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Synthesis and Evaluation of Novel Analogues of Ripostatins

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Abstract

Ripostatins are polyene macrolactones isolated from myxobacterium *Sorangium cellulosum*. They exhibit antibiotic activity by inhibiting bacterial RNA-polymerase through a binding site and mechanism different from those of current antibacterial drugs and thus serve as starting points for the development of new anti-infective agents with a novel mode of action. In this work, several derivatives of ripostatins were produced. The 15-desoxy-ripostatin A was synthesized using one-pot carboalumination/cross-coupling. The 5,6-dihydro-ripostatin A was constructed utilizing an intramolecular Suzuki cross-coupling macrolactonization approach, and 14,14'-difluororipostatin A and both epimeric 14,14'-difluororipostatins B were synthesized using a Reformatsky-type aldol addition of haloketone, Stille cross-coupling and ring-closing metathesis. RNAP-inhibitory and antibacterial activities are presented. Structure-activity relationships indicate that the monocyclic keto-ol form of ripostatin A is the active form of ripostatin A, that the ripostatin 5-6 unsaturation is important for activity, and that C14 geminal difluorination can be introduced into ripostatin B without loss of activity

Keywords

RNA polymerase inhibitors; natural products; SAR-studies; antibiotics; antibacterial agents

Introduction

Resistance of bacterial pathogens to current antibacterial drugs is an urgent problem.^[1] Bacterial RNA polymerase (RNAP) represents an attractive target for the development of new antibacterial agents effective against bacterial pathogens resistant against current antibacterial drugs.^[2] In particular, the RNAP “switch region SW1/SW2 sub-region,” a structural element that mediates opening and closing of the RNAP active-center cleft, has been shown to be the binding site for three classes of antibacterial natural products: ripostatins (Fig. 1, **1**, **1'**, and **2**), coralopyronins (Fig 1, **3**) and myxopyronins (Fig 1, **4**).^[2,3]

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In our pursuit of new lead structures for the development of novel antibacterial agents, we became attracted to ripostatins due to their interesting biological properties and challenging molecular architecture. Consequently, we developed a convergent approach to synthesis of the ripostatin scaffold and accomplished, concurrently with two other groups, the total synthesis of ripostatin B.^[4] We also reported the first total synthesis of ripostatin A. With a reliable synthetic strategy in hands, we next aimed to generate novel analogues of ripostatin A and ripostatin B for the elucidation of structure-activity relationship. In this article we provide a detailed account on our recent synthetic endeavours in this direction and also provide data on RNAP-inhibitory activity and antibacterial activity of analogues.

Results and Discussion

Though the preparation of 15-desoxyripostatin A was already reported by us earlier,^[4b] the amount of the compound produced was small. Thus, additional material was needed to perform a detailed biological evaluation of this analogue.

Therefore, we decided to optimize key steps in the synthesis and to streamline the overall sequence. In particular, we re-examined the one-pot carboalumination/cross-coupling reaction^[5] of the first triple bond (Scheme 1). Attempts to achieve this transformation at room temperature failed, but after additional experimentation, the desired cross-coupling was found to proceed smoothly upon heating to 50 °C. Encouraged by this result, we also investigated one-pot elaboration of the second triple bond. Gratifyingly, carboalumination of **6** under standard conditions followed by treatment of the vinylic aluminium species with an excess of allylbromide in the presence of palladium catalyst led yielded the desired product **7**.

The one-pot functionalisation of the second triple bond was particularly beneficial, since the cross-coupling of allyltributyl stannane proceeds significantly faster at the C3 iodoacrylic ester site than at the C8 terminal vinyl iodide. Following our route, intermediate **7** was converted into the final product, 15-desoxyripostatin A (**10**), in five steps with an overall yield of 50%.

As a next possible modification, we examined the replacement of the C5/C6 double bond with a saturated chain. We proposed that this modification would not significantly affect the conformation of the macrolactone ring. The cyclic methyl acetal **16** from our synthesis of ripostatin A^[6] was utilized here as an advanced intermediate. Suitable alken-terminated building blocks were prepared from the iodoacrylic acid **11** (Scheme 2). The best results were achieved using Pd(Ph₃P)₄-catalyzed Negishi coupling^[7] of the **11** with an excess of alkenyl zinc reagent.^[8] The corresponding methyl ester **15** was prepared via esterification/cross-coupling sequence.

The extended carboxylic acid fragment was appended to the acetal **16** by Mitsunobu esterification^[9] and the resulted ester was then introduced into hydroboration/Suzuki cross-coupling^[10] reaction conditions to give the macrolactone **18**, in moderate yield (Scheme 3). We also isolated some quantities of alcohol **19** following oxidative workup of the reaction.

The relatively low yield of the product from the intramolecular cross-coupling reaction prompted us to evaluate an alternative macrolactonisation-based approach (Scheme 4).^[11] No product was observed in an attempted cross-coupling reaction with carboxylic acid **12**. An attempted intermolecular cross-coupling with methyl ester **15** gave an excellent yield of the desired product **20**; however, the ester resisted subsequent saponification under numerous conditions.^[12] Reduction of the ester with LAH to **21** was possible, but attempts to convert **21** to the seco-acid **22** or directly to the macrolactone **18** via various oxidation methods were fruitless.

Unable to accomplish the ring closure via lactone bond formation, we next examined intramolecular Suzuki cross-coupling at the iodoacrylic ester terminus. Thus, cyclic acetal **16** was coupled first with alkenyl zinc reagent to introduce a four-carbon chain with terminal double bond (Scheme 5). Esterification of **23** in Mitsunobu or Yamaguchi conditions^[13] yielded the ester **24**, which upon standard conditions failed to provide the macrolactone **18** in even moderate yield.

Finally, selective cleavage of the primary TBS-ether of **18** from Scheme 3, followed by a sequence of Dess-Martin periodinane and Pinnick-Kraus^[14] oxidations, and hydrolysis of the methyl acetal upon purification of on reversed phase HPLC in the presence of formic acid, completed the synthesis of 5,6-dihydro-ripostatin A **25** (Scheme 6).

We then turned our attention toward fluorinated derivatives of ripostatins. We reasoned that introduction of a gem-difluoro-substitution at C-14 position of ripostatin A would stabilize the bicyclic hemiketal form of ripostatin A relative to the monocyclic keto-ol form of ripostatin A (see Fig. 1, **1** and **1'**) and, simultaneously, would prevent β -acetoxo elimination, which has been shown to be the major route of degradation of ripostatin A.^[15] Following our general synthetic logic, we synthesized the difluoroketone building block **29** using an organolithium reagent derived from the side chain alkyl iodide **28**^[4b] with the Weinreb amide of chlorodifluoroacetic acid (Scheme 7). Interestingly, in the case of the Weinreb amide of bromodifluoroacetic acid, a reversed halogen metal exchange was observed in the reaction with organolithium reagent.

Use of the difluoroketone **29** in the Reformatsky-type aldol addition according to the Ishihara^[16] protocol produced the desired product **34** in relatively low yield (Scheme 8). Attempts to optimise this reaction by varying reaction temperature and/or by addition of Lewis acids were unsuccessful.

We reasoned that the vinylic iodide functionality may interfere with the aldol reaction by concomitant formation of vinylzinc reagent and consequent polymerization of the aldehyde. Therefore, we changed the order of steps in our sequence and prepared the allyl-substituted aldehyde **36** from diol **35** via cross-coupling and subsequent periodate cleavage (Scheme 9). With aldehyde **36**, the aldol reaction proceeded in excellent yield and produced a 1:1.2 mixture of diastereomeric adducts. Subsequent treatment of this mixture with camphorsulfonic acid in MeOH in the presence of trimethyl orthoformate provided two cyclic hemiketals **38** and **39** instead of the expected methyl-acetal derivatives. The hemiketals were separated by flash chromatography, and their stereochemistries were assigned using

Rychnovsky acetonide method by analysis of ^{13}C -NMR spectra of compounds **41** and **42**.^[17] In both cases, the methyl groups and acetal carbon of acetonide moieties exhibited expected characteristic shift patterns (see Experimental Section).

