concerning bonding interactions that involve sulfur, Dr. M. T. Stubbs for his help in the crystal structure determinations, and Prof. G. A. Garcia for providing samples of tRNA.

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Synthesis and Characterisation of Acyl Carrier Protein Bound Polyketide Analogues

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Polyketide synthases (PKSs) are widespread in plants, bacteria and fungi.[1] They are responsible for the biosynthesis of an enormous range of organic compounds, many of which have important pharmaceutical and agrochemical properties.[2] Three types of synthases have been documented. Type I systems consist of very large multifunctional proteins which can be either processive (for example the modular systems responsible for macrolide synthesis)[3] or iterative (for example the lovastatin nonaketide synthase from *Aspergillus terreus*[4]). The iterative Type II PKS systems consist of complexes of monofunctional proteins exemplified by the actinorhodin (act) **1** PKS from *Streptomyces coelicolor*.[5] Type III systems are responsible for the synthesis of chalcones and stilbenes in plants and polyhydroxy phenols in bacteria.[6] All PKS possess the key β-ketoacyl synthase domain responsible for the C—C bond forming reaction. In Type I and Type II systems the growing acyl chain is covalently attached to the terminal thiol of a phosphopantetheine (PP) prosthetic group on an acyl carrier protein (ACP).

In the act PKS a minimal set of proteins has been identified which is capable of synthesising polyketides in vitro from malonyl CoA.[7] These proteins are KS_α, responsible for C—C bond formation, KS_β responsible inter alia for starter unit production[8] and the ACP (Figure 1).[9] We have extensively studied these components and their biochemical activities in vitro. In short, these proteins load malonyl units onto the terminal PP thiol of the ACP (Scheme 1).[10] KS_β then produces acetyl-ACP and KS_α performs seven decarboxylative condensations to produce a putative ACP-bound octaketide. The subsequent cyclisation and release of this octaketide, probably controlled by the minimal PKS, yields SEK4 (**2**) and SEK4b (**3**) as the products.

We studied the acyl ACP intermediates formed during PKS catalysis. Novel acyl ACPs could act as surrogate intermediates or starter units for PKS and may lead to the synthesis of novel compounds in vitro. Acyl ACPs could also be substrates for other

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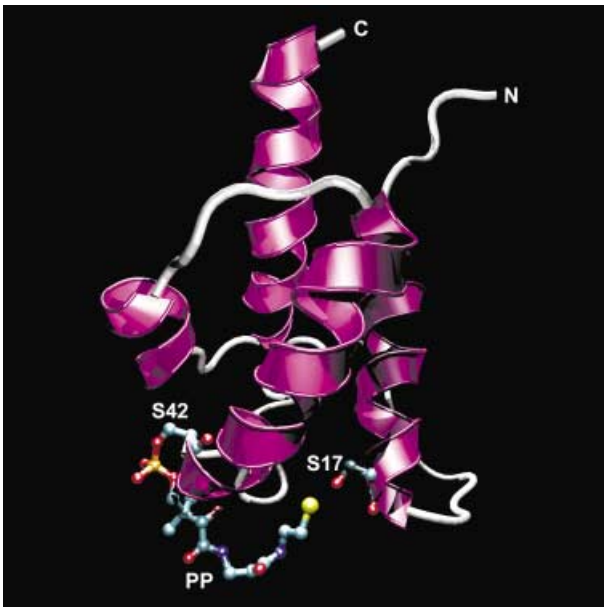
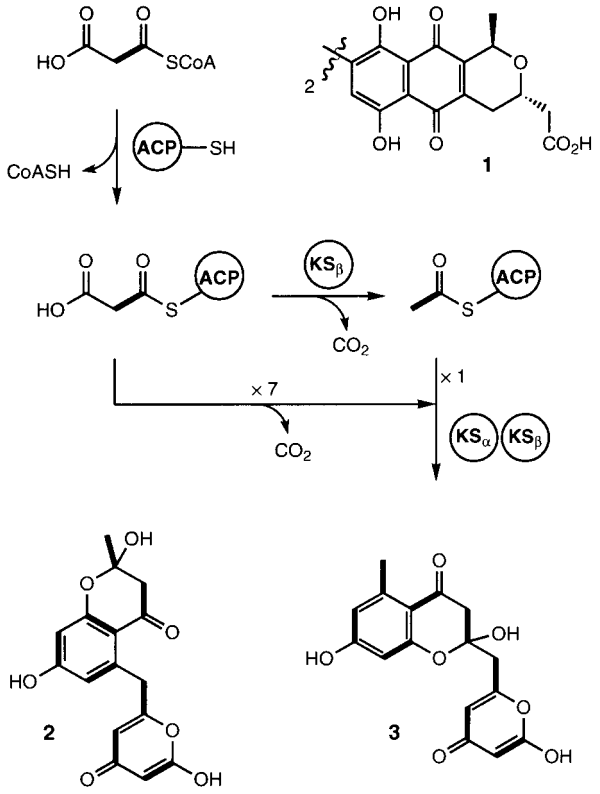


