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Dinucleotides containing two allyl groups by combinations of allyl phosphotriesters, 5-allyl-, 2′-*O*-allyl- and 2′-*arabino*-*O*-allyl uridine derivatives as substrates for ring-closing metathesis

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Abstract—Five different dinucleotides, each containing two allyl groups in various positions, were prepared and studied as substrates for ring-closing metathesis reactions. These dinucleotides were designed from appropriate nucleoside building blocks combining four different positions for the allyl group; the allyl phosphotriester linkage, 5-allyl-2′-deoxyuridine, and *ribo*- as well as *arabino*-configured 2′-*O*-allyluridine. Thus, convenient procedures for these building blocks were developed. From the dinucleotides, two new cyclic nucleotide structures were obtained; one connecting two adjacent nucleobase moieties and the other forming an unsaturated four-carbon linkage between the phosphate moiety and the adjacent pyrimidine nucleobase. The latter cyclic dinucleotide was also prepared with a saturated four-carbon linkage using a tandem ring-closing metathesis–hydrogenation procedure. This compound was found to be significantly more stable towards a nucleophilic ring-opening than its unsaturated counterpart.

1. Introduction

The application of ring-closing olefin metathesis (RCM) for the preparation of medium and large rings has been a major tool in organic and bioorganic chemistry.[1] In particular, the introduction of highly efficient and functional group tolerant ruthenium-based catalysts by Grubbs and co-workers has made metathesis technology generally attractive.[2] A large number of heterocyclic rings have been constructed by RCM,[1,3] and recently, the application of RCM for the synthesis of phosphorus heterocycles has been specifically reviewed.[4] In a bioorganic context, cyclic peptide or peptide mimetic structures have been achieved by RCM reactions.[5] In view of this, we have focused on the application of RCM in nucleic acid chemistry,[6–10] and as a result, conformationally restricted bi- and tricyclic nucleoside monomers[6] as well as di- and trinucleotides with large cyclic structures have been achieved.[7–10] In addition, other research groups have recently prepared different nucleoside derivatives by RCM based strategies.[11]

In the studies of nucleic acid chemical biology, a significant number of conformationally restricted nucleic acid fragments have been designed.[12] As a relatively new approach, however, the relationship between structure and function of nucleic acid secondary and tertiary structures[13] has motivated the preparation of nucleic acids with covalent intra- and interstrand linkages.[14] Sekine and co-workers have introduced a number of cyclic nucleotide and dinucleotide structures for mimicking nucleic acid secondary structures.[15] In these cases, linkages have been established between the 2′-position and the nucleobase in the two adjacent nucleosides in a dinucleotide or between the nucleobase and the adjacent phosphate moiety.[15] Recently, another example of a conformationally restricted dinucleotide for this purpose with a linkage between the phosphate and the adjacent 5′-position has been introduced.[16] In all these cases, the covalent linkages have been obtained by conventional phosphoramidite chemistry,[17] disulfide bond formation and/or peptide/amide bond formation.[14–16] We have concentrated on the development of a general methodology based on RCM for the construction of this type of conformationally restricted nucleic acid fragments. Thus, we have synthesised a series of diastereomeric dinucleotides with phosphotriester internucleotide linkages containing seven-membered rings.[7] These were prepared by an RCM reaction on a dinucleotide substrate in which two terminal double bonds have been introduced by a 5′-*C*-vinyl moiety and an allyl phosphotriester linkage.[7] In a

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similar way, a ring connecting the phosphotriester linkage in a dinucleotide with a nucleobase has been demonstrated,[8,9] and two adjacent phorphortriester linkages have been linked in a cyclic trinucleotide structure.[8] Thus, a tandem RCM and hydrogenation procedure efficiently mediated the preparation of cyclic dinucleotides with a butylene nucleobase phosphotriester connection, which was unobtainable by a conventional hydrogenation method.[9] In this paper, some of these results are reported with all experimental details, and the general study on exploring the scope of ring-closing metathesis in the construction of cyclic dinucleotides with large rings is continued. Thus, four different positions of terminal double bonds were investigated, the appropriate nucleoside building blocks were prepared, and a selection of the possible dinucleotides were synthesised and investigated as substrates for RCM. Hereby, two cyclic dinucleotide structures were obtained.

2. Results and discussion

Four different positions for the allyl groups were deduced for incorporation into dinucleotides; the allyl phosphotriester linkage **A**, 5-allyl-2′-deoxyuridine **B**, 2′-*O*-allyluridine **C**, and 2′-*arabino*-*O*-allyluridine **D** (Fig. 1). Among these, **A** and a 5-allyluridine counterpart of **B** have been applied in our earlier studies.[7,8] From these four different allyl positions, a series of 12 different dinucleotides might be constructed; (5′–3′) **AB**, **AC**, **AD**, **BB**, **BC**, **BD**, **CB**, **CC**, **CD**, **DB**, **DC** and **DD**, as **A** cannot logically be placed in the 3′-end of the dinucleotides. Nevertheless, we decided to make only a section of five of these dinucleotides representing all of the four different allyl positions in at least two dinucleotides each. Thus, **AB**,[9] **AD**, **BB**, **CB** and **CD** were produced and investigated as substrates for RCM reactions.

In order to make dinucleotides with an allyl phosphotriester linkage **A**, the phosphoramidite **1** (Scheme 1) was formed in two steps from thymidine as shown in the literature.[7,18]

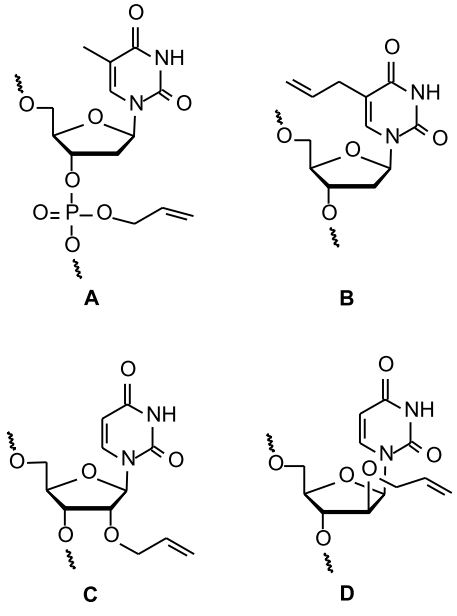
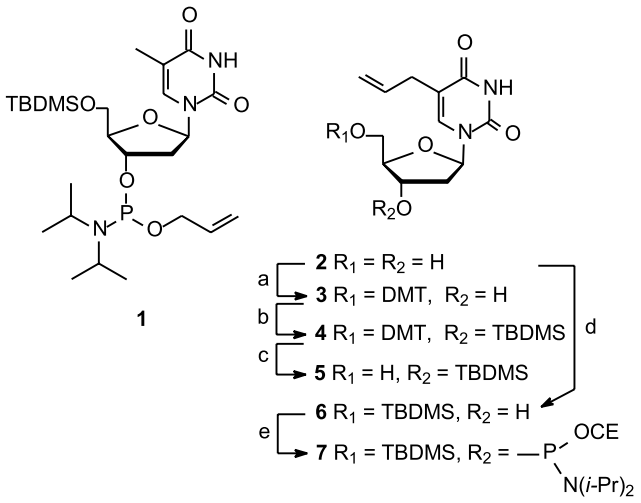


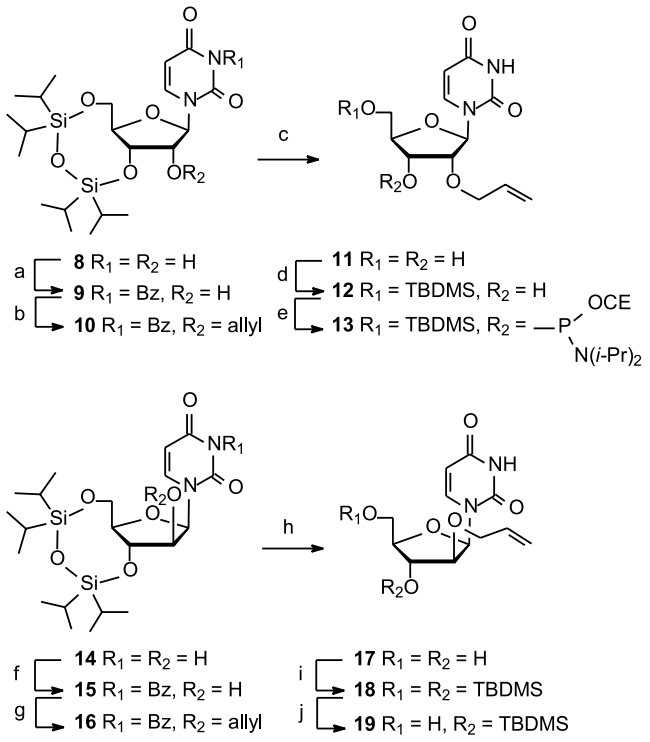
Figure 1. The four different positions for allyl groups in this study.



Scheme 1. Reagents and conditions: (a) DMTCl, $AgNO_3$, pyridine, 84%; (b) TBDMSCl, $AgNO_3$, pyridine, 86%; (c) *p*-TsOH, CH_2Cl_2, CH_3OH, 0 °C, 88%; (d) TBDMSCl, $AgNO_3$, pyridine, 42%; (e) CEOP(N(*i*-Pr)$_2$)$_2$, 4,5-dicyanoimidazole, CH_2Cl_2, CH_3CN, 75%. DMT=4,4′-dimethoxytrityl, TBDMS=*tert*-butyldimethylsilyl, CE=2-cyanoethyl.

Hereby, the appropriate building block for incorporation of **A** into dinucleotides by standard phosphoramidite chemistry[17] was formed. For the 5-allyluracil alternative **B**, 5-allyl-2′-deoxyuridine **2** was synthesised from 2′-deoxyuridine in two steps using a known procedure based on the formation of an organomercuri-intermediate followed by a transmetallation with Li_2PdCl_4 and allylation with allylchloride in methanol.[19] Subsequently, **2** was converted to the 3′-protected derivative **5** in three steps, that is, selective 5′-*O*-tritylation to give **3**, 3′-*O*-silylation to give **4**, both reactions accelerated by silver nitrate, and finally acidic detritylation to give the product **5** in 64% yield over the three steps (Scheme 1). Hereby, the building block for incorporating **B** in the 3′-end of the dinucleotides was formed.[9] Furthermore, **2** was selectively protected at the 5′-position as a silyl ether to give **6** and converted to the 3′-phosphoramidite **7** using a phosphordiamidite reagent and 4,5-dicyanoimidazole[20] as an activating reagent. This formed the building block for incorporating **B** in the 5′-end of the dinucleotides.

The formation of 2′-*O*-allyl-ribonucleosides, **C**, is well described in the literature.[21–24] Thus, Sproat et al. synthesised 2′-*O*-allyluridine[21] via a neutral palladium(0) catalysed allylation method[25] combined with a 5′,3′-TIPDS protection of the two alcohols and a 4-*O*-(2,6-dichlorophenyl) protection of the uracil moiety.[21] Later, it was demonstrated that the allylation can be performed with a simpler and cheaper 3-*N*-(4-*tert*-butylbenzoyl) group as protection of the uracil moiety.[22] Recently, also a 4-*O*-(2,6-dimethylphenyl) protection of the uracil has been successfully applied in the same reaction.[23] On the other hand, a conventional Williamson type allylation, that is, allyl bromide and sodium hydride, has been used with 5′-*O*-MMT-3′-*O*-TBDMS-uridine as substrate.[24] Thus, an allylation can be performed without a protecting group for the uracil, and we decided to attempt the same reaction with TIPDS-protected uridine **8** (Scheme 2). However, this led to only 20% of the 2′-*O*-allyl derivative due to a partial basic cleavage of the silyl ether moieties. Subsequently, we



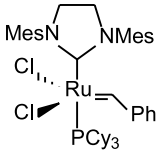
Scheme 2. Reagents and conditions: (a) BzCl, Bu_4NBr, CH_2Cl_2, aq Na_2CO_3, 77%; (b) allylethylcarbonate, tris(benzylidenacetone)dipalladium(0), bis(diphenylphosphino)butane, THF, 92%, reflux; (c) i. TBAF, THF, ii. aq NH_3, CH_3OH, 95%; (d) TBDMSCl, $AgNO_3$, pyridine, 79%; (e) CEOP(Cl)N(*i*-Pr)$_2$, EtN(*i*-Pr)$_2$, CH_2Cl_2, 65%; (f) i. TMSCl, Et_3N, CH_2Cl_2, ii. BzCl, EtN(*i*-Pr)$_2$, pyridine, iii. *p*-TsOH, CH_2Cl_2, 81%; (g) as (b), (h) as (c), 75%, two steps; (i) TBDMSCl, imidazole, DMF, 83%; (j) 80% aq AcOH, 73%. TBDMS=*tert*-butyldimethylsilyl, TMS=trimethylsilyl, CE=2-cyanoethyl.

decided to apply the neutral palladium(0) catalysed allylation method[25] with **8**. Thus, in this first attempt, nucleobase protection was avoided and, as expected, only the 3-*N*,2′-*O*-dialkylated product, or even the 3-*N*-monoalkylated product, was obtained. Therefore, we decided to follow the procedure by Sproat and co-workers[22] but with a conventional 3-*N*-benzoyl protection of the uracil instead of the 3-*N*-(4-*tert*-butylbenzoyl) group. Thus, the TIPDS-protected uridine **8** was treated with benzoyl chloride in a known phase transfer reaction with DCM/Na_2CO_3(aq) and tetrabutylammonium bromide as phase transfer catalyst to give **9** in 77% yield (Scheme 2).[26,27] The palladium(0) catalysed allylation procedure[25] applied with **9** afforded **10** in 92% yield. Subsequently, desilylation with TBAF in THF followed by debenzoylation with 25% ammonia in methanol gave 2′-*O*-allyluridine **11** in 95% yield, that is a conveniently 67% overall yield from **8**. Finally, **11** was reprotected as a silyl ether at the 5′-position to give **12** and converted to the 3′-phosphoramidite **13** by standard methods (Scheme 2). Hereby, the building block for incorporating **C** in the 5′-end of the dinucleotides was formed.

The formation of 2′-*O*-allyl-arabinouridine, **D**, has also been described in the literature.[28] However, only the conventional Williamson ether formation has been demonstrated using 5′,3′-*bis*-*O*-tetrahydropyranyl protected arabinouridine without protection of the uracil moiety to afford the 2′-*O*-allyl product in 40% yield.[28] Therefore, we decided to use the same procedure for allylation as applied with the *ribo*-configured uridine. Thus, the known TIPDS-protected *arabino*-configured uridine **14**[29] was treated with benzoyl chloride using the same phase transfer conditions as before but in this case only 34% yield of the 3-*N*-benzoylated product **15** was obtained due to a formation of a significant amount of the 3-*N*,2′-*O*-dibenzoylated product. Therefore, an in situ trimethylsilyl protection of the 2′-*O*-position was accomplished before a conventional *N*-benzoylation to give, after mild acidic desilylation, **15** in 81% yield over the three steps (Scheme 2). The palladium based allylation[22,25] afforded **16**, and after complete deprotection, the target 2′-*O*-allyl-arabinouridine **17** was obtained in 76% yield from **15**. Hereby, **17** has been obtained in approximately 53% overall yield from arabinouridine, that is, 45% from uridine counting the two step conversion of the 2′-configuration.[30] In comparison, the reported method,[28] albeit in fewer steps, gave **17** in only approximately 17% overall yield from uridine. Finally, **17** was reprotected as its *bis*-silyl ether **18** in 83% yield and selectively deprotected using mild acid to give **19** in 73% yield as the appropriate building block for incorporating **D** in the 3′-end of the dinucleotides.

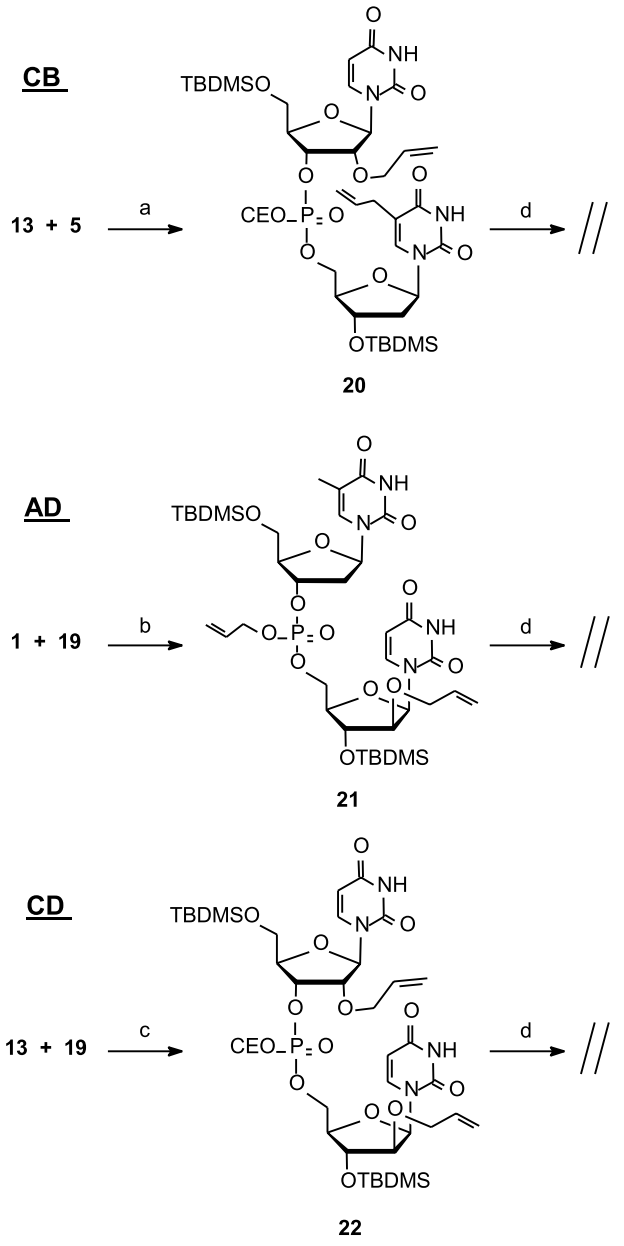
The five different protected dinucleotides **20**–**24** were made by the same general method using standard phosphoramidite chemistry[17] (Schemes 3–5). Thus, the appropriate 5′-alcohols and 3′-phosphoramidites were coupled with 1*H*-tetrazole as the activator followed by oxidation with *t*BuOOH. This oxidation reagent was efficient and easily handled, and the alternative standard reagent iodine was avoided due to the presence of double bonds. The general procedure afforded the dinucleotides in 60% to 100% yields. In all cases, the expected mixtures of two phosphorus epimers were obtained in approximately equimolar ratios as estimated from ^{1}H and ^{31}P NMR.

Subsequently, all the protected dinucleotides **20**–**24** were investigated as substrates for RCM reactions using 5–15 mol% of Grubbs second-generation catalyst **X** (Mes=2,4,6-trimethylphenyl, Cy=cyclohexyl),[2] in dichloromethane or 1,2-dichloroethane under reflux. We only investigated this catalyst, as this is by far the most generally successful according to the literature[1–4] as well as the most successfully applied in our lab for the synthesis of other dinucleotides with large rings.[7–10] We did not make a thorough investigation of a range of solvents, as dichloromethane and 1,2-dichloroethane have been the only successful solvents in our previous studies on nucleoside and nucleotide substrates.[6–10]



X

Of the five different dinucleotide substrates, however, neither **20**, **21** or **22** were good substrates for RCM reactions under these conditions (Scheme 3). In the case of **21** the substrate was converted to a highly polar material indicating polymerisation products, whereas both **20** and **22** could be re-isolated from the reaction mixtures. In the latter case,

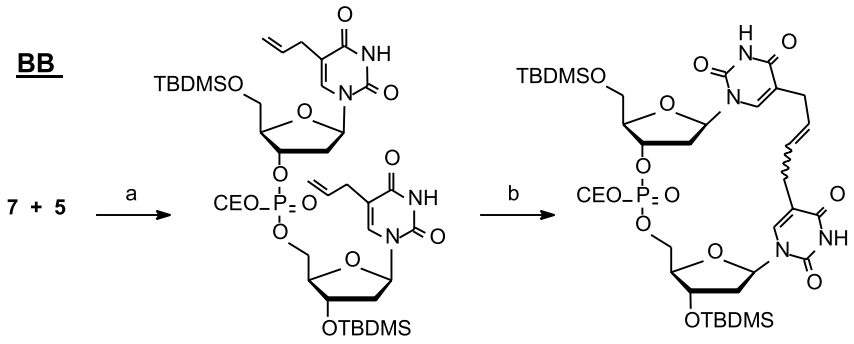


Scheme 3. Reagents and conditions: (a) i. 1*H*-tetrazole, CH_2Cl_2, CH_3CN, ii. *t*-BuOOH, CH_2Cl_2, CH_3CN, toluene, 100%; (b) 1*H*-tetrazole, CH_3CN, ii. *t*-BuOOH, CH_2Cl_2, toluene, 85%; (c) 1*H*-tetrazole, CH_2Cl_2, CH_3CN, ii. *t*-BuOOH, CH_2Cl_2, CH_3CN, toluene, 78%; (d) 5–15 mol% **X**, CH_2Cl_2/$ClCH_2CH_2Cl$/THF, reflux. TBDMS = *tert*-butyldimethylsilyl, CE = 2-cyanoethyl.

however, solubility problems forced us to investigate other solvents; toluene, ethyl acetate, acetonitrile/dichloromethane and THF, and with the latter, traces of a cyclised product was indicated from MS. On the other hand, the dinucleotide **23** was slowly converted to a ring-closed product **25** (Scheme 4). The best result was obtained with 5 mol% of the catalyst in dichloromethane at reflux from which **25** was isolated in 23% yield as a mixture of four stereoisomers in an approximately 1:1:4:6 ratio as deduced from the ^{31}P NMR spectrum. This indicates that both *E* and *Z*-configured products were obtained and also that the ratios are dependent on the configuration of the phosphorus and that the two isomers in the substrate reacted in different rates. Thus, 27% of the starting material was re-isolated with an approximately 1:10 ratio of phosphorus epimers. Nevertheless, the formation of a ring-closed product was also confirmed by MS showing the expected loss of ethylene, and by ^{1}H NMR demonstrating the conversion of terminal to internal double bonds.

Finally, the dinucleotide **24**[9] turned out to be the most efficient substrate for RCM. Thus, a treatment with 5 mol% of the catalyst in dichloromethane afforded **26** in 58% yield as a mixture of two phosphorus epimers in an equimolar ratio as deduced from ^{31}P NMR (Scheme 5). Again the product was also confirmed by MS and ^{1}H NMR showing the loss of ethylene and the conversion of terminal to internal double bonds. Thus, this RCM reaction was performed very smoothly, as it has also been demonstrated for its 3′-ribonucleosidic analogue.[8] Thus, **26** was the most easily obtained of the five projected cyclic dinucleotides, and subsequently, **26** has been further investigated for its stability towards ammonia.[9] Thus, ammonia is a standard reagent used in the final deprotection step in oligonucleotide synthesis, and as an allylic phosphotriester, **26** was expected to be labile towards nucleophiles. Thus, in an analytical experiment treating **26** with 32% aqueous ammonia for 24 h at room temperature, the allylic phosphotriester was found to react completely with ammonia at the allylic CH_2 group located next to the phosphate to give a zwitterionic ammonia adduct **27** as a dinucleotide with an achiral phosphordiester linkage in which also both TBDMS ethers were hydrolysed.[9] In order to increase the stability of the phosphotriester, we decided to prepare the saturated analogue of **26**. Thus, **24** was applied in a tandem RCM and hydrogenation reaction[31] by performing the RCM reaction as described earlier followed by hydrogenation of the reaction mixture in a Parr-bomb with 1000 psi H_2 at 50 °C. This gave the cyclic dinucleotide **28** in 63% yield as an equimolar mixture of two phosphorus epimers.[9] The protecting groups were subsequently removed by an acidic treatment to give the cyclic dinucleotide **29** in a quantitative yield. This dinucleotide was considerably more stable towards ammonia, as experiments showed that treatment with 32% NH_3(aq) at room temperature for 24 h resulted in only 10% conversion to the dinucleotide **30** as indicated by ^{31}P NMR and MS. A harsher treatment with 32% NH_3(aq) at 55 °C for 5 days resulted, however, in a complete conversion to the ammonia adduct **30**.

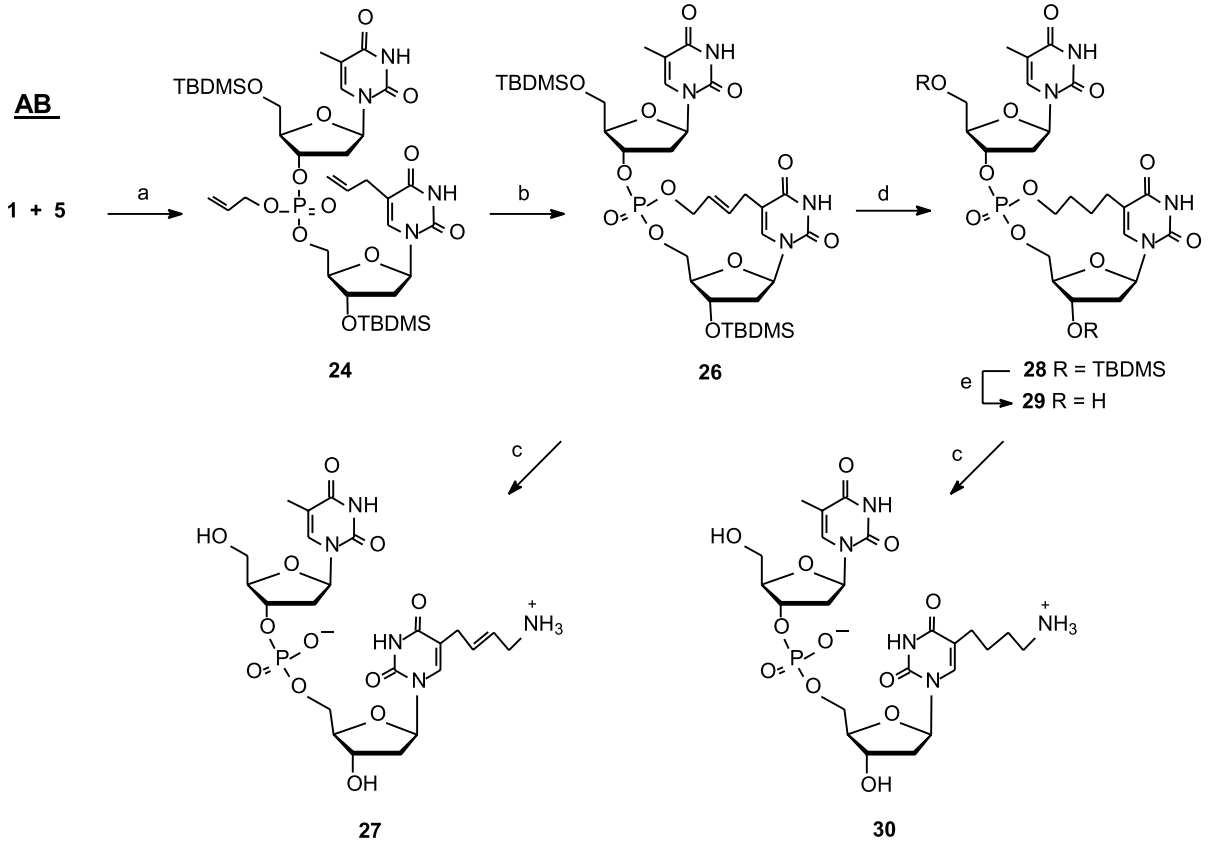
In summary, the present study has demonstrated that not all the five dinucleotide substrates were found to be substrates for RCM reactions. It could be argued, of course, that a longer range of catalysts and solvents should be included in the study. However, Grubbs second-generation catalyst **X** was in all cases tested by us[6–10,32] found to be superior to the first-generation catalyst ($(Cy_3P)_2RuCl_2CHPh$).[2] The recently commercialised Hoveyda–Grubbs second-generation catalyst[33] was not available, when most of the presented experiments were conducted. On the other hand, not even this catalyst has been found to be superior to **X** with dinucleotide substrates in our lab.[32] In the case of **21** Grubbs first-generation catalyst has been attempted, but only polymerisation was observed once again. Concerning solvents, we have attempted toluene in other studies[6–8] but this solvent has been incompatible with our nucleotide substrates. Furthermore, the major subject of this study has



Scheme 4. Reagents and conditions: (a) i. 1*H*-tetrazole, CH_3CN, ii. *t*-BuOOH, CH_3CN, toluene, 60%; (b) 5 mol% **X**, CH_2Cl_2, 23%, reflux. TBDMS=*tert*-butyldimethylsilyl, CE=2-cyanoethyl.

been to explore and compare different potential positions for introducing allyl groups into nucleotide building blocks and to compare different dinucleotides as RCM substrates. Thus, if a given substrate reacts very slowly with **X** or seems to prefer a polymerisation metathesis instead of a ring-closing reaction, no perspectives were seen in exploring a long range of reaction conditions. Therefore, we must conclude that only two of the five substrates, **23** and **24** could be transferred into the envisioned cyclic dinucleotides. Thus, the cyclic structures **25** and **26/29** can be used in the design of oligonucleotides that are conformationally restricted and potentially useful for targeting and mimicking nucleic acid secondary structures.

When comparing the four different building blocks **A**–**D** in Figure 1, all four have been very conveniently obtained and incorporated into dinucleotides. Thus, as an interesting spin-off from this study, very convenient procedures for preparing the two epimeric 2′-*O*-allyluridine **11** and 2′-*O*-allyl-arabinouridine **17** have been developed. Especially in the latter case, our procedure has surpassed the existing literature method. However, the efficient RCM reactions have been obtained only with **A** and **B**. Thus, taking in account our former studies,[7,8] the allyl group on the phosphotriester linkage is very reactive towards the catalyst and very well-positioned for performing ring-closing reactions. The 2′-*O*-*ribo* position of the allyl group, **C**, on



Scheme 5. Reagents and conditions: (a) 1*H*-tetrazole, CH_3CN, then *t*-BuOOH, toluene, 64%; (b) 5 mol% **X**, CH_2Cl_2, reflux, 58%; (c) 32% aq NH_3; (d) From **24**, 5 mol% **X**, CH_2Cl_2, reflux, then 1000 psi H_2, 50 °C, 63%; (e) 90% aq TFA, 100%. TBDMS=*tert*-butyldimethylsilyl.

the other hand, seems to be problematic. However, in other studies by us[32] and others,[11e] this position has been used to form cyclic nucleotide structures with large rings, and the reasons for the failure of **20** and **22** might be found in the particular conformational properties of these substrates. Similarly, the 2′-*O*-*arabino* position of the allyl group, **D**, has failed as demonstrated by the dinucleotides **21** and **22**. Nevertheless, reactivity between this allyl group and the catalyst is proven by the fact, that **21** is converted by polymerisation, probably by cross metathesis. Therefore, the reason for **21** being a bad substrate for RCM should be found in its conformational limitations making it impossible for the two double bonds (and their ruthenium [2+2]adducts) to approach and react in an intramolecular reaction. The 5-position of the pyrimidine, **B**, seems to be a good position for reaction with the catalyst **X**, and the possibility of making the very large ring structure in compound **25**, the largest of the projected rings in this study, might be partly driven by an ability to stack between the two pyrimidines. Furthermore, the planar geometry of these probably reduces any steric problems compared to the other positions for allyl groups. Nevertheless, also in this case, the reactivity is limited by geometry as illustrated by the fact that different reactivity was observed for the two phosphorus epimers. Thus, even though the selection of five dinucleotides that we choose for RCM studies led to the examination of each of the four allyl positions **A**–**D**, we cannot exclude the possibility that very efficient substrates and subsequent cyclic dinucleotides could be found with other combinations of **A**–**D**.

As evident from the present results, the butylene nucleobase to phosphotriester connection obtained in **29** has been efficiently obtained from the tandem RCM hydrogenation strategy. Further effort can now be put into separating the two phosphorus epimers of this compound and incorporating these into oligonucleotides. The stability of **29** towards ammonia supports these plans. Of the long range of possible cyclic dinucleotides investigated by this study, **29** is the most easily obtained and the obvious choice for further studies. Furthermore, the synthesis of other cyclic dinucleotides, constructed from allyl phosphotriester linkages and/or other positions for terminal alkene moieties[10,32] are in progress.

3. Conclusion

Five different dinucleotides combining four nucleotide building blocks with different allyl substituents have been explored as substrates for RCM reactions. Two cyclic dinucleotide structures were obtained and the one further elaborated to give a stable butylene connection between a nucleobase and a phosphate internucleotide linkage. Even though not all projected cyclisations could be performed in practice, more general knowledge within the scopes and limitation of the RCM based methodology towards conformationally restricted nucleic acid fragments has been obtained. Thus, we envision a large potential for constructing a plethora of cyclic dinucleotide structures mimicking a large range of nucleic acid secondary structures, and we expect the present RCM methodology to be a general future tool in nucleic acid chemical biology.

4. Experimentals

4.1. General

All commercial reagents were used as supplied. When necessary, reactions were performed under an atmosphere of nitrogen. Column chromatography was carried out on glass columns using silica gel 60 (0.040–0.063 mm). NMR spectra were recorded on a Varian Gemini 2000 spectrometer. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75.5 MHz, and ^{31}P NMR spectra at 121.5 MHz. Values for δ are in ppm relative to tetramethylsilane as internal standard or 85% H_3PO_4 as external standard. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode on a Kratos MS50TC spectrometer and MALDI mass spectra were recorded on an Ionspec Ultima Fourier Transform mass spectrometer. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen. Assignments of NMR spectra are based on 2D spectra and follow standard nucleoside style; that is, the carbon next to the nucleobase is assigned C-1′. For dinucleotides, the upper (5′) nucleotide is depicted U1 or T1 and the lower nucleoside U2 or T2.

4.1.1. Preparation of 5-allyl-2′-deoxy-5′-*O*-dimethoxytrityluridine (3). 5-Allyl-2′-deoxyuridine **2**[19] (0.700 g, 2.61 mmol) was coevaporated twice with anhydrous pyridine and redissolved in anhydrous pyridine (40 mL) in a darkened flask. $AgNO_3$ (0.557 g, 3.28 mmol) was added and the mixture was stirred for 5 min. DMTCl (1.099 g, 3.24 mmol) was added and the reaction mixture was stirred for 4 h, concentrated under reduced pressure and coevaporated with toluene. The residue was dissolved in ethyl acetate (50 mL), washed with a saturated aqueous solution of $NaHCO_3$ (50 mL) and brine (2×50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (1–4% CH_3OH and 1% pyridine in CH_2Cl_2) to give the product as a white foam (1.280 g, 86%) (Found C, 68.59; H, 6.07; N, 4.75%; $C_{33}H_{34}N_2O_7 \cdot 1/2H_2O$ requires C, 68.38; H, 6.08; N, 4.83%); mp=79–82 °C; R_f 0.23 (5% CH_3OH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 2.24–2.44 (2H, m, H-2′), 2.51–2.77 (2H, m, 5-CH_2), 3.33–3.43 (2H, m, H-5′), 3.78 (6H, s, OCH_3), 4.06 (1H, m, H-4′), 4.53 (1H, m, H-3′), 4.76–4.82 (2H, m, CH=CH_2), 5.60 (1H, m, CH=CH_2), 6.41 (1H, m, H-1′), 6.83 (4H, m, Ph), 7.16–7.41 (9H, m, Ph), 7.47 (1H, s, H-6), 9.33 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 30.5 (5-CH_2), 40.8 (C-2′), 55.2 (OCH_3), 63.5 (C-5′), 72.4 (C-3′), 84.8 (C-1′), 86.1 (Ph), 86.8 (C-4′), 113.23 (Ph), 113.71 (C-5), 116.65 (CH=CH_2), 125.3, 127.1, 127.9, 128.1, 128.2, 129.0, 130.1, 134.3, 135.4 (Ph), 135.4 (CH=CH_2), 136.1 (C-6), 144.3 (Ph), 150.4 (C-2), 158.6 (Ph), 163.1 (C-4); *m*/*z* FAB 570 (M).

4.1.2. Preparation of 5-allyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-5′-*O*-dimethoxytrityluridine (4). Compound **3** (0.490 g, 0.86 mmol) was dissolved in anhydrous pyridine (30 mL) in a darkened flask. $AgNO_3$ (0.165 g, 0.97 mmol) was added and the mixture was stirred for 5 min. TBDMSCl (0.145 g, 0.96 mmol) was added and the reaction mixture was stirred overnight, concentrated under reduced pressure and coevaporated with toluene. The residue was dissolved in ethyl acetate (50 mL), washed with a saturated aqueous

solution of $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (1–5% CH_3OH and 1% pyridine in CH_2Cl_2) to give the product as a white foam (0.495, 86%) (Found C, 66.96; H, 7.09; N, 3.84%; $C_{39}H_{48}N_2O_7Si\ H_2O$ requires C, 66.64; H, 7.16; N, 3.98%); mp = 60–64 °C; R_f 0.60 (5% CH_3OH in CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.02–0.16 (6H, m, $Si(CH_3)_2$), 0.81–0.97 (9H, m, $C(CH_3)_3$), 2.17–2.39 (2H, m, H-2′), 2.60–2.88 (2H, m, 5-CH_2), 3.28–3.48 (2H, m, H-5′), 3.82 (6H, s, OCH_3), 4.00 (1H, m, H-4′), 4.52 (1H, m, H-3′), 4.80–4.86 (2H, m, CH=CH_2), 5.67 (1H, m, CH=CH_2), 6.38 (1H, t, J = 6.7 Hz, H-1′), 6.82 (4H, m, Ph), 7.16–7.51 (9H, m, Ph), 7.55 (1H, s, H-6), 8.98 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −4.9, −4.7 ($Si(CH_3)_2$), 17.9 ($C(CH_3)_3$), 25.7 (C(CH_3)$_3$), 30.6 (C-2′) 41.3 (5-CH_2), 55.2 (OCH_3), 62.8 (C-5′), 72.1 (C-3′), 84.8 (C-1′), 86.7 (Ph), 87.4 (C-4′), 113.2 (Ph), 113.48 (C-5), 116.6 (CH=CH_2), 127.1, 127.7, 127.8, 127.9, 128.1, 128.2, 129.0, 129.1, 130.0, 134.4, 135.4 (Ph), 135.5 (CH=CH_2), 136.1 (C-6), 144.3 (Ph), 150.1 (C-2), 158.6 (Ph), 163.0 (C-4); m/z FAB 684 (M).

4.1.3. Preparation of 5-allyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridine (5). Compound **4** (0.450 g, 0.66 mmol) was dissolved in a mixture of CH_3OH and CH_2Cl_2 (2:3 v/v, 50 mL) and the solution was stirred at 0 °C. A solution of *p*-toluenesulfonic acid monohydrate (0.155 g, 0.82 mmol) in a mixture of CH_3OH and CH_2Cl_2 (2:3 v/v, 5 mL) was added over 5 min. The reaction mixture was stirred for 30 min and quenched by the addition of saturated aqueous ammonia (0.5 mL). The mixture was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL), washed with water (3×50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (1–3% CH_3OH in CH_2Cl_2) to give the product as a white foam (0.220 g, 88%); R_f 0.22 (5% CH_3OH in CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.01–0.13 (6H, m, $Si(CH_3)_2$), 0.83–0.96 (9H, m, $C(CH_3)_3$), 2.19–2.41 (2H, m, H-2′), 3.06–3.09 (2H, m, 5-CH_2), 3.72–3.76 (2H, m, H-5′), 3.92 (1H, m, H-4′), 4.50 (1H, m, H-3′), 5.11–5.18 (2H, m, CH=CH_2), 5.85 (1H, m, CH=CH_2), 6.15 (1H, t, J = 6.7 Hz, H-1′), 7.34 (1H, s, H-6), 9.01 (1H, s, NH); ^{13}C NMR ($CHCl_3$) δ −4.9, −4.7 $Si(CH_3)_2$), 18.0 ($C(CH_3)_3$), 25.7 (C(CH_3)$_3$), 30.5 (C-2′), 40.4 (5-CH_2), 62.0 (C-5′), 71.6 (C-3′), 87.2 (C-1′), 87.6 (C-4′), 113.5 (C-5), 117.6 (CH=CH_2), 134.2 (CH=CH_2), 137.6 (C-6), 150.2 (C-2), 163.1 (C-4); HiRes MALDI FT-MS m/z (M + Na) found/calcd 405.1828/405.1822.

4.1.4. Preparation of 5-allyl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridine (6). 5-Allyl-2′-deoxyuridine **2**[19] (0.979 g, 3.65 mmol) was coevaporated with anhydrous pyridine and redissolved in anhydrous pyridine (36 mL) in a darkened flask. $AgNO_3$ (0.503 g, 2.96 mmol) was added and the mixture was stirred for 5 min. TBDMSCl (0.235 g, 1.56 mmol) was added and the reaction mixture was stirred for 2 h. Another portion of TBDMSCl (0.339 g, 2.25 mmol) was added in 3 parts during 3 h, and the reaction was stirred for 5 h and quenched by the addition of CH_3OH (1 mL). The reaction mixture was concentrated under reduced pressure and coevaporated with toluene. The residue was dissolved in CH_2Cl_2 (80 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (40 mL) and brine (40 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by dry column chromatography (2–4% CH_3OH in CH_2Cl_2) to give the product as white foam (0.597 g, 42%) (Found C, 55.96; H, 8.02; N, 7.19%; $C_{18}H_{30}N_2O_5Si\cdot 1/4H_2O$ requires C, 55.86; H, 7.94; N, 7.23%); R_f 0.48 (10% CH_3OH in CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.07–0.10 (6H, m, $Si(CH_3)_2$), 0.91 (9H, s, $C(CH_3)_3$), 2.08 (1H, m, H-2′), 2.40 (1H, m, H-2″), 3.05–3.07 (2H, m, 5-CH_2), 3.81–3.83 (2H, m, H-5′), 4.04 (1H, m, H-4′), 4.40 (1H, m, H-3′), 5.06–5.12 (2H, m, CH=CH_2), 5.86 (1H, m, CH=CH_2), 6.34 (1H, m, H-1′), 7.42 (1H, s, H-6), 9.12 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.3, −5.1 ($Si(CH_3)_2$), 18.5 ($C(CH_3)_3$), 26.1 (C(CH_3)$_3$), 31.1 (C-2′), 41.1 (5-CH_2), 63.8 (C-5′), 72.7 (C-3′), 85.3 (C-1′), 87.2 (C-4′), 113.5 (C-5), 116.5 (CH=CH_2), 134.7 (CH=CH_2), 136.2 (C-6), 150.5 (C-2), 163.2 (C-4); HiRes MALDI FT-MS m/z (M + Na) found/calcd 405.1836/405.1816.

4.1.5. Preparation of 5-allyl-5′-*O*-(*tert*-butyldimethylsilyl)uridine-3′-*O*-(*N*,*N*-diisopropyl)-(2-cyanoethyl)phosphoramidite (7). Compound **6** (0.152 g, 0.40 mmol) was dissolved in anhydrous CH_2Cl_2 (2.5 mL) and a 0.5 M solution of 4,5-dicyanoimidazole (0.55 mL, 0.28 mmol) in CH_3CN was added. 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (0.126 g, 0.42 mmol) was added dropwise over 5 min and the reaction mixture was stirred at room temperature for 30 min. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (25 mL) and brine (25 mL). The combined aqueous phases were extracted with CH_2Cl_2 (30 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by dry column chromatography (50–60% ethyl acetate and 1% triethylamine in petrol ether) to give the product as a colourless oil and an epimeric mixture (0.173 g, 75%); R_f 0.36, 0.49 (75% ethyl acetate in petrol ether); ^1H NMR ($CDCl_3$) δ 0.10–0.11 (6H, m, $Si(CH_3)_2$), 0.88–0.98 (9H, m, $C(CH_3)_3$), 1.17–1.22 (12H, m, CH(CH_3)), 2.01–2.11 (1H, m, H-2′), 2.39–2.55 (1H, m, H-2″), 2.61–2.66 (2H, m, CH_2CN), 3.06–3.08 (2H, m, 5-CH_2), 3.56–3.91 (6H, m, H-5′, CH_2OP, 2×CH(CH_3)), 4.09–4.18 (1H, m, H-4′), 4.48–4.54 (1H, m, H-3′), 5.06–5.13 (2H, m, CH=CH_2), 5.80–5.91 (1H, m, CH=CH_2), 6.30–6.34 (1H, m, H-1′), 7.41, 7.45 (1H, 2 s, H-6), 8.27 (1H, br s, NH); ^{31}P NMR ($CDCl_3$) δ 149.45, 149.89; m/z FAB 583 (M + H).

4.1.6. Preparation of 2′-*O*-allyl-3-*N*-benzoyl-3′,5′-*O*,*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)uridine (10). Compound **9**[27] (0.496 g, 0.840 mmol) and allylethylcarbonate[34] (0.351 g, 2.70 mmol) was dissolved in anhydrous THF (4 mL). A solution of tris(dibenzylidenacetone)dipalladium(0) (0.012 g, 0.013 mmol, 1.5 mol%) and bis(diphenylphosphino)butane (0.038 g, 0.09 mmol, 10 mol%) in anhydrous THF (1 mL) was added. The reaction mixture was stirred at reflux for 45 min and then concentrated under reduced pressure. The residue was purified by column chromatography (4–50% ethyl acetate in petrol ether) to give the product as a white foam (0.487 g, 92%) (found: C, 59.19; H, 7.42; N, 4.43% $C_{31}H_{46}N_2O_8Si_2$ requires: C, 59.02; H, 7.35; N, 4.44%); R_f 0.71 (75% ethyl acetate in petrol ether); ^1H NMR ($CDCl_3$) δ 0.85–1.26 (28H, m, $SiCH(CH_3)_2$), 3.92 (1H, s, H-4′), 4.00 (1H, d, J = 14.0 Hz,

H-5′), 4.21–4.33 (5H, m, H-2′, H-3′, H-5″, 2′-OCH$_2$), 5.15 (1H, dd, J=1.5, 12.4 Hz, CH=CH_2), 5.31 (1H, dd, J=1.5, 17 Hz, CH=CH_2), 5.79 (1H, d, J=8.2 Hz, H-5), 5.76 (1H, s, H-1′), 5.87 (1H, m, CH=CH$_2$), 7.48–7.95 (m, 5H, Ph), 8.04 (1H, d, J=8.2 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 12.4, 12.8, 13.1, 13.4 (SiCH(CH$_3$)$_2$), 16.7, 16.8, 17.0, 17.1, 17.3, 17.3, 17.4, 17.5, 17.9 (SiCH(CH$_3$)$_2$), 59.4 (C-5′), 67.9 (C-3′), 71.2 (2′-OCH$_2$), 81.1 (C-4′), 82.0 (C-2′), 89.2 (C-1′), 101.45 (C-5), 117.4 (CH=CH$_2$), 129.2, 130.5, 131.3, 134.1 (Ph), 135.2 (CH=CH$_2$), 139.2 (C-6), 148.9 (C-2), 162.2 (C-4), 168.7 (C=O); m/z FAB 631 (M+H).

4.1.7. Preparation of 2′-*O*-allyluridine (11). Compound **10** (2.40 g, 3.80 mmol) was dissolved in THF (10 mL) and the solution was stirred at room temperature. A 1 M solution of TBAF in THF (8.6 mL, 8.6 mmol) was added over 5 min and the mixture was stirred for 30 min. A mixture of pyridine, CH_3OH and water (3:1:1 v/v, 10 mL) was added and the combined mixture was poured on 13 g amberlite IR-120® in pyridine, CH_3OH and water (3:1:1 v/v, 60 mL). The suspension was stirred for 30 min and filtered. The filtrate was concentrated under reduced pressure and the residue was dissolved in CH_3OH (50 mL) and added 25% aqueous ammonia (10 mL). The reaction mixture was stirred for 1 h 30 min, concentrated under reduced pressure and coevaporated with anhydrous ethanol. The residue was purified by dry column chromatography (0–10% CH_3OH in CH_2Cl_2) to give the product as a hygroscopic white foam (1.030 g, 95%); R_f 0.55 (10% CH_3OH in CH_2Cl_2); ^{1}H NMR (CD_3OD) δ 3.71–3.89 (2H, m, H-5′), 3.90–4.02 (2H, m, H-2′, H-4′), 4.20–4.24 (3H, m, H-3′, 2′-OCH$_2$), 5.13–5.34 (2H, m, CH=CH_2), 5.68 (1H, d, J=7.9 Hz, H-5), 5.87–5.98 (1H, m, CH=CH$_2$), 5.95 (1H, d, J=3.8 Hz, H-1′), 8.07 (1H, d, J=7.9 Hz, H-6); ^{13}C NMR (CD_3OD) δ 61.7 (C-5′), 70.0 (C-3′), 72.4 (2′-OCH$_2$), 82.5 (C-4′), 86.2 (C-2′), 89.2 (C-1′), 102.5 (C-5), 117.9 (CH=CH$_2$), 135.7 (CH=CH$_2$), 142.5 (C-6), 152.2 (C-2), 166.2 (C-4); HiRes MALDI FT-MS m/z (M+Na) found/calcd 307.0898/307.0901.

4.1.8. Preparation of 2′-*O*-allyl-5′-*O*-(*tert*-butyldimethylsilyl)uridine (12). Compound **11** (0.312 g, 1.10 mmol) was dissolved in anhydrous pyridine (9 mL). TBDMSCl (0.213 g, 1.41 mmol) and $AgNO_3$ (0.740 g, 4.36 mmol) were added, and the reaction mixture was stirred at room temperature for 24 h. The reaction was quenched by the addition of CH_3OH (1 mL) and the mixture was concentrated under reduced pressure and coevaporated with toluene. The residue was dissolved in ethyl acetate (75 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (50 mL) and brine (50 mL). The aqueous phase was extracted with ethyl acetate (50 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by dry column chromatography (0–6% CH_3OH in CH_2Cl_2) to give the product as a white foam (0.344 g, 79%) (Found C, 54.61; H, 7.70; N, 6.81%; $C_{18}H_{30}N_2O_6Si$ requires C, 54.25; H, 7.59; N, 7.03%); R_f 0.67 (10% CH_3OH in ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.12 (6H, s, Si(CH$_3$)$_2$), 0.93 (9H s, C(CH$_3$)$_3$), 2.64 (1H, d, J=7.9 Hz, 3′-OH), 3.84–3.92 (2H, m, H-5′), 4.01–4.07 (2H, m, 2′-OCH$_2$), 4.20–4.24 (2H, m, H-2′, H-4′), 4.40 (1H, m, H-3′), 5.23–5.33 (2H, m, CH=CH_2), 5.67 (1H, d, J=8.3 Hz, H-5), 5.89 (1H, m, CH=CH$_2$), 5.98 (1H, s, H-1′), 8.06 (1H, d, J=8.3 Hz, H-6), 9.07 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.6 (Si(CH$_3$)$_2$), 18.4 (C(CH$_3$)$_3$), 25.9 (C(CH$_3$)$_3$), 61.3 (C-5′), 67.9 (C-3′), 71.4 (2′-OCH$_2$), 81.5 (C-4′), 84.6 (C-2′), 87.3 (C-1′), 102.0 (C-5), 118.7 (CH=CH$_2$), 133.3 (CH=CH$_2$), 140.0 (C-6), 150.1 (C-2), 163.3 (C-4); HiRes MALDI FT-MS m/z (M+Na) found/calcd 421.1750/421.1765.

4.1.9. Preparation of 2′-*O*-allyl-5′-*O*-(*tert*-butyldimethylsilyl)uridine-3′-*O*-(*N*,*N*-diisopropyl)-(2-cyanoethyl)phosphoramidite (13). Compound **12** (0.123 g, 0.31 mmol) was dissolved in anhydrous CH_2Cl_2 (1.5 mL) and stirred at 0 °C. *N*,*N*-Diisopropylethylamine (0.30 mL, 0.227 g, 1.76 mmol) was added and chloro-*N*,*N*-diisopropylamino-(2-cyanoethyl)phosphine (0.20 g, 0.85 mmol) was added. The reaction mixture was stirred at 0 °C for 10 min and at room temperature for 5 h. The reaction mixture was cooled to 0 °C and another portion of chloro-*N*,*N*-diisopropylamino-(2-cyanoethyl)phosphine (0.20 g, 0.85 mmol) was added. The mixture was stirred at room temperature for 1.5 h and then quenched by the addition of water (1 mL). The mixture was diluted with ethyl acetate (10 mL) and washed with a 5% aqueous solution of $NaHCO_3$ (2× 25 mL) and brine (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (0.5% triethylamine and 50% ethyl acetate in petrol ether) to give the product as yellow foam and an epimeric mixture (0.120 g, 65%); R_f 0.36 (75% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.10–0.13 (6H, m, Si(CH$_3$)$_2$), 0.94–0.97 (9H, m, C(CH$_3$)$_3$), 1.11–1.23 (12H, m, CH(CH_3)$_2$), 2.59–2.66 (2H, m, CH$_2$CN), 3.59–3.70 (4H, m, CH$_2$OP, CH(CH$_3$)$_2$), 3.70–3.84 (2H, m, H-5′), 3.84–4.01 (2H, m, 2′-OCH$_2$), 4.17–4.32 (3H, m, H-2′, H-3′, H-4′), 5.16–5.33 (2H, m, CH=CH_2), 5.64–5.68 (1H, m, H-5), 5.85–6.92 (1H, m, CH=CH$_2$), 6.02–6.06 (1H, m, H-1′), 7.94–7.98 (2H, m, H-6); ^{31}P NMR ($CDCl_3$) δ 150.91, 150.97; m/z FAB 599 (M+H).

4.1.10. Preparation of 3-*N*-benzoyl-1-(3′,5′-*O*,*O*-(1,1,3,3-tetraisopropyldisiloxan-1,3-diyl)-β-D-arabinofuranosyl) uracil (15). Compound **14**[29] (5.33 g, 11.0 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL), stirred at 0 °C and added triethylamine (7.63 mL, 54.8 mmol). TMSCl (4.17 mL, 32.9 mmol) was added over 5 min and the reaction mixture was stirred at room temperature for 2 h. The mixture was cooled to 0 °C and added a 1.0 M aqueous solution of $NaHCO_3$ (100 mL). The organic phase was separated, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was redissolved in anhydrous pyridine (50 mL) and stirred at 0 °C. *N*,*N*-diisopropylethylamin (5.62 mL, 32.9 mmol) was added and the mixture was stirred for 10 min. Benzoyl chloride (3.81 mL, 32.9 mmol) was added and the reaction mixture was stirred at room temperature for 17 h. The reaction mixture was cooled to 0 °C and a 1.0 M aqueous solution of $NaHCO_3$ (100mL) was added. The organic phase was dried (Na_2SO_4), concentrated under reduced pressure and coevaporated with toluene (3× 10 mL). The residue was redissolved in CH_2Cl_2 (100 mL) and stirred at 0 °C. A solution of *p*-toluenesulfonic acid (4.72 g, 27.4 mmol) in THF (50 mL) was added and the reaction mixture was stirred for 10 min. Triethylamine (3.05 mL, 21.9 mmol) was added and the mixture was stirred for 15 min. A 1.0 M aqueous solution of $NaHCO_3$ (100mL) was added, and the organic phase was separated,

dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (5–40% ethyl acetate in petrol ether) to give the product as a white foam (5.21 g, 81%); mp = 50–53 °C; ^{1}H NMR ($CDCl_3$) δ 0.97–1.13 (28H, m, $CH(CH_3)_2$), 3.31 (1H, d, J = 4.6 Hz, 2′-OH), 3.73 (1H, m, H-4′), 4.05 (1H, dd, J = 2.5, 13.5 Hz, H-5′), 4.12 (1H, dd, J = 1.4, 13.5 Hz, H-5′), 4.16 (1H, t, J = 8.6 Hz, H-3′), 4.42 (1H, m, H-2′), 5.75 (1H, d, J = 8.2 Hz, H-5), 6.03 (1H, d, J = 6.2 Hz, H-1′), 7.80 (1H, d, J = 8.2 Hz, H-6), 7.93–7.42 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ 12.2, 12.8, 12.9, 13.3 ($CH(CH_3)_2$), 16.7, 16.8, 16.9, 17.1, 17.2, 17.3, 17.4 ($CH(CH_3)_2$), 60.0 (C-5′), 72.4 (C-3′), 75.4 (C-2′), 80.7 (C-4′), 101.3 (C-5), 128.3, 130.3, 131.3, 134.9 (Ph), 140.2 (C-6), 149.9 (C-2), 162.1 (C-4), 168.5 (C=O).

4.1.11. Preparation of 1-(2′-*O*-allyl-β-D-arabinofuranosyl)uracil (17). Compound **15** (5.21 g, 8.82 mmol) was dissolved in anhydrous THF (35 mL) and added allylethylcarbonate[34] (1.51 mL, 13.2 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.121 g, 0.132 mmol) and 1,4-bis(diphenylphosphino)butane (0.376 g, 0.88 mmol). The reaction mixture was stirred at reflux for 3 h and then concentrated under reduced pressure. The crude compound **16** was dissolved in THF (50 mL) and a 1.0 M solution of TBAF in THF (19.4 mL, 19.4 mmol) was added over 5 min. The reaction mixture was stirred for 15 min and then concentrated under reduced pressure. The residue was dissolved in CH_3OH (35 mL) and the solution was stirred at 0 °C. NH_3(g) was bubbled through the solution for 45 min. The mixture was concentrated under reduced pressure and the residue was dissolved in a mixture of pyridine, CH_3OH and water (3:1:1 v/v, 80 mL). The mixture was stirred with Dowex® (50 W×2) for 2 h and the resin was removed by filtration. The filtrate was concentrated under reduced pressure and coevaporated with toluene. The residue was purified by column chromatography (0–7% CH_3OH in CH_2Cl_2) to give the product as white solid (1.90 g, 76%); mp = 167–168 °C; ^{1}H NMR (CD_3OD) δ 3.34–3.83 (2H, m, H-5′), 3.87 (1H, m, H-4′), 3.97 (1H, m, 2′-OCH_2), 4.10 (1H, m, H-2′), 4.15 (1H, m, 2′-OCH_2), 4.20 (1H, t, J = 4.8 Hz, H-4′), 5.11–5.26 (2H, m, CH=CH_2), 5.69 (1H, d, J = 8.2 Hz, H-5), 5.97 (1H, m, CH=CH_2), 6.26 (1H, d, J = 5.2 Hz, H-1′), 7.85 (1H, d, J = 8.2 Hz, H-6); ^{13}C NMR (CD_3OD) δ 61.8 (C-5′), 72.6 (C-3′), 74.9 (2′-OCH_2), 84.6 (C-2′), 85.1 (C-4′), 85.7 (C-1′), 101.3 (C-5), 117.2 (CH=CH_2), 135.2 (CH=CH_2), 144.1 (C-6), 152.2 (C-2), 166.2 (C-4).

4.1.12. Preparation of 1-(2′-*O*-allyl-3′,5′-*O*,*O*-bis(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl)uracil (18). Compound **17** (0.200 g, 0.70 mmol) was dissolved in anhydrous DMF (7 mL) and added imidazole (0.480 g, 7.04 mmol) and TBDMSCl (0.530 g, 3.52 mmol). The reaction mixture was stirred for 24 h at room temperature, concentrated under reduced pressure and coevaporated with xylene (2.5 mL). The residue was dissolved in CH_2Cl_2 (20 mL), washed with water (2×20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (0–2% CH_3OH in CH_2Cl_2) to give the product as a colourless oil (0.298 g, 83%); ^{1}H NMR ($CDCl_3$) δ 0.09–0.13 (12H, m, $Si(CH_3)_2$), 0.87–0.93 (18H, m, $C(CH_3)_3$), 3.74–4.01 (6H, m, 2′-OCH_2, H-2′, H-4′, H-5′), 4.29 (1H, t, J = 5.2 Hz, H-3′), 5.11–5.20 (2H, m, CH=CH_2), 5.65–5.81 (2H, m, CH=CH_2, H-5), 6.26 (1H, J = 5.3 Hz, H-1′), 7.65 (1H, d, J = 8.4 Hz, H-6), 8.91 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, −4.9, −4.5 ($Si(CH_3)_2$), 17.9, 18.4 ($C(CH_3)_3$), 25.5, 25.6, 25.9 ($C(CH_3)_3$), 61.0 (C-5′), 72.3 (C-3′), 73.6 (2′-OCH_2), 83.2, 83.7, 83.9 (C-1′, C-2′, C-3′), 101.2 (C-5), 117.6 (CH=CH_2), 133.3 (CH=CH_2), 141.9 (C-6), 150.3 (C-2), 163.2 (C-4); *m/z* MALDI (M+Na) 535.

4.1.13. Preparation of 1-(2′-*O*-allyl-3′-*O*-(*tert*-butyldimethylsilyl)-β-D-arabinofuranosyl)uracil (19). Compound **18** (0.701 g, 1.37 mmol) was dissolved in 80% aqueous acetic acid (20 mL) and the reaction mixture was stirred at 60 °C for 2 h 30 min. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography (0–2% CH_3OH in CH_2Cl_2) to give the product as a white foam (0.399 g, 73%); mp = 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 0.11–0.16 (6H, m, $Si(CH_3)_2$), 0.87–0.94 (9H, m $C(CH_3)_3$), 2.23 (1H, m, 5′-OH), 3.78–3.98 (6H, m, 2′-OCH_2), 4.24 (1H, t, J = 3.8 Hz, H-3′), 5.15–5.21 (2H, m, CH=CH_2), 5.71 (1H, d, J = 8.2 Hz, H-5), 5.75 (1H, m, CH=CH_2), 6.27 (1H, d, J = 4.6 Hz, H-1′), 7.62 (1H, d, J = 8.2 Hz, H-6), 8.71 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ −4.9, −4.6 ($Si(CH_3)_2$), 17.9 ($C(CH_3)_3$), 25.6 ($C(CH_3)_3$), 61.4 (C-5′), 72.3 (C-3′), 75.1 (2′-OCH_2), 83.3, 84.3, 84.4 (C-1′, C-2′, C-4′), 101.2 (C-5), 118.3 (CH=CH_2), 132.9 (CH=CH_2), 142.0 (C-6), 150.2 (C-2), 163.0 (C-4); HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 421.1751/421.1765.

4.1.14. Preparation of (5-allyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxyuridin-5′-yl) (2′-*O*-allyl-5′-*O*-*tert*-butyldimethylsilyluridin-3′-yl) 2-cyanoethylphosphate (20). Compound **13** (0.103 g, 0.17 mmol) and compound **5** (0.073 g, 0.19 mmol) were coevaporated with anhydrous CH_2Cl_2 and dissolved in anhydrous CH_2Cl_2 (6 mL). A 0.45 M solution of 1*H*-tetrazole in CH_3CN (1.9 mL, 0.86 mmol) was added and the reaction mixture was stirred for 1 h. The reaction was quenched by the addition of CH_3OH (1 mL) and the mixture was concentrated under reduced pressure. The residue (crude phosphite, R_f 0.41 (75% ethyl acetate in petrolether)) was dissolved in a mixture of CH_2Cl_2 and CH_3CN (3:1 v/v, 8 mL) and cooled to 0 °C. A 3 M solution of *t*-BuOOH in toluene (0.3 mL, 0.9 mmol) was added and the reaction mixture was stirred for 1 h at 0 °C and for 2 h at room temperature. The reaction was quenched by the addition of CH_3OH (1 mL) and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (50% ethyl acetate in petrol ether and 10% CH_3OH in CH_2Cl_2) to give the product as a white foam (0.156 g, 100%); R_f 0.27 (75% ethyl acetate in petrol ether); ^{1}H NMR (CD_3CN) δ 0.10–0.12 (12H, m, $Si(CH_3)_2$), 0.81–0.92 (18H, m, $C(CH_3)_3$), 2.18–2.26 (2H, m, T2-H-2′), 2.78–2.82 (4H, m, 5-CH_2, CH_2CN), 3.83–4.43 (12H, m, U1-H-5′, U1-H-4′, U1-H-2′, U1-2′-OCH_2, T2-H-5′, T2-H-4′, T2-H-3′, CH_2OP), 4.87–4.92 (1H, m, U1-H-3′), 5.01–5.29 (4H, m, 2×CH=CH_2), 5.63–5.67 (1H, m, H-5), 5.78–5.94 (2H, m, 2×CH=CH_2), 5.99–6.02 (1H, m, U1-H-1′), 6.15–6.21 (1H, m, T2-H-1′), 7.31 (½H, s, T2-H-6), 7.38 (½H, s, T2-H-6), 7.72–7.75 (1H, m, H-6), 8.91–8.95 (2H, br s, 2×NH); ^{31}P NMR (CD_3CN) δ −1.74, −1.49; *m/z* ESI 918 (M+Na), 896 (M+H).

4.1.15. Preparation of allyl (2′-*O*-allyl-3′-*O*-(*tert*-butyldimethylsilyl)arabinouridin-5′-yl) (5′-*O*-*tert*-butyldimethylsilylthymidin-3′-yl) phosphate (21). Compound **19** (0.081 g, 0.20 mmol) was coevaporated with anhydrous CH_3CN and dissolved in anhydrous CH_3CN (3 mL). A 0.45 M solution of 1*H*-tetrazole in CH_3CN (0.68 mL, 0.30 mmol) was added and the mixture for stirred for 10 min. A solution of **1** (0.252 g, 0.46 mmol) in anhydrous CH_3CN (3 mL) was added and the reaction mixture was stirred for 2 h. The reaction was quenched by the addition of CH_3OH (0.5 mL) and the reaction mixture was concentrated under reduced pressure and coevaporated with CH_2Cl_2. The residue was dissolved in anhydrous CH_2Cl_2 (4 mL) and cooled to 0 °C. A 3 M solution of *t*-BuOOH in toluene (0.14 mL, 0.41 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of CH_3OH (1 mL) and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (0–2% CH_3OH in CH_2Cl_2) to give the product as a white foam and an epimeric mixture (0.148 g, 85%); mp = 70–71 °C; ^{1}H NMR ($CDCl_3$) δ 0.08–0.13 (12H, m, $Si(CH_3)_2$), 0.90–0.92 (18H, m, $C(CH_3)_3$), 1.90–1.94 (3H, br s, T1-CH_3), 2.00–2.06 (1H, m, T1-H-2′), 2.52–2.58 (1H, m, T1-H-2′), 3.90–4.28 (10H, m, U2-2′-OCH_2, U2-H-5′, U2-H-4′, U2-H-3′, U2-H-2′, T1-H-5′, T1-H-4′), 4.56-4.61 (2H, m, CH_2OP), 4.98-5.02 (1H, m, T1-H-3′), 5.14-5.21 (2H, m, U2-CH=CH_2), 5.29–5.43 (2H, m, T1-CH=CH_2), 5.68–5.80 (2H, m, U2-C*H*=CH_2, U2-H-5), 5.95–6.01 (1H, m, T1-C*H*=CH_2), 6.28–6.32 (1H, m, U2-H-1′), 6.33–6.39 (1H, m, T1-H-1′), 7.48–7.52 (1H, m, T1-H-6), 7.61–7.69 (1H, m, U2-H-6), 9.53–9.69 (2H, m, 2×NH); ^{31}P NMR ($CDCl_3$) δ −0.59; HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 879.3420/879.3404.

4.1.16. Preparation of (2′-*O*-allyl-3′-*O*-(*tert*-butyldimethylsilyl)arabinouridin-5′-yl) (2′-*O*-allyl-5′-*O*-*tert*-butyldimethylsilyluridin-3′-yl) 2-cyanoethylphosphate (22). Compound **13** (0.140 g, 0.23 mmol) and compound **19** (0.111 g, 0.28 mmol) were coevaporated twice with anhydrous CH_3CN and redissolved in anhydrous CH_3CN (7 mL). A 0.45 M solution of 1*H*-tetrazole in CH_3CN (2.6 mL, 1.17 mmol) was added and the reaction mixture was stirred for 2 h. The reaction was quenched by the addition of CH_3OH (0.5 mL) and the mixture was concentrated under reduced pressure. The residue was dissolved in a mixture of CH_2Cl_2 and CH_3CN (1:1 v/v, 16 mL) and stirred at 0 °C. A 3 M solution of *t*-BuOOH in toluene (0.38 mL, 1.14 mmol) was added and the reaction mixture was stirred at room temperature for 90 min. The reaction was quenched by the addition of CH_3OH (0.5 mL) and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (ethyl acetate) and precipitated twice from a mixture of ethyl acetate and petrol ether to give the product as a white powder and an epimeric mixture (0.181 g, 78%); R_f 0.27 (75% ethyl acetate in petrol ether); ^{1}H NMR (DMSO-d_6) δ 0.08–0.12 (12H, m, $Si(CH_3)_2$), 0.86–0.90 (18H, m, $C(CH_3)_3$), 2.92–2.96 (2H, m, CH_2CN), 3.77–4.28 (15H, m, U1-H-5′, U1-H-4′, U1-H-2′, U2-H-5′, U2-H-4′, U2-H-3′, U2-H-2′, CH_2OP, U1-2′-OCH_2, U2-2′-OCH_2), 4.82–4.86 (1H, m, U1-H-3′), 5.09–5.25 (4H, m, 2×C*H*=CH_2), 5.59–5.68 (2H, m, 2×H-5), 5.70–5.90 (2H, m, 2×C*H*=CH_2), 5.92–5.97 (1H, m, U1-H-1′), 6.21–6.23 (1H, m, U2-H-1′), 7.56–7.72 (2H, m, 2×H-6), 11.41–11.47 (2H, m, 2×NH); ^{31}P NMR (DMSO-d_6) δ −1.12; HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 934.3442/934.3462.

4.1.17. Preparation of (5-allyl-2′-deoxy-3′-*O*-*tert*-butyldimethylsilyluridin-5′-yl) (5-allyl-2′-deoxy-5′-*O*-*tert*-butyldimethylsilyluridin-3′-yl) 2-cyanoethylphosphate (23). Compound **7** (0.156 g, 0.268 mmol) and compound **5** (0.111 g, 0.290 mmol) were coevaporated twice with anhydrous CH_3CN and dissolved in anhydrous CH_3CN (3 mL). A 0.45 M solution of 1*H*-tetrazole in CH_3CN (3.0 mL, 1.35 mmol) was added and the reaction mixture was stirred for 30 min. The reaction was quenched by the addition of CH_3OH (0.6 mL) and the mixture was concentrated under reduced pressure. The residue (crude phosphite, R_f 0.23 (75% ethyl acetate in petrol ether)) was dissolved in anhydrous CH_3CN (3 mL) and a 3 M solution of *t*-BuOOH in toluene (0.45 mL, 1.35 mmol) was added. The reaction mixture was stirred for 1.5 h and added another portion of the 3 M solution of *t*-BuOOH in toluene (0.23 mL, 0.69 mmol). The reaction mixture was stirred for 30 min and the reaction was quenched by the addition of CH_3OH (0.5 mL). The mixture was concentrated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (50 mL), washed with a saturated aqueous solution of $NaHCO_3$ (25 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by dry column chromatography (50–0% petrol ether in ethyl acetate) to give the product as a white foam and an epimeric mixture (0.142 g, 60%); (Found C, 53.01; H, 7.32; N, 7.42%; $C_{39}H_{62}N_5O_{12}PSi_2$ requires C, 53.23; H, 7.10; N, 7.96%); R_f 0.36 (75% ethyl acetate in petrolether); ^{1}H NMR ($CDCl_3$) δ 0.10–0.11 (12H, m, $Si(CH_3)_2$), 0.89–0.91 (18H, m, $C(CH_3)_3$), 2.08–2.68 (4H, m, T1-H-2′, T2-H-2′), 2.75–2.80 (2H, m, CH_2CN), 3.00–3.08 (4H, T1-5-CH_2, T2-5-CH_2), 3.80–4.03 (4H, m, T2-H-5′, T2-H-4′, T1-H-4′), 4.20–4.30 (5H, m, T1-H-5′, T2-H-3′, CH_2OP), 4.41–4.45 (1H, m, T1-H-3′), 5.04–5.17 (4H, m, 2×CH=CH_2), 5.85–5.89 (2H, m, 2×C*H*=CH_2), 6.10–6.17 (1H, m, T1-H-1′), 6.27–6.32 (1H, m, T2-H-1′), 7.20–7.22 (1H, m, H-6), 7.39–7.41 (1H, m, H-6), 9.00–9.13 (2H, m, 2×NH); ^{31}P NMR ($CDCl_3$) δ −1.58, −1.44; HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 902.3581/902.3563.

