Stereocontrolled Total Synthesis of Apicularen A and Its $\Delta^{17,18}$ *Z* Isomer**

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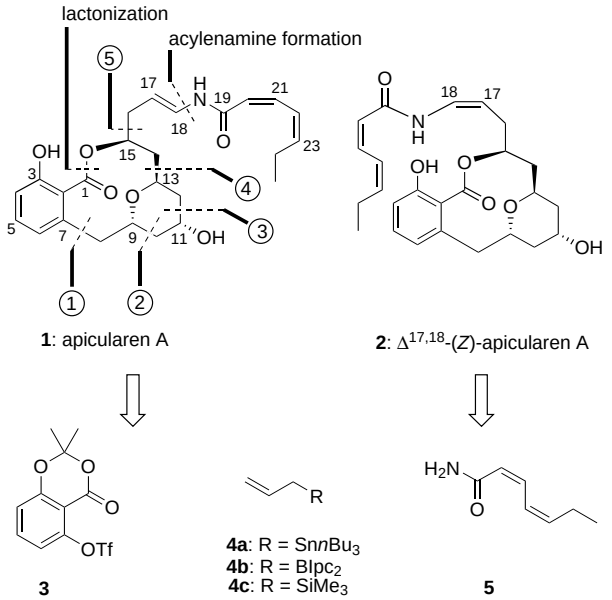
Apicularen A (**1**, Scheme 1) is a polyketide natural product with a novel molecular architecture and impressive antiproliferative properties against a series of human cancer cells including a drug-resistant line.[1,2] Recently isolated from various strains of the myxobacterial genus *Chondromyces* (i.e., *C. apiculatus*, *C. lanuginosus*, *C. pediculatus*, and *C. robustus*),[1] apicularen A possesses a structure characterized by a salicylic acid residue, a macrolide ring bridged by an oxygen atom in such a way as to form a tetrahydropyran system, and a 10-membered ring lactone bearing a side chain with a doubly unsaturated acylenamine moiety. Interestingly, biosynthetic studies revealed the incorporation of eleven intact acetate units into the molecule of apicularen A that account for the entire natural carbon skeleton of the molecule except for C-17 (which stems from glycine), C-18, and C-25 (which is derived from methionine).[2] Here we report a total synthesis[3] of apicularen A (**1**) and its $\Delta^{17,18}$ *Z* isomer (**2**) inspired by its polyacetate-based biosynthesis.



Scheme 1. Structures and retrosynthetic analysis of apicularen A (**1**) and its $\Delta^{17,18}$ *Z* isomer (**2**). ①–⑤: allylation–ozonolysis reiterations. Ipc = isopinocampheyl.

Scheme 1 traces retrosynthetically the origins of apicularen A (**1**) (and its isomer **2**) to key building blocks **3**, **4a**–**c**, and **5**, all of which are readily available.[4–6] In this analysis, we equated the introduction of an acetate unit to a two-step procedure involving allylation followed by ozonolysis. The five projected reiterations of the allylation–ozonolysis sequence are indicated on the structure of **1** and were to be performed in the designated order (① to ⑤). These steps not only would "mimic" nature's polyacetate biosynthetic pathway to **1**, but also would have the potential of yielding the correct stereochemistry at each chiral center of the target molecule through the judicious choice of appropriate reagents and conditions. Thus, while the first planned allylation[7] with allyltributyltin and a palladium catalyst should provide the required extension from the salicylic acid residue, the second and third allylations with Brown's reagent ($Ipc_2Ballyl$)[5] should install the C-9 and C-11 hydroxy groups in their absolute stereochemistry through the use of the appropriate enantiomer. The fourth allylation calls upon allyltrimethylsilane as a reagent to further extend the growing chain with concomitant formation of the C-13 stereocenter in a diastereocontrolled manner. The fifth allylation would require, once more, the use of Brown's reagent ($Ipc_2Ballyl$) for the construction of the final chiral center in its absolute stereochemical configuration. This strategy left the required ring-forming reactions and the stereoselective installment of the acylenamine side chain to be negotiated in the synthetic direction.

