Covalent double level dynamic combinatorial libraries: selectively addressable exchange processes

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MATERIAL AND METHODS

L-Cysteine ethyl ester hydrochlorid 98%, N-acetyl cysteine (4), di-tert-butyl dicarbonate (BOC) and 1-pentanethiol 98% (13) were purchased from Aldrich. 2-Mercaptobenzothiazole (MBT, 11) was purchased from Sigma. Hydrazine monohydrate, trifluoroacetic acid (TFA) and triethylamine (TEA) were purchased from Fluka.

Synthesis of building block 1

\[
\begin{align*}
10 & \xrightarrow{\text{DCM, r.t, 15 h}} 12 \\
12 & \xrightarrow{\text{DCM, r.t, 24 h}} 14 \\
13 & \xrightarrow{\text{SH}} 12 \\
14 & \xrightarrow{\text{TFA}} 15 \\
15 & \xrightarrow{\text{EDC, DMAP, Et3N}} 16 \\
16 & \xrightarrow{\text{DCM, r.t, 13 h}} 1
\end{align*}
\]

The thiol group of L-Boc-N-cysteine ethyl ester (10) (2.80 g, 11.3 mmol) was activated with MBT (11) (3.80 g, 11.3 mmol) in DCM (19 mL). The reaction stirred 15 h at room temperature. The resulting solution was washed with NaOH (2x), water (1x), and dried with Na 2SO4. L-Boc-N-cysteine ethyl ester-S-(1-mercaptobenzothiazole) (12) was purified by column chromatography and was obtained in a 47% yield (2.23 g).

L-Boc-N-cysteine ethyl ester-S-(1-mercaptobenzothiazole) (12)

\[\text{H NMR (300 MHz, CDCl}_3\text{) } \delta = 7.87 (2H, m, CC\text{HCH Ar}), 7.41 (2H, m, CCHC\text{H Ar}), 5.79 (1H, b.d, J = 6.8 Hz, NH), 4.65 (1H, d.t., J_1 = 4.9 Hz, J_2 = 6.8 Hz, H-\alpha), 4.23 (2H, q., J = 7.6 Hz, OCH}_2\text{CH}_3, 3.49 (2H, b.d., J = 4.9 Hz, CHCH}_2\text{S), 1.45 (9H, s., Boc), 1.26 (3H, t., J = 7.6 Hz, OCH}_3\text{CH}_2\text{S).}\]

\[\text{C NMR (75 MHz, CDCl}_3\text{) } \delta = 170.90 (S\text{CCH Ar), 170.15 (ester C=O), 155.12 (SCN Ar), 154.67 (C=O), 135.91 (N\text{CCH)), 126.34, 124.80 (CCHCH}_2\text{Ar), 122.31, 121.18 (CCHCH}, 80.35 ((CH}_3\text{)C), 62.12 (OCH}_2\text{CH}_3, 53.13 (C-\alpha), 42.16 (CCHCH}_3, 28.29 ((CH}_3\text{)C), 14.07 (OCH}_2\text{CH}_3).}\]

\[\text{HRMS-ESI calculated for C}_{17}\text{H}_{23}\text{N}_2\text{O}_4\text{S}_3\text{ [M+H]^+ 415.0820, found 415.0821.}\]

12 (265 mg, 0.62 mmol) and 1-pentanethiol (13) (80 µL, 0.62 mmol) were mixed in DCM (3 mL). The reaction was stirred for 24 h at room temperature. The resulting solution was diluted at 30 mL with DCM, washed with NaOH (2x) and water (2x), and dried with Na 2SO4. L-Boc-N-cysteine ethyl ester-S-(1-pentanethiol) (14) was purified by column chromatography and was obtained in a 94% yield (209 mg).

