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Absolute Stereochemistry of the Diepoxins

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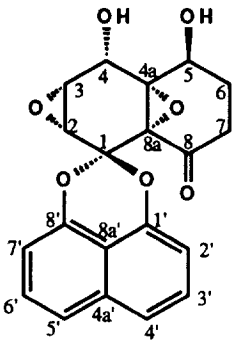
Abstract: *The exciton coupled CD method has been applied to determine the absolute configuration of the diepoxins, spiroketal-linked naphthodiepoxydecalinones of fungal origin. The CD spectra of the bis-dimethylaminobenzoate derivatives of the diepoxins η, ι and κ, reveal a positive chiral twist between the two substituted hydroxyl groups and thus infer the S configuration at both of these stereogenic centers. The absolute configuration of the remaining chiral centers is deduced from their relative configurations as established by X-ray diffraction of diepoxin κ. The twist boat conformation of the epoxycyclohexanone ring and the continued axial orientation of the substituents at C-4 and C-5 after dimethylaminobenzoate derivatization was corroborated by ^{1}H-NMR coupling constants.*

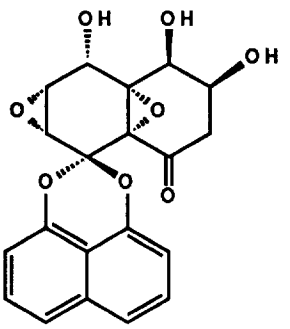
INTRODUCTION

Extracts from fermentation broths of a non-sporulating fungus, culture LL-07F275, contain novel metabolites with antifungal and antibacterial activity, antibiotic 07F275[1] and the diepoxins[2]. The latter represent a new class of biologically active compounds featuring the linkage of a naphthalene moiety to a decalin system via a spiroketal bridge. Members of this family differ from each other in their oxidation pattern and degree of oxygenation (Figure 1).
New components of this class have been discovered recently because of their broad range of biological activities. The diepoxins close relationship to the previously known antifungal preussomerins[3,4] was revealed in a publication covering the deoxypreussomerins[4], demonstrating that the structurally congruent deoxypreussomerins are precursors to the preussomerins. The deoxypreussomerins A and B are apparently identical with palmarumycin C_2 and CP_2, respectively, which are members of the palmarumycin[5] family, novel metabolites obtained from endophytic fungi, e.g. *Coniothyrium palmarum*. Two other compounds in this series, palmarumycins C_{14} and C_{13}, are reported to be identical with the diepoxins η(**1**) and ζ(**4**) respectively, although optical rotations of these particular palmarumycins were not published[5b]. Diepoxins η(**1**) and ζ(**4**) are identical with the antitumor compounds[6] SCH 53516

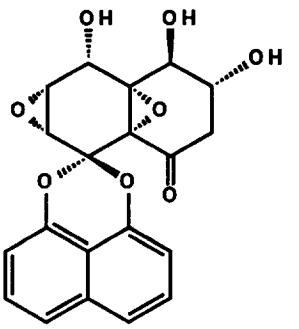
and SCH 53514, metabolites of the fungus *Nattrassia mangiferae*, as judged from published spectroscopic data including their positive optical rotations. The structure of cladospirone bisepoxide, a metabolite produced by the fungus *Cladosporium chlorocephalum*, initially published as a regio-isomer[7] of diepoxin ζ, was recently revised and presumed to be identical[8] with diepoxin ζ(4).



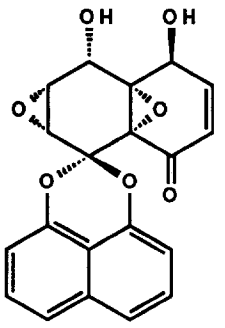
diepoxin η (1)



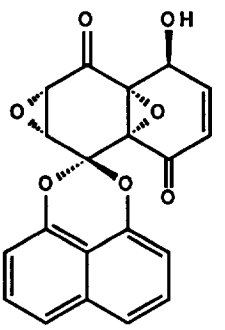
diepoxin γ (2)



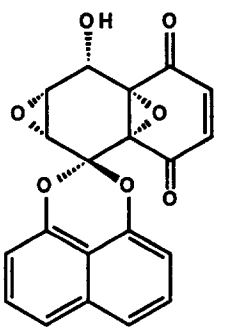
diepoxin δ (3)



diepoxin ζ (4)



diepoxin σ (5)



diepoxin ϕ (6)

Figure 1: Structures of selected diepoxins

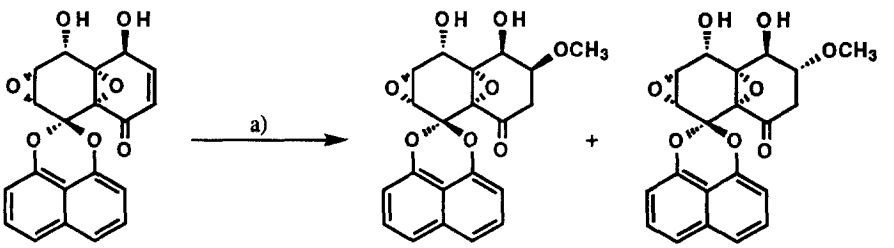
Although numerous diepoxin-type compounds have been reported over the last two-year period, their structures comprise relative configurations only and their absolute configurations remained unknown. The first report on the absolute configuration of a member[8] of this class of spiroketal linked bisdecalins appeared while this manuscript was being prepared. The absolute configuration of cladospirone bisepoxide was deduced from X-ray data of one of its derivatives bearing an iodine atom. Unfortunately, the optical rotation for cladospirone bisepoxide has not been reported and the possibility of cladospirone bisepoxide

and diepoxin ζ representing enantiomeric forms can therefore not be ruled out. Although naturally occurring enantiomers are rare, they do exist, as exemplified by the epimeric fungal products aspertetronin A[9a] and gregatin A[9b] or the enantiomers nanaomycin D[10] and kalafungin[10]. Herein we wish to report the absolute configuration of the diepoxins as determined by the exiton coupled CD method (ECCD[11]) and new members of the diepoxin family.