Figure 1. *Cartoon model of act ACP showing the location of the conserved Ser 42 with its attached PP prosthetic group and the site of the C17S mutation. The model was created by using act apo-ACP coordinates[9] and manually modelling the PP group between Ser 42 and Ser 17. The VMD[22] and Swiss PDB Viewer[23] programs were used to create the model and rendering was performed with the program POVRAY.*



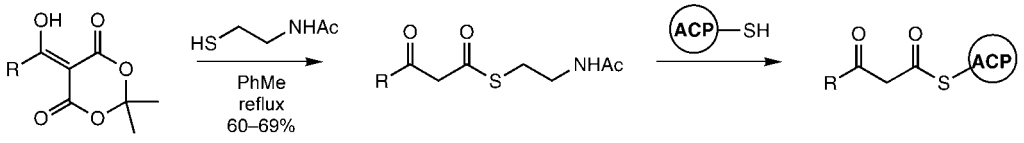
Scheme 1. *The biochemical roles of the constituent proteins of the actinorhodin minimal PKS.*

PKS-catalysed reactions such as keto-reduction or cyclisation.[5] We therefore set out to investigate the synthesis and properties of acyl ACP conjugates.

Several methods can be used for the acylation of ACPs. Acyl CoA derivatives can be used as the source of both PP and acyl groups as the enzyme holo-ACP synthase (ACPS) can transfer the acyl PP intact to the conserved serine of the apo-ACP.[11] Alternatively acyl imidazolides can transfer acyl groups to holo-ACP directly, but these compounds can be difficult to synthesise.[12] We have previously observed that β-oxoacyl groups such as malonyl and acetoacetyl can also transfer to act holo-ACP from either CoA or *N*-acetylcysteamine (NAC) thiolesters, but the generality of the reaction has not been explored.[13] We decided to investigate the scope of this reaction for the synthesis of potential PKS intermediates bound to ACP.

In order to explore this process in more detail we synthesised a range of acyl NACs to test their ability to load onto act ACP. Compounds **4a–d** and **4g** were prepared according to literature procedures from Meldrum's acid and the corresponding acyl chlorides.[14] The acyl Meldrum's acid derivatives then underwent thiolysis with NAC in refluxing toluene to give their respective novel β-oxoacyl *N*-acetylcysteamine thiolesters (SNACs) **5a–d** and **5g** in good yields after chromatography and recrystallisation (Scheme 2).[15] As expected, the thiolesters **5a–d** and **5g** existed as a mixture of keto (major) and enol (minor) tautomers in $CDCl_3$. We also synthesised malonyl SNAC (**5e**)[16] and acetoacetyl SNAC (**5f**)[17] by standard methods.

