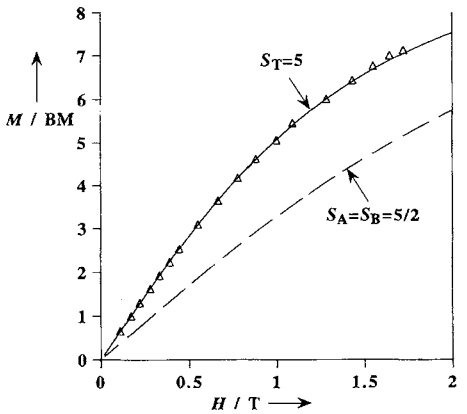

Figure 3. Dependence of the magnetization M of complex **1** on the field strength H at 4.4 K. Triangles: experimental data; solid line: Brillouin function for a spin of $S = 5$ with $g = 1.99$; dotted line: Brillouin function for two noninteracting local spins $S_A = S_B = 5/2$.

the Brillouin function for a spin of $S = 5$ with $g = 1.99$. The curve lies well above that for two independent local spins $S = 5/2$. The value of J is twice that reported for the parent bis(μ-end-on-azido)manganese(II) dimer $[Mn_2(terpy)_2(N_3)_4]\cdot 2H_2O$.[7] Because the angles at the azido bridge are similar (106.0(2)° in **1** and 104.6(1)° in the manganese(II) complex), the fact that the magnetically active 3d orbitals of Fe^{III} are lower in energy than those of Mn^{II}, and thus closer to the symmetry-adapted HOMOs of the bridging azide ligand, accounts for the greater ferromagnetic coupling in **1**. Further examples of iron(III) complexes with this structural type are needed to characterize the influence of the angle at the azido bridge on the nature and magnitude of the magnetic coupling.

Experimental Section

1: On attempting to prepare **2** by the diffusion technique described [9], polyhedral red-brown single crystals of **1** formed by slow oxidation (atmospheric oxygen) of aqueous solutions containing iron(II) chloride, bpym, and sodium azide. In the IR spectrum of **1** the $\nu_{as}(N_3)$ stretching mode appears as a very strong split band at 2040 and 2060 cm^{-1}, which is consistent with the presence of both end-on and terminal azide ligands. The ring-stretching mode of bpym appears as a quasi-symmetric doublet at 1570 and 1545 cm^{-1}, as observed in other complexes containing chelating bpym.

X-ray structure analysis of **1**: Siemens R3mV automatic diffractometer, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, graphite monochromator, 295 K. Lorentzian, polarization, and ψ-scan absorption corrections [14] were made. Data collection, solution, and refinement: ω-2θ, standard Patterson methods with subsequent Fourier recycling (SHELXTL-PLUS program) [15]. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms of the water molecules were located on a ΔF map and refined with constraints. The hydrogen atoms of the bpym ligand were set in calculated positions and refined as riding atoms. They were all refined isotropically. Final geometrical calculations and graphical manipulations were performed with the PARST program [16] and the XP utility of the SHELX-PLUS system, respectively. $C_{48}H_{40}Fe_4N_{54}O_2$ ($M_r = 1628.7$), monoclinic, space group $P2_1/c$, $a = 10.644(2)$, $b = 15.026(3)$, $c = 20.549(6)$ Å, $\beta = 99.86°$, $V = 3238.0(13)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.671$ g cm^{-3}, $\mu = 9.67$ cm^{-1}, $F(000) = 1652$, $3 < 2\theta < 54°$, crystal size $0.31 \times 0.27 \times 0.22$ mm. 7126 unique reflections, and 3939 assumed as observed with $I \geq 3\sigma(I)$, 487 parameters, $R = 0.050$, $R_w = 0.053$, and $s = 1.426$ with $w^{-1} = \sigma^2(F_o) + 0.0008(F_o)^2$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100168. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: int. code +(1223) 336-033; e-mail: deposit@chemcrys.cam.ac.uk).

