Synthesis of 3-(β-D-Ribofuranosyl)pyrazolo[3,2-*i*]-purine Derivatives and their Cytotoxic Activities

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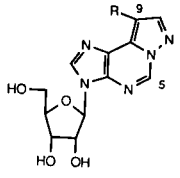
Received November 6, 1992

9-Amino-3-(β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**6**) has been prepared from a fully protected 3-(β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**2**) and the 9-bromo substituted derivative **3** by nitration, followed by reduction. Reaction of 9-bromo-3-(β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**1b**) with alkali gave the (pyrazol-3-yl)imidazole derivative, followed by diazocyclization with sodium nitrate to give 9-bromo-3-(β-D-ribofuranosyl)imidazolo[4,5-*d*]pyrazolo[2,3-*c*][1,2,3]triazine (**10**) after deacetylation. Compounds **6** and **10** exhibited cytotoxic activity against leukemia cells.

J. Heterocyclic Chem., **30**, 313 (1993).

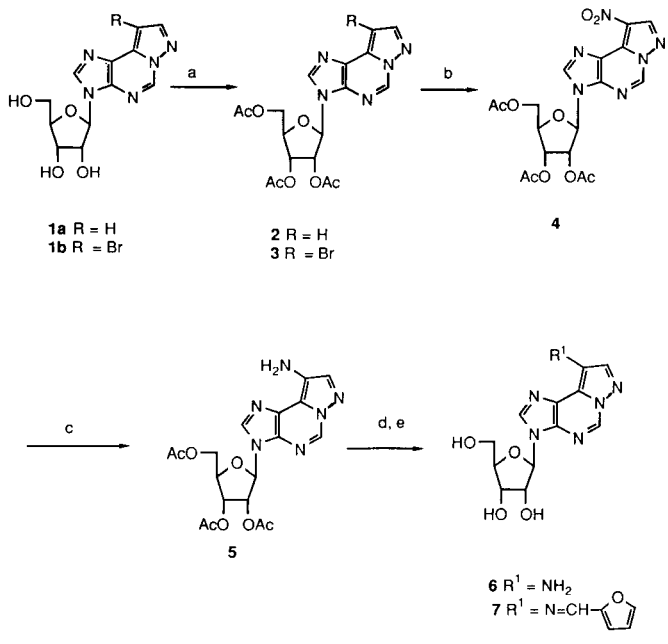
1,N^6-Ethenoadenosine derivatives are of biological interest for the studies of enzyme binding sites [2] or base-pair mismatches and mutation in bacteria [3]. Interestingly, 1,N^6-ethenoadenosine has not exhibited cytotoic activity, but 1,N^6-etheno-2-azaadenosine is active against a rat mammary tumor tissue culture line [4]. On the other hand, 9-deazatricyclicpurine derivatives, 3-(β-D-ribofuranosyl)-pyrazolo[3,2-*i*]purine (**1a**) and the 9-bromo derivative **1b**, exhibited cytotoxic activity against mouse leukemia L5178Y cells in culture (ID_{50} 0.21-0.26 ug/ml) [5]. Therefore, it seems that the nitrogen at position 9 of 1,N^6-ethenoadenosine has an important role in base-pair mismatches of DNA and RNA [3]. In order to elucidate the biological properties, we first tested for cytotoxic activity. In this paper, we report synthesis of 9-amino-3-(β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**6**) and 9-bromo-3-(β-D-ribofuranosyl)imidazolo[4,5-*d*]pyrazolo[2,3-*c*][1,2,3]triazine (**10**) and their cytotoxic activity against leukemia cells.



1a R = H
1b R = Br

3-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,2-*i*]-purine (**2**), which was prepared from **1a**, was nitrated with copper(II) nitrate trihydrate in acetic anhydride at room temperature to give the 9-nitro derivative **4** in 65% yield. Also by another route, **4** was prepared in 63% yield from **1b** by nitrodebromination [6] with copper(II) nitrate trihydrate. The nitro group of **4** was converted to the amino derivative **5** by hydrogenation. Attempts to reduce the nitro group with Fe in acetic acid gave the 9-*N*-acetyl derivative. Deacetylation of **5** with saturated ammonia gave 9-amino-3-(β-D-ribofuranosyl)-pyrazolo[3,2-*i*]purine (**6**). Also, reaction of **6** with 2-furaldehyde gave the azomethine derivative **7** after removal of the acetyl groups.

