Pergamon

Tetrahedron Letters 39 (1998) 8441–8444

TETRAHEDRON LETTERS

STRUCTURE ELUCIDATION OF SCH 49088, A NOVEL EVERNINOMICIN ANTIBIOTIC CONTAINING AN UNUSUAL HYDROXYLAMINO-ETHER SUGAR, EVERHYDROXYLAMINOSE

Anil K. Saksena,* Edwin Jao,* Bruce Murphy, Doris Schumacher, Tze-Ming Chan,* Mohindar S. Puar,* John K. Jenkins, Donal Maloney, Mayra Cordero, Birendra N. Pramanik,* Peter Bartner, Pradip R. Das, Andrew T. McPhail,† V. M. Girijavallabhan and Ashit K. Ganguly

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, N.J. 07033, U.S.A.
†Paul M. Gross Chemical Laboratories, Duke University, Durham, N.C. 27706, U.S.A.
(Dedicated to the memory of the late Professor Sir Derek Barton)

Received 17 August 1998; accepted 8 September 1998

Abstract: Degradation of Sch 49088 (**1**) provided a novel, highly functionalized O-substituted hydroxylamine-containing sugar, everhydroxylaminose **11** isolated as a β-OMe glycoside. Notably the two acid sensitive orthoester moieties in the intact antibiotic **1** co-exist in the presence of three phenolic groups and a free carboxylic acid group.

Ziracin **2**, a member of the everninomicin group of antibiotics produced by *Micromonospora Carbonacea*, is undergoing extensive clinical trials for its activity against methicillin resistant *Staphylococci* and vancomycin resistant *Enterococci.*[1] From the same fermentation, the nitroso **3**, hydroxylamino **4**, and amino **5** compounds have been isolated.[2] More recently we have isolated from this fermentation a polar, new antibiotic Sch 49088 (**1**),[3] as a white, amorphous solid, $C_{88}H_{123}NO_{39}Cl_2$ (M+Na$^+$ 1910.6904, calcd. 1910.6947),[4] $[\alpha]_D^{25}$ -44.4 (methanol). As in ziracin **2**,[5] the ^{13}C-nmr spectrum of **1**[6] indicated the presence of two orthoester carbons at δ119.2 and δ120.4. However, besides the absence of a nitro group in the infrared spectrum, there were other marked differences in the mass and nmr spectra of **1** compared to **2**. These included a signal at δ175.8 in **1** suggestive of a carboxylic acid or carboxamide carbon; also observed was an upfield shift of quarternary C65 from δ 89.9 in **2** to δ 59.8 in **1**.[7] Two sets of fragment ions of interest in the (HRFAB) mass spectrum of **1** at 954.3810 and 935.3396 suggested cleavage at the C16 orthoester as has been established for other everninomicins.[1] Fragmentation ions in the spectrum of **1** also showed identical sugar sequences from rings **A** to **J** as found in **2-5.** A new fragment peak at m/z 273.1860 ($C_{18}H_{25}O_2$) in **1** suggested its connectivity to ring **K** possibly through the quaternary carbon 65.

