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A 'click chemistry' approach to the efficient synthesis of modified nucleosides and oligonucleotides for PET imaging

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ABSTRACT

Different thymidine derivatives bearing either an iodoaryl moiety at the 5′ position or a dialkylsilyl group at the 3′ position have been efficiently synthesized as precursors for carbon-11 or fluorine-18 labeling, respectively. Furthermore, iodoarylated thymidine derivatives have been incorporated into oligonucleotides giving an original way to label them with carbon-11.

Positron Emission Tomography (PET) is a powerful imaging technique for clinical, medical, and biological investigations in various areas such as oncology, cardiology, and neurosciences. Due to the increasing need of this technique in in vivo biochemistry and medicine, the development of new tracers and radiolabeling strategies is always requested.[1–3] Nucleosides as PET tracers have recently been investigated by a number of institutions.[4] Among them, the [^{18}F]-fluorothymidine (FLT) has even become the second tracer, after [^{18}F]-fluorodeoxyglucose (FDG), for routine clinical PET. Furthermore, aptamers ('adaptable oligomers') can be generated against any small molecules or protein targets using a completely synthetic method (SELEX).[5] Thus, the use of aptamers for in vivo imaging is specially promising because of the wide range of possibilities available to introduce variations in their structure through defined chemical modifications. Only a very few examples of oligonucleotides labeling for PET were reported with gallium-68,[6] carbon-11,[7] and fluorine-18,[8] and to the best of our knowledge there is only one report of a fluorine-18 labeling attempt to an oligonucleotide aptamer.[9]

However, the chemistry of carbon-11 is nowadays representing an essential tool for the clinical research purpose.[10] In that way we recently developed a modified version of the Stille coupling allowing an efficient transfer of alkyl groups,[11] and the preparation of a [^{11}C]-methylstannate reagent[12] as well as its application to the labeling of functionalized quinolines.[13] In addition to the organotin-mediated carbon-11 transfer reaction, the use of organosilicon chemistry for fluorine-18 labelling of peptides has been recently described by Ametamey's group.[14,15] We then envisaged first the synthesis of modified thymidines bearing either an iodoaryl moiety or a dialkylsilyl group, and then the incorporation of iodoarylated thymidines into oligonucleotides (Fig. 1).

The 5′-iodoarylated thymidines **4**, **7,** and **9** were all synthesized from thymidine aldehyde **1**, prepared according to the Pfitzner–Moffatt procedure,[16,17] and by using a 3+2 cycloaddition of an azide with an alkyne so called 'click' chemistry as conjugation mode between the arm bearing an iodoaryl moiety and the nucleoside (Scheme 1). Two ways were explored by using either the azidothymidine **2** or the propargyl-thymidine **5**.[18] Thus, **2** was obtained in two steps: the first one being a Sakuraï reaction between **1** and the ω-bromoallyltrimethylsilane, easily prepared itself from ω-hydroxyallyltrimethyl-silane,[19] leading after treatment with $TiCl_4$, to the 5′-C(*S*)-bromopentenyl thymidine with a 55% overall yield.[20,21] Then, the azido group needed for 'click chemistry' was introduced by action of sodium azide in anhydrous dimethylformamide at 80 °C providing **2** in 89% yield. The 'click reaction' was then realized using the alkyne **3** as the partner, prepared from 4-iodophe-

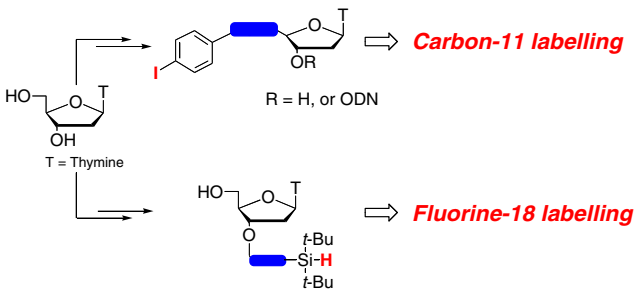

Figure 1. Modified nucleosides or oligonucleotides for PET imaging.

