Organic Process Research & Development **2004**, *8*, 883−888

An Improved Process for the Large-Scale Preparation of Antirheumatic Agent MX-68

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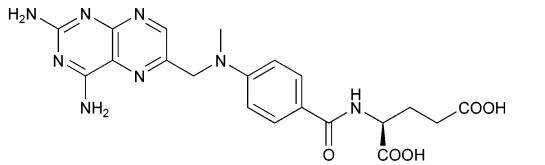
Abstract:

A large-scale preparation route of MX-68, a novel MTX derivative bearing a dihydro-2*H*-1,4-benzothiazine moiety and L-homoglutamic acid, is described. The original route that is a laboratory-scale synthesis for preclinical study has been improved. The improved process involves the following features: each step does not use haloalkane solvents, corrosive reagents, and chromatographic purification, and the formation of the major impurity at the final step is minimized. This improvement has enabled us to supply sufficient quantities of MX-68, which is required for both the toxicity test and the clinical study.

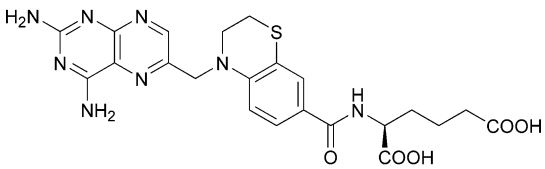
Introduction

Methotrexate (MTX), which has a high anti-folate activity, is noted for an antileukemic agent (Figure 1). Based on its biological profile, MTX is effective for the treatment of rheumatoid arthritis (RA),[1] psoriasis,[2] and other autoimmune diseases.[3,4] However, long-term MTX therapy is associated with some serious side effects, such as hepatic dysfunction[5] and lung fibrosis.[6] Therefore, our effort focused on the synthesis of novel MTX derivatives with an aim to develop safe and potent antirheumatic agents.

In this respect, we have already reported the synthesis, structure−activity relationships (SAR), and biology of the MTX derivatives.[7−10] *N*-[[4-[(2,4-Diaminopteridin-6-yl)-methyl]-3,4-dihydro-2*H*-1,4-benzothiazine-7-yl]carbonyl]-L-homoglutamic acid (MX-68), bearing dihydrobenzothiazine and L-homoglutamic acid in place of aminobenzoic acid and L-glutamic acid of MTX, respectively, exhibited potent antiproliferative effects on human synovial cells (hSC) and human peripheral blood mononuclear cells (hPBMC) obtained from RA patients and healthy volunteers.[10] In addition, MX-68 was shown to potently suppress progression of arthritis in a rat model. Importantly, MX-68 did not undergo polyglutamation, a function considered to be responsible not only for the potentiation of biological effects but also for the associated side effects.[10] The accumulation of the polyglutamated MTX, catalyzed by folylpolyglutamate synthetase (FPGS), causes cell death due to the disruption of reduced folate.[11]



MTX (1)



MX-68 (2)

Figure 1.

To supply sufficient quantities required for both the toxicity test and the clinical study, it was imperative to develop a large-scale preparation route. An original route of MX-68 for SAR studies is presented in Scheme 1. This route was designed based on the standard synthesis of MTX derivatives.[12]

As shown in Scheme 1, MX-68 was previously synthesized by hydrolysis of the diester **10** with NaOH in EtOH. The diester **10** was synthesized using the coupling reaction of 6-(bromomethyl)-2,4-diaminopteridine[13] with the amide **8**. The amide **8** was obtained by the reaction of the acid **4** prepared from *p*-aminobenzoic acid via seven steps using a

