SYNTHESIS OF [4,6-^{3}H]-2-PYRIDONE AND [^{3}H]-RS-91309

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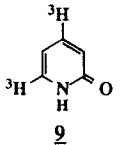
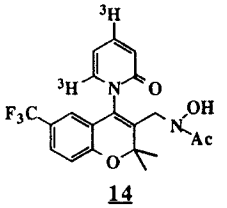
SUMMARY

We describe herein, a novel synthesis of the previously unreported compound, 4,6-dibromo-2-pyridone (**3**) and its use in the preparation of [^{3}H]-RS-91309, a potassium channel modulator. This key intermediate, (**3**), was reduced with carrier free tritium gas to furnish [4,6-^{3}H]-2-pyridone (**9**) having a specific activity of 50 Ci/mmole. Condensation of (**9**) with epoxide (**10**), followed by elaboration of the resulting chromene methyl group of (**12**) gave [^{3}H]-RS-91309 (**14**) whose specific activity was also 50 Ci/mmole. This chemistry, as well as the solution of several micro-scale related stoichiometry problems is discussed.

Key Words: [4,6-^{3}H-]2-pyridone-, 4,6-dibromopyridone, [^{3}H]-RS-91309, potassium channel.



9
[4,6-^{3}H]-2-pyridone



14
[^{3}H]-RS-91309

INTRODUCTION

A high specific activity analog of the potassium channel modulator [^{3}H]-RS-91309 was required for receptor binding and whole body autoradiography studies. Although the synthesis of [^{3}H]-2-pyridone had not been previously reported, this portion of the molecule seemed most amenable to labelling. The syntheses of target compounds (**9**) and (**14**), described below, serve as a vivid demonstration of how seemingly

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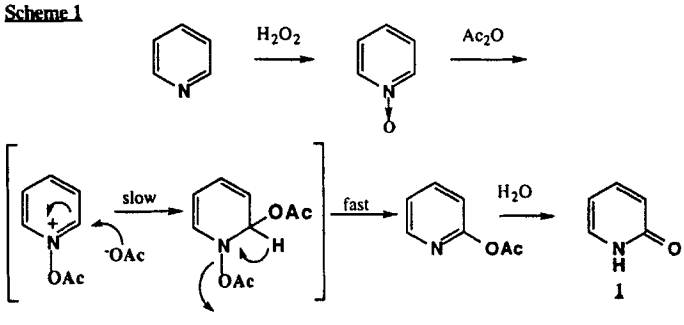
straightforward chemistry can present major problems when applied to micro-scale radiochemical processes.

For example, the general problem of selectivity, often controlled by stoichiometry on the gram and milligram scales, must be controlled by other means at smaller scales. Such lack of stoichiometric control may also result in the formation of by-products which are not normally encountered under stoichiometric conditions. Though these by-products can be thought of as a nuisance, their proper identification can give valuable insights into the reaction mechanism, and thus serve as a basis for the rational optimization of reaction conditions. The synthesis of [4,6-^{3}H]-2-pyridone (**9**) and its use in the construction of [^{3}H]-RS-91309 (**14**) required solutions to a variety of such micro scale related problems. The preparation and tritiation of the previously unreported precursor 4,6-dibromo-2-pyridone (**3**) and the transformation of a large scale synthesis of RS-91309 to one that was workable at the micro-scale are reported.

DISCUSSION

Our initial attempt to synthesize the key intermediate 4,6-dibromo-2-pyridone (**3**) was based on the known transformation of pyridine N-oxide to 2-pyridone (**1**)[1] as shown in **Scheme 1**.

