ChemPubSoc Europe

DOI: 10.1002/open.201402099

Open Access
ChemistryOPEN
Full Papers

Assessment of the Full Compatibility of Copper(I)-Catalyzed Alkyne-Azide Cycloaddition and Oxime Click Reactions for bis-Labelling of Oligonucleotides

Sandra Estalayo-Adriàn,[a] Rémy Lartia,[a] Albert Meyer,[b] Jean-Jacques Vasseur,[b] François Morvan,[b] and Eric Defrancq*[a]

The conjugation of oligonucleotides with reporters is of great interest for improving their intrinsic properties or endowing new ones. In this context, we report herein a new procedure for the bis-labelling of oligonucleotides through oxime ligation (Click-O) and copper(I)-catalyzed alkyne–azide cycloaddition (Click-H). 5′-Azido and 3′-aldehyde precursors were incorporated into oligonucleotides, and subsequent coupling reactions through Click-O and Click-H (or vice versa) were successfully achieved. In particular, we exhaustively investigated the full compatibility of each required step for both tethering strategies. The results demonstrate that click Huisgen and click oxime reactions are fully compatible. However, whilst both approaches can deliver the targeted doubly conjugated oligonucleotide, the route involving click oxime ligation prior to click Huisgen is significantly more successful. Thus the reactions investigated here can be considered to be key elements of the chemical toolbox for the synthesis of highly sophisticated bioconjugates.

Introduction

A major approach for improving oligonucleotide (ODN) properties is conjugation that is achieved by attaching molecules with relevant properties (pro-moiety) to ODN through covalent bonds. Conjugation aims to either improve existing oligonucleotide properties (e.g., stability, cell uptake, targeted delivery, etc.) or endow it with entirely new property (e.g., fluorescence). A large number of pro-moieties, such as antibodies, peptides and proteins, carbohydrates, lipids, polymers, fluorophores, and photoprobes, have been attached to ODNs to obtain conjugates for various applications.[1–3] In most cases, ODNs are conjugated at the 5′ or 3′ terminus because of the easier accessibility of the termini but conjugation at both extremities is also advantageous for purposes such as protection against nucleases and/or combination of reporter properties (e.g., fluorescent probe and cell penetrating peptide). Conjugation of a reporter group at the extremity is also expected to have a lesser impact on the hybridization properties than conjugation at an internal position.

Several synthetic approaches have been developed so far to prepare ODN conjugates, and these approaches can be grouped into two main categories: 1) on-support conjugation, where conjugation is carried out using support-bound ODNs, and 2) solution-phase conjugation, where ODNs are cleaved from the solid support, deprotected, and purified, prior to solution-phase conjugation. During the past decade significant efforts have been made to improve the coupling reaction, which should ideally meet click chemistry criteria.[4] In this context, the well-known copper(I)-catalyzed Huisgen 1,3-cycloaddition of azides and alkynes (CuAAC or Click-H) has emerged as a reaction of choice for ODN conjugation through 1,2,3-triazole linkages.[5] CuAAC reaction has been used for the successive incorporation of reporters at internal positions or at 3′, 5′ extremities.[6,7] In the same framework, bis-conjugation at both extremities has been accomplished through oxime ligation (Click-O) between aminooxy and aldehyde functions, using sequential conjugation/deprotection reactions.[8,9] Despite their respective benefits, click-O and click-H have seldom been used in conjunction for bis-conjugation.[10,11]

In a recent paper, we described the preparation of 5′-alkyne, 3′-diol ODN as a precursor for sequential bis-conjugation via click-O and Click-H reaction.[10] The present work aims at emphasizing this strategy by starting from another material, that is an ODN containing azide (for Click-H reaction) and aldehyde (for Click-O reaction) precursors for solution-phase conjugation. We investigated the full orthogonality of all reactions used for generating both 1,2,3-triazole and oxime bonds. In particular, we examined the stability of the azide and aldehyde functionalities during the oxidation (required for introduction of the al-

[a] S. Estalayo-Adriàn, Dr. R. Lartia, Prof. E. Defrancq
Département de Chimie Moléculaire UMR CNRS 5250
Université Grenoble Alpes
38041 Grenoble Cedex 9 (France)
E-mail: Eric.Defrancq@ujf-grenoble.fr

[b] A. Meyer, Dr. J.-J. Vasseur, Dr. F. Morvan
Institut des Biomolécules Max Mousseron, UMR 5247 CNRS
Université de Montpellier
34095 Montpellier Cedex 5 (France)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/open.201402099.

