SYNTHESIS OF NEW ANALOGS OF ECHINOCANDIN B BY ENZYMATIC DEACYLATION AND CHEMICAL REACYLATION OF THE ECHINOCANDIN B PEPTIDE: SYNTHESIS OF THE ANTIFUNGAL AGENT CILOFUNGIN (LY121019)

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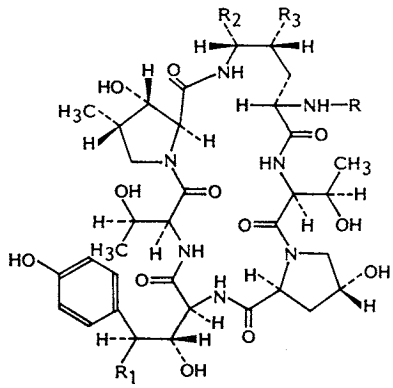
The antifungal antibiotic, echinocandin B (ECB), was modified by a sequential procedure in which the initial step involved enzymatic removal of the native *N*-linoleoyl group from the *N*-terminus using an *Actinoplanes utahensis* culture. The resulting product, ECB nucleus, was reacylated using active esters or acid halides of various substituted acids to give a series of ECB analogs. These analogs possessed anti-*Candida* activity both *in vitro* and *in vivo* (mice). Other studies have shown that one of these, cilofungin, the 4-*n*-octyloxybenzoyl-ECB analog (LY121019), has excellent anti-*Candida* activity, low toxicity and is superior to other available antifungal antibiotics.

Echinocandin B (ECB) is the major lipopeptide antifungal antibiotic of a complex produced by some species of *Aspergillus nidulans* and *Aspergillus rugulosus* (see Fig. 1).[1~4] This antibiotic is a hexapeptide cyclized by an *N*-acylaminal linkage between the *C*-terminus of the hydroxymethylproline and the ω-amino group of the *N*-terminal dihydroxyornithine residue. The *N*-terminal amino group is acylated with a linoleoyl group, which is characteristic of the echinocandin complex. Like A21978C, ECB was deacylated by *Actinoplanes utahensis* to give the corresponding deacylated peptide (**1**).[5,6] This report will present data concerning the characterization of the ECB peptide and its use in the synthesis of new analogs that possess interesting antifungal activity. These include cilofungin (**14**),

Fig. 1. The structures of the echinocandins.



Echinocandin B	R=Linoleoyl	R_1=OH	R_2=OH	R_3=OH
Echinocandin C	R=Linoleoyl	R_1=H	R_2=OH	R_3=OH
Echinocandin D	R=Linoleoyl	R_1=H	R_2=H	R_3=H

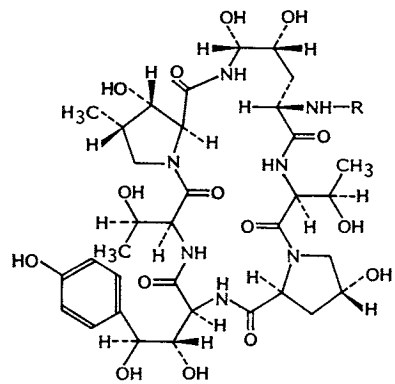
the 4-*n*-octyloxybenzoyl-ECB analog, which other studies have shown has promise as a new antifungal agent.[7~9]

Deacylation of Echinocandin B

The deacylation of ECB with *A. utahensis* results in loss of antimicrobial activity in the remaining peptide.[4] This deacylated peptide was obtained in 60~70% yield. Physical methods showed that this product was the peptide core of ECB. Comparison of the ^1H NMR spectrum of the peptide product with that of ECB showed that the key resonances corresponding to the linoleic acid side chain were no longer present and that no other structural features had been altered. The loss of the linoleoyl group was verified by mass spectral analysis. The product of this fermentation process had a new titratable group, (*pKa* 7.35) which was assigned to the *N*-terminal amino function of the ECB nucleus (**1**).

