DNA-NH₂酰化反应: 分子量还是结构占主导地位?

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摘要 DNA的化学修饰可以使DNA分子具有其他功能, 如构建DNA编码化合物库(DNA-encoded chemical libraries, DECLs)、制备用于生物传感的DNA探针、提高DNA适配体的结合活性等. 对DNA化学修饰反应效率已有的研究集中在对反应条件(溶剂、温度等)的优化, 为了确定影响修饰效率的重要因素, 我们以DNA-NH₂的酰化反应为模型进行了系统研究. 结果表明, 影响DNA-NH₂酰化反应的主要因素是DNA和酰化试剂的分子量. 相反, DNA的结构在反应中起着次要的作用.

关键词 DNA-NH₂酰化反应, 产率, 分子量, 结构, NHS 酯

DNA是脱氧核糖核酸(deoxyribonucleic acid)的缩写, 又称去氧核糖核苷酸, 是染色体的主要组成成分, 同时包含了生物体几乎全部的遗传信息. DNA分子的功能是贮存决定物种性状的几乎所有蛋白质和RNA分子的全部遗传信息. 但是随着DNA纳米技术的发展, 因为它具有稳定性、多样性以及特异性的特点, 人们发现DNA不仅可以作为生命遗传信息的载体, 还可以作为生物传感器^[1-5]、DNA分子机器^[6-10], 或作为模板指导化学合成反应^[11-14]等, 可以用于生物检测分析、疾病的治疗等各个领域.

对DNA进行各种化学修饰也是DNA纳米领域常用的手段. 如DNA荧光探针, 就是在DNA上连接荧光团或猝灭团等来观察荧光信号^[15-17]; 在DNA适配体上进行相应的化学修饰可以提高适配体对靶标物质的亲和力^[18]; 或是利用DNA化学修饰技术在

DNA上连接不同的有机小分子, 从而构建DNA编码化合物库(DECLs). 在DNA编码化合物库中, 每一个有机小分子都连着一特定唯一的DNA片段, 从而此DNA片段可以作为扩增识别的条形码, 可以通过聚合酶链反应扩增(PCR)和有效的高通量DNA测序方法读取, 可以将靶标配体或蛋白固定在固定相中, 进而高效快捷地筛选出与靶标物质相互作用的先导化合物^[19-27].

基于此, 分析影响DNA与有机小分子反应的主要因素, 对在DNA上进行化学修饰并提高其化学修饰效率有重要的意义. 已有的研究仅针对DNA与有机小分子反应的溶剂、温度等外在因素进行了优化^[28,29], 并没有探讨反应物分子自身的内在因素(分子量和分子构型)对反应的影响. 本文以DNA-NH₂与有机羧酸小分子进行DNA酰化反应为模型, 在反应

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物分子的分子层面系统地研究反应物分子量和分子构型对DNA修饰反应的影响。

1 实验

(i) 仪器与试剂. QYZH-10A纯水仪(重庆前沿水处理设备有限公司); SZCL-3A数显智能控温磁力搅拌器(巩义市予华仪器有限责任公司); RE-52AA旋转蒸发器(上海亚荣生化仪器厂); ZF-6型三用紫外分析仪(上海嘉鹏科技有限公司); AVANCE III核磁共振波谱仪(瑞士Bruker公司); 6510 Q-TOF质谱仪(安捷伦科技有限公司); JP-100S超声仪(深圳市洁盟清洗设备有限公司); 755B紫外吸收光度计(上海菁华科技仪器有限公司); 金属浴OSE-DB-01(天根生化科技(北京)有限公司); SI votex-genie 2涡旋振荡仪(美国Scientific Industries公司); 涡旋离心机D1008(大龙兴创实验仪器(北京)有限公司); 真空干燥离心机LNG-T86(太仓市华利达实验设备有限公司); 电泳装置HOEFER SE600(美国HOEFER公司); DY-7C电泳仪电源(北京市六一仪器厂); Canon LiDE 120扫描仪(佳能(中国)有限公司)。

