Natural Product Synthesis

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Total Synthesis of Phoslactomycin B and Its Biosynthetic Deamino Precursor**

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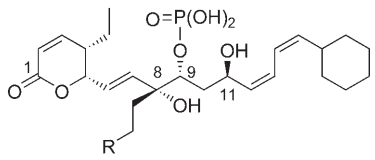
Phoslactomycins A–F and I are a unique class of compounds that contain a phosphate group and an amino group.[1–3] Similar structures are also seen in phosphazomycins C_1 and C_2,[4] leustroducsins A–C and H,[5] and fostriecin.[6] Antitumor, antibacterial, and antifungal activities have been shown for the phoslactomycins.[1a,2a] Inhibitory activity to human protein phosphatase 2A (PP2A) was later disclosed,[7] and the binding site that interacts with the phoslactomycins through the phosphate group was identified.[8] The carboxylate residue on the cyclohexane ring does not affect the activities, whereas the effect of other parts of the molecule on the activities has not been established. Herein, we describe the synthesis of phoslactomycin B (phospholine; **1**) and its deamino precursor **2**.[9]



phoslactomycin B (**1**): R = NH_2 (phospholine)
biosynthetic precursor **2**: R = OH

The synthesis of leustroducsin B was recently reported by Fukuyama and co-workers,[10] who introduced the specific *p*-(TBSO)C_6H_4CHO acetal protecting group (TBS = *tert*-butyldimethylsilyl), which was removed during the last stage of the synthesis, for the C8,9-diol unit. On the other hand, the synthesis of fostriecin has been demonstrated by several groups.[11,12] Thus, we initially attempted the synthesis of **1** by applying the transformations developed for the synthesis of fostriecin. However, such efforts were in vain owing to the inefficiency of the transformations[13] and the unexpected instability of the product.[14] We then examined another method that furnished **1** and **2** efficiently, as outlined below.

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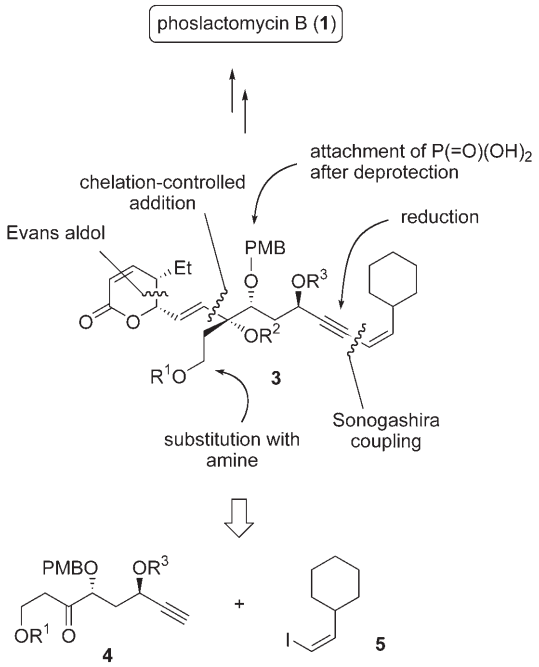
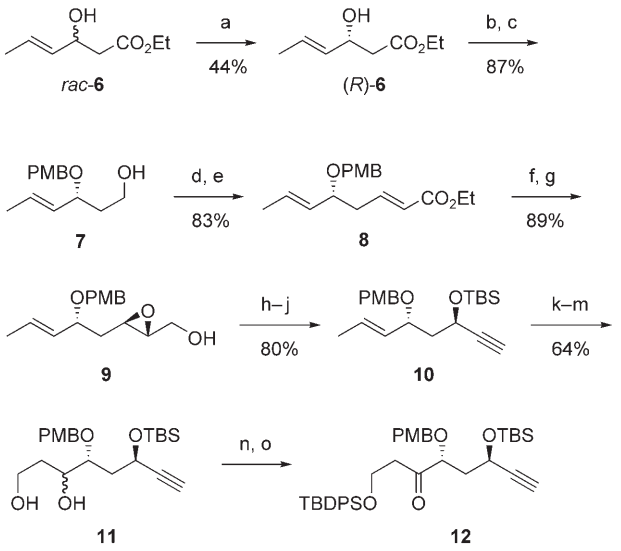
We envisioned that compound **3** would be transformed into **1** by reduction of the triple bond and introduction of the amino and phosphate groups (Scheme 1). To construct **3**, we chose a strategy using chelation-controlled addition of a vinyl anion to ketone **4**, followed by Evans aldol, lactone formation[10] and Sonogashira coupling with vinyl iodide **5**. Thus, the protecting group for the hydroxy group at C9 should be an ether to attain high selectivity in the chelation-controlled reaction and, furthermore, to be discriminated from the other hydroxy groups so as to introduce a phosphate group on it. Among possible candidates, we selected the *p*-methoxybenzyl (PMB) group.[12g]



