ARTICLE

OBC
www.rsc.org/obc

The synthesis and properties of oligoribonucleotide–spermine conjugates †

Andrew J. Marsh, David M. Williams and Jane A. Grasby *
Centre for Chemical Biology, Department of Chemistry, Krebs Institute, University of Sheffield, Brook Hill, Sheffield, UK S3 7HF. E-mail: j.a.grasby@sheffield.ac.uk; Fax: +44 (0)114 222 9346; Tel: +44 (0)114 222 9478

Received 22nd March 2004, Accepted 7th May 2004
First published as an Advance Article on the web 28th June 2004

Polyamines stabilise nucleic acids against chemical and enzymatic degradation, facilitate the formation of secondary and tertiary structures and enhance cellular uptake. Therefore methods for the syntheses of polyamine–nucleic acid conjugates are of interest. A route for the syntheses of RNA–spermine conjugates has been developed. The polyamine was introduced to the C-5 position of uridine *via* an ethyl tether and the molecule elaborated into a synthon suitable for oligoribonucleotide assembly. The resultant oligomers were components of the hairpin ribozyme. Characterisation of the spermine-conjugated catalytic RNA revealed that attachment of the polyamine was well tolerated in three of four positions, namely U41, U37 and U34, suggesting that conjugation to C-5 brings about minimal structural perturbation.

Introduction

Polyamines such as putrescine, spermidine and spermine are ubiquitous in biological systems. It is thought that they are involved in the proliferation and differentiation of cells, DNA replication, protein synthesis and the activity of several enzymes.[1] The interactions of polyamines with nucleic acids have been studied for many years. These polycationic molecules are known to stabilise DNA against thermal[2] and alkaline denaturation,[3] enzymatic degradation,[4] shear[5] and oxidative breakage,[6] radiation damage,[7] and intercalation of aromatic dyes.[8] Polyamines have also been shown to have stabilising effects on RNA–DNA hybrids, triple-helical DNA and double-helical secondary structures found in tRNA, rRNA and mRNA.[1,9] In addition, polyamines have been demonstrated to effect structural and conformational modifications in nucleic acids,[10,11] for example, allowing the hairpin ribozyme to fold into a catalytically competent structure in the absence of metal ions.[12] There is also evidence that polyamines can facilitate the cellular uptake of oligodeoxynucleotides (ODNs).[13–15] Since the therapeutic applications of ODNs as antisense and antigene agents rely on efficient cellular uptake of such molecules, polyamines and polyamine–ODN conjugates may be exploited to improve efficacy.

As a consequence of the properties of polyamines, methods for the synthesis of polyamine–ODN conjugates have received considerable interest.[16–35] A variety of nucleic acids with di- and poly-amines attached at different positions have been prepared. In several cases conjugation of a polyamine has been reported to stabilise the resultant ODN duplexes and triplexes compared to their unmodified counterparts. In addition, frequently the tethering of polyamines has also been found to confer nuclease resistance to the oligomer.

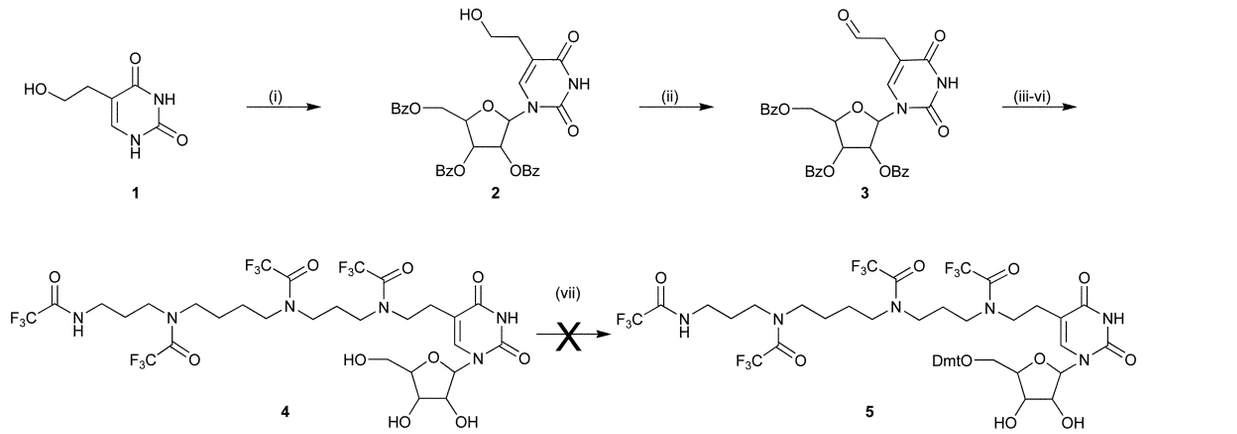
Various sites have been used to link polyamines to ODNs. Post-synthetic functionalisation with polyamines has been used to generate a 21-mer homopyrimidine ODN bearing a 5′-polyamine.[16] Chattopadhyaya and colleagues have prepared and attached a *C*-branched spermine derivative to the 5′- and 3′-termini of an ODN or internally to the 2′-position of *ara*-uridine *via* a phosphate linkage.[17,18]

Polyamines have also been introduced into ODNs *via* conjugation to the heterocyclic bases. In the pyrimidine series Ueda and co-workers introduced the diamine putrescine at the 5-position of thymidine *via* reduction of the Schiff base formed from a 5-formyl-2′-deoxyuridine derivative and *N*-phthaloylputrescine.[19] A similar strategy was used by Matsuda and colleagues to attach *syn*-norspermidine to the end of a butyl linker at the 5-position of 2′-deoxyuridine in a reductive amination step.[20] The same group also reported 2′-deoxyuridine analogues carrying either an *N*,*N*-bis(2-aminoethyl)aminoethylcarbamoyl or *N*,*N*-bis(3-aminopropyl)aminopropylcarbamoyl linker at the 5-position.[21] Kohgo *et al.* reported the incorporation into ODNs of a novel 2′-deoxyuridine derivative bearing a cyanomethoxycarbonylmethyl group at the 5-position. Post-synthetic functionalisation with monoamines, diamines and the polyamine *tris*(2-aminoethyl)amine was achieved under mild conditions.[22] A 2′-deoxyuridine derivatised at the 5-position with *tris*(2-aminoethyl)amine was also prepared by the same group from 5-methoxycarbonylmethyl-2′-deoxyuridine.[24] The same functionalisation and also the addition of spermine was attained *via* a post-synthetic method using analogous chemistry.[25] Similarly, Haginoya *et al.* used a post-synthetic modfication strategy reacting alkyldiamines with the convertible nucleoside unit 5-methoxycarbonyl-2′-deoxyuridine.[23] Prakash *et al.* tethered triethylenetetramine and spermine to the C-4 position of 2′-deoxy-5-methylcytidine *via* substitution of a 4-*O*-(2,4-dimethylphenyl) group.[26] Markiewicz *et al.* prepared 2′-deoxycytidine modified at the C-4 position with spermine following reaction with the 4-*p*-toluenesulfonate ester.[27] Reductive amination was employed to react 5-formyl-2′-deoxyuridine with putrescine and spermine, yielding conjugation at the 5-position *via* a methylene group.[28]

There have also been a number of polyamino–purine conjugates prepared recently. Schmid and Behr attached spermine to the N-2 position of 2′-deoxyguanosine in a post-synthetic fashion *via* substitution of fluorine.[29] Diaz *et al.* similarly linked spermine plus spermidine and 1-(3-aminopropyl)imidazole to the N-2 position of 2′-deoxyguanosine in a post-synthetic manner.[30] The Markiewicz lab described the synthesis of phosphoramidites of 2′-deoxyadenosine and 2′-deoxyguanosine with spermine at the N-6 and N-2 positions, respectively.[31,32] Potier *et al.* also synthesised a 2′-deoxyguanosine phosphor-

† Electronic supplementary information (ESI) available: MS and NMR spectra. See http://www.rsc.org/suppdata/ob/b4/b404150j/

DOI: 10.1039/b404150j



Scheme 1 Attempted synthesis of the uridine–spermine conjugate (**5**). *Reagents and conditions*: (i) 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, TMSCl, hexamethyldisilazane, $SnCl_4$, MeCN, room temperature, 80%; (ii) oxalyl chloride, DMSO, TEA, DCM, −60 °C, 36%; (iii) spermine, pyridine, methanol, room temperature; (iv) same pot $NaBH_4$; (v) NH_3 (aq.), room temperature; (vi) trifluoroacetic anhydride, pyridine, room temperature, 17% (four steps); (vii) DmtCl, pyridine, TEA, DMAP, $AgNO_3$, no reaction.

amidite with spermine at the N-2 position.[33,34] Shinozuka *et al.* used a post-synthetic strategy to obtain ODNs with a branched polyamine conjugated at the C-2 of 2′-deoxyguanosine.[35]

Ribozymes that are capable of the sequence-specific cleavage of RNA have potential uses *in vitro* as chemical nucleases and biosensors and *in vivo* as therapeutic agents for the controlled destruction of mRNA for gene inactivation and the targeted cleavage of viral, oncogene or mutant mRNA.[36–42] In analogy to ODNs, polyamine–catalytic RNA conjugates may have enhanced nuclease resistance, increased cellular uptake and a more stable tertiary structure. However, although the incorporation of a C5-(*N*-aminohexyl)carbamoyl-2′-OMe uridine monomer and its incorporation into DNA has been described recently,[43] there has, to our knowledge, been no description of the synthesis of polyamine–nucleic acid conjugates of RNA. Here we describe a method for the syntheses of RNA–spermine conjugates, prepared using a C-5 modified uridine derivative. The resultant oligomers are components of the hairpin ribozyme, and the properties of the resultant spermine-derivatised catalytic RNAs have been characterised.

