SYNTHESIS OF AN [^{125}I]-LABELLED DERIVATIVE OF MK-571, A TOOL FOR LTD$_4$ RECEPTOR STUDIES

Chun-Sing Li*, Yves Leblanc, Robert Zamboni and Robert N. Young

Merck Frosst Centre for Therapeutic Research, P.O. Box 1005,
Pointe Claire - Dorval, Québec, Canada H9R 4P8, Canada

SUMMARY

*An [^{125}I]-labelled derivative of MK-571 **2** was synthesized from the arylstanne derivative **11** in 50% radiochemical yield. Compound **2** is a useful tool for LTD$_4$ receptor studies. The arylstanne intermediate **11** was obtained from the coupling reaction of (±)-3-[[[3-[2-(7-chloro-2-quinolinyl)-(E)-ethenyl]phenyl][[3-[(3-aminopropyl)amino]-3-oxopropyl]thio]methyl]thio]propionic acid **10** with N-hydroxysuccinimidyl 4-trimethylstannylphenylacetate **3**. The synthesis of **3** could be easily achieved from 4-bromophenethyl alcohol.*

Key Words: Iodine-125, [^{125}I]-labelled derivative of MK-571, N-hydroxysuccinimidyl 4-trimethylstannyl-phenylacetate, leukotriene D$_4$ antagonist.

INTRODUCTION

Recently, a number of clinical studies have demonstrated that MK-571, **1**, is a potent and orally active LTD$_4$ receptor antagonist in normal[1] and asthmatic men,[2] that **1** inhibits both antigen-induced early- and late-phase responses[3] and exercise-induced bronchoconstriction in asthmatic patients,[4] and that **1** improves FEV$_1$ and symptom scores and reduces ß agonist usage in a 6-week study[5] in asthmatic subjects. The mechanism of action of this drug is related to its capacity to compete with leukotriene D$_4$ (LTD$_4$) for its receptor.[6] The high affinity of this drug for the LTD$_4$ receptor (IC$_{50}$ = 1 nM) makes it as an attractive tool to aid in the characterization of the LTD$_4$ receptor. A [^{35}S]-labelled MK-571 has been previously prepared for the receptor

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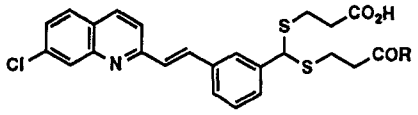
binding assay studies.[7] Herein, we describe the synthesis of a [^{125}I]-labelled analogue **2** (IC_{50} = 7nM), which would be suitable as a probe for the receptor isolation and studies.

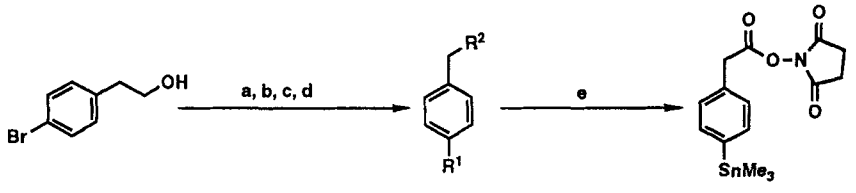


1 : R = NMe_2
2 : R = $-NH(CH_2)_3NHCOCH_2$-*p*-^{125}I-Ph

RESULTS AND DISCUSSION

For the preparation of the [^{125}I]-labelled compound **2**, we took advantage of the known arylstanane chemistry.[8] Arylstananes can be readily prepared from aryl bromide and regiospecifically converted to aryliodides under very mild reaction conditions. The synthesis of N-hydroxysuccinimidyl 4-trimethylstannylphenylacetate **3** was achieved as following (Scheme 1). The commercial available 4-bromophenethyl alcohol **4** was used as starting material

Scheme 1



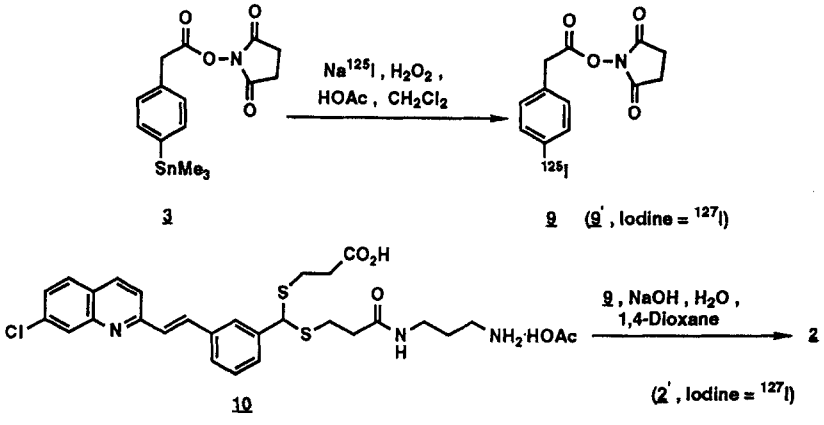
4 **5** : R^1 = Br ; R^2 = $-CH_2OSiMe_2t$-Bu **3**
6 : R^1 = $-SnMe_3$; R^2 = $-CH_2OSiMe_2t$-Bu
7 : R^1 = $-SnMe_3$; R^2 = $-CH_2OH$
8 : R^1 = $-SnMe_3$; R^2 = $-CO_2H$

