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DNA-Templated *N*(Me)-Alkoxyamine Glycosylation

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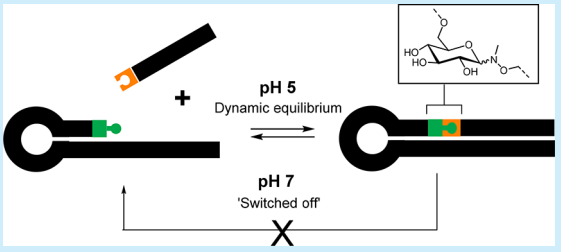
Supporting Information

ABSTRACT: The potential of *N*(Me)-alkoxyamine glycosylation as a DNA-templated ligation has been studied. On a hairpin stem-template model, a notable rate enhancement and an increased equilibrium yield are observed compared to the corresponding reaction without a DNA catalyst. The *N*-glycosidic connection is dynamic at pH 5, whereas it becomes irreversible at pH 7. The *N*(Me)-alkoxyamine glycosylation may hence be an attractive pH controlled reaction for the assembly of DNA-based dynamic products.

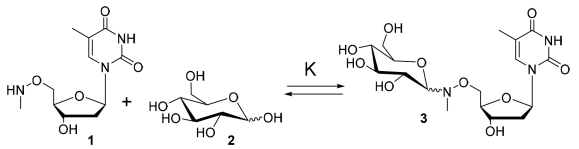


The concept of DNA-templated organic synthesis (DTS) has inspired researchers to apply a hybridization-driven proximity effect to various chemical reactions unrelated in structure to the DNA backbone for more than two decades.[1] Even reactions that are perceived to occur under dry conditions may work in aqueous media under hybridization-driven conditions. As an example, DNA-catalyzed glycosylation using aryl glycosides as donors has recently been described.[2] However, for the self-assembled DNA-based supramolecular constructs,[3,4] dynamic combinatorial libraries,[5] or for the models of self-replicating systems,[6−13] reversible dynamic reactions are of particular interest.[14−16] In addition, it would be beneficial if the reaction was biorthogonal and inducible by traceless stimuli, e.g., by a reasonable pH change,[17−22] oxidation,[23,24] or UV irradiation.[25] Imine formation, used extensively for the DNA-catalyzed sequence specific oligomerization of nucleic acid analogues,[26−30] partly meets these requirements, as the imine intermediates are irreversibly reduced to the stable alkylamine products (i.e., reductive amination). The boronic acid ligation[18−22] is, in turn, an excellent example of such a dynamic reaction, occurring between a 5′-ended boronic acid and a 3′-ended ribonucleotide under slightly basic conditions. Even autotemplated duplex self-assembly representing a model of sequence-defined synthetic polymers has been examined by bononucleic acids.[22]

The present study shows that *N*(Me)-oxyamine glycosylation is an attractive option as a dynamic, pH-controlled DNA-templated ligation. The reaction itself is known[31] and used for the preparation of various glycoconjugates.[31−37] Recently, real-time NMR studies of the reaction with different substrates have also been reported.[38] The reaction is advantageous since it is nearly biorthogonal, occurs in slightly acidic conditions and the products (i.e., *N*-alkoxyaminoglycosides) are virtually stable at neutral pH. Moreover, high anomeric selectivity may be observed (cf. Table 1: *β*-anomer with glucose).

To evaluate the central hypothesis, 5′-*O*-(methylamino)-thymidine (**1**) and appropriate building blocks for the automated DNA synthesis of 5′-*N*(Me)aminooxy- and 3′-D-glucose-modified oligonucleotides, i.e., phosphoramidite **4** and solid supported D-glucose **5**, were prepared. Syntheses of **1** and **4** are outlined in Scheme 1. The 5′-*O*-phthaliimido group (**6**) was introduced to thymidine by a published procedure.[39] The phthaloyl group of **6** was removed by hydrazinolysis, and the exposed aminooxy group was converted to oxime **7** by a one-pot treatment with formaldehyde. Reduction of **7** with $NaCNBH_3$ gave **1** in 86% overall yield (calculated from thymidine). The Fmoc protection and phosphitylation of the 5′-*O*-(methylamino) group and of the 3′-OH group, respectively, gave the phosphoramidite building block **4**. The preparation of solid supported D-glucose (**5**) is shown in Scheme 2. The anomeric hydroxyl group of D-glucose tetra acetate **9** was TBS protected, the acetyl groups were removed by a sodium methoxide-catalyzed transesterification, and the 6-OH group was selectively protected by the DMTr group to give **11** in 56% overall yield. Compound **11** was immobilized (loading of 20 μmol g^{-1}) to a long-chain alkylamino-modified controlled pore class (LCAA-CPG) via a one-pot conversion to a succinate and a subsequent amide coupling to LCAA-CPG

