Directed Lithiation of 3,5-Dichloroaniline and Its Application in the Synthesis of 1,3-Dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-b][5-^{14}C]quinazoline Monohydrochloride.

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SUMMARY

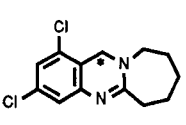
1,3-Dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-b][5-^{14}C]quinazoline monohydrochloride (1) was synthesized in 12 % overall yield starting from 3,5-dichloroaniline, and a radiochemical purity of 99.6 % was obtained. The synthetic sequence was facilitated by the regio-controlled preparation of 4,6-dichloroanthranilic acid (3) from 3,5-dichloroaniline using the t-butoxycarbonyl-protected aniline and t-butyllithium/tetramethylethylenediamine (t-BuLi/TMEDA) combination followed by carboxylation. The quinazoline monochloride 1 was constructed by condensation of 3 with 1-aza-2-methoxy1-cycloheptene and then reduction.

KEY WORDS: *cognition activating agent, directed lithiation, anthranilic acid, benzyne, 7-lithio-benzoxazole, and dichloroquinazolone.*

Introduction

Our investigations of cognition enhancing agents (1) required a methodology for the efficient synthesis of 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-b]quinazoline monohydrochloride **Figure 1**, labeled with ^{14}C at the C-5 position. The unlabeled compound was originally made from 3,5-dichloroaniline by sequential conversion to 4,6-dichloroisatin, 4,6-dichloroanthranilic acid, and dichloroquinazolone which in the final step was reduced to quinazoline hydrochloride (2).

Figure 1



Provided 4,6-dichloro-[carboxy-^{14}C]anthranilic acid (3) could be made, the synthesis of labeled quinazoline hydrochloride (1) may be achieved using the same sequence. Available literature (3) suggests that functional group directed ortho metalation of 3,5-dichloroaniline derivative may

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lead to the preparation of 4,6-dichloroanthranilic acid. However, the formation of a benzyne appears inherently favoured in 3,5-dichloroaniline derivative and the attainment of desired regiospecificity is similarly tenuous. We could conceivably mask the reactive C-4 position by converting 3,5-dichloroaniline to 3,5-dichloro-4-trimethylsilyl aniline, and then transform it to the target compound. By so doing, the unfavourable regioselective metalation otherwise constrained by the enhanced kinetic acidity of the 4-proton in the parent compound, would be eliminated. In addition, if the reaction temperature is sufficiently low, the formation of the benzyne species may be minimized. However, it is still not assured that the target compound 1 will be stable under the conditions required to regenerate the 4-proton.

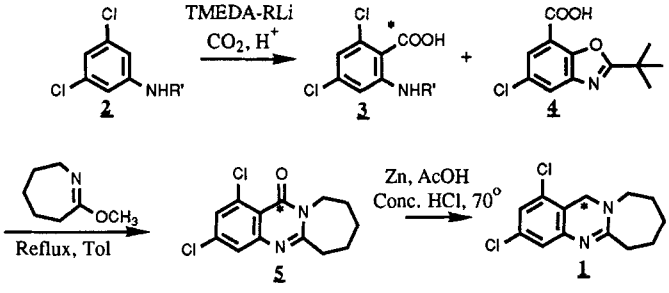
Results and Discussion

We preferred to develop a regiospecific synthesis of 4,6-dichloroanthranilic acid from 3,5-dichloroaniline derivative under conditions which would forestall benzyne formation. Accordingly, various organo-lithium reagents and additives were investigated at very low temperature in functional group-directed ortho lithiation reactions on 3,5-dichloroaniline. N-Pivaloyl 3,5-dichloroaniline and the N-Boc analog, N-(tert-butoxycarbonyl)-3,5-dichloroaniline, were selected for study. Both compounds were prepared by the standard method in which 3,5-dichloroaniline reacted with di-tert-butyl dicarbonate in refluxing THF to obtain N-(tert-butoxycarbonyl)-3,5-dichloroaniline and with trimethylacetyl chloride in THF at room temperature to make N-pivaloyl-3,5-dichloroaniline. It is known that tert-butyllithium (tert-BuLi) is a more effective reagent than either sec-butyllithium (sec-BuLi) or n-butyllithium (n-BuLi) in lithiation of simple N-(tert-butoxycarbonyl) aniline (4) . Under our conditions, n-BuLi was similarly ineffective without tetramethylethylenediamine (TMEDA) in metalating either N-pivaloyl- or N-(tert-butoxycarbonyl)-3,5-dichloroaniline. But in combination with TMEDA, both n-butyllithium and sec-butyllithium effected metalation yielding, upon sequential carboxylation and acidification, a mixture of N-pivaloyl-4,6-dichloroanthranilic acid (3) and an undesired product deduced to be 5-chloro-2-(1,1-dimethylethyl)-7-benzoxazole carboxylic acid (4) on the basis of spectroscopic data. The assigned structure was later confirmed by alternative synthesis (5) .

