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SYNTHESIS AND RADIOIODINATION OF 3-(*E*)-(2-IODOVINYL)-N-ACETYL-4-CYSTEAMINYLPHENOL, A PUTATIVE TYROSINASE SUBSTRATE FOR IMAGING NEURAL CREST TUMOURS

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KEYWORDS

3-Iodovinyl-N-acetyl-4-cysteaminylphenol, radioiodination, antimelanoma, tyrosinase, neural crest tumours, 4-SCAP

SUMMARY

The synthesis of 3-(*E*)-(2-iodovinyl)-N-acetyl-4-cysteaminylphenol (3-IV-N-Ac-4-SCAP) started from 3-iodophenol, which on condensation with cysteamine hydrochloride gave 3-iodo-4-cysteaminylphenol (**1**). Acetylation of **1** followed by selective deacetylation yielded 3-iodo-N-acetyl 4-cysteaminylphenol (3-I-N-Ac-4-SCAP; **3**). The stereospecific reaction of (*E*)-1-trimethylsilyl-2-tri-*n*-butyltinethylene with **3** afforded the vinyl-TMS precursor 3-(*E*)-(2-trimethylsilylvinyl)-N-acetyl-4-cysteaminylphenol (3-TMS-N-Ac-4-SCAP; **4**) in high yield. This TMS vinyl precursor is a useful synthon for high specific activity radioiodination. Iodination of the synthon 3-(*E*)-(2-trimethylsilylvinyl)-N-acetyl-4-cysteaminylphenol with iodine monochloride gave 3-IV-N-Ac-4-SCAP (**5**). High specific activity (no carrier added) 3-*(E)*-(2-[^{125}I]iodovinyl)-N-acetyl-4-cysteaminyl phenol was obtained in 85% radiochemical yield upon a 5 min exposure of a mixture of Na[^{125}I]I and 3-TMS-N-Ac-4-SCAP to 2450 MHz radiation in a microwave oven.

INTRODUCTION

One rational approach to the diagnosis and chemotherapy of melanoma and other tumours of neural crest origin is to target the biochemical pathway leading to melanin biosynthesis. A central enzyme in this pathway, tyrosinase, is highly expressed in malignant melanomas (Jimbow *et al*,

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1991). Research into targeted chemotherapy of malignant melanomas and neural crest tumors has led to the development of a class of 4-S-substituted phenols that are inhibitors of tyrosinase (Wick *et al*, 1977, 1978; Pawelek and Lerner, 1978; Fujita, 1980; McEwan *et al*, 1980). N-acetyl-4-cysteaminylphenol (N-Ac-4-SCAP), one of the most potent tyrosinase inhibitors in this class, causes selective destruction (necrosis) of melanocytes and significant regression of B16F10 melanoma in mice, (Jimbow *et al*, 1993; Miura *et al*, 1990; Alena *et al*, 1990; Parsons *et al* 1992). The radioactive analog of N-Ac-4-SCAP has been found to accumulate selectively in melanomas (Alena *et al*, 1994) and is therefore a putative agent for targeted diagnosis and radiotherapy when suitably radiolabelled. In keeping with the objectives to prepare an analogue that is both resistant to deiodination and easily prepared, the synthesis 3-TMSvinyl-N-Ac-4-SCAP, a synthon for the facile synthesis and radiolabelling of 3-(*E*)-(2-iodovinyl)-N-acetyl-4-cysteaminylphenol is now reported. The methodology is applicable to rapid, high specific-activity radioiodination.

EXPERIMENTAL

Materials and Methods. All chemicals used were of reagent grade. Anhydrous solvents, where used, were distilled fresh and stored under an inert atmosphere. The compounds were purified by column chromatography using Merck silica gel 60 (particle size 70-200 mesh ASTM) and the progress of reactions was monitored on Whatman MK6F (250 μm) micro tlc plates. ^{1}H and ^{13}C NMR spectra were acquired in deuterated chloroform on a Bruker AM-300 spectrometer; the chemical shifts are reported in δ (ppm) with respect to tetramethylsilane as internal standard. Mass spectra were provided by the Mass Spectrometry Laboratory, Dept. of Chemistry, University of Alberta. Sodium [^{125}I] iodide was purchased from Amersham International as a solution in 0.1N sodium hydroxide. Radiochemical products were purified by HPLC on a radial-pak C-18 analytical column. (*E*)-2-Tributylstannyl-1-trimethylsilylethylene was prepared using a published procedure (Cunico and Clayton, 1976).

