Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 7431–7434

Fluorous synthesis of minor groove binding agents related to distamycin

Sreeman K. Mamidyala and Steven M. Firestine*

Department of Pharmaceutical Sciences, Eugene Apllebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, MI 48201, USA

Received 11 July 2006; revised 16 August 2006; accepted 17 August 2006

Abstract—Minor groove binding agents related to distamycin have been shown to target specific DNA sequences with high affinity. We report a new method for the preparation of these agents using fluorous synthesis in which the fluorous tag is located on what will become the cationic tail of the molecule. We demonstrate that fluorous synthesis yields both simple and complex polyamides in good yields and in high purity.

Small molecules that can bind to a predefined target within DNA are important in a number of fields ranging from molecular biology to drug development. Of the compounds that target DNA, agents that bind to minor groove display the greatest ability to read the sequence of DNA. Naturally occurring minor groove binding agents, like distamycin or netropsin, possess limited sequence selectivity; however, researchers have modified these agents to create new molecules that have the ability to target any sequence combination.[1–4] These agents, termed polyamides or lexitropsins,[5] are composed of a variety of heterocycles joined by an amide bond to generate a polymer in which the presence of various hydrogen bond donors and acceptors is complementary to that found within the minor groove of DNA. These molecules also contain a positive charge which results in a favorable electrostatic interaction with DNA. Polyamides can bind to DNA in either a 1:1 or 2:1 stoichiometry with the 2:1 binding mode possessing the greatest sequence selectivity.[2] Polyamides and lexitropsins have found utility as gene regulators and as antimicrobial or antiviral agents.[2]

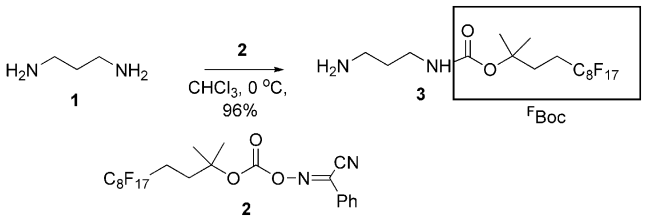
Given the utility of these agents, it is not surprising that a wide variety of methods exists for their synthesis. All methods rely upon amide bond formation between the amine of one heterocycle and the carboxylic acid or carboxylate derivative of the next heterocycle. Both solid- and solution-phase methods have been published and not surprisingly both methods borrow heavily from the literature on the synthesis of peptides.[6–9] For the solid-phase synthesis, both Boc- and Fmoc-protected heterocycles have been utilized and solid-phase synthesis can be automated and used in the synthesis of a wide range of polyamides.[6,9] Solution-phase methods utilizing either standard peptide coupling conditions or a haloform reaction have also been developed.[7,8]

To complement these methods, we have developed a fluorous synthesis of polyamides. Fluorous synthesis is compatible with a wide variety of chemistries and because molecules containing multiple fluorines preferentially bind to other polyfluorinated molecules, purification can be accomplished simply by passing the reaction mixture through a column containing fluorous silica.[10,11] Non-fluorous materials are washed away while polyfluorinated products are retained on the column. Elution of the products under specific solvent conditions provides a rapid and effective method for purification. Fluorous synthesis has been utilized in the synthesis of a wide range of molecules, including oligonucleotides,[12] peptides,[13] and carbohydrates.[14]

To conduct fluorous synthesis, a polyfluorinated tag must be present within the molecule of interest. We chose to incorporate the tag into what would eventually become the cationic tail that is present in all minor groove binding agents. Synthesis would then proceed

* Corresponding author. Tel.: +1 313 577 0455; fax: +1 313 577 2033; e-mail: sfirestine@wayne.edu

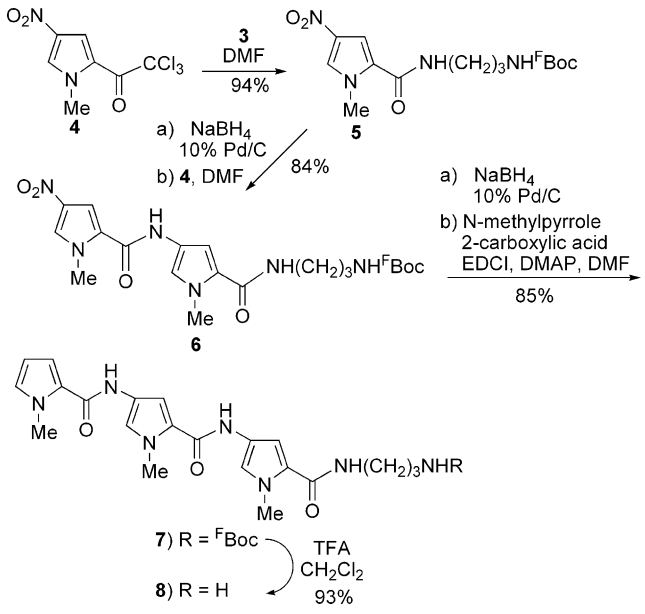
0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.08.058