tert-Butyllithium-tetramethylethylenediamine was the most suitable for our requirements and successful in accomplishing our target synthesis. At reaction temperature (-40° to -20° C) higher than in our conditions, N-pivaloyl-3,5-dichloroaniline tended to provide increased proportions of the benzoxazole product. Lithiation of N-pivaloyl-3,5-dichloroaniline with tert-BuLi-TMEDA as in

our protocol followed by the addition of CO_2 yielded a nearly (1:1) mixture of 5-chloro-2-(1,1-dimethylethyl)-7-benzoxazole carboxylic acid (4) and N-pivaloyl-4,6-dichloroanthranilic acid (3) (**scheme I** and notation b) which were separable by column chromatography. Under these same reaction conditions only 4, 6-dichloroanthranilic acid (3) (**scheme I** notation {c}) was obtained from N-(tert-butoxycarbonyl)-3,5-dichloroaniline. We did not observe the benzoxazole carboxylic acid 4 in either 'cold' or carbon-14 labeled preparation under these conditions. The investigation of N-(tert-butoxycarbonyl)-group (N-Boc) as ortho directing group in this system was in part prompted by a desire to take advantage of relative ease of preparation and hydrolytic cleavage of the group at the end of reaction sequence. The remarkable difference in product distribution between N-pivaloyl- and N-(tert-butoxycarbonyl)-protected 3,5-dichloroaniline was not anticipated.

Scheme I#

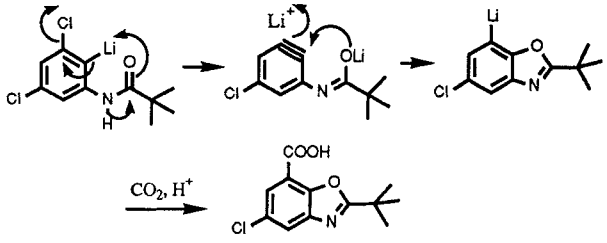


{a} Compd. (4) only when R' = Pivaloyl, RLi = n-BuLi
{b} Compds. (3) & (4) when R' = Pivaloyl , RLi = sec-BuLi, tert-BuLi
{c} Compd. (3) when R' = Boc , RLi = tert-BuLi

A divergent reaction pathway inducible by ortho directing group appears to account for the formation of benzoxazole carboxylic acid. N-Pivaloyl-3,5-dichloroaniline or the N-(tert-butoxycarbonyl) analog reacts with 2 molar equivalents of alkyllithium to form putative dilithio species. The secondary reaction pathway (**Scheme II**) prevails in N-pivaloyl-3,5-dichloroaniline and ultimately results in lithiation of aromatic ring carbon at a position meta to ' directing group' . In sequence lithium chloride is lost, a transient benzyne species is formed and rearranged to 7-lithio-benzoxazole. This pathway would be expected to be attenuated by a second lithium ion and a nucleophilic carbonyl oxygen as in the amide group. The carboxylation of the resulting lithio species would then furnish compound 4. Recently, Clark reported (6) the deliberate generation of benzyne species by similar but more severe conditions, utilizing this intramolecular trapping of a nucleophilic side chain by a benzyne species to make benzoxazole. Fisher (7) and subsequently

Richardson (5) have independently utilized this approach in the preparation of 1,2,3,4-tetrasubstituted benzenes by the addition of an electrophile to the 7-lithiobenzoxazole product of the intramolecular reaction. By carrying out reactions at low temperature (-95° C) and utilizing a potentially poorly nucleophilic ortho directing group, we sought to eliminate the benzyne pathway. Accordingly, the analogous dilithio species obtained from N-(tert-butoxycarbonyl)-3,5-dichloroaniline, being comparatively more stable, successfully inhibited the extrusion of lithium chloride to afford the compound 3 as would be expected from ortho metalation followed by carboxylation. We believe that N-pivaloyl may have been as effective an ortho metalation directing group as N-(tert-butoxycarbonyl). However, the divergent rearrangement reactions which N-pivaloyl group induces in these systems coupled with the rather harsh conditions required to deprotect the amide made N-(tert-butoxycarbonyl) group a better choice.

