Supporting Information

Nitroxide-labeled pyrimidines for noncovalent spin-labeling of abasic sites in DNA and RNA duplexes

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General

All commercial reagents were purchased from Sigma-Aldrich and used without further purification and all air and moisture sensitive reactions were performed in oven-dried reaction flasks, under an argon atmosphere. CH$_2$Cl$_2$, acetonitrile and pyridine were freshly distilled over calcium hydride before use; triethylamine was purchased anhydrous and stored over KOH pellets. Thin layer chromatography (TLC) was performed on glass backed TLC with extra hard layer (Kieselgel 60 F$_{254}$, 250µm, Silicycle) and compounds visualized by UV light. Silica gel (230-400 mesh, 60 Å) was purchased from Silicycle and used for flash column chromatography. The petroleum ether used for column chromatography was a fraction from distillation between 60 and 90 °C. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 MHz spectrometer and the chemical shifts were reported in parts per million (ppm) relative to the residual proton signal (for $^1$H NMR) and the carbon signal (for $^{13}$C NMR) of the deuterated solvents used [$d_6$-DMSO (2.50 ppm), CDCl$_3$ (7.26 ppm), $d_4$-MeOH (4.84 and 3.31 ppm)] for $^1$H NMR; [$d_6$-DMSO (39.52 ppm), CDCl$_3$ (77.0 ppm), $d_4$-MeOH (49.05 ppm)] for $^{13}$C NMR. All coupling constants were reported in Hertz. Molecular mass of organic compounds were determined by HR-ESI/ESI-MS (Bruker, MicroTof-Q). EPR samples were placed in 50 µL quartz capillary (BLAUBRAND intraMARK) and the EPR spectra recorded on a MiniScope MS200 (Magnettech Germany) spectrometer (100 kHz modulation frequency, 1.0 G modulation amplitude, and 2.0 mW microwave power). DNA oligonucleotides that contain abasic sites and unmodified DNAs were prepared by phosphoramidite chemistry, on an automated ASM800 DNA synthesizer (Biosset) using a trityl-off protocol and phosphoramidites with standard protecting groups on a 1.0 µmol scale (1000 Å CPG columns) and purified by following previously reported protocol.$^{1}$ The RNA oligonucleotides containing abasic sites and unmodified RNAs were purchased from RiboTask Aps (Denmark).

Note: Because of the paramagnetic nature of nitroxides, the NMR spectra of nitroxide compounds shows significant broadening of the signals and some peaks, particularly of nuclei close to the radical, are not seen in the spectra.
### List of abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DCE</td>
<td>1,2-dichloroethane</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>HMDS</td>
<td>1,1,1,3,3,3-hexamethyldisilazane</td>
</tr>
<tr>
<td>HR-ESI-MS</td>
<td>high-resolution electrospray ionization mass spectrometry</td>
</tr>
<tr>
<td>mCPBA</td>
<td>meta-chloroperbenzoic acid</td>
</tr>
<tr>
<td>TMS-Cl</td>
<td>trimethylsilyl chloride</td>
</tr>
<tr>
<td>TPS-Cl</td>
<td>2,4,6-triisopropylbenzenesulfonyl chloride</td>
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**Sample preparation for EPR measurements**

A stock solution of a spin label (200 μM) and a DNA or RNA (400 μM) oligomer containing an abasic site and its complementary strand (1:1.2) were mixed together and evaporated in SpeedVac. The resulting residue was dissolved in phosphate buffer (10 μL; 10 mM NaHPO₄, 100 mM NaCl, 0.1 mM Na₂EDTA, pH 7.0), annealed (annealing protocol: 90 °C for 2 min, 60 °C for 5 min, 50 °C for 5 min, 40 °C for 5 min, 22 °C for 15 min) and dried in SpeedVac. The residue was dissolved in an aqueous 30% ethylene glycol solution containing 2% DMSO (10 μL) and placed in a 50 μL quartz capillary (BLAUBRAND intraMARK) for the EPR measurements.
Binding of spin labels to an unmodified DNA duplex

Figure S1: EPR spectra of pyrimidine-derived spin labels in the presence of an unmodified 14-mer DNA duplex. 5’-d(GAC CTC GCA TCG TG)-3’ : 5’-d(CAC GAT GCG AGG TC)-3’. EPR spectra were recorded in a phosphate buffer pH 7.0, 30% ethylene glycol, 2% DMSO at −30 °C.
Binding of triazole-linked spin labels to a DNA duplex containing either A, C, T or G as an orphan base opposite the abasic site

