Synthesis of the Anticodon Hairpin $^{t}RNA_{f}^{Met}$ Containing *N*-{[9-(β-D-Ribofuranosyl)-9*H*-purin-6-yl]carbamoyl}-L-threonine (= N^6-{{[(1*S*,2*R*)-1-Carboxy-2-hydroxypropyl]amino}carbonyl}adenosine, t^6A)

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Dedicated to Prof. Dr. *Frank Seela* on the occasion of his 60th birthday

As part of our studies on the structure of yeast $^{t}RNA_{f}^{Met}$, we investigated the incorporation of *N*-{[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]carbamoyl}-L-threonine (t^6A) in the loop of a RNA 17-mer hairpin. The carboxylic function of the L-threonine moiety of t^6A was protected with a 2-(4-nitrophenyl)ethyl group, and a (*tert*-butyl)dimethylsilyl group was used for the protection of its secondary OH group. The 2′-OH function of the standard ribonucleotide building blocks was protected with a [(triisopropylsilyl)oxy]methyl group. Removal of the base-labile protecting groups of the final RNA with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and then with $MeNH_2$ was done under carefully controlled conditions to prevent hydrolysis of the carbamate function, leading to loss of the L-threonine moiety.

Introduction. – ^{t}RNAs contain a whole series of modified nucleotides which influence their structure and function [1]. The exact influence of the modified nucleotide on ^{t}RNA structure and on the recognition by ^{t}RNA synthetase is largely unknown. This situation is changing slowly because of both the progress in synthetic methodologies in obtaining RNA and the availability of high-resolution NMR instruments.

As a part of our studies on the structure of yeast $^{t}RNA_{f}^{Met}$, we plan to determine the solution conformation of the anticodon hairpin of this yeast ^{t}RNA. The nucleotide sequence of *Saccharomyces cerevisiae* $^{t}RNA_{f}^{Met}$ in the cloverleaf form is depicted in *Fig. 1*. [2] This $^{t}RNA^{Met}$ was purified from baker's yeast, and it can be converted to the formylated methionyl-$^{t}RNA_{f}$ by extracts of *E. coli* [2]. The same anticodon hairpin is also found in the initiator ^{t}RNA of *Torulopsis utilis* [3], a yeast belonging to *Fungi imperfecti*.

Both loop structures contain the modified-adenosine nucleoside t^6A (= *N*-{[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]carbamoyl}-L-threonine in the position next to the anticodon, t^6A representing an adenosine nucleoside substituted at the N^6-position with a L-threonine moiety *via* a carbonyl function [4]. Besides the occurrence in the above mentioned ^{t}RNA, a *N*-(9*H*-purin-6-ylcarbamoyl)-L-threonine ribonucleotide has also been discovered in several other ^{t}RNAs [5–10] and has been isolated from human urine [11][12]. The enzymatic synthesis of *N*-(9*H*-purin-6-ylcarbamoyl)-L-threonine riboside has been investigated [13][14]. The structure of *N*-(9*H*-purin-6-

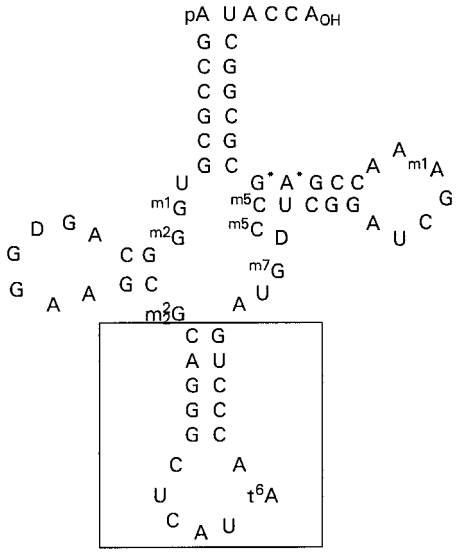


Fig. 1. *Nucleotide sequence of yeast* $^{t}RNA_{f}^{Met}$ *showing the hairpin structure with* $t^{6}A$ *in the position next to the anticodon*

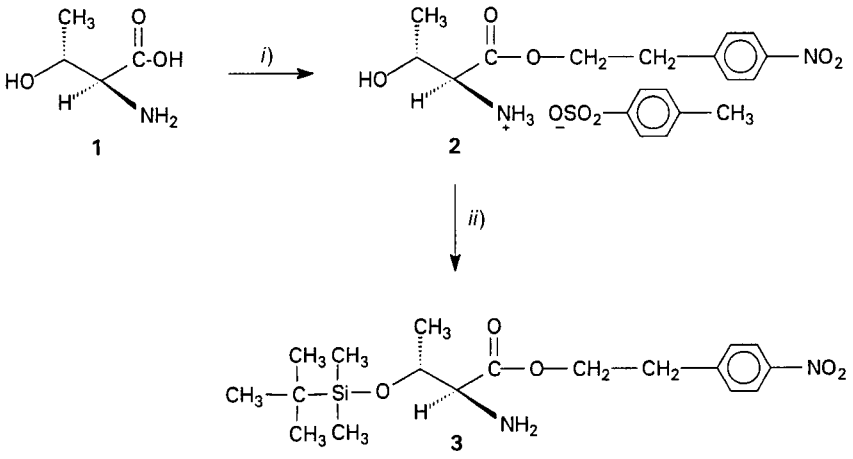
ylcarbamoyl)-L-threonine riboside was confirmed by X-ray diffraction [15][16]. It was observed that ^{t}RNAs with codons starting with adenine mostly contain $t^{6}A$ next to the 3′-side of the anticodon [6], and $t^{6}A$ is thought to stabilize U·A and U·G anticodon·codon base pairs being formed adjacent to the 5′-side of the modified nucleoside [17]. It was observed that $t^{6}A$-deficient ^{t}RNA is significantly less efficient in binding to ribosomes as compared to normal ^{t}RNA, and that $t^{6}A$ is required for proper codon·anticodon interaction [18]. Another study points to the importance of $t^{6}A$ in the recognition of the anticodon loop of $^{t}RNA_{1}^{Ile}$ by isoleucyl-^{t}RNA synthetase from *E. coli* [19]. NMR Studies point to the importance of $t^{6}A$ as a binding site for magnesium ion in ^{t}RNA [20][21].

