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Supplementary Material

Chemical Synthesis of an Enzyme Containing an Artificial Catalytic Apparatus

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Reagents. Boc-amino acids, 4-Me-benzylhydrylamine-resin (MBHA) and $-OCH_2$-Pam-resins (free $^{\alpha}$carboxyl peptides) and coupling reagents (HBTU and HATU) were purchased from Peptides International, Louisville, Kentucky; diethyl ether from Fisher; HF from Matheson Tri-Gas; *p*-cresol, triisopropylsilane, 2-bromoacetamide and *S*-trityl mercaptopropionic acid from Sigma-Aldrich; 4-mercaptophenylacetic acid was obtained from Sigma-Aldrich and purified by HPLC before use; tris(2-carboxyethyl)phosphine hydrochloride (TCEP) from Fluka; trifluoroacetic acid (TFA) from Halocarbon Products, New Jersey, *N,N*-diisopropylethylamine (DIEA) from Applied Biosystems.

Peptide Synthesis. Peptides were prepared manually by "in situ neutralization" Boc-chemistry stepwise solid phase peptide synthesis (SPPS) (Schnolzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Pept. Res. Therap.* **2007**, *13*, 31). Side-chain protection for amino acids was as follows: Arg(Tos), Asn(Xan), Asp(OcHex), Cys(4-CH_3Bzl), Glu(OcHex), Lys(2Cl-Z), Thr(Bzl), Tyr(Br-Z), Trp(CHO), His(Bom). Peptide segment [(7-OH)dihydroisoquinolinone,des(25-27)](1-40)-$^{\alpha}COSCH_2CH_2Arg_4$ was assembled on 4-Me-benzylhydrylamine-resin (MBHA) on a 0.15 mmol scale (resin loading: 1.0 mmol/g). The first Boc-Arg(Tos) was coupled twice. The *S*-trityl

mercaptopropionic acid (2.5-fold mol. excess) was coupled using the standard conditions used for Boc-protected amino acids. The trityl-group was removed using TFA : H_2O : triisopropylsilane (95 : 2.5 : 2.5 v/v/v) (1 flow wash and 2 min batch deprotection). After washing the resin with DMF, Boc-Gly was coupled twice using standard protocol and the remaining part of the sequence was assembled by conventional couplings, except the 7-OH-isoquinolinone building block **1** (160 mg, 0.321 mmol), which was coupled in ~2-fold mol. excess using coupling with HATU. The (Cys^{41}-99) segment was assembled on preloaded Boc-Phe-OCH_2-Pam-resin (resin loading: 0.6 mmol/g) using the standard coupling procedure. After chain assembly was complete, peptides were deprotected and simultaneously cleaved from the resin support by treatment with anhydrous HF containing 10% (v/v) *p*-cresol for 1 h at 0 ºC. After evaporation of the HF under reduced pressure, crude products were precipitated and triturated with chilled diethyl ether, and the peptide products were dissolved in 50% aqueous acetonitrile containing 0.1% TFA. Preparative HPLC of crude peptides after SPPS was performed on an Agilent 1100 prep system using in-house packed C4 (250 × 10 mm, Microsorb, 300 Å, 10 μm) columns. Peptides from SPPS and products from ligations were eluted from the column using an appropriate shallow gradient of acetonitrile/0.08% TFA versus water/0.1% TFA. Fractions containing the desired purified peptide product were identified by analytical LC-MS, combined and lyophilized. Segment (Cys^{41}-99)-COOH was purified two times.

Total chemical synthesis of the 99-residue HIV-1 protease polypeptide chains. In native chemical ligation, segments [(7-OH)dihydroisoquinolinone, des(25-27)](1-40)-$^{\alpha}COSCH_2CH_2COArg_4$ (12.6 mg, 2.5 μmol) and (Cys^{41}-99)-COOH (15.7 mg, 2.5 μmol) were dissolved in aqueous buffer (2.7 mL) comprising 6 M Gn·HCl, 0.2 M Na_2HPO_4 and 10 mM TCEP and 50 mM 4-mercaptophenylacetic acid at pH 7.0. After three hours 42 mg (300 μmol) of 2-bromoacetamide was added and the reaction mixture was stirred for 15 min at pH 6.7. Deformylation of Trp side chains was performed by treatment with a mixture of 2-mercaptoethanol and piperidine (1:1 (v/v), 4 mL) on ice for 15 min, and then neutralizing with HCl. The reaction mixture was diluted two-fold with buffer (6 M Gn·HCl, 0.2 M Na_2HPO_4) and purified by RP-HPLC on an in-house packed C4 (250 × 10 mm, Microsorb, 300 Å, 5 μm) column. Yield of isolated product: 8 mg (0.75 μmol,

30%). The product had the expected mass (10694.6 Da) by analytical LC-MS, with the MS data acquired across the entire UV peak.

Analytical RP-HPLC was performed on an Agilent 1100 system with in-house packed C4 and C18 silica columns (2.1 × 50 mm, Microsorb 300 Å, 3 μm) at flow rate of 0.5 mL/min at flow rate of 1.0 mL/min. Peptides were eluted from the column using a gradient of acetonitrile/0.08% TFA (solvent A) versus water/0.1% TFA (solvent B). Peptide masses were obtained using in-line electrospray MS detection with an Agilent 1100 LC/MSD ion trap.