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13. ABSTRACT *(Maximum 200 words)*

One hundred and ten candidate drugs or their intermediates have been prepared during the duration of this contract. Twenty-one of these materials are new to the chemical literature.

A new route has been developed for a large scale production of HI-6.

The synthesis of IND artelinic acid has been improved with large savings in material and labor costs.

The contol of purity of candidate drugs has been a prime objective of the contract. Rigorous detailed procedures have been developed to permit the definition of drug quality. Criteria used for purity definition consisted of elementary constitution analysis, chromatographic homogeneity, and infrared, ultraviolet and 500 MHz high resolution nmr spectra. If required, mass spectra, and high pressure liquid chromato-graphy (HPLC) was also used.

A broad spectrum of materials have been prepared during the six and one third year contract period. The type of requested materials were divided as follows: isoquinolinones, qinghaosu derivatives, quaternary anticholinesterase reacti-vators, triazines and derivatives, quinazolines and derivatives, camptothecin derivatives and intermediates, infectious disease related compounds and intermediates, chemical defense related compounds and intermediates and carbocyclic nucleosides.

14. SUBJECT TERMS Camptothecin and derivatives; 4',5'-(methylenedioxy)-2'-nitropropiophenone; 2'-amino-4',5'-(methylenedioxy)propiophenone; (S)-7-ethyl-7-hydroxy-10H-2,2-difluoro-1,3-dioxolo[4,5-g]-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-8,11-(7H,13H)-dione; 3,4-(difluoromethylenedioxy)-6-nitro-benzaldehyde; 6-amino-3,4-(difluoromethylenedioxy)benzaldehyde; N-(2-hydroxyethyl)-N-[2-(7-guaninyl)-ethyl]methylamine - a potential marker of efficacy for antivesicant agents; WR 99210 prodrug; analogs of phosphoramidon as metalloprotease inhibitors for botulinum toxin serotype B; artelinic acid; carbocyclic nucleosides.

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Jaroslav F. Novotny 4-12-99

Jaroslav F. Novotny, PI Date

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I SUMMARY

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A new route has been developed for a large scale production of HI-6.

The synthesis of IND artelinic acid has been improved with large savings in material and labor costs.

The contol of purity of candidate drugs has been a prime objective of the contract. Rigorous detailed procedures have been developed to permit the definition of drug quality. Criteria used for purity definition consisted of elementary constitution analysis, chromatographic homogeneity, and infrared, ultraviolet and 500 MHz high resolution nmr spectra. If required, mass spectra, and high pressure liquid chromatography (HPLC) was also used.

The following target compounds have been synthesized during this period: 3,4-dihydro-5-[3-(methylamino)propoxy]-1-(2*H*)-isoquinolinone, monohydrochloride; artemisitene; 1-(2-hydroxyiminomethyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane, dimethanesulfonate; artemisitene; *p*-hydroxylaminoheptanophenone, hydrochloride; α-artesunic acid; 1-(2-hydroxyiminomethyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride, monohydrate (HI-6); 1-[γ-(2',4',5'-trichlorophenoxy)propyloxy]-5-(2-propyl)biguanide, dihydrochloride; 1-[γ-(2',4',5'-trichlorophenoxy)propyloxy]-5-(2-propyl)biguanide 2.1 HCl, 0.5 H_2O; 2,5,5-trimethyl-2-[2'-(4"-carboxymethyl-1"-*R*-methyl-3"-trimethylsilylmethylenecyclohex-2"-yl)ethyl]-1,3-dioxane; 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-hydroxy-1,3,5-triazine, hydrobromide; 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4', 5'-trichlorophenoxy)-propyloxy]-1,3,5-triazine, hydrobromide; *O*-[2-[2-(diethyl-

amino)ethoxy]ethyl]-1-phenyl-1-cyclopentanemethanol, oxalate; 2,5,5-trimethyl-2-[2'-(4"-carboxymethyl-1"-*R*-methyl-3"-trimethylsilylmethylenecyclohex-2"-yl)ethyl]-1,3-dioxane; 7-(2-methylbenzyl)-7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine; (*RS*)-4-ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,10(6*H*)-dione; 7-[(2-trifluoromethylphenyl)methyl]-7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine; 1-(2-hydroxyiminomethyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride, monohydrate (HI-6); (*R*)-4-ethyl-1,4,7,8-tetrahydroxypyrano[3,4-*f*]indolizine- 3,6,10-trione; (*S*)-7-ethyl-7-hydroxy-14-methyl-10*H*-1,3-dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione, syn.: 7-methyl-10,11-(ethylenedioxy)-20(*S*)-camptothecin; [1*S*-(1α,2Z,3α,4β)]-4-methyl-3-[2-(2,5,5-trimethyl-1,3-dioxan-2-yl)ethyl]-2-[(trifluoromethylsilyl)-methylene]cyclohexaneacetic acid; (*S*)-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-8,11-(7*H*,13*H*)dione, syn.: 10,11-(methylenedioxy)-20(*S*)-camptothecin; (+)-1,3-dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(dibutylamino)propyl]phenanthrene, hydrochloride; dihydroartemisinin; 7-[(4-cyanophenyl)methyl]-8-methyl-7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine; (*S*)-7-,14-diethyl-7-hydroxy-10*H*-1,3-dioxolo[4,5-*g*]pyrano[3',4':6,7]indolizino-[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione, syn.: 7-ethyl-10,11-(methylenedioxy)-20(*S*)-camptothecin; (-)-1,3-dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(dibutylamino)propyl]-phenanthrene, hydrochloride; 7-[(4-cyanophenyl)methyl]-8-methyl-7*H*-pyrrolo[3,2-*f*]quinazoline-1,3-diamine; (*S*)-15-amino-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo-[4,5-*g*]pyrano[3',4':6,7]-indolizino[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione, syn.: 9-amino-10,11-(methylenedioxy)-20(*S*)-camptothecin; N-(2-hydroxy-ethyl)-N-[2-(7-guaninyl)ethyl]methylamine; 1,3-dichloro-6-trifluoromethyl-9-phenanthroic acid; 4-chloroimidazo[4,5-*c*]pyridine; 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichlorophenoxy)propyloxy]-1,3,5-triazine, hydrobromide; 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichloro-

phenoxy)propyloxy]-1,3,5-triazine (unpurified lot); 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichlorophenoxy)propyloxy]-1,3,5-triazine (purified lot); N-(2-hydroxyethyl)-N-[(2-(7-guaninyl)ethyl]methylamine; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione; WR99210 prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure); N^1-3,4-dichlorophenyl-N^5-isopropyldiguanide, hydrochloride; *L*-glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt; 6-cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine; 1-(3,4-dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)guanidine; *L*-glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt; *L*-glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt; 2-(guanin-7-yl)ethyl 2-hydroxyethyl sulfide; 1,3,5-triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester; N-(2-hydroxyethyl)-N-[2-(7-guaninyl)ethyl]methylamine; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]indolizine-3,6,10-trione; O-[[(1*R*)-N-[N-(phenylmethoxycarbonyl)glycyl]-1-aminoethyl]-methoxyphosphinyl]-(*R*)-lactic acid, methyl ester; O-[[(1*R*)-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; (*S*)-4-ethyl-1,4,7,8-tetrahydro-4-hydroxypyrano[3,4-*f*]indolizine-3,6,10-trione; 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; Fmoc-L-Gln(Trt)Ψ-($COCH_2$)-D,L-Phe; 1,9(2*H*,10*H*)-acridinedione, 7-chloro-3-(2,4-dichlorophenyl)-3,4-dihydro-; 1(2*H*)-acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-; product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine;

product(s) obtained by reacting EtMgBr with 7-chloro-3-(2,4-dichlorophenyl)-1(2*H*)-acridinone; Fmoc-L-Gln-(Trt)Ψ($COCH_2$)-D,L-Phe; product(s) obtained on condensation of 6-amino-piperonal with 2,5-dihydroxy-7-methyl-6-cyanoindolizine; (*S*)-15-chloro-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo-[4,5-*g*]pyrano-[3',4' : 6,7]indolizino[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione, syn.: 9-chloro-10,11-(methylenedioxy)-20(*S*)-camptothecin; (*S*)-7-ethyl-7-hydroxy-10*H*-2,2-difluoro-1,3-dioxolo-[4,5-*g*]pyrano[3',4' : 6,7]indolizino[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione, syn.: 10,11-(difluoromethylenedioxy)-20(*S*)-camptothecin; hexanoic acid, 3-ethyl-4-(3-methylphenyl)-; butyric acid, 4-(4-chlorophenyl)-4(*R*)-[10(β)-dihydroartemisininoxy]-; hexanoic acid, 4-ethyl-4-phenyl)-; artemisinin; dihydroartemisinin; artelinic acid, methyl ester; artelinic acid; artemisinin; dihydroartemisinin; artelinic acid; artelinic acid (cGMP); 3-deazaadenine; methyl 4-hydroxymethylbenzoate; artemisinin; artelinic acid (non cGMP); (+)-9-(*trans*-2', *trans*-3'-dihydroxycyclopent-4'-enyl)-3-deazaadenine; α-artelinic acid; (-)-9-(*trans*-2', *trans*-3'-dihydroxycyclopent-4'-enyl)-3-deazaadenine; β-artelinic acid, hemihydrate (cGMP).

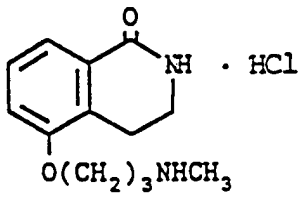
III. DISCUSSION

1. Chemistry

A broad spectrum of materials have been prepared during the six and one third year contract period. The type of requested materials were divided as follows: isoquinolinones, qinghaosu derivatives, quaternary anticholinesterase reactivators, triazines and derivatives, quinazolines and derivatives, camptothecin derivatives and intermediates, infectious disease related compounds and intermediates, chemical defense related compounds and intermediates and carbocyclic nucleosides.

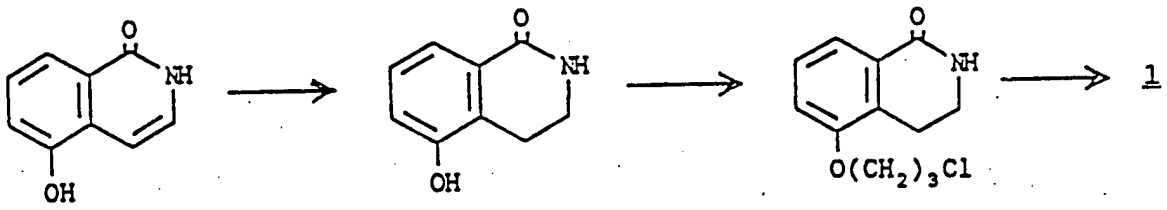
A. Isoquinolinones

The target compound 1 was prepared by



1

the following sequence of reactions. Commercially

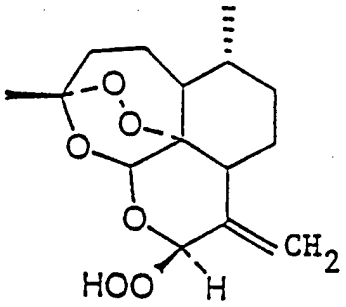


2 3 4

available dihydroxyisoquinoline 2 was reduced with 10% Pd/C to give 3 in 92.9% yield. The isoquinolinone 3 was alkylated with 1-bromo-3-chloropropane to give 4 in 80.2% yield. High pressure reaction with methylamine gave the target material in 87.3% yield. The overall yield was improved from 27.7 to 65.0%, i.e. by 135%.[1]

B. Qinghaosu Derivatives

The two target materials 5 & 6



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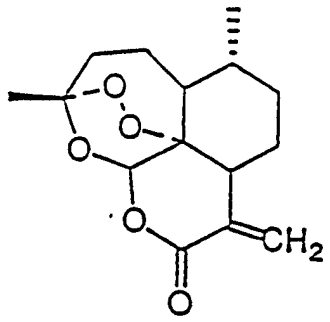
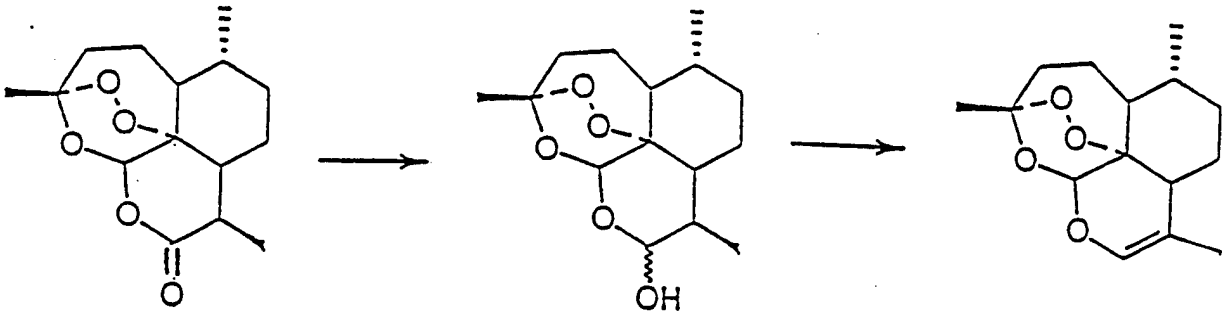


6

were prepared by the following sequence of reactions. Artemisinin 7 was reduced with

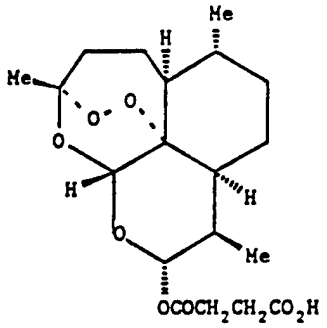


7 → 8 → 9

→ 5 → 6

sodium borohydride to give 8 in 92% yield. The material was dehydrated with $BF_3 \cdot Et_2O$ to give the olefin 9 in 72% yield[2]. A solution of 9 was irradiated while oxygen was bubbled through the solution to give the first target compound 5 in 52% yield[3]. Hydroperoxide 5 was converted to 6 by the action of Ac_2O. The yield was 96%.

The target compound 10 was prepared by



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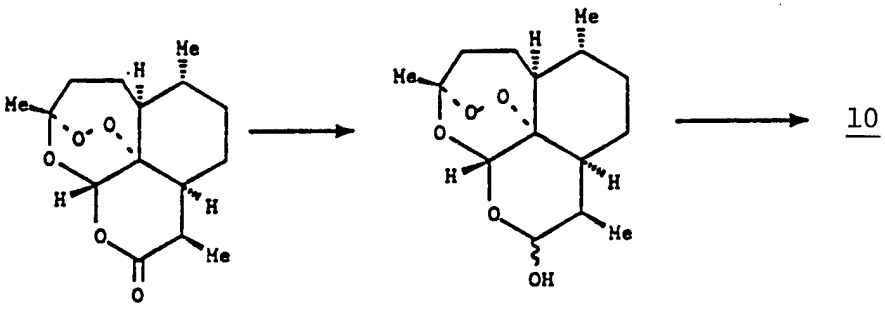
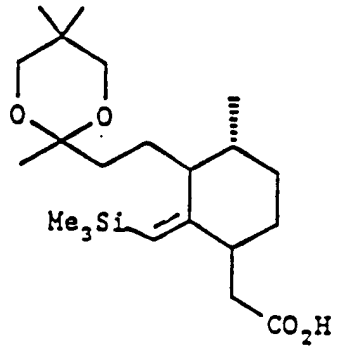
a two step synthesis as shown[4]. Artemisinin (7)



7 8

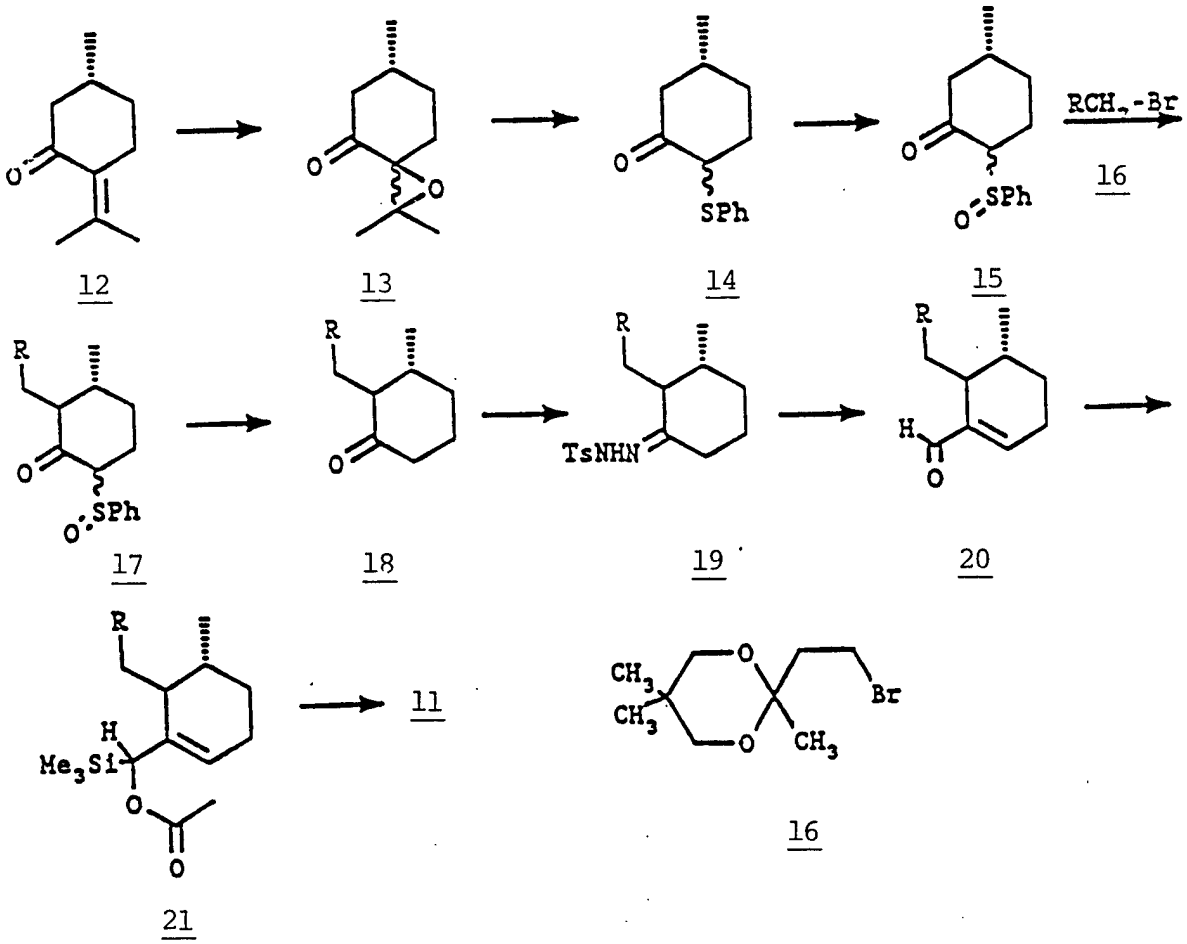
was reduced with sodium borohydride to give dihydroartemisinin 8 in 73.0% yield. The product 8 was reacted with succinic anhydride in the presence of pyridine to give 10 in 85.7% yield.

The target compound 11 was prepared by



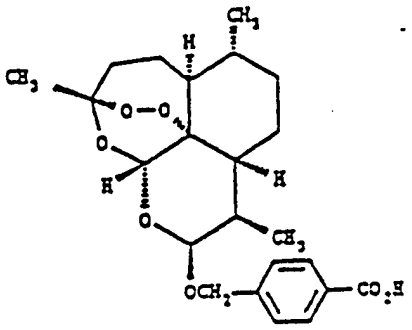
11

a ten-step synthesis, as shown below:[5,6]



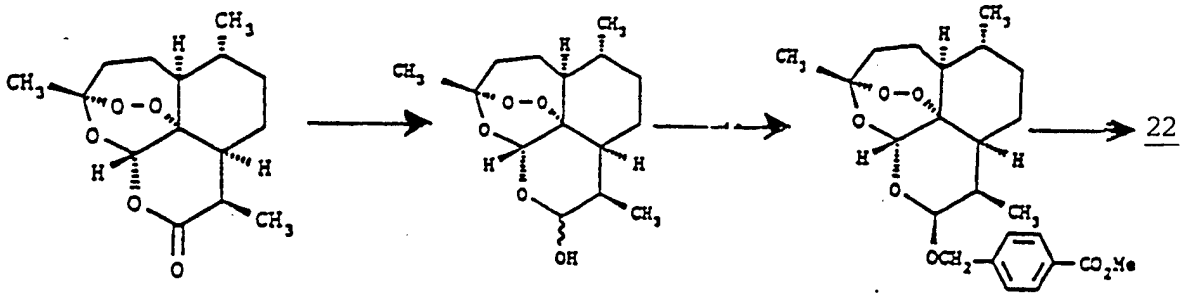
Pulegone 12 was oxidized with hydrogen peroxide to give a mixture of two pulegone oxides 13 in 98.2% yield. The mixture was reacted with thiophenol in base to give a mixture of sulfides 14 in 94.6% yield. A portion of the sulfides was oxidized with the magnesium salt of monoperoxyphthalic acid to give a mixture of sulfoxides 15 in 56.1% yield. The dioxane 16 was prepared from methyl vinyl ketone and neopentanediol in 60% yield. This was reacted with 15 to give 17 then 18 in 30.9% yield. The ketone 18 gave with tosylhydrazine the hydrazone 19 in 73.6% yield. This was converted to 20 in 66.3% yield. The aldehyde 20 was immediately converted to 21 in 73.1% yield. Rearrangement of 21 to target 11 was achieved in 55% yield.

Preparation of a reference sample of artelinic acid (22) was accomplished by the following sequence of reactions. Commercially available artemisinin (7)



22

was reduced with sodium borohydride to give a mixture of dihydroartemisinins (8) in 84% yield. This was not purified but immediately reacted with methyl 4-hydroxymethylbenzoate in the presence of boron trifluoride diethyl etherate to give the methyl ester of artelinic acid (23) in 67% yield. Hydrolysis of the ester with methanolic KOH at RT gave the target compound 22 in 86% yield. A portion (10.0 g) was transmitted to WRAIR.



7 8 23

In our preliminary (non-cGMP) efforts, each step of the reaction sequence was examined for potential optimization. The original literature procedure[7a,7b] called for a large excess of sodium borohydride in the first step of the sequence. We found that the molar ratio of sodium borohydride to artemisinin 7 could be decreased without effecting the yield of the product 8. Similarly, the amount of methanol was decreased by 50% without a decrease in product yield. Details of these changes are shown in Table 1.

