Supporting Information

Biomimetic Catalysis of Intermodular Aminoacyl Transfer

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Materials and Methods

Peptide synthesis. Peptides were synthesized using standard Fmoc chemistry with an Advanced Chemtech Peptide Synthesizer Model 348 Ω. A typical synthesis was performed on 0.07 mmol scale using 0.5-0.8 mmol/g Rink Amide MBHA resin (Novabiochem) for preparation of a C-terminal amide or Fmoc-Arg(Pbf)-Wang resin (Novabiochem) for preparation of a C-terminal acid. Standard side chain protecting groups included Arg(Pbf), Glu(OtBu), Asn(Trt), Ser(OtBu), Lys(Boc), His(Trt), Cys(Trt) or Cys(Acm), Asp(OtBu), and Gln(Trt). Chain elongations were achieved using DIC and HOBt in NMP with 65-minute couplings. Fmoc deprotection was achieved using 30% piperidine in NMP. Acetamidobenzoic acid (ABA) was coupled to the N-terminus of each peptide to facilitate ultraviolet detection. Peptides were cleaved from the resin with concomitant side chain deprotection by agitation in a solution of 95:2:2:1 trifluroacetic acid (TFA)/water/ethanedithiol (EDT)/triisopropylsilane (TIS) for four hours. The crude peptide was precipitated with ether, centrifuged, and washed twice with ether. After air-drying, the peptide was purified by preparative RP-HPLC using a Vydac C18 column and water/acetonitrile/TFA as eluent. Purified peptides were characterized by analytical HPLC and MALDI-TOF mass spectrometry. Analytical reverse-phase HPLC was performed using a Zorbax 300-SB C-8 column or a Zorbax 300-SB C-18 column connected to a Hitachi D-7000 HPLC system. Binary gradients of solvent A (99% H_2O, 0.9% acetonitrile, 0.1% TFA) and solvent B (90% acetonitrile, 9.9% H_2O, 0.07% TFA) were employed at a flow rate of 1.5 ml/min.

For the synthesis of side chain ester peptides **5** and **6**, Fmoc-Ser(Trt)-OH was used at the amino acid position eight. Subsequent to the incorporation of this residue, the resin was treated with 2:5:93 TFA/triethylsilane/DCM to deprotect selectively the Ser hydroxyl group. The esterification reaction was carried out on solid support using the symmetrical anhydride of Boc-Gly-OH (peptide **5**) or Boc-Ala-OH (peptide **6**), prepared just prior to the esterification using a DIC/HOBt mediated condensation in DCM. A catalytic amount of dimethylaminopyridine (DMAP) was included during the esterification, which proceeded for two hours at room temperature. Standard solid-phase peptide synthesis then proceeded as described above to complete the peptide.

To prepare sequence **10a**, Aba-Gly-SCH_2CO_2Me (32.4 mg, 100 μmol), PPh_3 (26 mg, 100 μmol), and DIEA (44 μL, 250 μmol) in DMF (2.0 mL) were added to peptide **10** (6.7 mg, 2.0 μmol). The reaction was stirred at room temperature. After three hours, the product was purified by preparative RP-HPLC to give **10a**. To prepare peptide **11a**, Boc-Gly-SCH_2CO_2Me (39.3 mg, 150 μmol) or Boc-Ala-SCH_2CO_2Me (27.7 mg, 100 μmol) was added to a DMF solution (3.0 mL) containing PPh_3 (39.3 mg, 150 μmol), DIEA (66 μL, 375 μmol), and peptide **11** (10 mg, 3.0 μmol). After stirring three hours, the product was purified by preparative RP-HPLC to give the Boc-protected derivatives of **11a**. The Boc protecting group was removed by treating the peptides with neat TFA for 30 minutes, and subsequent HPLC purification yielded **11a**.

Circular Dichroism. Circular dichroism spectroscopy (CD) measurements were obtained on an Aviv circular dichroism spectrometer model 62DS using 20 μM peptide in 10 mM 3-(N-morpholino)-propanesulfonic acid (MOPS) buffer at pH 7.0 with 0.5 mM dithiothreitol (DTT) as reductant. For thermal denaturation experiments, 3 M guanidinium hydrochloride was added to bring the T_m value below 100 °C. The T_m value for the 26-residue peptide **1** in the presence of 3 M GDN was 78 °C, which is somewhat lower than the reported value of 94 °C for the 33-residue GCN4-pLI (ref. S1) and likely reflects the lower thermodynamic stability of a shortened peptide.

Analytical ultracentrifugation, sedimentation velocity. A solution of peptide **1** (100 μM) was prepared in 250 mM HEPES buffer (pH 7.2) containing 10 mM tris-carboxylethylphosphine (TCEP) as reducing agent. Sedimentation velocity profiles were obtained on a temperature controlled Beckman XL-I analytical ultracentrifuge (Beckman Coulter, Fullerton, CA) equipped with an An50Ti rotor and a photoelectric scanner. A standard double sector cell, equipped with a 12 mm Epon centerpiece and quartz windows, was loaded with 420 μL of sample using a blunt-end microsyringe. Data at 290 nm were collected at rotor speeds of 3,000 rpm initially and 50,000 rpm in a continuous mode at 20 °C, with a step size of 0.002 cm.