RESULTS AND DISCUSSION

A series of diepoxins (**1** - **8**) has been isolated from cultures of the fungus LL-07F275 which grows as a mycelium sterilium. The occurrence of some of these compounds depends largely on fermentation conditions and isolation method. As previously reported[2a], the diepoxins η(**1**) and ζ(**4**) predominate early in the fermentation, whereas the oxidized derivatives such as diepoxin α and σ(**5**) occur almost exclusively at the end of the fermentation period. The components ι(**7**) and κ(**8**) can only be detected in fermentation extracts if methanol was used during the isolation procedure. This prompted the investigation of the influence of methanol on the principal diepoxins. Eventually, it was ascertained that methanol can undergo Michael addition to the enone function of diepoxin ζ(**4**).

When **4** was exposed to methanol under base-catalysis, the methoxylated derivatives **7** and **8** were obtained in 30% yield. Several by-products, such as diepoxin φ(**6**), were also formed during the reaction, presumably because oxygen was not excluded from the reaction mixture. Position and relative configuration of the methoxyl groups in **7** and **8** were determined through various NMR experiments such as COSY, HETCOR and HMBC, which ascertained that these compounds are epimeric at C-6. Since the structure of **8** was determined independently by X-ray diffraction analysis (Figure 2), the stereochemistries of **7** and **8** could be correlated and assigned unambiguously (Scheme 1).



diepoxin ζ (4) **diepoxin ι (7)** **diepoxin κ (8)**

4 : **3** ratio

a) CH_3OH, TEA(5%), rt, 5 h, 30%

Scheme 1. Base-catalyzed methylation of 4 yielding 7 and 8

Although compounds **7** and **8** together with the C-6 hydroxyl analogues, diepoxins γ(**2**) and δ(**3**), were originally isolated from fermentation broths, their synthesis under controlled conditions proved to be a valuable tool to correlate their structures and to assign the absolute configuration of various diepoxin components. Due to the fact that methanolic fermentation extracts contain the epimeric pair **7** and **8** in approximately the same relative ratio as they arise from the Michael addition, they are considered to be artifacts of the isolation procedure.

The relative configuration of the diepoxins[2] η(**1**), ζ(**4**), and σ(**5**) was deduced from NMR measurements in conjunction with X-ray data obtained on diepoxin κ(**8**). Only the X-ray projection provided unambiguous evidence for the C-4 hydroxyl group facing the same side of the molecule as the two epoxide groups (Figure 1). The "anti" relationship of the C-4 - and C-5 hydroxyl groups had been anticipated due to the lack of ROESY correlations between H-4 and H-5.

In order to determine the absolute configuration of the diepoxins by ECCD[11], compounds **1**, **7** and **8** were converted to their respective bis-dimethylamino-benzoate (DMAB) derivatives using the triazol/DBU method[12] (Scheme 2). DMAB was selected as the chromophore because its red-shifted UV absorption (λ_{max} 309 nm, ε = 28,000) would not couple with the preexisting naphthalene chromophore (λ_{max} 226 nm).

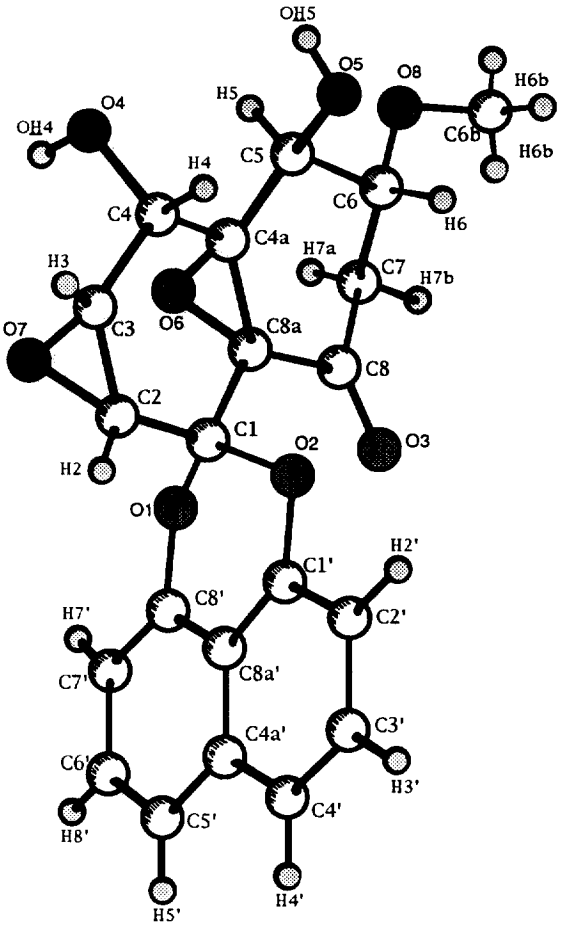
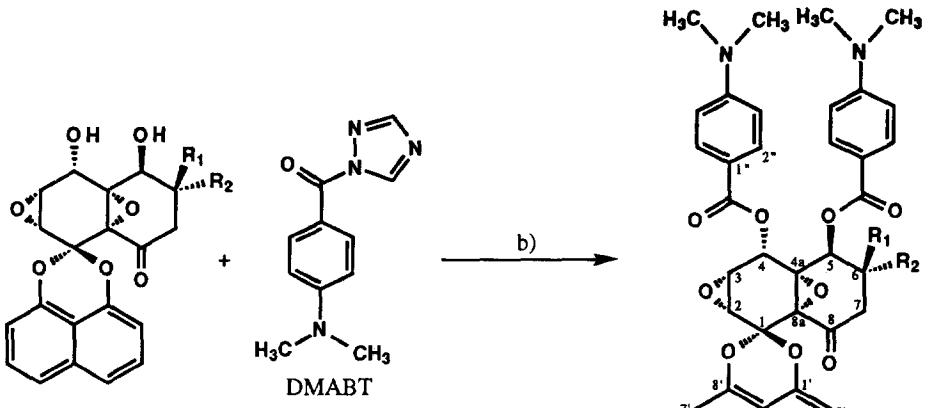