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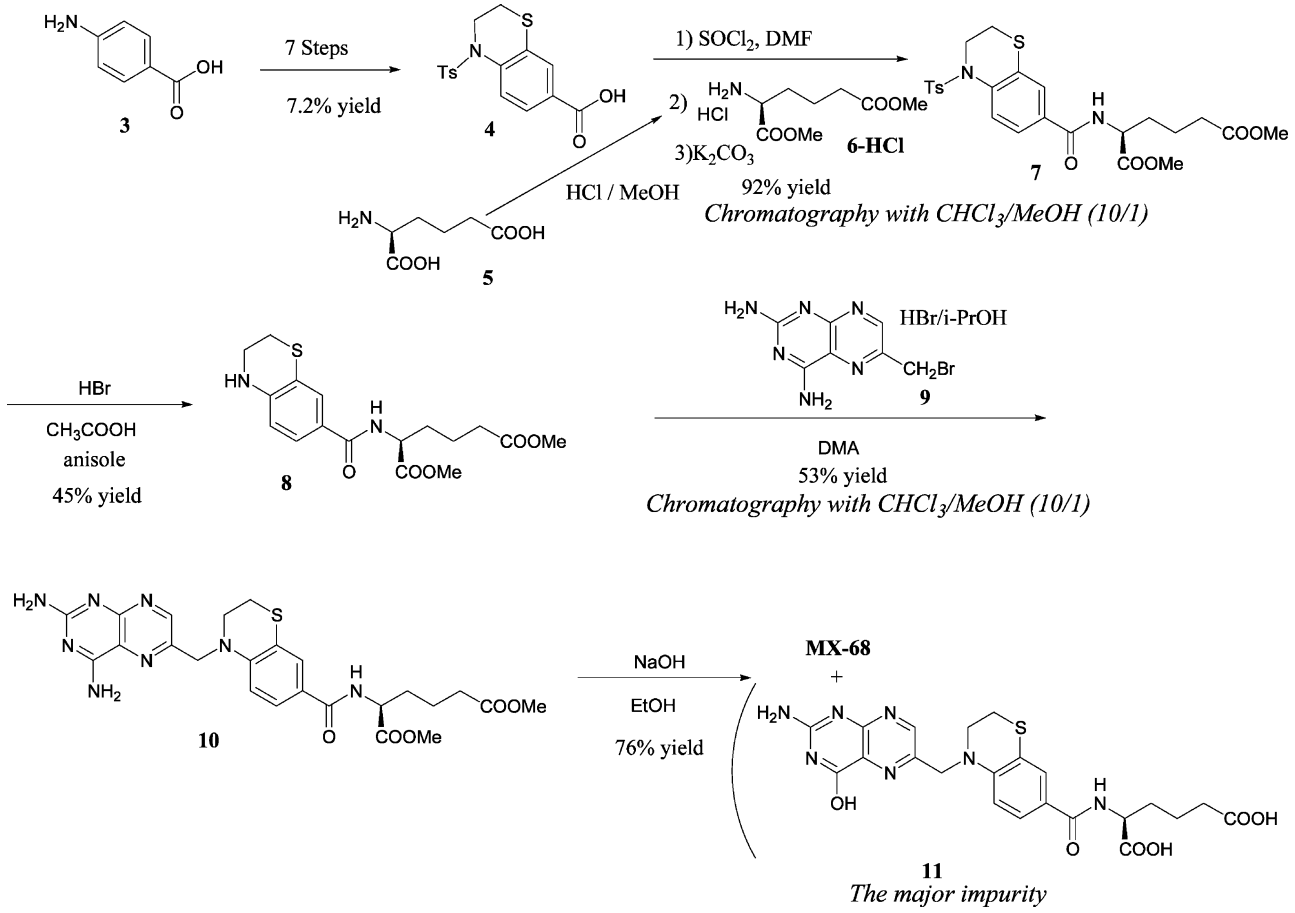
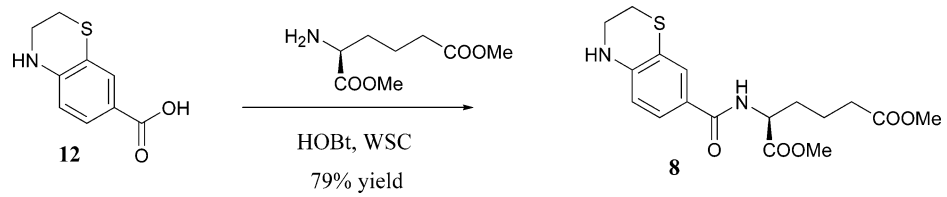
10.1021/op049867y CCC: $27.50
Published on Web 09/21/2004

Scheme 1. **Original route of MX-68 for preclinical study**



Scheme 2. **Improved synthesis of the amine 8 (pilot scale)**



slight modification of Wolfe's method,[14] with dimethyl L-homoglutamate hydrochloride **6-HCl**, followed by deprotection with HBr in acetic acid.

