Synthesis and Evaluation of Novel Fluorinated Methotrexate Derivatives for Application to Rheumatoid Arthritis Treatment

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An ongoing search for new antifolate drugs useful against rheumatoid arthritis (RA) led us to prepare new methotrexate (MTX) derivatives containing enantiomerically pure L-*erythro*- or L-*threo*-γ-fluoroglutamic acid. The derivatives in which the phenyl ring was replaced by a 3′-substituted phenyl or methylthiophene ring showed potent immunosuppressive activities, including *in vitro* inhibition of mitogen responses of both T and B cells and *in vivo* inhibition of antibody production in mice. These compounds also exhibited inhibitory activity in adjuvant arthritis in rats. Their toxicity was lower than that of MTX, which was probably due to the strong electronegativity of fluorine, which increases the acidity of the γ-carboxyl group and thereby decreases polyglutamylation in normal cells. These results revealed the potential of the fluorinated MTX derivatives as candidate drugs for the treatment of RA.

Introduction

Methotrexate (MTX, **1**, Figure 1), a potent antifolate in wide clinical use against various types of cancers, has also been used in the treatment of rheumatoid arthritis (RA) for more than 2 decades. During the 1980s, clinical trials of low-dose and weekly treatment have verified the efficacy for patients not treatable by conventional drugs such as nonsteroidal anti-inflammatory drugs.[1] Moreover, significant improvement of painful and swollen joints has been observed in such trials.[2] The Food and Drug Administration approved MTX for treating RA in 1988 and in recent years it has become a major therapeutic agent used in early stages of the disease in the United States and Europe. Now in Japan, phase III studies are being conducted in order to determine the adequate dosage.

The continued use of MTX in the treatment of RA has been implicated in a variety of adverse effects including gastrointestinal toxicity, stomatitis, hematologic toxicity, hepatotoxicity, and pulmonary toxicity. In addition, other undesirable side effects such as central nervous system[3] and cutaneous reactions[4] have been reported. Therefore, although MTX can be ranked among the most effective agents for the treatment of RA, particular caution and careful monitoring of the patients are needed during MTX therapy. These critical toxicities have been pointed out by only a few reports on the development of MTX derivatives useful for treating RA[5] compared with the huge number of derivatives aimed at application to cancer treatment.

Recently, poly-γ-glutamates of MTX derivatives biologically synthesized by folylpoly-γ-glutamate synthetase have been recognized as important determinants of cytotoxicity and therapeutic selectivity against malignant as compared with normal tissues in the treatment of cancers, because poly-γ-glutamates, having greater inhibitory activity against thymidylate synthase and aminoimidazolecarboxamide ribotide formyltransferase, exhibit prolonged cellular retention in comparison with the parent drug.[6] In order to reduce the formation of poly-γ-glutamates in normal cells, various

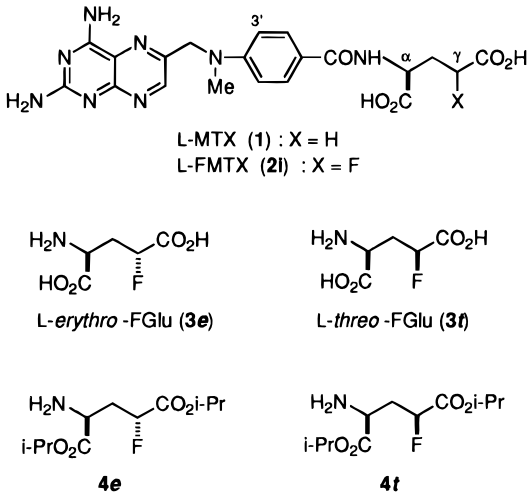

Figure 1.

modifications at the glutamic acid moiety, such as γ-carboxyl substitution with amide or peptide groups[7] and replacement of glutamic acid by other amino acids,[8] have been reported, aimed at generating a new class of antitumor agents.

We previously prepared several MTX derivatives containing α- or γ-substituted glutamic acid and found that γ-fluoromethotrexate (FMTX, **2i**) showed favorable features for high-dose treatment of MTX-resistant cancers.[9] These effects were probably due to the extreme electronegativity of fluorine causing acidity enhancement of the γ-carboxylic acid group and hence decreased its *in vivo* polyglutamate formation.[10] Since adverse effects of MTX in the treatment of RA are also assumed to be related to the accumulation of poly-γ-glutamate metabolites in normal tissues, we decided to extend this approach to the development of less toxic drugs to treat RA.

RA has been recognized as a chronic systemic autoimmune inflammatory disease, and immunological effects have been suggested to play an important role in the pathogenesis.[11] For example, proliferation of T cells triggering the release of tissue-damaging mediators from synovial cells, activation of T cells producing a variety of cytokines and eicosanoids, and antibody

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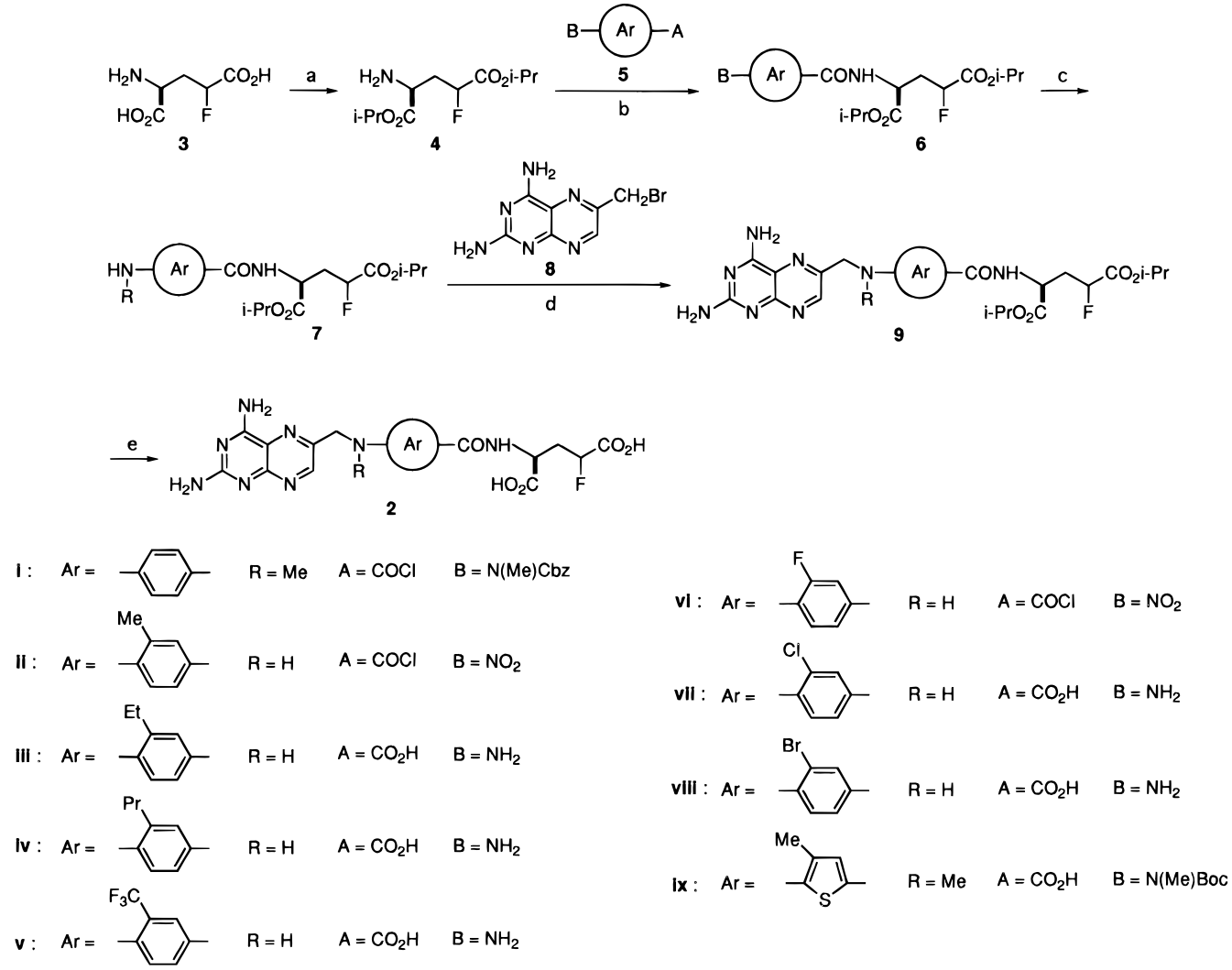
Scheme 1[a]



[a] (a) $SOCl_2$, *i*-PrOH; (b) see Table 1; (c) see Table 1; (d) see Table 1; (e) $Ba(OH)_2$, aq EtOH.

production by B cells resulting in formation of immune complexes and complement activation have been reported to be associated with the disease.

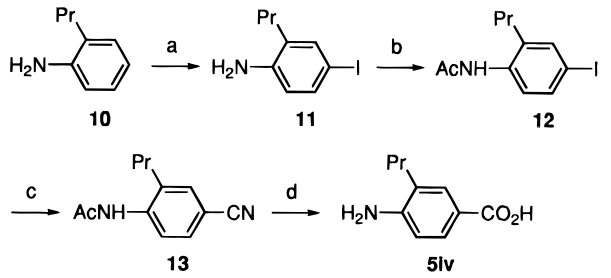
Consequently, although the precise mechanism for the efficacy of MTX in RA still remains obscure, we adopted immunological assays, *i.e.*, an *in vitro* inhibitory test against responses of both T and B cells to mitogens[12] and an *in vivo* inhibitory test against antibody production in mice for exploring new antifolate drugs useful for treating RA. Along this line, a number of MTX derivatives containing enantiomerically pure L-*erythro*- or L-*threo*-γ-fluoroglutamic acid (L-*erythro*-, L-*threo*-FGlu) were prepared. Among them, various compounds in which the phenyl ring was replaced by a 3′-substituted phenyl or a methylthiophene ring showed potent immunosuppressive activities and were selected for further evaluations such as against adjuvant arthritis in rats. The present paper describes the synthesis of these fluorinated MTX derivatives and their biological activities.

Chemistry

In considering the significant biological effect of chirality,[13] enantiomerically pure fluorinated MTX derivatives for the treatment of rheumatoid arthritis are of particular interest. Moreover, the MTX analogue containing D-glutamic acid has been reported to be a poor inhibitor of cell growth.[14] Therefore, we decided to synthesize FMTX derivatives in enantiomerically pure L-form. The synthesis of enantiomerically pure L-*threo*-FGlu (**3*t***) from L-4(*R*)-hydroxyproline has been reported by Hudlicky.[15] Recently, using this method, L-*threo*-FMTX (**2i*t***) has been prepared by Coward *et al.* and found to be a potent inhibitor of dihydrofolate reductase but inactive as a substrate for FPGS;[16] its *in vivo* activity has not been described. Consequently, in order to obtain the large amount of FMTX derivatives necessary for *in vivo* evaluation, we developed a practical way of preparing enantiomerically pure L-*erythro*- and L-*threo*-FGlu (**3*e*** and **3*t***) using aminoacylase.[17]

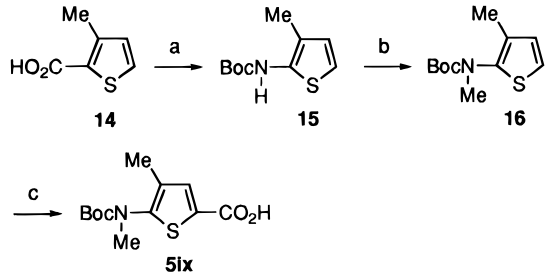
With substantial supplies of both enantiomerically pure glutamates **3*e*** and **3*t*** in hand, we sought to prepare both L-*erythro*- and L-*threo*-FMTX derivatives, for which purpose an efficient method would be to couple their protected derivatives **4*e*** and **4*t*** with a 4-aminopteroic acid derivative at a late stage of the synthesis. However, all efforts to use this approach were unrewarding.[18] Therefore, L-*erythro*- and L-*threo*-FGlu were incorporated at an early stage and the resulting compounds were manipulated using a procedure similar to that described in our previous report[9] regarding racemic FMTX **2i** as shown in Scheme 1.

Scheme 2[a]



[a] (a) I_2, $NaHCO_3$, aq MeOH; (b) Ac_2O, Py; (c) CuCN, DMF; (d) concd HCl.

Scheme 3[a]



[a] (a) DPPA, Et_3N, *t*-BuOH; (b) NaH, MeI, DMF; (c) LDA, CO_2, THF.

