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Application of Modified Flavone Closure for the Preparation of Racemic L86-8275

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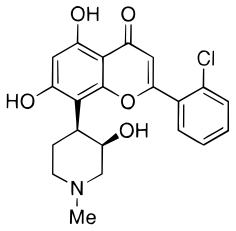
Abstract:

The laboratory preparation of racemic L86-8275 (1a) and the salt (1b) is described in 7% overall yield. Our method eliminated a number of chromatography steps from the patent procedure and improved the flavone-forming reaction by employing a stepwise mechanism. Solvent-free conditions for the demethylation step are also described.



1a L86-8275 (Flavopiridol)
1b Flavopiridol HCl

Figure 1.

Introduction

Polyhydroxylated flavones such as quercetin and genistein have recently been shown to have kinase inhibitory activity and antiproliferative effects on tumor cell lines after extended periods of incubation.[1] Further investigation of synthetic analogues of this class led to selection of L86-8275 (flavopiridol, (−) **1a**, Figure 1) to be advanced into phase I clinical trials for the treatment of human breast cancer.[1,2]

Despite these advancements, the only information available regarding the synthesis of free base L86-8275 (**1a**) and the HCl salt **1b** has been reported in three patent references.[3−5] It was our intention to develop scalable methods for the preparation of multigram quantities of racemic **1b** for further evaluation in *in vitro* and *in vivo* testing. The preparation of *o*-hydroxyacetophenone **2** has been reported in an earlier reference.[6] We found excellent overall yield reproducibility with the reported route (13.8 versus the published 18.8%). Additionally we found that two chromatograghic purifications could be eliminated without detriment to the overall product quality.[7] Therefore we saw **2** as an excellent starting point for our investigations.

Results and Discussion

Benzoylation of **2** using 2-chlorobenzoyl chloride provided the benzoate **3** in 76% yield. The ortho substitution between the benzoate functionality and the acetophenone set up a flavone-forming step that was reported earlier. Conditions for the cyclization called for reaction of **3** with sodium in dioxane or DMF at reflux to give a rearranged intermediate. Following extraction and volume consolidation, the adduct was treated with gaseous HCl to induce the dehydration to provide flavone **6**.[3] Our attempts to affect conversion of **3** to **6** using this procedure met with limited success and were not applicable for our scale-up purposes. We therefore investigated alternative conditions for the preparation of flavone **6** which might be better suited for functionality present in **3**. Methods involving the internal migration/dehydration strategy have been reported.[8,9] Our studies focused on selecting a method in which the intermediates could be characterized at each stage using NMR and mass spectroscopy (Scheme 1).

We were gratified to find that powdered KOH in hot pyridine smoothly isomerized the benzoate **3** to **4** in quantitative yield. The ^{1}H NMR purity of the crude **4** from the migration was quite high, despite the complexity of key signals. We could not assign the directionality of the keto−

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(7) Summary of yield and procedure improvements: Step 1 (trimethoxybenzene, 4-methylpiperdone condensation) 94% yield without recystallization. Step 2 (hydroboration, oxidation) 65% yield, scale-up was problematic because of the exothermicity during the quenching of the excess reagents required for convenient reaction times. We found it advantageous to limit the reaction size, since we observed a 20% decreased yield when the reaction was performed over 0.75 mol scale. We also incorporated an additional mild acid hydrolysis step, since up to 15% product loss was observed due to the formation of unhydrolyzed (water soluble) borate ester. We avoided column chromatography because of poor mass recovery. Step 3 (Swern oxidation) 71% yield used azeotropic drying with benzene before oxidation. Step 4 ($NaBH_4$ reduction) 45% yield, 7:4 ratio of cis/trans, formation of HCl salt showed appreciable solubility differences in acetone to aid separation. Step 5 (demethylation /Fries) 71% yield, the original procedure called for isolating the diacetate and performing a separate hydrolysis step. We found it more convenient to effect the hydrolysis of the phenolic acetate by quenching the reaction to pH 10 with sodium carbonate and increasing the exposure time before product extraction.