Results and discussion

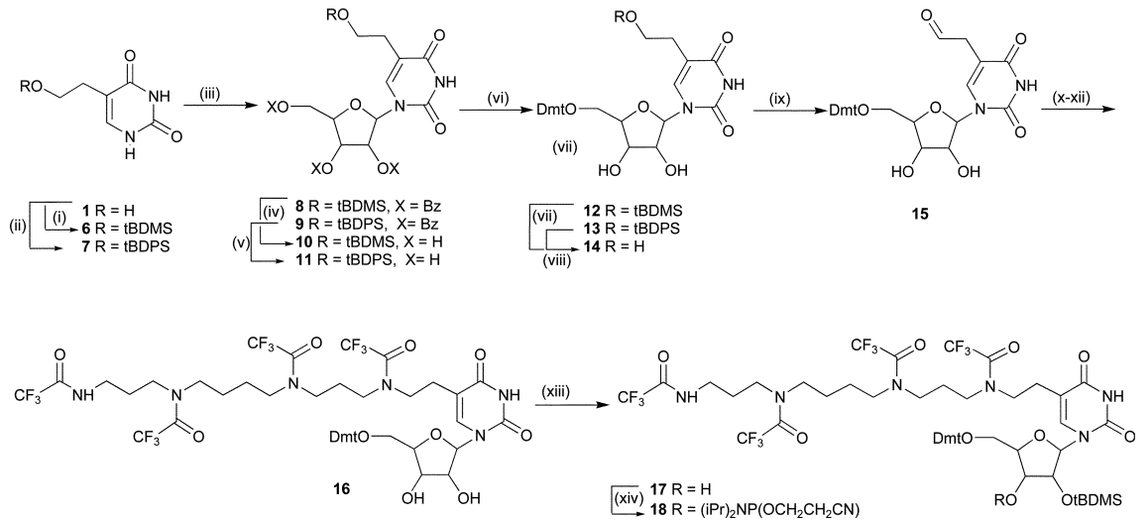
Synthesis of a spermine–uridine conjugate suitable for RNA synthesis

Several sites of attachment of polyamines to RNA are possible including the 5′- and 3′-termini, the 2′-hydroxyl group or other sites within the ribose unit, the phosphate or the nucleobase. Placement of a polyamine within a folded RNA structure is more likely to be achieved *via* internal, rather than 5′- or 3′-terminal, attachment. Since the 2′-hydroxyl group is commonly involved in RNA tertiary interactions, conjugation to this site may inhibit the formation of RNA three-dimensional structure. Other sites of sugar attachment are more synthetically demanding, whereas linking to the phosphate groups involves the formation of highly labile phosphate triesters. Thus we elected to create a polyamine conjugate linked to the nucleobase. The C-5 position of uridine was chosen for derivatisation since this does not interfere with the formation of Watson–Crick hydrogen bonds. When designing the uridine–spermine conjugate we considered an alkyl linker between the polyamine and the nucleobase to be vital to allow conformational flexibility of the entire polyamine. Thus, an ethyl linker was selected, since a high yielding and efficient synthesis of hydroxyethyluracil has been reported previously.[44]

Our first attempted route to a spermine–polyamine conjugate suitable for RNA synthesis is illustrated in Scheme 1. Hydroxyethyluracil (**1**)[44] was employed in a Vorbrüggen-type[45] coupling reaction to afford the perbenzoylated nucleoside **2** but in variable yield (30–80 %), possibly due to the poor solubility of the starting chromophore. As expected this procedure yielded only the β-isomer. Protection of the hydroxyl group of hydroxyethyluracil (**1**) was not required as this could be achieved by increasing the amount of trimethylsilyl chloride used to generate the silylated nucleobase. Subsequent oxidation of the hydroxyl group of **2** afforded the aldehyde **3** suitable for conjugation of the polyamine *via* reductive amination. Oxidation of the primary alcohol proceeded in highly variable but typically rather poor yield (35%) using Swern oxidation conditions, but failed entirely using pyridinium chlorochromate. Using a similar strategy to that described by Ueda and colleagues for the synthesis of a putrescinylthymidine derivative,[19] reaction of the aldehyde **3** with spermine to form a Schiff base, followed by reduction with $NaBH_4$ afforded a polyamine linked nucleoside. Without purification of the polyamine conjugate, the sugar benzoyl groups were removed with aqueous ammonia and then the amines were masked with trifluoroacetyl groups to produce the protected spermine conjugate **4**. The overall yield for these four steps was 17%. In addition to the target material bearing four trifluoroacetyl groups (compound **4**), significant amounts of a nucleoside with three trifluoroacetyl groups and one benzoyl group was also isolated. This latter side product most likely results from the transfer of a benzoyl group from the sugar to the polyamine and illustrates that it would be preferable to not have acyl hydroxyl protection in place during reductive amination. Attempts to introduce a dimethoxytrityl group to the spermine conjugate **4** to generate **5** proved unsuccessful, despite the addition of silver nitrate, DMAP, excess dimethoxytrityl chloride (DmtCl) and gentle warming. The tritylation failed for unknown reasons.

As an alternative route to the polyamine linker reagent, addition of the dimethoxytrityl group prior to introduction of the spermine moiety was investigated. In order to achieve selective 5′-*O*-dimethoxytritylation, the 5-hydroxyethyl side chain of compound **1** was protected with *tert*-butyldimethylsilyl chloride (tBDMSCl) to afford **6**, which was utilised in Vorbrüggen coupling to generate **8** (Scheme 2). The use of tBDMS-protected hydroxyethyluracil **6**, rather than hydroxyethyluracil itself, increased the yields and the reliability of the glycosyl bond-forming, probably due to the drastically improved solubility of the silylated nucleobase. The products of this reaction included both tBDMS-protected nucleoside **8** and some material that had lost the silyl protecting group. This latter material was therefore reprotected prior to isolation. This procedure gave reproducible yields of 68%.

The Lewis acid used in the coupling reaction presumably results in the partial removal of the tBDMS-protecting group from **8**. To increase the acid stability of the protecting group and avoid desilylation the use of the more acid-stable *tert*-



Scheme 2 Synthesis of the phosphoramidite monomer of the uridine–spermine conjugate (**18**). *Reagents and conditions*: (i) tBDMSCl, imidazole, DMF, room temperature, 98%; (ii) tBDPSCl, imidazole, DMF, room temperature, 99%; (iii) either **6** or **7**, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, TMSCl, hexamethyldisilazane, $SnCl_4$, MeCN, room temperature, 68% from **6**, 60% from **7**; (iv) NH_3 (MeOH), room temperature, 82%; (v) NH_3 (MeOH), room temperature, 72%; (vi) either **10** or **11**, DmtCl, pyridine, room temperature, 74% from **10**, 70% from **11**; (vii) TBAF, THF, room temperature, 98%; (viii) TBAF, THF, room temperature, 75%; (ix) Swern procedure **14**, oxalyl chloride, DMSO, TEA, DCM, −60 °C, 61%; Moffat procedure **14**, DMSO, pyridine, trifluoroacetic acid, room temperature, 42%; (x) spermine, pyridine, methanol, room temperature; (xi) same pot $NaBH_4$; (xii) trifluoroacetic anhydride, pyridine, room temperature, 57% (three steps); (xiii) tBDMSCl, $AgNO_3$, pyridine, THF, room temperature, 69%; (xiv) **17**, 2-cyanoethyl-*N*-diisopropylamine chlorophosphormamidite, diisopropylamine, DCM, room temperature, 86%.

butyldiphenylsilyl (tBDPS) group was investigated. However, yields of **9** from the glycosylation reaction using tBDPS-protected nucleobase **7** were an inferior 60%, although no silyl reprotection step was required prior to isolation. The reduced yield from this reaction might reflect the observed poorer solubility of silylated tBDPS-protected hydroxyuracil (**7**) compared with the tBDMS counterpart (**6**) or the increased steric bulk of the nucleophile. Therefore, despite the need for a reprotection step after the coupling reaction, tBDMS was used as the protecting group of choice for generation of the required intermediate compound **14**.

The benzoyl groups were efficiently removed from **8** and **9** by treatment with methanolic ammonia to yield **10** and **11**, allowing the introduction of a 5′-*O*-dimethoxytrityl (Dmt) group to generate **12** and **13** in good yield (70–75%). Treatment of **12** and **13** with tetrabutylammoniumfluoride (TBAF) removed the silyl protecting groups to yield the common 5′-*O*-Dmt intermediate **14**. Near quantitative yields (98%) for this reaction were obtained in the case of tBDMS protection, but tBDPS was removed less efficiently (70%). Selective oxidation of the nucleobase primary hydroxyl group of **14** to produce the 5′-aldehyde **15** was achieved in reasonable yield (61%) using a modified Swern oxidation procedure. During the Swern procedure, it was found essential to always keep the nucleoside in the presence of triethylamine, and after reaction subsequent manipulations were performed with cold solvents. These precautions greatly enhanced the yield of the reaction by preventing loss of the 5′-*O*-Dmt group. Pfitzner–Moffat oxidation was also suitable for this step but gave inferior yields (42%). Attempts to use Dess–Martin conditions, TPAP or SO_3/pyridine as the oxidant yielded only starting material. One-pot reductive amination, followed without purification by subsequent trifluoroacetylation in the presence of dry pyridine, yielded the trifluoroacetyl-protected spermine–uridine conjugate **16** in 57% yield. Selective introduction of a 2′-*O*-tBDMS group to generate **17** was achieved in good yield (69%) by treatment with tBDMSCl in the presence of silver nitrate. Finally, phosphitylation under standard conditions produced the target phosphoramidite **18** in 86% yield.

Curiously, ^{31}P NMR spectra of the phosphoramidite **18** demonstrated the presence of four peaks, rather than the expected two resonances of the phosphoramidite diastereoisomers (see ESI † for MS and NMR spectra). The ratio of these peaks was found to vary with the NMR solvent employed. Separation of the two diastereoisomers of the phosphoramidite observed on tlc result in ^{31}P NMR spectra that each contain two peaks. One possible explanation for the presence of the four NMR resonances is that a mixture of 2′-*O*-tBDMS-3′-*O*-phosphoramidite and 3′-*O*-tBDMS-2′-*O*-phosphoramidite have been isolated. Such a mixture would result if silyl migration had occurred during phosphitylation. The eventual consequences of the use of this mixture in oligoribonucleotide synthesis would be the generation of 2′–5′ linked RNA in addition to the normal 3′–5′ linked species. Alternatively, the four phosphorus resonances could be the result of the presence of more than one conformation of each phosphoramidite diastereoisomer. This might explain the differences in peak ratios observed in differing solvents, as the characteristics of a particular solvent might be expected to adjust conformational equilibria. Attempts to confirm the presence of a conformational equilibrium using variable-temperature NMR were unsuccessful due to decomposition of the phosphoramidite due to oxidation.

To investigate this further we therefore synthesised a ribonucleoside dimer, r(U*pG), where U* is the polyamine conjugate. ^{31}P NMR of this dimer indicated the presence of a single phosphorus species. Moreover when the dimer was subjected to enzymatic analysis[46] using RNase I and alkaline phosphatase it was completely degraded to guanosine and the uridine–polyamine conjugate **19** as monitored by RP HPLC. It is noteworthy that whilst this enzyme-catalysed reaction proceeds, it does so at a severely reduced rate suggesting that the polyamine modification confers some degree of nuclease resistance. Further studies of the substrate properties of the polyamine conjugates with a range of nucleases are in progress.

