Highly Efficient Synthesis of Allopurinol Locked Nucleic Acid Monomer by C6 Deamination of 8-Aza-7-bromo-7-deazaadenine Locked Nucleic Acid Monomer

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Abstract: An allopurinol locked nucleic acid (LNA) monomer was prepared by a novel strategy through C6 deamination of the corresponding 8-aza-7-bromo-7-deazaadenine LNA monomer with aqueous sodium hydroxide. An 8-aza-7-deazaadenine LNA monomer was also synthesized by a modification of the new synthetic pathway. *N*-Glycosylation at the 8-position was prevented by steric hindrance from the 7-bromo atom in the starting material 8-aza-7-bromo-7-deazaadenine. In the final step of the synthesis, the bromine was removed together with a benzyl protecting group by catalytic reduction with ammonium formate to give the required LNA monomers.

Key words: glycosylations, nucleobases, nucleosides, regioselectivity

In the last two decades, a great deal of interest has been focused on the synthesis and design of modified oligonucleotides, with the aim of developing strong recognition of complementary DNA or RNA sequences.[1–3] Many modified oligonucleotides containing bi- and tricyclic carbohydrate-modified nucleotides have been studied, and their duplexes with complementary nucleic acids have been shown to be much more stable than those of the corresponding unmodified duplexes.[3] Bicyclic ribonucleotides containing a 2′-*O*,4′-*C*-methylene bicyclic sugar moiety were named 'locked nucleic acids' (LNA) when they were introduced in 1998.[4] The corresponding oligonucleotides show unprecedented thermal stabilities when hybridized with complementary DNA and RNA strands, increasing the melting temperatures of the duplexes in comparison with those of their unmodified analogues.[4b,5–7] However, the thermal stability of oligonucleotide duplexes can be also be improved by modifying the nucleobase, as well as by modifying carbohydrate moiety. Replacing adenine with 8-aza-7-deazaadenine slightly increased the stability of duplexes, whereas the incorporation of 7-substituted derivatives leads to duplexes that are much more stable than their adenine-containing counterparts.[8,9]

The glycosylation of allopurinol is a problem in nucleoside synthesis, because the reaction lacks regioselectivity and gives poor yields.[10–13] To improve the regioselectivity of the reaction, allopurinol ribonucleoside have been synthesized from starting materials other than allopurinol.[13–15] For example, 4,6-dichloro-1*H*-pyrazolo[3,4-*d*]pyrimidine has been used in the presence of trimethylsilyl triflate as a catalyst, but additional synthetic steps are required to prepare the target compound.[15] Also, 4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine[13] and 4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine[14] have been glycosylated by using phase-transfer methods, but both the *N*-8 and *N*-9 glycosylated products were formed. This lack of regioselectivity also occurred in glycosylation of 8-aza-7-deazaadenine with 1,2-di-*O*-acetyl-3-*O*-benzyl-4-*C*-[(mesyloxy)methyl]-5-*O*-mesyl-D-*erythro*-pentofuranose (**2**) in the presence of trimethylsilyl triflate.[16] When we searched the literature for possible solutions to this problem, we found that 7-halogenated 8-aza-7-deazapurine-2,6-diamines can be glycosylated in the presence of the Lewis acid boron trifluoride etherate ($BF_3{\cdot}Et_2O$) to give the *N*-9 isomer exclusively.[17] Glycosylation at the *N*-8 position is prevented by the electron-withdrawing effect of the 7-halo group and by steric hindrance.[17] We therefore decided to use 8-aza-7-bromo-7-deazaadenine[18] (**1**) as a starting material for the synthesis of allopurinol LNA and 8-aza-7-deazaadenine LNA through a pathway involving an *N*-9 selective glycosylation followed by debromination.

We achieved selective *N*-9 glycosylation of 8-aza-7-bromo-7-deazaadenine (**1**) with 1,2-di-*O*-acetyl-3-*O*-benzyl-4-*C*-[(mesyloxy)methyl]-5-*O*-mesyl-D-*erythro*-pentofuranose (**2**)[4g] in the presence of two equivalents of tin(IV) chloride as a Lewis acid in refluxing acetonitrile. The nucleoside **3** (the *N*-9 isomer) was separated in 62% yield as the only product, and no traces of the *N*-8 isomer were detected (Scheme 1).