Table 1. N-Glycosylation of 5′-*O*-(Methylamino)thymidine (1) with D-Glucose (2)[a]



entry	pH	$t_{0.5}$	equilibrium yield (%)	β/α ratio	equilibrium constant, K (L mol^{-1})
1	4	30.8 ± 0.8 h	25.0	1:0	38.1
2	5	223 ± 8 h	35.1	1:0	65.6
3	6	95.6 ± 4.0 d	41.6	1:0	89.9

[a]Conditions: 5.0 mmol L^{-1} **1** and 10.0 mmol L^{-1} **2** in 0.1 mol L^{-1} sodium acetate or 2-(*N*-morpholino)ethanesulfonate, I = 0.1 mol L^{-1} (NaCl), pH = 4, 5 or 6, 24 °C.

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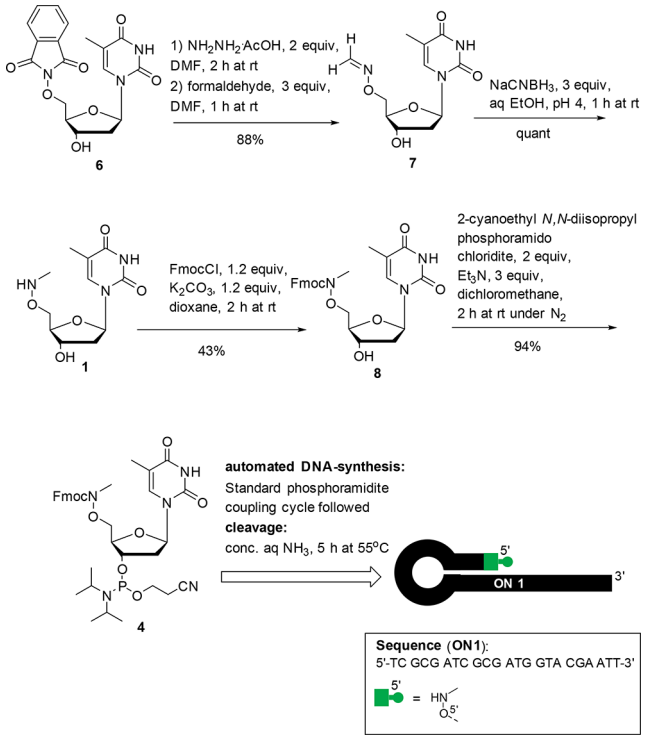
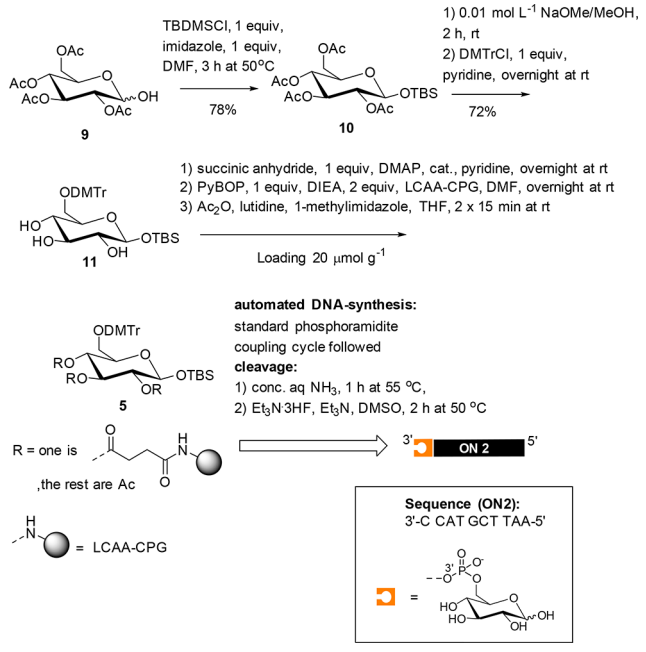
Scheme 1. Synthesis of 5′-*O*-(Methylamino)thymidine (1) and the Corresponding Phosphoramidite Building Block (4)



