Mild Condition Synthesis and Biological Activity: 1, 2, 3, 4-Tetrahydro-6-HydroxyBenz[*a*]anthracene-1, 7, 12-Trione

A. F. G. Masud Reza* and F. R. Alam

Abstract— The synthesis of new compounds with a broad spectrum of activity is caused by a large number of existing drugs with significant side effects, which is needed to minimize. The angucycline group is not only visual art but also have their own chemical properties like pharmaceutical. This group is the largest group and engineered natural products, rich in biological activities and chemical scaffolds. Mild condition, synthesis of 6-hydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene **6** through Krohn's method and simple aerial oxidation of this compounds **6** synthesized 1, 2, 3, 4-Tetrahydro-6-Hydroxy Benz[*a*]anthracene-1, 7, 12-Trione **7**, a basic skeleton of angucycline and tested antimicrobial activity, and found moderately biological active towards *Bacillus subtilis*, *Streptococcus-β-haemolitycus* and *Escherichia coli.*Quantum-mechanical calculations for compounds-substrates by means of computer modeling were carried out. The high degree of affinity to the chosen biological target gives us a promising opportunity in displaying antitumor activity of that compounds. By in silico studies the possibility of displaying biological activity of synthesized compounds was predicted.

Keywords — tetrahydro-β-naphthol, PIDA, sunlight, 6-hydroxy-1,2,3,4-tetrahydrobenz[a]anthracene.

1 INTRODUCTION

INTEREST in the synthesis of new compounds with a broad spectrum of activity is caused by a large number of existing drugs with significant side effects, which is needed to minimize. For example, hydrazine sulfate, used in the treatment of cancer at the same time is highly toxic. So, the challenge of scientists is to synthesize active substances that will show maximum pharmacological activity and minor side effects.

Angucycline group is not only visual art but also have their chemical properties like pharmaceutical. The angucycline group is the largest group and engineered natural products, rich in biological activities and chemical scaffolds. This stimulated synthetic creativity and biosynthetic inquisitiveness. The synthetic activity in the area of angucyclines began in 1976 with the synthesis of tetrangulol by Brown and Thomson [1].

The modern definition of angucyclines was introduced in 1984 after at least dozen of compounds of this class of antibiotics had already been published [2]. All angucyclines are secondary metabolites of some microorganism isolated either from various soil sample or from sallow sea mud[3]. Biosynthetic studies of Gould and co-workers [4] have revealed that angucyclines are derived from ten acetate[5] via polyketide pathway. The classification of angucyclines proposed by Rohr and Thriecke [6] in 1992. Angucyclines have a wide range of pharmacological properties, they are showing interesting biological activities, antitumor[7], hydroxylase[8]and /or monooxygenase inhibition[9], blood platelet aggregation[10], antibacterial[11] and antiviral[12] activity & antipyretic properties. It is isolated from plants and microorganisms are widely used for the treatment of cancerous tumors and parasitic diseases.

An overview of the existing methodologies and their strength and limitations are highlighted. Thomsons involved an intermolecular Michael type addition[1], Krohn et al[13] utilized an intramolecular nucleophilic displacement reaction, Uemura et al[14] used arene chromium tricharbonyl complex in the synthesis of rabelomycin and ochromycinone. Deshpande et al[15] have accomplished a total synthesis of brasiliquinone B following the similar strategy. Recently, Karsten Krohn[16] synthesised rabelomycin by biomimetic type angucycline from dekaketide.

The Diels-Alder reaction is one of the most convenient ways to construct six member condensed ring systems. In 1932, Carothers and

• *Department of Chemistry, Natural Science Group*
• *National University, Gazipur-1704, Bangladesh.*
**Email. masudrezanu@gmail.com*

Coffman[17] reported formation of benz[*a*]anthraquinone. The Diels-Alder reaction between push pull diene and juglone in the presence of $B(OAc)_3$ provided ochromycinone in an excellent yield [18]. Valderrama et al[19] have shown that the Diels Alder reaction between spirodienone and diene could be a viable route to the benz[*a*]anthraquinone skeleton, a different type of disconnection for the synthesis of angucyclines was employed by Suzuki et al [20].

