Halogenation of tubercidin by N-halosuccinimides. A direct route to 5-bromotubercidin, a reversible inhibitor of RNA synthesis in eukaryotic cells

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ABSTRACT

Tubercidin may be directly brominated by reaction with N-bromosuccinimide in DMF to give 5-bromotubercidin, a reversible inhibitor of RNA synthesis. When buffered with potassium acetate the major product is 6-bromotubercidin. 5,6-Dibromotubercidin is formed in minor amounts under both conditions. N-Chlorosuccinimide and tubercidin give 5-chlorotubercidin and 5,6-dichlorotubercidin.

INTRODUCTION

5-Bromotubercidin (5) has been suggested as a tool for the study of polynucleotide metabolism in eukaryotic cells. Reich and coworkers showed it to be a reversible inhibitor of heterogeneous nuclear RNA and ribosomal RNA synthesis in cultures of chick embryo fibroblasts, in addition to blocking viral RNA synthesis. The unavailability of 5 has been an important factor in its infrequent use. In contrast C-5 brominated pyrimidine nucleosides and C-8 brominated purine nucleosides have found extensive use in nucleic acid research. These are readily available by direct bromination of commonly available nucleosides. The only reported synthesis of 5 involved bromination of 4-chloro-7-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine by N-bromoacetamide in CH₂Cl₂, followed by treatment with methanolic NH₃. 5,6 5-Chlorotubercidin and 5-iodotubercidin were synthesized via the same intermediate.

N-Bromosuccinimide (NBS) in N,N-dimethylformamide (DMF) has been demonstrated to be a particularly mild combination of reagent and solvent for electrophilic bromination. The combination of NBS in DMF has been used successfully for the synthesis of 8-bromoguanosine. On the basis of these results the reaction of NBS with tubercidin was carefully examined.
RESULTS AND DISCUSSION

We have found that tubercidin (1) can be directly brominated or chlorinated under carefully controlled conditions. Treatment of tubercidin in DMF with N-bromosuccinimide at room temperature leads to two products, 5-bromotubercidin (2) and 5,6-dibromotubercidin (3) (Scheme I). These are readily separated by silica gel chromatography and purified by recrystallization from methanol. Chlorination of 1 by N-chlorosuccinimide (NCS) gives 5-chlorotubercidin (5) and 5,6-dichlorotubercidin (6) under similar conditions.

When the reaction mixture was buffered with potassium acetate the outcome was entirely different. The major product was now 6-bromotubercidin (4) which was isolated in 52% yield. By HPLC analysis the yield of 5-bromotubercidin in
this reaction mixture was less than 2%. 5,6-Dibromotubercidin was again a
minor product (3% yield).

In the absence of potassium acetate the amount of 6-bromotubercidin
formed in the reaction was less than 1%. Thus the reaction is exceptionally
selective for either 2′ or 4′ depending on the absence or presence of an acetate
buffer. We have also tried N-iodosuccinimide in DMF but got no reaction.
However, 5-iodotubercidin can be synthesized in two steps from tubercidin via
5-mercuritubercidin.9,10

Structure proofs were based on 1H NMR, 13C NMR, UV and elemental analy-
sis. NMR assignments were based on data obtained for a large number of C-5
substituted tubercidin analogs.10 The effect of the halogen at C-6 on the 1H
NMR signal of H-2′ is noteworthy. In one study the signal for H-2′ for adeno-
sine was observed at δ 4.15 in contrast to 8-bromoadenosine where the signal
fell at δ 4.77.11 Other sugar protons were not significantly shifted. We
have observed a shift of similar magnitudes for the H-2′ resonance between 5-
bromotubercidin and 5,6-dibromotubercidin and between 5-chlorotubercidin and
5,6-dichlorotubercidin (Table I). The C-6 position of tubercidin is located
in the same relative position as C-8 in adenosine. The large shift in the
H-2′ signal reflects the conformational change about the glycosidic bond in-
duced by addition of a bulky substituent.12

The UV maxima of 2′ and 4′ were in agreement with the values reported pre-
viously by Townsend and coworkers.5

