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Synthesis of a 1′-Aminomethylthymidine and Oligodeoxyribonucleotides with 1′-Acylamidomethylthymidine Residues

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Reported here is a 10-step synthesis of a phosphoramidite building block of 1′-aminomethylthymidine that starts from 2-deoxyribose. The framework of the branched aminonucleoside was elaborated from a known 1-cyano-1-bromo glycosyl donor, whose reaction with the silylated nucleobase furnished the 1′-cyanide, which was reduced to the desired aminomethylnucleoside. The *N*-allyloxycarbonyl (Alloc)-protected nucleoside was converted to a phosphoramidite building block and incorporated into the oligonucleotides 5′-GCAT*TATTAC-3′, and 5′-GCAT*TAT*TAC-3′, where T* denotes 1′-acylamidomethylthymidine residues. Removal of the Alloc protecting group and acylation with the residue of pyrene-1-yl-butanoic acid were achieved on support, using microwave irradiation to ensure full conversion. The UV-melting point of the duplex of the singly and doubly modified decamers with their fully complementary target sequence is 0.1−6.9 °C higher than that of the unmodified control duplex, depending on the salt concentration. This suggests that the aminomethyl linker may allow for the placing of a functional "payload" in the minor groove of DNA duplexes without disrupting the helix. Oligonucleotides thus endowed with functional modifications may become useful for biomedical applications.

Introduction

Oligodeoxynucleotides can be used as hybridization probes and biomedical agents. In their unmodified form, they can be employed in site-directed mutagenesis,[1] as primers for polymerase chain reactions,[2] and as immunostimulatory agents,[3] to name just three applications. Also numerous are the applications for modified oligonucleotides. For example, oligonucleotides with chemical modifications are routinely immobilized on surfaces to generate DNA microarrays (DNA chips).[4,5] A chemical modification can also increase the selectivity of oligonucleotide probes that bind a target sequence against a background of other sequences.[6] Further, chemical modifications have been developed that render oligonucleotides more resistant toward nuclease attack,[7] increase their affinity for target strands,[8] or both.[9]

Labeling oligonucleotides with dyes or radioactive elements facilitates their detection.[10] Most frequently, the dyes are attached to the termini of the oligonucleotides, using commercially available phosphoramidites of fluorophores featuring flexible alkyl linkers. Doubly dye-labeled oligonucleotides can be used as molecular beacons.[11] When repeated labeling is required or the termini have to remain unmodified, labeling has to occur in the interior of strands. In order not to interfere with the function of the oligonucleotides, most notably their ability to form Watson−Crick duplexes, the newly added moieties have to point away from the base pairing surface of the nucleobases. This can be achieved by labeling phosphorothioate backbones,[12] thus placing the label in the solvent. Alternatively, one may introduce substituents at the 5-position of pyrimidines and the 7- or 8-position of purine residues.[13] This places the substituents such that they point into the major groove of duplex DNA. If oligonucleotides were available that place substituents in *both* the major and the minor groove without disrupting duplexes, improved molecular recognition of

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(1) Zoller, M. J.; Smith, M. *Nucleic Acids Res.* **1982**, *10*, 6487−6500.
(2) Mullis, K. B. *Angew. Chem.* **1994**, *106*, 1271−1276; *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1209−1213.
(3) Dalpke, A. H.; Zimmermann, S.; Albrecht, I.; Heeg, K. *Immunology* **2002**, *106*, 102−112.
(4) Pirrung, M. C. *Angew. Chem.* **2002**, *114*, 1326−1341; *Angew. Chem., Int. Ed.* **2002**, *41*, 1277−1289.
(5) (a) Podyminogin; M. A., Lukhtanov, E. A.; Reed, M. W. *Nucleic Acids Res.* **2001**, *29*, 5090−5098. (b) Dombi, K. L.; Griesang, N.; Richert, C. *Synthesis* **2002**, 816−824 and references therein.
(6) (a) Kutyavin, I. V.; Afonina, I. A.; Mills, A.; Gorn, V. V.; Lukhtanov, E. A.; Belousov, E. S.; Singer, M. J. Walburger, D. K.; Lokhov, S. G.; Gall, A. A.; Dempcy, R.; Reed, M. W.; Meyer, R. B.; Hedgpeth, J. *Nucleic Acids Res.* **2000**, *28*, 655−661. (b) Dogan, Z.; Paulini, R.; Rojas Stütz, J. A.; Narayanan, S.; Richert, C. *J. Am. Chem. Soc.* **2004**, *126*, 4762−4763.
(7) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544−584.
(8) Christensen, N. K.; Petersen, M.; Nielsen, P.; Jacobsen, J. P.; Olsen, C. E.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 5458−5463.
(9) Reddy, P. M.; Bruice, T. C. *J. Am. Chem. Soc.* **2004**, *126*, 3736−3747.
(10) (a) *Oligonucleotides and Analogues, A Practical Approach*; Eckstein, F., Ed.; IRL Press: Oxford, 1991. (b) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: San Diego, 1995.
(11) (a) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303−308. (b) Ranasinghe, R. T.; Brown, L. J.; Brown, T. *Chem. Commun.* **2001**, 1480−1481. (c) Fang, X.; Liu, X.; Schuster, S.; Tan, W. *J. Am. Chem. Soc.* **1999**, *121*, 2921−2922.
(12) Fidanza, J. A.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1989**, *111*, 9117−9119.

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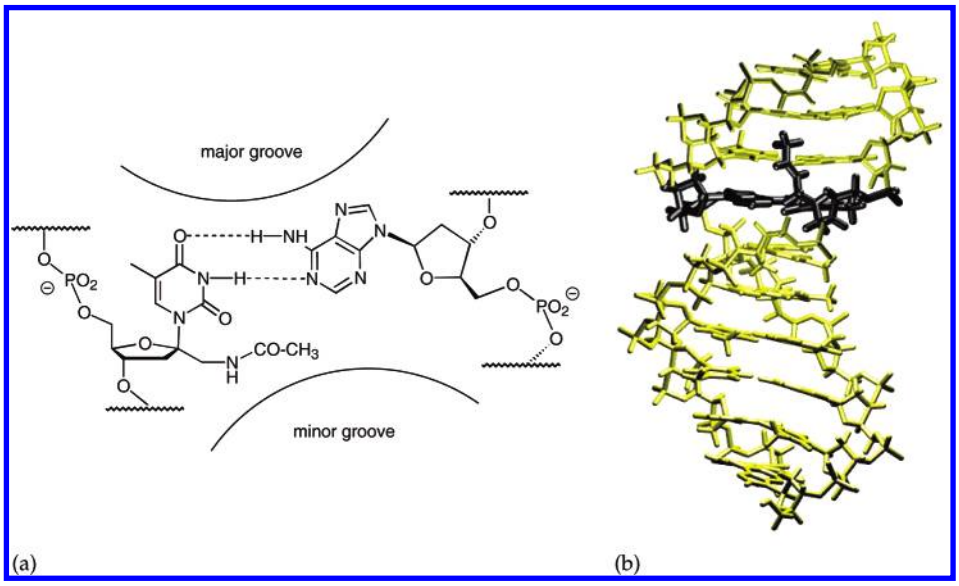


FIGURE 1. Structure of 1′-acetamidomethylthymidine-containing DNA. (a) Two-dimensional drawing of a base pair of the modified thymidine residue with a deoxyadenosine residue. (b) Structure of the DNA duplex 5′-GCAT*TATTAC-3′:5′-GTAATAATGC-3′, where T* denotes the 1′-acetamidomethylthymidine residue, as obtained through force field minimization using the program Macromodel. The nucleotides of the modified base pair are highlighted in black.

target strands could be envisioned, as well as improved molecular beacons or affinity labels for proteins that bind in either groove. Fluorophore-labeling at the 2′-position of oligonucleotides or analogues thereof has been reported,[14] though this point of attachment may change the conformation of duplexes toward the A-type conformation commonly found for RNA. Molecular modifications that place chemical appendages in the minor groove of DNA are less well-known. Minor groove binders have been appended through linkers originating in the termini of oligonucleotides[15,16] but not the interior of strands.

