Chemistry and folding of photomodulable peptides – stilbene and thioaurone-type candidates for conformational switches†

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Optimized synthetic strategies for the preparation of photoswitchable molecular scaffolds based on stilbene or on thioaurone chromophores and their conformationally directing properties, as studied by computations and by NMR spectroscopy, are addressed. For the stilbene peptidomimetics **1**, **2** and **3**, the length of connecting linkers between the chromophore and the peptide strands was varied, resulting in photochromic dipeptidomimetics with various flexibility. Building blocks of higher rigidity, based on *para*-substituted thioaurone (**4** and **6**) and *meta*-substituted thioaurone chromophores (**5** and **7**) are shown to have a stronger conformationally directing effect. Design, synthesis, theoretical and experimental conformational analyses are presented.

Introduction

Proteins capable of reversible photochemically driven conformational changes have attracted a lot of interest in recent years and were predicted to bear tremendous potential in the fields of data storage, molecular motors, protein and genetic engineering, in vivo protein tracking and fluorescent optical microscopy. Built-in switching units are expected to result in photoresponsive materials,[1] conformationally switchable nucleic acids,[2] antibodies,[3] and peptidomimetics.[4] Among examples from Nature, photoswitchable green fluorescent proteins (GFP) found in certain marine organisms have recently attracted much interest, including practical applications.[5] The demand for photomodulable units of improved and well-controlled properties motivated us to develop photoswitchable units that can straightforwardly be incorporated into functional peptides by standard automatable methods. Azobenzene chromophores have been successfully applied to control the structure of synthetic peptides and proteins,[6] however, their conformation inducing ability appeared to be limited, which in combination with their sensitivity to reducing agents and their unavoidable thermal isomerization make their use in control of protein function difficult if not impossible.[7] In contrast, in spite of their well-studied photochemistry[8] and successful incorporation into DNA,[2] examples for the use of stilbene derivatives in peptidomimetics are very rare.[9] Likewise, the thioaurone chromophore has only recently been proposed for this purpose.[10] Due to their greater chemical stability and resistance against thermal isomerization, stilbenes are promising alternatives to azobenzenes. Here, we propose a range of photochemically interconvertible stilbene and thioaurone derivatives, which are expected to induce turn-like conformations in their *Z* configurations, and open, linear geometries in their *E* configurations.[11,12] Their general synthetic strategies, suitable for the construction of phototriggerable, functional protein- as well as peptidomimetics, are exemplified by simple model compounds. In order to reduce complexity and focus on the role of the chromophore, apolar, non-aromatic amino acids were attached to the stilbene amino acid core.

The resulting peptidomimetics were, similar to numerous previously investigated compounds,[13,14] poorly soluble in water. Conformational studies were therefore perfomed in methanol and dimethylsulfoxide solutions. Here, we would like to stress that these compounds were designed to provide simplified models of the photoswitchable core of peptides and proteins with larger molecular weight. Their photochemical $E \rightarrow Z$ conversion as well as conformational properties, in comparison to those of the standard ^{D}Pro-Gly[13] and diphenylacetylene[15] turn mimetics (Scheme 1), is discussed.

Results and discussion

Synthesis

Based on computational design, *meta*,*meta*′-disubstituted stilbene-type turn-mimetics **1–3** (Scheme 1) encompassing methylene linkers of various length ($(CH_2)_n$, n = 0, 1, 2), providing different conformational flexibility, were prepared (Schemes 2–4). *E*-Isomers were synthesized and the *Z* analogues were obtained by photoisomerization of the former. Peptidomimetics **4–7**, incorporating the thioaurone chromophore were prepared in their thermally stable *Z* isomeric forms, and the *E* isomers were then obtained by analogous photochemical isomerization. Since peptides containing ^{D}Pro-Gly[13] or diphenylacetylene-type turn mimetics are known to fold in well-defined β-turn conformations,[15] mimetics **8–10** having identical amino acid strands to those attached to the stilbene and thioaurone units were synthesized as

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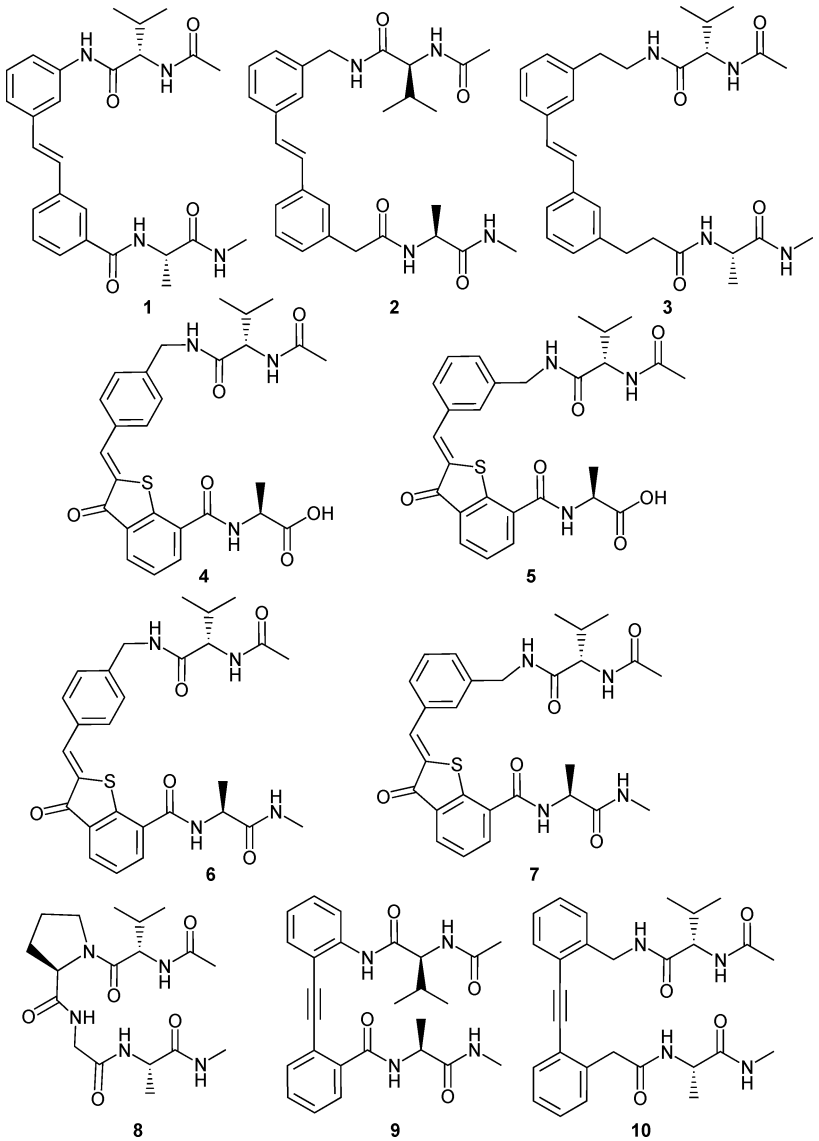


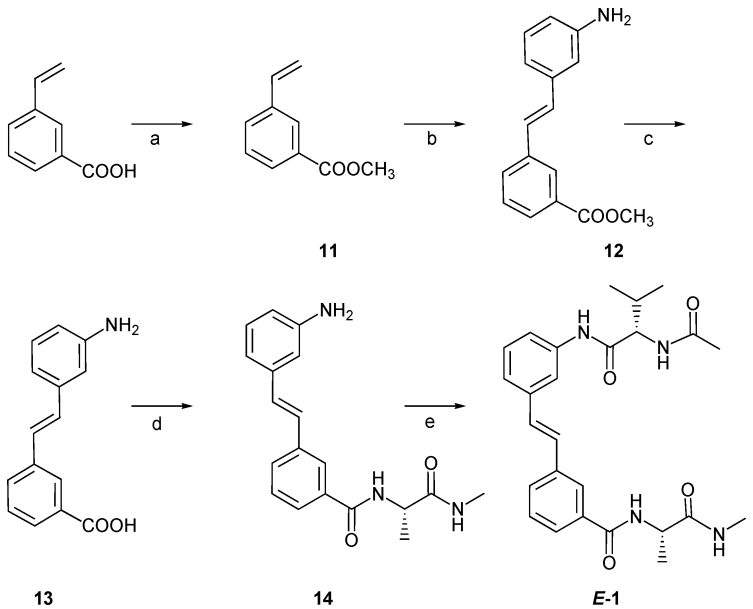
Scheme 1 Model peptidomimetics incorporating reversibly switchable chromophores (**1–7**) and β-turn mimetics (**8–10**).

reference compounds. Synthesis and structural studies of **9** have been published earlier[13] and are cited here for comparison.

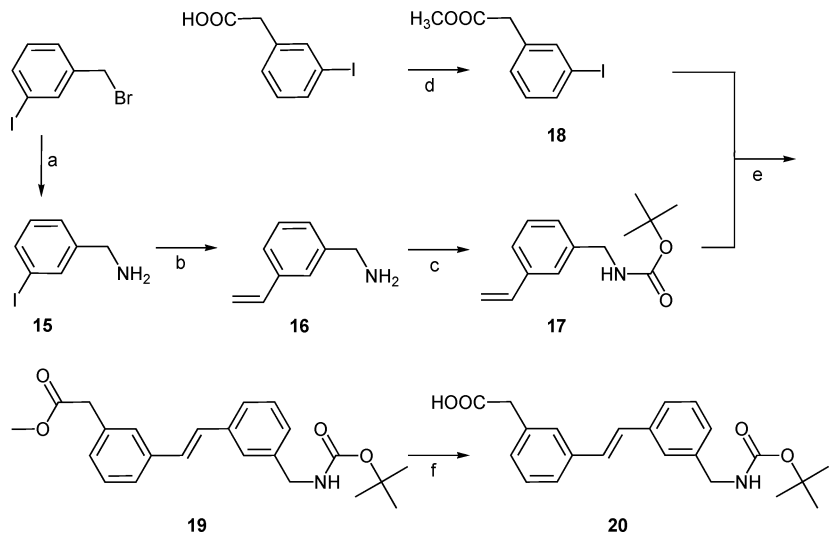
The anilinic amino group[15] of **13** was supposed to show reduced nucleophilicity as compared to typical amino acids, a fact known to cause difficulties in solid phase synthesis. Therefore, *E*-**1** was prepared in solution following the reaction path depicted in Scheme 2. A fast microwave mediated Heck-coupling[16] of **11** with the electron rich 3-iodoaniline resulted in a 2:18:80 mixture of the *Z*:internal:*E* products. The *E*-isomer was separated using column chromatography, its ester functionality was subsequently hydrolysed, and the zwitterionic **13** was then subjected to PyBOP mediated attachment of alanine methylamide, yielding **14**. Under these conditions, the anilinic amino group of **13** does not react, and therefore the coupling could be performed selectively.

In the next step, the electrone density of the amino group of **13** was increased, by activation with N,O-bis(trimethylsilyl)acetamide. Now the attachment of N-acetyl-valine was possible with HATU under mild basic conditions, yielding *E*-**1**. The product was isolated by precipitation from the reaction mixture and then investigated by ^{1}H NMR spectroscopy. Observation of only one set of signals ascertained that racemization at the valine C-α had not occurred. Obviously, a single set of signals could also result from complete inversion at this carbon, but this is highly unlikely. Analogous tri- or tetrapeptidomimetics are easily incorporatable into standard solid-phase peptide synthesis (SPPS).

The switchable units of **2** and **3** and the diphenylacetylene moiety of **10** were synthesized in solution and could subsequently be used in SPPS. As shown in Scheme 3, benzylic nucleophilic substitution[17] followed by a microwave-accelerated Stille reaction,[18] was utilized for the preparation of the central part of mimetic **2**. For the efficient Heck coupling[16] of **17** and **18**, leading to the artificial amino acid **20**, both Boc-protection of the amino group of **16**, yielding **17**, and esterification of the carboxyl group of 3-iodophenyl acetic acid to give **18** are indispensable. Base mediated hydrolysis of the resulting methyl



Scheme 2 Outline of the synthesis of **1**: (a) CH_3OH, conc. HCl, 70 °C, 14 h, 88%. (b) 3-Iodoaniline, $Pd(OAc)_2$, $(C_2H_5)_3N$, DMF, 150 °C, 20 min, 56%. (c) 6 M NaOH (aq), 100 °C, 6 h, 98%. (d) 2-Amino-*N*-methyl-propionamide, PyBOP, DIEA, r.t., 2 h, 17%. (e) 2-Acetylamino-3-methyl-butyric acid, N,O-bis(trimethylsilyl)acetamide, HATU, DIEA, r.t., 18 h, 49%.

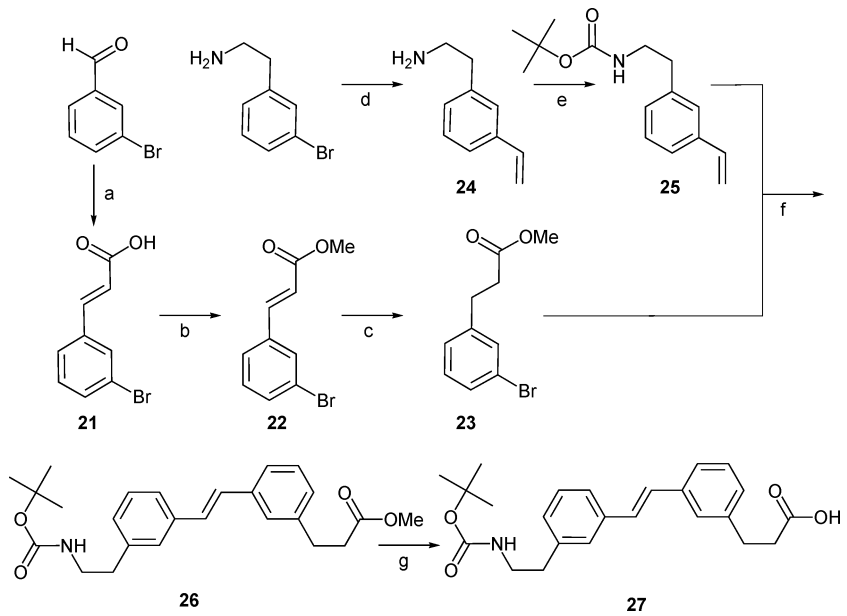


Scheme 3 Outline of the synthesis of **20**: (a) 1) $NaN(SiMe_3)_2$, HMDS, r.t., 24 h. 2) H^+, 63%. (b) $Bu_3SnCH{=}CH_2$, $Pd(PPh_3)_2Cl_2$, Et_3N, DMF, 130 °C, 15 min, 84%. (c) $O[CO_2C(CH_3)_3]_2$, CH_2Cl_2, K_2CO_3 in H_2O, r.t., 72 h, 68%. (d) CH_3OH, conc. HCl, r.t., 24 h, 98%. (e) $Pd(OAc)_2$, $(CH_3C_6H_4)_3P$, Et_3N, DMF, 90 °C, 15 min, 70%. (f) 6M NaOH (aq), 120 °C, 20 h, 34%.

