SYNTHESIS OF NEW FLUORESCENT NUCLEOSIDES, 3-β-D-RIBOFURANOSYLPYRAZOLO[3,2-i]PURINE DERIVATIVES AND THEIR CYTOTOXIC ACTIVITIES

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The novel nucleosides 3-β-D-ribofuranosylpyrazolo[3,2-i]purine (**8**) and their 9-substituted bromo, nitro and amino compounds (**3**, **6** and **11**) have been prepared from a fully protected 3-β-D-ribofuranosyl[3,2-i]purine-9-carboxyamide **1** by bromodeamidation (ipso bromination). Compounds **3**, **8** and **11** exhibited anti-leukemic activity against mouse leukemia L5178Y cells in culture, while the 9-substituted nitro, ester and amide compounds (**6**, **12** and **13**) showed no cytotoxity.

KEYWORDS pyrazolo[3,2-i]purine; bromodeamidation; nitrodebromination; nitration; fluorescence; mouse leukemia L5178Y; cytotoxic activity

Polycyclic nucleosides with five-membered rings fused to purines, 1,N^6-etheno-adenosine derivatives, are of biological interest for the studies of enzyme binding sites[2] or base-pair mismatches and mutation in bacteria.[3] For example, the etheno-substituted nucleosides are formed by reaction of chloroethylene oxide with adenosin residues in DNA or RNA, followed by oxidation in vivo.[4] The etheno-substituted deoxynucleosides showed misincorporation, and the hydrogen bonding of the 1,N^6-etheno-deoxyadenosine-deoxyguanosine base-pair at the position N-1 and N-9 was stabilized for formation of the double strand. Also, the etheno-substituted nucleosides were resistant to nuclease action. However, the compound does not exhibit cytotoxic activity.[5] In order to elucidate the biological properties, 9-deazatricyclicpurine derivatives, in which carbon is substituted at position 9 in the stead of nitrogen, were required. In this paper, synthesis of 3-β-D-ribofuranosylpyrazolo[3,2-i]purine and their 9-substituted derivatives and their cytotoxic activities against mouse leukemia L5178Y are reported.

Reaction of 3-[2,3-O-isopropylidene-5-O-(2-tetrahydropyranyl)-β-D-ribofuranosyl]-prazolo[3,2-i]purine-9-carboxyamide (**1**)[6a] with bromine for 3 h at room temperature gave **2** in 91% yield. The tetrahydropyranyl (THP) and isopropylidene protecting groups in **2** were removed with 40% trifluoroacetic acid to gave 9-bromo-3-β-D-ribofuranosylpyrazolo-[3,2-i]purine (**3**) [mp 195-197°C dec.; fluorescence λ max emission: 424 nm; fluorescence λ max excitation: 233 nm; ϕ_F=0.42,[7]] in 75% yield.[8] The structures of **2** and **3** were confirmed by ^{1}H-NMR[9] and mass spectroscopy. Attempts at direct amination of **1** using Hoffman rearrangement were unsuccessful. It is known that nitrodebrominations occur in hetero-aromatic compounds as "ipso nitration".[10] Therefore, product **2** could possibly be obtained by an "ipso bromination". Acetylation of **3** with acetic anhydride gave 9-bromo-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosylpyrazolo[3,2-i]purine (**4**). Reaction of **4** with copper(II) nitrate trihydrate in acetic anhydride gave **5** in 63% yield, the acetyl groups of which

