Novel uridin-2′-yl carbamates: synthesis, incorporation into oligodeoxyribonucleotides, and remarkable fluorescence properties of 2′-pyren-1-ylmethylcarbamate †

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A convenient method for the preparation of uridin-2′-yl carbamate derivatives is described. The stable 2′-*O*-(imidazol-1-ylcarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine, prepared from uridine, was treated with primary or secondary aliphatic amines to give 2′-carbamates in high yield. After 3′,5′-*O*-deprotection with triethylamine trihydrofluoride, 5′-*O*-dimethoxytritylation, and 3′-*O*-phosphitylation with bis(*N,N*-diisopropylamino)-2-cyanoethoxyphosphine, modified phosphoramidites were obtained and used in machine-assisted synthesis of modified oligodeoxynucleotides containing uridin-2′-yl carbamate residues bearing various *N*-substituents. The influence of uridin-2′-yl carbamate moieties on the stability of modified oligonucleotide duplexes with complementary DNA and RNA was studied by thermal denaturation experiments. Pyrene-modified oligonucleotides showed a considerable increase in fluorescence intensity upon hybridisation to complementary RNA and interesting binding properties when hybridised to a mismatched DNA.

Introduction §

Oligonucleotides conjugated with various ligands are the subject of extensive studies and applications in bioorganic chemistry, molecular biology and biotechnology. The particular site of ligand attachment depends on the nature of the biotarget. Studies we present here were motivated originally by a need to develop a convenient method for the introduction of various ligands into ribozymes. For example, attachment of ligands such as polyamines to the hairpin ribozyme might enhance its RNA cleavage potential.[1] Since nucleobases in ribozyme core regions are often involved in various non-covalent interactions important for maintaining structure and function,[2] the sugar part of nucleosides, particularly the 2′-hydroxy group, seemed to be an attractive position for ligand attachment, especially because 2′-ligand functionalisation has already proved valuable in applications such as fluorescence monitoring of the tertiary folding of RNAs.[3]

There are numerous examples of 2′-*O*-alkyl derivatives of nucleosides and their introduction into oligonucleotides.[4] A general and frequent shortcoming is a poor yield in the key step, 2′-*O*-alkylation. An alternative opportunity for 2′-functionalisation is *via* a carbamate function, easily generated from primary or secondary alcohols by successive treatment with 1,1′-carbonyldiimidazole (CDI) and an aliphatic amine. This chemistry has been used successfully for 5′-modification of oligonucleotides.[5] Aliphatic 5′-carbamates were reported to be stable under hot (55 °C) ammoniacal deprotection conditions in contrast to aniline-derived nucleoside 3′-carbamates.[6]

Oligonucleotides containing nucleoside 2′-carbamates have been reported previously.[7] Cytidin-2′-yl carbamate was obtained by CDI activation followed by amine treatment.[7b] Some uridin-2′-yl carbamate modifications were found to be detrimental to the stability of DNA–RNA duplexes.[7c,e] However, systematic studies and full synthetic details have not been reported yet. Moreover, the merit of 2′-carbamate functionalisation in many nucleic acid applications remained unexplored.

Here we describe the preparation of several uridin-2′-yl carbamates and their introduction into oligodeoxyribonucleotides and report on their hybridisation with complementary DNA and RNA. It was found that 2′-pyren-1-ylmethylcarbamate-modified oligonucleotides showed remarkable DNA mismatch affinity and enhanced fluorescent properties when bound to a complementary RNA.

Results and discussion

Synthesis of modified phosphoramidites

A general approach to the preparation of uridin-2′-yl carbamates and their corresponding phosphoramidites is outlined in Scheme 1. The starting 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine **1** was obtained from uridine as reported previously[8] and purified by column chromatography on silica gel in EtOAc–$CHCl_3$ (1 : 9, then 1 : 2 v/v). Subsequent reaction of **1** with CDI in dry DCM gave 2′-*O*-(imidazol-1-ylcarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine **2** in nearly quantitative yield. DCM proved to be the best solvent for imidazolide formation, whereas use of the more polar MeCN led to a much longer reaction time. Imidazolide **2** is stable during aqueous extraction, but is prone to hydrolysis under acidic or alkaline conditions. Some decomposition was observed also during silica gel chromatography. Note that the MALDI-TOF mass spectrum of compound **2** was obtained using the neutral

† Electronic supplementary information (ESI) available: Additional experimental data for compounds **3**, **5** and **6**. See http://www.rsc.org/suppdata/p1/b1/b111434b/

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§ Abbreviations: CDI 1,1′-carbonyldiimidazole, DIEA *N,N*-diisopropylethylamine, Dmt 4,4′-dimethoxytrityl, Piv pivaloyl = 2,2-dimethylpropanoyl, Tfa trifluoroacetyl, 2,4,6-THAP 2,4,6-trihydroxyacetophenone, 2,5-DHBA 2,5-dihydroxybenzoic acid, MALDI-TOF matrix-assisted laser desorption ionization time-of-flight.

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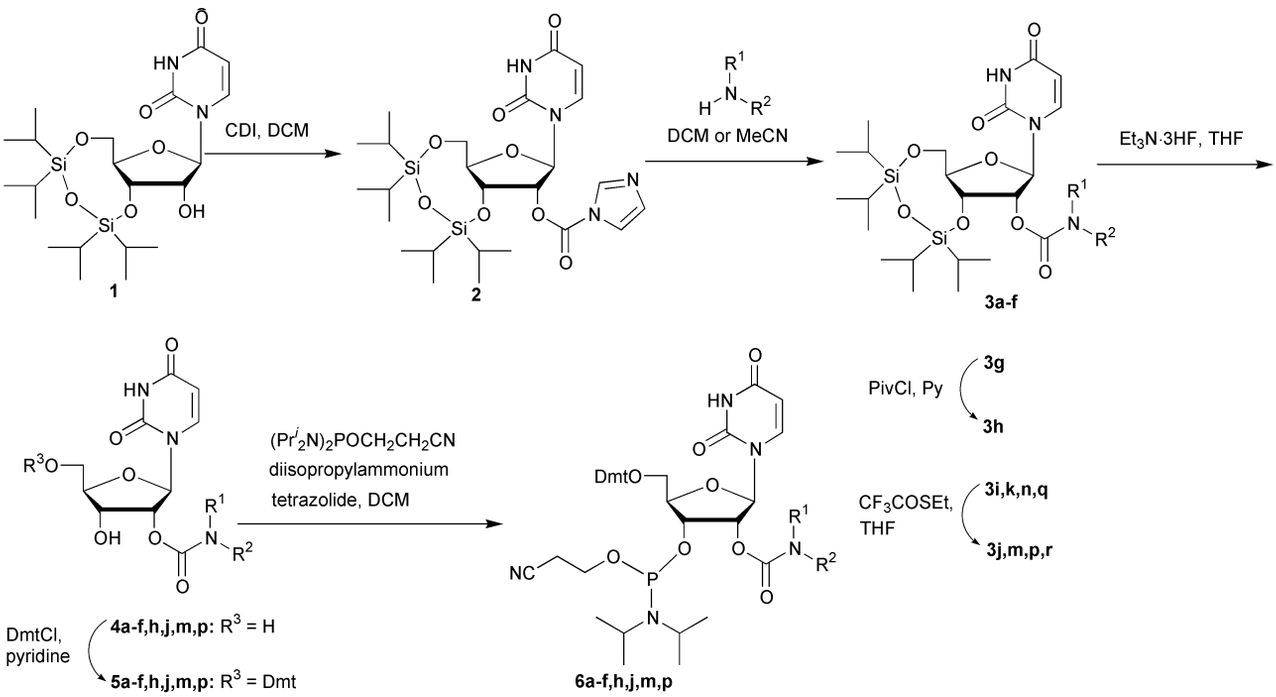

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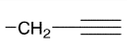
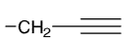
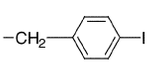
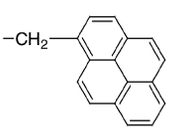
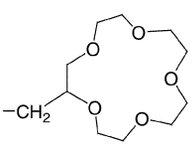
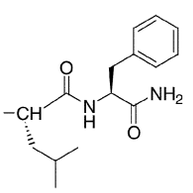
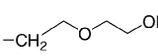
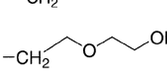
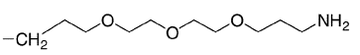
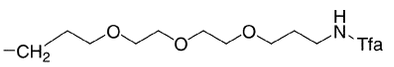
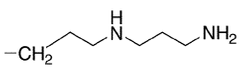
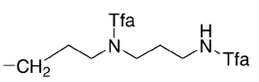
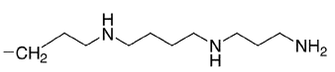
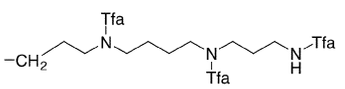
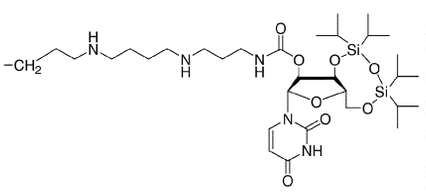
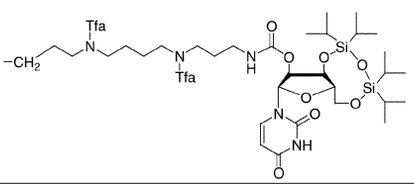
3a–6a	H	
3b–6b	Me	
3c–6c	H	
3d–6d	H	
3e–6e	H	
3f–6f	H	
3g	H	
3h–6h	H	

3i	H	
3j–6j	H	
3k	H	
3m–6m	H	
3n	H	
3p–6p	H	
3q	H	
3r	H	

Scheme 1

matrix 2,4,6-trihydroxyacetophenone (2,4,6-THAP) rather than the acidic 2,5-dihydroxybenzoic acid (2,5-DHBA), which is the standard matrix used for other 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine derivatives.

In many cases imidazolide **2** need not be isolated, but instead reacted *in situ* with an excess of the appropriate amine in dry DCM. In the case of amine hydrochlorides, 1.1 equivalents of DIEA was added to the reaction mixture. Reaction times differed vastly from one amine to another, and were found also to be solvent dependent. Whilst propargylamine (prop-2-ynylamine) reacted rapidly in DCM in less than an hour, *N*-methylpropargylamine, a secondary amine, required overnight reaction. 2-Aminomethyl-15-crown-5 reacted very slowly in DCM. To accelerate the reaction, the solvent was changed to MeCN and temperature increased to 55 °C. 3′,5′-*O*-Protected uridin-2′-yl carbamates **3** were isolated by column chromatography.

The reaction with polyamines, *N*-(3-aminopropyl)propane-1,3-diamine and spermine, gave exclusively primary amine-substituted products. This observation is in good agreement

with the recently reported high selectivity of the reaction of imidazolecarbonyl derivatives of alcohols with primary *vs.* secondary amines.[9]

The remaining amino functions in compounds **3i**, **3k**, **3n** were protected by the trifluoroacetyl group, which is used commonly for protection of aliphatic amino group in various phosphoramidite reagents, *e.g.* nucleoside reagents carrying polyamines.[10] The hydroxy group in compound **3g** was acylated with pivaloyl chloride to give ester **3h**.

Initially we used TBAF in THF to remove the Markiewicz' 3′,5′-*O*-silyl group from **3**. After 15–20 min, TLC showed smooth silyl deprotection, but also the appearance of a by-product with similar mobility. After several hours of TBAF treatment, an equilibrium was reached where a 1 : 1 ratio of desired product to by-product was obtained. The by-product was assumed to be the regioisomeric 3′-carbamate, generated by carbamoyl migration in the vicinal diol system. The possibility of carbamoyl migration has been discussed in a similar case,[7a] but the 3′-carbamate itself was not detected. Interestingly, these authors[7a] used a buffered TBAF–AcOH deprotection mixture. In other cases[7b,d] TBAF solution in THF was used. However in our hands, TBAF in THF always gave a mixture of products. Therefore we tried the alternative desilylating reagent, triethylamine trihydrofluoride. This gave only the desired 2′-carbamate product after overnight deprotection.