TABLE 1

Amounts used			Molar Ratio of		Yield of 8		Comments
7	$NaBH_4$	CH_3OH	7 :	$NaBH_4$	grams	%	
5 g	5 g	200 mL	1	7.5	3.6	70.6	Lit. method
5 g	5 g	200 mL	1	7.5	4.4	86.8	New work-up
5 g	2.5 g	200 mL	1	3.7	4.6	90.0	
5 g	1.75 g	200 mL	1	2.5	4.5	88.8	Longer time required
5 g	2.0 g	100 mL	1	2.9	4.6	90.0	Method to be used in scale-up
25 g	10 g	500 mL	1	2.9	23.2	91.7	

In the second step, a series of reaction conditions were explored which varied the ratio of dihydroartemisinin 8 to methyl 4-hydroxymethylbenzoate. The results are summarized on the following page in Table 2

TABLE 2

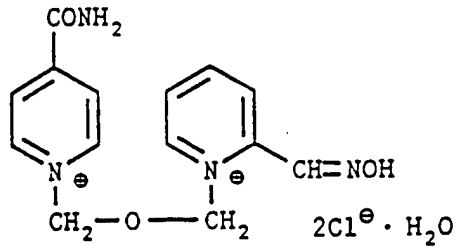
8	Amounts used: methyl ester	ether	Molar Ratio of 8 : methyl ester		Yield of 23: grams	%	Comments
4.1 g	8 g	450 mL	1	3.5	5.9	98.3	Lit. method
21.4 g	37 g	2200 mL	1	3	32.4	100.0	
2.0 g	2.33 g	200 mL	1	2	2.5	83.3	
2.0 g	2.33 g	100 mL	1	2	2.9	96.7	
2.0 g	1.74 g	50 mL	1	1.5	2.3	76.4	
2.0 g	2.33 g	50 mL	1	2	3.0	100.0	
2.0 g	1.74 g	50 mL	1	1.5	3.1	100.0	Method to be used in scale-up

The conditions described in the final entry of Table 2 were used in scale-up. The molar ratio of dihydroartemisinin 8 to methyl 4-hydroxymethylbenzoate was able to be decreased from 1:3½ to 1:1½, thus using only 43% of the amount of ester which would be required of the literature[4] method. The amount of ether used in this step could be decreased by over 75% with no change in product yield, so this modification was utilized as well. We found that the original conditions of hydrolysis in the final step (conversion of 23 to 22) to be satisfactory, so they remained unchanged.

Using these optimized conditions, 1575 g of artemisinin was reduced to give 1417.6 g (89.4%) of dihydroartemisinin 8. This material was reacted with methyl 4-hydroxymethylbenzoate in the presence of $BF_3 \cdot Et_2O$ to give 1441.5 g (66.0%) of methyl artelinate 23. Hydrolysis of 23 with KOH in methanol gave the target compound 22

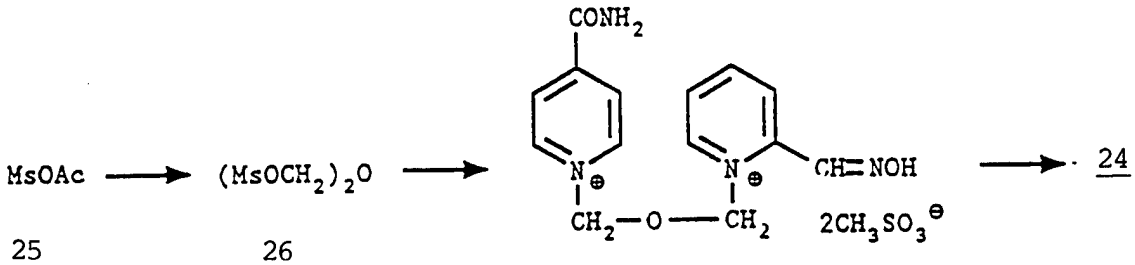
C. Quaternary Anticholinesterase Reactivators

The target compound 24 was prepared



24

by the following sequence of reactions.



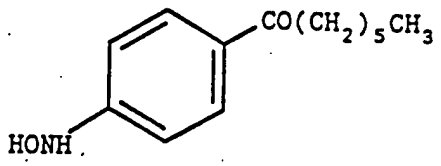
25 26 27 24

The mixed anhydride 25 was originally synthesized by reacting MsOH with AcOH in the presence of P_2O_5. The yields were moderate, and the workup tedious. The scale-up of this reaction was limited. In the new method acetic anhydride is used as the dehydrating agent. The acetic acid formed is removed in vacuo, and the product 25 is obtained in quantitative yield in a very short time. Approximately 8 kg of 25 could be produced in one man-day. The standard procedure to synthesize 26 was changed. Instead of reacting 25 with trioxane we have reacted trioxane with Ac_2O to give a mixture of $(AcOCH_2)_2O$ and $(AcO)_2CH_2$ in 1:1 ratio. These materials were not separated but immediately reacted with 25 to give 26 in ~50-60% yield; 9074.3 g of 26 was prepared. Preparation of 27 was scaled-up and its purification was improved. In all 1472.5 g of pure methanesulfonate 27 was converted to the chloride salt 24 on an anion exchange resin

($Cl^{\ominus}$ form) to give 1062.5 g of pure 24. This was crystallized from EtOH/H_2O 3:1 to obtain 1023.9 g of 24.

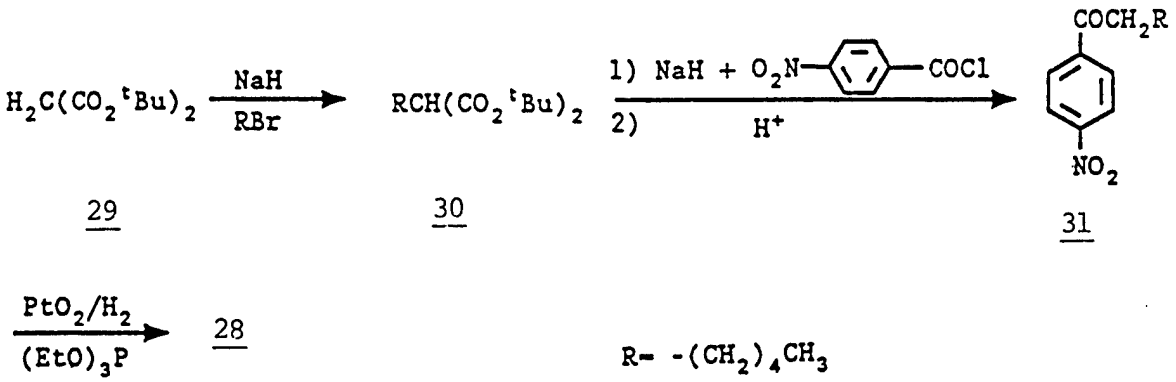
D. Anticyanide Intoxication Related Compounds

The target compound 28 was prepared



28

by the following reaction scheme. Approximately 1.9 kg of target 28 has been obtained prior to final purification by crystallization.

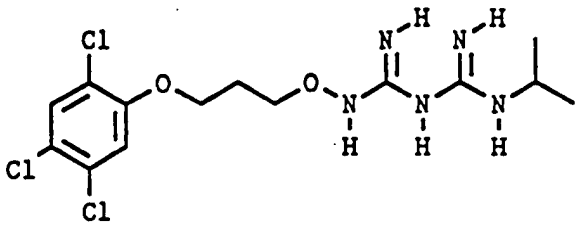


The crude free base (1888.8 g) was partially purified by chromatography to give 1437.7 g of 28. The purity of the product was unsatisfactory since the material was unstable to oxidation. After consultation with Dr. Robert Engle it was decided to prepare a suitable salt of the hydroxylamine. The HCl salt was found satisfactory. The material was divided into 100 g lots, and each lot was passed through a pad of silica gel (300 g) using hexane-EtOAc (3:1) as the eluent. Fractions containing product were combined then acidified with 7.15M HCl in EtOH (75 mL). The solid that separated was

collected, washed with ether, then dried; yield 841.8 g. A certain amount of material which contained additional impurity (~350 g) was set aside.

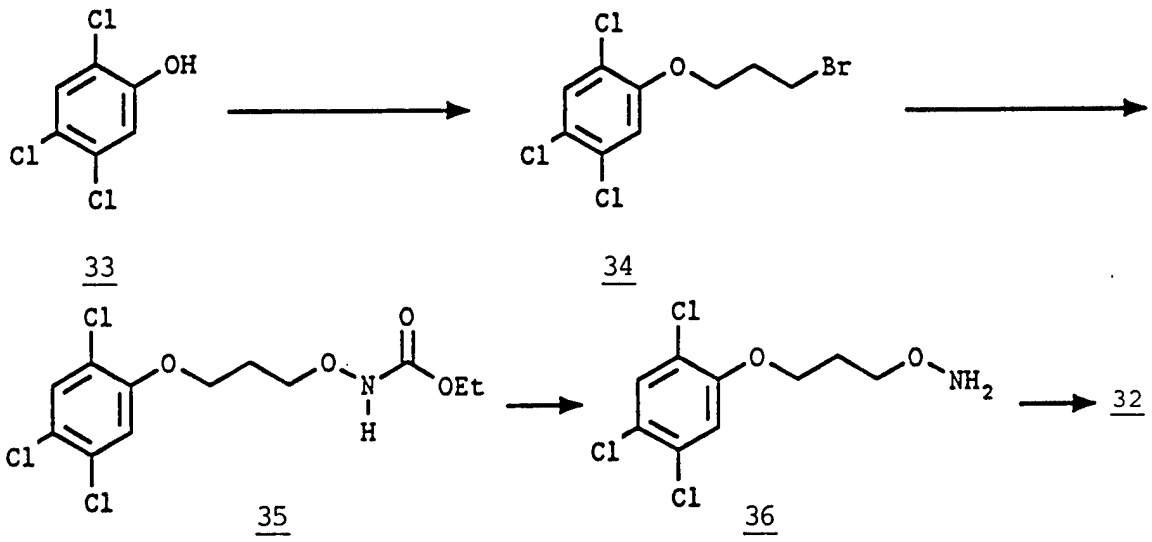
E. Triazines and Derivatives

The research compound 32 was prepared by the



32

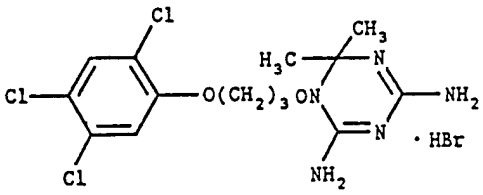
following sequence of reactions. The commercially available



33 34 35 36 32

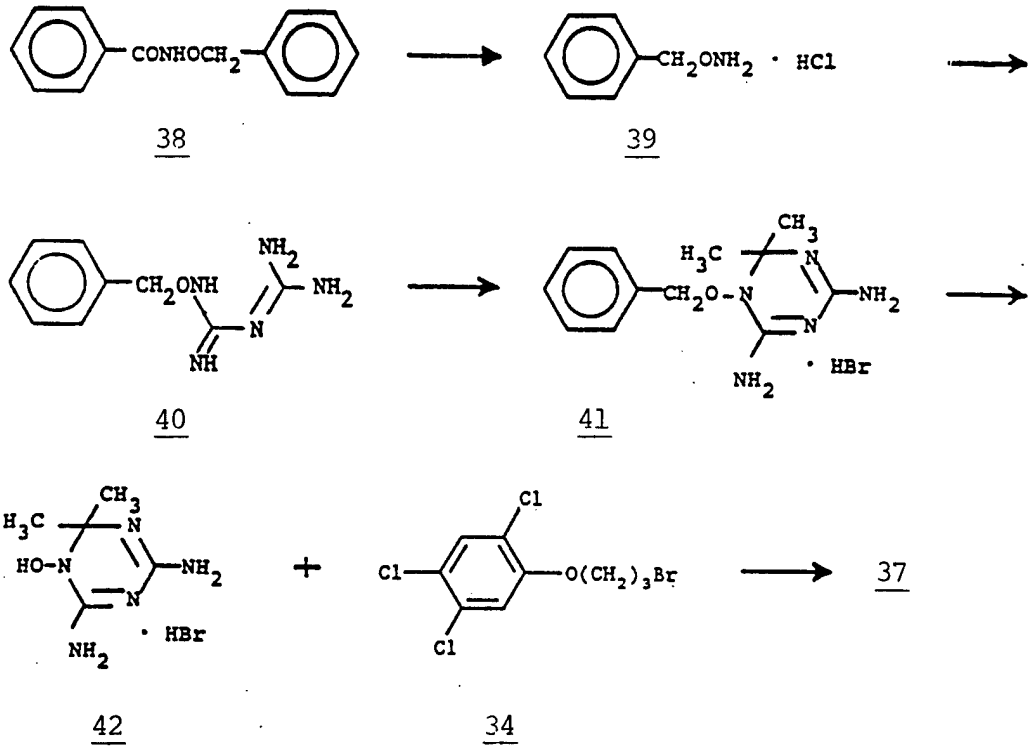
phenol 33 was first reacted with dibromopropane to give the ether 34 in 73% yield. The ether 34 gave on reaction with N-hydroxyurethane the carbamate ester 35 in quantitative yield. The ester 35 was hydrolyzed with potassium hydroxide to give the azane 36 in 67% yield. The material 36 was then alkylated with 2-propyldicyandiamide (prepared by a literature procedure[8] in 93% yield) to give the target material 1 in 57% yield.

The target compound 37 was prepared by a



37

seven step synthesis as shown below:

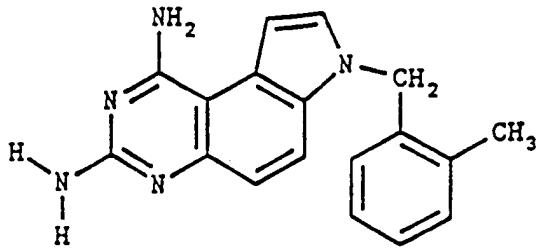


38 39 40 41 42 34 37

Benzhydroxamic acid was converted to N-benzyloxybenzamide 38 in 80% yield by a modified literature procedure[9] using NaOH as the base and methanol as the solvent. The benzamide 38 was hydrolyzed to give benzyloxyamine, hydrochloride 39 in 90% yield. The amine 39 was converted to benzyloxybiguanide (40) in 50% yield. The biguanide 40 was cyclized to 1-benzyloxy-4,6-diamino-1,2-dihydro-2,2-dimethyl-1,3,5-triazine, hydrobromide (41) in 54% yield by modifying the literature method[9], using methanol and HBr. The material 41 was reduced in 75% yield to 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-hydroxy-1,3,5-triazine, hydrobromide (42) using palladium on charcoal as the catalyst[10]. Triazine 42 and 3-(2',4',5'-trichlorophenoxy)propyl bromide (34) were combined to yield 37 in 52% yield by modifying the literature procedure.[9]

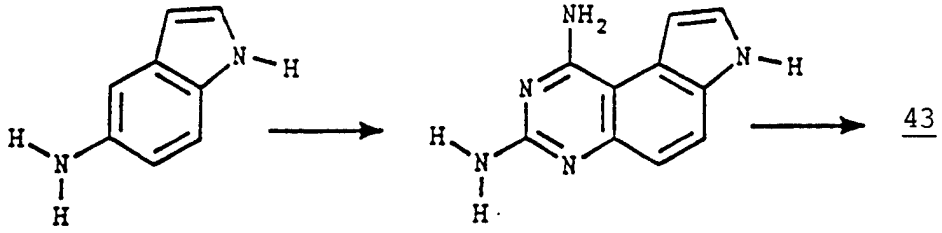
F. Quinazolines and Derivatives

The target compound 1 was



43

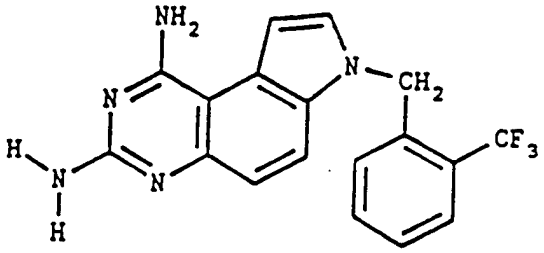
prepared by the following sequence of reactions.[11]



44 45 43

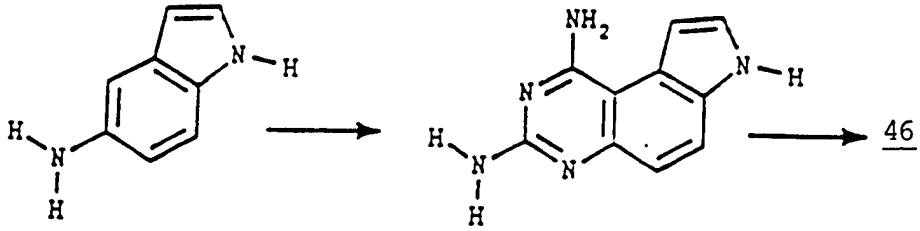
The commercially available 5-aminoindole 44 was converted to its hydrochloride, then reacted with sodium dicyanamide to give the quinazoline 45 in 60% yield. The material 45 was reacted with benzyl chloride to give the target material 43 in 59% yield.

The target compound 43 was



46

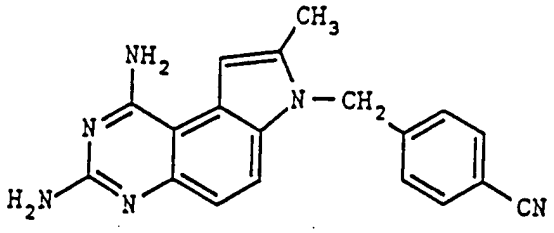
prepared by the following sequence of reactions.[11]



44 45 46

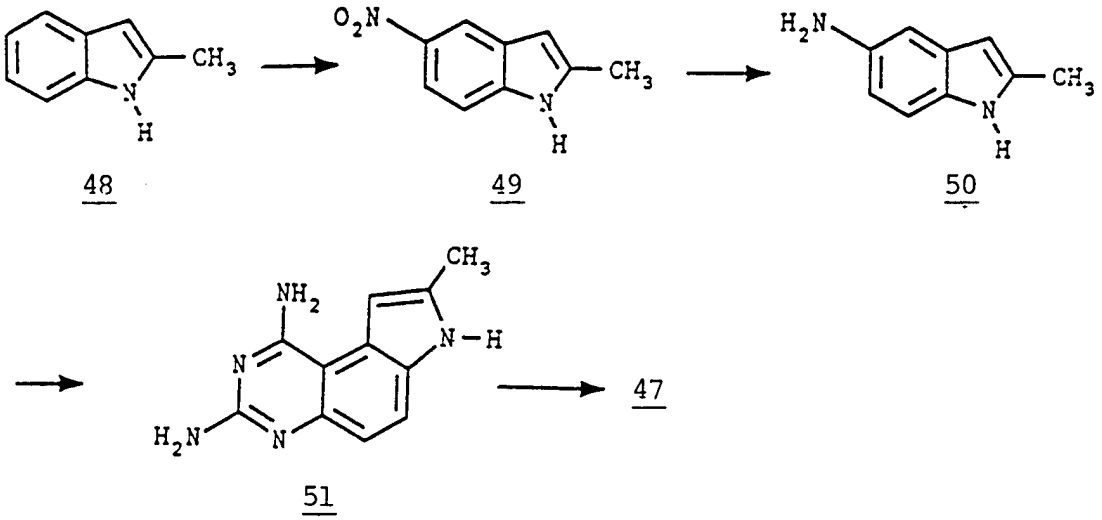
The commercially available 5-aminoindole 44 was converted to its hydrochloride, then reacted with sodium dicyanamide to give the quinazoline 45 in 60% yield. The material 45 was reacted with 2-trifluoromethylbenzyl bromide to give the target material 46 in 68% yield.

The target compound 47 was prepared by the



47

following sequence of reactions.[11]

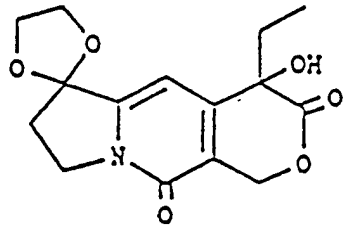


48 49 50 51 47

Nitration of 2-methylindole 48 gave 49 in 82.4% yield. Reduction of 49 with Fe/HOAc gave 50 in 56% yield. This was reacted with sodium dicyanamide to give the quinazoline 51 in 67% yield. Alkylation of 51 with *p*-cyanobenzyl bromide gave crude 47. The material was purified through its acetate salt.

