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Synthesis, kinase activity and molecular modeling of a resorcylic acid lactone incorporating an amide and a *trans*-enone in the macrocycle

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ABSTRACT

Details for the synthesis of a resorcylic acid lactone (RAL) incorporating a *trans*-enone and an amide in the macrocyclic ring are provided herein. The sequence included the assembly of three fragments by esterification, olefination, and lactamization. The RAL with the lactam was less potent as an inhibitor of kinases than other RALs investigated. The biological results were rationalized by docking and molecular dynamics simulations of the lactam bound to human ERK2 and comparison with hypothemycin.

1. Introduction

Kinase inhibition represents one of the most powerful approaches for the treatment of diseases related to intra-cellular signaling and cell cycle regulation.[1] Progress has been made and nine small-molecule inhibitors have been approved by the FDA over the last decade.[1b,2] However, the field still faces challenges. Kinase inhibitors often show off-target activity as well as activity against the primary intended target.[3] Knowledge of the kinase selectivity profile of small-molecule inhibitors and understanding the reasons for activity differences can facilitate the design of more optimally targeted inhibitors.

The resorcylic acid lactone (RAL) based polyketides hypothemycin,[4] LL-Z1640-2,[5] and L-783,277[6] (Fig. 1) are a structurally unique group of kinase inhibitors, with a built-in selectivity filter. The selective and powerful inhibition involves the Michael addition reaction of a protein thiol on to a *cis*-enone moiety of the macrolactone.[4] The covalent inactivation of the kinase is largely confined to ~10% of the kinome and specifically to kinases which bear a conserved cysteine residue. This corresponds to Cys166 in ERK2[4] and similarly conserved cysteine residues in other kinases.

Structure–activity relationship (SAR) studies based on hypothemycin and LL-Z1640-2 have included modifications of both the aliphatic and the aromatic portion of these natural products.[7] Recent research has demonstrated the importance of the hydroxyl group at the C-4′ position in order for there to be potent kinase inhibitory activity.[7a] There has also been the discovery of metabolically stabilized analogues with both in vitro and in vivo activities.[7c,8] As part of investigation of structure–activity relationships for the RALs,[9,10] we were interested in synthesis of the lactam **1**, where the hydroxyethylene group, which includes the 4′-hydroxy group, is replaced with an amide (Fig. 1). Although **1** contains a *trans*-enone, we believed based on precedent[10] that it could participate in a Michael addition reaction with conserved cysteines of certain kinases. The presence of the amide in the benzomacrolactone scaffold would possibly maintain the potential for hydrogen

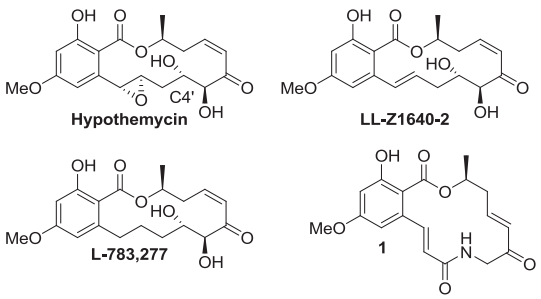

Fig. 1. Selected examples of natural RALs and **1**.

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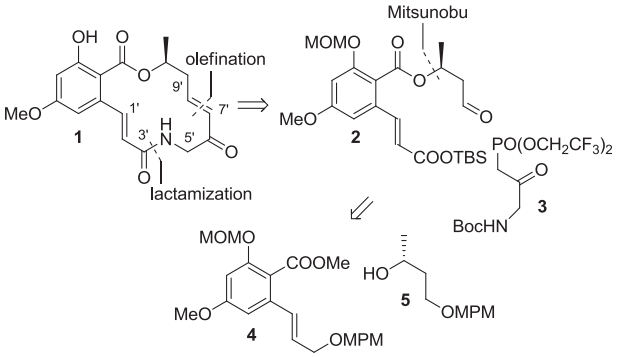
bonding with the backbone of the kinase and potentially mimic a hydrogen bonding interaction involving the 4′-OH of natural RALs. A synthetic route to **1** is described herein, which enabled the evaluation of its affinity as an inhibitor of kinases to be compared with natural RALs.

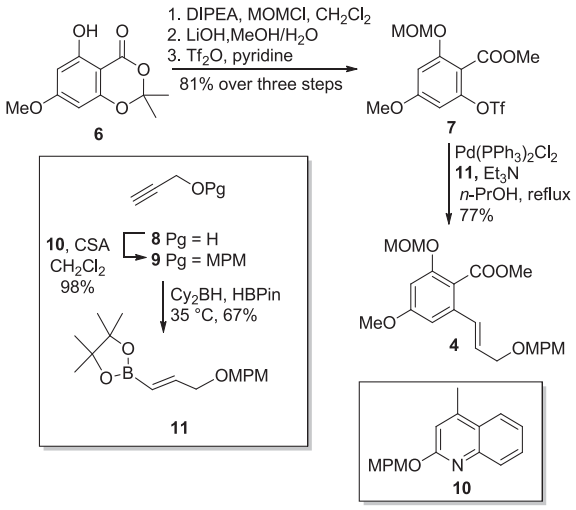
2. Synthesis

The synthetic route is summarized in Scheme 1 from the aldehyde **2**, which gives **1** after intermolecular olefination with the phosphonate **3** and subsequent lactam formation. The functionalized ester **2** was obtained from **4** and **5** via Mitsunobu esterification.[11]



Scheme 1. Retrosynthetic analysis.

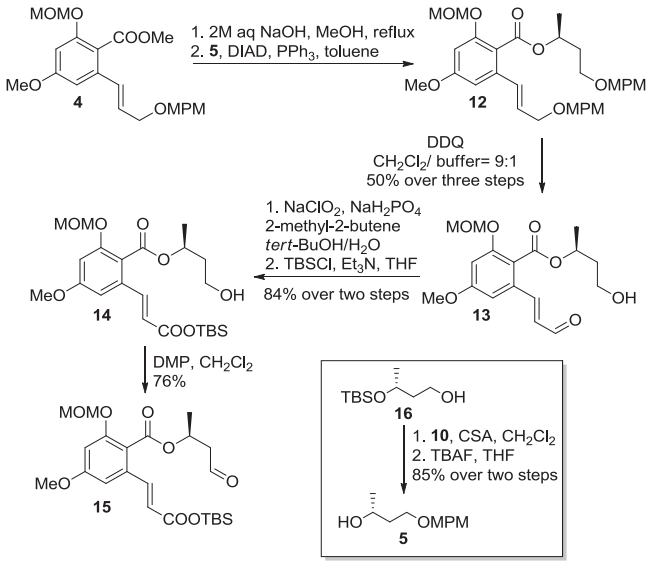
The synthesis of the aromatic fragment **4** is summarized in Scheme 2. Phenol **6** was prepared in two steps starting from 2,4,6-trihydroxybenzoic acid according to Sugawara and co-workers.[12] Next the conversion of **6** into the MOM ether followed by transesterification (LiOH, MeOH/H_2O) and then reaction with triflic anhydride in the presence of pyridine provided **7** (81% over three steps). The Suzuki–Miyaura coupling of the triflate **7** with boronate **11** was then considered. Propargyl alcohol **8** was converted into its MPM ether in excellent yield (>97%) using the Dudley reagent [2-(4-methoxybenzyloxy)-4-methylquinoline, **10**][13] under the Paquette conditions (CSA, CH_2Cl_2, room temperature).[14] Subsequent hydroboration of **9** with 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (pinacol borane) in the presence of a catalytic amount of dicyclohexylborane at 35 °C afforded **11** in satisfactory yield (67%). Subsequently the Suzuki–Miyaura-type coupling of **7**



