Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 1336–1339
Tetrahedron Letters

exo-*N*-[2-(4-Azido-2,3,5,6-tetrafluorobenzamido)ethyl]-dC: a novel intermediate in the synthesis of dCTP derivatives for photoaffinity labelling

Crina Cismaş [†], Thanasis Gimisis [*]

Organic Chemistry Laboratory, Department of Chemistry, University of Athens, 157 84 Athens, Greece

Received 17 November 2007; revised 14 December 2007; accepted 18 December 2007
Available online 23 December 2007

Abstract

An alternative route for the synthesis of a photoaffinity labelling (PAL) dCTP derivative is reported. This method involves the intermediacy of *exo*-*N*-[2-(4-azido-2,3,5,6-tetrafluorobenzamido)ethyl]-dC. The latter is prepared from the coupling of known *N*-(2-aminoethyl)-4-azido-2,3,5,6-tetrafluorobenzamide, prepared in an improved three-step sequence, with an activated 4-triazolyl derivative of dU, followed by deprotection. ^{19}F NMR spectroscopy proved extremely useful in following the synthetic transformations, and enabled control of any adventitious reduction of the azides.

Keywords: Photoaffinity labelling; dCTP Derivatives; 4-Azido-2,3,5,6-tetrafluorobenzamide derivatives; Synthesis; ^{19}F NMR

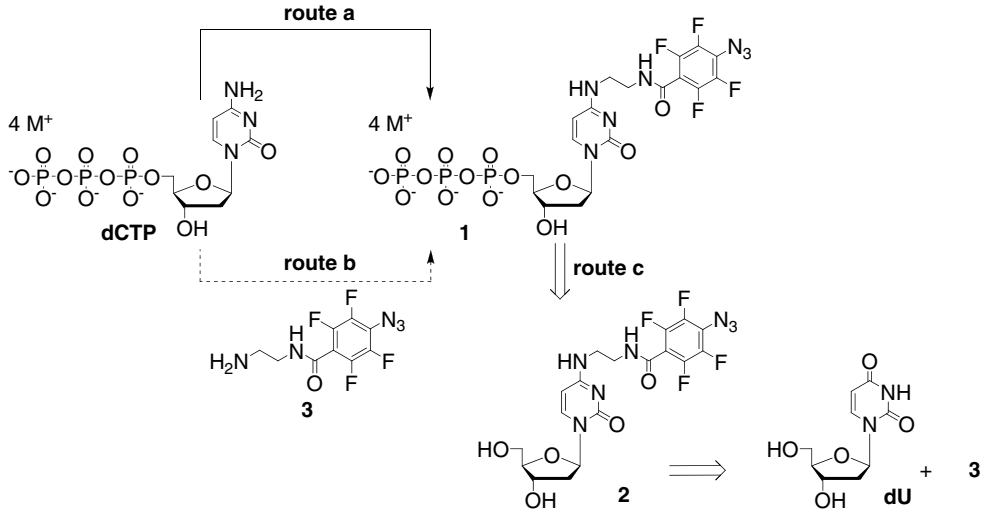
Modelling clustered DNA damage and repair requires oligonucleotides with DNA lesions in close proximity to each other, either in tandem, or on opposing DNA strands. These oligonucleotides act as model systems for clustered DNA damaged sites.[1] It has now been established that the overall repair of clustered sites is retarded when compared to the repair of the constituent isolated lesions, and the mechanism of repair is dependent on the lesions within the cluster, the interlesion distance and the orientation of the lesions to each other. However, little is known about which base excision repair proteins play the greatest role in the processing of clustered damage sites.

Photoaffinity labelling is a technique for marking the binding sites of proteins.[2] Perfluorophenyl azides have proven to be versatile labelling reagents and have already been used for the selective labelling of DNA binding proteins,[3–5] photoaffinity modification of human ribosome,[6,7] functionalization of bifunctional chelating agents for γ-imaging and radiotherapy[8] and, more recently, biotin-tagged photoaffinity probes.[9,10] A biological ligand modified with a photoactive moiety, such as the azido group, serves as a precursor of highly reactive intermediate nitrenes generated upon photolysis and capable of forming covalent crosslink bonds. By incorporating a photoaffinity labelling derivative of dCTP into the repair gap arising from the processing of a clustered damaged site, it is possible to capture base excision repair proteins involved in the repair of the cluster and compare them with the proteins involved in the repair of single lesions. In addition, capturing proteins at different times during the repair may provide insight into the dynamics of the repair processes.

