An Improved Synthesis of Anticancer Benzothiopyranoindazoles. An Efficient Large-Scale β-Aminoethylation Procedure [1]

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Improved processes for the synthesis of bulk quantities of the benzothiopyranoindazole clinical agent CI-958 and A-ring congeners is reported. The process chosen for scale-up operations achieves β-aminoethylation of an anilino precursor *via* a three-step sequence (acylation, reduction, deprotection) starting from *N*-(trityl)glycine. Detailed analytical data are reported for the target compounds and most intermediates, and detailed spectroscopy is given for CI-958.

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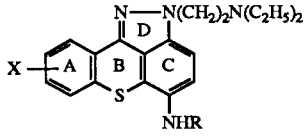
Introduction.

CI-958, **1a**, is a member of the benzothiopyranoindazoles, a new class of chromophore-modified anthracenediones related to the clinical anticancer agent mitoxantrone. In this structural class the quinone moiety, which is believed to be responsible for the cardiotoxicity of the anthracyclines, has been designed out. On the basis of its exceptional broad-spectrum *in vivo* murine anticancer activity, CI-958 has been chosen for clinical development [2].



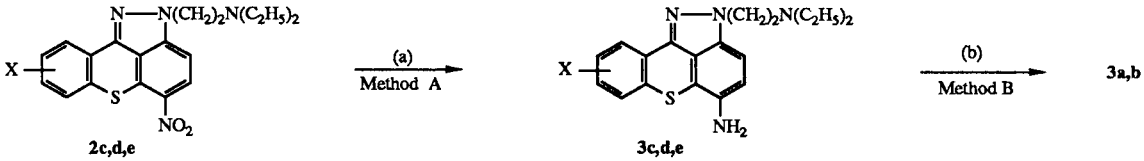
The preclinical development of CI-958 required large quantities of the drug as well as a reliable scale-up process. We desired a process that would utilize stable intermediates, which could be purified by conventional methods (crystallization), and provide high purity product.

This paper describes synthetic approaches to develop a suitable process for the large-scale preparation of CI-958 **1a**. The methodology has been extended to the synthesis of 9-hydroxy and deshydroxy A-ring congeners **1b** and **1c**, respectively, and utilizes a novel β-aminoethylation procedure.

Results and Discussion.

The 5-nitro precursors **2c-e** were synthesized as described before [2] and reduced to the anilino compounds **3c-e** by an improved procedure (Scheme 1). Hydrogenation of **2c-e** was carried out over 5% palladium on carbon in a methanol-water medium followed by precipitation of the dihydrochloride salt from the initial solution with two equivalents of hydrogen chloride. Demethylation of **3d,e** by boron tribromide furnished the intermediates **3a,b**.

Scheme 1



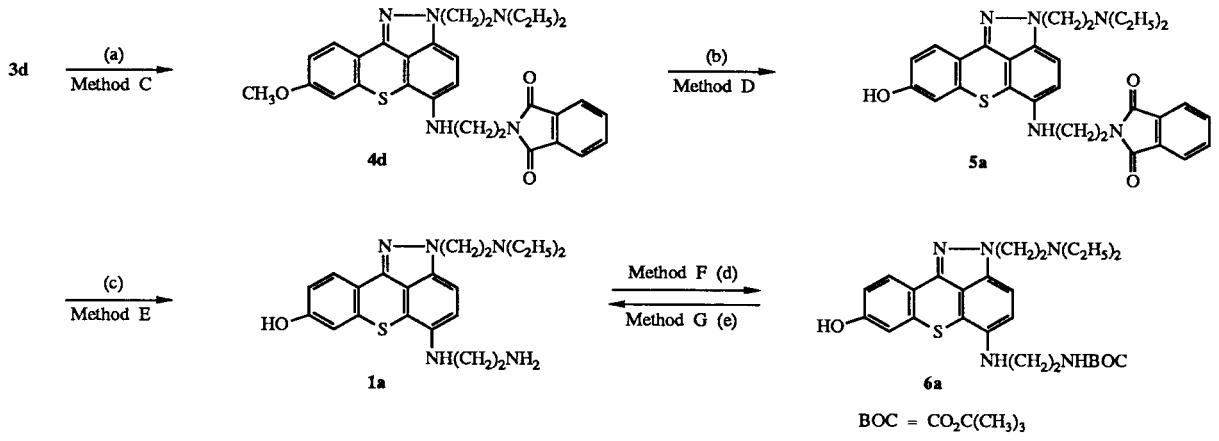
a: X = 8-OH
b: X = 9-OH
c: X = H
d: X = 8-OCH_3
e: X = 9-OCH_3

Reagents: (a) 5% Pd/C, H_2, CH_3OH-H_2O; (b) BBr_3, CH_2Cl_2-$ClCH_2CH_2Cl$

As shown before [2], two general approaches may be utilized to introduce the lower side chain. The initial synthesis employed an alkylation methodology starting with *N*-(β-bromoethyl)phthalimide (Scheme 2). This three-step process gave CI-958 in 38% overall yield, but only 96-97% pure. The major impurity, amine **3a**, resulted from lower side chain degradation during the final deprotection step. This degradation, which is favored in the presence of oxygen and light, impeded purification of CI-958 *via* crystallization. Acceptable purity (≥99%) was achieved by conversion of crude **1a** into the derivative **6a** followed by extensive chromatography, then deprotection of purified **6a** with gaseous hydrogen chloride in a methylene chloride-methanol mixture. Due to the low overall yield (~24%) and labor required, especially in the purification of **6a**, this process was considered impractical for large-scale preparations.

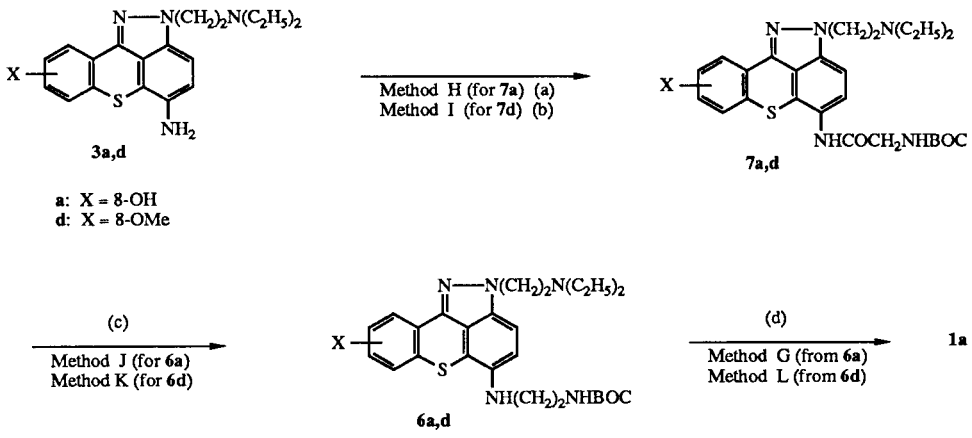
The introduction of the lower side chain *via* acylation methodology was previously performed on anilino intermediates **3** *via* a Schotten-Bauman reaction with *N*-(phthaloyl)glycinyl chloride or by bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP chloride) condensation with *N*-(*t*-butyloxycarbonyl)glycine [*N*-(*t*-BOC)glycine] [2 and references therein] (Scheme 3). Scale-up acylation *via* *N*-(phthaloyl)glycinyl chloride was not further evaluated because of the deprotection problems described above. Acylation of the 8-methoxy derivative **3d** with *N*-(*t*-BOC)glycine [2] is efficient because both protective groups can be removed in one step. Thus, double protected CI-958, **7d**, a crystalline solid that was 99.9% pure by hplc, was treated with boron tribromide in dichloromethane. This furnished CI-958 as the trihydrobromide salt that was only 96% pure by hplc and could be purified by recrystallization to desired specifications but with poor recovery. Since impuri-

Scheme 2



Reagents: (a) *N*-(2-bromoethyl)phthalimide, $NaHCO_3$; (b) BBr_3/CH_2Cl_2; (c) CH_3NHNH_2, CH_3OH; (d) $(BOC)_2O$, CH_2Cl_2, Et_3N; (e) EtOH, HCl (gas)

