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6'-Carbon-Substituted Carbocyclic Analogs of 2'-Deoxyribonucleosides - Synthesis and Effect on DNA/RNA Duplex Stability

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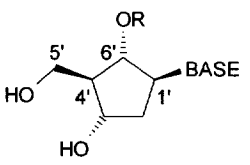
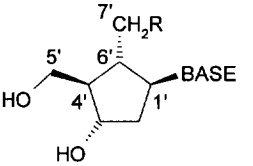
Abstract: *6'-α-Methyl and 6'-α-hydroxymethyl carbocyclic thymidines **11** and **22** have been synthesized via bicyclic lactone **2** and amines **9** and **19**, respectively, as key intermediates. Both nucleoside analogs were subsequently elaborated into 5'-O-DMTr protected 3'-phosphoramidites of 6'-α-methyl and 6'-α-hydroxymethyl carbocyclic thymidines as well as appropriately base protected 5-methyl 2'-deoxycytidines. Analysis of the RNA-binding affinity of modified oligodeoxyribonucleotides incorporating these 6'-substituted carbocyclic nucleoside analogs revealed a strongly position dependent effect on DNA/RNA duplex stability. While duplex stability is significantly reduced by point modifications at separated sequence positions, it is only marginally affected by stretches of contiguous modified building blocks.*

Introduction

The inhibition of gene expression by antisense oligonucleotides over the last few years has been widely recognized as a potentially very powerful alternative to classical drug design strategies.[1-4] By exploiting the inherent specificity of Watson-Crick duplex formation between complementary strands of nucleic acids this concept relies on the ability of a synthetic (antisense) oligonucleotide to bind to a selected target mRNA in a sequence-specific fashion and on the ensuing (specific) inhibition of protein synthesis that is often associated with such a binding event. However, in spite of its compelling conceptual simplicity antisense inhibition of protein expression in *biological systems* not only depends on an oligonucleotide's ability to bind to complementary RNA with high affinity and specificity, but on the complex interplay of a variety of different parameters.[1-4] One of the most fundamental prerequisites for biological activity to occur is a sufficient half-life of the oligonucleotide in a physiological environment, a provision that is not met by unmodified single stranded oligonucleotides (either DNA or RNA) that are based on a natural phosphodiester backbone. Thus, the successful implementation of an antisense based drug design and development strategy in a first step requires the design and synthesis of metabolically more stable, structurally modified oligonucleotides or oligonucleotide analogs, which also retain the ability to bind to complementary RNA with high affinity and in a highly sequence-specific manner. While numerous other approaches to address this problem have been described in the literature over the last five years,[5-8] we have recently reported on the synthesis of a series of 6'-O-substituted carbocyclic nucleosides **I** (*Figure 1*, R = H, alkyl) and their evaluation as potential building blocks for antisense oligonucleotides.[9,10]



I



II

Fig. 1

Oligonucleotides incorporating 6'-O-substituted carbocyclic nucleotide units generally exhibit improved nuclease resistance, but the presence of 6'-alkoxy substituents (**I**, R = alkyl) also leads to reduced RNA-binding affinity. In contrast, clusters of contiguous 6'-*hydroxy* substituted building blocks (**I**, R = H) were found to promote RNA-binding; however, at the same time these analogs were clearly least effective in protecting adjacent phosphodiester linkages from nucleolytic cleavage.

As an extension of the above study and in an attempt to develop carbocyclic nucleoside analogs that would be more suitable building blocks for antisense oligonucleotides (i. e. display a more favorable combination of effects on RNA-binding affinity and nuclease resistance) we have also investigated nucleoside analogs of type **II**, bearing carbon substituents attached to the 6'-position of the modified sugar moiety. In this paper we now wish to report on the synthesis of carbocyclic analogs of thymidine and 5-methyl 2'-deoxycytidine having either a simple methyl group or a hydroxymethyl group (as prototypes of hydrophobic and uncharged polar substituents, respectively) attached to the 6'-position as well as on the RNA-binding affinity of the corresponding oligonucleotides.

Carbocyclic analogs of 2'-deoxyribonucleosides bearing carbon substituents attached to the 6'-position have been rarely described in the literature.[11] One notable exception is 6'-α-methyl carbocyclic thymidine, whose synthesis has been reported by *Béres* and coworkers[12] employing the (commercially available) (+)-enantiomer of lactone **1**[13] as the key intermediate.

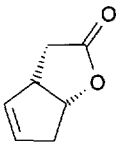
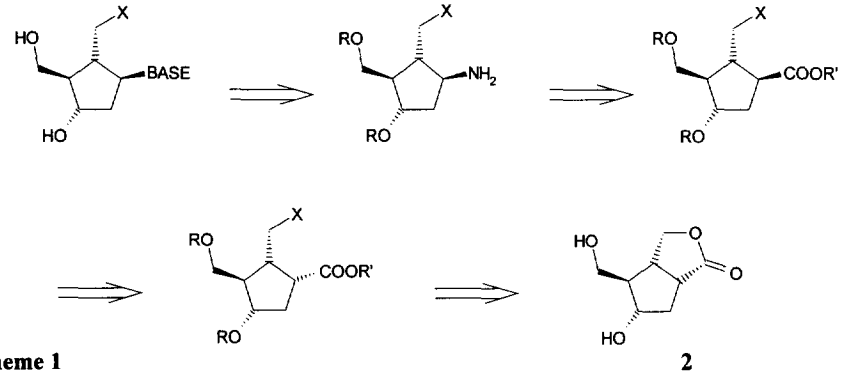


Fig. 2 **(+)-1**

However, our own retrosynthetic analysis of 6'-α-methyl as well as 6'-α-hydroxymethyl carbocyclic thymidine suggested that the bicyclic lactone (-)-**2**,[14] which we had previously used as the key intermediate in the synthesis of 1',6'-methano carbocyclic nucleoside analogs,[15] might in fact represent a more versatile precursor for the synthesis of our target compounds (*Scheme 1*, X = leaving group).

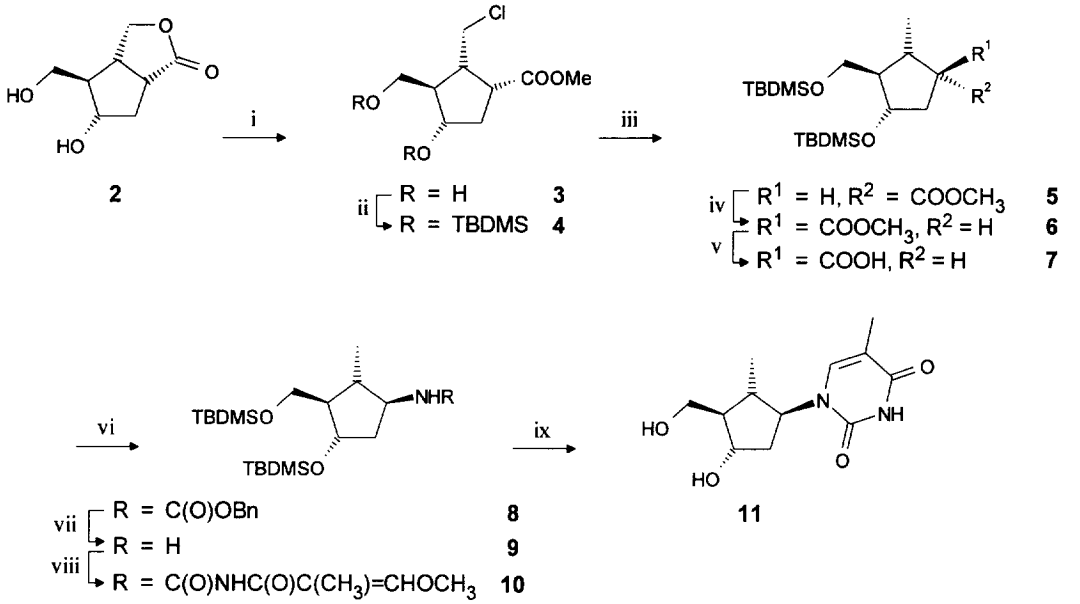


Scheme 1 **2**

Lactone (-)-**2** can be readily prepared from *cis*-tetrahydrophthalic acid anhydride even on large scale[14] and it can serve as a direct precursor for the synthesis of 6'-α-methyl as well as 6'-α-hydroxymethyl carbocyclic nucleosides without the need for degradative removal of an excess carbon atom as in *Béres'* strategy.[12] The apparent disadvantage of **2** as an intermediate in the synthesis of carbocyclic nucleoside analogs rests in the fact that entry into the natural β-series requires inversion of configuration at what is to become C-1' of the modified sugar unit, but we envisaged that this problem could be overcome by equilibration of an ester group formed upon opening of the lactone ring of **2** (with the protected carboxylic acid group serving as a masked amino group). As will be shown below, this equilibration approach did not prove to be successful in a practical sense; however, we have been able to devise alternative strategies that allowed for straightforward access to the desired carbocyclic nucleoside analogs with natural β-configuration.

Synthesis of Carbocyclic Thymidine Analogs

6'-α-Methyl carbocyclic thymidine. The synthesis of 6'-α-methyl carbocylic thymidine **11** from bicyclic lactone (-)-**2** is summarized in *Scheme 2*. The initial step in the sequence involves opening of the lactone ring with TMS-Cl in MeOH, which provided γ-chloromethyl ester **3** in 62% yield. In contrast to the corresponding γ-*bromo* ester,[15] **3** proved to be a rather stable compound and was readily converted to its bis-*tert*.-butyl-dimethylsilyl (TBDMS) ether **4** by treatment with N-TBDMS N-methyl acetamide[16] in 69% yield.



i. TMS-Cl, MeOH, $ZnCl_2$ (cat.), refl., 4h, 62%. ii. N-TBDMS N-Me acetamide, DMF, 60°, 16h, 69%. iii. Bu_3SnH, AIBN, NaI, DME, 80°, 6h, 94%. iv. a. LDA, THF, - 78°, 1h; b. H_2O, - 78° → RT, 59% (2 cycles). v. KOH, EtOH, refl., 3h, 91%. vi. a. DPPA, Et_3N, toluene, 0°, 30 min, RT, 1h; b. 80°, 1.75h; c. BnOH, 80°, 1.5h, 100°, 30 min, 92% (3 steps). vii. H_2, 10% Pd-C, toluene/MeOH 9/1, 1 atm., RT, 1h, 78%. viii. $CH_3OCH{=}C(CH_3)CONCO$, CH_2Cl_2, - 60° → RT, 30 min, 96%. ix. 0.2N HCl EtOH/H_2O 9/1, refl., 20h, 98%.

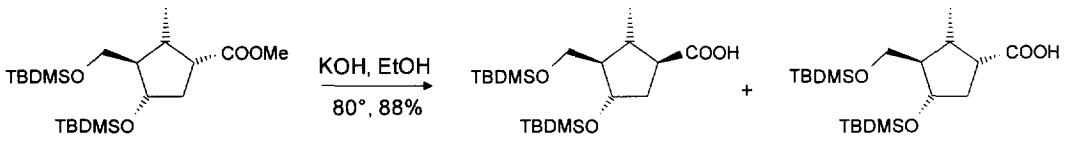
Scheme 2

The choice of this particular silylating agent was based on the results of extensive experimentation on the corresponding γ-bromo ester,[15] in which case N-TBDMS N-methyl acetamide had proven to be the most effective reagent for the introduction of the TBDMS protecting groups. Subsequent radical reduction of the chloromethyl group in **4** with Bu_3SnH/AIBN in the presence of 10 mol-% of NaI (dimethoxyethane, 80°) gave compound **5** in an excellent 94% yield, whereas no reaction occurred under identical conditions in the absence of NaI.

As indicated above, the synthesis of the desired carbocyclic analogs of natural β-nucleosides at this juncture required inversion of configuration at the ester α-carbon of **5**, which we had envisioned would be achievable by base catalyzed epimerization. However, treatment of **5** with 0.1 equiv. of potassium t-butoxide in t-BuOH for 18h at RT or with sodium methoxide in MeOH for 18h either at RT or at 50° only led to formation of a *ca.* 3:1 mixture of **5** and **6** (*in favor of* **5**), which renders the preparation of **6** *via* such a base catalyzed epimerization approach a rather low yielding and impractical process. We therefore resorted to a

kinetic deprotonation-reprotonation strategy, which involved formation of the ester enolate from **5** at - 78° (LDA, THF) and quenching of the enolate with water at the same temperature. This led to a *ca.* 3:1 mixture of **6** and **5**, respectively, from which **6** could be isolated in pure form by flash chromatography; the remaining material was resubjected to the deprotonation-reprotonation procedure, which finally provided pure **6** in 59% overall yield after two deprotonation-reprotonation cycles. Saponification of the ester group in **6** with KOH/EtOH gave acid **7** (91%) without any detectable epimerization. **7** was then converted to benzyloxycarbonyl protected amine **8** *via* Curtius rearrangement[17] and subsequent *in situ* quenching of the ensuing isocyanate with benzyl alcohol in 92% overall yield. Removal of the benzyloxycarbonyl group *via* catalytic hydrogenation provided amine **9**; reaction of **9** with β-methoxy α-methacryloyl isocyanate and cyclization of the resulting acryloyl urea under acidic conditions (2N aqu. HCl/EtOH 1/9, reflux),[18] which also led to concomitant cleavage of the TBDMS protecting groups, finally gave the desired 6'-α-methyl carbocyclic thymidine **11** in 94% overall yield from amine **9**. The relative stereochemistry in **11** was firmly established by NOE NMR experiments, with strong NOE's being observed between H-1' and H-4' as well as H-6' and H-5' and H-5" (nucleoside numbering system, *cf. Figure 1*), respectively, whereas no NOE could be detected between H-1' and H-3' or H-1' and H-5'/H-5".[19] The structure was further confirmed at a later stage by X-ray crystallography of a modified DNA/DNA duplex incorporating two 6'-α-methyl carbocyclic thymidine residue in each strand.[20] The conversion of **11** into protected analogs of thymidine and 5-methyl 2'-deoxycytidine suitable for oligonucleotide synthesis will be described below.

In the course of studies designed to assess the stereochemical integrity of the chiral center α to the ester group in **6** under the conditions of ester saponification we discovered that treatment of **5** with 3.5 equiv. of powdered KOH in EtOH at reflux temperature leads to a mixture of acids favoring the desired **7** by about 9:1 over its diastereoisomer **7a** (*Scheme 3*).

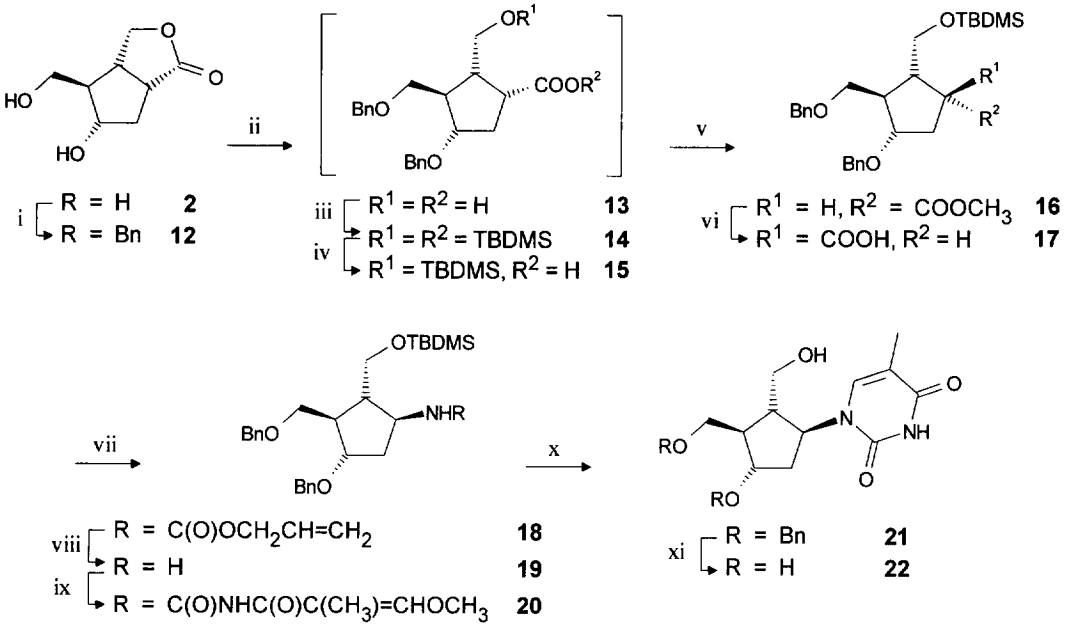


5 **7** **7a**

Scheme 3 9 : 1

This finding is reminiscent of an observation by *Meinwald* and *Gassman* on the behavior of *(-)-5,5-dimethylbicyclo[2,1,1]hexane-2β-carboxylic acid methyl ester*,[21] which would not undergo methoxide catalyzed epimerization even at elevated temperature, but upon ester cleavage with KOH/EtOH led to a mixture of carboxylic acids favoring the isomer with inverted stereochemistry α to the carboxyl group. Although chromatographic separation of **7** and **7a** proved not to be possible by flash chromatography on silica gel, the stereoselectivity of the saponification reaction can still be exploited for the synthesis of **11**, as isomer separation can be achieved at the stage of the final product. As will be shown in the following the above type of reaction also represents a key element in our synthesis of 6'-α-hydroxymethyl carbocyclic thymidine.

