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Solution and Solid-Phase Synthesis of Potent Inhibitors of Hepatitis C Virus NS3 Proteinase

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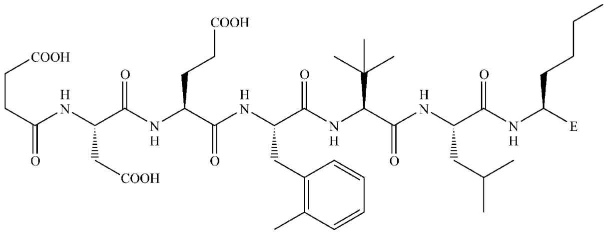
Abstract—A versatile route for the synthesis of homochiral α-ketoamide analogues of amino acids is described. Incorporation of this functionality into peptide sequences using either solution or solid-phase chemistry resulted in potent inhibitors of the Hepatitis C Virus NS3 proteinase. © 2002 Elsevier Science Ltd. All rights reserved.

Hepatitis C Virus (HCV) is the cause of the majority of cases of transfusion-associated hepatitis and a significant proportion of cases of community-acquired hepatitis worldwide. Infection by HCV frequently leads to persistent infections that result in a range of clinical conditions including an asymptomatic carrier state, severe chronic active hepatitis, cirrhosis and, in some cases, hepatocellular carcinoma.[1] The viral proteinase derived from the NS3 gene has proved a popular target for drug discovery programmes in this area[2,3] and indeed work in these laboratories, amongst others, has identified potent inhibitors based on the NS4A-4B cleavage site of the proteinase, for example, **1**–**3**.[4,5]



1. E=CHO
2. E=B(OH)$_2$
3. E=COCONHR

The development of α-ketoamide based inhibitors enabled exploration of space occupied by the prime-side residues of the substrate and resulted in inhibitors with significantly higher potency than had been previously observed. Our interest in α-ketoamides extended beyond those derived from readily available α-amino acids and therefore we sought to develop a simple synthesis of this functionality which did not require pre-synthesis of the amino acid. We also required a route which could be relied upon to introduce the stereochemistry in a controlled manner, would be sufficiently flexible to allow introduction of a variety of groups in either the N- or C-terminal directions and which could be adapted for use on solid support.

We were attracted by the work of Davies et al.,[6] who had devised a stereospecific route to β-amino-α-hydroxyesters and we modified this chemistry to suit our needs. Thus, Michael addition of lithium (*S*)-*N*-benzyl-α-methylbenzylamide to *tert*-butyl hept-2-enoate **4** followed by quenching of the resultant enolate with the Davis (1*S*)-(+)-(10-camphorsulphonyl)oxaziridine introduced all the required functionality and set up the required stereocentre[7] in one pot. Hydrogenolysis then afforded **5** which could be functionalised on either the N- or C-terminus. For example, cleavage of the *tert*-butyl ester followed by *N*-protection produced β-amino-α-hydroxyacid **6**. This was readily coupled to a variety of amines (exemplified here (Scheme 1) with (*S*)-(−)-1-phenylpropylamine) and the hydroxyl group then oxidised using Dess–Martin periodinane.[8] Thus, the desired α-ketoamide functionality was introduced without epimerisation of the sensitive adjacent chiral centre. Removal of the *N*-*tert*-butyloxycarbonyl protecting