Scheme 2 details the construction of advanced intermediate **14** from which both **1** and **2** would be derived. Acetonide triflate **3**[4] was coupled with allyltributyltin[7] under the influence of catalytic $[Pd(PPh_3)_4]$ to afford allyl compound **6** (99% yield) whose rupture with ozone led, upon reduction with PPh_3, to aldehyde **7** (92% yield). The addition of (−)-$Ipc_2Ballyl$ to **7** in diethyl ether at −100 °C produced the corresponding alcohol (70% yield, 95% *ee* by Mosher ester determination)[8] which was protected as its triethylsilyl derivative **8** (83% yield). Reiteration of the above ozonolysis–allylation sequence, this time with the + enantiomer of Brown's reagent ((+)-$Ipc_2Ballyl$), followed by mild acidic treatment (5% aqueous HCl) furnished diol **10** via intermediate aldehyde **9** (62% overall yield). Ozonolytic cleavage of the terminal olefin followed by acetylation of the resulting hydroxylactol resulted in the formation of diacetate **11** as a mixture of anomers (α:β ca. 3:1) in 83% yield. Anomeric allylation[9] of **11** with allyltrimethylsilane in the presence of $BF_3{\cdot}Et_2O$ lead stereoselectively to allyl derivative **12** (97% yield) with the desired *anti* stereochemistry. Finally, another ozonolysis–allylation sequence employing (+)-$Ipc_2Ballyl$ furnished the advanced intermediate **14** in 74% overall yield and in greater than 90% *de*.

Exposure of intermediate **14** to De Brabander's conditions[3a] (NaH, THF, 25 °C) for 2 h followed by addition of

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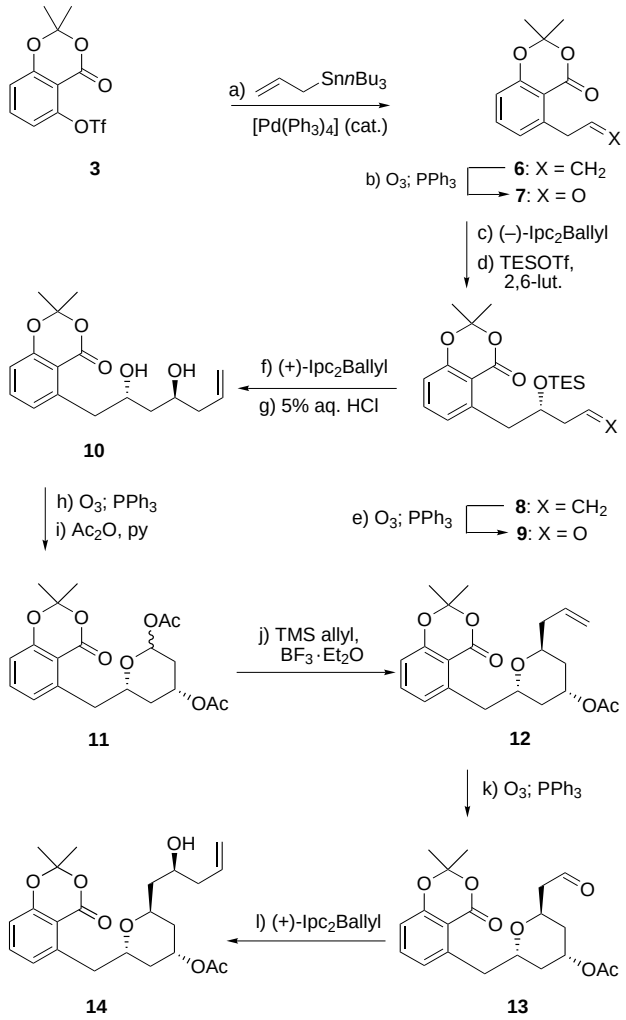
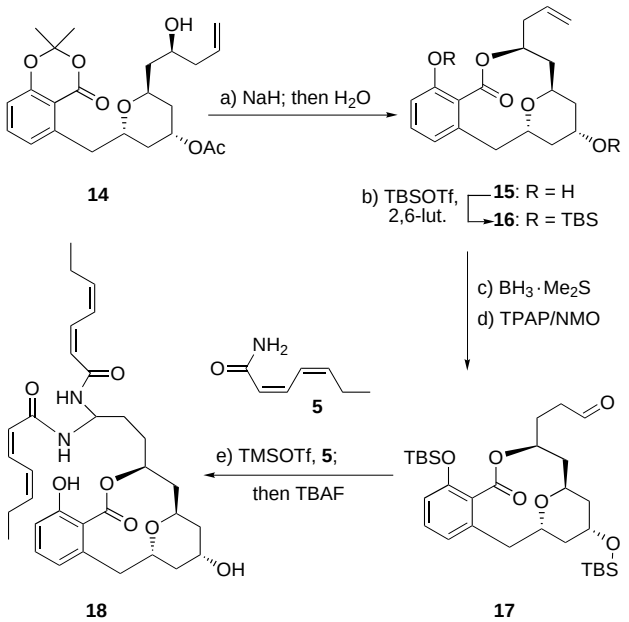


