Preparation of 17-amino-22-(4'-azido-3'-^{125}iodophenacyl)-17-demethoxygeldanamycin (1): An Ansamycin for Photoaffinity Labeling .

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SUMMARY

An azido-^{125}iodo-ansamycin (1) in the geldanamycin family was prepared in two steps in one reacton vessel from 17-amino-22-(4'-aminophenacyl)-17-demethoxygeldanamycin (3). The title compound was suitable for photoaffinity labeling proteins that interacted with ansamycins. A alternative synthesis is reported for preparation of unlabeled title compound in order to afford practical amounts of (1) for conventional biochemical studies.

Key Words: Ansamycin; Geldanamycin; Photoaffinity; ^{125}Iodine; Oncogene inhibition.

INTRODUCTION

Retroviruses are known to encode phosphoproteins with tyrosine kinase activity. These kinases and other receptor family tyrosine kinases are oncogene products, oncoproteins, that have been implicated in signal transduction pathways necessary for maintaining the malignant phenotype of cancer cells. Recently several small molecules have been discovered that inhibit these kinases. For example, in cells transformed by the oncogene *src* , the ansamycin herbimycin A has been shown to convert the transformed morphology to the normal morphology (1). Herbimycin A also was reported to cause a decrease in the p185 phosphoprotein in *erbB*-2 bearing SKBr-3 breast

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cancer cells (2). Geldanamycin, 17-amino-17-demethoxygeldanamycin (2) and other analogs were found to inhibit cell growth of SV40 transformed cells (3). 4,5-Dihydrogeldanamycin, a related ansamycin, was also shown to deplete p185 from *erbB-2* SKBr-3 cells (4). In addition, 17-amino-17-demethoxygeldanamycin (2) has been reported to inhibit RNA directed DNA polymerase (RDDP), a Rauscher Leukemia virus reverse transcriptase, as well as virus infected cell growth (5). Though efforts have been made to characterize the mechanisms of these effects, the molecular mechanism of ansamycins is unknown. Photoaffinity labeled compounds can be the focal tools for determining the mechanism by which small molecules exert their effects on cellular proteins. This paper describes the synthesis of a photoaffinity labeled ansamycin suitable for mechanistic studies with oncogene transformed tumor cells.

RESULTS AND DISCUSSION

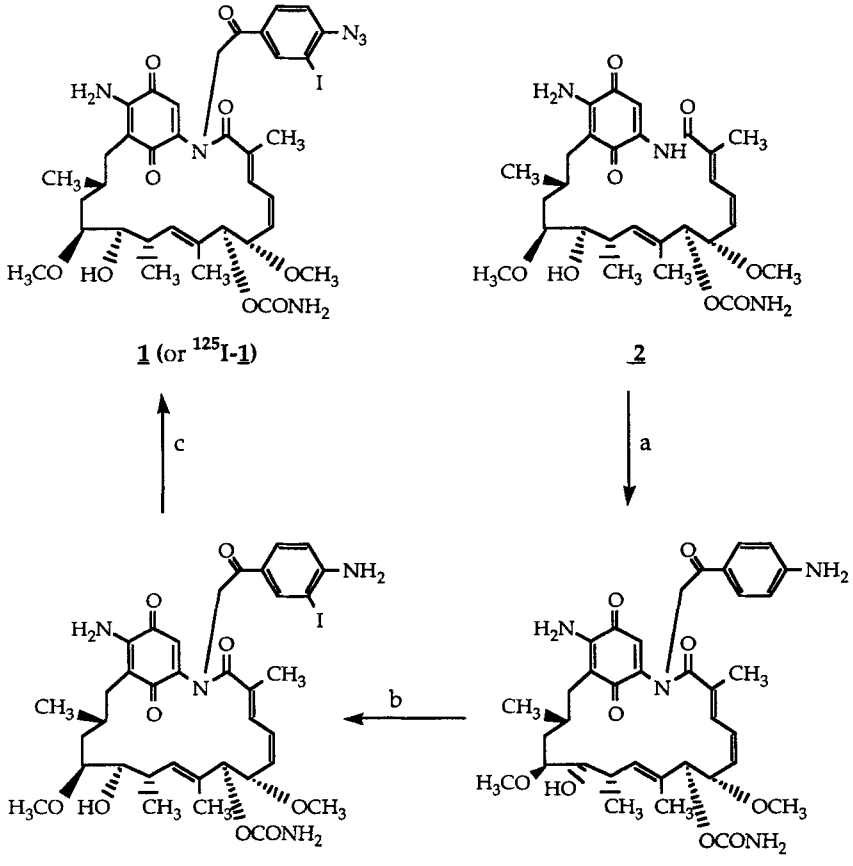
17-Amino-17-demethoxygeldanamycin (2) embraces a variety of functional groups suitable for chemical attachment of a photoaffinity moiety. 22-N-Alkylation was facile and promised to afford a non-hydrolyzable derivative. The phenacyl moiety was chosen for its ease of attachment and versatility for subsequent modification. Use of the 4-azido-3-^{125}iodo-phenyl moiety as a photolabile radiolabeling tool had been reported by Patel (6). An efficient synthesis of non-radioactive 17-amino-22-(4'-azido-3'-iodophenacyl)-17-demethoxygeldanamycin (1) was devised (Figure 1) in order to determine if it possessed sufficient biochemical activity (7). A slightly different radiolabeled synthesis was then designed to account for the particular needs of handling ^{125}I.

