BENZIMIDAZOLE RIBONUCLEOSIDES: OBSERVATION OF AN UNEXPECTED NITRATION WHEN PERFORMING NON-AQUEOUS DIAZOTIZATIONS WITH t-BUTYL NITRITE[1]

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Abstract. Mild, non-acidic conditions used in the non-aqueous diazotization of a 2-aminobenzimidazole nucleoside with *t*-butyl nitrite gave an unexpected 4-nitrobenzimidazol-2-one nucleoside which was not formed when primary alkyl nitrites were used. The isolated compounds were evaluated for activity against herpesviruses and for cytotoxicity against human foreskin fibroblast cells.

Benzimidazoles have demonstrated widespread biological activity ranging from antifilarial[2] to antiviral. Since the initial report[3] of activity for halogenated benzimidazole ribonucleosides against certain RNA viruses, considerable interest has been generated among analogs of 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB). Recently, we have found that polyhalogenated benzimidazole ribonucleosides are potent and selective inhibitors of human cytomegalovirus (HCMV) replication. In an effort to establish a structure-activity relationship in this specific area, we have been preparing and evaluating new benzimidazole ribonucleosides. It was during the preparation of these compounds that we encountered an interesting observation pertaining to non-aqueous diazotization.

Non-aqueous diazotization employs the known ability of alkyl nitrites to produce aryl radicals from arylamines[4]; the ensuing aryl radical can then abstract an atom from a suitable donor such as the solvent. Although an early use of non-aqueous diazotization using THF as the solvent involved the deamination of 2',3',5'-tri-O-acetyladenosine to furnish 2',3',5'-tri-O-acetylnebularine[5], more recent applications have involved the synthesis of various halogenated nucleosides[6]. The deamination-halogenation of purine nucleosides with alkyl nitrites in halogenated solvents has been reported to occur at the 6-position[7] and the 2-position[8,9] to provide various halogenated purine nucleosides. Although the synthesis of several 2-halogenated benzimidazole nucleosides has been reported,[10-12] the non-aqueous diazotization of benzimidazole nucleosides to afford the corresponding 2-halogenated nucleosides has not. We now wish to report on the unexpected results from a non-aqueous diazotization of a 2-amino-5,6-disubstituted benzimidazole riboside when using a tertiary alkyl nitrite and the antiviral evaluation of the isolated compounds.

