Synthesis of tritiated *N*-[4-(2,4-diaminopteridine-6-methyl)-3,4-dihydro-*2H*-1,4-benzothiazine-7-carbonyl]-L-homo glutamic acid (MX-68)

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SUMMARY

Synthesis of tritiated *N*-[4-(2,4-diaminopteridine-6-methyl)-3,4-dihydro-*2H*-1,4-benzothiazine-7-carbonyl]-L-homoglutamic acid, [9-$^3H_1$]MX-68 (**3**), is described. [9-$^3H_1$]MX-68 (**3**) was prepared *via* tritiation at the carbonyl group in 2,4-bis-(butanoylamino)-6-formylpteridine (**7**) with sodium borotritiide.

KEYWORDS: *N*-[4-(2,4-diaminopteridine-6-methyl)-3,4-dihydro-*2H*-1,4-benzothiazine-7-carbonyl]-L-homoglutamic acid, MX-68, methotrexate, [9-$^3H_1$]MX-68, sodium borotritiide

INTRODUCTION

Considerable attention has been paid to the synthesis of derivatives of methotrexate (MTX **1**, Figure 1) with the aim of developing antirheumatic agents which are less toxic than MTX.[1] We have successfully synthesized *N*-[4-(2,4-diaminopteridine-6-methyl)-3,4-dihydro-*2H*-1,4-benzothiazine-7-carbonyl]-L-homoglutamic acid **2** (MX-68)[2], which is impressively safe compared to its predecessor MTX and is currently under extensive preclinical investigations as a candidate antirheumatic agent. MX-68 has a homoglutamate group in place of the glutamate group in MTX and does not undergo polyglutamation; an effect considered to be responsible for potentiation of

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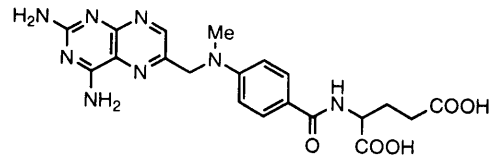
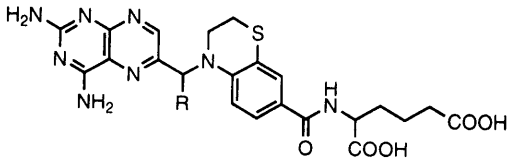
pharmacological effects and the associated toxicity of MTX.[3] Importantly however, MX-68 potently inhibited cell-proliferation *in vitro* and strongly suppressed mouse collagen arthritis *in vivo*.



1 Methotrexate



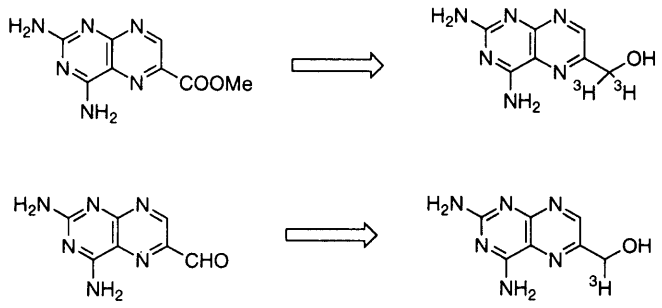
2 R = H; MX-68
3 R = ^{3}H; [9-$^3H_1$]MX-68

Figure 1

During the course of the preclinical development of this compound, synthesis of tritiated MX-68 was required for pharmacokinetic and metabolic studies. In this paper, we describe the synthesis of tritiated MX-68, [9-$^3H_1$]MX-68 (**3**), labeled at the methylene moiety adjacent to the pteridine ring of **2** *via* tritiation with NaB[$^3H_4$]. We adopted this route to facilitate the labeling of many other of our MTX derivatives which have differing biological activities.