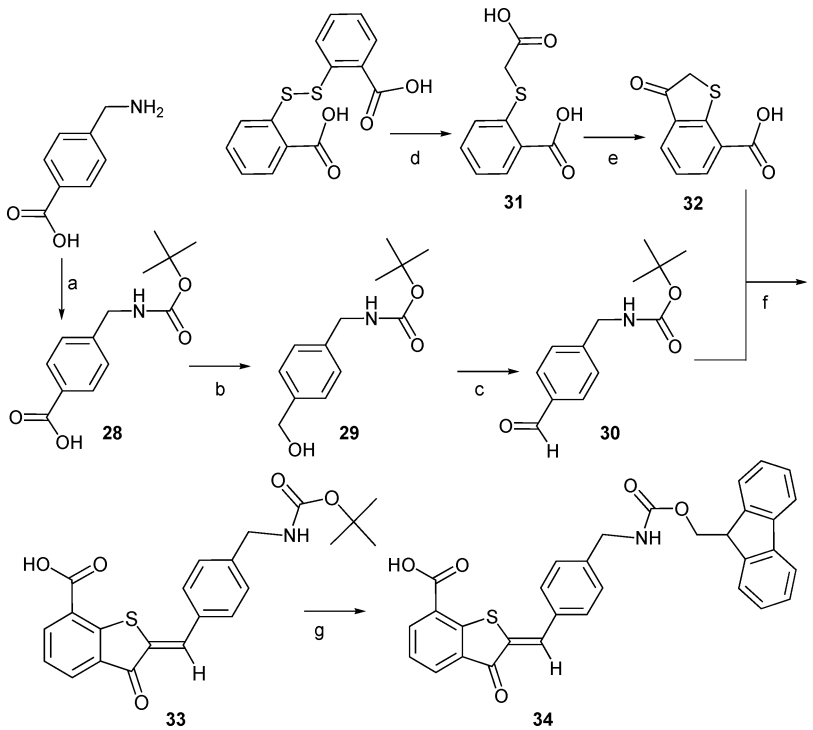
ester **19** yields **20**, a photochromic amino acid mimetic suitable for direct application in standard SPPS. The harsh conditions used for hydrolysis of **19** and also **12**, were required because of the low reactivity that is often encountered for benzylic and in particular aromatic esters. Peptidomimetic **3** was prepared using Knoevenagel condensation[19] followed by esterification of the carboxyl group, resulting in the α,β-unsaturated ester **22** (Scheme 4).

Since the reduction of a double bond in the presence of an aryl halide is known to be difficult, a nickelboride catalyzed route[20] was optimized for the reduction of **22** to **23**, giving 68% isolated yield and notably only 1.5% debrominated byproduct. Compound **26** was obtained by Heck coupling of **23** and **25** in a microwave reactor.[16] After several unsuccessful attempts to hydrolyse the ester **26** by conventional methods, the hydrolysis could be achieved with K-*tert*-BuO in diethyl ether, yielding **27**. The stilbene derivative artificial amino acid **27** could then be directly incorporated into **3** by standard SPPS. Details of the experimental procedure for the preparation of **27** are presented elsewhere.[12]

The syntheses of the thioaurone cores followed a previously reported general scheme *via* thioindoxyl (Schemes 5 and 6).[21] The aldehyde required for the preparation of **34** was obtained by the oxidation of the corresponding alcohol **29**.[22] Boc-protected thioaurone derivatives **33** and **39** were synthesized by an aldol condensation reaction between thioindoxyl **32** and the aldehydes **30** and **38**, respectively.[10] Here, conditions and synthetic strategy

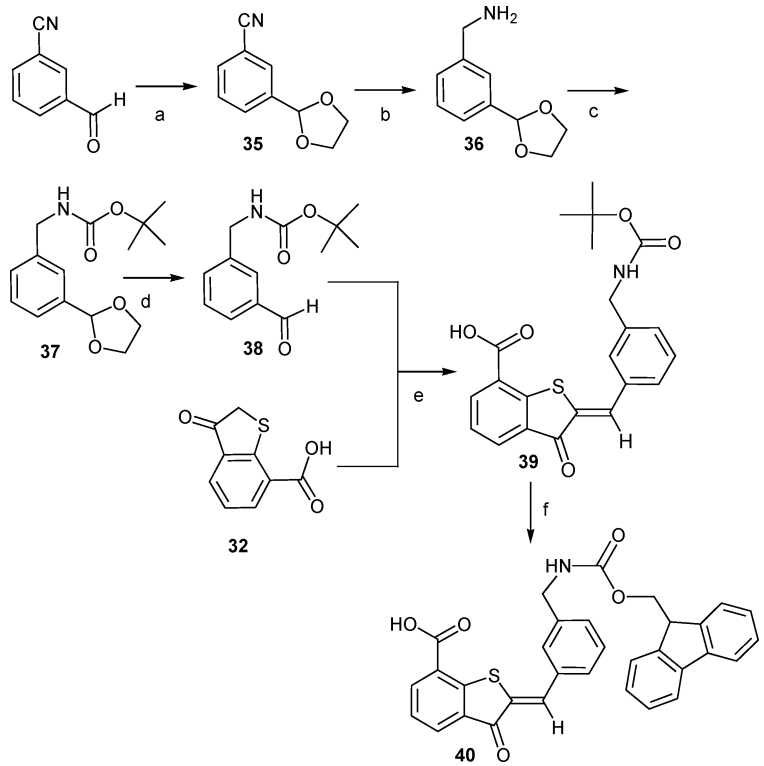


Scheme 4 Outline of the synthesis of **27**: (a) $CH_2(COOH)_2$, pyridine, piperidine, 100 °C, 1.5 h, 95%. (b) CH_3OH, conc. HCl, r.t., 16 h, 93%. (c) $Ni(OAc)_2$, $NaBH_4$, CH_3OH, EtOAc, H_2 (2 bar), 20 min, r.t., 68%. (d) $Bu_3SnCH{=}CH_2$, $Pd(PPh_3)_2Cl_2$, Et_3N, DMF, 130 °C, 25 min, 78%. (e) $O[CO_2C(CH_3)_3]_2$, CH_2Cl_2, K_2CO_3 in H_2O, r.t., 24 h, 70%. (f) $Pd(OAc)_2$, $(CH_3C_6H_4)_3P$, Et_3N, DMF, 120 °C, 30 min, 43%. (g) K-*tert*-BuO, Et_2O, 10.5 h, 99%.



Scheme 5 Outline of the synthesis of **34**: (a) 1) 1,4-dioxane, 1 M NaOH, $O[CO_2C(CH_3)_3]_2$, 0 °C, 40 min. 2) HCl, 81%. (b) LAH, THF, 40 °C o.n., 71%. (c) PCC, NaOAc, DCM, r.t. o.n., 72%. (d) 1) Na_2CO_3, $Na_2S_2O_4$, reflux 30 min. 2) $ClCH_2COOH$ neutralised with Na_2CO_3, reflux 1 h. 3) HCl to Congo red, 71%. (e) 1) $SOCl_2$, reflux 2 h. 2) $AlCl_3$, dichloroethane, r.t. o.n., 81%. (f) 1) 1% NaOH w/w, *t*-BuOH, 0 °C then reflux o.n. 2) HOAc, 81%. (g) 1) 50% TFA in DCM, 15 min. 2) Fmoc-Cl, Na_2CO_3, THF, 90%.

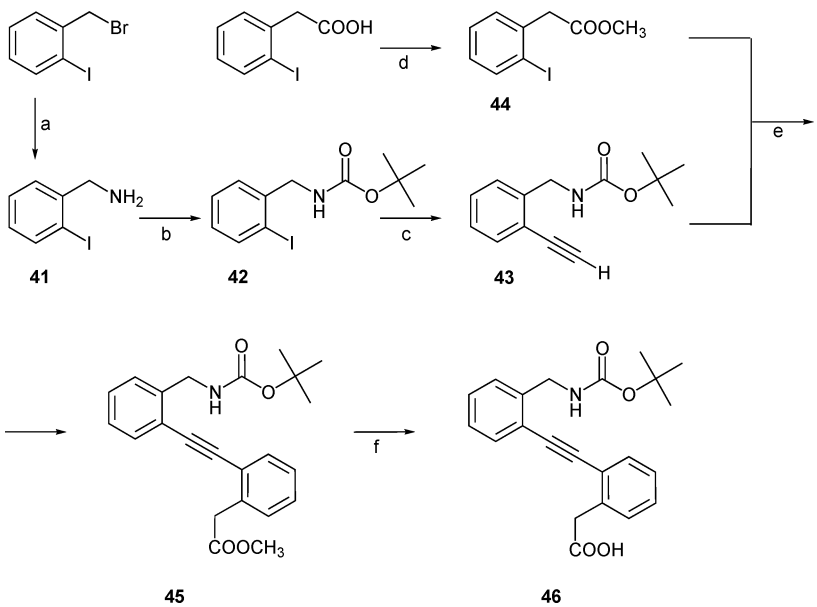
had to be altered from the literature procedure to achieve satisfactory results. Thus, an excess of thioindoxyl over aldehyde was used, and the reaction time was increased. To be able to use the thioaurone derivatives in SPPS, the protecting group had to be changed from Boc to Fmoc. Due to the instability of thioaurones under basic conditions, including primary but not secondary or tertiary amines, the synthetic strategy to prepare the peptides **4–7** had to be adjusted: Instead of using Boc-chemistry and the Kaiser oxime resin, as for **2** and **3**, a resin yielding the desired N-methylated amides after mild acidic cleavage was used, thus



Scheme 6 Outline of the synthesis of **40**: (a) Ethylene glycol, PTSA, reflux 18 h, 60% (b) LAH, THF, r.t., o.n. 40% (c) Anhyd. MeOH, TEA, $O[CO_2C(CH_3)_3]_2$, r.t., o.n., 60% (d) Acetone, water, cat. PTSA, r.t. o.n., 78% (e) 1) 1% NaOH w/w, *t*-BuOH, 0 °C then reflux o.n. 2) HOAc, 45%. (f) 1) 50% TFA in DCM, 15 min. 2) Fmoc-Cl, Na_2CO_3, THF, 70%.

avoiding the basic conditions required for the final cleavage of the peptidomimetic from the Kaiser oxime resin.

Reference compound **8** was prepared applying standard Boc-SPPS methodologies. The precursor of **10** was afforded in a similar reaction route to the one used for preparation of **2** (Scheme 7), but using *ortho*-substituted starting materials and rapid Sonogashira cross-couplings[13,23] instead of a Stille and a Heck reaction. The central part of compound **46** was obtained by benzylic nucleophilic



Scheme 7 Outline of the synthesis of **46**: (a) 1) $NaN(Si(CH_3)_3)_2$, hexamethyldisilazane, r.t., 40 h. 2) H^+, 50%. (b) $O[CO_2C(CH_3)_3]_2$, CH_2Cl_2, K_2CO_3 in H_20, r.t., 72 h, 60%. (c) 1) $(CH_3)_3Si–C{\equiv}CH$, $Pd(PPh_3)_2Cl_2$, CuI, $(C_2H_5)_2NH$, DMF, 120 °C, 6 min, 98%. 2) $KF{\cdot}2H_2O$, r.t., 14 h, 63%. (d) CH_3OH, HCl, r.t., 4 h, 99%. (e) $Pd(PPh_3)_2Cl_2$, CuI, $(C_2H_5)_2NH$, DMF, 120 °C, 6 min, 81%. (f) 6 M NaOH (aq), 120 °C, 2 h, 61%.

substitution, followed by Boc-protection of the amino group of **41**. It is worth mentioning that protection of the benzylamine of **41** with Boc instead of Fmoc, was necessary to facilitate the Sonogashira coupling. Furthermore, esterification of the carboxyl group of **44** was also required for efficient coupling of the two sides of **45**. Base mediated hydrolysis of the protected methyl ester yielded compound **46**, appropriate for incorporation into **10** by standard SPPS.

Photochemistry

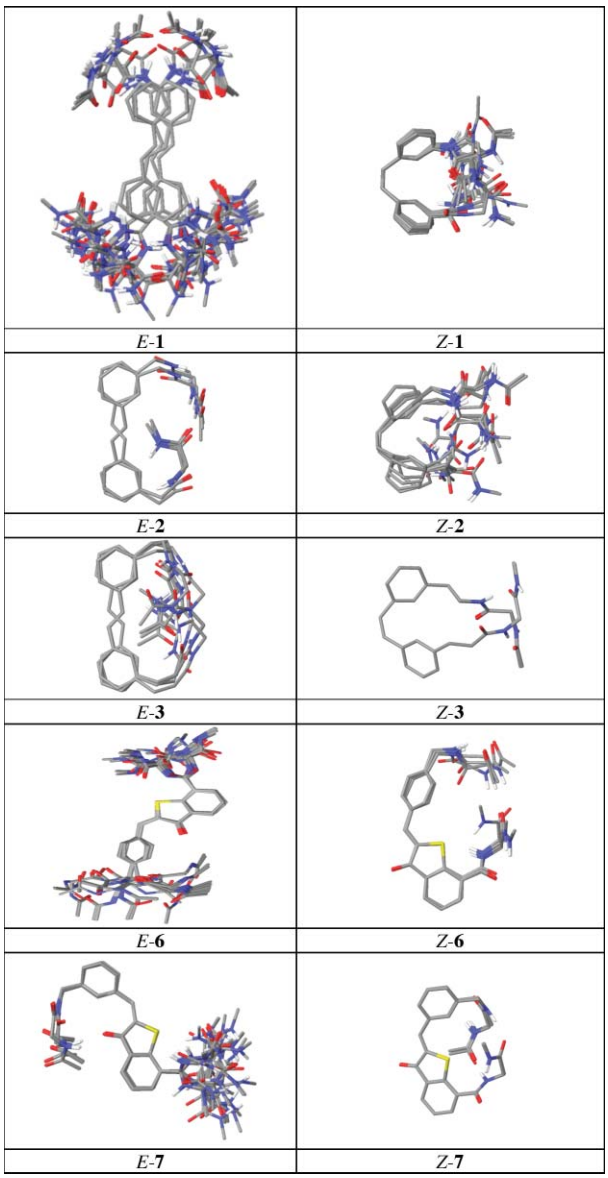
The photochemical properties of stilbene and thioaurone derivatives are well documented in the literature.[8,21b] The absorbance maxima of the π–π* transitions of the *trans*-double bonds in peptidomimetics **1–3** at approximately 300 nm indicated that *meta*-substituents do not significantly affect the λ_{max} of the stilbene double bond. Photoisomerizations were performed in acetonitrile solutions and the *E*/*Z* ratio was thereafter determined by ^{1}H NMR measurements. In contrast to azobenzenes[24] and thioaurones,[4,21b] thermal isomerization was absent for stilbene derivatives **1–3**.

Conversion between *E*-stilbene and *Z*-stilbene derivatives was achieved by irradiation at the absorption maxima of 300 nm and 280 nm, respectively. Isomerization of thioaurones was performed by non-selective irradiation, followed by investigation of the *E*-isomers, having half-lives of several hours.

Molecular mechanics calculations

Conformational analysis using molecular mechanics was used to predict the conformational population of the photoswitchable compounds **1–3**, **6** and **7**. The GB/SA $CHCl_3$ solvation model[25] was used as implemented in the program Macromodel 9.5.[26] Conformations within 25 kJ mol^{-1} of the global minimum were retained (Scheme 8). Interstrand hydrogen bonds and distances between $C_{\alpha,Ala}$–$C_{\alpha,Val}$ and the capping group C_{methyl}–C_{methyl} were recorded to identify folded or non-folded conformations. The presence of two hydrogen bonds (< 2.5 Å distance between H_{NH} and O, see Fig. 1) or an interstrand C_α–C_α distance of < 5.5 Å was used as criterion for beta sheet and/or folded conformations, respectively. The populations of folded conformations were estimated using the Boltzmann equation and are summarized in Table 1.

Compared to compound **8**, which has been included for comparison as a peptide predestined to generate β-hairpin motifs, the stilbene derivatives **1–3** show excellent preference for formation of turn-like motifs in their *Z* forms whereas their *E* isomers show larger interstrand distances. Compound **1** shows the most pronounced conformational alteration, while model compounds **2** and **3** display a tendency for intramolecular interchain interaction in both their *Z* and *E* isomers as a result of the high flexibility of their linker regions. It should be emphasized that the preferred geometry of *Z*-**2** and *Z*-**3** does not fully resemble the conformation of conventional turns, which is likely to be a result of the shortness of the amino acid strands present in the model systems. This is further supported by our previous studies which have shown that such flexible photochromic turn mimetics can provide a sufficient conformationally directing effect when they are incorporated into a medium sized peptide.[12] Switch candidates **6** and **7** show a pronounced difference between their respective



Scheme 8 Overlays of the most stable conformers of peptidomimetics with stilbene and thioaurone chromophores. Conformers within 5 kJ mol^{-1} of the global minimum are shown.