(a) t-$BuMe_2SiCl$; (b) Mg ; then Me_3SnCl ; (c) n-Bu_4NF , 79% from **4** ;
(d) Jones' reagent , 79% ; (e) DCC , N-Hydroxysuccinimide , 65% .

and the hydroxyl group was protected as a tert-butyldimethylsilyl ether **5**. The Grignard reagent derived from **5** was submitted to the stannylation condition (Me_3SnCl) to provide the arylstannyl intermediate **6**. Deprotection of the silyl group with tetra-butylammonium fluoride (78% from **4**) followed by Jones oxidation gave the desired 4-trimethylstannylphenylacetic acid **8** (79%). Esterification with N-hydroxysuccinimide then afforded the activated ester **3** in 65% yield.

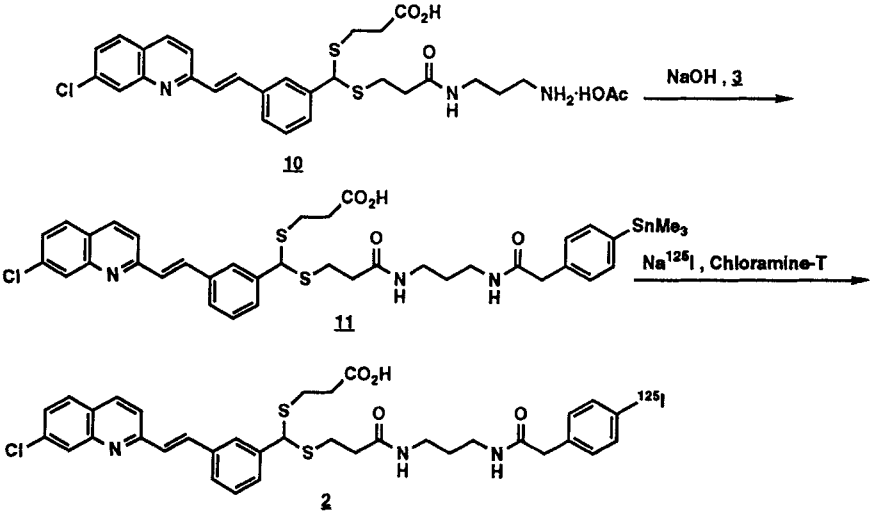
With the activated ester 3 on hand, two synthetic routes were considered for the synthesis of compound 2. The initial one (Scheme 2) was the radioiodination of 3 followed by direct coupling of the radioiodinated activated ester 9 with the amino side arm of 10.[9] This approach was suitable for avoiding the oxidative conditions for the radioiodination reaction, which might alter the dithioacetal linkage. However, the preparation of 9 from 3 under prolonged reaction time could lead to substantial hydrolysis of the activated ester. Although we were able

Scheme 2



to prepare the cold non-radioactive iodinated compound in this fashion, we found this approach unreliable for the production of the radiolabelled compound. Therefore, an alternative route (Scheme 3) was investigated.

Scheme 3



The amine **10** was coupled with the activated ester **3** to give the trimethylstannyl substituted MK-571 analogue **11**. It was our delight to find out that **11** smoothly underwent radioiodination with sodium [^{125}I] iodide and chloramine-T in DMF. The dithioacetal linkage was surprisingly stable under the reaction conditions. Purification by reverse phase HPLC then furnished the [^{125}I]-labelled compound **2** in 50% radio-chemical yield.

EXPERIMENTAL

Proton magnetic resonance spectra were obtained on a Bruker EM 250 or AM-300 instrument using the solvent indicated and tetramethylsilane as the internal standard. FAB mass spectra were obtained on a VG-ZAB-2F-HS mass spectrometer.

1-[(tert-Butyldimethylsilyl)oxy]-2-(4-bromophenyl)ethane (5)

A mixture of 4-bromophenethyl alcohol (8.8 g, 43.8 mmol) tert-butyldimethylsilyl chloride (7.2 g, 47.8 mmol), imidazole (4.5 g, 66.2 mmol) and 4-dimethylaminopyridine (100 mg) in DMF (50 mL) was stirred for 2 h, then diluted with water (200 mL) and extracted with diethyl ether (300 mL). The ethereal extract was washed with water (2x), dried (anhy. $MgSO_4$) and concentrated to yield the silylated product (12.9 g, 94%); ^{1}H NMR ($CDCl_3$) δ 7.40 (d, 2H, J=7.5Hz), 7.10 (d, 2H, J=7.5Hz), 3.80 (t, 2H, J=6.5Hz), 2.78 (t, 2H, J=6.5Hz), 0.88 (s, 9H), 0.00 (s, 6H).

