

The influence of dipole moments on the mechanism of electron transfer through helical peptides

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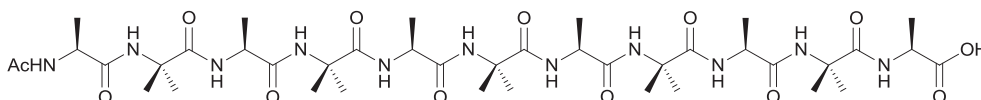
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1. Synthesis of the precursors of peptides 9 and 10

Preloaded chlorotriptyl resins as well as Fmoc-protected amino acids and solvents (peptide grade) used for the peptide synthesis were purchased from IRIS Biotech or Bachem. HCTU was ordered at Novabiochem. All reagents were used without purification. Chromatographic purification by HPLC was performed on a Waters Alliance 2690 using a reversed phase column (LiChrospher® 100, RP-18e, 5 μm) and HPLC-grade H_2O (0.1% TFA) and CH_3CN . For column chromatography silica60 purchased at Brunschwig was used. NMR-spectra were measured on a Bruker Avance III 300 MHz at 300 K. TMS or the solvent was used as internal standard.^[S1] The chemical shift is given in ppm and the coupling constants are given in Hz. Mass spectra were recorded on a Bruker esquire HCT with diluted solutions of the compounds in methanol.

1.1 Synthesis of the precursor of peptide 9

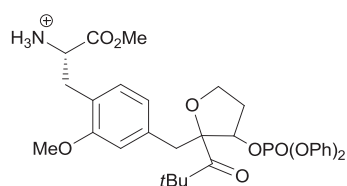
1.1.1 Synthesis of Ac-(Ala-Aib)₅-Ala-OH



Ac-(Ala-Aib)₅-Ala-OH was synthesized on a Multiple Peptide Synthesizer (Syro I) via the standard Fmoc-strategy using a preloaded 2-chlorotriptyl resin and HCTU as coupling reagent. After completion of the coupling/Fmoc-deprotection cycles the terminal amino group was acetylated using acetic anhydride and triethylamine in dichloromethane. Cleavage from the resin was achieved by using a solution of dichloromethane/TFA/trifluoroethanol 8:1:1 and the crude product was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 3:1) followed by precipitation from cold diethylether to yield 105 mg ($1.15 \cdot 10^{-4}$ mol) of Ac-(Ala-Aib)₅-Ala-OH as a colorless solid.

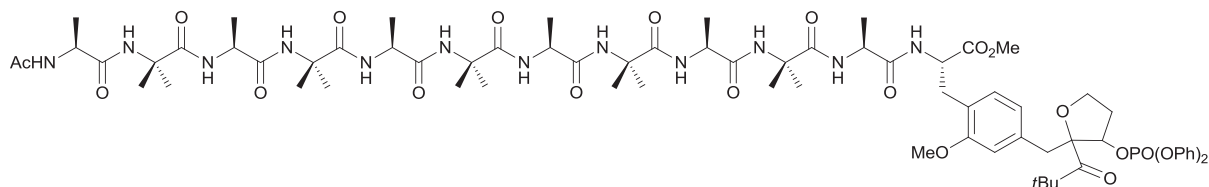
$R_f = 0.16$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 3:1); $^1\text{H-NMR}$ (300 MHz, DMSO-d_6): 8.53 (s, 1H, NH), 8.36 (d, 1H, $^3J_{\text{NH}/\alpha\text{-H}} = 6.0$ Hz, NH), 7.88-7.86 (m, 2H, 2x NH), 7.74-7.70 (m, 2H, 2x NH), 7.63 (s, 1H, NH), 7.32 (s, 1H, NH), 7.31 (d, 1H, $^3J_{\text{NH}/\alpha\text{-H}} = 9.0$ Hz, NH), 7.24 (d, 1H, $^3J_{\text{NH}/\alpha\text{-H}} = 9.0$ Hz, NH), 4.18-3.87 (m, 6H, 6x $\alpha\text{-H}$), 1.90 (s, 3H, Ac- CH_3), 1.63-1.07 (m, 48H, 10x Aib- CH_3 , 6x Ala- CH_3); ESI-MS: 935 $[\text{M}+\text{Na}]^+$, 479 $[\text{M}+2\text{Na}]^{2+}$.