Scheme 1. Retrosynthetic analysis of phoslactomycin B (**1**). PMB = *p*-methoxybenzyl.

The ketone synthon **4** was obtained in the form of compound **12**, and a Sharpless asymmetric epoxidation[15a,c] was used to install conveniently the hydroxy groups at C9 and C11 (Scheme 2). Thus, kinetic resolution of racemic alcohol *rac*-**6** by the asymmetric epoxidation produced (*R*)-**6** (> 97% *ee* by HPLC analysis of the derived benzoate using a chiral column) in 44% yield after easy separation of the epoxy alcohol coproduct (structure not shown). The hydroxy group of (*R*)-**6** was protected to afford the PMB ether, which was transformed into unsaturated ester **8** through a conventional sequence of reactions. Reduction of **8** with DIBAL-H gave the allylic alcohol, which was subjected to the asymmetric epoxidation[15b,c] to give epoxy alcohol **9** as a single diastereomer in 93% yield. The protocol of Yadav et al.[16] was applied to **9**, and subsequent ozonolysis of the resulting compound **10** produced an aldehyde, which upon aldol reaction with $LiCH_2CO_2Et$ followed by reduction with $LiAlH_4$ afforded diol **11** in 64% yield. Finally, selective protection of the primary alcohol as the TBDPS ether followed by Swern oxidation furnished the key ketone **12**.[17]



Scheme 2. Synthesis of ketone **12** (synthon **4** in Scheme 1). Reagents and conditions: a) *t*BuOOH (1 equiv), $Ti(O\mathit{i}Pr)_4$ (0.25 equiv), L-(+)-DIPT (0.30 equiv), 4Å M.S., −20°C, 30 h, 44%; b) $PMBOC(=NH)CCl_3$ (2 equiv), CSA (3 mol%); c) $LiAlH_4$ (0.7 equiv), 87% for 2 steps; d) $(COCl)_2$ (1.2 equiv), DMSO (3 equiv), Et_3N (4 equiv); e) $(EtO)_2P(O)CH_2CO_2Et$ (1.2 equiv), DBU (1.25 equiv), LiCl (1.3 equiv), MeCN, 83% for 2 steps; f) DIBAL-H (2.2 equiv), THF, 96%; g) *t*BuOOH (1.5 equiv), $Ti(O\mathit{i}Pr)_4$ (0.30 equiv), D-(−)-DIPT (0.36 equiv), 4-Å M.S. −20°C, 93%; h) PPh_3 (1.2 equiv), $NaHCO_3$ (0.22 equiv), CCl_4, 88%; i) *n*BuLi (3.14 equiv), −78°C, THF; j) TBSCl (1.2 equiv), imidazole (2 equiv), 91% for 2 steps; k) O_3, 2,6-lutidine (1.5 equiv), MeOH; l) $LiCH_2CO_2Et$ from LDA (2.1 equiv) and EtOAc (2.1 equiv), −78°C, THF; m) $LiAlH_4$ (1.5 equiv), THF, 64% for 3 steps; n) TBDPSCl (1.2 equiv), imidazole (2 equiv); o) $(COCl)_2$ (1.2 equiv), DMSO (3 equiv), Et_3N (5 equiv). DIPT = diisopropyl tartrate; M.S. = molecular sieves; CSA = (+)-10-camphorsulfonic acid; DMSO = dimethyl sulfoxide; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; DIBAL-H = diisobutylaluminum hydride; TBS = *tert*-butyldimethylsilyl; LDA = lithium diisopropylamide; TBDPS = *tert*-butyldiphenylsilyl.

