DOI: 10.1002/ejoc.200600747

# Solid-Phase Synthesis of Acid-Sensitive *N*-(2-Aminoethyl)glycine-PNA Oligomers by the Fmoc/Bhoc Strategy

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**Keywords:** Peptide nucleic acids / Solid-phase synthesis / Flavin / Oxetane / Electron transfer

In the context of investigation of nucleic acid-mediated excess electron transfer, a bis-functionalized (2-aminoethyl)glycine-PNA modified with a flavin and an oxetane moiety was synthesized. The solid-phase synthesis of the required PNA oligomer was especially intriguing because of the high acid sensitivity of the oxetane moiety, so the Fmoc/Bhoc strategy was adapted to the mild cleavage conditions of the Sieberamide resin. Along with smooth oligomerization conditions, the syntheses of oxetane- and flavin-functionalized Fmoc-protected (2-aminoethyl)glycine building blocks are reported.



## Introduction

*N*-(2-Aminoethyl)glycine-PNA was introduced in 1991, originally as a triple-strand binder to DNA.[1] The main intention was to generate an oligonucleotide surrogate that would function in the *antisense* or *antigene* strategy to control gene regulation, but this artificial oligomer has since become an important diagnostic tool and nucleic acid model thanks to the extraordinary binding properties of PNA to single- and double-stranded nucleic acids and the potential for triple strand formation, strand displacement, and strong mismatch discrimination.[2] Furthermore, (2-aminoethyl)glycine-PNA has been extensively used to address oligonucleotides with labels or functional units.[3] As part of our investigation of long-distance excess electron transfer, (2-aminoethyl)glycine-PNA needed to be functionalized with both an electron donor and an acceptor group (Figure 1).[4a] The PNA strand was used to address the PNA/DNA double or triple strands with the required modifica-

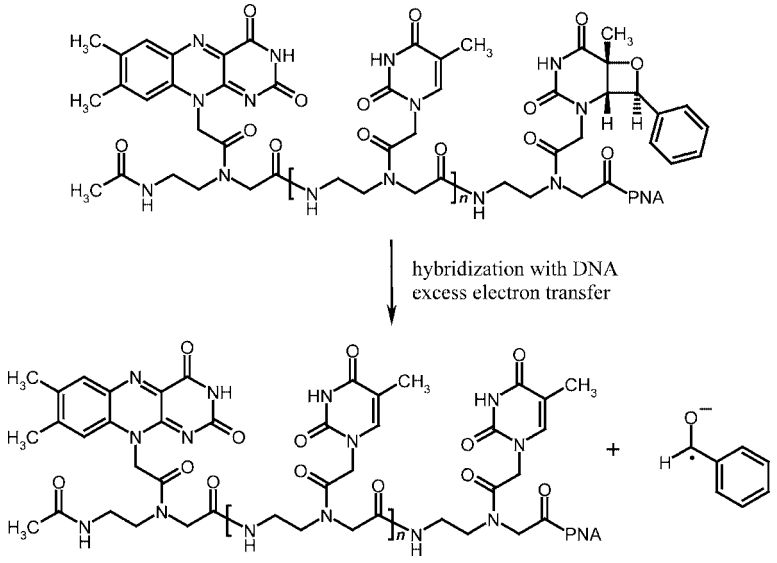


Figure 1. Bis-functionalized (2-aminoethyl)glycine-PNA oligomers for the investigation of excess electron transfer. A flavin chromophore serves for light-induced electron injection and a thymine oxetane for monitoring of anion migration through cycloreversion of the oxetane moiety[4] ($n$ = 1–8).

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tions, since PNA/DNA complexes are known to be structural mimics for the corresponding oligonucleotide pairing complexes in the B-DNA conformation.[5] Our electron transfer studies required the synthesis of bis-functionalized PNA oligomers containing a flavin moiety for "charge injection" and a highly acid-sensitive thymine oxetane for "charge trapping",[4] and so a technique for the preparation of bis-functionalized acid-sensitive (2-aminoethyl)glycine-PNA oligomers based on the Fmoc/Bhoc solid-phase peptide synthesis is presented, together with syntheses of the functionalized amino acid building blocks. In general, the optimization of the solid-phase methodology for PNA oligomers broadens the application of the Fmoc/Bhoc strategy, particularly with regard to the incorporation of highly acid-sensitive functionalities into (2-aminoethyl)glycine-PNA. It might also be applicable to the preparation of peptides modified with comparable acid-sensitive functionalities.

## Results and Discussion

For the synthesis of (2-aminoethyl)glycine-PNA, two strategies based on commercially available nucleo amino acids are known in the literature.[6] The Boc/Z strategy, with *N*-terminal Boc protection and use of the Z group for side chain protection, requires the removal of the Boc group with 95% TFA after each elongation cycle and TFMSA treatment during the final cleavage from solid support.[7] The Fmoc strategy, on the other hand, was developed for the synthesis of acid-sensitive oligomers, with the use of mild basic conditions (20% piperidine) for the removal of the Fmoc group. The Bhoc group used for side chain protection is usually removed with 95% TFA during the final cleavage from PEG-PS or Wang resin.[8]

For the synthesis of even more acid-sensitive oligomers such as the PNA/DNA chimera, the Fmoc/acyl and the Mmt/acyl strategies have been described for application in DNA synthesis,[9] although the relevant nucleo amino acids are not commercially available and require elaborate synthesis. For the synthesis of flavin-/oxetane-functionalized PNA neither the Fmoc/acyl nor the Mmt/acyl strategy appeared applicable, since the removal of the acyl protecting groups requires treatment with ammonia at elevated temperatures, which would be likely to cause degradation of the flavin and oxetane moieties, and so we decided to adapt the Fmoc/Bhoc strategy for acid-sensitive oligomers through the use of Sieberamide[10] or trityl resin.[11] An orientational experiment with the Fmoc/Bhoc monomers indicated that the Bhoc group could readily be removed within 20 min under the usual conditions for Sieberamide and trityl resin (1% TFA in DCM). The thymine oxetane moiety, representing the most acid-sensitive functionality in the oligomer, showed 10% hydrolysis within 20 min under the same conditions.

### Synthesis of Functionalized (2-Aminoethyl)glycine-PNA Building Blocks

The flavin-containing PNA building block **1** was synthesized from commercially available Fmoc-aeg-*t*Bu (**2**, Figure 2). The flavin moiety was generated by the alloxane ap-

