TECHNICAL NOTES

4-Azido[3,5-^{3}H]phenacyl Bromide, a Versatile Bifunctional Reagent for Photoaffinity Radiolabeling. Synthesis of Prostaglandin 4-Azido[3,5-^{3}H]phenacyl Esters

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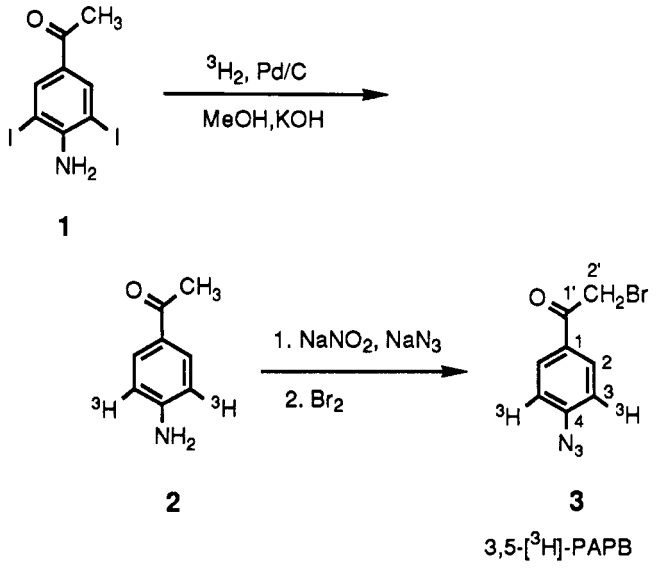
4-Azido[3,5-^{3}H]phenacyl bromide was synthesized in three steps from 4-amino-3,5-diiodoacetophenone and coupled to the prostaglandins PGE_2 and PGD_2 to provide potential photoaffinity compounds.

Since its introduction in 1973 (*1,2*), 4-azidophenacyl bromide (PAPB) has become a useful bifunctional reagent for the modification of both large and small molecules for photolabeling experiments (*3*). A current literature survey includes more than 25 photoaffinity studies using this compound. Generally, PAPB has been used to selectively alkylate reactive thiol residues in proteins, which have subsequently been used in photo-cross-linking experiments (*4, 5*). In analogous studies, it has been incorporated into polynucleotides via thiophosphate (*6*) and thiopyrimidine (*7*) linkages. Smaller molecules have been modified via attachment to thiol (*8*), carboxyl (*9*) and amino (*9, 10*) moieties to provide photolabile derivatives useful for labeling enzyme and receptor binding sites. In many cases, detection of the photolabeled compounds was achieved by using immunoassay techniques that were specific for each respective system.

We were interested in PAPB as a reagent to modify prostaglandin (PG) compounds for use in photoaffinity experiments (*11*). We reasoned that a suitable radioactive form of PAPB would provide an efficient way of introducing both the radio- and photolabile groups into the PG molecule. Radiolabeled PAPB has previously been reported as its 1′-^{14}C (*2*) and 2′-^{3}H (*12*) labeled forms. However, both derivatives were considered unsuitable for our purposes; the specific activity of the ^{14}C compound was too low, while synthesis of the ^{3}H compound required specialized handling. Also considered as unsuitable were any potential derivatives labeled with ^{125}I, since it has been noted that arylazido compounds substituted with iodine sometime result in low incorporation of the photoprobe (*13*).

We therefore decided to synthesize [3,5-^{3}H]PAPB (**3**), which was achieved by the sequence described in Scheme I. The tritium was introduced by catalytic dehalogenation of 4-amino-3,5-diiodoacetophenone (**1**; obtained by reaction of 4-aminoacetophenone with 2 equiv of iodine monochloride) using tritium gas and Pd/C catalyst in methanol/KOH.[1] Under these conditions the incorporation of tritium was high (specific activity of 50 Ci/mmol) and no concomitant reduction of the aryl ketone function was observed. Without purification, the tritiated 4-aminoacetophenone (**2**) was converted to [3,5-^{3}H]-PAPB (**3**) in a simple, high-yielding, two-step process.[2] As

Scheme I



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[1]The following was carried out by Amersham Corp., tritium labeling service (TR3 method): 4-amino-3,5-diiodoacetophenone (40 mg), 10% Pd/C (4 mg), methanol (8 mL), and 1 N KOH aqueous methanol solution (0.50 mL) were hydrogenated at room temperature under 1 atm of tritium gas for 3 h. The catalyst and solvent were removed, and the tritiated product **2** (14 mg, 5 Ci, 50 Ci/mmol) was taken up in ethanol (30 mL).

