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Phosphine-Free Stille–Migita Chemistry for the Mild and Orthogonal Modification of DNA and RNA

André Krause, Alexander Hertl, Fabian Muttach, and Andres Jäschke*[a]

Abstract: An optimized catalyst system of $[Pd_2(dba)_3]$ and $AsPh_3$ efficiently catalyzes the Stille reaction between a diverse set of functionalized stannanes and halogenated mono-, di- and oligonucleotides. The methodology allows for the facile conjugation of short and long nucleic acid molecules with moieties that are not compatible with conventional chemical or enzymatic synthesis, among them acid-, base-, or fluoride-labile protecting groups, fluorogenic and synthetically challenging moieties with good to near-quantitative yields. Notably, even azides can be directly introduced into oligonucleotides and (deoxy)nucleoside triphosphates, thereby giving direct access to "clickable" nucleic acids.

Introduction

Strategies for the functionalization of nucleic acids are of enormous interest for applications in modern molecular biotechnology[1] and medical research.[2] Although numerous methods exist to introduce modifications co- or postsynthetically into chemically synthesized oligonucleotides,[3] several limitations apply to chemosynthetic methods.[4] Co-synthetic approaches require the stability of the modified monomers under coupling and deprotection conditions.[5] Moreover, standard solid-phase synthetic procedures for oligonucleotides, and in particular for RNA, demand for a set of mutually orthogonal protecting groups, thereby restricting the use of acid-, base- and fluoride-labile moieties for conjugation purposes. Enzymatic strategies, in contrast, capitalize on the high accuracy and processivity of DNA and RNA polymerases,[6] but offer only limited opportunities for site-specific modification.

Pd^0-catalyzed cross-coupling reactions have become standard tools for the modification of nucleosides,[7] nucleotides[8] and oligonucleotides.[9] Yet, there are no reports on cross-coupling reactions on unprotected RNA, which is not surprising, given the need for strong bases in most of these reactions, inevitably leading to hydrolysis of the internucleotide bonds. Among the common reactions, the Stille–Migita coupling stands out for its mild reaction conditions,[10] encouraging us to attempt the coupling of stannylated moieties to 5-iodinated pyrimidine nucleosides and oligonucleotides without addition of bases.

[a] *Dipl.-Biochem. A. Krause, A. Hertl, F. Muttach, Prof. Dr. A. Jäschke*
Institute of Pharmacy and Molecular Biotechnology
Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg
jaeschke@uni-hd

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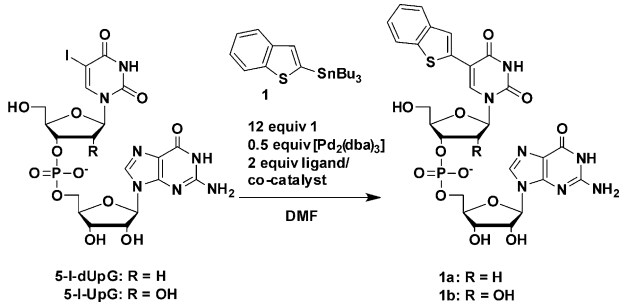
Results and Discussion

To start with a simple system, we investigated the Stille modification of ribo- or mixed ribo-/deoxyribodinucleotides (Scheme S1 in the Supporting Information), which we had recently identified as efficient "initiator dinucleotides"[11] that are selectively incorporated by RNA polymerases at the 5′ terminus of a transcript. In these molecules with the general structure 5′-(d)UpG-3′, the guanosine nucleoside is required for recognition by the polymerase, while the "carrier nucleoside" (d)U can be modified at the non-Watson–Crick face; preferably at position C-5. Ideally, the C-5 substituted target compound should be synthesized from a universal, unprotected dinucleotide precursor within a single selective and orthogonal transformation, giving access to a broad chemical space of modifications at minimal synthetic effort.

Due to the hydrolytic lability of RNA, a particularly mild catalytic system had to be identified (Table 1).

Stille–Migita reactions have previously been reported in polar solvent systems such as NMP and DMF with $[Pd_2(dba)_3]$ as a Pd^0 source and $P(Fu)_3$ or $AsPh_3$ as a ligand.[12] Compared to phosphine-based ligands, $AsPh_3$-based catalyst systems exhibit higher catalytic rates.[13] Cu^I as an additive was discussed to bind excessive ligand and thereby increase the activity of the catalytic Pd^0 species.[14] In an initial attempt, 5-I-dUpG in the presence of 0.5 equiv $[Pd_2(dba)_3]$, 2 equiv $AsPh_3$, 5 equiv CuI and 12 equiv of stannane **1**, reacted at 80 °C for 2 h, furnished deoxydinucleotide **1a** in 66% yield (Table 1, and Figure S1A in the Supporting Information). HPLC analysis indicated 34% unmodified starting material. Application of these conditions to ribodinucleotide 5-I-UpG resulted, however, in complete decomposition of the starting material (Figure S1B in the Supporting Information). A different catalytic system with $P(Fu)_3$ as a ligand[9d,12a] was only slightly more efficient, furnishing 10% of the desired product **1b** along with numerous decomposition products like guanosine and deglycosylated uracil derivative **1c**.

Table 1. Identification of a suitable catalyst system for the Stille–Migita reaction on the unprotected 5-I-(d)UpG dinucleotide.