Scheme II



Therefore, where secondary reactions involving benzyne intermediates are possible, the choice of an ortho directing functional group which is unlikely to be a reactive intramolecular nucleophile, may be critical to product obtained. In our case, N-(tert-butoxycarbonyl) group fulfilled this requirement and facilitated directed regiospecific ortho substitution of 3,5-dichloroaniline, providing in consequence a facile entry into 4,6-dichloroanthranilic acid. Applying the newly developed protocol, the requisite labeled acid 4,6-dichloro[carboxy-^{14}C]anthranilic acid was prepared in 65 % isolated yield from N-(tert-butoxycarbonyl)-3,5-dichloroaniline. Carbon-14 labeled 1,3-dichloro-7,8,9,10- tetrahydroazepino[2,1-b]quinazolin-12(6H)-one 5 was constructed from the acid by condensation with 1-aza-2-methoxy-1-cycloheptene. Reduction with Zn-acetic acid provided target compound 1. Purification of the compound 1 was best by column chromatography upon first isolation as the free base. Acidification followed by crystallization gave pure monohydrochloride 1.

In conclusion, we have developed a concise practical synthesis of 4,6-dichloroanthranilic acid by exploiting the ortho metalation directing effect of N-Boc group and its mild hydrolytic cleavage at the end of reaction. In this system, N-(tert-butoxycarbonyl) group proved superior to the

alternative N-pivaloyl amide which induced secondary reactions, and produced a mixture of compounds. We applied this finding to the regiospecific synthesis of ^{14}C-labeled 4,6-dichloroanthranilic acid from 3,5-dichloroaniline. In turn, the success provided a ready access to our target compound 1,3-dichloro-6,7,8,9,10,12-hexahydro-azepino[2,1-b][5-^{14}C]quinazoline monohydrochloride.

Experimental

General Methods.

All reactions were carried out under inert atmosphere. ^1H-NMR spectra were recorded with Varian (EM 390) 90 MHz spectrometer, a Gemini 200 MHz or a Varian XL 300 MHz spectrometer. Radiochemical purity of every labeled compound was determined by tlc radiochromatogram with Bioscan 200 imaging scanner. Radiochemical counting was performed on a Packard 574 liquid scintillation counter using Beckman Readi-Solv MP cocktail. HPLC analyses of final products were performed on a Waters Associates 600E system with on line Applied Biosystems 1000S diode array detector and either a β-RAM radioactivity detector or Radiomatic series A-200 radioactivity flow detector. Column chromatography was carried out on a Merck Kieselgel 60 (230μ). Mass spectral analysis was on a VG Masslab Trio-2 mass spectrometer (Fisons Instruments, Danvers, MA), operated in the electron impact mode (EI^+), with filament emission current and electron energy of 200μA and 70ev, respectively. A positive Fast Atom Bombardment (FAB) mass spectrum was obtained on a Finnigan MAT TSQ70 mass spectrometer, using a thioglycerol matrix and 1 milliampere of seven kilovolt xenon target gas. A negative electrospray ionization mass spectrum was obtained on a Fisons VG Trio-2000 quadrupole mass spectrometer, using 80:20 (acetonitrile:methanol) + 1 % acetic acid at a flow rate of 20 μL/min solvent system.

