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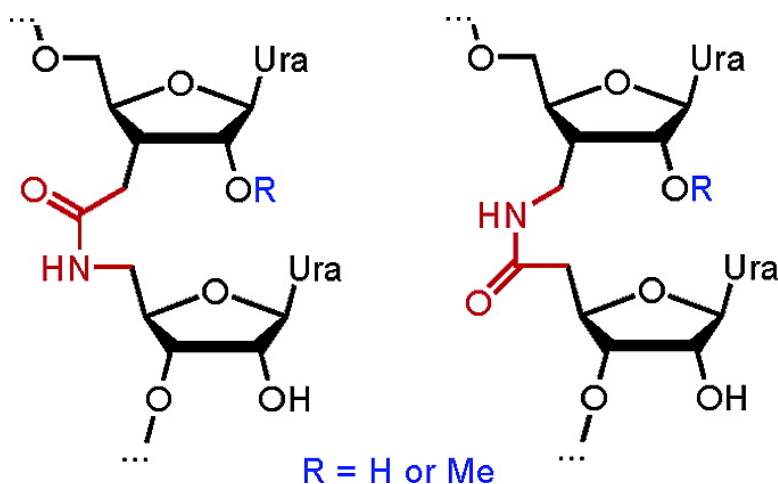

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Synthesis and Properties of RNA Analogues Having Amides as Interuridine Linkages at Selected Positions

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Abstract: Oligoribonucleotide analogues having amide internucleoside linkages (*AM1*: 3′-CH_2CONH-5′ and *AM2*: 3′-CH_2NHCO-5′) at selected positions have been synthesized and the thermal stability of duplexes formed by these analogues with complementary RNA fragments has been evaluated by UV melting experiments. Two series of oligomers with either 2′-OH or 2′-OMe vicinal to the amide linkages were studied. Monomeric synthons (3′ and 5′-C amines and carboxylic acids) were synthesized as follows: For synthesis of the *AM1* analogue, the known sequence of radical allylation followed by the cleavage of the double bond was adopted. For synthesis of the *AM2* analogue, novel routes via addition of nitromethane followed by conversion of the nitro function to either amino or carboxyl groups were developed. Coupling of monomeric amines and carboxylic acids followed by protecting group manipulation and phosphonylation gave dimeric 3′-hydrogenphosphonate building blocks for oligonucleotide synthesis. Monomeric model compounds having 3′-amide and 2′-OH or 2′-OMe groups were also prepared and their conformational equilibrium was determined by ^{1}H NMR. The *AM1* and *AM2* models showed equal preferences for the North conformers (at 40 °C, 88−89% with 2′-OH, and 92−93% with 2′-OMe). At physiological salt concentration (0.1 M NaCl) the duplexes between *AM1* modified oligonucleotides and RNA had stability similar to unmodified RNA-RNA duplexes (Δt_m= −0.2 to +0.7 °C per modification). However, the *AM2* modification resulted in substantial stabilization of duplexes: Δt_m= +1 to +2.4 °C per modification compared to all RNA. A 2′-O-methyl vicinal to the *AM2* linkage further increased the duplex stability. Our results suggest that RNA analogues having amide internucleoside bonds are very promising candidates for medicinal applications.

Introduction

Antisense therapy with synthetic oligonucleotides is a promising alternative to conventional chemotherapy of genetic disorders, cancer, and viral infections (such as HIV).[1] Important requirements for initial selection of potential antisense oligonucleotides are high stability toward nuclease degradation and high binding affinity to the intracellular target, usually a messenger RNA. Oligonucleotide analogues with dephospho internucleoside linkages could fulfill these requirements and are suggested as potential second generation antisense compounds.[2] The absence of the phosphodiester linkage must inherently ensure high nuclease stability of the modified oligonucleotide. The stability of duplexes formed by modified oligonucleotide and mRNA, however, is not so straightforward to foresee and usually has to be thoroughly examined for each particular dephospho linkage.

Of many nonionic oligodeoxynucleotide analogues screened (for recent reviews, see ref 2) those having amide[3−5] (3′-CH_2-CONH-5′ and 3′-CH_2NHCO-5′), methylene(methylimino)[6a,b] (3′-$CH_2N(CH_3)O$-5′), methylene(dimethylhydrazo)[6c] (3′-CH_2N-$(CH_3)N(CH_3)$-5′), formacetal[7,8] (3′-OCH_2O-5′) and thioformacetal[8] (3′-SCH_2O-5′) linkages give stable duplexes with

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(1) (a) Gewirtz, A. M.; Sokol, D. L.; Ratajczak, M. Z. *Blood* **1998**, *92*, 712−736. (b) *Antisense Drug Technology: Principles, Strategies, and Applications*; Crooke, S. T., Ed.; Marcel Dekker: New York, 2001; p 929.
(2) (a) De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366−374. (b) De Mesmaeker, A.; Altman, K.-H.; Waldner, A.; Wendeborn, S. *Curr. Opin. Struct. Biol.* **1995**, *5*, 343−355. (c) Sanghvi, Y. S.; Cook, P. D. In *Carbohydrate Modifications in Antisense Research*; Sanghvi, Y. S., and Cook, P. D., Eds.; ACS Symposium Series 580; American Chemical Society: Washington, DC, 1994; pp 1−22. (d) Freier, S. M., Altmann, K.-H. *Nucleic Acids Res.*, **1997**, *25*, 4429−4443. (e) Sanghvi, Y. S. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K., and Meth-Cohn, O., Eds.; Elsevier:, 1999; pp 285−311.
(3) For a review on amide substituted oligodeoxynucleotide analogues, see: De Mesmaeker, A.; Waldner, A.; Lebreton, J.; Fritsch, V.; Wolf, R. M. In *Carbohydrate Modifications in Antisense Research*; Sanghvi, Y. S., and Cook, P. D., Eds.; ACS Symposium Series 580; American Chemical Society: Washington, DC, 1994; pp 24−39.
(4) (a) Idziak, I.; Just, G.; Damha, M.; Giannaris, P. A. *Tetrahedron Lett.* **1993**, *34*, 5417−5420. (b) Lebreton, J.; Waldner, A.; Lesueur, C.; De Mesmaeker, A. *Synlett* **1994**, 137−140. (c) Mesmaeker, A.; Waldner, A.; Lebreton, J.; Hoffmann, P.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 226−229. (d) De Mesmaeker, A.; Lesueur, C.; Bevierre, M.-O.; Waldner, A.; Fritsch, V.; Wolf, R. M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2790−2794. (e) De Mesmaeker, A.; Lebreton, J.; Jouanno, C.; Fritsch, V.; Wolf, R. M.; Wendeborn, S. *Synlett* **1997**, 1287−1290.
(5) Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; De Mesmaeker, A. *Tetrahedron Lett.* **1994**, *35*, 5225−5228.
(6) (a) Vasseur, J.-J.; Debart, F.; Sanghvi, Y. S.; Cook, P. D. *J. Am. Chem. Soc.* **1992**, *114*, 4006−4007. (b) Morvan, F.; Sanghvi, Y. S.; Perbost, M.; Vasseur, J.-J.; Bellon, L. *J. Am. Chem. Soc.* **1996**, *118*, 255−256. (c) Sanghvi, Y. S.; Vasseur, J.-J.; Debart, F.; Cook, P. D. *Collect. Czech. Chem. Commun.* Special Issue **1993**, *58*, 158−162.

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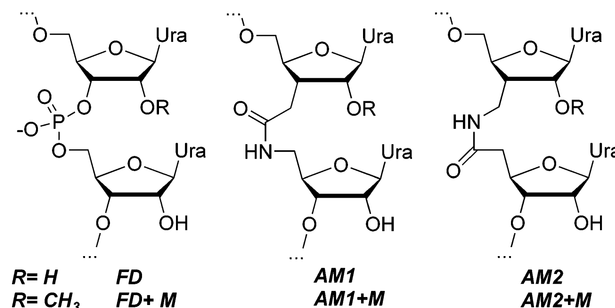
complementary RNA. In general, duplex stability is enhanced by modifications having restricted conformational freedom in the middle of the backbone: by restricted rotation around amide linkages and by other stereoelectronic effects.[2,9] Both shifting the amide bond by one atom along the backbone[10] and changing the backbone length[11] significantly decreases the stability of the duplex. The preference for North conformers in modified sugars has also been correlated with an increased stability of the A-type duplexes formed with RNA targets.[12]

Because RNA generally forms more stable duplexes than DNA both with RNA and DNA targets, oligoribonucleotide analogues having chemically and enzymatically stable internucleoside linkages may be better candidates also for therapy at the gene level than their deoxy counterparts. For example, a pentamer oligoribonucleotide (but not the corresponding deoxyoligoribonucleotide) hybridizes to a single-stranded DNA template in the open complex formed with RNA polymerase.[13a] Transcription inhibition with an oligoribonucleotide 2′-OMe analogue has been demonstrated by this approach.[13b] Thus, RNA analogues may be useful tools in both antisense applications and emerging new gene therapy approaches.

There are only a few reports on RNA analogues having dephospho linkages. Ribonucleoside dimers having thioformacetal[14] and sulfide[15] (3′-CH_2CH_2S-5′) linkages have been prepared and incorporated in oligonucleotides otherwise containing deoxynucleoside residues. Destabilization of such modified DNA-RNA duplexes was reported. The analysis of these data, however, is complicated because the effect of internucleoside linkage is not separated from the effect of alternating sugar composition: ribonucleoside dimers incorporated in otherwise oligodeoxynucleotides.[16] Uniformly modified oligoribonucleotides with dimethylene sulfone linkages (3′-$CH_2SO_2CH_2$-5′) have been prepared, but the strong self-association of this analogue apparently prevented hybridization with complementary RNA and DNA.[17] Uniformly modified riboadenosine pentamers with guanidine[18] (3′-NHC(=NH_2^+)NH-5′) and amide[19] (3′-CH_2-CONH-5′) linkages have been synthesized, however, the thermal stability of duplexes formed by these compounds has not been reported.



Model 16 5'-ApApGpCpGpApU**x**UpU**x**UpGpApCpApCpU
3'-UpUpCpGpCpUpApApApApCpUpGpUpGpA

Model 15 5'-ApCpApU**x**UpCpGpU**x**UpGpU**x**UpCpGpA
3'-UpGpUpApApGpCpApApCpApApGpCpU

Model 13 5'-U**x**UpU**x**UpU**x**UpU**x**UpU**x**UpU**x**UpU
3'-ApApApApApApApApApApApApA

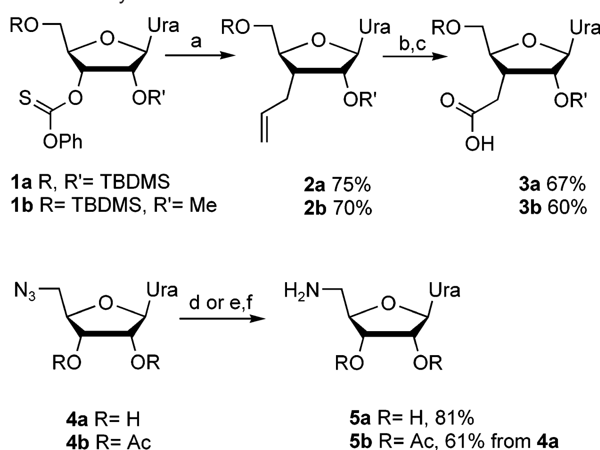
Figure 1. Oligoribonucleotide duplexes studied, **x** denotes position of the amide linkages, p denotes phosphodiesters.

We found that oligoribonucleotides where selected phosphodiester bonds were replaced by formacetal linkages had increased affinity to the complementary RNA fragments as compared to unmodified oligoribonucleotides.[20] In contrast, the formacetal modification in oligodeoxynucleotides is reported to decrease the stability of both DNA−RNA and DNA−DNA duplexes.[8,9] Encouraged by these results, we extended our studies to synthesize and investigate the properties of amide linked oligoribonucleotide analogues. Amide analogues were of particular interest because of: (1) potentially favorable hybridization properties, as expected from results in the deoxy series,[3−5] (2) automated solid phase synthesis of such analogues could be foreseen, similarly to peptide chemistry, (3) combining one type of amide linkage (our *AM1*) with 2′-O-methyl groups has been shown to increase affinity to RNA in mixed deoxyribo/ribo oligonucleotides[4d,e] and (4) uniformly modified, amide linked oligonucleotides could exhibit interesting properties, similarly to peptide nucleic acids (PNA, for reviews, see refs 2b,21).