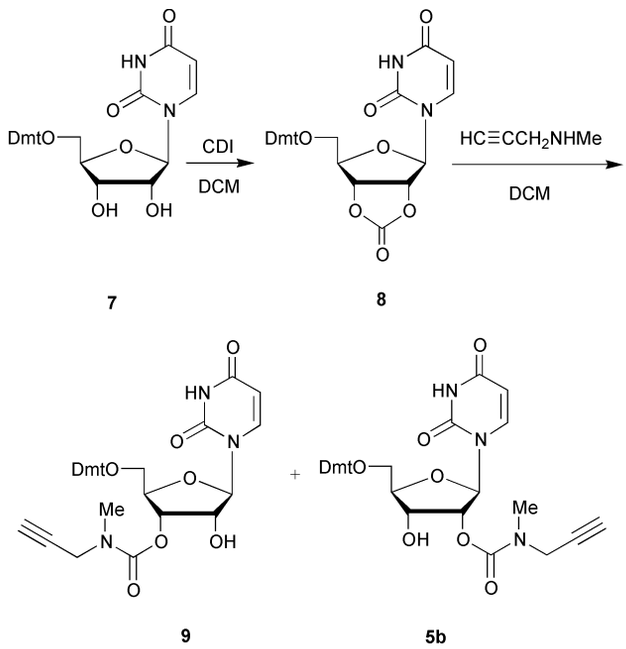
We were unable to obtain crystalline samples of uridin-2′-yl carbamates **4**, except in the case of aromatic derivatives **4c** and **4d**. Therefore crude compounds **4** (viscous oils) were used in the subsequent 5′-dimethoxytritylation to give derivatives **5** in high yield. The bulky 2′-carbamate moiety prevented subsequent dimethoxytritylation of the neighbouring 3′-hydroxy group, and 5′,3′-bis-dimethoxytritylated product was isolated only in the case of 15-crown-5 carbamate. No 2′–3′ migration was detected when pyridine was used as the solvent for dimethoxytritylation or in most cases during the column chromatography in the presence of MeOH and triethylamine. However, isolated compounds **5** showed a slow 2′–3′ migration under basic conditions or in protic solvents. Noteworthy, the pyrene derivative **5d** and the dipeptide derivative **5e** showed a considerable rate of isomerisation during column chromatography in the presence of triethylamine. Therefore pyridine was used as a neutralization additive during their purifications on silica gel.

5′-*O*-Dimethoxytrityluridin-2′-yl carbamates **5** were then phosphitylated with bis(*N,N*-diisopropylamino)-2-cyanoethoxyphosphine in DCM in the presence of diisopropylammonium tetrazolide to give phosphoramidites **6** which were isolated by column chromatography. None of the starting carbamate compounds **5** gave rise to isomerisation product during the phosphitylation reaction.

2′–3′ Migration of the carbamoyl group

Carbamates **4** and **5**, containing an adjacent 3′-OH group, are prone to isomerisation under alkaline conditions.[11] 5′-*O*-Dmt derivatives **5** upon ammonia treatment in aqueous MeOH gave 1 : 1 mixtures of two tritylated compounds with rather similar TLC mobilities. One spot was always the starting compound and the other was clearly different from 5′-*O*-dimethoxytrityluridine **7**. To confirm that the compound generated under migration conditions is the 3′-carbamate we set out to isolate it, confirm its structure by NMR, and prepare it by an independent method. Complete separation of the by-product by column chromatography appeared to be a difficult task. However, in the case of a carbamate derived from the secondary amine *N*-methylpropargylamine, the difference in chromatographic mobilities of the 5′-*O*-Dmt derivatives was sufficient for complete resolution on a preparative silica gel column. Therefore, a sample of compound **3b** was desilylated with TBAF and the resulting mixture was treated with DmtCl in pyridine to give 5′-*O*-Dmt-compounds **5b** and **9**, which were separated by column chromatography. ^{1}H NMR showed that the signal set obtained from **9** is similar to that obtained from **5b**, thus confirming that **5b** and **9** are isomers. MALDI-TOF mass spectral analysis also showed identical molecular masses.

For an independent route of synthesis, 5′-*O*-dimethoxytrityluridine **7**[12] was treated with CDI in dry DCM to give cyclic 2′,3′-carbonate **8** in quantitative yield (Scheme 2). The



Scheme 2

formation of 2′,3′-carbonates from CDI and 5′-*O*-tritylated nucleosides has been described before.[13] Carbonate **8** was reacted with *N*-methylpropargylamine to give a mixture of two regioisomeric carbamates **5b** and **9**. These were separated by column chromatography and proved to be identical to the samples obtained by the first route.

Oligonucleotide synthesis and thermal denaturation studies

2′-Modified nucleoside phosphoramidites **6** were used in solid phase oligodeoxyribonucleotide synthesis where the coupling time was increased to 10 min. The sequence chosen was a non-self-complementary 15-mer of mixed sequence d(CTCCCAGGCTCAAAT) (**ON2**). A deoxyuridine or a 2′-modified uridine was introduced in place of the internal thymidine (**ON4**–**ON14**), the thymidine close to the 5′-end (**ON15**–**ON23**), or at both sites (**ON24**–**ON31**). Also prepared were the complementary unmodified oligodeoxyribonucleotide d(ATTTGAGCCTGGGAG) (**ON1**) and oligoribonucleotide r(AUUUGAGCCUGGGAG) (**ON3**) as hybridisation targets. These syntheses allowed model DNA–DNA (**ON2**–**ON1**, T_m 57.0 °C) and DNA–RNA (**ON2**–**ON3**, T_m 59.2 °C) duplexes to be studied, into which 2′-modifications were incorporated into the same DNA strand. The series of 2′-modified oligonucleotides (Table 1) was characterised by MALDI-TOF mass spectrometry. In all cases, a 2′-carbamate function was found to be completely stable to the conditions of oligonucleotide synthesis as well as to final deprotection with concentrated aqueous ammonia (55 °C, 8–16 h). The pivalate ester obtained by use of phosphoramidite **3h** proved to be unexpectedly stable. Thus only after 4 days of hot ammoniacal treatment did the MALDI-TOF spectrum show complete ester hydrolysis in the doubly modified oligomer **ON28**.

The negative effect of a 2′-carbamate function on the thermal stability of a DNA–RNA duplex has been reported recently.[7c] Our data (Table 1) confirmed this tendency both in the DNA–DNA and DNA–RNA series. Incorporation of

Table 1 Properties of modified oligonucleotides and their duplexes with complementary DNA and RNA

#	Sequence, 5′ ⟵ 3′	*U* = U-2′-R; R =	MALDI MS $[M + H]^+$ (found/calculated)	Duplexes with **ON1**; d(ATTTGAGCCTGGGAG) T_m/ °C	Duplexes with **ON1**; d(ATTTGAGCCTGGGAG) ΔT_m/°C (per modification)	Duplexes with **ON3**; r(AUUUGAGCCUGGGAG) T_m/ °C	Duplexes with **ON3**; r(AUUUGAGCCUGGGAG) ΔT_m/°C (per modification)
ON4	CTCCCAGGC***U***CAAAT	H	4482.2/4483.9	56.4		59.1	
ON5		OMe	4515.9/4513.9	55.0	−1.4	59.6	+0.4
ON6		$OCONHCH_2C{\equiv}CH$	4581.2/4581.0	50.0	−6.4	54.5	−4.7
ON7		$OCON(Me)CH_2C{\equiv}CH$	4594.5/4595.0	49.0	−7.4	53.2	−6.0
ON8		$OCONHCH_2$(4-iodophenyl)	4759.9/4759.0	49.9	−6.5	54.7	−4.5
ON9		$OCONHCH_2$(pyren-1-yl)	4756.1/4757.2	54.7	−1.7	57.8	−1.4
ON10		$OCONHCH_2$([15-crown-5]-1-yl)	4772.3/4775.2	49.8	−6.6	53.9	−5.3
ON11		$OCONHCH_2CH_2OCH_2CH_2OH$	4628.2/4631.1	50.1	−6.3	53.7	−5.5
ON12		$OCONH(CH_2)_3[O(CH_2)_2]_3O(CH_2)_3NH_2$	4744.3/4746.2	49.8	−6.6	53.9	−5.3
ON13		$OCONH(CH_2)_3NH(CH_2)_3NH_2$	46.57.7/4657.1	51.5	−4.9	53.4	−5.8
ON14		$OCONH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	4927.9/4728.3	51.4	−5.0	55.1	−4.0
ON15	C***U***CCCAGGCTCAAAT	H	4482.0/4483.9	56.2		58.8	
ON16		OMe	4516.1/4513.9	55.1	−1.1	59.4	+0.6
ON17		$OCONHCH_2$(4-iodophenyl)	4759.6/4759.0	53.4	−2.8	54.4	−4.4
ON18		$OCONHCH_2$(pyren-1-yl)	4756.3/4757.2	57.5	+1.3	55.5	−3.3
ON19		$OCONHCH_2$([15-crown-5]-1-yl)	4772.3/4775.2	53.5	−2.8	54.8	−4.0
ON20		$OCONHCH_2CH_2OCH_2CH_2OH$	4627.3/4631.1	52.8	−3.4	56.2	−2.6
ON21		$OCONH(CH_2)_3[O(CH_2)_2]_3O(CH_2)_3NH_2$	4744.2/4746.2	52.6	−3.6	55.5	−3.3
ON22		$OCONH(CH_2)_3NH(CH_2)_3NH_2$	4657.3/4657.1	53.1	−3.1	55.9	−2.9
ON23		$OCONH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	4729.7/4728.3	54.1	−2.3	59.0	+0.2
ON24	C***U***CCCAGGC***U***CAAAT	H	4468.4/4469.9	56.1		58.1	
ON25		OMe	4531.5/4529.9	52.5	−1.8	60.7	+1.3
ON26		$OCONHCH_2$(pyren-1-yl)	5015.6/5016.5	52.6	−1.8	50.1	−4.0
ON27		$OCONHCH_2$([15-crown-5]-1-yl)	5050.2/5050.0	45.2	−5.5	48.6	−4.8
ON28		$OCONHCH_2CH_2OCH_2CH_2OH$	4759.3/4764.2	43.9	−6.6	49.8	−4.2
ON29		$OCONH(CH_2)_3[O(CH_2)_2]_3O(CH_2)_3NH_2$	4995.7/4994.5	45.0	−5.6	49.5	−4.3
ON30		$OCONH(CH_2)_3NH(CH_2)_3NH_2$	4818.2/4816.3	46.3	−4.9	50.6	−3.8
ON31		$OCONH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	4958.2/4958.6	47.3	−4.4	50.1	−4.0

Table 2 Thermal stabilities of mismatched duplexes with DNA

	DNA mismatch			
	ON35; d(ATTTG***C***GCCTGGGAG)		**ON36**; d(ATTTGAGCCTGGG***C***G)	
#	T_m/°C	ΔT_m/*°C[a]	T_m/°C	ΔT_m*/°C
ON4	47.0	−9.4	49.8	−6.6
ON5	45.2	−9.8	46.5	−8.5
ON6	42.9	−7.1	38.4	−11.6
ON7	42.8	−6.2	37.3	−11.7
ON8	46.4	−3.5	37.9	−12.0
ON9	52.9	−1.8	44.7	−10.0
ON10	43.8	−6.0	37.8	−12.0
ON11	42.9	−7.2	38.5	−11.6
ON12	43.1	−6.7	38.2	−11.6
ON13	44.7	−6.8	39.5	−12.0
ON14	45.9	−5.5	39.8	−11.6
ON15	46.4	−9.8	50.1	−6.1
ON16	44.2	−10.9	50.6	−4.5
ON17	40.9	−12.5	50.4	−3.0
ON18	44.8	−12.7	55.9	−1.6
ON19	41.4	−12.1	50.1	−2.4
ON20	41.0	−11.8	49.5	−3.3
ON21	40.9	−11.7	50.0	−2.6
ON22	40.9	−12.2	49.7	−3.4
ON23	40.9	−13.2	50.2	−3.9
ON24	46.1	−10.0	49.6	−6.5
ON25	42.4	−10.1	46.6	−5.9
ON26	51.6	−1.0	50.3	−2.3
ON27	37.3	−7.9	39.2	−6.0
ON28	36.7	−7.2	37.8	−6.1
ON29	37.4	−7.6	39.6	−5.4
ON30	38.6	−7.7	40.8	−4.5
ON31	40.2	−7.1	41.2	−6.1

[a] $\Delta T_m^* = \Delta T_m$ of mismatch contribution.

2′-*O*-methyluridine (oligomers **ON5**, **ON16** and **ON25**) in place of deoxyuridine was also somewhat destabilising for DNA–DNA duplexes, but slightly stabilising for DNA–RNA complexes. Most carbamate modifications result in a considerable decrease in T_m values of the duplexes but there was no correlation between the physical size of the carbamate *N*-substituent and the T_m of the corresponding duplexes. The highest destabilising effect was observed for the tertiary carbamate of *N*-methylpropargylamine (oligomer **ON7**) whereas the least destabilising was found for pyren-1-ylmethyl carbamate, especially in the case of the DNA–DNA duplex. As expected, a single modification close to the 5′-terminal position of the 15-mer (**ON17**–**ON23**) caused less destabilisation than one in the middle (**ON8**–**ON14**). In general, *internal* carbamate modifications are less destabilising for DNA–RNA than for DNA–DNA duplexes. By contrast, *near-terminal* carbamate modification is less destabilising for DNA–DNA duplexes.