The switch from preference for electrophilic substitution at C-6 in po-
tassium acetate buffered DMF to preference for C-5 substitution in unbuffered
DMF was unexpected. There is however a reasonable explanation for these re-
results. The reaction may go via electrophilic substitution since it proceeds
in the absence of free radical initiator and in the dark. When N-3 or N-4
are uncomplexed by Lewis acids, the N-4 amino can stabilize the incipient
carbonium ion resulting from attack of an electrophile at C-6. Direct reson-
ance stabilization of the sigma complex resulting from attack at C-6 is not
possible. Consequently C-6 substitution predominates in the presence of a
basic buffer (potassium acetate). On the other hand, in the absence of buffer
N-3 or N-4 may be a protonated or otherwise complexed by Lewis acids generated
from N-bromosuccinimide, and be unable to donate electron density via the π
system. Then C-5 by virtue of its conjugation to the nitrogen lone pair at
N-7 becomes the site of preferential attack. On the basis of these results,
C-5 substitution by other electrophiles under conditions where N-3 is either
protonated or otherwise complexed with a Lewis acid should predominate. Prob-
Table I. NMR Studies on 5-Halogenated and 5,6-Dihalogenated Tubercidin

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<tr>
<th>Compound</th>
<th>H-2</th>
<th>H-5</th>
<th>H-6</th>
<th>H-1'</th>
<th>H-2'</th>
<th>H-3'</th>
<th>H-4'</th>
<th>H-5'</th>
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</thead>
<tbody>
<tr>
<td>5-Bromotubercidin</td>
<td>8.15</td>
<td>7.64</td>
<td>6.10</td>
<td>4.38</td>
<td>4.67</td>
<td>3.66</td>
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<tr>
<td>6-Bromotubercidin</td>
<td>8.09</td>
<td>6.87</td>
<td>—</td>
<td>5.94</td>
<td>5.10</td>
<td>4.20</td>
<td>4.01</td>
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<td>5,6-Dibromotubercidin</td>
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<td>—</td>
<td>—</td>
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<td>4.22</td>
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<tr>
<td>5,6-Dichlorotubercidin</td>
<td>8.16</td>
<td>—</td>
<td>—</td>
<td>5.95</td>
<td>5.02</td>
<td>4.20</td>
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<tr>
<th>Compound</th>
<th>C-2</th>
<th>C-4</th>
<th>C-4a</th>
<th>C-5</th>
<th>C-6</th>
<th>C-7a</th>
<th>C-1'</th>
<th>C-2'</th>
<th>C-3'</th>
<th>C-4'</th>
<th>C-5'</th>
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<tbody>
<tr>
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<td>152.34</td>
<td>156.79</td>
<td>—b</td>
<td>—b</td>
<td>121.94</td>
<td>149.48</td>
<td>87.08</td>
<td>73.98</td>
<td>70.56</td>
<td>85.25</td>
<td>61.49</td>
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<td>151.32</td>
<td>156.33</td>
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<td>109.10</td>
<td>149.36</td>
<td>89.82</td>
<td>70.99</td>
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<td>85.90</td>
<td>62.17</td>
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<tr>
<td>5,6-Dibromotubercidin</td>
<td>152.22</td>
<td>156.12</td>
<td>102.42c</td>
<td>—b</td>
<td>112.00</td>
<td>149.24</td>
<td>91.16</td>
<td>71.26</td>
<td>70.74</td>
<td>86.17</td>
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<tr>
<td>5-Chlorotubercidin</td>
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<td>156.68</td>
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<td>149.19</td>
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<td>70.99</td>
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a Spectra were run in DMSO-d<sup>6</sup> and are referenced to internal TMS. H NMR spectra are at 60 mHz and C<sup>13</sup> NMR spectra at 25.2 mHz.
b Signal too weak to be observed.
c Assignment uncertain - may be either C-4a or C-5.
ably very few examples of C-6 substitution will be observed since most electrophilic substitutions require conditions and reagents that would either protonate or form a complex at N-3, N-4. Oddly enough the chlorination with N-chlorosuccinimide in DMF buffered with potassium acetate gave very little product and no 6-chlorotubercidin was isolated.

EXPERIMENTAL

Nuclear magnetic resonance spectra were taken on a Joel Model PS 100 Fourier Transform NMR and a Varian EM 360 NMR. Ultraviolet spectra were measured on a Cary 17 spectrometer in methanol. Column chromatography was done on E. Merck silica gel (70-230 mesh). Thin layer chromatography (TLC) was performed on Merck silica gel 60 F-254 0.20 mm plastic sheets (solvent system: chloroform; methanol; ammonia (75;25;0.5)). HPLC was done on a Waters μ-C-18 Bondapak column. (Solvent system: aqueous 0.01 M NH₄H₂PO₄; acetonitrile.) All reported products were homogeneous under these conditions on TLC and HPLC. Tubercidin was purchased from Upjohn, Fine Chemicals Division, Kalamazoo, MI. Elemental analyses were performed by the microanalytical laboratory at the University of California, Berkeley. Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected.