The utmost concern in the previous synthetic scheme was racemization. The diastereomeric composition of FMTX **2** was determined by HPLC using a reversed-phase octadecylsilica (ODS) column coated with L-stearoylcarnitine.[19] Since the analysis of enantiomers of **2** was rather difficult, the enantiomeric purity was determined on a chiral column after converting **2** to a dimethyl ester. These HPLC analyses suggested that several percent of racemization at the γ-position of FGlu occurred during hydrolysis of the isopropyl ester **9** with sodium or lithium hydroxide to give a mixture of diastereomers, while no racemization took place during the other steps, including the amide bond formation of step b. Although the use of a methyl ester would be desirable at the hydrolysis step, protection of the carboxyl group as isopropyl ester ***4e*** and ***4t*** was needed in order to prevent cyclization to a 2-pyrrolidone derivative in step b due to the inductive effect of fluorine. This racemization problem was overcome by using barium hydroxide at the hydrolysis step e.[20]

Preparation of 4-amino-3-propylbenzoic acid (**5iv**) was accomplished using a procedure similar to that described for the synthesis of 3-ethyl and 3-trifluoromethyl derivatives **5iii**[21] and **5v**[22] as shown in Scheme 2, *i.e.*, iodination of 2-propylaniline (**10**), protection of the primary amino group of **11** as an acetamide, conversion of iodide **12** to nitrile **13**, and hydrolysis of both amide and nitrile groups.

Methylthiophenecarboxylic acid **5ix** was prepared using a procedure similar to that described for the synthesis of a thiophene antifolate,[23] as shown in Scheme 3, *i.e.*, conversion of the carboxyl group of **14** to Boc-protected aminothiophene **15**, alkylation with iodomethane, and lithiation of **16** by LDA followed by the addition of carbon dioxide, though the yield of the last step c was rather low, probably because of the instability of the lithiomethylthiophene.

Table 1. Reagents of Scheme 1

compd	step b	step c	step d
i	$KHCO_3$, H_2O, CH_2Cl_2	HBr, HOAc	DMA
ii	Et_3N, DME	Zn, HOAc	DMA
iii	DEPC, Et_3N, DME	excluded	DMA
iv	DEPC, Et_3N, DME	excluded	DMA
v	DEPC, Et_3N, DME	excluded	DMA
vi	Et_3N, CH_2Cl_2	H_2, PtO_2, EtOAc	Proton-Sponge, DMA
vii	DEPC, Et_3N, DME	excluded	KI, DMA
viii	DEPC, Et_3N, DME	excluded	KI, DMA
ix	DEPC, Et_3N, DME	CF_3CO_2H, CH_2Cl_2	DMA

Table 2. *In vitro* Mitogen Responses and Primary Antibody Responses in Mice

	mitogen response IC_{50} (ng/mL)		antibody production		
compd	T cell[a]	B cell[b]	suppressive dose[c]	lethal dose[d]	safety index[e]
MTX	6.1	12.5	0.625	40	64
2i*e*	25.9	21.0	50	600	12
2i*t*	64.6	61.5	>400	–[f]	–
2ii*e*	7.8	9.9	0.31	12.5	40
2ii*t*	10.7	16.5	1.25	100	80
2iii*e*	3.8	9.7	0.625–1.25	100	80–160
2iii*t*	3.2	9.2	1.25–2.5	150	60–120
2iv*e*	5.3	22.2	5.0–10.0	200	20–40
2v*e*	8.0	14.3	5.0	–[f]	–
2v*t*	16.8	19.5	2.5–5.0	–[f]	–
2vi*e*	10.0	29.3	1.25	200	160
2vi*t*	9.4	22.7	1.25	200	160
2viii*e*	4.3	11.8	0.8	80	100
2viii*e*	2.1	8.2	2.5–5.0	>200	>40–80
2ix*e*	3.8	5.1	2.5–5.0	150	30–60
2ix*t*	11.5	14.9	10.0	>180	>18

[a] Inhibitory activity against Con A-stimulated T cell proliferation. [b] Inhibitory activity against LPS-stimulated B cell proliferation. [c] 75% suppression (mg/kg). [d] One of the mice died (mg/kg). [e] Ratio of lethal dose/suppressive dose value. [f] Not determined owing to insolubility.

Other 3-substituted 4-aminobenzoic acids derivatives were commercially available or prepared according to known procedures.[24]

Biological Evaluation and Discussion

For the primary screening test of immunological activities of the fluorinated MTX derivatives, the *in vitro* effects of MTX and these derivatives on the responses of both T and B cells to mitogens were examined.[12] As shown in Table 2, these compounds inhibited the proliferative responses of mouse spleen cells to T cell and B cell mitogens, concanavalin A (Con A) and lipopolysaccharide (LPS), respectively. Although the activity of FMTX **2i*e*** and **2i*t*** was several times weaker than that of MTX, the derivatives modified in the phenyl ring showed approximately the same potencies as MTX. These results suggested that introduction of a substituent at the 3′-position of the phenyl ring or replacement of the phenyl ring by the methylthiophene ring led to higher immunosuppressive activity. Moreover, the steric effects of this position seemed to be more important than the electronic factors for the activity, since electon-donating (**2ii**, **2iii**, **2iv**) and -withdrawing (**2v**, **2vi**, **2vii**, **2viii**) groups made no significant difference. As for the biological effect of the stereochemistry of fluorine substitution, both diastereomers showed almost equal potency, though the *erythro* diastereomers were a little more potent than the *threo* diastereomers in some cases, such as **2i** and **2ix**.[25] Therefore, the effects

Table 3. Secondary Antibody Responses in Mice

compd	antibody titer (log_2)[a]		
	0.625 mg/kg[b]	1.25 mg/kg[b]	2.5 mg/kg[b]
MTX	5.4 ± 0.6	4.2 ± 1.1	4.0 ± 0.7
2iii*e*	4.8 ± 0.8	3.8 ± 1.1	3.2 ± 1.3
2iii*t*	5.4 ± 0.9	5.2 ± 0.8	4.6 ± 1.8
2vi*e*	6.0 ± 0.7	5.4 ± 0.6	4.8 ± 0.8
2vi*t*	6.2 ± 0.8	6.8 ± 0.5	5.2 ± 0.5

[a] Mean titer ± SD. control, 7.5 ± 1.1. [b] Dose (5 days a week for 3 weeks).

Table 4. Adjuvant Arthritis in Rats

compd	ED_{50} (mg/kg)[a]	LD_{50} (mg/kg)	safety index[b]
MTX	0.058	0.82	14.1
2iii*e*	0.8	20.0	25.0
2iii*t*	0.89	10.9	12.2
2vi*e*	1.6	40.5	25.3
2vi*t*	0.62	29.0	46.8
2viii*e*	0.43	20.0	46.5
2ix*e*	0.8	46.0	57.5

[a] Inhibition of uninjected paw volume vs vehicle control. [b] Ratio of LD_{50}/ED_{50} value.

of the stereochemistry of the γ-position did not seem to be as significant as that of the α-position.

Next, to evaluate the primary immune responses to T cell dependent antigen, mice were immunized with sheep red blood cells (SRBC), and antibody production was determined after oral administration of the test compounds daily for 3 days. As shown in Table 2, the FMTX derivatives exhibited potent suppressive effects on the antibody production, though FMTX **2i*e*** and **2i*t*** had much lesser effects. The trends obtained in this *in vivo* assay and in the *in vitro* antimitogenic assay were similar. The lethal doses and safety index (lethal dose/suppressive dose) values obtained in the *in vivo* assay are also listed in Table 2. Several derivatives such as **2ii*t***, **2iii*e***, **2iii*t***, **2vi*e***, **2vi*t***, **2vii*e***, and **2viii*e*** had a greater safety index than MTX.

To evaluate secondary immune responses, mice were immunized with a low dosage of SRBC at 1-week intervals for 3 weeks, and antibody production was determined after oral administration of the test compounds **2iii*e***, **2iii*t***, **2vi*e***, and **2vi*t*** 5 days a week for 3 weeks. As shown in Table 3, these derivatives showed almost the same suppressive effects on antibody production as MTX. These results suggested that the FMTX derivatives also retained the potent immunosuppressive activity in secondary processes.

The effects of **2iii*e***, **2iii*t***, **2vi*e***, **2vi*t***, **2vii*e***, and **2ix*e*** on RA models were examined using adjuvant arthritis in rats. Although the potency of these derivatives was lower than that of MTX, they showed a significant reduction in paw swelling as well as an increase in body weight. The ED_{50} and LD_{50} values of the compounds and their safety index (LD_{50}/ED_{50}) are listed in Table 4. Compared with MTX, the FMTX derivatives exhibited a greater safety index. In addition, the inhibitory activity and toxicity of these derivatives gradually increased in a dose-dependent manner, whereas those of MTX rather abruptly increased over a narrow range. Since the potency of MTX is strong enough (dosage for RA, 7.5 mg/week),[26] the safety profile would be the most important factor for new drugs.

In order to confirm that the lower toxicity of these compounds was due to the decreased polyglutamate formation, the accumulation of **2iii*e***, **2iii*t***, **2vi*e***, and **2vi*t*** by EL4 cells was evaluated by exposing the cells to the test compounds for only 1 day. If these compounds were polyglutamylated, they should retain the ability to block cellular proliferation even after a 1-day exposure. While MTX inhibited the growth of EL4 cells with an IC_{50} of 1.57 μM under these conditions, the IC_{50} of the FMTX derivatives was >100 μM. These results suggested that the FMTX derivatives were not metabolized to the polyglutamates and were not retained within the cells, but still exhibited potent immunosuppressive and antirheumatic activities.

Conclusion

In this report, we have described the preparation of new MTX derivatives containing enantiomerically pure L-*erythro*- or L-*threo*-γ-fluoroglutamic acid as part of an ongoing search for less toxic drugs to treat RA. Several derivatives, in which the phenyl ring was replaced by a 3′-substituted phenyl or methylthiophene ring, showed potent immunosuppressive activities and inhibitory effects on adjuvant arthritis in rats and had a higher safety index than MTX. Their lower toxicity was probably due to the presence of the strongly electronegative fluorine atom, which increases the acidity of the neighboring γ-carboxyl group and is unfavorable for polyglutamylation.

From these results, the FMTX derivatives revealed their potential as new drug candidates for the treatment of RA. These studies also indicated that immunological assays such as the *in vitro* inhibitory test against responses of both T and B cells to mitogens and the *in vivo* inhibitory test against antibody production in mice are useful for evaluating the potential of MTX as antiarthritis drugs.

Experimental Section

General. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere with anhydrous solvents that had been dried over type 4A molecular sieves. Drying of an organic phase over anhydrous Na_2SO_4 is simply indicated by the word "dried". Column chromatography using Merck Silica gel 60 or a Merck Lobar column is referred to as "chromatography on silica gel". Melting points are uncorrected. ^1H and ^{13}C NMR data are presented in Table 5 and 6, respectively. ^1H NMR spectra were determined at 200 or 300 MHz. ^{13}C NMR spectra were determined at 50.3 MHz. Coupling values to fluorine of ^{13}C NMR signals are given in parentheses. Diastereomers of **2** were analyzed by HPLC using an L-column ODS (Waters, 15 × 0.46 cm) coated with L-stearoylcarnitine with 98:2 10 mM phosphate buffer (pH 7.0)−acetonitrile at 40 °C.[19] Enantiomers of the dimethyl esters of **2i*e*** and **2i*t***, which were prepared by thionyl chloride in MeOH, were analyzed by HPLC using Ultron ES-OVM (Shinwa, 15 × 0.46 cm) with 95:5 50 mM phosphate buffer (pH 6.0)−MeOH or using Ultron ES-CD (Shinwa, 15 × 0.46 cm) with 70:30 50 mM phosphate buffer (pH 6.0)−MeOH. Exact mass was determined from high-resolution liquid secondary ion mass spectra (HR-LSIMS) or fast atom bombardment mass spectra (HR-FABMS). Fractional moles of water found in analytical samples of antifolates could not be prevented in spite of drying *in vacuo* and were confirmed by the Karl Fischer method.

