Liquid-phase Synthesis of N-Functionalized Azanucleoside-incorporated Oligonucleotides and Development of Anodic C(sp^3)-H Acetoxylation Reaction for Direct Preparation of Azaribose

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General information

$^1$H and $^{13}$C NMR spectra were recorded in CDCl$_3$ and DMSO-$d_6$ with either JEOL ECA-600 spectrometer ($^1$H 600 MHz, $^{13}$C 150 MHz) or JEOL ECA-400 spectrometer ($^1$H 400 MHz, $^{13}$C 100 MHz). Mass spectra were obtained on JEOL JMS T-100LP mass spectrometer. Cyclic voltammetry (CV) was carried out ALS electrochemical analyzer 611 DN. Measurement of oxidation potential was carried out in 1.0 M LiClO$_4$-EtNO$_2$ or MeNO$_2$ solution in the presence of 50 mM acetic acid using glassy carbon as anode, platinum wire as cathode and Ag/AgCl as reference electrode. TCI precoated silica gel F254 plates (thickness 0.25 mm) was used for thin-layer chromatography (TLC) analysis. All materials were obtained from TCI Fine Chemicals, Wako Pure Chemical Industries, Kanto Chemical, and Sigma Aldrich and used without purification. Fluorescence spectrum was recorded by JASCO FP-8300.

Abbreviations: Acr, Acryloyl; Moc, Methoxycarbonyl; Boc$_2$O, Di-tert-butyl dicarbonate; TFA, Trifluoroacetic acid; THF, Tetrahydrofuran; DMAP, 4-Dimethylaminopyridine; Ac$_2$O, Acetic anhydride; A(Bz)$_6$, N$_6$-benzoyladenine; C(Bz)$_4$, N$_4$-benzoylcytosine; T, Thymine; BSA, N,O-Bis(trimethylsilyl)acetamide; TMSOTf, Trimethylsilyl trifluoromethanesulfonate, DMTr, Dimethoxytrityl; DIPCI: Diisopropylcarbodiimide, HOBt: 1-Hydroxybenzotriazole, ACSS: Alkyl-Chain-Soluble-Support, BMT: 5'-(benzylmercapto)-1H-tetrazole, NMI: N'-methylimidazole, DCA: Dichloroacetic acid, BPO: Butanone peroxide. CE: Cyanoethyl
Preparation of pyrenemethanethiol

![Scheme S1](image)

**pyren-1-ylmethyl carbamimidothioate hydrobromide (10)**

1-Pyrenemethanol (698 mg, 3 mmol) was suspended to toluene (30 mL) and cooled to 0 °C. PBr$_3$ (400 µL, 4.2 mmol) was dropwisely added to resulting solution and stirred for 2 h under Ar in dark. Resulting solution was neutralized with saturated NaHCO$_3$ aq. and extracted with toluene (15 mL x 3). The organic layer was washed with H$_2$O (15 mL x 2), brine (15 mL x 1) and dried over anhydrous MgSO$_4$. After concentrated in vacuo, resulting yellow solid was suspended in 95% EtOH-THF (v/v = 3:1, 20 mL). The solution was added thiourea (233.6 mg, 3.3 mmol) and stirred at reflux for 14 h under Ar in dark. Resulting mixture was cooled on ice and added diethyl ether (20 mL). The precipitate was filtrated and washed with cold ether. After dried in vacuo give compound 10 as white solid (2.8 mmol, 93% yield).

$^1$H-NMR (400 MHz, DMSO-d$_6$) δ 9.19 (s, 1H), 8.49 (d, J = 9.6 Hz, 1H), 8.12-8.39 (m, 9H), 5.28 (s, 2H)

$^{13}$C-NMR (151 MHz, DMSO-d$_6$) δ 169.38, 131.45, 131.11, 130.60, 129.14, 128.77, 128.67, 128.30, 127.97, 127.70, 127.05, 126.23, 126.12, 125.44, 124.61, 124.09, 123.49, 33.46