Scheme 2. Synthesis of the aromatic fragment **4**.

with boronate **11** catalyzed by $Pd(PPh_3)_2Cl_2$ efficiently proceeded to give the ester **4** (77%). Besides **4**, a small amount (8%) of the allylic alcohol derived from MPM protecting group removal was isolated during this reaction.

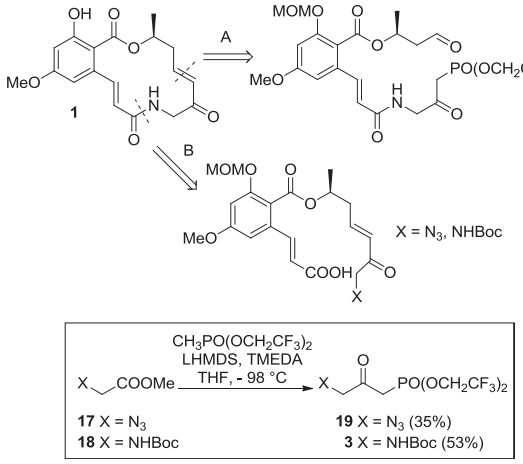
Hydrolysis of the ester **4** was followed by the Mitsunobu reaction of the resulting acid with alcohol **5** to give the MPM-diether **12** (Scheme 3). A previous process for the preparation of **5** had a relatively modest 68% yield.[9] Hence a two-step conversion of **16** into alcohol **5** was evaluated. Conversion of the alcohol **16** into its MPM ether (**10**, CSA) followed by removal of the TBS group using TBAF afforded **5** in 85% overall yield. 4-Methoxyphenylmethyl protecting group removal was next attempted by treating **12** with DDQ (2.5 equiv) in CH_2Cl_2/buffer of pH 7.6 (9:1). These conditions led to the formation of the aldehyde **13** in 50% overall yield starting from **4**. Oxidation of aldehyde **13** under Pinnick[15] conditions was next carried out and followed by chemoselective protection (TBSCl, Et_3N) of the resulting acid to smoothly afford **14** (84% over two steps). Subsequent treatment of **14** with the Dess–Martin periodinane[16] in anhydrous CH_2Cl_2 yielded aldehyde **15**.



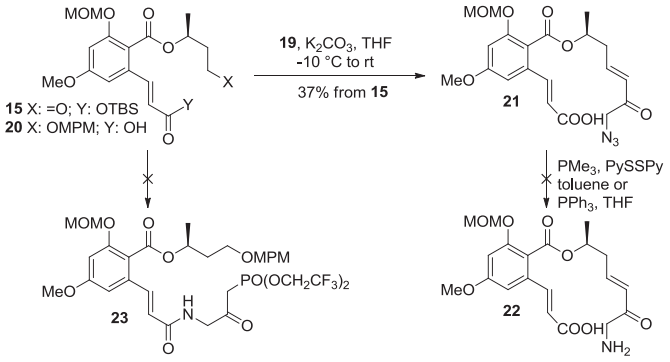
Scheme 3. Synthesis of aldehyde **15**.

With **15** in hand the formation of the macrocycle of **1** could conceptually be achieved in either of two ways (Scheme 4). The first (path A) involves amide formation followed by olefination; the second (path B) involves olefination followed by lactamization. Phosphonates **19** and **3** were both prepared with a view to establishing their usefulness for the formation of the macrocycle of **1**. The reaction of the enolate obtained from reaction of bis(2,2,2-trifluoroethyl)-methylphosphonate with LHMDS at −98 °C with esters **17**[17] and **18** in presence of TMEDA afforded **19** and **3**, respectively, in moderate yields (Scheme 4).

Treatment of the aldehyde **15** with the enolate of **19** (Scheme 5) afforded **21** as the *E*-isomer in modest yield (37%). However, the enone **21** could not be converted into **1**. Staudinger reduction of **21** using polymer-supported triphenylphoshine led to degradation of the starting material.[18] Conditions recently reported by Vilarrasa and co-workers, using PySSPy as a promoter for direct amide bond formation from carboxylic acids and azides, failed and did not lead to a macrocycle or **22**.[19] The same reaction conditions were investigated for intermolecular amide bond formation from **19** and **20** to give **23** but this approach was also unsuccessful (Scheme 5).



Scheme 4. Possible approaches to **1**. Preparation of **3** and **19**.

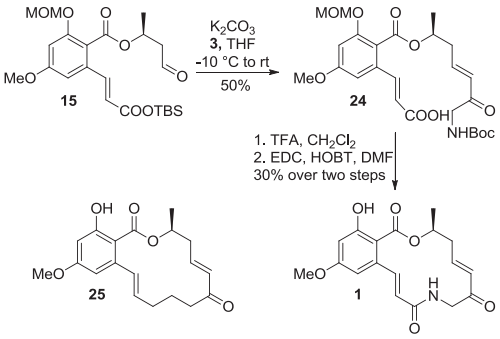


Scheme 5. Attempts to prepare **22** and **23**.