Figure S2: EPR spectra of triazole-linked spin labels in the presence of a 14-mer DNA duplex containing an abasic site. 5’-d(GAC CTC G__A TCG TG)-3’: 5’-d(CAC GAT XCG AGG TC)-3’, where __ is the abasic site and X is either A, C, T or G. EPR spectra were recorded in a phosphate buffer pH 7.0, 30% ethylene glycol, 2% DMSO at −30 °C.
**Experimental protocols for synthetic compounds**

**Compound 11a.** To a suspension of uracil 11 (0.500 g, 4.46 mmol) in 1,2-dichloroethane (10 mL), HMDS (3.8 mL, 18.1 mmol) and TMSCl (0.3 mL, 2.4 mmol) were added and refluxed for 4 h. The resulting reaction mixture was cooled to 50 °C, the solvent was removed under reduced pressure and 1,2-dichloroethane (10 mL) was added. The reaction mixture was cooled 24 °C and CH$_3$I (1.1 mL, 17.7 mmol) and I$_2$ (5 mg, 0.0197 mmol) were added and the solution refluxed for 24 h. The solvent was removed in vacuo and the residue purified by column chromatography using CH$_3$OH:CH$_2$Cl$_2$ (0:100 to 10:90) to afford 11a as a white solid (256 mg, 53% yield).

$^1$H-NMR (d$_6$-DMSO): δ 3.22 (s, 3H, CH$_3$), 5.51 (dd, $J$ = 7.81, 2.29 Hz, 1H, CH), 7.61 (d, $J$ = 7.81 Hz, 1H, CH), 11.21 (s, 1H, NH) ppm.

$^{13}$C-NMR (d$_6$-DMSO): δ 163.88, 151.25, 146.41, 100.48, 35.16 ppm.

**HR-ESI-MS:** Calculated for C$_5$H$_6$N$_2$O$_2$ 126.0429 m/z found 149.0323 [M + Na]$^+$. 
$^1$H NMR spectrum of 11a:

$^{13}$C NMR spectrum of 11a:
Compound 11b. To a solution of 11a (199 mg, 1.58 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL), TPS-Cl (360 mg, 2.39 mmol) and DMAP (19 mg, 0.16 mmol) and Et$_3$N (0.9 mL, 6.42 mmol) were added at 0 °C and the resulting reaction mixture was stirred for 4 h at 24 °C. The reaction mixture was diluted with CH$_2$Cl$_2$ (20 mL) and washed subsequently with water, aqueous NaHCO$_3$ (10 mL) and brine (10 mL). The organic layer was dried over Na$_2$SO$_4$ and the solvent was removed in vacuo. The residue was purified by column chromatography using CH$_3$OH:CH$_2$Cl$_2$ (0:100 to 2:98) to get 11b as a white solid (142.5 mg, 23% yield).

$^1$H-NMR (CDCl$_3$): δ 7.60 (d, $J$ = 7.04 Hz, 1H, ArCH), 7.20 (s, 2H, ArCH), 6.07 (d, $J$ = 7.03 Hz, 1H, ArCH), 4.24 (m, 2H, 2CH), 3.47 (s, 3H, CH$_3$), 2.90 (m, 1H, CH), 1.29 (d, $J$ = 8 Hz, 12H, 4CH$_3$) ppm.

HR-ESI-MS: Calculated for C$_{20}$H$_{28}$N$_2$O$_4$S 392.17 not found, compound is unstable.
$^1$H NMR spectrum of 11b:
Spin label 1. A solution of 11b (42.5 mg, 0.108 mmol) and 4-amino-TEMPO (29.6 mg, 0.173 mmol) and Et$_3$N (0.2 mL, 1.43 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) were refluxed for 12 h and diluted with CH$_2$Cl$_2$ (10 mL). The reaction mixture was washed with water and organic layer was dried over Na$_2$SO$_4$ and the solvent removed in vacuo to afford 1 as a white solid (11.9 mg, 39% yield).

$^1$H-NMR (CDCl$_3$): $\delta$ 1.24 (s, 1H), 3.2 (s, 3H, CH$_3$), 5.62 (bs, 1H, CH), 7.61(bs, 1H, CH) ppm.