HRMS (ESI$^+$) calc. for [C$_{18}$H$_{15}$N$_2$S]$^+$: 291.0956, Found: 291.0926

Melting point: 229 °C (decomposed)
1-pyrenemethanethiol (11)

Compound 10 (145.2 mg, 0.5 mmol) was suspended to 6% NaOH aq. (10 mL) and stirred at 43 °C for 1 h under Ar in dark. After cooled to room temperature, resulting mixture was acidified with 1 N HCl aq. (20 mL) and extracted with CHCl₃ (15 mL x 3). The organic layer was washed with H₂O (15 mL x 2) and dried over anhydrous MgSO₄. The crude product was purified by column chromatography on the silica gel with Hex:EtOAc = 10:1 as eluent give compound 11 as yellow solid (quantitative yield).

^1H-NMR (600 MHz, CDCl₃) δ 8.33 (d, J = 8.9 Hz, 1H), 8.13-8.22 (m, 4H), 7.96-8.07 (m, 4H), 4.49 (d, J = 7.6 Hz, 2H), 1.99 (t, J = 6.9 Hz, 1H)

^13C-NMR (151 MHz, CDCl₃) δ 134.63, 131.30, 130.82, 130.80, 127.97, 127.40, 127.32, 126.77, 126.07, 125.33, 125.24, 125.05, 122.84, 27.00

HRMS (DART⁺) calc. for [C₁₇H₁₃S]⁺: 249.0738, Found: 249.0746

Melting point: 126 °C
Synthesis of ACSS-azanucleoside unit

\((2R,3S,5S)-3\text{-acetoxyl}-5\text{-}(\text{5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl})\text{-}1\text{-}3\text{-ethylthio)-3,4-dihydropyrimidin-1(2H)-yl})\text{-}1\text{-}3\text{-propionamido)-3,4-dihydropyrimidin-1(2H)-yl})\text{-}3\text{-acetoxyl-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl})-1-(3-((pyren-1-ylmethyl)thio)propanoyl)pyrrolidin-2-yl)methyl acetate (2)\)

Compound 1 (0.27 mmol, 102 mg) was dissolved in CH\(_2\)Cl\(_2\)-MeOH (v/v = 1:1, 10 mL). Thiol 11 (0.8 mmol), DIPEA (1.2 mmol, 209 \(\mu\)L) was added to resulting solution and stirred for 12 h under Ar atmosphere in dark. Resulting mixture was concentrated in vacuo. The crude product was purified by column chromatography on the silica gel with Hex:EtOAc=1:5 as eluent give compound 2 as yellow solid (0.25 mmol, 93% yield).

\(^1\text{H-NMR (600 MHz, CDCl}_3\text{)}\)  \(\delta\) 9.43 (s, 1H), 8.25-8.35 (m, 1H), 7.86-8.22 (m, 8H), 7.65-7.68 (m, 1H), 7.50 (m, 1H), 7.08-7.11 (m, 1H), 6.31 (dd, \(J = 13.4, 7.9\) Hz, 1H), 5.06-5.23 (m, 1H), 4.28-4.60 (m, 3H), 4.16-4.23 (m, 1H), 2.48-2.90 (m, 4H), 1.76-2.11 (m, 10H)

\(^{13}\text{C-NMR (151 MHz, CDCl}_3\text{)}\)  \(\delta\) 171.33, 170.17, 170.08, 169.20, 163.45, 150.43, 135.20, 134.64, 132.05, 131.99, 131.91, 131.26, 131.15, 130.76, 130.68, 128.76, 128.72, 128.49, 128.42, 127.77, 127.67, 127.62, 127.44, 127.33, 127.29, 127.24, 126.17, 125.99, 125.42, 125.19, 125.11, 125.05, 124.61, 124.55, 124.50, 123.18, 123.09, 123.00, 110.95, 74.78, 74.17, 73.77, 73.63, 68.77, 68.58, 64.83, 63.09, 62.84, 62.42, 60.97, 38.87, 36.13, 35.21, 34.87, 34.78, 34.44, 32.88, 28.40, 27.19, 26.61, 20.88, 20.82, 20.75, 20.65, 20.58, 12.57, 12.50, 12.43