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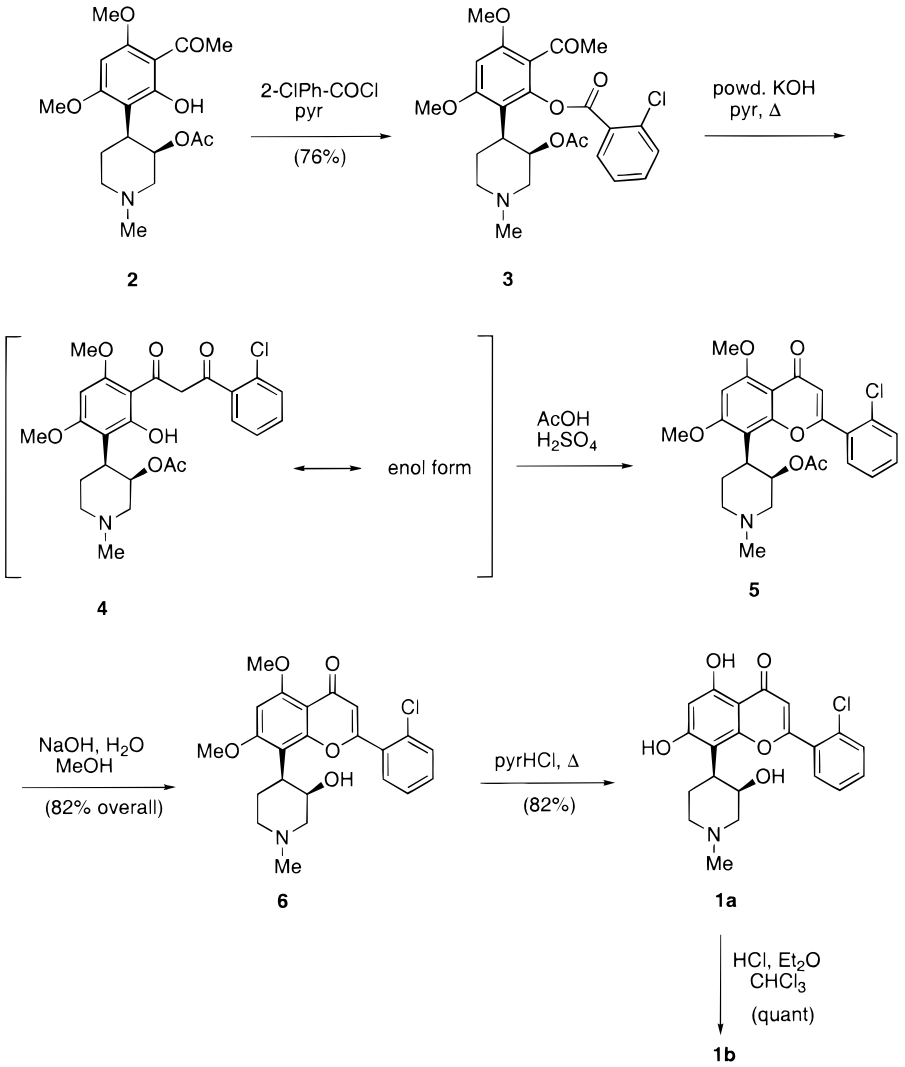
Scheme 1



enol tautomerism of the β-diketone structure, but could only infer that the extent of enolization was 64%, on the basis of the doubling of peaks in the ^{1}H NMR ($CDCl_3$) and the partial integrals of downfield signals for the tautomers. The next step in the flavone-forming reaction was the dehydration step, similar to the gaseous HCl conditions which were reported earlier. We found that catalytic sulfuric acid (concentrated) in acetic acid at 100 °C was an excellent alternative and provided **5** in quantitative yield. To our surprise, the anhydrous conditions for both the acyl migration (KOH, pyr) and the strongly acidic dehydration left the acetate group of **5** intact. Therefore, a separate hydrolysis step for cleavage of the acetoxy group was added to the reaction sequence. By the use of aqueous sodium hydroxide in MeOH, flavone **6** was isolated in 82% yield for the three-step sequence.