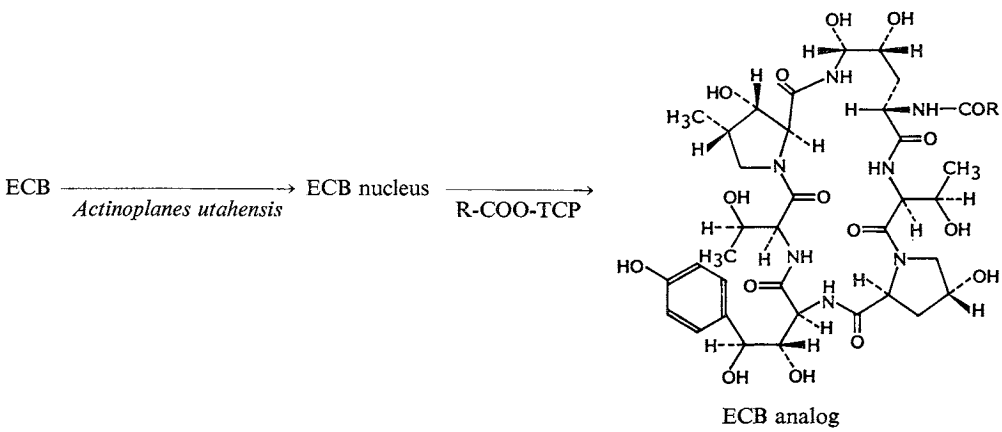
Reacylation of the ECB Nucleus

Selective acylation of the *N*-terminal amino group of the ECB nucleus in the presence of several hydroxyl groups could be readily accomplished by one of many methods (see Scheme 1). The acylation method was largely dictated by the availability of the acyl group. In general, the fatty acyl groups were introduced by a modified Schotten-Baumann reaction using buffered acetone, pyridine and the corresponding acid halide. Generally, the acylation was carried out using active esters which were obtained from the corresponding carboxylic acid. The trichlorophenylate (TCP) active ester was utilized most frequently although the reagent derived from *N*-hydroxybenztriazole was equally effective. The active ester and the ECB nucleus gave acylated products in moderate to high yields. The products were readily purified by chromatography



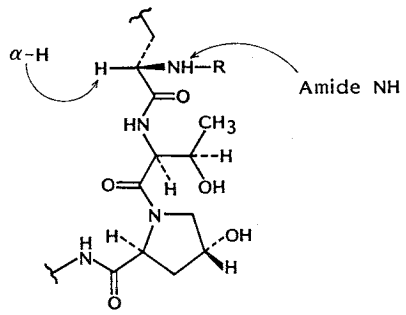
ECB nucleus (**1**) R=H
ECB (**2**) R=Linoleoyl
Cilofungin (**14**) R=4-*n*-Octyloxybenzoyl

Scheme 1. Synthesis of ECB analogs.



ECB analog

Fig. 2. ^{1}H NMR of the acylation of peptide **1**.



Peptide	R	δ values[a] (ppm)	
		α-H	Amide NH
ECB (**2**)	Linoleoyl	4.21	8.08
ECB nucleus (**1**)	H	3.23	—
Cilofungin (**14**)	4-*n*-Octyloxybenzoyl	4.38	8.38

[a] DMSO used as a solvent at 23°C.

and were generally of high final purity.

Acylation of ECB nucleus with 4-*n*-octyloxybenzoyl-TCP active ester gave the 4-*n*-octyloxybenzoyl-ECB analog, cilofungin (**14**), in 60% yield. The position of acylation in this analog was verified by ^{1}H NMR methods. Resonances in the vicinity of the *N*-terminal amino group are strongly influenced by the presence or lack of an acyl group at that position. Fig. 2 compares the resonances of the two protons in the dihydroxyornithine residue observed in the ECB, the ECB nucleus and the cilofungin molecules. Most notable is the shift of the signal for the proton on the α-carbon bearing the linoleoyl group from 4.21 to 3.23 ppm upon deacylation of ECB to ECB nucleus. Reacylation causes this resonance to shift back to lower field (4.38 ppm). The signal for the amide proton (8.08 ppm) formed by the α amino group of ECB is lost by deacylation and reestablished at 8.38 ppm in the reacylated product. These data were consistent with reacylation of the terminal amino group of the ECB nucleus in the same site occupied by linoleic acid in ECB. Further confirmation was obtained by regeneration of ECB by acylation of ECB nucleus with linoleoyl-TCP active ester.