In this paper, we report the synthesis of amide linked uridine-uridine dimers (with either 2′-OH or 2′-OMe vicinal to the amide linkages) and their incorporation in oligoribonucleotides (Figure 1). Novel synthetic routes toward 3′ and 5′-C one carbon extended nucleoside homologues are reported. UV melting experiments showed that both isomeric amides (*AM1* and *AM2*, see Figure 1) were well accommodated in RNA-RNA duplexes. Whereas *AM1* modified duplexes had an RNA affinity similar to that of nonmodified oligoribonucleotides (*FD*), the *AM2* modifications caused a large stabilization of up to over 2 °C per modification. The different stabilities of *AM1* and *AM2* suggest that amide modified oligoribonucleotides are interesting

(7) (a) Matteucci, M. *Tetrahedron Lett.* **1990**, *31*, 2385−2388. (b) Quaedflieg, P. J. L. M.; Pikkemaat, J. A.; van der Marel, G. A.; Kuyl-Yeheskiely, E.; Altona, C.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 15−21.
(8) (a) Jones, R. J.; Lin, K.-Y.; Milligan, J. F.; Wadwani, S.; Matteucci, M. D. *J. Org. Chem.* **1993**, *58*, 2983−2991. (b) Lin, K.-Y.; Pudlo, J. S.; Jones, R. J.; Bischofberger, N.; Matteucci, M. D.; Froehler, B. C. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1061−1064.
(9) De Mesmaeker, A.; Waldner, A.; Sanghvi, Y. S.; Lebreton, J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 395−398.
(10) (a) De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Synlett* **1993**, 733−736. (b) Lebreton, J.; De Mesmaeker, A.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Tetrahedron Lett.* **1993**, *34*, 6383−6386. (c) De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 873−878.
(11) Stork, G.; Zhang, C.; Gryaznov, S.; Schultz, R. *Tetrahedron Lett.* **1995**, *36*, 6387−6390. (b) De Mesmaeker, A.; Jouanno, C.; Wolf, R. M.; Wendeborn, S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 447−452.
(12) Griffey, R. H.; Lesnik, E.; Freier, S.; Sanghvi, Y. S.; Teng, K.; Kawasaki, A.; Wheeler, P.; Mohan, V.; Cook, P. D. In *Carbohydrate Modifications in Antisense Research*; Sanghvi, Y. S., and Cook, P. D., Eds.; ACS Symposium Series 580; American Chemical Society: Washington, DC, 1994; pp 212−224.
(13) (a) Perrin, D. M.; Mazumder, A.; Sadeghi, F.; Sigman, D. S. *Biochemistry* **1994**, *33*, 3848−3854. (b) Perrin, D. M.; Chen, C.-H. B.; Xu, Y.; Pearson, L.; Sigman, D. S. *J. Am. Chem. Soc.* **1997**, *119*, 5746−5747.
(14) Cao, X.; Matteucci, M. D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 807−810.
(15) (a) Meng, B.; Kawai, S. H.; Wang, D.; Just, G.; Giannaris, P. A.; Damha, M. J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 729−731. (b) Damha, M. J.; Meng, B.; Wang, D.; Yannopoulos, C. G.; Just, G. *Nucleic Acids Res.* **1995**, *23*, 3967−3973.
(16) For a systematic study of thermal stability of mixed ribo-deoxyribo sequences, see Hoheisel, J. D. *Nucleic Acids Res.*, **1996**, *24*, 430−432.
(17) Richert, C.; Roughton, A. L.; Benner, S. A. *J. Am. Chem. Soc.* **1996**, *118*, 8, 4518−4531.
(18) Dempcy, R. O.; Luo, J.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4326−4330. Kojima, N.; Bruice, T. C. *Org. Lett.* **2000**, *3*, 81−84. Kojima, N.; Szabo, I. E.; Bruice, T. C. *Tetrahedron* **2002,** *58,* 867−879.
(19) Robins, M. J.; Doboszewski, B. D.; Nilsson, B. L.; Peterson, M. A. *Nucleosides, Nucleotides & Nucleic Acids* **2000**, *19*, 69−86.
(20) Rozners, E.; Strömberg, R. *J. Org. Chem.* **1997**, *62*, 1846−1850.

Scheme 1. Synthesis of Monomers for *AM1* Modification[a]



[a] Reagents and conditions: (a) allyltributyltin, AIBN, 80 °C; (b) OsO_4, $NaIO_4$; (c) $NaClO_2$; for **5a** (d) Ph_3P, NH_3; for **5b** (e) acetyl chloride, pyridine; (f) Bu_3SnH, AIBN, 80 °C.

model systems for studies on factors that govern biopolymer recognition. In particular, we suggest that the difference in thermal stability is caused by different hydration of these analogs. Furthermore, amide linked RNA might find potential use as antisense compounds or as therapeutic ribozymes.

Results and Discussion

Syntheses of monomeric building blocks, carboxylic acids **3a,b** and **13**, and amines **5a,b** and **8a−d** are illustrated in Schemes 1-2. For the 3′-CH_2CONH-5′ linkage (abbreviated as *AM1*, Figure 1), the key transformations previously used by De Mesmaeker et al.[3] were successfully employed (Scheme 1). Free-radical allylation[4b,10a,11a,22] of 3′-O-phenoxythiocarbonyl derivatives **1a,b**[23] followed by cleavage of the double bond[24] with OsO_4 and $NaIO_4$, and oxidation of the intermediate aldehyde with $NaClO_2$[4b,c,25] gave the carboxylic acids **3a** and **3b** in 38 and 29% overall yields, respectively (4 steps, from 2′,5′-protected nucleosides[26]). Consistent with results previously reported in the deoxy series,[4b,10a,11a,22] the radical allylation gave preferably the 3′,4′-trans isomer shown in Scheme 1.[27]

Amines **5a,b** were readily synthesized by reduction of the known 5′-azido-5′-deoxyuridine[28] (Scheme 1). During radical reduction[29a] of 5′-azido-2′,3′-O-bis(acetyl)-5′-deoxyuridine **4b** (prepared by acetylation of **4a**), we observed some transacetylation yielding the *N*-acetyl byproduct. This side reaction resulted in somewhat lower yield of **5b** (70% vs ca. 90% reported in ref 29), but did not disturb further synthesis of the dimer **14a**. For synthesis of dimer **14b** we employed the 2′,3′-O-unprotected amine **5a**, prepared using triphenylphosphine reduction[29b] of **4a**. However, the high polarity of **5a** was somewhat inconvenient.

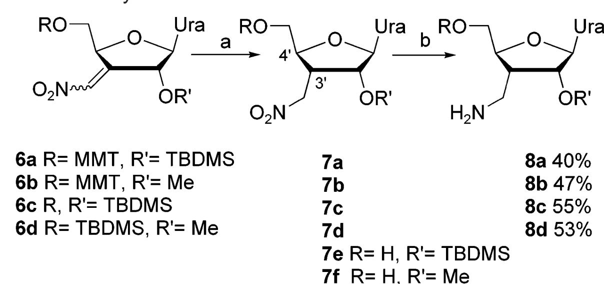
In contrast, the preparation of building blocks for the 3′-CH_2-NHCO-5′ linkage (abbreviated as *AM2*, Figure 1) was problematic. For preparation of 3′-CH_2NH_2 in the deoxy series, De Mesmaeker et al. used addition of styryltributyltin to 3′-C-centered radicals of protected deoxyribonucleosides.[5] In the ribo series, however, we found that the reaction was difficult to initiate and gave complex product mixtures. Synthesis via 3′-deoxy-3′-methylene derivatives (Wittig addition followed by hydroboration) was attempted but preliminary results gave poor stereoselectivity. A high stereoselectivity in this reaction has recently been reported but only for the ribonucleoside analogue (not the 2′-OMe), because the bulky 2′-TBDMS group is probably directing the stereoselectivity.[30]

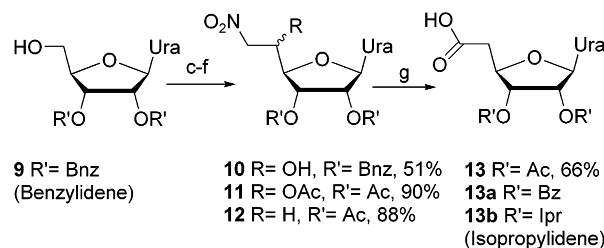
Of different one carbon homologization methods, the nitroaldol (Henry) reaction[31] seemed most promising because of the ease of carbon−carbon bond formation under relatively mild conditions. Only a few but encouraging examples of such reactions on carbohydrate derived ketones were known.[32] In an early report Rosenthal et al.[32a] described addition of nitromethane to 2′-keto xyloadenosine followed by hydrogenation of the nitro function to give 2′-CH_2NH_2 lyxoadenosine. More recently, Garg et al.[32b] reported addition of nitromethane to 3′-keto ribothymidine. Importantly, reduction of the double bond ($NaBH_4$/EtOH) in 3′- nitromethylene derivative gave a 2.5:1 mixture of 3′,4′-trans and cis 3′C−CH_2NO_2 ribothymidines.[32b]

Our syntheses of amines **8a−d** using the Henry reaction are illustrated in Scheme 2. Addition of nitromethane[32b] to appropriately protected 3′-keto nucleosides[33] gave the 3′-C-nitromethylene derivatives **6a−d** in 70−80% yields. Reduction of the double bond ($NaBH_4$/EtOH, 0 °C) gave inseparable mixtures of **7a−d** and their 3′,4′-cis isomers in ratios of 6:1 for **7a,c** and 15:1 for **7b,d**.[34] A brief investigation of the solvent effect (THF, EtOH, EtOEt, CH_2Cl_2, $ClCH_2CH_2Cl$, for detail, see Supporting Information) revealed that reduction in THF at −78 °C gave the best results, diastereomer ratio (dr) better than 95:5. For **6c** the use of $(Bu)_4NBH_4$ gave further significant improvement, dr better than 98:2. In the 2′-OMe series, however, the effects of solvent or reagent changes were insignificant: the best result for **6d** was dr 94:6 with $NaBH_4$ in THF at −78 °C.

(21) (a) Nielsen, P. E.; Haaima, G. *Chem. Soc. Rew.* **1997**, 73−78; Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2796−2823; Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624−630.
(22) Chu, C. K.; Doboszewski, B.; Schmidt, W.; Ullas, G. V.; Van Roey, P. *J. Org. Chem.* **1989**, *54*, 2767−2769.
(23) (a) Meier, C.; Huynh-Dinh, T. *Synlett* **1991**, 227−228. (b) Robins. M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059−4065.
(24) Sanghvi, Y. S.; Bharadwaj, R.; Debart, F.; De Mesmaeker, A. *Synthesis* **1994**, 1163−1166.
(25) Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567−569.
(26) Syntheses of TBDMS protected uridine derivatives were done using published procedures: Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. *Can. J. Chem.* **1982**, *60*, 1106−1113.
(27) Pure of **2a** and **2b** (3′,4′-trans isomers) were isolated after silica gel column chromatography. The configuration at C3′ was confirmed by NOESY spectra of the intermediate aldehydes. Informative NOEs were observed between H1′ and 3′-CH_2 and between H3′ and H6. Possible 3′,4′-cis isomers (less than 10% as judged by TLC) were not isolated.
(28) (a) Hata, T.; Yamamoto, I.; Sekine, M. *Chem. Lett.* **1975**, 977−980. (b) Yamamoto, I.; Sekine, M.; Hata, T. *J. Chem. Soc., Perkin Trans. 1* **1978**, 306−310.
(29) (a) Samano, M. C.; Robins, M. J. *Tetrahedron Lett.* **1991**, *32*, 6293−6296. (b) Mungall, W. S.; Greene, G. L.; Heavner, G. A.; Letsinger, R. L. *J. Org. Chem.* **1975**, *40*, 1659−1662.
(30) (a) Winqvist, A.; Strömberg, R. *Nucleosides, Nucleotides & Nucleic Acids*, **2001,** 1389−1392 (b) Winqvist, A.; Strömberg, R. *Eur. J. Org. Chem.* **2001,** 4305−4311.
(31) Rosini, G. In *Comprehensive Organic Synthesis, Part 2, Addition to C−X π Bonds*; Trost, B.; Fleming, I., Eds.; Pergamon Press: Oxford, 1991; pp 329−340.
(32) (a) Rosenthal, A.; Sprinzl, M.; Baker, D. A. *Tetrahedron Lett.* **1970**, *48*, 4233−4236. (b) Garg, N.; Hossain, N.; Plavec, J.; Chattopadhyaya *Tetrahedron*, **1994**, *50*, 4167−4178. (c) Albrecht, H. P.; Moffat, J. G. *Tetrahedron Lett.* **1970**, 1063−1066.
(33) (a) 3′-Keto derivatives were prepared from selectively protected nucleosides (see ref 26) using the published oxidation procedures: (a) DMSO/acetic anhydride or CrO_3, see Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron*, **1984**, *40*, 125−135. (b) Dess−Martin 12-I-5 periodane oxidant, see Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155−4156. Samano, V.; Robins, M. J. *J. Org. Chem.* **1990**, *55*, 5186−5188. For 5′-*O*-MMT protected derivatives we preferred the Dess−Martin oxidation.
(34) Ratios determined by ^{1}H NMR. The 3′,4′-trans configurations of the major isomers were confirmed by NOESY spectra of **7c, d** and **8a−d**. Informative NOEs were observed between H1′ and 3′-CH_2 and between H3′ and H6. As expected, no cross-peaks were detected for correlation between H1′ and H3′.

Scheme 2. Synthesis of Monomers for *AM2* Modification[a]





[a] Reagents and conditions: (a) $NaBH_4$ THF, −78 °C; (b) NiB_2, $NaBH_4$; (c) DMSO, DCC, $CHCl_2COOH$; (d) CH_3NO_2, $NaOCH_3$; (e) Ac_2O, $HClO_4$, 0 °C; (f) $NaBH_4$ ethanol-THF (1:1), 0 °C; (g) $NaNO_2$, AcOH, DMSO, 40 °C.

The unwanted 3′,4′-cis isomers were separated after further transformations.

Reduction of aliphatic nitro functions to give primary amines is a well-known reaction in organic chemistry.[35,36] However, its application to nucleoside derivatives could be problematic because of potential side reactions in the heterocyclic bases and steric hindrance from protecting groups used. For nucleosides only a few examples of successful catalytic hydrogenation of nitro groups (in the presence of acetic acid) are reported.[32a,c] We chose **7c** for initial studies on the reduction of nitro group. Catalytic hydrogenation (100 psi H_2, EtOH, 20 °C, 48 h) using either 10% Pd/carbon,[36a] Raney nickel[36b] or PtO_2[36c] catalysts gave no reaction. Hydrogenation using 10% Pd/carbon in the presence of acetic acid led to cleavage of the 5′-O-TBDMS group, whereas the nitrofunction remained intact. Loss of a primary TBDMS protection during hydrogenation has been previously observed by others.[37] Reactions with $LiAlH_4$,[36d] sodium bis-(2-methoxyethoxy)aluminum hydride (Red-Al),[36e] and $NaBH_4$ in the presence of 10% Pd/carbon catalyst[36f] gave complex mixtures containing also products from cleavage of the glycosidic linkage. Reductions with hydrazine hydrate and either nickel boride[36g] or Raney nickel[36h] as catalysts were slow. At elevated temperatures or high hydrazine concentrations complex mixtures were obtained.