The synthetic challenge for the incorporation of $t^{6}A$ into RNA is situated in selecting an appropriate way to synthesize the modified nucleosides and to introduce protecting groups which are stable during RNA synthesis and can be removed easily at the end of the synthesis.

Results and Discussion. – *Synthesis.* The naturally occurring nucleoside *N*-{[(9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]carbamoyl}-L-threonine ($t^{6}A$) has been synthesized before [22–26]. *Chheda* and *Hong* described the displacement of the ethoxy group of ethyl [9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purin-6-yl]carbamate with L-threonine followed by deprotection of the acetyl groups with NH_3/MeOH [22][25]. The same reaction scheme was used later by *Martin* and *Schlimme* [26]. The alternative of using an isocyanate intermediate of protected L-threonine was less successful [22]. As the α-amino group of L-threonine is more nucleophilic than the 6-amino group of the adenine base, we investigated the inverted approach and converted the protected adenosine to a N^{6}-isocyanate derivative.

The selection of the protecting groups for L-threonine is based on the following considerations: they should *a*) be stable during oligonucleotide synthesis, *b*) be easily removable after oligonucleotide synthesis with standard reagents, *c*) in the case of the carboxylic-acid protecting group, be removable before the ammonia-deprotection step of the oligonucleotide thus avoiding amide formation, and *d*) be removable without racemization of the L-amino acid. Therefore, we selected the (*tert*-butyl)dimethylsilyl group (^{t}BuMe$_2$SiCl) for protecting the secondary OH function of L-threonine and the 2-(4-nitrophenyl)ethyl group as a protecting group for the carboxylic acid. The latter protecting group can be introduced and removed without causing racemizing side reactions [27]. L-Threonine (**1**) was converted into its 2-(4-nitrophenyl)ethyl ester and isolated as *p*-toluenesulfonate salt **2** (*Scheme 1*) [27]. Protection of the secondary OH group with (*tert*-butyl)dimethylsilyl chloride in pyridine catalyzed by 1*H*-imidazole yielded the protected L-threonine **3**.

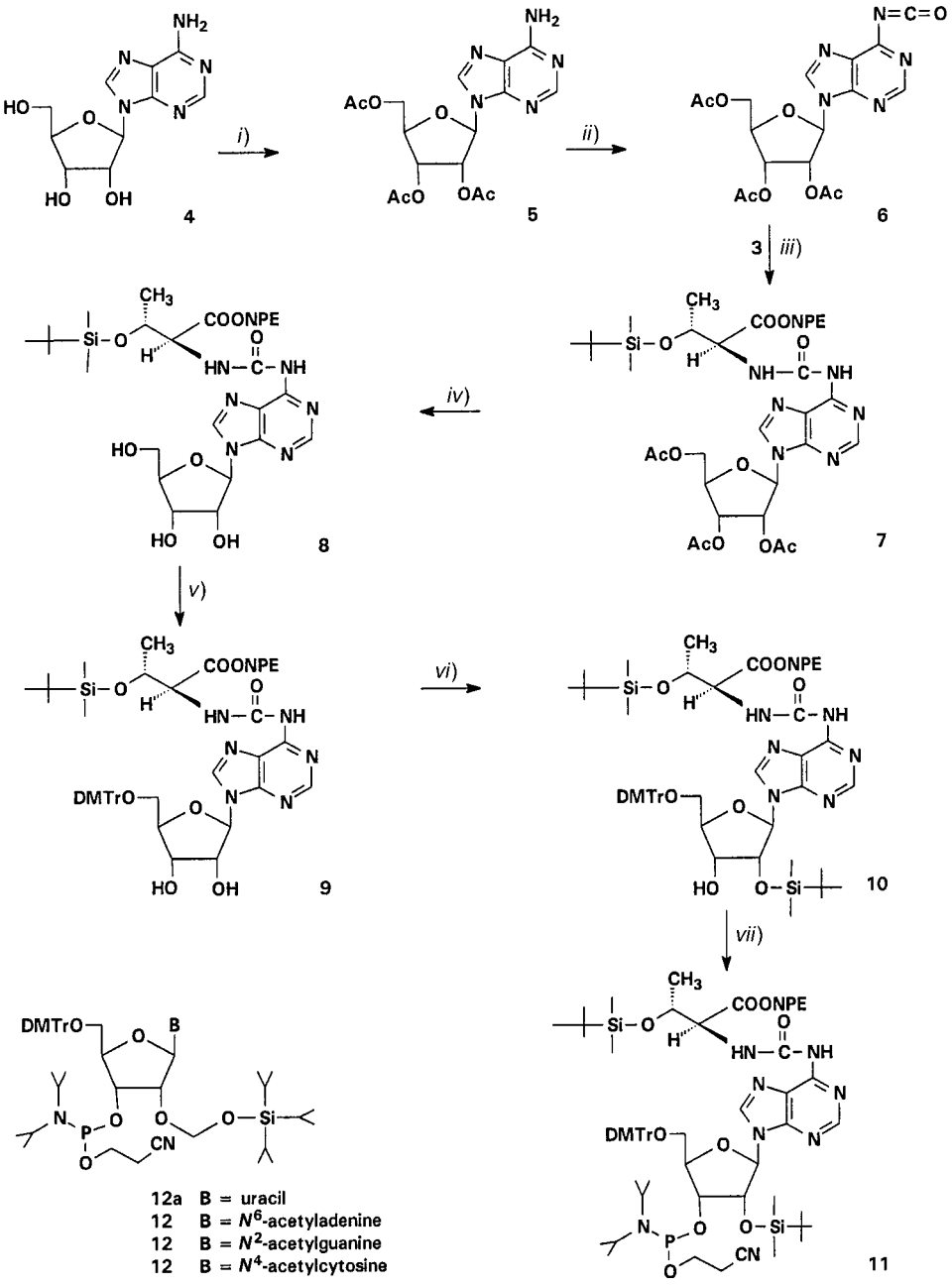
Scheme 1. *Synthesis of Protected* L-*Threonine*



i) 4-$NO_2C_6H_4(CH_2)_2OH$, TsOH, 105°, 17 h; 63%. *ii*) ^{t}BuMe$_2$SiCl, Py, 1*H*-imidazole, r.t., 17 h; 95%.