Inspection of the structure of B-form DNA duplexes indicates that a substituent replacing the hydrogen at the 1′-position of nucleotides should point into the minor groove. Duplexes with such a substituent, e.g., in the form of an acylamidomethyl group attached to a thymidine residue in the interior of one strand, do not show major distortions when subjected to force field minimization (Figure 1). This led us to pursue the synthesis of oligonucleotides that contain 1′-acylamidomethylthymidines. Thymidine as the nucleoside to be modified was chosen, because thymidine forms weaker base pairs with deoxyadenosine than deoxycytidines pairing with deoxyguanosines. Eventually, the weaker base pair may be stabilized through duplex-bridging interactions provided by a substituent introduced at the 1′-position. This might lead to isostable DNA,[17] i.e., DNA where the affinity for complementary strands does not depend on the sequence and where universally stringent hybridization conditions can be developed, even when complex mixtures of probes and target strands are present. Further, the thymidine residue has the advantage of lending itself more readily to synthetic transformations, as the nucleobase does not require a protecting group when generating building blocks for automatic DNA synthesis.

Here we report the synthesis of a 1′-aminomethylthymidine, together with that of oligonucleotides containing this nucleoside, prepared via the 3′-phosphoramidite of the aminomethylnucleoside. The synthesis of the oligonucleotides included the conversion of residues in the interior of protected oligonucleotides on solid supports in microwave-assisted conversions.[18]

Results

As mentioned above, 1′-aminomethylnucleosides of general structure **1** (Figure 2) were the key intermediate of the synthetic study. Nucleoside **1** should lead to **2** in no more than three steps, involving N-protection, 5′-protection, and phosphitylation. Several possible routes to aminomethylnucleoside **1** with either uracil or thymine as nucleobase were evaluated, as indicated in Figure 2 on the level of abridged retrosynthetic analyses. Route A employed the known[19,20] elaboration of tricyclic **3** from fructofuranose (**4**). This route has been used extensively for the synthesis of 1′-modified nucleic acids.[21−24] Al-

(13) Selected references: (a) Sando, S.; Abe, H.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 1081−1087. (b) Brakmann, S.; Löbermann, S. *Angew. Chem.* **2002**, *114*, 3350−3352; *Angew. Chem., Int. Ed.* **2002**, *41*, 3215−3217. (c) Rosenblum, B. B.; Lee, L. G.; Spurgeon, S. L.; Khan, S. H.; Menchen, S. M.; Heiner, C. R.; Chen, S. M. *Nucleic Acids Res.* **1997**, *25*, 4500−4504. (d) Khan, S. I.; Grinstaff, M. W. *J. Am. Chem. Soc.* **1999**, *121*, 4704−4705.

(14) Yamana, K.; Mitsui, T.; Nakano, H. *Tetrahedron* **1999**, *55*, 9143−9150.

(15) (a) Sinyakov, A. N.; Lokhov, S. G.; Kutyavin, I. V.; Gamper, H. B.; Meyer, R. B. *J. Am. Chem. Soc.* **1995**, *117*, 4995−4996. (b) Szewcyk, J. W.; Baird, E. E.; Dervan, P. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1487−1489. (c) Robles, J.; Rajur, S. B.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1996**, *118*, 5820−5821.

(16) Kutyavin, I. V.; Afonina, I. A.; Mills, A.; Gorn, V. V.; Lukhtanov, E. A.; Belousov, E. S.; Singer, M. J. Walburger, D. K.; Lokhov, S. G.; Gall, A. A.; Dempcy, R.; Reed, M. W.; Meyer, R. B.; Hedgpeth, J. *Nucleic Acids Res.* **2000**, *28*, 655−661.

(17) Nguyen, H. K.; Fournier, O.; Asseline, U.; Dupret, D.; Thuong, N. T. *Nucleic Acids Res.* **1999**, *27*, 1492−1498.

(18) Berlan, J. *Radiat. Phys. Chem.* **1995**, *45*, 581−589.

(19) Holy, A. *Nucleic Acids Res.* **1974**, *1*, 289−298.

(20) Ono, A.; Dan, A.; Matsuda, A. *Bioconjugate Chem.* **1993**, *4*, 499−508.

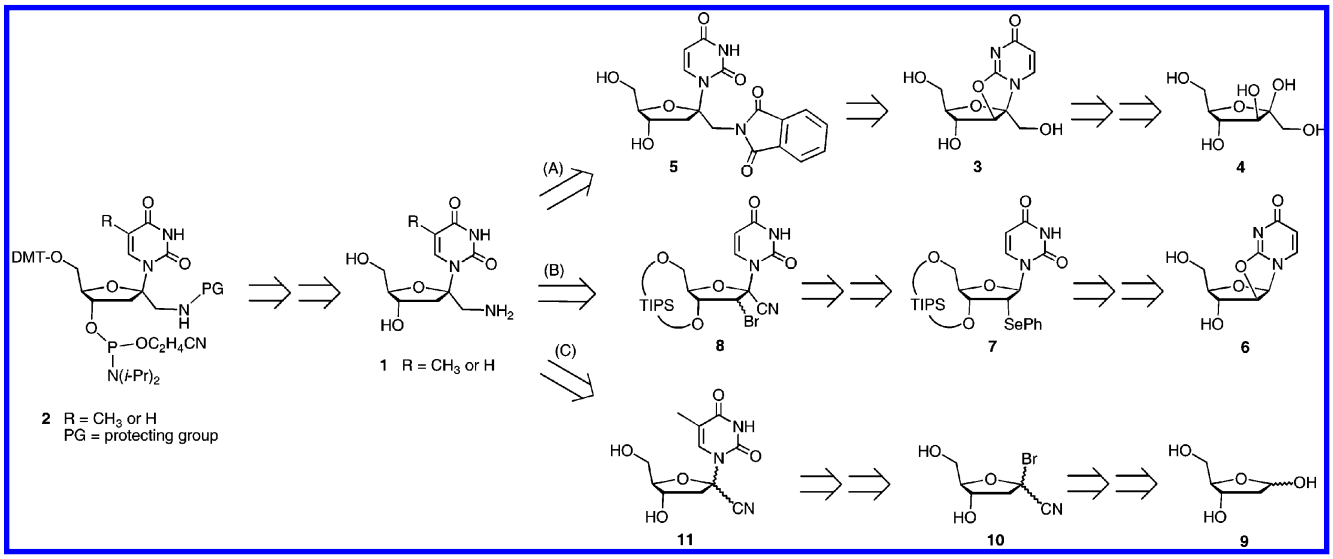


FIGURE 2.

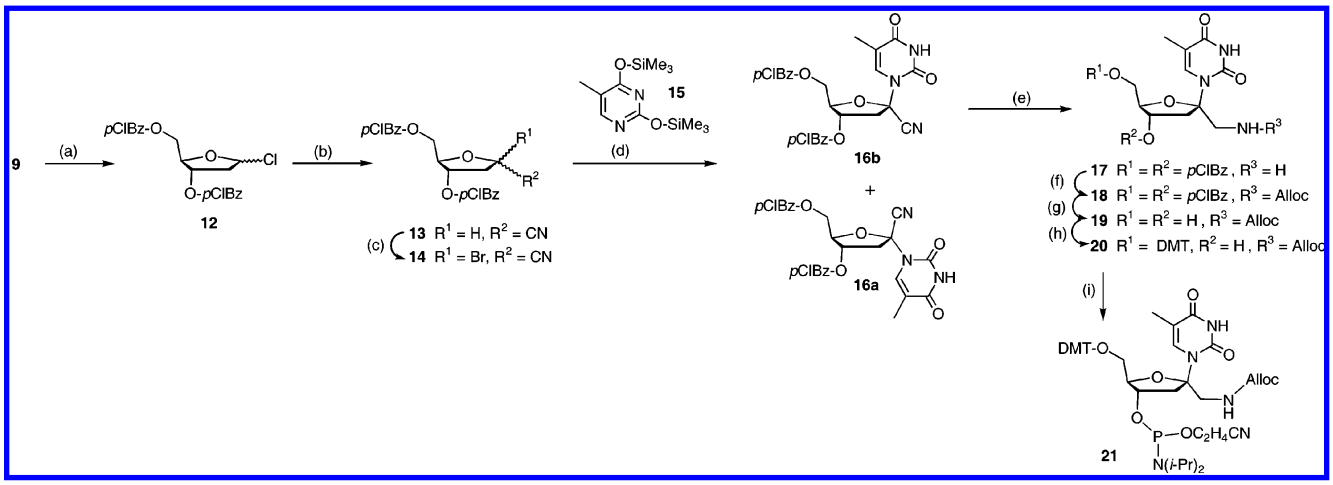
though syntheses for 1′-hydroxymethyl-2′-deoxyuridine and 1′-hydroxymethylthymidine have been reported, to the best of our knowledge, no synthesis of 1′-aminomethylthymidine is known. In our hands, the first step of Route A (addition of cyanamide to fructose) gave no more than 40% conversion, and purification of **3** required benzoylation followed by debenzoylation.[19] The total yield for **3** from **4** was thus lowered to 11%. Further, Mitsunobu reaction of **3** with phthalimide gave a difficult-to-separate mixture of regioisomers, with the 5′-substituted regioisomer of **5** as the predominant species. Route A was therefore not pursued further.

