

## 含亚甲基缩醛键的寡脱氧核苷酸的合成及杂交性质的初步研究

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**摘要:** 目的 本文设计合成了一类新型混合骨架的寡脱氧核苷酸(MBO)。方法 3'-O-(二苯膦酰氧)甲基缩醛核苷(1)在三甲基硅三氟甲磺酸酯(TMSOTf)条件下,与3'-位保护的脱氧核苷(2或6)缩合。得到的二聚(3)或三聚体(7)的5'-位经4,4'-二甲氧三苯甲基(DMT)保护,3'-位与(2-氰乙基 N,N-二异丙基)氯化亚磷酰胺缩合,然后应用标准固相DNA合成法掺入到寡核苷酸中。结果 本文合成了6条带亚甲基缩醛键的寡核苷酸(ODN II ~ ODN VII),考察了它们的杂交性质,测定了与互补DNA的解链温度 $T_m$ 值。结论 此类寡核苷酸平均每个亚甲基缩醛键的修饰,解链温度 $T_m$ 值下降约0.8~1.2℃,杂交亲和力与对照的天然磷酸二酯键的寡核苷酸相当。

**关键词:** 反义药物;寡脱氧核苷酸;解链温度;杂交

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## Synthesis and hybridizing properties of oligodeoxynucleotide analogs containing methyleneformacetal

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**Abstract:** **Aim** To design and synthesize a new mixed backbone oligonucleotide (MBO). **Methods** In the presence of trimethylsilyl trifluoromethane-sulfonate (TMSOTf), condensation of 3'-O-(diphenylphosphinyloxy) methyl acetal (1) with 3'-protected deoxynucleoside (2 or 6) afforded dimers (3) or trimers (7) respectively. 5'-Hydroxyl and 3'-hydroxyl groups of these acetal-linked oligomers were protected by 4,4'-dimethoxytriphenylmethyl (DMT) or by diisopropylamino- $\beta$ -cyanothoxyphosphine respectively. Then, compounds 5 and 9) were incorporated into oligonucleotides by using the standard solid-phase synthesis of DNA with the phosphoramidite method. **Results** Six new oligonucleotides (ODN II - ODN VII) containing methyleneformacetal have been synthesized. The melting temperatures ( $T_m$ ) of these ODNs with their DNA complements were determined. **Conclusion** The melting temperatures ( $T_m$ ) of these modified ODNs were lowered about 0.8 - 1.2 °C per methyleneformacetal modification. These new ODNs can hybridize to DNA with only slightly less affinity than a control phosphodiester ODN, yet more work is necessary to study these modified ODNs and their biological activities.

**Key words:** antisense drugs; oligodeoxynucleotide; melting temperature; hybridize

在反义药物的研究中,通常对磷酸二酯骨架进行修饰,以达到耐核酸酶、合适的膜通透性及提高与靶DNA杂交的亲和力等目的<sup>[1]</sup>。磷酸二酯键非桥键上的一个氧被硫取代可得到硫代寡核苷酸(PS-ODN)。PS-ODN因具有较好的抗酶解活性,已成为

目前最广泛研究的寡核苷酸(ODN)类似物之一。其中Vitravene是1998年上市的第一个反义药物,用于治疗艾滋病病人巨噬细胞病毒性视网膜炎<sup>[2]</sup>。然而,作为第一代的反义药物PS-ODN与互补RNA的结合亲和力弱于天然ODN,而且极易被胃和肠中的核酸酶降解,表现出非常差的口服生物利用度。另外,PS-ODN能与多种蛋白进行“非选择性”的结合,竞争性地抑制多种不同的酶,临床上高剂量(>10