Scheme 2. Synthesis of Solid-Supported D-Glucose (5)

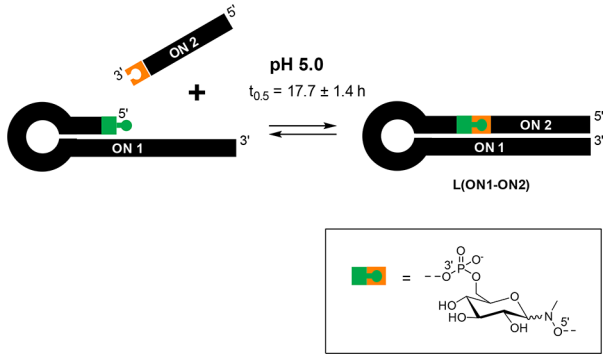


using PyBOP as an activator. The unreacted amino groups on the support (**5**) were capped by an acetic anhydride treatment.

Preliminary ligation experiments were first carried out without a DNA catalyst. Compound **1** was mixed with buffered solutions of D-glucose (**2**) at pH 4, 5, and 6, and reaction rates and equilibrium constants of the *N*-glycosylation (**3**) were determined (Table 1). Consistent with previous findings with *N*-methylethoxyamine,[38] lowering the pH from 6 to 4 accelerated the reaction, whereas the equilibrium yield decreased. Only β-anomer was detected in each experiment. It may also be worth noting that within the observed reaction rates the extent of the *N*-glycosylation would be unsubstantial at a micromolar concentration of the substrates (cf. the experiments below).

To demonstrate the DNA-templated *N*(Me)-alkoxyamine glycosylation, a simple hairpin stem-template architecture, similar to that has previously been used for the maleimide–thiol ligation,[40] was designed to provide the proximity effect between the 3′-reducing D-glucose and the 5′-*N*(Me) aminooxy group (Scheme 3). For that purpose, the phosphoramidite

Scheme 3. Hairpin Stem-Template Architecture Used for the Hybridization-Driven *N*(Me)-Alkoxyamine Glycosylation



building block of 5′-*O*-(methylamino)thymidine **4** and solid-supported D-glucose **5**, together with commercially available phosphoramidite building blocks, were used for the automated synthesis of 5′-*O*-(methyamino)oligonucleotide **ON1** and 3′-D-glucose-modified oligonucleotide **ON2**, respectively (Schemes 1 and 2, experimental details shown in the Supporting Information). As seen in the RP HPLC profiles of the crude product mixtures (Figure 1), the oligonucleotides (**ON1** and **ON2**) could be successfully synthesized. A buffered (pH 5) solution of the oligonucleotides (10 μmol L^{-1} **ON1** and 20 μmol L^{-1} **ON2**, at 24 °C) was then prepared, and the progress of the expected ligation was followed by an ion-exchange chromatography. As seen in the chromatograms (Figure 2), a product peak with the retention time of 23.8 min (together

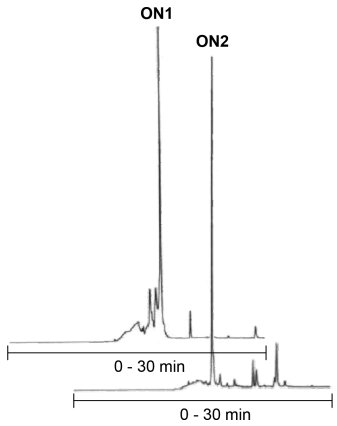


Figure 1. RP HPLC profiles of the crude product (**ON1** and **ON2**) mixtures. Conditions: analytical RP HPLC column (C18, 250 × 4.6 mm, 5 μm), gradient elution from 0 to 50% MeCN in 0.1 mol L^{-1} triethylammonium acetate (0–30 min), flow rate 1.0 mL min^{-1}, detection at 260 nm.