HR-ESI-MS: Calculated for C$_{14}$H$_{23}$N$_4$O$_2$ 279.1821 found m/z 302.1677 [M + Na]$^+$.
$^1$H NMR spectrum of 1:
**Compound 14:** A suspension of 5-iodocytosine (2 g, 8.43 mmol) in 1,2-dichloroethane (1,2-DCE) (20 ml) was treated with hexamethyldisilazane (HMDS) (5.3 ml, 25.31 mmol) and trimethylsilyl chloride (TMS-Cl) (0.53 ml, 4.21 mmol). The reaction mixture was refluxed until it became clear, cooled to 60 °C and the solvent was removed *in vacuo* to yield a colorless oil. The residue was dissolved in 1,2-DCE (20 ml) and treated with iodomethane (0.8 ml, 12.62 mmol) and a catalytic amount of I₂ (0.02 g, 0.08 mmol) at 25 °C. The reaction mixture was refluxed for 12 h and cooled to 25 °C prior to addition of H₂O (15 ml). The precipitate was filtered off and washed with water to give 14 as a white solid (1.1 g, 52% yield).

\[ ^1H \text{NMR (DMSO-}d_6\text{): } \delta \text{ 3.23 (s, 3H, CH}_3\text{), 8.13 (s, 1H, CH)} \]

\[ ^{13}C \text{NMR (DMSO-}d_6\text{): } \delta \text{ 163.61, 154.47, 153, 54.91, 36.46 ppm.} \]

**HR-ESI-MS:** Calculated for C₅H₆I₃N₃O 250.9556 found m/z 273.9433 [M + Na]⁺.
$^1$H NMR spectrum of 14:

$^{13}$C NMR spectrum of 14:
**Spin labels 2 and 3.** A solution of 13 or 14 (0.084 mmol) and nitroxide 15 (44 mg, 0.25 mmol) in DMF (2 mL) and Et₃N (2 mL) was degassed by bubbling argon through the solution for 5 min. Pd(PPh₃)₂Cl₂ (6 mg, 0.0084 mmol) and CuI (1.5 mg, 0.0084 mmol) were added and the resulting reaction mixture was stirred at 50 °C for 3 h. The solvent was removed *in vacuo* and the residue was purified preparative TLC using 5% CH₃OH in CH₂Cl₂ to give the spin labels as pale yellow solids (2, 7 mg, 30% yield; 3, 5 mg, 25% yield).

¹H-NMR (10% CD₃OD/CDCl₃) (2): δ 1.21 (bs, 1H, CH), 3.36 (s, 1H, CH), 7.60 (s, CH), 7.79 (s, NH) ppm.
HR-ESI-MS (2): Calculated for C₁₅H₂₀N₄O₂ 287.1508 m/z found 288.1581 [M + H]⁺.

¹H-NMR (CDCl₃) (3): δ 1.27 (s, 4H, CH₃), 3.47 (s, 3H, CH₃), 7.86 (s, CH) 8.65 (s, NH) ppm.
HR-ESI-MS (3): Calculated for C₁₆H₂₁N₄O₂ 301.1665 m/z found 302.1755 [M + H]⁺.
$^1$H NMR spectrum of 2:

$^1$H NMR spectrum of 3:
Compound 17. To a suspension of 5-iodo-uracil (16) (800 mg, 3.36 mmol) in 1,2-dichloroethane (10 mL), HMDS (2.0 mL, 8.4 mmol) and TMSCl (0.2 mL, 1.7 mmol) were added and the solution refluxed for 3 h under argon atmosphere. The reaction mixture was cooled to 50 °C, the solvent and excess silyl reagent was removed under reduced pressure. 1,2-Dichloroethane (10 mL) was added, followed by CH$_3$I (0.8 mL, 12.84 mmol) and I$_2$ (10 mg, 0.03 mmol) and the resulting reaction mixture was refluxed for 24 h. Solvent was removed under high vacuum and the residue purified by column chromatography using CH$_3$OH:CH$_2$Cl$_2$ (0:100 to 4:96) to afford 17 as a white solid (700 mg, 60% yield).

$^1$H-NMR (d$_6$-DMSO): δ 11.59 (s, 1H NH), 8.18 (s, 1H, CH), 3.23 (s, 3H, CH$_3$) ppm.

$^{13}$C-NMR (d$_6$-DMSO): δ 159.85, 150.69, 146.15, 94.01, 35.43 ppm.