As the use of the azide **19** was unproductive, we turned our attention to the Boc protected glycine derivative **3**. Amide bond formation (path A, Scheme 4) was first investigated. Reaction of the acyl chloride of **20** (prepared by treating **20** with oxalyl chloride in DMF/CH_2Cl_2) with in situ generated amine prepared from **3** by its reaction with NaI and DIPEA in MeOH/CH_3CN provided almost exclusively unreacted **20** together with decomposition products. A number of Boc-removal conditions (TFA/CH_2Cl_2, TBAF/THF, BBr_3/CH_2Cl_2) were screened with a view to isolate the amine from **3** but all proved unsuccessful or low yielding, leading to partial hydrolysis of the phosphonoester group and/or complex mixtures of products. The difficult removal of the Boc group from **3** and the very poor accessibility to the desired intermediate led us to exclude amide formation followed by olefination strategy to generate the macrocycle. Investigation of the intermolecular olefination reaction of the aldehyde **15** with phosphonate **3** and subsequent macrocyclisation was ultimately successful (Scheme 6). Reacting the anion of the Still–Gennari phosponate **3** (K_2CO_3, THF, −10 °C), with aldehyde **15**, led to the isolation of the *E*-enone **24** (50%); the *Z*-isomer was not observed in this reaction as might have been expected.[20] Partial removal of the MOM group from **24** was observed after chromatographic purification. The free phenol was usually present at a level ~10% with **24** after this reaction. The complete removal of the MOM and also the Boc groups from **24** was achieved using TFA. This was followed by lactamization (EDC, HOBT) to finally give the desired lactam **1** (30% from **24**).

3. Biological evaluation

With **1** in hand, it was screened against a panel of 12 kinases, all of which contained the important conserved cysteine residue and



Scheme 6. Synthesis of **1**.

which consequently have potential for covalent conjugation with RALs and the results are summarized in Table 1.[21] The IC_{50} values are compared with those for **25** and LL-Z1640-2, which showed inhibitory activity to these kinases. The lactam **1** inhibited kinases in the target subgroup and had IC_{50} values in the micromolar range. However **1** was generally significantly less potent than **25**, which has an ethylene group instead of the amide but also contains the *trans*-enone;[10] the introduction of the amide led to a loss of potency and also to a loss of selectivity within this subgroup of kinases. For example, **25** is 37 fold more active toward PDGFRα than VEGFR2, whereas **1** is ~1.6 fold less active toward PDGFRα than VEGFR2. The RALs **1** and **25** are both substantially less potent than LL-Z1640-2 in the enzymatic assays.

Table 1
Inhibition of kinases by **1** and **25**[a]

Compd	PDGFRα	VEGFR2	VEGFR3	MEK1	FLT3	c-kit
1	4.4	2.7	5.9	7.2	1.8	8.0
25	0.027	1.0	1.4	22	0.33	1.3
LL-Z1640-2	2.4×10^{-4}	4.9×10^{-4}	5×10^{-4}	0.002	0.0049	0.0096

[a] IC_{50} values (μM) from two independent experiments. No significant inhibition was observed at concentrations <100 μM for **1** for the following kinases: ERK2, ERK1, GSK3α, GSK3β, PDGFRβ, VEGFR1.

4. Molecular modeling

A molecular modeling study was carried out to investigate the binding of **1** to human ERK2 in order to try to obtain some insights into why it may not be as active as LL-Z1640-2 or its close structural analogue hypothemycin. The X-ray crystal structure of rat ERK2 bound to hypothemycin (PDB entry 3C9W)[22] formed a guiding point for the generation of a model of **1** (Fig. 1) bound with human ERK2. For docking (using MOE), a homology model of human ERK2 was generated and used throughout. Both the human and rat ERK2 enzyme structures show a high sequence similarity and the active site residues are highly conserved. The homology model was based on the X-ray structure of human ERK2 (PDB entry 2E1); the homology model was generated as the X-ray structure had a missing loop. Details for generation of this model are given in the Experimental section. Docking was then carried out and followed by molecular dynamics (MD) simulations using YASARA.

Compound **1** and hypothemycin were docked into the ATP binding site of the model of human ERK2 enzyme. The resulting docked complex showed that **1** had a completely different binding mode to ERK2 when compared to that of hypothemycin which orients in the pocket close to Met108 and with the double bond positions in close proximity to Cys166, thus enabling the formation of the covalent bond between ligand and protein. The energy minimized structure of **1** bound to the enzyme (Fig. 2) showed one

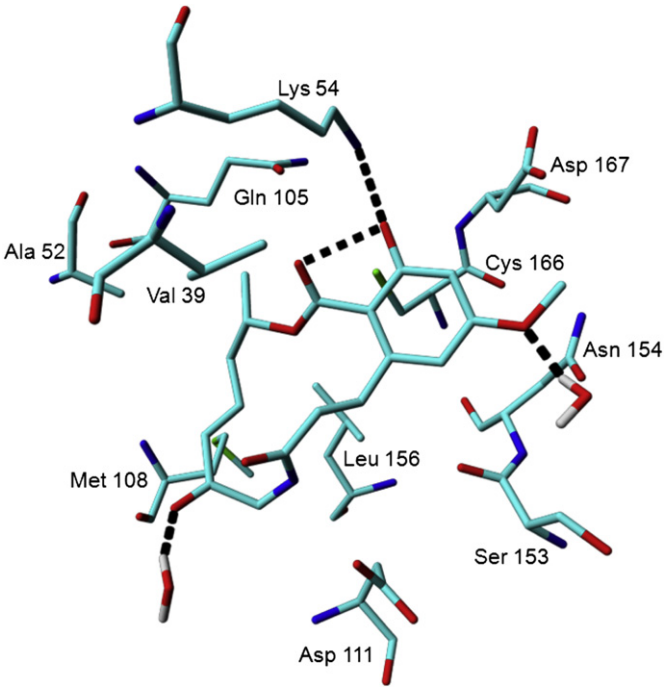


Fig. 2. Minimized structure of **1** docked into homology model of human ERK2. Hydrogen bonds are indicated with black dotted lines.

hydrogen bond between Lys54 and the phenol. Apart from the hydrogen bond with Lys54, no other hydrogen bonding was observed between **1** and ERK2. The methoxy and carbonyl groups formed hydrogen bonds with water molecules. The minimized complex of ERK2 and **1** was subjected to molecular dynamics (MD) for 6 ns. The final structure after MD did not show any additional hydrogen bonding interactions with the residues in the active site. The phenol, two carbonyl groups and the –NH group formed hydrogen bonds with water molecules. Overall, the major part of **1** is solvent exposed, which results in a much lower binding energy (52 kcal mol^{-1}) compared to hypothemycin (72 kcal mol^{-1}) for the non-covalent complexes. The distance between the reactive carbon of the enone and the sulfur atom of the cysteine residue (Cys166) for the final hypothemycin–ERK2 complex was 4.6 Å. The corresponding distance in the **1**–ERK2 complex was approximately 6 Å. This showed that hypothemycin is closer to the cysteine residue, which can thus aid in the Michael addition reaction leading to covalent bond formation to the cysteine in the active site and higher affinity for ERK2. These modeling generated observations provide an explanation for the low affinity observed for **1** for ERK2.