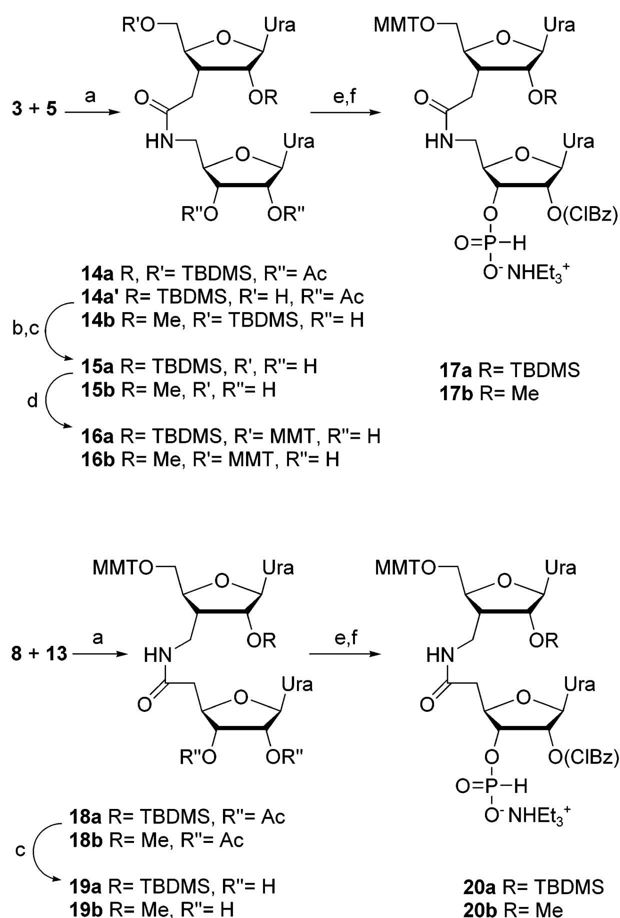
However, reduction with $NaBH_4$ (10 equiv) and nickel boride[38] (1 equiv.) in ethanol gave **8c** in ca. 50% isolated yield. The reduction sequence from **6** to **8** was further developed into a one pot procedure (see Experimental Section). The double bond in **6a**−**d** was reduced with $NaBH_4$ (4 equiv) in EtOH to give **7a**−**d** and successive addition of nickel boride (1.5 equiv for **6a,b** or 1 equiv. for **6c,d**)[38c] and $NaBH_4$ (10 equiv.) to the same reaction mixture gave **8a**−**d** in 40−50% overall yield from **6a**−**d**. Silica gel column chromatography yielded isomerically pure **8a**, whereas **8b**−**d** were obtained as still inseparable mixtures of 3′,4′-cis and trans isomers (see above). When the optimized conditions for the reduction of the double bond (see above) were used in the one pot procedure (see the Experimental Section) **8c** was obtained with dr 98:2, as expected. For the synthesis of *AM2* linked dimers we used **8a** and **8b** prepared using the $NaBH_4/NiB_2$ one pot procedure. Removal of the cis isomer of **8b** was achieved by chromatographic separation after synthesis of **18b**. In summary, addition of nitromethane to 3′-keto nucleosides followed by successive reduction of double bond and nitrofunction gave the required amines **8a**−**d** in 20−30% overall yields (5 steps, from the 2′,5′-protected nucleosides).

Our initial attempts to prepare carboxylic acid **13** using published methods for preparation of 5′-C one carbon homologues of nucleosides also met with limited success.[39] Instead, we developed a novel route based again on the addition of nitromethane followed by functional group interconversion (Scheme 2). Oxidation of 2′,3′-O−(benzylidene)uridine **9** and addition of nitromethane[40] was done in the same reaction mixture without isolation of the 5′-aldehyde. Acetylation of the 5′-OH under acidic conditions[40b] also conveniently cleaved the benzylidene protection and acetylated the 2′ and 3′ hydroxyls. Reductive removal of the 5′-O-acetyl group[40b,41] followed by transformation of the nitromethyl function[42] gave the carboxylic acid **13** in 27% overall yield (4 steps, from 2′,3′-O−(benzylidene)uridine).

DCC and hydroxybenzotriazol (HOBt) mediated coupling[43] of carboxylic acids **3a,b** and **13** with amines **5a,b** and **8a,b** gave the dimers **14a,b** and **18a,b**, respectively (Scheme 3). Selective removal of the primary 5′-O-TBDMS group (limited hydrolysis in 80% aqueous acetic acid), cleavage of the terminal 2′ and 3′-O-acetyl groups and protection of the 5′-OH as monomethoxytrityl (MMT) ether gave dimers **16a,b**. Dimers **19a,b** were obtained after treatment of **18a,b** with ammonia solution. Synthesis of the dimeric H-phosphonate building blocks **17a,b** and **20a,b** was achieved by one pot selective installation of the 2′-*ortho*-chlorobenzoyl (ClBz) group followed by 3′-O-phosphonylation as previously reported.[20]

(35) Larock, R. C. *Comprehensive Organic Transformations. A Guide to Functional Group Preparations*; VCH Publishers: New York, 1989.
(36) (a) Poos, G. I.; Kleis, J.; Wittekind, R.; Rosenau, J. D. *J. Org. Chem.* **1961**, *26*, 4898−4904. (b) Bachman, G. B.; Hass, H. B.; Platau, G. O. *J. Am. Chem. Soc.* **1954**, *76*, 3972−3974. (c) Secrist III, J. A.; Logue, M. V. *J. Org. Chem.* **1972**, *37*, 335−336. (d) Nystrom, R. F.; Brown, W. G. *J. Am. Chem. Soc.* **1948**, *70*, 3738−3740. (e) Butterick, J. R.; Unrau, A. M. *J. Chem. Soc. Chem. Commun.* **1974**, 307−308. (f) Petrini, M.; Ballini, R.; Rosini, G. *Synthesis* **1987**, 713−714. (g) Lloyd, D. H.; Nichols, D. E. *J. Org. Chem.* **1986**, *51*, 4294−4295. (h) Podona, T.; Guardiola-Lemaitre, B.; Caignard, D.-H.; Pfeiffer, B.; Renard, P.; Guillaumet, G. *J. Med. Chem.* **1994**, *37*, 1779−1793.
(37) Carreira, E. M.; Du Bois, J. *J. Am. Chem. Soc.* **1995**, *117*, 8106−8125.
(38) (a) Osby, J. O.; Ganem, B. *Tetrahedron Lett.* **1985**, *26*, 6413−6416. (b) for a recent examples of this reaction, see Corey, E. J.; Zhang, F.-Y. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1931−1934; Clive, D. L. J.; Bo, Y.; Tao, Y.; Daigneault, S.; Wu, Y.-J.; Meignan, G. *J. Am. Chem. Soc.* **1998**, *120*, 10 332−10 349. (c) The use of less $NaBH_4$ (5 equiv.) and nickel boride catalyst (0.5 equiv) gave a mixture of hydroxylamine (isolated yield 50%, for analytical data, see Supporting Information) and amine **8c** (isolated yield 12%).
(39) Carboxylic acid **13a** (Scheme 2) was obtained from 1, 2; 5, 6-di-*O*-(isopropylidene)glucose in a laborious multistep synthesis in 6% overall yield. Carboxylic acid **13b** was obtained from 2′,3′-*O*-(isopropylidene)-uridine in 19% overall yield, however, the stability of the isopropylidene protection complicated further operations. For synthetic details, see Supporting Information. Synthesis via Wittig addition of 1,3-dithia-2-cyclohexylidene triphenylphosphorane (see ref 5) failed because of instability of 2′,3′-protections (benzylidene or benzoyl) under conditions required to generate the carboxylic acid ($HgCl_2$ in $MeOH/H_2O$ followed by NaOH in H_2O).
(40) (a) Kappler, F.; Hampton, A. *J. Org. Chem.* **1975**, *40*, 1378−1385. (b) Mock, G. A.; Moffat, J. G. *Nucleic Acids Res.* **1982**, *10*, 6223−6234.
(41) Bachman, G. B.; Maleski, R. J. *J. Org. Chem.* **1972**, *37*, 2810−2814.
(42) Matt, C.; Wagner, A.; Mioskowski, C. *J. Org. Chem.* **1997**, *62*, 234−235.
(43) Rees, A. R.; Offord, R. E. *Biochem. J.* **1976**, *159*, 487−493.

Scheme 3. Synthesis of Dimeric Building Blocks Containing *AM1* or *AM2* Modifications[a]



[a] Reagents and conditions: (a) DCC, HOBt; (b) 80% AcOH, 50 °C; (c) NH_3/EtOH, 2:1; (d) MMTCl, pyridine; (e) *o*-Chlorobenzoyl chloride, 1.1 equiv., −78 °C; (f) PCl_3, imidazole, NEt_3, −78 °C; quenched with 2 M triethylammonium bicarbonate (aqueous), pH 7.5.

Three Model Oligoribonucleotides (Figure 1) having two (**Mod 16**), three (**Mod 15**) and six (**Mod 13**) internucleoside amide linkages (**x**) were prepared using dimers **17** or **20** and standard H-phosphonate oligoribonucleotide synthesis procedure.[44] Reference oligoribonucleotides (unmodified and having 2′-O-Me groups vicinal to the phosphodiester linkages **x**) and the complementary oligoribonucleotides were also synthesized via the H-phosphonate route[44] and the stabilities of the corresponding duplexes (Figure 1) were characterized by UV melting experiments.[20] Experiments were done at low (0.1 M NaCl) and high (1 M NaCl) salt concentrations; melting temperatures are collected in Table 1 and thermodynamic data in Table 2. For each model sequence two series of oligomers were studied: (1) in the ribo series *AM1* and *AM2*, were **x** (Figure 1) represents the corresponding amide linkage, were compared to unmodified phosphodiester oligoribonucleotide *FD*, and (2) in the corresponding 2′-O-methyl series *AM1+M* and *AM2+M* were compared to *FD+M* (in all series only the 2′-hydroxyls vicinal to **x** were methylated).

At low salt concentration (0.1 M NaCl, Table 1) the *AM1* modified duplexes showed a stability similar to that of the

(44) (a) Westman, E.; Sigurdsson, S.; Stawinski, J.; Strömberg, R. *Nucleic Acids Sym Ser.* **1994**, *31*, 25. (b) Strömberg, R.; Stawinski, J. *Current Protocols in Nucleic Acid Chemistry*, unit 3.4; John Wiley & Sons: New York, 2000. (c) Sarkar, M.; Sigurdsson, S.; Tomac, S.; Sen, S.; Rozners, E.; Sjöberg, B. M.; Strömberg, R.; Gräslund, A. *Biochemistry* **1996**, *35*, 4678−4688.

Table 1. Melting Temperatures of Oligonucleotide Duplexes[a]

		t_m (°C) 0.1 M NaCl	t_m (°C) 1 M NaCl
		Mod 16	
1	*FD*	55.9	70.3
2	*AM1*	55.7 (−0.1)	69.9 (−0.2)
3	*AM2*	60.3 (+2.2)	72.7 (+1.2)
4	*FD+M* [b]	57.4 (+0.8)	71.6 (+0.7)
5	*AM1+M* [b]	58.0 (+1.1)	70.9 (+0.3)
6	*AM2+M* [b]	59.4 (+1.8)	72.7 (+1.2)
		Mod 15	
7	*FD*	53.0	66.3
8	*AM1*	52.5 (−0.2)	64.1 (−0.8)
9	*AM2*	57.0 (+1.3)	67.5 (+0.4)
10	*FD+M* [b]	55.0 (+0.7)	68.7 (+0.8)
11	*AM1+M* [b]	54.4 (+0.5)	67.9 (+0.5)
12	*AM2+M* [b]	60.0 (+2.3)	71.2 (+1.6)
		Mod 13	
13	*FD*	13.3	30.4
14	*AM1*	17.2 (+0.7)	29.6 (−0.1)
15	*AM2*	27.5 (+2.4)	38.1 (+1.3)
16	*FD+M* [b]	19.1 (+1.0)	35.7 (+0.9)
17	*AM1+M* [b]	22.4 (+1.5)	32.1 (+0.3)
18	*AM2+M* [b]	31.6 (+3.1)	40.1 (+1.6)

[a] Δt_m per modification relative to the native RNA (*FD*) is given in brackets, [b] *+M* designates the 2-O-methyl series.

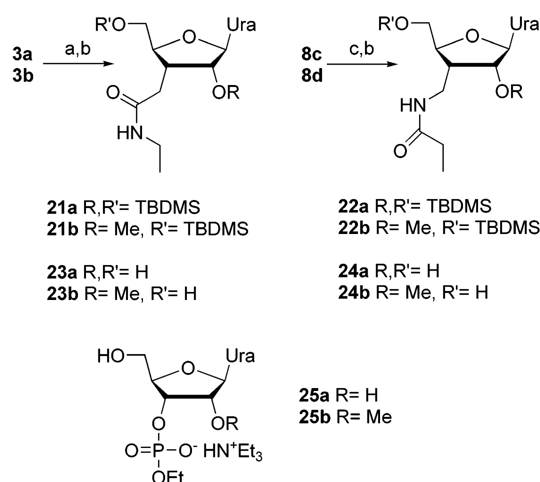
Table 2. Thermodynamic Parameters for the Formation of Oligonucleotide Duplexes at 0.1 M NaCl and 1 M NaCl (Mod 13 only)

		ΔH^o kcal/mol	$T\Delta S^o_{310}$ kcal/mol	ΔG^o_{310} kcal/mol
		Mod 16 [a]		
1	*FD*	−139.7	−123.1	−16.6
2	*AM1*	−127.8	−111.8	−16.1
3	*AM2*	−154.1	−134.7	−19.4
4	*FD+M* [b]	−144.9	−127.2	−17.7
5	*AM1+M* [b]	−127.6	−110.9	−16.7
6	*AM2+M* [b]	−134.5	−116.8	−17.7
		Mod 15 [a]		
7	*FD*	−130.1	−115.5	−14.6
8	*AM1*	−117.3	−103.1	−14.2
9	*AM2*	−123.8	−107.6	−16.1
10	*FD+M* [b]	−135.2	−119.1	−16.1
11	*AM1+M* [b]	−116.7	−101.8	−14.8
12	*AM2+M* [b]	−122.8	−105.8	−17.0
		Mod 13 [a]		
13	*FD*	−76.8	−74.6	−2.3
14	*AM1*	−89.0	−86.5	−2.6
15	*AM2*	−90.3	−84.5	−5.7
16	*FD+M* [b]	−77.3	−73.5	−3.8
17	*AM1+M* [b]	−82.1	−77.6	−4.5
18	*AM2+M* [b]	−88.7	−81.7	−7.0
		Mod 13 [c]		
19	*FD*	−99.5	−93.0	−6.5
20	*AM1*	−86.5	−80.0	−6.5
21	*AM2*	−96.6	−87.7	−8.9
22	*FD+M* [b]	−76.3	−68.1	−8.2
23	*AM1+M* [b]	−65.4	−57.8	−7.6
24	*AM2+M* [b]	−86.7	−77.2	−9.5

[a] 0.1 M NaCl, [b] *+M* designates the 2-*O*-methyl series, [c] 1 M NaCl

controls: Δt_m= −0.2 to +0.7 in the ribo series and, Δt_m= −0.2 to +0.5 in the 2′-O-methyl series. This result correlates well with previous observations in the deoxy series.[4] In contrast, the *AM2* modification gave substantially higher duplex stabilization: Δt_m= +1.3 to +2.4 in the ribo series and, Δt_m= +1.0 to +2.1 in the 2′-OMe series. This result is approaching the greatest positive effects observed when a phosphodiester bond in an

Scheme 4 . Synthesis of Model Compounds for Conformational Studies[a]



[a] Reagents and conditions: (a) DCC, HOBt, ethylamine; (b) tetrabutylammonium fluoride; (c) propionic anhydride.

oligonucleotide is replaced with a dephospho linkage[2,45] and is in contrast to the deoxy series where similar thermal stabilities of *AM1* and *AM2* modified duplexes were observed. [4,5]

At high salt concentration (1 M NaCl, Table 1) where electrostatic repulsion is greatly reduced, generally higher t_m values were observed. At 1 M NaCl the *AM1* modified duplexes showed a slight destabilization ($\Delta t_m = -0.8$ to -0.1) compared to the controls, whereas the stabilization caused by the *AM2* modification remained positive ($\Delta t_m = +0.4$ to $+1.3$), although less so than at lower salt concentration. The difference in Δt_m per modification compared to native RNA between high and low salt conditions ($\Delta\Delta t_m = 0.1$ to 1.2 for *AM1* and $\Delta\Delta t_m = 0.4$ to 1.4 for *AM2*) suggests that a substantial part of the gain in duplex stability at physiological salt concentration (0.1 M) is due to reduced electrostatic repulsion when replacing charged phosphodiester linkages with neutral amides. However, this is not responsible for all of the stabilization, especially in the case of the *AM2* modification.

