A NEW SYNTHETIC METHOD FOR THE SYNTHESIS OF HYDROXYLATED ISOQUINOLINES: PREPARATION OF METHYL 6,7- AND 7,8-DIHYDROXY-ISOQUINOLINE-3-CARBOXYLATES, POTENTIAL PROTEIN-TYROSINE KINASE INHIBITORS

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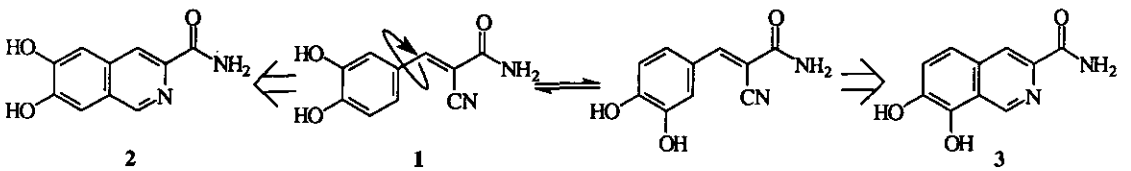
Abstract - Synthesis of potential protein-tyrosine kinase inhibitors (**6c**) and (**6e**) is presented as a general procedure for the preparation of polyhydroxylated isoquinolines. Key features of the method include selective hydroxyl protection of tetrahydroisoquinolines by *O*-acylation followed by aromatization using MnO_2. Quantitative deprotection of resulting *O*-acylisoquinolines is achieved using methanolic HCl.

Isoquinoline heterocycles are ubiquitous to a wide spectrum of natural products.[1] It has recently been shown that the hydroxylated isoquinoline-3-carboxyamide (**2**) possesses significant inhibitory activity against platelet-derived growth factor (PDGF) protein tyrosine kinase (PTK).[2] This is of particular note in that inappropriate or aberrant expression of PDGF[3] or other PTKs has been associated with a number of proliferative disorders, making inhibitors of PTKs potential candidates as anticancer therapeutics.[4,5] The 5,6-dihydroxyisoquinolinecarboxamide (**2**) can be viewed as a conformationally constrained analogue[2] of the more general class of styryl-based PTK inhibitors exemplified by α-cyanocinnamamide (**1**).[6] In a like manner, the 7,8-dihydroxyisoquinoline-carboxamide (**3**) represents a conformationally constrained mimetic of cinnamamide (**1**) having the phenyl ring rotated 180°. Evidence suggests that this second orientation may be preferred for PTK inhibition.[7]



2 **1** **3**

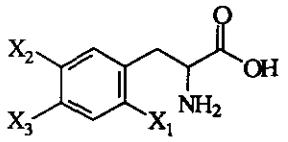
The fact that the number and pattern of hydroxyl groups can be extremely important to PTK inhibitory activity[6] highlights the need for a general synthetic methodology enabling the facile preparation of isoquinolines having defined hydroxylation patterns. Herein is reported the synthesis of both the 6,7- and 7,8-dihydroxy isomers of methyl isoquinoline-3-carboxylate (**6c** and **6e**, respectively) from a single starting material, 3,4-dihydroxyphenylalanine (DOPA, **4a**), using methodology which is applicable to the general synthesis of hydroxylated isoquinolines. Key features of the method include the use of MnO_2 for the aromatization of hydroxyl-containing isoquinolines after selective protection by acetylation. Deprotection to the phenolic isoquinolines is achieved quantitatively under mild conditions.

SYNTHESIS

A common approach for the synthesis of isoquinolines consists of the sequential use of a Pictet-Spengler cyclization of phenethylamines to tetrahydroisoquinolines[8] followed by dehydrogenation. The selection of this approach for the synthesis of **6c** and **6e** required careful consideration of several factors. First was the potential need for protecting the phenolic hydroxyls prior to the oxidative aromatization of the tetrahydroisoquinoline ring.[9–12] The problem with protecting phenolic groups prior to the Pictet-Spengler cyclization is that such protection might be incompatible with ring closure, especially when deactivating groups, such as bromine, are also present in the aromatic ring. Alternatively, if protection is delayed until after formation of the tetrahydroisoquinoline ring it must be performed selectively in the presence of the tetrahydroisoquinoline nitrogen. A second consideration was the direction of the Pictet-Spengler cyclization. The use of unsymmetrically substituted starting materials, such as DOPA, can result in the formation of isomeric products depending on whether ring closure occurs at the 2- or 6-position of the aromatic ring. In these situations the direction of cyclization is normally controlled by activating groups with ring closure frequently occurring <u>para</u> to one of these substituents. For the synthesis of the first target (**6c**), DOPA appeared to be the ideal starting material since ring closure is known to yield exclusively 6,7-dihydroxytetrahydroisoquinoline-3-carboxylate (**5a**)[12] which constitutes a direct precursor to **6c**.