The OH groups of adenosine were first protected as acetates ($\rightarrow$ **5**; *Scheme 2*) [26]. The 6-NH_2 function of the protected adenosine **5** was reacted wtih triphosgene under reflux. The isocyanate **6** was not isolated but was reacted directly with the protected L-threonine **3** to give **7** in low yield (19%). The acetyl groups of **7** could selectively be removed with methanolic ammonia solution at room temperature without concomitant conversion of the 2-(4-nitrophenyl)ethyl ester to an amide function ($\rightarrow$ **8**). Subsequent protection of the primary OH group with 4,4′-dimethoxytrityl chloride ((MeO)$_2$TrCl) in pyridine ($\rightarrow$ **9**; 94% yield), followed by reaction with ^{t}BuMe$_2$SiCl in THF in the presence of $AgNO_3$ and pyridine gave the 2′-*O*-silylated nucleoside derivative **10** (44% yield), besides the undesired 3′-*O*-silylated compound (30% yield). The modified building block **11** for oligonucleotide synthesis was obtained by phosphitylation of the 3′-OH group of according to **10** standard procedures as described before [28].

Scheme 2. *Synthesis of the Protected t^6A Phosphoramidites and Structure of the Protected Regular Nucleoside Phosphoramidites Used for Oligonucleotide Synthesis*



NPE = 4-NO_2–C_6H_4–CH_2CH_2, DMTr = (MeO_2)Tr, TOM = $(^iPr)_3SiOCH_2$

i) Ac_2O, Py, r.t., 17 h; 95%. *ii*) triphosgene, MeC_6H_5, 120°, 4 h. *iii*) MeC_6H_5, 80°, 17 h; 19% (for *ii*) and *iii*)). *iv*) NH_3, MeOH, r.t., 3 h; 100%. *v*) $(MeO)_2TrCl$, Py, CH_2Cl_2, 17 h, r.t.; 94%. *vi*) ^{t}BuMe$_2$SiCl, $AgNO_3$, THF, Py, 17 h, r.t.; 44%. *vii*) $^iPr_2EtN$, CH_2Cl_2, $(^iPr_2N)(OCH_2CH_2CN)PCl$, r.t.; 72%.

Oligoribonucleotide Synthesis and Characterization. Oligoribonucleotide synthesis is currently carried out with a 2′-*O*-(*tert*-butyl)dimethylsilyl protecting group. Due to the bulkiness of this protecting group, coupling times are rather long and coupling yields are lower than in oligodeoxyribonucleotide synthesis. Therefore, we used the recently developed [(triisopropylsilyl)oxy]methyl ((^{i}Pr)$_3$SiOCH$_2$; TOM) group to protect the 2′-OH function of the standard nucleosides (see amidites **12a**–**d**), allowing an efficient chemical synthesis of RNA. The (^{i}Pr)$_3$SiOCH$_2$ protecting group allowed us to carry out RNA synthesis with short coupling times (2 min) and high coupling yields (> 99%). The building blocks were used in 0.1M concentrations and were activated with 1-(benzylthio)-1*H*-tetrazole. Thus, a 17-mer corresponding to the anticodon loop of ^{t}RNA$_f^{Met}$ was synthesized in which the adenine base at the 11th position was replaced by the *N*-{[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]carbamoyl}(5′-CAGGGCU-CAU**t^{6}A**ACCCUG-3′). After '(MeO)$_2$Tr-off' synthesis, the solid-phase material was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in THF to remove the 2-(4-nitrophenyl)ethyl protecting groups, and then with MeNH$_2$ in EtOH/H$_2$O. The (^{i}Pr)$_3$SiOCH$_2$ groups were removed with Bu$_4$NF in THF. The oligoribonucleotide was desalted on a *Sephadex* (*G*10) column, purified on a *Dionex-Nucleopac-PA-100* column, and desalted again. The deprotection with DBU was necessary to obtain the correct final material; when this deprotection step was skipped and the protected oligoribonucleotide directly treated with MeNH$_2$, the methyl carboxamide of the t^{6}A insert was obtained. More vigorous deprotection conditions led to loss of the whole L-threonine moiety by hydrolysis of the carbamate function.

The modified RNA 17-mer was desalted with cation-exchange beads before it was submitted to analysis by mass spectrometry. *Fig. 2*,*a*, shows the ESI-MS with several peaks corresponding to multiply charged ions of the sample in the charge states [*M* – 4 H]$^{4-}$ to [*M* – 11 H]$^{11-}$. The Max Ent 1 processed spectrum (*Fig. 2*,*b*) indicates the experimental monoisotopic mass to be 5527.91 Da, confirming the identity of the RNA 17-mer (calc. 5527.80 Da).

Before starting NMR experiments, we determined the concentration-dependent T_m of the synthesized oligonucleotide. As can be seen in *Fig. 3*, the thermal stability of the oligomer did not change with increasing concentration of the oligonucleotide, indicating formation of a stable hairpin structure. Both T_m in 0.1M NaCl (64°) and the shape of the curve were independent of oligonucleotide concentration.

Conclusion. – The modified nucleoside. *N*-{[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]carbamoyl}-L-threonine (t^{6}A) was successfully incorporated into the loop of a hairpin RNA. The (*tert*-butyl)dimethylsilyl group was used for the protection of the secondary OH group of the modified nucleosides. The carboxy group was protected with a 2-(4-nitrophenyl)ethyl group. The 2′-*O*-{[(triisopropylsilyl)oxy]methyl}-protected regular nucleoside phosphoramidites were used as building blocks for RNA synthesis. The integrity of the final hairpin was established by ESI-MS. Thermal-stability studies indicated the stability of the RNA hairpin, which is now used for structural studies.

