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Improved synthesis of (5*Z*)-7-(3-endo-{(benzenesulfonamido)-bicyclo[2.2.1]heptyl}hept-5-enoic acid (S-145) derivatives and their iodine-125-labeled radioligands for the study of thromboxane A_2 receptor

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An improved synthetic scheme for (5*Z*)-7-{3-endo-[(benzenesulfonamido)-bicyclo[2.2.1]heptyl}-hept-5-enoic acid (S145) and its analogs has been designed. The procedure involves direct sulfonylation of 2-allyl-3-aminobicyclo[2.2.1]heptane intermediate followed by ozonolysis and addition of a C5 carboxyl unit. The yield of the final product was significantly improved. (5*Z*)-7-{3-endo-[(4-iodobenzensulfonamido)-bicyclo [2.2.1]heptyl}hept-5-enoic acid (HS-145) and (5*Z*)-7-{3-endo-[(4-hydroxy-benzensulfonamido)-bicyclo [2.2.1]heptyl}hept-5-enoic acid (HS-145) were synthesized directly without any protection and deprotection steps. [^{125}I](5*Z*)-7-{3-endo-[(4-iodobenzensulfonamido)- bicyclo[2.2.1]heptyl}hept-5-enoic acid ([^{125}I]HS-145) was prepared from IS-145 through an organotin intermediate and [^{125}I]sodium iodide with high specific radioactivity and good recovery of radioactivity. [^{125}I](5*Z*)-7-{3-endo-[(4-hydroxy 3-iodo-benzensulfonamido)-bicyclo[2.2.1]-heptyl}hept-5-enoic acid ([^{125}I]HS-145) was prepared by direct iodination with sodium iodide using a modified chloramine-T method. Both [^{125}I]HS-145 and [^{125}I]HS-145 were found to be valuable radioligands for studying thromboxane A_2 (TXA_2) receptor.

Key words: thromboxane; receptor

Introduction

Thromboxane A_2 (TXA_2) is known to be a potent inducer of platelet aggregation and vasoconstriction [1,2]. Its action is believed to be mediated by a specific receptor. Because of an extreme instability of thromboxane A_2, a variety of stable agonists and antagonists have been synthesized for studying the receptor. These include, but are not limited to, U46,619 [3] and I-BOP [4] for agonists and SQ29,548 [5], PTA_2 [6], 13-azaprostanoic acid [7] and S-145 [8] for antagonists. Among these ligands S-145 appears to have specifically high potency without any species differences in the affinity with thromboxane A_2 receptor [9]. Radiolabeled [^{3}H]S-145 and [^{125}I]HS-145 were found to be particularly valuable for studying thromboxane A_2 receptor because of low non-specific binding and high bind-

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Abbreviations: U46,619, 15(*S*)-hydroxy-11,9-epoxymethano-prosta-5*Z*, 13*E*-dienoic acid; PTA_2, 15(*S*)-hydroxy-11-dimethylmethano-11,12-methano-prosta-5*Z*,13*E*-dienoic acid; SQ29,548, [1*S*-[1α,2β(5*Z*),3β,4α]-7-[3-[[2-[(phenylamino)-carbonyl]hydrazino] methyl]-7-oxabicyclo-[2.2.1]hept-2-yl]-5-heptenoic acid; SQ28,668, [1*S*-[1α,2β(5*Z*),3β(1*E*,3*R*,4*S*), 4α]]-7-[3-(3-hydroxy-4-phenyl-1-pentenylo)-7-oxabicyclo-[2.2.1]-hept-2-yl]-5-heptenoic acid; SQ29,952 [1*S*-[1α,2β(5*Z*), 3β-(1*E*,3*R*,4*S*), 4α]]-7-[-3-3-hydroxy-4-(4-hydroxyphenyl)-1-pentenyl)-7-oxabicyclo-[2.2.1]hept-2-yl]-5-heptenoic acid; *S*-145, (5*Z*)-7-[3-endo-[(benzenesulfonamido)-bicyclo[2.2.1]-heptyl}-hept-5-enoic acid; HS-145, (5*Z*)-7-{3-endo-[(4-hydroxy-benzenesulfonamido)-bicyclo[2.2.1]-heptyl}-hept-5-enoic acid; IS-145, (5*Z*)-7-(3-endo-[(4-iodo-benzenesulfon-amido)-bicyclo-[2.2.1]-heptyl}-hept-5-enoic acid.