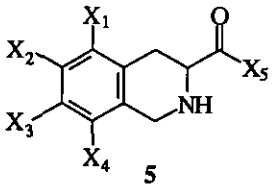
A more difficult problem is encountered when one wishes to use the same starting material to prepare the alternate and less favored 7,8-disubstituted tetrahydroisoquinoline (**5i**), which is a direct precursor to the second target (**6e**). Since the normal course of the Pictet-Spengler cyclization can be altered by blocking the preferred site of ring closure with bromine,[13] synthesis of the 7,8-disubstituted isoquinoline (**6e**) was initially approached using 2-bromo-4,5-dimethoxyphenylalanine (**4c**).[14] However, as mentioned previously as a possibility, the deactivating bromine atom prevented any cyclization to the desired 5-bromo-7,8-dimethoxytetrahydroisoquinoline-3-

carboxylate (**5f**) under Pictect-Spengler conditions. When the reaction was repeated using instead unprotected 2-bromo-4,5-dihydroxyphenylalanine (**4d**)[15] ring closure readily occurred yielding the expected 5-bromo-7,8-dihydroxytetrahydroisoquinoline (**5g**) which was isolated as the methyl ester (**5h**) following esterification with methanolic HCl. The desired 7,8-regioisomer (**5i**) was then easily obtained by hydrogenolysis of the bromine. For the ensuing aromatization reaction, protection of the phenolic groups in **5i** was a necessity since these groups were found to be extremely susceptible to oxidation. It was found that **5i** was particularly unstable in alkaline media but was reasonably stable under acidic conditions. This stability in acid offered the potential for selective protection of the phenolic hydroxyls in the presence of the basic tetrahydroisoquinoline nitrogen by maintaining the latter in its protonated form. After much experimentation it was found that treatment of **5i** with acetic anhydride in acetic acid in the presence of excess sulfuric acid afforded the desired 7,8-diacetoxy-protected derivative (**5j**). The use of HCl rather than sulfuric acid not only provided incomplete protection against acetylation on nitrogen, but also resulted in the precipitation of a partially acetylated intermediate.



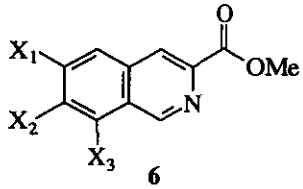
4

	X_1	X_2	X_3
a:	H	OH	OH
b:	H	OMe	OMe
c:	Br	OMe	OMe
d:	Br	OH	OH



5

	X_1	X_2	X_3	X_4	X_5
a:	H	OH	OH	H	OH
b:	H	OH	OH	H	OMe
c:	H	OMe	OMe	H	OH
d:	H	OMe	OMe	H	OMe
e:	H	OAc	OAc	H	OMe
f:	Br	H	OMe	OMe	OH
g:	Br	H	OH	OH	OH
h:	Br	H	OH	OH	OMe
i:	H	H	OH	OH	OMe
j:	H	H	OAc	OAc	OMe



6

	X_1	X_2	X_3
a:	OMe	OMe	H
b:	OAc	OAc	H
c:	OH	OH	H
d:	H	OAc	OAc
e:	H	OH	OH

For the aromatization of **5j** to the isoquinoline (**6d**), a cleaner and milder procedure was sought as an alternative to the use of sulfur in nitrobenzene at 140° C, employed in the aromatization of methyl 5,6-dimethoxytetrahydroisoquinoline-3-carboxylate (**5d**) to **6a**.[12] It was found that activated MnO_2, which had shown utility in the dehydrogenation of tetrahydro-ß-carbolines to ß-carbolines[16] converted **5j** to **6d** rapidly (2 h)

and cleanly in refluxing toluene. A secondary reaction arising from the intermolecular trans-acetylation of nitrogen, which resulted in an unreactive triacetylated product was limited by the use of a low substrate concentration, a large excess of MnO_2, and shorter reaction times. Pure **6d** could be obtained by simple filtration of the crude reaction mixture through a pad of silica gel. Finally, deprotection of **6d** was achieved by treatment with methanolic HCl at room temperature to give the desired target **6e** quantitatively as a crystalline solid.

