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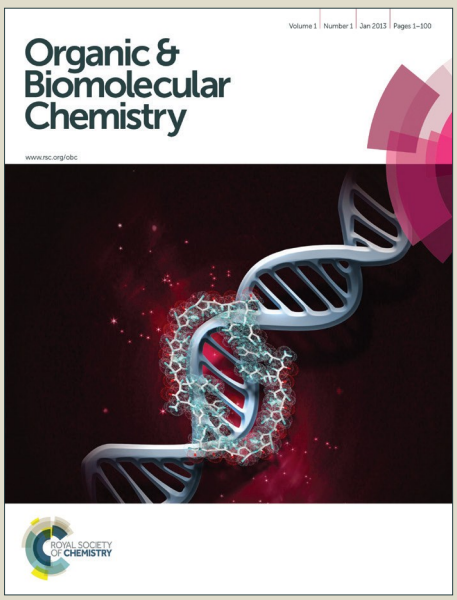

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ARTICLE

Efficient Synthesis of an *Exo*-enone Analogue of LL-Z1640-2 and Evaluation of its Protein Kinase Inhibitory Activities

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Stephanie Q. Wang,[a] Shermin S. Goh,[a] Christina L. L. Chai,*[,a, b] Anqi Chen*[,a]

An efficient synthesis of an *exo*-enone analogue (**5**) of resorcylic acid lactone (RAL) natural product LL-Z1640-2 (**1**) has been achieved using a Ni-catalysed regioselective reductive coupling macrocyclisation of alkyne-aldehyde as a key step. The synthetic route is significantly shorter than those for the natural product and avoids the isomerisation problem of the *cis*-double bond in the molecule. Preliminary biological evaluation showed the *exo*-enone analogue is a potent inhibitor of several important kinases relevant to cancer drug development.

Introduction

The resorcylic acid lactones (RALs) are a group of natural products isolated from the metabolites of various fungal strains, characterised by a β-resorcylic acid unit that is fused by a 14-membered macrolactone. These compounds possess important biological activities, especially as potent inhibitors of a range of protein kinases relevant to several important cancer targets.[1-6] Consequently, the RALs have been recognised as privileged structures for drug discovery.[7-15] For example, based on LL-Z1640-2 (also known as f152A1 or 5Z-7-oxozeaenol, **1**) E6201 (**2**) (**Fig. 1**) was discovered and shown to be a potent inhibitor of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK1, IC50 = 5.2 nM) and several other kinases.[16-18] This compound is currently under preclinical studies for the treatment of cancers[19] and in clinical trials for anti-inflammatory applications.[20] A unique feature of LL-Z1640-2 and a few other RALs such as L-783,277 (**3**) and hypothemycin (**4**) (**Fig. 1**) is their ability to bind irreversibly to a key cysteine residue located at the ATP binding pocket in a family of protein kinases,[21-22] thereby showing extremely high potencies.

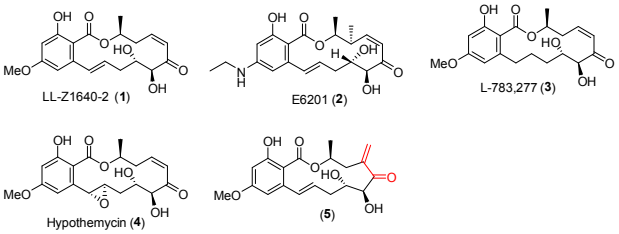


Fig. 1 RALs containing *cis*-enone and an *exo*-enone analogue (**5**).

The high activity of these RALs is attributed to the Michael reaction between *cis*-enone moiety and the cysteine thiol group, leading to protein-RAL covalent adducts.

One prominent problem associated with the synthesis of these *cis*-enone containing RALs, however, is the isomerisation of the *cis*-enone to the thermodynamically more stable *trans*-form,[23-25] which is known to be significantly less active.[26] Additionally, the synthetic routes to the *cis*-enone RALs are generally quite lengthy (often >15 steps) owing in part to the introduction of the *cis*-enone functionality.[1-6] In this context, we envisaged an *exo*-enone analogue such as (**5**), which could avoid the isomerisation problem whilst preserving the requisite reactivity as a Michael acceptor towards the cysteine residue at the ATP binding site of the kinases. Additionally, a more efficient route could be realised as steps involving introduction of the *cis*-enone functionality are avoided. In our continuing efforts directed towards the exploration of the therapeutic potentials of RALs,[27-30] we report herein the synthesis of an *exo*-enone RAL analogue (**5**) and evaluation of its protein kinase inhibitory activities.

Results and discussion

A convergent route to the *exo*-enone analogue (**5**) is outlined in **Fig. 2**. We envisaged that the *exo*-enone system can be converted from the corresponding macrocyclic *exo*-methylene allylic alcohol (**6**) which is to be formed by a Ni-catalysed, *exo*-selective reductive coupling macrocyclisation of alkyne-aldehyde based on an intermolecular reaction reported by Takai *et al*.[31] The cyclisation