RNase I catalyses the transesterification of RNA molecules to yield products terminating in a 2′,3′-cyclic phosphate and a 5′-hydroxyl group.[47] The cyclic phosphate is then hydrolysed to generate the 3′-phosphate.[47] Alkaline phosphatase then catalyses the conversion of the resultant nucleotides to the corresponding free nucleosides suitable for RP HPLC analysis. RNase I catalyses only the reaction of 3′–5′-phosphate diester linkages, thus implying that the dimer, r(U*pG), contained only these linked species. From this finding it can be concluded that the phosphoramidite only contains the correct 2′-*O*-tBDMS material. We therefore have ascribed the anomalous ^{31}P NMR spectra to conformational equilibria of the phosphoramidite

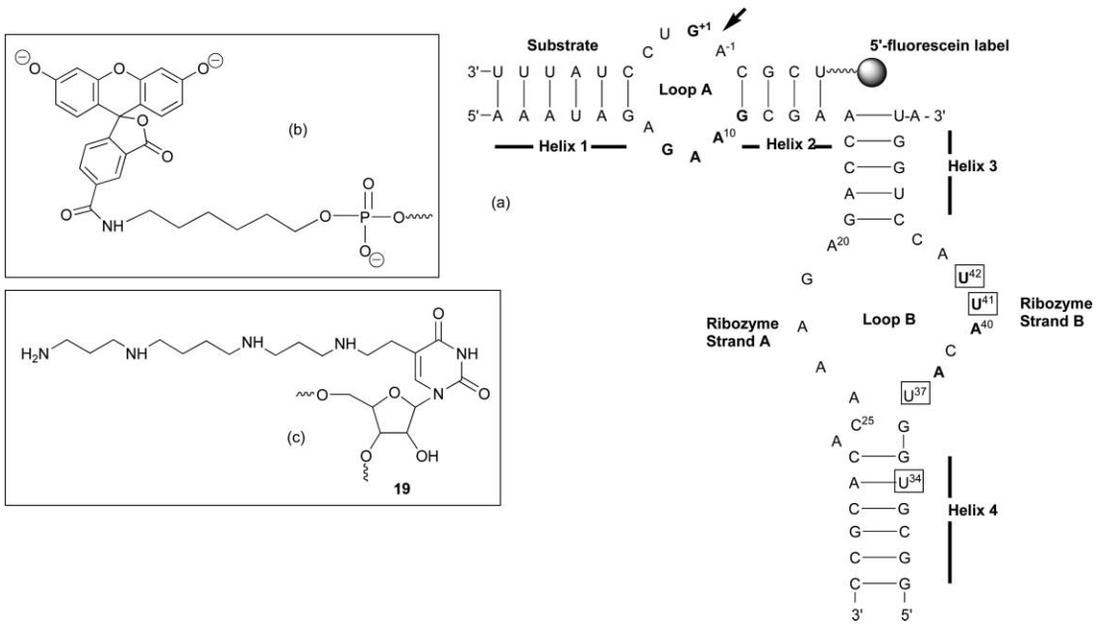


Fig. 1 (a) Three-stranded hairpin ribozyme used in these studies. Essential residues are shown in bold. An arrow indicates the site of reaction. Sites of modification are boxed. (b) 5′-Fluorescein label (5′-FAM). The substrate strand was fluorescently labelled using a 5′-fluorescein moiety to monitor the reaction. (c) The polyamine–uridine conjugate (U*) (**19**). Four separate modified ribozyme strands B were synthesised where the modified polyamine replaces U34, U37, U41 and U42 in (a) (boxed).

derivative **18**. Indeed, consistent with this are the findings that the anomeric proton signals for all of the compounds following introduction of spermine (compounds **16** onwards) display a more complex multiplet signal rather than the simple doublet seen for compound **15**. Furthermore, accurate mass data were consistent with those expected for compounds **16**–**18** and the mass spectra of these compounds indicated the absence of other mass ions. This allows the possibility of a mixture of two compounds that might be the result of formation of the Schiff base with either secondary or primary amino groups of the polyamine to be ruled out and is again consistent with a conformational equilibrium of a single species.

The synthesis of hairpin ribozymes containing the uridine–polyamine conjugate

The syntheses of oligoribonucleotides containing the polyamine conjugate were carried out using standard procedures employing 2′-*O*-TBDMS monomers. Relatively poor coupling yields were observed for the modified phosphoramidite **18** with a conventional tetrazole catalyst (81% by in-line trityl analysis), but the use of 5-(benzylmercapto)-1*H*-tetrazole improved coupling yields for both the modified (96%) and unmodified synthons (97%) in accordance with the observations of the Müller group.[48] Following conventional deprotection and purification, MALDI-TOF analysis revealed the presence of the desired modification and indicated that all the protecting groups had been removed.

To test the validity of conjugation of a polyamine to the C5-position of uridine in a complex RNA tertiary structure, four different hairpin ribozyme–spermine conjugates (Fig. 1) were created in which a uridine residue was replaced with a polyamine-bearing uridine derivative. The essential uridines, U41 and U42, were replaced with the spermine–conjugate in strand B of the ribozyme. In addition, two non-essential uridines U34 and U37 were also replaced.

Characterisation of the spermine-modified hairpin ribozymes

A three-stranded hairpin ribozyme was used in this study (Fig. 1). This consists of a substrate and two ribozyme strands, strand A and strand B. The sequence is identical to a ribozyme employed by Burke and co-workers and has been optimised to minimise alternative conformers.[49] A 5′-fluorescein moiety was added to the substrate to allow the amount of product to be quantified using a fluorescence detector following denaturing HPLC separation of the substrate and products.[50] The addition of a 5′-fluorophore to the hairpin ribozyme substrate has been shown previously not to change the catalytic parameters of the ribozyme.[51] The catalytic parameters of the ribozyme used in this study ($K_M = 7.1 \pm 1.6$ nM, $k_{cat} = 0.3 \pm 0.01$ min^{-1} with Mg^{2+} cofactor; $K_M = 5.4 \pm 1.4$ nM, $k_{cat} = 0.3 \pm 0.01$ min^{-1} with spermine as the sole cofactor) are similar to other three-stranded ribozymes.[12,52–54]

Table 1 Rate constants of unmodified and modified ribozymes with or without the presence of 10 mM Mg^{2+} at 100 nM [S] and 20 nM [E]

Modification	v/[E] (min^{-1})	
	With Mg^{2+}	Without Mg^{2+}
Unmodified	0.30	0.009
U*34	0.41	0.008
U*37	0.20	0.004
U*41	0.30	0.002
U*42	<0.01	<0.01

Evaluation of the polyamine hairpin ribozyme conjugates was carried out under standard hairpin ribozyme assay conditions (10 mM $MgCl_2$, 40 mM Tris HCl pH 7.5) with substrate and ribozyme concentrations of 100 and 20 nM, respectively. The results of this analysis are shown in Table 1. Substitution of either of U34, U37 or U41 with the polyamine conjugate [Fig. 1(c)] is tolerated well, producing rates of reaction comparable to the wild-type hairpin ribozyme. However, the replacement of U42 with the spermine–uridine derivative [Fig. 1(c)] was severely inhibitory to reaction. As U42 plays a critical role in the loop A–loop B interface, making five hydrogen bonds with other nucleosides within loops A and B,[55] it is likely that attachment of the polyamine prevents the correct folding of the ribozyme essential for catalysis. As exogenous spermine is capable of supporting hairpin ribozyme catalysis in the absence of divalent metal ions,[12] the rates of reaction of the spermine-modified hairpin ribozymes were also investigated in the absence of magnesium ions (Table 1). None of the conjugates produced a significant rate of reaction in the absence of divalent metal ions, suggesting that the polyamine is not correctly positioned to produce a catalytically competent structure in the

absence of divalent metal ions. The finding that in three of the four positions tested, attachment of spermine to the C-5 of uridine is tolerated, suggests that this is a suitable position for conjugation.

Conclusions

We have reported the synthesis of the phosphoramidite of a C5-modified uridine analogue that allows the preparation of RNA–spermine conjugates. The characterisation and catalytic properties of four different hairpin ribozymes containing the analogue in place of conserved uridines have been described. Three of the modified ribozymes show similar kinetic parameters to the wild-type sequence, whilst the substitution of U42 which is at the interface between loops A and B results in a large decrease in activity. However, the requirement for magnesium ion cofactors for all four spermine-modified ribozymes was not decreased compared to the wild-type ribozyme. Alternative sites of conjugation, longer tethers for linking to the polyamine and the use of *C*-branched polyamines may allow reduction or elimination of hairpin ribozyme divalent metal ion cofactor requirements. The finding that in three of the four positions tested, attachment of spermine to the C-5 of uridine is tolerated, suggests that this is a suitable position for conjugation. The ability to attach spermine to ribozymes without loss of activity may find application for the use of ribozymes *in vivo* due to the known propensity for the cellular uptake of polyamine–ODN conjugates.

Experimental

Dichloromethane (DCM), pyridine and acetonitrile were dried under reflux from calcium hydride and then distilled and stored over 3Å molecular sieves under argon. DMF and DMSO were obtained as anhydrous solvents from Aldrich. Triethylamine (TEA) and diisopropylethylamine (DIPEA) were dried by distillation from KOH pellets. Dry MeOH was obtained by distillation from magnesium turnings. Diethyl ether was distilled from sodium. All other reagents were purchased from commercial suppliers and used without purification. Silica gel for Flash Column Chromatography was used unless otherwise stated and was obtained from BDH (particle size 30–60 μm).

UV-visible data were obtained with a VARIAN CARY 50 Probe Spectrometer. Nuclear Magnetic Resonance (NMR) spectra were run on Bruker AC-250 and AMX-400 spectrometers. ^{1}H spectra were run at 250.13 or 400.13 MHz, respectively, ^{13}C spectra at 62.83 or 100.61 MHz, and ^{19}F spectra at 235.361 MHz. All coupling constants are quoted in Hertz.

5-(2-Hydroxyethyl)-2′,3′,5′-tri-*O*-benzoyl-uridine 2

5-(2-Hydroxyethyl)uracil **1** [44] (1.55 g, 9.9 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (5 g, 9.9 mmol) were suspended in dry acetonitrile (140 mL) under argon. Hexamethyldisilazane (2.51 mL, 11.9 mmol), trimethylsilyl chloride (1.51 mL, 11.9 mmol) and finally $SnCl_4$ (1.39 mL, 11.9 mmol) in dry acetonitrile (20 mL) were added. The resulting yellow solution was stirred 17 h at r.t. then partitioned between DCM (150 mL) and a saturated aq. $NaHCO_3$ solution (100 mL). The aqueous layer was then re-extracted with DCM (100 mL) and the combined organic layers washed with brine (50 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by silica gel chromatography (eluent 3% MeOH in DCM) to give a cream-coloured foam (4.79 g, 80%). ^{1}H NMR (250 MHz; $CDCl_3$): δ 2.19 (2H, m, C5-CH_2CH$_2$OH), 2.37 (1H, t, J = 5.8, C5-CH$_2$CH$_2$OH), 3.49 (2H, q, J = 5.8, C5-CH$_2$CH_2OH), 4.59 (1H, dd, J = 3.7, 11.6, 5′-H), 4.64 (1H, dd, J = 3.5, 5.6, 4′-H), 4.79 (1H, dd, J = 1.8, 11.3, 5′-H), 5.70 (1H, t, J = 6.1, 2′-H), 5.84 (1H, dd, J = 6.1, 3.4, 3′-H), 6.33 (1H, d, J = 6.4, 1′-H), 7.23–7.59 (10H, m, ArH and H6), 7.90 (4H, m, ArH), 8.06 (2H, m, ArH) and 8.73 (1H, s, NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ 30.4, 61.1, 64.0, 71.4, 73.4, 80.6, 87.3, 113.3, 128.4, 128.6, 128.9, 129.3, 129.7, 129.9, 130.0, 133.8, 137.0, 150.4, 164.0, 165.4; Electrospray-MS: 601 $(M + H)^+$, Acc. Mass: 601.1830, $C_{32}H_{29}N_2O_{10}$ requires 601.1822 deviation 1.4 ppm.