5. Summary and conclusions

The synthesis of a new resorcylic acid lactone lactam has been achieved providing a basis for the generation of new analogues of RALs for biological evaluation. Although **1** was not a very efficient inhibitor of kinases the study contributes to the structure–activity relationships for kinases. The modeling study provides a rationale for the lower affinity of **1**. Modeling of the *cis*-enone isomer of **1** to ERK2 (not shown herein) does indicate it should be a better inhibitor of ERK2. The synthesis of this isomer is currently being investigated. Such benzomacrolactone scaffolds are also of interest more widely for generation of compounds for screening and the synthetic work described herein provides a basis for further research in this regard.[23]

6. Experimental section

6.1. General experimental conditions

NMR spectra were recorded on 400 and 500 MHz spectrometers. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0) for ^1H and $CDCl_3$ (δ 77.0) for ^{13}C at 25 °C, unless otherwise stated. ^{13}C signals were assigned with the aid of DEPT-135, HSQC, and HMBC. ^1H signals were assigned with the aid of COSY. Coupling constants are reported in hertz. High-resolution mass spectra were measured using an ESI time-of-flight mass spectrometer and were measured in positive and/or negative mode as indicated. IR spectra were recorded on a Perkin–Elmer spectrum 1000 FT-IR spectrometer. Optical rotations were measured with a Schmidt & Haensch UniPol L1000 polarimeter. TLC were performed on aluminum sheets precoated with Silica Gel 60 (HF_{254}, E. Merck) and spots visualized by UV and charring with vanillin, molybdate, and/or ninhydrin solutions. Flash column chromatography was carried out using Silica Gel 60 (0.040–0.630 mm, E. Merck) and using a stepwise solvent polarity gradient correlated with TLC mobility. All moisture-sensitive reactions were performed under a nitrogen atmosphere unless otherwise specified. Anhydrous DMF and pyridine were used as purchased from Sigma–Aldrich. Dichloromethane and tetrahydrofuran were used as obtained from a Pure Solv™ solvent purification system. Petroleum ether is the fraction with bp 40–60 °C. Kinase assays were conducted as previously described.[24]

6.1.1. 4-Methoxy-2-methoxymethoxy-6-trifluoromethanesulfonyloxybenzoic acid methyl ester **7**. To a solution of phenol **6** (3.12 g, 13.8 mmol) and *N*,*N*-diisopropylethylamine (4.82 mL, 27.7 mmol) in CH_2Cl_2 (20 mL) was added MOMCl (1.26 mL, 16.6 mmol). The resulting mixture was stirred for 15 h at room temperature, then diluted with CH_2Cl_2, washed with 1 M aq HCl solution, dried (Na_2SO_4), and filtered. The volatile components were removed under diminished pressure. Chromatography of the residue (petroleum ether/EtOAc, 80:20–50:50) gave the MOM ether intermediate as a pale yellow oil (3.72 g, >95%); ^1H NMR (500 MHz, $CDCl_3$) δ 1.70 (s, 6H), 3.53 (s, 3H), 3.82 (s, 3H), 5.30 (s, 2H), 6.13 (d, *J* 1.5 Hz, 1H), 6.43 (d, *J* 1.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (C), 160.5 (C), 159.1 (C), 158.0 (C), 105.0 (C), 97.5 (C), 97.2 (CH), 95.0 (two signals, CH_2 and CH), 56.6 (CH_3), 55.7 (CH_3), 25.6 ($2\times CH_3$); HRMS calcd for $C_{13}H_{16}NaO_6$ $[M+Na]^+$ 291.0845, found 291.0861. Lithium hydroxide (0.670 g, 27.8 mmol) was added to a stirred suspension of this intermediate (3.72 g, 13.9 mmol) in a 2:1 mixture $MeOH/H_2O$ (36 mL). The reaction mixture was stirred for 90 min at room temperature, then acidified with 1 M aq HCl solution and extracted three times with EtOAc. The combined organic portions were dried (Na_2SO_4), filtered, and volatiles removed under diminished pressure. The methyl ester intermediate was obtained as a white solid and was used in the next step without further purification; ^1H NMR (500 MHz, $CDCl_3$) δ 3.51 (s, 3H), 3.80 (s, 3H), 3.92 (s, 3H), 5.18 (s, 2H), 6.17 (s, 2H), 11.87 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4 (C), 165.5 (C), 165.1 (C), 159.5 (C), 97.5 (C), 95.1, 95.0 (CH_2 and CH), 94.8 (CH), 56.4 (CH_3), 55.5 (CH_3), 52.2 (CH_3); HRMS calcd for $C_{11}H_{15}O_6$ $[M+H]^+$ 243.0869, found 243.0862. Triflic anhydride (3.01 mL, 17.9 mmol) was added dropwise to a stirred solution of this methyl ester in dry pyridine (20 mL). The resulting mixture was stirred at room temperature for 3 h and EtOAc was then added. The organic layer was washed twice with 1 M aq HCl solution, then dried (Na_2SO_4), filtered, and the solvent was removed under diminished pressure. Chromatography of the residue (petroleum ether/EtOAc, 85:15–60:40) afforded **7** as an off-white solid (4.20 g, 81% over two steps); ^1H NMR (500 MHz, $CDCl_3$) δ 3.50 (s, 3H), 3.83 (s, 3H), 3.91 (s, 3H), 5.21 (s, 2H), 6.49 (br s, 1H), 6.76 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.3 (C), 162.3 (C),

157.4 (C), 147.9 (C), 118.5 (q, *J* 318.5, CF_3), 110.8 (C), 101.5 (CH), 101.0 (CH), 95.2 (CH_2), 56.5 (CH_3), 55.9 (CH_3), 52.5 (CH_3); HRMS calcd for $C_{12}H_{13}F_3NaO_8S$ $[M+Na]^+$ 397.0181, found 397.0179.

