2-(4-Tolylsulfonyl)ethoxymethyl (TEM)—a new 2′-OH protecting group for solid-supported RNA synthesis†

Chuanzheng Zhou, Dmytro Honcharenko and Jyoti Chattopadhyaya*

Received 29th September 2006, Accepted 14th November 2006
First published as an Advance Article on the web 28th November 2006
DOI: 10.1039/b614210a

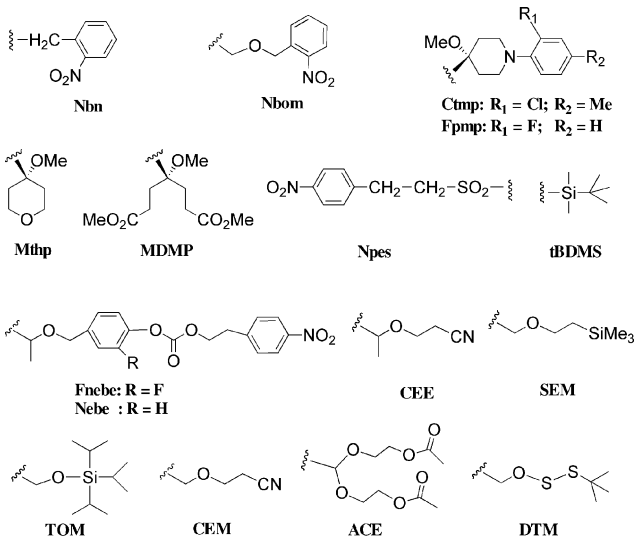
The 2-(4-tolylsulfonyl)ethoxymethyl (TEM) as a new 2′-OH protecting group is reported for solid-supported RNA synthesis using phosphoramidite chemistry. The usefulness of the 2′-*O*-TEM group is exemplified by the synthesis of 12 different oligo-RNAs of various sizes (14–38 nucleotides long). The stepwise coupling yield varied from 97–99% with an optimized coupling time of 120 s. The synthesis of all four pure phosphoramidite building blocks is also described. Two new reliable parameters, $\delta_{C2'} - \delta_{C3'}$ and $\delta_{H2'} - \delta_{H3'}$, have been suggested for the characterization of isomeric 2′-*O*-TEM and 3′-*O*-TEM as well as other isomeric mono 2′/3′-protected ribonucleoside derivatives. The most striking feature of this strategy is that the crude RNA prepared using our 2′-*O*-TEM strategy is sufficiently pure (>90%) for molecular biology research without any additional purification step, thereby making oligo-RNAs easily available at a relatively low cost, saving both time and lab resources.

Introduction

Recent development of RNA interference (RNAi),[1] and evidence that short interfering RNA (siRNA)[2] can effectively silence gene expression, have highlighted the need for dependable methodologies for the chemical synthesis of oligo-RNA sequences 20–25-nucleotides long. The most convenient way to obtain oligo-RNA is solid-supported synthesis using phorsphoramidite building blocks.[3] For this, the correct choice of a 2′-OH protecting group is necessary for the assembly of the desired RNA sequence, because the nature of this protecting group can influence considerably the coupling reaction time[4] and the coupling yield between the 3′-*O*-phosphoramidite monomer block and the 5′-OH terminal of the ribonucleotide chain on the solid support, as well as the purity of the final product. With the aim of circumventing this, the sterically less hindered and more reactive 3′-(2-cyanoethyl-*N*-ethyl-*N*-methylamino phosphoramidite), in conjunction with a 2′-*O*-tBDMS group, has been tried, but without favorable results.[4] Therefore, the design of an ideal 2′-OH protecting group for improving RNA synthesis has been a key issue during past 30 years or so.[5] The currently available 2′-OH protecting groups can be classified into the following types, depending upon the unblocking conditions (as shown in Scheme 1): (1) photosensitive groups such as Nbn[6] and Nbom,[7] (2) acid-labile acetal derivatives such as Mthp,[8] MDMP,[9,10] Ctmp and Fpmp,[11] (3) base-labile groups such as Npes,[12] Fnebe and Nebe,[13] (4) the reductively removable DTM group with a labile S–S bond,[14] (5) fluoride-labile groups such as tBDMS,[15–17] SEM,[18] or CEE,[19] which have been found to be useful in the solid-phase oligo-RNA synthesis.

An ideal 2′-OH protecting group should be:[20] (1) easy to introduce, (2) achiral, (3) unable to migrate to vicinal 3′-OH, (4) completely stable under the conditions required for the assembly of the fully desired RNA sequence (as well as for its subsequent unblocking and release from the solid support), (5) removable under conditions under which RNA is completely stable (the 3′→5′ phosphodiester should also not isomerize to a 2′→5′ linkage). Though the 2′-OH protecting groups shown in Scheme 1 are widely used, none of them completely fulfils the above criteria. For example, tBDMS, the most widely used 2′-OH protecting group, is unstable[21] in ammonia solution, giving chain cleavage[22] and the product of migration to 3′-OH,[23] as well as giving a relatively poor coupling yield after the required coupling time of *ca.* 10 min.[24] The recent appearance of 2′-*O*-ACE[25] (Scheme 1) gives considerable improvements in the synthesis of oligo-RNA compared to the 2′-*O*-tBDMS group, but this strategy is not compatible with a conventional automated synthesizer, and also makes it impossible to monitor the coupling reactions in a standard solid-phase synthesizer with a UV detector.[25]

Recently, some new 2′-OH protecting groups with a formaldehyde acetal linker such as (triisopropylsilyloxy)methyl (TOM)[26]



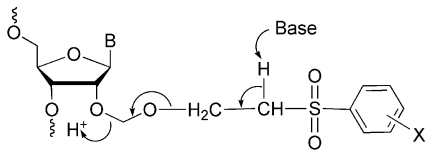
Scheme 1 The 2′-OH protecting groups used in oligo-RNA synthesis.

Department of Bioorganic Chemistry, Box 581, Biomedical Center, Uppsala University, S-751 23, Uppsala, Sweden. E-mail: jyoti@boc.uu.se

† Electronic supplementary information (ESI) available: Additional experimental information. See DOI: 10.1039/b614210a

and 2-cyanoethoxymethyl (CEM)[27] have been developed for solid-phase RNA synthesis. Using the 2′-*O*-TOM or 2′-*O*-CEM groups, an average coupling yield of 99% can be obtained after a coupling time of 60–150 s. Presumably, the formaldehyde acetal linker has relatively less steric hindrance toward the vicinal 3′-phosphoramidite, thereby giving a much higher coupling yield in a relatively short coupling time. As for the 2′-*O*-CEM, it can be unblocked through a β-elimination process with fluoride ion as the base. As expected, it is also cleaved to some extent during the ammonia treatment, which increases the possibility of chain cleavage, and may limit its further use for synthesis of larger oligo-RNAs. Clearly, reducing the acidity of the proton α to the -CN group in 2′-*O*-CEM (2′-*O*–$CH_2OCH_2CH_2CN$)[27] will increase its stability in the ammonia deprotection step.

We report here our efforts to modulate the base-lability of the α-proton by employing an appropriate substituent (X) in the aromatic moiety of 2-arylsulfonylethoxymethyl group as a potential 2′-OH protecting group (Scheme 2). Given the fact that the pK_a of the RCH_2SO_2Ph proton (pK_a = 27.9 in DMSO, R = PhO) is very similar to that of RCH_2CN (pK_a = 28.1 in DMSO, R = PhO),[28] introducing a *para*-methyl substituent (X = *p*-Me in Scheme 2) to the phenyl, as in *para*-tolylsulfonylethoxymethyl (TEM), is expected to reduce the acidity of the α-proton.[29] This electronic tuning may make TEM a more stable 2′-OH protecting group than CEM. This paper demonstrates the solid-supported chemical synthesis of RNA using TEM as a new 2′-OH protecting group. We also report the synthesis of all four native phosphoramidite building blocks, and their use in automated RNA synthesis, as well as the deblocking conditions to prepare pure oligo-RNA.



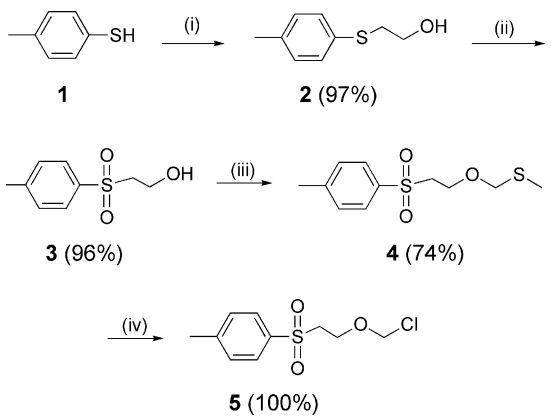
Scheme 2 The electronic nature of X dictates the fragmentation reactivity.

Results and discussion

(A) Synthesis of the phosphoramidite building blocks

The reagent, TEM-Cl (**5**), was synthesized using the strategy shown in Scheme 3: 2-(4-tolylthio)ethanol (**2**) was synthesized from 4-methylbenzenethiol (**1**).[30] The thioether **2** was first oxidized to sulfone **3** in 96% yield by 35% hydrogen peroxide in aqueous acetic acid (H_2O–AcOH, 1 : 1, reflux, 20 min). Then sulfone **3** was treated with a mixture of DMSO, acetic acid and acetic anhydride at room temperature for two days, followed by purification by silica gel column chromatography to give *O*,*S*-acetal **4** (74%). Finally, the reagent **5** was generated immediately before use by treatment of **4** with SO_2Cl_2 (1 equiv.) in CH_2Cl_2. This conversion of **4** → **5** was a quantitative reaction, and no further purification step was necessary.