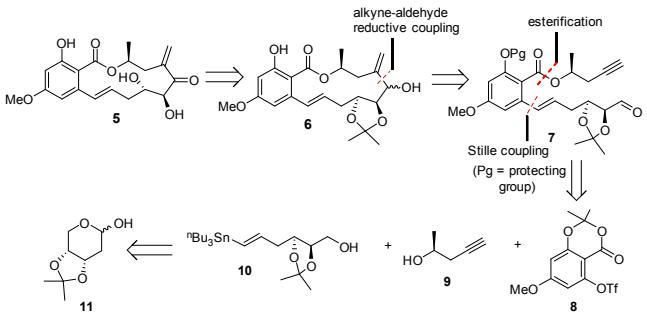


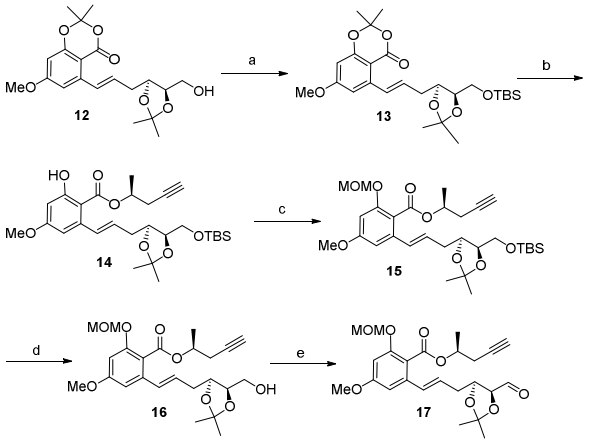
Fig. 2 Retrosynthetic analysis of *exo*-enone analogue (**5**).

[a] *Institute of Chemical and Engineering Sciences (ICES), Agency for Science, Technology and Research (A*STAR), 8 Biomedical Grove, Neuros #07-01, Singapore 138665. E-mail: chen_anqi@ices.a-star.edu.sg (A. Chen)*
[b] *Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543E-mail: phacllc@nus.edu.sg (C. L. L. Chai)*

† Electronic Supplementary Information (ESI) available: ^{1}H and ^{13}C NMR spectra of all new compounds and kinase profiling data (% control) for *exo*-enone **5** see DOI: 10.1039/x0xx00000x

precursor (**7**) can be conveniently accessed by coupling three fragments (**8**, **9**, **10**) as previously reported.[29] This involves a Stille coupling of aryl triflate (**8**) with vinyl stannane (**10**) followed by a transesterification on the protected resorcylate with (*S*)-pent-4-yn-ol (**9**) to introduce the top alkyne side chain. The vinyl stannane fragment (**10**),[29] which contains the requisite configurations of the two stereogenic hydroxy groups in **5**, can be readily prepared from 2-deoxy D-ribose acetonide (**11**) via a Colvin rearrangement[32,33] followed by hydrostannylation.

The synthesis of **5** commenced with the alkene (**12**) which is obtained in 3 steps from **11** by Stille coupling of the triflate (**8**) and the vinyl stannane (**10**). Transesterification of the TBS protected alcohol (**13**) with (*S*)-pent-4-yn-ol (**9**) in the presence of NaH installed the top alkyne side chain with concomitant phenol deprotection upon acidic workup, providing the fully assembled intermediate **14** in essentially quantitative yield. Reprotection of the phenol as its MOM ether followed by deprotection of the TBS protecting group provided the alcohol (**16**) in excellent yields for both steps. The alcohol was further oxidised with Dess Martin periodinane, leading to alkyne-aldehyde (**17**) ready for the macrocyclisation (Scheme 1).



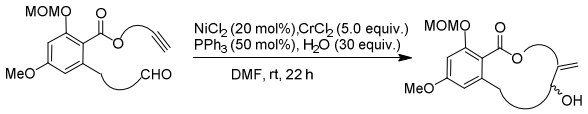
Scheme 1. Reagents and conditions: a) TBS-Cl, imidazole, DCM, rt, quant.; b) **9**, NaH, THF, 0 °C – rt, 98%; c) MOM-Cl, NaH, THF/DMF (3:1), rt, 92%; d) TBAF, THF, rt, 85%; e) Dess-Martin periodinane, DCM, rt, 75%.

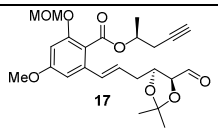
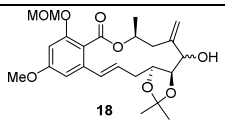
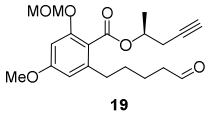
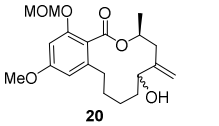
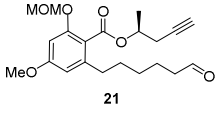
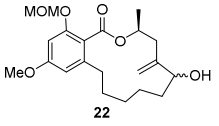
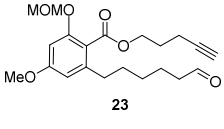
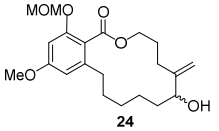
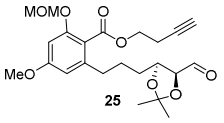
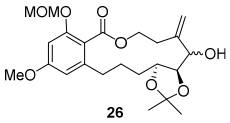
The crucial step of our proposed macrocyclisation of **17** requires an *exo*-selective reductive coupling of the alkyne and the aldehyde within the molecule. After assessing a number of potential methogologies for macrocyclisation,[31] a $NiCl_2$ mediated intermolecular version of reaction reported by Takai *et al*[32], which provides *exo*-methylene allylic alcohols in high regioselectivity (≥ 95:5), appeared to be most suitable although it has not been applied to macrocyclisation.[35]

