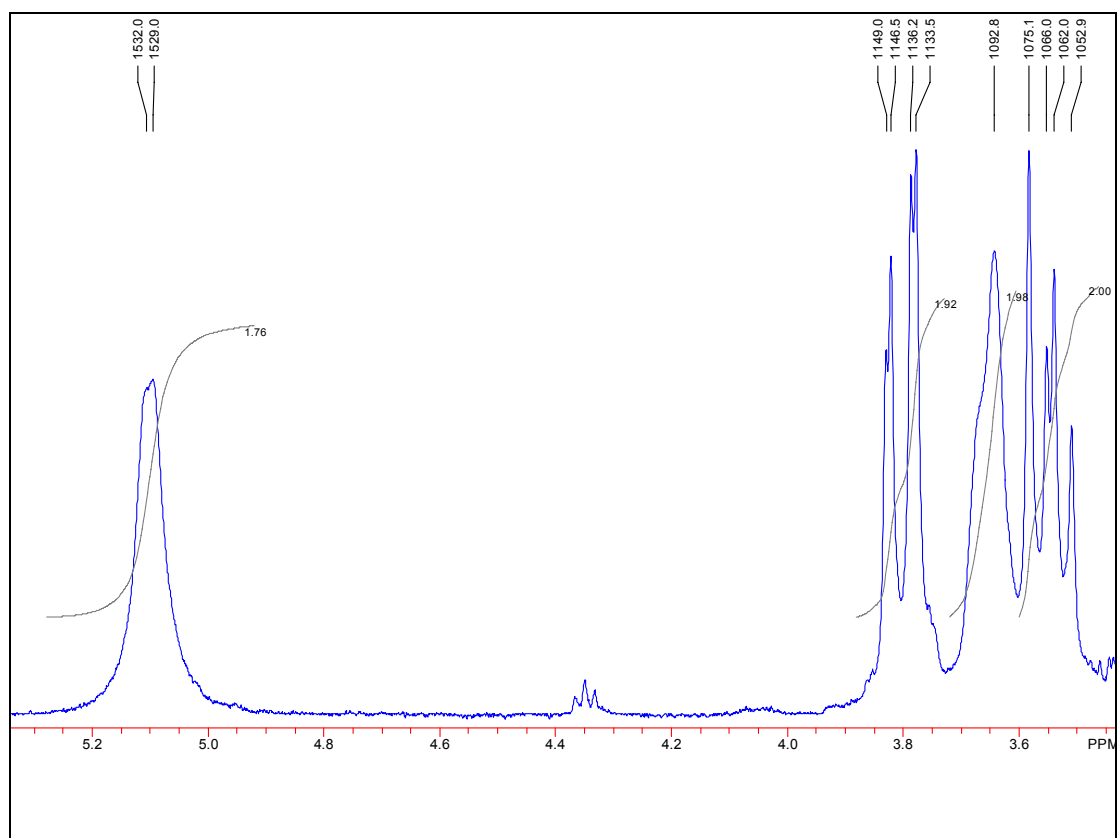

Synthetic nucleic acid secondary structures containing the four stereoisomers of 1,4-bis(thymine-1-yl)butane-2,3-diol

