RADIOIODODESTANNYLATION. CONVENIENT SYNTHESIS OF A HIGH AFFINITY THROMBOXANE A_2/PROSTAGLANDIN H_2 RECEPTOR ANTAGONIST.

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SUMMARY

Radioiodination of methyl-7-[(2R, 2S, 3S, 5R)-6,6-dimethyl-3-(4-trimethylstannylbenzenesulfonylamino) bicyclo[3.1.1]hept-2-yl]-5(Z)-heptenoate with [^{125}I] Na using a modification of the chloramine-T method in organic solvent is simple with high yields and site specific. The product, following hydrolysis of the ester, 7-[(2R, 2S, 3S, 5R)-6,6-dimethyl-3-(4-[^{125}I]-iodobenzenesulfonylamino) bicyclo[3.1.1]hept-2-yl]-5(Z)-heptenoic acid ([^{125}I]-ISAP), was purified by HPLC. The high specific activity and specific binding will make the ligand a useful tool for the characterization of thromboxane A_2/prostaglandin H_2 receptors.

Key words: [^{125}I]-ISAP, Thromboxane A_2/Prostaglandlin H_2 receptors, electrophilic destannylation.

INTRODUCTION

Since 1975 when the structure of unstable thromboxane A_2 (TXA_2), 1, was proposed (1), a variety of stable mimetics and antagonists have been prepared and utilized for the pharmacological study of thromboxane A_2/prostaglandin H_2 (TXA_2/PGH_2) receptors in many tissues (2). In addition, a number of tritiated and radioiodinated analogs have been synthesized and used in

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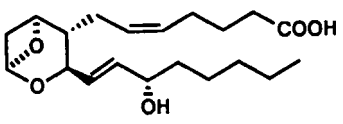
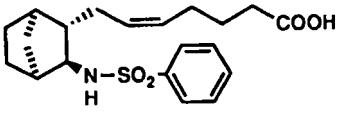
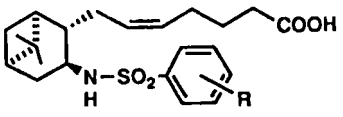
radioligand binding assays for the characterization and purification of this receptor (3-8). One of these ligands [^{3}H]S145, 2, has been shown to bind to the TXA_2/PGH_2 receptor with high affinity (0.5-3nM depending on tissue type)(6,8). However, the greater specific activity of [^{125}I] (2200 Ci/mmole) compared to [^{3}H] (20 Ci/mmole) provides a greater advantage in systems where receptor density is low and/or tissue protein is limiting. We set out therefore, to synthesize the iodinated pinane derivative, 4, (I-SAP). The pinane bicyclic nucleus was chosen in preference to the norbornyl system of S-145 because optically active starting materials are readily available and result in the synthesis of enantiomerically pure analogs. This negates the necessity of a stereoselective synthesis and/or a resolution step as is required for 2(9).



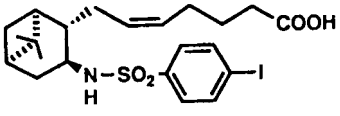
1 TXA_2



2 S-145



3a R=4-NH_2
3b R=3-NH_2



4 I-SAP

Nonradiolabelled I-SAP was shown to possess high affinity for the TXA_2/PGH_2 receptor. A K_d value of 142 ± 38 pM (N=3) was obtained in solubilized platelet membranes (10). In order to evaluate the potential use of the [^{125}I]labelled isotope of 4 as a probe to the TXA_2/PGH_2 receptor, we synthesized the intermediate amine 3a and performed a Sandmeyer reaction in order to substitute [^{125}I] for the amino group. Using various catalysts however, we found the yields to be very poor (2-10%) and highly variable. In addition, we made the 3-substituted amine, 3b, and following radioiodination, were unable to effect the removal of the amine by standard dediazotization procedures, a procedure that has worked well in the synthesis of a radioiodinated TXA_2/PGH_2 mimetic (7). We sought therefore an alternative route to [^{125}I]-ISAP.