In 1992 Gordon and Danishefsky[21] have fabricated a macrocyclic ring in presence of naphthoquinone and Fischer chromium carbene complex. Following this elegant work another methodology was developed by Chuang et al[22] who used Mn (III) acetate promoted free radical generation and cyclisation leading to benz[*a*]anthraquinone system. In another study, Murphy et al [23] have applied a similar method on bromonaphthoquinone having a peri methoxy group.

Various approaches to the synthesis of angucyclines have been undertaken in the past 30 years. However, the wide structural varieties and complexities of angucyclines provide ample scope for further studies towards discovering newer pharmaceutical chemistry such as biological properties.

2 EXPERIMENTAL

The melting point was recorded on an electro thermal melting point apparatus (Gallenkamp) and was uncorrected. Infrared spectra (IR) were recorded on FTIR-8400 and Perkin-Elmer 883 using KBr pellets for solids and neat for liquids and the characteristic peaks are expressed in cm^{-1}. ^{1}HNMR spectra were recorded on a 200 MHz (Bruker) spectrometer as solutions in ^{2}H-chloroform with tetramethylsilane as an internal standard. Chemical shifts are expressed in δunit and $^1H—^1H$ coupling constant in Hz. Thin layer chromatographic plates (0.25 mm thickness) were prepared by spreading a layer of silica gel (60 GF_{254}). E. Merck) on glass plates. For preparative layer chromatography (PLC) the layer was formed over a glass plate using water suspension of silica gel (60 GF_{254}).

5, 6, 7, 8- Tetrahydro-4a-methoxy naphthalene-2(4aH)-one 2

To a stirred solution of 5,6,7,8-tetrahydro-β-naphthol **1** (0.2 g, 1.35 mmol) in methanol (10 ml) at 0°C under N_2 atmosphere, was added PIDA (Phenyliodonium diacetate) (0.2 g, 0.62 mmol). This condition was maintained for about 30 min. and then the mixture was allowed to come to ambient temperature for 1 h. Bulk of methanol was evaporated under reduced pressure and the residue upon quick silica gel filtration afforded the enone **2** (69 % yield) as a neat liquid. IR: 1663, 1441, 1095.^{1}H NMR (CD_2Cl_2): δ= 6.67 (d, 1H, *J* = 10 4-H), 6.30 (dd, 1H, *J* = 10, 2, 3-H), 6.14 (m, 1H, 1-H), 3.04 (s, 3 H, -OMe), 2.31 (m, 2H, ring-CH_2), 2.00 (m, 3H, ring-CH_2), 1.60 (m, 1H, ring-CH_2), 1.32 (m, 2H, ring-CH_2).

3-Cyanophthalide 5

To a water solution of NaCN (10 g, 204 mmol) at 0 °C was added 3-hydroxyphthalide **3** (5 g, 33.3 mmol). Hydrochloric acid (32 %, 37.5 ml) was added slowly to this mixture and temperature mainted at 0°C for about 1 h or until no staring material could be detected. This mixture was then extracted with ethyl acetate (2 x75 mL) and the combined extract was washed with saturated brine, water, dried over Na_2SO_4 and its volume reduced to 75 ml. This solution then cooled to 0 °C and dicyclohexyl carbodiimide (DCC) (7.34 g. 36 mmol) was added to it. The resulting solution was stirred overnight at ambient temperature. After filtration of dicyclohexyl urea from the reaction mixture, the filtrate was evaporated to dryness and the residue was chromatographed on silica gel to give a solid which was recrystallized from ethyl acetate to give **5** as a white solid (77% yield) m.p. 66°C (lit. [28] 65-67°C). IR: 1772.5. ^{1}H NMR (CD_2Cl_2): δ= 8.00 (d, 1H, *J* = 6, Ar-H), 7.91 (t, 1H, *J* = 8.4, Ar-H) 7.70 (m, 2H, Ar-H), 6.07(s, 1H, -CHCN).

