *N*-(diphenylmethylene)glycinate (**4**). Different pathways for the introduction of a phenylseleno moiety α to the ketone function were investigated. (*S*)-Boc-*p*-[2′-(phenylselenenyl)propanoyl]phenylalanine (**11**) was easily obtained from (*S*)-Boc-*p*-(propanoyl)phenylalanine (**10**). However, the phenylseleno moiety α to the carbonyl group was found to be unstable to the conditions required for solid-phase peptide synthesis. Therefore, (2*S*)-Boc-*p*-[2′-(phenylselenenyl)propanoyl]phenylalanine (**11**) was transformed into Boc-*p*-[3′-(phenylselenenyl)propanoyl]phenylalanine (**13**), which was successfully incorporated into the sequence of a model peptide. The corresponding enone function generated under mild acidic conditions was found to be stable in aqueous solution at pH = 7, but suitably reactive upon irradiation. Thus, these phenylalanine analogs bearing an unsaturated ketone represent new photoreactive probes.

Introduction

Photoaffinity labeling is one of the most powerful techniques for receptor binding site mapping.[1−5] However, generalization of this methodology for the analysis of peptide/protein interactions is hampered by various difficulties. Firstly, the precursor containing the intended photoreactive species must be stable during peptide synthesis and should not disturb the recognition step between the peptide and its receptor. Secondly, the activated species generated by UV irradiation must be highly reactive, except with water molecules, sufficiently stable regarding eventual rearrangement or elimination reactions, and also marginally selective towards the different amino acid side chains that might be present in the binding site. The covalent bond resulting from the photolabeling must also be stable towards proteolysis or chemical degradation, and − finally − high cross-linking yields are required for development of this strategy with picomole amounts of receptor. Aryl azides, aryldiazirines, α-diazo-carbonyls, diazonium salts, enones, and benzophenones are the chemical functions that are classically activated under irradiation conditions, giving rise to nitrenes, carbenes, carbocations, and radicals.[6,7] All these probes have more or less the required properties for photoaffinity labeling. Four probes (aryl azide, aryldiazirine, α-diazocarbonyl, and benzophenone) have been incorporated into the same pentapeptide, thymopentin TP5, in order to compare their cross-linking properties in 1-propanol/water mixtures under identical irradiation conditions.[8] Carbenes reacted with the same efficiency with 1-propanol and with water, whereas nitrenes, less reactive towards water, afforded rearrangement products. In this comparative study, *p*-benzoylphenylalanine appeared to be the better photoprobe. The diradical triplet state of benzophenone abstracts a hydrogen either from a C−H bond to produce a stable diarylcarbinol or from an X−H bond (X = N, O, or S) to afford unstable (reversible) species.[9] The benzophenone moiety is generally introduced into a peptide as *p*-benzoylphenylalanine, although this hindered amino acid may hamper access of the biologically active peptide to its receptor.

In order to develop new photoprobes, we were interested in substitution of the *p*-benzoyl group attached to the aromatic nucleus with either a *p*-propanoyl or a *p*-propenoyl group. We expected that such substituents should give a

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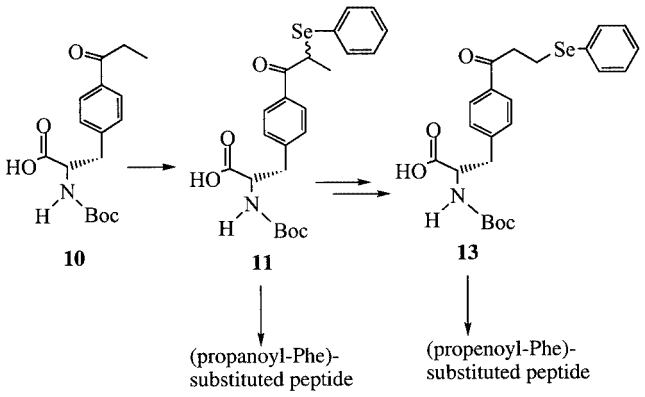
better probe than *p*-benzoylphenylalanine, at least with sterically hindered binding pockets.

UV irradiation of the *p*-propenoyl group, must generate a 1,4-diradical that must provide stable cross-linking. Furthermore, this enone group can also achieve cross-linking as a Michael acceptor. Phenyl alkyl ketone and enone moieties have been used as photoprobes in only a few cases. Acetophenone derivatives of guanosine-5′-triphosphate peptides for photoaffinity labeling of GTP-binding proteins and human serum albumin have been reported, and steroids bearing an enone function have been developed for photoaffinity labeling of sex hormone binding globulin.[12,13]

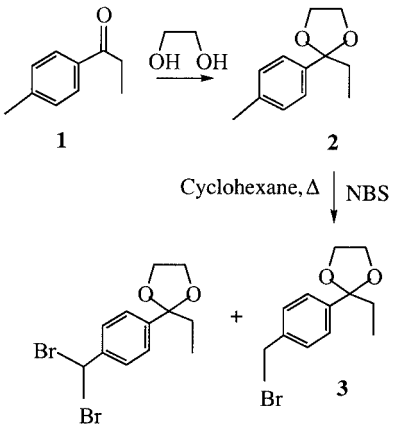
In this study, we report the syntheses of three *N*-protected phenylalanine derivatives: (*S*)-*p*-(propanoyl)phenylalanine (**10**), (*S*)-Boc-*p*-[2′-(phenylselenenyl)propanoyl]phenylalanine (**11**), and (*S*)-Boc-*p*-[3′-(phenylselenenyl)propanoyl]-phenylalanine (**13**) (Scheme 1). The protected amino acids **11** and **13** were introduced into the sequence of the model pentapeptide thymopentin TP5 (*N*-acetylated, *C*-terminal carboxamide), which, after HF cleavage, yielded peptides bearing either a *p*-propanoyl or a *p*-propenoyl group on the aromatic residue. We established that only the β-phenylseleno carbonyl derivative was usable as a precursor of an enone function in solid-phase peptide synthesis, the generation of this enone function being achievable under mild conditions. Finally, we demonstrated that this enone function, when incorporated into a peptide sequence, was stable in aqueous solution at pH = 7, but suitably reactive upon irradiation.