5-I-dUpG: R = H
5-I-UpG: R = OH

1a: R = H
1b: R = OH

Dinucleotide	Ligand/ co-catalyst	Yield [%][d]	Starting material [%][d]	Total side-product/ (G[e]/B[f]) [%][d]
5-I-dUpG[a]	$AsPh_3$/5 equiv CuI	66	34	–
5-I-UpG[a]	$AsPh_3$/5 equiv CuI	–	–	complete decay
5-I-UpG[a]	PFu_3/–	10	<1	89/(75/14)
5-I-UpG[a]	$AsPh_3$/–	68	1	31/(23/8)
5-I-dUpG[a]	$AsPh_3$/–	99	1	–
5-I-UpG[b]	$AsPh_3$/–	92	6	2/(2/0)
5-I-UpG[c]	$AsPh_3$/–	>95	4	–

[a] Reaction performed at 80 °C, 2 h; [b] reaction performed at 80 °C, 15 min; [c] reaction performed at 60 °C, 30 min; [d] based on integration of the UV signal at 254 nm; [e] guanosine; [f] deglycosylated uracil coupling product **1c**.

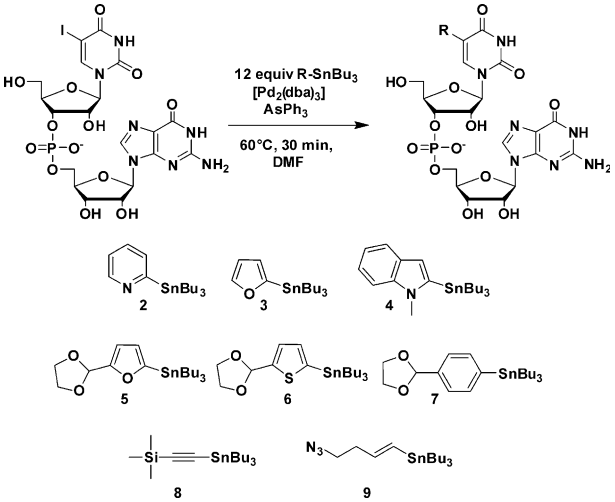
Considering the incompatibility of nucleic acids with various copper species,[15] we now tried a copper-free $[Pd_2(dba)_3]$/$AsPh_3$ catalytic system. Both 5-I-dUpG and 5-I-UpG were consumed quantitatively, resulting in near-complete product formation for 5-I-dUpG and 68% for 5-I-UpG. From this encouraging starting point, we attempted to further improve the yield of the desired products by varying temperatures and reaction times. Indeed, a reduced reaction time of 15 min at 80 °C resulted in 92% yield of **1b** at essentially suppressed dinucleotide decomposition, while near-quantitative conversion could be achieved at 60 °C within 30 min (Figure 1 and Figures S2 and S3 in the Supporting Information).

To investigate the generality of this approach, commercially available furan-, pyridine- and *N*-methylindole-derived stannanes were subjected to Stille–Migita coupling on 5-I-UpG (Table 2 and Figure S4 in the Supporting Information). The catalytically challenging pyridine-derived stannane **2**[16] yielded 23% of the desired coupling product, while near-quantitative product formation was obtained for stannanes **3** and **4**.

Since standard procedures in RNA oligonucleotide synthesis are associated with alkaline and acidic deprotection steps, as well as with exposure toward fluoride ions, the chemical space for the introduction of labile functionalities is limited. Yet, it is of considerable interest to generate RNAs bearing acid-, base- or fluoride-labile moieties for further site-specific transformations.[3c,17] We therefore synthesized stannanes of various aryl dioxolanes (**5**–**7**), as well as TMS-protected ethynyl stannane **8**. 5-I-UpG was converted quantitatively within 30 min at 0.5 equiv $[Pd_2(dba)_3]$ in the presence of stannanes **5** and **6** without any sign of dinucleotide decay (Figure S5 in the Supporting Information). Stannane **7** reacted slower, and a higher catalyst loading (1 equiv $[Pd_2(dba)_3]$) was required to reach near-quantitative consumption of the starting material. Besides the expected coupling product **7a** (44%) we observed additional species eluting as a broad peak (37%) during HPLC separation. High resolution ESI mass spectrometry revealed the masses and isotope patterns of three different Pd-containing compounds (Figure S6 in the Supporting Information), supporting the assumption of an oxidative addition intermediate where Pd^{II} is attached to C-5 of the 5′-uridine moiety, as well as a hydrated and a ligand-free analogue. For TMS-acetylene-derived stannane **8**, a moderate ratio of reduction to UpG (11%), associated with some guanosine formation (25%) was observed, yielding 51% of **8a**.

As^{III} exhibits a higher stability towards oxidation than P^{III} in phosphine-derived ligands. We therefore assumed that Staudinger-type reactions between phosphines and azides,[5,18] which prevent the direct

Table 2. Scope of the Stille–Migita reaction on unprotected 5-I-UpG.