This route involved several problems for a large-scale preparation, for example, use of corrosive reagents, low yield at the deprotection step, purification by column chromatography using $CHCl_3$ for the isolation of the diester **10**, and major impurity formation at the final step.

Herein, we would like to report a new route, which has been accomplished by improving the original process, specifically for a large-scale preparation of MX-68.

Results and Discussion

We chose an improvement of the original route because the problems for the large-scale preparation were clear. We focused on the solution of the problems mentioned above by the following methods: change of the corrosive reagents to other reagents, replacement of the protected amine **4** to an unprotected amine **12**, change of the chromatographic purification to crystallization, and improvement of the reaction condition at the final step to minimize the major impurity formation. Furthermore, these improvements were expected to lead to an increase in the total yield and provide a more environmentally friendly synthesis.

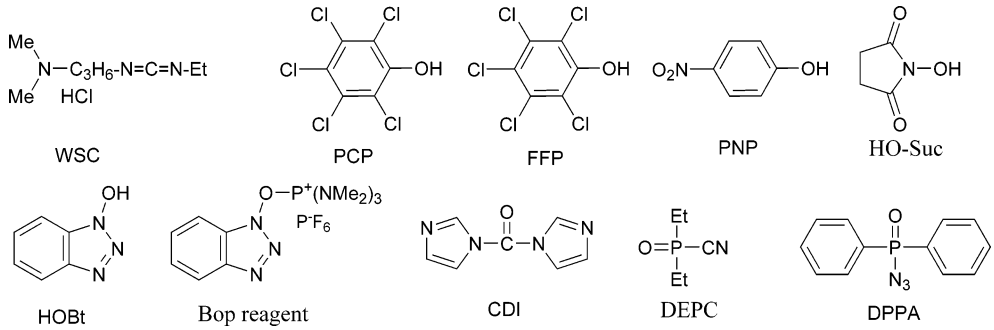
Alternative Preparation of the Amide 8. In our previous report,[10] the amide **8** was obtained from the *N*-protected amine **7** by removing the *p*-toluenesulfonyl group but in low yield. The reason for the low yield was inferred from the simultaneous hydrolysis of the methyl ester groups in the amide **8**, where the reaction was conducted under strong acidic conditions. Therefore, we investigated the coupling of the unprotected amine **12**, which was commercially available,[15] with the amino diester **6** (Scheme 2). Dimerization of the unprotected amine **12** was not thought to be a major side reaction because the amino group of the amino diester **6** was more nucleophilic than the secondary amine moiety of the acid **12** by MM2[16] and PM3[17] calculation.

(14) Wolfe, J. F. SRI International Technical Report, AFOSFR-TR80-1370, Order No. AD-A093879, 1980.
(15) Commercially available from Ube Industries, Ltd.

Table 1. **Coupling reaction of the unprotected amine 12 with the diester 6**

entry	coupling reagent[a]	temp (°C)	time (h)	yield (%)	HPLC area %
1	$SOCl_2$ (1.0 equiv)	5	2.5	55.0	97.5
2	PCP (1.2 equiv), WSC (1.2 equiv)	25	14.5	0	
3	PFP[18] (1.2 equiv), WSC (1.2 equiv)	27	21.0	25.9	
4	PNP (1.2 equiv), WSC (1.2 equiv)	28	22.5	0	
5	HOSuc (1.2 equiv), WSC (1.2 equiv)	26	18.5	10.5	
6	HOBt (1.2 equiv), WSC (1.2 equiv)	21	3.5	92.1	95.0
7	Bop reagent[19] (1.2 equiv)	rt	5.0	88.8	83.8
8	DEPC[20]	rt	4.0	90.7	93.0

[a] The coupling reagents were the following structures:



Thus, we carried out the coupling of the unprotected amine **12** with the diester **6** using several coupling reagents presented in Table 1. A similar condition of the original route using $SOCl_2$ resulted in an unsatisfied yield of the desired compound (run 1). When the unprotected amine **12** was converted to the corresponding active esters using PCP/WSC, PFP[18]/WSC, PNP/WSC, and HOSuc/WSC, the coupling reaction did not give the desired amide **8** in sufficient yields (runs 2−5). Using HOBt and WSC, however, the reaction proceeded for 3.5 h to give the amide **8** in over 92.1% yield (run 6). Reactions using BOP reagent[19] and DEPC[20] gave comparable results as in the case with HOBt and WSC (run 7, 8). Considering the price of reagents for a scale-up preparation and the product yield, we chose the coupling conditions of run 6 for the improved process of MX-68.