A direct boundary fitting approach was applied to the sedimentation velocity data. The program Svedberg developed by John Philo (ref. S2) was used in which multiple data sets (concentration vs. radial position) taken at various times during the experiment were simultaneously fit to approximate solutions

of the Lamm equation. This method is sensitive to the number of species present and is advantageous in that it allows for the calculation of the molecular weight of the sedimenting species using equation 1 since the sedimentation coefficient and diffusion coefficient can be obtained from the fitting analysis.

$$MW = sRT/D(1-\bar{\upsilon}\rho) \qquad \text{(eq. 1)}$$

where MW is the molecular weight, s is the sedimentation coefficient (in Svedbergs, 1 Svedberg = $1x10^{-13}$ sec), R is the universal gas constant (8.314 x 10^7 erg/mol), $\bar{\upsilon}$ is the partial specific volume (cm^3/g) and ρ is the solvent density (g/cm^3). The buffer density used was calculated from tabulated data to be 1.00848 g/cm^3. The data were fit to one or two non-interacting species. The best fit was to one non-interacting species with MW = 13.49 ± 0.03 kDa and s = 1.172 ± 0.000 Svedbergs (ref. S2), which corresponds well with the expected tetrameric mass of 13.42 kDa.

Sedimentation equilibrium. The hydrodynamic molecular weight of peptide **1** was confirmed by sedimentation equilibrium measurements carried out employing a temperature-controlled Beckman XL-I Analytical Ultracentrifuge equipped with an An-60 Ti rotor and a photoelectric scanner (Beckman Instrument Inc., Palo Alto, CA). Peptide **1** (50 μM) in buffer (300 mM HEPES pH 7, 10 mM TCEP) was loaded in a double sector cell equipped with a 12 mm Epon centerpiece and a sapphire optical window. The reference compartment was loaded with a matching solution without peptide. Samples (80 μL) were monitored employing a rotor speed of 3000 to 20000 rpm at 25 ºC and analyzed by a nonlinear squares approach using a single species model with the program Origin (Microcal Software Inc., Northampton, MA). The best fit yielded a MW of 12.6 kDa ±0.3 kDa which corresponds more closely with the tetrameric mass of 13.4 kDa than with the trimeric mass of 10.1 kDa.

Cbz-Gly-SNAC preparation. N-α-carboxybenzyl-glycine (Novabiochem, 2 mmol) was stirred in DCM (20 ml) with HOBt (2.1 mmol) for 10 min at 0 °C. After adding 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, Aldrich, 2.1 mmol) and stirring an additional 30 min, N-acetyl cysteamine (Aldrich, 2.3 mmol) was added and the ice bath was removed; the reaction stirred 6 hr. The organic solution was washed twice with 5% aqueous sodium bicarbonate, twice with water, once with brine, dried over $MgSO_4$, and concentrated by rotary evaporation. The compound was purified by preparative RP-HPLC chromatography for kinetic assays, giving a colorless solid in 57% yield. Observed ESI MS (m/z) 311.1067 Da (M + H), calculated average isotope composition for $C_{14}H_{19}N_2O_4S$ 311.1060 Da. ^{1}H NMR ($CDCl_3$, 300 Mhz) δ ppm 1.97 (3H, s), 3.07 (2H, t, *J* = 6.2 Hz) 3.43 (2H, br dd, *J* = 6.1 Hz) 4.12 (2H, d, *J* = 5.7 Hz) 5.15 (2H, s) 5.47 (1H, br s) 5.89 (1H, br s) 7.36 (5H, m); ^{13}C ($CDCl_3$, 100MHz) δ ppm 23.14, 28.30, 39.19, 50.68, 67.36, 128.10, 128.30, 128.54, 135.94, 156.25, 170.39, 198.04.

Cbz-L-Ala-SNAC preparation. Prepared as described for the Gly derivative using N-α-carboxybenzyl-L-alanine-OH (Novabiochem, 2 mmol). Observed ESI MS (m/z) 325 Da (M + H), calculated average isotope composition for $C_{15}H_{20}N_2O_4S$ 324.1 Da. ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 1.49 (3H, d, *J* = 6.2 Hz) 2.03 (3H, s) 3.12 (2H, t, *J* = 6.2 Hz) 3.49 (2H, m) 4.52 (1H, m) 5.21 (2H, s) 5.34 (1H, br d, *J* = 7.0 Hz) 5.97 (1H, br s) 7.44 (5H, m).

Cbz-L-Ser-SNAC preparation. Prepared as described for the Gly derivative using N-α-carboxybenzyl-L-serine(O-tBu)-OH (Novabiochem, 2 mmol). After aqueous workup of the reaction, the side chain protecting group was removed by stirring in 1:1 TFA/DCM for 2 h, and a portion of the resulting product was purified by preparative HPLC for kinetic assays. Observed ESI MS (m/z) 341 Da (M + H), calculated average isotope composition for $C_{15}H_{20}N_2O_5S$ 340.1 Da. ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 2.04 (3H, s) 3.01 (1H, m) 3.13 (1H, m) 3.48 (1H, m) 3.62 (1H, m) 3.88 (1H, dd, *J* = 3.7, 11.8 Hz) 4.21 (1H, m) 4.49 (1H, br d, *J* = 8.1 Hz) 5.23 (2H, s) 6.19 (1H, d, *J* = 8.4 Hz) 6.33 (1H, br s) 7.44 (5H, m).

