## Azobenzene-Based ω-Amino Acids and Related Building Blocks: Synthesis, Properties, and Application in Peptide Chemistry

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**Abstract:** Azobenzenes are widely used as optical triggers for the development of photoresponsive materials. Starting from the literature-known  $\omega$ -amino acids 4,4'-APB **1a** and 4,4'-AMPB **2a**, *meta*-substituted analogues were synthesized for the incorporation into peptides and proteins. While 4,4'-APB **1a** requires special chemistry for peptide synthesis, an Fmoc/*t*-Bu protecting group strategy is applicable for 3,3'-APB **1b**. The versatility of this and other novel azobenzene  $\omega$ -amino acids is demonstrated by the preparation of cyclic photoswitchable phosphopeptides with the binding motif pTyr-Val-Asn-Val. Photochromic properties of the azo subunits and of the corresponding peptides are also presented.

Key words: azo compounds, amino acids, peptides, photoswitch, photochromism

## Introduction

Photoswitchable compounds have experienced considerable interest for studying complex living systems using light.<sup>1</sup> One application is based on the modulation of the structure and chemical dynamics of peptides and proteins by site-specific incorporation of a photoswitchable subunit.<sup>2,3</sup> Functionalized photoswitches are also appealing candidates to broaden the range of scaffolds and templates for the design of peptidomimetics.<sup>4</sup>

With the aim to develop photoswitchable Grb2-SH2 antagonists for Ras signal transduction, we initiated a longterm research program directed towards the development of novel photoswitchable building blocks for the synthesis and screening of libraries of cyclic photoswitchable peptide ligands with the binding motif pTyr-Val-Asn-Val. In the past, Ettmayer and co-workers successfully demonstrated that a  $\beta$ -turn conformation centered around this binding motif in cyclic peptides is essential for high-affinity binding to the SH2-domain.<sup>5</sup> If this binding motif is incorporated into a photoswitchable cyclic peptide of suitable ring size, it should be possible to modulate the  $\beta$ turn structural element and thereby the binding event, through conformational changes arising from the lightinduced switching process within the photoswitchable hinge unit.

Our program is founded on the development of novel photoswitchable azobenzene-<sup>6,7</sup> and hemithioindigo-based<sup>8-11</sup> subunits for application in peptide synthesis. A selection of azobenzenes from our studies is shown in Scheme 1. The peptide library design is also based on variations in the ring size of the cyclic peptides and in the amino acid sequence surrounding the binding motif.<sup>10</sup> The first step within an iterative selection process towards photoswitch-



peptide assembly requires silylation, and amino acid fluorides for acylation



4,4'-APB-based peptidomimetic structural elements derived from N-arylation



examples for meta-substitution patterns with increased flexibility



Fmoc-based peptide assembly

Scheme 1 4,4'-APB-based peptidomimetics and analogues of 4,4'-APB 1a and 4,4'-AMPB 2a derived by *meta*-substitution: enhancing and suppressing resonance effects

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## **Biographical Sketches**



**Karola Rück-Braun** studied Chemistry at the University of Mainz where she received her Diploma in 1988 and her Ph.D. in 1992, working under the supervision of Prof. Horst Kunz. After postdoctoral studies with Prof. Steven V. Ley at the University of Cambridge (UK) in 1992–1993, she obtained her Habilitation at the University of

**Stefan Kempa** was born in Berlin in 1980. He studied Chemistry at the Technische Universität Mainz in 1998, and became a Professor of Organic Chemistry at the Technische Universität Berlin in 2000. Amongst other awards, she received the ADUC-Habilitation Award in 1998 and held a Habilitandenstipendium of the Deutsche Forschungsgemeinschaft (1993–1996). Her research interests are directed towards the synthesis of heterocy-

Berlin and obtained his Diploma in 2006. Currently he is carrying

cles and photoswitches and include metal-mediated reactions and the development of reaction cascades for the preparation of functionalized heterocycles. Current research topics are the development of photoswitches for biological applications and the design and synthesis of photoswitch-linker-conjugates for nanostructured surfaces.

out his Ph.D. studies in Prof. Rück-Braun's group.



**Beate Priewisch** was born in Berlin in 1976. She studied Chemistry at the Technische Universität Berlin and the Universidad de Complutense de Madrid, Spain, and obtained her Diploma in 2002 and her Ph.D. in 2006. Her research focused on the development of photoswitchable amino acids for the photomodulation of the conformation and activity of peptides. Since 2007 she is working as a patent attorney trainee at Polypatent, Overath.



Anja Richter was born in Berlin in 1979. She completed a training as a chemical laboratory assistant at the Schering AG in 2002. Thereafter she studied Chemistry at the Technische Universität Berlin and received her Diploma in 2007 under the supervision of Prof. Rück-Braun. As a scholarship holder of the Fond der Chemischen Industrie, she is currently working as a Ph.D. student in the research group of Prof. Herbert Waldmann at the Max-Planck-Institut für Molekulare Physiologie in Dortmund, focusing on the synthesis of natural products.



Sabine Seedorff was born in Berlin in 1978. She studied Chemistry at the Technische Universität Berlin and obtained her Diploma in 2006. Currently she is carrying out her Ph.D. studies in Prof. Rück-Braun's group in collaboration with the

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Lukas Wallach was born in Hydebreck/Cosel, Poland, in 1981. He studied Chemistry at the Technische Universität Berlin and obtained his Diploma in 2008. Currently he is carrying out his Ph.D. studies in Prof. Rück-Braun's group.

able cyclic phosphopeptides is directed towards the choice of suitable photoswitchable subunits, driven by the evaluation and optimization of synthetic routes. This step includes also the analysis of the photochromic properties of the subunits and of small linear peptides incorporating them.<sup>6,8,9</sup> The next step is the synthesis of small libraries of cyclic photoswitchable phosphopeptides and their characterization, for example, by CD spectroscopy.

Herein, we describe the synthesis of cyclic peptides c[-Gly-pTyr-Val-Asn-Val-Pro-Gly-Azo-] with novel azobenzene-based  $\omega$ -amino acids (Azo) as backbone constituents.<sup>10</sup> The research program is currently being extended by an activity analysis, and will be supplemented by the conformational analysis of selected members of the cyclic phosphopeptide library based on NMR spectroscopy.

Our studies towards novel azobenzene-based photoswitchable subunits started from the literature-known  $\omega$ -amino acids 4,4'-APB<sup>12,13</sup> **1a** and 4,4'-AMPB<sup>7,14,15</sup> **2a** (Scheme 1). Because of the poor nucleophilicity of the amino group in the more rigid  $\omega$ -amino acid 4,4'-APB **1a**, the typical peptide coupling reagents cannot be applied.<sup>12,13</sup> Instead, amide bond formation requires activation of the amino group by silylation, followed by acylation using amino acid fluorides. We herein report on alternatives to overcome these synthetic limitations.