6'-α-Hydroxymethyl carbocyclic thymidine. As it was the objective of this work to provide protected derivatives of 6'-α-methyl and 6'-α-hydroxymethyl carbocyclic nucleosides for the purpose of oligonucleotide synthesis (and not primarily the free nucleoside analogs), the synthesis of 6'-α-hydroxymethyl derivatives had to be based on a protecting group strategy that would allow for the differentiation of the primary hydroxyl groups on C-5' and C-7' (*cf. Figure 1*). Lactone (-)-**2** in this case was therefore protected as the corresponding bis-benzyl ether **12**, which was converted to hydroxy acid **13** by treatment with LiOH in THF/H_2O and subsequent acidic work-up (*Scheme 4*).



i. a. NaH, DMF, RT, 4h; b. BnBr, TBAI (0.1 equ.), RT, 18h, 54%. ii. 1M LiOH, DME/H_2O 1/1, RT, 1.5h. iii. TBDMS-Cl, imidazole, DMF, 0° → RT, 20h. iv. NaOMe/MeOH, RT, 2.5h. v. MeI, $KHCO_3$, 0° → RT, 21h, 69% (4 steps). vi. KOH/EtOH, refl., 3.5h, 91% (~ 8/1 mixture of distereoisomers). vii. a. DPPA, Et_3N, toluene, 0°, 30 min, RT, 1h; b. 80°, 2h; c. CH_2=$CHCH_2OH$, 80°, 1.5h, 100°, 30 min, 64% (3 steps). viii. $Pd(Ph_3)_4$ (cat), morpholine, THF, RT, 2h, 93%. ix. CH_3OCH=$C(CH_3)CONCO$, CH_2Cl_2, - 60° → RT, 30 min, 93%. x. 0.2N HCl EtOH/H_2O 9/1, refl., 20h, 85%. xi. H_2, 10% Pd-C, MeOH, RT, 3h, quant..

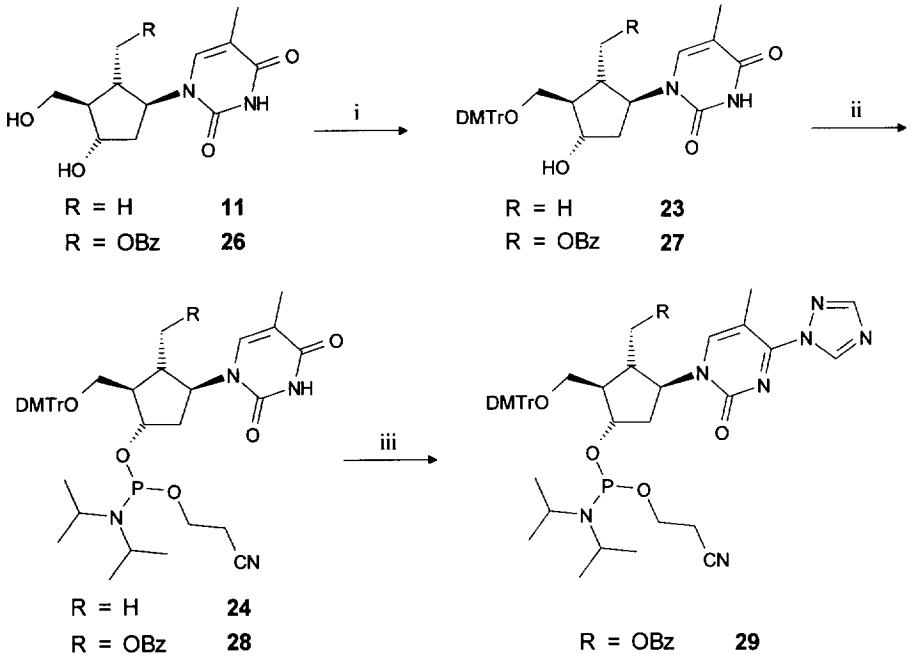
Scheme 4

Due to its pronounced tendency to re-cyclize to **2**,[22] hydroxy acid **13** after extractive work-up and without further purification was immediately treated with 3.0 equiv. of TBDMS-Cl to give fully protected cyclopentane derivative **14**.[23] Direct conversion of crude **14**[24] to γ-TBDMS-oxy acid **15** by treatment with NaOMe in MeOH (2.0 equiv.) followed by esterification with $CH_3I/KHCO_3$ in DMF finally gave methyl ester **16** in 69% overall yield from **12** without purification of intermediates **13**, **14**, and **15**. As indicated above, inversion of stereochemistry at the center α to the ester group in **16** could be achieved by treatment of this compound with KOH in EtOH at reflux temperature, which led to a ~ 8/1 mixture of carboxylic acids in favor of the desired isomer **17** (91%).[25] As this mixture of acids could not be resolved by silica gel chromatography on a preparative scale it was used as such in the subsequent *Curtius* rearrangement; *in situ* quenching of the isocyanate formed in the rearrangement step with allyl alcohol then led to a mixture of allyloxycarbonyl protected amines, from which the desired **18** could be isolated in diastereomerically and enantiomerically pure form in 64% yield (based on the mixture of starting carboxylic acids). After removal of the allyloxycarbonyl group with Pd(0) in THF in the presence of morpholine as allyl cation scavenger (93%)[26] the resulting amine **19** was converted to 3'-O, 5'-O-bis-benzyl protected 6'-α-hydroxymethyl carbocyclic thymidine **21** by treatment with β-methoxy α-methacryloyl isocyanate and subsequent acid catalyzed cyclization of the resulting acryloyl urea **20** (2N aqu. HCl/EtOH 1/9, reflux).[18, 27] On a small scale removal of the benzyl protecting groups in **21** gave free 6'-α-hydroxymethyl carbocyclic thymidine **22** in quantitative yield. The bulk of **21**, however, was used for the preparation of protected derivatives of **22** as

well as the corresponding 5-methyl 2'-deoxy cytidine analog for the purpose of oligonucleotide synthesis (*vide infra*).

Synthesis of Phosphoramidites

Thymidine Analogs. As shown in *Scheme 5*, 6'-α-methyl carbocyclic thymidine **11** could be readily converted to the corresponding 5'-O-(4,4'-dimethoxytrityl (DMTr))-3'-(2-cyanoethyl-N,N-diisopropylamino)-phosphite **24**[28] by treatment with DMTr-Cl in pyridine followed by phosphitylation with bis-(diisopropylamino)-2-cyanoethyl phosphite[29] in 61% overall yield.

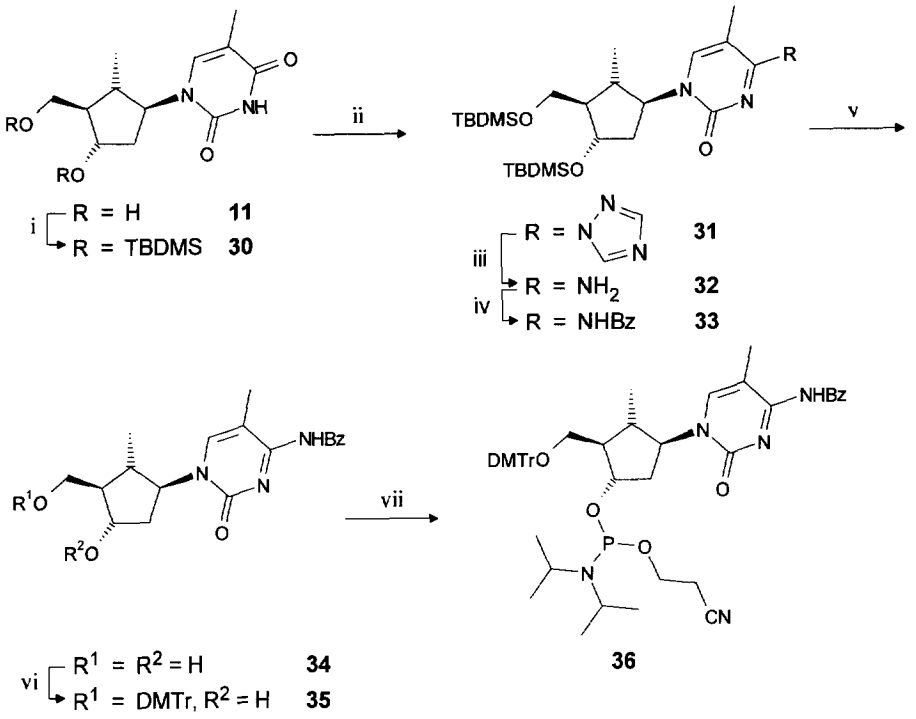


i. DMTr-Cl, Et_3N, DMAP (cat.), pyridine, RT. **23**: 81%; **27**: 86%. ii. $[(i\text{-}C_3H_7)_2N]_2POCH_2CH_2CN$, $(i\text{-}C_3H_7)_2NH_2{}^+CHN_4{}^-$, CH_2Cl_2, RT. **24**: 75%; **28**: 89%. iii. $POCl_3$, triazole, NEt_3, CH_3CN/ CH_2Cl_2 1/1, RT, 2.5h, 60%.

Scheme 5

In the case of 6'-α-hydroxymethyl thymidine incorporation into oligonucleotides also required appropriate protection of the primary hydroxyl group attached to C-7' (*cf. Figure 1*); for this reason bis-benzyl protected derivative **21** (*Scheme 4*) was initially converted to 7'-benzoyloxy derivative **26** (*Scheme 5*) by benzoylation with benzoic anhydride and subsequent removal of the benzyl protecting groups *via* catalytic hydrogenation (91%). Dimethoxytritylation and phosphitylation provided the corresponding phosphoramidite **28** in 77% yield.

5-Methyl 2'-deoxycytidine analogs. The transformation of 6'-α-methyl carbocylic thymidine **11** into the corresponding N^4-benzoyl 5-methyl 2'-deoxycytidine analog **34** and elaboration of the latter into the phosphoramidite **36** is summarized in *Scheme 6*.



i. TBDMS-Cl, imidazole, DMF, RT, 4h, 40°, 40 min, 96%. ii. 1,2,4-Triazole, Et_3N, $POCl_3$, CH_3CN, RT, 2.5h. iii. Dioxane/conc. aqu. NH_3 3/1, 40°, 16h, 77%. iv. Bz-Cl, Et_3N, DMAP (cat.), ether, RT, 1h, 91%. v. Bu_4NF, THF, 40°, 2h, 76%. vi. DMTr-Cl, Et_3N, DMAP (cat.), pyridine, RT, 3h, 90%. vii. $[(i\text{-}C_3H_7)_2N]_2POCH_2CH_2CN$, $(i\text{-}C_3H_7)_2NH_2{}^+CHN_4{}^-$, CH_2Cl_2, RT, 3h, 75%.

Scheme 6

After reprotection of **11** as its bis-TBDMS ether, transformation of this derivative into the corresponding 5-methylcytidine analog **32** was accomplished in 77% yield by a previously described two-step sequence,[30] involving preparation of the 4-triazolo derivative **31** ($POCl_3$, triazole, Et_3N) followed by displacement of the triazolo group with ammonia (conc. aqu. NH_3/dioxane 1/3, RT). Benzoylation of **32** with benzoyl chloride and subsequent removal of the TBDMS groups with tetrabutylammonium fluoride gave **34** (69%), which was then converted to the desired 5'-O-DMTr-protected phosphoramidite **36** in 68% yield by reaction with DMTr-Cl and subsequent phosphitylation.

Incorporation of 6'-α-hydroxymethyl carbocyclic 5-methyl 2'-deoxycytidine into oligonucleotides was chosen to be performed *via* a different strategy, which involved conversion of phosphoramidite **28** to 4-triazolo derivative **29** ($POCl_3$, triazole, Et_3N, 60%) (*Scheme 5*).[31] **29** can be considered as a surrogate of the corresponding 5-methyl 2'-deoxycytidine analog and upon treatment of the completed oligonucleotide with aqu. ammonia (required for release of the oligonucleotide from the solid support and concomitant removal of base and phosphate protecting groups) the 4-triazolo 5-methyl pyrimidin-2-one containing nucleotide units are converted to 5-methyl 2'-deoxycytidine residues.

Phosphoramidites **24**, **28**, **29**, and **36** were used for the synthesis of a number of different modified oligodeoxyribonucleotides employing standard solid-phase synthesis protocols[32] (with the exception of extended reaction times for those coupling steps involving modified building blocks). No special problems were encountered under those conditions for the coupling of modified phosphoramidites. For details regarding synthesis and characterization of oligonucleotides *cf.* "Experimental Part".

RNA Binding Affinity

As a first step in the evaluation of 6'-α-methyl and 6'-α-hydroxymethyl substituted carbocyclic nucleosides as potential building blocks for antisense oligonucleotides we have investigated their effects on RNA-binding affinity. In order to address this problem in a meaningful manner, several different sequences have been studied, containing between one and fourteen modified building blocks either as point modifications at separated sequence positions or as blocks of contiguous residues (*Table 1*).

Table 1: Relative RNA-binding affinities of modified oligodeoxyribonucleotides incorporating 6'-substituted carbocyclic thymidines and 5-methyl 2'-deoxycytidines (ΔT_m-values/ modification[a]).

R	TTTT**t**CTCTCTCTCT (**A**) [T_mWT = 52.7][b]	**t**CCAGG**t**G**t**CCGCA**t**C (**B**) [T_mWT = 62.3][b]	GCG**ttttttttt**GCG (**C**) [T_mWT = 48.5][b]	**ttttctctctctc**T (**D**) [T_mWT=58.2][b,c]
CH_3	- 0.1	- 1.5	- 0.2	- 0.5[d]
CH_2OH	- 0.1	- 1.0	+ 0.2	- 0.2

[a]Difference in melting temperature (ΔT_m) between the modified DNA/RNA duplex and the unmodified wild-type (WT) duplex divided by the number of modifications ($\Delta T_m = T_m - T_m$WT); **t**, **c** = modified thymidine, 5-methyl 2' -deoxycytidine. T_m's were determined in 10 mM phosphate buffer, pH 7, 100 mM Na^+; for details see ref. 33; [b]T_m of the corresponding unmodified wild-type duplex in °C. [c]WT-oligonucleotide contains 5-methyl 2'-deoxycytidine. [d]5'-**ttttctctctctct**-PO_3H_2.

As can be seen from *Table 1* incorporation of either 6'-α-methyl or 6'-α-hydroxymethyl carbocyclic thymidine into an oligodeoxyribonucleotide at separated sequence positions (sequence **B**) results in a pronounced decrease in DNA/RNA duplex stability as indicated by an overall decrease in melting temperature (T_m) of 6° and 4°, respectively. In contrast, the presence of stretches of contiguous modified building blocks as in sequences **C** and **D** leads to a much smaller effect on RNA-binding affinity and with the exception of sequence **D** in combination with 6'-α-methyl carbocyclic nucleotide units, the thermodynamic stability of the corresponding modified DNA/RNA duplexes is virtually identical with that of the unmodified wild-type duplex. 6'-Hydroxymethyl substituted building blocks appear to be generally less destabilizing than 6'-methyl analogs; this observation is reminiscent of the different effects of 6'-α-*hydroxy* and 6'-α-*methoxy* substituted carbocyclic nucleotides on RNA binding affinity (*vide supra*),[9] although the differences in the latter case are more pronounced than those shown in *Table 1*.

We have also investigated the effect of the above 6'-substituted carbocyclic building blocks on the biological activity of chimeric antisense oligonucleotides (containing modified building blocks only in the terminal regions of the sequence)[34] in cell culture (data not shown). The results of these experiments indicate that the presence of 6'-substituted nucleotide units in combination with a nuclease resistant phosphorothioate backbone does not lead to any significant change in antisense activity as compared to the (otherwise) unmodified parent 2'-deoxy phosphorothioate. In contrast, replacement of the phosphorothioate linkages between the modified building blocks by natural phosphodiester groups leads to a pronounced loss (> 10-fold) in antisense activity, which indicates that the degree of nuclease resistance conferred to an adjacent phosphodiester linkage by either type of 6'-substituted building block does not suffice for biological effects to be observable at reasonably low concentrations (< 1 μM).[35]

Conclusions

We have developed efficient strategies for the synthesis of 6'-α-methyl and 6'-α-hydroxymethyl carbocyclic thymidines **11** and **22**, respectively, that are based on lactone **2** as the central intermediate. We have also prepared appropriately protected and activated derivatives of **11** and **22** as well as the corresponding 5-methyl 2'-deoxycytidine analogs that have been successfully used for the synthesis of modified oligodeoxyribonucleotides. When present as stretches of contiguous nucleotide units both types of modified building blocks only marginally affect RNA-binding affinity, with 6'-α-hydroxymethyl substituted carbocyclic nucleotides generally leading to slightly higher DNA/RNA duplex stability than 6'-α-methyl analogs. Preliminary experiments on the biological activity of modified oligonucleotides incorporating 6'-α-methyl or hydroxymethyl substituted carbocyclic nucleotide units indicate that the degree of nuclease resistance conferred to an adjacent phosphodiester bond by these modifications may not suffice for biological activity at submicromolar concentrations.