Scheme 1

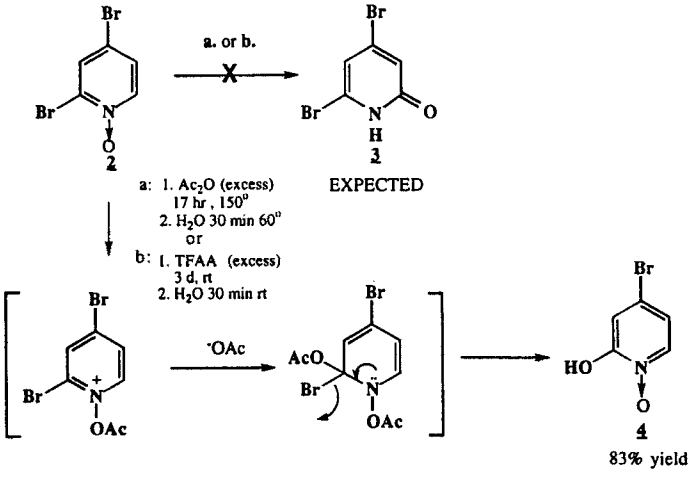


Extrapolating from the mechanism in **Scheme 1**, we expected that 2,4-dibromopyridine N-oxide (**2**), upon treatment with acetic anhydride, would give (**3**). However, an 83% yield of 2-hydroxy-4-bromopyridine N-oxide (**4**) was obtained instead, presumably by the mechanism proposed in **Scheme 2**. The mechanism is, essentially, the same as that in **Scheme 1**, except that acetate ion adds to C-2 which, due to the electronegative effect of the bromine, is more electrophilic than C-6. Rearomatization can then occur by loss of bromide ion, as shown, followed by hydrolysis of the acetate groups to give (**4**).

It seemed clear at this point that the mechanism shown in **Scheme 2** could be exploited to produce (**3**) by simply using the symmetrically disposed 2,4,6-tribromopyridine N-oxide (**6**) as the starting material. To that end, (**6**) was prepared, in 90% yield from 2,6-dibromo-4-nitropyridine N-oxide[2] (**5**). When (**6**) was treated with either acetic or trifluoroacetic anhydride, then hydrolyzed in a one pot reaction, as depicted in **Scheme 3**, the expected 4,6-dibromo-2-hydroxypyridine N-oxide (**7**) was, indeed, obtained in 90% yield. Titration of (**7**) with 20% aqueous $TiCl_3$ afforded a quantitative yield of 4,6-dibromo-2-pyridone (**3**).

In the course of our work, we found that treating 2,6-dibromo-4-nitropyridine N-oxide (**5**) with a large excess of acetylbromide while bubbling the reaction vigorously with

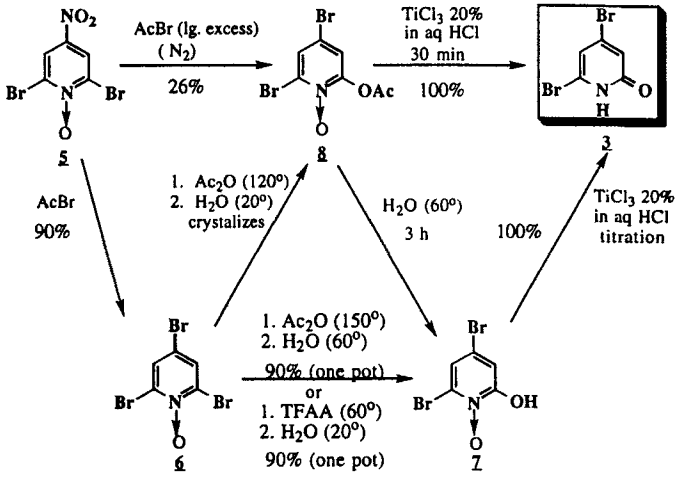
Scheme 2



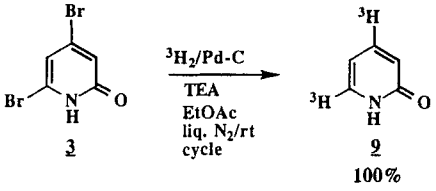
nitrogen furnished 4,6-dibromo-2-acetoxypyridine N-oxide (**8**) directly, albeit, in low yield. The structure of (**8**) was confirmed by independent synthesis from the known intermediate (**6**). Hydrolysis of (**8**) gave the previously identified intermediate (**7**). Stirring the O-acetate N-oxide (**8**) for 30 min with the 20% aqueous HCl solution of $TiCl_3$ resulted in quantitative hydrolysis of the acetate and concomitant N-deoxygenation to the tritiation substrate (**3**).