Cbz-L-Gln-SNAC preparation. Prepared as described for the Gly derivative using N-α-carboxybenzyl-L-glutamine(trityl)-OH (Novabiochem, 2 mmol). After aqueous workup of the reaction, the side chain protecting group was removed by stirring in 1:1 TFA/DCM for 2 h, and a portion of the resulting product was purified by preparative HPLC for kinetic assays. Observed ESI MS (m/z) 382 Da (M + H), calculated average isotope composition for $C_{17}H_{23}N_3O_5S$ 381.1 Da. ^{1}H NMR (DMSO, 400 MHz) δ ppm 1.67 (1H, m) 1.74 (3H, s) 1.93 (1H, m) 2.10 (2H, t, *J* = 7.4 Hz) 2.80 (2H, t, *J* = 7.3 Hz) 3.10 (2H, m) 4.08 (1H, m) 5.01 (2H, d, *J* = 5.6 Hz) 6.74 (1H, br s) 7.28 (5H, m).

Cbz-L-Glu-SNAC preparation. Prepared as described for the Gly derivative using N-α-carboxybenzyl-L-glutamic acid(O-tBu)-OH (Novabiochem, 2 mmol). After aqueous workup of the reaction, the side chain protecting group was removed by stirring in 1:1 TFA/DCM for 2 h. After solvent evaporation, the material was crystallized by dissolving to saturation in DMF, adding excess water containing 0.1 % TFA, and cooling. Observed ESI MS (m/z) 383 Da (M + H), calculated average isotope composition for $C_{17}H_{22}N_2O_6S$ 382.1 Da. ^{1}H NMR (DMSO, 400 MHz) δ ppm 1.71 (4H, m) 1.95 (1H, m) 2.29 (2H, t, *J* = 7.3 Hz) 2.86 (2H, t, *J* = 6.7 Hz) 3.13 (2H, m) 4.15 (1H, m) 5.01 (2H, s) 7.28 (5H, m).

Cbz-L-His-SNAC preparation. Prepared as described for the Gly, derivative using N-α-carboxybenzyl-L-histidine(trityl)-OH (prepared from Cbz-His-OH, 2 mmol). After aqueous workup of the reaction, the side chain protecting group was removed by stirring in 1:1 TFA/DCM for 2 h, and a portion of the resulting product was purified by preparative HPLC for kinetic assays. Observed ESI MS (m/z) 391 Da (M + H), calculated average isotope composition for $C_{18}H_{22}N_4O_4S$ 390.1 Da. ^{1}H NMR (DMSO, 400 MHz) δ ppm 1.75 (3H, s) 2.89 (3H, m) 3.12 (3H, m) 4.47 (1H, m) 4.99 (2H, s) 7.28 (7H, m).

Cbz-L-Thr-SNAC preparation. Prepared as described for the Gly derivative using N-α-carboxybenzyl-L-threonine(O-tBu)-OH (Novabiochem, 2 mmol). After aqueous workup of the reaction, the side chain protecting group was removed by stirring in 1:1 TFA/DCM for 2 h, and a portion of the resulting product was purified by preparative HPLC for kinetic assays. Observed ESI MS (m/z) 355 Da (M + H), calculated average isotope composition for $C_{16}H_{22}N_2O_5S$ 354.1 Da. ^{1}H NMR (DMSO, 400 MHz) δ ppm 1.03 (3H, d, *J* = 6.3 Hz) 1.73 (3H, s) 2.82 (2H, t, *J* = 6.9 Hz) 3.08 (2H, q, *J* = 6.5 Hz) 4.05 (2H, m) 5.05 (2H, s) 7.31 (5H, m).

Cbz-L-Leu-SNAC preparation. Prepared as described for the Gly derivative using N-α-carboxybenzyl-L-leucine-OH (Novabiochem, 2 mmol). Observed ESI MS (m/z) 367 Da (M + H), calculated average isotope composition for $C_{18}H_{26}N_2O_4S$ 366.2 Da. ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 0.96 (6H, d, *J* = 6.2 Hz) 1.96 (3H, s) 3.04 (2H, t, *J* = 6.2 Hz) 3.41 (2H, m) 4.41 (1H, m) 5.14 (2H, s) 5.78 (1H, br s) 7.35 (5H, m).

Boc-Gly-SCH$_2$CO$_2$Me preparation. Prepared as described for Cbz-Gly-SNAC using N-α-Boc-glycine-OH (Novabiochem, 2 mmol) and methyl thioglycolate in place of N-acetyl cysteamine. Observed SSI-MS (m/z) 285.7 Da (M + Na), calculated average isotope composition for $C_{10}H_{17}NO_5SNa$ 286.1 Da. ^{1}H NMR

($CDCl_3$, 400 MHz) δ ppm 1.45 (s, 9H), 3.70 (s, 2H), 3.72 (s, 3H), 4.07 (d, *J* = 6.0 Hz, 2H), 5.25 (br, 1H). MS (LC-MS).

Boc-Ala-SCH_2CO_2Me preparation. Prepared as described for Cbz-Gly-SNAC using N-α-Boc-L-alanine-OH (Novabiochem, 2 mmol) and methyl thioglycolate in place of N-acetyl cysteamine. Observed SSI-MS (m/z) 299.7 Da (M + Na), calculated average isotope composition for $C_{11}H_{19}NO_5SNa$ 300.1 Da. ^1H NMR ($CDCl_3$, 400 MHz) δ ppm, 1.37 (d, *J* = 7.2 Hz, 3H), 1.43 (s, 9H), 3.62 (d, *J* = 16.0 Hz, 1H), 3.71 (s, 3H), 3.72 (d, *J* = 15.6 Hz, 1H), 4.42-4.38 (m, 1H), 5.05 (d, *J* = 7.2 Hz, 1H).