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ing affinity with various receptor preparations [10]. Although the synthesis of S-145 and its analogs have been reported [8], we have improved the synthetic schemes and achieved better yield of the final products. Furthermore, we would like to report the preparation of a novel radioligand, [^{125}I]IS-145 that is particularly useful for studying thromboxane A_2 receptor.

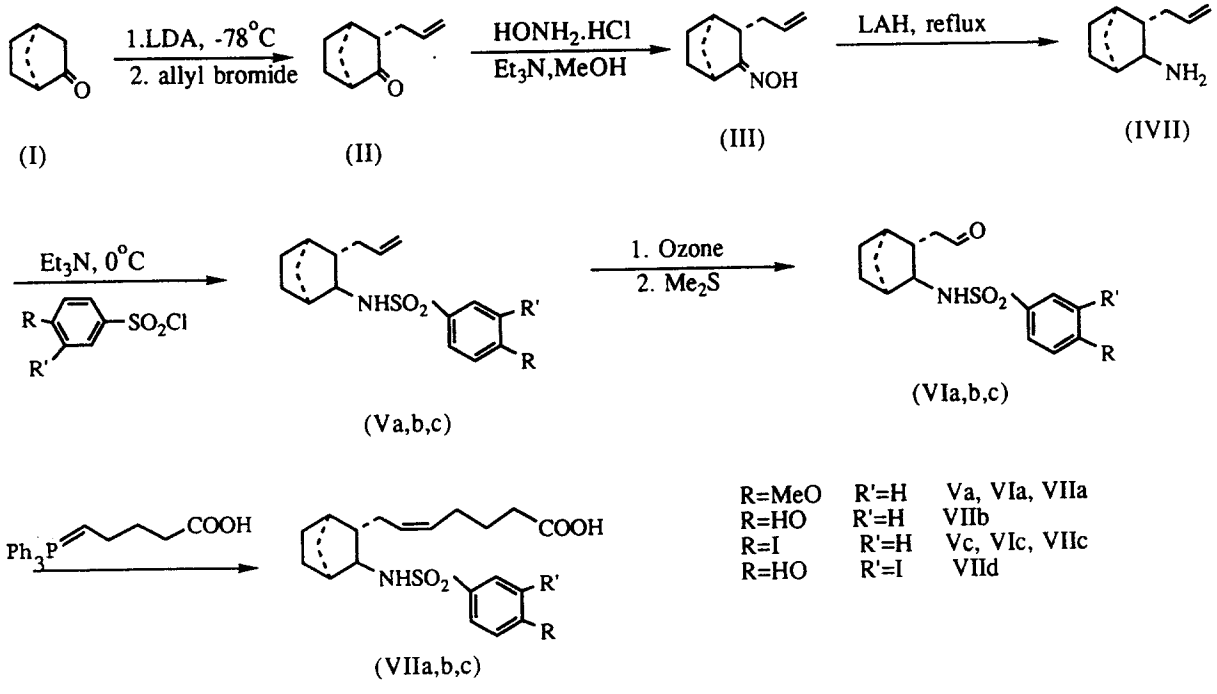
Experimental Procedure

Materials

Norcamphor, lithium diisopropyl amide, carboxy butylthphenylphosphonium bromide, lithium aluminum hydride, diazald, pipsyl chloride, sulfanilyl chloride, *p*-methoxybenzenesulfonyl chloride, di-*t*-butylpyrocarbonate, chloramine-T, thallium (III) trifluoroacetate, hexamethylditin, tetrakis(triphenyl)phosphine, palladium (0), ammonia and deuterated solvents were purchased from Aldrich. SQ29,548, SQ28,668 and SQ29,952 were gifts from Squibb. U46,619 and PTA_2 were purchased from Cayman Chemical Co. [^{3}H]SQ29,548 and [^{125}I]sodium iodide were obtained from DuPont-NEN. Human platelets were obtained from human volunteers. Whatman GF/C filters glass filter was supplied by Fisher. Phenylmethylsulfonyl chloride and indomethacin were obtained from Sigma.