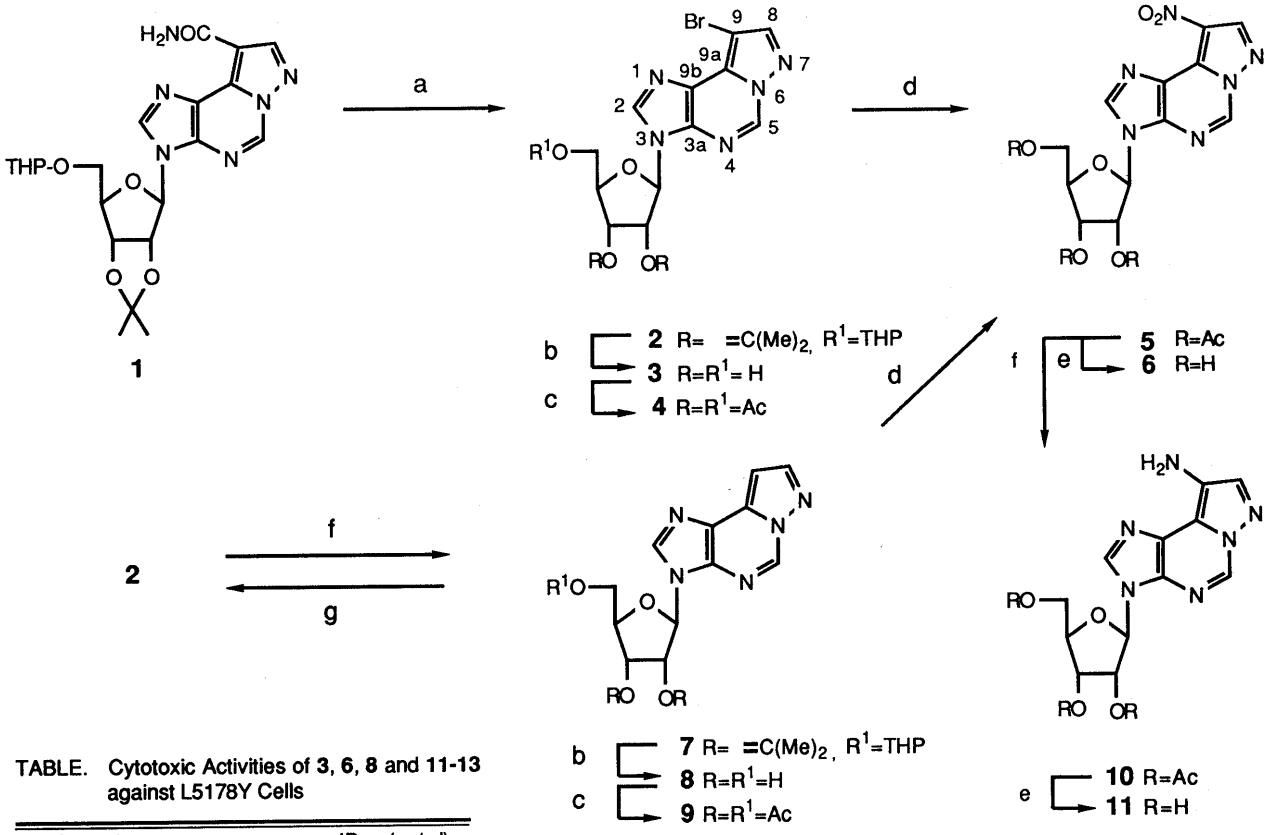

TABLE. Cytotoxic Activities of **3**, **6**, **8** and **11-13** against L5178Y Cells

Compd.	ID_{50} (μg/ml)
3	0.21
6	100
8	0.26
11	2.14
12	>100
13	>100
5-FU	0.20

Reagents:
a) Br_2, AcOEt-phosphate buffer (pH 6.9); b) 40% aq. CF_3COOH;
c) Ac_2O, pyridine; d) $Cu(NO_3)_2.3H_2O$, Ac_2O, r.t.; e) sat. NH_3 in MeOH;
f) H_2, Pd-C, AcOEt; g) Br_2, AcOEt.

were removed with ammonia to give **6** [mp 193-196°C dec.; IR: 1490 (NO_2), 1400 (NO_2) cm^{-1}.[11] It is known that nitrodebromination of pyrazole takes place in strong acid.[10] However, reaction with copper(II) nitrate trihydrate has been found to be a milder method.

