Cu(I)-catalysed N-H insertion in water: A new tool for chemical biology

Supporting Information

Kiril Tishinov, Na Fei, and Dennis G. Gillingham*

Department of Chemistry, University of Basel, 4056 Basel, Switzerland

* To whom correspondence should be addressed. Email: dennis.gillingham@unibas.ch
Table of contents

General experimental information S3
Screening for metal catalyst for ON alkylation S4
Aniline double alkylation with copper carbenoids S6
Chemical synthesis and characterization data S7-S47
Oligonucleotide synthesis and purification S48
Copper(I)-Catalyzed Alkylation of Oligonucleotides S49-S61
Copper(I)-Catalyzed Oligonucleotide Alkylation in the Presence of Proteins S62
Copper(I)-Catalyzed Tandem ‘Click’/Alkylation Reactions S63-S69
References S70
General

All reagents and solvents used were of analytical grade. Buffers were prepared with ultrapure water. All chemicals were purchased from Sigma-Aldrich, Fluka or Acros and were used as received. Analytical TLC was performed on Silica gel 60 F254 pre-coated aluminium sheets. Flash chromatography was performed on Silica gel 60 40-63 μm (230-400 mesh) (SiliCycle, Quebec). Solid-phase oligonucleotide synthesis was carried out on an Expedite 8909 nucleic acid synthesis system (PerCeptive Biosystems). 1H and 13C NMR spectra were acquired on a Bruker AvanceIII+ 500 MHz and Bruker AvanceIII 400 MHz spectrometers at 298 K. Chemical shifts relative to TMS were referenced to the solvent’s residual peak and are reported in ppm. ESI MS spectra were obtained on a Bruker Esquire3000plus spectrometer by direct injection in positive polarity of the ion trap detector. High resolution mass spectra were acquired on a Bruker maXis 4G QTOF ESI mass-spectrometer. MALDI TOF analyses were carried out on a Bruker Microflex mass-spectrometer in linear negative mode using 3-hydroxypicolinic acid/ammonium citrate, pH 6.0 or 2,4,6-trihydroxyacetophenone/ammonium citrate, pH 6.0 matrices. IR spectra were obtained on a Bruker ALPHA FT-IR spectrometer. Analytical HPLC analyses were carried out on an Agilent 1100 LC system equipped with a Chromolith Performance RP18e 4.6 × 100 mm column (Merck) using methods A or B, or with an Eclipse XDB-C18 4.6 × 150 mm column (Agilent), using method C. Method A: flow rate 1 ml/min, 100 mM triethylammonium acetate (pH 7.3)/acetonitrile gradient 0-16 % acetonitrile in 12 min, 16-80 % acetonitrile in 3 min, 80 % acetonitrile in 2 min. Method B: flow rate 1 ml/min, 100 mM triethylammonium acetate (pH 6.0)/acetonitrile gradient as in method A. Method C: flow rate 1 ml/min, 100 mM triethylammonium acetate (pH 7.3)/acetonitrile gradient 0-16 % acetonitrile in 18 min, 16-80 % acetonitrile in 4.5 min, 80 % acetonitrile in 3 min. In all methods detection was done by monitoring the absorbance of the column effluent at 254 nm. UPLC-MS was carried out on a Agilent 1290 Infinity system, equipped with a Zorbax Eclipse Plus C18 2.1 × 50 mm column (Agilent), coupled to an Agilent 6130 Quadrupole LC/MS. Elution was done with 0.1 % trifluoroacetic acid in water/acetonitrile system at a flow rate of 0.2 ml/min using method D: 0-54 % acetonitrile in 4 min, 54-95 % acetonitrile in 1 min, 95 % acetonitrile in 1 min, detection at 230 and 254 nm, and with ESI-MS in positive ion mode of the ion trap.
Table S1 | Screening for metal catalyst for ON alkylation

<table>
<thead>
<tr>
<th>Entry</th>
<th>Metal</th>
<th>Metal source</th>
<th>Number of alkylation products&lt;sup&gt;[a]&lt;/sup&gt;</th>
<th>ON conversion (%)&lt;sup&gt;[b]&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cu(II)</td>
<td>CuSO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>Ag(I)</td>
<td>AgBF&lt;sub&gt;4&lt;/sub&gt;</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Co(II)</td>
<td>CoCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Ni(II)</td>
<td>NiCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Fe(III)</td>
<td>FeCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

**Reaction conditions**: 5 mM oligonucleotide, 1 mM metal source, 50 mM methyl 2-diazo-2-(4-(((dimethylamino)methyl)phenyl)acetate, 100 mM MES, pH 6, 24 h, room temperature.

<sup>[a]</sup> Determined by HPLC-analysis of the reaction mixture.  
<sup>[b]</sup> Amount of the modified species as a percentage of the total amount of ON.
Figure S1 | HPLC traces of the screening for metal catalyst for ON alkylation using method A (see Table S1). (a) Starting oligonucleotide (b) Modified products (c) Non-converted diazo substrate
### Table S2 | Aniline double alkylation with copper carbenoids

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar</th>
<th>Ratio products 3:4[^a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Ar1" /></td>
<td>97:3</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="Ar2" /></td>
<td>94:6</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="Ar3" /></td>
<td>98:2</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Ar4" /></td>
<td>99:1</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Ar5" /></td>
<td>99:1</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Ar6" /></td>
<td>99:1</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Ar7" /></td>
<td>99:1</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Ar8" /></td>
<td>99:1</td>
</tr>
</tbody>
</table>

[^a]: Determined as ratios of the integration areas for the 230-nm absorbance peaks in the UPLC-MS analyses of the crude reaction mixtures.
Figure S2 | UPLC trace of the Cu(I)-catalyzed alkylation reaction of a series of \( p \)-substituted anilines with Dz-1 (Sections A-F correspond to entries 1-6 in Table S2). The singly (S) and doubly alkylated (D) anilines were identified by ESI-MS
Figure S3 | UPLC trace of the Cu(I)-catalyzed alkylation reaction of a series of 2- and 3-aminopyridine with Dz-1 (Sections A and B correspond to entries 7 and 8 in Table S2). The singly (S) and doubly alkylated (D) anilines were identified by ESI-MS.
Chemical syntheses

Synthesis of Diazo Substrates

The synthesis and characterization of the dimethylamino diazo substrate has already been described.1