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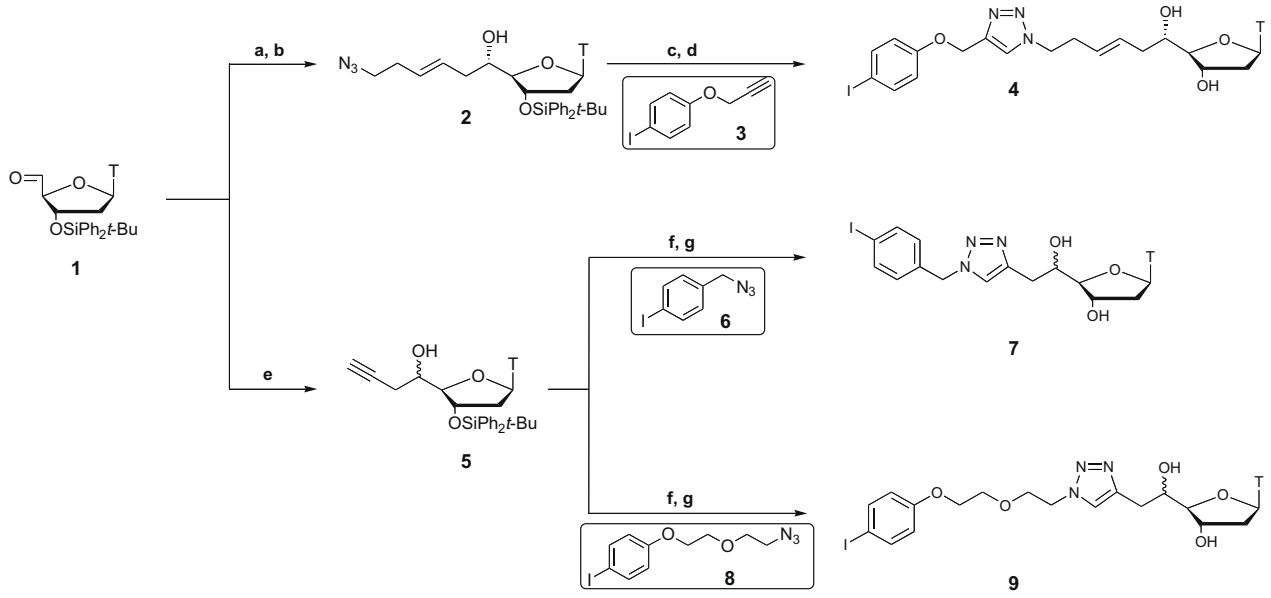
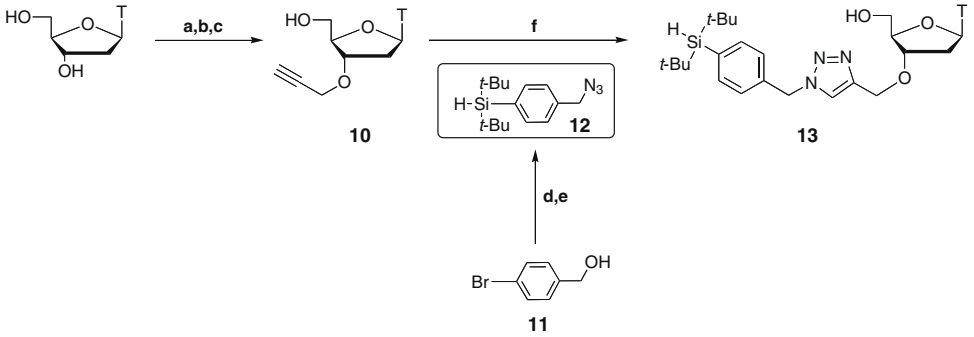


Scheme 1. Synthesis of 5′-iodoarylated thymidines **4**, **7**, and **9**. Reagents, conditions, and yields: (a) (i) ω-bromoallyltrimethylsilane, $BF_3.Et_2O$, CH_2Cl_2, −78 °C to rt, 4 h; (ii) $TiCl_4$, CH_2Cl_2, 0 °C, 5 min, 55%; (b) NaN_3, DMF, 80 °C, 3 h, 95%; (c) 1-iodo-4-(prop-2-ynyloxy)benzene **3**, $CuSO_4$ (20 mol %), sodium ascorbate (60 mol %), *t*-BuOH/H_2O (3:1), rt, 12 h, 89%; (d) NH_4F, MeOH, 60 °C, 24 h, 86%; (e) propargyl bromide, Zn (activated), THF, 10 °C, 5 min, 91% (*S*/*R*: 70:30); (f) $CuSO_4$ (20 mol %), sodium ascorbate (60 mol %), *t*-BuOH/H_2O (3:1), rt, 12 h, 84% starting from **6** and 80% from **8**; and (g) NH_4F, MeOH, 60 °C, 24 h, 85% starting from **6** and 79% from **8**.