Compound **5** has also been prepared by another route. Thus, hydrogenolysis of **2** over 5% Pd-C gave **7** in 95% yield, the protecting groups of which were removed with 40% trifluoroacetic acid to give 3-β-D-ribofuranosylpyrazolo[3,2-i]purine (**8**) in 72% yield [mp 211-213°C dec.; UV λ max MeOH (ε): 230 (26100), 258 (sh, 3700), 268 (5200), 279 (6210), 307 (5970) nm; fluorescence λ max emisson: 406 nm; fluorescence λ max excitation: 233 nm; ϕ_F=0.68].[12] In the ^{1}H-NMR spectrum a new signal appeared in the aromatic region at δ 6.85 (1H, dd, J = 2 Hz, J = 1 Hz, H-9). Bromination of **7** in ethyl acetate gave **2** in 97% yield. The compound was found to be identical to **2** by TLC and ^{1}H-NMR data. Acetylation of **8** with acetic anhydride gave **9**, which was treated with copper(II) nitrate trihydrate in acetic anhydride to give **5** in 58% yield. Catalytic hydrogenation of **5** over 5% Pd-C gave **10** in 62% yield as a foam. The acetyl groups of **10** were removed with ammonia to give **11** as slightly yellow needles [mp 221-223°C dec.; UV λ max MeOH (ε): 239 (17950), 300 (7400), 350 (3680) nm]. In the ^{1}H-NMR spectrum an amino signal was found at δ 4.26 (br s, 2H, NH_2).[13] These nucleosides were tested for cytotoxic activity against L5178Y Cells (Table). Compounds **3** and **8** exhibited strong cytotoxic activity similar to that

of 5-FU (5-fluorouracil). The amino compound **11** exihibited weak cytotoxic activity. On the other hand, ethyl 3-β-D-ribofuranosylpyrazolo[3,2-*i*]purine-9-carboxylate (**12**)[6a] and the amide **13**[6a] and **6** did not show cytotoxic activity.

In the present work novel nucleosides, 3-β-D-ribofuranosylpyrazolo[3,2-*i*]purine as a 1,N^6-ethenoadenosine analogue and the 9-substituted derivatives, have been prepared from **1** by a novel bromodeamidation reaction. Compounds **3** and **8** were found to exhibit cytotoxic activity, and these are a first example.

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7) The quantam yield (ϕ_F) was obtained from that of standard quinine sulfate in 0.025 M phosphate buffer, 21°C (J. A. Secrist III, J. R. Barrio, N. J. Leonard and G. Weber, Biochemistry, **11**, 3499 (1972).
8) Satisfactory elemental analyses were obtained for all the compounds.
9) ^{1}H-NMR spectrum (DMSO-d_6-D_2O) of **3**: δ 4.01 (1H, dd, J = 8 Hz, J=5 Hz, H-4'), 4.20 (1H, t, J = 5 Hz, H-3'), 4.56 (1H, t, J = 5 Hz, H-2'), 6.05 (1H, d, J = 5 Hz, H-1'), 8.32 (1H, s, H-8), 8.61 (1H, s, H-2), 9.48 (1H, s, H-5).
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11) ^{1}H-NMR (DMSO-d_6) for the aglycon moiety of **6**: δ 8.86 (1H, s, H-8), 9.01 (1H, s, H-2), 9.69 (1H, s, H-5).
12) ^{1}H-NMR (DMSO-d_6) for the aglycon moiety of **8**: δ 6.85 (1H, dd, J = 2 Hz, J = 1 Hz H-9), 8.23 (1H, d, J = 2 Hz, H-8), 8.57 (1H, s, H-2), 9.42 (1H, d, J = 1 Hz, H-5); ^{13}C-NMR (DMSO-d_6) for the aglycon moiety of **8**: δ 94.5 (^1J = 180.5 Hz, ^2J = 10.3 Hz, C-9), 123.9 (^3J = 11.8 Hz, C-9b), 136.2 (^3J = 11.7 Hz, C-3a), 136.8 (^2J = 4.4 Hz, ^3J = 4.4 Hz, C-9a), 138.0 (^1J = 214.2 Hz, C-5), 139.9 (^1J = 214.2 Hz, C-2), 145.2 (^1J = 184.9 Hz, ^2J = 4.4 Hz, C-8).
13) ^{1}H-NMR (DMSO-d_6) for aglycon moiety of **11**: δ 4.26 (2H, br s, NH_2), 7.76 (1H, s, H-8), 8.41 (1H, s, H-2), 9.04 (1H, s, H-5).

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