6-hydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene 6

To a stirred solution of $LiOBu^t$(0.328 g, 4.1 mmol) in THF (25 ml) under N_2 atmosphere at 0 °C was added cyanophthalide **5** (0.500 g, 3.12 mmol). A golden yellow colour was developed at this point. After 10 min. a solution of naphthalenone **2** (0.55 g, 3.09 mmol) in THF was introduced into the reaction mixture and quickly transferred the reaction flask to refrigerator. The resulting deep green colour mixture was kept -10°C for 2 h and then at ambient temperature for 4 h. It was then quenched with saturated NH_4Cl (7 ml) and concentrated to give a solid residue. This was filtered and washed with water several times. The

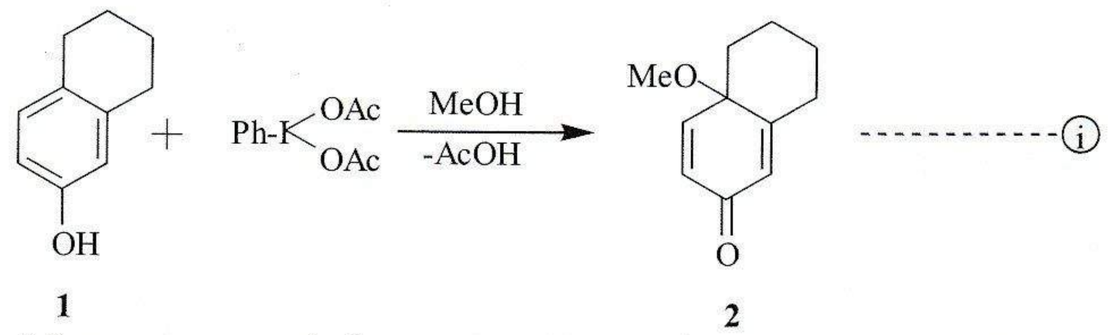
filtrate was extracted with ethyl acetate (3 x 25 ml).The combined extract was washed with brine (25 ml), water, dried (Na_2SO_4) and then concentrated to give a gummy solid which was combined with the first crop of the product and chromatographed to yield **6** as an orange colour solid in 82% yield. m.p. 143-145°C (lit.[27] 144-145°C). IR: 3440, 1634, 1582. ^{1}H NMR (CD_2Cl_2): δ= 13.05 (s, 1H, Ar-OH), 8.21 (m, 2H, 8, 11 -H), 7.74 (m, 2H, 9, 10 –H), 7.01 (s, 1H, 5 –H), 3.26 (br s, 2H, 1 –H), 2.88 (br s, 2H, 4 –H), 1.82 (m, 4H, 2,3 –H).

1,2,3,4-tetrahydro-6-hydroxybenz[*a*]anthracene-1,7,12-trione 7

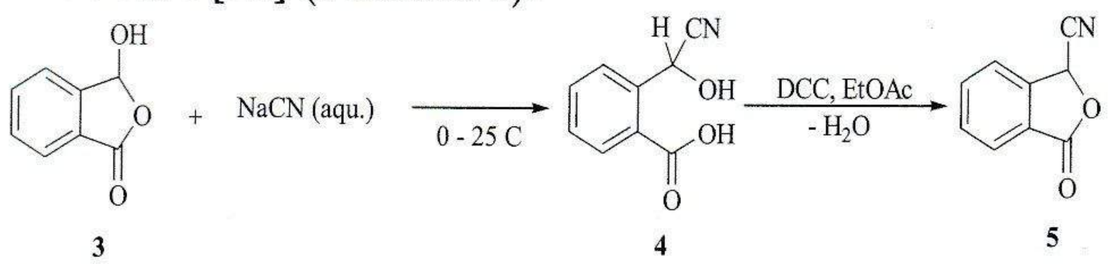
A solution of compound **6** (0.03 mmol) in distilled $CHCl_3$ (10 ml) was taken in a small conical flask and directly exposed it to sunlight. When the reaction deemed complete (5-6 h) by TLC, the solution was concentrated and chromatographed to furnish compound **7** as an orange-red crystals (93%), m.p. 185-186°C (lit. [27]186-187°C). IR: 3443, 1686, 1636, 1589. ^{1}H NMR (CD_2Cl_2): δ= 13.05 (s, 1H, Ar-OH), 8.40 (m, 2H, 8, 11 -H), 7.75 (m, 2H, 9, 10 –H), 7.00 (s, 1H, 5 –H), 2.82 (br s, 2H, A-ring –CH_2), 1.89 (m, 4H, A-ring –CH_2).