Construction of the triol **22**, which corresponds to the proposed key intermediate **3** in Scheme 1, was accomplished by the method summarized in Scheme 3. Addition of CH_2=CHMgBr to ketone **12** proceeded cleanly to furnish **13** as the sole product in 87% yield from diol **11**.[18,19] The stereochemistry at C8 was tentatively assigned as depicted, by analogy,[20] and was proved by completion of the synthesis. After protection of the OH group at C8 with TESOTf and 2,6-lutidine, the resulting compound **14** was subjected to ozonolysis in the presence of 2,6-lutidine to produce an aldehyde, which upon Horner–Wadsworth–Emmons reaction furnished α,β-unsaturated ester **15**. Thus, the stage was set for coupling with the remaining partners at the two ends to construct the full carbon skeleton. Installation of the fragment on the right at the acetylene carbon center by Sonogashira reaction[21] with *Z*-vinyl iodide **5** provided enyne **16** in 87% yield. Attachment of the lactone on the left was accomplished by using Evans aldol.[10,22] Thus, ester **16** was reduced to an alcohol and reoxidized to aldehyde **17**. Aldol reaction[23] of **17** with the boron enolate derived from **18** proceeded stereoselectively to afford an aldol, which was converted to lactone **22** by routine reactions through **19**–**21** (see Scheme 3).

Next, zinc-mediated reduction[24] of the triol **22** provided *Z*,*Z*-diene **23** with perfect *cis* selectivity according to ^{1}H NMR spectroscopic analysis. The remaining part of the synthesis involved integration of the amino and phosphate groups on the molecule. As removal of the TES protecting group of the hydroxy group at C8 at the last stage under various conditions was unsuccessful,[14] the more labile TMS group was next studied as the hydroxy-protecting group. Selective protection of the primary and secondary alcohols with TESCl afforded **24** in 85% yield. The remaining tertiary hydroxy group at C8 in **24** was then protected to afford TMS ether **25**, which was exposed to PPTS in THF/MeOH to yield **26** in 53% yield, along with recovered **25** in 38% yield (86% yield of **26** based on recovered **25**). Introduction of the amino group was successfully carried out with $HN(CO_2\text{-allyl})_2$ under Mitsunobu conditions (DIAD, PPh_3)[25] to furnish **27** in 73% yield. To install the phosphate group, the PMB group was removed with DDQ, which proceeded cleanly; subsequent phosphorylation of the resulting alcohol **28** with $(\mathit{i}Pr)_2NP(O\text{-allyl})_2$[26,12f] and 1*H*-tetrazole (then 35% H_2O_2) provided phosphate **29** in good yield. Deprotection of the silyl group (AcCl, CH_2Cl_2/THF/MeOH 5:5:1) produced diol **30** in 95% yield. Finally, all the allyl groups on the nitrogen and phosphorous atoms were removed cleanly by palladium-catalyzed reaction[27,28] with Bu_3SnH and H_2O at 0°C for 1.5 h to afford **1** in 88% yield after preparative TLC (silica gel, normal phase, MeOH/H_2O 8:1). The overall yield of **1** from *rac*-**6** in 38 steps was 0.9% (2% from (*R*)-**6**). Spectral data (^{1}H NMR (500 MHz), ^{13}C NMR (75 MHz), IR), optical rotation ($[\alpha]_D^{28} = +80$ (c = 0.03, MeOH)), and mobility on TLC of synthetic phoslactomycin B were identical in all respects to those reported for the natural material ($[\alpha]_D^{21} = +81$ (c = 1.0, MeOH)).[1b,2]

Next, we turned our attention to the synthesis of the deamino precursor **2**, which is the implicated intermediate in the biosynthesis of **1**.[9] Furthermore, stronger PP2A inhibitory activity was expected from the inhibitory study of the biotin-containing amide derivative at the amino group of **1**.[8] The synthesis started with selective removal of the PMB group of **25** by DDQ followed by assembly of the phosphate group to furnish phosphate **31** in 64% yield (Scheme 4). Desilylation of **31** under acidic conditions provided triol phosphate **32**, which upon palladium-catalyzed deallylation with Bu_3SnH and H_2O at 0°C for 1 h furnished the second target **2** in 77% yield after chromatography on silica gel.

