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TITLE: Preparation of Chemicals and Bulk Drug Substances for the
U.S. Army Drug Development Program

PRINCIPAL INVESTIGATOR: Jaroslav F. Novotny, Ph.D.

CONTRACTING ORGANIZATION: Starks Associates, Inc.
Buffalo, NY 14213

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Fort Detrick, Frederick, Maryland 21702-5012

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13. ABSTRACT (Maximum 200) A broad spectrum of chemical compounds for U. S. Army Drug Development Program were synthesized during this period. Nineteen candidate drugs were delivered. New synthetic methods were designed for eight products transmitted to WRAIR. The purity of all target compounds and intermediates were rigorously checked by a series of physical and chemical tests. Methods have been developed for compounds not previously recorded in the chemical literature. The cost for raw materials and labor were kept under strict control by minimizing the turn-around-time for each requested material. Continued on the next page.					
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I SUMMARY

A broad spectrum of chemical compounds for U. S. Army Drug Development Program were synthesized during this period. Nineteen candidate drugs were delivered. New synthetic methods were designed for eight products transmitted to WRAIR.

The purity of all target compounds and intermediates were rigorously checked by a series of physical and chemical tests. Methods have been developed for compounds not previously recorded in the chemical literature. The cost for raw materials and labor were kept under strict control by minimizing the turn-around-time for each requested material.

The following target compounds have been synthesized during this period: WR99210 prodrug, amide from $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure); N^1 -3,4-dichlorophenyl- N^5 -isopropyl-diguanide, hydrochloride; 1-(3,4-dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine; 6-cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine; 1,3,5-triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester; (S)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyran[3,4-f]indolizine-3,6,10-trione; (S)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyran[3,4-f]indolizine-3,6,10-trione; 1(2H)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; L-glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-L-phenylalanyl]-, trilithium salt; L-glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-L-phenylalanyl]-, diammonium salt; L-glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-L-phenylalanyl]-, trilithium salt; 2-(guanine-7-yl)ethyl 2-hydroxyethyl sulfide; N-(2-hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine; O-[[[(1R)-N-[N-(phenylmethoxycarbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(R)-lactic acid, methyl ester; O-[[[(1R)-N-[N-(phenylmethoxycarbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(R)-lactic acid, lithium salt; O-[(L)-1-[[N-(phenylmethoxycarbonyl)glycyl]-amino]ethyl]hydroxyphosphinyloxy]-L-lactic acid, dilithium salt.

II FOREWORD

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____For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

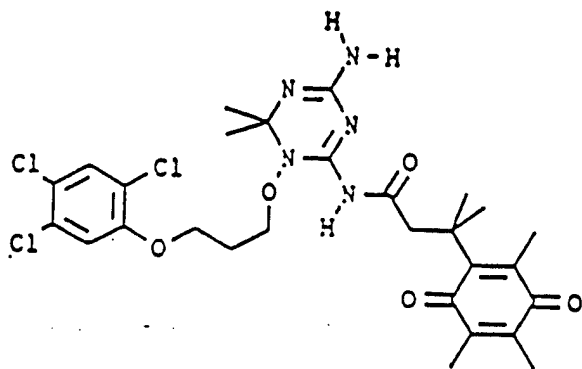
____In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Jaroslav F. Novotny 20/12/96
Jaroslav F. Novotny, PI Date

III. CUMULATIVE LIST OF REQUESTED TARGET COMPOUNDS DELIVERED TO
WALTER REED ARMY INSTITUTE OF RESEARCH FROM DECEMBER 1, 1995
TO NOVEMBER 30, 1996

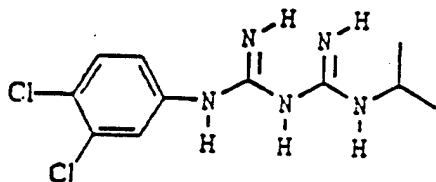
Compound

Cumulative No.*



1. WR99210 Prodrug;
amide from $\beta,\beta,2,4,5$ -
pentamethyl-3,6-dioxo-
1,4-cyclohexadiene-1-
propanoic acid
(intended structure)

1214



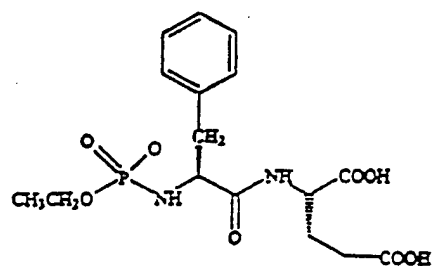
2. N¹-3,4-Dichlorophenyl-
N⁵-isopropyldiguanide,
hydrochloride

1215

*Additional information may be found in the Cumulative List
on page 7, this report.

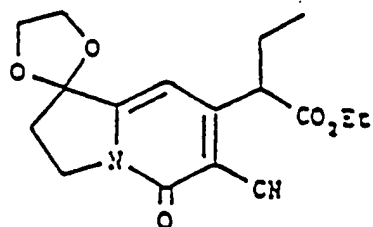
Compound

Cumulative No.



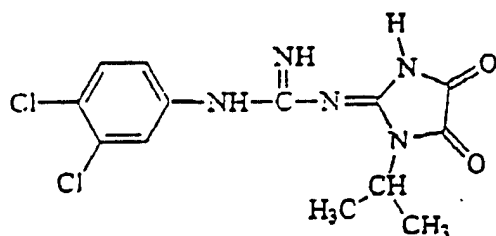
3. L-Glutamic acid, N-[N-
[ethoxyhydroxyphosphinyl]-
L-phenylalanyl]-,
trilithium salt

1216



4. 6-Cyano-7-[1'-(ethoxy-
carbonyl)propyl]-1,1-
(ethylenedioxy)-5-oxo-
1,2,3,5-tetrahydro-
indolizine

1217

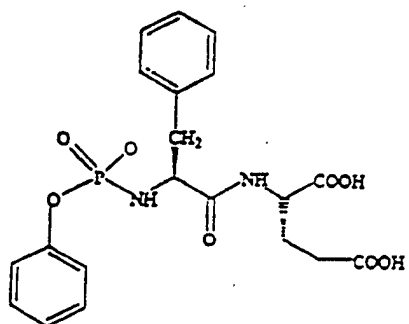


5. 1-(3,4-Dichlorophenyl)-
3-(1-isopropyl-4,5-
dioxo-2-imidazol-
idinylidene)guanidine

1218

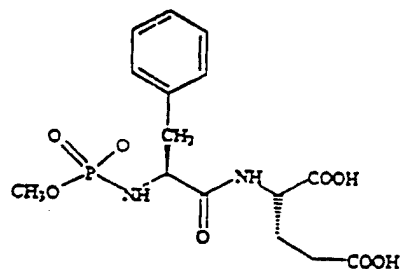
Compound

Cumulative No.



6. L-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-L-phenylalanyl]-, diammonium salt

1219

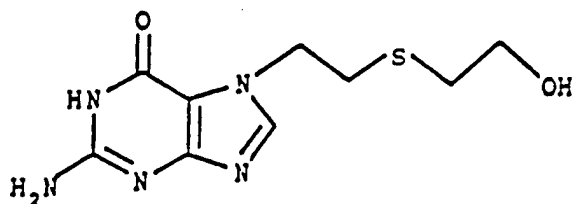


7. L-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-L-phenylalanyl]-, trilithium salt

1220

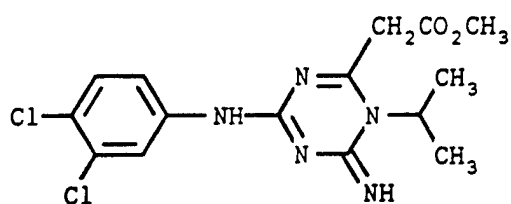
Compound

Cumulative No.



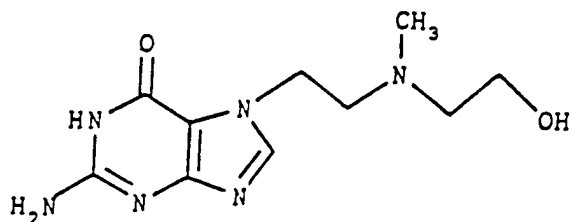
8. 2-(Guanin-7-yl)-
ethyl 2-hydroxy-
ethyl sulfide

1221



9. 1,3,5-Triazine-2-
acetic acid, 4-[(3,4-
dichlorophenyl)amino]-
1,6-dihydro-6-imino-1-
(1-methylethyl)-,
methyl ester

1222

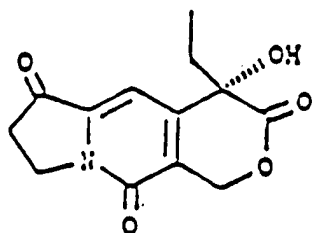


10. N-(2-Hydroxyethyl)-N-
[2-(7-guaninyl)ethyl]-
methylamine

1223

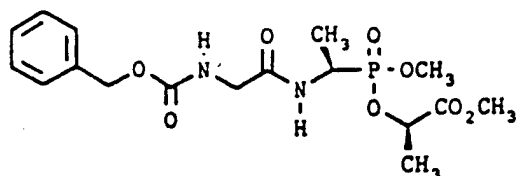
Compound

Cumulative No.



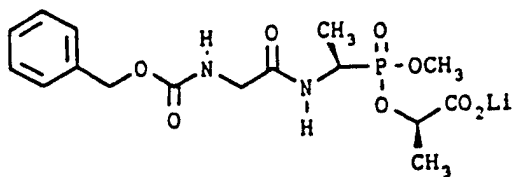
11. (S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano[3,4-f]indolizine-3,6,10-trione

1224



12. O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(R)-lactic acid, methyl ester

1225

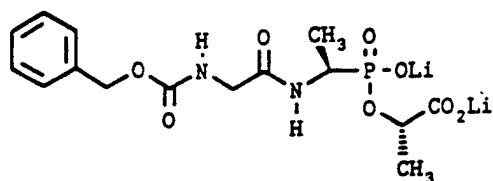


13. O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(R)-lactic acid, lithium salt

1226

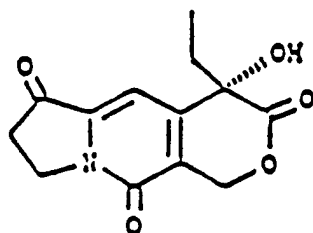
Compound

Cumulative No.



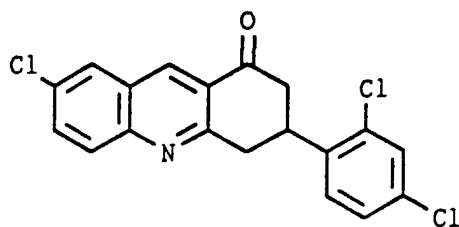
14. O-[(L)-1-[[N-(Phenylmethoxycarbonyl)glycyl]-amino]ethyl]hydroxyphosphinyloxy-L-lactic acid, dilithium salt

1227



15. (S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]indolizine-3,6,10-trione

1228



16. 1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-

1229

IV. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO
WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) FROM
DECEMBER 1, 1995 TO NOVEMBER 30, 1996

The previous Cumulative List covering the period from July 1, 1965 to June 30, 1973 may be found in Starks Associates, Inc. Final Comprehensive Report dated June 30, 1973 pages 54-97, Contract No. DA-49-193-MD-2751. The list covering the period from July 1, 1973 to September 28, 1979 may be found in Starks Associates, Inc. Final Comprehensive Report dated September 1979, pages 82-241, Contract No. DAMD17-73-C-3159. The list covering the period from September 29, 1979 to September 14, 1983 may be found in Starks Associates, Inc. Final Comprehensive Report dated September 1983, pages 56-163, Contract No. DAMD17-79-C-9170. The list covering the period from September 15, 1983 to March 14, 1989 may be found in Starks Associates, Inc. Final Comprehensive Report dated March 14, 1989, pages 55-116, Contract No. DAMD17-83-C-3206. The list covering the period from March 15, 1989 to November 30, 1992 may be found in Starks Associates, Inc. Final Comprehensive Report dated November 30, 1992, pages 35-58, Contract No. DAMD17-89-C-9058. The list covering the period from December 1, 1992 to November 30, 1993 may be found in Starks Associates, Inc., Annual Report dated November 30, 1993 pages 4-5, Contract No. DAMD17-93-C-3003. The list covering the period from December 1, 1993 to November 30, 1994 may be found in Starks Associates, Inc., Annual Report dated November 30, 1994 pages 4-5, Contract No. DAMD17-93-C-3003. The list covering the period from December 1, 1994 to November 30, 1995 may be found in Starks Associates, Inc. Annual Report dated January 1996 page 10-16, Contract No. DAMD17-93-C-3003.

<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
1211	6-Hydroxy-4,4,5,7,8-pentamethylhydro-coumarin	3.4 g	BN64767	279647	123
1212	$\beta,\beta,2,4,5$ -Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid	3.3 g	BN64776	279690	123
1213	Succinimidyl $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate	2.0 g	BN64785	280492	123

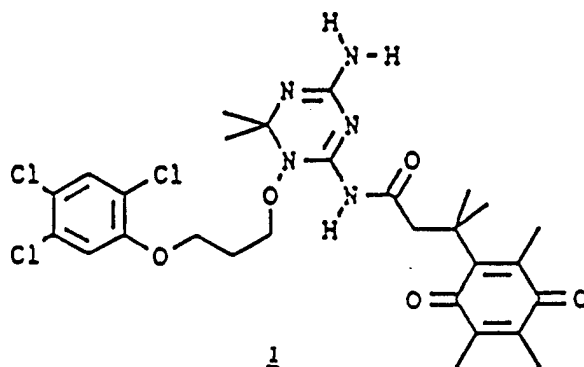
<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
1214	WR99210 Prodrug; amide from $\beta,\beta,2,4,5$ - pentamethyl-3,6-dioxo- 1,4-cyclohexadiene- 1-propanonic acid (intended structure)	1.4 g	BN64794	280493	123
1215	N ¹ -3,4-Dichlorophenyl- N ⁵ -isopropyldiguanide, hydrochloride	29.1 g	BN65111	042313	123
1216	L-Glutamic acid, N-[N- [ethoxyhydroxyphos- phanyl]-L-phenyl- alanyl]-, trilithium salt	1.0 g 20 mg	BN65102 BN65488	280451 280451	123 123
1217	6-Cyano-7-[1'-(ethoxy- carbonyl)propyl]-1,1- (ethylenedioxy)-5-oxo- 1,2,3,5-tetrahydro- indolizine	5.3 g	BN65120	280157	123
1218	1-(3,4-Dichlorophenyl)- 3-(1-isopropyl-4,5- dioxo-2-imidazol- idinylidene)guanidine	192.3 g 156.6 g	BN66369 BN66378	182393 182393	123 123
1219	L-Glutamic acid, N-[N- [phenoxyhydroxyphos- phanyl]-L-phenyl- alanyl]-, diammonium salt	700 mg 20 mg	BN66387 BN66396	280526 280526	123 123
1220	L-Glutamic acid, N-[N- [methoxyhydroxyphosphanyl]- L-phenylalanyl]-, trilithium salt	800 mg 20 mg	BN67473 BN67482	280527 280527	123 123
1221	2-(Guanin-7-yl)- ethyl 2-hydroxy- ethyl sulfide	50 mg	BN70069	280607	124
1222	1,3,5-Triazine-2- acetic acid, 4-[(3,4- dichlorophenyl)amino]- 1,6-dihydro-6-imino-1- (1-methylethyl)-, methyl ester	150 mg	BN70667	280640	124

<u>Cumu- lative No.</u>	<u>Compound</u>	<u>Amount</u>	<u>BN#</u>	<u>WR#</u>	<u>Starks Assoc. Report</u>
1223	N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]-methylamine	800 mg	BN70676	280419	124
1224	(S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano[3,4-f]indolizine-3,6,10-trione	10.2 g	BN72134	280463	125
1225	O-[[(1R)-N-[N-(Phenyl-methoxycarbonyl)glycyl]-1-aminoethyl]methoxy-phosphinyl]-(R)-lactic acid, methyl ester	20 mg	BN72143	280685	125
1226	O-[[(1R)-N-[N-(Phenyl-methoxycarbonyl)glycyl]-1-aminoethyl]methoxy-phosphinyl]-(R)-lactic acid, lithium salt	20 mg	BN72152	280686	125
1227	O-[(L)-1-[[N-(Phenyl-methoxycarbonyl)glycyl]-amino]ethyl]hydroxy-phosphinyloxy]-L-lactic acid, dilithium salt	30 mg 1.4 g	BN72634 BN78172	280693 280693	125 125
1228	(S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano[3,4-f]indolizine-3,6,10-trione	20.3 g	BN78921	280463	126
1229	1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-	0.2 g	BN81320	280850	126

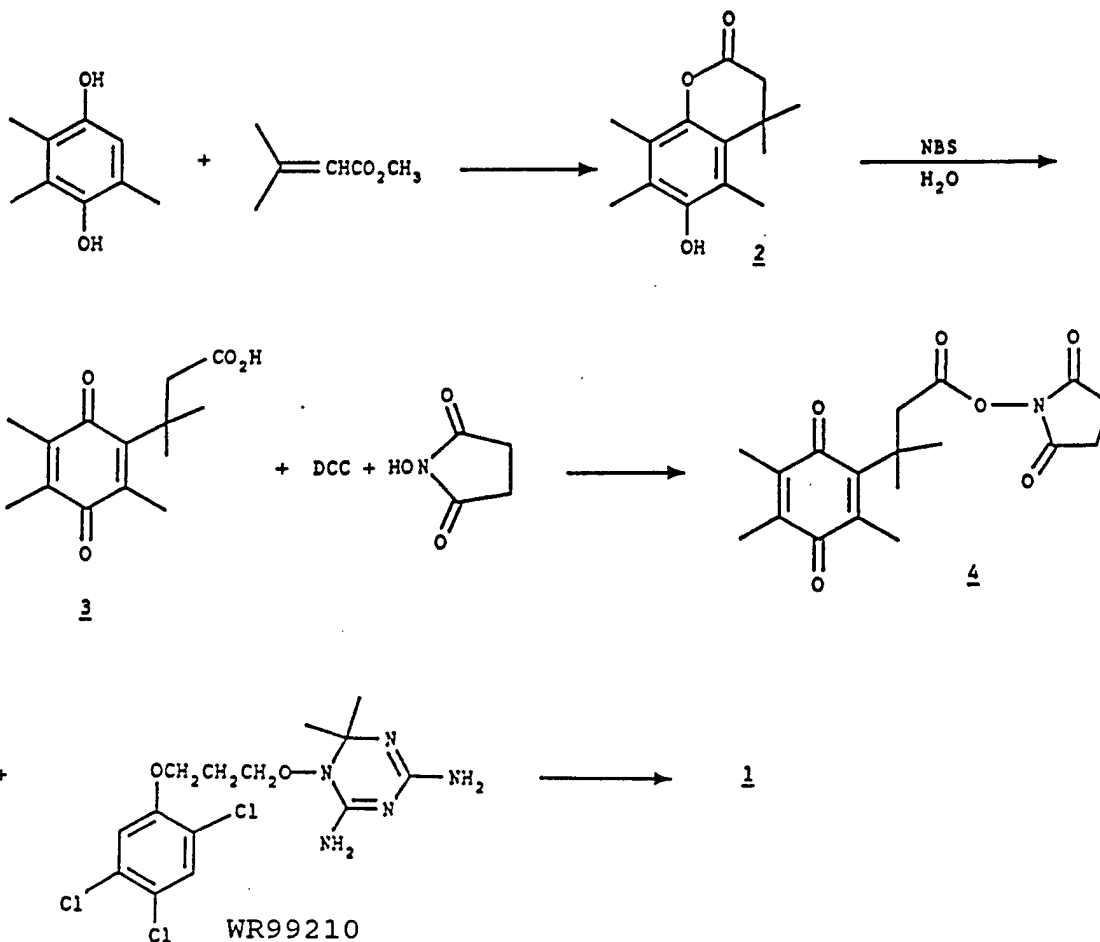
V. DISCUSSION OF RESEARCH AND TARGET COMPOUNDS

1. WR99210 Prodrug; amide from $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure)

The target compound 1 was prepared

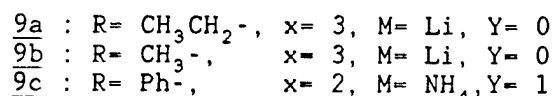
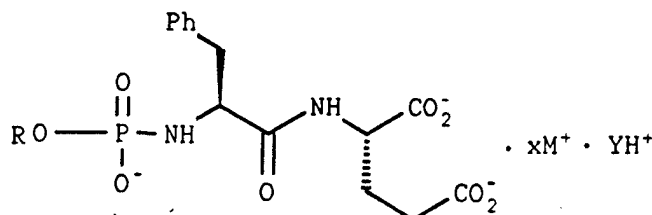


by the following sequence of reactions.^{1,2}



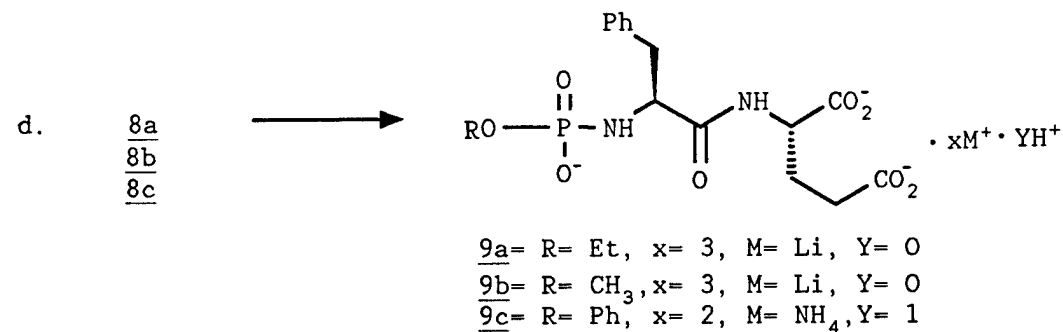
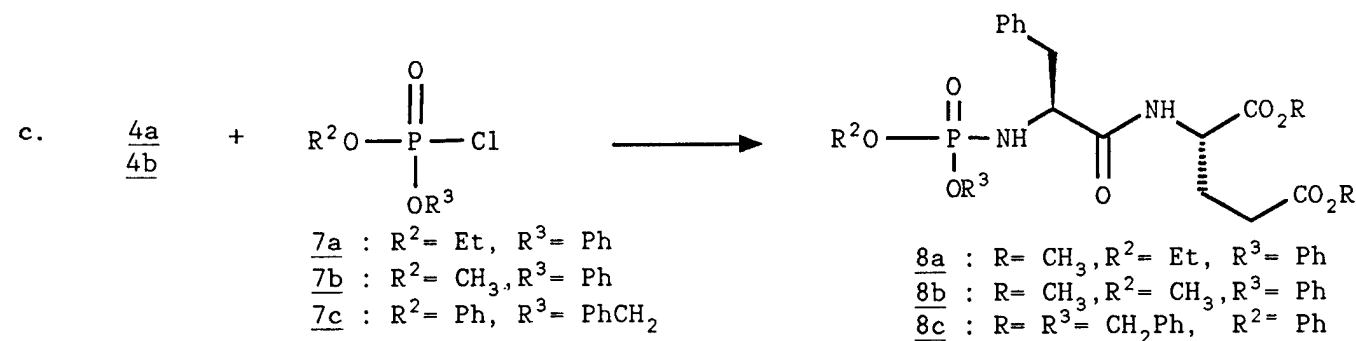
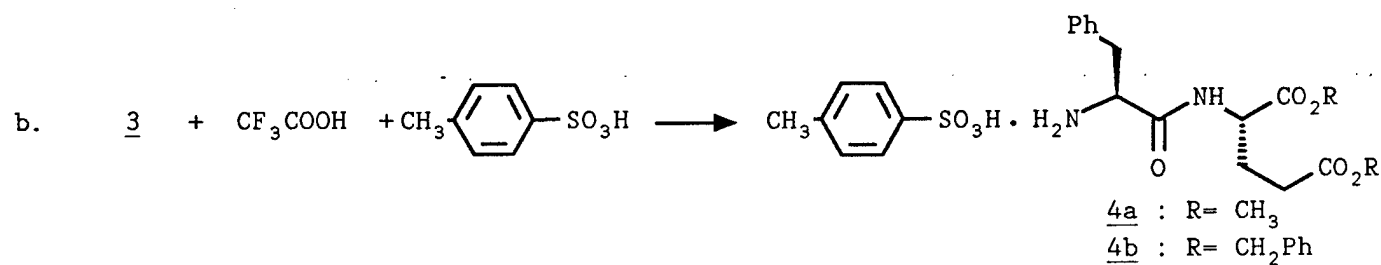
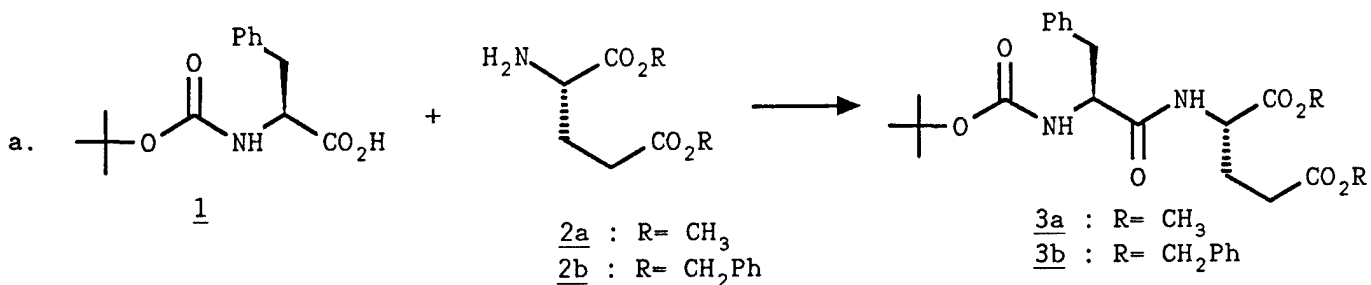
Trimethylhydroquinone was reacted with methyl 3,3-dimethylacrylate to give hydrocoumarin 2 in 56% yield. Reaction of 2 with N-bromosuccinimide in acetonitrile gave the propanoic acid 3 in 69.9% yield. Reaction of 3 with N-hydroxysuccinimide in the presence of DCC gave the succinimidyl ester 4 in 75.8% yield. Reaction of 4 with WR99210 (free base) gave as one of the products the N⁶ or N⁴ amide. The N⁴ amide shown represents the intended structure. Spectral data (NMR) are not in full accord with the intended structure and further characterization might be necessary. Please note that the NMR spectrum has changed subsequent to shipment.

2. a. *L*-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9a)
 - b. *L*-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt (9b)
 - c. *L*-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt (9c)
-



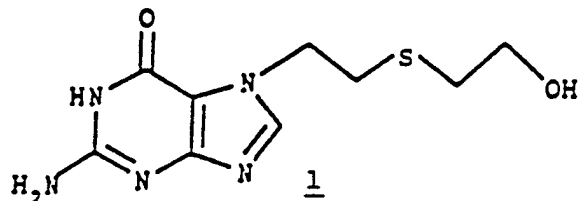
Dipeptides 4a and 4b were prepared in 77-91% and 90% yield, respectively, by coupling commercially available *l*-BOC-*L*-phenylalanine (1) with *L*-glutamic acid, dimethyl ester (2a) or dibenzyl ester (2b), using HBTU³, followed by removal of the *l*-butoxycarbonyl protecting group with trifluoroacetic acid to give 4a and 4b as tosylate salts⁴ (98% yield). Intermediates 8a and 8b were prepared in 26% and 18% yield by phosphorylation of 4a with chlorophosphates 7a and 7b, respectively. The protected phosphorylated dipeptides were hydrolyzed with 1.5M LiOH to give 9a and 9b as trilithium salts (88% and 92% yield)⁵, containing excess LiCl and H₂O.

Intermediate 8c was prepared in 23% yield by phosphorylation of 4b with chlorophosphate 7c. The tribenzyl protected phosphorylated dipeptide was debenzylated by transfer hydrogenation⁶ with 10% palladium on carbon, employing ammonium formate as the source of hydrogen, to give 9c as a diammonium salt (76% yield), containing excess H₂O.

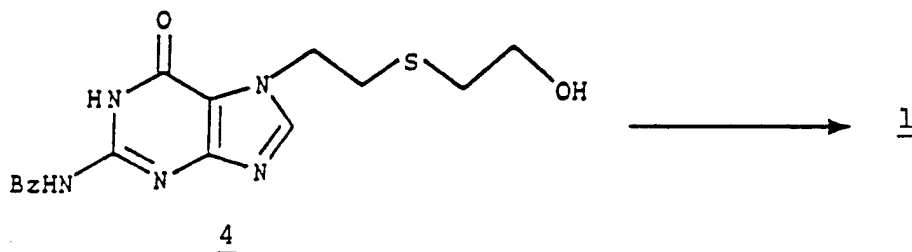
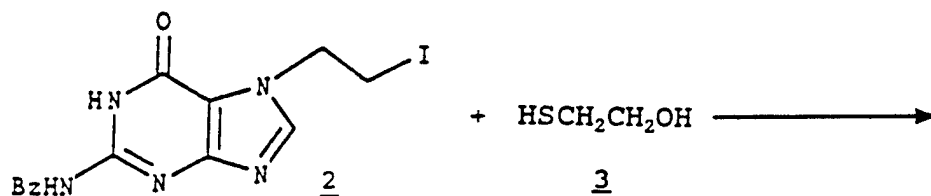


3. 2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (1)

The target compound 1 was prepared



from iodoethylpurinone 2 (prepared previously, please see Starks Associates Yearly Report, dated January 1996, p. 172, Contract DAMD17-93-C-3003) and 2-mercaptoethanol (3) using CH_3ONa as the base to give 4 in 94% yield.

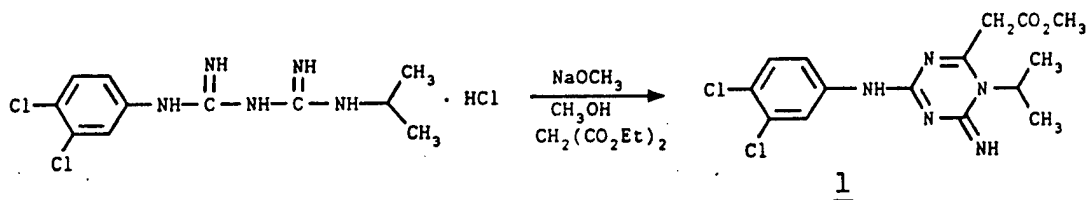


Material 4 was deprotected with boiling sodium methoxide in CH_3OH to yield the target material in 99% yield.

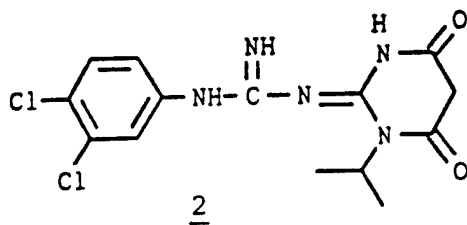
4. 1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester (1)

The target compound (tentative structure) was prepared by the following reaction sequence.

Reaction Sequence

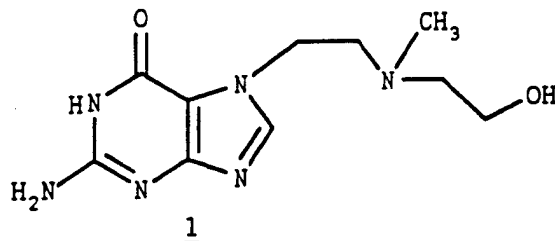


The original intent of this reaction sequence was to produce the pyrimidinone 2, however, none of this material could be isolated under these conditions.