In meta-substituted analogues, resonance effects of the amino group or the carboxyl group with the diazenyl linkage cannot operate (Scheme 1).<sup>6,11</sup> As a consequence we expected a higher nucleophilicity of the amino group in 3,3'-APB 1b. Therefore, a typical Fmoc-protocol should be applicable. Furthermore, this meta-substitution should also result in an increased conformational freedom of the azobenzene unit<sup>3,6,16,17</sup> leading to additional configurational isomers in both photoisomeric states. The axes for the rotation of the differently substituted phenyl rings and their dihedral angles  $\beta$  and  $\beta'$  for 3,3'-ABP **1b** are shown in Scheme 2. These rotary joints are responsible for a higher flexibility of meta-substituted azobenzenes. For 3,3'-AMPB **2b**, the flexibility is further increased by the methylene group as indicated by the additional axis with the dihedral angle  $\alpha$ . Following these considerations, we investigated the synthesis and the photochromic properties of a series of *meta*-substituted analogues of the  $\omega$ -amino acids 4,4'-APB **1a** and 4,4'-AMPB **2a**. However, during our search for alternative synthetic approaches towards 4,4'-azobenzene structural motifs, particularly suitable as ultra fast light-triggers for protein folding, we also studied the copper-catalyzed N-arylation of  $\alpha$ -amino acids with the bromo-substituted azobenzene precursor system **12** (Scheme 1).

Finally, we report on the successful synthesis and characterization of photoswitchable phosphopeptides derived from a small library approach towards four peptides incorporating the  $\omega$ -amino acids 4,4'-AMPB **2a**, 3,3'-AMPB **2b**, 3,4'-AMPB **2c**, and 3,3'-APB **1b**. These studies also include detailed discussions of photochromic properties of differently substituted azobenzenes presented herein.

# Synthesis and Peptide Assembling: 3,3'-APB 1b and Other Analogues

The preparation of 3,3'-APB **1b**, starting from 3-aminoaniline and 3-nitrosobenzoic acid in basic medium similar to the literature protocol<sup>13</sup> for 4,4'-APB **1a**, gave no conversion under various reaction conditions; redox processes between the reactants were observed instead. To reduce these side-reactions, the protected precursors **3** and **4** were employed in an alternative approach (Scheme 3), affording the amino acid derivative **5** in 53% yield. Simultaneous hydrolysis of the amide and the ester was achieved by treatment of **5** with aqueous 3 N HCl at 80 °C for 48 hours furnishing 3,3'-APB **1b**, quantitatively.

For the application in solid-phase synthesis, compound **1b** was converted into the Fmoc-protected building block **6** with FmocOSu in the presence of NaHCO<sub>3</sub> in dioxane–water in high yield by using a standard protocol.

However, an alternative synthetic route starting from nitrosonitrobenzenes 7 was also investigated (Scheme 4). Condensation of nitrosobenzene 7a with 3-aminobenzoic acid (8a) afforded the nitro-substituted azobenzene 9a in 93% yield. Subsequent reduction of compound 9a with sodium sulfide in dioxane–ethanol under reflux gave 3,3'-APB 1b in 75% yield. By following this two-step synthe-



Scheme 2 Flexibility induced by rotary joints of azobenzene hinge units illustrated for the *trans-* and *cis-*isomers of 3,3'-APB 1b and 3,3'-AMPB 2b



Scheme 3 Synthesis of Fmoc-3,3'-APB 6 for peptide chemistry

tic route, 3,4'-APB **1c** and 4,3'-APB **1d** (Scheme 4) were also successfully prepared in good yields via the intermediate nitro-substituted azobenzenes **9b** and **9c**.<sup>18</sup>





Scheme 4 Synthesis of APB analogues 1b–d by reduction of nitro precursors 9

To verify that the amino group of 3,3'-APB **1b** can be acylated by standard peptide coupling reagents, the synthesis of tripeptide **11** was carried out by solution-phase synthesis (Scheme 5). 3,3'-APB **1b** was treated with 1.5 equivalents of (*S*)-H-Val-O*t*-Bu using EDC in the presence of HOBt and DIPEA in DMF to obtain the dipeptide **10** in 82% yield. Acylation of **10** with Boc-Gly-OH using TCTU/HOBt/DIPEA furnished tripeptide **11** in 92% yield. According to these findings, the Fmoc-protected 3,3'-APB building block **6** was found to be suitable for solid-phase peptide synthesis using Fmoc-chemistry.

For the synthesis of peptides or peptidomimetics containing a 4,4'-azobenzene structural motif, we also investigated the copper(I)-catalyzed coupling procedures of bromide  $12^7$  with  $\alpha$ -amino acids (Scheme 6). All methods



Scheme 5 Peptide chain assembly of 3,3'-APB 1b by standard peptide chemistry

published in the literature for aryl halides represent catalytic examples of the long-known Ullmann-coupling without the need for disadvantageously high temperatures.<sup>19,20</sup> Ma and co-workers successfully demonstrated that these coupling procedures proceed without racemization.<sup>19</sup>

Treatment of bromide **12** with 1.5 equivalents of (*S*)-alanine in the presence of 10 mol% copper(I) iodide and 2 equivalents of  $K_3PO_4$  in 2-(dimethylamino)ethanol at 80 °C gave the coupling product **13b** in 65% isolated yield (Scheme 6). During storage of this product at -18 °C amine **14** was observed, presumably formed by decarbox-



by-product from (S)-Ala:



Scheme 6 Cu-catalyzed N-arylation towards 4,4'-APB-based peptidomimetic structural elements 13

ylation furnishing an imine intermediate, prior to hydrolysis to the corresponding aldehyde and amine byproducts. Analogously, reactions of compound 12 with (S)-valine and (S)-proline furnished 13a (67%) and 13c (66%). Prior to an optimization of the coupling protocol the photochromic properties of compound 13b were investigated, which are described below.

The synthesis of the two analogues, 3,3'-AMPB **2b** and 3,4'-AMPB **2c**, was carried out following our previously published procedure (Scheme 7) for the Fmoc-protected 4,4'-AMPB building block **17a**.<sup>7</sup> Condensation reactions of the Fmoc-protected anilines **15a**<sup>16</sup> and **15b**<sup>14</sup> with nitrosoarene **16** were carried out at room temperature in DMSO–glacial AcOH furnishing the isomeric Fmoc-protected  $\omega$ -amino acids **17b,c**. The Fmoc-group was removed by treatment with 20% aqueous NaOH–THF to give the  $\omega$ -amino acids **2b,c** in good overall yields.





**2b**:  $R^1 = CH_2NH_2$ ,  $R^2 = H$  (3,3'-AMPB) **2c**:  $R^1 = H$ ,  $R^2 = CH_2NH_2$  (3,4'-AMPB)



Synthetic studies towards a small library of peptides containing the pTyr-Val-Asn-Val binding motif was carried out with the Fmoc-protected building blocks Fmoc-4,4'-AMPB **17a**,<sup>7</sup> Fmoc-3,3'-AMPB **17b**, Fmoc-3,4'-AMPB **17c**, and Fmoc-3,3'-APB **6**. The solid-phase synthesis of the target phosphopeptides **20** is shown in Scheme 8.

