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A New Stereocontrolled Total Synthesis of the Mast Cell Inhibitory Alkaloid, (+)-Monanchorin, via the Wittig Reaction of a Stabilized Ylide with a Cyclic Guanidine Hemiaminal

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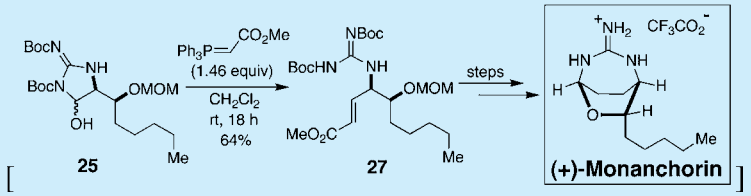
Supporting Information



ABSTRACT: An asymmetric total synthesis of the mast cell inhibitor (+)-monanchorin is reported in which a Sharpless AD on **11** and a cyclic sulfate ring opening with an azide feature as key steps. After further manipulation, a novel guanidine-controlled ester reduction provided the guanidine-hemiaminal **25** which underwent Wittig olefination to give **27**. Hydrogenation and a second guanidine-controlled reduction of the ester in **28**, to obtain aldehyde **29**, then set up a trifluoroacetic acid mediated cyclization to give (+)-monanchorin TFA salt.

Mast cells are leukocytes of bone marrow hematopoietic stem cell origin that contribute significantly to the establishment, growth, and metastatic spread of human tumors.[1] In this regard, the cytoplasm of mature mast cells is packed full of large secretory granules that can release a variety of powerful pro-angiogenic mediators when activated. These include fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), heparin, and angiopoietin-1 (Ang-1). Mast cells are also known to secrete tumor-promoting cytokines such as tumor necrosis factor-α (TNF-α), various interleukins (ILs), and granulocyte-colony stimulating factor (GM-CSF). They further release a range of tissue degrading and tissue remodeling proteases that include tryptase (mMCP-6), chymase (mMCP-4), the metalloexopeptidase carboxypeptidase A3 (CPA3), collagenases, cathepsins, and various matrix metalloproteases that include MMP9.

It is now well established that the chemokines CCL2 and CCL5, tumor-secreted stem cell factor (SCF, *c*-Kit ligand), and SCF expressed on the outer cell surface of human tumors can all lead to the chemotactic migration and local recruitment of immature mast cells to tumors,[1] and once there within the tumor microenvironment, immature mast cells can eventually differentiate, mature, and remain in a constant state of activation through the presence of host activators such as the C5a complement protein.[2] Activated mast cells can thus provide a rich and fertile secretory source of the above pro-angiogenic and tissue remodelling factors which, when in the vicinity of tumors, ultimately promote tumor vascularization, tumor growth, and eventual patient demise by accelerating metastatic spread.

It was Westphal[3] who first recognized that mast cells congregate in a large number at the juncture of developing solid tumors and normal healthy tissue, way back in 1891, and although mature mast cells do indeed populate many normal tissues, they generally do so only at a fairly low level, *except* for the lung and airways, the digestive tract, around neurons, in skin, or in wounds, where their presence in high abundance helps combat unwanted pathogens and parasites.

It appears that tumors and tumor-associated mast cells exert very profound effects on T-regulatory cell function that can result in host immunosuppression and further promote malignancy.[1,4] Indeed, the interactions between mast cells and regulatory T-cells appear to determine whether mast cells combat pathogens exclusively (which is their normal function) or whether they promote excessive tumor inflammation, tumor immunity, and aggressive tumor growth.

Of possible mechanistic relevance to the latter finding is the observation that tumor cells[5] and mast cells[6] both have the ability to transfer mRNA and microRNA, peptides, lipids, and nucleosides into other nearby cells via small nanosized vesicles known as exosomes.[5,6] The latter are released by a variety of cell types, to allow communication and signaling to occur between cells. In cases where mRNA is actually transferred and eventually transfected, exosomes do this by fusing to and eventually merging with target cells. Recipient cells are thus capable of being genetically reprogrammed by exosomes![6]

It is now fairly well established that tumor-secreted exosomes can upregulate the expression of cytokines such as phospho-