Synthesis of Echinocandin B Analogs

The methods outlined above provided a versatile process for the generation of new analogs of ECB. As with other lipopeptides, reacylation of the *N*-terminus of the peptide nucleus reconstitutes this activity.[5] Although the exact mechanism of action of lipopeptide antibiotics is not well understood, it appears the peptide core carries the information that determines the antimicrobial spectrum.[9,10] When attached to the proper acyl group the peptide is somehow optimally transported to the site of action.[10,11] The variation of the acyl group can be expected to influence the activity and other important properties such as toxicity. Therefore, several series of analogs of ECB having a variety of fatty acid acyl, aroyl and aralkanoyl groups at the *N*-terminus were synthesized by the methods described above and each analog characterized and tested for antimicrobial activity against *Candida albicans*. Most of the acyl groups were synthesized from available chemical sources. Aroyl and aralkanoyl groups bearing alkoxyphenyl functions were synthesized by alkylation of the appropriate hydroxyphenyl derivative. The alkylthiobenzoyl side chains were prepared from rearrange-

ment of *O*-(4-carbomethoxyphenyl)dimethylthiocarbamate to methyl 4-mercaptobenzoate which can be alkylated without isolation to the desired 4-(alkylthio)benzoic acid utilizing the method of NEWMAN and KARNES.[12] These acids were converted to the TCP active esters by the method discussed above. The active esters of the alkylthiobenzoic acids were oxidized with *m*-chloroperbenzoic acid to give the corresponding alkyl sulfonylbenzoyl-TCP esters. Each of these acyl groups were attached to ECB nucleus by the procedure outlined above. Each analog was purified by HPLC and characterized by mass spectral analysis. The analogs prepared in this study are listed in Tables 1 and 2, along with the appropriate characterization data.

Selective Evaluation of ECB Analogs

Reacylation of the ECB nucleus generally

Table 1. Identification parameters of fatty acid acyl ECB analogs.

	Fatty acid acyl analogs	FD-MS[a] (M+23 (Na^+))	Rf (reversed-phase TLC)[b]
3:	*n*-Dodecanoyl	1,002	—
4:	*n*-Tridecanoyl	1,016	0.62
5:	*n*-Tetradecanoyl	1,007 (no Na^+)	0.57
6:	*n*-Pentadecanoyl	1,044	0.46
7:	*n*-Hexadecanoyl	1,058	0.38
8:	*n*-Heptadecanoyl	1,071	0.33
9:	*n*-Octadecanoyl	1,086	0.25
10:	*n*-Nonadecanoyl	1,100	0.17
11:	*n*-Eicosanoyl	1,114	0.13
12:	*n*-Dicosanoyl	1,142	0.00

[a] Field desorption mass spectrum. The analogs give an (M+Na^+) mass ion.

[b] Rf by reversed-phase TLC on Whatman KC_{18} plates, (fluorescent indicator) using H_2O - MeOH - CH_3CN (1 : 2 : 2).

—: Data not available.

Table 2. Identification parameters of the ECB analogs.