Trial reductions of (**3**) with hydrogen and a variety of catalysts resulted in significant amounts of ring reduced products[3]. Addition of catalyst poisons was not effective in moderating the reduction. This problem was solved by cooling the reaction with liquid N_2 followed by thawing with an ambient temperature methanol bath. After a few minutes, an aliquot was removed[4], the reaction was frozen with liquid nitrogen, and the aliquot was analyzed by TLC. This process was repeated over a combined ambient temperature reaction time of about one hour, until the reaction was complete. The result, after chromatography, was a quantitative yield of [4,6-^{3}H]-2-pyridone (**9**) having a specific activity of 50 Ci/mmole (**Scheme 4**).

Scheme 3

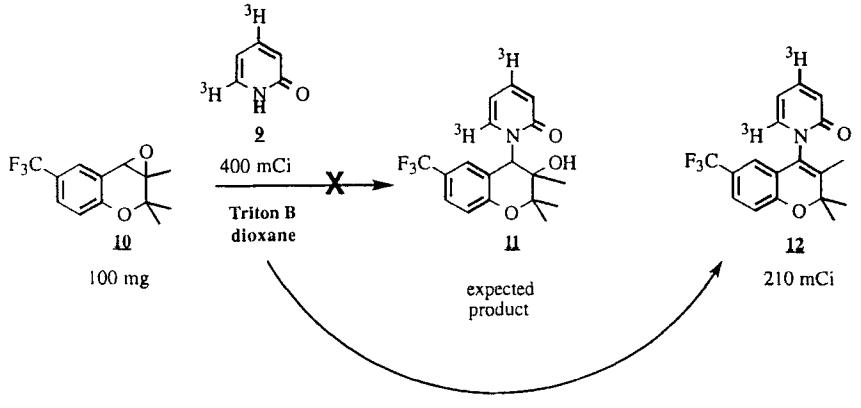


Scheme 4



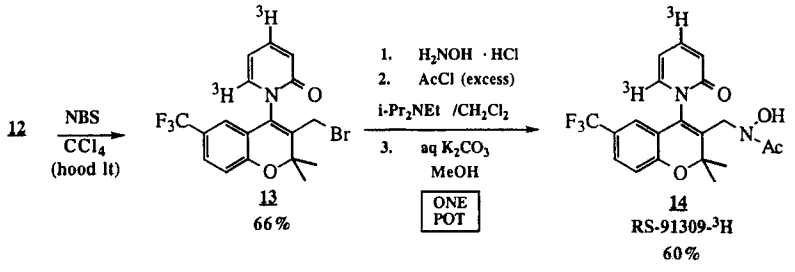
The coupling of pyridone with epoxide (**10**) worked well when pyridone was used in excess. However, when pyridone was the limiting reagent (**Scheme 5**), as would be the case if [^{3}H]-pyridone were used, the major products obtained did not coincide with any of the major products formed with excess pyridone. One of the two major products was N-benzylpyridone from the reaction of Triton B with pyridone. The other major product was identified as the elimination product (**12**) which was, coincidentally, the product of the following step in the kilo scale synthesis. The yield of the one pot epoxide coupling dehydration was comparable to the yield over two steps in the large scale process.

Scheme 5



The final two steps in the synthesis of RS-91309-^{3}H are shown in **Scheme 6**. Allylic bromination of (**12**) with NBS gave complex mixtures when the reaction was activated with either a radical initiator or sunlight. However, exposure of the reaction to ordinary fume hood lighting (100W incandescent bulb at about three feet) gave allylic bromomethyl compound (**13**) in 60-70% yield (no reaction occurred in the dark). Hydroxyamination and subsequent monoacylation were problematic because these reactions could not be run under stoichiometric conditions, at reasonable concentration, with the small amounts of (**13**) available,i.e., 60 mCi at 50 Ci/mmole (1.2×10^{-3} mmole, 0.52 mg). Despite the fact that (**13**) was unstable to a large excess of hydroxylamine, we found, that by carefully monitoring the reaction, hydroxyamination could be maximized after two hours. As a result, a one pot reaction was devised in which (**13**) was treated with an excess of hydroxylamine, then quenched with an excess of acetyl chloride to give the intermediate N,O-bis-acyl product. Addition of potassium carbonate served to selectively hydrolyze the O-acetate, affording [^{3}H]-RS-91309 (**14**) at 50 Ci/mmole in 60% yield after chromatographic purification.