Experimental Part

General. Solvents were generally of analytical grade (FLUKA) and used without further purification. If required, DMF, MeOH, and pyridine were dried over 3Å molecular sieves. Reactions were generally performed under dry argon. ^{1}H, ^{13}C, and ^{31}P spectra were recorded at 298K. For 250 MHz ^{1}H NMR spectra only coupling constants > 4Hz are reported. The multiplicity of ^{13}C signals was determined by DEPT experiments, with "*C*H" and "*C*q" referring to tertiary and quaternary C-atoms, respectively. The following abbreviations are used in this section: DMAP, N,N-dimethyl-4-aminopyridine; DMTr, 4,4'-dimethoxytrityl; FC, flash chromatography; RT, room temperature; TBDMS, *tert.*-butyl-dimethylsilyl.

(1R, 2S, 3R, 4S)-4-Hydroxy-3-hydroxymethyl-2-chloromethyl-cyclopentanecarboxylic acid methyl ester (**3**)
To an ice-chilled solution of *5-hydroxy-4-hydroxymethyl-hexahydro-cyclopenta[c]furan-1-one* **2** (3.0 g, 17.43 mmol) in *abs.* MeOH (60 ml) was added dropwise trimethylsilyl chloride (TMS-Cl) (22.0 ml, 174 mmol) over a period of 5 min followed by two crystals of $ZnCl_2$. The mixture was heated to reflux for 2h, after which period it was again cooled to 0° and additional TMS-Cl (5 ml, 39.5 mmol) was added. Heating at reflux temperature was then continued for another 2h, the solvent was evaporated, and the residue was twice co-evaporated with MeOH. Purification by FC in AcOEt/MeOH 9/1 gave 2.41 g of **3** (62%) as a viscous oil.
3: *^{1}H NMR* (250 MHz, $CDCl_3$). δ = 4.09 [dd, J = 14Hz, J = 6Hz, 1H, (C-4)*H*]; 3.85 - 3.55 [m, 7H, incl. s at 3.70, CH_2OH + CH_2Cl + COOCH_3]; 3.50 [s, br, 2H, O*H*]; 3.10 [dd, J = 10Hz, J = 6Hz, 1H, (C-1)*H*]; 2.40 [m, 1H]; 2.20 [m, 1H]; 2.10 - 1.90 [m, 2H]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 174.99 [*C*OOCH$_3$]; 74.56 [C-4]; 62.56 [*C*H$_2$OH]; 52.48; 51.37 [COO*C*H$_3$ + C-1]; 44.76 [*C*H]; 44.23 [*C*H$_2$Cl]; 43.43 [*C*H]; 36.34 [C-5]. *EI-MS* ($C_9H_{15}ClO_4$ 222.653): 206 (5) [M - H_2O for ^{37}Cl]; 208 (18) [M - H_2O for ^{35}Cl].

*(1S, 2S, 3R, 4S)-4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-chloromethyl-cyclopentanecarboxylic acid methyl ester* (**4**)
To a solution of **3** (3.10 g, 13.90 mmol) in DMF (30 ml) was added N-*tert.*-butyldimethylsilanyl-N-methyl-acetamide and the mixture was heated to 50° for 16h. It was then diluted with ether (200 ml) and extracted three times with 100 ml of H_2O. The organic extract was dried ($MgSO_4$), the solvent was evaporated and the residue was purified by FC in CH_2Cl_2 to give 4.35 g of **4** (69%) as a colorless liquid.
4: *^{1}H NMR* (250 MHz, $CDCl_3$). δ = 3.95 [dd, J = 17Hz, J = 7Hz, 1H, (C-4)*H*]; 3.60 [m, 7 H, incl. s at 3.60, CH_2OSi + CH_2Cl + COOCH_3]; 2.90 [dd, J = 17Hz, J = 10Hz, (C-1)*H*]; 2.40 [m, 1H]; 2.10 [m, 1H]; 1.95 - 1.80 [m, 2H]; 0.85 [s, 18H, SiC(CH_3)$_3$]; 0.00 [s, 12H, Si(CH_3)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 172.87 [*C*OOCH$_3$]; 71.36 [C-4]; 60.42 [*C*H$_2$OSi]; 51.95; 50.45 [COO*C*H$_3$ + C-1]; 44.47 [*C*H$_2$Cl]; 41.90 [*C*H]; 41.33 [*C*H]; 36.28 [C-5]; 24.66 [SiC(*C*H$_3$)$_3$]; 24.52 [SiC(*C*H$_3$)$_3$]; 17.00 [Si*C*(CH$_3$)$_3$]; 16.70 [Si*C*(CH$_3$)$_3$]. *EI-MS* ($C_{21}H_{43}ClO_4Si_2$ 451.141): 395 (45) [M - C(CH$_3$)$_3$ for ^{37}Cl]; 393 (100) [M - C(CH$_3$)$_3$ for ^{35}Cl].

*(1R, 2S, 3R, 4S)-4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentanecarboxylic acid methyl ester* (**5**)

To a solution of **4** (4.30 g, 9.53 mmol) in dimethoxyethane (95 ml) were added Bu_3SnH (5.06 ml, 19.09 mmol), NaI (0.143 g, 0.995 mmol), and AIBN (0.157 g, 0.955 mmol) and the mixture was heated to 80° for 6h. It was then poured into 200 ml of ether, and this solution was extracted three times with 50 ml of sat. aqu. $NaHCO_3$, dried ($MgSO_4$) and the solvent was evaporated. FC of the residue in CH_2Cl_2 furnished 3.72 g of **5** (94%) as a colorless liquid.

5: *^1H NMR* (250 MHz, $CDCl_3$). δ = 3.95 [dd, J = 18Hz, J = 8Hz, 1H, (C-4)*H*]; 3.65 [s, 3H, $COOCH_3$]; 3.60 [m, 2H, CH_2OSi]; 2.80 [dd, J = 17Hz, J = 8Hz, (C-1)*H*]; 2.17 [m, 1H]; 2.10 - 1.90 [m, 2H]; 1.50 [m, 1H]; 0.90 [d, J = 8Hz, 3H, (C-2)CH_3]; 0.85 [s, 18H, $SiC(CH_3)_3$]; 0.00 [s, 12H, $Si(CH_3)_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 175.12 [$COOCH_3$]; 72.46 [C-4]; 60.51 [CH_2OSi]; 56.39 [C-1]; 51.19 [$COOCH_3$]; 44.26 [C-3]; 36.31 [C-5]; 34.34 [C-2]; 25.93 [$SiC(CH_3)_3$]; 25.85 [$SiC(CH_3)_3$]; 18.29 [$SiC(CH_3)_3$]; 18.02 [$SiC(CH_3)_3$]; 15.98 [(C-2)CH_3]. *EI-MS* ($C_{21}H_{44}O_4Si_2$ 416.695): 401 (2) [M - CH_3]; 353 (100) [M - $C(CH_3)_3$].

*(1S, 2S, 3R, 4S)-4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentanecarboxylic acid methyl ester* (**6**)

A solution of diisopropylamine (0.779 ml, 5.50 mmol) in THF (50 ml) was cooled to -78°. To this was then added dropwise a 1.6M solution of n-BuLi in hexane (3.43 ml, 5.49 mmol) and the mixture was stirred at -78° for 1h. A solution of **5** (2.08 g, 4.99 mmol) in THF (5 ml) was then added dropwise over a period of 5 min and the reaction mixture was kept at -75° for 1.5h, after which period 1 ml of H_2O was added dropwise at the same temperature. After warming to RT (3h), 10 ml of sat. aqu. NH_4Cl were added and the solution was diluted with 300 ml of ether. The organic layer was separated and washed three times with 100 ml of H_2O. After drying ($MgSO_4$) and evaporation of solvent the residue was purified by FC in CH_2Cl_2 to give 0.766 g of pure **6** together with 1.071 g of a mixture of **6** and **5**. The mixture was again subjected to the deprotonation/reprotonation procedure. FC in CH_2Cl_2 yielded another 0.461 g of pure **6**. Total yield of **6**: 1.227 g (59%).

6: *^1H NMR* (250 MHz, $CDCl_3$). δ = 4.10 [dd, J = 12Hz, J = 6Hz, 1H, (C-4)*H*]; 3.65 [s, 3H, $COOCH_3$]; 3.60 [dd, J = 10Hz, J = 6Hz, 1H, CH_2OSi]; 3.50 [dd, J = 10Hz, J = 6Hz, 1H, CH_2OSi]; 2.60 [dd, J = 19Hz, J = 8Hz, (C-1)*H*]; 2.00 - 1.70 [m, 3H, (C-2)*H* + (C-5)H_2]; 1.50 [m, 1H, (C-3)H]; 1.10 [d, J = 7Hz, 3H, (C-2)CH_3]; 0.85 [s, 18H, $SiC(CH_3)_3$]; 0.00 [s, 12H, $Si(CH_3)_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 176.52 [$COOCH_3$]; 73.82 [C-4]; 61.90 [CH_2OSi]; 58.45 [C-1]; 51.58 [$COOCH_3$] ; 49.28 [C-3]; 38.49 [C-5]; 38.25 [C-2]; 25.96 [$SiC(CH_3)_3$]; 25.87 [$SiC(CH_3)_3$]; 19.30 [(C-2)CH_3]; 18.35 [$SiC(CH_3)_3$]; 18.01 [$SiC(CH_3)_3$]; - 4.59, - 4.72; - 5.42; - 5.52 [$Si(CH_3)_2$].

*(1S, 2S, 3R, 4S)-4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentanecarboxylic acid* (**7**)

To a solution of **6** (1.996 g, 4.79 mmol) in EtOH (48 ml) was added powdered KOH (0.942 g, 16.80 mmol) and the mixture was heated to reflux for 3h. The solution was then concentrated, ether (100 ml) and H_2O (150 ml) were added and the pH of the mixture was adjusted to 2.5 by addition of 2N HCl with vigorous stirring. The organic layer was separated and the aqueous solution was three times extracted with 50 ml of ether. The combined organic extracts were dried ($MgSO_4$), the solvent was evaporated, and the residue was purified by FC in CH_2Cl_2/ether 1/1 to give 1.77 g of **7** (91%) as a viscous oil.

7: *^1H NMR* (250 MHz, $CDCl_3$). δ = 11.85 [s, br, 1H, COO*H*]; 4.10 [dd, J = 12Hz, J = 6Hz, 1H, (C-4)*H*]; 3.60 [dd, J = 11Hz, J = 5Hz, 1H, CH_2OSi]; 3.50 [dd, J = 11Hz, J = 6Hz, 1H, CH_2OSi]; 2.60 [dd, J = 19Hz, J = 8Hz, (C-1)*H*]; 2.10 - 1.80 [m, 3H, (C-3)*H* + (C-5)H_2]; 1.50 [m, 1H, (C-2)*H*]; 1.10 [d, J = 7Hz, 3H, (C-2)CH_3]; 0.85 [s, 18H, $SiC(CH_3)_3$]; 0.00 [s, 12H, $Si(CH_3)_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 182.57 [COOH]; 73.71 [C-4]; 61.71 [CH_2OSi]; 58.41 [C-1]; 49.21 [C-3]; 38.38 [C-2]; 38.01 [C-5]; 25.92 [$SiC(CH_3)_3$]; 25.82 [$SiC(CH_3)_3$]; 19.29 [(C-2)CH_3]; 18.29 [$SiC(CH_3)_3$]; 17.97 [$SiC(CH_3)_3$]; *EI-MS*: ($C_{20}H_{42}O_4Si_2$ 402.670): 345 (79) [M - $C(CH_3)_3$].

*(1S, 2S, 3R, 4S)-[4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentyl]-carbamic acid benzyl ester* (**8**)

To an ice-chilled solution of **7** (1.84 g, 4.57 mmol) in toluene (46 ml) was added 95%-diphenylphosphoryl azide (DPPA) (1.14 ml, 5.03 mmol) and Et_3N (0.759 ml, 54.48 mmol) and the mixture was stirred at 0° for 30 min and for 1h at RT; it was then heated to 80° for 1.75h. After cooling to ~ 10°, dry benzyl alcohol (1.04 ml, 10.05 mmol) and catalytic amounts of dibutyltin dilaurate were added and heating to 80° was continued for 1.5h followed by heating to 100° for 30 min. The reaction mixture was then cooled to RT and diluted with 200 ml of ether. This solution was extracted once with 50 ml sat. aqu. $NaHCO_3$ and then twice with 50 ml of H_2O. The combined aqueous extracts were once back-extracted with 100 ml of ether. The combined organic extracts were dried ($MgSO_4$), the solvent was evaporated and the residue was purified by FC in CH_2Cl_2/ether 19/1 to give 2.15 g of **8** (92%) as an oil.

8 (All NMR signals broad and generally poorly resolved): *^1H NMR* (250 MHz, $CDCl_3$). δ = 7.40 - 7.20 [m, 5H, *H*-ar (Bn)]; 5.05 [s, 2H, CH_2 (Bn)]; 4.30 [d, J = 8Hz, 1H, N*H*]; 4.10 [m, br, 1H, (C-4)*H*]; 3.80 [m, br, 1H, (C-1)*H*]; 3.65 [d, J = 10Hz, 1H, CH_2OSi]; 3.50 [d, J = 10Hz, 1H, CH_2OSi]; 1.97 [m, 1H, (C-5)*H*-β]; 1.60 [m, 1H]; 1.45 ["s", br, 2 H]; 1.10 [d, J = 6Hz, 3H, (C-2)CH_3]; 0.85 [2 x s, 18H, $SiC(CH_3)_3$]; 0.00 [2 x s, 12H, $Si(CH_3)_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 155.99 [NH*C*(O)O]; 136.66 [*C*q (Bn)]; 128.47; 128.10 [*C*H-ar (Bn)]; 71.84 [C-4]; 66.25 [CH_2OBn]; 61.08 [CH_2OSi]; 56.84 [C-1]; 41.79 [C-5]; 40.89 [*C*-3, *C*-2]; 25.93 [$SiC(CH_3)_3$]; 25.83 [$SiC(CH_3)_3$]; 18.32 [$SiC(CH_3)_3$]; 17.97 [$SiC(CH_3)_3$]; 17.56 [(C-2)CH_3]. *EI-MS:* ($C_{27}H_{49}NO_4Si_2$ 507.803): 450 (92) [M - $C(CH_3)_3$].

*(1S, 2S, 3R, 4S)-4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentylamine* (**9**)

To a solution of **8** (2.13 g, 4.19 mmol) in toluene (42 ml + 3 drops of Et_3N was added 10% Pd-C and hydrogen gas was bubbled through the solution. After 45 min MeOH (4.2 ml) was added and hydrogenation was continued for additional 1.25h. The catalyst was removed by filtration through *Hyflo*, washed with AcOEt, and the filtrate was twice extracted with 100 ml of 0.2N NaOH. The combined aqueous extracts were twice back-extracted with AcOEt (50 ml). All the organic extracts were then combined and dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by FC in AcOEt/MeOH 4/1 → AcOEt/MeOH 3/2 to provide 1.23 g of **9** (78%) as a colorless oil.

9: *^1H NMR* (250 MHz, $CDCl_3$). δ = 4.10 [m, br, 1H, (C-4)*H*]; 3.60 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 3.50 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 2.95 [dd, J = 18Hz, J = 8Hz, 1H, (C-1)*H*]; 1.85 [m, 3H, NH_2 + (C-5)*H*-β]; 1.60 - 1.40 [m, 2H]; 1.30 [m, 1H]; 1.00 [d, J = 6Hz, 3H, (C-2)CH_3]; 0.85 [2 x s, 18H, $SiC(CH_3)_3$]; 0.00 [2 x s, 12H, $Si(CH_3)_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 71.11 [C-4]; 60.78 [CH_2OSi]; 56.86 [CH]; 56.79 [CH]; 43.44 [C-5]; 42.57 [C-2]; 24.97 [$SiC(CH_3)_3$]; 24.88 [$SiC(CH_3)_3$]; 17.34 [$SiC(CH_3)_3$]; 17.01 [$SiC(CH_3)_3$]; 16.09 [(C-2)CH_3]. *EI-MS:* ($C_{19}H_{43}NO_2Si_2$ 373.675): 373 (2) [M^+], 316 (19) [M - $C(CH_3)_3$].

