ORGANIC LETTERS

2007
Vol. 9, No. 10
2043−2046

Total Synthesis of Anibamine, a Novel Natural Product as a Chemokine Receptor CCR5 Antagonist

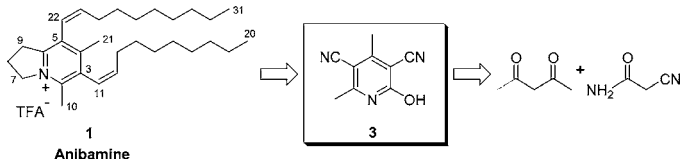
Guo Li,[†] Karen Watson,[‡] Robert W. Buckheit,[‡] and Yan Zhang*[,†]

Department of Medicinal Chemistry, Virginia Commonwealth University, 410 North 12th Street, P.O. Box 980540, Richmond, Virginia 23298-0540, and ImQuest BioSciences, Inc., 7340 Executive Way, Suite R, Frederick, Maryland 21704

yzhang2@vcu.edu

Received March 27, 2007

ABSTRACT



The total synthesis of anibamine, the first and only natural product known as a chemokine receptor CCR5 antagonist, is reported herein. Anibamine was synthesized from acetylacetone and cyanoacetamide in 10 steps.

Anibamine, a novel pyridine quaternary alkaloid recently isolated from *Aniba* sp.,[1,2] has been found to effectively bind to the chemokine receptor CCR5 with an IC_{50} at 1 μM in competition with ^{125}I-gp120, a HIV viral envelop protein binding to CCR5 with high affinity.[1] The chemokine receptor CCR5, a G-protein-coupled receptor, has been identified as an essential co-receptor for HIV virus entry to host cells.[3] Therefore, an antagonist of CCR5 receptor that would inhibit the cellular entry of human immunodeficiency virus type I (HIV-1) provides a new therapy choice for the treatment of HIV infection.[4]

Currently, all the known antagonists to CCR5 have been developed by optimization of the lead compounds from high-throughput screening, such as, SCH-D,[5] TAK220,[6] and UK427857[7] **(**Figure 1).

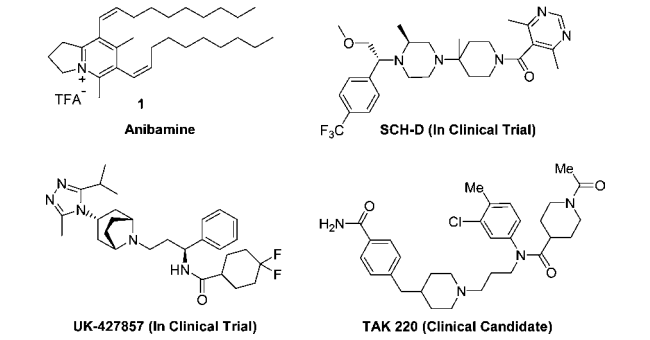


Figure 1. Anibamine and some known CCR5 antagonists

Anibamine is the first and the only natural product that has been identified as a CCR5 antagonist with high affinity.

* Address correspondence to this author.
[†] Virginia Commonwealth University.
[‡] ImQuest BioSciences, Inc.

(1) Jayasuriya, H.; Herath, K. B.; Ondeyka, J. G.; Polishook, J. D.; Bills, G. F.; Dombrowski, A. W.; Springer, M. S.; Siciliano, S.; Malkowitz, L.; Sanchez, M.; Guan, Z. Q.; Tiwari, S.; Stevenson, D. W.; Borris, R. P.; Singh, S. B. *J. Nat. Prod.* **2004**, *67*, 1036−1038.

(2) Klausmeyer, P.; Chmurny, G. N.; McCloud, T. G.; Tucker, K. D.; Shoemaker, R. H. *J. Nat. Prod.* **2004**, *67*, 1732−1735.

(3) (a) Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C. M.; Saragosti, S.; Lapouméroulie, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. J.; Doms, R. W.; Vassart, G.; Parmentier, M. *Nature* **1996**, *382*, 722−725. (b) Littman, D. R. *Cell* **1998**, *93*, 677−680.