HRMS (ESI\(^+\)) calc. for [C\(_{34}H_{33}N_3NaO_7S]\(^+\) : 650.1973, Found: 650.1930
1-((2S,4S,5R)-5-(((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-1-(3-((pyren-1-ylmethyl)thio)propanoyl)pyrrolidin-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (3)

Compound 2 (156 mg, 0.25 mmol) was dissolved to MeOH·H₂O (v/v = 10:1, 5 mL). K₂CO₃ (69.1 mg, 0.5 mmol) was added to solution and stirred at room temperature for 1 h in dark. The reaction mixture was neutralized with amberlite and filtrated. The filtrate was concentrated in vacuo and dissolved in CH₂Cl₂. Catalytic amount of DMAP, NEt₃ (116 µL, 0.83 mmol), DMTr-Cl (281.2 mg, 0.83 mmol) was added to solution and stirred at 45 °C for 12 h under Ar atmosphere in dark. The reaction was quenched with MeOH (15 mL) and stirred for 15 min. After concentrated in vacuo, the crude product was purified by column chromatography on the silica gel Hex:EtOAc=1:5 as eluent give a compound 3 as yellow solid (0.21 mmol, 84% yield).

¹H-NMR (600 MHz, CDCl₃) δ 10.00-10.49 (m, 1H), 7.80-8.30 (m, 10H), 7.34-7.41 (m, 1H), 7.25 (m, 3H), 7.07-7.22 (m, 4H), 6.68-6.82 (m, 5H), 6.02-6.31 (m, 1H), 4.30-4.48 (m, 4H), 3.59-3.78 (m, 8H), 3.29-3.41 (m, 1H), 2.45-2.94 (m, 5H), 1.87-2.38 (m, 4H)

¹³C-NMR (151 MHz, CDCl₃) δ 171.13, 170.99, 164.45, 164.15, 158.54, 158.40, 151.11, 151.02, 144.72, 144.10, 136.90, 135.61, 135.50, 135.17, 134.96, 131.59, 131.15, 130.76, 130.70, 130.67, 129.88, 129.85, 129.79, 128.72, 128.67, 127.94, 127.85, 127.77, 127.70, 127.64, 127.32, 127.13, 126.98, 126.81, 126.10, 125.89, 125.30, 125.26, 125.13, 125.07, 124.64, 124.61, 124.55, 124.51, 123.15, 113.21, 113.08, 109.71, 86.93, 86.42, 73.17, 71.92, 70.11, 68.26, 67.56, 63.06, 60.66, 55.12, 55.07, 55.05, 40.56, 38.24, 35.17, 34.77, 34.69, 27.89, 26.38, 12.41, 12.30

Compound 3 (62 mg, 0.073 mmol) was dissolved in CH₂Cl₂ (5 mL). NEt₃ (71.2 µL, 0.51 mmol), catalytic amount of DMAP, succinic anhydride (33 mg, 0.33 mmol) was added to solution and stirred at room temperature for 12 h under Ar atmosphere in dark. Resulting mixture was added saturated NH₄Cl aq. and extracted with CH₂Cl₂ (5 mL x 3). The organic layer was dried over anhydrous Na₂SO₄ and concentrated in vacuo. Resulting crude was dissolved in CH₂Cl₂ (5 mL) and DIPEA (38.3 µL, 0.22 mmol), ACSS • HCl (74.3 mg, 0.073 mmol), DIPCI (20.1 µL, 0.13 mmol), HOBt (12.2 mg, 0.09 mmol), catalytic amount of DMAP was added to solution. Resulting mixture was stirred at room temperature for 14 h. After the reaction, solvent was removed in vacuo to half volume and MeOH was added. After 2 times repeat this cycle, resulting precipitate was filtrated and washed with MeOH. The precipitate was collected and dried in vacuo. Compound 4 was obtained as white solid (0.06 mmol, 82% yield).