All four linear peptide precursors **18** were assembled on solid support in an automated synthesis. Commercially available preloaded chlorotrityl resin was used as a solid support to enable the cleavage of the side-chain protected peptide and to avoid diketopiperazine formation associated with proline as the starting amino acid. The Fmocgroup was used as the temporary protecting group and HCTU as the coupling reagent in the presence of DIPEA. The trityl group was employed as a side-chain protecting group for asparagine, and the dialkylamide protected building block Fmoc-pTyr(NMe<sub>2</sub>)<sub>2</sub>-OH was used to incorporate the phosphotyrosine. To avoid the formation of truncated sequences due to a back-folding of the growing peptide chain on the solid support, induced by the  $\beta$ -turn structural element, the assembly of the linear peptides was



Scheme 8 Solid-phase synthesis of a small library of cyclic photoswitchable peptide ligands 20 (details are provided in the Supporting Information)

carried out using NMP as a solvent, and all amino acids following the phosphotyrosine building block were coupled twice. Removal of the temporary Fmoc-protecting group was achieved using 40% piperidine in NMP. Acidic cleavage from the resin with a mixture of acetic acid, trifluoroethanol, and dichloromethane afforded the linear protected peptides 18a-d according to HPLC-ESI-MS analysis. These side-chain-protected peptides were cyclized using a threefold excess of PyBOP and HOBt in the presence of DIPEA in a solution of 3% NMP in dichloromethane ( $c = 4 \cdot 10^{-4} \text{ mol/L}$ ). Peptides **19a–c** were finally deprotected using 90% trifluoroacetic acid in water. 3,3'-AMPB-peptide **20b** and 3,3'-APB-peptide **20c** were purified by semi-preparative RP-HPLC. The 4,4'-AMPBpeptide 20a could not be isolated in pure form, and we have been unable to ascertain the reasons for this singular purification problem. The 3,4'-AMPB-peptide 19d was purified after the cyclization procedure by semi-preparative RP-HPLC and finally deprotected, affording the pure cyclic phosphopeptide 20d.

### **Conformational Analysis by CD Spectroscopy**

All three peptides **20b–d** were photoresponsive molecules undergoing cis-to-trans isomerization. However, CD spectroscopy revealed significant structural differences between the conformations of the dark-adapted and the irradiated states only for the 3,3'-APB-peptide 20c and the 3,4'-AMPB-peptide 20d (Figure 1). This might be due to a decreased flexibility of their photoswitchable subunits, and thus a larger impact of the isomerization process onto the peptide backbone, compared to the 3,3'-AMPB subunit of peptide 20b. The higher flexibility of the latter peptide can be explained by three independent rotary joints in the hinge 3,3'-AMPB 2b (methylene group and two *meta*-functionalized phenyl rings), in comparison to two rotary joints in the hinges 3,3'-APB 1b (two metafunctionalized phenyl rings) and 3,4'-AMPB 2c (methylene group and one meta-functionalized phenyl ring). For peptide 20b the CD spectra at the best resemble the characteristics of  $\alpha$ -helix-like spectra, generally assigned to type I  $\beta$ -turns, (maxima below 200 nm and negative bands in the 220-235 nm region)<sup>21</sup> shifted to longer wavelengths, which, however, are not altered upon irradiation with  $\lambda = 340$  nm (*cis*-pss; pss = photostationary state) or  $\lambda = 405 \text{ nm} (trans-pss)$ . In the *cis*-form the 3,3'-APB-peptide **20c** displayed the CD spectrum of a  $\beta$ -sheet-like spectrum<sup>22</sup> with a maximum at 199 nm and a strong minimum at 215 nm, whereas for the trans-isomer the CD spectrum revealed no significant signals of a defined peptide conformation. On the contrary, for both isomeric forms of the 3,4'-AMPB-peptide 20d maxima below 200 nm and minima at 219 nm were observed, also indicating β-sheet-like spectra. Upon photoisomerization from the *trans*-form to the *cis*-form a decrease of  $[\Theta]_{R,219}$  by  $3.5 \cdot 10^3$ deg·cm<sup>2</sup>·dmol<sup>-1</sup> was detected.

## **Photochromic Analysis**

The photochromic properties of a series of azobenzenes presented in this study were investigated in DMSO by means of UV/vis and <sup>1</sup>H NMR spectroscopy. All compounds in Table 1, apart from 3,4'-APB **1c**, belong to the

 Table 1
 Photochromic Properties of Selected Azobenzene Compounds in DMSO

Compound	λ <sub>max,trans</sub> (nm)	$\lambda_{\max,cis}^{a}$ (nm)	λ <sub>iso</sub> (nm)	ε <sup>b</sup>	τ <sub>1/2</sub> (h)
3,3'-APB 1b	325, 426	432	265	1.28	42 (49) <sup>c</sup>
3,4'-APB 1c	401	357, 447	360, 476	1.18	1.7
4,3'-APB 1d	333, 410	318, 436	280	1.98	23.6
<b>5</b> <sup>d</sup>	319, 444	433	276, 397	1.7	(246) <sup>c,d</sup>
peptide 20c	323, 437	427	265, 406	1.02	92 (205) <sup>e</sup>
4,4'-AMPB 2a	346, 453	290, 434	290, 406	1.74	37
3,4'-AMPB 2c	326, 446	433	275, 389	1.29	129
peptide <b>20d</b> <sup>f</sup>	331, 440	295, 432	281, 387	1.59	81 (231) <sup>e</sup>

<sup>a</sup>  $\lambda_{\max,cis}$  was determined in the pss upon light illumination at 325 nm (compound 1d), 405 nm (compound 1c), or 340 nm.

<sup>b</sup> For *trans*-isomers [10<sup>4</sup> L mol<sup>-1</sup>·cm<sup>-1</sup>].

<sup>c</sup>  $\tau_{1/2}$  was determined by <sup>1</sup>H NMR spectroscopy (200 or 400 MHz).

<sup>d</sup> Ac-3,3'-APB-OMe **5** in CH<sub>2</sub>Cl<sub>2</sub>.

 $^{e}$   $\tau_{1/2}$  was determined in H<sub>2</sub>O.

<sup>f</sup> H<sub>2</sub>O content in DMSO: 6%.

azobenzene-type of azoaromatics according to the classification of Rau,<sup>23</sup> showing two clearly separated absorption maxima, for the lowest lying n- $\pi^*$  transition centered around 430 nm and the  $\pi$ - $\pi$ \* transition at 320–350 nm. The light-induced trans-to-cis isomerization is readily achieved upon illumination at 325, 340, or 405 nm (Table 1 and Figure 2) and shows isosbestic points. 4,4'-APB 1a with donor/acceptor arrangement shows the characteristics of an aminoazobenzene, rather than the effects of a pseudo-stilbene-type azo compound.<sup>24</sup> For the transisomer, a close energetic proximity of the n- $\pi^*$  and the  $\pi$ - $\pi^*$ -transitions was observed.<sup>24</sup> However, peptides derived from 4,4'-APB 1a more or less exhibit the classical features of azobenzene-type azo compounds.<sup>12</sup> Peptidomimetic ligands obtained by N-arylation show the characteristics of a typical pseudo-stilbene-type azo compound, as exemplified for compound 13b (Scheme 6 and Figure 2). In dichloromethane, the absorption maximum is located around 397 nm and is shifted to 473 nm in DMSO, due to the increased solvent polarity. After light



Figure 1 CD-evaluation of the light-induced conformational changes in the photoswitchable peptides 20b-d

induced reversible *trans*-to-*cis* isomerization with  $\lambda = 365$ , 385, or 405 nm for 1–20 minutes in dichloromethane and with  $\lambda = 480$  nm for 3–60 minutes in DM-SO, a fast relaxation was observed, but the corresponding half-lives could not be determined with standard equipment. Thus, incorporation of azobenzene hinge units by N-arylation breaks new ground for light-induced changes of peptide and protein conformations with very short half-lives of the *cis*-isomeric states.