The apparent stability of the hemiketal form of the difluoro-derivatives **38** and **39** prompted us to proceed with the synthesis without protecting these intermediates as methyl acetals. Unexpectedly, inversion of the stereocenter at C-13 in hemiketal **39** using Mitsunobu esterification was accompanied by migration of the acyl group towards the 11-OH-position and thus only the C-11-OH ester **40** was isolated from reaction mixture. Cleavage of this ester under basic conditions led to the hemiketal **38**, thus confirming the inversion of the absolute configuration at C-13. A similar sequence of steps was performed using acetic acid with the intention to convert **39** to **38**, but the overall yield was only 40%. It turned out that the C-13 hydroxy group can be selectively acylated by activated anhydride of iodoacrylic acid **11** in relatively good yield using our modified Yamaguchi protocol (Scheme 10).

Subsequent transformations, including Stille cross-coupling^[18] and ring-closing metathesis,^[19] proceeded with generally high yields. Finally, deprotection of the primary hydroxyl group followed by a double oxidation gave rise to the 14,14'-difluororipostatin A (**45**). NMR-data indicate that, as expected, this compound exists predominantly in the bicyclic hemiketal form (form as in Fig. 1, **1**).

We next attempted to produce difluorinated derivatives of ripostatin B. First, in line with the earlier work on ripostatin A,^[15] we investigated a direct reduction of 14,14'-difluororipostatin A **45** with NaBH_4 . However, no reaction was observed under standard conditions. Reasoning that the bicyclic hemiketal form of **45** was too stable to permit reduction, we repeated the reaction in the presence of base, seeking to shift the equilibrium between bicyclic hemiketal and monocyclic keto-ol forms in the direction of the monocyclic keto-ol form to promote reduction. In the presence of stoichiometric amount of base, the two epimeric 14,14'-difluororipostatins B, 14,14'-difluororipostatin B1 (**46**) and 14,14'-difluororipostatin B2 (**47**), were obtained by reduction. The stereochemistries of **46** and **47** were tentatively assigned based on similarity of NMR spectra to the NMR spectrum of ripostatin B, activity data, and additional information from the X-ray structure of difluororipostatin B RNAP-complex.^[20] Confirmation of stereochemistries by Mosher ester analysis is in progress.

RNAP-inhibitor activities and antibacterial activities were measured as described.^[2b] RNAP-inhibitor activities and antibacterial activities are reported in Table 1 and Table 2, respectively. The 15-desoxyripostatin **10** and the 14,14'-difluoro-ripostatin A **25** exhibited no or poor RNAP-inhibitory activity (IC_{50} s at least approximately two orders of magnitude higher than IC_{50} for ripostatin A) and no or poor antibacterial activity (MICs at least approximately an order of magnitude higher than MIC for ripostatin A). 15-desoxyripostatin **10** and 14,14'-difluoro-ripostatin A **25** exist exclusively or predominantly in a bicyclic form, in contrast to ripostatin A, which exists as an equilibrium mixture of bicyclic and monocyclic forms (Fig. 1, **1** and **1'**). The poor biochemical and biological activities of these "bicyclic-trapped" analogs suggests that the bicyclic form of ripostatin A (**1**) is inactive and that the monocyclic form of ripostatin A (**1'**) is the active form of ripostatin A.

The 5,6-dihydro-ripostatin A **25** exhibited weak but clear RNAP-inhibitory activity (IC₅₀ approximately one order of magnitude higher than IC₅₀ for ripostatin A). We infer that the C5-C6 unsaturation is important for, but not indispensable for, interaction of ripostatin with RNAP. The 14,14'-difluoro-ripostatin B1 **46** exhibited RNAP-inhibitory and antibacterial activities comparable to those of ripostatin A and ripostatin B. The epimeric 14,14'-difluoro-ripostatin B2 **47** was significantly less potent than **46** in both RNAP-inhibitory and antibacterial activities. The high potency and high stability of 14,14'-difluoro-ripostatin B1 **46** have allowed a crystal structure of the corresponding RNAP-inhibitor complex to be determined, representing the first crystal structure of a RNAP-ripostatin complex.^[21] The excellent biochemical and biological activities of 14,14'-difluoro-ripostatin B1 (**46**), which exists exclusively in a monocyclic form, support the conclusion that the monocyclic form of ripostatin A (**1'**, Fig. 1) is the active form of ripostatin A.

Conclusion

The presented results demonstrate that our synthetic strategy can be utilized to prepare structurally modified analogues of ripostatins A and B. Although, difficulties were encountered en-route to the 5,6-dihydro-ripostatin A, the synthesis of gem-difluorinated derivatives of both ripostatin A and ripostatin B was straightforward.

The structure-activity relationships derived from analysis of RNAP-inhibitory and antibacterial activities of the resulting analogs indicate that that the bicyclic form of ripostatin A (Fig. 1, **1**) is inactive and that the monocyclic form of ripostatin A (Fig. 1, **1'**) is the active form of ripostatin A. The structure-activity relationships further indicate that the ripostatin 5-6 unsaturation is important for activity and that stability-enhancing gem-difluorination can be introduced at ripostatin B C14 without loss of activity.

Future work on ripostatin-based inhibitors of RNA-polymerase will be aimed at the development of more potent compounds, with support from *in silico* screening. Efforts will be focussed on analogs of ripostatin A trapped in the monocyclic state ("monocyclic-trapped" analogs, including 11-OH-methylated ripostatin A analogs) and on analogs of ripostatins A and B with modifications of the ripostatin phenylalkyl side chain. The outcomes of these studies will be reported in due course.

Experimental Section

General remarks

Optical rotations were determined on a Perkin-Elmer 241 instrument. NMR spectra were recorded in CDCl₃ and D₃COD on a Bruker AM 300, AM 400, AMX 500, DMX-600 and AMX 700 spectrometers. ESI mass spectra were obtained on a Finnigan MAT 95 spectrometer, high resolution data were acquired using peak matching (M/DM = 10000). Analytical TLC on aluminium sheets, silica gel Si 60 F254 (Merck), solvent: mixtures of ethyl acetate/petroleum ether, detection: UV absorption at 254 nm, dark blue spots on staining with cerium(IV)sulfate-phosphomolybdic acid in sulfuric acid followed by charring. Unless otherwise stated, all reactions were performed under inert gas blanket. All solvents used were commercial absolute solvents over molecular sieves with water content less than

50 ppm (e.g. from Acros Organics). Experimental procedures for compounds **8**, **9**, **10**, **20**, **21**, **23**, **24**, **27**, **29**, **31**, **32**, **36**, **40**, **44**, and some other intermediates are provided in the Supporting Information.

***tert*-butyldimethyl(((2*S*,4*R*,6*R*)-2-((*E*)-3-methyl-5-phenylpent-3-en-1-yl)-6-(prop-2-yn-1-yl)tetrahydro-2H-pyran-4-yl)oxy)silane (**6**)**

To a flame dried argon purged 25 mL round-bottom Schlenk-flask was added zirconocene dichloride (276.4 mg, 0.95 mmol), followed by the dropwise addition of trimethylaluminum (2.0 M solution in toluene, 3.8 mL, 7.6 mmol) and *iso*-BuOH (43.6 μ L, 0.47 mmol) at 0 °C. While stirring at 0 °C, the alkyne **5** (895.2 mg, 2.36 mmol) in DCM (5 mL) was added. The mixture was warmed to room temperature and stirred overnight, at which point carboalumination was complete, as determined by TLC. In a separate flask BnBr (0.34 mL, 2.86 mmol) was added to a solution of Pd(PPh₃)₄ (90.0 mg, 0.08 mmol) in THF (6 mL), and the yellow mixture was immediately transferred to the vinylalane solution *via* cannula and the reaction stirred at 50 °C. After 3 h, the reaction was diluted with ether (60 mL) and quenched with 1M HCl (60 mL). The aqueous layer was extracted with ether (3 $\times$ 45 mL), and the combined organic extracts were washed with saturated aqueous NaHCO₃ (40 mL) and brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo* to give the yellow oil engaged in the following step.