Preparation of (5Z)-7-[3-endo-[(4-iodobenzensulfonamido)-bicyclo[2.2.1]-heptyl]- hept-5-enoic acid: (Scheme 1)

Synthesis of 2-Allylnorcamphor (**II**). Norcamphor (I) (1.1 g) was dissolved in 50 ml of anhydrous tetrahydrofuran. The vessel was then flushed with nitrogen. The mixture was cooled to -78°C. One equivalent of 1.5 M lithium diisopropylamide tetrahydrofuran complex in *n*-hexane was added dropwise. The reaction mixture was stirred at the same temperature for another hour. Two equivalents of allyl bromide were added dropwise and it was allowed to warm up to room temperature. To the mixture 50 ml cold 1 M HCl was added and stirred for 20 min. The organic layer was washed with saturated brine and dried. Evaporation left the crude product that was pure enough for the following reaction. Analytical sam-



Scheme 1. Synthetic route to HS145 and analogs.

ple was obtained by column chromatography with silica gel using a 0–5% acetone-chloroform gradient. The desired product (1.24 g) was obtained.

I.R. ($CHCl_3$) cm^{-1}: 3460, 3080, 2960, 2880, 1780, 1680, 1440, 1080, 995, 910. NMR ($CDCl_3$): d 1.38–1.70 (m, 4H), 1.75–2.00 (m, 4H), 2.38–2.60 (m, 3H), 5.02 (m, 2H), 5.80 (m, 1H).

Synthesis of 2-exo-allyl-2(E,Z)-hydroxyiminobicyclo[2.2.1]-heptane (**III**). Three grams of allylnorcamphor (**II**) was dissolved in 50 ml of anhydrous methanol and 2.88 g of hydroxylamine hydrochloride was added to the solution. Three milliliters of triethylamine was added dropwise as the exothermic reaction of the mixture took place. The reaction was allowed to continue for another hour and was then evaporated under vacuum. The residue was dissolved in 50 ml chloroform and washed three times with 50 ml of distilled water. The organic layer was dried with magnesium sulfate and evaporated. Column chromatography with silica gel using 0–10% ethyl acetate in *n*-hexane as eluant yielded a mixture of the oximes (**III**). *Trans*- and *cis*-isomers could be separated with preparative thin-layer chromatography on silica gel plate with 5% ethyl acetate *n*-hexane and developed three times. The combined yield of *trans*- and *cis*-isomers was 2.89 g.

E-isomer (less polar fraction)

IR (neat): 3590, 3280, 3140, 3075, 1675, 1635 cm^{-1}. ^{1}H-NMR ($CHCl_3$): d 1.00–1.90 (m, 7H), d 1.91–2.65 (m, 4H), d 3.52 (br. s, 1H), d 4.86–5.25 (m, 2H), d 5.61–6.17 (m, 1H).

Z-isomer (more polar fraction)

IR (neat): 3590, 3280, 3140, 3085, 1678, 1641. ^{1}H-NMR ($CDCl_3$): d 1.12–2.01 (m, 7H), d 2.12–3.20 (m, 5H), d 4.90–5.22 (m, 2H) d 5.60–6.12 (m, 1H).

Synthesis of 2-allyl-3-[4-iodophenylsulfamido]-bicyclo[2.2.1]-heptane (V_c). The mixture of oximes (**III**) (1.5 g) was dissolved in 50 ml of tetrahydrofuran and cooled to 0°C. Fifteen milliliters of 1 M lithium aluminum hydride in tetrahydrofuran was added dropwise under vigorous stirring. The resulting mixture was stirred for 4 h and then refluxed overnight. The excessive reagent was destroyed carefully by adding 5 ml of ethyl acetate dropwise and stirring for 30 min. Saturated sodium sulfate solution was added dropwise until precipitation of the inorganic salts ceased. To the resulting slurry, 20 g of anhydrous magnesium sulfate was added. The solution was filtered and the solid cake was washed three times with 30 ml of ethyl acetate. The organic solvent was evaporated and crude amine (**IV**) was obtained as a pale yellow oil, which showed positive ninhydrin test. It was used without further purification.

