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# RADIOIODODESTANNYLATION. CONVENIENT SYNTHESIS OF A STABLE PENICILLIN DERIVATIVE FOR RAPID PENICILLIN BINDING PROTEIN (PBP) ASSAY

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SUMMARY

Radioiodination of *p*-(trimethylstannyl)penicillin V with [$^{125}$I]Na using a modification of the chloramine-T method is simple, high yielding, and site-specific. The structure and penicillin binding protein (PBP) affinity of *p*-[$^{125}$I]-penicillin V (IPV) are similar to penicillin G and the product can be used directly without purification in the PBP assay. Because of the high degree of stability toward autoradiolysis and equivalent PBP binding affinity, IPV can be used in place of [$^{3}$H]-penicillin G or [$^{14}$C]-penicillin G for these experiments.

**Key Words** *p*-[$^{125}$I]-penicillin V, penicillin binding proteins, electrophilic destannylation

## INTRODUCTION

The quantitative determination of β-lactam antibiotic binding affinity for subcellular targets, the penicillin binding proteins (PBP's), has become an important aspect of the *in vitro* characterization of these compounds (1). These experiments were originally executed using side chain carbonyl-labelled [$^{14}$C]-penicillin G (benzylpenicillin) as the imaging reagent (2). The low specific radioactivity of the reagent combined with the low copy number of essential PBP's in many bacteria resulted in long exposure times (months) required for autoradiography. Even with enhancing improvements such as fluorography (3) and film preflash (4), the required exposure times precluded the routine use of this important evaluation technique.

In order to shorten exposure times, a penicillin with higher specific radioactivity, [$^{3}$H]-penicillin G, has been employed (5). Although this material is commercially available,

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tedious synthetic manipulations required for its preparation and the autoradiolytic sensitivity of the product substantially increase the cost, while the autoradiographic time requirement is reduced minimally (weeks). Radioiodinated furazlocillin (6) and a radioiodinated derivative of ampicillin (7) provided access to penicillins with improved radiographic characteristics, but large differences in enzyme binding affinity relative to penicillin G rendered these compounds of questionable utility as general imaging reagents. Labia (8) has offered a practical solution with the synthesis of radioiodinated penicillin X (p-hydroxybenzylpenicillin) at high specific activity. Although this reagent was not characterized structurally and was quite susceptible to autoradiolysis, introduction of the label in the final step of a synthesis providing a close structural analog of penicillin G constituted an important advance in the effort to bring the PBP assay closer to routine use.

Electrophilic destannylation (9) offers several distinct advantages for the introduction of a radiolabel to chemically sensitive β-lactam molecules: 1) the labelling procedure may be performed in the last step, under extremely mild conditions, and with a very high degree of site selectivity thereby obviating the need for any purification of radioactive material 2) the requisite tin atom may be appended at any convenient point in the synthesis 3) the approach promises to be quite general with respect to both the electrophilic radiolabel and the β-lactam substrate. The preparation of an imaging agent with high specific activity, autoradiolytic stability, and a short radiographic exposure time requirement for use in PBP studies represents a worthy initial objective. This communication describes a convenient method for the site specific introduction of $^{125}I$ to penicillin V.

## DISCUSSION

During earlier investigations in these laboratories on labelling β-lactam molecules utilizing electrophilic destannylation, we noted that intermediates in the penicillin G series appeared to suffer more chemical lability than anticipated. We then speculated that the well-known autoradiolysis of tritiated and radioiodinated penicillin G may, in fact, be due to, or at least related to, the chemical lability of penicillin G itself. When analogous intermediates in the penicillin V series were synthesized, we observed that the chemical stability, as judged by relative decomposition rates in mildly acidic solution, was significantly greater. We were then prompted to investigate the potential for increased stability of $^{125}I$ derivatives in the penicillin V series.

The application of standard iodination conditions (sodium iodide and chloramaine-T) to *p*-(trimethylstannyl)penicillin V resulted in a quantitative conversion of the starting material to *p*-iodopenicillin V. For analytical convenience, the product was isolated as the tetrabutylammonium salt. A proton magnetic resonance spectrum of the crude product unambiguously confirmed the pure *ipso*-substitution specificity and the completeness of the iododestannylation process. In analogous fashion, radioiododestannylation with [$^{125}I$]Na (17.4 mCi/μg iodide) afforded the desired IPV. Stability of IPV is inversely proportional to storage concentration and specific activity. **At 37.5 Ci/mMol, 28 μg/mL and 4° C, the storage half-life of IPV, as judged by microbiological analysis, is 21 days.** At 37.5 Ci/mMol, only 15-24 hr exposure is

required for autoradiography in a typical PBP experiment. Thus, IPV represents a stable and practical reagent for rapid PBP assays.