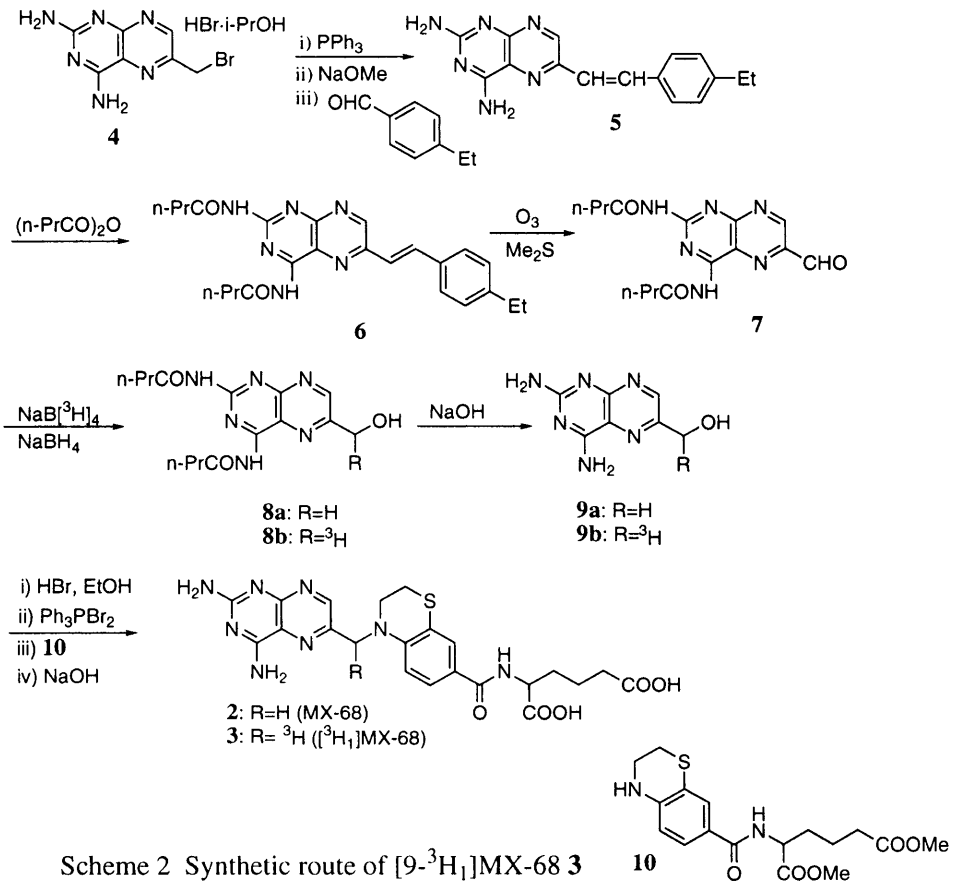
SYNTHESIS

Tritiation of MX-68 *via* reductive ditritiation of the ester group in 6-methoxycarbonyl-2,4-diaminopteridine[4] with $NaBH_4$ in MeOH, as shown in Scheme 1, resulted in recovery of the starting ester. Reasons for the unsuccessful reduction were thought to be poor solubility of the ester in MeOH and low reactivity of the carbonyl group, which is deactivated by many nitrogen in the pteridine ring. Tritiation was accomplished *via* monotritiation of the carbonyl group in 6-formyl-2,4-diaminopteridine derivative, which was prepared by wittig olefination and subsequent ozonolysis according to Taylor's method[5].



Scheme 1

The synthetic scheme of the target compound [9-^{3}H]MX-68 is given in Scheme 2. Treatment of 6-bromomethyl-2,4-diaminopteridine hydrobromide (**4**) with triphenylphosphine gave the phosphonium salt and the subsequent Wittig reaction with



Scheme 2 Synthetic route of [9-^{3}H$_1$]MX-68 **3**

4-ethylbenzoic aldehyde yielded olefin **5** in a mixture of *cis* and *trans* forms. In order to improve the solubility of **5** in routine organic solvents, the amino groups of this compound were acylated with butyric anhydride to give the lipophilic olefin **6**. The olefin moiety of the amide **6** was next oxidized by treatment with ozone to produce aldehyde **7**. Reduction of **7** with $NaB[^3H]_4$ (53.2 mCi/mmol) or $NaBH_4$ was performed in isopropanol to give the corresponding tritiated alcohol, **8a** or **8b**. Compounds **8a** or labeled alcohol **8b** were deacylated to yield acyl free pteridine **9a**[6] or **9b**.

Compounds **9a** or **9b** were converted to the monohydrobromide salt and brominated by treatment with dibromotriphenylphosphorane (PhP_3Br_2). Finally, amination of thus prepared bromide with **10**[7] and subsequent hydrolysis produced [9-^{3}H]MX-68 (**3**). Its specific radioactivity was found to be 180 mCi/mmol.

In conclusion, we synthesized the labeled MTX derivative, [9-^{3}H]MX-68 (**3**), *via* tritiation at the methylene moiety adjacent to the pteridine ring. The novel synthetic route, which we have used, is expected to have a wide range of application.

