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Function-Oriented Synthesis toward Peloruside A Analogues

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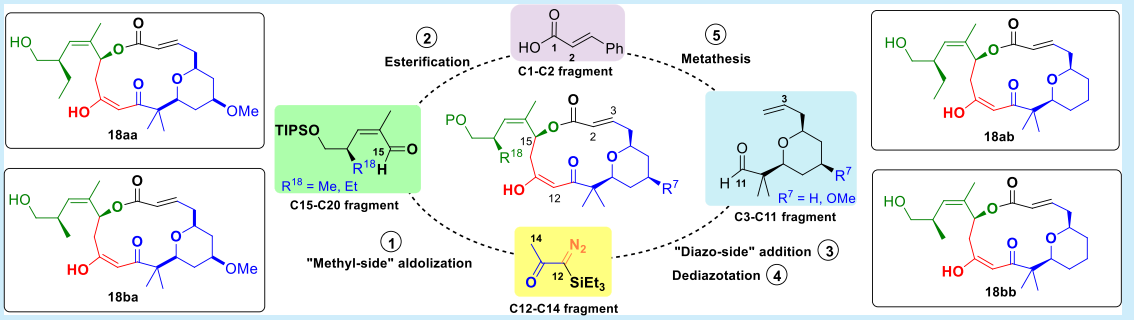
Supporting Information



ABSTRACT: A convergent and rapid synthesis of original C2,C3-unsaturated, C11,C13-keto−enol macrocycles with a peloruside A skeleton has been developed. These original unsaturated macrocycles constitute valuable platforms to access peloruside A analogues with high diversity. The four-fragment strategy implemented features two aldol-type couplings with the central C12−C14 building block TES-diazoacetone and a late-stage ring-closing metathesis. Enantiopure analogue **18ab** showed antiproliferative activity in the low micromolar range on NCI and MCF7 tumor cell lines.

Peloruside A (PelA) is a highly cytotoxic 16-membered macrolide, extracted in 2000 from the New Zealand marine sponge *Mycale hentscheli* (Figure 1).[1] Natural congener peloruside B was later discovered, displaying similar cytotoxicity.[2] Like paclitaxel, PelA acts as a potent microtubule stabilizing agent, stopping cell division in the G2/M phase.[3] Due to its unique nontaxoid binding-site on tubulin β,[4] PelA displays exciting properties like synergistic effects with taxanes and preserved efficiency in paclitaxel and epothilone-resistant cell lines.[5] More recently, PelA proved highly efficient in targeting tumoral angiogenesis.[6] Preliminary investigations on its potential role on neurodegenerative and autoimmune disorders also led to encouraging results.[7] Despite its promising pharmacological profile, preclinical trials on PelA stagnate due to short supply of the natural product.[7,8]

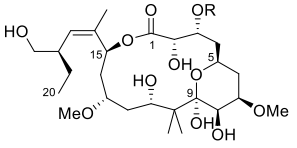


Figure 1. Structures of the marine sponge macrolides peloruside A (PelA) and peloruside B.

Six total syntheses have been achieved so far, providing enantiopure peloruside A on a milligram scale.[9] Around 20 analogues were reported, including natural congeners, analogues produced by hemisynthesis or resulting from total synthesis.[10] Our previous work in this field had resulted in the convergent total synthesis of a simplified C2,C3-unsaturated analogue, displaying antiproliferative activity around 15 μM on three tumor cell lines.[11] The available data on the structure−activity relationship (SAR) of PelA have been recently reviewed.[10a] Key structural elements have emerged, including the primary alcohol of the lateral chain and the embedded tetrahydropyran skeleton, though the influence of each

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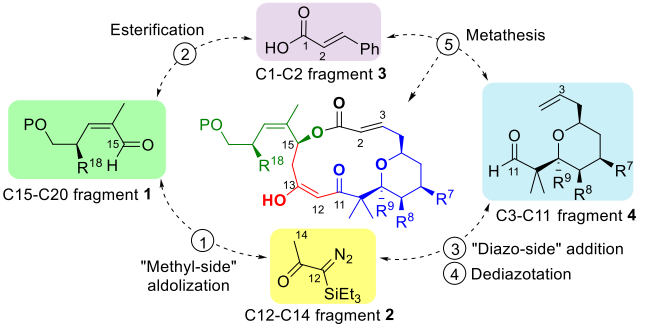
oxygenated substituent on this heterocycle is still unclear. Additionally, the impact of each oxygenated functionality along the macrolactone skeleton has not been systematically explored. The crystallographic structure of a PelA/tubulin complex was resolved in 2014,[4a] as well as dually bound PelA/Taxol to microtubules,[4b] providing structural information on the binding mode. Despite this propitious context, synthetic studies toward new PelA analogues have been scarcely reported since 2014.[10b,12] The lack of SAR study is undoubtedly due to the structural complexity of PelA, requiring challenging synthetic work for each single analogue.