Scheme 6



Careful analysis of reaction products resulted in the elucidation and use of reaction mechanisms to advantage. In this way, reactions were devised, and conditions were developed to achieve effective syntheses of [4,6-^{3}H]-2-pyridone and [^{3}H]-RS-91309 at very high specific activity.

EXPERIMENTAL

Carrier-free tritium gas was purchased in break-seal ampoules from New England Nuclear Corp., Boston, Mass. Standard reagents and solvents were used without purification, with the exception of N-bromosuccinimide which was recrystallized from water. Titanous chloride (20% aqueous solution) was obtained from Fisher Scientific (ST43-500). "Chromatotron" is a radial chromatography apparatus manufactured by Harrison Research, Palo Alto, CA. Radiochromatography was performed on either a Berthold Model LB 2760 or a Bioscan Model 200 scanner. Radioassays were obtained using a Packard Tri-Carb 4000 Series liquid scintillation counter. UV spectra were obtained using a Shimadzu Model UV-265 Recording Spectrophotometer. Nmr spectra were recorded on Bruker spectrometers either Model AM 300, ACF 300, or AMX 500. All ^{3}H-nmr spectra were run on the ACF 300. Mass Spectra were recorded on a Finnigan TSQ 70. Products were also identified by chromatographic mobility compared to authentic standards. HPLC analyses were performed using a Beckman System Gold equipped with a Model 166 variable wave length UV detector and a Model 171 radioisotope detector. Thin layer chromatography (TLC) was performed in a qualitative fashion, and not under controlled conditions. Therefore, R_f values are not reported since they would be quite variable.

4,6-Dibromo-2-hydroxypyridine N-oxide (7)

Tribromopyridine N-oxide (6) (63.2 mg, 0.19 mmole) was dissolved in excess (0.40 mL) acetic anhydride at 150° and stirred overnight. Water (0.5 mL) was added to the cooled reaction, and the mixture was heated at 60° for 30 min. The cooled reaction was extracted four times with 1.5 mL CH_2Cl_2. The combined organic phase was dried with Na_2SO_4 and evaporated to give 43.4 mg (90% yield) of 4,6-dibromo-2-hydroxypyridine N-oxide (7). A parallel reaction run for 24 hrs at ambient temperature in excess trifluoroacetic anhydride, followed by ambient temperature hydrolysis afforded a 90% yield of nearly pure product which was confirmed to be (7) by NMR and MS.

TLC: silica gel: 1% aqueous NH_4Cl in MeOH

^{1}H NMR: (300 MHz, $CDCl_3$) δ 6.780-6.787 (d, 1H), δ 6.906-6.913 (d, 1H).

MS: (EI) m/z (rel. inten.) 267 (M+, 56), 190 (40)

2-Acetoxy-4,6-dibromopyridine N-oxide (8)
The 2,6-dibromo-4-nitropyridine N-oxide (5) (0.81 g, 2.72 mmole) was suspended in 10 mL HOAc and the internal temperature was raised to 60°. Acetyl bromide (0.40 mL, 5.4 mmole) was added dropwise. The reaction was continuously bubbled with a moderate flow of nitrogen to remove NO_2 and the temp was raised to 80°. Over the next three hrs, an additional 3.2 mL of acetyl bromide was added until all of the substrate was consumed. The crude reaction mixture was concentrated at 60° to a gritty semi solid and redissolved in 5 mL CH_2Cl_2. This solution was extracted three times with 5 mL portions of sat. aqueous $NaHCO_3$, then back extracted with 10 mL CH_2Cl_2. The combined organic phase was dried with Na_2SO_4 and concentrated to dryness. The solid residue (0.81 g) was applied as a CH_2Cl_2 solution to a pre-equilibrated (10% EtOAc/hexane) 2 mm silica gel "Chromatotron" rotor and eluted with 10-20% EtOAc/hexane. A strong UV band (228 mg) corresponded to the R_f of the expected product 2,4,6-tribromopyridine N-oxide (6). A slightly less polar strong UV band (220 mg, 26% yield) was collected and confirmed to be 2-acetoxy-4,6-dibromopyridine N-oxide (8).