The amine was dissolved in 10 ml chloroform with the addition of 1.5 ml triethylamine. 1.1 equivalents of 4-iodobenzensulfonyl chloride were added. The mixture was stirred for 1 h at room temperature and 10 ml of 10% sodium bicarbonate was added. After an hour, the organic layer was separated and washed with 10 ml of 1 N HCl, then saturated brine. The organic layer was then dried and evaporated. Column chromatography with 0–10% acetone/chloroform mixture on silica gel yielded 56% of the intermediate sulfonamide (V_c).

IR (neat) cm^{-1}: 3380, 3080, 2930, 2850, 1560, 1350, 1170, 1000, 910. ^{1}H-NMR ($CDCl_3$): d 1.00–1.60 (m, 7H), 1.75–2.05 (m, 4H), 3.02 (dt 1H), 4.84 (m, 2H), 5.52 (m, 1H), 7.60 (m, 2H), 7.87 (m, 2H).

Wittig reaction and formation of (5Z)-7-[3-endo-[(4-iodobenzenesulfonamido)-bicyclo[2.2.1]-heptyl]-hept-5-enoic acid: (IS-145, **VII**$_c$). 2-Allyl-3-*p*-iodobenzenesulfonamido-norcamphor (**LIII**$_c$) (0.5 g) was dissolved in 50 ml of dichloromethane. The solution was cooled to -78°C in a dry ice-acetone bath. Ozone was passed through the mixture until a persistent blue color was observed. Dimethyl sulfide was added in excess to destroy the ozone and reduce the ozonide. The solution was evaporated at a high vacuum at less than 4°C in a water bath. 0.48 g (100%) of aldehyde (**LIV**$_c$) was obtained. The resulting aldehyde gave a single spot on TLC and a positive test with Purpald. It was used without further purification. IR (neat) cm^{-1}: 1680.

To 3 equivalents of carboxybutyltriphenylphosphonium bromide were added 20 ml of anhydrous tetrahydrofuran. The slurry was cooled to -78°C with a dry-ice-acetone bath. The flask

was then flushed with nitrogen. Six equivalents of potassium butoxide was added as a 1-M tetrahydrofuran solution. The solid gradually dissolved into an orange solution. The crude aldehyde in tetrahydrofuran was added dropwise. The mixture was stored in a -20°C freezer overnight. 50 ml of distilled water was added together with 30 ml of *n*-hexane. After stirring vigorously for 10 min, the organic layer was discarded and the aqueous layer was extracted with 50% ether in *n*-hexane. The aqueous phase was separated and acidified with 6 N hydrochloric acid to pH 3. The milky solution was extracted with ethyl acetate three times. The combined organic layer was dried and evaporated. The residue was then purified with column chromatography with 10% methanol in chloroform (with 1% formic acid) on a silica gel column. The purified products were esterified with diazomethane and analyzed with a C 18 column.

For the separation of *trans*- and *cis*-products, the methyl ester (from diazomethane) of the crude mixture was applied to a TLC plate and developed with 1% acetone in chloroform under continuous conditions for 6 h (until the two separated). The resulting methyl ester was hydrolyzed in a 14% ammonia water/methanol mixture. Pure ammonium salt of the product was obtained after evaporation. The yield was 46%.

IR (neat): 3500, 3380, 2500, 1712, 1154, 1102. ^{1}H-NMR (CD_3OD): d 0.85–2.50 (m, 15H), d 2.18 (t, J = 7, 1H), d 2.71 (td, J = 4,7 Hz, 1H), d 5.20 (m, 2H), d 5.69 (d, J = 7Hz, 1H), d 7.92–7.95 (td, 2H). ^{13}C NMR (CD_3OD): d 24.75, 26.62, 30.12, 32.52, 33.38, 35.11, 40.62, 40.87, 51.46, 55.24, 66.34, 128.64, 128.80, 128.98, 129.H, 129.11, 129.97, 156.12, 159.47, 174.07. MS-EI: *m/e* 503.0631 ($M^{+}\cdot$) (Calc $C_{20}H_{26}O_4NSI$ 503.0629).