6.1.2. 4-Methoxy-2-[3-(4-methoxybenzyloxy)-propenyl]-6-methoxymethoxy-benzoic acid methyl ester ***4***. A mixture of propargyl alcohol (1.16 mL, 20.0 mmol) and 2-(4-methoxybenzyloxy)-4-methylquinoline **10** (11.1 g, 39.8 mmol) was placed under an atmosphere of argon, dissolved in CH_2Cl_2 (40 mL), and treated with CSA (462 mg, 1.99 mmol) to afford a clear, bright yellow solution. The mixture was stirred for 45 h at room temperature, then volatiles were removed under diminished pressure. Chromatography of the residue (petroleum ether/EtOAc, 95:5) afforded **9** as a colorless oil (3.43 g, 98%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.45 (t, *J* 2.4 Hz, 1H), 3.80 (s, 3H), 4.14 (d, *J* 2.4 Hz, 2H), 4.54 (s, 2H), 6.88 (d, *J* 8.6 Hz, 2H), 7.28 (d, *J* 8.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.4 (C), 129.8 (2×CH), 129.3 (C), 113.8 (2×CH), 79.8 (C), 74.5 (CH), 71.1 (CH_2), 56.7 (CH_2), 55.3 (CH_3). Next the pinacol borane **11** was prepared. Dicyclohexylborane (1 M) was prepared by the addition of cyclohexene (0.200 mL, 0.002 mmol) to BH_3·THF (1 M solution, 1.00 mL, 1.00 mmol) at 0 °C and stirring for 45 min. Dicyclohexylborane (700 μL of a 1 M solution, 0.700 mmol) was then added in three portions to a mixture of **9** (1.00 g, 5.68 mmol) and pinacolborane (1.40 mL, 9.65 mmol). The reaction mixture was stirred for 24 h at 35 °C, before cooling to room temperature and bubbling air through the reaction for 2 h. Chromatography of the residue (petroleum ether/EtOAc, 90:10) gave **11** as a pale yellow oil (1.16 g, 67%); IR (neat, cm^{-1}): 3054, 2981, 2935, 2839, 1644, 1613, 1513, 1359, 1265, 1144, 1035, 849; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 (s, 12H), 3.80 (s, 3H), 4.07 (dd, *J* 1.5, 4.6 Hz, 2H), 4.46 (s, 2H), 5.71–5.77 (br d, 1H), 6.67 (dt, *J* 4.6 18.1 Hz, 1H), 6.87 (d, *J* 8.6 Hz, 2H), 7.27 (d, *J* 8.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1 (C), 149.3 (CH), 130.3 (C), 129.2 (2×CH), 119.2 (CH), 113.7 (2×CH), 83.2 (2×C), 72.0 (CH_2), 71.4 (CH_2), 55.2 (CH_3), 24.7 (4×CH_3). The pinacol borane **11** (1.15 g, 3.78 mmol), $Pd(PPh_3)_2Cl_2$ (483 mg, 0.688 mmol), triflate **7** (1.28 g, 3.44 mmol), and triethylamine (478 μL, 3.44 mmol) were added to a flask containing *n*-PrOH (30 mL) and stirred whilst heating at reflux for 4.5 h. Volatile components were evaporated under diminished pressure. The residue was partitioned between Et_2O and H_2O; the layers were separated and the organic phase was dried (Na_2SO_4), filtered, and the solvent was removed under diminished pressure. Chromatography of the residue (petroleum ether/acetone, 90:10–80:20) afforded **4** as a yellow oil (1.06 g, 77%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.47 (s, 3H), 3.81 (s, 6H), 3.88 (s, 3H), 4.14 (d, *J* 5.8 Hz, 2H), 4.48 (s, 2H), 5.16 (s, 2H), 6.26 (dt, *J* 5.8, 15.8 Hz, 1H), 6.61 (d, *J* 15.8 Hz, 1H), 6.63 (d, *J* 1.8 Hz, 1H), 6.70 (d, *J* 1.8 Hz, 1H), 6.89 (d, *J* 8.5 Hz, 2H), 7.29 (d, *J* 8.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.2 (C), 161.3 (C), 159.2 (C), 155.6 (C), 136.9 (C), 130.2 (C), 129.7 (CH), 129.4 (2×CH), 128.9 (CH), 116.7 (C), 113.8 (2×CH), 103.8 (CH), 101.3 (CH), 94.9 (CH_2), 71.8 (CH_2), 70.2 (CH_2), 56.2 (CH_3), 55.5 (CH_3), 55.3 (CH_3), 52.2 (CH_3); HRMS calcd for $C_{22}H_{26}NaO_7$ $[M+Na]^+$ 425.1576, found 425.1588.

6.1.3. (2R)-4-(4-Methoxybenzyloxy)-butan-2-ol ***5***. A mixture of alcohol **16**[9] (600 mg, 2.94 mmol) and 2-(4-methoxybenzyloxy)-4-methylquinoline **10** (1.64 g, 5.88 mmol) was placed under an atmosphere of argon, dissolved in CH_2Cl_2 (10 mL), and treated with CSA (68.2 mg, 0.294 mmol) to afford a clear, bright yellow solution. The mixture was stirred for 40 h at room temperature, then volatiles were removed under diminished pressure. Chromatography of the residue (petroleum ether/EtOAc, 98:2–95:5) afforded the MPM ether intermediate as a colorless oil (876 mg, 92%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04, 0.05 (2s, 6H), 0.88 (s, 9H), 1.13 (d, *J* 6.1 Hz, 3H), 1.66–1.75 (m, 2H), 3.45–3.56 (m, 2H), 3.80 (s, 3H), 3.93–4.01 (m, 1H), 4.38 (d, *J* 11.4 Hz, 1H), 4.44 (d, *J* 11.4 Hz, 1H), 6.87 (d, *J* 8.6 Hz, 2H), 7.25 (d, *J* 8.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1 (C), 130.7 (C), 129.3 (2×CH), 113.7 (2×CH), 72.6 (CH_2), 67.0 (CH_2), 65.7 (CH), 55.3 (CH_3), 39.6 (CH_2), 25.9 (3×CH_3), 24.1 (CH_3), 18.1 (C), −4.4 (CH_3), −4.8 (CH_3); HRMS calcd for $C_{18}H_{32}NaO_3Si$ $[M+Na]^+$ 347.2018, found 347.2023. TBAF (6.40 mL of a 1 M solution in THF, 6.40 mmol) was added to a stirred solution of TBS-ether in dry THF (15 mL). The resulting mixture was stirred at room temperature for 24 h, then EtOAc was added. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered, and the solvent evaporated under reduced pressure. Chromatography of the residue (petroleum ether/acetone, 90:10–85:15) gave **5** as a pale yellow oil (522 mg, 92%). The analytical data obtained for **5** were consistent with those reported in literature.[25]