Scheme 2. Construction of advanced intermediate **14**. a) Allyltributyltin (1.2 equiv), LiCl (3.0 equiv), $[Pd(PPh_3)_4]$ (0.02 equiv), THF, reflux, 12 h, 99%; b) O_3, CH_2Cl_2, −78°C, 1 h; then PPh_3 (4.0 equiv), 1 h, 92%; c) (−)-Ipc_2Ballyl (2.0 equiv), Et_2O, −100°C, 2 h, 70%; d) TESOTf (2.0 equiv), 2,6-lut (4.0 equiv), CH_2Cl_2, 25°C, 3 h, 83%; e) O_3, CH_2Cl_2, −78°C, 1 h; then PPh_3 (4.0 equiv), 1 h, 95%; f) (+)-Ipc_2Ballyl (2.0 equiv), Et_2O, −100°C, 2 h; g) 5% aq. HCl, 25°C, 4 h, 62% over 2 steps; h) O_3, CH_2Cl_2, −78°C, 1 h; then PPh_3 (4.0 equiv), 1 h; i) Ac_2O, py, 25°C, 1 h, 83% over 2 steps; j) allyltrimethylsilane (5.0 equiv), BF_3·Et_2O (1.1 equiv), CH_3CN, 0°C, 1 h, 97%; k) O_3, CH_2Cl_2, −78°C, 1 h, PPh_3 (4.0 equiv), 4 h, 98%; l) (+)-Ipc_2Ballyl (2.0 equiv), Et_2O, −100°C, 2 h, 74%. 2,6-lut = 2,6-lutidine; py = pyridine; TES = triethylsilyl; TMS = trimethylsilyl.

5.0 equiv of water and further stirring (8 h) at ambient temperature furnished the corresponding phenol–lactone **15** through ring closure, expulsion of acetone, and acetate cleavage, in 75% overall yield (Scheme 3). Compound **15** was then bis-silylated with TBSOTf in the presence of 2,6-lutidine to afford silyl derivative **16** (99% yield). Subsequent hydroboration to the corresponding primary alcohol (71% overall yield) followed by oxidation (99% yield) afforded aldehyde **17**. Finally, compound **17** reacted with unsaturated primary amide **5** in the presence of TMSOTf in 1,2-dichloroethane to furnish, upon addition of TBAF, the bis-amide derivative **18** in 75% overall yield. Attempts to convert this intermediate to apicularen A proved unsuccessful[10] and therefore a new approach was sought.



Scheme 3. Total synthesis of apicularen A bis-amide derivative **18**. a) NaH (7.0 equiv), THF, 25°C, 2 h; then H_2O (5.0 equiv), 8 h, 75%; b) TBSOTf (4.0 equiv), 2,6-lut (8.0 equiv), CH_2Cl_2, 25°C, 4 h, 99%; c) BH_3·Me_2S (5.0 equiv), THF, 25°C, ultrasound, 30 min; then $NaHCO_3$, H_2O_2, 1 h, 71%; d) TPAP (0.1 equiv), NMO (2.0 equiv), 4 Å MS, CH_2Cl_2, 25°C, 2 h, 99%; e) TMSOTf (0.5 equiv), **5** (2.0 equiv), 1,2-dichloroethane, 25°C, 12 h; then TBAF (5.0 equiv), 25°C, 1 h, 75%. TBS = *tert*-butyldimethylsilyl; OTf = trifluoromethanesulfonate; TPAP = tetra-*n*-propylammonium perruthenate; NMO = 4-methylmorpholine *N*-oxide; 4 Å MS = 4 Ångström molecular sieves; TBAF = tetra-*n*-butylammonium fluoride.