4-Iodo-2-propylaniline (11). To a solution of 5.0 g (37.0 mmol) of **10** in 25 mL of MeOH was added a solution of 5.0 g (59.5 mmol) of $NaHCO_3$ in 25 mL of water. After adding 8.4 g (33.1 mmol) of iodine portionwise over a 70-min period at 10 °C, the mixture was stirred for 30 min. The mixture was diluted with water and extracted with EtOAc. The organic solution was washed with 5% $Na_2S_2O_3$ solution and saturated

Table 5. ^{1}H NMR Data

compd	δ
11	($CDCl_3$) 0.99 (t, J = 7.4 Hz, 3H), 1.53−1.69 (m, 2H), 2.40 (t, J = 7.8 Hz, 2H), 3.63 (br s, 2H), 6.45 (d, J = 8.0 Hz, 1H), 7.26−7.33 (m, 2H)
12	($CDCl_3$) 0.98 (t, J = 7.4 Hz, 3H), 1.55−1.66 (m, 2H), 2.19 (s, 3H), 2.48 (t, J = 7.8 Hz, 2H), 6.97 (br s, 1H), 7.48−7.56 (m, 3H)
13	($CDCl_3$) 1.01 (t, J = 7.4 Hz, 3H), 1.61−1.72 (m, 2H), 2.24 (s, 3H), 2.57 (t, J = 7.8 Hz, 2H), 7.26 (br s, 1H), 7.46−7.53 (m, 2H), 8.18 (s, 1H)
5iv	($CDCl_3$) 1.01 (t, J = 7.4 Hz, 3H), 1.63−1.74 (m, 2H), 2.48 (t, J = 7.6 Hz, 2H), 6.65 (d, J = 9.0 Hz, 1H), 7.78−7.82 (m, 2H)
15	($CDCl_3$) 1.52 (s, 9H), 2.11 (s, 3H), 6.45 (br s, 1H), 6.69 (d, J = 3.6 Hz, 1H), 6.84 (d, J = 3.6 Hz, 1H)
16	($CDCl_3$) 1.41 (s, 9H), 2.06 (s, 3H), 3.18 (s, 3H), 6.71 (d, J = 3.6 Hz, 1H), 7.00 (d, J = 3.6 Hz, 1H)
5ix	($CDCl_3$) 1.43 (s, 9H), 2.09 (s, 3H), 3.22 (s, 3H), 7.56 (s, 1H)
6i*e*	($CDCl_3$) 1.24 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.35−2.85 (m, 2H), 3.36 (s, 3H), 4.80−5.18 (m, 4H), 5.19 (s, 2H), 6.98 (d, J = 6.6 Hz, 1H), 7.34 (s, 5H), 7.37 and 7.82 (AB q, J = 8.6 Hz, 4H)
7i*e*	($CDCl_3$) 1.24 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.29 (d, J = 6.2 Hz, 6H), 2.34−2.87 (m, 2H), 2.89 (s, 3H), 4.80−5.22 (m, 4H), 6.58 and 7.72 (AB q, J = 8.6 Hz, 4H), 6.83 (d, J = 6.6 Hz, 1H)
9i*e*	($CDCl_3$−CD_3OD) 1.23 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.28 (d, J = 6.2 Hz, 6H), 2.34−2.85 (m, 2H), 3.20 (s, 3H), 4.76 (s, 2H), 4.80−5.20 (m, 4H), 6.77 and 7.74 (AB q, J = 9.0 Hz, 4H), 6.99 (d, J = 6.4 Hz, 1H), 8.65 (s, 1H)
2i*e*	(DMSO-d_6) 2.10−2.50 (m, 2H), 3.21 (s, 3H), 4.55 (m, 1H), 4.79 (s, 2H), 5.07 (m, 1H), 6.82 and 7.71 (AB q, J = 9.0 Hz, 4H), 6.95 (br s, 2H), 7.88 (br s, 2H), 8.38 (d, J = 7.2 Hz, 1H), 8.58 (s, 1H)
6i*t*	($CDCl_3$) 1.20 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.29 (d, J = 6.4 Hz, 3H), 1.30 (d, J = 6.4 Hz, 3H), 2.38−2.85 (m, 2H), 3.36 (s, 3H), 4.84−5.18 (m, 4H), 5.19 (s, 2H), 6.84 (d, J = 6.6 Hz, 1H), 7.34 (s, 5H), 7.36 and 7.80 (AB q, J = 8.6 Hz, 4H)
9i*t*	($CDCl_3$−CD_3OD) 1.19 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.28 (d, J = 6.2 Hz, 6H), 2.25−2.70 (m, 2H), 2.88 (s, 3H), 4.83−5.20 (m, 4H), 6.58 and 7.68 (AB q, J = 8.6 Hz, 4H), 6.69 (d, J = 7.4 Hz, 1H)
2i*t*	(DMSO-d_6) 2.15−2.45 (m, 2H), 3.23 (s, 3H), 4.51 (m, 1H), 4.82 (s, 2H), 4.90 (m, 1H), 6.84 and 7.75 (AB q, J = 8.6 Hz, 4H), 7.20 (br s, 2H), 8.08 (br s, 2H), 8.43 (d, J = 8.6 Hz, 1H), 8.63 (s, 1H)
6ii*e*	($CDCl_3$) 1.24 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 6.3 Hz, 3H), 1.28 (d, J = 6.6 Hz, 3H), 1.29 (d, J = 6.6 Hz, 3H), 2.66 (s, 3H), 2.40−2.90 (m, 2H), 4.80−5.00 (m, 4H), 7.05 (br s, 1H), 7.72−7.84 (m, 2H), 8.02 (d, J = 8.4 Hz, 1H)
7ii*e*	($CDCl_3$) 1.24 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 6.3 Hz, 3H), 1.28 (d, J = 6.6 Hz, 3H), 1.29 (d, J = 6.3 Hz, 3H), 2.30 (s, 3H), 2.35−2.90 (m, 2H), 3.95 (br s, 2H), 4.80−5.20 (m, 4H), 6.67 (d, J = 7.8 Hz, 1H), 6.84 (d, J = 6.0 Hz, 1H), 7.51−7.60 (m, 2H)
9ii*e*	(DMSO-d_6) 1.15−1.18 (m, 12H), 2.22 (s, 3H), 2.23−2.48 (m, 2H), 4.46 (m, 1H), 4.56 (d, J = 5.7 Hz, 2H), 4.83−4.94 (m, 2H), 5.19 (m, 1H), 6.25 (br s, 1H), 6.58 (br s, 2H), 6.59 (d, J = 8.4 Hz, 1H), 7.52 (dd, J = 1.8 and 8.4 Hz, 1H), 7.57 (d, J = 1.8 Hz, 1H), 7.63 (br s, 2H), 8.33 (d, J = 7.5 Hz, 1H), 8.68 (s, 1H)
2ii*e*	(DMSO-d_6) 2.22 (s, 3H), 2.22−2.49 (m, 2H), 4.47 (m, 1H), 4.57 (d, J = 5.1 Hz, 2H), 5.07 (m, 1H), 6.24 (br s, 1H), 6.58 (d, J = 8.1 Hz, 1H), 6.76 (br s, 2H), 7.51−7.59 (m, 2H), 7.80 (br s, 2H), 8.28 (d, J = 7.8 Hz, 1H), 8.69 (s, 1H)
6ii*t*	($CDCl_3$) 1.24 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 6.3 Hz, 3H), 1.28 (d, J = 6.6 Hz, 3H), 1.29 (d, J = 6.3 Hz, 3H), 2.66 (s, 3H), 2.40−2.70 (m, 2H), 4.85−5.20 (m, 4H), 6.98 (br s, 1H), 7.70−7.86 (m, 2H), 8.03 (d, J = 8.4 Hz, 1H)
7ii*t*	($CDCl_3$) 1.24 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 6.3 Hz, 3H), 1.28 (d, J = 6.6 Hz, 3H), 1.29 (d, J = 6.3 Hz, 3H), 2.19 (s, 3H), 2.30−2.60 (m, 2H), 4.85−5.20 (m, 4H), 6.65−6.70 (m, 2H), 7.45−7.60 (m, 2H)
9ii*t*	(DMSO-d_6) 1.16−1.23 (m, 12H), 2.23 (s, 3H), 2.24−2.49 (m, 2H), 4.47 (m, 1H), 4.57 (d, J = 5.7 Hz, 2H), 4.85−5.12 (m, 3H), 6.26 (br s, 1H), 6.58 (br s, 2H), 6.60 (d, J = 8.4 Hz, 1H), 7.54−7.59 (m, 2H), 7.63 (br s, 2H), 8.43 (d, J = 8.1 Hz, 1H), 8.68 (s, 1H)
2ii*t*	(DMSO-d_6) 2.25 (s, 3H), 2.25−2.52 (m, 2H), 4.51 (m, 1H), 4.65 (d, J = 4.5 Hz, 2H), 4.92 (m, 1H), 6.34 (br s, 1H), 6.60 (d, J = 8.4 Hz, 1H), 7.57−7.64 (m, 2H), 7.80 (br s, 2H), 8.38 (d, J = 8.4 Hz, 1H), 8.78 (br s, 2H), 8.79 (s, 1H)
6iii*e*	($CDCl_3$) 1.52−1.61 (m, 15H), 2.74−2.91 (m, 4H), 4.27 (br s, 2H), 5.12−5.49 (m, 4H), 6.97 (m, 1H), 7.14 (d, J = 7.4 Hz, 1H), 7.78−7.94 (m, 2H)
9iii*e*	(DMSO-d_6) 1.17−1.22 (m, 15H), 2.28−2.66 (m, 4H), 4.50−4.64 (m, 3H), 4.84−4.97 (m, 2H), 5.22 (m, 1H), 6.40 (m, 1H), 6.59−6.66 (m, 3H), 7.53−7.67 (m, 4H), 8.39 (d, J = 7.2 Hz, 1H), 8.69 (s, 1H)
2iii*e*	(DMSO-d_6) 1.23 (t, J = 7.4 Hz, 3H), 2.00−2.51 (m, 2H), 2.62 (q, J = 7.4 Hz, 2H), 4.43−4.58 (m, 3H), 4.87 (m, 1H), 6.34 (m, 1H), 6.36−6.63 (m, 3H), 7.53−7.65 (m, 4H), 8.62 (d, J = 9.0 Hz, 1H), 8.67 (s, 1H)
6iii*t*	($CDCl_3$) 1.15−1.30 (m, 15H), 2.25−2.64 (m, 4H), 3.97 (br s, 2H), 4.79−5.18 (m, 4H), 6.68 (m, 1H), 7.21 (br s, 1H), 7.48−7.65 (m, 2H)
9iii*t*	(DMSO-d_6) 1.15−1.23 (m, 15H), 2.21−2.43 (m, 2H), 2.62 (q, J = 7.5 Hz, 2H), 4.41−4.58 (m, 3H), 4.83−5.18 (m, 3H), 6.39 (m, 1H), 6.42−6.63 (m, 3H), 7.53−7.66 (m, 4H), 8.45 (d, J = 7.6 Hz, 1H), 8.66 (s, 1H)
2iii*t*	(DMSO-d_6) 1.21 (t, J = 7.4 Hz, 3H), 2.16−2.27 (m, 2H), 2.61 (q, J = 7.4 Hz, 2H), 4.44−4.57 (m, 3H), 4.81 (m, 1H), 6.33 (m, 1H), 6.56−6.74 (m, 3H), 7.52−7.65 (m, 4H), 8.23 (d, J = 7.4 Hz, 1H), 8.66 (s, 1H)
6iv*e*	($CDCl_3$) 1.12 (t, J = 6.2 Hz, 3H), 1.33 (d, J = 6.2 Hz, 3H), 1.36 (d, J = 6.2 Hz, 3H), 1.45 (d, J = 6.2 Hz, 6H), 1.69−1.90 (m, 2H), 2.47−2.98 (m, 4H), 4.93−5.22 (m, 4H), 6.79 (d, J = 7.5 Hz, 1H), 6.97 (d, J = 6.2 Hz, 1H), 7.61−7.70 (m, 2H)
9iv*e*	(DMSO-d_6) 0.97 (t, J = 6.5 Hz, 3H), 1.14 (t, J = 6.2 Hz, 3H), 1.16 (d, J = 6.2 Hz, 3H), 1.21 (d, J = 6.2 Hz, 6H), 1.52−1.73 (m, 2H), 2.18−2.64 (m, 4H), 4.44−4.59 (m, 3H), 4.78−4.95 (m, 2H), 5.18 (m, 1H), 6.35 (br s, 1H), 6.54−6.63 (m, 3H), 7.46−7.55 (m, 2H), 7.63 (br s, 2H), 8.32 (d, J = 8.9 Hz, 1H), 8.63 (s, 1H)
2iv*e*	(DMSO-d_6) 0.99 (t, J = 7.4 Hz, 3H), 1.58−1.68 (m, 2H), 2.10−2.62 (m, 4H), 4.50−4.57 (m, 3H), 4.99 (m, 1H), 6.36 (br s, 1H), 6.57 (d, J = 8.4 Hz, 1H), 6.78 (br s, 2H), 7.51−7.56 (m, 2H), 7.73 (br s, 2H), 8.39 (d, J = 7.2 Hz, 1H), 8.66 (s, 1H)
6v*e*	($CDCl_3$) 1.24 (t, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.43−2.74 (m, 2H), 4.57 (br s, 2H), 4.82−5.16 (m, 4H), 6.76 (d, J = 8.6 Hz, 1H), 6.90 (d, J = 6.2 Hz, 1H), 7.77 (dd, J = 2.0 and 8.6 Hz, 1H), 7.97 (d, J = 2.0 Hz, 1H)
9v*e*	(DMSO-d_6) 1.14 (t, J = 6.2 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H), 1.18 (d, J = 6.2 Hz, 6H), 2.24−2.51 (m, 2H), 4.53 (m, 1H), 4.66 (d, J = 5.2 Hz, 2H), 4.78−4.96 (m, 2H), 5.20 (m, 1H), 6.64 (br s, 2H), 6.90−6.98 (m, 2H), 7.56 (br s, 2H), 7.87 (d, J = 8.2 Hz, 1H), 8.01 (s, 1H), 8.65 (s, 1H), 8.68 (d, J = 8.2 Hz, 1H)
2v*e*	(DMSO-d_6) 2.18−2.62 (m, 2H), 4.52 (m, 1H), 4.66 (d, J = 5.2 Hz, 2H), 4.84 (m, 1H), 6.67 (br s, 2H), 6.87−6.96 (m, 2H), 7.20 (br s, 2H), 7.89 (d, J = 8.0 Hz, 1H), 8.03 (s, 1H), 8.66 (s, 1H), 9.04 (d, J = 8.2 Hz, 1H)
6v*t*	($CDCl_3$) 1.21 (d, J = 6.2 Hz, 3H), 1.23 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.40−2.61 (m, 2H), 4.55 (br s, 2H), 4.84−5.18 (m, 4H), 6.73−6.78 (m, 2H), 7.25 (d, J = 8.6 Hz, 1H) 7.75 (dd, J = 2.0 and 8.6 Hz, 1H), 7.96 (d, J = 2.0 Hz, 1H)
9v*t*	(DMSO-d_6) 1.15 (d, J = 6.2 Hz, 3H), 1.17 (d, J = 6.2 Hz, 3H), 1.21 (d, J = 6.2 Hz, 6H), 2.17−2.68 (m, 2H), 4.50 (m, 1H), 4.67 (d, J = 5.4 Hz, 2H), 4.84−5.21 (m, 3H), 6.65 (br s, 2H), 6.93−6.99 (m, 2H), 7.58 (br s, 2H), 7.91 (d, J = 8.2 Hz, 1H), 8.04 (s, 1H), 8.66 (s, 1H), 8.76 (d, J = 8.0 Hz, 1H)
2v*t*	(DMSO-d_6) 2.15−2.38 (m, 2H), 4.53 (m, 1H), 4.66 (d, J = 5.2 Hz, 2H), 4.85 (m, 1H), 6.66 (br s, 2H), 6.86−6.98 (m, 2H), 7.62 (br s, 2H), 7.90 (d, J = 8.8 Hz, 1H), 8.04 (s, 1H), 8.58 (d, J = 7.4 Hz, 1H)

