The Synthesis of Conformationally Constrained Peptides and *Pseudo*-Peptides Incorporating an *Endo*-(2*S*, 3*R*)-2-Amino-3-Carboxy-Norborn-5-ene Residue

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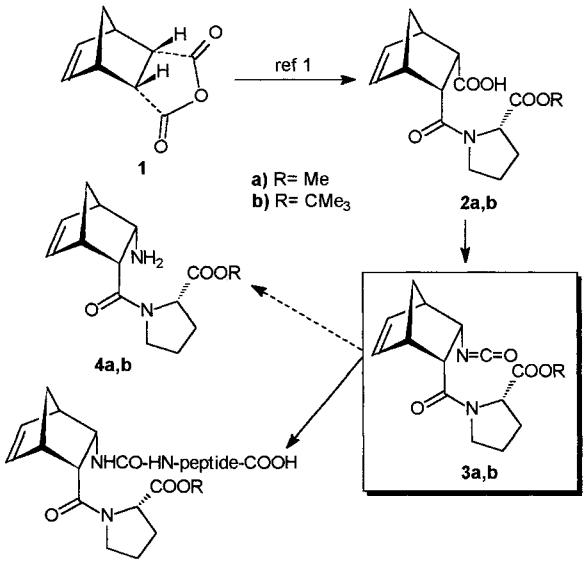
Abstract: Methodology has been developed to carry out a Curtius rearrangement on enantiomerically pure amido-acids derived from (*S*)-proline esters. The resulting isocyanates were used to prepare conformationally constrained peptides incorporating an *endo*-(2*S*, 3*R*)-2-amino-3-carboxy-norborn-5-ene residue.

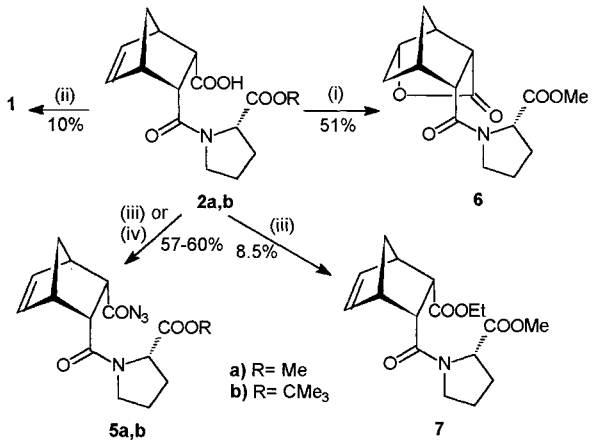
In recent publications[1], we have reported the facile desymmetrisation of *meso*-anhydrides utilising methyl (*S*)-prolinate as a chiral reagent. In this communication we report the application of this methodology to the synthesis of conformationally constrained *pseudo*-peptides starting from *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride **1** as outlined in Scheme 1. There is currently much interest in the synthesis and applications of 2-amino-3-carboxy-norborn-5-ene derivatives. Racemic syntheses of both the *endo*- and *exo-cis* isomers of this β-amino acid have been reported[2-5], along with methodology for their resolution[5]. An asymmetric synthesis of the *trans*-isomer based upon a chiral auxiliary controlled Diels-Alder reaction has also been reported[6], though no asymmetric synthesis of the *cis*-diastereomers has been described. The applications of this amino acid include their use as a turn inducer in synthetic peptides, and its use in the preparation of scaffolding for combinatorial peptide synthesis[3].

The synthetic approach we envisaged for this work involved the stereospecific conversion of the carboxylic acid functionality of compounds **2** into the corresponding isocyanates **3** *via* a Curtius[7] or related rearrangement. Isocyanates **3** would then be trapped with a peptide to give a *pseudo*-peptide incorporating a urea unit, or hydrolysed to the corresponding amines **4** which could be incorporated into peptides using the amine and masked acid functionalities.