© 2014 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

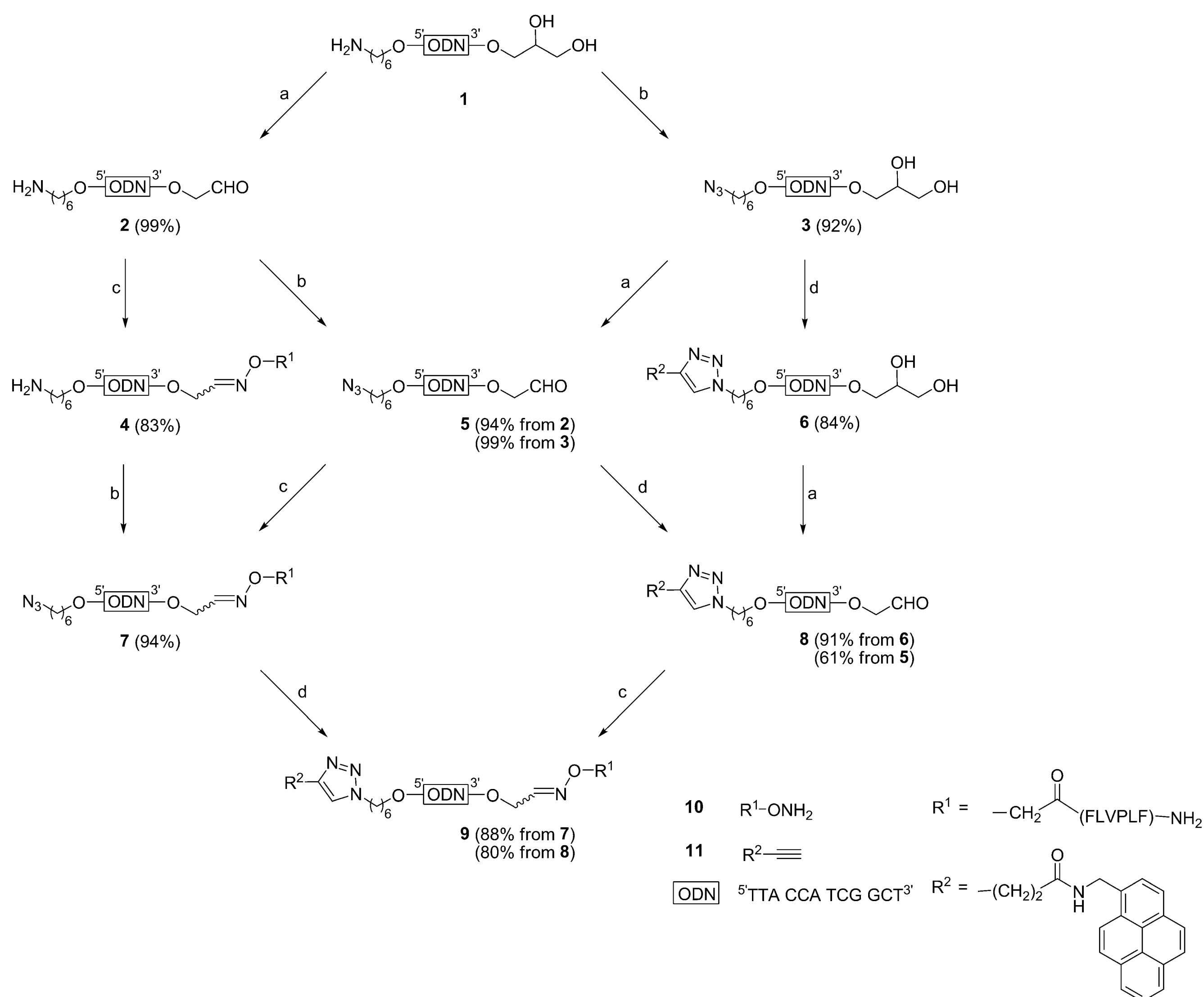
dehyde moiety from the diol precursor) and the azido-transfer reaction (required for introduction of the azide moiety from an amino precursor) steps, respectively.