The final steps required for completion of the synthesis of **1b** were demethylation of the methyl ether-protecting groups and HCl salt formation. Our initial attempts to deprotect **6** using boron tribromide using a 4-fold excess of reagent provided a mix of **6**, **1a** and, to our surprise a monomethylated derivative of **1a**. Therefore, the feasibility of scaling up the earlier reported protocol, which called for the use of pyridinium hydrochloride in quinoline, was examined in detail. The relatively small scale of the original procedure (<5 g) necessitated the use of quinoline as a solvent for the reaction, and thus, a silica gel column to facilitate its removal as an impurity. The advantage of the scale-up demethylation was the possibility of running the reaction in solvent-free conditions. Our initial trials showed that heating of **6** in 10 equiv of pyridinium hydrochloride at its melting point (ca. 210 °C) provided a stirable melt which served as the reaction solvent. Equally satisfying were the convenient reaction times (4 h) and the ease of work up. Quenching the free-flowing melt directly into water provided high purity **1a** after filtration. Neutralization and extraction provided additional product of acceptable quality after ether trituration to remove residual pyridine. The combined material did not require purification by silica gel. The demethylation was successfully conducted on 75 g scale and provided **1a** in 82% yield. The free base was treated with ethereal HCl in chloroform and afforded **1b** in 96% yield. Owing to the very clean demethylation procedure, the only procedure required for the isolation of **1b** was evaporation and trituration with ether. Our material was consistent with the data (mp) reported earlier for the compound.[3] By this

method, we have prepared research scale quantities of **1b** for use in pharmacological evaluation.

In conclusion, a six-step procedure for the preparation of **1b** has been described. Our method details the conversion of benzoate **3** through a high-yielding flavone process in which the intermediate stages were characterized, thus providing flavone **6** in high yield. Solvent-free conditions were developed for the demethylation reaction, which provided **1a** (Flavopiridol) and its salt **1b** on a laboratory scale.

Experimental Section

General Methods. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Unless otherwise noted, ^{1}H NMR (300 MHz) and ^{13}C NMR spectra (75 MHz) were recorded in $CDCl_3$, and chemical shifts are given in ppm on the δ scale from internal TMS. Mass spectra were obtained on either VG 70-VSE (High Res DCI) or Finnigan MAT 8230 (DCI) mass spectrometers.

Benzoate 3. A 5-L round-bottom flask was charged with **2**[6] (137.6 g, 0.39 mol) and pyridine (4.0 L) and cooled to 0 °C. 2-Chlorobenzoyl chloride (148.6 mL, 1.173 mol) was added (exothermic!) and the reaction allowed to reach rt, after which the mixture was stirred for 4 h. The reaction was poured into a stirred solution of Na_2CO_3 (saturated), with care taken to control the foaming which occurred. The mixture was extracted with $CHCl_3$ (3 × 500 mL), washed with H_2O (1×) and brine (1×), dried ($MgSO_4$), filtered through a mix of DARCO and Celite, and concentrated under reduced pressure. The residue was crystallized from a mixture of Et_2O and hexane to give **3** as a white powder (144.7 g) in 76% yield: mp 132−135 °C; TLC R_f = 0.53 (50% MeOH in EtOAc); ^{1}H NMR δ 8.10 (dt, J = 7.3, 0.9 Hz, 1H), 7.47 (m, 2H), 7.39 (m, 1H), 6.40 (s, 1H), 5.08 (br s, 1H), 3.90 (s, 6H), 3.22 (dt, J = 12.8, 2.7 Hz, 1H), 3.03 (m, 2H), 2.79 (m, 1H), 2.46 (s, 3H), 2.24 (s, 3H), 2.23 (m, 1H), 2.03 (s, 3H), 2.00 (m, 1H), 1.74 (br d, 1H); ^{13}C NMR δ 200.21, 171.18, 163.96, 160.79, 157.63, 147.85, 134.25, 133.11, 132.27, 131.13, 129.12, 126.92, 117.19, 114.94, 93.08, 71.01, 59.25, 56.88, 55.87, 55.79, 46.30, 37.94, 31.76, 25.05, 21.35; IR (KBr, cm^{-1}) 2940, 1750, 1728, 1690, 1606, 1240, 1136; MS (NH_3−CI) *m*/*e* 490 (M + H$^+$, 100%). Anal. Calcd for $C_{25}H_{28}ClNO_7$: C, 61.29; H, 5.76; N, 2.87. Found: C, 60.74; H, 5.63; N, 2.86.