Therefore, we were interested in the synthesis of the photoaffinity labelling (PAL) derivative of dCTP (**1**). The need to develop a new synthetic strategy for this compound emerged from the problems encountered when following the literature procedure (Scheme 1, route a).[11] The sequence of Scheme 1 could be reproduced by extending the reaction times (3 days vs 24 h), but, in our hands, the yields were unsatisfactory. Convergent to this, our attempt

* Corresponding author. Tel.: +30 210 727 4928; fax: +30 210 727 4761.
E-mail address: gimisis@chem.uoa.gr (T. Gimisis).
† Present address: Organic Chemistry Department, Babes-Bolyai University, 11 Arany Janos str., 400028 Cluj-Napoca, Romania.

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.12.083



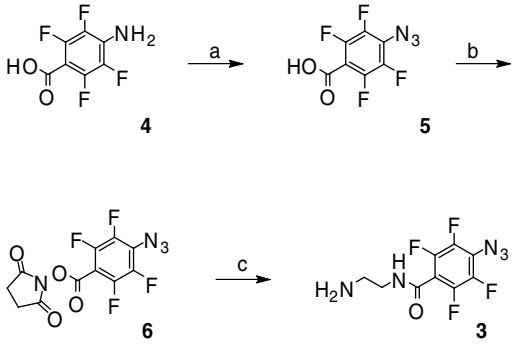
Scheme 1. Previous synthetic routes and current retrosynthetic analysis of PAL-dCTP (**1**).

for a one-step coupling to obtain the target derivative (Scheme 1, route b) met with little success because of the lability of the azide group in the reaction media. We found that, under the reaction conditions, the perfluoroazide derivative **3** reduced to the corresponding perfluoroaniline.

A retrosynthetic strategy for the synthesis of the target compound **1**, described in this Letter (route c), is also presented in Scheme 1. It relies on the intermediacy of a stable product such as **2,** whose protected precursor may be purified through normal phase chromatographic methods. A potential problem to overcome in this scheme would be the relative ease of reduction of the azide group, which has been mainly accomplished as presented below.

For the synthesis of compounds **6** and **3** (Scheme 2), we started from procedures described in the literature with essential changes that improved yield and selectivity.

Diazotation of the commercially available 4-amino-2,3,5,6-tetrafluorobenzoic acid with sodium nitrite, followed by displacement with NaN_3 was performed in trifluoroacetic acid[12] and afforded the aromatic azide in excellent yield after increasing the reaction time (Scheme 2).



Scheme 2. Reagents and conditions: (a) (i) 2 equiv $NaNO_2$, TFA, 1 h; (ii) 10 equiv NaN_3, Et_2O, 2 h (95%); (b) 1 equiv NHS, 1.02 equiv DCC, CH_3CN, overnight (90%); (c) 15 equiv ethylenediamine, CH_3CN, 0–5°C, 1 h (75%).

N-Hydroxy-succinimidyl ester **6**[13] was isolated in 90% yield with no traces of dicyclohexylurea by performing the reaction in acetonitrile instead of dichloromethane, followed by a standard ethyl acetate work-up. Monoacylation of ethylenediamine with derivative **6** gave a hard-to-separate 2.5:1 mixture of azide **3** and the corresponding reduced amine when following the literature procedure.[8] We overcame this problem by working at 0–5 °C, and selectively obtained the desired product, in an improved yield (Scheme 2).

A clear differentiation between those derivatives bearing the azide function and those with amine could be observed in ^{19}F NMR spectra. The signals corresponding to the two fluorine atoms next to azide (in the range −151 to −153 ppm) are at least 10 ppm shifted compared with the signals of the fluorine atoms next to a reduced amine group (in the range −164 to −166 ppm, Table 1). It is interesting to note that, although they are particularly useful, no ^{19}F NMR data had been previously reported for intermediates **5**, **6** or **3**.