Scheme 3



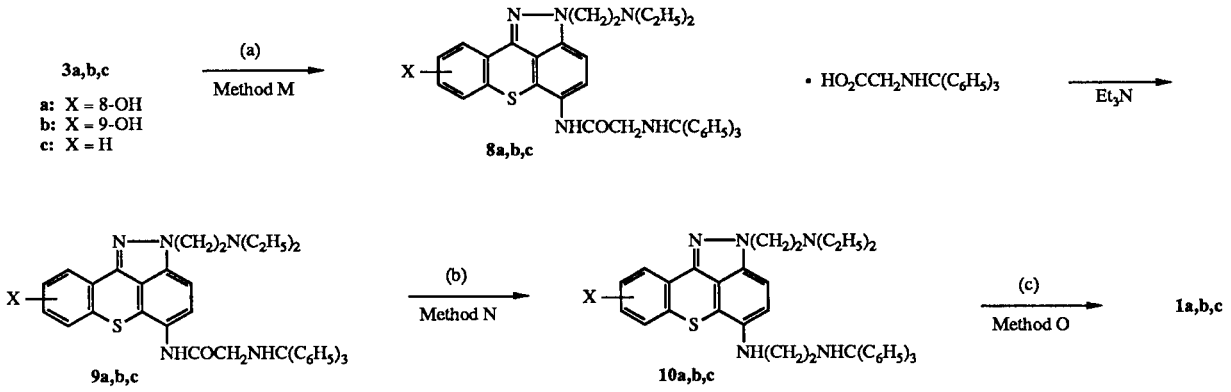
Reagents (a) CDI, DMA, *N*-(BOC)glycine; (b) BOP-chloride, *N*-(BOC)glycine, CH_2Cl_2; (c) Red-Al®, toluene; (d) BBr_3,CH_2Cl_2

ties were being introduced at the demethylation step, we opted to attempt acylation chemistry on the 8-hydroxy intermediate **3a**. Acylation of **3a** with *N*-(*t*-BOC)glycine and 1,1'-carbonyldiimidazole (CDI) in *N,N*-dimethylacetamide at room temperature followed by Red-Al® reduction of **7d** gave the non-crystalline *t*-BOC-protected CI-958, **6d**. Standard purification by column chromatography followed by hydrogen chloride deprotection as in Scheme 2 afforded pure CI-958 **1a**.

In an attempt to obtain a crystalline penultimate intermediate, we evaluated acylation *via* *N*-(trityl)glycine, which is commercially available in bulk quantity (Scheme 4). Acylation of the amine **3a** with *N*-(trityl)glycine and CDI in *N,N*-dimethylformamide gave a crystalline solid **8a** as the *N*-(trityl)glycinate salt. The structure of **8a** was confirmed by microanalyses and spectral data (ir, ^{1}H-nmr, ms). The ^{1}H-nmr spectrum of **8a** showed fifteen additional protons in the aromatic region, while ms (FAB) revealed only the molecular ion peak of the free base **9a**. The pure free base **9a** was obtained by recrystallization of **8a** from an acetonitrile:ethyl acetate mixture containing triethylamine. Red-Al® reduction of **9a** gave the penultimate **10a** as a crystalline solid that was 99.2% pure by hplc and contained $\leq$0.2% of the amine **3a** following recrystallization.

Whereas deblocking of the *t*-BOC-protected CI-958, **6d**, was achieved by gaseous hydrogen chloride in dichloromethane-methanol (Scheme 3), similar conditions did not cleave the *N*-trityl group of **10a**. Table 1 shows different conditions evaluated to achieve the deprotection in an optimum yield and purity. Each preceding run had certain limitations and required further modification until an acceptable procedure was developed (Entries 9-12). Deprotection in acetic acid-concentrated hydrochloric acid (Entry 2) gave pure product in good yield but the reaction was slow to reach completion. A trifluoroacetic acid-hydrochloric acid mixture (Entry 6) reduced the reaction time to 2-3 hours, however the level of amine **3a** impurity was too high (*ca.* 3%). Based on a literature report [3] of the use of 2,2,2-trifluoroethanol (TFE) as a solvent or cosolvent in the detritylation of some amino acids, we evaluated it with

Scheme 4



Reagents: (a) CDI, DMF, N-(trityl)glycine; (b) Red-Al®, toluene; (c) CH_2Cl_2, TFE, HCl

Table 1

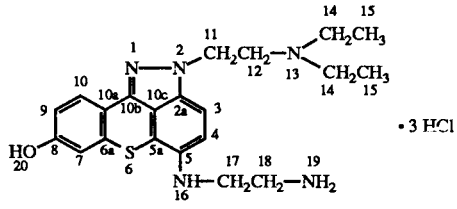
Conditions Evaluated for Deprotection of Penultimate Interndiate **10a**

Number	Reaction Mixture	Reaction Time hours	Yield, %	Purity by HPLC. % (% of **3a**)
1	EtOH:CH_2Cl_2/1:1 + HCl (gas)	24	NR	
	+ HCl(12 *N*)	96	~50% conversion	
2	AcOH:HCl(12 *N*)/1:1	24	82	99.65
3	AcOH:HCl(12 *N*)/6:4	48	82	99.28
4	AcOH:HCl(12 *N*)/9:1	48	88.7	99.5
5	AcOH:TFA:HCl(12 *N*)/3:2:1	24	86.7	99.17 (0.7)
6	TFA:HCl(12 *N*)/8:1	2.5	84	96.7 (3.0)
7	TFE:HCl(12 *N*)/10:1	2.5	83	99.7 (0.12)
8	CH_2Cl_2:TFE:HCl(12 *N*)/5:5:1	2.5	87	99.4 (0.2)
9	CH_2Cl_2:TFE:HCl(6 *N*):H_2O/2:1:1:3	2.0	89	99.2 (0.34)
10	CH_2Cl_2:TFE:HCl(6 *N*):H_2O/2:1:0.8:3.5	1	87.5	99.7 (0.13)
11	CH_2Cl_2:TFE:HCl(6 *N*):H_2O/2:1:0.8:4	1	87.2	99.86 (0.14)
12	CH_2Cl_2:TFE:HCl(9 *N*):H_2O/2:1:0.8:5	0.7	86.5	99.44 (0.31)

AcOH - acetic acid, TFA - trifluoroacetic acid, TFE - 2,2,2,-trifluoroethanol.

Table 2

NMR Chemical Shift Assignments (δ) for CI-958 **1a** Trihydrochloride [a]



Postition	^{1}H	^{13}C [b]
2a		120.1 (0)
3	7.38	105.9 (1)
4	7.38	119.9 (1)
5		137.0 (0)
5a		128.4 (0)
6a		133.3 (0)
7	6.93	112.7 (1)
8		158.2 (0)
9	6.83	115.4 (1)
10	7.81	124.9 (1)
10a		116.0 (0)
10b		140.5 (0)
10c		115.2 (0)
11	4.81	43.2 (2)
12	3.51	49.2 (2)
13	11.08 [c]	
14	3.16	46.5 (2)
15	1.21	8.4 (3)
16	8.95 [c]	
17	3.51	43.2 (2)
18	3.16	37.0 (2)
19	8.43 [c]	
20	8.95	

[a] Spectra acquired in dimethyl sulfoxide-d_6. [b] Number in parenthesis denote the number of protons attached to the carbon. [c] Protonation sites for HCl.