This research was supported by a grant for the K.U. Leuven (GOA 97/11). *Arthur Van Aerschot* is a research associate of the *Flemish Fund of Scientific Research*. We are grateful to Dr. *Jan Claereboudt*,

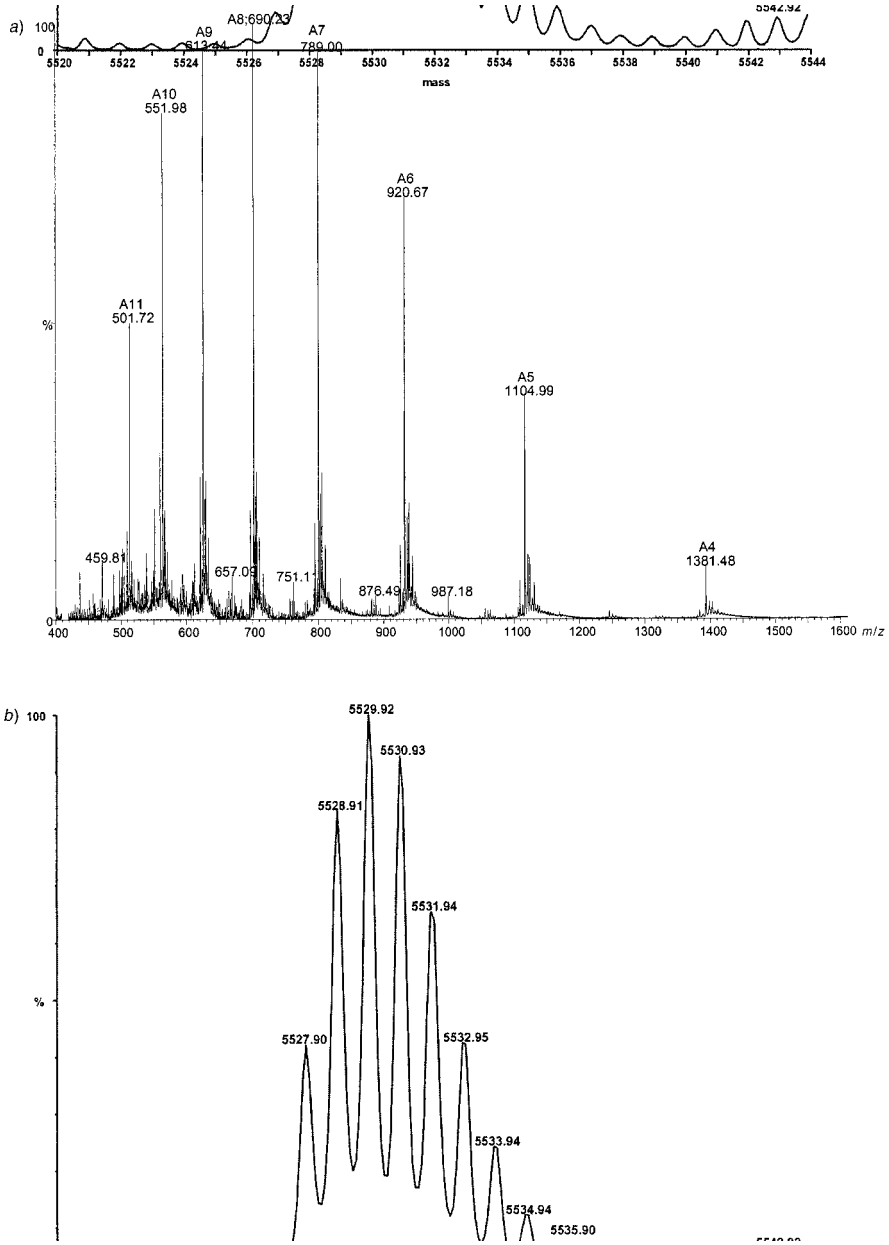


Fig. 2. a) *ESI-Mass spectrum of the RNA 17-mer corresponding to the anticodon loop of* $^{t}RNA_{f}^{Met}$ *in which the adenine base at position 11 is replaced by* N-*[[9-(β-*D*-ribofuranosyl)-9*H*-purin-6-yl]carbamoyl]-*L*-threonine* ($t^{6}A$) *and* b) *its Max Ent 1 processed spectrum, indicating the experimental monoisotopic mass.* See *Exper. Part* for details.

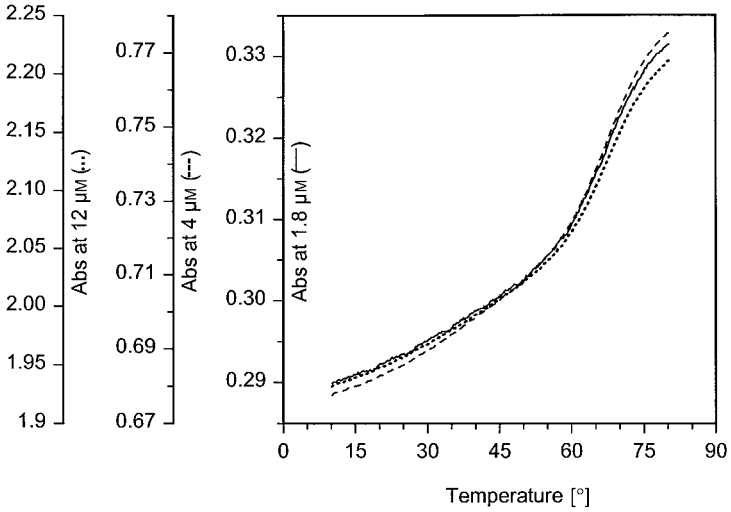


Fig. 3. *Concentration-dependent* T_m *of the RNA hairpin with incorporated t⁶A*

Micromass, Belgium, for all the efforts in providing us with the Q-TOF mass spectra. We thank *Chantal Biernaux* for editorial help.