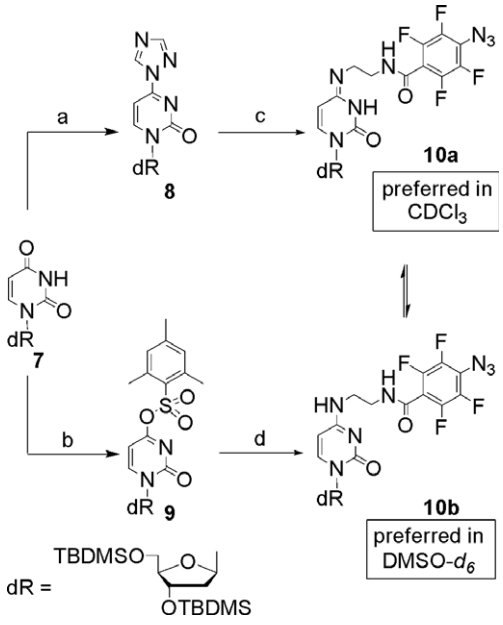
The TBDMS-protected deoxyuridine **7**[14] was chosen as starting material for the synthesis of the nucleoside building-block. The C-4 position on uracil was activated for nucleophilic substitution by conversion into either the triazolylpyrimidinone **8**[15] or the mesitylenesulfonyl derivative **9**[16] (Scheme 3, ^1H and ^{13}C NMR spectra are reported in the Supplementary data).

We next examined the method to obtain the new TBDMS-protected derivative **10** (see Supplementary data)

Table 1
^{19}F NMR shifts for compounds **4**, **5**, **6** and **3** (in $CDCl_3$)

Compd.	F_a	F_b
4	−162.60	−139.14
5	−151.08	−137.31
6	−150.26	−133.97
3	−150.99	−141.45

F_a represents the fluorine atoms *ortho* to the azide (or amino) groups.



Scheme 3. Reagents and conditions: (a) 4.4 equiv TBDMS–Cl, 8.8 equiv imidazole, dry DMF, overnight (96%), (b) 2.2 equiv MsCl, 0.25 equiv DMAP, Et_3N, anhyd DMF, 24 h (35%); (c) 1.5 equiv **3**, THF, 20 h (65%), (d) 2 equiv **3**, cat. K_2CO_3, CH_2Cl_2, 6 h (40%).

by the nucleophilic displacement of **8** or **9** with perfluoroazide **3**. The reaction of the mesitylene derivative **9** in the presence of a catalytic amount of K_2CO_3 yielded product **10** in moderate yield (40 %), even in the presence of a large excess of amine (10 equiv). A comparable yield was obtained when the triazolyl derivative **8** was reacted with amine **3,** in dry pyridine, but changing the solvent to tetrahydrofuran significantly increased the yield (65 %).

The ^1H NMR spectrum of compound **10** exhibited two sets of signals in $CDCl_3$, while just one set of sharp signals was observed in DMSO-d_6 solution. This indicated that **10** exists in a tautomeric equilibrium between 4-amino and the *E* and *Z* isomers of 4-imino forms in $CDCl_3$. A similar behaviour as well as isomeric ratio (3:1) was previously reported for a 4-amino-substituted pyrimidinone.[17] Interestingly, this pattern was better observed in the ^{19}F NMR spectra and the two sets of signals were registered in the $CDCl_3$ spectrum in a 3:1 ratio (Fig. 1).