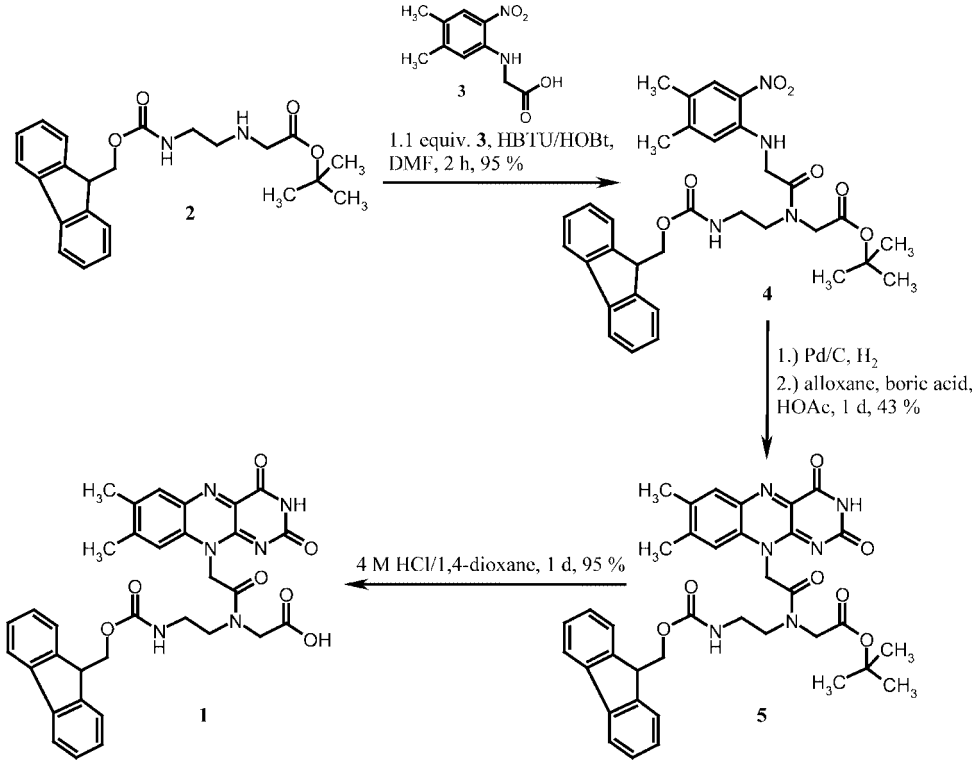


Figure 2. Synthesis of flavin building block **1** by the alloxane approach. (Dimethylnitrophenyl)glycine **3** was synthesized as described in the literature.[15]

  

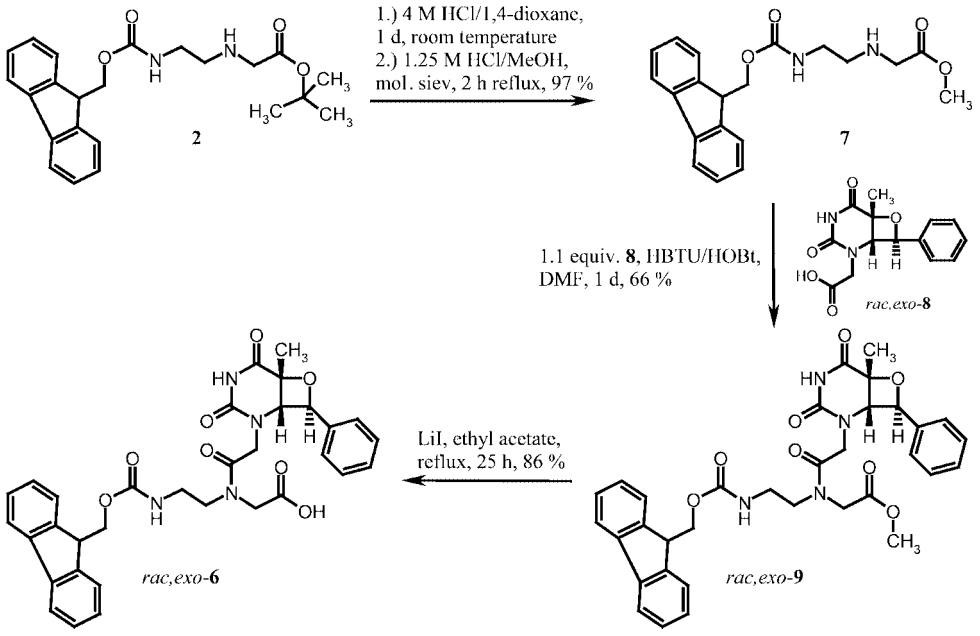


Figure 3. Synthesis of thymine oxetane building block *rac*,*exo*-**6**. Lithium iodide-mediated dealkylation of methyl ester **9** as reported by Fisher.[16]

proach after condensation with (dimethylnitrophenyl)glycine **3** by in situ HBTU/HOBt activation.[12] The nitro group in intermediate **4** was reduced with $H_2$ over Pd/C and then condensed with alloxane under boric acid catalysis conditions in an overall yield of 43%.[13] The corresponding building block **1** was obtained nearly quantitatively by acidolysis of *tert*-butyl ester **5** in 4 M HCl/1,4-dioxane.[14]

Synthesis of the oxetane-functionalized amino acid **6** by direct acylation of (2-aminoethyl)glycine was unsuccessful. Since it was necessary to avoid hydrolysis of the oxetane-functionalized amino acid under acidic conditions, the synthesis of the oxetane building block **6** was started from the Fmoc-protected (2-aminoethyl)glycine methyl ester **7**, which was in turn synthesized quantitatively from the *tert*-butyl ester **2** by transesterification (Figure 3). After condensation of thymine oxetane acetic acid *rac*,*exo*-**8** with Fmoc-aeg-OMe (**7**) by in situ HBTU/HOBt activation, the obtained methyl ester **9** was hydrolyzed in the same step by lithium iodide-mediated dealkylation with conservation of the Fmoc group in an 86% overall yield.[16] The mild hydrolysis of a methyl ester through dealkylation requires the presence of a γ-carbonyl group, as is the case for (2-aminoethyl)glycine-PNA building blocks in general. This method was clearly superior to basic hydrolysis (removal of the Fmoc group, attack on the oxetane) or hydrogenolytic cleavage of a *C*-terminal benzyl ester with $H_2$ over Pd/C (reductive oxetane opening),[17] so lithium iodide-mediated dealkylation seems suitable as a general approach for acid-sensitive (2-aminoethyl)glycine-PNA building blocks.

The thymine oxetane acetic acid *rac*,*exo*-**8** was synthesized as a racemic mixture from thymineacetic acid **10** and benzaldehyde (**11**) in a Paterno–Büchi reaction (Figure 4) and was obtained in an *exo* configuration, as indicated by the coupling constant ($^3J_{(H\text{-}6,benzyl\text{–}H)}$ = 6.6 Hz) and confirmed by 2D-NOESY NMR experiments.[18,19] The alternative regioisomer was not observed.[20] The thymine oxetane acetic acid was purified by precipitation and obtained in 28% yield.[21]

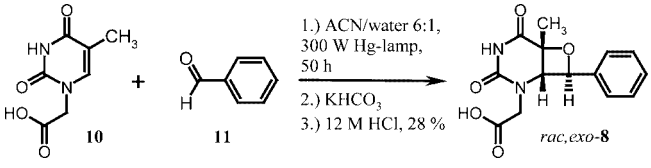


Figure 4. Synthesis of thymine oxetane acetic acid **8** through a Paterno–Büchi reaction.

## Oligomer Synthesis

The solid-phase peptide synthesis of oligomers **12**–**20** was performed manually in a plastic syringe on NovaSyn TG Sieberamide resin with use of a polyethylene glycol spacer to improve the solubility and swelling properties of the resin (Table 1). The resin was loaded to a low Fmoc-glycine capacity (0.1–0.2 mmol·g$^{-1}$); free amino termini were acetylated with acetic anhydride. PNA oligomer synthesis was performed with the commercially available Fmoc/Bhoc nucleo amino acids, the synthesized building blocks **1** and **6**, and L-Fmoc-Lys(Mtt)-OH by use of the HATU/HOAt activation technique. The synthesis was followed by UV monitoring of the dibenzofulvene released during the Fmoc deprotection,[22] which allowed optimization of the coupling conditions. In contrast with the incorporation of the flavin building block, which required double coupling and prolonged reaction times, the coupling of the oxetane building block was readily achieved. Both the flavin

  

building block and the Mtt-protected lysine were prone to acetylation during capping with acetic anhydride,[23] so a capping procedure based on Mmt chloride was used after incorporation of one of these amino acids.

