SYNTHESIS OF A DEOXYGUANOSINE-2-AMINO ADDUCT OF A TRIIMIDAZOLE-CONTAINING TALLIMUSTINE ANALOG

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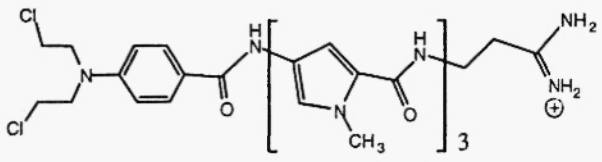
ABSTRACT: A covalent adduct, compound **4**, of a triimidazole-containing analog of tallimustine with the 2-amino group of deoxyguanosine was prepared and characterized.

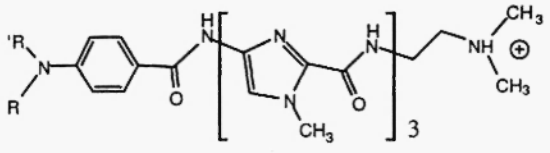
Small molecules, especially minor groove binding agents, that are capable of recognizing specific sequences of DNA are of significant interest. Such compounds have application in medical diagnostics,[1] research,[1] as well as therapeutics.[2] One group of compounds that has received a lot of attention is the distamycin class. While pyrrole-containing analogs of distamycin bind preferentially to the minor groove of AT-rich sequences,[3] their imidazole-containing counterparts are found to tolerate GC sites.[4] More recently compounds in this class have been demonstrated to interact with a strong sequence preference in the minor groove through the side-by-side binding motif.[5] These non-covalently reactive compounds are being developed as potential gene recognizing and controlling agents.[1,5]

In the mid-1980s, derivatives of distamycin bearing alkylating agents, such as a benzoic acid nitrogen mustard, were developed.[6] From them, tallimustine, **1**, was selected for clinical evaluation for the treatment of cancer.[7] The results showed tallimustine to have high myeloid suppressive toxicity.[7a] In 1993, we reported the preparation and biological testing of a triimidazole-containing analog of

tallimustine, **2**.[8] Even though agent **2** has similar cytotoxicity to tallimustine itself, it gave a different pattern of non-covalent sequence binding selectivity. For instance, tallimustine showed a preference for poly(dA-dT), but compound **2** preferred poly(dG-dC).[8] However, it was later discovered that both compounds **1**[9] and **2**[10] covalently reacted at similar consensus sequences, ca. 5′-TTTTGPu and 5′-TTTTAA, with alkylation at the underlined base. It was also demonstrated that even though agents **1** and **2** have two 2-chloroethyl groups, neither was able to effectively produce DNA-DNA interstrand crosslinks, unlike the simple nitrogen mustards such as benzoic acid mustard and chlorambucil.[9,10] This observation had prompted us[11] and others[12] to prepare and test analogs of tallimustine that contain only one 2-chloroethyl arm, i.e. a half-mustard. Our results showed that the half-mustard, **3**, was not only as cytotoxic as its full-mustard, **2**, but both compounds produced similar covalent sequence specificity for 5-TTTTGPu, including 5′-TTTTGG sites.[11] While the results indicated that compounds **1** - **3** interacted in the minor groove, and reaction at deoxyguanosine-N3 was inferred from thermally induced cleavage studies, alkylation at G-2-amino sites could not be totally ruled out.[10,11]