2′,3′,5′-Tri-*O*-benzoyluridine-5-acetaldehyde 3

Anhydrous DMSO (0.13 mL, 1.8 mmol) in dry DCM (0.4 mL) was added to a stirred solution of oxalyl chloride (0.08 mL, 0.9 mmol) in dry DCM (2 mL) under argon at −78 °C. After 2 min, hydroxy nucleoside **2** (dried over P_2O_5 under high vacuum) (0.5 g, 0.8 mmol) in dry DCM/DMSO (0.8 mL/0.5 mL) was added over 5 min, the solution was then stirred for 15 min and anhydrous TEA (0.58 mL, 4.2 mmol) was added. The reaction was then stirred for 5 min and allowed to warm to r.t. over 1 h before being partitioned between water (10 mL) and DCM (10 mL). The organic layer was then washed successively with 10 mL each of brine, 1% aq. HCl solution, water, dilute aq. Na_2CO_3 solution (5%) and finally water. The organic layer was then dried ($MgSO_4$) and evaporated and the crude product purified by silica gel chromatography (eluent gradient 10–25%, EtOAc in DCM) to yield **3** as a pale yellow solid (0.18 g, 36% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.94 (2H, s, C5-CH_2CHO), 4.57 (1H, dd, J = 3.5, 12.1, 5′-H), 4.65 (1H, dd, J = 3.5, 6.2, 4′-H), 4.82 (1H, dd, J = 2.6, 12.1, 5′-H), 5.69 (1H, t, J = 6.1, 2′-H), 5.85 (1H, dd, J = 6.0, 3.5, 3′-H), 6.36 (1H, d, J = 6.4, 1′-H), 7.28–7.60 (10H, m, ArH and H6), 7.90 (4H, m, ArH), 8.05 (2H, m, ArH), 8.53 (1H, s, NH) and 9.42 (1H, t, J = 1.1, C5-CH$_2$CHO); ^{13}C NMR (250 MHz, $CDCl_3$): δ 40.4, 64.0, 71.4, 73.7, 80.8, 87.5, 108.0, 128.6, 129.0, 129.4, 129.7, 129.9, 130.0, 133.8, 138.1, 150.1, 162.3, 165.4, 197.2; Electrospray-MS: 599 $(M + H)^+$, Acc. Mass: 599.1676, $C_{32}H_{27}N_2O_{10}$ requires 599.1666 deviation 1.7 ppm.

5-[15-Trifluoroacetamido-3,7,12-*tris*-(*N*-trifluoroacetyl)-3,7,12-triazapentadecyl]-uridine 4

Aldehyde nucleoside **3** (1 g, 0.3 mmol) was evaporated from dry pyridine (3 × 5 mL), then spermine (1.69 g, 8.4 mmol), anhydrous MeOH (5 mL) and dry pyridine (5 mL) were added and the solution stirred at r.t. under argon for 16 h until no starting material remained. Sodium borohydride (0.13 g, 3.5 mmol) was then added and after 1.5 h the solvent was evaporated. The resulting solid was then suspended in aq. NH_3 solution (S.G. = 0.88) (10 mL), stirred for 2.5 h and then evaporated to dryness. After co-evaporation with dry pyridine (3 × 5 mL) the material was suspended in dry pyridine (10 mL) and trifluoroacetic anhydride (5.66 mL, 40.1 mmol) added to the stirred solution at 0 °C under argon over 15 min. The reaction was then allowed to warm to r.t. and after 19 h was cooled in ice and MeOH (5 mL) was added. The solvent was then evaporated and the crude product partitioned between water (25 mL) and EtOAc (25 mL). The organic layer was dried ($MgSO_4$) and evaporated and the crude product purified by silica gel chromatography (eluent 5–25% MeOH in DCM) to yield a moist, orange solid. This material was purified twice by silica gel chromatography (eluent 2.5–25% MeOH in DCM) to yield **4** as a pale yellow foam (0.25 g, 17% yield). ^{1}H NMR (250 MHz, CD_3OD): δ 1.70 (4H, m, CH_2CH$_2$N), 1.87–2.15 (4H, m, CH_2CH$_2$N), 2.62–2.75 (2H, m, C5-CH_2CH$_2$N), 3.31–3.39 (2H, m, C5-CH$_2$CH_2N), 3.43–3.71 (12H, m, CH$_2$N), 3.75–3.86 (1H, m, 5′-H), 3.87–3.94 (1H, m, 5′-H), 4.02–4.14 (1H, m, 4′-H), 4.17–4.30 (2H, m, 2′-H and 3′-H), 5.94 (1H, d, J = 4.0, 1′-H), 8.05 (1H, d, J = 13.1, H6); ^{13}C NMR (250 MHz, CD_3OD): δ 24.9, 25.5, 26.8, 27.2, 29.2, 37.9, 38.3, 45.4, 45.8, 46.4, 47.1, 47.6, 62.1, 71.2, 75.9, 86.3, 90.6, 112.1, 113.7, 116.5, 119.4, 139.8, 152.4, 158.5, 163.4, 165.8; Electrospray-MS: 879 $(M + Na)^+$, Acc. Mass: 879.2182, $C_{29}H_{36}N_6O_{10}F_{12}Na$ requires 879.2199 deviation 1.9 ppm; λ_{max} (MeOH) 269 nm.

5-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-uracil 6

Hydroxyethyluracil **1** [44] (10 g, 64.1 mmol) was dissolved in anhydrous DMF (200 mL) under argon. Imidazole (17.5 g, 0.256 mol) and *tert*-butyldimethylsilyl chloride (tBDMSCl) (11.6 g, 76.9 mmol) were added and the reaction stirred at r.t. for 14 h after which further tBDMSCl (0.48 g, 3.2 mmol) was added and the reaction was left for 1 h. DCM (400 mL) was then added and the organic layer was washed with dilute aq. $NaHCO_3$ solution (5%) (2 × 200 mL) and brine (200 mL), then dried ($MgSO_4$) and evaporated. The residue was washed with water (1 L) before drying under a high vacuum over P_2O_5 for 5 h to give protected nucleobase **6** as a white crystalline solid (16.9 g, 97.7% yield). ^{1}H NMR (250 MHz, DMSO-d_6): δ 0.00 (6H, s, SiMe), 0.85 (9H, s, *t*-Bu), 2.36 (2H, t, J = 6.4, C5-CH_2CH$_2$OSi), 3.62 (2H, t, J = 6.5, C5-CH$_2$CH_2OSi), 7.24 (1H, s, H6), 10.70 (1H, s, NH), 11.05 (1H, s, NH); ^{13}C NMR (250 MHz, DMSO-d_6): δ −4.9, 18.3, 26.2, 30.0, 61.5, 109.0, 139.6, 151.8, 164.9; Electrospray-MS: 271 (M + H)$^+$, Acc. Mass: 271.1487, $C_{12}H_{23}N_2O_3Si$ requires 271.1478 deviation 3.2 ppm.

5-[2-(*tert*-Butyldiphenylsilanyloxy)ethyl]-uracil 7

Hydroxyethyluracil **1** [44] (4.5 g, 28.8 mmol) was dissolved in anhydrous DMF (45 mL) under argon. Imidazole (7.85 g, 0.12 mol) and *tert*-butyldiphenylsilyl chloride (tBDPSCl) (8.99 mL, 34.6 mmol) were added and the reaction stirred at r.t. for 17.5 h. EtOAc (100 mL) was then added and the organic layer washed with dilute aq. $NaHCO_3$ solution (5%) (2 × 50 mL) and brine (100 mL), then dried ($MgSO_4$) and evaporated. The residue was washed with hexane (4 × 50 mL) followed by water (4 × 50 mL) before drying under a high vacuum over P_2O_5 for 5 h to give protected nucleobase **7** as a white solid (11.3 g, 99% yield). ^{1}H NMR (250 MHz, DMSO-d_6): δ 0.97 (9H, s, *t*-Bu), 2.46 (2H, bs, C5-CH_2CH$_2$OSi), 3.70 (2H, bs, C5-CH$_2$CH_2OSi), 7.26 (1H, s, H6), 7.44 and 7.57 (10H, 2 × s, ArH), 10.71 (1H, s, NH), 11.03 (1H, s, NH); ^{13}C NMR (250 MHz, DMSO-d_6): δ 19.3, 27.1, 29.9, 62.5, 109.0, 128.3, 130.2, 133.6, 135.4, 139.7, 151.9, 164.9; Electrospray-MS: 395 (M + H)$^+$, Acc. Mass: 395.1793, $C_{22}H_{27}N_2O_3Si$ requires 395.1791 deviation 0.6 ppm.