As expected, the solid-phase synthesis succeeded easily, while the key problem turned out to be the cleavage of the oligomers. Cleavage under standard conditions (1% TFA, 4% TES in DCM) failed even when the TFA content was increased to 10%; this might have been due to extremely reduced solubility of the oligomer after Bhoc and Mtt removal in the cleavage solution. Another problem might be the reaction of the deprotected, nucleophilic oligomer with the xanthene cation of the Sieber linker, which was suggested by the absence of the typical red color of the xanthene cation.[10] Both problems were sufficiently solved by use of a HFIP-containing cleavage solution under continuous flow conditions (Figure 5).

To this end a solution of TFA (1%), TES (4%) in DCM/HFIP (2:1) was passed continuously through the resin by use of a burette with a flow of 1 mL min$^{-1}$. The oligomers were readily dissolved in this solution and cleavage was complete within 30 min. Without use of continuous flow, but under otherwise identical conditions, no cleavage was observed even after one day. All oligomers were synthesized on a 5 μmol scale and obtained in 5–10% yields after preparative HPLC separation. A loss of about 20% in yield was due to acid-mediated hydrolysis of the oxetane moiety. The integrity of the oxetane moieties in oligomers **12**–**20**

Table 1. Techniques used for manual solid-phase synthesis of bis-functionalized PNA by the Fmoc/Bhoc strategy on TG Sieberamide resin on a 5 μmol scale.

| Step | Reagent | | Comment |
|---|---|---|---|
| Deprotection | 20% piperidine/NMP | 3 × 3 min | Fmoc removal |
| Washing | i. NMP, 5% DIPEA, ii. DCM, iii. NMP | i.–iii. 5× | dibenzofulvene removal |
| Preactivation | 4 equiv. monomer, 3.9 equiv. HATU, 4 equiv. HOAt[a] | 5 min | in a separated vessel<br>in case of a second coupling 3 equiv. monomer, 2.9 equiv. HATU, 10 min for flavin and g |
| Coupling | preactivation mixture | 60 min | 2 × 120 min for flavin |
| Washing | i. NMP, 5% DIPEA, ii. DCM, iii. NMP | i. 2 × 5 min, ii. and iii. 5× | activated ester removal |
| Capping | i. $Ac_2O$/lutidine/NMP 1:1:8, *or*<br>ii. 0.5 M Mmt-Cl, 0.1 M DMAP, 0.5 M DIPEA, in NMP/pyridine 4:1 | i. 2 × 5 min<br>ii. 1 × 15 min | blocking of free *N*-termini<br>ii. starting with the incorporation of flavin or Lys(Mtt) |
| Washing | i. NMP, 5% DIPEA, ii. DCM,<br>iii. *tert*-butyl methyl ether, iv. NMP | i.–iv. 5× | |

[a] Fmoc-Lys(Mtt)-OH and Fmoc-Gly-OH were coupled by the HBTU/HOBt method.[24]

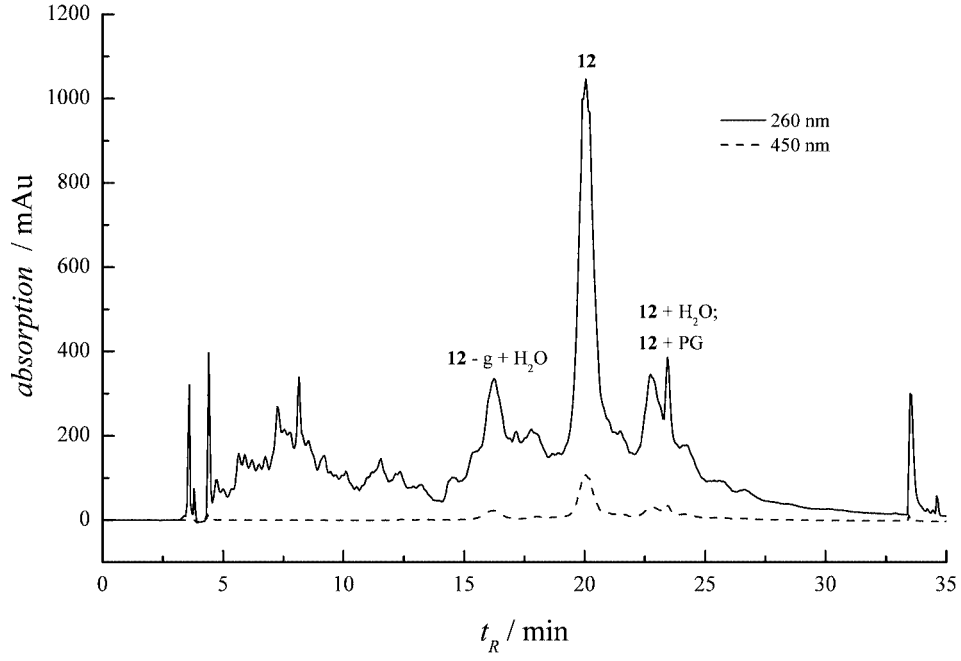


Figure 5. Preparative RP-HPLC analysis after cleavage of oligomer **12** with TFA (1%), TES (4%) in DCM/HFIP (2:1) under continuous flow conditions. Synthesis was performed as described in Table 1. The absorption at 260 nm (nucleobases) and 450 nm (flavin) is given. $t_R$ = retention time (10% B to 40% B in 25 min, B = acetonitrile/water 9:1 + 0.1% TFA, A = water + 0.1% TFA); PG = protecting group; g = aeg-guanine.

  

Table 2. Bis-functionalized PNA oligomers **12**–**20** synthesized for excess electron transfer experiments. High-resolution mass spectroscopy data (HR-ESI-MS) and the RP-HPLC retention times $t_R$ (analytical column, Vydac 25 cm, C-4, 5% → 40% B in 20 min, B = acetonitrile/water 8:2 + 0.1% TFA, A = water + 0.1% TFA) are shown. Flavin = aeg-flavin, Ox = aeg-(thymine oxetane); Uro = N=C[N(CH$_3$)$_2$]$_2$; t, a, c, g = aeg-(nucleobase), K = lysine, G = glycine.

| Sequence | HR-ESI-MS species | *m*/*z* calcd. | HPLC *m*/*z* found | $t_R$ [min] |
|---|---|---|---|---|
| Ac-*Flavin*-t$_2$-*Ox*-t$_2$-g$_2$-c-g$_2$-c-K$_2$-G-NH$_2$ (**12**) | $[M]^+$ | 3857.5725 | 3857.5734 | 16.04 |
| Ac-*Flavin*-t$_5$-g$_2$-c-g$_2$-c-K$_2$-NH$_2$ (**13**) | $[M + 3H]^{3+}$ | 1232.5103 | 1232.5099 | 13.60 |
| Uro-t$_5$-g$_2$-c–g$_2$-c-K$_2$-NH$_2$ (**14**) | $[M + 4H]^{4+}$ | 843.1183 | 843.1183 | 12.32 |
| Ac-*Flavin*-t-*Ox*-t$_7$-a-g-c-K$_2$-G-NH$_2$ (**15**) | $[M + 4H]^{4+}$ | 1019.1737 | 1019.1738 | 16.54 |
| Ac-*Flavin*-t$_2$-*Ox*-t$_6$-a-g-c-K$_2$-G-NH$_2$ (**16**) | $[M + 4H]^{4+}$ | 1019.1737 | 1019.1735 | 16.37 |
| Ac-*Flavin*-t$_4$-*Ox*-t$_4$-a-g-c-K$_2$-G-NH$_2$ (**17**) | $[M + 4H]^{4+}$ | 1019.1737 | 1019.1736 | 16.32 |
| Ac-*Flavin*-t$_6$-*Ox*-t$_2$-a-g-c-K$_2$-G-NH$_2$ (**18**) | $[M + 4H]^{4+}$ | 1019.1737 | 1019.1738 | 15.96 |
| Ac-*Flavin*-t$_8$-*Ox*-a-g-c-K$_2$-G-NH$_2$ (**19**) | $[M + 4H]^{4+}$ | 1019.1737 | 1019.1737 | 15.56 |
| Ac-*Flavin*-t$_9$-a-g-c-K$_2$-G-NH$_2$ (**20**) | $[M + 4H]^{4+}$ | 992.6633 | 992.6631 | 14.05 |

was confirmed by HR-ESI-MS (Table 2) and by subsequent excess electron transfer experiments, which are described elsewhere.[4a]

