Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2961–2966

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

CONFORMATIONALLY CONSTRAINED NO SYNTHASE INHIBITORS: RIGID ANALOGS OF L-*N*-IMINOETHYLORNITHINE

Jacques Eustache [#] [*], Alfred Grob [!], Charles Lam [!], Odile Sellier [#] and Gerhard Schulz [!].

[#] *Laboratoire de Synthèse Organique et Chimie Microbienne associé au CNRS. Université de Haute-Alsace, Ecole Nationale Supérieure de Chimie de Mulhouse. 3, Rue Alfred Werner, F-68093 Mulhouse cedex (France)*
[!] *Department of Dermatology, Novartis Forschungsinstitut Wien, Brunner Strasse 59, A-1230 Wien (Austria)*

Received 1 July 1998; accepted 23 September 1998

Abstract: The synthesis of eight rigid analogs of L-*N*-iminoethylornithine (L-NIO) is described. The compounds have been evaluated for their inhibition of inducible nitric oxide synthase. Preliminary structure-activity relationships are discussed.

1. Introduction

Since its identification in 1987 as the endothelium-derived relaxing factor (EDRF)[1], nitric oxide (NO) has emerged as an important biological messenger, involved in a wide variety of physiological and pathophysiological processes. In living organisms, NO is formed during the conversion of arginine into citrulline, catalyzed by specific enzymes, the NO synthases (NOS). In view of numerous potential therapeutic applications, the search for potent NOS inhibitors is currently the focus of considerable research activity.

Arginine and NOS inhibitors such as N^G-methylarginine (L-NMA), N^G-nitroarginine or L-*N*-iminoethylornithine (L-NIO) are believed to bind at the enzyme's active site in a similar way. The "bioactive conformation" of these flexible molecules, however, is still unclear, thus complicating the rational design of potent and selective NOS inhibitors. In order to help defining this bioactive conformation, we have synthesized several constrained analogs of L-NIO. The compounds have been examined for their inhibition of RAW cell NOS.

2. Chemistry

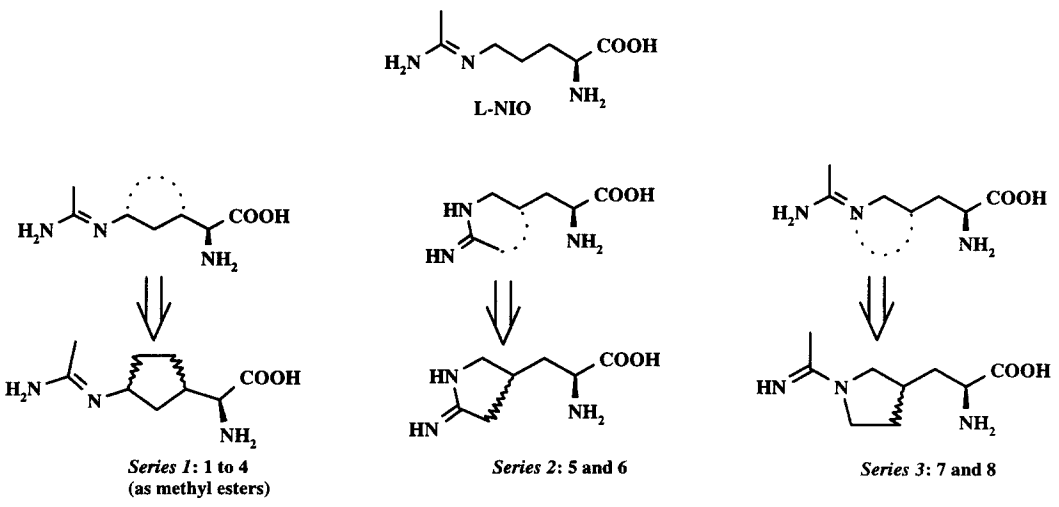
A L- (but not D)-α-aminoacid residue[2], and a basic (guanidino, amidino) group are believed to form the pharmacophore of most arginine-derived NOS inhibitors. At the onset of this program, the spatial relationship between these elements when binding to NOS was unknown, but previous studies had shown that enzyme inhibition was highly sensitive to steric factors.[3] This dictated the choice of our target molecules which had to obey the following criteria:

* *FAX : ..33 3 89 43 77 90 , e-mail : j.eustache@univ-mulhouse.fr*

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00532-0

1. The new analogs were to be basic, L-amino acids,
2. They should be conformationally constrained in order to represent different, minimally overlapping L-NIO conformer families.
3. The bulk inevitably introduced by the chemical modifications had to be kept minimal.