[2]To a 2.5-mL aliquot of the stock ethanol solution of tritiated **2** (500 mCi, 1.2 mg) was added unlabeled 4-aminoacetophenone (10.0 mg) and 5% aqueous H_2SO_4 (5 mL). The solution was cooled in ice, treated with aqueous $NaNO_2$ solution (18 mg in 200 μL), and stirred for 20 min before a chilled solution of NaN_3 (24 mg in 200 μL) was added. After stirring for a further 10 min, ether (10 mL) was added, and the phases separated. The organic layer was washed (2 × 3 mL water), dried ($MgSO_4$), and evaporated to give crude 4-azido[3,5-^{3}H]acetophenone. This residue was taken up in ether (1 mL) and treated with acetic acid (1 drop) and bromine solution (60 μL, 1 N CCl_4 solution). After 40 min, TLC indicated the bromination was complete [R_{fs} for 4-azidoacetophenone and monobromo and dibromo products (eluent, CH_2Cl_2) were 0.56, 0.74, 0.82; product ratios were 0.05, 0.90, 0.05, respectively]. Ether (15 mL) and water (3 mL) were added, and the organic layer separated and was dried. Removing of the solvent and purification by flash chromatography (1.5 × 30 cm column packed with 12 cm of silica; eluent, 1:1 CH_2Cl_2/hexane) gave [3,5-^{3}H]PAPB (**3**) [8.0 mg (38%), 160 mCi, 4.8 Ci/mmol; UV MeOH, λ_{max} 292 nm, $\epsilon = 2.08 \times 10^4$ (**2**)], which was taken up in toluene (5 mL) and stored at −78 °C.

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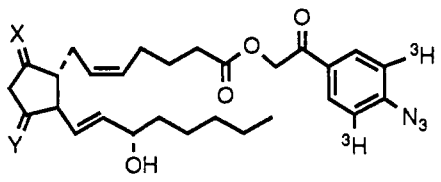
a bifunctional reagent, [3,5-^{3}H]PAPB has the following advantages: (i) Its synthesis is straightforward and inexpensive, producing large amounts of labeled material of high specific activity. (ii) [3,5-^{3}H]PAPB is stable to long-term storage at low temperature temperature and therefore, in principle, one sample can be utilized for a variety of labeling targets. Even unreactive amino and alcohol groups can be attached via linker groups (*5*).(iii) The presence of the radio- and photolabels on the same ring eliminates the possibility of them becoming separated in any postlabeling digestive workup.

We have used [3,5-^{3}H]PAPB to esterify the carboxyl functions of PGE_2 and PGD_2 to produce potential PG photoaffinity compounds **4** and **5**, respectively.[3] These PG compounds are currently being used in photolabeling experiments designed to characterize the structural aspects of PG synthase enzymes (*14*) and PG receptors (*15*).



4 X = O; Y = α-OH , β-H
5 X = α-OH, β-H; Y = O

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Note Added in Proof: The procedures described in footnotes 2 and 3 have been successfully repeated on a 10-fold smaller scale to allow the carrier-free synthesis of [3,5-^{3}H]PAPB and prostaglandin ester **4** at a specific activity of 50 Ci/mmol.

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[3] [3,5-^{3}H]PAPB (**3**; 4 mg, 16 mmol, 80 mCi) was treated with a solution of PGD_2 (10 mg, 28 mmol) in THF (0.50 mL) and diisopropylethylamine (5 μL, 29 mmol). The solution was stirred overnight at room temperature, the solvent was evaporated and the residue was purified by flash chromatography (1 × 20 cm column packed with 6 cm of silica; eluent, 1:1 ethyl acetate/hexane) to give PGD_2 4-azido[3,5-^{3}H]phenacyl ester **5** [4.8 mg, 45 mCi, 4.8 Ci/mmol]. PGE_2 4-azido[3,5-^{3}H]phenacyl ester **4** was synthesized by using the same procedure except the chromatography eluent was 4:1 ethyl acetate/hexane.

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