3 Results And Discussion

In our present study, we focused our attention to the synthesis of angucycline a new class of antibiotic, popularly employed for the treatment of tumor[24]. In consonance, we opted to utilize PIDA (Phenyliodonium diacetate) for the oxidation of tetrahydro-*β*-naphthol **1**. There exist many reagents and procedures in literature[25, 26] for the oxidation of phenols and *β*-naphthols. But PIDA oxidation is superior to all other reagents. It is mild and the reaction is generally carried out at ambient temperature in the presence of air and moisture[27]. Work-up procedure is very simple. No acid or alkali generally is required for quenching the reaction. To generate the demanded enone **2**,PIDA has been applied upon compound **1** as a test case. Exceptionally, it was found one equivalent of PIDA was sufficient for the complete oxidation of tetrahydro-*β*-naphthol **1**(equ. i).

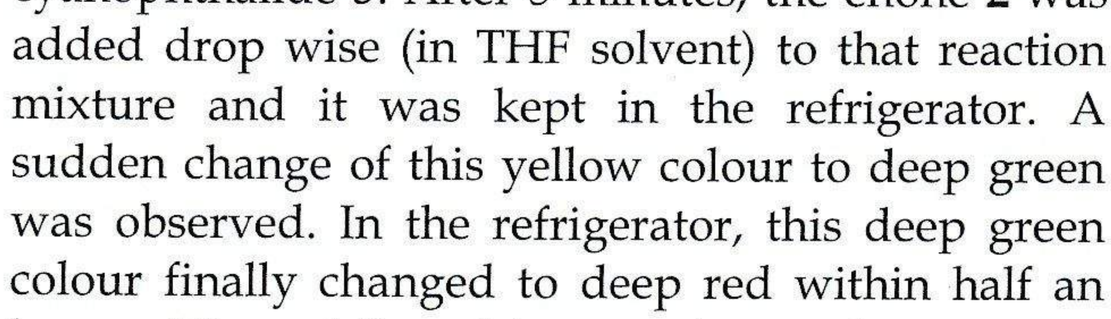


After successful attainment of the enone **2**, we prepared cyanophthalide **5** by the literature procedure[28]. In that particular literature KCN was used to form cyanohydrin with 3-hydroxy phthalalide **3**. But in our present work we have used NaCN to avoid excessive toxicity of KCN. The yield of the cyanophthalide is quite satisfactory (75%) and its melting point perfectly matched with the literature [28] (Scheme 1).