Substitution of 3′-CH_2 for 3′-O should shift the conformational equilibrium of modified sugars toward North[46] which in turn has been correlated with increased stability of the A-type duplexes formed by modified oligonucleotides with RNA targets.[12] To evaluate the changes in sugar conformation caused by the amide linkages we synthesized monomeric nucleoside models **23a,b** and **24a,b** (Scheme 4) and studied their conformational equilibrium by ^{1}H NMR.[47]

Amide modified models showed a greatly increased preference for North conformation: percentage of North conformers was for the 2′-OH models **23a** 88%, **24a** 89% and for the 2′-OMe models **23b** 92%, **24b** 93% (for full experimental data, see Supporting Information). In comparison, the equilibrium positions of the corresponding phosphodiester models **25a** and **25b** were 46 and 54% North, respectively. These results are also in good agreement with conformational studies on methylene(methylimino)[48] and methylene(dimethylhydrazo)[12] linked dimers. Because both amides caused the same conformational preferences of the sugar residues in the model compounds, the conformational preorganization induced by the 3′-CH_2 modification cannot be responsible for the observed differences in stability of *AM1* and *AM2* modified RNA duplexes. Before other interactions (see below) are better understood, direct correlation between stability of modified oligonucleotide duplexes and conformational preference of the corresponding nucleoside models is best used with caution.

Analysis of the thermodynamic data (Table 2) of the two different amide modifications suggests that in general *AM1* is more entropically favored, whereas the *AM2* modification is more enthalpically favored. This suggests that conformational preorganization could be a dominating factor with *AM1* and that some additional bonding interaction, e.g., direct or water mediated H-bonding, stabilizes the *AM2* duplexes. The thermal stability of modified nucleic acid duplexes should depend on many factors hydrogen bonding, preferred sugar conformation, hydration, electrostatic and steric factors, hydrophobic interactions, etc. Substitution of neutral dephosphono linkages for phosphodiesters is a radical change that should interfere with almost all of these factors. The example of the isomeric amides *AM1* and *AM2*, studied herein, is of particular interest: although many factors should be affected similarly, the net difference between these modifications is surprisingly large. In the deoxy series, molecular mechanics and molecular dynamics studies on both modifications give similar geometry and UV melting experiments give similar thermal stability.[4,5] Our results showed a very similar preference for North conformers in the different model compounds. For both *AM1* and *AM2* modified duplexes we found a similar gain in stability due to reduction of electrostatic repulsion (see above). Still, the *AM2* modified duplexes are much more stable than *AM1*, the difference being as large as 1 to 1.5 °C per modification.

To gain more insight into possible reasons for the greater stability of the *AM2* modification, we studied the dependence of melting temperatures of **Mod 13** on the concentration of different types of salts. At high concentrations (>1M) salts exert specific effects on the stability of biopolymer structures through indirect interaction with the surrounding aqueous solvent. According to the Hofmeister series,[49] kosmotropes (polar water structure-makers, e.g., sodium acetate) stabilize whereas chaotropes (water-structure breakers, e.g., sodium perchlorate) destabilize the native conformation of biopolymers. Sodium chloride has little effect on water structure. The comparison of *AM1* (triangles) and *AM2* (circles) melting points in the presence of added sodium chloride (empty markers) or sodium acetate (filled markers) is shown in Figure 2. According to the Hofmeister series,[49] **Mod 13** was expected to be more stable in sodium acetate than in sodium chloride solution. This was indeed observed for the unmodified RNA (data not shown) and the *AM1* modified oligonucleotide (Figure 2, filled vs empty triangles). In contrast, **Mod 13** with *AM2* modifications did not

(45) Cationic guanidine and *S*-methylthiourea modified DNA forms extremely stable 2: 1 complexes with natural DNA, see (a) Linkletter, B. A.; Szabo, I. E.; Bruice, T. C. *Nucleic Acids Res.* **2001,** *29,* 2370−2376. (b) Linkletter, B. A.; Szabo, I. E.; Bruice, T. C. *J. Am. Chem. Soc.* **1999,** *121,* 3888−3896. (c) Arya, D. P.; Bruice, T. C. *J. Am. Chem. Soc.* **1998,** *120,* 12 419−12 427.

(46) (a) Plavec, J.; Garg, N.; Chattopadhyaya, J. *J. Chem. Soc. Chem. Commun.* **1993**, 1011−1014. (b) For a discussion on the stereoelectronic effects in nucleosides and analogues, see also Plavec, J.; Tong, W.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **1993**, *115*, 9734−9746.

(47) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333−2344.

(48) Bhat, B.; Swayze, E. E.; Wheeler, P.; Dimock, S.; Perbost, M.; Sanghvi, Y. S. *J. Org. Chem.* **1996**, *61*, 8186−8199.

(49) (a) Cacace, M. G.; Landau, E. M.; Ramsden, J. J. *Quart. Rev. Biophysics* **1997**, *30*, 241−277. (b) Collins, K. D.; Washabaugh, M. W. *Quart. Rev. Biophysics* **1985**, *18*, 323−422.

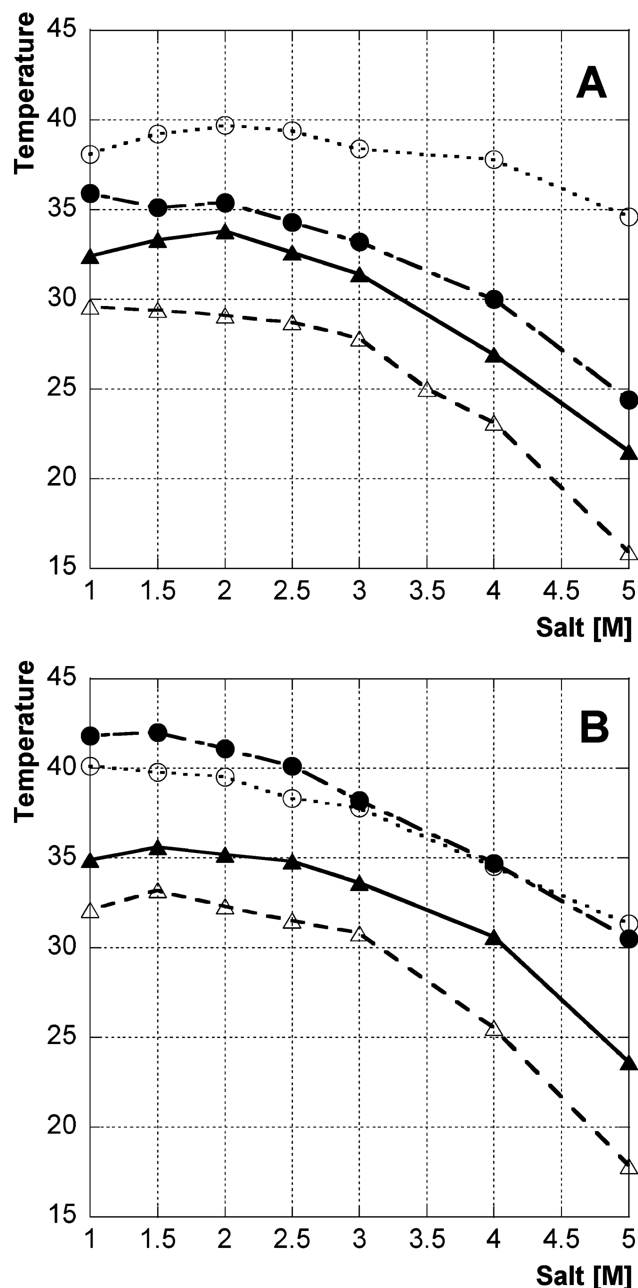


Figure 2. Dependence of melting points for AM1 (triangles) and AM2 (circles) modified Mod 13 on the concentration of sodium chloride (empty markers) and sodium acetate (filled markers). A Mod 13 in the 2′-OH series; B Mod 13 in the 2′-OMe series.

display a similar behavior, being more stable in sodium chloride solutions in the 2′-OH series (Figure 2A, filled vs empty circles). In the 2′−O-methyl series *AM2* also displayed an unusual behavior: the slight stabilization in sodium acetate vs sodium chloride disappeared as the concentration of salts increased beyond 3 M (Figure 2B, filled vs empty circles). Addition of sodium perchlorate (data not shown) strongly destabilized all sequences with no significant differences between *AM1* and *AM2*. These results suggested a substantial difference in the water structure surrounding the duplexes having *AM1* and *AM2* modifications.

Although more structural data have to be obtained to disclose the origin of the difference in behavior of duplexes containing *AM1* and *AM2* modified oligoribonucleotides, some suggestions can be made. The 2′-OH contributes to the higher thermal stability of RNA−RNA duplexes compared to DNA−DNA duplexes both entropically, by conformational preorganization of the ribose and enthalpically, by improvement of hydration.[50] The water mediated hydrogen bond network observed in crystal structures of A-RNA duplexes is suggested to play a crucial role in thermal stability.[51] Thus, we can suggest that the changes in hydration pattern of modified RNA−RNA duplexes are more favorable for *AM2* than for *AM1*. The enthalpical stabilization of *AM2* modified duplexes (when compared to *AM1*) supports this hypothesis. It could be that the amide linkage of the *AM2* modification interacts with the hydration network through hydrogen bonding, thereby stabilizing the duplex, but more structural data is needed to see if this is a viable hypothesis. Structural regularity, as in **Mod 13** having alternating amide and phosphodiester linkages, could be favorable for formation of well-defined hydration pattern. This would also explain why **Mod 13** showed generally higher Δt_m values (Table 1). A similar increase in stability when the number of periodic modifications are increased has also been observed by others.[6c]

At this stage, alternative explanations for the greater stability of the *AM2* modified duplexes are also feasible. Previous molecular dynamics studies in the deoxy series suggest that both *AM1* and *AM2* modified DNA−RNA duplexes adopt similar A-DNA like conformation having trans amide linkages.[4c,d,5] Although less likely, we cannot without structural data rule out the possibility that the 2′-oxygen in our models causes the alternative cis amide conformation in either *AM1* or *AM2* leading to different thermal stabilities of the modified RNA−RNA duplexes. We can also not rule out the possibility that the observed effects are either sequence specific or caused by the alternating nature of the phosphodiester and amide linkages. Analysis of high salt concentration experiments on **Mod 13** may also be complicated by a possible duplex−triplex equilibrium. It has been shown that short oligo(rU)-oligo(rA) form duplexes at low salt concentration, whereas increasing the salt concentration stabilizes triple helical structures.[52] In our melting experiments with **Model 13** we always observed single thermal transitions under all salt concentrations (0.01 to 5 M). However, duplex to single strands and triplex to duplex transitions in short oligonucleotides could overlap.[52] Therefore, we cannot rule out the possibility that increasing salt concentration causes *AM1* and/or *AM2* modified **Model 13** to adopt different higher order structures leading to different behavior in the high salt experiments. Whatever the cause, the higher thermal stability of *AM2* modified sequences in all models is nevertheless unambiguous.

Conclusions

Although there are many different sides to developing antisense and antigene compounds, favorable hybridization and stability toward nucleases are of major importance. The amide modifications studied herein are well tolerated in RNA−RNA duplexes and are potential candidates for application in oligonucleotide therapeutics: either as 3′- and 5′-end modifications in a “gapmer” antisense approach, or as uniformly modified oligonucleotides in alternative procedures (for recent example,

(50) (a) Egli, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1894−1909. (b) For a recent review on DNA hydration, see Berman, H. M. *Curr. Opin. Struct. Biol.* **1994**, *4*, 345−350.
(51) (a) Portmann, S.; Usman, N.; Egli, M. *Biochemistry* **1995**, *34*, 7569−7575. (b) Egli, M.; Portmann, S.; Usman, N. *Biochemistry* **1996**, *35*, 8489−8494.
(52) Porschke, D. *Biopolymers* **1971**, *10*, 1989−2013.

see ref 13). The excellent hybridization properties of *AM2* modified oligoribonucleotides (Δt_m = +1.0 to +2.4) make this analogue particularly interesting for further studies. This is to our knowledge the largest positive effect observed with nonionic oligonucleotide dephospho analogues studied so far. For comparison with other studies it should be noted that these Δt_m values are relative to an oligoribonucleotide reference as opposed to an oligodeoxyribonucleotide, which if used as reference would result in a higher Δt_m for the amide and OH or OMe modification as a whole.