Applying the reported conditions[32] to **17** under moderate dilution (0.013 M), gratifyingly provided the desired macrocyclisation product (**18**) in 57% isolated yield. The reaction is known to proceed via an alkenylnickel species formed by a regioselective hydronickelation of the alkyne. Presence of water is essential as it serves as a hydride source for the formation of nickel hydride required for hydronickelation of the alkyne whilst addition of a catalytic amount of triphenylphosphine accelerates the reaction and stabilises the catalyst, thereby preventing the formation of inactive nickel metal particles.[32]

The presence of the terminal alkene was evidenced by the ^{1}H-NMR spectrum which clearly showed the two characteristic geminal olefinic protons at 5.05 and 5.35 ppm. Additionally, the product was isolated as a single diastereomer although the absolute stereochemistry of the newly formed hydroxy group was not determined as it would be oxidised in the next step. The reaction was further applied to several other alkyne-aldehydes, providing the corresponding cyclisation products (**Table 1**) which were subsequently converted into the corresponding enones which are valuable compounds for structure-activity relationship studies.

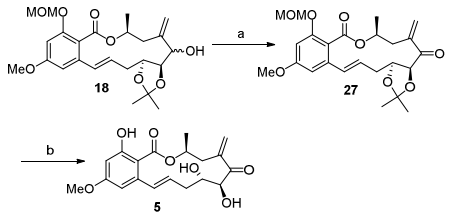
Table 1. Macrocyclisation of alkyne-aldehydes by $NiCl_2$ mediated reductive coupling.



Entry	Substrate	Product	Yield (%)
1			57
2			38
3			40
4			36
5			28

The allylic alcohol (**18**) was oxidised to enone (**27**) under the Dess-Martin conditions. Final global deprotection using HCl in methanol furnished the required *exo*-enone analogue (**5**) (Scheme 2). The structure of the product was confirmed by extensive NMR spectroscopic analysis.

Kinase inhibition activity of the *exo*-enone (**5**) was evaluated by screening against a panel of 62 selected kinases at 10 μM using



Scheme 2. Reagents and conditions: a) Dess-Martin periodinane, DCM, rt, 65%; b) HCl (2.0 M), MeOH, rt, HPLC purification, 61%.

DMSO as a negative control. The results showed strong inhibition of the compound against several kinases (Table 2) comparable to the natural product LL-Z1640-2 (**1**). These strongly inhibited kinases mainly fall into two families, viz TK (tyrosine kinase) and STE (homologs of yeast Sterile 7, Sterile 11, Sterile 20 kinases) and all belong to a kinase group that contain a cysteine residue at the ATP binding site.[21] Importantly, most of these kinases are validated targets for cancer drug development.[36-37] The binding constants (K_d) for these kinase (Table 2) showed that the analogue **5** is a potent inhibitor against these important kinases at nanomolar range (except for TGFBR2, entry 12) and is specially potent and selective for MEK kinases (MEK1, 2 and 5, entries 5-7) which are validated cancer targets.[38-39] These results indicate the *exo*-enone **5** is a promising compound for further development as a protein kinase inhibitor. Additionally, it appeared that the change of ring size from a 14-membered (**1**) to 13-membered (**5**) macrocycle does not have significant effects on activities. However, further work would be required to ascertain if this new compound indeed covalently binds to the cysteine residue at the ATP binding site.

Table 2. % Inhibition of selected kinases by compound **1** and **5** and binding constants (K_d) of **5**

Entry	Kinase	% Control by **1**[a,c]	% Control by **5**[b,c]	K_d (nM) of **5**[d]
1	FLT1 (VEGFR1)	0.5	1.8	340
2	FLT3	0.6	1.6	290
3	FLT4	0.3	0.5	300
4	KIT	0.6	0.4	590
5	MEK1 (MAP2K1)	0.1	0.65	12
6	MEK2 (MAP2K2)	0.1	1.3	25
7	MEK5 (MAP2K5)	0.1	0.8	5.7
8	MKNK2 (MNK2)	11.0	1.0	600
9	PDGFRA	1.4	0.45	220
10	PDGFRB	0.1	0	180
11	TGFBR2	0.9	3.6	2100
12	VEGFR2 (KDR)	5.2	4.6	520

[a] Data taken from Library of Integrated Network-based Cellular Signatures, Harvard Medical School.[40]

[b] Full screening results see Supplementary Information.

[c] Determined at 10 μM with DMSO as a negative control. A lower value indicates a stronger inhibition.

[d] K_ds are means of duplicate measurements of an 11-point 3-fold serial dilution with an initial concentration of **5** at 30,000 nM.

Conclusions

In conclusion, a novel *exo*-enone analogue of LL-Z1640-2 has been synthesised in a concise manner (10 steps from 2-deoxy D-ribose acetonide **11**) utilising a $NiCl_2$ mediated intramolecular reductive coupling macrocyclisation of an alkyne-aldehyde intermediate as the key step. The synthetic route is significantly shorter than those for the natural product and avoids the isomerisation problem of the *cis*-double bond in the molecule. The *exo*-enone analogue has been shown to be a highly potent inhibitor for several important kinases relevant to cancer targets and has the potential for further development.

Experimental

General: ^{1}H NMR and ^{13}C NMR spectra were recorded on an Ultra Shield Avance 400 plus spectrometer in deuterated chloroform unless otherwise stated with undeuterated chloroform residue as the reference. High resolution mass spectra were obtained by the electrospray ionization time-of-flight (ESI-TOF) mode on an Agilent 6210 mass spectrometer. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer using evaporated films. Optical rotations were measured using a JASCO P-1030 polarimeter. All air and/or moisture sensitive reactions were carried out under argon atmosphere in oven-dried glassware. Flash chromatography was performed with 40-63 μm silica gel (Merck).