Synthesis of (5Z)-7-[3-endo-[(4'-hydroxy-benzenesulfonamido)-bicyclo[2.2.1]-heptyl}-hept-5-enoic acid (HS-145, **VII$_b$**). (5Z)-7-[3-endo-[(4'-methoxybenzenesulfonamido)bicyclo[2.2.1]-heptyl}-hept-5-enoic acid was synthesized and converted to its corresponding methyl ester as above. The methyl ester (100 mg) was dissolved in 2 ml of dimethylsulfoxide and heated to 180°C for 16 h. The brownish solution was evaporated under high vacuum. The pure HS-145 was obtained by preparative TLC with silica gel and 10% methanol in chloroform with 1% formic acid. The yield was 58 mg.

IR (neat) cm^{-1}: 3550, 3400, 1715, 1160, 1091. ^{1}H-HMR (CD_3OD): d 1.02–2.24 (m, 17H), 2.87 (dt, 1H), 5.20 (m, 2H), 6.90 (d, 2H), 7.69 (d, 2H). ^{13}C-NMR (CD_3OD): d 24.75, 26.62, 30.12, 32.53, 33.38, 35.11, 40.62, 40.87, 51.46, 55.24, 66.34, 128.64, 128.80, 128.98, 129.11, 129.61, 129.97, 156.12, 159.47, 174.07. MS-FAB (glycerol): *m/e* 298 (M-PhOH$_2$) 40.9%, 392 (M-H-) 100%, 414 (M-H$_2$ + Na) 9.5%, 430 (M-H$_2$ + K. MS-EI: *m/e* 375.1507 (M-H$_2$O) (Calc. $C_{20}H_{25}O_4$ NS 375.1504)

Radioiodination of (5Z)-7-13-endo-[(4'-hydroxybenzenesulfonamido)-bicyclo[2.2.1]-heptyl}-hept-5-enoic acid (HS-145)

HS-145 (1 μg) was dissolved in 10 ml of 50 mM triethylamine hydrochloride solution (pH 9.0). One equivalent of chloramine-T in the same solution was added. [^{125}I]sodium iodide (10 μl) was added (10 μCi). The solution was sealed and placed in the dark overnight. Saturated sodium metabisulfite solution (1 ml) was added to stop the reaction. The solution was evaporated under a stream of nitrogen. The residue was extracted three times with 50 ml of methanol. The methanol extract was spotted on a TLC plate and developed with 10% methanol in chloroform with 1% formic acid. The product (**VII$_d$**) was located by autoradiography. 5.2 μCi of radioactivity were recovered, which co-migrated with the corresponding iodine-127 product (R_f 0.46).

^{1}H-NMR of [^{127}I]-HS145 (CD_3OD): d 1.20–2.70 (m, 17H), 3.08 (dt, 1H), 5.33 (m, 2H), 7.04 (d, 1H), 7.84 (d, 1H).

Radioiodination of (5Z)-7-[3-endo-[(4-iodobenzenesulfonamido)-bicyclo[2.2.1]-heptyl}-hept-5-enoic acid (Scheme 2)

IS-145 (1 mg) was dissolved in 1 ml of dioxane. One equivalent each of tetrakis(triphenylphosphine) palladium (0) and hexamethylditin were added. After refluxing under nitrogen for 2 h, the solution was evaporated and redissolved in 1 ml of

ethyl acetate. The solution was filtered with 5 g of celite. The celite pad was washed again with 10 ml of ethyl acetate. The organic layer was evaporated and redissolved in 1 ml of methanol. No attempt was made to purify the unstable organo-tin intermediate. Ten microliters of the solution was allowed to react with 1.1 equivalents of chloramine-T and 50 μCi of sodium [^{125}I]iodide at room temperature for 5 min. Fifty microliters of 1 N sodium hydroxide in methanol was added. The mixture was kept at room temperature for 4 h. The solution was applied to a silica gel TLC plate and developed with 10% methanol in chloroform with 1% formic acid. The radioactive band corresponding to the R_f value of IS-145 was collected. The yield was typically around 50% based on the recovery of radioactivity. MS-DCI of organotin intermediate: *m/z* 556 $(M + H)^+$, 492 $(M + H - Sn(CH_3)_3)^+$.