1.1.2 Synthesis of ^+H_3N -Inj-OMe



30 mg ($4.05 \cdot 10^{-5}$ mol) of **1** (R = Boc)^[S2] were dissolved in 2 mL of HCl/dioxane (4 M) and stirred for 30 min at room temperature (rt). The solvent was removed *in vacuo* and the dried crude product was used in the next step without further purification.

1.1.3 Synthesis of Ac-(Ala-Aib)₅-Ala-Inj-OMe (precursor of **9**)

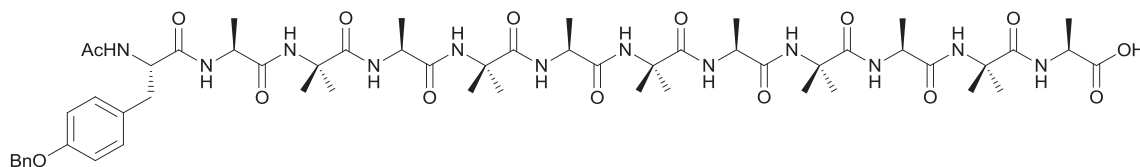


47 mg ($5.20 \cdot 10^{-5}$ mol) of Ac-(Ala-Aib)₅-Ala-OH were dissolved in 14 mL of DMF. 43 mg ($1.04 \cdot 10^{-4}$ mol, 2.0 eq) of HCTU and a solution of $4.05 \cdot 10^{-5}$ mol (0.8 eq) of ^+H_3N -Inj-OMe and 28 μ L ($1.65 \cdot 10^{-4}$ mol, 3.2 eq) of DIPEA in 1.6 mL of DMF were added. Since the reaction solution was not yet basic enough, another 28 μ L ($1.65 \cdot 10^{-4}$ mol, 3.2 eq) of DIPEA were added. It was stirred for 18 h at rt under a N₂-atmosphere and the solution was poured into a biphasic system of EtOAc and a saturated (sat.) aqueous NH₄Cl-solution. The organic layer was washed twice with a sat. NH₄Cl-solution and twice with a sat. NaHCO₃-solution, each followed by brine. The organic layer was dried over MgSO₄ and the solvent was removed *in vacuo*. The crude product was purified by HPLC [gradient: H₂O (0.1% TFA)/CH₃CN : 60% CH₃CN (0 min) \rightarrow 80% CH₃CN (16 min) \rightarrow 100% CH₃CN (18 min) \rightarrow 100% CH₃CN (20 min) \rightarrow 60% CH₃CN (25 min) \rightarrow 60% CH₃CN (30 min)] to yield 12 mg ($7.81 \cdot 10^{-6}$ mol, 19%) of Ac-(Ala-Aib)₅-Ala-Inj-OMe as a colorless solid.

HPLC: t_R = 12 min; ESI-MS: 1558 [M+Na]⁺, 791 [M+2Na]²⁺.

1.2 Synthesis of the precursor of peptide **10**

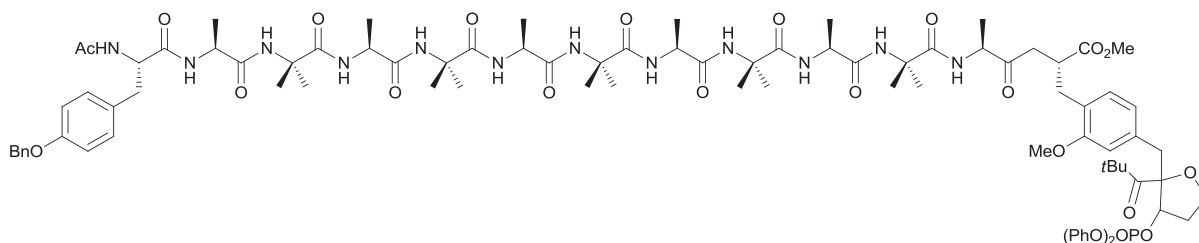
1.2.1 Synthesis of Ac-Tyr(OBn)-(Ala-Aib)₅-Ala-OH



