Supporting Information

The dithiane protected, tert-butyl ester benzoin was synthesized according to the methods of Rock and Chan[10] with minor variation. It was found that the overall yield was improved with the dithiane being hydrolyzed before the acylation step, hence the dithiane was removed with the addition of flouroboric acid and mercury(II)oxide. Activated esterification of tert-butyl ester protected CMB with bromoacetic acid was mediated by the addition of DCC. This was followed by the cleavage of the tert-butyl group with 50% TFA yielding the desired bromoacetylated linker. This derivatized benzoin serves as the sulfhydryl-selective cross-linking function for the preparation of the cyclic peptide.

Note: At this time selective loop formation of the peptide must be made synthetically due to the wide variety of nucleophilic side chains present. However, with recent advances in protein synthesis and semi-synthesis this technique can be applied to study virtually any protein. The synthesis of the peptide was performed using Fmoc-based chemistry, taking advantage of the flexibility it incurs on cysteine protection.

VHP-34 M12C prepared with the *N*-terminal bromoacetylated CMB group attached = (BrAc-CMB)-LSDEDFKAVFGCTRSAFANLPLWKQQLKKEKGL-NH_2. To minimize the differences between the VHP-35 and the relinearized peptide product, the N-terminal Phe in the VHP-35 was not included as part of the cVHP-34 M12C-CMB. This truncation was justified on the grounds that the photolysed benzofuran with its aromatic rings would roughly substitute for the terminal Phe in the native protein.

Synthesis of protected CMB

3'-(carboxymethoxy)benzoin-otBu

A solution of (±)-1-Hydroxy-1-[3'-((carboxymethoxy)tert-butyl)benzoin (5.0 g, 14.2 mmol) in 5 ml of THF is added to a solution of mercury(II)oxide (5.04 g, 23.3 mmol) and fluoroboric acid (12.26 ml, 35% aqueous soln.) in 15 ml of THF. After 5 minutes of stirring at room temperature the solution is filtered and concentrated to 5 ml. The crude product was brought up in ether and washed with 10% NaOH, 10% KI, and H_2O. Water was removed using a brine wash and treatment with $MgSO_4$. A off white solid (3.48 g, 72%) was obtained after solvent removal. (^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.896 (d, J = 6.9 Hz, 2 H), δ 7.525 (t, J = 7.5 Hz, 1 H), δ 7.394 (t, J = 7.20 Hz, 2 H), δ 7.239 (s, 2 H), δ 6.976 (d, J = 7.8 Hz, 1 H), δ 6.873 (t, 1 H), δ 6.811 (d, J = 8.1, 1 H), δ 5.903 (s, 1 H), δ 4.462 (s, 2 H), δ 1.456 (s, 9 H).

Synthesis of BrAc-CMB

[(±)-O-bromoacetyl-3'-(carboxymethoxy)benzoin]

A solution of 3'-(carboxymethoxy)benzoin-OtBu (685 mg, 2 mmol) and bromoacetic acid (694 mg, 5 mmol) was prepared in 10 mL DCM. This solution was treated with DCC (866 mg, 4.2 mmol). After 12 h, the resulting suspension was filtered, and the filtrate was poured into diethyl ether. The organic phase was washed with 5% sodium bicarbonate and water, dried with $MgSO_4$, and evaporated. The residue was

treated with 1:1 TFA / DCM for 45 min, then evaporated. The crude BrAc-CMB was purified by flash chromatography (silica gel, 1:1 hexanes / ethyl acetate) to yield 629 mg (70%) as an oil. R_f = 0.25 (1/1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.906 (d, *J* = 7.2 Hz, 2 H), δ 7.508 (t, *J* = 7.5 Hz, 1 H), δ 7.383 (t, *J* = 7.20 Hz, 1 H), δ 7.286 (t, 1 H), δ 7.103 (d, *J* = 5.70 Hz, 1 H), δ 7.056 (t, 1 H), δ 6.887 (d, *J* = 8.4 Hz, 1 H), δ 6.865 (s, 1 H), δ 4.672 (s, 2 H), δ 3.99 (d, *J* = 1.8 Hz,2 H); HRMS (FAB) *m/z* (MNa^+) cacld. 428.9950, obsd. 428.9957.

Peptide synthesis and cyclization

All peptides were synthesized using Fmoc chemistry on a Perceptive Biosystems model 9050 peptide synthesizer. Standard side chain protecting groups were used except for the mild acid labile Cys(Mmt). All couplings were performed using a 4-fold excess of a 0.3 M Fmoc-amino acid solution, preactivated with 1:1:1.5 HBTU / HOBt / DIEA. Fmoc groups were cleaved with 20% (v/v) piperidine in DMF. VHP-34 was prepared on a 0.2 mmol scale, using Novasyn TGR© Rink amide resin (Novabiochem). Once the synthesis was complete, the resin was rinsed with DCM and dried. A resin sample was cleaved using 95% TFA / 2.5% TIS / 2.5% water / 2.5% EDT, and the crude peptide isolated and characterized by MALDI-TOF MS ($[M+H]^+$ exp. 3883.51, found 3883.42). The peptide on resin (0.025 mmol) was capped with BrAc-CMB-OH (200 mg, 0.5 mmol) using 75 mL DIPCDI in 3 mL DMF until a ninhydrin test on a small aliquot indicated completion of the amide bond formation, 12 hrs. The resin was then rinsed with 3 x 5 mL DMF followed by 3 x 5 mL DCM. The cysteine Mmt protecting group was then selectively removed with 5 x 3 mL treatments (10 min ea.) of the resin with 2% TFA / 2%