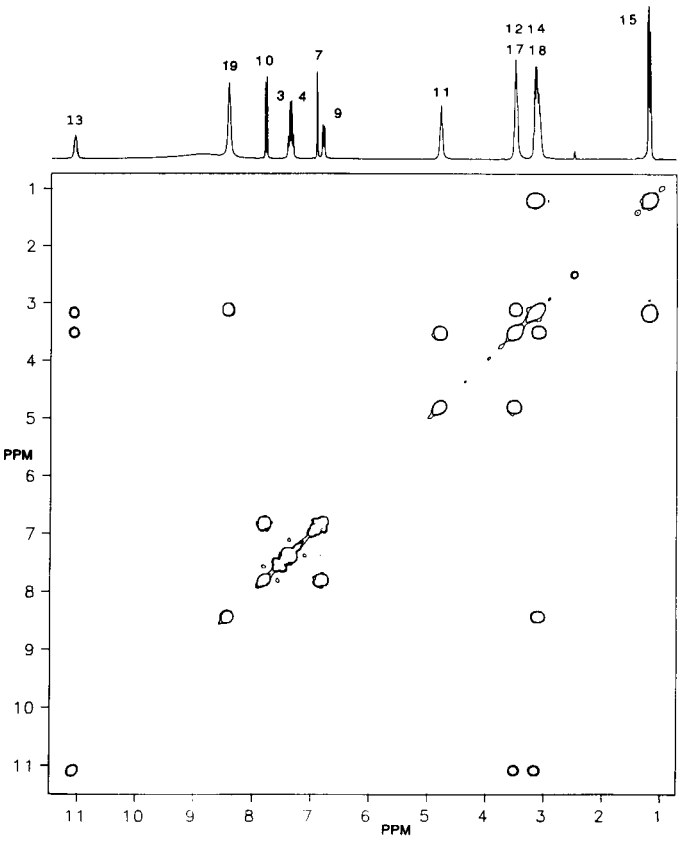


Figure 1. Contour plot of the COSY spectrum of CI-958 trihydrochloride in dimethylsulfoxide-d_6. The proton signals are labeled along the top axis. Off-diagonal peaks correlate scalar-coupled protons at the diagonal frequencies. See Experimental text for spectra parameters.

Table 3

IR and MS Assignments for CI-958 **1a** Trihydrochloride

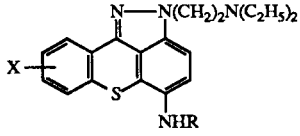
Wavenumber (cm^{-1})	Vibrational Assignments	Ion [a] (m/z)	Structural Assignment
3550	O-H stretch; alcohol group	397	$M^{+\bullet}$-(molecular ion of CI-958 free base)
3400-2400	N-H stretch; protonated primary, secondary, and tertiary amine groups	367	$[M-(\bullet CH_2NH_2)]^+$
		311	$[M-(\bullet CH_2N(CH_2CH_3)_2)]^{+\bullet}$
3080-2800	C-H stretch; methyl, methylene, and aromatic CH's	298	$[M-(CH_2{=}CHN(CH_2CH_3)_2]^{+\bullet}$
		280	$[M-(CH_3NH_2),\ (\bullet CH_2N(CH_2CH_3)_2)]^+$
1610-1590	N-H deformation; amine groups	268	$[M-(\bullet CH_2NH_2),\ (CH_2{=}CHN(CH_2CH_3)_2)]^+$
		252	$[M-(\bullet CH_2N(CH_2CH_3)_2),\ (\bullet NHCH_2CH_2NH_2)]^{+\bullet}$
1580	In-plane skeletal ring vibration; thioxanthene ring structure	86	$CH_2{=}N(CH_2CH_3)_2^+$
1477-1450	In-plane skeletal ring vibration; thioxanthene ring structure		
1384	Symmetrical C-H deformation; methyl groups		
1279-1249	C-N vibrations; primary and secondary amines		
1227	C-O stretch; alcohol group		

[a] Electron impact (70eV), VG Analytical 7070E-HF mass spectrometer using a desorption probe for sample introduction.

hydrochloric acid (Entry 7). The reaction was as rapid as that of Entry 6 while the impurity level was much lower (0.12%). However, the reaction mixture was an unworkable glue-type slurry. To improve the solubility of both the starting material and the product, methylene chloride and water were added. After adjustment of the ratio of the four components in the two-phase reaction mixture, a fast and complete deprotection to CI-958 **1a** was achieved. A small amount (1-2%) of ascorbic acid was added to the water layer to prevent oxidative degradation of the product. CI-958 was precipitated from the aqueous phase by dilution with hydrogen chloride/alcohol (ethanol or 2-propanol for a large scale). The overall yield of high purity **1a** (>99%) from nitro precursor **2a** by this route was 40%. With minor modifications, β-aminoethylation *via* *N*-(trityl)glycine was applied to the preparation of high purity CI-958 analogs **1b,c**.

Spectral characteristics of CI-958 **1a**, trihydrochloride, especially nmr, were studied in detail and are summarized in Tables 2 and 3. Where possible, the proton assignments were based on chemical shift and homonuclear coupling. A two-dimensional nuclear Overhauser (NOESY) [4] spectrum was used to identify protons that were close spatially, and a homonuclear correlation (COSY) [5] spectrum was

Table 4
Substituted Benzothopyranoindazoles



Method	Compound	X	R	mp, °C	Yield, %	Recyristallization Solvent	HPLC Purity, % (System)
A	**3d**	8-OMe	H	254-255.5	84	MeOH, Et_2O [a]	99.6 (Ala)
A	**3e**	9-OMe	H	>285 (d)	83	MeOH:2-PrOH: 1.5 M aq HCl/ 75:5:20	-
B	**3a**	8-OH	H	308-309 (d)	89	MeOH [a]	99.2 (B2c)
B	**3b**	9-OH	H	305 (d)	97	dichloroethane: CH_2Cl_2:MeOH (40:30:30)	98.4 (B2e)
A	**3c**	H	H	260-265	66	hot MeOH [a]	98.7 (F2e)
C	**4d**	8-OMe	$(CH_2)_2$NPht [b]	171-172.5	60	CH_3CN [a]	99.1 (Alb)
D	**5a**	8-OH	$(CH_2)_2$NPht	232.5-234	71	CH_2Cl_2, hexane [a]	98.9 (Alb)
E	**1a**	8-OH	$(CH_2)_2NH_2$	263-265 (d)	72	[c]	99.1 (E2m) [d]
F	**6a**	8-OH	$(CH_2)_2$NHBOC [e]	foam	79	-	99.4 (E2b)
G	**1a**	8-OH	$(CH_2)_2NH_2$	260-264 (d)	90	[c]	99.4 (E21) [d]
H	**7a**	8-OH	$COCH_2$NHBOC	198 (d)	91	hot CH_3CN [a]	94.7 (E21)
I	**7d**	8-OMe	$COCH_2$NHBOC	131-133	86	CH_3CN	
J	**6a**	8-OH	$(CH_2)_2$NHBOC	foam	57	-	97.3 (E2b)
K	**6d**	8-OMe	$(CH_2)_2$NHBOC	133-134	63	CH_3CN	99.9 (C2a)
L	**1a**	8-OH	$(CH_2)_2NH_2$	-	60	-	96.7 (E21)
M	**8a**	8-OH	$COCH_2$NHTrt [f]	199-200 (d)	80	CH_3CN:MeOH/2:8	
M	**9a**	8-OH	$COCH_2$NHTrt	214-216	73	MeOH [a]	99.6 (B2d)
M	**9b**	9-OH	$COCH_2$NHTrt	239-241	81	MeOH [a]	94.6 (B2d)
M	**9c**	H	$COCH_2$NHTrt	194-195	77	$CHCl_3$	99.4 (F2f)
N	**10a**	8-OH	$(CH_2)_2$NHTrt	198-200	78	CH_3OH:CH_3CN:EtOAc/ 1:1:1.7	99.2 (D2b)
N	**10b**	9-OH	$(CH_2)_2$NHTrt	239-241	76	MeOH	97 (B2d)
N	**10c**	H	$(CH_2)_2$NHTrt	142-155	89	EtOAc:MeOH	99.8 (F2f)
O	**1a**	8-OH	$(CH_2)_2NH_2$	262-264 (d)	83	[g]	99.8 (G2n)
O	**1b**	9-OH	$(CH_2)_2NH_2$	270 (d)	81	EtOH:0.1 M aq HCl/3:1	98.9 (B2c)
O	**1c**	H	$(CH_2)_2NH_2$	>200 (d)	85	[h]	99.8 (F2k)

[a] With trituration. [b] Phthaloyl. [c] Reprecipitated from water with ethanol and HCl/2-PrOH. [d] UV detection at 254 nm. [e] *t*-Butoxycarbonyl. [f] Trityl. [g] Reprecipitated from water with 2-PrOH, HCl/2-PrOH and ascorbic acid. [h] Crude product dissolved in EtOH:H_2O (4:1), solution treated with ca. 10% (w/w) ascorbic acid, then saturated with gaseous HCl.