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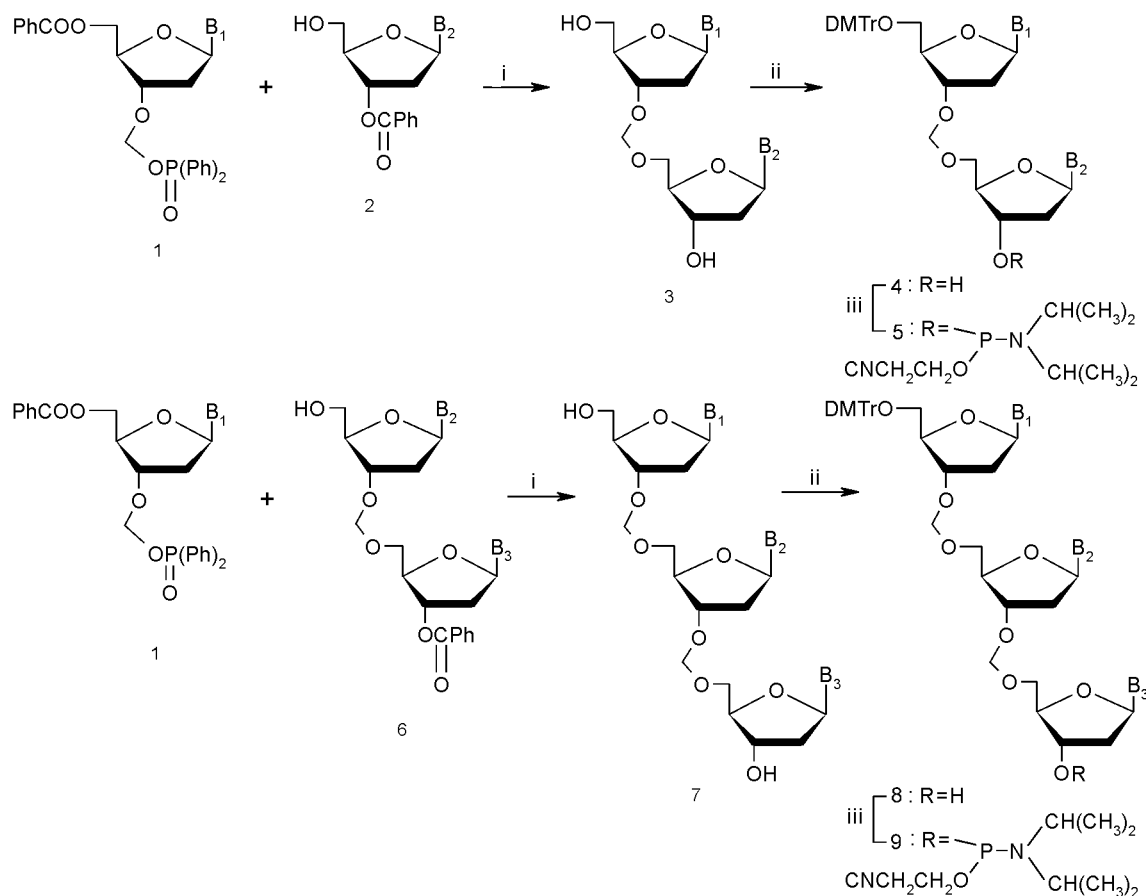
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mg•kg<sup>-1</sup>)时表现出毒副作用<sup>[3]</sup>。

因此,在反义化学的骨架修饰中必须要使反义化合物非常类似于天然的 ODN,从而保留杂交亲和力、特异性以及催化活性,即杂交后能够诱导 RNase H 裂解靶 RNA。通盘考虑这些反义性质,没有任何单一骨架修饰的化合物要优于 PS-ODN<sup>[3]</sup>。甲缩醛键由于能耐核酸酶、非手性、电中性且是磷酸二酯键的电子等排体,这些均克服了 PS-ODN 的缺陷。因此在以前研究的基础上<sup>[4,5]</sup>,作者设计合成了 5'-或 3'-端经甲缩醛键修饰,中间连着一段磷酸二酯键的混合骨架寡核苷酸(MBO),希望在不影响杂交亲和力的前提下既能增强整条 MBO 耐核酸酶的能力,又能保留诱导 RNase H 裂解双链中 RNA 的能力。