Ac-Tyr(OBn)-(Ala-Aib)₅-Ala-OH was synthesized on a Multiple Peptide Synthesizer (Syro I) via the standard Fmoc-strategy using a preloaded 2-chlorotrityl-resin and HCTU as coupling reagent. After completion of the coupling/Fmoc-deprotection cycles, the terminal amino group was acetylated using acetic anhydride and triethylamine in dichloromethane. Cleavage from the resin was achieved by using a solution of dichloromethane/TFA/trifluoroethanol 8:1:1 and the crude product was purified by column chromatography (CH₂Cl₂/MeOH 3:1) to yield 149 mg ($1.28 \cdot 10^{-4}$ mol) of Ac-Tyr(OBn)-(Ala-Aib)₅-Ala-OH as yellowish foam.

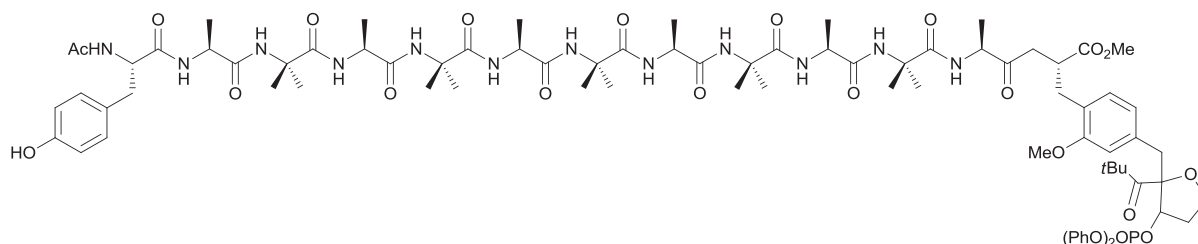
$R_f = 0.09$ (CH₂Cl₂/MeOH 3:1); ESI-MS: 1188 [M+Na]⁺.

1.2.2 Synthesis of Ac-Tyr(OBn)-(Ala-Aib)₅-Ala-Inj-OMe



96 mg ($8.24 \cdot 10^{-5}$ mol) of Ac-Tyr(OBn)-(Ala-Aib)₅-Ala-OH were dissolved in 22 mL of DMF. 69 mg ($1.67 \cdot 10^{-4}$ mol, 2.0 eq) of HCTU and a solution of $9.44 \cdot 10^{-5}$ mol (1.1 eq) of ⁺H₃N-Inj-OMe and 64.5 μL ($3.79 \cdot 10^{-4}$ mol, 4.6 eq) of DIPEA in 3.2 mL of DMF were added. Since the pH of the reaction solution was not yet basic another 64.5 μL ($3.79 \cdot 10^{-4}$ mol, 4.6 eq) of DIPEA were added and the reaction mixture was stirred for 18 h under a N₂-atmosphere. The solution was poured into a biphasic system of EtOAc/NH₄Cl (sat. aqueous solution) and the organic layer was washed twice with a sat. NH₄Cl-solution and twice with a sat. NaHCO₃-solution, each followed by brine. The organic layer was dried over Na₂SO₄ and the solvent was removed *in vacuo* to yield Ac-Tyr(OBn)-(Ala-Aib)₅-Ala-Inj-OMe as a yellow foam. The crude product was used in the next step without further purification.

