SYNTHESIS OF A 3-ACETOXY-6-PHENYLPYRONE AND ITS CONVERSION TO A PYRIDO[1,4]BENZODIAZEPINE

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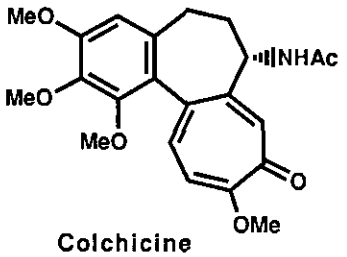
Abstract - 3-Acetoxy-6-(2-nitro-3,4,5-trimethoxyphenyl)-4-pyrone (5) was prepared via condensation of 3-acetoxy-4-methoxy-3-buten-2-one (2) with the appropriate benzoyl chloride and acid catalyzed cyclization. The intermediate pyrone was converted to the target, 6-oxo-2,5,9,10,11-pentamethoxy-1,2,3,6-tetrahydropyrido[1,6-d][1,4]benzodiazepine (1).

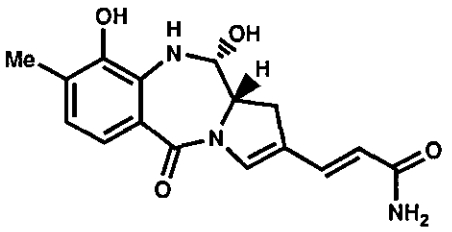
We recently desired the pyridobenzodiazepine 1 as part of a program toward the design of novel antitumor agents. A key element in the structure of 1 was the α–alkoxyamino functionality incorporated into the diazepine ring (ring B), similar to that found in anthramycin. Compounds in the anthramycin class of antitumor antibiotics are known to bind in the minor grove of DNA. The aminal functionality found in anthramycin[2] and related compounds has been shown to be important to the tight binding of these molecules to DNA.[3] Nmr data suggests that the aminal moiety is attacked by the amino group present in the 2 position of guanine to form a covalent attachment.[4] A second important subunit of molecule 1 was the 2-trimethoxyphenyl-5-methoxypyridone ring comprising rings A and C. Molecular modeling studies suggested that relevant oxygens in structure 1 had a similar orientation to those in the colchicine molecule. Colchicine has been shown to bind to tubulin, to prevent microtubule formation and thus prevent cell division.[5] By incorporating aminal functionality into a molecule resembling colchicine we hoped to produce a molecule which would bind to tubulin and form a covalent bond with nucleophiles present in the protein.

A pivotal intermediate in the preparation of the target was alkoxypyrone 5. Previously 3-alkoxy-4-pyrones have been prepared by the oxidation of furfuryl alchohols[6], however we wished to investigate the possibility of forming pyrone 5 by condensation of a benzoyl chloride with an appropriately substituted butenone. 3-Acetoxy-4-methoxy-3-buten-2-one (2) is readily available in two steps from commercially available 4-methoxy-3-buten-2-one.[7,8] Koreeda and

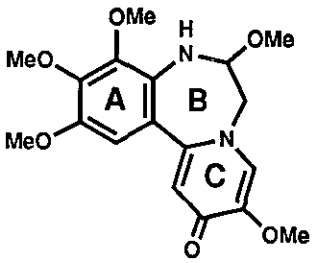
Ganem have shown that the lithium enolate of 4-methoxy-3-buten-2-one can be condensed with acid chlorides to give 2-substituted 4-pyrones.[9] To employ this strategy using the 3-acetoxy-substituted butenone **2** required selective deprotonation of the methyl group adjacent to the ketone carbonyl without deprotonation of the methyl group in the acetoxy moiety.