6.1.4. (2S)-4-Hydroxybutan-2-yl 4-methoxy-2-(methoxymethoxy)-6-[(1E)-3-oxoprop-1-en-1-yl]benzoate ***13***. Aq NaOH (2 M, 15.0 mL, 30.0 mmol) was added to a stirred solution of **4** (977 mg, 2.43 mmol) in MeOH (15 mL). The resulting mixture was stirred whilst heating at reflux until the starting material was consumed. The volatile components were evaporated under reduced pressure; the aqueous layer was acidified (final pH 5) with 1 M aq HCl solution, and extracted three times with $CHCl_3$. The combined organic portions were dried (Na_2SO_4), filtered, and the solvent was removed under diminished pressure to give a yellow wax containing the acid intermediate. The crude residue was used in the next step without further purification; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.51 (s, 3H), 3.79 (s, 3H), 3.84 (s, 3H), 4.17 (br d, *J* 5.9 Hz, 2H), 4.51 (s, 2H), 5.26 (s, 2H), 6.19 (dt, *J* 5.9, 15.7 Hz, 1H), 6.69 (br s, 1H), 6.74 (br s, 1H), 6.88 (d, *J* 7.8 Hz, 2H), 7.09 (d, *J* 15.7 Hz, 1H), 7.30 (d, *J* 7.8 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.7 (C), 162.2 (C), 159.2 (C), 156.7 (C), 141.0 (C), 131.0 (CH), 130.3 (C), 129.6 (2×CH), 129.5 (CH), 113.8 (2×CH), 113.0 (C), 106.1 (CH), 101.2 (CH), 95.6 (CH_2), 71.8 (CH_2), 70.2 (CH_2), 56.7 (CH_3), 55.6 (CH_3), 55.3 (CH_3); HRMS calcd for $C_{21}H_{24}NaO_7$ $[M+Na]^+$ 411.1420, found 411.1407. Next, to a mixture of the acid in dry toluene (23 mL), triphenylphosphine (1.27 g, 4.86 mmol), alcohol **5** (510 mg, 2.43 mmol), and DIAD (956 μL, 4.86 mmol) were added sequentially. After 30 min, EtOAc and brine were added. The layers were separated and the aqueous phase was extracted with EtOAc. The combined organic portions were dried (Na_2SO_4), filtered, and the solvent was removed under diminished pressure. Chromatography of the residue (petroleum ether/acetone, 95:5 to 80:20) afforded **12** as a pale yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (d, *J* 6.3 Hz, 3H), 1.82–1.92 (m, 1H), 1.92–2.03 (m, 1H), 3.43 (s, 3H), 3.55 (t, *J* 6.5 Hz, 2H), 3.79 (s, 6H), 3.80 (s, 3H), 4.11 (br d, *J* 5.8 Hz, 2H), 4.39 (d, *J* 11.5 Hz, 1H), 4.44 (d, *J* 11.5 Hz, 1H), 4.45 (s, 2H), 5.10 (d, *J* 6.9 Hz, 1H), 5.11 (d, *J* 6.9 Hz, 1H), 5.32–5.40 (m, 1H), 6.26 (dt, *J* 5.8, 15.7 Hz, 1H), 6.59–6.68 (m, 2H), 6.69 (d, *J* 2.0 Hz, 1H), 6.86 (d, *J* 8.6 Hz, 2H), 6.87 (d, *J* 8.7 Hz, 2H), 7.23–7.29 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2 (C), 161.1 (C), 159.2 (C), 159.1 (C), 155.4 (C), 136.6 (C), 130.4 (C), 130.2 (C), 129.6 (CH), 129.4 (2×CH), 129.2 (2×CH), 128.7 (CH), 117.3 (C), 113.8 (4×CH), 103.5 (CH), 101.1 (CH), 94.6 (CH_2), 72.7 (CH_2), 71.9 (CH_2), 70.2 (CH_2), 69.5 (CH), 66.5 (CH_2), 56.1 (CH_3), 55.5 (CH_3), 55.3 (2×CH_3), 36.1 (CH_2), 20.3 (CH_3); HRMS calcd for $C_{33}H_{40}NaO_9$ $[M+Na]^+$ 603.2570, found 603.2596. Purification of **12** proved to be difficult via chromatography. An unpurified sample of **12** was thus subjected to the following deprotection step. To a stirred CH_2Cl_2/phosphate buffer (pH 7.6) (9:1, 50 mL) emulsion containing **12**, DDQ (1.35 g, 5.95 mmol) was added in one portion at room temperature. After 3 h the mixture was diluted with CH_2Cl_2 and washed three times with water; the organic layer was dried (Na_2SO_4), filtered, and the solvent was removed under diminished pressure. Chromatography of the residue (petroleum ether/acetone, 90:10–75:25) gave **13** as a pale yellow oil (411 mg, 50% over three steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42 (d, *J* 6.3 Hz, 3H), 1.81–1.99 (m, 2H), 3.49 (s, 3H), 3.72–3.83 (m, 2H), 3.85 (s, 3H), 5.19 (d, *J* 7.1 Hz, 1H), 5.21 (d, *J* 7.1 Hz, 1H), 5.40–5.49 (m, 1H), 6.65 (dd, *J* 7.6, 15.8 Hz, 1H), 6.79 (d, *J* 1.8 Hz, 1H), 6.82 (d, *J* 1.8 Hz, 1H), 7.57 (d, *J*

15.8 Hz, 1H), 9.68 (d, *J* 7.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.4 (CH), 167.0 (C), 161.6 (C), 156.0 (C), 148.9 (CH), 134.1 (C), 131.1 (CH), 118.0 (C), 104.7 (CH), 104.0 (CH), 94.9 (CH_2), 70.4 (CH), 59.0 (CH_2), 56.4 (CH_3), 55.7 (CH_3), 38.8 (CH_2), 20.5 (CH_3); HRMS calcd for $C_{17}H_{22}NaO_7$ $[M+Na]^+$ 361.1263, found 361.1267.

6.1.5. (2S)-4-Hydroxybutan-2-yl 2-[(1E)-3-[(tert-butyldimethylsilyl) oxy]-3-oxoprop-1-en-1-yl]-4-methoxy-6-(methoxymethoxy)benzoate ***14***. To a solution of aldehyde **13** (388 mg, 1.15 mmol) in *t*-BuOH (18 mL) was added 2-methyl-2-butene (1.22 mL, 11.5 mmol). A solution of $NaClO_2$ (207 mg, 2.29 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (1.17 g, 7.47 mmol) in water (18 mL) was then added and the mixture was stirred overnight at room temperature. Brine was added and the mixture was extracted three times with EtOAc. The combined organic portions were dried (Na_2SO_4), filtered, and volatiles were evaporated under diminished pressure. The acid was obtained as a yellow wax and progressed to the next step without any purification. *tert*-Butylsilyl chloride (173 mg, 1.15 mmol) was added to a solution of acid and Et_3N (160 μL, 1.15 mmol) in dry THF (20 mL). The mixture was stirred at room temperature for 4 h, then EtOAc was added. The organic portion was washed with water, dried (Na_2SO_4), filtered, and volatiles were evaporated under reduced pressure. Chromatography of the residue (petroleum ether/acetone, 90:10) afforded **14** as a pale yellow oil (450 mg, 84% over two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.32 (s, 6H), 0.98 (s, 9H), 1.40 (d, *J* 6.2 Hz, 3H), 1.77–1.87 (m, 1H), 1.88–1.98 (m, 1H), 2.60–2.69 (m, 1H), 3.48 (s, 3H), 3.67–3.83 (m, 2H), 3.84 (s, 3H), 5.17 (d, *J* 6.9 Hz, 1H), 5.20 (d, *J* 6.9 Hz, 1H), 5.38–5.47 (m, 1H), 6.35 (d, *J* 15.7 Hz, 1H), 6.76 (br s, 2H), 7.66 (d, *J* 15.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.0 (C), 166.5 (C), 161.4 (C), 155.6 (C), 141.8 (CH), 134.3 (C), 122.9 (CH), 118.5 (C), 104.3 (CH), 103.3 (CH), 94.8 (CH_2), 70.0 (CH), 58.6 (CH_2), 56.3 (CH_3), 55.6 (CH_3), 38.6 (CH_2), 25.6 (3×CH_3), 20.5 (CH_3), 17.7 (C), −4.7 (2×CH_3); HRMS calcd for $C_{23}H_{36}NaO_8Si$ $[M+Na]^+$ 491.2077, found 491.2091.