Aba-Gly-SCH_2CO_2Me preparation. A solution of acetamidobenzoic acid (Aba) (895.9 mg, 5.0 mmol), HOBt (800.0 mg, 6.0 mmol), and EDC (1150 mg, 6.0 mmol) in DCM (50 mL) was stirred at 0°C for 30 minutes and then stirred at room temperature for another 30 minutes. H-Gly-OMe (627.5 mg, 5.0 mmol) was added and the reaction mixture was kept overnight. After concentration, the residue was taken up in ethyl acetate and washed once with one volume of 10% aqueous $NaHCO_3$. The organic layer was dried using anhydrous $MgSO_4$ and concentrated. The crude product was purified by flash chromatography to afford Aba-Gly-OMe (625.0 mg, 50%). ^1H NMR (400 MHz, CD_3OD) δ 7.80 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 8.8 Hz, 2H), 4.08 (s, 2H), 3.72 (s, 3H), 2.12 (s, 3H).

To a solution of Aba-Gly-OMe (200.0 mg, 0.8 mmol) in THF/H_2O (7.0 mL, v/v = 1/1), LiOH.H_2O (42.0 mg, 1.0 mmol) was added. The reaction was monitored by RP-HPLC. After completion, the solution was acidified with HCl (1 M) to pH 1, concentrated, and used in next step without further purification. ^1H NMR (400 MHz, CD_3OD/TMS) δ 7.70 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 8.8 Hz, 2H), 4.04 (s, 2H), 2.10 (s, 3H). MS (LC-MS) Observed SSI-MS (m/z) 236.6 Da (M + H), calculated average isotope composition for $C_{11}H_{12}N_2O_4$ 236.1 DA (M + H).

A solution of Aba-Gly-OH (crude product from above), HOBt (135.1 mg, 1.0 mmol), and EDC (191.7 mg, 1.0 mmol) in DCM/DMF (9 mL, v/v = 8/1) was stirred at 0°C for 30 minutes and then stirred at room temperature for another 30 minutes. $HSCH_2CO_2Me$ (89 μL, 1.0 mmol) was added, and the solution was kept at room temperature overnight. After concentration by rotary evaporation, the residue was taken up in ethyl acetate, washed once with one volume of 10% aqueous $NaHCO_3$, dried using anhydrous $MgSO_4$, and concentrated. The crude product was purified by flash chromatography to afford Aba-Gly-SCH_2CO_2Me (162 mg, 62% for two steps) ^1H NMR (400 MHz, CD_3OD/TMS) δ 7.81 (d, *J* = 8.8 Hz, 2H), 7.66 (d, *J* = 8.8 Hz, 2H), 4.25 (s, 2H), 3.72 (s, 2H), 3.67 (s, 3H), 2.10 (s, 3H). Observed SSI-MS (m/z) 324.3 Da (M + H), calculated average isotope composition for $C_{14}H_{16}N_2O_5S$ 324.1 DA (M + H).

Acyl Transfer Assay and Analysis. All reactions were carried out in 285 mM HEPES buffer at pH 7.0 in the presence of 10 mM TCEP as reductant and ~200 μM ABA as internal concentration standard. The exact concentration of ABA in buffer was determined prior to the reaction by ultraviolet absorbance at 270 nm on a Cary 100 Bio UV-Vis spectrophotometer using an extinction coefficient of 14,324 M^{-1} cm^{-1} for ABA at pH 7.0. Fresh peptide stock solutions were prepared daily by dissolving lyophilized peptide in the appropriate buffer. The concentration of peptide in the stock solution was determined by comparison of the HPLC peak integrations for peptide and ABA at 270 nm. Cbz-aminoacyl-SNAC stock solutions (40 mM) were prepared fresh daily in 4:1 reaction buffer/DMF (to aid solubility; final DMF concentration in the acyl transfer reaction was 5%). Reaction aliquots were quenched with 5% TFA to a final pH of ~1 and analyzed by analytical RP-HPLC. Product identities were confirmed by MALDI-TOF or SSI electrospray mass spectrometry and by comparison with independently prepared authentic samples.

For bimolecular reactions (second-order, units of M^{-1} s^{-1}) catalyzed via a substrate-catalyst complex (first-order, units of s^{-1}), the ratio of the catalyzed reaction to the non-catalyzed reaction is expressed in units of molarity and represents the effective concentration of the second substrate that would be needed in solution to mimic the reactivity of that substrate in the active site. For peptide **1** (k_2 = 9.2x10^{-4} s^{-1}), the effective concentration of active site Lys relative to control peptide **7** (k_2 = 1.7 x10^{-5} M^{-1} s^{-1}) is 54 M. This value is ~5x10^5 greater than the actual concentration present in solution (100 μM).