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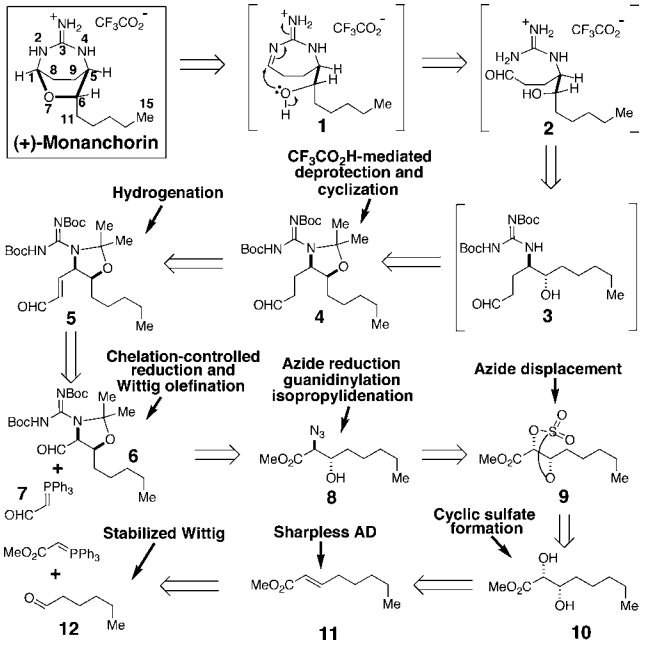
STAT3, phospho-SMAD2/3, IL-10,[4] and TGF-β1[4] in regulatory T-cells (nT-reg/iT-reg), to help diminish the immune response of cancer sufferers. Tumor-derived exosomes can likewise upregulate granzyme B, perforin, and membrane-associated death ligands such as FasL and TRAIL to activate apoptosis in CD8+-T cells.[4,7] It is possible that this immunosuppressive reprogramming of T-cells by tumor-derived exosomes[7] involves the direct transfer of genetic information via mRNA transfection, but it could also involve the donation of key reprogramming proteins from the exosome-budding tumors. It is thus conceivable that tumor-derived exosomes could reprogram mast cells to promote host immunosuppression, subvert T-regulatory cell function, and even themselves behave rather like malignant tumor cells.

In light of this, the selective, yet *controlled* inhibition of tumor-associated mast cell function (including exosome export) could go a significant way toward restoring the normal healthy immune response of some cancer patients, and possibly elicit a cancer cure, if allied with simultaneous antitumor drug therapy and surgery. However, such a hypothesis can only be tested properly, if selectively cytotoxic small molecule inhibitors of mast cell function can be identified, as opposed to simple mast cell stabilizers (which will only prevent mast cell degranulation, rather than actually stopping the mast cell exosome budding).

It was with these thoughts in mind that we became interested in the recent report by McKee and co-workers[8a] that the structurally novel bicyclic guanidine alkaloid, (+)-monanchorin, showed cytotoxicity (IC_{50} = 11.7 μg/mL) in the NCI high-throughput murine IC2 mast cell differential cytotoxicity assay.[8b] The latter screens molecules for their ability to selectively inhibit mast cells of the wild type, as well as those carrying the D814Y mutation (which is c-Kit activating and equivalent to that found in human D816Y mast cells). Notwithstanding this assay providing information about selective cytotoxicity, the original isolation team never revealed whether (+)-monanchorin was indeed selectively cytotoxic toward IC2 mast cells. Despite this, the very unusual structure of (+)-monanchorin still makes it a most alluring small molecule lead for further medicinal chemistry exploration and exploitation, and it was with the latter in mind that we ultimately decided to develop a new and highly practical asymmetric total synthesis of (+)-monanchorin to assist those efforts.

So far, only two total syntheses of (+)-monanchorin have been achieved. The first, by Snider and Yu,[9] unambiguously defined the bicyclic ring structure of the natural product and confirmed the absolute configuration as that depicted in Scheme 1. The Snider pathway utilized a Shi catalytic asymmetric epoxidation[10] to create the key chiral epoxide needed. This epoxide was then ring opened with azide ion to obtain an inseparable 1.25:1 mixture of two regioisomers which, after further synthetic manipulation, were ultimately separated at the very final stages of the synthesis, the major isomer being converted through to (+)-monanchorin by a novel acid-mediated bicyclization process. The other route, developed by Sutherland,[11] exploited a highly stereoselective Pd-catalyzed Overman trichloroacetimidate rearrangement[12] to set the nitrogen stereocenter present in the target (with 12:1 stereoselectivity), and although this did lead to a very elegant and efficient total synthesis, it did require the use of noxious and highly toxic trichloroacetonitrile in one of the steps. It also employed (*R*)-glycidol as its chiral starting material, which presently is sold at £18 a gram by the Aldrich Chemical Co. In

Scheme 1. Our Retrosynthetic Analysis of (+)-Monanchorin



light of these issues, we decided to embark on the development of a new, much more convenient and cheap, asymmetric total synthesis of (+)-monanchorin based upon the retrosynthetic approach outlined in Scheme 1.

