Tetrahedron Vol 45 No 16 pp 4939 to 4944, 1989
Printed in Great Britain

0040-4020/89 $3 00+ 00

THE TOTAL SYNTHESIS OF BISUCABERIN

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(*Received in USA 28 December* 1988)

Abstract The first total synthesis of 6,17-dihydroxy-1,6,12,17-tetraazacyclodocosane-2,5,13,16-tetrone (bisucaberin) is presented The synthetic scheme employed in this study illustrates the utility of O-benzyl-N-(*tert*-butoxycarbonyl)-N-(4-cyanobutyl)hydroxylamine 3, as an intermediate in the synthesis of both bisucaberin and desferrioxamine B The bisucaberin macrocyclic lactam precursor, a linear ω-amino acid 10, is constructed from the intermediates 3 and O-benzyl-N-(4-cyanobutyl)hydroxylamine 4, utilizing a series of sequential acylations and nitrile reductions Cyclization of 10 generates the 22 membered ring, 6,17-dibenzylbisucaberin 11 Deprotection of the hydroxamates in the last step affords the natural product, bisucaberin 1

Siderophores are a group of low molecular weight iron chelators produced by microorganisms for the purpose of accessing environmental iron, iron which exists largely in the insoluble ferric state Although there have been a substantial number of siderophores isolated and characterized, generally they fall into two main classes of metal ligands the catecholamides, e g parabactin, and the hydroxamates, e g desferrioxamine B[1]

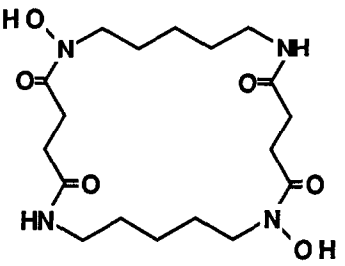
A large ring chelator belonging to the latter class of compounds has recently been isolated and characterized The structure of this dihydroxamate ligand, bisucaberin 1 (Figure 1) was determined through spectroscopy and X-ray crystallography[2] to be a 22 membered ring, containing two hydroxamate and two secondary amide functional groups Retrosynthetic analysis of this cyclic tetracoordinate chelator reveals that the molecule can be segmented into two repeating units, succinic acid and 1-amino-5-(hydroxyamino)pentane, i e , N-hydroxycadaverine The arrangement is such that the hydroxamate coordination sites are at opposite sides of the ring

The microbial iron chelator, bisucaberin 1 (Figure 1), isolated from the marine bacterium *Alteromonas haloplanktis*, was found to slow the growth of both L-1210 and IMC carcinoma cells with IC_{50}'s of 9 7 and 12 7 μM, respectively,[3,4] and to sensitize tumor cells to macrophage promoted cytolysis [3]

Interestingly, the biological activity of bisucaberin 1 is absent in nocardamine, desferrioxamine E, the analogous 33 membered trihydroxamate cyclic siderophore [3,5] Both bisucaberin and nocardamine bind with ferric ion to form red, water soluble complexes [3] This dichotomy in biological activity encouraged us to pursue a synthetic approach to 1 which would allow us to access both the parent molecule as well as structurally related species The long term goal of the program is aimed at the the evaluation of structure-activity relationships, which might shed light on the mechanism of bisucaberin's activity

Results and Discussion

Our total synthesis of chelator 1 is presented in Schemes 1 and 2 The construction of the acyclic precursor amino acid 10 is a unique example of the applicability of the intermediates and methodologies developed in our total synthesis of desferrioxamine B[6] to the production of additional hydroxamate ligands Furthermore, the bisucaberin synthesis offers an