Furthermore, to avoid use of the corrosive reagent for the large-scale preparation, and for environmental suitability, saturated HCl in MeOH was replaced with 2 equiv of MsOH in MeOH in the esterification of L-homoglutamic acid **5**. As a result, after the esterification was followed by neutralization with 2 equiv of Et_3N, the reaction mixture was directly used in the subsequent coupling reaction. This new esterification process could avoid the use of the corrosive reagent and at the same time shorten the operation time by eliminating the isolation process of the diester **6**.

Optimization of Preparation of the Diester 10. In the previous process, the amide **8** reacted with 2,4-diaminopteridine **9** in 16 times the volume of DMA for 3 days at 70 °C. Then, the mixture was extracted with $CHCl_3$, and the product was purified by silica gel column chromatography using $CHCl_3$/MeOH (10/1) to give the diester **10** in 53% yield. To scale-up this process, we tried to shorten the reaction time and to eliminate the chromatographic purification using $CHCl_3$, which was not suitable from an environmental standpoint. First, the amount of the solvent was examined, where the amount of DMA was changed from 16 times the volume into 5 times. As a result, the reaction could complete within 4 h. Next, the workup procedure was examined. Previously, the extractive workup required $CHCl_3$ because of the solubility of the diester **10**, and the extracts contained not only the desired compound **10** but also several unknown byproducts; therefore, chromatographic purification was required for the isolation of the diester **10**. To avoid the chromatographic purification, the isolation was conducted directly from the reaction mixture without the extraction. Upon addition of water and neutralization with 1 N NaOH, the crude product was obtained as a precipitate because the solubility of the HBr-free form of the diester **10** was lower than that of the HBr salt form in the DMA/water solvent system. Then, the obtained crude product was recrystallized 3 times from MeOH/water to give the desired compound **10** in 56.5% yield in pure form (Scheme 3).

Improvement of the Reaction Condition at the Final Step. In the previous synthesis, the diester **10** was hydrolyzed for over 12 h with 3.0 equiv of 1 N NaOH in 46 times the volume of EtOH at room temperature. After the concentration, the obtained residue was diluted with water and adjusted to pH 3.7 with 1 N HCl to give MX-68 in 76% yield. The major impurity obtained from this reaction was the byproduct **11**, which had a hydroxyl group instead of the 4-amino group on the 2,4-diaminopteridine moiety. The byproduct **11** was difficult to remove from the desired product, MX-68 (Scheme 1). In general, the 4-amino group of the 2,4-diaminopteridine

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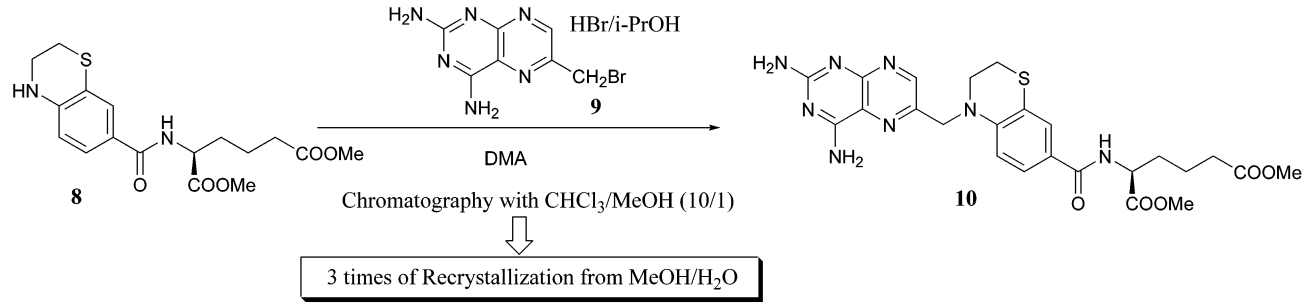
Scheme 3. **Optimized process of the diester 10 (pilot scale)**