The crude protected alkyne was dissolved in a mixture of THF (6 mL) and MeOH (5 mL) at 0 °C and solid potassium carbonate (654.0 mg, 4.73 mmol) was added in one portion. The suspension was stirred at room temperature for 3.5 h. Additional K₂CO₃ (108.0 mg, 0.78 mmol) was added and stirred for 0.5 h. The precipitate was filtered off, the filter cake was washed with ether (10 mL) and the filtrate concentrated *in vacuo*. The residue was purified by column chromatography (PE/Et₂O = 70:1) to give terminal alkyne **6** (520.9 mg, 1.26 mmol, 53%) as a colorless oil. *R*_f = 0.49 (PE/EtOAc = 25:1); ¹H-NMR (500 MHz, CDCl₃): δ = 0.05 (s, 6H), 0.88 (s, 9H), 1.16-1.25 (m, 2H), 1.50-1.57 (m, 1H), 1.67-1.73 (m, 1H), 1.71 (s, 3H), 1.76-1.80 (m, 1H), 1.98-2.02 (m, 2H), 2.06-2.18 (m, 2H), 2.28-2.33 (ddd, ²J = 16.63 Hz, ³J = 7.32 Hz, ⁴J = 2.75 Hz, 1H), 2.44-2.49 (ddd, ²J = 16.63 Hz, ³J = 5.80 Hz, ⁴J = 2.75 Hz, 1H), 3.21-3.26 (m, 1H), 3.32-3.39 (m, 1H), 3.35 (d, ³J = 7.17 Hz, 2H), 3.69-3.75 (m, 1H), 5.34-5.38 (m, 1H), 7.15-7.28 (m, 5H); ¹³C-NMR (125 MHz, CDCl₃): δ = -4.5, 16.2, 18.1, 25.9, 34.2, 34.3, 35.5, 40.8, 41.4, 68.7, 69.9, 73.8, 75.2, 80.9, 123.4, 125.7, 128.3, 128.4, 135.7, 141.8.

***tert*-butyldimethyl(((2*S*,4*R*,6*R*)-2-((*E*)-3-methyl-5-phenylpent-3-en-1-yl)-6-((*E*)-2-methylhexa-2,5-dien-1-yl)tetrahydro-2H-pyran-4-yl)oxy)silane (**7**)**

To a flame dried argon purged 25 mL round-bottom Schlenk-flask was added zirconocene dichloride (505.0 mg, 1.73 mmol) and Dichloromethane (0.5 mL), followed by the dropwise addition of trimethylaluminum (2.0 M solution in toluene, 2.6 mL, 5.20 mmol) and *iso*-BuOH (48.0 μ L, 0.52 mmol) at 0 °C. While stirring at 0 °C, the alkyne **6** (356.9 mg, 0.86 mmol) in DCM (3 mL) was added. The mixture was warmed to room temperature and stirred for 1 day, at which point carboalumination was complete, as determined by TLC. The solvent was removed under reduced pressure and replaced with THF (2 mL). In a separate flask allyl bromide (112 μ L, 1.29 mmol) was added to solution of Pd(PPh₃)₄ (60.0 mg, 0.05

mmol) in THF (3 mL), and the yellow mixture was immediately transferred to the vinylalane solution *via* canula and the reaction stirred at 60 °C. After 3 h, the reaction was diluted with ether (25 mL) and quenched with 1M HCl (25 mL). The aqueous layer was extracted with ether (3 × 15 mL), and the combined organic extracts were washed with saturated aqueous NaHCO₃ (20 mL) and brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (PE/Et₂O = 30:1) to provide 237.70 mg (0.51 mmol, 59%) of **7** as a colourless oil. **R_f** = 0.31 (PE/Et₂O = 30:1); ¹H-NMR (500 MHz, CDCl₃): δ = 0.04 (s, 6H), 0.87 (s, 9H), 1.12-1.22 (m, 2H), 1.50-1.57 (m, 1H), 1.61 (s, 3H), 1.63-1.72 (m, 1H), 1.70 (s, 3H), 1.75-1.78 (m, 1H), 2.03-2.19 (m, 4H), 2.26-2.30 (dd, ²J = 13.73 Hz, ³J = 7.17 Hz, 1H), 2.72-2.75 (m, 2H), 3.16-3.21 (m, 1H), 3.29-3.38 (m, 1H), 3.33-3.35 (d, ³J = 7.32 Hz, 2H), 3.67-3.73 (m, 1H), 4.91-4.94 (d, ³J = 10.07 Hz, 1H), 4.98-5.02 (d, ³J = 17.09 Hz, 1H), 5.21 (t, ³J = 7.32 Hz, 1H), 5.33 (t, ³J = 7.32 Hz, 1H), 5.74-5.82 (m, 1H), 7.15-7.28 (m, 5H); ¹³C-NMR (125 MHz, CDCl₃): δ = -4.5, 16.2, 16.5, 18.2, 25.9, 32.3, 34.3, 34.4, 35.6, 41.6, 41.8, 46.1, 69.2, 74.4, 75.0, 114.3, 123.2, 123.8, 125.7, 128.3, 133.4, 136.0, 137.2, 141.8; [α]_D = +2.57 (c = 2.61, DCM); HRMS (ESI): m/z calc. for C₃₀H₄₈O₂SiNa [M+Na]⁺: 491.3321, found: 491.3322.

(E)-3-(2-((tert-butyldimethylsilyl)oxy)ethyl)hepta-2,6-dienoic acid (**12**).

The zinc dust (1.28 g, 19.58 mmol) were added to a nitrogen purged flask, to which dry THF (8 mL) were then added, and irradiated in an ultrasonic cleaning bath for 15 min. Then 1,2-dibromoethane (76 μL, 0.88 mmol) was added and heated to 65 °C for 3 min. The reaction mixture was cooled to room temperature and chlorotrimethylsilane (111 μL, 0.88 mmol) was added. After stirring for 10 min, a solution of 4-iodobuten (3.20 g, 17.58 mmol) in THF (3 mL) was slowly added. The reaction mixture was stirred for 20 h at 36-39 °C and cooled to room temperature again. The clear and colorless solution was immediately transferred to a solution of carboxylic acid **11** (1.22 mg, 3.43 mmol) and Pd(PPh₃)₄ (0.24 g, 0.17 mmol) in Et₂O (20 mL) and DMF (10 mL) *via* cannula at 0 °C and then stirred at room temperature for 3 h. The reaction mixture was diluted with ether (20 mL) and quenched with saturated aqueous NH₄Cl (60 mL). The aqueous layer was extracted with ether (4 × 30 mL), and the combined organic extracts were washed with brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (PE/EtOAc = 5:1) to provide 0.79 g (2.78 mmol, 81%) of **12** as a yellow oil. **R_f** = 0.30 (PE/EtOAc = 5:1); ¹H-NMR (500 MHz, CDCl₃): δ = 0.04 (s, 6H), 0.88 (s, 9H), 2.20-2.25 (m, 2H), 2.36-2.39 (t, ³J = 6.56 Hz, 2H), 2.40-2.74 (m, 2H), 3.73-3.76 (t, ³J = 6.56 Hz, 2H), 4.94-4.97 (d, ³J = 10.22 Hz, 1H), 5.00-5.05 (d, ³J = 17.09 Hz, 1H), 5.70 (s, 1H), 5.79-5.87 (m, 1H); ¹³C-NMR (75 MHz, CDCl₃): δ = -5.3, 18.3, 25.9, 32.0, 32.6, 41.8, 61.4, 115.0, 116.9, 137.9, 163.6, 171.6; HRMS (ESI): m/z calc. for C₁₅H₂₈O₃SiNa [M+Na]⁺: 307.1705, found: 307.1706.