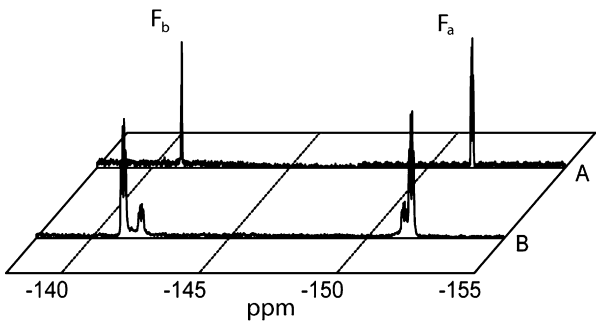
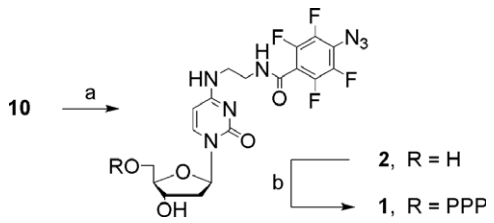


Fig. 1. Compound **10** ^{19}F NMR spectra in DMSO-d_6 (A) and $CDCl_3$ (B); F_a represents the fluorine atoms *ortho* to the azide group.



Scheme 4. Reagents and conditions: (a) $(HF)_n$/Py, THF, 2.5 h (50%), (b) (i) 1.3 equiv $POCl_3$, 1.5 equiv proton-sponge, 43 equiv triethyl phosphate, 3 h, 0 °C; (ii) 6.1 equiv Bu_3N, 2.4 equiv TBAP, 30 min. (15%).

For the cleavage of the TBS-protecting groups we found that the perfluorinated aromatic azide was not compatible with the more commonly used tetrabutylammonium fluoride (TBAF) reagent.[18,19] We isolated the new derivative **2** (Scheme 4) by using the milder $(HF)_n$/Py reagent[20] and the ^{19}F NMR spectrum showed that the azide function was stable under these conditions.

The final stage was the two-step, one-pot, triphosphorylation procedure in the presence of a proton-sponge[21] (Scheme 4), followed by semi-preparative HPLC purification of the reaction product. The reaction was followed by analytical HPLC to ensure the formation of the monophosphate intermediate and its conversion to the corresponding triphosphate.

The spectral data of the final compound **1** are comparable with those reported (^1H, ^{31}P NMR),[11] while the ^{19}F NMR spectrum confirms the presence of the azide group. The small differences in the proton NMR spectrum are probably due to the different counter-ion, that is, Na^+ versus Et_3NH^+ in our case.

The signals corresponding to the α and β phosphorus atoms in the ^{31}P NMR (pH 4) have similar shifts to the ones reported.[11] The γ phosphorus exhibited a 5 ppm high field shift. This is a well-documented shift,[22] attributable to the pH difference between the published (pH not reported) and our (pH 4) ^{31}P NMR spectra.

The expected two sets of signals are clearly shown in the ^{19}F NMR spectrum, with one at −144 ppm corresponding to the fluorine atoms (F_b) *ortho* to the amidic group and the other at −151 ppm corresponding to the fluorine atoms (F_a) next to the azide group.

Considering the above analyses, it was surprising to find that the ESI-MS exhibited molecular peak only for the reduced azide. Nevertheless, it has been reported that in the presence of a phosphoryl group, ESI induces the transformation of an azide to the corresponding amine.[23] This effect was not observed in the ESI-MS spectra of any of the other azides (compounds **3**, **5**, **6**, **10** and **2**), in this study.

In conclusion, we have developed an alternative route for the synthesis of PAL-dCTP derivative **1**. This involves the intermediacy of protected and free PAL-dC (compounds **10** and **2**, respectively), two new and easy-to-handle intermediates. ^{19}F NMR spectroscopy has proven extremely helpful in following the synthetic transformations

and in avoiding adventitious reduction of the azide to the corresponding amine. Compound **2** is useful for the preparation of the corresponding phosphoramidites and after insertion to PAL-oligonucleotides, together with **1**, in photoaffinity labelling studies.

Acknowledgements

The project was co-funded by the European Social Fund and National Resources (EPEAEK II, PYTHAGORAS). C.C. acknowledges financial aid by the European Communities' Marie Curie Research Training Network (Contract MRTN-CT-2003-505086 [CLUSTOXDNA]).

Supplementary data

Experimental procedures, ^{1}H NMR and ^{13}C NMR spectra of **9**, ^{19}F NMR spectra of **1**, **2** and **10**, HPLC trace and ^{31}P NMR spectrum of **1** and LR ESI-MS spectra of **1**, **2** and **10**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.12.083.

