Electronic Supplementary Information

Fast and efficient MCR-based synthesis of clickable Rhodamine tags for protein profiling

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**Synthesis of Ugi-reactive PEG linkers**

\[
\begin{align*}
\text{HO} & \quad (\text{O})_n \quad \text{O} \quad \text{OH} \\
\text{a} & \quad \downarrow \\
\text{N}_3 & \quad (\text{O})_n \quad \text{O} \quad \text{N}_3 \\
\text{b} & \quad \downarrow \\
\text{N}_3 & \quad (\text{O})_n \quad \text{O} \quad \text{NH}_2 \\
\text{c,d} & \quad \downarrow \\
\text{N}_3 & \quad (\text{O})_n \quad \text{O} \quad \text{NC} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia: n=2</td>
<td>93%</td>
</tr>
<tr>
<td>Ib: n=3</td>
<td>84%</td>
</tr>
<tr>
<td>Ic: n=4</td>
<td>87%</td>
</tr>
<tr>
<td>IIa: n=2</td>
<td>77%</td>
</tr>
<tr>
<td>IIb: n=3</td>
<td>43%</td>
</tr>
<tr>
<td>IIc: n=4</td>
<td>38%</td>
</tr>
<tr>
<td>IIIa: n=2</td>
<td>42%</td>
</tr>
<tr>
<td>IIIb: n=3</td>
<td>67%</td>
</tr>
<tr>
<td>IIIc: n=4</td>
<td>62%</td>
</tr>
</tbody>
</table>

\(^a\) Reagents and Conditions: (a) MsCl, Et\(_3\)N, THF, 0\(^\circ\)C/1h, RT/3h; Na\(_2\)N\(_3\), NaHCO\(_3\)
H\(_2\)O, 80\(^\circ\)C. (b) H\(_3\)PO\(_4\) (0.65 M), PPh\(_3\)/Et\(_2\)O, RT/24h. (c) ethyl formiate, reflux, 3h.
(d) POCl\(_3\), diisopropylamine, CH\(_2\)Cl\(_2\), RT/2h.

**General procedure A: Synthesis of diazido-PEG compounds (Ia-c).** Under a nitrogen atmosphere triethylamine (2.2 equiv.) dissolved in dry THF (20 mL) was added to a solution of glycol (n = 2, 3 or 4) and methanesulfonyl chloride (2.2 equiv.) dissolved in dry THF (80 mL) at 0 \(^\circ\)C. The resulting solution was stirred for 1 hour at 0 \(^\circ\)C and for another 3 hours at room temperature. Next, dest. H\(_2\)O (80 mL), solid sodium hydrogen carbonate (0.54 equiv.) and sodium azide (2.2 equiv.) were added to the reaction mixture. The organic solvent was removed under reduced pressure and the remaining aqueous solution was heated at 80 \(^\circ\)C during 12 hours. Afterwards the aqueous phase was extracted with dichloromethane (4 x 200 mL) and the combined extracts were dried over Na\(_2\)SO\(_4\). The organic solvent was removed \textit{in vacuo} to yield the diazido-PEG compounds Ia-c, which were stored at -30 \(^\circ\)C.
General procedure B: Staudinger reduction. Under a nitrogen atmosphere a suspension of the diazido-PEG in 0.65 M phosphoric acid (100 mL) was cooled to 0 °C. Triphenylphosphine (0.86 equiv.) dissolved in dry ether (100 mL) was added dropwise. After complete addition the reaction mixture was allowed to stir at room temperature for an additional 24 hours. The organic phase was separated and the aqueous phase was washed with ether (3 x 100 mL). To the aqueous phase solid potassium hydroxide (3.5 equiv.) was added and traces of organic solvent were removed under reduced pressure. The remaining aqueous solution was placed at 0 °C for 24 hours, after which a solid had formed, which was removed by filtration. The filtrate was transferred in a 4 M NaOH solution and extracted with dichloromethane (16 x 50 mL). The combined organic layers were dried over Na₂SO₄ and the solvent was removed under reduced pressure. The residue was purified on silica (CH₂Cl₂/MeOH/Et₃N 8:1:1) to yield the amine as a light yellow liquid, which was subsequently stored at -30 °C.