Using Patel's pH 7.5 buffered conditions, the NaI/Chloramine-T mediated direct iodination of 17-amino-22-(4'-aminophenacyl)-17-demethoxygeldanamycin (3) failed to give phenyl ring iodination. (8) However, treatment of (3) with KCl/ICl in methanol/water according to the method of Dahlbom (9) yielded 4'-amino-3'-iodo derivative (4) in 60% yield. Diazotization of (4) then gave (1) in 72% yield.

Because ^{125}ICl was not an attractive iodinating reagent in comparison with Na^{125}I/Chloramine-T, alternative reaction conditions were investigated involving the

latter reagents. It was found that iodine could be incorporated with approximately 50% conversion at pH 1-2 in methanol/water when a 5-fold excess of aniline (3) was employed.

Figure 1. Synthesis of Photoreactive Radioiodinated Ansamycin



a, 4'-aminophenacyl chloride, K-O-*t*-Bu, DMSO; b, KCl/ICl, MeOH/H_2O, or $Na^{125}I$ Chloramine-T, pH1-2, MeOH/H_2O; c, $NaNO_2$, HCl, NaN_3, MeOH/H_2O.

Isolation and purification of radiolabeled intermediate (^{125}I-4) would increase the hazard of preparing radiolabeled (1). To reduce the volume of radioactive waste generated in the synthesis, a sequential diazotization of (^{125}I-4) was performed in the same reaction vessel as used for iodination, thus affording a one pot preparation of (^{125}I 1) from (3) via (^{125}I-4). Purification of radiolabeled (1) was accomplished by direct application of the complete reaction mixture to reverse phase HPLC. (^{125}I-1) stored at 0.2

μM in tris buffer , pH 7.5, at -20 °C was chemically unstable, decomposing to ~15% of its original concentration after 20 days ($t_{1/2}$ ~ 7 days) (^{125}I $t_{1/2}$ = 60 days) as determined by reverse phase-HPLC with radiochemical detection.

EXPERIMENTAL

17-Amino-22-(4'-azido-3'-^{125}iodophenacyl)-17-demethoxygeldanamycin (1)

To a "V" type vial containing 10 mCi of $Na^{125}I$ (4.60 nmol, DuPont NEN cat. # NEZ-033L, ^{125}I = 2175 Ci/mmol) in 10^{-5} M NaOH were added compound (3) (157 μg, 0.23 μmol) dissolved in 78.5 μL of methanol, 143 μL (14.3 μmol) of 0.1 N HCl (10) and chloramine-T (143 μg, 0.52μmol) in 143 μL of water. After reacting 1 hour the reaction was terminated by addition of sodium metabisulfite (100μg, 0.52 μmol) in 100 μL of water. Then the reaction vessel was charged with 35 μL of 6N HCl (180 μmol) and 20 μL of 0.5M $NaNO_2$ (10 μmol), stirred for 15 minutes and charged with 20 μL of 0.5 M NaN_3 (10 μmol). After an additional 15 minutes the reaction was quenched by addition of 50 μL of 4 N NH_4OH (200 μmol). The entire reaction mixture was injected onto a reverse phase HPLC Waters μBondapack™ C18 analytical column eluted at 1 mL/min with a gradient of 40-60% methanol/water containing 0.1 M triethylamine and 0.1 M acetic acid over a 45 minute period. A UV and radiochemical detector indicated that pure product eluted at 60-65 minutes affording (^{125}I-1) in 1-2% overall yield based upon starting radioactivity. Fractions containing the (^{125}I-1) were collected, lyophilyzed, diluted to 0.2 μM in 1 mL of 10mM tris buffer at pH 7.5 and stored at -20 °C. (11)

17-Amino-22-(4'-aminophenacyl)-17-demethoxygeldanamycin (3)