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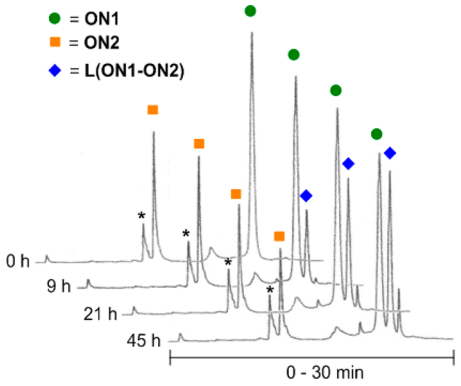

Figure 2. Ion-exchange HPLC chromatograms of the reaction mixture (Scheme 1. Time points at 0, 9.0, 21, and 45 h shown. **ON1**: t_r = 22.5 min, **ON2**: t_r = 12.2 min. (* = unidentified side product related to **ON2**), **L(ON1−ON2)**: t_r = 23.8 min (major) and 24.5 min (minor). Reaction conditions: 10 μmol L^{-1} **ON1** and 20 μmol L^{-1} **ON2** in 0.1 mol L^{-1} sodium acetate buffer, I = 0.1 mol L^{-1} (NaCl), pH 5.0, at 24 °C. HPLC conditions: an analytical monolithic ion-exchange column, flow rate 1.5 mL min^{-1}, detection at 260 nm., a gradient elution at 40 °C from 17 to 200 mmol L^{-1} $NaClO_4$ in 20 mmol L^{-1} Tris over 30 min.

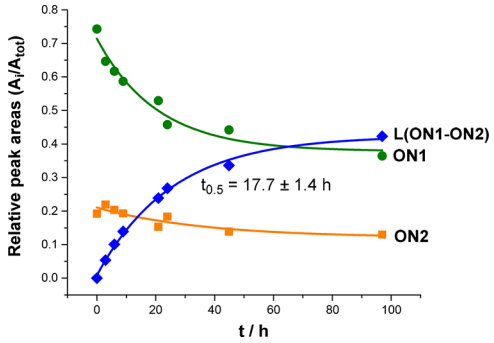

Figure 3. Reaction profiles of the DNA-templated *N*-glycosylation. (Note: relative peak areas described.)

with minor one at 24.5 min attributed to an external duplex) accumulated, which by MS (ESI-TOF) spectroscopy was confirmed to be the desired ligation product **L(ON1−ON2)** (Figure 4). The half-life of this model DNA-templated *N*-glycosylation was 17.7 ± 1.4 h, and the reaction remained at ca.

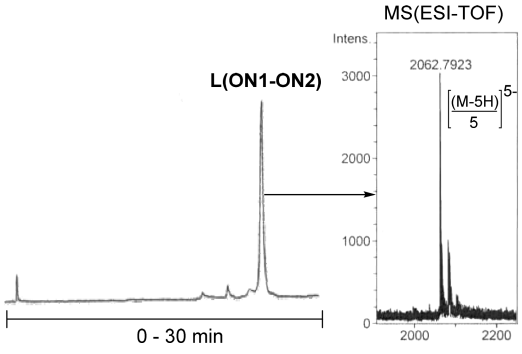

Figure 4. Ion-exchange HPLC chromatogram and MS(ESI-TOF) spectrum of isolated L(ON1−ON2). Sample of a buffered solution of 1 μmol L^{-1} isolated **L(ON1−ON2)** at pH 7.0 analyzed after 2 weeks. HPLC conditions as above in Figure 1. Calculated $[(M - 5H)/5]^{5-}$ for **L(ON1−ON2)**: 2062.7.

