

This article was downloaded by: [Université de Moncton]

On: 16 October 2014, At: 04:15

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides and Nucleotides

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/Incn19>

Efficient Automated Synthesis of Molecular Beacons

Bashar Mullah^a & Ken Livak^a

^a PE Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA, 94404, USA

Published online: 04 Oct 2006.

To cite this article: Bashar Mullah & Ken Livak (1999) Efficient Automated Synthesis of Molecular Beacons, Nucleosides and Nucleotides, 18:6-7, 1311-1312, DOI: [10.1080/07328319908044700](https://doi.org/10.1080/07328319908044700)

To link to this article: <http://dx.doi.org/10.1080/07328319908044700>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

EFFICIENT AUTOMATED SYNTHESIS OF MOLECULAR BEACONS

Bashar Mullah* and Ken Livak

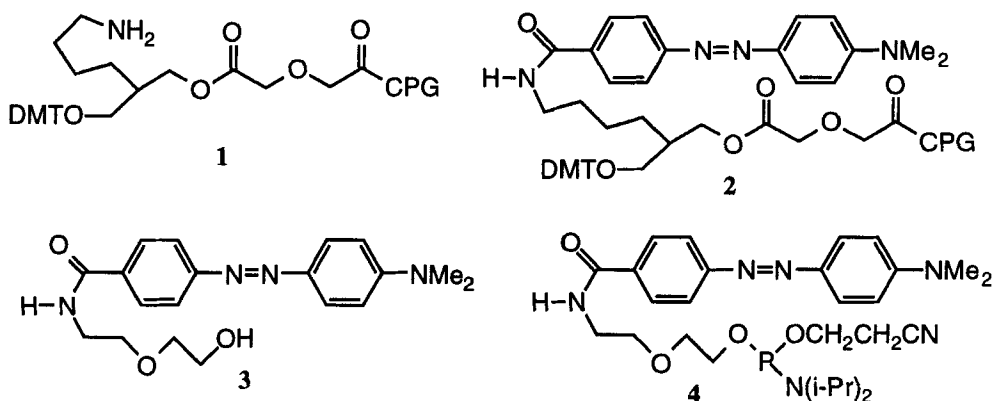
PE Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404, USA

ABSTRACT: Automated synthesis of molecular beacons using 4-(4-dimethylamino phenylazo) benzoic acid (dabcyl) support and phosphoramidite is described.

Molecular beacons are oligonucleotide probes bearing a fluorescent reporter and a non-fluorescent quencher dyes and are used for detection of specific nucleic acids sequences in hybridization based assays.¹ Molecular beacons possess a stem-loop structure. The stem brings reporter and quencher dyes in close proximity which results in quenching of the reporter by fluorescence resonance energy transfer.² The non-fluorescent quencher used in molecular beacons is 4-(4-dimethylamino phenylazo) benzoic acid (dabcyl). Reported synthesis of molecular beacons involves two consecutive reactions of deprotected oligonucleotide containing a sulfhydryl and a primary amino groups at two ends with activated dye derivatives.¹ Post synthesis addition of dyes to beacons involves labor intensive purification process and double dye-labeled beacons are isolated in low yield. Here we describe an efficient automated synthesis of molecular beacons.

We have reported automated synthesis of double dye-labeled oligonucleotide probes in high yield by using dye labeled solid supports and dye phosphoramidites.³ Only a few fluorescent dyes are commercially available as phosphoramidites.⁴ In an effort to automate and to improve the synthesis of molecular beacons we have derivatized dabcyl labeled solid support (**2**) and synthesized dabcyl phosphoramidite (**4**). Coupling of dabcyl N-hydroxysuccinimide ester with non-nucleosidic support (**1**) in presence of triethylamine in DMF gave dabcyl labeled CPG support (**2**). Dye coupling was determined to be 96-98%. Compound **3** was synthesized in 98% yield by treating 2-aminoethoxyethanol with dabcyl in presence of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and diisopropylethylamine in DMF. Reaction of **3** with 2-cyanoethyl tetraisopropyl-phosphorodiamidite in presence of tetrazole gave dabcyl phosphoramidite **4** in 68% yield

after chromatography. Dabcyl labeled CPG support was used with fluorescein phosphoramidites for the synthesis of fluorescein dye containing molecular beacons. For the synthesis of rhodamine dye containing beacons dabcyl phosphoramidite was used with rhodamine dye labeled supports. To investigate the quenching of fluorescein and rhodamine dyes by dabcyl, we have synthesized a number of molecular beacons having sequence, 5'>X GCGAG-CCCTTCTCTGTGGCCACTTCAGCCTG-CTCGC-Y<3'. For fluorescein beacons, X = FAM, TET, HEX and Y = dabcyl whereas for rhodamine beacons, X = dabcyl and Y = TMR, ROX. The T_m of the stem was found to be 60-62°C. Quenching efficiency of dabcyl was measured by recording fluorescence spectra of the beacons at 22 °C and 73°C respectively. At 22°C the intact stem keeps the reporter in close proximity to dabcyl, resulting in greatly reduced fluorescence of reporter due to quenching. The stem opens at 73°C and this causes the reporter to move



away from the quencher. As a result a significant increase in fluorescence of the reporter dye is observed. Fluorescein dye containing beacons showed 15-30 times increase in fluorescence at 73°C whereas rhodamine dye containing beacons showed 3 times increase in fluorescence at 73°C. The results indicated that dabcyl quenched fluorescein dyes better than rhodamine dyes. However, the quenching of rhodamine dyes was sufficient and these dyes could be used as reporters. The use of dabcyl support and amidite allows automated synthesis of molecular beacons containing fluorescein and rhodamine dyes as reporter.

REFERENCES

1. Tyagi, S.; Kramer, F. R., *Nature Biotechnology*, **1996**, *14*, 303-308.
2. Stryer, L., *Ann. Rev. Biochem.*, **1978**, *47*, 819-846.
3. (a) Mullah, B.; Andrus, A., *Tetrahedron Lett.*, **1997**, *38*, 5751-5754. (b) Mullah, B.; Livak, K.; Andrus, A.; Kenney, P., *Nucleic acids Res.*, **1998**, *26*, 1026-1031.
4. Applied Biosystems User Bulletin No. 78.