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Towards Artificial DNA-Repair Enzymes: Incorporation of a Flavin Amino Acid into DNA-Binding Oligopeptides**

Thomas Carell* and Jens Butenandt

UV irradiation of cells causes the formation of cyclobutane pyrimidine dimers by $[2\pi + 2\pi]$ cycloaddition of two pyrimidine bases that are adjacent to one another in the DNA double helix.[1, 2] To counteract the resulting lethal loss of genetic information, all organisms have developed sophisticated DNA-repair mechanisms to remove these DNA lesions and to restore the integrity of the genome.[3] Investigation of the inherited lethal disease *Xeroderma pigmentosum* revealed that insufficient DNA-repair capacities are directly involved in tumor genesis.[4, 5] DNA photolyases are ancient repair enzymes that are capable of specifically recognizing cyclobutane pyrimidine dimers, possibly by flipping the pyrimidine dimers out of the DNA double helix into a cage-like active site.[6] The enzymes

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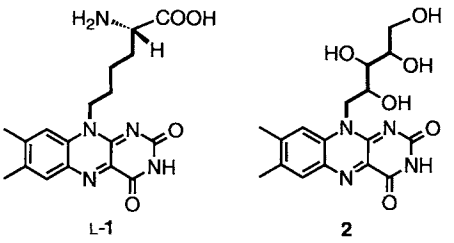
utilize multiple light-driven energy- and electron-transfer steps for repairing the dimer lesion. Initially, excitation of a flavin semiquinone ($FADH^{\bullet}$) yields the reduced flavin cofactor ($FADH^-$) by an electron transfer from Trp^{306}, which is more than 13 Å away from the flavin.[6] A second light-driven electron transfer from the $FADH^-$ to the bound cyclobutane pyrimidine dimer initiates monomerization (repair) of the dimer lesion. In addition to $FADH^-$ all known microbial photolyases contain a second cofactor (either a 8-hydroxy-5-deazaflavin or a methenyltetrahydrofolate), which acts as a photo antenna and transmits the required excitation energy to the flavin moiety.[6, 7]

To learn about these energy- and electron-transfer steps, we started a research program aimed at preparing small cofactor-containing model proteins that form stable and structurally well-defined complexes with DNA. These model proteins should allow rapid synthetic modification, for example incorporation of unnatural amino acids at various sites.

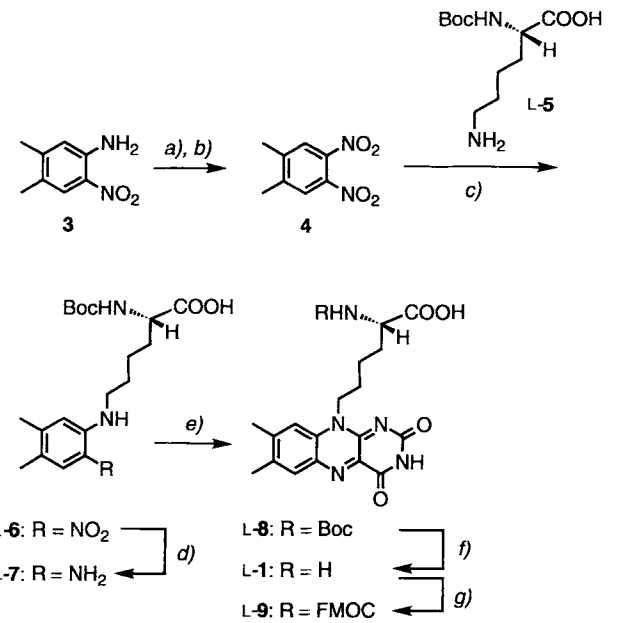
DNA-binding oligopeptides possessing the sequence of the DNA-binding domain of the helix-loop-helix transcription factors MyoD[8, 9] were prepared by solid-phase peptide synthesis. The DNA-repair function was included in these DNA-binding proteins by incorporation of the flavin amino acid L-**1**.[10] These



cofactor-modified oligopeptides were investigated for their ability to repair cyclobutane pyrimidine dimers in single-stranded DNA. A well-defined oligonucleotide containing a synthetic dimer lesion[11] was synthesized to enable quantification of the repair reaction and allow HPLC monitoring of potential side products that have been observed by irradiation of DNA in the presence of riboflavin **2**.[12]