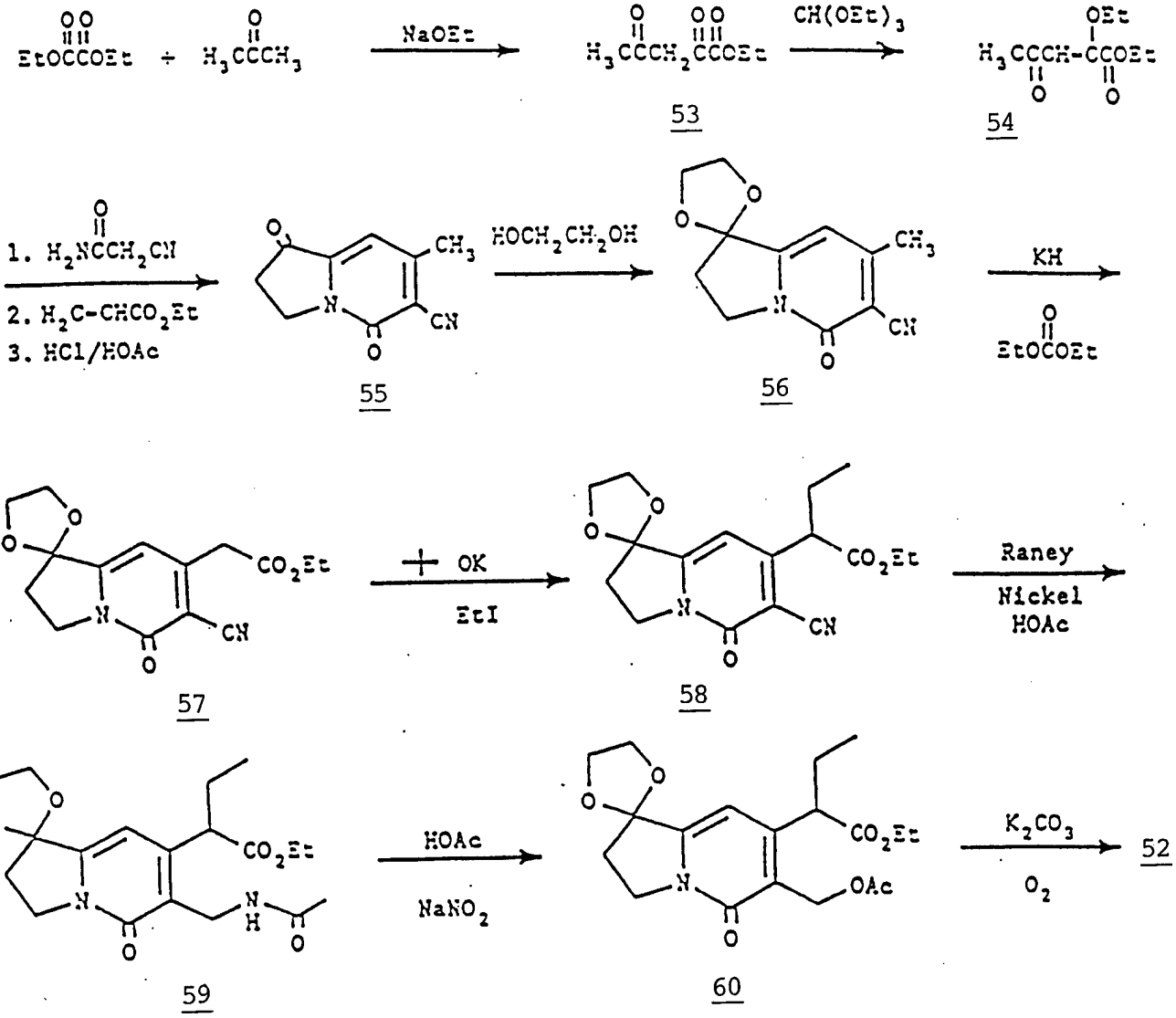
G. Camptothecin Derivatives and Intermediates

The racemic target ketal 52 was prepared

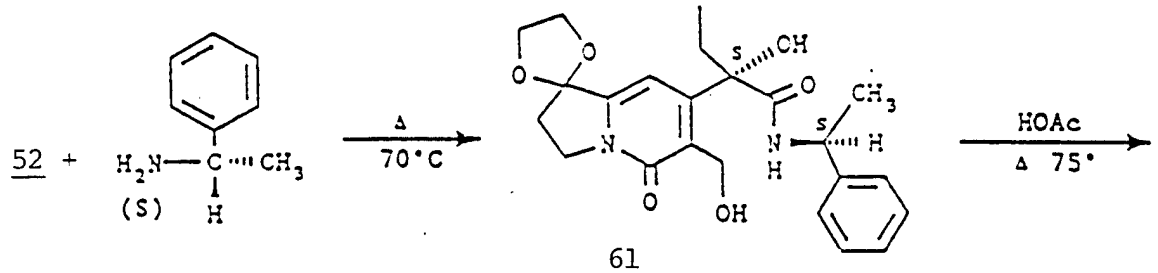


52

by a nine step literature[12-14] procedure shown below.

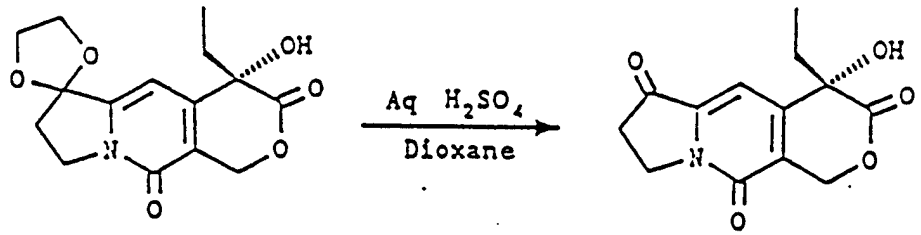


The racemic ketal 52 was reacted with (*S*)-(-)-α-methylbenzylamine to give the (*S*,*S*) and (*R*,*S*)-diastereomers 61 & 61a. These were separated by trituration and crystallization then hydrolyzed yielding optically active 62 and (*R*)-enriched 52. Material 62 was deprotected to give the target compound 63. The three-step sequence was repeated on (*R*)-enriched 52 with (*R*)-(+)-α-methylbenzylamine to give the (*R*,*R*)- and (*S*,*R*)-diastereomers. These were separated then hydrolyzed to give the target material 63a.



61

and (R,S)-Diastereomer 61a

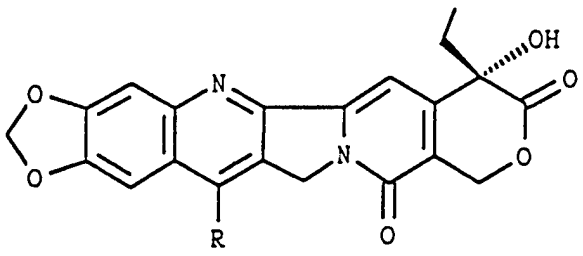


62

63 = S

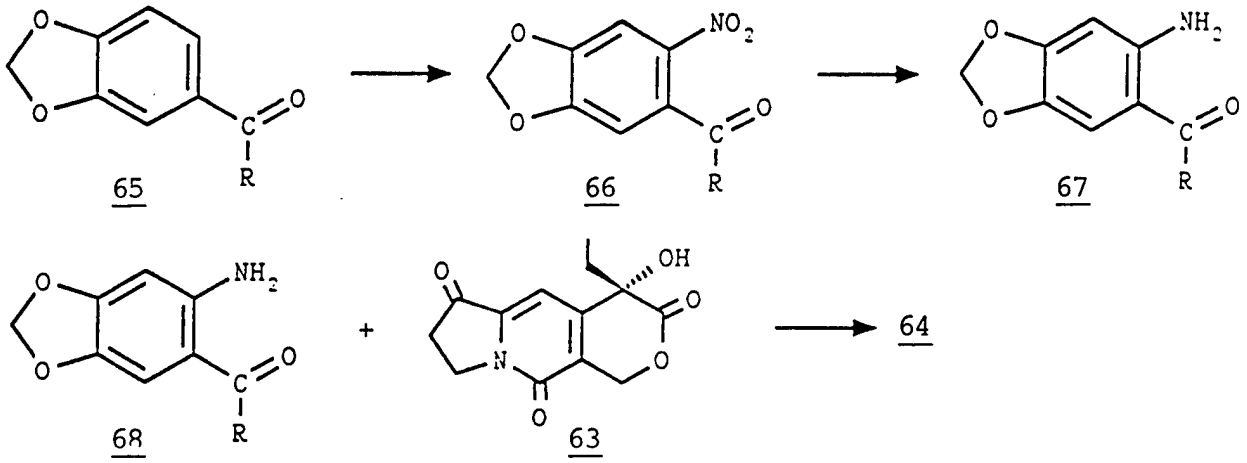
63a = R

The parent compound 64a was prepared[15] by reduction



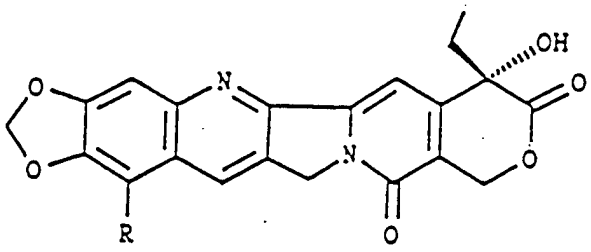
64a R = H
64b R = CH_3
64c R = C_2H_5

(with ferrous sulfate, heptahydrate) of commercially

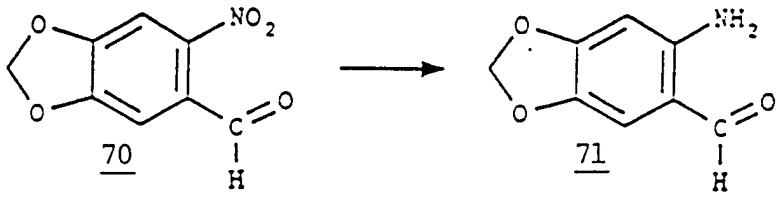


available 66 (R=H) (72.4% yield) followed by condensation with (*S*)-trione 63 (92.5% yield). Camptothecin 64b was prepared by nitration of acetophenone 65 (R=CH_3) (78% yield), followed by reduction with Fe/AcOH (74% yield) and condensation with (*S*)-trione 63. Propiophenone 65 (R=C_2H_5), starting material for 64c, was prepared by the Friedel-Crafts reaction of propionyl chloride on 1,3-benzodioxole in the presence of $SnCl_4$. The remainder of the synthesis was analogous to that for 64b.

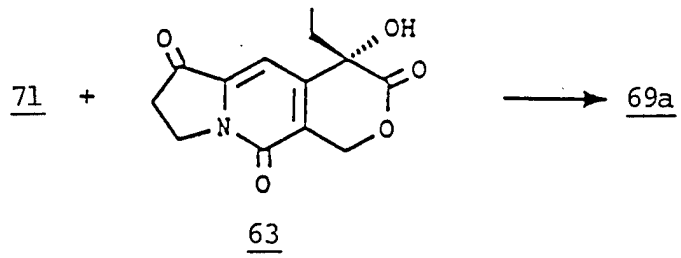
The parent compound 69a was prepared[15] by reduction (with ferrous sulfate, heptahydrate) of commercially available 70 (R=H) (72.4% yield) followed by condensation



69a : R = H
69b : R = NO_2
69c : R = NH_2



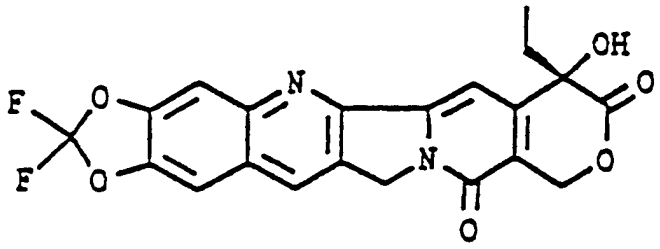
70 71



71 + 63 → 69a

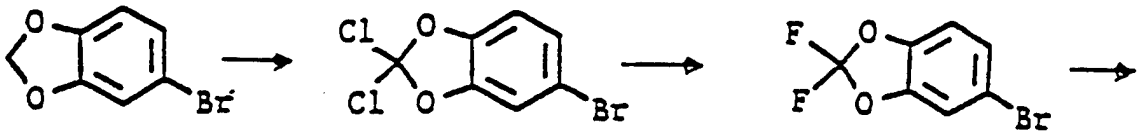
with (*S*)-trione 63 (92.5% yield). Camptothecin (69b) was prepared by nitration of 69a (72.7% yield). Reduction of 69b with stannous chloride gave the target compound 69c, in 52% yield.

The target compound 72 was prepared by the

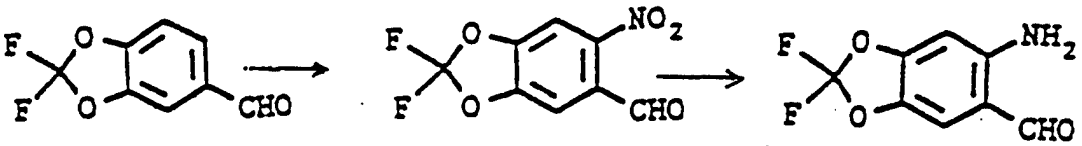


72

following sequence of reactions. Commercially available 3,4-(methylenedioxy)bromobenzene (73) was reacted with phosphorus pentachloride to give the dichloro derivative in 87% yield. Compound 74 gave with antimony trifluoride the difluoro compound 75 in 85% yield.

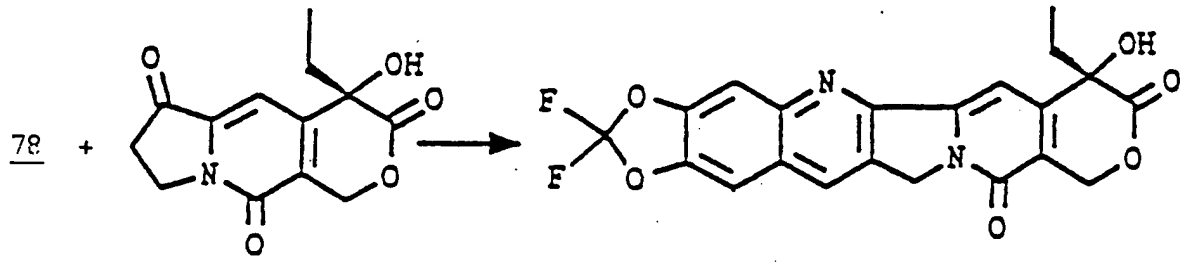


73 74 75



76 77 78

The reaction of 75 with butyllithium and DMF yielded the aldehyde 76 in 59% yield. Preparation of 74,75 & 76 has been described in the literature[16]. Aldehyde 76 was nitrated with 90% nitric acid and trifluoromethanesulfonic acid to give material 77 in 72% yield. The reaction of 77 with ferrous sulfate gave the amino compound 78 in 33% yield. Material 78 was condensed with

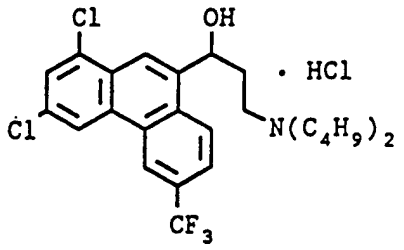


78 + 79 → 72

trione 79 to give the target material 72 in 37% yield. Materials 77,78, & 72 are unknown to the chemical literature.

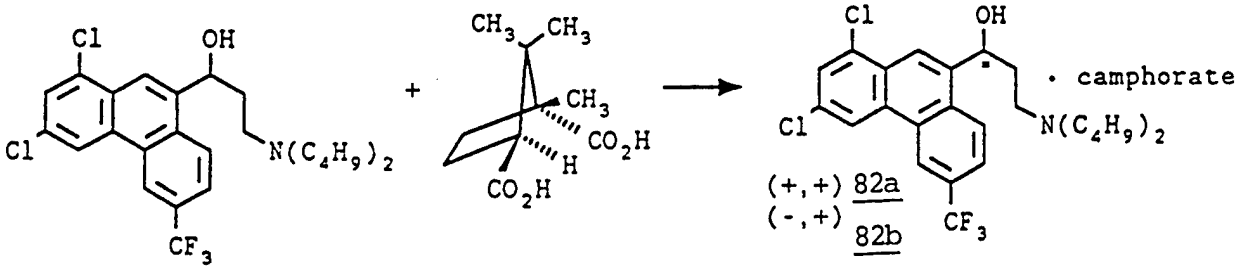
H. Infectious Disease Related Compounds and Intermediates

The racemic material 80[17] was first



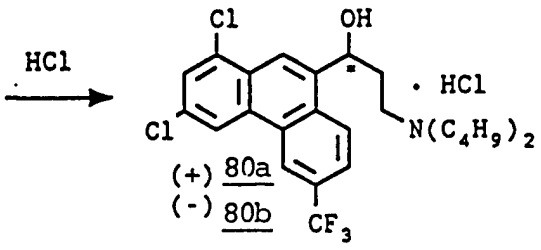
80

converted to its free base 81. This was reacted with (1*R*,3*S*)-(+)-camphoric acid to give a mixture of (+,+) and (-,+)-camphorate salts 82.

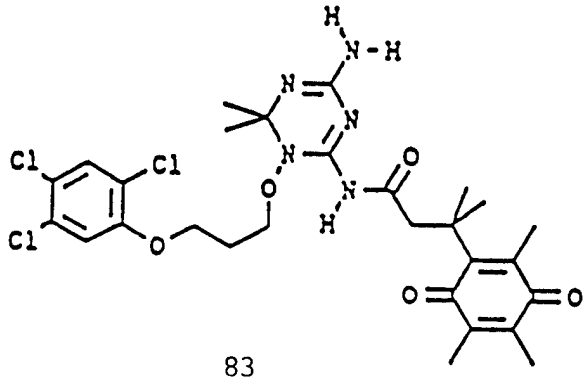


81

Repeated crystallization of these gave pure 82a and 82b. These materials were converted to (+) 80a and (-) 80b, respectively.

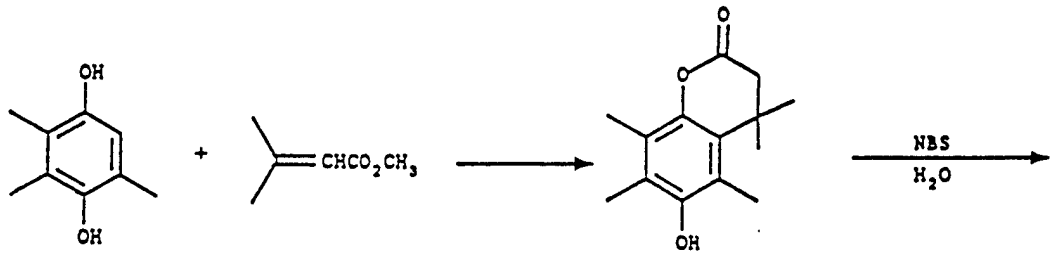


The target compound 83 was prepared

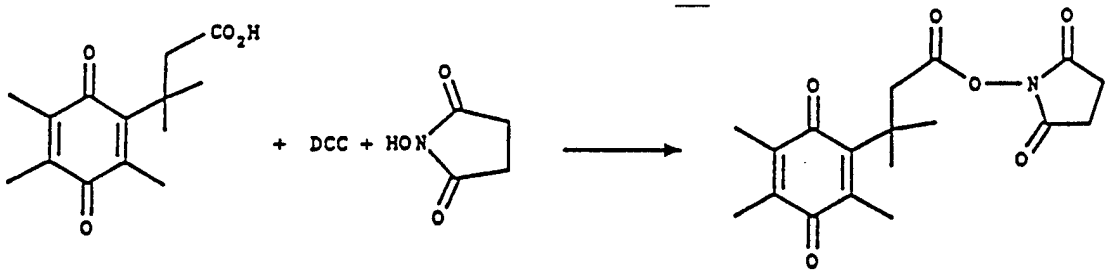


83

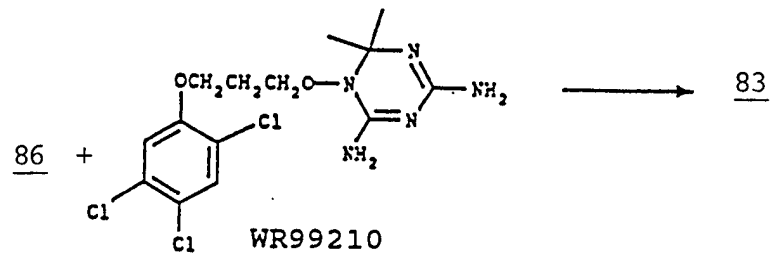
by the following sequence of reactions[11,15].



84



85 86

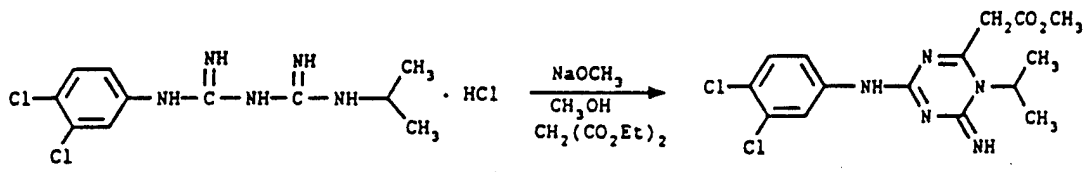


86 + WR99210 → 83

Trimethylhydroquinone was reacted with methyl 3,3-dimethylacrylate to give hydrocoumarin 84 in 56% yield. Reaction of 84 with N-bromosuccinimide in acetonitrile gave the propanoic acid 85 in 69.9% yield. Reaction of 85 with N-hydroxysuccinimide in the presence of DCC gave the succinimidyl ester 86 in 75.8% yield. Reaction of 86 with WR99210 (free base) gave as one of the products the N^6 or N^4 amide. The N^4 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.

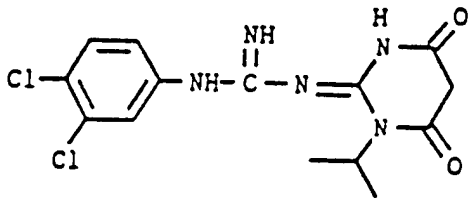
The target compound (tentative structure) was prepared by the following reaction sequence.

Reaction Sequence



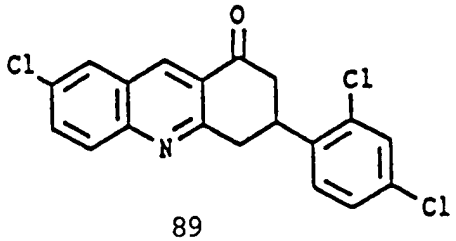
87

The original intent of this reaction sequence was to produce the pyrimidinone 88, however, none of this material could be isolated under these conditions.



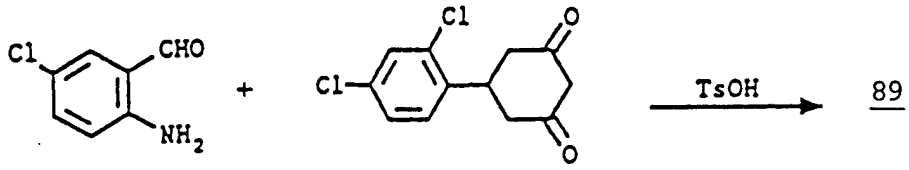
88

The target compound 89 was prepared by



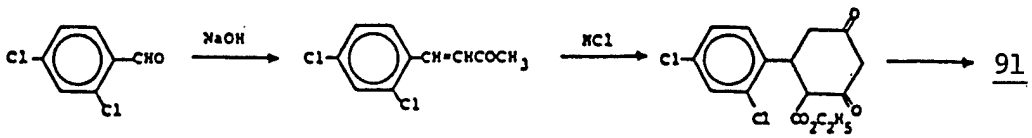
89

reacting 5-chloro-2-aminobenzaldehyde (90) with 5-(2,4-dichlorophenyl)cyclohexane-1,3-dione (91) in the presence of *p*-toluenesulfonic acid.



90 91

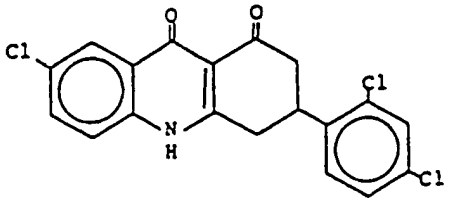
Compound 90 was obtained by reducing the corresponding nitro derivative with ferrous sulfate, heptahydrate. Material 91 was prepared by a three step synthesis shown below.[18] Dichlorobenzaldehyde 92 was reacted



92 93 94

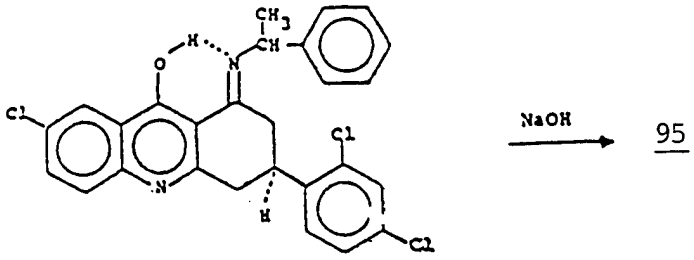
with acetone in the presence of base to give 93 in 67% yield. Reaction of 93 with diethyl malonate in the presence of sodium ethoxide gave 94 in 72% yield. Ester 94 was hydrolyzed and decarboxylated to give 91 in 79% yield.