Actinorhodin holo-ACP was prepared in the usual way by coexpression of actI ORF3(C17S) with *Escherichia coli* ACPS in an *E. coli* host, followed by purification to homogeneity.[18][19] The purified SNAC thiolesters **5a–g** were incubated with purified act C17S holo-ACP monomer (5mM **5a–g**, 100 μM ACP) in 50 mM



a : R = $PhCH_2$
b : R = CH_3CH_2
c : R = $CH_3(CH_2)_4$
d : R = $CH_3(CH_2)_5$
e : R = OH
f : R = CH_3
g : R = Ph

Scheme 2. *Synthesis of β-ketoacyl SNACs. See Experimental Section for yields and conditions.*

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phosphate buffer at pH 7.5. The reactions were followed by high-performance liquid chromatography (HPLC) analysis (Figure 2 A) and the purified proteins were examined by electrospray mass spectrometry (ESMS; Figure 2 B). In the majority of cases the acyl SNACS loaded smoothly onto the holo-ACP (Table 1) in 4 – 5 h to produce the β-ketoacyl ACPs **6 a** – **6 f** in approximately 50 % yield. Malonyl SNAC (**5 e**) was more reactive and the yield approached 100 %. After 4 h the proportion of acyl ACP fell, but by desalting the reaction mixture and treating it with a second portion of **5 a** – **f** the yield could be increased to 65 %.

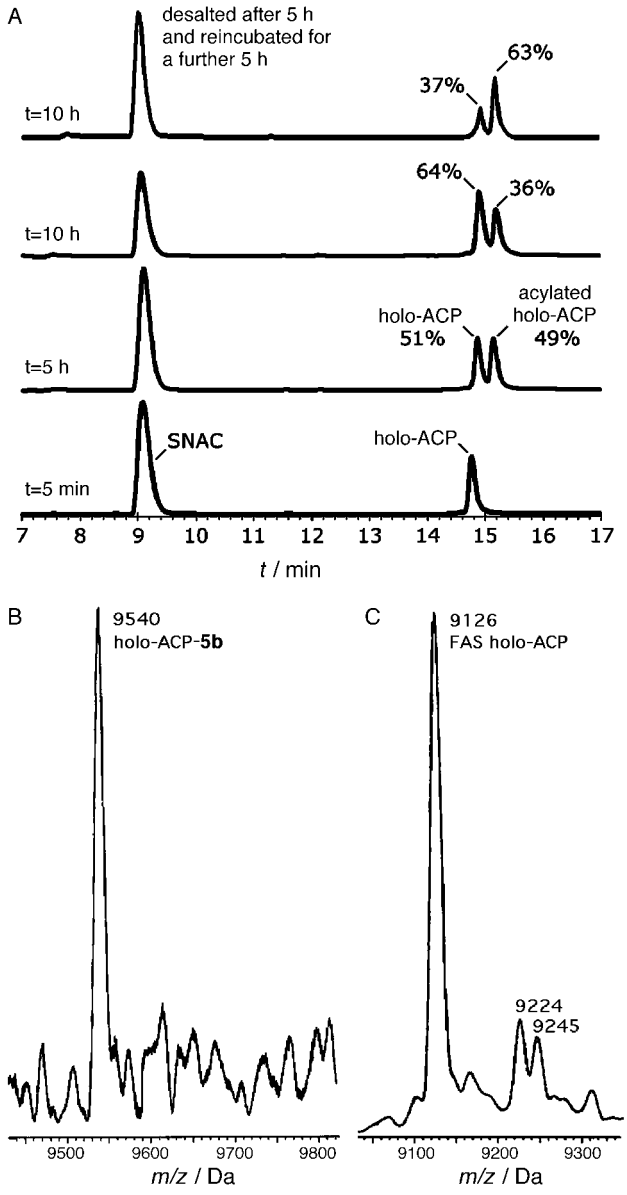

Figure 2. *A) HPLC trace of* act *ACP/**5 b** reaction mixture after 5 min, 5 h and 10 h. See Experimental Section for HPLC details. B) ESMS of **6 b**;* m/z *9540 = act holo-ACP-**5 b**. C) ESMS of* S. coelicolor *FAS ACP/**5 b** reaction mixture after 4 h (identical results were obtained in the presence of MCAT);* m/z *9126 = FAS holo-ACP; 9224 = FAS holo-ACP-**5 b**; 9245 = FAS holo-ACP-SNAC. See Table 1 for calculated* M_w.