5-Bromotubercidin (2). A solution of 178 mg (1.0 mmol) N-bromosuccinimide in 5 mL dry DMF was added dropwise to a stirred solution of 266 mg (1.0 mmol) tubercidin in 5 mL dry DMF at room temperature. The red mixture was stirred an additional 30 minutes, the DMF evaporated at reduced pressure, and the residue dissolved in methanol and evaporated with 3 g silica gel. Chromatography on 150 g silica gel eluting with a methanol-chloroform gradient (15-25% methanol; 900 mL total) gave, after crystallization from methanol, 83 mg (0.24 mmol, 24%) 5-bromotubercidin, $λ_{max}$ 275 nm. 5-Bromotubercidin softens at 191° and melts after much darkening and decomposition at 232-235°. Analysis calcd. for C₁₁H₁₃BrN₄O₄: C, 38.28; H, 3.80; N, 16.23; Br, 23.15. Found: C, 38.42; H, 4.00; N, 16.27; Br, 22.9.

6-Bromotubercidin (4) and 5,6-Dibromotubercidin (3). A solution of 3.56 g (20.0 mmol) N-bromosuccinimide in 20 mL dry DMF was added dropwise to a stirred solution of 2.66 g (10.0 mmol) tubercidin and 1.96 g (20.0 mmol) potassium acetate in 40 mL of dry DMF at 20°C. The red reaction mixture was stirred an additional 10 minutes, the DMF evaporated at reduced pressure, and the residue dissolved in methanol and evaporated with 10 g of silica gel. Chromatography on 300 g silica gel eluting with a methanol-chloroform gradient gave, after crystallization from methanol, 1.784 g (5.17 mmol, 51.7%) 6-bromotubercidin,
m.p. 197-200° d, and 123 mg (0.029 mmol, 2.9%) 5,6-dibromotubercidin, $\lambda_{max}^{\text{MeOH}}$ 284 nm, m.p. 225-226°. Analysis calcd. for C$_{11}$H$_{13}$BrN$_4$O$_4$ (4): C, 38.28; H, 3.80; N, 16.23; Br, 23.15. Found: C, 37.99; H, 4.05; N, 15.98; Br, 23.0.

Analysis calcd. for C$_{11}$H$_{12}$Br$_2$N$_4$O$_4$ (2): C, 31.16; H, 2.85; N, 13.21; Br, 37.69. Found: C, 31.45; H, 3.08; N, 12.86; Br, 37.5.

5-Chlorotubercidin (5) and 5,6-Dichlorotubercidin (6). A solution of 266 mg (2.0 mmol) N-chlorosuccinimide in 5 mL dry DMF was added dropwise to a stirred solution of 266 mg (1.0 mmol) tubercidin in 5 mL dry DMF at room temperature. The light orange reaction mixture was stirred an additional 2 hours, the DMF evaporated at reduced pressure, and the residue dissolved in methanol and evaporated with 3 g silica gel. Chromatography on 150 g silica gel eluting with a methanol-chloroform gradient gave, after crystallization from methanol, 120 mg (0.40 mmol, 40%) 5-chlorotubercidin, $\lambda_{max}^{\text{MeOH}}$ 275 nm, and 40 mg (0.12 mmol, 12%) 5,6-dichlorotubercidin, $\lambda_{max}^{\text{MeOH}}$ 281 nm, m.p. 124-125°. Nucleoside 4 softens at 191-192°C and melts with decomposition at 226-228°. Analysis calcd. for C$_{11}$H$_{13}$ClN$_4$O$_4$ (5): C, 44.23; H, 4.39; N, 18.09; Cl, 11.87; Found: C, 43.97; H, 4.46; N, 18.37; Cl, 12.1.

Analysis calcd. for C$_{11}$H$_{12}$Cl$_2$N$_4$O$_4$ (6): C, 39.42; H, 3.61; N, 16.72; Cl, 21.16. Found: C, 39.49; H, 3.76; N, 16.58; Cl, 21.2.

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REFERENCES