Stannane	$[Pd_2(dba)_3]$/ $AsPh_3$ [equiv]	Yield [%][a]	Starting material [%][a]	UpG [%][a,b]	Side-products/ G[c] [%][a]
2	0.5/2	20	14	59	7/2
2	1/4	23	3	63	11/3
3	0.5/2	>99	–	–	–
4	0.5/2	98	–	<2	–
5	0.5/2	99	–	<1	–
6	0.5/2	99	–	<1	–
7	0.5/2	8	81	<1	10/<1
7	1/4	44	5	11	40/3
8	0.5/2	51	<1	11	37/25
9	0.5/2	47	51	<1	<1
9	1/4	82	–	6	12/8

[a] Based on integration of the UV signal at 254 nm; [b] product of reduction; [c] guanosine.

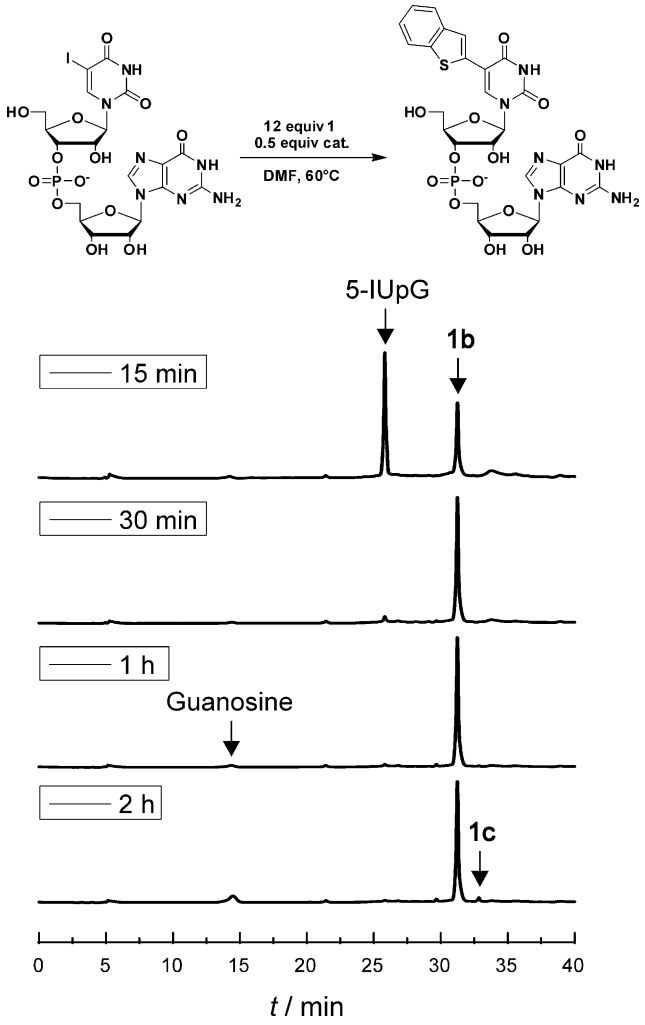


Figure 1. Optimization of reaction temperature and time for the Stille–Migita reaction between 5-I-UpG and **1** in the presence of 0.5 equiv $[Pd_2(dba)_3]$ and 2 equiv $AsPh_3$. HPLC analysis revealed near-quantitative coupling product formation at full dinucleotide stability at 60 °C for 30 min.

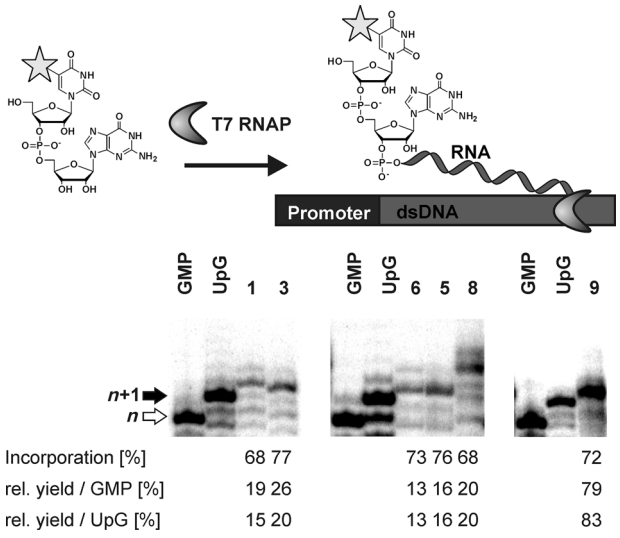


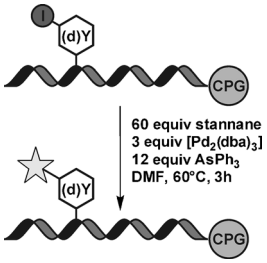
Figure 2. Enzymatic 5′ incorporation of modified 5-substituted UpG dinucleotides into RNA by T7 RNAP. A 19-nt test RNA was synthesized in vitro in the presence of C-5-substituted UpG dinucleotides or GMP and UpG as reference initiators. Open and filled arrows indicate the positions of unmodified and modified transcripts, respectively. Incorporation efficiencies and relative RNA yields were normalized to GMP and UpG are indicated below the corresponding lanes.

incorporation of azide groups in phosphoramidite solid phase synthesis, could be circumvented in an $AsPh_3$-based catalyst system. This would allow for the direct, postsynthetic introduction of azides into the dinucleotide using an azide-bearing stannane. Indeed, under the conditions optimized above, azido-stannane **9** was coupled to 5-I-UpG at 0.5 equiv $[Pd_2(dba)_3]$ loading with 47% efficiency, showing no sign of azide decomposition (Table 2). An increased catalyst loading of 1 equiv $[Pd_2(dba)_3]$ furnished the target compound in 82% yield (Figure S7 in the Supporting Information).