Although the synthesis of the apicularen derivative **18** was of interest from the chemical biology point of view (see below), the task of synthesizing the natural product (**1**) remained unfinished. Completion of the total synthesis required retreat back to advanced intermediate **14** (see Scheme 4) which was now silylated to afford terminal olefin **19** (94% yield). Ozonolytic cleavage of **19** (89% yield) followed by Takai iodo-olefination[11] ($CHI_3/CrCl_2$, 91% yield) led to (*E*)-vinyliodide **20** contaminated with ca. 10% of its *Z* isomer. Coupling of this mixture with primary amide **5** under the influence of copper(I) thiophene carboxylate[12] and Rb_2CO_3 furnished, stereospecifically, the sought-after $\Delta^{17,18}$-(*E*)-enamide derivative **21** together with its *Z* isomer **21′** (90% yield, based on 50% conversion, ca. 10:1 ratio). The two enamide isomers **21** (see Table 1 for selected data) and **21′** were chromatographically separated and converted individually to their respective final products. Thus, removal of the silicon protecting group from **21** was effected with TBAF in THF at ambient temperature to furnish key intermediate hydroxy acylenamine **22** in 80% yield. Finally, and in one stroke, precursor **22** was converted to apicularen A (**1**) in 50% overall yield by initial exposure to NaH in THF followed by addition of 5.0 equiv of water at ambient temperature through ring closure and global deprotection. $\Delta^{17,18}$-(*Z*)-Apicularen A (**2**, see Table for selected data) was prepared from **21′** similarly. Synthetic **1** exhibited identical chromatographic and spectroscopic data to those of an authentic sample.[13] Biological assays[14] revealed that whereas synthetic **1** exhibited

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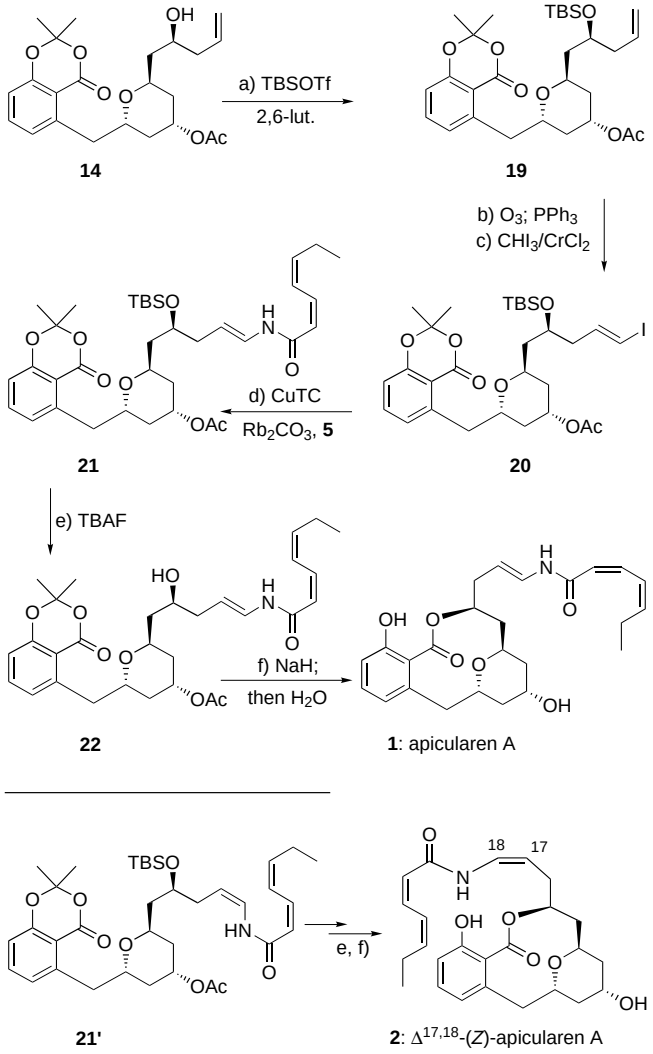