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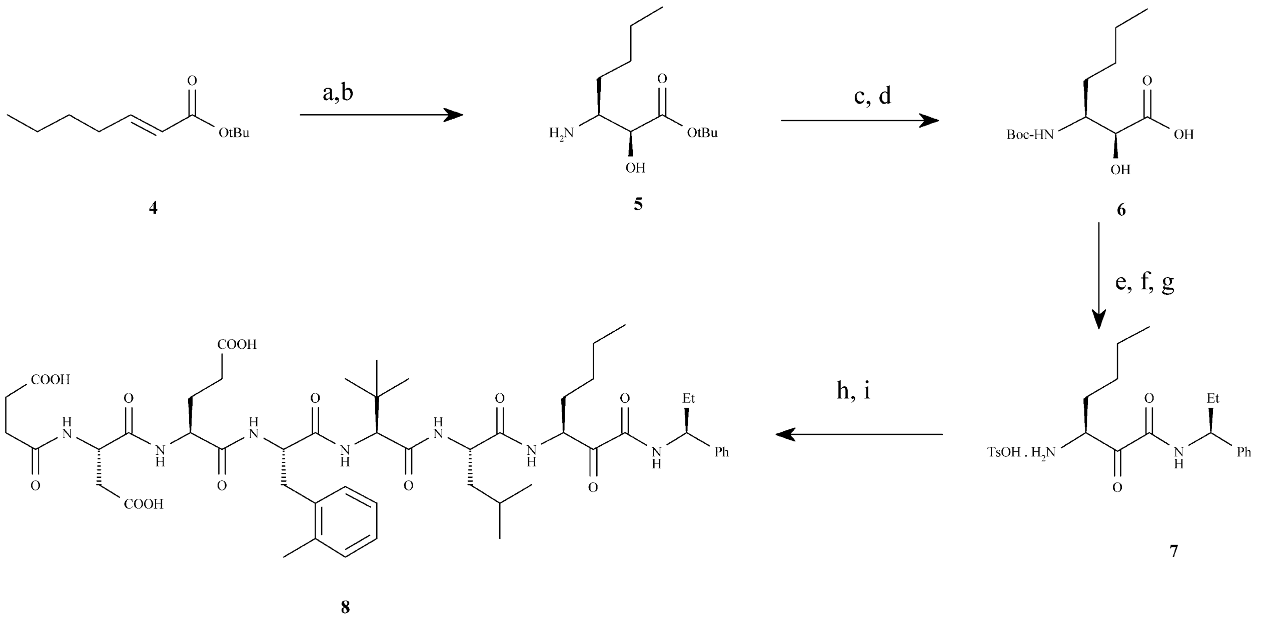
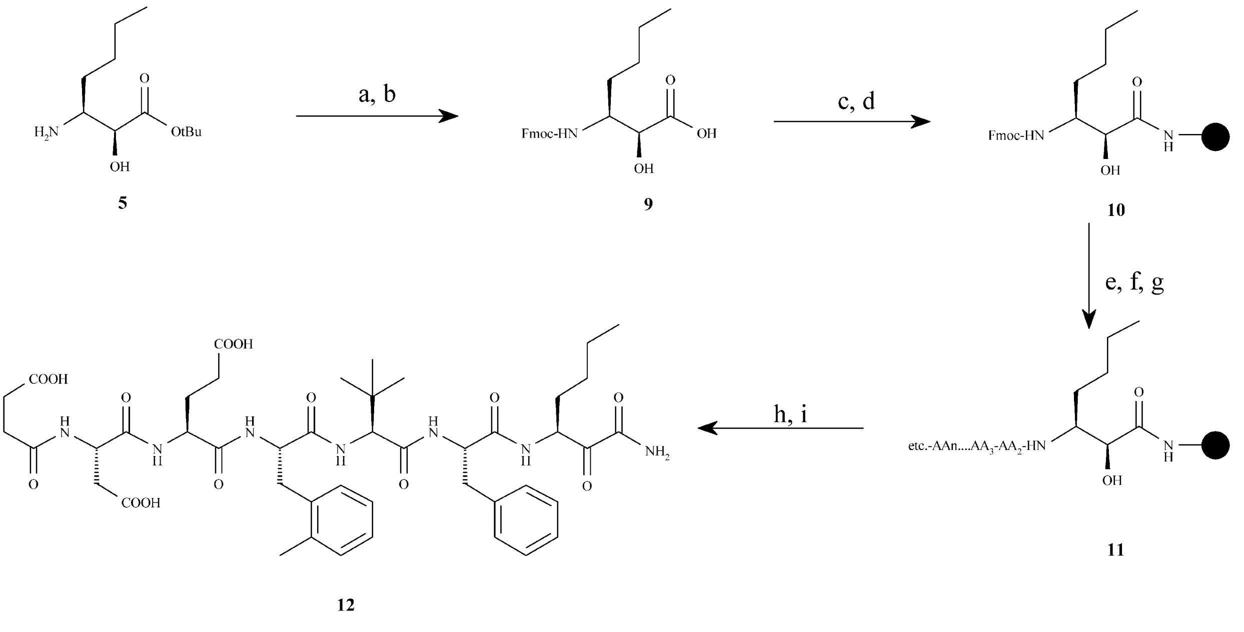


Scheme 1. (a) (i) (*S*)-*N*-Benzyl-α-methylbenzylamine, *n*-BuLi, THF, 0 °C, 45 min; (ii) add **4**, −78 °C, 2 h; (iii) (1*S*)-(+)-(10-camphorsulphonyl)oxaziridine, −78 °C, 1 h, 85%; (b) (i) H_2, Pd/C, 0.5 MPa, AcOH, 16 h; (ii) $NaHCO_3$ wash, 98%; (c) TFA, 30 min, 70%; (d) $(Boc)_2O$, $NaHCO_3$, H_2O, dioxan, 16 h, 100%; (e) (*S*)-(−)-1-phenylpropylamine, HOBt, $Me_2N(CH_2)_3NCNEt{\cdot}HCl$, CH_2Cl_2, 3 h, 76%; (f) Dess–Martin periodinane, CH_2Cl_2, 30 min, 70%; (g) TsOH; MeCN, 20 min; (h) Succ(O^tBu)-Asp(O^tBu)-Glu(O^tBu)-Phe(2-Me)-Val(3-Me)-Leu-OH, HOBt, $Me_2N(CH_2)_3NCNEt{\cdot}HCl$, NEM, CH_2Cl_2, 16 h, 32% over steps g and h; (i) TFA, 30 min, 47%.