Methyl (Z)-5-((tert-butyldimethylsilyl)oxy)-3-iodopent-2-enoate (**14**)

To a solution of EDC·HCl (139.6 mg, 0.73 mmol), acid **11** (235.8 g, 0.66 mmol) in DCM (2 mL) at 0 °C was added MeOH (45 μL, 1.11 mmol) and DMAP (8.1 mg, 66 μmol). The mixture was stirred at 0 °C for 0.5 h and warmed to room temperature and stirred for 3 h. Then the mixture was diluted with DCM (2 mL) and quenched with saturated aqueous NH₄Cl (3 mL). The aqueous layer was extracted with DCM (3 × 3 mL) and the combined

organic extracts were washed with brine (5 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash chromatography (PE/Et₂O = 14:1) to give **14** (200.6 mg, 0.54 mmol, 82%) as a yellow oil. *R*_f = 0.40 (PE/Et₂O = 12:1); ¹H-NMR (400 MHz, CDCl₃): δ = 0.07 (s, 6H), 0.89 (s, 9H), 2.91-2.94 (t, ³J = 5.92 Hz, 2H), 3.77 (s, 3H), 3.79-3.82 (t, ³J = 5.92 Hz, 2H), 6.43 (s, 1H); ¹³C-NMR (100 MHz, CDCl₃): δ = -5.3, 18.2, 25.8, 51.0, 51.6, 61.3, 117.2, 126.4, 164.8; HRMS (ESI): *m/z* calc. for C₁₂H₂₃IO₃SiNa [M+Na]⁺: 393.0359, found: 393.0366.

Methyl (*E*)-3-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)hepta-2,6-dienoate (**15**)

The zinc dust (0.62 g, 9.48 mmol) were added to a nitrogen purged flask, to which dry THF (5 mL) were then added, and irradiated in an ultrasonic cleaning bath for 15 min. Then 1,2-dibromoethane (33 μL, 0.41 mmol) was added and heated to 65 °C for 3 min. The reaction mixture was cooled to room temperature and chlorotrimethylsilane (52 μL, 0.41 mmol) was added. After stirring for 10 min, a solution of 4-iodobuten (1.50 g, 8.24 mmol) in THF (3 mL) was slowly added. The reaction mixture was stirred overnight at 38 °C and cooled to room temperature again to give 1 M solution of alkenyl zinc reagent. The zinc organic solution (1.5 mL, 1.5 mmol) was immediately transferred to the a solution of **14** (214 mg, 0.58 mmol) and Pd(PPh₃)₄ (54 mg, 47 μmol) in Et₂O (3.0 mL) and DMF (1.5 mL) *via* cannula at 0 °C and then stirred at room temperature for 1 h. Additional Pd(dppf)Cl₂ (34 mg, 47 μmol) was added, stirred at room temperature for 1 h and then at 50 °C for 50 min. The reaction mixture was quenched with saturated aqueous NH₄Cl (5 mL). The aqueous layer was extracted with ether (2 × 5 mL), and the combined organic extracts were washed with brine (7 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (PE/Et₂O = 18:1) to provide alkene **15** (97.8 mg, 0.33 mmol, 57%) as a colourless oil. *R*_f = 0.34 (PE/Et₂O = 18:1); ¹H-NMR (500 MHz, CDCl₃): δ = 0.03 (s, 6H), 0.87 (s, 9H), 2.19-2.24 (m, 2H), 2.33-2.36 (td, ³J = 6.56 Hz, ²J = 1.07 Hz, 2H), 2.69-2.72 (m, 2H), 3.67 (s, 3H), 3.71-3.74 (t, ³J = 6.71 Hz, 2H), 4.94-4.97 (d, ³J = 10.07 Hz, 1H), 5.01-5.05 (d, ³J = 17.09 Hz, 1H), 5.68 (s, 1H), 5.80-5.88 (m, 1H); ¹³C-NMR (125 MHz, CDCl₃): δ = -5.3, 18.3, 25.9, 31.8, 32.6, 41.5, 50.9, 61.5, 114.9, 116.9, 138.0, 160.7, 166.7; HRMS (ESI): *m/z* calc. for C₁₆H₃₀O₃SiNa [M+Na]⁺: 321.1862, found: 321.1866.

(2*S*,4*R*,6*R*)-6-((*E*)-3-iodo-2-methylallyl)-2-methoxy-2-((*E*)-3-methyl-5-phenylpent-3-en-1-yl)tetrahydro-2H-pyran-4-yl-(*E*)-3-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)hepta-2,6-dienoate (**17**)

To a solution of alcohol **16** (83.2 mg, 0.18 mmol), acid **12** (92.2 mg, 0.32 mmol) and PPh₃ (102.0 mg, 0.39 mmol) in dry benzene (1.5 mL) at 0 °C was added DIAD (77.0 μL, 0.39 mmol) and the reaction was stirred at room temperature for 2.0 h. The mixture was concentrated under reduced pressure and then the residue was immediately purified by flash chromatography (PE/Et₂O = 10:1) to provide ester **17** (117.6 mg, 0.16 mmol, 89%) as a colourless oil. *R*_f = 0.24 (PE/Et₂O = 10:1); ¹H-NMR (400 MHz, CD₃OD): δ = 0.11 (s, 6H), 0.94 (s, 9H), 1.50-1.88 (m, 5H), 1.78 (s, 3H), 1.95 (s, 3H), 2.03-2.10 (m, 3H), 2.25-2.30 (m, 2H), 2.36-2.49 (m, 4H), 2.71-2.87 (m, 2H), 3.14 (s, 3H), 3.37-3.39 (d, ³J = 7.63 Hz, 2H), 3.82-3.85 (t, ³J = 6.10 Hz, 2H), 4.04-4.10 (m, 1H), 4.97-5.10 (m, 3H), 5.38-5.42 (m, 1H), 5.78 (s, 1H), 5.83-5.95 (m, 1H), 6.10 (s, 1H), 7.15-7.30 (m, 5H); ¹³C-NMR (75 MHz,

CD₃OD): δ = -5.2, 16.3, 19.1, 25.2, 26.4, 32.6, 33.8, 34.4, 35.1, 35.4, 36.1, 36.4, 42.3, 46.0, 47.9, 62.3, 65.2, 67.7, 77.4, 100.8, 115.3, 119.2, 124.6, 126.7, 129.3, 129.4, 136.9, 139.3, 142.9, 146.2, 161.5, 167.6; $[\alpha]_D$ = +19.6 (c = 2.13, DCM); HRMS (ESI): m/z calc. for C₃₇H₅₇IO₅SiNa [M+Na]⁺: 759.2918, found: 759. 1919.

(1R,4E,10E,13R,15S)-5-(2-((tert-butylidimethylsilyl)oxy)ethyl)-15-methoxy-11-methyl-15-((E)-3-methyl-5-phenylpent-3-en-1-yl)-2,14-dioxabicyclo[11.3.1]heptadeca-4,10-dien-3-one (18)