5-Chloro-2-(1,1-dimethylethyl)-7-benzoxazolecarboxylic acid (4) and N-Pivaloyl-4,6-dichloroanthranilic acid (3)

To a solution of TMEDA (2.81 g, 3.65 mL, 24.2 mmol) in dry THF (40.0 mL) at -95° C (toluene-liq. N_2) under argon atmosphere was added in one portion sec-BuLi (18.8 mL, 24.5 mmol , 1.3 molar solution in hexane) and stirred for 1 hr . A solution of N-pivaloyl-3,5-dichloroaniline (2.88 g, 11.7 mmol) in dry THF (20.0 mL) was added dropwise to the reaction in 15 min. After stirring for 2 h at -95° C, the reaction was sealed and frozen in liq. N_2, and transferred to a vaccum line connected to a carbon dioxide generator. It degassed at liquid N_2 temperature and the CO_2 generated from $BaCO_3$ (2.30 g, 11.7 mmol) by the action of conc. H_2SO_4 (30.0 mL) was transferred

into the reaction flask. The liquid N_2 bath was replaced with dry ice-acetone bath, and reaction was allowed to proceed while the bath warmed up to room temperature. To the reaction was added 20 % AcOH in THF (10.0 mL) and then diluted with EtOAc (300 mL). It was washed with water, sat'd NaCl solution, and dried on anhydrous Na_2SO_4 to give a mixture that was separated by column chromatography on silica gel eluted with CH_2Cl_2:MeOH:NH_4OH (75: 25: 3) to give desired N-pivaloyl-4,6-dichloroanthranilic amide (25 %), crystallized from ether-hexane mixture. Proton nmr (DMSO-d_6) δ 11.84 (s), 8.32 (d J = 2.1 Hz), 7.27 (brs), 7.10 (d J = 2.1 Hz), 1.18 (s) ; MS (E^+) m/z 289 and other ions at 256, 245, 214, 205, 187, 161 153, 85 and 57. FAB, (+Q3MS) 290.4 (100), 274.3 (16.98), 256.5 (19.70), 212.7 (17.79), 154.5 (17.08), 126.6 (14.40), 116.8 (15.13). ES- MS 579.0 (14.82), 288.1 (100), 244.2 (15.34). IR (KBr) 3423-2853, 1668, 1610, and 1567 cm^{-1} . Further elution gave the undesired 5-chloro-2-(1,1-dimethylethyl)-7-benzoxazolecarboxylic acid (75 %) (crystallized from acetone). Proton nmr (DMSO-d_6) δ 14.30 - 13.20 (br), 8.09 (d, J = 2.3 Hz), 7.78 (d, J = 2.2 Hz), 1.43 (s); MS (E^+) m/z 253 and other ions at 238, 211, 197, 179, 153, 126, 97, 88, 62, and 57. FAB, (+ Q3MS) 254.5 (100), 217.5 (48.9), 181.4 12.53), 109.7 (33.15) . ES- MS 505.2 (36.92), 252.2 (100), 208.3 (8.35). IR (KBr) 3427- 2570, 1721 and 1621 cm^{-1}.

TMEDA-tert-BuLi Lithiation Reaction

A solution of TMEDA (2.81 g, 3.65 mL, 24.2 mmol) in dry THF (40.0 mL) at -95° C and tert-BuLi (14.88 mL, 25.3 mmol; 1.7 molar solution in hexane) similarly reacted with a solution of N-pivaloyl-3,5-dichloroaniline (2.88 g, 11.7 mmol) in dry THF (20.0 mL) followed by addition of CO_2 generated from $BaCO_3$ (2.30 g, 11.7 mmol) by the action of conc. H_2SO_4 (30.0 mL) as described in the above experiment. Workup followed by column chromatography gave an isomeric mixture of desired N-pivaloyl-4,6-dichloroanthranilic amide (42 %), crystallized from ether-hexane mixture, and followed by undesired 5-chloro-2-(1,1-dimethylethyl)-7-benzoxazolecarboxylic acid (56 %), crystallized from acetone.

4,6-Dichloro[carboxyl-^{14}C]anthranillic acid

To tetramethylethylenediamine (TMEDA) (3.25 g, 4.23 mL, 28.0 mmoL) in dry THF (20.0 mL), cooled to -95° C under argon atmosphere, was added tert-BuLi (1.7 M in hexane, 16.5 mL, 28.0 mmol) in one portion. After stirring for 1 h, a solution of N-(*tert*-butoxycarbonyl)-3,5-dichloroaniline (3.66 g, 14.0 mmol) in dry THF (30 mL) precooled to -95° C was added dropwise over 20 min. It was stirred at -95° C under argon atmosphere for a further 2 h, then frozen in liq. nitrogen and transferred to a vaccum line that was connected to a CO_2 generator. Carbon

