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TOTAL SYNTHESIS OF A NOVEL CYTOTOXIC METABOLITE GYMNASTATIN A

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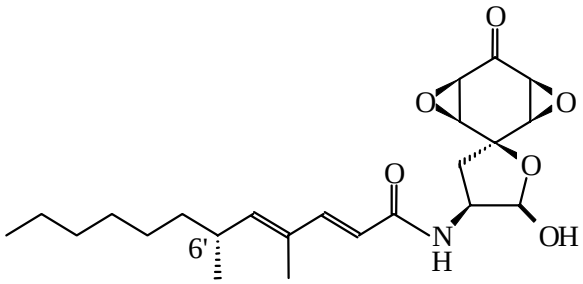
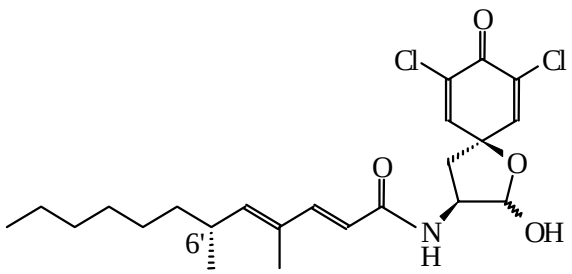
Abstract *- The first total synthesis of a novel, potent cytotoxic metabolite gymnastatin A is described.*

INTRODUCTION

As a part of our continued interest in natural products belonging to 1-oxaspiro[4.5]decane ring system, we earlier reported studies[1] on the total synthesis of aranorosin (**1**), associated with a potent cytotoxic activity. Recently, five metabolites belonging to the above class and designated as gymnastatin A-E, produced by the strain of *Gymnasella dankaliensis* isolated from the sponge *Halicondria japonica*, were reported.[2] Among the five metabolites, gymnastatin A (**2**) showed the strongest cytotoxicity (ED_{50} value 0.018 μg mL^{-1}) in the P388 lymphocyte test system in a cell culture.



Aranorosin (**1**)



Gymnastatin A (**2**)

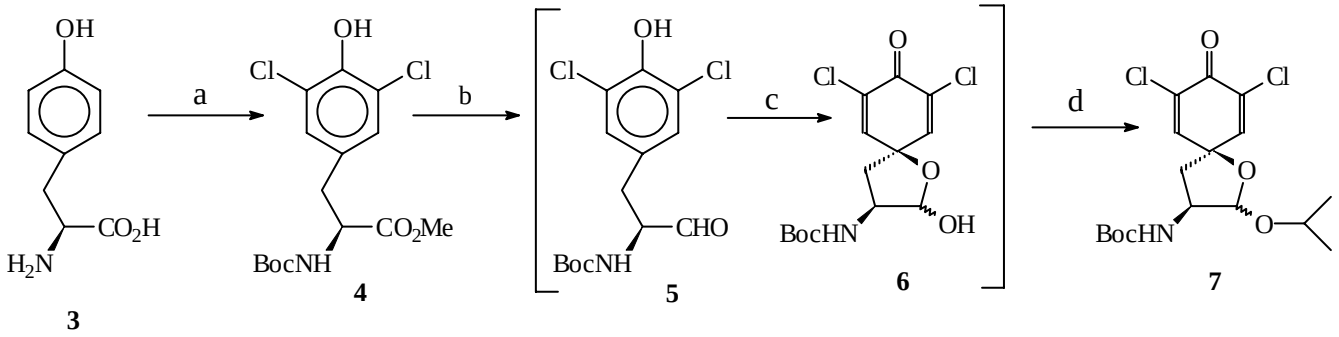
The absolute stereostructure of **2** except that of C-6' was elucidated. However, later studies[3] based on spectroscopic and chemical transformation established the stereochemistry at C-6' to be *R* which correlated with the side chain of aranorosin (**1**). Herein we report the first total synthesis of gymnastatin A (**2**).

RESULTS AND DISCUSSIONS

The synthetic strategy was based on oxidative cyclisation of 3,5-dichlorotyrosine derivative to obtain 1-oxaspiro[4.5]decane system. The introduction of C-12 side chain on nitrogen mediated by peptide bond

coupling should complete the total synthesis of gymnastatin A.