Colchicine



Anthramycin



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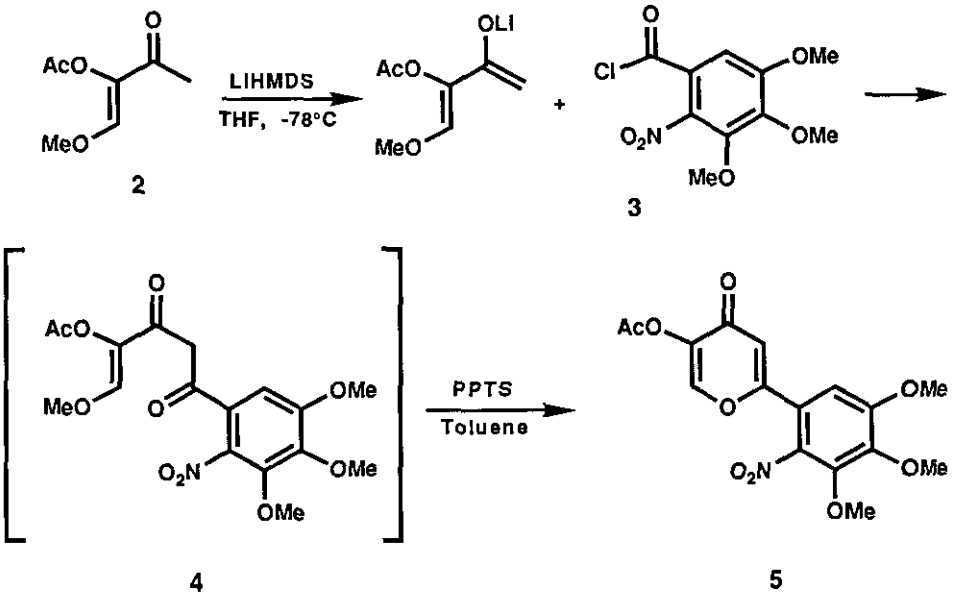
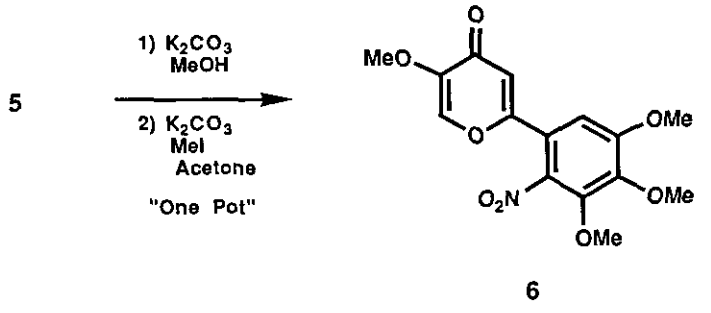
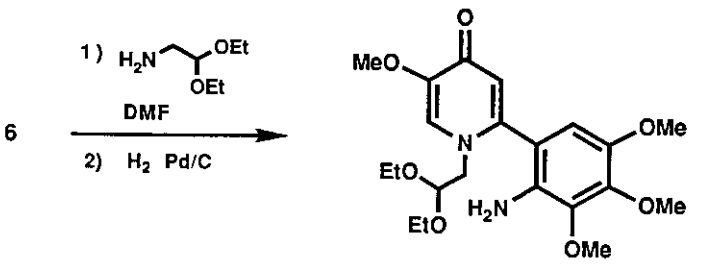
This was achieved by reaction of ketone **2** with lithium hexamethyldisilazide at -78°C. Two equivalents of the lithium enolate of ketone **2** were treated with one equivalent of benzoyl chloride **3** and the unisolated intermediate diketone **4** was cyclized with pyridinium p-toluenesulfonate to give a 45% overall yield of pyrone **5**. Cleavage of the acetate by the action of K_2CO_3 in methanol, evaporation of the solvent and treatment of the residue with methyl iodide in acetone resulted in a 95% yield of the methoxypyrone **6**.
Treatment of pyrone **6** with aminoacetaldehyde diethylacetal in moist DMF followed by catalytic hydrogenation produced pyridone **7**. Unfortunately all attempts to hydrolyze the acetal moiety of **7** resulted in either no reaction or in extensive decomposition of starting material. To solve this problem we chose an aldehyde synthon which could be unmasked using oxidative rather than

hydrolytic conditions. The N-allylpyridone **8** was prepared by reaction of allylamine with pyrone **6**. The terminal olefin of pyridone **8** was cleaved with catalytic osmium tetroxide in the presence of sodium periodate to give aldehyde **9** in 60% yield. The synthesis of pyridobenzodiazepine ring system was successfully completed by iron-acetic acid reduction of the aryl nitro group of **9**. The presumed aniline intermediate spontaneously cyclized to give the cyclic imine **10**.[10]







