Eur. J. Med. Chem. **24** (1989) 271–276

Original paper

Adamantylmethyl analogues of chloramphenicol

George B. MULLEN, David M. MARYNIAK, Lesley A. RADOV, Laura A. TRUSSO and Vassil St. GEORGIEV*

Departments of Organic Chemistry and Pharmacology, Fisons Pharmaceuticals, Divisional Research and Development, Rochester, NY 14623, USA

(Received July 12, 1988, accepted December 28, 1988)

Summary — The synthesis and biological activity of novel adamantylmethyl analogues of chloramphenicol are described. Substituting the polar *para*-nitro group on the phenyl ring with a non-polar lipophilic adamantylmethyl moiety resulted in a diminished antimicrobial activity. At a daily dose of 3 mg / kg the *threo*-4-[2′-tricyclo[3.3.1.1^{3,7}]decylidene)-methyl-1-[1″,3″-dihydroxy-2″-(α,α-dichloroacetamido)propyl]benzene caused a significant loss of humoral immunocompetence in the T-dependent Kennedy plaque assay in mice.

Résumé — **Analogues du chloramphénicol avec un reste adamantylméthyle.** *Le remplacement du groupe* p-*nitro polaire sur le noyau phényle par un reste non polaire lipophile adamantylméthyle diminue l'activité antimicrobienne. A une dose journalière de 3 mg / kg, le thréo-[(tricyclo-2′[3.3.1.1^{3,7}]-4-décylidène)méthyl]-4-[dihydroxy-1″, 3″-(α,α-dichloroacétamido)-2″-propyl]benzène entraîne une perte significative d'immunocompétence humorale dans les tests de la plaque Kennedy T-dépendant chez la souris.*

adamantylmethyl analogues of chloramphenicol / *threo*-chloramphenicol / antimicrobial activity / immunosuppressive activity

Introduction

A fermentation product of *Streptomyces venezuelae*, chloramphenicol **1** was the first antibiotic with a broad spectrum of activity against various Gram-positive and Gram-negative bacteria that was introduced into medicinal use [1]. Later, another synthetic analogue, thiamphenicol **2** was also reported to display a broad spectrum of antimicrobial activity [2]. However, it was only the *threo*-diastereomers of both compounds that were found active; the corresponding *erythro*-isomers were devoid of any meaningful therapeutic activity.

Among a number of unwelcome side effects associated with the biological activity of chloramphenicol, the aplastic and hypoplastic anemias and thrombo- and granulocytopenias are considered to be most dangerous. The substitution of the *para*-nitro group on the phenyl ring of chloramphenicol with other substituents (most notably a methylsulfonyl group) was clearly beneficial in decreasing the toxicity of the drug. The great majority of substituents used to replace the *para*-nitro group of **1** represent either other electron-withdrawing non-lipophilic groups (acetyl [3] and perchloryl [4]) or electron-withdrawing but lipophilic functions such as chlorine [5], bromine [5, 6] and fluorine [7–9].

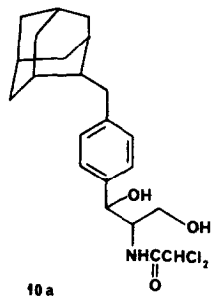
To our knowledge, there are few reports in the literature describing chloramphenicol analogues bearing electron-donating non-polar lipophilic functions. Therefore, it was of interest to prepare such derivatives and investigate the effect, if any, of a large lipophilic moiety on the nature and scope of antimicrobial activity. Based on our previous experience with the adamantane ring system [10–16], we decided to use the adamantylmethyl moiety as the non-polar lipophilic substituent in place of the *para*-nitro group of chloramphenicol.

In the present communication we report the synthesis and biological activity of 2 adamantylmethyl analogues of chloramphenicol, the *threo*- and *erythro*-4-{[2′-tricyclo-[3.3.1.1^{3,7}]decyl]methyl-(1″,3″-dihydroxy-2″-α,α-dichloroacetamido)propyl}benzenes (**10a** and **10b**, respectively), as well as 2 unsaturated analogues, the *threo*- and *erythro*-4-[(2′-tricyclo[3.3.1.1.^{3,7}]decylidene)methyl]-1-[1″,3″-dihydroxy-2″-(α,α-dichloroacetamido)propyl]benzenes **12a** and **12b**, respectively.