Scheme 1. Structures of the precursors **10**, **11**, and **13** of photoactivatable analogues of phenylalanine

Results

We have previously reported the synthesis of unnatural amino acids starting fromsultam *N*-(diphenylmethylene)glycinate.[14] Strong asymmetrical induction was observed with Oppolzer's sultam,[15,16] the levorotatory enantiomer of the sultam yielding the *S* configuration at the α-carbon for the monoalkylated amino acids. The difficulties encountered in the preparation of unnatural amino acids depend on the nature of the side chain, incorporated as an electrophile in the alkylation step, and are especially pronounced when the electrophile possesses an acidic proton, which imposes additional protection/deprotection steps. In this work, all these syntheses required the preparation of the same electrophile **3** (Scheme 2).



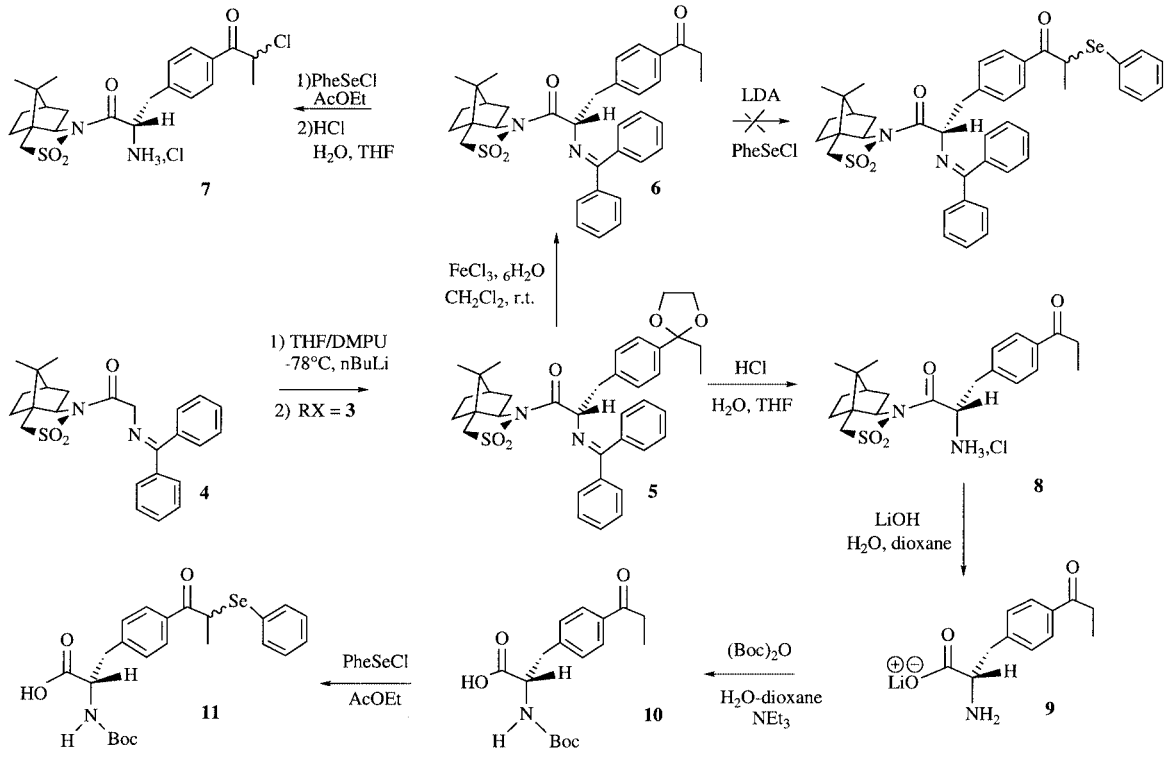
Scheme 2. Synthesis of 2-[4-(bromomethyl)phenyl]-2-ethyl-1,3-dioxolane (**3**)

Synthesis of 2-[*p*-(Bromomethyl)phenyl]-2-ethyl-1,3-dioxolane (3)

The electrophilic reagent **3** was prepared from *p*-methylpropiophenone as starting material, after protection of the ketone as a cyclic acetal (Scheme 2). Bromination under classical conditions (N-bromosuccinimide/dibenzoyl peroxide in carbon tetrachloride) gave a complex mixture of starting material **2**, ketone **1**, and the expected monobrominated product **3** contaminated with the dibrominated product, with brominated forms of ketone **1** also being identified. When this reaction was performed in cyclohexane the formation of ketone was prevented, and after optimization a mixture consisting of **3** (75%), dibrominated dioxolane (15%), and 10% of starting material **2** was obtained (NMR analysis of the crude mixture). After flash chromatography and crystallization, 2-[*p*-(bromomethyl)phenyl]-2-ethyl-1,3-dioxolane (**3**) was isolated in moderate yields (47%), but this yield surprisingly dropped to 25% when the reaction was tentatively scaled up to more than 5 mmol.

Synthesis of Boc-*p*-(Propanoyl)phenylalanine (10)

Alkylation of *N*-(diphenylmethylene)glycinate **4**[14] with 2-[*p*-(bromomethyl)phenyl]-2-ethyl-1,3-dioxolane **3** was performed after deprotonation by lithium diisopropylamide (LDA) in a THF/DMPU mixture, resulting in compound **5** (Scheme 3). NMR analysis showed that the alkylation was highly diastereoselective (*de* > 95%). Acidic hydrolysis of compound **5** afforded **8**, with a free amino group and a ketone, while removal of the sultam moiety under basic conditions yielded the free amino acid **9**, which was then Boc-protected with $(Boc)_2O$ (Scheme 3). Boc-*p*-(propanoyl)-phenylalanine **10** was obtained in an overall yield of 60%.