	FD-MS (M+1)	HPLC retention time (minutes)
4-Alkoxybenzoyl-ECB analogs		
13: *n*-Hexyl	1,024	—
14: *n*-Octyl (cilofungin)	1,052	1.83
15: *n*-Decyl	1,080	2.98
16: *n*-Dodecyl	1,107	6.73
17: *n*-Tetradecyl	1,135	12.69
4-(Alkylthio)benzoyl-ECB analogs		
18: *n*-Octyl	1,068	4.71
19: *n*-Decyl	1,096	9.90
20: *n*-Dodecyl	1,124	4.81
21: *n*-Tetradecyl	1,152	7.78
4-(Alkylsulfonyl)benzoyl-ECB analogs		
22: *n*-Octyl	1,083	—
23: *n*-Decyl	1,128	—
4-Amidobenzoyl-ECB analogs 4-*N*-acyl group		
24: Acetyl	980	3.37
25: *n*-Decanoyl	1,093	1.73
26: *n*-Dodecanoyl	1,121	—
27: *n*-Tetradecanoyl	1,149	4.52
28: *n*-Hexadecanoyl	1,177	6.21
4-Alkoxyphenylpropionyl-ECB analogs		
29: *n*-Butyl	1,024	1.92
30: *n*-Pentyl	1,038	2.31
31: *n*-Hexyl	1,052	5.00
32: *n*-Heptyl	1,066	4.04
33: *n*-Octyl	1,081	2.31
34: *n*-Dodecyl	1,136	6.15
4-Alkoxyphenylacetyl-ECB analogs		
35: *n*-Octyl	1,056	4.23
36: *n*-Dodecyl	1,123	16.35
4-Alkoxyphenoxyacetyl-ECB analogs		
37: *n*-Octyl	1,056	3.46
38: *n*-Decyl	1,123	5.19
4-Alkoxycinnamoyl-ECB analogs		
39: *n*-Hexyl	1,050	6.15
40: *n*-Octyl	1,078	18.27
41: *n*-Decyl	1,083	21.54

HPLC retention times were determined using (6.4×305 mm) C_{18} μBondapak resin (Waters Associates, Inc., Milford, Mass.) and elution with a solvent system comprised of H_2O - MeOH - CH_3CN (1 : 2 : 2) using a pressure of 105 kg/cm² with a flow rate of 3 ml/minute and a Waters 600A pump and a chart speed of 5 mm/minute. Eluent was monitored at 230 nm.

—: Data not available.

Table 3. *In vitro* anti-*Candida* activity of ECB analogs.

	MIC (μg/ml)		MIC (μg/ml)
Fatty acid acyl analogs		4-Amidobenzoyl-ECB analogs	
3: Dodecanoyl	1.25	**24**: Acetyl	>20
4: Tridecanoyl	0.625	**25**: *n*-Decanoyl	0.625
5: Tetradecanoyl	0.312	**26**: *n*-Dodecanoyl	0.625
6: Pentadecanoyl	0.312	**27**: *n*-Tetradecanoyl	1.25
7: Hexadecanoyl	0.312	**28**: *n*-Hexadecanoyl	0.312
8: Heptadecanoyl	0.625	4-Alkoxyphenylpropionyl-ECB analogs	
9: Octadecanoyl	0.625	**29**: *n*-Butyl	20
10: Nonadecanoyl	2.5	**30**: *n*-Pentyl	5
11: Eicosanoyl	5.0	**31**: *n*-Hexyl	0.625
12: Dicosanoyl	5.0	**32**: *n*-Heptyl	0.625
4-Alkoxybenzoyl-ECB analogs		**33**: *n*-Octyl	0.078
13: *n*-Hexyl	2.5	**34**: *n*-Dodecyl	0.312
14: *n*-Octyl (cilofungin)	0.156	4-Alkoxyphenylacetyl-ECB analogs	
15: *n*-Decyl	0.156	**35**: *n*-Octyl	1.25
16: *n*-Dodecyl	0.312	**36**: *n*-Dodecyl	0.312
17: *n*-Tetradecyl	2.5	4-Alkoxyphenoxyacetyl-ECB analogs	
4-(Alkylthio)benzoyl-ECB analogs		**37**: *n*-Octyl	0.312
18: *n*-Octyl	0.312	**38**: *n*-Decyl	0.156
19: *n*-Decyl	0.156	4-Alkoxycinnamoyl-ECB analogs	
20: *n*-Dodecyl	2.5	**39**: *n*-Hexyl	1.25
21: *n*-Tetradecyl	20	**40**: *n*-Octyl	0.156
4-(Alkylsulfonyl)benzoyl-ECB analogs		**41**: *n*-Decyl	0.312
22: *n*-Octyl	20		
23: *n*-Decyl	2.5		

