Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 12, pp. 1563-1566, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00271-0

AN EFFICIENT PREPARATION OF THE POTENT AND SELECTIVE PSEUDOPEPTIDE THROMBIN INHIBITOR, INOGATRAN

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Abstract. Inogatran is an effective and selective pseudopeptide inhibitor of thrombin that has been shown to be efficacious in a series of in vitro and in vivo models of thrombosis. Herein, we report an efficient and convergent chemical synthesis of Inogatran that is amenable to the preparation of multiple gram quantities.

Thrombin is a trypsin-like serine protease that plays a major role in blood coagulation. In particular, thrombin is responsible for conversion of the soluble plasma protein fibrinogen by cleavage of the Arg^{16}-Gly^{17} amide bond to the insoluble fibrin leading to clot formation. As with other serine proteases, thrombin utilizes a catalytic triad of amino acids (Ser^{195}, His^{57}, and Asp^{102}) to initiate this conversion. Thrombin plays a pivotal role in the critical balance between hemostasis and thrombolysis. Thus, inhibitors of thrombin may offer treatment for venous and arterial thrombosis, pulmonary embolism and restenosis following angioplasty.[2-4] In addition, thrombin has been implicated in atherosclerosis, inflammation, and neurodegenerative diseases.[3] Therefore, potent and specific inhibitors of thrombin are of significant pharmaceutical interest.

Several indirect and direct inhibitors have been reported.[2-4] The indirect inhibitors include coumarins, heparins and low molecular weight heparin. The most potent, direct, and selective thrombin inhibitor known is hirudin, an acidic 65 amino acid peptide isolated from the salivary gland of the medicinal leech.[5] Hirudin binds to both the active site and to a remote anion-binding exosite of thrombin. Several smaller analogues of hirudin known as "hirulogs" have also been described, however lack of oral bioavailability limits their therapeutic potential.[6]

Thus, low molecular weight direct inhibitors of thrombin are of considerable current interest. In general, these inhibitors were designed from the observation that the chloromethyl ketone of D-Phe-Pro-Arg (PPACK) could mimic the sequence of fibrinogen (thrombin's natural substrate).[7] Several X-ray structures have been recently solved with such inhibitors bound in the active site and these are being actively utilized in the structure-based design of more potent and selective analogues (For recent reviews, see refs. 2–4). Several of these inhibitors form a covalent bond with the Ser^{195} residue of thrombin. However, several common problems

have been encountered in the development of these small molecule inhibitors: (a) lack of selectivity for thrombin over other trypsin-like enzymes, (b) low oral bioavailability, (c) short duration of action, (d) rapid clearance, and (e) increased bleeding times.[2,3]

Recently, Inogatran (glycine, N-[2-[2-[[[3-[(aminoiminomethyl)amino]propyl]amino]-carbonyl]-1-piperidinyl]-1-(cyclohexylmethyl)-2-oxoethyl]-[*2R*-[*2S*]]), (**1**), a pseudopeptide, has been reported to be an effective, noncovalently bound, direct and selective inhibitor of thrombin in several in vitro and in vivo models of thrombosis (K_i = 15 nM for thrombin, 45 nM for trypsin).[8–15] Inogatran has been shown to inhibit arterial thrombosis more effectively than heparin or acetylsalicylic acid in a closed-chest porcine model[14] and at high doses only moderately increase bleeding times.[15] In addition, Inogatran has significant oral bioavailability in rats (32–51%) and dogs (34–44%).[9–11] The only reported synthesis of Inogatran is nonconvergent, has several low yielding steps and requires a final purification by reversed-phase high pressure liquid chromatography (HPLC).[8] Thus, in order to further evaluate the potential therapeutic utility of Inogatran as an antithrombotic agent an efficient synthesis of the molecule was required. Herein, we report an efficient and convergent chemical synthesis of Inogatran that is amenable to the preparation of multiple gram quantities.

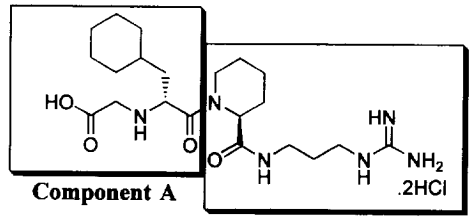
Results and Discussion

The synthesis of Inogatran was achieved by a convergent strategy, in which the molecule was divided into two components (**A** and **B**) (Scheme 1). Component **A** (**7**) was prepared in five steps (29% overall) starting from L-phenylalanine (**2**). L-Phenylalanine was converted to its *t*-butyl ester (**3**), hydrogenated (**4**) and alkylated with benzyl-2-bromoacetate to yield (**5**). The secondary amine was then protected with the benzyloxycarbonyl group to provide compound (**6**), which was converted to compound (**7**) by treatment with HCl. Component **B** (**12**) was prepared in four steps (61% overall) from Boc-1,3-diaminopropane (**8**). Compound (**8**) was sequentially guanylated, Boc deprotected, coupled to N^{α}Boc-pipecolic acid and Boc deprotected once more to yield (**12**) as an HCl salt. A number of conditions were explored to couple Components **A** and **B**. The optimum coupling reagent proved to be HATU [O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] to give (**13**), which upon hydrogenolysis, conversion to the HCl salt and lyophilization provided Inogatran (**1**) in quantitative yield without the necessity for additional purification (>98% pure by analytical reversed-phase HPLC).