Mikkel S. Christensen,^a Andrew Bond^b and Poul Nielsen^{a*}

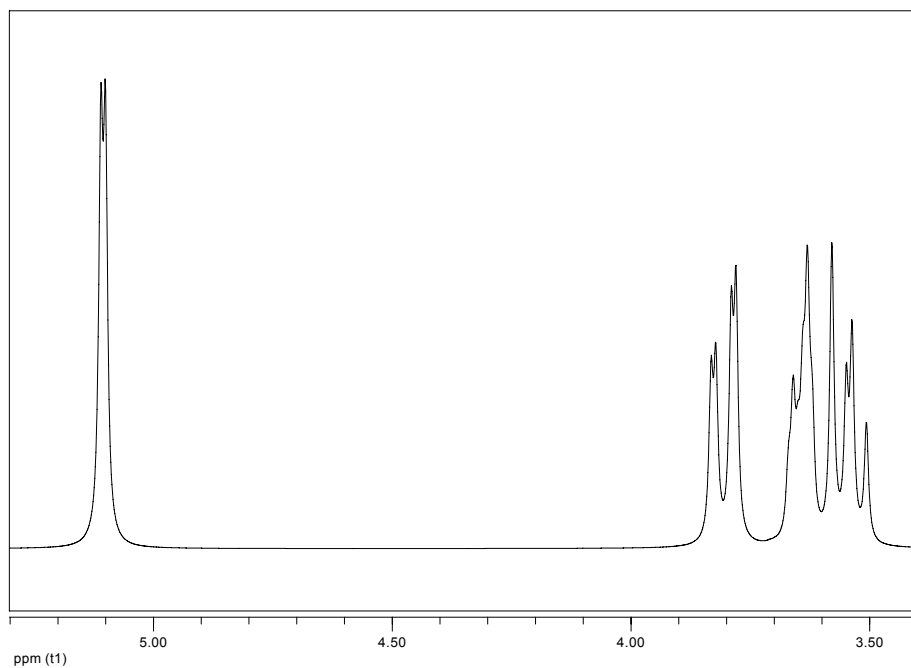
^aNucleic Acid Center, [†]^{a,b}Department of Physics and Chemistry, University of Southern Denmark, 5230 Odense M, Denmark. Fax: +45 66158780; Tel: +45 65502565; E-mail: pon@ifk.sdu.dk

Electronic Supplementary Information (ESI)

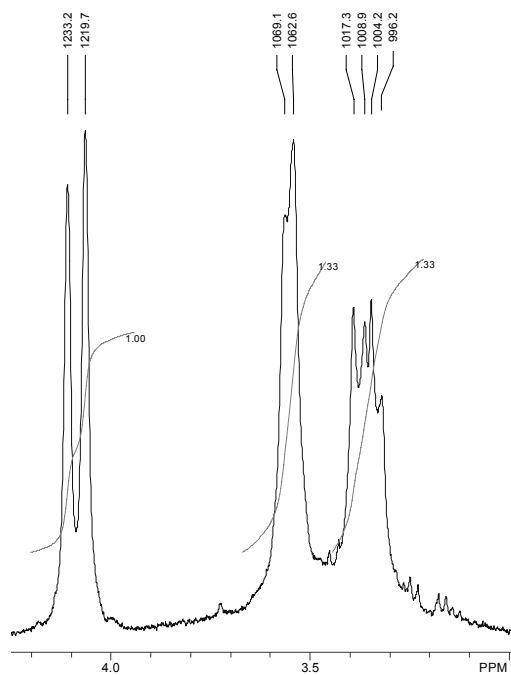
ESI-Figure 1 (a). ¹H-NMR spectrum of compound **13** expanded in the region of the strongly coupled system. The 8 s spectrum was acquired on a 300 MHz Varian GEMINI in DMSO-*d*₆.



ESI-Figure 1 (b). 300 MHz simulated spectrum for the spin system {ABCXA'B'C'X'} in compound **13** with 3 Hz line width and $J_{ab} = 13.0$ Hz, $J_{ac} = 9.1$ Hz, $J_{bc} = 2.6$ Hz, $J_{cx} = 3$ Hz and $J_{cc'} = 1-4$ Hz. The remaining coupling constants are assumed to be zero.



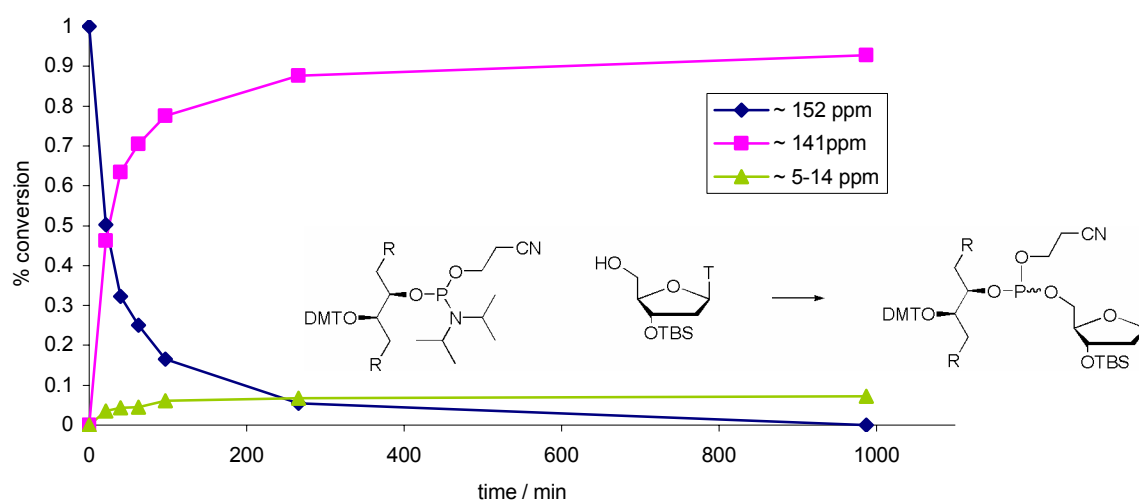
ESI-Figure 1 (c). ¹H-NMR spectrum of compound **14** expanded in the region of the strong coupled system. The 8 s spectrum was acquired on a 300 MHz Varian GEMINI in DMSO-*d*₆.