**¹H-NMR (600 MHz, CDCl₃)** δ 7.84-8.30 (m, 8H), 7.30 (m, 2H), 7.11-7.24 (m, 5H), 6.78 (dd, J = 8.6, 6.5 Hz, 3H), 6.58 (t, J = 6.9 Hz, 2H), 5.20 (m, 1H), 4.36-4.54 (m, 2H), 3.93-3.97 (m, 7H), 3.63-3.77 (m, 6H), 3.06-3.17 (m, 1H), 2.58-2.85 (m, 3H), 2.28-2.47 (m, 2H), 2.04-2.22 (m, 1H), 1.72-1.85 (m, 10H), 1.26-1.30 (m, 109H), 0.88 (dd, J = 7.6, 6.2 Hz, 11H)

**¹³C-NMR (151 MHz, CDCl₃)** δ 172.53, 172.13, 170.68, 170.53, 163.95, 158.58, 158.44, 153.18, 153.05, 150.49, 144.35, 143.82, 139.64, 135.47, 135.42, 135.34, 134.89, 134.83, 131.11, 130.74, 130.70, 129.86, 129.81, 129.78, 128.73, 128.61, 127.99, 127.84, 127.78, 127.73, 127.61, 127.31, 127.18, 127.02, 126.87, 126.03, 125.92, 125.22, 125.15, 125.06, 124.65, 124.56, 124.49, 123.10, 113.27, 113.13, 108.74, 105.57, 105.49, 87.18, 86.63, 76.43, 74.98, 73.45, 69.46, 69.21, 65.97, 64.32, 55.11, 55.05, 35.01, 34.62, 31.86, 30.25, 29.66, 29.60, 29.55, 29.36, 29.32, 29.27, 27.58, 26.28, 26.04, 22.63, 14.07, 12.48, 12.34

Deprotection process of DMTr group was carried out by general procedure. Compound 4 (84.3 mg, 0.052 mmol) was dissolved to CH$_2$Cl$_2$ (10 mL) and added 3% DCA solution of CH$_2$Cl$_2$ (4.1 mL) stirred at room temperature for 3 min, resulting solution was added MeOH and neutralized with NEt$_3$ (1.49 mmol, 209 µL). After concentrated in vacuo, resulting precipitate was filtrated and washed with MeOH. Compound 8 was obtained as pink solid (0.049 mmol, 94% yield).

$^1$H-NMR (600 MHz, CDCl$_3$) $\delta$ 8.69 (s, 1H), 8.30 (d, $J = 9.6$ Hz, 1H), 7.98-8.19 (m, 7H), 7.86-7.90 (m, 1H), 7.40 (m, 1H), 6.58 (m, 3H), 6.34 (m, 1H), 5.08-5.30 (m, 3H), 4.41-4.49 (m, 3H), 3.95 (q, $J = 6.4$ Hz, 8H), 3.70 (d, $J = 9.6$ Hz, 1H), 3.46 (s, 1H), 3.10-3.16 (m, 1H), 2.73-2.86 (m, 2H), 2.57 (m, 1H), 2.44 (m, 1H), 2.28-2.37 (m, 2H), 1.84 (s, 2H), 1.71-1.81 (m, 9H), 1.44 (q, $J = 7.3$ Hz, 7H), 1.27 (s, 93H), 0.88 (t, $J = 6.9$ Hz, 6H)

$^{13}$C-NMR (151 MHz, CDCl$_3$) $\delta$ 172.33, 171.76, 170.70, 170.14, 169.87, 168.61, 163.80, 153.23, 139.59, 135.56, 131.24, 131.02, 130.74, 128.81, 127.77, 127.36, 126.08, 125.32, 124.59, 123.07, 110.64, 105.64, 75.46, 73.53, 69.29, 67.62, 60.56, 53.41, 38.65, 34.78, 34.30, 31.91, 30.29, 29.71, 29.64, 29.41, 29.35, 28.32, 27.27, 26.08, 22.67, 14.10, 12.48

**Synthesis of aza-oligonucleotides by ACSS method**

5’-DMTr-d[A(Bz)T(Aza)]-3’-ACSS (9)

