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# LEUCINE-PHENYLALANINE DIPEPTIDE-BASED *N*-MESYLOXYSUCCINIMIDES: SYNTHESIS OF ALL FOUR STEREOISOMERS AND THEIR ASSAY AGAINST SERINE PROTEASES

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***Abstract:*** The four stereoisomers of 3-(*N*-acetylleucylamino)-3-benzyl-1-[(methylsulfonyl)oxy]succinimide have been prepared and shown to inhibit α-chymotrypsin and human neutrophil elastase. The structural and stereochemical features that affect the potency and selectivity of inhibition are discussed. 

A mechanism-based inactivator of an enzyme is unique in that it contains a built-in molecular switch that is activated by the normal catalytic action of the enzyme. This process releases latent reactivity within the active site to give specific and irreversible inactivation of the enzyme.[1,2] Serine proteases have proven to be a particularly popular target for many studies in this area, partly because the molecular details of their mechanism of action are so well understood,[3] but also since an over activity of some examples has been implicated in pathological conditions including emphysema, tumour invasion, and arthritis.[3] Many of the published mechanism-based inhibitors of serine proteases are simple amino acid mimetics with little peptidic character (e.g., **1**) which are potent against a number of proteases.[4] The inactivation of serine proteases by compounds of the type **1** is initiated by enzyme catalysed cleavage of the N-C2 imide bond. This is followed by a Lossen rearrangement (switch on) to liberate a highly electrophilic and enzyme bound isocyanate.[4] This isocyanate then acylates an available nucleophilic amino acid in the enzyme active site to give irreversible inactivation of the enzyme. We chose to target some amino acid-derived examples of **1**, which would allow their incorporation into peptide sequences specific for a given protease. The idea was to gain an insight into those structural features that contribute to potency and selectivity of inhibition rather than produce therapeutic agents. Recently, we reported a synthesis of the (*S*)-phenylalanine-based *N*-mesyloxysuccinimides **14** and **16**.[5] We now report the synthesis of their enantiomers **10** and **13** and results of the assay of all four stereoisomers, and the related compounds **15**[6] and **17**,[5] against α-chymotrypsin and human neutrophil elastase.

The key to the synthesis of **10** and **13** required the preparation and cyclisation of **5** to give the *N*-benzyloxysuccinimide **6** (Scheme 1). The absolute configuration of **5** was established using oxazolidinone chemistry pioneered by Seebach et al.[7] To this end, the *syn*-oxazolidinone **2**, derived from (*R*)-phenylalanine, was deprotonated with lithium hexamethyldisilazide (LiHMDS) and the resulting C4 anion was alkylated to give **3**. Deprotection and coupling with *O*-benzylhydroxylamine, in the presence of dicyclohexylcarbodiimide (DCC) and hydroxybenztriazole monohydrate (HOBT), gave **5**. Treatment with triethylamine at room temperature then gave the succinimide **6** which was *N*-deprotected to give the key free amine **7**.[8] Separate