*(1S, 2S, 3R, 4S)-[4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopenty]-3-(3-methoxy-2-methyl-acryloyl)-urea* (**10**)

To a solution of **9** (1.22 g, 3.26 mmol) in CH_2Cl_2 (20 ml) was added at - 60° distilled β-methoxymethacryloyl isocyanate[36] (0.598 g, 4.24 mmol). The reaction mixture was allowed to warm to RT over a period of ~ 30 min, diluted with ether (100 ml) and extracted twice with 50 ml of sat. aqu. $NaHCO_3$. The combined aqueous extracts were once back-extracted with 50 ml of ether. The organic extracts were then combined and dried ($MgSO_4$) and the solvent was evaporated. Purification of the residue by FC in CH_2Cl_2/ether 9/1 gave 1.62 g of **10** (96%) as a highly viscous oil.

10: *^1H NMR* (250 MHz, $CDCl_3$). δ = 9.30 [s, 1H, C(O)N*H*C(O)]; 8.70 [d, J = 8Hz, 1H, C(O)N*H*(C-1)]; 7.40 [=C*H*OCH_3]; 4.15 - 3.85 [m, 2H, (C-4)*H* + (C-1)*H*]; 3.85 [s, 3H, OCH_3]; 3.60 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 3.50 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 2.00 [ddd, 1H, J = 10Hz, J = 8Hz, J < 4Hz, (C-5)*H*-β]; 1.75 [s, 3H, =$C(CH_3)$]; 1.75 - 1.40 [m, 3H]; 1.00 [d, J = 6Hz, 3H, (C-2)CH_3]; 0.85 [2 x s, 18H, $SiC(CH_3)_3$]; 0.00 [2 x s, 12H, $Si(CH_3)_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 169.99 [*C*(O)NH]; 158.13 [=*C*HOCH_3];

154.81 [NHC(O)NH]; 71.75 [C-4]; 61.39 [OCH_3]; 61.16 [CH_2OSi]; 57.08 [CH]; 55.46 [CH]; 41.86 [C-5]; 40.94 [C-2]; 25.97 [$SiC(CH_3)_3$]; 25.85 [$SiC(CH_3)_3$]; 18.35 [$SiC(CH_3)_3$]; 18.00 [$SiC(CH_3)_3$]; 16.95 [(C-2)CH_3]. *EI-MS:* ($C_{25}H_{50}N_2O_2Si_2$ 514.794): 499 (2) [M - CH_3], 457 (100) [M - $C(CH_3)_3$].

(1S, 2S, 3R, 4S)-1-(4-Hydroxy-3-hydroxymethyl-2-methyl-cyclopentyl)-thymine (**11**)

A solution of **10** (1.60 g, 3.11 mmol) in 31 ml EtOH/3.1 ml 2N aqu. HCl was heated to reflux for 20h. The reaction mixture was then evaporated to dryness and the residue co-evaporated with EtOH (2x). Purification by FC in AcOEt/MeOH 9/1 gave 0.780 g of **11** (98%) as a white foam.

11: *^1H NMR* (500 MHz, CD_3OD). δ = 7.46 [d, J < 2Hz, 1H, (C-6)*H* thymine]; 4.70 [dd, br, J = 20Hz, J = 9Hz, 1H, (C-1)*H*]; 4.20 [m, 1H, (C-4)*H*]; 3.75 [dd, J = 10Hz, J = 5Hz, 1H, CH_2OH]; 3.67 [dd, J = 10Hz, J = 5Hz, 1H, CH_2OH]; 2.07 [ddd, 1H, J = 14Hz, J = 10Hz, J = 8Hz, (C-5)*H*-β]; 1.98 - 1.90 [m, 2H]; 1.89 [s, J < 2Hz, 3H, (C-5)CH_3 thymine]; 1.57 [td, J_t = 5Hz, J_d = 10Hz, (C-3)*H*]; 1.02 [d, J = 7Hz, 3H, (C-2)CH_3]. *^{13}C NMR* (62.5 MHz, CD_3OD. δ = 165.85 [C-4 thymine]; 152.99 [C-2 thymine]; 139.02 [C-6 thymine]; 111.45 [C-5 thymine]; 71.86 [C-4]; 61.63 [CH_2OH]; 61.53 [CH]; 56.77 [CH]; 40.16 [C-2]; 39.09 [C-5]; 16.17 [(C-2)CH_3]; 11.98 [(C-5)CH_3 thymine]. *FD-MS:* ($C_{12}H_{18}N_2O_4$ 254.268): 254 [M^+].

(4S, 5S, 7R, 8S)-5-Benzyloxy-4-benzyloxymethyl-hexahydro-cyclopenta[c]furan-1-one (**12**)

To a suspension of NaH (2.03 g, 84.5 mmol) in DMF (80 ml) was added dropwise with stirring (over a period of 15 min) a solution of *5-hydroxy-4-hydroxymethyl-hexahydro-cyclopenta[c]furan-1-one* **2** (6.61 g, 38.4 mmol) in DMF (20 ml). The mixture was stirred at RT for 4h, after which period was added benzyl bromide (10.15 ml, 85.5 mmol) followed by tetrabutylammonium iodide (0.142 g, 0.38 mmol). The reaction mixture was stirred at RT for further 18h and then poured into 300 ml ether/200 ml H_2O. The organic layer was separated and extracted two more times with 100 ml of ice-cold H_2O. Drying over $MgSO_4$ and evaporation of solvent provided an oily residue, which was purified by FC in ether. Re-chromatography of product containing fractions with ether/CH_2Cl_2 19/1 gave 7.27 g of **12** (54%) as an oil.

12: *^1H NMR* (250 MHz, $CDCl_3$). δ = 7.30 [m, 10H, *H*-ar (Bn)]; 4.55 [d, J = 12Hz, 1H, CH_2 (Bn)]; 4.30 [d, J = 12Hz, 1H, CH_2 (Bn)]; 4.45 [m, 3H, (C-3)*H* + CH_2 (Bn)]; 4.25 [dd, J = 9Hz, 1H, (C-3)*H*]; 3.90 [m, 1H, (C-5)*H*]; 3.35 [m, 2H, CH_2OBn]; 3.00 [m, 1H, (C-7)*H*]; 2.75 [m, 1H, (C-8)*H*]; 2.45 - 2.30 [m, 2H, (C-6)*H* + (C-4)*H*]; 2.10 [m, 1H, (C-6)*H*]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 179.5 [C-1]; 137.13 [Cq-ar (Bn)]; 137.07 [Cq-ar (Bn)]; 127.53; 127.44; 126.85; 126.66 [CH-ar (Bn)]; 81.1 [C-5]; 72.24 [CH_2Ph]; 72.18 [CH_2Ph]; 69.75 [OCH_2]; 69.53 [OCH_2]; 52.4 [C-7]; 41.6 [CH]; 39.7 [CH]; 32.9 [C-6]. *FD-MS* ($C_{22}H_{23}O_4$ 351.399): 352 [$(M + H)^+$].

*(1R, 2S, 3R, 4S)-4-Benzyloxy-3-benzyloxymethyl-2-(*tert.*-butyl-dimethyl-silanyloxymethyl)-cyclopentane-carboxylic acid (*tert.*-butyl-dimethyl-silanyloxymethyl) ester* (**14**)

To an ice-chilled solution of **12** (7.25 g, 20.6 mmol) in dimethoxyethane (80 ml) were added 79.4 ml of a 1M aqu. solution of LiOH and the mixture was stirred at RT for 1.5h. It was then poured into 200 ml AcOEt/40 ml H_2O, 60 ml of 10% citric acid (28.6 mmol) were added, and the mixture was vigorously stirred for 2 min. The organic layer was separated and the aqueous layer extracted twice with 100 ml of AcOEt. Drying of the combined AcOEt extracts over $MgSO_4$ and evaporation of solvent at 20° (!) gave hydroxy acid **13**, which was immediately dissolved in DMF (100 ml) at 0°; imidazole (7.07 g, 104 mmol) and TBDMS-Cl (9.4 g, 62.4 mmol) were added to this solution and the mixture was stirred at RT for 18h. It was then poured into 300 ml ether/200 ml ice-water; the organic layer was separated, twice extracted with 100 ml of ice-water, dried over $MgSO_4$ and the solvent was evaporated. FC of the residue in 0.1% Et_3N/CH_2Cl_2 gave 8.0 g of **14** (65%) as an oil (*cf.* ref. 24).

14: *^1H NMR* (250 MHz, $CDCl_3$). δ = 7.30 [m, 10H, *H*-ar (Bn)]; 4.60 [d, J = 11Hz, 1H, CH_2 (Bn)]; 4.50 [s, 2H, CH_2 (Bn)]; 4.45 [d, J = 11Hz, 1H, CH_2 (Bn)]; 3.90 [m, 1H, (C-4)*H*]; 3.70 [d, J = 7Hz, 2H, CH_2OSi]; 3.60 [dd, J = 8Hz, J = 4Hz, 1H, CH_2OBn]; 3.50 [dd, J = 8Hz, J = 4Hz, 1H, CH_2OBn]; 2.85 [m, 1H, (C-1)*H*]; 2.30 [m, 2H]; 2.15 [m, 1H]; 2.10 [m, 1H]; 0.95 [s, 9H, $SiC(CH_3)_3$]; 0.90 [s, 9H, $SiC(CH_3)_3$]; 0.30 [s, 6H, $Si(CH_3)_2$]; 0.00 [s, 6H, $Si(CH_3)_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 173.60 [COOSi]; 138.65 [Cq-ar (Bn)];

138.50 [*C*q-ar (Bn)]; 128.10; 128.06; 127.47; 127.29 [*C*H-ar (Bn)]; 80.31 [C-4]; 72.48 [O*C*H$_2$]; 71.04 [O*C*H$_2$]; 70.70 [O*C*H$_2$]; 63.03 [O*C*H$_2$]; 47.93 [*C*H]; 44.28 [*C*H]; 43.42 [*C*H]; 34.26 [C-5]; 25.73 [SiC(*C*H$_3$)$_3$]; 25.38 [SiC(*C*H$_3$)$_3$]; 18.03 [Si*C*(CH$_3$)$_3$]; 17.36 [Si*C*(CH$_3$)$_3$]. *FD-MS* ($C_{34}H_{53}O_5Si_2$ 597.927): 598 [M$^+$]; 541 [M - C(CH$_3$)$_3$].

*(1R, 2S, 3R, 4S)-4-Benzyloxy-3-benzyloxymethyl-2-(*tert.*-butyl-dimethyl-silanyloxymethyl)-cyclopentane-carboxylic acid methyl ester* (**16**)

A. To a solution of **14** (2.83 g, 4.73 mmol) in *abs.* MeOH (47 ml) was added NaOMe (0.397 g, 7.35 mmol) and the mixture was stirred at RT for 2h. The MeOH was then evaporated and the residue was redissolved in AcOEt (30 ml). A solution of 0.728 g (3.47 mmol) of citric acid in 20 ml of H_2O was added and the mixture was vigorously stirred for 2 min. The AcOEt layer was separated, washed twice with 20 ml of H_2O and dried ($MgSO_4$). Evaporation of solvent at RT gave 1.52 g of crude acid **15** as an oil.

15: *^{1}H NMR* (500 MHz, $CDCl_3$). δ = 7.30 - 7.05 [m, 10H, *H*-ar (Bn)]; 4.60 [d, J = 12Hz, 1H, C*H*$_2$ (Bn)]; 4.55 [d, J = 12Hz, 1H, C*H*$_2$ (Bn)]; 4.50 [s, 2H, C*H*$_2$ (Bn)]; 3.95 [dd, J = 12Hz, J = 5Hz, 1H, (C-4)*H*]; 3.75 [m, 2H, *CH*$_2$OSi]; 3.55 [dd, J = 9Hz, J = 4Hz, 1H, C*H*$_2$OBn]; 3.45 [dd, J = 9Hz, J = 4Hz, 1H, C*H*$_2$OBn]; 3.05 [dd, J =16Hz, J = 8Hz, 1H, (C-1)*H*]; 2.35 - 2.25 [m, 2H, (C-2)*H* + (C-5)*H*]; 2.15 [m, 2H, (C-3)*H* + (C-5)*H*]; 0.90 [s, 9H, SiC(C*H*$_3$)$_3$]; 0.05 [s, 6H, Si(C*H*$_3$)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 178.52 [*C*OOH]; 137.92 [*C*q-ar (Bn)]; 137.83 [*C*q-ar (Bn)]; 127.81; 127.20; 127.03; 127.00 [*C*H-ar (Bn)]; 79.88 [C-4]; 72.51 [O*C*H$_2$]; 70.92 [O*C*H$_2$]; 70.04 [O*C*H$_2$]; 62.45 [O*C*H$_2$]; 47.26 [*C*H]; 43.58 [*C*H]; 42.65 [*C*H]; 33.54 [C-5]; 25.33 [SiC(*C*H$_3$)$_3$]; 17.66 [Si*C*(CH$_3$)$_3$].

Solid powdered $KHCO_3$ (0.947 g, 9.46 mmol) was added to crude **15** followed by ice-chilled *abs.* DMF (20 ml) and CH_3I (0.471 ml, 7.57 mmol). The mixture was stirred at RT for 18h and then poured into 50 ml ether/30 ml ice-water. The organic layer was separated, twice extracted with 20 ml of ice-water and the solvent was evaporated. Purification of the residue by FC in 0.1% Et_3N/CH_2Cl_2 gave 1.29 g of **16** (55%) as an oil.

B. To a solution of 11.7 g of crude **14** (prepared from 5.93 g (16.8 mmol) of **12**, *vide supra*) in *abs.* MeOH (169 ml) was added NaOMe (1.83 g, 33.9 mmol) and the reaction mixture was stirred at RT for 2.5h. The MeOH was then evaporated and the residue (crude **15** as its sodium salt) was twice co-evaporated with *abs.* DMF and then redissolved in *abs.* DMF (50 ml). To this solution were added powdered $KHCO_3$ (3.38 g, 33.8 mmol) and CH_3I (2.5 ml, 27.04 mmol) (0°) and the mixture was stirred at RT for 18h. At this time additional $KHCO_3$ (3.38 g, 33.8 mmol) and CH_3I (2.5 ml, 27.04 mmol) were added and stirring was continued for 3 more h. The reaction mixture was then cooled to 0° and poured into 200 ml ether/100 ml ice-water. The organic layer was separated, twice extracted with 50 ml of ice-water and the solvent was evaporated. The yellow oily residue was purified by FC in 0.01% Et_3N/CH_2Cl_2 to give 5.69 g of **16** (69% based on **12**) as a slightly yellow oil.

16: *^{1}H NMR* (250 MHz, $CDCl_3$). δ = 7.30 [m, 10H, *H*-ar (Bn)]; 4.60 [d, J = 11Hz, 1H, C*H*$_2$ (Bn)]; 4.50 [s, 2H, C*H*$_2$ (Bn)]; 4.45 [d, J = 11Hz, 1H, C*H*$_2$ (Bn)]; 3.80 [dd, J = 17Hz, J = 7Hz, 1H, (C-4)*H*]; 3.75 - 3.65 [m, 5H incl. s at 3.65, *CH*$_2$OSiC(CH$_3$)$_3$ + COOC*H*$_3$]; 3.50 [m, 2H, C*H*$_2$OBn]; 2.85 [m, 1H, (C-1)*H*]; 2.35 [m, 1H]; 2.20 [m, 2H]; 2.05 [dd, J = 24Hz, J = 10Hz, 1H, (C-5)*H*]; 0.85 [s, 9H, SiC(C*H*$_3$)$_3$]; 0.00 [s, 6H, Si(C*H*$_3$)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 174.04 [*C*OOCH$_3$]; 138.73 [*C*q-ar (Bn)]; 138.56 [*C*q-ar (Bn)]; 128.27; 127.62; 127.47; 127.43 [*C*H-ar (Bn)]; 80.06 [C-4]; 73.00 [O*C*H$_2$]; 71.51 [O*C*H$_2$]; 70.60 [O*C*H$_2$]; 63.10 [O*C*H$_2$]; 51.39 [COO*C*H$_3$]; 47.69 [*C*H]; 43.96 [*C*H]; 42.20 [*C*H]; 34.11 [C-5]; 25.88 [SiC(*C*H$_3$)$_3$]; 18.24 [Si*C*(CH$_3$)$_3$].