The three series of molecules shown in figure 1 fulfill these requirements and were selected for the study.



Conformationally constrained L-NIO analogs.

Figure 1

The synthesis of compounds **1-2** (figure 2 and scheme 1) started from the known (3*S*) substituted cyclopentanone **9**.[4] Reduction of the carbonyl group in **9** ($NaBH_4$) predominently led to the (1*R*, 3*S*) (cis) substituted cyclopentanol **10**. Cleavage of the pyrazine ring using Schöllkopf's conditions and treatment of the resulting mixture with benzyl chloroformate (CBzCl) afforded, after chromatography, the protected hydroxy-L-aminoacid **11**. Treatment of **11** with *N,N'*-bis(benzyloxycarbonyl)acetamidine (BBA) gave amidine **12**.[5] Hydrogenolysis of the CBz groups yielded the desired methyl ester **1**. Starting from **10**, two successive Mitsunobu reactions, first with *p*-nitrobenzoic acid, then with BBA, gave **2** after hydrogenolysis of the CBz groups.

(3*R*) Cyclopentanone **9'** was prepared from (2*S*)-(-)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-pyrazine, using Schöllkopf's conditions. The sequence of reactions described above for the preparation of **11** led to the hydroxy-D-aminoacid derivative **11'**. After considerable experimentation, we found that, using a 1 M solution of sodium methoxide in methanol, equilibration of **11'** proceeded smoothly to yield a ca. 5 : 3 mixture of protected D- and L-amino acids **11'** and **17** which could be separated by chromatography. Recovered **11'** was recycled and the equilibration procedure was repeated twice to give **17** in 54% yield. Esters **3** and **4** were then prepared following the procedure used for **1** and **2**.[6]

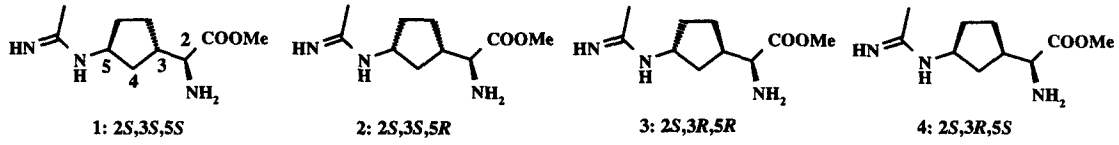
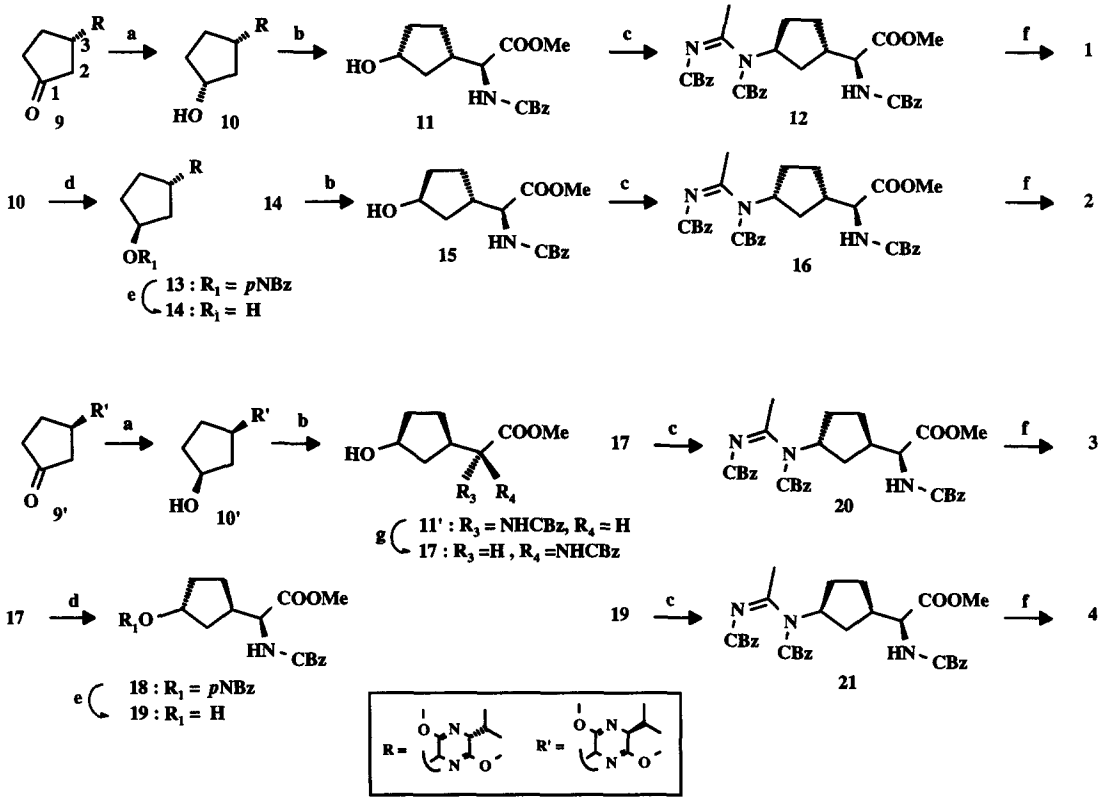