Although some explanations can be suggested, the difference between *AM1* and *AM2* modified duplexes is still puzzling and further structural studies are of great interest. We therefore believe that this should be carefully investigated in future structural studies that must include model systems with uniformly modified amide linked RNA. Efforts toward this goal are well underway and our current synthetic efforts focus on synthesis of oligoribonucleotides having all phosphodiesters replaced with amides.[53]

Experimental Section

General Methods. Pyridine, acetonitrile, and toluene (pa) were dried over 3 Å molecular sieves. Methylenechloride was dried over 4 Å molecular sieves. Triethylamine and DMSO were dried by refluxing with CaH_2 overnight followed by distillation. Pivaloyl chloride and PCl_3 were distilled. All other reagents and solvents were used as purchased. Triethylammonium bicarbonate buffer (pH ca. 7.5) was prepared by passing CO_2 (g) through a mixture of triethylamine and water until saturation. TLC was performed on Merck silica gel 60 F_{254} precoated plates using solvents A ($CHCl_3$/methanol, 9:1, v/v), B ($CHCl_3$/methanol, 4:1, v/v), C (toluene/ethyl acetate, 1:4, v/v), D ($CHCl_3$/methanol, 19:1, v/v), E (toluene/ethyl acetate, 4:1, v/v). Silica gel (35−70 μm) from Amicon Europe was used for column chromatography and the columns were run in the flash mode. Chloroform was passed through basic Al_2O_3 prior to use. NMR spectra were recorded on a JEOL GSX-270 spectrometer at 25 °C. Signals were assigned by ^{1}H-^{1}H and ^{13}C-^{1}H COSY experiments.

1-[3-*C*-Allyl-2,5-O-bis(*tert*-butyldimethylsilyl)-*β*-D-pentofuranosyl]-uracyl (2a). 1-[2,5-O-bis(*tert*-butyldimethylsilyl)-3-O-phenylthiocarbonyl-*β*-d-pentofuranosyl]-uracyl **1a** (6.10 g, 10 mmol, prepared from 2′,5′-O-bis(*tert*-butyldimethylsilyl)uridine[26] following the published procedures[23a]) was dissolved in dry toluene, tributylallyltin (12.5 mL, 40 mmol) was added and the solution was degassed by passing through dry nitrogen gas (ca. 30 min). AIBN (0.82 g, 5 mmol) was added, and the mixture was heated at 80 °C for 2 h. Another portion of AIBN (0.41 g, 2.5 mmol) was added and the mixture heated at 80 °C for 4 h. The mixture was cooled to 20 °C and purified by silica gel column chromatography (0−30% of ethyl acetate in toluene). Yield: 3.76 g, 75%, R_f = 0.36 (Solvent D), 0.38 (Solvent E), ^{1}H NMR ($CDCl_3$) δ: 9.85 (s, 1H, NH), 8.26 (d, J = 8.0 Hz, 1H, H6), 5.86−5.67 (m, 1H, CH_2=C*H*), 5.66 (s, 1H, H1′), 5.60 (dd, J_{NH-H5} = 1.8 Hz, 1H, H5), 5.12−5.02 (m, 2H, C*H*$_2$=CH), 4.26 (d, $J_{H2'-H3'}$ = 3.6 Hz, 1H, H2′), 4.06 (m, $J_{H3'-H4'}$ = 9.9 Hz, 1H, H4′), 4.17 and 3.74 (ABX, $J_{H5'-H5''}$ = 12.1 Hz, $J_{H4'-H5'}$ = 1.5 and < 1 Hz, 2H, H5′), 2.34 and 2.02 (2m, 2H, CH_2=CH−C*H*$_2$), 2.14 (m, 1H, H3′), 0.93 (s, 18H, CH_3), 0.28, 0.13, 0.12, 0.11 (4s, 12H, $SiCH_3$). ^{13}C NMR ($CDCl_3$) δ: 164.26 (C4), 150.61 (C2), 140.79 (C6), 135.80 (CH_2=*C*H), 116.72 (*C*H$_2$=CH), 101.10 (C5), 91.42 (C1′), 85.29 (C4′), 77.98 (C2′), 63.49 (C5′), 40.07 (C3′), 28.63 (CH_2=CH-*C*H$_2$), 26.02 (CH_3), 18.54, 18.27 (quaternary C in *t*-Bu), −4.00, −5.34, −5.42 ($SiCH_3$).

1-[3-C−Allyl-5-O-(*tert*-butyldimethylsilyl)-2-O-methyl-*β*-d-pentofuranosyl]-uracyl (2b) was synthesized from 1-[5-O-(*tert*-butyldimethylsilyl)-2-O-methyl-3-O-phenylthiocarbonyl-*β*-d-pentofuranosyl]-uracyl **1b** (prepared from 5′-O-(*tert*-butyldimethylsilyl)-2′-O-methyl-uridine[26] following the published procedures[23a]) as described above for **2a**. Yield: 70%, R_f= 0.52 (Solvent D), ^{1}H NMR ($CDCl_3$) δ: 8.47 (s, 1H, NH), 8.24 (d, J = 8.0 Hz, 1H, H6), 5.86 (s, 1H, H1′), 5.78−5.65 (m, 1H, CH_2=C*H*), 5.63 (dd, J_{NH-H5} = 2.2 Hz, 1H, H5), 5.13−5.02 (m, 2H, C*H*$_2$=CH), 4.15 (m, $J_{H5'-H5''}$ = 12.1 Hz, 1H, H5′), 4.00 (m, 1H, H4′), 3.76−3.70 (m, 2H, H5′, H2′), 3.57 (s, 3H, OCH_3), 2.40−2.20 (m, 2H, CH_2=CH−C*H*$_2$, H3′), 2.01 (m, 1H, CH_2=CH−C*H*$_2$), 0.93 (s, 9H, CH_3), 0.11 (s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3$) δ: 164.22 (C4), 150.54 (C2), 140.69 (C6), 135.81 (CH_2=*C*H), 117.00 (*C*H$_2$=CH), 101.41 (C5), 88.48, 86.22, 85.54 (C1′, C4′, C2′), 61.23 (C5′), 58.37 (OCH_3), 39.19 (C3′), 28.61 (CH_2=CH-*C*H$_2$), 26.05 (CH_3), 18.60 (quaternary C in *t*-Bu), −5.25, −5.39 ($SiCH_3$).

1-[2,5-O-bis(*tert*-Butyldimethylsilyl)-3-C-carboxymethyl-*β*-d-pentofuranosyl]-uracyl (3a) Compound **2a** (3.48 g, 7 mmol) and 4-methylmorpholine *N*-oxide (1.04 g, 7.7 mmol) were dissolved in dioxane (35 mL). A solution of OsO_4 (3.5 mL, 250 mg in 25 mL water, 0.14 mmol) was added, the reaction mixture was protected from light and stirred for 3 h (TLC, Solvent D). The mixture was diluted with $CHCl_3$ (200 mL) and extracted with saturated $NaHCO_3$ (aqueous) (2 × 200 mL). The organic layer was separated, dried over Na_2SO_4, evaporated and redissolved in dioxane (35 mL). A solution of $NaIO_4$ (1.65 g in 5 mL, 7.7 mmol) was added dropwise and the mixture was stirred for 3−4 h (TLC Solvent D). The mixture was diluted with $CHCl_3$ (200 mL) and extracted with saturated $NaHCO_3$ (aqueous) (2 × 200 mL). The organic layer was separated, dried over Na_2SO_4, evaporated and the residue was purified by silica gel column chromatography (0−40% of ethyl acetate in toluene). Yield: 2.87 g, 82%, R_f= 0.24 (Solvent D), 0.28 (Solvent E), ^{1}H NMR ($CDCl_3$) δ: 9.79 (s, 1H, COH), 9.68 (s, 1H, NH), 8.15 (d, J = 8.4 Hz, 1H, H6), 5.73 (s, 1H, H1′), 5.66 (dd, J_{NH-H5} = 1.6 Hz, 1H, H5), 4.44 (d, $J_{H2'-H3'}$ = 4.4 Hz, 1H, H2′), 4.04 (m, $J_{H3'-H4'}$ = 9.9 Hz, 1H, H4′), 4.13 and 3.69 (ABX, $J_{H5'-H5''}$ = 11.9 Hz, $J_{H4'-H5'}$ = < 1 Hz, 2H, H5′), 2.82 and 2.42 (ABX, J = 18.3, 9.5 and 4.2 Hz, 2H, C*H*$_2$COH), 2.61 (m, 1H, H3′), 0.92, 0.90 (2s, 18H, CH_3), 0.24, 0.11, 0.10, 0.05 (4s, 12H, $SiCH_3$). ^{13}C NMR ($CDCl_3$) δ: 199.66 (COH), 163.94 (C4), 150.56 (C2), 140.39 (C6), 101.51 (C5), 91.42 (C1′), 84.72 (C4′), 77.66 (C2′), 61.41 (C5′), 39.40 (*C*H$_2$COH), 35.05 (C3′), 25.99 (CH_3), 18.51, 18.19 (quaternary C in *t*-Bu), −4.26, −5.39, −5.45 ($SiCH_3$).

This material (2.25 g, 4.5 mmol) was dissolved in a mixture of DMSO (10 mL) and *tert*-butyl alcohol (20 mL) and a solution of $NaH_2PO_4 \times H_2O$ (0.21 g in 2 mL of water, 1.35 mmol) were added. A solution of $NaClO_2$ (0.72 g in 6 mL of water, 6.3 mmol) was added during 2 h under stirring and cooling (ice bath) and the mixture was further stirred for 4 h at 20 °C (TLC Solvent D). The mixture was diluted with $CHCl_3$ (200 mL) and extracted with saturated NaCl (3 × 200 mL, containing 0.5 mL of acetic acid). The organic layer was separated, dried over Na_2SO_4, evaporated and the residue was purified by silica gel column chromatography (0−60% of ethyl acetate in toluene containing 0.1% of acetic acid). Yield: 2.87 g, 82%, R_f = 0.20 (Solvent D). ^{1}H NMR ($CDCl_3$) δ: 10.14 (s, 1H, NH), 8.23 (d, J = 8.4 Hz, 1H, H6), 5.71 (s, 1H, H1′), 5.68 (d, 1H, H5), 4.44 (d, $J_{H2'-H3'}$ = 4.4 Hz, 1H, H2′), 4.04 (m, $J_{H3'-H4'}$ = 9.9 Hz, 1H, H4′), 4.14 and 3.72 (ABX, $J_{H5'-H5''}$ = 11.2 Hz, $J_{H4'-H5'}$ = < 1 Hz, 2H, H5′), 2.68 and 2.28 (ABX, J = 16.5 and <1 Hz, 2H, C*H*$_2$COOH), 2.51 (m, 1H, H3′), 0.92, 0.90 (2s, 18H, CH_3), 0.23, 0.11, 0.10, 0.08 (4s, 12H, $SiCH_3$). ^{13}C NMR ($CDCl_3$) δ: 176.81 (COOH), 164.76 (C4), 150.62 (C2), 140.98 (C6), 101.38 (C5), 91.63 (C1′), 84.63 (C4′), 77.82 (C2′), 61.31 (C5′), 37.01 (C3′), 29.23 (*C*H$_2$COOH), 25.98 (CH_3), 18.55, 18.20 (quaternary C in *t*-Bu), −4.28, −5.42, −5.53 ($SiCH_3$).

1-[5-O-(*tert*-Butyldimethylsilyl)-3-C-carboxymethyl-2-O-methyl-*β*-d-pentofuranosyl]-uracyl (3b) was prepared from **2b** using the same procedures as for **3a**. Intermediate aldehyde: yield: 70%, R_f = 0.28 (Solvent D), 0.45 (Solvent A), ^{1}H NMR ($CDCl_3$) δ: 9.86 (s, 1H, NH), 9.78 (s, 1H, COH), 8.12 (d, J = 8.1 Hz, 1H, H6), 5.91 (s, 1H, H1′),

(53) Rozners, E.; Liu, Y. *Org. Lett.* **2003**, *5*, 181−184.

5.65 (d, 1H, H5), 4.11 and 3.67 (ABX, $J_{H5'-H5''}$ = 12.1 Hz, $J_{H4'-H5'}$ = < 1 Hz, 2H, H5′), 3.98 (m, $J_{H3'-H4'}$ = 10.8 Hz, 1H, H4′), 3.93 (d, $J_{H2'-H3'}$ = 5.1 Hz, 1H, H2′), 3.50 (s, 3H, OCH_3), 2.81 and 2.39 (ABX, *J* = 17.8, 9.7 and 3.8 Hz, 2H, C*H*$_2$COH), 2.65 (m, 1H, H3′), 0.90 (s, 9H, CH_3), 0.09 (s, 6H, $SiCH_3$).

Carboxylic acid **3b**: yield 86%, R_f = 0.15 (Solvent D), 0.66 (Solvent B). ^{1}H NMR ($CDCl_3/CD_3OD$, 19:1) δ: 8.16 (d, *J* = 8.1 Hz, 1H, H6), 5.85 (s, 1H, H1′), 5.60 (d, 1H, H5), 4.10 and 3.66 (2m, 2H, H5′), 3.92 (m, 2H, H2′, H4′), 3.51 (s, 3H, OCH_3), 2.57 (m, 2H, C*H*$_2$COOH, H3′), 2.20 (m, 1H, C*H*$_2$COOH), 0.88 (s, 9H, CH_3), 0.08, 0.07 (2s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3/CD_3OD$, 19:1) δ: 174.14 (COOH), 164.26 (C4), 150.32 (C2), 140.58 (C6), 101.32 (C5), 88.58 (C1′), 85.89 (C4′), 84.75 (C2′), 60.96 (C5′), 58.34 (OCH_3), 35.97 (C3′), 28.63 (*C*H$_2$COOH), 25.91 (CH_3), 18.49 (quaternary C in *t*-Bu), −5.50 ($SiCH_3$).

Amines 5 were prepared from 5′-azido-5′-deoxyuridine[28] by reduction with triphenylphosphine[29b] (**5a**, 81%) or via acylation with acetyl chloride in CH_2Cl_2/pyridine, 9:1 and radical reduction with tributylstannane[29a] (**5b**, 61%, two steps).

