A New Approach to Tubacin

Jian Hong*, Xin Xu, Debasis Das*, Pengyu Yang, Shu-Hui Chen and Ge Li

Division of Discovery Chemistry Service, WuXi AppTec Inc., 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, P.R., China

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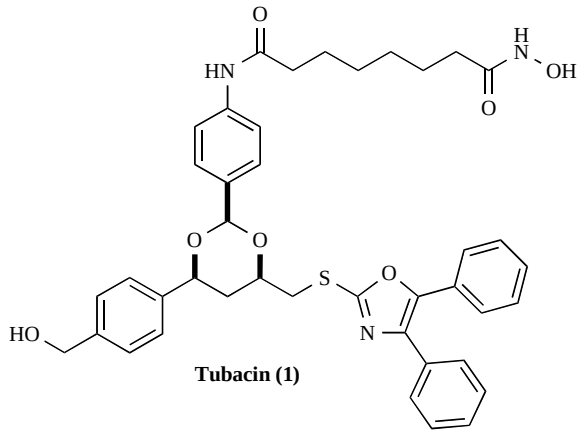
Abstract: A new simple synthesis of tubacin is reported. It entails the formation of the *syn*-1,3-diol unit and its stereoselective ketalization with a functionalized benzaldehyde derivative.

Keywords: Tubacin, histone deacetylases, allyl borane reaction, stereoselective ketal formation, solution phase synthesis, hydroxyamide synthesis.

INTRODUCTION

Every year throughout the world, around 10 million people are diagnosed with cancer. Cancer cells are highly dependent on protein degradation due to cell cycle, hypermutation and chromosomal rearrangement. Histone deacetylases (HDACs) [1] are nuclear zinc-dependent enzymes which catalyze deacetylation of N-acetyl-lysine residues and play an important role in a number of biological processes such as gene expression [2] and cell cycle. Compounds which inhibit HDACs can suppress the expression of certain genes thus resulting in an antitumor effect [3]. Therefore in recent years, a key success in cancer research was the discovery of novel HDAC inhibitors [4]. This resulted in the launch of a non-selective HDAC inhibitor Zolinza® by Merck Co. Still much research is being focused on the discovery of new selective HDAC inhibitors [5].



Tubacin (1)

Fig. (1).

Identified recently as a selective HDAC6 inhibitor by high-throughput synthesis [6], tubacin (1) (Fig. **1**) does not affect the level of histone acetylation in gene expression and cell cycle. As selective inhibitors of HDAC6 are used in the treatment of protein degradation disorders [7], tubacin may have therapeutic applications as antimetastatic and antiangiogenic agent. Since the discovery of tubacin, its demand has been increasing and the compound is used as a reference for the design of new HDAC inhibitors with enhanced activity and selectivity.

Challenges in the tubacin synthesis are the construction of the three-substituted 1,3-dioxane ring with all substituents in *syn* dispositions and finding a short route to its *syn*-1,3-diol unit. Schreiber and coworkers described a synthesis of tubacin on a solid support [8]. Herein we report our facile route to tubacin relying upon a simple formation of *syn*-1,3-diol unit and its stereoselective ketalization with a functionalized benzaldehyde derivative.

RESULTS AND DISCUSSION

Our route to tubacin is depicted in Scheme **1**. Thus, the commercially available 1,4-benzenedimethanol (**2**) was selectively mono-TBDPS-protected and oxidized to **3** in 92% yield. The reaction of (-)-B-allyldiisopinocampheylborane ((-)-Ipc_2BAll) [9] with the *para*-substituted benzaldehyde **3** led to the desired *S*-homoallylic alcohol **4** in 65% yield and 96% *ee*. Instead of targeting the chiral pure *syn*-epoxide, we have prepared epoxide **5** as a mixture of diastereomers *via m*-CPBA oxidation [10]. This mixture was then reacted with 4,5-diphenyloxazolin-2-thione [11] affording diastereomers **6a** (42%) and **6b** (36%) [12] which were separated by preparative HPLC. The absolute stereochemistry of the *syn*-1,3-diol **6a** was unambiguously determined based on ^{13}C NMR analysis of the corresponding acetonide **7a** [13]. The ^{13}C NMR chemical shifts of the two methyls and the central quaternary carbon of 19.1, 29.3 and 98.8 ppm, respectively, support a chair conformation and confirm the *syn*-1,3-orientation compared to the *anti*-diastereomer **7b** which has a boat conformation (with 24.1, 24.2 and 100.5 ppm, respectively).