The target compound 95 was prepared



95

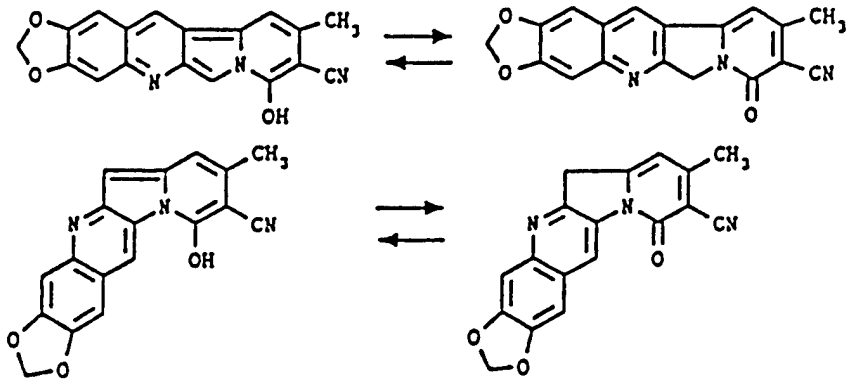
by hydrolyzing 96 with NaOH. Material 96 was



96

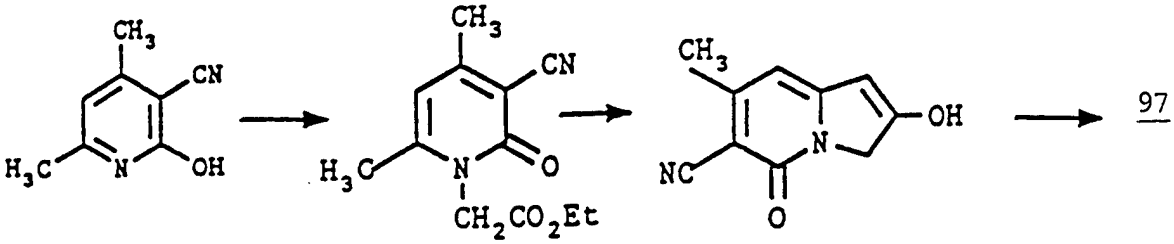
obtained from stock and was previously prepared from 95 and *e*-(-)-1-methylbenzylamine.[19]

Product 97 obtained on condensation of 6-aminopiperonal with compound (100).



Possible structure for 97

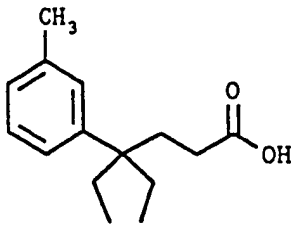
The above material was prepared by the following sequence of reactions. Commercially



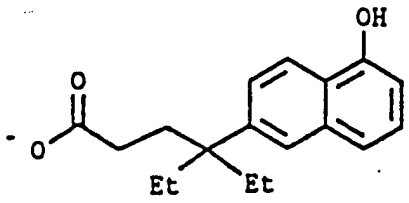
98 99 100

available 98 was reacted with ethyl bromoacetate in the presence of a base to give 99 in 39% yield. The action of sodium hydroxide on 99 gave 100 in 87% yield. Material 100 was condensed with 6-aminopiperonal in the presence of *p*-toluenesulfonic acid to give 97. A portion of this material was transmitted to WRAIR, although the product was not fully characterized.

The material 110b was isolated during an attempted synthesis of model compound 101. This preparation was a model for the synthesis of ligand 09E.

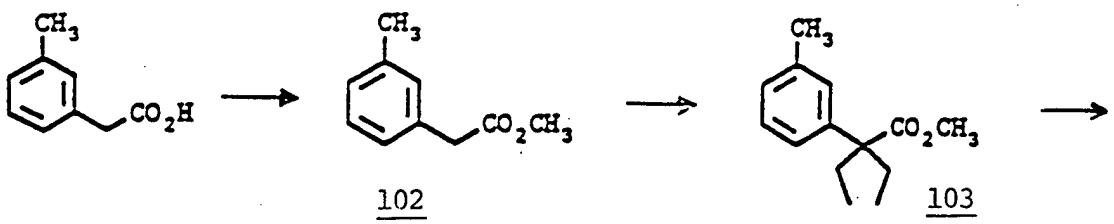


101

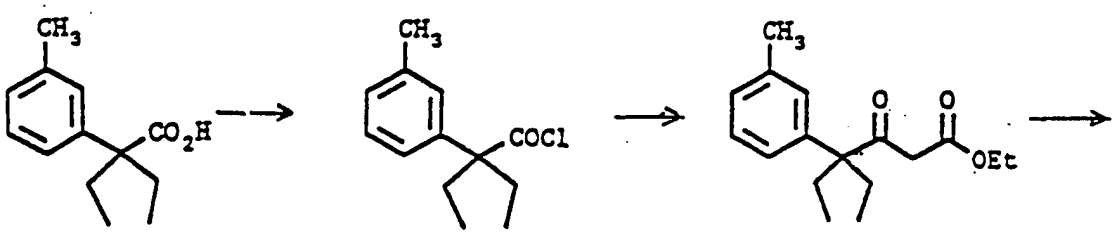


Ligand 09E

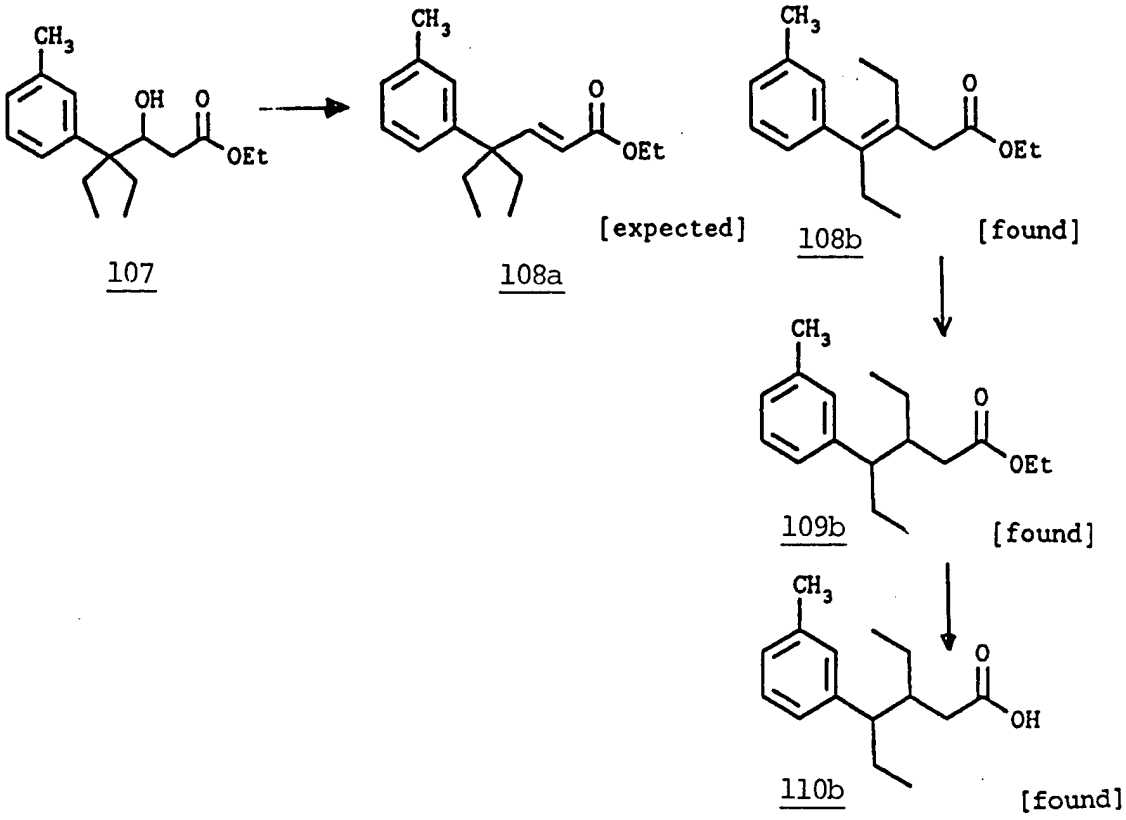
m-Tolylacetic acid was esterified to give the ester 102 in 99% yield. Material 102 was reacted with ethyl iodide to give 103 in 81% yield. The ester 103 was hydrolyzed to afford the acid 104 in 92% yield. The acid was converted to the acid chloride 105 then reacted with monoethyl malonate to give 106 in 40% yield. The ketoester 106 was reduced with sodium borohydride to give the hydroxy compound 107. Dehydration of 107 with *p*-toluene-sulfonic acid did not yield the expected 108a but rather the material 108b. Reduction of 108b to 109b followed by hydrolysis gave the material 110b.



102 103



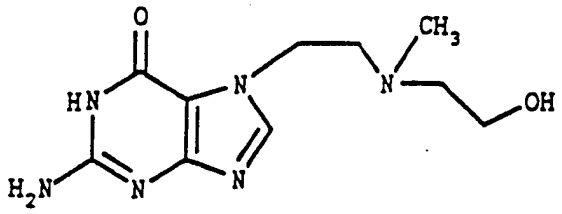
104 105 106



107 108a 108b [found] 109b [found] 110b [found]

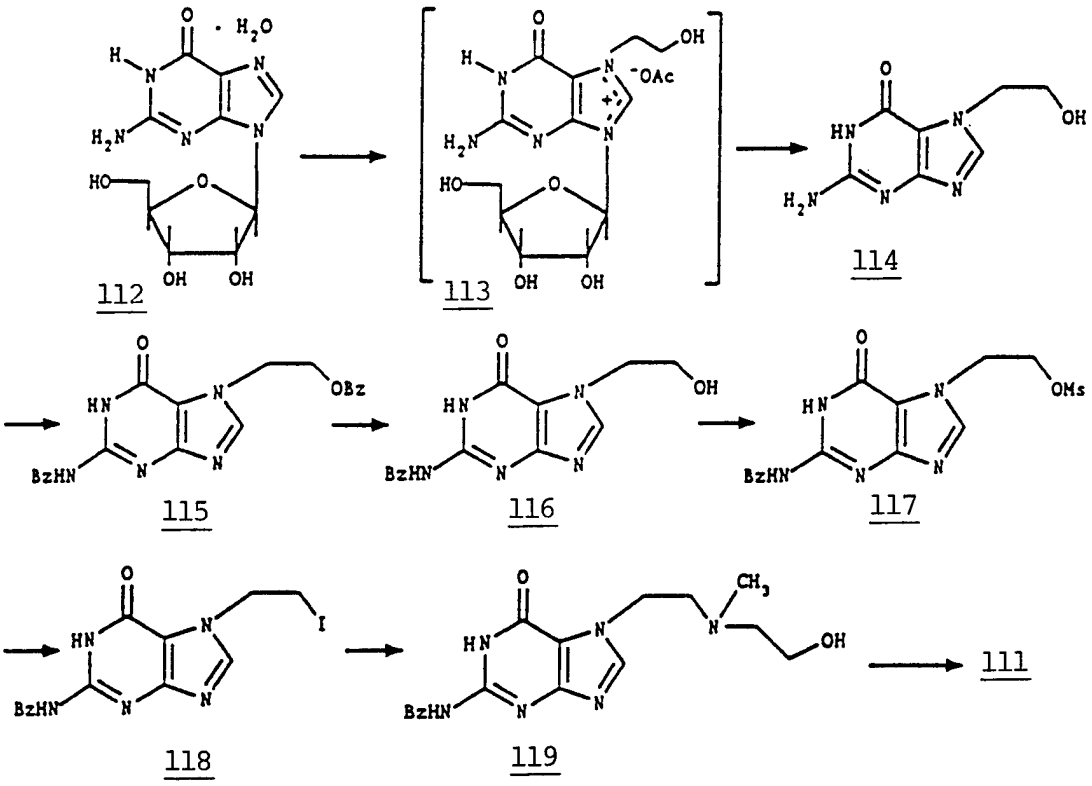
I. Chemical Defense Related Compounds and Intermediates

The material 111 was prepared by the



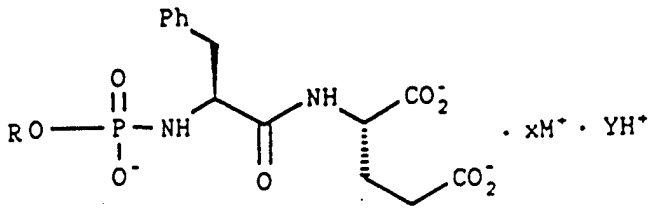
111

following sequence of reactions.[20-22] Guanosine hydrate was



reacted with ethylene oxide in acetic acid and the

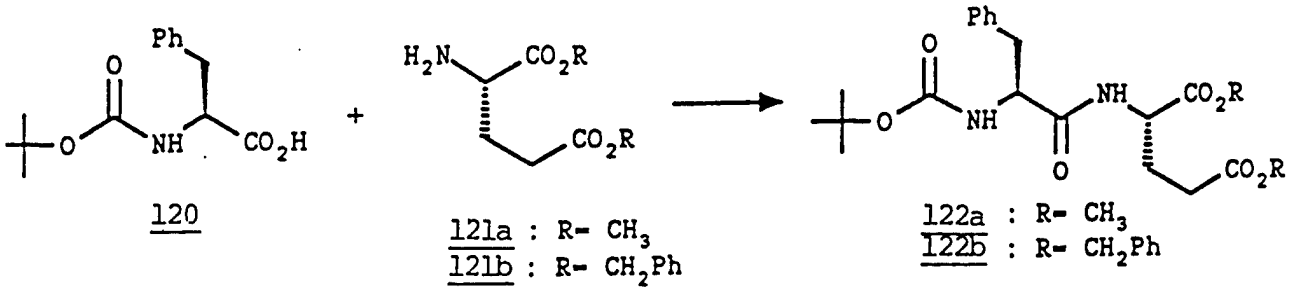
intermediate 113 was immediately hydrolyzed to give 114 in 71% yield. The amino- and hydroxy- functions of 114 were protected by the use of benzoyl cyanide to give 115 in 42% yield, then selectively deprotected to yield 116 in 72% yield. Reaction of 116 with MsCl gave 117, in 88% yield. Material 117, after conversion to the iodide, was reacted with commercially available 2-methylaminoethanol to give 119 in 84% yield. This material was deprotected with sodium methoxide to give crude target material 118, in 62% yield.



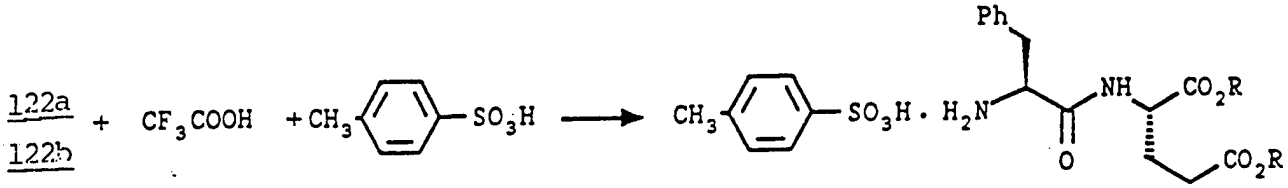
126a : R= CH_3CH_2-, x= 3, M= Li, Y= 0
126b : R= CH_3-, x= 3, M= Li, Y= 0
126c : R= Ph-, x= 2, M= NH_4, Y= 1

Dipeptides 123a and 123b were prepared in 77-91% and 90% yield, respectively, by coupling commercially available *t*-BOC-*L*-phenylalanine (120) with *L*-glutamic acid, dimethyl ester (121a) or dibenzyl ester (121b), using HBTU[23], followed by removal of the *t*-butoxycarbonyl protecting group with trifluoroacetic acid to give 123a and 123b as tosylate salts[24] (98% yield). Intermediates 125a and 125b were prepared in 26% and 18% yield by phosphorylation of 123a with chlorophosphates 124a and 124b, respectively. The protected phosphorylated dipeptides were hydrolyzed with 1.5M LiOH to give 126a and 126b as trilithium salts (88% and 92% yield)[25], containing excess LiCl and H_2O.

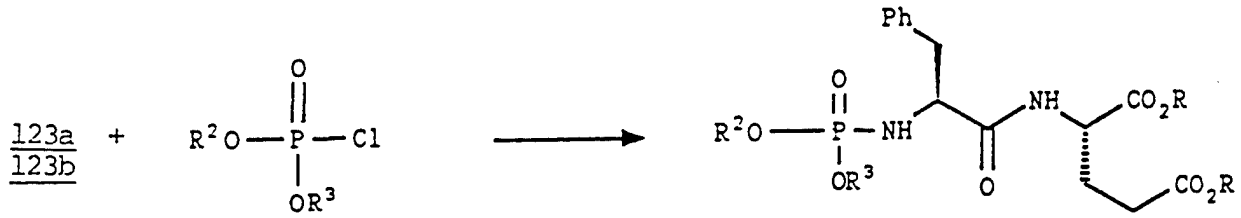
Intermediate 125c was prepared in 23% yield by phosphorylation of 123b with chlorophosphate 124c. The tribenzyl protected phosphorylated dipeptide was debenzylated by transfer hydrogenation[26] with 10% palladium on carbon, employing ammonium formate as the source of hydrogen, to give 126c as a diammonium salt (76% yield), containing excess H_2O.



120 + 121a : R- CH_3 / 121b : R- CH_2Ph → 122a : R- CH_3 / 122b : R- CH_2Ph

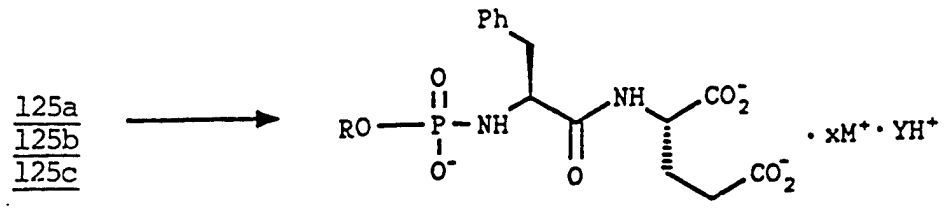


122a / 122b + CF_3COOH + CH_3-Ph-SO_3H → 123a : R- CH_3 / 123b : R- CH_2Ph



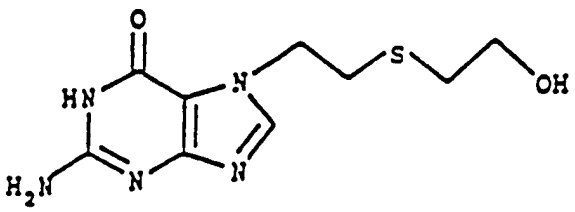
123a / 123b + $R^2O-P(=O)(OR^3)-Cl$ → 125a : R- CH_3, R^2- Et, R^3- Ph / 125b : R- CH_3, R^2- CH_3, R^3- Ph / 125c : R- R^3- CH_2Ph, R^2- Ph

124a : R^2- Et, R^3- Ph
124b : R^2- CH_3, R^3- Ph
124c : R^2- Ph, R^3- $PhCH_2$



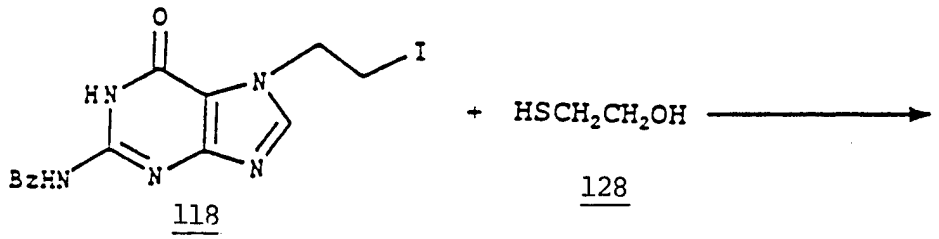
125a / 125b / 125c → 126a - R- Et, x- 3, M- Li, Y- 0
126b - R- CH_3, x- 3, M- Li, Y- 0
126c - R- Ph, x- 2, M- NH_4, Y- 1

The target compound 127 was prepared



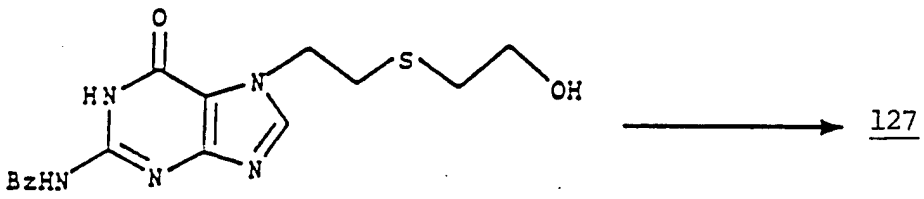
127

from iodoethylpurinone 118 (prepared previously, please see p.35 this report) and 2-mercaptoethanol (128) using CH_3ONa as the base to give 129 in 94% yield.



118

128

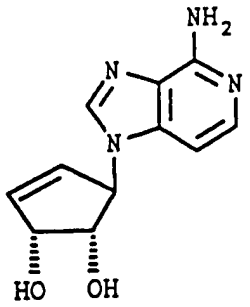


129

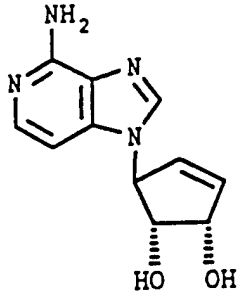
Material 129 was deprotected with boiling sodium methoxide in CH_3OH to yield the target material in 99% yield.