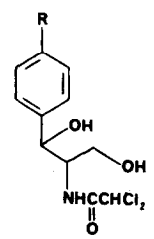
Chemistry and pharmacology

The synthesis of compounds **10a** and **10b** was straightforward, and involved in initial reaction of 2-adamantanone **3** with the Grignard reagent of α-chloro-*para*-xylene to furnish the 2-[(*para*-methylphenyl)methyl]tricyclo[3.3.1.1.^{3,7}]-

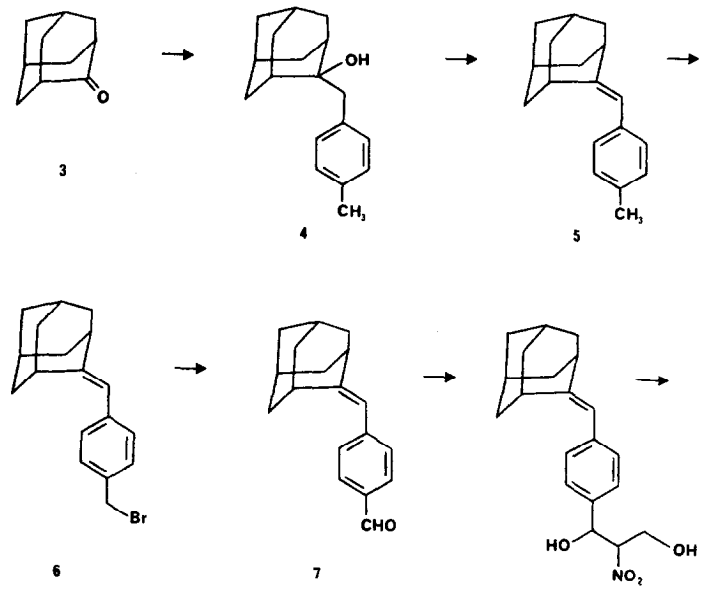
*Author to whom correspondence should be addressed.

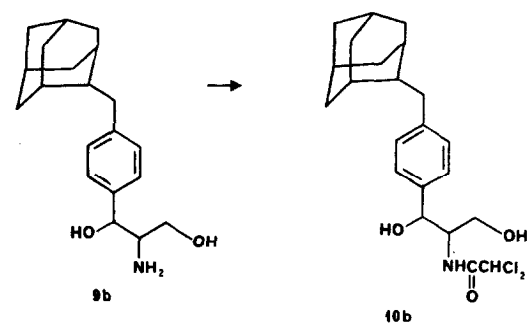


10a



1 R = NO_2
2 R = SO_2CH_3





Scheme 1.

decan-2-ol **4**. Dehydration with 85% phosphoric acid led to the preparation of the corresponding olefin derivative **5**. The latter was brominated, and the resulting bromomethyl compound **6** was oxidized to the 4-[(2′-tricyclo-[3.3.1.$1^{3,7}$]decylidene)methylene]benzaldehyde **7**. Reaction of **7** with 2-nitroethanol yielded the 1,3-dihydroxy-2-nitropropylphenyl derivative **8** as a diastereomeric mixture which was readily separated by flash chromatography on neutral silica gel. The *erythro*-isomer **8b** was hydrogenated over platinum catalyst to provide the corresponding amino analogue **9b**. Treatment of the latter with ethyl dichloroacetate completed the synthesis of *erythro*-4-{[2′-tricyclo[3.3.1.1.^{3,7}]decyl]methyl-(1″,3″-dihydroxy-2″-α,α-dichloroacetamido)propyl}benzene (**10b**; Scheme 1).

The *threo*-diastereomer **10a** was prepared in a similar way, starting with the nitrodiol precursor **8a**.

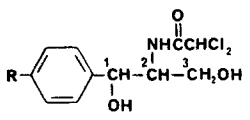
The configuration of the asymmetric centers of **10a** and **10b** was determined by interpretation of their ^{1}H NMR spectra and comparison with the corresponding values for *threo*-chloramphenicol (Table I). As seen from the Table, for *threo*-diastereomer **10a** the coupling constant $J_{1,2}$ (3.30 Hz) compared favorably to that of *threo*-chloramphenicol ($J_{1,2}$ = 2.74 Hz). The corresponding value for $J_{1,2}$ of *erythro*-**10b** was much larger (4.72 Hz). Furthermore, the protons at C-3 of *threo*-**10a** appeared as 2 doublets with coupling constants $J_{2,3}$ = 4.14 Hz, again similar to the pattern observed for the protons at C-3 of *threo*-chloramphenicol (2 doublets with $J_{2,3}$ = 5.46). Contrary to that, the protons at C-3 for the *erythro*-diastereomer **10b** appeared as doublets (ABX pattern) with coupling constants of $J_{2,3}$ = 3.94 and $J_{2,3'}$ = 3.55 Hz, and $J_{3,3'}$ =12.1 Hz.

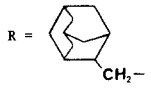
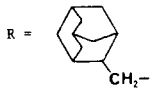
For the preparation of the *erythro*-4-[(2′-tricyclo[3.3.1.1.^{3,7}]-decylidene)methyl]-1-[1″,3″-dihydroxy-2″-(α,α-dichloroacetamido)propyl]benzene **12b**, the nitro group of *erythro*-**8b** was reduced in a selective manner by using aluminum amalgam. The resulting *erythro*-amino analogue **11b** was treated with ethyl dichloroacetate to provide **12b** (Scheme 2). The *threo*-diastereomer **12a** was synthesized in a similar way by starting with *threo*-nitro analogue **8a**.