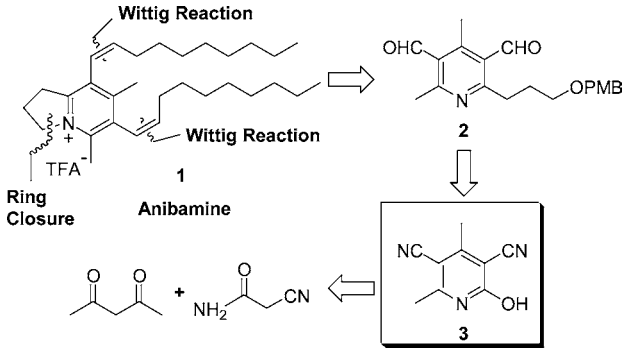
(4) (a) Reeves, J. D.; Piefer, A. J. *Drugs* **2005**, *65*, 1747−1766. (b) Este, J. A. *Curr. Med. Chem.* **2003**, *10*, 1617−1632. (c) Schwarz, M. K.; Wells, T. N. C. *Nat. Rev. Drug Discovery* **2002**, *1*, 347−358. (d) Lusso, P. *EMBO J.* **2006**, *25*, 447−456.

10.1021/ol070748n CCC: $37.00
Published on Web 04/21/2007

Clearly, anibamine possesses a novel structural skeleton compared with all other known CCR5 antagonists.[5] Thus the chemical synthesis of anibamine and its analogues may lead to a new class of AIDS therapeutic agents. Herein is the first report of the total synthesis of anibamine.

As shown below, anibamine has a unique structural skeleton, in which a pyridine quaternary ion is embodied by a fused five-member ring. Such a skeleton can also be called the 2,3-dihydro-*1H*-indolizinium ion. On the pyridine ring, two ten-carbon aliphatic chains are attached at positions 3 and 5 via cis double bonds. In addition, both positions 2 and 4 are occupied by methyl groups. The retrosynthetic analysis of anibamine is illustrated in Scheme 1.

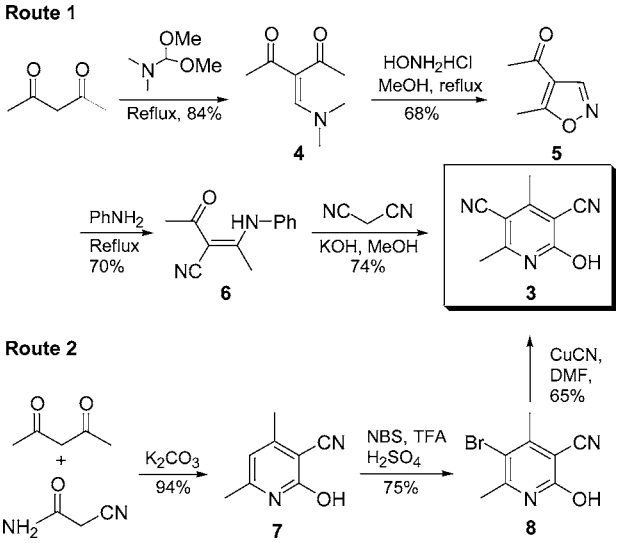
Scheme 1. The Retrosynthetic Analysis of Anibamine



The two ten-carbon side chains can be introduced by Wittig reactions between the Wittig reagent of 1-bromononane and the 3,5-dialdehyde intermediate **2**. Intermediate **2** can be prepared by the reduction of the 3,5-dicyano intermediate. The fused five-member-ring system can be achieved by introducing a three-carbon side chain at position 2, followed by the cyclization reaction to form the indolizinium ring. Apparently the substituted pyridine **3** is the most critical intermediate since it possesses all the potential functional groups.