Experimental Part

General. Anh. solvents were obtained as follows: DMF was dried over molecular sieves; CH_2Cl_2 was stored over P_2O_5, refluxed, and distilled; THF was stored over Na/benzophenone, refluxed, and distilled; pyridine was stored over CaH_2 refluxed, and distilled. Column chromatography (CC): *Acros* silica gel (0.060–0.200 nm). TLC: precoated *Macherey-Nagel Alugram SIL G/UV*$_{254}$ plates. ^{1}H- and ^{13}C-NMR Spectra: *Varian-Gemini-200* spectrometer; δ in ppm rel. to $SiMe_4$ as internal standard, J in Hz; the tyrosine numbering is used for the side chain at NH_2–C(6) of adenosine in **7**–**11**. Liquid secondary-ion mass spectra (LSI-MS): Cs^+ as primary-ion beam *Kratos-Concept-IH* (*Kratos;* Manchester, UK); solns. in NBA (= 3-nitrobenzyl alcohol), THGLY (= thioglycerol), of NPOE (= 2-nitrophenyl octyl ether); acceleration of secondary ions at 6 ZV; scans at 10 s/decade from *m*/*z* 1500 down to *m*/*z* 50. ESI-MS. *Micromass-Q-TOF* mass spectrometer (*Whytenshawe*, Manchester, UK) fitted with a standard electrospray-ion source.

L-*Threonine 2-(4-Nitrophenyl)ethyl Ester 4-Methylbenzenesulfonate Salt* (**2**) [27]. L-Threonine (500 mg, 4.2 mmol), 2-(4-nitrophenyl)ethanol (npe-OH; 12.6 mmol, 2.11 g) and TsOH (12.6 mmol, 2.40 g) were heated in toluene (100 ml) at 105° for 17 h in a *Dean-Stark* apparatus. The soln. was cooled to r.t., and Et_2O (25 ml) was added. The oily residue was decanted, and the upper layer was removed to collect the oil. Precipitation of **2** was realized by adding to the oil MeOH (25 ml) and Et_2O (100 ml): 1.17 g (63%) of **2**, identical to the compound described in [27]. ^{1}H-NMR ((D$_6$)DMSO): 1.14 (d, J = 6.6, Me(γ)); 2.29 (s, *Me*C$_6$H$_4$); 3.10 (t, J = 6.2, C*H*$_2$CH$_2$O); 3.89 (d, J = 4.0, CH(α)); 4.0–4.1 (m, CH(β)); 4.45 (t, J = 6.2, CH$_2$C*H*$_2$O); 5.6 (br. s, OH); 7.11 (d, J = 8.2, 2 arom. H(Ts)); 7.48 (d, J = 8.2, 2 arom. H(Ts)); 7.58 (d, J = 8.8, 2 arom. H(npe)); 8.1–8.2 (m, NH_3, 2 arom. H(npe)). ^{13}C-NMR ((D$_6$)DMSO): 19.96 (Me(γ)); 20.72 (*Me*C$_6$H$_4$); 33.83 (*C*H$_2$CHO); 57.87 (CH$_2$*C*H$_2$O); 64.91 (CH(α)); 65.43 (CH(β)); 123.52 (arom. C(npe)); 125.65 (arom. C(Ts)); 128.14 (arom. C(npe)); 130.38 (arom. C(Ts)); 137.73 (arom. C(Ts)); 146.32 (2 arom. C(Ts, npe)); 146.53 (arom. C(npe)); 168.23 (COO). LSI-MS (THGLY/NBA): 483 ($[M+K]^+$).

O-*[(*tert*-Butyl)dimethylsilyl]*-L-*threonine 2-(4-Nitrophenyl)ethyl Ester* (**3**). Compound **2** (500 mg, 1.15 mmol) was dissolved in dry pyridine (30 ml) and treated with one half of ^{t}BuMe$_2$SiCl (260 mg, 3.45 mmol) and 1*H*-imidazole (120 g, 3.45 mmol). After 10 min, the second half was added, and the reaction was allowed to continue for 17 h at r.t. The mixture was diluted with CH_2Cl_2 (500 ml) and washed successively with sat. $NaHCO_3$ soln. (2 × 300 ml) and H_2O (3 × 300 ml). The org. layer was dried, evaporated, co-evaporated with toluene and MeOH and purified by CC (silica gel, 0–6% MeOH/CH_2Cl_2): 416 mg (95%) **3**. Oil. ^{1}H-NMR

((D$_6$)DMSO): −0.17, −0.06 (2*s*, 2 MeSi); 0.75 (*s*, ^{t}BuSi); 1.11 (*d*, *J* = 6.2, Me(γ)); 1.6 (br. *s*, NH$_2$); 3.06 (*t*, *J* = 6.2, C*H*$_2$CH$_2$O); 3.17 (*d*, *J* = 2.4, CH(α)); 3.9–4.4 (*m*, CH(β), CH$_2$C*H*$_2$O); 7.57 (*d*, *J* = 8.8, 2 arom. H); 8.18 (*d*, *J* = 8.8, 2 arom. H). ^{13}C-NMR ((D$_6$)DMSO): −5.44, −4.47 (2 MeSi); 17.59 (Me$_3$*C*Si); 20.42 (Me(γ)); 25.58 (*Me*$_3$CSi); 34.14 (*C*H$_2$CH$_2$O); 59.99 (CH$_2$*C*H$_2$O); 64.21 (CH(α)); 69.74 (CH(β)); 123.61 (2 arom. C); 130.50 (2 arom. C); 146.89 (2 arom. C); 174.39 (COO). LSI-MS (THGLY): 383 ([*M* + H]$^+$). Anal. calc. for C$_{18}$H$_{30}$N$_2$O$_5$Si (382.5): C 56.52, H 7.90, N 7.32; found: C 56.81, H 7.72, N 7.18.