Diketone 4. A 2-L round-bottom flask equipped with an overhead stirrer was charged with **3** (194.7 g, 0.397 mol) and pyridine (700 mL) and heated to 55 °C. Freshly pulverized KOH (33.1 g, 0.589 mol) was added with stirring and the heating continued for 30 min. The resulting black mixture was poured into a mixture of H_2O (3.08 L) and HCl (concentrated, 560 mL) and allowed to stand for 30 min. While the mixture cooled, the pH was adjusted to 9 with Na_2CO_3 (saturated), and the aqueous layer was extracted with 5% MeOH in $CHCl_3$ (4 × 500 mL). The combined organic layers were washed with H_2O (2×) and brine (1×), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The yellow glassy solid **4** (198 g) was obtained and used without further purification. TLC R_f = 0.63 (50% MeOH in $CHCl_3$); ^{1}H NMR δ [k-keto form, e-enol form; ratio of 7/4 determined by comparison of integrals at δ 13.5 (k) and δ 4.6 (e)] 13.92 (s, 1He*), 13.54 (s, 1Hk*), 8.62 (br d, J = 4.0 Hz, 1H), 7.41−7.28 (m, 5H), 7.24 (s, 1He*), 5.93 (s, 1He), 5.84 (s, 1Hk), 5.16 (br s, 1He), 5.11 (br s, 1Hk), 4.62 (q, J = 16.5 Hz, 1Hk*), 3.88 (s, 3He), 3.85 (s, 3He), 3.84 (s, 3Hk), 3.62 (s, 3Hk), 3.32 (dt, J = 13.6, 3.3 Hz, 1H), 3.22−3.20 (m, 3H), 2.31 (m, 4H), 2.07 (m, 1H), 1.99 (s, 3H), 1.97 (s, 3H), 1.56 (m, 1H); *- exchangeable with D_2O; MS (NH_3−CI) *m*/*e* 490 (M + H$^+$, 100%).

Dehydration product 5. A 12-L round-bottom flask was charged with **4** (198 g, 0.397 mol), AcOH (5.50 L), and H_2SO_4 (concentrated, 55 mL) and heated to 100 °C. Heating was maintained for 2 h followed by cooling to rt overnight. The reaction was concentrated on a rotory evaporator, diluted with H_2O, and neutralized by the addition of Na_2CO_3 (saturated, approx 5 L) to pH 9. The aqueous layer was extracted with 5% MeOH in $CHCl_3$ (4 × 500 mL). The combined organics were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The product **8** was obtained as a foam (196 g) and used without further purification. TLC R_f = 0.80 (50% MeOH in $CHCl_3$); ^{1}H NMR δ 7.62 (m, 1H), 7.52 (m, 1H), 7.43 (m, 2H), 6.46 (s, 1H), 6.42 (s, 1H), 5.11 (br s, 1H), 4.00 (s, 3H), 3.94 (s, 3H), 3.51 (dt, J = 12.8, 3.0 Hz, 1H), 3.10 (m, 1H), 3.03 (br d, J = 11. 3 Hz, 2H), 2.30 (m, 1H), 2.27 (s, 3H), 2.04 (br t, J = 11.3 Hz, 1H), 1.91 (s, 3H), 1.71 (br d, J = 11.3 Hz, 1H); MS (NH_3−CI): *m*/*e* 472 (M + H$^+$, 100%).

Flavone 6. A 3-L round-bottom flask was charged with **5** (187.5 g, 0.39 mol), MeOH (500 mL), and H_2O (645 mL) followed by the addition of aqueous NaOH (50%, 440 mL). The reaction was stirred for 1 h at room temperature and diluted with H_2O. The aqueous layer was placed on a rotary evaporator to remove the volatiles and extracted with 5% MeOH in $CHCl_3$ (4 × 500 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The product was azeotroped with toluene, and several of the impurities could be removed by trituration of the glassy foam (158.9 g) with Et_2O. The product was obtained (140.2 g) in 82% yield: mp 126−134 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.89 (dd, J = 7.0, 2.2 Hz, 1H), 7.68 (dd, J = 7.7, 1.5 Hz, 1H), 7.57 (m, 2H), 6.67 (s, 1H), 6.34 (s, 1H), 4.07 (br s, 1H exchangeable with D_2O), 3.93 (s, 3H), 3.91 (s, 3H), 3.3.71 (br s, 1H), 3.23 (br d, J = 12.6 Hz, 1H), 2.92 (m, 1H), 2.80 (br d, J = 11.4 Hz, 2H), 2.13 (s, 3H), 2.04 (br d, J = 11 Hz, 1H), 1.84 (br t, J = 11 Hz, 1H), 1.45 (br d, J = 11.4 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 176.20, 162.91, 159.35, 157.60, 132.59, 131.67 (2×), 130.90, 128.19, 113.55, 111.20, 108.60, 94.03, 68.95, 63.16, 57.51, 56.76, 56.57, 46.58, 24.86 (3 carbons unaccounted for); IR (KBr, cm^{-1}) 3434 (br), 2936, 1652, 1598; MS (NH_3−CI) *m*/*e* 430 (M + H$^+$, 100%). Anal. Calcd for $C_{23}H_{24}ClNO_5 \cdot 0.5H_2O$ C, 62.94; H, 5.74; N, 3.19. Found: C, 63.02; H, 5.60; N, 3.15.

