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PII: S0040-4039(13)00980-5
DOI: http://dx.doi.org/10.1016/j.tetlet.2013.06.024
Reference: TETL 43077

To appear in: *Tetrahedron Letters*

Received Date: 7 May 2013
Revised Date: 3 June 2013
Accepted Date: 6 June 2013

Please cite this article as: Naidoo, J., Bemben, C.J., Allwein, S.R., Liang, J., Pieper, A.A., Ready, J.M., Development of a Scalable Synthesis of P7C3-A20, a Potent Neuroprotective Agent, *Tetrahedron Letters* (2013), doi: http://dx.doi.org/10.1016/j.tetlet.2013.06.024

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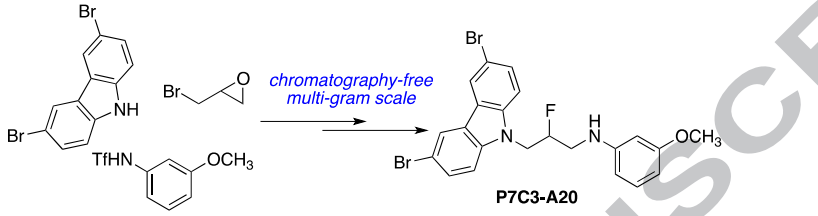

Tetrahedron Letters
journal homepage: www.elsevier.com

Development of a Scalable Synthesis of P7C3-A20, a Potent Neuroprotective Agent

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Neuroprotective
Synthesis
Carbazole
Fluorination

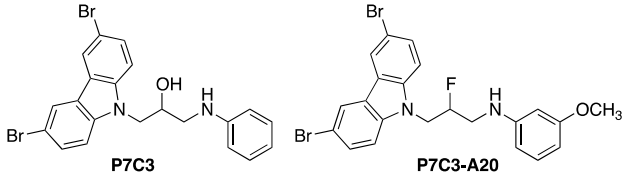
ABSTRACT

A scalable synthesis of the neuroprotective agent P7C3-A20 is described. The synthesis has provided hundred-gram batches of the final compound for biological evaluation in rodents primates. The synthesis can be performed without chromatographic purification of intermediates or the final product.

1. Introduction

The aminopropyl carbazole P7C3 was discovered in an unbiased, in vivo assay for neuroprotective and pro-neurogenic compounds.[1,2] A medicinal chemistry campaign led to the discovery of P7C3-A20, an analog displaying increased activity and an improved toxicity profile compared to P7C3.[3] In mice, both compounds were found to 1) protect mature spinal cord motor neurons from cell death in the G93A SOD1 transgenic mouse model of amyotrophic lateral sclerosis (ALS),[4] 2) protect dopaminergic neurons in the substantia nigra from toxicity associated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a model of Parkinson's disease,[5] 3) protect neuronal function and survival in a mouse model of traumatic brain injury,[6] and 4) enhance hippocampal neurogenesis.[1] P7C3 has also proven to be protective in a rat model of age-related cognitive decline[1] and a zebrafish model of retinal degeneration.[7]

To further study the neuroprotective effects and potential therapeutic utility of the P7C3 class of chemicals, multi-gram quantities of P7C3-A20 were required. However, our original synthesis yielded only hundreds of milligrams of final product and was not optimized for multi-gram preparations.[3] The requirement for multiple chromatographic purifications and the low solubility of several intermediates may have complicated large-scale synthesis. Here we describe a new synthesis that



requires no chromatography and has provided hundreds of grams of P7C3-A20.