Table 5
Elemental Analyses

Method	Compound	Molecular Formula	Calcd/Found C	H	N	S	Halogen	H_2O
A	3d	$C_{20}H_{24}N_4OS \cdot 1.9HCl \cdot 0.5H_2O \cdot 0.3CH_3OH$	53.42	6.21	12.28	7.03	14.76	
			53.71	6.31	12.03	7.16	15.07	
A	3e	$C_{20}H_{24}N_4OS \cdot 2HCl \cdot 0.6H_2O$	53.12	6.06	12.39	7.09	15.68	
			53.17	5.77	12.52	6.92	15.37	
B	3a	$C_{19}H_{22}N_4OS \cdot 1.9HBr \cdot 0.7H_2O \cdot 0.7CH_3OH$	43.56	5.21	10.31	5.90	27.95	2.32
			43.48	5.10	10.32	5.57	28.28 (Cl^-, 0.08)	2.25
A	3c	$C_{19}H_{22}N_4OS \cdot 1.9HCl \cdot 0.7H_2O$	54.29	6.07	13.13	7.63	16.02	
			54.45	6.05	13.25	7.76	16.39	
C	4d	$C_{30}H_{31}N_5O_3S$	66.52	5.77	12.93	5.80		
			66.50	5.80	13.01	6.44		
D	5a	$C_{29}H_{29}N_5O_3S \cdot 2HBr$	50.52	4.53	10.16	4.65	23.18	
			50.52	4.47	10.70	4.40	23.27	
E	1a	$C_{21}H_{27}N_5OS \cdot 2.9HCl \cdot 0.3H_2O$	49.59	6.04	13.77	6.30	20.21	1.06
			49.27	5.87	13.58	6.12	19.87	1.00
F	6a	$C_{26}H_{35}N_5O_3S \cdot 0.3H_2O$	62.19	7.13	13.95	6.39		
			61.82	7.09	14.07	6.86		
G	1a	$C_{21}H_{27}N_5OS \cdot 3HCl \cdot 0.5H_2O$	48.89	6.06	13.57	6.21	20.61	1.75
			49.12	6.03	13.57	6.14	20.81	1.40
H	7a	$C_{26}H_{33}N_5O_4S \cdot 0.5H_2O$	59.98	6.58	13.45	6.16		
			59.98	6.33	13.42	6.30		
I	7d	$C_{27}H_{35}N_5O_4S$	61.69	6.71	13.32	6.10		
			61.45	6.69	13.22	6.00		
J	6a	$C_{26}H_{35}N_5O_3S \cdot H_2O$	60.56	7.23	13.58	6.22		
			60.90	7.18	13.24	6.40		
K	6d	$C_{27}H_{37}N_5O_3S$	59.76	7.51	12.91	5.91		
			59.77	7.56	12.85	6.17		
L	1a	$C_{21}H_{27}N_5OS \cdot 2.9HBr \cdot 0.1H_2O$	39.78	4.79	11.05	5.06	36.55	
			39.52	4.81	10.78	5.21	36.77	
M	8a	$C_{61}H_{58}N_6O_4S \cdot 0.2H_2O$	75.16	6.04	8.62	3.29		
			74.83	6.08	8.63	3.70		
M	9a	$C_{40}H_{39}N_5O_2S \cdot 0.3H_2O$	72.85	6.06	10.62	4.86		0.86
			72.54	5.99	10.31	5.05		0.47
M	9b	$C_{40}H_{39}N_5O_2S \cdot 0.5CH_3OH$	72.62	6.17	10.45	4.79		
			72.55	5.89	10.63	4.61		
M	9c	$C_{40}H_{39}N_5OS \cdot 0.2H_2O$	74.90	6.19	10.92	5.00		
			74.86	6.09	10.83	5.09		
N	10a	$C_{40}H_{41}N_5OS$	75.08	6.46	10.94	5.01		
			75.20	6.41	10.94	4.73		
N	10b	$C_{40}H_{41}N_5OS$	75.08	6.46	10.94	5.01		
			74.87	6.56	10.90	5.18		
N	10c	$C_{40}H_{41}N_5S$	77.01	6.62	11.23	5.14		
			76.74	6.57	11.12	5.21		
O	1a	$C_{21}H_{27}N_5OS \cdot 2.9HCl \cdot 0.5H_2O$	49.24	6.08	13.67	6.26	20.07	1.76
			49.31	6.20	13.65	6.23	20.26	1.63
O	1b	$C_{21}H_{27}N_5OS \cdot 3HCl \cdot 1.5H_2O$	47.24	6.23	13.12	6.01	19.92	
			47.10	6.12	12.94	6.29	19.97	
O	1c	$C_{21}H_{27}N_5S \cdot 3HCl \cdot 0.9H_2O$	49.74	6.32	13.81	6.32	20.97	
			49.94	6.32	13.87	6.42	20.69	

used to identify the protons directly coupled to each other. The COSY spectrum is shown in Figure 1 with the proton assignments labeled along the top axis. The protonated carbon signals were assigned from the heteronuclear correlation (HETCOR) [6] experiment and the nonprotonated carbons were assigned by a long-range heteronuclear correlation (LR-HETCOR) [6] experiment. The LR-HETCOR is shown in Figure 2 with the critical crosspeaks labeled.

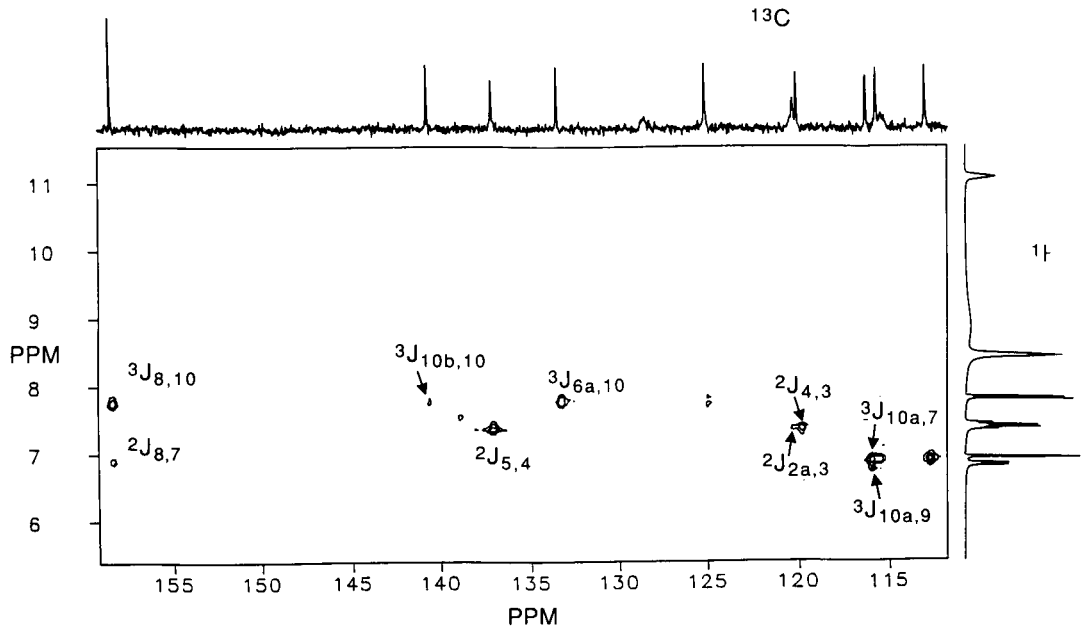


Figure 2. Contour plot of the ^{13}C-^{1}H LR-HETCOR spectrum of CI-958 trihydrochloride in dimethylsulfoxide-d_6. The one-dimensional proton and carbon spectra are plotted on the vertical and horizontal axis, respectively. The crosspeaks correlate proton and carbon signals that are 2-3 bonds apart. The labels have the format $^{k}J_{m,n}$ where k is the number of bonds between the proton and carbon nucleus, and m and n are the position numbers of the coupled carbon and proton nuclei, respectively. These correlations provide the basis for assigning nonprotonated carbons, and corroborate previous proton and carbon assignments. See Experimental text for spectra parameters.