Treatment of **1** with excess diazomethane gave **1a** (R = R' = Me) adding four methyl groups, three as methyl ethers and one as a methyl ester.[8] The presence of three phenolic groups on rings **A** and **I** may account for the addition of three of the methyl groups as methyl ethers. In the (HRFAB) mass spectrum of **1a**, a fragment peak at m/z 287.2012 ($C_{19}H_{27}O_2$) replaced the above m/z 273 fragment indicating addition of the remaining methyl functionality on the ring **K** side chain. Based on the well precedented Ganguly-Sarre degradation sequence,[1] two-phase acid hydrolysis of **1a** in ethyl acetate followed by treatment of the intermediate ester **6** with diazomethane in methanol gave the lactone **7** and compound **8** as major products, the latter being identical in all respects to an authentic sample obtained from ziracin **2**[1b] in the same manner. The lactone **7**, which contained the elements responsible for differences with ziracin **2**, is an amorphous solid, $C_{49}H_{69}NO_{15}Cl_2$ (M+H$^+$ 982.4113, calcd. 982.4123), $[\alpha]_D^{25}$ -46.9 ($CHCl_3$). As in **1a** , the mass spectrum of **7** had an important fragment peak at m/z 287.2012 ($C_{19}H_{27}O_2$) confirming the origin of the fragment ion m/z 273 derived from the parent antibiotic **1**. Detailed nmr studies of **7**[6,9] revealed that the $C_{19}H_{27}O_2$ side chain consists of a trisubstituted phenyl ring with a C_7H_{14} alkyl, a methyl and a *trans*-1,2-disubstituted double bond connected to a methylene carbonyl-methoxy unit.

Further degradation of **7** in refluxing methanol in the presence of p-toluene sulfonic acid provided a lipophilic monosugar α-methyl glycoside **9** $C_{28}H_{45}NO_6$ (M+H$^+$ 492.3311, calcd. 492.3325), $[\alpha]_D^{25}$ +11.1 ($CHCl_3$) and its β-

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01923-6

anomer **10** (M+H$^+$ 492.3311), $[\alpha]_D^{25}$ -45.8 ($CHCl_3$) as thick syrups in a 1:2 ratio.[10] Acid hydrolysis of **1** under identical conditions provided the same α- and β- OMe glycosides **9** (63-CH, dd, δ 4.4, J = 5.1, 1.7 Hz) and **10** (63-CH, d, δ 4.64, J = 4.5 Hz) respectively.[10] Their detailed mass and nmr spectral analysis confirmed that the $C_{18}H_{25}O_2$ fragment was attached to ring **K** through the nitrogen at the quaternary C-65, with all of the structural features mentioned above remaining intact during the degradation.

The connections between all subunits in **9** and **10**, especially the unusual C-N-O-C linkage, were elucidated by a series of selective INEPT experiments, which showed that the O-methyl at C-88, C-87 CH, and the C-86 CH are all long-range coupled to C-88. These protons are either two or three bonds from C-88. The C-71 CH was found to be two or three bonds from quaternary C-72 and C-73, one methine carbon (C-77) in the aromatic region, and two methylene carbons (C-79 and C-80) in the aliphatic region. In addition C-71 CH is not long-ranged coupled to C-65 at 59.7 ppm, which is four bonds away in **9** or **10**. Similar connectivities were found in the HMBC[5] spectrum of **1**.

Methylation of **10** with NaH/CH_3I in tetrahydrofuran yielded gem-dimethylated **12** $C_{30}H_{49}NO_6$ (M+H$^+$ 520.3648, calcd. 520.3639). A doublet of doublets at 3.24 ppm (2H) in the pmr spectrum of **10** was replaced by a singlet at 1.46 ppm (6H) in **12** indicating a methylene spacer between the C-86 and C-88 in the former. Hydrogenation of **10** (Pd/C) gave the dihydro sugar **13** $C_{28}H_{47}NO_6$ (M+H$^+$ 494.3458, calcd. 494.3481).

Basic hydrolysis of **10** (KOH/dioxane/H_2O) at room temperature provided everhydroxylaminose, the novel sugar carboxylic acid **11** $C_{27}H_{43}NO_6$ (M+H$^+$ 478.3159, calcd. 478.3169), $[\alpha]_D^{25}$ -53.3 ($CHCl_3$), as its β-OMe glycoside.[11] Treatment of **11** with diazomethane regenerated **10** confirming the presence of a methyl ester at C-88 and therefore a carboxy group in **11** and **1**.