40% equilibrium yield. If the reaction between **ON1** and **ON2** could be treated as a bimolecular reaction, these values correspond to a 6.3 × 10^3-fold rate enhancement and ca. 1000-fold higher equilibrium yield compared to the *N*-glycosylation between D-glucose (**2**) and 5′-*O*-(methylamino)-thymidine (**1**). However, the DNA-templated *N*-glycosylation is shifted toward an intramolecular reaction (i.e., the reaction is not concentration dependent) in which the reaction rate and equilibrium constant depend on the proximity effect between the reducing sugar and the *N*(Me)-aminooxy group. While the given half-life is correct, reporting of an accurate rate constant value for **ON1−ON2** ligation would require deeper understanding of the reaction mechanism, including kinetics of the DNA hybridization. To demonstrate further the reversibility of the ligation, **L(ON1−ON2)** was isolated and dissolved again in the same buffer at pH 5 (10 μmol L^{-1} **L(ON1−ON2)**). Decay of **L(ON1−ON2)** converting to **ON1** and **ON2** was observed following the half-life of 11.8 ± 1.0 h (cf. Supporting Information). Despite the known characteristics of the *N*-glycosidic connection,[31−38] the stability of the isolated ligation product **L(ON1−ON2)** was additionally studied at pH 7. The mixture of **L(ON1−ON2)** was found to be virtually intact, as expected (Figure 4: an HPLC profile of the **L(ON1−ON2)** mixture at pH 7 after 2 weeks). The "switched off-state" of this dynamic equilibrium may hence be obtained.

In summary, a DNA-templated *N*(Me)-oxyamine glycosylation has been described for the first time. A notable rate enhancement was observed compared to the nontemplated reaction at pH 5, as expected. The beneficial properties of the DNA-templated *N*(Me)-oxyamine glycosylation, i.e., a dynamic biorthogonal reaction that may switched on/off by a pH change, may find applications for many supramolecular purposes. Dynamic combinatorial libraries, self-assembled DNA-based constructs, nucleoside analogues, and a deeper understanding of the reaction kinetics and distance requirements, based on hybridization with different architectures and *N*(Me)-oxyamine glycosylation, are currently underway in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b00113.

