CSIRO PUBLISHING

Full Paper

www.publish.csiro.au/journals/ajc

Aust. J. Chem. **2010**, *63*, 227–235

Convergent Solution Phase Synthesis of Chimeric Oligonucleotides by a 2+2 and 3+3 Phosphoramidite Strategy

Chih-Hau Chen,[A] *Wei-Yu Chen,*[A] *Yu-Chie Chen,*[A] *Ming-Juan Lee,*[B] *Chyuan-Der Huang,*[B] *Kaushik Chanda,*[A] *and Chung-Ming Sun*[A,C]

[A]Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300-10, Taiwan.
[B]ScinoPharm Taiwan Ltd, Tainan 741-44, Taiwan.
[C]Corresponding author. Email: cmsun@mail.nctu.edu.tw

A chimeric oligonucleotide tetramer and hexamer were synthesized by the phosphoramidite approach using a 2+2 and 3+3 strategy, respectively. The concept of convergent synthesis provides an efficient route toward the synthesis of longer chimeric oligonucleotides, such as small interfering RNA oligonucleotides without the pollution of $n-1$ or shorter failures. This methodology offers an efficient and economical way to scale-up the synthesis of high purity oligonucleotides for clinical trials and commercial uses.

Manuscript received: 19 May 2009.
Manuscript accepted: 27 October 2009.

Introduction

Since Watson and Crick[1] first constructed the model of DNA, scientists have devoted themselves to revealing the mysteries of human life. Organic chemists, in particular, have tried to create artificial oligonucleotides to mimic natural oligonucleotides for medicinal applications. Consequently, many oligonucleotide synthesis strategies have been developed during recent decades such as the phosphotriester method,[2] the phosphite triester method,[3] the phosphoramidite method,[4] and the H-phosphonate method.[5] The importance of oligonucleotides was emphasized again by the understanding of RNA interference mechanism in *Caenorhabditis elegans* by Fire, a Nobel Prize winner in 2006.[6] Furthermore, the first oligonucleotide drug, Vitravene, which is the first antisence drug to treat cytomegalovirus (CMV) retinitis in people with AIDS was approved by the Food and Drug Administration (FDA) in 1998.[7] CALAA-01 is a nanoparticle-containing non-chemically modified small interfering RNAs developed for the treatment of cancer and is now in phase I clinical trials.[8] Because of these advanced milestones, many drug companies started to focus on the research of novel antisense oligonucleotide sequences for treating different diseases and to try to amplify the production of oligonucleotides for therapeutics and diagnostic demands.

Traditionally, oligonucleotides were synthesized using the phosphoramidite method by a solid phase automated synthesizer on a small scale with 0.1–10 μmol quantities. A small quantity of oligonucleotides is suitable for screening, biological testing, and preclinical trials, but not enough for clinical trials and commercialization. Another disadvantage is that solid phase synthesis often needs excess reagents to force the reaction to completion and limits this strategy for low cost production. Researchers have proposed many scale-up and purification methodologies during the past two decades. Bonora et al.[9] used poly(ethylene glycol) as a polymer support to prepare oligonucleotides of up to 20-mer. This approach can increase production yield to 100 μm with unavoidable intermediate product loss during the repeated precipitation and filtration steps. Vasseur[10] developed polymer-supported reagents to assist the synthesis of tri-nucleotides with 10 mmol productivity. This strategy is relatively economical and environmentally friendly because the polymer-supported reagents can be recycled. However, the oligonucletide synthesis in this approach is only up to trimers and final products may be polluted by H-phosphonate diester resulting from excess phosphoramidite. Damha and coworkers[11] used an ionic tag as a soluble support for oligoribonucleotides synthesis on a 1 g scale. Furthermore, the dimer phosphoramidites are sometimes used as building blocks to prepare chemically modified oligoribonucleotides.[12]

Results and Discussion

This study presents a novel convergent solution phase approach to synthesize oligonucleotides using the phosphoramidite method. Convergent 2+2 and 3+3 synthetic routes can minimize the number of synthesis steps and produce a high quality product that does not suffer from the pollution of $n-1$ failures. We first synthesized dimer-5′-OH and dimer phosphoramidite, and then coupled these two fragments into the tetramer. The tetramer also can be transformed into tetramer-5′-OH and tetramer phosphoramidite. These two moieties are joined to form an octamer after the coupling reaction. Hexamers, dodecamers, and longer oligonucleotides can be efficiently synthesized in the same manner (Fig. 1). Therefore, it is promising that the solution phase synthesis of oligonucleotides is most amenable to scale-up production.

© CSIRO 2010 10.1071/CH09298 0004-9425/10/020227

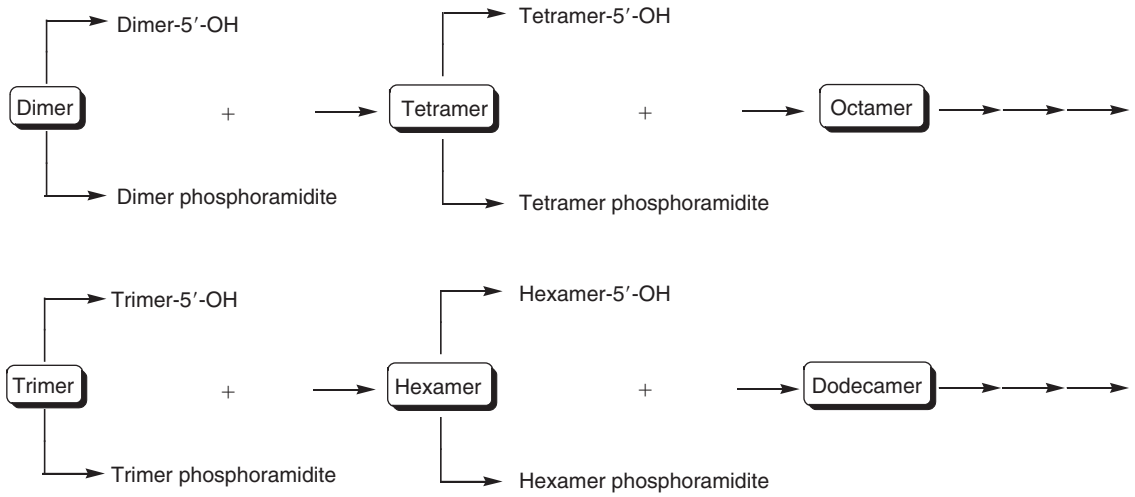
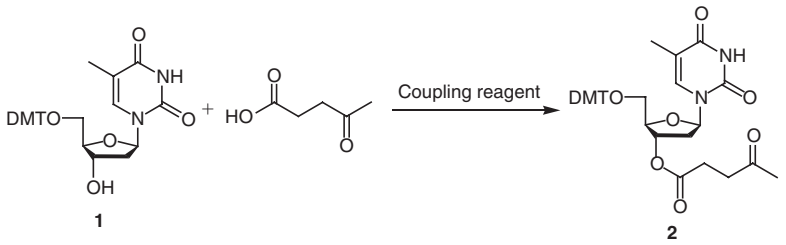
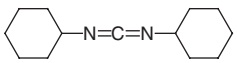
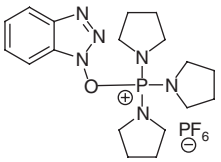
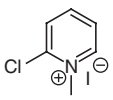
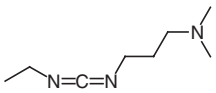
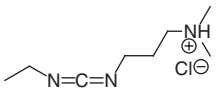


Fig. 1. The concept of convergent solution phase synthesis of chimeric oligonucleotides.