Scheme 1



Reagents and conditions: a) ref. 4; b) DIBAL-H, CH_2Cl_2, -78 ^{0}C, 1 h; c) $PhI(OCOCF_3)_2$, MeCN : H_2O (4:1), rt, 30 min; d) isopropanol, cat. CSA, rt, 24 h.

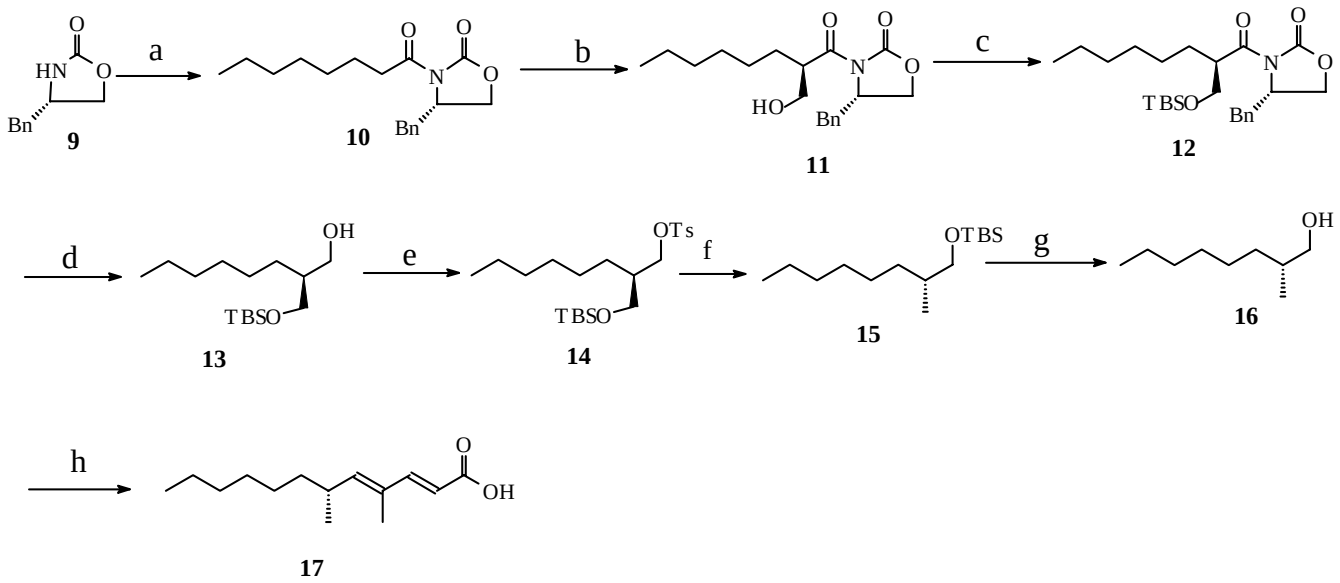
Chlorination of L-tyrosine (**3**) with chlorine in acetic acid at 0^0C furnished 3,5-dichloro-L-tyrosine which was subsequently esterified in the presence of MeOH and acetyl chloride followed by protection of the free amino group with $(Boc)_2O$ to give the *N*-Boc protected tyrosine derivative (**4**)[4] (Scheme 1). Conversion of the ester group of **4** into the corresponding aldehyde (**5**) was accomplished with DIBAL-H in toluene-CH_2Cl_2 at -78 ^{0}C.

Earlier we have reported[1] the oxidative cyclisation of tyrosine derivative[5-7] using bis(trifluoro-acetoxy)iodobenzene (PIFA) to afford the spirolactone derivative. A similar approach was sought for the preparation of the spirolactol derivative (**6**). Thus, compound (**5**) was reacted with PIFA in aqueous MeCN to afford the spirolactol derivative (**6**), *albeit in* 20% yield. For convenience it was decided to protect the lactol function in **6** as isopropyl acetal derivative (**7**) using isopropanol and catalytic CSA.