We next demonstrated that penicillin G, penicillin V, and *p*-iodopenicillin V all have very similar binding affinities for the essential PBP's of a variety of bacteria, including *E. coli, Providentia rettgeri, Staphylococcus aureus, Streptococcus pyogenes and Enterococcus faecalis* (10). It seemed reasonable to expect that penicillin V, labelled with $^{125}I$, would function as a useful imaging reagent for PBP studies. This expectation has been fully realized. The application of this technique to the synthesis of other labelled β-lactam antibiotics is being actively pursued.

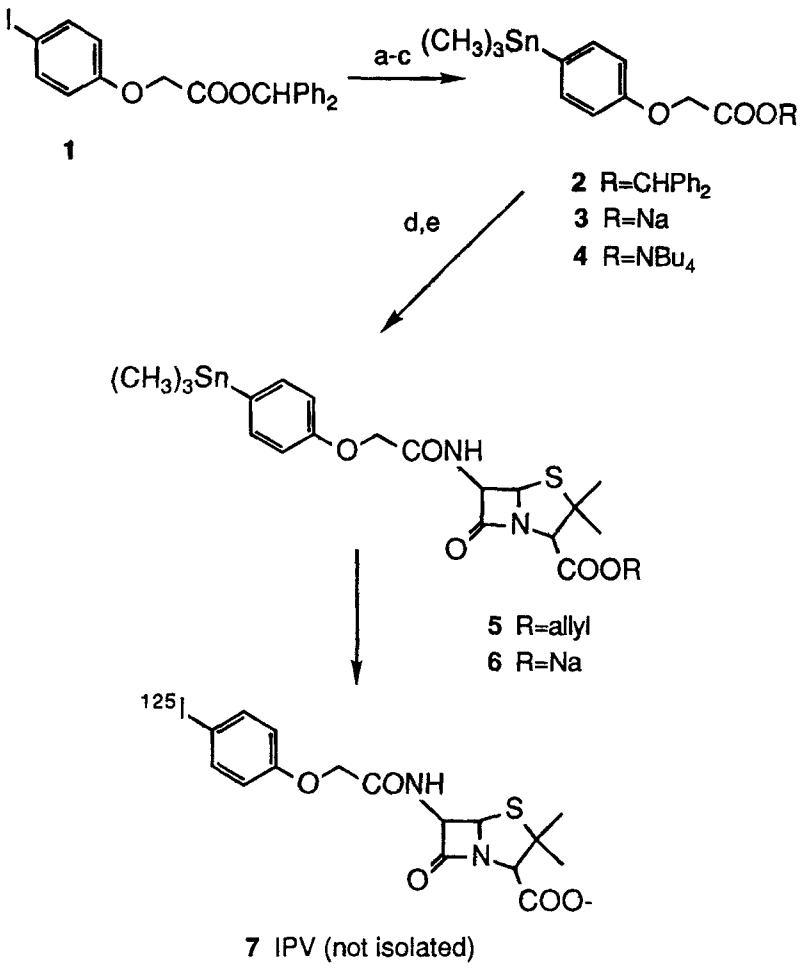
## EXPERIMENTAL

All reactions except the hydrolysis of **2** were carried out in dry glassware under an inert atmosphere. Satisfactory spectral data were obtained for all new compounds.

*Benzhydryl p-iodophenoxyacetate* (**1**). Benzhydrol (38.69 g, 210 mMol) and bromoacetic acid (34.74 g, 250 mMol) were dissolved in benzene (1000 mL). Toluenesulfonic acid monohydrate (2.00 g, 5 mole %) was added and the solution refluxed with stirring under a Dean-Stark water separator until water no longer distilled. The solution was cooled to room temperature and extracted once with pH 7 phosphate buffer and once with brine. Drying ($MgSO_4$) and concentration *in vacuo* afforded benzhydryl bromoacetate as a red-orange oil which was taken on without further purification. Sodium hydride (60% dispersion in mineral oil, 8.43 g, 211 mMol) was washed with pentane, slurried with benzene (75 mL) and DMF (75 mL), and cooled in an ice bath. A solution of *p*-iodophenol (47.17 g, 208 mMol) in benzene (125 mL) and DMF (125 mL) was added during 1/2 hr. When gas evolution ceased, the crude benzhydryl bromoacetate (63.46 g in 150 mL of benzene:DMF, 1:1) was added over 15 min. After stirring overnight at room temperature, the reaction mixture was diluted with ethyl acetate and washed sequentially: 2 x water, 1 x N HCl, 1 x saturated aq. sodium bicarbonate, 1 x brine. Drying ($MgSO_4$) and concentration *in vacuo* yielded an off-white solid. Recrystallization from methylene chloride/hexane afforded pure **1** (69.6 g, 75%).