## Conclusions

The synthesis of (2-aminoethyl)glycine-PNA functionalized both with a flavin and with an oxetane moiety was achieved by the Fmoc/Bhoc strategy on solid support. The problem of the high acid sensitivity of the oxetane unit was circumvented by careful optimization of the cleavage conditions, and the resulting solid-phase peptide synthesis methodology is applicable in general for the incorporation of acid-sensitive functionalities into (2-aminoethyl)glycine-PNA. The synthesis of the thymine oxetane building block through dealkylation of an intermediate methyl ester has been introduced as an advantageous alternative to the common approach for synthesis of Fmoc-protected acid-sensitive building blocks.[2a,9a,14]

## Experimental Section

**General Methods:** Infrared spectra were recorded on a Perkin–Elmer FT-IR 1600 instrument, NMR spectra were recorded on a Varian Unity 300 or a Varian Inova 600 spectrometer, and UV absorption spectra were collected with a Jasco V-550 spectrometer. Fluorescence emission and excitation spectra were collected with a Jasco FP 6200 instrument. ESI mass spectra were collected with a Finnigan LCQ iontrap spectrometer, while high-resolution mass spectra (HR-ESI) were recorded on a Bruker FTMS-7 APEX® IV 70e FT-ICR spectrometer. Reversed-phase HPLC was performed on a Pharmacia Biotech Äkta Basic 900 system [eluents: A: deionized water (18 MΩ·cm$^{-1}$) + 0.1% TFA; analytical run: B: acetonitrile/water 8:2 + 0.1% TFA; preparative run: B: acetonitrile/water 9:1 + 0.1% TFA]. Analytical HPLC was carried out on a Vydac 214TP54, 250 × 4.6 mm, 5 μm, 300 Å, C-4 (butyl) column (1 mL min$^{-1}$). Preparative HPLC was performed on a J'sphere ODS-H80, 250 × 20 mm ID, 8–4 μm, 8 nm, C18 (YMC) column (10 mL min$^{-1}$).

**Analytical Data:** The NMR analysis of compounds **1**, **4**, **5**, **6**, and **9** suffered from high rotation barriers caused by the tertiary amide bond of the *N*-acyl-(2-aminoethyl)glycine, which resulted in broadening and partial doubling of $^1$H and $^{13}$C NMR signals.[14] In most cases the rotamers have been obtained separately giving two complete sets of NMR assignments with the aid of H,H-COSY, HSQC, and HMBC experiments.

**Fmoc-aeg-flavin-OH (1):** Fmoc-aeg-flavin-O*t*Bu (**5**, 230 mg, 0.34 mmol) was suspended in DCM (4 mL), and HCl (4 M)/1,4-dioxane (8 mL) was added slowly. After stirring overnight at room temperature the resulting solution was mixed with *tert*-butyl methyl ether (60 mL, 0 °C), and precipitation of **1** was complete within 30 min in an ice bath. The solid product was precipitated by centrifugation and the liquid phase was separated. The precipitate **1** was washed twice by suspension in *tert*-butyl methyl ether (60 mL) and obtained as a yellow solid (200 mg, 0.32 mmol, 95%), which was used for solid-phase synthesis without further purification and was stored in the dark at –28 °C to prevent slow degradation of the Fmoc group. $R_F$ (ethyl acetate/methanol 95:5) = 0.05. $^1$H NMR (300 MHz, [D$_6$]DMSO, 35 °C): $\delta$ = 11.34 (s, 1 H, NH flavin), 7.90 (d, $^3J_{H,H}$ = 7.2 Hz, 1 H, CH Fmoc), 7.88 (s, 0.5 H, CH flavin), 7.86 (s, 0.5 H, CH flavin), 7.83 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, CH Fmoc), 7.66 (t, $^3J_{H,H}$ = 8.4 Hz, 2 H, CH Fmoc), 7.57 (s, 1 H, CH flavin), 7.39 (br. t, 2 H, CH Fmoc), 7.29 (br. d, 2 H, CH Fmoc), 5.68 (br. s, 1 H, CH$_2$ flavin), 5.51 (br. s, 1 H, CH$_2$ flavin), 4.41–4.19 (m, 4 H, CH Fmoc, CH$_2$ Fmoc, 1 H C*H*$_2$COOH), 4.02 (s, 1 H, C*H*$_2$COOH), 3.66 (br. t, 1 H, CH$_2$CH$_2$), 3.40 (br. t, 1 H, CH$_2$CH$_2$), 3.30 (br. t, 1 H, CH$_2$CH$_2$), 3.16 (br. t, 1 H, CH$_2$CH$_2$), 2.45 (s, 1.5 H, CH$_3$), 2.41 (s, 1.5 H, CH$_3$), 2.38 (s, 1.5 H, CH$_3$), 2.36 (s, 1.5 H, CH$_3$) ppm. $^{13}$C NMR (150 MHz, [D$_6$]DMSO, 35 °C): $\delta$ = 171.4/170.1 (COOH), 165.3/165.0 (CO), 160.0, 156.1, 155.1, 150.1, 146.7 (C flavin), 143.8 (2 × C Fmoc), 140.6 (2 × C Fmoc), 136.7, 135.8, 133.4, 131.1, 130.8 (CH flavin), 127.5 (2 × CH Fmoc), 127.0 (2 × CH Fmoc), 125.0 (2 × CH Fmoc), 120.0 (2 × CH Fmoc), 116.3 (CH flavin), 65.4 (CH$_2$ Fmoc), 49.7, 47.4, 46.7 (CH Fmoc), 46.0, 38.0, 20.5 (CH$_3$ flavin), 18.6 (CH$_3$ flavin) ppm. IR (KBr): $\tilde{\nu}$ = 3400, 1714, 1673, 1545, 1456, 1351, 1251, 1019, 867, 762, 741 cm$^{-1}$. UV/Vis (methanol/water 1:1): $\lambda_{max}$ = 445, 367, 266, 222 nm. Fluorescence: $\lambda_{exc}$ = 450, 370 nm, $\lambda_{max,em}$ = 525 nm. ESI-MS: *m*/*z* (%): 645.2 (90) $[M + Na]^+$, 621.1 (100) $[M - H]^-$, 1243.1 (30) $[2M - H]^-$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 623.2249 for C$_{33}$H$_{30}$N$_6$O$_7$; found: 623.2253.