5-{(*E*)-3-[(4*S*,5*R*)-5-{[(t-Butyldimethylsilyl)oxy]methyl}-2,2-dimethyl-1,3-dioxolan-4-yl]prop-1-en-1-yl}-7-methoxy-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (13). To a solution of the alcohol (**12**, prepared according to previously reported procedures,[29] 798 mg, 2.11 mmol) in anhydrous dichloromethane (20 mL) were added imidazole (228 mg, 3.35 mmol) followed by *t*-butyldimethylsilyl chloride (380 mg, 2.52 mmol). The reaction mixture was stirred at room temperature for 70 min. prior to the addition of DCM (30 mL) and saturated NH_4Cl solution (10 mL). The organic phase was separated and the aqueous phase was extracted with DCM (10 mL × 2). The combined organic layer was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel by gradient elution with EtOAc in petroleum ether to give compound **13** (1.04 g, quantitative) as a colourless oil; R_f = 0.47 (EtOAc : petroleum ether = 1:2); $[\alpha]_D^{20}$ = –33.6 (*c* = 1.1, $CHCl_3$); IR (film): ν_{max} 3308, 2958, 1739, 1649, 1609, 1574, 1380, 1318, 1256, 1216, 1160 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.58 (d, *J* = 15.8 Hz, 1H), 6.79 (d, *J* = 2.5 Hz, 1H), 6.34 (d, *J* = 2.7 Hz, 1H), 6.30 – 6.25 (m, 1H), 4.30 (m, 1H), 4.16 (m, 1H), 3.84 (s, 3H), 3.73 – 3.68 (m, 2H), 2.61 – 2.56 (m, 2H), 1.69 (s, 6H), 1.46 (s, 3H), 1.35 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.9, 160.4, 158.8, 143.9, 131.5, 130.3, 108.4, 108.2, 103.9, 100.4, 78.0, 77.4, 77.3, 62.1, 55.8, 33.3, 28.3, 26.1, 25.8, 25.7, 18.4, -5.21, -5.26; HRMS (ESI-TOF): *m/z* calcd. for $C_{26}H_{41}O_7Si^+$ $[M+H]^+$ 493.2616; found 493.2617.

(*S*)-Pent-4-yn-2-yl 2-{(*E*)-3-[(4*R*,5*R*)-5-{[(tert-butyldimethylsilyl)oxy]methyl}-2,2-dimethyl-1,3-dioxolan-4-yl]prop-1-en-1-yl}-6-hydroxy-4-methoxybenzoate (14). To a stirred, ice-cooled suspension of sodium hydride (60% in mineral oil , 270 mg, 6.75 mmol) in THF (15 mL) was added dropwise a solution of (*S*)-pent-4-yn-2-ol (9) (234 mg, 2.78 mmol) in THF (3 mL) via a cannula at 0 °C. The reaction mixture was stirred at 0 °C for 15 min before a solution of **13** (1.04 g, 2.11 mmol) in THF (5 mL) was added dropwise via a cannula. The reaction mixture was stirred at 0 °C for 60 min. before water (3 mL) was added dropwise to quench the reaction. The mixture was then diluted with ethyl acetate (25 mL) and adjusted to pH 6 with 1.0 M HCl solution. The phases were separated and the aqueous phase was extracted with diethyl ether (25 mL × 2). The combined organic phase was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel by gradient elution with ethyl acetate in petroleum ether to give compound **14** (1.07g, 98%) as a colourless oil; R_f = 0.51 (EtOAc : petroleum ether = 1:4); $[\alpha]_D^{20}$ = – 32.0 (*c* = 0.20, $CHCl_3$); IR (film):

ν_{max} 3314, 2954, 2929, 2857, 1652, 1610, 1574, 1463, 1318, 1256, 1215, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 11.62 (s, 1H), 7.13 (d, *J* = 15.5 Hz, 1H), 6.48 (d, *J* = 2.7 Hz, 1H), 6.38 (d, *J* = 2.6 Hz, 1H), 5.97 (m, 1H), 5.28 (m, 1H), 4.39 – 4.20 (m, 1H), 4.20 – 4.06 (m, 1H), 3.82 (s, 3H), 3.73 (m, 1H), 3.64 (dd, *J* = 10.4, 4.7 Hz, 1H), 2.62 (dd, *J* = 5.6, 2.7 Hz, 2H), 2.59 – 2.45 (m, 2H), 2.08 (t, *J* = 2.7 Hz, 1H), 1.47 (d, *J* = 6.3 Hz, 3H), 1.44 (s, 3H), 1.34 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 165.2, 164.2, 143.6, 133.4, 129.0, 108.6, 108.1, 103.9, 99.9, 79.8, 77.9, 77.4, 71.3, 70.3, 62.1, 55.6, 33.2, 29.9, 28.3 26.0, 25.7, 25.7, 25.6, 19.3, 18.4, -5.22, -5.28; HRMS (ESI-TOF): *m/z* calcd. for $C_{28}H_{42}NaO_7Si^+$ $[M+Na]^+$ 541.2592; found 541.2590.