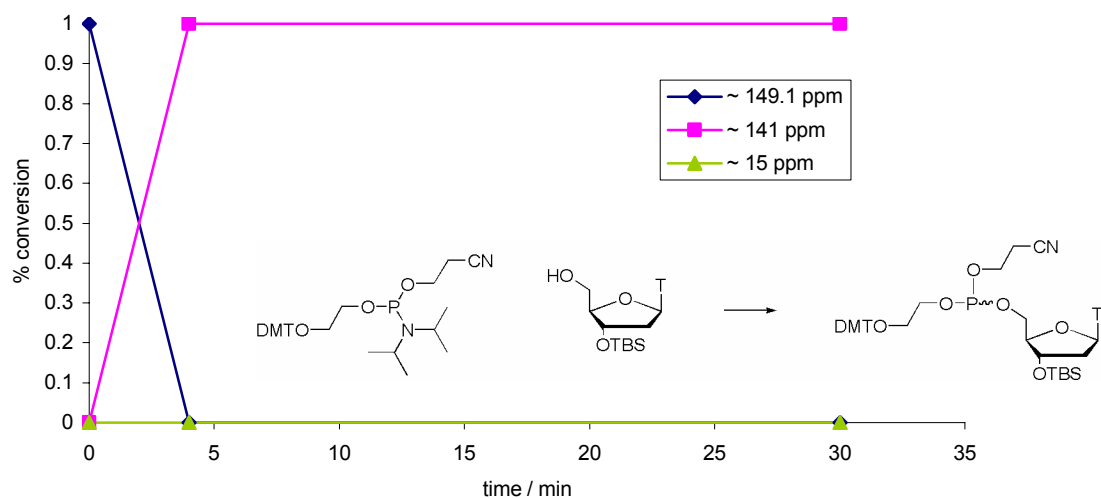
ESI-Figure 2. ^{31}P -NMR study of the coupling kinetics with 1-*H*-tetrazole. The spectra were acquired on a 200 MHz Varian GEMINI using external reference and a sealed capillary tube inside the NMR tube with $\text{DMSO-}d_6$ to provide a deuterium lock signal.

General description: To an NMR-tube with 20 mg (0.060 mmol) 3'-*O-tert*-butyldimethylsilyl-thymidine was added a 0.3 M solution of the phosphoramidite **8a** in MeCN (0.20 mL, 0.06 mmol), and the first spectrum of the amidite was acquired. Then a 0.45 M solution of 1-*H*-tetrazole in MeCN (0.20 mL, 0.090 mmol) was added to initiate the reaction. The first spectrum was acquired with 64 scans, which represents a time point approx. 5 min. after reaction start.

A) Coupling of double-headed D-threitol nucleoside with 1-*H*-tetrazole.

