Structural Assignment of 2,3,7,8-Tetrahydro-5*H*,10*H*-[1,5,3]dioxazepino[3,2-*c*]indolo[3,2-*g*]pteridin-7-one, a New Heterocyclic Ring System

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The structure of a new heterocyclic ring system, 2,3,7,8-tetrahydro-5*H*,10*H*-[1,5,3]dioxazepino[3,2-*c*]-indolo[3,2-*g*]pteridin-7-one, derived from isatins and 5-aminocytidine was assigned by establishing the regiochemistry with the aid of model reactions and ^{13}C nmr techniques.

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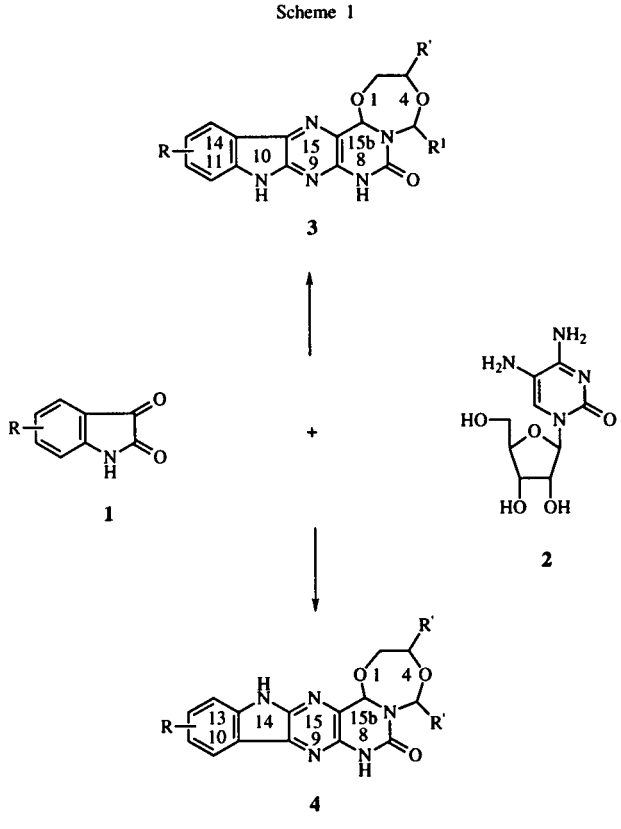
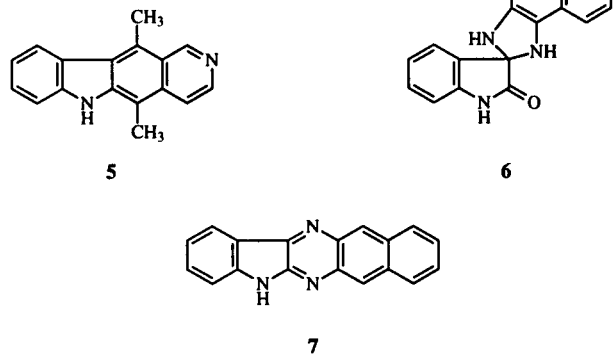
Introduction.

As part of our anticancer drug discovery program, we synthesized [1,2] a series of potential DNA-binding agents containing a new heterocyclic ring system, 2,3,7,8-tetrahydro-5*H*,10*H*-[1,5,3]dioxazepino[3,2-*c*]indolo[3,2-*g*]-pteridin-7-one (**3**), which is derived from isatins **1** and 5-aminocytidine (**2**) (Scheme 1). The reaction of **1** with **2** involves condensation of the diamine followed by formation of the 7-membered ring *via* nucleophilic attack by the 5'-OH of **2** at position 6 of the pyrimidine ring [2]. Theoretically, two possible regioisomers, the 5*H*,10*H*-isomer **3** and the 5*H*,14*H*-isomer **4**, could form in the condensation step (Scheme 1), but only one product was obtained. One of the two isomers, **3**, is structurally related to the anticancer drug ellipticine (**5**) [3,4] and was designed on the basis of this analogy. In this note, we describe the structural assignment of the product with the regiochemistry of **3**.