To a solution of 0.5M 9-BBN (100.0 μ L, 50 μ mol) at 0 °C was added a solution of iodide **17** (24.6 mg, 33 μ mol) in THF (0.6 mL). The reaction mixture was stirred for 1 h at 0 °C and for 2.5 h at room temperature. The colourless mixture was diluted with THF (4 mL) and added *via* cannula to a solution of Pd(PPh₃)₄ (2.3 mg, 2 μ mol), PPh₃ (0.8 mg, 3 μ mol) and 3 M K₃PO₄ (33 μ L, 99 μ mol) in THF (2 mL) in a separate flask over 1.5 h at 49 °C. After stirring for 1.5 h, additional Pd(PPh₃)₄ (2.4 mg, 2 μ mol) was added and stirred at 49 °C for 2 h. The violet mixture was cooled to the room temperature and treated with ethanolamine (10 μ L, 166 μ mol) for 20 min. Then the mixture was quenched with hexane (6 mL), water (4 mL) and birne (2 mL). The aqueous layer extracted with hexane (3 $\times$ 5 mL) and the combined organic extracts were washed with brine (7 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude material was purified by flash chromatography (PE/Et₂O = 5:1 with 0.1% Et₃N) to provide **18** (5.2 mg, 8.5 μ mol, 26%) as a colourless oil. **R_f** = 0.44 (PE/Et₂O = 4:1); ¹H-NMR (700 MHz, CD₃OD): δ = 0.11 (s, 6H), 0.94 (s, 9H), 1.15-1.21 (m, 1H), 1.27-1.43 (m, 2H), 1.46-1.52 (m, 1H), 1.59-1.64 (m, 1H), 1.67 (s, 3H), 1.79 (s, 3H), 1.77-1.93 (m, 3H), 1.95-1.98 (m, 1H), 2.02-2.16 (m, 6H), 2.23-2.29 (m, 2H), 2.32-2.41 (m, 2H), 2.98-3.02 (m, 1H), 3.23 (s, 3H), 3.38-3.39 (d, ³J = 7.53 Hz, 2H), 3.73-3.77 (m, 1H), 3.80-3.83 (m, 2H), 5.03-5.05 (m, 1H), 5.12-5.14 (m, 1H), 5.40-5.43 (m, 1H), 5.77 (s, 1H), 7.15-7.30 (m, 5H); ¹³C-NMR (125 MHz, CD₃OD): δ = -5.2, -5.2, 16.3, 18.6, 19.1, 26.4, 28.2, 30.8, 30.8, 32.9, 33.8, 34.4, 35.1, 36.6, 43.8, 45.0, 47.9, 62.2, 66.7, 69.1, 101.5, 119.0, 124.7, 126.7, 128.4, 129.3, 129.4, 133.6, 136.8, 142.8, 158.6, 169.2; $[\alpha]_D$ = +28.1 (c = 0.80, DCM); HRMS (ESI): m/z calc. for C₃₇H₅₈O₅SiNa [M+Na]⁺: 633.3951, found: 633. 3953.

(1R,4E,10E,13R,15S)-5-(2-hydroxyethyl)-15-methoxy-11-methyl-15-((E)-3-methyl-5-phenylpent-3-en-1-yl)-2,14-dioxabicyclo[11.3.1]heptadeca-4,10-dien-3-one

To a solution of silyl ether **18** (12.6 mg, 21 μ mol) in dry THF (0.35 mL) was added 20% Et₃N·3HF (0.17 mL, 208 μ mol) at 0 °C. After stirring for 2 days at 0 °C, the reaction mixture was put into a solution of NaHCO₃ (100 mg) in water (2.5 mL) and EtOAc (2.5 mL) at 0 °C. The aqueous layer was washed with EtOAc (3 $\times$ 2 mL). The combined organic extracts were washed with brine (2.5 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash chromatography (PE/EtOAc = 1.5:1) to give primary alcohol (6.3 mg, 13 μ mol, 60%) as a colorless oil. **R_f** = 0.20 (PE/EtOAc = 1.5:1); ¹H-NMR (700 MHz, CD₃OD): δ = 1.15-1.22 (m, 1H), 1.29-1.42 (m, 2H), 1.46-1.52 (m, 1H), 1.59-1.64 (m, 1H), 1.67 (s, 3H), 1.79 (s, 3H), 1.82-1.93 (m, 3H), 1.95-1.99 (m, 1H), 2.03-2.15 (m, 6H), 2.22-2.29 (m, 2H), 2.34-2.44 (m, 2H), 2.98-3.02 (m, 1H), 3.23 (s, 3H), 3.38-3.39 (d, ³J = 7.53 Hz, 2H), 3.68-3.76 (m, 3H), 5.04-5.05 (m, 1H), 5.13-5.15 (m, 1H), 5.40-5.42 (m, 1H), 5.79 (s, 1H), 7.16-7.29 (m, 5H); ¹³C-NMR (175 MHz, CD₃OD): δ =

16.3, 18.5, 28.2, 30.7, 30.9, 32.8, 33.8, 34.4, 35.1, 36.6, 36.6, 43.4, 45.0, 47.8, 60.9, 66.7, 69.2, 101.6, 118.9, 124.7, 126.7, 128.4, 129.3, 129.4, 133.6, 136.8, 142.8, 158.1, 169.2; $[\alpha]_D = +31.4$ ($c = 0.44$, DCM); HRMS (ESI): m/z calc. for $C_{31}H_{44}O_5Na$ $[M+Na]^+$: 519.3086, found: 519.3088.

2-((1*R*,4*E*,10*E*,13*R*,15*S*)-15-hydroxy-11-methyl-15-((*E*)-3-methyl-5-phenylpent-3-en-1-yl)-3-oxo-2,14-dioxabicyclo[11.3.1]heptadeca-4,10-dien-5-yl)acetic acid (25**)**

To a solution of previous alcohol (6.0 mg, 12 μ mol) in THF (0.6 mL) at 0 °C was added Dess-Martin periodinane (10.9 mg, 26 μ mol). The reaction mixture was stirred at room temperature for 15 min. Then 2,3-dimethyl-2-butene (0.2 mL) and *tert*-BuOH (0.6 mL) were added at 0 °C, followed by a solution $NaClO_2$ (4.6 mg, 51 μ mol) and $NaH_2PO_4 \cdot H_2O$ (9.0 mg, 65 μ mol) in water (0.4 mL). The mixture was stirred at room temperature for 20 min before saturated aqueous NH_4Cl (3 mL) and EtOAc (3 mL) were added. The phases were separated and the aqueous phase was extracted with EtOAc (3×2 mL). The combined organic layers were washed with brine (2 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by HPLC (VarioPrep 21 $\times$ 250 mm Nucleodur 5 μ C-18 (Machery-Nagel) column; eluent A: 5% CH_3CN with 1% formic acid in H_2O ; eluent B: CH_3CN ; linear gradient 20-85% B in 30 min, then 85-95% B in 20 min at 10 mL/min flow rate; t_R =36.0 min) to give the final product **25** (3.1 mg, 6.2 μ mol, 52%) as a mixture of tautomers (hemiacetal/keto-form = 1:1.5) as a colorless oil. $R_f = 0.29$ (DCM/MeOH = 11:1); HRMS (ESI): m/z calc. for $C_{30}H_{40}O_6Na$ $[M+Na]^+$: 519.2723, found: 519.2729.

(2*E*,10*R*,12*E*)-10-((*tert*-butyldimethylsilyl)oxy)-7,7-difluoro-8-hydroxy-3,12-dimethyl-1-phenylhexadeca-2,12,15-trien-6-one (37**)**

The acid-washed zinc dust (230.0 mg, 3.52 mmol) and CuCl (34.8 mg, 0.35 mmol) were added to a nitrogen purged flask, to which dry THF (1 mL) were then added, and irradiated in an ultrasonic cleaning bath for 0.5 h. Then solutions of ketone (243.0 mg, 0.89 mmol) in THF (1 mL) and aldehyde (299 mg, 1.06 mmol) in THF (1 mL) were added *via* cannula, followed by addition of TMSCl (10 μ L, 0.08 mmol). After stirring at 60 °C for 1 h, TMSCl (12 μ L, 0.09 mmol) was added again and the reaction was stirred at this temperature overnight. After completion of the reaction the precipitate was filtered off and the filter cake was washed with ether (20 mL). The filtrate was washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash chromatography (PE/Et₂O = 13:1) provide **37** as a 1:1.2 mixture (442.7 mg, 0.81 mmol, 92%) of two diastereomers as a colourless oil. $R_f = 0.41$ (PE/Et₂O = 8:1); HRMS (ESI): m/z calc. for $C_{30}H_{46}O_3F_2SiNa$ $[M+Na]^+$: 543.3082, found: 543.3084.