Synthesis of L-**1** was achieved in gram quantities by the route outlined in Scheme 1. In the first two steps 4,5-dimethyl-2-nitroaniline (**3**) was oxidized with Caro's acid and hydrogen peroxide/nitric acid to yield dinitro dimethyl benzene (**4**).[13] Reaction of **4** with the chiral building block *N*-α-Boc-L-lysin (L-**5**), which was chosen with an unprotected carboxylic acid to minimize base-induced racemization at the chiral α-CH center during the *ipso*-substitution reaction, afforded nitroaniline L-**6**. The reaction conditions were varied to further reduce the risk of racemization; optimal results were finally obtained with potassium acetate as the base in water/ethanol/*n*-butanol (1/1/2). Catalytic hydrogenation of L-**6** afforded the amino acid L-**7**, which was then treated with alloxane monohydrate to yield the Boc-protected flavin amino acid L-**8**.[14] Deprotection of L-**8** and reaction of the resulting unprotected flavin amino acid L-**1** with 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide (FMOC-OSu) and sodium hydrogen carbonate afforded the FMOC-protected L-flavin amino acid L-**9** in an overall yield of 25%.

To determine the enantiomeric purity of L-**1**, the corresponding D-amino acid D-**1** was prepared by the same procedure from the D-lysine building block D-**5**. The racemate was obtained by



Scheme 1. Synthesis of the flavin amino acid L-**1** and the FMOC-protected amino acid L-**9**: a) H_2SO_5, room temperature (RT), 80%; b) H_2O_2, HNO_3, RT, 85%; c) KOAc, H_2O, EtOH, *n*BuOH, 80 °C, 3 d, 60%; d) H_2, Pd/C, quant; e) HOAc, $B(OH)_3$, alloxane, RT, 65%; f) TFA, RT, quant; g) FMOC-OSu, $NaCO_3$, 95%.

mixing both enantiomers. Separation of the enantiomers was accomplished by thin-layer ligand-exchange chromatography on reverse phase CHIRALPLATES.[15] A dilution experiment, in which decreasing amounts of D-**1** were added to the corresponding L-form, and subsequent scanning of the obtained TLCs established that as little as 1% D-amino acid is detectable. Since the synthetic material L-**1** yields only one spot, it was synthesized with greater than 99:1 enantiomeric purity.

A standard manual FMOC solid-phase synthesis protocol with a Rink-Amide MBHA resin as the solid support was modified for the synthesis of the oligopeptides depicted in Figure 1.[16] The X-ray crystal structure of the helix-loop-helix transcription factor MyoD complexed with double-stranded DNA was analyzed for an initial design of the flavin peptides.[17] The side chain of Leu^{121} at the C-terminal end of the basic domain points towards the major groove; Leu^{121} was therefore replaced by L-**1**. The solid-phase synthesis of the flavin oligopeptide **P1** was performed in analogy to **P2**, which does not contain flavin. However, due to the slower coupling rates of L-**9** the coupling time for this amino acid was extended from 25 to 45 min, and the coupling repeated three times with a threefold excess of L-**9**. Capping procedures (for example acetic anhydride/Hünig's base), often performed after an elongation step, were omitted when possible after incorporation of the flavin amino acid. Cleavage of the final peptide from the solid support and removal of the side-chain protection groups required larger quantities of the scavengers triisopropyl silane (2 vol%) and, in the case of the cysteine-containing peptide **P3**, ethanedithiol (2 vol%) to obtain the flavin oligopeptides **P1** and **P3** in yields and with a purity comparable to those of the non-flavin peptide **P2**.

All peptides were purified by preparative reverse-phase HPLC and characterized by analytical HPLC as well as electrospray and laser-ionization mass spectrometry.[18] The UV/Vis spectrum of the flavin peptides feature the typical flavin absorption bands around 450 and 360 nm. The fluorescence spectra show the flavin fluorescence band at 550 nm.