resulted in reestablishment of anti-*Candida* activity, provided that the acyl group was sufficiently lipophilic. The MIC values are shown in Table 3. These analogs were studied further to evaluate the effect of structural modification of the acyl group on the substantial hemolytic properties of the parent ECB molecule. The discussion of the structure-activity relationships among these analogs appears in a separate report.[8,9,13] Investigations by GORDEE *et al.* indicate that cilofungin has a lower hemolytic potential and greater activity than parent ECB, as well as the best therapeutic index of all the analogs tested, including amphotericin B.[7,13] Cilofungin was tested against a large number of clinical isolates of *C. albicans* and was shown to be very effective with MIC values in the 0.156~1.25 μg/ml range as well as showing good *in vivo* activity in experimental fungal infections in mice (ED_{50} 7.6 mg/kg). On the basis of these data, cilofungin is currently undergoing advanced testing for possible clinical entry as an antifungal agent.

Experimental

Deacylation of ECB

The enzymatic deacylation of ECB by *A. utahensis* was carried out by the procedure described by BOECK *et al.*[4]

Purification of ECB Nucleus

Crude ECB nucleus (25 g) was dissolved in 300 ml of water - CH_3CN - AcOH - pyridine (96:2:1:1) and chromatographed using this solvent (7~8 liters) on a 4-liter stainless steel column filled with Lichroprep RP-18 (particle size 25~40 μm) (MC/B Manufacturing Chemists, Inc., Cincinnati, OH) and used as part of a Chromatospac Prep 100 unit. This column was operated at a pressure

of 6.3~7.0 kg/cm² at 60 ml/minute. The elution was monitored by an optical unit set at 280 nm and 500-ml fractions were collected every minute. Fractions were combined, concentrated and lyophilized. Analytical HPLC showed that the desired ECB nucleus had a retention time (K') of 11.52 minutes on a 4×300 mm column of Silica gel C_{18} using ammonium acetate - CH_3CN - water (1:2:97) at a rate of 3 ml/minute at 176 kg/cm² and a detection wavelength set at 230 nm. This procedure gave ECB nucleus as a white lyophilizate having the following properties:

Fast atom bombardment (FAB)-MS *m/z* 798 (M+H); IR ν_{max} (KBr) cm^{-1} 3340 (OH, H-bonded), 1660 and 1625 (several carbonyl groups); *pKa* (66% DMF) 7.35; UV $\lambda_{max}^{H_2O}$ nm (ε) 273 (1,053); $[\alpha]_D$ −36.20° (*c* 10, MeOH).

General Procedure for the Synthesis of 4-Amidobenzoic Acids

The 4-amidobenzoic acids used in this study were synthesized from the reaction of the appropriate acid chloride with 4-aminobenzoic acid. Each was readily available from commercial sources. The fatty acid chloride was added dropwise to the amino acid (1:1 mol ratio) in pyridine to give a molar concentration of the reactants of 0.1~0.2 M. After stirring for 3~6 hours the reaction mixture was poured into a large volume of water. The resulting solid precipitate was collected by filtration, dried and recrystallized from MeOH.

Synthesis of 2,4,5-Trichlorophenyl Esters of 4-Amidobenzoic Acids

The 4-amidobenzoic acid (1 mol), 3,4,5-trichlorophenol (TCPOH) (1 mol) and *N,N'*-dicyclohexylcarbodiimide (DCC) (1 mol) were dissolved in CH_2Cl_2, ether or THF and stirred at room temperature for 16~20 hours. The reaction mixture was filtered to remove the bulk of the dicyclohexylurea. The filtrate was evaporated to dryness and the product crystallized from either CH_3CN - water or Et_2O - petroleum ether. This procedure was equally applicable to the conversion of the other classes of carboxylic acids to the corresponding TCP esters.