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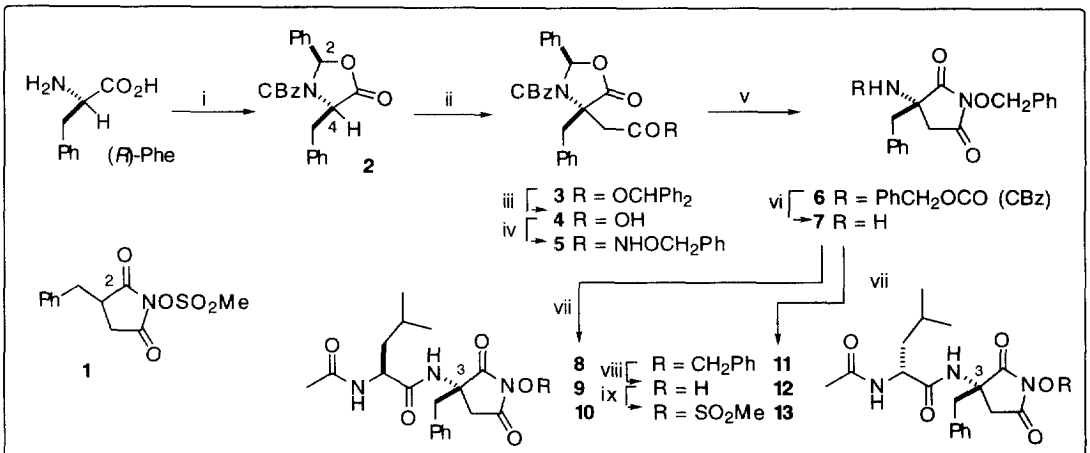
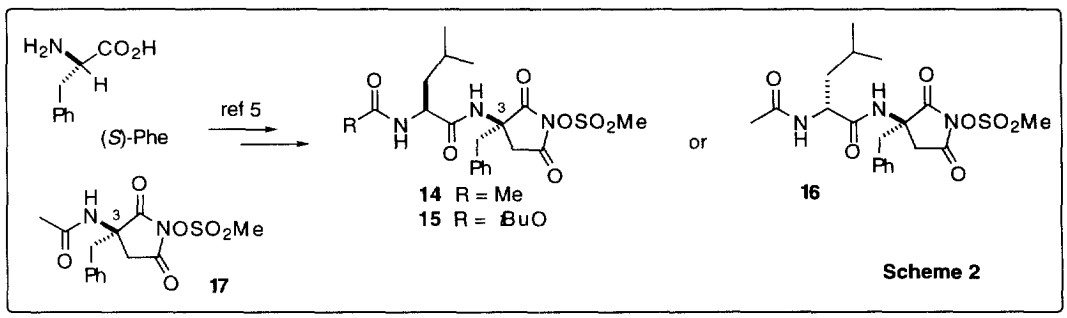



**Scheme 1.** *Reagents and conditions:* (i) NaOH, PhCHO; then CBzCl, -20 °C to 4 °C; (ii) LiHMDS, THF, -78 °C; then $BrCH_2CO_2CHPh_2$; (iii) TFA, $CH_2Cl_2$, 0 °C; (iv) $NH_2OCH_2Ph$, $Et_3N$, DCC, HOBT, $CH_2Cl_2$; (v) $Et_3N$, $CH_2Cl_2$, rt; (vi) PTSA, toluene, reflux; (vii) DCC, HOBT, $CH_2Cl_2$, *N*-acetyl-(*S*)-Leu (**8**) or *N*-acetyl-(*R*)-Leu (**11**); (viii) 10% Pd on C, $H_2$; (ix) $iPr_2EtN$, $MeSO_2Cl$, $CH_2Cl_2$, 0 °C

samples of **7** were coupled with (*S*)- and (*R*)-*N*-acetyl-leucine, in the presence of DCC and HOBT, to give **8** and **11**, respectively. A final sequence involving debenzylation, followed by reaction with mesyl chloride, gave the desired peptidomimetics **10** and **13**,[9] respectively. The stereoisomers **14** and **16** were prepared similarly from (*S*)-phenylalanine[5] and compounds **15**[6] and **17**[5] were prepared using related chemistry (Scheme 2).




**Scheme 2**

## Inhibition of serine proteases

Compounds **10**, **13**, **14**, and **15** gave time-dependent inhibition of α-chymotrypsin. Importantly, a lag period was not observed in the inhibition studies which is consistent with the *N*-(mesyloxy)succinimide, rather than a hydrolysis product, being the active species. In addition, no significant recovery of enzyme activity was observed with dialysis of the assay samples against phosphate buffer (pH 7.2) after more than 20 hours at 4 °C. These observations are consistent with irreversible inhibition of α-chymotrypsin where it is likely[4] that the C2 (rather than C5) carbonyl of the succinimides of the type **1**, **10**, and **13** is hydrolysed by the enzyme as the initial step in its inactivation. This carbonyl group is the mimic of the scissile peptide bond in the normal substrate.

