ORGANIC LETTERS

2010
Vol. 12, No. 5
916−919

Improved Synthesis of the Two-Photon Caging Group 3-Nitro-2-Ethyldibenzofuran and Its Application to a Caged Thymidine Phosphoramidite

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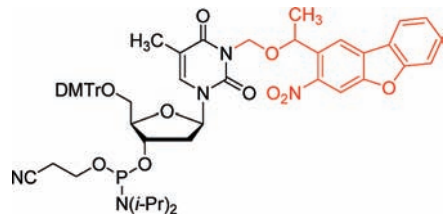
Received December 4, 2009

ABSTRACT



A new and efficient route to the recently reported 3-nitro-2-ethyldibenzofuran caging group was developed. Furthermore, its installation on a thymidine phosphoramidite is described. This caging group is efficiently removed through light-irradiation at 365 nm.

Photolabile protecting groups (caging groups) are important tools in the investigation of biological processes. Cleavage of caging groups by light (decaging) is a mild and noninvasive technique that is completely orthogonal to other chemical processes in a biological system. Moreover, caged molecules offer a unique way to study biological systems, because they allow for precise temporal and spatial control over the chemical and biological (re)activity of molecules. This approach has been successfully applied to the photochemical regulation of the activity of small molecules, peptides, proteins, and oligonucleotides.[1−4] Several caging strategies for oligomers have been developed: statistical caging of the DNA or RNA backbone,[5−7] incorporation of photocleavable linkers into the oligomer strand,[8−11] and incorporation of caged nucleotide building blocks into oligonucleotides.[12−18]

All base-caged phosphoramidites developed to date require UVA irradiation of approximately 360 nm for efficient decaging. Disadvantages of UVA light include limited tissue penetration, potential photodamage, and difficulties in achieving high-precision three-dimensional focusing of the photoexcitation process. A solution to these problems is the application of two-photon excitation techniques. Under two-photon irradiation conditions, a molecule that has an absorbance maximum at 350 nm can be excited by simultaneously absorbing two photons of precisely half the energy, i.e. 700 nm.[19,20] Light at near-IR or IR wavelengths (between 700 and 1000 nm) exhibits deeper tissue-penetration (e.g., up to

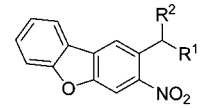
(1) *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*; Goeldner, M., Givens, R. A., Eds.; Wiley-VCH: Weinheim, Germany, 2005.
(2) Young, D. D.; Deiters, A. *Org. Biomol. Chem.* **2007**, *5*, 999–1005.
(3) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921.
(4) Lee, H.; Larson, D. R.; Lawrence, D. S. *ACS Chem. Biol.* **2009**, *4*, 409–427.
(5) Ando, H.; Furuta, T.; Tsien, R. Y.; Okamoto, H. *Nat. Genet.* **2001**, *28*, 317–325.
(6) Shah, S.; Rangarajan, S.; Friedman, S. H. *Angew. Chem., Int. Ed.* **2005**, *44*, 1328–1332.
(7) Nguyen, Q. N.; Chavli, R. V.; Marques, J. T.; Conrad, P. G., II; Wang, D.; He, W.; Belisle, B. E.; Zhang, A.; Pastor, L. M.; Witney, F. R.; Morris, M.; Heitz, F.; Divita, G.; Williams, B. R. G.; McMaster, G. K. *Biochim. Biophys. Acta* **2006**, *1758*, 394–403.
(8) Zhang, K.; Taylor, J. *J. Am. Chem. Soc.* **1999**, *121*, 11579–11580.
(9) Ordoukhanian, P.; Taylor, J. S. *Bioconjugate Chem.* **2000**, *11*, 94–103.
(10) Lenox, H. J.; McCoy, C. P.; Sheppard, T. L. *Org. Lett.* **2001**, *3*, 2415–2418.

10.1021/ol902807q
Published on Web 01/29/2010

8 mm in bovine muscle tissue),[21−23] causes less photodamage, and can be focused much more precisely (down to an effective excitation volume of 1 fL).[20,24]

