Stereospecific Synthesis of Tetradeuterated (R)- and (S)-ifosfamide

Jeff J.-H. Wang[†] and Kenneth K. Chan[‡,*]

[†]School of Pharmacy, University of Southern California, Los Angeles, CA 90033

[‡]Colleges of Pharmacy and Medicine, the Ohio State University, Columbus, OH 43210

Summary

The tetradeuterated analogues of (R)- and (S)-ifosfamide [(S)- and (R)-2-(2-chloro-2,2-dideuterioethyl)amino-3-(2-chloroethyl)-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide, **(S)- and (R)-1-d4**] were synthesized by modification of a literature procedure. The enantiomers of α–methylbenzylamine were employed as the resolving agents in the multi-step synthesis in a 9% overall yield. The selected labeled positions should minimize potential isotope effects useful for the investigation of stereoselective metabolism of ifosfamide by pseudoracemate methodology.

Key Words: ifosfamide, enantiomers, pseudoracemate, mass spectrometry

Introduction

It has now become clear that enantiomers of a chiral molecule may display major differences in metabolism, pharmacokinetics, and pharmacological effects (1). Ifosfamide (IF, **1**), a widely used alkylating agent and a structural isomer of the important drug cyclophosphamide (CP), possesses an asymmetric phosphorus atom, therefore existing in enantiomers (2-5). The clinically used form of IF is a racemic mixture. IF itself is not cytotoxic but requires hepatic activation (5,6). The most important metabolic pathway is 4-hydroxylation and generates 4-hydroxyifosfamide (4-OHIF) which may play an important role in transporting cytotoxic species across cells (7-9). Subsequent ring opening and cleavage of this metabolite generates iphosphoramide mustard (IPM) which is important for antitumor activity (10,11). Dealkylation on the 2-chloroethyl side chains generates N2- and N3-dechloroethyl ifosfamides (N2D and N3D) along with 2-chloroacetyl aldehyde which has been

* Author for correspondence

CCC 0362–4803/96/020105–11
©1996 by John Wiley & Sons, Ltd.
Received 10 April 1995
Revised 1 September 1995

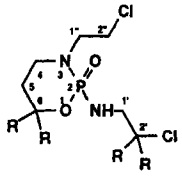
implicated in the involvement in neurotoxicity observed in patients receiving IF therapy (12,13). These various metabolic pathways may display stereoselectivity, which may link to the observed differences in the antitumor activity and/or toxicity between the enantiomers of IF (14). However, systematic investigations on the stereoselective metabolism and pharmacokinetics of IF have been scarce and the results obtained were inconclusive (15-17).

The use of a pseudoracemate (a 1:1 mixture of unlabeled and labeled enantiomers of a chiral drug) is a powerful tool to obtain reliable metabolic and pharmacokinetic data in stereoselective disposition study of chiral drugs. This methodology has been successfully used in several studies, including warfarin and CP (18-21). Several aspects for designing the labeled enantiomers must be considered (19). Firstly, the labeled compounds must possess physicochemical properties similar to the unlabeled molecules in order to have nearly identical behaviors such as distribution, extraction, and derivatization. For this reason, a minimal number of deuterium labels should be incorporated into the molecules, yet still allowing adequate mass discrimination between the labeled and unlabeled compounds. Secondly, the labeled positions should be remote from sites of metabolism to minimize metabolic isotope effect. Thirdly, the labels should be retained in the major metabolites so that the concentrations of these metabolites as well as their origin could be traced by a mass spectrometric method. Finally, the designed compounds should be synthetically achievable with reasonable efforts.

IF contains two chlorine atoms, which give rise to a cluster of isotope peaks at M, M+2, and M+4 in an approximate ratio of 9:6:1 under chemical ionization mass spectrometry. Such cluster ions may interfere with ions from deuterium labeling. For example, M+4 ion from $^{37}Cl_2$ of the unlabeled IF will interfere with IF labeled with 4 deuterium atoms. However, when IF was derivatized with silylating agent (MSTFA) and processed through GC/CIMS, the IF derivative was converted to a dehydrochlorinated cyclic compound probably due to thermal degradation. Therefore, in this case four deuterium atoms would be adequate for tracking the labeled enantiomer since the monitored ion would be 2 mass units higher than the ^{37}Cl ion of the unlabeled dehydrochlorinated IF.