Reaction Modeling. For sequences **1**-**6**, the concentrations of all peptide species were simultaneously fit using SIMFIT (ref. S3) based on the following minimal reaction model (peptide **1** used as example):

$$\mathbf{1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{1a} + \mathrm{RSH} \qquad (1)$$

$$\mathbf{1a} \xrightarrow{k_2} \mathbf{1b} \qquad (2)$$

$$\mathbf{1b} \underset{k_{-1}}{\overset{k_3}{\rightleftharpoons}} \mathbf{1c} + \mathrm{RSH} \qquad (3)$$

where species **1**, **1a**, **1b**, and **1c** are as shown in Figure 2 of the manuscript and RSH denotes N-acetyl cysteamine. Equations (1) and (3) describe the reversible, pseudo-first order reactions (substrate loading) to generate peptidyl-thiolester species and N-acetyl cysteamine (RSH). Rate constants for the forward reactions (k_1, k_3) were allowed to vary independently, while the reverse reactions were assumed to proceed at the same rate (k_{-1}). Equation (2) describes the irreversible aminoacyl transfer step of the reaction. Peptide folding/association events were not included in the reaction model. Root mean square (RMS) deviations from the model ranged from 5-15%.

For sequences **7**-**9**, for which transthiolesterification is not possible, a single reaction was employed for fitting (peptide **7** used as example):

$$\mathbf{7} \xrightarrow{k_2} \mathbf{7b} + \mathrm{RSH} \qquad (4)$$

where species **7** represents the unmodified peptide and **7b** represents the peptide irreversibly acylated by the Cbz-Gly-SNAC substrate under the pseudo-first order conditions of the reaction. RMS deviations from the model were ≤ 3%.

To fit the reactions occurring in heterotetrameric assemblies of peptides **9** and **12**, the following model was used:

$$\mathbf{12} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{12a} + \mathrm{RSH} \qquad (5)$$

$$\mathbf{12a} + \mathbf{9} \xrightarrow{k_2} \mathbf{12} + \mathbf{9b} \qquad (6)$$

where species **12**, **12a**, **9**, and **9b** are as shown in Figure 3 of the manuscript and RSH denotes N-acetyl cysteamine. Equation (5) describes the reversible, pseudo-first order substrate loading reaction to generate the peptidyl-thiolester species and N-acetyl cysteamine. Equation (6) describes the irreversible aminoacyl transfer step of the reaction. Peptide folding/association events were not included in the reaction model. RMS deviation from the model for the simultaneous fits of the five reactions shown in Figure 3c of the manuscript was 8%.

Supporting References:

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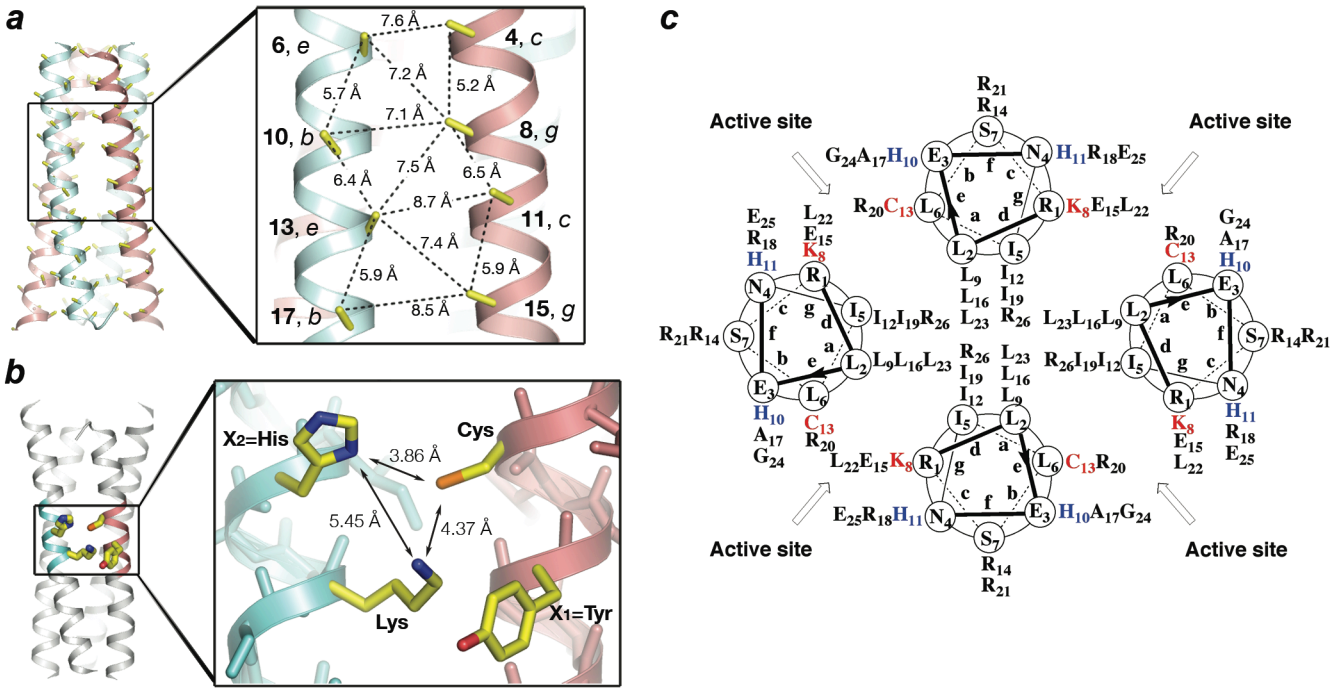

Figure S1. Structural representations of the designed aminoacyl transfer catalysts. a) A map of inter-residue distances measured between β-carbon atoms (yellow sticks) in the crystal structure of a homotetrameric GCN4-derived coiled-coil (ref. S1). Residues are labeled by number and heptad position. A number of different active site arrangements provide the desired β-carbon distance of 6-10 Å between aminoacyl-donor and –acceptor sites. b) A crystal structure (ref. S4) showing the location of the active site residues near the center of the coiled-coil assembly and a close-up view of active site inter-residue distances. Only one of the four symmetry-related active sites is shown for clarity. c) Helical wheel diagram for sequence **1** illustrates the location of the four symmetry-related active sites at the helical interfaces.