Scheme I



1a R = H
1b R = Br

2 R = H
3 R = Br

4

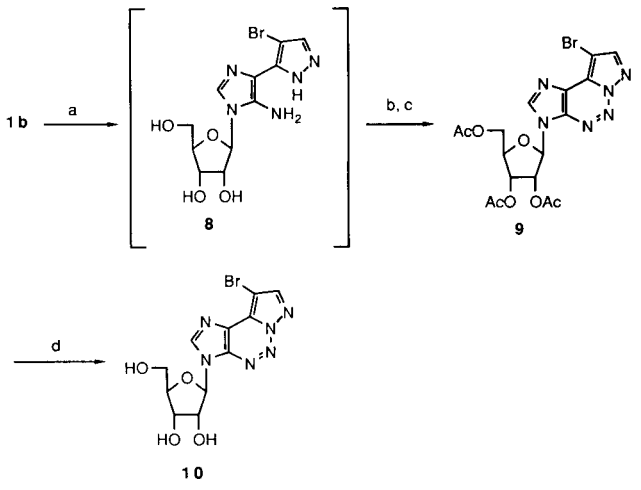
5

6 $R^1 = NH_2$
7 R^1 = N=CH-

Reagents: (a) Ac_2O, pyridine; (b) $Cu(NO_3)_2 \cdot H_2O$, Ac_2O; (c) H_2, 5% Pd-C; (d) sat NH_3, CH_3OH; (e) 2-furaldehyde

Reaction of 9-bromo-3-(β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**1b**) with sodium hydroxide gave the (pyrazolo-3-yl)imidazole derivative, which was cyclized with sodium nitrate in acetic acid [4], followed by acetylation to give 9-bromo-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazolo-[4,5-*d*]pyrazolo[2,3-*c*][1,2,3]triazine (**9**) in 63% yield after purification by silica gel column chromatography. In this reaction, after treatment with sodium nitrate, the purification of the product was difficult. Removal of the acetyl groups of **9** gave 9-bromo-3-(β-D-ribofuranosyl)imidazolo-[4,5-*d*]pyrazolo[2,3-*c*][1,2,3]triazine (**10**). The cytotoxic ac-

Scheme II



Reagents: (a) NaOH, H_2O (b) $NaNO_2$, CH_3COOH; (c) Ac_2O, pyridine; (d) sat NH_3, CH_3OH.

tivity of **6** exhibited an ID_{50} of 2.14 μg/ml against human leukemia CCRF-HSB-2 cells. On the other hand, compound **10** showed an ID_{50} of 4.7 μg/ml against mouse leukemia L5178Y cells.

In conclusion, it was found that the 9-substituted amino compound **6** and the 5-aza derivative **10** exhibited weaker cytotoxic activities than those of compounds **1a** and **1b**.

EXPERIMENTAL

All melting points are uncorrected. The ir spectra were taken on a JASCO A-102 spectrophotometer. The uv spectra were measured on a Hitachi EPS-3T spectrophotometer. The mass spectra (ms) were measured with a JEOL JMS-D300 spectrometer. The ^{1}H-nmr spectrometer recorded on a JEOL JNM-FX 100 spectrometer, using tetramethylsilanes as an internal standard. For the resonance signals the following abbreviations are used; s, singlet; t, triplet; m, multiplet; b, broad; dd, doublet of doublets.

3-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**2**).