*(1S, 2S, 3R, 4S)-4-Benzyloxy-3-benzyloxymethyl-2-(*tert.*-butyl-dimethyl-silanyloxymethyl)-cyclopentane-carboxylic acid* (**17**)

To a solution of **16** (5.69 g, 11.4 mmol) in *abs.* EtOH (114 ml) was added powdered KOH (2.23 g, 39.8 mmol) and the mixture was heated to reflux for 3.5h. The mixture was then concentrated and after cooling to RT was poured into 200 ml ether/100 ml ice-water; a solution of 4.19 g of citric acid in H_2O (50 ml) was then added and the mixture was vigorously stirred for 2 min. The organic layer was separated and the aqueous

layer was twice extracted with 50 ml of ether. Drying of the combined ethereal extracts ($MgSO_4$) and evaporation of solvent provided an oil, which was purified by FC in ether to give 5.04 g (91%) of a *ca.* 8/1 mixture of **17** and its C-1 epimer.

17 (spectra recorded on the mixture of **17** with its C-1 epimer; only signals for **17** are listed): *^1H NMR* (500 MHz, $CDCl_3$). δ = 7.30 [m, 10H, *H*-ar (Bn)]; 4.52 [d, J = 12Hz, 1H, C*H*$_2$ (Bn)]; 4.48 ["d", 2H, C*H*$_2$ (Bn)]; 4.44 [d, J = 12Hz, 1H, C*H*$_2$ (Bn)]; 3.99 [dd, J = 10Hz, J = 5Hz, 1H, *CH*$_2$OSi]; 3.84 [m, 1H, (C-4)*H*]; 3.59 ["t" (dd), J = 10Hz, 1H, *CH*$_2$OSi]; 3.44 [dd, J = 10Hz, J = 7Hz, 1H, C*H*$_2$OBn]; 3.36 [dd, J = 10Hz, J = 7Hz, 1H, C*H*$_2$OBn]; 2.98 [dd, J =18Hz, J = 9Hz, 1H, (C-1)*H*]; 2.23 [m, 1H, (C-5)*H*]; 2.15 [m, 2H, (C-2)*H* + (C-5)*H*]; 2.00 [m, 1H, (C-3)*H*]; 0.93 [s, 9H, SiC(C*H*$_3$)$_3$]; 0.13 [s, 6H, Si(C*H*$_3$)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 178.07 [*C*OOH]; 138.05 [*C*q-ar (Bn)]; 137.81 [*C*q-ar (Bn)]; 128.01; 127.94; 127.14; 127.08 [*C*H-ar (Bn)]; 80.68 [C-4]; 72.68 [O*C*H$_2$]; 70.75 [O*C*H$_2$]; 70.17 [O*C*H$_2$]; 66.16 [O*C*H$_2$]; 48.00 [*C*H]; 46.03 [*C*H]; 45.78 [*C*H]; 34.07 [C-5]; 25.41 [SiC(*C*H$_3$)$_3$]; 17.85 [Si*C*(CH$_3$)$_3$].

*(1S, 2S, 3R, 4S)-[4-Benzyloxy-3-benzyloxymethyl-2-(*tert.*-butyl-dimethyl-silanyloxymethyl)-cyclopentyl]-carbamic acid allyl ester* (**18**)

To an ice-chilled solution of the mixture of **17** and its C-1 epimer (4.19 g, 8.67 mmol) in toluene (86 ml) was added diphenylphosphoryl azide (DPPA) (2.06 ml, 9.54 mmol) and Et_3N (1.44 ml, 10.4 mmol) and the mixture was stirred at 0° for 30 min and for 1h at RT; it was then heated to 80° for 2h. After cooling to ~10°, allyl alcohol (1.30 ml, 19.07 mmol) and catalytic amounts of dibutyltin dilaurate were added and heating to 80° was continued for 1.5h followed by heating to 100° for 30 min. The reaction mixture was then cooled to RT and poured into 200 ml ether/50 ml sat. aqu. $NaHCO_3$. The ethereal layer was separated and washed twice with 50 ml of H_2O. Drying ($MgSO_4$) and evaporation of solvent provided an oily residue, which was purified by FC in CH_2Cl_2/ether 19/1 (0.1% Et_3N) to give 3.0 g of **18** (64% based on the mixture of **17** and its C-1 epimer) as an oil.

18 (NMR signals generally broad and poorly resolved): *^1H NMR* (250 MHz, $CDCl_3$). δ = 7.25 [m, 10H, *H*-ar (Bn)]; 5.85 [m, 1H, -C*H*= (allyl)]; 5.20 [m, 2H, =C*H*$_2$ (allyl)]; 4.55 - 4.45 [m, 5H, incl. s at 4.50, C*H*$_2$ (Bn) + C*H*$_2$ (Bn) + OC*H*$_2$ (allyl)]; 4.40 [d, J = 12Hz, 1H, C*H*$_2$ (Bn)]; 3.90 [m, 2H, (C-1)*H* + (C-4)*H*]; 3.65 [m, 2H, C*H*$_2$OSi]; 3.55 [dd, J = 12Hz, J = 4Hz, 1H, C*H*$_2$OBn]; 3.40 [dd, J = 12Hz, J = 4Hz, 1H, C*H*$_2$OBn]; 2.20 [m, 1H]; 2.05 [m, 1H]; 1.75 [m, 2H]; 0.85 [s, 9H, SiC(C*H*$_3$)$_3$]; 0.00 [s, 6H, Si(C*H*$_3$)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 154.69 [NH*C*(O)O]; 137.61 [*C*q-ar (Bn)]; 137.24 [*C*q-ar (Bn)]; 132.02 [-CH=CH$_2$]; 127.35; 127.26; 126.56; 126.52; 126.39 [*C*H-ar (Bn)]; 116.30 [-CH=*C*H$_2$]; 79.07 [C-4]; 70.75 [O*C*H$_2$]; 69.87 [O*C*H$_2$]; 64.22 [O*C*H$_2$]; 64.01 [br, O*C*H$_2$]; 52.56 [*C*H]; 48.24 [*C*H]; 46.50 [*C*H]; 37.48 [C-5]; 24.69 [SiC(*C*H$_3$)$_3$]; 17.17 [Si*C*(CH$_3$)$_3$]. *FD-MS* ($C_{31}H_{45}NO_5Si$ 539.731): 540 [M^+]; 482 [M - C(CH$_3$)$_3$].

*(1S, 2S, 3R, 4S)-4-Benzyloxy-3-benzyloxymethyl-2-(*tert.*-butyl-dimethyl-silanyloxymethyl)-cyclopentylamine* (**19**)

To a solution of **18** (3.0 g, 5.56 mmol) in *abs.* THF (60 ml) were added morpholine (4.84 ml, 55.6 mmol) and $Pd(PPh_3)_4$ (0.556 g, 0.482 mmol). After 2h stirring at RT the solvent was evaporated, the residue redissolved in hexane/ether 1/1, the solution filtered, and the filtrate evaporated. Purification of the residue by FC in AcOEt → AcOEt/MeOH 9/1 (0.1% Et_3N) gave 2.38 g of **19** (93%) as an oil.

19: *^1H NMR* (250 MHz, $CDCl_3$). δ = 7.30 [m, 10H, *H*-ar (Bn); 4.46 [d, 2H, C*H*$_2$(Bn)]; 4.42 [s, 2H, C*H*$_2$ (Bn)]; 3.85 [m, 1H, (C-4)*H*]; 3.75 [dd, J = 10Hz, J = 6Hz, 1H, C*H*$_2$OSi]; 3.60 [dd, J = 10Hz, J = 7Hz, 1H, C*H*$_2$OSi]; 3.45 [dd, J = 9Hz, J = 6Hz, 1H, C*H*$_2$OBn]; 3.40 - 3.25 [m, 2H, incl. dd at 3.38, J = 9Hz, J = 6Hz, C*H*$_2$OBn + (C-1)*H*]; 2.00 [m, 2H]; 1.75 [s, br, N*H*$_2$]; 1.50 [m, 2H,]; 0.85 [s, 9H, SiC(C*H*$_3$)$_3$]; 0.05 [s, 6H, Si(C*H*$_3$)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 139.61 [*C*q-ar (Bn)]; 139.26 [*C*q-ar (Bn)]; 129.03; 128.98; 128.30; 128.20; 128.05 [*C*H-ar (Bn)]; 81.34 [C-4]; 73.76 [O*C*H$_2$]; 72.52 [O*C*H$_2$]; 71.30 [O*C*H$_2$]; 66.58 [br, O*C*H$_2$]; 55.69 [*C*H]; 52.88 [*C*H]; 48.98 [*C*H]; 41.92 [C-5]; 24.69 [SiC(*C*H$_3$)$_3$]; 18.97 [Si*C*(CH$_3$)$_3$]. *FD-MS* ($C_{27}H_{41}NO_3Si$ 455.662): 456 [M^+]; 398 [M - C(CH$_3$)$_3$].

*(1S, 2S, 3R, 4S)-1-[4-Benzyloxy-3-benzyloxymethyl-2-(*tert.*-butyl-dimethyl-silanyloxymethyl)-cyclopentyl]-3-(3-methoxy-2-methyl-acryloyl)-urea* (**20**)

To a solution of **19** (2.61 g, 5.72 mmol) in CH_2Cl_2 (20 ml) was added at - 60° distilled β-methoxymethacryloyl isocyanate[36] (1.05 g, 7.45 mmol). The reaction mixture was allowed to warm to RT over a period of ~ 30 min and was then poured into 50 ml CH_2Cl_2/50 ml sat. aqu. $NaHCO_3$. The organic layer was separated and the aqueous solution was twice extracted with 30 ml of CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated. Purification of the residue by FC in ether/hexane 1/1 gave 3.18 g of **20** (93%) as an oil.

20: *^1H NMR* (500 MHz, $CDCl_3$). δ = 8.71 [d, J = 7Hz, 1H, (C-1)N*H*]; 7.90 [s, br, 1H, CH_3OCH=]; 7.30 [m, 10H, *H*-ar (Bn)]; 4.52 [d, J = 20Hz, 1H, CH_2 (Bn)]; 4.50 [d, J = 20Hz, 1H, CH_2 (Bn)]; 4.48 [d, J = 22Hz, 1H, CH_2 (Bn)]; 4.44 [d, J = 22Hz, 1H, CH_2 (Bn)]; 4.23 [quintett, J = 8Hz, 1H, (C-1)*H*]; 3.90 [m, 1H, (C-4)*H*]; 3.83 [s, 3H, OCH_3]; 3.72 [dd, J = 10Hz, J = 5Hz, 1H, CH_2OSi]; 3.67 [dd, J = 10Hz, J = 6Hz, 1H, CH_2OSi]; 3.57 [dd, J = 9Hz, J = 4Hz, 1H, CH_2OBn]; 3.42 [dd, J = 9Hz, J = 6Hz, CH_2OBn]; 2.11 [m, 2H, (C-3)*H* + (C-5)*H*-α]; 1.89 [m, 5H, incl. s at 1.88, CH_3OCH=C(CH_3)- + (C-2)*H* + (C-5)*H*-β]; 0.85 [s, 9H, Si(CH_3)$_3$]; 0.10 [s, 3H, Si(CH_3)$_2$]; 0.05 [s, 3H, Si(CH_3)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 169.23 [NH*C*(O)C(CH_3)=]; 157.41 [=*C*HOCH_3]; 154.02 [NH*C*(O)NH]; 137.95 [*C*q-ar (Bn)]; 137.14 [*C*q-ar (Bn)]; 127.56; 127.48; 126.91; 126.79; 126.71; 126.64 [*C*H-ar (Bn)]; 107.13 [C(O)*C*(CH_3)=]; 79.50 [C-4]; 72.69 [O*C*H$_2$]; 70.37 [O*C*H$_2$]; 69.88 [O*C*H$_2$]; 68.82 [br, O*C*H$_2$]; 60.67 [O*C*H$_3$]; 50.27 [*C*H]; 48.68 [*C*H]; 46.58 [*C*H]; 38.11 [C-5]; 25.13 [SiC(*C*H$_3$)$_3$]; 17.47 [Si*C*(CH$_3$)$_3$]; 8.04 [C(O)C(*C*H$_3$)=]. *FD-MS* ($C_{33}H_{48}N_2O_6Si$ 596.775): 597 [M^+]; 539 [M - C(CH_3)$_3$].

(1S, 2S, 3R, 4S)-1-(4-Benzyloxy-3-benzyloxymethyl-2-hydroxymethyl-cyclopentyl)-thymine (**21**)

A solution of **20** (3.10 g, 5.20 mmol) in 60 ml EtOH/6.6 ml 2N aqu. HCl was heated to reflux for 20h. The reaction mixture was then evaporated to dryness and the residue co-evaporated with EtOH (4x). Purification by FC in AcOEt gave 2.00 g of **21** (85%) as a white foam.

21: *^1H NMR* (250 MHz, $CDCl_3$). δ = 9.00 [s, 1H, N*H*]; 7.35 [m, 10H, *H*-ar (Bn)]; 7.05 [s, 1H, (C-6)*H* thymine]; 4.90 [q, J = 8Hz, 1H, (C-1)*H*)]; 4.52 [s, 2H, CH_2 (Bn)]; 4.52 [d, J = 12Hz, 1H, CH_2 (Bn)]; 4.45 [d, J = 12Hz, 1H, CH_2 (Bn)]; 3.95 [m, 1H, (C-4)*H*]; 3.90 [m, 1H, (C-4)*H*]; 3.60 [m, 4H, CH_2OBn + CH_2OH]; 3.10 [s, br, 1H, CH_2O*H*]; 2.25 - 2.05 [m, 4H, (C-2)*H* +(C-3)*H* + (C-5)H_2]; 1.80 [s, 3H, (C-5)CH_3 thymine]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 164.07 [C-4 thymine]; 151.80 [C-2 thymine]; 138.10 [*C*q-ar (Bn)]; 137.80 [*C*q-ar (Bn)]; 137.47 [C-6 thymine]; 128.49; 128.37; 127.82; 127.69; 127.61 [*C*H-ar (Bn)]; 111.27 [C-5 thymine]; 79.57 [C-4]; 73.41 [O*C*H$_2$]; 71.00 [O*C*H$_2$]; 70.86 [O*C*H$_2$]; 62.97 [O*C*H$_2$]; 57.47 [C-1]; 48.18 [*C*H]; 47.54 [*C*H]; 35.94 [C-5]; 12.38 [(C-5)*C*H$_3$ thymine]. *FD-MS* ($C_{26}H_{30}N_2O_5$ 450.493): 450 [M^+].

(1S, 2S, 3R, 4S)-1-(4-hydroxy-2,3-bis-hydroxymethyl-cyclopentyl)-thymine (**22**)

21 (80 mg, 0.177 mmol) was hydrogenated over 10% Pd-C in MeOH (5 ml) for 3h (RT, atmospheric pressure). The catalyst was then removed by filtration through *Hyflo*; evaporation of the filtrate gave a greyish residue, which was redissolved in AcOEt/MeOH. Filtration through *Hyflo* followed by evaporation of the filtrate provided **22** as a white solid in quantitative yield.

22: *^1H NMR* (250 MHz, CD_3OD). δ = 7.40 [s, 1H, (C-6)*H* thymine]; 4.75 [q, J = 10Hz, overlapping with H_2O signal, (C-1)*H*)]; 4.10 [m, 1H, (C-4)*H*]; 3.60 [septett, 2H, (C-3)CH_2OH]; 3.50 [d, J = 5Hz, 2H, (C-2)CH_2OH]; 2.10 [m, 2H]; 1.95 - 1.70 [m, 5H, incl. s at 1.80 ((C-5)CH_3 thymine)]. *^{13}C NMR* (62.5 MHz, CD_3OD). δ = 167.00 [C-4 thymine]; 153.62 [C-2 thymine]; 141.31 [C-6 thymine]; 111.63 [C-5 thymine]; 72.97 [C-4]; 63.82 [O*C*H$_2$]; 63.41 [O*C*H$_2$]; 58.75 [C-1]; 53.49 [*C*H]; 48.94 [*C*H]; 39.46 [C-5]; 12.44 [(C-5)*C*H$_3$ thymine].

(1S, 2S, 3R, 4S)-1-{4-Hydroxy-3-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-2-methyl-cyclopentyl}-thymine (**23**)

To a solution of **11** (0.554 g, 2.18 mmol) in pyridine (21 ml) were added Et_3N (0.422 ml, 3.06 mmol), DMTr-Cl (0.854 g, 2.52 mmol), and a catalytic amount of DMAP, and the mixture was stirred at RT for 4h. It

was then diluted with 100 ml of ether and poured into ice-water (100 ml). The organic layer was separated and the aqueous solution was three times extracted with 50 ml of ether. The combined organic extracts were then dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by two FC's in 0.1% Et_3N/AcOEt to furnish 0.998 g of **23** (81%) as a very light yellow foam.