(*S*)-Pent-4-yn-2-yl 2-[(*E*)-3-[(4*R*,5*R*)-5-[(tert-butyldimethylsilyloxy)methyl]-2,2-dimethyl-1,3-dioxolan-4-yl]prop-1-enyl]-4-methoxy-6-(methoxymethoxy)benzoate (15). To a stirred, ice-cooled suspension of sodium hydride (60% in mineral oil, 250 mg, 6.25 mmol) in THF (14 mL) and DMF (5 mL) was added dropwise the phenol **14** (1.07 g, 2.06 mmol) in THF (3 mL) via a cannula. The mixture was stirred at 0 °C for 15 min before methoxymethyl chloride (0.19 mL, 2.50 mmol) was added. The reaction was stirred at 0 °C for 40 min after which time water (10 mL) was added carefully dropwise. The mixture was diluted with diethyl ether (20 mL) and the phases were separated. The aqueous phase was extracted with diethyl ether (20 mL × 2). The combined organic phases were washed with brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel by gradient elution with ethyl acetate in petroleum ether to give compound **15** (1.06 g, 92%) as a colourless oil; R_f = 0.48 (EtOAc : petroleum ether = 1 : 5); $[\alpha]_D^{22}$ =– 25.0 (*c* = 0.80; $CHCl_3$); IR (film): ν_{max} 2955, 2928, 2856, 1730, 1602, 1579, 1464, 1260, 1215, 1155, 1102, 1049 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.69 (d, *J* = 2.2 Hz, 1H), 6.59 (d, *J* = 2.2 Hz, 1H), 6.52 (d, *J* = 15.7 Hz, 1H), 6.27 (m, 1H), 5.28 (m, 1H), 5.15 (s, 2H), 4.23 (m, 1H), 4.12 (m, 1H), 3.81 (s, 3H), 3.75 – 3.59 (m, 2H), 3.46 (s, 3H), 2.69 – 2.40 (m, 4H), 2.04 (t, *J* = 2.7 Hz, 1H), 1.59 (br, s, 1H), 1.44 (d, *J* = 6.3 Hz, 3H), 1.43 (s, 3H), 1.34 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 189.0, 167.3, 161.3, 155.6, 137.5, 130.6, 128.6, 116.7, 110.5, 108.2, 103.6, 100.8, 94.8, 80.0, 77.8, 77.4, 76.7, 70.8, 69.6, 61.9, 56.3, 55.6, 33.4, 28.3, 26.0, 25.7, 25.6, 19.2, 18.4, -5.22, -5.29; HRMS (ESI-TOF): *m/z* calcd. for $C_{30}H_{47}O_8Si^+$ $[M + H]^+$ 563.3035; found 563.3031.

(*S*)-Pent-4-yn-2-yl 2-[(*E*)-3-[(4*R*,5*R*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]prop-1-enyl]-4-methoxy-6-(methoxymethoxy)benzoate (16). To a stirred solution of **15** (1.03 g, 1.83 mmol) in THF (17 mL) was added tetrabutylammonium fluoride (3.65 mL of 1.0 M solution in THF, 3.65 mmol) at room temperature. The reaction was stirred at room temperature for 1 h before ethyl acetate (40 mL) and saturated ammonium chloride solution (15 mL) were added. The phases were separated and the aqueous phase was extracted with ethyl acetate (15 mL × 2). The combined organic phase was washed with brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel by gradient elution with ethyl acetate in petroleum ether to give the alcohol (**16**) (0.70 g, 85%) as a colourless oil; R_f = 0.39 (EtOAc : petroleum ether = 1 : 1); $[\alpha]_D^{22}$ = – 21.9 (*c* = 1.1, $CHCl_3$); IR (film): ν_{max} 3297, 2987, 1719, 1602, 1578, 1155, 1102, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.67 (d, *J* = 2.2 Hz, 1H), 6.60 (d, *J* = 2.2 Hz, 1H), 6.55 (d, *J* = 15.7 Hz, 1H), 6.19 (m, 1H), 5.36 – 5.22 (m, 1H), 5.15 (s, 2H), 4.29 (m, 1H), 4.20 (m, 1H), 3.80 (s, 3H), 3.68 (t, *J* = 5.8 Hz, 2H), 3.46 (s, 3H), 2.68 – 2.37 (m, 4H), 2.05 (t, *J* = 2.7 Hz, 1H), 1.87 (t, *J* = 6.0 Hz, 1H), 1.49 (s, 3H), 1.45 (d, *J* = 6.3 Hz, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = , 189.0, 167.3, 161.4, 155.7, 137.4, 129.5, 129.2, 116.6, 108.5, 103.8, 100.8, 94.8, 80.1, 77.9, 77.4, 76.5, 70.8, 69.7, 61.8, 56.3, 55.7, 33.3, 28.2, 25.7, 25.6, 19.3; HRMS (ESI-TOF): *m/z* calcd. for $C_{24}H_{33}O_8^+$ $[M + H]^+$ 449.2170; found 449.2156.