HR-ESI-MS: Calculated for C$_5$H$_5$IN$_2$O$_2$ 251.9396 m/z found 274.9341 [M + Na]$^+$. 
$^1$H NMR spectrum of 17:

$^{13}$C NMR spectrum of 17:
Spin labels 4 and 5. A solution of 16 or 17 (0.084 mmol) and nitroxide 15 (44 mg, 0.25 mmol) in DMF (2 mL) and Et$_3$N (2 mL) was degassed by bubbling argon through the solution for 5 min. Pd(PPh$_3$)$_2$Cl$_2$ (6 mg, 0.0084 mmol) and Cul (1.5 mg, 0.0084 mmol) were added and the resulting reaction mixture was stirred at 50 °C for 3 h. The solvent was removed in vacuo and the residue was purified by preparative TLC using 5% CH$_3$OH in CH$_2$Cl$_2$ to give spin labels as pale yellow solids (4, 7 mg, 30% yield; 5, 5 mg, 25% yield).

$^1$H-NMR (10% CD$_3$OD/CDCl$_3$) (4): $\delta$ 1.18 (s, 1H, CH), 3.32 (s, 2H, CH), 7.56 (s, 1H, CH), 7.75 (bs, ArCH) ppm.

HR-ESI-MS (4): Calculated for C$_{15}$H$_{18}$N$_3$O$_3$ 288.1348 m/z found 311.1240 [M + Na]$^+$.  

$^1$H-NMR (CDCl$_3$) (5): $\delta$ 1.26 (s, H, CH$_3$), 3.56 (s, 3H, CH$_3$), 8.10 (s, CH) ppm.

HR-ESI-MS (5): Calculated for C$_{16}$H$_{20}$N$_3$O$_3$ 302.1505 m/z found 325.1393 [M + Na]$^+$.  

$^{1}H$ NMR spectrum of 4:

$^{1}H$ NMR spectrum of 5:
Compound 18: A solution of 14 (250 mg, 0.99 mmol) in DMF (5 mL), pyridine (5 mL) was added benzoyl chloride (0.14 mL, 1.19 mmol) and the resulting reaction mixture was stirred for 12 h at 70 °C. The reaction mixture was cooled to 24 °C, diluted with CH₂Cl₂ (20 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was dried over Na₂SO₄ and the solvent was removed in vacuo. The residue was purified by column chromatography using 1% MeOH in CH₂Cl₂ to yield 18 as a pale yellow solid (100 mg, 30% yield).

$^1$H-NMR (CDCl₃): δ 3.47 (s, 3H, CH₃), 7.47-7.50 (m, 3H, ArCH), 7.77 (s, 1H, ArCH), 8.12 (d, $J = 8$, 2H, ArCH), 8.37 (d, $J = 8$, 2H, ArCH) ppm.

$^{13}$C-NMR (CDCl₃): δ 179.80, 157.79, 150.72, 148.51, 136.77, 133.87, 133.00, 130.37, 128.66, 128.40, 68.86, 37.05 ppm.

HR-ESI-MS: Calculated for C₁₂H₁₀N₃O₂ 354.9818 found 377.9696 [M + Na]⁺.
$^1$H NMR spectrum of 18:

$^{13}$C NMR spectrum of 18:
Spin label 6: To a solution of 18 (20 mg, 0.056 mmol) in DMF (2 mL) and Et$_3$N (2 mL), Pd(PPh$_3$)$_2$Cl$_2$ (4 mg, 0.0056 mmol) and Cul (2.1 mg, 0.011 mmol) were added. The resulting reaction mixture was treated with 15 (15 mg 0.084 mmol) and stirred for 12 h at 50 °C. The reaction mixture was diluted with absolute ethanol (2 mL) and stirred for 60 °C for 12 h. The resulting reaction mixture was cooled and the solvent was removed in vacuo. The residue was purified by preparative TLC to yield 6 as a pale yellow solid (12 mg, 70% yield).

$^1$H-NMR (CDCl$_3$): δ 1.30 (bs, 1H, CH$_3$), 1.72-1.78 (bd, 1H, CH$_2$), 3.47 (s, 2H, CH$_3$), 3.62 (s, 1H, CH) 7.86 (s, 1H, CH), 8.5 (s, 1H, CH) ppm.

HR-ESI-MS: Calculated for C$_{16}$H$_{21}$N$_4$O$_2$ 301.1665 found 302.1740 [M + 1]$^+$. 
$^1$H NMR spectrum of 6:
**Compound 19:** To a solution of 17 (160 mg, 0.78 mmol) in DMF (2 mL) and Et₃N (2 mL), Pd(PPh₃)₄ (85 mg, 0.078 mmol) and CuI (14 mg, 0.078 mmol) was added. The resulting reaction mixture was treated with trimethylsilyl acetylene (0.27 mL 1.95 mmol) and stirred for 2 h at 50 °C. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed subsequently with H₂O (10 mL x 2), aqueous NaHCO₃ solution and brine (10 mL each). The organic layer was dried over Na₂SO₄ and the solvent was removed *in vacuo*. The crude product was purified by column chromatography using a 99.5:0.5 to 98:2 CH₂Cl₂/MeOH gradient to yield 19 as a white solid (120 mg, 70% yield).