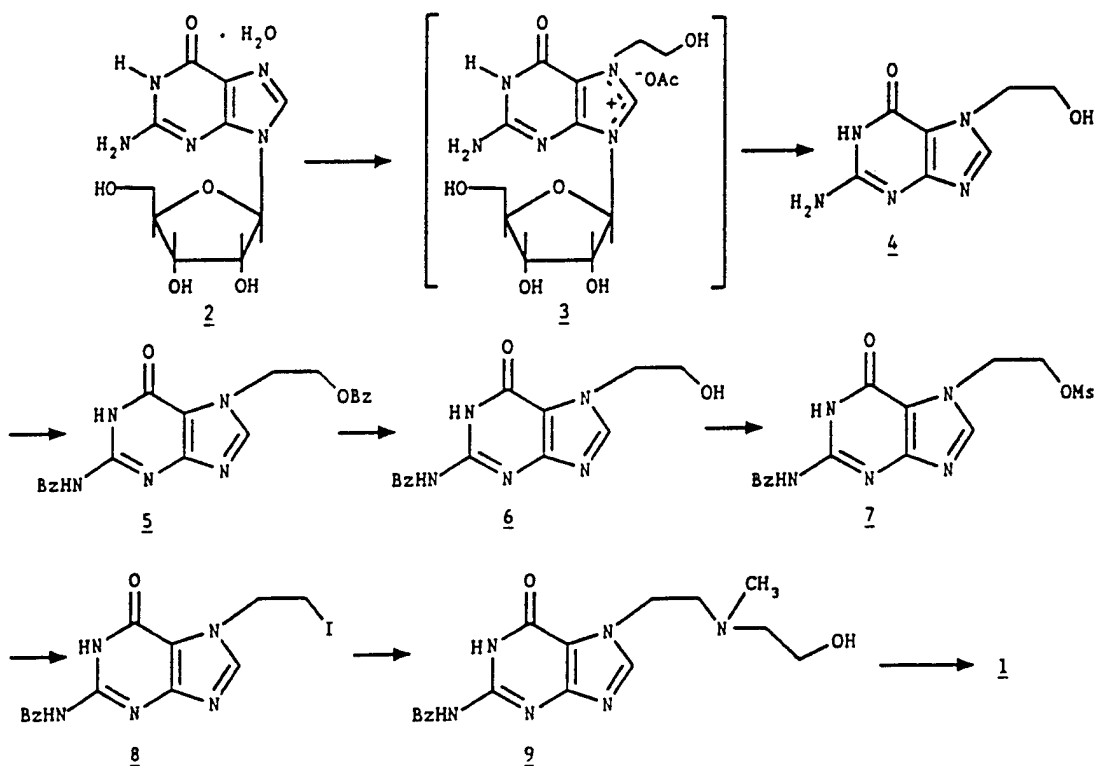


5. N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine (1)

The material 1 was prepared by the



following sequence of reactions.⁷⁻¹⁰ Guanosine hydrate was

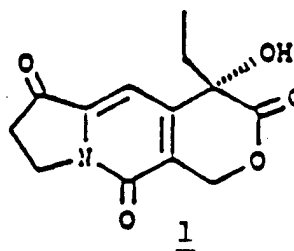


reacted with ethylene oxide in acetic acid and the

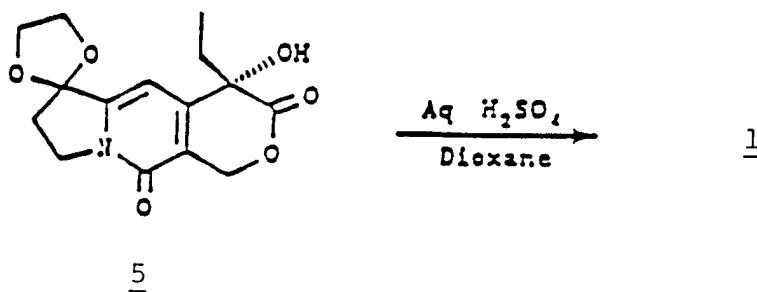
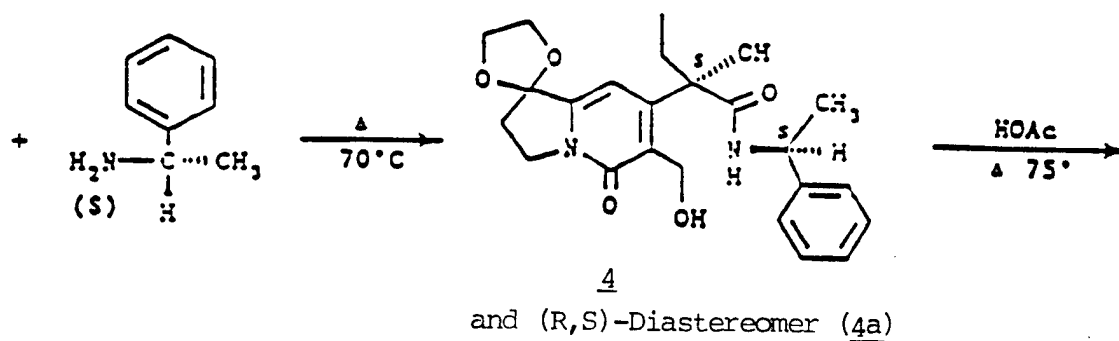
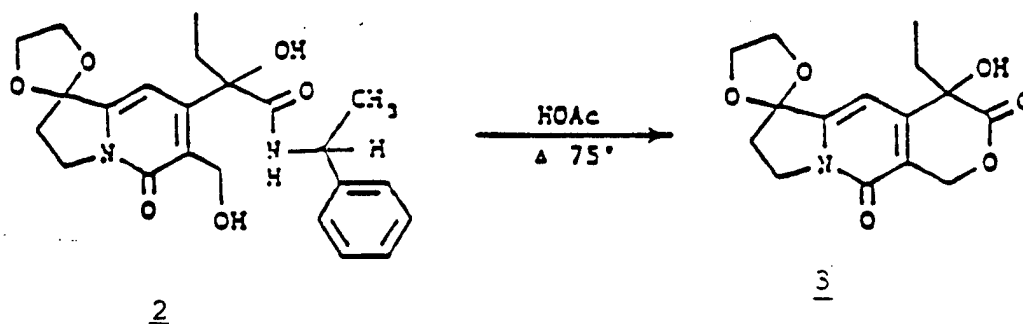
intermediate 3 was immediately hydrolyzed to give 4 in 71% yield. The amino- and hydroxy- functions of 4 were protected by the use of benzoyl cyanide to give 5 in 42% yield, then selectively deprotected to yield 6 in 72% yield. Reaction of 6 with MsCl gave 7 in 88% yield. Material 7 was reacted with NaI in acetone to give 8 in 88% yield. Compound 8 was reacted with commercially available 2-methylaminoethanol to give 9 in 84% yield. This material was deprotected with sodium methoxide to give crude target material 1 in 62% yield.

6. (S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]-indolizine-3,6,10-trione (1)

The target compound 1 was prepared



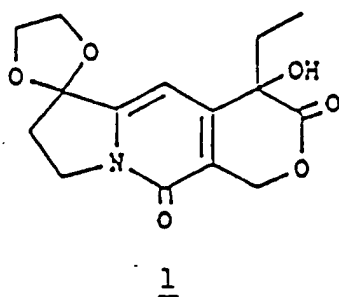
from crude intermediate 2 by the procedure shown below.



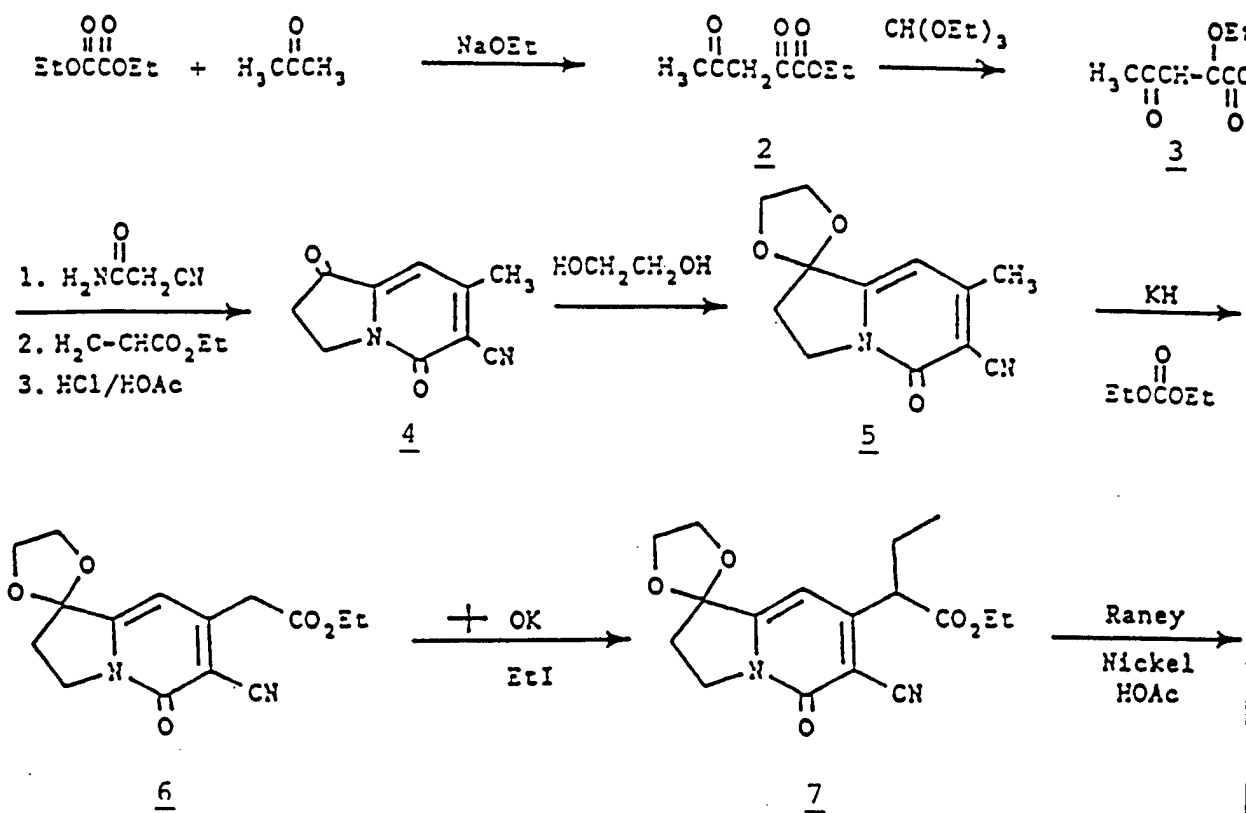
Compound 2 (saved from an earlier synthesis of the target material) was hydrolyzed yielding the racemic ketal 3. The ketal 3 was reacted with (*S*)-(-)- α -methylbenzylamine to give the (*S,S*)- and (*R,S*)-diastereomers 4 and 4a. These were separated by trituration and crystallization, then hydrolyzed to obtain optically active 5. Material 5 was deprotected to give the target compound 1.

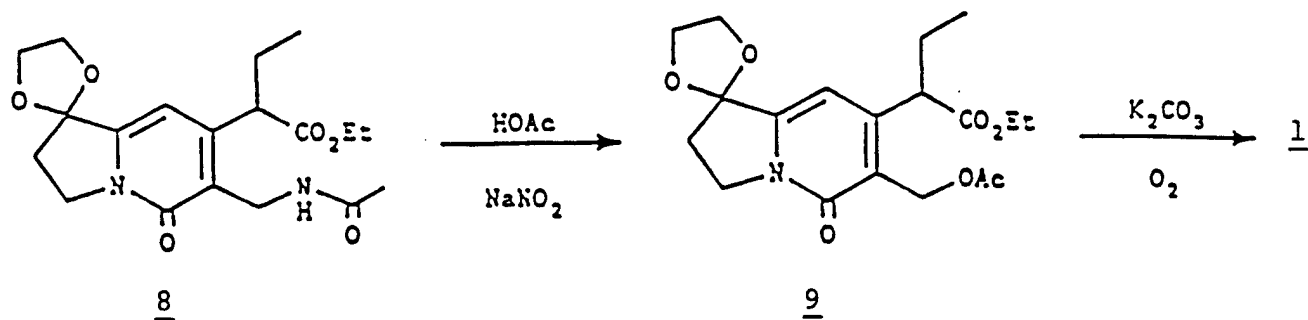
7. (S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]-indolizine-3,6,10-trione (12)

The racemic target ketal 1 was prepared

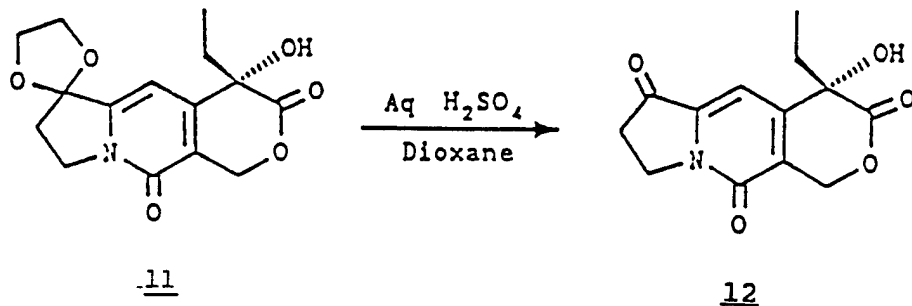
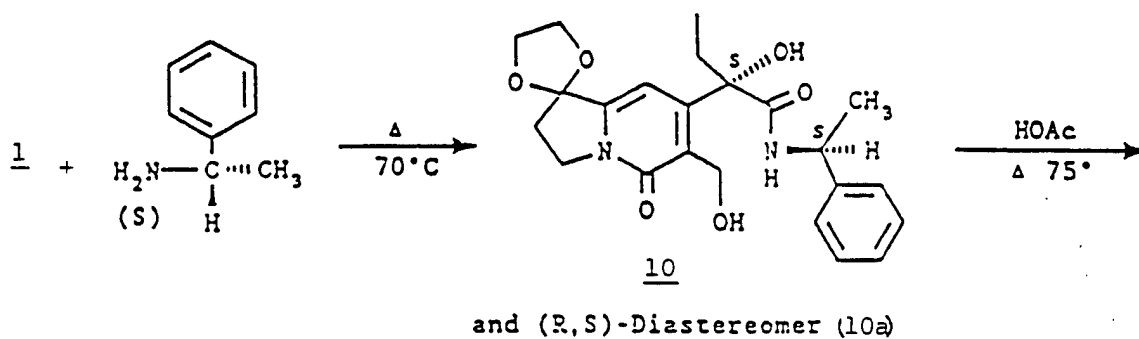


by a nine step literature¹¹⁻¹³ procedure shown below.





The racemic ketal 1 was reacted with (*S*)-(-)- α -methylbenzylamine to give the (*S,S*)- and (*R,S*)-diastereomers 10 & 10a. These were separated by trituration and crystallization then hydrolyzed yielding optically active 11 and (*R*)-enriched 1. Material 11 was deprotected to give the target compound 12.



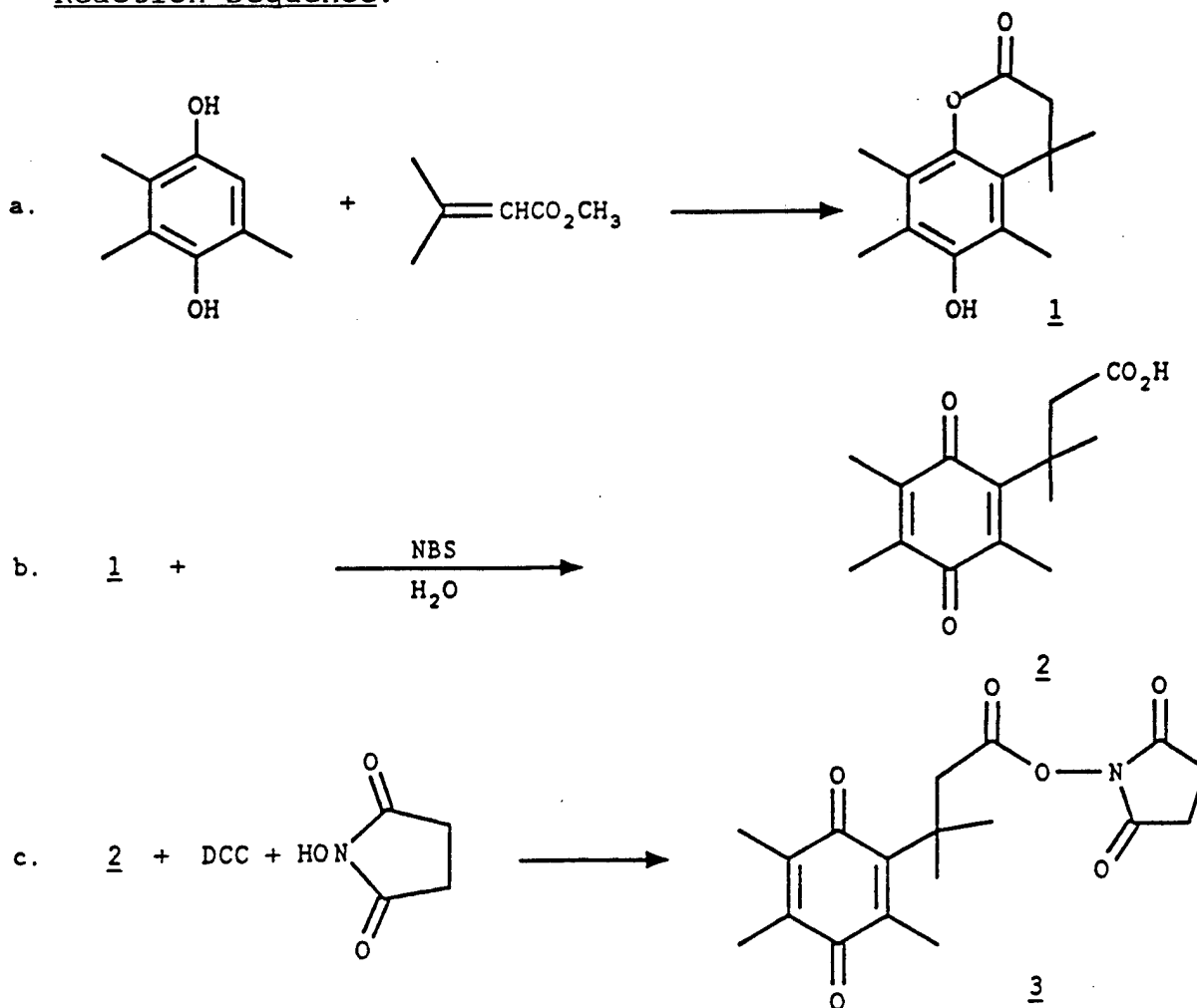
VI. RESEARCH AND KNOWN TARGET COMPOUNDS AND INTERMEDIATES COMPLETED AND DELIVERED TO WALTER REED ARMY INSTITUTE OF RESEARCH FROM DECEMBER 1, 1995 TO NOVEMBER 30, 1996

A. Infectious Disease Related Compounds and Intermediates

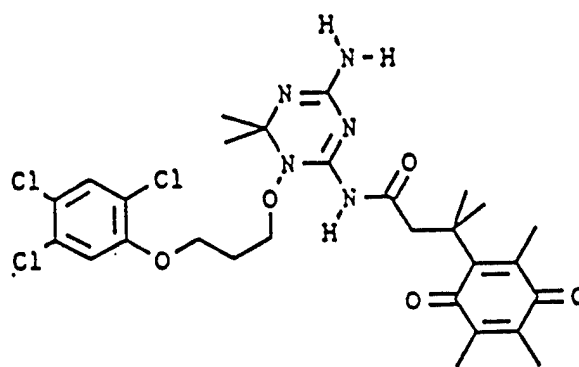
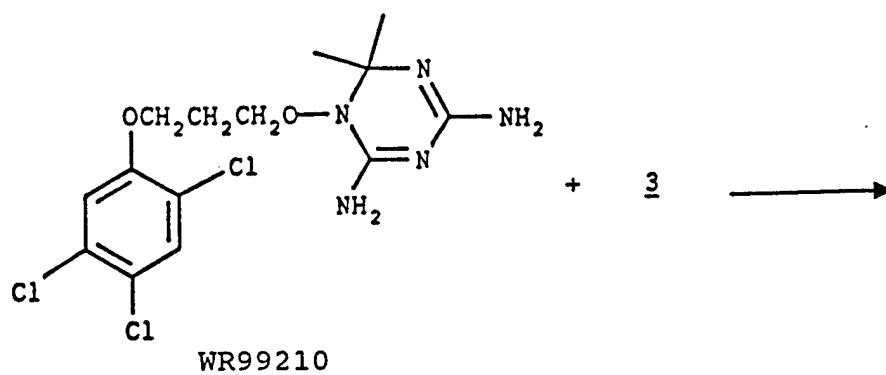
1. WR99210 Prodrug; amide from $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure)

The target compound 4 was prepared by the following sequence of reactions. Intermediates 1-3, which are new to the program, were transmitted to WRAIR.

Reaction Sequence:



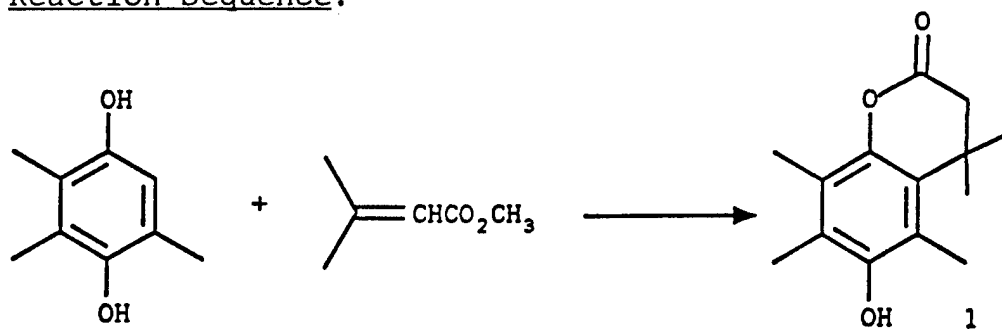
d.



4

1. 6-Hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (1)

Reaction Sequence:



Experimental

To heated (70°C) methanesulfonic acid (100 mL) was added 10 g (65.7 mmol) of trimethylhydroquinone and 8.5 g (9.8 mL, 74.5 mmol) of methyl 3,3-dimethylacrylate all at once, and the mixture was stored at 70°C for 90 min, cooled, poured into 1.25 L of H₂O, then extracted with EtOAc (3 x 500 mL). The extracts were washed with H₂O, saturated NaHCO₃, and NaCl solutions (500 mL each), then dried (MgSO₄). Solvent removal gave the crude lactone as a tan solid (15.5 g). The material was purified by chromatography on a column of silica gel (500 g), packed and eluted with CH₂Cl₂. The following fractions were collected (250 mL each): fractions 1-4 contained product and a faster running impurity (4.2 g); fractions 5-7 contained pure product (6.8 g) and fractions 8-14 contained product and a slower running impurity (3.8 g), total recovery 16.3 g. Product from fractions 5-7 (6.8 g) was recrystallized from hexane-EtOAc, yield 3.8 g (55.9% recovery), mp 185-185.5°C, literature^{14,15} mp 183-186°C and 186-187°C, respectively. A portion (3.4 g) will be transmitted to WRAIR, (Lot No. NJ14-149-2). Additional product (45 g) was obtained from another reaction. The material was suitable for further transformation.

Anal.

	<u>C</u>	<u>H</u>
Calc'd for $C_{14}H_{18}O_3$	71.77	7.74
Found	71.89	7.77

Spectral Data

Infrared (Nujol)

Major bands: 3420, 2910, 2840, 1730, 1640, 1600,
1445, 1400, 1365, 1355, 1300, 1280,
1245, 1195, 1170, 1155, 1110, 1070,
1020, 950, 900 cm^{-1} .

Ultraviolet (EtOH)

λ_{max} 210 nm (sh, $\log \epsilon$ 4.42); 240 nm (sh, 3.69);
288 nm (3.48).

Nuclear Magnetic Resonance ($CDCl_3$)

δ 4.72 (s, 1, OH); 2.54 (s, 2, H's at C-3); 2.36
(s, 3, CH_3 at C-8); 2.22 (s, 3, CH_3 at C-7); 2.19
(s, 3, CH_3 at C-5); 1.45 (s, 6, 2 x CH_3 at C-4).
 D_2O exchanges proton at 4.72 ppm.

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

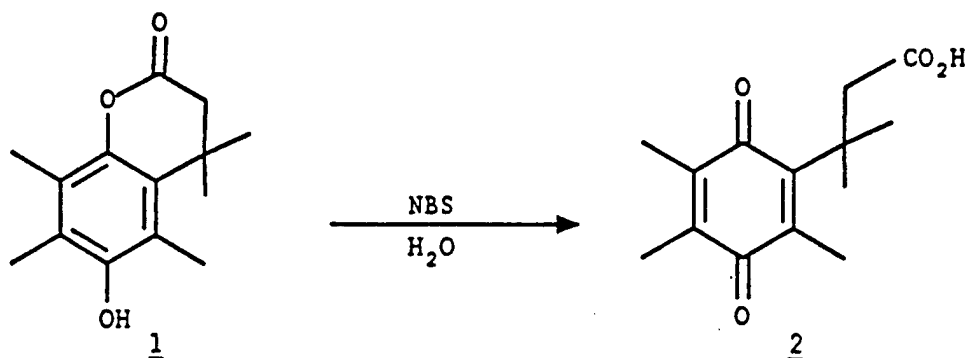
	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	CH ₂ Cl ₂	0.47	Homogeneous
2.	Ether	0.83	Homogeneous
3.	Ether-hexane (4:1)	0.63	Homogeneous

Source of Materials

1.	Methanesulfonic acid	Aldrich Chemical Co., Inc.
2.	Trimethylhydroquinone	Aldrich Chemical Co., Inc.
3.	Methyl 3,3-dimethylacrylate	Aldrich Chemical Co., Inc.
4.	EtOAc	J.T. Baker Chemical Co.
5.	NaHCO ₃	Aldrich Chemical Co., Inc.
6.	Silica gel	EM Laboratories
7.	CH ₂ Cl ₂	J.T. Baker Chemical Co.
8.	Hexane	J.T. Baker Chemical Co.

2. $\beta,\beta,2,4,5$ -Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (2)

Reaction Sequence:



Experimental

6-Hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (1)

Please refer to the preceding synthesis, this report.

$\beta,\beta,2,4,5$ -Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (2)

To a stirred solution of 6-hydroxy-4,4,5,7,8-pentamethylhydrocoumarin (1) (30.8 g, 131 mmol) in 1500 mL of 10% aq. acetonitrile was added dropwise, at 25°C, a solution of N-bromosuccinimide (24.6 g, 138 mmol) in 300 mL of acetonitrile. The mixture was stirred for an hour at 25°C, diluted with H₂O (3 L) and extracted with ether (3 x 1 L). The extracts were combined, washed with H₂O (1.5 L), brine (1.5 L), dried (MgSO₄), then concentrated in vacuo to a yellow oil (34.1 g). The material was recrystallized from acetone-hexane to give 23.0 g of yellow solid. The material was suitable for further transformation. A portion (18 g) was again recrystallized from acetone (50 mL) and hexane (300 mL); yield 13.8 g, mp 105-106°C; literature¹⁵ mp 101-103°C. A portion (3.3 g) will be transmitted to WRAIR (Lot No.

NJ14-153-3). The mother liquors from both crystallizations were concentrated, and the residue (13.7 g) was recrystallized from acetone (38 mL) and hexane (228 mL); yield, 11.8 g. The material was suitable for further transformation; total yield 30.6 g, 93.0%. Additional product (8.3 g) was obtained from a scouting run.

Anal.

	<u>C</u>	<u>H</u>
Calc'd for $C_{14}H_{18}O_4$	67.18	7.25
Found	67.23	7.27

Spectral Data

Infrared (Nujol)

Major bands: 2950, 2830, 2730, 2630, 1875, 1685, 1625, 1575, 1415, 1390, 1360, 1315, 1265, 1225, 1205, 1140, 1115, 1085, 1020, 855, 670 cm^{-1} .

Ultraviolet (EtOH)

λ_{max} 261 nm (log ϵ 4.28).

Nuclear Magnetic Resonance (CDCl_3)

δ 3.02 (s, 2H, C-2 CH_2); 2.14 (s, 3H, C-2' CH_3); 1.95 (d, 3H, $J=1.1$ Hz, C-4' or C-5' CH_3); 1.92 (d, 3H, $J=1.1$ Hz, C-4' or C-5' CH_3); 1.44 (s, 6H, C-3 CH_3 's).

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

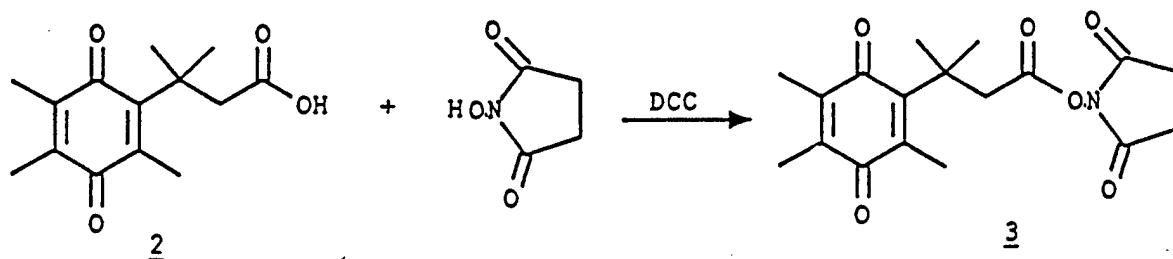
<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1. EtOAc-hexane (2:1)	0.51	Homogeneous
2. Ether-hexane (2:1)	0.27	Homogeneous
3. Ether	0.72	Homogeneous

Source of Materials

- | | | |
|----|---|----------------------------|
| 1. | 6-Hydroxy-4,4,5,7,8-pentamethyl-
hydrocoumarin | Starks Associates, Inc. |
| 2. | Acetonitrile | J.T. Baker Chemical Co. |
| 3. | N-Bromosuccinimide | Aldrich Chemical Co., Inc. |
| 4. | Ether | Fisher Scientific |
| 5. | Acetone | J.T. Baker Chemical Co. |
| 6. | Hexane | J.T. Baker Chemical Co. |

3. Succinimidyl $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate (3)

Reaction Sequence:



Experimental¹⁴

$\beta,\beta,2,4,5$ -Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (2)

Please refer to the preceding synthesis, this report.

Succinimidyl $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate (3)

Dicyclohexylcarbodiimide (5.0 g, 24.2 mmol) was added to a solution of quinone acid 2 (5.0 g, 20 mmol) and N-hydroxysuccinimide (2.53 g, 22 mmol) in 150 mL of dry THF at 0°C, and the mixture was stirred for 20 h at RT. The solid that separated (dicyclohexylurea) was filtered off, and the filtrate was concentrated in vacuo, diluted with EtOAc (50 mL) and again filtered. The filtrate was concentrated, and the solid residue (8.8 g) was chromatographed on a column of silica gel (500 g) using EtOAc-hexane (1:1) as the eluent.

Fractions 1-5 contained front-running impurity and were discarded. Fractions 5-10, containing product, were concentrated and the residue (6.4 g) was crystallized from EtOAc (40 mL); yield, 5.0 g. The material was suitable for further transformation. A portion (2.9 g) was recrystallized from EtOAc (20 mL) to give 2.4 g of pure material. A portion (2.0 g) will be transmitted to WRAIR (Lot No. NJ14-156-3). Additional product (31.6 g) was obtained from a larger run.

Anal.

		<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for	$C_{18}H_{21}NO_6$	62.24	6.09	4.03
	Found	62.01, 61.96	6.13, 6.14	3.98, 3.90

Spectral Data

Infrared (KBr)

Major bands: 1785, 1760, 1715, 1630, 1570, 1415, 1350, 1270, 1190, 1040 cm^{-1} .

Ultraviolet (Ethanol)

λ_{max} 260 nm ($\log \epsilon$ 4.20).

Nuclear Magnetic Resonance ($CDCl_3$)

δ 3.28 (s, 2H, CH_2); 2.78 (s, 4H, succinimidyl CH_2 's); 2.17 (s, 3H, 2- CH_3); 1.96 (t, 6H, $J = 0.8$ Hz, 4- and 5- CH_3 's); 1.53 (s, 6H, gem CH_3 's)

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

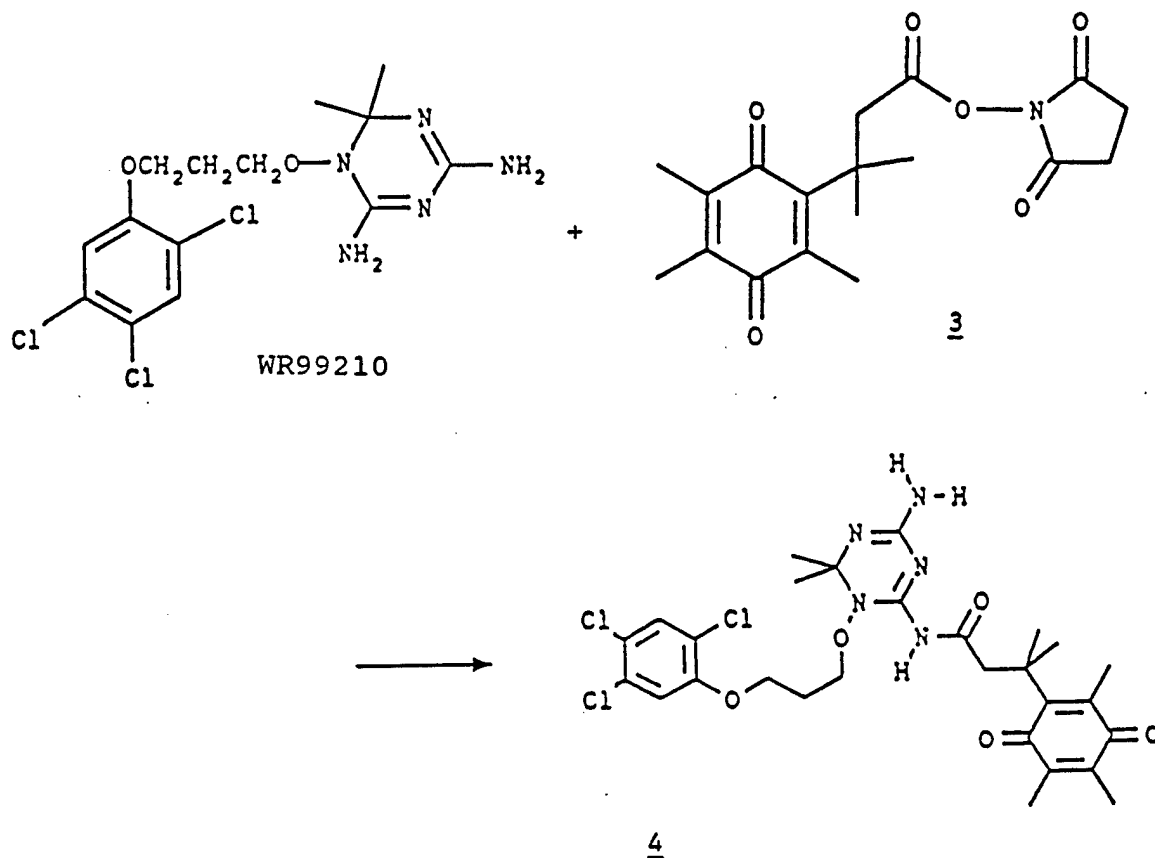
	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	EtOAc-hexane (2:1)	0.74	Homogeneous
2.	Ether-hexane (2:1)	0.20	Homogeneous
3.	Ether	0.72	Homogeneous

Source of Materials

1.	Dicyclohexylcarbodiimide	Aldrich Chemical Co., Inc.
2.	$\beta,\beta,2,4,5$ -Pentamethyl 3,6-dioxo-1,4-cyclohexadiene- 1-propanoic acid	Starks Associates, Inc.
3.	N-Hydroxysuccinimide	Aldrich Chemical Co., Inc.
4.	THF	Aldrich Chemical Co., Inc.
5.	EtOAc	J.T. Baker Chemical Co.
6.	Silica gel	EM Laboratories
7.	Hexane	J.T. Baker Chemical Co.

4. WR99210 Prodrug; amide from $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure) (4)

Reaction Sequence:



Experimental

Succinimidyl $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate (3)

Please refer to the preceding synthesis, this report.

WR99210 Prodrug; amide from $\beta,\beta,2,4,5$ -pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure) (4)

A suspension of WR99210 (23.8 g, 0.060 mol) and 3 (21.0 g, 0.060 mol) in DMF (400 mL) was heated at 60°C for 20 h,

under an argon atmosphere. The mixture was cooled and the solvent was removed in vacuo to yield a residue (51.7 g) as a thick brown oil. The oil was purified by chromatography on a column (500 g) of SiO₂ using EtOAc-hexane (1:1) as the eluent. Fractions containing product were combined then concentrated to a yellow oil (21.9 g). The oil was rechromatographed on a 2 kg silica gel column using the same solvent system. The following fractions were collected: 5-16 (7.6 g), 17-23 (5.8 g) & 24-32 (1.9 g). Fractions 5-16 (7.6 g) were again rechromatographed to give 2.4 g of product. Recrystallization from 2-propanol (20 mL) gave pure product (1.7 g, 70.8% recovery), mp 170-172°C¹⁶. A portion (1.4 g) was transmitted to WRAIR on December 13, 1995 (Lot No. NJ12-109-3). Note that this product, lot number NJ12-109-3, was never fully characterized due to apparent instability. See discussion on page 11 of this report.