Scheme 1



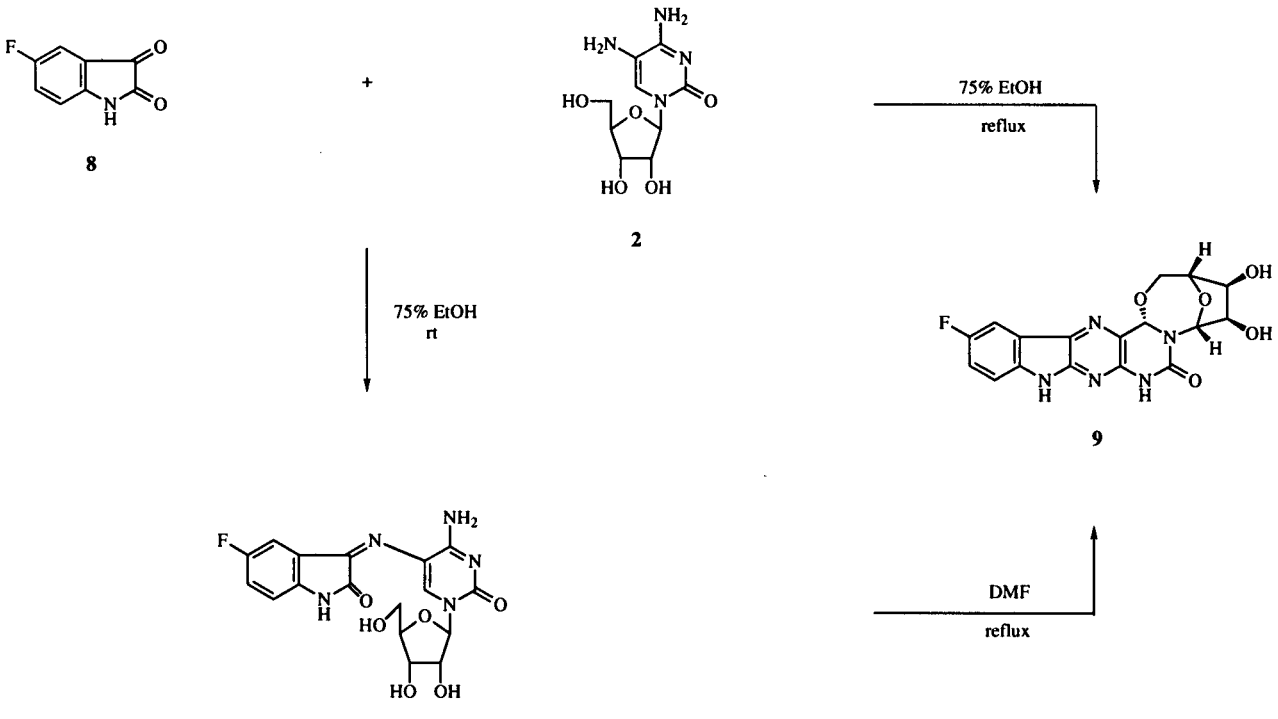


Results and Discussion.

When 5-fluoroisatin (**8**) was allowed to react with **2** in refluxing 75% ethanol, it gave product **9**, but at room temperature it produced a red precipitate **10**, which upon heating in dimethylformamide directly cyclized to **9** (Scheme 2). The Schiff-base structure of intermediate **10** was assigned by nmr (see below) and was suggested by the observation that the color of the reaction mixture changed from orange yellow to deep red. A similar development of a deep color was observed by Grantham *et al.* [5] during the formation of the anil between *N*-methylisatin and an aniline derivative. The formation of a Schiff-base intermediate was unexpected, because previous studies implicated an intermediate with a spiro structure in the condensation of *o*-phenylenediamine with substituted isatins [6,7]. The reaction conditions and the type of *o*-diamine, as well as the structure of the isatin derivatives used in the condensation greatly influenced the nature of the product [6,7]. For example, spiro **6** was produced when isatin was allowed to react with 1,2-diaminonaph-

thalene, whereas linear compound **7** was obtained as the sole isolated product in the case of 2,3-diaminonaphthalene.