1-[(tert-Butyldimethylsilyl)oxy]-2-(4-trimethylstannylphenyl)ethane (6)

To magnesium (1.0 g, 41.7 mmol) in THF (10 mL) with a small iodine crystal was added the aryl bromide **5** (~0.5 g). The reaction was started with the heating with a heat gun. More THF (40 mL) was added in one portion, the remaining aryl bromide (10.0 g, total 10.5 g; 33.3 mmol) was added dropwise over a period of ~15 min. After the addition was completed, the

mixture was refluxing for 15 min, then cooled to 0°C and trimethyltin chloride (7.0 g, 35.1 mmol) in THF (10 mL) was added. The mixture was stirred at room temperature for 30 min, quenched with water (160 mL) and extracted with diethyl ether (200 mL). The ethereal extract was washed with brine, dried (anhydrous $MgSO_4$) and concentrated. Chromatography over silica gel and elution with 3% EtOAc in hexanes gave 12.5 g (94%) of partially purified aryl tin product **6**; ^1H NMR ($CDCl_3$) δ 7.40 (d, 2H, J=5.5Hz), 7.20 (d, 2H, J=5.5Hz), 3.80 (t, 2H, J=6.5Hz), 2.80 (t, 2H, J=6.5Hz), 0.90 (s, 9H), 0.30 (s, 9H), 0.00 (s, 6H).

N-Hydroxysuccinimidyl 4-trimethylstannylphenylacetate (3)

A mixture of arylstanane **6** (3.5 g, 8.8 mmol) and tetrabutylammonium fluoride (10 mL, 1N in THF; 10 mmol) was stirred at room temperature for 3 h. Solvent was removed in vacuo. The residue was diluted with water and extracted with diethyl ether. The ethereal layer was separated, washed with water, dried over anhydrous magnesium sulfate and concentrated. Chromatography over silica gel and elution with hexanes-ethyl acetate (4:1) yielded 2.1 g (83%) of alcohol **7**; ^1H NMR ($CDCl_3$) δ 7.48 (d, 2H, J=7.0Hz), 7.24 (d, 2H, J=7.0Hz), 3.88 (m, 2H), 2.88 (t, 2H, J=6.5Hz), 1.48 (t, 1H, J=6.5Hz), 0.30 (s, 9H).

To a solution of alcohol **7** (1.8 g, 6.3 mmol) in acetone (60 mL) at 0°C was added Jones reagent (4.5 mL). The mixture was stirred for 1.5 h and quenched with saturated aqueous sodium bisulfite. The clear solution was decanted, diluted with ethyl acetate (100 mL), washed with brine (3x), dried over anhydrous magnesium sulfate and concentrated in vacuo to give 1.5 g (79%) of crude acid **8**; ^1H NMR ($CDCl_3$) δ 7.48 (d, 2H, J=7.0Hz), 7.28 (d, 2H, J=7.0Hz), 3.62 (s, 2H), 0.30 (s, 9H).

A mixture of above crude acid **8** (1.5 g, 5.0 mmol), N-hydroxysuccinimide (0.8 g, 7.0 mmol) and 1,3-dicyclohexylcarbodiimide (1.4 g, 6.8 mmol) in acetone (40 mL) was stirred at room temperature for 3 h. The white precipitate formed was filtered off. The filtrate was

evaporated in vacuo. Chromatography over silica gel and elution with 8% acetone in toluene afforded 1.3 g (65%) of activated ester **3**; ^{1}H NMR ($CDCl_3$) δ 7.50 (d, 2H, J=7.0Hz), 7.30 (d, 2H, J=7.0Hz), 3.94 (s, 2H), 2.84 (s, 4H), 0.30 (s, 9H).

N-Hydroxysuccinimidyl 4-iodophenylacetate (**9'**)

To a mixture of 0.5M sodium iodide (202 μL, 0.1 mmol; in 0.4M of pH 7.4 phosphate buffer) and acetic acid (15 μL) was added 30% H_2O_2 (20 μL, 0.18 mmol). The mixture was stirred for 1 min. Then a solution of activated ester **3** (20 mg) in CH_2Cl_2 (200 μL) was added. After further stirring for 3 min, TLC showed no starting material remained and the mixture was quenched with 2N aqueous sodium bisulfite. The methylene chloride layer was separated, washed with brine, dried over anhydrous magnesium sulfate and concentrated to give the iodide **9'** as a white solid (18 mg, quantitative); ^{1}H NMR ($CDCl_3$) δ 7.70 (d, 2H, J=7.5Hz), 7.10 (d, 2H, J=7.5Hz), 3.90 (s, 2H), 2.85 (s, 4H).