1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐(EDC, 纯度>98.0%)、*N*-羟基琥珀酰亚胺(NHS, 纯度>98.0%)、核酸染料(Stains-all, C₃₀H₂₇BrN₂S₂, 生物染色剂)和无水*N,N*-二甲基甲酰胺(DMF, 超干溶剂, 99.8%)均购买自Sigma-Aldrich(上海)贸易有限公司; 过硫酸铵(纯度≥98.5%)、*N,N,N',N'*-四甲基乙二胺(TEMED, 纯度>99.0%)均购买自北京鼎国昌盛生物技术有限责任公司; 三乙二醇单甲醚(纯度>95.0%)、壬乙二醇单甲醚(纯度>97.0%)和丁二酸酐(纯度>99.0%)均购买自上海阿拉丁试剂公司; 尿素(纯度>99.5%)、三羟甲基氨基甲烷(纯度≥99.0%)、乙二醇四乙酸二钠(纯度>99.0%)、硼酸(纯度≥99.5%)、丙烯酰胺(纯度≥99.9%)、甲叉双丙烯酰胺(纯度≥98.0%)均购买于生工生物工程(上海)股份有限公司。其余试剂(分析纯)均购买自成都市科龙化工试剂厂。所有试剂均未经过进一步纯化, 实验所用水为超纯水, 电阻率为18.25 MΩ cm。

(ii) DNA-NH₂酰化反应中有机羧酸小分子的合成及表征. 12-氧代-2,5,8,11-四氧杂十五烷-15-酸的合成. 如式(1)所示, 向三乙二醇单甲醚(1.00 g, 6.1 mmol)和丁二酸酐(1.22 g, 12.3 mmol)的混合溶液中, 缓慢滴加三乙胺(0.5 mL, 3.6 mmol), 将温度升高至

60℃反应, 并通过薄层色谱(TLC)监测, 5 h后停止反应. 经过柱层析(甲醇:三氯甲烷=1:9, 体积比)分离粗产物, 得到纯化后的目标产物12-氧代-2,5,8,11-四氧杂十五烷-15-酸1.05 g, 为淡黄色液体, 产率66%。

30-氧代-2,5,8,11,14,17,20,23,26,29-十氧杂三十四烷-33-酸的合成. 如式(2)所示, 向壬乙二醇单甲醚(0.50 g, 1.2 mmol)和丁二酸酐(0.24 g, 2.4 mmol)的混合溶液中, 缓慢滴加三乙胺(0.1 mL, 0.7 mmol), 将温度升高至60℃反应, 并通过薄层色谱(TLC)监测, 6 h后停止反应. 经过柱层析(乙酸乙酯:甲醇=3:7, 体积比)分离粗产物, 得到纯化后目标产物30-氧代-2,5,8,11,14,17,20,23,26,29-十氧杂三十四烷-33-酸0.43 g, 为淡黄色液体, 产率69%。

化合物利用核磁共振和质谱进行结构表征, 其表征结果如下。

12-氧代-2,5,8,11-四氧杂十五烷-15-酸, 微黄色液体, 产率为66%, ¹H NMR(400 MHz, CDCl₃) δ: 4.29~4.25(m, 2H), 3.71~3.65(m, 8H), 3.59(dd, *J*=5.7, 3.4 Hz, 2H), 3.39(s, 3H), 2.68~2.65(m, 4H)(图S1). ¹³C NMR(100 MHz, CDCl₃) δ: 175.63, 171.98, 71.84, 70.70, 70.44, 70.35, 68.97, 63.88, 58.88, 29.37, 29.04(图S2). ESI-HRMS *m/z*: 实测值263.1140[M-H]⁺; 计算值C₁₁H₁₉O₇: 263.1136(图S3)。

30-氧代-2,5,8,11,14,17,20,23,26,29-十氧杂三十四烷-33-酸, 微黄色液体, 产率69%, ¹H NMR(400 MHz, CDCl₃) δ: 4.28~4.25(m, 2H), 3.70(s, 1H), 3.68(d, *J*=3.0 Hz, 6H), 3.67~3.64(m, 24H), 3.63(s, 1H), 3.55(dd, *J*=5.8, 3.6 Hz, 2H), 3.38(s, 3H), 2.67~2.64(m, 4H)(图S4). ¹³C NMR(100 MHz, CDCl₃) δ: 174.30, 172.14, 71.93, 70.68, 70.57~70.46, 69.03, 63.88, 58.99, 29.57, 29.12(图S5). ESI-HRMS *m/z*: 实测值 527.2704 [M-H]⁺; 计算值C₂₃H₄₃O₁₃: 527.2709(图S6)。

