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Microwave-assisted synthesis of a triazole-linked 3′–5′ dithymidine using click chemistry

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Abstract

Synthesis of a triazole-linked 3′–5′ thymidine dimer making use of 1,3-dipolar cycloaddition is described. The azido-precursor was obtained by regioselective chlorination of thymidine, followed by azidation. The second precursor, a propargyl derivative, was obtained by selective 3′-O-alkylation of thymidine. Two 'click systems' were compared to obtain the desired dimer. These reactions were performed by microwave irradiation.

Keywords: Dithymidine; Click chemistry; Microwave; Selective chlorination

Oligonucleotides have attracted a lot of interest in biochemistry and genomic research.[1] Among their numerous applications, they could be used in antisens or triple helix therapy to inhibit RNA translation or DNA transcription.[2] One of the most radical modifications brought to the familiar backbone is the complete substitution of the phosphodiester bridge, to achieve stronger affinity for the nucleic acid target, and/or enhanced resistance to nucleases, or improved membrane permeability and cellular uptake. Our research effort in this area has focused on the synthesis of new 3′–5′ dinucleotide analogues. The need of a rapid, efficient and highly chemoselective reaction for the building of 3′–5′ dinucleosides led us to choose the 'click chemistry' reaction defined by Sharpless et al.[3] and Tornoe et al.[4] Microwave (MW) activation was used whenever possible (Schemes 1 and 2) in continuation of our programme aimed at studying the influence of microwave on organic synthesis.[5]

Precursors **2** and **4** were synthesized from thymidine. Selective chlorination of the primary hydroxyl group of thymidine was obtained with tosylchloride (1.5 equiv) in dry DMF giving after microwave activation (1 min, 80 °C, 300 W) 5′-chloro-5′-deoxythymidine **1** in 80% yield. According to McCormick,[6] the first step (Scheme 3) appears to be the formation of a *O*-(*p*-toluenesulfonyl)-*N*,*N*-dimethyl acetiminium salt (**A**).

The second step consists in a nucleophilic displacement of the tosylate group followed by an attack of the chloride ion on the iminium carbon, displacing the sulfonate group, with the subsequent displacement of chlorine by thymidine.

Then, the chloroiminium thymidine intermediate can be attacked by a chloride ion to produce 5′-chloro-5′-deoxythymidine. Iminium salt (**B**) was isolated, analysed and its structure confirmed.

5′-Chloro-5′-deoxythymidine **1** was then azidated using 15 equiv of sodium azide in DMF. After 3 min of microwave irradiation (80 °C, 300 W), we observed by TLC the conversion **1**→**2**: the two compounds had similar R_f values,[7] but compound **2** gave intense purple spots after spraying PPh_3/Et_2O followed by ninhydrin and heating. NMR