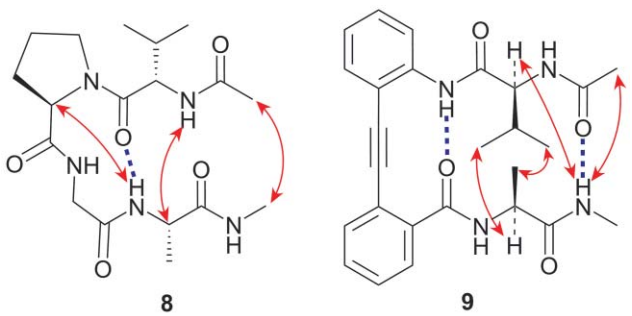
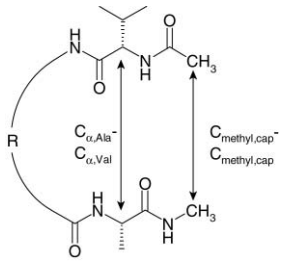


Fig. 1 Selected interstrand NOEs (arrows) and hydrogen bonds (dashed lines) observed in $CDCl_3$ solutions of **8** and **9**.

Z and *E* isomers. As for **1**, their superior rigidity is indicated by the pronounced absence (*i.e.*, relative amount ≤ 0.1%) of turn-like

Table 1 Relative amounts of folded conformations of the *Z* and *E* isomers of photoswitchable compounds **1–3**, **6–7**, and reference compound **8**



	Z		*E*	
	$C_{\alpha,Ala}$–$C_{\alpha,Val}$ < 5.5 Å (%)	C_{Me}–C_{Me} < 5.5 Å (%)	$C_{\alpha,Ala}$–$C_{\alpha,Val}$ < 5.5 Å (%)	C_{Me}–C_{Me} < 5.5 Å (%)
1	98	25	0	0
2	97	30	7	92
3	99	12	87	6
6	12	91	0	0
7	100	0	0	0.1
8	94	97	—	—

conformations for their *E* isomers. An interesting feature is the similar conformational behaviour of *Z*-**7** and *E*-**3**, as well as *Z*-**6** compared to *E*-**2**, revealing that this geometry can easily be reached from a tighter (*Z*-**2** or *Z*-**3**) or a more extended (*E*-**6** or *E*-**7**) state through photochemical induction. However, some of these turn motifs show a twisted geometry, which is also indicated by the presence of "crossing" hydrogen bonds (*e.g.*, in *Z*-**1**, see ESI for details). Thus, depending on the need of the intended application area a stilbene or a thioaurone-type switch should be chosen to provide an optimal conformational modulator.

Spectroscopic conformational analysis

Conformational preferences were further investigated by NMR spectroscopy. Structural assignment was performed using COSY,[27] NOESY,[28] ROESY,[29] and TOCSY[30] experiments. If necessary, solvent signals were suppressed using the WET presaturation scheme.[30] Similarly to other investigations,[4d,6b] the *Z*-stilbene isomers were studied in mixtures with the *E* isomers, obtained by photoisomerization of the latter. The molecular conformation in solution was examined by the study of amide temperature coefficients (Table S1, ESI) and by analysis of NOE cross-peak patterns.

The presence of β-hairpin populations of **9**, and of folded conformers of **8**, was indicated by interstrand NOEs (Fig. 1). The NOESY and $\Delta\delta/\Delta T$ values did not indicate folding for the DMSO or methanol solutions of stilbene derivatives **1–3** nor of the thioaurone derivatives **4–7**. This result is in excellent agreement with the extraordinarily high flexibility of low-molecular weight peptides, frequently mentioned in the literature.[31] Hence, small peptides can only be expected to fold into a well-defined structure when an exceptionally rigid conformational directing organic unit, such as the tolan moiety in **9**, is present in their sequence. The uncapped peptidomimetics **4** and **5**, having a free carboxy group, and their capped congeners **6** and **7**, showed similar chemical shifts, temperature coefficients and NOE patterns. Since the presence of an additional polar group would be expected to increase the tendency to form interstrand hydrogen bonds, the absence of such interactions further indicates the high conformational flexibility of the peptidomimetics.

Discussion

Our NMR investigations (measuring NOE, $\Delta\delta_{NH}/\Delta T$, and $\Delta\delta_{NH,solv}$) showed that the ^{D}Pro-Gly nucleated peptide **8** folds into a β-turn in methanol and chloroform solutions. However, in DMSO or D_2O:H_2O (1:1) solutions, no interchain NOEs could be detected, an observation in line with the well-known high flexibility and low folding propensity of small peptides. The more rigid diphenylacetylenic mimetic **9** formed a β-hairpin in both $CDCl_3$, DMSO, and methanol solutions.[13] The chemical shift alteration upon change of solvent suggested that compound **9** folded into a stable β-hairpin conformation, in which NH^{Ph} and NH^{CH3} were involved in intramolecular hydrogen bonding, while only a single interchain amide hydrogen bond (NH^{Ala}) was detectable for **8** ($\Delta\delta_{solv} = \delta_{DMSO} - \delta_{CDCl3}$ for the amide protons, which assumed the following values: $\Delta\delta_{solv}(NH^{Ph}) = 0.1$ (**9**); $\Delta\delta_{solv}(NH^{CH3}) = 0.01$ (**9**), 0.6 (**9**); $\Delta\delta_{solv}(NH^{Ala}) = 1.6$ (**9**), 0.1 (**8**); $\Delta\delta_{solv}(NH^{Val}) = 1.6$ (**9**), 0.4 (**8**); $\Delta\delta_{solv}(NH^{Gly}) = 0.8$ (**8**)). In the DMSO solution of the comparatively flexible non-switchable mimetic **10**, a non-folded structure was observed whereas in its methanol solution a complicated ^{1}H NMR spectrum was obtained, indicative for a mixture of low energy conformations. Hence, increased turn flexibility leads to decreased folding propensity.

In agreement with our computational studies, no sign of folding was observable for methanolic and DMSO solutions of neither *Z*-**1–3** (Table 1), nor for DMSO solutions of *Z*-**6** and *Z*-**7**. Here it should be noted that these model compounds are insoluble in chloroform, which should be taken in account when comparing computational and experimental results. Polar, hydrogen bond accepting and/or donating solvents are likely to interfere with intramolecular hydrogen bonding and thereby support the formation of unfolded conformations in solution.[32] Furthermore, water would not be an alternative choice of solvent for these compounds, since it is known to counteract the formation of, *e.g.*, β-turn motifs in small peptides.[33]

Investigations of the non-switchable model compounds **8–10** indicate that turn rigidity is a critical factor for hairpin folding. Thus, a thermodynamically stable β-hairpin conformation with two interchain hydrogen bonds was observed for the most rigid diphenylacetylene derivative **9**, but not for **10**.

Formation of one hydrogen bond (resulting in a β-turn) was indicated for the less rigid **8**, and no folding was observed for the flexible derivative **10**. If the flexibility is high, folding is disfavoured for entropic reasons.

We therefore conclude that the absence of any folded conformers in solutions of stilbene derivatives **2** and **3** is due to their flexible $(CH_2)_n$ linkers. Another factor likely to affect the formation of folded conformers is the orientation of the amide groups of the antiparallel chains of **1** and **2**. In native hairpins, hydrogen bonds were shown to be close to planar.[34] According to our computations, the N–H–O and N–O–C angles within the existing interchain hydrogen bonds, in partially folded single conformations, would be tilted for mimetics **1–2** (typically N–H–O < 155°, N–O–C < 135 °). On the other hand, the high flexibility of the $(CH_2)_2$ linkers of **3** simultaneously decreases its turn inducing properties through entropic factors, and allows its amino acid

strands to align themselves in optimal angles and distance for intramolecular hydrogen bonding. Thus, incorporation of the stilbene chromophore of **3** into a larger cyclic peptidomimetic resulted in a system for which photoisomerization between the *Z* and *E* configurations lead to reversible reorientation between a β-hairpin and random coil conformations.[12]

Conclusion

Synthetic strategies as well as conformationally directing properties of a set of small stilbene and thioaurone-based building blocks, aimed to be applied as photoswitchable nucleating cores of functional protein mimetics, have been investigated. Straightforward synthetic protocols were developed providing N-Boc and N-Fmoc protected amino acid analogues, which can be directly transferred to standard solid-phase peptide synthesis. Hence, using the proposed synthetic routes, building blocks resembling standard Fmoc- and Boc-protected amino acids can be prepared and straightforwardly applied for the synthesis of peptide and protein mimetics.

Theoretical conformational analysis indicated that these photochromic systems should be excellent conformational switching units when incorporated into a peptide environment. By small variation of chemical structure a wide range of different switching units were obtained. Amongst these, **1** is the most rigid, capable of reversible alternation between a turn-like motif and an open structure upon irradiation at 300 and 280 nm, respectively. Compounds **2** and **3** provide the possibility of photochemical switching between turn-like (*Z*, C_α–C_α < 5.5 Å) and open (*E*, C_α–C_α ~ 8.0 Å) geometries. The thioaurone derivatives **6** and **7** should provide access to more rigid open and turn-like motifs. Formation of turns under experimental conditions was not observed, as can be expected for small, linear peptides. However, by incorporation into suitable petidomimetic environments, they should have the potential to provide access to a variety of photoswitchable target molecules. This has recently been verified using a congener of **3**,[12] and further studies are currently in progress.

Experimental

Starting materials were purchased from commercial suppliers and were used without purification. Column chromatography was performed using Merck silica gel 60 (40–63μm) or Florisil (100–200 mesh). Thin-layer chromatography (TLC) was performed using aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm, E. Merck). Chromatographic spots were visualized by UV and/or spraying with an ethanolic solution of ninhydrin (2%), followed by heating.

Purification of the peptides was performed on a Gilson 321 HPLC system connected to a Vydac Protein & Peptide C18 (218TP) column (10 μm, 22 × 250 mm) using a gradient of MeCN in 0.1% aq. TFA (10–85% MeCN in 75 min) at a flow rate of 5 cm^3 min^{-1} and with detection by UV absorbance at 230 nm (LKB 2151 absorbance detector), or on a Gilson system (Gilson 231 XL Injector, 118 UV/VIS detector, Gilson 402 Syringe pump, Gilson 333 and 334 pumps and Gilson FC204) connected to a Grace Vydac C18 (22 × 250 mm, 5 µm) with a gradient of MeCN in 0.1% aq. TFA (20–60% MeCN in 60 min). The fractions were further analyzed by LC-MS using a Chromolith Performance RP-18e column (4.6 × 100 mm), or by analytical HPLC using a Chromolith Performance RP-18e column (4.6 × 100 mm). The procedure applied for microwave heating is described in ref 23. Photochemical reactions were performed on acetonitrile solutions under N_2 gas flow using an Oriel 1000 W Xe ARC light source and a 300 nm Oriel UV filter. The emitted light intensity was determined using a UV enhanced Si photodiode (5.8 mm^2) attached to a current meter. The thioaurone peptides **4–7** were isomerized for 15 min in an NMR-tube with a nonselective ACE Glass Incorporated 450 W UV-lamp connected to a 7830 Power supply. UV spectra were measured with a Varian Cary 3 spectrometer. IR spectra were obtained on a Perkin Elemer 1600 series FTIR instrument; recording 16 scans on 10 mol·dm^{-3} samples in $CHCl_3$ solution using a 1 mm cuvette (NaCl windows).

Mass spectra (EI, 70 eV) were obtained with a Hewlett Packard 5971 Series Mass Selective detector interfaced with a Hewlett Packard 5890 Series II Gas Chromatograph equipped with a DB-1 (25 m × 0.20 mm) capillary column. The ESI-MS data were obtained with a Finnigan ThermoQuest AQA mass spectrometer (ESI-MS 30eV, probe temperature 100 °C) equipped with a Gilson 322-H2 gradient pump system and an SB-C18 column. A water-acetonitrile-formic acid (0.05%) mobile phase was used with a gradient of 20% to 100% acetonitrile during 3–5 minutes. High resolution MS spectra were run on an Agilent LC/MSD TOF instrument (Agilent Technologies, Santa Clara, CA, USA). Detection was performed in positive ion mode. The voltage applied at the sampling capillary at the entrance of the mass spectrometer was 4.0 kV. Nitrogen at 300 °C and 7 L min^{-1} was used as drying gas, and nebuliser gas flow was at 15 L min^{-1}. Voltages fixed at fragmentor, skimmer and octopole guides were 215 V, 60 V and 250 V, respectively. The ion pulser at the TOF analyser was set up to a measurement frequency of 0.5 cycles/s. Agilent TOF software and Agilent QS software were used to record and analyse mass spectra, respectively. Standard autotune of masses was performed in the TOF-MS instrument before the experimental runs, and typical mass errors of 1 to 3 ppm were achieved in the calibration.

NMR spectra were recorded on a Varian UNITY INOVA spectrometer (^1H at 499.9 MHz), a Jeol JNM EX400 spectrometer (^1H at 399.8 MHz, ^{13}C at 100.5 MHz), a Varian UNITY spectrometer (^1H at 399.9 MHz, ^{13}C at 100.6 MHz), a Varian Mercury plus spectrometer (^1H at 300.0 MHz, ^{13}C at 75.4 MHz), or on a JEOL JNM EX270 spectrometer (^1H at 270.2 MHz, ^{13}C at 67.9 MHz). Data are reported in the following manner: chemical shift (integration, multiplicity, coupling constant if appropriate, assignment). Coupling constants (*J*) are reported in Hertz. Chemical shifts are referenced indirectly to TMS *via* the ^2H lock signal and the following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. NOE effects were measured from NOESY and ROESY spectra with mixing times between 0.4 and 1.5 s.

Melting points were determined in open capillaries using a Stuart Scientific melting point apparatus SMP10 and are uncorrected.

Amino acid analyses were performed at the Department of Biochemistry and Organic Chemistry, Biomedical Centre, Uppsala, Sweden, on 24 h hydrolyzates with an LKB 4151 Alpha Plus analyzer, using ninhydrin detection.

Conformational analysis was performed in MacroModel 9.5[26] using the OPLS-AA/L force field (OPLS 2005) and the $CHCl_3$ solvation model using the generalized Born solvent-accessible

surface area (GB/SA) method developed by Still *et al.*[25] The conformational search was conducted using the Systematic Unbound Multiple Minimum search method,[35] with 20,000 search steps. The number of torsion angles allowed to vary simultaneously during each search step ranged from 1 to n-1, where n is the total number of investigated torsion angles. Truncated Newton conjugate gradient (TNCG) energy minimization with a maximum of 500 iterations was used with the derivative convergence criterion set to 0.05 kJ mol^{-1} Å^{-1}. Two subsequent energy minimizations were performed on the found conformations using a derivative convergence criterion of 0.001 kJ mol^{-1} Å^{-1}, first using a maximum of 500 TNCG minimization steps, followed by a maximum of 10,000 PR conjugate gradient minimization steps. Conformations within 25 kJ mol^{-1} of the lowest energy minimum found were saved in the conformational search. The relative population of the found conformations was calculated using Eq 1,

$$\%population_i = \frac{e^{-\Delta E_i / RT}}{\sum_j e^{-\Delta E_j / RT}} \times 100 \quad (1)$$

where ΔE is the relative potential energy of the conformation, R is the universal gas constant and T is the temperature (T = 298 K was used in the calculations). The conformations were classified as folded or non-folded depending on interstrand hydrogen bond pattern and distance. Hydrogen bonds were defined as an acceptor atom and amide proton at a distance below 2.5 Å. The additional criteria for optimal hydrogen bonds in a beta sheet were defined as the angle N–H $\cdots$ O > 155° and angle N $\cdots$ O=C between 135–165°.[34] The criteria for folded conformations based on interstrand distance were C–C < 5.5 Å (see Table 1 for details).

