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The Synthesis of Thrombin Inhibitor L-370,518 via an α-Hydroxy-β-Lactam.

Kellie J. Cutrona and Philip E. J. Sanderson*

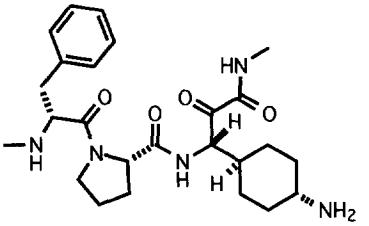
Department of Medicinal Chemistry,

Merck Research Laboratories, West Point, PA 19486, USA

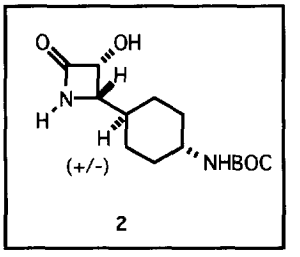
Abstract: We describe an efficient synthesis of thrombin inhibitor L-370,518 (1) via β-lactam **2**. The synthesis of **2** is carried out in six steps from 4-aminocyclohexane carboxylic acid, with a yield of 27%.

Tripeptide L-370,518 (**1**) is a thrombin inhibitor which binds reversibly to the enzyme with a K_i of 90 pM.[1] The molecule contains a novel C-terminal α-keto-β-amino acid residue which serves both as a serine trap and as an effective arginine mimic, conferring both potency and selectivity. The desirable properties of this residue necessitated the development of an efficient synthesis of a versatile precursor of it for use in further medicinal studies. In this communication we report a synthesis of such an intermediate and, for illustrative purposes, its conversion to **1**.

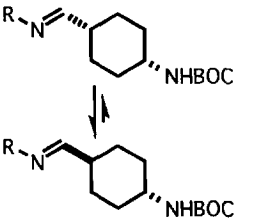
Typically a one-carbon chain extension from the α-amino acid is employed in the synthesis of α-keto-β-amino acids[2] and this method is generally useful when starting from readily available α-amino acids. However, a cumbersome synthesis results when the method is applied to **1**[1] since one of the principal challenges of the synthesis, the establishment of the *trans*-substitution across the cyclohexane,[3] still has to be addressed. So, instead, we chose to investigate a two-carbon 'Staudinger' disconnection via β-lactam **2**, knowing that this strategy



L-370,518 (**1**): K_i (thrombin) 90 pM



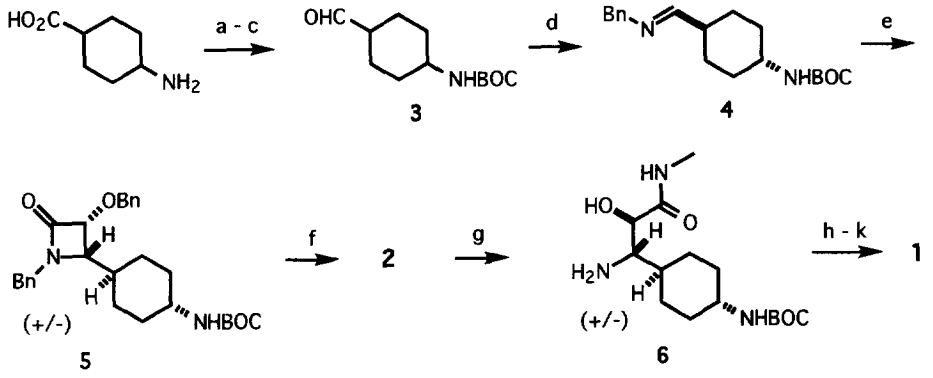
2



eq. 1

has been applied to the stereocontrolled synthesis of α-hydroxy-β-amino acids such as the taxol side chain.[4] In our case intermediate **2** is useful because the C-terminus is preactivated for amide formation. More importantly, the Staudinger strategy opens up the possibility of establishing the cyclohexane geometry by thermodynamic equilibration of an imine derived from protected *cis*-4-aminocyclohexane carboxaldehyde (eq. 1).

Hydrogenation of *para*-aminobenzoic acid[5] provided the starting 4-aminocyclohexane carboxylic acid as a mixture of isomers, *cis* predominating. This mixture was protected as its *t*-butoxycarbonyl (BOC) derivative under standard conditions and Weinreb amide formation followed by reduction with LAH[6] gave the aldehyde **3** still as a mixture with the *cis*-isomer predominating. In a key step we found that it was possible to make the benzylimine and equilibrate it *in situ* under very mild reaction conditions ($MgSO_4$, 1% acetic acid, methylene chloride, RT, 4 d) to give imine **4** as a 12:1 *trans:cis* mixture.[7] In contrast, *p*-anisidine under the same conditions gave very poor quality imine.



Reagents

(a) $(BOC)_2O$, KOH, H_2O, dioxane, 16 h (91%). (b) MeONHMe.HCl, Et_3N, EDC, DMF, 16 h. (c) LAH, Et_2O, -55° C to 5° C, 1 h (79%). (d) $BnNH_2$, $MgSO_4$, 0.01 equiv. AcOH, CH_2Cl_2, 4 d. (e) $BnOCH_2COCl$, Et_3N, CH_2Cl_2, -15° C to RT, 3 h. (f) Na, NH_3, -78° C, 15 min (37%). (g) $MeNH_2$, MeOH, 50° C, 16 h (96%). (h) BOC-N-Me-D-PhePro, EDC, Et_3N, HOBT, DMF, 16 h (67%). (i)$(COCl)_2$, DMSO, CH_2Cl_2, -78° C, 15 min then Et_3N -78° C to RT, 30 min. (j) TFA, CH_2Cl_2, 15 min. (k) C_{18} prep. HPLC (42%).