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for C ₂₈ H ₃₄ Cl ₃ N ₅ O ₅	53.64	5.47	11.17
Found	53.82	5.50	11.06

Spectral Data

Infrared (KBr)

ν 3296.1, 3196.5, 3073.2, 2974.6, 2924.5,
2870.1, 1707.1, 1666.1, 1639.1, 1580.2, 1466.7,
1379.6, 1329.8, 1279.9, 1240.6, 1181.8, 1136.2,
1081.2, 1059.6, 1026.8, 977.5, 938.9, 897.3,
844.9, 819.1, 745.5, 679.9, 649.8, 593.7,
414.2 cm⁻¹.

Ultraviolet (Ethanol)

$\lambda_{\max}$ 236 nm (sh, log ϵ 4.32); 289 nm (4.31);
295 nm (sh, 3.71).

Nuclear Magnetic Resonance (CDCl₃)

Spectral data (NMR) are not in full accord with the intended structure. Furthermore, the spectrum of the material has changed subsequent to shipment.

δ 10.34 (s, 1, NH); 7.42 (s, 1, ClCCHCCl); 7.25 (s, 2, NH₂); 7.22 (s, 1, ClC-CHCO); 4.25 (t of d, 1, J= 9.2/6.8 Hz, CH₂ON); 4.17 (t of d, 1, J= 9.2/6.6 Hz, CH₂ON); 4.07 (t of d, 1, J= 10.0/5.7 Hz, CH₂O); 3.99 (t of d, 1, J= 10.0/5.7 Hz, CH₂O); 3.00 (d, 1, J= 16.8 Hz CHCO); 2.26 (d, 1, J= 16.8 Hz, CHCO); 2.18 (m, 2, CH₂CH₂CH₂); 2.02 (d, 3, J= 0.9 Hz, quinone CH₃); 1.98 (d, 3, J= 0.9 Hz, quinone CH₃); 1.24 (d, 3, quinone CH₃); 1.17 (d, 6, 2 x CH₃, geminal); 1.1 (s, 3, triazine CH₃); 0.98 (s, 3, triazine CH₃).

Thin Layer Chromatography

EM precoated TLC plates, glass support, 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection-ultraviolet light.

	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	EtOAc-hexane (1:1)	0.42	Homogeneous
2.	EtOH-NH ₄ OH (20:1)	0.86	Homogeneous
3.	CH ₂ Cl ₂ -CH ₃ OH (19:1)	0.51	Homogeneous

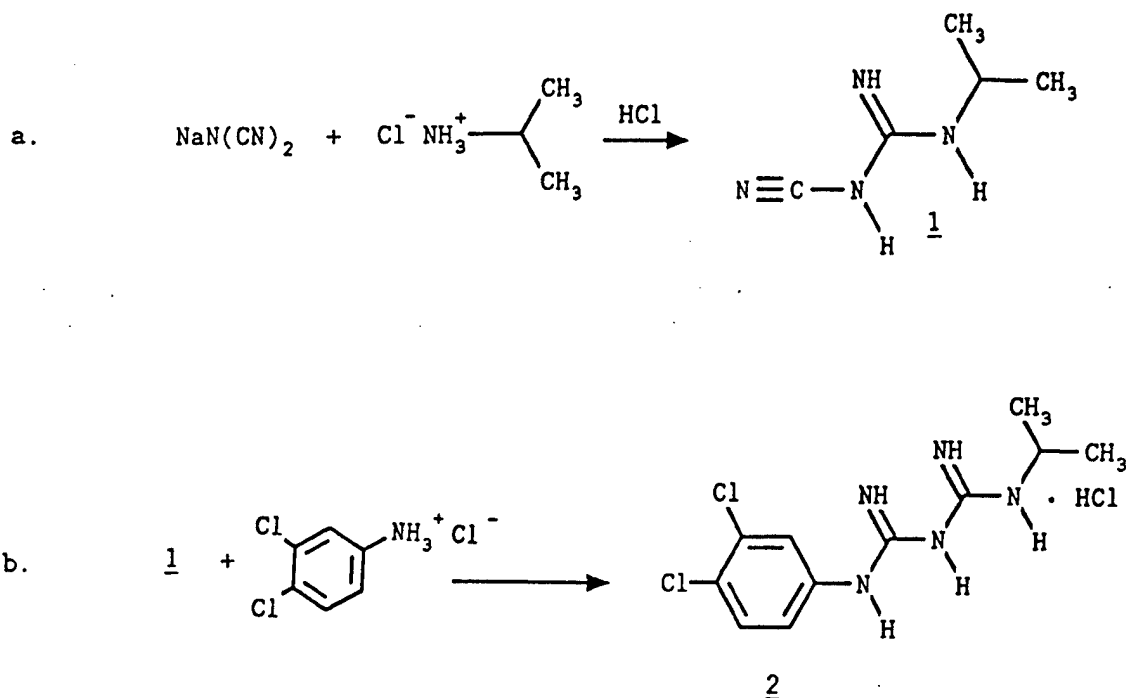
Source of Materials

- | | | |
|----|---|----------------------------|
| 1. | WR99210 | Starks Associates, Inc. |
| 2. | Succinimidyl $\beta,\beta,2,4,5$ -
pentamethyl-3,6-dioxo-
1,4-cyclohexadiene-1-
propanoate | Starks Associates, Inc. |
| 3. | DMF | Aldrich Chemical Co., Inc. |
| 4. | SiO ₂ | EM Laboratories |
| 5. | EtOAc | J.T. Baker Chemical Co. |
| 6. | Hexane | J.T. Baker Chemical Co. |
| 7. | 2-Propanol | Aldrich Chemical Co., Inc. |

5. N¹-3,4-Dichlorophenyl-N⁵-isopropyldiguanide, hydrochloride (chloroproguanil hydrochloride) (2)

The target compound 2 was prepared by the following sequence of reactions.

Reaction Sequence:



Isopropyl dicyandiamide (1)

Isopropylamine (100 mL, 1.17 mol) was added over a ten minute period to a solution of 10.2N hydrochloric acid (107.8 mL, 1.10 mol) and 1-butanol (1370 mL). The mixture was stirred and heated, then the water removed by distillation, at atmospheric pressure, through a Claisen head until the head temperature reached 116°C (950 mL). Additional 1-butanol (320 mL) and sodium dicyanamide (100 g, 96% purity, 1.08 mol) was added to the solution at room temperature. The reaction mixture was refluxed for 2.75 h, cooled to room temperature, then filtered. The insolubles (NaCl) were washed with 1-butanol (2 x 50 mL) then discarded. The filtrate, together with the washings, was concentrated in vacuo at 40°C to a

clear viscous oil (154 g). The oil was dissolved in dioxane (970 mL at 55°C). After cooling to room temperature, the white solid, that separated was collected, then dried (room temperature 4-5 h). NMR showed the material contained ca. 0.5 molar eq. of dioxane, 169 g (92%). Additional product of similar purity (428.6 g) was obtained from other runs.

Spectral Data

Infrared (Nujol)

Major bands: 3360, 3180, 3140, 2960, 2910, 2850, 2160(CN), 1670, 1620, 1540, 1470, 1455, 1425, 1380, 1370, 1290, 1260, 1170, 1130, 1110, 990, 980, 860 cm^{-1} .

Nuclear Magnetic Resonance (DMSO- d_6)

δ 6.75 (broad s, 1, NH); 6.55 (broad s, 2, 2xNH); 3.75 [m, 1, $\text{CH}(\text{CH}_3)_2$]; 3.40 (s, dioxane); 1.10 (d, 6H, 2 x CH_3).

N¹-3,4-Dichlorophenyl-N⁵-isopropyldiguanide, hydrochloride
(chloroproguanil hydrochloride) (2)

A solution of 3,4-dichloroaniline (159.2 g, 0.9826 mol), isopropyldicyandiamide hemisolvate (1) (168.9 g, 0.9922 mol of material containing 0.5 molar eq. of dioxane), 10.2N hydrochloric acid (97.3 mol, 0.9925 mol) and 2-ethoxyethanol (1.1 L) was refluxed for 70 min, and cooled to room temperature overnight. Ethyl acetate (1690 mL) was added dropwise to the stirred suspension over 2.5 h. After storing at room temperature for 4 h, the mixture was filtered, and the white crystals were washed with ethyl acetate (2 x 200 mL) then dried in vacuo at room temperature; yield 189.2 g (59%), m.p. 248-250°C; literature¹⁷ mp 246°C. Additional product of similar purity (496.6 g), mp 247-247.5°C (d) was prepared in a similar way. A portion (29.1 g) was transmitted to WRAIR on January 5, 1996 (Lot No. NJ19-28-1).

Anal.

	<u>C</u>	<u>H</u>	<u>Cl</u>	<u>N</u>
Calc'd for C ₁₁ H ₁₆ Cl ₃ N ₅	40.69	4.97	32.76	21.57
Found	40.43	4.85	32.80	21.37
	40.42	4.88	21.30	

Spectral Data

Infrared (Nujol)

Major bands: 3280, 3080, 2920, 2860, 1630, 1600, 1565, 1530, 1470, 1410, 1365, 1290, 1250, 1230, 1170, 1130, 1030, 870, 810 cm⁻¹.

Ultraviolet (MeOH)

$\lambda_{\max}$ 260 nm (log ϵ 4.37).

Nuclear Magnetic Resonance (DMSO-d₆)

δ 10.39 & 10.12 (2 broad s, 1H, ⁹NH); 8.1-8.0 (m, 1H, NH); 7.84 (d, 2, J= 2.5 Hz, H-5, H-6); 7.58 (d, 1, J= 8.7 Hz, H-2); 7.38 (s, 1 NH); 7.28 (s, 1, NH); 6.89 (s, 1, NH); 3.76 (m, 1, CH(CH₃)₂); 1.18 (d, 6, CH(CH₃)₂).

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - ultraviolet light.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
MeOH-NH ₄ OH (19:1)	0.16	Homogeneous

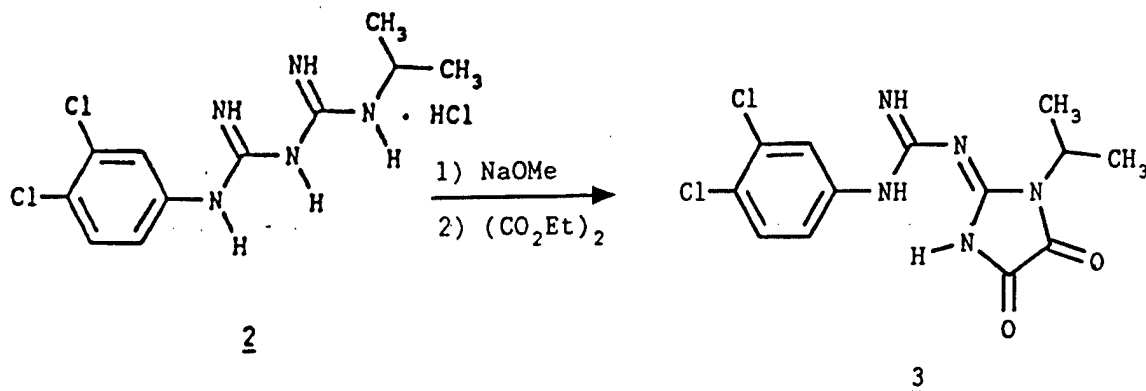
Source of Materials

1.	Isopropylamine	Aldrich Chemical Co., Inc.
2.	HCl	J.T. Baker Chemical Co.
3.	NaN(CN) ₂	Aldrich Chemical Co., Inc.
4.	Dioxane	Aldrich Chemical Co., Inc.
5.	3,4-Dichloroaniline	Aldrich Chemical Co., Inc.
6.	Isopropyldicyandiamide	Starks Associates, Inc.
7.	2-Ethoxyethanol	Aldrich Chemical Co., Inc.
8.	Ethyl acetate	EM Science Co.

6. 1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine (3)

The target compound 3 was prepared by the following reaction.

Reaction Sequence:



Experimental

N¹-3,4-Dichlorophenyl-N⁵-isopropyldiguanide, hydrochloride (chloroproguanil hydrochloride) (2)

Please refer to the preceding synthesis, this report.

1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine (3)

A suspension of N¹-3,4-dichlorophenyl-N⁵-isopropyldiguanide hydrochloride (2) (101.0 g, 0.311 mol), sodium methoxide (80.6 mL of 25 wt % solution in methanol, 0.35 mol) and absolute methanol (1050 mL) was brought to reflux, cooled to room temperature (20°C), then filtered. The insoluble solid was washed with absolute methanol (50 mL). The washings were combined with the filtrate, and to this solution was

added in one portion diethyl oxalate (42 mL, 0.311 mol). The suspension was stirred at 20°C for 1.5 h then filtered. The yellow solid was washed with methanol (4 x 60 mL) then dried in vacuo at room temperature to constant weight; 75.2 g (71%). The material was combined with 85 g of product of similar purity to give 160.2 g, mp 243-244°C(d); literature¹⁷ mp 248°C(d), 250-251°C(d) & 253-254°C(d). A portion (156.6 g) was transmitted to WRAIR on January 29, 1996 (Lot No. NJ19-54-1). Additional material (193.3 g), mp 245-246°C was obtained from another reaction. A portion (192.3 g) was transmitted to WRAIR on January 29, 1996 (Lot No. NJ19-40-1).

Anal.

	<u>C</u>	<u>H</u>	<u>Cl</u>	<u>N</u>
Calc'd for C ₁₃ H ₁₃ Cl ₂ N ₅ O ₂	45.63	3.83	20.72	20.47
Found (Lot No. NJ19-54-1)	45.70	3.84	20.81	20.45
(Lot No. NJ19-40-1)	45.70	3.83	20.55	20.63

Spectral Data

Infrared (Nujol)

The infrared spectra for both lots were identical to the spectrum of a reference sample provided to us by COL J. Scovill.

Major bands: 3320, 3220, 3120, 2900, 2840, 1760, 1730, 1650, 1600, 1470(b), 1370, 1320, 1310, 1255, 1220, 1180, 1130, 1105, 1070, 1025, 990, 940, 860, 800, 750 cm⁻¹.

Ultraviolet (Methanol)

Lot No. NJ19-40-1	$\lambda_{\max}$ 237 nm (log ϵ 4.19); 269 m μ (4.27).
Lot No. NJ19-54-1	$\lambda_{\max}$ 237 nm (log ϵ 4.17); 270 m μ (4.25).

Nuclear Magnetic Resonance (DMSO-d₆ & DCl)

The NMR spectra for both lots were identical to the spectrum of a reference sample provided to us by COL J. Scovill. All spectra show the material to be a mixture of several isomers.

δ 7.9-7.2 (m, 6H); 4.3-3.5 (m, 1H); 1.3 - 1.0 (m, 6H).

Thin Layer Chromatography

A satisfactory TLC system was not found because of the limited solubility of the material in most suitable solvents.

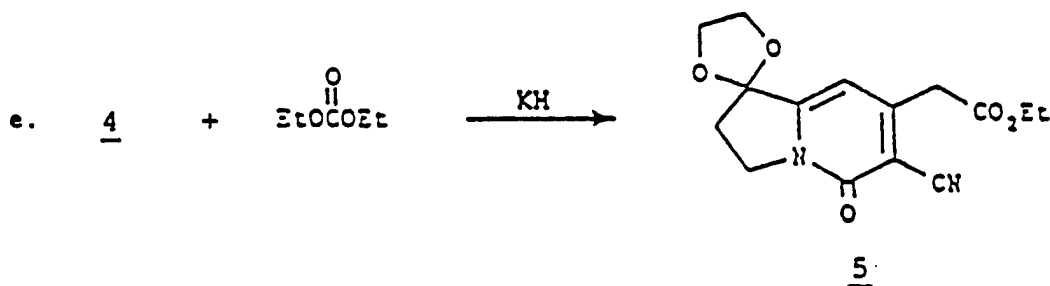
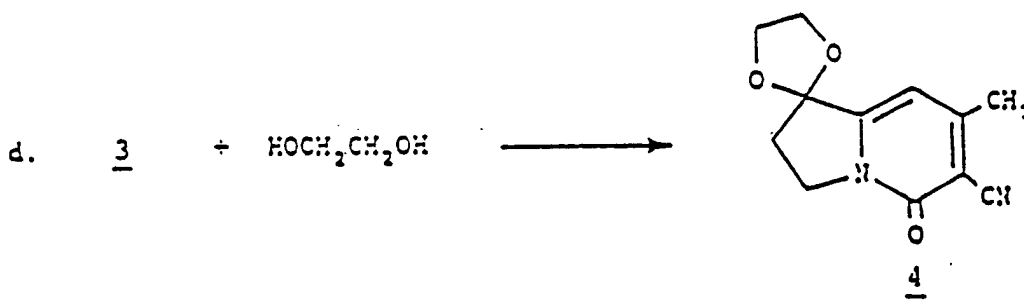
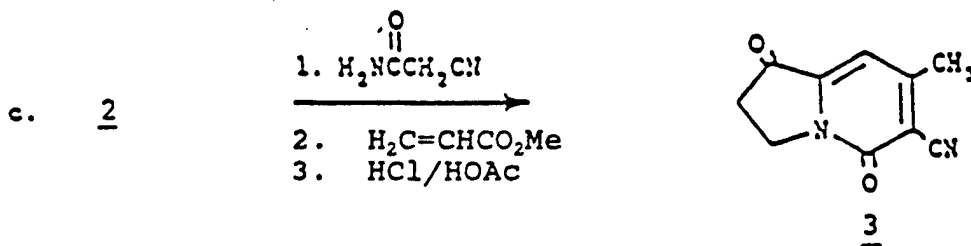
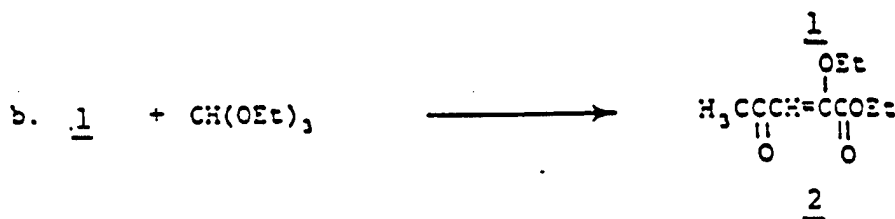
Source of Materials

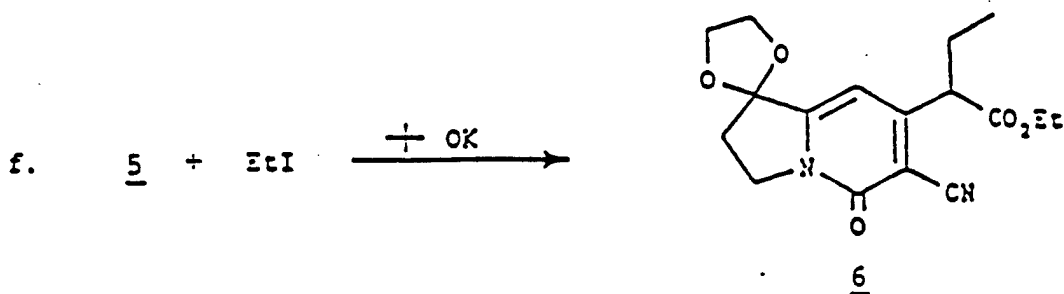
- | | |
|---|----------------------------|
| 1. N ¹ -3,4-Dichlorophenyl-N ⁵ -
isopropyldiguanide, HCl | Starks Associates, Inc. |
| 2. Sodium methoxide
(25% wt. solution in CH ₃ OH) | Aldrich Chemical Co., Inc. |
| 3. Diethyl oxalate | Aldrich Chemical Co., Inc. |
| 4. CH ₃ OH | J.T. Baker Chemical Co. |

7. 6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylene-dioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (6)

The intermediate (6) was prepared by the following sequence of reactions:

Reaction Sequence:





Experimental

The material was prepared as discribed on pp. 56-69, this report. A portion (5.3 g) was transmitted to WRAIR on December 21, 1996 (Lot No. NJ12-124-1).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_{17}H_{20}N_2O_5$	61.44	6.07	8.43
Found	61.51	6.07	8.42

Spectral Data

Infrared (Nujol)

Major bands: 2920, 2840, 2220, 1730, 1660, 1600, 1530, 1460, 1370, 1340, 1320, 1260, 1230, 1190, 1090, 1020, 930, 810, 770 cm^{-1} .

Ultraviolet (EtOH)

λ_{max} 216 nm (log ϵ 4.28); 335 nm (4.02); 340 nm (sh, 4.01).

Nuclear Magnetic Resonance (CDCl_3)

δ 6.40 (s, 1, H at C-8); 4.23-4.11 (m, 8, $\text{OCH}_2\text{CH}_2\text{O}$, $\text{CH}_3\text{CH}_2\text{CO}$, CH_2N); 3.93 (t, 1, $J = 7.60$, CHCH_2CH_3); 2.41 (t, 2, $J = 6.90$, CHCH_2CH_3); 2.12 (m, 1, NCH_2CH_2); 1.78 (m, 1, NCH_2CH_2); 1.26 (t, 3, $J = 7.10$, CH_3); 0.97 (t,

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; visulization - UV.

	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	EtOAc-hexane (4:1)	0.32	homogeneous
2.	CH ₂ Cl ₂ -EtOAc (7:3)	0.48	homogeneous
3.	CH ₂ Cl ₂ -acetone (4:1)	0.70	homogeneous

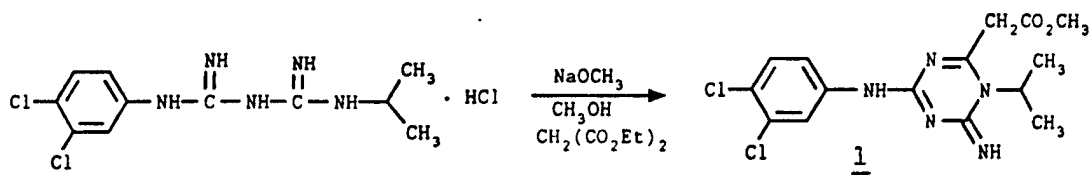
Source of Material

Please refer to pp. 67-69, this report.

8. 1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester (1)

The target compound (tentative structure) was prepared by the following reaction sequence.

Reaction Sequence



Experimental

1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester (1)

To a solution of N¹-3,4-dichlorophenyl-N⁵-isopropyl-diguanide hydrochloride (chloroproguanil hydrochloride) (1.00 g, 3.10 mmol) in methanol (10.0 mL) was added sodium methoxide in methanol (25 wt %, 0.8 mL, 3.50 mmol). The suspension was heated to reflux then cooled to 20°C and filtered. To the filtrate was added diethyl malonate (468 µL, 3.10 mmol) and the mixture was heated at reflux for 20 h. After cooling to 20°C, the reaction mixture was diluted with EtOAc (150 mL) and washed with 0.1N HCl (2 x 100 mL) and H₂O (100 mL), then dried (MgSO₄), filtered, and concentrated to give the crude product (0.4 g), which was purified by silica gel chromatography (1:1 EtOAc:hexanes, 40 g SiO₂) to give the triazine (tentative structure) 1 (230 mg 21%) as a pale yellow solid; mp 108-110°C. A portion (150 mg) was transmitted to WRAIR on May 22, 1996 (Lot No. NJ20-76-2).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>	<u>Cl</u>
Calc'd for $C_{15}H_{17}Cl_2N_5O_2$	48.66	4.63	18.92	19.15
Found	48.84	4.67	18.91	19.06

Spectral Data

Infrared (KBr)

Major bands: 3409, 3356, 2955, 2926, 2328, 2304,
1737, 1612, 1569, 1508, 1477, 1443,
1405, 1323, 1299, 1199, 1173, 1125,
994, 815, 497 cm^{-1} .

Nuclear Magnetic Resonance ($CDCl_3$)

(mixture of tautomers)

δ 8.07 and 7.87 (2 br s, 1H, C=NH); 7.34-6.97 (m, 3H, Ar); 5.23 and 5.16 (2 br s, 1H, NH); 4.20-4.08 (m, 1H, CH(CH₃)₂); 3.75 (s, 3H, CO₂CH₃); 3.63 and 3.56 (2 s, 2 H, CH₂); 1.25 and 1.22 (2 d, 6H, CH(CH₃)₂).

Mass Spectrum

Method of Ionization = Electrospray (positive)

Calc'd for $C_{15}H_{17}Cl_2N_5O_5$ = 369.1

Found: 370.1 (m + H)⁺.

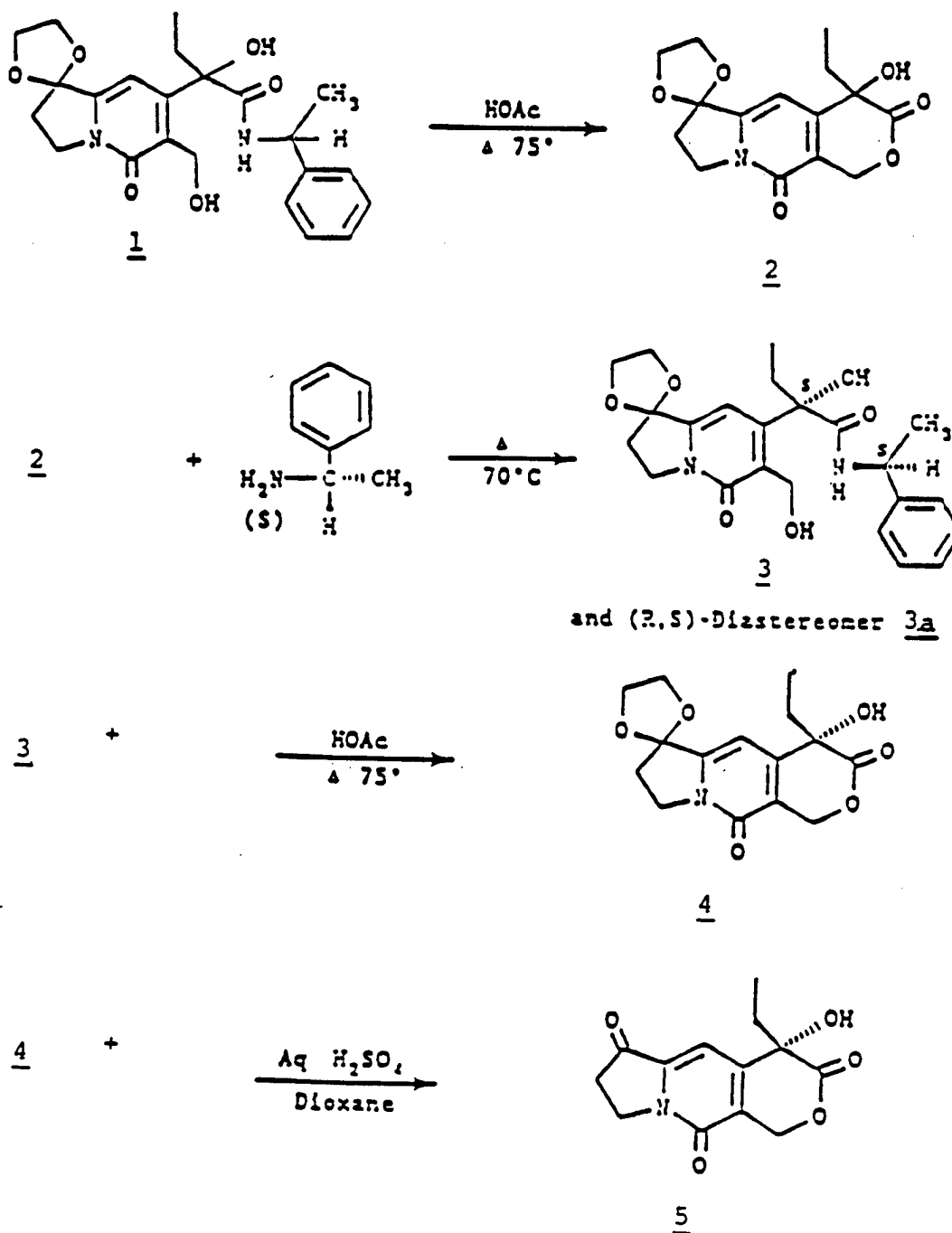
Source of Materials:

- | | |
|--|----------------------------|
| 1. Chloroproguanil
hydrochloride | Starks Associates, Inc. |
| 2. Methanol | J.T. Baker Chemical Co. |
| 3. Sodium methoxide
(25 wt % in CH ₃ OH) | Aldrich Chemical Co., Inc. |
| 4. Diethyl malonate | Aldrich Chemical Co., Inc. |
| 5. Ethyl acetate | E.M. Science |
| 6. Hydrochloric acid | J.T. Baker Chemical Co. |
| 7. Magnesium sulfate | J.T. Baker Chemical Co. |
| 8. Silica gel | E.M. Science |
| 9. Hexanes | J.T. Baker Chemical Co. |

9. (S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-f]-indolizine-3,6,10-trione (5)

The target compound 5 was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

(R,S)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]indolizine-3,10-(6H)-dione (2)

A solution of crude (R,S)-2-hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(R,S)- α -methylbenzyl]butyramide (1) (71.0 g, 0.16 mol) and AcOH (1.3 L) was stirred at 75°C for 3 h. The solvent was removed in vacuo and the last traces of AcOH were removed by codistillation with toluene (2 x 50 mL). The residue (107.7 g) was chromatographed on silica gel (1 kg) using CH₂Cl₂-acetone (4:1) as the eluent. Fractions containing product were combined and concentrated in vacuo to obtain 43.9 g of product suitable for further transformation.

(S)-2-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(S)-(-)- α -methylbenzyl]butyramide (3)

A solution of racemic 4-ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]indolizine-3,10-(6H)-dione (2) (14.2 g, 46.2 mmol) and (S)-(-)- α -methylbenzylamine (45.0 g, 0.372 mol), maintained under an argon atmosphere, was heated at 70°C for 20 h then the excess amine was removed by vacuum distillation. The oily residue (25.5 g) was suspended in toluene (150 mL), and the mixture was stirred for 2 h. The resulting solid was collected on a filter, washed with toluene (2 x 25 mL), hexane (2 x 25 mL), then dried; yield 9.3 g. This was slurried in CH₂Cl₂ (80 mL). The light suspension was filtered, and toluene (150 mL) was added to the filtrate. The mixture was stirred for 2 h. The precipitated solid was collected, washed with hexane (50 mL) and dried in vacuo to give 7.0 g (35.4%) of 3 as a white solid. Additional product (18.8 g) was obtained from a larger reaction. The material was suitable for further transformation.

(S)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]indolizine-3,10-(6H)-dione (4)

A solution of (S)-2-hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(S)-(-)- α -methylbenzyl]butyramide (3) (4.7 g, 10.97 mmol) and AcOH (100 mL) was stirred at 75°C for 2 h then the solvent was removed by evaporation. The residue was dissolved in EtOAc then chromatographed on silica gel (200 g) eluted with EtOAc-MeOH (10:1) to give 3.1 g (92%) of 4; m.p. 166-168°C $[\alpha]_D^{22} = +100.32^\circ$ (C= 1.00 in CHCl₃). An additional reaction was carried out to give a total of 12.1 g of (4) via this route.

(S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]-indolizine-3,6,10-trione (5)

A solution of (S)-4-ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]indolizine-3,10-(6H)-dione (4) (15.0 g, 48.8 mmol), H₂SO₄ (260 g), water (220 mL), and dioxane (390 mL) was heated at reflux for 80 min, cooled, then extracted with CH₂Cl₂ (3 x 500 mL). The combined organic layers were washed with water (2 x 200 mL). The aqueous layer was neutralized to pH6 with solid Na₂CO₃ and re-extracted with CH₂Cl₂ (2 x 200 mL). The combined organic layers were dried over MgSO₄ then concentrated in vacuo to a residue. The residue was dissolved in MeOH (200 mL) and charcoaled with carbon (1 g). The solvent was removed, and the residue was dissolved in CH₂Cl₂ (100 mL). The solid was precipitated by dropwise addition of hexane (100 mL). This was collected on a filter and dried in vacuo at 40°C to 10.3 g of 4; mp 170-172°C. Literature¹³ mp 174-176°C, $[\alpha]_D^{20} = 105.6^\circ$ (C= 0.765; CHCl₃/MeOH 4:1); literature¹³ $[\alpha]_D^{20} = +105.2^\circ$ (C= 0.49; CHCl₃/MeOH 4:1). A portion (10.2 g) was transmitted to WRAIR on June 19, 1996 (Lot No. NJ19-72-4).

Anal.

		<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for	$C_{13}H_{13}NO_5$	58.79	5.03	5.27
	• 0.13 H_2O			
Found		58.76	5.01	5.25

Spectral Data

Infrared (Nujol)

Major bands: 3400, 2900, 2840, 1740, 1660, 1610,
1455, 1385, 1345, 1315, 1260, 1230,
1200, 1150, 1100, 1055, 995 cm^{-1} .

Ultraviolet (EtOH)

λ_{max} 228 nm (sh, log ϵ 4.03); 260 nm
(sh, 3.39); 337 nm (3.88).

Nuclear Magnetic Resonance ($CDCl_3$)

δ 7.22 (s, 1, pyridone H); 5.66 (d, 1, $J=17.0$ Hz,
H at C-1); 5.24 (d, 1, $J=17.0$ Hz, H at C-1);
4.39 (t, 2, $COCH_2$); 3.76 (s, 1, OH); 2.96 (t, 2,
 NCH_2); 1.81 (q, 2, $\underline{CH_2}-CH_3$); 0.98 (t, 3, CH_3).

Source of Material

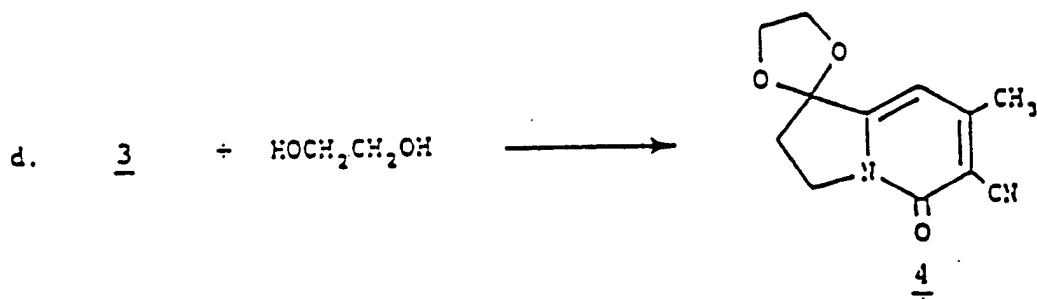
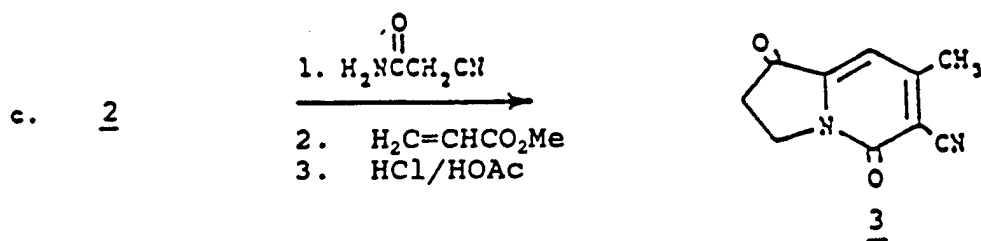
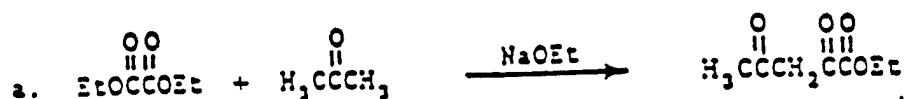
1. (*R,S*)-2-Hydroxy-2-[6-hydroxy-methyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*R,S*)- α -methylbenzyl]-butyramide
Starks Associates, Inc.
2. AcOH
J.T. Baker Chemical Co.
3. Toluene
J.T. Baker Chemical Co.