***N*-(4,5-Dimethyl-2-nitrophenyl)glycine (3):**[15] 3,4-Dimethyl-2-nitroaniline (8.0 g, 48 mmol) and bromoacetic acid (7.6 g, 50 mmol) were melted at 120 °C in an oil bath and stirred as long as the melt was liquid (1 h). It was necessary to ensure that the temperature never rose above 140 °C since product **3** tends to decarboxylate explosively. After cooling to room temperature, the resulting solid was dissolved in a mixture of ethyl acetate (300 mL) and aqueous KOH (1 M, 300 mL), the aqueous layer was separated, and the organic layer was extracted twice with aqueous KOH (1 M, each 200 mL). The combined aqueous layers were acidified with HCl

  

(12 M, pH 2) and the precipitated product was separated by filtration with a Buchner funnel. The crude product was recrystallized from acetic acid (70 mL) or ethanol and **3** was obtained as red crystals (3.77 g, 17 mmol, 35%). M.p. 204 °C. $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ = 7.90 (s, 1 H, aryl), 6.65 (s, 1 H, aryl), 4.12 (s, 2 H, $CH_2$), 2.28 (s, 3 H, $CH_3$), 2.19 (s, 3 H, $CH_3$) ppm. $^{13}$C NMR (50.3 MHz, [D$_6$]DMSO, 35 °C): $\delta$ = 171.2 (COOH), 147.2 (C aryl), 142.7 (C aryl), 129.1 (C aryl), 124.9 (C aryl), 124.4 (CH), 114.8 (CH), 44.16 ($CH_2$), 19.6 ($CH_3$), 17.5 ($CH_3$) ppm. IR (KBr): $\tilde{\nu}$ = 3364, 2917, 1636, 1588, 1508, 1392, 1221, 1147, 1057, 919, 762, 614 cm$^{-1}$. UV/Vis (methanol): $\lambda_{max}$ = 434, 292, 235 nm. ESI-MS: *m*/*z* (%) = 223.0 (70) [M – H]$^-$, 447.0 (100) [2M – H]$^-$, 225.1 (30) [M + H]$^+$. HR-ESI-MS: *m*/*z* [M + H]$^+$ = calcd. 225.0870; found: 225.0869.

**Fmoc-aeg-(dimethylnitrophenylglycine)-O*t*Bu (4):** *N*-(4,5-Dimethyl-2-nitrophenyl)glycine (**3**, 1.35 g, 6.0 mmol) and HOBt (1.12 g, 8.3 mmol) were dissolved in DMF abs. (50 mL), a solution of HBTU (2.20 g, 6.0 mmol) in DMF (5 mL) was added, and the system was stirred for 2 min. DIPEA (2.90 mL) was added and the solution was stirred for 2 min, after which Fmoc-aeg-O*t*Bu·HCl (**2**, 2.00 g, 4.6 mmol) in DMF (2 mL) was added and the system was stirred for a further 2 h. The solvent was removed in vacuo, the crude product was extracted with ethyl acetate (200 mL), and the organic layer was washed with aqueous HCl (0.1 M, 4 × 100 mL), brine (100 mL), sat. $NaHCO_3$ (4 × 100 mL), and brine (100 mL) and dried with $Na_2SO_4$. The title compound **4** (2.71 g, 4.37 mmol, 95%) was obtained after purification on silica gel (ethyl acetate/hexane 1:1) as an orange foam. $R_F$ (ethyl acetate/hexane 1:1) = 0.25. M.p. 98 °C. NMR: Two rotamers, A and B with A/B = 1.35 were analyzed separately. Rotamer A: $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 8.45 (br. s, 1 H, NH), 7.71 (s, 1 H, CH aryl), 7.55 (d, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 7.41 (d, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 7.26 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 7.15 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 6.24 (br. t, $^3J_{H,H}$ = 6.3 Hz, 1 H, NH), 6.16 (s, 1 H, CH aryl), 4.26 (d, $^3J_{H,H}$ = 7.2 Hz, 2 H, $CH_2$ Fmoc), 4.04 (t, $^3J_{H,H}$ = 7.2 Hz, 1 H, $CH_2CH$ Fmoc), 4.04 (s, 2 H, $CH_2$), 3.92 (s, 2 H, $CH_2$), 3.53 (t, $^3J_{H,H}$ = 5.5 Hz, 2 H, $CH_2$), 3.40 (m, 2 H, $CH_2$), 1.93 (s, 3 H, $CH_3$ aryl), 1.79 (s, 3 H, $CH_3$ aryl), 1.50–1.44 [m, 9 H, C($CH_3$)$_3$] ppm. $^{13}$C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta$ = 169.4, 168.4, 156.8, 146.9, 143.2, 141.9 (2 × C Fmoc), 140.9 (2 × C Fmoc), 130.0, 127.6 (2 × CH Fmoc), 126.9 (2 × CH Fmoc), 126.4 (CH aryl), 124.7, 124.4 (2 × CH Fmoc), 119.7 (2 × CH Fmoc), 114.0 (CH aryl), 82.8 [*C*($CH_3$)$_3$], 67.2 ($CH_2$ Fmoc), 49.6, 47.9, 46.8 ($CH_2CH$ Fmoc), 44.3, 38.9, 28.0 [C(*C*H$_3$)$_3$], 20.1 ($CH_3$ flavin), 18.3 ($CH_3$ flavin) ppm. Rotamer B: $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 8.45 (br. s, 1 H, NH), 7.88 (s, 1 H, CH aryl), 7.70 (d, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 7.54 (d, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 7.35 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 7.24 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, CH Fmoc), 6.39 (s, 1 H, CH aryl), 5.49 (br. t, $^3J_{H,H}$ = 6.0 Hz, 1 H, NH), 4.31 (d, $^3J_{H,H}$ = 7.2 Hz, 2 H, $CH_2$ Fmoc), 4.13 (t, $^3J_{H,H}$ = 7.2 Hz, 1 H, $CH_2CH$ Fmoc), 4.04 (s, 2 H, $CH_2$), 3.92 (s, 2 H, $CH_2$), 3.60 (t, $^3J_{H,H}$ = 5.4 Hz, 2 H, $CH_2$), 3.40 (m, 2 H, $CH_2$), 2.19 (s, 3 H, $CH_3$), 2.11 (s, 3 H, $CH_3$), 1.50–1.44 [m, 9 H, C($CH_3$)$_3$] ppm. $^{13}$C NMR (75.5 MHz, $CDCl_3$, 25 °C): $\delta$ = 169.2, 168.3, 156.8, 147.2, 143.8, 142.5 (2 × C Fmoc), 141.2 (2 × C Fmoc), 130.4, 127.6 (2 × CH Fmoc), 127.0 (2 × CH Fmoc), 126.6 (CH aryl), 125.0, 124.4 (2 × CH Fmoc), 119.9 (2 × CH Fmoc), 114.3 (CH aryl), 83.6 [*C*($CH_3$)$_3$], 66.8 ($CH_2$ Fmoc), 50.5, 48.2, 47.1 (CH Fmoc), 44.5, 39.0, 28.0 [C(*C*H$_3$)$_3$], 20.5 ($CH_3$ flavin), 18.5 ($CH_3$ flavin) ppm. IR (KBr): $\tilde{\nu}$ = 3423, 2976, 1722, 1633, 1567, 1509, 1397, 1237, 1155, 1042, 848, 742, 569 cm$^{-1}$. UV/Vis (methanol): $\lambda_{max}$ = 430, 301, 258, 215 nm. ESI-MS: *m*/*z* (%) = 625.2 (30) [M + Na]$^+$, 1226.9 (100) [2M + Na]$^+$, 647.0 (100) [M + HCOO]$^-$, 1248.5 (60) [2M + HCOO]$^-$. HR-ESI-MS: *m*/*z* [M + H]$^+$ = calcd. 603.2813; found: 603.2813.