Both 1-pyrenylmethyl and 4-iodobenzyl substituents are residues of a similar nature (arylmethyl) and have comparable molecular masses. It is interesting to compare data for oligonucleotides bearing these modifications. Not surprisingly, **ON9**–**ON1** is 4.8 °C more stable than **ON8**–**ON1** (*internal* position, DNA–DNA), **ON18**–**ON1** is 4.1 °C more stable than **ON17**–**ON1** (*near-terminal* position, DNA–DNA), **ON9**–**ON3** is 3.1 °C more stable than **ON8**–**ON3** (*internal* position, DNA–RNA) and **ON18**–**ON3** is 1.1 °C more stable than **ON17**–**ON3** (*near-terminal* position, DNA–RNA). Thus, despite the chemical similarity of pyrenyl and iodophenyl moieties, pyrene-modified duplexes are always more stable than those of iodophenyl (as well as most other substituents). The difference lies apparently in the planar polyaromatic structure of pyrene which is evidently more suitable for binding to nucleic acid duplexes. The nature of the interaction leading to partial compensation of carbamate destabilisation remains unclear. Pyrene itself is often thought of as a DNA intercalator and its interactions with nucleic acids have been studied spectroscopically.[14] However, at least three modes of free pyrene binding to DNA have been postulated,[14a] with groove binding being particularly favoured.[14c] From consideration of the B-DNA duplex structure, stacking between two adjacent base pairs (intercalation) and, more obviously, minor groove binding are both in principle feasible for a pyren-1-ylmethyl-2′-carbamate substituent. That pyrene is more stabilising than iodophenyl in the case of DNA–DNA rather than DNA–RNA duplexes, might suggest the better fit of pyrene into the DNA–DNA minor groove. In comparison with uridin-2′-ylpyren-1-ylmethylcarbamate modification with 2′-deoxyuridine, very little difference is seen for *internally* located pyrene substitution on DNA–DNA and DNA–RNA duplex stability (1.4 and 1.7 °C decrease in T_m, respectively). *Near-terminal* pyrene location, by contrast, gives +1.4 °C stabilisation for DNA–DNA and −3.3 °C destabilisation for DNA–RNA duplexes. These data show that the effect of *near-terminal* pyrene for DNA–DNA and DNA–RNA duplexes is very different. Thus pyrene is better able to exert its DNA–DNA binding effect near the end of the duplex, presumably because the greater flexibility in this region allows better minor groove placement of the pyrene.

A number of studies of pyrene attached to the 2′-position of individual nucleosides within duplexes have been published recently.[3,15] Pyrene as well as other polyaromatic compounds often show greater stabilising effect in DNA–DNA duplexes than in DNA–RNA duplexes.[15b,d] The pyrene 2′-carbamate modification is not an exception, giving a clear stabilising effect of pyrene superimposed on the destabilising influence of the carbamate linker.

A similar situation occurs in the case of polyamines, where a T_m increase due to phosphate charge neutralisation might be expected. In fact, the duplex-disturbing influence of carbamate is predominant, so that only in the case of *near-terminal* location of spermine in the RNA–DNA (**ON23**–**ON3**) duplex was there no decrease in T_m observed.

Doubly modified duplexes are more than additively ("cooperatively") destabilised. Again, in this case pyrene showed the lowest destabilising influence, especially for the DNA–DNA duplex, where it was of the same level as 2′-*O*-methyl substitution.

To evaluate the selectivity of binding of 2′-carbamate-modified oligonucleotides to a complementary DNA we carried out melting experiments using two mismatched oligodeoxyribonucleotides **ON35** and **ON36** as templates (Table 2) containing deoxycytidine in place of the normal deoxyadenosine. The overall effect of a dC : dU mismatch in the unmodified series (**ON4**, **ON15** and **ON24**) was found to be in the range of 6–10 °C, those in the middle of a sequence being considerably more destabilising. The incorporation of a single uridin-2′-yl carbamate directly opposite a deoxycytidine in otherwise complementary DNA led to a further decrease in stability, again more prominent when the mismatch is *internally* situated. However, most interesting behaviour was demonstrated by the pyren-1-ylmethyl carbamate, which appeared to selectively stabilise dC : *U* mismatch when placed directly opposite (*U* stands for uridinyl carbamate). In the case of *internal* pyrene (**ON9**–**ON35**), the pure mismatch effect is about 2 °C, whereas for *near-terminal* pyrene (**ON18**–**ON36**) the overall destabilisation is even less pronounced as compared to the unmodified duplex (Table 2). Our data further show this unusual stabilisation disappearing when the mismatch is situated elsewhere (**ON9**–**ON36** and **ON18**–**ON35** duplexes show a T_m decrease of 10–13 °C). The dC mismatch was also well-tolerated in doubly pyrenylated duplexes **ON26**–**ON35** and **ON26**–**ON36**. These results may be ascribed to 2′-pyrene actually replacing uridine in stacking with neighboring nucleobases. This proposal is confirmed by an efficient fluorescence quenching in the case of pyrene mismatches which is characteristic of pyrene being able to interact with DNA heterocycles (data not shown).

Thus, uridin-2′-yl carbamate modification in a DNA strand is usually destabilising for both DNA–DNA and DNA–RNA duplexes and therefore might be less suitable for antisense applications where higher stability of a duplex is preferential. However, the 2′-carbamate modification affords a convenient way to place various ligands in the minor groove of a DNA–DNA duplex (*e.g.* crown ether for metal ion binding, an aliphatic amino group for labelling with activated derivatives of fluorescent dyes). Other possible applications include the specific minor groove delivery of reactive functions (*e.g.* for directed cross-linking or conjugation with peptides), as well as modifications of flexible parts of structured RNA molecules, such as ribozymes, for studies of catalysis[1] or folding.[3] In this respect, we have incorporated some 2′-carbamate modified uridines into synthetic oligoribonucleotides (data not shown) and studies on such modified ribozymes will be reported later.

Pyrene absorbance and fluorescence in single-stranded oligonucleotides and duplexes

The introduction of a pyrene chromophore into oligonucleotides can be easily confirmed by the characteristic UV spectrum of modified oligonucleotides, showing pyrene absorbance bands in the region of 320–360 nm (Fig. 1a). The oligonucleotide-bound pyrene is spectrally distinct from the single nucleoside-bound. Curve 1 shown in Fig. 1b depicts the UV difference spectrum of oligonucleotides **ON26** and **ON18**, *i.e.* formally corresponds to the spectrum of a single oligonucleotide-bound pyrene. It can be seen clearly that the pyrene maxima either in pyrenyl oligonucleotides (Fig. 1a), or in this "oligonucleotide-bound pyrene" are shifted by 5–8 nm in comparison to pyrene in the pyrenyl nucleoside **4d** (Fig. 1b). Such a bathochromic shift correlates well with previously published data.[14c]

The melting of the duplexes carrying two pyrene residues (**ON26**–**ON1** and **ON26**–**ON3**) can be observed not only with

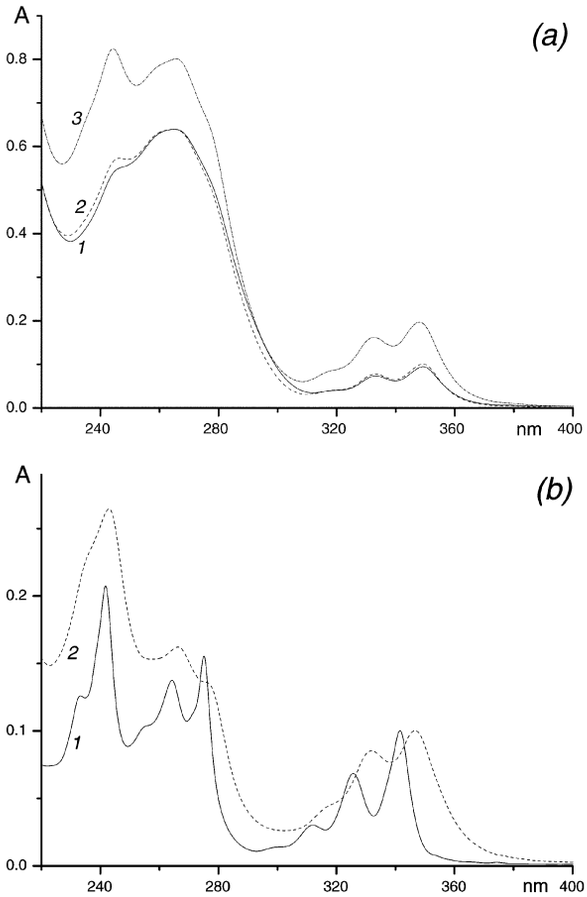


Fig. 1 (a) UV spectra of pyrene-modified oligonucleotides **ON9** (*1*), **ON18** (*2*) and **ON26** (*3*) in water, normalized by pyrene absorbance. (b) Comparison of the UV spectrum of pyrene-modified nucleoside **4d** (*1*) and the difference between UV spectra of oligonucleotides **ON26** and **ON18** (*2*) in water.

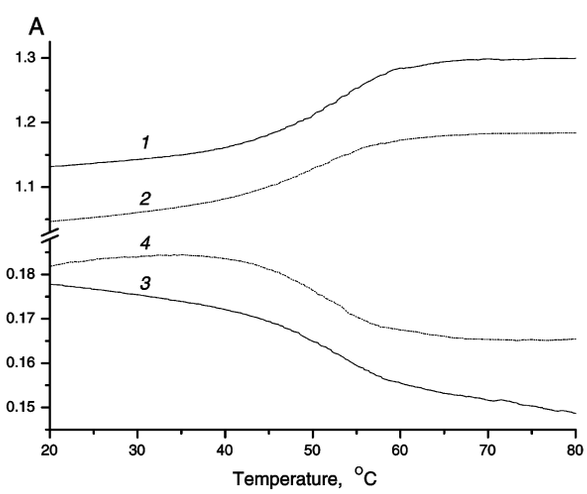


Fig. 2 Thermal denaturation curves of the duplexes **ON26** × **ON1** (*1,3*) and **ON26** × **ON3** (*2,4*) in hybridisation buffer detected at 260 (*1,2*) and 349 nm (*3,4*).

an increase of nucleoside absorbance at 260 nm, but also with a *decrease* in pyrene absorbance at 349 nm (Fig. 2). For the DNA–DNA duplex the inversion point of the curve at 52.3 °C is consistent with that of the nucleoside absorbance curve (52.6 °C). This result can be explained by the average distance between two chromophores in the flexible single stranded oligomer **ON26** being less than in the more rigid, extended duplex **ON26**–**ON1**. The increase of pyrene–pyrene distance in the duplex leads to an increase in their total "photon capture

section" and, as a consequence, an increase in the absorbance. In this simplified explanation, it is also assumed that pyrene molecules both in a single strand and in a duplex are capable of random rotation, which may not necessarily be the case here. For the DNA–RNA duplex T_m values calculated from nucleoside (50.1 °C) and pyrene absorbance (51.1 °C) are also in close agreement. Noteworthy, the "pyrene" melting curves for DNA–DNA and DNA–RNA duplexes have different shape thus confirming a different mode of interaction of a pyrene residue with a nucleic acid framework.

The fluorescence spectra of single-stranded pyrene oligonucleotides (**ON9**, **ON18**; Fig. 3a) show that fluorescence intensity (which correlates with quantum yield) is dependent on the modification site: *Internally* bound pyrene fluoresces approximately three times more intensively than near-terminal pyrene (note that the nucleobase context is the same in both cases). The spectrum of the doubly-modified oligomer **ON26** has a distinct broad band of excimer fluorescence in the region around 480 nm. This provides evidence for a conformation with a close coplanar arrangement of two pyrene residues (*e.g.* a metastable hairpin with two base pairs in the stem). When bound to a complementary DNA oligomer, the pyrene residues in **ON26** are unable to be brought closely together and the excimer fluorescence completely disappears (Fig. 3b).