General procedure C: Conversion of an amine into its corresponding isocyanide. A solution of amine in ethyl formiate (20 mL) was refluxed for 3 hours, followed by the evaporation of all volatiles to yield the corresponding formamide as a slightly red liquid. The liquid was dissolved in dry dichloromethane (60 mL) and placed under a nitrogen-atmosphere. Diisopropylamine (3 equiv.) was added and the reaction mixture was cooled to 0 °C. After the dropwise addition of phosphorus oxychloride (1.2 equiv.) the reaction mixture was warmed to room temperature and allowed to stir for an additional 2 hours. The reaction was quenched with a sodium carbonate solution (6.5 g in 35 mL dest. H₂O). The resulting suspension was stirred for further 30 minutes after which the mixture was diluted with water and the organic layer was separated. The aqueous phase was extracted with dichloromethane (3 x 40 mL). The combined organic layers were dried over Na₂SO₄ and the organic solvent was evaporated in vacuo. The remaining residue was purified by column chromatography.
1-Azido-2-[2-(2-azidoethoxy)ethoxy]ethane (Ia). Starting from tetraethylene glycol (17.3 mL, 100 mmol) Ia was obtained according to General Procedure A as a slightly yellow liquid (22.76 g, 93%). $^1$H-NMR (300 MHz, CDCl$_3$) δ: 3.32 (m, 4H), 3.61 (m, 12 H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ: 50.4, 69.8, 70.4.

2-[2-[2-(Azidoethoxy)ethoxy]ethoxy]ethanamine (IIa). A suspension of Ia (21.7 g, 88.8 mmol) in 0.65 M phosphonic acid (210 mL) was treated with triphenylphosphine as described in General Procedure B to yield after purification by column chromatography (CH$_2$Cl$_2$/MeOH/Et$_3$N 8:1:1) the desired amine IIa as a yellow liquid (14.99 g, 77%). TLC (CH$_2$Cl$_2$/MeOH/Et$_3$N 8:1:1) R$_f$ = 0.64; $^1$H-NMR (300 MHz, CDCl$_3$) δ: 2.44 (s, 2H), 2.87 (t, $^3$J = 5.3 Hz, 2H), 3.61 (m, 2H), 3.53 (m, 2H), 3.67 (m, 10 H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ: 41.4, 50.4, 61.1, 69.8, 70.3, 70.4, 71.0, 72.4, 72.9; ESI-MS m/z: 219.2 [M+H]$^+$. 

1-Azido-2-[2-(2-isocyanoethoxy)ethoxy]ethane (IIIa). Amine IIa (7.5 g, 34.4 mmol) was transformed into its corresponding isocyanide according to General Procedure C to yield compound IIIa as a yellow liquid (3.25 g, 41%) after column chromatography (EtOAc/Hex 4:1). TLC (EtOAc/Hex 4:1) R$_f$ = 0.67; $^1$H-NMR (300 MHz, CDCl$_3$) δ: 3.40 (m, 2H), 3.58 (m, 2H), 3.66-3.74 (m, 12H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ: 41.6, 50.5, 68.5, 69.9, 70.5, 70.6, 70.7.

1,14-Diazido-3,6,9,12-tetraoxatetradecane (Ib). Starting from pentaethylene glycol (20.0 g, 83.9 mmol) Ib was obtained according to General Procedure A as a slightly yellow liquid (20.29 g, 83%). $^1$H-NMR (300 MHz, CDCl$_3$) δ: 3.39 (m, 4H), 3.67 (m, 16 H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ: 50.5, 69.9, 70.5, 70.6.

14-Azido-3,6,9,12-tetraoxatetradecan-1-amine (IIb). A suspension of Ib (16.6 g, 57.6 mmol) in 0.65 M phosphonic acid (180 mL) was treated with triphenylphosphine as described in General Procedure B to yield after purification by column chromatography
(CH$_2$Cl$_2$/MeOH/ Et$_3$N 8:1:1) the desired amine IIb as a yellow liquid (6.49 g, 43%). TLC (CH$_2$Cl$_2$/MeOH/ Et$_3$N 8:1:1) $R_f$ = 0.58; $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$: 2.17 (s, 2H), 2.88 ($^3J = 5.3$ Hz, 2H), 3.40 (m, 2H), 3.53 (m, 2H), 3.68 (m, 14 H); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$: 41.6, 50.6, 70.0, 70.2, 70.5, 70.6, 70.7, 73.0; ESI-MS m/z: 263.2 [M+H]$^+$. 