(iii) DNA-NH₂酰化反应中不同DNA结构的设计与合成. DNA序列组成如下:

NH₂-A: 5'-NH₂-CTCTTCGAGGCGTCG-3', 15 nt;
NH₂-ABC: 5'-NH₂-CTCTTCGAGGCGTCGGCTCATTACTGTGCGGTATCCTGATCGAG-3', 45 nt; A': 5'-CGACGCCTCGAAGAG-3', 15 nt; B': 5'-GCACAGTA

AATGAGC-3', 15 nt; C': 5'-CTCGATCAGGATACC-3', 15 nt; A'B': 5'-GCACAGTAAATGAGCCGACGCCTCGAAGAG-3', 30 nt; B'C': 5'-CTCGATCAGGATACC GCACAGTAAATGAGC-3', 30 nt; A'B'C': 5'-CTCGATCAGGATACCGCACAGTAAATGAGCCGACGCCTCGAAGAG-3', 45 nt. 上述8条DNA链(NH₂-A; NH₂-ABC; A'; B'; C'; A'B'C'; B'C'; A'B'C')均购自生工生物工程(上海)股份有限公司. 其中5'端氨基修饰的DNA链的纯化方式为高效液相色谱(HPLC)纯化, 其余DNA链的纯化方式为聚丙烯酰胺凝胶电泳(PAGE)纯化.

DNA结构的设计. DNA引物分子NH₂-A的碱基序列(5'-NH₂-CTCTTCGAGGCGTCG-3', 15 nt)与DNA引物分子A'的碱基序列(5'-CGACGCCTCGAAGAG-3', 15 nt)是反向互补的(NH₂-A—A'), 同理, 其他DNA引物分子中对应的B, C区域的碱基序列分别与B'(蓝色, 15 nt)、C'(绿色, 15 nt)区域反向互补(B—B'; C—C'). 这8条单链的DNA两两相互组合, 得到不同的DNA结构a-i, 如图1所示.

a~i结构中(图1), 氨基基团均连接在DNA序列**A**区域的5'端. a和c分别是15个碱基和45个碱基的柔性单链DNA; b和d~i都含有互补的DNA双链, 互补双链的长度有15, 30和45 bp, 互补的区域有**A**区域(15 nt)、**B**区域(15 nt)、**C**区域(15 nt)、**AB**区域(30 nt)、**BC**区域(30 nt)以及**ABC**区域(45 nt). 设计目的为: (1) 观察氨基在柔性单链末端与在刚性双链末端对整个酰化反应动力学的影响; (2) 探究在此基础上延长柔性单链和刚性双链长度对其酰化反应动力学的变化.

图 1 9 种 DNA 构型的设计
Figure 1 Designs of nine DNA conformations

DNA结构的组装. 按照组装的DNA结构a~i(图1), 各取两条4 nmol的互补DNA分子(或4 nmol同一DNA分子), 加入20 μ L磷酸盐缓冲液(0.2 mol/L, pH 8.0), 使每条DNA链的浓度均为0.2 mmol/L. 进行DNA分子退火: 95 $^{\circ}$ C, 5 min; 65 $^{\circ}$ C, 30 min; 50 $^{\circ}$ C, 30 min; 37 $^{\circ}$ C, 30 min; 25 $^{\circ}$ C, 30 min. 4 $^{\circ}$ C储存2 h备用.

(iv) DNA-NH₂酰化反应. NHS酯的制备: 2,5-二氧代吡咯烷-1-基(2-(2-(2-甲氧基乙氧基)乙氧基)乙基)琥珀酸酯; 2,5-二氧代吡咯烷-1-基(2,5,8,11,14,17,20,23,26-壬氧基辛二糖-28-基)琥珀酸酯. 如式(3)所示, 分别取有机羧酸小分子(80 μmol), *N*-羟基琥珀酰亚胺(NHS, 80 μmol), 1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐(EDC, 80 μmol), 加入至200 μL无水DMF中, 涡旋振荡反应, 薄层色谱监测反应进度(展开剂: 乙酸乙酯), 反应6 h后停止. 将溶液离心, 取微量澄清溶液(用无水DMF稀释400倍至浓度为1.0 mmol/L)备用, 此溶液即为有机羧酸小分子的NHS酯溶液.