The preclinical study using [9-^{3}H]MX-68 is now underway in our company. And its results will be published elsewhere in the near future.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Spectra were recorded as follows: infrared (IR) spectra were recorded with a Hitachi 270-30 spectrometer; proton and ^{13}C nuclear magnetic resonance (NMR) spectra with a JEOL FX-200; high-resolution FAB mass spectra (HR-FAB-MS) with a VG Analytical VG11-250. Radioactivity was measured with an Aloka LSC-900. Column chromatography was carried out with Merck Kieselgel 60, 230-400 mesh. $NaB[^3H]_4$ (53.2 mCi/mmol) was purchased from Amersham Japan (lot No. 2745-269).

β-(2,4-Diamino-6-pteridinyl)-*p*-ethylstyrene (5) A mixture of **4** (1.0 g, 2.63 mmol) and triphenylphosphine (810 mg, 3.09 mmol) in dry dimethylformamide (DMF, 17 mL) was heated at 60°C for 3 h with stirring and then cooled to room temperature. To the mixture was added freshly prepared sodium methoxide (453 mg, 8.39 mmol) followed by *p*-ethylbenzaldehyde (750 mg, 5.60 mmol). The resulting mixture was stirred for 10 h, poured into water and extracted with $CHCl_3$-MeOH. The organic layer was dried over Na_2SO_4, filtered and concentrated. The obtained residue was chromatographed on silica gel with $CHCl_3$-MeOH (10:1) to give **5** (205 mg, 0.70 mmol, 27%) as a yellow powder. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, m), 2.68 (2H, m), 6.4-7.8 (6H, m), 8.55 (1H, s). HR-FAB-MS *m/z*: Calcd for $C_{16}H_{17}N_6$: MH^+, 293.1515. Found: 293.1579 (MH^+).

β-[2,4-Bis-(butanoylamino)-6-pteridinyl]-*p-trans*-ethylstyrene (6) A suspension of **5** (1.5 g, 5.40 mmol) in butyric anhydride (25 mL) was heated at 150°C for 2 h with stirring. After cooling, the reaction mixture was poured into n-hexane and precipitated solids were collected by filtration. The obtained solids were dried *in vacuo*

to give **6** (750 mg, 1.74 mmol, 34%) as a yellow powder. ^{1}H-NMR ($CDCl_3$) δ: 1.06 (6H, m), 1.28 (3H, t, *J*=7.6 Hz), 1.84 (4H, m), 2.71 (2H, q, *J*=7.6 Hz), 2.95 (2H, t, *J*=7.3 Hz), 3.13 (2H, t, *J*=7.3 Hz), 7.25 (1H, d, *J*=16.2 Hz), 7.28 (2H, d, *J*=8.1 Hz), 7.58 (2H, d, *J*=8.1 Hz), 7.81 (1H, d, *J*=16.2 Hz), 8.30 (1H, s), 9.16 (1H, s), 9.62 (1H, s). IR (KBr) cm^{-1}: 2960, 1710, 1680, 1600, 1580. HR-FAB-MS *m/z*: Calcd for $C_{24}H_{29}N_6O_2$: MH^+, 433.2352. Found: 433.2345 (MH^+).

2,4-Bis-(butanoylamino)-6-formylpteridine (7) To a solution of **6** (760 mg, 1.76 mmol) in CH_2Cl_2-MeOH (1:1, 200 mL) at -78°C was passed ozone and the mixture was stirred for 30 min at the same temperature. To the mixture was added dimethylsulfide (25 mL) and the resulting mixture was stirred for 2 h at 0°C, then concentrated, diluted with $CHCl_3$ and washed with brine. The $CHCl_3$ layer was dried over Na_2SO_4, filtered, concentrated and chromatographed on silica gel with 10% MeOH in $CHCl_3$ to give **7** (340 mg, 1.03 mmol, 59%) as a pale yellow powder. ^{1}H-NMR ($CDCl_3$) δ: 1.0-1.1 (6H, m), 1.7-1.9 (4H, m), 2.97 (2H, t, J=7.3 Hz), 3.10 (2H, t, J=7.3 Hz), 8.57 (1H, s), 9.58 (2H, s), 10.20 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 13.66, 13.69, 17.90, 18.11, 39.71, 40.23, 122.01, 143.21, 150.56, 157.00, 158.33, 158.38, 172.76, 173.38, 189.72. IR (KBr) cm^{-1}: 3500-3200, 2960, 1700, 1600, 1580. HR-FAB-MS *m/z*: Calcd for $C_{15}H_{19}N_6O_3$: MH^+, 331.1519. Found: 331.1560 (MH^+).