BISUCABERIN 1

Figure 1

$PhCH_2ONH_2 \cdot HCl$

↓

$PhCH_2ONHBOC$ 2

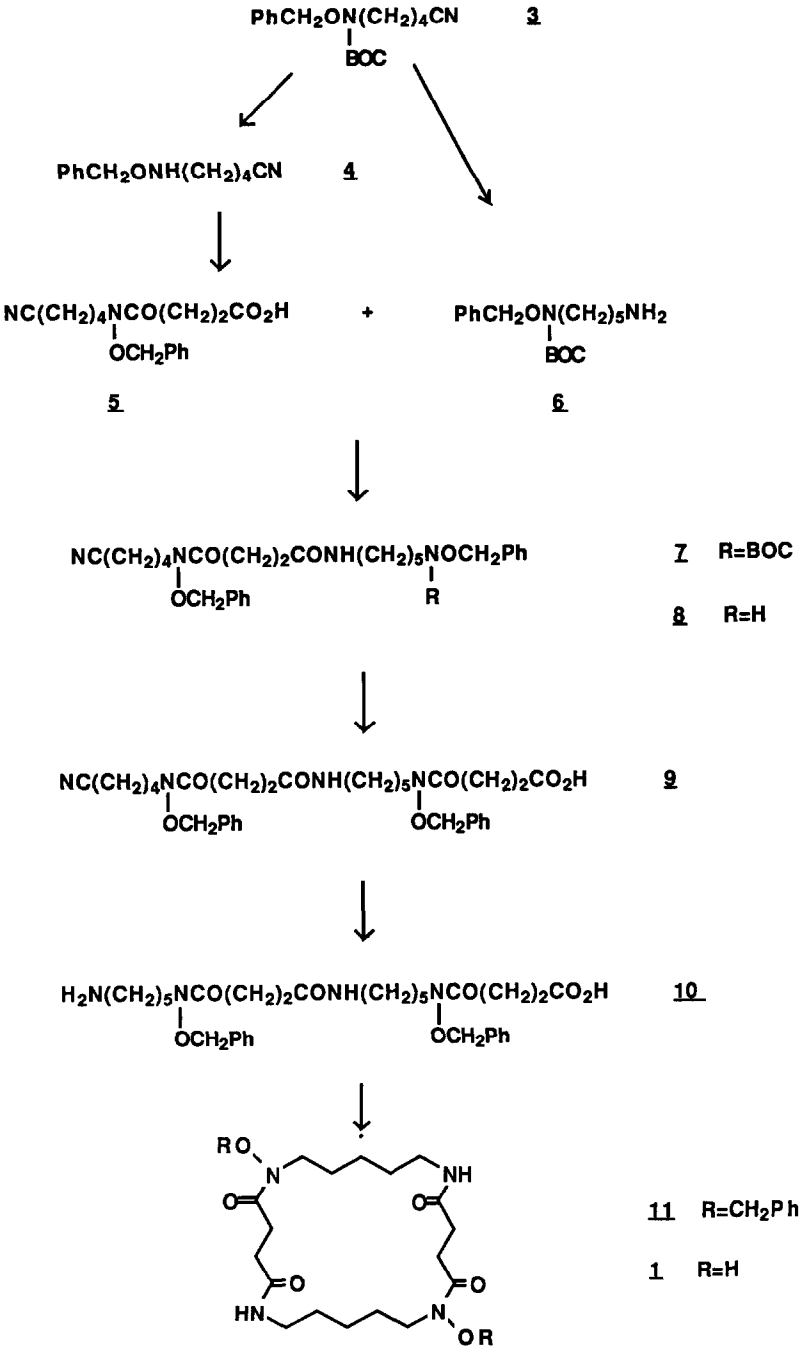
↓

$PhCH_2ON(CH_2)_4CN$ 3
|
BOC

↓

$PhCH_2ONH(CH_2)_4CN$ 4

Scheme 1


PhCH2ON(CH2)4CN
BOC
3
PhCH2ONH(CH2)4CN
4
NC(CH2)4NCO(CH2)2CO2H
OCH2Ph
5
+
PhCH2ON(CH2)5NH2
BOC
6
NC(CH2)4NCO(CH2)2CONH(CH2)5NOCH2Ph
OCH2Ph
R
7 R=BOC
8 R=H
NC(CH2)4NCO(CH2)2CONH(CH2)5NCO(CH2)2CO2H
OCH2Ph
OCH2Ph
9
H2N(CH2)5NCO(CH2)2CONH(CH2)5NCO(CH2)2CO2H
OCH2Ph
OCH2Ph
10
RO
N
NH
O
O
O
O
HN
N
OR
11 R=CH2Ph
1 R=H

Scheme 2

alternative method for accessing aminonitrile 4, a key starting material for both desferrioxamine B and bisucaberin (Scheme 1)