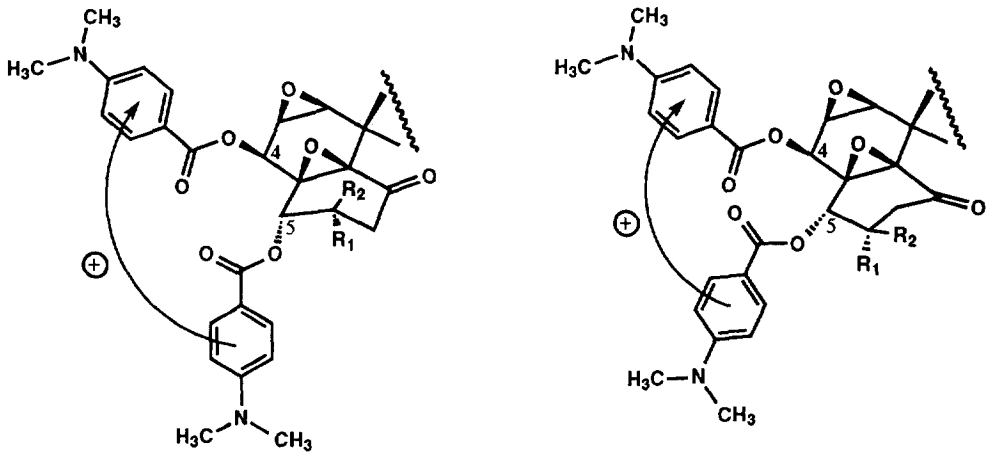
Figure 2: **PLUTO representation of X-ray data obtained for diepoxin κ(8)**



1(η): $R_1 = H$ $R_2 = H$
7(ι): $R_1 = OMe$ $R_2 = H$
8(κ): $R_1 = H$ $R_2 = OMe$

1a: $R_1 = H$ $R_2 = H$
7a: $R_1 = OMe$ $R_2 = H$
8a: $R_1 = H$ $R_2 = OMe$

Scheme 2: Derivatization of diepoxins η(1), ι(7) and κ(8)



Conformer I

1a: $R_1 = H$ $R_2 = H$
7a: $R_1 = OMe$ $R_2 = H$
8a: $R_1 = H$ $R_2 = OMe$

Conformer II

Figure 3: Two possible conformers of the diepoxin derivatives

On the basis of Dreiding Models, two conformations (I and II, Figure 3) for compounds **1a, 7a,** or **3a** are the most favored. Assuming S-configuration for C-4 and C-5, the C-5 OR-group in conformer **I** will be axial whereas in conformer **II** the same group will adopt an equatorial orientation (Figure 3). Although the alignment of the two DMAB chromophores, or more precisely, their electric dipole transition moments, would result in different dihedral angles between the ester groups in conformer I and II, both angles are *positive.* Accordingly, the respective *positive exciton couplets* would differ only in magnitude but not in sign. The observed bathochromic shift of 7 to 8 nm in the UV/VIS-spectra of **1a, 7a,** or **8a** by comparison to a non-coupled DMAB chromophore implies that the dihedral angle between the interacting DMAB substituents[13] is greater than 90°. This will only be the case, if compounds **1a, 7a,** or **8a** adopt conformation **I** (Figure 3).

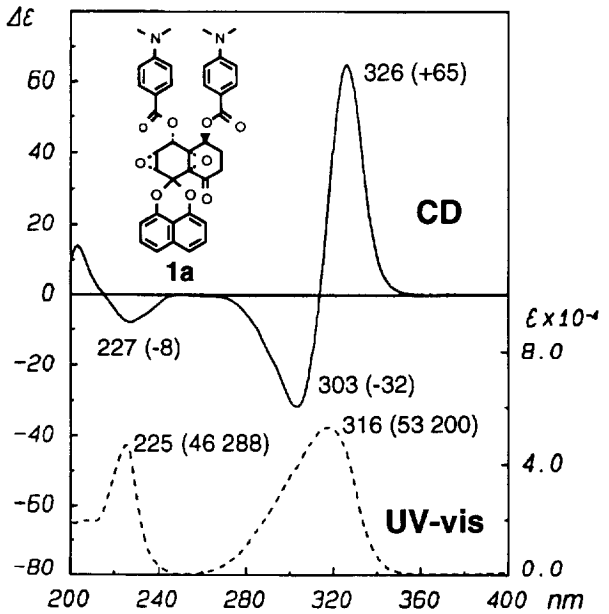


Figure 4: CD and UV spectra of 1a in acetonitrile

The CD spectra of the three derivatives **1a, 7a** and **8a** show identical curves (Figure 4) with intense *positive* exciton couplets (A = ca. +96) centered at ca. 310 nm. These *positive* exciton couplets are evidence for a *positive chirality* between the two DMAB-chromophores at C-4/C-5 in **1a, 7a** and **8a**. Consequently, the S-configuration can be assigned to C-4 and C-5. This assignment in conjunction with the X-ray data also establishes the absolute configuration for each of the remaining stereogenic centers as shown for the diepoxins **1 - 6** in Figure 1.

ECCD is a powerful method to determine absolute stereochemistry provided no ambiguity exists regarding the relative configuration and conformation. It is particularly useful when only a minuscule amount of sample is available, as it was the case with diepoxin κ. The determination of the absolute stereochemistry of the diepoxins proves that the structures of cladospirone bisepoxide and diepoxin ζ are indeed identical, as is SCH 53514 (positve optical rotation), whereas an uncertainty remains in regard to palmarumycin C_{13}.

MATERIAL AND METHODS

Instrumental. A Hewlett-Packard 1090M LC system with diode array detection employing a Zorbax RX (C8) reverse phase column (5μ, 4.6 x 250 mm), eluted isocratically with 45% aqueous methanol at 45°C, or, alternatively, with 30% aqueous acetonitrile, was used for analysis of fractions or to check the purity of isolated components. NMR spectra were obtained on a Bruker AMX 300 MHz, or a Varian VXR 400 MHz NMR instrument at ambient temperature. Chemical shifts of ^1H and ^{13}C NMR signals were determined in parts per million relative to TMS or referenced to the solvent signals of deutero-chloroform at δ 7.26 ppm (^1H) and δ 77.0 ppm (^{13}C) or deutero-methanol at δ 3.30 ppm (^1H) and δ 49.0 ppm (^{13}C) instead. UV spectra were obtained "on the fly" with a HP 1090M LC system with diode array detection, or recorded using either a Perkin Elmer Lambda 4B, or a Hewlett-Packard Model 8450A UV/VIS spectrophotometer λ_{max}[nm] (ε[$Lmol^{-1}cm^{-1}$]). CD spectra were measured in MeCN on a Jasco 720 spectropolarimeter, λ_{max}[nm] ($\Delta\varepsilon$[$Lmol^{-1}cm^{-1}$]); IR spectra were obtained on a Perkin Elmer 1600 FT-IR spectrophotometer, NaCl cell. Mass spectra were obtained on a JEOL DX 303HF spectrometer, using a 3-nitrobenzyl alcohol matrix, positive ion mode. The same procedure was applied for HRMS, using internal matching with matrix references at *m/z* 679.4119 and 635.3859.