In summary, a highly stereocontrolled and efficient total synthesis of phoslactomycin B (**1**) and its biosynthetic deamino precursor **2** was accomplished by selective protection of the hydroxy group at C9 using the PMB group, which not only provided high selectivity in the construction of the C8 stereocenter but also enabled easy installation of the phosphate group. Furthermore, the successful differentiation of the three other hydroxy groups was demonstrated, thus allowing modification of the functional groups and attachment of another functional group for the study of the structure–activity relationship of **1** and its derivatives and for the execution of chemical biology in this field.

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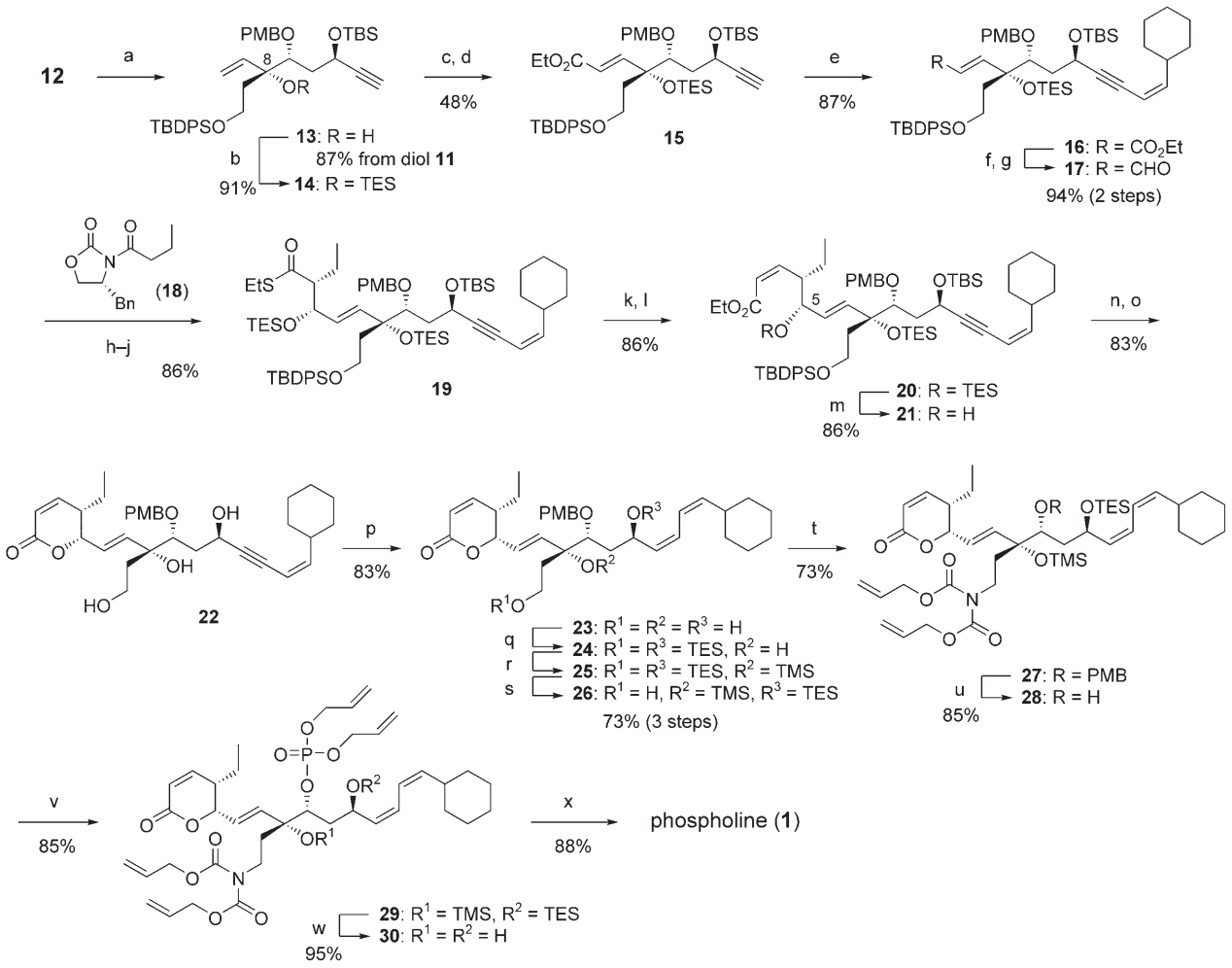
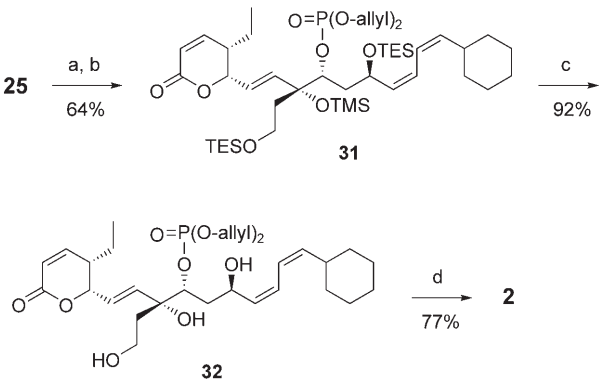