Scheme 3. Syntheses of Boc-*p*-(propanoyl)phenylalanine (**10**) and Boc-*p*-[2′-(phenylselenenyl)propanoyl]phenylalanine (**11**)

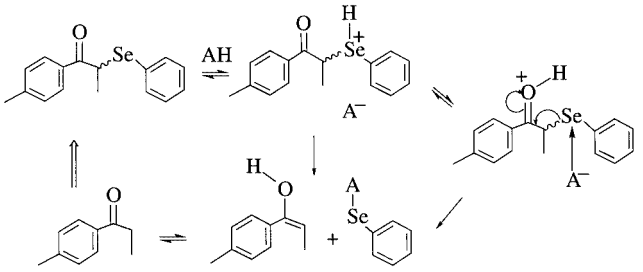
Synthesis of Boc-*p*-[2′-(Phenylselenenyl)propanoyl]-phenylalanine (11)

Different pathways for the introduction of the phenylseleno group were investigated (Scheme 3). Starting from derivative **5**, selective removal of the 1,3-dioxolane was performed by treatment with iron chloride hexahydrate in dichloromethane for 30 minutes.[17] After flash chromatography, compound **6** was obtained in 73% yield. The enolate of **6** was generated at low temperature (LDA/THF, −78°) and phenylselenenyl chloride was added,[18] but instead of the expected selenation product, only aldol condensation products were observed. Selenation of compound **6** under nonbasic conditions (EtOAc/PheSeCl) was examined,[19] but instead of the selenated product, compound **7** was isolated. This compound was the result of an unexpected halogenation in the position α to propiophenone, as demonstrated by mass spectrometry, and consisted of a mixture of two diastereoisomers in a 1:1 ratio (NMR analysis). We suspected that steric hindrance of the sultam and/or *N*-diphenylmethylene moieties was the origin of this opposite regioselectivity, since phenylselenyl chloride reacted as expected with Boc-*p*-(propanoyl)phenylalanine (**10**) under these conditions. The selenation product (*S*)-Boc-*p*-[2′-(phenylselenenyl)propanoyl]phenylalanine (**11**) was isolated in 56% yield after column chromatography, with (*S*)-Boc-*p*-(2′-chloropropanoyl)phenylalanine being only the major by-product. This reaction was also performed under acidic conditions (acetic acid) with similar efficiency. Altogether, these results confirmed that regioselective attack by phenylselenyl chloride depends strongly on steric effects.

Deselenation Reaction

(*S*)-Boc-*p*-[2-(phenylselenenyl)propanoyl]phenylalanine (**11**) was incorporated into position 5 of the pentapeptide thymopentin, TP5 = Ac-Arg[1]-Lys[2]-Asp[3]-Val[4]-Phe[5]-$CONH_2$ (see peptide synthesis). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) of the purified peptide indicated substitution of the phenylseleno group by one hydrogen. We therefore looked for the step at the origin of this deselenation reaction, with α-phenylselenenyl-3′-methylpropiophenone as a model compound. At room temperature, the loss of a phenylseleno group was found to be very slow under the conditions required for Boc deprotection (trifluoroacetic acid). The rate of this reaction was, however, faster (50% of phenylseleno removal) in HF (−5 °C to 0 °C for 60 minutes). Furthermore, in the presence of scavenger mixtures (dimethylsulfide and anisole in HF), conditions required for the cleavage of the peptide from the resin, complete loss of the phenylseleno group was observed. These data suggested that nucleophilic species such as the fluoride or trifluoroacetate ion or dimethyl sulfide may attack the selenium, resulting in the cleavage of the selenium−carbon bond (Scheme 4). Formation of the stabilized enol of the ketone should be the driving force for this elimination. Phenylseleno removal has also been observed during the conversion of selenoesters to methyl ketones after addition of diazomethane followed by workup with aqueous HBr.[20]

We postulated that shifting the phenylseleneno group to the β-position of the ketone function should provide a compound stable under acidic conditions. Indeed, the model

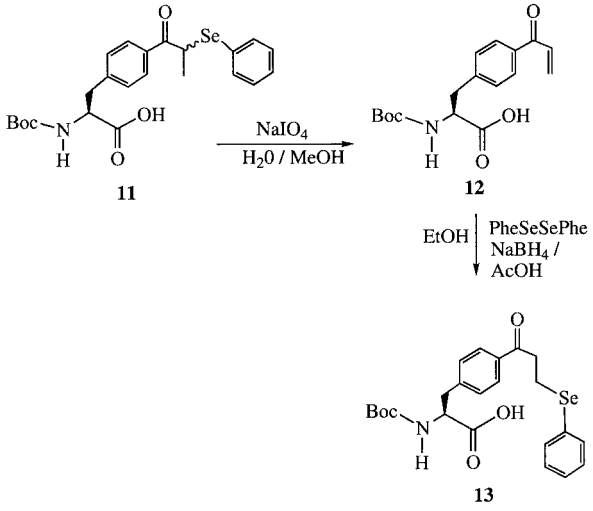


Scheme 4. Mechanism proposed for phenylselenyl substitution

compound β-(phenylselenenyl)-4′-methylpropiophenone was found to be stable under the different conditions listed above for peptide synthesis by a Boc strategy. However, β-(phenylselenenyl)-4′-methylpropiophenone was found to be unstable to piperidine treatment, precluding a Fmoc strategy for peptide synthesis. These results support the postulated mechanism.