Results and Discussion

The major advantage of the solution-phase conjugation approach is that a reporter molecule with even incompatible chemistries can be conjugated to oligonucleotide. Furthermore, the individual fragments can be prepared, purified, and characterized separately by using the most efficient chemistry available. In this context, conjugation through Click-H is frequently performed between an ODN bearing the alkyne moiety and an azide-containing reporter group since the stability of the azide moiety is not fully compatible with the classical phosphoramidite chemistry for ODN synthesis (due to the interfering Staudinger reaction that takes place between azides and P^{III} derivatives).[12,13] As a consequence, post-synthetic introduction of the azide function has been developed.[14,15]

Recently, we proposed the use of a diazo transfer (DZT) reagent that enables clean and efficient conversion of aminated ODNs into their azido counterparts under mild conditions.[16] The introduction of the azido moiety through DZT reaction is an interesting alternative, which could offer different ways for accessing bis-conjugated ODNs (Scheme 1). Indeed the oxime ligation could be first performed and followed by the CuAAC reaction (**1**→**2**→**4**→**7**→**9** route) or vice versa—that is, the CuAAC reaction followed by the oximation (**1**→**3**→**6**→**8**→**9** route). It is also noticeable that compound **5**, which could arise from **2** or **3** and bears both azide and aldehyde functional moieties, represents a key intermediate for both subsequent coupling reactions (**5**→**7**→**9** and **5**→**8**→**9** routes).

We thus investigated the chemical compatibility of the azide moiety and its introduction through the DZT reaction with all the required reactions for the introduction of a reporter group



Scheme 1. Synthesis of oligonucleotide bis-conjugates from 5′-amino, 3′-diol ODN **1**. Reaction a): diol oxidation; reaction b): diazo transfer (DZT); reaction c): oxime ligation; reaction d): CuAAC. For reagents and conditions, see Experimental Section.

 © 2015 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

through click-O strategy. In particular, the following key points were examined: 1) the stability of the sensitive aldehyde and the oxime bond during DZT (reaction b in Scheme 1)—that is, for **2**→**5** and **4**→**7** conversions, respectively; 2) the stability of the azide moiety during diol oxidation (reaction a in Scheme 1)—that is, for **3**→**5** conversion as well as during the oxime ligation (reaction c in Scheme 1)—that is, for **5**→**7** conversion.

For this purpose, 12-mer ODN **1** containing the four canonical nucleobases and 5′-amino and 3′-diol functions was prepared. Two reporters groups were chosen for subsequent conjugation reactions: a model hexapeptide (**10**) exhibiting an aminooxy function, as peptide–oligonucleotide conjugates (POCs) are promising biomolecular tools used in many purposes,[17] and alkyne-pyrene derivative **11** as this fluorophore is frequently used as a hybridization probe.[18] Compounds **10** and **11** were prepared by classical procedures (see the Supporting Information). Starting material 5′-amino, 3′-diol ODN **1** was synthesized using commercially available 5′-aminolinker phosphoramidite and glyceryl long-chain alkyl amine (LCAA)-controlled pore glass (CPG) support on a DNA synthesizer using phosphoramidite chemistry (for detailed protocols, see the Supporting Information).

Strategy starting from oxime ligation followed by CuAAC reaction

For this strategy, we envisioned two different routes. The first one (**1**→**2**→**4**→**7**→**9** route, Scheme 1) consists of a sequential approach—that is, the formation of the aldehyde by oxidation of the diol precursor then oximation followed by the formation of the azide moiety through DZT reaction and finally CuAAC reaction. In the second approach (**1**→**2**→**5**→**7**→**9** route, Scheme 1), key intermediate **5** (bearing both aldehyde and azide functionalities) is first formed for subsequent oximation and CuAAC reactions.

1→2→4→7→9 route

According to this route (Scheme 1), the diol functional group of ODN **1** was first oxidized by sodium *meta*-periodate to afford the corresponding 3′-aldehyde ODN (**2**), which was purified by size-exclusion chromatography to remove excess of sodium periodate. ODN **2** was further conjugated with aminooxy peptide **10** in ammonium acetate buffer at pH 4.5 to give ODN **4** within four hours in 83% yield (Table 1 and Figure S3 in the Supporting Information). It should be noted that the Schiff base, which could be formed through reaction of 5′-amino moiety with the 3′-aldehyde, did not perturb the oxime ligation; this might be due to the slightly acidic conditions required for oxime ligation leading to less reactive protonated 5′-amino moiety (on the contrary, the aminooxy moiety is mostly unprotonated under the conditions used here. A control reaction monitoring ODN **2** in solution over a 24-hour period did not shown the presence of any cyclic and/or dimeric species, which could be formed through Schiff base.