Scheme 3. Synthesis of phoslactomycin B (**1**) through the key intermediate **22** (compare **3** in Scheme 1). Reagents and conditions: a) CH_2=CHMgBr (2 equiv), −78 °C, THF, 87% from diol **11**; b) TESOTf (1.1 equiv), 2,6-lutidine (2 equiv), 91%; c) O_3, 2,6-lutidine (2 equiv), MeOH/*i*PrOH (1:1); d) $(EtO)_2P(O)CH_2CO_2Et$ (2.1 equiv), NaH (2 equiv), THF, 48% for 2 steps; e) **5** (1.2 equiv), $[Pd(PPh_3)_4]$ (5 mol%), CuI (15 mol%), *t*$BuNH_2$ (10 equiv), 87%; f) DIBAL-H (2.5 equiv), THF, 97%; g) $SO_3{\cdot}C_5H_5N$ (3 equiv), DMSO (30 equiv), Et_3N (10 equiv), 97%; h) **18** (1.55 equiv), Bu_2BOTf (1.5 equiv), $(iPr)_2NEt$ (2.2 equiv), −78 °C to RT, CH_2Cl_2; i) TESCl (2 equiv), C_5H_5N (15 equiv), 95% for 2 steps; j) EtSLi from EtSH (5 equiv) and *n*BuLi (3 equiv), 0 °C, 90%; k) DIBAL-H (1.3 equiv), −78 °C, toluene; l) $(PhO)_2P(O)CH_2CO_2Et$ (1.8 equiv), Bu_4NOH (1.75 equiv), THF, 86% for 2 steps; m) PPTS (3 mol%), MeOH/THF (3:1), 86%; n) $Ti(OiPr)_4$ (0.2 equiv), 80 °C, benzene, 94%; o) Bu_4NF (4 equiv), 88%; p) Zn activated with $BrCH_2CH_2Br$, $LiCuBr_2$, EtOH, reflux, 83%; q) TESCl (4 equiv), C_5H_5N (30 equiv), 0 °C, 85%; r) TMSOTf (4 equiv), 2,6-lutidine (30 equiv); s) PPTS (0.1 equiv), THF/MeOH (1:1), 86% for 2 steps based on recovered **25** (38%); t) DIAD (2.6 equiv), PPh_3 (2.4 equiv), $HN(CO_2$-allyl$)_2$ (1.3 equiv), toluene, −78 °C to 0 °C, 73%; u) DDQ (1.5 equiv), CH_2Cl_2/H_2O (19:1), 85%; v) $(iPr)_2NP(O\text{-allyl})_2$ (1.4 equiv), 1*H*-tetrazole (2 equiv) then 35% H_2O_2 (5 equiv), 85%; w) AcCl (0.6 equiv), 0 °C, CH_2Cl_2/THF/MeOH (5:5:1), 95%; x) $[PdCl_2(PPh_3)_2]$ (5 mol%), Bu_3SnH (5 equiv), H_2O (50 equiv), 0 °C, 1.5 h, CH_2Cl_2, 88%. Tf = trifluomethanesulfonyl; TES = triethylsilyl; Bn = benzyl; PPTS = pyridinium *p*-toluenesulfonate; TMS = trimethylsilyl; DIAD = diisopropyl azodicarboxylate; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.



