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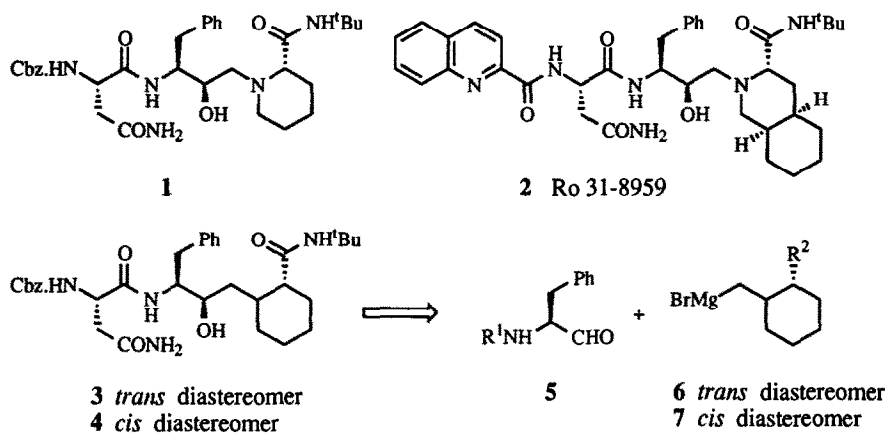
CARBOCYCLIC ANALOGUES OF HYDROXYETHYLAMINE CONTAINING INHIBITORS OF HIV PROTEINASE

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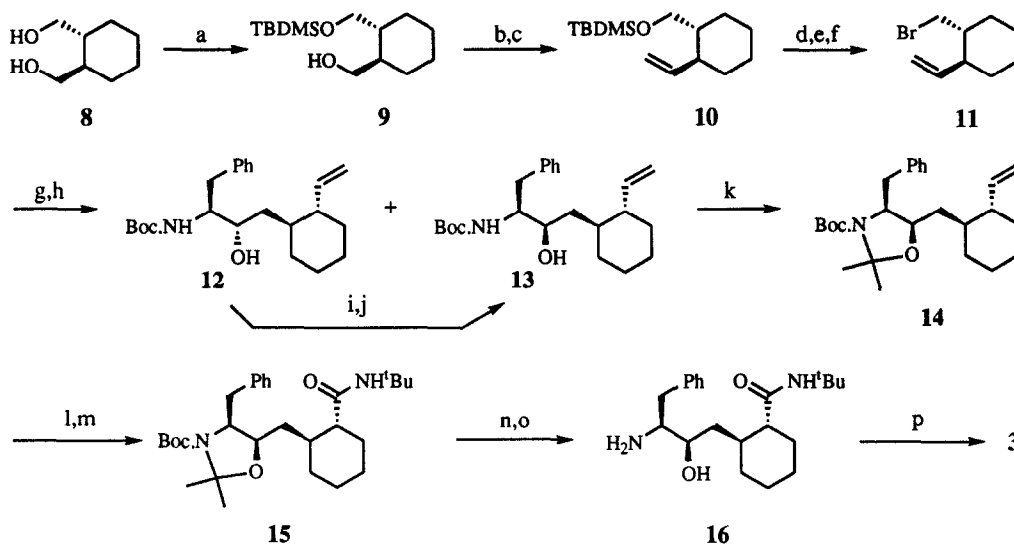
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Abstract: The cyclohexane analogues **3** and **4** of the piperidine carboxamide **1** have been synthesized. The *trans* isomer **3** is more potent than the hydroxyethylamine **1** versus HIV-1 proteinase, while the *cis* isomer **4** is less potent.

Since its discovery as the causative agent of AIDS, a considerable effort has been devoted to the elucidation of the replicative processes of the human immunodeficiency virus (HIV), particularly with the aim of identifying potential therapeutic targets. One of the most thoroughly studied targets is a virally encoded aspartic proteinase which is essential for production of mature infectious virions.¹ Numerous inhibitors of this enzyme have been reported in recent years, and much of this work has been reviewed.²⁻⁷ Most published approaches to the design of inhibitors of HIV proteinase involve the synthesis of mimetics of peptide substrates in which the scissile amide bond is replaced by a non-hydrolysable transition state isostere. We have reported a series of inhibitors based on the Phe-Pro cleavage sequences of HIV proteinase and which contain hydroxyethylamine transition state mimetics.⁸ Optimisation of activity has led to a number of highly potent and selective inhibitors of HIV proteinase which also exhibit potent *in vitro* anti-HIV activity. These include the piperidine **1**, as well as the decahydroisoquinoline analogue **2** (Ro 31-8959) which has become the first inhibitor of HIV-proteinase to enter clinical trials for the treatment of AIDS.