Preparation of human platelet suspension

Human venous blood (30 ml) was collected into one-tenth the volume of 3.8% of sodium citrate solution in a 50-ml plastic tube with cap. The platelet rich plasma (PRP) was prepared by centrifugation at 180 × *g* at room temperature for 10 min. Three milliliters of ACD (85 mM trisodium citrate, 111 mM dextrose and 71 mM citric acid) was added to the PRP to prevent platelet aggregation during manipulation. Platelets were then pelleted from PRP by centrifugation at 800 × *g* at room temperature for 10 min. The platelets were washed once with TES buffer containing 10 mM Tris–HCl, 1 mM EDTA and normal saline (pH 7.5) and were finally suspended in a proper volume of calcium and magnesium free Tyrode buffer.

Preparation of human platelet membranes

Platelets were disrupted by adding 3 vols. of hypotonic buffer (10 mM Tris–HCl (pH 7.4), 10 μM indomethacin, 50 mg/ml phenylmethylsulfonyl fluoride) and left standing on ice for 10 min. The mixture was then sonicated four times 10 s each with an ultrasonicator at a setting of 4. The homogenate was centrifuged at 100 000 × *g* for 30 min at 4°C. The pellet was resuspended in ice-cold 10 mM Tris–HCl (pH 7.4) buffer at a concentration of 15–25 mg protein/ml and frozen at -80°C until used.

Receptor binding studies

Ligand binding studies were conducted in 25 mM Tris–HCl (pH 7.4) buffer with 5 mM $CaCl_2$. Normally 100 μg of human platelet membranes and 0.1 nM (2000 Ci/mmol) of ^{125}I-labelled ligands were used per assay. Unlabeled compounds were added in various concentrations. The mixture was incubated at room temperature for 45 min. The reaction was terminated with the addition of 5 ml of ice-cold washing buffer (25 mM Tris–HCl, pH 7.4) and the solution was filtered under vacuum through a Whatman GF/C fiber glass filter. The filter was then washed twice with 5 ml washing buffer quickly. The radioactivity retained on the filter was counted with 10 ml of scintillation cocktail.

Results and Discussion

Synthesis of S-145 derivatives is shown in Scheme I. Significant modification of a previous procedure [8] was made. Norcamphor (**I**) was used as the starting material as before since the upper side chain could be easily constructed by adding a C2 unit and then a C5 unit. The lower sulfonamide could be easily prepared by simple functional group transformation. Protection and deprotection steps were avoided so to improve the yield and shortened the synthetic route.

Readily available allyl-amine (**IV**) was obtained from norcamphor as reported [8] with some modifications. Purification of the amine (**IV**) was difficult. However, direct sulfonamide formation with the crude amine gave a high yield of (**V**). Instead of consequential two-step epoxide formation and periodic acid oxidation of the allyl sulfonamide (**V**) [8], a more efficient one-step method of aldehyde (**V**) was searched for. Ozonolysis of the resulting sulfonamide gave 100% crude aldehyde (**VI**). It did not involve any tedious purification steps. On the other hand, work up of the reaction mixture after periodic acid oxidation was not easy. The resulting aldehyde tended to decompose during extraction and concentration steps, which decreased the overall yield. Aldehyde

(VI_c) underwent Wittig's reaction with a 78% yield of final product (VII_c) with *trans/cis* ratio about 1:5 as shown by HPLC analysis. The *trans*-isomer could be minimized by running the reaction at -25°C.

The synthesis of IS-145 was very efficient with about 20% overall yield in only six steps. The structure of the compound was verified by proton magnetic resonance spectrography and mass spectroscopy. The *cis* conformation of the double bond was easily verified by ^{1}H-NMR by its characteristic coupling constant. By judging the α-hydrogen of the amino group, the relationship of the phenylsulfonamide and heptenoic acid side chain was shown to be *cis*.