  0570-0833/97/3613-1462 $ 17.50+.50/0

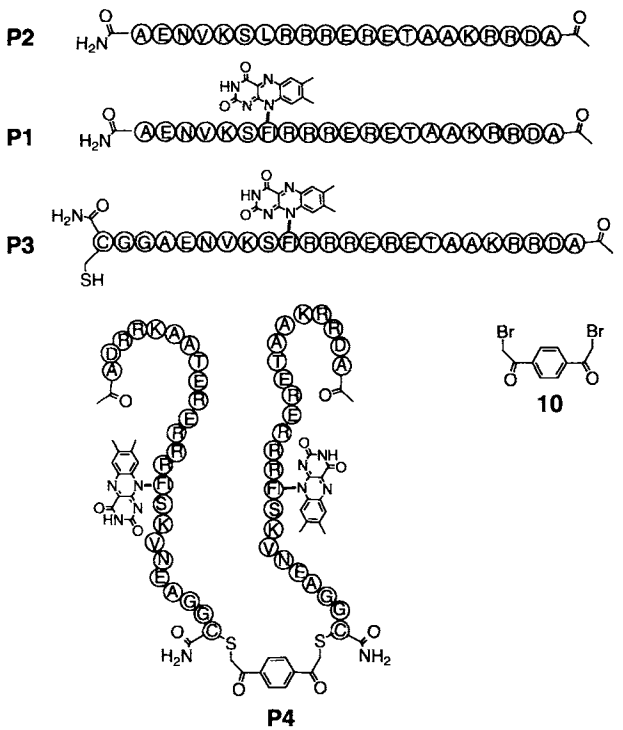
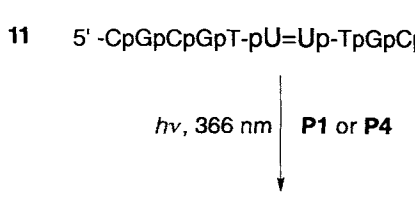


Figure 1. Sequences of the synthesized oligopeptide **P2** and the flavin-containing oligopeptides **P1** and **P3**. Presentation of the template **10** and the linked dimeric oligopeptide **P4**. Peptides were synthesized manually on Rink-Amide MBHA support (FMOC chemistry) with a HOBt/HBTU coupling protocol.

Maximizing the DNA-binding properties requires dimerization of the basic oligopeptides, for example, by linking the two peptides to a given template.[19] Such dimerized oligopeptides mimic the general feature of helix-loop-helix proteins to fold into an α-helical conformation and form stable, structurally well-defined complexes with their DNA targets.[19] To see if the novel flavin peptides are amenable to the required dimerization procedure, peptide **P3**, which possesses a Cys-Gly-Gly handle at the C-terminus, was synthesized.[19] The peptide was treated with the bis(bromo)acetyl benzene template **10**[20] under exclusion of oxygen (Figure 1) to yield a new peptide species, which was isolated by preparative reverse-phase HPLC.[18] The electro-spray mass spectrum confirmed the formation of template-linked **P4**.

To investigate if the flavin moiety within the transcription-factor fragments is a functional unit that can perform clean DNA repair, **P1** and **P4** were added to a solution containing the synthetic oligonucleotide **11**[11] (Scheme 2). The solutions were degassed with nitrogen and irradiated at 4 °C with daylight or with light of 366 nm. Ethylene diamine tetraacetic acid (EDTA)



Scheme 2. Repair of the synthetic oligonucleotide **11**. A lesion model compound (U=U) of the cyclobutane uracil dimer was incorporated into a specific site [11]. *The oligonucleotide product* **12** *of the reaction was independently synthesized and* co-injected to ensure that **12** was cleanly formed.

was added to the reaction buffer to photoreduce the isoalloxazin moiety. This resulted in the in situ conversion of the flavin into the fully reduced species, which is active in photolyases. We found that **P1** is able to *completely* convert the lesion-containing **11** into the repaired oligonucleotide **12**. The irradiation times required for the complete conversion varied from one to several hours, depending on the concentration of both peptides and the oligonucleotide substrate as well as on the ion strength of the reaction buffer. This indicates that a close association between the reaction partners, presumably based on interactions between the phosphodiester and arginine or lysine, is of crucial importance. However, the formed associates are still unstructured, since similar transcription-factor elements require double-stranded DNA for the formation of structurally well-defined complexes.[21]

Nevertheless, the repaired oligonucleotide **12** is cleanly formed upon irradiation without the appearance of cleaved DNA side products, which are generally obtained after irradiation of DNA in the presence of oxidized riboflavin.[12] The results of a long irradiation experiment are depicted in Figure 2 (10 μM **11** and 20 μM **P1**). Approximately 75% conversion of the starting material **11** into **12** was recorded after 6 h of irradiation. Further irradiation resulted in almost complete conversion into **12**. Independent syntheses of **12**[11] and co-injection confirmed that the new HPLC peak belongs to **12**.