Scheme 1. Synthesis of ^{F}Boc 1,3-diaminopropane.

from the C- to the N-terminus of the molecule. Since the fluorous tag would need to be removed before biological studies could be conducted, we focused on fluorinated protecting groups and choose to use fluorous Boc (^{F}Boc).[15] Treatment of excess 1,3-diaminopropane (**1**) with the commercially available ^{F}Boc reagent **2** yielded the desired mono-protected product **3** in 94% yield (Scheme 1). We observed no di-addition in this reaction. The free amine of **3** could then be attached to a heterocycle via standard coupling conditions to start the synthesis of the minor groove binding agent.

We initially focused on the synthesis of a three ring polyamide composed of only *N*-methylpyrrole units. This distamycin analogue was prepared as outlined in Scheme 2. The formation of the amide bonds was done using either the haloform reaction or standard peptide coupling conditions. We primarily used the haloform reaction for amide bond formation since the reaction requires no additional reagents, is simple to conduct and the required starting material is easy to acquire in large amounts and in good yield.[8] Reaction of **3** with 2 equiv of 4-nitro-2(-trichloroacetyl)-*N*-methylpyrrole (**4**) in dry DMF at room temperature gave the desired product **5** in 94% purified yield. Purification was accomplished using a fluorous silica gel chromatography. The crude reaction mixture was added to the fluorous silica column and the non-fluorous materials were eluted by washing the column with 80% MeOH/H_2O. The desired fluorous-containing material could be obtained by simply washing the column with 100% THF. This purification method was rapid (~10 min per compound) and generated pure material (>95%). This procedure was used for the purification of fluorous containing compounds.

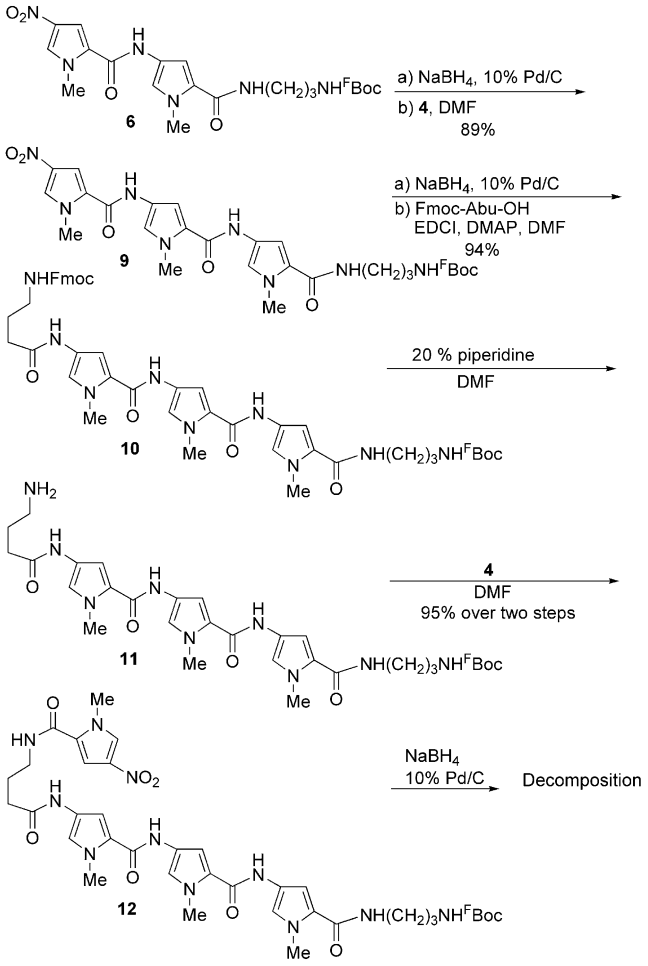


Scheme 2. Fluorous synthesis of tripeptide Py–Py–Py–NH_2.