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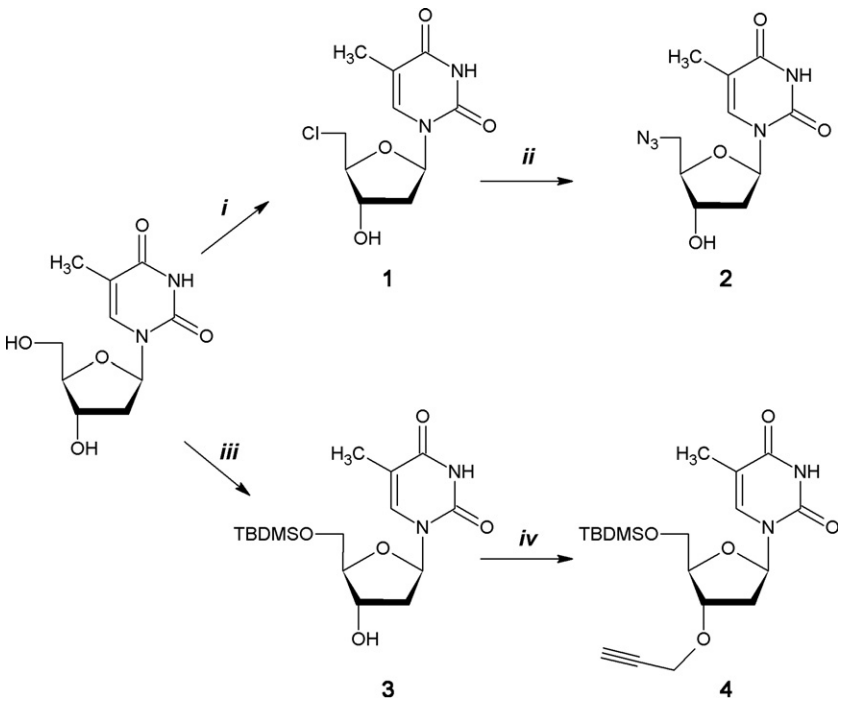
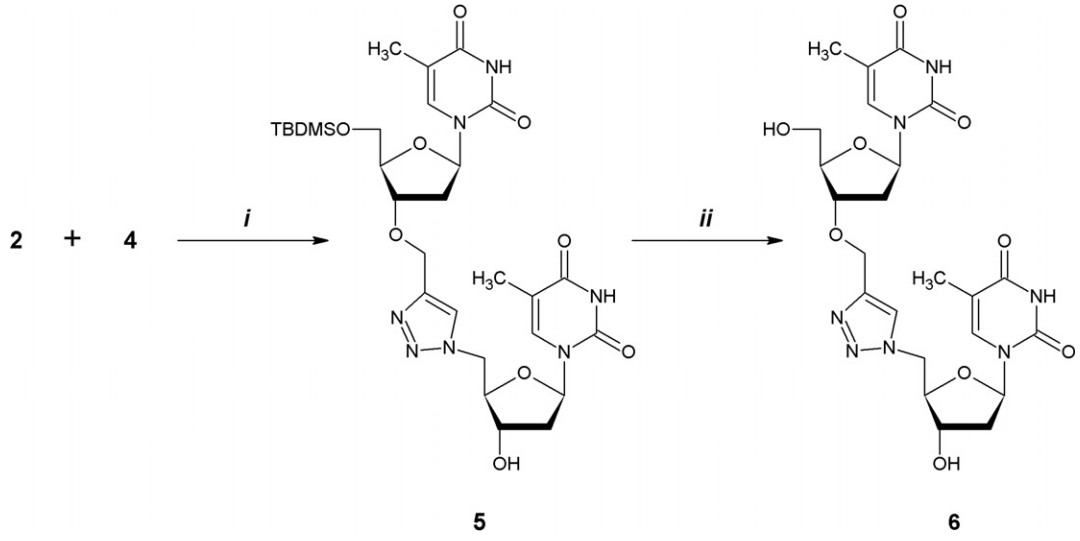


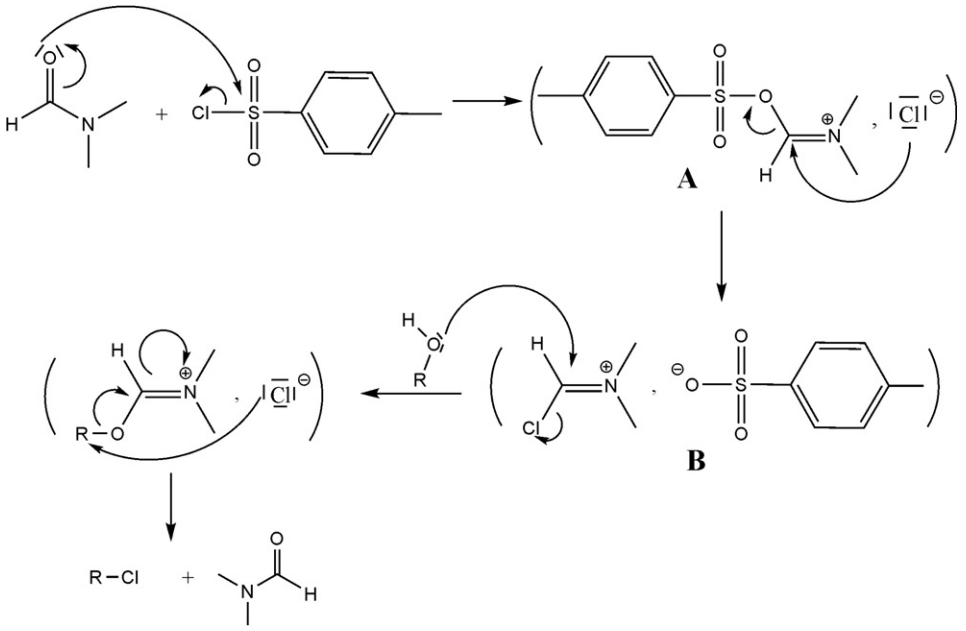
Scheme 1. Synthesis of azido- and propargyl-precursors (**2** and **4**). Reagents and conditions: (i) TsCl, DMF, MW (80 °C, 300 W, 1 min); (ii) NaN_3, DMF, MW (80 °C, 300 W, 3 min); (iii) TBDMSCl, DMAP, Pyr, 10 h; (iv) 1-NaH, THF, MW (40 °C, 200 W, 3 min), 2-propargylbromide, MW (40 °C, 200 W, 3 min).