5b ^{1}H NMR ($CDCl_3/CD_3OD$, 4:1) δ: 7.49 (d, *J* = 8.1 Hz, 1H, H6), 5.76 (d, $J_{H1'-H2'}$ = 5.1 Hz, 1H, H1′), 5.65 (d, 1H, H5), 5.34 (t, $J_{H2'-H3'}$ = 5.8 Hz, 1H, H2′), 5.22 (t, $J_{H3'-H4'}$ = 5.9 Hz, 1H, H3′), 3.98 (m, 1H, H4′), 2.94 and 2.84 (ABX, $J_{H5'-H5''}$ = 14.2 Hz, $J_{H4'-H5'}$ = 3.5 and 5.3 Hz, 2H, H5′), 2.01, 2.00 (2s, 6H, Ac). ^{13}C NMR ($CDCl_3/CD_3OD$, 4:1) δ: 170.11, 169.97 (C=O), 163.97 (C4), 150.43 (C2), 141.22 (C6), 102.93 (C5), 88.87 (C1′), 82.55 (C4′), 73.01, 70.28 (C2′, C3′), 42.23 (C5′) 20.34, 20.26 (CH_3 in Ac).

General Procedure for Synthesis of Amines 8. Ni_2B catalyst[38] was prepared as follows: $NiCl_2$-$6H_2O$ (238 mg, 1 mmol) was dissolved in anhydrous ethanol (10 mL), $NaBH_4$ (113 mg, 3 mmol) was added and the mixture was stirred at 0 °C for 30 min. Compounds **6a**−**d** (1 mmol, prepared as described in ref 32) were dissolved in cold (0 °C) anhydrous ethanol (15 mL), $NaBH_4$ (151 mg, 4 mmol) was added and the mixture was stirred at 0 °C for 2 h. A solution of freshly prepared Ni_2B catalyst (1.5 equiv. for **6a,b** or 1 equiv. for **6c,d**) was then added. After 45 min another portion of $NaBH_4$ (375 mg, 10 mmol) was added to the reaction mixture. After stirring at 20 °C for 8 h the mixture was diluted with $CHCl_3$ (100 mL) and extracted first with 10% citric acid (aqueous) (50 mL) and then with saturated $NaHCO_3$ (aqueous) (50 mL). Organic layer was separated, dried over Na_2SO_4, evaporated and the residue was purified by silica gel column chromatography (0−8% of methanol in $CHCl_3$).

8a yield 40%, R_f = 0.35 (Solvent A), ^{1}H NMR ($CDCl_3/CD_3OD$, 19:1) δ: 8.19 (d, *J* = 8.2 Hz, 1H, H6), 7.46−7.27, 6.88 (m, 14H, MMT), 5.72 (s, 1H, H1′), 5.29 (d, 1H, H5), 4.43 (d, *J* = 4.2 Hz, 1H, H2′), 4.14 (m, 1H, H4′), 3.82 (s, 3H, OCH_3), 3.72 and 3.35 (ABX, *J* = 11.5, 2.0, 2.9 Hz, 2H, H5′), 2.89 and 2.50 (ABX, *J* = 12.8, 8.6, 5.0 Hz, 2H, CH_2N), 2.37 (m, 1H, H3′), 0.92 (s, 9H, CH_3), 0.27, 0.18 (2s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3/CD_3OD$, 19:1) δ: 164.09 (C4), 150.60 (C2), 158.98, 143.98, 143.82, 134.77, 130.71, 128.63, 128.20, 127.44, 113.47 (MMT), 140.58 (C6), 101.47 (C5), 91.61 (C1′), 82.64 (C4′), 77.37 (C2′), 62.15 (C5′), 55.42 (OCH_3), 44.77 (C3′), 37.26 (CH_2N), 25.91 (CH_3), 18.24 (quaternary C in *t*-Bu), −4.01, −5.36 ($SiCH_3$). HRMS calcd for $C_{36}H_{45}O_6N_3Si$+Na 666.2975, found 666.2989.

8b yield 47% (dr 94:6), R_f = 0.10 (Solvent A), ^{1}H NMR ($CDCl_3/CD_3OD$, 19:1) δ: 8.19 (d, *J* = 8.1 Hz, 1H, H6), 7.44−7.28, 6.86 (m, 14H, MMT), 5.92 (s, 1H, H1′), 5.25 (d, 1H, H5), 4.08 (m, 1H, H4′), 3.93 (d, *J* = 4.2 Hz, 1H, H2′), 3.80 (s, 3H, OCH_3 in MMT), 3.68 and 3.30 (ABX, *J* = 11.3, 1.5, 2.5 Hz, 2H, H5′), 3.59 (s, 3H, OCH_3), 2.91 (m, 1H, CH_2N), 2.55 (m, 2H, CH_2N, H3′). ^{13}C NMR ($CDCl_3/CD_3OD$, 19:1) δ: 164.22 (C4), 150.51 (C2), 158.90, 143.85, 143.68, 134.67, 130.60, 128.52, 128.14, 127.41, 113.41 (MMT), 140.42 (C6), 101.70 (C5), 88.29, 85.78, 82.60 (C1′, C4′, C2′), 61.50 (C5′), 58.10, 55.35 (OCH_3), 43.37 (C3′), 36.84 (CH_2N). HRMS calcd for $C_{31}H_{33}O_6N_3$+Na 566.2267, found 566.2288.

8c yield 55% (dr 85:15), R_f = 0.25 (Solvent A), 1H NMR ($CDCl_3/CD_3OD$, 19:1) δ: 8.17 (d, *J* = 8.1 Hz, 1H, H6), 5.68 (s, 1H, H1′), 5.61 (d, 1H, H5), 4.39 (d, *J* = 4.0 Hz, 1H, H2′), 4.13 (m, 2H, H4′, H5′), 3.74 (m, 1H, H5′), 2.73 and 2.97 (ABX, *J* = 12.2, 8.4, 5.1 Hz, 2H, CH_2N), 2.23 (m, 1H, H3′), 0.92, 0.91 (2s, 18H, CH_3), 0.25−0.11 (4s, 12H, $SiCH_3$). ^{13}C NMR ($CDCl_3/CD_3OD$, 19:1) δ: 164.03 (C4), 150.60 (C2), 140.62 (C6), 101.28 (C5), 91.30 (C1′), 83.85 (C4′), 77.17 (C2′), 62.33 (C5′), 43.74 (C3′), 37.61 (CH_2N), 26.07, 25.88 (CH_3), 18.58, 18.39 (quaternary C in *t*-Bu), −4.15, −4.58, −5.39 ($SiCH_3$). HRMS calcd for $C_{22}H_{43}O_5N_3Si_2$+Na 508.2639, found 508.2653.

8d yield 53% (dr 85:15), R_f = 0.12 (Solvent A), 1H NMR ($CDCl_3/CD_3OD$, 19:1) δ: 8.19 (d, *J* = 8.1 Hz, 1H, H6), 5.90 (s, 1H, H1′), 5.61 (d, 1H, H5), 4.16−4.06 (m, 2H, H4′, H5′), 3.90 (d, *J* = 4.8 Hz, 1H, H2′), 3.73 (m, 1H, H5′), 3.60 (s, 3H, OCH_3), 2.97 and 2.76 (2m, 2H, CH_2N), 2.35 (m, 1H, H3′), 0.93 (s, 9H, CH_3), 0.18 (s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3/CD_3OD$, 19:1) δ: 164.30 (C4), 150.54 (C2), 140.47 (C6), 101.43 (C5), 88.13, 86.05, 83.71 (C1′, C4′, C2′), 61.61 (C5′), 58.13 (OCH_3), 42.21 (C3′), 37.00 (CH_2N), 25.99 (CH_3), 18.55 (quaternary C in *t*-Bu), −5.41, −5.48 ($SiCH_3$). HRMS calcd for $C_{17}H_{31}O_5N_3Si$+Na 408.1931, found 408.1928.

Compound **6c** (205 mg, 0.4 mmol) was dissolved in anhydrous THF (5 mL). The mixture was cooled on an acetone-dry ice bath (−78 °C) $(Bu)_4NBH_4$ (616 mg, 2.4 mmol) was added and the mixture was stirred at −78 °C for 5 h. The mixture was treated with $NaBH_4/Ni_2B$ (1 equiv.), stirred at 20 °C overnight, worked up and purified as described above to give **8c**, yield 101 mg (49%), dr 98:2.

2′,3′-O-Benzylidene-5′-C-(nitromethyl)uridine (10). 2′,3′-O−(Benzylidene)uridine[54] **9** (1.99 g, 6 mmol) was dried by evaporation of added dry toluene (50 mL) and dry acetonitrile (50 mL) and then dissolved in dry DMSO (15 mL). DCC (3.71 g, 18 mmol) and dichloroacetic acid (0.24 mL) were added and the mixture was stirred for 18 h at 20 °C. Nitromethane (30 mL), methanol (12 mL) and $NaOCH_3$ (9 mL, 30% in methanol) were mixed, stirred for 10 min at 20 °C and added to the reaction mixture. After stirring for 2 h at 20 °C the mixture was neutralized and the excess DCC was hydrolyzed by a careful addition (cooling on ice) of oxalic acid dihydrate (7.9 g, 63 mmol) in methanol (25 mL). The mixture was stirred for 30 min at 0 °C, filtered, the precipitate was washed with cold methanol (10 mL), and the filtrate was evaporated. The residue was dissolved in $CHCl_3$ (200 mL) and extracted with saturated $NaHCO_3$/saturated NaCl (aqueous) (1:1, 150 mL). Organic layer was separated, dried over Na_2SO_4, evaporated and the residue was purified by silica gel column chromatography (0−5% of methanol in $CHCl_3$, two purifications were necessary to remove nonnucleosidic contamination completely). Yield 1.21 g, 51% R_f = 0.24 (Solvent A), ^{1}H NMR (major diastereomer, $CDCl_3$) δ: 7.52−7.36 (m, 5H, Ar), 7.27 (d, *J* = 8.1 Hz, 1H, H6), 6.03 (s, 1H, benzylidene), 5.73 (d, 1H, H5), 5.59 (d, *J* = 1.8 Hz, 1H, H1′), 5.29 (m, 1H, H3′), 5.14 (m, 1H, H2′), 4.62−4.41 (m, 3H, $CHCH_2NO_2$), 4.08 (m, 1H, H4′). ^{13}C NMR (major diastereomer, $CDCl_3$) δ: 163.98 (C4), 150.98 (C2), 135.63, 130.17, 128.65, 126.82, 104.28 (benzylidene), 143.87 (C6), 103.28 (C5), 96.80 (C1′), 85.72 (C4′), 83.61 (C2′), 81.85 (C3′), 78.45 (CH_2NO_2), 68.61 (C5′).

2′,3′,5′-O-Triacetyl-5′-C-(nitromethyl)uridine (11). Compound **10** (0.99 g, 2.5 mmol) was dissolved in cold (0 °C) acetic anhydride (15 mL), $HClO_4$ (0.24 mL) was added and the mixture was stirred for 1 h at 0 °C. The mixture was diluted with $CHCl_3$ (100 mL), saturated $NaHCO_3$ (aqueous) (50 mL) and saturated NaCl (aqueous) (50 mL) were added, and the mixture was stirred for 30 min at 20 °C. Organic layer was extracted with saturated $NaHCO_3$/saturated NaCl (aqueous) (1:1, 100 mL), separated, dried over Na_2SO_4, evaporated, coevaporated with toluene (2 × 100 mL), and the residue was purified by silica gel column chromatography (0−6% of methanol in $CHCl_3$). Yield 0.97 g, 90% R_f = 0.42 (Solvent A), ^{1}H NMR (major diastereomer, $CDCl_3$) δ: 9.90 (s, 1H, NH), 7.21 (d, *J* = 8.1 Hz, 1H, H6), 5.85−5.75 (m, 3H, H1′, H5, H5′), 5.60−5.52 (m, 2H, H2′, H3′), 4.89−4.62 (m, 2H, CH_2-

(54) Prepared using the procedure described for 2′,3′-*O*-(*p*-anisylidene)uridine: Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1976**, *84*, 430−440.

NO_2), 4.27 (t, 1H, H4′), 2.12, 2.09 (2s 9H, acetyl). ^{13}C NMR (major diastereomer, $CDCl_3$) δ: 170.02, 169.86, 169.67 (C=O), 163.51 (C4), 150.32 (C2), 142.28 (C6), 103.46 (C5), 92.42 (C1′), 80.52 (C4′), 73.99 (CH_2NO_2), 72.45, 70.30 (C3′, C2′), 69.03 (C5′), 20.76, 20.57 (CH_3).

2′,3′-O-Diacetyl-5′-deoxy-5′-C-(nitromethyl)uridine (12). Compound **11** (1.08 g, 2.5 mmol) was dried by evaporation of added dry acetonitrile (30 mL) and then dissolved in cold (0 °C) absolute ethanol/THF (1:1, 20 mL). $NaBH_4$ (0.19 g 5 mmol) was added in small portions over 10 min and the mixture was stirred for 45 min at 0 °C. The reaction mixture was neutralized with acetic acid, diluted with $CHCl_3$ (100 mL), and extracted with saturated $NaHCO_3$/saturated NaCl (aqueous) (1:1, 100 mL). The organic layer was separated, dried over Na_2SO_4, evaporated, and the residue was purified by silica gel column chromatography (0−5% of methanol in $CHCl_3$). Yield 0.82 g, 88%, R_f = 0.42 (Solvent A), ^{1}H NMR ($CDCl_3$) δ: 7.20 (d, J = 8.1 Hz, 1H, H6), 5.74 (d, 1H, H5), 5.59 (d, $J_{H1'-H2'}$ = 4.0 Hz, 1H, H1′), 5.47 (dd, $J_{H2'-H3'}$ = 6.3 Hz, 1H, H2′), 5.24 (t, 1H, H3′), 4.53 (t, 2H, CH_2NO_2), 4.12 (m, 1H, H4′), 2.55−2.32 (m, 2H, H5′), 2.08, 2.07 (2s 6H, acetyl). ^{13}C NMR ($CDCl_3$) δ: 170.07, 169.96 (C=O), 163.57 (C4), 150.24 (C2), 141.77 (C6), 103.27 (C5), 91.48 (C1′), 78.44 (C4′), 72.97 (C2′), 72.48 (C3′), 71.54 (CH_2NO_2), 29.96 (C5′), 20.52 (CH_3).