Figure 2

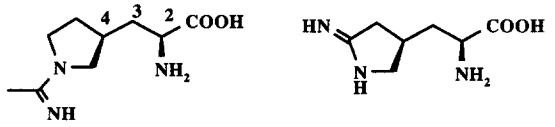


(a) $NaBH_4$, EtOH, r.t., 1 h, 66 % (**10**), 62 % (**10'**); (b) 1: HCl (0.25 N), THF, r.t., 4 h; 2: CBzCl, Et_3N, 0°C→20°C, 1 h, 72% (**11**), 57% (**11'**), 58% (**15**); (c)CBz-N=C(CH_3)NHCBz, PPh_3, DEAD, THF, 0°C→20°C, 1h, 42% (**12**), 41% (**16**), 50% (**20**) or 45% (**21**); (d) *p*-nitrobenzoic acid, PPh_3, DEAD, THF, 0°C→20°C, 1h, 97% (**13**), 92% (**18**); (e) NaOMe, MeOH, r.t, 95 % (**14**), 93% (**19**) ; (f) Pd 10% / C, EtOH, 1 h, 100%; (g) 1M NaOMe, MeOH, r.t., 1 h, 54%.

Scheme 1

We next turned our attention to series 2 and 3. The structural similarity of the target molecules **5** - **8** (figure 3), suggested that they could be derived from common synthetic intermediates. Our synthetic plans involved starting from a readily available L-amino acid derivative - in order to secure the required (2*S*) configuration - and constructing the key pyrrolidine ring found in **5** - **8**. The realization of this strategy is shown in scheme 2. The protected amino acid **22**,[7] was first treated with excess nitromethane. The resulting mixture of Michael adducts was purified by chromatography, using hexanes / diethylether as eluent. Reduction of the nitro group gave the corresponding γ-aminoesters. Brief refluxing in toluene and treatment of the mixture of lactames thus obtained by Lawesson reagent yielded thiolactames **23** and **24**. Ammonolysis of **23** and **24** afforded the corresponding cyclic amidines **25** and **26**.[8] Acidic cleavage of the protective groups in **25** and **26** gave amino acids **5** and **6** respectively.