Methyl 2-diazo-2-(4-(piperazin-1-ylmethyl)phenyl) acetate (6a). 4-(Bromomethyl)- phenylacetic acid (0.4 g, 1.75 mmol) was mixed with dry methanol (3 ml) and dry benzene (3 ml) under nitrogen. Trimethylsilyldiazomethane (2 mol/l in hexanes) was added drop-wise at continuous stirring until stable yellow color of the solution was obtained (the reaction vessel should be vented as gas evolution takes place). The mixture was stirred for 15 min at room temperature, then evaporated and dried under high vacuum to yield the ester as a colorless oil. The oil was dissolved in dry acetonitrile (5 ml), and added to a stirring mixture of N-Boc-piperazine (0.335 g, 1.8 mmol) and triethylamine (0.182 mg, 1.8 mmol) in 10 ml of dry acetonitrile under nitrogen. The mixture was stirred until completed as judged by TLC (30 min). The volatiles were removed under vacuum, and the residue was dissolved in dichloromethane (20 ml), washed with 2 × 20 ml of saturated sodium bicarbonate, dried with anhydrous sodium sulfate, evaporated and dried under high vacuum. It was then mixed with p-acetamidobenzenesulfonyl azide (0.462 g, 1.93 mmol) in 10 ml of dry acetonitrile under nitrogen. DBU (0.297 g, 1.93 mmol) was added to the solution with continuous stirring and the mixture was stirred for 24 h at room temperature. The mixture was then evaporated under vacuum and the residue purified by flash chromatography on Si60 in DCM/methanol to afford a yellow-orange oil. The oil was dissolved in dry dichloromethane (10 ml), mixed with freshly filtered through a short pad of Al2O3 2,6-lutidine (0.811 g, 7.6 mmol), and cooled on ice. Trimethylsilyl trifluoromethanesulfonate (0.85 g, 3.8 mmol) was introduced drop-wise and the mixture was stirred until complete as judged by TLC. It was then evaporated to dryness and the residue was purified by column chromatography on Si60 in dichloromethane/methanol to yield the final product as a yellow-brown solid (87 %). 1H NMR (500 MHz, CD3CN) δ/ppm: 7.50 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 3.81 (s, 3H), 3.73 (s, 2H), 3.27 – 3.21 (m, 4H), 2.87 – 2.76 (m, 4H). 13C NMR (126 MHz, CD3CN) δ/ppm: 165.41, 132.78, 130.11, 125.75, 123.98, 60.95, 51.73, 48.78, 43.57. MS (ESI) calc. for C14H19N4O2+: (M + H)+, 275.22; Found: (M + H)+, 275.0.
Cu(I)-catalysed N-H insertion in water

Tishinov, Fei & Gillingham

Electronic Supplementary Material (ESI) for Chemical Science
This journal is © The Royal Society of Chemistry 2013
Methyl 2-diazo-2-(4-((4-(prop-2-yn-1-yl)piperazin-1-yl)methyl)phenyl) acetate (6b). Compound 6a (80 mg, 0.29 mmol) was mixed with N,N-diisopropylethylamine (0.113 mg, 0.86 mmol) and 80 % (w/w) propargyl bromide in toluene (86 mg solution, 0.58 mmol) under nitrogen. The mixture was stirred until complete as judged by TLC (ca. 3 h). The reaction mixture was evaporated and the residue was purified by column chromatography on Si60 in dichloromethane/methanol to yield a red-orange oil (62 %). TLC (DCM-methanol 20:1 v/v) R_f=0.43. IR (film) 2810, 2084, 1702, 1512, 1435, 1150, 1009, 909, 730 cm^{-1}. ^1H NMR (500 MHz, CDCl_3) δ/ppm: 7.42 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 8.5 Hz, 2H), 3.86 (s, 3H), 3.50 (s, 2H), 3.29 (d, J = 2.5 Hz, 2H), 2.55 (d, J = 4.4 Hz, 8H), 2.24 (t, J = 2.4 Hz, 1H). ^13C NMR (126 MHz, CDCl_3) δ/ppm: 165.74, 136.03, 129.79, 124.04, 123.94, 78.94, 73.14, 62.45, 52.93, 52.02, 51.93, 46.83. HRMS (ESI) calc. for C_{17}H_{21}N_{4}O_{2}: (M + H)^+ , 313.1665; Found: (M + H)^+, 313.1659.
Prop-2-yn-1-yl 2-diazo-2-(4-((dimethylamino)methyl)phenyl) acetate (8). 4-(Bromomethyl)phenylacetic acid (0.117 g, 0.51 mmol) was mixed with propargyl alcohol (0.084 g, 1.5 mmol) and 1,2-dichloroethane (0.5 ml) were mixed in a 4-ml screw top vial with a teflon cap. A drop of concentrated sulfuric acid was added and the vial was closed tightly and heated at 80°C for 1 h. The mixture was then diluted with 3 ml of dichloromethane, washed with 2 × 4 ml of saturated sodium bicarbonate, dried with anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue was passed through a small pad of Si60 in dichloromethane/methanol 100:1 and the first fraction was evaporated and dried under high vacuum. The residue was mixed with ethanol (2 ml) and 4 ml of 5.6 M dimethyamine in ethanol were added. The mixture was kept for 1 h at room temperature, then taken up to dryness, re-dissolved in 5 ml of dichloromethane, washed with 2 × 5 ml of saturated sodium carbonate solution, then dried with anhydrous sodium sulfate, taken up to dryness and dried under high vacuum. The residue was dissolved in dry acetonitrile (5 ml) under nitrogen, and p-acetamidobenzenesulfonyl azide (0.135 g, 0.56 mmol) and DBU (0.154 g, 1.0 mmol) were then added, and the mixture was stirred for 24 h at room temperature. The mixture was then evaporated under vacuum and the residue purified by flash chromatography on Si60 in dichloromethane/methanol to afford a yellow-orange oil (55%). TLC (DCM-methanol 4:1 v/v) Rf=0.25. IR (film) 2768, 2084, 1701, 1513, 1336, 1240, 1144, 808 cm⁻¹. ¹H NMR (500 MHz, CD₃CN) δ/ppm: 7.44 (d, J = 8.5 Hz, 2H), 7.34 (d, J = 8.7 Hz, 2H), 4.86 (d, J = 2.5 Hz, 2H), 3.38 (s, 2H), 2.82 (t, J = 2.5 Hz, 1H), 2.16 (s, 6H). ¹³C NMR (126 MHz, CD₃CN) δ/ppm: 164.33, 137.60, 129.49, 123.96, 123.75, 77.93, 75.51, 63.19, 52.12, 44.53. HRMS (ESI) calc. for C₁₇H₂₂N₃O₂⁺: (M + H)⁺, 258.1243; Found: (M + H)⁺, 258.1237.
Methyl 2-(4-azidophenyl)-2-diazoacetate