A solution of **1a** (0.291 g, 1 mmole) and acetic anhydride (0.408 g, 4 mmoles) in pyridine (3 ml) was stirred for 4 hours at room temperature. The solvent was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate (50 ml) and washed with water, dried over sodium sulfate, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g) using 10% acetone in chloroform as the eluent, and the eluate was evaporated *in vacuo* to give **2** (0.400 g, 95%) as a colorless foam. The analytical sample was obtained from acetone-hexane by precipitation; ^{1}H-nmr (DMSO-d_6): δ 2.04 (s, 6H, 2 x $COCH_3$), 2.14 (s, 3H, $COCH_3$), 4.35-4.44 (m, 3H, H-4′,5′), 5.60 (dd, J = 7 Hz, J = 4 Hz, 1H, H-3′), 6.01 (t, J = 7 Hz, 1H, H-2′), 6.35 (d, J = 7 Hz, 1H, H-1′), 6.88 (dd, J = 2 Hz, J = 1 Hz, 1H, H-9), 8.25 (d, J = 2 Hz, 1H, H-8), 8.55 (s, 1H, H-2), 9.47 (d, J = 1 Hz, 1H, H-5); ms: m/z 417 (M^+), 159 (B + 1).

Anal. Calcd. for $C_{18}H_{19}N_5O_7$: C, 51.80; H, 4.59; N, 16.78. Found: C, 51.80; H, 4.73; N, 16.48.

9-nitro-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,2-*i*]-purine (**4**).

A solution of **2** (0.417 g, 1 mmole) and copper(II) nitrate trihydrate (0.241 g, 1 mmole) in acetic anhydride (5 ml) was stirred for 6 hours at room temperature. The insoluble material was filtered off, and the filtrate was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (20 ml) and washed with water, and the organic solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (70 g) using 4% acetone in chloroform as the eluent, and the eluate was evaporated *in vacuo* to give **4** (0.3 g, 65%) as a colorless foam; ir (potassium bromide): 1745 (CO), 1635, 1490 (NO_2), 1400 (NO_2) cm^{-1}; ^{1}H-nmr (DMSO-d_6): δ 2.05 (s, 6H, 2 x $COCH_3$), 2.14 (s, 3H, $COCH_3$), 4.30-4.54 (m, 3H, H-4′,5′), 5.61 (t, J = 5 Hz, 1H, H-3′), 5.98 (t, J = 5 Hz, 1H, H-2′), 6.44 (d, J = 5 Hz, 1H, H-1′), 8.82 (s, 1H, H-8), 9.02 (s, 1H, H-2), 9.74 (s, 1H, H-5); ms: m/z 462 (M^+), 204 (B + 1). The analytical sample was obtained from acetone-hexane by precipitation.

Anal. Calcd. for $C_{18}H_{18}N_6O_9$: C, 46.75; H, 3.92; N, 18.17. Found: C, 46.62; H, 4.13; N, 18.30.

9-Bromo-3-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,2-*i*]-purine (**3**).

A solution of **1b** (0.370 g, 1 mmole) and acetic anhydride (0.408 g, 4 mmoles) in pyridine (3 ml) was stirred for 3 hours at room temperature. The solvent was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate (50 ml). The organic layer was washed with water, dried over sodium sulfate, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel with chloroform as the eluent and the eluate was evaporated *in vacuo* to give **3** (0.496 g, 96%) as a colorless a foam: ^{1}H-nmr (DMSO-d_6): δ 2.04 (s, 6H, 2 x $COCH_3$), 2.13 (s, 3H, $COCH_3$), 4.20-4.45 (m, 3H, H-4′,5′), 5.60 (t, J = 6 Hz, 1H, H-3′), 5.98 (t, J = 6 Hz, 1H, H-2′), 6.36 (d, J = 6 Hz, 1H, H-1′), 8.35 (s, 1H, H-8), 8.60 (s, 1H, H-2), 9.47 (s, 1H, H-5); ms: m/z 497 (M^+), 495 (M^+), 237 (B + 1), 239 (B + 1).

Anal. Calcd. for $C_{18}H_{18}N_5O_7Br$: C, 43.56; H, 3.65; N, 14.10; Br, 16.10. Found: C, 43.59; H, 3.74; N, 14.01; Br, 15.93.

Nitrodebromination of **3**.

A solution of **3** (0.496 g, 1 mmole) and copper(II) nitrate trihydrate (0.241 g, 0.5 mmole) in acetic anhydride (8 ml) was stirred for 4 hours at room temperature, and then copper(II) nitrate trihydrate (0.120 g, 0.5 mmole) was added to the solution. The solution was stirred for 2 hours at room temperature. The insoluble material was filtered off, and the filtrate was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (50 ml), and washed with water, dried over sodium sulfate, and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (80 g) using 4% acetone in chloroform as the eluent, and the eluate was evaporated *in vacuo* to give 9-nitro-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**4**) (0.294 g, 63%) as a colorless foam. The compound was identical with that of an authentic sample prepared by reaction of **2** and copper(II) nitrate trihydrate.