Table 5. (Continued)

compd	δ
6vi*e*	($CDCl_3$) 1.23 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.45−2.81 (m, 2H), 4.83−5.18 (m, 4H), 7.14 (d, J = 6.2 Hz, 1H), 7.71−7.82 (m, 2H), 8.15 (m, 1H)
7vi*e*	($CDCl_3$) 1.23 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.28−2.84 (m, 2H), 4.08 (br s, 2H), 4.81−5.17 (m, 4H), 6.74−6.84 (m, 2H), 7.42−7.56 (m, 2H)
9vi*e*	($CDCl_3$−CD_3OD) 1.24 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.41−2.70 (m, 2H), 4.64 (s, 2H), 4.83−5.16 (m, 4H), 6.73 (m, 1H), 7.10 (d, J = 6.6 Hz, 1H), 7.50−7.61 (m, 2H) 8.81 (s, 1H)
2vi*e*	(DMSO-d_6) 2.14−2.51 (m, 2H), 4.52 (m, 1H), 4.57 (d, J = 5.0 Hz, 2H), 4.98 (m, 1H), 6.81−6.93 (m, 4H), 7.54−7.64 (m, 2H), 7.86 (br s, 2H), 8.63 (d, J = 7.2 Hz, 1H), 8.72 (s, 1H)
6vi*t*	($CDCl_3$) 1.24 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.47−2.66 (m, 2H), 4.86−5.20 (m, 4H), 7.00 (d, J = 7.4 Hz, 1H), 7.69−7.80 (m, 2H), 8.15 (m, 1H)
7vi*t*	($CDCl_3$) 1.23 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.40−2.61 (m, 2H), 4.08 (br s, 2H), 4.85−5.14 (m, 4H), 6.69 (d, J = 7.0 Hz, 1H), 6.77 (m, 1H), 7.43−7.55 (m, 2H)
9vi*t*	($CDCl_3$−CD_3OD) 1.15 (d, J = 6.2 Hz, 3H), 1.16 (d, J = 6.2 Hz, 3H), 1.18 (d, J = 6.2 Hz, 6H), 2.25−2.84 (m, 2H), 4.65 (s, 2H), 4.61−5.15 (m, 4H), 6.74 (m, 1H), 6.96 (d, J = 6.8 Hz, 1H), 7.48−7.60 (m, 2H) 8.82 (s, 1H)
2vi*t*	(DMSO-d_6) 2.18−2.33 (m, 2H), 4.53 (m, 1H), 4.57 (d, J = 5.0 Hz, 2H), 4.83 (m, 1H), 6.81−6.91 (m, 4H), 7.56−7.66 (m, 2H), 7.78 (br s, 2H), 8.49 (d, J = 8.0 Hz, 1H), 8.71 (s, 1H)
6vii*e*	($CDCl_3$) 1.24 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H), 2.39−2.79 (m, 2H), 4.41 (br s, 2H), 4.83−5.15 (m, 4H), 6.77 (d, J = 8.1 Hz, 1H), 6.81 (d, J = 6.0 Hz, 1H), 7.56 (m, 1H), 7.79 (d, J = 2.1 Hz, 1H)
9vii*e*	(DMSO-d_6) 1.16 (d, J = 6.2 Hz, 3H), 1.17 (d, J = 6.2 Hz, 3H), 1.19 (d, J = 6.2 Hz, 6H), 2.30−2.49 (m, 2H), 4.51 (m, 1H), 4.61 (d, J = 6.0 Hz, 2H), 4.85−4.95 (m, 2H), 5.20 (m, 1H), 6.60 (br s, 1H), 6.74−6.89 (m, 3H), 7.54−7.87 (m, 4H), 8.54 (d, J = 8.4 Hz, 1H), 8.68 (s, 1H)
2vii*e*	(DMSO-d_6) 2.18−2.54 (m, 2H), 4.55 (m, 1H), 4.61 (d, J = 5.2 Hz, 2H), 5.00 (m, 1H), 6.83−6.98 (m, 4H), 7.56−7.68 (m, 2H), 7.85 (br s, 2H), 8.61 (d, J = 7.0 Hz, 1H), 8.71 (s, 1H)
6viii*e*	($CDCl_3$) 1.15 (d, J = 6.2 Hz, 3H), 1.17 (d, J = 6.2 Hz, 3H), 1.21 (d, J = 6.2 Hz, 6H), 2.21−2.65 (m, 2H), 4.53 (m, 1H), 4.82−4.91 (m, 2H), 5.23 (m, 1H), 5.90 (br s, 2H), 6.78 (d, J = 9.8 Hz, 1H), 7.61 (m, 1H), 7.96 (d, J = 2.0 Hz, 1H), 8.51 (d, J = 7.8 Hz, 1H)
9viii*e*	($CDCl_3$) 1.13 (d, J = 6.2 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H), 1.19 (d, J = 6.2 Hz, 6H), 2.18−2.54 (m, 2H), 4.55 (m, 1H), 4.64 (d, J = 6.2 Hz, 2H), 4.79−4.90 (m, 2H), 5.22 (s, 1H), 6.62 (br s, 1H), 6.78−6.87 (m, 3H), 7.64−8.03 (m, 4H), 8.56 (d, J = 8.6 Hz, 1H), 8.71 (s, 1H)
2viii*e*	(DMSO-d_6) 2.08−2.52 (m, 2H), 4.55 (m, 1H), 4.61 (d, J = 5.6 Hz, 2H), 4.93 (m, 1H), 6.57 (br s, 1H), 6.69 (br s, 2H), 6.83 (d, J = 10.2 Hz, 1H), 7.44−7.78 (m, 3H), 8.04 (d, J = 2.0 Hz, 1H), 8.67 (br s, 1H), 8.69 (s, 1H)
6ix*e*	($CDCl_3$) 1.26 (d, J = 6.0 Hz, 3H), 1.27 (d, J = 6.3 Hz, 3H), 1.29 (d, J = 6.6 Hz, 3H), 1.30 (d, J = 6.6 Hz, 3H), 1.42 (s, 9H), 2.07 (s, 3H), 2.38−2.75 (m, 2H), 3.19 (s, 3H), 4.78−5.15 (m, 4H), 6.72 (d, J = 6.3 Hz, 1H), 7.26 (s, 1H)
7ix*e*	($CDCl_3$) 1.25 (d, J = 6.6 Hz, 3H), 1.26 (d, J = 6.6 Hz, 3H), 1.28 (d, J = 6.6 Hz, 3H), 1.29 (d, J = 6.3 Hz, 3H), 2.00 (s, 3H), 2.36−2.74 (m, 2H), 2.97 (s, 3H), 4.80−5.15 (m, 4H), 6.48 (d, J = 6.0 Hz, 1H), 7.20 (s, 1H)
9ix*e*	($CDCl_3$) 1.23−1.30 (m, 12H), 2.18 (s, 3H), 2.35−2.71 (m, 2H), 2.84 (s, 3H), 4.35 (s, 2H), 4.78−5.13 (m, 4H), 6.88 (d, J = 6.6 Hz, 1H), 7.30 (s, 1H), 8.80 (s, 1H)
2ix*e*	(DMSO-d_6) 1.90−2.20 (m, 2H), 2.14 (s, 3H), 2.78 (s, 3H), 4.31 (s, 2H), 4.40 (m, 1H), 4.83 (m, 1H), 7.48 (s, 1H), 8.60 (s, 1H)
6ix*t*	($CDCl_3$) 1.22−1.30 (m, 12H), 1.42 (s, 9H), 2.07 (s, 3H), 2.35−2.57 (m, 2H), 3.19 (s, 3H), 4.87−5.15 (m, 4H), 6.57 (d, J = 6.3 Hz, 1H), 7.23 (s, 1H)
7ix*t*	($CDCl_3$) 1.22−1.29 (m, 12H), 1.99 (s, 3H), 2.31−2.58 (m, 2H), 2.97 (d, J = 4.2 Hz, 3H), 3.96 (br s, 3H), 4.87−5.13 (m, 4H), 6.31 (d, J = 7.5 Hz, 1H), 7.17 (s, 1H)
9ix*t*	($CDCl_3$) 1.20−1.30 (m, 12H), 2.17 (s, 3H), 2.30−2.54 (m, 2H), 2.83 (s, 3H), 4.34 (s, 2H), 4.84−5.13 (m, 4H), 6.70 (d, J = 8.1 Hz, 1H), 7.26 (s, 1H), 8.80 (s, 1H)
2ix*t*	(DMSO-d_6) 2.10−2.30 (m, 2H), 2.15 (s, 3H), 2.70 (s, 3H), 4.32 (s, 2H), 4.45 (m, 1H), 4.86 (m, 1H), 7.53 (s, 1H), 8.61 (s, 1H)

$NaHCO_3$ solution and then dried and evaporated to afford 9.4 g (98%) of **11** as a dark colored oil: IR ($CHCl_3$) 3431, 3384 cm^{-1}. Anal. ($C_9H_{12}IN$) C, H, I, N.

4-Iodo-2-propylacetanilide (12). To a solution of 9.4 g (36.0 mmol) of **11** in 15 mL of toluene were added 7.0 mL (90 mmol) of pyridine and 7.0 mL (78 mmol) of acetic anhydride at 0 °C. The mixture was stirred for 14 h at 25 °C. The resulting precipitate was filtered and washed with toluene and water and then dried *in vacuo* to afford 9.4 g (86%) of **12** as colorless crystals: mp 180−181 °C; IR (KBr) 1688 cm^{-1}; HR-FABMS m/z 304.0191 $(M + H)^+$ (calcd for $C_{11}H_{15}INO$ m/z 304.0198).

4-(Acetylamino)-2-propylbenzonitrile (13). To a solution of 8.9 g (29.4 mmol) of **12** in 60 mL of DMF was added 3.9 g (44.1 mmol) of copper(I) cyanide. The mixture was stirred for 5 h at 160 °C. After cooling to 25 °C, the mixture was diluted with 150 mL of EtOAc and then filtered. The filtrate was washed with 4% aqueous ammonia, 1 N HCl solution, and water. The organic solution was concentrated and the residue was recrystallized from 1:4 EtOAc−hexane to afford 5.64 g (95%) of **13** as colorless crystals: mp 131−133 °C; IR (KBr) 2229, 1670 cm^{-1}. Anal. ($C_{12}H_{14}N_2O$) C, H, N.