Staudinger reaction of **4** with benzyloxyketene generated *in situ* from benzyloxyacetyl chloride and one equivalent of triethylamine[4a,8] gave poor yields of the β-lactam **5** and significant quantities of carboxaldehyde **3** were recovered. The dependence of the rate of the Staudinger reaction on solvent polarity and base stoichiometry has been investigated by Lynch.[10] We reasoned that we could increase the rate of ketene formation and its subsequent addition to the imine to generate the β-lactam by increasing the polarity of the reaction medium through the use of excess triethylamine. Side reactions would then be minimized, assuming that they were not similarly accelerated. Thus when we used ten equivalents of triethylamine the recovery of **3** was effectively suppressed. Analysis of the crude reaction mixture by ^{1}H-NMR and preparative HPLC revealed no evidence for the formation of product with substituents either *trans* on the β-lactam[4] or *cis* on the cyclohexane ring.

We found it expedient to submit the crude mixture from the Staudinger reaction to a dissolving metal reduction to remove both benzyl groups[11] since, after evaporation of the ammonia and an aqueous work-up, analytically pure **2** could be isolated in 37% yield from the aldehyde **3** *simply by triturating the crude material with methylene chloride.*

We feel that the simplicity of our route to **2** offsets any possible advantage of Ojima's asymmetric synthesis of α-hydroxy-β-amino acids.[12] However, we did investigate an asymmetric synthesis of **2** using the (S)-α-methylbenzylimine[4a,d] (equilibrated to an 8:1 *trans:cis* mixture across the cyclohexane). The diastereoselectivity in the Staudinger reaction was poor (ratio of the two *cis*-substituted lactams 2.2:1 by ^{1}H NMR) so this approach was not pursued further.

With the lactam preactivated for C-terminal amide formation and the two amino groups differentiated, the remaining steps to **1** were straightforward. The lactam was cleanly opened with methylamine in methanol to give methylamide **6**. This was coupled with BOC-N-methyl-D-PhePro[13] under standard conditions to give the tripeptide as a 1:1 diastereomeric mixture. Swern oxidation gave a mixture of the corresponding ketones. Finally deprotection (TFA) followed by chromatographic separation of the two diastereomers by C_{18} preparative HPLC (CH_3CN/H_2O/0.1% TFA)[1] completed the synthesis.[14]

Experimental Procedure for the Preparation of 2 from 3:
A mixture of **3** (11.37 g, 50.0 mmol), benzylamine (5.46 ml, 50.0 mmol), acetic acid (0.029 ml, 0.50 mmol) and anhydrous magnesium sulfate (12.04 g, 100 mmol) in methylene chloride (75 ml) was stirred under nitrogen for 4 days. The mixture was filtered through Celite and was evaporated *in vacuo* to give **4** (15. 8 g, 100%) as a solid.[7] A solution of benzyloxyacetyl chloride (6.94 ml, 44.0 mmol) in methylene chloride (20 ml) was added over 5 min to a stirred solution of **4** (12.66 g, 40.0 mmol) and triethylamine (55.75 ml, 0.40 mol) in methylene chloride (40 ml) under nitrogen. The mixture was warmed to RT and after 3 h water (10 ml) was added. The mixture was evaporated *in vacuo* to a syrup which was partitioned between ethyl acetate and 10% citric acid solution. The organic layer was washed with dilute $NaHCO_3$ solution and brine, dried (Na_2SO_4) and evaporated *in vacuo* to a solid (crude **5**,[9] 17.95 g). A solution of crude **5** (1.00 g,) in dry THF (12.5 ml) was added to a stirred solution of sodium (0.6 g) in ammonia (100 ml) at -78° C under nitrogen. After 15 min ammonium chloride powder (5.0 g) was added to give a white suspension. The ammonia was allowed to evaporate and the residue was partitioned between ethyl acetate and brine, adding sufficient water to dissolve the excess salts. The organic layer was dried and evaporated *in vacuo* to a foam. Trituration with methylene chloride gave **2** (233 mg, 37% from **3**); mp: 208-211° C; ^{1}H NMR (DMSO-d_6): δ 0.78-1.29 (5 H, m, cyclohexyl H's), 1.33 (9 H, s, *t*-Bu), 1.66-1.78 (4 H, m, cyclohexyl H's), 3.05 (1 H, dd, J = 9.3 and 4.6 Hz, C*H*CHOH), 3.12 (1H, m, C*H*NHBOC), 4.62 (1 H, dd, J = 7.9 and 4.6 Hz, C*H*OH), 5.85 (1 H, d, J = 7.9 Hz, OH), 6.63 (1 H, d, J = 7.9 Hz, NHBOC), 8.16 (1 H, s, lactam NH); Anal. Calc. for $C_{14}H_{24}N_2O_4$: C 59.14, H 8.51, N 9.85. Found: C 58.97, H 8.50, N 9.79.

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9. A sample was recrystallized (2:1 hexanes/ethyl acetate) to give pure **5**, mp: 136-137° C, ^{1}H NMR ($CDCl_3$): δ 1.01-1.10 (4 H, m, cyclohexyl H's), 1.43 (9 H, s, *t*-Bu), 1.70-2.05 (5 H, m, cyclohexyl H's), 3.27 (1 H, t, J = 5.2 Hz, C*H*CHOBn), 3.30 (1 H, br s, C*H*NHBOC), 4.02 (1 H, d, J = 14.9 Hz, benzylic H), 4.34 (1 H, br s, NH), 4.59 (1 H, d, J = 5.2 Hz, CHC*H*OBn), 4.71 (1 H, d, J = 11.9 Hz, benzylic H), 4.84 (1 H, d, J = 14.9 Hz, benzylic H), 4.93 (1 H, d, J = 11.9 Hz, benzylic H), 7.17-7.37 (10 H, m, Ph's).
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