Acetyl (Ac), dimethylaminomethylene (Dmf) and phenoxyacetyl (Pac) were used to protect N^4 of cytosine, N^2 of guanosine and N^6 of adenosine, respectively. These exocyclic amino-protected building blocks and uridine were converted to their respective 5′-*O*-DMTr derivatives according to the published procedures.[31–33] The appropriately protected ribonucleoside was protected at 2′-



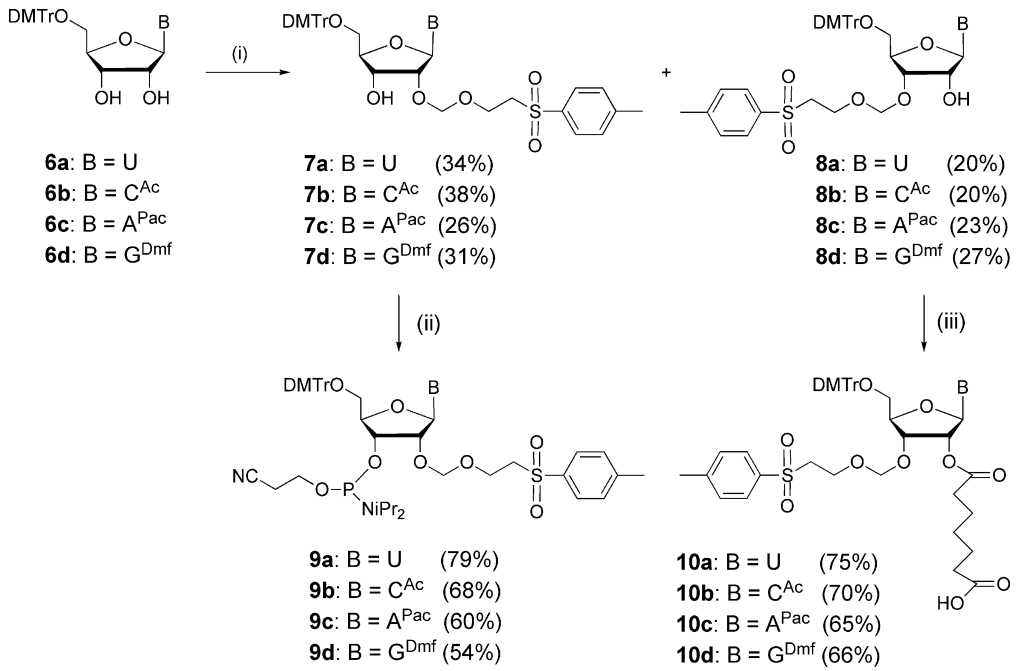
Scheme 3 *Reagents and conditions*: (i) 2-chloroethanol, 10 M aq. NaOH, ethanol, reflux, 2 h; (ii) 35% hydrogen peroxide, HOAc, H_2O, reflux, 20 min; (iii) DMSO, HOAc, Ac_2O, r.t., 48 h; (iv) SO_2Cl_2, CH_2Cl_2, r.t., 1 h.

OH by the TEM group by the procedure optimized by Pitsch and co-workers[26] to give a mixture of two 2′- and 3′-isomeric ribonucleosides **7** and **8** (Scheme 4) by reacting **6a/b/c/d** with Bu_2SnCl_2/^{i}Pr_2NEt in 1,2-dichloroethane at room temperature to form activated cyclic 2′-*O*,3′-*O*-dibutylstannylidene intermediate, followed by treatment with 1.3 equivalents of TEM-Cl (**5**) at 80 °C for 1 h. The two isomeric 2′-*O*- and 3′-*O*-TEM derivatives can be isolated by silica gel column chromatography. The first isomer eluting from the column was the predominant product, 2′-*O*-TEM ribonucleoside **7a/b/c/d** in 26–38% yield. The second-eluting isomer, **8a/b/c/d**, was isolated in 20–27% yield. These two isomers have been identified by NMR (see Section B). Compound **7a/b/c/d** was further converted to the corresponding 5′-*O*-DMTr-2′-*O*-TEM-ribonucleoside 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) **9a/b/c/d** (in 79/68/60/54% yield respectively) by using a literature procedure[34] (see the Experimental section).

(B) Identification of isomeric 2′-*O*-TEM and 3′-*O*-TEM ribonucleosides by NMR

When ribonucleoside **6a/b/c/d** was alkylated with TEM-Cl **5**, two monoalkylated 2′/3′-*O*-TEM isomers were obtained. The isomer with lower R_f was converted to corresponding pimelate **10a/b/c/d**, which showed a downfield shift of H-2′ in the ^{1}H NMR spectrum (data listed in Table 1), thereby showing that the compound with lower R_f is the 3′-*O*-TEM derivative.

The ^{1}H and ^{13}C NMR data of isomerically pure 2′- and 3′-*O*-TEM derivatives **7a–d** and **8a–d**, the 2′- and 3′-*O*-CEM derivatives **11a–c** and **12a–c**, as well as the 2′- and 3′-*O*-tBDMS ribonucleoside derivatives **13a–14c**, are listed in Table 1. It can be seen from Table 1, regardless of alkylation or silylation at the 2′-OH or 3′-OH, the $\delta_{H1'}$ values do not follow any systematic upfield or downfield shift, and in fact the difference between the $\delta_{H1'}$ values of the two isomers is negligible. On the other hand, the $^3J_{1',2'}$ values of all alkylated compounds **7a–12c** follow Reese's rule,[35] in that the $^3J_{1',2'}$ values of the 2′-isomers are smaller than those of the 3′-isomers, while this is not the case for the 2′,3′-isomeric silylated compounds **13a–14c**. A comparison of $\delta_{H2'}$ and $\delta_{H3'}$ showed that for all the mono-2′/3′-*O*-alkylated compounds **7a–12c**, alkylation causes an upfield shift of the proton attached to the site of 2′- or 3′-*O*-alkylation. The value of $\delta_{H2'} - \delta_{H3'}$ for the



Scheme 4 *Reagents and conditions*: (i) (a) Bu_2SnCl_2, $^iPr_2NEt$, $ClCH_2CH_2Cl$, r.t., 1 h, (b) compound **5**, 80 °C, 1 h, (ii) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, $^iPr_2NEt$, CH_2Cl_2, r.t., 2 h, (iii) pimelic acid, EDAC, DMAP, pyridine, r.t., 4 h.

2′-isomer is always smaller than that of the 3′-isomer. In contrast, a completely opposite rule can be drawn for the isomeric 2′/3′-*O*-silylated compounds **13a–14c**.

^{13}C NMR signals were also used by Ogilvie *et al.* to identify 2′-*O*- and 3′-*O*-silylated isomers.[36] They found that silylation at a sugar hydroxyl leads to a downfield shift of the sugar carbon to which it is attached. As shown in Table 1, we find that this rule also applies to all the isomeric 2′/3′-*O*-alkylated compounds **7a–12c**. Alternatively, the value of $\delta_{C2'} - \delta_{C3'}$ can be used as a more convenient identification parameter. The value of $\delta_{C2'} - \delta_{C3'}$ for the 2′-isomer is always larger than that of the corresponding 3′-isomer.

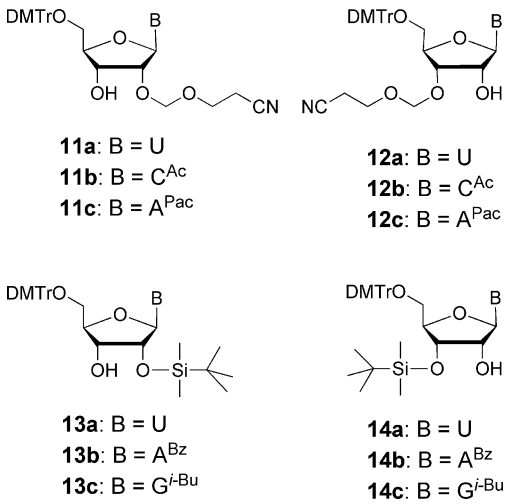
(C) Preparation of the solid support

Initially, we anchored the ribonucleoside to LCAA-CPG support through the succinate linker. Just as described by Pitsch,[26] the coupling yields for the first seven coupling steps were unsatisfactory. Pitsch and his co-workers have overcome this problem

Table 1 ^1H and ^{13}C NMR chemical shifts of isomeric 2′/3′-protected ribonucleoside derivatives

	$\delta_{H1'}$ ($^3J_{H1',H2'}$)	$\delta_{H2'}$	$\delta_{H3'}$	$\delta_{H2'} - \delta_{H3'}$	$\delta_{C2'}$	$\delta_{C3'}$	$\delta_{C2'} - \delta_{C3'}$
7a	5.90 (1.84)	4.21	4.48	−0.27	80.18	68.72	11.46
8a	5.90 (3.70)	4.34	4.27	0.07	74.53	75.90	−1.37
7b	5.88[b]	4.18	4.44	−0.26	80.11	67.81	12.3
8b	5.93 (2.10)	4.33	4.18	0.15	75.37	75.61	−0.24
7c	6.21 (3.18)	4.86	4.56	0.30	80.46	70.32	10.14
8c	6.04 (5.52)	4.91	4.44	0.47	74.35	77.25	−2.90
7d	6.05 (4.81)	4.66	4.47	0.19	80.03	70.41	9.62
8d	5.91 (5.33)	4.80	4.36	0.44	74.04	76.81	−2.77
11a[a]	5.98 (2.15)	4.29	4.50	−0.21	79.99	68.87	11.12
12a[a]	6.03 (3.52)	4.47	4.38	0.09	74.18	75.07	−0.89
11b[a]	5.95[b]	4.30	4.47	−0.17	79.73	67.93	11.8
12b[a]	5.93 (2.34)	4.39	4.28	0.11	75.16	75.10	0.06
11c[a]	6.25 (3.90)	4.92	4.58	0.34	80.14	70.38	9.76
12c[a]	6.06 (5.71)	4.93	4.50	0.43	74.36	76.90	−2.54
13a[a]	5.95 (2.65)	4.34	4.33	0.01	76.32	74.46	1.86
14a[a]	5.96 (4.17)	4.16	4.39	−0.23	71.30	75.29	−3.99
13b[a]	6.11 (5.34)	5.03	4.37	0.66	75.91	71.76	4.15
14b[a]	6.07 (4.93)	4.48	4.60	0.28	74.79	72.32	2.47
13c[a]	5.74 (7.42)	5.26	4.34	0.92	74.32	71.32	3.00
14c[a]	5.71 (5.97)	4.85	4.46	0.39	73.60	71.65	1.95
10a	—	5.37	4.43	—	73.97	73.54	—
10b	—	5.39	4.40	—	74.31	72.42	—
10c	—	5.83	4.71	—	74.37	73.96	—
10d	—	5.92	4.51	—	74.13	73.51	—

[a] Compounds **11a–14c** were synthesized according to the literature procedure;[27,37] their structures are shown in Scheme 5. [b] Broad singlet.



Scheme 5 Structures of isomeric mono-2′/3′-protected ribonucleosides.

by employing a longer linker. We also adopted this strategy, but a more convenient synthetic route was developed: pimelic acid was reacted directly with the protected ribonucleosides **8a–d** in the presence of 2 equivalents of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC) and DMAP in dry pyridine. This quick and clean reaction, completed in 4 h, was worked-up and purified by silica gel column chromatography to give the corresponding pimelates **10a–d** in 65–75% yield. The pimelates were subsequently immobilized to the LCAA-CPG support by a modification of the general procedure of Pon and Yu,[38] which involved shaking the mixture of pimelates, LCAA-CPG, benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexfluorophosphate (BOP), *N*-hydroxybenzotriazole (HOBt) and diisopropylethylamine (DIPEA) in acetonitrile for 2 h to give good loadings (20–25 μmol g^{-1}). Prolonging the reaction time overnight gave improved loadings (35 μmol g^{-1}).