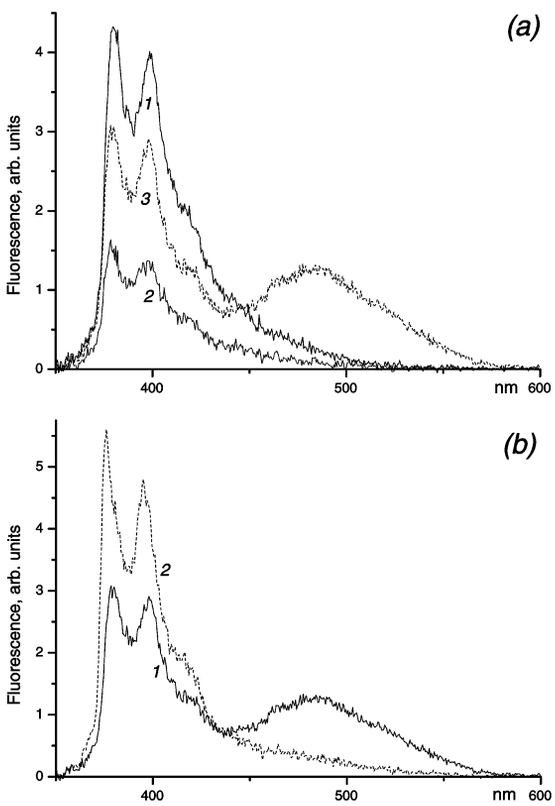


Fig. 3 (a) Fluorescence spectra of single stranded pyrene-modified oligonucleotides **ON9** (*1*, **ON18** (*2*) and **ON26** (*3*). (b) Fluorescence spectra of single stranded double-pyrene-modified oligonucleotides **ON26** (*1*) and is duplexed with complementary oligodeoxynucleotide **ON26** × **ON1** (*2*). Conditions: hybridisation buffer (see Experimental section), λ_{ex} 339 nm, concentrations 2×10^{-7} M.

This effect could be applied to the detection of complementary sequences by use of an excimer-forming double pyrene-labelled probe. This might be sequence-dependent and would require extensive experiments on probe structure optimisation (to design probes with maximal excimer fluorescence) and thorough controls (study of the influence of non-complementary and partly complementary sequences on excimer fluorescence).

The total fluorescence intensity upon duplex binding of singly pyrene-labelled oligomers with complementary DNA does not change very much (Fig. 4). By contrast, the binding to

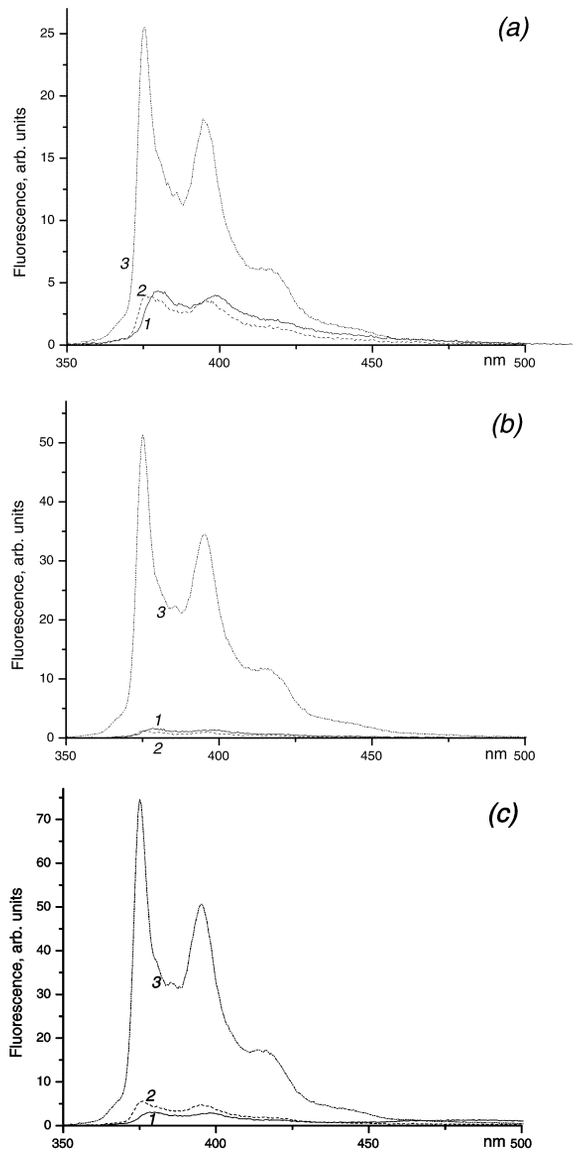


Fig. 4 (a) Fluorescence spectra of single stranded pyrene-modified oligonucleotide **ON9** (*1*) and its duplex with complementary DNA **ON9** × **ON1** (*2*) and RNA **ON9** × **ON3** (*3*). (b) Fluorescence spectra of single stranded pyrene-modified oligonucleotide **ON18** (*1*) and its duplex with complementary DNA **ON18** × **ON1** (*2*) and RNA **ON18** × **ON3** (*3*). (c) Fluorescence spectra of single stranded pyrene-modified oligonucleotide **ON26** (*1*) and its duplex with complementary DNA **ON26** × **ON1** (*2*) and RNA **ON26** × **ON3** (*3*). Conditions: hybridisation buffer (see Experimental section), λ_{ex} 339 nm, concentrations 2×10^{-7} M.

a complementary RNA gives a 5 (*internal* pyrene) to 30 fold (*near-terminal* pyrene) increase in fluorescence for singly pyrene-labelled (Fig. 4a–b) and 30-fold for doubly pyrene-labelled oligonucleotide (Fig. 4c). This provides evidence that the excited pyrene fluorophore (linked through 2′-carbamate) is effectively quenched by nucleobases in single-stranded DNA oligomers and in DNA–DNA duplexes, but not in DNA–RNA duplexes. Similar examples of fluorescence increases upon hybridisation with complementary RNA are demonstrated for probes containing 2′-*O*-attached pyrene.[15b,e]

To conclude, a convenient method of oligonucleotide 2′-modification is described. Most interesting properties were demonstrated by the 2′-*O*-(pyren-1-ylmethylcarbamoyl)-uridine-containing DNA oligomers. The pyrene modification

was able to stabilise selectively a duplex with a mismatched deoxycytidine directly opposite the carbamate-modified nucleoside. Moreover, the fluorophore showed a considerable fluorescence increase upon hybridisation with complementary RNA. The remarkable features of the 2′-*O*-(pyren-1-ylmethylcarbamoyl)uridine may therefore find application in the detection of RNA and possibly detection of point mutations in DNA.

Experimental

S-Ethyl trifluorothioacetate and 4-iodobenzylamine were from Lancaster Synthesis; *N,N*-diisopropylethylamine (DIEA), propargylamine, *N*-methylpropargylamine, 1-pyrenylmethylamine hydrochloride, 1-aminomethyl-15-crown-5, 2-(2-aminoethoxy)ethanol, 4,7,10-trioxatridecane-1,13-diamine, spermine, *N*-(3-aminopropyl)propane-1,3-diamine, Et_3N, triethylamine trihydrofluoride, and pivaloyl chloride were from Aldrich; 1,1-carbonyldiimidazole (CDI) was from Sigma, leucylphenylalaninamide hydrochloride was from Bachem, 4,4′-dimethoxytritylchloride (DmtCl) was purchased from Avocado; and bis(*N,N*-diisopropylamino)-2-cyanoethoxyphosphine from Fluka. 3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)uridine,[8b] 5′-*O*-(4,4′-dimethoxytrityl)uridine,[12] and diisopropylammonium tetrazolide[16] were prepared as described.

DCM (Fisher) was always used freshly distilled over CaH_2. Anhydrous pyridine was from Aldrich; THF (BDH) was freshly distilled over powdered $LiAlH_4$ and stored over 4 Å molecular sieves under nitrogen. Other solvents, toluene (BDH), chloroform, ethyl acetate, acetone, acetonitrile, hexane, absolute ethanol (Fisher) and methanol (Fluka) were used as received.

300 MHz ^{1}H and 121 MHz ^{31}P NMR spectra were recorded on a Bruker DRX 300 spectrometer. Chemical shifts (δ) for ^{1}H and ^{31}P are referenced to internal solvent resonances and reported relative to $SiMe_4$ and 85% aq. H_3PO_4, respectively. ^{1}H NMR coupling constants are reported in Hz and refer to apparent multiplicities. MALDI-TOF mass spectra were obtained on a Voyager-DE BioSpectrometry Workstation (PerSeptive Biosystems) in a positive ion mode. High-resolution ESI mass spectra were provided by the Department of Chemistry, University of Cambridge. TLC and column chromatographies were carried out with Macherey-Nagel silica gel (ALUGRAM™ SIL G/UV$_{254}$ and Kieselgel 60 0.040–0.063 mm). Thermal denaturation experiments with oligonucleotide duplexes were performed on a Perkin Elmer Lambda 40 UV/VIS Spectrometer with PTP 6 (Peltier Temperature Programmer). Fluorescence spectra were obtained using a Perkin Elmer LS 50B Luminescence Spectrometer.

Oligonucleotide Synthesis was carried out on a ABI 380B DNA/RNA synthesizer either on 0.2 or 1 μmol scale using 2′-deoxynucleoside phosphoramidites from Cruachem (Scotland) and 2′-TOM-ribonucleoside phosphoramidites from Glen Research (*via* Cambio), and standard synthetic procedures.[17] Ion exchange HPLC purification of oligoribonucleotide **ON3** was carried out using a NucleoPac PA-100 (Dionex) (9 × 250 mm) using gradients of sodium perchlorate (1 mM–400 mM) in a buffer of 20 mM Tris HCl (pH 6.8), 25% formamide, and at 280 nm wavelength. The mass of each oligonucleotide was checked by MALDI-TOF mass spectrometry on a Perseptive Biosystems Voyager DE mass spectrometer in positive ion mode using a mixture (1 : 1 v/v) of 2,6-dihydroxyacetophenone (40 mg cm^{-3} in MeOH) and aqueous diammonium hydrogen citrate (80 mg cm^{-3}) as a matrix premixed just before loading the samples onto a plate. Duplex stability studies were done in a buffer containing 100 mM NaCl, 10 mM Na–phosphate, 0.1 mM EDTA, pH 7.0.

2′-*O*-(Imidazol-1-ylcarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (2)

To a solution of **1** (2.434 g, 5.0 mmol) in dry DCM (50 cm^3), CDI (852 mg, 5.25 mmol) was added in one portion. TLC showed the conversion of the starting nucleoside into an imidazolide with lower mobility on TLC is complete after 0.5–2 h. The solution was washed with water (2 × 50 cm^3), dried (Na_2SO_4), evaporated, coevaporated with dry DCM, and the residue was dried *in vacuo* to give the imidazole derivative (2.872 g, 98.9%) as a white crystalline powder, pure according to TLC and NMR. R_f 0.38 (EtOAc), mp 193–195 °C (EtOAc–$CHCl_3$). MALDI-TOF (2,4,6-THAP): $[M + Na]^+$ calc. 603.77, found 604.58, $[M + K]^+$ calc. 619.88, found 620.55. ^{1}H-NMR: 11.44 (s, 1H, *H*-3), 8.39 (s, 1H, imidazole), 7.69 (s, 1H, imidazole), 7.64 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.09 (s, 1H, imidazole), 5.85 (s, 1H, *H*-1′), 5.79 (d, 1H, $J_{2',3'}$ = 5.4 Hz, *H*-2′), 5.61 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 4.74 (m, 1H, *H*-3′), 4.13–3.92 (m, 3H, *H*-4′, *H*-5′), 1.10–0.80 (m, 28H, Pr^i).

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)uridin-2′-yl carbamates (3)

A general procedure. To a solution of **2**, prepared as above from 5 mmol of **1**, the corresponding amine was added and the completion of reaction (from 1 h to several days) was monitored by TLC (EtOAc). The reaction mixture was diluted with DCM (50 cm^3), washed with water (100 cm^3), 5% citric acid (100 cm^3), and water (100 cm^3), then dried (Na_2SO_4), evaporated, and the residue was chromatographed on a silica gel column in an appropriate solvent system. Fractions containing product were combined, evaporated, and the residue was dried *in vacuo* to afford compounds listed below as white foamy solids.

2′-*O*-(Propargylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3a). Chromatography: stepwise gradient of 33⟶40% (v/v) EtOAc in $CHCl_3$. Yield 2.780 g (97.9%). R_f 0.49 ($CHCl_3$–EtOAc 1 : 1 v/v). ESI MS: $[M + Na]^+$ calc. 590.23, found 590.23. ^{1}H-NMR: 11.42 (s, 1H, *H*-3), 7.84 (t, 1H, *J* = 5.4 Hz, OCON*H*), 7.69 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 5.64 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.34 (d, 1H, $J_{2',3'}$ = 4.9 Hz, *H*-2′), 4.50 (m, 1H, *H*-3′), 4.34–3.88 (m, 2H, *H*-5′), 3.85–3.69 (m, 3H, CH_2N, *H*-4′), 3.06 (s, 1H, ≡C*H*), 1.10–0.80 (m, 28H, Pr^i).

2′-*O*-(*N*-Methyl-*N*-propargylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3b). Chromatography: EtOAc–$CHCl_3$, 1 : 2 v/v. Yield 2.827 g (97.2%). R_f 0.56 ($CHCl_3$–EtOAc, 1 : 1 v/v). ESI MS: $[M + Na]^+$ calc. 604.25, found 604.24. ^{1}H-NMR: 11.41 (s, 1H, *H*-3), 7.66 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 5.67 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.35 (m, 1H, *H*-2′), 4.53 (dd, 1H, $J_{2',3'}$ = 6.0 Hz, $J_{3',4'}$ = 7.9 Hz, *H*-3′), 4.17–3.83 (m, 5H, *H*-4′, *H*-5′, CH_2N), 3.21 (s, 1H, ≡C*H*), 2.93 (s, 1.8H), 2.84 (s, 1.2H) (NCH_3, rotamers), 1.07–0.82 (m, 28H, Pr^i).