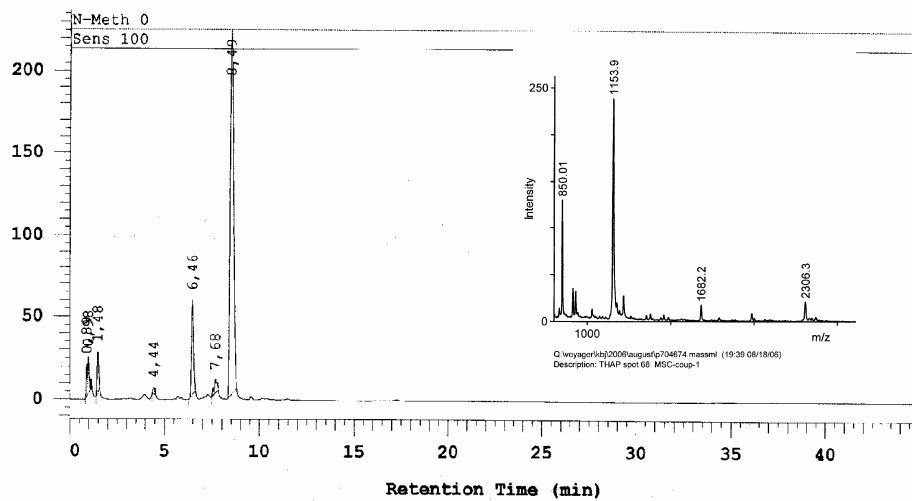


B) Coupling of ethylene glycol with 1-*H*-tetrazole.

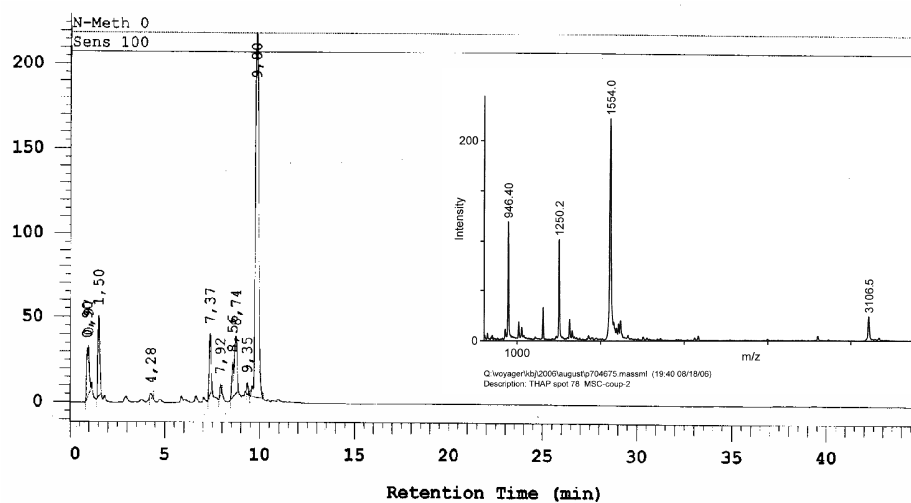


ESI-Figure 3 (a). Ion exchange chromatography and MALDI MS of small oligomers

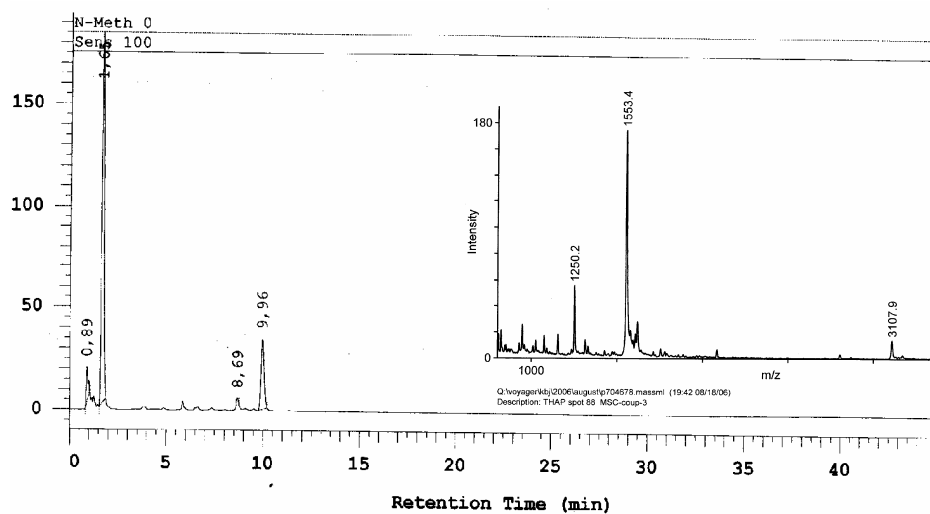
I) 5'-TTTT (m/z = 1154)