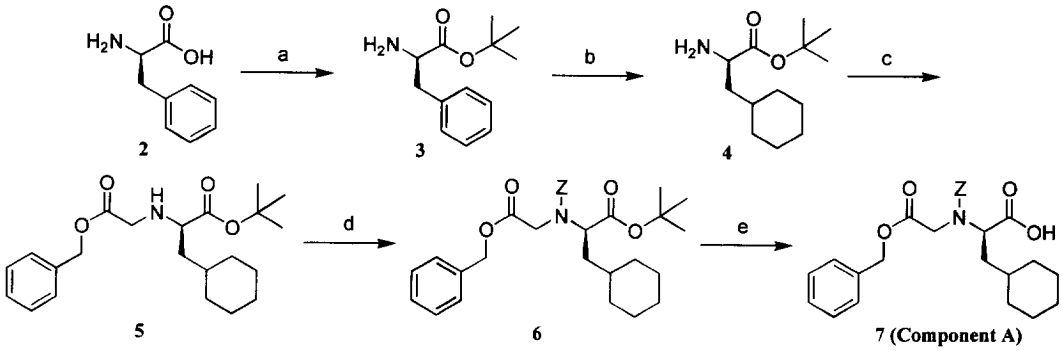
The lowest yielding step (62%) in this synthetic strategy is the alkylation of the relatively inexpensive L-cyclohexylalanine with benzyl-2-bromoacetate which is performed early in the sequence. In the previously reported preparation of Inogatran,[8] alkylation takes place later on an intermediate that requires several steps to prepare (Scheme 2). Also, epimerization at the alpha carbon of pipecolic acid has been reported to a problem for the previous preparation[8] however, with the current strategy no epimerization was observed by reversed-phase HPLC. Finally, this sequence allows for the preparation of Inogatran without the necessity for purification by reversed-phase HPLC.

Scheme 1

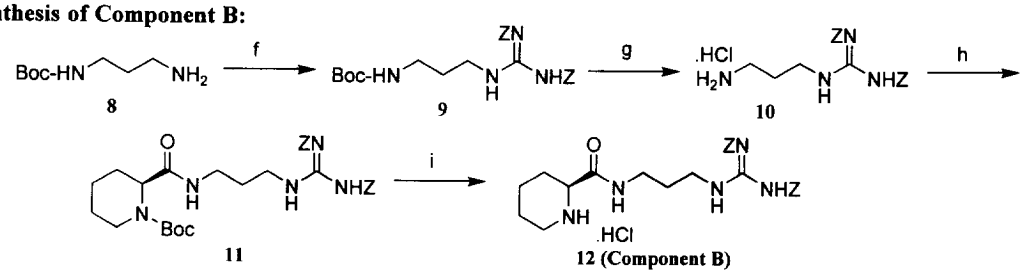
Inogatran.2HCl (1):



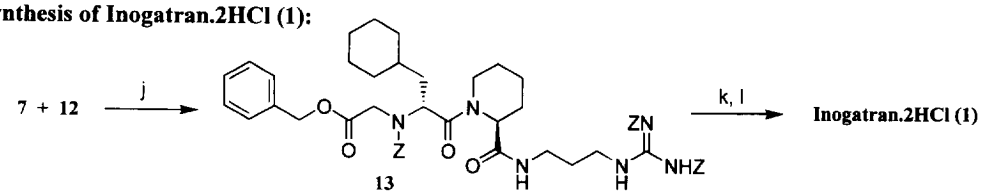
Synthesis of Component A:



Synthesis of Component B:

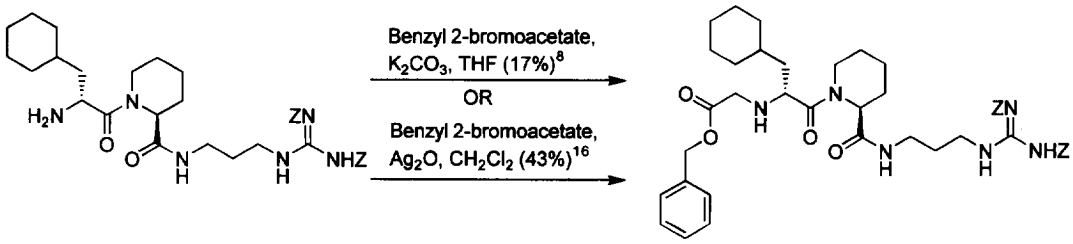


Synthesis of Inogatran.2HCl (1):



Conditions: (a) isobutylene, H_2SO_4, *p*-dioxane (72%); (b) H_2, 10% Rh-C, H_2SO_4, MeOH (75%); (c) benzyl 2-bromoacetate, K_2CO_3, MeOH (62%); (d) benzyl chloroformate, triethylamine, THF (86%); (e) 4 N HCl, *p*-dioxane (>99%); (f) bis-benzyloxycarbonyl-1*H*-pyrazole-1-carboxamidine, NMM, THF (75%); (g) 4 N HCl, *p*-dioxane, ethyl methyl sulfide (product was not isolated and used directly in the next step); (h) N^{α}Boc-pipecolic acid, BOP reagent [benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphate], NMM, DMF (85%, for steps g and h); (i) 4 N HCl, *p*-dioxane, ethyl methyl sulfide (95%); (j) HATU, DIEA, DMF (56%); (k) H_2, 10% Pd-C, MeOH, (100%); (l) 2 equiv 1 N aqueous HCl (100%).

Scheme 2



Inogatran (**1**), prepared by the strategy depicted in Scheme 1 has been shown to be consistent with the previously described structure by high-resolution proton NMR and electrospray mass spectrometry and homogenous by reversed-phase HPLC. In our hands, this synthetic sample of Inogatran was able to inhibit thrombin with a K_i of 3.0 nM, which compares favorably with literature values.[8–15]

Acknowledgments: The authors would like to thank the laboratory of Dr. Peter Winocour (BioChem Therapeutic, Inc.) for the pharmacological studies.

References and Notes

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(*Received in USA* 2 *May* 1997; *accepted* 15 *May* 1997)