The *N*-octanoyloxazolidinone[8,9] derivative (**10**) was synthesized from Evans oxazolidinone derivative (**9**) and octanoic acid (**8**) by a mixed anhydride method as depicted in Scheme 2. Subsequent condensation[10] of **10** with 1,3,5-trioxane in the presence of titanium tetrachloride and Hunig's base provided the alcohol (**11**). When the present work was undertaken the issue related to the stereochemical configuration at C-6' of **2** was not settled.[2] Therefore we planned to develop a strategy by which both the (6'*R*)- and (6'*S*)- side chains could be synthesized from a common precursor (**11**). Compound (**11**) can form a surrogate for both 6'*R* and 6'*S* chain of gymnastatin A. For example, reduction of hydroxylmethyl group at C-2 would lead to 6'*S* derivative (route b) whereas reduction of amide at C-1 (route a) would provide 6'*R* configuration (Figure 1). Since Numata *et al.*[3] conclusively established the stereochemistry of the side chain as *R*, route a should fulfil our requirement. Protection of free hydroxyl with TBS-Cl in the presence of imidazole gave rise to the silyl ether (**12**). Treatment of **12**

with $LiBH_4$ in ether cleaved the oxazolidinone ring and consequently reduced the carbonyl group to afford the alcohol (**13**). In order to deoxygenate the hydroxymethyl function, compound (**13**) was treated with *p*-toluenesulfonyl chloride and pyridine to afford **14**, which was reduced with $LiAlH_4$ in THF at 50 ^{0}C to give **15**. Removal of the TBS group from **15** with nBu_4NF in THF gave (2*R*)-2-methyloctanol (**16**) whose optical rotation and ^{1}H-NMR data were identical with the reported values.[7]

Scheme 2



Reagents and conditions: a) $C_7H_{15}CO_2H$ (**8**), ref. 8; b) $TiCl_4$, DIPEA, CH_2Cl_2, 1,3,5-trioxane, 0^{0}C-rt, 2 h; c) TBSCl, imidazole, CH_2Cl_2, rt, 1 h; d) $LiBH_4$, ether, water, rt, 1 h; e) *p*-TsCl, Py, CH_2Cl_2, rt, 5 h; f) $LiAlH_4$, THF, 50 ^{0}C, 4 h; g) 1M nBu_4NF, THF, rt, 1 h; h) ref. 1.

Conversion of **16** to (6*R*)-4,6-dimethyldodecadienoic acid (**17**) was carried out by the procedure reported from this laboratory.[1] Having obtained both the moieties *viz*. spirolactol derivative (**7**) and the 6*R*-side chain (**17**), our next concern was to develop a suitable procedure for the coupling reaction. Accordingly, the

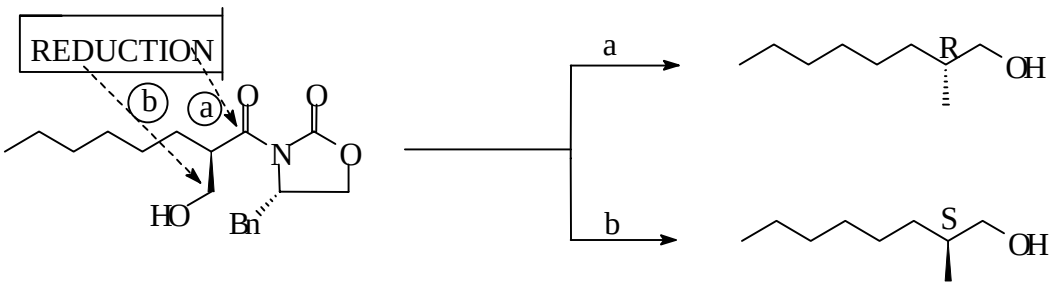
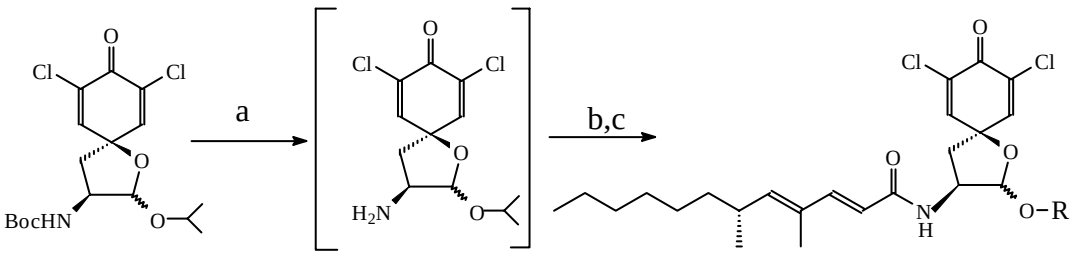