J. Carbocyclic Nuclesides

The enantiomeric carbocyclic nucleosides 130 and 131

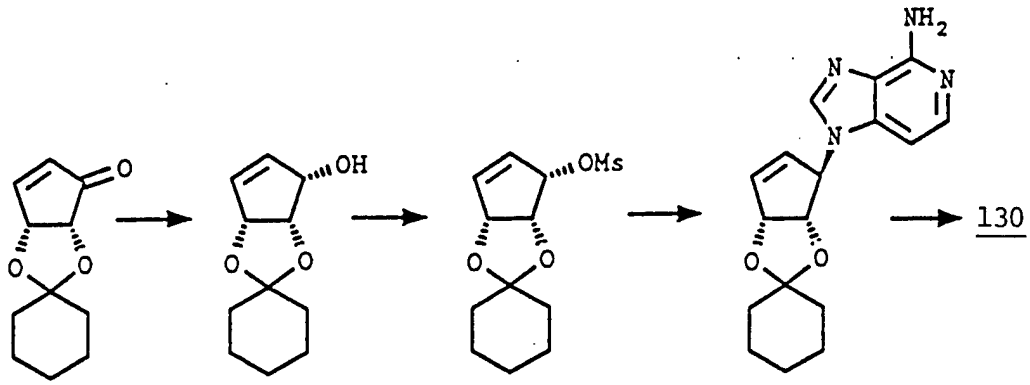


130



131

have been prepared from the enantiomeric cyclopentenone derivatives (132) as shown below:

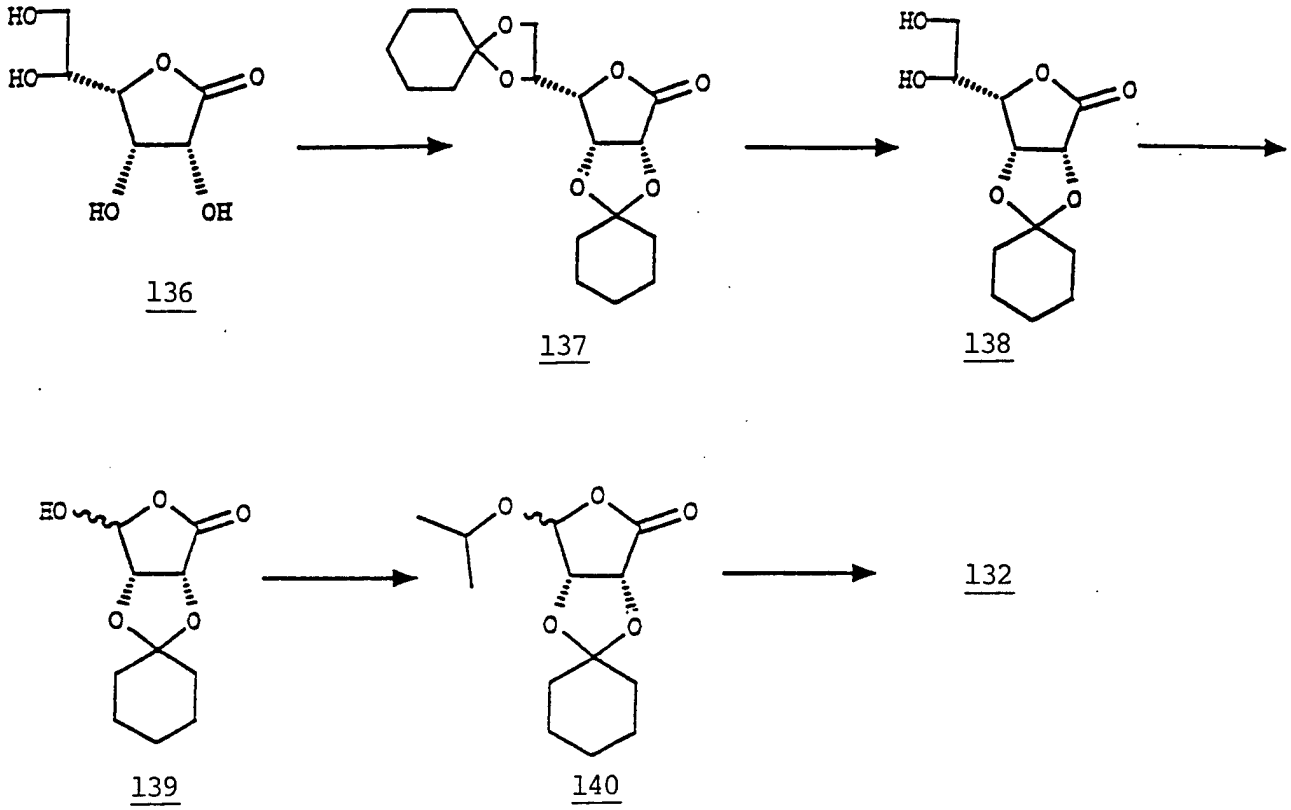


132 133 134 135 130

Compound 132 was synthesized by following a literature[27] preparative procedure.

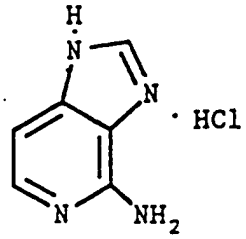
The enone 132 was reduced with $NaBH_4$ in the presence of $CeCl_3$ to give 133 in 80% yield. This was reacted with MsCl to give 134 in quantitative yield. Material 134 was condensed with deazaadenine, prepared in a seven step synthesis described previously[28], to give 135 in 70% yield. Removal of the protecting group from 138 gave the target material 130 in 90% yield. The material was transmitted to WRAIR.

Cyclopentenone 132 was prepared[27,29] from *D*-gulonolactone 136 as follows:



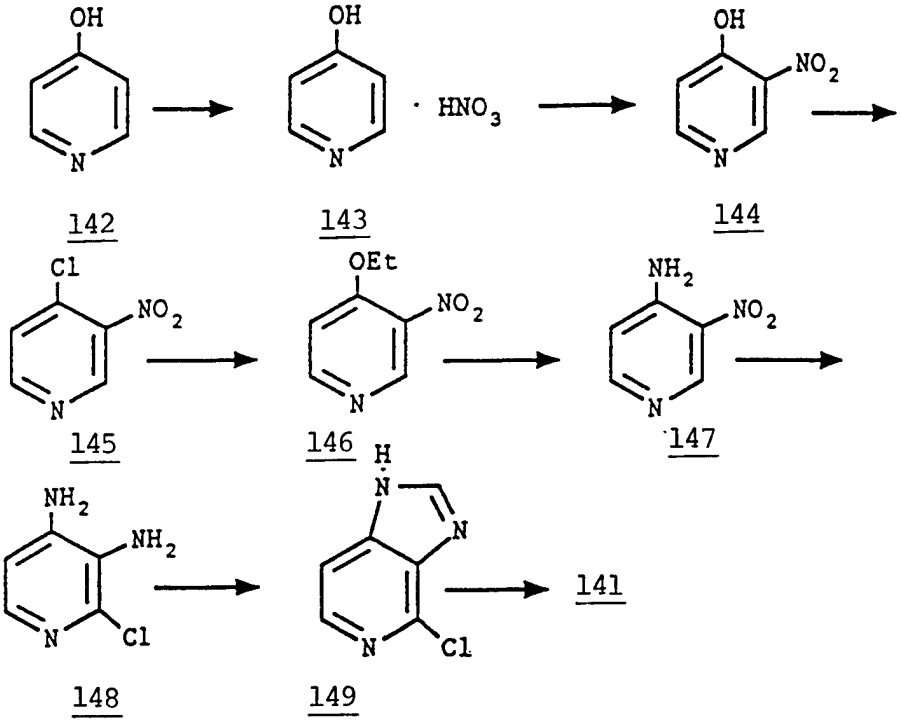
Compound 131 was prepared in a similar way starting from the enantiomeric cyclopentenone ((+)-132), which was prepared from *L*-gulonolactone.

The target compound 141 was prepared by



141

the following sequence of reactions.[30a,30b] Commercially



available 4-hydroxypyridine (142) was first converted to nitrate salt 143 in 100% yield. The nitrate salt was transformed to nitropyridine 144 in 71% yield using fuming HNO_3 and H_2SO_4. The hydroxy function of 144 was chlorinated with PCl_5 and the chloropyridine 145 was not isolated but immediately converted to more stable ethoxypyridine 146. The

yield over two steps was 93%. The ethoxy group of 146 was converted to amino function of 147 with NH_4OAc in 91% yield. Reduction of 146 with $SnCl_2$ gave diaminopyridine 148 in 84% yield which was cyclized with triethyl orthoformate to give 149 in 90% yield. The chloroimidazopyridine 149 was reacted with hydrazine, and the hydrazide reduced with RaNi to give the target compound 141.

2. Quality Control

The purity of all target compounds and intermediates are rigorously checked by a series of physical and chemical tests. The following information is routinely recorded for all materials:

1. elemental analysis
2. IR, UV, high resolution NMR and mass spectra
3. melting or boiling points
4. thin layer or vapor phase chromatograms, HPLC
5. solubilities
6. yields

Lot numbers and suppliers are recorded for raw materials employed in the preparation of all compounds. When indicated, storage stability of materials is determined along with tests for air, heat, and moisture sensitive compounds.

Detailed records are kept for the synthesis of each material. These include the number of runs, the size of each batch, quantities of solvents used in the reaction and for purification, the yields obtained in each run, and procedures used for the work-up. A reference sample for each transmitted material is retained in our laboratory.

3. Purity Aspects

To avoid particulate matter contamination of any synthesized material a program of precautions has been instituted for the laboratory and the bench chemist. The precautionary measures comprise:

a. periodic cleaning of walls, ceilings, windows, and hoods
b. daily cleaning of floors
c. daily trash removal
d. weekly polishing of asphalt floor tiles
e. exhaust vents in hoods
f. the use of gloss paint
g. filtration of solvents and reagents
h. cleanliness of reaction vessels and other equipment
i. suitable coverings to protect reaction mixtures
j. protection of openings in reaction apparatus
k. regular cleaning of bench areas and all working areas.

The chemical purity of all materials is determined by a series of tests discussed in the previous section under quality control.

4. Pilot Work

Three studies are made by the bench chemist for each compound assignment:

Run I - To determine that the literature method will operate as described

Run II - Alterations are made to permit scale-up

Run III - Scale-up run made to achieve eventual full scale use of 100-gallon Pfaudler, if necessary.

The pilot area, which contains a twenty-, fifty-, and hundred-gallon Pfaudler reactor, large capacity vacuum oven, efficient flash evaporator, and a facility for 50 and 72 liter reactions, has been used during the contract period. In the scale-up syntheses of IND materials, quantities of intermediates had to be synthesized in order to prepare kilograms of the final compound.

5. IND Approach

The synthesis of kilogram quantities of materials designed for preclinical and clinical trials requires precise development of each step of the reaction sequence, rigorous quality control on all intermediates, and an extensive record gathering on all phases of the production.

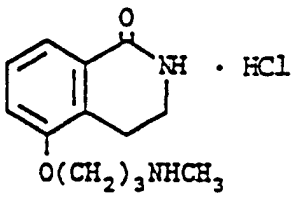
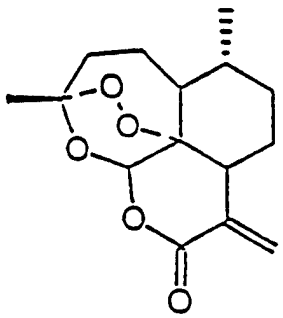
It is often necessary to modify existing synthetic routes or to develop new synthetic procedures. These innovations are usually required to make the large scale synthesis of IND materials feasible and safe.

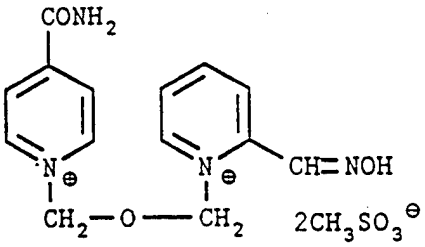
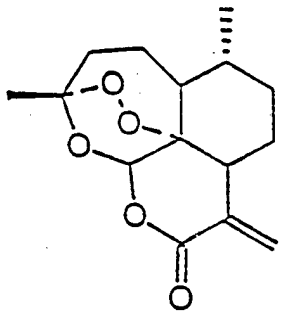
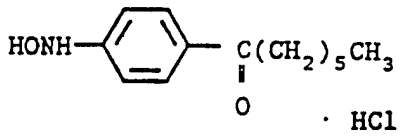
Each step of the reaction sequence is monitored closely by the quality control procedures described in section 2.

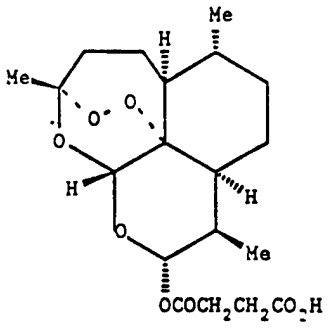
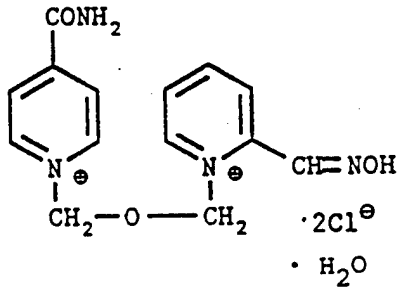
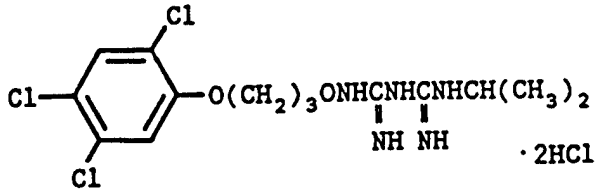
The gathered information is recorded in a table form for each phase of a target compound preparation. Each table includes the number of runs, the size and the amount of materials used in each run, the yields of crude and purified products along with other pertinent data such as lot numbers for all raw materials and detailed discussion of reaction conditions.

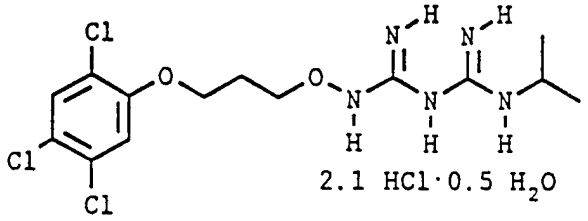
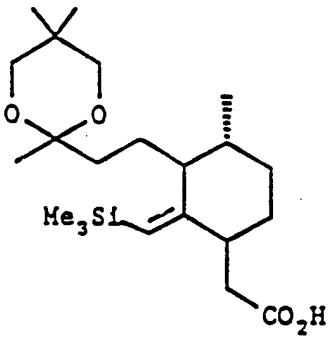
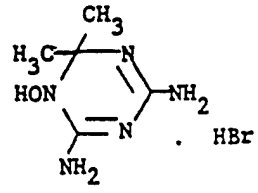
IV. CUMULATIVE LIST OF REQUESTED COMPOUNDS DELIVERED TO WALTER REED ARMY INSTITUTE OF RESEARCH (WRAIR) FROM DECEMBER 1, 1992 TO MARCH 31, 1999

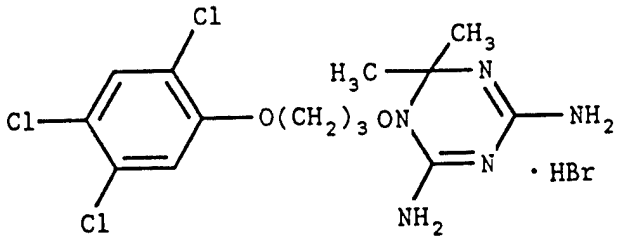
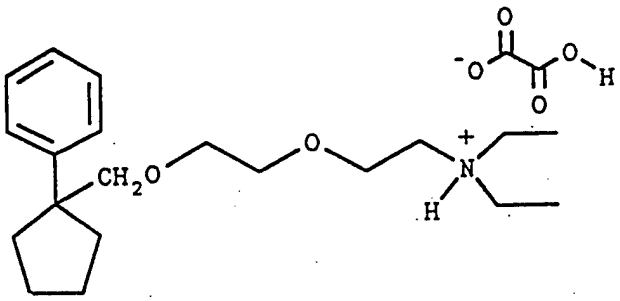
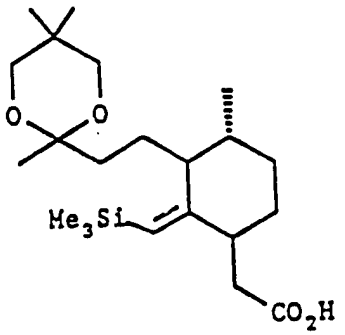
The previous Cumulative List covering the period from March 15, 1989 to November 30, 1992 may be found in Starks Associates, Inc. Final Report dated November 30, 1992, page 35, Contract No. DAMD-17-89-C-9058. The list covering the period from September 15, 1983 to March 14, 1989 may be found in Starks Associates, Inc. Final Report dated March 14, 1989, page 55, Contract No. DAMD17-83-C-3206. The list covering the period from September 29, 1979 to September 14, 1983 may be found in Starks Associates, Inc. Final Summary Report dated September 1983, page 56, Contract No. DAMD17-79-C-9170. The list covering the period from July 1, 1973 to September 28, 1979 may be found in Starks Associates, Inc. Final Summary Report dated September 1979, page 82, Contract No. DAMD17-73-C-3159. The list covering the period from July 1, 1965 to June 30, 1973 may be found in Starks Associates, Inc. Final Summary Report dated June 1973, page 54, Contract No. DA49-193-MD-2751.

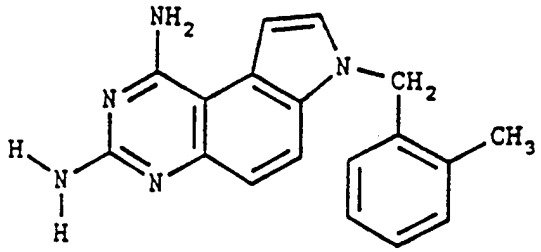
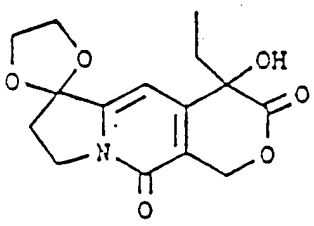
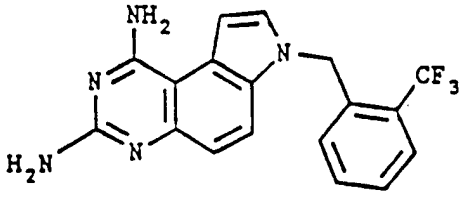
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1147	3,4-Dihydro-5-[3-(methylamino)propoxy]-1-(2*H*)-isoquinolinone, monohydrochloride	202.6 g	BM15465	276,504	111
					
1148	Artemisitene	2.2 g	BM16024	273,873	111

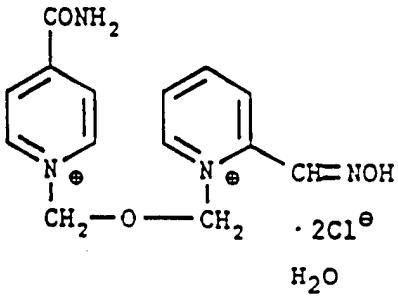
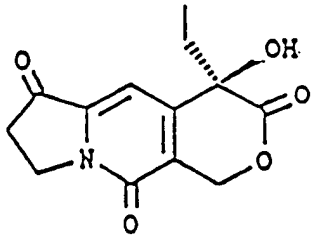
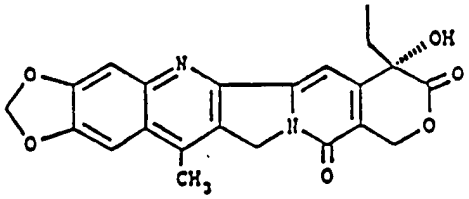
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1149	1-(2-Hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane, dimethanesulfonate	12.0 g	BM16659	249,655	112
					
1150	Artemisitene	15.2 g	BM16695	273,873	112
					
1152	*p*-Hydroxylamino-heptanophenone, hydrochloride	841.2 g	BM17076	272,677	112

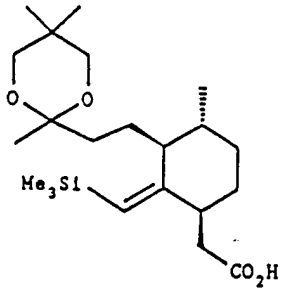
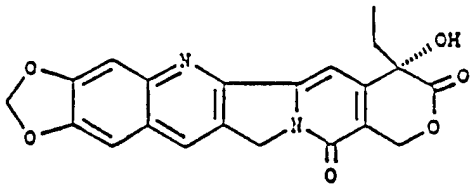
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1153	α-Artesunic acid	102.0 g	BM17174	256,283	112
					
1154	1-(2-Hydroxyiminomethyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride, monohydrate (HI-6)	1023.5 g	BM17567	249,655	112
					
1156	1-[γ-(2',4',5'-Trichlorophenoxy)propyloxy]-5-(2-propyl)-biguanide, dihydrochloride	2.4 g	BN34278	250,417	114

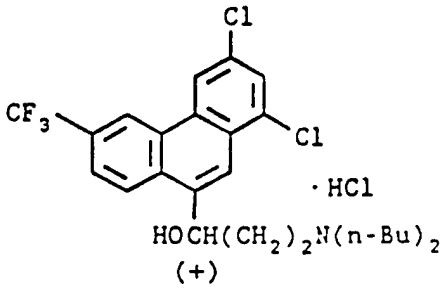
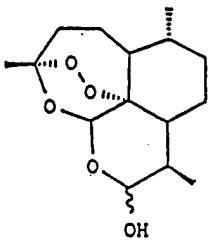
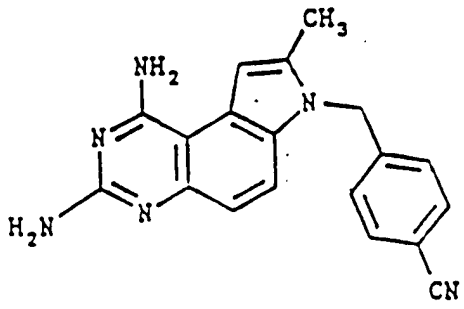
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
	 2.1 HCl·0.5 H_2O				
1157	1-[γ-(2',4',5'-Trichlorophenoxy)-propyloxy]-5-(2-propyl)biguanide, 2.1 HCl, 0.5 H_2O	65.9 g	BN35275	250,417	115
					
1158	2,5,5-Trimethyl-2-[2'-(4"-carboxymethyl-1"-*R*-methyl-3"-trimethylsilylmethylene-cyclohex-2"-yl)ethyl]-1,3-dioxane	101.2 g	BN36638	268,384	116
					
1159	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-hydroxy-1,3,5-triazine, hydrobromide	135.5 g	BN37420	99,152	116