In this, we proposed to create the guanidine-hydroxy-aldehyde **2** from the aldehyde **4** by treatment with anhydrous trifluoroacetic acid.[9,13] Compound **2** was then expected to cyclize and dehydrate to form the seven-membered guanidinium imine **1** which would subsequently undergo internal nucleophilic addition to provide (+)-monanchorin. We believed that aldehyde **4** would be derivable from the catalytic hydrogenation of enal **5** which itself would be available from the Wittig reaction of **6** with **7**. Aldehyde **6** would potentially derive from the α-azido ester **8** by azide reduction, guanidinylation, N,O-isopropylidenation, and chelation-controlled semireduction of the ester. A Sharpless AD[14] and a cyclic sulfate ring opening[15] were planned for the synthetic acquisition of **8**.

We commenced the above route with the Wittig reaction between *n*-hexanal and methyl triphenylphosphoranylidene acetate in CH_2Cl_2 which afforded the (*E*)-alkene **11** predominantly in 85% yield (Scheme 2). This was then subjected to Sharpless AD[13] with AD-mix-α to give the *syn*-diol **10** cleanly in 75−84% yield and 92−93% ee. The aforementioned ee estimate was based on NMR analysis of the (*R*)-Mosher ester of azido alcohol **8**, which itself was derived from **10** following cyclic sulfate formation and azide displacement under Sharpless' now standard ring-opening conditions, which furnished **8** exclusively. We progressed the synthesis by catalytically hydrogenolyzing **8** to obtain the amine **13** and guanidinylating the latter with **14** in the presence of stoichiometric silver(I) nitrate. Although both of these steps went well, the subsequent N,O-isopropylidenation of **15** to access **17** proved difficult to achieve, providing only the enol ether **16** in a most disappointing 34% yield. The reaction of **15** with dimethoxypropane and *p*-TsOH in dry acetone was also tested, but **17** was still not isolated.

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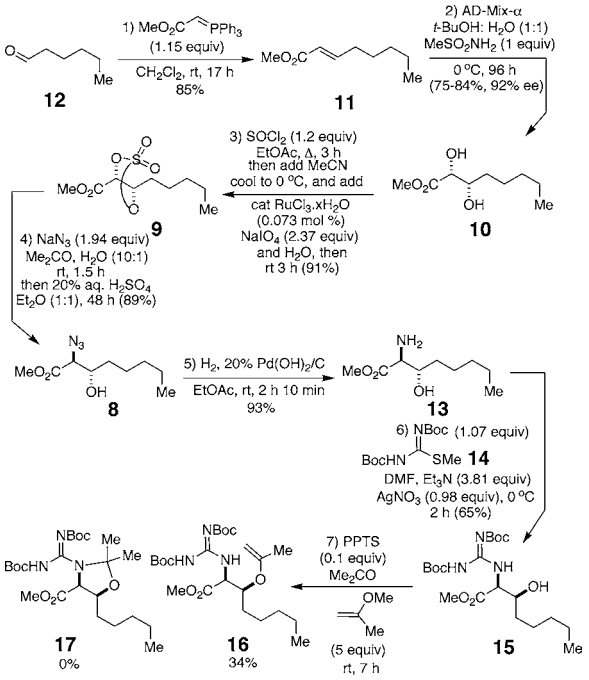
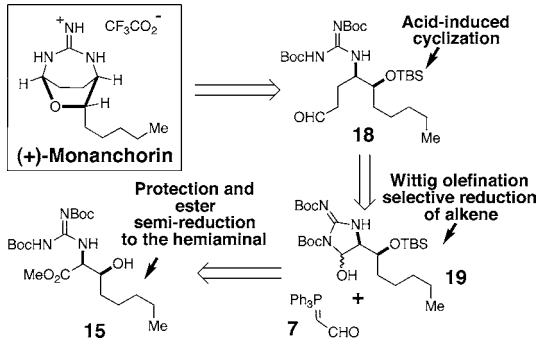
Scheme 2. Our Initial Attempt at Preparing Aldehyde 4