L-Boc-N-cysteine ethyl ester-S-(1-pentanethiol) (14)

\[\text{H NMR (300 MHz, CDCl}_3\text{) } \delta = 5.36 (1H, b.d., J = 7.0 Hz, NH), 4.59 (1H, d.t., J_1 = 5.4 Hz, J_2 = 7.0 Hz, CH-\alpha), 4.23 (2H, q., J = 7.1 Hz, OCH}_2\text{CH}_3, 3.15 (2H, d.d.d., J_1 = 5.4 Hz, J_2 = 5.4 Hz, J_3 = 13.5 Hz, CHCH}_2\text{S), 2.71 (2H, t., J = 7.4 Hz, S\text{CH}_2\text{CH}_3, 1.67 (2H, m, J = 7.4 Hz, S\text{CH}_2\text{CH}_3, 1.47 (9H, s., (CH}_3\text{)C), 1.36 (4H, m, CH}_2\text{CH}_2\text{CH}_3, 1.31 (3H, t., J = 7.2 Hz, CH}_2\text{CH}_2\text{CH}_3, 0.91 (3H, t., J = 7.2 Hz, OCH}_2\text{CH}_3).}\]

\[\text{C NMR (75 MHz, CDCl}_3\text{) } \delta = 170.81 (ester C=O),}\]
155.07 (carbamate C=O), 80.10 ((CH₃)₃C), 61.73 (OCH₂CH₃), 53.10 (C-α), 41.28 (CH₃S), 39.00 (SCH₂CH₂), 30.61 (SC₂H₂CH₂), 28.72 (CH₂CH₂CH₃), 28.30 ((CH₃)₃C), 22.26 (CH₂CH₂CH₃), 14.11 (CH₂CH₂C), 13.92 (OCH₂C).


14 was deprotected following conventional methods (91% yield). L-cysteine-S-(1-pentanethiol) (15) (195 mg, 0.77 mmol) was mixed with 4-dimethoxymethylbenzoic acid (16) (166 mg, 0.85 mmol), EDC (177 mg, 0.92 mmol), DMAP (9.76 mg, 0.08 mmol) and TEA (118 μL, 0.85 mmol) in DCM (5 mL). The reaction was stirred 13 h at room temperature. The resulting solution was diluted to 50 mL with DCM, washed with NaHCO₃ (2x), HCl (2x) and water (1x), and dried with Na₂SO₄. 4-dimethoxymethylbenzoic-L-cysteine-S-(1-pentanethiol) (1), was purified by column chromatography and was obtained in a 61% yield (202 mg).

4-dimethoxymethylbenzoic-L-cysteine-S-(1-pentanethiol) (1)

A NMR (300 MHz, CDCl₃) δ = 7.85 (2H, d., J = 8.5 Hz, CHCC=O Ar), 7.56 (2H, d., J = 8.5 Hz, CHCC=O Ar), 7.07 (1H, b.d., J = 7.2 Hz, H-α), 5.46 (1H, s., (CH₃O)₂CH), 5.10 (1H, d.t., J₁ = 5.2 Hz, J₂ = 7.2 Hz, H-α), 4.29 (2H, q., J = 7.1 Hz, OCH₂CH₃), 3.34 (6H, s., CH₃O), 3.34 (2H, d.d.d., J₁ = 4.9 Hz, J₂ = 4.9 Hz, J₃ = 14.1 Hz, CHC₂H₂S), 2.71 (2H, t., J = 7.6 Hz, SCH₂CH₂), 1.66 (2H, q., J = 7.3 Hz, SCH₂CH₂), 1.35 (4H, m., CH₂CH₂CH₃), 1.34 (3H, t., J = 7.1 Hz, OCH₂CH₃), 0.89 (3H, t., J = 7.1 Hz, OCH₂CH₃).

13C NMR (75 MHz, CDCl₃) δ = 170.51 (ester C=O), 166.70 (amide C=O), 141.88 (C=ONH), 133.75 ((CH₃O)₂C), 127.07 (4 CH Ar), 102.26 ((CH₃O)₂CH), 62.00 (CH₂CH₂CH₃), 52.58 (CH₂O + C-α), 40.51 (CH₂CH₂), 38.97 (SCH₂CH₂), 30.55 (SCH₂CH₂), 28.64 (CH₂CH₂CH₃), 22.22 (CH₂CH₂CH₃), 14.11 (CH₂CH₂CH₃), 13.88 (OCH₂CH₃).

HRMS-ESI calcd for C₂₀H₃₂NO₅S₂ [M+H]+ 430.1722, found 430.1730.

Synthesis of building blocks 2 and 3

Phenylacetic hydrazide (2)

A NMR (300 MHz, CDCl₃) δ = 7.37 (2H, d., J = 8.5 Hz, CHCC=O Ar), 7.35 (2H, t., CHCC=O Ar), 7.27 (1H, t., J = 8.5 Hz, CHCC=O Ar), 6.67 (1H, b.s., NH), 3.88 (2H, b.s., NH₂), 3.59 (2H, s., CH₂C=O Ar).