**1H-NMR (CDCl₃):** δ 0.20 (s, 9H, 3xC₃H₃), 3.38 (s, 3H, C₃H₃), 7.48 (s, CH), 9.38 (s, NH) ppm.

**13C-NMR (CDCl₃):** δ −0.06, 36.44, 95.05, 99.88, 100.14, 148.45, 150.24, 161.97 ppm.

**HR-ESI-MS:** Calculated for C₁₀H₁₄N₂O₂Si 222.0825 m/z found 223.0818 [M + 1]^+. 
$^1$H NMR spectrum of 19:

$^{13}$C NMR spectrum of 19:
**Compound 20:** To a solution of 19 (80 mg, 0.36 mmol) in conc. methanolic ammonia (10 mL) was stirred for 12 h at 24 °C. The solvent was removed *in vacuo* and the crude product was purified by column chromatography using a gradient of 99:1 to 95:5 CH₂Cl₂/MeOH to yield 20 as a pale yellow solid (30 mg, 55% yield).

**¹H-NMR (d₆-DMSO):** δ 3.24 (s, 3H, CH₃), 4.06 (s, ≡CH), 8.09 (s, CH) ppm.

**¹³C-NMR (d₆-DMSO):** δ 35.56, 76.36, 83.32, 96.23, 150.29, 150.55, 162.37 ppm.

**HR-ESI-MS:** Calculated for C₇H₆N₂O₂Si 150.0429 found m/z 173.0319[M + Na]+.
$^1$H NMR spectrum of 20:

$^{13}$C NMR spectrum of 20:
**Spin label 7**: To a suspension of 20 (7 mg, 0.046 mmol) in acetone (5 mL), CuI (2 mg, 0.01 mmol) and 21 (5 mg, 0.021 mmol) were added. Few drops of DMSO were subsequently added to completely dissolve 20. The reaction mixture was refluxed for 2 h and the solvent was removed *in vacuo*. The residue was purified by preparative TLC using 10% MeOH/CH$_2$Cl$_2$ to afford 7 as a pale yellow solid (10 mg, 65% yield).

$^1$H NMR (CDCl$_3$): $\delta$ 1.27 (bs, 4H), 3.57 (bs, 1H, CH$_3$), 4.69 (bs, 1H, CH), 8.10 (s, 1H, ArCH), 8.40 (s, 1H, ArCH) ppm.

HR-ESI-MS: Calculated for C$_{19}$H$_{21}$N$_6$O$_3$ 381.1675 and found $m/z$ 404.1574 [M + Na]$^+$. 
$^1$H NMR spectrum of 7:
**Compound 22:** To a solution of 19 (50 mg, 0.2249 mmol) in dry CH₂Cl₂ (5 mL), TPS-Cl (136 mg, 0.4498 mmol) was added at 0 °C. The resulting solution was treated with DMAP (3 mg, 0.0224 mmol) and Et₃N (0.13 mL, 0.8996 mmol) and stirred for 2 h at 0 °C. The reaction mixture was diluted with CH₂Cl₂ (10 mL) and washed with water, aqueous NaHCO₃ and brine (10 mL). The organic layer was dried over Na₂SO₄ and solvent was removed under reduced pressure. The crude product was purified by flash column chromatography using EtOAc/petroleum ether (10:90) to yield 22 as a white solid (55 mg, 55% yield).

**¹H-NMR (CDCl₃):** δ -0.21 (s, 9H, 3CH₃), 1.24 & 1.28 (2d, 18H, 6CH₃), 2.89 (m, 1H, CH), 3.44 (s, 3H, CH₃), 4.30 - 4.37 (m, 2H, CH), 7.19 (s, 2H, ArCH), 7.75 (s, 1H, CH) ppm.

**¹³C-NMR (CDCl₃):** δ -0.22, 23.60, 24.70, 29.69, 34.42, 38.76, 92.72, 93.54, 101.90, 124.16, 130.51, 151.61, 152.41, 153.23, 154.65, 165.67 ppm.