In this context, the development of a convergent synthetic strategy, allowing the late-stage functionalization of a synthetic platform, would be of high value. In 2012, Taylor's team reported a pioneering work in this field.[13] Their strategy relied on a ring-closing olefin metathesis (RCM) step in order to elaborate *Z*- and *E*-C12,C13 unsaturated PelA skeletons in 12 steps. Analogues resulting from functionalization of these synthetic platforms were not biologically evaluated due to chemical instability.

In the present work, we describe a four-fragment-based approach for the function-oriented synthesis of PelA analogues via the elaboration of stable C2,C3-unsaturated macrocyclic platforms (platform approach). Our convergent strategy relies on the five-step assembly of four fragments (Scheme 1). At

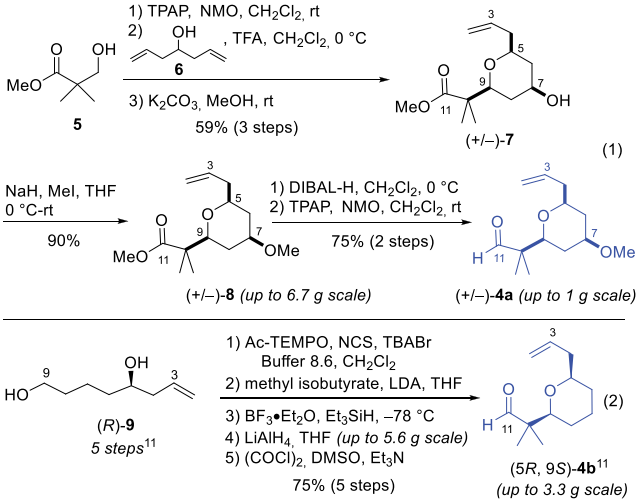
Scheme 1. Four-Fragment Diazo Strategy toward C2,C3-Unsaturated, C11,C13 Keto−Enol Macrocyclic Platforms



first, C15−C20 fragment **1** and C12−C14 fragment triethylsilyldiazoacetone **2** (TES-diazoacetone) would be coupled by "methyl-side" aldolization (step 1), followed by esterification of cinnamic acid (C1−C2 fragment **3**) with the resulting C-15 alcohol (step 2). Assembly of C3−C11 fragment **4** would be carried out by "diazo-side" addition (step 3), followed by dediazotation/1,2-H migration (step 4). A final RCM step would lead to the expected C2−C3 unsaturated macrocycles (step 5). This "diazo route" illustrates the synthetic potential of the sequential double cross-aldolization methodology we had previously developed on TES-diazoacetone.[14] Both the C11,C13 keto−enol moiety and the C2,C3 double bond constitute an attractive gateway to diversity on the macrolactone skeleton. Moreover, diversity could be generated through the modulation of the substituents R^7, R^8, and R^9 on fragment **4** and R^{18} on fragment **1**.

In order to enrich the SAR of PelA and to access potentially active simplified analogues, our diazo strategy was implemented with racemic C3−C11 fragment (±)-**4a** (R^7 = OMe, R^8 = R^9 = H) and enantiopure C3−C11 fragment (5*R*,9*S*)-**4b**[11] (R^7 = R^8 = R^9 = H) (Scheme 2). Original fragment (±)-**4a** was selected on the basis of crystallographic data,

Scheme 2. Synthesis of C3−C11 Fragments (±)-4a and (5*R*,9*S*)-4b[11]