Scheme 4. Total synthesis of apicularen A (**1**) and its $\Delta^{17,18}$ isomer (**2**). a) TBSOTf (2.0 equiv), 2,6-lut (4.0 equiv), CH_2Cl_2, 25 °C, 4 h, 94 %; b) O_3, CH_2Cl_2, −78 °C, 1 h; then PPh_3 (4.0 equiv), 1 h, 89 %; c) CHI_3 (4.0 equiv), $CrCl_2$ (12 equiv), THF, 25 °C, 12 h, 91 %; d) CuTC (1.0 equiv), Rb_2CO_3 (3.0 equiv), amide **5** (3.0 equiv), DMA, 90 °C, 12 h, 90 %; e) TBAF (5.0 equiv), THF, 25 °C, 8 h, 80 %; f) NaH (7.0 equiv), THF, 25 °C, 1 h; then H_2O (5.0 equiv), 25 °C, 4 h, 50 %. CuTC = copper(I) thiophene carboxylate; DMA = *N*,*N*-dimethylacetamide.

potent cytotoxicity against the 1A9 tumor cell line (IC_{50} = 0.42 nM), its bis-amide analogue **18** was devoid of such activity at concentrations up to 300 nM. The latter observation is not surprising given the likely importance of the enamide functionality of apicularen A (**1**) for biological activity. Interestingly, however, $\Delta^{17,18}$-(*Z*)-apicularen A (**2**) maintained considerable cytotoxicity (IC_{50} = 92 nM) against the 1A9 tumor cell line.

The described chemistry demonstrates a chemical equivalent to the polyacetate biosynthetic pathway to polyketides, and establishes an entry into designed apicularen analogues. Applications of the developed synthetic technology to structure–activity relationship studies and chemical biology investigations within the apicularen family are anticipated.

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Table 1. Selected physical properties of compounds **2** and **21**.

2: white solid; R_f = 0.32 (silica, EtOAc/hexanes 1:4); $[\alpha]_D^{20}$ = 10.0 (*c* = 0.2, acetone); IR (thin film): $\tilde{\nu}_{max}$ = 3354, 2955, 2919, 2849, 1719, 1702, 1684, 1655, 1661, 1237, 1619, 1578, 1508, 1461, 1420, 1372, 1290, 1208, 1102, 1078, 1055, 1020 cm^{-1}; ^1H NMR (500 MHz, [D_6]acetone): δ = 8.80 (br d, *J* = 10.7 Hz, 1H), 8.45 (s, 1H), 7.49 (ddd, *J* = 11.4, 11.4, 1.1 Hz, 1H), 7.10 (dd, *J* = 8.4, 7.4 Hz, 1H), 6.86–6.80 (m, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.69 (d, *J* = 7.4 Hz, 1H), 5.83 (d, *J* = 12.5 Hz, 1H), 5.82–5.76 (m, 1H), 5.47 (m, 1H), 4.81 (dt, *J* = 9.2, 7.5 Hz, 1H), 4.28–4.24 (m, 1H), 4.00–3.96 (m, 1H), 3.89–3.85 (m, 1H), 3.77 (d, *J* = 4.0 Hz, 1H), 3.35 (dd, *J* = 14.7. 10.3 Hz, 1H), 2.44–2.37 (m, 3H), 2.29–2.23 (m, 2H), 1.94–1.90 (m, 1H), 1.86–1.79 (m, 1H), 1.68–1.63 (m, 1H), 1.60–1.56 (m, 1H), 1.53–1.46 (m, 2H), 0.99 ppm (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, [D_6]acetone): δ = 169.3, 164.0, 154.2, 141.6, 140.2, 137.0, 130.2, 125.8, 125.4, 123.9, 122.2, 120.8, 114.3, 106.5, 73.8, 73.7, 67.7, 64.8, 40.1, 39.9, 39.5, 39.1, 32.1, 21.0, 14.3 ppm; HRMS (MALDI-FTMS), calcd for $C_{25}H_{31}NO_6$ [*M*+Na^+]: 464.2043, found: 464.2039