合成带有部分甲缩醛键的反义寡核苷酸,关键是合成(3' → 5')亚甲基缩醛连接的二聚、三聚等寡聚体的核苷。参照文献[5],3'-O-(二苯磷酰氧)甲基缩醛核苷(1)经三甲基硅三氟甲磺酸酯(TMSOTf)与 3'-位苯甲酰保护的核苷(2 或 6)缩合,引入(3' → 5')亚甲基缩醛键,分别合成二聚体(3)和三聚体脱氧核苷(7)。化合物 3 或 7 的 5'-位经 4,4'-二甲氧三苯甲基(DMTTr)保护后,3'-位与(2-氰乙基 N,N-二异丙基)氯化亚磷酰胺缩合反应,就可以用亚磷酰胺法(phosphoramidite method)和标准固相 DNA 合成仪来完成(图 1),化合物 4,7 和 8 的结构经<sup>1</sup>H NMR 和 FAB-MS 确证(表 1)。



i. TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, molecular sieves; KOH, methanol/dioxane, 0 °C; ii. DMTrCl, pyridine; iii. 2-cyanoethyl N,N-diisopropylchlorophosphoramidite, N,N-diisopropylethylamide

Figure 1 Route of synthesis of target compounds 4, 7 and 8

所合成 MBO 序列的选择<sup>[6]</sup>,用 Vitravene 中一段 15 个碱基的片段 5'-d(TTTGCTCTTCTTCTT)-3',与互补 DNA 的解链温度 T<sub>m</sub> 值见表 2。T<sub>m</sub> 值是寡核苷酸设计中的一个重要参数,即寡核苷酸与模板之间精确互补的情况下有 50%的寡核苷酸与模板配对,

而另外 50%的寡核苷酸处于解离状态时的温度, T<sub>m</sub> 值可以衡量寡核苷酸与靶 DNA 或 RNA 的杂交亲和力。由表中可以看出带亚甲基缩醛连接的寡核苷酸,平均每个修饰 T<sub>m</sub> 值下降约 0.8~1.2 °C,相当于磷硫酰修饰的寡核苷酸(1.0 °C)<sup>[7]</sup>。甲缩醛键修饰