Acylation of the ECB Nucleus

a) Modified Schotten-Baumann Methods: The ECB nucleus was dissolved in a mixture of 0.1 M KH_2PO_4 buffer, pH 7.5 (5~10 ml) and Me_2CO or MeOH (5~10 ml) and cooled in an ice bath. A solution of the desired acid chloride in Me_2CO (2~20 ml) was added dropwise over a 30-minute interval with intermittent adjustment of the pH (6.5~7.5) after each addition of acid chloride. The reaction was stirred for 1.5~3.0 hours during which time a precipitate formed which was removed by centrifugation. The supernatant was adjusted to pH 5.0~7.0 and extracted twice with Et_2O and the organic extract discarded. The aqueous layer was concentrated to remove the residual organic solvents and an equal volume of MeOH was added and extracted successively with $CHCl_3$ and with EtOAc and these organic extracts were combined and concentrated to dryness to give a crude alkanoyl derivative of the ECB nucleus. This derivative was purified by reversed-phase HPLC. The derivative was dissolved in 5~6 ml of MeOH and injected on a 1.6×109 cm stainless steel column packed with LP-1/C_{18} resin and eluted with a H_2O - MeOH - CH_3CN solvent system (1:2:2) at 70~105 kg/cm² at a flow rate of 10~12 ml/hour. The chromatography was monitored at 280 nm and the desired fractions were combined and concentrated under reduced pressure. TLC using reversed-phase plates (Whatman KC_{18}) and the H_2O - MeOH - CH_3CN solvent (1:2:2) was used to assess homogeneity. The products were also analyzed by field desorption mass spectrometry (FD-MS).

b) Acylation of the ECB Nucleus Using the 2,4,5-Trichlorophenyl Active Ester Method: A solution of ECB nucleus and the desired 2,4,5-trichlorophenyl active ester (1:1 or 1:2 molar ratio) in DMF was stirred for 15~18 hours at room temperature. The reaction mixture was taken to dryness under reduced pressure and the residue washed with Et_2O (2×) and with CH_2Cl_2 (2×) to remove soluble organic byproducts. The remaining residue was dissolved in EtOAc - MeOH (3:2) and chromatographed on a silica gel (Woehlm 70~150 mesh) column using this solvent. The progress of the separation was monitored by TLC (Merck) using EtOAc - MeOH (3:2) to develop the plates. Fractions containing the desired product were combined and evaporated under reduced pressure to give a residue. The purity of the product was assessed by analytical HPLC utilizing a C_{18} μBondapak resin (Waters Associates, Inc., Milford, Mass.) which was eluted with a solvent system comprised of

H_2O - MeOH - CH_3CN (1 : 2 : 2), 105 kg/cm^2 at a flow rate of 3 ml/minute using a Waters 600A pump. The column was monitored by a UV detector set at 230 nm. The products were also analyzed with FD-MS. The ECB analogs synthesized by this method are shown in Table 2.

Preparation of 4-*n*-Octyloxybenzoic Acid

A solution of 4-hydroxybenzoic acid (19.2 g, 150 mmol) in 10% NaOH (120 ml) was added to 480 ml of DMSO which had been heated to 80°C. *n*-Octylbromide (28.95 g, 150 mmol) was added dropwise to the solution and the reaction mixture stirred for 4 hours at room temperature. The reaction mixture was poured into ice water (1,200 ml) and acidified with 30 ml of conc HCl. After the precipitation was complete the resulting solid was removed by filtration, washed, dried and crystallized from CH_3CN - water: MP 97~98°C.

Anal Calcd for $C_{15}H_{22}O_3$: C 71.97, H 8.86.
Found: C 71.67, H 8.92.

The other *n*-alkyloxybenzoic acids were made by this procedure using the appropriate alkyl bromide.

2,4,5-Trichlorophenyl 4-*n*-Octyloxybenzoate

n-Octyloxybenzoic acid (2.5 g, 10 mmol), 2,4,5-trichlorophenol (1.97 g, 10 mmol) and *N,N'*-dicyclohexylcarbodiimide (2.06 g, 10 mmol) were dissolved in Et_2O (100 ml) and stirred for 18 hours at room temperature. The dicyclohexylurea was removed by filtration and the filtrate evaporated to an oil which crystallized from Et_2O - petroleum ether (1 : 2) to give 2,4,5-trichlorophenyl 4-*n*-octyloxybenzoate (1.82 g, 41%): MP 55~57°C; UV λ_{max}^{MeOH} nm (ε) 264 (18,652).

Anal Calcd for $C_{21}H_{23}O_3Cl_3$: Cl 24.75.
Found: Cl 24.50.

