From Static to Dynamic: Escaping Kinetic Traps in Hydrazone-Based Dynamic Combinatorial Libraries

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Electronic Supplementary Information

S1 General Experimental Procedures

All chemicals, unless otherwise stated, were purchased from Aldrich, Alfa Aesar, Lancaster or NovaBioChem and used as received. All solvents were distilled prior to use with the exception of deuterated solvents and HPLC or LC/MS grade solvents. $CDCl_3$ was purchased from Euriso-top. The HPLC and LC/MS grade MeOH and CH_3CN were purchased from Fisher. The water used in the eluent was purified by a Millipore system. HPLC-grade formic acid was purchased from Romil and LC/MS grade formic acid was purchased from Fluka. Column chromatography was carried out using silica gel 60 F (Merck). HPLC grade solvent from Fisher was used in the preparation of DCLs.

^{1}H and ^{13}C-NMR spectra were recorded on a Bruker DPX-400 spectrometer, operating at 400 MHz (^{1}H), 100 MHz (^{13}C). All spectra were obtained at 298 K and are referenced to the internal solvent residue. Chemical shifts (δ) are quoted in ppm and have uncertainties of ± 0.01 ppm for ^{1}H, and ± 0.05 ppm for ^{13}C. Coupling constants (*J*) are listed in Hz. The following abbreviations are used for convenience in reporting the multiplicity for NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; sep, septet; m, multiplet and br, broad.

Amino acid protons are labelled according to the traditional scheme: α, β, γ, and δ. The NMR data was processed using Bruker Topspin 2.0. Assignment of all ^{1}H and ^{13}C resonances was achieved using standard 2D NMR techniques; COSY, NOESY, HMQC and HMBC.

HPLC analysis was performed on an Agilent Technologies 1200 Series system coupled to a diode array UV/Vis detector. LC/MS was carried out on an Agilent 1100 LC/MSD trap XCT system operating in alternating ultrascan mode with nebuliser, 25 psi; dry gas, 8 $lmin^{-1}$; dry temperature, 340 °C; capillary, 3500 V; skimmer, 40 V; capillary exit, 241 V; Oct1 DC, 12V; Oct 1 DC, 4.45 V; Trap drive, 167.9 V; Oct RF, 210.4 Vpp; lens 1, -5; lens 2, -60; scan range, 100-2200 m/z; max accumulation time, 200 ms and smart target, 50000.

Separations of DCLs formed from building blocks **1**, **2** and **3** were achieved using a Waters Symmetry C_{18} 3.5 µm 4.6 × 150 mm column maintained at 45 °C. The mobile phase solutions prepared were 0.1% formic acid in H_2O (A) and 0.1% formic acid in MeOH (B). Analysis of macrocycle mixtures was achieved using the method outlined below.

Method A Injection Volume: 10 µl

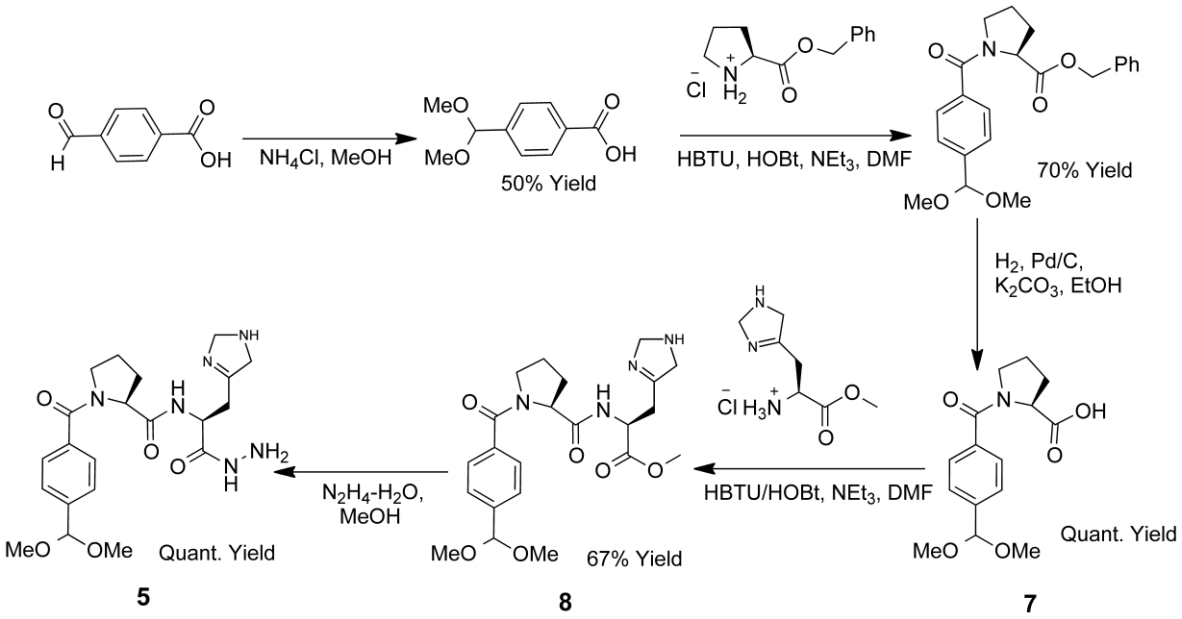
Time / min	% Solution A	% Solution B
0	50	50
3	30	80
10	27	83
17	0	100
20	0	100

Separations of DCLs formed from building blocks **4**, **5** and **6** were achieved using a Waters Symmetry Shield RP_{18} 5 µm 4.6 × 150 mm column maintained at 45 °C. The mobile phase solutions prepared were 0.1% formic acid in H_2O (A) and 0.1% formic acid in CH_3CN (B). Analysis of DCLs was achieved using the method outlined below.