21: colorless oil; R_f = 0.50 (silica, hexanes/EtOAc 2:1); $[\alpha]_D^{20}$ = −7.1 (*c* = 2.8, acetone); IR (thin film): $\tilde{\nu}_{max}$ = 3450, 3319, 2955, 2931, 2861, 1731, 1713, 1642, 1584, 1455, 1373, 1242, 1208, 1088, 1044, 838, 779 cm^{-1}; ^1H NMR (600 MHz, [D_6]acetone): δ = 9.05 (br d, *J* = 10.8 Hz, 1H), 7.55–7.50 (m, 2H), 7.08 (d, *J* = 7.4 Hz, 1H), 6.89 (d, *J* = 7.9 Hz, 1H), 6.84 (dd, *J* = 11.9, 11.4 Hz, 1H), 6.77 (dd, *J* = 14.5, 10.6 Hz, 1H), 5.80–5.76 (m, 1H), 5.73 (d, *J* = 11.4 Hz, 1H), 5.25 (dt, *J* = 14.5, 7.4 Hz, 1H), 5.04–4.97 (m, 1H), 4.21–4.17 (m, 1H), 3.94–3.89 (m, 1H), 3.50–3.46 (m, 1H), 3.41 (dd, *J* = 12.7, 3.5 Hz, 1H), 3.15 (dd, *J* = 12.9, 8.6 Hz, 1H), 2.29–2.24 (m, 2H), 2.16–2.04 (m, 2H), 2.06–2.04 (m, 1H), 2.0 (s, 3H), 1.84–1.79 (m, 1H), 1.76–1.74 (m, 1H), 1.70–1.67 (m, 1H), 1.69 (s, 3H), 1.65 (s, 3H), 1.47–1.41 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H), 0.83 (s, 9H), −0.6 ppm (s, 6H); ^{13}C NMR (150 MHz, [D_6]acetone) δ = 170.4, 163.5, 160.7, 157.8, 144.6, 141.3, 136.6, 135.8, 127.9, 125.8, 125.4, 120.9, 116.6, 113.4, 108.8, 105.8, 70.4, 70.0, 68.5, 68.0, 40.5, 39.5, 37.5, 35.4, 26.3, 26.2, 25.0, 21.2, 21.0, 18.5, 14.3, −4.4, −4.5 ppm; HRMS (MALDI-FTMS), calcd for $C_{36}H_{53}NO_8Si_2$ [*M*+Na^+]: 678.3432, found 678.3439

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[14] We thank Prof. Paraskevi Giannakakou and Aurora O'Brate of the Winship Cancer Institute, Emory University School of Medicine, for these biological assays.