Route B is based on transformations reported by Miyasaka and co-workers.[25−27] We employed a 3′,5′-TIPS protecting group scheme rather than one TBDMS group each for the 3′- and 5′-hydroxyl groups, hoping to avoid a low-yielding deprotection step.[27] Silyl protection of anhydrouridine **6**, opening of the tricyclic structure with phenylselenide to **7**, and oxidation with MCPBA followed by elimination proceeded uneventfully, following the protocols published by Miyasaka and co-workers. The mixture of diastereomers obtained upon addition of a bromine and a pivalate moiety[26] proved difficult to handle, however, and all attempts to generate **8** by treatment with cyanides and various activating agents failed.

Accordingly, Route C was evaluated next. It starts from deoxyribose (**9**) and involves the oxidation of a suitably protected 1-cyano-2-deoxyribose to a derivative of **10**,[28] followed by a base introduction reaction to give **11** as a mixture of anomers. The details of this synthesis, which led to the thymidine form of **2**, are given in Scheme 1. Starting from deoxyribose, chlorofuranoside **12** was prepared in three steps, following a literature protocol[29] that is an optimized version of Hoffer's original protocol.[30] Cyanide **13** could be prepared from **12** by treatment with NaCN in DME,[31] but this method occasionally suffered from partial conversion and some formation of the 2-benzyloxymethylfuran elimination product. The use of Et_2AlCN in THF[28] was considered, but the use of TMS-CN/BF_3·OEt_2 was pursued, as it had proven higher yielding in reactions with a similar chlorosugar.[32] In the event, cyanide **13** was obtained in 75% yield and was smoothly converted to bromofuranoside **14**, using the conditions of a literature protocol.[28] Bromosugar **14** was directly employed in the subsequent base introduction with silylated nucleobase **15** and Hg(II) cyanide as Lewis acid.[33] As expected for the 2-deoxyglycosyl donor, a mixture of the α- and β-anomers **16a**/**b** was obtained. The combined yield of the anomers was 69%, and either diastereomer was found at approximately the same percentage. Separation of approximately 30% of the anomers by flash chromatography required 50 g of silica per gram of crude, making it advisable to achieve full separation by rechromatographing mixed fractions. Assignment of the two anomers was chiefly based on ROESY NMR spectra (see Supporting Information). For example, the H6 proton of the nucleobase gave stronger NOEs to H5′/5″ in the case of **16b**, whereas the same proton gave a stronger NOE to H4′ in the case of **16a**. Nuclear Overhauser effects found for later intermediates of the synthesis confirmed the assignment.

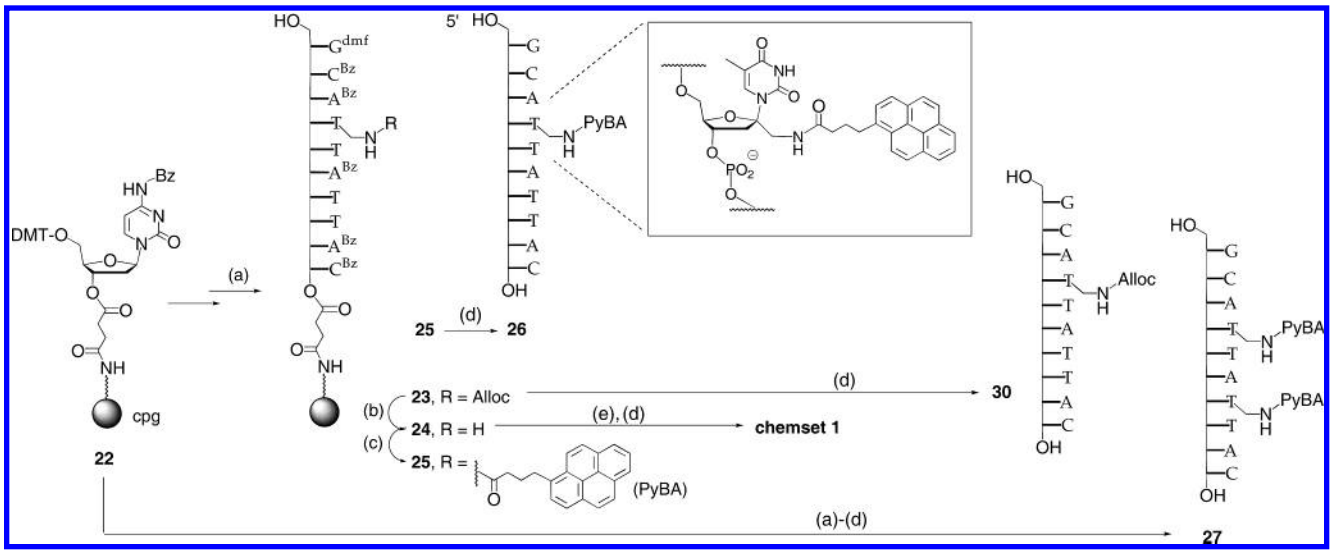
(21) Tatsuda, T.; Imao, K.; Suzuki, K. *Heterocycles* **1986**, *24*, 2133-2136.
(22) Yoshimura, Y.; Otter, B. A.; Ueda, T.; Matsuda, A. *Chem. Pharm. Bull.* **1992**, *40*, 1761−1769.
(23) Kværnø, L.; Nielsen C.; Wightman, R. H. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2903−2906.
(24) Kværnø, L.; Wightman, R. H.; Wengel, J. *J. Org. Chem.* **2001**, *66*, 5106−5112.
(25) Haraguchi, K.; Tanaka, H.; Hayakawa, H.; Miyasaka, T. *Chem. Lett.* **1988**, 931−934.
(26) Itoh, Y.; Haraguchi, K.; Tanaka, H.; Gen, E.; Miyasaka, T. *J. Org. Chem.* **1995**, *60*, 656−662.
(27) Yoshimura, Y.; Kano, F.; Miyasaki, S.; Ashida, N.; Sakata, S.; Haraguchi, K.; Ito, Y.; Tanaka, H.; Miyasaka, T. *Nucleosides Nucleotides* **1996**, *15*, 305−324.
(28) Lenox, H. J.; McCoy, C. P.; Sheppard, T. L. *Org. Lett.* **2001**, *3*, 2415−2418.
(29) Eger, K.; Jalalian, M.; Schmidt, M. *Tetrahedron* **1994**, *50*, 8371−8380.
(30) Fox, J. J.; Yung, N. C.; Wempen, I.; Hoffer, M. *J. Am. Chem. Soc.* **1961**, *83*, 4066−4068.
(31) (a) Bergstrom, D. E.; Zhang, P. *Tetrahedron Lett.* **1991**, *32*, 6485−6488. (b) Miller, T. J.; Farquar, H. D.; Sheybani, A.; Tallini, C. E.; Saurage, A. S.; Fronczek, F. R.; Hammer, R. P. *Org. Lett.* **2002**, *4*, 877−880.
(32) Jazouli, M.; Guianvarc'h, D. M.; Soufiaoui, K.; Bougrin, P.; Vierling, R.; Benhida, A. *Tetrahedron Lett.* **2003**, *44*, 5807−5810.
(33) Uteza, V.; Chen, G.-R.; Le Quan Tuoi, J.; Descotes, G.; Fenet, B.; Grouiller, A. *Tetrahedron* **1993**, *49*, 8579−8588.