1.2.3 Synthesis of Ac-Tyr-(Ala-Aib)₅-Ala-Inj-OMe (precursor of 10)



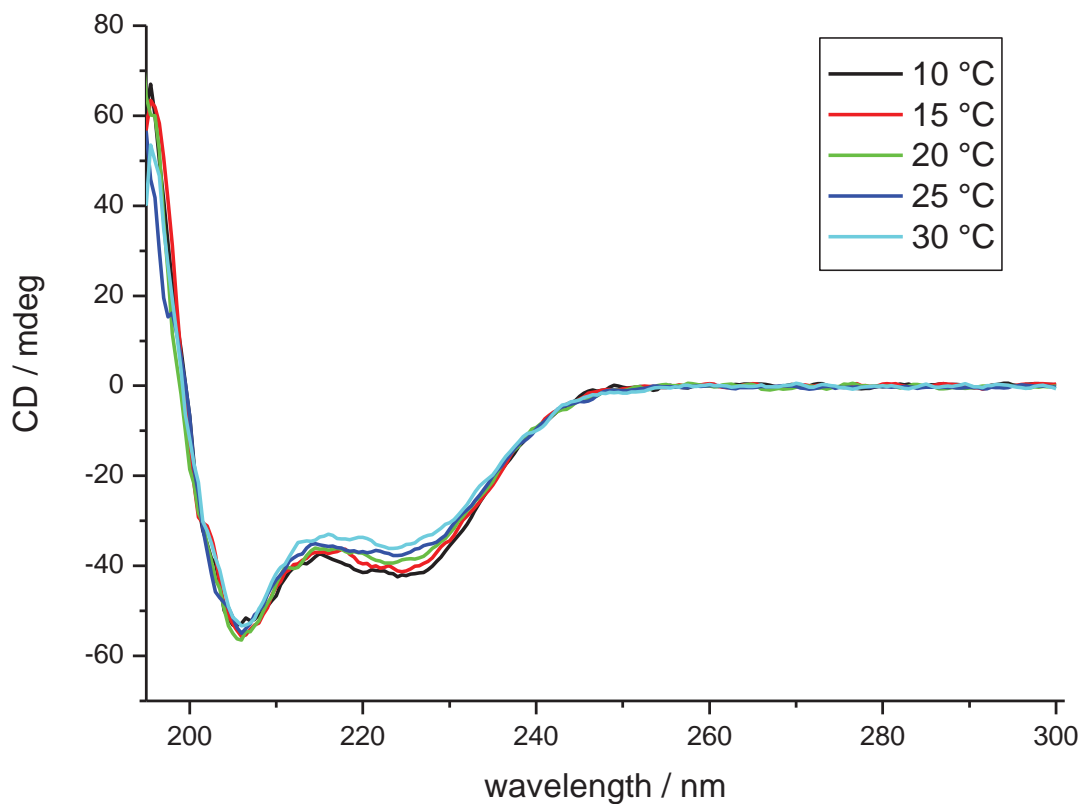
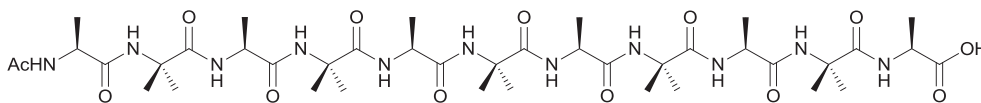
Ac-Tyr(OBn)-(Ala-Aib)₅-Ala-Inj-OMe was dissolved in 2.5 mL of MeOH. Under a N₂-atmosphere a catalytic amount of Pd/C was added. The N₂-atmosphere was exchanged against a H₂-atmosphere (1 bar) and the reaction mixture was stirred for 18 h. It was filtered over celite and the solvent of the filtrate was removed *in vacuo*. The crude product was purified by HPLC [gradient: H₂O (0.1% TFA)/CH₃CN : 60% CH₃CN (0 min) → 80% CH₃CN (16 min) → 100% CH₃CN (18 min) → 100% CH₃CN (20 min) → 60% CH₃CN (25 min) → 60% CH₃CN (30 min)] to yield 10 mg (5.89*10⁻⁶ mol, 7% over two steps) of Ac-Tyr-(Ala-Aib)₅-Ala-Inj-OMe as a colorless solid.

HPLC: t_R = 11 min; ESI-MS: 1699 [M+H]⁺, 850 [M+2H]²⁺.