TIS (v/v) in DCM. Deprotection was determined to be complete when the solution remained colorless after the addition of the deprotection mixture. The peptide was rinsed extensively with DCM, then cyclized for 24 h, by treating the resin with 5% (v/v) DIEA in NMP. After cyclization, the resin sample was rinsed with DCM, dried, then cleaved using 95% TFA / 2.5% TIS / 2.5% water / 2.5% EDT. The peptide products were collected by 4 cycles of precipitation into MTBE followed by centrifugation. MALDI-TOF MS, $[M+H]^+$ exp. 4191.8 found 4191.94 (monoisotopic),. The Crude peptide was dissolved in 50 mM Na phosphate buffer, pH 7.0, and any residual ether was removed under a stream of nitrogen prior to purification by RP-HPLC. All RP-HPLC was performed on a 10 mm x 250 mm C4 column (Phenomenex), using a gradient of 0.1% (w/v) TFA in water (solvent A), and 0.1% TFA in acetonitrile (solvent B).

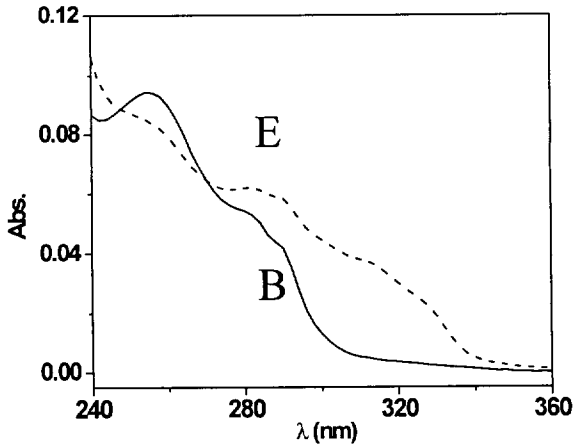

Figure 1. Steady-state photolysis of 28 μM cVHP M12C-CMB (**4**) in 10 mM $NaPO_4$ buffer, pH 7.4, irradiated from 300 to 400 nm using a filtered high-pressure mercury vapor arc lamp (same sample as in Figure 2 of the communication). cyclized form, irradiation time (s): (B) 0, (E) 90.

Steady state photolysis:

Samples where placed in a quartz cuvette irradiated with an Oriel 66011 Hg vapor arc lamp operating at 450 W and filtered through a 355 nm UG11 band pass filter. A sample of cVHP-34 M12C-CMB was dissolved in 10 mM sodium phosphate buffer, pH 7.0. The optical absorbance was monitored with an HP 8452 diode array spectrophotometer. The photolysis was judged to be complete when no further spectral changes occurred. HPLC analysis was performed using a phenomenex analytical C18 reverse phase column. MALDI-TOF MS, $[M+H]^+$ exp. 4191.8, found 4191.25 (average).

Circular Dichroism

Samples for CD Spectroscopy were brought up in 10 mM sodium phosphate buffer, pH 7.4. Spectra were recorded on an Aviv 62A DS spectrometer in a 1mm cell at 25 °C. The results are expressed as mean residue molar ellipticities [θ].

Photoacoustic Calorimetry and Photothermal Beam Deflection

Curve fitting and experimental setup were as previously described.[17]

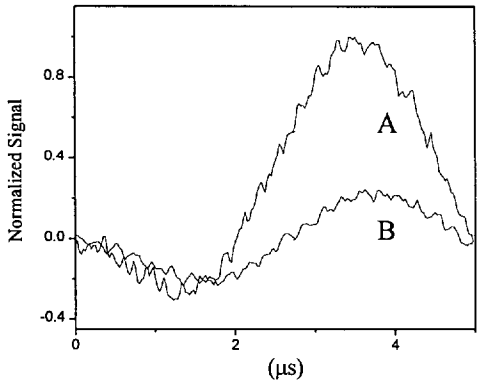

Figure 2. Graphical Representation of PAC signal. A.) Reference compound B.) Photolysis of cVHP-34 M12C-CMB.

Abbreviations: DIPCDI, diisopropylcarbodiimide; DIEA, diisoproplethylamine; FAB, fast atom bombardment; Fmoc, fluorenylmethoxycarbonyl; HBTU 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, N-Hydroxybenzotriazole monohydrate; TBTU, 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; Mmt, methoxytrityl; TGR©, Tentagel rink resin; DCC, N,N'-Dicyclohexylcarbodiimide; DMAP, 4-Dimethylaminopyridine.