In summary, we have described much improved processes for the bulk preparation of CI-958 and its analogs. The use of *N*-(trityl)glycine for β-aminoethylation of the anilino precursers *via* a three-step sequence (acylation, reduction, deprotection) gave high purity products by a conventional purification technique (crystallization). Detailed analytical data for the target compounds as well as for most of the intermediates are given in the Experimental and Tables 4 and 5, respectively.

EXPERIMENTAL

Melting points (mp) were taken on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared (ir) spectra were determined on a Digilab FTS-14 or Nicolet MX-1 FT-IR spectrometer system. Routine proton magnetic resonance (pmr) spectra were recorded on a Varian EM-390 or XL-200 spectrometer operating at 90 MHz or 200 MHz, respectively. Other nmr spectra for CI-958 trihydrochloride were acquired on a Varian XL300 equipped with a Varian 5 mm broadband switchable probe tuned for ^{13}C (75 MHz) on the observe coil and ^{1}H (300 MHz) on the decoupler coil. The sample concentration was between 20-60 mg in 0.7 ml of dimethyl sulfoxide-d_6. The ^{13}C-^{1}H correlation (HETCOR) spectrum was obtained in the absolute value mode by acquiring 128 blocks of 2048 complex data points over a ^{13}C sweep width of 5-165 ppm, and a ^{1}H sweep width of 0.7-11.4 ppm. The final delay was optimized for $^{1}J_{C\text{-}H}$ of 140 Hz. A total recycle time of 1.1 seconds was used between acquisitions and 128 scans were averaged for each block. A signal enhancement function was applied to the t_2 (carbon) fids (SE = 1.59, AF = 0.051). A similar function was applied to the t_1 (proton) interferograms (SE = 1.49, AF = 0.048). The long-range ^{13}C-^{1}H correlation (LR-HETCOR and XCORFE [7]) spectra were obtained with similar ω_2 parameters but with 256 blocks and 640 transients each. The final delays in the LR-HETCOR were set to optimize for J_{CH} = 11 Hz. The XCORFE experiment used a fixed evolution time of 3.93 ms and a 33 ms refocusing delay after the final BIRD pulse. Similar filters were applied to the t_2 fids (SE = 3.18, AF = 0.047) and the t_1 interferograms (SE = 3.18, AF = 0.047). The COSY and NOESY experiments were performed using 256 blocks of 1024 complex data points over the proton range of 0.7-11.4 ppm. A total of 16 transients were averaged for each block. The COSY and NOESY experiments were acquired in absolute value mode and phase sensitive mode, respectively. Chemical shifts are reported as δ units in parts per million downfield from internal tetramethylsilane, coupling constants are reported in Hz.

Combustion analyses were performed on a Perkin-Elmer Model 240, Control Equipment Corporation Model 240XA, or Carlo-Erba Model 1106 elemental analyzer. Water of crystallization was determined by Karl Fischer titration and sulfur was analyzed by ion exchange chromatography with a Dionex-4000 chromatograph and Dionex AS3 column. Halogens were determined by silver nitrate titration.

Chromatography was carried out with E. Merck products utilizing silica gel 60, catalog number 5789 for normal phase tlc, catalog number 7754-3 for open column chromatography (70-230 mesh) and catalog number 9385 for flash chromatography (230-400 mesh).

High pressure liquid chromatography (hplc) was carried out on

the following columns: A, Alltech Silica, 10 μ; B, Alltech, Econosil, C-18, 5 μ; C, Alltech, Econosphere, C-18, 5 μ; D, Alltech, Ultrasphere, C-18, 5 μ; E, Altex, C-18, 5 μ; F, Phenomenex, Ultremex-5, C-8; G, Lichospher RP-18e, 5 μ. Mobile phases utilized were: (1), absolute ethanol:chloroform:ammonium hydroxide in a ratio of a, 5:95:0.25; b, 10:90:0.25; (2), *x* parts of (methanol:acetonitrile) to *y* parts of 2.6 *p*H buffer prepared by mixing 2.8 g of (potassium dihydrogen phosphate and 7 ml of triethylamine in 1 l of water and adjusting *p*H to 2.6 with phosphoric acid. Ratios of x:y were as follows: a, 40 (0:100):60; b, 60 (90:10):40; c, 30 (80:20):70; d, 75 (80:20):25; e, 40(50:50):60, f, 65 (50:50):35; g, 25 (50:50):75; h, 65 (80:20):35; j, 60 (80:20):40; k, 25 (80:20):75; l, gradient 30 (90:10):70 at 0 minute, 60:40 at 30 minutes, 60:40 at 36 minutes; m, 30 (100:0):70; n, gradient 20 (50:50):80 at 0 to 3.5 minutes, linear increase to 70 (50:50):30 at 13.5 minutes, the same at 16 minutes. Flow rates were 1 or 1.5 ml/minutes and uv detection was at 233 nm if not indicated otherwise.

All solvents and reagents utilized in reactions were reagent grade. Charcoal refers to activated "Darco" G-60. *In vacuo* refers to 1.0-1.5 mm. All solvents were concentrated on a rotary evaporator at 30-40° (15-20 mm) unless noted otherwise.

Method A. 5-Amino-*N,N*-diethyl-8-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine Dihydrochloride (**3d**).

A mixture of *N,N*-diethyl-8-methoxy-5-nitro-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine hydrochloride, **2d** (354 g, 0.814 mole) and 15 g of 5% palladium on carbon was suspended in 3.5 liters of methanol and 0.87 liter of water, then hydrogenated at 50 psi and 25° for 20.4 hours. The catalyst was removed by filtration and the filter cake was washed with 1 liter of methanol. The combined filtrate and washings were adjusted to *p*H 2 with hydrogen chloride/2-propanol. The acidified mixture was refrigerated overnight. The precipitate was filtered, washed with ether (2 x 3 liters), and dried at 50°/220 mm/16 hours to give 303.6 g of the product **3d**.

Method B. 5-Amino-*N,N*-diethyl-8-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazole-2-ethanamine Dihydrobromide (**3a**).

A 12 liter four-neck flask was equipped with a mechanical stirrer, nitrogen inlet, dropping funnel with a rubber septum, and a condenser connected to a scubber. The methoxy compound **3d** (150 g, 0.34 mole) was suspended in 3 liters of a 1:1 mixture of dichloromethane:1,2-dichloroethane. Boron tribomide was transferred into the funnel with a flexneedle under argon and was added dropwise to the stirred slurry. The addition took about 40 minutes and the reaction temperature rose from 20° to 32°. The reaction mixture was heated at reflux on a steam bath overnight, monitoring by tlc, and quenched by the careful addition of 5 liters of methanol, then cooled to 30°. Tlc (4:1 dichloromethane: methanol) showed the complete consumption of starting material. Methanol (5 liters) was carefully added to the reaction mixture which was then refluxed for four hours (55-57°). The mixture was cooled to 25-30° and chilled overnight in an 2-propanol/ice bath. The cold mixture (−6°) was filtered with a candle filter, washed with 1 liter of cold methanol, transferred to a Buchner funnel using 1 liter of methanol, and filtered. The collected precipitate was air dried for 20 minutes, then dried at 200 mm/42°/overnight to give 156.4 g of the product **3a**.

Method C. 2-[2-[[2-[2-(Diethylamino)ethyl]-8-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-1*H*-isoindole-1, 3(2*H*)-dione (**4d**).