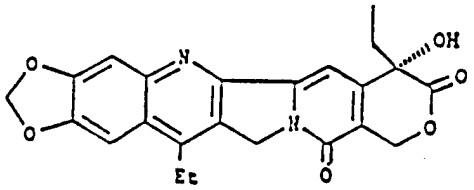
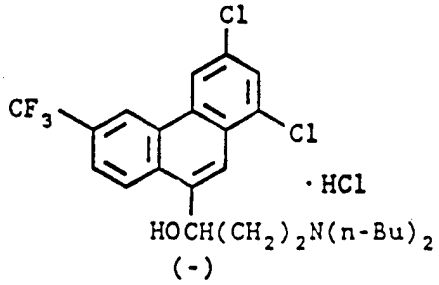
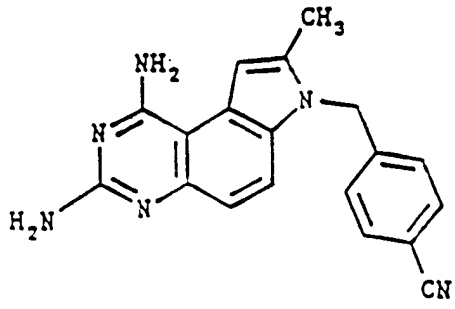
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1160	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichlorophenoxy)propyloxy]-1,3,5-triazine, hydrobromide	110.5 g	BN37859	99,210	116
					
1162	O-[2-[2-(Diethylamino)ethoxy]ethyl]-1-phenyl-1-cyclopentanemethanol, oxalate	7.7g	BN39086	279,852	117
					
1163	2,5,5-Trimethyl-2-[2'-(4"-carboxymethyl-1"-*R*-methyl-3"-trimethylsilylmethylene-cyclohex-2"-yl)ethyl]-1,3-dioxane	122.3 g	BN39844	268,384	117

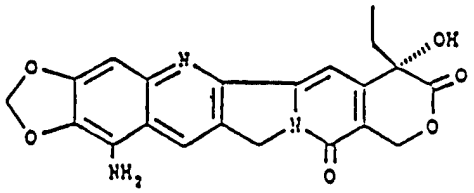
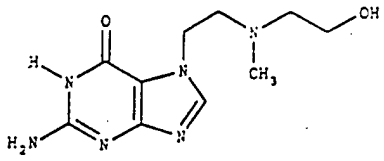
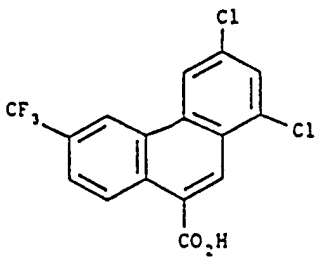
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1165	7-(2-Methylbenzyl)-7*H*-pyrrolo[3,2-*f*]-quinazoline-1,3-diamine	27.4 g	BN42583	228,275	118
					
1166	(*RS*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]indolizine-3,10(6*H*)-dione	21.4 g	BN42976	280,063	119
					
1167	7-[(2-Trifluoromethyl-phenyl)methyl]-7*H*-pyrrolo[3,2-*f*]-quinazoline-1,3-diamine	28.6 g	BN43115	227,825	119

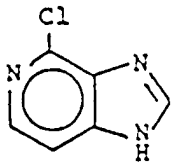
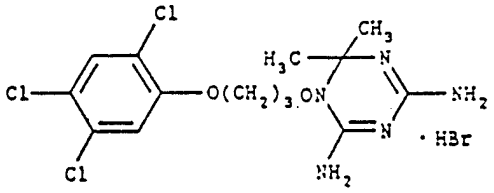
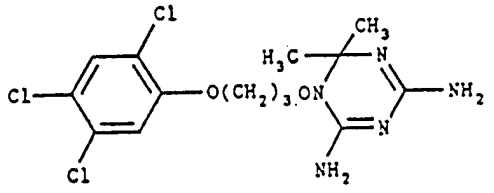
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1168	1-(2-Hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxa-propane dichloride, monohydrate (HI-6)	1232.0 g	BN44621	249,655	119
					
1174	(*R*)-4-Ethyl-1,4,7,8-tetrahydroxypyrano-[3,4-*f*]indolizine-3,6,10-trione	44.6 g	BN45011	280,154	119
					
1177	(*S*)-7-Ethyl-7-hydroxy-14-methyl-10*H*-1,3-dioxolo-[4,5-*g*]pyrano-[3',4':6,7]-indolizino[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione syn.: 7-Methyl-10,11-(ethylenedioxy)-20(*S*)-camptothecin	5.9 g	BN45913	279,781	120

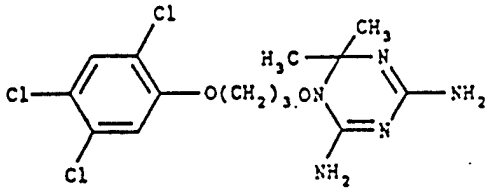
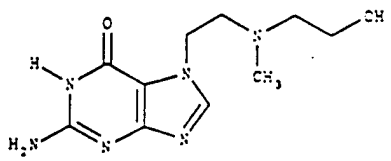
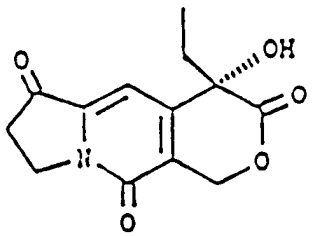
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1178	[1*S*-(1α,2Z,3α,4β)]-4-Methyl-3-[2-(2,5,5-trimethyl-1,3-dioxan-2-yl)ethyl]-2-[(trifluoromethylsilyl)-methylene]cyclohexane-acetic acid	33.8 g	BN45904	268,384	120
					
1180	(*S*)-7-Ethyl-7-hydroxy-10*H*-1,3-dioxolo[4,5-*g*]-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione syn.: 10,11-(Methylenedioxy)-20(*S*)-camptothecin	5.2 g	BN46643	279,775	120

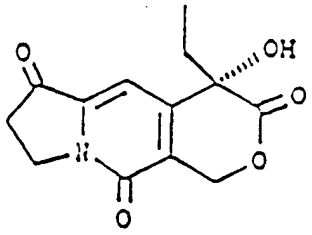
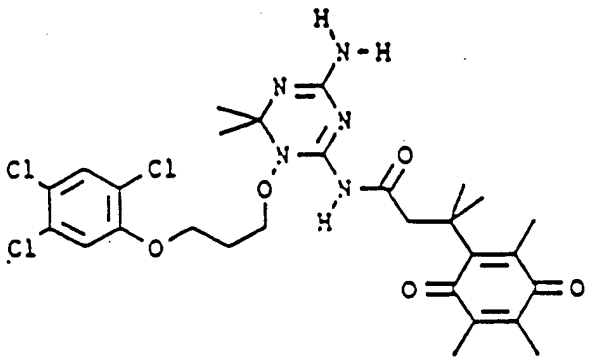
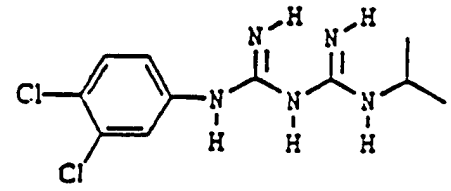
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1181	(+)-1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(dibutylamino)-propyl]phenanthrene, hydrochloride	1.2 g	BN46125	216,062	120
					
1182	Dihydroartemisinin	174.1 g	BN46116	253,997	120
					
1186	7-[(4-Cyanophenyl)-methyl]-8-methyl-7*H*-pyrrolo-[3,2-*f*]quinazoline-1,3-diamine	6.7 g	BN46661	232,155	120

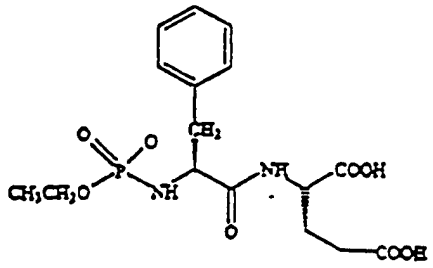
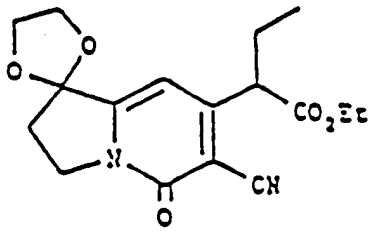
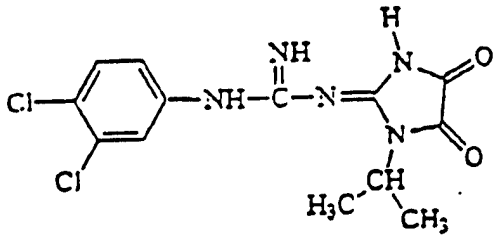
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1190	(*S*)-7,14-Diethyl-7-hydroxy-10*H*-1,3-dioxolo[4,5-*g*]-pyrano[3',4':6,7]indo-lizino[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione syn.: 7-Ethyl-10,11-(methylenedioxy)-20(*S*)-camptothecin	5.6 g	BN47060	279,778	120
					
1191	(-)-1,3-Dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(dibutyl-amino)propyl]phenan-threne, hydro-chloride	1.7 g	BN47051	216,063	120
					
1192	7-[(4-Cyanophenyl)-methyl]-8-methyl-7*H*-pyrrolo[3,2-*f*]quin-azoline-1,3-diamine	27.0 g	BN48816	232,155	120

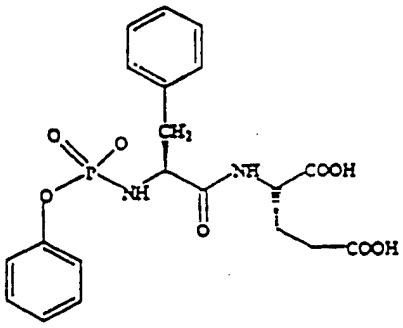
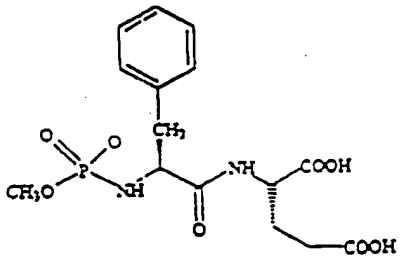
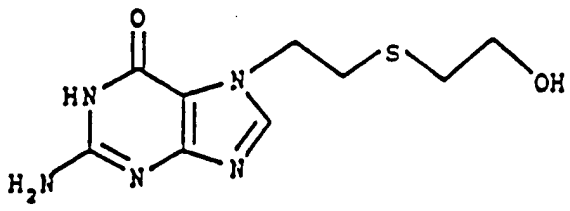
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1194	(*S*)-15-Amino-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo-[4,5-*g*]pyrano[3',4':6,7]-indolizino[1,2-*b*]quinoline-8,11(7*H*,13*H*)-dione syn.: 9-Amino-10,11-(methylenedioxy)-20(*S*)-camptothecin	4.8 g	BN57628	279,776	121
					
1200	N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)-ethyl]methylamine	50 mg	BN58269	280,419	121
					
1201	1,3-Dichloro-6-trifluoromethyl-9-phenanthroic acid	31.0 g	BN58349	150,238	122

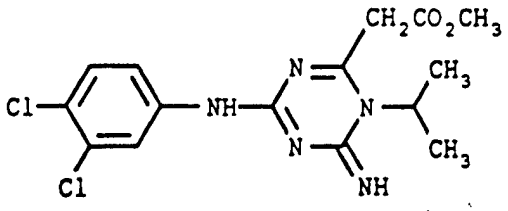
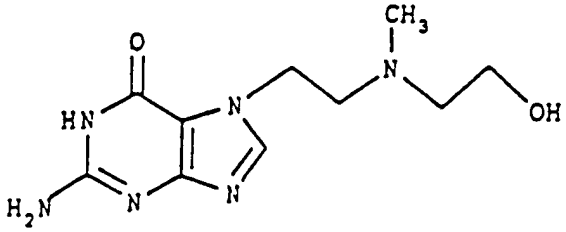
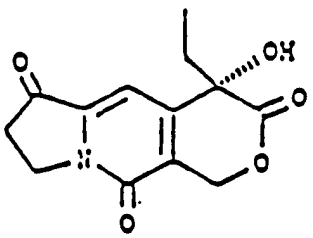
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1202	4-Chloroimidazo-[4,5-*c*]pyridine	200 mg			122
					
1203	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichlorophenoxy)-propyloxy]-1,3-5-triazine, hydrobromide	103.0 g	BN62174	99,210	122
					
1204	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichlorophenoxy)-propyloxy]-1,3,5-triazine (unpurified lot)	28.1 g	BN62585	99,210	122

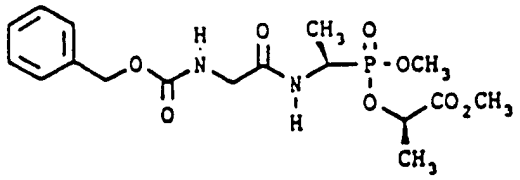
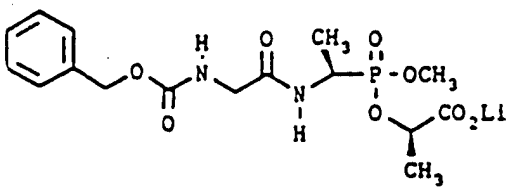
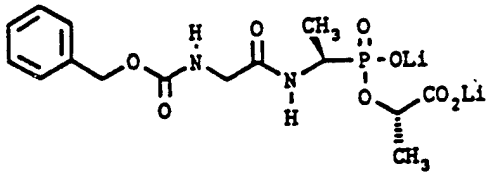
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1205	4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[γ-(2',4',5'-trichlorophenoxy)-propyloxy]-1,3,5-triazine (purified lot)	20.5 g	BN62594	99,210	122
					
1206	N-(2-Hydroxyethyl)-N-[(2-(7-guaninyl)ethyl]-methylamine	50 mg	BN63448	280,419	122
					
1207	(*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano-[3,4-*f*]indolizine-3,6,10-trione	7.2 g	BN63591	280,463	122

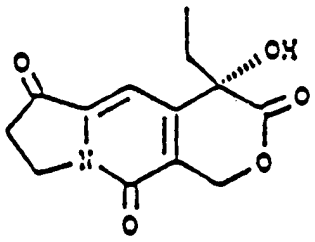
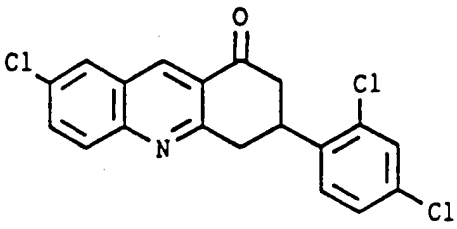
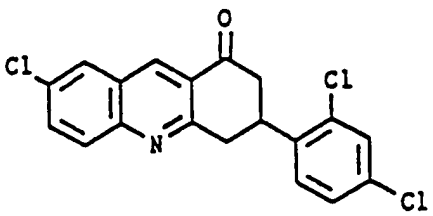
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1210	(*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano-[3,4-*f*]indol-izine-3,6,10-trione	4.0 g	BN63948	280,463	122
					
1214	WR99210 Prodrug; amide from β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid (intended structure)	1.4 g	BN64794	280,493	123
					
1215	N^1-3,4-Dichlorophenyl-N^5-isopropyldiguanide, hydrochloride	29.1 g	BN65111	042,313	123

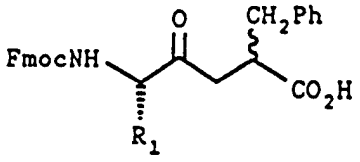
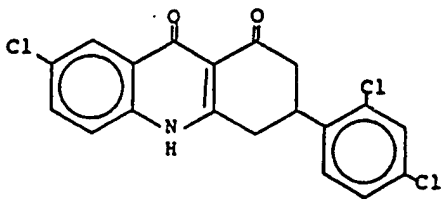
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1216	*L*-Glutamic acid, N-[N-[ethoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt	1.0 g 20 mg	BN65102 BN65488	280,451 280,451	123 123
					
1217	6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine	5.3 g	BN65120	280,157	123
					
1218	1-(3,4-Dichlorophenyl)-3-(1-isopropyl-4,5-dioxo-2-imidazolidinylidene)-guanidine	192.3 g 156.6 g	BN66369 BN66378	182,393 182,393	123 123

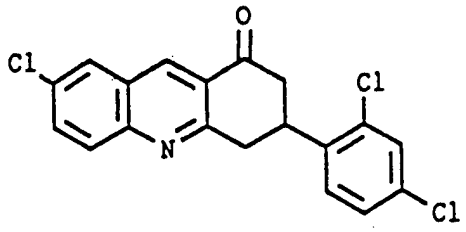
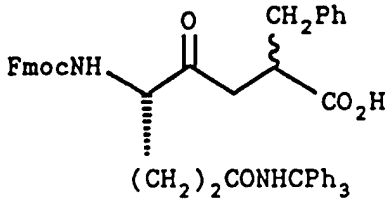
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1219	*L*-Glutamic acid, N-[N-[phenoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, diammonium salt	700 mg 20 mg	BN66387 BN66396	280,526 280,526	123 123
					
1220	*L*-Glutamic acid, N-[N-[methoxyhydroxyphosphinyl]-*L*-phenylalanyl]-, trilithium salt	800 mg 20 mg	BN67473 BN67482	280,527 280,527	123 123
					
1221	2-(Guanin-7-yl)-ethyl 2-hydroxyethyl sulfide	50 mg	BN70069	280,607	124

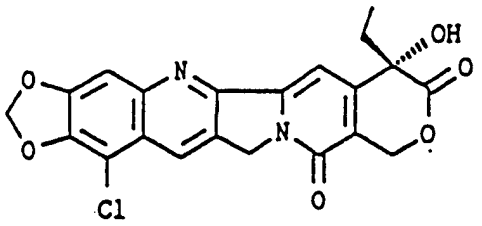
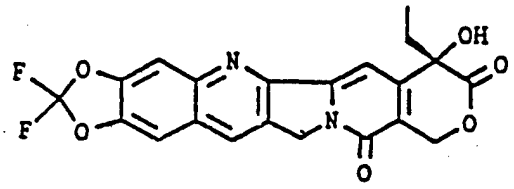
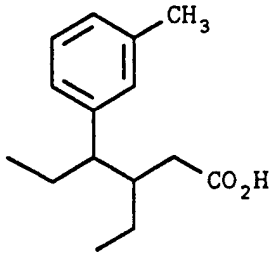
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1222	1,3,5-Triazine-2-acetic acid, 4-[(3,4-dichlorophenyl)amino]-1,6-dihydro-6-imino-1-(1-methylethyl)-, methyl ester	150 mg	BN70667	280,640	124
					
1223	N-(2-Hydroxyethyl)-N-[2-(7-guaninyl)ethyl]-methylamine	800 mg	BN70676	280,419	124
					
1224	(*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano-[3,4-*f*]indolizine-3,6,10-trione	10.2 g	BN72134	280,463	125

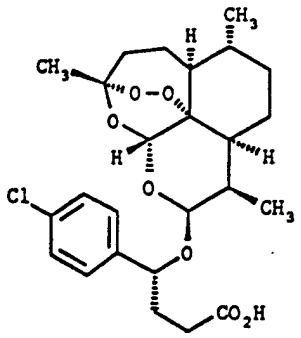
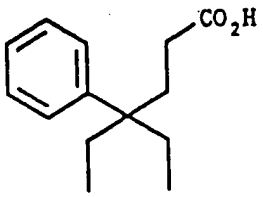
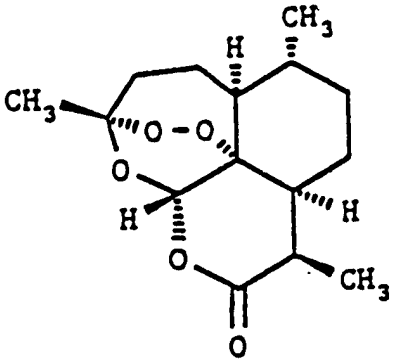
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1225	O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, methyl ester	20 mg	BN72143	280,685	125
					
1226	O-[[(1*R*)-N-[N-(Phenylmethoxycarbonyl)glycyl]-1-aminoethyl]methoxyphosphinyl]-(*R*)-lactic acid, lithium salt	20 mg	BN72152	280,686	125
					
1227	O-[(*L*)-1-[[N-(Phenylmethoxycarbonyl)glycyl-amino]ethyl]hydroxyphosphinyloxy]-(*L*)-lactic acid, dilithium salt	30 mg	BN72634	280,693	125
		1.4 g	BN78172	280,693	125

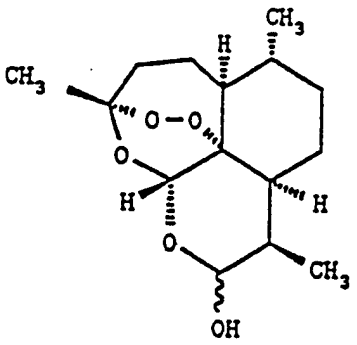
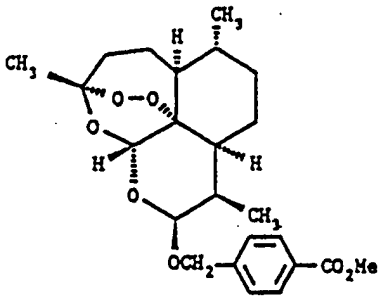
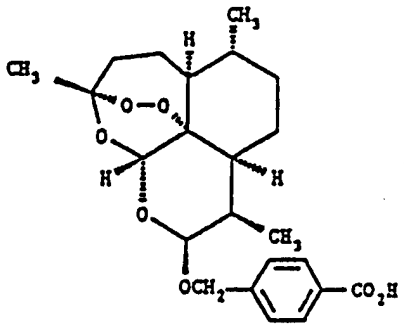
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1228	(*S*)-4-Ethyl-1,4,7,8-tetrahydro-4-hydroxy-pyrano[3,4-*f*]indolizine-3,6,10-trione	20.3 g	BN78921	280,463	126
					
1229	1(2*H*)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichloro-phenyl)-	0.2 g	BN81320	280,850	126
					
1230	1(2*H*)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-	2.1 g	BN83315	280,850	127

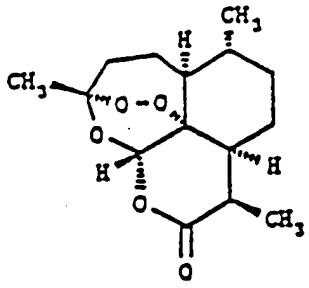
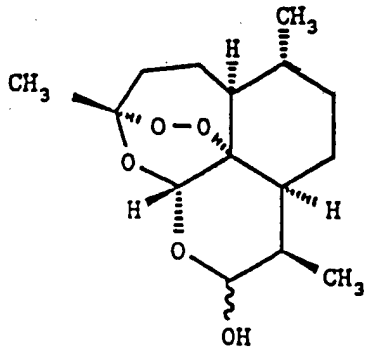
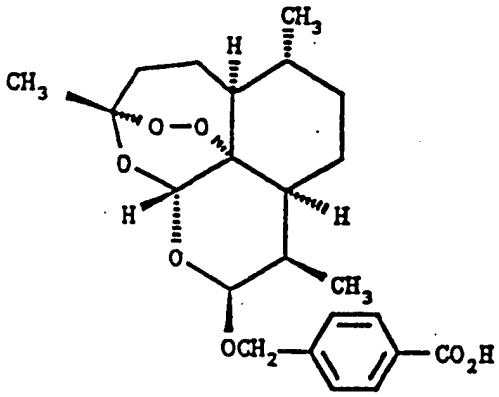
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1231	Fmoc-L-Gln(Trt)Ψ-($COCH_2$)-D,L-Phe	188 mg	BN84134	280,905	127
					
