Pergamon

Tetrahedron Letters 42 (2001) 2249–2251

TETRAHEDRON LETTERS

An efficient synthesis of a class of heterobifunctional photo-reactive crosslinkers, labels, and probes

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Received 6 October 2000; revised 17 January 2001; accepted 22 January 2001

Abstract—Aryl esters are selectively reduced with DIBAL-H in the presence of an azide functional group to provide access to 4-azido-2-alkoxybenzaldehydes in five steps with overall yields ranging from 72 to 78%. This methodology improves traditional approaches to this class of compounds, which suffer from poor overall yields (4–11%). Our approach can be used to synthesize a variety of new aryl azide cross-linkers, labels, and probes. © 2001 Elsevier Science Ltd. All rights reserved.

Heterobifunctional photoaffinity cross-linkers containing aryl azides, have simplified the study of protein–protein interactions in multi-subunit proteins[1] and have found use as photoaffinity probes/labels due to their ability to be made radioactive with high specific acitivities.[2] In contrast to homobifunctional reagents, heterobifunctional reagents allow the stepwise activation of crosslinking groups. Thus, a single subunit or small molecule can be activated with one functional group of a photo-reactive heterobifunctional reagent (Fig. 1). This protein or ligand can then be coupled to an adjacent protein or protein active site[3] via the selective activation of the remaining functional group of the bifunctional reagent.

Despite these useful applications, extensive employment of the 4-azido-2-alkoxybenzaldehydes has been hampered by the extremely laborious and inefficient synthesis of these reagents. In this letter, we describe a rapid and efficient synthesis of a series of derivatized azidobenzaldehydes for use as labeling and cross-linking reagents.

In our studies of new photolithographic methods, we envisioned using 4-azido-2-alkoxybenzaldehydes as agents for the manufacture of self-assembled monolayers (SAM). Ultimately, by taking advantage of the photoreactive properties of the azide functional group, the formed SAMs will undergo well-defined photoinduced reactions. Photopatterning a SAM in the presence of a

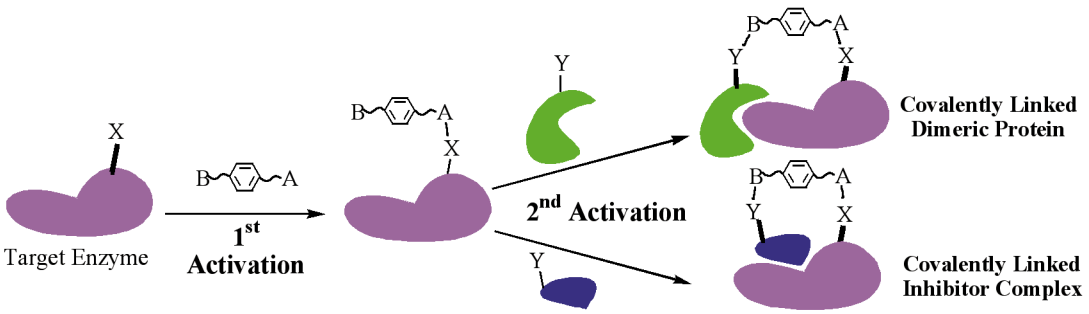

Figure 1.

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PII: S0040-4039(01)00131-9

chosen trapping agent, opens the possibility of tailoring surfaces with a variety of molecular functionalities.

Accordingly, we required multi-gram quantities of 4-azido-2-alkoxybenzaldehydes and found the previously described synthesis[1e–g,2d] to be plagued by the formation of undesired by-products and difficult purifications.[1e–g,2d,4] Due to the poor yields achieved using these methods, 4–11% for four steps, we conceived an improved route to these compounds that would provide both a facile set of reactions and a high overall yield for the sequence of steps.

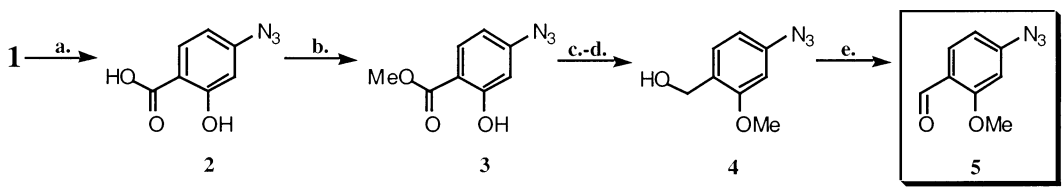
We found that the use of the methyl ester **3** (Scheme 1) rather than the mixed anhydride, could avoid the formation of the observed by-products of both a benzyl amine and alcohol. The later products are the result of over-reduction of the azide and acid functional groups within **2** using literature methods.[1e–g,2d,4] In this case, a milder reducing agent could be used en route to the desired aldehyde. Also, the diminished water solubility and ease of reduction compared to the acid derivative made the ester a desirable intermediate. To support work to this end, we had to be certain that the ester could be both synthesized in high yield and reduced selectively in the presence of the azide. These tasks were successfully completed.

We chose 4-azido-2-methoxybenzaldehyde **5** as our model compound for the synthesis of 4-azido-2-alkoxybenzaldehydes (Scheme 1). Synthesis of this compound would test if our approach would circumvent the aforementioned problems.

First, using a modified procedure of Smith and Brown,[5,6] 4-aminosalicylic acid **1** was diazotized with HNO_3 and H_2SO_4. Upon the introduction of NaN_3, the aryl azide **2** was formed in excellent yield without the need for the traditional recrystallization. The acid **2** was then converted to the methyl ester **3** without purification with $BF_3{\cdot}Et_2O$ and methanol.[6] Acid-catalyzed methanolysis yielded the desired ester in only modest yields, due to the formation of the unreacted acid **2** as well as the decarboxylated azide.[7] Alkylation of the free hydroxy group with methyl iodide (MeI) in refluxing acetone gave the methyl aryl ether in quantitative yield.[8] Treatment of the crude azide with DIBAL-H resulted in the selective reduction of the ester to yield the benzyl alcohol **4**. Oxidation of this free alcohol using Swern conditions[9] gave the desired 4-azido-2-methoxybenzaldehyde **5** in high yield after purification by flash chromatography. In addition, MnO_2[10] and Dess–Martin[11] reagents gave the aldehyde in quantitative yields. The overall process of converting the starting material to 4-azido-2-methoxybenzaldehyde **5**, includes five steps and one purification, and proceeds in 72–78% overall yield.[12]

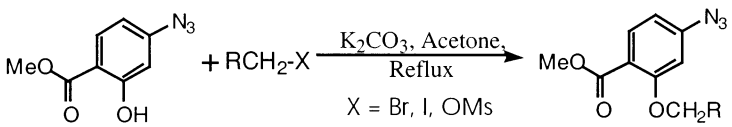
Table 1 summarizes the results of the reactions of other alkylating agents with **3** using K_2CO_3 in acetone. Since the reaction conditions were optimized, inspection of the table reveals both the alkyl iodide (entries **1**–**2**) and mesylates (entries **3**–**5**) afford the desired compound in high yields. The ideal reaction conditions for the alkylation include ten equivalents of the alkylating agent and five equivalents of the base, K_2CO_3. Alkylations employing the use of triethylamine as the base in DMF do not go to completion.

As expected, alkyl bromides could serve adequately in the desired alkylation, but required a longer reaction time (70 hours) and gave lower yields of the desired



Scheme 1. (a) HNO_2, NaN_3, 91%; (b) $BF_3{\cdot}Et_2O$, MeOH, reflux, 90%; (c) MeI, K_2CO_3, acetone, reflux; (d) DIBAL-H, CH_2Cl_2, −78°C, 95% (two steps); (e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78°C, 92%.

Table 1. Alkylating agents used for the derivatization of aryl azide **3** in Scheme 1



Entry	Substrate	Equivalents	Base/equivalents	Time (h)	Yield (%)
1	$CH_2{=}CH(CH_2)_7CH_2I$	10	K_2CO_3/5	7.5	98
2	$CH_2{=}CH(CH_2)_7CH_2I$	15	K_2CO_3/5	7.5	100
3	$CH_2{=}CH(CH_2)_7CH_2OMs$	10	K_2CO_3/5	17	99
4[a]	$OHC(CH_2)_8CH_2OMs$	10	K_2CO_3/5	45	80
5	$TBSOCH_2(CH_2)_8CH_2OMs\beta$	10	K_2CO_3/5	24	98

[a] The aldol product of acetone, the solvent, reacting with the free aldehyde was observed.

product (25–80% with 3–10 equivalents of $CH_2CH(CH_2)_7CH_2Br$) than the reactions with the corresponding alkyl iodide or mesylate (entries **1**–**5**). Further manipulation of the products isolated from these reactions, by the strategies described within, allow the possibility of using the azido-aldehyde derivatives as surface-bound reagents. Currently, we our modifying substrates of the type synthesized in Table 1 for their use in SAMs. In general, by simply changing the nature of the alkylating agent, one can gain access to numerous derivatives of 4-azido-2-alkoxybenzaldehydes. In summary, our facile, high yielding synthesis of 4-azido-2-methoxybenzaldehyde **5** provides a potentially useful approach to highly derivatized 4-azido-2-alkoxybenzaldehydes, and therefore, cross-linkers, labels, and probes.

We believe the one extra step in the synthesis of the mentioned class of azidobenzaldehydes is offset by the ease of the reactions performed and the high overall yield. In addition, to our knowledge, the reaction described here involving the selective reduction of an aryl ester in the presence of an aryl azide is the first example using DIBAL-H. This approach may prove useful in other systems.

Acknowledgements

Financial support from the National Science Foundation (NSF CTS-9816494) and (NSF CHE-9501728) (D.C.M.), the Alfred P. Sloan Foundation (D.C.M.), and the Rohm and Haas Company (D.C.M.) is gratefully acknowledged.

References

1. (a) Knapp, D. R.; Oatis, J. E. *Tetrahedron Lett*. **1998**, *39*, 1665; (b) Kominami, R.; Uchiumi, T. *EMBO J*. **1994**, *14*, 3389; (c) Burgess, R. R.; McMahan, S. A. *Biochemistry* **1994**, *33*, 12092; (d) Moazed, D.; Noller, H. F.; Roberston, J. M. *Nature* **1988**, *334*, 362; (e) Maassen, J. A.; Moller, W. *Eur. J. Biochem*. **1981**, *2*, 279; (f) Maassen, J. A. *Biochemistry* **1979**, *7*, 1288; (g) Maassen, J. A.; Moller, W. *J. Biol. Chem*. **1978**, *253*, 2777.
2. (a) Tumay, A. W.; Rajasekharan, R. *BBA-Mol. Cell Biol*. **1999**, *1*, 47; (b) Crocker, P. J.; Dwyer, L. D.; Watt, D. S.; Vanaman, T. C. *J. Biol. Chem*. **1992**, *267*, 22606; (c) Buchardt, O.; Henriksen, U. *Tetrahedron Lett*. **1990**, *31*, 2443; (d) Arevalo, M. A.; Ballesta, J. P. G.; Polo, F.; Tejedor, F. *J. Med. Chem*. **1989**, *32*, 2200; (e) Cooperman, B. S. *Methods Enzymol*. **1988**, *164*, 341.
3. (a) Antonovic, L.; Hodek, P.; Novak, P.; Smrcek, S.; Strobel, H. W.; Sulc, M. *Arch. Biochem. Biophys*. **1999**, *370*, 208; (b) Arevalo, M. A.; Ballesta, J. P. G.; Polo, F.; Tejedor, F. *J. Biol. Chem*. **1988**, *263*, 58.
4. **Conventional synthesis of 4-azido-2-alkoxybenzaldehydes**. (a) HNO_2, NaN_3, H_2O, 70%; (b) CDI (carbon diimidazole), dioxane; (c) $LiAlH_4$, Et_2O, −20°C, 11–25% (two steps); (d) R-I/R-Br, K_2CO_3/KOH, acetone/EtOH, 55–65%.
5. 4-Aminosalicylic acid **1** (5.0 g, 33 mmol) was dissolved in a solution of 25 mL H_2SO_4 and 130 mL deionized water in a 2 L roundbottomed flask. The resulting mixture was cooled to 0°C whereupon the amine was diazotized with a solution of $NaNO_2$ (2.8 g, 40 mmol) in 25 mL deionized water. After stirring for 1 hour at 0°C, a solution of NaN_3 (3.6 g, 56 mmol) in 20 mL deionized water was added dropwise to the chilled reaction. The suspension was kept in the ice-bath and stirred for 1 hour after the final addition, and was then allowed to stand overnight at room temperature. The reaction mixture was washed with ethyl acetate, then satd NaCl. After drying the organics, evaporation yielded 5.3 g (91%) of the azide **2** as an orange solid.
6. Kadaba, P. K. *Synth. Commun*. **1974**, *2*, 167.
7. Doub, L.; Schaefer, J. J. *J. Am. Chem. Soc*. **1949**, *71*, 3564.
8. Our work has shown both diazomethane and trimethylsilyldiazomethane can serve to transform acid **2** to the desired aryl ether **5**. This method is not applicable for the synthesis of 4-azido-2-alkoxybenzaldehydes in general, however, because it does not allow the formation of different aryl ethers. Our strategy allows the possibility to derivatize ester **3** with any number of groups.
9. Huang, S.-L.; Mancuso, A. J.; Swern, D. *J. Org. Chem*. **1978**, *43*, 2480.
10. Fatiadi, A. J. *Synthesis* **1976**, *2*, 65.
11. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc*. **1991**, *113*, 7277.
12. Satisfactory spectroscopic data was obtained for all intermediates. Ester **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, 3H), 6.53 (dd, 1H, $J=8.4$, 2.3 Hz), 6.62 (d, 1H, $J=2.3$ Hz), 7.81 (d, 1H, $J=8.6$ Hz), 10.93 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.33, 107.23, 109.28, 110.52, 129.11, 131.59, 162.92; HRMS m/z calcd for $C_8H_7N_3O_3$ (M^+): 193.0487, found 193.0492. Alcohol **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (s, 1H), 3.85 (s, 3H), 4.63 (s, 2H), 6.49 (d, 1H, $J=2.3$ Hz), 6.64 (dd, 1H, $J=8.0$ 2.0 Hz), 7.25 (d, 1H, $J=8.4$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.49, 61.44, 101.87, 110.64, 126.00, 129.83, 140.77, 158.51; HRMS m/z calcd for $C_8H_9N_3O_2$ (M^+): 179.0695, found 179.0694. Aldehyde **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 6.55 (d, 1H, $J=2.1$ Hz), 6.71 (ddd, 1H, $J=8.7$, 2.0, 0.8 Hz), 7.84 (d, 1H, $J=8.4$ Hz), 10.31 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.84, 102.37, 111.21, 121.97, 130.39, 147.71, 163.08, 188.24; HRMS m/z calcd for $C_8H_7N_3O_2$ (M^+): 177.0538, found 177.0538.