氨基-DNA与小分子NHS酯的酰化反应. 反应原理图如图2所示, 9组DNA-NH₂结构(20 μL, 0.2 mmol/L)分别与2种小分子NHS酯(20 μL, 1.0 mmol/L)反应, 在室温下涡旋振荡10 min, 离心后取上层液, 向其加入变性染料备用(以防止部分反应继续进行). 通过进行20%变性的聚丙烯酰胺凝胶电泳(20%变性PAGE)观察反应情况, 再用Image J图像分析软件计算DNA条带的灰度值.

2 结果与讨论

图3为两种小分子NHS酯分别与9种DNA-NH₂模型反应的20%变性聚丙烯酰胺凝胶图像. 通过20%变性聚丙烯酰胺凝胶分析, 在DNA-NH₂模型中, 单链DNA(single-strand DNA, ssDNA)构型(比如H₂N-**A**和NH₂-**ABC**)与有机羧酸小分子的反应物只存在两种DNA条带, 从上至下分别对应DNA-NH₂酰化产物和H₂N-ssDNA; 双链DNA-NH₂结构(比如b和d-i)与有机羧酸小分子的反应物存在3种DNA条带, 从上至下分别对应DNA-NH₂酰化产物、H₂N-ssDNA、对应的单链DNA互补链. 在图3(a)中, 由于有机羧酸小分子(PEG=3)的分子量较小, c-i与有机羧酸小分子(PEG=3)

图 2 DNA-NH₂ 酰化反应的原理图

Figure 2 DNA-NH₂ acylation reaction

图3 有机羧酸小分子NHS酯(PEG=3)(a)和(PEG=9)(b)与DNA-NH₂ 酰化反应的 20%变性聚丙烯酰胺凝胶图像

Figure 3 20% denaturing polyacrylamide gel images of acylation reaction of small organic carboxylic acid (PEG=3) (a) and (PEG=9) (b) with DNA-NH₂.

的酰化产物与 H_2N -ssDNA之间的分子量和分子构型接近,在电泳时间较短时,其所对应的DNA条带迁移不会完全分开,这样不利于运用Image J图像分析软件对这两个DNA条带的灰度进行分析.

通过延长一定的电泳时间,将DNA-NH₂酰化产物与H₂N-ssDNA的条带完全分开,结果如图4所示.可以发现,在c-i与有机羧酸小分子(PEG=3)的反应

物中, DNA-NH₂酰化产物与DNA-NH₂对应的DNA条带已基本分开, 可通过Image J图像分析软件对这两个DNA条带的灰度进行分析.

所有实验均重复3次,将3次实验的20%变性聚丙烯酰胺凝胶图像用Image J图像分析软件进行处理分析,得到相应DNA条带的灰度值,并计算出各个反应的产率,统计结果如表1所示.

图4 有机羧酸小分子NHS酯(PEG=3)与DNA-NH₂(c-i)酰化反应的20%变性聚丙烯酰胺凝胶图像

Figure 4 20% denaturing polyacrylamide gel images of acylation reaction of DNA molecules (c-i) with small organic carboxylic acid (PEG=3)

表1 有机羧酸小分子与DNA-NH₂酰化反应的产率

Table 1 The yields of acylation reaction of DNA-NH₂ with small organic carboxylic acid

序号	DNA样品	产率PEG-3(%)	产率PEG-9(%)
1	H ₂ N-A	50±0.3	43±0.3
2	H ₂ N-A-A ₁	43±0.5	33±0.4
3	H ₂ N-ABC	41±2.0	36±0.7
4	H ₂ N-ABC-A ₁	34±2.6	31±0.8
5	H ₂ N-ABC-B ₁	34±2.7	33±2.5
6	H ₂ N-ABC-C ₁	37±1.1	34±2.0
7	H ₂ N-ABC-A ₁ B ₁	31±0.7	30±0.4
8	H ₂ N-ABC-B ₁ C ₁	36±2.4	34±2.0
9	H ₂ N-ABC-A ₁ B ₁ C ₁	29±2.7	29±0.7