underlying the involvement of the C7-methoxy group in stabilizing lateral contact interactions with a second tubulin unit.[4] The synthesis of aldehydic fragment (±)-**4a** could be readily carried out in six steps (Scheme 2, eq 1). Convergent elaboration of the THP moiety was achieved through Prins cyclization, induced by excess trifluoroacetic acid,[15] between the aldehyde derived from alcohol **5** and commercial bis-homoallylic alcohol **6**. Methanolysis of the trifluoroacetate intermediate provided the racemic 7-hydroxytetrahydropyran (±)-**7** in 65% yield as a single 5,7-*cis*/5,9-*cis* diastereoisomer. Methylation of the resulting alcohol occurred in high yield, leading to tetrahydropyran (±)-**8**, on which the equatorial position of all substituents was clearly deduced from ^{1}H NMR analysis ($^3J_{6ax\text{-}5}$ = $^3J_{6ax\text{-}7}$ = 11.5 Hz; $^3J_{8ax\text{-}9}$ = 11.7 Hz). Aldehyde (±)-**4a** resulted from a classical reduction/oxidation sequence on methyl ester (±)-**8**. This straightforward synthesis could be conducted on a multigram scale. Enantiopure aldehyde (5*R*,9*S*)-**4b**[11] (Scheme 2, eq 2) was also selected as a valuable simplified C3−C11 fragment. Indeed, when the PelA polyoxygenated THP was replaced by the 7,8,9-trideoxy THP moiety, the resulting simplified analogue retained a hundred nanomolar range cytotoxity.[16] Preparation of enantiopure aldehyde (5*R*,9*S*)-**4b** was performed on a multigram scale, via intermediate (*R*)-**9**, according to the 10-step strategy developed in the course of our previous study.[11]

pro-Peloruside C15−C20 fragment (*R*)-**1a**[17] and *nor*-methyl C15−C19 fragment (*R*)-**1b** were selected to implement our strategy (Scheme 3). A robust nine-step synthesis of *pro*-peloruside fragment (*R*)-**1a** was reported by Taylor's team[17] based on a chiral oxazolidinone-controlled alkylation to produce intermediate (*R*)-**10** in five steps and a (*Z*)-selective Still−Gennari olefination. The above strategy was carried out, using an alternative Ando-type procedure[18a] with diaryl phosphonate **12**,[18b,c] to generate the trisubstituted alkene in high yield with a unique (*Z*)-geometry (Scheme 3, eq 1). C15−C20 fragment (*R*)-**1a** could thus be readily synthesized on a multigram scale.

nor-Methyl fragment (*R*)-**1b** (Scheme 3, eq 2) was elaborated in a context where structural modification on the C-18 residue has no precedent in the literature. Original aldehyde (*R*)-**1b**, while retaining a structure very close to the natural side chain, could advantageously be more readily

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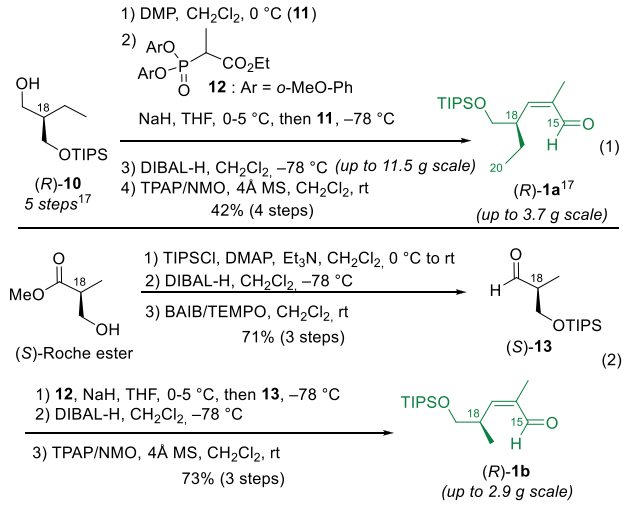
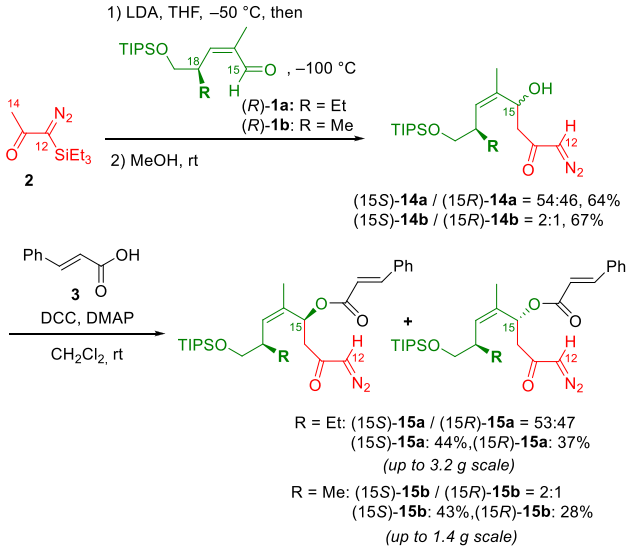
Scheme 3. Synthesis of C15−C20 Fragment (*R*)-1a[17] and *nor*-Methyl C15−C19 Fragment (*R*)-1b



synthesized in only six steps from (*S*)-Roche ester. This commercially available precursor was first transformed into the corresponding TIPS-protected aldehyde (*S*)-**13** in three steps. The latter underwent *Z*-selective olefination with phosphonate **12** to provide exclusively the expected (*Z*)-trisubstituted alkene. A reduction/oxidation sequence of the ester moiety led to the targeted aldehyde (*R*)-**1b**. This synthesis could be achieved on a multigram scale in 57% global yield over six steps.