Table 1. Reaction yields for ODN formation and mass spectrometry data for ODNs **1**–**9**.

ODN	Reaction	Yield[a] [%]	$[M+H]^+$ (*m/z*) calcd	exptl
1	–	–	3928.6	3928.2[b]
2	**1**→**2**	99	3896.5	3894.6[b]
3	**1**→**3**	92	3954.6	3953.7[b]
4	**2**→**4**	83	4685.5	4685.6[b]
5	**2**→**5**	94	3922.5	3921.3[b]
5	**3**→**5**	99	3922.5	3922.3[b]
6	**3**→**6**	84	4265.9	4264.7[c]
7	**4**→**7**	94	4711.5	4711.2[b]
7	**5**→**7**	86	4711.5	4710.8[b]
8	**5**→**8**	61	4233.9	4234.0[c]
8	**6**→**8**	91	4233.9	4234.0[b]
9	**7**→**9**	88	5022.9	5022.5[c]
9	**8**→**9**	80	5022.9	5023.0[b]

Data obtained from [a] HPLC, [b] ESI-MS, [c] MALDI-TOF MS analysis.

The DZT reaction was next performed with ODN **4**, using imidazole-1-sulfonyl azide hydrochloride as DZT reagent in presence of copper(II) sulfate at 60 °C for one hour.[16] As shown in the HPLC profile of crude mixture (Figure S4 in the Supporting Information), ODN **4** was transformed to azide-containing ODN **7** as the major compound (Table 1) demonstrating that the oxime bond is stable in these conditions. ODN **7** was next reacted with alkyne-pyrene **11** in the presence of copper(II) sulfate and sodium ascorbate for six hours at room temperature. It should be noted that microwave (MW) assistance could not be applied when an oxime bond is present as it is for **7** since it has been shown that the oxime linkage is unstable under such conditions.[10] The HPLC profile of the crude mixture (Figure S5 in the Supporting Information) showed the full conversion of compound **7** with the formation of bis-conjugate **9** as the major product and the presence of excess alkyne-pyrene **11**, which was easily removed by size-exclusion chromatography. Ultimately, compound **9** was purified by C18 reversed-phase HPLC.

1→2→5→7→9 route

ODN **5**, which contains both functionalities for oximation and CuAAC reactions, represents a key intermediate for the bis-conjugation. Two important points must be taken into account for validating this route: 1) the stability of the aldehyde moiety during the DZT reaction (reaction b from **2** to **5**), and 2) the stability of the azide moiety during the oximation reaction (reaction c from **5** to **7**).

The DZT reaction was performed with ODN **2** (thus in the presence of the aldehydic moiety at the 3′ terminus) under the same conditions as described above. The HPLC profile of the crude mixture shows the formation of a major product corresponding to ODN **5** (Figure S6 in the Supporting Information). Importantly, no degradation of the aldehydic moiety was observed as confirmed by electrospray ionization mass spectrometry (ESI-MS) analysis. After reversed-phase HPLC purification, ODN **5** was reacted with aminooxy peptide **10** affording ODN **7** in 86% yield. No degradation of the azide moiety was ob-

© 2015 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

served during the oximation step (Figure S7 in the Supporting Information). So-obtained ODN **7** was then conjugated with alkyne-pyrene **11** through a CuAAC reaction under the same conditions as outlined above affording bis-conjugate **9**; no difference in reactivity of ODN **7** obtained from this route (i.e., from **5**) was observed in comparison with ODN **7** obtained from the aforementioned route (i.e., from **4**).

Strategy starting from CuAAC reaction followed by oxime ligation

Again two different routes could be envisaged for this strategy. The first one (**1**→**3**→**6**→**8**→**9** route) involves a sequential approach—that is, the formation of the azide moiety then the conjugation through CuAAC reaction followed by the formation of the aldehyde by oxidation and finally the oximation. In the second approach (**1**→**3**→**5**→**8**→**9** route), key intermediate **5** is first formed from **3** for subsequent CuAAC reaction and oximation.