A solution of 441.4 g (1.0 mole) of **3d** in 8.9 liters of water was adjusted to *p*H 10 with 2.5 liters of 2*N* aqueous sodium hydroxide. The precipitated free base was extracted into 52 liters of dichloromethane. The organic phase was washed with water and concentrated to a residual solid that was dried by azeotropic distillation with 7.4 liters of toluene, then ground in a mortar to a fine powder. This was thoroughly mixed with 393.8 g (1.55 moles) of *N*-(2-bromoethyl)phthalimide and 109.2 g (1.3 moles) of sodium bicarbonate. The mixture was stirred at 100-106° for 19 hours, then cooled to 25°. The hardened solid mass was digested in a mixture of dichloromethane and water. The organic phase was washed with water, dried, and concentrated to a gummy residue that was triturated in diethyl ether. The solids were collected by filtration to give 545.5 g of crude product that was dissolved in 40 liters of acetonitrile. The solution was clarified with charcoal then maintained at 0-5° overnight. The precipitated solids were collected by filtration, washed with cold acetonitrile, and dried at 220 mm/37° to give 324.1 g of **4d**; ir (potassium bromide): 1770, 1710, 1604, 1527, 1469 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 0.84 (t, J = 7.0, 6H), 2.05-2.50 (m, 2H + dimethyl sulfoxide), 2.76 (t, J = 6.5, 2H), 3.17-3.39 (m, 4H), 3.76 (s, 3H), 4.23 (t, J = 6.4, 2H), 4.61 (t, J = 6.5, 1H), 6.81-7.08 (m, 4H), 7.76-7.83 (m, 5H).

Method D. 2-[2-[[2-[2-(Diethylamino)ethyl]-8-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-1*H*-isoindole-1, 3(2*H*)-dione Dihydrobromide (**5a**).

A solution of 324.1 g (0.598 mole) of **4d** in 7.45 liters of dichloromethane was treated dropwise over 2 hours with 2.1 liters (2.1 moles) of a one molar solution of boron tribromide in dichloromethane. The mixture was heated at reflux for 6.75 hours, treated with an additional 300 ml (0.3 mole) of boron tribromide solution, then heated further overnight. The refluxing mixture was treated cautiously with 1.86 liters of methanol, refluxed for an additional 12 hours, cooled, and stirred for 3 hours in an ice bath. The solids were collected by filtration, washed successively with dichloromethane and hexane, then dried at 220 mm/45° to give 291.8 g of **5a**; ir (potassium bromide): 1776, 1722, 1604, 1479, 1388 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 1.20 (t, J = 7, 6H), 3.18-3.30 (m, 4H), 3.42-3.43 (m, 2H), 3.56-3.64 (m, 2H), 3.8 (br s, 2H), 4.65 (br s, 2H), 5.8-6.2 (br s, 3H, exchangeable with deuterium oxide), 6.74 (br s, 2H), 7.08 (d, J = 8.2, 1H), 7.27 (d, J = 8.9, 1H), 7.75-7.91 (m, 5H), 9.32 (br s, 1H, exchangeable with deuterium oxide).

Method E. 5-[(2-Aminoethyl)amino]-2-[2-(diethylamino)ethyl]-2*H*-[1]-benzothiopyrano[4,3,2-*cd*]indazol-8-ol (**1a** from **5a**).

A suspension of 5 g (7.25 mmoles) of **5a** in 150 ml of methanol was treated dropwise with 9.6 ml of anhydrous methylhydrazine and the mixture was stirred at 25° for 21 hours. The solution was filtered through Celite® then concentrated to an oily residue which was cooled in an ice bath and treated with 30 ml of 2 *N* aqueous hydrochloric acid. The precipitated solids were filtered and the aqueous filtrate was successively heated at 45-50° for 10 minutes, stirred at 25° for 10 minutes, and cooled at 5° for 30 minutes. Additional precipitated solids were filtered and washed with 25 ml of chilled water. The aqueous filtrate was clarified

with charcoal, treated with 1 ml of Dowex 1X2-100 (Cl^-) ion exchange resin, and stirred at 5° for 50 minutes. The solution was filtered through Celite® and the filtrate was diluted with 1.3 volumes of absolute ethanol. The ice-cold solution was treated portionwise with 0.15 volume of a 10 *N* solution of hydrogen chloride in 2-propanol. After standing overnight at 0-5°, the solids were collected by filtration, washed successively with absolute ethanol, ether, then hexane, and dried to give 2.95 g (80%) of **1a**, mp 261-263° (dec). A 2.6 g sample was dissolved in a mixture of 20 ml of water and 80 ml of absolute ethanol. The ice-cold solution was treated as above with 15 ml of hydrogen chloride in 2-propanol, then maintained at 0-5° for 4 hours. Filtration of the precipitated solids followed by washings as described above and drying at 0.8 mm/50°/overnight gave 2.35 g (90%) of **1a**.

Method F. 1,1-Dimethylethyl [2-[[2-[2-(Diethylamino)ethyl]-8-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-carbamate (**6a**).

A slurry of 40 g (78.9 mmoles) of crude CI-958, **1a**•3HCl and 19 g (86.8 mmoles) of di-*t*-butyl dicarbonate in 500 ml of dichloromethane under nitrogen was cooled to 2° and a solution of 33 ml of triethylamine in 40 ml of dichloromethane was added dropwise. The temperature was increased slowly to 10°. After 1 hour, 2 ml of triethylamine in 10 ml of dichloromethane was added. Stirring was continued and a clear solution was formed after about 3.5 hours. The reaction was monitored by tlc (4:1 dichloromethane:methanol) and showed no starting material after 4.5 hours. The mixture was washed with 100 ml of 5% aqueous sodium bicarbonate, then with 150 ml of water. The water solution was extracted with dichloromethane (2 x 50 ml), the organic layers were combined, dried over magnesium sulfate for 2 hours, filtered, and concentrated to give 48.5 g of a foamy residue.

A glass column (150 x 15 cm) was packed as a slurry in dichloromethane with 6.2 kg of silica gel, then charged with the above crude material dissolved in 500 ml of dichloromethane. The column was eluted successively with 8 liters of dichloromethane, 30 liters of 95:5 dichloromethane:methanol and 130 liters of 94:6 dichloromethane:methanol. Two-liter fractions were collected and monitored by tlc (4:1 dichloromethane:methanol). The fractions containing the pure product (40 liters) were combined and concentrated to furnish 31.3 g of **6a** as a yellow foam; ir (potassium bromide): 1697, 1603, 1523, 1467 cm^{-1}; pmr (deuteriochloroform): δ 0.98 (t, J = 7, 6H), 1.40 (s, 9H), 2.63 (q, J_1 = 7, J_2 = 7.4, 4H), 2.94 (t, J = 6.3, 2H), 3.23-3.29 (m, 4H), 4.27 (t, J = 6.3, 2H), 5.05 (t, J = 5.2, 1H, exchangeable with deuterium oxide), 6.48-6.52 (m, 2H), 6.76 (s, 2H), 7.71 (d, J = 8.7, 1H).

Method G. Synthesis of CI-958 **1a**•3HCl from **6a**.

To an ice-cold solution of 29.4 g (59.1 mmoles) of *t*-BOC precursor **6a** and 800 ml of absolute ethanol was bubbled slowly anhydrous hydrogen chloride until the temperature reached 12°. The mixture was cooled to 3°, and the bubbling process was repeated three times, then the mixture was warmed to 25°. After stirring overnight, the solids were collected by filtration then stirred for 30 minutes at 5° with a solution of 250 ml of water and 800 ml of ethanol. The mixture was diluted with an additional 250 ml of ethanol and stirred at 5° for 5 hours, then at −5° for 30 minutes. The solids were collected by filtration, washed successively with diethyl ether then hexane, and dried over phosphorus pentoxide at 2 mm/40°/2 hours then at 2 mm/25°/48 hours to give 27.6 g of **1a**•3HCl.

Method H. 1,1-Dimethylethyl [2-[[2-[2-(Diethylamino)ethyl]-8-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]-2-oxoethyl]carbamate (**7a**).