Table 1. *ESMS and HPLC data for acyl-ACP conjugates.*

Species	*t* [min]	Calculated mass [Da]	Observed mass [Da]
act holo-ACP	14.8	9441	9441 ± 2.6
6 a	15.9	9601	9602 ± 1.1
6 b	15.2	9539	9540 ± 1.9
6 c	15.6	9581	9584 ± 3.3
6 d	16.6	9595	9595 ± 2.0
6 e	14.7	9527	9527 ± 1.2
6 f	–	9525	9527 ± 2.4
6 g	–	9587	–
act holo-ACP-SNAC	14.8	9558	9556 ± 3.6
FAS holo-ACP	–	9125	9126 ± 1.2
FAS holo-ACP-**5 b**	–	9223	9224 ± 1.2
FAS holo-ACP-SNAC	–	9242	9245 ± 1.2
malonyl FAS *holo*-ACP	–	9211	9212 ± 1.0

In the case of the phenylketone **5 g**, however, no acyl transfer was observed and the only newly formed protein product was a disulfide between ACP and NAC, observed after 24 h incubation. This is presumably formed after slow hydrolysis of **5 g** over time. Phenyl ketone **5 g** is more sterically restricted at the β-carbonyl group than the other compounds and its inability to acylate the act ACP may be indicative of specific substrate/protein interactions at this position. It is clear that the β-carbonyl group is important for transfer because we have previously shown that acetyl CoA and butyryl CoA cannot undergo this reaction.[13] The newly formed β-ketoacyl ACPs **6 a** – **f** were purified by a standard desalting procedure.

In parallel experiments we examined whether the *S. coelicolor* malonyl CoA: holo-ACP acyl transferase (MCAT)[20] from the fatty acid synthase (FAS) cluster could transfer β-ketoacyl groups from SNACs to ACPs. The *S. coelicolor* FAS ACP was expressed and purified analogously to the act ACP. The assays contained purified recombinant MCAT at 1nM, FAS ACP at 100 μM and **5 b** at 1mM.[21] The FAS ACP was examined by ESMS after 4 h. Interestingly some slow transfer was observed, showing 5 – 10 % formation of acyl ACP and an equivalent amount of NAC disulfide (Figure 2 C). However in a control reaction containing no MCAT, identical results were observed. In a further control reaction incubation of FAS ACP (100 μM) with MCAT (1 nM) and malonyl CoA (1 mM) led to full conversion into malonyl-ACP indicating that the MCAT was fully active (Table 1). Acetoacetyl SNAC (**5 f**) was not a substrate for MCAT transfer to FAS holo-ACP, but malonyl SNAC (**5 e**) could serve as a substrate for this reaction, albeit very much less efficiently than malonyl CoA (data not shown).

Thus the ability of act ACP to transfer β-carbonyl acyl groups from synthetic SNACS onto its PP thiol appears to be an extremely useful synthetic tool for the preparation of PKS intermediates and their analogues. Analogous SNACs not possessing β-carbonyl groups and compounds sterically more encumbered at the β-carbonyl are not substrates for this reaction, and the reaction is very slow with FAS ACP. The β-keto acyl SNACs do not appear to be substrates for MCAT-catalysed reactions. β-Keto acyl ACPs **6** are currently undergoing functional studies in PKS assays, as well as structural studies.

Experimental Section

HPLC was performed with Beckman System Gold pumps and a Phenomenex "Jupiter" column (250 × 4.6 mm C_5/300 Å) at a rate of 1 mL min^{-1}. Buffer A = 0.1% trifluoroacetic acid (TFA) in deionised water, buffer B = 0.05% TFA in CH_3CN (HPLC grade). Eluents: 0–2 min 10% B in A; 2–5 min 10–35% B; 5–15 min 35-52% B; 15–16 min 52–90% B; 16–17 min 90% B; 17–18 min 90–10% B; 18–22 min 10% B. Absorbance at 218 nM was monitored and product peaks were collected manually. The samples were freeze-dried, resuspended in water and analysed by ESMS.[16]