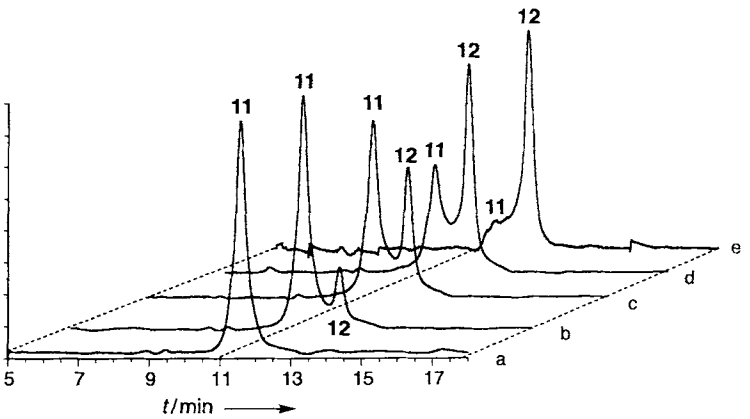


Figure 2. HPL chromatograms (a: 0 h; b: 1 h; c: 3 h, d: 6 h, e: 14 h) measured during the irradiation of a solution of oligonucleotide **11** (10 μM) in reaction buffer (12.5 mM Tris, 5 mM EDTA, 6 mM NaOH, pH 7.8) at 4 °C in the presence of **P1** (20 μM). Column: Lichrosphere C18, 100 Å, 0.4 × 250 mm. Mobile phase A: H_2O, 0.1 M NEt_3·HOAc. Mobile phase B: 80% CH_3CN, 20% H_2O, 0.1 M NEt_3·HOAc. Gradient: 0–30% B in 30 min. Injection volume: 20 μL.

Preliminary irradiation experiments with **P4** indicate that this dimeric oligopeptide performs the DNA-repair reaction faster than **P1**, even at lower substoichiometric concentrations (1–2 μM peptide, 10 μM DNA). Irradiation of DNA solutions containing either riboflavin or **P1** in the absence of EDTA caused decomposition of the oligonucleotide instead of repair. No repair was observed in the absence of the flavin peptide, in the presence of non-flavin-containing **P2**, or in the presence of reduced riboflavin under otherwise identical conditions (EDTA and damaged DNA in buffer solution). These control experiments prove that the prepared flavin-derived transcription-factor fragments **P1** and **P4** can perform primitive DNA repair. So far, reversion of pyrimidine dimers within a DNA strand has been reported with the electron-donating tripeptide Lys-Trp-Lys. Here, a maximum of 60% conversion was obtained, since the photo repair required a harmful wavelength of less than 300 nm.[22] More recently, diimine(9,10-phenanthrenequinone)-

rhodium complexes were reported to catalyze the repair of thymine dimers by electron abstraction from the DNA.[23] Therefore, the flavin oligopeptides represent the first compounds that can completely repair damaged oligonucleotides by simulating the light-driven electron-donating repair process of DNA photolyases.[24]

We have shown that the enantiomerically pure flavin-α-amino acid L-**1** is readily available in large quantities. The building block can be incorporated into oligopeptides by a slightly modified FMOC peptide synthesis protocol, and the peptides obtained are amenable to a dimerization procedure.[25, 26] Most importantly, the flavin peptides are able to cleanly repair a oligonucleotide containing a synthetic pyrimidine-dimer lesion. These results offer the possibility of constructing small, artificial DNA-binding and cofactor-containing proteins, which may help to elucidate the requirements for efficient energy- and electron-transfer reactions at the interface between proteins and DNA.

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Solid-Phase Synthesis of the CD52 Glycopeptide Carrying an N-Linked Core Pentasaccharide Structure**

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With the increased understanding of the biological functions of glycoproteins as recognition signals for various phenomena,[1] the synthesis of glycopeptides and glycoproteins has become the focus of numerous research efforts.[2] Among the various methods that have been developed for synthesizing glycopeptides or fragments of glycoproteins, solid-phase synthesis has proven to be most suitable for larger oligomers. Thus, this method has been applied to synthesize *O*-[3] and *N*-glycopeptides,[4] usually by using acetylated oligosaccharides as building blocks. However, all reported syntheses have thus far employed relatively simple oligosaccharide moieties, whereas naturally occurring glycoproteins may contain rather compli-

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