Scheme 1

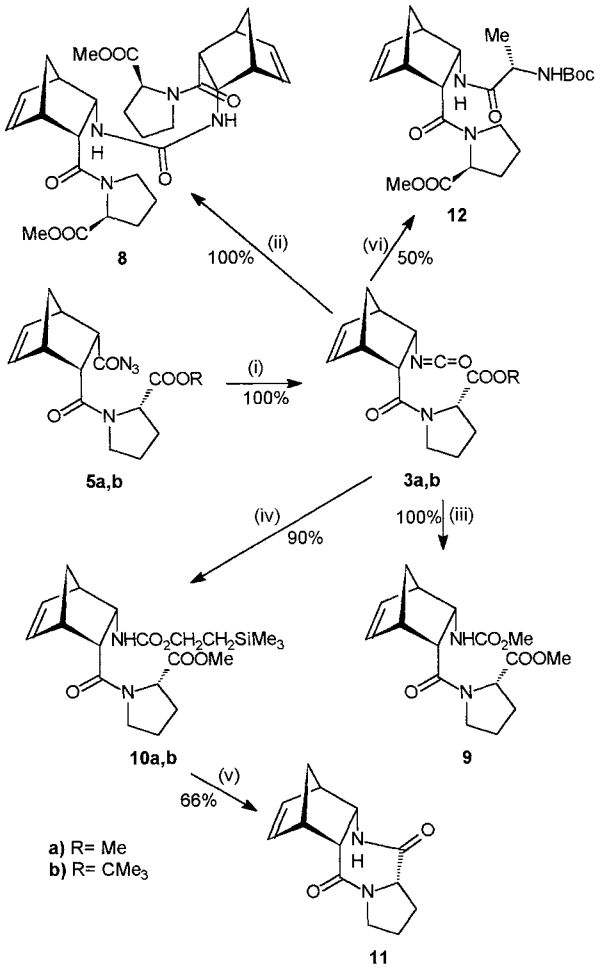
The conversion of acid **2a** into the corresponding acyl azide **5a** proved to be far from straight forward, and attempts to accomplish this transformation under classical conditions all proved unsuccessful. In particular, treatment of acid **2a** with conc. sulphuric acid and sodium azide[8] predictably gave lactone **6** (Scheme 2), whilst attempted formation *via* the acid chloride[9] (using thionyl chloride or oxalyl chloride) gave only anhydride **1**. Activation of the acid functionality *via* a mixed anhydride was more successful, with ethyl chloroformate / sodium azide[2,6,10] giving the desired acyl azide[11] **5a** in 60% yield, but always contaminated with 8.5% of the corresponding ethyl ester **7**. Reasoning that ester **7** was formed by attack of ethanol (liberated from the mixed anhydride) upon acyl azide **5a**, we investigated the use of alternative chloroformates. The more sterically hindered isobutyl chloroformate gave no better results, but gratifyingly the use of isopropenyl chloroformate[12] produced the desired acyl azide **5a** as the only isolated reaction product in 57% yield (Scheme 2). The success of reactions involving isopropenyl chloroformate can be ascribed to the fact that the only by-product of this reagent is non-nucleophilic acetone.



Scheme 2. *Reagents:* i, H_2SO_4/ NaN_3; ii, $SOCl_2$ or $(COCl)_2$; iii, EtOCOCl/ Et_3N/ NaN_3; iv, H_2C=CMeOCOCl/ Et_3N/ NaN_3

Having accomplished a high yielding synthesis of acyl azide **5a**, its stereospecific conversion into isocyanate **3a** was accomplished in quantitive yield simply by refluxing in benzene as shown in Scheme 3.[13] However, all attempts to hydrolyse isocyanate **3a** directly to amine **4a** were unsuccessful. Under acidic or basic conditions, extensive decomposition occurred, whilst neutral conditions gave urea **8** as the sole product in quantitive yield. Isocyanate **3a** could however be converted into urethane **9** simply by reaction with excess methanol, and this lead us to investigate a two step procedure for hydrolysis of isocyanate **3a**. Thus, reaction of **3a** with β-trimethylsilylethanol gave urethane **10a**. As expected, treatment of urethane **10a** with tetrabutylammonium fluoride resulted in rapid removal of the β-

