# SYNTHESIS OF A TRITIUM LABELLED ANALOG OF THE NOVEL HEMATOREGULATORY AGENT SB 209978.

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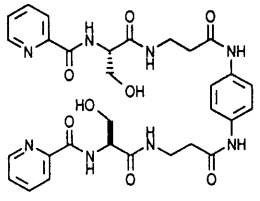
## SUMMARY

[$^{3}$H]-SB 209978 (**10**) was synthesized in 6 steps from phenylene diamine dihydrochloride. A key aspect was the regioselective synthesis of 6-chloro-picolinic acid (**7**). This was condensed with diamine **6** and the resulting product was deprotected to give **9**. Palladium catalyzed $^{3}$H-hydrogenolysis of the chloropyridine rings gave the final product **10**.

**Key Words:** [$^{3}$H]-SB 209978, 6-chloropicolinamide, catalytic $^{3}$H-hydrogenolysis, hematoregulatory agent.

## INTRODUCTION

N,N'-Bis (picolinoyl-L-seryl-β-alanyl)-1,4-phenylenediamine (SB 209978) is a novel immunoregulatory compound. In a murine stromal bone marrow cell line, C6.4, this compound induces the production of a hematopoietic synergistic factor (1). In studies designed to elucidate the mechanism of action of SB 209978, a $^{3}$H-labelled form of this compound was required. This paper presents the synthesis of [$^{3}$H]-SB 209978.




SB 209978

Figure 1

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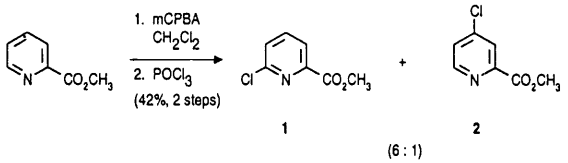
## RESULTS AND DISCUSSION

The pyridine rings of SB 209978 were the logical sites for tritium incorporation. A synthetic scheme was devised to introduce tritium in the last step thereby minimizing manipulations with radioactive material. To this end, methyl 6-chloropicolinate (**1**) was synthesized from methyl




eq. 1

picolinate by oxidation with mCPBA and treatment of the resulting N-oxide with $POCl_3$ (2). Also isolated from this reaction was a small amount of the 4-chloro-isomer **2** (equation 1).

The remainder of the tritiation substrate was synthesized as detailed in Scheme 1. Phenylenediamine dihydrochloride (**3**) was coupled to N-BOC-β-alanine using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBT) in $CH_2Cl_2$ to give **4**. This material was used without further purification. Removal of the BOC-protecting groups followed by coupling of the resulting diamine to Z-L-Ser(*t*-Bu)-OH with EDC and HOBT in DMF gave **5** (56 % yield from **3**). Treatment of **5** with hydrogen in the presence of palladium gave the diamine **6** (SB 216928) in high yield.

Basic hydrolysis of **1** followed by condensation with **6** in the presence of EDC gave the protected tritiation precursor **8**. Brief treatment of **8** with trifluoroacetic acid gave the diol **9** in high yield. Exposure of **9** to $^3H_2$ in the presence of 10% Pd on carbon in DMF gave the desired material. Purification by semi-preparative HPLC afforded **10** in high radiochemical purity (specific activity of 29 Ci/mmol at 99% radiochemical purity).

## EXPERIMENTAL

**General.** $^1H$ NMR spectra were recorded at 250 MHz on a Bruker AM250 instrument and at 400 MHz on a Bruker AM400 instrument. Chemical shifts are reported in part per million (ppm) downfield from internal tetramethylsilane. Analytical thin layer chromatography was performed using EM Reagents precoated silica gel 60 (0.25 mm thickness) glass plates. Compounds were visualized with phosphomolybdic acid, iodine or u.v. irradiation. Flash chromatography was