Seven carbon atoms of IF could be potentially labeled with deuterium atoms. However, certain positions are not suitable for deuterium labeling because they are sites for metabolic transformation and resultant isotope effects may complicate data interpretation. Among them are C4, C1', C1", and C5. The first three positions are involved in enzymatic hydroxylation and the last is involved in the formation of iphosphoramide mustard (5). C6 was then chosen as one of the labeling sites. The other site selected was C2' because of synthetic feasibility. Therefore, (R)- and (S)-2-(2-chloro-2,2-dideuterioethyl)amino-3-(2-chloroethyl)-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide

[**(R)**- and **(S)-1-d4**] were designed for the use in the pseudoracemate-mass spectrometric studies (see structure). Using these compounds, most of the metabolites could be traced by the deuterium labels.



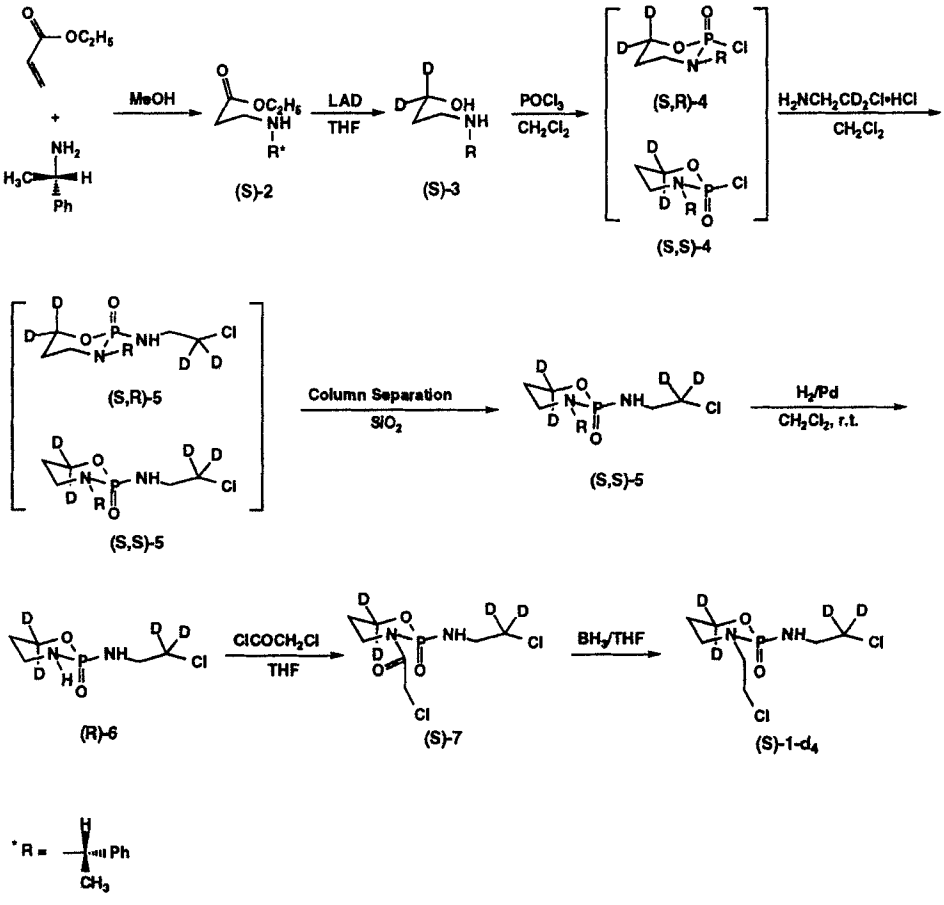
1 R = H

1-d_4 R = D

Results and Discussion

The stereospecific synthesis of the enantiomeric forms of IF was first reported in 1978 by Kinas *et al.* (22). The synthetic method was based on the introduction of an asymmetric α-methylbenzyl group to the exocyclic nitrogen atom of IF and, after separation of diastereomers, the removal of the N-attached chiral moiety. They found that the hydrogenation removal of the α-methylbenzyl group was very inefficient and (S)- and (R)-IF were obtained in very low yield (22,23). Pankiewicz *et al.* developed an alternative method (24). In their method, N-(S)–α–methylbenzyl-3-aminopropan-1-ol was first synthesized by reaction of (S)–α–methylbenzylamine with 3-chloroethylpropan-1-ol. Reaction of this amino alcohol with phosphorus oxychloride afforded a mixture of diastereomers of 2-chloro-3-(S)-α–methylbenzyl-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide in a ratio of 4:1. After condensation with aziridine (ethylenimine), this mixture gave diastereomers of phosphoroethylenimides in the same ratio. The predominant (S,S)-diastereomer was separated and treated with anhydrous hydrogen chloride to give (S)-2-(2-chloroethyl)amino-3-[(S)–α–methylbenzyl]tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide. Hydrogenolysis of this compound in ethanol for 2-4 days readily removed the methylbenzyl group on the endocyclic nitrogen and provided (R)-2-(2-chloroethyl)amino-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide with a yield of 73%. Upon acylation with chloroacetyl chloride and subsequent reduction with diborane in THF, it gave (S)-IF. (R)-IF was synthesized by a similar method using (R)-α-methylbenzylamine as the resolving agent. In all of these steps, no racemization occurred and regioselectivity was observed in some cases.

In order to adapt these synthetic routes to the present synthesis of labeled enantiomeric forms of IF, several modifications were made as outlined in Scheme 1 for the (S)-enantiomer. Ring deuterium labels must be introduced to the skeleton before ring closure. Thus, Michael addition of the (S)- amine with ethyl acrylate in methyl alcohol under reflux condition for 2 days led to the formation



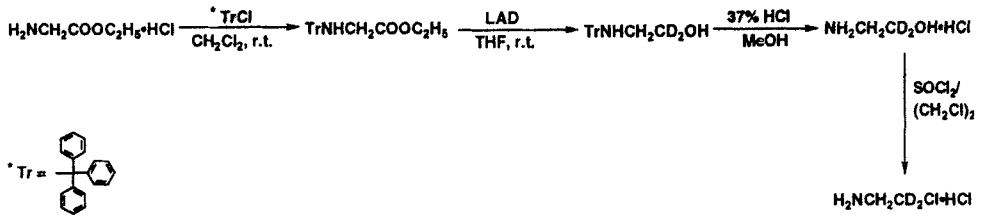
SCHEME 1

of ethyl N-(S)–α–methylbenzyl-3-aminopropionate [**(S)-2**], which was purified by vacuum distillation. Reduction of **(S)-2** with lithium aluminum deuteride (LAD) gave the corresponding alcohol, N-(S)–α–methylbenzyl-1,1-dideuterio-3-aminopropan-1-ol [**(S)-3**], one of the deuterium-labeled synthons. The reduction was carried out in anhydrous THF at room temperature. No major side product was detected by TLC and MS and the reaction was quantitative. Condensation of **(S)-3** with phosphorus oxychloride formed the oxazaphosphorine frame with a mixture of diastereomers of (S)- and (R)-2-chloro-3-[(S)–α–methylbenzyl]-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [**(S, S)-4** and **(S,R)-4**]. The reaction was carried out in CH_2Cl_2 at -78°C, and it was found that low temperature favored the formation of a diastereomer subsequently idnentified to be the desired **(S,S)-4**.

The side chain deuterium labeled synthon, 2-chloro-2,2-dideuterioethylamine hydrochloride, the synthesis of which is described later, was then added directly to the reaction mixture to give the