Scheme 1

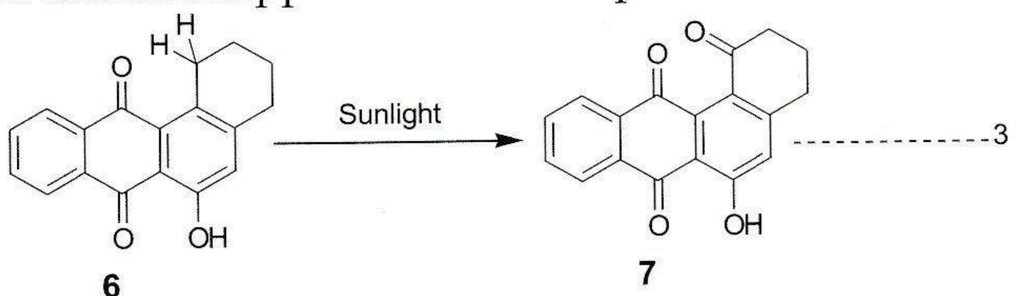
To construct the basic skeletone of an angucycline **6**, cyanophthalide **5** was reacted to a stirred THF solution of LiOBu^t base with enone **2** under N_2 prior at -10°C (equ.3). At the first stage of this reaction, a beautiful golden yellow colour was developed when LiOBu^t was added to THF solution of cyanophthalide **5**. After 5 minutes, the enone **2** was added drop wise (in THF solvent) to that reaction mixture and it was kept in the refrigerator. A sudden change of this yellow colour to deep green was observed. In the refrigerator, this deep green colour finally changed to deep red within half an hour. Meanwhile, this reaction mixture was removed from the refrigerator and it was kept outside for half an hour. Saturated NH_4Cl solution was used to quench the reaction mixture. The whole mass was concentrated and eventually, we obtained an orange colour solid. Column chromatography with silica gel (60-120 mesh) afforded beautiful orange colour crystals with 85% yield (equ. ii).



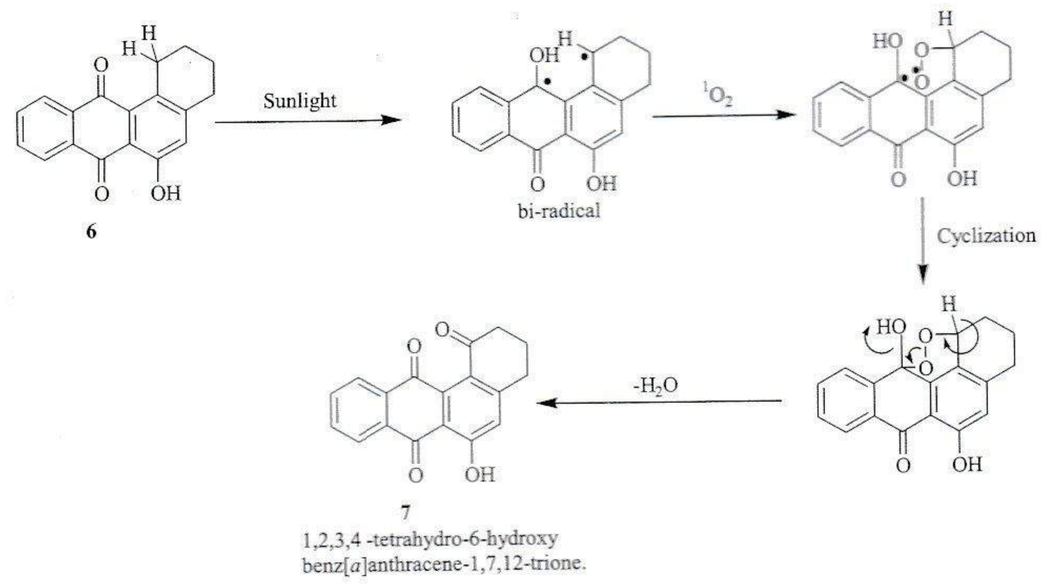
Infrared spectroscopy of this compound **6** indicated two sharp >C=O peaks at ν = 1634, 1582 cm^{-1} and one –OH broad peak at ν = 3440 cm^{-1} respectively. Proton magnetic resonance spectroscopy indicated an aromatic hydroxy group at δ = 13.05 ppm (due to H-bonding between >C=O of C-7 and –OH group of C-6 carbon atom) and eight aliphatic protons have

been found in the region δ = 3.26-1.82 ppm. With these peaks the aromatic protons were also observed in the region δ = 8.27-7.01 ppm.