E-Stilbene derivative (*E*-1)

A solution of 45.0 mg of **14** (0.14 mmol) in dichloromethane (1.0 cm^3) was treated with N,O-bis(trimethylsilyl)acetamide (0.07 cm^3, 0.28 mmol) for 30 minutes. 2-Acetylamino-3-methylbutyric acid (0.11 g, 0.70 mmol) was dissolved in a mixture of dichloromethane (2.0 cm^3), dimethylformamide (1.00 cm^3) and N,N-diisopropylethylamine (0.24 cm^3, 1.40 mmol). HATU (0.26 g, 0.70 mmol) was added and the mixture was stirred for 20 minutes. The two solutions were combined and the mixture was stirred for 18 h. Thereafter, the mixture was poured into 0.1 M HCl (10 cm^3) and was extracted three times with dichloromethane (10 cm^3). A precipitate was filtered off from the organic layer yielding a white solid (32.0 mg, 49%); mp 198–199 °C; amino acid analysis: Ala, 1.00; Val, 1.00; λ_{max} (CH_3OH)/nm 246, 292; $[\alpha]_D^{20}$ +13.45 (c 0.001 in MeOH); δ_H (499.5 MHz; CD_3OD:CH_3OH (1:1)) 1.03 (6H, 2d, *J* 6.8, CH_3^V), 1.46 (3H, d, *J* 7.2, CH_3^A), 2.03 (3H, s, CH_3^{CO}), 2.14 (1H, m, CH_β^V), 2.76 (3H, d, *J* 4.0, CH_3^{NH}), 4.28 (1H, dd, *J* 6.8, 7.7, CH_α^V), 4.55 (1H, dq, *J* 7.0, 7.2, CH_α^A), 7.25 (1H, d, *J* 16.5, CH), 7.26 (1H, d, *J* 16.5, CH), 7.31 (1H, t, *J* 7.9, ArH), 7.33 (1H, m, ArH), 7.43 (1H, m, ArH), 7.46 (1H, t, *J* 7.7, ArH), 7.71 (1H, m, ArH), 7.76 (1H, ddd, *J* 1.1, 1.6, 7.7, ArH), 7.85 (1H, dd, *J* 0.9, 1.5, ArH), 8.0 (1H, q, J 4.0, NH^{CH3}), 8.10 (1H, t, *J* 1.6, ArH), 8.18 (1H, d, *J* 6.8, NH^V), 8.54 (1H, d, *J* 7.2, NH^A), 9.96 (1H, br s, NH^{Ph}); δ_C (75 MHz; DMSO-d_6) 18.6, 19.1, 19.8, 23.0, 26.2, 31.1, 49.6, 59.4, 117.9, 119.6, 122.2, 125.9, 127.5, 128.6, 129.2, 129.7, 129.8, 130.0, 135.1, 137.4, 137.9, 139.8, 166.4, 170.0, 171.1, 173.2; *m/z* (ESI-MS, 30 eV) 465 [M + H]$^+$, 434, 255.

Z-Stilbene derivative (*Z*-1)

A solution of *E*-**1** in acetonitrile (24.00 cm^3, 2.0 mmol/dm^3 solution) was irradiated at 300 nm for 2 h under nitrogen gas flow. The solution was concentrated under reduced pressure resulting in 22.0 mg of a 7:3 mixture of the *cis*:*trans* isomers. Quantum yield: 5.7%. δ_H (499.5 MHz; CD_3OD:CH_3OH (1:1)) 0.96 (6H, 2d, *J* 6.4, CH_3^V), 1.41 (3H, d, *J* 7.4, CH_3^A), 2.01 (3H, s, CH_3^{CO}), 2.14 (1H, m, CH_β^V), 2.75 (3H, d, *J* 4.9, CH_3), 4.23 (1H, dd, *J* 7.3, 7.6, CH_α^V), 4.49 (1H, dq, *J* 7.0, 7.6, CH_α^A), 6.68 (1H, d, *J* 12.2, CH), 6.70 (1H, d, *J* 12.2, CH), 6.96 (1H, d, *J* 7.8, ArH), 7.19 (1H, t, *J* 7.8, ArH), 7.30 (1H, t, *J* 8.3, ArH) 7.36 (1H, d, *J* 7.8, ArH), 7.37 (1H, d, *J* 8.3, ArH), 7.47 (1H, br s, ArH), 7.68 (1H, d, *J* 8.3, ArH), 7.77 (1H, br s, ArH), 8.3 (1H, d, *J* 7.6, NH^A), 7.93 (1H, q, *J* 4.9, NH^{CH3}), 8.11 (1H, d, *J* 7.6, NH^V), 9.84 (1H, s, NH^{Ph}); *m/z* (ESI-MS, 30 eV) 465 [M + H]$^+$, 434, 255.

E-Stilbene derivative (*E*-2)

Compound **20** was incorporated into standard solid-phase peptide synthesis techniques using 4-nitro-benzophenoneoxime resin (1.1 mmol/g) on a 0.55 mmol scale. Standard Boc-Alanine or N-acetyl-Valine and PyBOP mediated couplings were applied. The Boc groups were deprotected with 50% trifluoroacetic acid in dichloromethane and cleavage was performed with 2 M methylamine in THF, purification by HPLC yielding white crystals (17.4 mg, 7%); amino acid analysis: Ala, 1.04; Val, 0.96; UV: λ_{max} (CH_3OH)/nm 203, 232, 297, 310, 323 nm; $[\alpha]_D^{20}$ −5.7° (c 0.008 in MeOH); δ_H (499.5 MHz; CD_3OD:CH_3OH (1:1)) 0.97 (3H, d, *J* 6.6, CH_3^V), 0.98 (3H, d, *J* 6.6, CH_3^V), 1.35 (3H, d, *J* 6.9, CH_3^A), 2.02 (3H, s, CH_3^{CO}), 2.10 (1H, dh, *J* 6.6, 7.4, CH_β^V), 2.73 (3H, d, *J* 2.7, CH_3^{NH}), 3.61 (1H, d, *J* 14.5, CH_2^{CO}), 3.61 (1H, d, *J* 14.5, CH_2^{CO}), 4.19 (1H, dd, *J* 7.4, 8.1, CH_α^V), 4.33 (1H, dq, *J* 6.6, 7.4, CH_α^A), 4.40 (1H, d, *J* 6.0, 15.0, CH_2^{NH}), 4.43 (1H, d, *J* 6.0, 15.0, CH_2^{NH}), 7.17 (1H, d, *J* 16.0, CH), 7.20 (1H, d, *J* 16.0, CH), 7.21 (2H, 2dd, *J* 7.5, 7.5, ArH), 7.31 (2H, 2t, *J* 7.5, ArH), 7.44 (2H, 2dt, *J* 1.5, 7.5, ArH), 7.51 (2H, 2t, *J* 1.5, ArH), 7.88 (1H, br q, *J* 2.7, NH^{CH3}), 8.05 (1H, d, *J* 8.1, NH^V), 8.28 (1H, d, *J* 6.6, NH^A), 8.56 (1H, t, *J* 6.0, NH^{CH2}); δ_C (75 MHz, CD_3OD:CH_3OH (1:1)) 17.0, 17.5, 18.5, 21.3, 25.2, 30.5, 42.2, 42.7, 49.4, 59.5, 125.0, 125.3, 125.4, 126.7, 127.1, 128.3, 128.6 (2C), 128.7 (2C), 136.0, 137.9, 139.1, 146.7, 172.3, 172.5, 174.4, 175.8; *m/z* (ESI-MS, 30 eV) 986 [2M + H]$^+$, 494 [M + H]$^+$, 258.

Z-Stilbene derivative (*Z*-2)

A solution of 8.2 mg (16.60 μmol) *E*-**2** in acetonitrile was irradiated at 300 nm for 1.5 h under nitrogen gas flow. The solution was concentrated under reduced pressure resulting in a 8.3:1.7 mixture of the *cis*:*trans* isomers. Quantum yield: 3.9%. δ_H (499.5 MHz; CD_3OD:CH_3OH (1:1)) 0.89 (6H, 2d, *J* 6.8, CH_3^V), 1.29 (3H, d, *J* 6.0, CH_3^A), 1.98 (1H, dh, *J* 6.8, 7.5, CH_β^V), 2.00 (3H, s, CH_3^{CO}), 2.72 (3H, d, *J* 5.0, CH_3^{NH}), 3.45 (1H, d, *J* 14.5, CH_2^{CO}), 3.48 (1H, d, *J* 14.5, CH_2^{CO}), 4.16 (1H, dd, *J* 7.5, 8.5, CH_α^V), 4.23–4.32 (3H, m, CH_2^{NH}, CH_α^A), 6.58 (1H, d, *J* 12.6, CH), 6.60 (1H, d, *J* 12.6, CH), 7.07–7.18 (8H, m, ArH), 7.88 (1H, br q, *J* 5.0, NH^{CH3}), 7.97 (1H, d, *J* 8.1, NH^V), 8.20 (1H, d, *J* 6.0, NH^A), 8.44 (1H, t, *J* 5.8, NH^{CH2}).

E-Stilbene derivative (*E*-3)

Stilbene derivative **27** was incorporated into solid-phase peptide synthesis using 4-nitro-benzophenoneoxime resin (1.1 mmol/g) on a 0.55 mmol scale. Boc-Ala, N-acetyl-Valine and PyBOP mediated couplings were applied. The Boc groups were deprotected with 50% trifluoroacetic acid in dichloromethane and cleavage was performed with 2 M methylamine in THF, purification on HPLC yielded white crystals (33.6 mg, 12%); amino acid analysis: Val, 1.04; Ala, 0.96; UV: λ_{max} (CH_3OH)/nm 205, 232, 298, 310, 323; $[\alpha]_D^{20} = -11.9°$ (c 0.004 in MeOH); δ_H (499.5 MHz; CD_3OD:CH_3OH (1:1)) 0.88 (3H, d, *J* 7.0, CH_3^V) 0.89 (3H, d, *J* 7.0, CH_3^V), 1.25 (3H, d, *J* 7.0, CH_3^A), 1.98 (3H, s, CH_3^{CO}), 2.01 (1H, dh, *J* 7.0, 7.4, CH_β^V), 2.57 (2H, dt, *J* 2.5, 8.3, CH_2^{CH2CO}), 2.69 (3H, d, *J* 4.5, CH_3^{NH}), 2.83 (2H, t, *J* 7.4, CH_2^{CH2NH}), 2.94 (2H, t, *J* 7.4, CH_2^{CO}), 3.51 (2H, m, CH_2^{NH}), 4.09 (1H, dd, *J* 7.4, 8.5, CH_α^V), 4.29 (1H, dq, *J* 5.5, 7.0, CH_α^A), 7.12 (2H, 2d, *J* 8.0, ArH), 7.15 (2H, m, CH), 7.27 (2H, 2t, *J* 8.0, ArH), 7.39 (2H, 2d, *J* 8.0, ArH), 7.42 (2H, br s, ArH), 7.64 (1H, br q, *J* 4.5, NH^{CH3}), 7.94, (1H, d, *J* 8.5, NH^V), 8.09 (2H, m, NH^A, NH^{CH2}); δ_C (75 MHz; CD_3OD:CH_3OH (1:1)) 17.1, 17.5, 18.7, 21.5, 25.4, 30.6, 31.5, 35.3, 37.4, 40.6, 49.1, 59.4, 124.4, 124.5, 126.5, 126.9, 127.7, 128.1, 128.5, 128.6, 128.72, 128.74, 137.80, 137.81, 139.7, 141.4, 172.1, 172.5, 173.8, 174.4; *m*/*z* (ESI-MS, 30 eV) 1042 $[2M + H]^+$, 521 $[M + H]^+$, 450.

Z-Stilbene derivative (*Z*-3)

A solution of compound *E*-**3** (18.1 mg) dissolved in acetonitrile was irradiated at 300 nm for 1.5 h under nitrogen gas flow. The solution was concentrated under reduced pressure resulting in a 49:51 mixture of the *cis*:*trans* isomers. Quantum yield: 7.8%. δ_H (499.5 MHz; CD_3OD:CH_3OH (1:1)) 0.89 (3H, d, *J* 6.5, CH_3^V), 0.90 (3H, d, *J* 6.5, CH_3^V), 1.25 (3H, d, *J* 7.5, CH_3^A), 1.98 (3H, s, CH_3^{CO}), 2.01 (1H, dh, *J* 6.5, 7.4, CH_β^V), 2.46 (1H, dt, *J* 2.5, 8.3, CH_2^{CH2CO}), 2.70 (3H, d, *J* 4.5, CH_3^{NH}), 2.83 (1H, t, *J* 7.4, CH_2^{CH2NH}), 2.94 (2H, t, *J* 7.4, CH_2^{CO}), 3.44 (2H, m, CH_2^{NH}), 4.09 (1H, dd, *J* 7.4, 8.5, CH_α^V), 4.29 (1H, dq, *J* 5.5, 7.5, CH_α^A), 6.57 (1H, d, *J* 13.2, CH), 6.59 (1H, d, *J* 13.2, CH), 7.05 (4H, m, ArH), 7.09 (2H, 2t, *J* 1.5, 6.5, ArH), 7.13–7.15 (2H, m, ArH), 7.71 (1H, br q, *J* 4.5, NH^{CH3}), 7.94 (1H, d, *J* 7.2, NH^V), 8.03 (1H, br t, *J* 5.0, NH^{CH2}), 8.06 (1H, d, *J* 7.5, NH^A).

Z-*para*-Thioaurone derivative (*Z*-4)

Compound **34** was incorporated into standard solid-phase peptide synthesis techniques using 2-chlorotrityl chloride resin (1.4 mmol/g) on 0.5 mmol scale. Standard Fmoc-protected monomers and PyBOP mediated couplings were applied. The Fmoc groups were deprotected with 20% piperidine in DMF and cleavage from resin was performed with 0.5% TFA in dichloromethane. After purification on HPLC and lyophilization a yellow fluffy solid was obtained (9.0 mg, 8%); amino acid analysis: Val, 0.92; Ala, 1.08; δ_H 499.9 MHz; DMSO-d_6) (0.85 (1H, dd, *J* 1.7, 6.9, CH_γ^V), 1.46 (1H, d, *J* 7.4, CH_β^A), 1.89 (3H, s, CH_3), 1.99 (1H, dq, *J* 6.9, CH_β^V), 4.16 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.34 (2H, d, *J* 6.1, CH_2), 4.48 (1H, q, *J* 7.4, CH_α^A), 7.45 (2H, XX′ part of AA′XX′, ArH), 7.55 (1H, t, *J* 7.7, ArH), 7.78 (2H, AA' part of AA′XX′, ArH), 7.87 (1H, s, C=CH), 7.91 (1H, d, *J* 8.5, NH^V), 8.05 (1H, dd, *J* 1.0, 7.7, ArH), 8.40 (1H, dd, *J* 1.2, 7.7, ArH), 8.55 (1H, br t, *J* 6.3, NH), 9.08 (1H, d, *J* 7.2, NH^A), 12.66 (1H, br s, OH^A); *m*/*z* (ESI-MS, 30 eV) 523.90 $[M + H]^+$, 540.95.

E-*para*-Thioaurone derivative (*E*-4)

The chemical shifts were elucidated from a spectrum with a 0.6:1 mixture of Z:E. δ_H (499.9 MHz; DMSO-d_6) 0.85 (1H, dd, *J* 1.7, 6.9, CH_γ^V), 1.43 (1H, d, *J* 7.4 CH_β^A), 1.89 (3H, s, CH_3), 1.98 (1H, dq, *J* 6.9, CH_β^V), 4.16 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.34 (2H, t, *J* 6.7, CH_2), 4.46 (1H, q, *J* 7.4, CH_α^A), 7.32 (2H, XX′ part of AA′XX′, ArH), 7.50 (1H, t, *J* 7.7, ArH), 7.59 (1H, s, C=CH), 7.89 (1H, d, *J* 8.5, NH^V), 8.16 (2H, AA' part of AA′XX′, ArH), 8.37 (1H, dd, *J* 1.0, 7.7, ArH), 8.51 (1H, br t, *J* 6.3, NH), 9.02 (1H, d, *J* 7.2, NH^A), 12.64 (1H, br s, OH^A).