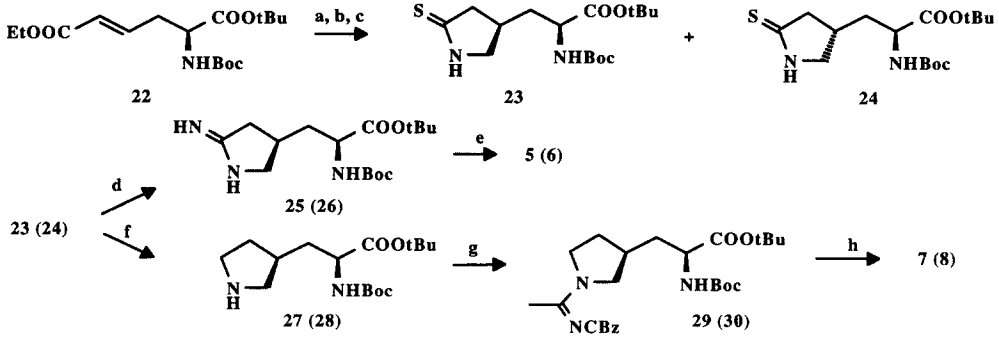
Desulfurization of the thiolactames **23** and **24** by nickel boride [9] yielded the protected cyclic amino acids **27** and **28**. These were readily converted into the amidine derivatives **29** and **30** by treatment with BBA. Sequential hydrogenolysis and acidic treatment provided the target molecules **7** and **8**.



7 (2*S*, 4*S*) (shown here)
8 (2*S*, 4*R*)

5 (2*S*, 4*R*) (shown here)
6 (2*S*, 4*S*)

Figure 3

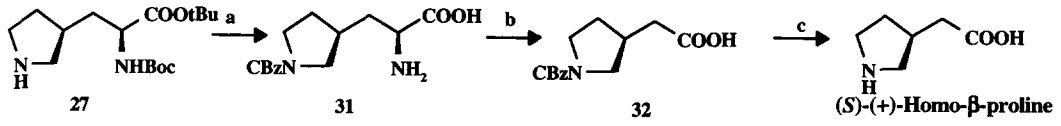


(a) CH_3NO_2, $Bu_4N^+F^-$, THF, -60°C , 10 min, r.t., 2.5 h, chromatography, 79%; (b) 1: Pd 10% / C, $HCOO^-$ NH_4^+, r.t., 0.5 h; 2: toluene, reflux, 3 h; (c) 1: Lawesson's reagent, r.t., 7 h; 2: chromatography, 22% (**23**), 25% (**24**); (d) NH_3, MeOH, r t., 11 days, 26% (**25**), 31% (**26**); (e) HCl (8N) in dioxane, r.t., 2 h, 90% (**5**, **6**); (f) $NiCl_2$, $NaBH_4$, MeOH-THF (1:1), 0°C, 15 min, 88% (**27**), 79% (**28**), (g) CBz-N=C(CH_3)NHCBz, CH_2Cl_2, r.t., 15 min, 86% (**29**), 68% (**30**); (h) 1: H_2, Pd 10% /C; 2: HCl (6N) in dioxane, 4°C, 16 h, 100% (**7**), 72% (**8**).

Scheme 2

Our final task was to determine the stereochemistry at C-4 in **27** and **28**, in order to assess the absolute configuration of compounds **5** - **8**. This was accomplished as shown in scheme 3.

Starting from **27**, protection of the free amino group as the corresponding CBz derivative and removal of the Boc and *tert*-butyl ester groups afforded **31**. Although a variety of methods have been reported for the decarboxylation of amino acids, compound **31** proved to be unusually resistant to most of them. Finally, the desired oxydative decarboxylation could be cleanly effected using silver picolinate[10] to give **32**. Cleavage of the CBz protecting group led to (*S*)-(+)-homo-β-proline[11] thus establishing that **27** had the (2*S*, 4*S*) configuration and allowing the attribution of absolute configurations to compounds **5** - **8**.



(a) CBzCl, Et_3N, THF, r.t., 1 h, 79%; (b) 1: HCl (6M) in dioxane, r.t., 2 h, 90%; 2: silver picolinate, water / benzene 1:1, reflux, 10 min; 3: $KMnO_4$, water / tBuOH, r.t., 5 min, 48%; (c) H_2, Pd 10% / C, EtOH / H_2O, 15 min, 100%.