17-amino-17-demethoxygeldanamycin (3) (0.501 g, 0.919 mmol) was dissolved in 10 mL anhydrous dimethylsulfoxide in flame dried glassware. Potassium t-butoxide (0.108 g, 0.961 mmol) was added and the solution stirred at room temperature under nitrogen for 30 minutes. 4-Aminophenacyl chloride (0.171 g, 1.01 mmol) was added and the solution stirred at room temperature for 3 hours. This solution was diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered and evaporated in vacuo; 0.457 g (73%): mp 188°C (dec); ^{1}H-NMR ($CDCl_3$) δ 0.71 (d, J = 8 Hz, 3H, 14-Me), 0.86 (br m, 1H, H-13), 1.04 (d, J = 8 Hz, 3H, 10-Me), 1.39 (s, 3H, 8-Me), 1.49 (br

m, 1H, H-13), 1.90 (dd, J = 13 Hz, 5 Hz, 1H, H-15), 2.00 (s, 3H, 2-Me), 2.22 (br m, 2H, H-10, H-14), 2.48 (br s, 1H, 11-OH), 2.92 (m, 2H, H-12 and H-15), 3.29 (s, 3H, OMe), 3.33 (s, 3H, OMe), 3.62 (d, J = 10 Hz, 1H, H-11), 4.28 (s, 2H, NH_2), 4.29 (t, J = 9 Hz, 1H, H-6), 4.37 (d, J = 18 Hz, 1H, α-CH_2), 4.75 (br s, 2H, NH_2), 5.03 (d, J = 9 Hz, 1H, 7-H), 5.18 (br s, 2H, NH_2), 5.21 (d, J = 9 Hz, 1H, H-9), 5.26 (t, J = 13 Hz, 1H, H-5), 5.95 (d, J = 18 Hz, 1H, α-CH_2), 5.96 (s, 1H, H-19), 6.49 (t, J = 10 Hz, 1H, H-4), 6.66 (d, J = 9 Hz, 2H, aromatic), 7.18 (d, J = 13 Hz, 1H, H-3), 7.78 (d, J = 9 Hz, 2H, aromatic); m/z 701 (M^+ + Na); ir (KBr, cm^{-1}) 1718, 1708, 1655, 1619, 1580; Calc: $C_{36}H_{46}N_4O_9 \bullet 0.5H_2O$ C, 62.86; H, 6.89; N, 8.15%. Found: C, 62.74; H, 6.56; N, 8.06%.

17-Amino-22-(4'-amino-3'-iodophenacyl)-17-demethoxygeldanamycin (4)

Potassium chloride (0.022 g, 0.29 mmol) was dissolved in 1 mL water and poured into iodine monochloride (0.043 g, 0.27 mmol). This mixture was poured into a solution of 17-amino-22-(4'-aminophenacyl)-17-demethoxygeldanamycin (3) (0.139 g, 0.2 mmol) dissolved in 24 mL 0.1N hydrochloric acid and ~3 mL methanol. After stirring for 1 hour at room temperature the reaction was quenched with sodium bisulfite, extracted with ethyl acetate, washed with 3N hydrochloric acid, saturated sodium bicarbonate, water, and brine, dried over magnesium sulfate, filtered and evaporated *in vacuo*. The residue was dissolved in 1 mL chloroform and precipitated with hexanes to give a salmon-colored solid, 0.099 g (60%): ^1H NMR ($CDCl_3$) δ 0.66 (d, J = 7 Hz, 3H, 14-Me), 0.75 (m, 1H, H-13), 0.98 (d, J = 6 Hz, 3H, 10-Me), 1.34 (s, 3H, 8-Me), 1.47 (m, 1H, H-13), 1.91 (m, 1H, H-15), 1.95 (s, 3H, 2-Me), 2.10-2.29 (br m, 2H, H-10 and H-14), 2.59 (s, 1H, 11-OH), 2.78-2.92 (m, 2H, H-12 and H-15), 3.25 (s, 3H, OMe), 3.29 (s, 3H, OMe), 3.59 (d, J = 10 Hz, 1H, H-11), 4.24 (t, J = 10 Hz, 1H, H-6), 4.32 (d, J = 18 Hz, 1H, α–CH_2), 4.56 (s, 2H, NH_2), 4.95 (d, J = 10 Hz, 1H, H-7), 5.04 (br s, 2H, NH_2), 5.15 (d, J = 11 Hz, 1H, H-9), 5.22 (t, J = 11 Hz, 1H, H-5), 5.58 (br s, 2H, NH_2), 5.88 (d, J = 18 Hz, 1H, α–CH_2), 5.89 (s, 1H, H-19), 6.37 (t, J = 12 Hz, 1H, H-4), 6.60 (dd, J = 9 Hz and 3 Hz, 1H, aromatic), 7.06 (d, J = 12 Hz, 1H, H-3), 7.71 (dd, J = 9 Hz and 2 Hz, 1H, aromatic), 8.22 (dd, J = 2 Hz and 3 Hz, 1H, aromatic); m/z 805 (M + H); ir (KBr, cm^{-1}) 1720, 1660, 1610, 1580; Calc ($C_{36}H_{45}IN_4O_9 \bullet 2H_2O$) C, 51.43; H, 5.88; N, 6.66%. Found C, 51.40; H, 5.31; N, 6.32%.

