

Synthesis of Fluorescent, Photolabile 3'-O-Protected Nucleoside Triphosphates for the Base Addition Sequencing Scheme

Mike B. Welch & Kevin Burgess

To cite this article: Mike B. Welch & Kevin Burgess (1999) Synthesis of Fluorescent, Photolabile 3'-O-Protected Nucleoside Triphosphates for the Base Addition Sequencing Scheme, Nucleosides and Nucleotides, 18:2, 197-201, DOI: [10.1080/15257779908043067](https://doi.org/10.1080/15257779908043067)

To link to this article: <http://dx.doi.org/10.1080/15257779908043067>



Published online: 04 Oct 2006.



Submit your article to this journal [↗](#)



Article views: 64



View related articles [↗](#)



Citing articles: 11 View citing articles [↗](#)

SYNTHESIS OF FLUORESCENT, PHOTOLABILE 3'-O-PROTECTED NUCLEOSIDE TRIPHOSPHATES FOR THE BASE ADDITION SEQUENCING SCHEME

Mike B. Welch and Kevin Burgess*

Department of Chemistry, Texas A & M University, PO Box 300012,
College Station, TX 77842-3012

ABSTRACT: The dansylated nucleoside triphosphates **1a** and **1b** were prepared as a prelude to investigating sequencing of DNA via a scheme that does not involve electrophoresis.

Throughput of sequence data is critical in The Human Genome Project and similar ventures. Gel electrophoresis is a "bottle-neck" in high throughput sequencing of DNA hence it is desirable to develop methods that avoid this technique. Several groups have been interested in an approach that we call, "The Base Addition Sequencing Scheme", BASS.¹⁻⁶ This involves modified nucleotides **I** that are: (i) incorporated by DNA replicating enzymes; (ii) spectroscopically distinct (at P*) so that the parent base (A, T, G, and C) can be differentiated; and, (iii) labile at the 3'-position such that the 3'-hydroxyl terminus of a polynucleotide can be regenerated. This scheme may be slower and sequence less bases per experiment than existing methods but, unlike methods involving gels, there is potential for multiple experiments to be run in parallel. Such combinatorial sequencing schemes may lead to significantly improved throughput. A major challenge in development of BASS, however, is that the 3'-blocking group must be