Figure 1

spirolactol derivative (**7**) was treated with CF_3CO_2H to cleave the *N*-Boc protecting group and then the free amine (**18**) was coupled with the acid (**17**) in the presence of a promoting agent, EDCI and catalytic DMAP to give **19** (Scheme 3).

Scheme 3



7 **18**

19 R = CHMe$_2$
2 R = H

Reagents and conditions: a) CF_3CO_2H, CH_2Cl_2, $0^0C \rightarrow$ rt, 30 min; b) EDCI, cat. DMAP, DMF, **17**, rt, 4 h; c) 70% CH_3CO_2H, cat. H_2SO_4, THF, 50 ^{0}C, 12 h.

Finally compound (**19**) was treated with 70% CH_3CO_2H in the presence of catalytic amount of H_2SO_4 in THF to deprotect the isopropyl group and obtain gymnastatin A (**2**). The optical rotation, ^{1}H-NMR and ^{13}C-NMR data for the synthetic product (**2**) were identical with the reported values of the natural product.[2] In the preceding lines we have described the first total synthesis of gymnastatin A by an efficient approach. Based on the strategy adopted it could be possible to obtain analogues of gymnastatin A for undertaking structure-activity relationship.

EXPERIMENTAL

(2*RS*,3*S*)-3-(*N*-*tert*-Butoxycarbonyl)amino-7,9-dichloro-2-isopropoxy-1-oxaspiro[4.5]-deca-6,9-dien-8-one (7**).**

Compound (**4**)[4] (5.0 g, 13.7 mmol) in CH_2Cl_2 (25 mL) was cooled to –78 ^{0}C and 1M solution of DIBAL-H in toluene (42.5 mL, 43.0 mmol) was added slowly maintaining the temperature. After 1 h at -78^{0}C, the reaction was quenched by addition of MeOH (1.5 mL) and saturated potassium ammonium tartarate. The organic layer was separated, washed with water, dried (Na_2SO_4) and concentrated to afford **5** (4.1 g) which was taken further for next reaction.

To the above product (**5**) (4.1 g) in MeCN: H_2O (15 mL, 4:1), PIFA (5.0 g, 11.6 mmol) was added and then stirred at rt for 30 min. MeCN was removed, the residue extracted with CH_2Cl_2 and the extract was successively washed with water, dried (Na_2SO_4) and concentrated. The resulting product was chromatographed on silica gel using EtOAc-light petroleum (1:4) to afford **6** (0.96 g, 20%), oil, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.50 (s, 9H), 2.61 (t, 1H, J= 10.6 Hz), 2.82 (dd, 1H, J= 10.6, 12.7 Hz), 4.45 (m, 1H), 5.25 (br s, 1H), 7.01 (d, 1H, J= 2.3 Hz), 7.17 (br s, 1H), IR (neat): 3392 (NH), 1716 (C=O) cm^{-1}, HRFABMS: Found 350.0569 (M^+) calcd for $C_{14}H_{17}NO_5Cl_2$: 350.0562.

Compound (**6**) (0.5 g, 1.5 mmol), CSA (25 mg) and isopropanol (5.0 mL) were stirred at rt for 24 h, basified with Et_3N and concentrated. The residue was partitioned between CH_2Cl_2-water. The organic layer was dried (Na_2SO_4), concentrated and the residue was chromatographed on silica gel using EtOAc-light petroleum (1:9) to give **7** (0.45 g, 80%), oil, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.22 (m, 6H), 1.45 (m, 9H), 2.10 (t, 1H, J= 12.2 Hz), 2.55 (m, 1H), 3.95 (m, 1H), 4.15 (br s, 1/2 H), 4.35 (br s, 1/2 H), 4.87 (d, 1H, J= 8.2 Hz), 5.16 (d, 1/2 H, J= 4.9 Hz), 5.24 (s, 1/2 H), 6.95 (s, 1H), 7.05 (br s, 1H), FABMS m/z M^+: 392, Anal. Calcd for $C_{17}H_{23}NO_5Cl_2$. C, 52.04; H, 5.86. Found: C, 52.20; H, 5.71.