Z-*meta*-Thioaurone derivative (*Z*-5)

Compound **40** was incorporated into standard solid-phase peptide synthesis techniques using 2-chlorotrityl chloride resin (1.4 mmol/g) on 0.5 mmol scale. Standard Fmoc-protected monomers and PyBOP mediated couplings were applied. The Fmoc groups were deprotected with 20% piperidine in DMF and cleavage from resin was performed with 0.5% TFA in dichloromethane. After purification on HPLC and lyophilization a yellow, fluffy solid was obtained (13.0 mg, 5%); amino acid analysis: Val, 0.92; Ala, 1.08; δ_H (499.9 MHz; DMSO-d_6) 0.85 (1H, dd, *J* 1.7, 6.9, CH_γ^V), 1.46 (1H, d, *J* 7.4, CH_β^A), 1.89 (3H, s, CH_3), 1.99 (1H, dq, *J* 6.9, CH_β^V), 4.16 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.33 (1H, dd, *J* 5.8, 15.4, CH_2), 4.37 (1H, dd, *J* 5.8, 15.4, CH_2), 4.48 (1H, q, *J* 7.4, CH_α^A), 7.39 (1H, m, ArH), 7.55 (1H, t, *J* 7.6, ArH), 7.56 (1H, t, *J* 7.6, ArH), 7.66 (1H, m, ArH), 7.73 (1H, m, ArH), 7.83 (1H, s, C=CH), 7.91 (1H, d, *J* 8.7, NH^V), 8.05 (1H, dd, *J* 1.0, 7.6, ArH), 8.41 (1H, dd, *J* 1.0, 7.6, ArH), 8.57 (1H, br t, *J* 5.8 Hz, NH), 9.08 (1H, d, *J* 7.4, NH^A), 12.66 (1H, br s, OH^A); *m*/*z* (ESI-MS, 30 eV) 523.90 $[M + H]^+$, 540.95.

E-*meta*-Thioaurone derivative (*E*-5)

The chemical shifts were elucidated from a spectrum with a 0.3:1 mixture of Z:E. δ_H (499.9 MHz; DMSO-d_6) 0.83 (1H, dm, *J* 6.9, CH_γ^V), 1.43 (1H, d, *J* 0.7, 7.4, CH_β^A), 1.87 (3H, d, *J* 0.9,CH_3), 1.99 (1H, dq, *J* 6.9, CH_β^V), 4.16 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.32 (1H, d, *J* 5.8, CH_2), 4.46 (1H, q, *J* 7.4, CH_α^A), 7.34 (1H, dm, *J* 7.6, ArH), 7.40 (1H, t, *J* 7.6, ArH), 7.50 (1H, t, *J* 7.6, ArH), 7.57 (1H, s, C=CH), 7.89 (1H, d, *J* 8.7, NH^V), 7.94 (1H, dt, *J* 1.0, 7.6, ArH), 7.95 (1H, m, ArH), 8.10 (1H, dm, *J* 8.05, ArH), 8.37 (1H, dt, *J* 1.0, 7.6, ArH), 8.51 (1H, br t, *J* 5.8, NH), 9.01 (1H, d, *J* 7.4, NH^A), 12.66 (1H, br s, OH^A).

Z-*para*-Thioaurone derivative (*Z*-6)

Compound **34** was incorporated into standard solid-phase peptide synthesis techniques using {3-[(methyl-Fmoc-amino)-methyl]-indol-1-yl}-acetyl AM resin (0.63 mmol/g) on 0.3 mmol scale. Standard Fmoc-protected monomers and PyBOP mediated couplings were applied. The Fmoc groups were deprotected with 20% piperidine in DMF and cleavage from resin was performed with 5% TFA in dichloromethane. After purification on HPLC and lyophilization a yellow, fluffy solid was obtained (29.0 mg,

17%); amino acid analysis: Val, 0.88; Ala, 1.12; δ_H (499.9 MHz; DMSO-d_6) 0.86 (1H, dd, *J* 2.2, 6.9, CH_γ^V), 1.37 (1H, d, *J* 7.4, CH_β^A), 1.89 (3H, s, CH_3), 1.99 (1H, q, *J* 6.9, CH_β^V), 2.62 (3H, d, *J* 4.6, CH_3), 4.17 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.34 (2H, d, *J* 6.0, CH_2), 4.48 (1H, q, *J* 7.4, CH_α^A), 7.55 (1H, t, *J* 7.7, ArH), 7.45 (2H, XX′ part of AA′XX′), 7.78 (2H, AA′ part of AA′XX′), 7.87 (1H, s, C=CH), 7.93 (1H, d, *J* 8.7, NH^V), 7.94 (1H, q, *J* 4.6, NH^{cap}), 8.04 (1H, dd, *J* 1.3, 7.7, ArH), 8.40 (1H, dd, *J* 1.3, 7.7, ArH), 8.57 (1H, br t, *J* 6.0, NH), 8.93 (1H, d, *J* 7.4, NH^A); *m/z* (ESI-MS, 30 eV) 537.11 $[M + H]^+$, 554.15, 1073.18 $[2M + H]^+$, 1090.22.

E-para-Thioaurone derivative (*E*-6)

The chemical shifts were elucidated from a spectrum of a 0.6:1 mixture of Z:E. δ_H (499.9 MHz; DMSO-d_6) 0.86 (1H, dd, *J* 2.2, 6.9, CH_γ^V), 1.35 (1H, d, *J* 7.4, CH_β^A), 1.88 (3H, s, CH_3), 1.98 (1H, q, *J* 6.9, CH_β^V), 2.60 (3H, d, *J* 4.6, CH_3), 4.16 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.3 (2H, d, J 6.0, CH_2), 4.46 (1H, q, *J* 7.4, CH_α^A), 7.32 (2H, XX′ part of AA′XX′), 7.48 (1H, t, *J* 7.7, ArH), 7.58 (1H, s, C=CH), 7.92 (1H, d, *J* 8.7, NH^V), 7.94 (1H, q, *J* 4.6, NH^{cap}), 7.94 (1H, dd, *J* 1.3, 7.7, ArH), 8.16 (2H, AA′ part of AA′XX′), 8.42 (1H, dd, *J* 1.3, 7.7, ArH), 8.54 (1H, br t, *J* 6.0, NH), 8.85 (1H, d, *J* 7.4, NH^A).

Z-meta-Thioaurone derivative (*Z*-7)

Compound **40** was incorporated into standard solid-phase peptide synthesis techniques using {3-[(methyl-Fmoc-amino)-methyl]-indol-1-yl}-acetyl AM resin (0.63 mmol/g) on 0.3 mmol scale. Standard Fmoc-protected monomers and PyBOP mediated couplings were applied. The Fmoc groups were deprotected with 20% piperidine in DMF and cleavage from resin was performed with 5% TFA in dichloromethane. After purification on HPLC and lyophilization a yellow, fluffy solid was obtained (29.0 mg, 11%); amino acid analysis: Val, 0.87; Ala, 1.12; δ_H (499.9 MHz; DMSO-d_6) 0.84 (1H, dd, *J* 6.9, 1.0, CH_γ^V), 1.37 (1H, d, *J* 7.4, CH_β^A), 1.89 (3H, s, CH_3), 2.01 (1H, q, *J* 6.9, CH_β^V), 2.62 (3H, d, *J* 4.6, CH_3), 4.15 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.37 (2H, dd, *J* 6.0, 15.4, CH_2), 4.33 (2H, dd, *J* 6.0, 15.4, CH_2), 4.48 (1H, q, *J* 7.4, CH_α^A), 7.39 (1H, dm, *J* 7.6, ArH), 7.55 (1H, t, *J* 7.6, ArH), 7.67 (1H, m, ArH), 7.73 (1H, dm, *J* 7.6, ArH), 7.83 (1H, s, C=CH), 7.92 (1H, d, *J* 8.7, NH^V), 7.94 (1H, q, *J* 4.6, NH^{cap}), 8.04 (1H, dd, *J* 1.2, 7.6, ArH), 8.45 (1H, dd, *J* 1.2, 7.6, ArH), 8.57 (1H, br t, *J* 6.0, NH), 8.93 (1H, d, *J* 7.4, NH^A); *m/z* (ESI-MS, 30 eV) 537.11 $[M + H]^+$, 554.15, 1073.18 $[2M + H]^+$, 1090.22.

E-*meta*-Thioaurone derivative (*E*-7)

The chemical shifts were elucidated from a spectrum of a 0.6:1 mixture of Z:E. δ_H (499.9 MHz; DMSO-d_6) 0.83 (dd, 1H, *J* 6.9, 1.0, CH_γ^V), 1.35 (1H, d, *J* 7.4, CH_β^A), 1.87 (3H, s, CH_3), 1.99 (1H, q, *J* 6.9, CH_β^V), 2.62 (3H, d, *J* 4.6, CH_3), 4.16 (1H, dd, *J* 6.9, 6.9, CH_α^V), 4.32 (2H, d, *J* 6.0, CH_2), 4.46 (1H, q, *J* 7.4, CH_α^A), 7.34 (1H, dm, *J* 7.6, ArH), 7.40 (1H, t, *J* 7.6, ArH), 7.49 (1H, t, *J* 7.6, ArH), 7.56 (1H, s, C=CH), 7.89 (1H, d, *J* 8.7, NH^V), 7.94 (1H, q, *J* 4.6, NH^{cap}), 7.94 (1H, dd, *J* 1.2, 7.6, ArH), 7.96 (1H, m, ArH), 8.10 (1H, dm, *J* 7.6, ArH), 8.42 (1H, dd, *J* 7.6, 1.2, ArH), 8.51 (1H, br t, *J* 6.0, NH), 8.85 (1H, d, *J* 7.4, NH^A).

CH_3CO-V-^{D}P-G-A-$NHCH_3$ (8)

The peptide **8** was prepared by standard solid-phase peptide synthesis techniques using 4-nitro-benzophenone-oxime resin (1.1 mmol/g) on 0.55 mmol scale with standard Boc-protected monomers or N-acetyl-Valine and PyBOP mediated couplings. For deprotection of the Boc groups 50% trifluoroacetic acid in dichloromethane was applied and cleavage was performed with 2M methylamine in THF, purification on HPLC yielding a colorless oil (85.0 mg, 39%); amino acid analysis: Val, 1.04; Pro, 1.01; Gly, 0.96; Ala, 0.99; $[\alpha]_D^{20}$ –9.97° (c 0.02 in MeOH); δ_H (499.5 MHz; CD_3OD:CH_3OH (1:1),) 0.97 (3H, d, *J* 6.5, CH_3^V), 1.00 (3H, d, *J* 6.5, CH_3^V), 1.42 (3H, d, *J* 7.0, CH_3^A), 2.00 (3H, s, CH_3^{CO}), 2.06 (1H, dh, *J* 6.5, 7.0, CH_β^V), 2.10–2.28 (4H, m, CH_β^P, $CH_{\beta'}^P$, CH_γ^P, $CH_{\gamma'}^P$), 2.70 (3H, d, *J* 4.0, CH_3^{NH}), 3.71 (1H, m, $CH_{\delta'}^P$), 3.79 (1H, dd, *J* 6.0, 17.0, $CH_{\alpha'}^G$), 3.86 (1H, dd, *J* 6.0, 17.0, CH_α^G), 3.94 (1H, m, CH_δ^P), 4.2 (1H, m, CH_α^P), 4.21 (1H, dq, *J* 7.0, 7.5, CH_α^A), 4.44 (1H, dd, *J* 7.0, 7.5, CH_α^V), 7.66 (1H, q, *J* 4.0, NH^{CH3}), 7.95 (1H, d, *J* 7.5, NH^A), 8.15 (1H, d, *J* 7.5, NH^V), 8.44 (1H, t, *J* 6.0, NH^G); δ_C (75 MHz; $CDCl_3$) 16.7, 19.1, 19.4, 22.4, 25.0, 26.4, 29.5, 30.4, 43.1, 48.1, 49.1, 58.2, 61.6, 170.1, 172.6, 172.7, 173.0, 173.5; *m/z* (ESI-MS, 30 eV) 796 $[2M + H]^+$, 398 $[M + H]^+$, 341.

Diphenylacetylene derivative (10)

Compound **46** was incorporated into standard solid-phase peptide synthesis techniques 4-nitro-benzophenoneoxime resin (1.1 mmol/g) on 0.55 mmol scale. Standard Boc-protected monomers or N-acetyl-Valine and PyBOP mediated couplings were applied. The Boc groups were deprotected with 50% trifluoroacetic acid in dichloromethane and cleavage was performed with 2M methylamine in THF, purification on HPLC and by recrystallization from a mixture of methanol, acetonitrile and trifluoroacetic acid yielding white crystals (76.0 mg, 28%); mp 246–248 °C; amino acid analysis: Val, 1.00; Ala, 1.00; $[\alpha]_D^{20}$ = 27.8° (c 0.001 in MeOH); δ_H (499.9 MHz; DMSO-d_6) 0.83 (6H, 2d, *J* 6.5, CH_3^V), 1.14 (3H, d, *J* 7.0, CH_3^A), 1.86 (3H, s, CH_3^{CO}), 1.97 (1H, dh, *J* 6.0, 7.5, CH_β^V), 2.54 (3H, d, *J* 5.4, CH_3^{NH}), 3.74 (1H, d, *J* 15.5, CH_{2b}^{CO}), 3.79 (1H, d, *J* 15.5, CH_{2a}^{CO}), 3.94 (1H, m, CH_δ^P), 4.20 (1H, m, CH_α^P), 4.20 (1H, dd, J 7.5, 9.0, CH_α^V), 4.23 (1H, dq, *J* 7.0, 7.2, CH_α^A), 4.47 (1H, dd, *J* 6.1, 15.5, NH^{CH2b}), 4.52 (1H, dd, *J* 6.1, 15.5, NH^{CH2a}), 7.79 (1H, q, *J* 5.4, NH^{CH3}), 7.93 (1H, d, *J* 9.0, NH^V), 8.20 (1H, d, J 7.2, NH^A),8.50 (1H, t, *J* 6.1, NH^{CH2}); δ_C (75 MHz; DMSO-d_6) 18.9, 19.1, 20.0, 26.2, 30.9, 35.0, 48.9, 58.6, 70.5, 91.4, 93.2, 121.8, 123.4, 127.4, 127.6, 127.9, 129.4, 130.6, 132.6, 132.7, 138.7, 141.1, 141.2, 169.9, 170.1, 172.1, 173.2; *m/z* (ESI-MS, 30 eV) 982 $[2M + H]^+$, 491 $[M + H]^+$, 420.

Methyl-3-vinyl-benzoate (11)

3-Vinyl-benzoic acid (2.00 g, 13.50 mmol) was dissolved in methanol (100 cm^3). Concentrated HCl (1.15 cm^3, 13.50 mmol) was added dropwise to the stirred solution. The reaction mixture was refluxed for 14 h, then the solvent was removed by rotary evaporation. The residual was dissolved in a mixture of water (20 cm^3) and diethyl ether (20 cm^3) and the phases were allowed to separate. The water phase was reextracted two times. The combined ether phases were concentrated on a rotary evaporator

giving 3-vinyl-benzoic acid methyl ester as an oil (1.94 g, 88%); δ_H (270.2 MHz; $CDCl_3$) 3.92 (3H, s, CH_3), 5.31 (1H, d, *J* 11.0, CH_2), 5.82 (1H, d, *J* 18.0, CH_2), 6.74 (1H, dd, *J* 11.0, 18.0, CH), 7.39 (1H, dd, *J* 8.9, 9.2, ArH), 7.58 (1H, d, *J* 9.2, ArH), 7.92 (1H, d, *J* 8.9, ArH), 8.08 (1H, br s, ArH); δ_C (67.9 MHz; $CDCl_3$) 56.6, 119.5, 131.7, 133.0, 133.2, 134.8, 134.9, 140.3, 142.2, 171.4; *m/z* (EI, 70 eV) 162 $[M + H]^+$, 131, 117, 103.