In order to move from small to larger RNAs, HPLC-purified coupling products of stannanes **1**, **3**, **5**, **6**, **8** and **9** with 5-I-UpG were then subjected to transcriptional priming experiments with T7 phage RNA polymerase (RNAP),[19] applying guanosine monophosphate (GMP) and the unmodified dinucleotide UpG[11,20] as reference initiator nucleotides. All dinucleotides were found to be accepted as transcriptional starters by T7 RNAP (Figure 2, Figure S8 and Table S1 in the Supporting Information) at similar incorporation ratios of 68 to 77%. Sterically demanding 5-substituents gave lower yields of the desired transcription product. The highest incorporation efficiency was observed for **9a**, which gave RNA yields similar to unmodified UpG. Thus, the Stille–Migita coupling products gave access to a diverse set of site-specifically modified RNA molecules.

The successful coupling on the dinucleotide system prompted us to investigate the expansion of this methodology to the functionalization of oligonucleotides. 5-I-dU-, 5-I-dC- or 5-I-U-bearing DNA and RNA sequences (Figure S9 in the Supporting Information) were synthesized by phosphoramidite chemistry and, prior to standard deprotection by ammonia, subjected to coupling reactions with the corresponding stannanes while still attached to the solid support (Table 3). 5-I-dU-bearing ON1 was found to couple with stannanes **1**, **8** and **9** in yields of 38, 54 and 57%, respectively (Figure S10 in the Supporting Information). On alkaline deprotection of the TMS-ethynyl-modified DNA precursor, the corresponding ethynyl-bearing oligonucleotide was generated, thus providing an efficient alternative to the direct incorporation of expensive 5-ethynyldeoxyuridine phosphoramidites[21] into oligonucleotides. Once more, azido-stannane **9** was found to be transferred to both terminal and internal positions of the oligonucleotide (Figures S11 and S12 in the Supporting Information). Coupling yields were found to be dependent on the accessibility of the iodinated residue, ranging from 23% for internal to 57% for terminal modifications. Double modification of ON4 yielded 24% of the target compound. 5′-modified 5-I-dU or 5-I-U-bearing RNA oligonucleotides exhibited similarly efficient coupling properties, yielding 61 and 59% of the respective coupling product (Figure S13 in the Supporting Information). Internally modified RNA oligonucleotides of identical base sequence to ON2, however, turned out to be essentially unreactive, most likely due to steric

Table 3. Synthesis of C-5-substituted DNA and RNA oligonucleotides conflicting with standard phosphoramidite synthesis and deprotection chemistry on solid support.



ON	Sequence	Stannane	Yield [%][c]	M_{calcd}	M_{found}
1[a]	dU*ATAGGAGCT	**1**	38	3183.5474	3183.5488
1[a]	dU*ATAGGAGCT	**8**	54	3075.5433[d]	3075.5440
1[a]	dU*ATAGGAGCT	**9**	57	3146.5918	3146.5775
2[a]	TATAdU*GAGCT	**9**	23	3121.5691	3121.5853
3[a]	TATAdU*GAGCTCAGCT	**9**	25	4645.8348	4645.8106
4[a]	dU*AdU*AGGAGCT	**9**	24	3227.6250	3227.6222
5[a]	C*ATAGGAGCT	**9**	46	3145.5949	3145.6083
6[a]	TATAC*GAGCT	**9**	23	3120.6018	3120.6030
7[a]	TATAC*GAGCTCAGCT	**9**	23	4644.8202	4644.8507
8[b]	dU*AUAGGAGCU	**9**	61	3262.5019	3262.5153
9[b]	U*AUAGGAGCU	**9**	59	3278.5196	3278.5102

[a] DNA oligonucleotide; [b] RNA oligonucleotide; [c] based on integration of UV signal at 254 nm; [d] alkaline deprotection of coupling product results in TMS-deprotection and yields the 5-ethynyl-modified conjugate.

shielding by the bulky 2′-O-TBDMS groups of protected RNAs on solid support. Thus, Stille–Migita chemistry provides an elegant route towards azido-modified oligonucleotides for click chemistry, a class of substances not easily accessible to solid-phase synthesis.