2′-*O*-(4-Iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3c). Chromatography: stepwise gradient of 25⟶33% (v/v) EtOAc in $CHCl_3$. Yield 3.491 g (93.6%). R_f 0.50 ($CHCl_3$–EtOAc, 1 : 1 v/v). MALDI-TOF (2,5-DHBA): $[M + Na]^+$ calc. 768.74, found 769.30, $[M + K]^+$ calc. 784.85, found 785.37. ^{1}H-NMR: 11.41 (s, 1H, *H*-3), 7.96 (br t, 1H, OCON*H*), 7.68 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.62 (d, 2H, *J* = 7.6 Hz, Ar*H*), 7.05 (d, 2H, *J* = 7.6 Hz, Ar*H*), 5.65 (s, 1H, *H*-1′), 5.58 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.35 (d, 1H, $J_{2',3'}$ = 5.4 Hz, *H*-2′), 4.48 (m, 1H, *H*-3′), 4.20–3.80 (m, 5H, CH_2Ar, *H*-4′, *H*-5′), 1.08–0.88 (m, 28H, Pr^i).

2′-*O*-(Pyren-1-ylmethylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3d). Chromatography: stepwise gradient of 25⟶30⟶33% (v/v) EtOAc in $CHCl_3$. Yield 3.492 g (93.9%). R_f 0.54 ($CHCl_3$–EtOAc, 1 : 1). MALDI-TOF (2,5-DHBA): $[M + Na]^+$ calc. 766.98, found 768.31. ^{1}H-NMR: 11.43 (s, 1H, *H*-3), 8.42 (d, 1H, $J_{9'',10'}$ = 9.3 Hz, pyrene *H*-10), 8.31–7.97 (m, 9H, Ar*H*, OCON*H*), 7.69 (d, 1H,

$J_{5,6}$ = 8.1 Hz, *H*-6), 5.68 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.40 (d, 1H, $J_{2',3'}$ = 5.8 Hz, *H*-2′), 4.91 (m, 2H, CH_2Ar), 4.66 (m, 1H, *H*-3′), 4.04 (m, 1H, $^2J_{5'a,5'b}$ = 12.6 Hz, $J_{4',5'a}$ = 3.2 Hz, *H*-5′a), 3.93–3.77 (m, 2H, *H*-4′, *H*-5′b), 1.04–0.67 (m, 28H, Pr^i).

2′-*O*-(1,4,7,10,13-Pentaoxacyclopentadecan-2-ylmethyl)-aminocarbonyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3e). The solution of **2** (1.162 g, 2.0 mmol) and 2-aminomethyl-15-crown-5 (514 mg, 2.0 mmol) in MeCN (25 cm^3) was kept at 55 °C for 96 h, then cooled, evaporated to dryness, and the residue was dissolved in EtOAc (100 cm^3), washed with water (100 cm^3), 5% citric acid (100 cm^3), and water (100 cm^3), dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (a stepwise gradient of 2 to 10% (v/v) MeOH in $CHCl_3$). Yield 1.165 g (76.4%). R_f 0.32 ($CHCl_3$–MeOH, 17 : 3). ESI MS: $[M + H]^+$ calc. 762.36, found 762.37. ^{1}H-NMR: 11.41 (s, 1H, *H*-3), 7.68 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.40 (t, 1H, *J* = 5.7 Hz, OCON*H*), 5.64 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.33 (d, 1H, $J_{2',3'}$ = 5.7 Hz, *H*-2′), 4.48 (dd, 1H, $J_{2',3'}$ = 5.7 Hz, $J_{3',4'}$ = 8.2 Hz, *H*-3′), 3.99 (m, 2H, $^2J_{5'a,5'b}$ = 12.9 Hz, $J_{4',5'a}$ = 2.7 Hz, $J_{4',5'b}$ = 4.1 Hz, *H*-5′), 3.84 (m, 1H, *H*-4′), 3.64–3.36 (m, 19H¶, C*H*(CH_2OCH_2)$_4$CH_2), 3.00 (m, 2H, CH_2N), 1.06–0.82 (m, 28H, Pr^i).

***N*α-[3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)uridin-2′-*O*-ylcarbonyl]-L-leucyl-L-phenylalaninamide (3f).** The solution of **2** (1.162 g, 2.0 mmol), H-Leu-Phe-NH_2 hydrochloride (942 mg, 3.0 mmol) and DIEA (0.61 cm^3, 3.5 mmol) in MeCN (25 cm^3) was kept at 55 °C for 72 h, then cooled, evaporated to dryness, and the residue was dissolved in EtOAc (100 cm^3), washed with water (100 cm^3), 5% citric acid (100 cm^3), and water (100 cm^3), dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (a stepwise gradient of 33⟶50% EtOAc in $CHCl_3$, then 33⟶50% acetone in $CHCl_3$–EtOAc (1 : 1 v/v)). Yield 610 mg (38.6%). R_f 0.45 (EtOAc). ESI MS: $[M + H]^+$ calc. 790.39, found 790.43. ^{1}H-NMR: 11.43 (s, 1H, *H*-3), 7.87 (d, 1H, *J* = 8.2 Hz, N*H*CHCH$_2$Ph), 7.70 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.44 (d, 1H, *J* = 8.4 Hz, OCON*H*), 7.39 (br s, 1H, NH_2), 7.19 (m, 5H, Ph), 7.06 (br s, 1H, NH_2), 5.64 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.35 (d, 1H, $J_{2',3'}$ = 5.1 Hz, *H*-2′), 4.47 (m, 2H, *H*-3′, C*H*CH$_2$Ph), 4.15–3.83 (m, 4H, *H*-4′, *H*-5′, C*H*Bu^i), 3.03–2.73 (m, 2H, ^2J = 13.4 Hz, J_{AX} = 4.7 Hz, J_{BX} = 9.1 Hz, CH_2Ph), 1.49 (m, 1H, CH$_2$C*H*Me$_2$), 1.29 (m, 2H, CH_2Pr^i), 1.10–0.72 (m, 34H, SiC*H*(CH_3)$_2$, CH$_2$CH(CH_3)$_2$).

2′-*O*-[2-(2-Hydroxyethoxy)ethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3g). Chromatography: $CHCl_3$–EtOAc–acetone, stepwise gradient 2 : 1 : 1⟶1 : 1 : 1⟶1 : 1 : 2 (v/v/v). Yield 2.269 g (73.5%). R_f 0.28 (EtOAc). ESI MS: $[M + Na]^+$ calc. 640.27, found 640.26. ^{1}H-NMR: 11.41 (s, 1H, *H*-3), 7.68 (d, 1H, $J_{5,6}$ = 7.9 Hz, *H*-6), 7.40 (br s, 1H, OCON*H*), 5.64 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 7.9 Hz, *H*-5), 5.31 (d, 1H, $J_{2',3'}$ = 5.4 Hz, *H*-2′), 4.55 (m, 2H, *H*-3′, O*H*), 4.12–3.79 (m, 3H, *H*-4′, *H*-5′), 3.53–3.27 (m, 6H¶, CH_2OCH_2CH_2), 3.12 (m, 2H, CH_2N), 1.10–0.82 (m, 28H, Pr^i).

2′-*O*-[2-(2-Pivaloyloxyethoxy)ethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine (3h). To the stirred solution of **3g** (2.162 g, 3.5 mmol) in dry DCM (20 cm^3), pyridine (1.0 cm^3, 12.5 mmol) and pivaloyl chloride (0.615 cm^3, 5 mmol) were added, and the reaction was kept at 20 °C until the starting compound disappeared (*ca.* 20 h). The mixture was diluted with DCM (100 cm^3), washed with water (100 cm^3), 5% $NaHCO_3$ (100 cm^3), and water (100 cm^3), dried (Na_2SO_4), evaporated to dryness, and the residue was chromatographed on silica gel (stepwise gradient of 10 to 50% (v/v) EtOAc in $CHCl_3$). Yield 1.977 g (80.5%). R_f 0.34 ($CHCl_3$–EtOAc, 1 : 1 v/v). ESI MS: $[M + Na]^+$ calc. 724.33, found 724.32. ^{1}H-NMR: 11.41 (s, 1H, *H*-3), 7.69 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.37 (br t, 1H, *J* = 4.6 Hz, OCON*H*), 5.64 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.31 (d, 1H, $J_{2',3'}$ = 4.9 Hz, *H*-2′), 4.50 (m, 1H, *H*-3′), 4.17–3.78 (m, 5H, *H*-4′, *H*-5′, CH_2OPiv), 3.59 (m, 2H, CH_2OCH$_2$), 3.40 (m, 2H¶, CH_2N), 3.10 (m, 2H, CH_2OCH$_2$), 1.12 (s, 9H, Bu^t), 1.07–0.83 (m, 28H, Pr^i).

2′-*O*-[13-(Trifluoroacetylamino)-4,7,10-trioxatridecan-1-yl-aminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3j). Compound **2** was prepared from **1** (1.703 g, 3.5 mmol) and CDI (592 mg, 3.65 mmol) in dry DCM (30 cm^3). The solution was added dropwise to the magnetically stirred, ice-cooled solution of 4,7,10-trioxatridecane-1,13-diamine (3.9 cm^3, 17.5 mmol) in DCM (100 cm^3). After 2 h, cooling was removed, and the mixture was kept overnight at 20 °C, then washed with water (150 cm^3), 5% $NaHCO_3$ (150 cm^3), and water (150 cm^3), dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in dry DCM (10 cm^3), and *S*-ethyl trifluorothioacetate (3.0 cm^3, 24 mmol) was added in one portion. The mixture was left overnight, then evaporated to dryness (*STENCH!*), and the residue was chromatographed on silica gel ($CHCl_3$–EtOAc, stepwise gradient 1 : 1⟶1 : 2⟶1 : 3⟶1 : 4, v/v) to give the product as a colourless oil (1.888 g, 65.1%). R_f 0.42 (EtOAc). ESI MS: $[M + Na]^+$ calc. 851.35, found 851.35. ^{1}H-NMR: 11.41 (s, 1H, *H*-3), 9.36 (br s, 1H, N*H*TFA), 7.69 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.36 (t, 1H, *J* = 5.7 Hz, OCON*H*), 5.64 (d, 1H, $J_{1',2'}$ = 1.8 Hz, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.31 (m, 1H, *H*-2′), 4.49 (dd, 1H, $J_{2',3'}$ = 5.0 Hz, $J_{3',4'}$ = 7.9 Hz, *H*-3′), 3.99 (m, 2H, $^2J_{5'a,5'b}$ = 13.0 Hz, $J_{4',5'a}$ = 2.7 Hz, $J_{4',5'b}$ = 4.4 Hz, *H*-5′), 3.82 (m, 1H, *H*-4′), 3.55–3.30 (m, 12H¶, (CH_2OCH_2)$_3$), 3.22 (apparent q, 2H, *J* = 6.5 Hz, CH_2NHTFA), 3.00 (m, 2H, OCONHCH_2), 1.69 (apparent quintet, 2H, *J* = 6.8 Hz, CH_2), 1.60 (apparent quintet, 2H, *J* = 6.6 Hz, CH_2), 1.06–0.81 (m, 28H, Pr^i).

2′-*O*-[4-Trifluoroacetyl-7-(trifluoroacetylamino)-4-azaheptan-1-ylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridine (3m). A solution of **2**, prepared from **1** (2.434 g, 5.0 mmol) and CDI (852 mg, 5.25 mmol) in dry DCM (50 cm^3), was added dropwise to the magnetically stirred, ice-cooled solution of *N*-(3-aminopropyl)propane-1,3-diamine (5.6 cm^3, 40 mmol) in DCM (100 cm^3). After 2 h cooling was removed, and the mixture was kept overnight at 20 °C, then washed with water (200 cm^3), 5% $NaHCO_3$ (200 cm^3), and water (200 cm^3), dried (Na_2SO_4), and evaporated to dryness. The residue was coevaporated with THF (30 cm^3), dissolved in dry THF (10 cm^3), and *S*-ethyl trifluorothioacetate (5.0 cm^3, 39 mmol) was added in one portion. After 20 h TLC (EtOAc) showed formation of one predominant product. The mixture was evaporated to dryness (*STENCH!*) and the residue was chromatographed on silica gel ($CHCl_3$–EtOAc, stepwise gradient 1 : 1⟶1 : 2⟶1 : 3 v/v) to give the product as a white foam (2.558 g, 61.2%). A sample of the product was triturated in DCM–hexane to give colourless crystals, mp 74–77 °C. R_f 0.69 (EtOAc). ESI MS: $[M + Na]^+$ calc. 858.30, found 858.30. ^{1}H-NMR: 11.42 (s, 1H, *H*-3), 9.51, 9.43 (2 br t, 1H, N*H*TFA, rotamers), 7.69 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 7.51, 7.45 (2m, 1H, OCON*H*, rotamers), 5.65 (d, 1H, $J_{1',2'}$ = 1.8 Hz, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.32 (m, 1H, *H*-2′), 4.50 (m, 1H, *H*-3′), 3.97 (m, 2H, $^2J_{5'a,5'b}$ = 12.5 Hz, $J_{4',5'a}$ = 4.2 Hz, $J_{4',5'b}$ = 2.4 Hz, *H*-5′), 3.83 (m, 1H, *H*-4′), 3.33 (m, 4H¶ CH_2NCH_2), 3.19 (m, 2H, CH_2NHTFA), 2.98 (m, 2H, OCONHCH_2), 1.74 (m, 4H, CH_2), 1.10–0.85 (m, 28H, Pr^i).