2. Secondary structure of the peptides

The secondary structures were investigated by CD-spectroscopy (Jasco J-810 circular dichroism spectropolarimeter) using solutions of the peptide in CH₃CN/H₂O 3:1, which is the solvent that was also used for the laser measurements. For a right-handed α -helix one expects two minima at 208 nm and 222 nm with the same intensity.^[S3] A right-handed 3₁₀-helical peptide shows a very similar behavior with a negative band at 207 nm and a shoulder at 222 nm with a ratio of [Θ]₂₂₂/[Θ]₂₀₈ = 0.4.^[S4]

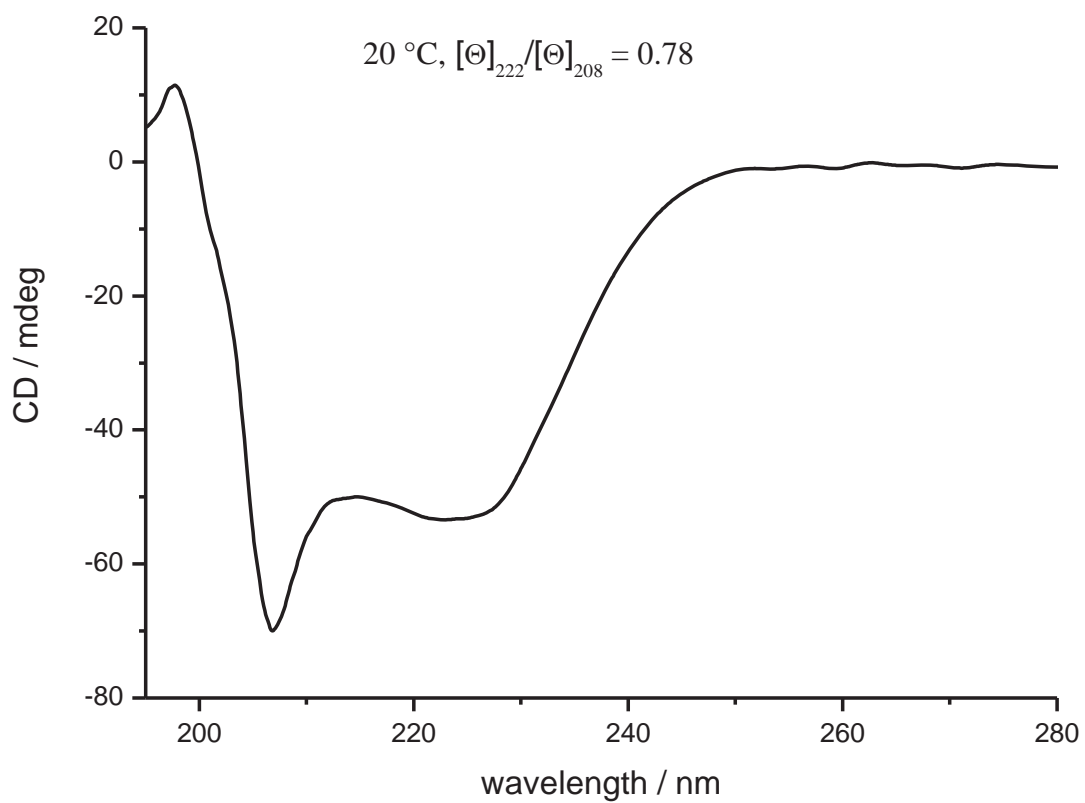
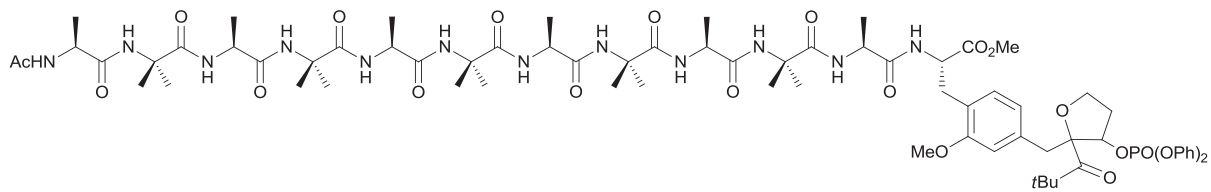
2.1 CD-spectra of Ac-(Ala-Aib)₅-Ala-OH



