COMMUNICATION

OBC
www.rsc.org/obc

Cross-metathesis coupling of sugars and fatty acids to lysine and cysteine

Andrea J. Vernall and Andrew D. Abell*
Department of Chemistry, University of Canterbury, Christchurch, New Zealand.
E-mail: andrew.abell@canterbury.ac.nz; Fax: +64-3-3642110; Tel: +64-3-3642818

Received 25th June 2004, Accepted 2nd August 2004
First published as an Advance Article on the web 10th August 2004

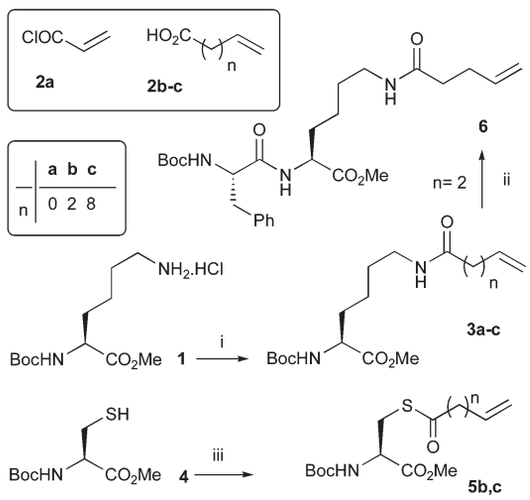
Attachment of an olefin tether to the side chain of either lysine or cysteine allows cross metathesis (CM) conjugation with olefin-containing sugar and fatty acid analogues.

The ability to link or conjugate a molecule to the side chain of lysine or cysteine, be it as a separate amino acid or as part of a peptide or protein, is fundamental to a number of important natural and non-natural processes. Examples include the biosynthesis of glycoproteins,[1] the definition of protein structure,[2] the preparation of haptens for monoclonal antibody production,[3] and the cross-linking of proteins associated with the formation of blood clots, collagen and food-stuffs.[4] Nature has evolved a number of general methods to achieve this goal, for lysine these include the Maillard reaction[4] and cross-linking catalysed by enzymes such as transglutaminase[5] and the formation of disulfide bonds in the case of cysteine. By contrast, synthetic chemists are essentially limited to the formation of an amide or glycosidic bond as a general method of conjugating an organic molecule to a lysine side chain,[6] and mimicking disulfide bond formation in the case of cysteine. These restrictions greatly limit the choice of conjugation partners.

We now report a simple sequence whereby an olefin tether is attached to the side chain of either lysine or cysteine to allow cross metathesis (CM)[7] conjugation with an olefin partner. CM conjugations of olefins to allylglycine[8] and vinylglycine[9] are known, however these non-natural amino acid are expensive and difficult to access enantiomerically pure, particularly in large quantities. By contrast, the method reported here uses cheap, natural amino acids (lysine and cysteine) to which is attached an olefin tether of variable length. These amino acids are often associated with cross-linking in their own right and as such bioconjugates resulting from CM of the olefin tethered derivatives, with biologically important olefins, should be of use in biosynthetic studies and as pharmaceutical targets.

N-Boc-L-lysine **1** and *N*-Boc-L-cysteine **4** were used to prepare the *N*- and *S*-acylated derivatives that bear an olefin tether of variable chain length for cross metathesis studies (see **3a**–**c** and **5b**,**c**, Scheme 1). To this end, N_α-Boc-L-Lys-OMe **1** was treated with EDCI/HOBt and either **2b** or **2c** to give N_ε-substituted alkyl amides **3b** and **3c**, respectively. The α,β-unsaturated amide derivative **3a** was synthesised by reaction of **1** with acryloyl chloride **2a** in the presence of base. Reaction of the corresponding acrylic acid was attempted using EDCI/HOBt without success. The corresponding *S*-acylated cysteines **5b** and **5c** were prepared by BOP-Cl mediated coupling of *N*-Boc-L-cysteine with the alkene acids **2b** and **2c**. Finally, the lysine-based dipeptide **6** was prepared to extend the investigation beyond a single amino acid-based substrate. The *N*-Boc protecting group of **3b** was removed on treatment with HCl gas and the resultant hydrochloride salt was coupled to *N*-Boc-L-Phe-OH to give **6** in 63% overall yield. The non-peptide, organic coupling partners were chosen to allow conjugation of lysine and cysteine amino acids to biologically important olefins such as those derived from sugars 2,3,4,6-tetra-*O*-benzyl-1-α-*C*-allylglucoside (**7**)[10] and fatty acids (**8**) (Fig. 1).