**HR-ESI-MS:** Calculated for C₂₅H₃₆N₂O₄Si 488.2165 found m/z 511.2016 [M + Na]⁺.
$^1$H NMR spectrum of 22:

$^{13}$C NMR spectrum of 22:
**Compound 23:** Compound 22 (100 mg, 0.2046 mmol) was dissolved in conc. methanolic ammonia (5 mL) and stirred for 12 h at 24 °C. The reaction mixture was concentrated and the crude material was purified by flash column chromatography using MeOH/CH₂Cl₂ (1:99 to 3:97) to afford 23 as a white solid (50 mg, 90% yield).

$^1$H-NMR (d$_6$-DMSO): $\delta$ 3.23 (s, 3H, CH₃), 4.33 (s, 1H, CH), 8.06 (s, 1H, ArCH) ppm.

$^{13}$C-NMR (d$_6$-DMSO): $\delta$ 36.90, 75.82, 85.74, 87.69, 151.10, 154.62, 164.73 ppm.

HR-ESI-MS: Calculated for C$_7$H$_7$N$_3$O 149.0589 found m/z 150.0662 [M + 1]$^+$. 
$^1$H NMR spectrum of 23:

$^{13}$C NMR spectrum of 23:
Spin label 8: To a solution of 23 (5 mg, 0.0335 mmol) in THF:H₂O:tBuOH:acetone (0.6 mL: 0.2 mL: 0.2 mL: 1 mL), 21 (7.6 mg, 0.335 mmol) was added, followed by CuI (2 mg, 0.0105 mmol). The resulting reaction mixture was stirred for 12 h at 70 °C and concentrated. The residue was purified by preparative TLC using 8% MeOH in CH₂Cl₂, giving 8 as a white solid (5 mg, 65% yield).

\(^1\)H NMR (CDCl₃ 5% CD₃OD): δ 1.21 (bs, 2H), 3.35 (bs, 1H, CH), 3.48 (bs, 3H, CH₃), 7.93 (s, 1H, ArCH), 8.04 (s, 1H, ArCH), 8.46 (s, 1H, ArCH) ppm.

HR-ESI-MS: Calculated for C₁₉H₂₂N₇O₂ 380.1835 found m/z 381.1908 [M + 1]⁺.
$^1$H NMR spectrum of 8:
**Compound 24:** A solution of 5-iodouracil (16) (1 g, 4.20 mmol) and K$_2$CO$_3$ (0.35 g, 2.5 mmol) in DMSO (10 ml) was stirred for an hour prior to addition of a solution of Z-protected 2-bromoethylamine (0.54 g, 2.10 mmol) in DMSO (50 ml). The resulting reaction mixture was stirred for 12 h at 23 °C, diluted with H$_2$O (50 ml) in an ice-bath, stirred for 5 min and extracted with CH$_2$Cl$_2$ (5 times x 50 mL). The organic layer was washed with water (50 mL) and brine (50 mL) and dried over Na$_2$SO$_4$. The residue was purified by column chromatography (1.5% MeOH in CH$_2$Cl$_2$) to yield the 24 as a white solid (0.45 g, 35% yield).

$^1$H NMR (CDCl$_3$, 10% CD$_3$OD): $\delta$ 3.33 (t, $J = 8$ Hz, 2H, CH$_2$), 3.77 (t, $J = 8$ Hz, 2H, CH$_2$), 5.01 (s, 2H, CH$_2$), 7.25 (m, 5H, ArCH), 7.62 (s, 1H, ArCH) ppm.

$^{13}$C NMR (CDCl$_3$, 10% CD$_3$OD): $\delta$ 149.67, 144.74, 136.22, 128.60, 128.46, 128.24, 127.98, 67.63, 67.03, 49.78, 49.57, 41.55, 39.36 ppm.

HR-ESI-MS: Calculated for C$_{14}$H$_{14}$IN$_3$O$_4$ 415.0029 found m/z 437.9901 [M + Na]$^+$. 
$^1$H NMR spectrum of 24:

$^{13}$C NMR spectrum of 24:
**Compound 25:** To a mixture of 24 (840 mg, 2.02 mmol), Pd(PPh₃)₂Cl₂ (142 mg, 0.20 mmol) and Cul (76 mg, 0.40 mmol) was added DMF (10 mL) and Et₃N (10 mL) and the solution degassed by bubbling argon through it for 10 min. The resulting reaction mixture was treated with trimethylsilyl acetylene (0.57 mL, 4.04 mmol) and stirred for 12 h at 25 °C. The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography using 99.5:0.5 to 99:1 CH₂Cl₂/MeOH to yield 25 as a pale brown solid (750 mg, 70% yield).