N^6-*{{{(1S,2R)-2-{[(*tert*-Butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}adenosine 2′,3′,5′-Triacetate* (**7**). A suspension of **5** [26] (2.40 g, 6.14 mmol) in dry toluene (300 ml) was treated with triphosgene (3.64 g, 12.28 mmol), and the mixture was heated under reflux (120°) for 4 h to give crude isocyanate **6**. The soln. was evaporated and then dissolved in anh. CH$_2$Cl$_2$/toluene 1:1 (60 ml). A soln. of **3** (1.76 g, 4.60 mmol) in dry toluene (30 ml) was added dropwise to the soln. of **6**, and the reaction was continued for 17 h at 80°. The brown mixture was evaporated and directly purified by CC (silica gel, 0–5% MeOH/CH$_2$Cl$_2$): 934 mg (19%) of pure **7**. ^{1}H-NMR ((D$_6$)DMSO): −0.03, 0.07 (2*s*, 2 MeSi); 0.91 (*s*, ^{t}BuSi); 1.26 (*d*, *J* = 6.2, Me(γ)); 2.09, 2.12, 2.16 (3*s*, 3 MeCO); 3.03 (*t*, *J* = 6.2, C*H*$_2$CH$_2$O); 4.3–4.6 (*m*, CH(α), CH(β), CH$_2$C*H*$_2$O, H–C(4′), 2 H–C(5′)); 5.68 (*t*, *J* = 4.9, H–C(3′)); 6.06 (*t*, *J* = 5.4, H–C(2′)); 6.24 (*d*, *J* = 5.4, H–C(1′)); 7.30 (*d*, *J* = 8.5, 2 arom. H (npe)); 7.92 (*d*, *J* = 8.5, 2 arom. H (npe)); 8.32, 8.48 (2*s*, H–C(2), H–C(8)); 8.61 (br. *s*, NH–C(6)); 10.03 (*d*, *J* = 9.0, NH–C(α)). ^{13}C-NMR ((D$_6$)DMSO): −5.55, −4.43 (2 MeSi); 17.69 (Me$_3$*C*Si); 20.31, 20.46, 20.64 (3 *Me*CO); 21.00 (Me(γ)); 25.44 (*Me*$_3$CSi); 34.66 (*C*H$_2$CH$_2$O); 59.58 (CH$_2$*C*H$_2$O); 63.04 (CH$_2$(5′)); 64.37 (CH(α)); 68.59 (CH(β)); 70.66 (CH(3′)); 72.93 (CH(2′)); 80.43 (CH(4′)); 86.68 (CH(1′)); 121.04 (C(5)); 123.50 (2 arom. C (npe)); 129.72 (2 arom. C (npe)); 141.89 (CH(8)); 145.56 (2 arom. C (npe)); 150.42 (CH(2)); 151.45 (C(4), C(6)); 154.40 (NHCONH); 169.48, 169.69, 170.51 (3 Me*C*O); 170.99 (*C*OOCH$_2$CH$_2$). LSI-MS (THGLY): 802 ([*M* + H]$^+$). Anal. calc. for C$_{35}$H$_{47}$N$_7$O$_{13}$Si (801.9): C 52.42, H 5.91, N 12.23; found: C 52.21, H 6.02, N 12.14.

N^6-*{{{(1S,2R)-2-{[(*tert*-Butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}adenosine* (**8**). A methanolic NH$_3$ soln. (100 ml) of **7** was stirred at r.t. for 3 h. Evaporation and purification by CC (silica gel, 0–10% MeOH/CH$_2$Cl$_2$) afforded pure **8** (598 mg, quant.). ^{1}H-NMR ((D$_6$)DMSO): −0.13, 0.01 (2*s*, 2 MeSi); 0.82 (*s*, ^{t}BuSi); 1.15 (*d*, *J* = 6.2, Me(γ)); 3.04 (*t*, *J* = 6.0, C*H*$_2$CH$_2$O); 3.5–3.8 (*m*, 2 H–C(5′)); 4.0 (*m*, H–C(4′)); 4.2 (*m*, H–C(3′)); 4.2–4.5 (*m*, CH(α)), CH(β), CH$_2$C*H*$_2$O); 4.6 (*m*, H–C(2′)); 5.12 (*t*, *J* = 5.5, OH–C(5′)); 5.22 (*d*, *J* = 4.8, OH–C(3′)); 5.52 (*d*, *J* = 5.6, OH–C(2′)); 6.01 (*d*, *J* = 5.2, H–C(1′)); 7.48 (*d*, *J* = 8.6, 2 arom. H (npe)); 8.00 (*d*, *J* = 8.6, 2 arom. H (npe)); 8.36, 8.70 (2*s*, H–C(2), H–C(8)); 9.86 (*d*, *J* = 9.2, NH–C(α)); 9.93 (br. *s*, NH–C(6)). ^{13}C-NMR ((D$_6$)DMSO): −5.68, −4.50 (2 MeSi); 17.44 (Me$_3$*C*Si); 20.90 (Me(γ)); 25.36 (*Me*$_3$CSi); 33.98 (C*H*$_2$CH$_2$O); 59.03 (CH$_2$C*H*$_2$O); 61.24 (CH$_2$(5′)); 64.82 (CH(α)); 68.34 (CH(β)); 70.26 (CH(3′)); 74.11 (CH(2′)); 85.67 (CH(4′)); 88.01 (CH(1′)); 121.13 (C(5)); 123.37 (2 arom. C(npe)); 130.35 (2 arom. C(npe)); 142.52 (CH(8)); 146.68 (2 arom. C(npe)); 150.29 (C(4), C(6)); 154.06 (NHCONH); 170.84 (*C*OOCH$_2$CH$_2$). LSI-MS (THGLY): 676 ([*M* + H]$^+$). Anal. calc. for C$_{29}$H$_{41}$N$_7$O$_{10}$Si (675.8): C 51.54, H 6.12, N 14.51; found: C 51.32, H 6.17, N 14.23.