Electrophilic destannylation (11,12) offers several distinct advantages for the introduction of a radiolabel: 1) The labelling procedure may be performed in the last step under very mild conditions and with a very high degree of site selectively 2) The requisite tin atom may be appended at any point in the synthesis 3) The approach should be quite general with respect to the radiolabel and the substrate. This communication describes a convenient method for introduction of [^{125} I] into a novel TXA_2 receptor antagonist to yield high specific activity and specific binding.

EXPERIMENTAL

Figure 1 shows the synthetic route and intermediates leading to I-SAP and [^{125}I]-ISAP. The synthesis of amine 5 was carried out as described previously (13). The structure of all compounds and intermediates was confirmed by ['H] NMR spectroscopy and direct chemical ionization mass spectroscopy (DCI-MS). The purity was checked by both thin layer chromatography in silica gel plates using chloroform-methanol (9.5:0.5) as the mobile phase and HPLC at 254nm on a Whatman

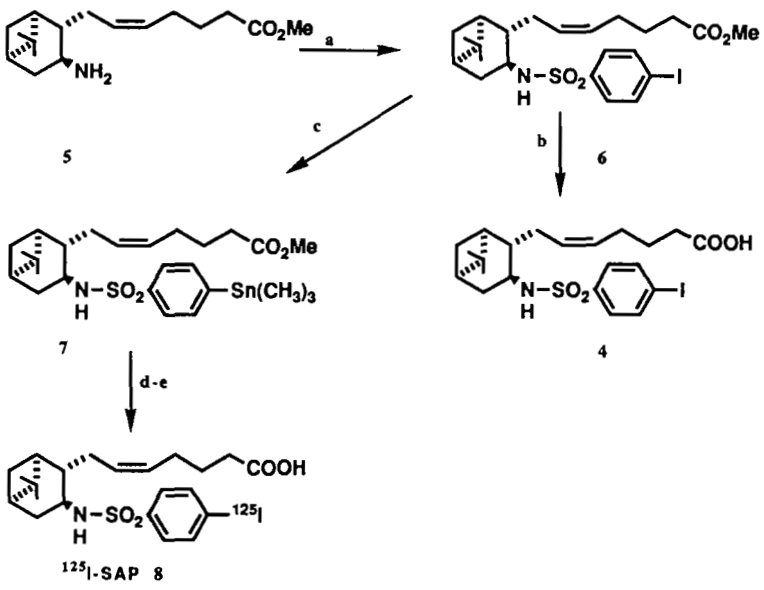


FIGURE 1. Synthetic route to I-SAP and [^{125}I]-ISAP. a) pipsyl chloride, toluene, triethylamine b) THF-0.2M LiOH (1:1). c) hexamethylditin, $Pd(Ph_3P)_4$, dry dioxane-reflux 2hr. d) MeOH, ^{125}INa Chloramine-T e) THF-2M LiOH (1:1) 1hr.

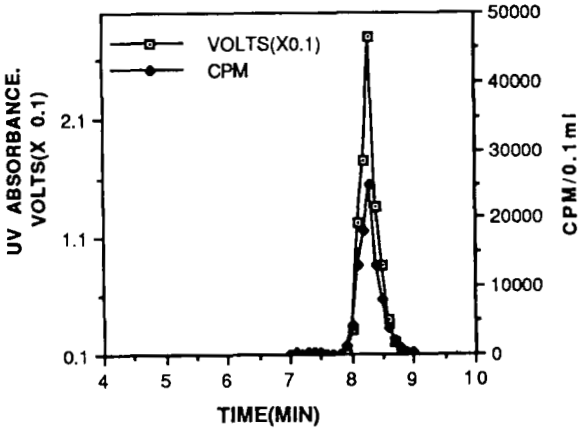


FIGURE 2. HPLC chromatogram of I-SAP and radioiodinated I-SAP. See experimental for details.

partisil-5 ODS-3 reverse phase column utilizing 68% MeOH-32% 0.1M ammonium acetate as the mobile phase at a flow rate of 1ml per minute. The final [^{125}I]-ISAP was purified by HPLC using the above described column conditions. Figure 2 shows an HPLC chromatogram of I-SAP authentic standard in conjunction with the elution profile of radioiodinated I-SAP.