¹H NMR (CDCl₃): δ 0.22 (s, 9H, 3CH₃), 3.45 (t, J = 8 Hz, 2H, CH₂), 3.86 (t, J = 8 Hz, 2H, CH₂), 5.09 (s, 2H, CH₂), 7.34 (m, 5H, ArCH), 9.09 (s, 1H, ArCH) ppm.

¹³C NMR (CDCl₃): δ 161.74, 156.80, 150.08, 148.19, 136.25, 132.29, 132.19, 132.11, 128.76, 128.71, 128.59, 128.43, 128.25, 100.32, 94.97, 67.29, 49.15, 39.82, -0.02 ppm.

HR-ESI-MS: Calculated for C₁₉H₂₃N₃O₄Si 385.1458 found m/z 408.1340 [M + Na]⁺.
$^1$H NMR spectrum of 25:

$^{13}$C NMR spectrum of 25:
Compound 26: Compound 25 (150 mg, 0.38 mmol) was dissolved in conc. methanolic ammonia (10 mL) and resulting solution was stirred for 12 h at 25 °C in a sealed tube. The solvent was removed in vacuo and the residue was purified by column chromatography using 0.5% - 2% MeOH in CH₂Cl₂ to yield 26 a pale yellow solid (80 mg, 90% yield).

¹H NMR (CDCl₃): δ 3.15 (s, 1H, CH), 3.36 (t, J = 8 Hz, 2H, CH₂), 3.89 (t, J = 8 Hz, 2H, CH₂), 5.02 (s, 2H, CH₂), 7.28 (m, 5H, ArCH), 7.51 (s, 1H, ArCH) ppm.

¹³C NMR (CDCl₃): δ 157.10, 148.87, 136.25, 128.59, 128.23, 127.95, 98.66, 96.11, 82.14, 74.34, 67.01, 39.31 ppm.

HR-ESI-MS: Calculated for C₁₆H₁₅N₃O₄ 313.1063 found m/z 336.0935 [M + Na]^+.
$^1$H NMR spectrum of 26:

13C NMR spectrum of 26:
Compound 27: To a solution of 26 (50 mg, 0.15 mmol) in acetone (5 mL) was added 21 (37 mg, 0.16 mmol) followed by CuI (13 mg, 0.06 mmol). The resulting reaction mixture was stirred for 12 h at 60 °C and concentrated. The residue was purified by flash column chromatography (2% MeOH in CH$_2$Cl$_2$) to afford 27 as a pale yellow solid (51 mg, 65% yield).

$^1$H NMR (CDCl$_3$): $\delta$ 1.26 (bs, 1H, CH), 3.63 (bs, 2H, CH$_2$), 4.05 (bs, 2H, CH$_2$), 5.09 (s, 2H, CH$_2$), 7.30 (m, 3H, ArCH), 8.44 (s, 1H, ArCH), 8.71 (s, 1H, ArCH) ppm.

$^{13}$C NMR (CDCl$_3$): $\delta$ 156.14, 149.68, 141.30, 139.08, 135.56, 127.94, 127.48, 119.43, 99.18, 66.42, 49.25, 39.66, 30.35, 29.06 ppm.

HR-ESI-MS: Calculated for C$_{28}$H$_{30}$N$_7$O$_5$ 544.2308 found m/z 567.2202 [M + Na]$^+$. 
$^1$H NMR spectrum of 27:

$^{13}$C NMR spectrum of 27:
**Spin label 9:** A solution of 27 (36 mg, 0.06 mmol) in MeOH (6 mL) was hydrogenated over 10% Pd/C (10 mg) for 2 h at 30 psi pressure. The resulting reaction mixture was filtered through celite bed and solvent was removed *in vacuo*. The residue was dissolved in MeOH (5 mL) and treated with Cu(OAc)$_2$·H$_2$O (5 mg, 0.002 mmol) and stirred for 1 h at 24 °C in the presence of air. The reaction mixture was concentrated and residue was purified by preparative TLC using 10% MeOH in CH$_2$Cl$_2$ containing 1% aq. ammonia to yield 9 as a white solid (19 mg, 75% yield).

$^1$H NMR (CDCl$_3$, 5% CD$_3$OD): $\delta$ 1.14 (bs, 1H), 3.25 (bs, 2H, CH$_2$), 3.29 (bs, 1H, CH), 3.86-3.95 (bs, 2H, CH$_2$), 4.15 (s, 2H, CH$_2$), 7.28 (bs, 1H, ArCH), 8.31 (s, 1H, ArCH), 8.67 (s, 1H, ArCH) ppm.