Synthesis of Boc-*p*-[3′-(phenylselenenyl)propanoyl]-phenylalanine (13)

Alkylphenyl selenoxides undergo facile *syn* elimination to form olefins,[21] this strategy having frequently been applied in total synthesis for introduction of unsaturation.[22] The one-pot strategy involves oxidation of the α-phenylselenocarbonyl compound to the corresponding selenoxide, which eliminates at room temperature to afford the desired enone. (*S*)-Boc-*p*-(propenoyl)phenylalanine **12** was cleanly obtained by treatment of (*S*)-Boc-*p*-[2-(phenylselenenyl)propanoyl]phenylalanine **11** with two equivalents of sodium periodate in MeOH/H_2O. The benzeneselenol generated by action of sodium borohydride on diphenyldiselenide in the presence of acetic acid[23] reacted by a 1,4-Michael addition to afford (*S*)-Boc-*p*-[3′-(phenylselenenyl)propanoyl]phenylalanine **13** (Scheme 5).



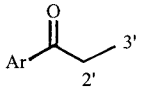
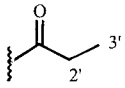
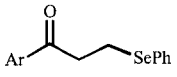
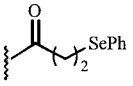
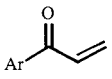
Scheme 5. Synthesis of Boc-*p*-[3′-(phenylselenenyl)propanoyl]phenylalanine (**13**)

Syntheses of Peptides I, II, and III

(*S*)-Boc-*p*-[2-(phenylselenenyl)propanoyl]phenylalanine (**11**) and (*S*)-Boc-*p*-[3′-(phenylselenenyl)propanoyl]phenylalanine (**13**) were incorporated into position 5 of the pentapeptide thymopentin (TP5 = Ac-Arg^1-Lys^2-Asp^3-Val^4-Phe^5-$CONH_2$).[7] This short peptide was used to test the stability of the functions present in **11** and **13** towards the conditions required for solid-phase peptide synthesis. The peptide syntheses were carried out with *p*-methylbenzhydrylamine resin, by a Boc protecting group strategy. *N*-α-Boc-amino acids in tenfold excess (except for **11** and **13**) were used, with dicyclohexylcarbodiimide/1-hydroxybenzotriazole as coupling agents. After the peptide resins had been treated with anhydrous HF, in the presence of scavengers (anisole and dimethylsulfide), at −5 °C to 0 °C for 60 minutes, the HF was evaporated and the peptide was extracted with 10% acetic acid. The lyophilized peptides were purified by preparative reversed-phase HPLC, and characterized by mass spectrometry and 2D NMR analyses. (*S*)-Boc-*p*-[2-(phenylselenenyl)propanoyl]phenylalanine (**11**) afforded a unique peptide **I**, whereas the incorporation of (*S*)-Boc-*p*-[3′-(phenylselenenyl)propanoyl]phenylalanine (**13**) resulted in a mixture of two peptides **II/III**. The proton resonance assignments of peptides **I**, **II**, and **III** (H_2O) were accomplished in a standard manner, by means of COSY and TOCSY experiments.[24] ^{1}H chemical shift assignments are shown in Table 1; the chemical shift values of the Hα protons were typical of unstructured peptides.[25] According to MALDI-TOF and NMR spectra, the phenyl ring of peptide **I** (*m*/*z* = 761.58), obtained from (*S*)-Boc-*p*-[2-(phenylselenenyl)propanoyl]phenylalanine **11**, bore an ethyl ketone function *para* to the aromatic ring [δ(H2′) = 3.0, δ(H3′) = 1.05]. Peptide **I** corresponds to [(*p*-propanoyl)Phe^5]TP5, a new type of photoreactive analogue of thymopentin.

The MALDI-TOF spectrum of the crude product obtained from (*S*)-Boc-*p*-[3′-(phenylselenenyl)propanoyl]-phenylalanine (**13**) featured two ions at *m*/*z* = 917.36 and *m*/*z* = 759.44. The isotopic distribution observed around *m*/*z* = 917.36 denoted the presence of selenium-containing peptide. After purification, the peptides corresponding to these ions (peptides **II** and **III**) were analyzed by NMR and MALDI-TOF. The chemical shifts of peptide **II** (*m*/*z* = 917.36), with δ(2′-H) = 3.2, δ(3′-H) = 3.36, indicated the presence of a phenylselenenyl group in the 3′-position. In aqueous solution at room temperature, peptide **II**, {[*p*-3′-(phenylselenenyl)propanoyl]Phe^5}TP5, rapidly lost its phenylseleno group, resulting in peptide **III** (*m*/*z* = 759.44). The presence of peptide **III** in the crude product of peptide **II** must result from oxidation and subsequent elimination of the phenylselenenyl group from peptide **II** during the workup (extraction of the peptide by 10% acetic acid). Indeed, oxidation of the peptide **II/III** mixture by $NaIO_4$ for 10 minutes, or air oxidation for 24 hours, exclusively provided peptide **III** [(*p*-propenoyl)Phe^5]TP5. The enone function in the *para* position of the aromatic ring of phenylalanine was characterized by NMR spectroscopy, especially