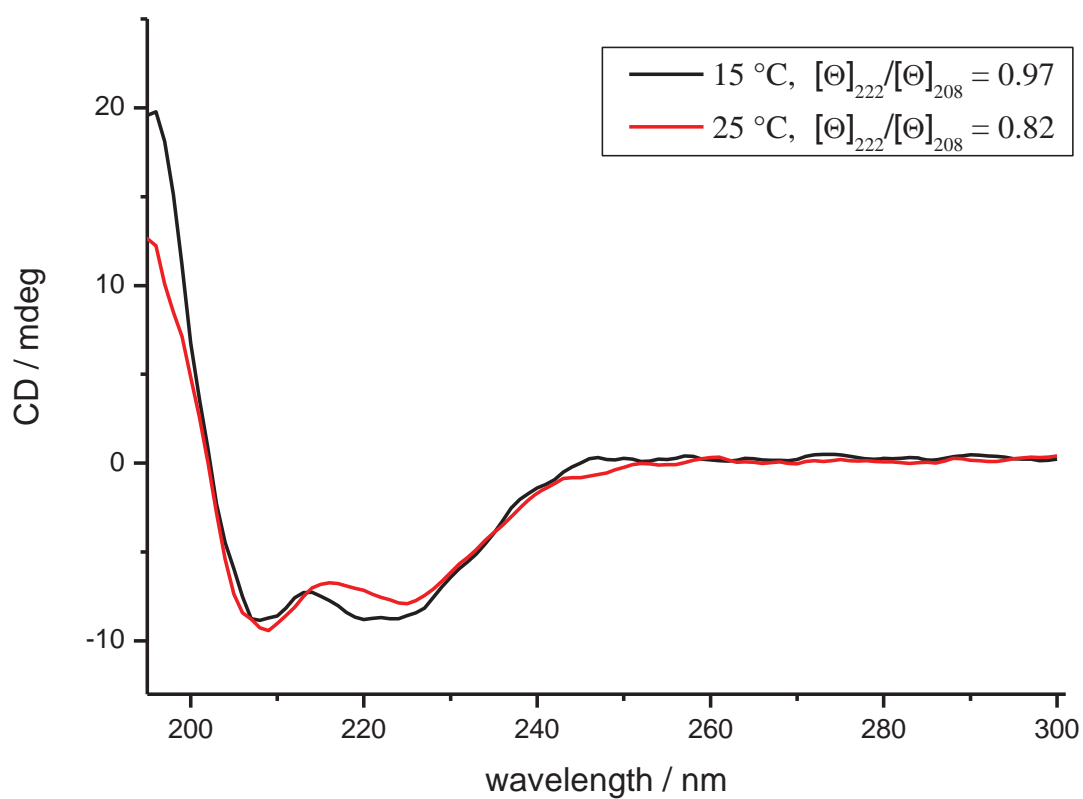
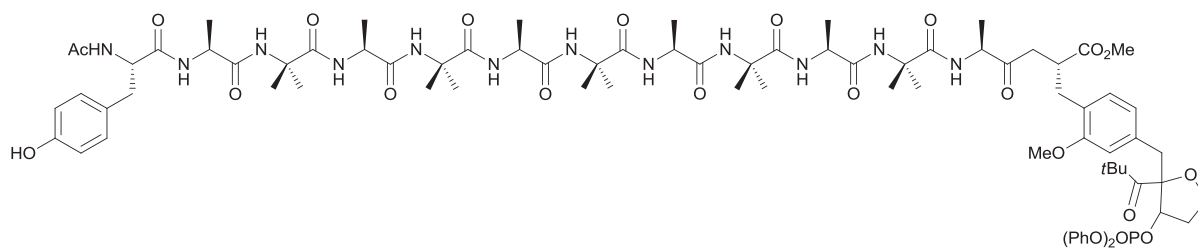
Temperature	$[\Theta]_{222}/[\Theta]_{208}$
10 °C	0.81
15 °C	0.77
20 °C	0.75
25 °C	0.73
30 °C	0.71

The $[\Theta]_{222}/[\Theta]_{208}$ ratios at different temperatures show that we obtained a mixture between α -helix and 3₁₀-helix in all cases, with an increasing amount of the 3₁₀-helix with rising temperature.