Scheme 4. **Improved process of MX-68 for large-scale preparation**

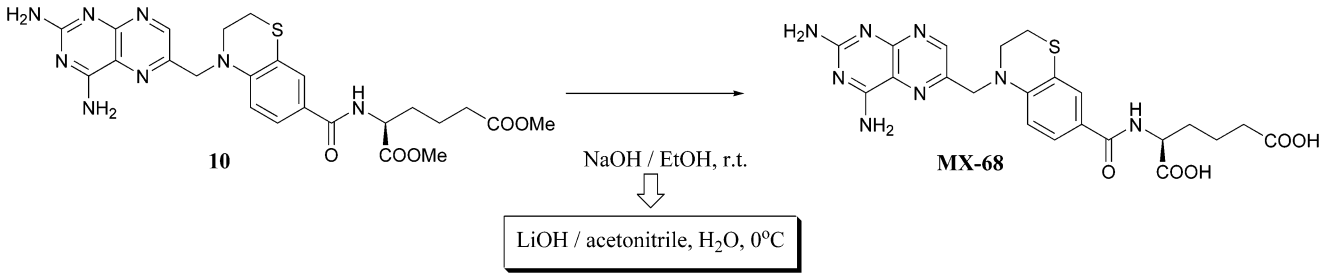


Table 2. **Racemization on hydrolysis of the diester 10**

entry	base	solvent	temp (°C)	ee of MX-68[a] (%)
1	1 N LiOH (3.3 equiv)	EtOH	20	97.0
2	1 N NaOH (3.3 equiv)	EtOH	20	96.8
3	1 N KOH (3.3 equiv)	EtOH	20	96.5

[a] The enantio excess (ee) was measured by HPLC after the obtained MX-68 was converted to the diester **10** with HCl/MeOH.

moieties can be easily substituted with a hydroxyl group in aqueous alkaline conditions.[21] To minimize the formation of the byproduct **11**, several reaction conditions, such as the reaction temperature and the reaction solvent system, were studied. At first, NaOH was replaced with LiOH as a base, because the hydrolysis using LiOH led to the least racemization as shown in Table 2. Next, EtOH was replaced with acetonitrile as a reaction solvent because of its sufficient solubility of the diester **10** and MX-68 hydrochloride salt. Furthermore, re-esterfication of MX-68 by EtOH was avoided during the hydrolysis and the HCl salt formation. As shown in Table 3, the reaction conducted at 0 °C in acetonitrile/H_2O (10/15) as a solvent system was found to be the best way relative to both the impurities and the reaction time. Upon addition of 1 N HCl to the mixture, the product could be isolated as an HCl salt. The HCl salt was neutralized with 1 N NaOH and followed by adjustment to pH 3.7 to give MX-68. By this procedure, MX-68 in 99.37% purity was obtained in 80% yield starting from 10 g of the diester **10**, and the major impurity contained was only 0.13%. Furthermore, the racemization did not occur in this condition. Hence, we confirmed the improved procedure using 2.5 kg of the diester **10**. As a result, the obtained HCl salt of MX-68 contained only 0.09% of the byproduct **11** (Scheme 4).

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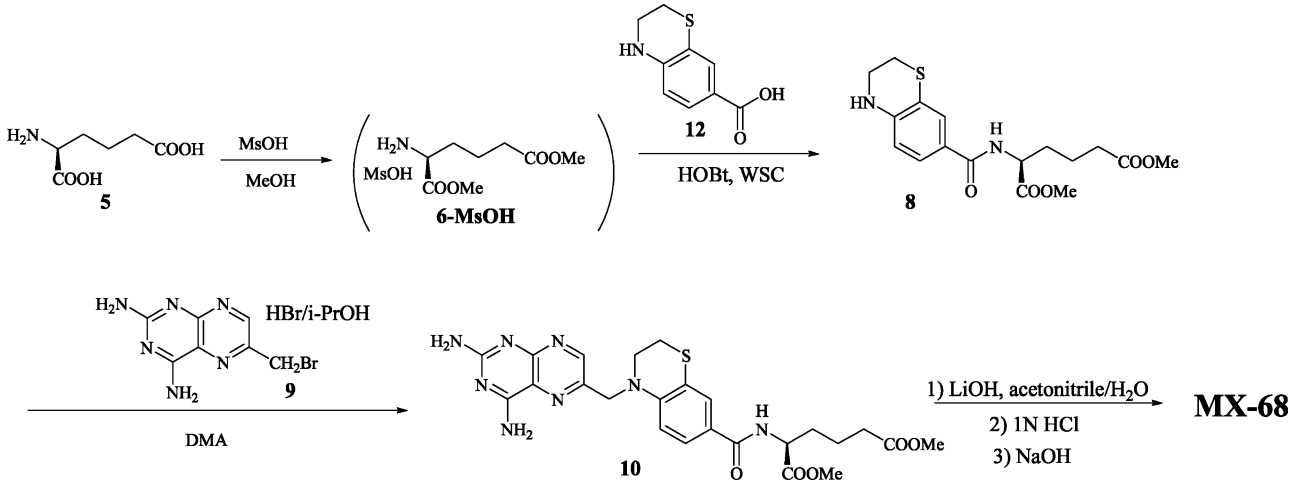
Table 3. **Major impurity formation on hydrolysis of the diester 10**