Table S1. Active sites, molar ellipticities, and sequences of the designed peptides.

Peptide	Active Site	$[\theta]^a$ (deg cm^2 dmol^{-1})	Sequence
1	K_HH_C	-25800	Aba-RLENILS**K**L**HH**I**C**RELARIRRLLGER-$CONH_2$
2	K_AH_C	-24300	Aba-RLENILS**K**L**AH**I**C**RELARIRRLLGER-$CONH_2$
3	K_HA_C	-23100	Aba-RLENILS**K**L**HA**I**C**RELARIRRLLGER-$CONH_2$
4	K_AA_C	-25200	Aba-RLENILS**K**L**AA**I**C**RELARIRRLLGER-$CONH_2$
5	(S^{Gly})_HH_C^b	N.D.	Aba-RLENILS**(S^{Gly})**L**HH**I**C**RELARIRRLLGER-$CONH_2$ ^{b}
6	(S^{Ala})_HH_C^b	N.D.	Aba-RLENILS**(S^{Ala})**L**HH**I**C**RELARIRRLLGER-$CONH_2$ ^{b}
7	SKL	N.D.	Aba-S**K**L-COOH
8	K_HH_S	-25000	Aba-RLENILS**K**L**HH**I**S**RELARIRRLLGER-$CONH_2$
9	K_AH_S	-25800	Aba-RLENILS**K**L**AH**I**S**RELARIRRLLGER-$CONH_2$
10	(C^{Acm})_HD_C^b	N.D.	Aba-RLENILS**C(Acm)**L**HD**I**C**RELARIRRLLGER-$CONH_2$
11	C_DH_(C^{Acm}) ^{b}	N.D.	Aba-RLENILS**C**L**DH**I**C(Acm)**RELARIRRLLGER-$CONH_2$
12	R_HA_C	-26700	Aba-RLENILS**R**L**HA**I**C**RELARIRRLLGER-$CONH_2$

^{a} Molar ellipticity values at 222 nm for peptides (20 μM) in pH 7.0 MOPS buffer (10 mM). N.D. = not determined. ^{b} (S^{Gly}) denotes glycyl-esterified Ser residue, (S^{Ala}) denotes alanyl-esterified Ser residue, (C^{Acm}) denotes acetamidomethyl-protected Cys residue.