3,4'-APB **1c** belongs to the aminoazobenzene-type azo aromatics,<sup>23</sup> however, peptides derived by amide bond formation should show the typical photochromic properties of azobenzene-type azo compounds, comparable to the peptides derived from 4,4'-APB **1a**.<sup>12</sup> Furthermore, from the data summarized in Table 1, for 3,3'-APB **1b** and

4,3'-APB **1d** a close proximity of the two absorption maxima is concluded. Strikingly, for both compounds a decrease of the intensities of the  $n-\pi^*$  transitions upon *trans*to-*cis* isomerization is observed. In comparison with all other compounds (Table 1) this effect correlates with the amino group in *meta*-position.

The thermal *cis*-to-*trans* isomerization of azobenzenetype azo compounds in solution follows first order kinetics, and the half-lives  $\tau_{1/2}$  of the *cis*-isomers presented in Table 1 were determined at 30 °C following the timedependent absorption changes by UV/vis spectroscopy (~1·10<sup>-4</sup> M). As expected, the half-life increased in the APB series with the amino group in *meta*-position (Table 1, compounds **1c** and **1b**). In the AMPB series, a strong increase of the half-life is also observed upon



Figure 2 Comparison of the UV/vis spectra of representative types of azobenzenes in DMSO

placement of the carboxyl group in *meta*-position (compounds **2a** and **2c**), and a similar trend is seen in the APB series (compounds **1d** and **1c**).

Furthermore, for 3,3'-APB peptide **20c** a half-life of 205 hours was determined in water, and for the 3,3'-APB derivative **5** a half-life of 246 hours was observed in dichloromethane (Table 1). Irradiation of peptide **20c** in DMSO- $d_6$  (5.8·10<sup>-2</sup> M) gave a pss containing 84% of the *cis*-isomer, as determined by <sup>1</sup>H NMR spectroscopy at room temperature using the NH of Gly3 as a probe. For the 3,4'-AMPB peptide **20d** a half-life of 231 hours was ascertained in water. Upon irradiation a pss containing 93% of the *cis*-isomer was reached under RP-HPLC conditions. In the dark adapted state, generally only the *trans*-isomers of all azobenzenes reported herein were observed. However, for the peptides **20** also traces of the *cis*-isomers have been detected in isolated cases by <sup>1</sup>H NMR spectroscopy.

### **Summary and Outlook**

In conclusion, we have developed practical synthetic routes for a series of novel meta-substituted azobenzenebased  $\omega$ -amino acid building blocks for site-specific incorporation into peptides. We have demonstrated that Fmoc-3,3'-APB 6 is well suited for an Fmoc-based peptide assembly on the solid support. The methylene group in the AMPB series and the *meta*-functionalized phenyl rings in the APB and the AMPB series are effective rotary joints for introducing flexibility. Using a library approach, these differences in conformational freedom could successfully be demonstrated by CD-evaluation of the lightinduced conformational changes of the peptide backbones of four cyclic phosphopeptides with the binding motif pTyr-Val-Asn-Val. Our future efforts will focus on additional concepts to further improve the properties of azobenzenes for biological applications, including strategies to increase solubility and redox-stability.

Chemicals, including compounds 3, 8a, and 8b, were obtained from Acros, Aldrich, Fluka, or Novabiochem and were used as supplied. Compounds 4, 7a, <sup>25</sup> 7b, <sup>25</sup> 12, 16, and 17a were prepared by following the literature procedure.<sup>7</sup> Solvents were distilled prior to use: CH<sub>2</sub>Cl<sub>2</sub> (P<sub>2</sub>O<sub>5</sub>), EtOAc (K<sub>2</sub>CO<sub>3</sub>), pentane (P<sub>2</sub>O<sub>5</sub>), MeOH, acetone. Solvents for chromatography were dried according to standard procedures and distilled prior to use.26All reactions were carried out under N<sub>2</sub>. <sup>1</sup>H NMR spectra were recorded at 400 MHz and <sup>13</sup>C NMR spectra at 100.6 MHz on a Bruker AM 400. Residual solvent protons were used as internal standards. All chemical shifts are given in ppm relative to TMS and coupling constants in Hz. Low- and high-resolution mass spectra were obtained on a Varian MAT 95S spectrometer with electron ionization, using an ionization potential of 70 eV. ESI-MS spectra were obtained on a Bruker Esquire 2000 with an ESI voltage of 4 kV and HRMS-ESI spectra on a Thermo Fisher Scientific LTQ Orbitrap XL with an ESI voltage of 5 kV. IR spectra were recorded on a Nicolet Avatar 360 spectrometer. CD spectra were recorded on a JASCO J-715 spectropolarimeter. Melting points were measured with a Büchi melting point apparatus according to Dr. Tottoli and are uncorrected. TLC was carried out on silica gel 60 F<sub>254</sub> (Merck) and was visualized under UV light (254 nm) or using ninhydrin stain. Column chromatography was performed on silica gel (ICN Biomedicals GmbH silica; 32–63  $\mu$ m, 60Å).

#### Methyl 3-[(3-Acetamidophenyl)diazenyl]benzoate (5)

Solid *N*-(3-aminophenyl)acetamide (**3**; 9.09 g, 60.6 mmol, 2.00 equiv) was added in one portion to a solution of **4** (5.00 g, 30.3 mmol) in glacial AcOH (300 mL). The mixture was stirred at r.t. for 11 d. H<sub>2</sub>O (300 mL) was then added, and the precipitate that formed was separated by filtration using a suction filter, washed with H<sub>2</sub>O (300 mL), and dried (desiccator, Sicacide). The solid was dissolved in EtOAc (300 mL), the mixture was filtered to remove an insoluble black-brown residue, and the filtrate was concentrated under reduced pressure. Recrystallization (CH<sub>2</sub>Cl<sub>2</sub>) gave **5** as an orange-yellow solid; yield: 4.80 g (53%); mp 143 °C;  $R_f = 0.67$  (EtOAc).

IR (ATR): 3304, 1724, 1672, 1598, 1547 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 2.23 (s, 3 H), 3.97 (s, 3 H), 7.41–7.83 (m, 5 H), 8.04–8.16 (m, 3 H), 8.53 (t, *J* = 1.7 Hz, 1 H).

<sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>): δ = 24.7, 52.5, 113.5, 119.8, 122.8, 124.2, 127.2, 129.3, 129.8, 131.3, 131.8, 138.9, 152.5, 153.0, 166.7, 168.7.

MS (190 °C): m/z (%) = 92 (44), 134 (100), 297 (80, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>: 297.1113; found: 297.1111.

#### 3-[(3-Aminophenyl)diazenyl]benzoic Acid (1b)

*Method 1*: Compound **5** (2.00 g, 6.72 mmol) was suspended in aq 3 N HCl (250 mL) and warmed to 80 °C. The resulting solution was stirred at 80 °C for 2 d. The mixture was allowed to attain r.t., and H<sub>2</sub>O (200 mL) was added. The precipitate was separated by filtration using a suction filter, washed with a small amount of cold H<sub>2</sub>O, and dried (desiccator, Sicacide). Recrystallization (EtOAc–MeOH) afforded **1b**·HCl as an orange solid; yield: 1.74 g (quant); mp 205 °C (dec.);  $R_f = 0.46$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 9:1).

IR (ATR): 3362, 2930, 1701, 691 cm<sup>-1</sup>.

<sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>): δ = 4.70 (br s, 3 H), 7.35 (d, J = 7.8 Hz, 1 H), 7.56–7.65 (m, 2 H), 7.72–7.80 (m, 2 H), 8.12–8.18 (m, 2 H), 8.37 (s, 1 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ = 113.7, 121.3, 122.4, 124.5, 127.5, 130.1, 130.7, 132.2, 132.3, 137.1, 151.7, 152.4, 166.6.

MS (200 °C): m/z (%) = 65 (58), 92 (100), 121 (24), 241 (53, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{13}H_{11}N_3O_2$ : 241.0851; found: 241.0853.

Method 2: Compound **9a** (50.0 mg, 184 µmol) was suspended in EtOH–dioxane (1:1; 3 mL). A solution of Na<sub>2</sub>S (32–38%, 140 mg, 1.79 mmol, 9.7 equiv) in H<sub>2</sub>O (1.5 mL) was added and the clear red solution was heated to 90 °C in a pressure vessel for 9.5 h. After cooling to r.t., EtOH and dioxane were removed under reduced pressure. Then H<sub>2</sub>O (10 mL) was added and the solution was adjusted to pH 5.5, using aq 1 N HCl. The precipitate was separated by filtration using a suction filter, washed with aq 1 N HCl (5 mL) and H<sub>2</sub>O (5 mL), and dried (desiccator, Sicacide). By dissolving this precipitate in EtOAc (50 mL) an insoluble blackish brown solid formed, which was filtered and discarded. Removal of the solvent under reduced pressure gave **1b** as an orange-yellow solid; yield: 33 mg (75%).

## 3-[(3-{[(9H-Fluoren-9-yl)methoxy]carbonylamino}phenyl)diazenyl]benzoic Acid (6)

Solid NaHCO<sub>3</sub> (782 mg, 9.32 mmol, 2.50 equiv) was added to a solution of 3,3'-APB-OH **1b** (900 mg, 3.73 mmol) in dioxane–H<sub>2</sub>O (1:1; 60 mL). A solution of *N*-(9*H*-fluoren-9-ylmethoxycarbonyloxy)succinimide (1.45 g, 4.29 mmol, 1.15 equiv) in dioxane (12

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mL) was added dropwise over a period of 10 min by syringe. The solution was stirred at r.t. for 36 h. Dioxane was removed under reduced pressure, and the precipitate was separated by filtration using a suction filter, washed with H<sub>2</sub>O (100 mL) and hexane–EtOAc (3:1, 100 mL), and dried. Compound **6** was obtained as a bright orange solid; yield: 1.50 g (87%); mp 214–216 °C;  $R_f = 0.75$  (EtOAc).

IR (ATR): 3309, 1700, 1600, 1544, 1222 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ):  $\delta = 4.34$  (t, J = 6.7 Hz, 1 H), 4.54 (d, J = 6.7 Hz, 2 H), 7.32–7.79 (m, 10 H), 7.91 (d, J = 7.4 Hz, 2 H), 8.10–8.15 (m, 3 H), 8.36 (t, J = 1.7 Hz, 1 H), 10.05 (s, 1 H), 13.35 (br s, 1 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 46.6, 65.7, 111.0, 118.1, 120.2, 122.3, 125.2, 125.9, 126.8, 127.2, 127.7, 129.3, 129.8, 131.9, 140.2, 140.8, 143.7, 145.2, 151.7, 152.4, 153.5, 167.4.

HRMS-ESI:  $m/z [M + H]^+$  calcd for  $C_{28}H_{22}N_3O_4$ : 464.16103; found: 464.15942.

#### 3-[(3-Nitrophenyl)diazenyl]benzoic Acid (9a)

Compound **7a** (4.80 g, 31.6 mmol, 2.00 equiv) was suspended in glacial AcOH (350 mL) and the suspension was sonified until a clear green solution was obtained. 3-Aminobenzoic acid (**8a**; 2.12 g, 15.8 mmol) was added, and the mixture was stirred at r.t. for 12 h. The precipitate was separated by filtration using a suction filter and washed with H<sub>2</sub>O (300 mL). Additional material precipitated from the mother liquor upon addition of H<sub>2</sub>O. The combined solid was recrystallized (MeOH) and washed thoroughly with Et<sub>2</sub>O to afford **9a** as an orange solid; yield: 4.00 g (93%); mp 223 °C;  $R_f = 0.48$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 9:1).

IR (ATR): 3081, 1687, 1530, 1352, 759, 681 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ): δ = 7.54 (t, J = 7.7 Hz, 1 H), 7.85–7.95 (m, 2 H), 8.11 (td, J = 7.6, 1.3 Hz, 1 H), 8.33–8.47 (m, 3 H), 8.57 (t, J = 2.0 Hz, 1 H).

<sup>13</sup>C NMR (50.3 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 115.8, 122.5, 125.8, 127.9, 130.0, 130.1, 131.2, 132.3, 132.7, 148.7, 151.5, 152.0, 166.5.

MS (180 °C): m/z (%) = 65 (100), 76 (70), 121 (94), 149 (22), 271 (26, [M]<sup>+</sup>).

HRMS: *m*/*z* [M]<sup>+</sup> calcd for C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>4</sub>: 271.0593; found: 271.0591.

#### 3-[(4-Nitrophenyl)diazenyl]benzoic Acid (9b)

4-Nitrosonitrobenzene (**7b**; 2.50 g, 16.4 mmol) was suspended in glacial AcOH (190 mL) and the suspension was sonified until a clear green solution was obtained. 3-Aminobenzoic acid (**8a**; 1.13 g, 8.22 mmol) was added, and the mixture was stirred at r.t. for 24 h. The precipitate was separated by filtration using a suction filter and washed with H<sub>2</sub>O (100 mL). Additional material precipitated from the mother liquor upon the addition of H<sub>2</sub>O, which was likewise separated by filtration using a suction filter and washed with H<sub>2</sub>O (100 mL). Both fractions were dried (desiccator, Sicacide), and recrystallized twice (acetone–MeOH, 5:1) to afford **9b** as an orange solid; yield: 1.84 g (83%); mp 242 °C;  $R_f = 0.43$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 9:1).

IR (ATR): 1682, 1523, 1346, 762, 689 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 7.78 (t, J = 7.81 Hz, 1 H), 8.13 (d, J = 8.87 Hz, 2 H), 8.18–8.23 (m, 2 H), 8.44–8.46 (m, 3 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ = 122.6, 123.6, 125.0, 127.8, 130.1, 132.3, 132.9, 148.7, 151.7, 154.8, 166.4.

HRMS: *m*/*z* [M]<sup>+</sup> calcd for C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>4</sub>: 271.0593; found: 271.0587.

Anal. Calcd for  $C_{13}H_9N_3O_4$ : C, 57.57; H, 3.34; N, 15.49. Found: C, 57.40; H, 3.69; N, 15.14.

#### 3-[(4-Aminophenyl)diazenyl]benzoic Acid (1c)

Compound 9b (600 mg, 2.21 mmol) was dissolved in EtOH-dioxane (1:1, 24 mL) to give a clear red solution. A solution of Na<sub>2</sub>S (32-38%, 1.64 g, 21.0 mmol, 9.5 equiv) in H<sub>2</sub>O (12 mL) was added, turning the solution dark green. After the mixture was heated to reflux for 3 h, it was cooled to r.t. and the organic solvents were removed under reduced pressure. Then H<sub>2</sub>O (20 mL) was added and the insoluble brown solid was filtered and discarded (pH 14). The filtrate was adjusted to pH 7, using aq 1 N HCl, and the precipitate formed was filtered and dried (desiccator, Sicacide). Additional material was collected from the mother liquor upon addition of more aq 1 N HCl (pH 5.5). Then the precipitates were dissolved separately in EtOAc (250 mL and 100 mL), and an insoluble blackishbrown solid was filtered and discarded from both fractions. The EtOAc fractions were combined and concentrated in vacuo to give **1c** as an orange-brown solid; yield: 376 mg (70%); mp 207–210 °C (dec.);  $R_f = 0.32$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 9:1).