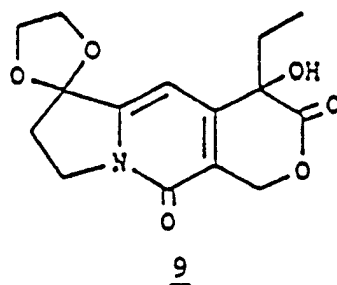
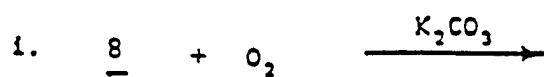
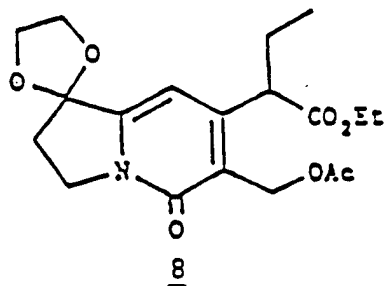
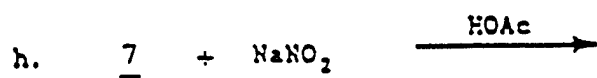
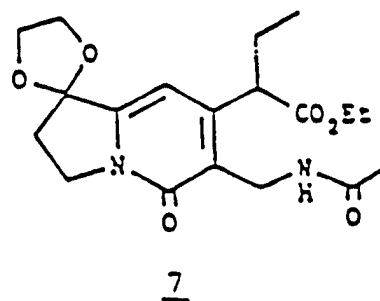
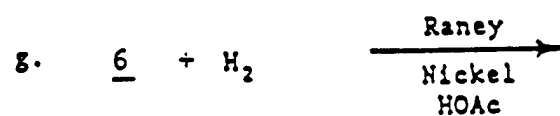
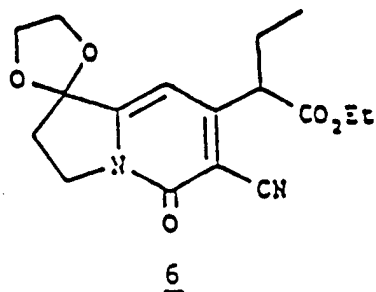
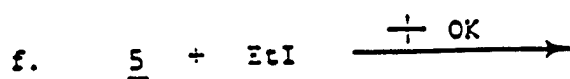
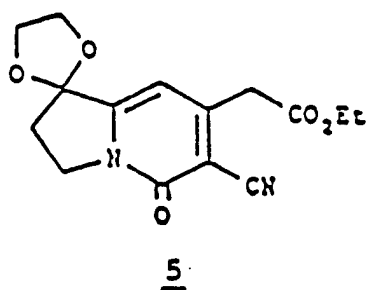
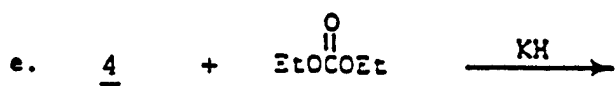
4.	Silica gel	EM Laboratories
5.	CH ₂ Cl ₂	J.T. Baker Chemical Co.
6.	Acetone	J.T. Baker Chemical Co.
7.	(R,S)-4-Ethyl-6,6-(ethylene-dioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]-indolizine-3,10-(6H)-dione	Starks Associates, Inc.
8.	(S)-(-)- α -Methylbenzylamine	Aldrich Chemical Co., Inc.
9.	Hexane	J.T. Baker Chemical Co.
10.	(S)-2-Hydroxy-2-[6-hydroxy-methyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(S)- α -methylbenzyl]-butyramide	Starks Associates, Inc.
11.	EtOAc	J.T. Baker Chemical Co.
12.	CH ₃ OH	J.T. Baker Chemical Co.
13.	(S)-4-Ethyl-6,6-(ethylene-dioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-f]-indolizine-3,10-(6H)-dione	Starks Associates, Inc.
14.	H ₂ SO ₄	J.T. Baker Chemical Co.
15.	Dioxane	J.T. Baker Chemical Co.
16.	Na ₂ CO ₃	Aldrich Chemical Co., Inc.

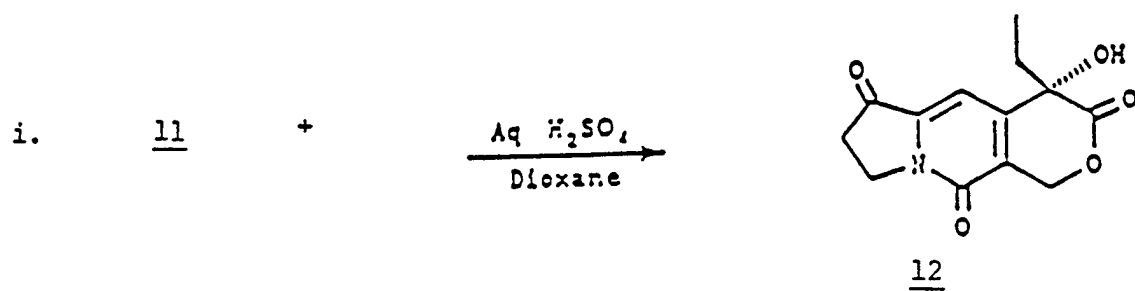
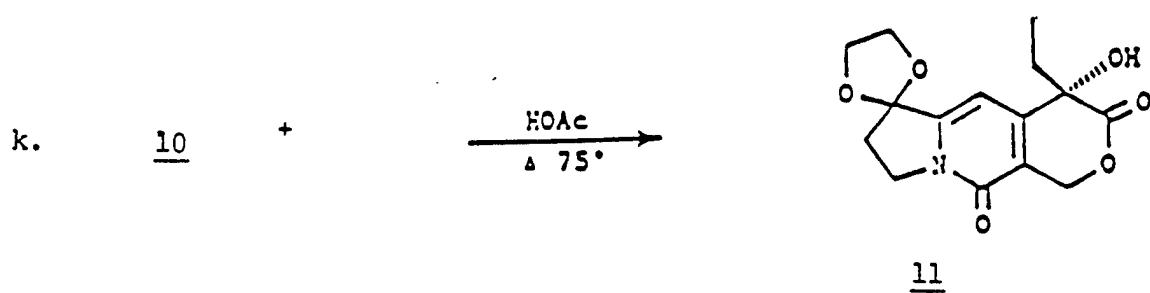
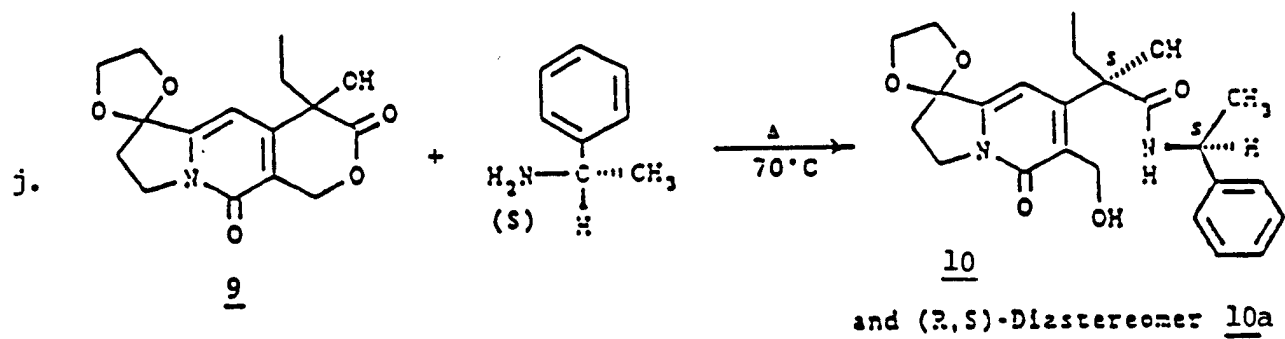
10. (S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-f]-indolizine-3,6,10-trione (12)

The target compound 12 was prepared by the following sequence of reactions.

Reaction Sequence:







Experimental

Ethyl acetopyruvate (1)

To a cold (0°C), stirred solution (under argon) of ethanolic NaOEt (5000 g of 21% in EtOH, 15.44 mol) in EtOH (6 L) was added a mixture of diethyl oxalate (2052 g; 14.04 mol) and acetone (814 g; 14.04 mol) during 1 h. The temperature was maintained between 0-5°C during the addition. The resulting suspension was stirred at ambient temperature for 16 h then separated by filtration. The collected solid was washed with Et₂O (2 x 2 L) then dried in vacuo at 40°C to give 2182.8 g (86.4%) of compound 1 as the sodium salt. This material was suspended (partial solution) in cold (0°C) H₂O then acidified with concentrated H₂SO₄. The resulting mixture was extracted with CH₂Cl₂ (2 x 10 L). The organic extracts were combined, washed with 5% aqueous NaHCO₃ (4 L), dried over Na₂SO₄ then concentrated in vacuo to a brown oil. This oil was vacuum distilled to give 1157.8 g (52.2%) of compound 1; bp 95-105°C at 15 mm. The material was suitable for further transformation.

Ethyl 2-ethoxy-4-oxopent-2-enoate (2)

A mixture of ethyl acetopyruvate (1) (1157.8 g, 7.32 mol), triethyl orthoformate (1191.5 g, 8.04 mol), ammonium chloride (79.2 g, 1.48 mol) and ethanol (2.1 L) was stirred at RT for 120 h then concentrated in vacuo to ~2 L. The solid was filtered off then washed with EtOH (250 mL). The filtrate and washings were combined then concentrated in vacuo to an oil. The oil was dissolved in ether (3 L), and the solution was washed with 2N K₂CO₃ (3 x 1 L), dried (Na₂SO₄), then concentrated (1357.6 g). The material was distilled in vacuo to yield 1225.8 g (89.9%) of material 2 as pale yellow oil, bp 110-115°/10 mm. The product was suitable for further transformation.

6-Cyano-1,5-dioxo-7-methyl-1,2,3,5-tetrahydroindolizine (3)

To a warm (45°C), stirred suspension of cyanoacetamide (142.9 g; 1.70 mol), anhydrous K₂CO₃ (230.5 g; 1.67 mol), and dry DMF (3 L) under argon was added ethyl 2-ethoxy-4-oxo-pent-2-enoate (2) (294 g; 1.58 mol) in a thin stream during 45 min. The resulting deep red suspension was stirred at 45°C for 65 h after which time TLC (toluene:dioxane:HOAc 90:25:4) analysis indicated the starting material had been consumed. The warm (45°C) solution was diluted with H₂O (57 mL) then methyl acrylate (990 mL, 947 g, 11 mol) was added in a thin stream during 45 min. The resulting red suspension was stirred at 45°C for 96 hours, cooled to room temperature then separated by filtration. The solid was washed on the funnel with DMF (2 x 250 mL) and acetone (3 x 250 mL), then suspended in H₂O (3 L). This suspension (partial solution) was acidified (pH~1) with conc. HCl. The suspended solid was collected on a funnel then washed with H₂O (2 x 250 mL). The wet cake (901 g) of the indolizine was dissolved in concentrated HCl (1.5 L) and glacial HOAc (1.5 L) then stirred at reflux for 18 h. TLC analysis (CHCl₃/acetone/MeOH, 15:4:1) showed completeness of reaction. The reaction mixture was cooled to room temperature then extensively extracted with CH₂Cl₂ (7 x 4 L). The organic extracts were washed with brine (16 L), dried over MgSO₄, then concentrated in vacuo to a residue. This material was triturated with hexane to give 95.1 g (32% overall yield from compound (2)) of the bicyclic ketone intermediate 3. Additional product (271.9 g) was obtained from other reactions. The material was suitable for further transformation.

6-Cyano-1,1-(ethylenedioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydroindolizine (4)

A stirred solution (containing minor amount of insolubles) of 6-cyano-1,5-dioxo-7-methyl-1,2,3,5-tetrahydroindolizine (3) (186.5 g; 0.99 mol) in CH_2Cl_2 (8 L) was warmed to 30°C then 17.5 g of charcoal was added. This suspension was stirred for 0.5 h then clarified by filtration. The temperature of the filtrate was adjusted to 25°C then ethylene glycol (134.7 g; 2.17 mol) and chlorotrimethylsilane (558 mL; 4.40 mol) were added in one portion. The reaction mixture was stirred at 25°C for 7 days. TLC analysis (CHCl_3 /acetone/MeOH 15:4:1) indicated completeness of reaction. The reaction mixture was clarified by filtration, washed with 5% NaOH (5 x 3.5 L), dried over MgSO_4 , then concentrated in vacuo to a residue. This residue was recrystallized from MeOH (5 L) to give 121.3 g (52.7%) of purified intermediate 4. Additional product (165.5 g) was obtained from another reaction. The material was suitable for further transformation.

6-Cyano-7-[(ethoxycarbonyl)methyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (5)

A stirred suspension of 6-cyano-1,1-(ethylenedioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydroindolizine (4) (111.0 g; 0.478 mol), NaH (60% min oil dispersion, 47.8 g; 1.194 mol) and anhydrous toluene (1000 mL) was heated under argon at reflux for 15 min, then a mixture of diethyl carbonate (148.7 g; 1.259 mol) and absolute EtOH (22.2 g; 28.3 mL; 0.482 mol) was added dropwise during 45 min. The reaction mixture (green suspension) was heated at reflux for 3 h then cooled to room temperature. The solid was collected on a funnel then washed on the funnel with toluene (3 x 200 mL). The solid was placed in a flask and glacial acetic acid (2 L) was slowly added

under a strong argon purge (some NaH decomposition is occurring in this step). The resulting mixture (mostly dissolved) was diluted with H₂O (1388 mL) then extracted with CH₂Cl₂ (3 x 2 L). The combined extracts were washed with brine (2 x 1500 L), dried over Na₂SO₄, then concentrated in vacuo to a green solid (147.5 g). This material was combined with product of similar purity (256.6 g) then chromatographed on a 22 kg column of silica gel eluted with CH₂Cl₂-CH₃OH (10:1) to give 309.4 g of purified product. The material was suitable for further transformation.

6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (6)

A stirred suspension of 6-cyano-7-[(ethoxycarbonyl)-methyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (5) (150.0 g, 0.493 mol) in dry 1,2-dimethoxyethane (2600 mL) under argon was cooled to -65°C, then potassium *t*-butoxide (52.6 g, 0.473 mol) was added in one portion. The reaction mixture was allowed to warm to 0°C then re-cooled to -65°C. Ethyl iodide (397.4 g, 2.55 mol) was added, and the reaction mixture was stirred at -65°C for 3 h, then at room temperature for 18 h. Quenching of the mixture was carried out by the slow addition of H₂O (900 mL). The resulting mixture was extracted with CH₂Cl₂ (3 x 1880 mL). The combined extracts were washed with brine (2 x 2000 mL), dried over Na₂SO₄, then concentrated in vacuo to a solid residue (168.0 g). Additional crude product (178.6 g) was obtained from another reaction. The lots were combined, then chromatographed on an 11 kg silica gel column eluted with EtOAc/hexanes (4:1), to give 272.8 g (80.4% overall) of intermediate 6. The material was suitable for further transformation.

6-(Acetamidomethyl)-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (7)

A stirred suspension of 6-cyano-7-[1'-(ethoxycarbonyl)-propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (6) (80.0 g; 0.24 mol), activated Raney nickel (47 mL of a slurry that had been washed with HOAc decantations (6x100 mL) and acetic anhydride/HOAc decantations (3:1, 6x100 mL)), and acetic anhydride/HOAc (3:1, 810 mL) was hydrogenated at 45°C and 70 psi hydrogen for 3.5 h. TLC analysis (CHCl₃/acetone/MeOH 15:4:1) indicated complete loss of the starting material. The catalyst was removed by filtration, and the filtrate was used directly in the next step without further isolation. Additional 6 (185.6 g) was reacted in a similar manner.

6-Acetoxymethyl-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (8)

The solution of 6-(acetamidomethyl)-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-7-[1'-(ethoxycarbonyl)-propyl]-5-oxo-1,2,3,5-tetrahydroindolizine (7) (90.5 g; 0.24 mol) from the previous step was cooled to 0°C, under argon, with magnetic stirring. Sodium nitrite (90.0 g, 1.3 mol) was added portionwise, during a 30 min period, while maintaining the internal temperature at 0-5°C. After complete addition, the reaction mixture was stirred at ambient temperature for 20 h, then concentrated in vacuo. The resulting residue was suspended in CCl₄ (1 L), heated at reflux for 3 h, then cooled to ambient temperature. The solution was washed with H₂O (3x1 L), dried over Na₂SO₄, then concentrated in vacuo to give 68.4 g of intermediate 8 as a brown oil. This crude material (95.7 g) was used directly in the next step without further purification or characterization. Additional 8 (241.2 g) was obtained from other reactions.

(R,S)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyran[3,4-f]indolizine-3,10(6H)-dione (9)

A stirred suspension of 6-acetoxymethyl-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine (8) (155.4 g, 0.41 mol), K₂CO₃ (68.1 g; 0.49 mol), and anhydrous methanol (4 L) was heated to 30°C. Oxygen was continuously passed through the reaction mixture for 3 h (then suspension became a solution as the reaction proceeded). TLC analysis (acetone/MeOH, 4:1) indicated completeness of reaction. The reaction mixture was acidified to pH 2 by the slow addition of 1M H₂SO₄, then concentrated in vacuo to a solid residue. This material was partitioned between H₂O (6 L) and CH₂Cl₂ (2 L). The aqueous layer was removed and extracted with CH₂Cl₂ (3 x 3 L). The combined organic layers were dried over Na₂SO₄, then chromatographed over silica (6 kg) eluted with CH₂Cl₂/acetone (4:1) to give 39.9 g of high purity tricyclic ketal 9. Additional product (37.3 g) was obtained from another reaction. The material was suitable for further transformation.

(S)-2-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine-7-yl]-N-[(S)-(-)-α-methylbenzyl]butyramide, monohydrate (10)

A solution of racemic (R,S)-4-ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyran[3,4-f]indolizine-3,10(6H)-dione (9) (77.2 g, 0.251 mol) and (S)-(-)-α-methylbenzylamine (381 g, 3.14 mol), maintained under an argon atmosphere, was heated at 75°C for 20 h then the excess amine was removed by vacuum distillation (333.2 g was recovered). The oily residue (132.5 g) was suspended in toluene (1.5 L), and the mixture was stirred for 2 h. The resulting solid was collected on a filter, washed with toluene, hexane, then dried; yield, 41.5 g (41.3%). The filtrate contains the more soluble (R)(S) diastereomer 10a. The material was suitable for further transformation.

(S)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-f]indolizine-3,10(6H)-dione (11)

A solution of (S)-2-hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(S)-(-)- α -methylbenzyl]butyramide (10) (41.2 g, 0.096 mol) and AcOH (1 L) was stirred at 75°C for 2 h then the solvent was removed by evaporation. The residue was dissolved in EtOAc-CH₃OH (10:1) then chromatographed on a column of silica gel (500 g) using EtOAc-CH₃OH (10:1) as the eluent. Fractions containing product were combined, concentrated in vacuo to give 28 g of crude 11. The material was recrystallized from EtOAc (870 mL) to give 24.6 g of pure 11. Additional product (11.0 g) was obtained previously. The material was suitable for further transformation.

(S)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-f]indolizine-3,6,10-trione (12)

A mixture of (11) (16.9 g, 55 mmol), H₂SO₄ (16 mL), H₂O (248 mL), and dioxane (390 mL) was heated under reflux for 80 min then cooled to RT. The mixture was extracted with CH₂Cl₂ (3 x 500 mL). The extracts were combined, then washed with H₂O (2 x 200 mL). The water washes were combined, neutralized to pH 6 with solid Na₂CO₃ then extracted with CH₂Cl₂ (2 x 200 mL). The CH₂Cl₂ extracts and washes were combined, washed with H₂O (200 mL), dried (MgSO₄), then concentrated in vacuo to give 10.1 g of amber colored residue. Additional crude product (15.1 g) was obtained from another reaction. The two lots were dissolved in CH₃OH (~500 mL), charcoaled (2 g), then concentrated in vacuo to a yellow solid. The solid was dissolved in CH₂Cl₂ (200 mL) and hexane (200 mL) was added. The solid that separated was collected then stored. The filtrate was concentrated and the process was repeated. The solids were combined then dried (40°C); yield, 20.5 g (67.2%); mp 222-223°C; literature¹⁸ mp 174-176°C; $[\alpha]_D^{20} = +103.8^\circ$

(C=0.814; CHCl₃/MeOH 4:1); literature¹⁸ $[\alpha]_D^{20} = +105.2^\circ$ (C=0.49; CHCl₃/MeOH 4:1). A portion (20.3 g) was transmitted to WRAIR on September 5, 1996 (Lot No. NJ24-38-1).

Anal.

		<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for	C ₁₃ H ₁₃ NO ₅	59.31	4.98	5.32
	Found	59.26	4.97	5.26

Spectral Data

Infrared (Nujol)

Major bands: 3400(b), 2900, 2840, 1730, 1645, 1500, 1445, 1405, 1390, 1370, 1330, 1300, 1250, 1220, 1185, 1140, 1080, 1045, 980, 935, 890, 810 cm⁻¹.

Ultraviolet (EtOH)

$\lambda_{\max}$ 222 nm (sh, log ϵ 4.05); 338 nm (3.90).

Nuclear Magnetic Resonance (CDCl₃)

δ 7.22 (s, 1, pyridone H); 5.65 (d, 1, J= 17.1 Hz, H at C-1); 5.23 (d, 1, J= 17.1 Hz, H at C-1); 4.34 (t, 2, J=6.1 Hz, COCH₂); 3.84 (s, 1, OH); 2.96 (t, 2, J=5.2 Hz, NCH₂), 1.81 (d of q, 2, J= 7.1/2.2 Hz, CH₃CH₂); 0.97 (t, 3, J=7.4 Hz, CH₃).

Source of Material

1. Sodium ethoxide in EtOH	Aldrich Chemical Co., Inc.
2. Ethanol	US Industrial Chem. Corp.
3. Diethyl oxalate	Aldrich Chemical Co., Inc.
4. Acetone	J.T. Baker Chemical Co.
5. Ether	Fisher Scientific
6. H ₂ SO ₄	J.T. Baker Chemical Co.
7. CH ₂ Cl ₂	J.T. Baker Chemical Co.
8. NaHCO ₃	Aldrich Chemical Co., Inc.
9. Ethyl acetopyruvate	Starks Associates, Inc.
10. Triethyl orthoformate	Aldrich Chemical Co., Inc.
11. NH ₄ Cl	Aldrich Chemical Co., Inc.
12. K ₂ CO ₃	Aldrich Chemical Co., Inc.
13. Ethyl 2-ethoxy-4-oxo- pent-2-enoate	Starks Associates, Inc.
14. Cyanoacetamide	Aldrich Chemical Co., Inc.
15. DMF	Aldrich Chemical Co., Inc.
16. Methyl acrylate	Aldrich Chemical Co., Inc.
17. HCl	General Chemical Co.
18. AcOH	General Chemical Co.
19. MgSO ₄	J.T. Baker Chemical Co.
20. Hexane	J.T. Baker Chemical Co.
21. 6-Cyano-1,5-dioxo-7- methyl-1,2,3,5-tetrahydro- indolizine	Starks Associates, Inc.
22. Ethylene glycol	Aldrich Chemical Co., Inc.
23. Chlorotrimethylsilane	Aldrich Chemical Co., Inc.
24. NaOH	J.T. Baker Chemical Co.

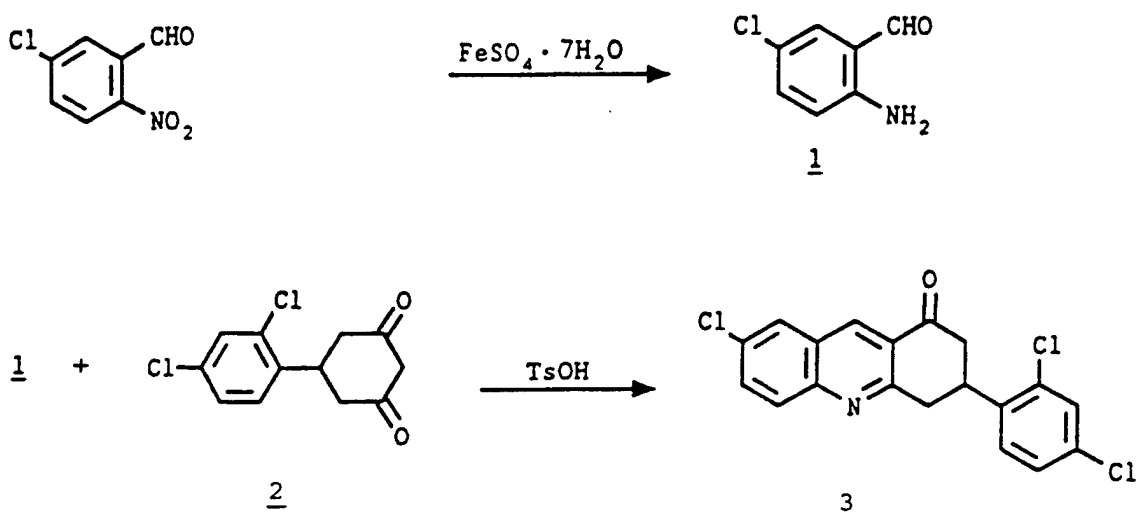
25.	MeOH	J.T. Baker Chemical Co.
26.	6-Cyano-1,1-(ethylene-dioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydro-indolizine	Starks Associates, Inc.
27.	NaH	Lancaster Synthesis
28.	Toluene	Aldrich Chemical Co., Inc.
29.	Diethyl carbonate	Aldrich Chemical Co., Inc.
30.	Na ₂ SO ₄	Aldrich Chemical Co., Inc.
31.	Silica gel	EM Laboratories
32.	6-Cyano-7-[(ethoxycarbonyl)methyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine	Starks Associates, Inc.
33.	1,2-Dimethoxyethane	Aldrich Chemical Co., Inc.
34.	Potassium <i>t</i> -butoxide	Aldrich Chemical Co., Inc.
35.	Ethyl iodide	Aldrich Chemical Co., Inc.
36.	EtOAc	J.T. Baker Chemical Co.
37.	6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine	Starks Associates, Inc.
38.	Raney Ni	Aldrich Chemical Co., Inc.
39.	Acetic anhydride	Aldrich Chemical Co., Inc.
40.	6-Acetamidomethyl)-1,1-(ethylene-dioxy)-7-[1'-(ethoxycarbonyl)propyl]-5-oxo-1,2,3,5-tetrahydro-indolizine	Starks Associates, Inc.
41.	Sodium nitrite	Aldrich Chemical Co., Inc.
42.	CCl ₄	Aldrich Chemical Co., Inc.
43.	6-Acetoxyethyl)-7-[1'-(ethoxy-carbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydro-indolizine	Starks Associates, Inc.
44.	O ₂	Linde

- | | | |
|-----|---|----------------------------|
| 45. | (RS)-4-Ethyl-6,6-(ethylene-dioxy)-1,4,7,8-tetrahydro-4-hydroxypyran[3,4-f]-indolizine-3,10-(6H)-dione | Starks Associates, Inc. |
| 46. | (S)-(-)- α -Methylbenzylamine | Aldrich Chemical Co., Inc. |
| 47. | (S)-4-Ethyl-6,6-(ethylene-dioxy)-1,4,7,8-tetrahydro-4-hydroxypyran[3,4-f]-indolizine-3,10-(6H)-dione | Starks Associates, Inc. |
| 48. | Dioxane | J.T. Baker Chemical Co. |

11. 1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

The target compound (3) was prepared by the following sequence of reactions.

Reaction Sequence:



Experimental

5-Chloro-2-aminobenzaldehyde (1)

To a boiling solution of ferrous sulfate, heptahydrate (53.0 g, 0.19 mol) in H_2O (250 mL) was added a solution of 5-chloro-2-nitrobenzaldehyde (5.0 g, 26.9 mmol) in 50% aqueous ethanol (250 mL). The solution was boiled for one minute and then concentrated ammonium hydroxide (70 mL) was added in 10 mL portions. After the addition the suspension was boiled for 10 min, filtered hot, and the solid was washed with boiling H_2O (2 x 100 mL). The filtrate and washings were combined, cooled, and the solid that separated was collected. This was dissolved in CH_2Cl_2 (100 mL), washed with H_2O (50 mL),

dried over Na_2SO_4 and after removal of solvent in vacuo 2.2 g of 1 was obtained, which was suitable for the next step.

5-(2,4-Dichlorophenyl)cyclohexane-1,3-dione (2)

Please refer to Starks Associates, Inc. Annual Summary Report dated June 1987 (July 1, 1986 to June 30, 1987) Contract DAMD17-83-C-3206, pg. 28.

1(2H)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)- (3)

To a mixture of 5-chloro-2-aminobenzaldehyde (1) (2.2 g, 14.1 mmol) and 5-(2,4-dichlorophenyl)cyclohexane-1,3-dione (2) (3.65 g, 14.2 mmol) in toluene (1 L) was added *p*-toluene-sulfonic acid monohydrate (200 mg) and the mixture was heated at slow reflux using Dean Stark apparatus to remove water. After 2 h of reflux the reaction was cooled and concentrated in vacuo. The residue was triturated with hot CH_3OH (100 mL). The solid was collected then crystallized from glacial acetic acid (150 mL); yield 1.7 g (32.0%) mp 222-223°C. A portion (200 mg) was transmitted to WRAIR on October 18, 1996 (NJ24-38-1).

Anal.

		<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for	$\text{C}_{19}\text{H}_{12}\text{Cl}_3\text{NO}$	60.59	3.21	3.72
	Found	60.33	3.35	3.63

Spectral Data

Infrared (KBr)

Major bands: 3430, 3060, 1690, 1610, 1590, 1555,
1475, 1450, 1430, 1395, 1370, 1300,
1270, 1230, 1205, 1190, 1130, 1100,
1070, 1050, 1015, 930 cm^{-1} .

Ultraviolet (CHCl_3)

λ_{max} 260 nm ($\log \epsilon$ 4.75); 325 nm (3.93).

Nuclear Magnetic Resonance (TFA-d)

δ 9.71 (s, 1, H-9); 8.46 (s, 1, H-8); 8.31 (m, 2, H-5 and H-6); 7.53 (d, 1, C-3'), 7.38 (m, 1, C-5'); 7.34 (d, 1, C-6'); 4.37 (m, 1, C-3); 3.97 (m, 2, C-4); 3.40 (m, 2, C-2).

Mass Spectrometry (Electron impact)

m/e [Rel. int., ID]; 379 [11, $(m+4)^+$]; 378 [10, $(m+3)^+$]; 377 [32, $(m+2)^+$]; 375 [32, m^+].

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; visulization - UV.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
Hexane-acetone (4:1)	0.36	Single spot
Hexane-EtOAc (2:1)	0.74	Single spot

Source of Material

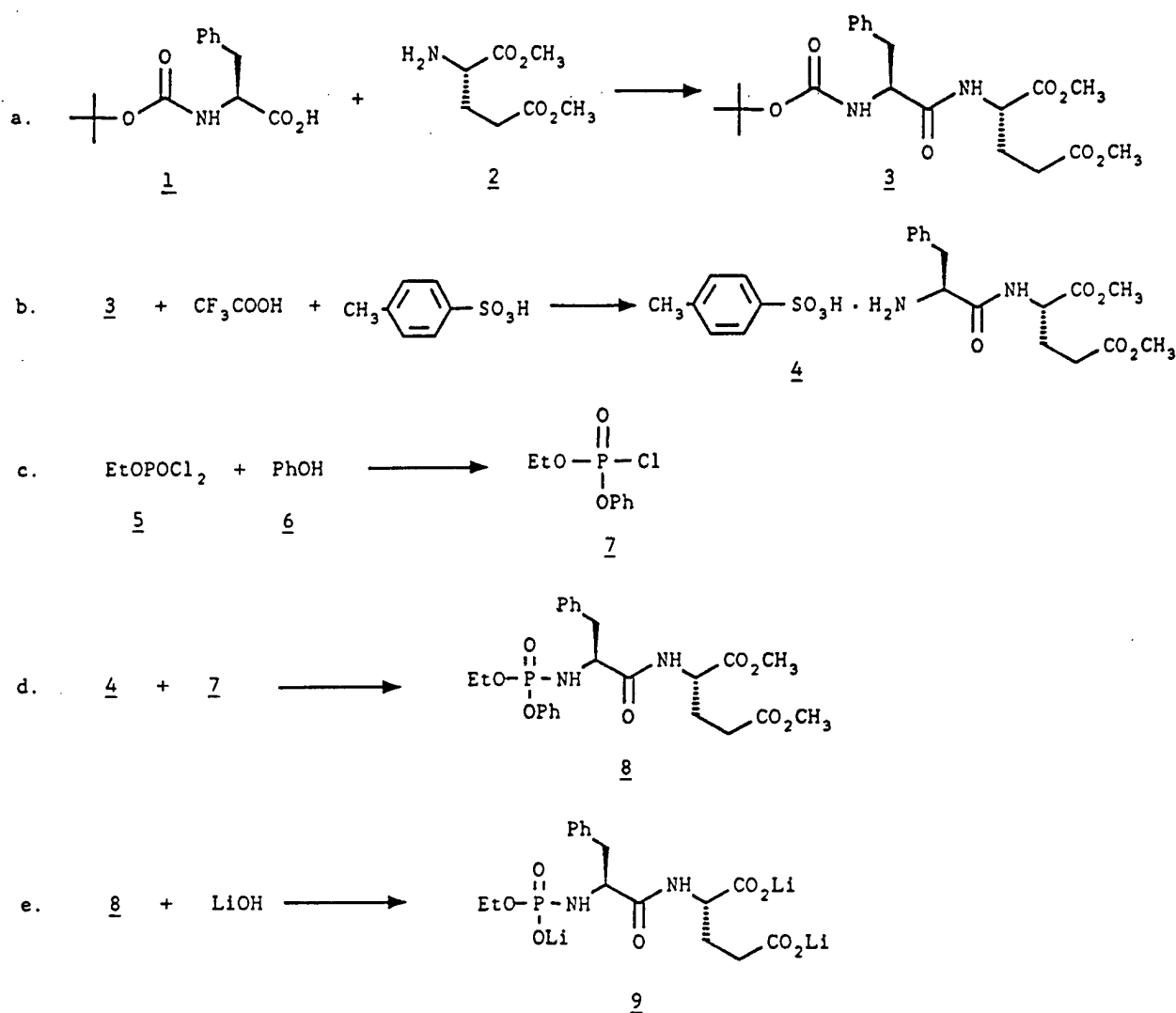
- | | | |
|----|--|----------------------------|
| 1. | 5-Chloro-2-nitrobenzaldehyde | Aldrich Chemical Co., Inc. |
| 2. | $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ | Sigma |
| 3. | CH_2Cl_2 | J.T. Baker Chemical Co. |
| 4. | Na_2SO_4 | J.T. Baker Chemical Co. |
| 5. | 5-(2,4-Dichlorophenyl)-
cyclohexane-1,3-dione | Starks Associates, Inc. |
| 6. | Toluene | J.T. Baker Chemical Co. |
| 7. | <i>p</i> -Toluenesulfonic acid | Aldrich Chemical Co., Inc. |
| 8. | Methanol | J.T. Baker Chemical Co. |
| 9. | Acetic acid | J.T. Baker Chemical Co. |

B. Chemical Defense Related Compounds and Intermediates

12. L-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-L-phenylalanyl]-, trilithium salt (9)

The target compound 9 was prepared by the following reaction sequence.