diastereomeric mixture, (S)- and (R)-2-(2-chloro-2,2-dideuterioethyl)amino-3-[(S)–α–methylbenzyl]-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [**(S,S)-5** and **(S,R)-5**]. The predominant diastereomer **(S,S)-5** appeared in solid state and was separated by silicic acid column chromatography. The minor diastereomer **(S,R)-5**, which appeared in liquid form, was found difficult to purify and therefore was discarded. The removal of the α-methylbenzyl group from **(S,S)-5** by hydrogenation in CH_2Cl_2 formed the (R)-enantiomer of 2-(2-chloro-2,2-dideuterioethyl)amino-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [**(R)-6**]. It was found that significant degradation products were detected when ethyl alcohol, the conventional solvent for hydrogenation, was used according to the literature procedure (24). Additionally, it was found necessary to wash the catalyst palladium on charcoal with hexane immediately before use. The reason for this effect is not known, presumably due to the renewal of the surface of the catalyst. Reaction of **(R)-6** with an equal molar amount of chloroacetyl chloride in THF at room temperature gave (S)-2-(2-chloro-2,2-dideuterioethyl)amino-3-(2-chloroacetyl)-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [**(S)-7**]. This process was regiospecific (24). Only the desired product was detected by TLC. There was no evidence of acylation on the exocyclic nitrogen nor diacylation. Subsequent reduction to **(S)-1-d4** required the use of an excess amount of diborane for complete reaction. **(R)-1-d4** was prepared similarly using (R)-α-methylbenzylamine as the starting marterial. Both **(R)-** and **(S)-1-d4** were obtained with isotopic purity of greater than 99.5% as determined by GC/MS analysis. Since most of the synthetic procedures adopted those published and no racemization was reported, the stereochemistry of the intermediates were first tentatively assigned based on the literature information and the optical rotation values were not measured. Their stereochemical assignments were verified based on the end products, the enantiomers of IF, which showed identifical optical rotation values to those reported (24).

The synthesis of 2-chloro-2,2-dideuterioethylamine hydrochloride was accomplished by an indirect method (Scheme 2). Direct reduction of ethyl glycine ester with LAD was not successful due to poor extraction efficiency. This might be due to a tight binding of the product with the inorganic salt. Thus, ethyl glycine ester hydrochloride was first treated with triphenylmethyl chloride in CH_2Cl_2 to give the highly lipophilic compound ethyl N-triphenylmethyl glycine ester, which was readily reduced by LAD at room temperature. The alcohol product was readily isolated by THF extraction in quantitative yield. The triphenylmethyl group was removed by concentrated hydrochloric acid (37%) in methanol under reflux condition to give 2,2-dideuterioethanolamine, which was readily crystallized



SCHEME 2

as the HCl salt in ethyl alcohol. Chlorination of this alcohol with thionyl chloride in 1,2-dichloroethane gave 2-chloro-2,2-dideuterioethylamine, which precipitated as its HCl salt. The yield was almost quantitative and the purity of the starting 2,2-dideuterioethanolamine was found to be the major determinant for the completion of chlorination.

Experimental

The entire synthesis was first evaluated repetitively using protiated starting materials. All melting points were measured on a Kofler hot stage and uncorrected. CH_2Cl_2, THF, $(CH_2Cl)_2$ were purchased from E. M. Science (Gibbstown, N.J.), distilled and dried by conventional methods before use. All chemicals were purchased from Aldrich Chemical Inc. (Milwaukee, WI).

Mass spectra were obtained on a Hewlett-Packard 5985A quadrupole mass spectrometer under chemical ionization (CI) using ammonia as the reagent gas. ^{1}HNMR spectra were recorded with a Bruker NR-250 FT NMR spectrometer and chemical shifts reported relative to internal tetramethylsilane. Optical activity measurements were made with a Perkin-Elmer 241 photopolarimeter. Silicic acid (70-230 mesh) was used for column chromatography and all TLC were performed on silicic acid plates.

Ethyl N-(S)-α-methylbenzyl-3-aminopropionate [(S)-2] and its enantiomer [(R)-2]. To a solution of ethyl acrylate (11.65 g, 116.5 mmol) in absolute ethyl alcohol (50 ml) was added a solution of S-(-)-α-methylbenzylamine (14.10 g, 116.5 mmol) in the same solvent (50 ml) at room temperature. The mixture was refluxed for 24 hrs. Ethanol was then removed by rotary evaporation under reduced pressure. The remaining oily residue (22.62 g) was distilled under vacuum (136°C/0.65 mm Hg, oil bath 150-160°C) to give **(S)-2** as a colorless liquid (15.88 g, 62%): Rf 0.72 (ethyl acetate); NMR ($CDCl_3$) δ 1.25 (t, J=6.8 Hz, 3H, -O-CH_2-$\underline{CH_3}$), 1.34 (d, J=6.6 Hz, 3H, -CH-$\underline{CH_3}$), 1.72 (bs, 1H, -NH-), 2.46 (t, J=6.5 Hz, 2H, -CO-$\underline{CH_2}$-), 2.70 (m, 2H, -NH-$\underline{CH_2}$-),