When screened for antimicrobial activity in the disc diffusion assay, the *threo*-diastereomer **10a** displayed weak activity at 500 μg/disc against *Staphylococcus aureus*, *S. pyogenes* and *Naisseria gonorrhoeae*; by comparison, when tested under similar conditions, cephalothin and gentamycin showed broad spectrum activity at 30 and 10 μg/disc, respectively. In the homogenized broth assay, both **10a** and **10b** were found to be weakly active at 1000 μg/ml against *Proteus vulgaris*, *Fusobacterium necrophorum* and *Clostridium histolyticum*, and *Salmonella pneumoniae*, *Klebsiella pneumoniae*, *Bacteriodes fragilis* and *Clostridium histolyticum*, respectively.

Over the years, the immunosuppressive activity of *threo*-chloramphenicol has been well documented [1]. Therefore, we decided to investigate the 2 unsaturated analogues **12a** and **12b** for potential immunosuppressive activity in the T-dependent Kennedy plaque assay in mice (Table II). As seen from the table, the *erythro*-diastereomer **12b** did not demonstrate any significant immunosup-

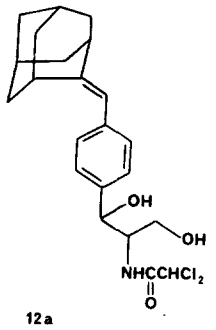
Table I. Assignment of protons in the ^{1}H NMR spectra (200 MHz) of *threo*-**10a**, *erythro*-**10b** and *threo*-chloramphenicol **1**.



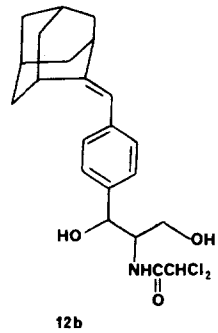
Compound	H(C-1)	H(C-2)	H(C-3)
threo-Chloramphenicol (R = NO_2)[a]	5.40 ppm (1 doublet) $J_{1,2}$ = 2.74 Hz	4.35 ppm (1 multiplet)	4.08 ppm (2 doublets) $J_{2,3}$ = 5.46 Hz
threo-10a R = 	5.11 ppm (1 doublet) $J_{1,2}$ = 3.30 Hz	4.08 ppm (1 multiplet)	3.89 ppm (2 doublets) $J_{2,3}$ = 4.14 Hz
erythro-10b R = 	5.16 ppm (1 doublet) $J_{1,2}$ = 4.72 Hz	4.26 ppm (1 multiplet)	4.06 ppm (1 doublet-doublet) $J_{2,3}$ = 3.94, 12.10 Hz 3.89 ppm (1 doublet-doublet) $J_{2,3}$ = 3.55, 12.04 Hz

[a]There is no report in the literature describing the ^{1}H NMR spectrum of *erythro*-chloramphenicol.

pressive activity. However, the *threo*-analogue **12a** caused a significant loss of humoral immunocompetence at a dose of 3 mg/kg. The immunosuppressive effect of **12a** was evident throughout the dose-range tested, although due to the small sample site and large variability within the samples, only the 3-mg/kg dose was statistically significant when compared to the controls.

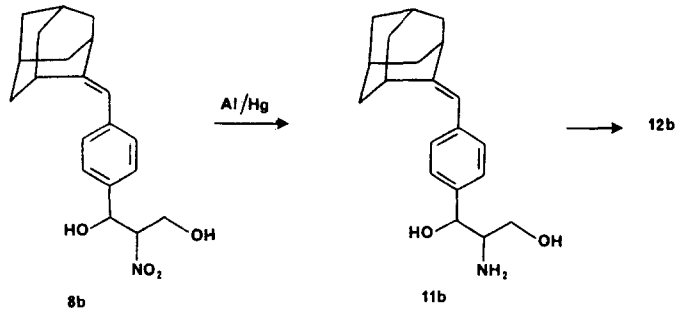


12a



12b

The overall results of the present study have demonstrated that replacing the polar *para*-nitro group on the phenyl ring of chloramphenicol with the non-polar lipophilic adamantylmethyl moiety was detrimental for the antimicrobial activity of the drug [17]. The unsaturated *threo*-analogue **12a** elicited immunosuppressive activity in the T-dependent Kennedy plaque assay in mice.



8b 11b

Scheme 2.

Table II. Effect of *threo*-**12a** and *threo*-**12b** on the humoral immunocompetence of mice sensitized with a T-dependent antigen.