4-Amino-2-propylbenzoic Acid (5iv). A mixture of 5.64 g (27.9 mmol) of **13** and 125 mL of concentrated HCl solution was stirred for 4 h at 100 °C. After cooling to 0 °C, the mixture was brought to pH 2.0 by adding 50% sodium hydroxide solution. After being stirred for 30 min, the resulting precipitate was filtered and washed with water and then dried to afford 4.68 g (94%) of **5iv** as colorless crystals: mp 109−111 °C; IR (KBr) 1668 cm^{-1}; HR-FABMS m/z 180.1006 $(M + H)^+$ (calcd for $C_{10}H_{14}NO_2$ m/z 180.0988).

2-[*N*-(*tert*-Butoxycarbonyl)amino]-3-methylthiophene (15). To a solution of 8.0 g (56.3 mmol) of **14** in 60 mL of *tert*-butyl alcohol were added 7.8 mL (59.2 mmol) of triethylamine and 12.5 mL (58.0 mmol) of diphenyl phosphorazidate. The mixture was refluxed for 18 h and then poured into ice water. The resulting precipitate was filtered and washed with water. The crude product was chromatographed on silica gel using 1:8 EtOAc−hexane to afford 8.0 g (67%) of **15** as colorless crystals: mp 76−77 °C; IR (KBr) 1694 cm^{-1}. Anal. ($C_{10}H_{15}NO_2S$) C, H, N, S.

2-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]-3-methylthiophene (16). To a suspension of 0.50 g (20.6 mmol) of sodium hydride in 11 mL of DMF was added 4.0 g (18.8 mmol) of **15** at 0 °C. The mixture was stirred for 30 min at 0 °C, and 1.18 mL (19.0 mmol) of iodomethane was added. The mixture was stirred for 18 h at 25 °C, diluted with water, and extracted with ether. The organic solution was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel using 1:30 EtOAc−hexane to afford 3.47 g (81%) of **16** as a colorless oil: IR (Nujol) 1704 cm^{-1}. Anal. ($C_{11}H_{17}NO_2S$) C, H, N, S.

5-[*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino]-3-methyl-2-thiophenecarboxylic Acid (5ix). To a solution of 2.5 mL (17.5 mmol) of diisopropylamine in 17 mL of THF was added 9.92 mL (16.0 mmol) of 1.6 M *n*-butyllithium in hexane at −50 °C. To this solution was added a solution of 13.4 g (15.0 mmol) of **16** in 17 mL of THF dropwise over a 30-min period at −60

Table 6. ^{13}C NMR Data

compd	δ
11	($CDCl_3$) 14.58, 22.14, 33.52, 80.36, 117.99, 129.83, 135.94, 138.30, 144.36
12	($CDCl_3$) 14.41, 23.18, 24.83, 33.44, 90.06, 90.09, 126.12, 136.19 (2C), 138.67, 168.15
13	($CDCl_3$) 13.86, 22.18, 24.69, 32.85, 107.95, 118.98, 122.57, 130.96 (2C), 133.08, 139.60, 168.43
5iv	($CDCl_3$) 14.56, 21.93, 33.48, 114.74, 119.22, 125.80, 130.46, 132.53, 149.94, 172.87
15	($CDCl_3$) 12.52, 28.21 (3C), 81.16, 117.38, 127.44, 133.49, 152.92, 158.79
16	($CDCl_3$) 12.82, 28.25 (3C), 38.39, 80.44, 121.22, 127.99, 132.04, 140.50, 155.02
5ix	($CDCl_3$) 13.07, 28.19 (3C), 38.37, 81.45, 127.21, 133.94, 136.18, 148.65, 154.19, 167.34
6i*e*	($CDCl_3$) 21.56, 21.66 (2C), 21.77, 34.28 (d, J = 20.6 Hz), 37.38, 49.89 (d, J = 1.6 Hz), 67.73, 69.89, 70.25, 85.99 (d, J = 184.2 Hz), 125.12 (2C), 127.80 (2C), 128.00 (2C), 128.19, 128.57 (2C), 130.55, 136.23, 146.53, 155.06, 166.36, 168.72 (d, J = 23.0 Hz), 170.90
9i*e*	($CDCl_3$−CD_3OD) 21.55, 21.62 (2C), 21.74, 34.39 (d, J = 20.0 Hz), 39.32, 49.64 (d, J = 2.4 Hz), 55.91, 69.23, 70.09, 86.10 (d, J = 183.5 Hz), 111.65 (2C), 121.51, 121.87, 128.94 (2C), 147.31, 149.70, 151.67, 155.05, 162.23, 162.87, 167.02, 168.96 (d, J = 23.1 Hz), 171.29
2i*e*	(DMSO-d_6) 33.86 (d, J = 20.6 Hz), 39.19, 48.71 (d, J = 1.6 Hz), 54.79, 86.55 (d, J = 181.7 Hz), 111.01, 111.13, 121.00, 121.42, 128.72, 128.90, 146.50, 149.08, 150.89, 154.05, 162.00, 162.59, 166.04, 170.74 (d, J = 23.0 Hz), 172.99
6i*t*	($CDCl_3$) 21.59, 21.63 (2C), 21.73, 34.62 (d, J = 20.7 Hz), 37.35, 49.80 (d, J = 1.7 Hz), 67.71, 69.97, 70.02, 86.48 (d, J = 185.9 Hz), 125.06 (2C), 127.85 (2C), 127.93 (2C), 128.19, 128.53 (2C), 130.54, 136.19, 146.44, 155.08, 166.50, 168.71 (d, J = 23.1 Hz), 170.75
9i*t*	($CDCl_3$) 21.57, 21.63 (2C), 21.73, 34.75 (d, J = 20.7 Hz), 39.25, 49.55 (d, J = 3.9 Hz), 55.88, 69.86, 69.97, 86.47 (d, J = 189.1 Hz), 111.58 (2C), 121.46, 121.95, 128.99 (2C), 147.23, 149.58, 151.64, 155.00, 162.34, 162.95, 167.20, 169.00 (d, J = 23.1 Hz), 171.32
2i*t*	(DMSO-d_6) 33.40 (d, J = 20.7 Hz), 39.84, 48.40 (d, J = 1.7 Hz), 54.72, 85.77 (d, J = 181.9 Hz), 110.99 (2C), 121.00, 121.53, 128.85 (2C), 147.66, 148.92, 150.83, 151.67, 160.34, 162.58, 166.31, 170.80 (d, J = 22.3 Hz), 173.06
6ii*e*	($CDCl_3$) 20.23, 21.58, 21.68 (2C), 21.78, 34.22 (d, J = 20.5 Hz), 50.16, 70.07, 70.54, 86.14 (d, J = 185.2 Hz), 124.99, 125.62, 131.83, 134.08, 137.41, 151.21, 165.30, 168.77 (d, J = 22.9 Hz), 170.74
7ii*e*	($CDCl_3$) 17.23, 21.57, 21.65 (2C), 21.77, 34.52 (d, J = 20.4 Hz), 49.72, 69.77, 70.00, 86.11 (d, J = 184.0 Hz), 114.01, 121.52, 123.03, 126.58, 129.89, 148.48, 167.11, 168.89 (d, J = 22.9 Hz), 171.34
9ii*e*	(DMSO-d_6) 17.58, 21.24, 21.34 (2C), 21.41, 33.50 (d, J = 21.7 Hz), 46.20, 48.67, 68.13, 68.98, 86.32 (d, J = 182.2 Hz), 108.48, 120.93, 121.00, 121.24, 126.90, 129.32, 146.59, 148.65, 149.47, 155.19, 162.74, 162.82, 166.37, 168.19 (d, J = 23.5 Hz), 170.83
2ii*e*	(DMSO-d_6) 17.58, 33.91 (d, J = 21.7 Hz), 46.19, 48.80, 86.56 (d, J = 181.0 Hz), 108.47, 121.04, 121.24, 121.30, 126.90, 129.33, 147.53, 148.49, 149.38, 153.44, 161.61, 162.74, 166.24, 170.73 (d, J = 22.9 Hz), 173.01
6ii*t*	($CDCl_3$) 20.19, 21.64, 21.68 (2C), 21.76, 34.38 (d, J = 20.5 Hz), 50.08, 70.11, 70.33, 86.58 (d, J = 185.8 Hz), 124.96, 125.63, 131.87, 134.03, 137.48, 151.23, 165.34, 168.61 (d, J = 22.9 Hz), 170.52
7ii*t*	($CDCl_3$) 17.22, 21.61, 21.67 (2C), 21.77, 35.02 (d, J = 20.4 Hz), 49.56, 69.77, 69.85, 86.57 (d, J = 184.6 Hz), 113.98, 121.48, 123.03, 126.57, 129.93, 148.43, 167.16, 168.89 (d, J = 23.5 Hz), 171.23
9ii*t*	(DMSO-d_6) 17.59, 21.22, 21.34 (2C), 21.41, 33.03 (d, J = 20.5 Hz), 46.20, 48.60, 68.13, 69.02, 85.80 (d, J = 182.2 Hz), 108.48, 120.92, 120.98, 121.27, 126.92, 129.33, 146.57, 148.69, 149.45, 155.18, 162.74, 162.81, 166.72, 168.34 (d, J = 22.8 Hz), 170.96
2ii*t*	(DMSO-d_6) 17.63, 33.36 (d, J = 21.6 Hz), 46.15, 48.38, 85.71 (d, J = 181.6 Hz), 108.50, 125.33 (3C), 126.96, 129.35, 148.13, 148.37, 149.22, 150.30, 158.00, 162.73, 166.56, 170.56 (d, J = 22.9 Hz), 173.03
2iii*e*	(DMSO-d_6) 13.55, 23.85, 34.41 (d, J = 21.1 Hz), 46.59, 49.35 (d, J = 1.5 Hz), 87.10 (d, J = 181.1 Hz), 109.16, 121.51, 121.81, 127.21, 127.30, 127.97, 148.16, 148.52, 149.69, 153.09, 161.54, 163.14, 166.73, 171.47 (d, J = 22.2 Hz), 173.56
6iii*t*	($CDCl_3$) 13.18, 22.03, 22.10 (2C), 22.19, 24.37, 35.45 (d, J = 19.6 Hz), 49.95 (d, J = 1.5 Hz), 70.20, 70.28, 85.43 (d, J = 185.1 Hz), 114.91, 123.73, 126.66, 127.77, 128.50, 147.99, 167.55, 169.29 (d, J = 23.1 Hz), 171.56
9iii*t*	(DMSO-d_6) 13.53, 21.79 (3C), 21.88, 23.86, 33.48 (d, J = 20.1 Hz), 46.62, 49.05 (d, J = 1.5 Hz), 68.62, 69.49, 86.25 (d, J = 182.6 Hz), 109.17, 121.35, 121.46, 127.21, 127.32, 128.00, 147.17, 148.39, 149.81, 155.55, 163.16, 163.19, 167.24, 168.80 (d, J = 23.1 Hz), 171.41
2iii*t*	(DMSO-d_6) 13.46, 23.77, 33.99 (d, J = 21.1 Hz), 46.48, 48.95 (d, J = 1.5 Hz), 86.43 (d, J = 181.2 Hz), 109.05, 121.31, 121.63, 127.07, 127.20, 127.86, 147.86, 148.11, 149.61, 153.99, 162.11, 163.03, 166.88, 171.59 (d, J = 21.2 Hz), 173.84
6iv*e*	($CDCl_3$) 14.59, 22.03, 22.12 (3C), 22.23, 33.72, 34.91 (d, J = 20.6 Hz), 50.14 (d, J = 1.5 Hz), 70.23, 70.48, 86.48 (d, J = 183.6 Hz), 115.02, 123.53, 126.39, 126.77, 129.51, 148.36, 167.53, 169.33 (d, J = 22.6 Hz), 171.75
9iv*e*	(DMSO-d_6) 14.24, 21.58, 21.70 (2C), 21.77, 21.81, 32.88, 33.77 (d, J = 20.6 Hz), 46.45, 48.90 (d, J = 3.0 Hz), 68.47, 69.31, 84.60 (d, J = 181.6 Hz), 109.16, 121.22 (2C), 125.61, 127.15, 128.92, 147.15, 148.33, 149.64, 155.36,163.03 (2C), 166.72, 168.52 (d, J = 24.1 Hz), 171.19
2iv*e*	(DMSO-d_6) 14.29, 21.85, 32.89, 34.52 (d, J = 22.1 Hz), 46.47, 49.50 (d, J = 3.0 Hz), 87.44 (d, J = 180.6 Hz), 109.14, 121.27, 121.52, 125.67, 127.11, 128.91, 147.59, 148.18, 149.58, 154.60, 162.52, 163.01, 166.50, 171.60 (d, J = 22.1 Hz), 173.59
6v*e*	($CDCl_3$) 21.96, 22.05 (2C), 22.15, 34.78 (d, J = 20.1 Hz), 50.21 (d, J = 1.5 Hz), 70.30, 70.53, 86.49 (d, J = 185.1 Hz), 112.98 (q, J = 30.7 Hz), 117.00, 122.63, 125.10 (q, J = 254.4 Hz), 127.07 (q, J = 5.6 Hz), 132.17, 148.10, 166.25, 169.19 (d, J = 23.1 Hz), 171.17
9v*e*	(DMSO-d_6) 21.19, 21.30 (2C), 21.36, 33.21 (d, J = 21.1 Hz), 45.77, 48.64 (d, J = 3.0 Hz), 68.25, 68.94, 86.17 (d, J = 182.1 Hz), 111.00 (q, J = 30.4 Hz), 111.86, 120.36, 120.76, 124.62 (q, J = 272.1 Hz), 126.10 (q, J = 3.2 Hz), 132.86, 145.59, 147.16, 149.23, 155.13,162.60, 162.80, 165.01, 168.09 (d, J = 23.1 Hz), 170.53
6v*t*	($CDCl_3$) 21.98, 22.07 (2C), 22.17, 35.06 (d, J = 19.6 Hz), 50.18 (d, J = 1.5 Hz), 70.32, 70.37, 86.87 (d, J = 165.0 Hz), 113.21 (q, J = 30.2 Hz), 117.01, 122.60, 124.97 (q, J = 256.8 Hz), 127.21 (q, J = 1.5 Hz), 132.24, 148.17, 166.41, 169.28 (d, J = 23.1 Hz), 171.23
9v*t*	(DMSO-d_6) 21.30 (3C), 21.36, 32.87 (d, J = 20.6 Hz), 45.77, 48.69, 68.28, 69.02, 85.68 (d, J = 181.6 Hz), 111.04 (q, J = 29.5 Hz), 111.86, 120.36, 120.77, 125.95 (q, J = 254.3 Hz), 126.30 (q, J = 3.2 Hz), 132.86, 145.59, 147.16, 149.21, 155.14, 162.60, 162.80, 165.40, 168.25 (d, J = 23.1 Hz), 170.65
7vi*e*	($CDCl_3$) 21.98, 22.07 (2C), 22.17, 35.06 (d, J = 19.6 Hz), 50.18 (d, J = 1.5 Hz), 70.32, 70.37, 86.87 (d, J = 165.0 Hz), 113.21 (q, J = 30.2 Hz), 117.01, 122.60, 124.97 (q, J = 256.8 Hz), 127.21 (q, J = 1.5 Hz), 132.24, 148.17, 166.41, 169.28 (d, J = 23.1 Hz), 171.23
9vi*e*	(DMSO-d_6) 21.30 (3C), 21.36, 32.87 (d, J = 20.6 Hz), 45.77, 48.69, 68.28, 69.02, 85.68 (d, J = 181.6 Hz), 111.04 (q, J = 29.5 Hz), 111.86, 120.36, 120.77, 125.95 (q, J = 254.3 Hz), 126.30 (q, J = 3.2 Hz), 132.86, 145.59, 147.16, 149.21, 155.14, 162.60, 162.80, 165.40, 168.25 (d, J = 23.1 Hz), 170.65
2vi*e*	(DMSO-d_6) 34.56 (d, J = 20.6 Hz), 46.03, 49.94 (d, J = 2.0 Hz), 87.56 (d, J = 152.4 Hz), 111.49, 113.53 (d, J = 31.8 Hz), 121.40, 121.85 (d, J = 5.6 Hz), 125.19, 139.36 (d, J = 12.0 Hz), 146.76, 149.92, 150.33 (d, J = 239.4 Hz), 154.91, 162.81, 163.06, 163.18, 163.41, 165.25