Progressing towards tubacin (Scheme **2**), the synthesis of fragment **9** was accomplished in good yield by coupling 4-aminobenzyl alcohol (**8**) with octanoyl monomethyl ester

*Address correspondence to these authors at the Division of Discovery Chemistry Service, WuXi AppTec Inc., 288 Fute Zhong Road, Waigaoqiao Free Trade Zone, Shanghai 200131, P.R.China; Tel: +86-21-58682139, +86-21-58683340;
E-mails: hong_jian@wuxiapptec.com, debasis_das@wuxiapptec.com

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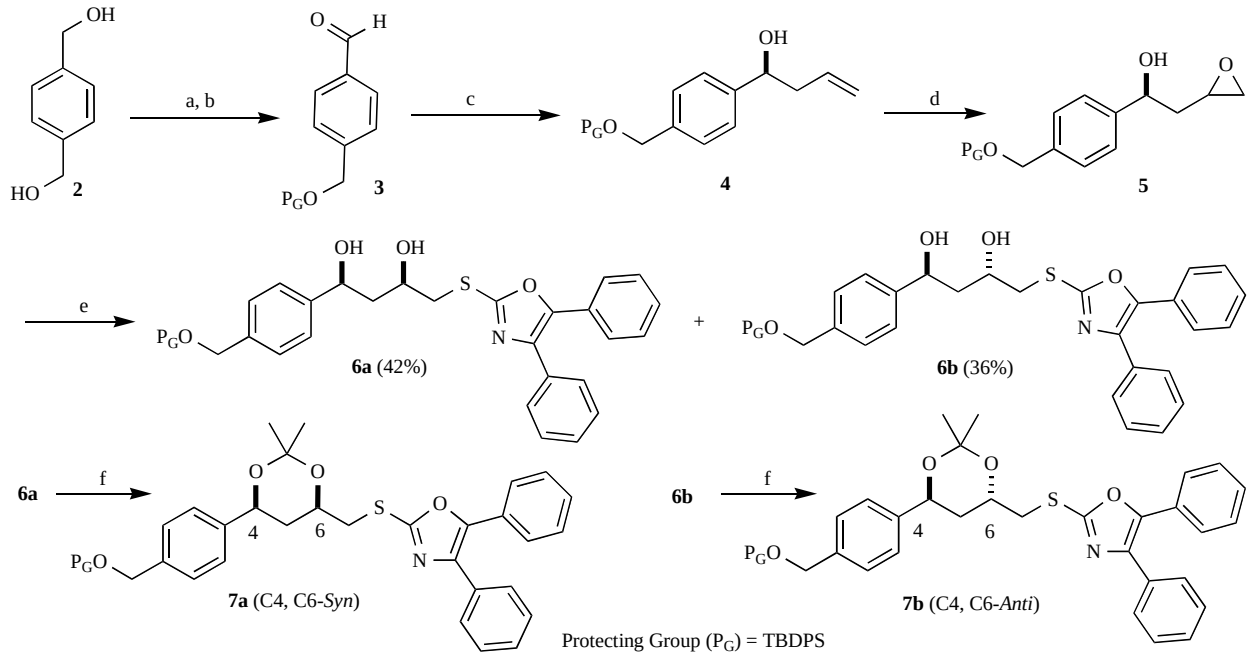
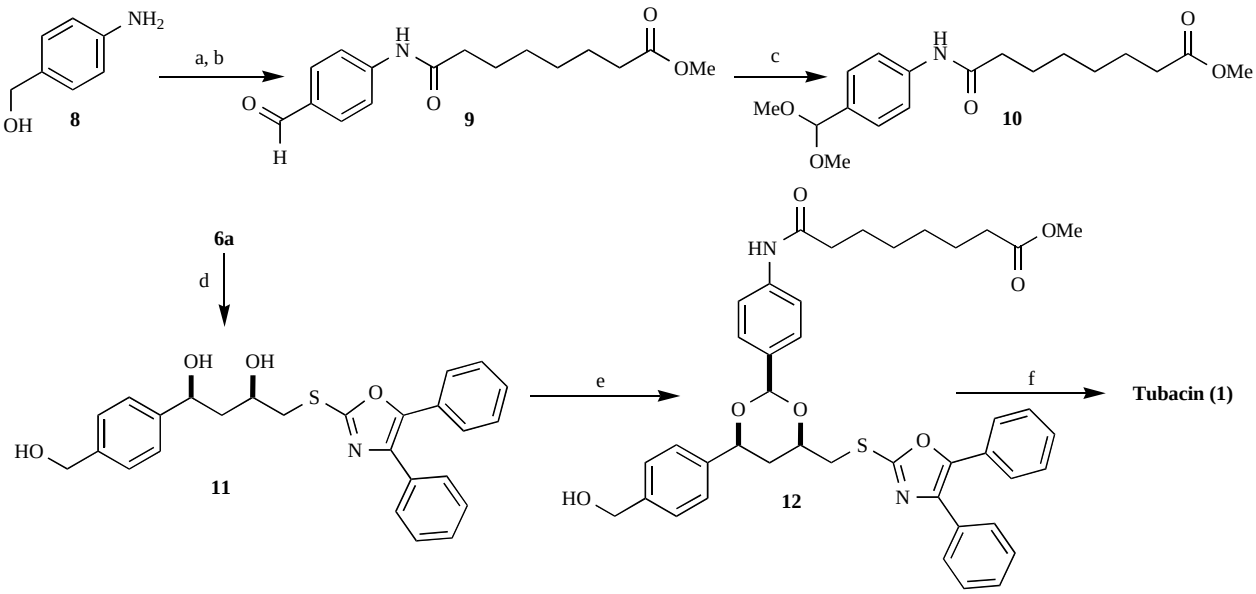