3.77 (q, J=6.6 Hz, 1H, -CH-CH_3), 4.13 (q, J=6.8 Hz, 2H, -O-CH_2-CH_3), 7.22-7.40 (m, 5H, 5 x -Ph-H); MS (CI) *m/z* 222 (MH^+). Similarly, starting with R-(+)-α-methylbenzylamine, **(R)-2** was obtained in 64 % yield, with same TLC and NMR data.

N-(S)-α-Methylbenzyl-1,1-dideuterio-3-aminopropan-1-ol [(S)-3] and its enantiomer [(R)-3]. To a cooled (-78°C) suspension of LAD (0.76 g, 18.1 mmol) in THF (50 ml) was added dropwise a solution of **(S)-2** (4.00 g, 18.1 mmol) in THF (10 ml). The temperature of the reaction mixture was then raised to ambient temperature (22°C) and the stirring was continued for 1 hr. Thereafter, water (0.8 g) in THF (50 ml) was added to decompose the excessive hydride. Precipitated solid was removed by filtration and washed with THF (50 ml x 3). The filtrate and washings were combined and concentrated *in vacuo* to give **(S)-3** as a pale yellow liquid (3.19 g, 98%): Rf 0.13 (ethyl acetate); NMR ($CDCl_3$) δ 1.36 (d, J=6.6 Hz, 3H, -CH-CH_3), 1.40-1.92 (m, 3H, -NH-, -CH_2-CH_2-CD_2-), 2.60-2.85 (m, 2H, -NH-CH_2-), 3.73 (q, J=6.6 Hz, 1H, -CH-CH_3), 7.20-7.42 (m, 5H, 5 x -Ph-H); MS (CI) *m/z* 182 (MH^+). Starting with **(R)-2**, in an analogous procedure, **(R)-3** was obtained in 99%, with spectroscopic data identical to those of the **(S)-3**.

(S)-2-(2-Chloro-2,2-dideuterioethyl)amino-3-[(S)-α-methylbenzyl]-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [(S,S)-5]and its enantiomer [(R,R)-5]. To a cooled (-78°C) solution of **(S)-3** (3.19 g, 17.6 mmol) and triethylamine (3.56 g, 35.2 mmol) in CH_2Cl_2 (50 ml) was added dropwise a solution of phosphorus oxychloride (2.70 g, 17.6 mmol) in CH_2Cl_2 (20 ml). The reaction mixture was stirred for 1 hr at -78°C, and then another hr at room temperature. The progress of the reaction was monitored by TLC and only one major product was detected. When the starting material disappeared, 2-chloro-2,2-dideuterioethylamine hydrochloride (2.08 g, 17.6 mmol) was added to the reaction mixture, followed by triethylamine (3.56 g, 35.2 mmol) in 20 ml of CH_2Cl_2. Stirring was continued at room temperature for 1 hr, then the reaction mixture was heated to reflux for 24 hrs. Precipitated triethylamine hydrochloride was removed by suction filtration and the filtrate was washed with distilled water (20 ml x 3). The organic phase was dried over anhydrous sodium sulfate. After filtration, the solvent in the filtrate was evaporated *in vacuo*. The residue (6.01 g) was purified by silicic acid chromatography with CH_2Cl_2-acetone as the eluant to give the presumed **(S,S)-5** as colorless crystals (2.16 g, 40%): m.p. 114-116°C; Rf 0.62 (CH_2Cl_2-acetone 1:1); NMR ($CDCl_3$) δ 1.53 (d, J=6.6, 3H, -CH-CH_3), 1.61-1.80

(m, 2H, C5-H) 2.69-2.85 (m, 1H, NH), 2.90-3.10 (m, 2H, C4-H), 3.20-3.40 (m, 2H, -$\underline{CH_2}$-CD_2-Cl), 4.87-5.02 (m, 1H, -$\underline{CH}$-CH_3), 7.22-7.58 (m, 5H, Ph-H); MS (CI) *m/z* 307 (MH^+). In an analogous manner, the presumed **(R,R)-5** was prepared (44%): m.p. 113-116°C; with spectroscopic data identical to those of **5** with the presumed **(S,S)** configuration.