Table 6 (Continued)

compd	δ
6vi*t*	($CDCl_3$) 22.07, 22.11 (2C), 22.18, 34.11 (d, J = 19.6 Hz), 50.63, 70.66, 70.95, 86.94 (d, J = 186.2 Hz), 118.31 (q, J = 23.1 Hz), 123.36 (d, J = 4.8 Hz), 127.04 (d, J = 3.2 Hz), 139.58, 140.77 (d, J = 7.2 Hz), 155.81 (d, J = 266.5 Hz), 164.25, 168.95 (d, J = 23.6 Hz), 170.59
7vi*t*	($CDCl_3$) 22.03, 22.19 (2C), 22.30, 35.15 (d, J = 21.1 Hz), 50.71, 69.76, 71.03, 86.46 (d, J = 184.6 Hz), 115.51 (d, J = 33.5 Hz), 115.91 (d, J = 19.2 Hz), 123.89 (d, J = 12.0 Hz), 124.32 (d, J = 7.2 Hz), 138.90 (d, J = 13.6 Hz), 151.09 (d, J = 240.2 Hz), 166.41, 169.24 (d, J = 23.1 Hz), 171.39
9vi*t*	(DMSO-d_6) 21.69 (3C), 21.77, 33.24 (d, J = 19.6 Hz), 46.01, 49.05 (d, J = 2.5 Hz), 68.61, 69.40, 86.05 (d, J = 183.6 Hz), 111.62, 114.04 (d, J = 19.2 Hz), 121.26, 121.37, 125.37, 139.65 (d, J = 12.0 Hz), 146.35, 149.96, 150.31 (d, J = 238.6 Hz), 155.63, 163.29, 163.37, 165.98, 168.64 (d, J = 23.1 Hz), 171.13
2vi*t*	(DMSO-d_6) 34.16 (d, J = 22.1 Hz), 46.03, 49.34 (d, J = 6.0 Hz), 86.73 (d, J = 183.6 Hz), 111.48, 113.91 (d, J = 19.9 Hz), 121.28, 121.65 (d, J = 5.5 Hz), 125.01 (d, J = 7.9 Hz), 139.31 (d, J = 12.7 Hz), 146.60, 149.80, 150.21 (d, J = 238.2 Hz), 154.81, 162.70, 162.93 (d, J = 12.7 Hz), 165.54, 172.01, 173.59
6vii*e*	($CDCl_3$) 22.01, 22.12 (2C), 22.23, 34.82 (d, J = 20.1 Hz), 50.22, 70.34, 70.64, 86.46 (d, J = 184.1 Hz), 108.90, 115.05, 124.54, 128.01, 132.65, 147.85, 166.08, 169.23 (d, J = 23.1 Hz), 171.56
9vii*e*	(DMSO-d_6) 21.23, 21.32 (2C), 21.40, 33.28 (d, J = 21.1 Hz), 45.87, 48.71, 68.22, 68.97, 86.21 (d, J = 183.1 Hz), 110.64, 117.27, 120.87, 121.80, 127.88, 128.37, 145.79, 146.16, 149.45, 149.82, 155.18, 162.68, 162.82, 165.00, 168.14 (d, J = 22.6 Hz), 170.60
2vii*e*	(DMSO-d_6) 34.51 (d, J = 20.6 Hz), 45.84, 49.62 (d, J = 4.0 Hz), 87.90 (d, J = 180.6 Hz), 110.56, 110.72, 114.59, 117.29, 120.87, 121.50, 122.26, 127.66, 128.37, 145.90, 149.38, 154.81, 162.63, 164.50, 174.44
6viii*e*	(DMSO-d_6) 22.03, 22.12 (2C), 22.23, 34.82 (d, J = 20.6 Hz), 50.24 (d, J = 1.5 Hz), 70.34, 70.65, 86.46 (d, J = 184.6 Hz), 108.91, 115.07, 124.56, 128.03, 132.65, 147.82, 166.08, 169.23 (d, J = 23.1 Hz), 171.56
9viii*e*	(DMSO-d_6) 21.72, 21.82, 21.88, 23.86, 33.74 (d, J = 21.6 Hz), 46.48, 49.15 (d, J = 3.5 Hz), 68.73, 69.48, 86.70 (d, J = 182.6 Hz), 108.08, 111.19, 122.88, 128.60, 128.95, 132.14 (2C), 147.30, 147.52, 149.33, 149.82, 163.13, 165.33, 168.61 (d, J = 23.1 Hz), 171.06
2viii*e*	(DMSO-d_6) 34.46 (d, J = 20.6 Hz), 46.49, 49.72 (d, J = 3.5 Hz), 87.47 (d, J = 181.1 Hz), 108.13, 111.14, 121.39, 123.24, 128.90, 132.17, 146.81, 147.40, 149.84, 154.55, 162.54, 163.11, 165.16, 171.72 (d, J = 22.3 Hz), 173.50
6ix*e*	($CDCl_3$) 13.09, 21.54, 21.64, 21.67, 21.75, 28.22 (3C), 34.41 (d, J = 20.5 Hz), 38.39, 49.76, 69.89, 70.29, 81.22, 85.99 (d, J = 184.6 Hz), 130.05, 132.38, 133.54, 145.52, 154.33, 161.63, 168.72 (d, J = 23.5 Hz), 170.82
9ix*e*	($CDCl_3$) 13.59, 21.22, 21.33 (2C), 21.39, 33.39 (d, J = 20.4 Hz), 43.22, 48.58, 59.69, 68.33, 69.05, 86.19 (d, J = 182.8 Hz), 121.24, 124.32, 126.83, 131.65, 144.62, 149.91, 155.26, 156.97, 161.26, 162.78, 162.93, 168.11 (d, J = 23.4 Hz), 170.47
2ix*e*	(DMSO-d_6) 13.51, 34.17 (d, J = 21.5 Hz), 43.16, 49.28, 59.74, 87.19 (d, J = 181.1 Hz), 121.19, 124.52, 127.43, 131.29, 144.83, 149.86, 154.71, 156.63, 160.96, 162.53, 162.68, 171.21 (d, J = 22.3 Hz), 172.89
6ix*t*	($CDCl_3$) 13.07, 21.64, 21.70 (2C), 21.76, 28.20 (3C), 34.87 (d, J = 19.9 Hz), 38.38, 49.66, 70.00, 70.09, 81.18, 86.47 (d, J = 185.7 Hz), 130.02, 132.43, 133.50, 145.54, 154.34, 161.65, 168.74 (d, J = 23.4 Hz), 170.69
9ix*t*	(DMSO-d_6) 13.57, 21.33 (2C), 21.39 (2C), 32.94 (d, J = 19.9 Hz), 43.19, 48.54, 59.72, 68.37, 69.09, 85.68 (d, J = 182.8 Hz), 121.24, 124.34, 126.71, 131.74, 144.64, 149.94, 155.27, 157.10, 161.61, 162.78, 162.93, 168.27 (d, J = 22.9 Hz), 170.63
2ix*t*	(DMSO-d_6) 13.54, 33.67 (d, J = 21.7 Hz), 43.19, 48.61, 59.78, 85.98 (d, J = 182.2 Hz), 121.25, 124.52, 127.29, 131.44, 144.90, 149.93, 154.79, 156.82, 161.31, 162.59, 162.76, 170.86 (d, J = 21.7 Hz), 172.80

°C. After being stirred for 30 min, crushed dry ice was added at −50 °C. The mixture was allowed to warm to 25 °C and stirred for 18 h. The mixure was poured into water, brought to pH 5.0 by adding citric acid, and then extracted with $CHCl_3$. The organic solution was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel using 1:20 MeOH−$CHCl_3$ to afford 1.51 g (37%) of **5ix** as colorless crystals: mp 133−134 °C; IR (KBr) 1715, 1666 cm^{-1}. Anal. ($C_{12}H_{17}NO_4S$) C, H, N, S.

N*-[4-[[(Benzyloxy)carbonyl]methylamino]benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6i*e*).** To a solution of 2.68 g (9.39 mmol) of **4*e·HCl[17] in 20 mL of CH_2Cl_2 was added a solution of 7.25 g (75.1 mmol) of potassium bicarbonate in 40 mL of water at 0 °C. After addition of 3.42 g (11.3 mmol) of **5i** in 20 mL of CH_2Cl_2, the mixture was stirred for 24 h at 25 °C and then diluted with EtOAc. The organic solution was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel using 1:2 EtOAc−hexane to afford 4.14 g (85%) of **6i*e*** as colorless crystals: mp 78−79 °C; $[\alpha]^{24}_D$ +21.4° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1731, 1702, 1663 cm^{-1}. Anal. ($C_{27}H_{33}FN_2O_7$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9i*e*).** A solution of 4.04 g (7.82 mmol) of **6i*e in 25 mL of 30% hydrogen bromide in acetic acid was stirred for 4 h at 25 °C. After adding 250 mL of ether at 0 °C, the mixture was stirred for 1 h and then left for 24 h. The organic layer was decanted and then the residue was washed with ether three times. The resulting residue was dissolved in EtOAc. The organic solution was washed with saturated $NaHCO_3$ solution and brine and then dried and evaporated to afford *N*-[4-(methylamino)benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic acid α,γ-diisopropyl ester (**7i*e***), which was used for the next reaction without further purification.