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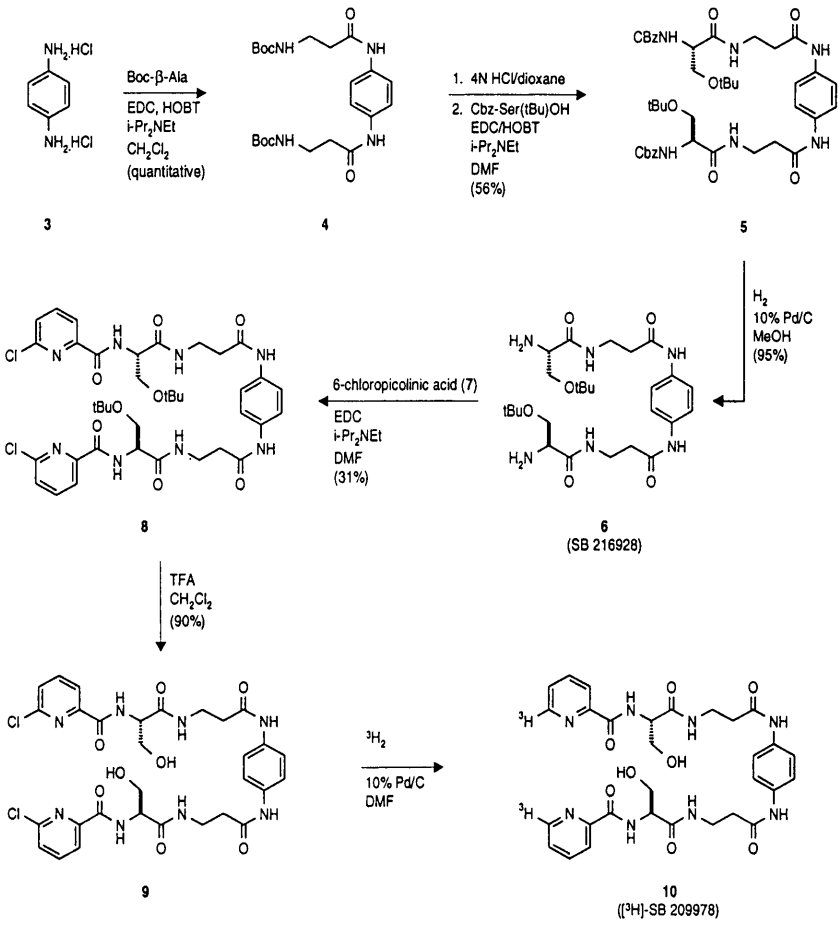


Scheme 1

performed with EM Reagents silica gel 60 (230 - 240 mesh). Radioactivity was determined with a Beckman LS 6000SC liquid scintillation counter using Beckman Ready Safe as the counting medium. HPLC was conducted using a β-RAM radioactivity flow detector and a Hamilton PRP™-1 column, 10 μm, 10 mm x 25 cm.

**Methyl 6-chloropicolinate (1).** To a solution of methyl picolinate (0.63 g, 4.60 mmol) in $CH_2Cl_2$ (18 mL) was added mCPBA (0.99 g, 5.74 mmol). After 2 days at RT, the reaction was quenched by diluting with $CH_2Cl_2$ (100 mL) and adjusting the pH to 8 with sat $NaHCO_3$. The organic layer was washed with sat $NaHCO_3$ (50 mL) and dried over $Na_2SO_4$. Removal of the solvent gave 0.50 g (71 %) of the N-oxide as a white solid. TLC and $^1H$ NMR analysis revealed

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that this material was of sufficient purity to carry onto the next step. $^1$H NMR (250 MHz, $CDCl_3$) δ 8.25 (m, 1H), 7.60 (m, 1H), 7.35 (m, 2H), 4.00 (s, 3H). TLC: $R_f$ 0.15 (10% MeOH/EtOAc, u.v. visualization)