2.2 CD-spectrum of Ac-(Ala-Aib)₅-Ala-Inj-OMe (precursor of **9**)



2.3 CD-spectra of Ac-Tyr-(Ala-Aib)₅-Ala-Inj-OMe (precursor of **10**)

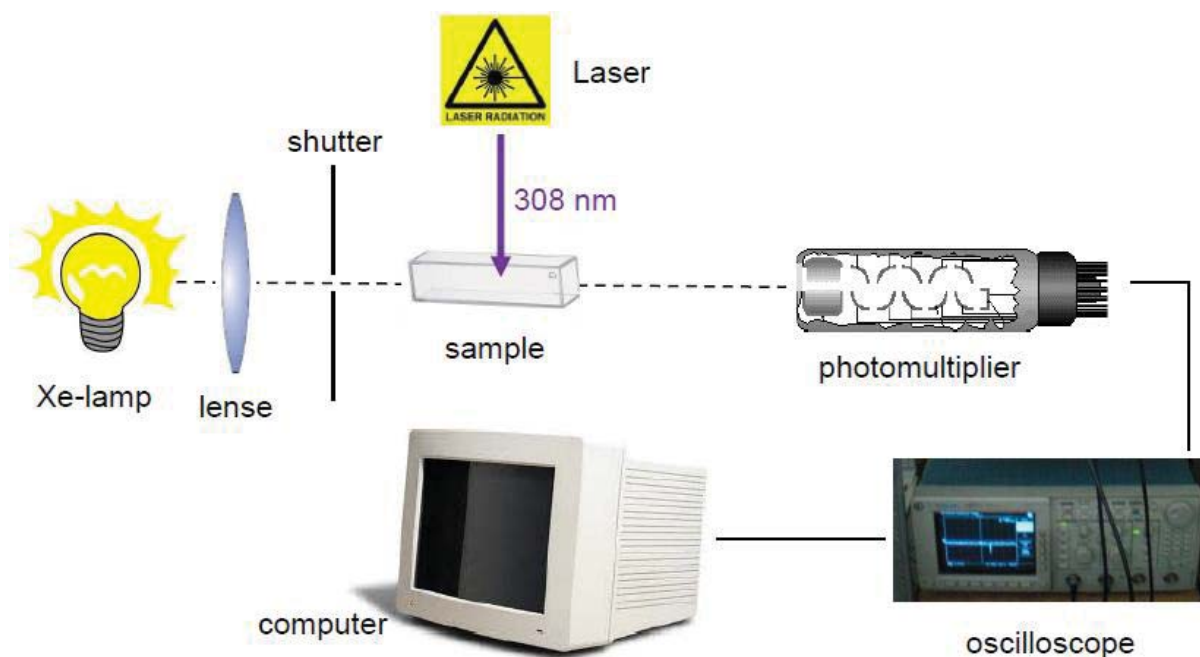


3. Measurement of the ET rates

Two different setups were used. In all cases the solutions were degassed and the concentrations of the peptides are given in the publication or in the cited literature.