Table 1. Different coupling reagents were tested for levulinylation



1 2

Condition	Coupling reagent	Solvent	Yield [%]
A		CH_2Cl_2	0
B		CH_2Cl_2	0
C		CH_3CN/dioxane	0
D		Dioxane	98
E		Dioxane	91

Beginning with a simple case, the 2+2 synthesis of oligonucleotides was started from a 10 g scale. This study develops the knowledge of oligonucleotide synthesis for subsequent, more challenging tasks. The 3′-hydroxy group of thymidine **1** was first protected with levulinic acid and dicyclohexylcarbodiimide (DCC) as a coupling reagent. However, we failed to the protect 3′ secondary alcohol with levulinic acid even under refluxing conditions.[13] Other coupling reagents have been tested in this coupling reaction, including PyBop, Mukaiyama reagent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (Table 1).[14] Excellent results were obtained when EDC (yield 98%) or EDCI (yield 91%) (conditions D and E) were employed.[15] All the reactions depicted herein were monitored by TLC, ^{1}H and ^{31}P NMR spectroscopy, and matrix-assisted

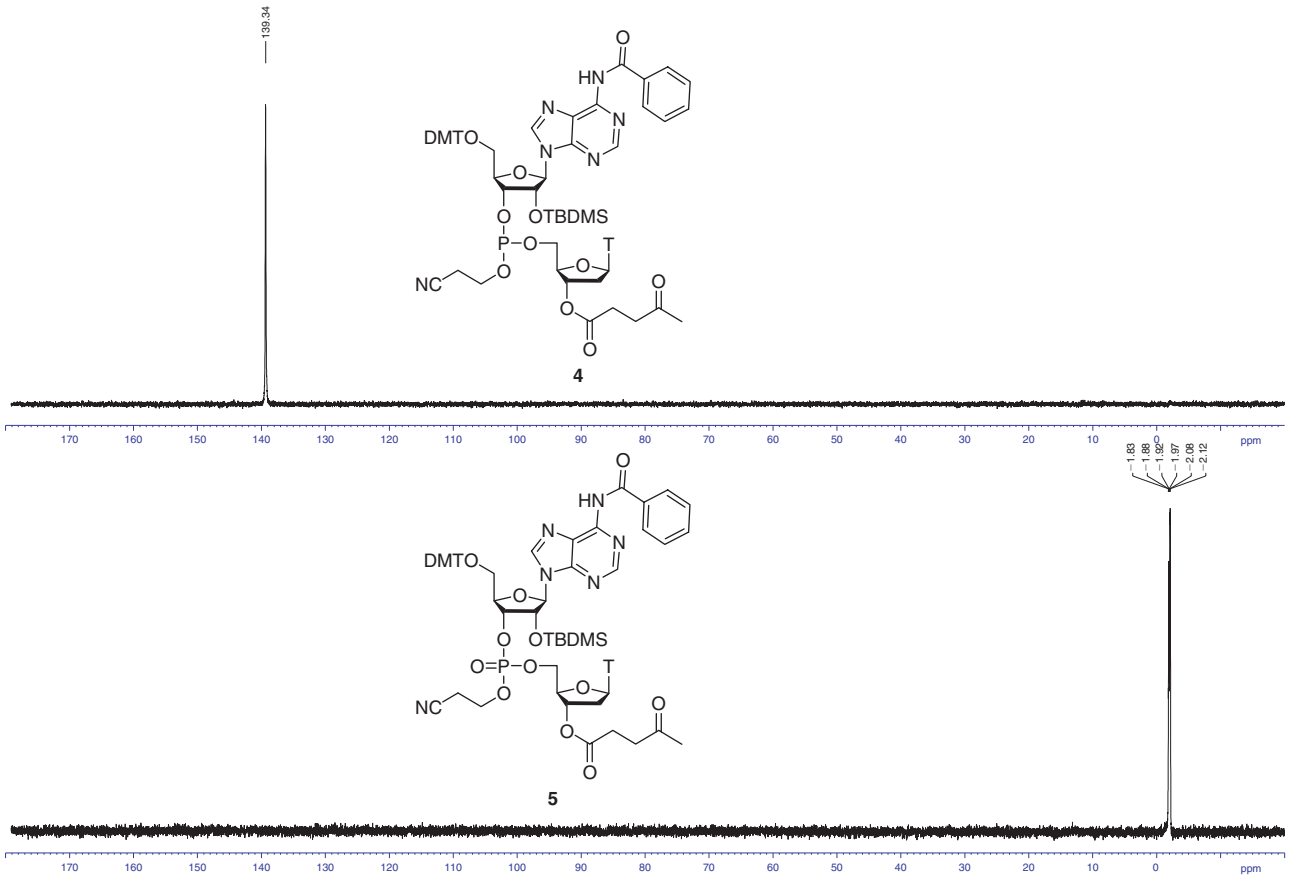


Fig. 2. Progress of the oxidation monitored by ^{31}P NMR spectroscopy.

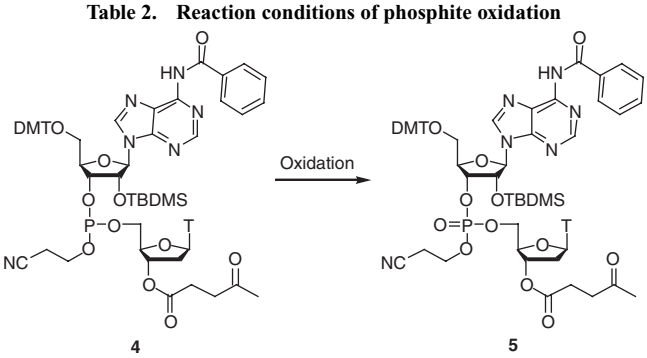
laser desorption ionization (MALDI) mass spectra. After levulinylation, three additional signals appeared in the ^{1}H NMR spectrum (2.75, 2.50, and 2.15 ppm, as shown in the Accessory Publication). Detritylation of **2** with 3% trichloroacetic acid in dichloromethane afforded 5′-OH thymidine **3**. Three sets of proton signals disappeared in the ^{1}H NMR spectrum (7.5–7.2, 6.9, and 3.75 ppm) after detritylation. Condensation of **3** with phosphoamidite rA in the presence of activator, 5-(benzylthio)-1*H*-tetrazole, resulted in the phosphite triester **4**, which was then further oxidized to phosphate triester **5** subsequently. After condensation, the crude product was purified with neutral silica gel and the column was also deactivated with 3% pyridine in hexane. The eluent was also prepared with 1% pyridine to preserve the acid-sensitive phsophite triester bond.

A small amount of phosphite trimester **4** was decomposed in silica gel during purification which caused the moderate yield. The signal of phosphite triester appeared at 140 ppm in the ^{31}P NMR spectrum after condensation, and it was shifted to 0 ppm after oxidation (Fig. 2). Three different reaction conditions were investigated in the oxidation reaction as described in Table 2.

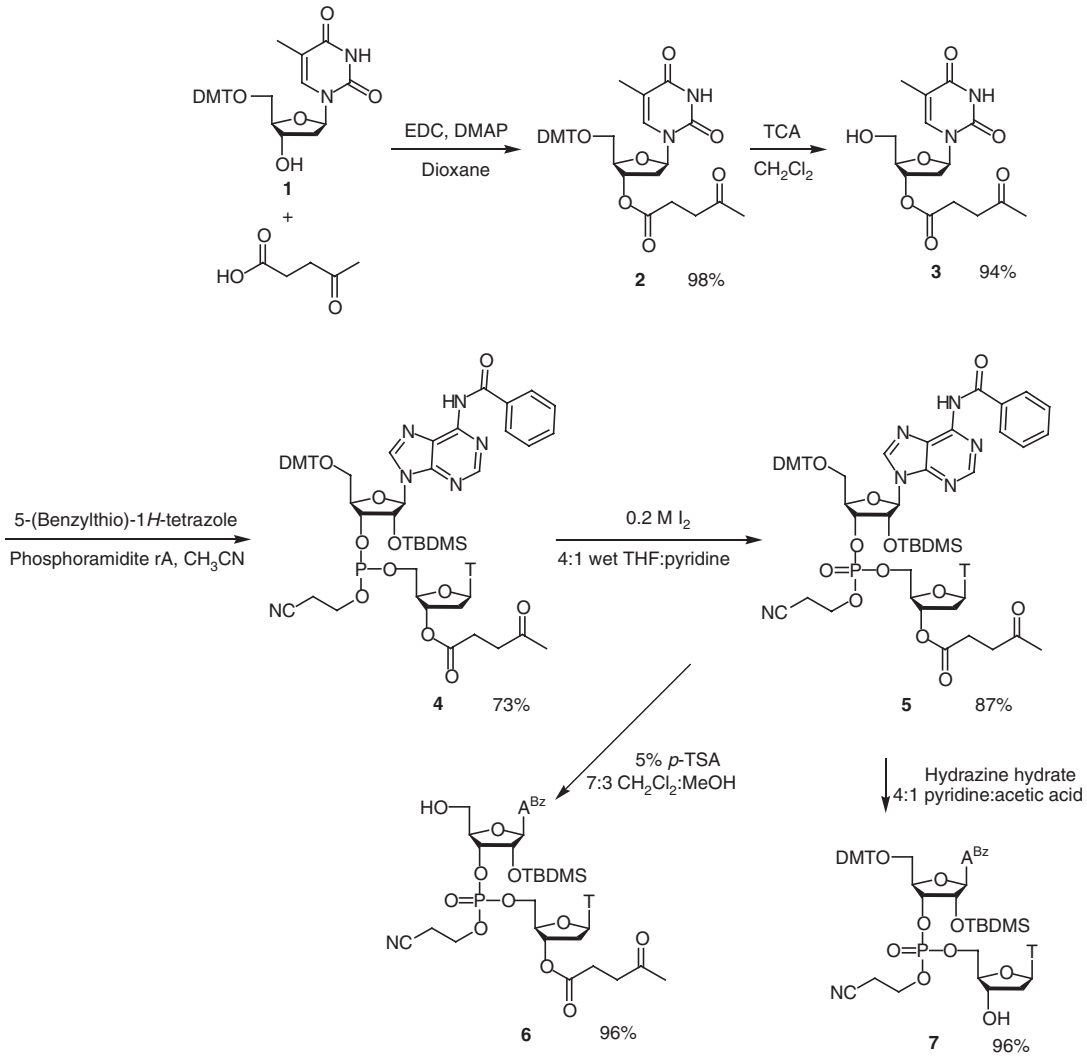
Optimal results were obtained with I_2 in wet tetrahydrofuran (THF) as an oxidative reagent and only extraction is enough to yield a pure product without further complicated column purification.[14] Furthermore, products obtained from condition F and G must be purified by column chromatography, which is easily decomposed in silica gel during the purification. The detritylation of **5** was successfully conducted in 5% *p*-toluenesulfonic acid (Scheme 1). The common trichloroacetic acid was not chosen as a detritylation reagent here because it may destroy the P–O bonds. Prior to transfer of the 3′ position protecting group to levulinyl group, the deprotection of the 3′ position was examined with 3′ methyl succinate-protected $A_{o=p}T$ dimer.

After reaction with hydrazine hydrate, only the adenine deprotection product was obtained and the 3′ protecting group remained intact. Compound **5** was delevulated with hydrazine hydrate and the precursor **7** for the dimer phosphoramidite was obtained (Scheme 1).

Table 2. Reaction conditions of phosphite oxidation



Condition	Reagent	Solvent	Yield [%]
F	80% TBHP in DTBP	CH_3CN	15
G	5.5 M TBHP in decane	CH_3CN	18
H	0.2 M I_2	1/4 pyridine/wet THF	89



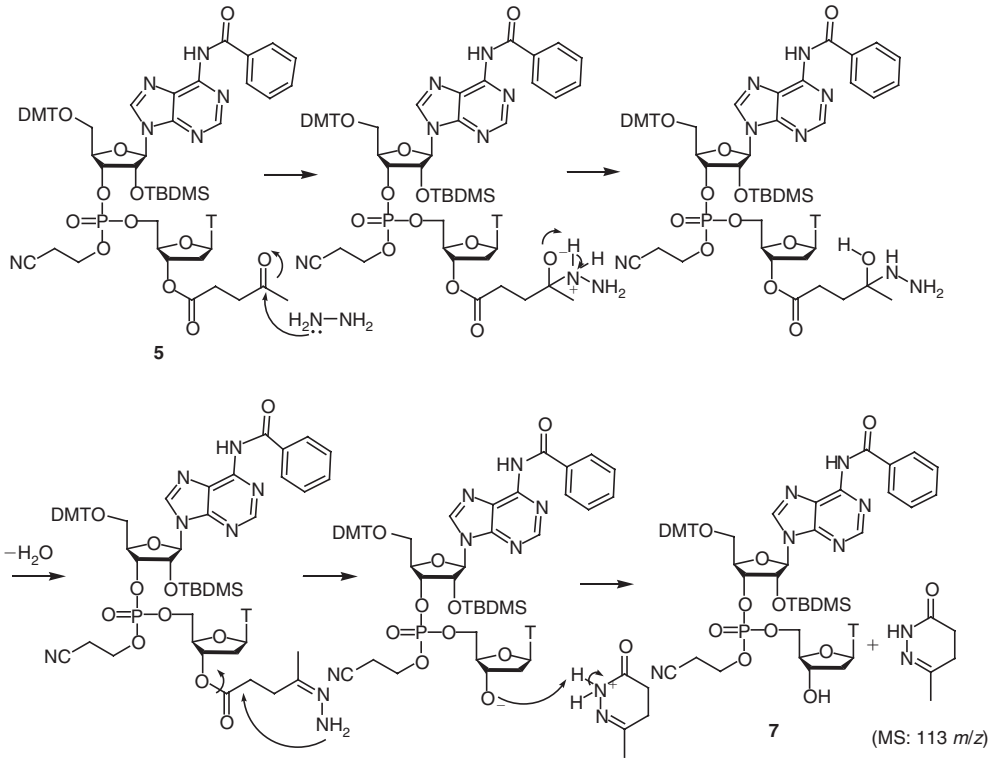
Scheme 1. Synthesis of 5′-OH dimer **6** and 3′-OH dimer **7**.

The proposed mechanism (Scheme 2) began with the hydrazine attack on the ketone of the levulinyl group. The other amine of the hydrazine cleaved the ester bond of **5**, which led to the formation of **7** and 6-methyl-4,5-dihydropyridazin-3(2*H*)-one (ESI^+: 113 *m*/*z*). Dimer phosphoramidite **8** was synthesized under two different conditions. Basic conditions were proceeded with chloro(2-cyanoethoxy)-(diisopropylamino) phosphine and disopropylethylamine. In an alternative approach, tetraisopropylphosphorodiamidite and 5-(benzylthio)-1*H*-tetrazole were employed (Table 3, conditions I and J). The low yields of these methods were due to the sensitivity of phosphoramidite to the weakly acidic silica gel during column purification. The ^{31}P signal of phosphoramidite was seen at around 150 ppm after phosphitylation (Fig. 3). Dimer 5′-OH **6** was condensed with dimer phosphoamidite **8** to generate the tetramer phosphite triester **9** and 5′-OH tetramer **11** was obtained after oxidation and detritylation (Scheme 3).

In the foregoing content, we have achieved the solution phase 2+2 chimeric oligonucleotide synthesis, but purifying the product remained a barrier. As the length of the oligonucleotides increased, the products were more easily decomposed by the silica gel. Despite the low yield from the products decomposing on the column, this problem can be solved by using other purification methods such as extraction or using cellulose or polystyrene as purification media. The total yield for the three steps sequence including 2+2 coupling, iodine-induced oxidation, and tetramer detritylation was improved to 81% just by extraction and filtration without column purification. The more challenging solution phase 3+3 oligonucleotide synthesis began with the condensation of **6** with phosphoamidite rG to obtain the trimer phosphite triester **12**. In a similar manner, phosphite triester **12** was oxidized to form phosphate triester **13**, which immediately underwent detritylation to generate 5′-OH trimer **14**. The 3′-levulinyl group of **13** was also removed by hydrazine hydrate (Scheme 4). Trimer 3′-OH **15** was transformed into trimer phosphoramidite **16** by reacting **15** with the same phosphitylation reagents described previously. Condensation of the 5′-OH trimer **14** and trimer phosphoramidite **16** provided the hexamer phosphite triester **17**, which was oxidized and detritylated to form 5′-OH hexamer **19** (Scheme 5).

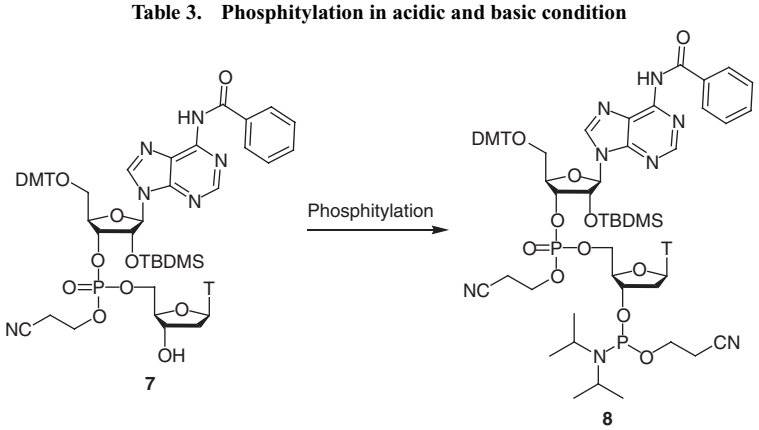
Conclusion

In summary, the synthesis of oligonucleotide tetramer and hexamer through the solution phase 2+2 and 3+3 strategy has been achieved. This convergent synthesis established a practical method for the large-scale synthesis of oligonucleotides. It reduced the synthetic steps for constructing longer oligonucleotides and reduced the time needed to reach



Scheme 2. Mechanism of delevulinylation of dinucleotide **5**.

Table 3. Phosphitylation in acidic and basic condition



Condition	Reagent	Solvent	Time [h]	Yield [%]
I	2-Cyanoethyl *N*,*N*,*N*′,*N*′-tetra-isopropylphosphordiamidite, 5-(benzylthio)-1*H*-tetrazole	CH_2Cl_2	6	41
J	2-Cyanoethyl *N*,*N*-diisopropyl-chlorophosphoramidite, diisopropylethylamine	THF	1	58

targeted molecules. Moreover, this methodology avoided the products from the pollution of $n-1$ or shorter failures and it is applicable for scale-up production to fit clinical trials and commercialization.

Experimental

General

^{1}H NMR spectra were recorded at 300 MHz (Bruker DRX-300) and the chemical shifts were measured from the solvent peak as an internal standard (CD_2HCN in CD_3CN) as an external standard. ^{13}C and DEPT NMR spectra were recorded at 75 MHz (Bruker DRX-300) and the chemical shifts were measured from the solvent peak (CD_2HCN in CD_3CN) as an internal standard. ^{31}P NMR spectra were recorded at 65 MHz and the chemical shifts were measured from 85% H_3PO_4 as an external standard. Acetonitrile was distilled from CaH_2 and stored under nitrogen. TLC was performed on Merck Kieseigel 60 F254 precoated glass plates. Column chromatography was performed with silica gel SiliaFlash, G60 (Silicycle Co. Ltd). The MALDI time-of-flight (TOF) mass spectrometry was carried out by use of a Bruker Daltonics Biflex III (Leipzig, Germany).

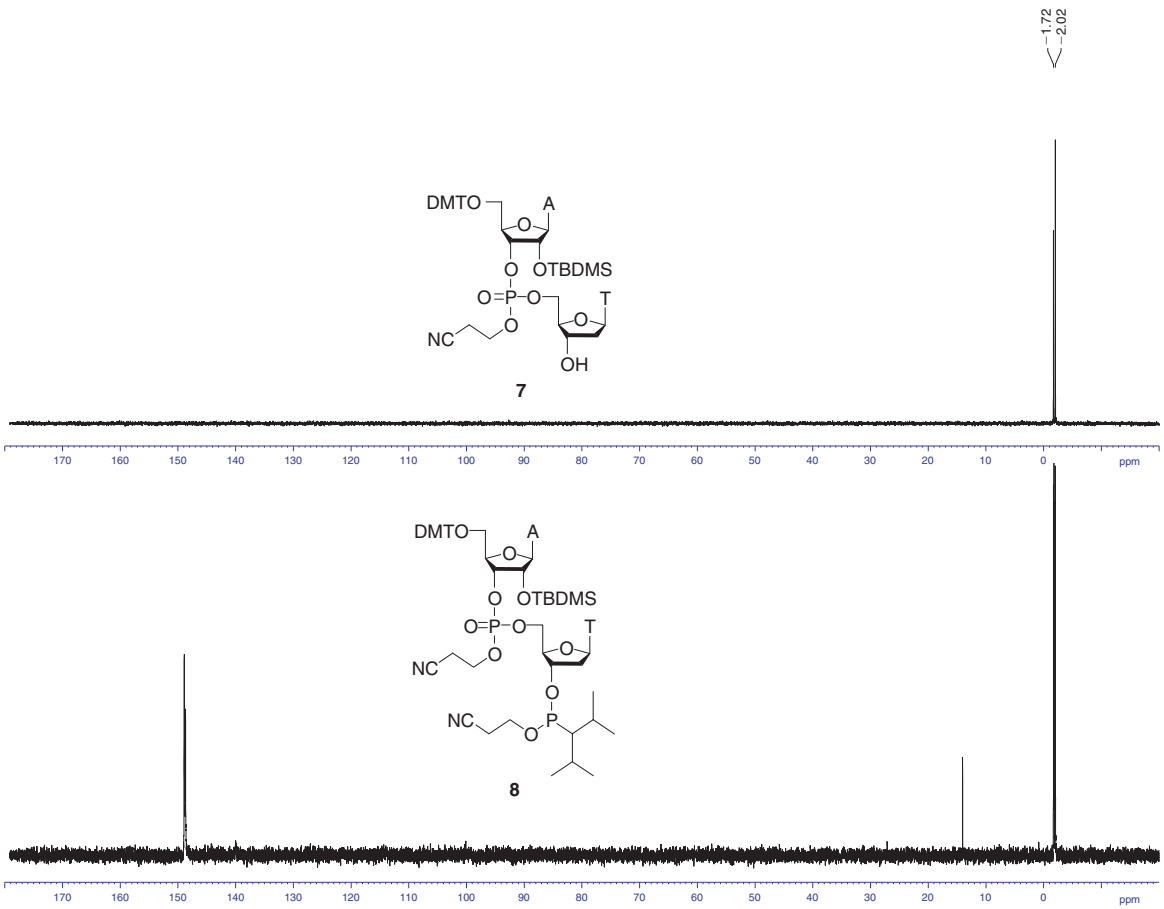


Fig. 3. Progress of the phosphitylation monitored ^{31}P NMR spectroscopy.

*Synthesis of 5′-O-Dimethoxytrityl-3′-O-levulinoyl thymidine (**2**)*

To a solution of 5′-*O*-dimethoxytritylthymidine (10.0 g, 18.4 mmol) in dioxane (132 mL), levulinic acid (4.2 g, 36.0 mmol), EDC (5.8 g, 36.0 mmol), and dimethylaminopyridine (DMAP, 0.22 g, 1.8 mmol) were added. After stirring for 2.5 h, TLC analysis (5% MeOH/DCM) indicated complete conversion of the starting material. The solvent was evaporated by vacuum, and the residue was dissolved in dichloromethane (200 mL). After washing of the organic phase with water, 10% $KHSO_4$, and 10% $NaHCO_3$ (three times), the organic phase was dried ($MgSO_4$), filtered, and concentrated to give a foam (**2**), which was used directly in the next reaction. δ_H (300 MHz, CD_3CN): 10.02 (br, 1H), 7.58 (s, 1H), 7.49 (d, *J* 7.5, 2H), 7.43–7.21 (m, 7H), 6.9 (d, *J* 8.7, 4H), 6.35 (t, *J* 6.4, 1H), 5.46 (s, 1H), 4.13 (s, 1H), 3.77 (s, 6H), 3.63 (s, 2H), 3.42 (dd, *J* 19.0 and 7.7, 2H), 2.76 (t, *J* 6.2), 2.54 (t, *J* 6.4, 2H), 2.54–2.36 (m, 2H), 2.14 (s, 3H), 1.50 (s, 3H). δ_C (DEPT, 75 MHz, CD_3CN): 207.3 (C_q), 178.2 (C_q), 164.6 (C_q), 159.2 (C_q), 151.2 (C_q), 145.3 (C_q), 136.2 (CH), 136.1 (C_q), 135.9 (C_q), 130.5 (CH), 128.5 (CH), 128.4 (CH), 127.5 (CH), 117.8 (C_q), 113.7 (CH), 111.2 (C_q), 87.1 (C_q), 84.8 (CH), 84.1 (CH), 75.4 (CH), 67.1 (CH_2), 55.4 (CH_3), 54.8 (C_q), 37.9 (CH_2), 37.5 (CH_2), 29.4 (CH_3), 28.3 (CH_2), 11.8 (CH_3).

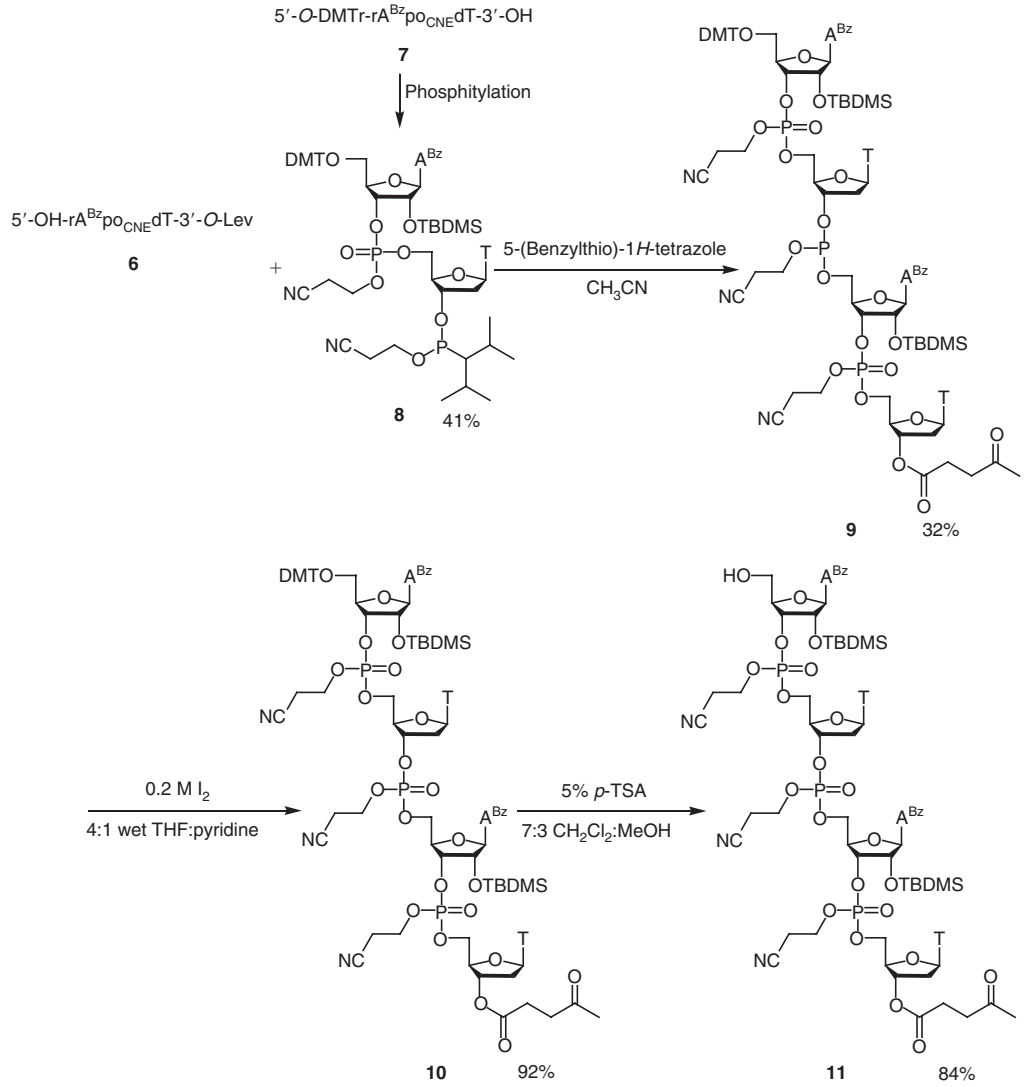
*Synthesis of 5′-Hydroxyl-3′-O-levulinoyl-thymidine (**3**)*

To a solution of 5′-*O*-dimethoxytrityl-3′-*O*-levulinoyl-thymidine (**2**) (11.6 g, 18.0 mmol) in dichloromethane (200 mL), 6% trichloroacetic acid in dichloromethane (200 mL) was added at 0°C for 1 h. After reaction completion, solvent was removed and the crude product was subjected to column purification. First, 33% hexane in ethyl acetate was used as eluent to remove the byproduct and then 100% ethyl acetate was used to elute product **3**. The product was obtained as a white foam in 94% yield. δ_H (300 MHz, CD_3CN): 7.64 (s, 1H), 6.22 (t, *J* 7.0, 1H), 5.26 (d, *J* 1.6, 1H), 4.03 (d, *J* 2.2, 1H), 3.75 (d, *J* 2.1, 2H), 2.77 (t, *J* 6.2, 2H), 2.53 (t, *J* 6.5, 2H), 2.36–2.25 (m, 2H), 2.14 (s, 3H), 1.85 (s, 3H). δ_C (DEPT, 75 MHz, CD_3CN): 208.0 (C_q), 173.2 (C_q), 165.1 (C_q), 151.7 (C_q), 137.2 (CH), 118.3 (C_q), 111.3 (C_q), 87.7 (CH), 85.6 (CH), 75.7 (CH), 62.7 (CH_2), 38.3 (CH_2), 37.7 (CH_2), 29.8 (CH_3), 28.7 (CH_2), 12.5 (CH_3).

General Procedures for the Condensation, Oxidation, Detritylation, Delevulinylation, and Phosphitylation Reaction

Condensation

5′-OH oligonucleotide (16.9 mmol, 1.0 equiv), phosphoramidite (22.0 mmol, 1.3 equiv), and activator, 5-(benzylthio)-1*H*-tetrazol (84.5 mmol, 5.0 equiv) was added in a 500 mL flask under nitrogen. Acetonitrile was added and the reaction mixture was stirred for 30 min until reaction completion. Acetonitrile was removed by rotary evaporation and the crude product was purified by column chromatography (column was packed with 5% pyridine in hexane). 5′-O-DMTr-rA^{Bz}p$_{CNE}$dT-3′-O-Lev (**4**): δ_P (65 MHz, CD_3CN): 139.3, 139.3. 5′-O-DMTr-rA^{Bz}po$_{CNE}$dTp$_{CNE}$rA^{Bz} po$_{CNE}$dT-3′-O-Lev (**9**): δ_P (65 MHz, CD_3CN): 140.0, 140.0, 139.8, 139.8,



Scheme 3. Synthesis of dimer phosphoramidite **8** and 5′-OH tetramer **11**.

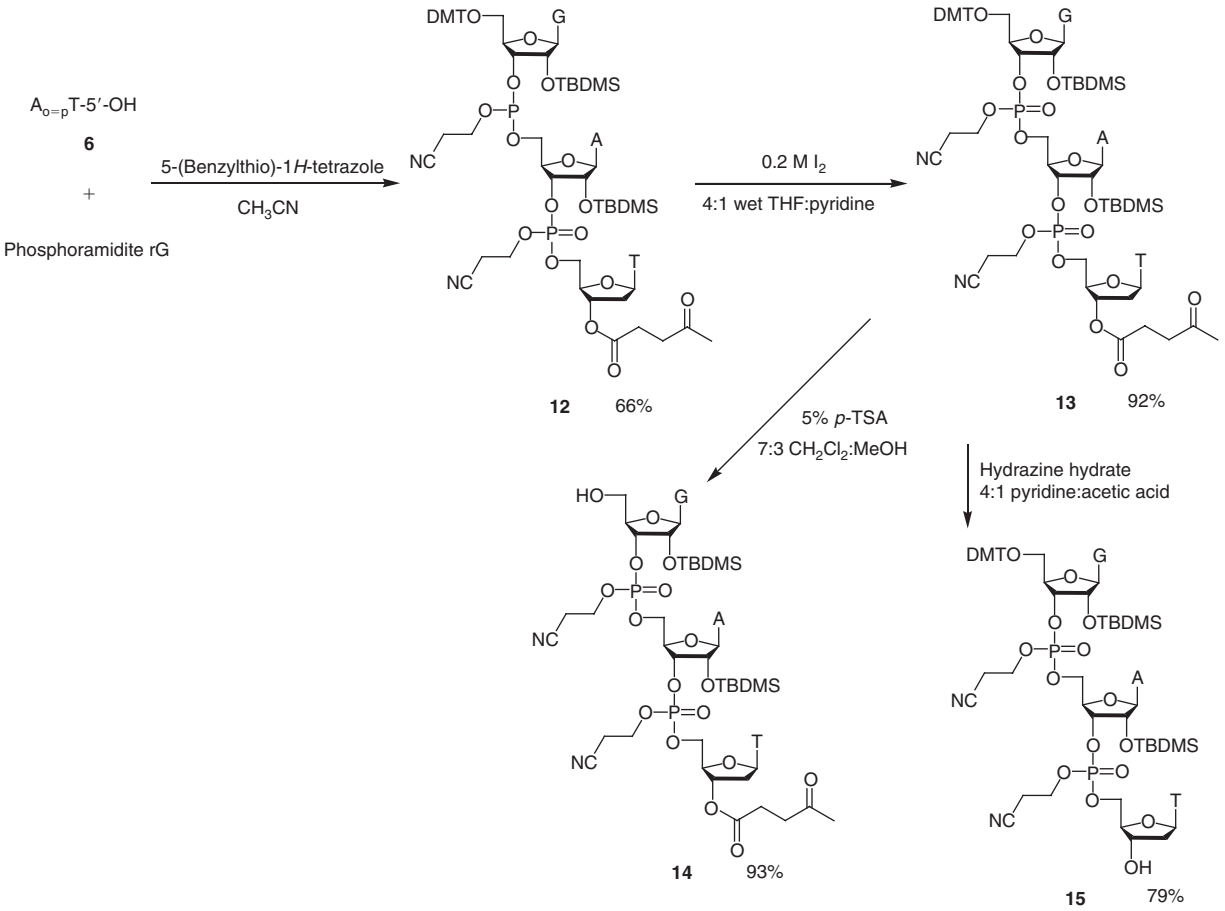
139.6, 139.6, 139.6, 139.4, −1.7, −1.8, −1.8, −1.9, −1.9, −2.0, −2.0, −2.1, −2.1. *m/z* (MALDI TOF): Anal. Calc. for $C_{101}H_{120}N_{17}O_{29}P_3Si_2$ 2183.72. Found 2206.75 $(M+Na)^+$. 5′-O-DMTr-rG^{iBu}p$_{CNE}$rA^{Bz}po$_{CNE}$dT- 3′-O-Lev (**12**): δ_P (65 MHz, CD_3CN) 139.6, 139.5, 139.5, 139.4, −1.9, −2.0, −2.3, −2.4. 5′-O-DMTr-rG^{iBu}po$_{CNE}$rA^{Bz}po$_{CNE}$dTp$_{CNE}$rG^{iBu}po$_{CNE}$rA^{Bz}po$_{CNE}$dT-3′-O-Lev (**17**): δ_P (65 MHz, CD_3CN): 140.3, 140.3, 140.0, 140.0, 139.8, 139.7, 139.7, 139.1, −1.7, −1.9, −1.9, −2.0, −2.1, −2.2, −2.2, −2.2, −2.4, −2.4.

Oxidation

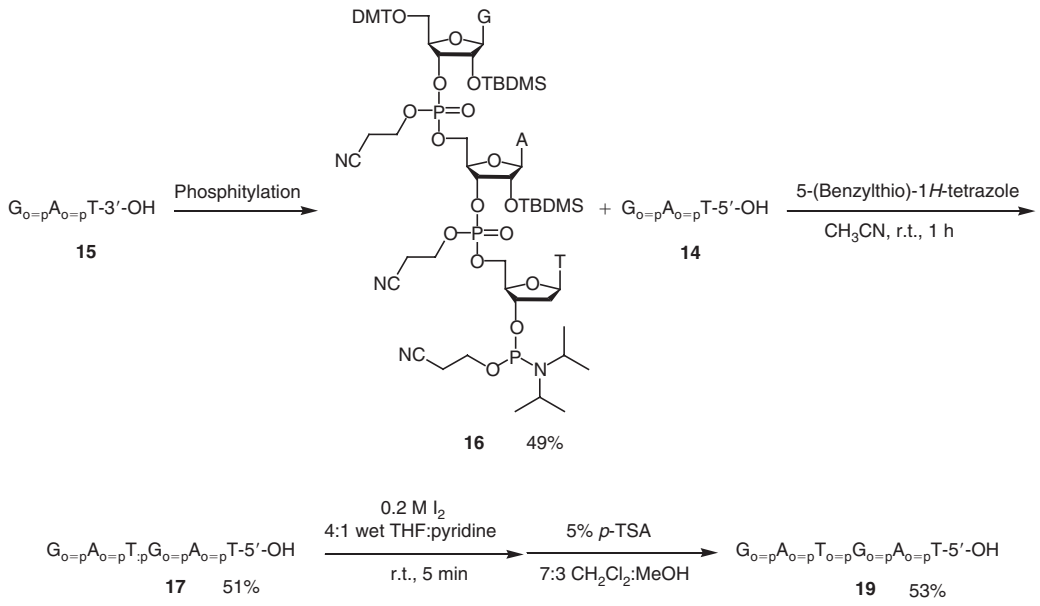
A solution of 0.2 M I_2 in 4/1 THF/pyridine was added to the phosphite trimester and the reaction mixture was stirred for 10 min. The reaction was quenched by extraction with 1 M $Na_2S_2O_3$, 10% $KHSO_4$, 10% $NaHCO_3$, and a mixture of brine/water (1/1), the organic layer was collected and dried by $MgSO_4$. After filtration and solvent removal, product was obtained as a brown foam. 5′-O-DMTr-rA^{Bz}po$_{CNE}$dT-3′-O-Lev (**5**): δ_P (65 MHz, CD_3CN): −1.9, −2.1. *m/z* (MALDI TOF): Anal. Calc. for $C_{62}H_{71}N_8O_{16}PSi$: 1242.45. Found 1265.00 $(M+Na)^+$. 5′-*O*-DMTr-rA^{Bz}po$_{CNE}$dTp$_{CNE}$rA^{Bz}po$_{CNE}$dT-3′-O-Lev (**10**): δ_P (65 MHz, CD_3CN): −1.7, −1.8, −2.0, −2.1, −2.3, −2.4, −2.5, −2.5. 5′-O-DMTr-rG^{iBu}po$_{CNE}$rA^{Bz}po$_{CNE}$dT-3′-O-Lev (**13**): δ_P (65 MHz, CD_3CN): −1.9, −2.0, −2.1, −2.2. *m/z* (MALDI TOF): Anal. Calc. for $C_{83}H_{102}N_{14}O_{24}P_2Si_2$: 1796.62. Found 1819.39 $(M+Na)^+$. 5′-O-DMTr-rG^{iBu}po$_{CNE}$rA^{Bz}po$_{CNE}$d Tpo$_{CNE}$rG^{iBu}po$_{CNE}$rA^{Bz}po$_{CNE}$dT-3′-O-Lev (**18**): δ_P (65 MHz, CD_3CN): −1.7, −1.8, −1.9, −2.0, −2.0, −2.1, −2.2, −2.2, −2.2, −2.4, −3.0, −3.0, −3.1. *m/z* (MALDI TOF): Anal. Calc. for $C_{143}H_{182}N_{29}O_{46}P_5Si_4$: 3308.06. Found 3331.51 $(M+Na)^+$.

Detritylation for Dimer or Longer Oligoribonucleotide

To the solution of phosphate triester in 7/3 dichloromethane/methanol at 0°C, 10% *p*-toluenesulfonic acid in dichloromethane/methanol (7/3) was added and the resulting mixture was stirred for 30 min at 0°C. After completion, water was added and the resulting mixture was vigorously stirred for 10 min at 0°C. A saturated $NaHCO_3$ aqueous solution was added and again, the reaction mixture was stirred strongly for 10 min at 0°C. The reaction mixture was then washed with saturated $NaHCO_3$ solution, 0.2 M $Na_2S_2O_3$, and 0.5 M NaCl. The organic



Scheme 4. Synthesis of 5′-OH trimer **14** and 3′-OH trimer **15**.



Scheme 5. Synthesis of trimer phosphoramidite **16** and 5′-OH hexamer **19**.

layer was separated and dried over anhydrous $MgSO_4$. After filtration the solvent was evaporated under rotary evaporation. The crude product was purified by column choromatography or ether precipitation. The product was obtained as a white solid. 5′-OH-rGiBupoCNErABzpoCNEdT-3′-O-Lev (**14**): *m/z* (MALDI TOF): Anal. Calc. for $C_{62}H_{84}N_{14}O_{22}P_2Si_2$: 1494.49. Found 1517.07 $(M + Na)^+$. 5′-OH-rGiBupoCNErABzpoCNEd TpoCNErGiBupoCNErABzpoCNEdT-3′-O-Lev (**19**): *m/z* (MALDI TOF): Anal. Calc. for $C_{122}H_{164}N_{29}O_{44}P_5Si_4$: 3005.93. Found 3029.35 $(M + Na)^+$.

Delevulinylation

To the solution of phosphate triester in 4/1 pyridine/acetic acid, hydrazine monohydrate (1.5 equiv) was added and reacted for 2 h. After reaction completion, acetylacetone (2.0 equiv) was added to quench the excess hydrazine, and the mixture was stirred for 10 min. The solvents were removed under vacuum and the residue was dissolved in dichloromethane and extracted with water, 10% $KHSO_4$ (two times), 10% $NaHCO_3$ (two times), and brine. After drying by $MgSO_4$ and filtration, the solvents were removed by rotary evaporation. The crude product was purified by column chromatography (column was packed with 3% pyridine in hexane). 5′-O-DMTr-rA^{Bz}po$_{CNE}$dT-3′-OH (**7**): *m*/*z* (MALDI TOF): Anal. Calc. for $C_{57}H_{65}N_8O_{14}PSi$: 1144.41. Found 1145.70 $(M+H)^+$. 5′-*O*-DMTr-rG^{iBu}po$_{CNE}$rA^{Bz}po$_{CNE}$dT-3′-OH (**15**): *m*/*z* (MALDI TOF): Anal. Calc. for $C_{78}H_{96}N_{14}O_{22}P_2Si_2$: 1698.58. Found 1721.32 $(M+Na)^+$.

Basic Conditions for Phosphitylation

To a solution of 3′-OH oligonucleotides (1.0 equiv) in THF under nitrogen at 0°C, *N*,*N*-diisopropylethylamine (3.0 equiv) and chloro(2-cyanoethoxy)(diisopropylamino) phosphine (1.5 equiv) were added and the reaction mixture was stirred for 1 h. After completion, the mixture was washed with 5% aqueous $NaHCO_3$ and dried over $MgSO_4$. The crude product was purified by column chromatography (column was packed with 3% pyridine in hexane) and product was obtained as yellow form. 5′-O-DMTr-rA^{Bz}po$_{CNE}$dT-3′-O-po$_{CNE}$N(i-Pr)$_2$ (**8**): δ_P (65 MHz, CD_3CN) 148.9, 148.9, 148.8, 148.7, −1.7, −1.8, −1.9, −2.0. *m*/*z* (MALDI TOF): Anal. Calc. for $C_{66}H_{82}N_{10}O_{15}P_2Si$: 1344.52. Found 1345.60 $(M+H)^+$. 5′-*O*-DMTr-rG^{iBu}po$_{CNE}$rA^{Bz}po$_{CNE}$dT-3′-O-po$_{CNE}$N(i-Pr)$_2$ (**16**): δ_P (65 MHz, CD_3CN) 148.9 148.9, 148.9, 148.8, 148.8, 148.7, 148.6, 148.5, −1.7, −1.8, −1.8, −1.9, −1.9, −2.0, −2.0, −2.1. *m*/*z* (MALDI TOF): Anal. Calc. for $C_{87}H_{113}N_{16}O_{23}P_3Si_2$: 1898.69. Found 1921.66 $(M+Na)^+$.

Acidic Conditions for Phosphitylation

To a solution of 3′-OH oligonucleotides (1.0 equiv) in anhydrous dichloromethane, 2-cyanoethyl tetraisopropylphosphorodiamidite (1.3 equiv), and 5-(benzylthio)-1*H*-tetrazol (0.4 equiv) were added at ambient temperature. After 5 h, the mixture was washed with 5% aqueous $NaHCO_3$ and dried over $MgSO_4$. The crude product was purified by column chromatography (column was packed with 3% pyridine in hexane) and product was obtained as yellow form.

Acknowledgements

The authors are thankful to National Science Council of Taiwan (NSC) and ScinoPharm® Taiwan for financial assistance and National Center for High-Performance Computing for computer time and facilities.

References

[1] J. D. Watson, F. H. C. Crick, *Nature* **1953**, *171*, 737. doi:10.1038/171737A0

[2] (a) H. G. Khorana, *Pure Appl. Chem.* **1968**, *17*, 349. doi:10.1351/PAC196817030349
(b) H. G. Khorana, *Biochem. J.* **1968**, *109*, 709.
(c) K. L. Agarwal, A. Yamazaki, P. J. Cashion, H. G. Khorana, *Angew. Chem. Int. Ed.* **1972**, *11*, 451. doi:10.1002/ANIE.197204511

[3] (a) R. L. Letsinger, J. L. Finnan, G. A. Heavner, W. B. Lunsford, *J. Am. Chem. Soc.* **1975**, *97*, 3278. doi:10.1021/JA00844A090
(b) R. L. Letsinger, W. B. Lunsford, *J. Am. Chem. Soc.* **1976**, *98*, 3655. doi:10.1021/JA00428A045

[4] S. L. Beaucage, M. H. Caruthers, *Tetrahedron Lett.* **1981**, *22*, 1859. doi:10.1016/S0040-4039(01)90461-7

[5] P. J. Garegg, T. Regberg, J. Stawinski, R. Strömberg, *Chem. Scr.* **1985**, *25*, 280.

[6] A. Fire, S. Q. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, C. C. Mello, *Nature* **1998**, *391*, 806. doi:10.1038/35888

[7] http://www.isispharm.com/vitravene.html (accessed 21 July 2009).

[8] I. Fraser-Moodie, *Types of Targeted Therapeutics*, in *The Future of Targeted Therapeutics* **2008**, Ch. 3 (Business Inslights Ltd: West Indies).

[9] (a) G. M. Bonora, C. L. Scremin, F. P. Colonna, A. Garbesi, *Nucleic Acids Res.* **1990**, *18*, 3155. doi:10.1093/NAR/18.11.3155
(b) G. M. Bonora, G. Biancotto, M. Maffini, C. L. Scremin, *Nucleic Acids Res.* **1993**, *21*, 1213. doi:10.1093/NAR/21.5.1213
(c) G. M. Bonora, R. Rossin, S. Zaramella, D. L. Cole, A. Eleuteri, V. T. Ravikumar, *Org. Process Res. Dev.* **2000**, *4*, 225. doi:10.1021/OP990096L

[10] C. Dueymes, A. Schönberger, I. Adamo, A.-E. Navarro, A. Meyer, M. Lange, J.-L. Imback, F. Link, F. Morvan, J.-J. Vasseur, *Org. Lett.* **2005**, *7*, 3485. doi:10.1021/OL0511777

[11] (a) R. A. Donga, S. M. Khaliq-Uz-Zaman, T.-H. Chan, M. J. Damha, *J. Org. Chem.* **2006**, *71*, 7907. doi:10.1021/JO061279Q
(b) R. A. Donga, T.-H. Chan, M. J. Damha, *Can. J. Chem.* **2007**, *85*, 274. doi:10.1139/V07-022

[12] (a) T. Murata, S. Iwai, E. Ohtsuka, *Nucleic Acids Res.* **1990**, *18*, 7279. doi:10.1093/NAR/18.24.7279
(b) K. Sato, K. Seio, M. Sekine, *J. Am. Chem. Soc.* **2002**, *124*, 12715. doi:10.1021/JA027131F

[13] (a) J. H. van Boom, P. J. M. Burgers, *Tetrahedron Lett.* **1976**, *17*, 4875. doi:10.1016/S0040-4039(00)78935-0
(b) G. Kumar, M. S. Poonian, *J. Org. Chem.* **1984**, *49*, 4905. doi:10.1021/JO00199A032
(c) D. E. Bergstrom, P. W. Shum, *J. Org. Chem.* **1988**, *53*, 3953. doi:10.1021/JO00252A014

[14] J. G. Lackey, D. Sabatio, M. J. Damha, *Org. Lett.* **2007**, *9*, 789. doi:10.1021/OL0629521

[15] M. C. de Koning, A. B. T. Ghisaidoobe, H. I. Duynstee, P. B. W. Ten kortenaar, D. V. Filippov, G. A. van der Marel, *Org. Process Res. Dev.* **2006**, *10*, 1238. doi:10.1021/OP060133Q