3-Iodo-4-cysteaminylphenol (1). A mixture of 3-iodophenol (2.20 g; 10 mmol) and cysteamine hydrochloride (22.3 g; 100 mmol) was dissolved in aqueous hydrobromic acid (47% w/v; 100 mL) and heated at 110 °C (16 h). A tlc examination of the reaction mixture at this point showed the formation of three products, subsequently determined to be **1** (major) along with some 2- and 6- positional isomers (minor). The reaction mixture was cooled and the hydrobromic acid was removed under reduced pressure. The resulting viscous mass was repeatedly extracted into warm acetone, to remove unreacted cysteamine hydrochloride. The acetone phase was evaporated and loaded onto a silica gel column. Elution with ether:acetone:aqueous ammonia (70:30:1.5; v/v) afforded **1** (1.1 g; 36%): ^{1}H NMR (CD_3COCD_3) δ(ppm) 2.06 (*br*, D_2O exchangeable,1H, O*H*), 3.0-3.14 (*q*, J=6.0, 2H, S-C*H*$_2$), 3.42-3.51 (*m*, 2H, C*H*$_2$-NH), 6.85 (*d*, $J_{5,6}$=8.0 of *d*, $J_{2,6}$=2.0, 1H, ***H-6***), 7.35 (*d*, $J_{6,5}$=8.0 Hz, 1H, ***H-5***), 7.38 (*br*, D_2O exchangeable, 1H, N*H*) and 7.40 (*d*, $J_{6,2}$=2.0 Hz, 1H, ***H-2***).

3-Iodo-2-cysteaminylphenol (1a) was isolated (10%) from the column during purification of **1**: ^{1}H NMR (CD_3COCD_3) δ(ppm) 2.94 (*br*, 1H, exchanges with deuterium oxide, O*H*), 2.97 (*t*, JCH_2-CH_2=6.0, 2H, S-C*H*$_2$), 3.40 (*t*, JCH_2-CH_2=6.0, 2H, C*H*$_2$-NH), 6.88 (*d*, $J_{5,6}$=8.0 of *d*, $J_{6,4}$=2.0, 1H, ***H-4***), 6.94 (*d*, $J_{4,5}$=8.0 of *d*, $J_{5,6}$=7.5, 1H, ***H-5***), 7.36 (*br*, 1H, exchanges with D_2O N*H*) and 7.40 (*d*, $J_{5,6}$=7.5 of *d*, $J_{4,6}$=2.0, 1H, ***H-6***).

3-Iodo-6-cysteaminylphenol (1b) was similarly recovered (12%) from the column during isolation of **1**: ^{1}H NMR (CD_3COCD_3) δ(ppm) 3.02 (*t*, JCH_2-CH_2=6.0, 2H, S-C*H*$_2$), 3.04 (*br*, 1H, O*H*, exchanges with D_2O), 3.39 (*br*, *t*, 2H, C*H*$_2$-NH), 7.05 (*d*, $J_{5,4}$=8.0 of *d*, $J_{2,4}$=2.0, 1H, ***H-4***), 7.14 (*d*, $J_{4,5}$=8.0, 1H, ***H-5***), 7.22 (*d*, $J_{4,2}$=2.0, 1H, ***H-2***).