E-3-(3′-Amino-phenylethenyl)-benzoic acid methyl ester (12)

Methyl-3-vinyl-benzoate (1.00 g; 6.17 mmol), 3-iodoaniline (0.65 cm^3, 5.61 mmol), $Pd(OAc)_2$ (50.00 mg, 0.22 mmol), triethylamine (1.40 cm^3, 9.81 mmol), and dimethylformamide (1.50 cm^3) was added to a Smith Process Vial and was heated to 150 °C for 20 min with microwave irradiation. The procedure was repeated two times and the reaction mixtures were combined. The mixture was filtered through Celite to a separation funnel with dichloromethane (25 cm^3). 0.1 M HCl (25 cm^3) was added and the phases were allowed to separate. The aqueous phase was reextracted two times. Thereafter water (25 cm^3) was added to the combined organic layers. The water phase was extracted with three portions of dichloromethane (25 cm^3) before the organic layers were concentrated on a rotary evaporator. The residue was purified by column chromatography using hexane:ethyl acetate:dichloromethane: triethylamine (3:1:1:0.05) eluent mixture. The fractions containing product were concentrated on a rotary evaporator giving *E*-3-(3-amino-phenylethenyl)-benzoic acid methyl ester as yellow solid (2.38 g, 56%); ν_{max} ($CHCl_3$)/cm^{-1} 3451, 3401, 3027, 2977, 1718, 1619, 1288, 1238; UV: (CH_3OH)/nm 232, 294; δ_H (270.2 MHz; $CDCl_3$) 3.92 (3H, s, CH_3), 6.62 (1H, ddd, *J* 1.0, 2.1, 7.8, ArH), 6.82 (1H, t, *J* 2.1, ArH), 6.92 (1H, dd, *J* 1.7, 7.6, ArH), 7.05 (1H, d, *J* 17.6, CH), 7.06 (1H, d, *J* 17.6, CH), 7.14 (1H, t, *J* 7.8, ArH), 7.39 (1H, t, *J* 7.7, ArH), 7.63 (1H, dt, *J* 1.0, 7.8, ArH), 7.86 (1H, dt, *J* 1.7, 7.6, ArH), 8.12 (1H, t, *J* 1.7, ArH); δ_C (67.9 MHz; $CDCl_3$) 52.1, 113.7, 115.6, 118.2, 127.3, 127.4, 128.3, 128.6, 129.5, 129.8, 130.3, 130.8, 137.5, 137.9, 145.1, 167.2; *m/z* (EI, 70 eV) 253, 220, 193, 177, 165. HRMS, *m/z* = 210.1248 ($[M + H]^+$), $C_{15}H_{16}N$ requires 210.1282.

E-3-(3′-Amino-phenylethenyl)-benzoic acid (13)

3-(3′-Amino-phenylethenyl)-benzoic acid methyl ester (600.0 mg, 2.37 mmol) and aqueous 6M NaOH (50 cm^3) were stirred in a round bottom flask at 100 °C for 6 h. The heating was turned off and the reaction mixture was stirred at room temperature for 14 h. The mixture was poured into water in a separation funnel and the pH was decreased to 7 using 5 M HCl (aq). A yellow solid was filtered off and by adding additional HCl the pH was increased to 4, yielding yellow precipitate (280.0 mg, 98%); δ_H (270.2 MHz; CD_3OD) 6.63 (1H, ddd, *J* 1.0, 2.3, 7.8, ArH), 6.90 (1H, dt, *J* 1.5, 7.6, ArH), 6.95 (1H, t, *J* 2.3, ArH), 7.08 (1H, t, *J* 7.8, ArH), 7.11 (1H, d, *J* 16.5, CH), 7.12 (1H, d, *J* 16.5, CH), 7.33 (1H, t, *J* 7.6, ArH), 7.55 (1H, dt, *J* 1.0, 7.8, ArH), 7.81 (1H, dt, *J* 1.5, 7.6, ArH), 8.12 (1H, t, *J* 1.5, ArH); δ_C (67.9 MHz; CD_3OD) 113.1, 114.9, 116.8, 126.9, 127.7, 127.8, 127.9, 128.0, 129.0 (2C), 137.2, 138.4, 138.3, 147.6, 174.0; *m/z* (ESI-MS, 30 eV) 240 $[M + H]^+$, 214. HRMS, *m/z* = 240.0970 ($[M + H]^+$), $C_{15}H_{14}NO_2$ requires 240.1025.

E-3-[2′-(3″-Amino-phenyl)-vinyl]-N-(1‴″-methyl-carbamoyl-ethyl)-benzamide (14)

3-(3′-Amino-phenylethenyl)-benzoic acid (0.20 g, 0.84 mmol) was dissolved in DIEA (1.50 cm^3) and dichloromethane (15 cm^3). PyBOP (0.87 g, 1.67 mmol) was added and the mixture was stirred for 2 minutes. Then, a solution of 2-amino-*N*-methyl-propionamide (170.0 mg, 1.67 mmol), diisopropyl-ethylamine (0.50 cm^3), dichloromethane (2.5 cm^3) and dimethylformamide (5 cm^3) was added and the mixture was stirred for 2 h. The reaction mixture was poured into 0.1 M HCl (10 cm^3). The mixture was extracted three times with dichloromethane (10 cm^3). The combined organic layers were washed with conc. aqueous $NaHCO_3$ (10 cm^3), re-extracting the aqueous phase twice with dichloromethane. Thereafter, water (10 cm^3) was added to the organic layer. The water phase was extracted with three portions of dichloromethane (10 cm^3) before the organic layers were concentrated on a rotary evaporator. The residue was purified by column chromatography using ethyl acetate:diethylamine:methanol (1:0.01:0.01) eluent mixture. The residual was precipitated from chloroform yielding a white solid (45.00 mg, 17%); δ_H (270.2 MHz; $CDCl_3$) 1.44 (3H, d, *J* 6.9, CH_3^{NH}), 2.76 (3H, s, CH_3^{CO}), 4.65 (1H, q, *J* 6.9, CH_α^A), 6.62 (1H, ddd, *J* 1.0, 2.0, 7.9, ArH), 6.83 (1H, t, *J* 2.0, ArH), 6.90 (1H, ddd, *J* 1.0, 2.0, 7.9, ArH), 7.00 (1H, d, *J* 16.6, CH), 7.01 (1H, d, *J* 16.6, CH), 7.11 (1H, t, *J* 7.9, ArH), 7.34 (1H, t, *J* 7.8, ArH), 7.38 (1H, br q, *J* 6.9 NH^{CH3}), 7.55 (1H, ddd, *J* 1.2, 1.7, 7.8, ArH), 7.64 (1H, ddd, *J* 1.2, 1.7, 7.8, ArH), 7.68 (1H, d, *J* 6.9 NH^A), 7.91 (1H, t, *J* 1.7, ArH); δ_C (67.9 MHz; $CDCl_3$) 18.2; 26.0, 49.0, 113.3, 115.3, 117.7, 125.1, 125.9, 127.3, 128.7, 129.5, 129.6, 129.9, 133.8, 137.7, 137.8, 145.8, 167.3, 173.3; *m/z* (ESI-MS, 30 eV) 324 $[M + H]^+$, 293, 265, 222.

3-Iodobenzylamine (15)

A mixture of 3-iodobenzyl bromide (5.00 g, 16.84 mmol), sodium bis(trimethylsilyl)amide (9.26 g, 50.51 mmol) and hexamethyldisilazan (24.86 cm^3, 117.87 mmol) was stirred for 72 h. The crude mixture was extracted with conc. $NaHCO_3$ and CH_2Cl_2, re-extracting the water phase three times. Then, the combined organic phases were extracted with aqueous HCl (5M, pH = 2). A precipitate was formed that could be dissolved in a mixture of $CHCl_3$ and 1M HCl. By adding NaOH pellets, the pH of the combined water phase was increased to 14, and thereafter it was extracted with CH_2Cl_2. The organic phase was filtered through $MgSO_4$ and concentrated under reduced pressure yielding a yellowish oil (2.47 g, yield: 63%); ν_{max} (neat)/cm^{-1} 3280, 3051, 2853, 1589, 1561, 1061, 994, 880, 769, matching literature data;[36] δ_H (270.2 MHz; $CDCl_3$) 1.53 (2H, s, NH_2), 3.64 (2H, s, CH_2), 6.92 (1H t, *J* 7.7, ArH), 7.12 (1H ddd, *J* 0.7, 1.8, 7.7 ArH), 7.43 (1H ddd, *J* 0.7, 0.6, 7.7, ArH), 7.54 (1H dd, *J* 0.6, 1.8, ArH); δ_C (100.5 MHz; $CDCl_3$) 45.8, 94.9, 126.0, 130.0, 135.8, 136.1, 145.7; *m/z* (EI, 70 eV) 233 $[M + H]^+$, 217, 127, 106, 77.

3-Vinylbenzylamine (16)

3-Iodobenzylamine (0.50 g, 2.15 mmol), tributylvinyltin (1.02 g, 3.22 mmol), $PdCl_2(PPh_3)_2$ (30.1 mg, 0.04 mmol), lithium chloride (272.8 mg, 6.44 mmol) and DMF (1.5 cm^3) were mixed in a Smith Process Vial and irradiated at 130 °C for 20 minutes with microwaves, repeating the procedure five times (the total amount

of 3-iodobenzylamine used being 2.47 g). The combined products from five runs were filtered through Celite and extracted with conc. aqueous $NaHCO_3$. Thereafter, the organic phase was extracted with 1M aqueous HCl and the water phase was kept. By adding NaOH pellets, the pH of the aqueous solution was increased to 14, followed by extraction with CH_2Cl_2. The organic phase was filtered through $MgSO_4$ and concentrated under reduced pressure yielding a yellowish oil (1.18 g, 84%); δ_H (270.2 MHz; $CDCl_3$) 1.66 (2H, s, NH_2), 2.71 (2H, s, CH_2), 5.14 (1H, d, *J* 10.9 CH_2), 5.67 (1H, d, *J* 18.0, CH_2), 6.61 (1H, dd, *J* 10.9, 18.0, CH), 7.0–7.2 (3H, m, ArH), 7.83 (1H, br s, ArH); δ_C (67.9 MHz; $CDCl_3$) 45.3, 112.8, 123.9, 125.7, 127.6, 127.8, 135.8, 136.6, 142.6. *m/z* (ESI-MS, 30 eV) 132 $[M + H]^+$, 115, 105, 77, 51.

(3-Vinyl-benzyl) carbamic acid *tert*-butyl ester (17)

3-Vinylbenzylamine (1.18 g, 8.91 mmol) was dissolved in 15 cm^3 CH_2Cl_2 and was mixed with an aqueous solution of di-*tert*-butyldicarbonate (2.46 g, 26.73 mmol) and potassium carbonate (3.69 g, 26.73 mmol). The reaction mixture was stirred for 3 h, then an additional 2.46 g di-*tert*-butyldicarbonate was added and the mixture was stirred for a further 72 h. The organic phase was separated and extracted with water, and the water phase was three times re-extracted with dichloromethane. Thereafter the product was purified on a Silica 60 column using hexane:ethylacetate (9:1) eluent mixture, yielding a yellowish oil (805.9 mg, 68%); ν_{max} $(CHCl_3)/cm^{-1}$ 3345, 2974, 2923, 1699, 1514, 1370, 1252, 1168; δ_H (270.2 MHz; $CDCl_3$) 1.44 (9H, s, CH_3^{Boc}), 4.25 (2H, s, CH_2), 5.2 (1H, s, NH), 5.22 (1H, d, *J* 17.9, CH), 5.72 (1H d, *J* 10.9, CH), 6.68 (1H, dd, *J*, 10.9, 17.9, CH), 7.15 (1H, m, ArH), 7.28–7.30 (3H, m, ArH); δ_C (67.9 MHz, $CDCl_3$) 28.1 (3C), 44.3, 79.1, 113.8, 124.8, 125.0, 126.6, 128.5, 136.4, 137.5, 139.1, 155.8; *m/z* (ESI-MS, 30 eV) 467 $[2M + H]^+$, 260, 234 $[M + H]^+$, 219.

Methyl-3-iodophenyl acetic acid (18)[37]

3-Iodophenylacetic acid (2.26 g, 8.62 mmol) was stirred in a mixture of 15 cm^3 methanol and 10 drops of concentrated aqueous HCl for 24 h. The solvent was evaporated, the residue dissolved in dichloromethane and washed with water, then filtrated through $MgSO_4$ and concentrated under reduced pressure yielding a colorless oil (2.34 g, 98%); ν_{max} $(CHCl_3)/cm^{-1}$ 3054, 2993, 2948, 1727, 1560; δ_H (399.8 MHz; $CDCl_3$) 3.52 (2H, s, CH_2), 3.64 (3H s, CH_3), 7.00 (1H, ddd, *J* 0.5, 7.8, 8.1, ArH), 7.21 (1H, d, *J* 1.2, 1.7, 7.8, ArH), 7.55 (1H, ddd, *J* 1.2, 2.2, 8.1, ArH), 7.61 (1H, ddd, *J* 0.5, 1.7, 2.2, ArH); δ_C (100.5 MHz, $CDCl_3$) δ_C 40.6, 52.3, 94.7, 128.8, 130.4, 136.3, 136.4, 138.3, 171.4; *m/z* (EI, 70 eV) 276 $[M + H]^+$, 217, 107, 90.

Methyl-(3-{2′-[3″-(*tert*-butoxycarbonylamino-methyl)-phenyl]-vinyl}-phenyl)-acetate (19)

Methyl-3-iodophenyl acetic acid (830.7 mg, 3.01 mmol), (3-vinyl-benzyl)-carbamic acid *tert*-butyl ester (637.4 mg, 2.73 mmol), $Pd(OAc)_2$ (18.4 mg, 0.08 mmol), tri-o-tolyl-phosphine (49.9 mg, 0.16 mmol), triethylamine (1.40 cm^3; 1.61 mmol), and dimethyl-formamide (1.50 cm^3) were stirred in a Smith Process Vial at 90 °C for 15 min in the microwave cavity. The resulting mixture was filtered through Celite into a separation funnel, then extracted with dichloromethane (25 cm^3) and 0.1 M HCl (25 cm^3), re-extracting the aqueous phase twice. The combined organic layers were washed three times with water (25 cm^3) and concentrated under reduced pressure. The residue was purified by column chromatography using hexane:ethyl acetate:dichloromethane:triethylamine, (3:1:1:0.05) eluent mixture, yielding a yellowish oil (696.9 mg, 70%); ν_{max} $(CHCl_3)/cm^{-1}$ 3446, 3032, 2971, 2894, 1731, 1501, 1251, 1171; δ_H (399.8 MHz; $CDCl_3$) 1.41 (9H, s, CH_3^{Boc}), 3.56 (2H, br s, CH_2^{NH}), 3.62 (3H, s, CH_3), 4.23 (2H, s, CH_2^{CO}), 5.25 (1H, br s, NH), 6.80–7.10 (2H, m, ArH), 7.09 (2H, 2d, *J* 6.8, ArH), 7.20–7.25 (2H, m, ArH), 7.27–7.35 (4H, m, ArH); δ_C (100.5 MHz, $CDCl_3$) 28.4 (3C), 41.1, 44.6, 52.0, 79.4, 125.4, 125.5, 125.6, 126.8, 127.5, 128.5, 128.6, 128.8, 128.9, 129.0, 134.5, 137.5, 137.6, 139.7, 156.1, 171.9; *m/z* (ESI-MS, 30 eV) 763 $[2M + H]^+$, 707, 663, 607, 382 $[M + H]^+$, 367.

(3-{2′-[3″-(*tert*-Butoxycarbonylaminomethyl)-phenyl]-vinyl}-phenyl) acetic acid (20)

A solution of **19** (969.6 mg, 1.82 mmol) in 50 cm^3 6 M aqueous NaOH solution was refluxed for 24 h. Thereafter, the solution was acidified to pH = 3 with 5.0 M aqueous HCl and extracted with CH_2Cl_2. The organic phase was filtered through $MgSO_4$ and concentrated under reduced pressure yielding a white solid (230.8 mg, 0.63 mmol, 34%); ν_{max} $(CHCl_3)/cm^{-1}$ 3446, 3026, 2977, 2934, 1703, 1497, 1234; δ_H (399.8 MHz; $CDCl_3$) 1.48 (9H, s, CH_3^{Boc}), 3.67 (2H, s, CH_2^{NH}), 4.33 (2H, s, CH_2^{CO}), 4.94 (1H, br s, NH), 7.08 (2H, m, 2CH), 7.18 (2H, d, *J* 7.2, ArH), 7.28–7.34 (2H, m, ArH), 7.37–7.43 (4H, m, ArH), 9.77 (1H, br s, COOH); δ_C (100.5 MHz, $CDCl_3$) 28.5 (3C), 41.2, 44.8, 79.8, 125.6, 125.7, 125.8, 126.9, 127.6, 128.7, 128.8, 128.9, 129.0, 129.1, 134.0, 137.6, 137.7, 139.4, 156.1, 177.1; *m/z* (ESI-MS, 30 eV) 757 $[2M + Na]^+$, 735 $[2M + H]^+$, 390 $[M + Na]^+$, 353, 312.