1232	1,9(2*H*,10*H*)-Acridinedione, 7-chloro-3-(2,4-dichlorophenyl)-3,4-dihydro-	2.1 g	BN85284	243,246	127

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1233	1(2*H*)-Acridinone, 7-chloro-3,4-dihydro-3-(2,4-dichlorophenyl)-	5.0 g	BN85293	280,850	127
1234	Product(s) obtained on condensation of 6-aminopiperonal with 6-cyano-2,5-dihydroxy-7-methylindolizine	200 mg	BN85471	280,993	127
1235	Product(s) obtained by reacting EtMgBr with 7-chloro-3-(2,4-dichlorophenyl)-1(2*H*)-acridinone	15 mg	BN85480	280,994	127
					
1236	Fmoc-L-Gln(Trt)Ψ($COCH_2$)-D,L-Phe	630 mg	BN87644	281,074	128
1237	Product(s) obtained on condensation of 6-amino-piperonal with 2,5-dihydroxy-7-methyl-6-cyanoindolizine	100 mg			128

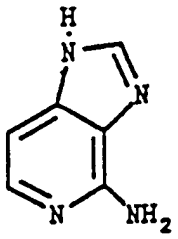
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1238	(*S*)-15-Chloro-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo-[4,5-*g*]pyrano[3',4' : 6,7]-indolizino[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione Syn.: 9-chloro-10,11-(methylenedioxy)-20(*S*)-camptothecin	3.9 g	BN89353	279,773	129
					
1239	(*S*)-7-Ethyl-7-hydroxy-10*H*-2,2-difluoro-1,3-dioxolo[4,5-*g*]pyrano-[3',4' : 6,7]indolizino-[1,2-*b*]quinoline-8,11-(7*H*,13*H*)-dione Syn.: 10,11-(Difluoro-methylenedioxy)-20(*S*)-camptothecin	700 mg	BN89684	281,187	129
					
1240	Hexanoic acid, 3-ethyl-4-(3-methylphenyl)-	25 mg	BN89693	281,181	129

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1241	Butyric acid, 4-(4-chlorophenyl)-4(*R*)-[10(β)-dihydroartemisininoxy]-	25.2 g	BN92092	280,325	129
					
1242	Hexanoic acid, 4-ethyl-4-phenyl-	99 mg	BN92743	281,380	130
					
1243	Artemisinin	10.0 g	BN92752	249,309	130

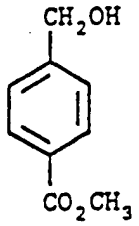
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1244	Dihydroartemisinin	10.0 g	BN95075	253,997	130
					
1245	Artelinic acid, methyl ester	12.0 g	BN95066	255,608	130
					
1246	Artelinic acid	10.0 g	BN95084	255,663	130

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1247	Artemisinin	10.0 g	BN97453	249,309	131
					
1248	Dihydroartemisinin	10.0 g	BN97462	253,997	131
					
1249	Artelinic acid	10.0 g	BN97471	255,663	131

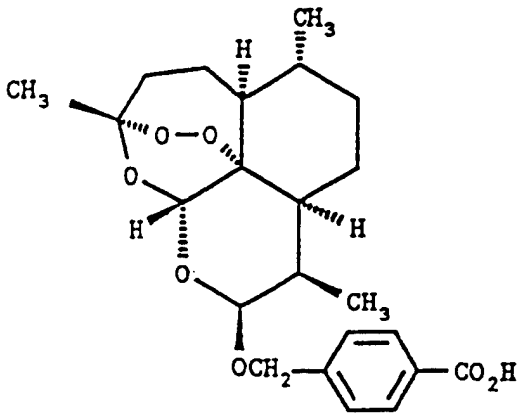
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report



*3-Deazaadenine

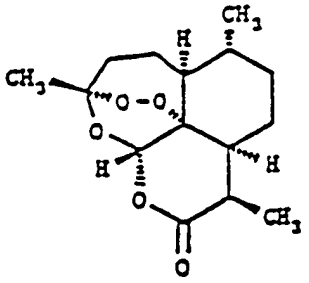
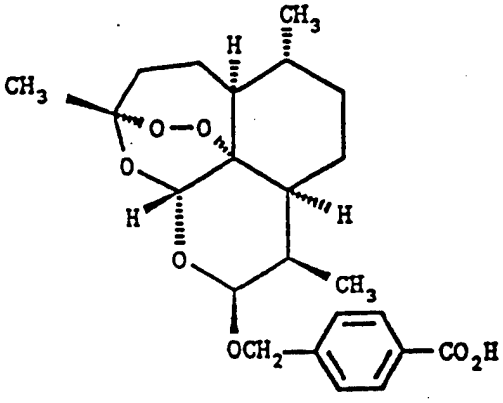
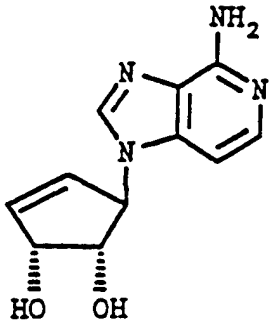


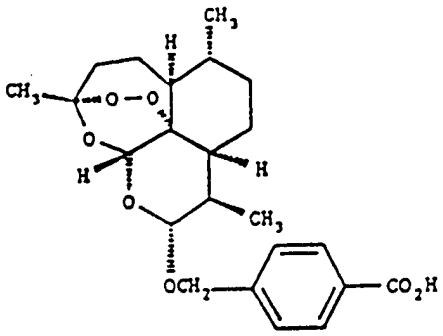
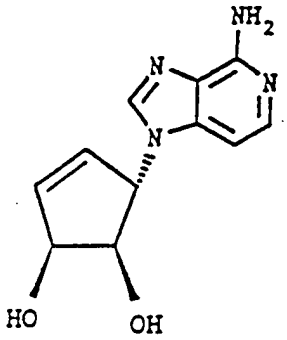
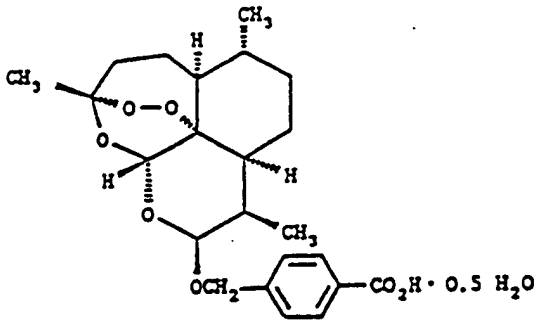
*Methyl 4-hydroxymethyl-benzoate



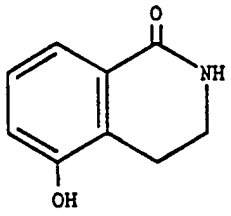
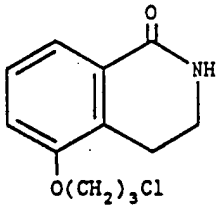
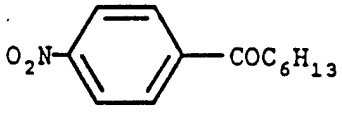
1250	Artelinic acid (cGMP)	14.9 g	BP11207	255,663	134

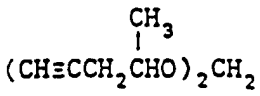
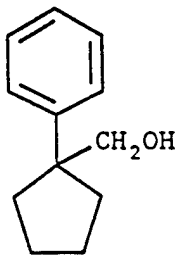
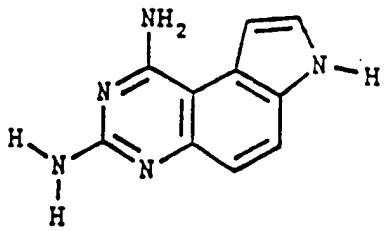
*These materials were used in the synthesis of 1253 and 1252, respectively.

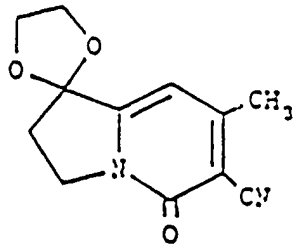
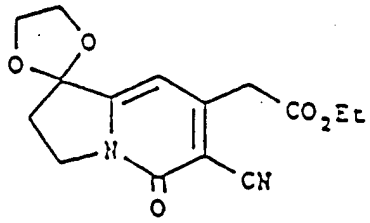
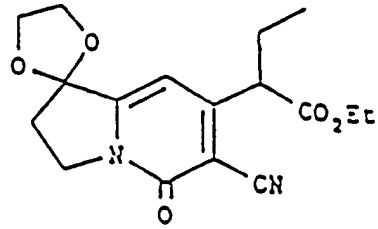
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1251	Artemisinin	10.0 g	BP11216	249,309	134
					
1252	Artelinic acid (non cGMP)	1084.0 g	BP11387	255,663	134
					
1253	(-)-9-(*trans*-2', *trans*-3'-Dihydroxy-cyclopent-4'-enyl)-3-deazaadenine	8.25 g	BP11396	273,938	134

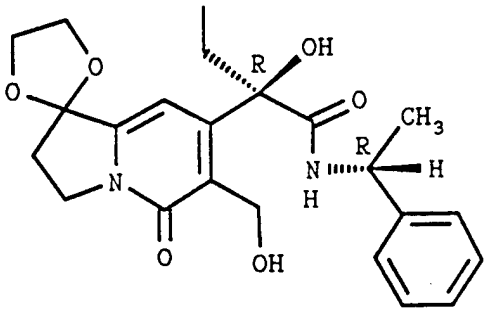
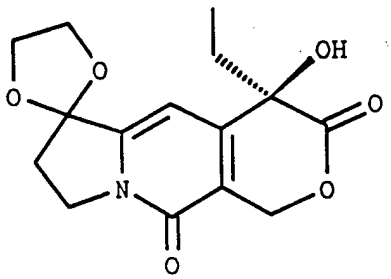
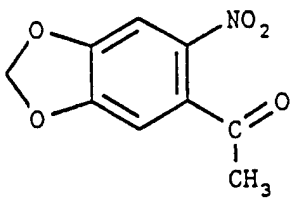
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1254	α-Artelinic acid	13.1 g	BP12106	282,644	135
					
1255	(+)-9-(*trans*-2', *trans*-3'-Dihydroxy-cyclopent-4'-enyl)-3-deazaadenine	4.1 g	BP12222	273,938	135
					
1256	β-Artelinic acid, hemihydrate (cGMP)	3900.0 g	BP12419	255,663	135

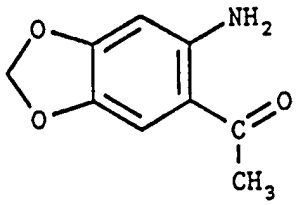
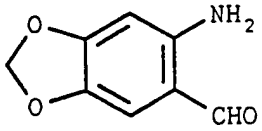
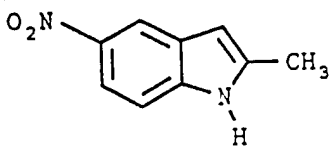
V. CUMULATIVE LIST OF INTERMEDIATES DELIVERED TO WALTER REED ARMY INSTITUTE of RESEARCH FROM DECEMBER 1, 1992 TO MARCH 31, 1999

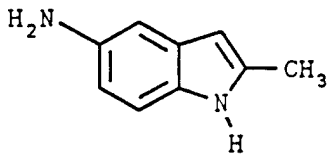
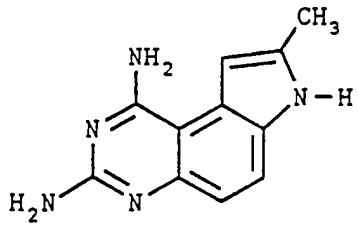
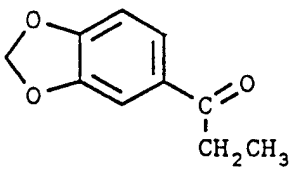
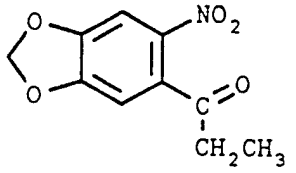
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
1145	 3,4-Dihydro-5-hydroxy-1-(2*H*)-isoquinolinone	6.0 g	BM15447	279,388	111
1146	 3,4-Dihydro-5-(3-chloropropoxy)-1-(2*H*)-isoquinolinone	3.0 g	BM15456	279,389	111
1151	 *p*-Nitroheptanophenone	6.0 g	BM17183	279,478	112

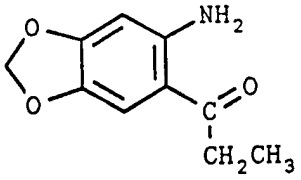
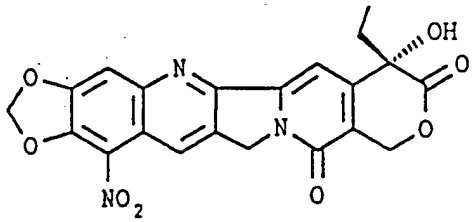
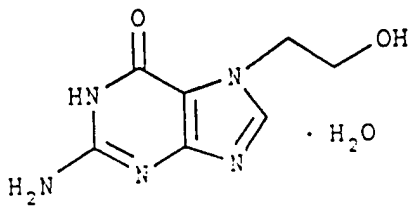
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1155	Bis(1-methyl-3-butynyloxy)methane	4.3 g	BM18617	279,567	113
					
1161	1-Phenylcyclopentane-methanol	1.5 g	BN39077	208,340	117
					
1164	7*H*-Pyrrolo[3,2-*f*]-quinazoline-1,3-diamine	2.1 g	BN42574	221,152	118

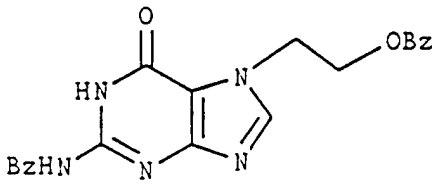
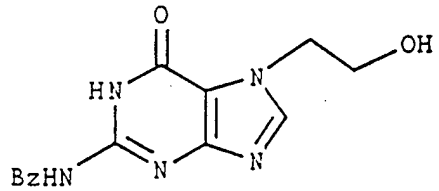
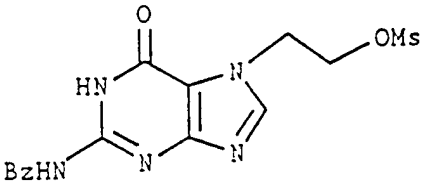
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1169	6-Cyano-1,1-(ethylenedioxy)-7-methyl-5-oxo-1,2,3,5-tetrahydroindolizine	3.1 g	BN45020	290,155	119
					
1170	6-Cyano-7-[(ethoxycarbonyl)methyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine	3.2 g	BN45039	280,156	119
					
1171	6-Cyano-7-[1'-(ethoxycarbonyl)propyl]-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizine	4.4 g	BN45048	280,157	119

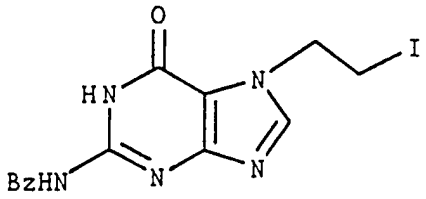
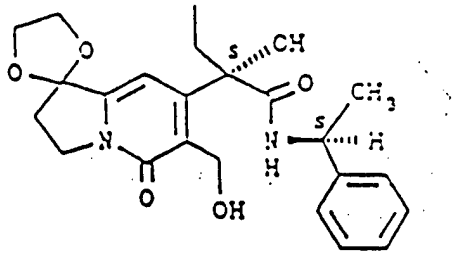
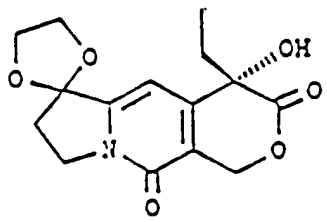
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1172	(*R*)-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydro-indolizin-7-yl]-N-[(*R*)-(+)-α-methyl-benzyl]butyramide, monohydrate	2.4 g	BN44998	280,152	119
					
1173	(*R*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]indolizine-3,10(6*H*)-dione	2.4 g	BN45002	280,153	119
					
1175	4',5'-(Methylene-dioxy)-2'-nitro-acetophenone	0.8 g	BN45922	280,206	120

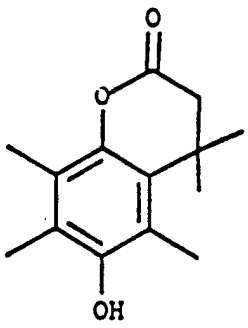
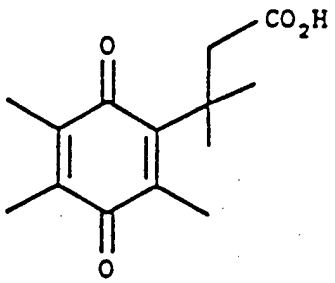
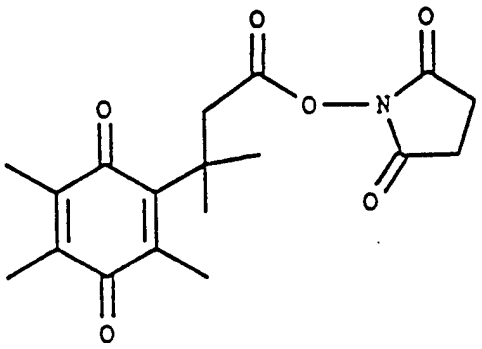
Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
1176	 2'-Amino-4,5'-(methylenedioxy)-acetophenone	1.2 g	BN45931	280,207	120
1179	 6-Aminopiperonal	1.9 g	BN46652	119,620	120
1183	 2-Methyl-5-nitroindole	1.8 g	BN46689	280,245	120

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1184	5-amino-2-methyl-indole	1.8 g	BN46670	112,305	120
					
1185	8-Methyl-7*H*-pyrrolo-[3,2-*f*]quinazoline-1,3-diamine	2.0 g	BN46698	229,014	120
					
1187	3',4'-(Methylene-dioxy)propiophenone	2.0 g	BN47097	280,253	120
					
1188	4',5'-(Methylene-dioxy)-2'-nitro-propiophenone	2.2 g	BN47088	280,252	120

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1189	2'-Amino-4',5'-(methylenedioxy)-propiophenone	1.7 g	BN47079	280,248	120
					
1193	(*S*)-15-Nitro-7-ethyl-7-hydroxy-10*H*-1,3-dioxolo-[4,5-*g*]pyrano[3',4':6,7]-indolizino[1,2-*b*]-quinoline-8,11(7*H*,13*H*)-dione syn.: 10,11-(Methylene-dioxy)-9-nitro-20(*S*)-camptothecin	1.2 g	BN57619	280,411	121
					
1195	2-Amino-7-(2-hydroxy-ethyl)purin-6-one, monohydrate	1.0 g	BN58223	211,546	121

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1196	2-Benzamido-7-[2-(benzoyloxy)ethyl]-purin-6-one	1.0 g	BN58232	280,416	121
					
1197	2-Benzamido-7-[2-hydroxyethyl)purin-6-one	1.0 g	BN58241	280,417	121
					
1198	2-Benzamido-7-[2-[(methanesulfonyl)-oxy]ethyl]purin-6-one	1.0 g	BN57646	280,395	121

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1199	2-Benzamido-7-(2-iodoethyl)purin-6-one	1.0 g	BN58250	280,418	121
					
1208	(*S*)-2-Hydroxy-2-[6-hydroxymethyl-1,1-(ethylenedioxy)-5-oxo-1,2,3,5-tetrahydroindolizin-7-yl]-N-[(*S*)-(-)-α-methylbenzyl]butyramide, monohydrate	3.1 g	BN63957	280,474	122
					
1209	(*S*)-4-Ethyl-6,6-(ethylenedioxy)-1,4,7,8-tetrahydro-4-hydroxypyrano-[3,4-*f*]indolizine-3,10(6*H*)-dione	2.8 g	BN63939	280,472	122

Cumulative No.	Compound	Amount	BN#	WR#	Starks Assoc. Report
					
1211	6-Hydroxy-4,4,5,7,8-pentamethylhydrocoumarin	3.4 g	BN64767	279,647	123
					
1212	β,β,2,4,5-Pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid	3.3 g	BN64776	279,690	123
					
1213	Succinimidyl β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoate	2.0 g	BN64785	280,492	123

VI. BIBLIOGRAPHY OF REPORTS

The work on the subject contract has been summarized in the following reports.

Annual Report

Dated	
November 30, 1993	December 1, 1992 - November 30, 1993 (includes contents of Quarterly Reports numbers 111-114)
November 30, 1994	December 1, 1993 - November 30, 1994 (includes contents of Quarterly Reports numbers 115-118)
January, 1996	December 1, 1994 - November 30, 1995 (includes contents of Quarterly Reports numbers 119-122)
December, 1996	December 1, 1995 - November 30, 1996 (includes contents of Quarterly Reports numbers 123-126)
December, 1997	December 1, 1996 - November 30, 1997 (includes contents of Quarterly Reports numbers 127-130)
December, 1998 (submitted and returned)	December 1, 1997 - November 30, 1998 (includes contents of Quarterly Reports numbers 131-134)
	December 1, 1998 - February 28, 1999 Quarterly Report number 135
	March 1, 1999 - March 31, 1999 no additional work was carried out.