3-Iodo-N,O-di-acetyl-4-cysteaminylphenol (2). This was prepared by addition of acetic anhydride (2.5 equivalents) to a solution of 3-iodo-4-cysteaminylphenol (0.5 g; 1.7 mmol) in anhydrous pyridine (Ac_2O : pyridine; 1:4 v/v). The solution was stirred for 4 h (25 °C), at which time tlc showed complete conversion of the starting material to a single product. Ice was added to decompose excess acetic anhydride and the solvent was evaporated *in vacuo* to form a viscous mass which was coevaporated with toluene, redissolved in a minimum quantity of dichloromethane and loaded onto a silica gel column. An elution with methanol in chloroform (1% v/v) yielded the fully acetylated product (**2**), yield, 0.52 g (81%); ^{1}H NMR ($CDCl_3$) δ(ppm) 1.86 (*s*, 3H, NHCOC***H***$_3$), 2.24 (*s*, 3H, OCOC***H***$_3$), 3.40 (*q*, JCH$_2$-C***H***$_2$=7.5 Hz, 2H, SC***H***$_2$), 3.80 (*t*, JCH$_2$-C***H***$_2$=7.5 Hz, 2H, NC***H***$_2$), 7.18 (*d*, $J_{5,6}$=8.0 of *d*, $J_{2,6}$=2.7, 1H, ***H-6***), 7.20 (*d*, $J_{6,2}$=2.7, 1H, **H-2**), 7.55 (*d*, $J_{6,5}$=8.0, 1H, ***H-5***) and 7.60 (*br*, 1H, N***H***, D_2O exchangeable).

3-Iodo-N-acetyl-4-cysteaminylphenol (3) was prepared by stirring **2** (200 mg; 0.53 mmol) in methanolic ammonia (2 M) for 3 h at 25 °C. The methanol was evaporated and the crude product was purified on a silica gel column. An eution with methanol (2.5%) in chloroform afforded **3** (130 mg; 57%): m.p. 119 °C;^{1}H NMR (CD_3COCD_3) δ(ppm) 1.87 (*s*, 3H, C***H***$_3$), 2.94 (*t*, JCH$_2$-CH$_2$=7.1, 2H, S-C***H***$_2$), 3.04 (*br*, 1H, O***H***, exchanges with D_2O), 3.26-3.38 (two *t*, merged, JCH$_2$-CH$_2$=7.1, 2H, N-C***H***$_2$), 6.88 (*d*, $J_{5,6}$=8.8 of *d*, $J_{2,6}$=2.7, 1H, ***H-6***), 7.38 (*br*, 1H, N***H***, D_2O exchangeable), 7.41 (*d*, $J_{6,2}$=2.7, 1H, ***H-2***), 7.46 (*d*, $J_{6,5}$=8.8, 1H, ***H-5***); ^{13}C NMR (CD_3COCD_3) δ(ppm) 22.89 (*C*H$_3$), 35.53 (S*C*H$_2$), 39.26 (*C*H$_2$NH), 39.37 (***C-3***), 104.71 (***C-4***), 117.39 (***C-5***), 127.51 (***C-2***), 133.38 (***C-6***), 157.94 (***C-1***), 172.19 (*C*=O); analysis for C10H12INO2S, calc. C, 35.61; H, 3.56; N, 4.15; found C,35.53; H, 3.56 and N, 4.15.