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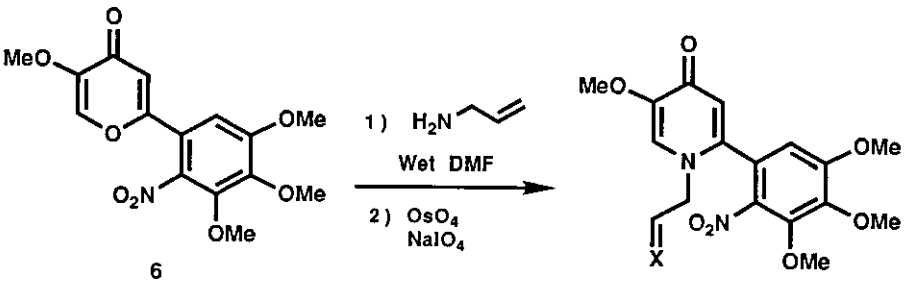
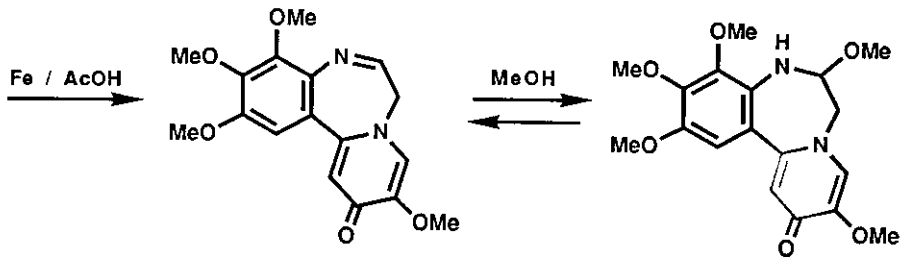
Methanol readily added to the imine double bond to produce the desired α-methoxyamine 1. The equilibrium behavior of 1 and 10 as observed by ^1H nmr was consistent with the imine-α-methoxyamine equilibrium observed for anthramycin and its analogues.[11] While the imine 10 and α-methoxyamine 1 showed similar aminal-imine equilibrium behavior to anthramycin and its analogues, it did not have any of the biological properties associated with this class of antitumor antibiotics. The molecule 1 was found to only bind weakly to tubulin.[12]



6

8 X=CH_2

9 X=O



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We have demonstrated that 3-acetoxy-4-methoxy-3-buten-2-one (**2**) can be condensed with a benzoyl chloride giving facile entry into 6-aryl-3-alkoxy-4-pyrone systems such as **5**. Such a protocol should be applicable to the synthesis of many 6-substituted 3-alkoxy-4-pyrones. The preparation of diazepine **1** from 4-pyrone **5** represents a successful strategy for the synthesis of highly substituted pyrido[1,2-d][1,4]benzodiazepines.

EXPERIMENTAL

3-Acetoxy-6-(2-nitro-3,4,5-trimethoxyphenyl)-4-pyrone (5).