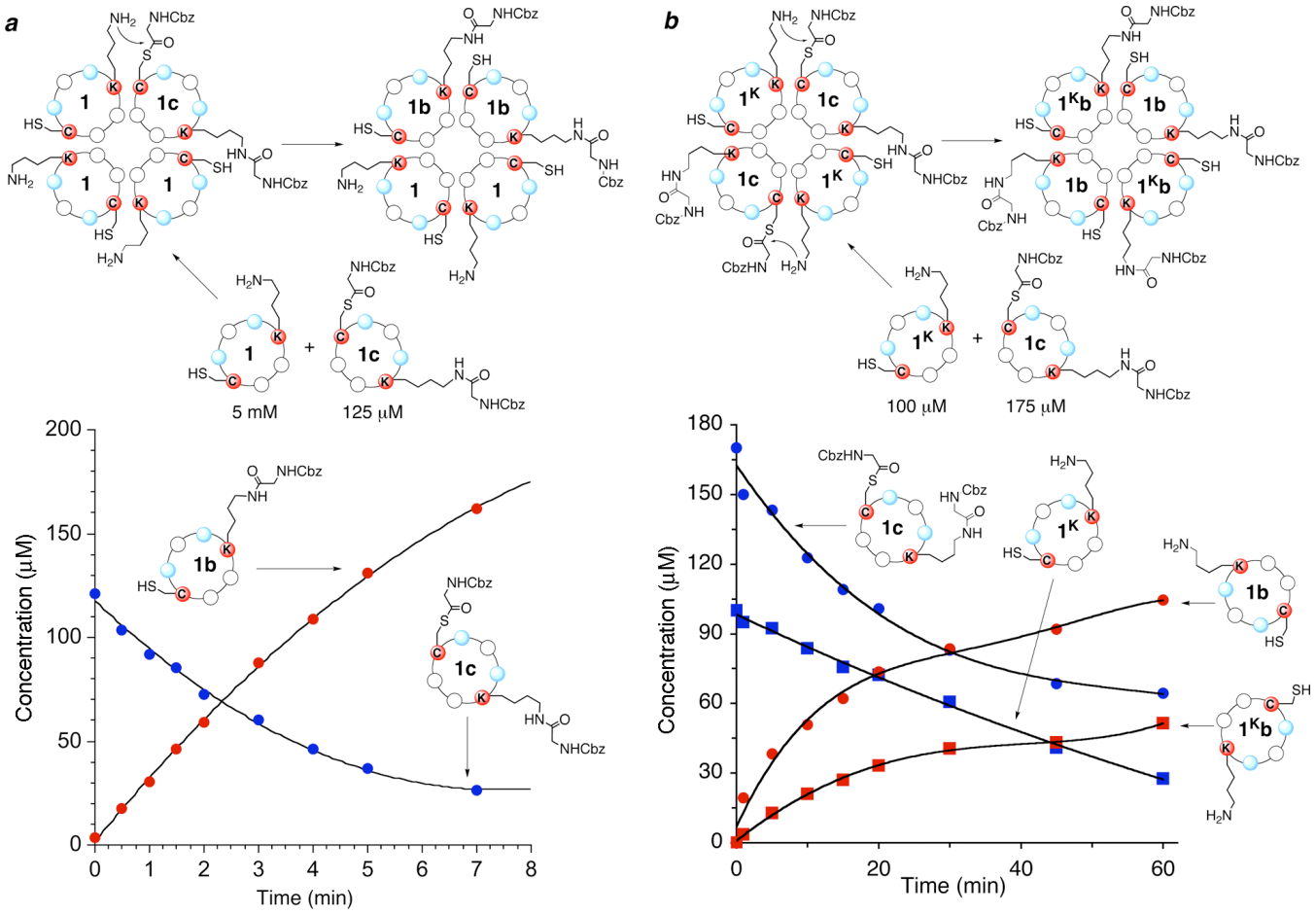

Figure S2. Establishing the interhelical mode of aminoacyl transfer in tetrameric coiled-coil assemblies. a) Peptide concentration as a function of time is shown for the pseudo-first order reaction of purified peptides **1c** (125 μM) and **1** (5 mM) under standard buffer conditions (without the Cbz-Gly-SNAC substrate). Note that approximately two equivalents of **1b** are produced for each equivalent of **1c** used. The tetrameric assembly shown represents only one of a number of possible coiled-coil assemblies in solution. b) To further support the interhelical aminoacyl transfer mechanism, we prepared peptide $\mathbf{1^K}$, which contains an extra N-terminal lysine residue to afford this peptide a distinct mass and HPLC retention time from sequence **1**. Mixing peptides $\mathbf{1^K}$ (100 μM) and **1c** (175 μM) under standard reaction conditions (without the Cbz-Gly-SNAC substrate) led to the rapid formation of aminoacylated products $\mathbf{1^Kb}$ and **1b**. The tetrameric assembly shown represents only one of a number of possible coiled-coil assemblies in solution. These findings strongly supporting the interhelical mechanism, as sequence $\mathbf{1^Kb}$ cannot form via an intrahelical transfer.

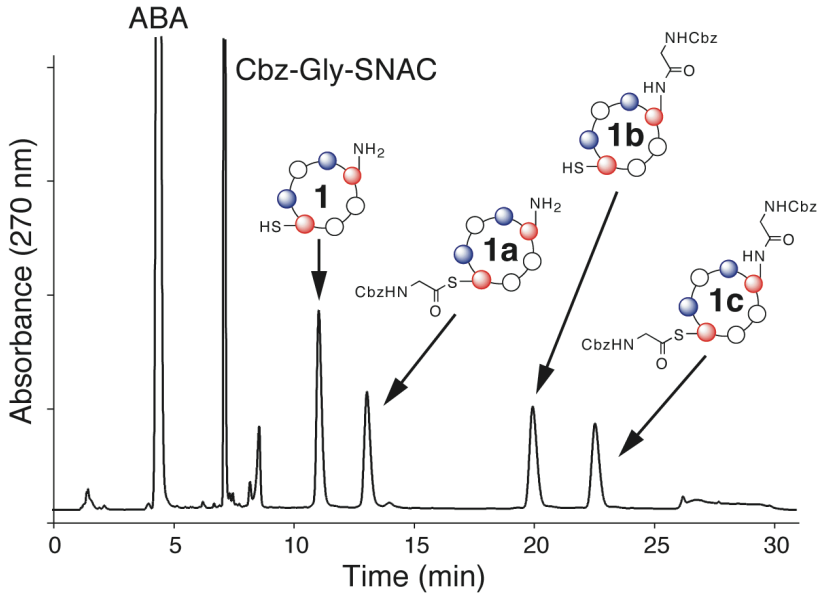

Figure S3. Example HPLC trace for an aminoacyl transfer reaction. Shown is a 30-minute time point from peptide **1** reacting with Cbz-Gly-SNAC under standard conditions (100 μM peptide, 10 mM Cbz-Gly-SNAC, 285 mM HEPES pH 7.0, 10 mM TCEP, 200 μM ABA). Absorbance was monitored at 270 nm. Product peptide masses, determined by MALDI-TOF MS, were consistent with expected values.