从表1中可以发现, (1) 影响DNA-NH₂酰化反应的主要因素是反应物(DNA-NH₂和羧酸分子)的分子量, 其分子量越大, 所对应的反应速率越慢. 可以从以下两点说明: 对于相同DNA-NH₂而言, 它与低分子量的有机羧酸分子(PEG=3)的反应产率高于与高分子量的有机羧酸分子(PEG=9)的反应产率; 对于相同有机羧酸分子而言, 它与低分子量的DNA-NH₂的反应产率高于与高分子量的DNA-NH₂的反应产率. 表1结果表明, 随着DNA-NH₂的分子量增加, 其对应与有机羧酸分子酰化反应的产率也呈现出减少的趋势. 这可能是因为反应物分子的分子质量越小, 反应物分子就能在溶液中相对有着更强的扩散游离作用, 有更多的机会相互碰撞而促进反应发生. 相反, 分子质量越大, 反应物分子在溶液中的扩散游离作用较小, 相互碰撞的机会减少, 而减慢反应速率. (2) DNA的结构也会一定程度上影响DNA-NH₂酰化反应. 在表1中, H₂N-ABC-A₁, H₂N-ABC-B₁, H₂N-ABC-C₁都

含有单链和双链的DNA结构(15 bp), 并且有近乎相似的分子量, 但是氨基基团的局部环境却有一定差别, H₂N-ABC-A₁中氨基基团局部相连的是15个碱基对的双链, 它的酰化反应产率较低; H₂N-ABC-B₁中氨基基团局部相连的是15个碱基的单链, 它的酰化反应产率相对增加; H₂N-ABC-C₁中氨基基团局部相连的是30个碱基的单链, 它的酰化反应产率增加明显. 对于H₂N-ABC-A₁B₁和H₂N-ABC-B₁C₁的反应, 也有相同的趋势. 这可能是由于单链DNA的空间排列柔韧并灵活, 它能快速地调整构象, 因此氨基基团与单链相连接时, 在溶液中单链的灵活性能快速的变化氨基的位置, 从而促进反应发生, 加快反应速率. 相应地, 氨基基团与双链相连接时, 双链的柔韧性不足, 不能有效地促进反应发生, 所以其反应速率相对较慢.

3 结论

本文利用9种不同分子量不同DNA结构的DNA-NH₂与有机羧酸分子(PEG=3, 9)进行酰化反应, 前期通过有机合成反应自主合成有机羧酸分子, 利用核磁共振仪、质谱仪对化合物进行表征; 通过DNA自组装反应制备得9种DNA-NH₂分子. 对这两种反应分子进行一系列DNA-NH₂酰化修饰反应, 通过20%变性聚丙烯酰胺凝胶电泳进行表征, Image J图像分析软件计算条带灰度从而计算反应产率, 系统地讨论反应物分子的分子质量和DNA结构这两个内在因素对整个DNA-NH₂酰化反应的影响.

此实验表明, 影响DNA-NH₂酰化反应的主要因素是反应物分子的分子质量, 分子质量越大, 其对应的反应速率越慢, 反应产率越低. DNA的分子构型也

会在一定程度上影响DNA-NH₂酰化反应,氨基基因连接在柔韧的单链DNA上,它能促进氨基基团的游离性,促进反应进行,加快反应速率.现阶段的研究结果表明,在DNA分子的修饰反应中,应该首先避免反应物分子的分子质量过大,这样会明显降低反

应产率;其次要将DNA上所要修饰的部分与韧性的单链DNA连接,使得修饰基团在柔韧的单链上可以增加其反应机会,促进反应发生.如果以上两点都无法满足,这时应该增加反应时间或者增加反应物分子的摩尔浓度,方可促进反应.