IR (ATR): 3369, 1694, 1625, 1599, 1506 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 6.20 (s, 2 H), 6.67–6.69 (m, 2 H), 7.63 (t, *J* = 7.79 Hz, 1 H), 7.68–7.71 (m, 2 H), 7.95–7.98 (m, 2 H), 8.24 (t, *J* = 1.85 Hz, 1 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ = 113.4, 121.5, 125.5, 126.6, 129.6, 129.7, 131.9, 142.7, 152.5, 153.3, 167.0.

MS (130 °C): m/z (%) = 92 (100), 121 (48), 241 (55, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{13}H_{11}N_3O_2$ : 241.0851; found: 241.0847.

#### 4-[(3-Aminophenyl)diazenyl]benzoic Acid (1d)

Compound **9c** (600 mg, 2.21 mmol) was suspended in EtOH–dioxane (1:1, 24 mL). A solution of Na<sub>2</sub>S (32–38%, 1.64 g, 21.0 mmol, 9.5 equiv) in H<sub>2</sub>O (12 mL) was added and the clear red solution was heated to reflux for 3 h. Cooling to r.t. and removal of the organic solvents in vacuo caused the formation of a red precipitate. After addition of H<sub>2</sub>O (20 mL), the insoluble solids were filtered and discarded (pH 14). The filtrate was adjusted to pH 7, using aq 1 N HCl, and the precipitate was filtered and dried (desiccator, Sicacide). Additional material was collected from the mother liquor upon addition of more aq 1 N HCl (pH 3.5). Then the precipitates were dissolved separately in EtOAc (250 mL and 100 mL), and an insoluble blackish-brown solid was filtered and discarded from both fractions. The EtOAc fractions were combined and concentrated in vacuo to give **1d** as an orange-red solid; yield: 407 mg (76%); mp 227–229 °C (dec.);  $R_f = 0.38$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 9:1).

IR (ATR): 3347, 3063, 1681, 1623, 1601 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ): δ = 5.51 (br s, 2 H), 6.78–6.80 (m, 1 H), 7.10–7.14 (m, 2 H), 7.25 (t, J = 7.83 Hz, 1 H), 7.90 (d, J = 8.35 Hz, 2 H), 8.12 (d, J = 8.25 Hz, 2 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ = 105.1, 112.9, 117.9, 122.3, 129.6, 130.5, 132.4, 149.7, 152.9, 154.3, 166.6.

MS (140 °C): m/z (%) = 65 (57), 92 (100), 121 (28), 241 (75, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{13}H_{11}N_3O_2$ : 241.0851; found: 241.0855.

#### **Dipeptide 10**

EDC (357 mg, 1.87 mmol, 1.50 equiv), HOBt (252 mg, 1.87 mmol, 1.50 equiv), DIPEA (326  $\mu$ L, 241 mg, 1.24 mmol, 1.00 equiv), and compound **1b** (300 mg, 1.24 mmol) were successively added to a solution of (*S*)-valine *tert*-butyl ester hydrochloride (392 mg, 1.87 mmol, 1.50 equiv) in DMF (25 mL) at 0 °C. Then the mixture was allowed to attain r.t. and was stirred for 36 h. Afterwards, CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added, and the organic layer was washed with aq 0.5 N HCl (2 × 50 mL), aq NaHCO<sub>3</sub> (50 mL), H<sub>2</sub>O (50 mL), and brine (50 mL), dried (MgSO<sub>4</sub>), and concentrated in vacuo. H<sub>2</sub>O (50 mL)

was added to the oily residue, and a red precipitate was separated by decanting the supernatant mother liquor. The precipitate was dissolved in MeOH (20 mL) and reprecipitated by the addition of H<sub>2</sub>O, twice. Lyophilization gave compound **10** as an orange-red solid; yield: 402 mg (82%); mp 55 °C;  $[\alpha]_D^{20}$  +61.2 (*c* = 0.49, CHCl<sub>3</sub>);  $R_f = 0.69$  (EtOAc-pentane, 3:2).

IR (ATR): 3432, 2932, 1719, 1653, 1602, 1526 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz,  $CDCl_3$ ):  $\delta = 1.00-1.06$  (m, 6 H), 1.50 (s, 9 H), 2.25–2.32 (m, 1 H), 3.87 (br s, 2 H), 4.68–4.73 (m, 1 H), 6.79–6.83 (m, 2 H), 7.22–7.26 (m, 1 H), 7.31 (t, J = 7.80 Hz, 1 H), 7.36–7.39 (m, 1 H), 7.59 (t, J = 7.78 Hz, 1 H), 7.94–7.96 (m, 1 H), 8.01–8.03 (m, 1 H), 8.29 (d, J = 1.40 Hz, 1 H).

<sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>): δ = 18.0, 19.1, 28.2, 32.0, 57.9, 82.4, 107.6, 115.4, 118.4, 121.5, 125.6, 129.6, 130.0, 135.5, 147.4, 152.7, 153.6, 166.6, 171.4.

MS (190 °C): m/z (%) = 57 (24), 92 (48), 224 (100), 295 (24), 396 (15, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{22}H_{28}N_4O_3$ : 396.2161; found: 396.2162.

#### **Tripeptide 11**

TCTU (112 mg, 315 µmol, 2.50 equiv), HOBt (43.0 mg, 315 µmol, 2.50 equiv), DIPEA (55.0 µL, 41.0 mg, 315 µmol, 2.50 equiv), and peptide **10** (50.0 mg, 1.26 µmol) were successively added to a stirred solution of Boc-Gly-OH (55.0 mg, 315 µmol, 2.50 equiv) in DMF (2.50 mL) at 0 °C. Then the mixture was allowed to attain r.t. and stirred at this temperature for 26 h. Afterwards, CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added, and the organic layer was washed with aq 0.5 N HCl (2 × 10 mL), aq NaHCO<sub>3</sub> (10 mL), H<sub>2</sub>O (50 mL), and brine (50 mL), dried (MgSO<sub>4</sub>), and concentrated in vacuo. H<sub>2</sub>O (10 mL) was added to the oily residue, and a red precipitate was separated by decanting the supernatant mother liquor. The precipitate was dissolved in MeOH (5 mL) and reprecipitated by the addition of H<sub>2</sub>O, twice. Lyophilization gave compound **11** as an amorphous orange-red foam; yield: 66 mg (92%);  $R_f = 0.76$  (pentane–EtOAc, 2:3).