Table 1 Physical and spectra data of compounds 4a - d, 7a - c and 8a - c

No. *	Structure	Molecular formula	MP/ °C	Yield/ %	<sup>1</sup> H NMR/ δ( DMSO d <sub>6</sub> )	FAB- MS m/z
4a	B <sub>1</sub> = T B <sub>2</sub> = T	C <sub>42</sub> H <sub>46</sub> N <sub>4</sub> O <sub>12</sub>	118 - 121	90	1.56 and 1.96(6H, 2s, -CH <sub>3</sub> × 2), 2.29 - 2.36(2H, m, 2'-H <sub>ab</sub> ), 2.56 - 2.66(2H, m, 2'-H <sub>ab</sub> ), 3.49 - 3.58(2H, m, 5'-H <sub>ab</sub> ), 3.94(8H, s, -OCH <sub>3</sub> × 2, 5'-H <sub>ab</sub> ), 4.15 - 4.35(2H, m, 4'-H × 2), 4.42 - 4.44 and 4.73 - 4.75(2H, 2m, 3'-H × 2), 4.96 - 5.04(2H, m, -OCH <sub>2</sub> O), 6.41 - 6.47(2H, m, 1'-H × 2), 7.02 - 7.05(4H, m, Ar H), 7.40 - 7.71(10H, m, 6-H, Ar H × 2), 7.86(1H, s, 6-H)	799( M + H <sup>+</sup> )
4b	B <sub>1</sub> = T B <sub>2</sub> = C <sup>Bz</sup>	C <sub>48</sub> H <sub>49</sub> N <sub>5</sub> O <sub>12</sub>	120 - 123	83	1.67(3H, s, -CH <sub>3</sub> ), 2.30 - 2.63(4H, m, 2'-H <sub>ab</sub> × 2), 3.65 - 3.87(4H, m, 5'-H <sub>ab</sub> × 2), 3.90(6H, s, -OCH <sub>3</sub> × 2), 4.19 - 4.26(2H, m, 4'-H × 2), 4.48 - 4.56(2H, 2m, 3'-H × 2), 4.96 - 5.02(2H, m, -OCH <sub>2</sub> O), 6.32 - 6.35(2H, m, 1'-H × 2), 7.05 - 8.11(20H, m, Ar H × 4, 5-H/dC, 6-H/T), 8.25(1H, d, J = 7.5 Hz, 6-H/dC)	888( M + H <sup>+</sup> )
4c	B <sub>1</sub> = C <sup>Bz</sup> B <sub>2</sub> = T	C <sub>48</sub> H <sub>49</sub> N <sub>5</sub> O <sub>12</sub>	129 - 130	85	1.42(3H, s, -CH <sub>3</sub> ), 2.00 - 2.31(4H, 2m, 2'-H <sub>ab</sub> × 2), 3.18 - 3.27(2H, m, 5'-H <sub>ab</sub> ), 3.70(8H, s, -OCH <sub>3</sub> × 2, 5'-H <sub>ab</sub> ), 4.00 - 4.07(2H, m, 4'-H × 2), 4.14 - 4.47(2H, m, 3'-H × 2), 4.78(2H, AB, J = 7.0, 10.0 Hz, -OCH <sub>2</sub> O), 5.37(1H, s, OH), 6.12 - 6.20(2H, m, 1'-H × 2), 6.84 - 8.01(20H, m, Ar H × 4, 5-H/dC, 6-H/dT), 8.11(1H, d, J = 7.5 Hz, 6-H/dC)	888( M + H <sup>+</sup> )
4d	B <sub>1</sub> = C <sup>Bz</sup> B <sub>2</sub> = C <sup>Bz</sup>	C <sub>54</sub> H <sub>52</sub> N <sub>6</sub> O <sub>12</sub>	95 - 97	80	1.51 - 1.61(2H, m, 2'-H <sub>ab</sub> ), 1.89 - 2.02(2H, m, 2'-H <sub>ab</sub> ), 3.25 - 3.46(4H, m, 5'-H <sub>ab</sub> × 2), 3.75(6H, s, -OCH <sub>3</sub> × 2), 3.88 - 4.01(2H, m, 4'-H × 2), 4.11 - 4.35(2H, m, 3'-H × 2), 4.75(2H, AB, J = 7.0, 10.0 Hz, -OCH <sub>2</sub> O), 5.96 - 6.03(2H, m, 1'-H × 2), 6.87 - 8.03(20H, m, Ar H × 4, 5-H/dC × 2), 8.07(1H, d, J = 7.5 Hz, 6-H/dC), 8.12(1H, d, J = 7.5 Hz, 6-H/dC)	977( M + H <sup>+</sup> )
7a	B <sub>1</sub> = T B <sub>2</sub> = T B <sub>3</sub> = T	C <sub>32</sub> H <sub>42</sub> N <sub>6</sub> O <sub>15</sub>	98 - 100	90	1.78(9H, s, -CH <sub>3</sub> × 3), 2.01 - 2.24(6H, m, 2'-H <sub>ab</sub> × 3), 3.57 - 3.69(6H, m, 5'-H <sub>ab</sub> × 3), 3.86 - 4.30(6H, m, 3'-H × 3, 4'-H × 3), 4.77(4H, 2AB, -OCH <sub>2</sub> O × 2), 5.14(1H, t, J = 5.0 Hz, 5'-OH), 5.36(1H, d, J = 4.3 Hz, 3'-OH), 6.11 - 6.20(3H, m, 1'-H × 3), 7.51, 7.53 and 7.69(3H, 3s, 6-H × 3), 11.31, 11.33 and 11.35(3H, 3s, NH × 3)	751( M + H <sup>+</sup> )