Treatment of nucleoside **3** with 2 M aqueous sodium hydroxide in 1,4-dioxane with stirring at room temperature for one hour resulted in deacetylation and ring closure of the sugar moiety to give the LNA monomer **4** in 88% yield. No chromatography was necessary during the workup, and the product could be used in the next step without additional purification. Benzoate displacement of the 5′-mesylate group in **4** gave the corresponding 5′-*O*-benzoyl LNA monomer **5** in 84% yield (Scheme 1).

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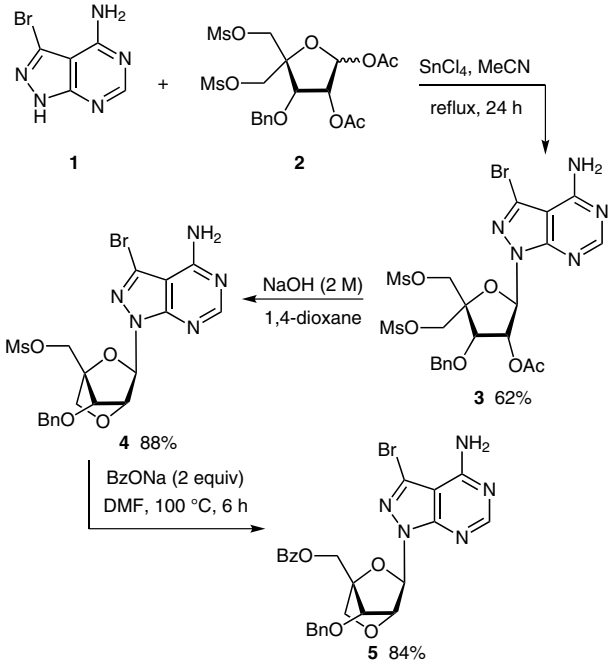
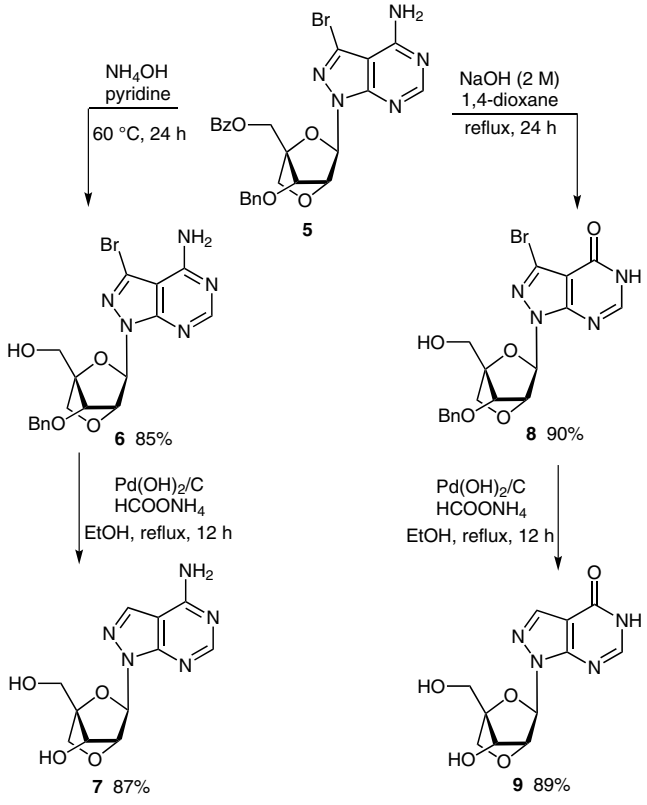


Scheme 1

Enzymatic C6 deamination of adenosine analogues is a well-known reaction.[19–25] Density functional theory calculations have shown that 8-aza-7-deazapurine is more readily hydrated than are simple purines.[25] Furthermore, the hydrate of 8-aza-7-bromo-7-deazapurine has been shown to be more stable than that of its 7-unsubstituted analogue.[25] Accordingly, we expected C6 deamination of 8-aza-7-bromo-7-deazaadenine to take place on treatment with hydroxide ion. Indeed, when compound **5** was refluxed in 2 M aqueous sodium hydroxide and 1,4-dioxane for 24 hours, both 5′-O-debenzoylation and C6 deamination occurred to give the deprotected keto derivative **8** in about 90% yield, but when compound **5** was treated with a mixture of concentrated ammonium hydroxide and pyridine at 60 °C for 24 hours, the 5′-O-debenzoylated product **6** was obtained in 85% yield, and no C6 deamination occurred (Scheme 2).