2′-*O*-[4,9-Bis(trifluoroacetyl)-12-(trifluoroacetylamino)-4,9-diazadodecan-1-ylaminocarbonyl]-3′,5′-*O*-(tetraisopropyl-disiloxane-1,3-diyl)uridine (3p). A solution of **2** (5 mmol, prepared *in situ* as above) in dry DCM (50 cm^3) was added dropwise to a stirred, ice-cooled solution of spermine (2.63 g,

¶ Calculated value; the signal of water is also present in the region.

13 mmol) in DCM (20 cm^3). After 2 h the cooling was removed and the mixture was stirred overnight, diluted with $CHCl_3$ (150 cm^3), washed with 20% NaCl (2 × 200 cm^3), dried (Na_2SO_4), and evaporated to dryness. The residue was trifluoroacetylated as above, and the desired compound was isolated using chromatography on silica gel (stepwise gradient of 50⟶30⟶20⟶0% $CHCl_3$ in EtOAc, v/v). Yield 2.457 g (49.0%), R_f 0.67 (EtOAc). MALDI-TOF (2,5-DHBA): $[M + H]^+$ calc. 1004.07, found 1004.02, $[M + Na]^+$ calc. 1025.35, found 1025.64, $[M + K]^+$ calc. 1041.33, found 1041.36. ^{1}H-NMR: 11.42 (s, 1H, *H*-3), 9.51, 9.44 (2 br t, 1H, N*H*TFA, rotamers), 7.69 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.51, 7.45 (2t, 1H, *J* = 5.2 Hz, OCON*H*, rotamers), 5.65 (s, 1H, *H*-1′), 5.59 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.32 (m, 1H, *H*-2′), 4.49 (m, 1H, *H*-3′), 3.98 (m, 2H, $^2J_{5'a,5'b}$ = 12.6 Hz, $J_{4',5'a}$ = 4.2 Hz, *H*-5′), 3.83 (m, 1H, *H*-4′), 3.40–3.28 (m, 8H¶, CH_2N(TFA)CH_2), 3.20 (m, 2H, CH_2-NHTFA), 2.98 (m, 2H, OCONHCH_2), 1.86–1.60 (m, 4H, CH_2CH$_2$NH), 1.51 (m, 4H, CH$_2$CH_2CH_2CH$_2$), 1.06–0.80 (m, 28H, Pr^i).

Uridin-2′-yl carbamates (4)

A general procedure.

To a solution of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-uridin-2′-yl carbamate (2 mmol) in THF (5 cm^3) in a screw-top Teflon flask (Nalgene) was added triethylamine trihydrofluoride (0.814 cm^3, 5 mmol) and the mixture was left for 6 h or overnight at room temperature (the completion of deprotection was checked by TLC (15% MeOH in $CHCl_3$, v/v)), then diluted with hexane (25 cm^3). The upper layer was discarded, and the residue (oil or solid) was washed with 1 : 1 (v/v) toluene–hexane mixture (3 × 25 cm^3) by decantation, triturated in absolute ethanol (5 cm^3), and the crystalline product was filtered off, washed with EtOH (5 cm^3), Et_2O (10 cm^3), and dried *in vacuo*.

2′-*O*-(4-Iodobenzylaminocarbonyl)uridine (4c). Yield 993 mg (98.6%). R_f 0.34 ($CHCl_3$–MeOH, 17 : 3), mp 192–197 °C (EtOH). MALDI-TOF (2,5-DHBA): $[M + Na]^+$ calc. 526.24, found 527.55. ^{1}H-NMR: 11.36 (s, 1H, *H*-3), 7.90 (m, 2H, *H*-6, OCON*H*), 7.65 (d, 2H, *J* = 7.4 Hz, Ar*H*), 7.02 (d, 2H, *J* = 7.4 Hz, Ar*H*), 5.99 (m, 1H, *H*-1′), 5.66 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.52 (d, 1H, *J* = 4.4 Hz, 3′-O*H*), 5.18 (m, 1H, 5′-O*H*), 5.05 (m, 1H, *H*-2′), 4.25–4.05 (m, 3H, *H*-3′, CH_2N), 3.88 (m, 1H, *H*-4′), 3.59 (m, 2H, *H*-5′).

2′-*O*-(Pyren-1-ylmethylaminocarbonyl)uridine (4d). Yield 467 mg (93.5%). R_f 0.37 ($CHCl_3$–MeOH, 17 : 3), mp 200–208 °C (EtOH). MALDI-TOF (2,5-DHBA): $[M + H]^+$ calc. 502.50, found 502.34, $[M + Na]^+$ calc. 524.48, found 525.60, $[M + K]^+$ calc. 540.59, found 541.68. ^{1}H-NMR: 11.38 (s, 1H, *H*-3), 8.42–7.90 (m, 11H, *H*-6, OCON*H*, A*H*), 6.03 (d, 1H, $J_{1',2'}$ = 5.7 Hz, *H*-1′), 5.68 (d, 1H, $J_{5,6}$ = 7.8 Hz, *H*-5), 5.57 (m, 1H, 3′-O*H*), 5.21 (m, 1H, 5′-O*H*), 5.14 (m, 1H, *H*-2′), 4.91 (m, 2H, CH_2Ar), 4.24 (m, 1H, *H*-3′), 3.91 (m, 1H, *H*-4′), 3.60 (m, 2H, *H*-5′).

5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-yl carbamates (5)

A general procedure.

The crude oily uridin-2′-yl carbamates from the general procedure above (washed with 1 : 1 (v/v) toluene–hexane mixture) were coevaporated with toluene (3 × 20 cm^3), pyridine (3 × 20 cm^3), dissolved in dry pyridine (15 cm^3), cooled in an ice bath, and DmtCl (845 mg, 2.5 mmol) was added in one portion. The reaction was monitored by TLC and further portions of DmtCl (170 mg, 0.5 mmol) were added every 4 h until the starting nucleoside disappears (the amount of DmtCl is dependent on the residual HF removal rate and varied usually from 3 to 4 mmol). After completion of the reaction, the excess of DmtCl was quenched with MeOH (1 cm^3), and after 10 min the mixture was diluted with $CHCl_3$ (100 cm^3), washed with water (100 cm^3), 5% $NaHCO_3$ (100 cm^3), and water (100 cm^3), then dried (Na_2SO_4), evaporated, coevaporated with toluene (3 × 25 cm^3) and the residue was chromatographed on silica gel column in an appropriate solvent system. Fractions containing product were combined, evaporated, and the residue was dried *in vacuo* to afford compounds listed below as amorphous solids.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(propargylaminocarbonyl)-uridine (5a). Chromatography: stepwise gradient of 1⟶3⟶5% MeOH in $CHCl_3$ + 0.5% Et_3N (v/v/v). Yield 1.156 g (92.1%), R_f 0.60 (EtOAc). ESI MS: $[M + Na]^+$ calc. 650.21, found 650.20. ^{1}H-NMR: 11.38 (br s, 1H, *H*-3), 7.85 (t, 1H, *J* = 5.7 Hz, OCON*H*), 7.66 (m, 1H, *H*-6), 7.40–7.19 (m, 9H, Ar*H*), 6.89 (d, 4H, *J* = 8.7 Hz, Ar*H*), 5.79 (d, 1H, *J* = 5.7 Hz, 3′-O*H*), 5.75 (s, 1H, *H*-1′), 5.39 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.11 (m, 1H, *H*-2′), 4.35 (m, 1H, *H*-3′), 4.06 (m, 1H, *H*-4′), 3.79 (m, 2H, CH_2N), 3.73 (s, 6H, CH_3), 3.40–3.15 (m, 3H¶, *H*-5′, ≡C*H*).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[*N*-methyl-*N*-propargyl-aminocarbonyl]uridine (5b). Chromatography: stepwise gradient of 1⟶2% MeOH in $CHCl_3$–EtOAc (1 : 1) + 0.5% Et_3N (v/v/v/v). Yield 1.097 g (85.5%), R_f 0.73 (EtOAc). ESI MS: $[M + Na]^+$ calc. 664.23, found 664.22. ^{1}H-NMR: 11.39 (s, 1H, *H*-3), 7.70 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.41–7.19 (m, 9H, Ar*H*), 6.89 (d, 4H, *J* = 8.6 Hz, Ar*H*), 5.89 (d, 1H, $J_{1',2'}$ = 3.3 Hz, *H*-1′), 5.54 (m, 1H, 3′-O*H*), 5.39 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.15 (m, 1H, *H*-2′), 4.32 (m, 1H, *H*-3′), 4.12–3.93 (m, 3H, *H*-4′, CH_2N), 3.73 (s, 6H, OCH_3), 3.35–3.16 (m, 3H¶, *H*-5′, ≡C*H*), 2.92 (s, 1.8H), 2.85 (s, 1.2H), (NCH_3, rotamers).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(4-iodobenzylamino-carbonyl)uridine (5c). Chromatography: stepwise gradient of 0.5⟶1⟶1.5⟶2% MeOH in $CHCl_3$–EtOAc (1 : 1) + 0.5% Et_3N (v/v/v/v). Yield 1.378 g (85.5%), R_f 0.75 (EtOAc). ESI MS: $[M + Na]^+$ calc. 828.14, found 828.14. ^{1}H-NMR: 11.42 (s, 1H, *H*-3), 7.95 (t, 1H, *J* = 6.0 Hz, OCON*H*), 7.70 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 7.66 (d, 1H, *J* = 8.2 Hz, Ar*H*), 7.40–7.18 (m, 9H, Ar*H*), 7.06 (d, 2H, *J* = 8.2 Hz, Ar*H*), 6.88 (d, 4H, *J* = 8.8 Hz, Ar*H*), 5.92 (d, 1H, $J_{1',2'}$ = 4.8 Hz, *H*-1′), 5.61 (d, 1H, $J_{3',OH}$ = 5.6 Hz, 3′-O*H*), 5.38 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.17 (apparent t, 1H, $J_{1',2'} = J_{2',3'}$ = 4.8 Hz, *H*-2′), 4.33 (m, 1H, *H*-3′), 4.14 (d, 2H, *J* = 6.0 Hz, NCH_2), 3.98 (m, 1H, *H*-4′), 3.73 (s, 6H, CH_3), 3.35–3.16 (m, 2H¶, *H*-5′).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(pyren-1-ylmethylamino-carbonyl)uridine (5d). Chromatography: stepwise gradient of 0.5⟶1⟶1.5% MeOH in $CHCl_3$ + 0.5% pyridine (v/v/v). Yield 1.482 g (92.2%), R_f 0.75 (EtOAc). ESI MS: $[M + Na]^+$ calc. 826.27, found 826.28. ^{1}H-NMR: 11.44 (s, 1H, *H*-3), 8.41 (d, 1H, $J_{9'',10''}$ = 9.3 Hz, *H*-10″), 8.32–8.00 (m, 9H, Ar*H*, OCON*H*), 7.73 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.42–7.10 (m, 9H, Ar*H*), 6.88 (d, 4H, *J* = 8.6 Hz, Ar*H*), 5.97 (d, 1H, $J_{1',2'}$ = 4.6 Hz, *H*-1′), 5.65 (d, 1H, $J_{3',OH}$ = 5.6 Hz, 3′-O*H*), 5.39 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.26 (m, 1H, *H*-2′), 4.95 (d, 2H, *J* = 5.3 Hz, NCH_2), 4.38 (m, 1H, *H*-3′), 3.99 (m, 1H, *H*-4′), 3.72 (s, 6H, CH_3), 3.35–3.15 (m, 2H¶, *H*-5′).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(1,4,7,10,13-pentaoxa-cyclopentadecan-2-ylmethylaminocarbonyl)uridine (5e). Chromatography: stepwise gradient of 3⟶4.5⟶6⟶9% MeOH in $CHCl_3$–EtOAc (2 : 1) + 0.5% Et_3N (v/v/v/v). Yield 1.248 g (85.5%), R_f 0.29 ($CHCl_3$–MeOH, 17 : 3). ESI MS: $[M + Na]^+$ calc. 844.33, found 844.32. ^{1}H-NMR: 11.39 (s, 1H, *H*-3), 7.69 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.41–7.12 (m, 10H, Ar*H*, OCON*H*), 6.89 (d, 4H, *J* = 8.8 Hz, Ar*H*), 5.90 (d, 1H, $J_{1',2'}$ = 4.7 Hz, *H*-1′), 5.53 (m, 1H, 3′-O*H*), 5.38 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.15 (m, 1H, *H*-2′), 4.32 (m, 1H, *H*-3′), 3.97 (m, 1H, *H*-4′), 3.73 (s, 6H, CH_3), 3.69–3.16 (m, 21H¶, *H*-5′, C*H*(CH_2OCH_2)$_4$CH_2), 3.02 (m, 2H, CH_2N).