The synthesis of nitrile 4 begins with the conversion of O-benzylhydroxylamine hydrochloride to its crystalline N-(*tert*-butoxycarbonyl) derivative 2 [7,8] This is accomplished by reaction of the hydroxylamine salt with di-*tert*-butyl dicarbonate (NEt_3, aqueous THF) The carbamate 2 thus readily obtained is next N-alkylated with 5-chlorovaleronitrile (NaH, DMF, NaI) to give intermediated nitrile 3 in 87% yield Exposure of 3 to trifluoroacetic acid (TFA) results in collapse of 3 to carbon dioxide, isobutylene and O-benzyl-N-(4-cyanobutyl)hydroxylamine 4 in 75% yield The nitrile 4 has been previously utilized in our laboratories in the total synthesis of desferrioxamine B,[6] however the present route to this compound is an efficient alternative to our previous method The earlier synthesis required the preparation of 4-cyanobutanal and its reductive amination by O-benzylhydroxylamine hydrochloride using sodium cyanoborohydride [6] This aldehyde is only accessible in low yield and is somewhat unstable Next, amine 4 is acylated upon heating with succinic anhydride in pyridine to yield nitrile acid 5 as before[6] (Scheme 2)

N-(*tert*-butoxycarbonyl) nitrile 3 is selectively hydrogenated to generate primary amine 6 in 83% yield with W-2 grade Raney nickel in methanolic ammonia Coupling of acid 5 with primary amine 6 (DCC, catalytic DMAP) affords nitrile 7 (65%) In terms of the basic synthon construct, only the second succinate unit remains to be annealed

The *tert*-butoxycarbonyl group of 7 is cleaved by brief exposure of the molecule to trifluoroacetic acid to give secondary amine 8 (83%) This amine is next acylated with succinic anhydride in pyridine to afford nitrile-acid 9 in 96% yield, thus completing the framework of the molecule Hydrogenation of the nitrile group in 9 (Raney nickel, methanolic ammonia) furnishes ω-amino acid 10 in 65% yield Thus the acyclic precursor to bisucaberin 1 has been formed efficiently by adapting methodologies developed for our desferrioxamine B synthesis

At this point all that remained was the cyclization of 10 and the unmasking of the hydroxamates Of the many methods available for the cyclization of large ring lactams, the Yamada reagent,[9] that is diphenylphosphoryl azide, offered the most promise Diphenylphosphoryl azide was introduced as a peptide coupling reagent and has recently proved useful in the formation of functionalized macrocyclic lactams, which are similar in size to ours [10] Thus, the azide reagent (1 2 equivalent) was added to a solution of amino acid 10 in DMF followed by stirring for three days at 0 °C The hydroxamate protected macrocycle, 6,17-dibenzylbisucaberin 11 was obtained in 43% chromatographed yield Finally, the benzyl groups of 11 were cleanly removed under a hydrogen atmosphere (10% Pd-C, CH_3OH, 1 atm) to give bisucaberin 1 The final product was identical with a sample of natural bisucaberin[11] by silica gel TLC and high field ^1H and ^{13}C NMR

Our synthetic route permits the efficient production of homologues of bisucaberin 1 The length of each of the four methylene chains in cyclic chelator 1 can be varied in order to tailor its size to a given metal and to determine structure-activity relationships N-(*tert*-butoxycarbonyl) amine 2 can be monoalkylated as in Scheme 1 with commercially available $Cl(CH_2)_nCN$, n=1-3, to shorten either N-hydroxydiamine chain or with 7-bromoheptanenitrile to lengthen it Moreover, acylation of amine 4 with glutaric anhydride in place of succinic anhydride would give the homologue of nitrile acid 5 Thus the length of each methylene chain in key segments 5 and 6 in Scheme 2 could be adjusted to provide a variety of both symmetrical and unsymmetrical bisucaberin homologues Currently we are measuring the Fe(III) and Cu(II) bisucaberin metal binding constants as well as evaluating the mechanism of the compound's biological action

Experimental

All reagents were purchased from Aldrich Chemical Company and were used without further purification Sodium sulfate was employed as a drying agent, and Fisher Optima grade solvents were routinely used Melting points are uncorrected Silica gel 60 (70-230 mesh), obtained from EM Science, Darmstadt, West Germany, was used for column chromatography Preparative layer chromatography was carried out on silica gel GF plates (2 mm thick) purchased from

Analtech, Newark, DE Proton NMR spectra were recorded on a Varian EM-390 or a Nicolet NT-300 instrument and, unless otherwise noted, were run in $CDCl_3$ with chemical shifts given in parts per million downfield from an internal tetramethylsilane standard (coupling constants are in hertz) Chemical shifts are reported with HOD (δ 4.8) as the standard for samples run in D_2O IR spectra were recorded on a Perkin-Elmer 1420 Ratio Recording Infrared or a Beckman AccuLab 3 spectrophotometer Mass spectra were carried out on a Kratos MS 80 or a Fennigan 4516 instrument Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA

O-Benzyl-N-(*tert*-butoxycarbonyl)hydroxylamine (2) was prepared from O-benzylhydroxylamine hydrochloride and di-*tert*-butyl dicarbonate (NEt_3, aqueous THF) by the literature method mp 46-48 °C (lit 45-47 °C) [8]

O-Benzyl-N-(*tert*-butoxycarbonyl)-N-(4-cyanobutyl)hydroxylamine (3). Sodium iodide (84 mg, 0 56 mmol) and then sodium hydride (80% oil dispersion, 0 49 g, 16 3 mmol) were added to 2 (2 68 g, 12 0 mmol) in dry DMF (40 mL) After stirring for 15 min, 5-chlorovaleronitrile (1 5 mL, 13 3 mmol) was added, and the suspension heated at 80-85 °C for 4 h under argon After cooling, the reaction was quenched with H_2O (100 mL), then extracted with ether (4x75 mL) The combined organic layers were washed with 100 mL each of 1% aqueous Na_2SO_3, H_2O and brine and then concentrated to give 4 39 g crude product Column chromatography with 4 5% EtOAc/$CHCl_3$ produced 3 17 g of 3 (87% yield) NMR δ 1 5-1 75 (s+m,13 H), 2 3 (t, 2 H), 3 4 (t, 2 H), 4 77 (s, 2 H), 7 3 (s, 5 H) Anal calcd for $C_{17}H_{24}N_2O_3$ C, 67 08, H, 7 95, N, 9 20 Found C, 67 19, H, 7 99, N, 9 11

O-Benzyl-N-(4-cyanobutyl)hydroxylamine (4). Trifluoroacetic acid (TFA, 16 mL) was added to 3 (2 59 g, 8 51 mmol), and the solution stirred at room temperature for 20 min (Drierite tube) Excess TFA was removed on the rotovap, saturated $NaHCO_3$ (50 mL) was added and the product was extracted into ether (3x50 mL) After a brine wash (50 mL), the organic extracts were concentrated to yield 1 77 g crude product Column chromatography with 3% EtOH/$CHCl_3$ furnished 1 31 g of 4 (75% yield), which has been previously prepared [6] NMR δ 1 5-1 7 (m, 4 H), 2 16-2 35 (m, 2 H), 2 78-2 98 (m, 2 H), 4 66 (s, 2 H), 5 45 (br s, 1 H), 7 28 (s, 5 H)

N-(Benzyloxy)-N-(4-cyanobutyl)succinamic acid (5) was prepared by the method developed in this laboratory [6] A sample of 5 was crystallized from n-hexane/ethyl acetate, mp 71 °C

O-Benzyl-N-(5-aminopentyl)-N-(*tert*-butoxycarbonyl)hydroxylamine (6). Raney nickel (W-2 grade, 0 98 g) and concentrated NH_4OH (1 6 mL) were added to a solution of 3 (0 57 g, 1 87 mmol) in methanol (9 5 mL) in a 250 mL Parr bottle The suspension was cooled to 0 °C, and ammonia was gently bubbled in for 10 min Hydrogenation was carried out on a Parr shaker for 3 5 h at 55-58 psi The catalyst was filtered off (Celite), and the filtrate was concentrated to give 0 61 g crude product Column chromatography using 5% concentrated NH_4OH/CH_3OH yielded 0 48 g of 6 (83% yield) NMR δ 1 25-1 72 (m, 17 H), 2 64 (t, 2 H, J=7), 3 39 (t, 2 H, J=7), 4 78 (s, 2 H), 7 31 (s, 5 H) Anal calcd for $C_{17}H_{28}N_2O_3$ C, 66 21, H, 9 15, N, 9 08 Found C, 66 24, H, 9 18, N, 9 05