Surprisingly, in the presence of sunlight and in chloroform solution, the compound **6** undergoes a quick change. TLC monitoring in chloroform petroleum ether (1:5) solvent system, it was observed a different growing spot showing a different R_f-value relative to the starting tetracyclic compound **6.** Observing this change of compound **6** inspired us to keep all the compound in sunlight in chloroform solution. Within six hours, the whole mass had been converted into a new spot. After that,chloroform was evaporated and a deep red colour product was obtained. IR spectra of this compound indicated a new >C=O peak in the region of ν = 1686 cm^{-1}.In proton magnetic resonance spectra, two hydrogen atoms were missing from the aliphatic regions. Literature[29] showed that this type of compound undergoes a quick change in sunlight in $CHCl_3$ solution due to the presence of γ-proton with respect to >C=O group at C-12 carbon. This is clearly a Norrish type-II reaction happened in the compound **6.**



Proposed mechanism, In the presence of sunlight and air, the γ-proton is first abstracted by the >C=O group at C-12 carbon atom and formed a bi-radical. This bi radical trapped the singlet oxygen ($^1O_2$) to form a cyclic peroxide. In sunlight, this cyclic peroxide underwent quick disruption and eventually eliminated a molecule of water from the peroxide and thus introduced a new >C=O group at C-1 carbon. The mechanism may be in the following manner (Scheme 2).



Scheme 2

4 BIOLOGICAL EVALUATIONS

Angucycline are showing antibacterial activity[30] therefore, we opted to utilize compound **7** directly to some antibacterial tests. Interestingly, this compound **7** beautifully responded to these biological tests, it's showed clear antimicrobial activity against the selected test pathogen in comparison with that of standard kanamycin (Table 1) by the disk diffusion assay [31].However, the activity profile of compound **7** was nicely against *Bacillus subtilis, Streptococcus β haemolitycus* and *Escherichia coli.* In comparison with other pathogens.

Table 1: Antimicrobial activity of 1, 2, 3, 4-Tetrahydro-6-Hydroxy Benz[*a*]anthracene-1, 7, 12-Trione **7** and standard Kanamycin (K)

Bacterial Strains	Diameter of zone of inhibition (mm)		
	100 µg/disc	150 µg/disc	K 30 µg/disc
Bacillus subtilis	11	11	34
Streptococcus-β-haemolitycus	12	13	30
Escherichia coli	10	12	23

The minimum inhibitory concentration of the compound **7** was determined against *Bacillus subtilis* and *Escherichia coli* by serial dilution technique [31]. The MIC levels of the compound **7** were found 64 µg/mL against both *Bacillus subtilis* and *Escherichia coli* (Table 2).

Table 2 : The MIC values of the compound **7**

Test Organism	MIC values (µg/mL)
Bacillus subtilis	64
Escherichia coli	64

Having carried out in silico studies by molecular docking, we could predict the possibility of showing

the biological activity of compounds. As a result of docking studies the highest affinity of the studied compounds to a fragment of DNA was established. Accordingly, low levels of binding compounds with fragment tubulin, protein PPARγ were found. In Fig. 1&2, it was shown the compound-hit 1, 2, 3, 4-Tetrahydro-6-Hydroxy Benz[*a*]anthracene-1, 7, 12-Trione **7** in the field of DNA binding fragment (crystallographic model 2DES) and in compare with the selective inhibitor Morpholine-Doxorubicin. There was the possibility