Tallimustine, **1**

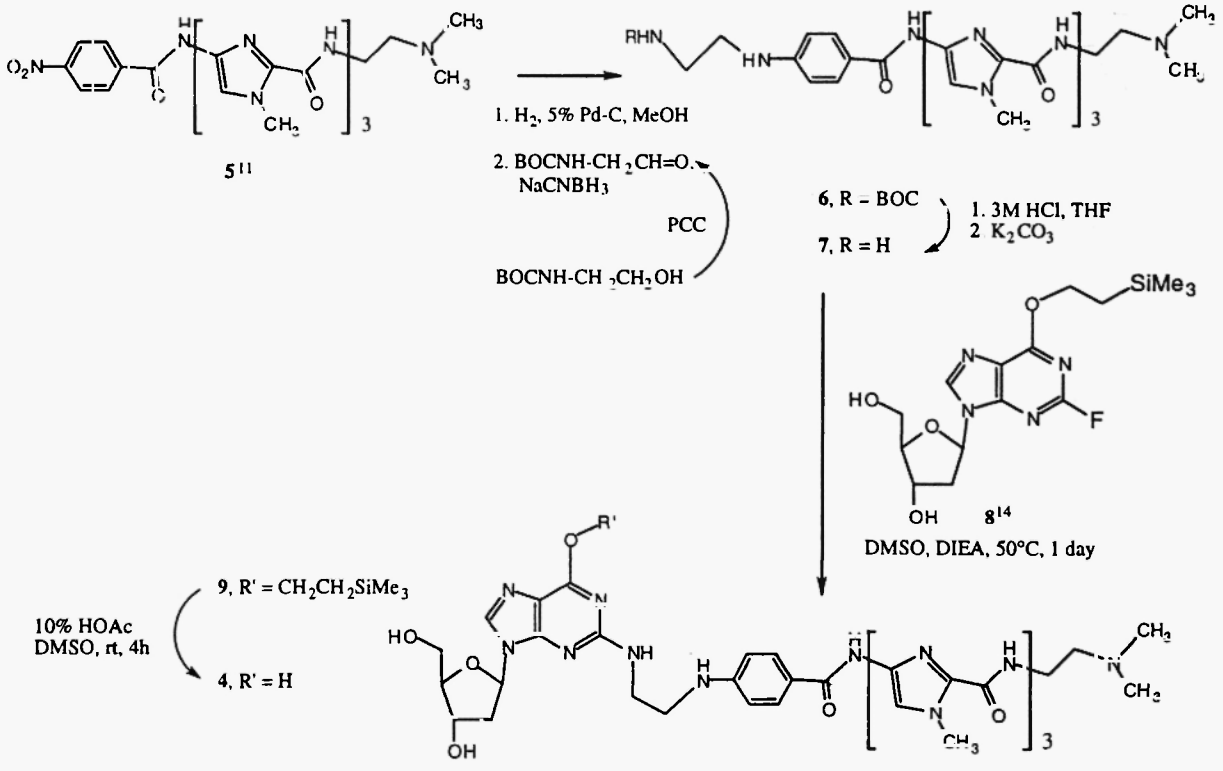


2, R, R' = CH_2CH_2Cl
3, R = CH_2CH_2Cl, R' = H
4, R = $CH_2CH_2NH_2$, R' = H

Figure 1. Structures of tallimustine and its triimidazole-containing analogs.

As part of our continuing studies on the molecular pharmacology of the tallimustine class of compounds, we were interested in isolating nucleoside adducts of half-mustard **3**, by initially reacting the drug with calf thymus DNA followed by enzymatic digestion.[13] To facilitate these studies, we needed to have an authentic sample of a deoxyguanosine-2-amino adduct of compound **3**. Accordingly, in this

communication, we report the synthesis and characterization of a deoxyguanosine-2-amino adduct, **4**.



Scheme 1. Synthesis of the deoxyguanosine-2-amino adduct **4**. DIEA is N,N-diisopropyl-N-ethylamine.

The synthetic approach for the preparation of adduct **4** is depicted in Scheme 1. Catalytic hydrogenation of compound **5**[11] gave an amine intermediate. This amine was reductively alkylated with N-BOC-acetaldehyde (freshly prepared from PCC oxidation of N-BOC-ethanolamine) in the presence of $NaCNBH_3$ and acetic acid to produce compound **6** in 10 percent yield. The BOC protecting group was readily removed by treatment with 3M HCl, followed by quenching with anhydrous K_2CO_3, giving amine **7** in quantitative yield. Reaction of amine **7** with protected 2-fluoroinosine **8**[14] in DMSO and diisopropylethylamine at 50°C gave conjugate **9** which was purified on a silica gel column using an acetonitrile: water: ammonium

hydroxide (90: 6: 4) solvent system (36 percent yield). The silyl protecting group of compound **9** was eventually removed by treatment with acetic acid to give the desired adduct **4**, which was purified by reversed phase HPLC (31 percent yield based on HPLC).[15] Electrospray mass spectral analysis of compound **4** gave a M+H peak at 870.4 amu, consistent with the moleular formula. The structure was ascertained by complete assignment of its 400 MHz ^{1}H-NMR spectrum[16] (Figure 2) using a COSY experiment.