(2*S*,4*R*,6*R*)-3,3-difluoro-2-((*E*)-3-methyl-5-phenylpent-3-en-1-yl)-6-((*E*)-2-methylhexa-2,5-dien-1-yl)tetrahydro-2H-pyran-2,4-diol (38**) and (2*S*,4*S*,6*R*)-3,3-difluoro-2-((*E*)-3-methyl-5-phenylpent-3-en-1-yl)-6-((*E*)-2-methylhexa-2,5-dien-1-yl)tetrahydro-2H-pyran-2,4-diol (**39**)**

To a solution of silyl ether **37** (760.3 mg, 1.46 mmol) in dry MeOH (8 mL) was added a solution of CSA (215.0 mg, 0.92 mmol) in MeOH (2 mL) at 0 °C. The reaction was stirred at this temperature for 4.5 h, before Et₃N (150 μ L) was added. Then the mixture was

quenched with a mixture of pH 7 buffer solution (10 mL), ether (50 mL) and water (50 mL). The aqueous layer extracted with Et₂O (3 × 50 mL) and the combined organic extracts were washed with brine (50 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude material was purified by flash chromatography (PE/Et₂O = 2.5:1 to 1:1) to provide cyclic hemiketals **38** (264.3 mg, 0.65 mmol, 45%) and **39** (326.4 mg, 0.80 mmol, 55%) as a colourless oil. **38**: *R*_f = 0.40 (PE/Et₂O = 2:1); ¹H-NMR (500 MHz, CDCl₃): δ = 1.62 (s, 3H), 1.76 (s, 3H), 1.80-2.00 (m, 4H), 2.10-2.14 (m, 1H), 2.18-2.24 (m, 1H), 2.27-2.34 (m, 2H), 2.72-2.75 (m, 2H), 3.35 (d, ³J = 7.17 Hz, 2H), 4.11-4.15 (m, 1H), 4.22-4.27 (m, 1H), 4.93 (d, ³J = 10.07 Hz, 1H), 5.00 (d, ³J = 17.09 Hz, 1H), 5.24 (t, ³J = 7.17 Hz, 1H), 5.43 (t, ³J = 7.32 Hz, 1H), 5.73-5.81 (m, 1H), 7.15-7.29 (m, 5H); ¹³C-NMR (125 MHz, CDCl₃): δ = 16.2, 16.4, 30.9, 31.1, 32.3, 34.3, 35.9, 44.8, 62.6, 68.5, 68.7, 68.8, 69.0, 97.8, 98.0, 98.2, 112.1, 114.1, 114.2, 114.5, 116.1, 124.1, 124.6, 125.9, 128.3, 128.5, 132.5, 136.6, 137.0, 141.4; [α]_D = +22.9 (c = 3.85, DCM); HRMS (ESI): *m/z* calc. for C₂₄H₃₂O₃F₂Na [M+Na]⁺: 429.2217, found: 429.2214. **39**: *R*_f = 0.16 (PE/Et₂O = 2:1); ¹H-NMR (700 MHz, CDCl₃): δ = 1.50-1.56 (m, 1H), 1.57 (s, 3H), 1.78 (s, 3H), 1.92-2.03 (m, 3H), 2.11 (dd, ²J = 13.77 Hz, ³J = 5.81 Hz, 1H), 2.18-2.22 (m, 1H), 2.27 (dd, ²J = 13.77 Hz, ³J = 7.53 Hz, 1H), 2.32-2.36 (m, 1H), 2.71 (t, ³J = 6.67 Hz, 2H), 3.34-3.35 (d, ³J = 7.31 Hz, 2H), 4.06-4.09 (m, 1H), 4.21-4.26 (m, 1H), 4.92 (d, ³J = 10.11 Hz, 1H), 4.98 (d, ³J = 16.99 Hz, 1H), 5.21-5.23 (m, 1H), 5.47 (t, ³J = 7.31 Hz, 1H), 5.72-5.78 (m, 1H), 7.13-7.28 (m, 5H); ¹³C-NMR (125 MHz, CDCl₃): δ = 16.0, 16.4, 29.7, 31.7, 32.3, 34.4, 37.4, 45.0, 66.3, 66.5, 66.6, 66.7, 97.5, 97.7, 97.7, 97.9, 114.5, 115.6, 117.5, 117.6, 119.6, 124.8, 125.4, 126.0, 128.3, 128.6, 132.4, 136.7, 137.0, 141.1; [α]_D = +24.0 (c = 4.07, DCM); HRMS (ESI): *m/z* calc. for C₂₄H₃₂O₃F₂Na [M+Na]⁺: 429.2217, found: 429.2215.

(E)-1-((4*R*,6*R*)-2,2-dimethyl-6-((*E*)-2-methylhexa-2,5-dien-1-yl)-1,3-dioxan-4-yl)-1,1-difluoro-5-methyl-7-phenylhept-5-en-2-one (41)

To a solution of cyclic hemiketal **38** (25.4 mg, 62 μmol) in DCM (0.7 mL) was added 2,2-Dimethoxypropane (150 μL, 1.22 mmol) followed by addition of CSA (4.0 mg, 17 μmol) at 0 °C. The reaction was warmed to the room temperature and stirred for 18 h. After Et₃N (6 μL) was added, the mixture was quenched with a mixture of pH 7 buffer solution (0.5 mL), DCM (2 mL) and water (2 mL). The aqueous layer was extracted with DCM (2 × 3 mL) and the combined organic extracts were dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude material was purified by flash chromatography (PE/Et₂O = 25:1) to provide acetone **41** (23.6 mg, 53 μmol, 85%) as a colorless oil. *R*_f = 0.28 (PE/Et₂O = 22:1); ¹H-NMR (500 MHz, CD₃OD): δ = 1.31 (s, 3H), 1.39-1.46 (m, 1H), 1.41 (s, 3H), 1.58-1.61 (dt, ²J = 12.82 Hz, ⁴J = 2.59 Hz, 1H), 1.68 (s, 3H), 1.77 (s, 3H), 2.13-2.17 (m, 1H), 2.25-2.29 (m, 1H), 2.35-3.38 (t, ³J = 7.32 Hz, 2H), 2.79-2.81 (m, 2H), 2.85-2.89 (m, 2H), 3.38-3.39 (d, ³J = 7.32 Hz, 2H), 4.10-4.15 (m, 1H), 4.44-4.52 (m, 1H), 4.95-4.98 (m, 1H), 5.03-5.07 (m, 1H), 5.26-5.29 (m, 1H), 5.38-5.42 (m, 1H), 5.80-5.88 (m, 1H), 7.15-7.29 (m, 5H); ¹³C-NMR (125 MHz, CD₃OD): δ = 16.5, 16.6, 19.8, 29.3, 30.2, 33.2, 33.2, 35.1, 38.2, 47.6, 68.2, 71.0, 71.2, 71.3, 71.5, 100.7, 114.8, 114.9, 116.8, 116.8, 118.8, 125.3, 125.8, 126.9, 129.4, 129.5, 133.7, 135.7, 138.2, 142.7, 202.0, 202.2, 202.3, 202.5; [α]_D = +16.3 (c = 2.19, DCM). HRMS (ESI): *m/z* calc. for C₂₇H₃₆O₃F₂Na [M+Na]⁺: 464.2530, found: 464.2530.

(E)-1-((4S,6R)-2,2-dimethyl-6-((E)-2-methylhexa-2,5-dien-1-yl)-1,3-dioxan-4-yl)-1,1-difluoro-5-methyl-7-phenylhept-5-en-2-one (42)