Compound[a]	No. of experiments[b]	Dose (mg/kg)	No. of plaque-forming cells (PFC) / 10^5 splenocytes ± SE	Response (%[c])
Control	3	–	86.8 ± 5.6	100.0
6-Mercaptopurine	3	60.0	44.7 ± 5.5	50.0[d]
threo-**12a**	3	1.0	64.8 ± 5.3	73.0
	3	3.0	62.5 ± 3.6	71.0[d]
	3	6.0	69.0 ± 3.5	78.0
	3	12.0	66.4 ± 2.1	75.0
erythro-**12b**	3	1.0	74.9 ± 4.2	85.0
	3	3.0	88.2 ± 10.0	99.6
	3	6.0	74.8 ± 4.9	84.0
	3	12.0	71.8 ± 5.1	81.0

[a]6-Mercaptopurine was administered intraperitoneally on days 1 and 2 after the antigen (SRBC) challenge. Compounds **10** were administered on days 1, 2 and 3 after the antigen (SRBC) challenge. [b]In each experiment 4 replicate samples were averaged. [c]The observed activity was expressed as a percentage of the control response. [d]The response decreased significantly when compared with the controls; $P \leq 0.050$ using analysis of variance, followed by the Newman–Keuls test.

Experimental protocols

Chemistry

The melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on a Nicolet MX-1 FT spectrometer as KBr discs. The ^{1}H NMR spectra were obtained on a Varian EM-360 A (60 MHz) or a Bruker–IBM 200 Fourier transform (200 MHz) instrument. All spectra were consistent with the assigned structures. Elemental analyses were within the acceptable limits of 0.4% of theory.

2-[(4-Methylphenyl)methyl]tricyclo[3.3.1.1^{3,7}]decan-2-ol **4**
Compound **4** was prepared by a modified procedure of the method of Keul [18] as follows. Under a nitrogen atmosphere, 17.3 g (0.711 mol) of magnesium turnings were covered with 50 ml of anhydrous ether. While stirring vigorously, a solution of 100.0 g (0.711 mol) of α-chloro-*p*-xylene in 300 ml of anhydrous ether was added dropwise over a period of 3 h with external cooling (ice-water bath). The resulting dark-colored suspension was stirred for 30 min, then a solution of 93.8 g (0.624 mol) of 2-adamantanone **3** in 1 liter of anhydrous ether was added over a period of 3 h. The reaction mixture was stirred at room temperature overnight, then quenched by cautious addition of 500 ml of 2 N hydrochloric acid. The organic layer was washed sequentially with water, saturated aqueous sodium chloride and then dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure and crystallization of the resulting solid from pentane gave 144.0 g of alcohol **4** (mp 70–73°C) which was used in the next step without further purification. ^{1}H-NMR (60 MHz, $CDCl_3$): 1.43 ppm (s, 1H, OH); 1.50–2.25 ppm (m, 14H); 2.32 ppm (s, 3H, $ArCH_3$); 2.92 ppm (s, 2H, $ArCH_2$); 7.10 ppm (s, 4H).

2-[(4-Methylphenyl)methylene]tricyclo[3.3.1.1^{3,7}]decane **5**
Compound **5** was obtained by a procedure similar to that described by Keul [18]. Alcohol **4** (131.6 g, 0.513 mol) was suspended in 1 liter of 85% phosphoric acid and the suspension was heated to 120°C for 3 h with vigorous stirring.

After cooling to ambient temperature, the reaction mixture was poured into ice-water that resulted in the precipitation of a white solid. The latter was collected by suction filtration, then taken up in ether and washed with water and saturated aqueous sodium chloride and dried over anhydrous magnesium sulfate. Crystallization from cold acetone furnished 107.1 g of the olefin derivative **5** as white needles melting at 55–57°C. ^{1}H NMR (60 MHz, $CDCl_3$): 1.72–2.07 ppm (m, 12H); 2.32 ppm (s, 3H, $ArCH_3$); 2.37–2.55 ppm (m, 1H, CH-C=C); 3.05–3.25 ppm (m, 1H, CH-C=C); 6.15 ppm (s, 1H, C=CH). Olefin **5** was used in the next step without further purification.

4-[(2'-Tricyclo[3.3.1.1^{3,7}]decylidene)methylene]benzaldehyde **7**
The preparation of benzaldehyde **7** was carried out by a procedure similar to that described by Nace and Monagle [19], as follows. Under a nitrogen atmosphere, 12.58 g (0.071 mol) of *N*-bromosuccinimide and 0.47 g (0.002 mol) of benzoyl peroxide were added successively to a solution of 11.97 g (0.050 mol) of olefin **5** in 150 ml of carbon tetrachloride. The resulting suspension was refluxed for 3 h, then cooled to ambient temperature and filtered to remove the succinimide. The filtrate was washed sequentially with saturated aqueous sodium bicarbonate, water and saturated aqueous sodium chloride, and then dried over anhydrous sodium sulfate. A small sample of the crude bromo derivative **6** was purified by flash chromatography on neutral silica gel using hexane as eluent; mp 41–44°C (methanol). ^{1}H NMR (60 MHz, $CDCl_3$): 1.77–2.10 ppm (m, 12H); 2.40–2.57 ppm (m, 1H, CH-C=C); 3.03–3.23 ppm (m, 1H, CH-C=C); 4.47 ppm (s, 2H, CH_2Br); 6.15 ppm (s, 1H, C=CH); 7.16 ppm (d, 2H, J=8 Hz); 7.31 ppm (d, 2H, J=8 Hz).