Representative procedure: 3-oxo-4-phenylbutanoic acid SNAC (5a): A solution of NAC (0.23 g, 1.95 mmol) in toluene (5 mL) was added to a solution of phenylacetyl Meldrum's acid (**4a**; 0.50 g, 1.95 mmol) in toluene (5 mL), and the reaction mixture was stirred under nitrogen at 80 °C for 5 h. Once judged to be complete by thin-layer chromatography (TLC), the solvent was removed in vacuo and the crude product was purified by flash column chromatography (EtOAc) then recrystallised from EtOAc/petrol to afford the desired compound as a colourless crystalline solid (0.34 g, 1.21 mmol, 62%). mp 80.5–81.5 °C; IR (solid): $\tilde{\nu}_{max}$ = 1716 (m, C=O), 1687, 1635 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 1.96 (s, 3H, $COCH_3$), 3.06 (t, ^3J(H,H) = 6.3 Hz, 2H, SCH_2), 3.44 (m, 2H, CH_2NH), 3.70 (s, 2H, $COCH_2CO$), 3.81 (s, 2H, ArCH_2CO), 5.99 (br s, 1H, CH_2NH), 6.81–7.84 (m, 5H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 22.9 (CH_3CO), 28.9 (CH_2), 38.9 (CH_2), 50.1 (CH_2), 55.9 ($COCH_2CO$), 126.6 (Ar C), 127.5 (Ar CH), 128.0 (Ar CH), 128.7 (ArCH), 170.6 (amide C=O), 192.0 (thiolester C=O), 199.8 (ketone C=O); MS (ES$^+$) *m/z* (%): 280 (100) $[M+H]^+$, 302 (37) $[M+Na]^+$; elemental analysis calcd (%) for $C_{14}H_{17}NO_3S$: C 60.19, H 6.13, N 5.01; found: C 60.30, H 6.33, N 5.04.

3-oxopentanoic acid SNAC (5b): By using the procedure described above **5b** (36%) was obtained as a colourless solid. mp 54.5–55.5 °C; IR (nujol): $\tilde{\nu}_{max}$ = 1721 (m, C=O), 1687, 1682, 1652 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 1.08 (m, 3H, CH_3CH_2), 1.98 (s, 3H, $COCH_3$), 2.57 (m, 2H, CH_3CH_2CO), 3.11 (m, 2H, SCH_2), 3.44 (m, 2H, CH_2NH), 3.71 (s, 2H, $COCH_2CO$), 6.19 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 17.3 (CH_3CH_2), 22.9 (CH_3CO), 29.0 (CH_2), 36.6 (CH_2), 40.0 (CH_2), 56.8 ($COCH_2CO$), 170.3 (amide C=O), 192.2 (thiolester C=O), 202.6 (ketone C=O); MS (ES$^+$) *m/z* (%): 218 (100) $[M+H]^+$, 240 (37) $[M+Na]^+$; elemental analysis calcd (%) for $C_9H_{15}NO_3S$: C 49.75, H 6.96, N 6.45; found: C 49.59, H 6.86, N 6.37.

3-oxo-octanoic acid SNAC (5c): By using the procedure described above **5c** (69%) was obtained as a colourless solid. mp 87-88 °C; IR (nujol): $\tilde{\nu}_{max}$ = 1715 (m, C=O), 1687, 1635 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 0.89 (m, 3H, CH_3CH_2), 1.30 (m, 4H, 2 × CH_2), 1.59 (m, 2H, CH_2), 1.98 (s, 3H, $COCH_3$), 2.53 (2H, t, ^3J(H,H) = 7.2 Hz, CH_2CO), 3.09 (m, 2H, SCH_2), 3.45 (m, 2H, CH_2N), 3.70 (s, 2H, $COCH_2CO$), 6.19 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 13.9 (CH_3), 22.3 (CH_3), 23.3 (CH_2), 28.5 (CH_2), 28.7 (CH_2), 31.3 (CH_2), 39.1 (CH_2), 43.4 (CH_2), 56.8 ($COCH_2CO$), 170.4 (amide C=O), 192.3 (thiolester C=O), 202.2 (ketone C=O); MS (ES$^+$) *m/z* (%): 260 (100) $[M+H]^+$, 282 (37) $[M+Na]^+$; elemental analysis calcd (%) for $C_{12}H_{21}NO_3S$: C 55.57, H 8.16, N 5.40; found: C 55.67, H 8.29, N 5.33.