N^6-*{{{(1S,2R)-2-{[(*tert*-Butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}-5′*-O-*(4,4′-dimethoxytrityl)adenosine* (**9**). To a cooled (0°) soln. of **8** (854 mg, 1.26 mmol) in dry pyridine (15 ml), a soln. of 4,4′-dimethoxytrityl chloride (596 mg, 1.76 mmol) in dry CH$_2$Cl$_2$ (5 ml) was added dropwise. After 15 min at 0°, the mixture was allowed to warm to r.t. and the reaction continued for 17 h. MeOH (5 ml) was added, the soln. evaporated, and the residue dissolved in CH$_2$Cl$_2$ (200 ml). The org. layer was washed successively with sat. NaHCO$_3$ soln. (2 × 150 ml), brine (2 × 150 ml), and H$_2$O (2 × 150 ml), and then dried, evaporated, and co-evaporated. Purification by CC (silica gel, 0–3% MeOH/CH$_2$Cl$_2$ 1% Et$_3$N) afforded 1.16 g (94%) of **9**. ^{1}H-NMR ((D$_6$)DMSO): −0.13, 0.01 (2*s*, 2 MeSi); 0.82 (*s*, ^{t}BuSi); 1.18 (*d*, *J* = 5.8, Me(γ)); 3.03 (*t*, *J* = 6.2, C*H*$_2$CH$_2$O); 3.3 (*m*, 2 H–C(5′)); 3.70 (*s*, 2 MeO); 4.1 (*m*, H–C(4′)); 4.3–4.5 (*m*, H–C(3′), CH(α), CH(β), CH$_2$C*H*$_2$O); 4.8 (*m*, H–C(2′)); 5.26 (*d*, *J* = 5.8, OH–C(3′)); 5.61 (*d*, *J* = 5.6, OH–C(2′)); 6.03 (*d*, *J* = 4.0, H–C(1′)); 6.7–7.4 (*m*, 13 H, (MeO)$_2$*Tr*); 7.48 (*d*, *J* = 8.8, 2 arom. H (npe)); 7.99 (*d*, *J* = 8.8, 2 arom. H (npe)); 8.25, 8.59 (2*s*, H–C(2), H–C(8)); 9.88 (*d*, *J* = 9.2, NH–C(α)); 9.98 (br. *s*, NH–C(6)). ^{13}C-NMR ((D$_6$)DMSO): −5.68, −4.50 (2 MeSi); 17.44 (Me$_3$*C*Si); 20.93 (Me(γ)); 25.36 (*Me*$_3$CSi); 34.01 (C*H*$_2$CH$_2$O); 55.08 (2 MeO); 59.02 (CH$_2$*C*H$_2$O); 63.76 (CH$_2$(5′)); 64.85 (CH(α)); 68.37 (CH(β)); 70.35 (CH(3′)); 73.14 (CH(2′)); 83.34 (CH(4′)); 85.58 (CH(1′)); 88.89 ((MeO)$_2$*Tr*); 113.23, 126.77, 127.89, 129.89, 135.75, 158.24 (arom. C(MeO)$_2$*Tr*)); 120.88 (C(5)); 123.40 (2 arom. C (npe)); 130.41 (2 arom. C (npe)); 143.13 (CH(8)); 145.13 (CH(2)); 146.35, 146.74 (2 arom. C (npe)); 150.50 (C(4), C(6)); 154.11 (NHCONH); 170.93 (*C*OOCH$_2$CH$_2$). LSI-MS (THGLY) 1000 ([*M* + Na]$^+$). Anal. calc. for C$_{50}$H$_{59}$N$_7$O$_{12}$Si (978.1): C 61.40, H 6.08, N 10.02; found: C 61.21, H 5.81, N 9.83.

2′-O-*[(*tert-*Butyl)dimethylsilyl]*-N^6-*{{{(1S,2R)-2-[[*(tert-*butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}-5′*-O-*(4,4′-dimethoxytrityl)adenosine* (**10**). To a soln. of **9** (1.10 g, 1.13 mmol) in dry THF (15 ml), $AgNO_3$ (230 mg, 1.35 mmol) was added. The mixture was sonicated and stirred 10 min, before ^{t}BuMe$_2$SiCl (221 mg, 1.47 mmol) was added. After 17 h at r.t., TLC indicated incomplete reaction. Thus, more $AgNO_3$ (54 mg, 0.32 mmol), ^{t}BuMe$_2$SiCl (51 mg, 0.34 mmol), and pyridine (100 µl) were added. After 3 h more, the mixture was filtered through *Celite* into a sat. $NaHCO_3$ soln. and extracted with CH_2Cl_2 (300 ml). The org. layer was washed with sat. $NaHCO_3$ soln. (2 × 200 ml), brine (2 × 200 ml), and H_2O (2 × 200 ml), dried, and evaporated. Purification by CC (silica gel, 0–2% MeOH/$CHCl_3$ + 1% Et_3N) followed by purification with a *Chromatotron*® apparatus (4-mm layer; eluants: 35, 45, 55, 65, and 75% AcOEt/hexane) afforded pure **10** (539 mg, 44%). ^{1}H-NMR ((D$_6$)DMSO): −0.14, −0.04, −0.01 (3*s*, 4 MeSi); 0.74, 0.79 (2*s*, 2 ^{t}BuSi); 1.16 (*d*, *J* = 6.2, Me(*γ*)); 3.02 (*t*, *J* = 5.7, C*H*$_2$CH$_2$O); 3.3 (*m*, 2 H–C(5′)); 3.70 (*s*, 2 MeO); 4.1 (*m*, H–C(4′)); 4.2–4.4 (*m*, H–C(3′), CH(*α*), CH(*β*), CH$_2$C*H*$_2$O); 4.98 (*t*, *J* = 5.0, H–C(2′)); 5.19 (*d*, *J* = 5.4, OH–C(3′)); 6.04 (*d*, *J* = 4.8, H–C(1′)); 6.7–7.5 (*m*, 15 arom. H, (MeO)$_2$*Tr*, npe); 7.98 (*d*, *J* = 8.8, 2 arom. H (npe)); 8.25, 8.61 (2*s*, H–C(2), H–C(8)); 9.87 (*d*, *J* = 8.8, NH–C(*α*)); 10.02 (br. *s*, NH–C(6)). ^{13}C-NMR ((D$_6$)DMSO): −5.71, −5.35, −4.83, −4.56 (4 MeSi); 17.41, 17.84 (2 Me$_3$*C*Si); 20.87 (Me(*γ*)); 25.27, 25.58, 25.82 (2 *Me*$_3$CSi); 33.99 (*C*H$_2$CH$_2$O); 55.05 (2 MeO); 58.99 (CH$_2$*C*H$_2$O); 63.7 (CH$_2$(5′)); 64.85 (CH(*α*)); 68.34 (CH(*β*)); 70.22 (CH(3′)); 74.74 (CH(2′)); 83.79 (CH(4′)); 85.64 (CH(1′)); 88.77 ((MeO)$_2$*Tr*); 113.20, 126.77, 127.86, 129.89, 135.66, 158.27 (arom. C ((MeO)$_2$*Tr*)); 120.94 (C(5)); 123.34 (2 arom. C (npe)); 130.38 (2 arom. C (npe)); 143.16 (CH(8)); 145.19 (CH(2)); 146.32, 146.71 (2 arom. C (npe)); 150.29, 150.53 (C(4), C(6)); 154.06 (NHCONH); 170.90 (*C*OOCH$_2$CH$_2$): LSI-MS (THGLY): 1114 ([*M* + Na]$^+$). Anal. calc. for $C_{56}H_{73}N_7O_{12}Si_2$ (1092.4): C 61.57, H 6.74, N 8.98; found: C 61.36, H 6.51, N 8.82.