entry	solvent	temp (°C)	time (h)	HPLC area % of **11** in a reaction mixture
1	EtOH/H_2O (20/5)	0	8	0.41
2	EtOH/H_2O (20/5)	20	6	0.61
3	acetonitrile/H_2O (10/15)	0	3	0.27
4	acetonitrile/H_2O (10/15)	10	2	0.35
5	acetonitrile/H_2O (10/15)	25	1	0.48

Conclusions

We have developed a large-scale preparation route of MX-68, a novel MTX derivative, by improving the original route for used SAR studies. Because the *N*-protected amine **4**, used in the original process as a starting material, was replaced with the unprotected amine **12**, the deprotection step using HBr/AcOH, a corrosive reagent, was eliminated. Therefore, the new route is suitable for a large-scale preparation. As a result of optimizing the reaction conditions and improving the isolation method of the compound **10**, no chromatographic purification was required for the coupling reaction of the amine **8** with the 2,4-diaminopteridine **9**. Furthermore, minimizing the formation of the major byproduct **11** contaminating the final product of MX-68 was achieved by optimizing the hydrolysis conditions of the diester **10** (Scheme 5). These improved hydrolysis conditions may be generally applied to the synthesis of other MTX derivatives because the common 4-amino group of the 2,4-diaminopteridine moieties may be easily substituted with a hydroxyl group under the hydrolysis conditions.[21] Thus, the present improved process provided us with MX-68 in overall yield of 37% from the unprotected amino acid **12** and was able to supply sufficient quantities required for both the toxicity test and the clinical study of MX-68.

Scheme 5. **Improved route of MX-68 for large-scale preparation**



Experimental Section

^{1}H NMR spectra were recorded on a JEOL model JNM-EX270 NMR spectrometer with Me_4Si as the reference. Infrared spectra were run on a Hitachi model 270-3 infrared spectrometer, and EI mass spectra were recorded on a Shimadzu GCMS-QP1000 instrument. FAB and HR-FAB mass spectra were recorded on a VG Analytical VG11-250 instrument. Melting points were taken on a Yanaco Model MP apparatus. TLC was routinely performed on a Merck Kieselgel 60 F254.

HPLC analyses were performed on a SHIMADZU LC-10 or LC-6 system according to the following conditions. Method A: column, TSK-gel ODS-80 250 mm × 4.6 mm i.d.; eluent, 300:700:1 acetonitrile/water/TFA; flow rate 1.0 mL/min; wavelength 254 nm. Method B: column, YMC-Pack A-302 S-5 120A ODS-A, 150 mm × 4.6 mm i.d.; eluent, 400:600:1 acetonitrile/water/TFA; flow rate 1.0 mL/min; wavelength 254 nm. Method C: YMC-Pack AM-312 S-5 120A ODS-AM, 150 mm × 4.6 mm i.d.; eluent, 100:450:0.55 acetonitrile/water/TFA; flow rate 1.0 mL/min; wavelength 254 nm.