(D) Automated RNA synthesis

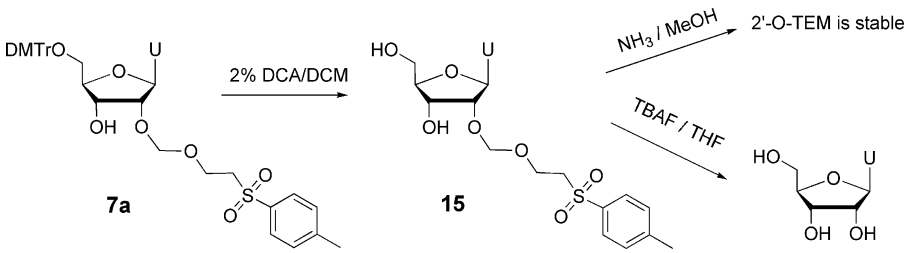
We first synthesized U_{12} under different conditions to optimize the synthesis cycle. This oligonucleotide was synthesized on an Applied Biosystems 392 DNA/RNA synthesizer with common DNA synthesis reagents. 2′-*O*-Succinate-**8a** CPG was used as the solid support and a 1.0 μmol RNA synthesis cycle was applied but with a modified coupling time. The average stepwise yield (ASWY), obtained by a detritylation assay, was used to evaluate the synthesis efficiency. We first tried synthesis with 4,5-dicarbonitrile-1*H*-imidazole (DCI) ($pK_a = 5.2$),[39] 5-(ethylthio)-1*H*-tetrazole (ETT) ($pK_a = 4.28$) and 5-(benzylthio)-1*H*-tetrazole (BTT) ($pK_a = 4.08$)[40] as the activator with coupling time of 120 s; ETT gave the best result. Since an activator acts as an acid to protonate, and thereby activate, the phosphoramidites, the more acidic the activator is, the faster the coupling reaction should be.[41] On the other hand, as the activator becomes increasingly acidic, it can also lead to some concomitant loss of the 5′-*O*-DMTr protecting group during coupling, which explains why ETT, despite being less acidic than BTT, gives a better coupling yield in the RNA synthesis. Next, with ETT as the activator, we tried different coupling times (60, 90 and 120 s), and found that the ASWY improved with longer coupling time. However, a coupling time of 150 s or 200 s did not improve the yields. We therefore settled for 120 s as the coupling time for the synthesis. During the final optimization experiments, we found the first 7 couplings always gave a poor coupling yield, and after that the ASWY increased steadily. A pimelated CPG (with a chain three methylenes longer) instead of the conventional succinate (Scheme 4) improved the coupling yield only slightly.

On the basis of the above experiments, 12 different oligo-RNAs (sequences shown in Table 2) were synthesized to test the efficacy of TEM as a 2′-OH protecting group. The poly-U synthesis always gave a perfect coupling yield, whereas the synthesis of mixed RNA sequences (**ON3–12**), particularly the one involving G-phosphoramidite, generally gave poorer yields. The average stepwise yield for each synthesis is also listed in Table 2.

(E) Post-synthesis deprotection treatment

Prior to deprotection of the oligo-RNAs, we tested the stability of 2′-*O*-TEM in ammonia. Compound **7a** was treated with 3% $Cl_2CHCOOH$ in CH_2Cl_2 to give compound **15** (Scheme 6). Compound **15** was first treated with 33% $MeNH_2$ in EtOH at room temperature for 24 h. The reaction was monitored by TLC, and it was found about 10% loss of 2′-*O*-TEM has taken place (the TLC is shown in the Supporting information). This suggests that TEM is not stable under highly alkaline conditions involving $MeNH_2$/EtOH. Anhydrous methanolic ammonia (25% NH_3/MeOH)[22] was then tried. The reaction mixture was kept at 55 °C for 21 h, and very small amount of TEM cleavage was observed. If the reaction was allowed to proceed at room temperature, the amount of 2′-*O*-TEM cleavage was very low. Therefore NH_3/MeOH was chosen for exocyclic amino deprotection. In view of the fact that about 5% cleavage of 2′-*O*-CEM is found to take place under the same conditions, it is concluded that 2′-*O*-TEM is more stable in ammonia solution, just as we expected at the outset.

In order to find an optimal condition to remove 2′-*O*-TEM, compound **15** was treated separately with neat $Et_3N{\cdot}3HF$ and 1 M TBAF/THF. It was found that only 1 M TBAF/THF is an



Scheme 6 Stability of 2′-*O*-TEM upon ammonia and fluoride treatment.

Table 2 Average stepwise and overall yields and mass of synthesized RNAs using the 2′-*O*-TEM protecting group

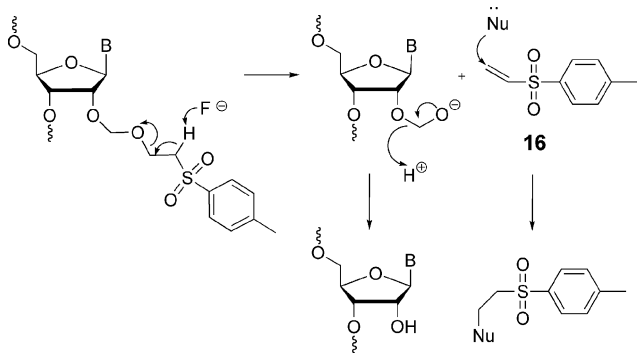
Oligo-RNAs	RNA sequence	[MH]$^+$ (calcd)	[MH]$^+$ (found)	Yield (%)[a]	Crude product[b] (Overall yield (%))
ON1[c]	5′-HO-U_{20} -3′-OH	6062.42	6064.33	99.1	102 OD (53%)
ON2[c]	5′-HO-U_{38} -3′-OH	11683.37 [M + 5Na]$^+$	11682.17 [M + 5Na]$^+$	98.4	144 OD (40%)
ON3[c]	5′-r(GACGUAAACGG CCACAAGUUC)	6719.16	6720.07	97.8	112 OD (54%)
ON4[c]	5′-r(ACUUGUGGCCG UUUACGUCGC)	6643.00	6643.92	98.0	124 OD (64%)
ON5[d]	5′-CGCGC U U G C 3′-GCGCG U G A G	5779.53	5777.13	97.5	21 OD (65%)
ON6[d]	5′-CGCGC U U 3′-GCGCG G C	4453.74	4451.56	97.0	17 OD (69%)
ON7[d]	5′-CGCGC G A 3′-GCGCG A A	4523.84	4521.69	97.0	20 OD (78%)
ON8[d]	5′-CGCGC A G U C 3′-GCGCG A U G A	5786.57	5784.14	97.6	18 OD (53%)
ON9[d]	5′-CGCGC A G U A 3′-GCGCG A U G	5481.39	5479.18	97.2	21 OD (64%)
ON10[d]	5′-CGCGC A G U 3′-GCGCG A U G	5152.18	5150.11	97.4	19 OD (63%)
ON11[d]	5′-CGCGC G U 3′-GCGCG U G	4493.76	4491.68	97.3	17 OD(68%)
ON12[d]	5′-r(GAAGAAAAAA UGAAG)	4918.14	4915.51	97.0	24 OD(68%)

[a] Average stepwise coupling yield. [b] Overall crude product yields were measured at 260 nm UV absorption. [c] Oligonucleotides were synthesized on a 1 μmol scale. [d] Oligonucleotides were synthesized on a 0.2 μmol scale.

efficient reagent for the deprotection of the 2′-*O*-TEM group, in that the deprotection was found to be complete in 5 min at room temperature.

Therefore, the oligonucleotide-anchored solid supports were first treated with 25% NH_3/MeOH at room temperature for 20 h, and then at 40 °C for 4 h to ensure the complete removal of the exocyclic amino protecting groups. After solvent removal and drying by co-evaporation with dry THF, the samples were treated with 1 M TBAF/THF for 20 h at room temperature to make sure that all the 2′-*O*-TEM groups were removed. After desalting through a NAP-10 column and Sep-Pak column, the crude products were subjected to PAGE or HPLC analysis. The PAGE diagrams (see Fig. 1) together with HPLC profiles (see Fig. S1.2†) show that the crude products of U_{20} and U_{38} are more than 80% pure.

However, just as in the CEM strategy, deprotection of the mixed oligo-RNA sequence using the same deprotection procedure did not produce satisfactory results. This was because of the formation of the adducts owing to the side-reaction of the purine bases as nucleophiles (at N7, N3, and N1) with the α,β-unsaturated sulfone **16**, generated by fluoride ion treatment (Scheme 7). The formation



Scheme 7 The mechanism of adduct formation.

of these adducts has been evidenced by denatured PAGE as well as by MALDI-TOF MS analysis.