(*S*)-Pent-4-yn-2-yl 2-[(*E*)-3-[(4*R*,5*S*)-5-formyl-2,2-dimethyl-1,3-dioxolan-4-yl]prop-1-en-1-yl]-4-methoxy-6-(methoxymethoxy)benzoate (17). To a solution of **16** (100 mg, 0.22 mmol) in acetone (4 mL) was added Dess-Martin periodinane (190 mg, 0.45 mmol) and the white suspension was stirred at room temperature for 5 h. The reaction mixture was filtered through a silica gel pad, washed with acetone. Saturated $Na_2S_2O_3$ solution (3 mL) was added to the filtrate and the phases were separated. The aqueous phase was extracted with ethyl acetate (8 mL × 2). The combined organic phase was washed with saturated $NaHCO_3$ solution (5 mL). The organic phase was then dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel by gradient elution with ethyl acetate in petroleum ether provided the aldehyde **17** (74 mg, 75%) as a colourless oil. R_f = 0.54 (EtOAc : petroleum ether = 2 : 1); $[\alpha]_D^{22}$ = – 17.5 (*c* = 0.80, $CHCl_3$); IR (film): ν_{max} 2936, 1726, 1602, 1265, 1155, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 9.69 (d, *J* = 3.0, 1H), 6.66 (d, *J* = 2.2 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 6.53 (d, *J* = 15.7 Hz, 1H), 6.16 (m, 1H), 5.34 – 5.21 (m, 1H), 5.15 (s, 2H), 4.49 – 4.37 (m, 1H), 4.37 – 4.26 (m, 1H,), 3.81 (s, 3H), 3.46 (s, 3H), 2.65 – 2.33 (m, 4H), 2.03 (t, *J* = 2.7 Hz, 1H), 1.561 (s, 3H), 1.46 (d, *J* = 6.3 Hz, 3H), 1.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 202.3, 161.5, 155.7, 137.2, 130.1, 128.4, 110.9, 103.9, 100.9, 94.8, 82.0, 78.3, 77.4, 70.8, 69.7, 56.3, 55.7, 33.7, 27.6, 25.7, 25.4, 19.3; HRMS (ESI-TOF): *m/z* calcd. for $C_{24}H_{31}O_8^+$ $[M + H]^+$ 447.2013; found 447.2024.

(3a*R*,7*S*,16a*R*,*E*)-4-Hydroxy-12-methoxy-10-(methoxymethoxy)-2,2,7-trimethyl-5-methylene-4,5,6,7,16,16*a*-hexahydrobenzo[*c*][1,3]dioxolo[4,5-*h*][1]oxacyclotridecin-9(3*aH*)-one (18). To a reaction mixture of **17** (87 mg, 0.20 mmol), triphenylphosphine (26 mg, 0.10 mmol), $CrCl_2$ (120 mg, 0.98 mmol) and $NiCl_2$ (5.3 mg, 0.040 mmol) in DMF (13 mL) was added H_2O (1.2 mL of a 1.0 M in DMF, 6.0 mmol). The reaction mixture was stirred at room temperature for 22 h. Brine (6 mL) was subsequently added and the reaction mixture was extracted with diethyl ether (20 mL × 3). The combined organic phase was brine, dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel by gradient elution with ethyl acetate in petroleum ether provided the alcohol **18** (51 mg, 57 %) as colourless oil. R_f = 0.58 (EtOAc : petroleum ether = 2:1); $[\alpha]_D^{20}$ = + 40.2 (*c* = 1.2, $CHCl_3$); IR (film): ν_{max} 2958, 2924, 2854, 1718, 1601, 1577, 1456, 1377, 1263, 1212, 1156, 1100, 1047 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.63 (d, *J* = 2.3 Hz, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 6.20 – 6.06 (m, 2H), 5.40 – 5.31 (m, 2H), 5.19 (d, *J* = 6.8 Hz, 1H), 5.15 (d, *J* = 6.8 Hz, 1H), 5.06 (s, 1H), 4.65 – 4.58 (m, 1H), 4.54 (d, *J* = 9.4 Hz, 1H), 4.47 (dd, *J* = 7.1, 1.2 Hz, 1H), 3.81 (s, 3H), 3.46 (s, 3H), 3.10 (m, 1H)), 2.86 (dd, *J* = 15.3, 2.0 Hz, 1H), 2.75 (m, 1H), 2.51 (dd, *J* = 15.4, 5.5 Hz, 1H), 2.41 (d, *J* = 9.5 Hz, 1H), 1.55 (s, 3H), 1.41 (s, 3H), 1.23 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.4, 161.6, 155.8, 144.7, 138.5, 130.8, 129.0, 116.0, 107.6, 105.0, 101.2, 95.1, 76.1, 75.5, 72.0, 69.7, 56.3, 55.6, 39.4, 32.8, 26.7, 23.9, 19.0; HRMS (ESI-TOF): *m/z* calcd. for $C_{24}H_{33}O_8^+$ $[M + H]^+$ 449.2170; found 449.2160.

(3*S*)-6-Hydroxy-12-methoxy-14-(methoxymethoxy)-3-methyl-5-methylene-3,4,5,6,7, 8,9,10-octahydro-1*H*-benzo[*c*][1]oxacyclododecin-1-one (20). Followed the procedure

for **18**, alkynal **19** (78.3 mg, 0.22 mmol) provided alcohol **20** (30.8 mg, 38 %) as a colourless oil. R_f = 0.56 (EtOAc : petroleum ether = 2:1); IR (film): ν_{max} 3456, 3016, 3005, 2971, 2945, 1739, 1728, 1606, 1441, 1366, 1355, 1229, 1217 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.52 (d, *J* = 2.2 Hz, 1H), 6.38 (d, *J* = 2.2 Hz, 1H), 5.31 (m, 1H), 5.16 (d, *J* = 6.7 Hz, 1H), 5.12 (d, *J* = 6.7 Hz, 1H), 5.03 (s, 1H), 4.90 (s, 1H), 4.23 (t, *J* = 6.0 Hz, 1H), 3.78 (s, 3H), 3.47 (d, *J* = 5.3 Hz, 3H), 2.82 (d, *J* = 14.9 Hz, 1H), 2.73 (m, 1H), 2.57 – 2.46 (m, 1H), 2.36 (dd, *J* = 14.9, 8.6 Hz, 1H), 1.82 – 1.60 (m, 3H), 1.34 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.5, 161.2, 155.1, 148.0, 142.3, 119.2, 113.8, 107.7, 99.3, 94.8, 74.6, 71.3, 56.3, 55.5, 41.0, 34.0, 29.8, 26.7, 23.1, 20.0; HRMS (ESI-TOF): *m/z* calcd. for $C_{20}H_{28}NaO_6^+$ $[M + Na]^+$ 387.1778, found: 387.1788.