The simplicity of this approach, wherein phenolic protection is performed after the Pictet-Spengler cyclization, and the ease with which the 7,8-dihydroxy isomer was obtained led us to apply this technique to the preparation of the 5,6-dihydroxyisoquinoline (**6c**). Previous workers have shown that the Pictet-Spengler cyclization performed with 3,4-dimethoxyphenylalanine (**4b**) provides 5,6-dimethoxytetrahydroisoquinoline-3-carboxylate (**5c**), which after conversion to the methyl ester (**5d**) can be aromatized to methyl 5,6-dimethoxyisoquinoline-3-carboxylate (**6a**) *via* the sulfur / nitrobenzene oxidation methodology.[12] An advantage of the new route over this older approach is the ready availability of the starting material, DOPA, and the fact that the penultimate intermediate, 5,6-diacetoxyisoquinoline (**6b**), could be deprotected under milder conditions than the corresponding dimethoxy compound (**6a**). Indeed, methyl 5,6-dihydroxytetrahydroisoquinoline-3-carboxylate (**5b**)[12] was selectively *O*-acetylated as described above and readily aromatized to the isoquinoline (**6b**) by oxidation with MnO_2. In this instance, formation of the triacetylated, dead-end intermediate was more noticeable than in the previous case of the 7,8-diacetoxy isomer (**5j**). Deprotection to **6c** was accomplished similarly, and the compound was isolated quantitatively as a crystalline solid. This technique should be of general value in the regiochemically controlled preparation of hydroxylated isoquinolines.

EXPERIMENTAL

Removal of solvents was performed by rotary evaporation under reduced pressure at 45 °C. Silica gel filtration was carried out using a 6.5 cm diameter x 3 cm high pad of tlc grade silica gel (5-25μ; Aldrich). Melting points were determined on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA., USA. Positive ion fast atom bombardment mass spectra were acquired with a VG Analytical 7070E mass spectrometer using either a nitrobenzyl alcohol or glycerol matrix operated under the control of a VG 2035 data system . ^{1}H-Nmr data were obtained on a Bruker AC250 (250 MHz) using DMSO-d_6 as solvent.

Methyl 5-bromo-7,8-dihydroxytetrahydroisoquinoline-3-carboxylate hydrochloride (5h•HCl): To a solution of 2-bromo-4,5-dihydroxyphenylalanine[15] (**4d**) (38.4 g, 139 mmol) in 1 N HCl (200 ml) is added formaldehyde (30 ml, 37% in H_2O) and the mixture is stirred at room temperature under argon overnight. The resulting suspension is reduced in volume to a slurry, mixed with ice-cold H_2O (50 ml), and filtered. The light brown filter cake is washed with ice-cold H_2O (2 x 50 ml), taken to dryness twice from anhydrous MeOH (2 x 200 ml), then refluxed with methanolic HCl (200 ml, 2 h). The resulting suspension is reduced in volume to a slurry, resuspended in MeOH (50 ml), filtered and the filter cake washed with MeOH (2 x 50 ml) to yield product **5h**•HCl as off-white crystals; 19.6 g (42%), mp 221-223°C; m/z = 302/304 $(M+H)^+$; Anal. Calcd for $C_{11}H_{12}NO_4Br•HCl•CH_3OH$: C, 38.89; H, 4.62; N, 3.78. Found: C, 38.80; H, 4.65; N, 3.72; ^{1}H-nmr δ: = 2.86 (dd, J = 16.9, 11.2 Hz, 1H, H-4_a), 3.12 (dd, J = 16.9, 5.2 Hz, 1H, H-4_b), 3.84 (s, 3H, OCH_3), 4.04 (d, J = 16.4 Hz, 1H, H-1_a), 4.27 (d, J = 16.4 Hz, 1H, H-1_b), 4.54 (dd, J = 11.2, 5.2 Hz, 1H, H-3), 7.08 (s, 1H, aromatic-H), 9.40 (s, 1H, OH), 9.99 (br s, 2H, NH_2), 10.13 (s, 1H, OH).