TLC: silica gel: EtOAc-hexane (1:4)
MS (6): (EI) m/z (rel. inten.) 329 (M^+, 42), 224 (20)
MS (8): (EI) m/z (rel. inten.) 311 (M^+, 18), 188 (12)
^1H NMR (8): (300 MHz, $CDCl_3$) δ 6.652-6.660 (d, 1H), δ 6.906-6.913 (d, 1H)

4,6-Dibromo-2-pyridone (3), via (7)
The 4,6-dibromo-2-hydroxypyridine N-oxide (7) (7.3 mg) was dissolved in 0.5 mL acetonitrile and titrated with 0.1 mL of commercial aqueous $TiCl_3$ reagent until the purple color of the reagent persisted (quantitative reaction by tlc). The reaction was diluted with 1 mL CH_2Cl_2 and 3 mL saturated aqueous $NaHCO_3$ to pH 5-5.5. The aqueous phase was extracted with three equal volumes of CH_2Cl_2 and the combined organic phase was dried with Na_2SO_4 and concentrated to dryness to afford 3.6 mg white crystalline solid.

TLC: silica gel: MeOH-Et_2O-Toluene (1:1:2)
NMR: (300 MHz, $CDCl_3$) δ 6.912-6.917 (d, 1H), δ 6.993-6.997 (d, 1H)
MS: (EI) m/z (rel. inten.) 253 (M^+, 98), 172 (100)

4,6-Dibromo-2-pyridone (3), via (8)
The substrate (8) (178 mg , 0.572 mmole) was dissolved in 5 mL CH_3CN. Excess (2 mL) 20% $TiCl_3$/aq HCl was added dropwise and the reaction was stirred at ambient temp for four hrs to a single more polar spot on TLC. The reaction was diluted with 20 mL CH_2Cl_2 and the pH was adjusted to 5-6 with about 25 mL sat. aq $NaHCO_3$. The mixture was extracted three times with 20 mL portions of CH_2Cl_2 and the combined organic phase was dried with Na_2SO_4 then concentrated to a white crystalline solid (3) (144.7 mg, quantitative yield).

TLC: MeOH-Et_2O-Toluene (1:1:2) from mini work up: pH 6-7 aq $NaHCO_3$/CH_2Cl_2

[4,6-^{3}H]-2-Pyridone (9)
A solution of 12.2 mg (0.048 mmole) 4,6-dibromo-2-pyridone (3) and 25 µL TEA in 3-4 mL of EtOAc was injected under vacuum onto 7 mg 10% Pd-C contained in a 10 cc septum side-arm flask. Carrier free tritium gas (10 Ci) was transferred onto the degassed mixture, frozen in liquid nitrogen. The reaction was warmed to ambient temperature with a methanol bath. After about 15 minutes an aliquot was removed and

the reaction was immediately refrozen with liquid nitrogen. After tlc analysis, the process was repeated until the reaction was judged to be complete (a total of about 1 hr at ambient temp). Volatiles were removed by vacuum transferring about 1/3 of the solvent to a waste bulb. The catalyst was removed by canula transfer of the reaction mixture through a septum equipped disposable Whatman "syringeless filtering device" (cat. no. AV125UNAO, with the plunger removed). Labile tritium was removed by concentrating the methanolic filtrate to dryness four times from methanol. The residue was filtered through a 15 cc bed of silica gel contained in a 25 cc disposable pipet by eluting with (1:3) MeOH-EtOAc to remove TEA•^{3}HCl. The pure [4,6-^{3}H]-2-pyridone (**8**) (2.35 Ci) had a specific activity of 50 Ci/mmole (by MS).

radio-TLC: silica gel: MeOH-EtOAc (1:2), MeOH-Et_2O-Toluene (1:1:2)

^{3}H NMR: (300 MHz, C_6D_6) δ 7.4 (s decoupled, ^{1}^{3}H), δ 7.5 (s decoupled, ^{1}^{3}H)

^{3}H MS: (EI) m/z (rel. inten.) 99 (M+, 100),

4-N-([4',6'-^{3}H]-2'-pyridon)yl-6-trifluoromethyl-2,2,3-trimethyl-3-chromene (12)