(3*S*)-6-Hydroxy-13-methoxy-15-(methoxymethoxy)-3-methyl-5-methylene-4,5,6,7,8,9,10,11-octahydrobenzo[*c*][1]oxacyclotridecin-1(3*H*)-one (22). Followed the procedure for **18**, alkynal **21** (82 mg, 0.22 mmol) provided alcohol **22** (33 mg, 40%) as a colourless oil; R_f = 0.60 (EtOAc : petroleum ether = 2:1); IR (film): ν_{max} 3453, 2936, 2865, 1718, 1606, 1377, 1266, 1154, 1038 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): diastereomeric mixture (~1.7:1) δ = 6.53 (d, *J* = 2.2 Hz, 1H), 6.38 (d, *J* = 1.8 Hz, 1H), 5.68 – 5.47 (m, 1H), 5.23 – 5.10 (m, 4H), 4.20 – 4.07 (m, 1H), 3.78 (s, 3H), 3.46 (s, 3H), 2.66 – 2.40 (m, 3H), 2.29 – 2.16 (m, 1H), 1.76 – 1.23 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$): diastereomeric mixture δ = 168.2, 161.0, 155.4, 148.0, 141.9, 118.5, 114.3, 111.3, 107.4, 106.7, 99.9, 94.5, 73.0, 71.9, 56.1, 55.3, 39.3, 34.5, 32.7, 31.6, 31.1, 30.7, 29.2, 28.4, 25.8, 25.3, 22.9, 21.6, 20.9, 20.1; HRMS (ESI-TOF): *m/z* calcd. for $C_{21}H_{30}NaO_6^+$ $[M + Na]^+$ 401.1935, found: 401.1933.

7-Hydroxy-14-methoxy-16-(methoxymethoxy)-6-methylene-3,4,5,6,7,8,9,10,11,12-decahydro-1*H*-benzo[*c*][1]oxacyclotetradecin-1-one (24). Followed the procedure for **18**, alkynal **23** (129 mg, 0.34 mmol) provided alcohol **24** (46 mg, 36%) as a colourless oil; R_f = 0.38 (EtOAc : petroleum ether = 1:1); IR (film): ν_{max} 3443, 2934, 2858, 1719, 1606, 1586, 1466, 1320, 1267, 1213, 1194, 1154, 1104, 1044 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.55 (d, *J* = 2.2 Hz, 1H), 6.43 (d, *J* = 2.2 Hz, 1H), 5.15 (s, 2H), 5.13 (s, 1H), 4.89 (s, 1H), 4.56 (m, 1H), 4.25 (m, 1H), 4.13 (m, 1H), 3.79 (s, 3H), 3.47 (s, 3H), 2.61 – 2.42 (m, 2H), 2.17-1.96 (m, 2H), 2.05 – 1.96 (m, 2H), 1.74 – 1.58 (m, 3H), 1.50-1.39 (m, 3H), 1.34-1.20. (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.4, 161.4, 155.8, 142.9, 117.4, 108.0, 99.2, 99.4, 95.0, 83.2, 75.3, 69.2, 63.6, 63.1, 56.3, 55.5, 33.7, 32.8, 31.3, 29.3, 27.9, 25.6, 15.4; HRMS (ESI-TOF): *m/z* calcd. for $C_{21}H_{31}O_6^+$ $[M+H]^+$ 379.2115, found: 379.2120.

(3a*R*,16a*S*)-4-Hydroxy-12-methoxy-10-(methoxymethoxy)-2,2-dimethyl-5-methylene-4,5,6,7,14,15,16,16a-octahydrobenzo[*c*][1,3]dioxolo[4,5-*h*][1]oxacyclotridecin-9(3*aH*)-one (26). Followed the procedure for **18**, alkynal **25** (89 mg, 0.20 mmol) provided alcohol **26** (25 mg, 28%) as a colourless oil. R_f = 0.50 (EtOAc : petroleum ether = 1:1); IR (film): ν_{max} 3498, 2938, 1724, 1606, 1585, 1258, 1155 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.57 (d, *J* = 2.3 Hz, 1H), 6.36 (d, *J* = 2.2 Hz, 1H), 5.30 (s, 1H), 5.26 (s, 1H) 5.15 (s, 2H), 5.03 – 4.93 (m, 1H), 4.29 (m, 1H), 4.17 – 4.11 (m, 2H), 4.11 – 4.04 (m, 1H), 3.78 (s, 3H), 3.48 (s, 3H), 2.79 (s, 1H), 2.70 – 2.52 (m, 3H), 2.43-2.34 (m, 1H), 1.72 – 1.62 (m, 2H), 1.49 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.0, 161.4, 155.8, 143.5, 142.1, 117.8, 115.6, 108.5, 108.0, 99.7, 95.0, 78.0, 77.4, 72.7, 61.5, 56.3, 55.5, 34.6, 30.7, 28.8, 28.4, 25.8; HRMS (ESI-TOF): *m/z* calcd. for $C_{23}H_{32}NaO_8^+$ $[M + Na]^+$ 459.1989, found: 459.1996.