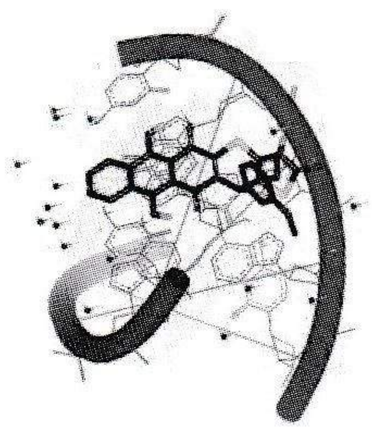


Fig.1

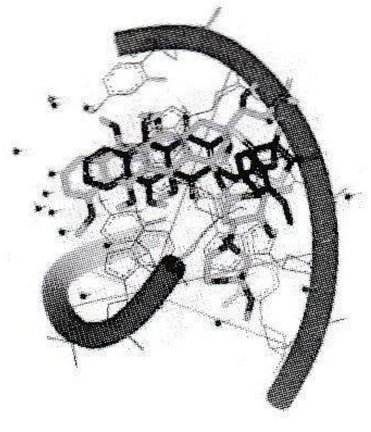


Fig.2

a) **Fig. 1.** The compound-hit **7** in the field of binding fragment DNA (crystallographic model 2DES). b) **Fig. 2.** The compound-hit **7** in the field of binding fragment DNA (crystallographic model 2DES) and compared with selective inhibitor Morpholine-Doxorubicin.

of π-π binding between the fragments of compound 1, 2, 3, 4-Tetrahydro-6-Hydroxy Benz[*a*]anthracene-1, 7, 12-Trione **7** and fragments of purine nucleotides, and also hydrophobic interactions of Morpholine-Doxorubicin and DNA.

Analyzing the results of obtained scoring functions we can talk about the highest binding of compound **7** with a fragment of DNA. The high degree of affinity to the chosen biological target gives us a promising opportunity in displaying antitumor activity of that compounds.

5 CONCLUSION

We have synthesized 1,2,3,4-tetrahydro-6-hydroxybenz[*a*]anthracene-1,7,12-trione **7**, a basic skeleton of angucycline by simple aerial oxidation of 6-hydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene **6** and tested antimicrobial activity, found this compound **7** is moderately biological active. Quantum-mechanical calculations for compounds-substrates by means of computer modeling were carried out. The high degree of affinity to the chosen biological target gives us a promising opportunity in displaying antitumor activity of that compounds.

REFERENCES

[1] P. M. Brown and R. H. Thomson, *J. Chem. Soc.,Perkin Trans 1*, 997, 1976.

[2] H. Drautz, H. Zahner,J. Rohr and A. Zeeck, *J. Antibiot.*, **39**, 1657, 1984.

[3] T. Okazaki, T. Kitahara, and Y. Okami, *J. Antibiot.*, **28**, 176, 1975.

[4] S. J. Gould, X-c. Cheng, and C. Melville, *J. Am. Chem. Soc.*, **116**, 1804, 1994.

[5] a) T. J. Simpson, *Nat. Prod. Rep.*, 1985, **2**, 321. b).T. J. Simpson, *Nat. Prod. Rep.*, **4**, 339,1987.

[6] J. Rohr and R. Thireike, *Nat. Prod. Rep.*, **9**, 103, 1992.

[7] a). S. Omura, H. Tanaka, R. Oiwa, J. Awaya, R. Masuma, and K. Tanaka, *J. Antibiot.*, **30**, 908, 1977. b). J. H. Wilton, D. C. Cheney, G. C. Hokanson, J. C. French, H. Cun-heng, and J. Clardey, *J. Org. Chem.*, **50**, 3936, 1985.c). Lili Zhu, Bohdan Ostash, Uwe Rix, Mohammad Nur-e-Alam, Almuth Mayers, Andriy Luzhetskyy, Carmen Mendez, Jose A. Salas, Andreas Bechthold, Victor Fedorenko, and Ju¨ rgen Rohr, *J. Org. Chem.*, **70**, 631-638, 2005.

[8] K. U. Bindseil, P. Hug, H. H. Peter, F. Petersen, B. E. Roggo, *J. Antibiot.*,**48**, 457, 1995.

[9] K. Ohta, E.Mizuta, H. Okazaki, T. Kishi, *J. Antibiot.*, **32**, 4350,1984.

[10] S. Omura, A. Nakagawa, N. Fukamachi, S. Miura, Y. Takahashi, K. Komiyama, Y. Kobayashi, *B. J. Antibiot.*, **41**, 812, 1988.

[11] S. J. Gould, X.-c. Cheng, *J. Org. Chem.*, **59**, 400, 1994.