3-oxononanoic acid SNAC (5d): By using the procedure described above **5d** (43%) was obtained as a colourless solid. mp 88–90 °C; IR (nujol): $\tilde{\nu}_{max}$ = 1716 (m, C=O), 1687, 1636 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 0.82 (m, 3H, CH_3CH_2), 1.22 (m, 6H, 3 × CH_2), 1.50 (m, 2H, CH_2), 1.91 (s, 3H, $COCH_3$), 2.45 (m, 2H, CH_2CO), 3.02 (m, 2H, SCH_2), 3.38 (m, 2H, CH_2N), 3.70 (s, 2H, $COCH_2CO$), 6.19 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 13.9 (CH_3), 22.3 (CH_3), 23.3 (CH_2), 28.5 (CH_2), 28.7 (CH_2), 31.3 (CH_2), 31.4 (CH_2), 39.1 (CH_2), 43.4 (CH_2), 56.8 ($COCH_2CO$), 170.4 (amide C=O), 192.3 (thiolester C=O), 202.2 (ketone C=O); MS (ES$^+$) *m/z* (%): 274 (100) $[M+H]^+$, 296 (37) $[M+Na]^+$; elemental analysis calcd (%) for $C_{13}H_{23}NO_3S$: C 57.11, H 8.48, N 5.12; found: C 57.23, H 8.80, N 5.38.

3-oxo-3-phenylpropionic acid SNAC (5g): By using the procedure described above **5g** (71%) was obtained as a colourless solid. mp 87–88 °C; IR (nujol): $\tilde{\nu}_{max}$ = 3309, 3061, 2919, 1646, 1606, 1574, 1544, 1494; ^{1}H NMR (270 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 1.99 (s, 3H, CH_3CO), 3.15 (m, 2H, CH_2), 3.50 (m, 2H, CH_2), 4.26 (2H, s, $COCH_2CO$), 6.15 (br s, 1H, NH), 7.45 (m, 3H, Ar), 7.8 (m, 1H, Ar), 7.95 (m, 1H, Ar); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS, keto isomer): δ = 23.2 (CH_3), 28.1 (CH_2), 39.2 (CH_2), 53.6 ($COCH_2CO$), 126.6 (2 × ArCH), 128.9 (2 × ArCH), 132.0 (ArCH), 132.6 (ArC), 169.4 (CONH), 192.0 (CO), 192.8 (COS); MS (ES$^+$) *m/z* (%): 266 (100) $[M+H]^+$, 288 (5) $[M+Na]^+$, 531 (12) $[M_2+H]^+$; elemental analysis calcd (%) for $C_{13}H_{15}NO_3S$: C 58.85, H 5.70, N 5.28; found: C 59.23, H 5.92, N 5.29.

3-oxo-4-phenylbutanyl-ACP (6a): A solution containing **5a** (5 mM) and act C17S holo-ACP (100 μM) in phosphate buffer (50 mM, pH 7.5, 1 mL final volume) was incubated at 30 °C for 6 h. The progress of the reaction was monitored hourly by reversed-phase HPLC (50 μL injected onto a C_5 column and eluted according to the standard protocol). A single peak was collected and analysed by ESMS. See Table 1 for HPLC and ESMS data. When no further reaction was observed the reaction mixture was desalted by standard procedures (Pharmacia Hiprep 26/10 column eluted at 5mL min^{-1}).