In the CEM strategy,[27] 10% n-propylamine and 1% bis(2-mercaptoethyl) ether was used as scavenger for the active α,β-unsaturated compound (acrylonitrile), and thereby adduct formation was suppressed efficiently. However, it should be kept in

Table 3 Conditions used for deblocking **ON3** and **ON4**[a] treatment

Entry	Oligonucleotide	Step I: NH_3	Step II: 2′-Deprotection	Isolated yield (%)	HPLC profile[b]
1	**ON4**	Condition 1	Condition 4	53.7	Fig. S3.1
2	**ON4**	Condition 1	Condition 5	48.6	Fig. S3.2
3	**ON3**	Condition 1	Condition 5	57.4	Fig. S3.11
4	**ON4**	Condition 1	Condition 6	50.4	Fig. S3.3
5	**ON3**	Condition 1	Condition 7	43.7	Fig. S3.4
6	**ON4**	Condition 1	Condition 8	27.9	Fig. S3.5
7	**ON4**	Condition 1	Condition 9	[c]	Fig. S3.6
8	**ON4**	Condition 1	Condition 10	[c]	Fig. S3.7
9	**ON4**	Condition 1	Condition 11	[c]	Fig. S3.8
10	**ON3**	Condition 1	Condition 12	49.1	Fig. S3.9
11	**ON4**	Condition 2	Condition 6	34.9	Fig. S3.10
12	**ON3**	Condition 3	Condition 6	52.1	Fig. S3.12

[a] Condition 1: 25% NH_3/MeOH, r.t., 20 h, 40 °C, 4 h; Condition 2: 25% NH_3/MeOH (with 1% CH_3NO_2), r.t., 20 h, 40 °C, 4 h; Condition 3: 25% NH_3/MeOH, 55 °C, 3 h; Condition 4: 1 M TBAF/THF (with 10% n-propylamine and 1% bis(2-mecaptoethyl) ether), 24 h; Condition 5: 1 M TBAF/THF (with 10% n-propylamine and 1% bis(2-mecaptoethyl) ether), 4 h; Condition 6: 1 M TBAF/THF (with 10% n-propylamine and 1% CH_3NO_2), 4 h; Condition 7: 1 M TBAF/THF (with 10% DBU and 1% CH_3NO_2), 4 h; Condition 8: 1 M TBAF/THF (with 10% piperidine and 1% CH_3NO_2), 4 h; Condition 9: 1 M TBAF/THF (with 10% CH_3NO_2), 4 h; Condition 10: 1 M TBAF/THF (with 1% n-propylamine and 10% CH_3NO_2), 4 h; Condition 11: 1 M TBAF/THF (with 10% n-propylamine and 10% CH_3NO_2), 4 h; Condition 12: 1 M TBAF/THF (with 10% n-propylamine), 4 h. [b] HPLC conditions: RP-column, 0–40 min, buffer C → C/D 8 : 2. All of the HPLC profiles can be found in Supporting information.† [c] No pure product obtained.

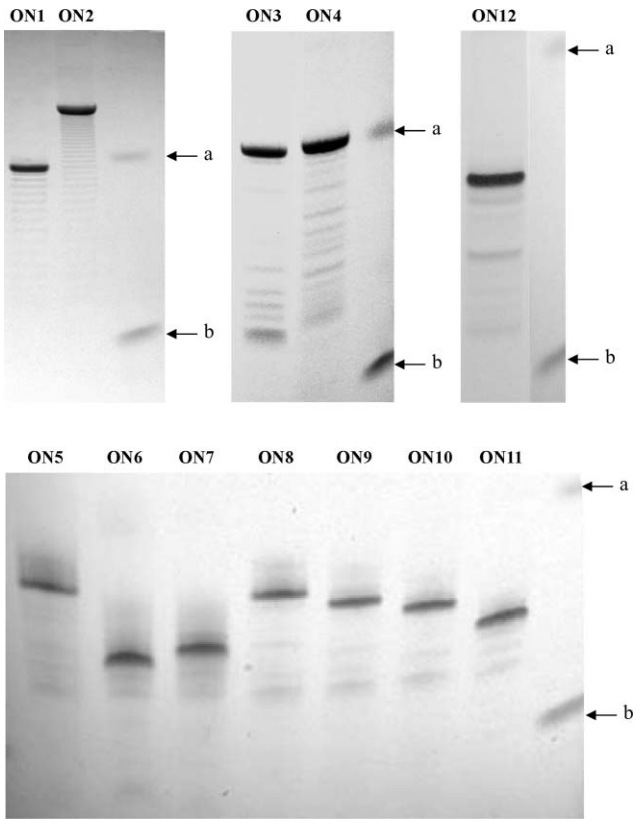


Fig. 1 20%-Denatured PAGE diagrams of the crude products directly after deprotection. a: Xylene cyanol blue. b: Bromophenol blue.

mind that n-propylamine also acts as a moderate base and can promote chain cleavage. So, in the hope of developing an improved scavenger, we tested several different deprotecting conditions for 2′-*O*-TEM with **ON3** and **ON4** as the substrates. The results are listed in Table 3. Comparison of these data suggests the following:

(1) Deprotecting 2′-*O*-TEM with TBAF/THF is a fast process. Reaction periods of 4 h and 24 h give nearly the same yields.

(2) Amine-promoted chain cleavage is significant. When more basic amines such as DBU or piperidine were used, considerably more cleavage was observed.

(3) n-Propylamine itself is an efficient scavenger. In the absence of bis(2-mercaptoethyl)ether, the deprotecting reaction is cleaner.

(4) CH_3NO_2 was reported as a scavenger for acrylonitrile in the presence of amines such as NH_3/MeCN, $MeNH_2/H_2O$–EtOH–MeCN,[42] but this is not the case in the presence of TBAF in THF. On the contrary, when CH_3NO_2 was used in conjunction with n-propylamine, it can inhibit the activity of n-propylamine.

(5) The 2′-*O*-TEM group is found to be stable in ammonia. Treating the oligonucleotide with NH_3/MeOH at room temperature for 20 h, then 40 °C for 4 h (or 55 °C for 3 h) gave similar results, which again proves the enhanced stability of 2′-*O*-TEM in NH_3/MeOH.

(6) Up until now, 1 M TBAF/THF with 10% n-propylamine and 1% bis(2-mercaptoethyl) ether has remained the preferred reagent for deprotecting the 2′-*O*-TEM group from oligo-RNAs. However, recent unpublished data from this lab suggests that 1 M TBAF/THF with 10% morpholine at room temperature seems to be a better deprotection agent. This will be reported in due course.

We then deprotected all of our oligo-RNAs, **ON3–12**, in the following manner: (i) treatment with methanolic ammonia (RT, 20 h followed by 40 °C for 4 h) as described above, and (ii) treatment with 1 M TBAF/THF containing 10% n-propylamine and 1% bis(2-mercaptoethyl) ether for 20 h at room temperature to give full-length oligo-RNAs in good purity. The PAGE pictures of the crude products (>90% pure, which are acceptable for enzymological work, see below) are shown in Fig. 1. These oligo-RNAs were subsequently examined by MALDI-TOF MS, which confirmed the chemical integrity of all the synthesized RNAs (see Table 2 and Fig. S1.3–13†).

(F) Enzymatic digestion of the crude RNA directly after deprotection without involving any purification step

RNA synthesis with the TEM strategy described above can indeed give products with high purity. To see whether the crude product

is pure enough for biological research, RNA 15 nucleotides long (**ON12** in Table 2) was synthesized and, after deprotecting and desalting (see Fig. 1 for purity of the crude product), applied directly in an RNase H digestion study (Fig. S4†). It may be noted that the synthesized crude RNA (compare its relative purity (~90%) with that of the purified RNA in Fig. S5†) has nearly the same cleavage efficiency as the pure RNA (compare the RNase H promoted relative degradation rates in Fig. 2). This suggests that the crude RNA prepared by our TEM strategy is satisfactory for most biological experiments. To our knowledge, it is the first approach that can provide crude RNA for biological studies directly without a laborious, time-consuming purification step.

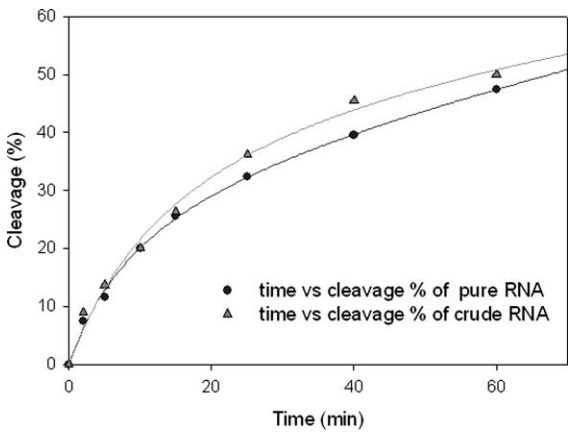


Fig. 2 RNA cleavage efficiency of pure RNA (Fig. S5†) and crude RNA (**ON12** in Fig. 1) with RNase H (for the purity of pure RNA see the PAGE picture in Fig. S5). Conditions of cleavage reactions: pure or crude RNA (0.1 μM) and complementary DNA (1 μM) in buffer containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM $MgCl_2$ and 0.1 mM DTT at 21 °C; 0.06 U of RNase H in a total reaction volume of 30 μL.

The crude **ON12** was also subjected to digestion by phosphodiesterase I (from *Crotalus adamanteus* venom) and shrimp alkaline phosphatase. The products were analyzed by RP-HPLC, and the profile is shown in Fig. 3. The crude products are completely digested to give pure nucleosides, which strongly suggests that the oligo-RNA is in a biologically active form and that no modified base is present.

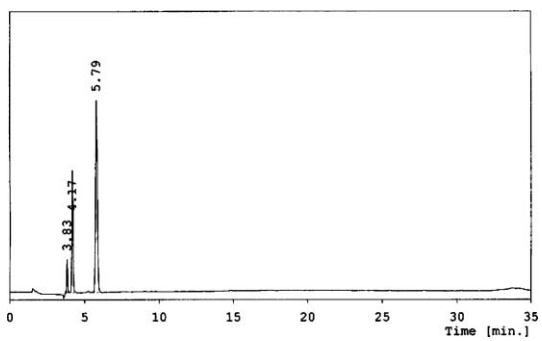


Fig. 3 Reversed-phase HPLC profile of the products obtained by digestion of crude **ON12** (R_t = 14.12 min) in Fig 1 with a mixture of phosphodiesterase I and alkaline phosphatase. The peaks correspond to uridine (3.83 min), guanosine (4.17 min) and adenosine (5.79 min). HPLC conditions: C_{18} RP column, 100 × 4.6 mm, 1 ml min^{-1}, r.t., 0–10 min, buffer C → C/D 9 : 1, 10–35 min, buffer C/D 9 : 1 → C/D 2 : 8.

Conclusions

The conclusions of our study are as follows:

(1) A new set of NMR trends, involving $\delta_{H2'} - \delta_{H3'}$ and $\delta_{C2'} - \delta_{C3'}$, was identified to characterize monomeric 2′-*O*- and 3′-*O*-alkylated or silylated ribonucleosides. The proposed parameters are more regular and reliable than earlier reported parameters such as $\delta_{H1'}$, $J_{H1',H2'}$, $\delta_{C3'}$ and $\delta_{C2'}$.

(2) TEM has been developed as a new 2′-OH protecting group for solid-supported oligo-RNA synthesis. With this methodology, RNA synthesis can be carried out on a standard solid-support synthesizer with high average coupling yield and a coupling time of only 120 s.

(3) The advantages of our 2′-*O*-TEM-based strategy for RNA synthesis are the stability of the 2′-*O*-TEM group upon ammonia treatment, the simpler post-synthesis deprotection procedure and the higher purity of the crude product than that of RNA prepared using the 2′-*O*-cyanoethoxymethyl (2′-*O*-CEM) group.