2,4-Bis-(butanoylamino)-6-hydroxymethylpteridine (8a) To a solution of **7** (40 mg, 0.12 mmol) in CH_2Cl_2 (1.0 mL) at -10°C was added a solution of $NaBH_4$ in iso-propanol (0.03 M, 1.4 mL, 0.42 mmol) over 15 min. The mixture was stirred for the next 30 min at the same temperature and then poured into aqueous NH_4Cl solution, extracted with $CHCl_3$, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained residue was chromatographed on preparative TLC (silica gel) with $CHCl_3$-MeOH (10 :1) as an eluent to give **8a** (25 mg, 0.075 mmol, 63%) as a pale yellow powder. ^{1}H-NMR ($CDCl_3$-CD_3OD) δ: 1.06 (6H, m), 1.82 (4H, m), 2.69 (2H, t, J=7.3 Hz), 2.79 (2H, t, J=7.3 Hz), 4.98 (2H, s), 9.06 (1H, s). IR (KBr) cm^{-1}: 3500-3200, 2960, 1730, 1680, 1550. HR-FAB-MS *m/z*: Calcd for $C_{15}H_{21}N_6O_3$: MH^+, 333.1675. Found: 333.1687 (MH^+).

2,4-Bis-(butanoylamino)-6-(hydroxy[$^3H_1$]methyl)pteridine (8b) To a suspension of **7** (200 mg, 0.61 mmol) in CH_2Cl_2 at -10°C was added a solution of $NaB[^3H]_4$ (500 mCi, 53.2 Ci/mmol) in iso-propanol (0.31 mL). The mixture was stirred for 10 min at the same temperature. To the mixture was added a solution of $NaBH_4$ (6.08 mg, 0.16 mmol) in isopropanol (5.3 mL) and the resulting mixture was further stirred for 10 min at -10°C. The reaction mixture was then poured into aqueous NH_4Cl and extracted with $CHCl_3$. The extract was dried over Na_2SO_4, filtered and concentrated. The obtained residue was recrystallized from n-hexane-$CHCl_3$-MeOH to give **8b** (88 mg, 0.27 mmol, 43%, 163 mCi, 611 mCi/mmol). Its TLC Rf value was identical with the authentic sample **8a**.

2,4-Diamino-6-hydroxymethylpteridine[6] (9a) A solution of **8a** (100 mg, 0.31 mmol) and 1N NaOH (0.9 mL, 0.90 mmol) in dimethylformamide (DMF, 3.0 mL) was stirred for 4 h at room temperature. The resulting solid was collected by filtration. The obtained solid was washed with water and MeOH, successively, and dried *in vacuo* to give **9a** (46 mg, 0.24 mmol, 77%) as an orange powder. ^{1}H-NMR (CF_3CO_2D) δ: 5.27 (2H, m), 9.08 (1H, s).

2,4-Diamino-6-hydroxy[$^3H_1$]methylpteridine (9b) A solution of **8b** (88 mg, 0.27 mmol, 163 mCi) and 1N NaOH (0.79 mL) in DMF (3.0 mL) was stirred at room temperature for 4 h. The resulting solid was collected by filtration and washed with water, MeOH and $CHCl_3$, successively. The obtained solid was dried *in vacuo* to give **9b** (18 mg, 0.094 mmol, 35%, 40 mCi, 425 mCi/mmol) as an orange powder. Its TLC Rf value was identical with the authentic sample **9a**.