Carboxylic acid 13. Compound **12** (0.80 g, 2.15 mmol) was dried by evaporation of added dry acetonitrile (30 mL) and then dissolved in dry DMSO (5 mL). $NaNO_2$ (0.69 g, 10 mmol) and acetic acid (1.8 mL, 33 mmol) were added and the mixture was stirred at 40 °C for 30 h. Water (5 mL) was added, pH was adjusted to ca. 4.5 with 1 M HCl, and the mixture was partitioned between CH_2Cl_2 (100 mL) and saturated NaCl (aqueous) (50 mL). The water layer was extracted with CH_2Cl_2 (4 × 50 mL). Combined organic layers were separated, dried over Na_2SO_4, evaporated and the residue was purified by silica gel column chromatography (0−10% of methanol in $CHCl_3$). Yield 0.51 g, 66% R_f = 0.20 (Solvent B), ^{1}H NMR ($CDCl_3$) δ: 7.44 (d, J = 8.1 Hz, 1H, H6), 5.95 (d, $J_{H1'-H2'}$ = 5.9 Hz, 1H, H1′), 5.74 (d, 1H, H5), 5.45−5.31 (m, 2H, H2′, H3′), 4.31 (m, 1H, H4′), 2.80 (d, J = 5.1 Hz, 2H, H5′), 2.07, 2.05 (2s, 6H, CH_3). ^{13}C NMR ($CDCl_3$) δ: 172.95, 170.06, 169.87 (C=O), 163.71 (C4), 150.47 (C2), 140.98 (C6), 103.47 (C5), 87.53 (C1′), 79.01, 72.77, 72.23 (C4′, C3′, C2′), 36.59 (C5′), 20.72, 20.59 (CH_3).

General Procedure for Synthesis of Amide Linked Dinucleosides. Carboxylic acid (1 mmol) and 1-hydroxybenzotriazole (0.135 g 1 mmol) were dried by evaporation of added dry acetonitrile (2 × 30 mL) and were then dissolved in dry CH_2Cl_2 (10 mL). DCC (0.213 g, 1 mmol) was added, and the mixture was stirred at 20 °C for 30 min. The mixture was filtered, and the required amine (1 mmol) and triethylamine (0.14 mL, 1.5 mmol) were added. The mixture was stirred at 20 °C for 4 h (TLC, Solvents B and C), diluted with CH_2Cl_2 (40 mL) and extracted with saturated $NaHCO_3$ (aqueous) (2 × 50 mL). The organic layer was separated, dried over Na_2SO_4, evaporated, and purified by silica gel column chromatography using the solvent systems specified below.

14a 50−100% of ethyl acetate in toluene, yield 0.56 g, 68%, R_f = 0.22 (Solvent D), 0.29 (Solvent C), ^{1}H NMR ($CDCl_3$)[55] δ: 9.97, 9.74 (2s, 2H, NH), 8.17 (d, J = 8.1 Hz, 2H, H6), 7.33 (d, J = 8.1 Hz, 2H, H6*), 5.81 (m, 2H, H5*, H1′), 5.68 (m, 2H, H5, H1′*), 5.59 (t, 1H, H2′*), 5.36 (t, 1H, H3′*), 4.51 (m, 1H, H2′), 4.25 (m, 1H, H4′*), 4.06 (m, 2H, H4′, H5′), 3.79 (m, 2H, H5″, H5′*), 3.52 (m, 1H, H5″*), 2.68 (m, 2H, H3′, CH_2CO), 2.26 (m, 1H, CH_2CO), 2.15, 2.13 (2s, 6H, CH_3CO), 0.97, 0.92 (2s, 18H, *t*-Bu), 0.20, 0.15, 0.09 (3s, 12H, $SiCH_3$). ^{13}C NMR ($CDCl_3$)[55] δ: 171.59, 170.00, 169.84 (C=O), 163.92, 163.49 (C4), 150.70, 150.35 (C2), 142.30 (C6*), 140.71 (C6), 103.06 (C5*), 101.68 (C5), 91.82 (C1′*), 90.36 (C1′), 84.60 (C4′), 80.66 (C4′*), 77.47 (C2′), 72.79 (C2′*), 70.82 (C3′*), 62.74 (C5′), 40.42 (C5′*), 37.98 (C3′), 31.55 (CH$_2$CO), 26.01, 25.85 (CH_3), 20.53 (CH$_3$CO), 18.50, 18.09 (quaternary C in *t*-Bu), −4.55, −5.36, −5.42, −5.50 ($SiCH_3$). HRMS calcd for $C_{36}H_{57}O_{13}N_5Si_2$ 823.3491, found 823.3508.

(55) * indicates resonances from protons and carbons in 5′-yl unit of the dimer.

14b a modified procedure was used: amine **5a** was added in dry DMF (10 mL), after stirring for 4 h, the solvent was evaporated (no aqueous workup) and the residue was purified by silica gel column chromatography (2−10% of methanol in $CHCl_3$), yield 0.35 g, 47%, R_f = 0.45 (Solvent B), ^{1}H NMR ($CDCl_3$/CD_3OD, 4:1)[55] δ: 8.09 (d, J = 8.6 Hz, 2H, H6), 7.36 (d, J = 8.0 Hz, 2H, H6*), 5.76 (s, 1H, H1′), 5.61 (d, 1H, H5*), 5.52 (d, 1H, H5), 5.46 (d, $J_{H1'-H2'}$ = 4.0 Hz, H1′*), 4.20 (m, 1H, H2′*), 4.03−3.87 (m, 4H, H3′*, H4′, H4′*, H5′), 3.77 (d, $J_{H2'-H3'}$ = 5.2 Hz 1H, H2′), 3.62−3.53 (m, 2H, H5″, H5′*), 3.40 (s, 3H, OCH_3), 3.34−3.24 (m, 1H, H5″*), 2.56 (m, 1H, H3′), 2.37, 2.08 (2m, 2H, CH_2CO), 0.82 (s, 9H, *t*-Bu), 0.01 (s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3$/CD_3OD, 4:1)[55] δ: 172.03 (C=O), 164.48, 164.24 (C4), 150.77, 150.48 (C2), 142.19 (C6*), 140.55 (C6), 102.29 (C5*), 101.18 (C5), 93.12 (C1′*), 88.12 (C1′), 85.67 (C2′), 84.78, 82.49 (C4′, C4′*), 73.18 (C2′*), 70.59 (C3′*), 61.09 (C5′), 57.88 (OCH_3), 40.75 (C5′*), 36.32 (C3′), 30.60 (CH$_2$CO), 25.74 (CH_3), 18.32 (quaternary C in *t*-Bu), −5.72 ($SiCH_3$).

18a 50−100% of ethyl acetate in toluene, yield 0.85 g, 86%, R_f = 0.21 (Solvent D), 0.40 (Solvent C), 1H NMR ($CDCl_3$)[55] δ: 7.99 (d, J = 8.2 Hz, 1H, H6), 7.39−7.14 (m, 13H, MMT, H6*), 6.83 (m, 2H, MMT), 5.72 (d, $J_{H1'-H2'}$ = 2.0 Hz, 1H, H1′), 5.66 (d, $J_{H1'-H2'}$ = 4.6 Hz, 1H, H1′*), 5.59−5.53 (m, 2H, H2′*, H5*), 5.31 (t, 1H, H3′*), 5.18 (d, 1H, H5), 4.38 (m, 2H, H4′*, H2′), 4.18 (m, 1H, H4′), 3.77 (s, 3H, OCH_3), 3.63−3.36 (m, 3H, H5′, CH_2NH), 3.15 (m, 1H, CH_2NH), 2.59−2.44 (m, 3H, H5′*, H3′), 2.06 (2s, 6H, CH_3CO), 0.86 (s, 9H, *t*-Bu), 0.15, 0.07 (2s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3$)[55] δ: 170.09, 169.95, 169.84 (C=O), 163.79, 163.44 (C4), 150.63 (C2), 142.44 (C6*), 140.50 (C6), 158.98, 143.85, 143.66, 134.58, 130.66, 128.58, 128.20, 127.50, 113.47 (MMT), 103.07 (C5*), 101.88 (C5), 91.50 (C1′*), 91.01 (C1′), 87.53 (MMT), 82.34 (C4′), 79.26, 77.45 (C2′, C4′*), 72.93 (C3′*), 72.80 (C2′*), 62.96 (C5′), 55.37 (OCH_3), 41.86 (C3′), 38.91 (C5′*), 36.10 (CH_2NH), 25.83 (CH_3), 20.56 (CH$_3$CO), 18.10 (quaternary C in *t*-Bu), −4.41, −5.38 ($SiCH_3$). HRMS calcd for $C_{50}H_{59}O_{14}N_5Si$+Na 1004.3726, found 1004.3714.

18b 0−10% of methanol in $CHCl_3$, yield 0.77 g, 87%, R_f = 0.18 (Solvent D), 0.45 (Solvent C), ^{1}H NMR ($CDCl_3$)[55] δ: 8.07 (d, J = 8.1 Hz, 1H, H6), 7.41−7.18 (m, 13H, MMT, H6*), 6.83 (m, 2H, MMT), 5.83 (s, 1H, H1′), 5.65−5.55 (m, 3H, H1′*, H2′*, H5*), 5.32 (t, 1H, H3′*), 5.19 (d, 1H, H5), 4.45 (m, 1H, H4′*), 4.10 (m, 1H H4′), 3.86 (m, 1H, H2′), 3.76 (s, 3H, OCH_3), 3.60−3.20 (m, 4H, H5′, CH_2NH), 3.50 (s, 3H, 2′-OCH_3), 2.71−2.53 (m, 3H, H5′*, H3′), 2.06, 2.05 (2s, 6H, CH_3CO). ^{13}C NMR ($CDCl_3$)[55] δ: 170.04, 169.50, 169.40 (C=O), 164.03, 163.49 (C4), 150.86, 150.51 (C2), 142.39 (C6*), 140.47 (C6), 158.90, 143.95, 143.68, 134.62, 130.65, 128.55, 128.20, 127.41, 113.47 (MMT), 103.21 (C5*), 101.70 (C5), 91.34 (C1′*), 88.56 (C1′), 87.43 (MMT), 85.95 (C2′), 82.52 (C4′), 79.63 (C4′*), 72.83 (C3′*, C2′*), 61.82 (C5′), 58.24 (2′-OCH_3), 55.38 (OCH_3), 40.56 (C3′), 39.00 (C5′*), 34.98 (CH_2NH), 20.63, 20.54 (CH$_3$CO). HRMS calcd for $C_{45}H_{47}O_{14}N_5$+Na 904.3017, found 904.3018.

Selective Cleavage of 5′-O-(*tert*-Butyldimethylsilyl) Protection. Dimers **14a** and **14b** were dissolved in 80% acetic acid (aqueous) (20 mL/mmol) and the solutions were heated at 50 °C for 3 h. The solvent was evaporated and the residue was dried by coevaporating with absolute ethanol (2 × 20 mL). Dimer **14a′** was purified by silica gel column chromatography (0−15% of methanol in $CHCl_3$), yield 59%, R_f= 0.42 (Solvent A). Later fractions gave fully desilylated dimer 34%, R_f = 0.17 (Solvent A). Crude **15b** was used in subsequent steps.

Cleavage of Acetyl Protections. Dimers **14a′**, **18a**, and **18b** were dissolved in 32% NH_3/ethanol, 2:1 (20 mL/mmol) and kept at 20 °C for 6 h. The solvent was evaporated. Crude **15a** was dissolved in water (20 mL/mmol), freeze-dried and used in subsequent steps. **18a** and **18b** were dried by evaporation of added absolute ethanol (2 × 20 mL) and purified by silica gel column chromatography: **19a** 0−12% of methanol in $CHCl_3$, yield 85%, R_f = 0.38 (Solvent A); **19b** 5−15% of methanol in $CHCl_3$, yield 69%, R_f = 0.66 (Solvent B).

5′-O-Monometoxytritylation. Dimers **15a** and **15b** were reacted with 4-monomethoxytrityl chloride (1.1 equiv) according to the standard procedure,[56] and the products were purified by silica gel column chromatography (0−10% of methanol in $CHCl_3$): **16a**, yield 74% (43%, three steps from **14a**), $R_f = 0.29$ (Solvent A); **19b** yield 60%, two steps from **14b**, $R_f = 0.63$ (Solvent B).

Synthesis of H-Phosphonates 17a,b and 20a,b was done as previously reported.[20]

17a yield 69%, $R_f = 0.24$ (Solvent B), ^1H NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 8.11 (d, J = 8.1 Hz, 1H, H6), 7.91 (d, J = 7.7 Hz, 1H, *o*-ClBz), 7.43−7.23 (m, 16H, Ar, H6*), 6.85 (m, 2H, MMT), 6.82 (d, J = 640 Hz, 1H, PH), 5.89 (bs, 1H, H1′*), 5.73 (s, 1H, H1′), 5.69 (d, J = 8.1 Hz, 1H, H5*), 5.57 (m, 1H, H2′*), 5.21 (d, 1H, H5), 4.91 (p, 1H, H3′*), 4.49 (m, 1H, H2′), 4.23 (m, 1H, H4′*), 4.08 (m, 1H, H4′), 3.78 (s, 3H, OCH_3), 3.63−3.55 (m, 3H, H5′*, H5′), 3.34 (m, 1H, H5″), 2.97 (q, J = 7.3 Hz, 6H, NCH_2), 2.73 (m, 1H, H3′), 2.49 and 2.07 (2m, 2H, CH_2CO), 1.24 (t, 9H, CH_3), 0.86 (s, 9H, *t*-Bu), 0.19, 0.06 (2s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 171.33 (C=O in amide), 164.21, 164.13, 163.64 (C4, C4*, C=O in *o*-ClBz), 150.50, 150.42 (C2, C2*), 158.73, 143.87, 143.68, (MMT) 141.57, 140.76 (C6, C6*), 134.64, 133.99, 133.34, 132.08, 131.10, 130.57, 128.49, 128.00 127.25, 126.84, 113.30 (MMT, *o*-ClBz), 102.99, 101.32 (C5, C5*), 91.23 (C1′), 89.69 (C1′*), 87.29 (MMT), 83.32 (C4′), 81.89 (C4′*), 77.39 (C2′), 74.55 (C2′*), 71.26 (C3′*), 62.01 (C5′), 55.24 (OCH_3), 45.77 (NCH_2), 40.67 (C5′*), 38.32 (C3′), 30.57 (CH_2CO), 25.80 (*t*-Bu), 18.00 (quaternary C in *t*-Bu), 8.67 (CH_3), −4.53, −5.53 ($SiCH_3$). HRMS calcd for $C_{53}H_{59}O_{15}N_5ClSiP$ 1099.3203, found 1099.3279.

17b yield 66%, $R_f = 0.25$ (Solvent B), ^1H NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 8.14 (d, J = 8.4 Hz, 1H, H6), 7.92 (d, J = 7.7 Hz, 1H, *o*-ClBz), 7.51−7.22 (m, 16H, Ar, H6*), 6.86 (m, 2H, MMT), 6.84 (d, J = 636 Hz, 1H, PH), 6.00 (d, $J_{H1'-H2'}$ = 5.1 Hz, 1H, H1′*), 5.87 (s, 1H, H1′), 5.74 (d, J = 8.4 Hz, 1H, H5*), 5.52 (t, 1H, H2′*), 5.27 (d, 1H, H5), 4.88 (p, 1H, H3′*), 4.27 (m, 1H, H4′*), 4.04 (m, 1H, H4′), 3.91 (m 1H, H2′), 3.80 (s, 3H, OCH_3), 3.80−3.29 (m, 4H, H5′*, H5′), 3.52 (s, 3H, 2′-OCH_3), 2.71 (q, J = 7.4 Hz, 7H, NCH_2, H3′), 2.43 and 2.04 (2m, 2H, CH_2CO), 1.11 (t, 9H, CH_3). ^{13}C NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 171.68 (C=O in amide), 164.24, 163.62 (C4, C4*, C=O in *o*-ClBz), 150.65, 150.30 (C2, C2*), 158.76, 143.87, 143.68, (MMT) 141.19, 140.46 (C6, C6*), 134.71, 134.06, 133.35, 132.11, 131.14, 130.57, 128.63, 128.49, 128.06 127.25, 126.84, 113.33 (MMT, *o*-ClBz), 103.17, 101.44 (C5, C5*), 89.03, 88.63 (C1′, C1′*), 87.28 (MMT), 85.76 (C2′), 83.82 (C4′), 82.25 (C4′*), 74.57 (C2′*), 71.31 (C3′*), 61.33 (C5′), 58.06 (2′-OCH_3), 55.28 (OCH_3), 45.85 (NCH_2), 40.39 (C5′*), 37.69 (C3′), 30.80 (CH_2CO), 9.99 (CH_3).

20a yield 56%, $R_f = 0.26$ (Solvent B), ^1H NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 8.07 (d, J = 8.1 Hz, 1H, H6), 7.93 (d, J = 7.7 Hz, 1H, *o*-ClBz), 7.42−7.21 (m, 16H, Ar, H6*), 6.85 (m, 2H, MMT), 6.79 (d, J = 641 Hz, 1H, PH), 5.89 (bs, 1H, H1′), 5.73 (s, 1H, H1′*), 5.61 (m, 1H, H2′*), 5.55 (d, J = 8.1 Hz, 1H, H5*), 5.16 (d, 1H, H5), 4.90 (p, 1H, H3′*), 4.45 (m, 2H, H2′, H4′*), 4.18 (m, 1H, H4′), 3.78 (s, 3H, OCH_3), 3.63−3.37 (m, 3H, H5′, CH_2NH), 3.15 (m, 1H, CH_2NH), 2.97 (q, J = 7.3 Hz, 6H, NCH_2), 2.74−2.58 (m, 3H, H3′, H5′*), 1.19 (t, 9H, CH_3), 0.86 (s, 9H, *t*-Bu), 0.17, 0.09 (2s, 6H, $SiCH_3$). ^{13}C NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 170.61 (C=O in amide), 164.32, 164.08, 163.81 (C4, C4*, C=O in *o*-ClBz), 150.62, 150.48 (C2, C2*), 158.85, 143.95, 143.60, (MMT) 142.30, 140.66 (C6, C6*), 134.59, 134.05, 133.38, 132.22, 131.14, 130.60, 128.49, 128.09, 127.36, 126.90, 113.38 (MMT, *o*-ClBz), 102.81, 101.57 (C5, C5*), 90.91 (C1′), 90.30 (C1′*), 87.40 (MMT), 82.25 (C4′), 80.17 (C4′*), 77.04 (C2′), 74.83 (C2′*), 72.67 (C3′*), 62.61 (C5′), 55.27 (OCH_3), 45.72 (NCH_2), 41.54 (C3′), 38.59 (C5′*), 35.76 (CH_2NH), 25.75 (*t*-Bu), 18.01 (quaternary C in *t*-Bu), 8.51 (CH_3), −4.52, −5.47 ($SiCH_3$). HRMS calcd for $C_{53}H_{59}O_{15}N_5ClPSi$+2Na 1144.2920, found 1144.2915.

20b yield 75%, $R_f = 0.27$ (Solvent B), ^1H NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 8.13 (d, J = 8.1 Hz, 1H, H6), 7.95 (d, J = 7.7 Hz, 1H, *o*-ClBz), 7.65 (d, J = 8.1 Hz, 1H, H6*), 7.47−7.21 (m, 15H, Ar), 6.87 (m, 2H, MMT), 6.86 (d, J = 635 Hz, 1H, PH), 6.04 (bd, 1H, H1′*), 5.88 (s, 1H, H1′), 5.57 (d, 2H, H5*, H2′*), 5.21 (d, 1H, H5), 4.90 (p, 1H, H3′*), 4.58 (m, 1H, H4′*), 4.12 (m, 1H, H4′), 3.92 (m, 1H, H2′), 3.79 (s, 3H, OCH_3), 3.62−3.37 (m, 4H, CH_2NH, H5′), 3.56 (s, 3H, 2′-OCH_3), 2.90 (q, J = 7.4 Hz, 6H, NCH_2), 2.86−2.70 (m, 3H, H3′, H5′*), 1.19 (t, 9H, CH_3). ^{13}C NMR ($CDCl_3/CD_3OD$, 9:1)[55] δ: 170.07 (C=O in amide), 164.14, 163.78 (C4, C4*, C=O in *o*-ClBz), 150.64, 150.46 (C2, C2*), 158.82, 144.03, 143.71, (MMT) 141.39, 140.47 (C6, C6*), 134.67, 134.11, 133.30, 132.27, 131.14, 130.63, 128.79, 128.52, 128.12 127.28, 126.90, 113.41 (MMT, *o*-ClBz), 103.00, 101.51 (C5, C5*), 88.78, 88.51 (C1′, C1′*), 87.29 (MMT), 85.49 (C2′), 82.52 (C4′), 80.90 (C4′*), 74.83 (C2′*), 72.51 (C3′*), 61.72 (C5′), 58.13 (2′-OCH_3), 55.32 (OCH_3), 45.74 (NCH_2), 40.46 (C3′), 38.59 (C5′*), 34.76 (CH_2-NH), 8.92 (CH_3). HRMS calcd for $C_{48}H_{47}O_{15}N_5ClP$+Na 1022.2395, found 1022.2433.

Oligonucleotides were synthesized, purified and analyzed as previously reported.[20] MALDI-TOF MS and enzymatic degradation followed by RP HPLC analysis data are included in Supporting Information (Table 4). Dimers **17a,b** and **20a,b** were used under standard coupling conditions. Oligonucleotides bearing 2′-*O*-TBDMS protections were deprotected as follows: after removal of the acyl protections and cleavage of the oligomer from polymeric support (32% NH_3/EtOH 3:1, for 8 h at 20 °C) the ammonia solution was lyophilized, the residue was dissolved in neat triethylamine trihydrofluoride[57] (0.3 mL, Aldrich) and kept overnight at 20 °C. Water (1 mL) was added, the aqueous phase was extracted with ethyl acetate (4 × 1 mL), and lyophilized. Further purification and analysis were done as reported.[20]

Thermal Melting and Hybridization Thermodynamics. Absorbance vs temperature profiles were measured at 260 nm on a Varian Cary 3 spectrophotometer in buffers 10 mM sodium phosphate (pH 7.2), 0.1 mM EDTA, 2 μM of each oligonucleotide (analogue and complementary RNA) and various concentrations of added sodium salts (chloride, acetate, perchlorate). Extinction coefficients were calculated from the nearest-neighbor approximation.[58] The temperature was increased at a rate of 0.2 °C per minute (control runs at a rate of 0.1 °C per minute gave essentially the same results) and data points were collected every 0.1 °C. A thermostatable multicell (2 × 6) block was used to simultaneously monitor up to five samples, the sixth cell was used for internal temperature control. At temperatures below 15 °C the sample compartment was flushed with dry nitrogen gas. The melting curves for all models uniformly showed single thermal transitions with a well-defined lower and upper baseline over all experimental conditions (for **Model 13** 0.01 to 5 M Na^+) allowing us to fit the data to a two-state model. The melting temperatures and thermodynamic parameters (Tables 1 and 2) were obtained using Varian Cary software, Version 2.5. The experimental absorbance vs temperature curves were converted into fractions of strands remaining hybridized (α) vs temperature curve by fitting the melting profile to a two-state transition model, with linearly sloping lower and upper baselines. The t_m's were obtained directly from the temperature at $\alpha = 0.5$. The thermodynamic parameters were determined from van't Hoff plot (ln K vs $1/T$) with ($-\Delta H/R$) as the slope and ($\Delta S/R$) as the intercept. Values of K (equilibrium constant) were determined at each temperature using equation $K = \alpha/(Ct/n)^{n-1}\alpha^n)$ where Ct is the total strand concentration and n is the molecularity of the reaction. Reported values are the average of at least three experiments.

Synthesis and Conformational Analysis of Monomeric Models 23a,b and 24a,b. Carboxylic acids **3a,b** were coupled with ethylamine

(56) Connolly, B. A. In *Oligonucleotides and Analogues: A Practical Aproach*; Eckstein, F., Ed.; IRL Press: Oxford, 1991; pp 161−162.

(57) (a) Gasparutto, D.; Livache, T.; Bazin, H.; Duplaa, A.-M.; Guy, A.; Khorin, A.; Molko, D.; Roget, A.; Teoule, R. *Nucleic Acids Res.* **1992**, *20*, 5159−5166. (b) Westman, E.; Strömberg, R. *Nucleic Acids Res.* **1994**, *22*, 2430−2431.

(58) Puglisi, J. D.; Tinoco, I., Jr. *Methods in Enzymology* **1989**, *180*, 304−324.

using the HOBt/DCC procedure outlined above. Amines **8c,d** were reacted with propionic anhydride (for full procedures, see Supporting Information). The TBDMS groups were removed in 80% acetic acid (aqueous) at 50 °C for 24 h. Preparation of compounds **25a,b** has been previously reported.[20] Purification of the products (RP HPLC) and NMR experiments were done as previously reported.[20] For experimental coupling constants, see Tables 5−8 in the Supporting Information. The equilibrium between North and South conformers was estimated using a straightforward approximation- South (%)= $({}^3J_{H1'-H2'} /({}^3J_{H1'-H2'} + {}^3J_{H3'-H4'})) \times 100$.[59] To ensure that the results obtained in D_2O are representative for water buffers, control NMR experiments were also done in buffers used in UV melting studies containing only 10% D_2O (both at 1.0 and 0.1 M NaCl and at 40, 60 and, 80 °C). No significant differences were observed in these experiments (typically deviations within ±0.3 Hz).

23a ^{1}H NMR (D_2O, 270 MHz, 40 °C) δ: 7.96 (d, J = 8.1 Hz, 1H, H6), 5.83 (d, 1H, H5), 5.77 (d, 1H, H1′), 4.38 (m, 1H, H2′), 4.13−4.06 (m, 1H, H4′), 3.92 and 3.72 (ABX, $J_{H5'-H5''}$ = 13.0 Hz, 2H, H5′), 3.16 (m, 2H, NCH_2), 2.51−2.29 (m, 3H, CH_2CO, H3′), 1.07 (t, J = 7.5 Hz, 3H, CH_3).

23b ^{1}H NMR (D_2O, 270 MHz, 40 °C) δ: 7.99 (d, J = 8.2 Hz, 1H, H6), 5.90 (d, 1H, H1′), 5.84 (d, 1H, H5), 4.08−4.02 (m, 1H, H4′), 3.96 (m, 1H, H2′), 3.90 and 3.72 (ABX, $J_{H5'-H5''}$ = 13.3 Hz, 2H, H5′), 3.49 (s, 3H, OCH_3), 3.17 (m, 2H, NCH_2), 2.56−2.28 (m, 3H, CH_2CO, H3′), 1.08 (t, J = 7.3 Hz, 3H, CH_3).

24a ^{1}H NMR (D_2O, 270 MHz, 40 °C) δ: 7.94 (d, J = 8.1 Hz, 1H, H6), 5.82 (d, 1H, H5), 5.77 (d, 1H, H1′), 4.39 (m, 1H, H2′), 4.18−4.11 (m, 1H, H4′), 3.96 and 3.73 (ABX, $J_{H5'-H5''}$ = 13.1 Hz, 2H, H5′), 3.43 and 3.31 (ABX, J = 8.1, 6.0 and 14.0 Hz, 2H, NCH_2), 2.41−2.30 (m, 1H, H3′), 2.23 (q, J = 7.6 Hz, 2H, CH_2CO), 1.06 (t, 3H, CH_3).

24b ^{1}H NMR (D_2O, 270 MHz, 40 °C) δ: 7.97 (d, J = 8.0 Hz, 1H, H6), 5.90 (d, 1H, H1′), 5.83 (d, 1H, H5), 4.09 (m, 1H, H4′), 4.03 (m, 1H, H2′), 3.96 and 3.73 (ABX, $J_{H5'-H5''}$ = 13.0 Hz, 2H, H5′), 3.51 (s, 3H, OCH_3), 3.44 and 3.27 (ABX, J = 8.2, 5.9 and 13.9 Hz, 2H, NCH_2), 2.49−2.38 (m, 1H, H3′), 2.22 (q, J = 7.7 Hz, 2H, CH_2CO), 1.08 (t, 3H, CH_3).

(59) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973** *95*, 2333−2344.

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Supporting Information Available: Experimental procedures and spectral data (^{1}H and ^{13}C NMR) not included in Experimental Section, Tables with experimentally obtained spin−spin coupling constants for model compounds **23a,b** and **24a,b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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