Hydrogenation of **4**.

A solution of **4** (0.327 g, 0.7 mmole) in benzene-ethyl acetate (1:1 v/v, 40 ml) was hydrogenated over 5% Pd-C (160 mg) at atmospheric pressure for 5 hours at room temperature. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*.

The residue was purified by column chromatography on silica gel (30 g) using 2% methanol in chloroform as the eluent, and the eluate was evaporated *in vacuo* to give 9-amino-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**5**) as a foam, yield 94 mg (62%); ^{1}H-nmr (deuteriochloroform): δ 2.09 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 2.15 (s, 3H, Ac), 3.52 (br s, 2H, NH_2), 4.39-4.55 (m, 3H, H-4′, H-5′), 5.61-5.70 (m, 1H, H-3′), 5.97 (t, J = 5 Hz, 1H, H-2′), 6.19 (d, J = 5 Hz, 1H, H-1′), 7.77 (s, 1H, H-8), 7.98 (s, 1H, H-2), 8.81 (s, 1H, H-5); ms: m/z 432 (M^+), 174 (B + 1).

Anal. Calcd. for $C_{18}H_{20}N_6O_7$: C, 49.99; H, 4.66; N, 19.43. Found: C, 49.62; H, 4.48; N, 19.34.

9-Amino-3-(β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**6**).

A solution of **5** (0.215 g, 0.5 mmole) in saturated ammonia in methanol (5 ml) was allowed to stand for 15 hours at room temperature in a sealed tube. The solvent was evaporated *in vacuo*, and the resulting precipitate was collected by filtration. Recrystallization of **6** from methanol-water gave slightly yellow needles, mp 221-223° dec; uv (methanol): λ max (ε) 239 (17900), 300 (7400), 350 (3680) nm; ^{1}H-nmr (DMSO-d_6): δ 3.56-3.70 (m, 2H, H-5′), 3.94-4.02 (m, 1H, H-4′), 4.11-4.19 (m, 1H, H-3′), 4.26 (br s, 2H, NH_2), 4.55 (dd, J = 6 Hz, J = 5 Hz, 1H, H-2′), 5.02 (t, J = 6 Hz, 1H, OH), 5.18 (d, J = 5 Hz, 1H, OH), 5.48 (d, J = 5 Hz, 1H, OH), 5.96 (d, J = 5 Hz, 1H, H-1′), 7.76 (s, 1H, H-8), 8.41 (s, 1H, H-2), 9.04 (s, 1H, H-5); ms: m/z 306 (M^+), 174 (B + 1).

Anal. Calcd. for $C_{12}H_{14}N_6O_4$: C, 47.05; H, 4.61; N, 27.44. Found: C, 47.25; H, 4.62; N, 27.16.

9-Furylazomethino-3-(β-D-ribofuranosyl)pyrazolo[3,2-*i*]purine (**7**).

A solution of **5** (0.216 g, 0.5 mmole) and 2-furaldehyde (0.072 g, 0.75 mmole) in toluene (5 ml) was heated for 1 hour at 100°. After cooling, the solvent was evaporated *in vacuo*. The residue was passed through Florisil (50 g) using 8% methanol in chloroform as the eluent, and the eluant was evaporated *in vacuo*. The residue was used in the next step without purification. The residue was dissolved in saturated methanolic ammonia (5 ml), and allowed to stand at room temperature for 15 hours. The solvent was evaporated *in vacuo*, and the residue was recrystallized from methanol to give **7** (0.124 g, 64%) as slightly yellow needles, mp 224-227° dec; uv (methanol): λ max (ε) 230 (16900), 270 (10960 sh), 279 (13300), 349 (20000 sh), 365 (22600), 384 (16000 sh) nm; ^{1}H-nmr (DMSO-d_6): δ 3.57-3.73 (m, 2H, H-5′), 3.99 (m, 1H, H-4′), 4.20 (dd, J = 10 Hz, J = 5 Hz, 1H, H-3′), 4.56 (dd, J = 10 Hz, J = 5 Hz, 1H, H-2′), 5.07 (t, J = 5 Hz, 1H, OH), 5.23 (d, J = 5 Hz, 1H, OH), 5.57 (d, J = 5 Hz, 1H, OH), 6.08 (d, J = 5 Hz, 1H, H-1′), 6.71 (dd, J = 3 Hz, J = 1 Hz, 1H, furan), 7.08 (d, J = 3 Hz, 1H, furan), 7.92 (d, J = 1 Hz, 1H, furan); ms: m/z 384 (M^+), 252 (B + 1).

