Derivation of the equation for an equilibrium ratio of products in a dynamic combinatorial library.

Consider a common starting material, SM, in equilibrium with two inhibitors, A and B, which can each bind reversibly to a target, T, to form complexes T¥A and T¥B

\[
\begin{align*}
    \text{SM} & \rightleftharpoons K_{sA} A + T \rightleftharpoons K_{aA} T¥A \\
    B + T & \rightleftharpoons K_{sB} B + T \rightleftharpoons K_{aB} T¥B
\end{align*}
\]

(1)

(2)

Equations 3-5 define respectively \( K_{sA} \), the equilibrium constant for synthesis of inhibitor A, \( K_{aA} \), the equilibrium constant binding of inhibitor A to the target, and \([A_T]\), the total of bound and unbound forms of A.

\[
K_{sA} = \frac{[A]}{[SM]} \quad (3)
\]

\[
K_{aA} = \frac{[T \cdot A]}{[T][A]} \quad (4)
\]

\[
[A_T] = [A] + [T \cdot A] \quad (5)
\]

Combining these equations and solving for \([A_T]\) yields equation 6.
\[ [A_r] = K_{aA}[SM][K_{aA}[T]+1] \quad (6) \]

Dividing this equation by an analogous equation for \([B_r]\) yields equation 7.

\[ \frac{[A_r]}{[B_r]} = \frac{K_{aA}(K_{aA}[T]+1)}{K_{aB}(K_{aB}[T]+1)} \quad (7) \]

Under typical conditions (tight binding and an excess of target at a high concentration), the product of the association constant (\(K_{aA}\) or \(K_{aB}\)) and free target concentration is much greater than one so the equation simplifies to equation 8, below.\(^1\)

\[ \frac{[A_r]}{[B_r]} = \frac{K_{aA} \times K_{aA}}{K_{aB} \times K_{aB}} \quad (8) \]

Characterization Data:

**EtO2C-(4’-SO2NH2)Phe-Phe-O-t-butyl (3a).** Prepared as 3b to afford to afford 173 mg (72%). \(^1\)H NMR (CDCl3) \(\delta\) 7.80 (d, 2H, \(J = 7.8\) Hz), 7.33-7.24 (m, 5H), 7.09 (d, 2H, \(J = 6.0\) Hz), 6.41 (d, 1H, \(J = 5.7\) Hz), 5.19 (d, 1H, \(J = 6.1\) Hz), 4.96 (s, 2H), 4.75-4.61 (m, 1H), 4.52-4.38 (m, 1H), 4.10-4.03 (q, 2H, \(J = 6.9\) Hz), 3.20-2.96 (m, 4H), 1.39 (s, 9H), 1.21 (t, 3H, \(J = 6.8\) Hz). \(^{13}\)C NMR (CDCl3) \(\delta\) 170.34, 170.11, 142.21, 140.84, 136.03, 130.38, 129.65, 128.67, 127.31, 126.96, 82.93, 77.44, 61.76, 53.86, 38.40, 38.21, 28.16, 14.69. Analysis calculated for C\(_{25}\)H\(_{33}\)N\(_3\)O\(_7\)S C, 57.79; H, 6.40; N, 8.09. Found: C, 57.71; H, 6.34; N, 7.96.

**EtO2C-(4’-SO2NH2)Phe-Leu-O-t-butyl (3c).** Prepared as 3b to afford 227 mg (78%). \(^1\)H NMR ((CD\(_3\))\(_2\)CO) \(\delta\) 7.81 (d, 2H, \(J = 8.4\) Hz), 7.55 (m, 2H), 7.48 (d, 2H, \(J = 8.1\) Hz), 6.51 (m, 1H), 6.31 (m, 1H), 4.50 (m, 1H), 4.39 (m, 1H), 4.00-3.95, (q, 2H, \(J = 5.7\)), 3.32-3.26 (dd, 1H, \(J = 13.8\) and 3.9 Hz), 3.05-2.97 (dd, 1H, \(J = 13.6\) and 9.6 Hz), 1.75-1.64 (m, 2H), 1.61-1.56 (m, 2H), 1.45 (s, 9H), 1.12 (t, 3H, \(J = 7.4\) Hz), 0.94-0.90 (m, 7H). \(^{13}\)C NMR ((CD\(_3\))\(_2\)CO) \(\delta\) 171.8, 171.0, 156.4, 82.93, 77.44, 61.76, 53.86, 38.40, 38.21, 28.16, 14.69. Analysis calculated for C\(_{25}\)H\(_{33}\)N\(_3\)O\(_7\)S C, 57.79; H, 6.40; N, 8.09. Found: C, 57.71; H, 6.34; N, 7.96.

\(^1\) If the binding is not tight or the target is not in excess at high concentration, then the concentrations of the inhibitors will be more similar than that discussed in the text above and it will be even harder to distinguish which is the better inhibitor.
Analysis calculated for C_{22}H_{35}N_{3}O_{7}S: C, 54.42; H, 7.26; N, 8.65. Found: C, 54.28; H, 1.27; N, 8.46.

