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Dipeptide vinyl sultams: Synthesis via the Wittig–Horner reaction and activity against papain, falcipain-2 and *Plasmodium falciparum*

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Abstract—The synthesis of phosphonate derivatives of *N*-phenyl- and *N*-benzyl-γ- and δ-sultams, and their application in the Wittig-Horner reaction with *N*-Boc-L-phenylalanine aldehyde to afford *E*- and *Z*-isomers, are described. These compounds were further processed to provide five dipeptide vinyl sultams, which were found to be inactive against papain at concentrations up to 50 μM. In contrast, vinyl sultams demonstrated weak activity against recombinant falcipain-2 and *Plasmodium falciparum* W2.

Malaria is the major life-threatening parasitic disease in tropical and sub-tropical regions. Worldwide, there are at least 300 million acute cases of malaria and more than 1 million deaths each year, mostly young children infected with *Plasmodium falciparum*.[1] With the rapid spread of multidrug-resistant *P. falciparum* strains, the development of safe and effective antimalarials has become an important strategy towards achieving effective control of malaria. Cysteine proteases regulate a broad spectrum of physiological functions in mammals, parasitic protozoa, plants and yeast. In humans, elevated levels of these enzymes can lead to disease states such as osteoporosis, rheumatoid arthritis and cancer.[2,3] In parasites, they play a crucial role in metabolism and reproductive function.[2,3] Falcipain-2 is a cysteine protease stored in the acidic food vacuole of *P. falciparum* and is likely involved in the hydrolysis of haemoglobin that produces free amino acids required for parasite survival.[4–6] Disruption of this haemoglobin degradation pathway is lethal to the parasite and thus compounds designed to inhibit falcipain-2 present excellent opportunities for developing antimalarial drug candidates.

Vinyl sulfones (VS) and their analogues, such as sulfonamides and sulfonates, have been reported as a promising class of inhibitors for parasitic cysteine proteases. They act by irreversibly alkylating the active site cysteine residue via conjugate addition.[2,3,7]

Developing conformationally restricted analogues is a commonly used approach to probe the binding site of receptors and to further improve activity.[8] Despite the recent finding that cyclic vinyl sulfones derived from the Bsmoc amino-protecting group are irreversible inhibitors of papain and cathepsin B,[9] the design of conformationally restricted analogues of sulfones as cysteine protease inhibitors is still an underexplored field. In this context, we now report the synthesis of the vinyl sultam scaffold as a cyclic isostere of vinyl sulfonamides, and thus as a building block for a new family of irreversible cysteine protease inhibitors. Vinyl sultams containing an exocyclic double bond are also structurally similar to α-methylene lactones, which are known Michael acceptors for thiols.[10,11] With the aim of designing effective inhibitors for falcipain-2, the vinyl sultams were attached to a peptide sequence. The chosen sequence was Mu-Leu-Phe (Mu = 4-morpholinecarbonyl), by analogy with the vinyl sulfone Mu-Leu-Hph-VS-Ph, which was considered an optimal inhibitor for this enzyme (IC_{50} 0.003 μM).[2] A vinyl sultam with the Ac-Phe-Phe moiety was also prepared based on the success of the vinyl sulfones Cbz-Phe-Hph-VS-R against cruzain, a related cysteine protease from *Trypanosoma*

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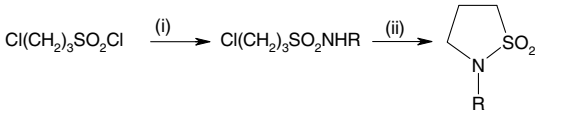
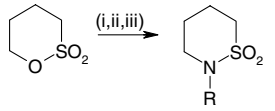
cruzi.[2] The sultam nitrogen atom carried either a benzyl or phenyl group. The evaluation of the target compounds against falcipain-2, papain and the chloroquine-resistant *P. falciparum* strain W2 is also reported.

Synthesis involved the preparation of the γ- and δ-sultam cores **1** and **2** (Schemes 1 and 2), using appropriate modifications of previously described methods.[12,13] Incorporation of the peptide moiety proceeded according to the general sequence reported for the analogous vinyl sulfones,[14] which involve as the key step a Wittig-Horner reaction (Scheme 3). This method has been widely applied to the synthesis of α,β-unsaturated sulfonate derivatives,[6,14,15] but to the best of our knowledge, not to sultams; reported synthesis of vinyl derivatives of the latter involves: (i) the direct coupling by an aldol-like reaction, followed by dehydration[16] and (ii) the intramolecular Heck reaction.[17] In order to follow the Wittig-Horner approach, the anion of **2a** was generated (2 equiv *n*-BuLi, dry THF, rt, N_2) and allowed to react with diethyl chlorophosphate to afford compound **4a** (method A).[15,18] However, when this procedure was applied to the other sultams it furnished either decomposition or poor yields (Table 1). For these reasons, conditions B and C were investigated. In general, we found LDA at −78 °C to afford higher yields of the sultam phosphonates than BuLi either at −78 °C or room temperature. Phosphonates **3** and **4** were condensed with *N*-Boc-L-phenylalanine aldehyde (**5**), prepared from *N*-Boc-L-phenylalanine via formation of the *N*,*O*-dimethylhydroxamate.[19] The reaction provided *E*- and *Z*-vinyl sultams **6**–**9** (Table 2). The *E*/*Z* ratio is consistent with the general observation that phosphonates with alkyl groups give mostly the thermodynamically more stable *E*-olefins.[20,21]