Dimethyl *N*-[(3,4-Dihydro-2*H*-1,4-benzothiazin-7-yl)-carbonyl]-L-homoglutamate (8). Methyl orthoformate (7.82 kg, 73.7 mol) was added to a mixture of L-homoglutamic acid (**5**) (4.95 kg, 30.7 mol) and MsOH (5.90 kg, 61.4 mol) in MeOH (61.4 L), and the mixture was refluxed for 7−8 h. After completion of the reaction, the reaction mixture was cooled to ambient temperature. Next, a mixture of 3,4-dihydro-2*H*-1,4-benzothiazine-7-carboxylic acid (**12**) (6.00 kg, 30.7 mol), HOBt (5.64 kg, 36.8 mol), and WSC (7.06 kg, 36.8 mol) in THF (61.3 mL) was stirred for 2 h at 20−30 °C, and successively added to it was the crude dimethyl L-homoglutamate methanesulfonic acid salt in MeOH and Et_3N (11.2 kg, 110.7 mol). Then, the mixture was stirred for 2.5 h at 20−30 °C. After completion of the reaction, water was added, and the resulting mixture was cooled to at 0 °C. The obtained precipitate was collected by filtration, dried, suspended in MeOH/MeCN at 5 °C for 1 h, and dried to afford 8.87 kg (78.8% yield) of the product **8** with an HPLC purity of 99.3% (Method A). ^{1}H NMR (DMSO-d_6): δ 1.5−1.7 (m, 2H, hGlu-β), 1.7−1.9 (m, 2H, hGlu-γ), 2.33 (t, 2h, J = 7.3 Hz, hGlu-δ), 2.95−2.98 (m, 2H, −CH_2S-), 3.5−3.6 (m, 2H, −NCH_2-), 3.58 (s, 3H, hGlu-α-OCH_3), 3.63 (s, 3H, hGlu-δ-OCH_3), 4.3−4.4 (m, 1H, hGlu-α), 6.51 (d, 1H, J = 8.0 Hz, Ar), 7.40 (dd, 1H, J = 2.0, 8.6 Hz, Ar), 7.52 (d, 1H, J = 2.0 Hz, Ar), 8.26 (d, 1H, J = 7.3 Hz, −CONH-). IR (KBr): cm^{-1} 3320, 1720, 1630, 1590. MS: m/z 366 (M^+), 178, 122, 59. HR-MS calcd for $C_{17}H_{22}N_2O_5S$: M, 366.1249. Found: 366.11249 (M^+).

Dimethyl *N*-[[4-[(2,4-Diaminopteridin-6-yl)methyl]-3,4-dihydro-2*H*-1,4-benzothiazin-7-yl]carbonyl]-L-homoglutamate (10). A mixture of **8** (3.50 kg, 9.55 mol) and **9** (4.70 kg, 10.5 mol) in DMA (17.5 L) was stirred at 70−80 °C for 4 h. Then, the reaction mixture was quenched by the addition of water (47.6 L) and washed with EtOAc (47.6 L). To the separated aqueous layer were added water (51.5 L) and 1 N NaOH (12.0 L), successively. After 1 h of stirring of the mixture at ambient temperature, the precipitate formed was collected by centrifugation and dried over 50 °C for 7 h to afford 5.96 kg of the crude product. A half amount of the crude product (2.92 kg) was dissolved in MeOH (87.6 L), and undissolved materials were removed by filtration. To the filtrate were added MeOH (29.2 L) and water (93.4 L) dropwise, and the mixture was cooled to 5 °C. After 1 h of stirring, the obtained precipitate was collected by centrifugation. The precipitate was dissolved in MeOH (93.4 L) at 45 °C. The MeOH solution was cooled to 30 °C, and to it was added water (74.8 kg) dropwise. The mixture was cooled to 5 °C and was stirred for 1 h. The precipitate formed was collected by centrifugation. Furthermore, the precipitate was dissolved in MeOH (84.1 L) at 50 °C. The solution was cooled to 30 °C, and water (67.3 L) was added dropwise. Then, the mixture was cooled to 5 °C. After 1 h of stirring, the obtained precipitate was collected by centrifugation and dried over 50 °C to afford 1.46 kg (56.1% yield) of the desired product **10** with an HPLC purity of 98.1% (Method B). ^{1}H NMR (DMSO-d_6): δ 1.5−1.7 (m, 2H, hGlu-β 1.7−1.8 (m, 2H, hGlu-γ), 2.32 (t, 2h, J = 7.3 Hz, hGlu-δ), 3.15−3.19 (m, 2H, −CH_2S−), 3.57 (s, 3H, hGlu-α-OCH_3), 3.61 (s, 3H, hGlu-δ-OCH_3), 3.9−4.0 (m, 2H, −NCH_2−), 4.3−4.4 (m, 1H, hGlu-α), 4.78 (s, 2H, pteridine−CH_2), 6.64 (brs, 2H, −NH_2), 6.81(d, 1H, J = 8.9 Hz, benzothiazine−Ar),