1→3→6→8→9 route

According to this route, the DZT reaction was first performed with ODN **1** in the same conditions as described above to afford the corresponding 5′-azido-containing ODN (**3**). The HPLC profile of the crude mixture showed the formation of a single product without any degradation of the diol moiety (Figure S8 in the Supporting Information). ODN **3** was then reacted with alkyne-pyrene **11** using copper(II) sulfate and sodium ascorbate for 30 minutes at 60 °C under MW assistance to speed up the reaction. The HPLC profile (Figure S9 in the Supporting Information) and matrix-assisted laser desorption/ionization (MALDI)-time of flight (ToF) MS analysis of the crude mixture confirmed the formation of ODN **6**. Subsequent oxidation of the 3′-diol moiety was performed as detailed above to afford the corresponding 3′-aldehyde ODN (**8**) in quantitative yield (Figure S10 in the Supporting Information). After desalting by size-exclusion chromatography, 3′-aldehyde ODN **8** was further conjugated with aminooxy peptide **10** to give final bis-conjugate **9** (Figure S11 in the Supporting Information).

1→3→5→8→9 route

Key intermediate **5** could also be produced from oxidation of ODN **3**. In this case, the critical point to be taken into account is the stability of the azide moiety during the periodate oxidation of the diol (a large excess of $NaIO_4$ is indeed required for the efficient conversion of the diol to the aldehyde). As confirmed by HPLC and ESI-MS analyses, the azide moiety remains stable under these harsh oxidative conditions—ODN **5** was quantitatively formed (Figure S12 in the Supporting Information).

According to this strategy, click-H should be performed with ODN **5**, which contains the rather unstable aldehyde moiety. For these reasons, CuAAC reaction was carried out at room temperature without MW assistance for six hours. Nevertheless, the HPLC profile of the crude mixture (Figure S13 in the Supporting Information) revealed the presence of some impurities at longer retention times. MALDI-TOF MS characterization confirmed the formation of **8** but also the presence of 5′-pyrene-ODN-3′-monophosphate ($[M+H]^+$ *m/z* calcd: 4191.9, found: 4191.6) and 5′-pyrene-ODN-3′-OH ($[M+H]^+$ *m/z* calcd: 4111.9, found: 4108.9) demonstrating the partial instability of the aldehyde function during the CuAAC reaction. It should be noted that the stronger Lewis acid Cu^{2+} might not be involved in this degradation due to the experimental conditions (i.e., preactivation of $CuSO_4$/ascorbate reagent before addition). Consequently, the yield of formation of conjugate **8** from ODN **5** was lower in comparison with the yield of **8** obtained from ODN **6** (61 % vs 91 % yield). ODN **8** was next engaged in a click-O reaction with aminooxy peptide **10** to afford the final bis-conjugate **9** (it was noted that no difference of reactivity of ODN **8** obtained from this route (i.e., from **5**) was observed in comparison with ODN **8** obtained from the aforementioned route (i.e., from **6**).

Oligonucleotides **1–9** could all be easily purified by reversed-phase HPLC and obtained with high purity (see Figure S14 in the Supporting Information). They were all characterized by mass spectrometry analysis that showed an excellent agreement between the experimentally determined molecular weights and the calculated values (Table 1).

Conclusion

The present work describes different alternatives to access bis-conjugate oligonucleotides by using oxime and CuAAC click chemistries starting from the common 3′-diol, 5′-amino bifunctionalized oligonucleotide precursor (**1**), which is readily accessible from commercially available reagents. The introduction of the reactive moiety (i.e., the aldehyde and the azido moieties through reactions a and b in Scheme 1, respectively) was found to be compatible with the other groups within the oligonucleotide. Thus the oxidation of the diol moiety (reaction a) to afford the aldehydic group affects neither the integrity of the azide nor the triazole linkage (**3**→**5** and **6**→**8** steps, respectively). In the same way, the DZT reaction (reaction b) to introduce the azide moiety does not affect the integrity of the aldehydic group nor the oxime linkage (**2**→**5** and **4**→**7** steps, respectively).