SCHEME 1[a]



[a] Reagents and conditions: (a) (1) HCl, MeOH, (2) *p*ClBzCl, pyridine, (3) HCl, AcOH (80% over 3 steps); (b) TMSCN, $BF_3{\cdot}OEt_2$ (75%); (c) NBS, benzoyl peroxide, CCl_4 (quant); (d) **15**, $Hg(CN)_2$, MeCN (69% for anomeric mixture, 27% **16a**, 30% **16b**); (e) $NaBH_4$, TFA, THF; (f) Alloc-Cl, THF (70% over 2 steps); (g) NaOMe, MeOH (97%); (h) DMT-Cl, DMAP, TEA, THF (74%); (i) Cl-P(N*i*Pr$_2$)OC$_2$H$_4$CN, DIEA, MeCN (71%).

SCHEME 2[a]



[a] Reagents and conditions: (a) Standard DNA synthesis, including extension cycle(s) with **21**; (b) $Pd(PPh_3)_4$, $[NEt_2H_2][HCO_3]$, DMF, microwave irradiation 200 W, 80 °C, 10 min; (c) HOBT, HBTU, DIEA, pyrene butyric acid, DMF, microwave irradiation 150−200 W, 80 °C, 10 min; (d) NH_4OH, rt; (e) HOBT, HBTU, DIEA, mixture of carboxylic acids, DMF, microwave irradiation 150−200 W, 80 °C, 10 min.

Amine **17** was obtained through reduction of **16b** with sodium trifluoroacetoxy borohydride,[34] prepared in situ from $NaBH_4$ and TFA. The free amine was Alloc-protected in situ to avoid handling losses of this very polar compound during purification. An Fmoc group for N-protection proved inferior, as it was partially lost during the subsequent steps, even after careful optimization of the reaction conditions. Alloc-protected **18**, obtained in 70% over two steps, was readily debenzoylated to **19**, and 5′-re-protected with a dimethoxytrityl group to give **20**. Phosphitylation of the latter provided key building block **21** in 6% overall yield from **9**.

With phosphoramidite **21** in hand, oligodeoxyribonucleotides with one or two modified thymidine residues could be prepared (Scheme 2). Whereas chain assembly starting from support **22** proceeded in high yield, the on-support removal of the Alloc group of **23** initially proved difficult. Exposing the fully assembled strand on solid support to $Pd(PPh_3)_4$ and diethylammonium bicarbonate in CH_2Cl_2, conditions successfully employed for Alloc removal on cpg in earlier cases,[35] led to little **24**, as determined by MALDI-TOF mass spectra of crudes obtained after treatment with saturated aqueous ammonia. Heating to 40 °C for up to 3 d, combined with repeated sonications for 30 min, did not lead to clean conversions either. Subsequent acylation attempts with HBTU/HOBT-activated pyrene butyric acid, a lipophilic acid that can readily be detected in the UV−vis range, did not give satisfactory quantities of **25**, as only small peaks for **26** were observed in MALDI mass spectra of

(34) Iyer, R. P.; Phillips, L. R.; Egan, W. *Synth. Commun.* **1991**, *20*, 2053−2063.

(35) Mokhir, A. A.; Connors, W. H.; Richert, C. *Nucleic Acids Res.* **2001**, *29*, 3674−3684.

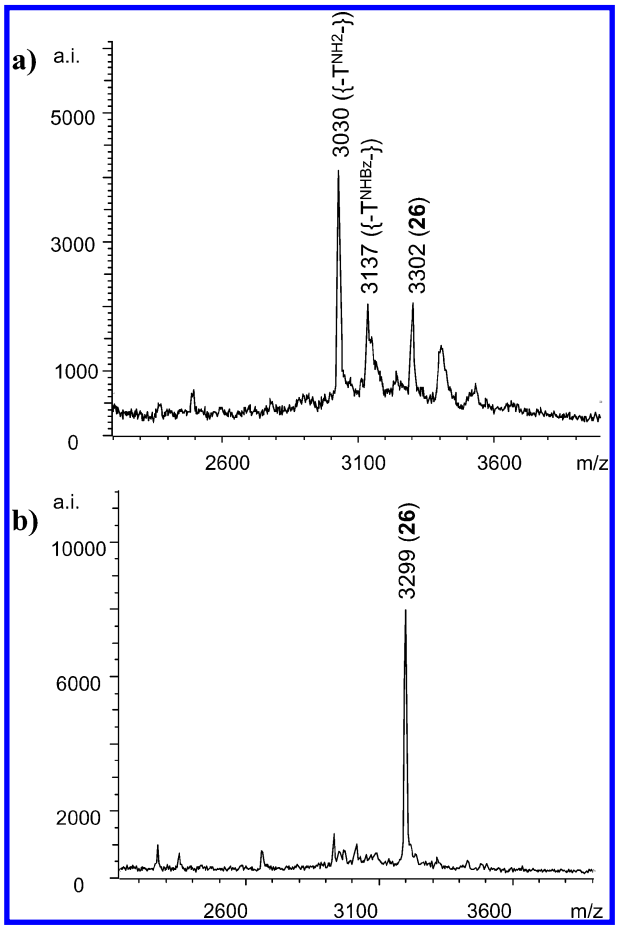


FIGURE 3. MALDI-TOF mass spectra of crude **26** as obtained after deprotection with NH_4OH. (a) Deprotection of **23** and acylation of **24** without microwave irradiation (3 d reaction time for each reaction and sonication of 5 × 30 min at 40 °C). (b) Deprotection and acylation for 10 min with microwave irradiation (200 W, 80 °C max). Parentheses show peak assignments, with side products resulting from incomplete acylation of **24**, indicated as {-T^{NH2}-} and from blocking of **24** through benzoyl migration, presumably from neighboring residues, as {-T^{NHBz}-}.

deprotected crudes (Figure 3). This seemed to suggest that the steric hindrance caused by the tightly packed strands on the non-swellable support was overwhelming.

Rather than reverting to discontinuous syntheses, where chain assembly is interrupted after coupling of each modified residue, followed by individual Alloc removal and acylation prior to continuation of the chain assembly, we chose to employ irradiation with microwaves to assist the reactions in the interior of the strands. Although the effect of microwave irradiation on the heterogeneous reaction mixture was small with CH_2Cl_2, probably because of the low boiling point, 150- to 200-W irradiation (limited by a maximum temperature of 80 °C in the flask) for 10 min was sufficient for full Alloc removal when DMF was employed as solvent. Without subsequent acylation, MALDI spectra of crudes showed contamination of the deprotected amine with products carrying an acetyl or benzoyl group. These were most likely the result of the migration of protecting groups to the primary amine of the modified thymidine residue in the presence of base. Labile acetyl groups may have been introduced through the capping steps, and the benzoyl groups may have migrated from the exocyclic amino groups of adenine and cytosine nucleobases.

When performed with microwave irradiation using an instrument designed for organic synthesis (Discover, CEM Inc.), the acylation of amine **24** with the active ester of pyrene butyric acid gave surprisingly pure **25**, however. The MALDI-TOF mass spectrum of a representative crude of **26**, obtained after deprotection and removal from the cpg, is shown in Figure 3b. High-yielding conversions were confirmed when crude **26** was analyzed by HPLC, where the peak of the product dominated. An exploratory synthesis of doubly modified **27** was performed, using the same conditions for on-support modification as for **26**. Again, MALDI-TOF mass spectra of crudes showed successful double Alloc removal and doubly acylation, even though one of the thymdines to be modified was only the fourth residue from the surface of the solid support.