Methyl-7-[(2R,2S,3S,5R)-6,6-dimethyl-3-(4-iodobenzenesulfonylamino)-bicyclo[3.1.1]hept-2-yl]-5(Z)-heptenoate 6

Amine methyl ester 5 (50 mg, 180 umoles) was dissolved in 2 ml of dry toluene and 1.2 equivalents of triethylamine added. To this mixture was added 59 mg (200 umoles) of pipsyl chloride and the solution was stirred overnight at room temperature. The toluene was evaporated under a nitrogen stream and the residue taken up in chloroform-methanol (9.5:0.5) and flash chromatographed to yield 84% of a glass solid.DCI-MS:m/z $546(M+H)^+$,$563(M+NH_4)^+$,$420(M+H-I)^+$.

Methyl-7-[(2R,2S,3S,5R)-6,6-dimethyl-3-(4-trimethylstannylbenzenesulfonylamino) bicyclo[3.1.1]hept-2-yl]-5(Z)-heptenoate 7

To a solution of 6 (25 mg, 46 umoles) in dry dioxane (2 ml) was added hexamethylditin (7.5 mg, 50 umoles) and 2 mg. (3 mole %) of $Pd(PPh_3)_4$. The reaction was refluxed for 3 hours, cooled to room temperature, filtered through celite and the residue evaporated to dryness under a nitrogen stream. Flash chromatagraphy was carried out with silica gel permeated with pyridine and eluted with chloroform-methanol (9.5:0.5) to give 62% yield of the trimethyltin derivative 7. DCI-MS: m/z $584(M+H)^+$ $601(M+NH_4)^+$, $520(M+H-Sn(CH_3)_3)^+$. This product was dissolved in dry hexane at a concentration of 2 mM and stored at -20°C under an argon atmosphere. This served as the precursor for introduction of [^{125}I].

7-[(2R, 2S, 3S, 5R)-6,6-dimethyl-3-(4-iodobenzenesulfonylamino) bicyclo[3.1.1]hept-2-yl]-5(Z)-heptenoic acid (I-SAP) 4

10 mg (18 umoles) of methyl ester 6 was quantitatively converted to the free acid 4 by THF:0.2N LiOH (1:1) at room temperature for 15 hours. DCI-MS: m/z $532(M+H)^+$, $549(M+NH_4)^+$, $406(M+H-I)^+$.

7-[(2R, 2S, 3S, 5R)-6,6-dimethyl-3-(4-[^{125}I]-iodobenzenesulfonylamino) bicyclo[3.1.1]hept-2-yl]-5(Z)-heptenoic acid ([^{125}I]-ISAP) 8

2 nmoles of 7 was evaporated under argon and 25 ul of MeOH added. To this was added 1 mCi [^{125}I]-NaI followed by 10ul of chloramine-T dissolved in 200 mM phosphate buffer at pH 7.5 (5 mg chloramine-T/ml). After 4 min, 10 ul of THF and 10 ul of 2N LiOH (in water) was added. The hydrolysis of the methyl ester was complete after 1 hr. The reaction mixture was injected onto a Whatman ODS-3 reverse column and the product eluated with 68% MeOH-32% 0.1M NH4Ac. Under these conditions, the product eluted at 9-10 minutes and co-migrated with I-SAP by HPLC and TLC. The yield based on starting [^{125}I] was 69 +/- 5 % (N=4). A theoretical specific activity of 2200 Ci/mmole can be assigned since [^{125}I]-ISAP can be seperated from starting material.

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