N-(4-Cyanobutyl)-3-[[5-[(benzyloxy)-*tert*-butoxy-carbonylamino]pentyl]carbamoyl]-O-benzylpropionohydroxamic acid (7). A solution of 5 (0 52 g, 1 71 mmol) in dry CH_2Cl_2 (50 mL) was added to 6 (0 48 g, 1 56 mmol) and 4-dimethylaminopyridine (DMAP, 31 mg, 0 25 mmol) After cooling the solution to 0 °C under N_2, 1,3-dicyclohexylcarbodiimide (DCC, 0 39 g, 1 89 mmol) was introduced, and stirring was continued for 45 min at 0 °C, then for 19 h at room temperature After cooling the mixture to 0 °C, the solid was filtered off and washed with CH_2Cl_2 (4x10 mL) The filtrate ceded 1 38 g material, which was chromatographed eluting with 5% EtOH/$CHCl_3$ to give 0 93 g product Water (25 mL) was added, followed by extraction with $CHCl_3$ (4x25 mL) The combined organic extracts were washed with 30 mL each 5% $NaHCO_3$ (2x) and H_2O, and evaporated to give 0 95 g product Column chromatography using 4% EtOH/$CHCl_3$, followed by preparative layer chromatography on part of the eluted material gave 0 72 g product, which still contained DCU This was dissolved in $CHCl_3$ and filtered (Celite, 2x) to give 0 600 g of 7, for a 65% yield NMR δ 1 18-1 88 (m, 19 H), 2 19-2 52 (m, 4 H), 2 77 (t, 2 H, J=7), 3 01-3 72 (m, 6 H), 4 78 and 4 82 (2 s, 4 H), 5 84 (br s, 1 H), 7 2-7 4 (m, 10 H) A sample of 7 (38 mg) was subjected to preparative layer chromatography (4% EtOH/$CHCl_3$) to give an analytical sample of 7 (28 mg) Anal calcd for $C_{33}H_{46}N_4O_6$ C, 66 64, H, 7 80, N, 9 42 Found C, 66 48, H, 7 88, N, 9 39

N-(4-Cyanobutyl)-3-[[5-[(benzyloxy)amino]pentyl]carbamoyl]-O-benzylpropionohydroxamic acid (8). TFA (9 mL) was added to 7 (0 560 g, 0 94 mmol), and the solution stirred for 20 min at room temperature After removal of the excess TFA, saturated $NaHCO_3$ (35 mL) was added to the residue, which was extracted with $CHCl_3$ (3x60 mL) The organic phase was washed with H_2O (50 mL) and concentrated to give 0 51 g crude product Preparative layer chromatography (2 plates) using 4% EtOH/$CHCl_3$ gave 0 388 g of 8 (83%) NMR δ 1 2-1 8 (m, 10 H), 2 2-3 3 (m, 10 H), 3 62 (t, 2 H, J=7), 4 63 (s, 2 H), 4 82 (s, 2 H), 5 5 (br s, 1 H), 5 87 (br s, 1H), 7 15-7 35 (m, 10 H) Anal calcd for $C_{28}H_{38}N_4O_4$ C, 67 99, H, 7 74, N, 11 33 Found C, 67 82, H, 7 79, N, 11 29

5,16-Bis(benzyloxy)-20-cyano-4,12,15-trioxo-5,11,16-triazaeicosanoic acid (9) A solution of 8 (0 365 g, 0 738 mmol) and succinic anhydride (0 113 g, 1 13 mmol) in pyridine (10 mL) was heated at 106 °C for 1 5 h under N_2 After removing the pyridine *in vacuo*, the residue was diluted with ether (25 mL), and then extracted with saturated $NaHCO_3$ (3x25 mL) The combined aqueous portion was extracted further with ether (2x25 mL), then cautiously acidified with cold 6 N HCl (20 mL) Extraction with $CHCl_3$ (3x40 mL), followed by a final water wash (40 mL) and solvent removal led to 0 424 g of 9 (96% yield) NMR δ 1 25-1 84 (m, 10 H), 2 2-2 92 (m, 10 H), 3 05-3 33 (m, 2 H), 3 5-3 8 (m, 4 H), 4 80 and 4 83 (2 s, 4 H), 6 6 (br s, 1 H), 7 34 (s, 10 H) Anal calcd for $C_{32}H_{42}N_4O_7$ C, 64 63, H, 7 12, N, 9 42 Found C, 64 70, H, 7 16, N, 9 39