*p-(Trimethylstannyl)phenoxyacetic Acid Benzhydryl Ester* (**2**). To a solution of **1** (1.11 g, 2.5 mMol) in dry dioxane (5 mL) was added hexamethylditin (847 mg, 2.6 mMol) and $Pd(PPh_3)_4$ (87 mg, 3 mole %). The reaction mixture was refluxed for 2 hr, cooled to room temperature, filtered through celite, and concentrated to give **2** as an oil which was used without purification.

*p-(Trimethylstannyl)phenoxyacetic Acid Tetrabutylammonium Salt* (**4**). Aqueous sodium hydroxide (3 mL of a 5N solution) was added to a solution of crude **2** in 21 mL of ethanol. The reaction mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with water (100 mL) and extracted once with ether. Tetrabutylammonium hydrogen sulfate (1.36 g, 4 mMol) was neutralized with excess aq. $NaHCO_3$ and added to the aqueous phase. The mixture was extracted with methylene chloride, dried ($Na_2SO_4$), and concentrated to give **4** as a waxy solid (1.40 g, 100 %).




a) hexamethylditin, Pd(0), dioxane,100°C b) NaOH, aq. ethanol, 25°C c) tetrabutylammonium hydrogen sulfate, pH 7 buffer/methylene chloride ~99% from 1 d) 2-chloro-4,6-dimethoxy-*s*-triazine, N-methylmorpholine, penicillin nucleus allyl ester, methylene chloride, 25°C, 68% e) sodium 2-ethylhexanoate, Pd(0), ethyl acetate/methylene chloride, 25°C, 97%

*p-(Trimethylstannyl)penicillin V Allyl Ester* (**5**). To a solution of **4** (2.82 g, 5.27 mMol) in dry methylene chloride (42 mL) was added 2-chloro-4,6-dimethoxy-*s*-triazine (11) (1.47 g, 8.38 mMol) using methylene chloride (4 mL) to aid the transfer. The reaction mixture was stirred at room temperature for 1.5 hr and cooled to 5°C. Tosylate salt of 6-aminopenicillanic acid allyl ester (12) (3.96 g, 8.88 mMol) in methylene chloride (23 mL) was added with additional methylene chloride (23 mL) to aid the transfer. N-methylmorpholine (1.8 mL, 19.3 mMol) was added and the mixture was stirred at room temperature for 5.5 hr. The reaction mixture was diluted with ethyl acetate, washed three times with pH 4 acetate buffer (0.5M), and then washed repeatedly with saturated sodium bicarbonate solution until gas evolution ceased. After a brine wash, the organic phase was dried ($Na_2SO_4$) and concentrated to an orange syrup. The crude

product was rapidly chromatographed over silica gel (which had been slurried with acetone, filtered and dried) using 20% ethyl acetate in hexane as eluant. Pure **5** (0.940 g, 68 %) was isolated as a yellow syrup.

*p-(Trimethylstannyl)penicillin V Sodium Salt* (**6**). By the method of McCombie and Jeffrey (13), **5** (0.50 g, 1.07 mMol) was converted to **6** with sodium 2-ethylhexanoate used in place of potassium 2-ethylhexanoate (0.463 g, 97%). The final product was collected as a pure (>95% by nmr), amorphous white solid which is stable indefinitely when stored at -20°C under inert atmosphere and for at least two weeks when stored at room temperature in the air.

*Radioiodopenicillin V (IPV)* (**7**). A 20 µl aliquot from a solution of **6** (1.31 mg/0.456 mL) in 0.2 M phosphate buffer at pH=7.5 was diluted with 50 µl of buffer and added to a cold (0° C) solution of $Na^{125}I$ (37.3 µL of buffer plus 61.7 µl of 64.8µCi/µL stock solution in buffer prepared from NEN lot #I061588A $Na^{125}I$, specific activity = 17.4 mCi/µg iodide). A 20 µL aliquot of freshly prepared chloramine-T solution (2.38 mg/1.19 mL in buffer) was added. After 2 min, a 10 µL portion of NaI (2 mg/mL in buffer) was added and the reaction mixture allowed to stand a 0° C for an additional 15 min. Finally, the reaction was terminated by the addition of 20 µL of sodium *meta*-bisulfite solution (4.68 mg/2.34 mL in water). The solution thus obtained was stored at 4° C and diluted as necessary for the PBP experiments.

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