Given this disappointing result, we decided to change tack, and return to the hydroxy-guanidine **15** (Scheme 3). We

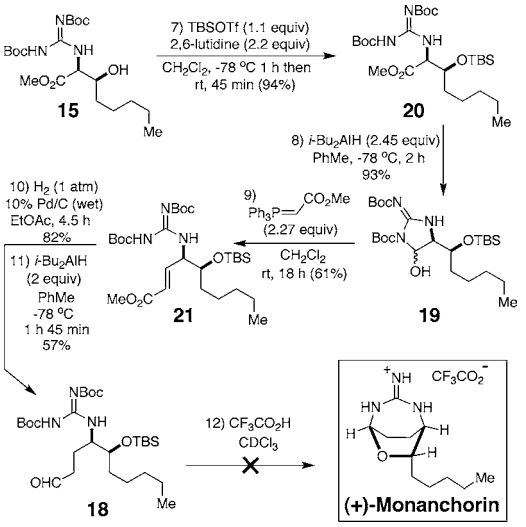
Scheme 3. A Revised Retrosynthetic Plan



reasoned that if the hydroxyl group of **15** could be protected as an OTBS ether, we might be able to effect a controlled semi-reduction of the ester to the aldehyde, which could be immediately intercepted by the pendant guanidine group to give the guanidine hemiaminal **19**. A Wittig reaction with **7** might then yield an enal whose olefin could be reduced selectively. Acid-mediated deprotection could then possibly complete the pathway to (+)-monanchorin in a manner analogous to that shown in Scheme 1. Although this was a very bold and direct synthetic plan, *a search of the literature did not reveal a single precedent for performing a Wittig olefination on an uncharged cyclic guanidine-hemiaminal like **19** with a stabilized phosphorus ylide,*[16] and a second issue that could potentially arise, if this reaction did succeed, was *in situ* Michael cyclization of the liberated guanidine anion[17] onto the product enal. Despite these significant risks, the great brevity and overall simplicity of this route still attracted us toward it.

Alcohol **15** was thus protected with TBSOTf (1.1 equiv) and 2,6-lutidine (2.2 equiv) in dry CH_2Cl_2 (Scheme 4) at −78 °C for 1 h and rt for a further 45 min. Fortunately, the potentially

Scheme 4. Attempted Execution of the Revised Plan



cleavable Boc groups remained intact and **20** was isolated in good yield. Slow addition of DIBAL (2.45 equiv) to a −78 °C solution of **20** in PhMe and stirring for a total of 2 h then effected a highly efficient reduction of the ester to give the cyclized guanidine-hemiaminal **19** in 93% yield after aqueous workup. Unfortunately, the cyclic hemiaminal **19** failed to react with ylide **7** under a range of conditions. We therefore attempted the olefination of **19** with the more nucleophilic methyl triphenylphosphoranylidene acetate, and to our delight, this reaction proceeded successfully over 18 h at rt to give the enoate **21** in 61% yield. The alkene in **21** was thereafter reduced to the alkane, and another cyclic guanidine-intercepted ester reduction was attempted with DIBAL at low temperature. Again, this selective reduction worked out well, with aldehyde **18** arising. At this point in the route, we were confident that we could complete the synthesis of (+)-monanchorin by a simple CF_3CO_2H-induced O-desilylation and bicyclization (or vice versa), but unfortunately, the TBS group did not cleave during this attempted ring-closure process.