5-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-2′,3′,5′-tri-*O*-benzoyl-uridine 8

Compound **6** (16.5 g, 61 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (30.8 g, 61 mmol) were suspended in dry acetonitrile (900 mL) under argon. Hexamethyldisilazane (16.7 mL, 79.3 mmol), trimethylsilyl chloride (10.1 mL, 79.3 mmol) and finally $SnCl_4$ (8.57 mL, 73.2 mmol) in dry acetonitrile (90 mL) were then added. The resulting yellow solution was stirred for 16 h at r.t. then DCM (750 mL) was added, followed by washing with saturated aq. $NaHCO_3$ solution (600 mL). The aqueous layer was further extracted with DCM (200 mL) and the combined organic layers washed with brine (300 mL), dried ($MgSO_4$) and evaporated to give a pale yellow solid (44.3 g). This was dissolved in dry DMF (500 mL) and stirred at r.t. under argon with tBDMSCl (6.68 g, 44.3 mmol) and imidazole (10.1 g, 0.148 mol). Further amounts of tBDMSCl (2 × 1.11 g, 7.38 mmol) were added after 3 and 20 h respectively. After 24 h the reaction was partitioned between DCM (800 mL) and dilute aq. $NaHCO_3$ solution (5%) (500 mL), and the organic layer was washed further with aq. $NaHCO_3$ solution (5 %) (500 mL) and brine (300 mL), then dried ($MgSO_4$) and evaporated, co-evaporating with toluene (3 × 100 mL). This gave a cream-coloured solid (41 g) which was purified by silica gel chromatography (eluent 0–5% MeOH in DCM) yielding protected nucleoside **8** as a cream-coloured foam (29.7 g, 68% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.00 (6H, s, SiMe), 0.85 (9H, s, *t*-Bu), 2.13–2.23 (1H, m, C5-CH_2CH$_2$OSi), 2.37–2.47 (1H, m, C5-CH_2CH$_2$OSi), 3.65 (2H, t, J = 6.1, C5-CH$_2$CH_2OSi), 4.68–4.87 (3H, m, 4′-H and 5′-H), 5.79 (1H, t, J = 6.1, 2′-H), 5.94 (1H, dd, J = 3.8, 6.2, 3′-H), 6.36 (1H, d, J = 6.1, 1′-H), 7.30 (1H, s, H6), 7.37–7.67 (10H, m, ArH), 7.96–7.99 (3H, m, ArH), 8.14–8.18 (2H, m, ArH), 8.49 (1H, s, NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ −6.5, 18.2, 25.9, 30.3, 60.7, 64.0, 71.3, 73.6, 80.5, 88.0, 113.0, 128.5, 128.7, 128.7, 129.3, 129.7, 129.8, 129.9, 133.5, 133.7 133.7, 137.1, 150.5, 163.5, 165.3, 165.3, 166.1; Electrospray-MS: 715 (M + H)$^+$, Acc. Mass: 715.2712, $C_{38}H_{43}N_2O_{10}Si$ requires 715.2687 deviation 3.5 ppm.

5-[2-(*tert*-Butyldiphenylsilanyloxy)ethyl]-2′,3′,5′-tri-*O*-benzoyl-uridine 9

Compound **7** (11.2 g, 28.6 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (14.4 g, 28.6 mmol) were suspended in dry acetonitrile (420 mL) under argon. Hexamethyldisilazane (7.23 mL, 34.3 mmol), trimethylsilyl chloride (4.35 mL, 34.3 mmol) and finally $SnCl_4$ (4.05 mL, 34.3 mmol) in dry acetonitrile (40 mL) were added. The cloudy solution was then stirred overnight at r.t. before heating to an oil bath temperature of 70 °C over 1 h. Some material dissolved but a precipitate remained so the reaction was worked up by addition of DCM (350 mL) and saturated aq. $NaHCO_3$ solution (275 mL). The aqueous layer was further extracted with DCM (150 mL) and the combined organic extracts were washed with brine (150 mL), dried ($MgSO_4$) and evaporated. Following silica gel chromatography (eluent 0–4% MeOH in DCM) the protected nucleoside **9** was obtained as a white foam (14.4 g, 60% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.92 (9H, s, *t*-Bu), 2.08–2.18 (1H, m, C5-CH_2CH$_2$OSi), 2.30–2.40 (1H, m, C5-CH_2CH$_2$OSi), 3.66 (2H, t, J = 5.8, C5-CH$_2$CH_2OSi), 4.55–4.69 (3H, m, 4′-H and 5′-H), 5.69 (1H, t, J = 5.8, 2′-H), 5.80–5.84 (1H, m, 3′-H), 6.17 (1H, d, J = 5.8, 1′-H), 7.17–7.55 (20H, m, ArH and H6), 7.85–8.06 (7H, m, ArH and NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ 19.1, 26.9, 30.3, 61.5, 64.1, 71.4, 73.7, 80.5, 88.5, 112.8, 127.7, 128.6, 128.8, 129.4, 129.7, 129.9, 130.0, 130.1, 133.6, 133.7, 133.8 135.6, 137.2, 150.3, 163.1, 165.3, 166.1; Electrospray-MS: 839 (M + H)$^+$, Acc. Mass: 839.3031, $C_{48}H_{47}N_2O_{10}Si$ requires 839.3000 deviation 3.7 ppm.

5-[2-(*tert*-Butyldimethylsilanyloxy)ethyl]-uridine 10

Protected nucleoside **8** (29.7 g, 41.6 mmol) was treated with saturated ammonia in MeOH (400 mL) and stirred at r.t. for 30 h. The solvent was then evaporated to give an orange-coloured oil which was purified by silica gel chromatography (eluent 4–10% MeOH in DCM) which gave debenzoylated nucleoside **10** as a white foam (13.8 g, 82% yield). ^{1}H NMR (250 MHz, CD_3OD): δ 0.00 (6H, s, SiMe), 0.84 (9H, s, *t*-Bu), 2.47 (2H, t, J = 6.4, C5-CH_2CH$_2$OSi), 3.67–3.74 (3H, m, C5-CH$_2$CH_2OSi and 5′-H), 3.78–3.84 (1H, dd, J = 2.7, 11.9, 5′-H), 3.94–3.98 (1H, m, 4′-H), 4.10–4.17 (2H, m, 2′-H and 3′-H), 5.86 (1H, d, J = 4.3, 1′-H), 7.84 (1H, s, H6); ^{13}C NMR (250 MHz, CD_3OD): δ −5.0, 19.2, 26.6, 31.6, 62.3, 62.5, 71.4, 75.8, 86.4, 90.6, 112.4, 140.1, 152.6, 165.9; Electrospray-MS: 403 (M + H)$^+$, Acc. Mass: 403.1909, $C_{17}H_{31}N_2O_7Si$ requires 403.1901 deviation 2.2 ppm.

5-[2-(*tert*-Butyldiphenylsilanyloxy)ethyl]-uridine 11

Protected nucleoside **9** (12.7 g, 15.1 mmol) was treated with saturated ammonia in MeOH (130 mL) and stirred at r.t. for 23 h. Evaporation of the solvent and purification by silica gel chromatography (eluent 1:1 DCM/EtOAc to EtOAc) gave **11** as a white solid (5.82 g, 72% yield). ^{1}H NMR (250 MHz, CD_3OD): δ 1.10 (9H, s, *t*-Bu), 2.54 (2H, t, J = 6.0, C5-CH_2CH$_2$OSi), 3.68–3.75 (1H, dd, J = 3.1, 12.2, 5′-H), 3.80–3.85 (3H, m, C5-CH$_2$CH_2OSi and 5′-H), 3.98–4.00 (1H, m, 4′-H), 4.12–4.17 (2H, m, 2′-H and 3′-H), 5.87 (1H, d, J = 4.3, 1′-H), 7.29–7.63 (10H, m, ArH), 7.86 (1H, s, H6); ^{13}C NMR (250 MHz, CD_3OD): δ 20.1, 27.6, 31.7, 62.4, 63.0, 71.5, 75.8, 86.5, 90.8, 112.5, 128.9, 130.9, 134.8, 134.9, 136.7, 140.1, 152.6, 165.7;

Electrospray-MS: 527 (M + H)$^+$, Acc. Mass: 527.2209, $C_{27}H_{35}N_2O_7Si$ requires 527.2214 deviation 0.8 ppm.

5-[2-(*tert*-Butyldimethylsilanyloxy)ethyl)]-5′-*O*-(4,4′-dimethoxytrityl)-uridine 12

Nucleoside **10** (5.76 g, 14.3 mmol) was treated with DmtCl (5.81 g, 17.2 mmol) in dry pyridine (55 mL) under argon at r.t. After 18 h a further amount of DmtCl (0.97 g, 2.86 mmol) was added. After 1 h, water (150 mL) and DCM (150 mL) were added and the aqueous layer extracted further with DCM (2 × 150 mL). The combined organic layers were dried ($MgSO_4$) and evaporated and the residual solvent removed by co-evaporation with toluene (2 × 50 mL). Purification by silica gel chromatography (eluent 0–3% MeOH in DCM containing 0.2% TEA) gave **12** as a yellow foam (7.51 g, 74% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.00 (6H, s, SiMe), 0.74 (9H, s, *t*-Bu), 1.96–2.07 (1H, m, C5-CH_2CH$_2$OSi), 2.25–2.35 (1H, m, C5-CH_2CH$_2$OSi), 3.27–3.32 (1H, dd, J = 3.1, 10.7, 5′-H), 3.38–3.43 (1H, dd, J = 2.3, 10.5, 5′-H), 3.45–3.57 (2H, m, C5-CH$_2$-CH_2OSi), 3.71 (6H, s, ArOCH_3), 4.16–4.20 (2H, m, 3′-H and 4′-H), 4.27 (1H, t, J = 4.3, 2′-H), 5.76 (1H, d, J = 4.0, 1′-H), 6.76 (4H, d, J = 8.9, ArH), 7.11–7.34 (10H, m, ArH and H6), 7.53 (1H, s, NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ −5.4, 18.2, 25.9, 30.4, 55.2, 61.0, 62.8, 70.4, 75.3, 83.9, 86.7, 90.5, 112.0, 113.3, 127.0, 128.0, 130.1, 135.4, 135.5, 137.3, 144.5, 151.3, 158.6, 164.1; Electrospray-MS: 705 (M + H)$^+$, Acc. Mass: 705.3228, $C_{38}H_{49}N_2O_9Si$ requires 705.3207 deviation 2.9 ppm.

5-[(2-*O*-*tert*-Butyldiphenylsilanyloxy)ethyl]-5′-*O*-(4,4′-dimethoxytrityl)-uridine 13

Compound **11** (5.65 g, 10.7 mmol) was treated with DmtCl (4.36 g, 12.9 mmol) in dry pyridine (50 mL) under argon at r.t. Two further amounts of DmtCl (0.36 g, 1.08 mmol) were added after 21 and 28 h respectively. After 50 h the mixture was partitioned between water (100 mL) and DCM (100 mL) and the aqueous layer further extracted with DCM (2 × 50 mL) and the organic layers were combined and dried (Na_2SO_4) and evaporated. Residual solvent was removed by evaporation with toluene (2 × 50 mL) to yield a yellow foam/solid which was purified by silica gel chromatography twice (eluent 0–5% MeOH in DCM containing 0.2% TEA) to yield **13** as a cream-coloured solid (6.21 g, 70% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.96 (9H, s, *t*-Bu), 1.98–2.09 (1H, m, C5-CH_2-CH$_2$OSi), 2.29–2.40 (1H, m, C5-CH_2CH$_2$OSi), 3.27–3.41 (2H, m, 5′-H), 3.59–3.75 (8H, m, C5-CH$_2$CH_2OSi, ArOCH_3), 4.14–4.18 (3H, m, 2′-H, 3′-H and 4′-H), 5.69 (1H, d, J = 3.4, 1′-H), 6.72 (4H, d, J = 8.5, ArH), 7.10–7.50 (21H, m, ArH, H6 and NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ 19.2, 26.9, 30.3, 55.2, 61.9, 62.9, 70.3, 75.1, 84.0, 86.7, 90.8, 111.7, 113.3, 127.0, 127.6, 128.0, 128.1, 129.6, 129.6, 130.1, 133.6, 133.8, 135.5, 135.6, 137.3, 144.6, 151.2, 158.6, 163.8; Electrospray-MS: 829 (M + H)$^+$, Acc. Mass: 829.3500, $C_{48}H_{53}N_2O_9Si$ requires 829.3520 deviation 2.5 ppm.