The crude N-oxide obtained above (0.46 g, 3.00 mmol) was dissolved in freshly distilled $POCl_3$ (3.40 mL, 36.5 mmol). The reaction was heated at 80 °C for 17 h. After cooling to RT, the bulk of the $POCl_3$ was removed under reduced pressure. Ice was added to the residue and the mixture was allowed to stand for 4 h. The mixture was partitioned between $CH_2Cl_2$ (50 mL) and sat $NaHCO_3$ (100 mL). The organic phase was separated and the aqueous phase was further extracted with $CH_2Cl_2$ (2 x 50 mL). The combined organic extracts were washed with brine (50 mL) and dried over $Na_2SO_4$. Removal of solvent gave a yellow solid (0.39 g). TLC and $^1$H NMR analysis revealed that this was a mixture of two regio-isomeric products. The products were separated by flash chromatography (30% to 50% EtOAc/hexane, silica gel) to give 0.26 g (51%) of the 6-chloro-isomer and 0.04 g (8%) of the 4-chloro-isomer. Both compounds were white solids. Methyl 6-chloropicolinate: $^1$H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.0 Hz, 1H), 7.84 (t, J = 7.9 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 4.01 (s, 3H). MS (ES+) m/z 172.0 ($M+H^+$), 194.0 ($M+Na^+$), 365 ($2M+Na^+$). TLC: $R_f$ 0.2 (20% EtOAc/hexane, u.v. visualization). Methyl 4-chloropicolinate: $^1$H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 5.2 Hz, 1H), 8.15 (d, J = 2.0 Hz, 1H), 7.50 (dd, J = 5.3, 1.9 Hz, 1H), 4.03 (s, 3H). TLC: $R_f$ 0.07 (20% EtOAc/hexane, u.v. visualization).

**N,N'-Bis(BOC-β-Ala)-1,4-phenylenediamine (4).** To a suspension of phenylene diamine dihydrochloride (5.00 g, 27.6 mmol) and BOC-β-Ala (11.5 g, 60.8 mmol) in $CH_2Cl_2$ (150 mL) at 0 °C was added Et*N*-$Pr_2$ (10.6 mL, 60.8 mmol), HOBT (8.20 g, 60.7 mmol) and EDC (11.6 g, 60.5 mmol). The reaction was allowed to warm to RT. After 2 days, the reaction was quenched by pouring into a swirled mixture of $Et_2O$ (500 mL), 1N HCl (100 mL) and $H_2O$ (100 mL). The resulting precipitate was collected and further rinsed with water. Drying under vacuum gave 10.8 g (87%) of **4** as a white solid. This material was judged to be of sufficient purity by $^1$H NMR analysis and was used in the next step without further purification. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.85 (s, 2H), 7.49 (s, 4H), 6.86 (broad s, 2H), 3.19 (dd, *J* = 13.2, 6.7 Hz, 4H), 2.43 (t, *J* = 7.1 Hz, 4H), 1.37 (s, 18H). MS (ES +) m/z 451.2 ($M+H^+$), 250.0 (M-2BOC). TLC: $R_f$ = 0.71 (10 % MeOH/EtOAc, silica gel, faintly u.v. active).

**N,N'-Bis(CBZ-L-Ser(t-Bu)-β-Ala)-1,4-phenylenediamine (5).** A solution of 4N HCl/dioxane (5.00 mL, 20.0 mmol) was added to **4** (0.50 g, 1.11 mmol) at 0 °C. The resulting suspension was allowed to warm to RT and maintained there for 1 h. The solvent was removed *in*

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*vacuo* and the residue was dissolved in DMF (5 mL). $EtNiPr_2$ (0.85 mL, 4.88 mmol), Z-L-Ser(t-Bu)-OH (0.72 g, 2.44 mmol), HOBT (0.33 g, 2.44 mmol) and EDC 0.47 g, 2.45 mmol) were sequentially added. After stirring at RT for 24 h, the reaction was poured into a mixture of water (50 mL) and EtOAc (50 mL). The resulting precipitate was collected, washed with water and dried under vacuum to 0.50 g (56 %) of **5** as a white solid. $^1H$ NMR analysis revealed that this material was of sufficient purity to be used in the next step. $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 9.00 (s, 2H), 8.03 (m, 2H), 7.50 (s, 4H), 7.33 (m, 10H), 7.22 (d, J = 8.2 Hz, 2H), 5.03 (m, 4H), 4.05 (m, 2H), 3.50 - 3.25 (m, 8H), 2.45 (m, 4H), 1.03 (s, 18H). TLC: $R_f$ = 0.58 (10 % MeOH/EtOAc, silica gel, u.v. active, stains with $I_2$ and PMA).

