ORGANIC LETTERS

2012
Vol. 14, No. 17
4678–4681

Total Synthesis of Pentosidine

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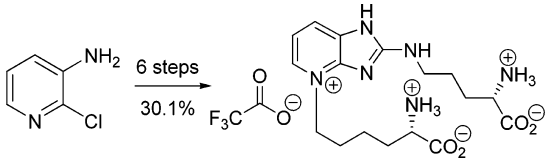
Received July 31, 2012

ABSTRACT



Pentosidine, a biologically important advanced glycation endproduct, has been accessed in a rapid, high-yielding manner. The synthesis was accomplished via a six-step sequence starting with 3-amino-2-chloropyridine and features a palladium-catalyzed tandem cross-coupling/cyclization to construct the imidazo[4,5-*b*]pyridine core.

Pentosidine (**1**) is an advanced glycation endproduct (AGE) containing an imidazo[4,5-*b*]pyridine core that has attracted interest as a biochemical marker.[1] It was discovered as an extracellular protein cross-link by Monnier in 1989 and is one of only a handful of characterized AGEs.[2] Pentosidine is a naturally occurring biological fluorophore and, as such, has found use in noninvasive diagnostics. It has been reported as a chemical marker of diabetic complications, kidney dysfunction, oxidative stress, aging, and age-related diseases.[3−10] Recently, Veralight Inc. has begun marketing a device that utilizes the spectroscopic properties of AGEs to carry out a noninvasive method of detecting type II diabetes.[11−14] Biosynthetically, pentosidine is believed to be a protein cross-link derived from a post-translational Maillard reaction of arginine and lysine residues with a pentose.[15] There is also evidence that it is both a singlet oxygen sensitizer and an antioxidant.[16,17] Pentosidine is available commercially; however its low supply and corresponding high price make it difficult to study.[18] Therefore, our goal was to develop a straightforward route to pentosidine that would allow us to explore the properties of this molecule.

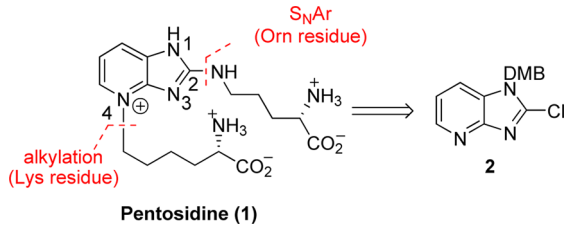
From a synthetic point of view pentosidine (**1**) presents an interesting structural target. The imidazo[4,5-*b*]pyridine core has attracted only casual interest in the literature, with few direct efforts at preparation.[19−27] The challenges inherent in this molecular architecture are derived from the need to generate this moiety in a cost efficient and chemically straightforward manner. There have been several published preparations of pentosidine to date,

(1) Dyer, D. G.; Dunn, J. A.; Thorpe, S. R.; Bailie, K. E.; Lyons, T. J.; McCance, D. R.; Baynes, J. W. *J. Clin. Invest.* **1993**, *91*, 2463–2469.
(2) Sell, D.; Monnier, V. *J. Biol. Chem.* **1989**, *264*, 21597–21602.
(3) Sugimoto, K.; Yasujima, M.; Yagihashi, S. *Curr. Pharm. Des.* **2008**, *14*, 953–961.
(4) Tsukahara, H. *Curr. Med. Chem.* **2007**, *14*, 339–351.
(5) Cho, S.-J.; G. RoCman, F.; Yeboah; Konishi, Y. *Curr. Med. Chem.* **2007**, *14*, 1653–1671.
(6) Wihler, C.; Schäfer, S.; Schmid, K.; Deemer, E.; Münch, G.; Bleich, M.; Busch, A.; Dingermann, T.; Somoza, V.; Baynes, J.; Huber, J. *Diabetologia* **2005**, *48*, 1645–1653.
(7) Takahashi, M. *Curr. Rheumatol. Rev.* **2006**, *2*, 319–324.
(8) Treweek, J. B.; Dickerson, T. J.; Janda, K. D. *Acc. Chem. Res.* **2009**, *42*, 659–669.
(9) Thomas, M. C.; Forbes, J. *The Maillard Reaction*; The Royal Society of Chemistry: 2010, pp 85−91.
(10) Sell, D. R.; Nagaraj, R. H.; Grandhee, S. K.; Odetti, P.; Lapolla, A.; Fogarty, J.; Monnier, V. M. *Diabetes Metab. Rev.* **1991**, *7*, 239–251.
(11) Maynard, J. D.; Rohrscheib, M.; Way, J. F.; Nguyen, C. M.; Ediger, M. N. *Diabetes Care* **2007**, *30*, 1120–1124.
(12) Ediger, M. N.; Maynard, J. D. *Rev. Endocrinol.* **2007** (June), p 62.
(13) Conway, B.; Edmundowicz, D.; Matter, N.; Maynard, J.; Orchard, T. *Diabetes Technol. Ther.* **2010**, *12*, 339–345.
(14) Felipe, D. L.; Hempe, J. M.; Liu, S.; Matter, N.; Maynard, J.; Linares, C.; Chalew, S. A. *Diabetes Care* **2011**, *34*, 1816–1820.
(15) Grandhee, S.; Monnier, V. *J. Biol. Chem.* **1991**, *266*, 11649–11653.
(16) Deng, B.; Simental, A.; Lutz, P.; Shaheen, G.; Chaiken, J. Proc. SPIE 8219, *82190D* 2012.
(17) Deng, B.; Wright, C.; Lewis-Clark, E.; Shaheen, G.; Geier, R.; Chaiken, J. *Proc. SPIE 7560, 75600P* **2010**.
(18) Available from PolyPeptide Group, 2 mg for $414.00 as the TFA salt.
(19) Senanayake, C. H.; Fredenburgh, L. E.; Reamer, R. A.; Liu, J.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. *Tetrahedron Lett.* **1994**, *35*, 5775–5778.
(20) Batt, D. G.; Houghton, G. C. *J. Heterocycl. Chem.* **1995**, *32*, 963–969.

10.1021/ol3021226
Published on Web 08/21/2012

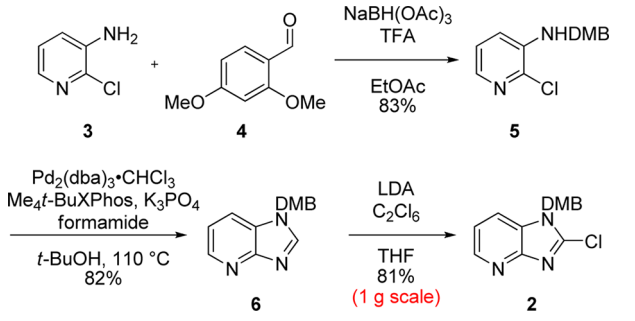
including two synthetic routes. In 1989 Sell and Monnier carried out a biomimetic synthesis by heating lysine, arginine, and ribose in an aqueous environment and then subjecting the mixture to HPLC purification to give pentosidine in 0.02% yield.[2,15] Recently Cravotto and co-workers improved upon this by utilizing protected amino acids under microwave irradiation.[28] However these methods, while requiring few synthetic steps, require HPLC purification and are low yielding. The first total synthesis of pentosidine was published by Shioiri and co-workers in 1991[29,30] requiring 15 total steps and HPLC purification. More recently Sayre's research group published an approach to the total synthesis of pentosidine.[31,32] Although this route is shorter, the expensive 2,3-diaminopyridine was used as a starting material.

Scheme 1. Retrosynthesis



Retrosynthetically we envisioned disconnections at C2 and N4, leaving an imidazo[4,5-*b*]pyridine core with an electron-donating protecting group at N1 (Scheme 1). This protection scheme is required so that N4 will be activated for selective alkylation. Without N1 or N3 being blocked, a mixture of N1, N3, and N4 alkylation products is obtained.[21] Additionally, Shioiri demonstrated that electron-withdrawing groups deactivated the imidazo-[4,5-*b*]pyridine for alkylation at any position.[29] These disconnections would allow the introduction of protected ornithine and lysine residues, thus avoiding asymmetric reactions and chiral auxiliaries by generating the stereocenters from the chiral pool. Recently we reported the preparation of imidazo[4,5-*b*]pyridines using a cross-coupling/cyclization strategy, which allowed the use of 3-amino-2-chloropyridine as our starting material.[26] Using this route, we would avoid using 2,3-diaminopyridine which is known to be problematic to functionalize in a regioselective manner.[29,30,33]

Scheme 2. Preparation of Imidazo[4,5-*b*]pyridine Core

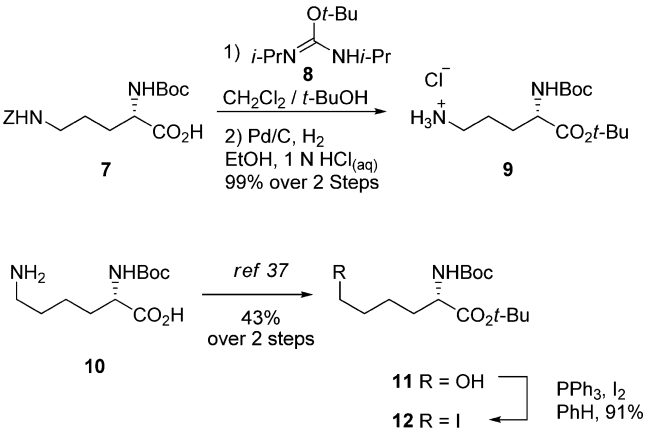


Our synthesis started from commercially available amino-2-chloropyridine **3**,[34] which was protected as its 2,4-dimethoxybenzyl (DMB) amine (**5**) via a reductive amination (Scheme 2).[33,35] We then applied our recently reported palladium-catalyzed amide coupling/cyclization methodology to generate the core imidazo[4,5-*b*]pyridine in a single step.[26] High yields have consistently been obtained for this reaction on a 2.5−5 g scale. Compound **6** was then chlorinated at the 2-position using hexachloroethane, giving chloro-azole **2** in 81% yield.[36] This three-step sequence rapidly assembles the activated imidazo[4,5-*b*]pyridine core in high yield on a gram scale.

Ornithine residue **9** was prepared in excellent yield from commercially available Boc-Orn(Z)-OH, by esterification with isourea **8** followed by Cbz cleavage with Pd/C and H_2 (Scheme 3). The lysine fragment can be prepared from Boc-Lys(H)-OH via alcohol **11** using the method of Adamczyk.[37] Alcohol **11** was then transformed to the desired iodide under Appel conditions in 91% yield. This short, two-step route avoids the seven-step sequence pursued by Shioiri. In addition the stereocenter was installed from commercially available lysine **10**.[29,30]

(21) Khanna, I. K.; Weier, R. M.; Lentz, K. T.; Swenton, L.; Lankin, D. C. *J. Org. Chem.* **1995**, *60*, 960–965.
(22) Senanayake, C. H.; Fredenburgh, L. E.; Reamer, R. A.; Liu, J.; Roberts, F. E.; Humphrey, G.; Thompson, A. S.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J.; Sinkai, I. *Heterocycles* **1996**, *42*, 821–830.
(23) Grimmett, M. R. *Imidazole and Benzimidazole Synthesis*; Academic Press: San Diego, 1997.
(24) Deghati, P. Y. F.; BierÃugel, H.; Wanner, M. J.; Koomen, G.-J. *Tetrahedron Lett.* **2000**, *41*, 569–573.
(25) Yıldırım, I.; Kandemirli, F.; Demir, E. *Molecules* **2005**, *10*, 559–571.
(26) Rosenberg, A. J.; Zhao, J.; Clark, D. A. *Org. Lett.* **2012**, *14*, 1764–1767.
(27) Bukhryakov, K.; Kurkin, A.; Yurovskaya, M. *Chem. Heterocycl. Compd.* **2011**, *47*, 533–557.
(28) Visentin, S.; Medana, C.; Barge, A.; Giancotti, V.; Cravotto, G. *Org. Biomol. Chem.* **2010**, *8*, 2473–2477.
(29) Sugiyama, H.; Yokokawa, F.; Shioiri, T.; Katagiri, N.; Oda, O.; Ogawa, H. *Tetrahedron Lett.* **1999**, *40*, 2569–2572.
(30) Yokokawa, F.; Sugiyama, H.; Shioiri, T.; Katagiri, N.; Oda, O.; Ogawa, H. *Tetrahedron* **2001**, *57*, 4759–4766.
(31) Liu, Y.; Zhang, W.; Sayre, L. M. *J. Heterocycl. Chem.* **2011**, *48*, 426–433.
(32) Liu, Y.; Zhang, W.; Sayre, L. M. *J. Heterocycl. Chem.* **2010**, *47*, 683–686.
(33) Leahy, D. K.; Desai, L. V.; Deshpande, R. P.; Mariadass, A. V.; Rangaswamy, S.; Rajagopal, S. K.; Madhavan, L.; Illendula, S. *Org. Process Res. Dev.* **2012**, *16*, 244–249.
(34) Available from Matrix Scientific for $133/250 g
(35) McLaughlin, M.; Palucki, M.; Davies, I. W. *Org. Lett.* **2006**, *8*, 3307–3310.
(36) Strekowski, L.; Kiselyov, A. S. *e-EROS Encyclopedia of Reagents for Organic Synthesis*; Wiley: New York, 2001.
(37) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. *Tetrahedron: Asymmetry* **1999**, *10*, 775–781.
(38) Hong, Y.; Senanayake, C. H.; Xiang, T.; Vandenbossche, C. P.; Tanoury, G. J.; Bakale, R. P.; Wald, S. A. *Tetrahedron Lett.* **1998**, *39*, 3121–3124.

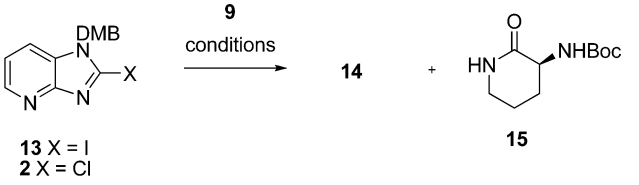
Scheme 3. Preparation of Amino Acid Derived Side Chains



Functionalization of C2 with the ornithine side-chain **9** proved unexpectedly complicated (Table 1). We first explored palladium catalyzed methods developed by Senanayake for the synthesis of the 2-aminobenzimidazole, Norastemizole.[38,39] Unfortunately, only low yields of the desired product **14** were obtained (entries 1−5). Having previously reported C2 functionalization using an S_NAr reaction with an iodide analogous to **13**, we returned to this for installation of the ornithine residue.[26] Initial attempts proved unsatisfactory due to the propensity of ornithine **9** to cyclize and form δ-lactam **15** (entry 6).[40] Switching to DMF as the solvent resulted in the dimethylamine adduct as the only isolable addition product (entry 7). The halide was changed from iodine to the more electronegative chlorine **2** to increase the reactivity of the azole for nucleophilic attack. Use of **2** combined with a higher loading of ornithine **9** led to formation of desired product **14** in 87% yield (entries 8 and 9). Efforts to lower the equivalents of ornithine **9** using either fluoride[41] or DABCO[42−44] as nucleophilic catalysts did not improve the yield or selectivity (entries 10 and 11). Using this optimized procedure, we were able to synthesize >1 g of **14** in a single run.

As aforementioned, electron-donating substituents installed at C2 and N1 activate N4 for alkylation. Iodide **12** and imidazo[4,5-*b*]pyridine **14** were refluxed in THF to provide iodide salt **16**, the fully protected pentosidine (Scheme 4). Purification of pyridinium salts is known to be problematic;[45] however, to our delight, **16** was easily purified using standard silica gel chromatography. With pure protected pentosidine **16** in hand, a

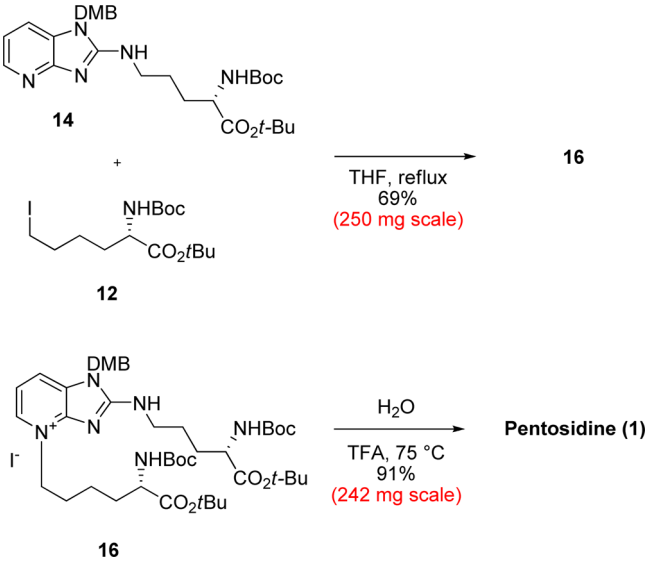
Table 1. Optimization of C2 Functionalization



entry	X	conditions[c]	solvent	yield (%)	14:15
1	I	A	toluene	10	1:0
2	I	B	*t*-AmOH	0	–
3	Cl	A	toluene	13	1:0
4	Cl	B	*t*-AmOH	39	1:1
5	Cl	B	toluene	44	2:1
6	I	C	EtOH	0	–
7	I	C	DMF	0[d]	–
8[a]	Cl	D	EtOH	31	2:1
9[a]	Cl	D	*n*-BuOH	87	1:0[b]
10	Cl	E	TGME	0	–
11	I	F	DMA	0	–

[a] 2.0 equiv of Orn. [b] After purification, 1:1 ratio prior to purification. [c] Conditions A: 1.5 mol % $Pd_2(dba)_3$, 4.5 mol % BINAP, NaO*t*-Bu, reflux. Conditions B: 1.5 mol % $Pd_2(dba)_3$, 4.5 mol % BippyPhos, K_3PO_4, reflux. Conditions C: Na_2CO_3, reflux. Conditions D: $EtN(iPr)_2$, reflux. Conditions E: KF, 2,6-lutidine, 120 °C. Conditions F: cat. DABCO, Na_2CO_3. [d] Dimethylamine addition observed.

Scheme 4. Final Assembly



global deprotection was accomplished by heating in aqueous TFA. Following this protocol, pentosidine (**1**) was obtained as the TFA salt without the need for tedious HPLC purification.[46] In our hands, we were

(39) Hong, Y.; Tanoury, G. J.; Wilkinson, H. S.; Bakale, R. P.; Wald, S. A.; Senanayake, C. H. *Tetrahedron Lett.* **1997**, *38*, 5607–5610.
(40) Chulin, A. N.; Rodionov, I. L.; Baidakova, L. K.; Rodionova, L. N.; Balashova, T. A.; Ivanov, V. T. *J. Pept. Sci.* **2005**, *11*, 175–186.
(41) Senanayake, C. H.; Hong, Y.; Xiang, T.; Wilkinson, H. S.; Bakale, R. P.; Jurgens, A. R.; Pippert, M. F.; Butler, H. T.; Wald, S. A. *Tetrahedron Lett.* **1999**, *40*, 6875–6879.
(42) Linn, J. A.; McLean, E. W.; Kelley, J. L. *J. Chem. Soc., Chem. Commun.* **1994**, 913–914.
(43) Shi, Y.-J.; Humphrey, G.; Maligres, P. E.; Reamer, R. A.; Williams, J. M. *Adv. Synth. Catal.* **2006**, *348*, 309–312.
(44) Bita, B. *Eur. J. Chem.* **2010**, *1*, 54–60.
(45) Bluhm, L. H.; Li, T. *Tetrahedron Lett.* **1998**, *39*, 3623–3626.

able to prepare 112 mg of pentosidine in a single-run over the six-step sequence.

In summary, we report a short, rapid total synthesis of pentosidine that utilizes a highly efficient synthesis of imidazo[4,5-*b*]pyridine **2**. Use of highly economical S_NAr and alkylation chemistry allows for a scalable and reproducible synthetic route. This approach provides pentosidine with only six steps in the longest linear sequence (ten total steps) in 30.1% yield. The high efficiency of the synthesis, low-cost of the starting materials, and lack of toxic reagents make this a practical method for research scale synthesis of pentosidine, thereby allowing further studies of this interesting natural product.

(46) The spectroscopic data (^{1}H and ^{13}C NMR as well as absorbance and fluorescence) were found to be in agreement with the published spectra obtained by Sell and Monnier. See ref 2.

Acknowledgment. We thank Syracuse University and LighTouch Medical Inc. for generous financial support of this research. We also thank Professor Joe Chaiken (Syracuse University) for his interest in this research, bringing the pentosidine problem to our attention, and many helpful discussions. Professors Chisholm, Hahn, and Totah and the Clark research group (Syracuse University) are also thanked for many helpful discussions.

Supporting Information Available. Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.