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Larry L. Klein [a] & Valentina Petukhova [a]
[a] Institute for Tuberculosis Research, University of Illinois at Chicago , Chicago , Illinois , USA
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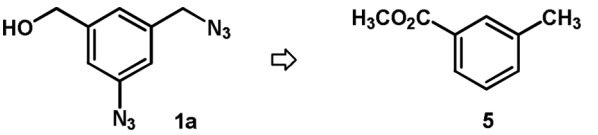
SYNTHESIS OF TRIFUNCTIONAL BIS-AZIDE PHOTOAFFINITY PROBE

Larry L. Klein and Valentina Petukhova
Institute for Tuberculosis Research, University of Illinois at Chicago, Chicago, Illinois, USA

GRAPHICAL ABSTRACT



Abstract *Methyl 3-azidomethyl-5-azido-benzoate (**1b**) and its corresponding hydroxymethyl reduction product (**1a**) have been utilized for the synthesis of valuable nonradioactive photoaffinity probes. Previous preparations of (**1a**) depend upon a nonselective monoactivation of a bis-1,3-hydroxymethylaryl intermediate, leading to the expected statistical range of products/starting material. We report an alternate synthetic approach to these 1,3,5-trisubstituted molecules using recently described C-H activation and boronate refunctionalization methods.*

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Keywords Azide; borylation; photoaffinity

INTRODUCTION

Photoaffinity labeling is a useful tool for investigation of ligand–protein interaction.[1] This process involves the use of a binding substrate (probe) that has been modified with a photoactive functional group. Upon irradiation, this group produces a chemoreactive center (e.g., carbene, nitrene), which can form a covalent bond to proximal atoms in the protein, thus establishing these proteins as potential binding targets for this molecule. The actual photoactive groups have included benzophenones,[2] diazirines,[1,3] and azides,[4] among others, with the former being less reactive/more selective and the latter being more reactive/less selective. Various nonradioactive methods have been used to identify these proteins and usually involve a second chemical connection ("tagging") to rhodamines/fluoresceins,[5]

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Address correspondence to Larry L. Klein, Institute for Tuberculosis Research, University of Illinois at Chicago, 833 S. Wood Street, Chicago, IL 60612, USA. E-mail: llk@uic.edu

biotin,[6] or fluorous-related[7] derivatives to isolate the "tagged" protein via visual or affinity-binding techniques.

DISCUSSION

In recent years, molecules containing a bisazide moiety such as **1a–c** (Fig. 1) have been utilized as a photoaffinity probes[4] to identify or characterize proteins involved in calcium ion release[8] and HDAC8 inhibitors.[9] In these cases, the ester or sulfonate group functionality serves as this probe's point of attachment via amide or ether linkage, respectively. For this tagging process to be successful, the attachment must occur on the ligand at sites that do not interfere with binding to the protein. The aryl azide in **1c** is selectively photoactivated in the presence of the more stable benzyl azide[4] and forms the covalent link to the protein. Subsequent Huisgen reaction of the benzyl azide with a pendant alkyne group of the visualizing agent is carried out to assist in the detection/isolation of the probe-linked protein. The 1,3,5-substitution pattern was key to isolating the three orthogonal synthetic processes for this novel moiety.

The synthesis of probes **1a** and **1b** have been reported[4] and modified in later work.[10] In both cases, the key limiting reaction involves a statistical monofunctionalization of the intermediate diol **2**, which in turn is prepared from commercially available diester **3**. We were in need of this useful probe and realized that this nonselective reaction of **2** could be avoided through the use of recently evolving technologies of C-H activation and boronaterefunctionalization and so report an alternate synthesis of **1** herein.

Malezcka and Smith[11] and Hartwig[12] reported an efficient regioselective formation of 1,3,5-trisubstituted aromatic molecules via the use of iridium-based catalyzed borylation reaction. Treatment of methyl 3-methylbenzoate under modified borylation conditions[13] led to a good yield of the desired regioisomer **6** (Scheme 1). Although consistent reports of the yield of **6** were 91–96%, using non-Schlenk techniques and microwave irradiation, we were only able to obtain 48% yield on a multigram scale. With this key intermediate in hand, we found that radical bromination[14] could be successfully carried out to afford **7**, containing a small amount of the purported α,α-dibromide after recrystallization from cyclohexane. Because of some instability, this material was used directly for subsequent bromide displacement with sodium azide to give the corresponding monoazide **8**. Recent

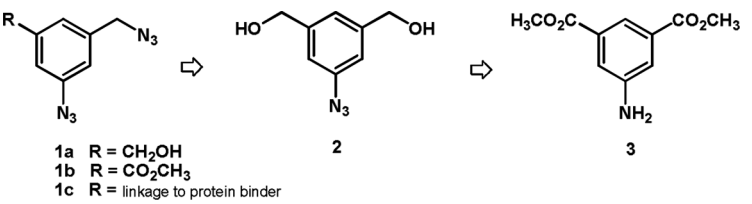
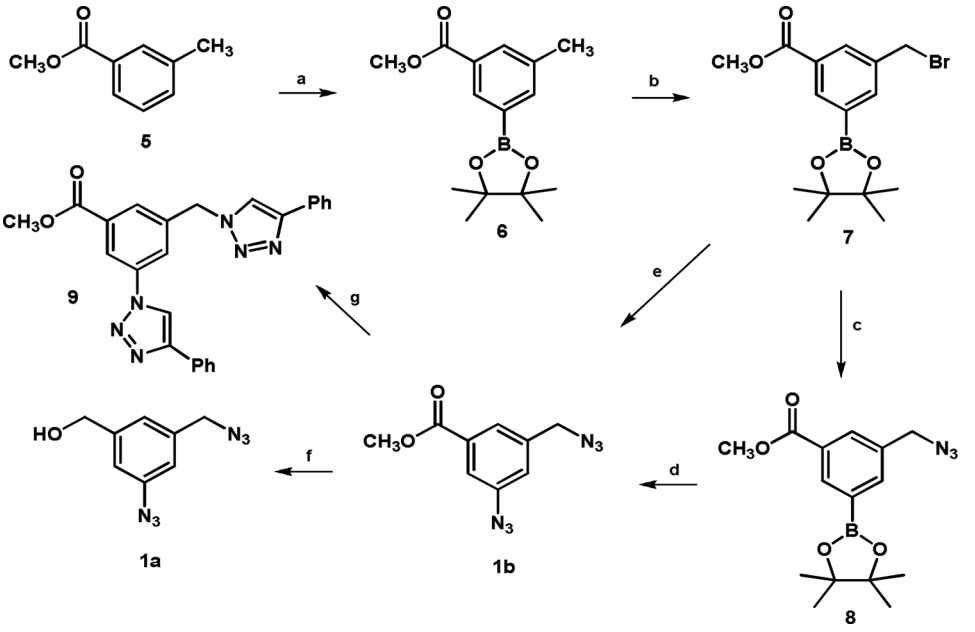


Figure 1. Structure of photoaffinity label.



Scheme 1. Alternate synthesis of **1a**. Reagents: (a) B_2Pin_2, $[COD(OMe)Ir]_2$, dtbpy, hexane; (b) NBS, Bz_2O_2, CCl_4; (c) 1.5 eq. NaN_3, MeOH; (d) 1.5 eq. NaN_3, 0.1 eq. CuOAc, MeOH; (e) 3 eq. NaN_3, 0.1 eq. CuOAc, MeOH; (f) 4 eq. DiBAH (1 M in hexanes); (g) 2 eq. PhC≡CH, 0.4 eq. sodium ascorbate, 0.1 eq. Cu_2SO_4, CH_3CN/H_2O.

work in the area of copper-catalyzed refunctionalization of boronates with a wide variety of nucleophiles[15] suggested that the aryl azide could be formed in a subsequent step from substrates such as **8**.

Treatment of azido-boronate **8** with sodium azide in the presence of catalytic copper acetate smoothly led to the known bis-azido-ester **1b**[4] in good yield. To further optimize this process, bromide **7** was directly reacted with 3 equivalents of sodium azide in the presence of copper acetate to afford **1b** in 65% yield without any intervening chromatography. Because the azide products produced no parent ion under standard mass spectrometry (MS) conditions, further structure proof for **1b** was obtained by derivatizing this compound via "Click" chemistry to give the bis-adduct **9**. A sample of **1b** was treated under standard Huisgen conditions (2 eq. phenyl acetylene, 0.4 eq. sodium ascorbate, 0.1 eq. copper sulfate, in 10% aqueous acetonitrile stirred at room temperature) for 2 days. The solvents were evaporated, and the residue was purified to give an analytical sample of bis-triazole **9**.[16]

Although the ester **1b** has been coupled via the Staudinger–Bertozzi methodology to give amide-linked probes,[17] our work required the corresponding ether linkage arising from coupling of our protein-binding entity to the benzyl tosylate. Through the action of diisobutylaluminum hydride, ester **1b** was reduced to the desired alcohol **1a** in 74% yield from which the tosylate or mesylate intermediate can be accessed as per literature.[4,10]

CONCLUSION

This alternate route provides probe **1a–c** from easily available starting materials by taking advantage of the continuously evolving C-H activation and boronate-functionalization protocols.

SUPPORTING INFORMATION

Synthetic experimental details and ^{1}H and ^{13}C NMR characterizations of **1a**, **1b**, **6**, **7**, and **8** are available online. HRMS details for bis-"click" adduct of **1b** (cpd. **9**) are also included.

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16. The residue was purified via preparative thin-layer chromatography (TLC) (0.5-mm plate) using ethyl acetate–hexanes (1:1) to give an analytical sample of bis-triazole **9**. HRMS calculated for $C_{25}H_{20}N_6O_2$ ($M+H^+$) 437.1721; found 437.1720.
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