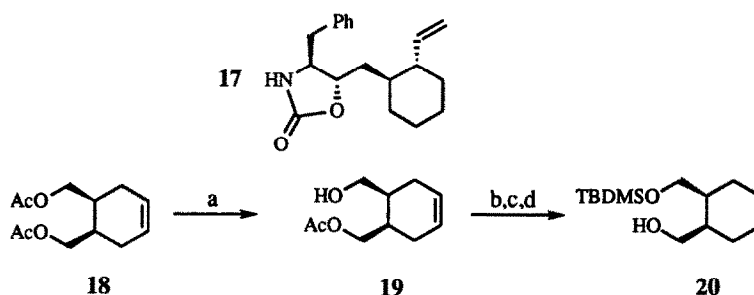
The distance between P₁ and P_{1'} residues is greater in hydroxyethylamines than in substrates or most transition state mimetics. It has been suggested⁹ that the high potency of these inhibitors may be due to the fact that rather than acting as mimetics of the amide hydrate transition state, they mimic the transition state involved in a later, rate-determining step in which a proton is transferred from the amide hydrate to one catalytic Asp residue, while a second proton is transferred from the other catalytic Asp to the departing basic nitrogen. Scission of the carbon-nitrogen bond may have occurred to a considerable extent at this stage. To successfully mimic this later transition state a greater separation of hydroxyl bearing carbon atom and amino group would be necessary. It was therefore of interest to determine whether the presence of a basic nitrogen atom in this position makes a significant contribution to the activity of hydroxyethylamines. Accordingly we wished to synthesize the isosteric carbocyclic analogues **3** and **4** having either *trans*- or *cis*-disubstituted cyclohexane residues instead of the piperidine carboxamide moiety of **1**. In addition, the preferred configuration at the newly introduced carbon atom in **3** and **4** would provide evidence for the binding configuration at the pyramidal nitrogen atom in **1**, which, at the outset of this work, was not known in the absence of any X-ray crystallographic data. We now wish to report the synthesis and HIV-1 proteinase inhibitory activity of the carbocyclic derivatives **3** and **4**.



Reagents: (a) NaH, THF, then TBDMSCl; (b) (COCl)₂, DMSO, CH₂Cl₂; (c) MePh₃PBr, *n*-BuLi, hexane, Et₂O; (d) Bu₄NF, THF; (e) MsCl, pyr.; (f) LiBr, DMF; (g) Mg, THF; (h) Boc.Phe.H, THF; (i) PDC, DMF; (j) NaBH₄, EtOH; (k) Me₂C(OMe)₂, TsOH; (l) KMnO₄, Aliquat 336, C₆H₆, AcOH, H₂O; (m) ^tBuNH₂, DCCI, HOBT, DMF; (n) TsOH, MeOH; (o) HCl, EtOAc; (p) Cbz.Asn.OC₆F₅, dioxan.

Our synthetic strategy was based on reaction of *trans*- or *cis*-disubstituted cyclohexylmethyl Grignard derivatives **6** and **7** with a suitably protected phenylalanine derivative. The *trans*-disubstituted cyclohexane **11** was derived from commercially available *rac*-1,2-cyclohexane-dicarboxylic acid. Resolution as the (+)- α -methylbenzylamine salt¹⁰ and reduction with lithium aluminium hydride gave the *R,R*-diol **8**. Attempted preparation of the monosilyl ether **9** under conventional conditions (1 equiv. TBDMSCl, imidazole, DMF) gave

