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Asymmetric synthesis and conformational analysis of the two enantiomers of the saturated analog of the potent thrombin inhibitor MOL-376

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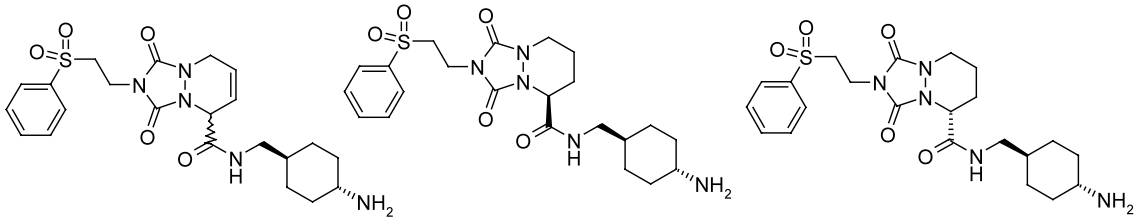
Abstract—Asymmetric synthesis of the two enantiomers of a small molecule thrombin inhibitor is described. The key step in the synthesis is the glucose-directed chiral induction in the hetero Diels–Alder cycloaddition step. Conformational analysis indicates that the *S*-enantiomer is a better fit for the idealized β-strand conformation. © 2002 Elsevier Science Ltd. All rights reserved.

We have been working for the past several years to develop mimetics of common secondary structure motifs such as β-strand, reverse turns and α-helices that can be used as novel therapeutic agents.[1–3] Thrombin is a serine protease that plays a key role in the blood coagulation cascade leading to thrombus formation. X-Ray crystallography studies of several proteases including thrombin have highlighted the fact that an extended strand motif is adapted by the inhibitor/substrate in the active site.[4] We have designed and synthesized a number of small molecule β-strand mimetics that adopt the bioactive conformation[5] and developed MOL-376 as a potent and selective thrombin inhibitor ($K_i = 1.2$ nM). The saturated analog of MOL-376 also showed very good activity. In this paper we describe the asymmetric synthesis of the two enantiomers **1** and **2** of saturated analog of MOL-376 (Fig. 1).

Stoodley and co-workers have shown that (*E*)-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxybuta)-1,3-diene displayed good diastereofacial selectivity in Diels–Alder reactions with cyclic dienophiles like urazoles.[6] We applied this reaction to the synthesis of product **1** as outlined below (Scheme 1).

Reaction of the monosodium salt of malonaldehyde[7] **3** with acetobromoglucose **4** in DMSO solution[8] afforded aldehyde **5**. Treatment of the aldehyde with phosphorane **6** at room temperature[6c] furnished the *trans*,*trans*-diene **7** which was obtained as a major diastereomer in 88% yield. The diastereomerically pure diene **7** was then obtained by crystallization from a methylene chloride–ether–hexane system (48% yield). Diels–Alder reaction of diene **7** with urazole **8** in the presence of iodobenzene diacetate[1] (IBD) in methylene chloride (crude dr 96:4)



MOL-376 **1** **2**

Figure 1.

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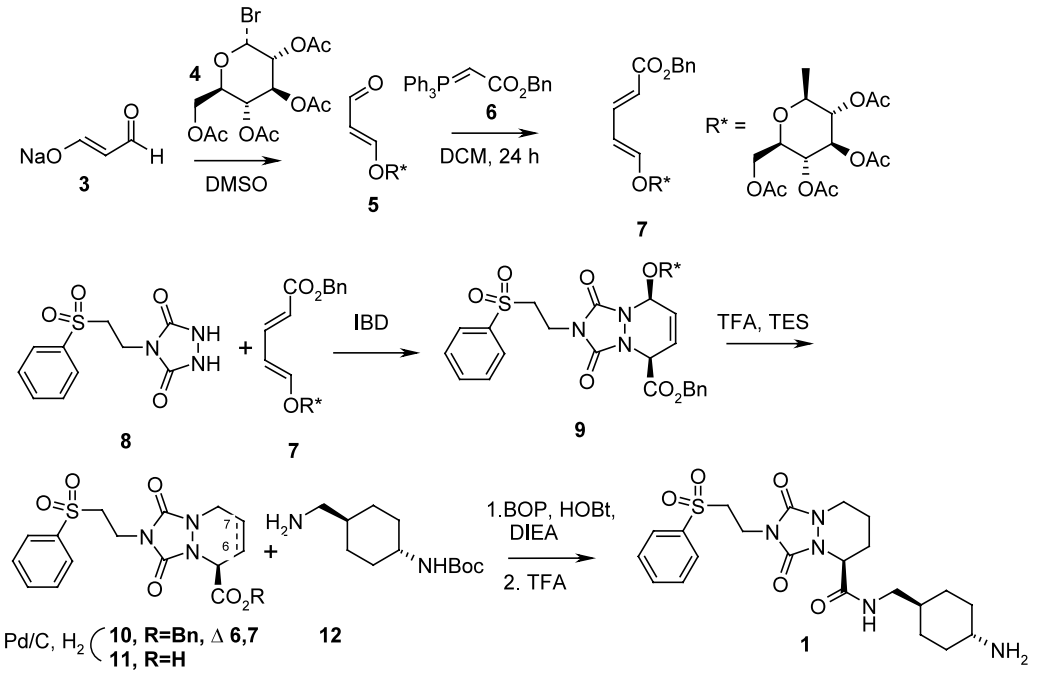