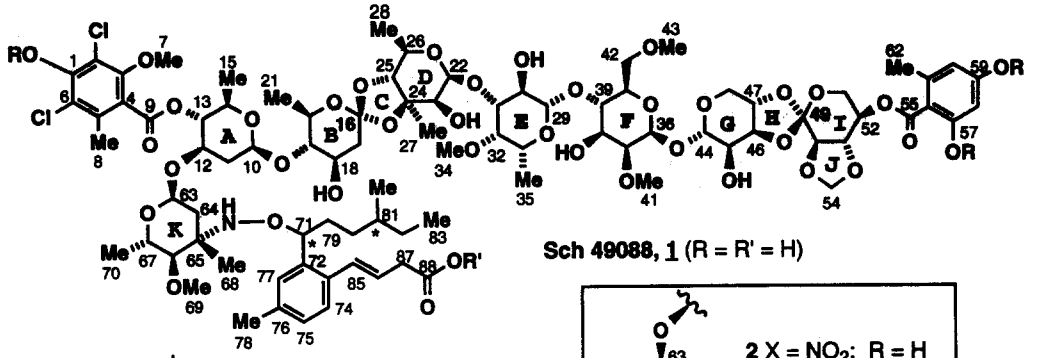
As expected, reductive N-O bond cleavage of both **9** and **10** in methanolic alkaline solutions with nickel/aluminium alloy[12,13] conveniently provided the corresponding amino sugars **15** and **16** and the lipophilic aromatic acid **14**, as waxy crystals, m.p. 50-51°C, $C_{18}H_{21}NO_4$ (M+Na$^+$ 315.1933, calcd. 315.1936), $[\alpha]_D^{25}$ -16.9 ($CHCl_3$).[14] Acetylation of the α-anomer **15** with acetic anhydride yielded the crystalline **17** m.p. 158-159°C, $C_{11}H_{21}NO_4$ (M+Na$^+$ 254.1370, calcd. 254.1368), $[\alpha]_D^{25}$ +60.5 ($CHCl_3$), which was identical in all respects (including single crystal X-ray data)[15] to the same compound isolated from evernitrose.[1b] Thus the stereochemistry of the new sugar, everhydroxylaminose **11** (isolated as β-OMe glycoside) at C65, C66 and C67 could be assigned in a relative as well as an absolute sense.[11]

We have reported on the determination of the absolute stereochemistry at the C16 orthoester of the everninomicin antibiotics.[1a] As can be seen from a comparision of ^1H and ^{13}C chemical shifts of **1** and **2** (rings **B**, **C** and **D**), there can be little doubt that the same connectivity and stereochemical relationship exists at C16 in the structure of **1**. (Table)

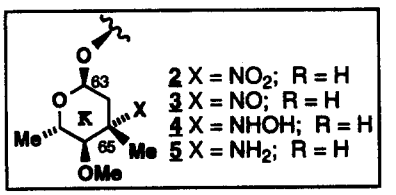
In summary, Sch 49088 (**1**) is a novel everninomicin antibiotic containing a unique O-alkylated hydroxylamine substituted sugar subunit. In *in vitro* antibacterial assays against *Staphylococci* as well as *Enterococci*, **1** was found to be only weakly active compared to ziracin (**2**), **3**, **4** and **5**.[1,2]

References and Notes:

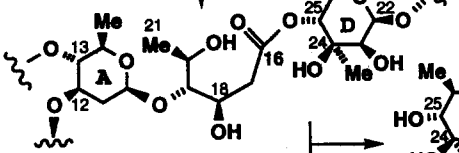
1. (a) A. K. Ganguly, J. L. McCormick, T-M. Chan, A. K. Saksena and P. R. Das, *Tetrahedron Lett.*, **1997**, 38, 7989; (b) A. K. Ganguly, B. Pramanik, T-M. Chan, Y.T. Liu, J. Morton and V. Girijavallabhan, *Heterocycles*, **1989**, 28, 83; (c) V. M. Girijavallabhan and A. K. Ganguly, *Kirk-Othmer Encyclopedia of Chemical Technology*, **1992**, 4th ed., Vol. 3, 259.
2. A. K. Ganguly. V. M. Girijavallabhan, G. H. Miller and O. Z. Sarre, *J. Antibiotics*, **1982**, 35, 561.
3. Isolation of **Sch 49088**: The fermentation broth[2] was adjusted to pH 10.5 and centrifuged to remove mycelia; the aqueous phase was adjusted to pH 7 and the resulting precipitate collected by filtration and vacuum dried at room temperature. A 40 g portion of this crude powder was chromatographed over silica gel (3.2 Kg). The column was eluted with 20% acetone/CH_2Cl_2 (28 L), 30% acetone/CH_2Cl_2 (40 L), acetone (28 L) and 5% H_2O/acetone (20 L). Fractions containing Sch 49088 (100% acetone and 5% H_2O/acetone) were combined and evaporated to dryness *in vacuo* to provide almost pure (TLC)material (18.3 g). A 7 g portion of this product was rechromatographed over silica gel (300 g) using 4-5% methanol/methylene chloride as



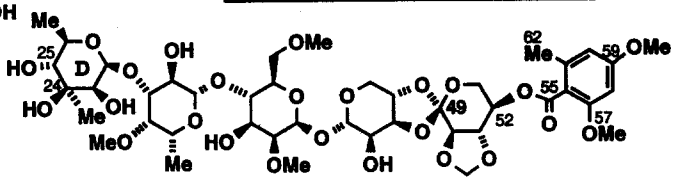
Sch 49088, 1 (R = R' = H)



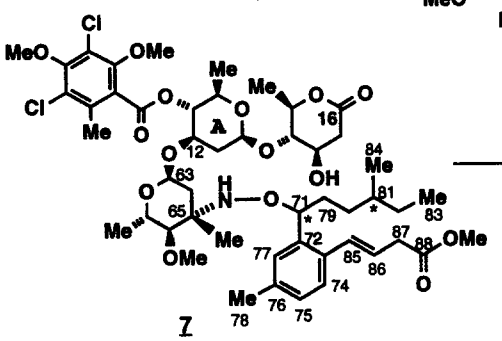
2 X = NO_2; R = H
3 X = NO; R = H
4 X = NHOH; R = H
5 X = NH_2; R = H



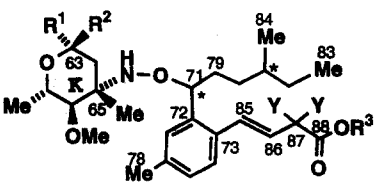
6 R = R' = Me



8



7

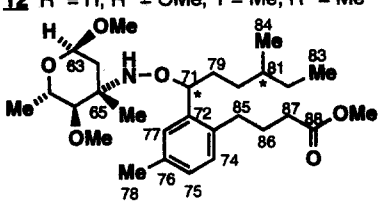


9 R^1 = OMe; R^2 = H; Y = H; R^3 = Me
10 R^1 = H; R^2 = OMe; Y = H; R^3 = Me
11 R^1 = H; R^2 =OMe; Y = H; R^3 = H
12 R^1 = H; R^2 = OMe; Y = Me; R^3 = Me

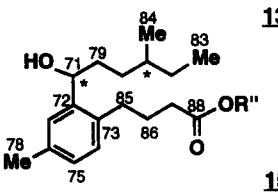
Table (in ppm)

Carbon	1		2	
	^{13}C*	^{1}H**	^{13}C*	^{1}H**
16	120.37	---	120.40	---
17	39.77	1.81, 2.41	39.79	1.81, 2.41
18	68.15	3.92	68.16	3.93
19	88.21	3.07	88.18	3.07
20	70.09	3.84	70.05	3.84
21	17.78	1.25	17.77	1.25
22	101.12	5.03	101.02	5.03
23	72.94	4.08	72.95	4.08
24	80.42	---	80.50	---
25	78.43	3.91	78.45	3.93
26	68.76	3.82	68.80	3.82
27	18.76	1.35	18.80	1.35
28	18.55	1.34	18.46	1.34