Reaction Sequence:



Experimental⁵

L-Glutamic acid, N-[N-[*tert*-butoxycarbonyl]-L-phenyl-
alanyl]-, dimethyl ester (3)

To a stirring suspension of N- α -*t*-butoxycarbonyl-L-phenylalanine (1) (5.0 g, 18.85 mmol), L-glutamic acid dimethyl ester hydrochloride (2) (4.8 g, 22.62 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (7.15 g, 18.85 mmol), and N-hydroxybenzotriazole hydrate (HOBt·H₂O) (2.55 g, 18.85 mmol) in dichloromethane (52 mL), at 7-10°C under argon, was added dropwise N,N-diisopropylethylamine (14.8 mL, 84.8 mmol), while maintaining an internal temperature of 10-12°C. After addition, the mixture was stirred at ambient temperature for 22 h. The mixture was concentrated to an oil, then dissolved in ethyl acetate (100 mL) and washed with 5% HCl (aq) (2 x 25 mL), H₂O (2 x 50 mL), sat. NaHCO₃ (aq) (2 x 50 mL), and brine (100 mL). The organic layer was dried (MgSO₄), filtered, and concentrated to give a light yellow oil (11 g), which was purified by flash column chromatography (300 g of flash SiO₂) eluted with 2:3 EtOAc:hexanes to give 6.1 g (77%) of pure 3.

Spectral Data:Nuclear Magnetic Resonance (CDCl₃)

δ 7.31-7.18 (m, 5H, Ar); 6.52-6.50 (m, 1H);
5.11-4.79 (m, 1H); 4.56-4.46 (m, 1H); 4.40-4.30
(m, 1H); 3.70 (s, 3H); 3.66 (s, 3H); 3.08-3.04
(m, 2H); 2.20-2.15 (m, 3H); 1.95-1.91 (m, 1H);
1.40 (s, 9H).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - anisaldehyde spray.

<u>Eluent</u>	<u>R_f Value</u>	<u>Comment</u>
EtOAc-hexanes (2:3)	0.29	Homogeneous

L-Glutamic acid, N-[L-phenylalanyl]-, dimethyl ester tosylate (4)

To an ice-chilled flask containing L-glutamic acid, N-[N-[*tert*-butoxycarbonyl]-L-phenylalanyl]-, dimethyl ester (4.9 g, 11.6 mmol) was added ice-cold trifluoroacetic acid (27 mL) with stirring under argon. The mixture was stirred at 0°C for 15 min, then *p*-toluenesulfonic acid monohydrate (2.2 g, 11.6 mmol) was added and the mixture was stirred at ambient temperature for 1 h. Concentrated to give an oil which was then coevaporated with CH₂Cl₂ (150 mL). The resulting oil was triturated with *tert*-butyl methyl ether (100 mL) and stirred for 1 h. The suspension was suction filtered and the filter cake was washed with Et₂O (150 mL) and dried in vacuo to give pure **4** (5.6 g, 98%) as a white solid. An additional reaction was performed to give a total of 6.8 g of pure **4**.

Spectral Data:

Nuclear Magnetic Resonance

δ 7.95-7.72 (m, 4H, Ar); 7.14 (s, 5H, Ar); 4.70 (m, 1H); 4.38 (m, 1H); 3.63 (s, 3H); 3.41 (s, 3H); 3.30-3.19 (m, 2H); 2.34 (s, 3H); 2.33-2.18 (m, 2H); 2.05-1.80 (m, 2H).

L-Glutamic acid, N-[N-[ethoxyphenoxyphosphinyl]-L-phenylalanyl]-, dimethyl ester (8)

To a solution of ethyl dichlorophosphate (5) (1.44 mL, 12.13 mmol) in CH₂Cl₂ (240 mL) at 0°C under argon was added a solution of anhydrous phenol (6) (1.2 g, 12.74 mmol) and triethylamine (1.8 mL, 12.9 mmol) in CH₂Cl₂ (120 mL) over 0.5 h. The mixture was stirred at 0°C/0.5 h and then at ambient temperature for 2 h, to give a solution of 7. To this solution was added a suspension of L-glutamic acid, N-[L-phenylalanyl]-, dimethyl ester tosylate (6.3 g, 12.74 mmol), triethylamine (1.9 mL, 13.63 mmol), and 4-dimethylaminopyridine (0.147 g, 1.20 mmol) in anhydrous THF (120 mL). The suspension was stirred at ambient temperature for 20 h, then concentrated to a residue which was dissolved in EtOAc (500 mL) and washed with 0.1N HCl (2 x 250 mL), sat. NaHCO₃ (2 x 250 mL), 50% NaCl (2 x 250 mL), and brine (250 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated to give a crude product (4.2 g) which was purified by flash chromatography (250 g flash SiO₂, 3:1 EtOAc:hexanes) to give pure 8, as a mixture of diastereomers (1.6 g, 26%).

Spectral Data:

¹H Nuclear Magnetic Resonance (CDCl₃)

Diastereomeric mixture:

δ 7.35-7.10 (m, 10H); 6.92-6.90 (2d, 1H);
4.56-4.50 (m, 1H); 4.13-3.90 (2m, 3H); 3.69-3.64
(4s, 6H); 3.38-3.15 (2m, 2H); 2.98-2.82 (2m, 1H);
2.26-2.11 (m, 3H); 1.89-1.87 (m, 1H); 1.29-1.26
(t, 3H).

³¹P Nuclear Magnetic Resonance (CDCl₃)

Diastereomeric mixture:

δ 2.86 (s, 1P); 2.75 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (positive)

Calc'd for C₂₄H₃₁N₂O₈P = 506.2

Found: 507.4 (m + H)

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - anisaldehyde
spray.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-hexanes (3:1)	0.28	Homogeneous

L-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-L-phenyl-
alanyl]-, trilithium salt (9)

To a solution of L-glutamic acid, N-[N-[ethoxyphenoxy-
phosphinyl]-L-phenylalanyl]-, dimethyl ester (1.6 g, 3.16
mmol) in THF (160 mL) and H₂O (80 mL) was added 1.5M LiOH (9.8
mL, 14.7 mmol), and the solution was refluxed for 8 h. After
cooling to room temperature, 0.985N HCl (5.3 mL) was added and
the solvents were evaporated at reduced pressure. Water (3
x 160 mL) was added and removed at reduced pressure (for
azeotropic removal of phenol). The residue was coevaporated
with anhydrous CH₃CN (3 x 80 mL) (for azeotropic removal of

H₂O), then pumped dry in vacuo to give 9, as a white solid (1.5 g, 88%, based on a molecular formula of C₁₆H₂₀Li₃N₂O₈P · 1.7 LiCl·2.75 H₂O). A portion (1.0 g) was transmitted to WRAIR on January 11, 1996 (Lot No. NJ20-27-1).

Anal.

	<u>C</u>	<u>H</u>
Calc'd for C ₁₆ H ₂₀ Li ₃ N ₂ O ₈ P	35.47	4.74
·1.7 LiCl·2.75 H ₂ O		
Found	35.49	4.70
	35.40	4.72

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

δ 7.25-7.13 (m, 5H, Ar); 4.00-3.98 (dd, 1H, CH);
3.80-3.78 (dd, 1H, CH); 3.58-3.47 (m, 2H, CH₃CH₂);
3.00-2.88 (m, 2H, PhCH₂); 1.88-1.81 (m, 3H, CH-CH₂-
CO); 1.74-1.72 (m, 1H, CH-C-CO); 1.00-0.97 (t, 3H,
CH₃CH₂).

³¹P Nuclear Magnetic Resonance (D₂O)

δ 6.56 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (negative)

Calc'd for C₁₆H₂₃N₂O₈P = 402.12 (free acid)

Found: 401.4 (m-H), 407.2 (m-2H+Li),
413.4 (m-3H+2Li).

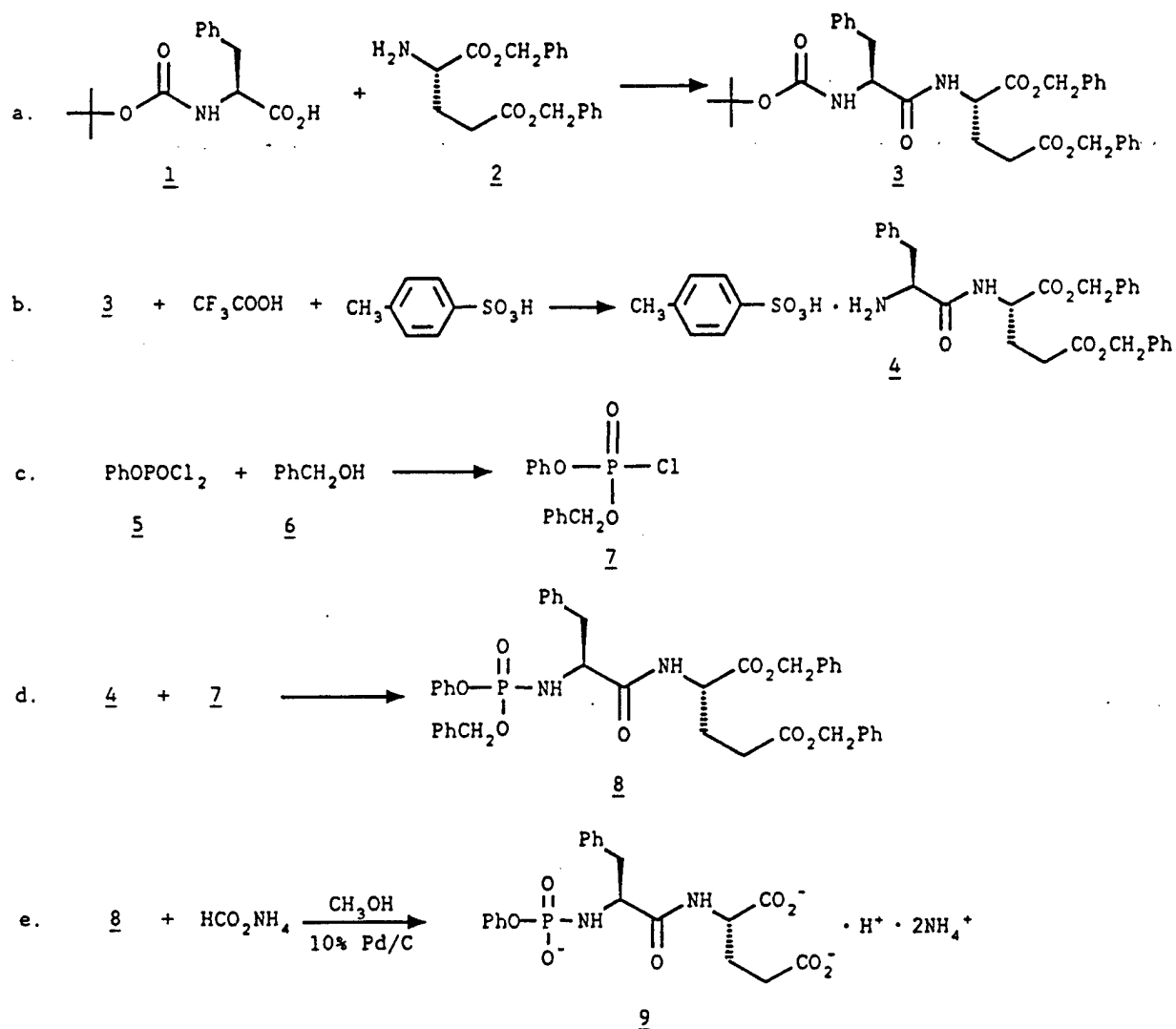
Source of Materials

1. N- α - <i>t</i> -butoxycarbonyl-L-phenylalanine	Nova Biochem
2. L-Glutamic acid dimethyl ester hydrochloride	Sigma Chemical Co.
3. HBTU	Nova Biochem
4. HOBT·H ₂ O	Nova Biochem
5. N,N-Diisopropylethylamine	Aldrich Chemical Co., Inc.
6. Dichloromethane	J.T. Baker Chemical Co.
7. EtOAc	EM Science
8. Hexanes	EM Science
9. Hydrochloric acid	J.T. Baker Chemical Co.
10. Sodium bicarbonate	J.T. Baker Chemical Co.
11. Magnesium sulfate	J.T. Baker Chemical Co.
12. Flash silica gel	E.M. Science
13. Trifluoroacetic acid	Aldrich Chemical Co., Inc.
14. <i>p</i> -Toluenesulfonic acid monohydrate	Kodak
15. <i>tert</i> -Butyl methyl ether	Aldrich Chemical Co., Inc.
16. Diethyl ether	Fisher Scientific
17. Ethyl dichlorophosphate	Aldrich Chemical Co., Inc.
18. Phenol	Aldrich Chemical Co., Inc.
19. Triethylamine	Aldrich Chemical Co., Inc.
20. 4-Dimethylaminopyridine	Reilly Industries
21. THF	Aldrich Chemical Co., Inc.
22. NaCl	Cargill
23. Sodium sulfate	Aldrich Chemical Co., Inc.
24. LiOH	Aldrich Chemical Co., Inc.
25. Hydrochloric acid, 0.985N	Aldrich Chemical Co., Inc.
26. CH ₃ CN	Aldrich Chemical Co., Inc.

13. L-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-L-phenylalanyl]-, diammonium salt (9)

The target compound 9 was prepared by the following reaction sequence.

Reaction Sequence:



Experimental^{5,6}

L-Glutamic acid, N-[N-[*tert*-butoxycarbonyl]-L-phenyl-alanyl]-, dibenzyl ester (3)

To a stirring suspension of N- α -*t*-butoxycarbonyl-L-phenyl-alanine (1) (5 g, 11.3 mmol), L-glutamic acid, α,γ -dibenzyl ester tosylate (2) (11.3 g, 22.62 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (7.15 g, 18.85 mmol), and N-hydroxybenzotriazole hydrate (HOBt·H₂O) (2.55 g, 18.85 mmol) in dichloromethane (52 mL), at 7-10°C under argon, was added dropwise N,N-diisopropylethylamine (14.8 mL, 84.8 mmol), while maintaining an internal temperature of 10-12°C. After addition, the mixture was stirred at ambient temperature for 22 h. The mixture was concentrated to an oil, then dissolved in ethyl acetate (250 mL) and washed with 5% HCl (aq) (2 x 100 mL), H₂O (2 x 100 mL), sat. NaHCO₃ (aq) (2 x 100 mL), and brine (200 mL). The organic layer was dried (MgSO₄), filtered, and concentrated to give a solid which was triturated and suction filtered with *t*-BuOCH₃ (120 mL) for 1 h. Dried in vacuo to give pure 3 (9.7 g, 90%).

Spectral Data:Nuclear Magnetic Resonance (CDCl₃)

δ 7.35-7.15 (m, 15H, Ar); 6.54-6.52 (m, 1H);
5.16-5.07 (m, 3H); 4.90 (m, 1H); 4.61 (m, 1H);
4.33 (m, 1H); 3.05-3.02 (m, 2H); 2.34-2.18
(m, 3H); 1.96-1.94 (m, 1H); 1.38 (s, 9H).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - anisaldehyde spray.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-hexanes (1:2)	0.42	Homogeneous

L-Glutamic acid, N-[L-phenylalanyl]-, dibenzyl ester tosylate (4)

To an ice-chilled flask containing L-glutamic acid, N-[N-[*tert*-butoxycarbonyl]-L-phenylalanyl]-, dibenzyl ester (8.7 g, 15.14 mmol) was added ice-cold trifluoroacetic acid (40 mL) with stirring under argon. The mixture was stirred at 0°C for 30 min, then *p*-toluenesulfonic acid monohydrate (2.88 g, 15.14 mmol) was added and the mixture was stirred at ambient temperature for 1 h. Concentrated to give an oil which was then coevaporated with CH₂Cl₂ (150 mL). The residue was triturated with *tert*-butyl methyl ether (200 mL) and stirred for 1 h. The suspension was suction filtered and the filter cake was washed with Et₂O (50 mL) and then dried in vacuo to give pure 4 (9.6 g, 98%) as a white solid.

Spectral Data:Nuclear Magnetic Resonance (CDCl₃)

δ 7.92-7.03 (m, 19H, Ar); 5.04 (s, 2H, PhCH₂); 4.85 (s, 2H, PhCH₂); 4.66 (m, 1H, CH); 4.41 (m, 1H, CH); 3.40-3.35 (br m, 1H); 3.25-3.10 (m, 2H, PhCH₂); 2.27-2.17 (m, 2H); 2.25 (s, 3H); 2.00-1.87 (m, 2H).

L-Glutamic acid, N-[N-[benzyloxyphenoxyphosphinyl]-L-phenylalanyl]-, dibenzyl ester (8)

To a solution of phenyl dichlorophosphate (5) (2.2 mL, 14.7 mmol) in CH₂Cl₂ (300 mL) at 0°C under argon was added a solution of benzyl alcohol (6) (1.7 mL, 16.4 mmol) and triethylamine (2.9 mL, 20.8 mmol) in CH₂Cl₂ (150 mL) over 0.5 h. The mixture was stirred at 0°C/0.5 h and then at ambient temperature for 2 h, to give a solution of 7. To this solution was added a suspension of L-glutamic acid, N-[L-phenylalanyl]-, dibenzyl ester tosylate (9.5 g, 14.7 mmol), triethylamine (7.2 mL, 51.7 mmol), and 4-dimethylaminopyridine (287 mg, 2.35 mmol) in anhydrous THF (150 mL). The suspension was stirred at ambient temperature for 22 h, then concentrated to a residue which was dissolved in EtOAc (1 L) and washed with 0.1N HCl (2 x 500 mL), sat. NaHCO₃ (2 x 500 mL), 50% NaCl (2 x 500 mL), and brine (500 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated to give a crude product (10.6 g) which was purified by flash chromatography (300 g, flash SiO₂, 1:1 EtOAc:hexanes) to give pure 8, as a mixture of diastereomers (2.3 g, 23%).

Spectral Data:

¹H Nuclear Magnetic Resonance (CDCl₃)

Diastereomeric mixture:

δ 7.37-6.79 (m, 25H); 5.08-5.00 (m, 7H);
4.90 and 4.70 (2m, 1H); 4.56-4.53 (m, 1H);
3.33-3.29 (m, 1H); 3.26-3.13 (2m, 1H); 2.87-
2.82 (m, 1H); 2.23-2.10 (m, 3H); 1.88-1.84
(m, 1H).

³¹P Nuclear Magnetic Resonance (CDCl₃)

Diastereomeric mixture:

δ 2.99 (s, 1P); 2.97 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - I₂.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc:hexanes (1:1)	0.32	Homogeneous

L-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-L-phenylalanyl]-, diammonium salt (9)

A mixture of L-glutamic acid, N-[N-[benzyloxyphenoxyphosphinyl]-L-phenylalanyl]-, dibenzyl ester (1.8 g, 2.50 mmol), ammonium formate (2.1 g, 33.3 mmol) and 10% palladium on carbon (1.8 g) in methanol (45 mL) was stirred at ambient temperature, under argon, for 24 h. The mixture was filtered through a pad of celite and concentrated to give a residue, which was dissolved in H₂O (25 mL) and again filtered through a pad of celite. The aqueous filtrate was concentrated in vacuo and the white solid residue was coevaporated with H₂O (12 x 35 mL) to azeotropically remove ammonium formate. The residue was then coevaporated with CH₃CN (3 x 50 mL), to azeotropically remove H₂O, to give 9 (0.95 g, 76% based on a molecular formula of C₂₀H₂₉N₄O₈P·0.8H₂O). A portion (720 mg) was transmitted to WRAIR on January 30, 1996 (Lot No. NJ20-45-1).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_{20}H_{29}N_4O_8P \cdot 0.8 H_2O$	48.15	6.18	11.23
Found	48.15	5.77	10.61
	48.05	5.82	10.58

Spectral Data

FT-Infrared (KBr)

Major bands: 3296, 3227, 3062, 3028, 2921, 2857,
1932, 1707, 1645, 1592, 1542, 1492,
1453, 1408, 1320, 1255, 1202, 1069,
889, 764, 545 cm^{-1} .

^1H Nuclear Magnetic Resonance (D_2O)

δ 7.23-6.97 (m, 10H, Ar); 3.94-3.85 (2m, 2H, 2CH);
2.97-2.75 (m, 2H, PhCH₂); 1.97-1.94 (m, 2H,
C-CH₂-CO); 1.84-1.64 (2m, 2H, -CH₂-C-CO).

A trace of ammonium formate is also seen at δ 8.28.

^{31}P Nuclear Magnetic Resonance (D_2O)

δ 2.67 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (negative)

Calc'd for $C_{20}H_{23}N_2O_8P$ = 450.1 (free acid)

Found: 449.2 (m-H), 355.1 (m-2H-PhO).

Source of Materials

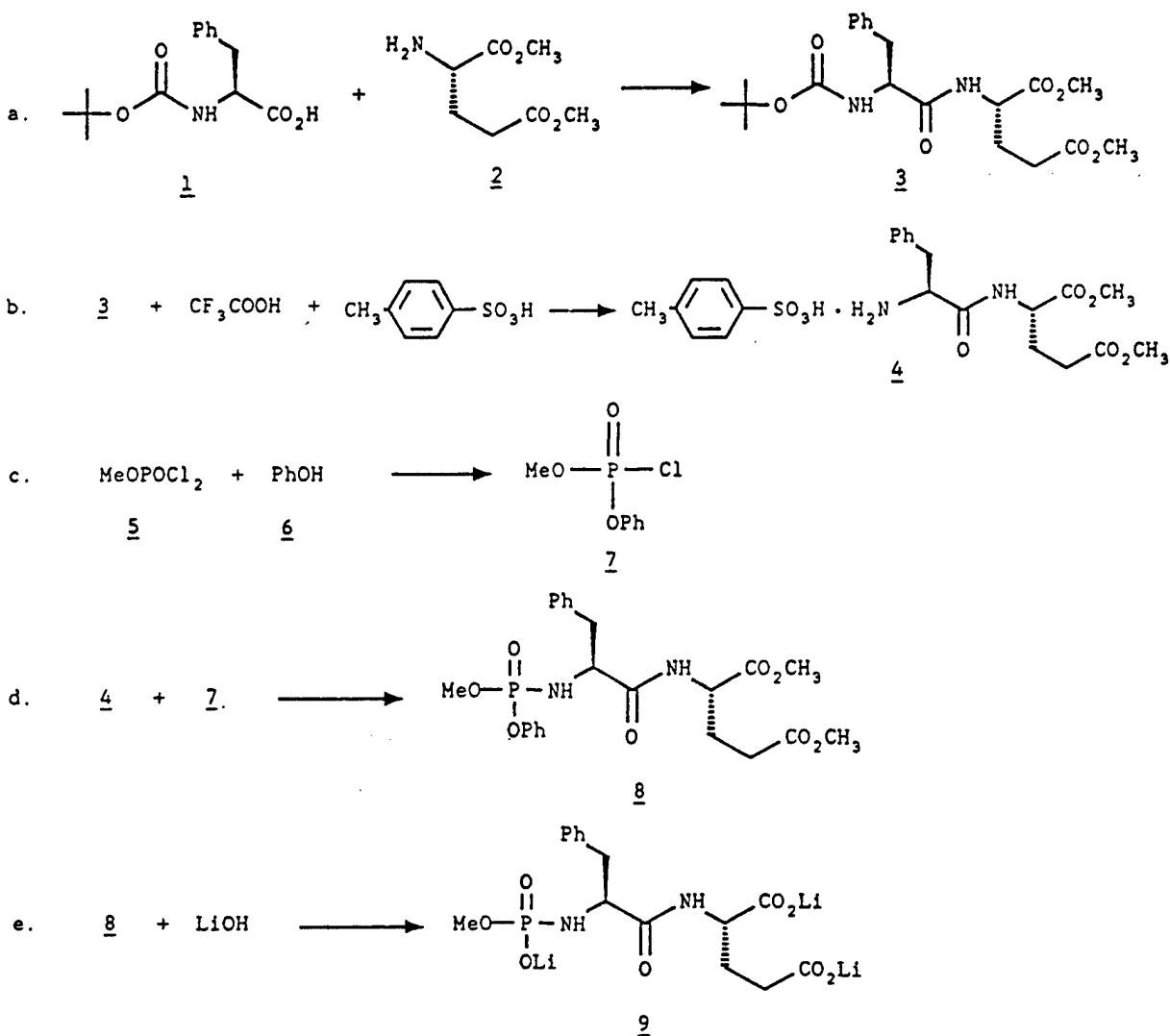
1. N- α - <i>t</i> -butoxycarbonyl-L-phenylalanine	Nova Biochem
2. L-Glutamic acid, α,γ -dibenzyl-ester tosylate	Bachem Bioscience Inc.
3. HBTU	Nova Biochem
4. HOBT $\cdot$ H ₂ O	Nova Biochem
5. N,N-Diisopropylethylamine	Aldrich Chemical Co., Inc.
6. Dichloromethane	J.T. Baker Chemical Co., Aldrich Chemical Co., Inc.
7. EtOAc	EM Science
8. Hexanes	EM Science
9. Hydrochloric acid	J.T. Baker Chemical Co.
10. Sodium bicarbonate	J.T. Baker Chemical Co.
11. Magnesium sulfate	J.T. Baker Chemical Co.
12. Flash silica gel	E.M. Science
13. Trifluoroacetic acid	Aldrich Chemical Co., Inc.
14. <i>p</i> -Toluenesulfonic acid monohydrate	Kodak
15. <i>tert</i> -Butyl methyl ether	Aldrich Chemical Co., Inc.
16. Diethyl ether	Fisher Scientific
17. Phenyl dichlorophosphate	Aldrich Chemical Co., Inc.
18. Benzyl alcohol	Aldrich Chemical Co., Inc.
19. Triethylamine	Aldrich Chemical Co., Inc.
20. 4-Dimethylaminopyridine	Reilly Industries
21. THF	Aldrich Chemical Co., Inc.
22. NaCl	Cargill
23. Sodium sulfate	Aldrich Chemical Co., Inc.
24. Ammonium formate	Aldrich Chemical Co., Inc.

- | | | |
|-----|-------------------------|----------------------------|
| 25. | CH_3OH | J.T. Baker Chemical Co. |
| 26. | CH_3CN | Aldrich Chemical Co., Inc. |
| 27. | 10% Palladium on carbon | Aldrich Chemical Co., Inc. |

14. L-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-L-phenylalanyl]-, trilithium salt (9)

The target compound 9 was prepared by the following reaction sequence.

Reaction Sequence:



Experimental⁵

L-Glutamic acid, N-[N-[*tert*-butoxycarbonyl]-L-phenyl-alanyl]-, dimethyl ester (3)

To a stirring suspension of N- α -*t*-butoxycarbonyl-L-phenyl-alanine (1) (10.0 g, 37.7 mmol), L-glutamic acid dimethyl ester hydrochloride (2) (9.6 g, 45.3 mmol), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (14.3 g, 37.7 mmol), and N-hydroxybenzotriazole hydrate (HOBt·H₂O) (5.1 g, 37.7 mmol) in dichloromethane (106 mL), at 7-10°C under argon, was added dropwise N,N-diisopropylethylamine (29.6 mL, 170 mmol), while maintaining an internal temperature of 10-12°C. After addition, the mixture was stirred at ambient temperature for 22 h. The mixture was concentrated to an oil, then dissolved in ethyl acetate (200 mL) and washed with 5% HCl (aq) (3 x 100 mL), H₂O (2 x 100 mL), sat. NaHCO₃ (aq) (2 x 100 mL), and brine (100 mL). The organic layer was dried (MgSO₄), filtered, and concentrated to give a solid (15.3 g), which was triturated with *t*-BuOCH₃ (30 mL) for 1 h, then diluted with pet ether (100 mL) and suction filtered and dried in vacuo to give pure 3 (14.4 g, 91%).

Spectral Data:

Nuclear Magnetic Resonance (CDCl₃)

δ 7.31-7.18 (m, 5H, Ar); 6.52-6.50 (m, 1H);
4.96-4.94 (m, 1H); 4.58-4.53 (m, 1H); 4.35-4.33
(m, 1H); 3.70 (s, 3H); 3.66 (s, 3H); 3.08-3.01
(m, 2H); 2.36-2.14 (m, 3H); 1.98-1.90 (m, 1H);
1.41 (s, 9H).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - anisaldehyde spray.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-hexanes (1:1)	0.51	Homogeneous

L-Glutamic acid, N-[L-phenylalanyl]-, dimethyl ester tosylate (4)

To an ice-chilled flask containing L-glutamic acid, N-[N-[*tert*-butoxycarbonyl]-L-phenylalanyl]-, dimethyl ester (14.4 g, 34.1 mmol) was added ice-cold trifluoroacetic acid (80 mL) with stirring under argon. The mixture was stirred at 0°C for 30 min, then *p*-toluenesulfonic acid monohydrate (6.49 g, 34.1 mmol) was added and the mixture was stirred at ambient temperature for 1 h. Concentrated to give an oil which was then coevaporated with CH₂Cl₂ (2x200 mL). The resulting oil was triturated with *tert*-butyl methyl ether (200 mL) and stirred for 1 h. The suspension was suction filtered and the filter cake was washed with Et₂O (2x50 mL) and dried in vacuo to give pure 4 (16.5 g, 98%) as a white solid.

Spectral Data:

Nuclear Magnetic Resonance (CDCl₃)

δ 7.93-7.72 (m, 4H, Ar); 7.14 (s, 5H, Ar); 4.70 (m, 1H); 4.38 (m, 1H); 3.62 (s, 3H); 3.42 (s, 3H); 3.30-3.14 (m, 2H); 2.34 (s, 3H); 2.32-2.18 (m, 2H); 2.01-1.85 (m, 2H).

L-Glutamic acid, N-[N-[methoxyphenoxyphosphinyl]-L-phenylalanyl]-, dimethyl ester (8)

To a solution of methyl dichlorophosphate (5) (1.0 mL, 10.0 mmol) in CH₂Cl₂ (200 mL) at 0°C under argon was added a solution of anhydrous phenol (6) (0.99 g, 10.5 mmol) and triethylamine (1.54 mL, 11.0 mmol) in CH₂Cl₂ (100 mL) over 45 min. The mixture was stirred at 0°C/2 h to give a solution of 7. To this solution at 0°C was added a suspension of L-glutamic acid, N-[L-phenylalanyl]-, dimethyl ester tosylate (5.19 g, 10.5 mmol), triethylamine (1.54 mL, 11.0 mmol), and 4-dimethylaminopyridine (0.122 g, 1.0 mmol) in anhydrous THF (100 mL). The suspension was warmed to ambient temperature and stirred for 20 h, then concentrated to a residue which was dissolved in EtOAc (1 L) and washed with 0.1N HCl (2 x 200 mL), sat. NaHCO₃ (2 x 200 mL), 50% NaCl (2 x 200 mL), and brine (200 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated to give a crude product (3.1 g) which was purified by column chromatography (90 g, SiO₂, EtOAc) to give pure 8, as a mixture of diastereomers (0.9 g, 18%). An additional reaction was performed to give a total of 1.8 g of pure 8.

Spectral Data:

¹H Nuclear Magnetic Resonance (CDCl₃)

Diastereomeric mixture:

δ 7.33-7.04 (m, 11H); 4.55-4.53 (m, 1H);
4.12-4.10 (m, 1H); 3.73-3.63 (m, 9H); 3.57-
3.55 (d, 1H); 3.21-3.10 (m, 1H); 3.08-2.90
(m, 1H); 2.28-2.10 (m, 3H); 1.90-1.87 (m, 1H).

³¹P Nuclear Magnetic Resonance (CDCl₃)

Diastereomeric mixture:

δ 4.12 (s, 1P); 3.97 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - I₂.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc	0.46	Homogeneous

L-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-L-phenylalanyl]-, trilithium salt (9)

To a solution of L-glutamic acid, N-[N-[methoxyphenoxyphosphinyl]-L-phenylalanyl]-, dimethyl ester (1.62 g, 3.29 mmol) in THF (162 mL) and H₂O (81 mL) was added 1.5M LiOH (10.2 mL, 15.3 mmol), and the solution was refluxed for 8 h. After cooling to room temperature, 0.985N HCl (5.513 mL) was added and the solvents were evaporated at reduced pressure. Water (3 x 160 mL) was added and removed at reduced pressure (for azeotropic removal of phenol). The residue was coevaporated with anhydrous CH₃CN (3 x 100 mL) (for azeotropic removal of H₂O), then pumped dry in vacuo to give 9, as a white solid (1.6 g, 92%, based on a molecular formula of C₁₅H₁₈Li₃N₂O₈P 0.55CH₃CN·1.5 LiCl·1.9 H₂O). A portion (820 mg) was transmitted to WRAIR on February 7, 1996 (Lot No. NJ20-57-1).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $C_{15}H_{18}Li_3N_2O_8P$	36.73	4.49	6.78
$\cdot 0.55 CH_3CN \cdot 1.5LiCl \cdot 1.9H_2O$			
Found	36.78	4.53	6.82
	36.68	4.54	6.78

Spectral Data

FT-Infrared (KBr)

Major bands: 3383, 2950, 2849, 2361, 1616, 1538,
1420, 1211, 1087, 1054, 961, 913, 841,
776, 702, 542 cm^{-1} .