Chromene epoxide (**10**) (102 mg, 0.396 mmole) and 50 μL Triton B was added to a solution of [^{3}H]-pyridone (ca. 425 mCi) in 1.0 mL dioxane. The reaction was stirred under nitrogen at 110° for two hours. The cooled reaction was diluted with EtOAc and extracted with water and brine. The organic phase was dried with Na_2SO_4 and combined with a similarly treated 300 mCi batch. Purification by radial chromatography (2 mm silica gel rotor, 2-10% MeOH/(1:1) EtOAc-hexane) afforded 210 mCi desired (**12**). Also, recovered were 60 mCi [^{3}H]-pyridone (**9**), 35 mCi of alcohol (**11**), and 210 mCi of by-product N-benzylpyridone-^{3}H.

radio-TLC: silica gel: MeOH-Et_2O-Toluene (1:1:2), MeOH-EtOAc (3:97)

4-N-([4',6'-^{3}H]-2'-pyridon)yl-6-trifluoromethyl-2,2-dimethyl-3-bromomethyl-3-chromene (13)

The methanol-ethyl acetate-hexane (1:5:5) solution of substrate (**12**) (ca. 62 mCi) was evaporated to dryness and redissolved in 1.0 mL CCl_4. N-bromosuccinimide (10 mg) was added and the heterogeneous mixture was purged with nitrogen. The reaction was stirred at ambient temperature under hood lighting for four hours. Water (2 mL) and 5% aqueous $FeSO_4$ (2 mL) were added and the mixture was extracted with 0.2% TEA in CCl_4 and back extracted with water. The organic phase was concentrated and applied to a silica gel column (1 cm^2 x 14 cm SiO_2) prepared and eluted with 0.2% TEA in CCl_4. Fractions containing product were combined to afford 41 mCi of >90% pure allylic bromide (**13**).

radio-TLC: silica gel: acetone-CH_2Cl_2 (1:9)

4-N-([4',6'-^{3}H]-2'-pyridon)yl-3-(N-acetyl-N-hydroxyamino)methyl-2,2-dimethyl-6-trifluoromethyl-3-chromene (14) RS-91309-^{3}H

A solution of allylic bromide (**13**) (36 mCi) diluted with CCl_4 and 0.2% TEA was concentrated to dryness and redissolved in 2 mL dry methylene chloride. Diisopropylethylamine (100 μL) and $H_2NOH \cdot HCl$ (10 mg) were added and the solution was concentrated to 0.5 mL. Maximum product formation occurred after 2 hr. The reaction was diluted with 1 mL dry CH_2Cl_2 and was cooled to -78°. A solution of acetyl chloride-CH_2Cl_2 (1:9) (200 μL) was added dropwise and the reaction was stirred for one hr until diacetylation was complete by radio-tlc [silica gel: acetone-CH_2Cl_2 (1:9)]. Water (1 mL) was added and the reaction was warmed to ambient

temperature. After bubbling with nitrogen to evaporate CH_2Cl_2, 100 mg K_2CO_3 was added. Following dilution with 3 mL of methanol, the reaction was stirred for about two hr until selective O-deacetylation was complete by radio-tlc [silica gel: THF-hexane (6:4)]. The reaction was partitioned between 20 mL EtOAc and 3 mL water + 3 mL saturated NaCl then further washed with water. The organic phase was dried with brine and Na_2SO_4, concentrated to dryness, and redissolved in 0.5 mL CH_3CN and 0.5 mL water. Purification by preparative HPLC (Vydac "protein & peptide C-18", 33% acetonitrile / water) afforded 22 mCi (60 % yield) of pure (>99%) RS-91309-[^{3}H] (14) having a specific activity of 49 Ci/mmole.

radio-TLC (13, and intermediates): silica gel: acetone-CH_2Cl_2 (1:9), MeOH-CH_2Cl_2 (1:9)
radio-TLC (14): silica gel: 0.3% TEA in MeOH-EtOAc (3:97), THF-hexane (6:4)
HPLC: Zorbax RX-C8 4.6mm x 250mm 33:67 (ACN: 0.03M TEAP, pH 3),
1 mL/min, 220 nm.

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