Scheme 1. Reagents and conditions: (**a**) TBDPSCl, imidazole, DMF, 50 °C, 16 h, 42%; (**b**) MnO_2, CH_2Cl_2, rt, 12 h, 92%; (**c**) (-)-Ipc_2BAll, Dioxane, Et_2O, -100 °C, 2 h, 65%; (**d**) *m*-CPBA, $CHCl_3$, rt, 24 h, 75%; (**e**) 4,5-diphenyloxazole-2(3H)-thione, DIEA, DMF, rt, 78%; (**f**) $Me_2C(OMe)_2$, TsOH, rt, 12 h, 76-80%.



Scheme 2. Reagents and conditions: (**a**) Methyl 8-chloro-8-oxooctanoate, Et_3N, CH_2Cl_2, rt, 10 h, 61%; (**b**) MnO_2, CH_2Cl_2, rt, 16 h, 90%; (**c**) $HC(OMe)_3$, TsOH, MeOH, reflux, 2 h, 94%; (**d**) TBAF, THF; (**e**) **10**, PPTS, DMF, rt, 12 h, 74%; (**f**) $NH_2OH.HCl$, KOH, MeOH, rt, 46%.

acid chloride, followed by oxidation. However, as the condensation of diol **6a** with aldehyde **9** did not proceed well, the aldehyde was converted into its acetal **10**. Several failed attempts to couple **6a** with **10** under different conditions pushed us to resort to **11** which was prepared by TBAF-deprotection of **6a**. Thus, acetal **10** was easily coupled with **11** in the presence of pyridinium *p*-toluenesulfonate (PPTS) to afford the desired compound **12** in 74% yield. Interestingly, the ketal formation was completely stereoselective as only product **12** was formed. The absolute configuration of the newly generated chiral center was established by NOE experiment [14]. Finally, treatment of **12** with hydroxylamine led smoothly to tubacin in a moderate 46% yield [15].

CONCLUSION

In summary, we have achieved the synthesis of tubacin in a simple and efficient way. This new synthetic route is based on the stereoselective preparation of the *syn*-1,3-diol fragment of tubacin and on its successful stereoselective ketalization with a benzaldehyde derivative. Our simple

approach could be extended to the synthesis of other tubacin analogues such as tropcin and histacin.