Racemic L86-8275 (1a). A 500-mL round-bottom flask was charged with **6** (50 g, 0.116 mol) and pyridine hydrochloride (134 g, 1.163 mol) and heated in an oil bath to 210−220 °C for 4 h. While still molten (CAUTION), the

reaction mixture was poured into ice water, and the aqueous layer was adjusted to pH 7−8 using $NaHCO_3$ (saturated). The resulting solid was filtered (25.9 g), and the filtrate was extracted with 10% MeOH in $CHCl_3$, the combined organic layers were washed with H_2O, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. This residue was triturated with Et_2O to remove residual pyridine and combined with the initial filtered solid and heated in a vacuum oven at 50 °C. The product (38.3 g) was isolated in 82% yield: mp 148−155 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.76 (dd, J = 7.8, 1.9 Hz, 1H), 7.67 (dd, J = 7.8, 1.1 Hz, 1H), 7.59 (dt, J = 7.3, 1.9 Hz, 1H), 7.53 (dd, J = 7.3, 1.1 Hz, 1H), 6.31 (s, 1H), 5.77 (s, 1H), 4.01 (br s, 1H), 3.32 (br d, 1H), 3.21 (m, 2H), 2.92 (d, J = 11.7 Hz, 1H), 2.82 (br t, 2H), 2.62 (s, 3H), 1.42 (br d, 1H); ^{13}C NMR (DMSO-d_6) δ 180.36, 174.29, 161.36, 160.46, 155.79, 132.66, 132.03, 131.79, 131.13, 128.28, 109.92, 108.40, 102.68, 100.73, 68.15, 61.07, 55.50, 44.13, 36.45, 23.05 (1 carbon unaccounted for); IR (KBr, cm^{-1}) 3414 (br), 2938, 1658, 1610, 1578; MS (NH_3−CI) *m*/*e* 402 (M + H$^+$, 100%). Anal. Calcd for $C_{21}H_{20}ClNO_5$: C, 62.77; H, 5.03; N, 3.50. Found: C, 62.12; H, 5.10; N, 3.33.

***cis*-5,7-Dihydroxy-2-[2-chlorophenyl]-8-[4-(3-hydroxy-1-methyl)-piperidinyl]-4H-1-benzopyran-4-one hydrochloride (1b).** A solution of **1a** (23.6 g, 0.059 mol) in $CHCl_3$ (500 mL) was heated to reflux to effect dissolution, and after the mixture cooled to room temperature, ethereal HCl (200 mL, 1M) was added, and stirring continued for 15 min. The volatiles were removed by rotary evaporation, and the residue was triturated with ether. The resulting **1b** was filtered as a brown solid (26.0 g) and was obtained in quantitative yield. The product from three similar runs were homogenized by trituration with ether. The solid (77.4 g) was finally dried in a vacuum oven at 100 °C: mp 185−200 °C (lit.[3] 198−200 °C); ^{1}H NMR (DMSO-d_6) δ 12.98 (s, 1H), 11.50 br, 1H), 9.68 (br, 1H), 7.90 (m, 1H), 7.70 (m, 1H), 7.67−7.61 (m, 2H), 6.60 (s, 1H), 6.53 (s, 1H), 4.07 (br s, 1H), 3.39 (m, 5H), 3.05 (m, 2H), 2.73 (s, 3H), 1.82 (br d, J = 12.1 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 182.26, 163.64, 162.81, 160.37, 156.70, 132.99, 131.97, 131.88, 131.43, 131.05, 128.61, 110.93, 106.60, 104.53, 100.02, 66.48, 59.80, 54.95, 43.69, 36.48, 22.27; IR (KBr, cm^{-1}) 3600−2400 (br), 1660, 1614, 1576, 1432, 1354; MS (NH_3−CI) *m*/*e* 402 (M + H$^+$, 100%). Elemental analysis indicated a small amount of HCl was trapped in the solid. The sample was dissolved in water and lyophilzed overnight. Anal. Calcd for $C_{21}H_{20}ClNO_5$ HCl: C, 57.55; H, 4.84; N, 3.21; Cl, 16.18. Found: C, 57.56; H, 4.71; N, 3.14; Cl, 16.33.

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