(3a*S*,7*S*,16a*R*,*E*)-12-Methoxy-10-(methoxymethoxy)-2,2,7-trimethyl-5-methylene-6,7,16,16a-tetrahydrobenzo[*c*][1,3]dioxolo[4,5-*h*][1]oxacyclotridecine-4,9(3*aH*,5*H*)-dione (27). To a solution of **17** (34 mg, 0.075 mmol) in DCM (5 mL) was added Dess-Martin periodinane (96 mg, 0.23 mmol). The white suspension was stirred at room temperature for 5 h. The reaction mixture was filtered through a silica gel pad, washed with acetone. Subsequently, saturated $Na_2S_2O_3$ solution (3 mL) was added to the filtrate and the phases were separated. The aqueous phase was extracted with ethyl acetate (8 mL × 2). The combined organic phase was washed with saturated $NaHCO_3$ solution. The organic phase was then dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the crude product by flash column chromatography on silica gel by gradient elution with ethyl acetate in petroleum ether afforded the protected enone (**27**) (22 mg, 65%) as a colourless oil. R_f = 0.30 (EtOAc : petroleum ether = 1:1); $[\alpha]_D^{23}$ = –11.9 (*c* = 0.90, $CHCl_3$); IR (film): ν_{max} 2962, 2924, 2850, 1723, 1689, 1600, 1262, 1214, 1156, 1105, 1048 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.60 (d, *J* = 2.3 Hz, 1H), 6.40 (d, *J* = 2.2 Hz, 1H), 6.38 – 6.26 (m, 2H), 6.22 (d, *J* = 1.4 Hz), 5.77 (m, 1H), 5.47 (m, 1H), 5.25 – 5.10 (m, 3H), 4.61 (m, 1H), 3.78 (s, 3H), 3.47 (s, 3H), 3.02 – 2.84 (m, 1H), 2.76 – 2.61 (m, 1H), 2.61 – 2.49 (m, 1H), 2.40 – 2.30 (m, 1H), 1.61 (s, 3H), 1.43 (*J* = 6.3 Hz, 3H), 1.40 (s, 3H) ; ^{13}C NMR (100 MHz, CD_2Cl_2): δ = 196.7, 167.1, 161.6, 155.7, 144.9, 138.5, 131.3, 128.5, 116.7, 109.6, 105.8, 101.1, 95.3, 77.2, 71.8, 56.5, 55.8, 34.8, 30.0, 27.0, 25.3, 21.2; HRMS (ESI-TOF): *m/z* calcd. for $C_{24}H_{31}O_8^+$ $[M + H]^+$ 447.2013; found 447.2010.

(3*S*,7*S*,8*R*,*E*)-7,8,15-Trihydroxy-13-methoxy-3-methyl-5-methylene-4,5,8,9-tetrahydrobenzo[*c*][1]oxacyclotridecine-1,6(3*H*,7*H*)-dione (5). To a solution of **27** (21 mg, 0.047 mmol) in MeOH (4.0 mL) was added HCl (0.60 mL of a 2.0 M aqueous solution, 1.2 mmol). The reaction mixture was stirred at room temperature for 3.5 days. Saturated $NaHCO_3$ solution was then added until pH 7 was attained. The reaction mixture was concentrated under reduced pressure and further extracted with ethyl acetate (5 mL × 3). The combined organic phase was washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The crude produce was purified by semi-preparatory HPLC (C18, 5 μM, 100 × 10 mm; gradient from 20% – 80% acetonitrile in water; flow rate 5mL/min, R_t = 16 min) to give *exo*-enone (**5**) (10.4 mg, 61%) as a white solid. R_f = 0.26 (EtOAc : petroleum ether = 1:1); $[\alpha]_D^{22}$ = –107.6 (*c* = 0.80, $CHCl_3$); IR (film): ν_{max} 3454, 2978, 1647, 1608, 1255, 1160 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 11.78 (s, 1H), 6.75 (dd, *J* = 15.4, 1.5 Hz, 1H), 6.39 (d, *J* = 2.6 Hz, 1H), 6.33 (s, 1H), 6.30 (dd, *J* = 2.7, 0.6 Hz, 1H), 6.01 (d, *J* = 2.2 Hz, 1H), 5.67 (m, 1H), 5.29 (m, 1H), 4.71 (dd, *J* = 8.6, 3.4 Hz, 1H), 4.03 (m, 1H), 3.80 (s, 3H), 3.67 (br, 1H), 3.06 (dd, *J* =15.6, 12 Hz, 1H), 2.58 – 2.11 (m, 1H), 2.47 – 2.14 (m, 1H), 1.51 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 202.6, 171.5, 165.5, 164.3, 143.5, 143.2, 134.0, 128.3, 127.6, 109.2, 103.8, 100.3, 73.8, 73.1, 72.3, 55.6, 38.0, 33.9, 20.8; HRMS (ESI-TOF): *m/z* calcd. for $C_{19}H_{23}O_7^+$ $[M + H]^+$ 363.1438, found: 363.1433.

Kinase profiling: Kinase profiling of *exo*-enone **5** was carried out against a panel of 62 selected kinases at 10 μM using DMSO as a negative control. The experiment was conducted by DiscoveRX® using the KINOMEscan® technology.[41]

Kd determination: Binding constants (K_d) of *exo*-enone **5** against 12 screened kinases with <5% control were determined by DiscoveRX® using the company's technology.[41]

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