Methyl 7,8-dihydroxytetrahydroisoquinoline-3-carboxylate hydrochloride (5i•HCl): A suspension of methyl 5-bromo-7,8-dihydroxytetrahydroisoquinoline-3-carboxylate hydrochloride (**5h**•HCl) (5.85 g, 17.3 mmol) in MeOH (250 ml) is hydrogenated in a Parr apparatus (600 mg of 10% Pd•C; 40 psi H_2 with replenishing of H_2 after 15 min). After 3 h the mixture is filtered through celite and taken to dryness. The resulting solid is re-evaporated from methanolic HCl (100 ml) then crystallized from MeOH/acetone to provide a white crystalline solid (2.72 g) which when combined with additional crystalline material obtained by working up the filtrate (1.69 g) yields product **5i**•HCl as a white crystalline solid: (4.41 g, 97 %), mp 216-218°C; m/z = 224 $(M+H)^+$; High resolution mass spectrum calculated for $C_{11}H_{14}NO_4$ $(M+H)^+$: 224.092. Found: 224.096; ^{1}H-nmr δ: = 3.02 (dd, J = 16.4, 10.8 Hz, 1H, H-4_a), 3.15 (dd, J = 16.4, 5.0 Hz, 1H, H-4_b), 3.18 (s, 3H, OCH_3), 4.05 (d, J = 16.2 Hz, 1H, H-1_a), 4.24 (d, J = 16.2 Hz, 1H, H-1_b), 4.98 (dd, J = 10.8, 5.0 Hz, 1H, H-3), 6.55 (d, J = 8.2 Hz, 1H, aromatic-H), 6.76 (d, J = 8.2 Hz, 1H, aromatic-H), 9.03 (s, 1H, OH), 9.50 (s, 1H, OH), 10.00 (br s, 2H, NH_2).

Methyl 7,8-diacetoxyisoquinoline-3-carboxylate (6d): A suspension of methyl 7,8-dihydroxytetrahydroisoquinoline-3-carboxylate hydrochloride (**5i**•HCl) (2.59 g, 10.0 mmol) in AcOH (50 ml) with 96 % H_2SO_4 (2.04 g, 20 mmol) is warmed briefly until a solution is formed. To the still warm solution is added acetic anhydride (3.78 ml, 40 mmol) and the light yellow solution is stirred at room temperature (1 h). The solution is diluted with ether (200 ml) and cooled on ice, providing a white precipitate which is triturated with

EtOAc (100 ml) and collected by filtration (2.80 g). The solid is partitioned between ice-cold aqueous $NaHCO_3$ (50 ml) and EtOAc (3 x 50 ml), dried ($MgSO_4$) and taken to dryness to yield crude methyl 7,8-diacetoxytetrahydroisoquinoline-3-carboxylate (**5j**) as an oil (2.38 g, 78%). A solution of **5j** (4.35 g, 14.2 mmol) in toluene (200 ml) is stirred at reflux temperature (1.5 h) under argon with activated MnO_2 (14.2 g). The mixture is filtered hot through celite and taken to dryness, yielding a light brown crystalline solid (2.65 g). The crude product is taken up in $CHCl_3$ and filtered through a silica gel pad with the aid of additional $CHCl_3$, yielding **6d** as beige colored crystals: 1.92 g (35% overall), mp 162-164°C (ether); m/z = 304 $(M+H)^+$; Anal. Calcd for $C_{15}H_{13}NO_6 \cdot 1/4\ H_2O$: C, 58.44; H, 4.58; N, 4.54. Found: C, 58.76; H, 4.29; N, 4.55; ^{1}H-nmr: δ = 2.39 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 3.96 (s, 3H, OCH_3), 7.89 (d, J = 8.9 Hz, 1H, aromatic-H), 8.25 (d, J = 8.9 Hz, 1H, aromatic-H), 8.76 (s, 1H, aromatic-H), 9.49 (s, 1H, aromatic-H).