References and notes

1. Shikazono, N.; Pearson, C.; O'Neill, P.; Thacker, J. *Nucleic Acids Res.* **2006**, *34*, 3722–3730.
2. For an excellent introduction to the topic see: Soundararajan, N.; Liu, S. H.; Soundararajan, S.; Platz, M. S. *Bioconjugate Chem.* **1993**, *4*, 256–261.
3. Dezhurov, S. V.; Khodyreva, S. N.; Plekhanova, E. S.; Lavrik, O. I. *Bioconjugate Chem.* **2005**, *16*, 215–222.
4. Lavrik Olga, I.; Kolpashchikov Dmitry, M.; Prasad, R.; Sobol Robert, W.; Wilson Samuel, H. *Nucleic Acids Res.* **2002**, *30*, 7371–7373.
5. Lavrik, O. I.; Prasad, R.; Beard, W. A.; Safronov, I. V.; Dobrikov, M. I.; Srivastava, D. K.; Shishkin, G. V.; Wood, T. G.; Wilson, S. H. *J. Biol. Chem.* **1996**, *271*, 21891–21897.
6. Demeshkina, N. A.; Laletina, E. S.; Meschaninova, M. I.; Repkova, M. N.; Ven'yaminova, A. G.; Graifer, D. M.; Karpova, G. G. *Mol. Biol.* **2003**, *37*, 132–139.
7. Demeshkina, N.; Laletina, E.; Meschaninova, M.; Ven'yaminova, A.; Graifer, D.; Karpova, G. *Biochim. Biophys. Acta* **2003**, *1627*, 39–46.
8. Rajagopalan, R.; Kuntz, R. R.; Sharma, U.; Volkert, W. A.; Pandurangi, R. *J. Org. Chem.* **2002**, *67*, 6748–6757.
9. Han, S.-Y.; Park, S.-S.; Lee, W. G.; Min, Y. K.; Kim, B. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 129–133.
10. Han, S.-Y.; Choi, S. H.; Kim, M. H.; Lee, W. G.; Kim, S. H.; Min, Y. K.; Kim, B. T. *Tetrahedron Lett.* **2006**, *47*, 2915–2919.
11. Wlassoff, W. A.; Dobrikov, M. I.; Safronov, I. V.; Dudko, R. Y.; Bogachev, V. S.; Kandaurova, V. V.; Shishkin, G. V.; Dymshits, G. M.; Lavrik, O. I. *Bioconjugate Chem.* **1995**, *6*, 352–360.
12. Pinney, K. G.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *56*(9), 3125–3133.
13. Keana, J. F. W.; Cai, S. X. *J. Org. Chem.* **1990**, *55*, 3640–3647.
14. Zhang, W.; Rieger, R.; Iden, C.; Johnson, F. *Chem. Res. Toxicol.* **1995**, *8*, 148–156.
15. Quinn, J. R.; Zimmerman, S. C. *J. Org. Chem.* **2005**, *70*, 7459–7467.
16. Bischofberger, N. *Tetrahedron Lett.* **1987**, *28*, 2821–2824.
17. Katoh, A.; Hida, Y.; Kamitani, J.; Ohkanda, J. *J. Chem. Soc., Dalton Trans.* **1998**, 3859–3864.
18. Golinski, M.; Heine, M.; Watt, D. S. *Tetrahedron Lett.* **1991**, *32*, 1553–1556.
19. Chehade, K. A. H.; Spielmann, H. P. *J. Org. Chem.* **2000**, *65*, 4949–4953.
20. Huang, Y.; Torres, M. C.; Iden, C. R.; Johnson, F. *Bioorg. Chem.* **2003**, *31*, 136–148.
21. Kovacs, T.; Otvos, L. *Tetrahedron Lett.* **1988**, *29*, 4525–4528.
22. Yoza, N.; Ueda, N.; Nakashima, S. *Fresenius J. Anal. Chem.* **1994**, *348*, 633–638.
23. Xiao, Q.; Ju, Y.; Yang, X.; Zhao, Y.-F. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1405–1410.