Scheme 2

Removal of the 3′-*O*-benzyl group and debromination of compounds **6** and **8** was achieved through catalytic transfer hydrogenation by stirring the compounds with ammonium formate and palladium(II) hydroxide/carbon in refluxing methanol. This gave the target products **7** and **9**, respectively (Scheme 2). The ^{1}H NMR and ^{13}C NMR spectra of compound **7** agreed with previously published data,[16] and the NMR spectra of the allopurinol part of **9** corresponded well with previously published data for allopurinol nucleosides.[13]

In summary, we have developed a highly efficient method for synthesizing the allopurinol LNA monomer **9** through C6 deamination of the 8-aza-7-bromo-7-deazaadenine LNA monomer **5**. The new method helps to overcome problems associated with lack of regioselectivity and low yields in glycosylation of allopurinol. In parallel, a new synthetic pathway for 8-aza-7-deazaadenine LNA monomer **7** was developed, in which the corresponding bromo derivative **6** was debrominated after reduction with palladium(II) hydroxide/carbon and ammonium formate.

NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer at 400 MHz for ^{1}H and at 101 MHz for ^{13}C with TMS as an internal standard; chemical shifts (δ) are quoted in ppm. Electrospray ionization high-resolution mass spectra were recorded on a PE SCIEX API Q-Star Pulsar spectrometer. For accurate determinations of ion masses, the $[M + H]^+$ or $[M + Na]^+$ ion was peak matched by calibration with NaI.

(2*R*,3*R*,4*S*)-2-(4-Amino-3-bromo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-4-(benzyloxy)-5,5-bis[(mesyloxy)methyl]tetrahydrofuran-3-yl Acetate (3)

8-Aza-7-bromo-7-deazaadenine[18] (**1**; 2.83 g, 13.22 mmol) and 1,2-di-*O*-acetyl-3-*O*-benzyl-4-*C*-[(mesyloxy)methyl]-5-*O*-mesyl-D-*erythro*-pentofuranose[4g] (**2**; 6.75 g, 13.22 mmol) were placed in a dried flask under argon. Anhyd MeCN (50 mL) was added by syringe, and the mixture was stirred for 15 min. $SnCl_4$ (3.10 mL, 26.4 mmol) was then added by syringe, and the mixture was refluxed for 24 h then cooled to r.t. The reaction was quenched with sat. aq $NaHCO_3$ (50 mL), and the mixture was extracted with EtOAc (3 × 100 mL). The organic layers were combined, dried (Na_2SO_4), concentrated, and purified by flash column chromatography [silica gel, MeOH–CH_2Cl_2 (1:25)] to give a white solid; yield: 62% (5.50 g); mp 90–92 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.26 (s, 1 H), 7.44–7.28 (m, 5 H), 6.45 (d, *J* = 2.6 Hz, 1 H), 5.80 (dd, *J* = 5.5, 2.6 Hz, 1 H), 4.89 (d, *J* = 5.5 Hz, 1 H), 4.69 (d, *J* = 11.2 Hz, 1 H), 4.62 (d, *J* = 11.1 Hz, 1 H), 4.53 (d, *J* = 11.1 Hz, 1 H), 4.47 (d, *J* = 11.0 Hz, 1 H), 4.38 (d, *J* = 10.7 Hz, 1 H), 4.29 (d, *J* = 10.8 Hz, 1 H), 3.21 (s, 3 H), 3.12 (s, 3 H), 2.09 (s, 3 H).

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^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.37, 157.39, 157.29, 154.75, 137.00, 128.28, 128.16, 127.89, 120.60, 99.70, 86.03, 83.04, 78.21, 73.35, 73.23, 68.33, 68.04, 36.79, 36.52, 20.42.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{22}H_{26}BrN_5NaO_{10}S_2$: 686.0197; found: 686.0172.