5-(2-Hydroxyethyl)-5′-*O*-(4,4′-dimethoxytrityl)-uridine 14

From 5-[(2-*tert*-Butyldimethylsilanyloxy)ethyl]-5′-*O*-(4,4′-dimethoxytrityl)-uridine 12. Tetrabutylammonium fluoride (TBAF) (1 M solution in THF, 14 mL, 14 mmol) was added to nucleoside **12** (8.2 g, 11.6 mmol) under argon at r.t. Over a 42 h period, the reaction was stirred and further TBAF solution (11.7 mL, 11.7 mmol) was added in five portions. The reaction was worked up by addition of water (250 mL) and DCM (250 mL) and the aqueous layer was further extracted with DCM (2 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated and the residue purified by silica gel chromatography (eluent 5–7% MeOH in DCM containing 0.2% TEA) to give **14** as a cream-coloured foam (6.7 g, 98 % yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.90 (2H, bs, C5-CH_2CH$_2$OH), 3.25–3.43 (4H, m, C5-CH_2CH$_2$OH and 5′-H), 3.68 (6H, s, ArOCH_3), 4.07 (1H, bs, 4′-H), 4.29 (2H, bs, 2′-H and 3′-H), 5.86 (1H, d, J = 3.4, 1′-H), 6.74 (4H, d, J = 8.6, ArH), 7.09–7.31 (10H, m, ArH and H6), 7.53 (1H, s, NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ 30.2, 55.2, 61.0, 63.0, 70.6, 74.7, 83.8, 86.8, 89.0, 112.5, 113.3, 127.1, 128.0, 128.2, 130.2, 135.4, 135.5, 137.8, 144.4, 151.3, 158.6, 165.0; Electrospray-MS: 613 (M + Na)$^+$, Acc. Mass: 613.2164, $C_{32}H_{34}N_2O_9Na$ requires 613.2162 deviation 0.3 ppm.

From 5-[(2-*tert*-Butyldiphenylsilanyloxy)ethyl]-5′-*O*-(4,4′-dimethoxytrityl)-uridine 13. TBAF (1 M solution in THF, 10.7 mL, 10.7 mmol) was added to nucleoside **13** (5.9 g, 7.12 mmol) under argon at r.t. The solution was stirred over a 42 h period and a further 4.27 mL (4.27 mmol) TBAF were added in 1.42 mL (1.42 mmol) aliquots. The reaction was then worked up by addition of water (150 mL) and DCM (150 mL). The aqueous layer was further extracted with DCM (2 × 50 mL) and the organic layers were combined, dried (Na_2SO_4) and evaporated. Purifiaction by silica gel chromatography (eluent 4–6% MeOH in DCM containing 0.2% TEA) gave **13** as a cream-coloured foam (3.1 g, 75% yield) with identical spectral data to those reported above.

5′-*O*-(4,4′-Dimethoxytrityl)-uridine-5-acetaldehyde 15

***Via* modified Swern oxidation.** A two-necked 100 mL round bottom flask fitted with a 50 mL dropping funnel was charged with argon and cooled to −60 °C in a dry ice/chloroform bath. Dry DCM (6.5 mL) followed by oxalyl chloride (0.18 mL, 2.04 mmol) were added with stirring, followed by dry DMSO (0.29 mL, 4.04 mmol) in dry DCM (2.5 mL). After 2 min, nucleoside **14** (1.09 g, 1.86 mmol) in dry DCM (11 mL) containing dry TEA (0.32 mL, 2.27 mmol) was added *via* the dropping funnel over 5 min. After a further 15 min dry TEA (1.01 mL, 7.25 mmol) was added and 5 min later the reaction was partitioned between cold saturated aq. $NaHCO_3$ solution (50 mL) and cold DCM (20 mL). The aqueous layer was further extracted with cold DCM (2 × 20 mL) and the combined organic layers were co-evaporated with toluene (10 mL). Purification by silica gel chromatography (eluent 3% MeOH in DCM containing 0.1% TEA) gave a pale yellow foam that was partitioned between saturated aq. $NaHCO_3$ solution (50 mL) and DCM (20 mL). The aqueous layer was further extracted with DCM (2 × 20 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated to give **15** as a pale yellow foam (0.67 g, 61% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.48 (2H, bs, C5-CH_2CHO), 3.34 (1H, d, J = 8.9, 5′-H), 3.48 (1H, d, J = 8.9, 5′-H), 3.70 (6H, s, ArOCH_3), 4.09–4.11 (1H, m, 4′-H), 4.30–4.41 (2H, m, 2′-H and 3′-H), 5.90 (1H, d, J = 3.4, 1′-H), 6.74 (4H, d, J = 8.5, ArH), 7.14–7.29 (10H, m, ArH and H6), 7.82 (1H, s, NH), 9.21 (1H, s, C5-CH$_2$C*H*O); ^{13}C NMR (250 MHz, $CDCl_3$): δ 40.5, 55.3, 62.4, 70.2, 75.2, 83.8, 86.8, 89.7, 107.4, 113.4, 127.3, 128.1, 128.3, 130.2, 135.1, 138.9, 144.2, 151.0, 158.7, 163.7, 197.8; Electrospray-MS: 611 (M + Na)$^+$, Acc. Mass: 611.1990, $C_{32}H_{32}N_2O_9Na$ requires 611.2006 deviation 2.6 ppm.

***Via* Moffat oxidation.** Nucleoside **14** (0.25 g, 0.42 mmol) was treated with dry DMSO (7 mL), dry pyridine (38 µl, 0.47 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (0.35 g, 1.69 mmol) and finally trifluoroacetic acid (16 µl, 0.21 mmol). The solution was stirred at r.t. under argon for 18 h before partitioning between DCM (20 mL) and water (20 mL). The organic layer was washed with water (20 mL) then dried (Na_2SO_4) and evaporated. Purification by silica gel chromatography (eluent 1–10% MeOH in DCM containing 0.2% TEA) gave compound **15** as a yellow foam (0.1 g, 42% yield) with identical spectral data to those reported above.

5-[15-Trifluoroacetamido-3,7,12-*tris*-(*N*-trifluoroacetyl)-3,7,12-triazapentadecyl]-5′-*O*-(4,4′-dimethoxytrityl)-uridine 16

Compound **15** (0.67 g, 1.14 mmol) was treated with spermine (1.15 g, 5.7 mmol) in dry pyridine and MeOH (3.5 mL each) under argon at r.t. After stirring for 15 h, $NaBH_4$ (91 mg, 2.4 mmol) was added and after 1 h the reaction was evaporated. DMAP (6.9 mg, 0.06 mmol) in dry pyridine (14 mL) was then added and the solution cooled in an ice bath. Trifluoroacetic anhydride (3.7 mL, 26.2 mmol) was then added dropwise over 15 min and the reaction was allowed to warm to r.t. over 1 h. Saturated aq. $NaHCO_3$ solution (50 mL) was then carefully added with cooling and the product extracted into DCM (3 × 20 mL). The combined organic layers were then evaporated to give a red/brown oil which was purified by silica gel chromatography (eluent 2–50% MeOH in DCM containing 0.1% TEA) to give the polyamine conjugate **16** as a pale brown foam (0.75 g, 57% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.60–1.96 (9H, m, CH_2CH_2N), 2.15–2.28 (1H, m, CH_2CH_2N), 3.06–3.62 (16H, m, CH_2CH_2N and 5′-H), 3.77 (6H, s, ArOCH_3), 4.20 (1H, bs, 4′-H), 4.35–4.38 (2H, m, 2′-H and 3′-H), 5.89–5.92 (1H, m, 1′-H), 6.80–6.84 (4H, m, ArH), 7.20–7.40 (10H, m, ArH and H6), 7.55–7.84 (2H, m, NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ 23.4, 25.3, 26.0, 26.3, 29.3, 36.2, 36.6, 43.4, 44.1, 45.4, 45.6, 46.8, 54.8, 61.0, 70.0, 74.9, 83.7, 86.4, 89.9, 109.9, 110.5, 112.9, 114.2, 117.1, 120.2, 126.8, 127.6, 129.7, 134.8, 137.3, 143.9, 150.6, 156.4, 156.8, 157.1, 158.3, 163.4; ^{19}F NMR (250 MHz, $CDCl_3$): (^{1}H-decoupled) δ −76.5, −76.4, −76.4, −69.6, −69.5, −69.5, −69.4, −69.4, −69.3, −69.3, −69.3; (^{1}H-coupled) δ −76.5, −76.4, −76.4, −69.6, −69.5, −69.5, −69.4, −69.4, −69.4, −69.3, −69.3, −69.3, −69.3, −69.2; Electrospray-MS: 1181 (M + Na)$^+$, Acc. Mass: 1181.3536, $C_{50}H_{54}N_6O_{12}F_{12}Na$ requires 1181.3506 deviation 2.6 ppm.

5-[15-Trifluoroacetamido-3,7,12-*tris*-(*N*-trifluoroacetyl)-3,7,12-triazahexadecyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-*tert*-butyldimethylsilyl-uridine 17

Compound **16** (1.32 g, 1.14 mmol) and silver nitrate (0.58 g, 3.41 mmol) were dissolved in dry pyridine (6.5 mL) under argon at r.t. with stirring. Dry THF (19.5 mL) and then tBDMSCl (0.51 g, 3.41 mmol) were added and the reaction was stirred for 0.5 h. The reaction mixture was filtered and the filtrate was dissolved in 5% aq. $NaHCO_3$ solution (50 mL) and extracted with DCM (3 × 20 mL). The organic layers were combined and evaporated and the residue purified by silica gel chromatography (eluent 20% EtOAc in DCM/0.01% TEA to EtOAc/0.01% TEA) to afford **17** as a cream-coloured foam (1 g, 69% yield). ^{1}H NMR (250 MHz, $CDCl_3$): 0.09 (6H, s, SiCH_3), 0.85 (9H, s, *t*-Bu), 1.54–1.90 (9H, m, CH_2CH_2N), 2.05–2.2 (1H, m, CH_2CH_2N), 2.57–2.62 (1H, m, 3′-OH), 3.15–3.55 (16H, m, CH$_2$CH_2N), 3.72 (6H, s, ArOCH_3), 4.07–4.09 (1H, m, 4′-H), 4.15–4.31 (1H, m, 3′-H), 4.35–4.39 (1H, m, 2′-H), 5.84–5.92 (1H, m, 1′-H), 6.73–6.79 (4H, m, ArH), 7.08–7.43 (10H, m, ArH and H6), 7.56–7.71 (1H, m, NH), 8.97–9.18 (1H, m, NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ −5.2, 18.0, 23.8, 24.7, 25.7, 26.4, 26.7, 28.3, 36.5, 37.0, 43.6, 44.2, 45.0, 45.9, 47.1, 55.2, 62.7, 70.9, 75.9, 83.8, 86.9, 88.4, 110.4, 111.2, 114.9, 117.8, 120.8, 113.2, 127.2, 127.7, 128.1, 129.1, 130.1, 135.0, 137.4, 144.3, 150.1, 157.1, 157.3, 157.7, 158.7, 163.2; Electrospray-MS: 1295 (M + Na)$^+$, Acc. Mass: 1295.4377, $C_{56}H_{68}N_6O_{12}F_{12}$-NaSi requires 1295.4371 deviation 0.5 ppm.

5-[15-Trifluoroacetamido-3,7,12-*tris*-(*N*-trifluoroacetyl)-3,7,12-triazapentadecyl]-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-*tert*-butyldimethylsilyl-3′-*O*-(2-cyanoethyl diisopropylphosphoramidite) 18

To compound **17** (0.3 g, 0.24 mmol) in dry DCM (5 mL) were added dry DIPEA (0.25 mL, 1.42 mmol) and chloro(2-cyanoethyl)(diisopropylamino)phosphine (0.16 mL, 0.71 mmol) under argon at r.t. After stirring for 15 h the reaction was quenched by addition of polymer-bound benzyl alcohol (0.27 g, 0.54 mmol), filtered and the filtrate then partitioned between DCM (10 mL) and saturated aq. $NaHCO_3$ solution (10 mL). The aqueous layer was further extracted with DCM (2 × 10 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated to give a yellow oil. Purification by silica gel chromatography [eluent (dried over Na_2SO_4 prior to use) 40% EtOAc in DCM/1% TEA to EtOAc/1% TEA 1%] gave the phosphoramidite **18** as a white foam (0.3 g, 86% yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 0.07 (6H, m, SiMe), 0.83–0.88 (9H, m, *t*-Bu), 1.04–1.22 [12H, m, 2 × NCH(CH_3)$_2$], 1.55–2.20 (10H, m, CH_2CH$_2$N), 2.23–2.39 [1H, m, NCH(CH$_3$)$_2$], 2.56–2.61 [1H, m, NCH(CH$_3$)$_2$], 3.01–3.89 (20H, m, OCH$_2$CH$_2$CN, CH$_2$CH_2CN and 5′-H), 3.71 (6H, s, ArOCH_3), 4.18–4.42 (3H, m, 2′-H, 3′-H and 4′-H), 5.88–5.96 (1H, m, 1′-H), 6.73–6.78 (4H, m, ArH), 7.20–7.57 (11H, m, ArH, H6 and NH), 7.68–7.70 (1H, m, NH); ^{13}C NMR (250 MHz, $CDCl_3$): δ −5.2, 17.6, 20.0, 21.1, 22.5, 23.4, 26.1, 29.3, 24.2, 25.3, 36.1, 36.6, 42.4, 42.8, 43.0, 43.3, 44.8, 45.5, 46.7, 54.8, 57.5, 62.3, 72.0, 75.5, 82.7, 86.4, 108.5, 109.6, 112.9, 114.5, 117.4, 120.0, 122.0, 124.5, 126.9, 127.6, 129.8, 134.7, 143.8, 149.6, 137.1, 156.6, 157.3, 158.4, 162.6; ^{31}P NMR (250 MHz, $CDCl_3$): δ 149.9, 150.3, 150.4 and 150.5; Electrospray-MS: 1473 (M + H)$^+$, 1496 (M + Na)$^+$; 1471 (M − H)$^-$, Acc. Mass: 1473.5636, $C_{65}H_{86}N_8O_{13}F_{12}SiP$ requires 1473.5630 deviation 0.4 ppm.

5-(15-Amino-3,7,12-triazapentadecyl)-uridine 19

Nucleoside **4** (0.1 g, 0.12 mmol) was treated with methanolic ammonia (100 mL) at 55 °C for 20 h. The solvent was then removed *in vacuo* and the residue was partitioned between water (10 mL) and EtOAc (10 mL). The aqueous layer was further extracted with EtOAc (2 × 10 mL) before it was evaporated to dryness *in vacuo* to yield the deprotected polyamine conjugate **19** as a brown oil (40 mg, 72 % yield). This was used as an HPLC standard. ^{1}H NMR (250 MHz, CD_3OD): δ 1.80 (4H, m, CH_2CH$_2$N), 2.05–2.18 (4H, m, CH_2CH$_2$N), 2.69–2.75 (2H, t, J = 6.7, C5-CH_2CH$_2$N), 3.02–3.24 (14H, m, CH$_2$N), 3.73–3.79 (1H, dd, J = 2.9, 12.4, 5′-H), 3.85–3.91 (1H, dd, J = 2.6, 12.4, 5′-H), 4.00–4.02 (1H, m, 4′-H), 4.15–4.22 (2H, m, 2′-H and 3′-H), 5.89 (1H, d, J = 4.0, 1′-H), 8.04 (1H, s, H6); Electrospray-MS: 473 (M + H)$^+$, Acc. Mass: 473.3073, $C_{21}H_{41}N_6O_6$ requires 473.3088 deviation 3.1 ppm.

Oligoribonucleotide synthesis

RNA synthesis was carried out on an ABI-294 synthesiser on a 1 μmol scale using 2′-*O*-*tert*-butyldimethylsilyl-3′-*O*-(2-cyanoethyl diisopropylphosphoramidite) monomers and solid supports with phenoxyacetyl protection for adenosine and guanosine and acetyl protection for cytidine (Glen Research). 5′-FAM substrates were prepared using 5′-fluorescein reagent (Glen Research) (Fig. 1b). The syntheses were carried out using standard RNA synthesis procedures[46] except for the use of 5-benzylmercaptotetrazole (EMP Biotech) instead of tetrazole.[48] Deprotection of the oligoribonucleotides was carried out by treatment with $NH_{3\ (aq.)}$:EtOH (3:1) (1 mL) at 55 °C for 6 h and then TBAF (1 mL) for 20 h followed by desalting using Sephadex NAP10 columns.

The following oligonucleotides were prepared, where U* represents a polyamine-conjugated uridine synthesised using phosphoramidite **18**: 5′-FAM substrate, 14-mer, M = 4882.08, $E_{260\ nm}$ = 146.6 μM^{-1} cm^2 (5′-FAM-UCG CAG UCC UAU UU-3′); ribozyme strand A, 32-mer, M = 10 369.48, $E_{260\ nm}$ = 401.2 μM^{-1} cm^2 (5′-AAA UAG AGA AGC GAA CCA GAG AAA CAC ACG CC-3′); ribozyme strand B, 21-mer, M = 6729.09, $E_{260\ nm}$ = 238.2 μM^{-1} cm^2 (5′-GGC GUG GUA CAU UAC CUG GUA-3′); modified ribozyme strand B synthesised using phosphoramidite **18** in place of a single uridine residue, M = 6957.44, $E_{260\ nm}$ = 238.2 μM^{-1} cm^2, U*^{34} where U^{34} is replaced by the conjugate U*, U*^{37} where U^{37} is replaced by the conju-

gate, U^{*41} where U^{41} is replaced by the conjugate and U^{*42} where U^{42} is replaced by the conjugate.

r(U*G) Dimer

The dimer r(U*G) was prepared on an ABI-294 synthesiser as described above, employing polyamine monomer **18**. Following detritylation the functionalised solid support was heated in conc. aq. NH_3:EtOH (3:1) at 55 °C for 6 h. After removal of the support the solution was evaporated and the residue treated with TEA·3HF (1 mL) for 20 h. After quenching with water (0.2 mL) the sample was precipitated by addition of 1-butanol (15 mL) at −20 °C. The sample was then recovered by centrifugation for 10 min at 3500 rpm and the solvent decanted. The residue was dissolved in sterile water and the resulting material was purified by reverse-phase (RP) HPLC on a Jones C18 column (25 cm × 4.6 mm). Buffer A = 100 mM triethylammonium acetate solution, pH 7, buffer B (100 mM triethylammonium acetate solution, pH 7, 50% acetonitrile) with a gradient of 0–15% B in 20 min. The retention time of the dimer was 13.5 min. The sample was concentrated to 500 µl to give pure dimer (4.9 OD_{260} units). MALDI-TOF MS: 819 $(M + H)^+$. ^{31}P NMR (250 MHz, D_2O): δ −0.49.

Enzymatic digestion of r(U*G) dimer

0.25 OD_{260} units of r(U*G) dimer were incubated with 10 µl of RNase I (1000 units, New England Biolabs) in a solution of 10 mM Tris HCl (pH 8.0) and 100 mM NaCl at 37 °C. After 3 h the reaction solution was adjusted to 10 mM $MgCl_2$ and 1 µl alkaline phosphatase (0.1 units, Sigma) was added. Monitoring by HPLC, further aliquots of RNase I (total volume used was 18 µl) were added over 34 h until the dimer was completely digested.

The reaction was monitored by RP HPLC using the same gradient as employed for purification of the dimer. Retention times: 5-(15-amino-3,7,12-triazapentadecyl)-uridine (**19**) 3.8 min, r(U*G) dimer 13.5 min, rG 15.5 min.

Purification of oligoribonucleotides

Modified and unmodified ribozyme strands A and B were purified by denaturing preparative 20% PAGE, excised from the gel and extracted by soaking overnight in ammonium acetate buffer [1–2 mL, 0.5 M NH_4OAc, 1 mM EDTA (pH 6.5)] and then desalted using Sep-Pak columns.

The 5′-FAM substrate was purified by reverse-phase HPLC (RP HPLC): on a Jones C18 column (25 cm × 4.6 mm) using the following conditions: buffer A: 100 mM triethylammonium acetate solution, pH 6.5, 5% acetonitrile; buffer B: 100 mM triethylammonium acetate, solution pH 6.5, 65% acetonitrile. Gradient of 10–75% B in 25 min: retention time approx. 18 min. Following RP HPLC purification, the oligonucleotide was desalted by extensive dialysis against water.

All oligoribonucleotides were analysed by MALDI-TOF MS and molecular weights were found to be within 4 Da of the calculated value.