23: *^1H NMR* (250 MHz, $CDCl_3$). δ = 7.40 - 7.25 [m, 9H, *H*-ar (DMTr)]; 6.95 [s, 1H, (C-6)*H* thymine]; 6.85 [d, J = 7 Hz, 4H, *H*-ar (DMTr, *ortho* to OCH_3)]; 4.75 [dd, J = 20Hz, J = 8Hz, 1H, (C-1)*H*]; 4.30 [dd, J = 14Hz, J = 6Hz, 1H, (C-4)*H*]; 3.80 [s, 6H, O*C*H_3]; 3.45 [dd, J = 8Hz, J < 4Hz, 1H, CH_2ODMTr]; 3.10 [dd, J = 8Hz, J = 6Hz, 1H, CH_2ODMTr]; 2.20 - 2.00 [m, 2H]; 2.00 - 1.70 [m, 5H, incl. s at 1.90 ((C-5)CH_3 thymine)]; 0.95 [d, J = 6Hz, 3H, (C-2)CH_3]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 162.88 [C-4 thymine]; 157.52 [*C*q-ar (DMTr, *C*-OCH_3]; 150.44 [C-2 thymine]; 143.80 [*C*q-ar (DMTr, *C*-CH(PhOCH_3)$_2$)]; 135.89 [C-6 thymine]; 135.00; 134.79 [2x *C*q-ar (DMTr, *para* to OCH_3]; 128.96; 127.02; 126.90; 125.88 [*C*H-ar (DMTr)]; 112.19 [*C*H-ar (DMTr, *ortho* to OCH_3)]; 110.25 [C-5 thymine]; 85.47 [*C*q (DMTr, *C*Ar$_3$)]; 72.77 [C-4]; 62.88 [*C*H_2ODMTr]; 59.62 [C-1]; 54.19 [O*C*H_3]; 52.99 [C-3]; 39.16 [C-2]; 37.21 [C-5]; 15.18 [(C-2)*C*H_3]; 11.58 [(C-5)*C*H_3 thymine]. *EI-MS:* ($C_{33}H_{36}N_2O_6$ 556.623): 556 (2.5) [M^+].

*(1S, 2R, 3S, 4S)-Diisopropyl-phosphoramidous acid 2-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-3-methyl-4-(thymidin-1-yl)-cyclopentyl ester (***24***)*

To a solution of diisopropylammonium tetrazolide[29] (0.358 g, 2.08 mmol) in CH_2Cl_2 (12 ml) was added dropwise 2-cyanoethoxy-bis(diisopropylamino)-phosphine (0.576 g, 1.91 mmol) followed by a solution of **23** (0.970 g, 1.71 mmol) in CH_2Cl_2 (12 ml). The mixture was stirred at RT for 2h and then poured into 70 ml CH_2Cl_2/70 ml sat. aqu. $NaHCO_3$. The organic layer was separated and the aqueous solution was three times extracted with 50 ml of CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by FC in toluene/AcOEt 1/1 (0.1% Et_3N) to give 1.10 g of **24** as a white foam. This material was dissolved in CH_2Cl_2 (2 ml) and carefully poured into pentane (70 ml, 0°, vigorous stirring), which provided 0.990 g of **24** (75%) as an amorphous white solid.

24 (mixture of two diastereoisomers): *^1H NMR* (250 MHz, $CDCl_3$). δ = 9.45 [s, br, 1H, N*H*]; 7.55 - 7.25 [m, 9H, *H*-ar (DMTr)]; 7.05 [s, 1H, (C-6)*H* thymine]; 6.90 - 6.80 [m, 4H, *H*-ar (DMTr, *ortho* to OCH_3)]; 4.80 [m, 1H, (C-4)*H*]; 4.60, 4.45 [2x m, 1H, (C-1)*H*]; 3.85 - 3.50, 3.35. 3.20 [3x m, 10H, 1H, 1H, incl. s at 3.80, O*C*H_3 + *C*H_2ODMTr + POCH_2 + N*H*$(CH_3)_2$ (2x)]; 2.60 [t, 1H, CH_2CN]; 2.45 [t, 1H, CH_2CN]; 2.30 - 1.75 [m, 7H, incl. "d" at 1.90, (C-5)CH_3 thymine + (C-5)H_2 + (C-3)*H* + (C-2)*H*]; 1.30 - 1.05 [m, 12H, NC*H*$(CH_3)_2$]; 0.95 [d, br, 3H, (C-3)CH_3]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 163.07 [C-4 thymine]; 157.73 [*C*q-ar (DMTr, *C*-OCH_3]; 150.80, 150.63 [C-2 thymine]; 144.28 [*C*q-ar (DMTr, *C*-CH(PhOCH_3)$_2$)]; 136.10, 136.03 [C-6 thymine]; 135.42, 135.35, 135.29 [2x *C*q-ar (DMTr, *para* to OCH_3]; 129.41; 128.26; 128.48; 127.42; 127.04; 126.05; 124.52 [*C*H-ar (DMTr)]; 117.11, 116.94 [*C*N]; 112.34 [*C*H-ar (DMTr, *ortho* to OCH_3)]; 110.57, 110.51 [C-5 thymine]; 85.31, 85.23 [*C*q (DMTr, *C*Ar$_3$)]; 73.39, 73.15, 72.61, 72.35 [C-1]; 60.72, 59.96 [*C*H_2ODMTr]; 59.66 [br, C-4]; 57.54, 57.23, 56.92 [PO*C*H_2]; 54.46 [O*C*H_3]; 53.07 [br, C-2]; 42.49, 42.29 [N*C*H$(CH_3)_2$]; 39.19, 38.86 [C-3]; 37.56, 37.15 [C-5]; 23.99, 23.88, 23.77, 23.65 [NCH$(CH_3)_2$]; 19.77, 19.65, 19.60, 19.49 [*C*H_2CN]; 15.80, 15.65 [(C-3)*C*H_3]; 11.77 [(C-5)*C*H_3 thymine]. 31P NMR (101.3 MHz, $CDCl_3$): 147.85.

(1S, 2R, 3S, 5S)-Benzoic acid 3-benzyloxy-2-benzyloxymethyl-5-(thymin-1-yl)- cyclopentylmethyl ester (**25**)

To a solution of **21** (1.91 g, 4.24 mmol) in CH_2Cl_2 (30 ml) were added pyridine (0.855 ml, 10.6 mmol), benzoic anhydride (1.20 g, 5.31 mmol) and a catalytic amount of DMAP. After stirring at RT for 18h, additional 0.30 g of DMAP were added and the reaction mixture was heated to reflux for 4 h. After cooling to RT MeOH (5 ml) was added and stirring was continued for 15 more min. The mixture was then poured into 50 ml CH_2Cl_2/50 ml sat. aqu. $NaHCO_3$ and the organic layer was separated. The aqueous solution was twice extracted with 50 ml of CH_2Cl_2 each, the combined organic extracts were dried (MgSO4) and the solvent was evaporated. After two co-evaporations with toluene the residue was purified by FC in AcOEt to give 2.14 g of **25** (91%) as a crystalline solid.

25: *^1H NMR* (250 MHz, $CDCl_3$). δ = 8.30 [s, br, 1H, N*H*]; 7.90 [d, J = 7Hz, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.50 [t, J = 6Hz, 1H, *H*-ar (Bz, *para* to C=O)]; 7.30 [m, 12H, *H*-ar (Bz + Bn)]; 7.00 [s, 1H, (C-6)*H* thymine]; 5.05 [q, J = 8Hz, 1H, (C-5)*H*)]; 4.55 - 4.45 [m, 6H, CH_2 (Bn) (2x) + CH_2OBz]; 4.05 [m, 1H, (C-3)*H*]; 3.65 [octett, 2H, CH_2OBn]; 2.60 [m, 1H]; 2.30 - 2.10 [m, 3H]; 1.70 [s, 3H, (C-5)CH_3 thymine]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 166.30 [Ph*C*(O)]; 163.80 [C-4 thymine]; 150.94 [C-2 thymine]; 138.08 [*C*q-ar (Bn)]; 137.97 [*C*q-ar (Bn)]; 137.88 [C-6 thymine]; 133.11 [*C*H-ar (Bz, *ortho* to C=O)]; 129.64 [*C*q-ar (Bz)]; 129.43; 128.49; 128.40; 127.81; 127.63 [*C*H-ar (Bz, Bn)]; 111.01 [C-5 thymine]; 79.86 [C-3]; 73.42 [CH_2OBz]; 70.87 [OCH_2 (Bn)]; 70.75 [OCH_2 (Bn)]; 65.99 [CH_2OBn]; 59.45 [C-5]; 47.64 [*C*H]; 43.31 [*C*H]; 36.23 [C-4]; 12.29 [(C-5)CH_3 thymine]. *FD-MS* ($C_{33}H_{34}N_2O_6$ 554.597): 554 [M^+].

(1S, 2R, 3S, 5S)-Benzoic acid 3-hydroxy-2-hydroxymethyl-5-(thymin-1-yl)-cyclopentylmethyl ester (**26**)

25 (2.14 g, 3.86 mmol) was hydrogenated over 10% Pd-C in MeOH (38 ml) for 5h (RT, atmospheric pressure). After addition of 20 ml of AcOEt the catalyst was removed by filtration through *Hyflo* and washed with AcOEt/MeOH 1/1. Evaporation of the filtrate gave 1.48 g of crude **26** (quant.) as a white foam, which was directly used in the next step.

26: *^1H NMR* (250 MHz, DMSO-d_6). δ = 11.20 [s, br, 1H, N*H*]; 7.85 [d, J = 8Hz, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.67 [t, J = 6Hz, 1H, *H*-ar (Bz, *para* to C=O)]; 7.65 [s, 1H, (C-6)*H* thymine]; 7.45 [t, J = 8Hz, 2H, *H*-ar (Bz, *meta* to C=O)]; 5.05 [q, J = 9Hz, 1H, (C-5)*H*)]; 4.90 [d, 1H, (C-3)O*H*]; 4.80 [s, br, 1H, CH_2O*H*]; 4.45 [dd, J = 10Hz, J = 5Hz, 1H, CH_2OBz]; 4.25 [dd, J = 10Hz, J = 8Hz, 1H, CH_2OBz]; 4.05 [m, 1H, (C-3)*H*]; 3.60 [s, br, 2H, CH_2OH]; 2.35 [m, 1H, (C-1)*H*]; 2.10 [m, 1H, (C-4)*H*]; 1.90 - 1.70 [m, incl. s at 1.80, 5H, (C-5)CH_3 thymine + (C-2)*H* + (C-4)*H*]. *^{13}C NMR* (62.5 MHz, CD_3OD). 167.26 [Ph*C*(O)]; 165.82 [C-4 thymine]; 152.64 [C-2 thymine]; 139.73 [C-6 thymine]; 133.96 [*C*H-ar (Bz, *para* to C=O)]; 130.68 [*C*q-ar (Bz)]; 130.05; 129.21 [*C*H-ar (Bz)]; 111.41 [C-5 thymine]; 72.31 [C-3]; 67.63 [CH_2OBz]; 62.74 [CH_2OH]; 59.90 [C-5]; 52.53 [*C*H]; 44.32 [*C*H]; 39.40 [C-4]; 11.99 [(C-5)CH_3 thymine]. *FD-MS* ($C_{19}H_{22}N_2O_6$ 374.377): 374 [M^+].

(1S, 2R, 3S, 5S)-Benzoic acid 2-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-3-hydroxy-5-(thymin-1-yl)-cyclopentylmethyl ester (**27**)

To a solution of **26** (1.43 g, 3.82 mmol, twice evaporated from *abs.* pyridine) in pyridine (20 ml) were added Et_3N (0.739 ml, 5.35 mmol), DMTr-Cl (1.50 g, 4.42 mmol), and a catalytic amount of DMAP, and the mixture was stirred at RT for 18h. It was then poured into AcOEt (200 ml) and the solution was three times washed with 50 ml of water. The combined aqueous extracts were once re-extracted with 100 ml of AcOEt, the combined organic extracts were dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by FC in 0.1% Et_3N/AcOEt; fractions containing impure product were combined separately and re-chromatographed in the same solvent system. In total 2.21 g of **27** (86%) were obtained as a light yellow foam.

27: *^1H NMR* (500 MHz, $CDCl_3$). δ = 8.00 [s, br, 1H, N*H*]; 7.87 [d, J = 8Hz, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.56 [t, J = 8Hz, 1H, *H*-ar (Bz, *para* to C=O)]; 7.40 [m, 4H, *H*-ar (Bz, DMTr)]; 7.30 [m, 6H, *H*-ar (DMTr)]; 7.23 [t, J = 7Hz, 1H, *H*-ar (DMTr)]; 6.94 [s, 1H, (C-6)*H* thymine]; 6.83 [d, J = 9Hz, 4H, *H*-ar (DMTr, *ortho* to OCH_3)]; 4.92 [q, J = 9Hz, 1H, (C-5)*H*)]; 4.39 [m, 1H, (C-3)*H*]; 4.29 [septett, 2H, CH_2OBz]; 3.78 [s, 6H, OCH_3]; 3.58 [dd, J = 9Hz, J = 5Hz, 1H, CH_2ODMTr]; 3.23 [dd, J = 9Hz, J = 7Hz, 1H, CH_2ODMTr]; 2.43 [m, 1H, (C-1)*H*]; 2.32 [d, 1H, (C-3)O*H*]; 2.25 [m, 1H, (C-4)*H*-β]; 2.14 [m, 1H, (C-4)*H*-α]; 2.02 [m, 1H, (C-2)H]; 1.96 [s, 3H, (C-5)CH_3 thymine]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 166.34 [Ph*C*(O)]; 163.96 [C-4 thymine]; 158.71 [*C*q-ar (DMTr, *C*-OCH_3]; 151.14 [C-2 thymine]; 144.88 [*C*q-ar (DMTr, *C*-CH(PhOCH_3)$_2$)]; 137.83 [C-6 thymine]; 136.03; 137.80 [2x *C*q-ar (DMTr, *para* to OCH_3]; 133.32 [*C*H-ar (Bz, *para* to C=O)]; 129.73 [*C*q-ar (Bz)]; 130.16; 129.58; 128.18; 127.14 [*C*H-ar (Bz, DMTr)]; 113.43 [*C*H-ar (DMTr, *ortho* to OCH_3)]; 111.40 [C-5 thymine]; 86.93 [*C*q (DMTr, *C*Ar_3)]; 74.06 [C-3]; 66.10 [CH_2OBz]; 60.61 [CH_2ODMTr]; 58.72 [C-5]; 55.36 [OCH_3]; 50.62 [*C*H]; 44.08 [*C*H]; 39.02 [C-4]; 12.54 [(C-5)CH_3 thymine].

(1S, 2R, 3S, 5S)-Benzoic acid 2-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-3-[diisopropylamino-(3-nitrilo-propoxy)-phosphanyloxy]-5-(thymin-1-yl)-cyclopentylmethyl ester (**28**)

To a solution of diisopropylammonium tetrazolide[29] (0.673 g, 3.91 mmol) in CH_2Cl_2 (15 ml) was added dropwise 2-cyanoethoxy-bis(diisopropylamino)-phosphine (1.08 g, 3.59 mmol) followed by a solution of **27** (2.20 g, 3.25 mmol) in CH_2Cl_2 (15 ml). The mixture was stirred at RT for 3h and then poured into 100 ml CH_2Cl_2/100 ml sat. aqu. $NaHCO_3$. The organic layer was separated and the aqueous solution was twice extracted with 50 ml of CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by FC in ether/AcOEt 3/1 (0.1% Et_3N) to give 2.55 g of **28**. Of this material 1.0 g were directly used for the preparation of **29**, whereas the remainder was dissolved in ether (5 ml) and precipitated by dropwise addition of pentane (150 ml, 0°, vigorous stirring). Due to indications of minute quantities of a phosphorous containing impurity in the ^{31}P NMR spectrum, the precipitated material was re-chromatographed in ether/AcOEt 3/1 (0.1% Et_3N). Precipitation from an ethereal solution by dropwise addition of pentane finally provided 1.20 g of pure **28** (89%) as an amorphous white solid.