HR-ESI-MS: Calculated for C$_{20}$H$_{24}$N$_7$O$_3$ 410.1914 found m/z 411.1946 [M + 1]$^+$. 
\(^1\)H NMR spectrum of 9:
**Compound 28:** A solution of 25 (250 mg, 0.46 mmol) in dry CH$_2$Cl$_2$ (20 mL) treated with TPS-Cl (589 mg, 1.94 mmol) and DMAP (8 mg, 0.064 mmol) at 0 °C. The resulting reaction mixture was stirred for 5 min and Et$_3$N (0.36 mL, 2.59 mmol) was added. The resulting solution was stirred for 2 h at 24 °C before dilution with CH$_2$Cl$_2$ (20 mL) and washed with water (20 mL), aqueous NaHCO$_3$ (20 mL) and brine (20 mL). The organic layer was dried over sodium sulphate and the solvent was removed under reduced pressure. The residue was dissolved in conc. methanolic NH$_3$ (7 mL) and stirred for 12 h in a sealed tube. The solvent was removed in vacuo and the residue was purified by column chromatography using 3% MeOH in CH$_2$Cl$_2$ to afford 28 as a pale yellow solid (50 mg, 25-30% yield).

$^1$H-NMR (CDCl$_3$): δ 3.24 (t, $J = 4$ Hz, 2H, CH$_2$), 3.32 (t, $J = 4$ Hz, 2H, CH$_2$), 3.38 (s, 1H, CH), 4.97 (s, 2H, CH$_2$), 7.22 (m, 5H, ArCH), 7.56 (s, 1H, ArCH) ppm.

$^{13}$C-NMR (CDCl$_3$): δ 157.19, 150.02, 136.31, 128.45, 128.04, 127.73, 90.31, 84.52, 73.83, 66.75, 49.99, 39.38 ppm.

HR-ESI-MS: Calculated for C$_{16}$H$_{16}$N$_4$O$_3$ 312.1222 found m/z 313.1257 [M + 1]$^+$. 
$^1$H NMR spectrum of 28:

$^{13}$C NMR spectrum of 28:
**Compound 29**: To a solution of 28 (41 mg, 0.13 mmol) in acetone (10 mL), compound 21 (40 mg, 0.17 mmol) was added followed by Cul (12.5 mg, 0.06 mmol). The resulting reaction mixture was stirred for 12 h at 60 °C and concentrated *in vacuo*. The residue was purified by flash column chromatography (3% MeOH in CH₂Cl₂) to afford 29 as a pale yellow solid (55 mg, 65% yield).

$^1$H NMR (CDCl₃): δ 0.94 (bs, 1H, CH), 1.31 (bs, 2H, CH₂), 3.6-3.97 (bs, 2H, CH₂), 5.04 (bs, 2H, CH₂), 7.17 (m, 4H, ArCH), 8.71 (s, 2H, ArCH) ppm.

HR-ESI-MS: Calculated for C$_{28}$H$_{31}$N$_8$O$_4$ 543.2468 found m/z 566.2202 [M + Na]$^+$. 
$^1$H NMR spectrum of 29:
Spin label 10: A solution of 29 (30 mg, 0.05 mmol) in MeOH (20 mL) was hydrogenated over 10% Pd/C (10 mg) for 2 h at 30 psi. The resulting reaction mixture was filtered through a celite bed and the solvent was removed in vacuo. The residue was dissolved in MeOH (10 mL) and treated with Cu(OAc)$_2$$\cdot$H$_2$O (5 mg, 0.002 mmol) and stirred for 1 h at 24 °C. The reaction mixture was concentrated and the residue was purified by preparative TLC using 10% MeOH in CH$_2$Cl$_2$ containing 1% aq. NH$_3$ to yield 10 as a white solid (20 mg, 75% yield).

$^1$H NMR (CDCl$_3$, 10% CD$_3$OD): $\delta$ 0.96 (bs, 1H), 3.14 (bs, 2H, CH$_2$), 4.00 (bs, 2H, CH$_2$), 4.34 (bs, 2H, CH$_2$), 8.49 (bs, 1H, ArCH), 8.95 (s, 1H, ArCH) ppm.

HR-ESI-MS: Calculated for C$_{20}$H$_{25}$N$_8$O$_2$ 409.2100 found m/z 410.2173 [M + 1]$^+$. 
$^1$H NMR spectrum of 10:
References