N-[4-(2,4-Diaminopteridine-6-methyl)-1,4-dihydro-*2H*-benzothiazine-7-carbonyl]-L-homoglutamic acid (2, MX-68) A suspension of **9a** (100 mg, 0.52 mmol) in EtOH (10 mL) was heated at 70°C with stirring. To the resulting suspension

was added 47% HBr (60 μL) and the mixture was refluxed for 15 min. The resulting solid was collected by filtration and dried *in vacuo*. To the suspension of the solid in DMF (1.0 mL) was added Ph_3PBr_2 (278 mg, 0.66 mmol) and the mixture was stirred 4 h at room temperature to give a dark orange solution. To the solution was added **10** (81 mg, 0.22 mmol) and this mixture was stirred at 70°C for 3 days. The reaction mixture was then poured into 5% $NaHCO_3$ aqueous solution, extracted with $CHCl_3$, dried over Na_2SO_4, filtered and concentrated. The residue was chromatographed on silica gel with $CHCl_3$-MeOH (10:1) to give the diester of **2** as an orange powder. Subsequently, a mixture of the obtained powder (67 mg) and 1N NaOH (0.37 mL, 0.37 mmol) in EtOH (3.0 mL) was stirred overnight at room temperature. The mixture was then concentrated under reduced pressure to 1.0 mL, adjusted to pH 3.7 with 1N HCl. A precitated solid was collected by filtration and dried *in vacuo* to yield **2** (27 mg, 0.05 mmol, 10%) as an orange solid. ^{1}H-NMR (DMSO-*d6*) δ: 1.7-2.2 (m, 4H), 2.50 (t, 2H, *J*=7.2 Hz), 3.46 (m, 2H), 4.23 (m, 2H), 4.58 (m, 1H), 5.05 (s, 2H), 7.09 (d, 1H, *J*=8.9 Hz), 7.73 (m, 2H), 8.49 (d, 1H, *J*=7.6 Hz), 8.95 (s, 1H). IR (KBr) cm^{-1}: 3500-3300, 1640, 1590, 1500. FAB-MS *m/z*: 513 (MH^+). mp 200-203°C (dec). Anal. Calcd for $C_{22}H_{24}N_8O_5S \cdot 1/2H_2O$: C, 48.97; H, 5.04; N, 20.77; S, 5.94. Found: C, 49.27; H, 5.29; N, 20.88; S, 6.16.

N-[4-(2,4-Diaminopteridine-6-[$^3H_1$]methyl)-1,4-dihydro-*2H*-benzothiazine-7-carbonyl]-L-homoglutamic acid (3, [9-^{3}H]MX-68) To a suspension of **9a** (82 mg, 0.43 mmol) and **9b** (18 mg, 0.094 mmol) in EtOH (10 mL) at 70°C was added 47% HBr (60 μL) and the mixture was stirred for 15 min. The resulting solid was collected by filtration and dried *in vacuo*. To a suspension of the solid in DMF (1.7 mL) was added Ph_3PBr_2 (463 mg, 1.08 mmol) and the mixture was stirred for 4 h at room temperature. To the reaction mixture was then added **10** (135 mg, 0.37 mmol) stirred at 70°C for 3 days. The resulting mixture was then poured into 5% $NaHCO_3$ aqueous solution, extracted with $CHCl_3$, dried over Na_2SO_4, filtered and concentrated. The residue was chromatographed on silica gel with $CHCl_3$-MeOH (10:1) to give the diester of **3** as an orange powder. Subsequently, a mixture of the obtained powder (96 mg) and 1N NaOH (0.53 mL, 0.53 mmol) in EtOH (4.3 mL) was stirred overnight at room temperature. The mixture was then concentrated under reduced pressure to 1.0 mL, adjusted to ph 3.7 with 1N HCl. A precipitated solid was collected by filtration and dried *in vacuo* to yield **3** (68 mg, 0.13 mmol, 25%, 24 mCi, 180 mCi/mmol) as an orange solid. Its TLC Rf value was identical with the authentic sample **2**.

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REFERENCES

1. Matsuoka H., Kato N., Tsuji K., Maruyama N., Suzuki H., Mihara M., Takeda Y and Yano K. -- Chem. Pharm. Bull. 44: 1332 (1996)

2. Mihara M., Urakawa K., Takagi N., Moriya Y., Takeda Y., Ohsugi Y. -- Arthritis. Rheum. 38: S280 (1995)

3. Chabner B. A., Allegra C. J., Curt G. A., Clendenin N. J., Baram J. and Koizumi S. -- J. Clin. Invest. 76: 907 (1985)

4. Sato N. and Saito N. -- J. Heterocyclic Chem. 25: 1737 (1988)

5. Taylor E. C. and Ray P. S. -- Synth. Commun. 17: 1865 (1987)

6. Piper J. R. and Montgomery J. A. -- J. Org. Chem. 42: 208 (1977)

7. Ohi N., Suzuki H. -- PCT Int. Application, WO-9414810.