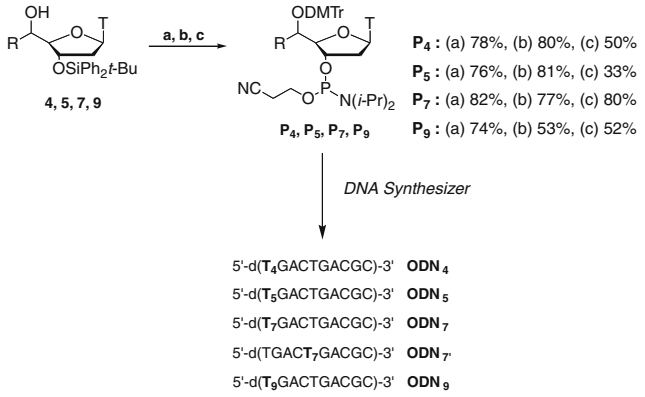
nol and propargyl bromide via a Williamson reaction, providing the corresponding triazolyl derivative in 89% yield. Finally, the deprotection of the silylated group, with NH_4F in methanol at 60 °C, allowed us to get the desired iodoarylated thymidine **4** in 86% yield**.** On the other hand, the alkyne-thymidine **5** was obtained in 91% yield, in a 70:30 *S*/*R* ratio, by treating **1** with propargyl bromide, in the presence of activated zinc. At this stage, the two diastereoisomers are separable by column chromatography, but we decided to use the mixture for the next steps. **5** was then engaged into two 'click reactions' involving two azides according to a procedure described by Thomson et al.:[22] **6** prepared from 4-iodobenzylic alcohol and **8** prepared by azidation of the alcohol resulting from the Williamson reaction between monotosylated diethylene glycol[23] and 4-iodophenol. Thus, the corresponding triazoles were obtained in 84% and 80% yields, respectively, and the deprotection of the 3′ position, using NH_4F in MeOH led, finally, to **7** and **9** in 85% and 79% yields, respectively.

Besides the compounds envisaged for the carbon-11 labeling, we have also developed a synthesis, involving the 'click chemistry', to modify a thymidine at the 3′ position with a di-*tert*-butylsilyl group in order to label it with fluorine-18 (Scheme 2). Thus, the introduction of an alkyne group at the 3′ position of a thymidine was done in three steps, involving the protection of the primary alcohol in 5′ with TBDPS, the propargylation, using the propargyl bromide, and finally the deprotection of the 5′ position leading to the desired compound **10**, in a 61% overall yield.[24] On the other hand, the suitable azide partner was obtained in two steps from 4-bromobenzyl alcohol **11**, prepared following a variation of Schreiber's Letter.[25] The first one was the introduction of the silyl group, by treating the lithiated derivative with di-*tert*-butylchlorosilane, leading to compound **12** in 50% yield. Then, the 'click reaction' between **10** and **12** was done under classical conditions, leading to the desired 3′-silylated thymidine **13** in 77% yield. Of course, the two strategies are versatile authorizing labeling with carbon-11 or fluorine-18 in both 3′ and 5′ positions. Indeed, azide **12** can react with propargyl-thymidine **5** giving access to the 5′-silylated thymidines and in the same way azide **6** can react with propargyl-thymidine **10** giving the 3′-iodoarylated thymidines.