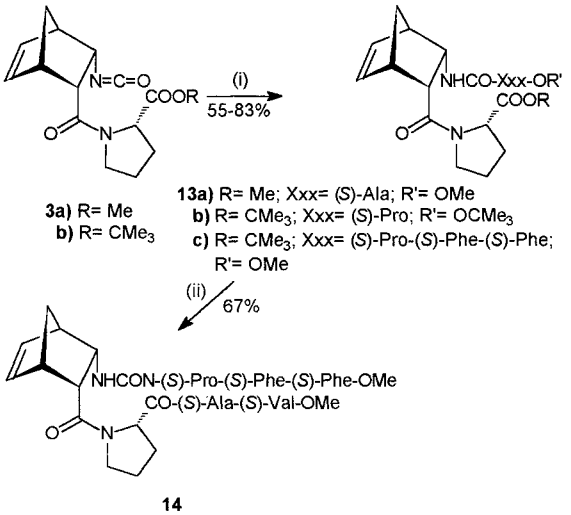
trimethylsilylethoxycarbonyl protecting group. However, under the basic reaction conditions the amine cyclised to give 7-membered ring *bis*-lactam **11** as the sole isolated product. Attempts to remove the β-trimethylsilylethoxycarbonyl protecting group under acidic conditions were also unsuccessful. To prevent the intramolecular cyclisation, we also investigated the use of a sterically hindered *t*-butyl ester for the proline ester, aiming to produce amine **4b**. Desymmetrisation of anhydride **1** using *t*-butyl (*S*)-prolinate proceeded in 68% yield, giving amido acid **2b** as an 8:1 ratio of diastereomers which were separable by trituration with diethyl ether. However, although the synthesis from acid **2b** through acyl azide **5b** and isocyanate **3b** to urethane **10b** proceeded as expected, treatment of **10b** with tetrabutylammonium fluoride again resulted in cyclisation to *bis*-lactam **11**.



Scheme 3. *Reagents*; i, Δ / C_6H_6; ii, THF / H_2O (1:1); iii, MeOH; iv, $Me_3SiCH_2CH_2OH$ / Δ / C_6H_6; v, Bu_4NF / THF; vi, *N*-Boc-(*S*)-Ala / Et_3N / Δ

Finally, we discovered that it was possible to prepare peptides directly from isocyanates **3**, without needing to prepare amino esters **4**. Thus reaction of isocyanate **3a** with *N*-Boc-(*S*)-alanine and triethylamine in refluxing benzene[14] lead directly to the desired tripeptide **12**. Hence we were able to prepare a conformationally constrained tripeptide incorporating a β-amino acid in just four steps from *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride **1**.

Isocyanates **3a,b** were also found to react with esters of amino acids and peptides as shown in Scheme 4, thus providing a short and novel synthesis of *pseudo*-peptides composed of a number of α-amino acids, a conformationally constrained β-amino acid (*endo*-(2*S*, 3*R*)-2-amino-3-carboxy-norborn-5-ene) and a urea unit.[15] Thus reaction of **3a** with (*S*)-alanine methyl ester gave **13a**,[16] whilst reaction of **3b** with (*S*)-proline *t*-butyl ester gave **13b** and reaction of **3b** with Pro-Phe-Phe-OMe gave **13c**. In the latter case, deprotection of the *t*-butyl ester followed by coupling to Ala-Val-OMe using water soluble carbodiimide and HOBt gave *pseudo*-heptapeptide **14**.



Scheme 4. *Reagents*; i, Xxx-OR'; ii, a) CF_3CO_2H b) H_2N-(*S*)-Ala-(*S*)-Val-OMe / EDC / HOBt / Et_3N

In conclusion, we have developed a short synthetic procedure for the synthesis of peptides and *pseudo*-peptides incorporating the conformationally constrained β-amino acid *endo*-(2*S*, 3*R*)-2-amino-3-carboxy-norborn-5-ene as a turn inducer. Further work on the conformational analysis of these peptides and on the synthetic utility of the desymmetrisation reaction is in progress and will be reported in due course.