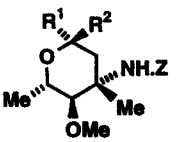
*δ (± 0.1 ppm); **δ (± 0.02 ppm)



13



14 R"=H



15 R^1 = OMe; R^2 = H; Z= H
16 R^1 = H; R^2 = OMe; Z= H
17 R^1 = OMe; R^2 = H; Z= Ac

eluent. Fractions (100 mL each) were collected and monitored by TLC (SiO_2, 10% methanol/CH_2Cl_2). Fractions 15-18 were combined and evaporated *in vacuo* to provide a white amorphous solid which was dissolved in a minimum of ethyl acetate and precipitated with efficient stirring into n-heptane. Pure Sch 49088 was dried in vacuum and obtained as a white powder (3.8 g). To prevent decomposition this material was stored as a dry powder at -40°C.

4. FAB mass spectra were obtained on the JEOL JMS-HX110A instrument equipped with a FAB gun using xenon gas. The use of DMSO as the solvent and 3NBA as the matrix with NaCl addition gave the best results producing abundant sodiated molecular ions. Negative ion FAB-MS analysis was performed in the DMSO-3NBA matrix without the addition of NaCl. High resolution fragment ions spectra (positive and negative FAB) were utilized to identify sugar sequences. The peak matching experiments were performed at a mass resolving power of 5000. Detailed mass and nmr spectral discussion will be presented elsewhere.
5. T-M. Chan, R. M. Osterman, J. B. Morton and A. K. Ganguly, *Magnetic Resonance in Chemistry*, **1997**, 35, 529.
6. NMR spectra were obtained on a Varian XL-400 instrument, operating at a frequency of 400 MHz for ^1H and 100.6 MHz for ^{13}C. Complete NMR assignments are based on COSY, NOESY, HETCOR, HMBC and SINEPT experiments according to reference 5. Selective spectral data is given here.
7. Numbering system follows according to reference 1c and has been used consistently including the degradation products except the amino sugars **15** , **16** and the acetamido derivative **17**.
8. All degradation experiments were conducted with **1a** due to its improved solubility and stability to storage compared to **1**.
9. Lactone **7**: ^1H NMR [$CDCl_3$] δ 0.80 (m, 70-CH_3, 83-CH_3, 84-CH_3), 1.00 and 1.42 (m, 80-CH_{2A}, 80-CH_{2B}), 1.05 (s, 68-CH_3), 1.10 and 1.25 (m, 82-CH_{2A}, 82-CH_{2B}), 1.29 (m,81-CH), 1.41 (d, 21-CH_3), 1.42 (d, 15-CH_3), 1.50 and 2.10 (d and dd 64-CH_{2A}, 64-CH_{2B}), 1.64 and 1.72 (m, 79-CH_{2A}, 79-CH_{2B}), 1.76 and 2.39 (m, 11-CH_{2A}, 11-CH_{2B}), 2.31 (s, 78CH_3), 2.36 (s, 8-CH_3), 2.56 and 3.09 (dd 17-CH_{2A}, 17-CH_{2B}), 3.02 (d, 66-CH), 3.22 (dd, 87-CH_2), 3.32 (dd, 19-CH), 3.43 (s, 69-OCH_3), 3.48 (m, 67-CHO), 3.60 (m, 14-CHO), 3.70 (s, 88-COOCH_3), 3.87 (s, 7-OCH_3) 3.91 (s, 1-C-OCH_3), 3.92 (m, 12-CHO), 3.99 (m, 18-CHO), 4.18 (m, 20-CHO), 4.21 (s, exchangeable), 4.59 (dd, 10-CHO), 4.71 (t, 71-CHO), 4.86 (d, 63-CHO), 4.94 (t, 13-CHO), 5.14 (broad, exchangeable), 6.08 (dt, 86-CH), 6.80 (d, 85-CH), 7.01 (d, 75-CH), 7.03 (s, 77-CH), 7.32 (d, 74-CH).