[3(2'*S*), 4*S*]-3-(2-Hydroxymethyl-1-oxooctyl)-4-phenylmethyl-2-oxazolidinone (11).

To a solution of oxazolidinone derivative (**10**) (5.0 g, 16.5 mmol) in dry CH_2Cl_2 (25 mL) at 0^0C, 1M solution of $TiCl_4$ in CH_2Cl_2 (17.3 mL 17.3 mmol) was added followed by, after 15min, with diisopropylethylamine (3.4 mL, 18.6 mmol). The reaction mixture was stirred for 1 h at 0^0C, 1,3,5-trioxane (3.0 g, 33.3 mmol) in CH_2Cl_2 (10.0 mL) was added followed by 1M solution of $TiCl_4$ in CH_2Cl_2 (17.3 mL, 17.3 mmol). The reaction was quenched after 2 h with saturated NH_4Cl solution and layers separated. The organic layer was washed with water, dried (Na_2SO_4) and concentrated to afford a residue which was purified on silica gel using EtOAc-light petroleum (1:3) to give **11** (4.7 g, 85%) as a thick syrup, $[\alpha]_D$ + 60° (c 1.7, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 400 MHz) : δ 0.83 (t, 3H, J= 6.5 Hz), 1.24 (m, 10H), 2.75 (t, 1H, J= 11.3 Hz), 3.24 (d, 1H, J= 11.3 Hz), 3.77 (m, 2H), 3.86 (m, 1H), 4.09 (m, 2H), 4.64 (m, 1H) 7.20 (m, 5H), HRFABMS: Found 333.1923 (M^+) calcd for $C_{19}H_{27}NO_4$: 333.1940.

(2*R*)-2-*tert*-Butyldimethylsilyloxymethyl-1-octyl-4-methyl-1-benzenesulfonate(14).

Compound (**11**) (7.0 g, 21.0 mmol), imidazole (3.3 g, 46.0 mmol), and TBSCl (3.8 g, 25.2 mmol) in CH_2Cl_2 (40 mL) was stirred for 1 h and worked-up in the usual fashion to afford a residue which was purified on silica gel using EtOAc-light petroleum (1:4) to give **12** (8.2 g, 87%), oil, $[\alpha]_D$ + 30° (c 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.06 (s, 6H), 0.83 (br s, 12H), 1.38 (br s, OH), 2.66 (dd, 1H, J= 8.5, 12.8 Hz), 3.29 (dd, 1H, J= 3.3, 12.8 Hz), 3.76 (dd, 1H, J= 4.2, 8.5 Hz), 3.87 (t, 1H, J= 8.5 Hz), 4.04 (m, 1H), 4.12 (m, 2H), 4.68 (m, 1H), 7.25 (m, 5H), HRFABMS: Found 448.2870 (M^++1) calcd for $C_{25}H_{42}NO_4Si$: 448.2881.

Compound (**12**) (8.2 g, 18.3 mmol), lithium borohydride (0.43 g, 19.7 mmol), ether (30 mL) and water (0.33 mL) were stirred at rt for 1 h, diluted with 1M sodium hydroxide solution and layers separated. The organic layer was washed with water, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using EtOAc-light petroleum (1:9) to afford **13** (3.7 g, 74%), oil, $[\alpha]_D$ - 12° (c 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.06 (s, 6H), 0.83 (br s, 12H), 1.27 (br s, 10H), 1.68 (br s, 1H), 2.66 (br s, 1H),3.57 (dd, 1H, J= 8.5, 10.6 Hz), 3.68 (m, 1H), 3.78 (dd, 1H, J= 4.2, 10.6 Hz), FABMS m/z M^+: 274.