Table 1. Chemical shifts of peptides I, II, and III in ^2H_2O at 298 K

Residue	Peptide	α-H	β-H	γ-H	Other	
Ac	**I, II, III**				(Me) 1.94	
Arg	**I**	4.14	1.70/1.55	1.65	δ 3.08	
	II	4.19	1.11/1.58	1.68	δ 3.08	
	III	4.19	1.75/1.57	1.65	δ 3.08	
Lys	**I**	4.20	1.66	1.30	δ 1.65	ε 2.90
	II	4.27	1.68	1.35	δ 1.66	ε 2.90
	III	4.26	1.65		δ 1.65	ε 2.91
Asp	**I**	4.50	2.60			
	II	4.49	2.54			
	III	4.49	2.52			
Val	**I**	3.89	1.85	0.62		
	II	3.92	1.86	0.62		
	III	3.93	1.87	0.64		
Phe-*p*-X	**I**	4.62	3.20/2.95		(o) 7.85	(m) 7.36
	II	4.61	3.20/2.95		(o) 7.86	(m) 7.39
	III	4.63	3.20/2.95		(o) 7.74	(m) 7.31
	**I**					(2') 3.0 (3') 1.05
	**II**					(2') 3.2 (3') 3.36 (*o*)7.45, (*m*, *p*) 7.25
					(Se-Ph)	
	**III**					(2') 7.19 (3'-*cis*) 6.02 (3'-*trans*) 6.32

by three resonances at δ(2′-H) = 7.19, δ(3′*trans*-H) = 6.32, and δ(3′*cis*-H) = 6.02.

Photolysis of [(*p*-Propenoyl)Phe^5]TP5

The stability of [(*p*-propenoyl)Phe^5]TP5 was followed by recording HPLC and 2D NMR spectra over 48 hours, since the amino group of the Lys moiety in the TP5 sequence could give rise to 1,4-addition on the enone, which is an efficient Michael acceptor. At pH = 7, no 1,4-Michael addition was observed. UV irradiation (HPR 125-W lamp at 365 nm) of [(*p*-propenoyl)Phe^5]TP5 peptide **III** and [(*p*-benzoyl)Phe^5]TP5 was carried out in water under the same irradiation conditions (lamp, time, temperature, concentration) as used in a procedure previously[8] described for photolabeled analogs of TP5. The photolyzed peptides were analyzed by reversed-phase HPLC and MALDI-TOF and/or ESI mass spectrometry. As already reported,[8] [(*p*-benzoyl)Phe^5]TP5 was completely transformed within 40 min into at least 15 photolysis products, with six major products. Under the same conditions, [(*p*-propenoyl)-Phe^5]TP5 was less reactive, about one third of the peptide being converted into a major compound after 40 min, while extended irradiation (100 min) resulted in complete disappearance of peptide **III**. The less hydrophobic peptide formed upon irradiation {elution at 7.35 min, compared to 12.55 min for [(*p*-propenoyl)Phe^5]TP5} had the same mass (*m*/*z*: 759.32) as peptide **III** (*m*/*z*: 759.44), suggesting intramolecular insertion into a X−H bond. ESI MS/MS sequencing allowed the identification of the *N*-terminal residues Ac-Arg and Lys; further sequencing failed.

Conclusion

Three optically active phenylalanine analogues − (*S*)-*p*-(propanoyl)phenylalanine (**10**), (*S*)-Boc-*p*-[2′-(phenylselenenyl)propanoyl]phenylalanine (**11**), and (*S*)-Boc-*p*-[3′-(phenylselenenyl)propanoyl]phenylalanine (**13**) − have been prepared by deprotonation/alkylation of the sultam *N*-(diphenylmethylene)glycinate (**4**). The introduction of analogs **11** and **13** into a model pentapeptide thymopentin TP5 by solid-phase peptide synthesis with a Boc strategy has demonstrated that an α-phenylseleno ketone moiety cannot be used as a precursor of an enone function. However, we have shown that an enone can be generated in a peptide sequence by incorporation of a β-phenylseleno carbonyl group on the aromatic nucleus of phenylalanine. A model peptide containing this enone function, [(*p*-propenoyl)Phe^5]TP5, proved to be sufficiently chemically stable to be used for photoaffinity labeling. (*p*-Propenoyl)Phe-substituted TP5 appeared to be less reactive than (*p*-benzoyl)Phe-substituted TP5 upon irradiation. This slightly higher stability might be a benefit for high affinity peptide/protein complexes.

Experimental Section

Syntheses of Phenylalanine Analogues. 2-Ethyl-2-tolyl-1,3-dioxolane (2): 4′-Methylpropiophenone (15.2 g, 102 mmol), ethylene glycol (12.56 g, 202 mmol), and *p*-toluenesulfonic acid (640 mg) in toluene (200 mL) were placed in a round-bottomed flask fitted with a Dean−Stark water separator and a reflux condenser. The reaction mixture was heated until no more water could be collected (24 hours). The solution was washed twice with a 5% sodium bicarbonate solution, dried ($MgSO_4$), and concentrated to give 16.9 g of a colorless oil (86%). R_f = 0.44 (5% ethyl acetate/cyclohexane). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.35 (d, 2 H, *ortho* aromatic), 7.25 (d, 2 H, *meta* aromatic), 4.05 (m, 2 H, dioxolane), 3.80 (m, 2 H, dioxolane), 2.35 (s, 3 H, $C_6H_4CH_3$), 1.90 (q, 2 H, CH_2-CH_3), 0.90 (t, 3 H, CH_2-CH_3). $C_{12}H_{16}O_2$: C,75.07, H 8.43; Found: calcd. C 74.97, H 8.39.

2-(*p*-Bromomethyl-phenyl)-2-ethyl-1,3-dioxolane (3): A mixture of dioxolane **2** (546 mg, 2.83 mmol), *N*-bromosuccinimide (750 mg, 4.24 mmol), and benzoyl peroxide (100 mg) in 25 mL of cyclohexane in the presence of 3Å molecular sieves was heated under reflux for 4 hours. The reaction mixture was then filtered through a celite pad, washed with a 5% sodium bicarbonate solution, dried ($MgSO_4$), and concentrated. After short chromatography on silica gel (cyclohexane/EtOAc/NEt_3: 95:5/few drops), **3** crystallized after standing for 24 hours at 4 °C. Crystals were collected and were washed twice with pentane (365 mg, 47%). R_f = 0.44 (5% ethyl acetate/cyclohexane); m.p. 65−67 °C (recrystallization from diethyl ether/pentane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.45 (d, 2 H, *ortho* aromatic), 7.35 (d, 2 H, *meta* aromatic), 4.50 (s, 2 H, CH_2Br), 4.00 (m, 2 H, dioxolane), 3.80 (m, 2 H, dioxolane), 1.90 (q, 2 H, CH_2-CH_3), 0.90 (t, 3 H, CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 143.3, 137.5, 129.1, 126.7, 110.9, 65.0, 33.7, 33.6, 8.2. $C_{12}H_{15}BrO_2$: calcd. C 53.10, H 5.57; Found: calcd. C 53.02, H 5.56.