N-(4-Octyloxybenzoyl)-ECB Nucleus (**14**)

A solution of ECB nucleus (14.2 g, 17.8 mmol), 2,4,5-trichlorophenyl 4-*n*-octyloxybenzoate (15.31 g, 35.7 mmol) in DMF (150 ml) was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure and the residue was washed twice each with Et_2O and with CH_2Cl_2 to remove the soluble byproducts. The residue was dissolved in 25% EtOAc - MeOH (80 ml) and purified by preparative HPLC using a Prep LC/System 500 unit (Waters Associates, Inc., Milford, Mass.) on a silica gel cartridge and eluting stepwise with 20% to 40% MeOH - EtOAc. The fractions were analyzed and pooled as in the general procedure outlined above. Fractions devoid of ECB nucleus were pooled and lyophilized to give *N*-(4-octyloxybenzoyl)-ECB nucleus (7.13 g, 38%): FD-MS *m/z* 1,052 (M+Na).

Anal Calcd for $C_{49}H_{71}N_7O_{17}\cdot H_2O$: C 56.14, H 7.02, N 9.35.
Found: C 56.62, H 6.94, N 9.10.

Preparation of 4-(Alkylthio)benzoic Acids

The 4-(alkylthio)benzoic acids employed in these studies were prepared according to the following general procedure:

Methyl 4-hydroxybenzoate (1 equivalent) was added dropwise to a suspension of sodium hydride (1 equivalent, 50% mineral oil dispersion) in DMF (100 ml/50 mmol) which had been cooled to 0°C. The reaction mixture was stirred under an inert atmosphere until the evolution of hydrogen gas subsided. The resulting solution of sodium 4-carbomethoxyphenolate was treated with *N,N*-dimethylthiocarbamoyl chloride (one equivalent) in one portion. The resulting suspension was heated to 70°C for 1~3 hours and then poured into a large excess of 1% KOH. The suspension was extracted twice with toluene - hexane (4 : 1). The organic extract was dried over $MgSO_4$, filtered and evaporated to dryness. The resulting oil was purified by chromatography over silica gel using 2% MeOH in CH_2Cl_2 to give *O*-(4-carbomethoxyphenyl)dimethylthiocarbamate (mp 111~113°C). This product was heated under a nitrogen atmosphere at 220°C for 30~60 minutes to give *S*-(4-carbomethoxyphenyl)dimethylthiocarbamate which was crystallized from MeOH: MP 108~110°C.

Anal Calcd for $C_{11}H_{13}NO_3S$: C 55.21, H 5.48, N 5.85, S 13.40.
Found: C 55.23, H 5.29, N 6.12, S 13.61.

This product was dissolved in DMSO which contained two equivalents of 10% NaOH. The mixture

was heated to 65~85°C and the appropriate alkyl bromide (1 equivalent) was added. Heating was continued for an additional 2~4 hours and the reaction products poured into a large excess of water. A precipitate formed upon acidification which was collected by filtration. 4-(Alkylthio)benzoic acid was then crystallized from MeOH. This procedure was used to synthesize the following 4-(alkylthio)-benzoic acids having the appropriate mass spectral molecular weights and empirical formulae:

4-(*n*-Octylthio)benzoic acid: MW 266.
Anal Calcd for $C_{15}H_{22}O_2S$: C 67.63, H 8.32, S 12.04.
Found: C 67.85, H 8.05, S 12.26.

4-(*n*-Decylthio)benzoic acid: MW 294.
Anal Calcd for $C_{17}H_{26}O_2S$: C 69.34, H 8.90, S 10.89.
Found: C 69.56, H 8.89, S 10.81.

4-(*n*-Dodecylthio)benzoic acid: MW 322.
Anal Calcd for $C_{19}H_{30}O_2S$: C 70.76, H 9.38, S 9.94.
Found: C 70.97, H 9.60, S 9.67.

4-(*n*-Tetradecylthio)benzoic acid: MW 350.
Anal Calcd for $C_{21}H_{32}O_2S$: C 71.95, H 9.78, S 9.15.
Found: C 72.01, H 9.54, S 8.50.