Scheme 4. Synthesis of biosynthetic precursor **2**. Reagents and conditions: a) DDQ (1.2 equiv), CH_2Cl_2/H_2O (19:1), 87%; b) $(iPr)_2NP(O\text{-allyl})_2$ (1.4 equiv), 1*H*-tetrazole (2 equiv) then 35% H_2O_2 (4 equiv), 73%; c) AcCl (0.6 equiv), CH_2Cl_2/THF/MeOH (5:5:1), 0 °C, 92%; d) $[PdCl_2(PPh_3)_2]$ (5 mol%), Bu_3SnH (2.5 equiv), H_2O (30 equiv), CH_2Cl_2, 0 °C, 1 h, 77%.

Keywords: asymmetric synthesis · natural products · phoslactomycins · phospholine · total synthesis

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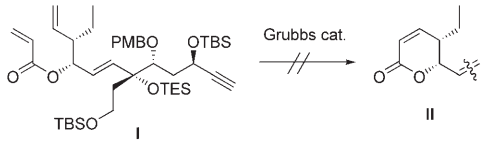
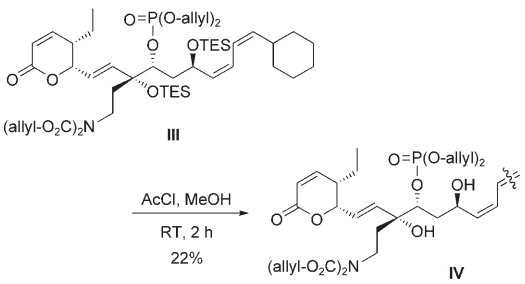
[13] Ring-closing metathesis of **I** with the Grubbs catalysts (first- and second-generation complexes) under various conditions was unsuccessful.



I II

[14] Deprotection of TES ether **III** with AcCl (0.5 equiv) in MeOH afforded a mixture of products, from which **IV** was isolated only in 22% yield. Other conditions (Bu_4NF, HF, $HF{\cdot}C_5H_5N$) resulted in decomposition.



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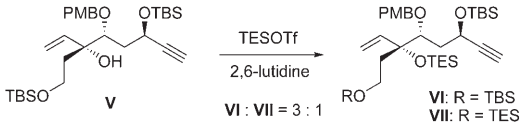
[17] A similar ketone with a TBS protecting group was synthesized from diol **11** and converted into alcohol **V** as well. However, subsequent protection with TESOTf produced a 3:1 mixture of **VI** and **VII** (compare with **13**→**14** in Scheme 3).



[18] A similar reaction with CH_2=CHLi (CH_2=CH$S n Bu_3$, *n*BuLi) afforded a mixture of products.

[19] Addition of the anion from (*E*)-ICH_2=$CHCH_2OH$ (*n*BuLi then $MgBr_2$) also proceeded stereoselectively. However, this alternative was not adopted owing to the requirement of additional steps for the stereoselective preparation of this alcohol from propargyl alcohol in four steps: 1) CrO_3, H^+; 2) HI; 3) MeOH, H^+; 4) DIBAL-H.

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[28] Deprotection of a model compound, (allyl-$O_2C)_2N(CH_2)_4OP$(=O)(O-allyl)$_2$, using HCO_2H, Et_3N, and a palladium catalyst under standard conditions[27b,c] afforded the Et_3N complex, probably $[H_2N(CH_2)_4OP(O)(OH)(O^-)](Et_3NH^+)$, even after chromatography.