The $k_{obs}$/[I] values were determined[10] for each of the inhibitors of α-chymotrypsin while the inhibition of human neutrophil elastase was determined simply as a percentage of activity remaining after 30 min incubation of the inhibitor with the enzyme (see Table 1). From these results it is clear that the C3 configuration of the inhibitor, which is defined by the phenylalanine used in its synthesis, has an important influence on the relative potency against α-chymotrypsin and human neutrophil elastase. Compounds with the (*R*)-configuration at C3 (**14-17**) were the most potent against α-chymotrypsin. An (*R*)-configuration at this centre[11] is analogous to that of natural (*S*)-phenylalanine which is found in substrates of α-chymotrypsin. Of these compounds, **16** is the most potent with a $k_{obs}$/[I] value of 244. This compares to a reported[4] value of 9000 for the simple analogue **1**, where both the (*R*)- and (*S*)-enantiomers are equally potent.[12] Compounds **1** are also very potent inhibitors of human neutrophil elastase such that they are general inhibitors of serine proteases. Interestingly, those compounds with the alternative (*S*)-configuration at C3 (**10** and **13**) were the least potent against α-chymotrypsin but the most potent against human neutrophil elastase (Table 1). Work is in progress to further understand the important observation that selectivity for α-chymotrypsin vs human neutrophil elastase is influenced by the configuration of the P1 amino acid.

A comparison of the inhibitory data for compounds **14-17** (Table 1) gives a measure of the importance of the P2 substituent[13] (i.e., the group attached to the *N*-terminus of the phenylalanine) since all these compounds have the same (*R*)-configuration at C3. Leucine was chosen at P2 since it is known to bind favourably to α-chymotrypsin.[14] Perhaps unexpectedly, those compounds containing natural (*S*)-leucine (**14** and **15**) were less potent against α-chymotrypsin than **16**, which has non-natural (*R*)-leucine at the P2 position. The *N-tert*-butoxycarbonyl (BOC) and *N*-acetyl compounds **14** and **15** show similar activity against α-chymotrypsin. Compound **17**, which lacks an amino acid at P2, is slightly more activity against α-chymotrypsin. The effect of the P2 residue on activity against human neutrophil elastase is less obvious where it should be noted that leucine is not a preferred amino acid at this position.

**Table 1.** Inhibition of α-chymotrypsin and human neutrophil elastase

| Compound | Configuration C3 | Leu | Enzyme inhibition α-chymotrypsin $k_{obs}$/[I][a] ($M^{-1}s^{-1}$) | elastase[b] |
|---|---|---|---|---|
| **10**[c] | S | S | 28 | 24% |
| **13**[c] | S | R | 9 | 6% |
| **14**[d] | R | S | 170 | 63% |
| **15**[d] | R | S | 180 | 48% |
| **16**[d] | R | R | 244 | 72% |
| **17**[d] | R | - | 217 | 56% |

[a] determined by the incubation method, the higher the number the more potent the compound[10]
[b] activity remaining after 30 min incubation, the lower the number the more potent the compound
[c] derived from (*R*)-Phe; [d] derived from (*S*)-Phe

In summary, although the peptidomimetics **10** and **13-17** are weaker inhibitors of α-chymotrypsin than the simple analogues **1** (presumably since C3 is fully substituted) they do show comparable activity to other reported mechanism-based inhibitors of α-chymotrypsin.[15] The configuration of the peptidic sequence in

our compounds has been shown to influence their potency and also their selectivity for one serine protease over another. Further work is in progress to understand all these preliminary observations. It should be noted that inhibition data are dependant on the exact conditions of the assay and direct comparisons with literature data are problematic.

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8. The configuration of **7** is defined by the absolute configuration at C2 of **2**, i.e. this centre governs which face of the planar C4 enolate is alkylated in step ii of Scheme 1 to give **3** and hence **6** and **7**. The C2 configuration of **2** is, in turn, determined by the configuration of the phenylalanine used in its preparation.[5b,7]
9. Compounds **10** and **13**, and their synthetic intermediates, gave specific rotations of comparable magnitude but opposite in sign to their reported[5] enantiomers. It is noteworthy that the chemical shifts and multiplicity of many of the resonances in the $^1$H NMR spectra of **8-13** were dependent on the concentration of the sample used in the analysis.
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11. The benzyl group of the phenylalanine-based imide ring is assumed to bind in the S1 binding pocket of α-chymotrypsin, which is known to prefer aromatic amino acids at this P1[13] position.[14]
12. It is also well documented that the greater the substitution on a cyclic compound (e.g. as in a succinimide) the less prone it is to ring-opening (see Allinger, N. L.; Zalkow, V. *J. Org. Chem.* **1960**, *25*, 701). On this basis, at least, it might be expected that compounds of the type **10** and **13** would be less susceptible than **1** to the ring-opening step required to give enzyme inactivation.
13. For a definition of this nomenclature see: Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
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