3.1 Experiments with compounds **7**, **8**, **9** (without aromatic electron donor)

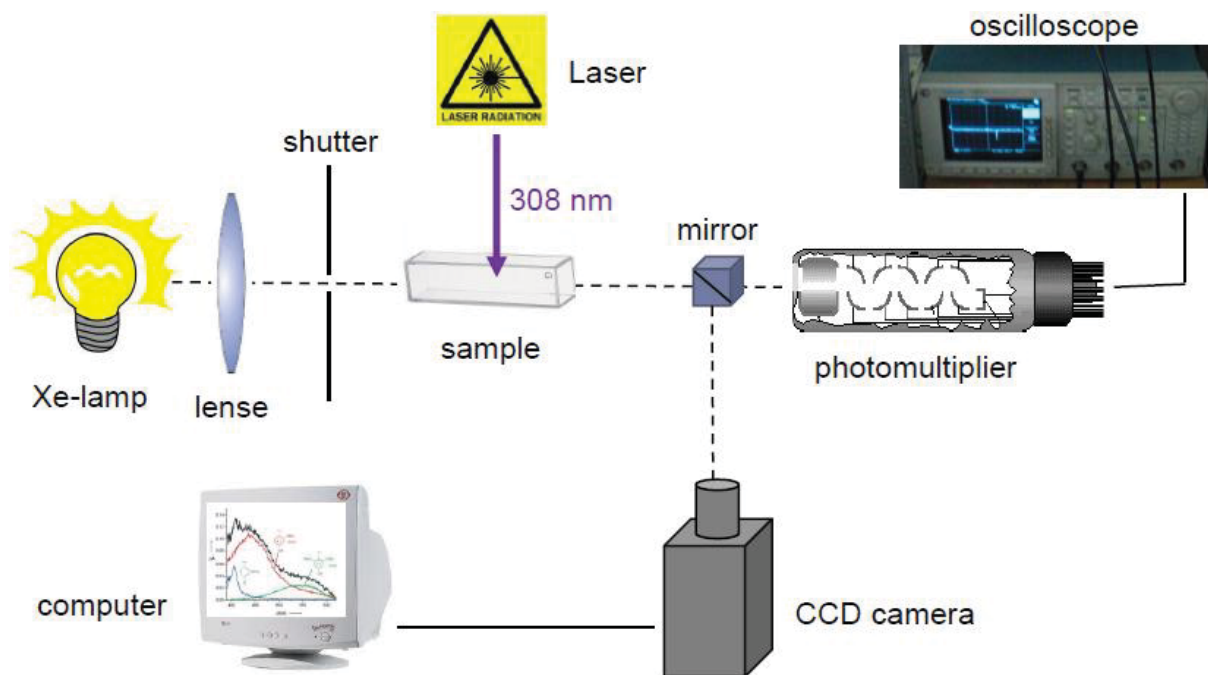
The injector radical cation (**4**) was generated by a Lambda Physics XeCl excimer laser at 308 nm. A Xe-lamp perpendicular to the laser in combination with a shutter and photomultiplier connected to an oscilloscope was employed to record the decreasing UV/vis-signal at 440 nm. The obtained graph was used to calculate the rates by applying a pseudo-first-order kinetic.



3.2 Experiments with compound **10** (with aromatic electron donor)

The absorption maxima of the aromatic radical cation **7** ($\lambda_{\text{max}} = 450 \text{ nm}$) and of the tyrosyl radical ($\lambda_{\text{max}} = 410 \text{ nm}$) lead to an overlap of their UV/vis-spectra. Therefore we measured the UV/vis-spectra of the reaction intermediates at different times. The injector radical cation (**4**) was generated by a Lambda Physics XeCl excimer laser at 308 nm. A Xe-lamp perpendicular to the laser flash in combination with a shutter and a CCD camera were used to record the transient absorption spectra at different times after the laser flash (40-500 ns). For the

determination of the delay a photomultiplier connected to an oscilloscope was used. By deconvolution the relative yields of the reactive intermediates at these times were determined. The treatment of the obtained data was described before.^[S5]



S1 H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512.

S2 R. Glatthar, M. Spichty, A. Gugger, R. Batra, W. Damm, M. Mohr, H. Zipse and B. Giese, *Tetrahedron*, 2000, **56**, 4177; M. Cordes, O. Jacques, A. Köttgen, C. Jasper, H. Boudebous and B. Giese, *Adv. Synth. Catal.*, 2008, **350**, 1053.

S3 G. Hungerford, M. Martinez-Insua, D. J. S. Birch and B. D. Moore, *Angew. Chem. Int. Ed.*, 1996, **35**, 326.

S4 C. Toniolo, A. Polese, F. Formaggio, M. Crisma and J. Kamphuis, *J. Am. Chem. Soc.*, 1996, **118**, 2744.

S5 J. Gao, P. Müller, M. Wang, S. Eckhardt, M. Lauz, K. M. Fromm, B. Giese, *Angew. Chem. Int. Ed.*, **2011**, *50*, 1926.