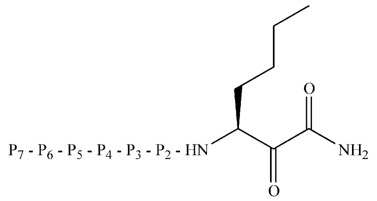
Scheme 2. (a) Fmoc-NSu, $NaHCO_3$, pH 9–10, water, dioxan; (b) TFA, CH_2Cl_2 (1:1), 30 min, 61% over steps a and b; (c) RINK amide MBHA resin, TBTU, NEM, DMF, 1 h; (d) AcOH, TBTU, NEM, DMF, 1 h; (e) piperidine, DMF (1:4), 5 min; (f) Fmoc-amino acid, TBTU, NEM, DMF, 1 h; (g) repeat steps e and f as required; (h) Dess–Martin periodinane, CH_2Cl_2, 1 h; (i) TFA, water (19:1), 10 min.

group, coupling to the C-terminus of a peptide (exemplified here with a protected pentapeptide) followed by global deprotection with TFA afforded the desired potent inhibitor **8** (IC_{50} 11 nM).[9]

This route allowed rapid access to peptides containing α-ketoamides and was particularly useful if the peptides required for coupling were readily available. However, we required a more flexible route to allow straightforward modification of the peptide residues. Solid-phase technology seemed particularly suited to our needs.[10]

Hydroxyester **5** was converted to hydroxyacid **9** which was then coupled to RINK amide MBHA resin[11] using TBTU in DMF. Any unfunctionalised linker amines were then capped as their acetamides. Removal of the

Table 1.



P_7	P_6	P_5	P_4	P_3	P_2	Yield/20 mg resin (mg)	Purity (%)	IC_{50} (nM)
Succ	Asp	Glu	Phe(2-Me)	Val(3-Me)	Ala(3-thienyl)	3.9	80	130
Succ	Asp	Glu	Phe(2-Me)	Gly(Phe)	Leu	5.1	90	42
Succ	Asp	Glu	Tyr(O-Bn)	Val(3-Me)	Leu	3.9	80	110
Succ	Asp	D-Val	Phe(2-Me)	Val(3-Me)	Leu	3.9	90	20
Succ	Asp	D-Ser(OBn)	Phe(2-Me)	Val(3-Me)	Leu	3.7	70	29
Succ	Trp	Glu	Phe(2-Me)	Val(3-Me)	Leu	2.6	80	41
(2-naphthyl)-Ac	Asp	Glu	Phe(2-Me)	Val(3-Me)	Leu	2.3	80	90

Fmoc protecting group produced a resin loading of 0.34 mmol/g as judged by quantitation of the released dibenzofulvene at 301 nm. The free amine could then be functionalised using routine solid-phase peptide chemistry[12] (exemplified here (Scheme 2) using Fmoc-L-phenylalanine, Fmoc-3-methyl-L-valine, Fmoc-2-methyl-L-phenylalanine, Fmoc-*O*-*tert*-butyl-L-α-glutamic acid, Fmoc-*O*-*tert*-butyl-L-α-aspartic acid and *tert*-butyl hydrogen succinate). Dess–Martin oxidation introduced the keto-functionality and subsequent TFA mediated cleavage from the resin and concomitant global deprotection afforded the α-ketoamide inhibitor as a white solid.

Over 130 compounds were synthesised employing this route using an Advanced ChemTech 496 synthesiser. Typically, 20 mg of resin afforded 2–4 mg of product with a purity of $>70\%$ as judged by LC-MS. Examples are shown in Table 1 together with their inhibitory activities.

The synthetic routes described here provided rapid access to a range of structurally diverse peptide based α-ketoamides. The central β-amino-α-hydroxyester moiety was introduced in one synthetic step and was readily elaborated either in solution or on solid support. The resultant products are inhibitors of the HCV NS3 proteinase and they demonstrated a range of activities which allowed the structure–activity relationships to be rapidly established for this series.

Acknowledgements

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