(R)-2-(2-Chloro-2,2-dideuterioethyl)amino-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [(R)-6] and its enantiomer [(S)-6]. The presumed **(S,S)-5** (2.16 g, 7.1 mmol) was dissolved in CH_2Cl_2 (50 ml). After addition of freshly hexane washed 10% palladium on charcoal (1.0 g), the solution was hydrogenated at room temperature for 48 hrs under ambient pressure. The progress of reaction was followed by TLC. When the reaction was completed, the catalyst was removed by filtration, and the solvent was removed *in vacuo*. The residue (1.25 g) was purified by crystallization in acetone to give the presumed **(R)-6** as colorless needles (0.98 g). The mother liquor was concentrated and chromatographed on a silicic acid column with CH_2Cl_2-acetone (4:1) as the eluant to give an additional amount of the product (0.20 g). The presumed **(R)-6** was obtained in 76% yield: m.p. 107-109°C; Rf 0.18 (CH_2Cl_2-acetone 1:3); NMR (acetone-d_6) δ 1.58-1.85 (m, 2H, C5-H), 3.10-3.28 (m, 4H, C4-H, C1'-H), 3.60-4.08 (m, 2H, 2x NH); MS *m/z* 203 (MH^+). Hydrogenolysis of the presumed **(R,R)-5** was performed similarly and the presumed **(S)-6** (80%, m.p. 107-108°C) was obtained. The spectroscopic data were identical to those of the **6** with the presumed **(R)** configuration.

(S)-2-(2-Chloro-2,2-dideuterioethyl)amino-3-chloroacetyl-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [(S)-7] and its enantiomer [(R)-7]. To a solution of the presumed **(R)-6** (0.93 g, 4.60 mmol) in THF (20 ml), was added dropwise chloroacetyl chloride (0.52 g, 4.60 mmol) at 0°C. The reaction mixture was stirred for 5 hrs at room temperature. The progress of acetylation was followed by TLC. When the starting material disappeared, THF was evaporated. The residue was redissolved in 20 ml of CH_2Cl_2 and washed with distilled water (10 ml x 2). The organic phase was dried over anhydrous sodium sulfate. After filtration, the solvent in the filtrate was removed *in vacuo*. The residue was purified on a short silicic acid column with CH_2Cl_2-acetone (4:1) as the eluant to afford the presumed **(S)-7** as colorless crystals (1.05 g, 82%): m.p. 84-86°C (acetone-ethyl ether); Rf 0.72 (CH_2Cl_2-acetone 1:1); NMR ($CDCl_3$) δ 1.90-2.20 (m, 2H, C5-H), 3.25-3.39 (m, 3H, C4-H, C1'-H), 3.40-3.70 (m, 1H, NH), 4.42-4.58 (m, 1H, C4-H), 4.50 (d,

J=14.4 Hz, 1H, C2"-H), 4.71 (d, J=14.4 Hz, 1H, C2"-H); MS (CI) *m/z* 279 (MH^+). Similarly, the presumed **(R)-7** was obtained in 80% yield: m.p. 85-86°C. The spectroscopic data were identical to those of **7** with the presumed **(S)** configuration.