3-(E)-(2-Trimethylsilylvinyl)-N-acetyl-4-cysteaminylphenol (4). (*E*)-2-Tributylstannyl-1-trimethylsilylethylene (189 mg; 0.48 mmol) was added to a solution of 3-iodo-N-acetyl-cysteaminylphenol (59 mg; 0.177 mmol) and bistriphenylphosphine palladium (II) chloride (18 mg; 0.026 mmol) in anhydrous acetonitrile (10 mL) under an inert atmosphere . The mixture was stirred at 80° C for 10 h, at which time tlc showed complete disappearance of the starting material and a new product at a higher R_f value. The solvent was evaporated *in vacuo* and the crude product was purified on a silica gel column using chloroform:methanol (98:2; v/v) as eluting solvent to afford **4** (20 mg.,40%). ^{1}H NMR ($CDCl_3$) δ(ppm) 0.17 (*s*, 9H, C***H***$_3$ of TMS), 1.74 (*s*, broad, O***H***), 1.94 (*s*, 3H, COC***H***$_3$), 2.82 (*t*, JCH$_2$-CH$_2$= 6.0 Hz, 2H, SC***H***$_2$), 3.34 (*d*, JCH$_2$-CH$_2$=6.0 Hz of *d*, J_{gem}= 11.5 Hz, 2H, NC***H***$_2$), 5.88 (*broad*, D_2O exchangeable, 1H, N***H***), 6.38 (*d*, $J_{vic.}$=18.5 Hz, 1H, -CH=C***H***X), 6.68 (*d*, $J_{5,6}$=8.0 Hz of *d*, $J_{2,6}$=2.5 Hz, 1H, ***H-6***), 7.07 (*d*, $J_{6,2}$= 2.5 Hz, 1H, ***H-2***), 7.31 (*d*, $J_{6,5}$=8.0 Hz, 1H, ***H-5***) and 8.49 (*d*, $J_{vic.}$=18.5,1H, ArC***H***=CHI); ^{13}C NMR ($CDCl_3$)-δ 1.91 (TMS *C*H$_3$), 23.07 (CO*C*H$_3$), 35.40 (S*C*H$_2$), 38.78 (*C*H$_2$NH), 117.75 (***C-6***), 120.28 (***C-2***), 122.22 (***C-4***), 132.75 (***C-5***), 135.55 (CH=*C*HTMS), 141.42 (*C*H=CHTMS), 142.02 (***C-3***), 157.32 (***C-1***) and 171.05 (*C*=O); FAB for $C_{15}H_{23}NO_2SSi$, exact mass calc., 309.4; found, 310.1 (17.98%).

3-(E)-(2-Iodovinyl)-N-acetyl-4-cysteaminylphenol (5). Iodine monochloride (17.0 mg; 0.01 mmol) was added to a solution of **4** (21.0 mg; 0.067 mmol) in anhydrous dichloromethane (4.0 mL). The mixture was stirred and maintained at 80° C for 4h. Tlc at this point showed complete disappearance of the silylated compound and a new product at a higher R_f value. The solvent was removed *in vacuo* on a rotary evaporator and the resulting viscous mass was purified on preparative tlc using $CHCl_3$:MeOH (98:2; v/v) to afford the iodinated product **5** (12.0 mg; 47 %); ^{1}H NMR ($CDCl_3$) δ(ppm) 2.04 (*s*, 3H, COC***H***$_3$), 2.86 (*t*, JCH$_2$-CH$_2$=6.0 Hz, 2H, SC***H***$_2$), 3.34 (*d*, JCH$_2$-CH$_2$=6.0 Hz of *d*, J_{gem}=9.0 Hz, 2H, C***H***$_2$NH), 5.86 (*broad*, D_2O exchangeable, 1H, N***H***), 6.75 (*d*, $J_{5,6}$=8.0 Hz of *d*, $J_{2,6}$=2.5 Hz, 1H, ***H-6***), 6.79 (*d*, J_{vic}=15.0 Hz, 1H, CH=C***H***I), 6.89 (*d*, $J_{6,2}$=2.5 Hz, 1H, ***H-2***), 7.36 (*d*, $J_{6,5}$=8.0 Hz, 1H, ***H-5***) and 7.99 (*d*, J_{vic}=15.0 Hz, 1H, C***H***=CHI); ^{13}C NMR ($CDCl_3$) δ(ppm) 23.27 (TMS CH_3 carbons), 31.23 (CO*C*H$_3$), 35.76 (S*C*H$_2$), 38.55