IR (ATR): 3322, 1715, 1690, 1648 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 1.00$  (d, J = 6.6 Hz, 3 H), 1.02 (d, J = 6.8 Hz, 3 H), 1.44 (s, 9 H), 1.45 (s, 9 H), 2.25–2.33 (m, 1 H), 4.00–4.07 (m, 2 H), 4.72 (dd, J = 8.7, 4.8 Hz, 1 H), 5.70 (t, J = 5.6 Hz, 1 H), 7.29 (d, J = 8.5 Hz, 1 H), 7.40 (t, J = 8.0 Hz, 1 H), 7.53 (t, J = 7.8 Hz, 1 H), 7.62 (d, J = 7.9 Hz, 1 H), 7.80 (d, J = 7.2 Hz, 1 H), 7.86 (s, 1 H), 7.95 (dt, J = 7.8, 1.6 Hz, 2 H), 8.32 (s, 1 H), 9.04 (br s, 1 H).

 $^{13}\text{C}$  NMR (100.6 MHz, CDCl<sub>3</sub>):  $\delta$  = 18.1, 19.1, 28.1, 28.4, 31.9, 51.2, 58.1, 80.5, 82.5, 112.9, 120.3, 122.4, 122.8, 124.9, 129.5, 129.7, 130.0, 135.3, 138.7, 152.2, 152.7, 156.7, 166.9, 168.4, 171.5.

MS (380 °C): m/z (%) = 120 (100), 553 (3, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{29}H_{39}N_5O_6$ : 553.2900; found: 553.2901.

## Copper-Catalyzed N-Arylation of Amino Acids; General Procedure

Compound **12** (150 mg, 415 µmol), the appropriate amino acid (623 µmol, 1.5 equiv), and  $K_3PO_4$  (176 mg, 829 µmol, 2.0 equiv) were suspended in 2-(dimethylamino)ethanol (420 µL). The vial was flushed with  $N_2$ , and CuI (8.00 mg, 42 µmol, 0.1 equiv) was added under  $N_2$ . The mixture was stirred and heated to 80 °C whereupon the solids completely dissolved. Stirring was continued until no further conversion was detected (TLC). The mixture was then allowed to attain r.t., and  $H_2O$  (15 mL) and EtOAc (15 mL) were added. The aqueous layer was separated and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (2 × 20

mL), dried (MgSO<sub>4</sub>), and concentrated in vacuo. The residue was purified by column chromatography (silica gel).

### (S)-2-(4{[4-(*tert*-Butoxycarbonyl)phenyl]diazenyl}phenylamino)-3-methylbutanoic Acid (13a)

The crude product was purified by chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 40:1 to 9:1) to obtain **13a** as a red solid; yield: 110 mg (67%); mp 66 °C;  $R_f = 0.45$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 9:1).

IR (ATR): 3369, 2929, 1710, 1599, 1291, 1136 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.07–1.12 (m, 6 H), 1.61 (s, 9 H), 2.21–2.30 (m, 1 H), 4.01–4.07 (m, 1 H), 6.68 (d, *J* = 8.3 Hz, 2 H), 7.81 (t, *J* = 8.3 Hz, 4 H), 8.07 (d, *J* = 8.3 Hz, 2 H).

<sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>): δ = 18.6, 19.2, 28.2, 31.4, 81.3, 113.0, 122.0, 125.7, 130.4, 132.5, 145.4, 150.2, 155.4, 165.5.

MS (180 °C): m/z (%) = 120 (100), 297 (80), 351 (62), 397 (49, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{22}H_{27}N_3O_4$ : 397.2002; found: 397.2002.

#### (S)-2-(4{[4-(*tert*-Butoxycarbonyl)phenyl]diazenyl}phenylamino)propanoic Acid (13b)

The crude product was purified by chromatography on silica gel (EtOAc, then EtOAc–MeOH, 19:1 to 1:1) to obtain **13b** as a brownred solid; yield: 99.0 mg (65%); mp 63 °C (dec);  $R_f = 0.59$  (EtOAc–MeOH, 1:1).

IR (ATR): 3375, 2977, 1709, 1599, 1394, 1292, 1135, 1116 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz,  $CDCl_3$ ):  $\delta = 1.59$  (d, J = 7.00 Hz, 3 H), 1.62 (s, 9 H), 4.29 (q, J = 7.0 Hz, 1 H), 5.57 (br s, 1 H), 6.68 (d, J = 8.9 Hz, 2 H), 7.83–7.88 (m, 4 H), 8.08–8.10 (m, 2 H).

<sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>): δ = 18.7, 28.2, 51.4, 81.3, 112.9, 122.0, 125.7, 130.3, 132.5, 145.5, 149.4, 155.3, 165.5, 178.6.

MS (170 °C): m/z (%) = 92 (100), 297 (41), 369 (22, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{20}H_{23}N_3O_4$ : 369.1689; found: 369.1689.

#### (S)-2-(4{[4-(*tert*-Butoxycarbonyl)phenyl]diazenyl}phenyl)pyrrolidine-2-carboxylic Acid (13c)

The crude product was purified by chromatography on silica gel (EtOAc, then EtOAc–MeOH, 85:15 to 1:1) to obtain **13c** as a red solid; yield: 108 mg (66%); mp 168 °C;  $R_f = 0.17$  (EtOAc–MeOH, 5:1).

IR (ATR): 3365, 2925, 1709, 1598, 1369 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 1.62$  (s, 9 H), 2.05–2.22 (m, 2 H), 2.26–2.42 (m, 2 H), 3.48 (q, J = 8.3 Hz, 1 H), 3.66 (td, J = 8.6, 3.1 Hz, 1 H), 4.40 (dd, J = 8.6, 2.4 Hz, 1 H), 6.64 (d, J = 9.0 Hz, 2 H), 7.84 (d, J = 8.4 Hz, 2 H), 7.90 (d, J = 9.0 Hz, 2 H), 8.08 (d, J = 8.4 Hz, 2 H).

<sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>): δ = 23.7, 28.2, 30.9, 48.6, 60.6, 81.2, 112.1, 121.9, 125.6, 130.3, 132.3, 144.4, 149.4, 155.4, 165.5, 178.7.

MS (200 °C): m/z (%) = 55 (100), 144 (56), 294 (26), 395 (10, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for C<sub>22</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>: 395.1845; found: 395.1850.

#### tert-Butyl 4-[(4-Aminophenyl)diazenyl]benzoate (14)

Mp 103 °C;  $R_f = 0.71$  (EtOAc–hexane–AcOH, 50:50:0.5).

IR (ATR): 3372, 2976, 2930, 1698, 1599, 1507 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.62 (s, 9 H), 6.75 (dt, *J* = 8.84, 2.43 Hz, 2 H), 7.82–7.86 (m, 4 H), 8.09 (dt, *J* = 8.48, 1.97 Hz, 2 H).

<sup>13</sup>C NMR (100.6 MHz, CDCl<sub>3</sub>): δ = 28.2, 81.2, 114.6, 122.0, 125.6, 130.3, 132.5, 145.6, 150.2, 155.3, 165.4.

MS (140 °C): m/z (%) = 92 (100), 120 (57), 297 (47, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{17}H_{19}N_3O_2$ : 297.1477; found: 297.1480.

#### 3-{[3-({[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}methyl)phenyl]diazenyl}benzoic Acid (17b)

A suspension of 3-nitrosobenzoic acid (**16**; 5.50 g, 36.5 mmol) in AcOH–DMSO (1:1, 500 mL) was sonified until a clear green solution was obtained. Then the carbamate **15a** (6.28 g, 18.2 mmol) was added as a solid, and the resulting soln was stirred at r.t. for 13 d (TLC monitoring). H<sub>2</sub>O (700 mL) was added, and the resulting precipitate was filtered, thoroughly washed with H<sub>2</sub>O, dried under reduced pressure, and recrystallized several times from acetone–H<sub>2</sub>O to afford **17b** as a brown solid; yield: 5.35 g (62%); mp 171–173 °C (dec.);  $R_f = 0.45$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 9:1).