Treatment of 2-amino-5,6-dichloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)benzimidazole[13] (1) with 10 equiv of *t*-butyl nitrite in CH_2I_2 under anhydrous conditions for 24 h at 110 °C gave (after solvent removal via a Kugelrohr apparatus and column chromatography) a bright-yellow product. A mass spectrum (FAB+) of the product identified a molecular ion at *m/z* 506 instead of the expected *m/z* 571 for 5,6-dichloro-2-iodo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)benzimidazole (3). The structure of this unexpected product (*m/z* 506) was subsequently elucidated from spectral data[14] as follows: The ^{1}H NMR (CDCl₃) spectrum of the product revealed an exchangeable proton at δ 10.05 (one proton, D_2O exchangeable) and only one peak (one proton) in the aromatic region at δ 7.56. The ^{13}C NMR spectrum revealed the presence of a urea-like carbon at δ 152.70. These data indicated that changes or substitutions had occurred on both the benzo and imidazo moieties of the benzimidazole
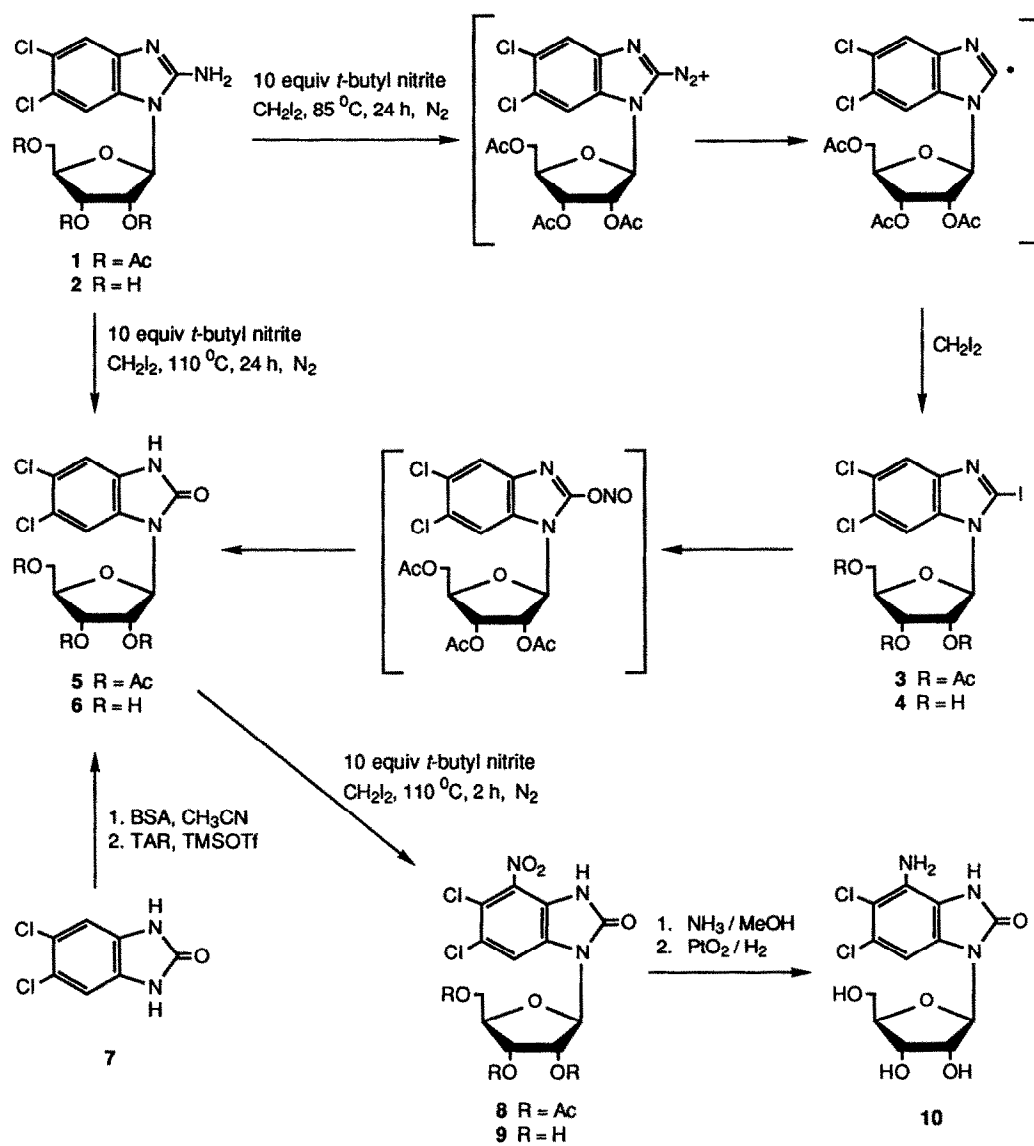
ring and established that the product was not the expected compound **3**. This led us to the conclusion that the unexpected product was a trisubstituted benzimidazol-2-one nucleoside with what we presumed to be a NO_2 group residing on the benzene moiety (compound **8**, IR (KBr): 1542 cm^{-1}).

The site of functionalization on the benzene portion of **8** was determined by an NOE experiment. Irradiation of the lone aromatic proton (δ 7.56) gave a 6.7% NOE enhancement of H-2' and a 6.6% NOE enhancement of H-1'. These observations indicated that **8** was indeed a 4-substituted benzimidazol-2-one. The NO_2 group could be bonded through either a nitrogen or an oxygen atom and required further structural confirmation. The possibility of O-nitrosation was excluded by effecting a reduction of **9** to **10** which provided an amino substituent, and not a hydroxyl group. A quantitative removal of the acetyl protecting groups from **8** was followed by a catalytic hydrogenation under H_2 in the presence of PtO_2 in ethanol to give **10** in 92% yield[15].

In an effort to elucidate the mechanism of this reaction by isolating some of the intermediates, the temperature was lowered to 85 °C. Within 5 min, the starting benzimidazole **1** had been consumed and a new product had been formed. After 20 min, three products could be detected by TLC. At 3 h, with all three products still prevalent, the solvent was removed and the three compounds were isolated by column chromatography. Spectral analysis[14, 16, 17] of these compounds allowed us to assign their structures as **3**, **5**, and **8**. By monitoring the progress of the reaction by TLC, we were able to determine that the reaction proceeded sequentially with the initial formation of **3**, then **5**, and finally **8**. Clearly, the expected 2-iodobenzimidazole nucleoside **3** had formed rather quickly, and the continued exposure of this compound to the reaction conditions led to the displacement of the 2-iodo group and ultimately the benzimidazol-2-one nucleoside **5**. Compound **5** was then nitrated *in situ* giving the novel 4-nitrobenzimidazol-2-one nucleoside **8**.

An alternative synthesis[17] of compound **5** by ribosylation of the known 5,6-dichlorobenzimidazol-2-one[18] (**7**) helped to establish its role as an intermediate. When **5** was treated with 10 equiv of *t*-butyl nitrite in diiodomethane at 110 °C, a complete conversion to compound **8** was observed within 2 h without the detection of any other intermediates. Compound **8** was isolated from this reaction in 86% yield after recrystallization.