2. Results and Discussion

The initial synthesis of P7C3-A20 involved the fluorination of a secondary alcohol in which an aniline NH was protected with a 4-nitrobenzene sulfonyl (4-Ns) group (**3c**, Scheme 1). Fluorination on substrates possessing the aniline NH (**3a**) proved problematic, but the Ns group endowed intermediates with poor solubility and led to reaction mixtures requiring chromatographic purification. Accordingly, our strategy to optimize the synthesis of P7C3-A20 centered on the identification of an aniline protecting group that would facilitate fluorination. To this end, a variety of N-protected amino alcohols were prepared as shown in

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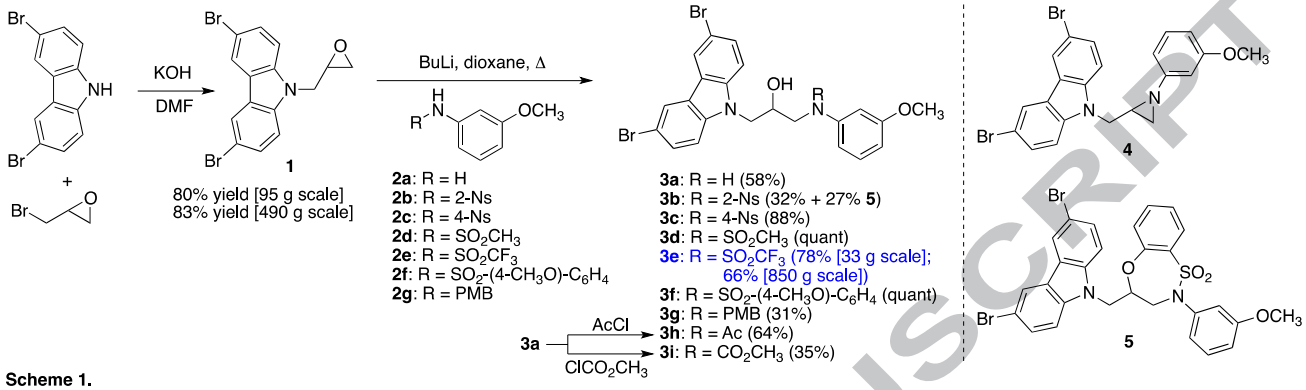
Scheme 1. Condensation of dibromocarbazole with epibromohydrin proceeded cleanly, and epoxide **1** was isolated in high yield on large scale by trituration with ethyl acetate.

Epoxide **1** was opened with *m*-anisidine (**2a**) and a variety of protected variants thereof. Optimal conditions involved a slight excess of BuLi in dioxane under elevated temperatures. As described below, the triflate **2e** ultimately proved optimal. It was prepared from *m*-anisidine and triflic anhydride in 77% yield on an 18 g scale and 73% yield on a 510 g scale. Addition to epoxide **1** was performed at 90 °C in the presence of 1.3 equiv of triflate **2e** and 1.8 equiv of BuLi. With less base we observed substantial quantities of a side product that we characterized as aziridine **4**.[8] On moderate scale, we isolated 23 g of **3e** (55%) through precipitation from the crude reaction mixture. An additional 10 g (23%) was isolated chromatographically. On a larger scale the reaction also proceeded smoothly, affording the ring-opened product in 66% yield after trituration with CH_2Cl_2/Hexanes (3:2 v:v, 850 g). Epoxide opening with most other anisidine derivatives and anisidine itself generally proceeded smoothly. However, 2-Ns anisidine (**2b**) provided nearly equal quantities of the desired ring-opened product (**3b**) and a cyclized side product **5**, which presumably arises from intramolecular S_NAr substitution of the nitro group.[9]

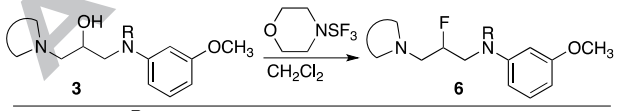


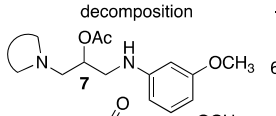
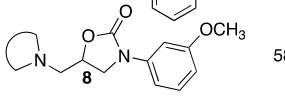
Scheme 1.

With access to a variety of N-protected amino alcohols (**3b-g**) we evaluated fluorination of the secondary alcohols with morphoDAST (Table 1). While unprotected amino alcohol **3a** produced complex mixtures in the presence of mophoDAST, several sulfonamides were fluorinated in high yields. In particular, triflate **6e** was isolated in excellent yield after simple aqueous workup on both a 22 g and 905 g scale. In contrast, an alkyl protected analog, **3g**, yielded a complex mixture under these reactions conditions. Likewise, the corresponding acetamide (**3h**)[10] and carbamate (**3i**) were converted to the O-acetate (**7**) and cyclic urea (**8**), likely through substitution of an activated alcohol with the carbonyl of the protecting group.

Finally, we explored conditions to remove the sulfonyl protecting groups. Previously, we described removal of the 4-Ns group with mercaptoacetic acid,[3] but, as discussed above, the Ns-containing intermediates were not ideal for large-scale preparations. The mesyl (**6d**) and methoxybenezene sulfonyl (**6f**) groups were resistant to all acidic, basic or reductive removal conditions explored. However, treatment of triflate **6e** with Red-Al (sodium bis(2-methoxyethoxy)aluminum hydride) at 60 °Ccleaved the triflate but generated several side products (Scheme 2). Acidification of the crude reaction mixture generated the HCl salt of P7C3-A20, and allowed the subsequent removal of unreacted starting material, dibromocarbazole, and a compound we assigned as an elimination product (**9**). This salt was free-based with saturated bicarbonate solution, and the resulting solid was triturated with CH_2Cl_2/Hexanes (3:7 v:v) to remove des-brominated impurities (**10**). In this way, we obtained 69% yield of 98% pure material on a 10.6 g scale. On an 850 g scale the free-based P7C3-A20 was initially precipitated from ethyl acetate/hexanes (1:6 v:v). The resulting product was recrystallized from hot ethanol, which afforded the product in 25% yield (168 g). On this larger scale, which was attempted only once, a significant exotherm was noted such that the internal temperature exceeded 90 °C. Side products formed under these conditions likely account for the lower yield on the 850 g scale reaction. Accordingly, caution is warranted during the addition of Red-Al on large scale.

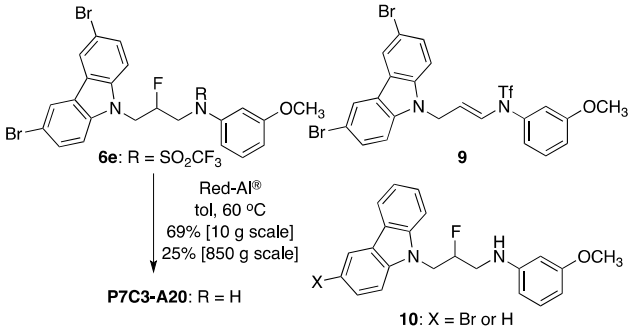
Table 1. Fluorination of amino alcohols **3**.[a]



Entry	R	Product	Yield (%)
1	H	**6a**	<10
2	4-Ns	**6c**	>88[a]
3	SO_2CH_3	**6d**	quant
4	SO_2CF_3	**6e**	quant [22 g scale] 98 [905 g scale]
5	SO_2-(4-CH_3O)-C_6H_4	**6f**	94
6	PMB	decomposition	–
7	Ac		64
8	CO_2CH_3		58

[a]Reactions carried out at room temperature with [3] = 0.1M.
[b]Crude **6c** was deprotected to yield P7C3-A20 in 88% yield from **3c**.

Scheme 2.



The synthetic material made from the route describe herein was evaluated for its effect on adult hippocampal neurogenesis. Mice treated intraperitoneally with 20 mg/kg/d for 7 days were found to have approximately twice the number of new hippocampal neurons as untreated mice, consistent with observations from the original synthetic route.[1-3,11]

3. Conclusion

The P7C3 class of compounds displays encouraging neuroprotective activity. They have proven effective in several animal models of neurodegenerative disease, and P7C3-A20 represents a valuable tool compound to explore the utility of this class of chemicals. The scalable synthesis described here should facilitate those endeavors.

Acknowledgments

This work was supported by the Edward N. and Della C. Thome Memorial Foundation and the Welch Foundation (I-1612) (to JMR). Additional support was received from The Hartwell Foundation and NIH (NIMH R01 MH087986) to AAP.

Supplementary Data

Supplementary data associated with this article, including experimental procedures can be found in the online version.

1. Pieper, A. A.; Xie, S.; Capota, E.; Estill, S. J.; Zhong, J.; Long, J. M.; Becker, G. L.; Huntington, P.; Goldman, S. E.; Shen, C.-H.; Capota, M.; Britt, J. K.; Kotti, T.; Ure, K.; Brat, D. J.; Williams, N. S.; MacMillan, K. S.; Naidoo, J.; Melito, L.; Hsieh, J.; De, B. J.; Ready, J. M.; McKnight, S. L. *Cell* **2010**, *142*, 39.
2. McKnight S.L.; Pieper A.A.; Ready J.M.; De Brabander, J. US Patent 8,362,277, 2010.
3. MacMillan, K. S.; Naidoo, J.; Liang, J.; Melito, L.; Williams, N. S.; Morlock, L.; Huntington, P. J.; Estill, S. J.; Longgood, J.; Becker, G. L.; McKnight, S. L.; Pieper, A. A.; De Brabander, J. K.; Ready, J. M. *J. Am. Chem. Soc.* **2011**, *133*, 1428.
4. Tesla, R.; Wolf, H. P.; Xu, P.; Drawbridge, J.; Estill, S. J.; Huntington, P.; McDaniel, L.; Knobbe, W.; Burket, A.; Tran, S.; Starwalt, R.; Morlock, L.; Naidoo, J.; Williams, N. S.; Ready, J. M.; McKnight, S. L.; Pieper, A. A. *Proc. Natl. Acad. Sci.* **2012**, *109*, 17016.
5. De Jesus-Cortes, H.; Xu, P.; Drawbridge, J.; Estill, S. J.; Huntington, P.; Tran, S.; Britt, J.; Tesla, R.; Morlock, L.; Naidoo, J.; Melito, L. M.; Wang, G.; Williams, N. S.; Ready, J. M.; McKnight, S. L.; Pieper, A. A. *Proc. Natl. Acad. Sci.* **2012**, *109*, 17010.
6. Blaya1, M. O.; Bramlett, H. M.; Naidoo, J.; Pieper, A. A.; Dietrich, W. D. *submitted for publication*.
7. Asai-Coakwell, M.; March, L.; Dai, X. H.; DuVal, M.; Lopez, I.; French, C. R.; Famulski, J.; De Baere, E.; Francis, P. J.; Sundaresan, P.; Sauve, Y.; Koenekoop, R. K.; Berry, F. B.; Allison, W. T.; Waskiewicz, A. J.; Lehmann, O. J. *Hum Mol Genet* **2013**, *22*, 1432.
8. The mechanism by which excess BuLi prevents aziridination is unclear. Formation of aziridine **4** may involve cyclization of an O-triflate. In this scenario, BuLi may cleave any O-triflate prior to cyclization, although no data exists to support this proposal.
9. Cleator, E.; Baxter, C. A.; O'Hagan, M.; O'Riordan, T. J. C.; Sheen, F. J.; Stewart, G. W. *Tetrahedron Lett* **2010**, *51*, 1079.
10. Morcuende, A.; Ors, M.; Valverde, S.; Herradon, B. *J. Org. Chem.* **1996**, *61*, 5264.
11. Mice (n=6) treated with 20 mg/k/d P7C3-A20 prepared as described herein were found to have 31.6±1.1 x10^6 new neurons/mm^3 in the dentate gyrus. Vehicle treated mice had 14.5±1.1 x 10^6 neurons/mm^3. See reference 1 for experimental details.