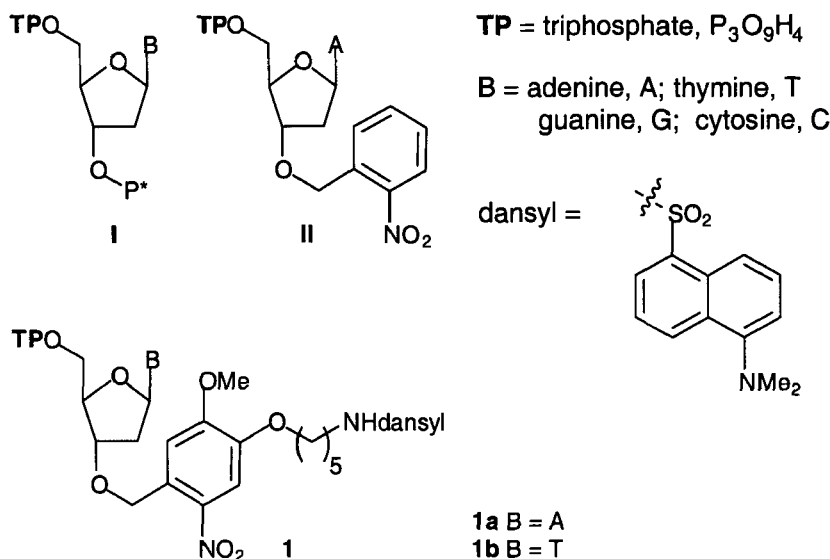
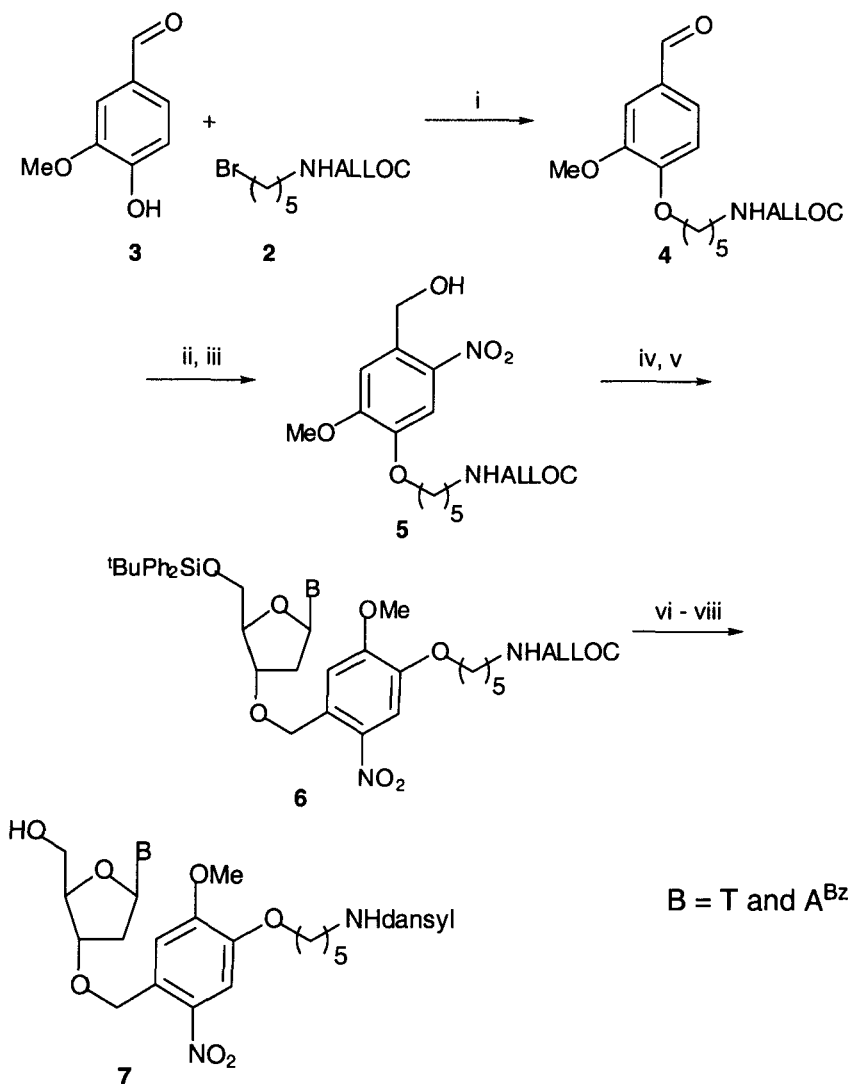


Diagram 1

removed in an aqueous medium using reagents/conditions that do not denature or modify double strand DNA. To address this issue, the focus of our efforts has been on photolabile protection. Earlier work from these labs proved that 3'-O-(2''-nitrobenzyl)adenosine triphosphate **II** could be incorporated by a DNA polymerase.¹ In an extension of these studies, we now describe syntheses of two systems **1a** and **1b** containing a 3'-O-(2-nitrobenzyl) group modified to carry a fluorescent label (Diagram 1).

The synthetic approach to target molecules **1a** and **1b** has parallels with chemistry developed for solid phase photolabile linkers.^{7,8} Details of the synthetic route used are given in Scheme 1. The most difficult step in this procedure was coupling of a benzylic halide to the 3'-hydroxyl group of a nucleoside derivative. Development of conditions for this step required considerable experimentation.