**Fmoc-aeg-flavin-O*t*Bu (5):** Fmoc-aeg-(dimethylnitrophenylglycine)-O*t*Bu **4** (1.50 g, 2.5 mmol) was dissolved in glacial acetic acid (30 mL) and the system was flushed with nitrogen. Pd/C (450 mg, 15 mol-%) was added and hydrogen was bubbled slowly through the solution until the yellow color of the starting material had disappeared (2 h). The reaction mixture was degassed with nitrogen and filtered through celite into a brown glass flask, alloxane (0.52 g, 3.2 mmol) and boric acid (50 mg) were added, the solution was stirred overnight, and the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (200 mL), washed (3 × 1 M aqueous HCl, 1 × brine, 3 × 0.5 M aqueous $KHCO_3$, 2 × brine), and dried with $Na_2SO_4$, and the obtained product was purified on silica gel (ethyl acetate/methanol 95:5) to provide compound **5** (0.71 g, 1.05 mmol, 43%) as a yellow solid. $R_F$ (ethyl acetate, 2% methanol) = 0.23. M.p. 174 °C. NMR: Two rotamers A and B with A/B = 1.33 were analyzed separately. Rotamer A: 1H NMR (600 MHz, [D$_6$]DMSO, 35 °C): $\delta$ = 11.33 (s, 1 H, NH), 7.89 (s, 1 H, CH aryl), 7.82 (d, $^3J_{H,H}$ = 7.8 Hz, 2 H, CH Fmoc), 7.67–7.64 (m, 2 H, CH Fmoc), 7.50 (br. t, 1 H, NH), 7.48 (s, 1 H, CH aryl), 7.40–7.35 (m, 2 H, CH Fmoc), 7.29 (t, $^3J_{H,H}$ = 7.8 Hz, 2 H, CH Fmoc), 5.67 (br. s, 2 H, $CH_2$ flavin), 4.41 (s, 2 H, $CH_2$), 4.39 (d, covered, 2 H, $CH_2$ Fmoc), 4.24 (br. t, 1 H, CH Fmoc), 3.65 (br. t, 2 H, $CH_2$), 3.43 (m, 2 H, $CH_2$), 2.40 (s, 3 H, $CH_3$ flavin), 2.36 (s, 3 H, $CH_3$ flavin), 1.36 [s, 9 H, C($CH_3$)$_3$] ppm. $^{13}$C NMR (150 MHz, [D$_6$]DMSO, 35 °C): $\delta$ = 169.1, 165.3, 159.6, 156.1, 155.1, 150.2, 146.7, 143.8 (2 × C Fmoc), 140.7 (2 × C Fmoc), 136.7, 135.8, 133.5, 131.2, 130.8 (CH flavin), 127.5 (2 × CH Fmoc), 127.0 (2 × CH Fmoc), 125.0 (2 × CH Fmoc), 120.0 (2 × CH Fmoc), 116.1 (CH flavin), 81.0 [*C*($CH_3$)$_3$], 65.5 ($CH_2$ Fmoc), 50.3, 47.5, 46.7 (CH Fmoc), 45.9, 38.9, 27.6 [C(*C*H$_3$)$_3$], 20.4 ($CH_3$ flavin), 18.7 ($CH_3$ flavin) ppm. Rotamer B: $^1$H NMR (600 MHz, [D$_6$]DMSO, 35 °C): $\delta$ = 11.33 (s, 1 H, NH), 7.89 (s, 1 H, CH flavin), 7.85 (d, $^3J_{H,H}$ = 7.8 Hz, 2 H, CH Fmoc), 7.67–7.64 (m, 2 H, CH Fmoc), 7.42 (s, 1 H, CH flavin), 7.40–7.35 (m, 2 H, CH Fmoc), 7.29 (t, $^3J_{H,H}$ = 7.8 Hz, 2 H, CH Fmoc), 7.23 (br. t, 1 H, NH), 5.47 (br. s, 2 H, $CH_2$ flavin), 4.30 (d, $^3J_{H,H}$ = 7.2 Hz, 2 H, $CH_2$ Fmoc), 4.19 (t, $^3J_{H,H}$ = 7.2 Hz, 1 H, $CH_2CH$ Fmoc), 3.97 (s, 2 H, $CH_2$), 3.37 (t, $^3J_{H,H}$ = 6.0 Hz, 2 H, $CH_2$), 3.14 (m, 2 H, $CH_2$), 2.45 (s, 3 H, $CH_3$), 2.36 (s, 3 H, $CH_3$), 1.52 [s, 9 H, C($CH_3$)$_3$] ppm. $^{13}$C NMR (150 MHz, [D$_6$]DMSO, 35 °C): $\delta$ = 167.8, 165.1, 159.6, 156.5, 155.2, 150.1, 146.6, 143.8 (2 × C Fmoc), 140.7 (2 × C Fmoc), 136.8, 135.9, 133.4, 131.1, 130.9 (CH flavin), 127.5 (2 × CH Fmoc), 127.0 (2 × CH Fmoc), 125.0 (2 × CH Fmoc), 120.0 (2 × CH Fmoc), 116.4 (CH flavin), 82.4 [*C*($CH_3$)$_3$], 65.4 ($CH_2$ Fmoc), 48.9, 47.3, 46.7 (CH Fmoc), 45.4, 38.1, 27.7 [C(*C*H$_3$)$_3$], 20.4 ($CH_3$ flavin), 18.6 ($CH_3$ flavin) ppm. IR (KBr): $\tilde{\nu}$ = 3407, 2977, 1715, 1673, 1582, 1546, 1242, 1155, 1017, 861, 740 cm$^{-1}$; UV/Vis (methanol/water 1:1): $\lambda_{max}$ = 445, 367, 266, 222 nm. Fluorescence: $\lambda_{exc}$ = 450, 365 nm, $\lambda_{max,em}$ = 525 nm. ESI-MS: *m*/*z* (%) = 701.2 (100) [M + Na]$^+$, 1379.0 (90) [2M + Na]$^+$, 677.2 (40) [M – H]$^-$, 1355.2 (100) [2M – H]$^-$. HR-ESI-MS: *m*/*z* [M + H]$^+$ = calcd. 679.2875; found: 679.2871.

**Fmoc-aeg-(thymine oxetane)-OH (*rac*,*exo*-6):** Fmoc-aeg-(thymine oxetane)-OMe *rac*,*exo*-**9** (660 mg, 1.05 mmol) was dissolved in ethyl acetate (20 mL), lithium iodide (500 mg, 3.7 mmol) was added, and the mixture was heated at reflux for 25 h. Ethyl acetate (200 mL) was added and the solution was washed with $NaHSO_3$ solution (3 × 100 mL 0.5 M aqueous $NaHSO_3$/HCl, pH 2) and brine (2 × 100 mL sat. aqueous NaCl/HCl, pH 2) and dried with $Na_2SO_4$. The resulting crude product (654 mg) was purified on RP-18 silica gel (600 mL, methanol/water 6:4) and compound **6** (550 mg, 0.90 mmol, 86%) was obtained as a white solid. $R_F$ (ethyl

  

acetate/methanol/water/acetic acid 10:1:1:0.5) = 0.66. M.p. 196 °C (decomp.). $^1$H NMR (600 MHz, $CD_3OD$): $\delta$ = 7.77 (d, $^3J_{H,H}$ = 7.2 Hz, 2 H, Fmoc), 7.60 (m, 2 H, Fmoc), 7.43–7.21 (m, 9 H, 4 H Fmoc, 5 H aryl), 5.75–5.72 (d, $^3J_{H,H}$ = 6.6 Hz, 1 H, CH thymine), 4.50–3.70 (m, 2 H, $CH_2$ thymine), 4.32–4.25 (m, 2 H, $CH_2$ Fmoc), 4.20–4.10 (m, 2 H, CH Fmoc, CH thymine), 4.10–3.95 (m, 2 H, $CH_2$COOMe), 3.42–3.37 (m, 1 H, $CH_2$–$CH_2$), 3.34–-3.31 (m, 1 H, $CH_2$–$CH_2$), 3.23–3.11 (m, 2 H, $CH_2$–$CH_2$), 1.72 (s, 1.5 H, $CH_3$ thymine), 1.70 (s, 1.5 H, $CH_3$ thymine) ppm. $^{13}$C NMR (150 MHz, $CD_3OD$): $\delta$ = 2×172.7 (C-4 thymine, COOH), 170.2 (C), 158.8 (CO-Fmoc), 153.4 (C-2 thymine), 145.3 (2×C Fmoc), 142.6 (2×C Fmoc), 140.6 (C aryl), 129.7 (3×CH aryl), 128.8 (2×CH Fmoc), 128.1 (2×CH Fmoc), 127.2 (2×CH aryl), 126.2 (2×CH Fmoc), 120.9 (2×CH Fmoc), 87.4 (CH–O thymine), 79.0 (C thymine), 67.8 ($CH_2$ Fmoc), 67.0 (CH thymine), 51.8 ($CH_2$), 49.5 ($CH_2$), 49.0 ($CH_2$), 48.4 (CH Fmoc), 39.6 ($CH_2$), 23.1 ($CH_3$ thymine) ppm. IR (KBr): $\tilde{\nu}$ = 3422, 1715, 1475, 1276, 1216, 759, 551 cm$^{-1}$. UV/Vis (methanol): $\lambda_{max}$ = 214, 263, 289, 299 nm. ESI-MS: *m*/*z* (%): 613.1 (30) $[M + H]^+$, 1247.1 (80) $[2M + Na]^+$; 611.1 (70) $[M - H]^-$, 1223.1 (100) $[2M - H]^-$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 613.2293 for $C_{33}H_{32}N_4O_8$; found: 613.2292.