Given this situation, and the fact that Sutherland[11] had successfully cleaved a MOM-ether with dry CF_3CO_2H[13] during the final step of his (+)-monanchorin synthesis, we reasoned that the use of a MOM-ether might produce a more satisfactory outcome in our projected route. Accordingly, we returned once more to the azido-alcohol **8** (Scheme 5), protected its hydroxyl as a MOM-ether with MOMCl and Hunig's base, and hydrogenolyzed the azido group of **22** with 20% $Pd(OH)_2$ on carbon in EtOAc. Amine **23** was then reacted with *N,N*-bis-Boc-S-methylisothiourea **14** in the presence of $AgNO_3$ and Et_3N at 0 °C. The result was the guanidine ester **24**, which was formed in 76% overall yield over three steps. DIBAL-reduction now proceeded successfully, delivering the guanidine hemiaminal **25** in 79% yield. We next investigated its Wittig olefination with the stabilized ylide **7**. Not surpisingly, many sets of reaction conditions failed to produce a satisfactory result, but a partial success was eventually achieved when this reaction was conducted in PhMe with 1.5 equiv of **7** at 70 °C in a sealed tube over 18 h. However, the yield of enal **26** was typically poor (31%) and there was a significant amount of accompanying degradation; it also proved hard to completely purify **26** from the triphenylphosphine oxide byproduct!

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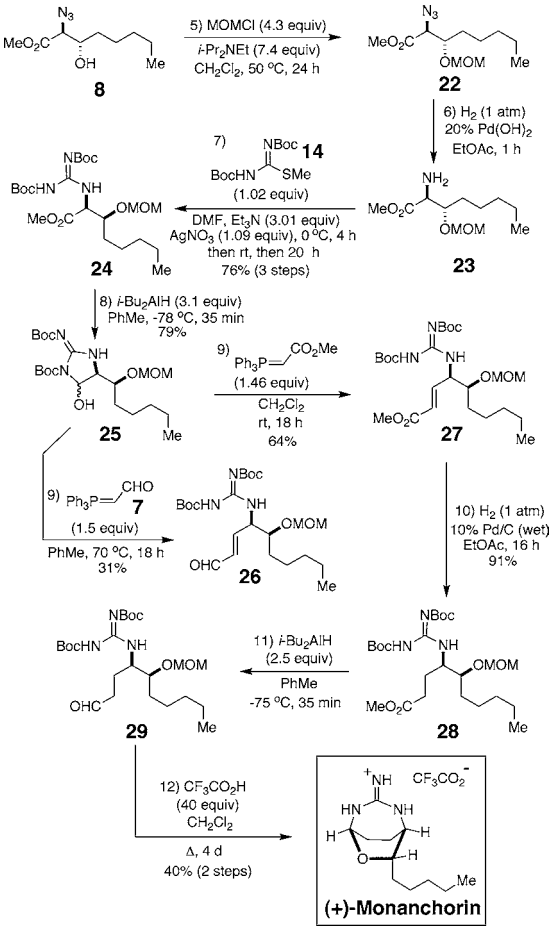
Scheme 5. A New Total Synthesis of (+)-Monanchorin



Fortunately, the room temperature Wittig reaction of methyl triphenylphosphoranylidene acetate with the guanidine-hemiaminal **25** proceeded cleanly when conducted in CH_2Cl_2 at rt for 18 h; a 64% yield of the desired enoate **27** was obtained. As was the case with **21**, enoate **27** had to be used *immediately* for the subsequent catalytic hydrogenation step, without any undue delay, otherwise self-destructive internal Michael addition and polymerization gradually started to consume it (when in highly concentrated form). The hydrogenation step gave the methyl ester **28** in 91% yield.

A selective −75 °C semireduction of the methyl ester in **28** was now attempted with DIBAL (2.5 equiv) in order to obtain the seven-membered hemiaminal, which underwent spontaneous ring opening *upon aqueous workup* to give the open-chain aldehyde **29** (presumably to relieve ring strain). Unfortunately, all of our different attempts to purify this compound by SiO_2 flash chromatography led to a much more polar pair of products being formed, which we suspected were the seven-membered cyclic guanidine hemiaminals. Given the difficulties associated with purifying this mixture, we eventually decided to treat crude **29** directly with CF_3CO_2H in CH_2Cl_2 and, after 4 d at reflux, the TFA-salt of (+)-monanchorin was isolated in 40% overall yield, after SiO_2 flash chromatography. Importantly, the product had NMR spectra and spectral data that matched closely with those published by Snider[9] and Sutherland.[11]

In summary, we have developed a new and fully stereocontrolled asymmetric synthesis of the mast cell inhibitory alkaloid, (+)-monanchorin, that delivers this molecule as its TFA salt in high ee and high overall yield (7.2%, 12 steps).

■ ASSOCIATED CONTENT

Supporting Information

NMR and IR spectra and HRMS and full experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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