6.1.6. (2S)-4-Oxobutan-2-yl 2-[(1E)-3-[(tert-butyldimethylsilyl)oxy]-3-oxoprop-1-en-1-yl]-4-methoxy-6-(methoxymethoxy)benzoate ***15***. Dess–Martin periodinane (390 mg, 0.920 mmol) was added to **14** (214 mg, 0.457 mmol) stirred in dry CH_2Cl_2 (20 mL). The resulting mixture was stirred at room temperature for 30 min, then loaded onto a short column of silica gel and eluted with a mixture of petroleum ether/acetone (90:10) to afford **15** (162 mg, 76%) as a colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.32 (s, 6H), 0.98 (s, 9H), 1.46 (d, *J* 6.3 Hz, 3H), 2.69 (ddd, *J* 1.3, 5.3 16.9 Hz, 1H), 2.82 (ddd, *J* 2.5, 7.2, 16.9 Hz, 1H), 3.46 (s, 3H), 3.83 (s, 3H), 5.16 (br s, 2H), 5.61–5.70 (m, 1H), 6.33 (d, *J* 15.7 Hz, 1H), 6.75 (s, 2H), 7.64 (d, *J* 15.7 Hz, 1H), 9.79–9.81 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.3 (CH), 166.3 (C), 166.1 (C), 161.5 (C), 155.9 (C), 141.5 (CH), 134.6 (C), 123.2 (CH), 117.6 (C), 104.3 (CH), 103.0 (CH), 94.6 (CH_2), 67.2 (CH), 56.2 (CH_3), 55.6 (CH_3), 49.4 (CH_2), 25.6 (3×CH_3), 20.2 (CH_3), 17.7 (C), −4.8 (2×CH_3); HRMS calcd for $C_{23}H_{35}O_8Si$ $[M+H]^+$ 467.2101, found 467.2107. Product, which was slightly contaminated with by-products from the periodinane was used in the subsequent step.

6.1.7. Bis(2,2,2-trifluoroethyl) 3-azido-2-oxopropylphosphonate ***19***. LHMDS (1.95 mL of 1 M in THF, 1.95 mmol) was added dropwise to a solution of bis(2,2,2-trifluoroethyl)methylphosphonate (338 mg, 1.30 mmol) and TMEDA (295 μL, 1.95 mmol) in dry THF (3 mL), which had been pre-cooled to −98 °C. After 15 min, a solution of ester **17**[17] (50.0 mg, 0.433 mmol) in THF (1 mL) was added and the reaction mixture was stirred at −98 °C for 100 min. The reaction mixture was then warmed to room temperature, diluted with Et_2O, washed with water and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography of the residue (petroleum ether/EtOAc, 90:10–60:40) gave **19** as a yellow oil (52.1 mg, 35%); IR (neat, cm^{-1}): 2976, 2922, 2113, 1732, 1418, 1294, 1173, 1072; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.34 (d, *J* 22.1 Hz, 2H), 4.10 (s, 2H), 4.42–4.51 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.2 (d, *J* 7.0 Hz, C), 122.3 (dq, *J* 8.0, 276.0 Hz, 2×CF_3), 62.7 (dq, *J* 5.5, 37.9 Hz, 2×CH_2), 58.4 (d, *J* 5.2 Hz, CH_2), 38.8 (d, *J* 138.1 Hz, CH_2); HRMS calcd for $C_7H_7F_6N_3O_4P$ $[M-H]^-$ 342.0078, found 342.0087.

6.1.8. tert-Butyl 3-(bis(2,2,2-trifluoroethoxy)phosphoryl)-2-oxopropylcarbamate ***3***. LHMDS (3.58 mL of 1 M in THF, 3.58 mmol) was added dropwise to a solution of bis(2,2,2-trifluoroethyl)methylphosphonate (619 mg, 2.38 mmol) and TMEDA (537 μL, 3.58 mmol) in dry THF (6 mL), which had been pre-cooled to −98 °C. After 15 min, a solution of Boc-glycine methyl ester **18** (151 mg, 0.800 mmol) in THF (2 mL) was added and the reaction mixture was stirred at −98 °C for 100 min. The reaction mixture was then warmed to room temperature, diluted with Et_2O, washed with water and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography of the residue (petroleum ether/acetone, 90:10–80:20) gave **3** as a pale yellow oil (176 mg, 53%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 9H), 3.31 (d, *J* 21.9 Hz, 2H), 4.09 (d, *J* 4.6 Hz, 2H), 4.40–4.50 (m, 4H), 5.16 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.4 (d, *J* 6.6 Hz, C), 155.7 (C), 122.4 (dq, *J* 8.2, 276.0 Hz, 2×CF_3), 80.5 (C), 62.6 (dq, *J* 5.6, 38.0 Hz, 2×CH_2), 51.4 (d, *J* 6.0 Hz, CH_2), 38.6 (d, *J* 138.6 Hz, CH_2), 28.2 (3×CH_3); HRMS calcd for $C_{12}H_{17}F_6NO_6P$ $[M-H]^-$ 416.0698, found 416.0700.