Determination of Michaelis–Menten parameters

With $MgCl_2$. A stock solution of the wild-type ribozyme strands A and B (50 nM each) in 40 mM Tris HCl (pH 7.5) and a separate stock solution of the 5′-FAM substrate (1 µM) were prepared. The ribozyme solution was denatured at 90 °C for 1 min and then placed in a water bath set at 37 °C for 10 min. The concentration of $MgCl_2$ in the ribozyme stock solution was adjusted to 10 mM and it was incubated for a further 15 min at 37 °C. During this 15 min the substrate stock solution was denatured at 90 °C for 1 min and then placed in the water bath set at 37 °C for 10 min. The appropriate quantity of ribozyme was added to the reaction mixtures containing $MgCl_2$ (final concentration 10 mM) and Tris HCl (pH 7.5) (final concentration 40 mM) before initiating the reaction with the suitable amount of substrate and brief vortexing. The final concentrations of substrate were 1–400 nM and for the ribozyme 0.1–20 nM. The reactions were monitored by the removal of an appropriately sized aliquot at suitable time intervals. The samples were immediately quenched by addition to 50 mM EDTA (pH 7.5) (5 × aliquot volume). The samples were analysed by denaturing HPLC (dHPLC) (DNA Sep™ Column, Transgenomic Inc.) using the following conditions: buffer A: 2.5 mM tetrabutylammonium bromide, 0.1% acetonitrile, 2 mM EDTA, pH 7.5; buffer B: 2.5 mM tetrabutylammonium bromide, 70% acetonitrile, 2 mM EDTA, pH 7.5. The retention time of the fluorescent product and substrate were approximately 5 and 7.5 min, respectively. The initial rates of reaction at the different substrate concentrations were determined and the kinetic parameters were calculated by non-linear regression fitting to the Michaelis–Menten equation [eqn. (1)] using KaleidaGraph (Synergy Software)

$$\frac{v}{[E]} = \frac{k_{cat}[S]}{K_M + [S]} \quad (1)$$

where v = initial rate of reaction, [E] = total concentration of ribozyme, [S] = concentration of substrate.

With spermine. The experiments were done in the same way as above with spermine (10 mM) replacing $MgCl_2$.

Rate of reaction of the polyamine-modified hairpin ribozymes

The four modified ribozymes were tested for cleavage activity with and without the presence of 10 mM $MgCl_2$ using 100 nM substrate and 20 nM ribozyme in 40 mM Tris HCl pH 7.5. The reactions were carried out in the same way as the Michaelis–Menten experiments above except for the experiments with no $MgCl_2$ where water was added to the ribozyme instead.

Acknowledgements

We are grateful to Dr Brian Taylor and Ms Sue Bradshaw for obtaining NMR spectra and we thank Ms Elaine Frary for her expert technical assistance. We are grateful to the BBSRC/EPSRC for an award of a BMS studentship to A. J. M. and to Dr T. Shibata for valuable discussions. J. A. G. is a BBSRC Advanced Research Fellow.

References

1 C. W. Tabor and H. Tabor, *Ann. Rev. Biochem.*, 1984, **53**, 749–790.
2 D. Esposito, P. DelVecchio and G. Barone, *J. Am. Chem. Soc.*, 1997, **119**, 2606–2613.
3 P. F. Cavanaugh, Z. P. Pavelic and C. W. Porter, *Cancer Res.*, 1984, **44**, 3856–3861.
4 U. Bachrach and G. Eilon, *Biochim. Biophys. Acta*, 1969, **179**, 494–496.
5 D. T. Hung, L. J. Marton, D. F. Deen and R. H. Shafer, *Science*, 1983, **221**, 368–370.
6 H. C. Ha, N. S. Sirisoma, P. Kuppusamy, J. L. Zweier, P. M. Woster and R. A. Casero, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 11 140–11 145.
7 T. Douki, Y. Bretonniere and J. Cadet, *Radiat. Res.*, 2000, **153**, 29–35.
8 A. K. Abraham and A. Pihl, *Trends Biochem. Sci.*, 1981, **6**, 106–107.
9 M. H. Hou, S. B. Lin, J. M. P. Yuann, W. C. Lin, A. H. J. Wang and L. S. Kan, *Nucleic Acids Res.*, 2001, **29**, 5121–5128.
10 P. M. Vertino, R. J. Bergeron, P. F. Cavanaugh and C. W. Porter, *Biopolymers*, 1987, **26**, 691–703.
11 B. G. Feuerstein, L. D. Williams, H. S. Basu and L. J. Marton, *J. Cell. Biochem.*, 1991, **46**, 37–47.
12 D. J. Earnshaw and M. J. Gait, *Nucleic Acids Res.*, 1998, **26**, 5551–5561.
13 R. M. Thomas, T. Thomas, M. Wada, L. H. Sigal, A. Shirahata and T. J. Thomas, *Biochemistry*, 1999, **38**, 13 328–13 337.

14 C. A. Stein, *Biochim. Biophys. Acta - Gene Struct. Expression*, 1999, **1489**, 45–52.
15 M. Saminathan, T. Antony, A. Shirahata, L. H. Sigal, T. Thomas and T. J. Thomas, *Biochemistry*, 1999, **38**, 3821–3830.
16 C. H. Tung, K. J. Breslauer and S. Stein, *Nucleic Acids Res.*, 1993, **21**, 5489–5494.
17 C. Sund, N. Puri and J. Chattopadhyaya, *Tetrahedron*, 1996, **52**, 12275–12290.
18 C. Sund, N. Puri and J. Chattopadhyaya, *Nucleosides Nucleotides*, 1997, **16**, 755–760.
19 T. Takeda, K. Ikeda, Y. Mizuno and T. Ueda, *Chem. Pharm. Bull.*, 1987, **35**, 3558–3567.
20 H. Nara, A. Ono and A. Matsuda, *Bioconjugate Chem.*, 1995, **6**, 54–61.
21 Y. Ueno, M. Mikawa and A. Matsuda, *Bioconjugate Chem.*, 1998, **9**, 33–39.
22 S. Kohgo, K. Shinozuka, H. Ozaki and H. Sawai, *Tetrahedron Lett.*, 1998, **39**, 4067–4070.
23 N. Haginoya, A. Ono, Y. Nomura, Y. Ueno and A. Matsuda, *Bioconjugate Chem.*, 1997, **8**, 271–280.
24 K. Shinozuka, M. Matsukura, T. Okamoto and H. Sawai, *Nucleosides Nucleotides*, 1998, **17**, 2081–2084.
25 K. Shinozuka, A. Umeda, T. Aoki and H. Sawai, *Nucleosides Nucleotides*, 1998, **17**, 291–300.
26 T. P. Prakash, D. A. Barawkar, V. Kumar and K. N. Ganesh, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1733–1738.
27 W. T. Markiewicz, P. Godzina, M. Markiewicz and A. Astriab, *Nucleosides Nucleotides*, 1998, **17**, 1871–1880.
28 P. Godzina and W. T. Markiewicz, in *Synthetic oligonucleotide combinatorial libraries 4. Synthesis of 5-polyaminomethyl-2'-deoxyuridines*, A. Holy and M. Hocek, ed., Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, 1999, pp. 79–82.
29 N. Schmid and J. P. Behr, *Tetrahedron Lett.*, 1995, **36**, 1447–1450.
30 A. R. Diaz, R. Eritja and R. G. Garcia, *Nucleosides Nucleotides*, 1997, **16**, 2035–2051.
31 W. T. Markiewicz, P. Godzina and M. Markiewicz, *Nucleosides Nucleotides*, 1999, **18**, 1449–1454.
32 P. Godzina, K. Adrych-Rozek and W. T. Markiewicz, *Nucleosides Nucleotides*, 1999, **18**, 2397–2414.
33 P. Potier, A. Adib, A. Kochkine, I. Huc and J. P. Behr, *Nucleosides Nucleotides*, 1999, **18**, 1467–1468.
34 P. Potier, A. Abdennaji and J. P. Behr, *Chem.-Eur. J.*, 2000, **6**, 4188–4194.
35 K. Shinozuka, M. Onodera, H. Ikeda and H. Sawai, *Chem. Lett.*, 2002, 200–201.
36 K. Oshima, H. Kawasaki, Y. Soda, K. Tani, S. Asano and K. Taira, *Cancer Res.*, 2003, **63**, 6809–6814.
37 M. Iyo, H. Kawasaki and K. Taira, *Curr. Opin. Mol. Ther.*, 2002, **4**, 154–165.
38 M. Cieslak, J. Niewiarowska, M. Nawrot, M. Koziolkiewicz, W. J. Stec and C. S. Cierniewski, *J. Biol. Chem.*, 2002, **277**, 6779–6787.
39 A. S. Lewin and W. W. Hauswirth, *Trends Mol. Med.*, 2001, **7**, 221–228.
40 T. Toyoda, Y. Imamura, H. Takaku, T. Kashiwagi, K. Hara, J. Iwahashi, Y. Ohtsu, N. Tsumura, H. Kato and N. Hamada, *FEBS Lett.*, 2000, **481**, 113–116.
41 H. Unwalla and A. C. Banerjea, *Biochem. J.*, 2001, **357**, 147–155.
42 J. Kurreck, B. Bieber, R. Jahnel and V. A. Erdmann, *J. Biol. Chem.*, 2002, **277**, 7099–7107.
43 T. Ito, Y. Ueno, Y. Komatsu and A. Matsuda, *Nucleic Acids Res.*, 2003, **31**, 2514–2523.
44 J. D. Fissekis and F. Sweet, *J. Org. Chem.*, 1973, **28**, 264–269.
45 H. Vorbruggen and B. Bennua, *Chem. Ber.-Recl.*, 1981, **114**, 1279–1286.
46 M. J. Gait, C. Pritchard and G. Slim, in *Oligoribonucleotide Synthesis*, F. Eckstein, ed., IRL Press, Oxford, 1991, pp. 25–48.
47 P. F. Spahr and B. R. Hollingworth, *J. Biol. Chem.*, 1961, **236**, 823–831.
48 R. Welz and S. Müller, *Tetrahedron Lett.*, 2002, **43**, 795–797.
49 K. J. Hampel, N. G. Walter and J. M. Burke, *Biochemistry*, 1998, **37**, 14672–14682.
50 D. V. Patel, M. R. Tock, E. Frary, M. Feng, T. J. Pickering, J. A. Grasby and J. R. Sayers, *J. Mol. Biol.*, 2002, **320**, 1025–1035.
51 C. Schmidt, R. Welz and S. Müller, *Nucleic Acids Res.*, 2000, **28**, 886–894.
52 K. Young, J. S. Vyle, T. J. Pickering, M. A. Cohen, S. C. Holmes, O. Merkel and J. A. Grasby, *J. Mol. Biol.*, 1999, **288**, 853–866.
53 J. A. Grasby, K. Mersmann, M. Singh and M. J. Gait, *Biochemistry*, 1995, **34**, 4068–4076.
54 S. Schmidt, L. Beigelman, A. Karpeisky, N. Usman, U. S. Sorensen and M. J. Gait, *Nucleic Acids Res.*, 1996, **24**, 573–581.
55 P. B. Rupert and A. R. Ferre-D'Amare, *Nature*, 2001, **410**, 780–786.