1-Azido-14-isocyano-3,6,9,12-tetraoxatetradecane (IIIb). Amine IIb (4.0 g, 15.3 mmol) was transformed into its corresponding isocyanide according to General Procedure C to yield IIIb as a yellow liquid (2.78 g, 67%) after column chromatography (EtOAc/petrol ether 4:1). TLC (EtOAc/petrol ether 4:1) $R_f$ = 0.26; $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$: 3.39 ($^3J = 5.3$ Hz, 2H), 3.58 (m, 2H), 3.67 (m, 16H); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$: 41.7, 50.6, 68.5, 69.9, 70.5, 70.6, 70.8. ESI-MS m/z: 273.2 [M+H]$^+$, 295.2 M+Na$^+$. 

1,17-Diazido-3,6,9,12,15-pentaoxaheptadecane (Ic). Starting from commercially available hexaethylene glycol (13 mL, 46.6 mmol) diazide Ic was obtained according to General procedure A as slightly yellow liquid (14.35 g, 87%). $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$: 3.39 ($^3J = 5.0$ Hz, 4H), 3.67 (m, 20 H); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$: 50.6, 70.0, 70.5, 70.6. 

17-Diazido-3,6,9,12,15-pentaoxaheptadecan-1-amine (IIc). A suspension of Ic (13.33 g, 40.1 mmol) in 0.65 M phosphonic acid (100 mL) was treated with triphenylphosphine as described in General Procedure B to yield after purification by column chromatography (CH$_2$Cl$_2$/MeOH/ Et$_3$N 8:1:1) the desired amine IIc as a light yellow liquid (4.61 g, 38%). TLC (CH$_2$Cl$_2$/MeOH/ Et$_3$N 8:1:1) $R_f = 0.57$; $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$: 1.92 (s, 2H), 2.88 ($^3J = 5.3$ Hz, 2H), 3.40 (t, $^3J = 5.3$ Hz, 2H), 3.53 (t, $^3J = 5.3$ Hz, 2H), 3.67 (m, 18 H); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$: 41.6, 50.6, 69.9, 70.2, 70.5, 70.6, 73.1; ESI-MS m/z: 307.0 [M+H]$^+$. 

1-Azido-17-isocyano-3,6,9,12,15-pentaoxaheptadecane (IIIc). Amine IIc (3.9 g, 12.7 mmol) was transformed into its corresponding isocyanide according to General Procedure C.
to yield compound IIIc as a yellow liquid (2.38 g, 63%) after column chromatography (EtOAc/Hex 4:1). TLC (EtOAc/Hex 4:1) $R_f = 0.30$; $^1$H-NMR (300 MHz, CDCl$_3$) $\delta$: 3.39 (m, 2H), 3.58 (m, 2H), 3.66-3.74 (m, 20H); $^{13}$C-NMR (75 MHz, CDCl$_3$) $\delta$: 41.7, 50.6, 68.6, 70.0, 70.5, 70.6, 70.8; ESI-MS m/z: 317.2 [M+H]$^+$, 339.2 M+Na$^+$.
- $^1$H and $^{13}$C spectra of Ugi-reactive PEG linkers

- Compound Ia
**Compound IIa**

![Graph showing spectral data for Compound IIa.](image)
- Compound IIIa
- Compound Ib

![Chemical Shift Graph](image)

![Chemical Shift Graph](image)
- Compound IIb

**Chemical Shift (ppm)**

- 3.70
- 3.88
- 4.06
- 4.44
- 5.76

**Normalized Intensity**

- 3.39
- 2.05
- 2.01
- 2.08
- 4.43

**Chemical Shift (ppm)**

- 6.00
- 7.86
- 7.99
- 8.01
- 8.05

**Normalized Intensity**

- 41.63
- 50.64
- 69.97
- 70.23
- 70.66
- 73.04
- 76.57
- 76.99
- 77.42
- Compound IIIb
- Compound Ic
- Compound IIc
- Compound IIIc
- $^1$H and $^{13}$C spectra of Ugi-modified rhodamine dyes
  - Compound 1
- **Compound 2**

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### Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

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- Compound 3

![Graph showing chemical shift and normalized intensity]
- Compound 4a

![NMR Spectra of Compound 4a](image1)

![NMR Spectra of Compound 4a](image2)
- Compound 4b

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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- Compound 6

![NMR spectrum of Compound 6](image)
- Compound 7

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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- **Compound 8**

![Graph 1](attachment:BRS391_20110427_01.png)

![Graph 2](attachment:BRS391_20110427_01.png)