***N*-[*N*′-(Diphenylmethylene)-(*S*)-*p*-(2-ethyl-1,3-dioxolan-2-yl)-phenylalanyl]-(−)-10,2-bornane Sultam (5):** Precursor **4** (2.18 g, 5 mmol, previously dried) was dissolved under argon in freshly distilled THF (15 mL) and DMPU (10 mL). The mixture was stirred at −78° C, and LDA (2.75 mL, 2 M solution in *n*-heptane/THF, 5.5 mmol) was added. After 15 min, the electrophile **3** (2.02 g, 7.5 mmol) in 3 mL of dry THF was added. The mixture was first stirred for 1 hour at −78° C and allowed to warm up to room temperature over one hour. Neat acetic acid (315 μL, 5.5 mmol) and diethyl ether (10 mL) were added. The mixture was washed three times with saturated NH_4Cl, and the organic layer was dried ($MgSO_4$) and concentrated. The oily residue was purified by chromatography on silica gel (20% ethyl acetate/cyclohexane) to give a colorless oil (1.7 g, 54%). R_f = 0.16 (30% ethyl acetate/cyclohexane). $[\alpha]_D^{20}$ = −57 (*c* = 1.14, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.44 (d, 2 H, aromatic), 7.41 (d, 2 H, aromatic), 7.70−7.15 (10 H, aromatic benzophenone imine), 4.89 [dd, 1 H, C(O)C*H*CH_2], 4.05 (m, 2 H, dioxolane), 3.90 (m, 1 H, CC*H*N), 3.75 (m, 2 H, dioxolane), 3.45 [m, 2 H, C(O)CHCH_2], 3.37 (dd, 2 H, CH_2SO_2), 2.0 (q, 1 H, CH_2CH_3), 2.10−1.72 (m, 5 H, C*H*HCH_2C*H*C*H*H or CH_2C*H*HC*H*C*H*H), 1.4−1.5 (m, 2 H, C*H*HCH_2CHC*H*H or CH_2C*H*HCHC*H*H), 0.93 (s, 3 H, CH_3CCH_3), 0.90 (t, 3 H, CH_2CH_3), 0.83 (s, 3 H, CH_3CCH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 170.5, 140.5, 139.3, 136.5, 135.5, 130.0−125.5, 110.5, 67.0, 64.9, 64.3, 52.9, 48.2, 47.5, 44.3, 40.7, 38.1, 33.2, 32.5, 26.2, 20.4, 19.7, 7.7.

(*S*)-*p*-(Propanoyl)phenylalanyl-(−)-10,2-bornane Sultam (8): Compound **5** (3.53 g, 6.05 mmol) was stirred overnight at room temperature in a mixture (1:3) of THF/1 N HCl (30 mL). THF was removed under reduced pressure, and the aqueous layer was washed three times with diethyl ether. The aqueous layer was concentrated to give 2.8 g of a yellow oil (98% yield). R_f = 0.45 (methanol/chloroform/acetic acid, 90:10:1); mass spectrometry (DCI/NH_3): MH^+ *m*/*z* = 419. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.61 (m, 3 H, NH_3), 7.92 (d, 2 H, aromatic), 7.53 (d, 2 H, aromatic), 4.88 [m, 1 H, C(O)C*H*CH_2], 4.10 (m, 1 H, CC*H*N), 3.47 (dd, 2 H, CH_2SO_2), 3.40 [m, 2 H, C(O)CHH_2], 2.98 (dd, 2 H, CH_2H_3), 1.8−2.0 (m, 5 H, CH_2C*H*HC*H*C*H*H or CH*H*CH_2C*H*C*H*H), 1.31 (m, 2 H, CH_2CH*H*CHCH*H* or C*H*HCH_2CHCH*H*), 1.23 (t, 3 H, CH_2CH_3), 1.07 (s, 3 H, CH_3CH_3), 0.84 (s, 3 H, CH_3CCH_3).

(*S*)-Boc-*p*-(Propanoylphenyl)alanine (10): Compound **8** was dissolved in 1,4-dioxane (15 mL) in the presence of monohydrated lithium hydroxide (440 mg, 10.5 mmol). The reaction mixture was stirred at room temperature for 4 days. The resulting precipitate was collected by filtration and washed twice with diethyl ether. This solid (compound **9**) was dissolved in water (4 mL). 1,4-Dioxane (2 mL) and triethylamine (840 μL, 6 mmol) was added to the mixture, which was cooled at 0° C prior to the addition of Boc_2O (1.20 g, 5.5 mmol) in 3 mL of 1, 4-dioxane. The solution was stirred at room temperature overnight. After concentration under reduced pressure to remove the dioxane, water (5 mL) was added. This basic aqueous layer was washed twice with diethyl ether, and diluted with 10 mL of EtOAc. HCl (3 N) was then added slowly at 0° C until pH = 2 was reached. The aqueous layer was extracted twice with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated to give an oil, which was purified by short flash chromatography on silica gel (CH_2Cl_2/MeOH/AcOH, 98:2/0.1) to give 963 mg of a colorless oil (60% overall yield for the three steps). R_f = 0.49 (chloroform methanol/acetic acid, 95:5/0.5). $[\alpha]_D^{20}$ = 50 (*c* = 0.73, $CHCl_3$); mass spectrometry (DCI/NH_3): MNH_4^+ *m*/*z* = 334, ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of *cis*/*trans* isomers): δ = 7.93 (d, 2 H, *ortho* aromatic), 7.29 (d, 2 H, *meta* aromatic), 6.33 (m, 0.4 H, N*H*), 4.99 (d, 0.6 H, N*H*), 4.66 (m, 0.6 H, C(O)C*H*CH_2), 4.47 [m, 0.4 H, C(O)C*H*CH_2], 3.29 [m, 1 H, C(O)CHCH*H*], 3.15 [m, 1 H, C(O)CHC*H*H], 3.01 (2 H, CH_2CH_3), 1.43 (s, 5.4 H, Boc), 1.31 (s, 3.6 H, Boc), 1.24 (t, 3 H, CH_2CH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 200.9, 175.7, 155.4, 141.5, 135;7, 129.7, 128.4, 80.5, 54.1, 46.3, 31.8, 28.3, 8.3.