To a solution of the above product in 25 mL of *N*,*N*-dimethylacetamide was added 2.64 g (6.67 mmol) of **8**.[27] The mixture was stirred for 18 h at 50 °C. After adding 300 mL of water at 0 °C, the mixture was stirred for 5 h and left for 24 h. The resulting precipitate was filtered and dissolved in 1:9 MeOH−$CHCl_3$. The organic solution was washed with saturated $NaHCO_3$ solution and water and then dried and concentrated. The residue was chromatographed on silica gel using 1:10 MeOH−$CHCl_3$ to afford 2.87 g (67%) of **9i*e*** as a yellow powder: mp 152−154 °C; $[\alpha]^{24}_D$ +7.2° (*c* 1.0, MeOH−$CHCl_3$); IR (KBr) 1735, 1655 cm^{-1}. Anal. ($C_{26}H_{33}FN_8O_5$·0.4H_2O) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2i*e*).** To a suspension of 1.13 g (2.04 mmol) of **9i*e in 25 mL of ethanol was added a solution of 1.6 g (5.1 mmol) of barium hydroxide octahydrate in 25 mL of water at 0 °C. After being stirred for 5 h at 25 °C, ethanol was evaporated. The mixture was brought to pH 3.0 by adding 1 N HCl solution. The precipitate was filtered and washed with water and then dried *in vacuo* at 40 °C to afford 0.84 g (87%) of **2i*e*** as a yellow powder: mp >253 °C; $[\alpha]^{25}_D$ +20.9° (*c* 1.0, 0.1 N NaOH); IR (KBr) 1639 cm^{-1}. HPLC t_R = 6.3 min; HPLC of the dimethyl ester on Ultron ES-OVM, t_R = 28.8 min, on Ultron ES-CD, t_R = 17.8 min. Anal. ($C_{20}H_{21}FN_8O_5$·2.3H_2O) C, H, F, N.

N*-[4-[[(Benzyloxy)carbonyl]methylamino]benzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6i*t*).** The procedure described for the preparation of **6i*e was used: colorless oil; $[\alpha]^{23.5}_D$ +15.5° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1731, 1701, 1665 cm^{-1}. Anal. ($C_{27}H_{33}FN_2O_7$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9i*t*).** The procedure described for the preparation of **9i*e was used.

***N*-[4-(Methylamino)benzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic acid α,γ-diisopropyl ester (7i*t*)** was used for the next reaction without further purification.

9i*t*: mp 158−159 °C; $[\alpha]^{25}_D$ +9.8° (*c* 1.0, MeOH−$CHCl_3$); IR (KBr) 1736, 1633 cm^{-1}. Anal. ($C_{26}H_{33}FN_8O_5 \cdot 0.5H_2O$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]-benzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid (2i*t*).** The procedure described for the preparation of **2i*e was used: mp >255 °C; $[\alpha]^{24}_D$ +11.4° (*c* 1.0, 0.1 N NaOH); IR (KBr) 1638 cm^{-1}. HPLC t_R = 11.6 min; HPLC of the dimethyl ester on Ultron ES-OVM, t_R = 25.6 min, on Ultron ES-CD, t_R = 23.5 min. Anal. ($C_{20}H_{21}FN_8O_5 \cdot 1.8H_2O$) C, H, F, N.

N*-(3-Methyl-4-nitrobenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6ii*e*).** The procedure described for the preparation of **6i*e was done using triethylamine and DME: mp 70−71 °C; $[\alpha]^{22}_D$ +35.0° (*c* 0.5, $CHCl_3$); IR (KBr) 1749, 1726, 1645, 1528, 1345 cm^{-1}. Anal. ($C_{19}H_{25}FN_2O_7$) C, H, F, N.

N*-(4-Amino-3-methylbenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (7ii*e*).** To a solution of 904 mg (2.19 mmol) of **6ii*e in 22 mL of acetic acid was added 1.7 g of zinc dust. The mixture was stirred for 2.5 h at 25 °C and filtered. The filtrate was concentrated *in vacuo*. The residue was poured into saturated $NaHCO_3$ solution and then extracted with $CHCl_3$. The organic solution was washed with saturated $NaHCO_3$ solution and brine and then dried and concentrated. The residue was chromatographed on silica gel using 1:3 EtOAc−hexane to afford 671 mg (80%) of **7ii*e*** as colorless crystals: mp 121−122 °C; $[\alpha]^{22}_D$ +28.2° (*c* 0.5, $CHCl_3$); IR (KBr) 1762, 1724, 1638 cm^{-1}. Anal. ($C_{19}H_{27}FN_2O_5$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-methylbenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9ii*e*).** The procedure described for the preparation of **9i*e was used: mp 234−236 °C; $[\alpha]^{23}_D$ +10.0° (*c* 0.5, DMSO); IR (KBr) 1735, 1633 cm^{-1}; HR-LSIMS *m*/*z* 557.2637 $(M + H)^+$ (calcd for $C_{26}H_{34}FN_8O_5$ *m*/*z* 557.2634).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-methylbenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2ii*e*).**[28] The procedure described for the preparation of **2i*e was used: mp >270 °C; IR (KBr) 1639 cm^{-1}. HPLC t_R = 6.5 min; HR-LSIMS *m*/*z* 473.1695 $(M + H)^+$ (calcd for $C_{20}H_{22}FN_8O_5$ *m*/*z* 473.1695).

N*-(3-Methyl-4-nitrobenzoyl)-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6ii*t*).** The procedure described for the preparation of **6i*e was done using triethylamine and DME: colorless oil; $[\alpha]^{23}_D$ +19.2° (*c* 0.5, $CHCl_3$); IR (film) 1738, 1652, 1527, 1347 cm^{-1}. Anal. ($C_{19}H_{25}FN_2O_7$) C, H, F, N.

N*-(4-Amino-3-methylbenzoyl)-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (7ii*t*).** The procedure described for the preparation of **7ii*e was used: mp 91−92 °C; $[\alpha]^{22}_D$ +24.0° (*c* 0.5, $CHCl_3$); IR (KBr) 1743, 1717, 1636 cm^{-1}. Anal. ($C_{19}H_{27}FN_2O_5$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-methylbenzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9ii*t*).** The procedure described for the preparation of **9i*e was used: mp 233−235 °C; $[\alpha]^{23}_D$ +14.3° (*c* 0.5, DMSO); IR (KBr) 1735, 1633 cm^{-1}; HR-LSIMS *m*/*z* 557.2631 $(M + H)^+$ (calcd for $C_{26}H_{34}FN_8O_5$ *m*/*z* 557.2634).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-methylbenzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid (2ii*t*).**[28] The procedure described for the preparation of **2i*e was used: mp >270 °C; IR (KBr) 1638 cm^{-1}. HPLC t_R = 7.1 min; HR-LSIMS *m*/*z* 473.1694 $(M + H)^+$ (calcd for $C_{20}H_{22}FN_8O_5$ *m*/*z* 473.1695).

N*-(4-Amino-3-ethylbenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6iii*e*).** The procedure described for the preparation of **6iii*t was used: mp 58−59 °C; HR-FABMS *m*/*z* 397.2130 $(M + H)^+$ (calcd for $C_{20}H_{30}FN_2O_5$ *m*/*z* 397.2139).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-ethylbenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9iii*e*).** The procedure described for the preparation of **9i*e was used: mp 231−233 °C; HR-FABMS *m*/*z* 571.2827 $(M + H)^+$ (calcd for $C_{27}H_{36}FN_8O_5$ *m*/*z* 571.2793).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-ethylbenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2iii*e*).** The procedure described for the preparation of **2i*e was used: mp 239−242 °C; $[\alpha]^{23}_D$ +7.6° (*c* 0.5, DMSO). HPLC t_R = 13.2 min; HR-FABMS *m*/*z* 487.1880 $(M + H)^+$ (calcd for $C_{21}H_{24}FN_8O_5$ *m*/*z* 487.1854).

N*-(4-Amino-3-ethylbenzoyl)-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6iii*t*).** To a solution of 2.60 g (9.08 mmol) of **4*t·HCl[17] in 45 mL of DMF was added a solution of 1.5 g (9.08 mmol) of **5iii**. After cooling to −40 °C, 1.7 mL (10.9 mmol) of diethyl cyanophosphonate (DEPC) and 5.06 mL (36.3 mmol) of triethylamine were added. The mixture was allowed to warm to 25 °C and stirred for 14 h. The mixture was poured into water and extracted with EtOAc. The organic solution was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel using 1:2 EtOAc−hexane to afford 2.63 g (73%) of **6iii*t*** as colorless crystals: mp 59−61 °C; $[\alpha]^{23}_D$ +20.9° (*c* 0.5, $CHCl_3$); IR (KBr) 1731, 1632 cm^{-1}. Anal. ($C_{20}H_{29}FN_2O_5$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-ethylbenzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9iii*t*).** The procedure described for the preparation of **9i*e was used: mp 234−236 °C; $[\alpha]^{23}_D$ +13.4° (*c* 0.5, DMSO); IR (KBr) 1737, 1627 cm^{-1}. Anal. ($C_{27}H_{35}FN_8O_5 \cdot 0.6H_2O$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-ethylbenzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid (2iii*t*).** The procedure described for the preparation of **2i*e was used: mp 254−256 °C; $[\alpha]^{23}_D$ +15.6° (*c* 0.5, DMSO); IR (KBr) 1638 cm^{-1}. HPLC t_R = 15.0 min; HR-FABMS *m*/*z* 487.1876 $(M + H)^+$ (calcd for $C_{21}H_{24}FN_8O_5$ *m*/*z* 487.1854).

N*-(4-Amino-3-propylbenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6iv*e*).** The procedure described for the preparation of **6iii*t was used: mp 94−95 °C; $[\alpha]^{22}_D$ +24.1° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 1733, 1653 cm^{-1}. Anal. ($C_{21}H_{31}FN_2O_5$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-propylbenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9iv*e*).** The procedure described for the preparation of **9i*e was used: mp 227−229 °C; $[\alpha]^{23}_D$ +10.2° (*c* 0.5, DMSO); IR (KBr) 1735, 1626 cm^{-1}. Anal. ($C_{28}H_{37}FN_8O_5 \cdot 0.3H_2O$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-propylbenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2iv*e*).** The procedure described for the preparation of **2i*e was used: mp >270 °C; $[\alpha]^{23}_D$ +15.9° (*c* 0.5, DMSO); IR (KBr) 1638 cm^{-1}. HPLC t_R = 32.0 min; HR-FABMS *m*/*z* 501.2028 $(M + H)^+$ (calcd for $C_{22}H_{26}FN_8O_5$ *m*/*z* 501.2010).

N*-[4-Amino-3-(trifluoromethyl)benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6v*e*).** The procedure described for the preparation of **6iii*t was used: colorless oil; $[\alpha]^{23}_D$ +15.4° (*c* 0.5, $CHCl_3$); HR-FABMS *m*/*z* 437.1697 $(M + H)^+$ (calcd for $C_{19}H_{25}F_4N_2O_5$ *m*/*z* 437.1699).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-(trifluoromethyl)benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9v*e*).** The procedure described for the preparation of **9i*e was used: mp 241−243 °C; $[\alpha]^{22}_D$ +9.1° (*c* 0.5, DMSO); HR-FABMS *m*/*z* 611.2379 $(M + H)^+$ (calcd for $C_{26}H_{31}F_4N_8O_5$ *m*/*z* 611.2353).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-(trifluoromethyl)benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2v*e*).**[28] The procedure described for the preparation of **2i*e was used: mp >270 °C. ^1H NMR (DMSO-d_6) δ 2.18−2.62 (m, 2H), 4.52 (m, 1H), 4.66 (d, *J* = 5.2 Hz, 2H), 4.84 (m, 1H), 6.67 (br s, 2H), 6.87−6.96 (m, 2H), 7.20 (br s, 2H), 7.89 (d, *J* = 8.0 Hz, 1H), 8.03 (s, 1H), 8.66 (s, 1H), 9.04 (d, *J* = 8.2 Hz, 1H); HPLC t_R = 17.8 min; HR-FABMS *m*/*z* 527.1424 $(M + H)^+$ (calcd for $C_{20}H_{19}F_4N_8O_5$ *m*/*z* 527.1415).