The remaining crude bromide **6** was dissolved in 250 ml of anhydrous dimethyl sulfoxide under a nitrogen atmosphere. Sodium bicarbonate (5.06 g, 0.060 mol) was added and the suspension was stirred at 120°C for 3 h. Upon cooling to ambient temperature, the reaction mixture was poured into ice-water and extracted with methylene dichloride. The organic layer was washed with water and saturated aqueous sodium chloride, and then dried over anhydrous magnesium sulfate. The product was purified by flash chromatography on neutral silica gel using hexane–ethyl acetate (19:1) as eluent, to provide (after crystallization from pentane) 8.11 g of aldehyde **7** as light yellow solid, mp 77–78.5°C. Anal. calcd for $C_{18}H_{20}O$: C: 85.67; H: 7.99; found: C: 85.60; H: 8.00. IR (KBr): 2924 (s); 2913 (s); 2848 (m); 1688 (s); 1650 (m); 1600 (s); 1562 (m); 1446 (m); 1305 (m); 1214 (m); 1200 (m); 1167 (m); 875 (m); 841 (m)and 823 (m) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): 1.79–2.01 ppm (m, 12H); 2.53 ppm (m, 1H, CH-C=C); 3.16 ppm (m, 1H, CH-C=C); 6.22 ppm (s, 1H, C=C); 7.34 ppm (d, 2H, J=8.2 Hz); 7.80 ppm (d, 2H, J=8.3 Hz); 9.96 ppm (s, 1H, CHO).

2-{[4-(1,3-Dihydroxy-2-nitropropyl)phenyl]methylene}tricyclo[3.3.1.1^{3,7}]-decane **8**
Compound **7** (9.57 g, 0.0379 mol) was suspended in 50 ml of anhydrous methanol under a nitrogen atmosphere. Then 2-nitroethanol (3.2 ml, 0.0446 mol) was added, followed by a solution of sodium methoxide in methanol (prepared from 1.12 g (0.050 mol) of sodium metal and 10 ml of anhydrous methanol). The resulting solution was stirred at ambient temperature for 1 h, during which time a precipitate formed. The latter was filtered off and the solid was washed first with methanol, and then with ether. Next, the solid was dissolved in 25 ml of glacial acetic acid, diluted with water, and extracted with ether. The organic layer was washed sequentially with water and saturated aqueous sodium chloride, then dried over anhydrous magnesium sulfate, and the solvent removed under reduced pressure. Purification of the residual oil by flash chromatography on neutral silica gel using hexane-ethyl acetate (3:2) as eluent, furnished: (a) 2.38 g of *threo*-**8a** (higher R_f value) as a light yellow oil; (b) 4.13 g of a mixture of *threo*-**8a** and *erythro*-**8b** (lower R_f value); and (c) 3.30 g of *erythro*-**8b** as a light yellow oil; the combined yield of diastereomers **8a** and **8b** was 67%.

threo-**8a**: ^{1}H NMR (60 MHz, $CDCl_3$): 1.70–2.05 ppm (m, 12H); 2.33–2.53 ppm (m, 1H, CH-C=C); 2.98–3.17 ppm (m, 1H, CH-C=C); 3.53–3.87 ppm (m, 2H, 2 OH); 4.50–4.97 ppm (m, 1H, $CHNO_2$); 5.13–5.30 ppm (m, 1H, C*H*-OH); 6.08 ppm (s, 1H, C=CH); 7.17 ppm (s, 4H). *erythro*-**8b**: ^{1}H NMR (60 MHz, $CDCl_3$): 1.77–2.03 ppm (m, 14H); 2.40–2.53 ppm (m, 1H, CH-C=C); 2.98–3.18 ppm (m, 1H, CH-C=C); 4.00–4.25 ppm (m, 2H, CH_2-OH); 4.48–4.77 ppm (m, 1H, $CHNO_2$); 5.38 ppm (d, 1H, J=4 Hz, C*H*-OH); 6.12 ppm (s, 1H, C=CH); 7.20 ppm (s, 4H).