Experimental details for the synthesis of **1**, **4**, and **5** and automated synthesis of **ON1** and **ON2**. HPLC analysis of **L(ON1−ON2)** decay. Orbitrap-Q-Exactive HRMS spectrum of **L(ON1−ON2)** (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Li, X.; Liu, D. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 4848.
(2) Hesser, A. R.; Brandsen, B. M.; Walsh, S. M.; Wang, P.; Silverman, S. K. *Chem. Commun.* **2016**, *52*, 9259.
(3) Iwaura, R.; Hoeben, F. J. M.; Masuda, M.; Schenning, A. P. H. J.; Meijer, E. W.; Shimizu, T. *J. Am. Chem. Soc.* **2006**, *128*, 13298.
(4) O'Reilly, R. K.; Turberfield, A. J.; Wilks, T. R. *Acc. Chem. Res.* **2017**, *50*, 2496.
(5) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652.
(6) Ficht, S.; Mattes, A.; Seitz, O. *J. Am. Chem. Soc.* **2004**, *126*, 9970.
(7) Schrum, J. P.; Ricardo, A.; Krishnamurthy, M.; Blain, J. C.; Szostak, J. W. *J. Am. Chem. Soc.* **2009**, *131*, 14560.
(8) Leu, K.; Kervio, E.; Obermayer, B.; Turk-MacLeod, R. M.; Yuan, C.; Luevano, J.-M, Jr.; Chen, E.; Gerland, U.; Richert, C.; Chen, I. A. *J. Am. Chem. Soc.* **2013**, *135*, 354.
(9) Zhang, S.; Blain, J. C.; Zielinska, D.; Gryaznov, S. M.; Szostak, J. W. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 17732.
(10) Kaiser, A.; Richert, C. *J. Org. Chem.* **2013**, *78*, 793.
(11) Chaput, J. C.; Switzer, C. *J. Mol. Evol.* **2000**, *51*, 464.
(12) Chen, J. J.; Cai, X.; Szostak, J. W. *J. Am. Chem. Soc.* **2009**, *131*, 2119.
(13) Heuberger, B. D.; Switzer, C. *Org. Lett.* **2006**, *8*, 5809.
(14) Crane, E. A.; Gademann, K. *Angew. Chem., Int. Ed.* **2016**, *55*, 3882.
(15) Walker, S. I.; Grover, M. A.; Hud, N. V. *PLoS One* **2012**, *7*, e34166.
(16) Engelhart, A. E.; Hud, N. V. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, a002196.
(17) Bean, H. D.; Anet, F. A. L.; Gould, I. R.; Hud, N. V. *Origins Life Evol. Biospheres* **2006**, *36*, 39.
(18) Luvino, D.; Baraguey, C.; Smietana, M.; Vasseur, J.-J. *Chem. Commun.* **2008**, 2352.
(19) Martin, A. R.; Barvik, I.; Luvino, D.; Smietana, M.; Vasseur, J.-J. *Angew. Chem., Int. Ed.* **2011**, *50*, 4193.
(20) Martin, A. R.; Mohanan, K.; Luvino, D.; Floquet, N.; Baraguey, C.; Smietana, M.; Vasseur, J.-J. *Org. Biomol. Chem.* **2009**, *7*, 4369.
(21) Barbeyron, R.; Martin, A. R.; Vasseur, J.-J.; Smietana, M. *RSC Adv.* **2015**, *5*, 105587.
(22) Barbeyron, R.; Vasseur, J.-J.; Smietana, M. *Chem. Sci.* **2015**, *6*, 542.
(23) Krishnan-Ghosh, Y.; Whitney, A. M.; Balasubramanian, S. *Chem. Commun.* **2005**, 3068.
(24) Ura, Y.; Beierle, J. M.; Leman, L. J.; Orgel, L. E.; Ghadiri, M. R. *Science* **2009**, *325*, 73.
(25) Fujimoto, K.; Matsuda, S.; Takahashi, N.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5646.
(26) Gartner, Z. J.; Liu, D. R. *J. Am. Chem. Soc.* **2001**, *123*, 6961.
(27) Engelhart, A. E.; Cafferty, B. J.; Okafor, C. D.; Chen, M. D.; Williams, L. D.; Lynn, D. G.; Hud, N. V. *ChemBioChem* **2012**, *13*, 1121.
(28) Li, X.; Zhan, Z.-Y.; Knipe, R.; Lynn, D. G. *J. Am. Chem. Soc.* **2002**, *124*, 746.
(29) Kleiner, R. E.; Brudno, Y.; Birnbaum, M. E.; Liu, D. R. *J. Am. Chem. Soc.* **2008**, *130*, 4646.
(30) Rosenbaum, M.; Liu, D. R. *J. Am. Chem. Soc.* **2003**, *125*, 13924.
(31) Peri, F.; Dumy, P.; Mutter, M. *Tetrahedron* **1998**, *54*, 12269.
(32) Ishida, J.; Hinou, H.; Naruchi, K.; Nishimura, S.-I. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1197.
(33) Langenhan, J. M.; Endo, M. M.; Engle, J. M.; Fukumoto, L. L.; Rogalsky, D. R.; Slevin, L. K.; Fay, L. R.; Lucker, R. W.; Rohlfing, J. R.; Smith, K. R.; Tjaden, A. E.; Werner, H. M. *Carbohydr. Res.* **2011**, *346*, 2663.
(34) Teze, D.; Dion, M.; Daligault, F.; Tran, V.; André-Miral, C.; Tellier, C. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 448.
(35) Bohorov, O.; Andersson-Sand, H.; Hoffmann, J.; Blixt, O. *Glycobiology* **2006**, *16*, 21C.
(36) Huang, M. L.; Cohen, M.; Fisher, C. J.; Schooley, R. T.; Gagneux, P.; Godula, K. *Chem. Commun.* **2015**, *51*, 5326.
(37) Cló, E.; Blixt, O.; Jensen, K. J. *Eur. J. Org. Chem.* **2010**, *3*, 540.
(38) Baudendistel, O. R.; Wieland, D. E.; Schmidt, M. S.; Wittmann, V. *Chem. - Eur. J.* **2016**, *22*, 17359.
(39) Perbost, M.; Hoshiko, T.; Morvan, F.; Swayze, E.; Griffey, R. H.; Sanghvi, Y. S. *J. Org. Chem.* **1995**, *60*, 5150.
(40) Gartner, Z. J.; Liu, D. R. *J. Am. Chem. Soc.* **2001**, *123*, 6961.

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