Iodinated MK-571 analogue (**2'**)

A mixture of amino acid **10** (50 mg, 0.083 mmol), iodide **9'** (33 mg, 0.092 mmol) and triethylamine (50 μL, 0.36 mmol) in 1,4-dioxane (2 mL) was stirred at room temperature in the dark for 24 h. Solvent was evaporated in vacuo. The residue was dissolved in 75% aqueous methanol (1 mL) and purified by reverse phase high performance liquid chromatography ($MeOH/H_2O/NH_4OAc/HOAc$ = 85/15/0.2/0.05, μBondapak C_{18} (10 μm, 25 mm x 100 mm), 10 mL/min, λ = 340 nm) to give 36 mg (55%) of coupling product **2'**; ^{1}H NMR (DMSO - d_6) δ 8.70-7.0 (m, 15H), 5.32 (s, 1H), 3.40 (s, 2H), 3.10-2.25 (m, 12H), 1.50 (m, 2H); FAB mass spectrum m/e 788 (M+H).

Preparation of tin derivative **11**

To the amino acid **10** (50 mg, 0.083 mmol) in 1,4-dioxane (2 mL) was added 1N aqueous sodium hydroxide until dissolution. The activated ester **3** was then added. After completion, the

reaction mixture was purified by flash chromatography (5% MeOH in CH_2Cl_2) to afford 36 mg of **11**.

^{125}I-Labelled Compound **2**

To the tin derivative **11** (2 mg) in DMF (100 µL) was added $Na^{125}I$ (2mCi in 40 µL of 0.4M phosphate buffer pH 8.0). A solution of chloramine-T in DMF (3 µL of a 20 mg/mL DMF solution) was then introduced at room temperature. After further stirring for 30 min, the mixture was quenched with a saturated aqueous solution of sodium bisulfite (10 µL) followed by the addition of DMF (100 µL). The whole mixture was then purified by reverse phase HPLC ($MeOH/H_2O/NH_4OAc/HOAc$ = 70/30/0.2/0.05 and 0.001% of 2-mercaptoethanol, Nova-Pak C_{18} (4 µm, 8 mm x 100 mm), 2 mL/min, λ = 280 nm): radiochemical yield: 50%.

REFERENCES

1. Kips, J.C., Joos, G., Margolskee, D., Delepeleire, I., Rogers, J.D., Paumels, R., and Van Der Straeten, M. - Am. Rev. Respir. Dis. 139: A63 (1989).

2. Kips, J.C., Joos, G.F., De Lepeleire, I., Margolskee, D.J., Buntinx, A., Paumels, R.A., Van Der Straeten, M.E. - Am. Rev. Respir. Dis. 144: 617 (1991).

3. Hendeles, L., Davison, D., Blake, K., Harman, E., Cooper, R., Margolskee, D. - J. Allergy Clin. Immunol. 85: 197 (1990).

4. Manning, P.J., Watson, R.M., Margolskee, D.J., Williams, V.C., Schwartz, J.I., O'Byrne, P.M. - N. Engl. J. Med. 323: 1736 (1990).

5. Margolskee, D., Bodman, S., Dockhorn, R., Israel, E., Kemp, J., Mansmann, H., Minotti, D.A., Spector, S., Stricker, W., Tinkelman, D., Townley, R., Winder, J., Williams, V.C. - J. Allergy Clin. Immunol. 87: 309, Abstract 677 (1990).

6. Gauthier, J.Y., Jones, T., Champion, E., Charette, L., Dehaven, R., Ford-Hutchinson, A.W., Hoogsteen, K., Lord, A., Masson, P., Piechuta, H., Pong, S.S., Springer, J.P., Thérien, M., Zamboni, R. and Young, R.N. - J. Med. Chem. 33: 2841 (1990).

7. Williams, H.W.R., Young, R.N. and Zamboni, R. - J. Labelled Compd. Radiopharm. 28: 297 (1990).

8. Kabalka, G.W. - Tetrahedron. 45: 6601 (1989).

9. Compound 10 was prepared from the corresponding diacid with 1,3-diaminopropane. Zamboni, R., Belley, M., Champion, E., Charette, L., DeHaven, R., Frenette, R., Gauthier, J.Y., Jones, T.R., Léger, S., Masson, P., McFarlane, C.S., Metters, K., Pong, S.S., Piechuta, H., Rokach, J., Thérien, M., Williams, H.W.R., and Young, R.N. - J. Med. Chem. 35: 3832 (1992).