erythro-*4-{[2'-Tricyclo[3.3.1.1^{3,7}]decyl]methyl-(1'',3''-dihydroxy-2''- α,α-dichloroacetamido)propyl}benzene* **10b**
A solution of 0.92 g (2.68 mmol) of *erythro*-**8b** in 25 ml of glacial acetic acid was hydrogenated in a Parr apparatus over 0.12 g of platinum oxide for 6 h. The reaction mixture was filtered through a bed of Celite and the solvent was removed under reduced pressure. The residual oil was suspended in water, then neutralized with potassium carbonate, and extracted with methylene dichloride. The organic extract was washed with water and dried over anhydrous magnesium sulfate. Following the removal of the solvent under reduced pressure, the amine derivative **9b** was obtained as a yellow oil. The crude amine **9b** was suspended in 10 ml of anhydrous methanol and 2.0 ml of ethyl dichloroacetate were added. The mixture was refluxed for 8 h under a nitrogen atmosphere. Upon cooling to ambient temperature the solvent was removed under reduced pressure and the remaining oily material was flash-chromatographed on neutral silica gel using hexane–ethyl acetate (1:1) as eluent. Crystallization from ethyl acetate gave 0.38 g of *erythro*- **10b** as white solid melting at 174–177°C (decomp.). Anal. calcd. for $C_{22}H_{29}NO_3Cl_2$: C: 61.97; H: 6.86; Cl: 16.63; N: 3.29; found: C:61.66; H: 6.95; Cl: 16.30; N: 3.25. IR (KBr): 3520–3180 (br m, OH, NH); 3085 (m); 2904 (s); 2850 (m); 1665 (s); 1579 (m); 1030 (m); and 815 (m) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$/TFA): 1.60–2.05 ppm (m, 16H); 2.76 ppm (d, 2H, J=7.8 Hz, Ar-CH_2-adamantyl); 3.90 ppm (dd, 1H, J=3.5, 12.0 Hz, CH-*H*CH-OH); 4.06 ppm (dd, 1H, J=4.0, 12.0 Hz, CH-HC*H*-OH); 4.26 ppm (ddd, 1H, J=3.5, 4.0, 4.7 Hz, C*H*-NH); 5.16 ppm (d, 1H, J=4.7 Hz, Ar-C*H*-OH); 6.08 ppm (s, 1H, $COCHCl_2$); 7.24 ppm (d, 2H, J=8.4 Hz); 7.26 ppm (s, 1H, NHCO); 7.28 ppm (d, 2H, J=8.4 Hz).

Compounds **8–10** were obtained by procedures similar to those described by Countroulis *et al.* [20].

threo-*4-{[2'-Tricyclo[3.3.1.1.^{3,7}]decyl]methyl-(1'',3''-dihydroxy-2'' -α,α-dichloroacetamido)propyl}benzene* **10a**
Compound **10a** was prepared by a procedure similar to that of **10b**. Yield: 0.55 g (19%) as a light yellow oil following purification by flash chromatography on neutral silica gel using hexane–ethyl acetate (1:1) as eluent. IR (KBr): 3560–3240 (br m, OH, NH); 2906 (s); 2850 (s); 1677 (s); 1558 (m); 1098 (m); 1050 (m); and 809 (m) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): 1.63–2.03 ppm (m, 16H); 2.72 ppm (d, 2H, J=7.8, Ar-

CH_2); 3.89 ppm (d, 2H, J=4.1 Hz, CH_2-OH); 4.08 ppm (dd, 1H, J=3.3, 4.1 Hz, C*H*-NH); 5.10 ppm (d, 1H, J=3.3 Hz, C*H*-OH); 5.84 ppm (s, 1H, $COCHCl_2$); 7.16 ppm (d, 2H, J=8.1 Hz); 7.25 ppm (d, 2H, J=8.1 Hz); 7.26 ppm (s, 1H, NHCO).

erythro-*4-[2'-Tricyclo[3.3.1.1.^{3,7}]decylidene)methyl]-1-[1'',3'' -dihydroxy-2''-(α,α-dichloroacetamido)propyl]benzene* **12b**

Aluminum amalgam was prepared by adding 1.20 g (0.044 mol) of aluminum foil (1/4 inch squares) to a solution of 0.50 g (0.002 mol) of mercuric chloride in 75 ml of water, under a nitrogen atmosphere. After 10 min of stirring at ambient temperature, the solvent was decanted and the amalgam was washed sequentially with water (50 ml), ethanol (50 ml) and ether (50 ml), then covered with ether and cooled in an ice bath. A solution of 1.67 g (0.005 mol) of *erythro*-**8b** in 20 ml of ether was added to the aluminum amalgam and the reaction mixture was warmed to ambient temperature over a 30-min period, then refluxed for 1 h. Upon cooling to room temperature, the mixture was filtered through a bed of Celite and the filtrate was concentrated under reduced pressure to give the amine derivative **11b**. The latter was dissolved in 25 ml of methanol and 10 equiv of ethyl dichloroacetate in 25 ml of methanol were added. The reaction mixture was refluxed under a nitrogen atmosphere for 15 h, then cooled to ambient temperature and concentrated *in vacuo*. Flash chromatography on neutral silica gel using hexane–ethyl acetate (1:1) as eluent provided 0.78 g (56%) of *erythro*-**12b**, mp = 170–172°C (ethyl acetate). Anal. calcd. for $C_{22}H_{27}Cl_2NO_3$: C: 62.27; H. 6.41; Cl: 16.71; N: 3.30; found: C: 62.22; H: 6.45; Cl: 16.56; N: 3.28. IR (KBr): 3560–3180 (br s, OH, NH); 3087 (m); 2910 (s); 2848 (m); 1664 (s); 1578 (m); 1034 (m); 869 (m); and 811 (m) cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6/D_2O): 1.68–1.98 ppm (m, 12H); 2.44–2.51 ppm (m, 1H, CH-C=C); 3.02–3.09 ppm (m, 1H, CH-C=C); 3.50 (ddd, 1H, J=3.9, 5.5, 11.0 Hz, *H*CH-OH); 3.66 ppm (ddd, 1H, J=5.5, 6.1, 11.0 Hz, HC*H*-OH); 3.86 ppm (dddd, 1H, J=3.9, 6.1, 7.2, 8.8 Hz, C*H*-NHCO); 4.61 ppm (dd, 1H, J=4.4, 7.2 Hz, C*H*-OH); 4.72 (t, 1H, J=5.5 Hz, CH_2-O*H*); 5.49 (d, 1H, J=4.4 Hz, CH-O*H*); 6.15 ppm (s, 1H, $COCHCl_2$); 6.34 ppm (s, 1H, C=CH); 7.08 ppm (d, 2H, J=7.7 Hz); 7.24 ppm (d, 2H, J=7.7 Hz); 8.34 ppm (d, 1H, J=8.8 Hz, CH-N*H*CO).