The main characteristic of a good two-photon caging group is a high two-photon decaging cross-section δ_u, which is defined as a product of a two-photon absorbance cross-section δ_a and a two-photon decaging quantum yield Q_u.[25] Ideally δ_u should exceed 0.1 Goeppert-Mayer (GM) for biological experiments in order to avoid potential tissue damage caused by high laser powers.[25−27] The two most commonly employed two-photon caging groups are 6-bromo-7-hydroxycoumarin-4-methyl (Bhc), with a δ_u of 0.72 GM at 740 nm,[25] and 8-bromo-7-hydroxyquinoline (BHQ), with a δ_u of 0.59 GM at 740 nm.[28] Both have been employed to cage a variety of substrates, including carboxylic acids, phosphates, carbonates, carbamates, diols, aldehydes, and ketones.[24,25,27−31] Bhc and BHQ caging groups decage according to a solvent-assisted heterolysis mechanism.[24,26] This makes them unsuitable for direct caging of substrates with high pK_a values,[32,33] such as alcohols, phenols, amines, and amides, hampering their application in the caging of nucleotide bases. Recently, Furuta and co-workers have prepared a pair of Bhc-caged nucleosides through the installation of the Bhc group via a carbamate linkage at the 6-NH_2 of deoxyadenosine and the 4-NH_2 of deoxycytidine.[31] However, the caging group carbamate linkage in the corresponding phosphoramidites would hydrolyze during the final step (base deprotection with NH_4OH, or $MeNH_2$) of the automated oligonucleotide synthesis (as observed by us for similar structures; data not shown), and neither compound has been converted into a phosphoramidite. Additionally, the overall yields for the synthesis of Bhc-caged nucleosides were low due to difficulties associated with the carbamate formation.

To develop a suitable and easily accessible two-photon caged phosphoramidite for DNA synthesis, we decided to combine our 6-nitropiperonyloxymethyl (NPOM) caging group strategy[17,18,34,35] with the recently discovered 3-nitro-2-ethyldibenzofuran (NDBF) group (**1**) (Figure 1), which



1: $R^1 = OH$, $R^2 = CH_3$
2: $R^1 = Br$, $R^2 = CH_3$
3: $R^1 = Br$, $R^2 = H$
4: $R^1 = OCH_2Cl$, $R^2 = CH_3$

Figure 1. The NDBF caging group derivatives **1**−**4**.

decages via a classical Norrish type II mechanism, enabling efficient caging and decaging of less acidic functional groups,[33] while displaying an excellent δ_u of 0.6 GM (at 710 nm).

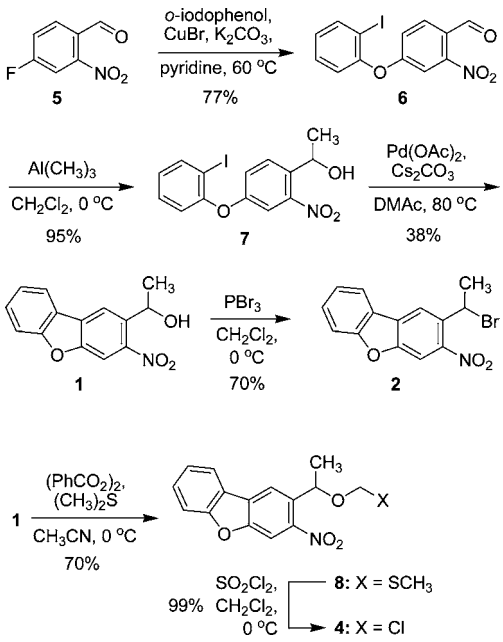
Despite its excellent photochemical properties and potential to cage diverse substrates, no application of the NDBF group has been reported since its discovery. We believe that this is due to difficulties accessing it synthetically. Reported syntheses of the alcohol **1** and the bromides **2**−**3** (Figure 1) are rather low yielding and often involve laborious and difficult purification steps.[33,36] In addition to developing our own synthetic path to **1**−**2**, we have also synthesized the chloromethyl ether **4** (Figure 1), which was modeled after the NPOM caging group for caging of nitrogen heterocycles such as nucleotide bases.[17,18,34]

The previously published synthesis, starting from dibenzofuran, produced (3-nitrodibenzofuran-2-yl)-ethanol (**1**) in 4% total yield over 6 steps, and 1-bromo-1-(3-nitrodibenzofuran-2-yl)ethane (**2**) in 10% overall yield over 4 steps.[33,36] An alternative synthetic strategy leading to the caging group 1-bromo-1-(3-nitrodibenzofuran-2-yl)-methane (**3**), starting from 1-chloro-2-nitrobenzene and *p*-cresol, afforded the final product **3** in only 1% total yield over 6 steps.[36]

We developed an optimized synthetic route to the NDBF group **4** (Scheme 1), enabling us to synthesize sufficient quantities of the caged phosphoramidite **11** to be used in automated DNA synthesis. Our approach starts from com-