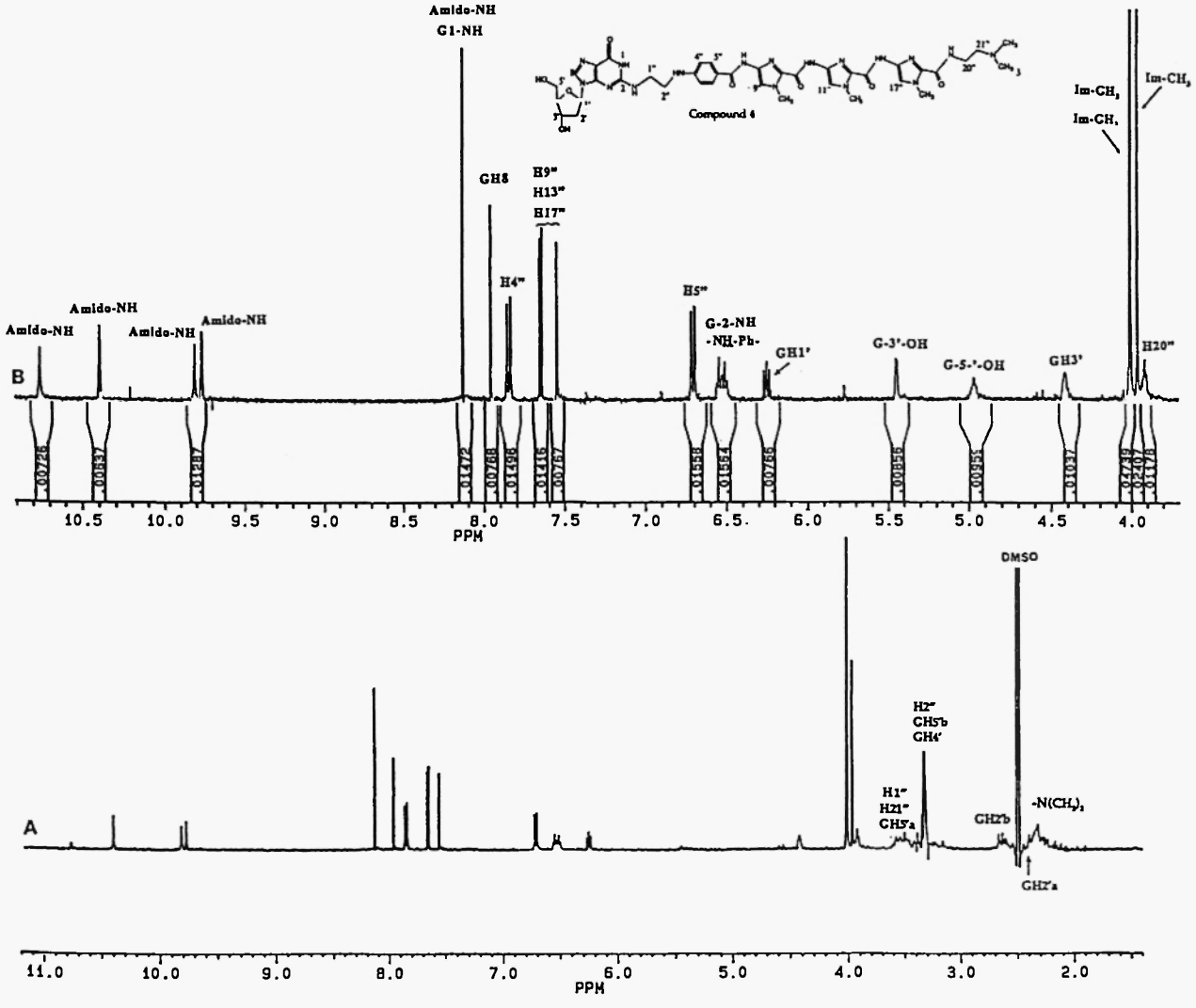


Figure 2. 400 MHz ^{1}H-NMR spectra of adduct **4**.

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15. RP-HPLC was done on a YMC-C18-ODS-AQ semi-preparative column, 310 nm, solvent A: 0.1 N ammonium formate, solvent B: acetonitrile. Gradient: 20-30% B in 5 minutes, then 30-35% B in 15 min. Retention time 10.64 min.
16. 400 MHz ^{1}H-NMR spectrum of compound **4** (DMSO-d_6): 10.72 (s, 1H, amido-NH), 10.34 (s, 1H, amido-NH), 9.74 (s, 1H, amido-NH), 9.70 (s, 1H, amido-NH), 8.12 (s, 2H, amido-NH and G-N(1)H), 7.94 (s, 1H, GH8), 7.83 (d, 8.0, 2H, Ph-H4"), 7.64, 7.63 and 7.53 (three singlets, 1H each, Im-H9", H11" and 17"), 6.63 (d, 8.0, 2H, Ph-H5"), 6.53 and 6.47 (both t br, 1H each, G-2-NH and –NH-Ph), 6.24 (t, 6.9, 1H, H1'), 5.40 (d, 3.5, 1H, G-3'-OH), 4.93 (t br, 3.8, 1H, G-5'-OH), 4.41 (m, 1H, H3'), 4.00 (s, 6H, two Im-CH_3), 3.96 (s, 3H, Im-CH_3), 3.91 (m, 2H, H20"), 3.55 (m, 3H, H21" and H5'a), 3.49 (m, 2H, H1"), 3.30 (m, 4H, H4', H5'b and H2"), 2.62 (m, 1H, H2'a), 2.30 (m, 1H, H2'b), 2.30 (s br, 6H, -$N(CH_3)_2$).

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