^1H Nuclear Magnetic Resonance (D_2O)

δ 7.28-7.09 (m, 5H, Ar); 4.03-4.01 (dd, 1H, CH);
3.84-3.80 (dd, 1H, CH); 3.22-3.20 (m, 3H, CH₃O);
3.01-2.83 (m, 2H, PhCH₂); 1.96 (CH_3CN); 1.93-1.85
(m, 3H, CH-CH₂CO); 1.77-1.73 (m, 1H, CH-C-CO).

^{31}P Nuclear Magnetic Resonance (D_2O)

δ 7.74 (s, 1P).

Mass Spectrum

Method of Ionization = Electrospray (negative)

Calc'd for $C_{15}H_{21}N_2O_8P$ = 388.1 (free acid)

Found: 387.0 (m-H), 393.2 (m-2H+Li),
399.2 (m-3H+2Li).

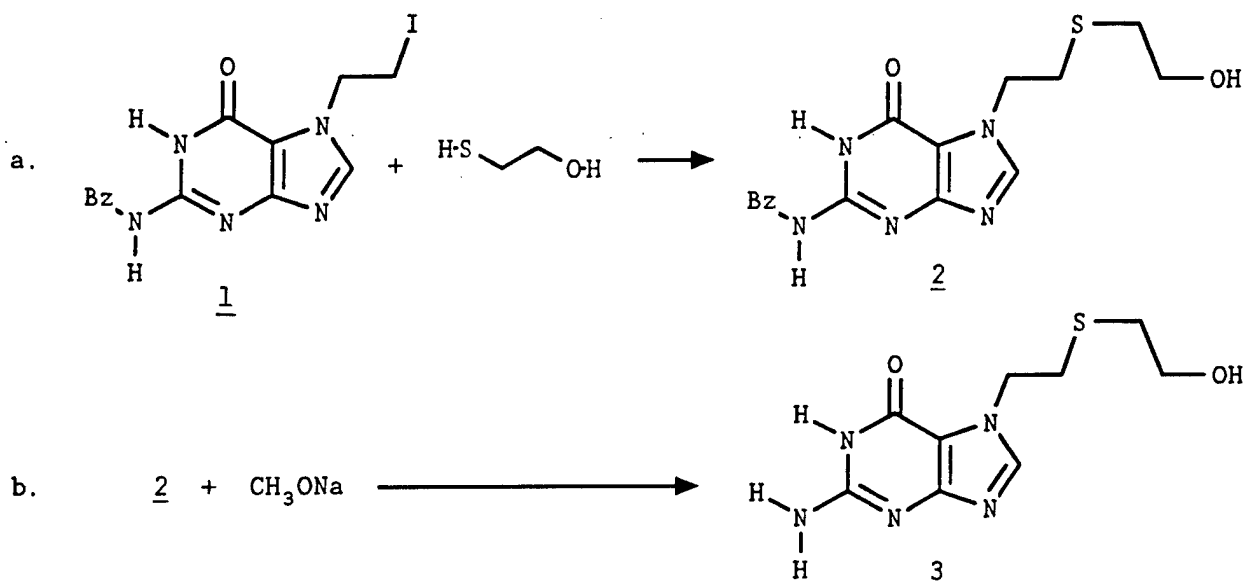
Source of Materials

1. N- α - <i>t</i> -butoxycarbonyl-L-phenylalanine	Nova Biochem
2. L-Glutamic acid dimethyl ester hydrochloride	Sigma Chemical Co.
3. HBTU	Nova Biochem
4. HOBT $\cdot$ H ₂ O	Nova Biochem
5. N,N-Diisopropylethylamine	Aldrich Chemical Co., Inc.
6. Dichloromethane	J.T. Baker Chemical Co.
7. EtOAc	EM Science
8. Hexanes	EM Science
9. Hydrochloric acid	J.T. Baker Chemical Co.
10. Sodium bicarbonate	J.T. Baker Chemical Co.
11. Magnesium sulfate	J.T. Baker Chemical Co.
12. Silica gel	E.M. Science
13. Trifluoroacetic acid	Aldrich Chemical Co., Inc.
14. <i>p</i> -Toluenesulfonic acid monohydrate	Kodak
15. <i>tert</i> -Butyl methyl ether	Aldrich Chemical Co., Inc.
16. Diethyl ether	Fisher Scientific
17. Ethyl dichlorophosphate	Aldrich Chemical Co., Inc.
18. Phenol	Aldrich Chemical Co., Inc.
19. Triethylamine	Aldrich Chemical Co., Inc.
20. 4-Dimethylaminopyridine	Reilly Industries
21. THF	Aldrich Chemical Co., Inc.
22. NaCl	Cargill
23. Sodium sulfate	Aldrich Chemical Co., Inc.
24. LiOH	Aldrich Chemical Co., Inc.
25. Hydrochloric acid, 0.985N	Aldrich Chemical Co., Inc.
26. CH ₃ CN	Aldrich Chemical Co., Inc.

2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (3)

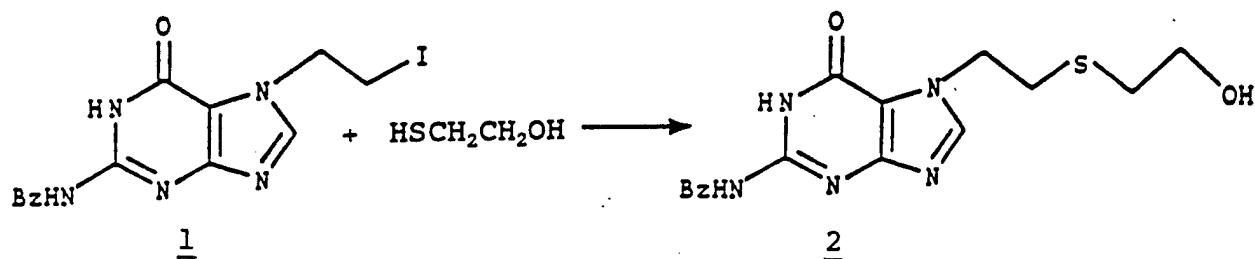
The target compound 3 was prepared by the following sequence of reactions. Intermediate 2 which is new to the Program will be transmitted to WRAIR.

Reaction Sequence:



15a. 2-(2-Benzamido-7-(2-iodoethyl)purin-6-one)ethyl 2-hydroxyethyl sulfide (2)

Reaction Sequence



Experimental

2-(Benzamido)-7-(2-iodoethyl)purin-6-one (1)

Please refer to p. 106, this report.

2-(2-Benzamido-7-(2-hydroxyethyl)purin-6-one)ethyl 2-hydroxyethyl sulfide (2)

To a solution of 2-mercaptoethanol (1.22 g, 15.6 mmol) in DMSO (30 mL) was added a 25% w/w solution of sodium methoxide in methanol (3.38 g soln = 0.845 g, 15.6 mmol). The mixture was stirred at room temperature for 30 minutes then compound 1 (6.4 g, 15.6 mmol) was added and quantitatively rinsed into the flask with DMSO (20 mL); a temperature rise of +4.5°C was observed. The mixture was stirred 30 min after which time TLC indicated some starting material 1 still remained. In a separate container were mixed 2-mercaptoethanol (0.55 mL, 7.8 mmol), DMSO (2 mL) and 25% sodium methoxide in methanol (1.69 g, 7.8 mmol), stirred 15 min then added to the reaction mixture. After stirring 40 min TLC indicated that most, but not all of the starting material 1 was gone. Again, mixed 2-mercaptoethanol (0.28 mL, 3.9 mmol), DMSO (1 mL) and 25% sodium methoxide in methanol (0.21 g, 3.9 mmol), stirred 15 min and added to the reaction mixture. After stirring 30 min, TLC indicated no starting

material 1 remained. The reaction mixture was concentrated to 5.30 g oil, redissolved in CH_2Cl_2 :MeOH (9:1) (30 mL) and applied to a 250 g column of silica gel. The column was eluted with CH_2Cl_2 :MeOH (9:1). Fractions containing the pure product (2) were concentrated to 2.13 g white solid. Fractions containing the product (2) plus some impurities were concentrated to a solid, triturated in CH_2Cl_2 filtered and dried to 2.88 g pure compound 2, to give a total of 5.01 g (13.9 mmol, 89.1%) compound 2. An additional 6.87 g compound 2 was obtained from a second reaction. A portion (2.7 g) will be transmitted to WRAIR (Lot No. NJ11-143-2), the remainder was used with the next reaction.

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>	<u>S</u>
Calc'd for $\text{C}_{16}\text{H}_{17}\text{N}_5\text{O}_3\text{S}$	53.47	4.77	19.49	8.92
Found	53.25	4.84	19.59	9.06

Spectral Data

Infrared (Nujol)

Major bands: 3370, 3170, 3100, 3050, 1690, 1675, 1660, 1605, 1555, 1540, 1450, 1420, 1370, 1350, 1270, 1225, 1200, 1125, 1085, 1070, 1035, 895, 775, 685 cm^{-1} .

Ultraviolet (MeOH)

λ_{max} 232 nm ($\log \epsilon$ 3.78); 278 nm ($\log \epsilon$ 3.72).

Nuclear Magnetic Resonance ($\text{DMSO}-d_6$)

δ 12.41 (s, 1, NH); 11.89 (s, 1, Ar NH); 8.28 (s, 1H, Ar); 8.10 (t, 2H, Bz); 7.72-7.70 (d, 1H, Bz); 7.61 (t, 2H, Bz); 4.84 (t, 1H, OH); 4.52 (t, 2H, CH_2); 3.61-3.57 (q, 2H, CH_2); 3.06 (t, 2H, CH_2); 2.64 (t, 2H, CH_2).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection ultraviolet light.

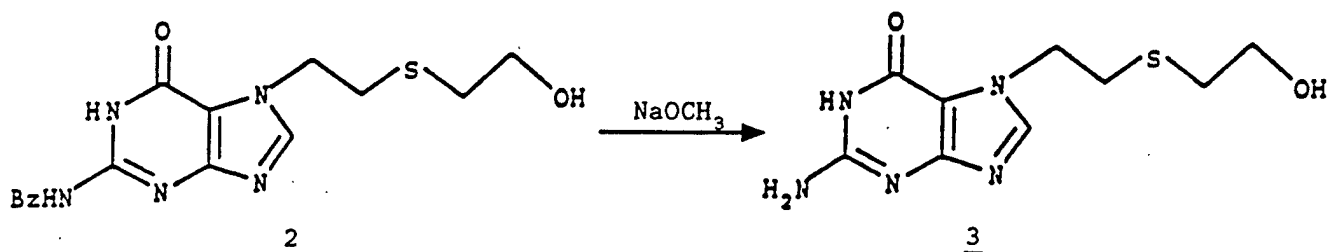
<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1. CH ₂ Cl ₂ :MeOH (9:1)	0.20	Homogeneous
2. CH ₂ Cl ₂ :EtOH (4:1)	0.57	Homogeneous
3. EtOAc:MeOH (19:1)	0.11	Homogeneous

Source of Material

1. Dichloromethane	J.T. Baker Chemical Co.
2. Methyl sulfoxide	Aldrich Chemical Co., Inc.
3. 2-Mercaptoethanol	Aldrich Chemical Co., Inc.
4. Methanol	J.T. Baker Chemical Co.
5. Sodium methoxide 25% w/w in methanol	Aldrich Chemical Co., Inc.
6. Silica gel	E.M. Science

15b. 2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (3)

Reaction Sequence:



Experimental

2-(2-Benzamido-7-guanylyl)ethyl 2-hydroxyethyl sulfide (2)

Please refer to the preceding synthesis, this report.

2-(Guanin-7-yl)ethyl 2-hydroxyethyl sulfide (3)

To a stirred suspension/solution of 2 (4.38 g, 12.2 mmol) in methanol (500 mL) was added a solution of 25% w/w sodium methoxide in methanol (12.5 g solution, 57.8 mmol). The stirred mixture was heated at reflux (66°C) for 5½ h, then allowed to cool while standing overnight. The absence of starting material 2 was confirmed by TLC and the mixture was concentrated to 9.90 g oily white solid. The solid was triturated in ether (approx. 70 mL) then collected. The recovered white powder was dissolved in water (20-25 mL) and filtered to remove any foreign matter. The aqueous solution (filtrate) was carefully neutralized with conc. HCl (approx. 4.3 mL) and then filtered. The collected white precipitate was dried in vacuo at 50°C to yield 3.08 g solid. The solid was suspended in CH₂Cl₂ with stirring then let stand overnight. The suspension was then filtered and the collected solid dried to 3.03 g compound 3 (11.9 mmol, 97.4%). An additional

1.29 g compound 3 was obtained from a scouting run. Both lots (4.32 g) were combined and stirred in 2 L boiling methanol for 20 min, then filtered while still hot. (The insoluble material was dried to 1.15 g off-white solid and consisted of compound 3 and some impurities). The clear filtrate was allowed to cool overnight. The crystals which had formed were collected by filtration and dried in a vacuum oven at 65°C to give 2.32 g pure compound 3, lot no. NJ11-147-4 a portion of which (50 mg) was submitted to WRAIR on April 10, 1996.

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>	<u>S</u>
Calc'd for $C_9H_{13}N_5O_2S$	42.35	5.13	27.43	12.56
Found	42.43	5.09	27.32	12.46

Spectral Data

Infrared (Nujol)

Major bands: 3400, 3300, 2940, 2740, 1685,
1665, 1610, 1550, 1440, 1400,
1365, 1345, 1220, 1090, 1055,
1045, 870, 845, 770 cm^{-1} .

Ultraviolet (MeOH)

λ_{max} 210 nm (sh, $\log \epsilon$ 4.30); 232 nm
(sh, 3.91); 287 nm (3.86).

Nuclear Magnetic Resonance (DMSO- d_6)

δ 10.76 (s, 1, NH); 7.96 (s, 1, CH at C-8);
6.16 (s, 2, NH_2 at C-2); 4.81 (s, 1, OH);
4.37 (t, 2, CH_2).

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection ultraviolet light.

	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	EtOAc:MeOH (2:1)	0.22	Trace impurity at Rf 0.88
2.	EtOH	0.22	Trace impurity at Rf 0.80
3.	CH ₂ Cl ₂ :MeOH (2:1)	0.41	Trace impurity at Rf 0.97

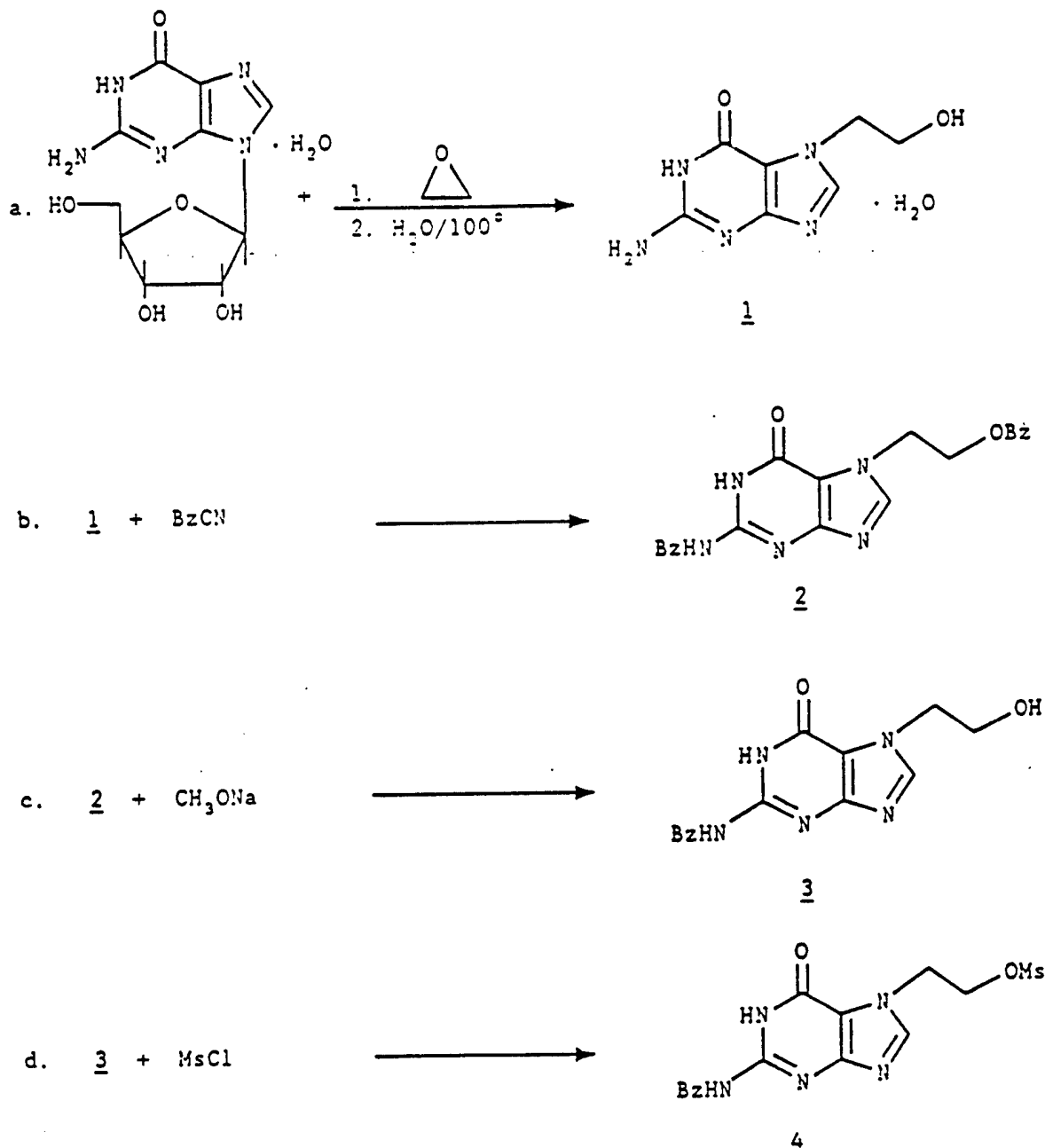
Source of Material

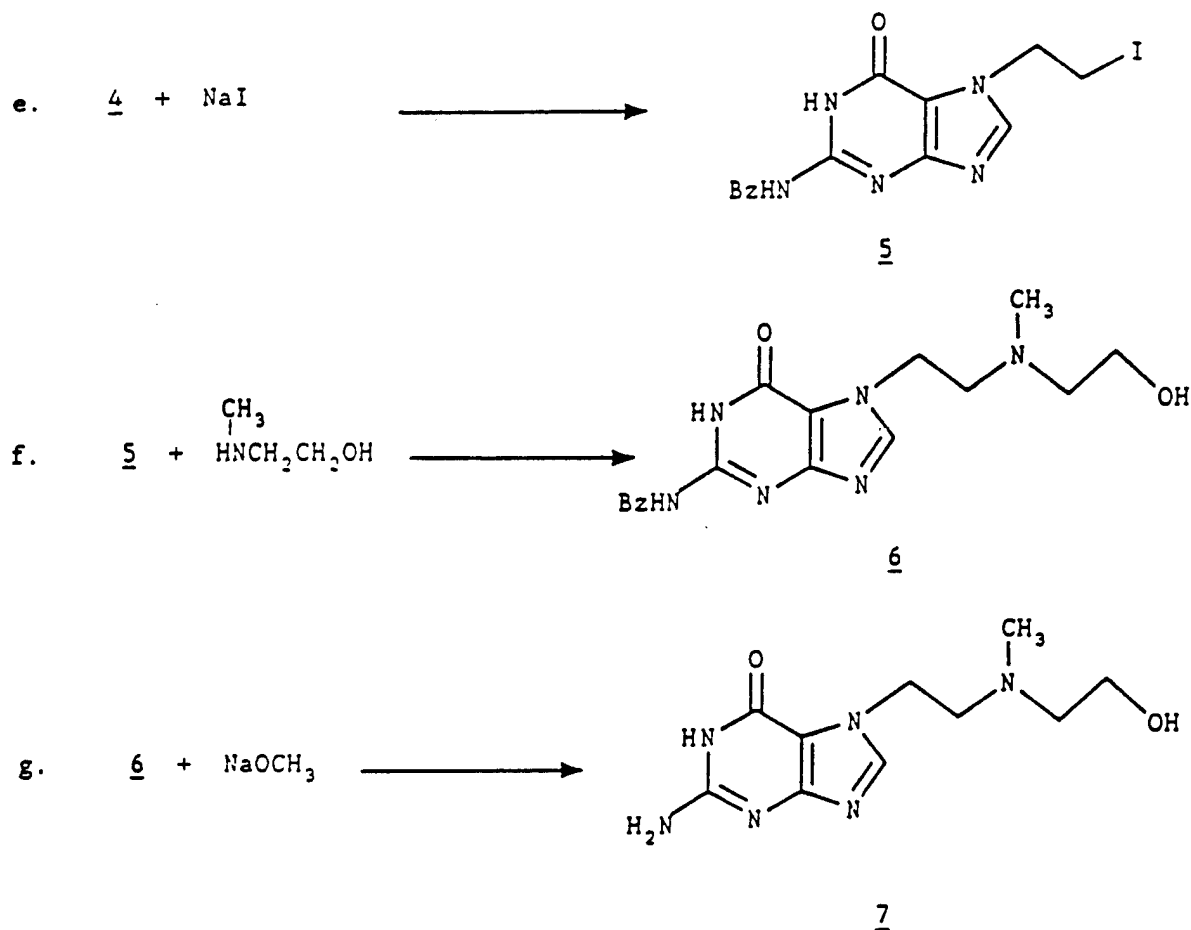
1.	Dichloromethane	J.T. Baker Chemical Co.
2.	Hydrochloric acid	J.T. Baker Chemical Co.
3.	Diethyl ether	Fisher Scientific
4.	Methanol	J.T. Baker Chemical Co.
5.	Sodium methoxide in methanol	Aldrich Chemical Co., Inc.

16. N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine (7)

The target compound 7 was prepared by the following sequence of reactions.

Reaction Sequence:





Experimental

2-Amino-7-(2-hydroxyethyl)purin-6-one, monohydrate (1)

Guanosine hydrate (100.0 g, 353 mmol) was suspended in AcOH (2 L) and stirred, under argon atmosphere, for several minutes. Ethylene oxide (151.9 g, 3.45 mol) was added, and the suspension was stirred at RT for 16 h. A solution was obtained. Excess ethylene oxide and acetic acid was removed in vacuo, and the residue was dissolved in H₂O (1000 mL), heated under reflux for 3 h, then cooled. The solid that separated was collected on a filter, washed with cold H₂O (200 mL), then recrystallized from H₂O (16 L) to give 1 as white

needles (63.9 g, 84.4%) mp >300°C; literature^{1,2} mp >300°C and >325°C, respectively. The material was suitable for further transformation.

2-Benzamido-7-[2-(benzoyloxy)ethyl]purin-6-one (2)

Material 1 (58.9 g, 0.276 mol) was suspended in dry pyridine (1237 mL) containing benzoyl cyanide (119.0 g, 0.907 mol). After the addition of diisopropylethylamine (35.8 g, 0.277 mol) the mixture was stirred at 75°C for 2 3/4 h, then at room temperature overnight. The reaction mixture was quenched by addition of H₂O (620 mL). The resulting precipitate was collected by filtration, washed with CH₂Cl₂ (3 x 190 mL) and dried in vacuo to give 91.0 g solid, mp 249-250°C; literature¹ mp 259-260°C. TLC (EtOAc:MeOH 9:1) showed one major spot corresponding to the product and one minor spot corresponding to the starting material. High field NMR confirmed both the identity of the product and the presence of starting material. Additional material (8.4 g) was obtained from another reaction. The material was suitable for further transformation.

2-Benzamido-7-(2-hydroxyethyl)purin-6-one (3)

A 25% solution of sodium methoxide in CH₃OH (16.0 g, 74.0 mmol) was diluted with CH₃OH (400 mL) then cooled to 0°C. To the solution was added 2 (10.0 g, 24.8 mmol), and the solution was stirred at 0°C for 1 h, under an argon atmosphere, at which time concentrated HCl was added until neutral to pH paper. The neutralized solution was stored at 0°C overnight and then filtered. The precipitate was washed with CH₃OH (40 ml) then dried; yield, 6.1 g (82.2%); mp 222-225°C; literature¹ mp 234-235°C. An infrared spectrum matched previous spectra for this compound and TLC (EtOH:MeOH 9:1)

shows one major spot corresponding to the product and two minor spots representing trace impurities. Additional product (56.2 g) was obtained from two larger reactions. The material was suitable for further transformation.

2-Benzamido-7-[2-[(methanesulfonyl)oxy]ethyl]purin-6-one (4)

Material 3 (6.1 g, 20.4 mmol) was dissolved in pyridine (305 mL). Methanesulfonyl chloride (7.25 g, 63.3 mmol) was added, and the mixture was stirred, under an argon atmosphere, at room temperature overnight. The precipitate which had formed was collected by filtration, washed with a few mL of ethanol and dried to 7.5 g solid. The filtrate was diluted with H₂O (600 mL), and the mixture was extracted with CH₂Cl₂:CH₃OH=9:1. The combined extracts were dried (Na₂SO₄) then concentrated in vacuo to 3.1 g solid. The two solids were combined and recrystallized from ethanol; three crops of material were collected, combined, and triturated in ethanol then dried to give 5.6 g (14.8 mmol, 72.8%). Additional product (65.4 g) was obtained from a scouting run and a larger reaction. The material was suitable for further transformation.

2-Benzamido-7-(2-iodoethyl)purin-6-one (5)

Compound 4 (10.0 g, 26.5 mmol) was added to NaI (55.0 g, 366.9 mmol) dissolved in 400 mL acetone. The suspension was stirred under reflux, in an argon atmosphere. After 4 h TLC of reaction showed the presence of starting material. Another 10.0 g of NaI was added and the reaction refluxed for a further 3 h, when the reaction was seen to be complete by TLC. The solvent was evaporated and the residue was partitioned between 10% CH₃OH in CH₂Cl₂ (1.2 L) and water (1 L). The organic portion was separated and washed with H₂O (500 mL),

dried over Na_2SO_4 and concentrated to dryness in vacuo to obtain 9.9 g (91.3%) of product. Additional product (4.1 g) was obtained from a scouting run. The material was suitable for further transformation.

N-(2-Hydroxyethyl)-N-[2-(2-benzamido-7-guaninyl)ethyl]-methylamine (6)

A stirred mixture of 2-benzamido-7-(2-iodoethyl)purin-6-one (5) (4.0 g, 9.77 mmol), methylaminoethanol (0.73 g, 9.77 mmol), and anhydrous potassium carbonate (1.35 g, 9.77 mmol) and 200 mL of acetonitrile was heated under reflux for 70 h. The solvent was removed on a rotary evaporator after cooling. The light brown residue was chromatographed on a column of silica gel (250 g), using CH_2Cl_2 -MeOH (4:1) then CH_2Cl_2 -MeOH (3:1) as the eluent. Fractions containing product were combined and concentrated to give 2.4 g of solid. Another 9.9 g of material of similar purity was obtained from a larger run. These were combined and rechromatographed on 1 kg SiO_2 with CH_2Cl_2 -MeOH (9:1) as eluent to obtain 9.8 g of white solid. The material was suitable for further transformation.

N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine (7)

N-(2-Hydroxyethyl)-N-[2-(2-benzamido-7-guaninyl)ethyl]-methylamine 6 (2.0 g, 5.86 mmol) was suspended in 20 mL of MeOH and 6.0 mL of 25% sodium methoxide in MeOH was added. The reaction mixture was heated under reflux for 6 h, under argon atmosphere. The solvent was removed in vacuo to give a residue (3.5 g). The residue was applied on a column of silica gel (200 g), and eluted with CH_2Cl_2 -MeOH (4:1), CH_2Cl_2 -MeOH (3:2). Fractions containing product were combined and evaporated to dryness to obtain 0.5 g product with 97.5% purity. This was combined with 1.64 g of material of similar

purity obtained from a larger run and chromatographed twice on silica gel with CH_2Cl_2 -MeOH (4:1), CH_2Cl_2 -MeOH (3:1) as eluent. Fractions containing product were combined and evaporated to dryness. The residue was dissolved in 25 mL H_2O and treated with activated carbon, then crystallized from EtOH- H_2O (1:1; 20 mL) to give 840 mg of pure product; mp 246-248°C (d). A portion (800 mg) was transmitted to WRAIR on May 23, 1996. (Lot No. NJ12-147-1).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd for $\text{C}_{10}\text{H}_{16}\text{N}_6\text{O}_2$	47.61	6.39	33.31
Found	47.69	6.41	33.25

Spectral Data

Infrared (KBr)

Major bands: 3280, 3130, 2860, 1670, 1550, 1535, 1465, 1380, 1270, 1215, 1090, 1025 cm^{-1} .

Ultraviolet (Ethanol)

λ_{max} 238 nm (log ϵ 3.80); 285 nm (3.87).

Nuclear Magnetic Resonance ($\text{DMSO}-d_6$)

δ 10.73 (s, 1, NH); 7.94 (s, 1, H at C-8); 6.12 (s, 2, NH_2); 4.32 (t, 1, OH); 4.28 (t, 2, $J = 6.3$ Hz, guanine $-\text{CH}_2$); 3.40 (m, 2, $J = 6.2, 5.6, 6.3$ Hz, CH_2OH); 2.77 (t, 2, $J = 6.2$ Hz guanine $-\text{CH}_2-\text{CH}_2$); 2.47 (t, 2, $J = 6.4$ Hz); $\text{CH}_2\text{CH}_2\text{OH}$); 2.26 (s, 3, CH_3).

Assay by HPLC

Column: Zorbax C ODS, 4.6 x 250 mm.
Mobile Phase: 20% CH₃OH/80% 0.05M Ammonium formate.
Flow rate: 1 mL/min
Solvent Delivery System: Waters 600E
Detector: 285 nm

Based on the HPLC trace the product NJ12-147-1 was pure.

Thin Layer Chromatography

Merck precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - UV light.

	<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
1.	CH ₂ Cl ₂ -MeOH (1:1)	0.20	elongated spot streaks
2.	MeOH-NH ₄ OH (10:1)	0.80	Homogeneous

Source of Materials:

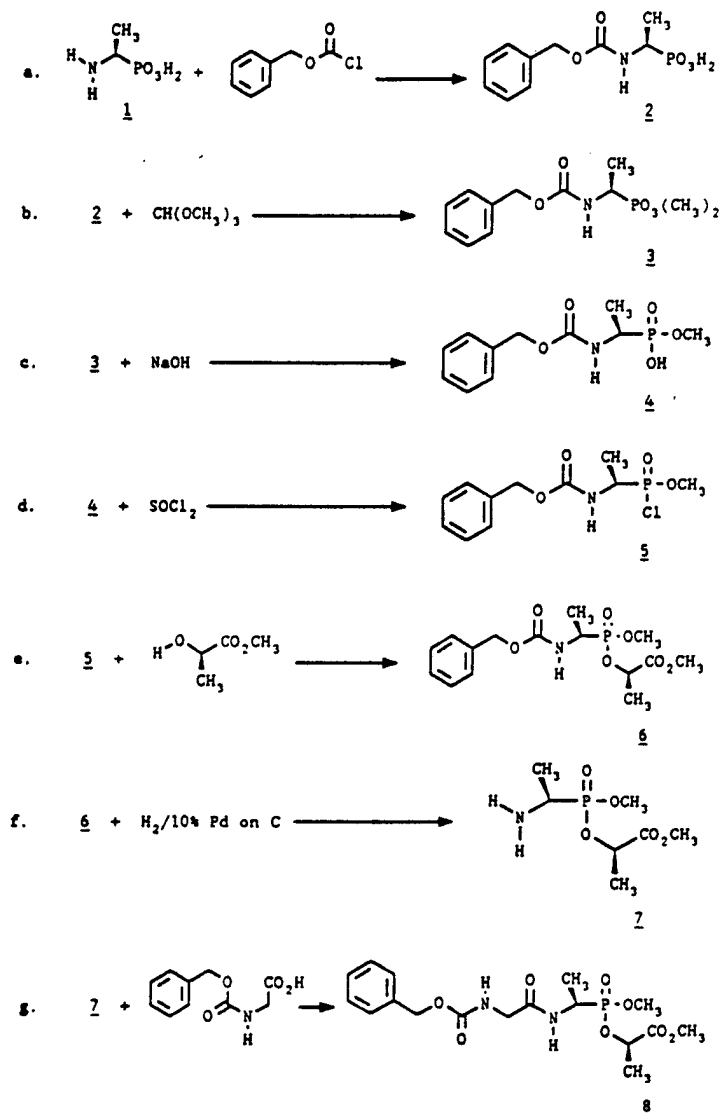
- | | | |
|-----|--|----------------------------|
| 1. | Guanosine hydrate | Aldrich Chemical Co., Inc. |
| 2. | AcOH | J.T. Baker Chemical Co. |
| 3. | Ethylene oxide | Eastman Kodak |
| 4. | 2-Amino-7-(2-hydroxy-ethyl)purin-6-one, monohydrate | Aldrich Chemical Co., Inc. |
| 5. | Pyridine | Aldrich Chemical Co., Inc. |
| 6. | Benzoyl cyanide | Aldrich Chemical Co., Inc. |
| 7. | DMAP | Aldrich Chemical Co., Inc. |
| 8. | CHCl ₃ | Aldrich Chemical Co., Inc. |
| 9. | CH ₃ CN | Aldrich Chemical Co., Inc. |
| 10. | 2-Benzamido-7-[2-benzoyloxy)ethyl]-purin-6-one | Starks Associates, Inc. |
| 11. | Sodium methoxide (25% in CH ₃ OH) | Aldrich Chemical Co., Inc. |
| 12. | HCl | J.T. Baker Chemical Co. |
| 13. | MeOH | J.T. Baker Chemical Co. |
| 14. | 2-Benzamido-7-(2-hydroxy-ethyl]purin-6-one | Starks Associates, Inc. |
| 15. | Methanesulfonyl chloride | Aldrich Chemical Co., Inc. |
| 16. | CH ₂ Cl ₂ | Aldrich Chemical Co., Inc. |
| 17. | EtOH | US Industrial. |
| 18. | Banzamido-7-[2-[(methane-sulfonyl)oxy]ethyl]-purin-6-one | Starks Associates, Inc. |
| 19. | Acetone | J.T. Baker Chemical Co. |
| 20. | NaI | J.T. Baker Chemical Co. |

- 22. Methylaminoethanol Aldrich Chemical Co., Inc.
- 23. Potassium carbonate J.T. Baker Chemical Co.
- 24. Silica gel EM Laboratories
- 25. N-(2-Hydroxyethyl)-N-[2-(2-benzamido-7-guaninyl)-ethyl]methanamine Starks Associates, Inc.

17. O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(R)-lactic acid, methyl ester (8)

Intermediate 8 has been prepared by the following reaction sequence. 20 mg of 8 was transmitted to WRAIR on June 27, 1996 (Lot No. NJ20-146-2).