With **26** and **27**, a set of melting curve analyses were performed to test the effect of the 1′-modification on the duplex with complementary strand 5′-GTAATAATGC-3′ (**28**). The results of the UV-melting experiments are compiled in Table 1. It can be discerned that the modification increases the melting points and that this effect is stronger for the doubly modified strand, with a ΔT_m of up to 6.9 °C over the control (3.4 °C per modification). This suggests that the acylamidomethyl modification at the 1′-position of the thymidine residues is tolerated by the helix, as also suggested by the unchanged sigmoidal appearance of the melting curve (Figure 4). The thermodynamic signatures (Table 1) show that duplexes carrying the 1′-acylamidomethyl substituent experience a decreased entropic penalty for duplex formation. This suggests that the 1′-substituent may help to preorganize **26** and **27** toward duplex formation. The Alloc-protected strand, **30**, on the other hand, leads to lower UV-melting points. The same is true for oligonucleotides with 1′-carbamoylmethylthymidine residues that are known from the literature.[20]

Finally, we have tested the suitability of our method for preparing oligonucleotides with other 1′-acylamidomethylthymidines. For this, solid support **24** was acylated with hippuric acid, 3-indolepropionic acid, *N*α-Boc- and *β*-*O*-benzyl-protected L-serine, the quinolone cinoxacin, and dehydrocholic acid. This diverse set of carboxylic acid building blocks was successfully used in a microwave-assisted coupling reaction. The ratio of the acids was chosen such that it creates a reactivity-adjusted mixture.[37] Figure 5 shows that even the mixed coupling, with its much higher propensity for side reactions, gives a clean conversion, resulting in a combinatorial library directly suitable for spectrometrically monitored selection assays (SMOSE).[38]

Discussion

Reactions in the interior of fully assembled and protected oligonucleotides on nonswellable supports are difficult. This is why we[35] and others[13d] have employed

(36) McDowell, J. A.; Turner, D. H. *Biochemistry* **1996**, *35*, 14077−14089.
(37) Dombi, K. L.; Steiner, U. E.; Richert, C. *J. Comb. Chem.* **2003**, *5*, 45−60.
(38) Berlin, K.; Jain, R. K.; Tetzlaff, C.; Steinbeck, C.; Richert, C. *Chem. Biol.* **1997**, *4*, 63−77.

TABLE 1. UV-Melting Points and Thermodynamic Values for DNA Duplex Formation

duplex	[NaCl]/[$MgCl_2$][a]	T_m (°C)[b]	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/K·mo)l	$\Delta G°$ (kcal/mol)[c]
29:**28** (control)	-/-	16.6 ± 1.4	−77.8 ± 8.4	−239.3 ± 28.0	−3.6 ± 0.4
	100 mM/10 mM	33.5 ± 0.5	−81.4 ± 1.9	−236.2 ± 6.1	−8.2 ± 0.2
	1 M/10 mM	37.5 ± 0.3	−76.0 ± 1.6	−215.5 ± 5.2	−9.2 ± 0.1
30:**28**	-/-	9.4 ± 0.3	−129.0 ± 10.2	−427.4 ± 36.5	3.6 ± 1.1
	100 mM/10 mM	21.2 ± 0.4	−72.0 ± 5.4	−215.5 ± 18.7	−5.2 ± 0.4
	1 M/10 mM	23.8 ± 0.2	−69.0 ± 4.0	−203.3 ± 13.5	−6.0 ± 0.2
26:**28**	-/-	17.8 ± 0.8	−76.0 ± 5.1	−232.1 ± 17.5	−4.1 ± 0.4
	100 mM/10 mM	34.2 ± 0.8	−76.6 ± 2.0	−220.0 ± 7.3	−8.4 ± 0.3
	1 M/10 mM	37.6 ± 1.4	−67.3 ± 10.9	−187.2 ± 34.0	−9.2 ± 0.4
27:**28**	-/-	23.5 ± 1.0	−65.8 ± 0.6	−192 ± 1.0	−6.1 ± 0.3
	100 mM/10 mM	39.2 ± 0.8	−73.3 ± 0.7	−205 ± 3.0	−9.6 ± 0.2
	1 M/10 mM	41.5 ± 0.7	−68.8 ± 1.1	−189 ± 4.0	−10.1 ± 0.2

[a] In 10 mM PIPES (pH 7) + salt concentrations given. [b] Mean of four values ± SD at 1.6 μM strand concentration. [c] Calculated on the basis of curve fitting with Meltwin[36] for 37 °C.

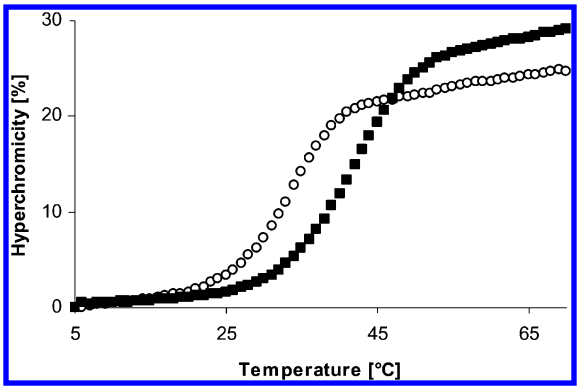


FIGURE 4. Representative UV-melting curves of the duplexes formed by doubly modified oligonucleotide **27** (■) or unmodified 5′-GCATTATTAC-3′ (○) with their fully complementary strands at 1.6 μM strand concentration, 100 mM NaCl, 10 mM $MgCl_2$, and 10 mM PIPES buffer, pH 7.

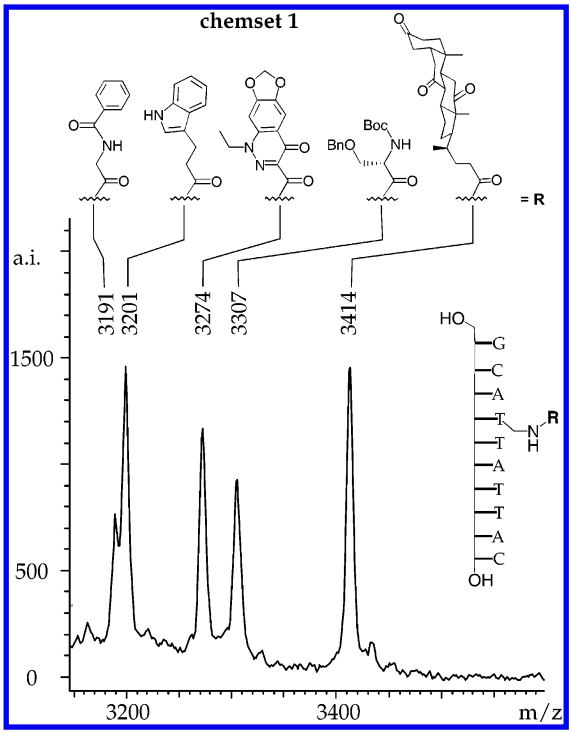


FIGURE 5. MALDI-TOF mass spectrum of the pseudomolecular ions ([M − H]$^-$) of the members of **chemset 1**, as obtained after acylation with a reactivity-adjusted mixture of activated caboxylic acids and deprotection.

interrupted syntheses for internal residues to be modified in the past. Initially, we were hesitant to apply microwave irradiation, because it was not clear why it may be more successful than conventional heating.[39] In the present case, microwave irradiation not only accelerated a Pd(0)-catalyzed transallylation and an amide-forming reaction to an extent that made them synthetically useful, but also led to cleaner reactions (Figure 3). The microwave-assisted heating apparently breaks up the inaccessibility of the reaction sites, so that the desired intermolecular reactions prevail and competing reactions, such as protecting group migrations from neighboring residues, are suppressed. Similar effects may be observed for other conversions that would otherwise be difficult to perform with partially protected oligonucleotides on solid support. Other reports on successful microwave-assisted transformations of nucleic acids on solid supports are beginning to emerge,[40] and so are reports on microwave-assisted Pd-mediated reactions.[41]

The advantage of the post-assembly derivatization of oligonucleotides on solid support is not just a more convenient procedure for the assembly of oligomers but also the fact that the residues introduced do not have to be compatible with subsequent extension steps. For example, alcohol groups on the newly attached moieties will be tolerated when introduced after completion of chain assembly but lead to side reactions with phosphoramidites in approaches that involve interrupted chain assembly. One may argue that introducing acyl groups during interrupted chain assembly has the advantage that different acyl groups can be introduced at different sites of an oligomer, but the same may be achieved by employing orthogonal protecting groups for the different modified nucleosides.