To a solution of cyclic hemiketal **39** (8.4 mg, 21 μ mol) in DCM (0.5 mL) was added 2,2-dimethoxypropane (86 μ L, 700 μ mol) followed by addition of a solution of CSA (0.55 mg, 2.3 μ mol) in DCM (0.1 mL). The reaction was warmed to the room temperature and stirred for 22 h. After Et₃N (5 μ L) was added, the mixture was quenched with a mixture of pH 7 buffer solution (0.5 mL), DCM (2 mL) and water (2 mL). The aqueous layer extracted with DCM (2 $\times$ 2 mL) and the combined organic extracts were washed with brine (7 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude material was purified by flash chromatography (PE/Et₂O = 20:1) to provide acetone **42** (4.8 mg, 11 μ mol, 51%) as a colorless oil. *R*_f = 0.48 (PE/Et₂O = 9:1); ¹H-NMR (700 MHz, CD₃OD): δ = 1.28 (s, 3H), 1.30 (s, 3H), 1.64-1.69 (m, 1H), 1.67 (s, 3H), 1.77 (s, 3H), 2.05-2.09 (m, 1H), 2.18-2.20 (m, 1H), 2.27-2.30 (m, 1H), 2.36-2.38 (m, 2H), 2.79-2.81 (t, ³J = 6.67 Hz, 2H), 2.87-2.89 (m, 2H), 3.38-3.39 (d, ³J = 7.53 Hz, 2H), 3.98-4.02 (m, 1H), 4.31-4.37 (m, 1H), 4.96-4.98 (m, 1H), 5.03-5.06 (m, 1H), 5.26-5.29 (m, 1H), 5.39-5.42 (m, 1H), 5.81-5.86 (m, 1H), 7.15-7.29 (m, 5H); ¹³C-NMR (175 MHz, CD₃OD): δ = 16.4, 16.5, 24.9, 24.9, 31.1, 33.2, 35.1, 37.9, 46.7, 66.7, 68.0, 68.2, 68.2, 68.4, 102.5, 114.8, 115.8, 117.2, 117.2, 118.7, 125.4, 125.6, 126.9, 129.4, 129.5, 134.0, 135.6, 138.2, 142.7, 202.0, 202.2, 202.2, 202.3. [α]_D = +31.4 (c = 0.44, DCM); HRMS (ESI): *m/z* calc. for C₂₇H₃₆O₃F₂Na [M+Na]⁺: 464.2530, found: 464.2531.

(Z)-((2S,4R,6R)-3,3-difluoro-2-hydroxy-2-((E)-3-methyl-5-phenylpent-3-en-1-yl)-6-((E)-2-methylhexa-2,5-dien-1-yl)tetrahydro-2H-pyran-4-yl) 5-((tert-butyldimethylsilyl)oxy)-3-iodopent-2-enoate (43)

To a solution of vinyl carboxyl acid **11** (540.2 mg, 1.52 mmol) in toluene (5 mL) was added NEt₃ (254.0 μ L, 1.82 mmol) followed by 2, 4, 6-trichlorobenzoyl chloride (284.0 μ L, 1.82 mmol) at 0 °C. After stirring for 2 h, a solution of alcohol **38** (228.3 mg, 0.56 mmol) and DMAP (22.5 mg, 0.18 mmol) in toluene (2.5 mL) were added. After stirring for 1 h, additional DMAP (22.8 mg, 0.19 mmol) was added and the reaction was stirred at 0 °C for 2 h. The mixture was quenched with a solution of Ether (5 mL) and 3% aqueous NaHCO₃ (5 mL), then followed by addition of pH 7 buffer solution (10 mL) and ether (10 mL). The phases were separated and the aqueous phase was extracted with ether (3 $\times$ 15 mL). The combined organic extracts were washed with brine (25 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was purified by flash chromatography (PE/Et₂O = 7:1 with 0.5% Et₃N) to give ester **43** (313.6 mg, 0.42 mmol, 75%) as a colourless oil. *R*_f = 0.35 (PE/Et₂O = 7:1); ¹H-NMR (500 MHz, CDCl₃): δ = 0.05 (s, 6H), 0.86 (s, 9H), 1.61 (s, 3H), 1.75 (s, 3H), 1.80-1.97 (m, 4H), 2.08-2.33 (m, 4H), 2.71-2.78 (m, 2H), 2.88-2.94 (m, 2H), 3.35 (d, ³J = 6.87 Hz, 2H), 3.79 (t, ³J = 6.10 Hz, 2H), 4.21-4.26 (m, 1H), 4.91-5.01 (m, 2H), 5.21-5.24 (m, 1H), 5.36-5.43 (m, 2H), 5.72-5.80 (m, 1H), 6.49 (s, 1H), 7.15-7.28 (m, 5H); ¹³C-NMR (125 MHz, CDCl₃): δ = -5.3, 16.2, 16.5, 18.2, 25.9, 31.3, 31.7, 32.3, 33.8, 34.3, 44.6, 51.3, 61.0, 63.2, 68.5, 68.7, 68.9, 69.0, 96.9, 97.1, 97.3, 111.5, 113.4, 113.6, 114.5, 115.5, 120.4, 123.9, 124.8, 125.7, 125.8, 128.3, 128.4, 132.3, 136.4, 136.9, 141.5, 162.2; [α]_D = -17.8 (c = 3.15, DCM); HRMS (ESI): *m/z* calc. for C₃₅H₅₁O₅F₂ISiNa [M+Na]⁺: 767.2416, found: 767.2413.

2-((1R,4E,7E,10E,13R,15S)-16,16-difluoro-15-hydroxy-11-methyl-15-((E)-3-methyl-5-phenylpent-3-en-1-yl)-3-oxo-2,14-dioxabicyclo[11.3.1]heptadeca-4,7,10-trien-5-yl)acetic acid (45)

To a solution of alcohol (33.0 mg, 63.9 μmol) in THF (1.5 mL) at 0 °C was added Dess-Martin periodinane (123.6 mg, 291.4 μmol). The reaction mixture was stirred at room temperature for 80 minutes. Then 2,3-dimethyl-2-butene (0.5 mL) and *tert*-BuOH (1.5 mL) were added at 0 °C, followed by a solution NaClO₂ (28.8 mg, 318.4 μmol) and NaH₂PO₄·H₂O (56.4 mg, 408.7 μmol) in water (1.2 mL). The mixture was stirred at room temperature for 40 min before saturated aqueous NH₄Cl (10 mL) and EtOAc (10 mL) were added. The phases were separated and the aqueous phase was extracted with EtOAc (3 $\times$ 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was purified by HPLC (VarioPrep 21 $\times$ 250 mm Nucleodur 5 μ C-18 (Machery-Nagel) column; eluent A: 5% CH₃CN with 1% formic acid in H₂O; eluent B: CH₃CN; linear gradient 20-85% B in 30 min, then 85-95% B in 20 min at 10 mL/min flow rate; *t_R*=23.3 min) to give carboxylic acid **45** (9.4 mg, 17.7 μmol , 28%) as a colorless oil. *R_f* = 0.39 (DCM/MeOH = 10:1); ¹H-NMR (700 MHz, CD₃OD): δ = 1.56-1.61 (m, 4H), 1.73 (s, 3H), 1.76-1.84 (m, 2H), 1.88-1.92 (m, 1H), 2.19-2.27 (m, 2H), 2.34-2.37 (m, 2H), 2.51-2.58 (m, 2H), 2.63-2.68 (m, 1H), 3.13-3.20 (m, 2H), 3.34-3.35 (d, ³J = 7.53 Hz, 2H), 3.92-3.95 (dd, ³J = 13.34 Hz, ³J = 6.02 Hz, 1H), 4.06-4.10 (m, 1H), 4.90-4.93 (m, 1H), 5.14-5.20 (m, 2H), 5.34-5.41 (m, 2H), 5.93 (s, 1H), 7.11-7.24 (m, 5H); ¹³C-NMR (125 MHz, CD₃OD): δ = 16.5, 18.5, 31.3, 32.8, 33.2, 35.2, 35.3, 35.6, 43.2, 45.3, 67.1, 71.4, 71.6, 71.7, 71.9, 98.5, 98.7, 98.7, 98.9, 114.3, 116.3, 116.5, 118.4, 120.5, 124.6, 126.5, 126.9, 127.8, 129.4, 129.5, 129.9, 133.9, 137.2, 143.0, 154.2, 167.0, 173.6; [α]_D = -33.7 (*c* = 0.92, DCM); HRMS (ESI): *m/z* calc. for C₃₀H₃₆O₆F₂Na [M+Na]⁺: 553.2378, found: 553.2379.