In contrast, the compatibility of the coupling reaction (reactions c and d) with the reactive aldehyde or azide function shows some differences. The azide group was found to be fully compatible with the conditions required for oxime ligation (step **5**→**7**, 86 % yield), whereas the aldehyde moiety was rather affected during the CuAAC reaction (step **5**→**8**, 61 % yield). Hence the overall yield for the synthesis of bis-conjugate **9** from **1** using the strategy starting from oxime ligation followed by CuAAC reaction (**1**→**2**→**4**→**7**→**9** and **1**→**2**→**5**→**7**→**9** routes) is almost the same (≈70 % yield). On the contrary, the overall yield using the strategy starting from CuAAC reaction followed by oxime ligation route gave an overall yield of around 56 % for **1**→**3**→**6**→**8**→**9** route (which is quite acceptable) whereas it drops to 45 % for **1**→**3**→**5**→**8**→**9** route.

© 2015 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

In conclusion, the results reported herein demonstrate that both click Huisgen and click oxime reactions are fully compatible. However, whilst both approaches can deliver the targeted doubly functionalized ODN, the route involving click oxime ligation prior to click Huisgen is significantly more successful. These reactions thus represent key elements of the chemical toolbox for the synthesis of highly sophisticated bioconjugates.

Experimental Section

General protocol for diol oxidation (reaction a in Scheme 1)

ODN **1** (2 mg, 521 nmol) was dissolved in aq $NaIO_4$ (2.2 mg, 1 mL, ~20 equiv), and the resulting solution was stirred for 30 min at RT. The reaction mixture was then purified on a gravity-flow steric-exclusion column. Fractions were gathered, frozen, and evaporated to dryness affording aldehyde-containing conjugate **2** (515 nmol, 99%). The same protocol was applied for ODNs **3** and **6** to afford conjugates **5** and **8**, respectively (for yields, see Table 1).

General protocol for diazo transfer (reaction b in Scheme 1)

ODN **4** (0.42 mg, 89 nmol) was dissolved in 50 mм $NaHCO_3$ buffer (pH 8.5)/CH_3CN mixture (200 µL, 1:1 *v/v*). Then, 100 mм aq $CuSO_4$ (2 µL) and an aliquot (10 µL) of imidazole-1-sulfonyl azide hydrochloride solution (25 mg in 1 mL $MeOH/H_2O$, 1:1 *v/v*) were added. The resulting solution was heated at 65 °C for 1 h. The crude mixture was pre-purified on a gravity-flow steric-exclusion column. Fractions containing product were subjected to reversed-phase C_{18} HPLC purification. Fractions were gathered, frozen, and lyophilized three times to afford azide-containing conjugate **7** (84 nmol, 94%). The same protocol was applied for oligonucleotides **1** and **2** to afford conjugates **3** and **5**, respectively (for yields, see Table 1).

General protocol for oxime ligation (reaction c in Scheme 1)

Oligonucleotide **2** (0.94 mg, 242 nmol) was dissolved in 0.4 м ammonium acetate buffer (pH 4.6, 820 µL). Then, aminooxy peptide **10** (0.89 mg, 1.10 µmol, 4.6 equiv) dissolved in H_2O/CH_3CN (60 µL, 1:2 *v/v*) was added, and the resulting mixture was stirred at RT for 4 h. The reaction was purified by reversed-phase C_{18} HPLC to give conjugate **4** (200 nmol, 83%). The same protocol was applied for ODNs **5** and **8** to afford conjugates **7** and **9**, respectively (for yields, see Table 1).

General procedure for Cu^I-catalyzed azide–alkyne 1,3-dipolar cycloaddition (reaction d in Scheme 1)

In solution under microwaves activation: To a solution of oligonucleotide **3** (0.30 mg, 75 nmol) in H_2O (190 µL), DMF (50 µL), alkyne-pyrene derivative **11** (100 mм in DMF, 375 nmol, 4 µL), freshly prepared aq $CuSO_4$ (40 mм, 400 nmol, 10 µL) and sodium ascorbate (100 mм, 2 µmol, 20 µL) were added to obtain a final volume of 270 µL. The resulting preparation was treated by microwave irradiation for 30 min at 60 °C in a microwave synthesizer (Anton–Paar Monowave 300). The mixture was then desalted by size-exclusion chromatography using a NAP 10 column, and the crude product was purified by reversed-phase C_{18} HPLC to afford oligonucleotide conjugate **6** (63 nmol, 84%).