[12] T. Sasaki, S. Gomi, M. Sezaki, Y. Takeuchi, Y. Kodoma, and K. Kawamura, *J. Antibiot.*, **41**, 843, 1988.

[13] K. Krohn, W. Droge and F. Hintze, *Anal. Quim.*, **91**, 388, 1995.

[14] a). M. Uemura, K. Take, Y. Hayashi, *J. Chem. Soc. Chem. Commun.*, 858, 1983. b). M. Uemura, K. Take, K. Isobe, T. Minami, and Y. Hayashi, *Tetrahedron.*, **41**, 5771, 1984.

[15] M. L. Patil, H. B. Borate, D. E. Ponde, B. M. Bhawal, and V. H. Deshpande, *Tetrahedron Lett.*, **40**, 4437, 1999.

[16] a). Karsten Krohn, Ulrich Flörke, Christian Freund, and Nasir Hayat, *Eur. J. Org. Chem.*, 1627-1632, 2000. b). Karsten Krohn, *Eur. J. Org. Chem.*, 1351-1362, 2002.

[17] W. H. Carothers and D. D. Coffman, *J. Am. Chem. Soc. Chem. Commune.*, **54**, 4071, 1993.

[18] A. Guingant and M. M. Barreto, *Tetrahedron Lett.*, **28**, 3107, 1987.

[19] J. A. Valderrama, C. D. Pessoa-Mahana and R. Tapia, *j. Chem. Soc., Perkin Trans 1*, 3521, 1994.

[20] a). T. Matsumoto, T. Sohma, H. Yamaguchi, S. Kurata, K. Suzuki, *Synlett.*, 263, 1995. b). T. Matsumoto, T. Sohma, H Yamaguchi, S. Kurata, K. Suzuki, *Tetrahedron*, **51**, 7347, 1995.

[21] D. M. Gordon, S. J. Danishefsky and G. K. Schulte, *J. Org. Chem.*, **52**, 7052, 1992.

[22] a). C-P. Chuang and S-F. Wang, *Tetrahedron.*, **54**, 1043, 1998. b). C-P. Chuang and S-F Wang, *Tetrahedron Lett.*, **35**, 4365, 1994.

[23] W. S. Murphy, D. Nevile, and G. Ferguson, *Tetrahedron Lett.*, **37**, 7615, 1996.

[24] G. Matsuo, Y. Miki, M. Nakata, S. Matsumura, and K. Toshima, *J. Chem. Soc., Chem. Commun.*, 225, 1996.

[25] U. K. Mallik and A. K. Mallik, *Ind. J. Chem. Soc.*, **31**, 696, 1992.

[26] A. Pelter and S. M. A. Elegendy, *J. Chem. Soc.,Perkin Trans 1.*, 1891, 1993.

[27] Dipakranjan Mal , Harendra N. Roy, Nirmal K. HaTra and Susanta Adhikari, *Tetrahedron*, **53** (6), 2177-2184, 1997.

[28] J. N. Freskos, G. W. Morrow and J. S. Swenton, *J. Org. Chem.*, **50**, 805, 1985.

[29] K. Krohn, A. Ballwanz, and W. Baltus, *Liebigs. Ann. Chem.*, 911, 1993.

[30] a). A. W. Beur, W. M. M. Kirby, J. C. Sherris, M. Turck, *Am. J. clin. Pathol.*, **44**,493,1966. b). Shoji, Junichi et al. Studies on antibiotics from the *genus Bacullus* XX isolation of two new polymycin group antibiotics, *J. Antibiot*, **30** (12), 1029 (Eng), 1977.

[31] a). Roland Reiners, Antibiotics, chemotherapeutic agent and Development of chemotherapy, *Antibiotics An Introduction*. Roche scientific service, Switzerland, 2-9 Detection of antibiotic activity 21, 1982. b). V. E. Tayler, L. R. Brady, J. E. P. Robbers, Ninth edition, Lea and febiger Philadelphia, 321, 1988.