(11) Tang, X.; Dmochowski, I. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 3523–3526.
(12) Kröck, L.; Heckel, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 471–473.
(13) Mayer, G.; Krock, L.; Mikat, V.; Engeser, M.; Heckel, A. *ChemBioChem* **2005**, *6*, 1966–1970.
(14) Tang, X. J.; Dmochowski, I. J. *Org. Lett.* **2005**, *7*, 279–282.
(15) Höbartner, C.; Silverman, S. K. *Angew. Chem., Int. Ed.* **2005**, *44*, 7305–7309.
(16) Ting, R.; Lermer, L.; Perrin, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 12720–12721.
(17) Lusic, H.; Young, D. D.; Lively, M. O.; Deiters, A. *Org. Lett.* **2007**, *9*, 1903–1906.
(18) Lusic, H.; Lively, M. O.; Deiters, A. *Mol. Biosyst.* **2008**, *4*, 508–511.
(19) Denk, W.; Strickler, J. H.; Webb, W. W. *Science* **1990**, *248*, 73–76.
(20) Piston, D. W. *Trends Cell Biol.* **1999**, *9*, 66–69.
(21) Fisher, W. G.; Partridge, W. P.; Dees, C.; Wachter, E. A. *Photochem. Photobiol.* **1997**, *66*, 141–155.
(22) Kollias, N.; Zonios, G.; Stamatas, G. N. *Vib. Spectrosc.* **2002**, *28*, 17–23.
(23) Detty, M. R.; Gibson, S. L.; Wagner, S. J. *J. Med. Chem.* **2004**, *47*, 3897–3915.
(24) Zhu, Y.; Pavlos, C. M.; Toscano, J. P.; Dore, T. M. *J. Am. Chem. Soc.* **2006**, *128*, 4267–4276.
(25) Furuta, T.; Wang, S. S.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1193–1200.
(26) Furuta, T.; Noguchi, K. *Trac-Trends Anal. Chem.* **2004**, *23*, 511–519.
(27) Furuta, T.; Takeuchi, H.; Isozaki, M.; Takahashi, Y.; Kanehara, M.; Sugimoto, M.; Watanabe, T.; Noguchi, K.; Dore, T. M.; Kurahashi, T.; Iwamura, M.; Tsien, R. Y. *ChemBioChem* **2004**, *5*, 1119–1128.
(28) Fedoryak, O. D.; Dore, T. M. *Org. Lett.* **2002**, *4*, 3419–3422.
(29) Lu, M.; Fedoryak, O. D.; Moister, B. R.; Dore, T. M. *Org. Lett.* **2003**, *5*, 2119–2122.
(30) Suzuki, A. Z.; Watanabe, T.; Kawamoto, M.; Nishiyama, K.; Yamashita, H.; Ishii, M.; Iwamura, M.; Furuta, T. *Org. Lett.* **2003**, *5*, 4867–4870.
(31) Furuta, T.; Watanabe, T.; Tanabe, S.; Sakyo, J.; Matsuba, C. *Org. Lett.* **2007**, *9*, 4717–4720.
(32) Schmidt, R.; Geissler, D.; Hagen, V.; Bendig, J. *J. Phys. Chem. A* **2007**, *111*, 5768–5774.
(33) Momotake, A.; Lindegger, N.; Niggli, E.; Barsotti, R. J.; Ellis-Davies, G. C. R. *Nat. Methods* **2006**, *3*, 35–40.
(34) Lusic, H.; Deiters, A. *Synthesis* **2006**, 2147–2150.
(35) Young, D. D.; Deiters, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2658–2661.
(36) Ellis-Davies, G. C. R.; Momotake, A. U.S. Patent Appl. 20080188645, 2008.