Two synthetic routes were explored to prepare the intermediate **3**, as shown in Scheme 2. In the first route, the condensation reaction between acetylacetone and *N*,*N*-dimethylformide dimethyl acetal provided compound **4**.[8] Then **4** was refluxed with hydroamine hydrochloride in methanol to give compound **5**.[9] Next **5** was coupled with aniline[10] to provide compound **6**. Finally the cyclization reaction between **6** and malononitrile[11] led to the intermediate **3** in an overall 30% yield. In the second route, using the

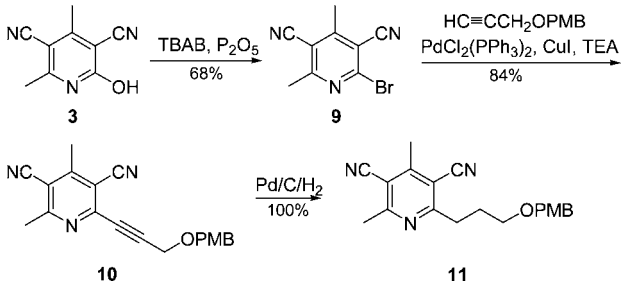
Scheme 2. The Chemical Synthesis of Intermediate **3**



procedure of Haley et al.,[12] the condensation reaction between acetylacetone and cyanoacetamide gave compound **7**, which was brominated with NBS in TFA and H_2SO_4 to give **8**.[13] Next, the nucleophilic cyanization of compound **8** was achieved by refluxing with cuprous cyanide in DMF to give **3**.[13] Here, the total yield was 46% over 3 steps. The first route is one step longer than the second one, yet is more convenient for large-scale synthesis. Since two cyano groups can be introduced subsequently in the second route, it may provide the possibility of attaching two different side chains on positions 3 and 5. This will be critical to anibamine analogue synthesis in the future.

The bromination[14] of **3** with TBAB/P_2O_5 led to the 2-bromo-substituted intermediate **9** (Scheme 3). The pal-

Scheme 3. The Synthesis of Intermediate **11**

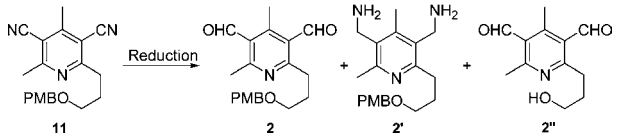


ladium-catalyzed alkynylation reaction of compound **9** with protected propargyl alcohol gave compound **10**.[15,16]

(5) Palani, A.; Tagat, J. R. *J. Med. Chem.* **2006**, *49*, 2851−2857.
(6) Imamura, S.; Ichikawa, T.; Nishikawa, Y.; Kanzaki, N.; Takashima, K.; Niwa, S.; Iizawa, Y.; Baba, M.; Sugihara, Y. *J. Med. Chem.* **2006**, *49*, 2784−2793.
(7) Wood, A.; Armour, D. *Prog. Med. Chem.* **2005**, *43*, 239−271.
(8) Schenone, P.; Mosti, L.; Menozzi, G. *J. Heterocycl. Chem.* **1982**, *19*, 1355−1361.
(9) Menozzi, G.; Schenone, P.; Mosti, L. *J. Heterocycl. Chem.* **1983**, *20*, 645−648.
(10) Alberola, A.; Antolin, L. F.; Gonzalez, M. A.; Pulido, F. J. *J. Heterocycl. Chem.* **1986**, *23*, 1035−1038.
(11) Alberola, A.; Antolin, L. F.; Gonzalez, M. A.; Laguna, M. A.; Pulido, F. J. *Heterocycles.* **1987**, *25*, 393−397.
(12) Haley, C. A. C.; Maitland, M. *J. Chem. Soc.* **1951**, 3155−3174.
(13) Mal, P.; Lourderaj, U.; Parveen; Venugopalan, P.; Moorthy, N. J.; Sathyamurthy, N. *J. Org. Chem.* **2003**, *68*, 3446−3453.
(14) Kato, Y.; Okada, S.; Tomimoto, K.; Mase, T. *Tetrahedron Lett.* **2001**, *42*, 4849−4851.
(15) Lautens, M.; Yoshida, M. *J. Org. Chem.* **2003**, *68*, 762−769.
(16) Negishi, E.; Anastasia, L. *Chem. Rev.* **2003**, *103*, 1979−2017.