Finally, we investigated whether this chemistry could be exploited to directly functionalize (deoxy)nucleoside triphosphates,[22] a delicate class of compounds that rapidly decomposes outside a narrow range of pH, salt and solvent conditions. Recently, Rao et al.[23] described the synthesis of an azido-modified UTP analogue over six synthetic steps. Applying a nonprotic solvent system of DMF/DMSO (1:1), Stille cross-coupling on 5-I-dCTP with 0.5 equiv $[Pd_2(dba)_3]$ and 2 equiv $AsPh_3$ at 60 °C for 45 min gave 5-((*E*)-4-azidobut-1-enyl)-dCTP (5-N_3-dCTP) in only one step in 54% isolated yield (Figure 3A). HPLC analysis indicated clean and quantitative conversion of the starting material without any side-product formation or decay (Figure 3B). Spectroscopic properties were found to be identical to those of 5-N_3-dCTP synthesized according to the conventional route from 5-N_3-dC with subsequent triphosphorylation (Figure 3C). 5-N_3-dCTP was subjected to PCR experiments with the KOD-XL DNA polymerase and 5-N_3-dCTP as the sole dCTP source (Figure 3D). The amount of amplified DNA in PCR reactions using 5-N_3-dCTP was found to be comparable to that of dCTP-containing reactions. The amplicon could be biotinylated quantitatively by SPAAC-biotinylation with sulfo-DBCO-biotin (**S11**), yielding a DNA-conjugate of higher molecular weight. On addition of streptavidin, a quantitative gel shift was observed, proving the reactivity of the azide handle. Under identical cross-coupling conditions the ribo-analogue 5-N_3-CTP was directly synthesized from 5-I-CTP at an isolated yield of 58% (Figure S14A in the Supporting Information). It was found to be a well-accepted substrate for the T7 RNAP, allowing the in vitro synthesis of internally modified RNA (Figure S14B–D in the Supporting Information).

Conclusion

In conclusion, our study explores, for the first time, the scope and utility of the Stille–Migita reaction on mono-, di-, and oligonucleotides and the utilization of the reaction products by DNA and RNA polymerases. The unique conditions of the Stille reaction allow for the direct introduction of sensitive functionalities that would be incompatible with the traditional procedures of solid phase oligonucleotide synthesis and phosphoramidite chemistry. A careful choice of the ligand conferred stability onto azide moieties under coupling conditions as well, providing a novel and general approach for the synthesis of "clickable" DNA and RNA by chemical or chemoenzymatic means. Triorganotin, as well as triorganoarsenic compounds have been subject to debates for their toxicity (see the Supporting Information). Work with these compound classes should always be carried out with care under appropriate laboratory conditions. Mildness, reliability and tolerance towards most functional groups make us expect Stille–Migita chemistry to become a standard tool for the synthesis of complex oligonucleotides and nucleoside triphosphates.

Experimental Section

Stille–Migita coupling on 5-I-dUpG and 5-I-UpG

In a 1 mL vial, 5-I-dUpG or 5-I-UpG (1 μmol) and stannane (12 μmol) were dissolved in DMF (250 μL). In a second vial a ten-fold-concentrated $[Pd_2(dba)_3]/AsPh_3$ catalyst solution in DMF (500 μL) was prepared under alternating vacuum/argon cycles. Premixed catalyst solution (50 μL) was transferred to the degassed reaction mixture under argon. The reaction was allowed to proceed at temperatures and times indicated for the individual experiment. Reactions were partitioned between water (500 μL) and DCM (500 μL, for acid-sensitive compounds 500 μL buffer A and 500 μL A-buffered DCM) and the organic layer was extracted with water (2×500 μL). Reaction products were analyzed by HPLC (gradient buffer A/buffer B; 0 min: 2% buffer B; 10 min: 2% buffer B; 20 min: 10% buffer B; 45 min: 100% buffer B; 52 min: 2% buffer B). Yields were derived by integration of UV signals at 254 nm. For preparative purpose, coupling products were purified by semipreparative HPLC (gradient buffer A/buffer B; 0 min: 10% buffer B; 10 min: 25% buffer B; 25 min 60% buffer B; 30 min: 100% buffer B; 35 min: 10% buffer B; 40 min: 10% buffer B).

Stille–Migita coupling on solid support

In a 1 mL vial, CPG-bound DNA or RNA, corresponding to about 200 nmol, and stannane (12 μmol) were suspended in 250 μL DMF. In a second vial $[Pd_2(dba)_3]$ (5.8 mg, 6 μmol) and $AsPh_3$ (7.8 mg,