Experimental Procedures

(2R,3R,4S,4aS,5S,8aR)-3,4,7-Trihydro-4,5-dihydroxy-6-methoxy-spiro[2,3:4a,8a-diepoxy-naphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (7 and 8)

Diepoxin ζ (**4**) (20.0 mg, 54 μmol) was dissolved in 20 mL of CH_3OH and 1 mL of triethylamine (TEA) was added, stirred up and set aside. The solution slowly turned a reddish-brown color. The progess of the reaction was monitored by HPLC and stopped after 5 hours by evaporating the solvents *in vacuo*. The oily residue was reconstituted in acetone and spotted onto a preparative TLC plate (Whatman PLK5F) using CH_2Cl_2/MeOH in a ratio of 7 : 3 as developing solvent. Five UV-active bands were detected on the plate. The bands were marked and removed with the silica gel, loaded into extraction vessels and washed with MeOH to remove the adsorbed components. Band 1 (most polar) contained 2.1 mg material, band 2 - 2.4 mg (**8**), band 3 - 3.2 mg (**7**), band 4 - 2.2 mg, and band 5 - 2.9 mg material.

Diepoxin ι -- (2R,3R,4S,4aS,5S,8aR)-3,4,7-Trihydro-4,5-dihydroxy-6R-methoxy-spiro[2,3:4a,8a-diepoxynaphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (7):
UV (MeOH): 226 (59,230), 299(8,000), 313 (5,900), 328 (4,800).
^{1}H-NMR ($CDCl_3$): 2.61 (*H*-7b, dd, $^2J_{7b/7a}$ = 17.1 Hz, $^3J_{7b/6}$ = 9.5 Hz), 2.76 (*H*-7a, dd, $^2J_{7a/7b}$ = 17.1 Hz, $^3J_{7a/6}$ = 5.0 Hz), 2.86 (O*H*-4, d, $^3J_{4/4OH}$ = 11.2 Hz), 2.95 (O*H*-5, d, $^3J_{5/5OH}$ = 3.4 Hz), 3.42 (OC*H*$_3$, s), 3.50 (*H*-3, dd, $^3J_{2/3}$ = 4.2 Hz, $^3J_{3/4}$ = 3.0 Hz), 3.54 (*H*-2, d, $^3J_{2/3}$ = 4.2 Hz), 3.73 (*H*-6, m, $^3J_{5/6}$ = 3.4 Hz, $^3J_{6/7a}$ = 5.0 Hz, $^3J_{6/7b}$ = 9.5 Hz), 4.83 (*H*-5, dd, $^3J_{5/6}$ = 3.0 Hz, $^3J_{5/5OH}$ = 3.4Hz), 4.90 (*H*-4, dd, $^3J_{3/4}$ = 3.0 Hz, $^3J_{4/4OH}$ = 11.2 Hz), 6.96 (*H*-2' or 7', dd, ^3J = 7.5 Hz, ^4J = 0.8 Hz), 7.09 (*H*-2' or 7', dd, ^3J = 7.2 Hz, ^4J = 1.1 Hz), 7.41 (*H*-3' or 6', dd, ^3J = 7.5 Hz, ^3J = 8.3 Hz), 7.45 (*H*-3' or 6', dd, ^3J = 7.2 Hz, ^3J = 8.4 Hz), 7.51 (*H*-4' or 5', dd, ^3J = 8.3 Hz, ^4J = 0.8 Hz), 7.52 (*H*-4' or 5', dd, ^3J = 8.4 Hz, ^4J = 1.1 Hz).
^{13}C NMR ($CDCl_3$): 38.7 (t, C-7), 54.8 (d, C-2), 55.3 (d, C-3), 56.7 (q, O<u>C</u>H$_3$), 62.8 (d, C-4), 63.5 (C-8a), 64.5 (d, C-5), 69.2 (C-4a), 73.6 (d, C-6), 94.6 (C-1), 109.0 (d, C-2' or 7'), 109.6 (d, C-2' or 7'), 112.2 (C-8a'), 120.97 (d, C-4' or 5'), 121.04 (d, C-4' or 5'), 127.2 (d, C-3' or 6'), 127.7 (d, C-3' or 6'), 134.2 (C-4a'), 145.3 (C-1' or 8'), 145.5 (C-1' or 8'), 194.7 (C-8);
^{1}H-NMR (CD_3OD): 2.49 (*H*-7b, dd, $^2J_{7b/7a}$ = 17.9 Hz, $^3J_{7b/6}$ = 10.1 Hz), 2.67 (*H*-7a, dd, $^2J_{7a/7b}$ = 17.9 Hz, $^3J_{7a/6}$ = 5.9 Hz), 3.39 (OC*H*$_3$, s), 3.42 (*H*-3, d, $^3J_{3/4}$ = 2.2 Hz), 3.43 (*H*-2), 3.74 (*H*-6, m, $^3J_{5/6}$ = 2.8 Hz, $^3J_{6/7a}$ = 5.9 Hz, $^3J_{6/7b}$ = 10.1 Hz), 4.81 (*H*-4, d, $^3J_{3/4}$ = 2.2 Hz), 4.84 (*H*-5, dd, $^3J_{5/6}$ = 2.8 Hz), 6.94 (*H*-2' or 7', dd, ^3J = 7.5 Hz, ^4J = 0.5 Hz), 7.00 (*H*-2' or 7', dd, ^3J = 7.4 Hz, ^4J = 0.8 Hz), 7.45 (*H*-3' or 6', dd, ^3J = 7.5 Hz, ^3J = 8.5 Hz), 7.47 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J = 8.3 Hz), 7.54 (*H*-4' and *H*-5', two dd).
^{13}C NMR (CD_3OD): 40.2 (t, C-7), 54.7 (d, C-2), 56.6 (d, C-3), 56.7 (q, O<u>C</u>H3), 63.2 (d, C-4), 64.3 (d, C-5), 65.1 (C-8a), 70.8 (C-4a), 74.8 (d, C-6), 96.5 (C-1), 109.8 (d, C-2' or 7'), 110.2 (d, C-2' or 7'), 113.4 (C-8a'), 121.9 (two d, C-4' and C-5'),128.5 (d, C-3' or 6'), 128.7 (d, C-3' or 6'), 135.7 (C-4a'), 146.9 (C-1' or 8'), 147.2 (C-1' or 8'), 198.2 (C-8).
MF: $C_{21}H_{18}O_8$; MS(nTSP): m/z 398 $[M]^-$.
crystal (MeOH) m.p. = 242 °C.