The final diazotization product obtained with *t*-butyl nitrite was in sharp contrast to the results obtained when a primary alkyl nitrite was used. The treatment of compound **1** with either amyl or isoamyl nitrite under
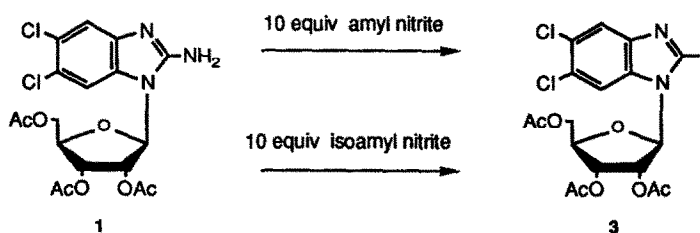
the same reaction conditions gave only the expected 5,6-dichloro-2-iodo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)benzimidazole (**3**) in 55% and 63% yields, respectively. The reaction product composition did not change even after 12 h. We also noted that treatment of **5** with 10 equiv of isoamyl nitrite at 110 °C in diiodomethane did not lead to the formation of any products even after 24 h. While it is widely accepted that the

pyrolysis and photolysis of *t*-butyl nitrite results in the formation of *t*-butoxyl radical and nitric oxide, the *in situ* nitration of compound 8 from 5 suggests that a different type of thermal decomposition is occurring. We can propose that the tertiary alkyl nitrite undergoes thermal decomposition giving rise to products which are more stable than their counterparts when primary alkyl nitrites are used. These thermal decomposition products are responsible for the observed displacement of the 2-iodo group of compound 3 and the *in situ* nitration of compound 5.

The protected nucleosides 1, 3, 5, and 8 were treated for 16 h with a methanolic ammonia solution (prepared by saturating methanol with ammonia gas at 0 °C) to achieve a removal of the acetyl protecting groups and give the unprotected nucleosides 2, 4, 6, and 9, respectively. The unprotected nucleosides were evaluated for their ability to inhibit HCMV replication using plaque and yield reduction assays[19,20] and for cytotoxicity against uninfected human foreskin fibroblasts (HFF) cells. These data were compared to results we have obtained with the 2-unsubstituted 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (DRB) and the clinically approved anti-HCMV agent DHPG. Of the compounds tested, 2 was somewhat active against HCMV but also cytotoxic in HFF cells, indicating that activity against the virus was a consequence of cell toxicity. Compound 4 was active against HCMV in both the plaque reduction and yield reduction assays and demonstrated some activity against HSV-1.

These data suggest that the type of substituents on the 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole moiety is critical for optimal antiviral activity. Those compounds having a benzimidazol-2-one functionality were devoid of any activity. When compared to the 2-unsubstituted compound (DRB), we found that the introduction of an amino group at the 2-position did not alter the anti-HCMV activity or the cytotoxicity. However, introduction of an iodine atom at the 2-position improved the selectivity by decreasing the cytotoxicity observed on uninfected HFF cells.

ANTIVIRAL EVALUATION

Compound	50 % Inhibitory Concentration, µM			
	HCMV Plaque	HCMV Yield[a]	HSV-1	HFF[b]
2	40	--	50	32
4[c]	30	26	21	127
6	>100	--	>100	100
9	>100	--	>100	100
10	>100	--	>100	>100
DRB[19,c]	42	19	30	24
DHPG	7.9[d]	2.0[c]	3.5[c]	>100[d]

[a]90% inhibitory concentration. [b]Visual cytotoxicity in HFF cells scored at 30-fold magnification at time of HCMV plaque enumeration. [c]Results are the average of 3 or more experiments. [d]Results are the average of 50 or more experiments.

While gaining insight into the structural modifications that may be made on benzimidazole nucleosides in order to optimize activity, we have encountered an interesting synthetic observation. We found that the application of a non-aqueous diazotization method on benzimidazole nucleosides with *t*-butyl nitrite has furnished an unexpected nitrated compound. These rather mild, non-acidic conditions make it an attractive and exploitable approach towards the selective functionalization of benzimidazole nucleosides. We are currently exploring the general use of this novel nitration method toward the synthesis of various structurally related heterocycles.