To our delight, the hydrogenation of compound **10** under Pd/C in methanol at room temperature gave intermediate **11** in quantitative yield. After that, several different reducing agents were applied to prepare intermediate **2**. These results are summarized in Table 1.

Table 1. Preparation of Intermediate **2**



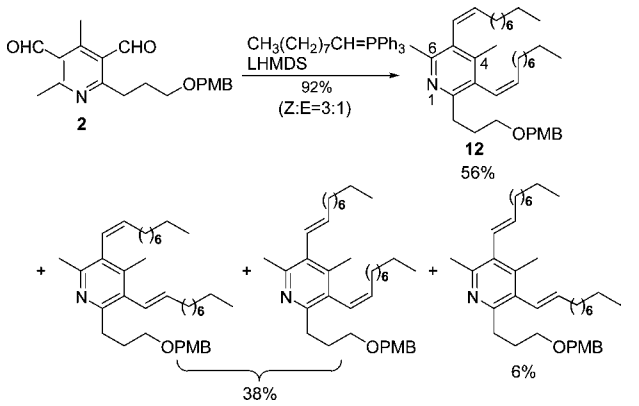
entry	reduction condition	product	yield (%)
1	Raney-Ni, NaH_2PO_2, Py/AcOH/H_2O	no reaction	
2	Raney-Ni, NaH_2PO_2, AcOH/H_2O	**2′**	70
3	Raney-Ni, 5 bar of H_2, AcOH	**2′**	95
4	Raney-Ni, 1 bar of H_2, AcOH	**2′**	90
5	Raney-Ni, 75% HCOOH aq	**2″**	13
6	DIBAL-H, THF, 0 °C to reflux	no reaction	
7	**DIBAL-H, toluene, 0 °C**	**2**	**77**

With use of Raney-Ni[17,18] as the reduction reagent in different solvent systems, the 3,5-diaminomethyl-substituted product (**2′**) was the only one except for the procedure with HCOOH as solvent, which led to compound **2″** in 13% yield. When DIBAL-H was used as the reduction reagent in toluene, the desired product (**2**) was obtained in 77% separation yield,[19] in contrast to the unsuccessful reaction in THF. Our explanation of this result is that the activity of the electrophilic reduction reagent DIBAL-H in toluene is higher than that in the comparatively more polar solvent THF.[20,21]

To introduce the aliphatic side chains onto positions 3 and 5, the Wittig reaction was explored under different basic conditions, such as NaH/toluene,[22] NaH/DMSO,[23] LHMDS,[24] and *n*-BuLi.[25] No reaction was observed when NaH was used as the base in toluene. A trace amount of the target product was observed while most of the starting material decomposed under NaH/DMSO and *n*-BuLi conditions. Finally, intermediate **12** was obtained as the major product when LHMDS was adopted as the base. We noticed that the crude dialdehyde **2** can be used in this step without further purification after the DIBAL-H reduction reaction, and the combined yield was 71% over two steps from compound **11**.

All four possible isomers were observed as products of this reaction (Scheme 4). Various chromatographic conditions have been applied yet no successful separation of any one of these four isomers has occurred. Therefore, after primary purification, the mixture was used for the next step. By calculating the ratios of the integrates of the methyl group at position 6 on the pyridine ring in the ^{1}H NMR spectrum, the composition of these four isomers in the mixture were determined, and compound **12** was characterized as the major one.

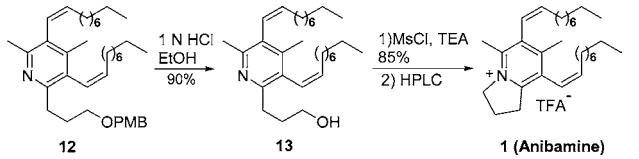
Scheme 4. The Wittig Reaction



Interestingly, the most common PMB deprotection reagent, DDQ, was proved inefficient in both CH_2Cl_2/H_2O[26,27] and CH_2Cl_2/ buffer (pH 7) system[28] for compound **12**. In fact, the PMB group was removed quickly with reasonable yield under acidic conditions to give compound **13**.[29]

The five-member-ring closure was achieved by treating compound **13** with methanesulfonyl chloride and triethylamine at room temperature.[30,31] The crude product was purified by preparative HPLC with the previously reported condition.[1] Both anibamine (**1**) and its (11*E*,22*E*) isomer were isolated as the trifluoroacetic acid salts. The spectral properties of anibamine were compared with those in the literature and no significant differences were observed.