28 (mixture of two diastereoisomers): *^1H NMR* (250 MHz, $CDCl_3$). δ = 8.25 [s, br, 1H, N*H*]; 7.85 [m, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.60 - 7.20 [m, 12H, *H*-ar (Bz, DMTr)]; 7.02 [s (2x), 1H, (C-6)*H* thymine]; 6.85 [m, 4H, *H*-ar (DMTr, *ortho* to OCH_3)]; 5.02 [quintett, J = 8Hz, 1H, (C-5)*H*)]; 4.50 [m, 1H, (C-3)*H*]; 4.30 [m, 2H, CH_2OBz]; 3.90 - 3.40 [m, 11H, OCH_3 (2x) + CH_2ODMTr + POCH_2 + NC*H*$(CH_3)_2$]; 3.30 [m, 1H, NC*H*$(CH_3)_2$]; 2.65 - 2.40 [m, incl. t at 2.61, t at 2.48, 3H, CH_2CN + (C-4)*H*]; 2.30 - 2.05 [m, 3H, (C-4)*H* + (C-2)*H* + (C-1)*H*]; 1.72 [s, br, 3H, (C-5)CH_3 thymine]; 1.20 - 1.05 [m, 12H, N(CH(CH_3)$_2$)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 166.13 [Ph*C*(O)]; 163.69 [C-4 thymine]; 158.53 [*C*q-ar (DMTr, *C*-OCH_3]; 150.95, 150.76 [C-2 thymine]; 144.79 [*C*q-ar (DMTr, *C*-CH(PhOCH_3)$_2$)]; 137.58, 137.38 [C-6 thymine]; 135.90, 135.86, 135.70 [2x *C*q-ar (DMTr, *para* to OCH_3]; 133.10 [*C*H-ar (Bz, *para* to C=O)]; 129.67 [*C*q-ar (Bz)]; 130.04; 129.42; 128.38; 128.15; 128.08; 127.93; 126.92 [*C*H-ar (Bz, DMTr)]; 117.89, 117.66 [*C*N]; 113.20 [*C*H-ar (DMTr, *ortho* to OCH_3)]; 111.14 [C-5 thymine]; 86.54 [*C*q (DMTr, *C*Ar_3)]; 75.03, 74.77, 74.63, 74.18 [C-3]; 66.27, 66.08 [*C*H_2OBz]; 63.04, 62.63 [*C*H_2ODMTr]; 58.56, 58.36 [C-5]; 58.21, 58.06, 57.92 [POCH_2]; 55.19 [OCH_3]; 49.66 [*C*H]; 43.55, 43.24, 43.06 [*C*H]; 38.45, 38.43 [C-4]; 24.72, 24.62, 24.46 [N(CH(*C*H_3)$_2$)$_2$]; 20.40, 20.33, 20.21 [*C*H_2CN]; 12.29 [(C-5)CH_3 thymine]. ^{31}P NMR (101.3 MHz, $CDCl_3$). δ = 147.83, 147.64.

(1S, 2R, 3S, 5S)-Benzoic acid 2-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-3-[diisopropylamino-(3-nitrilo-propoxy)-phosphanyloxy]-5-(5-methyl-2-oxo-4-[1,2,4]-triazol-1-yl-pyrimidin-1-yl)-cyclopentylmethyl ester (**29**)

To a solution of 1,2,4-triazole (1.77 g, 25.65 mmol) in acetonitrile/CH_2Cl_2 1/1 (20 ml) were added Et_3N (3.63 ml, 26.22 mmol) and $POCl_3$ (0.260 ml, 2.85 mmol) (formation of a precipitate) and the mixture was stirred at RT for 30 min. **28** (1.00 g, 1.14 mmol) was then added at 5° and the reaction mixture was stirred at RT for 2.5h. It was then poured into 200 ml CH_2Cl_2/10 ml Et_3N and this solution was extracted three times with 100 ml of H_2O. After drying ($MgSO_4$) the solvent was evaporated and the residue was purified by FC in AcOEt/ether 1/3 (0.1% Et_3N). Product containing fractions were combined, the solvent was evaporated, and the residue was dissolved in ether (5 ml). This solution was added dropwise to pentane (120 ml) (0°, vigorous stirring) and after 15 min at 0° the precipitate was collected by filtration. This procedure provided 0.633 g of **29** containing a trace amount of **28**. Re-chromatography of this material in AcOEt/ether 1/1 (0.1% Et_3N) gave 0.566 g of pure **29** (60%) as an amorphous white solid.

29 (mixture of two diastereoisomers): *^1H NMR* (250 MHz, $CDCl_3$). δ = 9.15 ["d", 1H, C*H*-triazolo]; 8.10 [s, 1H, C*H*-triazolo]; 7.80 [m, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.65 ["d", 1H, *H*-ar (Bz, *para* to C=O)]; 7.50 - 7.20 [m, 12H, *H*-ar (Bz, DMTr) + (C-6)*H* pyrimidine]; 6.90 [m, 4H, *H*-ar (DMTr, *ortho* to OCH_3)]; 5.10 [m, 1H, (C-5)*H*)]; 4.70 - 4.40 [m, 2H, (C-3)*H* + CH_2OBz]; 4.30 [m, 1H, CH_2OBz]; 3.90 - 3.30 [m, 12H, OCH_3 (2x) + CH_2ODMTr + POCH_2 + NC*H*$(CH_3)_2$ (2x)]; 2.80 [m, 1H]; 2.60 [t, 1H, CH_2CN]; 2.50 - 2.05 [m, 7H, CH_2CN + (C-4)H_2 + (C-2)*H* + (C-1)*H* + (C-5)CH_3 pyrimidine]; 1.20 - 1.10 [m, 12H, N(CH(*C*H_3)$_2$)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 166.74 [Ph*C*(O)]; 163.69 [C-4 pyrimidine]; 159.15 [*C*q-ar (DMTr, *C*-OCH_3]; 155.00 [C-2 pyrimidine]; 153.88 [*C*H-triazolo]; 149.68 [*C*H-triazolo]; 145.50 [C-6 pyrimidine]; 145.39

[*C*q-ar (DMTr, *C*-CH(PhOCH$_3$)$_2$)]; 136.54, 136.49, 136.42 [*C*q-ar (DMTr, *para* to OCH$_3$, Bz]; 133.69 [*C*H-ar (Bz, *para* to C=O)]; 129.67 [*C*q-ar (Bz)]; 130.77; 130.71; 130.02; 128.84; 128.56; 127.54 [*C*H-ar (Bz, DMTr)]; 118.58 [*C*N]; 113.83 [*C*H-ar (DMTr, *ortho* to OCH$_3$)]; 106.52 [C-5 pyrimidine]; 87.19 [*C*q (DMTr, *C*Ar$_3$)]; 75.48, 74.77, 74.50 [C-3]; 67.11, 66.78 [*C*H$_2$OBz]; 63.46, 63.07 [*C*H$_2$ODMTr + (C-5)]; 59.06, 58.75, 58.44 [PO*C*H$_2$]; 55.85 [O*C*H$_3$]; 50.45 [*C*H]; 44.42, 44.21 [*C*H]; 43.87, 43.67 [N(*C*H(CH$_3$)$_2$)$_2$]; 39.44, 39.14 [C-4]; 24.74, 24.63, 24.41 [N(CH(*C*H$_3$)$_2$)$_2$]; 20.56, 20.44, 20.35, 20.23 [*C*H$_2$CN]; 16.79 [(C-5)*C*H$_3$ pyrimidine]. ^{31}P NMR (101.3 MHz, CDCl$_3$). δ = 147.63, 147.49.

*(1S, 2S, 3R, 4S)-1-[4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentyl]-thymine* (**30**)

To a solution of crude **11** (0.719 g, 2.82 mmol; twice co-evaporated with AcOEt) in *abs.* DMF (30 ml) were added imidazole (0.577 g, 8.49 mmol) and TBDMS-Cl (1.02 g, 6.79 mmol) and the mixture was stirred at RT for 4h followed by 40 min at 40°. It was then poured into ether (100 ml) and the solution was three times extracted with 20 ml of H$_2$O. The organic layer was dried (MgSO$_4$) and the solvent was evaporated. The residue was purified by FC in ether (0.1% Et$_3$N) to give 1.31 g of **30** (96%, based on **10**) as a viscous oil which solidified upon standing.

30: *^{1}H NMR* (250 MHz, CDCl$_3$). δ = 9.50 [s, br, 1H, N*H*]; 6.90 [s, 1H, (C-6)*H* thymine]; 4.75 [q, J = 9Hz, 1H, (C-1)*H*]; 4.20 [q, 1H, (C-4)*H*]; 3.70 [dd, J = 10Hz, J < 4Hz, 1H, C*H*$_2$OSi]; 3.55 [dd, J = 10Hz, J < 4Hz, 1H, C*H*$_2$OSi]; 2.00 - 1.80 [m, 6H, incl. s at 1.90 ((C-5)C*H*$_3$ thymine)]; 1.55 [m, 1H]; 1.00 [d, J = 7Hz, 3H, (C-2)C*H*$_3$]; 0.85 [s, br, 18H, SiC(C*H*$_3$)$_3$]; 0.00 [2 x s, Si(C*H*$_3$)$_2$]. *^{13}C NMR* (62.5 MHz, CDCl$_3$). δ = 163.05 [C-4 thymine]; 150.58 [C-2 thymine]; 135.89 [C-6 thymine]; 110.26 [C-5 thymine]; 70.56 [C-4]; 59.59 [C-1]; 59.43 [*C*H$_2$OSi]; 55.23 [C-3]; 38.71 [C-5]; 37.59 [C-2]; 25.02 [SiC(*C*H$_3$)$_3$]; 24.91 [SiC(*C*H$_3$)$_3$]; 17.38 [Si*C*(CH$_3$)$_3$]; 17.09 [Si*C*(CH$_3$)$_3$]; 15.47 [(C-2)*C*H$_3$]; 11.75 [(C-5)*C*H$_3$ thymine]. *EI-MS:* (C$_{24}$H$_{46}$N$_2$O$_4$Si$_2$ 482.744): 425 (100) [M - C(CH$_3$)$_3$].

*(1S, 2S, 3R, 4S)-1-[4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentyl]-4-[1,2,4]-triazol-1-yl-1*H*-pyrimidine-2-one* (**31**)

To a solution of **30** (1.19 g, 2.47 mmol) in *abs.* acetonitrile (30 ml) were added Et$_3$N (7.90 ml, 57.1 mmol) and 1,2,4-triazole (3.84 g, 55.6 mmol). POCl$_3$ (0.552 ml, 6.03 mmol) was then added dropwise over a period of 10 min (formation of a precipitate) and the mixture was stirred at RT for 2.5h. After this period it was poured into 300 ml CH$_2$Cl$_2$/30 ml Et$_3$N and the solution was extracted with 100 ml of sat. aqu. NaHCO$_3$. After back-extraction of the aqueous solution with twice 100 ml of CH$_2$Cl$_2$ the combined organic extracts were dried and the solvents were evaporated to provide a solid residue. This was dissolved in AcOEt (100 ml) and the solution was twice washed with 50 ml of H$_2$O. It was then dried (MgSO$_4$) and the solvent was evaporated. FC of the residue in ether (0.1% Et$_3$N) gave **31** as a white foam.

31: *^{1}H NMR* (250 MHz, CDCl$_3$). δ = 9.20 [s, 1H, C*H*-triazolo]; 8.05 [s, 1H, C*H*-triazolo]; 7.55 [s, 1H, (C-6)*H* pyrimidine]; 4.90 [q, J = 8Hz, 1H, (C-1)*H*]; 4.30 [q, 1H, (C-4)*H*]; 3.70 [dd, J = 10Hz, J < 4Hz, 1H, C*H*$_2$OSi]; 3.60 [dd, J = 10Hz, J < 4Hz, 1H, C*H*$_2$OSi]; 2.40 [s, 3H, (C-5)C*H*$_3$ pyrimidine]; 2.15 - 1.90 [m, 3H]; 1.60 [m, 1H]; 1.00 [d, J = 7Hz, 3H, (C-2)C*H*$_3$]; 0.80 [s, br, 18H, SiC(C*H*$_3$)$_3$]; 0.00 [2 x s, Si(C*H*$_3$)$_2$]. *^{13}C NMR* (62.5 MHz, CDCl$_3$). δ = 157.13, 155.00 [C-4, C-2 pyrimidine]; 153.30 [*C*H-triazolo]; 147.69 [*C*H-triazolo]; 144.88 [C-6 pyrimidine]; 105.82 [C-5 pyrimidine]; 71.45 [C-4]; 63.41 [C-1]; 60.19 [*C*H$_2$OSi]; 56.93 [C-3]; 40.37 [C-5]; 39.33 [C-2]; 25.85 [SiC(*C*H$_3$)$_3$]; 25.73 [SiC(*C*H$_3$)$_3$]; 18.21 [Si*C*(CH$_3$)$_3$]; 17.14 [Si*C*(CH$_3$)$_3$]; 17.14; 16.43 [(C-2)*C*H$_3$; (C-5)*C*H$_3$ pyrimidine]. *EI-MS:* (C$_{26}$H$_{47}$N$_5$O$_3$Si$_2$ 533.807): 476 (100) [M - C(CH$_3$)$_3$].

*(1S, 2S, 3R, 4S)-1-[4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentyl]-5-methyl cytosine* (**32**)

To a solution of the above material in dioxane (30 ml) was added conc. aqu. NH$_3$ (10 ml) and the solution was stirred at 40° for 16h. It was then evaporated and the residue was dissolved in AcOEt. This solution was twice extracted with 50 ml of H$_2$O, the combined aqueous extracts were once back-extracted with 50 ml of

AcOEt, the organic extracts were dried ($MgSO_4$) and the solvent was evaporated. FC of the residue in AcOEt/MeOH 4/1 (0.1% Et_3N) gave 0.912 g of **32** (77%, based on **30**) as a white foam.
32: *^1H NMR* (250 MHz, $CDCl_3$). δ = 6.90 [s, 1H, (C-6)*H* methylcytosine]; 4.85 [q, J = 10Hz, 1H, (C-1)*H*]; 4.20 [q, 1H, (C-4)*H*]; 3.70 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 3.55 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 2.05 - 1.75 [m, 6H, incl. s at 1.90 ((C-5)CH_3 methylcytosine)]; 1.55 [m, 1H]; 1.00 [d, J = 7Hz, 3H, (C-2)CH_3]; 0.90 [s, 9H, SiC(CH_3)$_3$]; 0.85 [s, 9H, SiC(CH_3)$_3$]; 0.00 [2 x s, Si(CH_3)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 164.24 [C-4 methylcytosine]; 156.42 [C-2 methylcytosine]; 138.37 [C-6 methylcytosine]; 105.82 [C-5 methylcytosine]; 70.72 [C-4]; 60.19 [C-1]; 59.64 [CH_2OSi]; 55.69 [C-3]; 38.35 [C-5]; 38.13 [C-2]; 25.13 [SiC(CH_3)$_3$]; 25.02 [SiC(CH_3)$_3$]; 17.47 [SiC(CH$_3$)$_3$]; 17.18 [SiC(CH$_3$)$_3$]; 15.60 [(C-2)CH_3]; 12.54 [(C-5)CH_3 methylcytosine]. *EI-MS:* ($C_{24}H_{47}N_3O_3Si_2$ 481.771): 424 (100) [M - C(CH$_3$)$_3$].

*(1S, 2S, 3R, 4S)-N-{[4-(*tert.*-Butyl-dimethyl-silanyloxy)-3-(*tert.*-butyl-dimethyl-silanyloxymethyl)-2-methyl-cyclopentyl]-5-methyl-2-oxo-1,2-dihydro-pyrimidin-4-yl}-benzamide* (**33**)
To a solution of **32** (0.892 g, 1.85 mmol) in ether (20 ml) were added Et_3N (0.425 ml, 3.07 mmol), benzoylchloride (0.258 ml, 2.22 mmol), and a catalytic amount of DMAP. The solution was stirred at RT for 1h, diluted with 100 ml of ether, washed twice with 50 ml of sat. aqu. $NaHCO_3$ and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by FC in CH_2Cl_2 (0.1% Et_3N) → CH_2Cl_2/ether 19/1 (0.1% Et_3N) to give 0.988 g of **33** (91%) as a white foam.
33: *^1H NMR* (250 MHz, $CDCl_3$). δ = 13.30 [s, br, 1H, N*H*]; 8.30 [d, J = 8Hz, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.50 - 7.35 [m, 3H, *H*-ar (Bz)]; 7.10 [s, 1H, (C-6)*H* methylcytosine]; 4.80 [q, J = 10Hz, 1H, (C-1)*H*]; 4.25 [q, 1H, (C-4)*H*]; 3.75 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 3.60 [dd, J = 10Hz, J < 4Hz, 1H, CH_2OSi]; 2.10 [s, 3H, (C-5)CH_3 methylcytosine]; 2.10 - 1.85 [m, 3H]; 1.60 [m, 1H]; 1.05 [d, J = 7Hz, 3H, (C-2)CH_3]; 0.85 [s, 9H, SiC(CH_3)$_3$]; 0.80 [s, 9H, SiC(CH_3)$_3$]; 0.00 [2 x s, Si(CH_3)$_2$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 179.28 [*C*(O)NH]; 159.36 [C-4 methylcytosine]; 148.68 [C-2 methylcytosine]; 137.84 [C-6 methylcytosine]; 137.09 [*C*q-ar (Bz)]; 132.08 [*C*H-ar (Bz, *para* to C=O]; 129.63; 127.85 [*C*H-ar (Bz)]; 111.84 [C-5 methylcytosine]; 71.34 [C-4]; 61.18 [C-1]; 60.20 [CH_2OSi]; 56.24 [C-3]; 39.56 [C-5]; 38.50 [C-2]; 25.73 [SiC(CH_3)$_3$]; 25.60 [SiC(CH_3)$_3$]; 18.07 [SiC(CH$_3$)$_3$]; 17.77 [SiC(CH$_3$)$_3$]; 16.28 [(C-2)CH_3]; 13.45 [(C-5)CH_3 methylcytosine]. *EI-MS:* ($C_{31}H_{51}N_3O_4Si_2$ 585.875): 528 (100) [M - C(CH$_3$)$_3$].