Each of these acids were readily converted to the corresponding 2,4,5-trichlorophenyl 4-(alkylthio)-benzoate active esters by the general procedure outlined above.

Preparation of 2,4,5-Trichlorophenyl 4-(Alkylsulfonyl)benzoate Active Esters

A solution of 2,4,5-trichlorophenyl 4-(alkylthio)benzoate (1 equivalent) in CH_2Cl_2 (2 mmol/10 ml) was cooled in an ice bath and treated with *m*-chloroperbenzoic acid (1 equivalent). The reaction mixture was allowed to warm to room temperature (15 minutes), washed twice with 0.1 N NaOH and the organic phase dried over Na_2SO_4. The resulting product was crystallized from CH_3CN and re-oxidized as above to insure complete conversion. The product was purified, as before, by recrystallization from CH_3CN to give the following pure 2,4,5-trichlorophenyl 4-alkylsulfonylbenzoates having the correct FD-MS molecular weight and empirical formulae.

TCP 4-(octylsulfonyl)benzoate: MW 478.
Anal Calcd for $C_{21}H_{23}Cl_3O_4S$: C 54.61, H 5.02.
Found: C 54.89, H 4.90.

TCP 4-(decylsulfonyl)benzoate: MW 506.
Anal Calcd for $C_{23}H_{27}Cl_3O_4S$: C 54.61, H 5.38.
Found: C 54.90, H 5.45.

These active esters were used to prepare the corresponding ECB analogs by the general procedure outlined above.

Preparation of 4-*n*-Alkoxyphenyl Substituted Carboxylic Acids and Their TCP Esters

The 4-*n*-alkoxyphenyl substituted carboxylic acids used in this study were synthesized by the *O*-alkylation of their corresponding 4-hydroxyphenyl acids which were obtained from commercial sources using the following conditions: The desired 4-hydroxyphenyl acid (20 mmol) was dissolved in 100 ml of DMSO and 23.5 ml of 10% KOH. The solution was heated to 80°C for 6 hours. The reactions were monitored by TLC on silica gel plates (Merck) that were developed with 30% EtOAc in toluene for disappearance of starting materials. The reaction mixture was poured into 800 ml of ice water, acidified with conc HCl to pH 2.5 to give a white precipitate. After filtration and drying, this solid was dissolved in boiling Et_2O and filtered. The solution was concentrated to ~25 ml and petroleum ether added to give a white crystalline solid. After precipitation was complete, the crystals were filtered and washed with a small amount of cold petroleum ether. The following 4-alkyloxy-phenylalkanoic and -phenylalkenoic and their TCP esters were prepared by this method:

4-*n*-Octyloxyphenylacetic acid: mp 82°C (TCP mp 70°C), 4-*n*-dodecyloxyphenylacetic acid: mp 81°C (TCP mp 66°C), 4-*n*-butyloxyphenylpropionic acid: mp 93°C (TCP mp 63°C), 4-*n*-hexyloxyphenyl-propionic acid: mp 85°C (TCP mp 57~58°C), 4-*n*-hexyloxyphenylpropionic acid: mp 85°C (TCP mp 57~58°C), 4-*n*-octyloxyphenylpropionic acid: mp 70~71°C (TCP mp 54~56°C), 4-*n*-dodecyloxy-phenylpropionic acid: mp 84~86°C (TCP mp 65~67°C), 4-*n*-hexyloxycinnamic acid: mp 179°C (TCP

mp 92°C), 4-*n*-octyloxycinnamic acid: mp 167~168°C (TCP mp 96°C), 4-*n*-decyloxycinnamic acid: mp 135~137°C (TCP mp 88~90°C), 4-*n*-octyloxyphenoxy acetic acid: mp 115°C, 4-*n*-decyloxyphenoxyacetic acid: mp 118°C (TCP mp 62~64°C).

These acids were converted to the corresponding TCP active esters by the general procedure outlined above. Both acids and corresponding TCP active esters had the expected molecular weight (FD-MS) and ^{1}H NMR properties. TCP esters were generally used immediately after a single recrystallization provided purity criteria were met.

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