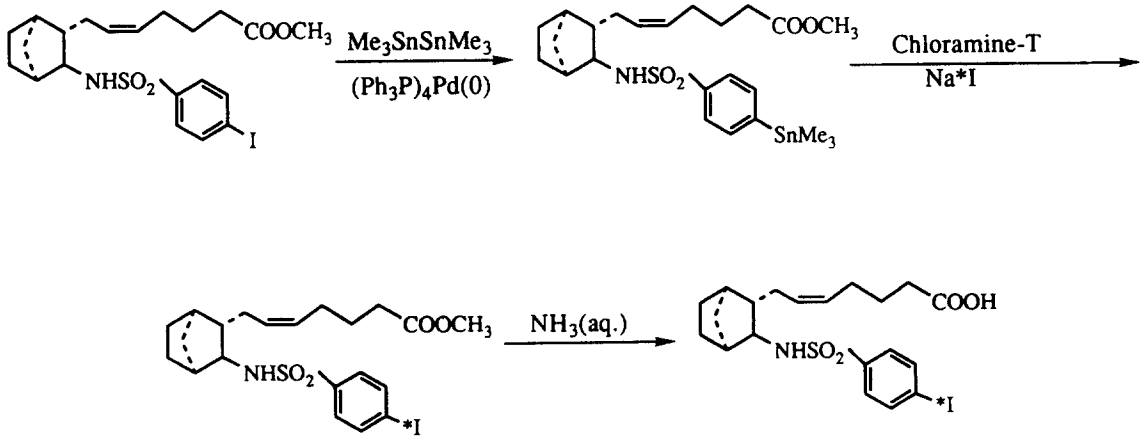
Synthesis of different analogs was generally carried out by using different sulfonyl chlorides for sulfonamide formation. For instance, HS-145 (VII_b) was synthesized using methoxybenzenesulfonyl chloride. The methoxy group of (VII_a) was demethoxylated by potassium cyanide in boiling dimethyl sulfoxide in 60% yield.

Two iodine-125-labeled thromboxane A_2 antagonists were synthesized for the characterization of the thromboxane A_2 receptor. The two compounds were [^{125}I]HS-145 (VII_d*) and [^{125}I]IS-145 (VII_c*) was also synthesized (Scheme II).

Radioiodination of HS-145 using traditional chloramine-T in buffered solution resulted in only 5% yield of the labeled compound. Peracetic acid, thallium(III) trifluoroacetate and enzymatic methods were found to be unsatisfactory. However, radio-iodination with one equivalent of chloramine-T in a pH 9 triethylamine hydrogen chloride buffer with sodium [^{125}I]-iodide for 16 h, yielded the target compound in 70% yield.

In the synthesis of radioactive IS-145, we also encountered some problems. At the beginning, we planned to use the (5*Z*)-7-{3-endo-[(4-aminoenzenesulfonamido)-bicyclo[2.2.1]- heptyl}-hept-5-enoic acid as the starting material. Through diazotization and displacement of diazo group by iodide, the desired ligand might be obtained. However, in practice, [^{125}I]IS-145 was isolated only in 2% yield after tedious purification. In order to overcome this problem, we looked into the possibility of substituting organometallic group with the iodine-125 atom. The trimethyltin group was particularly attractive. The organometallic intermediate can be obtained from the methyl ester of IS-145 under the action of tetrakis(triphenylphosphine)-palladium (0) and hexamethylditin [11,12]. The unstable intermediate then reacted with iodosonium ion to yield [^{125}I]IS-145 as the sole product in 50% yield after hydrolysis.

The failure of the diazotization-substitution approach was thought to arise from the acid labile double bond. As reported by Mais et al. [12], diazotization led to the formation of cyclic lactone on the upper side chain when they attempted to



Scheme 2. Radioiodination of IS145 via organotin intermediate.

generate an azido group from aniline group of a very similar compound (a pinane derivative of (5Z)-7-{3-endo-[(4-amino- benzenesulfonamido)-bicyclo[2.2.1]-heptyl}-hept-5-enoic acid). It was possible that the same case applied here. It was reported that the ratio of nitrous acid to the aniline was very crucial in a diazotization reaction with an optimum ratio of about 2:1 [13]. At high dilution reaction like ours, it was almost impossible to control the volatile nitrous acid level in any meaningful way. The present method allowed selective iodination at the *para* position with a high yield.