threo-*4-[(2'-Tricyclo[3.3.1.1.^{3,7}]decylidene)methyl]-1-[1'',3''-dihydroxy-2''-(α,α-dichloroacetamido)propyl]benzene* **12a**

Compound **12a** was obtained by a procedure similar to that of **12b**. mp = 162–167°C (ethyl acetate). Anal. calcd. for $C_{22}H_{27}Cl_2NO_3$: C: 62.27; H: 6.41; Cl: 16.71; N: 3.30; found: C: 62.61; H: 6.54; Cl: 16.18; N: 3.23. IR (KBr): 3600–3180 (br s, OH, NH); 2906 (s); 2847 (s); 1675 (s); 1527 (m); 1446 (m); 1060 (m); 1040 (m); and 812 (m) cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6/D_2O): 1.69–1.99 ppm (m, 12H); 2.46–2.52 ppm (m, 1H, CH-C=C); 3.04–3.10 ppm (m, 1H, CH-C=C); 3.31 ppm (ddd, 1H, J=5.5, 6.6, 11.0 Hz, *H*CH-OH); 3.55 ppm (ddd, 1H, J=6.6, 7.2, 11.0 Hz, HC*H*-OH); 3.86 ppm (dddd, 1H, J=3.3, 5.5, 7.2, 7.7 Hz, C*H*-NHCO); 4.87 ppm (t, 1H, J=6.6 Hz, CH_2-OH); 4.88 ppm (dd, 1H, J=3.3, 4.4 Hz, C*H*-OH); 5.67 (d, 1H, J=4.4 Hz, CH-O*H*); 6.15 ppm (s, 1H, $COCHCl_2$); 6.55 ppm (s, 1H, C=CH); 7.08 ppm (d, 2H, J=7.7 Hz); 7.24 ppm (d, 2H, J=7.7 Hz); 8.23 ppm (d, 1H, J=7.7 Hz, CH-N*H*CO).

Pharmacology

In vitro *screening for antimicrobial activity**

The *in vitro* screening of analogues **10a** and **10b** was accomplished by using modified procedures of those described by Cleeland and Grunberg [21].

Disc diffusion test. Discs were prepared by placing 0.02 ml of the test drug at 50 times the desired potency. Filter paper discs (7 mm in diameter) were prepared with 15 drug potencies ranging from 640–1.25 μg/disc. Inocula from at least 4 well isolated colonies of each organism were suspended in a small volume of saline. The suspensions were diluted with saline to match the turbidity of the McFarland standard of 1.0 by using a spectrometer. Agar plates were adjusted to a pH of 7.3 ± 0.2. Then a sterile cotton swab was dipped into the inoculum and the excess removed by rotating the swab against the inside wall of the tube. The entire surface of the agar plate was inoculated, and 15 discs were placed on the plate not more than 15 min after the time of inoculation. Potencies of the discs ranged from 0–640 μg (the 0-μg disc was used as the control). After 24 h of incubation at 37°C, the plates were examined and the diameter of the zone of growth inhibition was recorded in mm.

Broth dilution test

The bacteria were grown overnight in broth, diluted to 10^{-2} or 10^{-1}, and adjusted to a pH of 7.3 ± 0.2. *B. fragilis* and *C. hystolyticum* were grown overnight at 37°C in cooked meat media and then diluted to 10^{-1}. Fifteen serial 2-fold dilutions of the test drugs were made in 2-ml vol of broth resulting in final concentrations ranging from 100–0.006 μg/ml. All tubes containing test compounds (including a drug-free control tube) were inoculated with 0.05 ml of inoculum. Since dimethyl sulfoxide was used as solvent in the preparation of the drug samples, a solvent control tube was also inoculated (a media control tube was not inoculated). After incubation at 37°C for 18–24 h, each tube was examined for visible growth. The lowest concentration at which no visible growth appeared was determined as the minimum concentration (MIC) in μG/ml.