References and Notes

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13) Compound 1 was synthesized in 51% yield by ribosylation of 2-amino-5,6-dichlorobenzimidazole (Horner, J. K. and Henry, D. W., *J. Med. Chem.*, **1968**, *11*, 946) with bis(trimethylsilyl)acetamide (BSA), timethylsilyl trifluoromethanesulfonate (TMSOTf) and tetra-O-acetyl-β-D-ribofuranose (TAR) in refluxing acetonitrile. mp: 88-89 °C. ^{1}H NMR (CDCl$_3$): δ 7.42 (s, 1H), 7.27 (s, 1H), 5.87 (d, 1H, 1'-H, 6.83 Hz), 5.58 (s, 2H, Ar-NH$_2$), 5.47 (t, 1H, 2'-H), 5.37 (dd, 1H, 3'-H), 4.48 (dm, 2H, 5'-H), 4.35 (m, 1H, 4'-H), 2.17 (s, 3H), 2.15 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (CDCl$_3$): δ 170.04, 169.56, 169.11, 154.57, 141.64, 132.53, 125.90, 123.45, 117.72, 109.49, 85.78, 80.99, 70.95, 69.83, 62.91, 20.74, 20.54, 20.20. MS (FAB+): *m/z* 460.

14) **Compound 8:** Yield from **1:** 33%. mp: 186-187 °C. IR (KBr): 1542 cm^{-1}. ^{1}H NMR (DMSO-d$_6$): δ 10.05 (s, 1H, N-H), 7.56 (s, 1H, Ar-H), 5.96 (d, 1H, 1'-H, 6.59 Hz), 5.65 (t, 1H, 2'-H), 5.46 (dd, 1H, 3'-H), 4.41 (dm, 2H, 5'-H), 4.35 (m, 1H, 4'-H), 2.20 (s, 3H), 2.15 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (DMSO-d$_6$): δ 170.37, 169.84, 169.74, 152.70, 131.11, 128.63, 127.93, 125.07, 120.53, 114.90, 84.85, 80.13, 70.37, 69.94, 63.14, 20.89, 20.49, 20.34. MS (EI with DCI probe): *m/z* 506.

15) **Compound 10:** mp: 235-236 °C. ^{1}H NMR (DMSO-d$_6$): δ 10.62 (s, 1H, N-H), 7.15 (s, 1H, Ar-H), 5.59 (d, 1H, 1'-H, 7.37 Hz), 5.44 (bs, 2H, NH$_2$, D$_2$O exchangeable), 5.16 (d, 1H, 2'-OH), 5.05 (m, 2H, 3'-OH and 5'-OH), 4.43 (q, 1H, 2'-H), 4.04 (m, 1H, 3'-H), 3.82 (m, 1H, 4'-H), 3.58 (m, 2H, 5'-H). ^{13}C NMR (DMSO-d$_6$): δ 153.41, 129.89, 127.34, 124.26, 113.93, 109.12, 101.22, 86.05, 85.07, 70.18, 69.71, 61.60. MS (EI with DCI probe): *m/z* 349.

16) **Compound 3:** Isolated as a foam. ^{1}H NMR (CDCl$_3$): δ 7.81 (s, 1H), 7.75 (s, 1H), 6.07 (d, 1H, 1'-H, 7.02 Hz), 5.43 (m, 2H, 2'-H and 3'-H), 4.47(dm, 2H, 5'-H), 4.35 (m, 1H, 4'-H), 2.27 (s, 3H), 2.14 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (CDCl$_3$): δ 170.25, 169.50, 168.95, 145.43, 132.27,127.68, 120.72, 112.53, 103.82, 90.03, 80.61, 70.89, 69.31, 62.79, 20.89, 20.39, 20.07. MS (FAB): *m/z* 445. MS(DCI with ammonia): *m/z* 571. MS (EI 70 EV): *m/z* 570.

17) **Compound 5:** 5,6-Dichlorobenzimidazol-2-one (3.0 g, 15 mmol) was dissolved in dry acetonitrile (150 mL) and stirred in an inert atmosphere at 60 °C. BSA (4.03 mL, 16 mmol) was added and the mixture was stirred for 10 min. 1,2,3,5-Tetra-O-acetyl-β-D-ribofuranose (4.7 g, 15 mmol) and TMSOTf (3.14 mL, 16 mmol) were added to the clear solution and the mixture was allowed to stir at 60 °C for 1 h and then for 16 h at room temperature. The mixture was concentrated under reduced pressure and separated on a silica gel column to yield 3.57g (52.3%) of 5,6-dichloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)benzimidazol-2-one. mp: 177-178 °C. ^{1}H NMR (CDCl$_3$): δ 10.37 (s, 1H), 7.33 (s, 1H), 7.19 (s, 1H), 5.97 (d, 1H, 1'-H, 6.75 Hz), 5.73 (t, 1H, 2'-H), 5.49 (dd, 1H, 3'-H), 4.41 (m, 3H, 4'-H and 5'-H), 2.21 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (CDCl$_3$): δ 170.51, 169.68, 169.58, 154.64, 127.59, 127.36, 126.38, 125.31, 111.61, 111.34, 84.48, 79.67, 70.16, 69.96, 63.14, 20.92, 20.53, 20.37. MS (EI with DCI probe): *m/z* 460.

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