***N*α-[5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-*O*-ylcarbonyl]-L-leucyl-L-phenylalaninamide (5f).** Chromatography: stepwise gradient of 1⟶2⟶3% MeOH in $CHCl_3$–EtOAc (2 : 1) + 0.5% pyridine (v/v/v/v). Yield 706 mg (83.1%), R_f 0.45 (EtOAc). MALDI-TOF (2,4,6-THAP): $[M + Na]^+$ calc. 872.35, found 872.62, $[M + K]^+$ calc. 888.32, found 888.62. ^{1}H-NMR: 11.45 (s, 1H, *H*-3), 7.90 (d, 1H, J = 8.4 Hz, N*H*CHCH$_2$Ph), 7.72 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 7.48–7.10 (m, 12H, Ar*H*, NH_2, OCON*H*), 6.89 (d, 4H, J = 8.8 Hz, Ar*H*), 5.90 (d, 1H, $J_{1',2'}$ = 4.4 Hz, *H*-1′), 5.52 (d, 1H, $J_{3',OH}$ = 5.9 Hz, 3′-O*H*), 5.43 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.13 (m, 1H, *H*-2′), 4.47 (m, 2H, *H*-3′, C*H*CH$_2$Ph), 4.37 (m, 1H, *H*-3′), 4.17 (m, 1H, C*H*Bu^i), 3.98 (m, 1H, *H*-4′), 3.73 (s, 6H, OCH_3), 3.52–3.10 (m, 2H, *H*-5′), 3.05–2.70 (m, 2H, CH_2Ph), 1.57–1.22 (m, 3H, CH_2C*H*Me$_2$), 0.86–0.69 (m, 6H, CH(CH_3)$_2$).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[2-(2-pivaloyloxyethoxy)-ethylaminocarbonyl]uridine (5h). Chromatography: stepwise gradient of 0.5⟶1⟶1.5⟶2% MeOH in $CHCl_3$–EtOAc (2 : 1) + 0.5% Et_3N (v/v/v/v). Yield 1.377 g (90.4%), R_f 0.60 (EtOAc). ESI MS: $[M + Na]^+$ calc. 784.31, found 784.30. ^{1}H-NMR: 11.40 (s, 1H, *H*-3), 7.69 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 7.41–7.19 (m, 10H, Ar*H*, OCON*H*), 6.89 (d, 4H, J = 8.8 Hz, Ar*H*), 5.90 (d, 1H, $J_{1',2'}$ = 5.0 Hz, *H*-1′), 5.53 (d, 1H, $J_{3',OH}$ = 5.7 Hz, 3′-O*H*), 5.38 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.12 (apparent t, 1H, $J_{1',2'} = J_{2',3'}$ = 5.0 Hz, *H*-2′), 4.32 (m, 1H, *H*-3′), 4.11 (m, 2H, CH_2OPiv), 3.97 (m, 1H, *H*-4′), 3.73 (s, 6H, OCH_3), 3.57, 3.43 (2m, 4H, CH_2OCH_2), 3.27–3.02 (m, 4H, *H*-5′, CH_2N), 1.11 (s, 9H, Bu^t).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[13-(trifluoroacetylamino)-4,7,10-trioxatridecan-1-ylaminocarbonyl]uridine (5j). Chromatography: stepwise gradient of 1⟶2⟶3⟶3.5% MeOH in $CHCl_3$–EtOAc (2 : 1) + 0.5% Et_3N (v/v/v/v). Yield 1.427 g (80.3%), R_f 0.22 (EtOAc). ESI MS: $[M + Na]^+$ calc. 911.33, found 911.33. ^{1}H-NMR: 11.39 (s, 1H, *H*-3), 9.36 (br s, 1H, N*H*TFA), 7.70 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 7.41–7.19 (m, 10H, Ar*H*, OCON*H*), 6.89 (d, 4H, J = 8.8 Hz, Ar*H*), 5.89 (d, 1H, $J_{1',2'}$ = 5.0 Hz, *H*-1′), 5.54 (d, 1H, $J_{3',OH}$ = 5.6 Hz, 3′-O*H*), 5.38 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.13 (apparent t, 1H, $J_{1',2'} = J_{2',3'}$ = 5.0 Hz, *H*-2′), 4.32 (m, 1H, *H*-3′), 3.96 (m, 1H, *H*-4′), 3.73 (s, 6H, CH_3), 3.53–3.36 (m, 12H, (CH_2OCH_2)$_3$), 3.28–3.16 (m, 4H, *H*-5′, CH_2NHTFA), 3.02 (m, 2H, OCONHCH_2), 1.75–1.56 (m, 4H, CH$_2$CH_2CH$_2$).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-trifluoroacetyl-7-(trifluoroacetylamino)-4-azaheptan-1-ylaminocarbonyl]uridine (5m). Compound **5m** was isolated by chromatography using a stepwise gradient of 1⟶2⟶4% MeOH in $CHCl_3$–EtOAc (2 : 1) + 0.5% Et_3N (v/v/v/v); yield 1.556 g (86.8%), white amorphous solid, R_f 0.49 (EtOAc). ESI MS: $[M + Na]^+$ calc. 918.28, found 918.27. ^{1}H-NMR: 11.37 (br s, 1 H, H-3) (exchangeable with D_2O), 9.51, 9.46 (2 br s, 1 H, N*H*TFA, rotamers), 7.69 (m, 1 H, H-6), 7.53–7.19 (m, 10 H, Ar*H* (Dmt), OCON*H*), 6.89 (m, 4 H, Ar*H* (Dmt)), 5.92 (m, 1 H, H-1′), 5.56 (m, 1 H, 3′-OH) (exchangeable with D_2O), 5.37 (m, 1 H, H-5), 5.15 (m, 1 H, H-2′), 4.34 (m, 1 H, H-3′), 3.97 (m, 1 H, H-4′), 3.73 (s, 6 H, CH_3), 3.40–3.14 (m, 8 H¶, H-5′, CH_2NTFA), 3.00 (m, 2 H, OCONHCH_2), 1.73 (m, 4 H, CH$_2$CH_2CH$_2$).

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4,9-bis(trifluoroacetyl)-12-(trifluoroacetylamino)-4,9-diazadodecan-1-ylaminocarbonyl]-uridine (5p). Chromatography: stepwise gradient of 1⟶2⟶5% MeOH in $CHCl_3$–EtOAc (2 : 1) + 0.5% Et_3N (v/v/v/v). Yield 1.773 g (83.4%), R_f 0.50 (EtOAc). MALDI-TOF (2,4,6-THAP): $[M + Na]^+$ calc. 1085.33, found 1085.28, $[M + K]^+$ calc. 1101.31, found 1100.91. ^{1}H-NMR: 11.40 (s, 1H, *H*-3), 9.51, 9.44 (2m, 1H, N*H*TFA rotamers), 7.69 (m, 1H, *H*-6), 7.51–7.18 (m, 10H, Ar*H*, OCON*H*), 6.88 (d, 4H, J = 8.8 Hz, Ar*H*), 5.92 (m, 1H, *H*-1′), 5.56 (m, 1H, 3′-O*H*), 5.37 (m, 1H, *H*-5), 5.15 (m, 1H, *H*-2′), 4.34 (m, 1H, *H*-3′), 3.96 (m, 1H, *H*-4′), 3.73 (s, 6H, CH_3), 3.44–3.14 (m, 12H¶, *H*-5′, CH_2NTFA), 3.01 (m, 2H, OCONHCH_2), 1.73 (m, 4H, CH_2CH$_2$NH), 1.52 (m, 4H, CH$_2$CH_2CH_2CH$_2$).

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)uridin-2′-yl carbamates (6)

A general procedure.

5′-*O*-(4,4′-Dimethoxytrityl)uridin-2′-yl carbamates **5** (1.0 mmol) were coevaporated with dry DCM (2 × 20 cm^3), dissolved in dry DCM, diisopropylammonium tetrazolide (171 mg, 1.0 mmol) and bis(*N,N*-diisopropylamino)-2-cyanoethoxyphosphine (0.38 cm^3, 1.2 mmol) were added, and the mixture was stirred under nitrogen for 2 h. After conversion of the starting compound **5** is complete (monitoring by TLC, 25% acetone in $CHCl_3$ + 1% Et_3N v/v/v, pair of diastereomers), the mixture was diluted with $CHCl_3$, washed with 5% $NaHCO_3$ (100 cm^3), 20% NaCl (100 cm^3), dried over Na_2SO_4, evaporated to dryness, and the residue was chromatographed on a silica gel column in an appropriate solvent system. Fractions containing product were combined, evaporated, and the residue was dried *in vacuo* to afford compounds listed below as white amorphous solids.

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(propargylaminocarbonyl)uridine (6a). Chromatography: 33⟶50⟶100% EtOAc in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 1.029 g (82.8%). "Faster moving" diastereomer: R_f 0.56. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 828.87, found 828.67. ^{1}H-NMR: 11.45 (s, 1H, *H*-3), 7.95 (m, 1H, OCON*H*), 7.72 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 7.41–7.18 (m, 9H, Ar*H*), 6.88 (d, 4H, J = 8.5 Hz, Ar*H*), 5.89 (d, 1H, $J_{1',2'}$ = 5.0 Hz, *H*-1′), 5.43 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.33 (apparent t, 1H, $J_{1',2'} = J_{2',3'}$ = 5.0 Hz, *H*-2′), 4.56 (m, 1H, *H*-3′), 4.15 (m, 1H, *H*-4′), 3.85–3.68 (m, 8H, OCH_3, CH_2N), 3.63–3.44 (m, 4H, POCH_2, C*H*CH$_3$), 3.34–3.22 (m, 2H¶, *H*-5′), 3.09 (t, 1H, ^4J = 2.3 Hz, ≡C*H*), 2.58 (t, 2H, J = 6.1 Hz, CH_2CN), 1.19–0.95 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 150.784. "Slower moving" diastereomer: R_f 0.41. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 828.87, found 828.76. ^{1}H-NMR: 11.44 (s, 1H, *H*-3), 7.90 (t, 1H, J = 5.7 Hz, OCON*H*), 7.73 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-6), 7.41–7.18 (m, 9H, Ar*H*), 6.87 (m, 4H, Ar*H*), 5.91 (d, 1H, $J_{1',2'}$ = 5.1 Hz, *H*-1′), 5.42 (d, 1H, $J_{5,6}$ = 8.1 Hz, *H*-5), 5.36 (apparent t, 1H, $J_{1',2'} = J_{2',3'}$ = 5.1 Hz, *H*-2′), 4.55 (m, 1H, *H*-3′), 4.10 (m, 1H, H-4′), 3.82–3.68 (m, 10 H, OCH_3, CH_2N, POCH_2), 3.46 (m, 2H, C*H*CH$_3$), 3.35–3.21 (m, 2H¶, *H*-5′), 3.12 (m, 1H, ≡C*H*), 2.74 (t, 2H, J = 5.8 Hz, CH_2CN), 1.19–0.89 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 150.729.

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(*N*-methyl-*N*-propargylamino-carbonyl)uridine (6b). Chromatography: 30⟶50⟶70% EtOAc in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 1.004 g (79.5%), R_f 0.58, 0.69. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 842.89, found 842.89. ^{1}H-NMR: 11.42 (s, 1H, *H*-3), 7.70 (m, 1H, *H*-6), 7.42–7.16 (m, 9H, Ar*H*), 6.87 (m, 4H, Ar*H*), 5.90 (m, 1H, *H*-1′), 5.47–5.18 (m, 2H, *H*-5, *H*-2′), 4.55 (m, 1H, *H*-3′), 4.20–3.92 (m, 3H, *H*-4′, CH_2N), 3.73 (s, 6H, OCH_3), 3.62–3.18 (m, 7H¶, POCH_2, C*H*CH$_3$, *H*-5′, ≡C*H*), 2.94–2.78 (m, 3H, NCH_3), 2.74, 2.56 (2m, 2H, CH_2CN), 1.20–0.88 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 150.892 (26%), 150.764 (30%), 150.391 (44%), diastereomers and rotamers.