IR (ATR): 3406, 3320, 3066, 2667, 1699, 1600 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ):  $\delta = 4.25$  (t, J = 6.8 Hz, 1 H), 4.32 (d, J = 6.0 Hz, 2 H), 4.38 (d, J = 6.9 Hz, 2 H), 7.31 (t, J = 7.4 Hz, 2 H), 7.40 (t, J = 7.4 Hz, 2 H), 7.45 (d, J = 7.6 Hz, 1 H), 7.57 (t, J = 8.2 Hz, 1 H), 7.71 (d, J = 7.3 Hz, 2 H), 7.74 (t, J = 7.8 Hz, 1 H), 7.82–7.84 (m, 2 H), 7.89 (d, J = 7.5 Hz, 2 H), 8.00 (t, J = 6.0 Hz, 1 H), 8.11–8.14 (m, 2 H), 8.39 (t, J = 1.8 Hz, 1 H).

<sup>13</sup>C NMR (50.3 MHz, DMSO- $d_6$ ): δ = 43.5, 46.8, 65.4, 120.1, 120.8, 121.8, 122.3, 125.1, 127.1, 127.4, 127.6, 129.5, 130.0, 130.6, 131.9, 132.2, 140.8, 141.5, 143.9, 151.9, 156.4, 166.7.

MS (240 °C): m/z (%) = 165 (22), 178 (100), 196 (21), 477 (1, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{29}H_{23}N_3O_4$ : 477.1689; found: 477.1697.

#### 3-{[4-({[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}methyl)phenyl]diazenyl}benzoic Acid (17c)

A suspension of 3-nitrosobenzoic acid (**16**; 4.76 g, 31.5 mmol) in AcOH–DMSO (1:1, 580 mL) was sonified until a clear green soln was obtained. Then the carbamate **15b** (5.40 g, 15.7 mmol) was added as a solid in several portions, and the resulting soln was stirred at r.t. for 3 d (TLC monitoring). H<sub>2</sub>O (380 mL) was added to the brownish-black solution and then the aqueous layer was extracted with EtOAc (3 × 400 mL). The combined organic layers were washed with brine (200 mL), dried (MgSO<sub>4</sub>), and concentrated in vacuo. After addition of H<sub>2</sub>O, the precipitate formed was filtered, thoroughly washed with H<sub>2</sub>O, and recrystallized several times from acetone to afford **17c** as a light orange solid; yield: 5.53 g (74%, purity >95%); mp 212–215 °C (dec.);  $R_f = 0.64$  (EtOAc). A portion of the product (176 mg, 369 µmol) was purified by column chromatography on silica gel (EtOAc + 0.1% AcOH) to afford highly pure **17c** (133 mg, 76%, 278 µmol).

IR (ATR): 3312, 3065, 2971, 2635, 1700, 1604 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ):  $\delta = 4.25$  (t, J = 6.7 Hz, 1 H), 4.30 (d, J = 6.0 Hz, 2 H), 4.40 (d, J = 6.7 Hz, 2 H), 7.34 (t, J = 6.8 Hz, 2 H), 7.40–7.45 (m, 4 H), 7.71–7.75 (m, 3 H), 7.89 (d, J = 7.3 Hz, 2 H), 7.90 (d, J = 8.3 Hz, 2 H), 7.97 (t, J = 6.2 Hz, 1 H), 8.11–8.15 (m, 2 H), 8.39 (s, 1 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO- $d_6$ ): δ = 43.5, 46.8, 65.3, 120.1, 122.2, 122.8, 125.1, 127.0, 127.3, 127.6, 127.9, 129.9, 131.7, 132.4, 140.8, 143.9, 144.0, 150.8, 151.9, 156.4, 166.8.

MS (200 °C): m/z (%) = 165 (67), 178 (100), 196 (21), 477 (<1, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{29}H_{23}N_3O_4$ : 477.1689; found: 477.1680.

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#### 3-{[(3-Aminomethyl)phenyl]diazenyl}benzoic Acid (2b); Typical Procedure

To a solution of the Fmoc-protected  $\omega$ -amino acid **17b** (240 mg, 503  $\mu$ mol) in THF (10 mL) was added 20% aq NaOH (10 mL) and the resulting mixture was stirred vigorously at r.t. for 48 h (TLC monitoring). The layers were separated, the organic layer was discarded, and the aqueous layer was acidified (pH 1) by addition of aq 1 N HCl. The precipitate was separated by filtration using a suction filter, washed with a small amount of cold H<sub>2</sub>O and lyophilized, to afford **2b** as a yellow solid; yield: 55.0 mg (86%); mp 253 °C;  $R_f = 0.17$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH–AcOH, 50:48:2).

IR (ATR): 3081, 1687, 1530, 1352 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  = 4.17 (s, 2 H), 7.67 (t, J = 7.7 Hz, 1 H), 7.73–7.78 (m, 2 H), 7.96 (d, J = 7.9 Hz, 1 H), 8.10 (s, 1 H), 8.13–8.17 (m, 2 H), 8.40 (t, J = 1.7 Hz, 1 H), 8.57 (br s, 2 H), 13.3 (br s, 1 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ = 41.8, 122.3, 123.0, 123.1, 127.3, 129.8, 130.1, 132.1, 132.4, 132.5, 135.7, 151.80, 151.82, 166.6.

MS (330 °C): *m*/*z* = 77 (55), 89 (22), 106 (100), 121 (46), 255 (92, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{14}H_{13}N_3O_2$ : 255.1008; found: 255.1006.

#### 3-{[(4-Aminomethyl)phenyl]diazenyl}benzoic Acid (2c)

A solution of the Fmoc-protected  $\omega$ -amino acid **17c** (200 mg, 419  $\mu$ mol) in THF (16 mL) was treated with 20% aq NaOH (16 mL) for 48 h. Then aq 2 M HCl was added (pH 1), and the resulting precipitate was filtered. The crude material was washed with small amounts of cold H<sub>2</sub>O and THF and dried under reduced pressure, to afford **2c** as a yellow solid; yield: 74.0 mg (69%); mp >253 °C (dec.);  $R_f = 0.12$  (CH<sub>2</sub>Cl<sub>2</sub>–MeOH–AcOH, 50:48:2).

IR (ATR): 2920, 1691, 1626, 1592, 1529, 1388 cm<sup>-1</sup>.

<sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ): δ = 4.14 (s, 2 H), 7.73–7.78 (m, 3 H), 7.98 (d, J = 8.4 Hz, 2 H), 8.12–8.17 (m, 2 H), 8.39 (t, J = 1.7 Hz, 1 H), 8.59 (br s, 2 H).

<sup>13</sup>C NMR (100.6 MHz, DMSO-*d*<sub>6</sub>): δ = 41.7, 122.2, 122.8, 127.5, 130.0, 130.1, 132.0, 132.3, 138.0, 151.6, 151.8, 166.7.

MS (250 °C): *m*/*z* = 65 (22), 89 (100), 106 (58), 121 (22), 255 (50, [M]<sup>+</sup>).

HRMS: m/z [M]<sup>+</sup> calcd for  $C_{14}H_{13}N_3O_2$ : 255.1008; found: 255.1007.

**Supporting Information** for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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