1a R = Ph, 49% (2 steps)
1b R = Bz, 79% (2 steps)

Scheme 1. Reagents and conditions: (i) $PhNH_2$, TEA, DCM, reflux, 1 h or $BzNH_2$, DCM, −10 °C, 1 h; (ii) NaOH, TEA, MeOH, reflux.



2a R = Ph, 43% (3 steps)
2b R = Bz, 44% (3 steps)

Scheme 2. Reagents and conditions: (i) RNH_2, 100 °C; (ii) $POCl_3$, reflux; (iii) K_2CO_3, AcOEt, rt.

The geometry of the double bond comes from the examination of the chemical shifts of vinyl and methine protons (Table 3). This process has been reported as a reliable criterion for stereochemical assignment of α,β-unsaturated sulfonates:[15] as a result of the deshielding effect of the sulfonyl group, the vinyl proton resonance

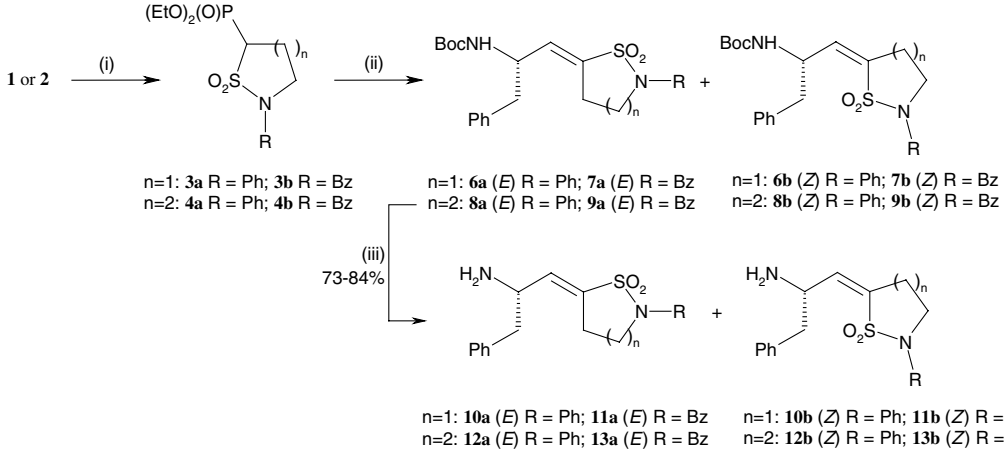
Table 1. Conditions for the synthesis of sultam phosphonates **3** and **4**

Phosphonate	Yield (%)		
	Method A	Method B	Method C
3a	27	56	65
3b	0	—	43
4a	48	—	45
4b	15	—	34

Method A: *n*-BuLi, dry THF, rt, N_2; method B: *n*-BuLi, dry THF, −78 °C, N_2; method C: LDA, dry THF, −78 °C, N_2.

Table 2. Yields and ratio of *E*/*Z* isomers

Phosphonate	Product	*E*/*Z*	Yield (%)	*E*/*Z* ratio
3a	**6a**	*E*	60	79:21
	6b	*Z*	16	
3b	**7a**	*E*	58	67:33
	7b	*Z*	29	
4a	**8a**	*E*	65	90:10
	8b	*Z*	7	
4b	**9a**	*E*	54	86:14
	9b	Z	9	



n=1: **3a** R = Ph; **3b** R = Bz
n=2: **4a** R = Ph; **4b** R = Bz

n=1: **6a** (*E*) R = Ph; **7a** (*E*) R = Bz
n=2: **8a** (*E*) R = Ph; **9a** (*E*) R = Bz

n=1: **6b** (*Z*) R = Ph; **7b** (*Z*) R = Bz
n=2: **8b** (*Z*) R = Ph; **9b** (*Z*) R = Bz

n=1: **10a** (*E*) R = Ph; **11a** (*E*) R = Bz
n=2: **12a** (*E*) R = Ph; **13a** (*E*) R = Bz

n=1: **10b** (*Z*) R = Ph; **11b** (*Z*) R = Bz
n=2: **12b** (*Z*) R = Ph; **13b** (*Z*) R = Bz

Scheme 3. Reagents and conditions: (i) LDA, THF, −78 °C, $(EtO)_2POCl$, N_2; (ii) *n*-BuLi, THF, Boc-Phe-CHO (**5**), −78 °C to rt, N_2; (iii) TFA 50% (w/v DCM solution), rt, 20 min.