To an ice cold solution of 4-aminophenylacetic acid (14, 2.0 g, 13.2 mmol) in concentrated HCl (20 mL) was added an aqueous solution of NaNO₂ (13.3 mmol, 0.92 g in 70 mL water) slowly with stirring. After 15 min, an aqueous solution of NaN₃ (132 mmol, 8.6 g in 200 mL of water) was added at 0 °C over a period of 15 min after which the reaction mixture was stirred at 25 °C for 15-20 min. The reaction mixture was extracted with EtOAc (3×50 mL), the organic phase was separated and dried over Na₂SO₄, and the solvent was removed in vacuum to give brown crystals (93%). The 2-(4-azidophenyl) acetic acid (0.21g, 1.18mmol) was dissolved in 7ml benzene/methanol (1:1) under nitrogen and 0.6 ml 2M TMS-CH₂N₂ in diethyl ether was added drop-wise at continuous stirring until the pale orange color of the solution persisted. During the addition of the reagent gas evolution took place so the reaction mixture was vented with a needle. The mixture was stirred for another 15 min, later evaporated to dryness. It was partitioned with DCM and 1M HCl, the organic layer was wash with saturated Na₂CO₃, and dried over MgSO₄, and evaporated to a colorless oil (88%). 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (0.164ml) in THF (0.8 ml) was added to a solution of the methyl arylacetate (0.186g, 1 mmol) and p-acetamidobenzenesulfonylazide (p-ABSA) (0.264g, 1mmol) in THF (2 ml) over 2 min at room temperature. The reaction mixture was stirred for an additional 13 h, later quenched by addition of saturated aqueous ammonium chloride. The aqueous layer was extracted with ethyl acetate (12 ml). The combined organic layers were washed with water (8 ml) and brine (5 ml), then dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel. (Hexane: ethyl acetate =12:1). ¹H NMR (500 MHz, CDCl₃) δ/ppm: 7.49 – 7.44 (m, 2H), 7.08 – 7.02 (m, 2H), 3.87 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ/ppm: 165.57, 137.66, 125.39 , 121.95, 119.68, 52.11. IR (υ): 2078 (broad strong), 1687 (strong), 1500 (strong), 1283 (medium), 830 (medium).
Cu(I)-catalysed N-H insertion in water (Supporting Information)
General procedure for copper(I)-catalyzed aniline NH-insertion with methyl 2-diazo-2-(4-((dimethylamino)methyl)phenyl)acetate. Methyl 2-diazo-2-(4-((dimethylamino)methyl)phenyl)acetate\textsuperscript{1} (1, 0.082 mmol) and the respective aniline or aminopyridine (0.090 mmol) were mixed in tert-butanol (0.33 ml) under nitrogen, 500 mM MES buffer, pH 6.0 (0.65 ml), 50 mM CuSO\textsubscript{4} in water (0.016 ml) and water (0.47 ml) were then added, the mixture was stirred, and 200 mM sodium ascorbate in water (0.16 ml) was introduced. The mixture was stirred for 1 h (compounds 3a-g) or overnight (compounds 3h and 3i) at room temperature. Equal volume of 10 % (w/v) K\textsubscript{2}CO\textsubscript{3} was added, and the mixture was extracted with 3× 4 ml of dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, evaporated to dryness under vacuum and the residue purified by column chromatography on Si60 in DCM/methanol to yield the desired product.
Methyl 2-((dimethylamino)methyl)phenyl)-2-(phenylamino) acetate (3a). Light yellow oil (85%). TLC (DCM-methanol 20:1 v/v) R<sub>f</sub>=0.38. IR (film) 2768, 1735, 1601, 1504, 1432, 1309, 1253, 1170, 1018, 857, 746, 691, 508 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ/ppm: 7.45 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 7.12 (dd, J = 8.5, 7.4 Hz, 2H), 6.70 (dd, J = 7.3, 7.3 Hz, 1H), 6.56 (d, J = 7.7 Hz, 2H), 5.07 (d, J = 6.0 Hz, 1H), 4.93 (d, J = 5.8 Hz, 1H), 3.73 (s, 3H), 3.44 (s, 2H), 2.26 (s, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ/ppm: 172.49, 146.07, 138.71, 136.65, 129.80, 129.36, 127.35, 118.23, 113.50, 63.93, 60.62, 52.93, 45.39. HRMS (ESI) calc. for C<sub>18</sub>H<sub>23</sub>N<sub>2</sub>O<sup>+</sup>: (M + H)<sup>+</sup>, 299.1760; Found: (M + H)<sup>+</sup>, 299.1754.
Methyl 2-(4-((dimethylamino)methyl)phenyl)-2-(methyl(phenyl)amino) acetate (3b).
Colorless oil (82 %). TLC (DCM-methanol 20:1 v/v) R<sub>f</sub>=0.33. **IR (film)** 2767, 1742, 1570, 1503, 1169, 1106, 1002, 749, 691 cm<sup>-1</sup>. **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ/ppm: 7.30 (d, J = 8.2 Hz, 2H), 7.27 – 7.18 (m, 4H), 6.84 (d, J = 8.0 Hz, 2H), 6.80 – 6.74 (m, 1H), 5.62 (s, 1H), 3.75 (s, 3H), 3.44 (s, 2H), 2.75 (s, 3H), 2.25 (s, 6H). **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ/ppm: 172.41, 149.85, 138.34, 134.78, 129.47, 129.31, 128.43, 118.08, 113.45, 65.48, 63.78, 52.02, 45.26, 34.46. **HRMS (ESI)** calc. for C<sub>19</sub>H<sub>25</sub>N<sub>2</sub>O<sub>2</sub>: (M + H)<sup>+</sup>, 313.1916; Found: (M + H)<sup>+</sup>, 313.1914.
Methyl 2-(4-((dimethylamino)methyl)phenyl)-2-((4-methoxyphenyl)amino) acetate (3c).
Colorless oil (75 %). TLC (DCM-methanol 20:1 v/v) R_f=0.35. IR (film) 1735, 1510, 1235, 1033, 818 cm⁻¹ ¹H NMR (500 MHz, CDCl₃) δ/ppm: 7.44 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 6.72 (d, J = 8.9 Hz, 2H), 6.53 (d, J = 8.9 Hz, 2H), 5.01 (d, J = 6.4 Hz, 1H), 4.64 (d, J = 6.4 Hz, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 3.44 (s, 2H), 2.26 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ/ppm: 172.61, 152.52, 140.21, 138.41, 136.75, 129.68, 127.27, 114.84, 114.77, 63.80, 61.43, 55.69, 52.74, 45.25. HRMS (ESI) calc. for C₁₉H₂₅N₂O₃+: (M + H)+, 329.1865; Found: (M + H)+, 329.1865.
Supporting Information

Cu(I)-catalysed N-H insertion in water

Electronic Supplementary Material (ESI) for Chemical Science
This journal is © The Royal Society of Chemistry 2013
Methyl 2-((4-chlorophenyl)amino)-2-(4-((dimethylamino)methyl)phenyl) acetate (3d).

Colorless oil (77 %). TLC (DCM-methanol 20:1 v/v) Rf=0.35. **IR (film)** 1735, 1598, 1498, 1312, 1171, 1018, 815 cm⁻¹. **1H NMR** (500 MHz, CDCl₃) δ/ppm: 7.42 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.05 (d, J = 8.9 Hz, 2H), 6.46 (d, J = 8.9 Hz, 2H), 5.02 (d, J = 6.0 Hz, 1H), 4.97 (d, J = 5.9 Hz, 1H), 3.73 (s, 3H), 3.44 (s, 2H), 2.26 (s, 6H). **13C NMR** (126 MHz, CDCl₃) δ/ppm: 172.08, 144.44, 138.73, 136.06, 129.75, 129.09, 127.19, 122.75, 114.50, 63.78, 60.46, 52.92, 45.28. **HRMS (ESI)** calc. for C₁₈H₂₂ClN₂O₂⁺: (M + H)⁺, 333.1370; Found: (M + H)⁺, 333.1363.
Ethyl 4-((1-(4-((dimethylamino)methyl)phenyl)-2-methoxy-2-oxoethyl)amino) benzoate (3e). Colorless oil (70%). TLC (DCM-methanol 20:1 v/v) Rf=0.33. IR (film) 1737, 1697, 1603, 1523, 1265, 1169, 1018, 839, 769, 698 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ/ppm: 7.81 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 6.51 (d, J = 8.9 Hz, 2H), 5.41 (d, J = 5.8 Hz, 1H), 5.12 (d, J = 5.8 Hz, 1H), 4.28 (q, J = 7.1 Hz, 2H), 3.74 (s, 3H), 3.43 (s, 2H), 2.25 (s, 6H), 1.32 (t, J = 7.1 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ/ppm: 171.74, 166.67, 149.43, 138.90, 135.70, 131.41, 129.79, 127.15, 119.70, 112.32, 77.30, 77.05, 76.79, 63.75, 60.25, 59.81, 53.04, 45.28, 14.42. HRMS (ESI) calc. for C₂₁H₂₇N₂O₄⁺: (M + H)⁺, 371.1971; Found: (M + H)⁺, 371.1971.
Methyl 2-(4-((dimethylamino)methyl)phenyl)-2-((4-(trifluoromethyl)phenyl)amino) acetate (3f). Colorless oil (74%). TLC (DCM-methanol 20:1 v/v) Rf = 0.30. IR (film) 1737, 1616, 1532, 1315, 1105, 1064, 825 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ/ppm: 7.42 (d, J = 8.1 Hz, 2H), 7.35-7.32 (m, 4H), 6.55 (d, J = 8.5 Hz, 2H), 5.33 (d, J = 5.7 Hz, 1H), 5.08 (d, J = 5.7 Hz, 1H), 3.75 (s, 3H), 3.44 (s, 2H), 2.26 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ/ppm: 171.74, 148.21, 138.83, 135.65, 129.79, 127.11, 126.57 (q, J = 4 Hz), 124.76 (q, J = 271 Hz), 119.61 (q, J = 33 Hz), 112.58, 63.71, 59.88, 53.02, 45.24. HRMS (ESI) calc. for C₁₉H₂₂F₃N₂O₂⁺: (M + H)⁺, 367.1633; Found: (M + H)⁺, 367.1633.
Methyl 2-((4-acetylphenyl)amino)-2-(4-((dimethylamino)methyl)phenyl) acetate (3g).

Colorless oil (75%). TLC (DCM-methanol 20:1 v/v) R\textsubscript{f}=0.30. IR (film) 2769, 1737, 1660,1594, 1524, 1420, 1357, 1270, 1171, 1018, 825, 591 cm\textsuperscript{-1}. H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) ppm: 7.75 (d, \(J=8.8\) Hz, 2H), 7.41 (d, \(J=8.1\) Hz, 2H), 7.32 (d, \(J=8.2\) Hz, 2H), 6.52 (d, \(J=8.9\) Hz, 2H), 5.51 (d, \(J=5.7\) Hz, 1H), 5.13 (d, \(J=5.8\) Hz, 1H), 3.75 (s, 3H), 3.43 (s, 2H), 2.45 (s, 3H), 2.25 (s, 6H). C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) ppm: 196.38, 171.63, 149.68, 139.01, 135.57, 130.65, 129.83, 127.52, 127.13, 112.32, 63.75, 59.72, 53.10, 45.28, 26.05. HRMS (ESI) calc. for C\textsubscript{28}H\textsubscript{32}N\textsubscript{2}O\textsubscript{3}: (M + H), 341.1865; Found: (M + H), 341.1863.
Methyl 2-(4-((dimethylamino)methyl)phenyl)-2-(pyridin-2-ylamino) acetate (3h). Colorless oil (51 %). TLC (DCM-methanol 20:1 v/v) R_f=0.25. **IR (film)** 2768, 1737, 1600, 1480, 1169, 1018, 772 cm^{-1}. **^1H NMR** (500 MHz, CD_{3}CN) δ/ppm: 7.98 (dd, J = 5.6, 1.9 Hz, 1H), 7.41 (d, J = 8.0 Hz, 3H), 7.32 (d, J = 8.3 Hz, 2H), 6.71 – 6.53 (m, 2H), 5.88 (s, 1H), 5.55 (d, J = 7.0 Hz, 1H), 3.64 (s, 3H), 3.41 (s, 2H), 2.17 (s, 6H). **^13C NMR** (126 MHz, CD_{3}CN) δ/ppm: 172.70, 157.46, 147.45, 139.46, 137.11, 136.27, 129.35, 127.57, 113.45, 109.04, 63.20, 58.26, 51.91, 44.48. **HRMS (ESI)** calc. for C_{17}H_{22}N_{3}O_{2}: (M + H)^{+}, 300.1712; Found: (M + H)^{+}, 300.1706.
Tishinov, Fei & Gillingham

Cu(I)-catalysed N-H insertion in water

(Supporting Information)
Methyl 2-(4-((dimethylamino)methyl)phenyl)-2-(pyridin-3-ylamino) acetate (3i). Yellow oil (76%). TLC (DCM-methanol 20:1 v/v) R_f=0.23. IR (film) 2769, 1737, 1587, 1480, 1171, 1017, 795, 708 cm⁻¹. ¹H NMR (500 MHz, CD₃CN) δ/ppm: 8.06 (s, 1H), 7.89 (s, 1H), 7.44 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H), 7.05 (s, 1H), 6.92 (d, J = 6.6 Hz, 1H), 5.48 (d, J = 7.1 Hz, 1H), 5.20 (d, J = 7.3 Hz, 1H), 3.67 (s, 3H), 3.44 (s, 2H), 2.19 (s, 6H). ¹³C NMR (126 MHz, CD₃CN) δ/ppm: 171.86, 139.08, 138.83, 136.26, 136.22, 129.57, 127.36, 123.71, 119.20, 62.95, 59.48, 52.36, 44.31. HRMS (ESI) calc. for C₁₇H₂₂N₃O₂⁺: (M + H)⁺, 300.1712; Found: (M + H)⁺, 300.1709.
Methyl 2-(4-((dimethylamino)methyl)phenyl)-2-(4-((1-(3-hydroxypropyl)-1H-1,2,3-triazol-4-yl)phenyl)amino) acetate (5). Methyl 2-diazo-2-(4-((dimethylamino)methyl)phenyl) acetate (1, 0.064 mmol), 4-ethynylaniline (0.071 mmol) and 3-azidopropan-1-ol (0.077 mmol) were mixed in tert-butanol (0.45 ml) under nitrogen, and 500 mM MES buffer, pH 6.0 (0.514 ml), 50 mM CuSO$_4$ in water (0.013 ml) and water (0.245 ml) were then added, the mixture was stirred, and 400 mM sodium ascorbate in water (0.064 ml) was introduced. The mixture was stirred for overnight at room temperature. Then an equal volume of 10 % (w/v) K$_2$CO$_3$ was added, and the mixture was extracted with 3× 3 ml of dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, evaporated under vacuum and the residue purified by column chromatography on Si60 in dichloromethane/methanol to yield the desired product as a colorless oil (62 %). TLC (DCM-methanol 20:1 v/v) $R_f$=0.10. IR (film) 2948, 1735, 1617, 1564, 1502, 1456, 1317, 1172, 1041, 798 cm$^{-1}$. $^1$H NMR (500 MHz, MeOD) δ/ppm: 8.09 (s, 1H), 7.59 – 7.46 (m, 4H), 7.34 (d, $J$ = 8.2 Hz, 2H), 6.70 (d, $J$ = 8.8 Hz, 2H), 5.23 (s, 1H), 4.50 (t, $J$ = 7.0 Hz, 2H), 3.71 (s, 3H), 3.58 (t, $J$ = 6.1 Hz, 2H), 3.55 (s, 2H), 2.29 (s, 6H), 2.17 – 2.08 (m, 2H). $^{13}$C NMR (126 MHz, MeOD) δ/ppm: 173.80, 149.32, 148.29, 138.70, 138.13, 131.23, 128.77, 127.63, 121.07, 120.92, 114.75, 64.22, 61.50, 59.31, 53.05, 48.26, 45.00, 33.99. HRMS (ESI) calc. for C$_{23}$H$_{30}$N$_{5}$O$_{3}^+$: (M + H)$^+$, 424.2349; Found: (M + H)$^+$, 424.2343.
Methyl 2-(4-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-(phenylamino)acetate
Methyl 2-(4-azidophenyl)-2-diazoacetate (14 mg, 0.064 mmol), aniline (6.57 mg, 0.071 mmol) and propargyl alcohol (3.6 mg, 0.064 mmol) were mixed in tert-butanol (1.37 ml) under nitrogen, and 500 mM MES buffer, pH 6.0 (0.514 ml), 100 mM CuSO4 in water (0.032 ml) were then added, the mixture was stirred, and 400 mM sodium ascorbate in water (0.32 ml), 0.5 ml water was introduced. The mixture was stirred for overnight at room temperature. Then an equal volume of 10 % (w/v) K2CO3 was added, and the mixture was extracted with 3× 6 ml of dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, evaporated under vacuum and the residue purified by column chromatography on Si60 in dichloromethane/methanol to yield the desired product as white solid, (70 %) TLC (DCM-methanol 30:1 v/v) Rf=0.31. IR (film) 3279, 3119, 1731, 1516, 1191, 1011, 765, 693 cm⁻¹. ¹H NMR (500 MHz, CDCl3) δ/ppm: 8.18 (s, 1H), 8.19 (s, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.11 (dd, J = 8.5, 7.5 Hz, 2H), 6.65 (dt, J = 8.5, 1.1 Hz, 3H), 5.30 (d, J = 5.7 Hz, 1H), 4.70 (s, 2H), 3.70 (s, 3H). ¹³C NMR (126 MHz, CDCl3) δ/ppm: δ 172.74, 149.89, 147.26, 139.58, 137.98, 130.10, 129.81, 122.04, 121.72, 121.63, 118.88, 114.42, 60.58, 56.52, 53.40. HRMS (ESI) calc. for C18H19N4O3⁺: (M + H)⁺, 339.1379; Found: (M + H)⁺, 339.1452.
Methyl 2-(4-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-((4-iodophenyl)amino)acetate

Methyl 2-(4-azidophenyl)-2-diazoacetate (14mg, 0.064mmol), 4-iodoaniline (15.5mg, 0.071mmol) and propargyl alcohol (3.6mg, 0.064mmol) were mixed in tert-butanol (1.37 ml) under nitrogen, and 500 mM MES buffer, pH 6.0 (0.514 ml), 100 mM CuSO$_4$ in water (0.032 ml) were then added, the mixture was stirred, and 400 mM sodium ascorbate in water (0.32 ml), 0.5 ml water was introduced. The mixture was stirred for overnight at room temperature. Then an equal volume of 10% (w/v) K$_2$CO$_3$ was added, and the mixture was extracted with 3× 6 ml of dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, evaporated under vacuum and the residue purified by column chromatography on Si60 in dichloromethane/methanol to yield the desired product as a white solid (70%). TLC (DCM-methanol 30:1 v/v) $R_f$=0.23. IR (film) 3368, 3151, 1734, 1497, 1184, 1059, 1005, 928, 749 cm$^{-1}$. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$/ppm: 8.18 (s, 1H), 7.80 (d, $J = 8.7$ Hz, 2H), 7.71 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.66 (d, $J = 8.4$ Hz, 2H), 7.12 – 7.05 (m, 1H), 6.49 – 6.37 (m, 2H), 5.68 (d, $J = 5.8$ Hz, 1H), 5.35 (d, $J = 5.8$ Hz, 1H), 4.69 (d, $J = 5.5$ Hz, 2H), 3.73 (s, 3H), 3.30 (t, $J = 5.7$ Hz, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$/ppm: 172.28, 149.92, 145.93, 140.17, 138.84, 138.15, 130.41, 129.67, 121.86, 120.66, 112.97, 85.99, 60.68, 56.52, 53.87. HRMS (ESI) calc. for C$_{18}$H$_{18}$IN$_4$O$_3$+: (M + H)$^+$, 465.0345; Found: (M + H)$^+$, 465.0418.
Cu(I)-catalysed N-H insertion in water

(Supporting Information)
Methyl 2-(4-((dimethylamino)methyl)phenyl)-2-((4-(4-hydroxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)amino)acetate

Methyl 2-diazo-2-(4-((dimethylamino)methyl)phenyl) acetate (30mg, 0.128mmol), aniline (20.61mg, 0.15mmol) and propargyl alcohol (7.2mg, 0.128mmol) were mixed in tert-butanol (0.8 ml) under nitrogen, and 500 mM MES buffer, pH 6.0 (0.93 ml), 100 mM CuSO4 in water (0.064 ml) were then added, the mixture was stirred, and 400 mM sodium ascorbate in water (0.64 ml), 0.12ml water were introduced. The mixture was stirred for overnight at room temperature. Then an equal volume of 10 % (w/v) K2CO3 was added, and the mixture was extracted with 3× 6 ml of dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, evaporated under vacuum and the residue purified by column chromatography on Si60 in dichloromethane/methanol to yield the desired product as a colorless oil (62 %). TLC (DCM-methanol 10:1 v/v) Rf=0.45. IR (film) 3366, 3292, 1728, 1525, 1223, 860, 830, 515cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ/ ppm: 8.00 (s, 1H), 7.59 (d, J = 8.2 Hz, 2H), 7.49 (dd, J = 18.1, 8.6 Hz, 4H), 6.75 (d, J = 9.0 Hz, 2H), 5.30 (s, 1H), 4.66 (s, 2H), 4.18 (s, 2H), 3.70 (s, 3H), 2.71 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ/ppm: 172.43, 147.64, 140.31, 132.44, 131.29, 129.21, 129.09, 122.98, 118.33, 114.55, 60.93, 60.67, 56.56, 53.47, 42.71. HRMS (ESI) calc. for C₂₁H₂₆N₅O₃⁺: (M + H)⁺, 396.1957; Found: (M + H)⁺, 396.2030.
Cu(I)-catalysed N-H insertion in water (Supporting Information)
Methyl 2-(4-(4-((dimethylamino)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-2-(phenylamino)acetate

Methyl 2-(4-azidophenyl)-2-diazoacetate (0.100 mmol), aniline (0.110 mmol) and N,N-dimethylprop-2-yn-1-amine (0.1 mmol) were mixed in tert-butanol (2 ml) under nitrogen, and 500 mM MES buffer, pH 6.0 (0.8 ml), 100 mM CuSO4 in water (0.05 ml) were then added, the mixture was stirred, and 400 mM sodium ascorbate in water (0.5 ml), 0.63 ml water was introduced. The mixture was stirred for overnight at room temperature. Then an equal volume of 10 % (w/v) K2CO3 was added, and the mixture was extracted with 3× 6 ml of dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, evaporated under vacuum and the residue purified by column chromatography on Si60 in dichloromethane/methanol to yield a white solid (63%).

TLC (DCM-methanol 20:1 v/v) Rf = 0.31. IR (film) 3366, 3292, 1728, 1525, 1223, 860, 830, 515 cm⁻¹.

1H NMR (500 MHz, CDCl₃) δ/ppm: 8.14 (s, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 8.6 Hz, 2H), 7.14 – 7.07 (m, 2H), 6.68-6.64 (m, 3H), 5.39 (d, J = 7.1 Hz, 1H), 5.29 (d, J = 7.2 Hz, 1H), 3.69 (s, 3H), 3.59 (s, 2H), 2.23 (s, 6H).

13C NMR (126 MHz, CDCl₃) δ/ppm: 172.74, 147.26, 146.66, 139.48, 138.03, 130.09, 129.79, 122.49, 121.62, 121.55, 118.87, 118.32, 114.42, 60.58, 54.77, 53.39, 45.22.

HRMS (ESI) calc. for C₂₀H₂₄N₅O₂⁺: (M + H)⁺, 366.1852; Found: (M + H)⁺, 366.1925
### Cu(I)-catalysed N-H insertion in water

**Electronic Supplementary Material (ESI) for Chemical Science**

This journal is © The Royal Society of Chemistry 2013

---

<table>
<thead>
<tr>
<th>6.00</th>
<th>2.07</th>
<th>2.98</th>
<th>1.01</th>
<th>0.93</th>
<th>2.96</th>
<th>1.94</th>
<th>2.09</th>
<th>1.97</th>
<th>0.97</th>
</tr>
</thead>
</table>

---

| 45.22 | 53.39 | 54.77 | 60.58 | 114.42 | 118.32 | 118.87 | 121.55 | 121.62 | 122.49 | 129.79 | 130.09 | 138.03 | 139.48 | 146.66 | 172.74 |
|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|

---

$\text{NH}$

$\text{O}$

$\text{ONN}$

---

Page S45
Methyl 2-(4-((1-(2-oxo-2-(4-(5-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoyl)piperazin-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)methylphenyl)-2-(phenylamino) acetate (7). Methyl 2-diazo-2-(4-(prop-2-yn-1-yl)piperazin-1-yl)methylphenylacetate (6b, 0.058 mmol) and aniline (0.063 mmol) were mixed in tert-butanol (0.406 ml) under nitrogen, and 500 mM MES buffer, pH 6.0 (0.464 ml), 50 mM CuSO₄ in water (0.012 ml) and 1-(2-azidoacetyl)-4-(D-biotinyl)piperazine (0.069 mmol) dissolved in water (0.220 ml) were then added, the mixture was stirred, and 400 mM sodium ascorbate in water (0.058 ml) was introduced. The mixture was stirred for overnight at room temperature. Then an equal volume of 10 % (w/v) K₂CO₃ was added, and the mixture was extracted with 3× 3 ml of dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate, evaporated under vacuum and the residue purified by column chromatography on Si60 in dichloromethane/methanol to yield the desired product as a colorless oil (70 %). TLC (DCM-methanol 4:1 v/v) Rf=0.25. IR (film) 1665, 1500, 1421, 1215, 1006, 825, 751, 693 cm⁻¹. ¹H NMR (500 MHz, MeOD) δ/ppm: 7.89 (s, 1H), 7.48 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 7.07 (dd, J = 8.5, 7.5 Hz, 2H), 6.66-6.62 (m, 3H), 5.51 (s, 2H), 5.16 (s, 1H), 4.56 – 4.45 (m, 1H), 4.32 (dd, J = 7.7, 4.5 Hz, 1H), 3.77 – 3.58 (m, 13H), 3.54 (s, 2H), 3.22 (dd, J = 7.9, 5.5 Hz, 1H), 2.94 (dd, J = 12.8, 4.9 Hz, 1H), 2.79 – 2.41 (m, 10H), 1.84 – 1.58 (m, 4H), 1.55 – 1.45 (m, 2H). ¹³C NMR (126 MHz, MeOD) δ/ppm: 172.73, 165.13, 165.04, 164.70, 142.85, 137.03, 136.96, 129.66, 128.61, 127.19, 125.95, 117.48, 113.27, 61.95, 61.88, 60.32, 60.23, 55.63, 52.11, 51.90, 51.58, 50.61, 48.45, 45.00, 44.79, 44.62, 44.27, 41.99, 41.60, 41.10, 40.85, 39.66, 32.23, 32.18, 28.48, 28.15, 24.84. HRMS (ESI) calc. for C₃₉H₅₃N₁₀O₅S⁺: (M + H)⁺, 773.3921; Found: (M + H)⁺, 773.3921.
Cu(I)-catalysed N-H insertion in water (Supporting Information)
Oligonucleotide synthesis and purification

Solid-phase oligonucleotide synthesis was carried out on 1-μmol CPG columns using standard phosphoramidite chemistry with 0.3 M 5-benzylthio-1-H-tetrazole as activator. The DNA oligonucleotides were cleaved from the support with 32 % (v/v) aqueous ammonia for 2 h at room temperature and deprotected for 18 h at 55 °C, then freeze-dried and purified by micropreparative HPLC using method A. The RNA oligonucleotides were synthesized using TBDMS protection strategy for the 2'-OH group. Cleavage from the support was done in 32 % aqueous ammonia/ethanol 3:1 (v/v) for 2 h at room temperature followed by deprotection for 12 h at 55 °C. The samples were freeze-dried and the TBDMS groups were cleaved with triethylamine/triethylamine trihydrofluoride/N-methylpyrrolidine 1.5:2:3 (v/v/v) for 2 h at 65 °C. The crude oligoribonucleotides were isolated by precipitation with 3 M NaOAc (1/10 volume) and n-butanol (4 volumes) for 2 h at -78 °C followed by centrifugation (15,000 × g, 10 min). The pellets were washed with 70 % (v/v) ethanol and dried under high vacuum. The samples were further purified by micropreparative HPLC using method A. The identity of all synthesized oligonucleotides was confirmed by ESI or MALDI TOF MS.
General procedure for Cu(I)-catalyzed oligonucleotide modification using α-diazocarbonyl compounds. Typically 10 or 20 μl reaction mixtures containing 5 mM oligonucleotide, 500 μM CuSO₄, 2.5 mM THPTA² and 50 mM α-diazocarbonyl compound, and 10 mM sodium ascorbate in 100 mM MES buffer, pH 6.0 were kept at 20 °C for 24-72 h. Analysis of the reaction products was carried out by micropreparative HPLC-separation of 5-μl aliquots of the reaction mixtures using methods A or C. The analysis of the modification reactions of d(ATG C) and d(ATG) was done in two different buffer systems (methods A and B) in order to fully resolve some of the modification products from the unreacted diazo substrate. The collected peak fractions were further assessed by ESI-MS or MALDI TOF. The designations of the chromatographic peak fractions (Supplementary Figures S1, 3, 6, 8-16) indicate the molecular mass of the species, and the number of alkylations per molecule of oligonucleotide (in parenthesis).

The singly charged ions of the modified products of the three trimers d(TAT), d(TGT) and d(TCT) from the ESI-MS were subjected to tandem mass spectrometry. The designation of the fragment ions was done using the nomenclature proposed by McLuckey et al.³
Figure S4 | HPLC trace of the modification reaction of a d(TAT) with Dz-1 (HPLC analysis by method A). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.

Figure S5 | ESI-MS (A) and MS/MS analysis (B) of the singly-modified d(TAT)
Figure S6 | HPLC trace of the modification reaction of a mixture of d(TGT) with Dz-1 (HPLC analysis by method A). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.
Figure S7 | ESI-MS (A, C) and MS/MS analysis (B, D) of the singly-modified d(TGT)
Figure S8 | ESI-MS (A) and MS/MS analysis (B) of the doubly-modified d(TGT)
Figure S9 | HPLC trace of the modification reaction of a d(TCT) with Dz-1 (HPLC analysis by method A). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.
Figure S10 | ESI-MS (A) and MS/MS analysis (B, C) of the singly-modified d(TCT)
Figure S11 | HPLC trace of the modification reaction of d(ATG C) with Dz-1 (HPLC analysis by method A). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.

Figure S12 | HPLC trace of the modification reaction of d(ATG C) with Dz-1 (HPLC analysis by method B). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.
Figure S13 | ESI-MS of singly (A), doubly (B) and triply modified (C) d(ATG C) with Dz-1
Figure S14 | HPLC trace of the modification reaction of d(T₄) with Dz-1 (HPLC analysis by method A). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.

Figure S15 | ESI-MS of singly modified d(T₄) with Dz-1
Figure S16  |  HPLC trace of the modification reaction of d(ATG) with Dz-1 (HPLC analysis by method A). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.

Figure S17  |  HPLC trace of the modification reaction of a d(ATG) with Dz-1 (HPLC analysis by method B). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.
Figure S18 | ESI-MS of singly (A), doubly (B) and triply modified (C) d(ATG) with Dz-1. The doubly and triply modified species were observed as triethylammonium adducts.
Figure S19  |  HPLC trace of the modification reaction of r(ACU GCU C) with Dz-1 (HPLC analysis by method C). The peak labels refer to the masses observed in the ESI-MS for those peaks. The (+1), (+2), or (+3) label indicates singly-, doubly-, or triply alkylated products.

Figure S20  |  ESI-MS of singly modified r(ACU GCU C) with Dz-1
Cu(I)-catalyzed oligonucleotide modification using α-diazocarbonyl compounds in the presence of proteins. Protein concentration was determined from the absorbance at 279 or 280 nm using the following extinction coefficients: 43 824 M⁻¹cm⁻¹ for bovine serum albumin⁴, 38 940 M⁻¹cm⁻¹ for lysozyme⁵, and 41 820 M⁻¹cm⁻¹ for streptavidine⁶. Typically 20 μl reaction mixtures containing 5 mM d(TAT), 1 mM protein of interest, 500 μM CuSO₄, 2.5 mM THPTA, 50 mM α-diazocarbonyl compound, and 10 mM sodium ascorbate in 100 mM MES buffer, pH 6.0 were kept at 20 ºC for 24 h. Analysis of the reaction products was carried out by HPLC using method A. Aliquots (5-μl) of the reaction mixtures were then separated micropreparatively and the collected peak fractions were further assessed by ESI-MS. The effect of the protein on the modification reaction was estimated by comparison of the conversion of the oligonucleotide and the diazo substrate in the presence of protein with a control sample.

Table S3  Cu(I)-Catalyzed alkylation of d(TAT) in the presence proteins*

<table>
<thead>
<tr>
<th>Entry</th>
<th>Protein</th>
<th>Conversion of d(TAT), %</th>
<th>Conversion of the diazo substrate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>41</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>Bovine serum albumin</td>
<td>&lt;1</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>Lysozyme</td>
<td>37</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>Streptavidin (wild type)</td>
<td>33</td>
<td>82</td>
</tr>
</tbody>
</table>

* The peaks for the proteins were not observed under the analysis conditions used. Therefore, the HPLC-traces were exactly the same as the one for the modification of d(TAT) described in the previous section. A separate injection under acidic conditions shows the protein to be unmodified.
Cu(I)-catalyzed oligonucleotide tandem alkylation/click reaction using propargyl-tagged α-diazocarbonyl compound 8 and 3-azidoprop-1-ol. Typically 10 or 20 μl reaction mixtures containing 5 mM oligonucleotide, 500 μM CuSO₄, 2.5 mM THPTA, 50 mM propargyl-tagged α-diazocarbonyl compound 8, 55 mM of the azide and 10 mM sodium ascorbate in 100 mM MES buffer, pH 6.0 were kept at 20 °C for 24 h. Analysis of the reaction products was carried out by micropreparative HPLC-separation of 5-μl aliquots of the reaction mixtures using method A. The collected peak fractions were further assessed by ESI-MS or MALDI TOF.

Cu(I)-catalyzed oligonucleotide tandem alkylation/click reaction using propargyl-tagged α-diazocarbonyl compound 8 and 1-(2-azidoacetyl)-4-(D-biotinyl)piperazine. Typically 10 or 20 μl reaction mixtures containing 2.5 mM oligonucleotide, 250 μM CuSO₄, 1.25 mM THPTA, 25 mM propargyl-tagged α-diazocarbonyl compound 8, 25 mM of the azide and 10 mM sodium ascorbate in 100 mM MES buffer, pH 6.0 were kept at 20 °C for 24 h. Analysis of the reaction products was carried out by micropreparative HPLC-separation of 5-μl aliquots of the reaction mixtures using method A. The collected peak fractions were further assessed by ESI-MS or MALDI TOF.
Figure S21 | HPLC trace of the tandem alkylation/'click' reaction of d(ATG) with propargyl-tagged α-diazocarbonyl compound 8 and 3-azidoprop-1-ol (HPLC analysis by method C). The number of alkylation-click fragments per molecule ON is given in parenthesis.
Figure S22 | ESI-MS of +1 Alk-Me (A), +1 Alk/Click (B), +2 Alk/Click (C), and +3 Alk/Click (D) modified d(ATG)
Figure S23  |  HPLC trace of the tandem alkylation/’click’ reaction of d(ATG C) with propargyl-tagged α-diazocarbonyl compound 8 and 1-(2-azidoacetyl)-4-D-biotinylpiperazine (HPLC analysis by method C). The number of alkylation-click fragments per molecule ON is given in parenthesis.
Figure S24 | MALDI TOF of the products from the tandem alkylation/'click’ reaction of d(ATGC) with propargyl-tagged α-diazocarbonyl compound 8 and 1-(2-azidoacetyl)-4-D-biotinylpiperazine (see Figure S23)
Figure S25 | HPLC trace of the tandem alkylation/'click' reaction of d(GGA GGC) with propargyl-tagged α-diazocarbonyl compound 8 and 3-azidoprop-1-ol (HPLC analysis by method C). The number of alkylation-click fragments per molecule ON is given in parenthesis. All fractions contained traces of the starting ON due to smearing of its peak.
Figure S26 | MALDI TOF of the products from the tandem alkylation/‘click’ reaction of d(GGA GGC) with propargyl-tagged α-diazocarbonyl compound 8 and 3-azidoprop-1-ol (see Figure S25)
References