(*S*)-Boc-*p*-[2′-(Phenylselenenyl)propanoyl]phenylalanine (11): A solution of Boc-amino acid **10** (341 mg, 1.05 mmol) in EtOAc (2 mL) in the presence of phenylselenyl chloride (222 mg, 1.15 mmol) was stirred under argon at room temperature for 72 hours. The precipitate was filtered off and the filtrate was concentrated and purified by careful flash chromatography on silica gel (CH_2Cl_2/MeOH/AcOH, 96:4/0.1) to give 280 mg (56%) of a yellow oil. R_f = 0.36 (chloroform/methanol/acetic acid, 95:5/0.5); mass spectrometry (DCI/NH_3): MH^+ *m*/*z* =478, MNH_4^+ *m*/*z* = 495. ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of two diastereoisomers): δ = 7.8 (d, 2 H, *ortho* aromatic), 7.5 (d, 2 H, *meta* aromatic), 7.22 (m, 5 H, aromatic Se-Ph), 6.36 (d, 0.33 H, N*H*), 5.06 (m, 0.66 H, N*H*), 4.67 [m, 1.66 H, C(O)C*H*CH_2, PhSeC*H*CH_3], 4.40 [0.33 H, m, C(O)CHCH*H*], 3.27 [m, 1 H, C(O)CHC*H*H], 3.13 [0.66 H, m, C(O)CHCH*H*], 2.98 [0.33 H, m, C(O)CHCH*H*], 1.63 (d, 3 H, PheSeCHCH_3), 1.43 (s, 5.2 H, Boc), 1.35 (s, 3 H, Boc). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ = 175.2, 155.2, 136.5−126.6, 81.8, 80.3, 55.5, 53.9, 39.5, 39.2, 37.6, 28.1, 20.9, 17.0.

(*S*)-Boc-*p*-(Propenoyl)phenylalanine (12): Sodium periodate (118 mg, 0.55 mmol) was added to a solution of Boc-amino acid **11** (131 mg, 0.27 mmol) in MeOH (2.2 mL) and H_2O (860 μL). The reaction mixture was stirred for 2 hours at room temperature in the dark. The precipitate was removed by filtration and rinsed

with MeOH (2 × 5 mL). The solution was concentrated and then diluted with EtOAc (15 mL). The organic layer was washed with $KHSO_4$ solution (pH = 2), dried ($MgSO_4$), and concentrated. We were unable to achieve complete purification of compound **12** by chromatography, R_f = 0.22 (dichloromethane/methanol/acetic acid: 95:4:0.5). Thus, compound **12** was characterized by ^1H NMR ($CDCl_3$, 250 MHz,), purity estimated around 90%, δ = 7.86 (d, 2 H, *ortho* aromatic), 7.22 (d, 2 H, *meta* aromatic), 7.18 [dd, 1 H, CC(O)C*H*CH$_2$], 6.42 [dd, 1 H, CC(O)CHC*H*H], 5.91 [dd, 1 H, C(O)CHCH*H*], 5.05 (dd, 1 H, N*H*), 4.61 [m, 1 H, NC(O)C*H*CH$_2$], 3.30 [m, 1 H, NC(O)CHC*H*H] 3.18 [m, 1 H, N(O)CHCH*H*], 1.37 (br. s, 9 H, Boc).

(*S*)-Boc-*p*-[3′-(Phenylselenenyl)propanoyl]phenylalanine (13): The above crude **12** (130 mg, 0.41 mmol) was dissolved in absolute ethanol (2.5 mL), and diphenyldiselenide (168 mg, 0.54 mmol) was added. Sodium borohydride (41 mg, 1.07 mmol) was then added (caution, hydrogen evolution), and the reaction mixture was stirred at 0 °C for ten minutes. Neat acetic acid (109 μL, 1.89 mmol) was added and the mixture was stirred for 3 hours at room temperature. The reaction mixture was diluted with 10 mL of EtOAc and washed with a saturated $KHSO_4$ solution, and the combined organic layers were dried ($MgSO_4$). After concentration, the residual oil was purified by short column chromatography (CH_2Cl_2/MeOH/AcOH, 96:4:0.1) to afford 65 mg (50% from compound **11**) of **13** as a yellow oil. R_f = 0.36 (chloroform methanol/acetic acid: 95:5:0.5). $[\alpha]_D^{20}$ = 17 (c = 1, CH_2Cl_2); mass spectrometry (DCI/NH_3): MH^+ m/z = 478, MNH_4^+ m/z = 495. ^1H NMR ($CDCl_3$, 250 MHz): δ = 7.83 (d, 2 H, *ortho* aromatic), 7.43 (d, 2 H, *meta* aromatic), 7.19 (m, 4 H, aromatic Se-*Ph*), 6.30 (m, 0.33 H, N*H*), 4.91 (d, 0.66 H, N*H*), 4.61 (m, 0.66 H, C(O)C*H*CH$_2$), 4.36 (m, 0.33 H, C(O)C*H*CH$_2$), 3.35 [m, 3 H, C(O)CHC*H*H, C*H*$_2$CH$_2$SePh or CH$_2$C*H*$_2$SePh], 3.28 [m, 3 H, C(O)CHCH*H*, C*H*$_2$CH$_2$SePh or CH$_2$C*H*$_2$SePh], 1.34 (br. s, 6 H, Boc), 1.23 (br. s, 3 H, Boc). ^{13}C NMR ($CDCl_3$, 62.9 MHz): δ = 135.4, 132.8, 129.7, 129.3, 128.4, 127.1, 77.3, 55.0, 39.4, 29.7, 28.3, 21.1.