(4) Furthermore, the crude RNA obtained by our 2′-*O*-TEM-based strategy is of high purity, which, after desalting, can be directly used in biological research without further purification, which is an important advantage over other strategies based on either 2′-*O*-tBDMS,[15–17] 2′-*O*-TOM,[26] 2′-*O*-CEM,[27] 2′-*O*-ACE,[25] or 2′-*O*-Fpmp.[20]

Experimental

Chromatographic separations were performed on Merck G60 silica gel. Thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F_{254} glass-backed plates. ^{1}H NMR spectra were recorded at 270.1 MHz and 500 MHz respectively, using TMS (0.0 ppm) as internal standards. ^{13}C NMR spectra were recorded at 67.9 MHz, 125.7 MHz and 150.9 MHz respectively, using the central peak of $CDCl_3$ (76.9 ppm) as an internal standard. ^{31}P NMR spectra were recorded at 109.4 MHz using 85% phosphoric acid as an external standard. Chemical shifts are reported in ppm (δ scale). MALDI-TOF mass spectra were recorded in the positive ion mode for oligonucleotides and for other compounds as indicated. The mass spectrometer was externally calibrated with a peptide mixture using α-cyano-4-hydroxycinnamic acid as matrix. Anion exchange (AE) HPLC: Luna 5 μ, NH_2, 100 Å, 150 × 4.6 mm. Flow 1 ml min^{-1} at room temperature, UV detector with detecting wavelength of 260 nm. Buffer A: 20 mM $LiClO_4$, 20 mM NaOAc in H_2O–CH_3CN (9 : 1), pH 6.5 with AcOH. Buffer B: 600 mM $LiClO_4$, 20 mM NaOAc in H_2O–CH_3CN (9 : 1), pH 6.5 with AcOH. Reversed-phase (RP) HPLC: Kromasil 100, C18, 5 μ, 100 × 4.6 mm. Flow 1 ml min^{-1} at room temperature, UV detector with detecting wavelength of 260 nm. Buffer C: 0.1 M TEAA in H_2O–CH_3CN (95 : 5). Buffer D: 0.1 M TEAA in H_2O–CH_3CN (50 : 50). Polyacrylamide gel electrophoresis (PAGE): 20% acrylamide (acrylamide/bis-acrylamide 29 : 1), 7 M urea, TBE buffer.

2-(4-Tolylthio)ethanol (2)

To a solution of 4-methylbenzenethiol (**1**, 50 g, 0.394 mol) and 2-chloroethanol (47.6 ml, 0.71 mol) in ethanol (400 ml) was added dropwise aqueous 10 M NaOH (39.4 ml, 0.394 mol) over 1 h. The reaction mixture was refluxed for 2 h, cooled and concentrated

in vacuo. The residue was diluted with AcOEt, washed with H_2O and dried over $MgSO_4$. After filtration, the filtrate was evaporated *in vacuo* to give 76.6 g (97%) of **2** as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.32 (s, 3H), 3.06 (t, $J = 5.9$ Hz, 2H), 3.70 (t, $J = 5.9$ Hz, 2H), 7.11 (d, $J = 9.1$ Hz, 2H), 7.30 (d, $J = 8.1$ Hz, 2H).

2-(4-Tolylsulfonyl)ethanol (3)

To a solution of **2** (20 g, 0.119 mol) in AcOH (50 ml) and H_2O (50 ml) in an ice bath was added hydrogen peroxide (30%, 36.5 ml) dropwise over half an hour. The mixture was then refluxed for 20 min and cooled. $NaHCO_3$ was added to the reaction mixture to neutralize this solution, followed by AcOEt. The organic layer was separated, washed with brine and water, and concentrated *in vacuo* to give 22.9 g (96.3%) of **3** as a white solid. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.46 (s, 3H), 3.33 (t, $J = 5.4$ Hz, 2H), 3.99 (t, $J = 5.4$ Hz, 2H), 7.39 (d, $J = 8.0$ Hz, 2H), 7.81 (d, $J = 8.3$ Hz, 2H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 21.7, 56.4, 58.3, 128.0, 130.1, 136.0, 145.2.

4-Tolylsulfonylethyl methylthiomethyl ether (4)

A solution of **3** (2 g, 10 mmol) in dimethyl sulfoxide (28 ml, 40 mmol) was treated with acetic anhydride (11.4 ml, 20 mmol) and acetic acid (19 ml, 20 mmol). The mixture was stirred at room temperature for 48 h and then added dropwise to an aqueous solution of $NaHCO_3$. After stirring for 1 h, the reaction mixture was extracted with ethyl acetate. The organic layer was separated, washed with saturated aqueous NaCl solution and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residual oil was chromatographed on a short column (ethyl acetate–cyclohexane, 1 : 5 to 2 : 5) to give 1.935 g (74%) of **4** as a colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.08 (s, 3H), 2.46 (s, 3H), 3.40 (t, $J = 6.3$ Hz, 2H), 3.88 (t, $J = 6.3$ Hz, 2H), 4.54 (s, 3H), 7.36 (d, $J = 7.9$ Hz, 2H), 7.81 (d, $J = 8.3$ Hz, 2H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 14.1, 21.7, 56.2, 61.4, 75.6, 128.1, 129.9, 136.8, 144.9.

4-Tolylsulfonylethoxymethyl chloride (5)

4-Tolylsulfonylethyl methylthiomethyl ether (**4**, 1.78 g, 6.8 mmol) was dissolved in CH_2Cl_2 (20 ml). Keeping the solution in an ice-bath, SO_2Cl_2 (0.95 g, 6.8 mmol) was added dropwise, and the reaction was allowed to proceed for 2 h at room temperature. After evaporation of the solvent under reduced pressure, 1.69 g (99%) of **5** was obtained as a colorless oil. This was used for next step of the synthesis without further purification. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.46 (s, 3H), 3.44 (t, $J = 6.2$ Hz, 2H), 4.03 (t, $J = 6.2$ Hz, 2H), 5.35 (s, 3H), 7.36 (d, $J = 8.0$ Hz, 2H), 7.80 (d, $J = 7.5$ Hz, 2H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 21.7, 55.7, 63.7, 82.0, 128.1, 129.9, 136.5, 145.0.

5′-*O*-(Dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]-uridine (7a) and 5′-*O*-(dimethoxytrityl)-3′-*O*-[2-(4-tolyl-sulfonyl)ethoxymethyl]uridine (8a)

To a solution of 5′-*O*-DMTr-uridine (3.83 g, 7 mmol) in CH_2Cl_2 (30 ml) was added diisopropylethylamine (4.2 ml, 24.7 mmol) and dibutyltin dichloride (2.55 g, 8.4 mmol), and the reaction was allowed to proceed at room temperature for 1 h. The mixture was then heated to 80 °C, 4-tolylsulfonylethoxymethyl chloride (**5**) (2.5 g, 9.1 mmol) added dropwise, and the mixture stirred at 80 °C for 1 hour. The mixture was allowed to cool down and saturated $NaHCO_3$ was added. After shaking vigourously, the resulting turbid solution was filtered through a Celite pad. CH_2Cl_2 was added to the filtrate, and the organic layer was separated and dried over $MgSO_4$. The residue, after evaporation of the solvent, was applied chromatographed on a short column (CH_2Cl_2 with 1% Et_3N, ethyl acetate from 0 to 40%). The first-eluted isomer was **7a** (1.83 g, 34.4%). ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.43 (s, 3H), 3.40 (t, $J = 5.8$ Hz, 2H), 3.54 (t, $J = 2.7$ Hz, 2H), 3.79 (s, 6H), 3.94 (m, 1H), 4.05–4.16 (m, 2H), 4.21 (dd, $J = 1.8$, 2.0 Hz, 1H, H-2′), 4.48 (dd, $J = 5.4$, 5.5 Hz, 1H, H-3′), 4.80 (d, $J = 6.8$, 1H), 4.98 (d, $J = 6.8$, 1H), 5.28 (d, $J = 6.3$, 1H), 5.90 (d, $J = 1.8$, 1H, H-1′), 6.84 (d, $J = 8.9$, 4H), 7.23–7.40 (m, 11H), 7.78 (d, $J = 8.3$, 2H), 7.96 (d, $J = 8.2$, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 55.3, 56.2, 61.4, 61.8, 68.7 (C-3′), 80.2 (C-2′), 83.2, 87.1, 88.1, 95.1, 102.2, 113.3, 127.2, 127.9, 128.0, 128.2, 130.0, 130.1, 130.2, 135.1, 135.3, 136.8, 140.0, 144.4, 145.0, 150.1, 158.8, 163.0. MALDI-TOF MS: $[M + Na]^+$ 781.20, calcd 781.25. The second-eluted isomer was **8a** (1.07 g, 20.2%). ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.41 (s, 3H), 3.30–3.39 (m, 3H), 3.57 (dd, $J = 2.5$, 2.3 Hz, 1H), 3.79 (s, 6H), 3.87 (t, $J = 5.2$ Hz, 1H), 3.99 (t, $J = 6.1$ Hz, 1H), 4.22 (t, $J = 5.3$ Hz, 1H), 4.27 (t, $J = 5.0$ Hz, 1H, H-3′), 4.34 (d, $J = 3.9$, 1H, H-2′), 4.72 (s, 2H), 5.35 (d, $J = 8.1$, 1H), 5.90 (d, $J = 3.7$, 1H, H-1′), 6.84 (d, $J = 8.2$, 4H), 7.23–7.37 (m, 11H), 7.75 (d, $J = 8.3$, 2H), 7.83 (d, $J = 8.1$, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 55.3, 56.1, 61.9, 62.0, 74.5 (C-2′), 75.9 (C-3′), 81.7, 87.1, 89.8, 95.6, 102.4, 113.3, 127.2, 127.9, 128.0, 128.2, 129.9, 130.1, 130.2, 135.1, 135.2, 136.7, 140.0, 144.2, 145.0, 150.6, 158.8, 163.0. MALDI-TOF MS: $[M + Na]^+$ 781.21, calcd 781.25.