[(1*R*,3*R*,4*R*,7*S*)-3-(4-Amino-3-bromo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-7-(benzyloxy)-2,5-dioxabicyclo[2.2.1]hept-1-yl]methyl Mesylate (4)

2 M aq NaOH (10 mL) was added to a solution of acetate **3** (5.0 g, 7.52 mmol) in 1,4-dioxane (40 mL). The solution was stirred at r.t. for 1 h, and then the solvents were removed under reduced pressure. The residue was suspended in CH_2Cl_2 (150 mL), washed with sat. aq $NaHCO_3$ (150 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give a white solid; yield: 88% (3.50 g). This was used in the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.32 (s, 1 H), 7.45–7.26 (m, 5 H), 6.36 (s, 1 H), 5.00 (s, 1 H), 4.78 (d, *J* = 11.8 Hz, 1 H), 4.72 (d, *J* = 11.8 Hz, 1 H), 4.57 (d, *J* = 12.1 Hz, 1 H), 4.51 (d, *J* = 12.1 Hz, 3 H), 4.48 (s, 1 H), 4.19 (d, *J* = 7.9 Hz, 1 H), 4.01 (d, *J* = 7.9 Hz, 1 H), 3.02 (s, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.42, 157.38, 155.16, 136.95, 128.61, 128.50, 128.33, 120.19, 100.49, 84.62, 84.38, 79.02, 78.08, 72.72, 72.46, 65.82, 37.77.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{19}H_{21}BrN_5O_6S$: 526.0390; found: 526.0388.

[(1*R*,3*R*,4*R*,7*S*)-3-(4-Amino-3-bromo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-7-(benzyloxy)-2,5-dioxabicyclo[2.2.1]hept-1-yl]methyl Benzoate (5)

NaOBz (1.75 g, 12 mmol) was added to a solution of mesylate **4** (3.2 g, 6 mmol) in anhyd DMF (50 mL). The mixture was stirred at 100 °C for 6 h then cooled to r.t., filtered, and concentrated under reduced pressure. The residue was suspended in EtOAc (100 mL), washed with sat. aq $NaHCO_3$ (3 × 50 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give the desired product as a white solid; yield: 84% (2.80 g).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31 (s, 1 H), 7.94 (d, *J* = 7.2 Hz, 2 H), 7.55 (t, *J* = 7.4 Hz, 1 H), 7.43–7.28 (m, 7 H), 6.42 (s, 1 H), 5.19 (s, 1 H), 4.81–4.66 (m, 3 H), 4.57–4.42 (m, 2 H), 4.25 (d, *J* = 7.8 Hz, 1 H), 4.08 (d, *J* = 7.7 Hz, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.98, 157.37, 157.26, 155.18, 137.13, 133.11, 129.82, 128.51, 128.40, 128.15, 127.87, 127.57, 120.01, 100.49, 84.71, 84.52, 78.88, 78.05, 72.64, 72.62, 60.55.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{25}H_{23}BrN_5O_5$: 552.0877; found: 552.0865.

[(1*S*,3*R*,4*R*,7*S*)-3-(4-Amino-3-bromo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-7-(benzyloxy)-2,5-dioxabicyclo[2.2.1]hept-1-yl]methanol (6)

Concd aq NH_4OH (8 mL) was added to a solution of benzoate **5** (0.55 g, 1 mmol) in pyridine (4 mL), and the solution was heated at 60 °C for 24 h. The mixture was then concentrated under reduced pressure, co-evaporated with toluene, and purified by column chromatography [silica gel, CH_2Cl_2–MeOH (30:1)] to give a white solid; yield: 85% (0.38 g); mp 160–162 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.26 (s, 1 H), 7.46–7.29 (m, 5 H), 6.13 (s, 1 H), 4.96 (t, *J* = 5.2 Hz, 1 H), 4.81 (s, 1 H), 4.66 (s, 2 H), 4.35 (s, 1 H), 3.96 (d, *J* = 7.9 Hz, 1 H), 3.83 (d, *J* = 7.9 Hz, 1 H), 3.74 (dd, *J* = 5.2, 12.7 Hz, 1 H), 3.68 (dd, *J* = 12.9, 5.2 Hz, 1 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 157.32, 157.13, 154.64, 137.70, 128.19, 128.16, 127.85, 119.76, 99.31, 87.00, 83.70, 78.41, 77.35, 72.04, 71.17, 57.14.

HRMS (ESI): *m/z* [M + Na]$^+$ calcd for $C_{18}H_{18}BrN_5NaO_4$: 470.0434; found: 470.0431.