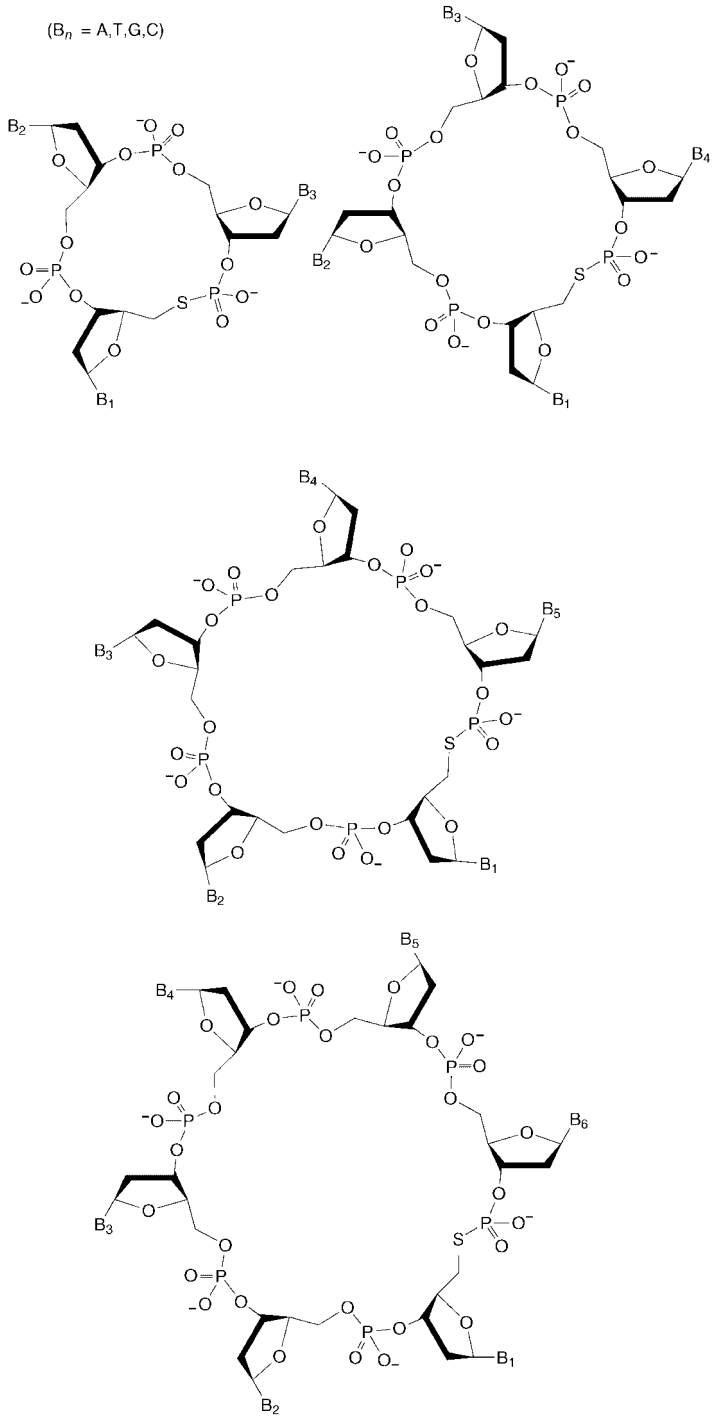
Efficient and Simple Solid-Phase Synthesis of Short Cyclic Oligodeoxynucleotides Bearing a Phosphorothioate Linkage**

Michael Smietana and Eric T. Kool*

There has been an increasing interest of late in the unusual chemical and biological properties of synthetic cyclic DNAs and RNAs.[1] Such molecules are distinct from standard linear oligonucleotides in several respects: they are often unusually good substrates for polymerase enzymes, they are remarkably stable in biological media, and they show unusual recognition abilities with other nucleic acids.[2] Such properties have been reported for a wide range of such molecules, from larger cyclic sequences (e.g., >100 nt in size),[3] to intermediate-sized compounds (e.g., 18–72 nt in size),[4] and even to the smallest ones (2–10 nt in size).[5] As for this latter class of molecules, cyclic dinucleotides have been suggested as promising biological agents. For instance, *c*(GG) is an activator of cellulose synthase in *Acetobacter xilinum*,[5c] and *c*(UU) and *c*(AU) are inhibitors of DNA-dependent RNA polymerase of *E. coli*.[5d] The hypothesized application of short cyclic oligonucleotides in elucidating enzyme mechanisms and as lead structures for development of new drugs,[6] calls for the definition of an easy and efficient production of such compounds.

So far, several methods have been proposed for the synthesis of short cyclic oligonucleotides, in solution using the phosphotriester[7–9] or H-phosphonate[10,11] method, or on polymeric support.[12–14] Unfortunately, these procedures have two main drawbacks that limit their use: they are not compatible with the more common phosphoramidite chemistry, and they require additional protection and deprotection steps. Moreover, the yield for cyclization never exceeded 50% using those approaches. De Napoli et al. wisely aimed to circumvent these problems by the use of a glass (CPG) support for a general synthesis of cyclic oligonucleotides. However, cyclization yields were low (20%) even in the case of very short oligomers (2–4 residues).[15] The useful solid support developed by Pedroso et al.[12] offers a moderate to good 50% yield for the smallest cycles; however, only the T-support is commercially available.

In this study, we describe the first solid-phase synthesis of cyclic oligonucleotides using the standard β-cyanoethylphosphoramidite method. Oligodinucleotides **1** and larger cyclic oligomers (Scheme 1) bearing one 5′-bridging phosphorothioate linkage are obtained in good to excellent yields. The



Scheme 1. Cyclic oligonucleotides (trimer through hexamer).

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