**Fmoc-aeg-OMe·HCl (7):** Fmoc-aeg-*t*Bu·HCl (**2**, 500 mg, 1.15 mmol) was suspended in DCM (10 mL) and dissolved slowly by addition of HCl (4 M) in 1,4-dioxane (15 mL). After complete conversion (TLC), the solvent was removed in vacuo, and the residue was dissolved in HCl in methanol (1.25 M, 20 mL) and heated at reflux after addition of a *small* amount of mol. sieves and toluene (20 mL) for 4 h *without* stirring. The mol. sieves were filtered off, the solvent was removed in vacuo, the residue was partitioned between DCM (100 mL) and aqueous $NaHCO_3$ (0.5 M, 100 mL), the aqueous layer was extracted with DCM (3×70 mL), and the combined organic layers were washed with aqueous $NaHCO_3$ (0.5 M, 2×100 mL) and brine (100 mL) and dried with $Na_2SO_4$. After addition of HCl in MeOH (1.25 M, 2 equiv.) the solvent was removed and **7** (438 mg, 1.12 mmol, 97%) was obtained as a white solid. $R_F$ (ethyl acetate/methanol 7:1) = 0.43 (**11**), 0.62 (**6**), 0.25 (Fmoc-aeg-OH). M.p. 100 °C. $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ = 7.77 (d, $^3J_{H,H}$ = 7.5 Hz, 2 H, Fmoc), 7.63 (d, $^3J_{H,H}$ = 7.5 Hz, 2 H, Fmoc), 7.37 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, Fmoc), 7.29 (t, $^3J_{H,H}$ = 7.5 Hz, 2 H, Fmoc), 4.38 (d, $^3J_{H,H}$ = 6.3 Hz, 2 H, $CH_2$ Fmoc), 4.19 (t, $^3J_{H,H}$ = 6.3 Hz, 1 H, CH Fmoc), 4.00 (s, 2 H, C$H_2$COOMe), 3.81 (s, 3 H, $OCH_3$), 3.45 (t, $^3J_{H,H}$ = 5.5 Hz, 2 H, $CH_2$–$CH_2$), 3.20 (d, $^3J_{H,H}$ = 5.5 Hz, 2 H, $CH_2$–$CH_2$) ppm. $^{13}$C NMR (75.5 MHz, $CD_3OD$): $\delta$ = 168.0 (*C*OOMe), 159.4 (CO-Fmoc), 145.1 (2×C Fmoc), 142.6 (2×C Fmoc), 128.8 (2×CH Fmoc), 128.1 (2×CH Fmoc), 126.1 (2×CH Fmoc), 120.9 (2×CH Fmoc), 68.1 ($CH_2$ Fmoc), 53.5 ($CH_3O$), 49.2 ($CH_2$), 48.2 (CH Fmoc), 48.1 ($CH_2$), 38.1 ($CH_2$) ppm. IR (KBr): $\tilde{\nu}$ = 3462, 2953, 1743, 1539, 1450, 1255, 742, 583 cm$^{-1}$. UV/Vis (MeOH): $\lambda_{max}$ = 214, 263, 299 nm. ESI-MS: *m*/*z* (%) = 355.1 (100) $[M + H]^+$, 708.7 (45) $[2M + H]^+$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 355.1652; found: 355.1653.

**Thymine Oxetane Acetic Acid (*rac,exo*-8):** Thymineacetic acid[25] (**10**, 4.00 g, 21.7 mmol) and benzaldehyde (**11**, 10 mL, 95 mmol) were suspended in acetonitrile (240 mL) in a Pyrex flask. With vigorous stirring, water was added (40 mL) until a clear solution was obtained, and the solution was flushed with argon. The reaction mixture was stirred and exposed to the unfiltered output of a 300 W Hg-lamp for 50 h under argon. The solvent was removed, the residue was dissolved in ethyl acetate (500 mL) and filtered, the organic layer was extracted with aqueous $KHCO_3$ (0.5 M, 5×100 mL), the combined aqueous layers were acidified with HCl (12 M, pH 2) at 0 °C, and the precipitated title compound **8** (1.81 g, 6.24 mmol, 28%) was filtered off, washed with aqueous HCl (0.1 M) at 0 °C, and dried in vacuo. If the purity was not sufficient, the precipitation was repeated. M.p. 240 °C. $^1$H NMR (300 MHz, [$D_6$]-DMSO, 35 °C): $\delta$ = 12.81 (s, 1 H, OH), 10.84 (s, 1 H, NH), 7.46–7.31 (m, 5 H, CH aryl), 5.64 (d, $^3J_{H,H}$ = 6.6 Hz, 1 H, O–CH), 4.35 (d, $^3J_{H,H}$ = 6.6 Hz, 1 H, H-6), 4.09 (d, $^2J_{H,H}$ = 17.7 Hz, 1 H, $CH_2$), 3.80 (d, $^2J_{H,H}$ = 17.4 Hz, 1 H, $CH_2$), 1.66 (s, 3 H, $CH_3$) ppm. $^{13}$C NMR (75.5 MHz, [$D_6$]DMSO, 35 °C): $\delta$ = 170.3, 170.2, 151.3 (C-2), 139.2 (C aryl), 128.5 (2×CH aryl), 128.3 (2×CH aryl), 126.4 (CH aryl), 85.5 (O–CH), 77.1 (C-5), 64.3 (CH-6), 48.2, 22.3 ($CH_3$ flavin) ppm. IR (KBr): $\tilde{\nu}$ = 3430, 1717, 1671, 1489, 1406, 1282, 1237, 886, 774 cm$^{-1}$; UV/Vis (methanol/water 1:1): $\lambda_{max}$ = 225 nm. ESI-MS: *m*/*z* (%): 602.9 (100) $[2M + Na]^+$, 289.1 (100) $[M - H]^-$, 578.9 (90) $[2M - H]^-$. HR-ESI MS: *m*/*z* $[M + H]^+$ = calcd. 291.0976; found: 291.0977.