Kennedy plaque assay

The humoral immunocompetence was measured using a procedure similar to that described by Garvey *et al.* [22]. For the IgM production in female C3H mice, the T-dependent antigen sheep erythrocytes (SRBC; 0.2 ml of a 20% suspension) were administered intraperitoneally (ip) on day 0. The mice were treated with the test compounds (1.0–12.0 mg/kg, ip), once daily on days +1, +2 and +3. Twenty-four hours after the final doses were implemented, the animals were sacrificed, the spleens were removed aseptically, and the number of plaque-forming cells (PFC) were enumerated. The statistical significance (P value) was determined by the analysis of variance, followed by the Newman–Keuls test [23]. The higher (6 and 12 mg/kg) and lower (1.0 mg/kg) doses for *threo*-**10a** are not statistically significant since their P values were at the 0.055 level and just over the accepted limit of P = 0.050 – a comparison of the observed immunosuppression at the 4 dose levels using the analysis of variance and the Newman–Keuls test showed that, in fact, the biological activity of *threo*-**10a** at all 4 levels may be considered quantitatively similar and thus not dose-related. Due to inadequate quantities, compounds **10a** and **10b** were not evaluated in this assay.

*The *in vitro* antimicrobial screening of analogues **10a** and **10b** was carried out by Dr. Stanley D. Allen in the Laboratory Animal Research Center at the Utah State University, Logan, Utah.

References

1 Georgiev V. St. (1983) *in: Survey of Drug Research in Immunologic Disease*. S. Karger, Basel, vol. 2, pp. 138–152
2 Greogiev V. St. (1983) *in: Survey of Research in Immunologic Disease*. S. Karger, Basel, vol. 2, pp. 153–156
3 Georgiev V. St. (1983) *in: Survey of Drug Research in Immunologic Disease*. S. Karger, Basel, vol. 2, pp. 137–138
4 Ziebell G., Gross H. & Bradley G. (1983) *Pharmazie* 38, 587
5 Badiger V.V. & Jamakhandi M.Y.M. (1977) *J. Indian Chem. Soc.* 54, 391
6 Badiger V.V. & Jamakhandi M.Y.M. (1971) *Indian J. Chem.* 9, 198
7 Neu H.C., Fu K.P. & Kung K. (1980) *Curr. Chemother. Infect. Dis. Proceedings 11th Int. Congr. Chemother.* (J.D. Nelson & C. Grassi, eds.), American Society of Microbiology, Washington DC, pp. 446–447
8 Schafer T.W., Moss E.L., Jr., Nagabhushan T.L. & Miller G.H. (1980) *Curr. Chemother. Infect. Dis. Proceedings 11th Int. Congr. Chemother.* (Nelson J.D. & Grassi C., eds.), American Society of Microbiology, Washington DC, pp. 444–446
9 Ziebell G., Bradler G., Nagel T. & Scholz M. (1978) *Abh. Akad. Wiss. DDR Abt. Math. Naturwiss. Tech.* 233
10 Georgiev V. St., Radov L.A., Kinsolving C.R., Griffith R.C., Zazulak W.I., Kamp D.K., Trusso L.A. & Mack R.A. (1986) *Eur. J. Med. Chem. Chim. Ther.* 21, 315
11 Georgiev V. St., Mullen G.B. & Acker C.G. (1986) *Heterocycles* 24, 751
12 Mullen G.B. & Georgiev V. St. (1986) *Heterocycles* 24, 3441
13 Mullen G.B. & Georgiev V. St. (1986) *Heterocycles* 24, 3447
14 Georgiev V. St., Acker C.G. & Kinsolving C.R. (1987) *Heterocycles* 26, 469
15 Georgiev V. St., Bennett G.A., Radov L.A., Kamp D.K. & Trusso L.A. (1987) *Arch. Pharm. (Weinheim)* 320, 465
16 Mullen G.B., Swift P.A. & Georgiev V. St. (1987) *J. Pharm. Sci.* 76, 930
17 Mullen G.B. & Georgiev V. St. (1988) *J. Pharm. Sci.* 77, 643 (preliminary communication)

18 Keul H. (1975) *Chem. Ber.* 108, 1198
19 Nace H.K. & Monagle J.J. (1959) *J. Org. Chem.* 24, 1792
20 Countroulis J., Rebstrock M.C. & Crooks H.M., Jr. (1949) *J. Am. Chem. Soc.* 71, 2463
21 Cleeland R. & Grunberg E. (1980) *Antibiotics in Laboratory Medicine* (V. Lorian, ed.), Williams & Wilkins, Baltimore
22 Garvey J.S., Cremer N.E. & Sussdorf D.H. (1977) *Methods in Immunology*, Benjamin, Reading, Massachusetts, pp. 424–428
23 Miller R.G., Jr. (1981) *Simultaneous Statistical Inference*. Springer-Verlag, NY, pp. 81–90