(1*S*,3*R*,4*R*,7*S*)-3-(4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptan-7-ol (7)

Bromo compound **6** (0.35 g, 0.78 mmol) was suspended in MeOH (10 mL), and the mixture was cooled 0 °C. 20% $Pd(OH)_2$/C (0.25 g) was added in portions during 10 min and then the mixture was allowed to stand for 10 min. HCO_2NH_4 (0.75 g) was added in small portions, and then the mixture was allowed to warm to r.t. for 1 h. The solution was then refluxed for 6 h, more HCO_2NH_4 (0.5 g) was added, and the mixture was refluxed for a further 6 h. The hot solution was filtered through a Celite pad that was washed with boiling MeOH. Evaporation of the filtrate gave white crystals; yield: 87% (0.19 g); mp 244–246 °C (MeOH).

^{1}H NMR (400 MHz, CD_3OD): δ = 8.20 (s, 1 H), 8.13 (s, 1 H), 6.25 (s, 1 H), 5.11 (s, 1 H), 4.37 (s, 1 H), 4.10 (d, *J* = 8.0 Hz, 1 H), 3.95 (d, *J* = 8.0 Hz, 1 H), 3.88 (s, 2 H).

^{13}C NMR (100 MHz, CD_3OD): δ = 159.86, 157.16, 155.22, 134.81, 101.84, 88.95, 85.93, 82.14, 73.82, 73.12, 59.45.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{11}H_{14}N_5O_4$: 280.1040; found: 280.1036.

1-{(1*S*,3*R*,4*R*,7*S*)-7-(Benzyloxy)-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]hept-3-yl}-3-bromo-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8)

2 M aq NaOH (25 mL) and H_2O (7 mL) were added to a solution of benzoate **5** (2.7 g, 4.9 mmol) in 1,4-dioxane (25 mL). The mixture was refluxed for 24 h, cooled to r.t., and neutralized with AcOH (4 mL). Sat. aq $NaHCO_3$ (25 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 100 mL). The organic layers were combined, dried (Na_2SO_4), and concentrated under reduced pressure to give a white solid; yield: 90% (2.00 g). The product was used in the next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.17 (s, 1 H), 7.45–7.31 (m, 5 H), 6.07 (s, 1 H), 4.79 (s, 1 H), 4.71 (d, *J* = 11.9 Hz, 1 H), 4.67 (s, 1 H), 4.66 (d, *J* = 11.9 Hz, 1 H), 4.50 (s, 1 H), 3.96 (d, *J* = 8.0 Hz, 1 H), 3.83 (d, *J* = 7.9 Hz, 1 H), 3.74 (d, *J* = 12.8 Hz, 1 H), 3.69 (d, *J* = 12.6 Hz, 1 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 156.32, 153.47, 150.12, 137.66, 128.18, 127.87, 127.61, 122.64, 104.79, 87.24, 83.97, 78.34, 77.32, 72.02, 71.21, 57.09.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{18}H_{18}BrN_4O_5$: 449.0455; found: 449.0445.

1-[(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]hept-3-yl]-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (9)

20% $Pd(OH)_2$/C (1.28 g) and HCO_2NH_4 (3.8 g) were added to a suspension of compound **8** (1.8 g, 4 mmol) in MeOH (30 mL), and the solution was refluxed for 6 h. More HCO_2NH_4 (1.5 g) was then added and the mixture was refluxed for a further 6 h. The hot solution was filtered through a Celite pad that was washed with boiling MeOH. Evaporation of the filtrate gave white crystals; yield: 89% (1.00 g); mp 196–198 °C (MeOH).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.35 (br s, 1 H), 8.19 (s, 1 H), 8.14 (s, 1 H), 6.07 (s, 1 H), 5.70 (br s, 1 H), 4.89 (s, 1 H), 4.84 (br s, 1 H), 4.32 (s, 1 H), 3.96 (d, *J* = 7.8 Hz, 1 H), 3.80 (d, *J* = 7.8 Hz, 1 H), 3.73 (d, *J* = 12.7 Hz, 1 H), 3.68 (d, *J* = 12.7 Hz, 1 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 157.00, 152.69, 148.69, 135.59, 105.91, 87.58, 83.80, 79.91, 71.88, 71.57, 57.54.

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{11}H_{13}N_4O_5$: 281.0880; found: 281.0871.

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