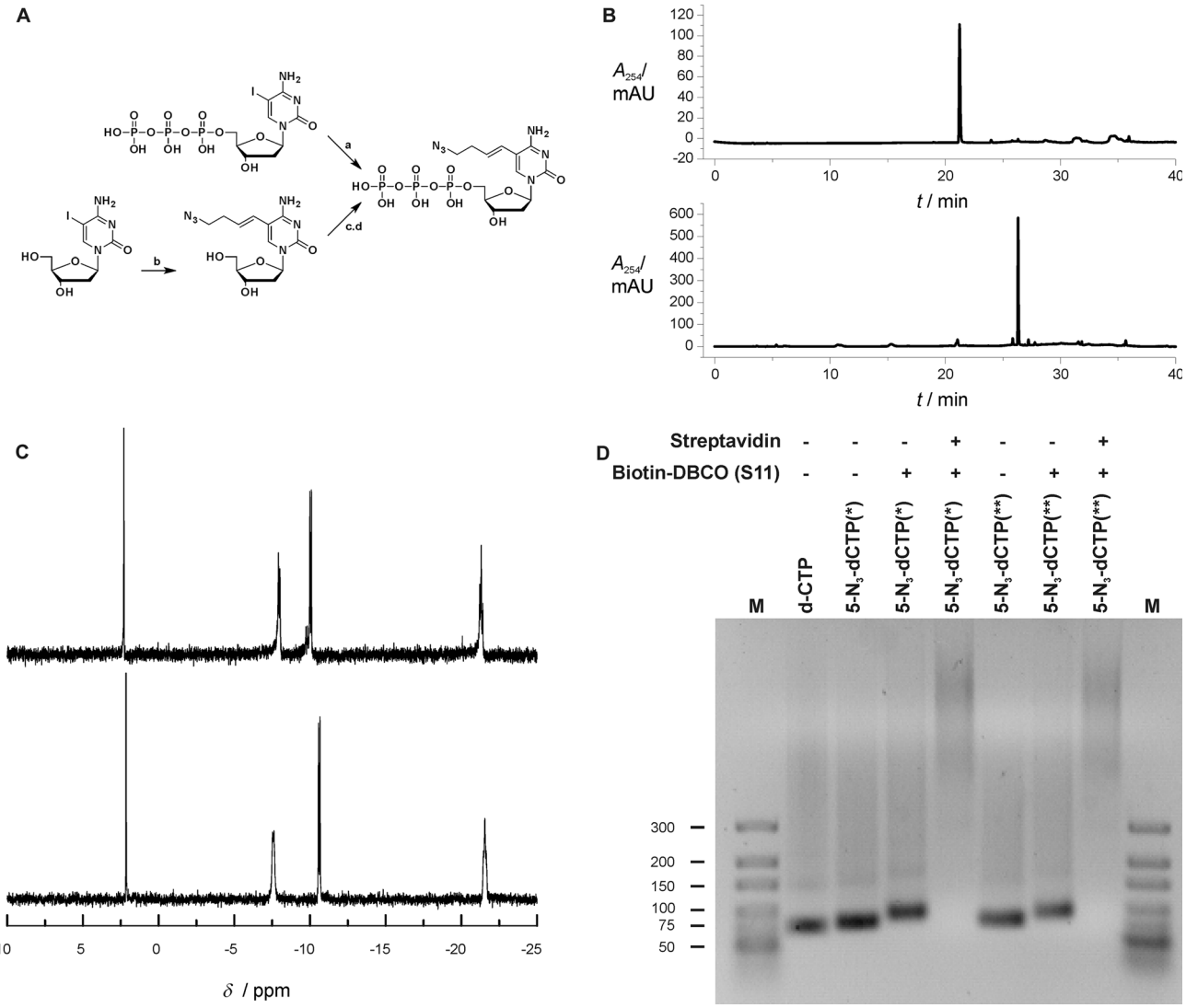


Figure 3. A) Alternative routes for the synthesis of 5-N_3-dCTP by Stille–Migita coupling: a) 5 equiv **9**, 0.5 equiv [$Pd_2(dba)_3$], 2 equiv $AsPh_3$, DMF/DMSO (1:1), 60 °C, 45 min, 54%; b) 1.2 equiv **9**, 5% [$Pd_2(dba)_3$], 20% $AsPh_3$, DMF, 80 °C, 3 h, 85%; c) 1.2 equiv $POCl_3$, $PO(OMe)_3$, 0 °C, 1 h; d) i. 5 equiv $(NHBu_3)H_2P_2O_7$, 4.2 equiv NBu_3, DMF, 0 °C, 2 h; ii. TEAB pH 7.5, 40%. B) HPLC chromatogram of 5-I-dCTP (upper graph) and reaction mixture after 45 min (lower graph). C) Comparison of ^{31}P NMR spectra of 5-N_3-dCTP synthesized by Stille coupling on 5-I-dCTP (upper graph) and triphosphorylation of 5-N_3-dC (lower graph). D) PCR-amplification of a 70-mer dsDNA template by KOD-XL DNA polymerase using 5-N_3-dCTP as sole dCTP source. Batches of conventionally (*) and post-synthetically obtained (**) 5-N_3-dCTP were applied. 5-N_3-dC-modified DNA was sequentially SPAAC-biotinylated using sulfo-DBCO-biotin (**S11**) and incubated with streptavidin. Stepwise bandshifts indicate successful incorporation of 5-N_3-dCTP and efficient SPAAC-modification of the azide moiety.

24 μmol) were dissolved in DMF (500 μL). The catalyst solution was degassed, back-filled with argon and 50 μL (0.6 μmol $[Pd_2(dba)_3]$ and 2.4 μmol $AsPh_3$) were transferred to the reaction mixture that was subsequently degassed and back-filled with argon again. The mixture was stirred at 60 °C for 3 h and diluted to 500 μL with DMF. The CPG material was recovered by filtration through a centrifuge filter and washed with 100 mM EDTA (2×500 μL, pH 8), DMF (2×500 μL) and diethyl ether (2×200 μL). Oligonucleotides were deprotected in 28–30% aqueous NH_3 (200 μL) at room temperature for 2 h for 5-I-(d)U-bearing sequences or at 55 °C for 3 h for 5-I-dC-modified sequences. The aqueous solutions were recovered and the CPG material was washed with water (3×200 μL). The combined aqueous layers were washed with $CHCl_3$ (200 μL). Excessive ammonia was removed by evaporation in a rotary concentrator. RNA oligonucleotides were desilylated in DMF/$Et_3N{\cdot}3HF$ (1:1, 120 μL) at 65 °C for 2 h. Isopropoxytrimethylsilane (240 μL) was added and the mixture was incubated for 10 min, followed by addition of Et_2O (1 mL) at RT. A precipitate formed that was collected by centrifugation and washed with Et_2O (300 μL). The air-dried pellet was redissolved in water (200 μL). Analytics was performed as described above for 5-I-(d)UpG (gradient of buffer A/buffer B; 0 min: 10% buffer B; 10 min: 10% buffer B; 35 min: 35% buffer B; 40 min: 100% buffer B; 45 min: 10% buffer B).