Scheme 5. The Synthesis of Anibamine



The anti-HIV activity of anibamine and its (11*E*,22*E*) isomer was evaluated. In an assay that determines inhibition

(17) Rapold, T.; Senn, M. U.S. Patent, 5,384,403, 1995.
(18) Heffner, R. J.; Jiang, J. J.; JoulliG, M. M. *J. Am. Chem. Soc.* **1992**, *114*, 10181−10189.
(19) Song, Z. J.; Zhao, M.; Desmand, R.; Devine, P.; Tschaen, D. M.; Tillyer, R.; Frey, L.; Heid, R.; Xu, F.; Foster, B.; Li, J.; Reamer, R.; Volante, R.; Grabowski, E. J. J.; Dolling, U. H.; Reider, P. J. *J. Org. Chem.* **1999**, *64*, 9658−9667.
(20) Bajwa, N.; Jennings, M. P. *J. Org. Chem.* **2006**, *71*, 3646−3649.
(21) Paquette, L. A.; Zhao, M. *J. Am. Chem. Soc.* **1993**, *115*, 355−356.
(22) Wightman, R. H.; Cresp, T. M.; Franz, S. *J. Am. Chem. Soc.* **1976**, *98*, 6052−6053.
(23) Kato, K.; Ohkawa, S.; Terao, S.; Terashita, Z.; Nishikawai, K. *J. Med. Chem.* **1985**, *28*, 287−294.
(24) Kan, T.; Fujiwara, A.; Kobayashi, H.; Fukuyama, T. *Tetrahedron* **2002**, *58*, 6267−6276.
(25) (a) Kiær, A.; Kjær, D.; Skrydstrup, T. *Tetrahedron* **1986**, *42*, 1439−1448. (b) Heathcock, C. H.; Finkelstein, B. L; Jarvi, E. T.; Radel, P. A.; Hadley, C. R. *J. Org. Chem.* **1988**, *53*, 1922−1942.

of HIV-1_{BaL} attachment to GHOST R5 cells, anibamine showed an EC_{50} at 0.6 μM and its (11*E*,22*E*) isomer did at 0.8 μM.[32]

In summary, anibamine was synthesized from acetylacetone and cyanoacetamide in 10 steps with 7.9% overall yield, or from acetylacetone and *N*,*N*-dimethylformide dimethyl acetal in 11 steps with 5.1% overall yield. To our knowledge, this is the first report of the total synthesis of anibamine. The above synthetic routes also offer the opportunity to prepare anibamine analogues for further biological evaluation and SAR study for their anti-HIV activity.

(26) Oikawa, Y.; Yoshioka, T.;Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885−888.
(27) Horita, K.; Yoshioka, T.; Tanaka, T.; Oikawa, Y.; Yonemitsu, O. *Tetrahedron* **1986**, *42*, 3021−3028.
(28) Yu, W. S.; Zhang, Y.; Jin, Z. D. *Org. Lett.* ***2001***, *3*, 1447−1450.
(29) Jenkins, D. J.; Riley, A. M.; Potter, B. V. L. *J. Org. Chem*. **1996**, *61*, 7719−7726.
(30) Ciufolini, M. A.; Roschangar, F. *J. Am. Chem. Soc.* **1996**, *118*, 12082−12089.
(31) Bates, R. W.; Boonsombat, J. *J. Chem. Soc., Perkin Trans.* **2001**, *1*, 654−656.
(32) Dextran sulfate were provided as a relevant positive control compound for the individual assays, with anti-HIV activity of EC_{50} at 14.5 μM.

Acknowledgment. The project was partially supported by DHHS AI069975.

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

OL070748N