Reaction Sequence



Experimental¹⁹⁻²²

[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)phosphonic-acid (1) (3.06 g, 24.5 mmol), sodium hydrogen carbonate (4.11 g, 48.9 mmol), sodium carbonate (5.19 g, 49.0 mmol), and 2*N* sodium hydroxide (25 mL, 50.0 mmol) at 5-10°C was added benzyl chloroformate (3.6 mL, 25.2 mmol) slowly by syringe. Two additional 3.6 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2*N* NaOH (26 mL) and H₂O (100 mL) to completely dissolve the mixture. This aqueous solution was washed with Et₂O (2 x 75 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 75 mL). The combined organic layer was washed with brine (75 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (5.0 g, 79%) as a white foam which solidified. Additional reactions were performed to give a total of 17.5 g of 2.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 10.0-9.60 (br m, 2H, -P(OH)₂); 7.26 (s, 5H, Ph-);
5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-CH₂);
4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, CH₃).

Dimethyl [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-
phosphonate (3)

A solution of [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (6.0 g, 23.2 mmol) in trimethyl orthoformate (185 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/CH₂Cl₂, 300 g SiO₂) to give 3 (3.2 g, 48%) as a colorless oil which solidified on standing at 0°C. Additional reactions were performed to give a total of 8.1 g of 3.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, PhCH₂-);
5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH);
3.75-3.73 (2s, 6H, 2 x OCH₃); 1.41-1.37 (dd, 3H,
CH₃).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 28.25 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
CH ₂ Cl ₂ -EtOH (19:1)	0.32	Homogeneous

Methyl hydrogen[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.4 mmol) in CH₃OH (11 mL) and 2N NaOH (7.26 mL, 14.5 mmol) was stirred at room temperature for 24 h. After dilution with H₂O (30 mL), the solution was washed with Et₂O (2 x 30 mL), acidified with 6N HCl to pH 1-2, and extracted with CH₂Cl₂ (4 x 75 mL). The combined CH₂Cl₂ was dried (MgSO₄), filtered, and concentrated to give a residue which was coevaporated with Et₂O (2 x 20 mL) and then dried in vacuo to give 4 (2.28 g, 89%) as a white solid. Additional reactions were performed to give a total of 5.4 g of 4.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 10.1 (br, 1H, -P(OH)); 7.33 (s, 5H, Ph-);
5.80-5.20 (br, 1H, NH); 5.11 (s, 2H, PhCH₂-);
3.90-4.16 (br, 1H, CH); 3.70 (d, 3H, -OCH₃);
1.36-1.32 (dd, 3H, CH₃).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 27.75 (s, 1P) (A small shoulder is also seen at
δ 26.96, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453,
1374, 1302, 1260, 1231, 1159, 1114,
1047, 991, 810, 696, 549 cm⁻¹.

O-[[(1R)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxy-phosphinyl]-(R)-lactic acid, methyl ester (6)

A solution of methyl hydrogen[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.9 mmol) in CH₂Cl₂ (60 mL) was treated with 2M thionyl chloride in CH₂Cl₂ (5.54 mL, 11.1 mmol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (2.9 g, 100%, crude product). This material was dissolved in CH₂Cl₂ (54 mL), chilled to 0-5°C under an argon atmosphere and a solution of methyl (R)-(+)-lactate (1.027 mL, 10.75 mmol, 98%) and Et₃N (1.76 mL, 12.6 mmol) in CH₂Cl₂ (27 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 64 h. The solution was concentrated to give a residue which was dissolved in EtOAc (100 mL) and washed with H₂O (50 mL), sat. NaHCO₃ (50 mL), 3M HCl (50 mL), and brine (75 mL), then dried (MgSO₄), filtered and concentrated to give the crude product (3.0 g, 85%) as a tan oil, which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 80 g SiO₂) to give pure 6 (2.6 g, 73%), as a colorless oil. Additional reactions were performed to give a total of 4.7 g of 6 (2.9 g of 6 is currently available for conversion to 8).

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 7.36-7.30 (m, 5H, Ph-); 5.45 (br d, 0.5H, NH); 5.14-4.93 (3m, 3.5H, PhCH₂ + CH + 0.5 NH); 4.35-4.10 (2m, 1H, CH); 3.83-3.75 (4s, 6H, -CO₂CH₃ and P-OCH₃); 1.57-1.50 (2d, 3H, -OCH(CH₃)CO-); 1.43-1.37 (dddd, 3H, -NCH(CH₃)P).

³¹P Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

 δ 27.78 (s); 26.73 (s).Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-hexanes (85:15)	0.36	Homogeneous

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(R)-lactic acid methyl ester (8)

A solution of O-[[(1R)-N-(phenylmethoxycarbonyl)-1-amino-ethyl]methoxyphosphinyl]-(R)-lactic acid, methyl ester (1.0 g, 2.78 mmol) in EtOAc (50 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (0.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (700 mg, 3.35 mmol) in THF (20 mL) at 0°C under argon was added 4-methylmorpholine (368 μ l, 3.35 mmol) followed by isobutyl chloroformate (435 μ L, 3.35 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (368 μ l, 3.35 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (15 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. NH₄Cl (3 mL). The suspension was filtered and the funnel was washed with THF (2 x 25 mL). The combined filtrate was concentrated and the

residue dissolved in CH_2Cl_2 (100 mL) and washed with 1.0M HCl (2 x 50 mL), sat. NaHCO_3 (2 x 50 mL), and brine (100 mL). The CH_2Cl_2 layer was dried (MgSO_4), filtered and concentrated to give the crude product (1.1 g, 95%), which was purified on a silica gel column (97:3 EtOAc:EtOH, 60 g SiO_2) to give pure **8** (0.9 g, 78%) as a colorless viscous oil. A portion (20 mg) was transmitted to WRAIR on June 27, 1996 (Lot No. NJ20-146-2).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd. for $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_8\text{P}$	49.04	6.05	6.73
Found	48.92	6.03	6.67

Spectral Data

^1H Nuclear Magnetic Resonance (CDCl_3)

(Mixture of two diastereomers at phosphorus)
 δ 7.35 (s, 5H, Ph-); 6.84 and 6.45 (2 br d, 1H, NH); 5.51 and 5.46 (2m, 1H, NH); 5.13 (s, 2H, Ph CH_2 -); 5.01 and 4.94 (2m, 1H, $-\text{OCH}(\text{CH}_3)\text{CO}_2-$); 4.60 and 4.53 (2m, 1H, $-\text{NHCH}(\text{CH}_3)\text{P}-$); 3.99-3.85 (m, 2H, $-\text{NHCH}_2\text{CO}-$); 3.83-3.73 (m, 6H, $-\text{CO}_2\text{CH}_3$ and $\text{P}-\text{OCH}_3$); 1.56-1.52 (2d, 3H, $-\text{OCH}(\text{CH}_3)\text{CO}-$); 1.39-1.34 (m, 3H, $-\text{NHCH}(\text{CH}_3)\text{P}-$).

^{31}P Nuclear Magnetic Resonance (CDCl_3)

(Mixture of two diastereomers at phosphorus)
 δ 27.83 (s); 26.38 (s).

FT - IR (thin film on KBr plate)

Major bands: 3288, 3065, 2957, 2855, 1722, 1680, 1537, 1454, 1379, 1309, 1232, 1101, 1043, 996, 859, 827, 744, 701, 558, 460 cm^{-1} .

Mass Spectrum

Method of Ionization = DEI (low temp)

Calc'd for $C_{17}H_{25}N_2PO_8$ = 416.1

Found: 416, (m)⁺.

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-EtOH (97:3)	0.26	Homogeneous

Source of Materials

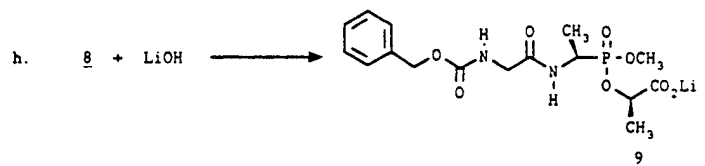
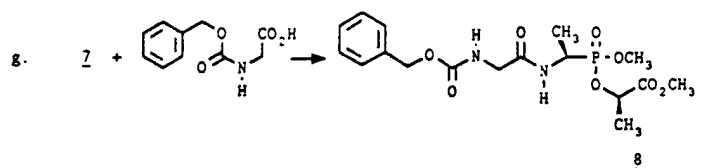
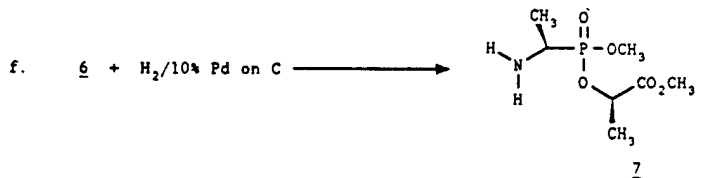
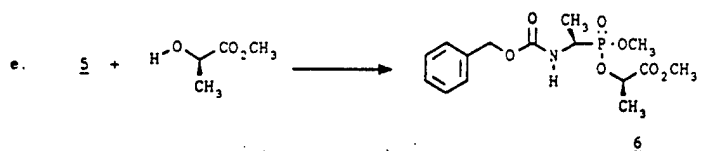
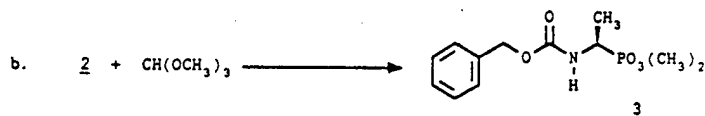
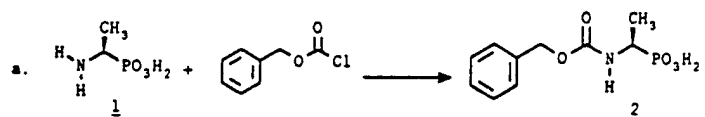
1. (R)-(-)-(1-Aminoethyl)- phosphonic acid	Aldrich Chemical Co., Inc.
2. Sodium hydrogen carbonate	J.T. Baker Chemical Co.
3. Sodium carbonate	J.T. Baker Chemical Co.
4. Sodium hydroxide	J.T. Baker Chemical Co.
5. Benzyl chloroformate	Aldrich Chemical Co., Inc.
6. Ether	Fisher Scientific
7. Hydrochloric acid	J.T. Baker Chemical Co.
8. Ethyl acetate	E.M. Science
9. Molecular sieves (4Å)	Aldrich Chemical Co., Inc.
10. Trimethyl orthoformate	Fluka
11. Silica gel	E.M. Science
12. Ethanol	US Industrial Chem. Corp.
13. Dichloromethane	J.T. Baker Chemical Co.
14. Methanol	J.T. Baker Chemical Co.

15.	Thionyl chloride (2M in CH_2Cl_2)	Aldrich Chemical Co., Inc.
16.	Methyl (R)-(+)-lactate	Aldrich Chemical Co., Inc.
17.	Triethylamine	Aldrich Chemical Co., Inc.
18.	Magnesium sulfate	J.T. Baker Chemical Co.
19.	Hexanes	J.T. Baker Chemical Co.
20.	10% Palladium on carbon Inc.	Aldrich Chemical Co.,
21.	Hydrogen (gas)	Matheson
22.	Celite	Manville
23.	Carbobenzyloxyglycine	Aldrich Chemical Co., Inc.
24.	Tetrahydrofuran	Aldrich Chemical Co., Inc.
25.	4-Methylmorpholine	Aldrich Chemical Co., Inc.
26.	Isobutyl chloroformate	Aldrich Chemical Co., Inc.
27.	Ammonium chloride	Aldrich Chemical Co., Inc.

18. O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(R)-lactic acid, lithium salt (9)

Compound 9 has been prepared by the following reaction sequence. 20 mg of 9 was transmitted to WRAIR on June 27, 1996 (Lot No. NJ22-21-1).

Reaction Sequence



Experimental¹⁹⁻²²[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)phosphonic-acid (1) (3.06 g, 24.5 mmol), sodium hydrogen carbonate (4.11 g, 48.9 mmol), sodium carbonate (5.19 g, 49.0 mmol), and 2*N* sodium hydroxide (25 mL, 50.0 mmol) at 5-10°C was added benzyl chloroformate (3.6 mL, 25.2 mmol) slowly by syringe. Two additional 3.6 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2*N* NaOH (26 mL) and H₂O (100 mL) to completely dissolve the mixture. This aqueous solution was washed with Et₂O (2 x 75 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 75 mL). The combined organic layer was washed with brine (75 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (5.0 g, 79%) as a white foam which solidified. Additional reactions were performed to give a total of 17.5 g of 2.

Spectral Data¹H Nuclear Magnetic Resonance (CDCl₃)

δ 10.0-9.60 (br m, 2H, -P(OH)₂); 7.26 (s, 5H, Ph-);
 5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-CH₂);
 4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, dd,
 3H, CH₃).

Dimethyl [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-phosphonate (3)

A solution of [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (6.0 g, 23.2 mmol) in trimethyl orthoformate (185 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/CH₂Cl₂, 300 g SiO₂) to give 3 (3.2 g, 48%) as a colorless oil which solidified on standing at 0°C. Additional reactions were performed to give a total of 8.1 g, of 3.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, PhCH₂-);
5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH);
3.75-3.73 (2s, 6H, 2 x OCH₃); 1.41-1.37 (dd, 3H, CH₃).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 28.25 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
CH ₂ Cl ₂ -EtOH (19:1)	0.32	Homogeneous

Methyl hydrogen[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.4 mmol) in CH₃OH (11 mL) and 2N NaOH (7.26 mL, 14.5 mmol) was stirred at room temperature for 24 h. After dilution with H₂O (30 mL), the solution was washed with Et₂O (2 x 30 mL), acidified with 6N HCl to pH 1-2, and extracted with CH₂Cl₂ (4 x 75 mL). The combined CH₂Cl₂ was dried (MgSO₄), filtered, and concentrated to give a residue which was coevaporated with Et₂O (2 x 20 mL) and then dried in vacuo to give 4 (2.28 g, 89%) as a white solid. Additional reactions were performed to give a total of 5.4 g of 4.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 10.1 (br, 1H, -P(OH)); 7.33 (s, 5H, Ph-);
5.80-5.20 (br, 1H, NH); 5.11 (s, 2H, PhCH₂-);
3.90-4.16 (br, 1H, CH); 3.70 (d, 3H, -OCH₃);
1.36-1.32 (dd, 3H, CH₃).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 27.75 (s, 1P) (A small shoulder is also seen at
δ 26.96, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453,
1374, 1302, 1260, 1231, 1159, 1114,
1047, 991, 810, 696, 549 cm⁻¹.

O-[[(1R)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxy-phosphinyl]-(R)-lactic acid, methyl ester (6)

A solution of methyl hydrogen[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (2.7 g, 9.9 mmol) in CH₂Cl₂ (60 mL) was treated with 2M thionyl chloride in CH₂Cl₂ (5.54 mL, 11.1 mmol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (2.9 g, 100%, crude product). This material was dissolved in CH₂Cl₂ (54 mL), chilled to 0-5°C under an argon atmosphere and a solution of methyl (R)-(+)-lactate (1.027 mL, 10.75 mmol, 98%) and Et₃N (1.76 mL, 12.6 mmol) in CH₂Cl₂ (27 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 64 h. The solution was concentrated to give a residue which was dissolved in EtOAc (100 mL) and washed with H₂O (50 mL), sat. NaHCO₃ (50 mL), 3M HCl (50 mL), and brine (75 mL), then dried (MgSO₄), filtered and concentrated to give the crude product (3.0 g, 85%) as a tan oil, which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 80 g SiO₂) to give pure 6 (2.6 g, 73%), as a colorless oil. Additional reactions were performed to give a total of 4.7 g of 6 (2.9 g of 6 is currently available for conversion to 8).

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 7.36-7.30 (m, 5H, Ph-); 5.45 (br d, 0.5H, NH); 5.14-4.93 (3m, 3.5H, PhCH₂ + CH + 0.5 NH); 4.35-4.10 (2m, 1H, CH); 3.83-3.75 (4s, 6H, -CO₂CH₃ and P-OCH₃); 1.57-1.50 (2d, 3H, -OCH(CH₃)CO-); 1.43-1.37 (dddd, 3H, -NCH(CH₃)P).

³¹P Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 27.78 (s); 26.73 (s).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-hexanes (85:15)	0.36	Homogeneous

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(R)-lactic acid methyl ester (8)

A solution of O-[[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(R)-lactic acid methyl ester (1.0 g, 2.78 mmol) in EtOAc (50 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (0.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (700 mg, 3.35 mmol) in THF (20 mL) at 0°C under argon was added 4-methylmorpholine (368 µl, 3.35 mmol) followed by isobutyl chloroformate (435 µL, 3.35 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (368 µl, 3.35 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (15 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. NH₄Cl (3 mL). The suspension was filtered and the funnel was washed with THF (2 x 25 mL). The combined filtrate was concentrated and the residue dissolved in CH₂Cl₂ (100 mL) and washed with 1.0M HCl

(2 x 50 mL), sat. NaHCO_3 (2 x 50 mL), and brine (100 mL). The CH_2Cl_2 layer was dried (MgSO_4), filtered and concentrated to give the crude product (1.1 g, 95%), which was purified on a silica gel column (97:3 EtOAc:EtOH, 60 g SiO_2) to give pure 8 (0.9 g, 78%) as a colorless viscous oil.

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd. for $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_8\text{P}$	49.04	6.05	6.73
Found	48.92	6.03	6.67

Spectral Data

^1H Nuclear Magnetic Resonance (CDCl_3)

(Mixture of two diastereomers at phosphorus)

δ 7.35 (s, 5H, Ph-); 6.84 and 6.45 (2 br d, 1H, NH); 5.51 and 5.46 (2m, 1H, NH); 5.13 (s, 2H, PhCH_2 -); 5.01 and 4.94 (2m, 1H, $-\text{OCH}(\text{CH}_3)\text{CO}_2$ -); 4.60 and 4.53 (2m, 1H, $-\text{NHCH}(\text{CH}_3)\text{P}$ -); 3.99-3.85 (m, 2H, $-\text{NHCH}_2\text{CO}$ -); 3.83-3.73 (m, 6H, $-\text{CO}_2\text{CH}_3$ and $\text{P}-\text{OCH}_3$); 1.56-1.52 (2d, 3H, $-\text{OCH}(\text{CH}_3)\text{CO}$ -); 1.39-1.34 (m, 3H, $-\text{NHCH}(\text{CH}_3)\text{P}$ -).

^{31}P Nuclear Magnetic Resonance (CDCl_3)

(Mixture of two diastereomers at phosphorus)

δ 27.83 (s); 26.38 (s).

FT - IR (thin film on KBr plate)

Major bands: 3288, 3065, 2957, 2855, 1722, 1680, 1537, 1454, 1379, 1309, 1232, 1101, 1043, 996, 859, 827, 744, 701, 558, 460 cm^{-1} .

Mass Spectrum

Method of Ionization = DEI (low temp)

Calc'd for $C_{17}H_{25}N_2PO_8$ = 416.1

Found: 416, (m)⁺.

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-EtOH (97:3)	0.26	Homogeneous

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(R)-lactic acid, lithium salt (9)

To a solution of O-[[(1R)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-(R)-lactic acid methyl ester (78 mg, 0.187 mmol) in THF (2 mL) and deionized H₂O (1 mL) was added a solution of LiOH·H₂O in deionized H₂O (1.5M, 125 μL, 0.203 mmol). After stirring at room temperature for 45 min., the mixture was concentrated to dryness and coevaporated with CH₃CN (3 x 10 mL) to give lot no. NJ22-21-1 as a white powder (70 mg, 92%). A portion (20 mg) was transmitted to WRAIR on June 27, 1996 (Lot No. NJ22-21-1).

Anal.

	<u>C</u>	<u>H</u>
Calc'd. for $C_{16}H_{22}LiN_2O_8P \cdot 0.8LiOH \cdot 0.35H_2O$	44.31	5.46
Found	44.35 44.33	5.44 5.49

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

(Mixture of two diastereomers at phosphorus)

δ 7.31-7.29 (m, 5H, Ph-); 5.02 (s, 2H, PhCH₂-);
4.36 (m, 1H, -OCH(CH₃)CO₂-); 3.73 (s, 2H,
-NHCH₂CO-); 3.70-3.54 (m, 4H, -NHCH(CH₃)P- and
P-OCH₃); 1.36-1.32 (m, 3H, -OCH(CH₃)CO-); 1.25-1.22
(m, 2.2H, -NHCH(CH₃)P-, major conformer); 1.14 (m,
0.8H, -NHCH(CH₃)P-, minor conformer).

³¹P Nuclear Magnetic Resonance (D₂O)

(Mixture of two diastereomers at phosphorus)

δ 27.96 (s); 27.49 (s).

Mass Spectrum

Method of Ionization = Electrospray (negative)

Calc'd for C₁₆H₂₂N₂O₈P = 402.12 (free acid)

Found: 401.4 (m-H)⁻.

Source of Materials

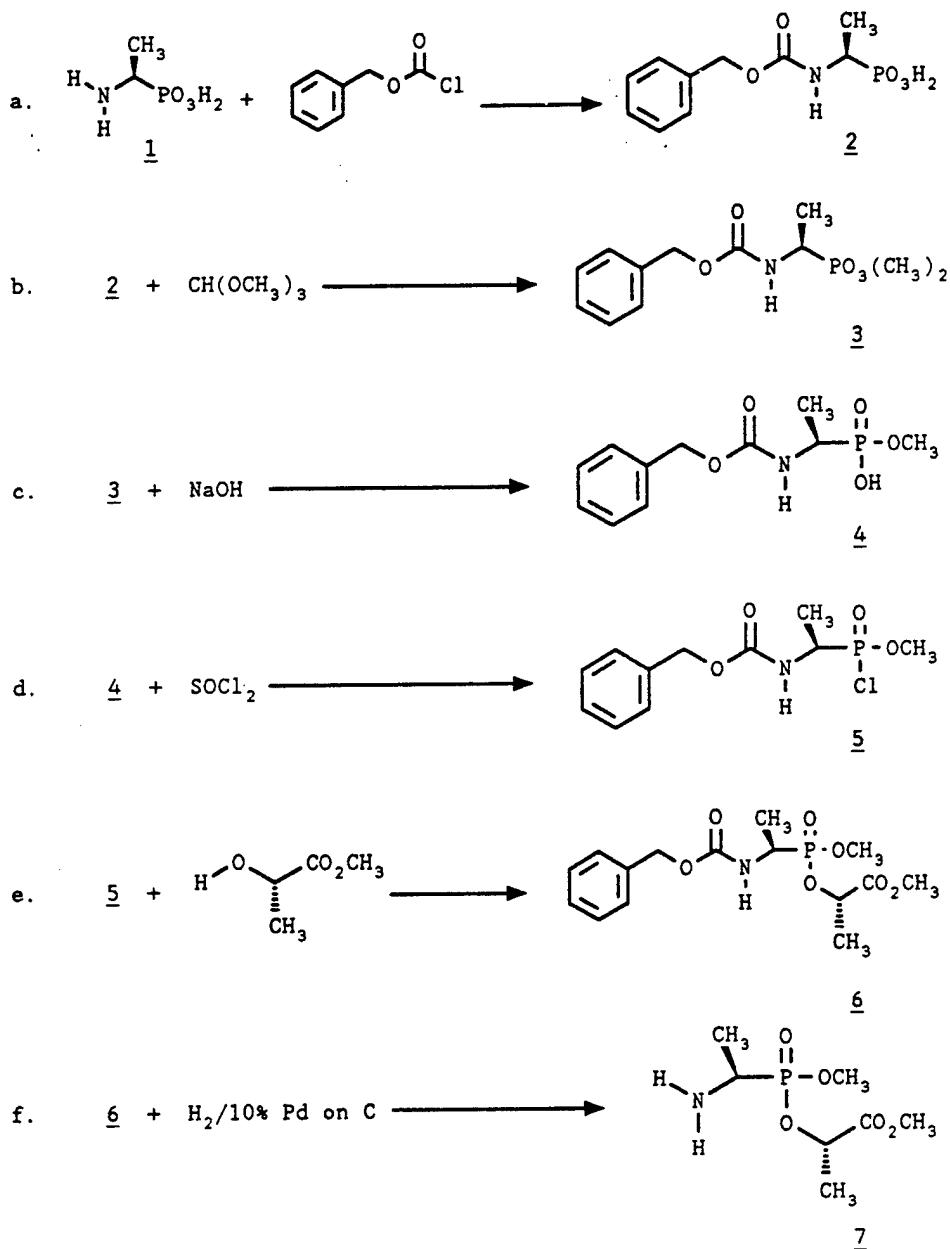
- | | |
|---|----------------------------|
| 1. (R)-(-)-(1-Aminoethyl)-
phosphonic acid | Aldrich Chemical Co., Inc. |
| 2. Sodium hydrogen
carbonate | J.T. Baker Chemical Co. |
| 3. Sodium carbonate | J.T. Baker Chemical Co. |
| 4. Sodium hydroxide | J.T. Baker Chemical Co. |
| 5. Benzyl chloroformate | Aldrich Chemical Co., Inc. |
| 6. Ether | Fisher Scientific |
| 7. Hydrochloric acid | J.T. Baker Chemical Co. |
| 8. Ethyl acetate | E.M. Science |
| 9. Molecular sieves (4Å) | Aldrich Chemical Co., Inc. |
| 10. Trimethyl orthoformate | Fluka |

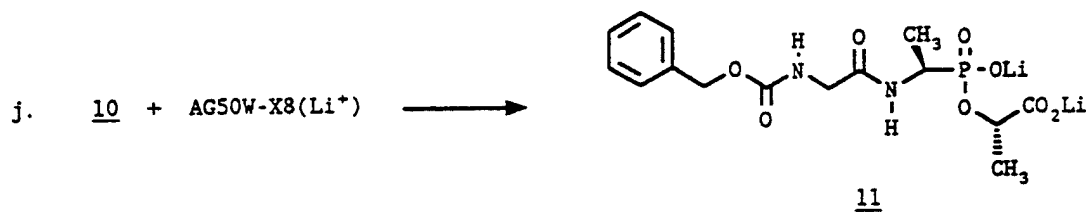
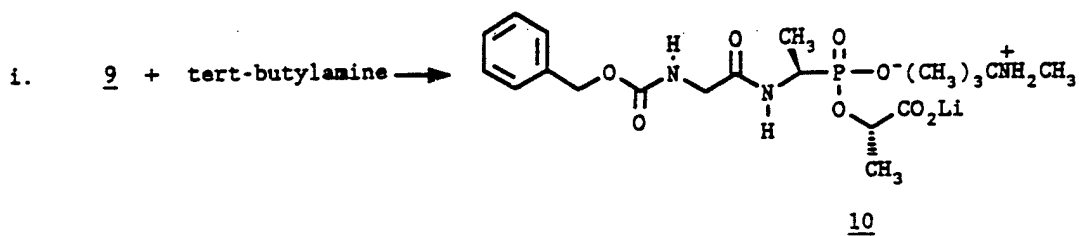
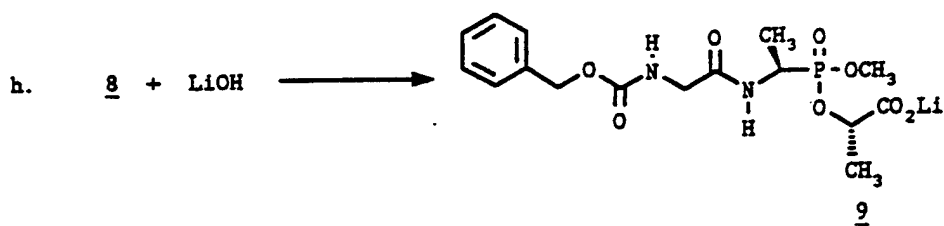
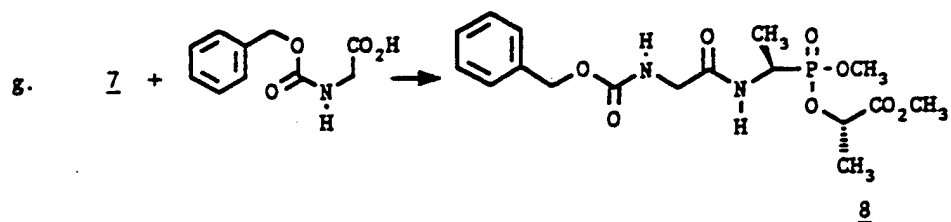
11. Silica gel	E.M. Science
12. Ethanol	US Industrial Chem. Corp.
13. Dichloromethane	J.T. Baker Chemical Co.
14. Methanol	J.T. Baker Chemical Co.
15. Thionyl chloride (2M in CH ₂ Cl ₂)	Aldrich Chemical Co., Inc.
16. Methyl (R)-(+)-lactate	Aldrich Chemical Co., Inc.
17. Triethylamine	Aldrich Chemical Co., Inc.
18. Magnesium sulfate	J.T. Baker Chemical Co.
19. Hexanes	J.T. Baker Chemical Co.
20. 10% Palladium on carbon	Aldrich Chemical Co., Inc.
21. Hydrogen (gas)	Matheson
22. Celite	Manville
23. Carbobenzyloxyglycine	Aldrich Chemical Co., Inc.
24. Tetrahydrofuran	Aldrich Chemical Co., Inc.
25. 4-Methylmorpholine	Aldrich Chemical Co., Inc.
26. Isobutyl chloroformate	Aldrich Chemical Co., Inc.
27. Ammonium chloride	Aldrich Chemical Co., Inc.
28. Lithium hydroxide monohydrate	Aldrich Chemical Co., Inc.
29. Acetonitrile	Aldrich Chemical Co., Inc.

19a. O-[[*(L)*-1-[[*N*-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyloxy]-*L*-lactic acid, dilithium salt (**11**)

Compound **11** has been prepared by the following reaction sequence. 30 mg of **11** was transmitted to WRAIR on July 23, 1996 (Lot No. NJ22-52-1).

Reaction Sequence





Experimental¹⁹⁻²²

[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)phosphonic acid (1) (6.07 g, 48.5 mmol), sodium hydrogen carbonate (8.16 g, 97.1 mmol), sodium carbonate (10.3 g, 97.2 mmol), and 2*N* sodium hydroxide (48.8 mL, 97.6 mmol) at 5-10°C was added benzyl chloroformate (7.2 mL, 50.4 mmol) slowly by syringe. Two additional 7.2 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2*N* NaOH (15 mL) and H₂O (150 mL) to completely dissolve the mixture. This aqueous solution was washed with Et₂O (2 x 100 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 120 mL). The combined organic layer was washed with brine (120 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (11.3 g, 90%) as a white foam which solidified. An additional reaction was performed to give a total of 20.0 g of 2.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 10.0-9.60 (br m, 2H, -P(OH)₂); 7.26 (s, 5H, Ph-); 5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-CH₂); 4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, dd, 3H, CH₃).

Dimethyl [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-phosphonate (3)

A solution of [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (8.7 g, 33.6 mmol) in trimethyl orthoformate (265 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/CH₂Cl₂, 367 g SiO₂) to give 3 (5.1 g, 53%) as a colorless oil which solidified on standing at 0°C. An additional reaction was performed to give a total of 10.1 g, of 3.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, PhCH₂-);
5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH);
3.75-3.73 (2s, 6H, 2 x OCH₃); 1.41-1.37 (dd, 3H, CH₃).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 28.24 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
CH ₂ Cl ₂ -EtOH (19:1)	0.32	Homogeneous

Methyl hydrogen[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (5.0 g, 17.4 mmol) in CH₃OH (20.5 mL) and 2N NaOH (13.5 mL, 27.0 mmol) was stirred at room temperature for 24 h. After dilution with H₂O (50 mL), the solution was washed with Et₂O (2 x 60 mL), acidified with 6N HCl to pH 1-2, and extracted with CH₂Cl₂ (4 x 100 mL). The combined CH₂Cl₂ was washed with brine (100 mL), dried (Na₂SO₄), filtered, and concentrated to give a residue which was coevaporated with Et₂O (20 mL) and then dried in vacuo to give 4 (4.3 g, 90%) as a white solid, mp 119-122°C. An additional reaction was performed to give a total of 9.0 g of 4.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.33 (s, 5H, Ph-); 6.89 (m, 1H, -P(OH)); 5.73 (m, 0.2H, NH, minor conformer); 5.27 (m, 0.8H, NH, major conformer); 5.11 (s, 2H, PhCH₂-); 4.16 (m, 0.8H, CH, major conformer); 4.02 (m, 0.2H, CH, minor conformer); 3.70 (d, 3H, -OCH₃); 1.36-1.32 (dd, 2.4H, J = 7.0, 16.7, CH₃, major conformer); 1.25 (m, 0.6H, CH₃, minor conformer).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 28.63 (s, ~80%, major conformer); 27.89 (br, ~20%, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453, 1374, 1302, 1260, 1231, 1159, 1114, 1047, 991, 810, 696, 549 cm⁻¹.

O-[[*(1R)*-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-L-lactic acid, methyl ester (6)

A solution of methyl hydrogen[*(1R)*-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4.5 g, 16.47 mmol) in CH₂Cl₂ (100 mL) was treated with 2M thionyl chloride in CH₂Cl₂ (9.225 mL, 18.45 mmol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (4.8 g, 100%, crude product). This material was dissolved in CH₂Cl₂ (45 mL), chilled to 0-5°C under an argon atmosphere and a solution of (-)-methyl L-lactate (1.712 mL, 17.92 mmol), and Et₃N (2.927 mL, 21.00 mmol) in CH₂Cl₂ (45 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 5 days. The solution was concentrated to give a residue which was dissolved in EtOAc (150 mL) and washed with H₂O (75 mL), sat. NaHCO₃ (75 mL), 3M HCl (75 mL), and brine (75 mL), then dried (MgSO₄), filtered and concentrated to give the crude product (5.3 g, 90%) as a tan oil. An additional reaction was performed to give a total of 9.9 g of crude product which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 300 g SiO₂) to give pure 6 (9.0 g, 80%), as a colorless oil.

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd. for C ₁₅ H ₂₂ NO ₇ P •0.22H ₂ O	49.59	6.23	3.86
Found	49.57	6.15	3.81

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 7.36-7.30 (m, 5H, Ph-); 5.55 (br d, 0.5H, NH);
5.15-4.92 (3m, 3.5H, PhCH₂ + CH + 0.5 NH); 4.35-4.10
(2m, 1H, CH); 3.82 and 3.73 (2d, 3H, J= 10.9, and
10.9); 3.76 (s, 3H); 1.56 (d, 1.5H, J= 6.9); 1.42
(m, 4.5H).

³¹P Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 27.71 (s); 26.83 (s).

FT - IR (neat)

Major bands: 3255, 3036, 2990, 2957, 1758, 1721,
1536, 1454, 1380, 1304, 1233, 1184,
1103, 1046, 997, 914, 859, 827, 801,
745, 699, 606, 549 cm⁻¹.