Scheme 2



The formation of the Schiff-base intermediate provided a means of solving the problem of regioisomers. If it could be determined which amino group of **2** was involved in the formation of the Schiff-base, it should be possible to deduce which regioisomer was produced, since only a single isomer was isolated [8].

We selected the anil **11** derived from 5-fluoroisatin (**8**) and aniline (**12**) as a model for ^{13}C nmr analysis (Figure 1). The presence of a fluorine atom allowed identification of the carbon signals derived from isatin through carbon-fluorine coupling constants. In this way, the remaining signals in the aromatic region for the base could be assigned. By comparing of the ^{13}C nmr spectra of **12** and the anil **11**, it was found that after condensation, the signals from the *ortho*- and *para*-position had significant down-field shifts from 114.4 ppm to 117.2 ppm and from 116.2 ppm to 125.3 ppm, respectively. In contrast, the *ipso*-position had a small up-field shift from 144.4 ppm to 143.3 ppm, while the *meta*-position did not change significantly. Thus, anil formation is accompanied by characteristic changes in the nmr pattern of aniline that, by analogy, could help to identify which of the two NH_2-groups of **2** reacts to form **10**.

A comparison of the ^{13}C nmr spectra of cytidine (**13**) with 5-aminocytidine (**2**) revealed that the signal from C-6 had a significant up-field shift, from 143.2 ppm to 127.9 ppm, caused by introduction of the 5-amino group, whereas signals from C-2 and C-4 had a relatively small

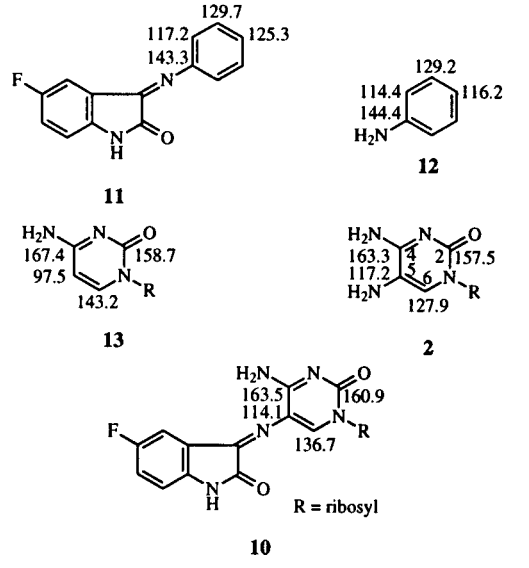


Figure 1. Carbon-13 nmr chemical shifts of compounds **2**, **10**, **11**, **12** and **13**.

up-field shift from 158.7 ppm to 157.5 ppm and 167.4 ppm to 163.3 ppm, respectively. Thus, the signal is more affected at C-6 than at C-4 by substitution at C-5.

A comparison of the ^{13}C nmr spectrum of **10** with that of 5-aminocytidine (**2**) revealed that upon Schiff-base

formation the signal from C-6 of cytosine had a large down-field shift of 9 ppm from 127.9 to 136.7 ppm. The signal from C-2 showed a 3 ppm down-field shift and that from C-4 changed very little. In contrast, the signal at C-5 shifted up-field by 3 ppm from 117.2 to 114.1 ppm during the reaction. The large down-field shift at C-6 and the up-field shift at C-5 suggest that Schiff-base formation occurs at C-5 and not at C-4, indicating that the 5-amino group is the more reactive of the two. Since it is the more electrophilic carbonyl [10] at the 3 position of **8** that reacts with the 5-amino group of **2**, the results are consistent with the proposed structure of **10**. Therefore, it can be concluded that product **9** formed from intermediate **10** must be the desired 5*H*,10*H*-isomer **3**.