poor yields, but application of a recently described procedure for monosilylation of diols through prior formation of the monosodium alkoxide salt¹¹ gave **9** in almost quantitative yield. Preliminary studies using cyclohexylmethyl Grignard derivatives which contained a potential carboxamide function in the form of benzyloxymethyl or silyloxymethyl substituents gave discouraging results. We therefore prepared the bromide **11** having a vinyl substituent which could be oxidised to a carboxyl group at a later stage. Reaction of the Grignard derivative of **11** with *N*-Boc.phenylalanine gave the alcohols **12** and **13** in the ratio 73:27. That the major diastereomer was the *S*-alcohol **12** arising from chelation control¹² was shown by cyclisation (^tBuOK, THF, reflux, 50 min) to the 2-oxazolidinone **17**. Vicinal 4- and 5-hydrogens in the ¹H nmr spectrum of **17** showed a coupling constant of 5.9 Hz. *Cis*-disubstituted oxazolidinones show coupling constants of 9–10 Hz, while *trans* isomers have values of 4–6 Hz.^{13,14} In addition, an NOE was observed between the benzylic methylene group and the 5-hydrogen. To obtain the desired *R*-alcohol **13** the *S*-isomer **12** was oxidised to the corresponding ketone which was reduced to give a mixture of **12** and **13** in which **13** predominated (24:76). Protection as the oxazolidine **14** was followed by oxidation of the vinyl group under phase transfer conditions¹⁵ and conversion to the amide **15**. Stepwise removal of protecting groups and coupling with Cbz.asparagine gave the target compound **3**.



Reagents: (a) PPL; (b) TBDMSCl, imidazole DMF; (c) NaOH; (d) H₂, Pd-C

For synthesis of the *cis* diastereomer **4** the homochiral cyclohexene **19** was obtained through hydrolysis of the *meso* diacetate **18** using porcine pancreatic lipase.¹⁶ The silyl ether **20** was subjected to a similar sequence of reactions to that described for **11**. In the case of the *cis*-disubstituted cyclohexane derivatives both the Grignard reaction with Boc.phenylalanine and reduction of the product of oxidation of the minor alcohol occurred with a higher degree of stereoselectivity, giving mixtures of *R* and *S*-alcohols in the ratios 14:86 and 84:16 respectively.

HIV-1 proteinase inhibition and *in vitro* anti-HIV activity were determined for **3** and **4** as previously described⁸ (Table 1). The *trans*-cyclohexane **3** was an order of magnitude more potent than the piperidine **1**, while the *cis* analogue **4** was less potent. As it was known from our previous studies of SAR's that replacement of the Cbz group of these inhibitors by a quinoline-2-carbonyl residue enhances potency, the corresponding *trans*-cyclohexane analogue **21** was synthesized. As expected, this was somewhat more potent than **3**, and was also more potent than the piperidine **22**. In terms of both HIV proteinase inhibitory and antiviral activity **21** was comparable to Ro 31-8959.

These results show that the basic nitrogen atom of these hydroxyethylamines does not contribute to enzyme binding, and that these inhibitors need not therefore be considered as mimetics of the late intermediate on

the reaction pathway. The preference for the *trans* stereochemistry in the cyclohexane ring suggests that the piperidine moiety in **1** also binds in a *trans* diequatorial fashion, the pyramidal nitrogen atom having the *S* configuration. This is the same configuration as was subsequently found in the X-ray crystal structure of the decahydroisoquinoline Ro 31-8959 **2** complexed with HIV-1 proteinase,¹⁷ and is opposite to that found in a hydroxyethylamine having Pro at P₁', and extended as far as P₃'.¹⁸

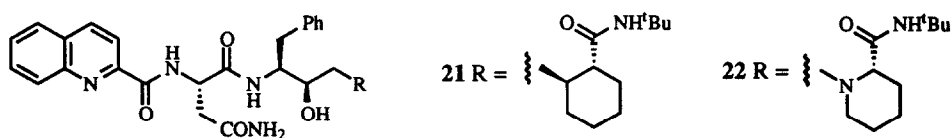


Table 1. HIV Proteinase Inhibition and Antiviral Activity

Compound	HIV Proteinase IC ₅₀ (nM)	Antiviral Activity IC ₅₀ (nM)
1	18	300
3	2.0	40
4	57	1000
21	1.4	4.0
22	2.0	17
2 (Ro 31-8959)	<0.4	2.0

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