Details of the synthesis shown in Scheme 1 are as follows. The allyloxycarbonyl (ALLOC) protected bromide **2** was used to alkylate the vanillin derivative **3** to give the aldehyde **4**. Nitration then borohydride



Scheme 1. Reagents and conditions: i, K_2CO_3 , KI, MeCN, reflux, 12 h (>95 %); ii, HNO_3 , Ac_2O , 0 °C; iii, NaBH_4 , EtOH, 0 °C, 2 h then 25 °C, 2 h (53 % for ii + iii); iv, CBr_4 , PPh_3 , EtOAc, 25 °C, 1 h; v, 5'-O-*tert*-butyldiphenylsilylnucleoside, NaI, Bu_4NOH , CH_2Cl_2 , 1 M NaOH, 25 °C, 16 h (iv + v, 49 % for B = A^{Bz} , 88 % for B = T); vi, cat. $\text{Pd}(\text{PPh}_3)_4$, THF, HNEt_2 , 25 °C, 2.5 h; vii, dansyl-Cl, NEt_3 , cat. 4-DMAP, PhMe/THF (1:1), 25 °C, 1 h; viii, Bu_4NF , THF, 25 °C, 11 min for T, and 10 min at 0 °C then 1 h at 25 °C for A^{Bz} ; (vi – viii, 69 % for B = A^{Bz} , 66 % for B = T).

reduction of this gave the benzylic alcohol **5**, which emerged as a key intermediate in this work. Coupling of benzyl alcohol **5** to the 3'-hydroxyl group of nucleoside derivatives was difficult. After considerable experimentation, it was found that conversion to the benzylic bromide, then alkylation *under phase transfer conditions* worked whereas many obvious alternative approaches did not. Removal of the ALLOC group from the coupled products **6**, dansylation, then removal of the 5'-silyl protecting group gave the desired nucleosides **7**.

Triphosphorylation of nucleosides **7**, and of many other unnatural nucleosides prepared in these labs, is experimentally difficult and tends to give poor yields. Ultimately, we settled on the protocol developed by Eckstein and co-workers,⁹ although none of the methods attempted were entirely satisfactory. Fortunately, only small amounts of the product are required for feasibility tests in bioassays. Debenzoylation of the adenosine derivative **7a** was performed after the triphosphorylation sequence (NH₄OH, 60 °C, 3 h). Both final products, **1a** and **1b**, were purified via chromatography, first on DEA cellulose, then by RP HPLC.

Preliminary tests¹ of compounds **1a** and **1b** as substrates for polymerases (Klenow, rTth DNA polymerase, and Vent (exo-) DNA polymerase) did not show evidence for incorporation. Further experiments are in progress, including modeling studies to attempt to dock **1a** and **1b** in the active sites of polymerases. The latter experiments should highlight bad interactions that must be avoided to advance this work further.

Acknowledgments. We thank Richard Gibbs of Baylor College of Medicine for valuable advice. Support for this work was provided by The Texas Technology Program, The Robert A. Welch Foundation, and The National Institutes of Health (HG/GM01745-01). KB thanks the NIH Research Career Development Award, and The Alfred P. Sloan Foundation for a fellowship.

REFERENCES

1. Metzker, M. L.; Raghavachari, R.; Richards, S.; Jacutin, S. E.; Civitello, A.; Burgess, K.; Gibbs, R. A. *Nucl. Acids Res.* **1994**, *22*, 4259.
2. Tsien, R. Y.; Ross, P.; Fahnestock, M.; Johnston, A. J. WO Patent 91/06678, 1991.
3. Cheeseman, P. C. US Patent 5,302,509, 1994.
4. Wilhelm, A. Germany Patent DE 41 41 178 A1, 1993.
5. Rosenthal, A.; Close, K.; Brenner, S. WO Patent 93/21340, 1993.
6. Canard, B.; Sarfati, R. S. *Gene* **1994**, *148*, 1.
7. Yoo, D. J.; Greenberg, M. M. *J. Org. Chem.* **1995**, *60*, 3358.
8. Holmes, C. P. *J. Org. Chem.* **1997**, *62*, 2370.
9. Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1989**, *54*, 631.

received 7/16/98

accepted 11/23/98