A mixture of 85.6 g (0.52 mole) of 1,1′-carbonyldiimidazole in 465 ml of *N,N*-dimethylacetamide was treated portionwise with 87.5 g (0.50 mole) of [(1,1-dimethylethoxy)carbonyl]glycine. The solution was stirred for 30 minutes then treated with 154.6 g (0.282 mole) of **3a** (as a salt with 2.4 equivalents of hydrogen bromide). The mixture was stirred at 25° for 21 hours, concentrated to a viscous oil under vacuum, then diluted with 10% aqueous sodium bicarbonate. The aqueous phase was extracted with three portions of dichloromethane and the combined extracts (1.05 liters) were washed with water then diluted with 650 ml of methanol. The solution was stirred at 25° for 64 hours then concentrated to a residual solid that was triturated with hot acetonitrile. The solids were collected by filtration, washed with acetonitrile, and air-dried at 25°/3 days to give 133.1 g of **7a**; ir (potassium bromide): 1684, 1663, 1600, 1516, 1157 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 0.84 (t, J = 7.1, 6H), 1.41 (s, 9H), 2.37-2.58 (m, 4H), 2.82 (t, J = 6.0, 2H), 3.74 (d, J = 6.0, 2H, collapses to s, 2H, with deuterium oxide), 4.33 (t, J = 6.0, 2H), 6.65-6.87 (m, 2H), 7.01-7.26 (m, 3H, collapses to m, 2H, with deuterium oxide) 7.81 (d, J = 8.6, 1H), 9.46 (s, 1H, exchangeable with deuterium oxide), 10.01 (s, 1H, exchangeable with deuterium oxide).

Method I. 1,1-Dimethylethyl [2-[[2-[2-(Diethylamino)ethyl]-8-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]-2-oxoethyl]carbamate (**7d**).

A mixture of 44.96 g (0.1 mole) of **3d**, 30.6 g (0.175 mole) of [(1,1-dimethylethoxy)carbonyl]glycine, 44.53 g (0.175 mole) of bis-(2-oxo-3-oxazolidinyl)phosphinic chloride, 76 ml (0.436 mole) of *N,N*-diisopropylethylamine, and 449 ml of dichloromethane was stirred for 24 hours at 25°. The solution was washed successively with 1 *N* aqueous potassium carbonate and water, dried, and concentrated to a residual solid. Trituration in 2-propanol followed by recrystallization from acetonitrile gave 45.1 g of **7d**. A small portion was further purified by flash silica gel chromatography eluting with 20:1 dichloromethane:methanol. Pure product fractions were concentrated to a solid which was crystallized from acetonitrile to give analytically pure **7d**; ir (potassium bromide): 1728, 1715, 1670, 1606, 1549, 1237 cm^{-1}; pmr (deuteriochloroform): δ 0.94 (t, J = 7.0, 6H), 1.45 (s, 9H), 2.51 (q, J = 7.0, 4H), 2.83 (t, J = 7.4, 2H), 3.73 (s, 3H), 3.94 (d, J = 5.9, 2H), 4.22 (t, J = 7.0, 2H), 5.37 (br s, 1H, exchangeable with deuterium oxide), 6.67-6.83 (m, 3H), 7.18-7.28 (m, 1H), 7.73 (br s, 1H, exchangeable with deuterium oxide), 7.88 (d, J = 8.5, 1H).

Method J. 1,1-Dimethylethyl [2-[[2-[2-(Diethylamino)ethyl]-8-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]ethyl]-carbamate (**6a**) from Amide Precursor **7a**.

An 80° solution of 479 ml (1.63 moles) of a 3.4 molar solution of sodium bis(2-methoxyethoxy)aluminum hydride in toluene was treated portionwise over 1 hour with 160.1 g (312 mmoles) of amide **7a**. Following addition, the solution was cooled to 25° and 200 ml of ethyl acetate was added dropwise followed by cautious addition of 25 ml of saturated aqueous ammonium chloride. The mixture was stirred for 18 hours, diluted with 1 liter of water, and filtered through Celite® . The filtrate was concentrated to an aqueous phase that was extracted with three portions of dichloromethane. The combined extracts were washed with water, dried, and evaporated to a residual oil that was purified by flash chro-

matography on 4.2 kg of silica gel eluting with 93:7 dichloromethane:methanol. Pure product fractions were concentrated to leave 89.3 g of **6a** as a foam.

Method K. 1,1-Dimethylethyl [2-[[2-[2-(Diethylamino)ethyl]-8-methoxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]amino]-ethyl]carbamate (**6d**).

An 80° solution of 49.9 g (95 mmoles) of amide **7d** in 120 ml of toluene was treated dropwise over 2.75 hours with 142.6 ml (475 mmoles) of a 3.4 molar solution of sodium bis(2-methoxyethoxy)-aluminum hydride in toluene. Following addition, the solution was ice-cooled and treated cautiously with 20 ml of saturated aqueous ammonium chloride, then 200 ml of water. The mixture was filtered through Celite® and the filtrate was concentrated to an aqueous solution that was extracted with three portions of dichloromethane. The combined extracts were washed with water, dried, and concentrated to a residual solid that was purified by flash silca gel chromatography eluting with 93:7 dichloromethane:methanol. Pure product fractions were concentrated to a solid that was crystallized from acetonitrile to give 30.7 g of **6d**; ir (potassium bromide): 1688, 1605, 1522, 1251 cm^{-1}; pmr (deuteriochloroform): δ 1.03 (t, J = 7.2, 6H), 1.48 (s, 9H), 2.60 (q, J = 7.0, 4H), 2.93 (t, J = 7.4, 2H), 3.12 (br s, 1H, exchangeable with deuterium oxide), 3.27-3.50 (m, 4H), 3.84 (s, 3H), 4.33 (t, J = 7.5, 2H), 4.90 (br s, 1H, exchangeable with deuterium oxide), 6.73-7.00 (m, 4H), 7.98 (d, J = 8.5, 1H).

Method L. Synthesis of CI-958 **1a** from Methoxy Precursor **6d**.

To a solution of 1 g (1.95 mmoles) of **6d** in 30 ml of dichloromethane under nitrogen, was added 9.8 ml (9.77 mmoles) of a 1 molar solution of boron tribromide in dichloromethane resulting in a yellow suspension. The mixture was refluxed for 24 hours, cooled to room temperature, and treated cautiously with 10 ml of methanol. The mixture was refluxed for 16 hours, filtered under argon, and the collected solids were washed with 5 ml of methanol, then dichloromethane. The precipitate was dried at 220 mm/60°/36 hours to afford 1.11 g of a slightly yellow solid, mp 284° dec, 91.8% pure by hplc. This material was dissolved in 8.7 ml of water and 30 ml of absolute ethanol while slightly warming the mixture. The solution was treated with 5.8 ml of 2.6 molar hydrogen bromide in 2-propanol and refrigerated overnight. The precipitate was filtered, washed with 9.5 ml of ethanol containing 0.5 ml of the hydrogen bromide solution, and dried as above to give 1.01 g of the product, mp 282° (dec), 95.33% pure by hplc. Further purification of the product as above but with charcoal clarification afforded 0.751 g of CI-958 **1a** trihydrobromide as a white solid.

Method M. *N*-[2-[2-(Diethylamino)ethyl]-8-hydroxy-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-5-yl]-2-[(triphenylmethyl)amino]-acetamide (**9a**).

A 22 liter four-neck flask was equipped with a mechanical stirrer, condenser, and nitrogen inlet, then charged with 560 g (3.45 moles) of 1,1'-carbonyldimmidazole dissolved in 4 liters of *N,N*-dimethylformamide. *N*-(Trityl)glycine (950 g, 2.99 moles) was added portionwise as a solid over 30 minutes. The dark mixture was stirred under nitrogen for 2 hours, then powdered amine **3a** (809 g, 1.567 moles) was added portionwise to the mixture over 30 minutes to give a dark brown solution. After 18 hours of stirring under nitrogen at room temperature, the mixture was examined by tlc (4:1 dichloromethane:methanol) which showed only a trace of starting material. The solution was concentrated to give a dark oil. The oil was poured into 16 liters of water with vigorous stirring to furnish a cream solid that was collected by filtration, washed thoroughly with water, and dried at 12 mm/40°/20 hours under a stream of nitrogen to give 4.5 kg of the crude wet product **8a**.

A small sample of **8a** was recrystallized and analyzed; ir (potassium bromide): 1664, 1605, 1531, 1492, 1448 cm^{-1}; pmr (d_6-dimethyl sufoxide + deuterium oxide): δ 0.90 (t, J = 7.0, 6H), 2.52-2.58 (m, 2H + dimethyl sulfoxide), 2.84 (s, 2H), 2.94 (br s, 4H), 3.64-3.65 (DOH), 4.36 (br t, 2H), 6.79-6.86 (m, 2H), 7.20-7.51 (m, 32H), 7.85 (d, J = 8.2, 1H).