Scheme 1.

followed by crystallization (methylene chloride–hexane system) afforded the cycloadduct **9** in 84% yield ($[\alpha]_D^{20}$ −15.8, *c* 1.0, $CHCl_3$). The diastereoselection in this type of Diels–Alder reaction involving cyclic aza dienophiles was reported by Stoodley to be higher than their olefinic counterparts and was rationalized by Stoodley and co-workers[6d] to arise by an *endo* attack of the cyclic dienophile to the less hindered top face of the solution preferred conformer, as depicted in Fig. 2.

When **9** was treated with triethyl silane and TFA in methylene chloride overnight it underwent a reductive cleavage reaction to afford the desired compound **10** in 81% yield ($[\alpha]_D^{20}$ −200.9, *c* 1.0, $CHCl_3$). Hydrogenation of the cycloadduct **10** afforded the acid **11**, which was further purified by crystallization (methylene chloride–hexane system). The crystallized acid **11** ($[\alpha]_D^{20}$ −61.5, *c* 1.0, CH_3OH) was then coupled with the *trans*-monoBoc protected diamine **12** in the presence of BOP, HOBt and DIEA followed by purification and removal of Boc group to give enantiomers **1** and **2**[9] in the ratio 98:2 (96% ee) in 62% yield ($[\alpha]_D^{20}$ −31.0, *c* 1.0, CH_3OH).

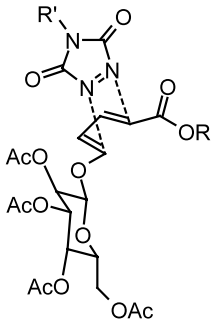
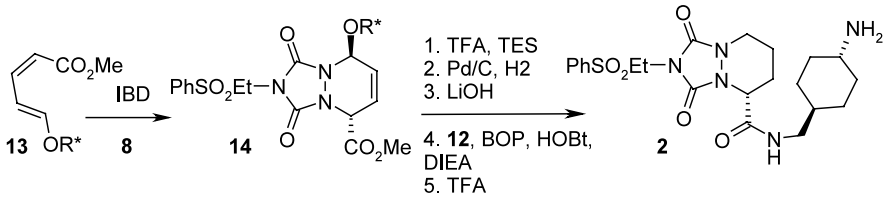


Figure 2.

The enantiomeric ratio of the product was determined by chiral HPLC[10] and compared with a racemic mixture prepared without using the sugar auxiliary.

In order to obtain **2**, the *trans* cycloadduct **14** must be synthesized. For this the *cis*,*trans*-diene **13** was synthesized from aldehyde **5** using a procedure reported by Still.[11] The crude diene, containing a trace of the *trans*,*trans*-diene **7**, was crystallized (methylene chloride–ether–hexane system) to obtain diastereomerically pure diene **13** which underwent cycloaddition with compound **8** (crude dr 92:8) to afford the *trans* product **14** (95% yield, $[\alpha]_D^{20}$ +113.1, *c* 1.0, $CHCl_3$). Removal of the sugar auxiliary as described above followed by hydrogenation of the double bond, hydrolysis of the methyl ester (0.2N LiOH, THF), coupling with *trans*-amine **12** and removal of the Boc group afforded product **2** with traces of **1** in the ratio 95:5 (90% ee, $[\alpha]_D^{20}$ +30.7, *c* 1.0, CH_3OH) (Scheme 2).[11]

Monte Carlo conformational analysis for the core bicyclic templates in Mol-376, **1**, and **2** was carried out using MMFF94/GBSA-water[12] as implemented in MacroModel (Version 7.1, Schrodinger, Inc.).[13] The overlay of the energy-minimized structures was based on X-ray data of MOL-376 bound to thrombin, where the anchoring of the cyclohexyl amine P1 to Asp-189 in the thrombin specificity pocket, was preserved. Two hydrogen-bond formations involved in binding to thrombin were also taken into account for the overlay of the templates. Overlay of the lowest energy conformations of MOL-376 with *S* configuration at C5 position and **1** (Fig. 3) shows the differences in the template conformation and in the positioning of the P3 methyl group. The P3 substituent differed in the directions by approximately 15°. While this difference may be small at the urazole moiety, the distance is significant at the



Scheme 2.