The current approach of modifying aminomethyl nucleosides on solid support lends itself readily to combinatorial chemistry. Therefore, it may lead to an efficient search for new modified oligonucleotides with increased affinity for target strands, particularly when combined with mass spectrometrically monitored selection experiments (SMOSE). Such searches have been performed with oligonucleotides acylated at one of the termini.[42] To allow generation of a wide range of modified residues, it

(39) Adam, D. *Nature* **2003**, *421*, 571−572.
(40) Andrzejewska, M., Kaminski, J.; Kazimierczuk, Z. *Nucleosides Nucleotides Nucleic Acids* **2002**, *21*, 73−78.
(41) Fürstner, A.; Seidel, G. *Org. Lett.* **2002**, *4*, 541−543.

is desirable to append diversity elements via amide bonds, as carboxylic acids form a large structure space of commercially available building blocks. The diversity will increase if other nucleobases than thymidine are being furnished with 1′-acylamidomethyl substituents. Deoxycytidine residues with such substituents may be readily prepared by treating **21** with 1,2,4-1*H*-triazole, $POCl_3$, and triethylamine[43] and liberating the cytosine from the triazolide thus prepared during the deprotection with aqueous ammonia.[44]

Besides the synthetic issues, one may ask how well the 1′-aminomethyl group fulfills the role of a tether that allows presenting substituents to the minor groove of a regular Watson−Crick helix without introducing a steric conflict. The data available thus far show good cooperativity in the thermal dissociation, as evidenced by melting curves of modified and unmodified duplex that are almost identical (Figure 4). Further, there is no drop in the melting point for the modified duplex **26**:**28**, whose acyl group is not expected to stabilize the duplex through binding in the minor groove, though one cannot rule out intercalation. Pyrene is a chromophore that is readily detected and that does not require protecting groups. As a result of its lipophilicity, it facilitates purification of modified strands carrying it. Other oligonucleotides containing 1′-acylamidomethylthymidines may carry pendant groups for electron transfer or Förster fluorescence resonance energy transfer studies.[45] Thus, oligonucleotides containing 1′-acylamidomethylthymidine residues may provide an entry into richly functionalized nucleic acids that act as functional hybridization probes.

Experimental Section

General. Anhydrous solvents were purchased over molecular sieves and used without further purification. HOBT (1-hydroxybenzotriazole) and HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate) were from Advanced ChemTech (Louisville, KY). Phosphoramidites (dA^{Bz}, dC^{Bz}, dC^{Bz}cpg, dG^{dmf}, dT), as well as other reagents for DNA synthesis, were from Proligo (Hamburg, Germany). Reactions involving controlled pore glass (cpg) were performed in polypropylene reaction chambers for DNA synthesis (Prime Synthesis, Aston, PA). Oligonucleotides were purified by HPLC on 250 mm × 4.6 mm Nucleosil C4 or 250 mm × 4.6 mm Nucleosil C18 columns, using a gradient of CH_3CN in 0.1 M triethylammonium acetate, pH 7, and detection at 260 nm. MALDI-TOF spectra were recorded in positive, linear mode in the case of small molecules, and in negative, linear mode in the case of oligonucleotides. MALDI matrixes were 2,5-dihydroxybenzoic acid (0.1 M in CH_3CN) for small molecules and a mixture of 2,4,6-trihydroxyacetophenone (0.3 M in EtOH) and diammonium citrate (0.1 M in H_2O) (2:1 v/v) for oligonucleotides.

3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranosyl Chloride (12). Compound **12** was prepared following a literature protocol[29] on a 0.15 mol scale and was obtained in 80% yield over three steps.

3,5-Bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranosyl Cyanide (13). To a stirred solution of **12** (6 g, 14 mmol) in anhydrous CH_2Cl_2 (80 mL) were added TMS-CN (2.1 g, 2.8 mL, 21 mmol, 1.5 equiv) and $BF_3{\cdot}OEt_2$ (3.97 g, 3.5 mL, 28 mmol, 2 equiv). The reaction mixture was stirred for 12 h. After complete conversion, the reaction was quenched with saturated ammonium bicarbonate solution (80 mL) and stirred for additional 30 min. The slurry was extracted with CH_2Cl_2 (5 × 300 mL). The combined organic phases were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (silica, hexane/EtOAc 4:1) to give 4.36 g (10.4 mmol, 75%) of **13** as a pale yellow solid (mixture of anomers). An analytical sample was rechromatographed, yielding a pure fraction of the fast eluting anomer. TLC (hexane/EtOAc 4:1) R_f 0.42, 0.34. ^{1}H NMR (500 MHz, $CDCl_3$, fast eluting anomer) δ 8.02 (d, J = 8.5 Hz, 2H, Ar-H), 7.94 (d, J = 8.5 Hz, 2H, Ar-H), 7.43 (dd, J = 7.2 Hz, J = 1.3 Hz, 2H, Ar-H), 5.62−5.61 (m, 1H, H3), 4.94 (dd, J = 9.1 Hz, J = 6.9 Hz, 1H, H1), 4.62−4.58 (m, 1H, H4), 4.56−4.52 (m, 2H, H5), 2.79−2.65 (m, 2H, H2). ^{13}C NMR (126 MHz, $CDCl_3$, fast eluting anomer) δ 165.3, 164.8, 140.3, 139.9, 131.1, 131.1, 129.0, 128.9, 127.8, 127.3, 117.9, 83.6, 77.4, 77.1, 76.9, 75.6, 65.9, 64.0, 37.6. MALDI-TOF MS m/z 443.2 ($[M + Na]^+$).

1-Bromo-3,5-bis-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranosyl Cyanide (14). Compound **14** was prepared from **13** following a literature protocol and was obtained in quantitative yield. It was used in the subsequent reaction without recrystallization.[28]

1′-Cyano-3′,5′-bis-*O*-(*p*-chlorobenzoyl)thymidine (16). To a stirred solution of thymine (568 mg, 4.5 mmol) in CH_3CN (6 mL) were added HMDS (1.5 g, 2.0 mL, 9.4 mmol, 2.1 equiv) and TMS-Cl (1.0 g, 1.2 mL, 9.4 mmol, 2.1 equiv). The resulting slurry was stirred for 5 min. TMS-OTf (244 mg, 213 μL, 1.1 mmol, 0.25 equiv) was added under stirring, and the reaction mixture was heated to reflux for 6 h to give a clear solution of 5-methyl-2,4-bis(trimethylsilyloxy)pyrimidine (**15**). The solution was evaporated to dryness, and the residue was redissolved in CH_3CN (10 mL). A solution of previously vacuum-dried **14** (1.5 g, 3 mmol) in anhydrous CH_3CN (15 mL) was stirred under presence of 4Å molecular sieves (50 mg) for 30 min. Then $Hg(CN)_2$ (1.1 g, 1.4 equiv) and the freshly prepared solution of **15** in CH_3CN were added, and the reaction mixture was stirred for 3 d at room temperature. The solids were filtered off and washed with CH_3CN. The filtrate was evaporated, and the residue was redissolved in $CHCl_3$ (30 mL). The resulting solution was washed once with 50% KI in half-saturated sodium chloride solution and once with brine. The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (100 g silica, CH_2Cl_2/EtOAc 85:15) yielding 1.1 g (2.1 mmol, 69%) total of the of the title compound as off-white solid. Eluting first was a fraction of **16a** (351 mg, 0.64 mmol, 21%), followed by a mixture of anomers (295 mg, 0.54 mmol, 18%) and a fraction of **16b** (469 mg, 0.9 mmol, 30%). Analytical data for **16a**: TLC (CH_2Cl_2/EtOAc 85:15) R_f 0.45. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.94 (bs, 1H, NH), 8.04 (d, J = 8.8 Hz, 2H, Ar-H), 7.69 (d, J = 8.8 Hz, 2H, Ar-H), 7.47 (s, 1H, H6), 7.47 (d, J = 8.8 Hz, 2H, Ar-H), 7.37 (d, J = 8.8 Hz, 2H, Ar-H), 5.70 (d, J = 5.7 Hz, 1H, H3′), 4.95 (m, 1H, H4′), 4.76 (dd, J = 12.6 Hz, J = 3.5 Hz, 1H, H5′), 4.65 (dd, J = 12.6 Hz, J = 4.4 Hz, 1H, H5′), 3.46 (d, J = 15.7 Hz, 1H, H2′), 3.28 (dd, J = 15.7 Hz, J = 6.0 Hz, 1H, H2′), 1.91 (s, 3H, H7). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.2, 164.4, 163.8, 149.6, 140.8, 140.4, 132.6, 131.2, 129.2, 129.1, 127.3, 126.6, 115.0, 111.5, 87.7, 87.7, 74.2, 63.4, 44.8, 12.7. MS (MALDI-TOF) m/z 566.2 ($[M + Na]^+$). Analytical data for **16b**: TLC (CH_2Cl_2/EtOAc 85:15) R_f 0.39. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (bs, 1H, NH), 8.08 (d, J = 8.8 Hz, 2H, Ar-H), 7.87(d, J = 8.8 Hz, 2H, Ar-H), 7.49 (d, J = 8.8 Hz, 2H, Ar-H), 7.48 (s, 1H, H6), 7.45 (d, J = 8.8 Hz, 2H, Ar-H), 5.72 (d, J = 8.0 Hz, 1H, H3′), 4.97 (m, 1H, H4′), 4.91 (dd, J = 12.9 Hz, J = 3.5 Hz, 1H, H5′), 4.59 (dd, J = 12.9 Hz, J = 3.8 Hz, 1H, H5′), 3.75 (d, J = 15.4 Hz, 1H, H2′), 2.76 (dd, J = 15.4