Methyl 7,8-dihydroxyisoquinoline-3-carboxylate hydrochloride (6e•HCl): Methyl 7,8-diacetoxyisoquinoline-3-carboxylate (**6d**) (1.87 g, 6.17 mmol) is suspended in anhydrous MeOH (10 ml) and the mixture is saturated with HCl gas. The bright yellow solution is stirred at room temperature under argon overnight. The resulting thick suspension of yellow crystals is cooled on ice, filtered and washed with MeOH, yielding **6e**•HCl as yellow crystals: 1.50 g (95%), mp >280°C (decomp); m/z = 220 $(M+H)^+$; Anal. Calcd for $C_{11}H_9NO_4 \cdot HCl$: C, 51.68; H, 3.94; N, 5.48. Found: C, 51.47; H, 3.98; N, 5.38; ^{1}H-nmr δ: = 3.99 (s, 3H, OCH_3), 7.81 (s, 2H, 2 aromatic-H), 8.78 (s, 1H, aromatic-H), 9.48 (s, 1H, aromatic-H).

Methyl 5,6-diacetoxyisoquinoline-3-carboxylate (6b): A suspension of methyl 5,6-dihydroxytetrahydroisoquinoline-3-carboxylate hydrochloride[12] (**5b**•HCl) (2.59 g, 10.0 mmol) in AcOH (50 ml) with 96 % H_2SO_4 (2.04 g, 20 mmol) is warmed briefly until a solution is formed. The solution is cooled to rt and acetic anhydride is added (3.78 ml, 40 mmol). A white precipitate is formed, which on brief warming redissolves. The reaction is stirred at room temperature (1 h) and additional acetic anhydride is added (944 mg, 10.0 mmol) to drive it to completion. The reaction is terminated after 15 min by the addition of ether (200 ml). The resulting white suspension is cooled on ice and ether is removed by decantation. The residue is triturated with hot EtOAc (100 ml), then collected by filtration and washed with EtOAc to yield crude methyl 5,6-diacetoxytetrahydroisoquinoline-3-carboxylate hydrogen sulfate (**5e**•H_2SO_4) as a white crystalline solid (3.64 g, 90 %). Partition of **5e**•H_2SO_4 (5.80 g, 14.3 mmol) between ice-cold aqueous $NaHCO_3$ and EtOAc (3 x 100 ml) yields the free base as an oil (3.53 g), which is stirred at reflux (3 h) in toluene (200 ml) under argon in the presence of activated MnO_2 (11.0 g). The reaction mixture is filtered hot through celite and evaporated to a

colored crystalline solid which is purified by filtration through a silica pad using $CHCl_3$. Product (**6b**) is obtained as a light pink crystalline solid (1.00 g, 21%). An analytical sample is obtained by triturating with ether; mp 169-170°C; m/z = 304 $(M+H)^+$; Anal. Calcd for $C_{15}H_{13}NO_6$: C, 59.41; H, 4.32; N, 4.62. Found: C, 59.46; H, 4.34; N, 4.58; ^{1}H-nmr δ: = 2.40 (s, 6H, $2CH_3$), 3.95 (s, 3H, OCH_3), 8.18 (s, 1H, aromatic-H), 8.22 (s, 1H, aromatic-H), 8.70 (s, 1H, aromatic-H), 9.43 (s, 1H, aromatic-H).

Methyl 5,6-dihydroxyisoquinoline-3-carboxylate hydrochloride (6c•HCl): Methyl 5,6-diacetoxyisoquinoline-3-carboxylate (**6b**) (606 mg, 2.00 mmol) is suspended in anhydrous MeOH (10 ml) and the mixture is saturated with HCl gas. The light orange solution is stirred at room temperature under argon overnight. The resulting thick suspension of crystals is filtered yielding **6c**•HCl as light pink crystals: 504 mg (98%), mp >280°C (decomp); m/z = 220 $(M+H)^+$; Anal. Calcd for $C_{11}H_9NO_4$•HCl•1/4 H_2O: C, 50.78; H, 4.07; N, 5.38. Found: C, 50.81; H, 4.45; N, 5.08; ^{1}H-Nmr δ: = 4.02 (s, 3H, OCH_3), 7.71 (s, 2H, 2 aromatic-H), 7.83 (s, 1H, aromatic-H), 8.75 (s, 1H, aromatic-H), 9.41 (s, 1H, aromatic-H).

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