Scheme 2. Click chemistry and deprotection. Reagents and conditions: (i) (cf Table 1) CuI (1 equiv), DIPEA (3 equiv), DMF; or $CuSO_4$ (0.01 equiv), Na ascorbate (0.1 equiv) H_2O/EtOH; (ii) TBAF (1.2 equiv), THF, rt, 7 h.

analysis of reaction the products indicated that the reaction had proceeded to virtual completion; after purification, compound **2** was obtained in 91% yield. Precursor **4** was synthesized first by selective protection of the 5′-hydroxyl group with *tert*-butyldimethylsilyl chloride (1.2 equiv) in dry pyridine in the presence of DMAP (0.05 equiv)[8] giving 5′-*tert*-butyldimethylsilylthymidine **3** in high yield (98%). The 3′-hydroxyl was then alkylated using Chattopadhyaya's method with NaH (2.5 equiv) and propargylbromide (2.5 equiv) in THF[9] and MW activation to give compound **4** in quantitative yield.

The final step consisted in the Huisgen's 1,3-dipolar cycloaddition[10] between compounds **4** and **2**, using copper salts as catalysts. Two different Cu salts, CuI and $CuSO_4$, have been tested and click reactions were realised with either classical heating or microwave activation.[11] Respective yields and reaction times are summarized in Table 1.

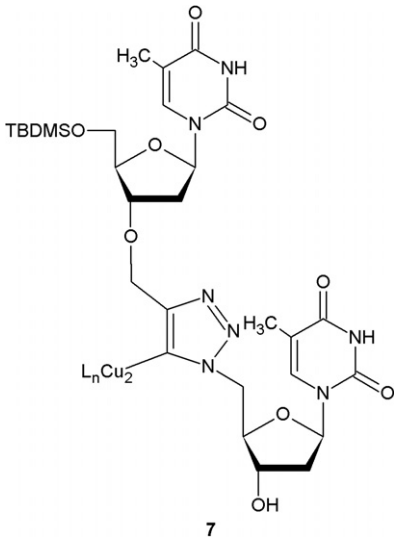


Scheme 3. Proposed mechanism of selective chlorination.

Table 1
Selected results of click reactions

Click system	Activation	Reaction time	Isolated yields (%)
CuI (1 equiv), DIPEA (3 equiv), DMF	rt	24 h	36
	rt	72 h	36
	Δ: 80 °C	5 h	61
	MW: 80 °C, 200 W	1 min	66
	MW: 80 °C, 200 W	3 min[a]	85
$CuSO_4$ (0.01 equiv), Na ascorbate (0.1 equiv) H_2O/EtOH	rt	24 h	40
	rt	72 h	44
	Δ: 80 °C	5 h[a]	80
	MW: 80 °C, 200 W	1 min	62
	MW: 80 °C, 200 W	3 min[a]	80

[a] Reaction times allowing virtual reaction completion (checked by TLC), longer reaction times gave no increase in reaction yields.



Scheme 4. Hypothetical structure of secondary product **7**.

Results indicate that microwave activation gave good yields after only 3 min of reaction. With CuI system, TLC shows total conversion into the click product. Furthermore we observed the formation of a side product at room temperature. In this case TLC showed two products with different R_f values.

Mass and ^{1}H NMR analyses of the isolated secondary product (**7**) are consistent with a supposed structure (Scheme 4), in accordance with the formation of an instable intermediate described in the literature.[12] In the ^{1}H NMR spectrum the triazole hydrogen does not show up at 8.09 ppm and, furthermore, the ^{13}C NMR signal of the corresponding carbon is shifted from 124.7 ppm in compound **5** to 147.3 ppm in compound **7**, which could be explained by the electro-attractive effect of bound copper. Mass analysis confirmed the presence of two bound copper atoms in the dimer structure ($m/z = 787.5$). After three months at room temperature, TLC analysis shows that this secondary product spontaneously evolved towards compound **5** that displays an increased charring intensity.

Click reaction using $CuSO_4$ gave a single compound. We observed in this case that classical heating (80 °C, 5 h) and

microwave activation (80 °C, 200 W, 3 min) gave identical results (80% yield). The last step is a deprotection of the 5′-alcohol function to give the expected dimer **6** in 70% yield (Scheme 2).

In conclusion, we have investigated a total synthesis of a 3′–5′ thymidine dimer using a 'click chemistry' reaction with MW irradiation to reduce reaction time. This work is presently continuing towards tri- and tetra-nucleoside synthesis with promising results.

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Supplementary data

Supplementary data (spectroscopic data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.12.012.

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