N*-[4-Amino-3-(trifluoromethyl)benzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6v*t*).** The procedure described for the preparation of **6iii*t was used: colorless oil; $[\alpha]^{22}_D$ +8.0° (*c* 0.5, $CHCl_3$); HR-FABMS *m*/*z* 437.1690 $(M + H)^+$ (calcd for $C_{19}H_{25}F_4N_2O_5$ *m*/*z* 437.1699).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-(trifluoromethyl)benzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9v*t*).** The procedure described for the preparation of **9i*e was used: mp 237−239 °C; $[\alpha]^{22}_D$ +9.5° (*c*

0.5, DMSO); IR (KBr) 1735, 1622 cm^{-1}; HR-FABMS *m/z* 611.2362 $(M + H)^+$ (calcd for $C_{26}H_{31}F_4N_8O_5$ *m/z* 611.2353).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-(trifluoromethyl)benzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid (2v*t*).**[28] The procedure described for the preparation of **2i*e was used: mp >270 °C; IR (KBr) 1632 cm^{-1}. HPLC t_R = 18.5 min; HR-FABMS *m/z* 527.1427 $(M + H)^+$ (calcd for $C_{20}H_{19}F_4N_8O_5$ *m/z* 527.1415).

N*-(3-Fluoro-4-nitrobenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6vi*e*).** The procedure described for the preparation of **6i*e was done using triethylamine and CH_2Cl_2. mp 86−87 °C. HR-FABMS *m/z* 417.1483 $(M + H)^+$ (calcd for $C_{18}H_{23}F_2N_2O_7$ *m/z* 417.1473).

N*-(4-Amino-3-fluorobenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (7vi*e*).** A solution of 980 mg (2.35 mmol) of **6vi*e in 25 mL of EtOAc was hydrogenated using 60 mg of platinum(IV) oxide for 4 h. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was chromatographed on silica gel using 1:1 EtOAc−hexane to afford 818 mg (100%) of **7vi*e*** as colorless crystals: mp 102−104 °C; $[\alpha]^{22}_D$ +24.6° (*c* 0.5, $CHCl_3$); HR-FABMS *m/z* 387.1718 $(M + H)^+$ (calcd for $C_{18}H_{25}F_2N_2O_5$ *m/z* 387.1732).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-fluorobenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9vi*e*).** The procedure described for the preparation of **9i*e was done using Proton-Sponge: mp 212−214 °C; $[\alpha]^{22}_D$ +6.1° (*c* 0.5, DMSO). HR-FABMS *m/z* 561.2376 $(M + H)^+$ (calcd for $C_{25}H_{31}F_2N_8O_5$ *m/z* 561.2385).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-fluorobenzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2vi*e*).** The procedure described for the preparation of **2i*e was used: mp >270 °C; $[\alpha]^{22}_D$ +26.9° (*c* 0.5, DMSO); HPLC t_R = 5.7 min; HR-LSIMS *m/z* 477.1450 $(M + H)^+$ (calcd for $C_{19}H_{19}F_2N_8O_5$ *m/z* 477.1446).

N*-(3-Fluoro-4-nitrobenzoyl)-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6vi*t*).** The procedure described for the preparation of **6i*e was done using triethylamine and CH_2Cl_2: mp 88−90 °C; $[\alpha]^{22}_D$ +17.0° (*c* 0.5, $CHCl_3$); IR (KBr) 1730, 1650, 1543, 1353 cm^{-1}. Anal. ($C_{18}H_{22}F_2N_2O_7$) C, H, F, N.

N*-(4-Amino-3-fluorobenzoyl)-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (7vi*t*).** The procedure described for the preparation of **7vi*e was used: mp 101−103 °C; $[\alpha]^{22}_D$ +20.8° (*c* 0.5, $CHCl_3$); IR (KBr) 1735, 1636 cm^{-1}. Anal. ($C_{18}H_{24}F_2N_2O_5$) C, H, F, N.

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-fluorobenzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9vi*t*).** The procedure described for the preparation of **9i*e was done using Proton-Sponge: mp 215−217 °C; $[\alpha]^{22}_D$ +12.1° (*c* 0.5, DMSO); IR (KBr) 1735, 1624 cm^{-1}; HR-FABMS *m/z* 561.2394 $(M + H)^+$ (calcd for $C_{25}H_{31}F_2N_8O_5$ *m/z* 561.2386).

N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]amino]-3-fluorobenzoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid (2vi*t*).** The procedure described for the preparation of **2i*e was used: mp >270 °C; $[\alpha]^{22}_D$ +28.5° (*c* 0.5, DMSO); IR (KBr) 1618 cm^{-1}. HPLC t_R = 6.0 min; HR-FABMS *m/z* 477.1461 $(M + H)^+$ (calcd for $C_{19}H_{19}F_2N_8O_5$ *m/z* 477.1446).

N*-(4-Amino-3-chlorobenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6vii*e*).** The procedure described for the preparation of **6iii*t was used: mp 124−125 °C; $[\alpha]^{22}_D$ +27.0° (*c* 1.0, $CHCl_3$); IR (KBr) 1765, 1725, 1629 cm^{-1}; HR-FABMS *m/z* 403.1421 $(M + H)^+$ (calcd for $C_{18}H_{25}ClFN_2O_5$ *m/z* 403.1436).

N*-[3-Chloro-4-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9vii*e*).** The procedure described for the preparation of **9i*e was done using potassium iodide: mp 224−226 °C; $[\alpha]^{22}_D$ +7.4° (*c* 0.5, DMSO); IR (KBr) 1736, 1631 cm^{-1}; HR-FABMS *m/z* 577.2108 $(M + H)^+$ (calcd for $C_{25}H_{31}{}^{35}ClFN_8O_5$ *m/z* 577.2090).

N*-[3-Chloro-4-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2vii*e*).** The procedure described for the preparation of **2i*e was used: mp >270 °C; IR (KBr) 1630 cm^{-1}; HR-FABMS *m/z* 493.1148 $(M + H)^+$ (calcd for $C_{19}H_{19}{}^{35}ClFN_8O_5$ *m/z* 493.1151); HPLC t_R = 11.8 min.

N*-(4-Amino-3-bromobenzoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6viii*e*).** The procedure described for the preparation of **6iii*t was used: mp 121−123 °C; $[\alpha]^{22}_D$ +25.7° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 1733, 1657 cm^{-1}. Anal. ($C_{18}H_{24}BrFN_2O_5$) C, H, Br, F, N.

N*-[3-Bromo-4-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9viii*e*).** The procedure described for the preparation of **9i*e was done using potassium iodide: mp 213−215 °C; $[\alpha]^{22}_D$ +11.3° (*c* 0.5, DMSO); IR (KBr) 1735, 1635 cm^{-1}; HR-FABMS *m/z* 621.1583 $(M + H)^+$ (calcd for $C_{25}H_{31}{}^{79}BrFN_8O_5$ *m/z* 621.1585).

N*-[3-Bromo-4-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2viii*e*).** The procedure described for the preparation of **2i*e was used: mp >270 °C; $[\alpha]^{23}_D$ +10.5° (*c* 0.5, DMSO); IR (KBr) 1637 cm^{-1}. HPLC t_R = 16.9 min; HR-FABMS *m/z* 537.0676 $(M + H)^+$ (calcd for $C_{19}H_{19}{}^{79}BrFN_8O_5$ *m/z* 537.0646).

N*-[5-[(*tert*-Butoxycarbonyl)methylamino]-4-methyl-2-thenoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6ix*e*).** The procedure described for the preparation of **6iii*t was used: colorless oil; $[\alpha]^{23}_D$ +19.5° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 1731, 1700, 1654 cm^{-1}; HR-LSIMS *m/z* 503.2226 $(M + H)^+$ (calcd for $C_{23}H_{36}FN_2O_7S$ *m/z* 503.2225).

N*-(4-Methyl-5-(methylamino)-2-thenoyl)-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (7ix*e*).** To a solution of 410 mg (0.90 mmol) of **6ix*e in 10 mL of CH_2Cl_2 was added 10 mL of trifluoroacetic acid. The mixture was stirred for 1 h at 25 °C and concentrated *in vacuo*. The residue was poured into saturated $NaHCO_3$ solution and then extracted with EtOAc. The organic solution was washed with saturated $NaHCO_3$ solution and brine and then dried and concentrated. The residue was chromatographed on silica gel using 7:50 EtOAc-$CHCl_3$ to afford 590 mg (100%) of **7ix*e*** as a colorless oil: IR ($CHCl_3$) 1733, 1634 cm^{-1}; HR-LSIMS *m/z* 403.1705 $(M + H)^+$ (calcd for $C_{18}H_{28}FN_2O_5S$ *m/z* 403.1702).

N*-[5-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]-4-methyl-2-thenoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9ix*e*).** The procedure described for the preparation of **9i*e was used: mp 182−183 °C; IR (KBr) 1735, 1628 cm^{-1}; HR-LSIMS *m/z* 577.2356 $(M + H)^+$ (calcd for $C_{25}H_{34}FN_8O_5S$ *m/z* 577.2355).

N*-[5-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]-4-methyl-2-thenoyl]-(α*S*,γ*R*)-γ-fluoroglutamic Acid (2ix*e*).**[28] The procedure described for the preparation of **2i*e was used: mp >270 °C; IR (KBr) 1626 cm^{-1}. HPLC t_R = 18.4 min; HR-LSIMS *m/z* 493.1418 $(M + H)^+$ (calcd for $C_{19}H_{22}FN_8O_5S$ *m/z* 493.1417).

N*-[5-[(*tert*-Butoxycarbonyl)methylamino]-4-methyl-2-thenoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (6ix*t*).** The procedure described for the preparation of **6iii*t was used: colorless oil; $[\alpha]^{23}_D$ +13.4° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) 1732, 1700, 1655 cm^{-1}; HR-LSIMS *m/z* 503.2223 $(M + H)^+$ (calcd for $C_{23}H_{36}FN_2O_7S$ *m/z* 503.2225).

N*-(4-Methyl-5-(methylamino)-2-thenoyl)-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (7ix*t*).** The procedure described for the preparation of **7ix*e was used: colorless solid; IR (KBr) 1739, 1618 cm^{-1}; HR-LSIMS *m/z* 403.1703 $(M + H)^+$ (calcd for $C_{18}H_{28}FN_2O_5S$ *m/z* 403.1702).

N*-[5-[(2,4-Diamino-6-pteridinyl)methyl]methylamino]-4-methyl-2-thenoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid α,γ-Diisopropyl Ester (9ix*t*).** The procedure described for the preparation of **9i*e was used: mp 188−189 °C; IR (KBr) 1737, 1628 cm^{-1}; HR-LSIMS *m/z* 577.2356 $(M + H)^+$ (calcd for $C_{25}H_{34}FN_8O_5S$ *m/z* 577.2355).

N*-[5-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]-4-methyl-2-thenoyl]-(α*S*,γ*S*)-γ-fluoroglutamic Acid (2ix*t*).**[28] The procedure described for the preparation of **2i*e was used: mp >270 °C; IR (KBr) 1637 cm^{-1}. HPLC t_R = 18.8 min; HR-LSIMS *m/z* 493.1413 $(M + H)^+$ (calcd for $C_{19}H_{22}FN_8O_5S$ *m/z* 493.1417).

***In Vitro* Mitogen Responses.** The effects on mitogen responses were determined as described in our previous report.[12]

Primary Antibody Responses in Mice. A procedure similar to that reported from these laboratories was used.[29]

Female BDF_1 mice (6 weeks old) were immunized intravenously with 1×10^8 SRBC. A sodium salt of test compound dissolved in water was administered *per os* from day 1 to day 3. The mice were sacrificed on day 7 and the blood was collected. Two-fold dilutions of 0.05 mL of test serum were prepared in phosphate-buffered saline containing 5×10^{-5} M 2-mercaptoethanol in 96-well microtiter plates to which 0.05 mL of 1% suspension of SRBC was added, and then the plates were incubated at 25 °C for 14 h. The hemagglutination titers were determined visually.

Secondary Antibody Responses in Mice. Female BDF_1 mice (6 weeks old) were immunized intravenously with 1×10^5 SRBC on day 0, 7, and 14. The sodium salt of the test compound dissolved in water was administered *per os* 5 days a week from day 0 (total, 15 days). The mice were sacrificed on day 20 and antibody production was determined as described above.

Adjuvant Arthritis in Rats. A procedure similar to that described by Winder was used.[30] Female Lewis rats (7 weeks old), weighing 140−160 g, were sensitized by a subcutaneous injection of 0.05 mL of liquid paraffin containing 0.5 mg of *Mycobacterium butyricum* in the left hind paw. A 0.6% suspension of the test compound in arabic gum was administered *per os* five days a week from day 1 (total, 12 days). Paw volumes were measured until day 17 with a plethysmometer and the effect of inhibition of uninjected paw volume versus the vehicle control was evaluated.

Accumulation in EL4 Cells. EL4 thymoma cell line from C57BL/6 mice was put in each well of a 96-well microtiter plate in one 0.1-mL scale containing 4×10^4 cells. The test compound in 0.1 mL of DMSO was added to each well in such a manner that its final concentration was in the range of 0−50 μg/mL. After 1 day incubation at 37 °C in a humidified atmosphere of air containing 5% carbon dioxide, the proliferation response was determined as described in our previous report.[12]

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