Deselenation Studies: The stability of model phenylselenyl compounds was followed directly by NMR before and after acidic treatment with trifluoroacetic acid, hydrogen fluoride, and hydrogen fluoride/anisole/dimethyl sulfide (10:2.5:0.25 per 0.5 mmol).

Preparation of the Model Compounds: α-Phenylselenenyl-4′-methylpropiophenone was prepared by the procedure previously reported.[3] ^1H NMR ($CDCl_3$, 400 MHz,): δ = 7.65 (d, 2 H, *ortho* aromatic), 7.49 (d, 2 H, *meta* aromatic), 7.31 (m, 5 H, aromatic Se-*Ph*), 4.70 (q, 1 H, PhSeC*H*CH$_3$), 2.43 (s, 3 H, C$_6$H$_4$C*H*$_3$), 1.67 (d, 3 H, PhSeCHC*H*$_3$). β-Phenylselenenyl-4′-methylpropiophenone was prepared by the procedure described for **13**. ^1H NMR ($CDCl_3$, 400 MHz,): δ = 7.83 (d, 2 H, *ortho* aromatic), 7.54 (d, 2 H, *meta* aromatic), 7.30 (m, 5 H, aromatic Se-*Ph*), 3.41 [m, 2 H, C(O)C*H*$_2$CH$_2$SePh], 3.26 [m, 2 H, C(O)CH$_2$C*H*$_2$SePh], 2.42 (s, 3 H, C*H*$_3$).

Peptides Syntheses: Peptide synthesis was carried out manually on a 0.1 mmol scale, with a *p*-methylbenzhydrylamine resin (MBHA resin, typical substitution 0.77 mmol/g of resin) according to the procedure previously described for the Boc strategy.[26] Standard procedures for *tert*-butoxycarbonyl-protected amino acids were used, with dicyclohexylcarbodiimide/1-hydroxybenzotriazole activation (10 equiv. for conventional Boc amino acids and one equiv. of Boc-amino acid **11** or **13**). The activated Boc-amino acid **11** or **13** was coupled onto the resin for 24 hours and the residual unchanged amino groups were acetylated until negative by the Kaiser test. After completion of the peptide sequence, the last *N*-α-Boc protecting group was removed and the N-terminus was acetylated. The peptidyl-MBHA resin was then treated with liquid HF at −20 °C (30 min) and 0 °C (30 min) in the presence of 1.5 mL of anisole, 0.25 mL of dimethyl sulfide per gram of peptidyl resin. The crude peptides were purified by HPLC with an Applied Biosystems apparatus, using a 10 × 250 mm Brownlee column packed with Aquapore Octyl, 20 μm pore size. The separation was accomplished with acetonitrile gradients in aqueous trifluoroacetic acid (0.1%) at a flow rate of 6 mL/min, with UV detection at 220 nm. Analytical monitoring was carried out by reversed-phase analytical HPLC on a LiChrosphor 100 RP-8e column (Merck) with acetonitrile gradients in aqueous trifluoroacetic acid (A = 0.1% TFA, B = 40% A and 60% CH_3CN) at a flow rate of 1.5 mL/min with UV detection at 210 nm. Yields of purified peptides were between 13% and 20%. The purities of the collected fractions (between 95% and 98%) were determined by HPLC in the gradient mode (from 20% to 80% B in 30 min). Retention times: t_R = 12.3 min peptide **I**; t_R = 25.4 min peptide **II**; t_R = 12.4 min peptide **III**.

Structural Determinations of Peptides

MALDI-TOF spectra were obtained on a Voyager Elite mass spectrometer (PerSeptive Biosystems), with α-cyano-4-hydroxycinnaminic acid as matrix, at concentrations around $5 \cdot 10^{-2}$ M in acetonitrile/water (4:1, v/v). The positive MALDI mass spectrum gave a unique ion at m/z = 761.58 for peptide **I**, m/z = 917.36 for peptide **II**, and m/z = 759.44 for peptide **III**.

NMR experiments were carried out on 2 mM solution in ^2H_2O at 298 K. NMR spectra were recorded on Bruker AM 500 or DMX 500 spectrometers and were processed with an Aspect 3000 computer (AM 500) or with the Bruker UXNMR software (DMX 500), Table 1. One-dimensional spectra were acquired over 16 K data points with a spectral width of 5000 Hz. Solvent suppression was achieved by presaturation during the relaxation delay (1.5 s). Two-dimensional experiments were acquired in the absolute mode for COSY and in phase-sensitive mode for TOCSY; TOCSY experiments were carried out with mixing times of 40 ms. Typically, 400 to 600 increments were acquired over a spectral width of 5000 Hz. Prior to Fourier transformation in $t2$ and $t1$, the free induction decays were zero-filled and multiplied by a $\pi/4$-shifted sine-bell function.

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