After obtaining modified monomers, the next step was to introduce the corresponding phosphoramidites into oligonucleotides (Scheme 3). Thus we have envisaged to synthesize 10-mer oligonucleotidic sequences, composed of the four bases and including



Scheme 2. Synthesis of 3′-silylated thymidine **13**. Reagents, conditions, and yields: (a) TBSCl, pyridine, rt, 24 h, 95%; (b) NaH, propargylbromide, THF, rt, 12 h, 76%; (c) PTSA, MeOH, rt, 24 h, 97%; (d) (i) NaH, THF, 50 °C, 3 h, (ii) *t*-BuLi, THF, −78 °C, (iii) $ClSiH(t\text{-}Bu)_2$, 50%; (e) DBU, DPPA, DMF, 60 °C, overnight, 80%; and (f) 1-(azidomethyl)-4-(di-*tert*-butylsilyl)-benzene **12**, $CuSO_4$ (20 mol %), sodium ascorbate (60 mol %), *t*-BuOH/H_2O (3:1), rt, overnight, 77%.



Scheme 3. Synthesis of phosphoramidites $\mathbf{P_4}$, $\mathbf{P_5}$, $\mathbf{P_7}$, and $\mathbf{P_9}$. Reagents and conditions: (a) DMTrCl, $AgNO_3$, collidine, THF, rt, 12 h; (b) TBAF, THF, rt, 12 h; and (c) (2-cyanoethyl)(*N*,*N*-diisopropylamino)chlorophosphite, $(i\text{-Pr})_2$EtN, THF, rt, 45 min.

either phosphoramidites $\mathbf{P_4}$, $\mathbf{P_5}$, $\mathbf{P_7}$, or $\mathbf{P_9}$ (the modified thymidine being noted $\mathbf{T^*}$ in the sequence) at the 5′ position (5′-d($\mathbf{T^*}$GACTGACGC)-3′) or even in the middle of the sequence with $\mathbf{P_7}$ (5′-d(TGAC$\mathbf{T^*}$GACGC)-3′). To do that, each monomer was converted first into the corresponding phosphoramidites, in a three-step procedure (Scheme 3).

The first one was the protection of the 5′ position with a DMTr (*p*-dimethoxytrityl) group.[21] The second step was the deprotection of the hydroxyl group at the 3′ position, and finally, the third one was the introduction of the phosphoramidite function at the 3′ position leading to $\mathbf{P_4}$, $\mathbf{P_5}$, $\mathbf{P_7}$, and $\mathbf{P_9}$. Oligonucleotides were then synthesized on 1 μmol scale by a standard phophophoramidite methodology. On the basis of trityl assays and materials recovered, $\mathbf{P_4}$, $\mathbf{P_5}$, $\mathbf{P_7}$, and $\mathbf{P_9}$ were incorporated with the same efficiency as the commercial phosphoramidites. The five oligonucleotides were then deprotected and removed from the solid support with concentrated ammonia. After HPLC purification, the isolated yields were in the range of those classically obtained for modified oligonucleotides. $\mathbf{ODN_4}$, $\mathbf{ODN_5}$, $\mathbf{ODN_{7,}}$ and $\mathbf{ODN_9}$ were characterized by mass spectroscopy (MALDI-TOF).

In conclusion, 5′- and 3′-modified original thymidines, envisaged as precursors for either carbon-11 or fluorine 18 labeling, can be prepared in a very efficient way by using 'click chemistry' as the conjugation step. By the way, we have already obtained good results in terms of methyl transfer with **4**, **7,** and **9** by applying our modified Stille's coupling reaction[11,12] and preliminary results, concerning the fluorination of **13**, were obtained by applying the conditions described by Ametamey's group[12] are particularly encouraging. These results will be reported in due course. Iodoarylated monomers have been successfully introduced into oligonucleotides, leading to various substrates usable to investigate our [^{11}C]-methyl group transfer methodology. Finally $\mathbf{ODN_5}$, the oligonucleotide in which the modified thymidine bears a pendant terminal alkyne, will be used as a versatile substrate to test direct 'click reactions'[18,26] either with **6**, **12** or with other suitable azido partners offering a very original and powerful way to get various precursors, from the same oligonucleotide, either for the carbon-11 or for fluorine-18 labeling.

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Supplementary data

Supplementary data (experimental details of chemistry and full characterizations of compounds **1**–**13** as well as the oligonucleotides $\mathbf{ODN_4}$, $\mathbf{ODN_5}$, $\mathbf{ODN_7}$, $\mathbf{ODN_{7'}}$ and $\mathbf{ODN_9}$) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.120.

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