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Cellular Uptake Studies with β-Peptides

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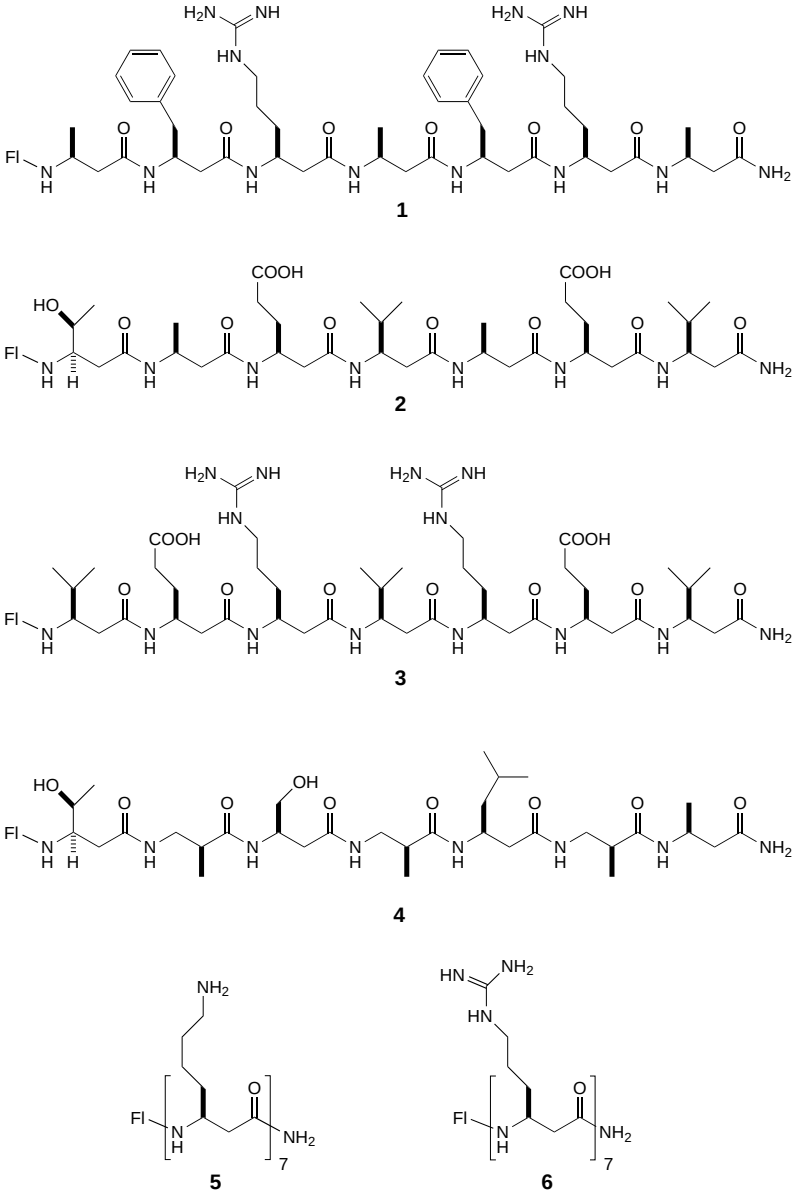
β-Peptides have been shown to fold into stable secondary structures similar to those observed in natural peptides and proteins.[1] The finding that β-peptides are completely stable to proteolytic degradation renders them candidates for use as peptidomimetics.[2] In fact, β-peptides have been demonstrated to mimic α-peptidic hormones,[3] to inhibit cholesterol uptake,[4] and to possess antimicrobial[5] and antiproliferative properties.[6]

The bioavailability of pharmacologically active compounds, for instance, peptides and proteins, depends significantly on their physical properties, as their uptake correlates with solubility in the polar extracellular medium and passive diffusion through the nonpolar lipid bilayer. Recently, methods have been developed for the delivery of proteins, biological active compounds, and DNA into living cells with the help of membrane-permeable carrier peptides (oligomers containing basic side chains).[7]

In order to determine the structural requirements for cellular uptake of β-peptides, a series of fluorescein-labeled β-peptides **1** – **6** has been prepared (Scheme 1). The substitution pattern of the β-peptides was selected for the following reasons: the β-



Scheme 1. *Molecular formulas of the investigated fluorescein-labeled β-peptides* ***1*** *–* ***6****. Fl = fluorescein.*

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