(S)-2-(2-Chloro-2,2-dideuterioethyl)amino-3-(2-chloroethyl)-6,6-dideuterio-tetrahydro-2H-*1,3,2*-oxazaphosphorin-2-oxide [(S)-1-d4] and its enantiomer [(R)-1-d4]. To a cooled (-78°C) solution of **(S)-7** (1.00 g, 3.60 mmol) in THF (10 ml) was added a 1M solution of diborane in THF (14 ml, 4:1 excess). The reaction mixture was stirred at ambient temperature for 1 hr. After the reduction was completed (TLC), 1.5 ml of distilled water in 10 ml of THF was added to decompose the remaining diborane. The solvent in the mixture was evaporated *in vacuo* and the residue was dissolved in CH_2Cl_2. After washing with water (10 ml), the organic phase was dried over anhydrous sodium sulfate and concentrated. The crude material (1.12 g) was purified by silicic acid chromatography with CH_2Cl_2:acetone:methanol (80:6:1) as the eluant. **(S)-1-d4** (0.58 g) was obtained in 61% yield: m.p. 63-64°C (ethyl ether); Rf 0.33 (CH_2Cl_2-acetone 1:1); NMR ($CDCl_3$) δ 1.86-2.05 (m, 2H, C5-H), 3.10-3.60 (m, 7H, NH, C4-H, C1'-H, C1"-H), 3.66 (t, J=6.4 Hz, 2H, C2"-H); MS (CI) *m/z* 265 (MH^+); $[\alpha]^{25}_D$ -38.8° (c 0.35, MeOH). **(R)-1-d4** was obtained in a similar yield from **(R)-7**: m.p. 63-64°C; $[\alpha]^{25}_D$ +38.9° (c 0.5, MeOH) with spectroscopic data identical to those of **(S)-1-d4**. The optical activity values were identical to those of literature (24). This establishes the stereochemical assignment of the end products and by inference those of the precursors. GC/MS of IF showed $[MH\text{-}HCl]^+$ at m/z 225 for IF-d_0 and at m/z 229 for IF-d4. The ratio of ion intensity of m/z 229 to the summation of those from 225 to 229 provided the isotope purity which indicated 99.5%.

Ethyl N-triphenylmethyl glycine ester. To a suspension of ethyl glycine ester hydrochloride (15.00 g, 107.5 mmol) and triphenylmethyl chloride (30.00 g, 107.5 mmol) in CH_2Cl_2 (200 ml) was added triethylamine (21.61 g, 215.1 mmol) in 50 ml CH_2Cl_2 at 0°C. After the completion of addition, the mixture was heated to reflux for 20 hrs. Precipitated triethylamine hydrochloride was removed by filtration and the filtrate was washed with water (50 ml x 3). The organic phase was dried over anhydrous sodium sulfate. After filtration, the solvent in the filtrate was evaporated *in vacuo* to give the crude product (37.00 g, 100%), which was crystallized in CH_2Cl_2-acetone (1:1) to give fine cubic crystals (35.20g): m.p. 100-105°C; Rf 0.58 (CH_2Cl_2); NMR ($CDCl_3$) δ 1.28 (t, J=7.2 Hz, 3H, $-CH_3$), 1.68 (bs, NH), 2.84 (s, 1H, -NH-CH-CO-), 3.14 (s, 1H, -NH-CH-CO-), 4.05 (q, J=7.2 Hz, 2H, $-CH_2-CH_3$), 7.15-7.50 (m, 15H, Ph-H); MS (CI) *m/z* 346 (MH^+).

N-Triphenylmethyl-2,2-dideuterioethanolamine. To a cooled (-78°C) suspension of LAD (3.04 g, 72.4 mol) in THF (50 ml), was added slowly a solution of ethyl N-triphenylmethyl glycine ester (50.00 g, 144.8 mmol) in THF (100 ml). The temperature was then raised to room temperature while stirring was maintained for 2 hrs. Water (10 ml) was added to destroy the remaining hydride. The slurry material was extracted with THF (50 ml x 3). The organic extract was dried over anhydrous sodium sulfate for 2 hrs. After filtration, the solvent in the filtrate was removed *in vacuo* to give the product (46.4 g, 100 %): m.p. 53-60°C; Rf 0.06 (CH_2Cl_2); NMR ($CDCl_3$) δ 1.68 (bs, 2H, -OH, -NH-), 2.34 (s, 2H, -CH_2-), 7.14-7.48 (m, 15H, -Ph-H); MS (CI) *m/z* 306.

2,2-Dideuterioethanolamine hydrochloride. To a solution of N-trityl-2,2-dideuterioethanolamine (46.41 g, 152.2 mmol) in methanol (200 ml) was added 37% hydrochloric acid (12 ml). The mixture was refluxed for 20 hrs. Methanol was removed by rotary evaporation. Water (50 ml) was added to the residue, and the lipophilic by-products were removed with CH_2Cl_2 (50 ml x 3) extraction. The resulting aqueous solution was treated with charcoal. After filtration, the solvent in the filtrate was evaporated *in vacuo* to give the crude amine salt. The product was crystallized in ethyl alcohol to give colorless crystals (14.8 g, 98%): m.p. 82-84°C (lit. 84-86°C for unlabeled compound); Rf 0.09 (acetone-methanol 1:1); MS (CI) *m/z* 64 (MH^+ of free amine).