A 72 liter flask fitted with a stirrer, condenser, and nitrogen inlet was charged with crude **8a**, 48 liters of methanol, 8 liters of ethyl acetate and 1 kg of triethylamine. The mixture was heated over a steam bath at 78° for 3 hours. The reaction mixture was cooled to −1° with a 2-propanol/ice bath, and filtered overnight with a candle filter. The precipitate was washed in the flask with methanol (2 x 8 liters). The solid was collected by filtration, washed with methanol (4 x 250 ml), and dried under nitrogen at 12 mm/40°/overnight to furnish 744 g of the product **9a**; ir (potassium bromide): 1667, 1604, 1529, 1472 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 0.86 (t, J = 7.0, 6H), 2.42-2.52 (m, 2H + dimethyl sulfoxide), 2.84 (t, J = 6.3, 2H), 2.97 (d, J = 7.0, 2H, s with deuterium oxide), 3.58 (t, J = 7.4, 1H), 4.35 (t, J = 5.9, 2H), 6.75-6.84 (m, 2H), 7.15-7.52 (m, 17H), 7.84 (d, J = 8.5, 1H), 9.59 (s, 1H, exchangeable with deuterium oxide), 10.05 (s, 1H, exchangeable with deuterium oxide).

Method N. 2-[2-(Diethylamino)ethyl]-5-[[2-[(triphenylmethyl)amino]ethyl]amino]-2*H*-[1]benzothiopyrano[4,3,2-*cd*]indazol-8-ol (**10a**).

A 22 liter four-necked flask was equipped with a condenser, mechanical stirrer, nitrogen inlet, thermometer with Therm-o-Watch® controller, and heating mantle. The flask was charged with 7 liters of toluene and 560 ml of a 3.4 *N* toluene solution of sodium bis(2-methoxyethoxy)aluminum hydride, and the mixture was heated to 70° under nitrogen. The solid amide **10a** (350 g, 0.535 mole) was added portionwise. The dark-red mixture was stirred at 80° for 4.5 hours then cooled to room temperature overnight. Tlc (4:1 dichloromethane:methanol) showed the absence of starting material. The reaction mixture was treated carefully with about 25 ml of water, resulting in a vigorous reaction, then with additional water (3.5 liters). The mixture was transferred into a 30 liter beaker, the layers were separated, and the aqueous phase was extracted with ethyl acetate, then with ethyl acetate:tetrahydrofuran (1:1). The organic layers were separated batchwise in a 6-liter separatory funnel and washed with water (1 liter per funnel) to neutral *p*H. The combined organic layers (62 liters total) were dried over sodium sulfate (200 g per 10 liters) overnight, and concentrated to a solid which was azeoptropically dried with 5 liters of toluene. Six liters of methanol was added to the almost dry solid residue, and the mixture was refluxed in the rotary evaporator at atmospheric pressure for 45 minutes. The flask was then left at *ca.* 3° overnight. The solid was collected by filtration, washed with cold methanol (2 x 0.5

liter), and dried in a vacuum oven at 40°/10 mm/overnight to give 316.7 g (93%) of crude **10a**, mp 175.5-178°, 98.3% pure by hplc.

A 20 g sample of this material was recrystallized from 800 ml of 5:5:6 methanol:acetonitrile:ethyl acetate to give two crops of the product; 16.26 g (81%), mp 203-205°, 99.18% pure by hplc and 2.9 g (15%), mp 201-203°, 98.52% pure by hplc. After drying overnight in a vacuum oven (200 mm/45°/15 hours), both crops contained some residual acetonitrile. This was clearly indicated by ^{1}H-nmr and microanalysis. After additional drying at 1.5 mm/45°/15 hours, both samples gave a satisfactory microanalysis and were free of acetonitrile.

Recrystallization of Bulk Material.

A 72 liter five-necked round bottom flask was equipped with a condenser, mechanical stirrer, thermocouple, and nitrogen inlet. The crude compound **10a** (1.201 kg combined from several runs) was placed in the flask under nitrogen, mixed with 29 liters of 1:1 methanol:acetonitrile, and the mixture was heated to 61° over a steam bath. Ethyl acetate (24 liters) was added and the mixture was heated at reflux for 1 hour. The hot solution was filtered into three 20 liter carboys and then cooled overnight at 2°. The highly crystalline precipitate was collected by filtration in a 3 liter fritted glass funnel, washed with cold methanol (3 x 0.25 liter), and dried in a Buflovac® oven at 35°/12 mm/15 hours. The insoluble material left in the 72 liter flask was dissolved in a 2:1:4 methanol:acetonitrile:ethyl acetate mixture at reflux, then the solution was filtered and cooled as above to give 81 g of a second portion of material. After additional cooling, the mother liquors gave two more crops. The above lots were dried, analyzed, blended, and screened through a Number 20 sieve to give 1.013 kg (84%) of the penultimate **10a**; ir (potassium bromide): 1604, 1556, 1518, 1489, 1462 cm^{-1}; pmr (d_6-dimethyl sulfoxide): δ 0.88 (t, J = 7.0, 6H), 2.21 (m, 2H, br t with deuterium oxide), 2.43-2.54 (m, 2H + dimethyl sulfoxide), 2.81 (t, J = 6.6, 2H), 3.28 (t, J = 4.5, 2H), 4.27 (t, J = 6.4, 2H), 4.33 (br t, 1H, exchangeable with deuterium oxide), 6.68-7.43 (m, 19H), 7.74 (d, J = 8.5, 1H), 9.90 (s, 1H, exchangeable with deuterium oxide).

Method O. Synthesis of CI-958 **1a**•3HCl from Trityl Penultimate **10a**.

A 20 liter flask was equipped with a stirrer, thermometer, addition funnel and argon inlet. The vessel was thoroughly purged with argon and charged with 2.75 g of ascorbic acid, 166.4 g (0.26 mole) of **10a**, 2.56 liters of dichloromethane and 1.29 liters of 2,2,2-trifluoroethanol. The mixture was stirred at room temperature for 5 minutes to give a yellow solution. Concentrated hydrochloric acid (721 ml) was added over 2 minutes, turning the solution immediately black. Stirring was continued for 16 minutes to give a greenish-yellow suspension. Degassed distilled water (2.6 liters) was added and the mixture was stirred for 15 minutes. More degassed distilled water (3.9 liters) was added and the mixture was stirred for 20 minutes to give a yellow suspension. A third portion (1 liter) of degassed distilled water was added, the mixture was stirred for 10 minutes, and the phases were separated. The mixture was transferred to a 20 liter separating funnel under argon and the organic phase was discarded. The orange aqueous layer was washed with dichloromethane (3 x 1.5 liters), treated with 2.75 g of ascorbic acid, and diluted first with 20 liters of 2-propanol then with 650 ml of hydrogen chloride/2-propanol (7.72 molar). The resulting suspension was kept at 25° for 120 hours. The pale yellow precipitate was collected by filtration, then dissolved under argon in 975 ml of degassed distilled water containing 1.75 g of ascorbic acid to give a bright yellow solution that was filtered under argon and diluted with 3.18 liters of 2-propanol. Hydrogen chloride/2-propanol (7.72 molar, 195 ml) was added, the suspension was thoroughly mixed, and allowed to stand overnight at room temperature. The cream-colored precipitate was collected by filtration, washed with 1 liter of 2-propanol and dried *in vacuo* at 30° over phosphorous pentoxide (nitrogen bleed) to give 120 g of **1a**•3HCl•3H_2O.

The water content was reduced to less than 2% (CI-958•3HCl•0.5H_2O) by slurrying the solid with 1 liter of 2-propanol for 30 minutes, collecting the solid by filtration under argon, washing with 865 ml of 2-propanol, and drying over phosphorous pentoxide at 100 mm/30°/4 days, then at 5 mm/3 days to give 111.7 g of **1a** as a pale yellow powder.

REFERENCES AND NOTES

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