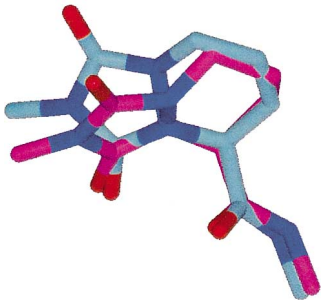


Figure 3. Comparison of the lowest energy conformations of MOL-376 (with P1 and P3 truncated for clarity) in cyan and saturated β-strand template **1** with *S* configuration in magenta. Superposition was carried out at three heavy atom positions to preserve the hydrogen-bonds and P1 binding to the thrombin active site (see text). Nitrogens and oxygens are color coded as blue and red, respectively.

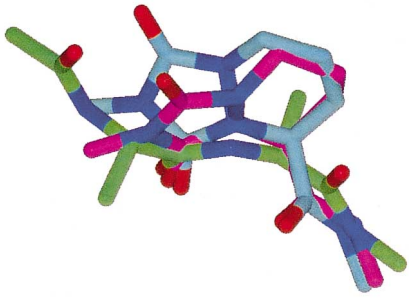


Figure 4. Comparison of the lowest energy conformation of the saturated β-strand template **1** in magenta against MOL-376 in cyan and the idealized antiparallel β-strand in green. RMSD value at nine heavy atom positions along with the strand backbone was found to be 0.47 Å.

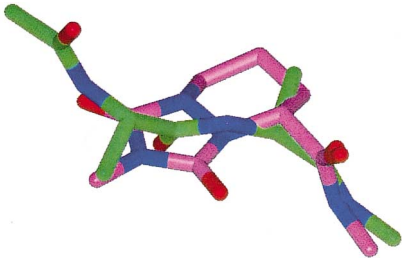


Figure 5. Comparison of the lowest energy conformation of the saturated β-strand template **2** in pink against the idealized antiparallel β-strand in green.

far end of the P3 group as it extends along the hydrophobic cleft of thrombin. *S*-Isomer **1** was also compared with an idealized β-strand conformation (in green) at nine heavy atom positions along with the strand backbone with RMSD value of 0.47 Å (Fig. 4). In contrast, the *R*-isomer, **2**, did not fit very well with the idealized β-strand conformation, as shown in Figure 5. From this analysis, we expect that the bioactive enantiomer of the saturated template is the *S*-isomer, **1**.

In summary, a facile asymmetric synthesis of both enantiomers of saturated MOL-376 was accomplished. The enantiomer **1** was synthesized from diene **7** in five steps and 30% overall yield while enantiomer **2** from diene **15** in six steps and 27% overall yield.

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9. For compound **1**: δ ^{1}H NMR (400 MHz, CD_3OD) 8.05 (t, $J=6$ Hz, 1H), 7.97–7.95 (m, 2H), 7.78–7.4 (m, 1H), 7.76–7.64 (m, 2H), 4.57 (dd, $J=5.2$ and 2.8 Hz, 1H), 3.87 (t, $J=6.4$, 3H), 3.67–3.63 (m, 2H), 3.14–2.98 (m, 4H), 2.91–2.86 (m, 1H), 2.26–2.21 (m, 1H), 2.04–1.78 (m, 7H), 1.63–1.29 (m, 4H), 1.1–1.03 (m, 2H); δ ^{13}C NMR (400 MHz, CD_3OD) 170.46, 154.68, 152.52, 139.84, 135.29, 130.53, 129.2, 56.88, 52.80, 51.46, 46.24, 46.11, 43.26, 37.98, 34.56, 31.42, 29.66, 26.77, 20.73; LCMS RT = 0.71, 478.2 (M^++H^+). For compound **2**: δ ^{1}H NMR (400 MHz, CD_3OD) 8.06 (t, $J=6$ Hz, 1H), 7.95 (d, $J=8$ Hz, 2 HZ), 7.76 (t, $J=7.6$ Hz, 1H), 7.65 (t, $J=7.6$ Hz, 2H), 4.57 (dd, $J=5.2$ and 3.2 Hz, 1H), 3.87 (t, $J=6.8$ Hz, 3H), 3.67–3.63 (m, 2H), 3.13–3.01 (m, 4H), 2.2–2.1 (m, 1H), 2.04–1.78 (m, 7H), 1.66–1.29 (m, 4H), 1.09–1.02 (m, 2H); δ ^{13}C NMR (400 MHz, CD_3OD) 170.14, 154.39, 152.18, 139.56, 134.98, 130.24, 128.88, 56.59, 52.53, 51.15, 45.93, 45.81, 42.93, 37.66, 34.25, 31.1, 29.34, 26.48, 20.43; LCMS RT = 0.72, 478.2 (M^++H^+).
10. Enantiomers separated on a ChromTech Chiral-AGP column, 4.0×100 mm using an isocratic 5% ACN, 95% NaH_2PO_4 at pH 7.0 at a flow rate of 0.9 ml/min.
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