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补充材料

- 图 S1 12-氧代-2,5,8,11-四氧杂十五烷-15-酸的氢谱图
- 图 S2 12-氧代-2,5,8,11-四氧杂十五烷-15-酸的碳谱图
- 图 S3 12-氧代-2,5,8,11-四氧杂十五烷-15-酸的质谱图
- 图 S4 30-氧代-2,5,8,11,14,17,20,23,26,29-十氧杂三十四烷-33-酸的氢谱图
- 图 S5 30-氧代-2,5,8,11,14,17,20,23,26,29-十氧杂三十四烷-33-酸的碳谱图
- 图 S6 30-氧代-2,5,8,11,14,17,20,23,26,29-十氧杂三十四烷-33-酸的质谱图
- 图 S7 2,5-二氧代吡咯烷-1-基(2-(2-(2-甲氧基乙氧基)乙氧基)乙基)琥珀酸酯的氢谱图
- 图 S8 2,5-二氧代吡咯烷-1-基(2-(2-(2-甲氧基乙氧基)乙氧基)乙基)琥珀酸酯的碳谱图
- 图 S9 2,5-二氧代吡咯烷-1-基(2,5,8,11,14,17,20,23,26-壬氧基辛二糖-28-基)琥珀酸酯的氢谱图
- 图 S10 2,5-二氧代吡咯烷-1-基(2,5,8,11,14,17,20,23,26-壬氧基辛二糖-28-基)琥珀酸酯的碳谱图

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Summary for “DNA-NH₂ 酰化反应：分子量还是结构占主导地位？”

DNA-NH₂ acylation reaction: Molecular weight or conformation dominates?

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DNA has the unique chemical and physical characteristics as a carrier of genetic information. With the development of structural DNA nanotechnology, it has been recognized that DNA can perform versatile functions with promising applications in nanomachine, computing, sensing and nanoarchitectures. In addition, the chemical incorporation with various functional groups brings in a diverse range of additional properties to DNA molecules for broadened applications. Therefore, post-synthetic modification of DNA has been widely used to introduce additional functionalities to DNA molecules, for example, design and synthesis of DNA-encoded library, preparation of DNA-based probes for biosensing, improving binding activities of DNA aptamers, etc. The modification efficiency is determined by both the intrinsic factors of the molecules involved (DNA and reagents) and the external factors (experimental conditions). However, previous studies on the modification focused on the optimization of reaction conditions. To determine the important intrinsic factors that affect modification efficiency, we have conducted a systematic study on the reaction with a model system—acylation of amino-DNA. Nine amino-modified DNAs with two different DNA conformations (a–i) were reacted with two carboxylic acids with different molecule weights (12-oxo-2,5,8,11-tetraoxapentadecan-15-oic acid, M_w 264.1209, and 30-oxo-2,5,8,11,14,17,20,23,26,29-decaoxatritriacontan-33-oic acid, M_w 528.2782), respectively, and characterized by polyacrylamide gel electrophoresis (PAGE). The bands were analyzed and calculated by Image J. In the present study, three factors are considered for the design. First, acylation is one of the most widely methods used for chemical modifications of DNA. The acylation reactions afford amides and these amides binding to DNA form more structurally complex and diverse DNA building blocks. Therefore, we explored the acylation reaction between amino-modified DNA with carboxylic acids. Second, two DNA conformations (single-stranded random coils, ssDNAs, and rigid, double-stranded DNA duplexes, dsDNA) were included. Single-stranded DNAs are more flexible than rigid DNA duplexes and thus can provide more variability in terms of modulating orientation of the reaction group to facilitate reactions. Third, the two small organic molecules with a varying number of polyethylene glycol (PEG) units were used, because PEG has been widely used in the field of chemical modification of biomacromolecules due to its excellent properties including a wide range of solubility, predominant bio-compatibility, good stability, low toxicity and no irritation, etc. We, therefore, systematically studied the DNA modification reaction to investigate the influences of two intrinsic factors (M_w and conformation) on the reaction. The primary factor for DNA-NH₂ acylation reaction is molecular weight (M_w). The higher the M_w of the reagents (both small molecules and DNAs) are, the slower the reactions are. DNA conformation plays a much minor role in the reaction. Compared with rigid dsDNA duplexes, flexible ssDNA chains facilitate reactions. Based on the current study, we would suggest for post-synthetic modification of DNA: (1) Keep the molecular weights of all reagents (both small molecules and DNA molecules) to the minimal in the design to allow fast diffusion. (2) Keep the DNA strands near the modification location to be single stranded to facilitate orientation adjustment. If high molecular weight and rigid conformation (duplex) are not avoidable, reaction time and/or reagent concentration should be elongated to ensure the modification efficiency.

DNA-NH₂ acylation, modification efficiency, molecular weight, conformation, NHS ester

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