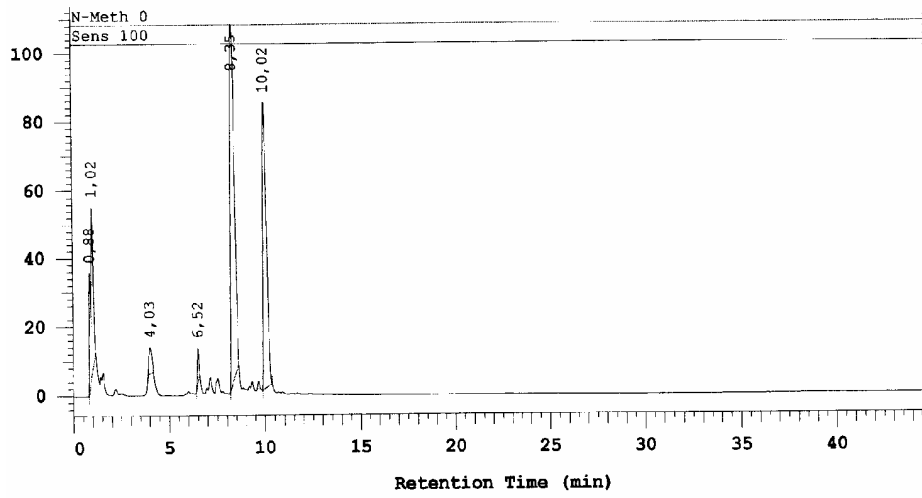
II) 5'-TTXTT (m/z = 1554) activator: pyrHCl



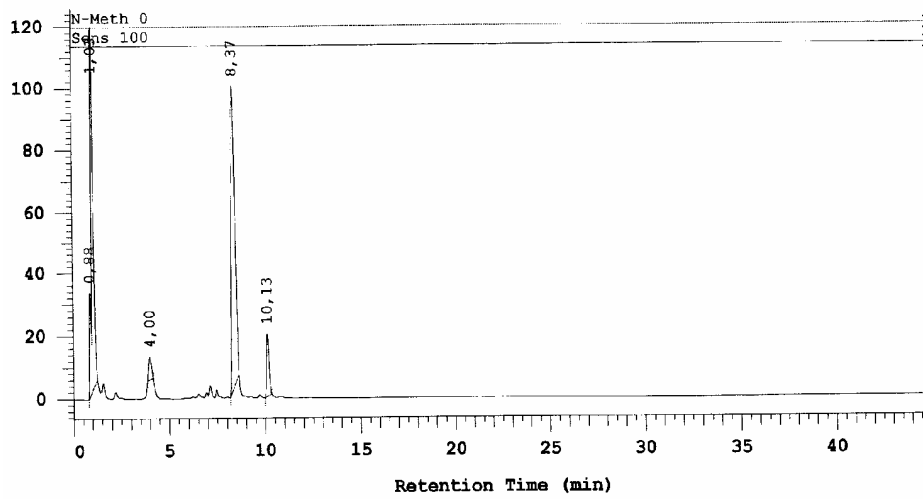
III) 5'-TTXTT activator: 1H-tetrazole



II spiked with I



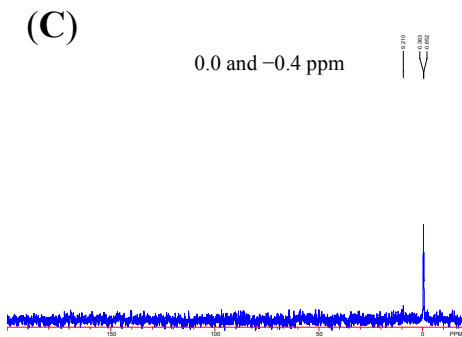
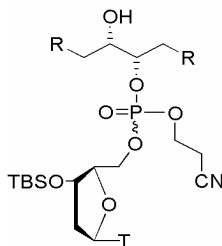
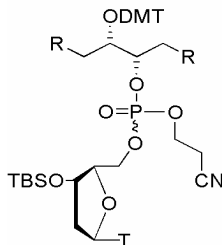
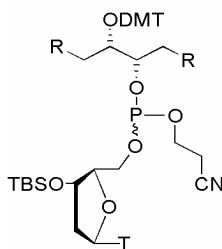
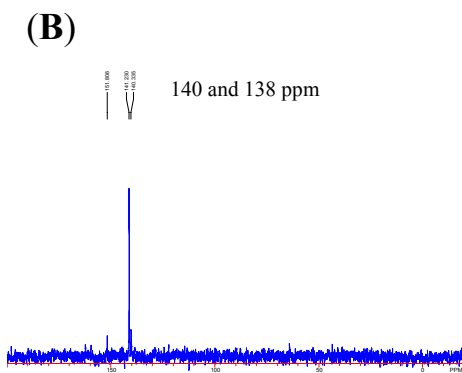
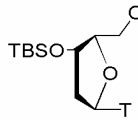
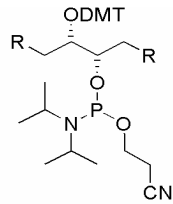
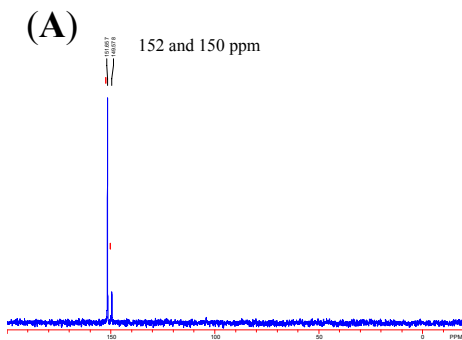
III spiked with I