**N,N'-Bis(L-Ser(t-Bu)-β-Ala)-1,4-phenylenediamine (6).** A solution of **5** (0.16 g, 0.20 mmol) in MeOH (2 mL) and DMF (2 mL) was purged with $H_2$. 10 % Pd on carbon (ca. 5 mg) was added and the reaction vessel was fitted with a balloon filled with $H_2$. After 1.5 h, the reaction vessel was flushed with argon to remove the $H_2$ and diluted with EtOAc (20 mL). The reaction mixture was filtered through a bed of Celite® to remove the catalyst. Removal of the solvent *in vacuo* gave 0.11 g (95 %) of pure **6** as a white solid. $^1H$ NMR (400 MHz, MeOH-$d_4$) δ 7.52 (s, 4H), 3.60 - 3.40 (m, 10H), 2.58 (t, J = 6.5 Hz, 4H), 1.13 (s, 18H). TLC: $R_f$ = 0.35 (10 % MeOH/EtOAc, silica gel, u.v. active, stains with $I_2$).

**N,N'-Bis[(6-Chloropicolinoyl)-L-Ser(t-Bu)-β-Ala]-1,4-phenylenediamine (8).** A solution of **6** (20.0 mg, 37.0 μmol), 6-chloropicolinic acid (**7**) (12.7 mg, 80.0 μmol), EDC (15.3 mg, 80.0 μmol) and $EtNiPr_2$ (20.0 μL, 115 μmol) in DMF (1 mL) was stirred at room temperature for 20 h. Water (2 mL) was then added and the reaction mixture extracted with EtOAc (3 x 10 mL). The combined extracts were dried over $MgSO_4$ and evaporated *in vacuo* to give a light oil. Semi-preparative HPLC ($t_R$ = 17.1 min, solvent gradient 5% to 50% acetonitrile/water + 0.1% TFA at 2 mL/min over 20 min, Hamilton PRP™-1 column , 8 mm x 25 cm) gave 8.0 mg (31%) of the desired material. MS (ES+) m/z 814.2 ($M+H^+$).

**N,N'-Bis[(6-Chloropicolinoyl)-L-Ser-β-Ala]-1,4-phenylenediamine (9).** To a solution of **8** (8.0 mg, 9.8 μmol) in $CH_2Cl_2$ (1 mL) was added trifluoroacetic acid (250 μL). After 20 h at room temperature, the solvent was removed *in vacuo* to leave a clear oil. Trituration with acetonitrile/water (1:1 v/v, 1 mL) gave a white solid which was collected and dried under high vacuum giving 6.2 mg (90 % ) of the desired material. This material was pure by HPLC analysis. MS (ES+) m/z 702.2 ($M+H^+$). HPLC: $t_R$ = 17.8 min (solvent gradient 5% to 50% acetonitrile/water + 0.1% TFA at 2 mL/min over 20 min, Hamilton PRP™-1 column , 8 mm x 25 cm)

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**[$^3$H]-SB 209978 (10).** Compound **9** (0.97 mg, 1.4 μmol), 5% Pd/C (1 mg) and $Et_3N$ (50 μL) in DMF (400 μL) was treated with tritium gas (4.6 Ci) using a tritium vacuum line for 20 h. The catalyst was removed by filtration and rinsed with methanol. The crude product was purified by semi-preparative HPLC ($t_R$ = 10.5 min, 15 % acetonitrile/water + 0.1% TFA at 2 mL/min, Hamilton PRP™-1 column, 8 mm x 25 cm) to give 2.6 mCi of the desired material with a specific activity of 29 Ci/mmol (as determined by mass spectral analysis) and at 99 % radiochemical purity. Using the HPLC method described above, **10** co-eluted with an authentic sample of non-tritiated SB 209978.

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