N^6-Acetyl-5′-*O*-(dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)ethoxy-methyl]cytidine (7b) and N^6-acetyl-5′-*O*-(dimethoxytrityl)-3′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]cytidine (8b)

N^4-Acetyl-5′-*O*-DMTr-cytidine (3.95 g, 6.7 mmol) was treated as described for **7a** and **8a** to give **7b** (2.04 g, 38.1%) and **8b** (1.08 g, 20.1%). Compound **7b**: ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.20 (s, 3H), 2.41 (s, 3H), 2.96 (broad, 1H), 3.43 (m, 2H), 3.54 (dd, $J = 2.4$ Hz, 2.4 Hz, 1H), 3.61 (dd, $J = 2.0$ Hz, 1.6 Hz, 1H), 3.81 (s, 6H), 3.92 (q, $J = 5.8$ Hz, 1H), 4.07–4.13 (m, 2H), 4.18 (d, $J = 5.1$ Hz, 1H, H-2′), 4.44 (t, 1H, H-3′), 4.83 (d, $J = 6.6$ Hz, 1H), 5.13 (d, $J = 6.6$ Hz, 1H), 5.88 (s, 1H, H-1′), 6.86 (d, $J = 8.9$ Hz, 4H), 7.09 (d, $J = 7.9$ Hz, 1H), 7.30–7.44 (m, 11H), 7.78 (d, $J = 8.3$ Hz, 2H), 8.47 (d, $J = 7.5$ Hz, 1H), 9.17 (s, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 24.9, 55.3, 56.3, 60.9, 61.7, 67.8 (C-3′), 80.1 (C-2′), 82.9, 87.1, 89.8, 94.9, 96.5, 113.3, 127.2, 128.0, 128.1, 129.9, 130.1, 135.3, 135.5, 136.8, 144,3, 144.7, 144.9, 155.0, 158.7162.6, 170.2. MALDI-TOF MS: $[MH]^+$ 800.21, calcd 800.28. Compound **8b**:^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.21 (s, 3H), 2.42 (s, 3H), 3.35 (dd, $J = 2.9$ Hz, 2.2 Hz, 1H), 3.44 (q, $J = 3.9$ Hz, 2H), 3.60 (dd, $J = 2.0$ Hz, 2.3 Hz, 1H), 3.81 (s, 7H), 4.03 (q, $J = 4.7$ Hz, 1H), 4.18 (dd, $J = 5.0$, 5.4 Hz, 1H, H-3′), 4.33 (m, 2H, H-2′, 4′), 4.61 (d, $J = 7.0$ Hz, 1H), 5.68 (d, $J = 7.0$ Hz, 1H), 5.93 (d, $J = 2.1$ Hz, 1H, H-1′), 6.84 (d, $J = 8.8$ Hz, 4H), 7.17 (d, $J = 7.5$ Hz, 1H), 7.23–7.34 (m, 11H), 7.78 (d, $J = 8.3$ Hz, 2H), 8.35 (d, $J = 7.5$ Hz, 1H), 8.99 (s, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.7, 25.0, 55.3, 56.1, 61.6, 61.9, 75.4, 75.6, 82.1, 87.1, 92.6, 95.7, 96.7, 113.4, 127.3, 128.0, 128.1, 128.2, 130.0, 130.2, 135.2, 135.3, 136.7, 144,2, 144.7, 144.9, 156.0, 158.8, 162.5, 170.2. MALDI-TOF MS: $[MH]^+$ 800.23, calcd 800.28.

N^6-Phenoxyacetyl-5′-*O*-(dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]adenosine (7c) and N^6-phenoxyacetyl-5′-*O*-(dimethoxytrityl)-3′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]adenosine (8c)

N^6-Phenoxyacetyl-5′-*O*-DMTr-adenosine (0.9 g, 1.2 mmol) was treated as described for **7a** and **8a** to give **7c** (0.31 g, 26.4%) and **8c** (0.265 g, 22.8%). Compound **7c**: ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.41 (s, 3H), 3.28 (t, $J = 3.0$ Hz, 2H), 3.40–3.54 (m, 2H), 3.76–3.86 (m, 7H), 3.99–4.04 (m, 1H), 4.25 (t, 1H), 4,56 (t, 1H, H-3′), 4.76–4.86 (m, 5H, H-2′ included), 6.21 (d, $J = 3.2$ Hz, 1H, H-1′), 6.78 (d, $J = 8.9$ Hz, 4H), 7.07 (m, 3H), 7.23–7.41 (m, 13H), 7.74 (d, $J = 8.2$ Hz, 2H), 8.23 (s, 1H), 8.69 (s, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 55.2, 56.1, 61.9, 63.0, 68.1, 70.3 (C-3′), 80.5 (C-2′), 84.1, 86.7, 87.3, 96.0, 113.2, 115.0, 122.5, 127.0, 127.8, 127.9, 128.2, 129.9, 130.1, 135.6, 136.7, 142.2, 144.5, 145.0, 148.4, 152.6, 157.0, 158.6, 166.6. MALDI-TOF MS: $[MH]^+$ 916.18, calcd 916.01. Compound **8c**: ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.42 (s, 3H), 3.27–3.34 (m, 3H), 3.47 (dd, $J = 4.0$, 4.2 Hz, 1H), 3.77 (s, 6H), 3.87–4.03 (m, 2H), 4.32 (t, $J = 3.7$ Hz, 1H), 4.44 (t, $J = 4.4$ Hz, 1H, H-3′), 4,72–4.86 (m, 4H), 4.91 (t, $J = 5.2$ Hz, 1H, H-2′) 6.04 (d, $J = 5.5$ Hz, 1H, H-1′), 6.78 (d, $J = 8.9$ Hz, 4H), 7.03–7.37 (m, 16H), 7.75 (d, $J = 8.2$ Hz, 2H), 8.22 (s, 1H), 8.72 (s, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 55.2, 56.1, 61.7, 63.0, 68.1, 74.3 (C-2′), 77.2 (C-3′), 83.2, 86.7, 89.5, 95.5, 113.2, 115.0, 122.5, 127.0, 127.9, 128.0, 129.8, 130.0, 135.4, 135.5, 136.7, 142.1, 144.4, 145.0, 148.4, 151.5, 152.4, 157.0, 158.6, 166.6. MALDI-TOF MS: $[MH]^+$ 916.30, calcd 916.01.

N^2-(*N*,*N*-Dimethylaminomethylene)-5′-*O*-(dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]guanosine (7d) and N^2-(*N*,*N*-dimethylaminomethylene)-5′-*O*-(dimethoxytrityl)-3′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]guanosine (8d)

N^2-(*N*,*N*-Dimethylaminomethylene)-5′-*O*-DMTr-guanosine (4.5 g, 7 mmol) was treated as described for **7a** and **8a** to give **7d** (1.88 g, 31.4%) and **8d** (1.60 g, 26.7%). Compound **7d**: ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.38 (s, 3H), 3.03 (s, 3H), 3.07 (s, 3H), 3.27 (t, $J = 5.2$ Hz, 2H), 3.42 (broad, 2H), 3.71–3.76 (m, 7H), 4.0 (q, $J = 5.1$ Hz, 1H), 4.23 (broad, 1H), 4.47 (broad, 1H, H-3′), 4.66 (t, $J = 5.0$ Hz, 1H, H-2′), 4.82 (dd, $J = 6.9$, 7.0 Hz, 2H), 6.05 (d, $J = 4.8$ Hz, 1H, H-1′), 6.80 (d, $J = 8.6$ Hz, 4H), 7.18–7.74 (m, 14H), 8.52 (s, 1H), 9.25 (broad, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 35.1, 41.3, 53.4, 55.2, 61.8, 63.6, 70.4 (C-3′), 80.0 (C-2′), 83.7, 85.6, 86.7, 95.3, 113.3, 120.6, 127.7, 127.8, 127.9, 128.1, 129.8, 130.0, 130.1, 135.5, 135.6, 136.0, 136.7, 144.5, 145.0, 150.4, 157.0, 157.9, 158.4, 158.6. MALDI-TOF: $[MH]^+$ 853.33, calcd 853.32. Compound **8d**: ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 2.38 (s, 3H), 2.99 (s, 3H), 3.03 (s, 3H), 3.28–3.39 (m, 4H), 3.76 (s, 6H), 3.85–4.05 (m, 2H), 4.22 (t, $J = 3.9$ Hz, 1H), 4.36 (t, $J = 4.8$ Hz, 1H, H-3′), 4.70–4.82 (m, 3H, H-2′, OCH_2O), 5.91 (d, $J = 5.3$ Hz, 1H, H-1′), 6.78 (d, $J = 8.7$ Hz, 4H), 7.17–7.75 (m, 14H), 8.44 (s, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 35.1, 41.3, 55.2, 56.2, 61.7, 63.2, 74.0 (C-2′), 76.8 (C-3′), 82.2, 86.5, 88.2, 95.5, 113.2, 120.4, 127.8, 127.9, 128.1, 129.8, 129.9, 130.0, 135.5, 135.6, 136.5, 136.7, 144.5, 144.9, 150.2, 156.7, 157.9, 158.1, 158.6. MALDI-TOF: $[MH]^+$ 853.30, calcd 853.32.

5′-*O*-(Dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]-uridine 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (9a)

A solution of compound **7a** (544 mg, 0.72 mmol) in dichloromethane (5 ml) was treated with *N*,*N*-diisopropylethylamine (0.3 ml, 1.79 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.24 ml, 1.1 mmol) was added dropwise. The reaction was allowed to proceed for 2 h at room temperature. After quenched with MeOH (0.5 ml), the mixture was diluted with dichloromethane and washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, chromatographed on a short column (cyclohexane with 1% Et_3N, ethyl acetate from 10% to 50%) to give the phosphoramidite **9a** (0.54 g, 78.6%). ^{31}P NMR (109.4 MHz, $CDCl_3$ + DABCO): 149.90, 151.00. MALDI-TOF: $[MH]^+$ 959.16, calcd 959.36.

N^6-Acetyl-5′-*O*-(dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)-ethoxymethyl]cytidine 3′-(2-cyanoethyl-*N*,*N*-diisopropyl-phosphoramidite) (9b)

Compound **7b** (1.23 g, 1.54 mmol) was treated as described for **9a**. Short column chromatography (cyclohexane with 1% Et_3N, acetone from 20% to 50%) gave **9b** (1.08 g, 67.9%). ^{31}P NMR (109.4 MHz, $CDCl_3$ + DABCO): 150.16, 151.69. MALDI-TOF MS: $[MH]^+$ 1000.29, calcd 1000.39.

N^6-Phenoxyacetyl-5′-*O*-(dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]adenosine 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (9c)

Compound **7c** (0.246 g, 0.26 mmol) was treated as described for **9a**. Short column chromatography (petroleum ether with 1% Et_3N, acetone from 20% to 40%) gave **9c** (0.18 g, 60.0%). ^{31}P NMR (109.4 MHz, $CDCl_3$ + DABCO): 148.66, 148.79. MALDI-TOF: $[MH]^+$ 1115.99, calcd 1116.42.