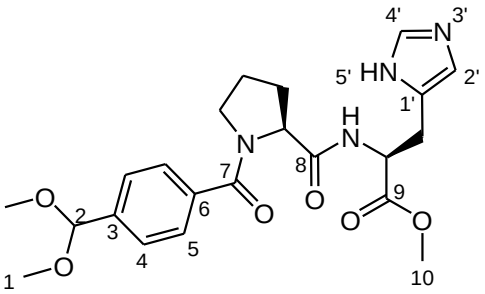
Method B Injection Volume: 5 µl

Time / min	% Solution A	% Solution B
0	99	1
14	0	100
15	99	1

S2 Synthesis of Building Block 5



*(4-(Dimethoxymethyl)benzoyl)-proline-histidine methyl ester (**8**)*



To a solution of HBTU (147 mg, 0.387 mmol) and HOBt (52 mg, 0.39 mmol) in DMF (1 ml) was added a solution of **7**[1] (113 mg, 0.385 mmol) in DMF (1.6 ml). After stirring for 5 minutes, L-H-His-OMe·2HCl (93 mg, 0.39 mmol) was added and, after a further 5 minutes stirring, triethylamine (0.17 ml) was added. The mixture was stirred for 3 hours and then the solvent was evaporated *in vacuo*. The residue was dissolved in MeOH (10 ml) and the pH was adjusted to pH 10 by addition of a solution of NaOMe (1 g / 10 ml in MeOH). The methanol was then evaporated *in vacuo* leaving a white residue. The residue was extracted with dichloromethane (3×10 ml). The combined extracts were filtered and evaporated *in vacuo* to yield a pale yellow oil. Purification was achieved by column

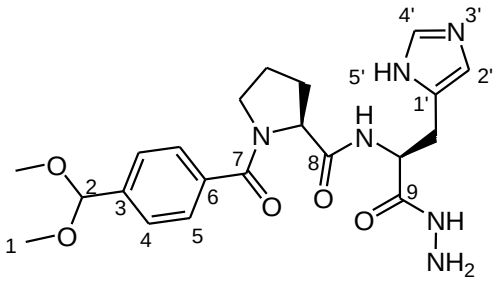
chromatography (silica, 10 % MeOH in CH_2Cl_2) to yield **8** as a pale yellow oil (114 mg, 67%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 2H, $^3J_{H4\text{-}H5}$ = 8.3 Hz, **H**5), 7.56 (s, 1H, **H**4′), 7.52 (d, 2H, $^3J_{H5\text{-}H4}$ = 8.3, **H**4), 6.90 (d, 1H, $^3J_{\alpha H\text{-}His\text{-}\alpha NH\text{-}His}$ = 7.5, α**N**H of His), 6.74 (s, 1H, **H**2′), 5.41 (s, 1H, **H**2), 4.84-4.80 (m, 1H, α**H** of His), 4.50-4.45 (m, 1H, α**H** of Pro), 3.77 (s, 3H, **H**10), 3.74-3.67 (m, 1H, δ**H** of Pro), 3.61-3.55 (m, 1H, δ**H** of Pro), 3.34 (dd, 1H, $^2J_{\beta H'\text{-}His\text{-}\beta H\text{-}His}$ = 15.6 Hz, $^3J_{\alpha H\text{-}His\text{-}\beta H\text{-}His}$ = 4.3 Hz, β**H** of His), 3.34 (s, 6H, **H**1), 3.18 (dd, 1H, $^2J_{\beta H\text{-}His\text{-}\beta H'\text{-}His}$ = 15.6 Hz, $^3J_{\alpha H\text{-}His\text{-}\beta H'\text{-}His}$ = 4.3 Hz, β**H**′ of His), 2.31-2.22 (m, 1H, β**H** of Pro), 2.21-2.10 (m, 2H, β**H** of Pro and γ**H** of Pro), 1.92-1.82 (m, 1H, γ**H** of Pro) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.19 (**C**8), 171.09 (**C**9), 170.05 (**C**7), 140.85 (**C**3), 136.08 (**C**4′), 135.81 (**C**6), 127.48 (**C**5), 126.98 (**C**4), 124.78 (**C**2′), 102.64 (**C**2), 61.40 (α**C** of Pro), 53.51 (α**C** of His), 52.92 (**C**1), 52.86 (**C**10), 50.89 (δ**C** of Pro), 29.56 (β**C** of Pro), 27.25 (β**C** of His), 25.79 (γ**C** of Pro) ppm.