To a flame dried 250 ml 3 neck flask which had been purged with nitrogen was added THF (90 ml) and hexmethyldisilazane (5.28 ml, 25.1 mmol). The solution was cooled to -78°C, and treated with n-butyllithium (16.2 ml, 1.5M solution in hexane). After 20 min 3-acetoxy-4-methoxy-3-buten-2-one (**2**)[7] (3.96 g, 25.1 mmol) was added in a solution of THF (16 ml). After another 20 min the enolate solution was treated with 2-nitro-3,4,5-trimethoxybenzoyl chloride (**3**)[13] (3.45 g, 12.6 mmol) in THF (16 ml) and the reaction mixture was warmed to -15°C over 30 min. The reaction mixture was quenched with 10% HCl and allowed to come to room temperature for 1 h. The phases were separated and the organic layer was diluted with 200 ml of ethyl ether and extracted with 10% HCl (50 ml). The combined aqueous phases were extracted with 3 X 100 ml of ether. The combined organic extracts were washed with brine and dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude residue was heated to reflux for 1 h in 100 ml of toluene in the presence of pyridinium *p*-toluenesulfonate (0.5 g). The toluene was removed under reduced pressure and the residue was taken up in 200 ml of $CHCl_3$. The solution was washed with sodium bicarbonate and brine and dried over $MgSO_4$. The solution was concentrated to 50 ml and 50 ml of ether was added. After storing overnight in the freezer the product was collected by filtration (1.58 g). The mother liquor was concentrated and the residue was chromatographed on silica gel (25% EtOAc in $CHCl_3$) to obtain an additional amount of the product (0.38 g), total yield 43%. mp 168-169°C ($CHCl_3$, Et_2O), ^{1}H-nmr (300MHz, $CDCl_3$) δ 7.89(s, 1H), 6.80(s, 1H), 6.70(s, 1H), 4.04(s, 3H), 3.97(s, 3H), 3.94(s, 3H), 2.34(s, 3H); ^{13}C-nmr (75 MHz, $CDCl_3$) δ 172.0, 167.5, 160.6, 155.1, 147.9, 146.9, 145.1, 141.2, 119.9, 115.7, 107.0, 62.6, 61.3, 56.5, 20.3; ir (cm^{-1}, $CHCl_3$) 3010, 2950, 1780, 1660, 1570, 1530, 1360, 1180, 1120; EIMS, 365 M^+, 323 M^+-C_2H_2; Anal.

ACKNOWLEDGEMENT

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to 0°C and treated with 50 ml of a 0.5 M aqueous solution of $Na_2S_2O_5$ which had also been cooled to 0°C. The resulting solution was extracted twice with 50 ml portions of chloroform. The aqeous layer was adjusted to pH 11 with sodium hydroxide, saturated with sodium chloride, and extracted with six 50 ml portions of chloroform. The chloroform extracts from the basic solution were dried with magnesium sulfate and evaporated to give the product as a light orange oil (0.177 g, 60% yield). This material was used in the next reaction without further purification. The aldehyde appears to exist in a hydrated or enolic form prior to chromatography. Ir (cm^{-1}, $CHCl_3$) 3100 (broad), 2980, 2940, 1625, 1580, 1530, 1490; EIMS m/z, (%) 378(24%), 336(base) M^+-C_2H_3O. If the sample was subjected to silica gel chromatography (2% methanol in chloroform), the nmr was indicative of the aldehydic form of the molecule (300MHz, $CDCl_3$) δ 9.63(s, 1H), 6.88(s, 1H), 6.53(s, 1H), 6.29(s, 1H), 4.67(s, 2H), 4.04(s, 3H), 3.97(s, 3H), 3.87(s, 3H), 3.82(s, 3H).

6-Oxo-2,5,9,10,11-pentamethoxy-1,2,3,6-tetrahydropyrido[1,6 d][1,4]-benzodiazepine (1).

The nitro aldehyde **9** (89 mg, 0.23 mmol) was dissolved in 2.5 ml of methanol and to the solution was added 0.1 ml of acetic acid and 50 mg of iron powder. The mixture was heated to reflux for 6 h. The solution was cooled, diluted with ethyl acetate (20 ml) and filtered through celite. The filtrate was evaporated, taken up in chloroform (20 ml) and filtered again through celite. This filtrate was stripped and the residue was chromatographed on silica gel (3-8% MeOH in $CHCl_3$ gradient elution) to give a light yellow solid (28 mg, 33% yield), mp 185°C (dec.). The product isolated chromatogaphically appeared to be a mixture of 2 compounds. Addition of MeOH-d_4 caused dissapearence of signals at δ 8.15(t, J=5Hz, 1H) and δ 5.57(br s, 2H). These signals where assigned as the protons on the imine carbon and the adjacent methylene carbon in compound **10**. The spectrum after addition of the MeOH-d_4 is as follows: δ 7.38(s, 1H), 6.76(s, 1H), 6.63(s, 1H), 5.03(dd, J=8, 4 Hz, 1H), 4.14(dd, J=14, 4 Hz, 1H), 3.95(s, 6H), 3.91(s, 3H), 3.88(s, 3H), the other methylene hydrogen signal is obscured by the methoxy groups; ir (cm^{-1}, $CHCl_3$) 3370, 2990, 2950, 1625, 1570, 1480, 1220, 1100; uv λ^{MeOH} nm: 320(sh, 5790), 283(12,700), 256(sh, 19,700), 239(28,000), HREIMS Calcd for $C_{17}H_{18}N_2O_5$ 330.1216, Found 330.1224.