Condensation process with phosphoramidite was carried out by general procedure. Compound 8 (0.049 mmol, 79.4 mg) was dissolved in CH₂Cl₂ (4.5 mL). Resulting solution was added 0.25 M BMT solution in MeCN (500 μL), dA(Bz) phosphoramidite (0.1 mmol, 85.8 mg), and stirred for 10 min under Ar. 2.4 mL of capping solution (premix of 25 μL pyridine, Ac₂O, NMI in CH₂Cl₂) was added and stirred for 10 min. 2.7 mL of oxidation premix (23 μL of 55% BPO sol. in CH₂Cl₂) was added and stirred for 30 min. After these reactions, reaction mixture was added MeOH. After concentrated in vacuo, precipitate was filtrated and washed with MeOH. Compound 9 was obtained as pink solid (0.047 mmol, 96% yield).

**1H-NMR (600 MHz, CDCl₃)** δ 8.63-8.65 (m, 1H), 7.87-8.28 (m, 9H), 7.32-7.61 (m, 4H), 7.18-7.25 (m, 2H), 6.77 (m, 3H), 6.56-6.59 (m, 2H), 5.26 (m, 1H), 4.65 (s, 1H), 4.34-4.42 (m, 1H), 3.93-3.96 (m, 6H), 3.72-3.77 (m, 4H), 3.37-3.63 (m, 5H), 2.50-2.81 (m, 4H), 1.99-2.33 (m, 2H), 1.72-1.83 (m, 7H), 1.60 (s, 34H), 1.45 (s, 6H), 1.25-1.29 (m, 90H), 0.87 (t, J = 6.9 Hz, 10H)

**13C-NMR (101 MHz, CDCl₃)** δ 169.23, 158.64, 153.26, 145.78, 143.88, 139.72, 137.99, 136.93, 132.50, 130.03, 128.84, 128.11, 127.92, 127.35, 125.27, 124.60, 113.20, 92.35, 86.47, 77.61, 73.53, 69.32, 55.24, 34.94, 31.92, 29.72, 29.66, 29.36, 26.10, 22.68, 14.11

**HRMS (ESI+)** calc. for [C₁₄₀H₁₉₀N₁₁NaO₁₉PS]⁺: 2415.3595, Found: 2415.3577
Same elongation cycle was carried for compound 9 with dC(Bz) phosphoramidite and gave 5 as white solid. (94% yield)

$^1$H-NMR (600 MHz, CDCl$_3$) δ 8.74 (m, 1H), 7.86–8.27 (m, 9H), 7.36–7.60 (m, 4H), 6.57–6.59 (m, 2H), 5.29 (m, 1H), 4.16–4.44 (m, 3H), 3.91–3.96 (m, 7H), 3.53–3.83 (m, 5H), 3.13 (q, $J$ = 7.3 Hz, 1H), 2.65–2.82 (m, 5H), 2.17 (s, 1H), 1.72–1.83 (m, 23H), 1.45 (s, 6H), 1.25–1.33 (m, 105H), 0.88 (t, $J$ = 6.9 Hz, 10H)

$^{13}$C-NMR (151 MHz, CDCl$_3$) δ 170.56, 157.93, 153.27, 139.76, 135.25, 132.93, 128.79, 128.01, 127.77, 127.33, 126.13, 125.37, 124.62, 123.14, 105.71, 73.56, 69.34, 45.43, 31.93, 30.33, 29.73, 29.67, 29.44, 29.38, 26.12, 22.70, 14.13, 8.51

$^1$H and $^{13}$C-NMR spectra

**Compound 10**
Compound 11

- Parts per Million (Proton):
  - X-axis: 0 to 10.0
  - Y-axis: 0 to 100

- Parts per Million (Carbon13):
  - X-axis: 0 to 140.0
  - Y-axis: 0 to 100

Diagram includes chemical structure and peaks with corresponding abundances.
Compound 2

[Image of a compound structure with chemical bonds and labels such as AcO, S, N, NH, and O]

[Graph with peaks at various X: parts per Million : Proton and X: parts per Million : Carbon13 values]
Compound 3
Compound 8
Compound 9
Compound 10