VII. BIBLIOGRAPHY OF PUBLICATIONS AND MEETING ABSTRACTS

U.S. Army Medical Research and Materiel Command
Procedings of the 1996 Medical Defense Bioscience
Review, Vol II, p. 937

Assay for Guanine N-7 Nitrogen Mustard Adduct: A Potential Marker of Efficacy for Antivesicant Agents

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Department of Pharmacology, Division of
Experimental Therapeutics, WRAIR, Washington, DC
and Starks Associates, Inc., Buffalo, NY

ABSTRACT

Nitrogen mustard (HN2) is a chemotherapy alkylating agent that causes vesication. A possible mechanism for nitrogen mustard cytotoxicity is its ability to form adducts with DNA. These adducts have been shown to include N-(2-hydroxyethyl)-N-(2-(7-guaninyl)ethyl)-methylamine (N-7-G), N-(2-hydroxyethyl)-N-(2-(3-adeninyl)ethyl)-methylamine, and the bis(2-(7-guaninyl)ethyl)methylamine. The most prominent adduct produced is N-7-G. We used this adduct to develop an assay to quantitate the level of DNA damage from nitrogen mustard. An authentic N-7-G was synthesized by a 7-step procedure to yield pure material without interferring products. N-7-G was analyzed using a HPLC technique. A C8 column with a mobile phase of 40:60 methanol and .05 M ammonium formate with a flow rate of .8 ml/min was used. UV absorbence set at 284 nm was used for detection. The adduct was found to elute at 5 minutes (Rt) with an internal standard which eluted at 10 minutes (Rt). The assay was able to detect down to 10 ng/ml of adduct from 1 mg/ml of calf thymus DNA. Nitrogen mustard (HN2) was reacted with DNA at 37°C for 4 hours and then heat treated at 70°C for 30 minutes. Adducts were separated from the DNA using Sephadex columns. This assay was able to detect levels of the N-7-G from nitrogen mustard reacted calf thymus DNA. The compound from the DNA mustard reaction was confirmed to be N-7-G by retention time, UV spectra and mass spectra. This technique will be expanded for use to determine DNA adducts in *in vitro* human tissue preparations and in an *in vivo* pig model.

U.S. Army Medical Research and Materiel Command
Procedings of the 1996 Medical Defense Bioscience
Review, Vol II, p. 1608-1615

ANALOGS OF PHOSPHORAMIDON AS METALLO-PROTEASE INHIBITORS FOR BOTULINUM TOXIN SEROTYPE B

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James D. Nicholson[2], Brennie E. Hackley[3]
and Michael Adler[3]

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[2]DAKKRO Corp., Edgewood, MD 21014 and [3]U.S. Army Medical
Research Institute of Chemical Defense, APG, MD 21010-5425

ABSTRACT

Botulinum toxin B (BoNT/B) is one of three serotypes frequently associated with human intoxications and the first one for which a mechanism of action was discovered (cleavage of the synaptic vesicle protein, synaptobrevin). Exposure to BoNT/B results in fatigue, muscle weakness, paralysis and respiratory collapse. Once symptoms appear, no specific treatments are available for counteracting the actions of this or other botulinum toxins. The rational design and synthesis of analogs of phosphoramidon, N-[(α-L-rhamnospyranosyloxy)hydroxyphosphinyl]-L-Leu-L-Trp, may provide potent and specific metalloprotease inhibitors for BoNT/B. Our particular interest is in L-Phe-L-Glu analogs of phosphoramidon. Preparation of phosphoramidon analogs is difficult because of the synthetic challenges associated with the phosphorus-linked glycopeptide. Recently, it was reported that replacement of the rhamnose moiety in phosphoramidon by simple alkyl groups resulted in metalloprotease inhibitors with potencies similar to the parent compound. These provided more attractive synthetic targets. We thus undertook the synthesis of rhamnose-free phosphoramidon analogs using the L-Phe-L-Glu dipeptide moiety. The alkoxy analogs were prepared by phosphorylation of L-Phe-L-Glu$(OMe)_2$ with methyl (or ethyl) phenyl phosphorochloridate followed by alkaline hydrolysis. The phenoxy analog was prepared by phosphorylation of L-Phe-L-Glu$(OCH_2Ph)_2$ with benzyl phenyl phosphorochloridate followed by hydrogenolysis of the benzyl groups.

This work was supported in part by the U.S. Army Medical Research and Materiel Command under Contracts DAMD17-93-C-3003 (Starks) and DAAD05-93-D-7025 (DAKKRO).

INTRODUCTION

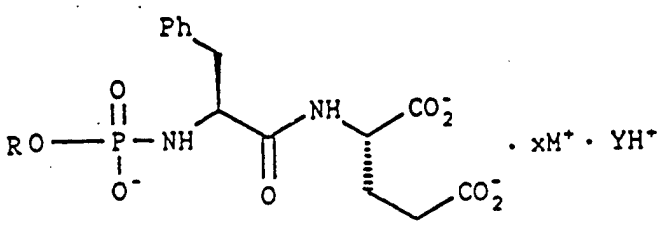
Botulinum neurotoxin (BoNT) inhibits acetylcholine release from motor nerve terminals resulting in a flaccid paralysis of skeletal muscle. The paralytic action of BoNT leads to a characteristic set of symptoms which generally include diplopia, dysphagia, generalized muscle weakness, muscle paralysis and death. Each of the seven serotypes of BoNT consists of a light chain of ~50 kD that has zinc metalloprotease activity and cleaves one of three proteins associated with transmitter release, synaptobrevin, SNAP-25 and syntaxin. The light chain is linked by a single disulfide bond and noncovalent forces to a heavy chain of ~100 kD that is responsible for the binding of the complex to the nerve terminal and for internalization of the light chain into the cytosol.

Although the incidence of botulism from foodborne sources has decreased in recent years, BoNT continues to be a potential military threat and a significant public heath problem. Our current treatment of BoNT intoxication is largely symptomatic, directed at maintaining respiratory and cardiovascular function. Since botulism leads to protracted paralysis with long-lasting consequences, it is important to develop effective pharmacological treatments for BoNT toxicity.

The present effort represents a rational approach towards the design of selective metalloprotease inhibitors for BoNT/B; a task that is rendered especially difficult by the absence of detailed x-ray crystallographic data on the BoNTs. The inhibitors were modelled after the structure of phosphoramidon, a naturally occurring metalloprotease inhibitor. Three compounds have been synthesized to date, all of which contain the dipeptide Phe-Glu coupled to a substituted phosphoryl group. These compounds are expected to bind to the active site of BoNT/B, interact with the active site zinc and inhibit the catalytic activity of BoNT/B.

SYNTHETIC METHODOLOGY

Our synthetic strategy of preparing rhamnose-free phosphoramidon analogs is based upon the recent work of Bertenshaw[1] and colleagues who prepared a series of phosphorylated L-Leu-L-Trp analogs. Our work diverged from that of Bertenshaw through the incorporation of the L-Phe-L-Glu moiety in place of L-Leu-L-Trp and through the development of a procedure to make the phenoxy analog.

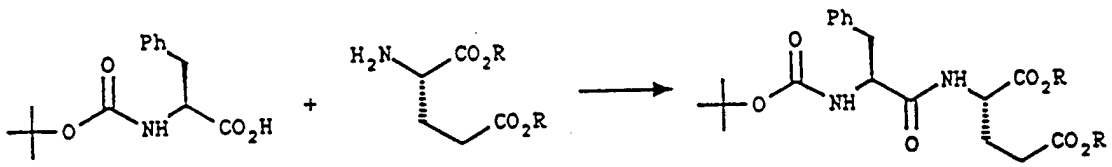


9a : R= CH_3CH_2-, x= 3, M= Li, Y= 0
9b : R= CH_3-, x= 3, M= Li, Y= 0
9c : R= Ph-, x= 2, M= NH_4, Y= 1

Dipeptides 4a and 4b were prepared in 77-91% and 90% yield, respectively, by coupling commercially available t-BOC-L-phenylalanine (1) with L-glutamic acid, dimethyl ester (2a) or dibenzyl ester (2b), using HBTU[2], followed by removal of the t-butoxycarbonyl protecting group with trifluoroacetic acid to give 4a and 4b as tosylate salts[3] (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), containing excess LiCl and H_2O.

Intermediate 8c was prepared in 23% yield by phosphorylation of 4b with chlorophosphate 7c. The tribenzyl protected phosphorylated dipeptide was debenzylated by transfer hydrogenation[4] with 10% palladium on carbon, employing ammonium formate as the source of hydrogen, to give 9c as a diammonium salt (76% yield), containing excess H_2O.

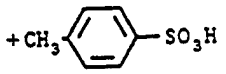
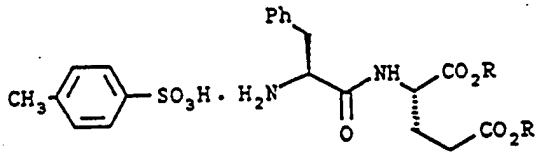
REACTION SEQUENCE

a. 

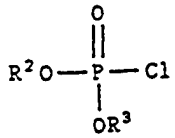
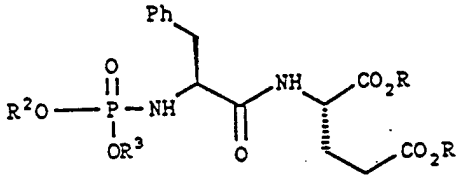
1

2a : R= CH_3
2b : R= CH_2Ph

3a : R= CH_3
3b : R= CH_2Ph

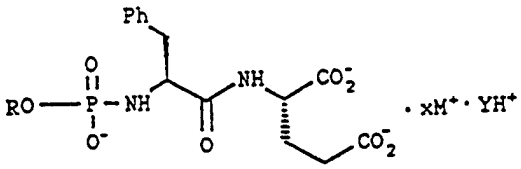
b. 3 + CF_3COOH +  

4a : R= CH_3
4b : R= CH_2Ph

c. 4a / 4b +  

7a : R^2= Et, R^3= Ph
7b : R^2= CH_3, R^3= Ph
7c : R^2= Ph, R^3= $PhCH_2$

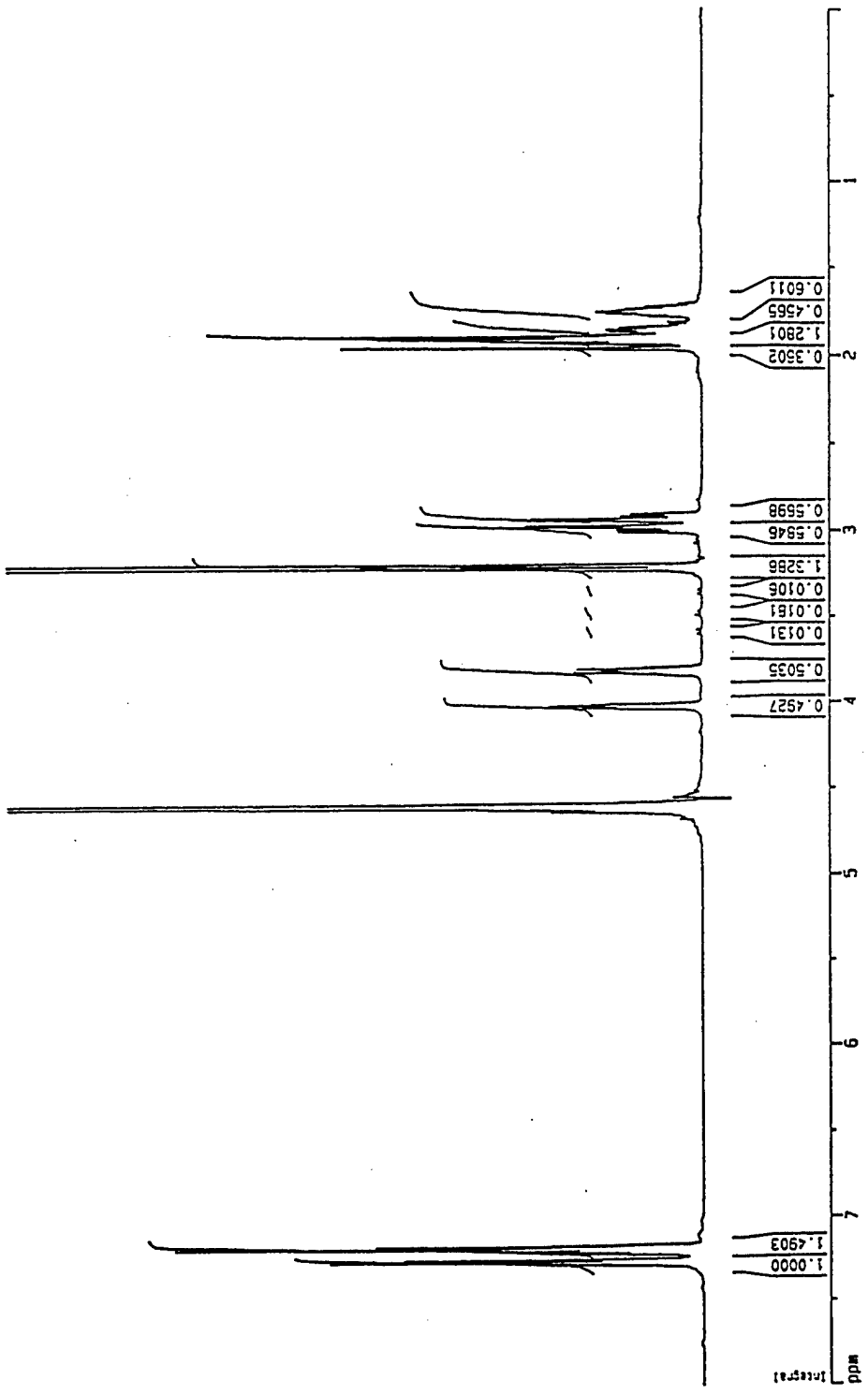
8a : R= CH_3, R^2= Et, R^3= Ph
8b : R= CH_3, R^2= CH_3, R^3= Ph
8c : R= R^3= CH_2Ph, R^2= Ph

d. 8a / 8b / 8c 

9a= R= Et, x= 3, M= Li, Y= 0
9b= R= CH_3, x= 3, M= Li, Y= 0
9c= R= Ph, x= 2, M= NH_4, Y= 1

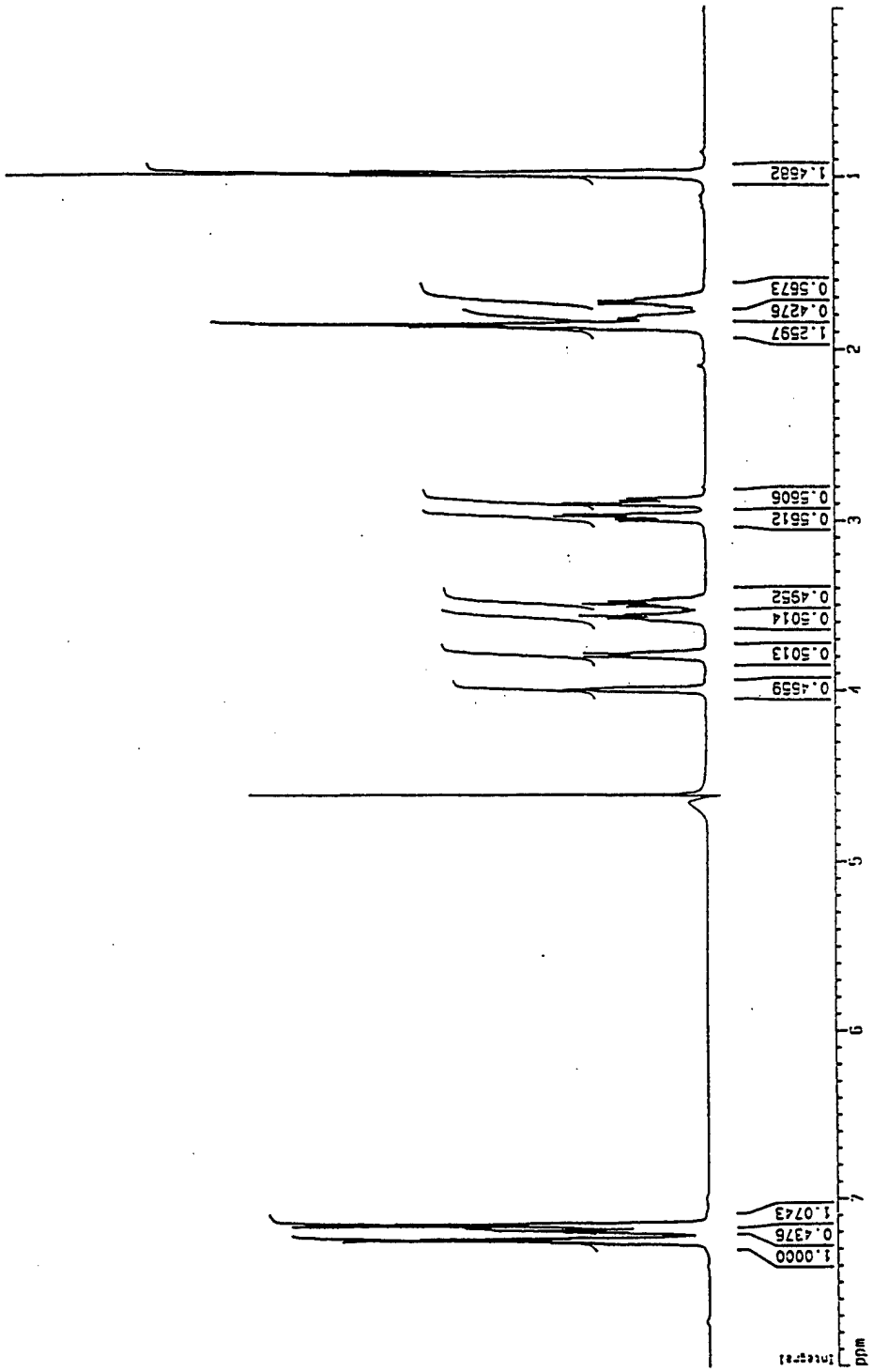
^{1}H Nuclear Magnetic Resonance (D_2O)

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



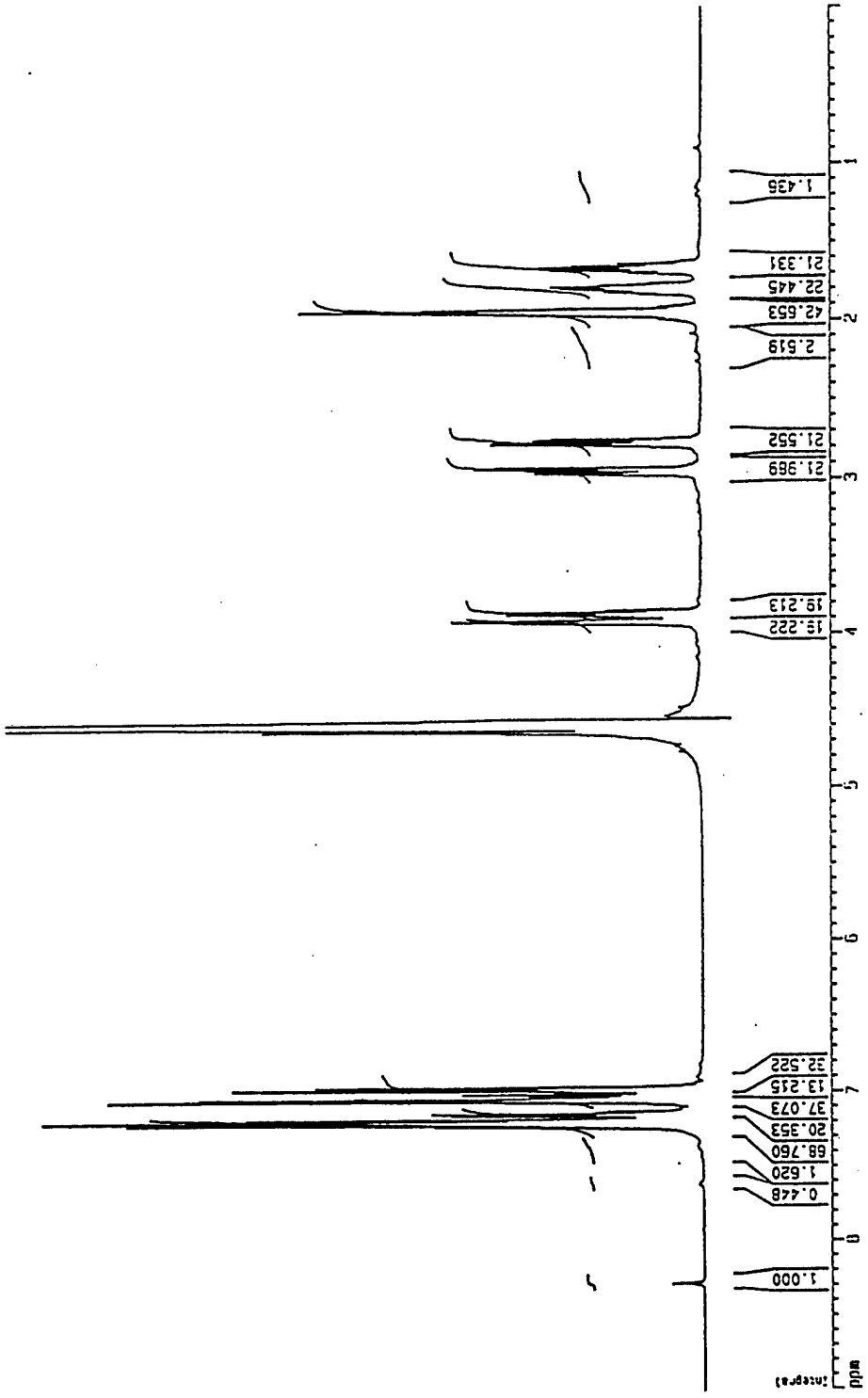
^{1}H Nuclear Magnetic Resonance (D_2O)

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



^{1}H Nuclear Magnetic Resonance (D_2O)

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



TEST SYSTEMS FOR EVALUATING THE METALLOPROTEASE INHIBITORS

- Cell-free systems

The inhibitors will be tested for their ability to slow or block the cleavage of synaptobrevin 2 (or an appropriate fragment of synaptobrevin 2) using capillary electrophoresis for detection (See poster by Asermely, Nowakowski, Courtney and Adler for details).

- *Aplysia californica* buccal ganglia

The inhibitors will be co-applied with BoNT/B light chain by microinjection of the driver cell while monitoring evoked synaptic currents (IPSCs) in the follower cell. (See poster by Apland, Filbert, Adler, Ferrer-Montiel and Montal for details of the preparation). In the absence of metalloprotease inhibitors, micro-injection of BoNT/B light chain produces ~90% inhibition of IPSC amplitudes in <3 hr. The putative inhibitors are expected to prevent or slow the inhibition of IPSC amplitudes mediated by BoNT/B light chain.

- Primary cultures and clonal cell-lines

If found effective, the compounds will be tested for acute toxicity on primary cortical or cultures and clonal NG108-15 neuroblastoma-glioma cells. Toxicity will be assessed by alterations in electrical excitability and by increases in reactivity to propidium iodide.

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VIII. LIST OF PERSONNEL

The following tecnical personnel was assigned to syntheses which have been requested by Walter Reed Army Institute of Research: Dr. J.F. Novotny (Supervisor), Dr. S.K. Chadda, Dr. Liang-quan Li, Dr. C.T. Kane, Jr., Mr. P.F. Rakowski, Mr. D.R. Saunders, Mr. J.J. Hurney and Mr. T.A. Donavan Jr.

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