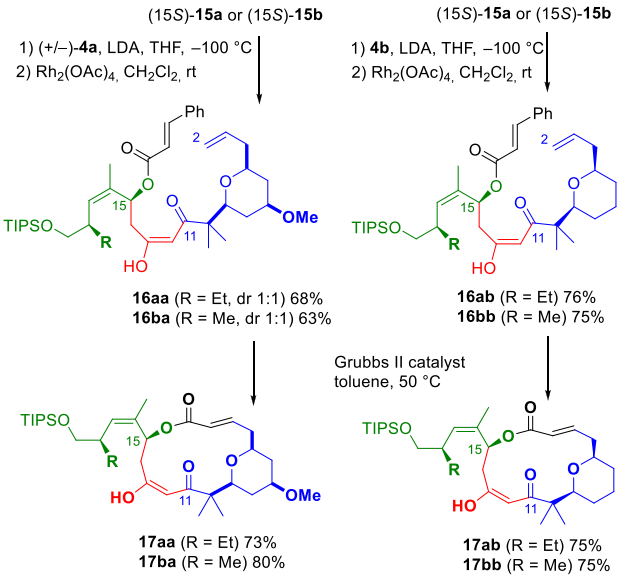
Coupling of C15−C20 fragments **1** with TES-diazoacetone **2** (C12−C14 fragment)[14] and cinnamic acid **3** (C1−C2 fragment) was then performed (Scheme 4).

Scheme 4. Coupling between C12−C14, C15−C20, and C1−C2 Fragments



At first, low-temperature LDA-induced methyl-side aldolizations were performed between aldehydes (*R*)-**1a** or (*R*)-**1b** and TES-diazoacetone **2**, prepared in three steps on a multigram scale.[19] Desilylation of any remaining C-protected diazoaldols was ensured by methanolysis on the crude products, providing aldols **14a** (R = Et) and **14b** (R = Me) as inseparable mixtures of two diastereoisomers. Absolute configurations of major (15*S*)-**14** and minor (15*R*)-**14** diastereoisomers were attributed on the basis of Mosher's esters study on the diastereoisomeric mixtures (see the SI).[20] Pleasingly, esterification of cinnamic acid **3** with alcohols **14a** and **14b** under Steglich conditions allowed clean separation of the resulting (15*S*)-**15** and (15*R*)-**15** diastereoisomers in both series. The cinnamic moiety constituted in our hands a stable surrogate to the acrylic moiety, whose introduction failed whatever the conditions used (acryloyl chloride/DIPEA or acrylic acid/DCC/DMAP). Assembly of C3−C11 pyran fragments (±)-**4a** and (5*R*, 9*S*)-**4b** with diazoketones (15*S*)-**15a** and (15*S*)-**15b** was then carried out via low-temperature, LDA-induced, diazoside addition (Scheme 5).[21] This step constituted a key feature

Scheme 5. Coupling of C3−C11 Fragments 4 and Ring-Closing of the Macrocyclic Platforms



in the establishment of our diazo strategy, as it was known to be particularly suitable for hindered tetrahydropyranic aldehydes.[14] Reaction time and temperature were optimized in order to prevent simultaneous elimination of the cinnamic ester moiety (leading to the unwilled C14,C15-unsaturated, highly conjugated system). The mixture of diastereoisomers resulting from this coupling was directly subjected to a dediazotation/1,2-H migration process catalyzed by $Rh_2(OAc)_4$,[14,22] leading to a diastereomeric mixture of keto−enols **16aa** and **16ba** and to enantiopure keto−enols **16ab** and **16bb**. The tautomeric structure of the sole keto−enols **16** observed in $CDCl_3$ was deduced from HMBC experiments (see the SI). Ring-closing metatheses of dienes **16** were best carried out using Grubbs II catalyst in toluene (10^{-3} M) at 50 °C, providing macrocyclic platforms **17aa** and **17ba** as a 1:1 mixture of two diasteroisomers[23] and enantiopure macrocycles **17ab** and **17bb** in good yields. The C2,C3 double bond was generated with a unique (*E*)-geometry ($^3J_{2-3}$ = 15.8 Hz). The convergent diazo strategy was thus validated with the elaboration of stable macrocyclic platforms **17aa**−**17bb**, involving 12 or 15 steps for the longest linear sequence (from a commercially available substrate), on a 180−500 mg scale.