Scheme 3

3. Biological evaluation

The compounds were evaluated for their ability to inhibit iNOS obtained from RAW 264.7 cells.[12] The results are reported in table 1.[13]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
L-NMA	25 ± 1.4	**4**	>200
L-NIO	9 ± 0.4	**5**	25 ± 1.5
1	50 ± 3.1	**6**	36 ± 2.9
2	47 ± 1.9	**7**	>200
3	>200	**8**	40 ± 3.6

Table 1

Within the first series of L-NIO analogs, **1** and **2** show almost identical levels of NOS inhibition, indicating that, whereas the (3*S*) configuration is required for biological activity, the configuration at C-5 has no importance. Inspection of molecular models shows that it is possible, while superimposing the amino acid moieties in **1** and **2**, to find conformations in which the terminal amidine nitrogens, are also superimposed. The crude model thus obtained cannot readily accomodate **3** and **4** (i.e when allowing the amino acid moieties of **1**, **2**, **3** and **4** to overlap, superimposition of the corresponding amidine nitrogen atoms is not possible). This may suggest that **1** and **2** can

adopt conformations similar to putative "active conformations" of arginine or arginine-derived analogs whereas **3** and **4** cannot. In series 3, only compound **8** with the (4*R*) configuration shows biological activity. Compounds **5** and **6** have similar activities and are the most potent among the new synthetic compounds. This finding is somewhat surprising and may suggest an alternate mode of binding for these two compounds.

At this very early stage, it would be premature to speculate on the detailed structural requirements for biological activity within these series of compounds. However, taken together, the results observed for **1** and **2** and **8**, may be indicative of preferred orientations of the L-NIO amidine moiety when binding to NOS. That none of the active compounds reaches the activity of the parent molecule, L-NIO, is not surprising, taking into account the preliminary character of the present study. Our results, however, suggest directions for further optimisation and may be included in modelling studies aimed at better defining a possible common pharmacophore for arginine and arginine-like inhibitors.

Aknowledgment: We thank the "Fondation de l'Ecole Nationale Supérieure de Chimie de Mulhouse" for a fellowship to Odile Sellier.

References and notes

1. Palmer, R.M.J.: Ferridge, A.G., Moncada, S. *Nature* **1987**, *327*, 524.
2. Few isosteric replacements of the α–aminoacid group have been reported : Hallinan, E.A.: Tsymbalov, S.; Finnegan, P.M.; Moore, W.M.; Jerome, G.M.; Currie, M.G.; Pitzele, B.S. *J. Med. Chem.* **1998**, *41*, 775.
3. For example, the drastic loss of activity observed between N^G-methyl and N^G-butyl arginine. Stuehr, D.J.; Griffith, O.W. *Adv. Enzymol Relat. Areas Mol. Biol.* **1992**, *65*, 287.
4. Schöllkopff, U.; Pettig, D.; Schulze, E.; Klinge, M.; Egert, E.; Benecke, B. and Noltemeyer, M. *Angew. Chem.* **1988**, *100*, 1238.
5. Eustache, J. and Grob, A. *Tetrahedron Lett.* **1995**, *36*, 2045.
6. For enzymic assays, the methyl esters **1-4** were converted to the corresponding free acids by acidic hydrolysis using aqueous HCl (6 M), 30 min.
7. Ramsamy, K.; Olsen, R.K. and Emery, T. *Synthesis* **1982**, *1*, 42.
8. Papandreou, G.; Tong, M.K.; Ganem, B. *J. Am. Chem. Soc.* **1993**, *115*, 11682.
9. Guziec, Jr. F.S.; Wasmund, L.M. *Tetrahedron Lett.* **1990**, *31*, 23.
10. Clarke, T.G.; Hampson, N.A.;.Lee, J.B.; Morley, J.R.; Scanlon, B. *J. Chem. Soc. (C)* **1970**, 815.
11. Nielsen, L.; Brehm, L.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1990**, *33*, 71.
12. Rosenkranz-Weiss, P.; Sessa, W.C.; Milstien, S.; Kaufman, S.; Watson, C.A.; Pober, J.S.. *J. Clin. Invest.* **1994**, *93*, 2236.
13. The IC_{50}s are the mean of 3 independent experiments.