EXPERIMENTAL

Melting points were determined in open-end capillary tubes on a Mel-Temp apparatus and are uncorrected. Proton and carbon-13 nmr were recorded on Varian Gemini 300 spectrometer and chemical shifts are reported in ppm relative to tetramethylsilane. Analyses (tlc) were performed on Uniplate GHLF silica gel (Analtech). Solvents and reagents were purchased from Aldrich, Fluka and Sigma. Elemental analyses were carried out by Atlantic Microlab, Inc.

5-Aminocytidine (**2**).

By the reported procedure [9], compound **2** was synthesized in 40% yield from 5-bromocytidine as a pale yellow solid, mp 220-222° (lit. 220°); ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 3.55-3.64 (m, 2H, 5'-H), 3.75 (m, 1H, 3'-H), 3.85-3.95 (m, 2H, 2',4'-H), 4.95 (m, 2H, 3',5'-OH), 5.20 (d, 1H, J = 4.7 Hz, 2'-OH), 5.78 (d, 1H, J = 3.6 Hz, 1'-H), 6.65 (br s, 1H, NH), 7.01 (s, 1H, 6-H), 7.30 (br s, 1H, NH). ^{13}C nmr (dimethyl-d_6 sulfoxide): δ 62.3 (C5'), 71.0 (C2'), 75.3 (C3'), 85.4 (C4'), 91.1 (C1'), 117.2 (C5), 127.9 (C6), 157.5 (C2), 163.3 (C4).

5*H*,10*H*-3(*R*),5(*R*),15b(*S*),16(*R*),17(*R*)-13-Fluoro-16,17-dihydroxy-3,5-ethano-2,3,7,8-tetrahydro[1,5,3]dioxazepino[3,2-*c*]-indolo[3,2-*g*]pteridin-7-one (**9**).

Method A.

The mixture of **8** (165 mg, 1 mmole) and **2** (258 mg, 1 mmole) in 75% ethanol was heated at 80° for 10 hours. The pale yellow crystals formed during this period were collected, washed with ethanol and water to afford compound **9** as a light yellow solid (185 mg, 48%), mp 245-250° dec; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 3.94 (s, 2H, 2-H), 4.18 (m, 1H, 16-H), 4.24 (m, 1H, 17-H), 4.35 (s, 1H, 3-H), 5.06 (d, 1H, J = 6.5 Hz, 16-OH), 5.26 (d, 1H, J = 5.0 Hz, 17-OH), 5.53 (s, 1H, 5-H), 6.15 (s, 1H, 15b-H), 7.36 (dt, 1H, J = 2.2 and 8.8 Hz, 12-H), 7.51 (dd, 1H, J = 4.4 and 8.8 Hz, 11-H), 7.86 (dd, 1H, J = 8.4 and 1.8 Hz, 14-H), 10.74 (s, 1H, 8-NH), 12.13 (s, 1H, 10-NH); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ 71.1 (s, C2), 71.5 (s, C17), 76.2 (s, C16), 85.0 (s, C3), 89.5 (s, C5), 94.0 (s, C15b), 105.9 (d, J = 24.2 Hz, C14), 113.5 (d, J = 8.9 Hz, C11), 115.5 (d, J = 25.7 Hz, C12), 120.5 (d, J = 9.6 Hz, C14a), 125.1 (s, C15a), 129.7 (d, J = 4.2 Hz, C10a), 136.4 (s, C14b), 142.6 (s, C9a), 145.9 (s, C7), 149.6 (s, C8a), 157.8 (d, J = 235.5 Hz, C13).

Anal. Calcd. for $C_{17}H_{14}N_5O_5F\cdot H_2O$: C, 50.37; H, 3.95; N, 17.28. Found: C, 50.72, H, 3.86; N, 16.92.

Method B.

The suspension of the adduct intermediate **10** (110 mg, 0.28 mmole) in dimethylformamide (10 ml) was heated to reflux with vigorous stirring. The red anil slowly dissolved to give an orange solution. When tlc showed that no starting material remained, the solution was evaporated to a small volume (1.5 ml). The pale solid which formed was separated, washed with ethanol and dried to afford compound **9** (77 mg, 77%), which was found to be identical to the product obtained by method A.