2-((3E,6E,9E,12R,14R)-14-((R,E)-1,1-difluoro-2-hydroxy-5-methyl-7-phenylhept-5-en-1-yl)-12-hydroxy-10-methyl-2-oxooxacyclotetradeca-3,6,9-trien-4-yl)acetic acid (46) and 2-((3E,6E,9E,12R,14R)-14-((S,E)-1,1-difluoro-2-hydroxy-5-methyl-7-phenylhept-5-en-1-yl)-12-hydroxy-10-methyl-2-oxooxacyclotetradeca-3,6,9-trien-4-yl)acetic acid (47)

To a suspension of NaBH₄ (2.00 mg, 52 μmol) and K₂CO₃ (1.60 mg, 12 μmol) in EtOH (200 μL) and H₂O (100 μL) at 0 °C was added a solution of **45** (6.80 mg, 13 μmol) in THF (200 μL). After stirring at 0 °C for 4.5 h, additional NaBH₄ (2.00 mg, 52 μmol) was added. The reaction was warmed to the room temperature and stirred for 1 h. The mixture was diluted with water (1 mL) and acidified with 0.1 M HCl to the pH 6. Then the mixture was quenched with EtOAc (3 $\times$ 3 mL). The combined organic layers were washed with brine (2 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue was purified by HPLC (VarioPrep 21 $\times$ 250 mm Nucleodur 5 μ C-18 (Machery-Nagel) column; eluent A: 5% CH₃CN with 1% formic acid in H₂O; eluent B: CH₃CN; 60% B in 5 min, then linear gradient 60-80% B in 35 min at 10 mL/min flow rate; *t_R*=27.1 min and 32.7 min) to give carboxylic acid **46** (1.76 mg, 3.3 μmol , 25%) and **47** (0.85 mg, 1.6 μmol , 12%) as a colorless oil. **47**: *R_f* = 0.33 (DCM/MeOH = 7:1); ¹H-NMR (700 MHz, CD₃OD): δ = 1.60-1.62 (m, 1H), 1.61 (s, 3H), 1.77 (s, 3H), 1.71-1.86 (m, 2H), 2.03-2.07 (m, 2H), 2.13-2.15 (m, 2H), 2.32-2.34 (m, 1H), 2.57-2.61 (m, 1H), 2.70-2.75 (m, 2H), 3.19-3.25 (m, 2H), 3.39-3.40

(d, $^3J = 7.31$ Hz, 2H), 3.89-3.92 (m, 1H), 3.99-4.02 (m, 1H), 4.07-4.12 (m, 1H), 5.13-5.18 (m, 1H), 5.24-5.26 (t, $^3J = 7.31$ Hz, 1H), 5.37-5.41 (m, 1H), 5.42-5.44 (t, $^3J = 7.31$ Hz, 1H), 5.49-5.53 (m, 1H), 5.85 (s, 1H), 7.17-7.30 (m, 5H); HRMS (ESI): m/z calc. for $C_{30}H_{38}O_6F_2Na$ $[M+Na]^+$: 555.2534, found: 555.2536. **46**: $R_f = 0.25$ (DCM/MeOH = 7:1); 1H -NMR (700 MHz, CD_3OD): $\delta = 1.56$ (s, 3H), 1.63-1.68 (m, 1H), 1.78-1.81 (m, 1H), 1.79 (s, 3H), 1.86-1.91 (m, 1H), 2.01-2.05 (m, 1H), 2.14-2.26 (m, 3H), 2.32-2.36 (m, 1H), 2.52-2.54 (m, 1H), 2.61-2.65 (m, 1H), 2.65-2.69 (m, 1H), 3.18-3.28 (m, 2H), 3.38-3.43 (m, 2H), 3.82-3.86 (m, 1H), 3.94-3.97 (m, 1H), 3.99-4.03 (m, 1H), 5.34-5.46 (m, 5H), 5.77 (s, 1H), 7.16-7.29 (m, 5H); ^{13}C -NMR (125 MHz, CD_3OD): $\delta = 16.2, 16.8, 28.6, 32.1, 34.4, 35.1, 35.6, 36.4, 49.5, 50.9, 65.3, 68.7, 68.9, 69.0, 69.1, 69.8, 69.9, 70.1, 119.7, 121.8, 123.3, 124.7, 125.2, 126.4, 126.8, 129.3, 129.4, 130.7, 135.6, 136.5, 142.9, 158.0, 165.5, 190.7$; HRMS (ESI): m/z calc. for $C_{30}H_{38}O_6F_2Na$ $[M+Na]^+$: 555.2534, found: 555.2537.

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Figure 1.
Natural products targeting “switch region” of RNA-polymerase.

Scheme 1.

Synthesis of desoxyripostatin A via one-pot carboalumination/cross-coupling. Cp = cyclopentadienyl, Bn = benzyl, TBAF = tetra-*n*-butylammonium fluoride, DIAD = diisopropyl azodicarboxylate, **11** = 1,1,1,3,3,3-hexafluoro-4,4-dimethyl-2-pyridylidene-1,2,3,4-tetrahydro-5H-pyrimidin-5-one, PPTS = pyridinium *p*-toluenesulfonate, DMP = Dess-Martin periodinane, Grubbs II = [1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium, TBS = *t*-butyldimethylsilyl.

Scheme 2.

Synthesis of alkene-terminated acrylate fragments. DMAP = 4-dimethylaminopyridine, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, dppf = 1,1'-bis(diphenylphosphino)ferrocene, TBS = *t*-butyldimethylsilyl.

Scheme 3.

Synthesis of intramolecular Suzuki cross-coupling. DIAD = diisopropyl azodicarboxylate, 9-BBN = 9-Borabicyclo[3.3.1]nonane, TBS = *t*-butyldimethylsilyl.

Scheme 4.

Attempted macrolactonisation approach. 9-BBN = 9-Borabicyclo[3.3.1]nonane, TBS = *t*-butyldimethylsilyl.

Scheme 5.

Alternative intramolecular Suzuki cross-coupling approach. TCBC = 2,4,6-trichlorobenzoyl chloride, DMAP = 4-dimethylaminopyridine, dppf = 1,1'-bis(diphenylphosphino)ferrocene, 9-BBN = 9-Borabicyclo[3.3.1]nonane.

Scheme 6.

Completion of 5,6-dihydro-ripostatin A (**25**). DMP = Dess-Martin periodinan.

Scheme 7.

Synthesis of difluoroketone building block. EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

Scheme 8.
Attempted aldol reaction with aldehyde **33**.

Scheme 9.

Synthesis and stereochemical assignment of cyclic hemiketals by aldol reaction with difluorochloroketone **32**. CSA = 10-camphorsulfonic acid, DIAD = diisopropyl azodicarboxylate, TBS = *t*-butyldimethylsilyl.

Scheme 10.

Completion of difluoro-ripostatin A. TBS = *t*-butyldimethylsilyl, TCBC = 2,4,6-trichlorobenzoyl chloride, DMAP = 4-dimethylaminopyridine, DMP = Dess-Martin periodinane, Grubbs II = [1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium.

Scheme 11.
Synthesis of difluoro-ripostatin B and its epimer.

Table 1

RNAP-inhibitory activities.

compound	IC50 <i>Escherichia coli</i> RNAP (μ M)	IC50 <i>Staphylococcus aureus</i> RNAP (μ M)	IC50 <i>Mycobacterium tuberculosis</i> RNAP (μ M)
ripostatin A (1)	0.026	0.88	270
ripostatin B (2)	0.018	0.081	300
15-desoxy-ripostatin A (10)	>25	160	70
5,6-dihydro-ripostatin A (25)	0.11	nt ^a	nt
14,14'-difluororipostatin A (45)	38	65	68
14,14'-difluororipostatin B1 (46)	0.035	0.068	3000
14,14'-difluororipostatin B2 (47)	3.1	45	200

^a nt, not tested.

Table 2

Antibacterial activities.

compound	MIC <i>Escherichia coli</i> D21f2tolC (µg/ml)	MIC <i>Staphylococcus aureus</i> ATCC 12600 (µg/ml)
ripostatin A (1)	0.39	12.5.
ripostatin B (2)	0.39	6.25
15-desoxy-ripostatin A (10)	12.5	>50
14,14'-difluororipostatin A (45)	12.5	50
14,14'-difluororipostatin B1 (46)	0.78	12.5
14,14'-difluororipostatin B2 (47)	6.25	>50