Diepoxin κ -- (2R,3R,4S,4aS,5S,8aR)-3,4,7-Trihydro-4,5-dihydroxy-6S-methoxy-spiro[2,3:4a,8a-diepoxynaphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (8):
UV (MeOH): 226 (59,230), 299(8,000), 313 (5,900), 328 (4,800).
^{1}H-NMR (CD_3OD): 2.66 (2*H*-7, m, $^3J_{7/6}$ = 6.0 Hz), 3.39 (OC*H*$_3$, s), 3.41 (*H*-3, d, $^3J_{3/4}$ = 2.0 Hz), 3.41 (*H*-2), 3.56 (*H*-6, m, $^3J_{5/6}$ = 4.8 Hz, $^3J_{6/7}$ = 6.0 Hz), 4.41 (*H*-5, dd, $^3J_{5/6}$ = 4.8 Hz), 4.88 (*H*-4, d, $^3J_{3/4}$ = 2.0 Hz), 6.94 (*H*-2' or 7', dd, ^3J = 7.5 Hz, ^4J = 0.5 Hz), 7.01 (*H*-2' or 7', dd, ^3J = 7.4 Hz, ^4J = 0.8 Hz), 7.45 (*H*-3' or 6', dd, ^3J = 7.5 Hz, ^3J = 8.5 Hz), 7.48 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J = 8.3 Hz), 7.55 (*H*-4' and *H*-5', two dd).
^{13}C NMR (CD_3OD): 40.7 (t, C-7), 54.5 (d, C-2), 56.7 (d, C-3), 57.6 (q, O<u>C</u>H3), 63.4 (d, C-4), 63.1 (C-8a), 67.0 (d, C-5), 69.9 (C-4a), 81.5 (d, C-6), 96.6 (C-1), 109.8 (d, C-2' or 7'), 110.3 (d, C-2' or 7'), 113.4 (C-8a'), 121.9 (two d, C-4' and C-5'), 128.5 (d, C-3' or 6'), 128.7 (d,C-3' or 6'), 135.7 (C-4a'), 146.9 (C-1' or 8'), 147.2 (C-1' or 8'), 199.2 (C-8).
MF: $C_{21}H_{18}O_8$; MS(nTSP): m/z 398 $[M]^-$.
crystal (MeOH) m.p. = 158 °C, resolidifies then m.p. = 232 °C.

X-ray - Diepoxin κ crystallized from methanol solution in colorless needles (m.p. 158 °C and 232 °C). A crystal having the approximate dimensions 0.50 x 0.10 x 0.07 mm was selected for X-ray diffraction. Crystal survey, unit cell determination, and data collection were performed using copper Kα radiation at a temperature of 23 ± 1°C.
The structure was solved by direct methods and refined by full-matrix least-squares and difference Fourier methods. All non-hydrogen atoms were refined anisotropically. The

hydrogen atoms attached to the oxygen atoms were located from difference Fourier maps and refined isotropically. The positions of the remaining hydrogens were calculated assuming ideal geometries. The absolute configuration was set arbitrarily.
One molecule formed a monoclinic unit cell with the dimensions: a = 25.484 (1), b = 5.711 (1), c = 13.824 (1) Å, and V1766.7 (4) $Å^3$. For Z =4 and F.W. = 398.37, the calculated density is 1.497 g/cm^3.
Of the 1516 reflections which were collected, 1477 were unique ($R_{int.}$ = 0.013). The intensities of the three representative reflections which were measured after every 150 reflections remained constant throughout data collection indicating crystal and electronic stability.