21-Amino-5,16-bis(benzyloxy)-4,12,15-trioxo-5,11,16,-triazaheneicosanoic acid (10) Raney nickel (W-2 grade, 0 58 g) and concentrated NH_4OH (1 6 mL) were added to 9 (0 402 g, 0 676 mmol) in CH_3OH (10 mL) in a 250

mL Parr bottle A stream of ammonia was bubbled through the mixture at 0 °C for 10 min Hydrogenation at 55-58 psi was carried out on a Parr shaker for 3 h The catalyst was filtered off (Celite), the solids were washed with CH_3OH, and solvent was stripped off to give 0 45 g crude product Purification by column chromatography (0 4% concentrated NH_4OH/CH_3OH) furnished 0 265 g of 10 as an amorphous white solid for a 65% yield NMR (D_2O) δ 1 12-1 88 (m, 12 H), 2 27-3 27 (m, 12 H), 3 54-3 85 (m, 4 H), 4 97 (s, 4 H), 7 5 (s, 10 H) Anal calcd for $C_{32}H_{46}N_4O_7$ C, 64 19, H, 7 74, N, 9 36 Found C, 63 96, H, 7 75, N, 9 29

6,17-Bis(benzyloxy)-1,6,12,17-tetraazacyclodocosane-2,5,13,16-tetrone (11). Amino acid 10 (0 227 g, 0 379 mmol) was dissolved in distilled DMF (50 mL) and the solution was cooled to 0 °C under N_2 Diphenylphosphoryl azide (0 10 mL, 0 46 mmol) was added by syringe, and the solution was stirred at 0-5 °C for 3 days After removal of the bulk of the solvent *in vacuo*, water (15 mL) was added, and the product was extracted with $CHCl_3$ (4x15 mL) The organic extracts were washed with water (15 mL) and concentrated to give 0 46 g product Preparative layer chromatography (2 plates), eluting with 4% EtOH/$CHCl_3$, produced 0 095 g 11 (43%) NMR (300 MHz) δ 1 1-1 9 (m, 12 H), 2 52 (t, 4 H, J=7), 2 82 (t, 4 H, J=7), 3 08-3 2 (m, 4 H), 3 6-3 75 (m, 4 H), 4 79 (s, 4 H), 6 61 (br s, 2 H), 7 32 (m, 10 H), EIMS calcd for $C_{32}H_{44}N_4O_6$ 580, found 580 (M) Anal calcd for $C_{32}H_{44}N_4O_6$ C, 66 19, H, 7 64, N, 9 65 Found C, 66 03, H, 7 68, N, 9 55 A sample of 11 was recrystallized from ethyl acetate/ethanol, mp 203-204 °C

6,17-Dihydroxy-1,6,12,17-tetraazacyclodocosane-2,5,13,16-tetrone (bisucaberin) (1) Glassware was soaked in 3 N HCl, rinsed with distilled water and CH_3OH and oven dried Compound 11 (0 078 g, 0 134 mmol) was suspended in CH_3OH (50 mL), 10% Pd-C (0 11 g) was added and hydrogenation was carried out at 1 atm for 6 h The reaction mixture was warmed and the catalyst was filtered and rinsed with hot CH_3OH Solvent removal afforded 48 mg of 1 (89%) Silica gel TLC (10% $CH_3OH/CHCl_3$) showed a red spot (ethanolic $FeCl_3$ staining) with R_f=0 54, R_f (lit)=0 52 [2] The sample (42 mg) was recrystallized from CH_3OH to give 29 mg crystalline 1 mp 208 5-210 °C (dec), mp (lit) 180 °C (dec),[2] IR (KBr) 3290, 3130, 2960, 2890, 1635, 1600, cm^{-1}, NMR (d_6-DMSO, 300 MHz) δ 1 07-1 6 (m, 12 H), 2 28 (t, 4 H, J=7), 2 59 (t, 4 H, J=7), 2 97-3 07 (m, 4 H), 3 49 (t, 4 H, J=6), 7 65 (m, 2 H), 9 57 (s, 2 H), ^{13}C NMR (d_6-DMSO, 75 MHz) δ 22 44, 25 50, 27 79, 28 15, 30 56, 37 91, 39 50 (septuplet, d_6-DMSO standard), 46 32, 171 45 (C=O), 171 80 (C=O), FABMS calcd for $C_{18}H_{32}N_4O_6$ 400, found 401 (M+1) Anal calcd for $C_{18}H_{32}N_4O_6$ C, 53 99, H, 8 05, N, 13 99 Found C, 54 03, H, 8 12, N, 13 90 The high field NMR spectra were identical to those of authentic 1 [11] The two samples were identical by cospotting on silica gel TLC (20% EtOH/$CHCl_3$, 10% $CH_3OH/CHCl_3$ and EtOAc)

Acknowledgments We thank Dr Yoshiro Okami of the Institute of Microbial Chemistry, Tokyo, Japan, for providing a sample of bisucaberin Financial support was provided by the National Institutes of Health, Grant GM-34897-05

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