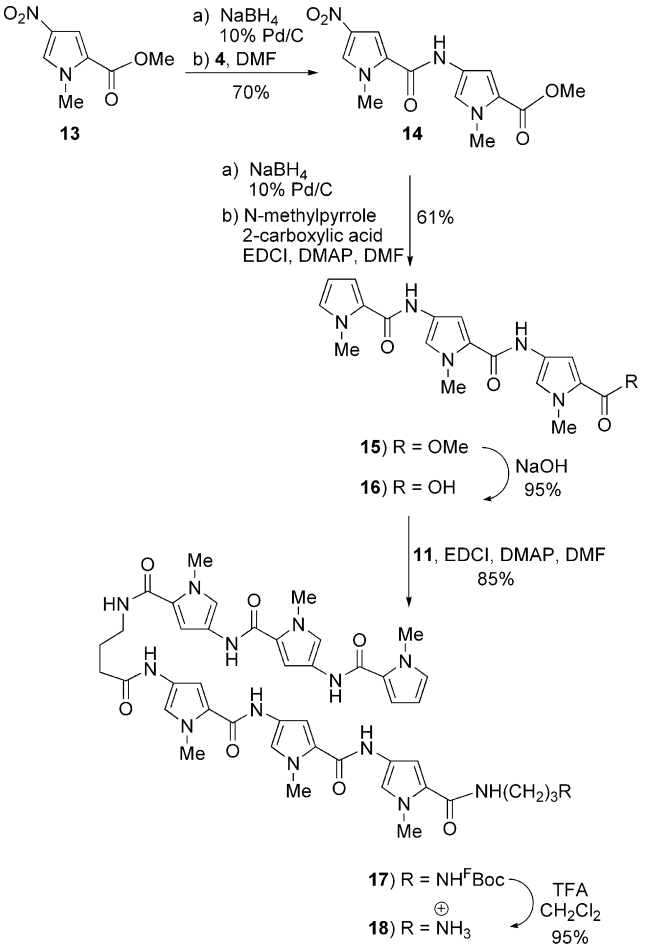
The conversation of the nitro group in **5** to the amine for the next reaction could be accomplished by standard hydrogenation conditions (H_2, Pd/C); however, we found that this method was not reproducible on a large scale. Thus, we investigated other methods to reduce the nitro group. We found that reduction of **5** with $NaBH_4$, Pd/C in ethyl acetate/MeOH at 0 °C gave quantitative conversion to the amine within 1 h.[16] The resulting amine was immediately reacted with **4** to generate the dipeptide NO_2–Py–Py–$NH(CH_2)_3NH^{F}$Boc (**6**) in 84% purified yield. Reduction of **6** followed by coupling with 2 equiv of *N*-methylpyrrole 2-carboxylic acid under standard peptide coupling conditions (EDCI, DMAP, DMF) generated the tripeptide, Py–Py–Py–$NH(CH_2)_3NH^{F}$Boc (**7**), in 85% yield. Removal of the ^{F}Boc under acidic conditions gave the final product, Py–Py–Py–$NH(CH_2)_3NH_2$ (**8**), in 93% yield.

The fluorous synthesis of the tripeptide gave an overall yield of 67% to **7** and 61% to **8**. Furthermore, we were able to recover unreacted heterocycle from the reaction simply by evaporating the material which did not bind to the fluorous silica column. In most cases, the recovered heterocycle was pure enough to be used without additional purification.

Previous studies have shown that the 2:1 binding mode of distamycin and related analogues provides the greatest degree of sequence selectivity.[2] Using this principle, Dervan and co-workers have developed a number of agents in which two polyamides are covalently attached to each other via a linker molecule. The location and nature of the linkage has been widely explored, but the most common is the hairpin motif in which the N-terminus of one polyamide is connected to C-terminus of the second.[1] The most common attachment is through the use of γ-aminobutyric acid (γ). Given the increased sequence selectivity and binding affinity that hairpin polyamides possess over single polyamides like **8**, we decided to examine the ability of fluorous synthesis to prepare hairpin polyamides (Scheme 3). The dipeptide **6** was reduced to the amine which was reacted with **4** to generate the tripeptide NO_2–Py–Py–Py–$NH(CH_2)_3NH^{F}$Boc (**9**) in 89%. The tripeptide **9** was reduced to the amine and reacted with Fmoc-γ-aminobutyric acid under peptide coupling conditions to yield the product FmocNH–γ-Py–Py–Py–$NH(CH_2)_3NH^{F}$Boc (**10**) in 94% yield. Fmoc deprotection of **10** was conducted using basic conditions to generate the free amine **11**. Reaction of **11** with **4** gave the peptide NO_2–Py–Py–Py–$NH(CH_2)_3NH^{F}$Boc (**12**) in 95% yield. However, conversation of **12** into the necessary amine resulted in decomposition. We believe that the decomposition was due to the instability of the amine and we are currently exploring alternative methods to produce and stabilize the amine.



Scheme 3. Attempted stepwise fluorous synthesis of hairpin polyamide.



Scheme 4. Fluorous synthesis of hairpin polyamide.

Given this problem with the stepwise approach, we examined an alternative method to the preparation of the desired hairpin polyamide (Scheme 4). Reduction of methyl 4-nitro-*N*-methylpyrrole-2-carboxylate (**13**) using $NaBH_4$ generated the amine which was reacted with **4** to produce the dipeptide in modest yield. Reduction of **14** followed by coupling with *N*-methylpyrrole-2-carboxylic acid gave the protected tripeptide, Py–Py–Py–OMe (**15**), which upon hydrolysis yielded the acid **16** in 80% yield. The acid was reacted with **11** to generate the desired protected hairpin polyamide **17** in 85% yield. Removal of the ^{F}Boc protecting group was accomplished under acidic conditions to generate the final product **18** in 95% yield. NMR analysis of **18** indicated that the material was 90% pure after passage through a fluorous silica column to remove the fluorous residue. Normal silica gel column was used to purify the material further. The synthesis of **18** could be accomplished in a 71% overall yield starting from **6** or in a 47% overall yield starting from **5**.

In conclusion, we have demonstrated the utility of fluorous synthesis for the preparation and isolation of minor groove binding polyamides containing *N*-methylpyrrole. We found that the simple isolation of the intermediates in the synthetic scheme was advantageous when compared to conventional purification methods. While the fluorous synthesis of polyamides offers several apparent advantages over conventional methods of synthesis, we should point out two caveats to our work. First, we have examined only the incorporation of *N*-methylpyrrole into the polyamide. Previous investigators have noted that the insertion of *N*-methylimidazole into the polyamide can be challenging.[8] Second, the synthetic method outlined here can only generate one specific cationic tail. However, it has been well established that the presence of additional hydrophobic groups on the cationic tail can enhance binding affinity and aid in the transport of these molecules through the cell wall.[17] Thus, the method presented here would not provide a convenient route to the synthesis of numerous derivatives of the tail portion of the molecule. We are currently working on synthetic methods that address these two issues and will report on our results in due course.

Acknowledgements

The authors acknowledge the support of Wayne State University for the funding of this project.

References and notes

1. Melander, C.; Burnett, R.; Gottesfeld, J. M. *J. Biotechnol.* **2004**, *112*, 195–220.
2. Dervan, P. B.; Doss, R. M.; Marques, M. A. *Curr. Med. Chem. Anti-Cancer Agents* **2005**, *5*, 373–387.
3. Reddy, B. S.; Sondhi, S. M.; Lown, J. W. *Pharmacol. Ther.* **1999**, *84*, 1–111.
4. Bailly, C.; Chaires, J. B. *Bioconjugate Chem.* **1998**, *9*, 513–538.
5. Lown, J. W. *Anticancer Drug Des.* **1988**, *3*, 25–40.
6. Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141–6146.
7. Boger, D.; Fink, B.; Hedrick, M. *J. Am. Chem. Soc.* **2000**, *122*, 6382–6394.
8. Xiao, J.; Yuan, G.; Huang, W.; Chan, A. S. C.; Lee, K. L. D. *J. Org. Chem.* **2000**, *65*, 5506–5513.
9. Wurtz, N. R.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *Org. Lett.* **2001**, *3*, 1201–1203.
10. Curran, D. P. In *Handbook of Fluorous Chemistry*; Gladysz, J. A., Curran, D. P., Horvath, I. T., Eds.; Light Fluorous Chemistry—A User's Guide; VCH: Weinheim, 2004; pp 128–155.
11. Zhang, W. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 784–797.
12. Pearson, W. H.; Berry, D. A.; Stoy, P.; Jung, K. Y.; Sercel, A. D. *J. Org. Chem.* **2005**, *70*, 7114–7122.
13. Mizuno, M.; Goto, K.; Miura, T.; Hosaka, D.; Inazu, T. *Chem. Commun. (Cambridge)* **2003**, 972–973.
14. Ko, K. S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162–13163.
15. Luo, Z.; Williams, J.; Read, R. W.; Curran, D. P. *J. Org. Chem.* **2001**, *66*, 4261–4266.
16. Beria, I.; Baraldi, P. G.; Cozzi, P.; Caldarelli, M.; Geroni, C.; Marchini, S.; Mongelli, N.; Romagnoli, R. *J. Med. Chem.* **2004**, *47*, 2611–2623.
17. Khalaf, A. I.; Waigh, R. D.; Drummond, A. J.; Pringle, B.; McGroarty, I.; Skellern, G. G.; Suckling, C. J. *J. Med. Chem.* **2004**, *47*, 2133–2156.