Diepoxin η -- (2R,3R,4S,4aS,5S,8aR)-3,4,6,7-Tetrahydro-4,5-dihydroxy-spiro[2,3:4a,8a-diepoxy-naphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (1):
[α] ^{25}D(MeOH) = +23±8 (c=0.3%); UV (MeOH): 225 (60,100), 297 (8,200), 305sh (6,000), 313 (5,900), 320sh (3,200), 327 (4,800). IR (KBr): 3478s, 3353br, 3019, 2962, 2943, 2915, 1728s, 1637, 1611s, 1587s, 1415s, 1382s, 1347, 1281s, 1188, 1160, 1139, 1095, 1077, 1034, 1000, 948, 918, 847, 818, 755 cm^{-1}.
^{1}H-NMR (CD_3OD): 1.81 (*H*-6b, m, $^2J_{6b/6a}$ = 13.6 Hz, $^3J_{5/6b}$ = 3.0 Hz, $^3J_{6b/7a}$ = 5.5 Hz, $^3J_{6b/7b}$ = 6.6 Hz), 2.02 (*H*-6a, m, $^2J_{6a/6b}$ = 13.6 Hz, $^3J_{5/6a}$ = 3.0 Hz, $^3J_{6a/7a}$ = 2.3 Hz, $^3J_{6a/7b}$ = 12.4 Hz), 2.55 (*H*-7b, m, $^2J_{7b/7a}$ = 18.1 Hz, $^3J_{7b/6a}$ = 12.4 Hz, $^3J_{7b/6b}$ = 6.6 Hz), 2.30 (*H*-7a, m, $^2J_{7a/7b}$ = 18.1 Hz, $^3J_{7a/6b}$ = 5.5 Hz, $^3J_{7a/6a}$ = 2.3 Hz), 3.40 (*H*-2, d), 3.41 (*H*-3, $^3J_{3/4}$ = 2.7 Hz), 4.70 (*H*-5, ddd, $^3J_{5/6}$ = 3.0 Hz), 4.85 (*H*-4, d, $^3J_{3/4}$ = 2.7 Hz), 6.92 (*H*-2' or 7', dd, ^3J = 7.4 Hz, ^4J = 0.7 Hz), 7.00 (*H*-2' or 7', dd, ^3J = 7.3 Hz, ^4J = 1.1 Hz), 7.42 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J = 8.3 Hz), 7.46 (*H*-3' or 6', dd, ^3J = 7.3 Hz, ^3J = 8.3 Hz), 7.52 (*H*-4' or 5', dd), 7.53 (*H*-4' or 5', dd).
^{13}C NMR (CD_3OD): 25.3 (t, C-6), 33.7 (t, C-7), 54.7 (d, C-2), 56.6 (d, C-3), 62.9 (d, C-4), 63.8 (d, C-5), 65.0 (C-8a), 71.0 (C-4a), 96.4 (C-1), 109.7 (d, C-2' or 7'), 110.2 (d, C-2' or 7'), 113.4 (C-8a'), 121.79 (d, C-4' or 5'), 121.84 (d, C-4' or 5'), 128.5 (d, C-3' or 6'), 128.7 (d, C-3' or 6'), 135.6 (C-4a'), 146.9 (C-1' or 8'), 147.2 (C-1' or 8'), 199.9 (C-8).
MF: $C_{20}H_{16}O_7$; MS(nTSP): m/z 368 $[M]^-$. crystal (MeOH) m.p. = 250 °C.

Diepoxin γ -- (2R,3R,4S,4aS,5S,8aR)-3,4,7-Trihydro-4,5,6R-trihydroxy-spiro[2,3:4a,8a-diepoxy-naphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (2):
UV (MeOH): 226 (59,200), 299 (8,000), 313 (5,900), 328 (4,800).
CD (MeOH): 203 (3.16), 213 (5.30), 228 (-1.96), 242 (-0.41), 260 (-0.06), 298 (1.69), 329sh (0.54).
^{1}H-NMR (CD_3OD): 2.55 (2*H*-7, m, $^3J_{7/6}$ = 7.3 Hz), 3.43 (*H*-2), 3.43 (*H*-3, d, $^3J_{3/4}$ = 2.1 Hz), 4.06 (*H*-6, m, $^3J_{5/6}$ =2.8Hz, $^3J_{6/7}$ = 7.3 Hz), 4.62 (*H*-5, d, $^3J_{5/6}$ = 2.8 Hz), 4.82 (*H*-4, d, $^3J_{3/4}$ = 2.1 Hz), 6.94 (*H*-2' or 7', dd, ^3J = 7.5 Hz, ^4J = 0.7 Hz), 7.00 (*H*-2' or 7', dd, ^3J = 7.4 Hz, ^4J = 0.9 Hz), 7.45 (*H*-3' or 6', dd, ^3J = 7.5 Hz, ^3J = 8.4 Hz), 7.48 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J = 8.4 Hz), 7.54 (*H*-4' or 5', dd), 7.55 (*H*-4' or 5', dd).
^{13}C NMR (CD_3OD): 42.7 (t, C-7), 54.7 (d, C-2), 56.7 (d, C-3), 63.2 (d, C-4), 65.2 (d, C-6), 65.4 (C-8a), 67.4 (d, C-5), 71.0 (C-4a), 96.5 (C-1), 109.8 (d, C-2' or 7'), 110.2 (d, C-2' or 7'), 113.4 (C-8a'), 121.8 (d, C-4' or 5'), 121.9 (d, C-4' or 5'), 128.5 (d, C-3' or 6'), 128.7 (d, C-3' or 6'), 135.7 (C-4a'), 146.9 (C-1' or 8'), 147.2 (C-1' or 8'), 198.5 (C-8).
MF: $C_{20}H_{16}O_8$; MS(nTSP): m/z 384 $[M]^-$.

Diepoxin δ -- (2R,3R,4S,4aS,5S,8aR)-3,4,7-Trihydro-4,5,6S-trihydroxy-spiro[2,3:4a,8a-diepoxy-naphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (3):
UV (MeOH): 226 (59,200), 299 (8,000), 313 (5,900), 328 (4,800).
CD (MeOH): 201 (2.38), 214 (5.22), 229 (-2.03), 243 (-0.39), 260 (-0.09), 298 (1.16), 328sh (0.17).

^{1}H-NMR (CD_3OD): 2.61 (*H*-7a, dd, $^2J_{7a/7b}$ = 16.0 Hz, $^3J_{7a/6}$ = 5.4 Hz), 2.67 (*H*-7b, dd, $^2J_{7a/7b}$ = 16.0 Hz, $^3J_{7b/6}$ = 6.8 Hz), 3.42 (*H*-2), 3.42 (*H*-3, d, $^3J_{3/4}$ = 1.3 Hz), 3.87 (*H*-6, m, $^3J_{5/6}$ =5.2 Hz, $^3J_{7a/6}$ = 5.4 Hz, $^3J_{6/7b}$ = 6.8 Hz), 4.30 (*H*-5, d, $^3J_{5/6}$ = 5.2 Hz), 4.85 (*H*-4, d, $^3J_{3/4}$ = 1.3 Hz), 6.94 (*H*-2' or 7', dd, ^3J = 7.5 Hz, ^4J = 0.6 Hz), 7.00 (*H*-2' or 7', dd, ^3J = 7.4 Hz, ^4J = 0.8 Hz), 7.45 (*H*-3' or 6', dd, ^3J = 7.5 Hz, ^3J = 8.3 Hz), 7.47 (*H*-3' or 6', dd, ^3J = 7.3 Hz, ^3J = 8.3 Hz), 7.55 (*H*-4' and *H*-5', two dd).
^{13}C NMR (CD_3OD): 43.5 (t, C-7), 54.5 (d, C-2), 56.6 (d, C-3), 63.4 (d, C-4), 71.7 (d, C-6), 65.4 (C-8a), 69.3 (d, C-5), 71.8 (C-4a), 96.6 (C-1), 109.8 (d, C-2' or 7'), 110.2 (d, C-2' or 7'), 113.4 (C-8a'), 121.9 (d, C-4' and C-5'), 128.5 (d, C-3' or 6'), 128.7 (d, C-3' or 6'), 135.7 (C-4a'), 147.0 (C-1' or 8'), 147.2 (C-1' or 8'), 199.1 (C-8).
MF: $C_{20}H_{16}O_8$; MS(nTSP): m/z 384 $[M]^-$.
crystal (MeOH) m.p. = 241 °C.