13C NMR (75 MHz, CDCl₃) δ = 171.69 (C=O), 134.99 (CH₂C), 127.07 (4 CH Ar), 102.26 ((CH₃O)₂CH₂), 62.00 (CH₂CH₂), 52.58 (CH₂O + C-α), 40.51 (CH₂CH₂), 38.97 (SCH₂CH₂), 30.55 (SCH₂CH₂), 28.64 (CH₂CH₂CH₃), 22.22 (CH₂CH₂CH₃), 14.11 (CH₂CH₂CH₃), 13.88 (OCH₂CH₃).


Benzoic hydrazide (3)

A NMR (300 MHz, CDCl₃) δ = 7.76 (2H, d.d., J₁ = 7.6 Hz, J₂ = 1.6 Hz, CHCHCH), 7.51 (2H, m., CHCH), 7.44 (1H, t., J = 1.6 Hz, CHCHCH), 7.42 (1H, b.s., NH), 4.13 (b.s., NH₂).

13C NMR (75 MHz, CDCl₃) δ = 131.75 (CHCHCH=O), 127.85 (2 CHCHCH), 128.34 (2 CHCH), 126.80 (C), 40.89 (CH₂).
HPLC ANALYSIS

HPLC was performed using a Hewlett-Packard 1050 instrument, coupled to a HP 1050 DAD. Acetonitrile was acquired from Tedia and formic acid from Merck. The water used was Milli Q.

HPLC Parameters
Injection volume: 5 μL
Flow rate: 1.000 mL/min
Column: Waters Symmetry C18, 250 × 4.6 mm, 5 μm.
Mobile phase: MilliQ water with 0.1% formic acid (solvent A) and acetonitrile:milliQ water (95:5) with 0.05% formic acid (solvent B)

Gradient elution

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LC data for preparation of libraries starting with hydrazone exchange followed by disulfide exchange and vice versa

Figure S1.
Chromatogram of a mixture made of aldehyde/disulfide 1, phenyl acetic hydrazide (2), bencil hydrazide (3) and N-acetyl cysteine (4), each one 5mM in chloroform with 40 eq of TFA (1 day) and alkalised with 43 eq of TEA (3 days, a) and chromatogram of a mixture made of aldehyde/disulfide 1, benzyhydrazide (2), phenylhydrazide (3) and N-acetyl cysteine (4), each one 5mM in chloroform with 3 eq of TEA (3 days), and acidified with 40 eq of TFA (1 day, b).

HPLC peaks were assigned by preparation of the individual components of the mixture. The presence of the expected compounds in the mixture was confirmed by ESI-MS using a Varian 1200 instrument (positive mode).
The procedure for the exchange experiments entailed dissolution of the building blocks 1 and 2 (5 mM) in CHCl₃ containing TFA (75 mM). The reaction was stirred at room temperature for 24 h. TEA (appropriate amount) followed by one equivalent of building block 3 were added and the reaction was kept stirring at room temperature for another 24 hours and analyzed by HPLC. a) 15 equivalents of TFA. b) 15 equivalents of TFA and 5 equivalents of TEA. c) 15 equivalents of TFA and 10 equivalents of TEA. d) 15 equivalents of TFA and 15 equivalents of TEA. e) 15 equivalents of TFA and 20 equivalents of TEA. f) 15 equivalents of TFA and 30 equivalents of TEA.
HPLC chromatograms of the mixtures used to elaborate Figure 2

The procedure involved dissolution of the building blocks 1 and 2 (5 mM) in CHCl₃ containing TFA (75 mM). The reaction was stirred at room temperature for 24 h. TEA (appropriate amount) followed by one equivalent of building block 4 were added and the reaction was kept stirring at room temperature for another 24 hours and analyzed by HPLC. a) 15 equivalents of TFA. b) 15 equivalents of TFA and 5 equivalents of TEA. c) 15 equivalents of TFA and 10 equivalents of TEA. d) 15 equivalents of TFA and 15 equivalents of TEA. e) 15 equivalents of TFA and 20 equivalents of TEA. f) 15 equivalents of TFA and 30 equivalents of TEA.