N^2-(*N*,*N*-Dimethylaminomethylene)-5′-*O*-(dimethoxytrityl)-2′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]guanosine 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (9d)

Compound **7d** (0.965 g, 1.13 mmol) was treated as described for **9a**. Short column chromatography (CH_2Cl_2 with 1% Et_3N, acetone from 10% to 30%) gave **9d** (0.64 g, 53.9%). ^{31}P NMR (109.4 MHz, $CDCl_3$ + DABCO): 148.67, 148.90. MALDI-TOF MS: $[MH]^+$ 1053.38, calcd 1054.17.

5′-*O*-(Dimethoxytrityl)-3′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]-uridine 2′-*O*-pimelate (10a)

To a solution of **8a** (0.5 g, 0.66 mmol) in dry pyridine (10 ml) was added pimelic acid (258 mg, 1.61 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC, 284 mg, 1.48 mmol) and 4-(dimethylamino)pyridine (82 mg, 0.67 mmol). The reaction was allowed to proceed at room temperature for 4 h. After evaporation of solvent, the residue was diluted with CH_2Cl_2, washed with H_2O twice, saturated $(NH_4)_2CO_3$ once, H_2O once, dried over $MgSO_4$ and chromatographed on a short column (dichloromethane with 1% Et_3N, methanol from 1% to 5%) to give **10a** (448 mg, 75.4%). ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 1.32 (m, 2H), 1.63 (m, 4H), 2.21 (t, $J = 6.9$ Hz, 2H), 2.36 (t, $J = 6.6$ Hz, 2H), 2.43 (s, 3H), 3.19 (t, $J = 3.8$ Hz, 2H), 3.37 (dd, $J = 1.9$ Hz, 1H), 3.58 (dd, $J = 1.8$ Hz,

1H), 3.73 (t, $J = 4.7$ Hz, 2H), 3.79 (s, 6H), 4.14 (t, $J = 2.4$ Hz, 1H), 4.43 (t, $J = 5.1$ Hz, 1H, H3′), 4.54 (dd, $J = 7.1$ Hz, 2H), 5.29 (d, $J = 8.1$ Hz, 1H), 5.37 (t, $J = 4.8$ Hz, 1H, H2′), 6.11 (d, $J = 4.7$ Hz, 1H), 6.84 (d, $J = 8.0$ Hz, 4H), 7.22–7.34 (m, 11H), 7.72 (m, 3H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.7, 24.7, 25.7, 28.9, 33.8, 36.8, 55.3, 56.0, 61.6, 62.2, 73.5 (C3′), 74.0 (C2′), 82.3, 86.6, 87.2, 94.9, 102.7, 113.3, 127.3, 127.9, 128.0, 128.3, 129.9, 130.1, 130.2, 135.0, 139.5, 144.0, 144.8, 150.3, 158.8, 163.2, 172.6, 179.8. MALDI-TOF MS: $[M + Na]^+$ 1023.16, calcd 1023.31.

N^6-Acetyl-5′-*O*-(dimethoxytrityl)-3′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]cytidine 2′-*O*-pimelate (10b)

Compound **8b** (150 mg, 0.19 mmol) was treated as described for **10a** to give **10b** (123 mg, 70.0%). ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 1.37 (m, 1H), 1.64 (m, 2H), 2.21 (s, 3H), 2.31 (t, $J = 6.6$ Hz, 1H), 2.37 (t, $J = 3.4$ Hz, 1H), 2.42 (s, 3H), 3.16 (t, $J = 3.7$ Hz, 2H), 3.39 (dd, $J = 2.0$ Hz, 1H), 3.67 (m, 3H), 3.81 (s, 6H), 4.19 (t, $J = 6.9$ Hz, 1H), 4.40 (m, 2H), 4.53 (d, $J = 7.1$ Hz, 1H), 5.39 (t, $J = 4.3$ Hz, 1H, H2′), 6.1 (d, $J = 2.1$ Hz, 1H, H1′), 6.85 (d, $J = 7.4$ Hz, 4H), 7.02 (d, $J = 7.5$, 1H), 7.71 (d, $J = 8.2$, 2H), 8.27 (d, $J = 7.6$, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.7, 24.7, 24.8, 24.9, 28.6, 33.8, 34.8, 55.3, 56.1, 61.4, 61.7, 72.4 (C3′), 74.3 (C2′), 81.7, 87.3, 88.8, 94.9, 96.8, 113.4, 123.8, 127.5, 128.0, 128.1, 128.4, 130,0, 130.2, 130.3, 135.1, 136.1, 136.9, 143.9, 144.7, 144.9, 149.8, 158.9, 162.8, 170.6, 172.2, 178.4. MALDI-TOF MS: $[M + Na]^+$ 964.31, calcd 965.04.

N^6-Phenoxyacetyl-5′-*O*-(dimethoxytrityl)-3′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]adenosine 2′-*O*-pimelate (10c)

Compound **8c** (214 mg, 0.23 mmol) was treated as described for **10a** to give **10c** (160 mg, 64.8%). ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 1.32 (m, 1H), 1.61 (m, 2H), 2.29–2.39 (m, 2H), 2.42 (s, 3H), 3.22 (t, $J = 5.8$ Hz, 2H), 3.33 (dd, $J = 3.8$ Hz, 1H), 3.56 (dd, $J = 3.1$ Hz, 1H), 3.77 (m, 8H), 4.27 (m, 1H), 4.57 (s, 2H), 4.71 (t, $J = 5.5$ Hz, 1H, H3′), 4.86 (s, 2H), 5.83 (t, $J = 4.4$ Hz, 1H, H2′), 6.26 (d, $J = 4.1$ Hz, 1H, H1′), 6,79 (d, $J = 8.8$ Hz, 1H), 7.04 (m, 3H), 7.15–7.39 (m, 13H), 7.73 (d, $J = 8.3$ Hz, 2H), 8.27 (s, 1H), 8.74 (s, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 24.3, 24.4, 28.2, 33.6, 33.7, 55.4, 56.0, 61.7, 62.5, 68.2, 73.9 (C3′), 74.4 (C2′), 82.7, 86.6, 86.8, 95.2, 113.2, 115.2, 123.0, 127.8, 128.1, 129.8, 130.1, 130.3, 135.4, 135.5, 136.8, 141.8, 144.3, 145.0, 148.5, 151.4, 153.0, 157.1, 158.7, 166.8, 172.4, 176.6. MALDI-TOF MS: $[M]^+$ 1058.36, calcd 1058.38

N^2-(*N*,*N*-Dimethylaminomethylene)-5′-*O*-(dimethoxytrityl)-3′-*O*-[2-(4-tolylsulfonyl)ethoxymethyl]guanosine 2′-*O*-pimelate (10d)

Compound **8d** (165 mg, 0.19 mmol) was treated as described for **10a** to give **10d** (126 mg, 65.6%). ^{1}H NMR (270 MHz, $CDCl_3$ + DABCO): δ 1.35 (m, 1H), 1.61 (m, 2H), 2.20 (t, $J = 7.3$ Hz, 1H), 2.38 (m, 4H), 3.07 (s, 3H), 3.11 (s, 3H), 3.15 (t, $J = 2.4$ Hz, 2H), 3.26 (dd, $J = 2.6$ Hz, 1H), 3.43 (dd, $J = 2.2$ Hz, 1H), 3.71–3.77 (m, 8H), 4.21 (m, 1H), 4.51 (m, 3H), 5.92 (t, $J = 3.2$ Hz, 1H, H2′), 5.98 (d, $J = 2.3$ Hz, 1H, H1′), 6.79 (m, 4H), 7.21–7.38 (m, 11 H), 7.71 (m, 3H), 8.58 (s, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$ + DABCO): δ 21.6, 24.8, 25.8, 29.0, 33.9, 35.2, 36.9, 41.3, 55.3, 55.9, 61.5, 62.7, 73.5 (C3′), 74.1 (C2′), 81.7, 86.5, 86.6, 95.0, 113.2, 127.0, 127.8, 127.9, 128.1, 129.9, 130.0, 135.5, 136.5, 136.8, 144.3, 145.5, 152.2, 156.9, 157.6, 158.5, 158.6, 172.6, 179.6. MALDI-TOF MS: $[M]^+$ 995.37, calcd 995.10.

2′-*O*-[2-(4-Tolylsulfonyl)ethoxymethyl]uridine (15)

Compound **7a** (115 mg, 0.15 mmol) was treated with 3% DCA/DCM (5 ml), and the reaction was allowed to proceed at room temperature for 5 min. The mixture was then diluted with ethyl acetate, washed with saturated $NaHCO_3$, and dried over $MgSO_4$. Short column chromatography (CH_2Cl_2–MeOH, 9 : 1, v/v), gave compound **15** (23 mg, 33%). ^{1}H NMR (270 MHz, CD_3OD): δ 2.42 (s, 3H), 3.50 (t, $J = 5.7$ Hz, 2H), 3.73 (dd, $J = 2.6$ Hz, 1H), 3.83–3.97 (m, 4H), 4.09 (t, $J = 4.2$ Hz, 1H), 4.19 (t, $J = 5.4$ Hz, 1H), 4.71 (dd, $J = 7.0$ Hz, 1H), 5.67 (d, $J = 8.1$ Hz, 1H), 5.88 (d, $J = 3.7$ Hz, 1H), 7.40 (d, $J = 7.8$ Hz, 2H), 7.77 (d, $J = 8.0$ Hz, 2H), 8.02 (d, $J = 8.0$ Hz, 1H). ^{13}C NMR (67.9 MHz, CD_3OD): δ 20.28, 55.76, 60.38, 61.87, 68.85, 79.20, 84.72, 88.01, 94.72, 101.30, 127.90, 129.57, 137.09, 141.11, 144.95, 150.82, 164.83. MALDI-TOF MS: $[MH]^+$ 457.22, calcd 457.12.

Preparation of the solid support

To a solution of **10a–d** (0.1 mmol) in dry acetonitrile (20 ml) was added LCAA-CPG (1 g, Biotech company), and then diisopropylethylamine (1.74 ml, 10 mmol), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexfluorophosphate (BOP, 84 mg, 0.2 mmol) and *N*-hydroxybenzotriazole (27 mg, 0.2 mmol). After shaking at room temperature for 2 h, this reaction mixture was filtered, washed in turn with acetonitrile, CH_2Cl_2, methanol, CH_2Cl_2 and diethyl ether. The solid was then suspended in dry pyridine (20 ml) with acetic anhydride (2.25 ml) and 4-(dimethylamino)pyridine (DMAP, 465 mg), and shaken for 2 h at room temperature. After filtration, the solids were washed in turn with pyridine, toluene, CH_2Cl_2, methanol, CH_2Cl_2 and diethyl ether, and dried over P_2O_5 under high vacuum. The loadings, determined by detritylation assays, were 20–25 μmol g^{-1}.