Scheme 1. Synthesis of NDBF Based Caging Groups **1**, **2**, and **4**

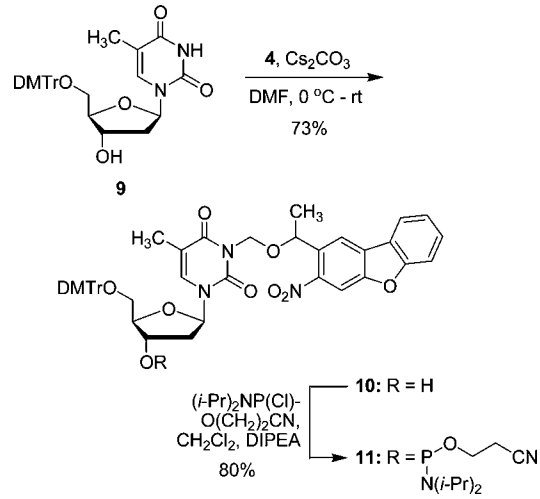


mercially available 4-fluoro-2-nitrobenzaldehyde (**5**) and 2-iodophenol by employing an Ullmann coupling, using CuBr and K_2CO_3 in pyridine, at 60 °C, to form the diaryl ether **6** in 77% yield. The aldehyde **6** was subsequently alkylated with $AlMe_3$ in DCM, affording the secondary alcohol **7** in 95% yield. The bottleneck of the synthesis was the formation of the dibenzofuran ring, which we accomplished via a Heck reaction by heating **7** in the presence of catalytic amounts of $Pd(OAc)_2$, using Cs_2CO_3 as a base in DMAc at 80 °C. The caging group **1** was isolated as the sole regioisomer in 38% yield (28% total yield over three steps). Unfortunately, attempts to improve the yield of the ring-closure reaction failed. Substituting Cs_2CO_3 for alternative bases (K_2CO_3, DIPEA, DBU) and changing the solvent (DMSO, toluene, THF) all provided lower yields. Increasing the temperature or employing microwave irradiation also resulted in lower yields, and the formation of multiple side products hampering purification. Also, cyclization attempts with the aldehyde **6** led to diminished yields. Subsequently, the alcohol **1** was converted to a bromide with PBr_3 in DCM, at 0 °C, in 70% yield, thus affording the caging group **2** in 19% over 4 steps. Alternatively, the caging group **1** was protected as a methylthiomethyl ether **8** in 70% yield, using Me_2S and benzoyl peroxide in CH_3CN, at 0 °C. The MTM-derivative **8** was then converted to the caging group **4**, employing SO_2Cl_2 in DCM, at 0 °C. The caging group **4** was directly used in the caging of thymidine without purification.

The synthetic path to **1**, **2**, and **4** presented herein is substantially shorter and offers markedly improved yields. Our synthesis also avoids the need for laborious purifications required for the separation of different regiosomers and multiple reaction side products.

To prepare an NDBF-caged thymidine phosphoramidite for DNA synthesis, we reacted 5′-*O*-(4,4′-dimethoxytrityl)thymidine (**9**, synthesized in one step from thymidine following a literature procedure[37]) with the caging group **4** (Scheme 2), using Cs_2CO_3 in DMF, at 0 °C to rt, obtaining

Scheme 2. Synthesis of the NDBF-Caged Thymidine Phosphoramidite **11**



10 in 73% yield. Gratifyingly, the free 3′-OH group was unreactive under those conditions, thus obviating additional protecting group chemistry. The NDBF-caged thymidine **10** was then converted to the phosphoramidite **11** in 80% yield with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, employing DIPEA as a base in DCM, at rt.

The stability of the NDBF-caged thymidine toward DNA synthesis was then studied. The installed caging group was found to be completely stable under several different conditions commonly used for the removal of protecting groups from the 5′-OH and the nucleotide bases, as well as oligonucleotide cleavage from the support resin: (a) concentrated NH_4OH, 65 °C, 16 h; (b) 33% $MeNH_2$ in EtOH, 55 °C, 16 h; and (c) 5% TFA in DCM, rt, 1 h (see Supporting

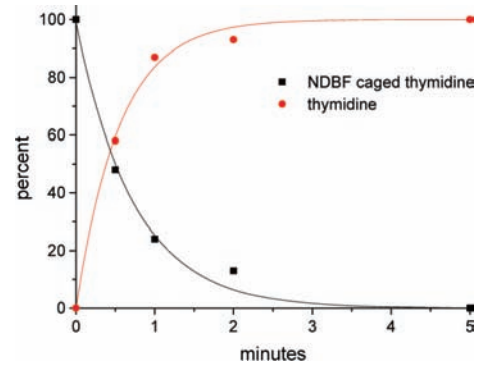


Figure 2. Decaging timecourse of NDBF-thymidine.

Information).[17] Decaging of the NDBF-thymidine was tested in CH_3OH (0.1 mM) by conducting a time course of UV irradiation over 5 min (hand-held Spectroline ENF-280C UV lamp, at 365 nm, 23 W) followed by HPLC analysis. It was found that NDBF-thymidine decages rapidly with a half-life of about 30 s (see Figure 2 and Supporting Information).

In summary, we have developed an improved synthetic route to the recently discovered two-photon caging group 3-nitro-2-ethyldibenzofuran (NDBF). Due to its favorable photochemical fragmentation mechanism, we were able to apply the NDBF group in the successful caging of a thymidine phosphoramidite. This caging moiety is stable to standard DNA synthesis conditions and thus can be readily incorporated into the synthesis of photocontrollable oligonucleotides for biological applications.

Acknowledgment. Financial support from the National Institutes of Health (R01GM079114) is acknowledged. A.D. is the recipient of a Beckman Young Investigator Award, a Cottrell Scholar Award, an NSF CAREER Award, and a TEVA USA Scholars Grant.

Supporting Information Available: Synthetic protocols and analytical data, stability tests and decaging experimentals, and copies of ^1H and ^{13}C NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL902807Q

(37) Lan, T.; McLaughlin, L. W. *J. Am. Chem. Soc.* **2000**, *122*, 6512–6513.