2′-Deoxy-5-((*E*)-4-azidobut-1-enyl)cytidine (5-N_3-dC)

5-I-dC (95 mg, 0.27 mmol), stannane **9** (125 mg, 0.33 mmol), $AsPh_3$ (16.5 mg, 55 μmol) and $[Pd_2(dba)_3]$ (12.5 mg, 13.5 μmol) were dissolved in anhydrous DMF (5.5 mL). The solution was degassed under vacuum and back-filled with argon. After being stirred at 80 °C for 3 h, the solvent was removed and the residue was subjected to flash column chromatography on a 12 g silica column

(gradient DCM/MeOH (1:0) to (7:1)), yielding 73 mg (85%) of the title compound. $R_f = 0.26$ (DCM/MeOH 95:5); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 8.07$ (s, 1H, H6), 6.34 (d, $J = 15.7$ Hz, 1H, H7), 6.15 (t, $J = 6.5$ Hz, 1H, H1′), 5.86 (dt, $J = 15.7$ Hz, 6.9 Hz, 1H, H8), 5.19 (d, $J = 4.3$ Hz, 1H, O*H*-3′), 5.07 (t, $J = 4.8$ Hz, 1H, O*H*-5′), 4.26–4.21 (m, 1H, H3′), 3.78 (q, $J = 3.5$ Hz, 1H, H4′), 3.74–3.60 (m, 1H, H5a′), 3.60–3.50 (m, 1H, H5b′), 3.42 (t, $J = 6.9$ Hz, 2H, H10), 2.38 (q, $J = 7.0$ Hz, 2H, H9), 2.14 (ddd, $J = 13.0$, 6.0, 3.8 Hz, 1H, H2a′), 2.02 ppm (td, $J = 13.0$, 6.4 Hz, 1H, H2b′); ^{13}C NMR (126 MHz, [D$_6$]DMSO): $\delta = 163.1$ (C4), 154.2 (C2), 137.1 (C6), 125.6 (C8), 123.1 (C7), 103.9 (C5), 87.1 (C4′), 85.0 (C1′), 69.9 (C3′), 60.8 (C5′), 49.9 (C10), 40.6 (C2′), 32.1 ppm (C9); HR-ESI-TOF-MS: *m/z* calcd for $[M+Na^+]$ $C_{13}H_{18}N_6O_4Na_1$ 345.1282, found 345.1277.

2′-Deoxy-5-((*E*)-4-azidobut-1-enyl)cytidine 5′-*O*-triphosphate (5-N$_3$-dCTP)

Method A: 5-N$_3$-dC (40 mg, 0.125 mmol) was dried at 80 °C under high vacuum and suspended in freshly distilled PO(MeO)$_3$ (315 μL). The mixture was cooled to 0 °C and POCl$_3$ (13.5 μL, 0.15 mmol) was added, followed by stirring for 2 h at 0 °C. An ice-cold solution of $(NBu_3H)_2H_2P_2O_7$ (340 mg, 0.62 mmol) and NBu$_3$ (125 μL, 0.52 mmol) in anhydrous DMF (1.25 mL) was added and the reaction mixture was stirred for 1 h at 0 °C. The reaction was stopped by adding 2 M aqueous TEAB (850 μL, pH 7.5). The solvents were partially removed by threefold co-evaporation with water. The residue was subjected to ion-exchange chromatography on a Sephadex A25 column (40 mL) in a linear gradient of TEAB in water (pH 7.5, 50 mM to 1.2 M over 4 h). Product-containing fractions were freeze-dried and purified by reversed phase chromatography on a 23 g C18 column, gradient buffer A/buffer B (0–5 min: 0% buffer B; 45 min: 45% buffer B, 1% per min). Yield: 38 mg, 40%. *Method B*: 5-I-dCTP (14.4 mg, 14.5 μmol) and stannane **9** (28.1 mg, 72.5 μmol) were mixed in anhydrous DMSO (375 μL) in a sealed 1 mL vial under argon atmosphere. In a separate vial, [Pd$_2$(dba)$_3$] (13.3 mg, 14.5 μmol) and AsPh$_3$ (17.8 mg, 58 μmol) were dissolved in anhydrous DMF (750 μL), degassed and back-filled with argon. The catalyst solution (375 μL) was added to the reaction mixture, followed by purging with argon and stirring at 60 °C for 45 min. Analytical HPLC revealed near-quantitative consumption of the starting material. The reaction mixture was diluted to 4.5 mL in buffer A and extracted with A-buffered CHCl$_3$ (3×1.5 mL). The aqueous phase was freeze-dried and 5-N$_3$-dCTP was purified by semipreparative HPLC, yielding 7.5 mg (54%) of the title compound as triethylammonium salt. ^{1}H NMR (500 MHz, D$_2$O): $\delta = 7.88$ (s, 1H, H6), 6.32 (t, $J = 6.9$ Hz, 1H, H1′), 6.27 (d, $J = 15.7$ Hz, 1H, H7), 6.14 (dt, $J = 15.6$, 6.8 Hz, 1H, H8), 4.68–4.60 (m, 1H, H3′), 4.43–3.97 (m, 3H, H4′, H5′), 3.52–3.48 (m, 2H, H10), 3.19 (q, $J = 6.9$ Hz, 12H, N-(C*H*$_2$-CH$_3$)$_3$), 2.52 (q, $J = 6.2$ Hz, 2H, H9), 2.41 (ddd, $J = 14.1$, 6.4, 3.5 Hz, 1H, H2a′), 2.36–2.31 (m, 1H, H2b′), 1.27 ppm (t, $J = 7.3$ Hz, 18H, N-(CH$_2$-C*H*$_3$)$_3$); ^{13}C NMR (126 MHz, D$_2$O): $\delta = 164.8$ (C4), 157.5 (C2), 138.4 (C6), 132.6 (C8), 122.2 (C7), 108.4 (C5), 86.6 (C1′), 86.2 (d, $J = 9.3$ Hz, C4′), 69.3 (C3′), 65.9 (d, $J = 5.3$ Hz, C5′), 50.7 (C10), 47.2 (*N*-(C*H*$_2$-CH$_3$)$_3$), 39.9 (C2′), 32.8 (C9), 8.8 ppm (*N*-(CH$_2$-C*H*$_3$)$_3$); ^{31}P NMR (202 MHz, D$_2$O, 10 mM phosphate buffer pD = 7.0 ($\delta = 2.35$ ppm)): $\delta = -7.37$ (d, $J = 17.6$ Hz, Pγ), −10.41 (d, $J = 19.7$ Hz, Pα), −21.35 ppm (t, $J = 18.5$ Hz, Pβ); HR-ESI-TOF-MS: *m/z* calcd for $[M-H^-]$ $C_{13}H_{20}N_6O_{13}P_3$ 561.0307, found 561.0326.