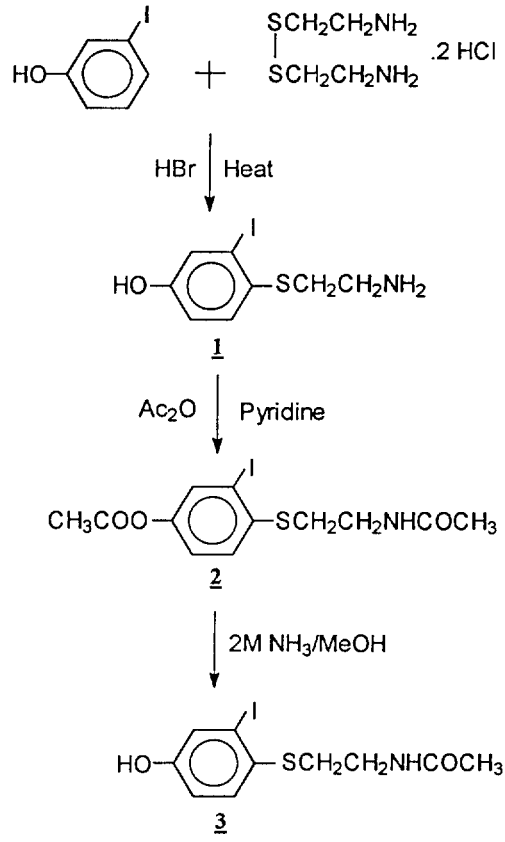
($NHCH_2$), 79.34 (CH=*C*HI), 113.37 (*C*H=CHI), 116. 54 (*C-5*), 136.44 (*C-6*), 143.24 (*C-2*), 180.00 (*C*=O). C-1, C-3 and C-4 were embeded in the base line. +ve **FAB** for $C_{12}H_{14}INO_2S$; exact mass calc., 363.2; found, 363.7 (5.67%).

Radioiodination. Aqueous sodium (^{125}I) iodide (4.5 μCi in 0.1N NaOH) was evaporated to dryness under a stream of nitrogen in a 1 mL Reactivial™, followed by the addition of a solution of **4** in 2-pentanone (100 μL). The contents were shaken for 5 min. and then exposed for 5 min to 2450 MHz microwave radiation in a domestic microwave oven set at "medium". The reaction vial was removed from the microwave oven and cooled, and the mixture analyzed on a μbond radial pak C-18 reverse phase column at 260 nm. The mixture was eluted with methanol:water = 70:30 (v/v) at a flow rate of 1.5 mL / min. The fractions (1.5 mL) were collected every minute and counted on a Beckman γ- scintillation counter. The pure product eluted at a retention time of 7.7 min. The radiochemical yield was 85% and the specific activity of the product was 780 mCi/mmol.

DISCUSSION

3-(*E*)-(2-Trimethylsilylvinyl)-N-acetyl-4-cysteaminylphenol (3-TMS vinyl-N-Ac-4-SCAP;**4**), the synthon for **5**, was synthesized by stereospecific condensation of 3-iodo-N-acetyl-4-cysteaminylphenol (**3**) with (*E*)-(1-tributylstannyl-2-trimethylsilyl)ethylene (Scheme 2). **3** was obtained by reaction of 3-iodophenol with cysteamine di-hydrochloride in the presence of hydrobromic acid. This reaction also yielded 2-cysteaminylphenol and 6-cysteaminylphenol, the positional isomers of **1**, in minor quantities. 3-I-N-Ac-4-SCAP was observed to decompose extensively during chromatographic purification and storage in a polar solvent, most likely via intramolecular deiodination elimination. On acetylation with acetic anhydride in anhydrous pyridine, **1** gave 3-iodo-N,O-diacetyl-4-S-cysteaminylphenol, (**2**), which was selectively deacetylated *in situ* to afford **3** (Scheme 1). 3-(*E*)-(2-trimethylsilylvinyl)-N-acetyl-4-cysteaminylphenol retained the *E* configuration of the starting reagent, with only a small quantity of the *Z*-isomer being formed. The solvents used in the reaction and in the purification have been reported to play a significant role in determining the ratio of the isomers isolated (Cunico and Clayton, 1976), with the *E* isomer slowly converting to *Z* in protic solvents. It was observed that elution with 5% MeOH in chloroform from a silica gel column afforded mainly the *Z* isomer of the silylated compound (the observed coupling constant of vinylic protons for this geometric isomer was 12 Hz, indicating their *cis* orientation) in which vinylic protons were present as a doublet at δ 5.32.