4.1.18. Preparation of allyl (5-allyl-2′-deoxy-3′-*O*-*tert*-butyldimethylsilyluridin-5′-yl) (5′-*O*-*tert*-butyldimethylsilylthymidin-3′-yl) phosphate (24). Compound **5** (0.188 g, 0.49 mmol) and compound **1** (0.520 g, 0.96 mmol) were coevaporated twice with anhydrous CH_3CN (10 mL) and dissolved in anhydrous CH_3CN (25 mL). A 0.45 M solution of 1*H*-tetrazole in CH_3CN (5.3 mL, 2.38 mmol) was added over 5 min and the reaction mixture was stirred for 1.5 h at room temperature. A 3 M solution of *t*-BuOOH in toluene (0.82 mL, 2.46 mmol) was added, and the mixture was stirred for another 1.5 h. The reaction was quenched by the addition of CH_3OH (0.25 mL), and the mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2×20 mL) and brine (20 mL). The aqueous phase was extracted with ethyl acetate (30 mL) and the combined organic phases were dried (Na_2SO_4) and concentrated under

reduced pressure. The residue was purified by column chromatography (20–70% ethyl acetate in petrol ether) to give the product as a white foam and an epimeric mixture (0.266 g, 64%); mp = 74–76 °C; R_f 0.34 (75% ethyl acetate in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.01–0.12 (12H, m, $Si(CH_3)_2$), 0.83–0.92 (18H, m, $C(CH_3)_3$), 1.90–1.94 (3H, m, CH_3), 2.08–2.19 (1H, m, T1-H-2′), 2.21–2.29 (2H, m, T2-H-2′), 2.50–2.58 (1H, m, T1-H-2″), 3.08–3.11 (2H, m, T2-5-CH_2), 3.85–3.92 (T1-H-5′), 4.01–4.03 (1H, m, T2-H-4′), 4.20–4.29 (3H, m, T2-H-5′, T1-H-4′), 4.41–4.44 (1H, m, T2-H-3′), 4.55–4.60 (2H, m, CH_2OP), 5.00–5.04 (1H, T1-H-3′), 5.09–5.17 (2H, m, T2-CH=C*H*$_2$), 5.28–5.41 (2H, m, T1–CH=C*H*$_2$), 5.84–6.00 (2H, m, 2×C*H*=CH_2), 6.17–6.25 (1H, m, T2-H-1′), 6.32–6.37 (1H, m, T1-H-1′), 7.24–7.28 (1H, m, T2-H-6), 7.46–7.48 (1H, m, T1-H-6), 8.76–8.87 (2H, m, 2×NH); ^{31}P NMR ($CDCl_3$) δ −0.61; HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 863.3454/863.3454.

4.1.19. Preparation of cyclic 5′-*O*,3′-*O*-*bis*-TBDMS protected dUpU containing a 5-to-5 (*E/Z*)-2-butenelinker (25). Compound **23** (26 mg, 0.030 mmol) was dissolved in anhydrous CH_2Cl_2 (3 mL). Grubbs second-generation catalyst $\mathbf{X}^2$ (1.4 mg, 1.6 μmol) was added and the reaction mixture was stirred at reflux for 22 h. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (2–8% CH_3OH in CH_2Cl_2) to give the product as a white powder and a mixture of diastereomers (6 mg, 23%) as well as starting material **23** (7 mg, 27%); R_f 0.28 (10% CH_3OH in CH_2Cl_2); ^{31}P NMR ($CDCl_3$) δ −2.01 (1), −0.90 (0.13), −0.56 (0.61), 0.02 (0.17); HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 874.3199/874.3250.

4.1.20. Preparation of cyclic 5′-*O*,3′-*O*-bis-TBDMS protected dTpU containing a phosphate-to-5 (*E/Z*)-2-butenelinker (26). Compound **24** (0.052 g, 0.062 mmol) was dissolved in anhydrous CH_2Cl_2 (6 mL). Grubbs second-generation catalyst $\mathbf{X}^2$ (2.8 mg, 3.2 μmol, 5.2 mol%) was added and the reaction mixture was stirred at reflux for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography (10–0% petrol ether in ethyl acetate) to give the product as a white foam and an epimeric mixture (29 mg, 58%); R_f 0.29 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.10–0.13 (12H, m, $Si(CH_3)_2$), 0.89–0.94 (18H, m, $C(CH_3)_3$), 1.90–1.94 (3H, m, T1-CH_3), 2.06–2.30 (3H, m, T2-H-2′, T1-H-2′), 2.50–2.60 (1H, m, T1-H-2″), 3.10–3.14 (2H, m, T2-5-CH_2), 3.80–3.96 (2H, m, T1-H-5′), 3.98–4.49 (5H, m, T1-H-4′, T2-H-5′, T2-H-4′, T2-H-3′), 4.51–4.90 (2H, m, CH_2OP), 5.01–5.05 (1H, m, T1-H-3′), 5.84–5.89 (1H, m, CH=C*H*CH_2OP), 5.91–5.98 (1H, m, C*H*=$CHCH_2OP$), 6.36–6.42 (2H, m, T1-H-1′, T2-H-1′), 7.37–7.51 (2H, m, T1-H-6, T2-H-6), 9.02–9.20 (2H, m, NH); ^{31}P NMR ($CDCl_3$) δ −0.65, 2.01. HiRes MALDI FT−MS *m/z* (M+Na) found/calcd 835.3148/835.3141; IR (KBr) ν cm^{-1}: 3441, 3065, 2954, 2930, 2857, 1694, 1471, 1276, 1100, 1006, 987, 837, 780.

4.1.21. Analytical formation of Tp-5-(4-amino-2-butenyl)-2′-deoxyuridine (27). Compound **26** (2 mg) was dissolved in 32% aqueous NH_3 (1 mL) for 24 h at room temperature. The reaction mixture was concentrated under reduced pressure to give the product as a white foam; ^{1}H NMR (DMSO-d_6) δ 1.78 (3H, s, CH_3), 1.91–2.18 (3H, m, T1-H-2′, T2-H-2′), 2.24–2.33 (1H, m, T1-H-2″), 2.92–3.09 (2H, m, T2-5-CH_2), 3.40–3.43 (2H, m, CH_2N), 3.51–3.59 (2H, m, T1-H-5′), 3.75–4.31 (5H, m, T2-H-5′, T1-H-4′, T2-H-4′, T2-H-3′), 4.60–4.70 (1H, m, T1-H-3′), 5.20–5.32 (2H, m, 2×OH), 5.69–5.78 (1H, m, CH=C*H*CH_2N), 5.89–5.97 (1H, m, C*H*=$CHCH_2N$), 6.14–6.18 (1H, m, T1-H-1′), 6.25 (1H, t, J=7.4 Hz, T2-H-1′), 7.68 (1H, s, T2-H-6), 7.70 (1H, s, T1-H-6); ^{31}P NMR (DMSO-d_6) δ −0.86; MALDI-MS *m/z*: 601.27 (MH^+), 623.19 (MNa^+).

4.1.22. Preparation of cyclic 5′-*O*,3′-*O*-bis-TBDMS protected dTpU containing a phosphate-to-5 butanelinker (28). Compound **24** (0.255 g, 0.303 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL) and Grubbs second-generation catalyst $\mathbf{X}^2$ (14.0 mg, 16 μmol, 5.4 mol%) was added. The reaction mixture was stirred at reflux for 3.5 h and then bubbled with hydrogen for 5 min. The mixture was placed in an autoclave at 1000 psi hydrogen at 50 °C overnight. The mixture was concentrated under reduced pressure and the residue was purified by column chromatography (20–0% petrol ether in ethyl acetate) to give the product as a white foam and an epimeric mixture (0.156 g, 63.0%); R_f 0.29 (1% CH_3OH in ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.10–0.13 (12H, m, $Si(CH_3)_2$), 0.89–0.93 (18H, m, $C(CH_3)_3$), 1.55–1.85 (4H, m, $CH_2CH_2CH_2CH_2OP$), 1.92–1.94 (3H, m, CH_3), 2.10–2.58 (6H, m, T1-H-2′, T2-H-2′, T2-5-CH_2), 3.85–4.01 (4H, m, T1-H-5′, CH_2OP), 4.09–4.44 (3H, m, T2-H-4′, T2-H3′, T1-H-4′), 4.45–4.60 (2H, m, T2-H-5′), 4.95–5.05 (1H, m, T1-H-3′), 6.29–6.40 (2H, m, T2-H-1′, T1-H-1′), 7.39–7.52 (2H, m, T1-H-6, T2-H-6), 9.13–9.24 (2H, m, 2×NH); ^{31}P NMR ($CDCl_3$) δ −0.81, 1.29; HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 837.3336/837.3298.

4.1.23. Preparation of cyclic dTpU containing a phosphate-to-5 butanelinker (29). Compound **28** (0.145 g, 0.178 mmol) was dissolved in a 90% aqueous solution of trifluoroacetic acid (5 mL) and stirred for 3 h. The mixture was concentrated under reduced pressure and coevaporated with 99.9% CH_3CH_2OH (3×5 mL) and with CH_3OH (5 mL) to give the product as a white powder and an epimeric mixture (0.104 g, 100%); ^{1}H NMR (CD_3OD) δ 1.56–1.75 (4H, m, $CH_2CH_2CH_2CH_2OP$), 1.77–1.80 (3H, s, CH_3), 2.08–2.50 (6H, m, T1-H-2′, T2-H-2′, T2-5-CH_2), 3.69–3.73 (2H, m, T1-H-5′), 3.90–4.42 (7H, m, T1-H-4′, T2-H-5′, T2-H-4′, T2-H-3′, $POCH_2$), 4.99–5.02 (1H, m, T1-H-3′), 6.18–6.28 (2H, m, T1-H-1′, T2-H-1′), 7.40 (½H, s, T2-H-6), 7.50 (½H, s, T2-H-6), 7.69–7.70 (1H, m, T1-H-6); ^{31}P NMR (CD_3OD) δ −1.05, 0.01 (^{1}H and ^{31}P NMR data from DMSO-d_6 has been published in Ref. 9); HiRes MALDI FT-MS *m/z* (M+Na) found/calcd 609.1584/609, 1568; IR (KBr) ν cm^{-1}: 3435, 1690, 1472, 1277, 1022, 784.

4.1.24. Analytical formation of Tp-5-(4-aminobutanyl)-2′-deoxyuridine (30). Compound **29** (2 mg) was dissolved in 32% aqueous NH_3 (1 mL) for 5 days at 55 °C. The reaction mixture was concentrated under reduced pressure to give the product as a white foam; ^{1}H NMR (DMSO-d_6) δ 1.51–1.56 (4H, m, $CH_2CH_2CH_2CH_2N$), 1.78 (3H, s, CH_3), 2.03–2.10 (2H, m, T2-H-2′), 2.11–2.19 (2H, m, T2-5-CH_2), 2.23-2.28 (2H, m, T1-H-2′), 2.81–2.84 (2H, m, CH_2N), 3.58–3.62 (2H, m, T1-H-5′), 3.81–3.88 (2H, m, T2-H-5′), 3.94–3.96 (2H, m, T1-H-4′, T2-H-4′), 4.30–4.32 (1H, m, T2-H-3′), 4.63–4.67 (1H, m, T1-H-3′), 5.31 (1H, br, OH),

6.16 (1H, t, $J=6.7$ Hz, T1-H-1′), 6.26 (1H, t, $J=7.0$ Hz, T2-H-1′), 7.70 (1H, s, T1-H-6), 7.73 (1H, s, T2-H-6), 8.2–9.2 (3H, br, NH_3); ^{31}P NMR (DMSO-d_6) δ −0.82; MALDI-MS *m/z*: 603 (M^+), 625 (MNa^+); IR (KBr) ν cm^{-1}: 3400, 2255, 2128, 1684, 1469, 1277, 1203, 1026, 825, 763.

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