RNA synthesis and purification

All the RNAs were assembled on a Applied Biosystems 392 DNA/RNA synthesizer. All syntheses were carried out in trityl-off mode; the synthesis cycle and reagents can be found in the Supplementary information†. After the assembly, the solid supports were removed from the cartridges and treated with 25% NH_3/MeOH (4 ml) at room temperature for 20 h, and then at 40 °C for 4 h. The supernatant solutions were then separated from the solid supports, and evaporated to dryness. After co-evaporation with dry THF twice, they were treated with 1 M tetrabutylammonium fluoride in THF (this solution was dried with 4 Å molecular sieves overnight before use) containing 10% n-propylamine and 1% bis(2-mercaptoethyl) ether at room temperature for 20 h. The reactions were quenched by the addition of an equal volume of doubly distilled water and applied to a NAP-10 column according to the manufacturer's instructions, with doubly distilled water as the eluting buffer. To ensure the complete removal of fluoride, the products were passed through a Sep-Pak cartridge.

RNase H digestion assays

Escherichia coli RNase H (5 units μL^{-1}, specific activity 420 000 units mg^{-1}, molecular weight 21 000 g mol^{-1}), T4

polynucleotide Kinase (30 units μL^{-1}) and [γ-^{32}P]ATP were purchased from Amersham Pharmacia Biotech (Sweden). The pure 15-mer RNA (**ON12**) was from IBA BioTAGnology (received in crude form and purified by PAGE; the purity is shown in Fig. S5†). Synthesis of the complementary DNA was carried out as previously described.[43] **ON12** was synthesized, deprotected, desalted by NAP-10 column and Sep-Pak cartridge as in the description above, to give the crude **ON12**, which was used directly for RNase H digestion. The RNA was 5′-end labeled with ^{32}P using T4 polynucleotide kinase, [γ-^{32}P]ATP by standard procedure.

The RNase H digestion of synthesized crude RNA and pure RNA was carried out according to the following procedure: target pure RNA (0.1 μM) or crude RNA (0.1 μM) (specific activity 70000 cpm) and 10-fold excess of complementary DNA (1 μM) were incubated in a buffer, containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM DTT at 21 °C in the presence of 0.06 U *E. coli* RNase H. Prior to the addition of the enzyme reaction components were pre-annealed in the reaction buffer by heating at 80 °C for 4 min followed by 1.5 h equilibration at 21 °C. Total reaction volume was 30 μL. Aliquots of 3 μL were taken after 2, 5, 10, 15, 25, 40 and 60 min and the reactions were terminated by mixing with stop solution (7 μL), containing 0.05 M EDTA, 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene cyanole in 80% formamide. The samples were subjected to 20% 7 M urea PAGE and visualized by autoradiography. Quantitation of cleavage products was performed using a Molecular Dynamics PhosphorImager.

Phosphodiesterase digestion assays

Shrimp Alkaline Phosphatase and Phosphodiesterase I (Crotalus adamanteus Venom) are from Amersham Pharmacia Biotech, Sweden. A reaction mixture containing 20 mM Tris-HCl (pH 8.0), 10 ml $MgCl_2$, Shrimp Alkaline Phosphatase (0.5 unit) and Phosphodiesterase I (Crotalus adamanteus Venom) (0.4 unit) was added to crude **ON12** (1 OD unit at 260 nm) with a total reaction volume of 30 μL. The reaction mixture was incubated at 37 °C for 24 h and subjected to HPLC analysis directly (0.2 OD per injection).

Acknowledgements

Generous financial support from the Swedish Natural Science Research Council (Vetenskapsrådet), the Swedish Foundation for Strategic Research (Stiftelsen för Strategisk Forskning) and the EU-FP6-funded RIGHT project (project no. LSHB-CT-2004-005276) is gratefully acknowledged.

References

1 A. Fire, S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver and C. C. Mello, *Nature*, 1998, **391**, 808–811.
2 S. M. Elbashir, J. Harborth, W. Lendechel, A. Yalcin, K. Weber and T. Tuschl, *Nature*, 2001, **411**, 494–498.
3 C. B. Reese, *Org. Biomol. Chem.*, 2005, **3**, 3851–3868.
4 D. Gasparutto, D. Molko and R. Téoule, *Nucleosides Nucleotides*, 1990, **9**, 1087–1098.
5 C. B. Reese, 'Protection of 2′-hydroxy functions of ribonucleosides' in *Current Protocols in Nucleic Acid Chemistry*, ed. S. L. Beaucage, D. E. Bergstrom, G. D. Glick and R. A. Jones, John Wiley & Sons, New York, 2000.
6 S. G. Chaulk and A. M. MacMillan, *Nucleic Acids Res.*, 1998, **26**, 3173–3178.
7 M. E. Schwartz, R. R. Breaker, G. T. Asteriadis, J. S. de Bear and G. R. Gough, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 1019–1024.
8 C. B. Reese, R. Saffhill and J. E. Sulston, *J. Am. Chem. Soc.*, 1967, **89**, 3366–3368.
9 A. Sandstroem, M. Kwiatkowski and J. Chattopadhyaya, *Acta Chem. Scand., Ser. B*, 1985, **39**, 273–290.
10 A. Sandstroem, M. Kwiatkowski and J. Chattopadhyaya, *Nucleosides Nucleotides*, 1985, **4**, 177–181.
11 W. Lloyd, C. B. Reese, Q. Song, A. M. Vandersteen, C. Vistina and P. Z. Zhang, *J. Chem. Soc., Perkin Trans. 1*, 2000, 165–176.
12 M. Pfister, H. Schirmeister, M. Mohr, S. Farkas, K. P. Stengele, T. Reiner, M. Dunkel, S. Gokhale, R. Charubala and W. Pfleiderer, *Helv. Chim. Acta*, 1995, **78**, 1705–1737.
13 S. Matysiak and W. Pfleiderer, *Helv. Chim. Acta*, 2001, **84**, 1066–1085.
14 A. Semenyuk, A. Foldesi, T. Johansson, C. Estmer-Nilsson, P. Blomgren, M. Brannvall, L. A. Kirsebom and M. Kwiatkowski, *J. Am. Chem. Soc.*, 2006, **128**, 12356–12357.
15 K. K. Ogilvie, K. L. Sadana, E. A. Thompson, M. A. Quilliam and J. B. Westmore, *Tetrahedron Lett.*, 1974, **33**, 2861–2863.
16 K. K. Ogilvie, N. Theriault and K. L. Sadana, *J. Am. Chem. Soc.*, 1977, **99**, 7741–7743.
17 N. Usman, K. K. Ogilvie, M. Y. Jiang and R. J. Cedergren, *J. Am. Chem. Soc.*, 1987, **109**, 7845–7854.
18 T. Wada, M. Tobe, T. Nagayama, K. Furusawa and M. Sekine, *Tetrahedron Lett.*, 1995, **36**, 1683–1684.
19 T. Umemoto and T. Wada, *Tetrahedron Lett.*, 2004, **45**, 9529–9531.
20 M. V. Rao, C. B. Reese, V. Schehlmann and P. S. Yu, *J. Chem. Soc., Perkin Trans. 1*, 1993, 43–55.
21 J. Stawinski, R. Strömberg, M. Thelin and E. Westman, *Nucleic Acids Res.*, 1988, **16**, 9285–9308.
22 T. Wu, K. K. Ogilvie and R. T. Pon, *Nucleic Acids Res.*, 1989, **17**, 3501–3517.
23 S. S. Jones and C. B. Reese, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2762–2764.
24 S. A. Scaringe, C. Francklyn and N. Usman, *Nucleic Acids Res.*, 1990, **18**, 5433–5441.
25 S. A. Scaringe, F. E. Wincott and M. H. Caruthers, *J. Am. Chem. Soc.*, 1998, **120**, 11820–11821.
26 S. Pitsch, P. A. Weiss, L. Jenny, A. Stutz and X. Wu, *Helv. Chim. Acta*, 2001, **84**, 3773–3795.
27 T. Ohgi, Y. Masutomi, K. Ishiyama, H. Kitagawa, Y. Shiba and J. Yano, *Org. Lett.*, 2005, **7**, 3477–3480.
28 F. G. Bordwell, *Acc. Chem. Res.*, 1988, **21**, 456–463.
29 S. Josephson, N. Balgobin and J. Chattopadhyaya, *Tetrahedron Lett.*, 1981, **22**, 4537–4540.
30 M. Jain, J. Fan, N. Z. Baturay and C. H. Kwon, *J. Med. Chem.*, 2004, **47**, 3843–3852.
31 K. A. Watanabe and J. J. Fox, *Angew. Chem., Int. Ed. Engl.*, 1966, **5**, 579–580.
32 T. L. Sheppard, A. T. Rosenblatt and R. Breslow, *J. Org. Chem.*, 1994, **59**, 7243–7248.
33 C. Chaix, A. M. Duplaa, D. Molko and R. Téoule, *Nucleic Acids Res.*, 1989, **17**, 7381–7393.
34 L. J. McBride and M. H. Caruthers, *Tetrahedron Lett.*, 1983, **24**, 245–248.
35 H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. E. Sulston and D. R. Trentham, *Tetrahedron*, 1966, **22**, 705–710.
36 K. K. Ogilvie, A. L. Schifman and C. L. Penney, *Can. J. Chem.*, 1979, **57**, 2230–2238.
37 J. Milecki, E. Zamaratski, T. V. Maltseva, A. Földesi, R. Adamiak and J. Chattopadhyaya, *Tetrahedron Lett.*, 1999, **55**, 6603–6622.
38 R. T. Pon and S. Yu, *Tetrahedron Lett.*, 1997, **38**, 3331–3334.
39 C. Vargeese, J. Carter, J. Yegge, S. Krivjansky, A. Settle, E. Kropp, K. Peterson and W. Pieken, *Nucleic Acids Res.*, 1998, **26**, 1046–1050.
40 E. Lieber and T. Enkoji, *J. Org. Chem.*, 1961, 4472–4479.
41 B. H. Dahl, J. Nielsen and O. Dahl, *Nucleic Acids Res.*, 1987, **15**, 1729–1743.
42 T. Umemoto and T. Wada, *Tetrahedron Lett.*, 2005, **46**, 4251–4253.
43 P. I. Pradeepkumar, E. Zamaratski, A. Földesi and J. Chattopadhyaya, *J. Chem. Soc., Perkin Trans. 2*, 2001, 402–408.