3-[(Cytidin-5-yl)imino-5-fluoro-2,3-dihydro-1*H*-indol-3-one (**10**).

To a solution of 5-aminocytidine (**2**) (258 mg, 1 mmole) in 75% ethanol was added 5-fluoroisatin (**8**) (165 mg, 1 mmole) at room temperature with stirring. The resulting red solution was stirred until no starting material was detected by tlc (about 2 days). The red solid formed was collected and washed with water and ethanol to afford **10** (268 mg, 70%), mp 268° dec; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 3.61 (m, 2H, 5"-H), 3.85 (m, 1H, 3"-H), 3.98 (m, 2H, 2" and 4"-H), 4.80 (brs, 1H, 5"-OH), 5.05 (d, 1H, J = 4.4 Hz, 3"-OH), 5.40 (d, 1H, J = 5.1 Hz, 2"-OH), 5.90 (d, 1H, J = 4.2 Hz, 1"-H), 6.85 (dd, 1H, J = 4.3 and 8.4 Hz, 7-H), 7.20 (t, 1H, J = 8.0 Hz, 6-H), 7.55 (br s, 1H, NH), 7.75 (m, 2H, NH and 4-H), 9.29 (s, 1H, 6'-H), 11.00 (s, 1H, 1-NH); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ 61.5 (s, C5"), 70.0 (s, C2"), 74.3 (s, C3"), 84.5 (s, C4"), 89.5 (s, C1"), 109.9 (d, J = 24.8 Hz, C4), 111.3 (s, C7), 114.1 (s, C5'), 118.7 (d, J = 23.9 Hz, C6), 124.6 (d, J = 8.6 Hz, C3a), 136.7 (s, C6'), 139.8 (s, C2), 147.2 (d, J = 2.3 Hz, C7a), 153.9 (s, C3), 158.3 (d, J = 237 Hz, C5), 160.9 (s, C2'), 163.5 (s, C4').

Anal. Calcd. for $C_{17}H_{16}N_5O_6F$: C, 50.37; H, 3.95; N, 17.28. Found: C, 50.57; H, 4.10;N, 17.19.

5-Fluoro-3-phenylimino-2,3-dihydro-1*H*-indol-2-one (**11**).

The mixture of aniline (80 mg, 0.86 mmole) and **8** (100 mg, 0.61 mmole) in ethanol (4 ml) was refluxed for 5 hours. The red solution was then cooled to room temperature. Yellow crystals formed on standing. The crystals were filtered, recrystallized from ethanol to give the anil **11** (125 mg, 86%), mp 234-236°; ^{1}H nmr (dimethyl-d_6 sulfoxide): δ 5.94 (dd, 1H. J = 8.4 and 2.6 Hz, 4-H), 6.90 (dd, 1H, J = 8.4 and 4.3 Hz, 7-H), 6.98 (d, 2H, J = 8.2 Hz, 2',6'-H), 7.23 (dd, 1H, J = 8.4 and 2.6 Hz, 6-H), 7.28 (t, 1H, J = 7.8 Hz, 4'-H), 7.49 (t, 2H, J = 7.8 Hz, 3',5'-H), 11.02 (s, 1H, NH); ^{13}C nmr (dimethyl-d_6 sulfoxide): δ 111.9 (d, J = 25.4 Hz, C4), 112.7 (d, J = 7.6 Hz, C7), 116.01 (d, J = 8.1 Hz, C3a), 117.2 (s, C2', C6'), 120.9 (d, J = 23.6 Hz, C6), 125.3 (s, C4'), 129.7 (s, C3', C5'), 143.3 (s, C1'), 150.1 (s, C2), 154.6 (d, J = 2.3 Hz, 7a), 156.7 (d, J = 236 Hz, C5), 163.5 (s, C3).

Anal. Calcd. for $C_{14}H_9N_2FO$: C, 70.00; H, 3.78; N, 11.66. Found: C, 69.90; H, 3.92; N, 11.41.

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