Acknowledgements

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(13) Isopropenyl chloroformate (0.4ml, 3.75mmol) was added to a mixture of amido acid **2a** (1g, 3.41mmol) and Et_3N (1ml) in dry THF (15ml) at -20°C. An aqueous solution of NaN_3 (0.55g, 8.5mmol) was then added at -10°C, the temperature was gradually raised to room temperature, and stirred for 1 hour. The reaction mixture was diluted with H_2O and the product was extracted three times with EtOAc. The organic phase was washed (Na_2CO_3, water, brine), dried ($MgSO_4$), filtered and concentrated *in vacuo* to leave acyl azide **5a** (0.57g) as a white powder. **5a** was dissolved in anhydrous benzene (10ml), and heated at reflux for 2 hours. The solvent was evaporated *in vacuo* to leave isocyanate **3a** (0.5g, 53%) as a clear oil which solidified on drying. $[\alpha]_D^{22}$ -56.1° (c=1, $CHCl_3$); δ_H 1.34 (1H, d *J* 9.2Hz, $CHCH_2CH$), 1.59 (1H, d *J* 9.2Hz, $CHCH_2CH$), 1.9-2.3 (4H, m, CH_2CH_2), 3.1-3.3 (3H, m, $COCHCHCH_2CH$), 3.78 (3H, s, OCH_3), 3.5-3.7 (2H, m, NCH_2), 4.29 (1H, dd *J* 8.8, 3.7Hz, CHN=C), 4.49 (1H, dd *J* 8.3, 4.0Hz, NCH), 6.08 (1H, dd *J* 5.3, 3.0Hz, =CH), 6.80 (1H, dd *J* 5.7, 3.6Hz, =CH); m/z (CI, NH_3) 308 ($M+NH_4^+$, 4), 291 (MH^+, 100); Found 291.1345 ($C_{15}H_{18}N_2O_4$ requires 291.1345).

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(16) Triethylamine (0.5ml) was added to a cooled (0°C) suspension of isocyanate **3a** (0.5g, 1.7mmol) and (*S*)-alanine methyl ester hydrochloride (0.36g, 2.6mmol) in CH_2Cl_2 (8ml). The reaction mixture was stirred at room temperature for 18 hours, and was subsequently washed (0.5M HCl, Na_2CO_3, H_2O) and dried ($MgSO_4$). The solvent was evaporated *in vacuo* and the residue subjected to flash chromatography (EtOAc) to give **13a** (0.4g, 61%) as a white solid. $[\alpha]_D^{22}$ -53.1° (c=1, $CHCl_3$); δ_H 1.32 (3H, d *J* 7.2Hz, $CHCH_3$), 1.35 (1H, d *J* 8.8Hz, $CHCH_2CH$), 1.48 (1H, d *J* 8.8Hz, $CHCH_2CH$), 1.9-2.2 (4H, m, CH_2CH_2), 3.0-3.2 (2H, m, $CHCH_2CH$), 3.28 (1H, dd *J* 9.1, 3.1Hz, $CHCHCO$), 3.5-3.8 (2H, m, NCH_2), 3.70 (6H, s, 2x OCH_3), 4.35 (1H, dd *J* 8.2, 4.9Hz, CH_2CHN), 4.40 (1H, pent *J* 7.3, CHMe), 4.74 (1H, d *J* 7.1Hz, $NHCHMe$), 4.80 (1H, dt *J* 9.5, 3.8Hz, $CHCHN$), 5.28 (1H, d *J* 9.5Hz, $CHCHNH$), 6.10 (1H, dd *J* 5.4, 3.0Hz, =CH), 6.49 (1H, dd *J* 5.5, 3.0Hz, =CH); m/z (CI, NH_3): 394 (MH^+, 100), 328 (17), 291 (39); Found 394.1978 ($C_{19}H_{28}N_3O_6$ requires 394.1978).