(42) (a) Altman, R. K.; Schwope, I.; Sarracino, D. A.; Tetzlaff, C. N.; Bleczinski, C. F.; Richert, C. *J. Comb. Chem.* **1999**, *1*, 493−508. (b) Mokhir, A. A.; Tetzlaff, C. N.; Herzberger, S.; Mosbacher, A.; Richert, C., *J. Comb. Chem.* **2001**, *3*, 374−386. (c) Connors, W. H.; Narayanan, S.; Kryatova, O. P.; Richert, C. *Org. Lett.* **2003**, *5*, 247−250. (d) Narayanan, S.; Gall, J.; Richert, C. *Nucleic Acids Res.* **2004**, *32*, 2901−2911.

(43) Webb, T. R.; Matteucci, M. D. *J. Am. Chem. Soc.* **1986**, *108*, 2764−2765.

(44) Martin, P. *Helv. Chim. Acta* **1995**, *78*, 486−504.

(45) Clegg, R. M. *Methods Enzymol.* **1992**, *211*, 353−388.

Hz, J = 6.0 Hz, 1H, H2′), 1.72 (s, 3H, H7). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.1, 164.8, 162.7, 149.0, 140.7, 140.1, 132.8, 131.4, 130.7, 129.3, 129.2, 127.1, 126.9, 115.7, 111.6, 87.5, 86.8, 74.8, 63.0, 46.7, 12.6. MS (MALDI-TOF) m/z 566.2 ([M + Na]$^+$).

β-1′-[*N*-Allyloxycarbonyl(aminomethyl)]-3′,5′-bis-*O*-(*p*-chlorobenzoyl)thymidine (18). To a stirred solution of **16b** (527 mg, 1 mmol) in anhydrous THF (25 mL) was added $NaBH_4$ (113 mg, 3 mmol, 3 equiv). To the resulting slurry was added TFA (177 mg, 155 μL, 1.5 mmol, 1.5 equiv). After brief gas evolution, a clear solution formed, which was stirred at room temperature for 12 h. After complete conversion, excess $NaBH_4$ was hydrolyzed with water (5 mL), and Alloc-Cl (241 mg, 198 μL, 2 mmol, 2 equiv) was added. (Attention: older samples of Alloc-Cl may be under substantial pressure and may contain highly toxic decomposition compounds.) The resulting solution was stirred for 24 h at room temperature. The solution was diluted with water (5 mL) and extracted trice with $CHCl_3$ (3 × 30 mL). The combined organic phases were dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (silica, CH_2Cl_2/MeOH 99:1), yielding 442 mg of **18** (0.70 mmol, 70%). TLC ($CHCl_3$/EtOH 95:5) R_f 0.48. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.10 (bs, 1H), 7.98 (d, J = 8.6 Hz, 2H), 7.82 (d, J = 8.6 Hz, 2H), 7.52 (s, 1H), 7.43 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 6.07 (bs, 1H), 5.80 (m, 1H), 5.56 (d, J = 6.6 Hz, 1H), 5.22 (d, J = 17.2 Hz, 1H), 5.15 (d, J = 10.4, 1H), 4.81−4.70 (m, 2H), 4.52−4.41 (m, 3H), 4.07−3.93 (m, 1H), 3.73 (dd, J = 14.2 Hz, J = 6.6 Hz, 1H), 3.02 (d, J = 15.6 Hz, 1H), 2.92 (dd, J = 15.6 Hz, J = 6.6 Hz, 1H), 1.67 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 165.0, 164.7, 156.8, 150.3, 140.22, 140.16, 139.6, 136.5, 133.0, 132.7, 131.2, 130.7, 129.04, 129.01, 128.5, 128.2, 127.5, 117.8, 108.9, 107.6, 99.0, 84.3, 76.0, 67.4, 65.8, 46.2, 42.5, 29.5, 23.8, 12.5. MS (MALDI-TOF) m/z 655.4 ([M + Na]$^+$).

β-1′-[*N*-Allyloxycarbonyl(aminomethyl)]thymidine (19). To a stirred solution of **18** (220 mg, 0.35 mmol) in methanol (5 mL) was added a solution of NaOMe in methanol (1 M, 163 μL, 0.19 mmol, 0.6 equiv), and the resulting solution was stirred at room temperature for 6 h. The solution was neutralized by adding Dowex 50 (H^+ form), and the resulting mixture was stirred for 30 min. The ion-exchange resin was filtered off and washed with methanol, and the filtrates were combined and evaporated. The residue was chromatographed (silica, CH_2Cl_2/MeOH 85:15) to give 115 mg (0.32 mmol, 91%) of **19**. TLC ($CHCl_3$/EtOH 85:15) R_f 0.5. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (s, 1H, H6), 7.36 (t, J = 6.6 Hz, 1H,), 5.83−5.75 (m, 1H, CH_2═C*H*), 5.65 (bs, 1H, N*H*-Alloc), 5.21 (dd, J = 17.3 Hz, J = 1.6 Hz, 1H, CH═CH_2), 5.15 (dd, J = 10.4, J = 1.6 Hz, 1H, CH═CH_2), 4.41 (m, 1H, O-CH_2), 4.29 (dd, J = 13.8, J = 5.6 Hz, 1H, O-CH_2), 4.10 (m, 1H, H3′), 3.97 (m, 1H, H4′), 3.80 (dd, J = Hz, J = 6.6 Hz, 1H, -CH_2-NH-), 3.46−3.34 (m, 2H, H5′), 3.32−3.35 (m, 1H, -CH_2-NH-), 2.61 (dd, J = 14.5, J = 6.0, 1H, H2′), 2.28 (dd, J = 14.5 Hz, J = 3.1 Hz, 1H, H2′), 1.70 (s, 3H, H7). ^{13}C (126 MHz, $CDCl_3$) δ 168.0, 164.4, 164.3, 150.9, 150.1, 134.3, 134.0, 131.5, 117.8, 109.4, 109.3, 93.2, 91.8, 88.2, 87.4, 75.3, 70.6, 70.5, 67.3, 63.8, 61.4, 60.5, 59.0, 53.4, 48.6, 47.1, 42.6, 12.9. MS (MALDI-TOF) m/z 680.5 ([M + Na]$^+$).