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[12] Preparation of **6**: To a solution of 4,5-diphenyloxazole-2(3H)-thione (1.0 g, 4.0 mmol) in DMF (5 mL), diisopropyl ethyl amine (DIEA) (1.5 mL) was added followed after 15 min by compound **5** (1.5 g, 3.5 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 12h then poured onto water (100 mL) and extracted with ethyl acetate (3x50 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue was passed through a silica gel pad (eluting with petroleum ether: ethyl acetate = 1:1) to give mixture **6** (1.85 g, 78% yield) as a yellow oil. Preparative HPLC afforded pure diastereomers **6a** and **6b**. **6a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ= 1.16 (s, 9H), 1.92-2.19 (m, 2H), 3.30-3.45 (m, 2H), 4.37-4.44 (m,1H), 4.78 (s, 2H), 5.05 (dd, 1H, J_1 = 2.4, J_2 = 11.6 Hz), 7.34-7.42 (m, 16H), 7.53-7.57 (m, 2H), 7.64-7.70 (m, 6H). **6b**: ^{1}HNMR (400 MHz, $CDCl_3$): δ = 1.09 (s, 9H), 1.99-2.17 (m, 2H), 3.35-3.45 (m, 2H), 4.38-4.43 (m, 1H), 4.77 (s, 2H), 5.12 (dd, 1H, J_1 = 2.8, J_2 = 8 Hz), 7.34-7.45 (m, 16H), 7.50-7.57 (m, 2H), 7.65-7.72 (m, 6H).

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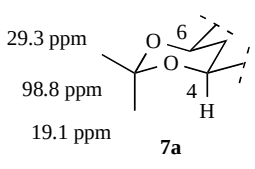

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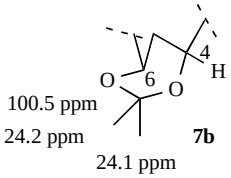
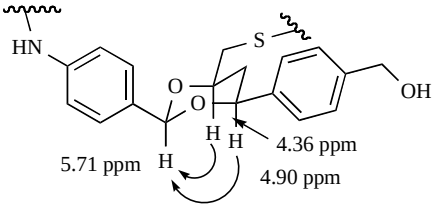


7b

[14] Synthesis of **12**: To a solution of compound **11** (100 mg, 0.23 mmol) in DMF (3.0 mL) were added compound **10** (337 mg, 1.0 mmol) and pyridinium *p*-toluenesulfonate (PPTS) (100 mg, 0.40 mmol). The mixture was stirred at room temperature for 12 h. Water (30 mL) was added and the mixture was extracted with ethyl acetate (3x50 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel (ethyl acetate: CH_2Cl_2 = 1:6, then, petroleum ether: ethyl acetate = 1:1) to afford ester **12** (103 mg, 74% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.35-1.36 (m, 4H), 1.61-1.71 (m, 4H), 1.82 (m,1H), 2.06-2.12 (m, 2H), 2.31 (t, 4H, *J* = 7.2 Hz), 3.51 (d, 2H, *J* = 6 Hz), 3.67 (s, 3H), 4.36 (m, 1H), 4.66 (s, 2H), 4.90 (d, 1H, *J* = 11.2 Hz), 5.71 (s, 1H), 7.32-7.41 (m, 10H), 7.51-7.56 (m, 6H), 7.61-7.69 (m, 2H).



Characteristic NOEs of **12**

[15] Synthesis of tubacin (**1**): To hydroxylamine hydrochloride (3.0 g, 43 mmol) in methanol (7.5 mL) KOH (2.4 g, 43 mmol) in methanol (14 mL) was added. The mixture was stirred at room temperature for 1 h and filtered. The prepared NH_2OH solution (4.0 mL, 2.0 M in methanol, 8.0 mmol) was added to compound **12** (0.27 g, 0.40 mmol), followed by a KOH solution (0.8 ml, 1.0 M in methanol, 0.8 mmol). The mixture was stirred at room temperature for 16 h then concentrated. Water (10 mL) was added to the residue and extracted with ethyl acetate (3x20 mL). The organic phase was dried over anhydrous Na_2SO_4, concentrated and the residue purified by chromatography on silica gel (MeOH: CH_2Cl_2 = 1:30 then 1:10) affording tubacin (110 mg, 46% yield) as a white solid. 1H NMR (400 MHz, CD_3OD): δ = 1.37-1.38 (m, 4H), 1.61-1.69 (m, 4H), 1.81 (m, 1H), 2.03 (m, 1H), 2.09 (t, 2H, *J* = 7.2 Hz), 2.33 (t, 2H, *J* = 7.2), 3.52 (m, 2H), 4.38 (m, 1H), 4.58 (s, 2H), 4.95 (dd, 1H, J_1 = 1.8, J_2 = 11.0 Hz), 5.71 (s, 1H), 7.32-7.40 (m, 12H), 7.44-7.48 (m, 6H). ESI-MS m/z calcd for $C_{41}H_{43}N_3O_7S$: 721.28; found: 722.3 $(M+1)^+$.