Mass Spectrum

Method of Ionization = DEI

Calc'd for C₁₅H₂₂NO₇P = 359

Found: 359 (m⁺), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-hexanes (85:15)	0.41	Homogeneous

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(L)-lactic acid methyl ester (8)

A solution of O-[[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(L)-lactic acid methyl ester (3.0 g, 8.35 mmol) in EtOAc (150 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (1.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (2.092 g, 10.00 mmol) in THF (60 mL) at 0°C under argon was added 4-methylmorpholine (1.10 mL, 10.0 mmol) followed by isobutyl chloroformate (1.30 mL, 10.0 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (1.10 mL, 10.0 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (45 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. NH₄Cl (9 mL). The suspension was filtered and the funnel was washed with THF (2 x 50 mL). The combined filtrate was concentrated and the residue dissolved in CH₂Cl₂ (150 mL) and washed with 1.0M HCl (2 x 75 mL), sat. NaHCO₃ (2 x 75 mL), and brine (150 mL). The CH₂Cl₂ layer was dried (MgSO₄), filtered and concentrated to give the crude product (3.5 g, 100%), which was purified on a silica gel column (92:8 EtOAc:EtOH, 105 g SiO₂) to give pure 8 (2.9 g, 83%) as a colorless viscous oil.

Anal.

	<u>C</u>	<u>H</u>	<u>6N</u>
Calc'd. for C ₁₇ H ₂₅ N ₂ O ₈ P •0.6H ₂ O	47.80	6.18	6.56
Found	47.82	6.15	6.59
	47.75	6.16	6.54

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 7.35 (m, 5H, Ph-); 7.15 and 6.65 (2m, 1H, NH); 5.49 (m, 1H, NH); 5.13 (s, 2H, PhCH₂-); 5.05 and 4.90 (2m, 1H, -OCH(CH₃)CO₂-); 4.62-4.54 (m, 1H, -NHCH(CH₃)P-); 3.97-3.85 (m, 2H, -NHCH₂CO-); 3.82-3.73 (m, 6H, -CO₂CH₃ and P-OCH₃); 1.57 and 1.52 (2d, 3H, -OCH(CH₃)CO-); 1.42-1.38 (m, 3H, -NHCH(CH₃)P-).

³¹P Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 27.49 (s); 26.26 (s).

FT - IR (thin film on KBr plate)

Major bands: 3279, 3064, 2956, 2855, 1757, 1678, 1532, 1453, 1379, 1308, 1234, 1101, 1039, 996, 859, 827, 740, 699, 586, 559, 451 cm⁻¹.

Mass Spectrum

Method of Ionization = DEI

Calc'd for C₁₇H₂₅N₂PO₈ = 416.1

Found: 416 (m⁺), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-EtOH (92:8)	0.43, 0.40	Two overlapping spots (two diastereomers)

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-L-lactic acid, lithium salt (9)

To a solution of O-[[(1R)-N-[N-phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-L-lactic acid, methyl ester (100 mg, 0.24 mmol) in THF (3 mL) and deionized H₂O (1.5 mL) was added a solution of LiOH·H₂O in deionized H₂O (1.5M, 160 μ L, 0.24 mmol). After stirring at room temperature for 45 min, the mixture was concentrated and the residue was coevaporated with CH₃CN (2 x 15 mL) to give 9 (98 mg, 100%), as a white solid.

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

(Mixture of two diastereomers at phosphorus).

δ 7.30-7.27 (m, 5H, Ph-); 5.01 (s, 2H, PhCH₂-); 4.40-4.32 (m, 1H, -OCH(CH₃)CO-); 3.73 and 3.72 (2s, CH₂, -NCH₂CO-); 3.64 (d, 3H, J= 10.7, -P(OCH₃)); 3.69-3.54 (m, 1H, -NCH(CH₃)P-); 1.33 (dd, 3H, J= 6.6, 18, 8, -OCH(CH₃)CO-); 1.24 (dd, 2.2H, J= 4.8, 11.6, -NCH(CH₃)P-, major conformer); 1.19-1.10 (m, 0.8H, -NCH(CH₃)P-, minor conformer).

³¹P Nuclear Magnetic Resonance (D₂O)

(Mixture of two diastereomers at phosphorus)

δ 27.72 (s) and 27.64 (s).

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-hydroxyphosphinyl]-L-lactic acid, monolithium N-methyl-*t*-butylammonium salt (10)

A solution of O-[[(1R)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-L-lactic acid, lithium salt (88 mg, 0.216 mmol) in *tert*-butylamine (freshly distilled from KOH) (9 mL) and 1,4-dioxane (4.5 mL) was heated at reflux for 24 h under argon. After cooling to room temperature, the suspension was concentrated to give a white powder, which was dried in vacuo for 2 h to give 10 (104 mg, 100%).

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

δ 7.30 (m, 5H, Ph-); 5.01 (s, 2H, PhCH₂-); 4.42 (m, 1H, -OCH(CH₃)CO-); 3.95 (m, 1H, -NCH(CH₃)P-); 3.71 (s, 2H, -NCH₂CO-); 1.27 (d, 3H, J= 6.6, -OCH(CH₃)CO-); 1.22 (s, 12H, (CH₃)₃C-NH₂CH₃); 1.15 (dd, 2.4H, J= 7.2, 15.2, -NCH(CH₃)P-, major conformer); 1.05 (m, 0.6H, -NCH(CH₃)P-, minor conformer).

³¹P Nuclear Magnetic Resonance (D₂O)

δ 20.64 (s).

O-[(L)-1-[[N-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyl]-L-lactic acid, dilithium salt (11)

Bio Rad AG50W-X8 (H⁺) resin was converted to the lithium form by suspending AG50W-X8(H⁺) resin (400 g) in 1.0 M LiOH (172 g LiOH·H₂O dissolved in 4 L of deionized H₂O) for 1.0 h, followed by suction filtration and washing of the resin with

deionized H₂O until the pH of the filtrate was neutral. The moist resin was stored at 0°C in an amber bottle. A solution of O-[[(1R)-N-[N-(phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-hydroxyphosphinyl]-L-lactic acid, monolithium N-methyl-*t*-butylammonium salt (98 mg, 0.203 mmol) in deionized H₂O (1.0 mL) was loaded to a column of AG50W-X8 (Li⁺) resin (10 mL bed volume, 1 x 13 cm), packed and eluted with deionized H₂O. Twenty fractions were collected (1.0 mL/fraction). Product found in fractions 6 and 7 (UV active and I₂ staining) was concentrated to a residue and coevaporated with CH₃CN (2 x 2 mL) and Et₂O (2 mL) to give the target 11 (50 mg, 62%), as a white solid. A portion (30 mg) was transmitted to WRAIR on July 23, 1996 (Lot No. NJ22-52-1).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd. for C ₁₅ H ₁₉ Li ₂ N ₂ O ₈ P • 1.0 H ₂ O	42.71	5.11	6.64
Found	42.72	5.12	6.64
	42.67	5.17	6.59

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

δ 7.31-7.27 (m, 5H, Ph-); 5.01 (s, CH₂, PhCH₂-); 4.42 (m, 1H, -OCH(CH₃)CO-); 3.95 (m, 1H, -NCH(CH₃)P-); 3.71 (s, 2H, -NCH₂CO-); 1.26 (d, 3H, J= 6.8, -OCH(CH₃)CO-); 1.15 (dd, 2.3H, J= 7.2, 15.2, -NCH(CH₃)P-, major conformer); 1.04 (m, 0.7H, -NCH(CH₃)P-, minor conformer).

Source of Materials

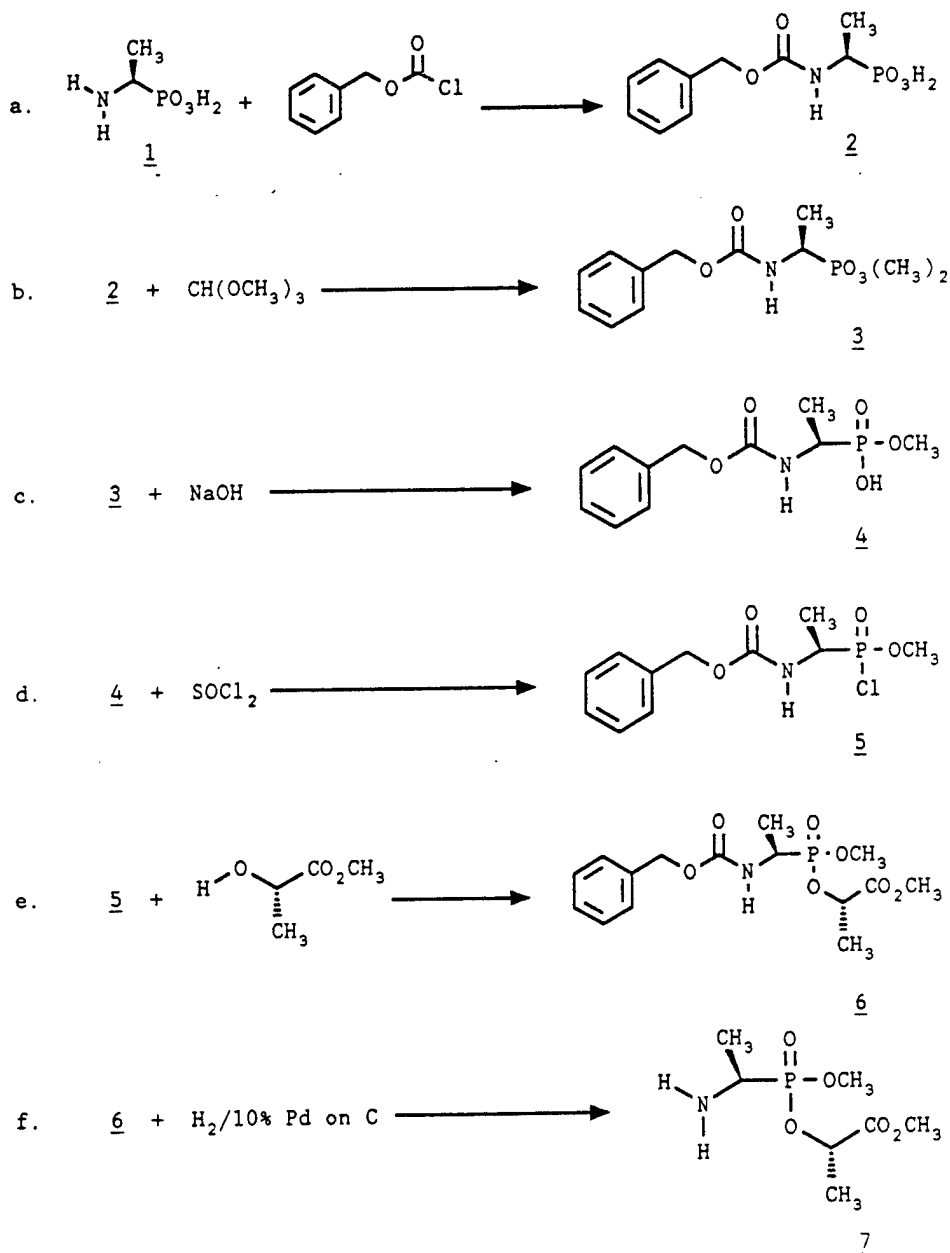
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2. Sodium hydrogen carbonate	J.T. Baker Chemical Co.
3. Sodium carbonate	J.T. Baker Chemical Co.
4. Sodium hydroxide	J.T. Baker Chemical Co.
5. Benzyl chloroformate	Aldrich Chemical Co., Inc.
6. Ether	Fisher Scientific
7. Hydrochloric acid	J.T. Baker Chemical Co.
8. Ethyl acetate	E.M. Science
9. Molecular sieves (4Å)	Aldrich Chemical Co., Inc.
10. Trimethyl orthoformate	Fluka
11. Silica gel	E.M. Science
12. Ethanol	US Industrial Chem. Corp.
13. Dichloromethane	J.T. Baker Chemical Co.
14. Methanol	J.T. Baker Chemical Co.
15. Thionyl chloride (2M in CH ₂ Cl ₂)	Aldrich Chemical Co., Inc.
16. (-)-Methyl L-lactate	Fluka
17. Triethylamine	Aldrich Chemical Co., Inc.
18. Magnesium sulfate	J.T. Baker Chemical Co.
19. Hexanes	J.T. Baker Chemical Co.
20. 10% Palladium on carbon	Aldrich Chemical Co., Inc.
21. Hydrogen (gas)	Matheson
22. Celite	Manville
23. Carbobenzyloxyglycine	Aldrich Chemical Co., Inc.
24. Tetrahydrofuran	Aldrich Chemical Co., Inc.
25. 4-Methylmorpholine	Aldrich Chemical Co., Inc.

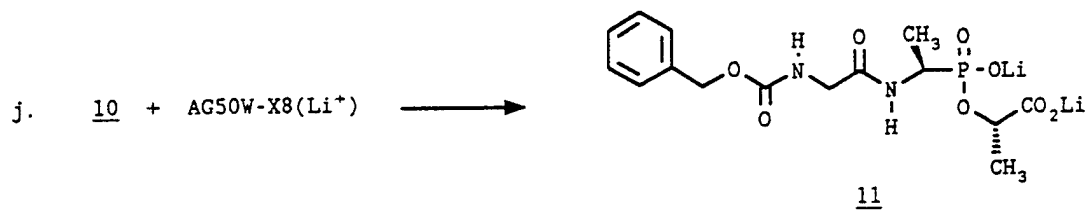
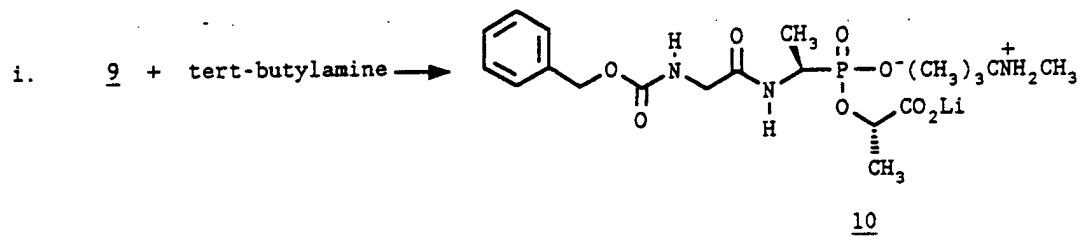
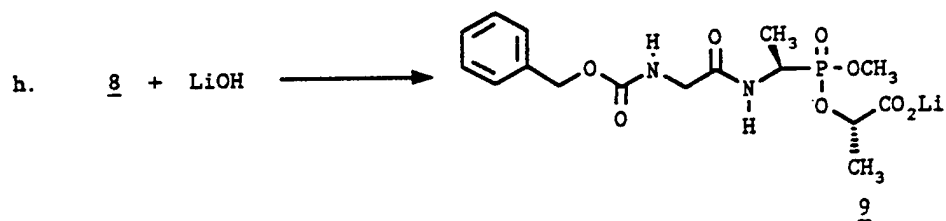
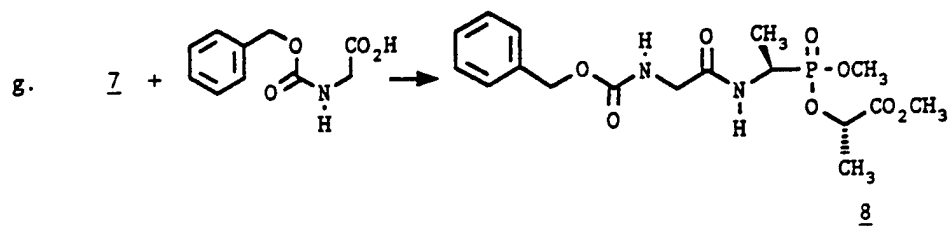
26.	Isobutyl chloroformate	Aldrich Chemical Co., Inc.
27.	Ammonium chloride	Aldrich Chemical Co., Inc.
28.	Lithium hydroxide monohydrate	Aldrich Chemical Co., Inc.
29.	Acetonitrile	Aldrich Chemical Co., Inc.
30.	<i>tert</i> -Butylamine	Aldrich Chemical Co., Inc.
31.	1,4-Dioxane	Aldrich Chemical Co., Inc.
32.	AG50W-X8(H ⁺) resin	Bio-Rad Laboratories
33.	Diethyl ether	Fisher Scientific

19b. O-[[*(L)*-1-[[*N*-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyloxy]-*L*-lactic acid, dilithium salt (**11**)

Compound **11** has been prepared by the following reaction sequence. 1.4 g of **11** was transmitted to WRAIR on August 7, 1996 (Lot No. NJ22-57-1).

Reaction Sequence





Experimental¹⁹⁻²²[(1*R*)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (2)

To a stirred slurry of (*R*)-(-)-(1-aminoethyl)-phosphonic-acid (1) (6.07 g, 48.5 mmol), sodium hydrogen carbonate (8.16 g, 97.1 mmol), sodium carbonate (10.3 g, 97.2 mmol), and 2*N* sodium hydroxide (48.8 mL, 97.6 mmol) at 5-10°C was added benzyl chloroformate (7.2 mL, 50.4 mmol) slowly by syringe. Two additional 7.2 mL portions of benzyl chloroformate were added at 1.0 h intervals, and then the mixture was stirred at room temperature for 20 h. To the white suspension was added enough 2*N* NaOH (15 mL) and H₂O (150 mL) to completely dissolve the mixture. This aqueous solution was washed with Et₂O (2 x 100 mL), acidified to pH 1-2 with conc. HCl, and then extracted with EtOAc (3 x 120 mL). The combined organic layer was washed with brine (120 mL), dried (4Å molecular sieves), filtered, and concentrated to give 2 (11.3 g, 90%) as a white foam which solidified. An additional reaction was performed to give a total of 20.0 g of 2.

Spectral Data¹H Nuclear Magnetic Resonance (CDCl₃)

δ 10.0-9.60 (br m, 2H, -P(OH)₂); 7.26 (s, 5H, Ph-);
5.65 (br m, 1H, NH); 5.11-4.92 (m, 2H, Ph-CH₂);
4.10-3.90 (br m, 1H, -CH); 1.28-1.24 (dd, 3H, dd,
3H, CH₃).

Dimethyl [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]-phosphonate (3)

A solution of [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonic acid (8.7 g, 33.6 mmol) in trimethyl orthoformate (265 mL) was heated to reflux for 48 h. After cooling, the solution was concentrated to give a residue, which was purified by silica gel chromatography (5% EtOH/CH₂Cl₂, 367 g SiO₂) to give 3 (5.1 g, 53%) as a colorless oil which solidified on standing at 0°C. An additional reaction was performed to give a total of 10.1 g, of 3.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.35 (s, 5H, Ph-); 5.12 (s, 2H, PhCH₂-);
5.00-4.95 (br d, 1H, NH); 4.25-4.15 (br m, 1H, CH);
3.75-3.73 (2s, 6H, 2 x OCH₃); 1.41-1.37 (dd, 3H, CH₃).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 28.24 (s, 1P).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>R_f Value</u>	<u>Comment</u>
CH ₂ Cl ₂ -EtOH (19:1)	0.32	Homogeneous

Methyl hydrogen[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4)

A solution of dimethyl [(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (5.0 g, 17.4 mmol) in CH₃OH (20.5 mL) and 2N NaOH (13.5 mL, 27.0 mmol) was stirred at room temperature for 24 h. After dilution with H₂O (50 mL), the solution was washed with Et₂O (2 x 60 mL), acidified with 6N HCl to pH 1-2, and extracted with CH₂Cl₂ (4 x 100 mL). The combined CH₂Cl₂ was washed with brine (100 mL), dried (Na₂SO₄), filtered, and concentrated to give a residue which was coevaporated with Et₂O (20 mL) and then dried in vacuo to give 4 (4.3 g, 90%) as a white solid, mp 119-122°C. An additional reaction was performed to give a total of 9.0 g of 4.

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

δ 7.33 (s, 5H, Ph-); 6.89 (m, 1H, -P(OH)); 5.73 (m, 0.2H, NH, minor conformer); 5.27 (m, 0.8H, NH, major conformer); 5.11 (s, 2H, PhCH₂-); 4.16 (m, 0.8H, CH, major conformer); 4.02 (m, 0.2H, CH, minor conformer); 3.70 (d, 3H, -OCH₃); 1.36-1.32 (dd, 2.4H, J = 7.0, 16.7, CH₃, major conformer); 1.25 (m, 0.6H, CH₃, minor conformer).

³¹P Nuclear Magnetic Resonance (CDCl₃)

δ 28.63 (s, ~80%, major conformer); 27.89 (br, ~20%, minor conformer).

FT - IR (KBr)

Major bands: 3283, 2643, 2301, 1689, 1543, 1453, 1374, 1302, 1260, 1231, 1159, 1114, 1047, 991, 810, 696, 549 cm⁻¹.

O-[[(1R)-N-(Phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(L)-lactic acid, methyl ester (6)

A solution of methyl hydrogen[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]phosphonate (4.5 g, 16.47 mmol) in CH_2Cl_2 (100 mL) was treated with 2M thionyl chloride in CH_2Cl_2 (9.225 mL, 18.45 mol) and stirred for 4 h at room temperature. The solvent was removed and the residue was pumped dry in vacuo for 0.5 h to give the chloridate 5 as a yellow oil (4.8 g, 100%, crude product). This material was dissolved in CH_2Cl_2 (45 mL), chilled to 0-5°C under an argon atmosphere and a solution of (-)-methyl L-lactate (1.712 mL, 17.92 mmol), and Et_3N (2.927 mL, 21.00 mmol) in CH_2Cl_2 (45 mL) was added dropwise. Stirred at 5°C for 15 min then stirred at RT for 5 days. The solution was concentrated to give a residue which was dissolved in EtOAc (150 mL) and washed with H_2O (75 mL), sat. NaHCO_3 (75 mL), 3M HCl (75 mL), and brine (75 mL), then dried (MgSO_4), filtered and concentrated to give the crude product (5.3 g, 90%) as a tan oil. An additional reaction was performed to give a total of 9.9 g of crude product which was purified by silica gel chromatography (85:15 EtOAc:hexanes, 300 g SiO_2) to give pure 6 (9.0 g, 80%), as a colorless oil.

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd. for $\text{C}_{15}\text{H}_{22}\text{NO}_7\text{P}$ •0.22 H_2O	49.59	6.23	3.86
Found	49.57	6.15	3.81

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 7.36-7.30 (m, 5H, Ph-); 5.55 (br d, 0.5H, NH);
5.15-4.92 (3m, 3.5H, PhCH₂ + CH + 0.5 NH); 4.35-4.10
(2m, 1H, CH); 3.82 and 3.73 (2d, 3H, J= 10.9, and
10.9); 3.76 (s, 3H); 1.56 (d, 1.5H, J= 6.9); 1.42
(m, 4.5H).

³¹P Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 27.71 (s); 26.83 (s).

FT - IR (neat)

Major bands: 3255, 3036, 2990, 2957, 1758, 1721,
1536, 1454, 1380, 1304, 1233, 1184,
1103, 1046, 997, 914, 859, 827, 801,
745, 699, 606, 549 cm⁻¹.

Mass Spectrum

Method of Ionization = DEI

Calc'd for C₁₅H₂₂NO₇P = 359

Found: 359 (m⁺), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm,
0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtOAc-hexanes (85:15)	0.41	Homogeneous

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(L)-lactic acid, methyl ester (8)

A solution of O-[[(1R)-N-(phenylmethoxycarbonyl)-1-aminoethyl]methoxyphosphinyl]-(L)-lactic acid methyl ester (3.0 g, 8.35 mmol) in EtOAc (150 mL) was hydrogenated at 15 psi. for 1.0 h over 10% palladium on carbon (1.5 g) (Parr hydrogenation apparatus). The suspension was filtered through a celite pad; the pad was washed with EtOAc (2 x 50 mL) and the combined filtrates were concentrated to give crude 7 as a colorless oil, which was used immediately in the next step below.

To a solution of carbobenzyloxyglycine (2.092 g, 10.00 mmol) in THF (60 mL) at 0°C under argon was added 4-methylmorpholine (1.10 mL, 10.0 mmol) followed by isobutyl chloroformate (1.30 mL, 10.0 mmol). After stirring at 0°C for 15 min, additional 4-methylmorpholine (1.10 mL, 10.0 mmol) was added followed by a solution of the crude amine 7 (prepared above) in THF (45 mL). After stirring at 0°C for 3 h, the reaction was quenched by the addition of sat. NH₄Cl (9 mL). The suspension was filtered and the funnel was washed with THF (2 x 50 mL). The combined filtrate was concentrated and the residue dissolved in CH₂Cl₂ (150 mL) and washed with 1.0M HCl (2 x 75 mL), sat. NaHCO₃ (2 x 75 mL), and brine (150 mL). The CH₂Cl₂ layer was dried (MgSO₄), filtered and concentrated to give the crude product (3.5 g, 100%), which was purified on a silica gel column (92:8 EtOAc:EtOH, 105 g SiO₂) to give pure 8 (2.9 g, 83%) as a colorless viscous oil.

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd. for C ₁₇ H ₂₅ N ₂ O ₈ P •0.6H ₂ O	47.80	6.18	6.56
Found	47.82	6.15	6.59
	47.75	6.16	6.54

Spectral Data

¹H Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 7.35 (m, 5H, Ph-); 7.15 and 6.65 (2m, 1H, NH); 5.49 (m, 1H, NH); 5.13 (s, 2H, PhCH₂-); 5.05 and 4.90 (2m, 1H, -OCH(CH₃)CO₂-); 4.62-4.54 (m, 1H, -NHCH(CH₃)P-); 3.97-3.85 (m, 2H, -NHCH₂CO-); 3.82-3.73 (m, 6H, -CO₂CH₃ and P-OCH₃); 1.57 and 1.52 (2d, 3H, -OCH(CH₃)CO-); 1.42-1.38 (m, 3H, -NHCH(CH₃)P-).

³¹P Nuclear Magnetic Resonance (CDCl₃)

(Mixture of two diastereomers at phosphorus)

δ 27.49 (s); 26.26 (s).

FT - IR (thin film on KBr plate)

Major bands: 3279, 3064, 2956, 2855, 1757, 1678, 1532, 1453, 1379, 1308, 1234, 1101, 1039, 996, 859, 827, 740, 699, 586, 559, 451 cm⁻¹.

Mass Spectrum

Method of Ionization = DEI

Calc'd for C₁₇H₂₅N₂PO₈ = 416.1

Found: 416 (m⁺), 91 (100%).

Thin Layer Chromatography

EM precoated TLC plates, glass support 5 cm x 10 cm, 0.25 mm silica gel 60F-254; detection - iodine stain.

<u>Eluent</u>	<u>Rf Value</u>	<u>Comment</u>
EtoAc-EtoH (92.8)	0.43, 0.40	Two overlapping spots (two diastereomers)

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(L)-lactic acid, lithium salt (9)

To a solution of O-[[(1R)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-(L)-lactic acid methyl ester (2.5 g, 6.04 mmol) in THF (75 mL) and deionized H₂O (37.5 mL) was added a solution of LiOH·H₂O in deionized H₂O (1.5M, 4.0 mL, 6.0 mmol). After stirring at room temperature for 45 min, the mixture was concentrated and the residue was coevaporated with CH₃CN (5 x 75 mL) and Et₂O (100 mL) to give 9 (2.4 g, 98%), as a white solid.

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

(Mixture of two diastereomers at phosphorus).

δ 7.30-7.27 (m, 5H, Ph-); 5.01 (s, 2H, PhCH₂-); 4.40-4.32 (m, 1H, -OCH(CH₃)CO-); 3.73 and 3.72 (2s, CH₂, -NCH₂CO-); 3.64 (d, 3H, J= 10.7, -P(OCH₃)); 3.69-3.54 (m, 1H, -NCH(CH₃)P-); 1.33 (dd, 3H, J= 6.6, 18.8, -OCH(CH₃)CO-); 1.24 (dd, 2.2H, J= 4.8, 11.6, -NCH(CH₃)P-, major conformer); 1.19-1.10 (m, 0.8H, -NCH(CH₃)P-, minor conformer).

³¹P Nuclear Magnetic Resonance (D₂O)

(Mixture of two diastereomers at phosphorus)

δ 27.70 (s) and 27.61 (s).

O-[[(1R)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-hydroxyphosphinyl]-L-lactic acid, monolithium N-methyl-*t*-butylammonium salt (10)

A solution of O-[[(1R)-N-[N-(phenylmethoxycarbonyl)-glycyl]-1-aminoethyl]methoxyphosphinyl]-L-lactic acid, lithium salt (2.4 g, 5.88 mmol) in *tert*-butylamine (freshly distilled from KOH) (250 mL) and 1,4-dioxane (125 mL) was heated at reflux for 24 h under argon. After cooling to room temperature, the suspension was concentrated to a residue which was coevaporated with Et₂O (4 x 50 mL) to give a white solid, which was dried in vacuo for 2 h to give 10 (2.8 g, 99%).

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

δ 7.30 (m, 5H, Ph-); 5.01 (s, 2H, PhCH₂-); 4.42 (m, 1H, -OCH(CH₃)CO-); 3.95 (m, 1H, -NCH(CH₃)P-); 3.71 (s, 2H, -NCH₂CO-); 1.27 (d, 3H, J= 6.6, -OCH(CH₃)CO-); 1.22 (s, 12H, (CH₃)₃C-NH₂CH₃); 1.15 (dd, 2.4H, J= 7.2, 15.2, -NCH(CH₃)P-, major conformer); 1.05 (m, 0.6H, -NCH(CH₃)P-, minor conformer).

³¹P Nuclear Magnetic Resonance (D₂O)

δ 20.61 (s).

O-[(L)-1-[[N-(Phenylmethoxycarbonyl)glycylamino]ethyl]-hydroxyphosphinyl]-L-lactic acid, dilithium salt (11)

Bio Rad AG50W-X8 (H⁺) resin was converted to the lithium form by suspending AG50W-X8(H⁺) resin (400 g) in 1.0 M LiOH (172 g LiOH·H₂O dissolved in 4 L of deionized H₂O) for 1.0 h, followed by suction filtration and washing of the resin with deionized H₂O until the pH of the filtrate was neutral (ca. pH 7). The moist resin was stored at 0°C in an amber bottle. A solution of O-[(1R)-N-[N-(phenylmethoxycarbonyl)glycyl]-1-aminoethyl]hydroxyphosphinyl]-L-lactic acid, monolithium N-methyl-*t*-butylammonium salt (2.8 g, 5.8 mmol) in deionized H₂O (50 mL) was loaded to a column of AG50W-X8 (Li⁺) resin (300 mL bed volume, 3 x 42 cm), packed and eluted with deionized H₂O. Twenty fractions were collected (15 mL/fraction). Product found in fractions 9 - 12 (UV active and I₂ staining) was concentrated to a residue and coevaporated with CH₃CN (5 x 20 mL) and Et₂O (5 x 20 mL) to give the target 11 (1.6 g, 56%), as a white solid. A portion (1.4 g) was transmitted to WRAIR on August 7, 1996 (Lot No. NJ22-57-1).

Anal.

	<u>C</u>	<u>H</u>	<u>N</u>
Calc'd. for C ₁₅ H ₁₉ Li ₂ N ₂ O ₈ P ·1.0H ₂ O	43.08	5.06	6.70
Found	43.04	5.08	6.77
	42.95	5.11	6.72

Spectral Data

¹H Nuclear Magnetic Resonance (D₂O)

δ 7.28 (m, 5H, Ph-); 4.98 (s, CH₂, PhCH₂-);
4.41 (m, 1H, -OCH(CH₃)CO-); 3.95 (m, 1H,
-NCH(CH₃)P-); 3.69 (s, 2H, -NCH₂CO-); 1.25 (d,
3H, J= 6.8, -OCH(CH₃)CO-); 1.15 (dd, 2.4H,
J= 7.2, 15.2, -NCH(CH₃)P-, major conformer);
1.02 (m, 0.6H, -NCH(CH₃)P-, minor conformer).

³¹P Nuclear Magnetic Resonance (D₂O)

δ 20.63 (s).

³¹C Nuclear Magnetic Resonance (D₂O)

δ 179.44, 170.52, 157.76, 147.45, 135.63,
128.14, 127.75, 127.09, 71.10, 66.63, 43.05,
42.45 (d, J= 152.2), 19.66, 14.90.

FT - IR (KBr)

Major bands: 3384, 2985, 2941, 1707, 1654, 1607,
1544, 1456, 1418, 1348, 1267, 1201,
1067, 1048, 977, 885, 809, 778, 738,
698, 576, 502 cm⁻¹.

Mass Spectrum

Method of Ionization = FAB (Glycerol matrix)

Calc'd for C₁₅H₁₉Li₂N₂O₈P = 400.1

Found: 401.1 (m+H)⁺, 450.2 (m+2H-CO₂+Glycerol)⁺,
493.1 (m+H+Glycerol)⁺.

Source of Materials

1. (R)-(-)-(1-Aminoethyl)-phosphonic acid	Aldrich Chemical Co., Inc.
2. Sodium hydrogen carbonate	J.T. Baker Chemical Co.
3. Sodium carbonate	J.T. Baker Chemical Co.
4. Sodium hydroxide	J.T. Baker Chemical Co.
5. Benzyl chloroformate	Aldrich Chemical Co., Inc.
6. Ether	Fisher Scientific
7. Hydrochloric acid	J.T. Baker Chemical Co.
8. Ethyl acetate	E.M. Science
9. Molecular sieves (4Å)	Aldrich Chemical Co., Inc.
10. Trimethyl orthoformate	Fluka
11. Silica gel	E.M. Science
12. Ethanol	US Industrial Chem. Corp.
13. Dichloromethane	J.T. Baker Chemical Co.
14. Methanol	J.T. Baker Chemical Co.
15. Thionyl chloride (2M in CH ₂ Cl ₂)	Aldrich Chemical Co., Inc.
16. (-)-Methyl L-lactate	Fluka
17. Triethylamine	Aldrich Chemical Co., Inc.
18. Magnesium sulfate	J.T. Baker Chemical Co.
19. Hexanes	J.T. Baker Chemical Co.
20. 10% Palladium on carbon	Aldrich Chemical Co., Inc.
21. Hydrogen (gas)	Matheson
22. Celite	Manville
23. Carbobenzyloxyglycine	Aldrich Chemical Co., Inc.
24. Tetrahydrofuran	Aldrich Chemical Co., Inc.
25. 4-Methylmorpholine	Aldrich Chemical Co., Inc.
26. Isobutyl chloroformate	Aldrich Chemical Co., Inc.

27.	Ammonium chloride	Aldrich Chemical Co., Inc.
28.	Lithium hydroxide monohydrate	Aldrich Chemical Co., Inc.
29.	Acetonitrile	Aldrich Chemical Co., Inc.
30.	<i>tert</i> -Butylamine	Aldrich Chemical Co., Inc.
31.	1,4-Dioxane	Aldrich Chemical Co., Inc.
32.	AG50W-X8(H ⁺) resin	Bio-Rad Laboratories
33.	Diethyl ether	Fisher Scientific

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