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(4-iodobenzylaminocarbonyl)-uridine (6c). Chromatography: stepwise gradient of 20⟶25% acetone in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 674 mg (67%), R_f 0.47, 0.62. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 1006.84, found 1008.17. ^{1}H-NMR: 11.46 (s, 1H, *H*-3), 8.04 (m, 1H, OCON*H*), 7.73–7.61 (m, 3H, Ar*H*, *H*-6), 7.42–7.18 (m,

9H, Ar*H*), 7.07 (m, 2H, Ar*H*), 6.87 (m, 4H, Ar*H*), 5.93 (m, 1H, *H*-1′), 5.46–5.28 (m, 2H, *H*-5, *H*-2′), 4.53 (m, 1H, *H*-3′), 4.12 (m, 3H, *H*-4′, NCH_2), 3.78–3.19 (m, 12H¶, POCH_2, C*H*N, *H*-5′, OCH_3), 2.68, 2.58 (2m, 2H, CH_2CN), 1.23–0.89 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 149.274.

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(pyren-1-ylmethylaminocarbonyl)uridine (6d). Chromatography: 5⟶10⟶20⟶25% acetone in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 978 mg (97.4%), R_f 0.55, 0.68. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 1005.08, found 1006.67. ^{1}H-NMR: 11.47 (s, 1H, *H*-3), 8.41 (d, 1H, $J_{9'',10''}$ = 9.3 Hz, *H*-10″), 8.36–7.98 (m, 9H, pyrene *H*-2″–*H*-9″, OCON*H*), 7.73 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.43–7.17 (m, 9H, Ar*H*), 6.87 (d, 4H, *J* = 8.5 Hz, Ar*H*), 5.95 (d, 1H, $J_{1',2'}$ = 5.1 Hz, *H*-1′), 5.42 (m, 2H, *H*-5, *H*-2′), 4.91 (d, 2H, *J* = 5.3 Hz, NCH_2), 4.57 (m, 1H, *H*-3′), 4.17 (m, 1H, *H*-4′), 3.71 (s, 6H, OCH_3), 3.42–3.35 (m, 6H, POCH_2, C*H*N, *H*-5′), 2.88, 2.58 (2t, 2H, *J* = 5.8 Hz, CH_2CN, diastereomers), 1.17, 1.02–0.89 (2m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 149.295.

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(1,4,7,10,13-pentaoxacyclopentadecan-2-ylmethylaminocarbonyl)uridine (6e). Chromatography: stepwise gradient of 20⟶50⟶66% acetone in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 955 mg (93.4%), R_f 0.11, 0.24. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 1023.09, found 1024.61, $[M + Na]^+$ calc. 1045.07, found 1046.01, $[M + K]^+$ calc. 1061.18, found 1062.02. ^{1}H-NMR: 11.44 (br s, 1H, *H*-3), 7.70 (m, 1H, *H*-6), 7.49–7.15 (m, 10H, Ar*H*, OCON*H*), 6.87 (m, 4H, Ar*H*), 5.91 (m, 1H, *H*-1′), 5.46–5.25 (m, 2H, *H*-5, *H*-2′), 4.53 (m, 1H, *H*-3′), 4.11 (m, 1H, *H*-4′), 3.80–3.19 (m, 31H¶, POCH_2, C*H*N, *H*-5′, C*H*(CH_2OCH_2)$_4$CH_2, OCH_3), 3.00 (m, 2H, CH_2N), 2.75, 2.59 (2m, 2H, CH_2CN), 1.20–0.90 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 149.280, 149.275 (diastereomers).

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[2-(2-pivaloyloxyethoxy)ethylaminocarbonyl]uridine (6h). Chromatography: acetone–$CHCl_3$ (1 : 1) + 1% Et_3N (v/v/v). Yield 947 mg (98.4%), R_f 0.43, 0.57. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 963.04, found 964.82. ^{1}H-NMR: 11.44 (s, 1H, *H*-3), 7.70 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.47–7.19 (m, 10H, Ar*H*, OCON*H*), 6.88 (m, 4H, Ar*H*), 5.90 (m, 1H, *H*-1′), 5.43 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.30 (m, 1H, *H*-2′), 4.55 (m, 1H, *H*-3′), 4.10 (m, 3H, *H*-4′, CH_2OCO), 3.72 (s, 6H, OCH_3), 3.62–3.01 (m, 12H¶, POCH_2, C*H*N, *H*-5′, NCH_2CH_2OCH_2), 2.74, 2.57 (2m, 2H, CH_2CN), 1.23–0.90 (m, 21H, CHCH_3, Bu^t). ^{31}P NMR (CD_3CN): 149.488, 149.416, 149.351 (diastereomers and rotamers).

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[13-(trifluoroacetylamino)-4,7,10-trioxatridecan-1-ylaminocarbonyl]uridine (6j). Chromatography: stepwise gradient of 20⟶33⟶50⟶66% acetone in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 1.069 g (98.2%), R_f 0.20, 0.34. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 1090.11, found 1092.09. ^{1}H-NMR: 11.44 (s, 1H, *H*-3), 9.37 (br s, 1H, N*H*TFA), 7.71 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.47–7.19 (m, 10H, Ar*H*, OCON*H*), 6.87 (m, 4H, Ar*H*), 5.91 (m, 1H, *H*-1′), 5.42 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.31 (m, 1H, *H*-2′), 4.55 (m, 1H, *H*-3′), 4.12 (m, 1H, *H*-4′), 3.80–3.16 (m, 24H¶, (CH_2OCH_2)$_3$, POCH_2, C*H*N, *H*-5′, OCH_3), 3.01 (m, 2H, OCONHCH_2), 2.74, 2.57 (2m, 2H, CH_2CN), 1.74–1.55 (m, 4H, CH$_2$CH_2CH$_2$), 1.18–0.92 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 149.346, 149.275 (diastereomers).

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[4-trifluoroacetyl-7-(trifluoroacetylamino)-4-azaheptan-1-ylaminocarbonyl]uridine (6m). Chromatography: 20⟶33⟶66% acetone in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 1.396 g (93.8%), R_f 0.14, 0.25. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 1097.02, found 1097.45. ^{1}H-NMR: 11.40 (br s, 1H, *H*-3), 9.52, 9.44 (2 br s, 1H, N*H*TFA, rotamers), 7.70 (m, 1H, *H*-6), 7.53 (m, 1H, OCON*H*), 7.41–7.15 (m, 9H, Ar*H*), 6.87 (m, 4H, Ar*H*), 5.92 (m, 1H, *H*-1′), 5.45–5.22 (m, 2H, *H*-5, *H*-2′), 4.54 (m, 1H, *H*-3′), 4.12 (m, 1H, *H*-4′), 3.80–3.09 (m, 18H¶, OCH_3, POCH_2, C*H*N, *H*-5′, CH_2NTFA), 2.99 (m, 2H, OCONHCH_2), 2.74, 2.57 (2m, 2H, CH_2CN), 1.73 (m, 4H, CH$_2$CH_2CH$_2$), 1.20–0.87 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 150.741 (54%), 150.658 (22%), 150.581 (24%), diastereomers and rotamers.

3′-*O*-(*N,N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[4,9-bis(trifluoroacetyl)-12-(trifluoroacetylamino)-4,9-diazadodecan-1-ylaminocarbonyl]uridine (6p). Chromatography: stepwise gradient of 20⟶50% acetone in $CHCl_3$ + 1% Et_3N (v/v/v). Yield 1.242 g (98.3%), R_f 0.18, 0.32. MALDI-TOF (2,6-DHAP-citrate): $[M + H]^+$ calc. 1264.15, found 1264.67. ^{1}H-NMR: 11.36 (br s, 1H, *H*-3), 9.52, 9.45 (2 br s, 1H, N*H*TFA, rotamers), 7.69 (m, 1H, *H*-6), 7.53 (m, 1H, OCON*H*), 7.41–7.16 (m, 9H, Ar*H*), 6.87 (m, 4H, Ar*H*), 5.92 (m, 1H, *H*-1′), 5.44–5.21 (m, 2H, *H*-5, *H*-2′), 4.53 (m, 1H, *H*-3′), 4.12 (m, 1H, *H*-4′), 3.80–3.12 (m, 22H¶, OCH_3, POCH_2, C*H*N, *H*-5′, CH_2NTFA), 2.99 (m, 2H, OCONHCH_2), 2.74, 2.57 (2m, 2H, CH_2CN), 1.74 (m, 4H, CH_2CH$_2$NH), 1.51 (m, 4H, CH$_2$CH_2CH_2CH$_2$), 1.19–0.86 (m, 12H, CHCH_3). ^{31}P NMR (CD_3CN): 150.758 (56%), 150.651 (20%), 150.584 (24%), diastereomers and rotamers.

5′-*O*-(4,4′-Dimethoxytrityl)uridine-2′,3′-cyclic carbonate (8)

To a solution of 5′-*O*-(4,4′-dimethoxytrityl)uridine (2.733 g, 5.0 mmol) in DCM (50 cm^3), CDI (892 mg, 5.5 mmol) was added in one portion. After 2 h TLC (EtOAc) showed completion of the reaction. The mixture was diluted with $CHCl_3$ (100 cm^3), washed with water (150 cm^3), 5% citric acid (150 cm^3), and water (150 cm^3), dried (Na_2SO_4), evaporated, and the residue was chromatographed on a short silica gel column (stepwise gradient of EtOAc 1 : 1⟶3 : 2⟶2 : 1 in $CHCl_3$ + 0.5% pyridine, v/v/v). Yield 2.857 g (99.8%), white solid. ESI MS: $[M + Na]^+$ calc. 595.55, found 595.17. ^{1}H-NMR: 11.45 (s, 1H, *H*-3), 7.76 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.39–7.16 (m, 9H, Ar*H*), 6.86 (m, 4H, Ar*H*), 5.99 (d, 1H, $J_{1',2'}$ = 1.6 Hz, *H*-1′), 5.64 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.59 (dd, 1H, $J_{1',2'}$ = 1.6 Hz, $J_{2',3'}$ = 7.6 Hz, *H*-2′), 5.20 (dd, 1H, $J_{2',3'}$ = 7.6 Hz, $J_{3',4'}$ = 4.2 Hz, *H*-3′), 4.41 (m, 1H, *H*-4′), 3.72 (s, 6H, OCH_3), 3.37 (m, 1H¶, $^2J_{5'a,5'b}$ = 10.0 Hz, $J_{4',5'a}$ = 7.4 Hz, *H*-5′a), 3.13 (m, 1H, $^2J_{5'a,5'b}$ = 10.0 Hz, $J_{4',5'b}$ = 4.6 Hz, *H*-5′b).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(*N*-methyl-*N*-propargylaminocarbonyl)uridine (9)

To a solution of **8** (1.145 g, 2.0 mmol) in dry DCM (30 cm^3) *N*-methylpropargylamine (0.422 cm^3, 5.0 mmol) was added. The reaction was monitored by TLC ($CHCl_3$–acetone, 4 : 1 v/v). After one week the starting carbonate was consumed. The mixture was diluted with $CHCl_3$ (50 cm^3), washed with water (100 cm^3), 5% citric acid (100 cm^3), and water (100 cm^3), dried (Na_2SO_4), evaporated, and the residue was chromatographed on silica gel using a stepwise gradient of 0.5⟶1.0⟶1.5⟶3.0% MeOH in $CHCl_3$–EtOAc (1 : 1) + 0.5% Et_3N (v/v/v/v). Compound **5b** (131 mg, 10.2%) was eluted first. Compound **9** (1.058 g, 82.4%) was obtained as a white amorphous solid, R_f 0.64 (EtOAc). ESI MS: $[M + Na]^+$ calc. 664.23, found 664.22. ^{1}H-NMR: 11.37 (br s, 1H, *H*-3), 7.69 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-6), 7.40–7.27 (m, 5H, Ar*H*), 7.22 (d, 4H, *J* = 8.6 Hz, Ar*H*), 6.89 (d, 4H, *J* = 8.6 Hz, Ar*H*), 5.89–5.71 (m, 2H, 2′-OH, *H*-1′), 5.42 (d, 1H, $J_{5,6}$ = 8.0 Hz, *H*-5), 5.07 (m, 1H, *H*-3′), 4.40–3.95 (m, 4H, *H*-2′, *H*-4′, CH_2N), 3.70 (s, 6H, OCH_3), 3.38–3.15 (m, 3H¶, *H*-5′, ≡C*H*), 2.90, 2.86 (2s, 3H, NCH_3, rotamers).

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