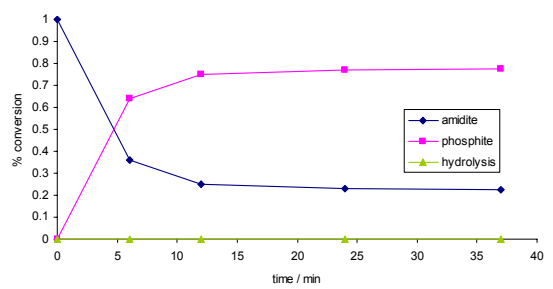
ESI-Figure 4. ^{31}P -NMR study of the coupling sequence using pyrHCl. The 128 s spectra were acquired on a 200 MHz Varian GEMINI using external reference and a sealed capillary tube inside the NMR tube with $\text{DMSO-}d_6$ to provide a deuterium lock signal.

In an NMR-tube, compound **8b** (0.16 g, 0.15 mmol) and 3'-*O*-*tert*-butyldimethylsilylthymidine (0.064 g, 0.18 mmol) was dissolved in MeCN (600 μL), and the first spectrum (**A**) of the amidite was acquired. Then a 0.5 M solution of pyrHCl in MeCN (0.43 mL, 0.22 mmol) was added and another spectrum (**B**) of the condensed phosphite product was acquired after 3 h. However, as shown in the figure (**D**), the reaction is complete after ca. 10 min. As a residue of amidite appeared in the spectrum the reaction was left for two days, but no further reaction was observed. Then, a 5.5 M solution of *tert*-butylhydrogenperoxide in decane (0.05 mL, 0.28 mmol) was added and a spectrum (**C**) was acquired after 3 h.

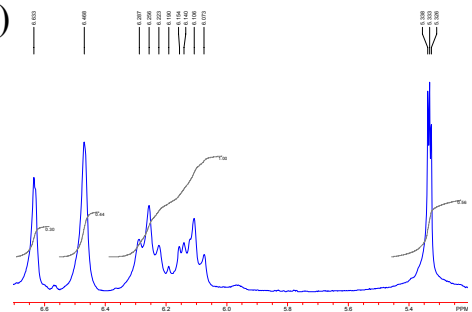
The following detritylation was monitored by ^1H -NMR by dissolving the dinucleotide (47 mg) in CD_2Cl_2 and recording a spectrum (**E**). Then a catalytic amount of trichloroacetic acid was added and the spectrum (**F**) was acquired after 10 min.



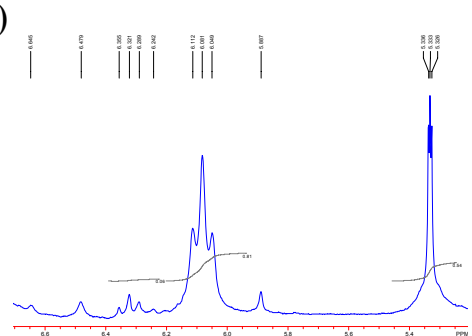
(D)



(E)



(F)



ESI-Figure 5 (supporting Figure 5 in the paper). ^{31}P -NMR study of the detritylation step using trichloroacetic acid in CD_2Cl_2 at $T = 24^\circ\text{C}$. ^{31}P NMR ($t = 2$ h) and curve showing the development in the reaction mixture based on ^{31}P NMR, $\delta \sim 0$ corresponds to the detritylated product, $\delta \sim 15$ to the cyclic phosphate and $\delta \sim -1$ corresponds to hydrolysis of the cyclic phosphate.