   ^{13}C NMR [$CDCl_3$] CH_3 δ 11.4 (C-83), 17.9, 18.1, 18.2, 18.3, 18.8, 19.2 (C-84), 21.4; CH_2 29.3 (C-82), 32.7, 33.4, 36.1, 36.5, 38.0, 38.6 (C-87); CH 34.4 (C-81; OCH_3) 51.9, 60.7, 61.3, 62.0; tert-C 59.6 (C-65); CHO 66.0 (C-67), 67.3 (C-18), 71.5, 71.7, 74.4, 75.7 (C-13), 82.3 (C-66), 83.3, 86.0 (C-71, C-14), 94.2 (C-63), 101.0 (C-10); tert-C 121.3, 122.7 (C-86), 125.7, 126.2, 126.6 (C-74), 127.3 (C-77),128.1 (C-75), 131.1 (C-85), 132.6, 135.3, 139.5, 153.3 (C-1/C-3), 154.7 (C-1/C-3), 165.6 (C-9/C-16), 172.0 (C-88).
10. Isolation of **9** and **10**: A solution of the lactone **7** (0.34 g) in dry methanol (30 ml) containing p-toluene sulfonic acid (0.018 g) was refluxed for 18 hrs. After cooling $NaHCO_3$ (0.025 g) was added, the reaction mixture stirred for ~30 min and evaporated to dryness *in vacuo*. The residue was dissolved in minimum CH_2Cl_2 and chromatographed over silica gel (50 g). The column was successively eluted with n-hexane and increasing concentration of ethyl acetate (5%, 8%, 10%, 12%) in n-hexane. Appropriate fractions were combined (TLC) to provide the less polar **10** (β-anomer, 0.099 g) and the more polar **9** (α-anomer, 0.056 g).
    β-anomer **10**: ^1H NMR [$CDCl_3$] δ 3.71 (s, 88-OCH_3), 3.24 (dd, J = 7.2, 1.5 Hz; 87-CH_2), 6.08 (dt, 15.5, 7.2, 7.2 Hz; 86-CH), 6.84 (broad d, J = 15.5 Hz; 85-CH), 7.34 (d, J = 7.8 Hz; 74-CH), 7.08 (broad s, 77-CH), 7.03 (dd, J = 7.8, 1.8 Hz; 75-CH_2), 2.34 (s, 78-CH_3), 4.75 (dd, J = 7.4, 5.9 Hz; 71-CH), 1.65, 1.78 (m, 79-CH_2), 1.05, 1.45 (m, 80-CH_2), 1.3 (m, 81-CH), 1.1, 1.3 (m 82-CH_2), 0.81 (t, J = 7.2; 83-CH_3), 0.82 (t, J = 6.8 Hz; 84-CH_3), 4.64 (d, J = 4.5 Hz; 63-CH), 2.15 (dd, J = 4.5, 13.8 Hz; 64-CH_{2A}), 1.56 (d, J =13.8 Hz; 64-CH_{2B}), 3.16 (d, J = 9.8 Hz; 66-CH), 3.67 (m, 67-CH), 1.28 (d, J = 6.1 Hz; 70-CH_3), 1.1 (s, 68-CH_3), 3.54 (s, 69-OCH_3), 3.28 (s, 63-OCH_3).
    ^{13}C NMR [$CDCl_3$] δ 51.8 (C-88-OCH_3), 172.0 (C-88), 38.5 (C-87), 122.6 (C-86), 131.1 (C-85), 132.5 (C-73), 139.4 (C-72), 126.5 (C-74), 127.2 (C-77), 128.0 (C-75), 137.2 (C-76), 21.3 (C-78), 83.1 (C-71), 33.3 (C-79), 32.7 (C-80), 34.4 (C-81), 29.2 (C-82), 11.3 (C-83), 19.1 (C-84), 98.7 (C-63), 38.1 (C-64), 59.7 (C-65), 82.8 (C-66), 65.2 (C-67), 18.5 (C-70), 18.1 (C-68), 61.4 (C-69), 54.6 (C-63-OCH_3).
11. Everhydroxylaminose β-methyl glycoside **11**: Except for the absence of C-88-OCH_3, the ^1H and ^{13}C NMR spectra of **10** and **11** were virtually identical. At this stage we do not know the absolute stereochemistry at C-71 and C-81. Early attempts to provide a crystalline derivative of either **11** or **14** failed to provide crystals suitable for X-ray analysis.
12. G. Lunn, E. B. Sansone, L. K. Keefer, *Synthesis*, **1985**, 1104.
13. Ni-Al alloy reduction of **9**: Ni-Al alloy (3.5 g) was added in portions to a solution of **9** (0.35 g) in methanol (20 ml) and IN KOH (14 ml). The reaction mixture was stirred at room temperature for ~15 hrs. Additional Ni-Al alloy (1 g) was added and the mixture stirred for another 8 hrs. At the end of this time the reaction mixture was filtered through celite to remove inorganic salts and washed with methanol/water (1:1, ~10 mL). The combined filtrates were evaporated to dryness *in vacuo* and residue taken up in water (~15 mL, pH ~14). The aqueous solution was extracted with CH_2Cl_2 (2 x 10 mL), the organic phase dried (Na_2SO_4) and evaporated to provide the amino sugar **15** (α-anomer ; 0.106 g) as a thick syrup. The remaining aqueous phase was adjusted to pH 2 by addition of citric acid and extracted with ethyl acetate (2 x 10 mL). The ethyl acetate phase was washed with brine (~5 mL), dried (Na_2SO_4) and evaporated to dryness *in vacuo* to provide the aromatic acid **14** (0.249 g)as an oil.
    Similar treatment of **10** with Ni-Al alloy provided the amino sugar **16** (β-anomer) and the same aromatic acid **14.**
14. Aromatic acid **14**: ^1H NMR [$CDCl_3$] δ 2.42 (t, J = 7.2 Hz; 87-CH_2), 1.92 (quintet, J = 7.6 Hz; 86-CH_2), 2.67 (m, 85-CH_2), 7.29 (broad s, 74-CH), 7.04 (d, J = 7.8 Hz; 77-CH), 7.01 (dd, J = 1.5, 7.8 Hz; 75-CH), 2.33 (s, 78-CH_3), 4.89 (dd, J = 7.6, 5.5 Hz; 71-CH), 1.72 (m, 79-CH_2), 1.1, 1.55 (m, 80-CH_2), 1.45 (m, 81-CH), 1.15, 1.45 (m, 82-CH_2), 0.85 (t, J = 7 Hz; 83-CH_3), 0.86 (d, J = 6.7 Hz; 84-CH_3).
    ^{13}C NMR [$CDCl_3$] δ 178.8 (C-88), 33.4 (C-87), 26.4 (C-86), 31.2 (C-85), 134.8 (C-73), 142.4 (C-72), 129.5 (C-74), 126.3 (C-77), 128.1 (C-75), 136.2 (C-76), 21.1 (C-78), 70.7 (C-71), 36.3 (C-79), 33.1 (C-80), 34.4 (C-81), 29.3 (C-82), 11.3 (C-83), 19.2 (C-82). **Note**: Concomitant reduction of C85, C86 double bond could not be avoided under the N-O bond cleavage conditions.
15. Atomic parameters bond lengths, bond angles and torsion angles for **17** have been deposited at the Cambridge Crystallographic Data Center, Cambridge, CB2 1EZ, U.K. Any request should be accompanied by full literature citation for this communication.