To a solution of **13** (2.0 g, 7.3 mmol) in dry CH_2Cl_2 (10 mL), pyridine (1.0 mL) and PTSCl (1.9 g, 10.0 mmol) were added. The reaction was stirred for 5 h at rt, diluted with CH_2Cl_2 and washed with saturated

$NaHCO_3$ solution, water, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using EtOAc-light petroleum (1:6) to afford **14** (2.8 g, 90%), oil, $[\alpha]_D$ -17° (c 1.2, $CHCl_3$),^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.06 (s, 6H), 0.84 (br s, 9H), 0.91 (t, 3H, J= 6.5 Hz), 1.25 (br s, 10H), 1.75 (m, 1H), 2.47 (s, 3H), 3.42 (dd, 1H, J= 5.5, 9 Hz), 3.56 (dd, 1H, J= 4.6, 9 Hz), 4.00 (d, 2H, J = 4.55 Hz), 7.34 (d, 2H, J= 8.86 Hz), 7.80 (d, 2H, J= 8.86 Hz), HRFABMS: Found 429.2481 (M^++1) calcd for $C_{22}H_{41}O_4SSi$: 429.2494.

(2*R*)-2-Methyloctan-1-ol (16).

A solution of **14** (2.0 g, 4.7 mmol) and $LiAlH_4$ (0.21 g, 5.60 mmol) in dry THF (12 mL) was heated at 50 ^{0}C for 4 h. The reaction mixture was quenched by slow addition of 15 % NaOH solution. The solution was filtered and filtrate concentrated. The residue was chromatographed on silica gel using EtOAc-light petroleum (1:9) to afford **15** (0.98 g, 82%) which was dissolved in THF (5 mL) and then 1M solution of Bu_4NF in THF (4.2 mL, 4.2 mmol) introduced. After 1 h at rt, saturated NH_4Cl solution was added, THF removed under reduced pressure and the aqueous layer extracted with ethyl acetate. The organic layer was washed with water, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using EtOAc-light petroleum (1:4) to give **16** (0.49 g, 87%), oil, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (m, 6H), 1.25 (m, 10H), 1.64 (m, 1H), 2.50 (br s, 1H), 3.41 (m, 2H); $[\alpha]_D$ + 10° (c 1.0, CH_2Cl_2), lit.,[7] $[\alpha]_D$ + 10.3° (c 1.0, CH_2Cl_2).

(2*E*,4*E*,6*R*)-*N*-[(2*RS*,3*S*)-7,9-Dichloro-2-isopropoxy-8-oxo-1-oxaspiro[4.5]deca-6,9-di-en-3-yl]-4,6-dimethyl-2,4-dodecadienamide (19).

To a stirred solution of **7** (1.3 g, 3.29 mmol) in dry CH_2Cl_2 (10 mL), cooled at 0 ^{0}C, was slowly added CF_3CO_2H (5.0 mL). The reaction mixture was brought to rt and stirred for 30 min. The solution was concentrated to give **18** as a salt. This was dissolved in anhydrous DMF (3 mL) and the pH of the solution was adjusted to 8 by adding dry Et_3N. In a separate flask, compound (**17**) (0.6 g, 2.7 mmol), EDCI (0.51 g, 2.7 mmol) and cat. DMAP were taken in DMF (5 mL) and then the mixture was stirred for 30 min till a clear solution was obtained. To this was added the solution containing compound (**18**). After 4 h, the reaction mixture was diluted with CH_2Cl_2, washed with water. The organic layer was dried (Na_2SO_4) and evaporated. Chromatographic purification on silica gel using EtOAc-light petroleum (1:6) gave **19** (0.78 g, 71 %) as a thick syrup, $[\alpha]_D$ - 6° (c 1.6, $CHCl_3$), ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, 3H, J= 6.5 Hz), 0.99 (d, 3H, J= 6.7 Hz), 1.10-1.26 (m, 10H), 1.30 (d, 6H, J= 6.8 Hz), 1.78 (s, 3H), 2.03-2.30 (m, 1H), 2.37-2.77 (m, 2H), 3.90-4.10 (m, 1H), 4.50 (m, 1/3H), 4.78 (m, 2/3H), 5.20 (d, 2/3H, J= 4.5 Hz), 5.30 (s, 1/3H), 5.65 (d, 1H, J= 11.0 Hz), 5.72 (d, 1H, J= 16 Hz), 5.89 (d, 1H, J= 8.2 Hz), 6.96 (d, 1H, J= 2.3 Hz), 7.03 (d, 1H, J= 2.3 Hz), 7.25 (d, 1H, J=16 Hz), HRFABMS : Found (M^++1) 499.2263 calcd for $C_{26}H_{38}NO_4Cl_2$: 499.2256; Anal. Calcd for $C_{26}H_{37}NO_4Cl_2$: C, 62.65; H, 7.42. Found: C, 62.33; H, 7.28.