7b	B <sub>1</sub> = C <sup>Bz</sup> B <sub>2</sub> = T B <sub>3</sub> = T	C <sub>38</sub> H <sub>45</sub> N <sub>7</sub> O <sub>15</sub>	108 - 111	85	1.78(6H, s, -CH <sub>3</sub> × 2), 2.13 - 2.28(6H, m, 2'-H <sub>ab</sub> × 3), 3.62 - 3.70(6H, m, 5'-H <sub>ab</sub> × 3), 3.89 - 4.32(6H, m, 3'-H × 3, 4'-H × 3), 4.79(4H, br, -OCH <sub>2</sub> O × 2), 6.12 - 6.18(3H, m, 1'-H × 3), 7.38(1H, d, J = 7.5 Hz, 5-H/dC), 7.49 - 7.63(5H, m, Ar H), 7.99 and 8.02(2H, 2s, 6-H/T × 2), 8.39(1H, d, J = 7.5 Hz, 6-H/dC), 11.31 and 11.35(2H, 2s, NH × 2)	840( M + H <sup>+</sup> )
7c	B <sub>1</sub> = T B <sub>2</sub> = C <sup>Bz</sup> B <sub>3</sub> = T	C <sub>38</sub> H <sub>45</sub> N <sub>7</sub> O <sub>15</sub>	104 - 107	87	1.76 and 1.77(6H, 2s, -CH <sub>3</sub> × 2), 2.08 - 2.25(6H, m, 2'-H <sub>ab</sub> × 3), 3.56 - 3.59(2H, m, 4'-H × 2), 3.68 - 3.94(6H, m, 5'-H <sub>ab</sub> × 3), 4.21 - 4.31(4H, m, 3'-H × 3, 4'-H), 4.78 - 4.82(4H, m, -OCH <sub>2</sub> O × 2), 5.12(1H, t, J = 5.0 Hz, 5'-OH), 5.35(1H, d, J = 4.3 Hz, 3'-OH), 6.11 - 6.21(3H, m, 1'-H × 3), 7.50(1H, d, J = 7.5 Hz, 5-H/dC), 7.60 - 7.68(5H, m, Ar H), 7.99 and 8.01(2H, 2s, 6-H/T × 2), 8.21(1H, d, J = 7.5 Hz, 6-H/dC)	840( M + H <sup>+</sup> )
8a	B <sub>1</sub> = T B <sub>2</sub> = T B <sub>3</sub> = T	C <sub>53</sub> H <sub>60</sub> N <sub>6</sub> O <sub>17</sub>	102 - 105	81	1.67, 1.86 and 1.91(9H, 3s, -CH <sub>3</sub> × 3), 2.15 - 2.68(6H, m, 2'-H <sub>ab</sub> × 3), 3.90(6H, m, -OCH <sub>3</sub> × 2), 3.91 - 4.68(11H, m, 3'-H × 2, 4'-H × 3, 5'-H <sub>ab</sub> × 3), 4.93(4H, 2AB, -OCH <sub>2</sub> O × 2), 5.62 - 5.64(1H, m, 3'-H), 6.26 - 6.34(2H, m, 1'-H × 2), 6.50 - 6.53(1H, m, 1'-H), 7.29 and 7.38(2H, 2s, 6-H × 2), 6.94 - 8.03(14H, m, Ar H × 3, 6-H)	1053( M + H <sup>+</sup> )
8b	B <sub>1</sub> = C <sup>Bz</sup> B <sub>2</sub> = T B <sub>3</sub> = T	C <sub>59</sub> H <sub>63</sub> N <sub>7</sub> O <sub>17</sub>	110 - 112	78	1.85 and 1.93(6H, 2s, -CH <sub>3</sub> × 2), 2.24 - 2.61(6H, m, 2'-H <sub>ab</sub> × 3), 3.91(6H, m, -OCH <sub>3</sub> × 2), 3.94 - 4.10(4H, m, 5'-H <sub>ab</sub> × 2), 4.27 - 4.67(7H, m, 3'-H × 2, 4'-H × 3, 5'-H <sub>ab</sub> ), 4.86 - 4.97(4H, m, -OCH <sub>2</sub> O × 2), 5.67 - 5.75(1H, m, 3'-H), 6.26 - 6.40(2H, m, 1'-H × 2), 6.51 - 6.55(1H, m, 1'-H), 6.93 - 8.01(21H, m, Ar H × 4, 6-H/T × 2, 5-H/dC), 8.17(1H, d, J = 7.5 Hz, 6-H/dC)	1142( M + H <sup>+</sup> )
8c	B <sub>1</sub> = T B <sub>2</sub> = C <sup>Bz</sup> B <sub>3</sub> = T	C <sub>59</sub> H <sub>63</sub> N <sub>7</sub> O <sub>17</sub>	108 - 110	75	1.69 and 1.86(6H, 2s, -CH <sub>3</sub> × 2), 2.25 - 2.60(6H, m, 2'-H <sub>ab</sub> × 3), 3.90(6H, m, -OCH <sub>3</sub> × 2), 3.91 - 4.11(4H, m, 5'-H <sub>ab</sub> × 2), 4.32 - 4.60(7H, m, 3'-H × 2, 4'-H × 3, 5'-H <sub>ab</sub> ), 4.82 - 4.90(4H, 2AB, -OCH <sub>2</sub> O × 2), 5.63 - 5.67(1H, m, 3'-H), 6.23 - 6.42(3H, m, 1'-H × 3), 6.95 - 8.04(21H, m, Ar H × 4, 6-H/T × 2, 5-H/dC), 8.15(1H, d, J = 7.5 Hz, 6-H/dC)	1142( M + H <sup>+</sup> )

\* C, H, N analyses were within 0.5 % of calculated values

后  $T_m$  的下降可能是由于在脱氧核糖中 2'-位没有取代, 3'-O 和 4'-O (环上氧) 的兔耳效应形成较为稳定的 C2'-内向构象, 不利于与互补 RNA 或 DNA 形成更为稳定的 A-型双键(图 2)<sup>[8]</sup>。目前我们正在研究 2'-取代后亚甲基缩醛键连接的寡核苷酸, 希望得到满意的结果。

**Table 2 Melting temperatures ( $T_m$ ) of modified and unmodified oligonucleotides hybridized to their complementary oligodeoxynucleotide strands**

Modified ODN	Sequence	$T_m/^\circ\text{C}$	$\Delta T_m^*$
ODN I (control)	5'-TTTGCTCTCTCTCTF-3'	55.0	-
ODN II	5'-TTTGCF- <i>m</i> CTTCTCTCF-3'	54.1	-0.9
ODN III	5'-TTTGCF- <i>m</i> C- <i>m</i> TTCTCTCF-3'	53.4	-0.8
ODN IV	5'-T- <i>m</i> T- <i>m</i> TGCTCTCTCTCF-3'	52.6	-1.2
ODN V	5'-TTTGCTCTCTTC- <i>m</i> T- <i>m</i> T3'	53.0	-1.0
ODN VI	5'-T- <i>m</i> TTGCTCTCTCTCF- <i>m</i> T3'	52.6	-1.2
ODN VII	5'-T- <i>m</i> T- <i>m</i> TGCTCTCTTC- <i>m</i> T- <i>m</i> T3'	50.6	-1.1
ODN VIII	5'-T-s-T-s-T-s-G-s-G-s-T-s-C-s-T-s-T-s' s-C-s-T-s-T-s-C-s-T-s-T-s'	40.0	-1.0

*m*: Methyleneformacetal; *s*: Phosphorothioate modified; \*  $\Delta T_m$ : The decrease in  $T_m$  per modification

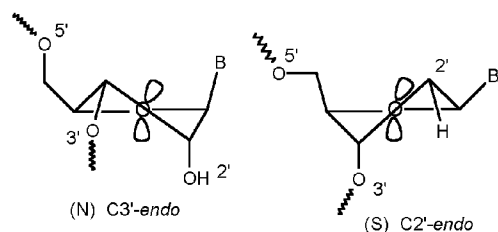


Figure 2 C3'-endo and C2'-endo sugar conformations typical of RNA and DNA, respectively

### 实验部分

熔点用 RY-2 型熔点仪测定, 温度未校正。<sup>1</sup>H NMR 用 Bruker AC-P300 型核磁共振仪, TMS 为内标。FAB-MS 用 MAT212 型质谱仪。元素分析用 Perkin Elmer 2400 型元素分析仪。柱色谱用硅胶 H, 薄层色谱用硅胶 GF<sub>254</sub> 均为青岛海洋化工厂产品。吡啶和二氯甲烷分别用 CaH<sub>2</sub> (5 g·L<sup>-1</sup>) 和 P<sub>2</sub>O<sub>5</sub> (5 g·L<sup>-1</sup>) 回流 3 h, 蒸馏后用分子筛(0.4 nm) 保存。起始原料 5'-O 苯甲酰-3'-O (二苯基磷酰氧) 亚甲基脱氧核苷(1)、3'-O 苯甲酰脱氧核苷(2)、脱氧核苷二聚体(3)和 3'-O 苯甲酰脱氧核苷二聚体(6)按文献[5]合成。