2′-O-*[(*tert-*Butyl)dimethylsilyl]*-N^6-*{{{(1S,2R)-2-[[*(tert-*butyl)dimethylsilyl]oxy}-1-{[2-(4-nitrophenyl)ethoxy]carbonyl}propyl}amino}carbonyl}-5′*-O-*(4,4′-dimethoxytrityl)adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**11**). A soln. of **10** (0.40 mmol) in CH_2Cl_2 (5 ml) was phosphitylated with *N*,*N*-diisopropylethylamine (3 equiv.) and 2-cyanoethyl diisopropylphosphoramidochloridite (1.5 equiv.) The reaction was difficult to follow as only a slight change of R_f was seen on TLC in several systems. Therefore, after 2 h at r.t., an extra equiv. of both reagents was added, and after further 2 h, the mixture was quenched by addition of H_2O. The mixture was partitioned between CH_2Cl_2 (50 ml) and sat. aq. $NaHCO_3$ soln. (30 ml), the org. phase washed with brine (2 × 30 ml), dried (Na_2SO_4), and evaporated, and the residue purified by flash chromatography (silica gel (30 g), hexane/acetone/Et_3N 68 : 30 : 2) and precipitation in cold hexane: 0.29 mmol (72%) of **11**. LSI-MS (NPOE): 1293 ([*M* + H]$^+$; for $C_{65}H_{90}N_9O_{13}PSi_2$, calc. 1291.593). ^{31}P-NMR: (external ref. H_3PO_4): 149.75, 151.55.

Oligoribonucleotide Synthesis. The oligoribonucleotide containing the L-threonine-modified adenosine nucleoside was prepared on a *Pharmacia-Gene-Assembler-Special*-DNA/RNA synthesizer at 1.5-µmol scale by solid-phase 2-cyanoethyl phosphoramidite chemistry with the 2′-*O*-[(triisopropylsilyl)oxy]methyl (^{i}Pr$_3$SiOCH$_2$; TOM) protecting group for the standard RNA monomers **12a**–**d**. The synthesis was performed '(MeO)$_2$Tr-off' on LCAA CPG (500 Å) with standard RNA cycles, except for the shorter coupling times (2 min) for TOM phosphoramidite chemistry. Coupling time for the modified phosphoramidite **11** and the subsequent TOM amidite was 10 min. (Benzylthio)-1*H*-tetrazole (0.35M) was used as activator. Average coupling efficiency for the synthesis was 99.3% as determined by the release of the 4,4-dimethoxytrityl cation. The solid-phase material was treated with 10% DBU in THF (1 ml) for 90 min at 45° to remove the 2-(4-nitrophenyl)ethyl protecting group. After lyophilization, the solid phase was reacted with 10M $MeNH_2$ in 50% aq. EtOH (500 µl) at r.t. for 4.5 h. The oligoribonucleotide in $MeNH_2$ soln. was decanted, the solid support washed with sterile H_2O (2 × 200 µl) and then the soln. evaporated in a *Speedvac* concentrator. Desilylation of the 2′-*O*-[(triisopropylsilyl)oxy]methyl protecting groups was achieved by dissolving the dried oligoribonucleotide in 1M Bu_4NF in THF (500 µl) at r.t. for 13.5 h. *Tris* buffer (pH 7.4, 500 µl) was added, and the oligoribonucleotide was desalted by CC (*Sephadex* (*G10*) sterile H_2O). The fully deprotected oligoribonucleotide was purified by a further CC (*Dionex Nucleopac PA-100* (4 × 250 mm), 50°, gradient 10 → 500 mM sodium perchlorate (10–35%) with in 30 min), 10 mM *Tris*, and 5M urea at pH 8.0). The desired product was eluted at 24 min at a flow rate of 1 ml/min. Then, the oligoribonucleotide was again desalted by CC (*Sephadex* (*G10*)) and lyophilized on a *Speedvac* concentrator.

For ESI-MS, the RNA sample was dissolved in H_2O and desalted by means of *Dowex-50W* cation-exchange beads. The supernatant was diluted by the addition of an equal volume of both MeCN and a 1% NH_3 soln. The *Q-TOF* mass spectrometer was operated in the negative mode with a source temp. of 80°, a counter current gas flow rate of 40 l/h and a potential of *ca.* 3200 V applied to the source needle (sample flow rate 7 µl/min). The instrument was calibrated with a two-point calibration using the singly charged ion of the monomer and dimer of raffinose.

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Received September 15, 1999