Gymnastatin A (2).

To a solution of **19** (0.70 g, 1.4 mmol) in THF (5 mL) was added 70% acetic acid (3 mL) and catalytic

H_2SO_4. After being stirred at 50 ^{0}C for 12 h, the mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4) and the mixture was concentrated. The crude mixture was purified on silica gel using EtOAc-light petroleum (1:3) to obtain **2** (0.32 g, 50 %) as a white solid, mp 70 °C, $[\alpha]_D$ – 3.9° (c 0.7, $CHCl_3$), lit.,[2] $[\alpha]_D$ -3.8° (c 0.8, $CHCl_3$). IR (neat): 3378, 3274, 1698, 1654, 1608 cm^{-1}, ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87 (t, 3H, J= 6.5 Hz), 0.96 (d, 3H, J= 6.6 Hz), 1.3 (m, 10H), 1.77 (s, 3H), 2.20 (t, 1H, J= 12.8 Hz), 2.50 (m, 1H), 2.60 (dd, 2/3H, J= 8.3, 12.8 Hz), 2.80 (dd, 1/3H, J= 8.3, 12.8 Hz), 4.57 (m, 2/3H), 4.75 (m, 1/3H), 5.54 (m, 1H), 5.75 (d, 1H, J= 15.7 Hz), 5.75 (d, 1H, J= 10 Hz), 6.00 (d, 1H, J= 8.2 Hz), 7.00 (d, 1H, J= 2.9 Hz), 7.08 (d, 1H, J=2.9 Hz), 7.25 (d, 1H, J= 15.7 Hz). ^{13}C ($CDCl_3$, 50 MHz) : δ 172.1, 169.5, 167.7, 149.7, 147.7, 146.3, 144.7, 131.0, 130.5, 130.5,117.9, 103.1, 96.3, 81.0, 79.7, 58.9, 51.0, 41.0, 38.4, 36.8, 32.7, 31.0, 28.9, 26.8, 22.1, 20.3, 14.7, 13.1, HRFABMS : Found (M^+) 456.1676 calcd for $C_{23}H_{31}NO_4Cl_2$: 456.1708.

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REFERENCES

1. A. V. Rama Rao, M. K. Gurjar, and P. A. Sharma, *Tetrahedron Lett*., 1991, **32**, 6613.
2. A. Numata, T. Amagata, K. Minoura, and T. Ito, *Tetrahedron Lett.*, 1997, **38**, 5675.
3. A. Numata, T. Amagata, K. Minoura, M. Doi, and T. Ohta, J. *Chem. Soc*., *Perkin Trans*. 1, 1998, 3585.
4. P. A. Sharma, *Ph.D. Thesis*, Bombay University, Bombay, 1992.
5. P. McKillop, L. McLaren, R. J. K. Taylor, R. J. Watson, and N. Lewis, *Tetrahedron Lett*., 1993, **34**, 5519.
6. P. McKillop, L. McLaren, R. J. K. Taylor, R. J. Watson, and N. Lewis, *Synlett*, 1992, 201.
7. P. Wipf, Y. Kim, and P. C. Fritch, *J. Org. Chem*., 1993, **58**, 7195.
8. D. A. Evans and J. R. Gage, *Org. Synth*., 1989, **68**, 77.
9. J. J. Plattner, A. K. L. Fung, J. A. Parks, R. J. Pariza, S. R. Cowley, A. G. Pernet, P. R. Bunnell and P. W. Dodge, *J. Med. Chem*., 1984, **27**, 1016.
10. D. A. Evans, F. Urpi, T. C. Somers, S. Clark and M. T. Bilodeau, *J. Am. Chem. Soc*., 1990, **112**, 8215.