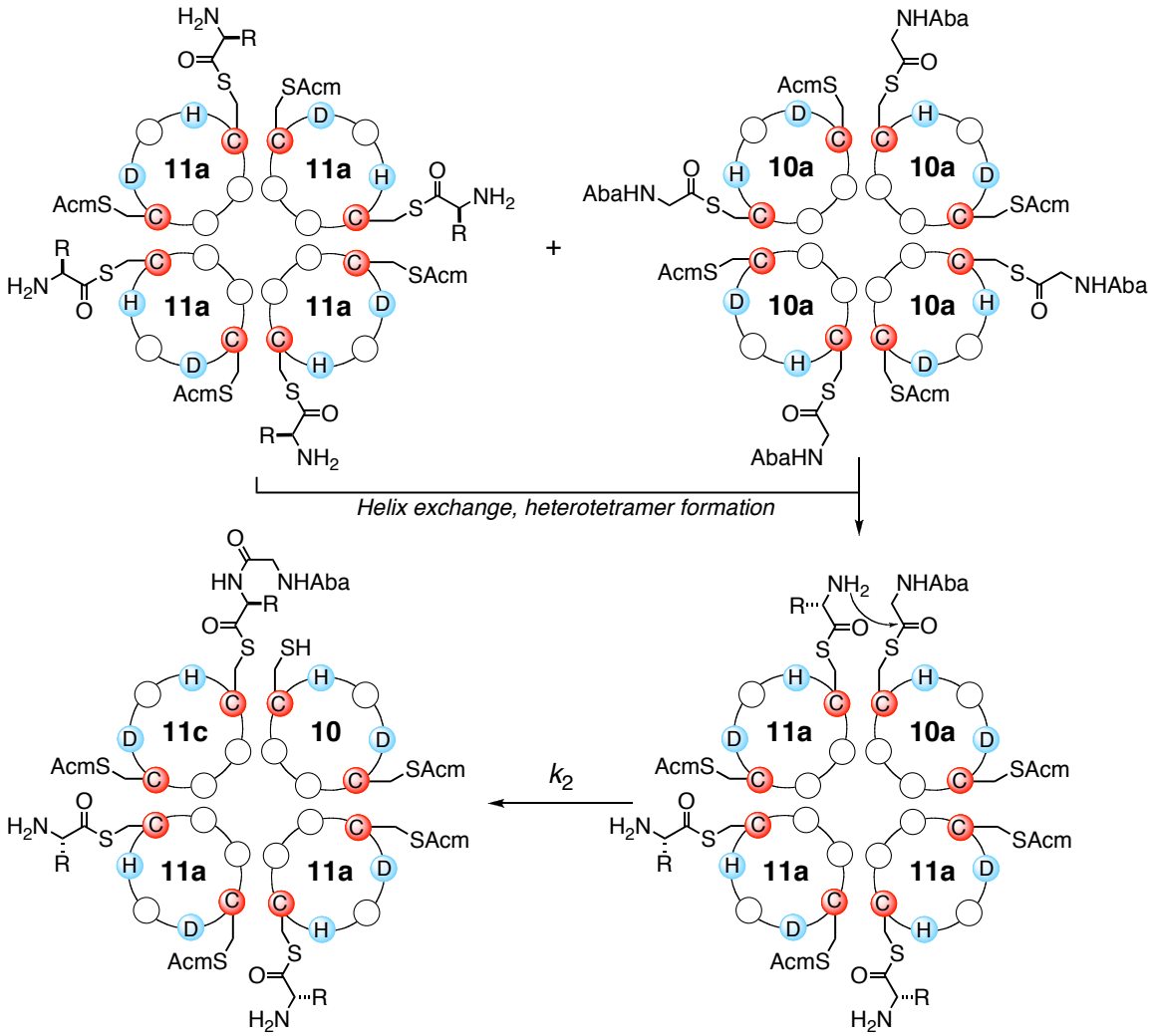

Figure S4. Reaction profile for aminoacyl transfer in Type-III active site designs (showing entire heterotetramer cross-section). Notably, aminoacyl transfer cannot occur within the parallel homotetrameric assemblies of **11a** or **10a**, but can only proceed when heterotetramer formation juxtaposes the two distinct sequences. The heterotetrameric assembly shown represents the statistically most predominant coiled-coil assembly in solution competent for aminoacyl transfer.

Table S2. Rate constants for the reaction of various aminoacyl-thiolester substrates with peptide **1**.[a]

Cbz-AA-SNAC	Side chain	k_1 (10^{-3} sec^{-1})	k_2 (10^{-4} sec^{-1})
glycine	-H	1.3	9.2
L-alanine	$-CH_3$	0.4	1.0
L-serine	$-CH_2OH$	0.06	2.1
L-glutamine	$-CH_2CH_2CONH_2$	0.05	3.2
L-glutamic acid	$-CH_2CH_2COOH$	0.06	2.6
L-threonine	$-CH(OH)CH_3$	0.04	1.3
L-leucine	$-CH_2CH(CH_3)_2$	0.03	1.6

[a] Reaction conditions are as described in Figure 2 of the manuscript.

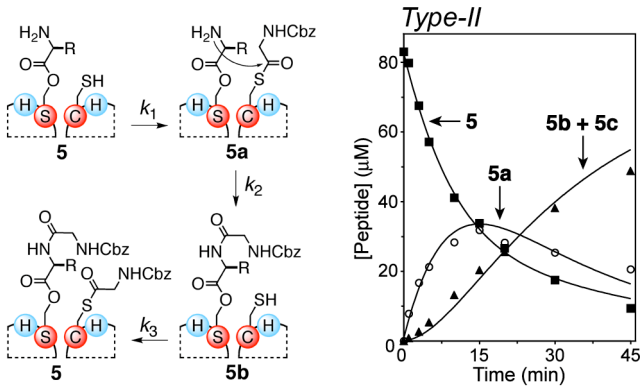

Figure S5. (left) Aminoacyl loading and intermodular acyl transfer reactions schemes and (right) product formation in time for the Type-II active sites.