**Fmoc-aeg-(thymine oxetane)-OMe (*rac,exo*-9):** Thymine oxetane acetic acid (*rac*,*exo*-**8**, 600 mg, 2.07 mmol), Fmoc-aeg-OMe·HCl (**7**, 734 mg, 1.88 mmol), HBTU (784 mg, 2.07 mmol), and HOBt (508 mg, 3.76 mmol) were dissolved at 0 °C in DMF (25 mL), DIPEA (1.45 mL) was added, and the resulting solution was stirred (14 h) at room temperature. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate (100 mL), washed (3×100 mL 0.5 M aqueous $NaHCO_3$, 100 mL brine, 3×100 mL 1 M aqueous HCl, 100 mL brine), and dried with $Na_2SO_4$. The crude product was purified on silica gel (300 mL, ethyl acetate), and compound **9** (780 mg, 1.24 mmol, 66%) was obtained as a white solid. $R_F$ (ethyl acetate) = 0.40. M.p. 104 °C. NMR: Two rotamers A and B with A/B = 3:1. $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ = 7.77 (d, $^3J_{H,H}$ = 7.5 Hz, 2 H, Fmoc), 7.60 (m, 2 H, Fmoc), 7.43–7.21 (m, 9 H, 4×H Fmoc, 5×H aryl), 5.75 (d, $^3J_{H,H}$ = 6.3 Hz, 0.75 H, CH thymine), 5.73 (d, $^3J_{H,H}$ = 6.3 Hz, 0.25 H, CH thymine), 4.50–3.80 (m, 2 H, $CH_2$ thymine), 4.32–4.25 (m, 2 H, $CH_2$ Fmoc), 4.20–4.15 (m, 1 H, CH Fmoc), 4.15–4.05 (m, 3 H, CH thymine, C$H_2$COOMe), 3.69 (s, 2.25 H, $OCH_3$), 3.61 (s, 0.75 H, $OCH_3$), 3.36–3.32 (m, 2 H, $CH_2$–$CH_2$), 3.21–3.11 (m, 2 H, $CH_2$–$CH_2$), 1.72 (s, 3 H, $CH_3$ thymine) ppm. $^{13}$C NMR (75.5 MHz, $CD_3OD$): $\delta$ = 172.7 (C-4 thymine), 171.4 (*C*OOMe), 170.2 (C), 158.8 (CO Fmoc), 153.3 (C-2 thymine), 145.3 (2×C Fmoc), 142.6 (2×C Fmoc), 140.6 (C aryl), 129.8 (3×CH aryl), 128.8 (2×CH Fmoc), 128.2 (2×CH Fmoc), 127.2 (2×CH aryl), 126.2 (2×CH Fmoc), 120.9 (2×CH Fmoc), 87.3 (CH benzyl thymine), 79.2 (C thymine), 67.7 ($CH_2$ Fmoc), 67.0 (CH thymine), 52.8 ($CH_3O$), 50.4 ($CH_2$), 49.2 ($CH_2$), 48.8 ($CH_2$), 48.4 (CH Fmoc), 39.9 ($CH_2$), 23.0 ($CH_3$ thymine) ppm. IR (KBr): $\tilde{\nu}$ = 3412, 1716, 1473, 1276, 1212, 759, 530 cm$^{-1}$. UV/Vis (MeOH): $\lambda_{max}$ = 214, 263, 289, 299 nm. ESI-MS: *m*/*z* (%) = 649.3 (25) $[M + Na]^+$, 1275.0 (100) $[2M + Na]^+$, 1251.0 $[2M - Na]^-$. HR-ESI-MS: *m*/*z* $[M + H]^+$ = calcd. 627.2450; found: 627.2449.

**Solid-Phase Peptide Synthesis:** Oligomers **12**–**20** were synthesized manually on a 5 μmol scale in a 2 mL plastic syringe. Commercially available nucleo amino acids Fmoc-aeg-thymine-OH, Fmoc-aeg-guanine(Bhoc)-OH, Fmoc-aeg-adenine(Bhoc)-OH, Fmoc-aeg-cytosine(Bhoc)-OH (purchased from Applied BioSystems), amino acids Fmoc-lysine(Mtt)-OH, Fmoc-glycine-OH (purchased from NovaBiochem), and the synthesized buildings blocks Fmoc-aeg-flavin-OH (**1**) and Fmoc-aeg-oxetane-OH (**6**) were used. The syntheses were performed on NovaSyn TG Sieberamide resin (0.15 mmol·g$^{-1}$), which was swollen in DCM (1 h) before the first amino acid was attached. The ordinary amino acids Fmoc-Lys(Mtt)-OH and Fmoc-Gly-OH were coupled by the HBTU/HOBt method with four equivalents relative to the resin. All nucleo amino acids were coupled with four equivalents relative to the resin by the HATU/HOAt technique, which required the preactivation (5 min) of the monomers in a separating vessel. The removal of

  

the Fmoc group was followed by UV monitoring of the released dibenzofulvene at 290 nm. For incorporation of flavin, coupling g on g and t on g, double coupling (2 × 3 equiv.) and prolonged coupling times (2 × 120 min) were used.

**Synthesis Procedure:** *Deprotection:* 2 × 4 min 20% piperidine in NMP; *washing:* 5 × NMP, 2 × DCM, 5 × NMP; *coupling of ordinary amino acids:* 4 equiv. amino acid, 4 equiv. HBTU/HOBt, 10 equiv. DIPEA, in NMP, final concentration of amino acid 0.2 M, 45 min; *coupling of nucleo amino acids:* 4 equiv. amino acid, 3.9 equiv. HATU/HOAt, 0.20 M DIPEA, 0.30 M lutidine in NMP, final concentration of amino acid 0.1 M, 5–10 min preactivation, 60 min coupling, for double coupling 3 equiv. amino acid, for flavin 2 h coupling time; *washing:* 5 × 5% DIPEA in NMP, 2 × 5 min NMP (for removal of active ester); *capping:* before incorporation of flavin or Lys(Mtt) 2 × 5 min 10% $Ac_2O$/20% lutidine in NMP, after incorporation of flavin or Lys(Mtt) 1 × 15 min 0.5 M Mmt-Cl, 0.1 M DMAP, 0.5 M DIPEA in NMP/pyridine, 4:1; *washing:* 5 × 5% DIPEA in NMP, 5 × *tert*-butyl methyl ether, 2 × NMP.

**Cleavage Procedure:** *Washing:* 5 × 10 min NMP, 5 × DCM, 5 × *tert*-butyl methyl ether, 5 × switching between DCM and *tert*-butyl methyl ether; *swelling:* 30 min DCM; *cleavage:* HFIP/DCM, 1:2 10 min for removal of Mmt and Mtt groups; 1% TFA, 4% TES in HFIP/DCM, 1:2, slowly dropping over the resin from a burette (1 mL min$^{-1}$), 20–30 min, color change of the resin to deep red within 5 min; drying in vacuo, pay attention to the instability of the oxetane under the cleavage conditions (20% degradation in 45 min). *Purification:* Solvation of the crude product in 0.5–1 mL 1% acetic acid, microfiltration, purification by preparative HPLC 10% B to 40% B in 25 min $t_R$ ca. 21 min (B = acetonitrile/water 9:1 + 0.1% TFA, A = water + 0.1% TFA), lyophilization and storage in brown caps at –28 °C.

**Abbreviations:** aeg = (2-aminoethyl)glycine, Bhoc = benzhydryloxycarbonyl, Boc = *tert*-butyloxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl, HFIP = 1,1,1,3,3,3-hexafluoropropan-2-ol, HATU = *O*-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HBTU = *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOAt = 1-hydroxy-7-azabenzotriazole, HOBt = 1-hydroxybenzotriazole, Mmt = monomethoxytrityl, Mtt = monomethyltrityl, TES = triethylsilane, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonic acid, Z = benzyloxycarbonyl.

## Acknowledgments

This work was supported by the Volkswagen-Stiftung and the Deutsche Forschungsgemeinschaft (GRK 782). We are grateful for a fellowship from the Fonds der Chemischen Industrie for T. S.

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Received: August 23, 2006
Published Online: November 27, 2006

  