Calcd for $C_{16}H_{15}NO_9$: C, 52.61, H, 4.14, N, 3.85. Found: C, 52.55, H, 4.19, N, 3.78.

3-Methoxy-6-(2-nitro-3,4,5-trimethoxyphenyl)-4-pyrone (6).
A slurry of the acetoxypyrone **5** (1.10 g, 3.0 mmol) in methanol (70 ml) was treated with potassium carbonate (1.5 g) and allowed to stir for 15 min. The methanol was removed under reduced pressure and the resulting residue was stirred in reluxing acetone (100 ml) containing methyl iodide (5 ml) for 2 h. The acetone and excess methyl iodide were removed under reduced pressure and the residue was partitioned between water and chloroform. The organic solution was washed with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure to give methoxypyrone **6** as an off white solid (0.984 g, 98% yield). mp 174-176°C ($CHCl_3$, Hexane); ^{1}H-nmr (300MHz, $CDCl_3$) δ 7.56(s, 1H), 6.77(s, 1H), 6.65(s, 1H), 4.02(s, 3H), 3,97(s, 3H), 3.96(s, 3H), 3.79(s, 3H); ir (cm^{-1},$CHCl_3$)3000, 2950, 1620, 1590; HREIMS Calcd for $C_{15}H_{15}NO_8$ 337.0798, Found, 337.0782

3-Methoxy-6-(2-nitro-3,4,5,-trimethoxyphenyl)-1-(2-propenyl)-4-pyridone (8).
Under an atmosphere of nitrogen the methoxypyrone **6** (0.945 g, 2.8 mmol) was dissolved in a solution of 9 ml of DMF, 9 ml of water and 9 ml of allylamine. The mixture was heated to 60°C for 3.5 h during which time the solids were gradually dissolved and the solution became dark red. The volatiles were removed under vacuum and the residue was chromatographed on silica gel (gradient elution 1-5% methanol in chloroform in 5 stages) to give the desired pyridone (**8**) as an orange oil (0.553 g, 53% yield). ^{1}H-Nmr (300 MHz, $CDCl_3$) δ 7.06(s, 1H), 6.59(s, 1H), 6.29(s, 1H), 5.90(ddt, J=17, 10, 6 Hz, 1H), 5.34(dd, J=10, 0.6 Hz, 1H), 5.16(dd, J=17, 0.6 Hz, 1H), 4.36(dd, J=16, 6 Hz, 1H), 4.28(dd, J=16, 6 Hz, 1H), 4.04(s, 3H), 3.98(s, 3H), 3.91(s, 3H), 3.84(s, 3H); ir (cm^{-1}, $CHCl_3$) 2970, 2940, 1620, 1580, 1525, 1470, 1340, 1220, 1110, 1010, 990, 930, 900, 850; HREIMS Calcd for $C_{18}H_{20}N_2O_7$ 376.1270, Found 376.1256.

1-(2-Ethanone)-3-methoxy-6-(2-nitro-3,4,5-trimethoxyphenyl)-4-pyridone (9).
The N-allylpyridone **8** (0.293 g, 0.78 mmol) was dissolved in 20 ml of 1:1 THF-water. Sodium periodate (0.500 g, 2.34 mmol) and a catalytic amount of osmium tetroxide (20 mg, 0.08 mmol) were added and the reaction mixture was stirred at room temperature for 2.5 h. The reaction mixture was filtered, cooled