Diepoxin ζ -- (2R,3R,4S,4aS,5S,8aR)-3,4-Dihydro-4,5-dihydroxy-spiro[2,3:4a,8a-diepoxynaphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (4):
[α] ^{25}D(MeOH) = +75±8 (c=0.3%). UV (MeOH): 225 (61,800), 298 (8,200), 313 (5,900), 327 (4,800).
CD (MeOH): 207(-11.9), 219 (1.05), 229(-4.97), 245 (9.8), 285 (-0.84), 326sh (2.6), 346 (4.5).
IR (KBr): 3418br, 2972, 2929, 2873, 1694s, 1609s, 1586s, 1414s, 1381s, 1276s, 1183, 1138, 1118, 1090, 1036, 1012, 945, 820, 758 cm^{-1}.
^{1}H-NMR (CD_3OD): 3.43 (*H*-2), 3.44 (*H*-3, d, $^3J_{3/4}$ = 2.14 Hz), 4.79 (*H*-5, dd, $^3J_{5/6}$ = 4.9 Hz, $^4J_{5/7}$ = 0.7 Hz), 5.09 (*H*-4, d, $^3J_{3/4}$ = 2.14 Hz), 5.88 (*H*-7, dd, $^3J_{7/6}$ = 10.6 Hz, $^4J_{5/7}$ = 0.7 Hz), 6.71 (*H*-6, dd, $^3J_{6/7}$ = 10.6 Hz, $^3J_{5/6}$ = 4.9 Hz), 6.91 (*H*-2' or 7', dd, ^3J = 7.4 Hz, ^4J = 0.5 Hz), 7.02 (*H*-2' or 7', dd, ^3J = 7.3 Hz, ^4J = 0.9 Hz), 7.43 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J = 8.3 Hz), 7.47 (*H*-3' or 6', dd, ^3J = 7.3 Hz, ^3J = 8.3 Hz), 7.53 (*H*-4' or 5', dd), 7.54 (*H*-4' or 5', dd).
^{13}C NMR (CD_3OD): 54.6 (d, C-2), 56.7 (d, C-3), 62.2 (d, C-4), 62.5 (d, C-5), 64.2 (C-8a), 72.0 (C-4a), 96.5 (C-1), 109.7 (d, C-2' or 7'), 110.2 (d, C-2' or 7'), 113.4 (C-8a'), 121.9 (two d, C-4' and C-5'), 127.1 (d, C-7), 128.5 (d, C-3' or 6'), 128.7 (d, C-3' or 6'), 135.7 (C-4a'), 144.9 (d, C-6), 146.9 (C-1' or 8'), 147.2 (C-1' or 8'), 189.9 (C-8).
MF: $C_{20}H_{14}O_7$; MS(nTSP): m/z 366 $[M]^-$; MS(FAB): m/z 367 $[M+H]^+$; FAB-HRMS: calcd. for $C_{20}H_{14}O_7$ =*m/z* 366.0739; obs. m/z 366.0737.

Diepoxin φ -- (2R,3R,4S,4aS,5S,8aR)-3,4-Dihydro-4-hydroxy-spiro[2,3:4a,8a-diepoxynaphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-5,8-dione (6):
UV (MeOH): 226 (61,450), 298 (8,350), 307 (5,700), 313 (5,950), 327 (5,000).
^{1}H-NMR ($CDCl_3$): 3.57 (*H*-2, d, $^3J_{2/3}$ = 4.2 Hz), 3.54 (*H*-3, dd, $^3J_{2/3}$ = 4.2 Hz, $^3J_{3/4}$ = 2.9 Hz), 5.19 (*H*-4, d, $^3J_{3/4}$ = 2.9 Hz), 6.55 (*H*-7, dd, $^3J_{7/6}$ = 10.7 Hz), 6.60 (*H*-6, d, $^3J_{6/7}$ = 10.7 Hz), 6.98 (*H*-2' or 7', d, ^3J = 7.5 Hz), 7.11 (*H*-2' or 7', dd, ^3J = 7.4 Hz, ^4J = 0.5 Hz), 7.44 (*H*-3' or 6', dd, ^3J = 7.5 Hz, ^3J = 8.3 Hz), 7.48 (*H*-3' or 6', dd, ^3J = 7.3 Hz, ^3J = 8.4 Hz), 7.55 (*H*-4' or 5', dd), 7.56 (*H*-4' or 5', dd).
^{13}C NMR ($CDCl_3$): 53.9 (d, C-2), 55.8 (d, C-3), 63.2 (d, C-4), 63.3 (C-8a), 63.5 (C-4a), 94.0 (C-1), 109.1 (d, C-2' or 7'), 109.8 (d, C-2' or 7'), 111.9 (C-8a'), 121.2 (two d, C-4' and C-5'), 127.3 (d, C-3' or 6'), 127.7 (d, C-3' or 6'), 134.1 (C-4a'), 135.3 (d, C-6), 137.4 (d, C-7), 144.9 (C-1' or 8'), 145.1 (C-1' or 8'), 185.4 (C-8), 192.4 (C-5).
MF: $C_{20}H_{12}O_7$; MS(nTSP): m/z 364 $[M]^-$.