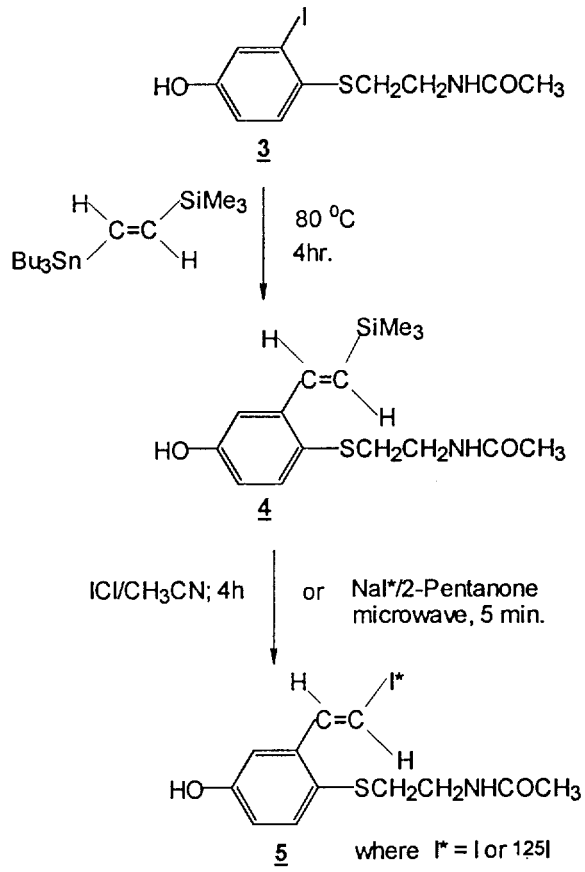
The silyl derivative **4** decomposed slowly at room temperature (22° C). Reaction of **4** with iodine monochloride in anhydrous dichloromethane, afforded 3-(*E*)-(2-iodovinyl)-N-acetyl-4-cysteaminylphenol (**5**) in satisfactory yield (48%) (Scheme 2). Replacement of the trimethylsilyl group with iodine affects the neighboring electronic environment substantially. ^1H NMR of **5** shows a strong deshielding effect on the both C'-1 and C'2 (vinyl) protons, with downfield shifts of 0.50 and 0.41 ppm, respectively, in comparison to the same protons in the trimethylsilyl precurser. The phenyl C-2 proton was shifted upfield by 0.18 ppm. The introduction of iodine significantly shields the C'-2 (vinyl), so that it appears at δ 79.34 ppm in the ^{13}C spectrum, in comparison to the silyl precursor, in which it resonates at 135.55 ppm. The C'-1 (vinyl), at δ 141.42 ppm in the silylated compound, was slightly downfield at δ 143.24, and C-2 and C-6 were shifted upfield by 3.7 and 4.4 ppm, respectively, when iodine replaced the TMS.



Scheme 1. Synthesis of 3-Iodo-4-N-acetyl cysteaminyl phenol

The procedure adopted for radioiodination is based on microwave-induced displacement of the vinylic-TMS group by iodide. The microwave reaction proceeds rapidly (5 min.) and does not give rise to any radiolabelled side products. The HPLC chromatogram of the hot reaction mixture showed only two radioactive fractions, corresponding to radioiodide at the column void volume (approx. 2 min) and radioiodinated **5** (7.7 min). Unreacted **4** appeared at a retention time of 14.3 min.

A comparison of the electrophilic replacement of trimethylsilyl group by iodine using iodine monochloride with microwave radiation indicates that microwave-induced displacement almost doubles the chemical yield of iodinated compound, and that no other side product is formed under these reaction conditions. The microwave method therefore appears to be advantageous for small scale iodinations and radioiodinations. Very high specific activities can be achieved by this approach, and losses of radioactivity are reduced by avoiding the chemical generation of volatile electrophilic radioiodinating species normally generated in electrophilic demetalation radioiodinations.



Scheme 2: Synthesis of 3-Iodovinyl-N-acetyl-4-cysteaminylphenol its ^{125}I analog

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