4-(N-*tert*-Butoxycarbonylaminomethyl)benzoic acid (28)[22a]

To a solution of 4-(aminomethyl)benzoic acid (10.00 g, 66.2 mmol), 1,4-dioxan (125 cm^3), water (60 cm^3) and 1 M NaOH (64 cm^3, 64 mmol) were added. To the mixture di-*tert*-butylpyrocarbonate (15.9 g, 72.8 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 40 min, then stirred at r.t. for another 30 min. The volume of the solution was reduced to 70 cm^3, then ethyl acetate (150 cm^3) was added. The mixture was acidified to pH 2 with 5 M HCl (20 cm^3). The white precipitate was dissolved in ethyl acetate and the solution was washed with water. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield a white solid. Recrystalization from ethyl acetate (120 cm^3) gave **28** as white colorless needles (13.5 g, 81%); mp 167–168 °C (Lit.:[22a] 167–168 °C); δ_H (499.9 MHz; $CDCl_3$) 1.47 (9H, s, $(CH_3)_3$), 4.40 (2H, d, *J* 6.1, CH_2), 4.94 (1H, br s, NH), 7.38 (2H, m, XX′ part of AA′XX′), 8.07 (2H, m, AA' part of AA′XX′); δ_C (125 MHz; $CDCl_3$) 28.5, 44.5, 80.1, 127.4, 128.4, 130.7, 145.3, 156.1, 171.0.

4-(N-*tert*-Butoxycarbonylaminomethyl)benzyl alcohol (29)[22a]

Lithiumaluminium hydride (0.40 g, 10.35 mmol) was suspended in dry THF (70 cm^3). The protected benzoic acid **28** was slowly added and the reaction mixture was stirred over night at 40 °C. The reaction was quenched by addition of water and the pH was adjusted to 5 by addition of acetic acid. The organic phase was

separated and the remaining aqueous phase was extracted with diethyl ether (4 × 50 cm^3). Unreacted carboxylic acid **28** was removed by extracting the combined organic phases with aqueous $NaHCO_3$. The combined organic layers were dried over anhydrous Na_2SO_4, filtrated and evaporated under reduced pressure to give a white solid (1.09 g, 44%); mp 100–101 °C (Lit.:[22a] 100–101 °C); δ_H (399.9 MHz; $CDCl_3$) 1.47 (9H, s, $(CH_3)_3$); 4.31 (2H, d, *J* 6.4, CH_2), 4.68 (2H, s, CH_2OH), 4.88 (1H, br s, NH), 7.28 (2H, m, XX′part of AA′XX′), 7.34 (2H, m, AA′part of AA′XX′); δ_C (100.5 MHz; $CDCl_3$) 28.6, 44.6, 79.7, 65.2, 127.4, 127.8, 138.5, 140.2, 156.1.

4-(N-*tert*-Butoxycarbonylaminomethyl)benzaldehyde (30)[22d]

The alcohol **29** (1.24 g, 5.23 mmol) was dissolved in dry dichloromethane (25 cm^3). Sodium acetate (0.12 g, 1.46 mmol) followed by PCC (0.97 g, 4.49 mmol) were added to the solution. The black mixture was stirred in the dark for 15 h. Diethyl ether was added (30 cm^3) and the mixture was triturated and filtered through a pad of cellite. The filtrate was washed with water (50 cm^3) and the resulting aqueous phase was extracted with ether (70 cm^3). The combined organic layers were dried over anhydrous Na_2SO_4, filtrated and evaporated under reduced pressure. The resulting greenish solid was purified by flash chromatography (20% EtOAc to 50% in pentane and 5% TEA) to give a off white solid (0.9 g, 72%); mp 83–84 °C (Lit.:[22d] 82–84 °C); δ_H (399.9 MHz, $CDCl_3$) 1.45 (9H, s, $(CH_3)_3$), 4.39 (2H, s, CH_2NH), 5.01 (1H, br s, NH), 7.44 (2H, m, XX′ part of AA′XX′), 7.84 (2H, m, AA′ part of AA′XX′), 10.0 (1H, s, CHO); δ_C (100.5 MHz; $CDCl_3$) 29.0, 44.9, 80.3, 128.1, 130.5, 136.0, 146.6, 156.2, 192.6.

o-Carboxyphenylmercaptoacetic acid (31)[21a]

To a solution of anhydrous sodium carbonate (20 g) in water (150 cm^3), 2,2-dithiodisalicylic acid (10 g, 32.6 mmol) and sodium dithionite (15 g, 86.2 mmol) were added. The mixture was refluxed for 30 min under the formation of sulfur dioxide. A solution of chloroacetic acid (15 g, 159 mmol) was neutralized with sodium carbonate, then added to the reaction mixture and refluxing was continued for 1 h. The mixture was allowed to cool to room temperature before acidified with hydrochloric acid (conc.) against Congo red. The yellow precipitate was recrystallized from H_2O, removing the green Congo red particles by filtration of the warm solution. Yellow powdery solid (9.77 g, 71%); mp 219–221 °C (Lit.:21[a] 217–218 °C); δ_H (399.8 MHz; CD_3OD) 3.77 (1H, s, CH_2); 5.01 (2H, br s, CH_2COOH), 7.21 (1H, ddd, *J* 1.3, 7.2, 7.8, ArH), 7.43 (1H, ddd, *J* 0.5, 1.3, 8.2 ArH), 7.48 (1H, ddd, *J* 1.6, 7.2, 8.2, ArH), 7.98 (1H, ddd, *J* 0.5, 1.6, 7.8, ArH); δ_C (100.5 MHz; CD_3OD) 34.5, 124.2, 125.7, 128.1, 131.4, 132.4, 140.8, 168.5 (CO), 172.1 (CO).

3-Oxo-2,3-dihydro-benzo[b]thiophene-7-carbonylchloride (32)[21b]

Following a general literature procedure,[21b] *o*-carboxyphenyl mercaptoacetic acid **31** (3.00 g, 14.1 mmol) was refluxed in $SOCl_2$ (18 cm^3) under the formation of SO_2. The excess of $SOCl_2$ was removed by distillation under reduced pressure. The resulting gold coloured acid chloride was dissolved in 1,2-dichloroethane (30 cm^3). To the cold solution (< 0 °C), $AlCl_3$ was added in 4 portions (4.70 g, 35.2 mmol). The green mixture was stirred under nitrogen with cooling another 20 min, then stirred at r.t. for 14 h. The reaction was quenched with ice/water and the orange mixture was extracted with DCM (3 × 50 cm^3). The DCM solution was washed with water, dried over anhydrous Na_2SO_4, filtrated and evaporated to give the product as an orange solid (2.44 g, 81%); mp 110–111 °C; δ_H (499.9 MHz; $CDCl_3$) 8.06 (1H, dd, *J* 1.3, 7.7, H-5), 7.43 (t, 1H, *J* 7.7, H-6), 3.83 (s, 2H, H-2), 8.54 (1H, dd, *J* 1.3, 7.7, H-4), δ_C (100.6 MHz; $CDCl_3$) 39.9, 125.3, 128.7, 132.7, 133.2, 141.0, 158.3, 166.2, 198.9 (COCl).

2-[1′-{4″-(*tert*-Butoxycarbonylamino-methyl)-phenyl}-meth-(*Z*)-ylidene]-3-oxo-2,3-dihydro-benzo[b]thiophene-7-carboxylic acid (33)[10b]

To a mixture of thioindoxyl **32** (3.94 g, 18.53 mmol), 1% NaOH w/w (110 cm^3) and *t*-BuOH (19 cm^3), 4-(N-*tert*-butoxyaminomethyl)benzaldehyde **30** (2.08 g, 8.82 mmol) in *t*-BuOH (27 cm^3) was added at 0 °C. The orange mixture was refluxed over night under nitrogen atmosphere. The mixture was cooled to r.t. and acidified with acetic acid (pH 1–2). The reaction mixture was extracted with ethyl acetate (3 × 50 cm^3). The combined organic phases were washed with water, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an orange solid. After flash chromatography (Florisil, 1:1 EtOAc:pentane and 1% HOAc) the thioaurone **33** was obtained as an orange solid (3.0 g, 84%); δ_H (499.9 MHz; CD_3OD) 1.47 (9H, s, $(CH_3)_3$), 4.30 (1H, br s, CH_2), 7.33 (1H, t, *J* 7.5, H-5), 7.42 (2H, m, XX′ part of AA′XX′), 7.81 (2H, m, AA′part of AA′XX′), 7.86 (1H, s, C=CH), 7.94 (1H, dd, *J* 1.4, 7.5, H-4), 8.27 (1H, dd, *J* 1.4, 7.5, H-6), δ_C (125 MHz; CD_3OD) 28.8, 44.9, 96.8, 126.2, 128.7, 128.9, 132.4, 132.6, 134.0, 134.3, 134.6, 134.8, 137.8, 143.7, 149.1, 153.2 (Boc-CO), 172.3 (COOH), 191.3 (CO).

2-[1′-{4″-[(9‴H-Fluoren-9‴-ylmethoxycarbonylamino)-methyl]-phenyl}-meth-(*Z*)-ylidene]-3-oxo-2,3-dihydro-benzo[b]thiophene-7-carboxylic acid (34)[38]

Thioaurone derivative **33** (2.4 g, 5.8 mmol) was stirred with 50% TFA in DCM (40 cm^3) for 15 min. The solution was concentrated, yielding a dark red oily residue. The remains were mixed with a mixture of 10% aq Na_2CO_3 and THF (80 cm^3) and 9-fluorenylmethyl chloroformate (1.8 g, 7.0 mmol) was added. After three days of stirring at r.t., the pH was adjusted to pH 1 by the addition of HCl (conc.). The mixture was extracted with DCM and the emulsions were removed by centrifugation. The combined organic phases were dried over anhydrous Na_2SO_4, filtrated and evaporated to give an orange solid. After flash chromatography (Florisil, gradient of $CHCl_3$ with 0.1% MeOH to 1:1 MeOH:THF) the product **34** was obtained yellow-orange solid (2.8 g, 90% yield over two steps); δ_H (499.9 MHz; CD_3OD) 4.23 (1H, t, *J* 7.2, Fmoc-CH), 4.34 (2H, br s, CH_2NH), 4.46 (2H, d, *J* 7.2, Fmoc-CH_2), 7.33–7.33 (2H, m, ArH), 7.36–7.43 (5H, m, ArH) 7.67 (2H, d, *J* 7.6, Fmoc-ArH), 7.81 (2H, d, *J* 7.6, Fmoc-ArH), 7.80 (2H, m, AA′part of AA′XX′), 7.87 (1H, s, C=CH), 8.00 (1H, dd, *J* 1.4, 7.6 Hz ArH), 8.28 (1H, dd, *J* 1.4, 7.6 ArH, H7); δ_C (125 MHz; CD_3OD) 45.1, 48.8 (CH_2), 67.6 (OCH_2), 120.9, 126.1, 126.3, 128.1, 128.7, 128.8, 129.0, 132.5, 132.7, 134.0 (=CH), 134.2, 134.5, 134.8, 137.8, 142.7, 143.2, 145.3, 149.1, 159.0 (CO) 172.3 (CO), 191.2 (CO).

3-(1,3-dioxolan-2-yl)benzonitrile (35)[10a]

3-Cyanobenzaldehyde (2.50 g, 19.1 mmol) was dissolved in toluene (70 cm^3) and treated with ethylene glycol (7.50 cm^3, 134 mmol) followed by PTSA (2.70 g, 14.3 mmol). The solution was refluxed in a Dean Stark apparatus for 18 h. After cooling to r.t. the solution was washed with 5% aq $NaHCO_3$ (50 cm^3). The yellow organic phase was separated and dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow oil. The residue was purified by bulb to bulb distillation (140 °C, 0.7 mbar) and the colourless oil obtained was dissolved in diethyl ether and washed with a 10% aq sodium bisulfite solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give colourless oil (2.00 g, 60%, 92% purity according to the ^1H NMR spectra); δ_H (499.9 MHz; $CDCl_3$) 4.04–3.93 (4H, m, CH_2), 5.73 (1H, s, CH), 7.41–7.38 (1H, m, ArH), 7.57–7.54 (1H, m, ArH), 7.64–7.62 (1H, m, ArH), 7.70 (1H, m, ArH); δ_C (125 MHz; $CDCl_3$) 65.4, 102.2, 112.4, 118.5, 129.2, 130.2, 130.9, 132.6, 139.7.

3-(1,3-dioxolan-2-yl)benzylamine (36)[10a]

Lithium aluminium hydride (0.50 g, 13.3 mmol) was mixed with dry THF (40 cm^3) under nitrogen at 0 °C. The benzonitrile (2.00 g, 12.0 mmol) was dissolved in THF (2.5 cm^3) and added dropwise to the suspension with a speed of 0.8 cm^3/h. The mixture was stirred over night at r.t., before quenching with water (0.50 cm^3), 15% aq NaOH (0.50 cm^3) and water (1.50 cm^3). The mixture was extracted with diethyl ether and the aqueous phase was extracted with more diethyl ether (3×20 cm^3). The combined organic layers were washed with water, then brine, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to give a yellow oil (0.85 g, 40%); δ_H (499.9 MHz; $CDCl_3$) 3.82 (2H, br s, CH_2NH), 4.12–3.95 (m, 4H, CH2), 5.76 (s, 1H, CH), 7.41–7.27 (m, 4H, ArH); δ_C (125 MHz; $CDCl_3$) 46.1, 65.2, 103.5, 124.8, 124.9, 127.8, 128.5, 138.1, 143.4.

***tert*-Butyl[3-(1,3-dioxolan-2-yl)benzyl]carbamate (37)**[10a]

The 3-(1,3-dioxolan-2-yl)benzylamine **36** (3.92 g, 21.9 mmol) was dissolved in anhydrous MeOH (35 cm^3), before the addition of triethyl amine (3.1 cm^3, 21.9 mmol). The yellow solution was cooled to 0 °C before the addition of di-*t*-butyl dicarbonate (6.21 g, 28.4 mmol). The solution was stirred for a couple of days at r.t. under nitrogen. The mixture was diluted with some diethyl ether and the organic phase was separated and washed with saturated NH_4Cl. The organic layers were washed with water, then brine, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo* to give a light yellow oil. The product **37** was recrystallized from diethyl ether and pentane (2:1) (4.93 g, 81%); δ_H (499.9 MHz; $CDCl_3$) 1.46 (9H, s, $(CH_3)_3$), 4.15- 4.00 (4H, m, 2 x CH_2), 4.32 (2H, br d, *J* 5.7, CH_2NH), 4.86 (1H, br s, NH), 5.79 (1H, s, CH), 7.41–7.27 (4H, m, ArH); δ_C (125 MHz; $CDCl_3$) 28.9 (CH_3), 45.1, 65.8 (CH_2), 79.8, 103.9 (CH), 126.0 (2C), 128.6, 138.6, 138.9, 139.5, 155.9 (CO).

***tert*-Butyl(3-Formylbenzyl)carbamate (38)**[10a]

The carbamate **37** (1.47 g, 5.26 mmol) was dissolved in acetone (16 cm^3) to form a yellow solution. After the addition of water (2 cm^3) and a catalytic amount of PTSA (3 mg, 15.8 μmol) the solution was stirred over night at 30 °C under nitrogen. After evaporation of the solvents, the residu was dissolved in DCM (20 cm^3) and washed with 10% w/w aq. $NaHCO_3$. The organic layers were washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo, yielding a slighly yellowish oil. After purification by flash chromatography (silica, 1:1 EtOAc:pentane), compound **38** was obtained as a solid (0.79 g, 64%); δ_H (499.9 MHz; $CDCl_3$) 1.43 (9H, s, $(CH_3)_3$), 4.35 (2H, d, *J* 6.0, CH_2NH), 5.16 (1H, br s, NH), 7.46 (1H, m, ArH), 7.53 (1H, m, ArH), 7.74 (2H, m, ArH), 9.96 (1H, s, CHO); δ_C (125 MHz; $CDCl_3$) 28.4 (CH_3), 44.1 (CH_2), 79.8 (quart. C), 128.2, 128.8, 129.3, 133.5, 136.7 (ipso C), 140.5 (ipso C), 156.0 (CO), 192.3 (CHO).