6.1.9. (2E)-3-[2-({[(2S,4E)-7-{[(tert-Butoxy)carbonyl]amino}-6-oxohept-4-en-2-yl]oxy}carbonyl)-5-methoxy-3-(methoxymethoxy) phenyl]prop-2-enoic acid ***24***. Potassium carbonate (62.1 mg, 0.450 mmol) was added to a stirred solution of phosphonate **3** (125 mg, 0.300 mmol) in dry THF (15 mL), which had been pre-cooled to −10 °C. The resulting mixture was stirred at −10 °C for 20 min, then aldehyde **15** (140 mg, 0.300 mmol) dissolved in dry THF (2 mL) was added. The mixture was allowed to gradually reach room temperature and stirring was prolonged overnight. 1 M aq HCl solution was added; the layers were separated and the aqueous phase was extracted with EtOAc. The combined organic portions were dried (Na_2SO_4), filtered, and the solvent was removed under diminished pressure. Chromatography of the residue (petroleum ether/acetone, 70:30–0:100) gave **24** as a yellow wax (75.0 mg, 50%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (d, *J* 6.3 Hz, 3H), 1.47 (s, 9H), 2.59–2.65 (m, 2H), 3.47 (s, 3H), 3.84 (s, 3H), 4.29 (br d, *J* 4.4 Hz, 2H), 5.17 (d, *J* 6.9 Hz, 1H), 5.20 (d, *J* 6.9 Hz, 1H), 5.29–5.38 (m, 1H), 5.47–5.54 (m, 1H), 6.18 (d, *J* 16.1 Hz, 1H), 6.37 (d, *J* 15.7 Hz, 1H), 6.77 (s, 2H), 6.89 (dt, *J* 7.0, 16.1 Hz, 1H), 7.72 (d, *J* 15.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.9 (C), 168.4 (C), 166.3 (C), 161.5 (C), 156.5 (C), 155.9 (C), 143.4 (CH), 142.4 (CH), 134.1 (C), 130.5 (CH), 121.1 (CH), 118.1 (C), 104.1 (CH), 103.5 (CH), 94.8 (CH_2), 80.7 (C), 70.8 (CH), 56.3 (CH_3), 55.6 (CH_3), 47.9 (CH_2), 39.1 (CH_2), 28.3 (3×CH_3), 20.1 (CH_3); HRMS calcd for $C_{25}H_{32}NO_{10}$ $[M-H]^-$ 506.2026, found 506.2007.

6.1.10. (3S,5E,11E)-16-Hydroxy-14-methoxy-3-methyl-3,4,8,9-tetrahydro-1H-2,9-benzoxazacyclotetradecine-1,7,10-trione ***1***. Trifluoroacetic acid (200 μL) was added to a stirred solution of **24** (3.6 mg, 0.007 mmol) in dry CH_2Cl_2 (5 mL). The resulting mixture was stirred at room temperature for 2 h, then volatile components were evaporated under reduced pressure. The residue was dissolved in DMF (10 mL) and HOBT (2.0 mg, 0.015 mmol) and EDC (2.9 mg, 0.015 mmol) were added and the resulting mixture was stirred overnight at room temperature. EtOAc was then added, the organic solution was washed with 1 M aq HCl solution and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Chromatography of the residue (petroleum ether/acetone, 80:20–60:40) gave **1** as an amorphous white solid (0.75 mg, 30% over two steps); $[\alpha]_D^{20}$ −51.7 (*c* 0.156, $CHCl_3$); IR (neat, cm^{-1}): 3406 (br), 2925, 2853, 1645, 1258, 1162; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (d, *J* 6.3 Hz, 3H), 2.48 (dddd, *J* 1.1, 4.5, 6.6, 15.2 Hz, 1H), 2.68 (ddd, *J*

3.8, 8.2, 15.2 Hz, 1H), 3.84 (s, 3H), 4.19–4.28 (m, 2H), 5.44–5.53 (m, 1H), 6.15–6.24 (m, 1H), 6.27 (d, *J* 16.0 Hz, 1H), 6.31 (br d, *J* 16.3 Hz, 1H), 6.46 (br s, 1H), 6.48 (d, *J* 2.5 Hz, 1H), 6.77–6.87 (m, 1H), 7.77 (d, *J* 16.0 Hz, 1H), 11.76 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.7 (C), 170.3 (C), 168.2 (C), 165.7 (C), 164.3 (C), 141.8 (CH), 140.4 (CH), 139.3 (C), 132.6 (CH), 124.8 (CH), 108.2 (CH), 104.1 (C), 101.5 (CH), 71.7 (CH), 55.6 (CH_3), 49.0 (CH_2), 38.0 (CH_2), 18.9 (CH_3); HRMS calcd for $C_{18}H_{18}NO_6$ $[M-H]^-$ 344.1134, found 344.1138.

6.2. Preparation of human ERK2 for docking and molecular dynamics

The crystal structure of nonphosphorylated human ERK2 complexed with FR148083 was retrieved from the protein data bank, PDB ID: 2E14.[26] The crystal structure has a missing activation loop in the region from residues Pro176 to Tyr187. Homology modeling was therefore performed for the entire human ERK2 amino acid sequence (FASTA sequence, downloaded from NCBI protein database) using the automated homology modeling module in YASARA[27] with the 2E14 crystal structure as the template. The obtained homology model was further minimized with the AMBER 03 force field[28] using YASARA, and used as a receptor for the subsequent dockings. Although the homology model has 10 additional residues toward the N-terminal compared to the 2E14 template, the 2E14 crystal structure numbering scheme has been used throughout.

6.3. Docking

Models of **1** and hypothemycin were built in the molecular operating environment (MOE) program,[29] minimized with the MMFF94x force field,[30] and used as starting structures for docking. The compounds were docked using the alpha PMI (principle moments of inertia) placement method, which is suitable for docking ligands to tight receptor pockets, and affinity dG scoring methods. The obtained docking results were further refined using the MMFF94x force field refinement method. For each system, the pose with highest possible scoring value and simultaneous proper alignment in the active site was selected, and the docked enzyme–ligand complexes used as starting structures for energy minimization and MD simulations. Hundred docked poses were initially selected for each system. Following force field refinement, the poses that deviated significantly or moved out of the active site after minimization were discarded, and the ones remaining in the active site retained.

6.4. Molecular dynamics simulations

Each docked enzyme–ligand complex was placed in a cubic box of size 76×70×85 Å with periodic boundary conditions and solvated with approximately 5800 TIP3P[31] water molecules. Counter ions Na^+ and Cl^- were randomly placed to neutralize the cell, and provide a physiologically relevant salt concentration of 0.9% (mass percent). Long-range interactions were treated with the Particle Mesh Ewald algorithm[32] with a cut off value of 7.86 Å. All complexes were initially subjected to steepest descent energy minimization using the AMBER 03 force field. After removing the conformational stress through equilibration simulations, production simulations were carried out with a multiple time step of 1.333 fs and with snapshots saved in the trajectory every 4 ps. All simulations were carried out at constant pressure and temperature (298 K) conditions, NPT, for a period of 6 ns. Covalent bonds involving hydrogens and the bond angles of water were constrained throughout the simulations. After the 6 ns MD simulations, the structures were minimized again, giving the *final* structures for which the binding energies were calculated. All the energy minimizations and MD simulations were carried out using the YASARA program.

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Supplementary data

^{1}H and ^{13}C NMR spectra for all the new compounds described herein and standard operating procedures (SOPs) for kinase assays are available free of charge via the Internet. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.04.082.

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