(2R,3R,4S,4aS,5S,8aR)-3,4,6,7-Tetrahydro-4,5-di-(4"-dimethylbenzoyl)-spiro[2,3:4a,8a-diepoxy-naphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (1a):
To a solution of **1** (1.0 mg, 2.7 µmol) and p-dimethylaminobenzoyltriazol[14] (1.75 mg, 8.1 µmol) in CH_2Cl_2 (1 mL) a solution of DBU (1.23 mg, 8.1 µmol) in CH_2Cl_2 (0.1 mL) was added. After

stirring at ambient temperature for 15min, the reaction mixture was diluted with CH_2Cl_2 (9 mL), extracted with sat. aq. $NaHCO_3$ solution (3 x 10 mL), washed with brine (3 x 10 mL), dried (Na_2SO_4) and evaporated (15 Torr and 0.01 Torr). Purification by preparative TLC (CH_2Cl_2) afforded **1a** (1.5 mg, 84%) as a slightly yellow powder. TLC (silica gel, CH_2Cl_2): *Rf* 0.19.
UV(MeCN): 224 (46,288), 316 (53,200). CD (MeCN): 227 (-8.0), 303 (-31.9), 326 (+64.6)
IR (CCl_4): 2956, 2928s, 2844, 1716s, 1604s, 1525, 1414, 1363, 1316, 1266s, 1177, 1149, 1135, 1089, 945 cm^{-1}.
^{1}H-NMR ($CDCl_3$): 2.07 - 2.31 (2*H*-6, br m), 2.52 - 2.55 (2*H*-7, m), 3.08 (2s, N(CH_3)$_2$), 3.10 (2s, N(CH_3)$_2$), 3.55 (*H*-2, d, $^3J_{2/3}$ = 4.2 Hz), 3.62 (*H*-3, dd, $^3J_{2/3}$ = 4.2 Hz, $^3J_{3/4}$ = 2.7 Hz), 5.86 (*H*-5, t, $^3J_{5/6}$ = 3.0 Hz), 6.14 (*H*-4, d, $^3J_{3/4}$ = 2.7 Hz), 6.67 (*H*-3" and *H*-5", 2d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 9.0 Hz), 6.71 (*H*-3" and *H*-5", 2d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 9.0 Hz), 7.02 (*H*-2' or 7', d, ^3J = 7.4 Hz), 7.15 (*H*-2' or 7', d, ^3J = 7.4 Hz), 7.47 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J= 8.4 Hz), 7.49 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J = 8.3 Hz), 7.55 (*H*-4' and *H*-5', 2 d, ^3J· = 8.4 Hz), 7.95 (*H*-2" and *H*-6", 2d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 8.9 Hz), 8.02 (*H*-2" and *H*-6", 2d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 8.9 Hz).
MS(FAB): 664 (20), 663 (45), 662 (100, [M^+·]), 661 (40); FAB-HRMS: calcd. for $C_{38}H_{34}N_2O_9$ =*m/z* 662.2264; obs. m/z 662.2254.

(2R,3R,4S,4aS,5S,8aR)-3,4,7-Trihydro-4,5-di-(4"-dimethylbenzoyl)-6S-methoxy-spiro[2,3:4a,8a-diepoxynaphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (8a):
A mixture (ca. 2 mg) containing some **8** was converted by the same procedure to 2.4 mg crude product, which was purified by preparative TLC (silica gel, CH_2Cl_2, 2x) and HPLC (normal phase, gradient CH_2Cl_2 to CH_2Cl_2/MeOH 40:1 in 20 min) to give **8a** (26 µg, 37.5 nM). TLC (silica gel, CH_2Cl_2): *Rf* 0.19.
UV (MeCN): 224 (46,288), 317 (53,200). CD (MeCN): 224 (-6.7), 303 (-32.0), 326 (+64.3).
MS (CI, {NH_3}): 712 (15), 711 (45), 710 (100, [*M* + NH_4]$^+$), 709 (24), 694 (16), 693 (35, [*M* + H]$^+$), 692 (14).

(2R,3R,4S,4aS,5S,8aR)-3,4,7-Trihydro-4,5-di-(4"-dimethylbenzoyl)-6R-methoxy-spiro[2,3:4a,8a-diepoxynaphthalene-1(2H),2'naphtho[1,8-de]-[1,3]dioxin]-8(5H)-one (7a):
By the same procedure **7** (0.8 mg, 2.0 µmol) was converted to 1.6 mg (115%) crude product, which was purified by preparative TLC (silica gel, CH_2Cl_2, 2 x) to give **7a** (1.0 mg, 72%) as a slightly yellow powder. TLC (silica gel, CH_2Cl_2): *Rf* 0.20.
UV (MeCN): 224 (46,288), 317 (53,200). CD (MeCN): 223 (-6.8), 303 (-30.9), 326 (+64.7).
^{1}H-NMR ($CDCl_3$): 2.64 - 2.84 (H-7, m), 2.88 - 2.93 (H-7, m), 3.08 (2 x 2s, N(CH_3)$_2$), 3.35 (s, OCH_3), 3.52 (*H*-2, d, $^3J_{2/3}$ = 4.3 Hz), 3.61 (*H*-3, dd, $^3J_{2/3}$ = 4.3 Hz, $^3J_{3/4}$ = 2.8 Hz), 3.97 - 4.01 (*H*-6, m), 6.04 (*H*-5, d, $^3J_{5/6}$ = 2.9 Hz), 6.22 (*H*-4, d, $^3J_{3/4}$ = 2.8 Hz), 6.67(*H*-3" and *H*-5", 2 d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 9.0 Hz), 6.69 (*H*-3" and *H*-5", 2 d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 9.0 Hz), 7.02 (*H*-2' or 7', d, ^3J = 7.4 Hz), 7.13 (*H*-2' or 7', d, ^3J = 7.3 Hz), 7.47 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J= 8.4 Hz), 7.49 (*H*-3' or 6', dd, ^3J = 7.4 Hz, ^3J = 8.3 Hz), 7.54 (*H*-4' and *H*-5', 2 d, ^3J· = 8.4 Hz), 7.92 (*H*-2" and *H*-6", 2d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 9.0 Hz), 8.03 (*H*-2" and *H*-6", 2d, $^3J_{2"/3"} = {}^3J_{5"/6"}$ = 8.9 Hz).
MS(FAB): 695 (12), 694 (45), 693 (100, [*M* + H]$^+$), 692 (40). FAB-HRMS: calcd. for $C_{39}H_{36}N_2O_{10}$ =*m/z* 692.2370; obs. m/z 692.2377.

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14. The triazole was prepared following the protocol of Cai, et al in Ref. 12.

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