2-[1′-{3″-(*tert*-Butoxycarbonylamino-methyl)-phenyl}-meth-(*Z*)-ylidene]-3-oxo-2,3-dihydro-benzo[b]thiophene-7-carboxylic acid (39)[10a]

To a mixture of thioindoxyl **32** (3.11 g, 14.6 mmol), 1% NaOH w/w (87 cm^3) and *t*-BuOH (14.5 cm^3), *t*-Butyl(3-Formylbenzyl)carbamate 38 (1.64 g, 6.97 mmol) in *t*-BuOH (14.5 cm^3) was added at 0 °C. The orange mixture was refluxed over night under nitrogen atmosphere. The mixture was cooled to r.t. and acidified with acetic acid (pH 1–2). The red precipitate was filtrated and purified by flash chromatography (Florisil, 20% MeOH in EtOAc) to give the thioaurone **39** as an orange solid (1.29 g, 45%); δ_H (499.9 MHz; CD_3OD) 1.46 (9H, s, $(CH_3)_3$), 4.30 (2H, br s, CH_2), 7.35 (1H, dd, *J* 2.5, 7.6, ArH), 7.36 (1H, t, *J* 7.5, ArH), 7.45 (1H, t, *J* 7.6, ArH), 7.67 (1H,br s, ArH), 7.75 (1H, br d, *J* 7.6, ArH) 7.83 (1H, s, C=CH), 7.93 (1H, dd, *J* 1.4, 7.5, ArH), 8.29 (1H, dd, *J* 1.4, 7.5, ArH); δ_C (100.6 MHz; CD_3OD) 29.8, 44.9, 80.4, 126.2, 129.1, 129.9, 130.1, 130.5, 131.3, 132.6, 134.0, 134.2, 134.9, 136.2, 137.9, 142.1, 149.2, 158.5 (CO), 172.4 (COOH), 191.2 (CO).

2-[1′-{3″-[(9‴H-Fluoren-9‴-ylmethoxycarbonylamino)-methyl]-phenyl}-meth-(*Z*)-ylidene]-3-oxo-2,3-dihydro-benzo[b]thiophene-7-carboxylic acid (40)

Thioaurone derivative **39** (1.0 g, 2.43 mmol) was stirred with 50% TFA in DCM (10 cm^3) for 15 min. The solution was concentrated, yielding a dark red oily residue. The remains were mixed with a mixture of 10% aq Na_2CO_3 and THF (18 cm^3) and 9-fluorenylmethyl chloroformate (0.75 g, 2.88 mmol) was added. After three days of stirring at r.t., the pH was adjusted to pH 1 by the addition of HCl (conc.). After separation of the organic phase, the aqueous phase was extracted with DCM. The combined organic phases were dried over anhydrous Na_2SO_4, filtrated and evaporated to give an orange solid. After flash chromatography (Florisil, gradient of $CHCl_3$ with 0.1% MeOH to 1:1 MeOH:THF) the product **40** was obtained as a yellow solid (0.9 g, 70% yield over two steps); mp 203–205 °C; ν_{max} (neat)/cm^{-1} 3326, 1599, 1403, 1262, 1046, 758; δ_H (499.9 MHz; 1:3 CD_3OD:$CDCl_3$) 4.20 (1H, br t, *J* 6.5, Fmoc-CH), 4.37 (2H, br s, Fmoc-CH_2), 4.42 (2H, d, *J* 6.9, CH_2), 7.26–7.23 (2H, m, ArH), 7.35–7.31 (3H, m, ArH), 7.39 (1H, t, *J* 7.6, ArH), 7.43 (1H, t, *J* 7.6, ArH), 7.60–7.58 (2H,m, ArH), 7.62 (1H, br s, ArH), 7.72–7.68 (3H, m, ArH), 7.91 (1H, s, C=CH),

8.07 (1H, dd, *J* 1.2, 7.6, ArH), 8.30 (1H, dd, *J* 1.3, 7.6 Hz, ArH); δ_C (100.6 MHz; 1:3 CD_3OD:$CDCl_3$) 46.2, 49.1 (CH_2), 68.5 (OCH_2), 121.6, 126.7, 127.1, 129.8, 129.4, 131.0, 131.1, 131.6, 131.9, 132.0, 132.2, 133.8, 133.9, 133.9, 136.3, 136.4, 139.0, 141.7, 143.1, 145.6, 150.5, 159.0 (CO), 169.3 (CO), 190.7 (CO).

2-Iodobenzylamine (41)

2-Iodobenzyl bromide (5.00 g, 16.84 mmol), N,N-bistrimethylsilylamide (9.26 g, 50.50 mmol) and hexamethyldisilazane (25 cm^3) were mixed and stirred at room temperature for 40 h. The reaction mixture was transferred to a separation funnel with dichloromethane (25 cm^3) and was extracted three times with conc. $NaHCO_3$ (aq) (25 cm^3). 5 M HCl (25 cm^3) was added to the dichloromethane phase and the mixture was extracted three times. NaOH pellets were added slowly to the combined acidic layers until pH 11. The basic aqueous layer was extracted three times with dichloromethane (25 cm^3). Thereafter the combined organic layers were concentrated on a rotary evaporator giving 2-iodobenzylamine as brown oil (1.95 g, 50%); ν_{max} ($CHCl_3$)/cm^{-1} 3051, 2957, 1588, 1467, 1432, 1011; δ_H (270.2 MHz $CDCl_3$) 2.43 (2H, br s, NH_2), 3.86 (2H, s, CH_2), 6.94 (1 H, ddd, *J* 1.2, 6.7, 7.9, ArH), 7.32 (1 H, ddd, *J* 2.3, 6.7, 7.9, ArH), 7.37 (1H, dd, *J* 2.3, 7.9, ArH), 7.82 (1H, dd, *J* 1.2, 7.9, ArH); δ_C (67.5 MHz $CDCl_3$) 51.1, 127.9, 128.5, 128.6, 128.7, 139.4, 144.5; *m/z* (EI, 70 eV) 233 $[M + H]^+$, 127, 106, 77, 51.

2-Iodobenzyl-carbamoyl acid *tert*-butyl ester (42)

2-Iodobenzylamine (1.75 g, 7.50 mmol) dissolved in dichloromethane (20 cm^3) was mixed with K_2CO_3 (3.11 g, 22.49 mmol) in water (20 cm^3), then di-tert-butyldicarbonate (9.82 g, 44.98 mmol) was added and stirred at room temperature for three days. The organic phase was separated and the water phase was extracted with dichloromethane (20 cm^3) twice. The dichloromethane phase was concentrated and purified by column chromatography yielding yellowish crystals (1.49 g, 60%); ν_{max} ($CHCl_3$)/cm^{-1} 3446, 3015, 1708, 1522, 1497, 1218, 1167; δ_H (270.2 MHz; $CDCl_3$) 1.45 (9H, s, CH_3^{Boc}), 4.32 (2H, s, CH_2), 5.03 (1H, br s, NH), 6.96 (1H, t, *J* 7.9, ArH), 7.29–7.37 (2H, m, ArH), 7.81 (1H, d, *J* 7.9, ArH); δ_C (67.9 MHz; $CDCl_3$) 28.4 (3C), 49.3, 79.6, 128.5, 129.1 (2C), 132.7, 139.3, 140.9, 155.6; *m/z* (ESI-MS, 30 eV) 667 $[2M + H]^+$, 360, 334 $[M + H]^+$, 319, 275.

(2-Ethynyl-benzyl)-carbamoyl acid *tert*-butyl ester (43)

A mixture of **42** (0.40 g, 1.20 mmol), ethynyl-trimethyl-silane (0.19 cm^3, 1.32 mmol), $Pd(PPh_3)_2Cl_2$ (16.80 mg, 23.94 μmol), CuI (9.10 mg, 47.78 μmol), diethylamine (1.50 cm^3) and dimethylformamide (0.50 cm^3) was added to a Smith Process Vial and was stirred at 120 °C for 6 min in the microwave cavity. The combined products of three runs were filtered through celite into a separation funnel. Dichloromethane (25 cm^3) and conc. $NaHCO_3$ (aq) (25 cm^3) was added and the phases were separated, re-extracting the aqueous phase twice. Thereafter, the organic phase was washed with water (25 cm^3), re-extracting the water phase three times. The organic phase was concentrated and purified by flash chromatography using hexane:ethyl acetate (9:1) eluent mixture yielding (2-trimethylsilylethynyl-benzyl)-carbamoyl acid *tert*-butyl ester as a brown oil (1.07 g, 98%); ν_{max} ($CHCl_3$)/cm^{-1} 3450, 3016, 2976, 2401, 2154, 1713, 1505, 1218; δ_H (270.2 MHz; $CDCl_3$) 0.25 (9H, s, $Si(CH_3)_3$); 1.43 (9H, s, CH_3^{Boc}), 4.43 (2H, br d, *J* 5.6, CH_2), 5.13 (1H, br s, NH), 7.17–7.32 (3H, m, ArH), 7.42 (1H, dd, *J* 1.3, 7.3, ArH); δ_C (67.9 MHz; $CDCl_3$) δ_C 0.22 (3C), 28.2 (3C), 43.1, 79.1, 99.2, 102.7, 121.7, 126.8, 127.5, 128.7, 132.2, 140.9, 155.7; *m/z* (ESI-MS, 30 eV) 304 $[M + H]^+$, 248, 204. The (2-trimethylsilylethynyl-benzyl)-carbamoyl acid *tert*-butyl ester (1.07 g, 3.53 mmol) was dissolved in methanol (25 cm^3) and $KF{\cdot}2H_2O$ (1.00 g, 10.60 mmol) was added. The mixture was stirred for 14 h at room temperature. The solvent was removed and the residue was extracted with dichloromethane (20 cm^3) and water (20 cm^3), re-extracting the water phase twice. The combined organic layers were concentrated and the residue purified by column chromatography using hexane:ethyl acetate (9:1) eluent mixture yielding a brown solid (0.51 g, 63%); ν_{max} ($CHCl_3$)/cm^{-1} 3450, 3301, 3004, 2980, 2101, 1709, 1507, 1364, 1251, 1168; δ_H (270.2 MHz; $CDCl_3$) 3.31 (1H, s, CH), 1.41 (9H, s, CH_3^{Boc}), 4.42 (2H, s, CH_2), 5.14 (1H, br s, NH), 7.17 (1H, t, *J* 7.3, ArH), 7.25–7.33 (2H, m, ArH), 7.44 (1H, d, *J* 7.3, ArH); δ_C (67.9 MHz; $CDCl_3$) 28.0 (3C), 42.9, 79.1, 81.0, 81.8, 120.6, 126.8, 127.5, 128.7, 132.5, 141.1, 155.5; *m/z* (ESI-MS, 30 eV) 232 $[M + H]^+$, 217, 176, 132.

2-Iodophenyl-acetic acid methyl ester (44)[39]

To 2-Iodophenyl-acetic acid (2.50 g; 9.54 mmol) dissolved in methanol (100 cm^3) conc. aqueous HCl (0.81 cm^3; 9.54 mmol) was added dropwise and the mixture was stirred for 4 h at room temperature. The solvent was concentrated under reduced pressure. The residual was poured into water (20 cm^3) and extracted three times with dichloromethane (20 cm^3). The organic phase was concentrated yielding a yellow oil (2.61 g, 99%); ν_{max} ($CHCl_3$)/cm^{-1} 2948, 1741, 1436, 1218, 1167, 1016; δ_H (270.2 MHz; $CDCl_3$) 3.72 (3H, s, CH_3), 3.81 (2H, s, CH_2), 6.97 (1H, ddd, *J* 1.3, 2.5, 7.9, ArH), 7.27–7.35 (2H, m, ArH), 7.84 (1H, dd, *J* 1.3, 7.9, ArH); δ_C (67.9 MHz; $CDCl_3$) δ_C 46.0, 52.1, 100.9, 128.4, 128.8, 130.5, 137.6, 139.4, 170.8; *m/z* (EI, 70 eV) 276 $[M + H]^+$, 217, 149, 121.

Methyl-{2-[2′-(*tert*-Butoxycarbonylamino-methyl)-phenyl-ethynyl]-phenyl}-acetate (45)

A solution of **44** (260 mg, 1.11 mmol), methyl-2-iodophenylacetate (330 mg, 1.22 mmol), $Pd(PPh_3)_2Cl_2$ (15.60 mg, 22.23 μmol), CuI (8.50 mg, 44.63 μmol), diethylamine (1.50 cm^3) and dimethylformamide (0.50 cm^3) was mixed in a Smith Process Vial and stirred at 120 °C for 6 min in a microwave cavity. The procedure was repeated one time and the mixtures were combined by filtering them through Celite into a separation funnel. The mixture was extracted using dichloromethane (25 cm^3) and conc. $NaHCO_3$ (aq) (25 cm^3) re-extracting the aqueous phase twice. The organic phase was washed with water (25 cm^3), re-extracting the water phase three times. Then, the organic phase was concentrated under reduced pressure yielding a yellowish solid (0.68 g, 81%); ν_{max} ($CHCl_3$)/cm^{-1} 3452, 3020, 2981, 2400, 1732, 1709, 1505, 1214, 1167; δ_H (270.2 MHz; $CDCl_3$) 1.44 (9H, s, CH_3^{Boc}), 3.69 (3H, s, CH_3), 3.91 (2H, s, CH_2), 4.51 (2H, s, CH_2), 5.21 (1H, br s, NH), 7.24–7.36 (5H, m, ArH), 7.42 (1H, d, *J* 7.3, ArH), 7.55 (2H, m,

ArH); δ_C (67.9 MHz; $CDCl_3$) 28.4 (3C), 40.0, 43.5, 52.2, 79.5, 91.4, 91.9, 122.0, 123.3, 127.3, 128.1 (2C), 128.8 (2C), 129.9, 132.4 (2C), 135.8, 140.5, 155.9, 171.6; *m/z* (ESI-MS, 30 eV) 380 $[M + H]^+$, 280, 263.

{2-[2′-(*tert*-Butoxycarbonylamino-methyl)-phenylethynyl]-phenyl}-acetic acid (46)

Compound **45** (1.34 g, 3.52 mmol) and 6 M NaOH (aq) (100 cm^3) were mixed in a round bottom flask. The suspension was stirred at 120 °C for 2 h. Thereafter the mixture was poured into water in a separation funnel and 5 M HCl (aq) was added to decrease pH to 7, and was extracted with dichloromethane three times. The pH of the aqueous phase was first adjusted to pH 5, then pH 3, using 1 M HCl (aq), and the aqueous phase was extracted at each pH three times with dichloromethane. The combined organic layers were concentrated, filtered through a silica gel plug and recrystallized from methanol giving a white solid (0.82 g, 61%); mp 155–156 °C; ν_{max} ($CHCl_3$)/cm^{-1} 3446, 3357, 3067, 2929, 1709, 1503, 1165; δ_H (399.8 MHz; Acetone-d_6) 1.46 (9H, s, CH_3^{Boc}); 3.98 (2H, s, CH_2), 4.59 (2H, d, *J* 6.0, CH_2), 6.60 (1H, br d, *J* 6.0, NH), 7.31–7.48 (6H, m, ArH), 7.60 (1H, dd, *J* 1.6, 7.6, ArH), 7.66 (1H, dd, *J* 1.7, 7.6, ArH); δ_C (100.5 MHz; Acetone-d_6) 27.8, 39.6, 42.6, 78.2, 91.2, 92.4, 121.6, 123.6, 126.9, 127.1, 128.7, 128.8, 130,4, 132.1, 132.3, 137.1, 141.6, 156.1, 171.6; *m/z* (ESI, 30 eV) 366 $[M + H]^+$, 310, 266, 214, 199. HRMS, *m/z* = 388.1464 ($[M + Na]^+$), $C_{22}H_{23}NO_4Na$ requires 388.1525.

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