Anal. Calcd. for $C_{17}H_{16}N_6O_5$: C, 53.12; H, 4.20; N, 21.87. Found: C, 52.97; H, 4.14; N, 21.66.

9-Bromo-3-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazolo[4,5-*d*]pyrazolo[2,3-*c*][1,2,3]triazine (**9**).

A solution of **1b** (0.246 g, 0.66 mmole) and sodium hydroxide (80 mg, 0.66 mmole) in water (5 ml) was stirred for 5 hours at room temperature. The solution was neutralized with 1% aqueous hydrochloric acid and evaporated *in vacuo*. The residue was dissolved in acetic acid (3 ml), and sodium nitrate was added to the solution. The mixture was stirred for 2 hours at room temperature, and the solution was evaporated *in vacuo*. After drying, the solution was dissolved in pyridine (1 ml) and acetic anhydride (3 ml), and the solution was stirred for 3 hours at room temperature. The solvent was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel using 3% acetone in chloroform, and the eluate was evaporated *in vacuo* to give **9** (0.204 g, 63%) as a foam; ^{1}H-nmr (DMSO-d_6): δ 2.05 (s, 6H, 2 x $COOCH_3$), 2.14 (s, 3H, $COCH_3$), 4.32-4.52 (m, 3H, H-4′, H-5′), 5.67 (t, J = 6 Hz, 1H, H-3′), 6.03 (t, J = 6 Hz, 1H, H-2′), 6.57 (d, J = 6 Hz, 1H, H-1′), 8.56 (s, 1H, H-2), 8.98 (s, 1H, H-8); ms: m/z 498 (M^+), 496 (M^+), 214 (B + 1), 212 (B + 1).

Anal. Calcd. for $C_{17}H_{17}N_6O_7Br$: C, 41.06; H, 3.44; N, 16.90. Found: C, 41.04; H, 3.22; N, 16.64.

9-Bromo-3-(β-D-ribofuranosyl)imidazolo[4,5-*d*]pyrazolo[2,3-*c*]-[1,2,3]triazine (**10**).

A solution of **9** (0.144 g, 0.5 mmole) in saturated ammonia in methanol (20 ml) was allowed to stand for 15 hours at 0° in a sealed tube. The solvent was evaporated *in vacuo*, and the resulting precipitate was collected by filtration. Recrystallization of from methanol gave **10** (75 mg, 70%) as colorless needles, mp 186-189° dec; uv (methanol): λ max (ε) 251 (24300 sh), 283 (3700), 360 (1800) nm; ^{1}H-nmr (DMSO-d_6): δ 3.62-3.77 (m, 2H, H-5′), 3.99-4.12 (m, 1H, H-4′), 4.28 (dd, J = 10 Hz, J = 4 Hz, 1H, H-3′), 4.59 (dd, J = 10 Hz, J = 4 Hz, 1H, H-2′), 5.09 (t, J = 5 Hz, 1H, OH), 5.26 (d, J = 5 Hz, 1H, OH), 5.68 (d, J = 5 Hz, 1H, OH), 6.25 (dd, J = 4 Hz, 1H, H-1′), 8.52 (s, 1H, H-2), 9.04 (s, 1H, H-8); ms: m/z 370 (M^+), 372 (M^+), 212 (B + 1), 214 (B + 1).

Anal. Calcd. for $C_{11}H_{11}N_6O_4Br \cdot 1/5H_2O$: C, 35.26; H, 3.33; N, 22.65; Br, 21.32. Found: C, 35.43; H, 3.31; N, 22.06; Br, 21.39.

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