17-Amino-22-(4'-azido-3'-iodophenacyl)-17-demethoxygeldanamycin (1)

17-Amino-22-(4'-amino-3'-iodophenacyl)-17-demethoxygeldanamycin (4) (0.036 g, 0.44 mmol) was dissolved in 90 mL methanol, cooled to 0 °C and shielded from light. To this solution was added 45 mL 1N hydrochloric acid and 45 mL 0.5N sodium nitrite. After 15 minutes of stirring, 45 mL 0.5N sodium azide was added and the reaction stirred another 15 minutes at 0 °C. The resulting solution was extracted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered and stripped to give a red glass which was dissolved in 1 mL ethyl acetate and precipitated with hexane. 0.027 g (72%): ^{1}H NMR ($CDCl_3$) δ 0.69 (d, J = 7 Hz, 3H, 14-Me), 0.79 (m, 1H, H-13), 1.03 (d, J = 8 Hz, 3H, 10-Me), 1.50 (s, 3H, 8-Me), 1.89 (m, 2H, H-13 and H-15), 1.99 (s, 3H, 2-Me), 2.11-2.32 (br m, 2H, H-10 and H-14), 2.53 (br s, 1H, 11-OH), 2.81-2.95 (m, 2H, H-12 and H-15), 3.28 (s, 3H, OMe), 3.32 (s, 3H, OMe), 3.62 (d, J = 9 Hz, 1H, H-11), 4.22 (t, J = 12 Hz, 1H, H-6), 4.41 (d, J = 17 Hz, 1H, α–CH_2), 4.80 (br s, 2H, NH_2), 5.02 (d, J = 9 Hz, 1H, H-7), 5.16-5.31 (br m, 4H, NH_2, H-9, and H-5), 5.92 (s, 1H, H-19), 5.99 (d, J = 18 Hz, 1H, α–CH_2), 6.40 (t, J = 14 Hz, 1H, H-4), 7.03 (d, J = 14 Hz, 1H, H-3), 7.12 (m, 1H, aromatic), 8.00 (m, 2H, aromatic), 8.40 (m, 1H, aromatic); ir (KBr, cm^{-1}) 2110, 1722, 1680, 1661, 1590; m/z 852 (M^+ + Na - H); Calc ($C_{36}H_{43}IN_6O_9 \bullet 4H_2O$) C, 47.14; H, 5.78; N, 9.16%. Found C, 47.15; H, 5.14; N,7.71%.

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References

1. Uehara Y., Hori M., Takeuchi T., Umezawa H. - Jpn. J. Cancer Res 76: 672 (1985).
2. Miller P., DiOrio C., Cullen W., Moyer J.D. - Amer. Assoc. Cancer Res. *Proceedings* 34: 344 (1993).
3. Sasaki K., Yasuda H., Onodera K. -J. Antibiotics 32: 849 (1993).

4. Cullen W., Jefferson M., Moyer J., Moyer M., Sciavolino F. - CSM/SIM Meeting Toronto, Abst. (1993).
5. Li L.H., Clark T.D., Cowie C.H., Rinehart K.L. - Cancer Treatment Reports 61: 815 (1977).
6. Patel A., Craig R.H., Daluge S.M., Linden J. - Molecular Pharmacology 33: 585 (1988).
7. Results of these studies will be reported shortly.
8. One explanation for the failure of the Patel iodination procedure at pH 7.5 could be that an N-iodo intermediate is formed at that pH which is stable and does not decompose to the desired C-iodo product. This latter reaction requires acid catalysis in this specific case.
9. Dahlbom R., and Mollberg R. - Acta Chem. Scand. 16: 655 (1962).
10. The authors recommend using phosphoric acid rather than HCl in this step in order to eliminate the contamination of the reaction mixture with chlorinated phenacyl products generated during the initial halogenation step.
11. Compound (1) was the last material to elute from the HPLC column under these conditions, as determined by both radio- and UVdetection. Compounds (4) (retention time 33 min) and (3) (retention time 22 min) were potential components of the reaction mixture along with numerous other materials that arose from incomplete reaction or, more likely, undesired reactions at either step in the one-pot preparation. Pure nonradioactive (1), prepared from pure (4), was used to identify HPLC separation conditions that avoided the need for an involved purification process. The low yield of the procedure is probably due to the inefficiency of the iodination using the chloramine-T procedure in combination with unoptimized diazotization conditions where the chloramine-T reactants, by-products and products halogenation side reactions were still present in the reaction mixture. The efficiency of the one step purification of the complex reaction mixture obtained in our one-pot procedure and the need to obtain only very small quantities of labeled (1) made practical the acceptance of the observed low yield.