5-((*E*)-4-Azidobut-1-enyl)cytidine 5′-*O*-triphosphate (5-N$_3$-CTP)

The reaction was carried out as for 5-N$_3$-dCTP, method B. 5-I-CTP (14.1 mg, 13.9 μmol) and stannane **9** (28.1 mg, 72.5 μmol) were mixed in anhydrous DMSO (365 μL) in a sealed 1 mL vial under argon atmosphere. [Pd$_2$(dba)$_3$] (13.3 mg, 14.5 μmol) and AsPh$_3$ (17.8 mg, 58 μmol) were dissolved in anhydrous DMF (730 μL) in a separate vial. The vial was degassed and back-filled with argon. The catalyst solution (365 μL) was added to the reaction mixture, followed by purging with argon and stirring at 60 °C for 45 min. Analytical HPLC revealed near-quantitative consumption of the starting material. The reaction mixture was diluted to 4.5 mL in buffer A and extracted with A-buffered CHCl$_3$ (3×1.5 mL). The aqueous phase was freeze-dried and the residue was purified by reversed-phase chromatography on a 23 g C18 column, gradient buffer A/buffer B (0–5 min: 0% buffer B; 45 min: 45% buffer B, 1% per min). Yield: 7.2 mg, 58%. ^{1}H NMR (500 MHz, D$_2$O): $\delta = 7.90$ (s, 1H, H6), 6.27 (d, $J = 15.82$ Hz, 1H, H7), 6.18 (dt, $J = 15.7$, 6.74 Hz, 1H, H8), 6.03 (d, $J = 5.1$ Hz, 1H, H1′), 4.44 (t, $J = 5.0$ Hz, 1H, H3′), 4.40 (t, $J = 5.2$ Hz, 1H, H2′), 4.35–4.18 (m, 3H, H4′ and H5′), 3.56–3.44 (m, 2H, H10), 3.20 (q, $J = 7.3$ Hz, 18H, N-(C*H*$_2$-CH$_3$)$_3$), 2.55–2.51 (m, 2H, H9), 1.28 ppm (t, $J = 7.3$ Hz, 27H, N-(CH$_2$-C*H*$_3$)$_3$); ^{13}C NMR (126 MHz, D$_2$O): $\delta = 164.0$ (C4), 156.9 (C2), 137.55 (C6), 131.9 (C8), 121.3 (C7), 107.8 (C5), 88.9 (C1′), 83.1 (d, $J = 9.2$ Hz,1C, C4′), 74.0 (C2′), 69.5 (C3′), 65.0 (d, $J = 5.2$ Hz, C5′), 50.1 (C10), 46.6 (*N*-(CH$_2$-CH$_3$)$_3$), 32.2 (C9), 8.2 ppm (*N*-(CH$_2$-C*H*$_3$)$_3$); ^{31}P NMR (202 MHz, D$_2$O, 10 mM phosphate buffer pD = 7.0 ($\delta = 2.35$ ppm)): $\delta = -8.34$ (d, $J = 19.6$ Hz, Pγ), −9.56 (d, $J = 20.2$ Hz, Pα), −21.00 ppm (t, $J = 19.6$ Hz, Pβ); HR-ESI-TOF-MS: *m/z* calcd for $[M-H^-]$ $C_{13}H_{20}N_6O_{14}P_3$ 577.0256, found 577.0266.

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