2-Chloro-2,2-dideuterioethylamine hydrochloride. To a cooled (0°C) suspension of 2,2-dideuterioethanolamine hydrochloride (7.45 g, 75.6 mmol) in 1,2-dichloroethane (50 ml) was added dropwise thionyl chloride (18.0 g, 151.3 mol). After the completion of addition, the reaction mixture was stirred at room temperature for 0.5 hr, and then it was heated gradually to 50-60°C. The progress of chlorination was monitored by TLC. The precipitated product (8.82 g, 100%) was collected by filtration and washed with CH_2Cl_2 and acetone. Crystallization in ethyl alcohol gave colorless crystals: m.p. 141-143°C (lit. 143-146°C for unlabeled compound); Rf 0.58 (acetone-methanol 1:1); MS *m/z* 82 (MH^+ of the free amine).

References

1. Ariens E.J. -Med. Res. Rev., 6:451 (1986).
2. Arnold H., Bourseaux F., and Brock N. -Nature, 181:931 (1958).
3. Arnold H., Bourseaux F., and Brock N. -Arzneim.-Forsch., 11:143 (1961).

4. Asta-Werke A.-G. -Chem. Abs., 73:44892d (1970).
5. Sladek N.E. -Pharmacol. Ther., 37:301 (1988).
6. Schoenike S.E. and Dana W.J. -Clin. Pharm., 9:179 (1990).
7. Connors T.A., Cox P.J., Farmer P.B., Foster A.B., and Jarman M. -Biochem. Pharmcol., 23:115 (1974).
8. Allen L.M. and Creaven P.J. -Cancer Chemother. Rep., 56:603 (1972).
9 Takamizawa A., Iwata T., and Matsumoto S. -Chem. Pharm. Bull., 25:2900 (1977).
10. Struck R.F., Dykes D.J., Corbett T.H., Suling W.J., and Trader M.W. -Br. J. Cancer, 47:15 (1983).
11. Bryant B.M., Jarman M., Baker M.H., Smith I.E., and Smyth J.F. -Cancer Res., 40:4734 (1980).
12 Goren M.P. -J. Chromatogr., 570:351 (1991).
13. Goren M.P., Wright R.K., Pratt C.B., and Pell F.E. -Lancet, 2:1219 (1986).
14. Kusnierczyk H., Radzikowski C., Paprocka M., Budzynski W., Rak J., Kinas R., Misiura K., and Stec W. -J. Immunopharmacol., 8:455 (1986).
15. Farmer P.B. -Biochem. Pharmacol., 37:145 (1988).
16 Wainer I.W., Granvil C.P., Wang T., and Batist G. -Cancer Res., 54:4393 (1994).
17. Kaijser G.P., Beijnen J.H., Bult A., and Underberg W.J.M. -Anticancer Res., 14:517 (1994).
18. Misiura K., Okruszek A., Pankiewicz K., Stec W.J., Czownicki Z., and Utracka B. -J. Med. Chem., 26:674 (1983).
19. Trager W.F. -J. Clin. Pharmacol., 26:443 (1986).
20. Cox P.J., Farmer P.B., Foster A.B., Griggs L.J., Jarman M., Kinas R., Pankiewicz K., and Stec W.J. -Biomed. Mass Spectrom., 4:371 (1977).
21. Cox P.J., Farmer P.B., Jarman M., Kinas R.W., and Stec W.J. -Drug Metab. Dispos., 6:617 (1978).
22. Kinas R., Pankiewicz K., Stec W.J., Farmer P.B., Jarman M., and Foster A.B. -Bull. Acad. Pol. Sci., 26:39 (1978).
23. Ludeman S.M., Barlett D.L., and Zon G. -J. Org. Chem., 44:1163 (1979).
24. Pankiewicz K., Kinas R., Stec W.J., Foster A.B., Jarman M., and Van Maanen J.M.S. -J. Am. Chem. Soc., 101:7712 (1979).