EtO_2C-(4'-SO_2NH_2)Phe-Pro-O-t-butyl (3d). Prepared as 3b to afford 312 mg (67%). ^1H NMR ((CD_3)_2SO) δ 7.69 (d, 2H, J = 7.6 Hz), 7.48 (d, 2H, J = 7.0 Hz), 7.36 (d, 2H, J = 7.2 Hz), 7.28 (s, 2H), 4.36 (m, 1H), 4.19 (m, 1H), 3.85 (t, 2H, J = 6.7 Hz), 3.65 (m, 2H), 2.97-2.94 (dd, 1H, J = 11.7 and 4.2 Hz), 2.82-2.74 (dd, 1H, J = 11.1 and 9.2 Hz), 2.14 (m, 1H), 1.90 (m, 2H), 1.77 (m, 1H), 1.35 (s, 9H), 1.04 (t, 3H, J = 7.3 Hz). ^13C NMR ((CD_3)_2SO) δ 171.6, 170.4, 156.8, 143.0, 142.7, 130.5, 126.1, 81.0, 60.5, 60.1, 54.7, 47.1, 36.4, 29.2, 28.3, 25.3, 15.2. Analysis calculated for C_{18}H_{27}N_{3}O_{7}S: C, 50.34; H, 6.34; N, 9.78. Found: C, 50.33; H, 6.35; N, 9.73.

EtO_2C-(4'-SO_2NH_2)Phe-Phe-OH (4a). Prepared as 4b to afford 270 mg (68%). ^1H NMR (CD_3OD) δ 8.19 (d, 1H, J = 9.3 Hz), 7.80 (d, 2H, J = 8.4 Hz), 7.38 (d, 2H, J = 9.3 Hz), 7.27-7.21 (m, 5H), 7.07 (d, 1H, J = 8.7 Hz), 4.68-4.63 (m, 1H), 4.40-4.35 (q, 2H, J = 6.1 Hz), 3.24-3.18 (dd, 1H, J = 13.9 and 5.2 Hz), 3.18-3.11 (dd, 1H, J = 14.3 and 5.5 Hz), 3.04-2.97 (dd, 1H, J = 13.9 and 8.2 Hz), 2.88-2.80 (dd, 1H, J = 13.9 and 9.7 Hz), 1.18-1.14 (t, 3H, J = 6.9 Hz). ^13C NMR (CD_3CD) δ 173.1, 172.3, 157.2, 142.3, 137.0, 129.8, 129.2, 128.3, 126.3, 126.0, 60.9, 55.9, 53.9, 37.6, 37.2, 13.7. HR-CIMS (m/z): [MH+] calculated for C_{21}H_{26}N_{3}O_{7}S, 464.1491; found, 464.1501.

EtO_2C-(4'-SO_2NH_2)Phe-Leu-OH (4c). Prepared as 4b to afford 174 mg (80%). ^1H NMR (CD_3CD) δ 7.82 (d, 2H, J = 8.4 Hz), 7.68 (d, 1H, J = 8.1 Hz), 7.45 (d, 2H, J = 8.1 Hz), 4.47-4.42 (m, 2H), 4.01-3.95 (q, 2H, J = 6.4 Hz), 3.31-3.29 (m, 1H), 3.25-3.31 (dd, 1H, J = 13.9 and 4.9 Hz), 2.95-2.87 (dd, 1H, J = 13.9 and 9.7 Hz), 1.71-1.62 (m, 2H), 1.18-1.13 (t, 3H, J = 7.1 Hz), 0.97-0.91 (m, 6H). ^13C NMR (CD_3CD) δ 174.6, 172.3, 157.2, 142.3, 137.0, 129.8, 129.2, 128.3, 126.3, 126.0, 60.9, 55.8, 50.9, 40.4, 37.7, 24.8, 22.2, 20.6, 13.7. HR-CIMS (m/z): [MH+] calculated for C_{18}H_{28}N_{3}O_{7}S, 430.1648; found, 430.1654.

EtO_2C-(4'-SO_2NH_2)Phe-Pro-OH (4d). Prepared as 4b except recrystallized from iso-propanol / hexanes to afford 94.5 mg (55%). ^1H NMR (CD_3OD) δ 7.83 (d, 2H, J = 8.1 Hz), 7.50 (d, 2H, J =
= 8.4 Hz), 7.25 (d, 1H, \( J = 3.9 \) Hz), 4.66-4.61 (dd, 1H, \( J = 8.8 \) and 5.5 Hz), 4.47-4.43 (dd, 1H, \( J = 8.4 \) and 3.9 Hz), 4.02-3.95 (q, 2H, \( J = 7.2 \) Hz), 3.80-3.75 (m, 1H), 3.56-3.51 (m, 1H), 3.32-3.29 (m, 1H), 3.21-3.14 (dd, 1H, \( J = 13.9 \) and 5.2 Hz), 2.96-2.89 (dd, 1H, \( J = 13.8 \) and 8.7 Hz), 2.27-2.21 (m, 1H), 2.05-1.96 (m, 2H), 1.19-1.16 (t, 3H, \( J = 7.2 \) Hz. \( \text{\textsuperscript{13}C NMR (CD}_3\text{CD}) \) \( \delta \) 174.0, 171.1, 157.3, 141.9, 130.1, 129.1, 128.2, 126.0, 60.8, 59.4, 53.9, 37.0, 29.0, 24.6, 13.7. HR-CIMS (m/z): \([\text{MH}^+]\) calculated for \( \text{C}_{17}\text{H}_{24}\text{N}_3\text{O}_7\text{S} \), 414.1335; found, 414.1325.