(1S, 2S, 3R, 4S)-N-[(4-Hydroxy-3-hydroxymethyl-2-methyl-cyclopentyl)-5-methyl-2-oxo-1,2-dihydro-pyrimidin-4-yl]-benzamide (**34**)
To a solution of **33** (0.960 g, 1.64 mmol) in THF (20 ml) was added 1M tetrabutylammonium fluoride/THF (6.56 ml, 6.56 mmol) and the solution was stirred for 2h at 40°. After addition of silica gel (5 g, 0.04 μ) the solvent was evaporated and the material adsorbed to the silica gel was purified by FC in AcOEt/MeOH 4/1 to give 0.580 g of impure **34**. This material was dissolved in hot MeOH (10 ml), water was added until the solution became turbid, and the mixture was kept at RT for 18h. Filtration provided 0.446 g of **34** (76%) as white crystals.
34: *^1H NMR* (250 MHz, DMSO-d_6). δ = 8.20 [d, br, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.90 - 7.35 [s, br, 1H, *H*-ar (Bz, *para* to C=O)]; 7.60 - 7.40 [m, 3H, *H*-ar (Bz) + (C-6)*H* methylcytosine]; 4.80 [d, J = 5Hz, (C-4)O*H*]; 4.70 [m, 1H, (C-1)*H*]; 4.60 [t, J < 4Hz, CH$_2$O*H*]; 4.05 [m, br, 1H, (C-4)*H*]; 3.50 [m, 2H, CH_2OH]; 2.10 - 1.85 [m, 6H, incl. s at 2.05 ((C-5)CH_3 methylcytosine)]; 1.50 [m, 1H]; 0.95 [d, J = 7Hz, 3H, (C-2)CH_3]. *^{13}C NMR* (62.5 MHz, DMSO-d_6). δ = 178.07 [*C*(O)NH]; 159.31 [C-4 methylcytosine]; 148.33 [C-2 methylcytosine]; 140.79 [C-6 methylcytosine]; 136.84 [*C*q-ar (Bz)]; 132.30 [*C*H-ar (Bz, *para* to C=O]; 129.21; 128.17 [*C*H-ar (Bz)]; 110.13 [C-5 methylcytosine]; 69.88 [C-4]; 60.88 [C-1]; 60.55 [CH_2OH]; 55.92 [C-3]; [C-5, C-2 underneath DMSO signal around 40]; 16.30 [(C-2)CH_3]; 12.64 [(C-5)CH_3 methylcytosine]. *EI-MS:* ($C_{19}H_{23}N_3O_4$ 357.387): 357 (36) [M^+].

(1S, 2S, 3R, 4S)-N-{{3-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4-Hydroxy-2-methyl-cyclopentyl}-5-methyl-2-oxo-1,2-dihydro-pyrimidin-4-yl}-benzamide (**35**)

To a solution of **34** (0.420 g, 1.18 mmol) in pyridine (12 ml) were added Et_3N (0.229 ml, 1.65 mmol), DMTr-Cl (0.478 g, 1.41 mmol), and a catalytic amount of DMAP, and the mixture was stirred at RT for 3h. It was then diluted with 100 ml of ether and extracted three times with 50 ml of H_2O. The combined aqueous extracts were once back-extracted with 50 ml of ether. The combined organic extracts were then dried ($MgSO_4$), the solvent was evaporated, and the residue was twice co-evaporated with toluene. Purification by FC in 0.1% Et_3N/ether and re-chromatography of impure fractions in the same solvent system in total furnished 0.704 g of **35** (90%) as a white foam.

35: *^1H NMR* (250 MHz, $CDCl_3$). δ = 8.30 [d, J = 9Hz, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.55 - 7.20 [m, 12H, *H*-ar (Bz + DMTr)]; 7.10 [s, 1H, (C-6)*H* methylcytosine]; 6.85 [d, J = 7Hz, 4H, *H*-ar (DMTr, *ortho* to OCH_3)]; 4.70 [q, J = 9Hz, 1H, (C-1)*H*]; 4.30 [q, br, J = 6Hz, 1H, (C-4)*H*]; 3.80 [s, 6H, OCH_3]; 3.50 [dd, J = 9Hz, J < 4Hz, 1H, CH_2ODMTr]; 3.15 [dd, J = 9Hz, J = 7Hz, 1H, CH_2ODMTr]; 2.65 [d, br, (C-4)O*H*]; 2.15 - 2.05 [m, 5H, incl. s at 2.10 ((C-5)CH_3 methylcytosine)]; 1.95 - 1.70 [m, 2H]; 0.95 [d, J = 6Hz, 3H, (C-2)CH_3]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 179.54 [*C*(O)NH]; 159.52 [C-4 methylcytosine]; 158.56 [*C*q-ar (DMTr, *C*-OCH_3]; 148.77 [C-2 methylcytosine]; 144.7 [*C*q-ar (DMTr, *C*-CH(PhOCH$_3$)$_2$)]; 138.32 [C-6 methylcytosine]; 137.18 [*C*q-ar (Bz)]; 135.93; 135.69 [2x *C*q-ar (DMTr, *para* to OCH_3]; 132.36 [*C*H-ar (Bz, *para* to C=O]; 129.95; 129.84; 128.08; 128.00; 126.95 [*C*H-ar (Bz + DMTr)]; 113.23 [*C*H-ar (DMTr, *ortho* to OCH_3)]; 112.22 [C-5 methylcytosine]; 86.59 [*C*q (DMTr, *C*Ar$_3$)]; 74.11 [C-4]; 64.06 [*C*H$_2$ODMTr]; 61.73 [C-1]; 55.22 [O*C*H$_3$]; 53.79 [C-3]; 40.28 [C-2]; 38.06 [C-5]; 16.25 [(C-2)*C*H$_3$]; 13.62 [(C-5)*C*H$_3$ methylcytosine].

(1S, 2R, 3S, 4S)-Diisopropyl-phosphoramidous acid 4-(4-benzoylamino-5-methyl-2-oxo-2H-pyrimidin-1-yl)-2-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-3-methyl-cyclopentyl ester (**36**)

To a solution of diisopropylammonium tetrazolide[29] (0.217 g, 1.26 mmol) in CH_2Cl_2 (11 ml) was added dropwise 2-cyanoethoxy-bis(diisopropylamino)-phosphine (0.353 g, 1.17 mmol) followed by a solution of **35** (0.700 g, 1.06 mmol) in CH_2Cl_2 (11 ml). The mixture was stirred at RT for 3h and then poured into 50 ml CH_2Cl_2/50 ml sat. aqu. $NaHCO_3$. The organic layer was separated and the aqueous solution was two times extracted with 50 ml of CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by FC in toluene/AcOEt 1/1 (0.1% Et_3N) to give 0.735 g of **36** as a white foam. This material was dissolved in CH_2Cl_2 (2 ml) and carefully poured into pentane (70 ml, 0°, vigorous stirring), which provided 0.575 g of **36** (75%) as an amorphous white solid.

36 (mixture of two diastereoisomers) *^1H NMR* (250 MHz, $CDCl_3$).: δ = 13.30 [s, br, 1H, N*H*]; 8.30 [d, br, 2H, *H*-ar (Bz, *ortho* to C=O)]; 7.55 - 7.35 [m, 13H, *H*-ar (Bz, DMTr) + (C-6)*H* methylcytosine]; 6.85 [d, J = 7Hz, 4H, *H*-ar (DMTr, *ortho* to OCH_3)]; 4.85 [m, 1H, (C-4)*H*]; 4.65, 4.55 [2x m, 1H, (C-1)*H*]; 3.90 - 3.50, 3.55 [2x m, incl. s at 3.80, 10H, 1H, O*C*H$_3$ + *C*H$_2$ODMTr + POC*H*$_2$ + NC*H*(CH$_3$)$_2$]; 3.20 [m, 1H, NC*H*(CH$_3$)$_2$]; 2.60, 2.50 [2x t, J = 6Hz, 2H, *C*H$_2$CN]; 2.35 - 2.00 [m, 6H, incl. "d" at 2.10 ((C-5)*C*H$_3$ methylcytosine)]; 1.85 [m, 1H]; 1.25 - 1.05 [m, 12H, NCH(*C*H$_3$)$_2$]; 0.95 [d, J = 6Hz, 3H, (C-3)*C*H$_3$]. *^{13}C NMR* (62.5 MHz, $CDCl_3$). δ = 179.53 [*C*(O)NH]; 159.60 [C-4 methylcytosine]; 158.48 [*C*q-ar (DMTr, *C*-OCH_3]; 148.46 [C-2 methylcytosine]; 145.00 [*C*q-ar (DMTr, *C*-CH(PhOCH$_3$)$_2$)]; 138.29 [C-6 methylcytosine]; 137.30 [*C*q-ar (Bz)]; 136.18; 136.10; 136.02 [2x *C*q-ar (DMTr, *para* to OCH_3]; 132.31 [*C*H-ar (Bz, *para* to C=O]; 130.16; 130.11; 129.85; 126.25; 128.18; 128.08; 127.83; 126.83 [*C*H-ar (Bz + DMTr)]; 117.75 [*C*N]; 112.09 [*C*H-ar (DMTr, *ortho* to OCH_3)]; 112.31, 112.23 [C-5 methylcytosine]; 86.06, 85.99 [*C*q (DMTr, *C*Ar$_3$)]; 74.22, 73.98, 73.37, 73.12 [C-1]; 61.39, 61.25 [C-4]; 60.58 [*C*H$_2$OH]; 58.25, 57.95, 57.58 [POC*H*$_2$]; 55.24, 55.21 [O*C*H$_3$]; 53.89 [C-2]; 43.23, 43.03, [NC*H*(CH$_3$)$_2$]; 40.20, 39.77 [C-3]; 38.43, 38.04 [C-5]; 24.78; 24.66; 24.55; 24.43 [NCH(*C*H$_3$)$_2$]; 20.65, 20.28 [*C*H$_2$CN]; 16.65, 16.52 [(C-3)*C*H$_3$]; 13.53 [(C-5)*C*H$_3$ methylcytosine]. *^{31}P NMR* (101.3 MHz, $CDCl_3$): 147.99, 147.863.

Oligonucleotide Synthesis. Oligonucleotides were synthesized on an ABI 394b DNA synthesizer using long-chain alkylamino controlled pore glass (CPG, 500 Å) and standard phosphoramidite chemistry.[32] Bases

were protected by benzoyl (dC, dA) and isobutyryl (dG) groups. Extended coupling times of 10 - 12 min were employed for modified phosphoramidites **24**, **28**, **29**, and **36**, and no significant reduction in coupling efficiency was observed under these conditions. After completion of chain assemblage the 5'-O-DMTr-protected product was released from the support with concomitant cleavage of all protecting groups by treatment of the support-bound oligonucleotide with conc. aqu. NH_3 for 16h at 55°. The 5'-O-DMTr-protected products were purified by RP-HPLC (4.6 x 200 mm *Hyposil* RP C-18 column; gradient from from 15% B to 45% B over 60 min; A = 50 mM triethylammonium acetate, pH 7; B = 50 mM triethylammonium acetate, pH 7, in 70% aqu. acetonitrile) and the 5'-O-DMTr group was subsequently removed by treatment with 80% aqu. acetic acid for 30 min at RT. The deprotection mixture was evaporated to dryness and the residue was twice co-evaporated with EtOH/H_2O 2/1. It was then redissolved in 2 ml of H_2O, extracted twice with ether and the solution was lyophilized. According to gel electrophoresis or capillary electrophoresis (CE) the fully deprotected oligonucleotides were at least 95% pure. The structures of sequences **A** as well as **D** containing 6'-α-methyl substituted building blocks were additionally confirmed by MALDI-TOF MS.[37] **A**-6'-α-CH_3: Found: 4431.6; calc. [M - H]: 4433.0. **D**-6'-α-CH_3: Found: 4755.4; calc. [M - H]: 4755.9 (3'-monophosphate, *cf. Table 1*). **A**-6'-α-CH_2OH: Found: 4452.4; calc. [M - H]: 4449.0.

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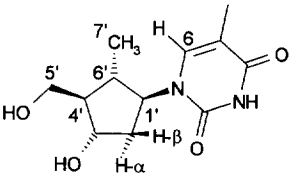
19. In addition, strong NOE's of about equal intensity were observed between C(7')H_3 and (C-1')*H* as well as between (C-7')H_3 and (C-4')*H*, respectively.



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Strong NOE's were also detected between (C-6)*H* of the thymine base and (C-2')*H*-β and (C-6')*H*, respectively. Finally an NOE was observed between (C-1')*H* and (C-2')*H*-α, whereas none was detectable between (C-1')*H* and (C-2')*H*-β, clearly indicating a *cis* relationship between (C-1')*H* and (C-2')*H*-α.

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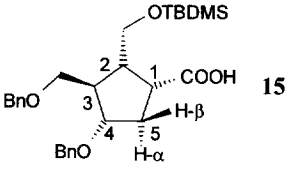
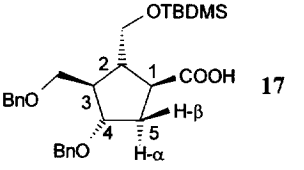
24. In principle, **14** can be isolated by flash chromatography on silica gel and in two separate experiments was obtained in 66% (7.25 g scale) and 46% (2.7 g scale) yield after chromatographic purification. However, as purificaton of **14** was always accompanied by the formation of **12** and several (unidentified) decomposition products (in a third experiment **14** could not be recovered intact from the column at all), the conversion of **12** into **16** without purification of intermediates proved to be the most efficient strategy for the prepartion of the latter compound. Upon standing at RT pure **14** slowly reverted to **12**.
25. The assignment of relative stereochemistry in **17** was based on NOE NMR experiments. Although the relative (and also absolute) configuration in **15** directly follows from the configuration of lactone **2**, for comparative purposes it was also additionally confirmed by NOE measurements.



15



17

For **17** irradiation of (C-1)*H* led to strong NOE's on (C-2)CH_2OTBDMS and also (C-5)*H*-α, whereas no NOE was observed between (C-1)*H* and (C-5)*H*-β. In contrast, for **15** a strong NOE was observed between (C-1)*H* and (C-5)*H*-β, but none was detected for (C-1)*H* and (C-5)*H*-α. The intensity of the (C-1)H/(C-5)H-β NOE in **15** is comparable to that between (C-4)*H* and (C-5)*H*-β, clearly indicating a *cis* relationship for the former pair of protons. While a NOE was in fact observed between (C-1)*H* and (C-2)CH_2OTBDMS

also in **15**, it was of much lower intensity than the one found in **17**. In addition, a strong NOE between (C-4)*H* and (C-1)*H* was observed in **15**, while this NOE was absent in **17**.

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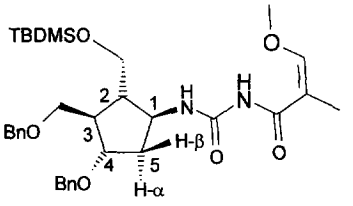
27. The relative stereochemistry of acryloyl-urea **20** was again confirmed by NOE NMR experiments.



A strong NOE was observed between (C-1)N*H* and (C-2)*H*, which is strongly indicative of a *trans* relationship between the ureido-group and the (C-2)CH_2OTBDMS substituent. In addition, strong NOE's were also detected between (C-2)CH_2OTBDMS and (C-1)*H* as well as between (C-1)*H* and (C-5)*H*-α. No NOE was observable between (C-1)*H* and (C-5)*H*-β. It should also be noted that the qualitatively similar effects of 6'-α-methyl and 6'-α-hydroxymethyl carbocyclic nucleosides on the RNA-binding affinity of the corresponding oligodeoxyribonucleotides (*vide supra*) strongly suggest that they exhibit the same stereochemistry at C-1' (nucleoside nomenclature, *cf. Figure 1*). As indicated in the text, the β-configuration of 6'-α-methyl carbocyclic thymidine has been rigorously established by X-ray crystallography of a modified DNA/DNA duplex incorporating two thymidine units in each strand.[20] Regarding the configuration of the double bond in **20**, this was not explicitly determined. However, based on data that we have obtained for related compounds the stereochemistry is likely to be as drawn.

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35. In *in vitro* experiments using 10% heat inactivated fetal calf serum as nuclease source 5'-TCCAGGTGTCCG**ttt**C-3' (**t** = 6'-α-methyl carbocyclic thymidine) was found to be 14 times more stable to degradation by 3'-exonucleases than the unmodified (phosphodiester based) parent oligonucleotide.
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