In solution at RT: To a solution of oligonucleotide **5** (0.54 mg, 137 nmol) in H_2O (190 µL) were added DMF (50 µL), alkyne-pyrene derivative **11** (100 mм in DMF, 700 nmol, 7 µL), freshly prepared aq $CuSO_4$ (40 mм, 720 nmol, 18 µL) and sodium ascorbate (100 mм, 3.4 µmol, 34 µL) to obtain a final volume of 300 µL. The coupling reaction was monitored by reversed-phase C_{18} HPLC. After 6 h, the mixture was desalted by size-exclusion chromatography using a NAP 10 column, and the crude product was purified by reversed-phase C_{18} HPLC to afford conjugate **8** (83 nmol, 61%). The same protocol was applied for oligonucleotide **7** to afford conjugate **9** (45 nmol, 88%).

The oligonucleotide synthesis, reversed-phase HPLC conditions and profiles, and additional synthetic details can be found in the Supporting Information.

Acknowledgements

This work was supported by Labex ARCANE (ANR-11-LABX-0003–01). The Nanobio-ICMG platform (FR 2603) is acknowledged for providing synthesis and purification of oligonucleotides facilities as well as for ESI-MS analysis. F.M. is a member of the French National Institute of Health and Medical Research (Inserm).

Keywords: click chemistry · conjugates · CuAAC reactions · oligonucleotides · oxime ligation

[1] F. Amblard, J. H. Cho, R. F. Schinazi, *Chem. Rev.* **2009**, *109*, 4207–4220.
[2] R. L. Juliano, X. Ming, O. Nakagawa, *Acc. Chem. Res.* **2012**, *45*, 1067–1076.
[3] Y. Singh, P. Murat, E. Defrancq, *Chem. Soc. Rev.* **2010**, *39*, 2054–2070.
[4] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; *Angew. Chem.* **2001**, *113*, 2056–2075.
[5] A. H. El-Sagheer, T. Brown, *Chem. Soc. Rev.* **2010**, *39*, 1388–1405.
[6] P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell, *Angew. Chem. Int. Ed.* **2008**, *47*, 3442–3444; *Angew. Chem.* **2008**, *120*, 3491–3493.
[7] S. De Tito, F. Morvan, A. Meyer, J. J. Vasseur, A. Cummaro, L. Petraccone, B. Pagano, E. Novellino, A. Randazzo, C. Giancola, D. Montesarchio, *Bioconjugate Chem.* **2013**, *24*, 1917–1927.
[8] O. P. Edupuganti, Y. Singh, E. Defrancq, P. Dumy, *Chem. Eur. J.* **2004**, *10*, 5988–5995.
[9] O. P. Edupuganti, O. Renaudet, E. Defrancq, P. Dumy, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2839–2842.
[10] A. Meyer, N. Spinelli, P. Dumy, J.-J. Vasseur, F. Morvan, E. Defrancq, *J. Org. Chem.* **2010**, *75*, 3927–3930.
[11] M. Karskela, M. Helkearo, P. Virta, H. Lonnberg, *Bioconjugate Chem.* **2010**, *21*, 748–755.
[12] T. Wada, A. Mochizuki, S. Higashiya, H. Tsuruoka, S. Kawahara, M. Ishikawa, M. Sekine, *Tetrahedron Lett.* **2001**, *42*, 9215–9219.
[13] C. Coppola, L. Simeone, L. De Napoli, D. Montesarchio, *Eur. J. Org. Chem.* **2011**, 1155–1165.
[14] J. Lietard, A. Meyer, J.-J. Vasseur, F. Morvan, *J. Org. Chem.* **2008**, *73*, 191–200.
[15] a) C. C. Y. Wang, T. S. Seo, Z. M. Li, H. Ruparel, J. Y. Ju, *Bioconjugate Chem.* **2003**, *14*, 697–701; b) J. Chao, W.-Y. Huang, J. Wang, S.-J. Xiao, Y.-C. Tang, J.-N. Liu, *Biomacromolecules* **2009**, *10*, 877–883.
[16] R. Lartia, P. Murat, P. Dumy, E. Defrancq, *Org. Lett.* **2011**, *13*, 5672–5675.
[17] K. Lu, Q.-P. Duan, L. Ma, D.-X. Zhao, *Bioconjugate Chem.* **2010**, *21*, 187–202.
[18] M. E. Østergaard, P. J. Hrdlicka, *Chem. Soc. Rev.* **2011**, *40*, 5771–5788.

Received: November 3, 2014
Published online on December 10, 2014

 © 2015 The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim