Check for updates

View Article Online
View Journal

Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: S. Fedorka, L. M. V. Tillekeratne, W. Taylor, A. Al-Hamashi, K. So, Ibtissam. Gad, R. Shah, Veronika. Kholodovych and Hanan. .Alqahtani, *Org. Biomol. Chem.*, 2018, DOI: 10.1039/C7OB03086J.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the author guidelines.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the ethical guidelines, outlined in our author and reviewer resource centre, still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C7OB03086J

Small-molecule anticancer agents kill cancer cells by harnessing reactive oxygen species in an iron-dependent manner

Sara R. Fedorka[a], Kevin So[a], Ayad Al-Hamashi[a], Ibtissam Gad[b], Ronit Shah[b], Veronika Kholodovych[b], Hanan D. Alqahtani[b], William R. Taylor[b]* and L. M. Viranga Tillekeratne[a]*

[a]Department of Medicinal and Biological Chemistry, College of Pharmacy and Pharmaceutical Sciences, The University of Toledo, 2801, W. Bancroft Street, Toledo, Ohio 43606, United States
[b] Department of Biological Sciences, College of Natural Sciences and Mathematics, The University of Toledo, 2801, W. Bancroft Street, Toledo, Ohio 43606, United States
*These authors contributing equally to this work and are co-corresponding authors.

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Organic & Biomolecular Chemistry Accepted Manuscript

Abstract:

In the course of generating a library of open-chain epothilones, we discovered a new class of small molecule anticancer agents that has no effect on tubulin but instead kills selected cancer cell lines by harnessing reactive oxygen species in an iron-dependent manner. Results of the preliminary studies are consistent with the recently described cell death mechanism ferroptosis. Studies are in progress to confirm ferroptosis as the cell death mechanism and to identify the specific molecular targets of these small molecule anticancer agents.

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Introduction

Epothilones **1**,**2** (Fig. 1), first isolated from the myxobacterium *Sorangium cellulosum* in the 1980s,[1] are potent cytotoxic agents[2] that induce apoptosis through microtubule stabilization like the blockbuster drug paclitaxel.[3,4] Since their isolation, they received the attention of the scientific community due to their potent cytotoxicity against a variety of cancer cell lines including breast, lung, and prostate cancers.[2] Both paclitaxel and epothilone molecules bind to the α/β tubulin heterodimer subunits increasing tubulin aggregation to microtubules, while decreasing α/β heterodimer disassociation from microtubules.[3]

We previously reported the synthesis and biological activity of a new class of structurally simplified open-chain epothilones **3** and **4** (Fig. 1)[5] in which the C9-C15 sector of the epothilone molecules (**1** and **2** , Fig. 1)[2–4] has been completely deleted, while retaining the two biologically relevant sectors C1-C8 and C16-C20 connected by a rigid cyclopentene molecular scaffold for conformational stability.

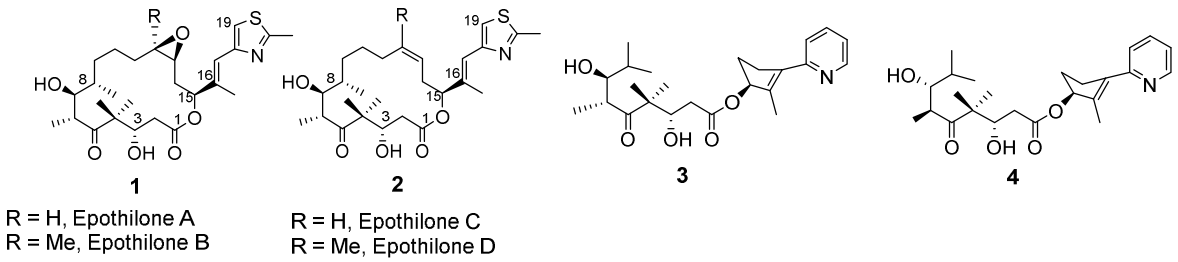


Fig. 1. Natural epothilones **1**,**2** and synthetic open-chain epothilones **3**,**4**.

The diastereomer **3**, with stereochemical assignments as in natural epothilones, had weak but selective activity against SNB-75 (GI_{50} = 21.9 μM) CNS cancer and OVCAR-4 (GI_{50} = 45.1 μM) ovarian cancer cell lines. A computational study performed by Rusinska-Roszak et al[6] suggested a high degree of conformational similarity between these open-chain molecules and natural epothilones and confirmed that conformational requirements for tubulin binding are

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

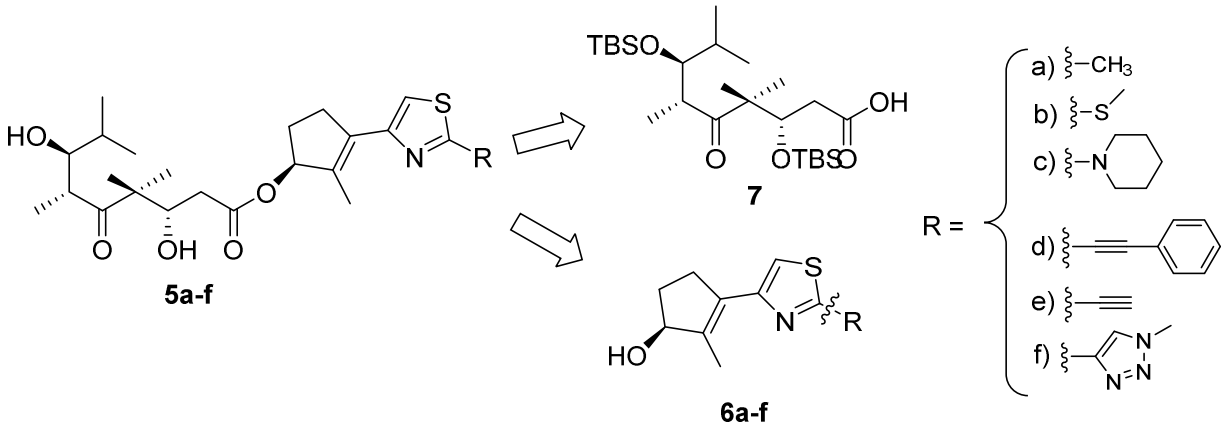
Organic & Biomolecular Chemistry Accepted Manuscript

being met with the open-chain analogues. In carrying out a structure-activity relationship study of these molecules, we focused on the substituent at the 2-positon of the aromatic ring as the rest of the structure embodied most of the important structural features important for activity. Thiazole was chosen as the aromatic moiety to be consistent with the thiazole of natural epothilones. Thus, we designed a library of open-chain epothilone analogues **5a-f** (Scheme 1) with a variety of 2-substituted thiazole rings to investigate their effects on biological activity. One of the compounds showed strong cytotoxic activity on several cell lines in the NCI 60 human tumor cell line screen tested at 10^{-5} M. Follow up studies in our laboratory traced the observed activity to a part of the molecule which kills cells by a nonapoptotic mechanism of action. Preliminary studies show that these compounds do not target tubulin, but kills cells by a mechanism that shows the hallmarks of the recently described cell death mechanism "ferroptosis",[7] characterized by dependence on iron and ROS.

Results and Discussion

Chemistry

Compounds **5a-f** were derived by the esterification of the carboxylic acid **7** with 2-substituted thiazole cyclopentenols **6a-f** as shown in the retrosynthetic analysis in Scheme 1.

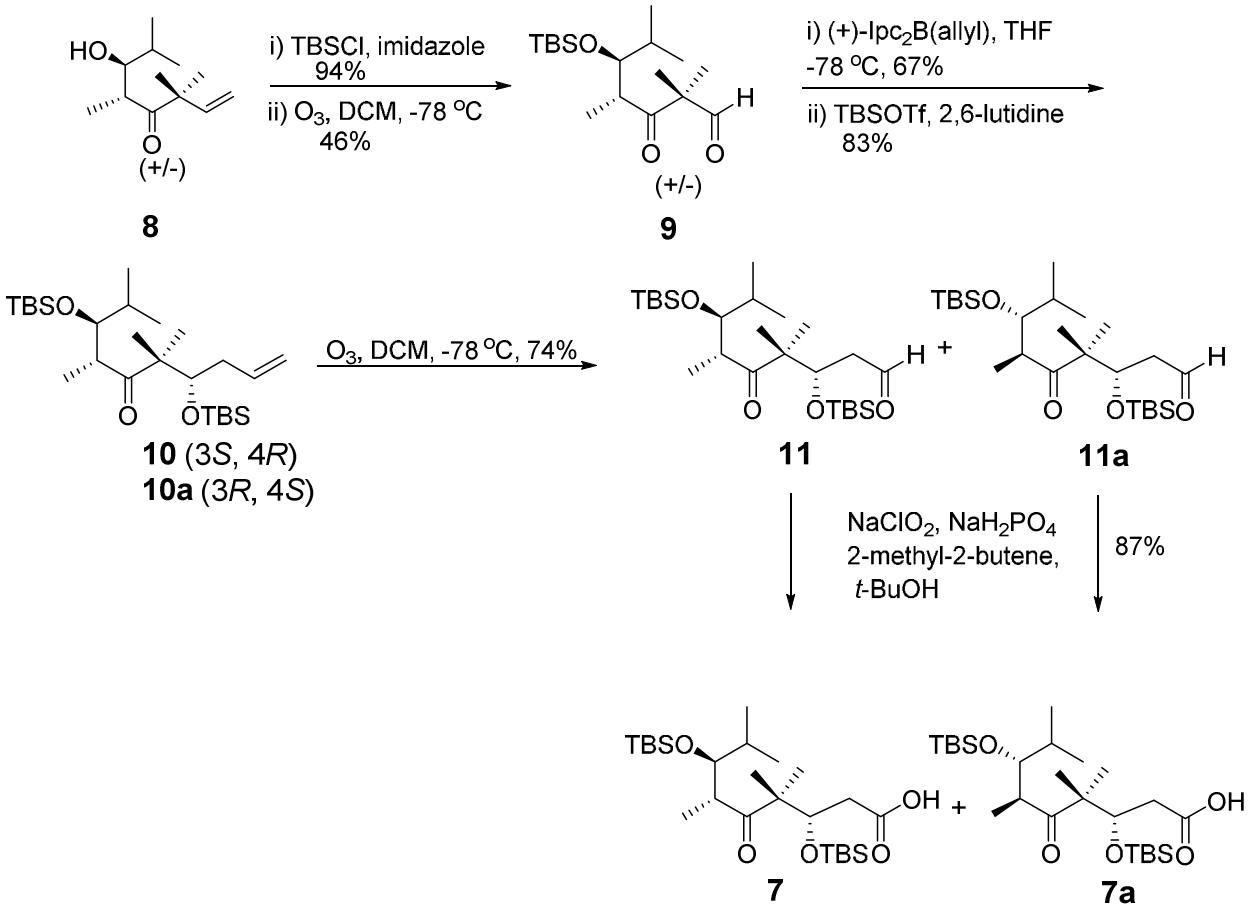


View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Scheme 1. Retrosynthetic analysis of open-chain epothilones

We synthesized the carboxylic acid **7** as reported previously (scheme 2),[5] with the exception that the two diastereomers were separated efficiently by flash chromatography at the aldehyde stage. This was found to be easier and more efficient than separating the two carboxylic acids as reported earlier. The two aldehydes were then separately converted to the corresponding carboxylic acids **7** and **7a** by Pinnick oxidation.[8]



Scheme 2. Synthesis of carboxylic acids **7** and **7a**

The synthesis of cyclopentenols bearing aromatic moieties **6a-f** began with dibromothiazole **12**, which by nucleophilic substitution, metal-halogen exchange followed by

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

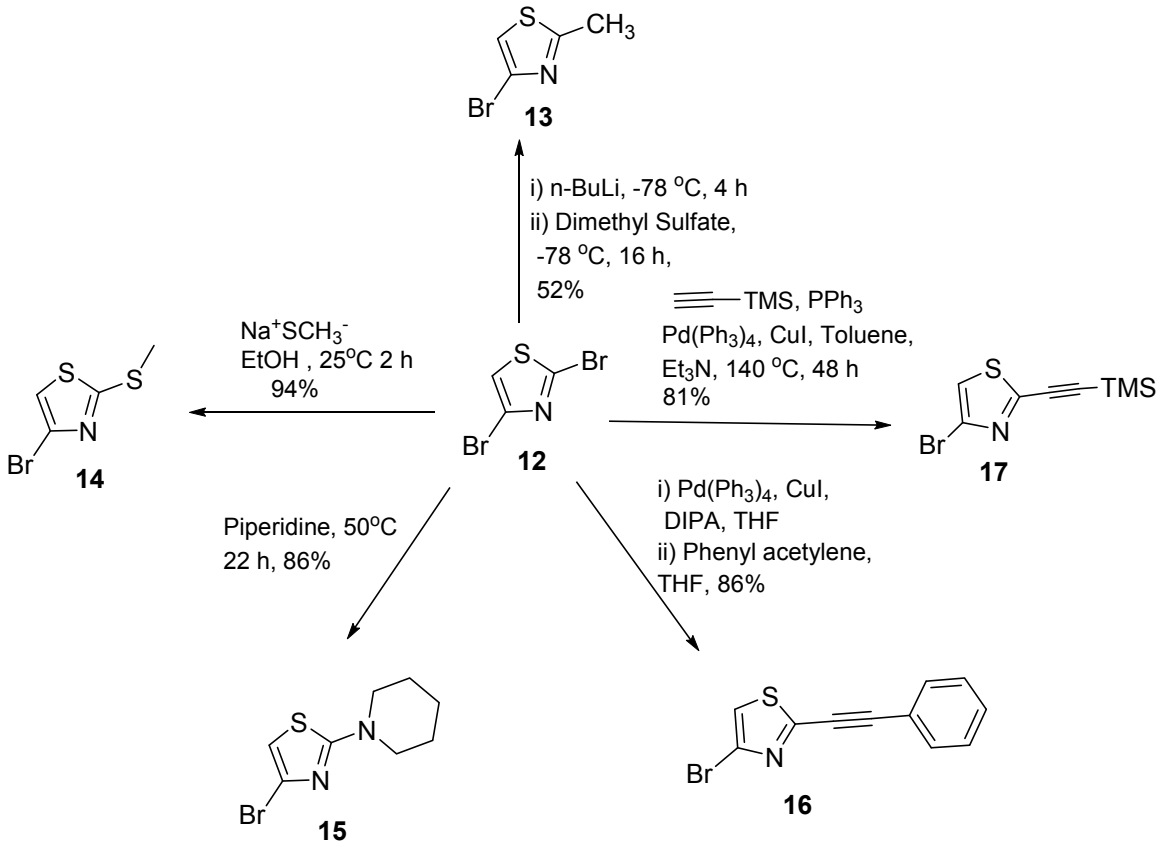
Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

alkylation or Sonogashira coupling, produced 2-substituted 4-bromothiazole derivatives **13-17** in good yields (scheme 3).[9–11] They were treated with *n*-butyllithium followed by trimethyltin chloride at -78^{o}C to produce the corresponding tin derivatives (Scheme 4a). *t*-Butyllithium proved more effective for the stannylation of the TMS-acetylene derivative **17**. The tin derivatives were partially purified by passing through a plug of silica gel deactivated with 5% triethylamine in hexanes and were immediately subjected to Stille coupling with iodocyclopentenone **19** in the presence of palladium catalyst to obtain the aryl-substituted cyclopentenones **20a-e** in good yields (schemes 4).[11] The TMS group of **20e** was removed with K_2CO_3 in methanol to obtain **21** prior to reduction (Scheme 4b). Cyclopentenones **20a-d** and **21** were selectively reduced using *(R)*-CBS catalyst to obtain cyclopentenols **6a-e**.[5] The stereochemistry of **6a-f** was confirmed using the Mosher ester model.[12,13] Enantioselective reduction of the piperonyl ketone **20c** to alcohol **6c** was not successful and the product was obtained as a racemic mixture, which was used in the next reaction without separation. Compound **21** was subjected to click chemistry with azidotrimethylsilane and copper iodide to obtain the triazole ketone **22** (scheme 5).[14]

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

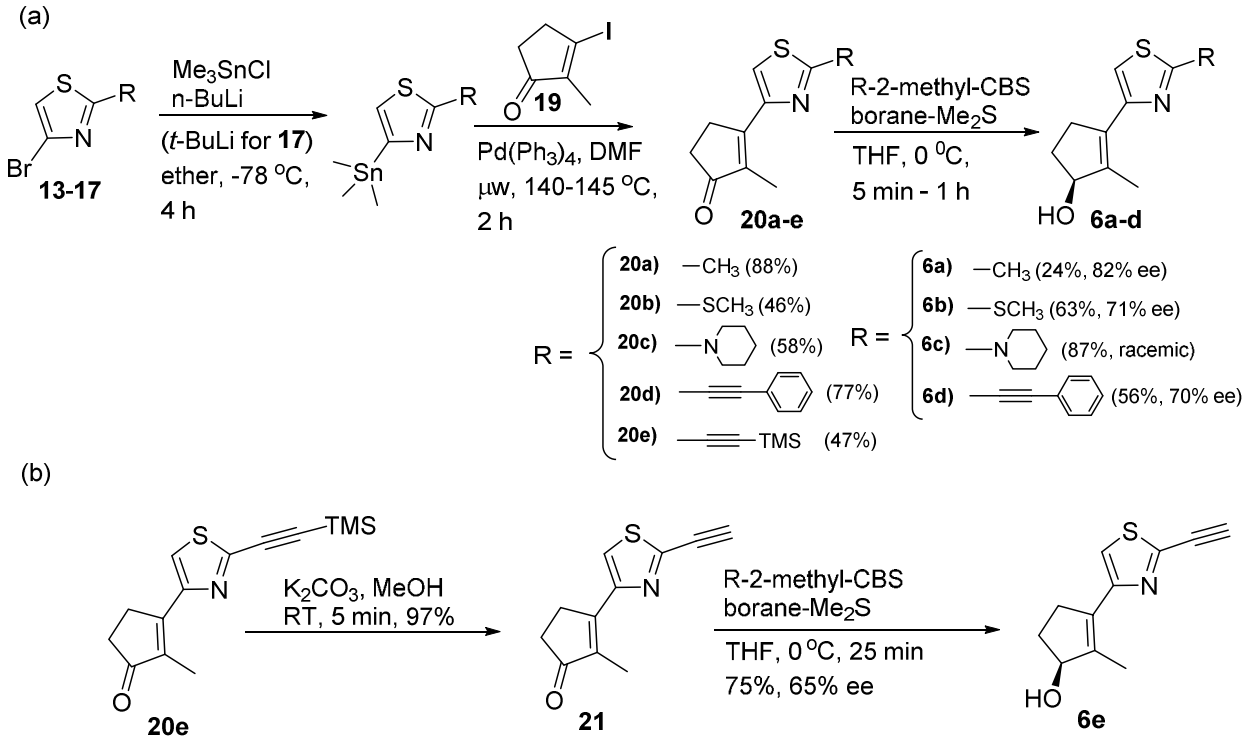


Scheme 3. Synthesis of 2-substituted-bromothiazoles

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

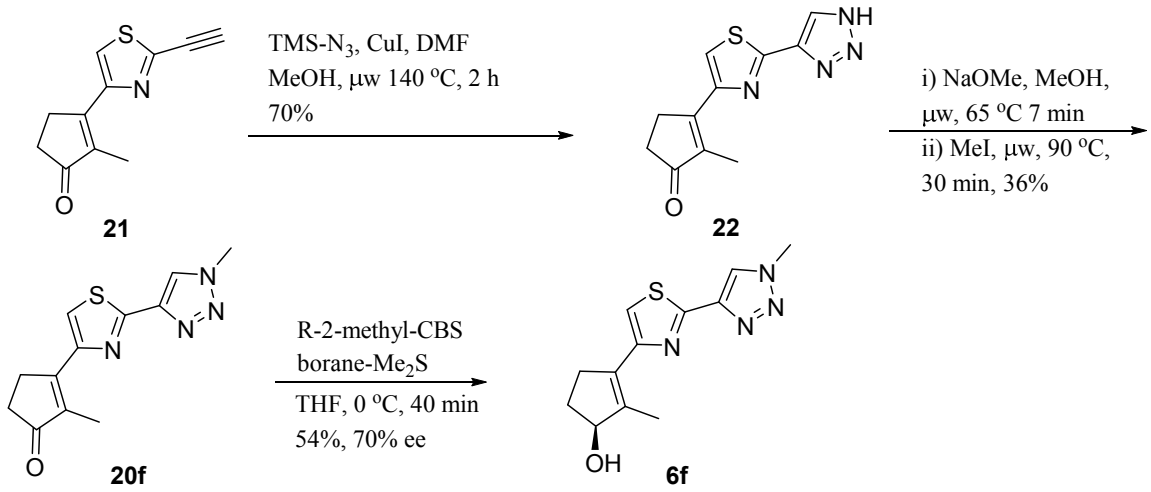


Scheme 4. Synthesis of 2-substituted cyclopentenols **6a-e**

The triazole product **22** was insoluble in most organic solvents except for large quantities of methanol that precluded CBS reduction. It was methylated[15] with MeI, and subjected to CBS reduction to obtain **6f**.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

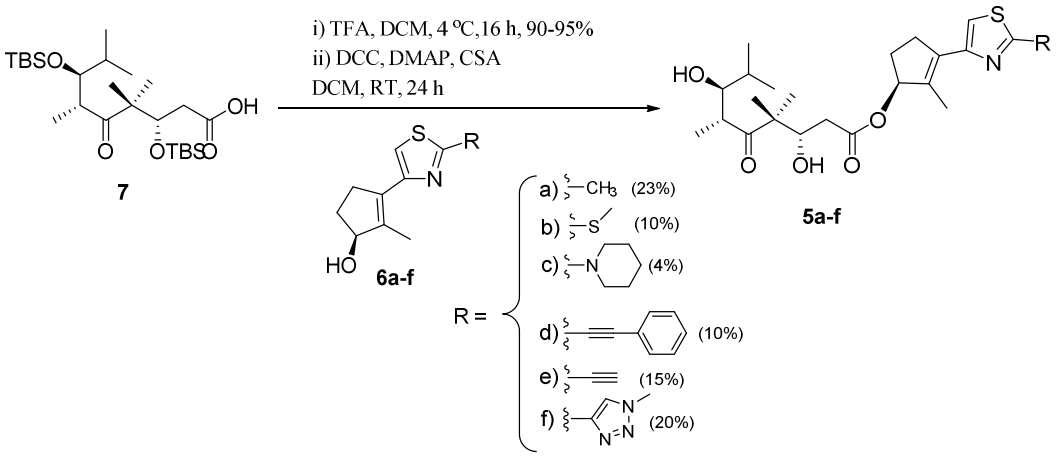


Scheme 5. Synthesis of cyclopentenol **6f**

We previously reported that desilylation of the TBS groups after esterification of **7** resulted in cleavage of the ester moiety.[5] Therefore, as before the silyl groups were first removed with TFA and the desilylated carboxylic acid was esterified with cyclopentenols **6a-f** using DCC and DMAP to yield open-chain epothilones **5a-f** (Scheme 6). The purification of the final products was carried out on preparative TLC plates, which were first deactivated with 5% Et_3N-hexanes to yield the final products **5 a-f**. Other purification methods including column chromatography on silica gel and reversed phase HPLC resulted in decomposition of the product and in most cases, the corresponding alcohol was isolated as a breakdown product.

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.
Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J



Scheme 6. Synthesis of target open-chain epothilone molecules

Biological studies

The compounds were tested in the NCI 60 cell line one-dose screen at 10^{-5} M. The compounds with methyl (**5a**), thiomethoxy (**5b**), piperidinyl (**5c**) and substituted triazole (**5f**)- thiazole rings showed no activity on any of the cell lines. Interestingly, the two alkyne analogues with acetylene and phenyl acetylene substituted thiazole rings showed activity against some cell lines. The phenylacetylene derivative (**5d**) inhibited the growth of non-small cell lung cancer HOP-62 (35%), non-small cell lung cancer NCI-H226 (45%), melanoma UACC-62 (40%) and renal cancer A498 (78%) cell lines. The acetylene derivative (**5e**) showed 75% growth inhibition against non-small cell lung cancer cell line HOP-62 and 25% growth inhibitory activity against CNS cancer U251 cell line. What was more striking was its lethal effect on non-small cell lung cancer NCI-H522 (-62%), melanoma LOX IMVI (-23%), ovarian cancer IGROV1 (-32%) and renal cancer UO-31 (-36%). Intrigued by the remarkable lethality of the analogue **5e** to NCI-H522 lung cancer cell line, we repeated this analysis. NCI-H522 cells were highly sensitive to

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

analogue **5e** (LC_{50} of ~0.6 μM) whereas HOP-62 cells were less sensitive (LC_{50} of ~30 μM) (Supplementary Fig. S1). These results show that analogue **5e** with a linear terminal alkyne substituent group at the 2-position of the thiazole ring has a profound and selective lethal effect on NCI-H522 cells.

During their synthesis, esters **5a-f** were found to be rather unstable and underwent ready hydrolysis in the presence of nucleophilic solvents like water and methanol. We therefore speculated that the observed cytotoxicity of compound **5e** may in fact, be due to compound **6e** formed by hydrolysis of the ester **5e** in the assay media, possibly aided by cellular esterases. As speculated, compound **6e** showed similar potency as **5e** in the cellular assay and also had a similar selectivity profile (see below). Therefore, our subsequent studies focused on **6e**. Comparison of several cancer cell lines exposed to **6e** for three days indicated an LC_{50} of 1.5 μM for NCI-H522, and an LC_{50} of 3.6 μM for the fibrosarcoma cell line HT1080 (Fig. 2). Colon cancer cell line HCT116, lung cancer HOP-62 and breast cancer cell line MCF7 were relatively unaffected by compound **6e** (Fig. 2). The large difference in sensitivities of these various cell lines suggests that **6e** is not a general toxin, but relies on metabolic and/or genetic factors in certain tumor cells to allow sensitivity.

We then synthesized the (*R)*-enantiomer **23** and the racemic alcohol **24** (Fig. 3) using *(S)*-CBS catalyst and direct $NaBH_4$ reduction, respectively. We also synthesized the corresponding oxime **25** by reaction of the ketone **21** with hydroxylamine hydrochloride and NaOAc in methanol (Fig. 3). The three compounds killed NCI-H522 cells with equal efficacy compared to **6e** and had LC_{50} values of 2.6 μM, 2.5 μM and 2.3 μM, respectively. The ketone **21** was less lethal with an LC_{50} value of 8.0 μM (Supplementary Fig. S1). These results show that cytotoxic

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

effect is independent of the stereochemistry of the alcohol function and that a H-bond donor is probably preferred over a H-bond acceptor at this position.

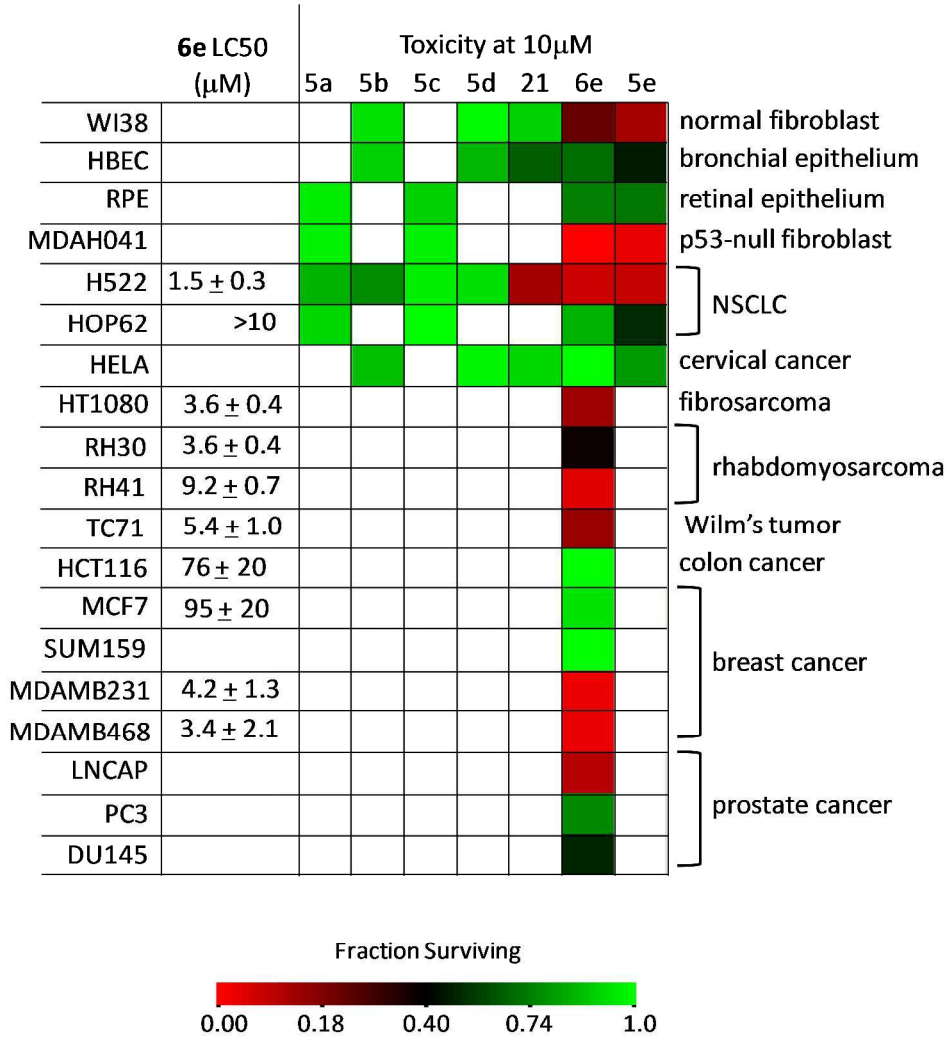


Fig. 2. Survey of cytotoxicity. Cells were exposed to the indicated compounds at 10 μM and analyzed by methylene blue staining 3 days later. LC_{50} values of various cell lines determined 3 days post treatment. Cells were treated one day after plating. Cell viability was determined using methylene blue staining.

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

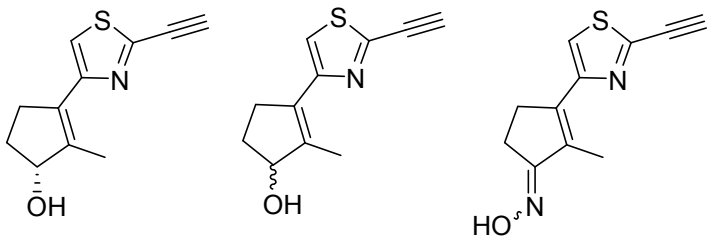


Figure 3. Other analogues synthesized

Rapid, non-apoptotic cell death in response to compounds 5e and 6e

Initial time-lapse microscopy indicated that 50% of cells were killed by **5e** in ~10 h and 100% by 18 hours (Fig. 4A). The rapid mode of killing of NCI-H522 cells by **5e** suggests that the mechanism of cell death is unlikely to be cell cycle-dependent. Similar rapid death was observed with the smaller **6e** compound. Immunofluorescence analysis showed that compound **6e** had no overt effects on tubulin in interphase or mitosis, suggesting that the compound does not mimic epothilone in mechanism of action (Fig. 4B). Furthermore, NCI-H522 cells were able to progress through mitosis in the presence of **6e (**Supplementary Fig. S2**)**. The rapid mode of cell death suggested that **6e** might directly disrupt mitochondrial function; however, membrane potentials were intact until late in the death process (Supplementary Fig. S3). Additional time-lapse microscopy indicated that cell death was not associated with membrane blebbing (Fig. 4C). Cells lost contact with the substratum shortly before losing membrane integrity and pyknotic nuclei were not evident (Fig. 4D). Inhibiting caspases with ZVAD blocked killing by taxol but not compound **6e** (Fig. 4E). These observations suggest that **6e** does not induce apoptosis.

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.
Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

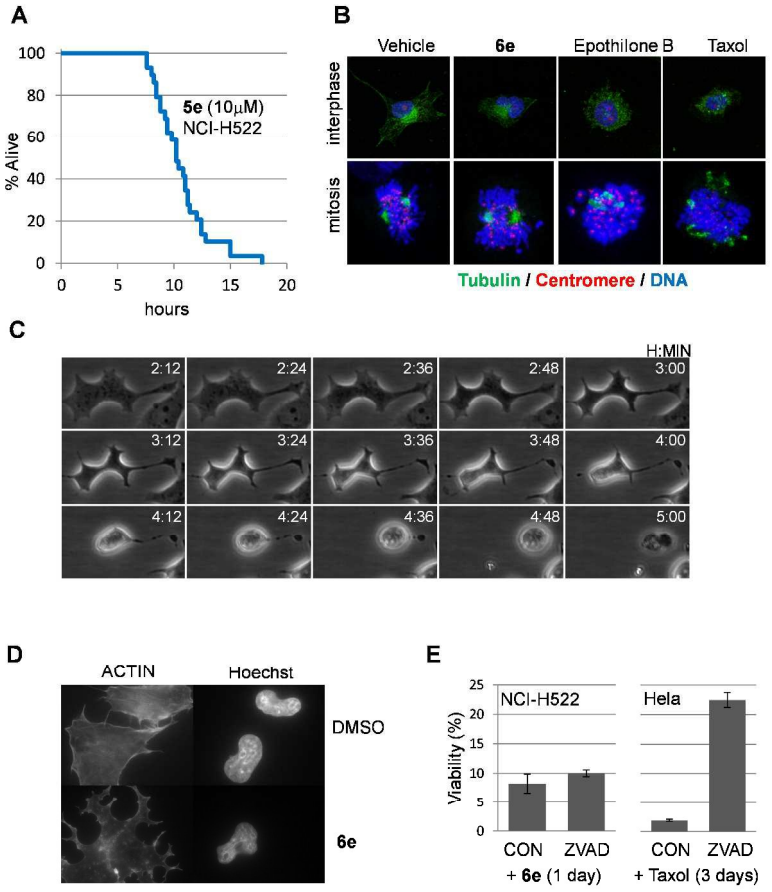


Fig. 4. Rapid cell death induced by compound **5e** and **6e**. (A) NCI-H522 cells were analyzed by time-lapse microscopy to determine time of death. Imaging was started at the time of drug addition. (B) **6e** does not affect tubulin. NCI-H522 were exposed to 10 μM **6e** for 6 hours and then analyzed by confocal immunofluorescence with antibodies against alpha-tubulin and borealin (to detect centromeres). DNA was stained with Hoechst 33342. For comparison, cells were exposed to either epothilone B or taxol for 6 hours before analysis. (C) Morphological features of **6e**-induced death. NCI-H522 cells exposed to compound **6e** at 10 μM were analyzed by time-lapse microscopy. Cell death is characterized by loss of contact with the substratum

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

followed by loss of membrane integrity. (D) Example of cytoplasmic shrinkage during cell death in the presence of 10 μM **6e**. Immunofluorescence was performed as described in "B", except cells were stained with phalloidin to detect actin. A wide-field image is shown. (E) Lack of protective effect of ZVAD on **6e**-induced death. Cells were co-treated with ZVAD and either 10 μM compound **6e**, or 10 μM taxol. Survival was assessed using methylene blue staining of fixed adherent cells.

Ferroptotic cell death in cells exposed to compound 6e

Next, we investigated potential non-apoptotic mechanisms of death in response to compound **6e**. Additional studies suggest that these compounds kill cells by inducing ferroptosis, a novel iron-dependent, non-apoptotic form of cell death.[7] Ferroptosis is a recently described mechanism of death characterized by a dependence on iron and ROS. Similarly to erastin, a prototypical inducer of ferroptosis, iron chelators ciclopirox olamine and hydroxyurea abolished killing of NCI-H522 cells by **6e** (Fig. 5A). Sensitivity was enhanced by adding ferric citrate, suggesting that iron is the relevant cation (Fig. 5B). Death of NCI-H522 cells induced by **6e** was also blocked by the free radical scavengers trolox and butylated hydroxyanisole (Fig. 5A). Thus, compound **6e** relies on ROS for cytotoxic activity. ROS are under multiple levels of control in cells.[16] Elevation of ROS, to the extent that they overwhelm antioxidant capacity, can induce damage to proteins, lipids, and nucleic acids causing cell death. Cellular sources of ROS include incomplete reduction of O_2 during electron transport to form superoxide and direct generation of superoxide by the membrane bound NADPH oxidases.[16] Inhibiting NADPH oxidases using diphenyleneiodonium chloride blocked killing by **6e**, suggesting that these enzymes are

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.
Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Organic & Biomolecular Chemistry Accepted Manuscript

important providers of ROS needed for killing (Fig. 5A). As predicted, exposure of NCI-H522 for 6 hours to compound **6e** increased the level of intracellular ROS (Fig. 5C, D). ROS elevation by **6e** was dependent on iron and NADPH oxidase and was blocked by trolox (Fig. 5D).

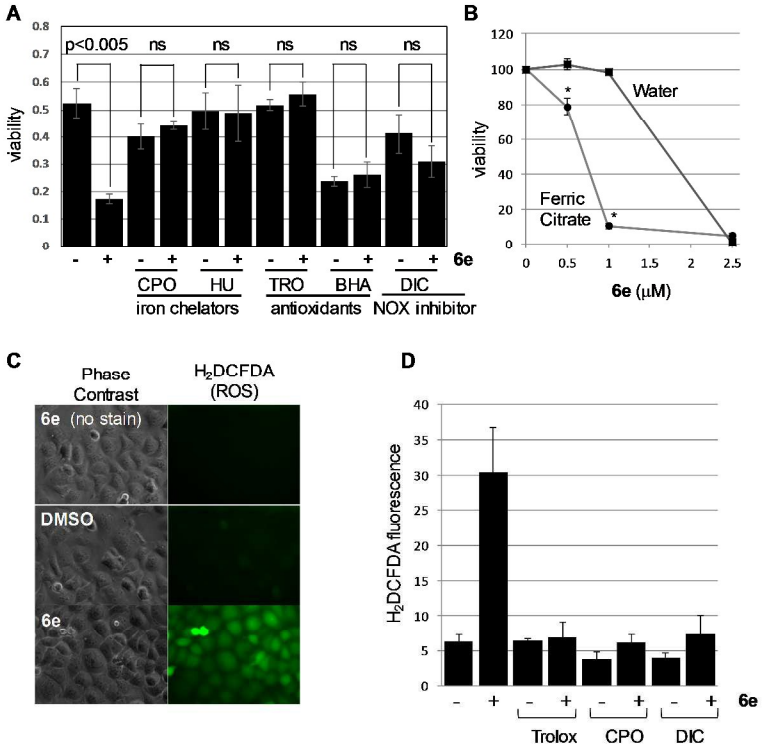


Fig. 5. Compound **6e** induces ferroptosis. (A) Effect of ferroptosis modulators on **6e** toxicity. NCI-H522 were exposed to 10 μM **6e** with or without the compounds indicated. Viability was analyzed 24 hours later using methylene blue. CPO: Ciclopirox olamine; HU: Hydroxyurea; BHA: Butylated hydroxyanisole; DIC: diphenyleneiodonium chloride. All modulators eliminated **6e**-induced killing (only **6e** alone [+] versus DMSO [-] was significantly different using a student's t-test). (B) Elevating iron enhances **6e** toxicity. NCI-H522 cells were exposed to **6e** with or without 25 μM ferric citrate. Viability was determined by methylene blue staining 2 days

View Article Online
DOI: 10.1039/C7OB03086J

later. (C) Elevation of ROS in NCI-H522 cells exposed to 10 μM compound **6e** for 4 hours. Representative microscopic fields of H2DCFDA-stained cells are shown. Dye was extracted and quantified in (D).

Conclusions

During the course of our studies to develop structurally simplified epothilone analogues, we discovered a new class of small molecules that kill selected cancer cell lines by a novel mechanism of action. The cytotoxic activity is cell cycle-independent and does not involve apoptosis. The compounds had no effect on tubulin or mitochondrial function. Preliminary studies show that they harness reactive oxygen species to kill cancer cells in an iron-dependent manner showing the hallmarks of ferroptosis, a recently reported mechanism of action. Studies are in progress to further confirm the ferroptotic cell death mechanism and to determine the specific molecular targets of these promising "drug-like" small molecule anticancer agents.

Experimental Section

General Information

All reactions were carried out under nitrogen using anhydrous solvents unless otherwise noted. Tetrahydrofuran (THF) was distilled from Na and benzophenone under nitrogen prior to use. NMR spectra were recorded on Varian VXRS 400 MHz, Varian INOVA 600 MHz and Bruker Avance 600 MHz spectrometers and calibrated using residual undeuterated solvent as internal reference. Optical rotations were recorded on Rudolph AUTOPOL III and Rudolph AUTOPOL IV 589/586 polarimeters. High-resolution mass spectra (HRMS) were recorded on a LCT Electrospray mass spectrometer at the Central Instrument Facility, Mass Spectrometry

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.
Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Organic & Biomolecular Chemistry Accepted Manuscript

Laboratory, the Wayne State University, Detroit, Michigan) and on a Micromass Q-Tof II Electrospray mass spectrometer at the Mass Spectrometry and Proteomics Facility, the Ohio State University, Columbus, Ohio. Ozone was generated on a Welsbach Model T-408 commercial ozone generator. A Biotage Initiator was used in microwave synthesis. Crude products were purified by flash column chromatography on silica gel (standard grade, 40-63 μm, 230 x 400 mesh) purchased from Sorbent Technologies and using RediSep prepacked cartridges from Teledyne ISCO Inc. on a Combiflash Companion. 1000 μ Uniplates purchased from Analtech Inc. were used in preparative thin layer chromatography using commercial solvents as specified. HPLC analyses were performed on a Waters 1525 Binary Pump system with Waters 2487 Dual Wavelength Absorbance Detector on a Supleco C_{18} reverse phase column (5 μm, 15 cm x 4.6 mm) and on a Symmetry C18 reverse phase column (5 μ, 15 cm x 4.6 mm) using a linear gradient of 60-100% acetonitrile in water over 10-20 min; flow rate of 1 mL/min and UV detection at 254 nm. Structural integrity and purity of the test compounds were determined by the composite of ^{1}H and ^{13}C NMR, HRMS and HPLC and were found to be > 95% pure.

A. Synthesis of (*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononoic acid (7) and the (*3S,6S,7R*) diastereomer (7a).

(*3S,6R,7S*)-3,7-bis(tert-butyldimethylsilyloxy)-4,4,6,8-tetramethyl-5-oxononanal (11) and (*3S,6S,7R*)-3,7-bis(tert-butyldimethylsilyloxy)-4,4,6,8-tetramethyl-5-oxononanal (11a)

Ozone was passed through a solution of diastereomers (*7S*)-3,7-dihydroxy-2,4,6,6-tetramethyldec-9-en-5-one (**10**)[8] (1.00 g, 2.12 mmol, 1 equiv) in DCM (12 mL) at -78 ^{o}C until

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

the reaction mixture reached a dark blue color. Nitrogen was bubbled through the solution for 15 min and triphenylphosphine (612 mg, 2.33 mmol, 1.1 equiv) was added. The reaction mixture was stirred at room temperature overnight and concentrated in vacuo. The mixture of aldehydes was separated by flash column chromatography on silica gel in 10% DCM-hexanes, monitored by NMR, to obtain the (*3S,6R,7S*) diastereomer **11** (150 mg), the mixture of the two aldehydes (416 mg), followed by (*3S,6S,7R*) diastereomer **11a** (245 mg) as colorless oils with an overall yield of 69%.

Data for the (*3S,6R,7S*) diastereomer **11**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 1H), 4.57 (t, *J* = 5.2 Hz, 1H), 3.70 (d, *J* = 7.6 Hz, 1H), 3.07-3.00 (m, 1H), 2.51-2.37 (m, 3H), 1.43-1.33 (m, 1H), 1.17 (m, 3H), 1.03 (m, 3H), 0.66 (m, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 218.18, 201.55, 77.85, 70.42, 53.92, 49.24, 45.44, 33.25, 26.43, 26.20, 26.12, 22.71, 21.31, 19.44, 18.76, 18.31, 16. 16.035, -3.24, -3.51, -3.10, -4.22.

Data for the (*3S,6S,7R*) diastereomer **11a:** ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.71 (s, 1H), 4.45 (t, *J* = 4.8 Hz, 1H), 3.70 (d, *J* = 6.8 Hz, 1H), 3.05-3.01 (m, 1H), 2.48-2.32 (m, 3H), 1.44-1.38 (m, 1H), 1.14 (s, 3H), 0.98 (d, *J* = 8.0 Hz, 3H), 0.81 (m, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 218.98, 201.47, 77.87, 71.25, 53.80, 49.79, 46.09, 46.05, 39.92, 32.89, 29.92, 26.46, 26.17, 26.10, 26.06, 24.12, 21.51, 18.791, 18.74, 18.32, 16.09, 15.86, -3.15, -3.46, -3.86, -4.28.

(*3S,6R,7S*)-3,7-bis(tert-butyldimethylsilyloxy)-4,4,6,8-tetramethyl-5-oxononanoic acid (7).

To a solution of aldehyde **11** (710 mg, 1.50 mmol, 1 equiv) and 2-methyl-2-butene (7.6 mL) in *t*-butanol (32 mL) was added a solution of $NaClO_2$ (1.244 g, 13.76 mmol, 9.1 equiv) and

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

NaH_2PO_4 (1.248 g, 10.38 mmol, 6.9 equiv) in water (9 mL) dropwise. The reaction mixture was stirred for 1 h at RT and the reaction was quenched with saturated aqueous ammonium chloride (40 mL) and water (40 mL). The reaction mixture was extracted with EtOAc (3 x 15 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was taken directly to the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.52-4.49 (m, 1H), 3.73 (dd, *J* = 8.0 Hz, 2.0 Hz, 1H), 3.09-3.01 (m, 1H), 2.47 (dd, *J* = 16.4 Hz, 3.6 Hz, 1H), 2.33 (dd, *J* = 16.4 Hz, 6.8 Hz, 1H), 1.46-1.38 (m, 1H), 1.16 (m, 3H), 1.01 (m, 3H), 0.98-0.82 (m, 9H), 0.03 (m, 6H).

(*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid.

A solution of the carboxylic acid **7** (350 mg, 0.72 mmol) in DCM (15 mL) was cooled to 0 ^{o}C. TFA (3.0 mL, 20% in DCM) was added and the reaction mixture was stirred at 4 ^{o}C for 25 h. Water was added (5 mL) and the reaction was evaporated to dryness under reduced pressure. The residue was dried azeotropically with toluene to obtain the desilylated *(3S,6R,7S)* carboxylic acid (168 mg, 90%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.23 (dd, *J* = 10.0 Hz, 2.4 Hz, 1H), 3.33 (dd, *J* = 8.8 Hz, 1.6 Hz, 1H), 3.26-3.21 (m, 1H), 2.51-2.37 (m, 3H), 1.69-1.63 (m, 1H), 1.15 (d, *J* = 1.6 Hz, 6H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 222.47, 177.25, 76.79, 72.47, 52.33, 41.079, 36.71, 30.66, 21.61, 19.54, 19.30, 19.17, 10.61.

(*3S,6S,7R*)-3,7-bis(tert-butyldimethylsilyloxy)-4,4,6,8-tetramethyl-5-oxononanoic acid (7a).

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

To a solution (*3S,6S,7R*)-3,7-bis(tert-butyldimethylsilyloxy)-4,4,6,8-tetramethyl-5-oxononanal **11 a** (282 mg, 0.569 mmol, 1 equiv) and 2-methyl-2-butene (3.0 mL) in *t*-butanol (12 mL) was added a solution of $NaClO_2$ (497 mg, 5.50 mmol, 9.1 equiv.) and NaH_2PO_4 (497 mg, 4.15 mmol, 6.9 equiv.) in water (4 mL) dropwise. The reaction was stirred at RT for 1 h and the reaction was quenched with saturated aqueous ammonium chloride (15 mL) and water (15 mL). The crude reaction mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was passed through a silica gel plug to obtain the (*3S,6S,7R*) carboxylic acid **7a** (241 mg, 87%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.39 (m, 1H), 3.72 (dd, *J* = 8.0 Hz, 1.2 Hz, 1H), 3.08 (m, 1H), 2.46 (dd, *J* = 16.4 Hz, 2.8 Hz, 1H), 2.29 (dd, *J* = 16.4 Hz, 6.8 Hz, 1H), 1.45-1.42 (m, 1H), 1.17 (m, 3H), 1.08 (m, 3H), 0.88-0.73 (m, 9H), 0.03 (m, 6H); ^{13}C ($CDCl_3$, 100 MHz): δ 218.51, 178.65, 77.87, 73.40, 53.81, 46.07, 40.43, 32.94, 26.48, 26.22, 23.73, 21.53, 19.04, 18.80, 18.41, 16.18, 15.90, -3.18, -3.46, -4.05.

(*3S,6S,7R*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid

A solution the carboxylic acid **7a** (882 mg, 1.81 mmol) in DCM (58 mL) was cooled to 0 ^{o}C and TFA (11.6 mL, 20% in DCM) was added. The reaction mixture was stirred at 4 ^{o}C for 25 h. Water (15 mL) was added and the reaction mixture was evaporated to dryness under reduced pressure. The residue was dried by azeotropically with toluene to obtain the desilylated *(3S,6S,7R)* carboxylic acid (450 mg, 95%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.26 (dd, *J* = 10.0 Hz, 2.4 Hz, 1H), 3.30-3.22 (m, 2H), 2.53-2.40 (m, 2H), 1.70-1.63 (m, 1H), 1.20 (s, 3H), 1.15 (s, 3H), 1.06 (d, *J* = 7.2 Hz, 3H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.86 (d, *J* = 7.2 Hz, 3H); ^{13}C

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

NMR ($CDCl_3$, 100 MHz): δ 222.61, 176.67, 76.58, 72.44, 52.16, 41.33, 36.38, 30.82, 21.60, 19.66, 19.21, 19.05, 10.54.

B. Synthesis of 2-substituted thiazole cyclopentenols (6a- f).

2-Methyl-4-bromothiazole (13).

Synthesized using conditions described by Hofle et al.[10] (0.765, 52%) reddish oil: TLC R_f = 0.68 (20% EtOAc-hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04 (s, 1H), 2.71 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.20, 123.94, 116.30, 19.41.

2-(Methylthio)-4-bromothiazole (14).

Synthesized using conditions described by Nicolaou et al.[11] (1.415 g, 94%) yellow solid: TLC R_f = 0.48 (2% EtOAc-hexanes); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.05 (s, 1H), 2.68 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 124.44, 115.68, 16.85.

2-(Piperidin-1-yl)-4-bromothiazole (15).

Synthesized using conditions described by Nicolaou et al.[11] (1.30, 86%) yellow solid: TLC R_f= 0.43 (5% EtOAc-hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.35 (s, 1H), 3.42 (d, J = 5.6 Hz, 4H), 1.63 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.03, 121.70, 102.97, 49.26, 25.14, 24.08.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

2-(Phenylethynyl)-4-bromothiazole (16).

A mixture of 2,4-dibromothiazole **12** (2.92 g, 12.0 mmol, 1 equiv), $Pd(Ph_3)_4$ (0.685 g, 0.60 mmol, 5 mol%), and CuI (0.228 g, 0.12 mmol, 10 mol %) was placed in a three-necked round bottom flask under nitrogen. Anhydrous THF (30 mL) was added, followed by *N,N*-diisopropylamine (2.55 mL, 18.02 mmol, 1.5 equiv). A solution of phenylacetylene (2.0 g, 17.98 mmol, 1.5 equiv) in anhydrous THF (6 mL) was slowly added to the reaction mixture via a syringe pump over 7 h. The reaction mixture was stirred for an additional 9 h. Water (50 mL) was added and the crude reaction mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel in 5% EtOAc in hexanes and recrystallized from EtOAc-hexanes to obtain product **16** (3.174 g, 86%) as brown crystals: TLC R_f= 0.59 (10 % EtOAc-hexanes); mp 79-80 ^oC; IR ν_{max}: 3300, 3000, 2900, 2350 cm^{-1}; ^1H NMR (600 MHz, acetone-d_6): δ 7.79 (s, 1H), 7.66 (m, 2H), 7.51 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.93, 132.249, 130.12, 128.78, 126.11, 121.10, 118.82, 95.69, 81.60; HRMS (m/z): $[M + Na]^+$ calcd for $C_{11}H_6NSBr$, 285.9302; found, 285.9314.

2–((Trimethylsilyl)ethynyl)-4-bromothiazole (17).

A mixture of 2,4-dibromothiazole **12** (3.000 g, 12.34 mmol, 1 equiv), triphenylphosphine (486 mg, 1.851 mmol, 5 mol%), CuI (120 mg, 0.617 mmol, 5 mol%), and $Pd(Ph_3)_2Cl_2$ (120 mg, 0.173 mmol, 1.4 mol %) was placed in a three-neck round bottom flask under nitrogen. Anhydrous toluene (42 mL) was added followed by anhydrous Et_3N (2.2 mL, 15.04 mmol, 1.3 equiv) and

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

trimethylsilylacetylene (2.6 mL, 18.51 mmol, 1.5 equiv). The reaction mixture was refluxed at 140 ^{o}C for 2 d. It was poured into water (50 mL) and extracted with EtOAc (3x 20 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel in DCM-hexanes to obtain **17** (2.63 g, 81%) as a brownish/red solid: TLC R_f = 0.72 (10 % EtOAc-hexanes); mp 37 ^{o}C; IR ν_{max}: 3300, 2900, 2250 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18 (s, 1H), 0.24 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.54, 125.87, 118.85, 103.03, 95.52, -0.41; HRMS (m/z): $[M + H]^+$ calcd for $C_8H_{11}NSSiBr$, 259.9565; found, 259.9565.

2-Methyl-3-(2-methylthiazol-4-yl)cyclopent-2-enone (20a).

A solution of 2-methyl-4-bromothiazole **15** (351 mg, 1.97 mmol, 1 equiv) in anhydrous ether (3 mL) was cooled to -78 ^{o}C and *n*-BuLi (1.30 mL of 2.5 M solution in THF, 3.35 mmol, 1.7 equiv) was added dropwise. The reaction mixture was stirred at -78 ^{o}C for 2 h and a solution of trimethytin chloride (785 mg, 3.94 mmol, 2 equiv) in anhydrous ether (2 mL) was added dropwise. The reaction mixture was stirred for an additional 1 h and allowed to warm to room temperature slowly. It was diluted with hexanes, passed through a silica gel plug deactivated with 5% Et_3N-hexanes, and eluted with EtOAc. The partially purified product was concentrated in vacuo to obtain a reddish oil which was immediately taken to the next step. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.19 (s, 1H), 2.75 (s, 3H), 0.33 (t, *J* = 3.6 Hz, 9H). A mixture of 3-iodo-2-methylcyclopent-2-enone **10** (350 mg, 1.57 mmol, 0.8 equiv) and $Pd(Ph_3)_4$ (455 mg, 0.394 mmol, 20 mol%) was placed in a μw vial under nitrogen. A solution of the tin derivative prepared above in anhydrous DMF (4.5 mL) was added via a syringe, and the vial was heated for

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

2 h 15 min at 145 ^{o}C in the microwave synthesizer. The reaction mixture was poured into water (15 mL) and extracted with EtOAc (3 x 5 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel in EtOAc-hexanes and recrystallized from EtOAc-hexanes to obtain **20 a** (336 mg, 88%) as tan crystals: TLC R_f = 0.39 (40% EtOAc-hexanes); mp 89-90 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (s, 1H), 2.95-2.92 (m, 2H), 2.73 (s, 3H), 2.49-2.47 (m, 2H), 2.09 (t, J = 2.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.24, 165.80, 158.67, 152.28, 136.60, 120.32, 33.89, 28.24, 19.56, 10.33; HRMS (m/z): $[M + Na]^+$ calcd for $C_{10}H_{11}NOS$, 216.0459; found, 216.0461.

2-Methyl-3-(2-(methylthio)thiazol-4-yl)cyclopent-2-enone (20b).

A solution of 2-thiomethyl-4-bromothiazole **14** (300 mg, 1.43 mmol, 1 equiv) in anhydrous ether (10 mL) was cooled to -78 ^{o}C. *n*-BuLi (1.80 mL, 1.6 M in hexanes, 2.86 mmol, 2 equiv) was added dropwise and the reaction mixture was stirred at -78 ^{o}C for 2 h. A solution of trimethyltin chloride (708 mg, 3.57 mmol, 2.5 equiv) in anhydrous ether (3 mL) was added dropwise. The reaction mixture was stirred at -78 ^{o}C for an additional 1 h and was allowed to warm slowly to room temperature. The reaction mixture was diluted with hexanes, passed through a silica gel plug deactivated with 5% Et_3N-hexanes, and eluted with EtOAc. The partially purified product was concentrated in vacuo to obtain a reddish oil which was taken immediately to the next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (s, 1H), 2.69 (s, 3H), 0.32 (t, J = 28.0 Hz, 9H). 3-Iodo-2-methylcyclopent-2-enone **10** (318 mg, 1.43 mmol, 1 equiv) and $Pd(Ph_3)_4$ (330 mg, 0.286 mmol, 20 mol%) were placed in a μw vial under nitrogen. A solution of the tin derivative prepared

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

above in anhydrous DMF (15 mL) was added via syringe, and the vial was heated for 2 h in the microwave synthesizer at 145 ^{o}C. The reaction mixture was poured into water (30 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The product was purified by flash chromatography on silica gel in EtOAc-hexanes, and recrystallized from EtOAc-hexanes to obtain the product **20b** (148 mg, 46%) as a pale yellow solid: TLC R_f = 0.60 (40% EtOAc-hexanes); mp 102 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.46 (s, 1H), 2.92-2.90 (m, 2H), 2.73 (s, 3H), 2.52-2.50 (m, 2H), 2.14 (t, J = 2.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 210.27, 166.73, 157.83, 152.64, 137.03, 119.64, 33.93, 27.92, 16.71, 10.40; HRMS (m/z): $[M + Na]^+$ calcd for $C_{10}H_{11}NOS$, 248.0180; found, 248.0189.

2-Methyl-3-(2-(piperidin-1-yl)thiazol-4-yl)cyclopent-2-enone (20c).

A solution of 2-piperdin-1-yl-4-bromothiazole **15** (550 mg, 2.22 mmol, 1 equiv) in anhydrous ether (19 mL) under nitrogen was cooled to -78 ^{o}C. *n*-BuLi (1.60 mL, 1.6 M in hexanes, 2.66 mmol, 1.2 equiv) was added dropwise and the reaction mixture was stirred at -78 ^{o}C for 2 h. A solution of trimethyltin chloride (590 mg, 3.33 mmol, 1.5 equiv) in anhydrous ether (7 mL) was added dropwise. The reaction mixture was stirred at -78 ^{o}C for an additional 1 h and was allowed to warm slowly to room temperature. The reaction mixture was diluted with hexanes, passed through a silica gel plug deactivated with 5% Et_3N-hexanes, and eluted with EtOAc. The partially purified product was concentrated in vacuo to obtain a yellowish oil which was taken immediately to the next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.56 (s, 1H), 3.47 (m, 4H), 1.65 (m, 6H), 0.27 (t, J = 20 Hz, 9H). 3-Iodo-2-methylcyclopent-2-enone **10** (338 mg, 1.52 mmol, 1

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

equiv) and $Pd(Ph_3)_4$ (352 mg, 0.304 mmol, 20 mol%) were placed in a μw vial under nitrogen. A solution of the tin derivative prepared above in anhydrous DMF (10 mL) was added via syringe. The vial was heated for 2 h in the microwave synthesizer at 140 ^oC. The reaction mixture was poured into water (20 mL) and extracted with EtOAc (3 x 8 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel in EtOAc-hexanes and recrystallized from EtOAc-hexanes to obtain **20 c** (341 mg, 58%) as yellow crystals: TLC R_f = 0.57 (40% EtOAc-hexanes); mp 108 ^oC; ^1H NMR (600 MHz, $CDCl_3$): δ 6.88 (s, 1H), 3.49 (m, 4H), 2.85 (m, 2H), 2.46 (m, 2H), 2.13 (t, *J* = 2.0 Hz, 3H), 1.66 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 221.25, 210.71, 170.84, 159.11, 149.68, 136.08, 109.85, 49.76, 33.93, 27.60, 25.33, 24.37, 10.39. HRMS (m/z): $[M + Na]^+$ calcd for $C_{14}H_{18}N_2O$, 285.1038; found, 285.1046.

2-Methyl-3-(2-(phenylethynyl)thiazol-4-yl)cyclopent-2-enone (20d).

A solution of 2-(phenylethynyl)-4-bromothiazole **16** (1.000 g, 3.78 mmol, 1 equiv) in anhydrous ether (15 mL) was cooled to -78 ^oC and *n*-BuLi (2.3 mL, 2.5 M in hexanes, 5.67 mmol, 1.5 equiv) was added dropwise. The reaction mixture was stirred at -78 ^oC for 2 h and a solution of trimethyltin chloride (1.503 g, 7.56 mmol, 2 equiv) in anhydrous ether (5 mL) was added dropwise. The reaction mixture was stirred for an additional 1 h at -78 ^oC before it was allowed to warm to room temperature slowly. The reaction mixture was diluted with hexanes, passed through a silica gel plug deactivated with 5% Et_3N-hexanes, and eluted with EtOAc. The partially purified product was concentrated in vacuo to obtain a reddish oil which was used immediately in the next step. ^1H NMR (400 MHz, $CDCl_3$): δ 7.59 (m, 2H), 7.38 (m, 3H), 0.37

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

(t, J = 27.6 Hz, 9H). 3-Iodo-2-methylcyclopent-2-enone **10** (678 mg, 3.02 mmol, 0.8 equiv) and $Pd(Ph_3)_4$ (873 mg, 0.756 mmol, 20 mol%) were placed in a μw vial under nitrogen. A solution of the tin derivative prepared above in anhydrous DMF (15 mL) was added via syringe. The vial was heated at for 2 h 15 min in the microwave synthesizer at 145 ^oC. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (3 x 20 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel in EtOAc-hexanes and recrystallized from EtOAc-hexanes to obtain the product **20d** (816 mg, 77%) as a brown solid: TLC R_f = 0.45 (40% EtOAc-hexanes); mp 117 ^oC; IR ν_{max}: 3320, 3200, 2900, 2250, 1800, 1750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.64 (s, 1H), 7.61 (m, 2H), 7.38 (m, 3H), 3.03 (m, 2H), 2.54 (m, 2H), 2.13 (t, J = 2.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.09, 157.99, 153.08, 148.76, 137.61, 132.27, 130.03, 128.76, 121.87, 121.24, 95.08, 82.16, 33.96, 28.40, 10.44; HRMS (m/z): $[M + Na]^+$ calcd for $C_{17}H_{13}NOS$, 302.0616; found, 302.0620.

2-Methyl-3-(2-((trimethylsilyl)ethynyl)thiazol-4-yl)cyclopent-2-enone (20e).

A solution of 2–((trimethylsilyl)ethynyl)-4-bromothiazole **17** (100 mg, 0.384 mmol, 1 equiv) in anhydrous ether (3 mL) under nitrogen was cooled to -78 ^oC and t-BuLi (240 μL, 1.6 M, 0.786 mmol, 1 equiv) was added dropwise. The reaction was stirred at -78 ^oC for 1 h and a solution of trimethyltin chloride (150 mg, 0.768 mmol, 2 equiv) in anhydrous ether (2 mL) was added via syringe. The reaction mixture was stirred at -78 ^oC for an additional 1 h and was allowed to warm to room temperature slowly. The reaction mixture was diluted with hexanes, passed through a silica gel plug deactivated with 5% Et_3N-hexanes, and eluted with EtOAc. It was

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

concentrated in vacuo to obtain a yellow oil, which was immediately taken to the next step. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.33 (s, 1H), 0.34 (t, *J* = 14.8 Hz, 9H), 0.25 (m, 3H). 3-Iodo-2-methylcyclopent-2-enone **10** (83 mg, 3.84 mmol, 1 equiv) and $Pd(Ph_3)_4$ (88 mg, 0.078 mmol, 20 mol%) were placed in a μw vial under nitrogen. A solution of tin derivative prepared above in anhydrous DMF (2 mL) was added via syringe. The μw vial was heated for 2 h 15 min in the microwave synthesizer at 145 ^{o}C. The reaction mixture was poured into water (30 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel in EtOAc-hexanes and recrystallized from DCM-hexanes to obtain **20e** as tan star shaped crystals (50 mg, 47%): TLC R_f = 0.63 (40% EtOAc-hexanes); mp 108-110 ^{o}C; IR ν_{max}: 3300, 2900, 2200, 1750 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.58 (s, 1H), 2.99 (t, *J* = 2.0 Hz, 2H), 2.52 (m, 2H), 2.10 (d, *J* = 2.0 Hz, 3H), 0.28 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 209.71, 157.66, 152.65, 148.22, 137.37, 121.79, 102.05, 96.15, 33.74, 28.18, 10.23, -0.46; HRMS (m/z): $[M + Na]^+$ calcd for $C_{14}H_{17}NOSSi$, 298.0698; found, 298.0680.

3-(2-Ethynylthiazol-4-yl)-2-methylcyclopent-2-enone (21).

A solution of 2-methyl-3-(2-((trimethylsilyl)ethynyl)thiazol-4-yl)cyclopent-2-enone **20e** (50 mg, 0.182 mmol, 1 equiv) and K_2CO_3 (3 mg, 0.022 mmol, 12 mol%) in methanol (1 mL) was stirred for 5 min. The crude product was poured into water (8 mL) and extracted with EtOAc (3 x 3 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The product was recrystallized from DCM-hexanes to obtain **21** as tan needles: TLC R_f = 0.33 (40% EtOAc-hexanes); mp 138-139 ^{o}C; IR: 3000, 2900, 2250, 1750 cm^{-1}; ^{1}H NMR

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

(400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 3.52 (s, 3H), 2.99-2.96 (m, 2H), 2.53-2.51 (m, 2H), 2.11 (t, *J* = 2.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 209.98, 157.56, 153.08, 147.50, 137.84, 121.90, 83.24, 76.31, 33.92, 28.31, 10.38; HRMS (m/z): $[M + Na]^+$ calcd for $C_{11}H_9NOS$, 226.0303; found, 226.0298.

3-(2-(1H-1,2,3-Triazol-4-yl)-2-methylcyclopent-2-enone (22).

3-(2-Ethynylthiazol-4-yl)-2-methylcyclopent-2-enone **21** (60 mg, 0.295 mmol, 1 equiv) and CuI (3 mg, 0.015 mmol, 5 mol%) were placed in a μw vial under nitrogen. Anhydrous DMF (3.6 mL) and anhydrous MeOH (440 μL, 9:1 ratio DMF/MeOH) were added, followed by azidotrimethylsilane (58 μL, 0.443 mmol, 1.5 equiv). The vial was heated for 2 h in the microwave synthesizer at 140 ^oC. The reaction mixture was poured into water (10 mL) and extracted with EtOAc (3 x 4 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude compound was purified by flash chromatography on silica gel in EtOAc-hexanes and recrystallized from methanol to obtain product **22** (51 mg, 70%) as a white solid: TLC R_f = 0.34 (50% EtOAc-hexanes); mp 241-243 ^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 8.25 (S, 1H), 7.67 (s, 1H), 3.04-2.98 (m, 2H), 2.57-2.55 (m, 2H), 2.20 (d, *J* = 4.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 208.87, 158.46, 157.43, 152.32, 141.26, 135.32, 141.26, 135.47, 122.25, 33.25, 27.46, 9.99; HRMS (m/z): $[M + Na]^+$ calcd for $C_{11}H_{10}N_4OS$, 269.0473; found, 269.0489.

2-Methyl-3-(2-(1-methyl-1H-triazol -4-yl)thiazol-4-yl)cyclopent-2-enone (20f).

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

3-(2-(1H-1,2,3-Triazol-4-yl)-2-methylcyclopent-2-enone **22** (25 mg, 0.102 mmol, 1 equiv) and NaOMe (5 mg, 0.100 mmol, 0.99 equiv) were placed in a μw vial under nitrogen and anhydrous MeOH (2 mL) was added. The reaction mixture was heated for 7 min in the microwave synthesizer at 65 ^{o}C and methyl iodide (7 μL, 0.112 mmol, 1.1 equiv) was added. The reaction mixture was reheated in the microwave synthesizer for 30 min at 90 ^{o}C. The insoluble starting material was filtered off using DCM. Purification by preparative thin-layer chromatography on silica gel in 60% EtOAc-hexanes gave the product **20f** (9.6 mg, 36%) as a white solid: TLC R_f= 0.44 (50% EtOAc-hexanes); mp 154-156 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.62 (s, 1H), 4.25 (s, 3H), 3.02-2.99 (m, 2H), 2.55-2.53 (m, 2H), 2.17 (t, J = 2.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.21, 158.19, 158.03, 153.40, 143.07, 137.36, 132.36, 120.48, 42.30, 33.93, 28.18, 10.41; HRMS (m/z): $[M + Na]^+$ calcd for $C_{12}H_{12}N_4OS$, found 283.0630; found, 283.0620.

(*S*)-2-Methyl-3-(2-methylthiazol-4-yl)cyclopent-2-enol (6a).

A solution of 2-methyl-3-(2-methylthiazol-4-yl)cyclopent-2-enone **20a** (100 mg, 0.517 mmol, 1 equiv) and (*R*)-2-methyl-CBS-oxazaborolidine (29 mg, 0.103 mmol, 20 mol %) in anhydrous THF (2 mL) was cooled to 0 ^{o}C. A solution of borane-Me_2S (260 μL of 2.0 M solution in THF, 0.517 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 0 ^{o}C for 1 h and the reaction was quenched by slow addition of water (5 mL) at 0 ^{o}C. The reaction mixture was extracted with EtOAc (3 x 2 mL), and the combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by preparative thin-layer chromatography on silica gel in 50% EtOAc-hexanes to obtain the product **6a** (25 mg,

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

24%) as white crystals: TLC R_f = 0.34 (40% EtOAc-hexanes); mp 70-71 °C; $[\alpha]^{25}_D$ = -18.0° (c = 0.75, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.94 (s, 1H), 4.72 (d, *J* = 5.6 Hz, 1H), 2.86-2.77 (m, 1H), 2.70 (s, 3H), 2.65-2.57 (m, 1H), 2.41-2.32 (m, 1H), 2.13 (d, *J* = 0.4 Hz, 3H), 1.76-1.66 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.98, 153.10, 138.67, 131.66, 115.26, 82.38, 32.91, 32.72, 19.55, 13.36; HRMS (m/z): $[M + Na]^+$ calcd for $C_{10}H_{11}NOS$, 218.0616; found, 218.0620.

(*S*)-2-Methyl-3-(2-(methylthio)thiazol-4-yl)cyclopent-2-enol (6b).

A solution of 2-methyl-3-(2-(methylthio)thiazol-4-yl)cyclopent-2-enone **20b** (100 mg, 0.444 mmol, 1 equiv) and (*R*)-2-methyl-CBS-oxazaborolidine (25 mg, 0.089 mmol, 20 mol %) in anhydrous THF (2 mL) was cooled 0 °C. A solution of borane-Me_2S (220 μL of 2.0 M solution in THF, 0.444 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and the reaction was quenched by slow addition of water (5 mL) at 0 °C. The reaction mixture was extracted with EtOAc (3 x 2 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by preparative thin-layer chromatography on silica gel in 60% EtOAc-hexanes to obtain **6b** (64 mg, 63%) as a white solid: TLC R_f = 0.48 (40% EtOAc-hexanes); mp 46-49 °C; $[\alpha]^{25}_D$ = -5.8° (c = 1.40, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.95 (s, 1H), 4.71 (m, 1H), 2.83-2.76 (m, 1H), 2.70 (s, 3H), 2.61-2.55 (m, 1H), 2.42-2.34 (m, 1H), 2.18 (s, 3H), 1.77-1.69 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.25, 153.35, 139.23, 130.69, 114.73, 82.15, 32.52, 32.45, 16.69, 13.41; HRMS (m/z): $[M + H]^+$ calcd for $C_{10}H_{13}NOS_2$, 228.0517; found, 228.0525.

2-Methyl-3-(2-(piperidin-1-yl)thiazol-4-yl)cyclopent-2-enol (6c).

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

A solution of 2-methyl-3-(2-(piperidin-1-yl)thiazol-4-yl)cyclopent-2-enone **20c** (100 mg, 0.380 mmol, 1 equiv) and (*R*)-2-methyl-CBS-oxazaborolidine (21 mg, 0.076 mmol, 20 mol%) in anhydrous THF (4 mL) was cooled to 0 ^{o}C. A solution of borane-Me_2S (190 μL of 2.0 M solution in THF, 0.380 mmol, 1 equiv) was slowly added. The reaction was stirred at 0 ^{o}C for 1 h before the reaction was quenched by slow addition of water (8 mL). The reaction mixture was extracted with EtOAc (3 x 3 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by preparative thin-layer chromatography on silica gel in 50% EtOAc-hexanes to obtain racemic **6c** yellow crystals (88 mg, 87%): TLC R_f = 0.53 (40% EtOAc-hexanes); mp 93-95 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.35 (s, 1H), 4.67 (t, *J* = 5.2 Hz, 1H), 3.44 (m, 4H), 2.76-2.72 (m, 1H), 2.52-2.49 (m, 1H), 2.36-2.30 (m, 1H), 2.20 (s, 3H), 1.71-1.62 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.88, 150. 137.98, 131.63, 104.57, 82.52, 49.65, 32.63, 32.13, 25.31, 24.38, 13.35; HRMS (m/z): $[M + H]^+$ calcd for $C_{14}H_{20}N_2OS$, 265.1375; found, 265.1375.

(*S*)-2-Methyl-3-(2-(phenylethynyl)thiazol-4-yl)cyclopent-2-enol (6d).

A solution of 2-methyl-3-(2-(phenylethynyl)thiazol-4-yl)cyclopent-2-enone **20d** (100 mg, 0.358 mmol, 1 equiv) and (*R*)-2-methyl-CBS-oxazaborolidine (20 mg, 0.072 mmol, 20 mol %) in anhydrous THF (2 mL) was cooled to 0 ^{o}C. A solution of borane-Me_2S (179 μL of 2.0 M solution in THF, 0.358 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 0 ^{o}C for 5 min before being quenched by slow addition of water (4 mL) at 0 ^{o}C. The crude reaction mixture was extracted with EtOAc (3 x 1 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

preparative thin-layer chromatography on silica gel in 20% EtOAc-DCM to obtain product **6d** (56 mg, 56%) as a red oil: TLC R_f = 0.55 (20 % EtOAc-DCM); $[\alpha]^{25}_D$ = + 3.5^o (c = 0.650, $CHCl_3$); IR v_{max}: 3350, 2900, 2300 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.59 (m, 2H), 7.38 (m, 3H), 7.15 (s, 1H), 4.74 (d, *J* = 3.6 Hz, 1H), 2.89 (m, 1H), 2.69 (m, 1H), 2.41 (m, 1H), 2.15 (s, 3H), 1.76 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.17, 147.79, 139.98, 132.19, 131.14, 129.70, 128.68, 121.658, 117.38, 93.95, 82.78, 82.31, 32.99, 32.69, 13.51; HRMS (m/z): $[M + H]^+$ calcd for $C_{17}H_{15}NOS$, 282.0953; found, 282.0953.

(*S*)-3-(2-Ethynylthiazol-4-yl)-2-methylcyclopent-2-enol (6e).

A solution of 3-(2-ethynylthiazol-4-yl)-2-methylcyclopent-2-enone **20e** (95 mg, 0.340, 1 equiv) and (*R*)-2-methyl-CBS-oxazaborolidine (19 mg, 0.072 mmol, 20 mol %) in anhydrous THF (2 mL) was cooled to 0 ^{o}C. A solution of borane-Me_2S (170 μL of 2.0 M solution in THF, 0.340 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 0 ^{o}C for 25 min and the reaction was quenched by slow addition of water (5 mL) at 0 ^{o}C. The reaction mixture was extracted with EtOAc (3 x 2 mL). The combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by preparative thin-layer chromatography on silica gel in 25 % EtOAc-DCM to obtain the partial purified product **6e** (72 mg, 75.1%) as a reddish/brown solid: TLC R_f = 0.58 (40% EtOAc-DCM); mp 69-71 ^{o}C; $[\alpha]^{25}_D$ = -12.8^o (c =1.50, $CHCl_3$); IR v_{max}: 3300, 2900, 2300 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.13 (s, 1H), 4.73 (d, *J* = 4.4 Hz, 1H), 3.44 (s, 1H), 2.88-2.81 (m, 1H), 2.67-2.60 (m, 1H), 2.43-2.34 (m, 1H), 2.14 (d, *J* = 8.8 Hz, 3H), 1.78-1.70 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz):

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

δ 154.15, 146.52, 140.24, 130.83, 117.48, 82.30, 82.14, 32.91, 32.67, 13.49; HRMS (m/z): [M + Na]$^+$ calcd for $C_{11}H_{11}NOS$, 228.0458; found, 228.0459.

(*S*)-2-Methyl-3-(2-(1-methyl-1H-1,2,3-triazol-4-yl)thiazol-4-yl)cyclopent-2-enol (6f).

A solution of 2-methyl-3-(2-(1-methyl-1H-1,2,3-triazol-4-yl)thiazol-4-yl)cyclopent-2-enone **20f** (20 mg, 0.071 mmol, 1 equiv) and (*R*)-2-methyl-CBS-oxazaborolidine (5 mg, 0.015 mmol, 20 mol %) in anhydrous THF (1.5 mL) was cooled to 0 ^{o}C. A solution of borane-Me_2S (40 μL of 2.0 M solution in THF, 0.077 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 0 ^{o}C for 40 min and quenched by the slow addition of water (5 mL). The reaction mixture was extracted with EtOAc (3 x 2 mL), and the combined organic extract was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by preparative thin-layer chromatography on silica gel in 5% MeOH-DCM to obtain **6f** (10 mg, 54%) as a white powder: TLC R_f = 0.51 (60% EtOAc-hexanes); mp 140-141 ^{o}C; $[\alpha]^{25}_D$ = -5.9^o (c = 0.540, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.12 (s, 1H), 4.75 (d, *J* = 5.4 Hz, 1H), 4.24 (s, 3H), 2.87-2.85 (m, 1H), 2.67-2.65 (m, 1H), 2.43-2.40 (m, 1H), 2.22 (s, 3H), 1.78-1.74 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.26, 154.27, 143.57, 139.66, 132.20, 131.09, 115.85, 82.37, 42.21, 32.82, 32.71, 13.46; HRMS (m/z): [M + Na]$^+$ calcd for $C_{12}H_{14}N_4OS$, 285.0786; found, 285.0776.

C. Synthesis of open-chain epothilones (5a-f).

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.
Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

(*3S,6R,7S*)-((*S*)-2-methyl-3-(2-methylthiazol-4-yl)cyclopent-2-enyl) 3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoate (5a).

A mixture of (*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid (20 mg, 0.077 mmol, 1equiv), (*S*)-2-methyl-3-(2-methylthiazol-4-yl)cyclopent-2-enol **6a** (15 mg, 0.077 mmol, 1 equiv), DCC (20 mg, 0.100 mmol, 1.3 equiv), DMAP (1 mg, 0.008 mmol, 10 mol %), and CSA (4 mg, 0.015 mmol, 20 mol %) in anhydrous DCM (300 μL) was stirred under nitrogen overnight. The reaction mixture was filtered through a cotton plug and purified by preparative thin-layer chromatography on silica gel plates (deactivated with 5% Et_3N-hexanes) in 25% EtOAc-hexanes to obtain **5a** (5 mg, 23%) as a clear oil: TLC R_f = 0.44 (40% EtOAc-hexanes); $[\alpha]^{25}_D$= +5.3° (c = 0.300, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.99 (s, 1H), 5.79 (s, 1H), 4.25-4.22 (m, 1H), 3.36-3.23 (m, 4H), 2.89-2.84 (m, 1H), 2.70 (s, 3H), 2.66 (m, 1H), 2.51-2.34 (m, 3H), 2.08 (s, 3H), 1.86-1.78 (m, 1H), 1.71-1.60 (m, 1H), 1.16 (s, 6H), 1.05 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 222.37, 173.29, 165.15, 152.50, 134.67, 134.12, 116.09, 85.53, 76.64, 72.78, 72.71, 52.40, 41.04, 37.10, 33.55, 30.79, 29.53, 21.69, 19.75, 19.61, 19.33, 19.28, 19.23, 13.69, 10.64; HRMS (m/z): $[M + Na]^+$ calcd for $C_{23}H_{35}NO_5S$, 460.2134; found 460.2144.

(*3S,6R,7S*)-((*S*)-2-methyl-3-(2-(methylthio)thiazol-4-yl)cyclopent-2-enyl) 3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoate (5b).

A mixture of (*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid (66 mg, 0.253 mmol, 1 equiv), (*S*)-2-methyl-3-(2-(methylthio)thiazol-4-yl)cyclopent-2-enol **6b** (78 mg, 0.343 mmol, 1.4 equiv), DCC (75 mg, 0.328 mmol, 1.3 equiv), DMAP (32 mg, 0.253 mmol, 1 equiv),

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

and anhydrous Et_3N (64 μL, 0.506 mmol, 2 equiv) in anhydrous DCM (300 μL) was stirred under nitrogen for overnight. The reaction mixture was filtered through a cotton plug and purified by successive preparative thin-layer chromatography on silica gel (deactivated with 5% Et_3N-hexanes) in 40% EtOAc-hexanes, 90% EtOAc-hexanes, 85% EtOAc-hexanes to obtain **5b** (12 mg, 10%) as a clear oil: TLC R_f = 0.56 (40% EtOAc-hexanes); $[\alpha]^{25}_D$= -4.6^o (c = 0.600, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.99 (s, 1H), 5.80 (t, *J* = 3.2 Hz, 1H), 4.26-4.22 (m, 1H), 3.35-3.22 (m, 4H), 2.87-2.80 (m, 1H), 2.69 (s, 3H), 2.67-2.62 (m, 1H), 2.51-2.35 (m, 3H), 2.12 (s, 3H), 1.86-1.78 (m, 1H), 1.69-1.64 (m, 1H), 1.16 (s, 6H), 1.04 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 222.38, 173.24, 165.59, 152.76, 134.73, 133.90, 85.46, 76.62, 72.75, 72.62, 52.36, 41.02, 37.08, 37.05, 33.14, 30.74, 29.47, 21.67, 21.65, 19.68, 19.29, 19.24, 19.19, 16.73, 13.72, 13.70, 10.60; HRMS (m/z): $[M + Na]^+$ calcd for $C_{23}H_{35}NO_5S_2$, 492.1854; found, 492.1858.

(*3S,6R,7S*)-((*R*)-2-methyl-3-(2-(piperidin-1-yl)thiazol-4-yl)cyclopent-2-enyl) 3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoate compound with (*3S,6R,7S*)-((*S*)-2-methyl-3-(2-(piperidin-1-yl)thiazol-4-yl)cyclopent-2-enyl) 3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoate (5c).

A mixture of (*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid (44 mg, 0.170 mmol, 1 equiv), 2-methyl-3-(2-(piperidin-1-yl)thiazol-4-yl)cyclopent-2-enol **6c** (45 mg, 0.170 mmol, 1 equiv), DCC (46 mg, 0.221 mmol, 1.3 equiv), DMAP (21 mg, 0.170, 1 equiv), and anhydrous Et_3N (47 μL, 0.340 mmol, 2 equiv) in anhydrous DCM (400 μL) was stirred under nitrogen overnight. The reaction mixture was filtered through a cotton plug and purified by

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

successive preparative thin-layer chromatography on silica gel (deactivated with 5% Et_3N-hexanes) in 30% EtOAc-hexanes, 50% EtOAc-hexanes to obtain a diasteromeric mixture **5c** (3 mg, 4%) as a yellow oil: TLC (20% EtOAc-hexanes); $[\alpha]^{25}_D$= -1.2^o (c = 0.650, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.39 (s, 1H), 5.77 (s, 1H), 4.21 (d, *J* = 10.2 Hz, 1H), 3.44 (m, 4H), 3.34-3.28 (m, 4H), 2.78 (m, 1H), 2.59 (m,1H), 2.50-2.36 (m, 3H), 2.13 (s, 3H), 1.82-1.79 (m, 1H), 1.63 (m, 6H), 1.15 (s, 6H), 1.04 (d, *J* = 8.0 Hz, 3H), 1.00 (d, *J* = 8.0 Hz, 3H), 0.85 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 222.31, 173.35, 173.32, 170.90, 149.47, 134.77, 134.72, 133.34, 133.28, 105.46, 105.42, 85.82, 76.60, 72.73, 72.66, 52.38, 52.34, 49.71, 40.10, 37.10, 37.02, 32.82, 30.74, 29.44, 25.388, 25.35, 24.42, 21.68, 21.64, 19.72, 19.22, 19.18, 13.66, 13.64, 10.61; HRMS (m/z): $[M + Na]^+$ calcd for $C_{27}H_{42}N_2O_5S$, 529.2712; found, 529.2729.

(*3S,6R,7S*)-((*S*)-2-methyl-3-(2-(phenylethynyl)thiazol-4-yl)cyclopent-2-enyl) 3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoate (5d).

A mixture of (*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid (52 mg, 0.199 mmol, 1equiv), (*S*)-2-methyl-3-(2-(phenylethynyl)thiazol-4-yl)cyclopent-2-enol **6d** (55 mg, 0.199 mmol, 1 equiv), DCC (53 mg, 0.259 mmol, 1.3 equiv), DMAP (25 mg, 0.199 mmol, 1 equiv), and anhydrous Et_3N (55 μL, 0.398 mmol, 2 equiv) in anhydrous DCM (400 μL) was stirred under nitrogen overnight. The reaction mixture was filtered through a cotton plug and purified by successive preparative thin-layer chromatography on silica gel (deactivated with 5% Et_3N-hexanes) in 40% EtOAc-hexanes, 5% EtOAc-hexanes, 25% EtOAc-hexanes to obtain **5d** (10 mg, 10%) as a white semisolid: TLC R_f = 0.45 (40% EtOAc-hexanes); $[\alpha]^{25}_D$ = -13.5^o (c = 0.275, $CHCl_3$); IR ν_{max}: 3500, 3200, 2900 , 2100 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (dd,

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

J = 7.6 Hz, *J* = 5.6 Hz, 2H), 7.39-7.33 (m, 3H), 7.19 (s, 3H), 5.82 (s, 1H), 4.27-4.22 (m, 2H), 3.35-3.22 (m, 4H), 2.93-2.89 (m, 1H), 2.79-2.71 (m, 1H), 2.52-2.36 (m, 3H), 2.10 (s, 3H), 1.89-1.82 (m, 1H), 1.70-1.64 (m, 1H), 1.16 (s, 6H), 1.05 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 222.34, 173.20, 153.50, 147.97, 135.49, 134.06, 132.20, 129.76, 128.70, 121.59, 118.06, 94.12, 85.30, 82.68, 76.62, 72.76, 72.69, 52.37, 41.03, 37.07, 33.61, 30.76, 29.49, 21.68, 19.70, 19.32, 19.27, 19.21, 13.79, 10.62; HRMS (m/z): $[M + Na]^+$ calcd for $C_{30}H_{37}NO_5S$, 546.2290; found 546.2285.

(*3S,6R,7S*)-((*S*)-3-(2-ethynylthiazol-4-yl)-2-methylcyclopent-2-enyl) 3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoate (5e).

A mixture of (*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid (38 mg, 0.146 mmol, 1 equiv), (*S*)-3-(2-ethynylthiazol-4-yl)-2-methylcyclopent-2-enol **6e** (30 mg, 0.146 mmol, 1 equiv), DCC (39 mg, 0.190 mmol, 1.3 equiv), DMAP (4 mg, 0.029 mmol, 20 mol %), and CSA (2 mg, 0.007 mmol, 5 mol %) in anhydrous DCM (300 μL) was stirred under nitrogen overnight. The reaction mixture was filtered through a cotton plug and purified by successive preparative thin-layer chromatography on silica gel (deactivated with 5% Et_3N-hexanes) in 10% AcCN-DCM, 50% DCM-hexanes, 10% acetone-hexanes to obtain **5e** (10 mg, 15%) as a colorless oil: TLC R_f= 0.69 (50% acetone-hexanes); $[\alpha]^{25}_D$ = -6.3^o (c = 0.600, $CHCl_3$); IR ν_{max}: 3300, 3050, 2900, 2100, 1750 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17 (s, 1H), 5.81 (s, 1H), 4.25-4.22 (m, 1H), 3.45 (s, 1H), 3.35-3.20 (m, 4H), 2.92-2.89 (m, 1H), 2.78-2.70 (m, 1H), 2.51-2.35 (m, 3H), 2.15 (s, 3H), 1.85-1.82 (m, 1H), 1.70-1.57 (m, 1H), 1.16 (s, 6H), 1.05 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

222.37, 173.18, 153.48, 146.71, 135.78, 133.71, 118.20, 85.24, 82.31, 76.80, 76.62, 72.75, 72.68, 52.36, 41.03, 37.06, 33.51, 30.75, 29.46, 21.67, 19.70, 19.27, 19.20, 13.75, 10.61; HRMS (m/z): $[M + Na]^+$ calcd for $C_{24}H_{33}NO_5S$, 470.1977; found 470.1957.

(*3S,6R,7S*)-((*S*)-2-methyl-3-(2-(1-methyl-1H-1,2,3-triazol-4-yl)thiazol-4-yl)cyclopent-2-enyl) 3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoate (5f).