In order to check the specificity of [^{125}I]IS-145, a very simple binding experiment was performed. As shown in Fig. 1, the binding of the ligand was shown to be saturable and obeyed general receptor ligand binding kinetics. It was revealed that 1.9 pmol of thromboxane A_2 receptor was presented per mg of human platelet membranes. The dissociation constant, K_D of the ligand was 46 nM, which was very similar to that obtained by the displacement study with [^{3}H]SQ29,548. The non-specific binding when used at 0.1 nM was typically 5% under our experimental conditions. The specific binding of the ligand was displaceable by a variety of structurally unrelated but well-studied thromboxane A_2 agonists and antagonists in a dose-dependent manner. (The IC_{50} values of these compounds were summarized in Table I.) Thus, it showed that it was very promising, with respect to its use in the characterization of the receptor.

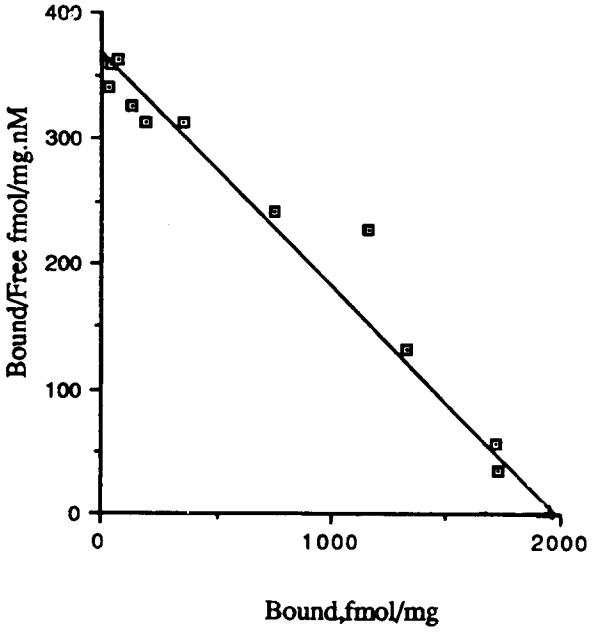


Fig. 1. Scatchard analysis of [^{125}I]IS-145 binding to human platelet membranes. Various concentrations of [^{125}I]IS-145 were incubated with human platelet membrane for 45 min at room temperature. The mixture was then filtered and retained radioactivity was determined as described in Materials and Methods. Non-specific binding was taken as the retained radioactivity in the presence of 1 μM of [^{127}I]IS-145.

Similarly, [^{125}I]HS-145 was prepared and used to characterize the receptor in human platelet membranes. Preparation of [^{125}I]HS-145 was previously reported [10]. We employed both labeled ligands to examine the specificity of the binding. Agonists like U46,619 or antagonist like SQ29,548 displaced the binding completely in a dose dependent manner. The K_I values obtained by calculation from the corresponding IC_{50} values was shown to be in close agreement with published values as shown in Table I. We may conclude that both [^{125}I]IS-145 and [^{125}I]HS-145 are very selective and highly potent radioactive ligands useful for the characterization of the thromboxane A_2 receptor. The characterization of the pharmacological properties of IS-145 will be described elsewhere [14].

TABLE I

IC_{50} values (nM) of thromboxane A_2 analogs against [^{125}I]IS-145 and [^{125}I]IS-145 for receptor binding in human platelet membranes

Ligands	[^{125}I]IS-145	[^{125}I]HS-145
S145	0.9	1.5
HS-145	1.4	1.1
IS-145	6.4	5.8
SQ29,548	26.4	20.5
SQ29,952	181.7	203.4
SQ28,668	706.2	650.7
PTA_2	435.3	343.8
U46,619	334.4	216.7

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