β-1′-[*N*-Allyloxycarbonyl(aminomethyl)]-5′-(dimethoxytrityl)thymidine (20). A mixture of DMT-Cl (265 mg, 0.78 mmol, 2 equiv) and DMAP (4.8 mg, 0.04 mmol, 0.1 equiv) was dried at 0.1 Torr and then added to a stirred solution of **19** (141 mg, 0.39 mmol) and NEt_3 (108 μL, 0.78 mmol, 2 equiv) in anhydrous THF (5 mL). The solution was stirred at room temperature for 24 h. The reaction mixture was diluted with CH_2Cl_2 and washed twice with saturated $NaHCO_3$ solution and once with brine. The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (silica, pretreated with CH_2Cl_2/MeOH/TEA 96:1:3, elution with CH_2Cl_2/MeOH 99:1) to give 188 mg **20** (0.29 mmol, 74%) as a solid. TLC ($CHCl_3$/EtOH 95:5) R_f 0.5. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (s, 1H, H6), 7.32−7.17 (m, 5H, Ar-H), 7.20 (dt, J = 8.8 Hz, J = 2.8, 4H, Ar-H), 6.78 (dt, J = 8.8 Hz, J = 2.8, 4H, Ar-H), 5.85−5.77 (m, 1H, CH_2═C*H*), 5.65 (bs, 1H, N*H*-Alloc), 5.23 (d, J = 17.3 Hz, 1H, CH═CH_2), 5.15 (dd, J = 10.4, J = 1.3 Hz, 1H, CH═CH_2), 4.40 (dd, J = 13.5, J = 5.7 Hz, 1H, O-CH_2), 4.48 (dd, J = 13.5, J = 5.6 Hz, 1H, O-CH_2), 4.36 (t, J = 4.7, 1H, H3′), 4.32 (d, J = 4.7, 1H, H2′), 4.04 (dd, J = 14.5 Hz, J = 8.2 Hz, 1H, -CH_2-NH-), 3.76 (d, J = 1.3 Hz, 6H, DMT-OMe), 3.54 (dd, J = 14.5 Hz, J = 7.2 Hz, 1H, -CH_2-NH-), 3.20 (dd, J = 10.3 Hz, J = 4.7 Hz, 1H, H5′), 3.04 (dd, J = 10.3 Hz, J = 3.8 Hz, 1H, H5′), 2.61 (dd, J = 14.4, J = 6.0, 1H, H2′), 2.28 (dd, J = 14.4 Hz, J = 3.1 Hz, 1H, H2′), 1.70 (s, 3H, H7). MS (MALDI-TOF) m/z 680.5 ([M + Na]$^+$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.5, 158.6, 150.4, 144.4, 137.1, 135.5, 135.4, 132.7, 129.9, 127.89, 127.85, 127.0, 117.8, 113.2, 108.5, 98.8, 88.1, 86.7, 72.7, 65.8, 63.4, 55.2, 45.9, 45.1, 12.6, 10.7.

β-1′-[*N*-Allyloxycarbonyl(aminomethyl)]-5′-(dimethoxytrityl)thymidin-3′-*O*-yl-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (21). To a stirred solution of **20** (90 mg, 0.14 mmol) and DIEA (74 μL, 0.42 mmol, 3 equiv) in anhydrous CH_3CN (1.5 mL) was added 2-cyanoethyl-*N*,*N*-diisopropyl-chlorophosphoramidite (41 μL, 0.19 mmol, 1.33 equiv). The solution was stirred for 1.5 h. After complete conversion, CH_2Cl_2 (60 mL) was added, and the solution was washed twice with a saturated $NaHCO_3$ solution (2 × 50 mL) and once with brine (50 mL). The organic phase was dried over Na_2SO_4, and the solvent was evaporated to an approximate volume of 0.5 mL. This solution was added dropwise to pentane (35 mL) and stored at 7 °C overnight. The supernatant was aspired, and the oily residue was dried in vacuo. The title compound was obtained as pale yellow foam (83 mg, 0.10 mmol, 71%). ^{31}P NMR (202 MHz, $CDCl_3$) 148.3, 147.8. MALDI-TOF MS m/z 897.2 [M + K]$^+$.

DNA Synthesis (Compound 23). Oligodeoxynucleotides were prepared on a 1-μmol scale, following the protocol recommended by the manufacturer of the synthesizer (8909 Expedite DNA synthesizer, system software 2.01). The coupling of modified phosphoramidite **21** to solid-support-bound DNA was carried out in a polypropylene vessel using a 0.12 M solution of the phosohoramidite in activator solution (250 μL). The oligodeoxynucleotides were cleaved from the solid support and deprotected with 30% aqueous ammonia for 12 h.

Removal of Alloc Groups (23 to 24). Solid support **23** (5 mg, approximately 0.14 μmol loading) was treated with a mixture of $Pd[PPh_3]_4$ (7.5 mg, 20.5 μmol) and diethylammonium bicarbonate (7.5 mg, 56 μmol) in DMF (0.6 mL) in a tapered Pyrex vial. This mixture was irradiated in a microwave synthesis system (Discover, CEM) for 10 min with up to 200 W and an upper temperature limit of 80 °C. The supernatant was removed, and the solid support was washed with DMF (3 × 1 mL) and once with CH_2Cl_2 (1 mL), followed by drying at 0.1 Torr.

Coupling of Pyrene Butyric Acid (24 to 25). A mixture of HOBT (15.3 mg, 100 μmol), HBTU (34.1 mg, 90 μmol), and pyrene butyric acid (29 mg, 100 μmol) was dissolved in DMF (600 μL). To this was added DIEA (80 μL, 486 μmol), and the resulting solution was shaken for 10 min. The solution was added to a tapered Pyrex vial together with support **24** (2.0 mg, 0.05 μmol DNA loading) and irradiated in a microwave synthesis system for 10 min at 200 W maximum and 80 °C upper temperature limit. After removal of the supernatant, the solid support was washed with DMF (3 × 1 mL) and then with CH_2Cl_2 (2 × 1 mL), followed by drying at 0.1 Torr.

Coupling of Pyrene Butyric Acid (to give 27). Pyrene butyric acid (29 mg, 100 μmol) was activated with a mixture of HOBT (15.3 mg, 100 μmol), HBTU (34.1 mg, 90 μmol) and DIEA (80 μL, 486 μmol) in DMF (600 μL) and was coupled to **24** (5.0 mg, 0.14 μmol DNA loading) as described for **25**.

GCAT$^{AM-PyBA}$TATTAC (26). HPLC: CH_3CN gradient 0% for 5 min to 27% in 55 min, t_R = 54.5 min. MALDI-TOF MS calcd for [M − H]$^-$ 3301.4, found 3300.9. Yield 3.0 nmol (6%).

GCAT$^{AM-PyBA}$TAT$^{AM-PyBA}$TAC (27). HPLC: CH_3CN gradient 0% for 5 min to 37% in 45 min, t_R = 39.0 min. MALDI-TOF MS calcd for $[M - H]^-$ 3600.8, found 3599.4. Yield 14.8 nmol (12%).

Preparation of 30. A sample of **23** (5.0 mg, 0.14 μmol DNA loading) was treated with 30% aqueous ammonia for 12 h to yield the Alloc-protected oligonucleotide, which was purified by HPLC analogously to **26**.

GCAT$^{AM-Alloc}$TATTAC (30). HPLC: CH_3CN gradient 0% for 5 min to 20% in 40 min, t_R = 34.0 min. MALDI-TOF MS calcd for $[M - H]^-$ 3115.7, found 3113.9. Yield: 54.8 nmol (38%).

Chemset 1. A mixture of hippuric acid (0.7 mg, 3.6 μmol), 3-indolepropionic acid (0.2 mg, 1 μmol), Boc-Ser(Bzl)-OH (1.4 mg, 4.8 μmol), dehydrocholic acid (2.37 mg, 5.9 μmol), and cinoxacin (0.5 mg, 1.7 μmol) in DMF (300 μL) was prepared from stock solutions and activated with HOBT (2.7 mg, 20 μmol), HBTU (6.8 mg, 18 μmol), and DIEA (8 μL, 46 μmol) and coupled to **24** (1.0 mg, 0.03 μmol DNA loading) as described for **25**. The coupling step was repeated once. MALDI-TOF MS calcd for ($[M - H]^-$) 3192.4/3202.2/3275.4/3308.5/3415.9, found 3191.2/3201.0/3274.3/3307.2/3414.6.

UV-Melting Experiments. UV-melting experiments were acquired at 260 nm wavelength and 1 cm path length at heating and cooling rates of 1 °C/min, using solutions of equimolar concentrations of the DNA strands in 10 mM PIPES (piperazine-*N*,*N*′-bis(ethane sulfonic acid)) buffer, pH 7, in deionized water and the salt concentrations given in Table 1. The melting temperatures and the thermodynamic data were determined with the program MeltWin 3.0.

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Supporting Information Available: Proton NMR spectra of **13**, **16a/b**, **18**, **19**, and **20**; ROESY NMR spectra of **16a/b**; and MALDI-TOF mass spectra of **26**, **27**, and **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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