7.43 (dd, 1H, J = 2.3, 8.9 Hz, benzothiazine−Ar), 7.60 (d, 1H, J = 2.3 Hz, benzothiazine−Ar), 8.36 (d, 1H, J = 7.6 Hz, −CONH−), 8.64 (s, 1H, pteridine−Ar). IR (KBr): cm^{-1} 3500−3200, 1740, 1630. Fab-MS: m/z 541 (MH^+). HR-Fab-MS: calcd for $C_{24}H_{29}N_8O_5S$: MH^+, 541.1981. Found: 541.1989 (MH^+).

***N*-[[4-[(2,4-Diaminopteridin-6-yl)methyl]-3,4-dihydro-2*H*-1,4-benzothiazine-7-yl]carbonyl]-L-hoglutamic Acid (2, MX-68). A. Laboratory-Scale preparation.** To a suspension of **10** (10.0 g, 18.5 mmol) in acetonitrile/water (1/1, 200 mL) was added 1.22 N LiOH (50 mL) at 0 °C, and the mixture was stirred for 4 h at the same temperature. Then, water (240 mL) was added, and the reaction mixture was warmed to 5−10 °C, acidified with 1 N HCl (111 mL), stirred for 30 min at the same temperature, cooled to 0 °C, and stirred for 0.5 min at the same temperature. The obtained precipitate was collected by filtration to afford *N*-[[4-[(2,4-diaminopteridin-6-yl)methyl]-3,4-dihydro-2*H*-1,4-benzothiazine-7-yl]carbonyl]-L-homoglutamic acid hydrochloride. Next, the obtained hydrochloride salt was dissolved in 1 N NaOH at 10 °C. The solution was adjusted to pH 3.7 with 1 N HCl and stirred for 1 h at ambient temperature. The obtained precipitate was collected by filtration, washed with water (740 mL), and dried at 50 °C to afford 7.56 g (79.7% yield) of the product **2** with an HPLC purity of 99.4% (Method C). The product contained 0.11% of the major byproduct **11** by HPLC analysis. ^{1}H NMR (DMSO-d_6): δ 1.5−1.7 (m, 2H, hGlu-β), 1.7−1.8 (m, 2H, hGlu-γ), 2.22 (t, 2h, J = 7.3 Hz, hGlu-δ), 3.15−3.18 (m, 2H, $-CH_2S-$), 3.9−4.0 (m, 2H, $-NCH_2-$), 4.2−4.3 (m, 1H, hGlu-α), 4.77 (s, 2H, pteridine$-CH_2$), 6.80 (d, 1H, J = 8.6 Hz, benzothiazine−Ar), 7.44 (dd, 1H, J = 2.0, 8.6 Hz, benzothiazine−Ar), 7.60 (d, 1H, J = 2.0 Hz, benzothiazine−Ar), 8.22 (d, 1H, J = 7.6 Hz, −CONH−), 8.67 (s, 1H, pteridine−Ar). IR (KBr): cm^{-1} 3500−3300, 1640, 1590, 1500. FAB-MS: m/z 513 (MH^+). HR−FAB-MS calcd for $C_{22}H_{25}N_8O_5S$: MH^+, 513.1669. Found: 513.1675 (MH^+). Mp: 200−203 °C. Anal. ($C_{22}H_{24}N_8O_5S\cdot{}^1/_2H_2O$) C, H, N, S.

B. Scale-up Preparation of MX-68 until MX-68 Hydrochloride Salt. According to the laboratory-scale preparation method, the diester **10** (2.50 kg) was converted to MX-68 hydrochloride salt with HPLC purity of 99.6% and with contamination of 0.09% of **11** by HPLC analysis (Method C). Further conversion was not carried out.

Acknowledgment

We gratefully thank Dr. Hiroharu Matsuoka and Dr. Utaka Miura for analytical support and Dr. Tomoyasu Iwaoka, Mr. Masahiro Shimizu, Mr. Akira Hiraide, and Mr. Teizo Shinozaki for discussions about the chemistry and large-scale operations. We also acknowledge Dr. Kunio Tamura for his valuable suggestion and encouragement in the preparation of this paper.

Received for review July 8, 2004.

OP049867Y