A mixture of (*3S,6R,7S*)-3,7-dihydroxy-4,4,6,8-tetramethyl-5-oxononanoic acid (55 mg, 0.201 mmol, 1 equiv), (*S*)-2-methyl-3-(2-(1-methyl-1H-1,2,3-triazol-4-yl)thiazol-4-yl)cyclopent-2-enol **6f** (52 mg, 0.201 mmol, 1 equiv), DCC (54 mg, 0.261 mmol, 1.3 equiv), DMAP (5 mg, 0.040 mmol, 20 mol %), and CSA (2 mg, 0.010 mmol, 5 mol %) in anhydrous DCM (400 μL) was stirred under nitrogen overnight. The reaction mixture was filtered through a cotton plug and purified by successive preparative thin-layer chromatography on silica gel (deactivated with 5% Et_3N-hexanes) in 40% EtOAc-hexanes, 35% acetone-hexanes to obtain **5f** (21 mg, 20%) as a colorless oil: TLC R_f= 0.44 (40 % EtOAc-Hex); $[\alpha]^{25}_D$ = -4.5° (c = 1.17, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.16 (s, 1H), 5.82 (d, *J* = 2.4Hz, 1H), 4.26 (d, *J* = 2.4 Hz, 1H), 4.18 (s, 3H), 3.35-3.23 (m, 4H), 2.94-2.88 (m, 1H), 2.75-2.71 (m, 1H), 2.52-2.34 (m, 3H), 2.15 (s, 3H), 1.89-1.81 (m, 1H), 1.71-1.60 (m, 1H), 1.16 (s, 1H), 1.04 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 222.35, 173.22, 157.40, 153.59, 143.46, 135.08, 134.04, 132.19, 116.60, 85.40, 76.60, 72.74, 72.66, 52.36, 42.23, 41.01, 37.07, 33.43, 30.74, 29.50, 21.66, 19.69, 19.30, 19.25, 19.17, 13.74, 13.71, 10.61; HRMS (m/z): $[M + Na]^+$ calcd for $C_{25}H_{36}N_4O_5S$, 527.2304; found, 527.2308.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

E. Synthesis of alcohols (23) and (24) and oxime (25)

(R)-3-(2-Ethynylthiazol-4-yl)-2-methylcyclopent-2-enol (23)

A mixture of 3-(2-ethynylthiazol-4-yl)-2-methylcyclopent-2-enone **21** (0.0258 g, 0.127 mmol, 1 equiv), *(S)*-(-)-2-methyl-CBS-oxazaborolidine (0.008 g, 0.029 mmol, 22.7 mol%), and anhydrous THF (0.6 mL) was cooled to 0°C. A solution of BH_3-Me_2S (0.063 mL of 2 M solution in THF, 0.126 mmol, 0.99 equiv) was added dropwise via syringe and the reaction mixture was stirred at 0°C for 25 min. The reaction was quenched by slow addition of water (~2 mL) at 0°C. The reaction mixture was extracted with EtOAc three times. The combined EtOAc extract was washed with brine and dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to obtain a yellow oil. The crude product was purified by flash chromatography on silica gel in 15% acetone/hexanes. The product was further purified by preparative TLC in 25% acetone/hexanes to obtain analog **23** as a yellow oil (0.0237 g, 91%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 4.80 (brs, 1H), 3.50 (s, 1H), 2.93 (m, 1H), 2.72 (m, 1H), 2.47 (m, 1H), 2.18 (s, 3H), 1.81 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 13.45, 32.67, 32.92, 76.95, 82.10, 82.29, 117.44, 130.85, 140.29, 146.55, 154.22. HRMS (m/z): $[M + Na]^+$ calcd for $C_{11}H_{11}NOS$, 228.0459; found 228.0452

2-Methyl-3-(2-((trimethylsilyl)ethynyl)thiazole-4-yl)cyclopent-2-enol (24)

A mixture of 3-(2-ethynylthiazol-4-yl)-2-methylcyclopent-2-enone **21** (0.0259 g, 0.127 mmol, 1 equiv) and methanol (~0.5 mL) was cooled to 0°C with stirring. Sodium borohydride (0.0064 g, 0.169 mmol, 1.328 equiv) was added and the reaction was monitored by TLC. An additional portion of sodium borohydride (0.0056 g, 0.148 mmol, 1.162 equiv) was added to the reaction

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

mixture and stirring was continued for another 40 min at 0°C. Excess methanol was removed on a rotary evaporator. Water (~2 mL) was added to the residue and extracted with EtOAc three times. The combined EtOAc extract was washed with brine, dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by flash chromatography on silica gel in 25% EtOAc/hexanes to obtain analog **24** as a yellow solid (0.0161 g, 62%); mp 84-85°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (s, 1H), 4.80 (brs, 1H), 3.50 (s, 1H), 2.91 (m, 1H), 2.71 (m, 1H), 2.45 (m, 1H), 2.17 (s, 3H), 1.79 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 13.45, 32.67, 32.92, 76.94, 82.12, 82.28, 117.44, 130.84, 140.30, 146.55, 154.22. HRMS (m/z): $[M + Na]^+$ calcd for $C_{11}H_{11}NOS$, 228.0459; found 228.0452

3-(2-Ethynylthiazol-4-yl)-2-methylcyclopent-2-enone oxime (25)

A mixture of 3-(2-ethynylthiazol-4-yl)-2-methylcyclopent-2-enone **(4a)** (0.0255 g, 0.126 mmol, 1 equiv), sodium acetate (0.0248 g, 0.303 mmol, 2.409 equiv), hydroxylamine hydrochloride (0.0102 g, 0.147 mmol, 1.17 equiv), methanol (1 mL), and water (0.25 mL) was heated at 70°C with stirring for 28 h. Methanol was removed on a rotary evaporator. Water (2 mL) was added to the residue and extracted with DCM three times. The combined DCM extract was washed with brine and dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The crude product was purified by flash chromatography on silica gel in 1% methanol/DCM to obtain analog **32** as a yellow solid (0.0179 g, 65%); mp 189-191°C (decomp.); ^{1}H NMR (600 MHz, acetone-*d*$_6$) δ 9.86 (s, 1H), 7.74 (s, 1H), 4.35 (s, 1H), 2.88 (m, 2H), 2.73 (m, 2H), 2.20 (s, 3H). ^{13}C NMR (150 MHz, acetone-d_6) δ 12.05, 24.90, 32.19, 77.81, 84.83, 120.66, 135.94, 141.87, 147.92, 155.09, 168.54. HRMS (m/z): $[M + Na]^+$ calcd for $C_{11}H_{10}N_2OS$, 219.0592; found 219.0594

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Biological Studies:

Materials and Methods

Cell Lines and Culture Conditions. Cell lines were cultured in a humidified 37 ^{o}C atmosphere containing 10% CO_2 in Dulbecco's Modified Eagle's Medium (Mediatech, Inc.) supplemented with 10% fetal bovine serum (Atlanta Biologicals) and 1000 U/ml of both Penicillin and Streptomycin (Mediatech). To determine viability 50,000 cells were plated in each well of a 24-well plate. Compounds were added 1 day post plating and cells were stained after 3 days with a saturated solution of methylene blue in 50% ethanol. Plates were rinsed and retained dye quantified by absorbance at 630 nm. Results are representative of at least two independent experiments. Some experiments were repeated using MTS dye (CellTitre96, Promega) with similar results.

Microscopy. For immunofluorescence microscopy, cells were fixed with 2% formaldehyde in phosphate buffered saline (PBS) for 15 minutes, followed by permeabilization [150 mM NaCl, 10 mM Tris (pH 7.7), 0.1% Triton X-100, and 0.1% BSA] for 9 minutes. Fixed cells were blocked with PBS containing 0.1% BSA for 1 h at room temperature. Cells were then stained with antibodies to alpha-tubulin and borealin.[17] Antibodies were visualized by incubating samples with Alexafluor-conjugated secondary antibodies (Invitrogen). DNA was visualized by staining with Hoechst 33342. Images were captured using a Leica SP8 confocal microscope. Time-lapse microscopy was carried out using an Olympus IX81 inverted microscope with an environmental chamber heated to 37 ^{o}C. Images were captured at defined intervals using an Photometrics Coolsnap HQ2 camera.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Supporting Information Available

^{1}H and ^{13}C NMR spectra of all intermediates and final products, and materials and methods of biological studies. This material is available free of charge via the internet at http://pubs.acs.org.

Corresponding Authors

*Phone: 419 530-1983: E-mail: ltillek@utoledo.edu

*Phone: 419 530-1966: E-mail: william.taylor3@utoledo.edu

CONFLICTS OF INTEREST

LMVT, SRF and WRT are coinventors of a patent application that covers these anticancer agents.

ACKNOWLEDGEMENTS:

We thank the Elsa U. Pardee Foundation and the deArce-Koch Memorial Endowment Fund for initial seed funding for the project and Michael Bekier for confocal imaging. This project was partially supported by grant NIH R15 GM100440-01 to WRT.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

REFERENCES:

(1) Hoefle, G.; Bedorf, N.; Gerth, K.; Reichenbach, H. Epothilone derivatives, 1993.

(2) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. Chemical Biology of Epothilones. *Angew. Chem., Int. Ed.* **1998**, *37* (15), 2014–2045.

(3) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. Epothilones, a New Class of Microtubule-Stabilizing Agents with a Taxol-like Mechanism of Action. *Cancer Res.* **1995**, *55* (11), 2325–2333.

(4) Kowalski, R. J.; Giannakakou, P.; Hamel, E. Activities of the Microtubule-Stabilizing Agents Epothilones A and B with Purified Tubulin and in Cells Resistant to Paclitaxel (Taxol). *J. Biol. Chem.* **1997**, *272* (4), 2534–2541.

(5) Alhamadsheh, M. M.; Hudson, R. A.; Tillekeratne, L. M. V. Design, Total Synthesis, and Evaluation of Novel Open-Chain Epothilone Analogues. *Org. Lett.* **2006**, *8* (4), 685–688.

(6) Kamel, K.; Rusinska-Roszak, D. A Computational Study of Open-Chain Epothilone Analogue. *Int. J. Quantum Chem.* **2008**, *108* (5), 967–973.

(7) Dixon, S. J.; Lemberg, K. M.; Lamprecht, M. R.; Skouta, R.; Zaitsev, E. M.; Gleason, C. E.; Patel, D. N.; Bauer, A. J.; Cantley, A. M.; Yang, W. S.; Morrison 3rd, B.; Stockwell, B. R. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. *Cell* **2012**, *149* (5), 1060–1072.

(8) Bal, B. S.; Childers Jr., W. E.; Pinnick, H. W. Oxidation of A,β-Unsaturated Aldehydes. *Tetrahedron* **1981**, *37* (11), 2091–2096.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

(9) Geronikaki, A.; Vasilevsky, S.; Hadjipavlou-Litina, D.; Lagunin, A.; Poroikov, B. V. Synthesis and Anti-Inflammatory Activity of Ethynylthiazoles. *Chem. Heterocycl. Compd. (N. Y., NY, U. S.)* **2006**, *42* (5), 675–680.

(10) Karama, U.; Hofle, G. Synthesis of Epothilone 16,17-Alkyne Analogs by Replacement of the C13-C15(O)-Ring Segment of Natural Epothilone C. *Eur. J. Org. Chem.* **2003**, No. 6, 1042–1049.

(11) Nicolaou, K. C.; King, N. P.; Finlay, M. R. V; He, Y.; Roschangar, F.; Vourloumis, D.; Vallberg, H.; Sarabia, F.; Ninkovic, S.; Hepworth, D. Total Synthesis of Epothilone E and Related Side-Chain Modified Analogues via a Stille Coupling Based Strategy. *Bioorg. Med. Chem.* **1999**, *7* (5), 665–697.

(12) Dale, J. A.; Mosher, H. S. Nuclear Magnetic Resonance Enantiomer Regents. Configurational Correlations via Nuclear Magnetic Resonance Chemical Shifts of Diastereomeric Mandelate, O-Methylmandelate, and Alpha -Methoxy-Alpha -Trifluoromethylphenylacetate (MTPA) Esters. *J. Amer. Chem. Soc.* **1973**, *95* (2), 512–519.

(13) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. High-Field FT NMR Application of Mosher's Method. The Absolute Configurations of Marine Terpenoids. *J. Am. Chem. Soc.* **1991**, *113* (11), 4092–4096.

(14) Jin, T.; Kamijo, S.; Yamamoto, Y. Copper-Catalyzed Synthesis of N-Unsubstituted 1,2,3-Triazoles from Nonactivated Terminal Alkynes. *Eur. J. Org. Chem.* **2004**, No. 18, 3789–3791.

Organic & Biomolecular Chemistry Accepted Manuscript

View Article Online
DOI: 10.1039/C7OB03086J

(15) Belletire, J. L.; Bills, R. A.; Shackelford, S. A. Practical Methylation Procedure for 1H-1,2,4-Triazole. *Synth. Commun.* **2008**, *38* (5), 738–745.

(16) Wallace, D. C.; Fan, W.; Procaccio, V. Mitochondrial Energetics and Therapeutics. *Annu Rev Pathol* **2010**, *5*, 297–348.

(17) Kaur, H.; Stiff Andrew, C.; Date Dipali, A.; Taylor William, R. Analysis of Mitotic Phosphorylation of Borealin. *BMC Cell Biol* **2007**, *8*, 5.

Published on 30 January 2018. Downloaded by Fudan University on 30/01/2018 17:41:55.

Organic & Biomolecular Chemistry Accepted Manuscript

Table of Content Graphic