Exact mass: Calculated: 445.2087; Found: 445.2076 ($M+H^+$).

*(4-(Dimethoxymethyl)benzoyl)-proline-histidine-NHNH$_2$ (**5**)*



Hydrazine monohydrate (0.50 ml) was added to a solution of **8** (50 mg, 0.12 mmol) in methanol (0.50 ml). The solution was stirred overnight and then the solvent evaporated *in vacuo* to quantitatively yield **5** as a colourless solid.

^{1}H-NMR (500 MHz, MeOD-d_4) δ 7.64 (d, 2H, $^3J_{H4\text{-}H5}$ = 8.0 Hz, **H**5), 7.54 (d, 2H, $^3J_{H5\text{-}H4}$ = 8.0 Hz, **H**4), 7.52 (d, 1H, $^3J_{H5'\text{-}H4'}$ = 0.9 Hz, **H**4′), 6.62 (s, 1H, **H**2′), 5.44 (s, 1H, **H**2), 4.60 (dd, 1H, $^3J_{\beta H\text{-}His\text{-}\alpha H\text{-}His}$ = 7.1 Hz, $^3J_{\beta H'\text{-}His\text{-}\alpha H\text{-}His}$ = 6.0 Hz, α**H** of His), 4.55 (dd, 2H, $^3J_{\beta H\text{-}Pro\text{-}\alpha H\text{-}Pro}$ = 8.3 Hz, $^3J_{\beta H'\text{-}Pro\text{-}\alpha H\text{-}Pro}$ = 5.7 Hz, α**H** of Pro), 3.74-3.66 (m, 1H, δ**H** of Pro), 3.61-3.55 (m, 1H, δ**H** of Pro), 3.33 (s, 6H, **H**1), 3.08 (m, 2H, β**H** of His), 2.36-2.27 (m, 1H, β**H** of Pro), 2.03-1.91 (m, 2H, β**H** of Pro and γ**H** of Pro), 1.91-1.85 (m, 1H, γ**H** of Pro) ppm.

(Spectrum assigned for the major conformer: 20% of a minor conformational isomer was observed.)

^{13}C-NMR (100 MHz, MeOD-d_4) δ 174.27 (**C**8), 172.26 (**C**9), 172.11 (**C**7), 142.16 (**C**3), 137.28 (**C**6), 136.27 (**C**4′), 134.38 (**C**1′), 128.41 (**C**5), 127.87 (**C**4), 118.48 (**C**2′), 104.08 (**C**2), 62.39 (α**C** of Pro), 53.64 (α**C** of His), 53.32 (**C**1), 51.78 (δ**C** of Pro), 30.83 (β**C** of Pro), 30.02 (β**C** of His), 26.31 (γ**C** of Pro) ppm.

Exact mass: Calculated 445.2199; Found: 445.2182 ($M+H^+$).

S3 Set-up and Analysis of Libraries

*S3.1 Libraries with building blocks **1** and **2**, macrocycle **(1·2)$_2$** and 4-methylbenzhydrazide (**3**) or aniline*

A solution of acetic acid (0.5% (v/v)) in $CHCl_3$ (100 ml) was prepared. This solution was used to prepare the following four solutions:

I. Fc-[CO-Val-$NHNH_2$]$_2$ (**1**) and isophthalaldehyde (**2**) (each 1 mM, 5 ml);
II. **(1·2)$_2$** (0.5 mM, 5 ml);
III. 4-methylbenzhydrazide (**4**) (20 mM, 1 ml);
IV. aniline (100 mM, 10 ml).

These four solutions (I-IV), as well as the solution of only 0.5% acetic acid in $CHCl_3$ were then mixed according to the table below to generate six 600 µl libraries, which were stirred for 2 weeks. All solutions were monitored by HPLC-MS after 5 and 11 days using HPLC method A.

Library	Building blocks or **(1·2)$_2$** solutions	Additive	0.5% acetic acid solution
1	300 µl of I	-	300 µl
2	300 µl of I	150 µl of III	150 µl
3	300 µl of I	150 µl of IV	150 µl
4	300 µl of II	-	300 µl
5	300 µl of II	150 µl of III	150 µl
6	300 µl of II	150 µl of IV	150 µl

*S3.2 Libraries with building blocks **4**, **5**, and 4-hydroxybenzhydrazide (**6**)*

The following three solutions were prepared in sodium formate buffer (18 mM, pH 3):

I. building block **4** (1 mM, 10 ml);

II. building block **5** (1 mM, 10 ml);

III. building block **4** (0.5 mM) and building block **5** (0.5 mM) in 10 ml.

Samples of solutions I (2 ml) and II (2 ml) were stirred overnight and then the two were mixed together to form solution IV (4 ml). Four 1 ml libraries were then set up according to the table below. The libraries were analysed by LC/MS after periodically and after 14 days using Method B

Library	Solution	Additive
1	1 ml of III	-
2	1 ml of IV	-
3	1 ml of III	1.2 mg of **6**
4	1 ml of IV	1.2 mg of **6**

1. S. M. Voshell, S. J. Lee and M. R. Gagné, *J. Am. Chem. Soc*., 2006, **128**, 12422-12423.