dioxide generated from $BaCO_3$ (2.65 g, 14.0 mmol {containing Ba*CO_3 250 mCi }) was transferred into the reaction flask. The liquid nitrogen bath was replaced with dry ice-acetone bath, and reaction was allowed to proceed at -78° C followed by gradual warming (ca. 5 h) to room temperature. Solvent was removed and the residual solid was taken up in CH_2Cl_2 (200 mL) and saturated NH_4Cl (10.0 mL), stirred for 10 min and the organic layer was separated. The aqueous layer was extracted repeatedly with methylene chloride and then brought to neutral pH by dropwise addition of 6N HCl. It was further extracted with ethyl acetate and the combined organic extract was dried and evaporated to dryness. Analysis by tlc showed a mixture of the protected and free acid (67:33) . Deprotection was done by stirring in ethyl acetate (80 mL) and 6N HCl (25.0 mL) at 60° C overnight. After adjusting to pH 7, the solvent was stripped, and product was chromatographed on C_{18} silica column eluted with 20% methanol in an 8% solution of ammonium hydroxide to give 4,6-dichloroanthranilic acid (1.86g, 65%). Proton nmr (DMSO-d_6), δ 10.0 – 7.0 (br), 6.76 (d, J = 2.0 Hz), 6.67 (d, J = 1.97 Hz); Ms m/z 205 (M^+) and other ions at 187, 160, 124, 97, 90, 73, and 62.

1,3-Dichloro-7,8,9,10-tetrahydroazepino[2,1-b]-5-[5-^{14}C]quinazolin-12(6H)-one (5)

4,6-Dichloroanthranilic acid (1.2 g) in dry toluene (100 mL) was refluxed and residual water was removed with the aid of a Dean Stark. A solution of 1-aza-2-methoxy-1-cycloheptene (8.0 mL) in dry toluene (25.0 mL) was added in portions over 1h, followed by additional 45 min reflux and the solvent was removed under reduced pressure. The product was chromatographed on silica gel column eluted with 25 % ethyl acetate in hexane to give (690 mg, 42 %) after crystallization, specific acitivity 65.8 μCi/mg . Proton nmr ($CDCl_3$) 7.48 d; 7.40 d; 4.33 brs; 3.0 brm; 1.83 brs.

1,3-Dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-b][5-^{14}C]quinazoline monohydrochloride (1).

Quinazolin-12(6H)-one (690 mg, 2.43 mmol) in glacial acetic acid (35.0 mL), was brought to 70° C in an oil bath and excess zinc dust (1.7 g, 26.0 mmol) was added. A solution of conc. HCl (4.2 mL) in glacial acetic acid (10.0 mL) was added over a period of 20 min to the mechanically stirred reaction mixture while maintaining internal temperature of 70° C . After a further stirring for 25 min, excess zinc was removed by filtration, the filtrate was concentrated and residual acid removed by azeotrope with toluene to give an oil. It was dissolved in water (10.0 mL) and THF (120 mL) was added. The pH was adjusted to between13 and 14 by dropwise addition of 50 % NaOH solution. Solid anhydrous K_2CO_3 followed by the addition of Celite and filtered. The solvent

was removed and the product was chromatographed on silica gel column, eluted with ethyl acetate:chloroform:triethylamine (60:40:1). The oil obtained was acidified with 6N HCl and crystallized from ethanol to give 1,3-dichloro-6,7,8,9,10,12-hexahydroazepino[2,1-b] [5-^{14}C]quinazoline monohydrochloride as a white solid (323 g, 44 %), specific activity 59.11μCi/mg, TLC silica gel 60 F-254 ($CHCl_3$: MeOH: NH_4OH; 300:25:1), Rf 0.63, RCP 100%; Hplc RCP 99.64%, CP 99+% on Alltech Econosil C_{18}, 10 μ, 4.6 mm x 250 mm, eluted with 0.05 M ammonium phosphate: CH_3CN (60:40) at a flow rate of 1.0 mL/ min, and detection at uv 225 nm. Proton nmr ($CDCl_3$) 14.01 brs; 7.52 s; 7.06 s; 4.81 s; 3.76 brd; 3.2 brm and 1.85 s. MS m/z at 267 (M^+), and others ions at 239, 225, 212, 123, 69 and 55.

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