Finally, the deprotection of the primary hydroxyl group on the lateral side chain of macrocyclic platforms **17** was achieved using $Et_3N{\cdot}3HF$[24] or TBAF/AcOH,[25] leading to the

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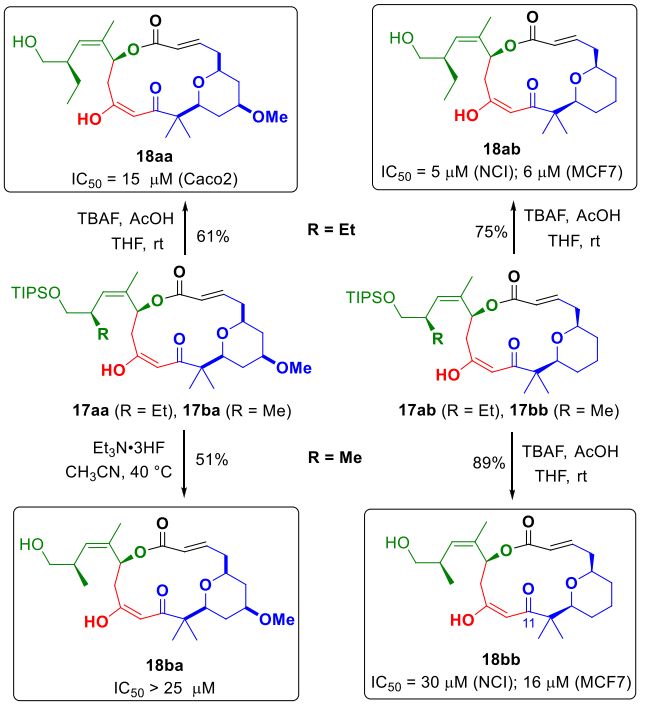
corresponding original peloruside A unsaturated analogues **18** (Scheme 6).[23] In vitro cytotoxic properties of these

Scheme 6. Deprotection of the Macrocyclic Platforms and Significant in Vitro Cytotoxic Activities



compounds were evaluated on seven tumor cell lines: Huh7 and NCI (liver), Caco2 and HCT116 (lung), PC3 (prostate), and MCF7 and MDA-MB-231 (breast). Enantiopure analogue **18ab**, featuring the ethyl-substituted lateral chain, was active in a low micromolar range on two tumor cell lines (NCI: IC_{50} = 5 μM; MCF7: IC_{50} = 6 μM), while its congener with the methyl chain **18bb** was less active (NCI: IC_{50} = 30 μM; MCF7: IC_{50} = 16 μM). Interestingly, the 7,8,9-trideoxy-THP moiety did not wipe out the cytotoxicity. Therefore, even though our analogues proved only slightly active, this observation is in accordance with Altmann's team results.[16] Analogues **18aa** and **18ba**, although obtained as 1:1 diastereoisomeric mixtures, were also tested, in order to gain a first-order approximation of their cytotoxic properties.[26] Only **18aa** displayed a weak activity (Caco2: IC_{50} = 15 μM).[27]

To conclude, a function-oriented strategy toward original PelA analogues has been developed. The convergent elaboration of stable unsaturated macrocyclic platforms with a PelA skeleton has been achieved, involving 12 or 15 steps in the longest linear synthetic sequence. The four-fragment diazo strategy implemented allows introduction of diversity by modulating the substituents on the C3−C11 and C15−C20 fragments. The low-micromolar cytotoxicity effects of analogue **18ab** is encouraging and seems to highlight the importance of the ethyl residue on the lateral chain. Further functionalization of the macrocyclic platforms is under investigation for positions C2, C3, C11, and C13 to provide a library of PelA analogues in order to enrich the SAR studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b00413.

Synthetic procedures and ^{1}H and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(21) Minor diastereoisomers (15*R*)-**15a** and (15*R*)-**15b**, produced in significant amounts, will constitute valuable C15−C20 fragments for the future elaboration of a library of PelA analogues in order to complete the SAR studies.

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(26) Ongoing synthetic efforts focus on the elaboration of enantiopure C3-C11 fragment **4** in order to access the corresponding stereopure macrocyclic platforms and to provide accurate biological evaluation of analogues **18aa** and **18ba**.

(27) All the IC_{50} values and the protocol used for in vitro assays of cell proliferation inhibition are described in the SI. The compounds were first tested at a unique dose of 25 μM, and IC_{50} was then calculated for the active ones.

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