#### 1 (2-氰乙基 N, N-二异丙基) 氯化亚磷酸胺

参照文献[9]方法, 收集 103 ~ 105 °C/11 Pa 馏分, 收率 50%。

#### 2 5'-O (4,4'-二甲氧三苯甲基)-脱氧核苷二聚体(4)

将化合物 3 (1.0 mmol) 和 4,4'-二甲氧三苯甲基氯 0.405 g (1.2 mmol) 加入到干燥吡啶 12 mL 中, 室温搅拌过夜。溶液蒸干, 产物经硅胶柱纯化得到白色固体 4。

#### 3 5'-O (4,4'-二甲氧三苯甲基)-3'-O (2-氰乙基 N, N-二异丙基) 亚磷酸胺脱氧核苷二聚体(5)

完全干燥的化合物 4 (1.0 mmol) 溶于无水二氯甲烷 5 mL 中。氮气保护下依次加入 N, N-二异丙基乙胺 0.8 mL (4.6 mmol) 和 (2-氰乙基 N, N-二异丙基) 氯化亚磷酸胺 0.473 g (2 mmol)。室温搅拌 30 min 后, 加入二氯甲烷 40 mL, 有机层用 5% 碳酸氢钠溶液 40 mL 洗涤, Na<sub>2</sub>SO<sub>4</sub> 干燥, 减压蒸除有机溶剂, 残余物经硅胶柱纯化, 用二氯甲烷和正己烷重结晶, 得白色固体 5。

#### 4 脱氧核苷三聚体(7)

将化合物 1 (1.0 mmol) 和化合物 6 (1.0 mmol) 溶于干燥二氯甲烷 10 mL 中。室温氮气保护下, 用 0.4 nm 分子筛干燥 2 h, 搅拌滴加 TMSOH (0.33 g, 1.5 mmol)。反应 0.5 h, 过滤, 用饱和碳酸氢钠 10 mL 洗涤, 水层用二氯甲烷 2 × 10 mL 萃取后, 合并有机层, 干燥(用 MgSO<sub>4</sub>), 浓缩。残余物溶于甲醇-二氧六环 20 mL (3:1) 中, 冷却至 0 °C, 加入 1 mol·L<sup>-1</sup> 氢氧化钾溶液 5 mL, 搅拌 30 min, 反应液用 732 型树脂中和至中性, 过滤, 减压浓缩, 粗品经硅胶柱纯化, 得白色固体 7。

#### 5 5'-O (4,4'-二甲氧三苯甲基)-脱氧核苷三聚体(8)

制备方法同化合物 4。

#### 6 5'-O (4,4'-二甲氧三苯甲基)-3'-O (2-氰乙基 N, N-二异丙基) 亚磷酸胺脱氧核苷三聚体(9)

制备方法同化合物 5。

#### 7 解链温度 ( $T_m$ ) 测定<sup>[10]</sup>

将等摩尔的修饰寡核苷酸(ODN II ~ ODN VIII) 及互补 DNA 溶于含有 1 mmol·L<sup>-1</sup> EDTA, 10 mmol·L<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub>, 0.14 mol·L<sup>-1</sup> NaCl 的溶液中, 配成约为 2.5 ~ 3.0 μmol·L<sup>-1</sup> 浓度, pH 值为 7.4。将样品在 90 °C 加热 5 min, 然后自然冷却至室温(约 20 °C)。将以上褪火的样品转移至 1 cm 石英比色杯中, 用 UV-260 紫外可见分光光度计测定其吸光度, 20 ~ 90 °C 每隔 1 °C 记录一次。以吸光度对温度作图绘制熔解曲线, 再由熔解曲线计算  $T_m$  值。同

样方法测定磷酸二酯寡核苷酸(ODN)的  $T_m$  值作参比,分别计算寡核苷酸平均每个甲缩醛或磷硫酰的修饰,解链温度  $T_m$  值的变化。

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