The cross metathesis reactions as shown in Schemes 2 and 4 were carried out using Grubb's 2nd generation catalyst **9**[11] with 1 equiv. of the amino acid-based substrate and 3 equiv. of **7** or **8**. The reactions were carried out under a flow of nitrogen to facilitate removal of



Scheme 1 (i) DIEA, CH_2Cl_2, **2a** (**3a**, 78%); EDCI, HOBt, DIEA, CH_2Cl_2 and **2b** (**3b**, 99%), or **2c** (**3c**, 58%); (ii) HCl gas, then *N*-Boc-L-Phe, EDCI, HOBt, DIEA, CH_2Cl_2 (55%); (iii) BOP-Cl, TEA, CH_2Cl_2 and **2b** (**5b**, 93%) or **2c** (**5c**, 82%).

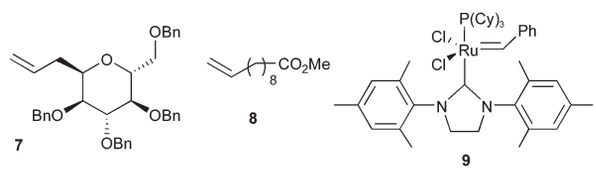


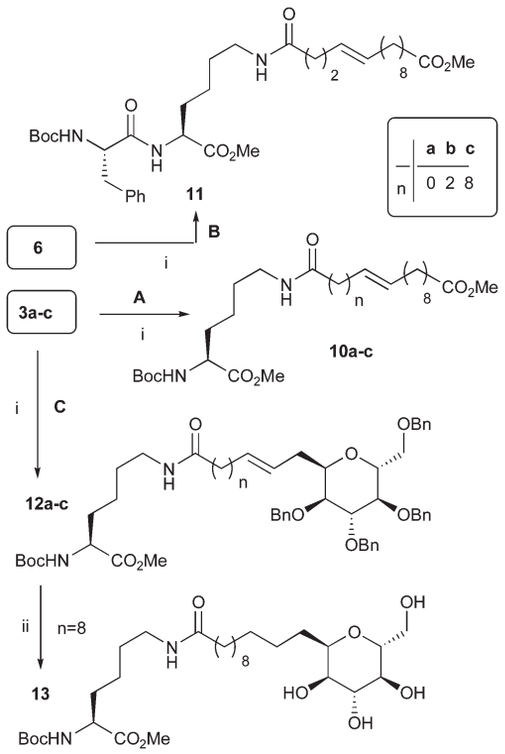
Fig. 1 Structure of the olefin coupling partners and CM catalyst.

the ethylene by-product to help drive the reaction to completion. The reaction mixtures were worked-up by adding DMSO followed by silica gel chromatography and the thus obtained cross coupled products were essentially pure by ^{1}H NMR spectroscopy.[12] Yields have not been optimized and some homodimer products derived from **8** were also isolated in its couplings with **3a**–**c** and **5c**.

The fatty acid analogue **8** was successfully coupled to the lysine-based olefins **3a**, **3b** and **3c** to give **10a** (69%), **10b** (45%) and **10c** (64%), respectively, pathway **A** Scheme 2. The higher yield of **10a**, relative to **10b**, is consistent with literature where it has been noted that cross metathesis reactions involving an α,β-unsaturated coupling partner, as in **3a**, are favored due to effective chelation of this moiety to the catalyst.[13] The extended tether of **3c** would also appear to be favored for cross metathesis couplings where **10c** was obtained in good yield. The lysine containing dipeptide **6** was also coupled with **8** to give **11** in 39%, thus demonstrating applicability of the methodology to systems other than simple side chain *N*-acylated lysines, Scheme 2 pathway **B**. Future extension of this methodology to more complex peptides will be linked to ongoing development of metathesis catalysts with improved efficiency in aqueous media.[14]

Next we coupled **3a** to the sugar analogue **7** to give the conjugate **12a** in 55%, Scheme 2 pathway **C**. The side chain *N*-acylated lysines **3b** and **3c**, also underwent cross metathesis with **7** to give **12b** (33%) and **12c** (65%), respectively. Here again superior yields were observed in reactions of **3a** and **3c** relative to **3b**. The sugar conjugate **12c** was subsequently treated with Pd on C and hydrogen

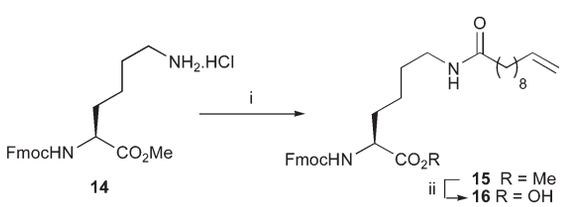
DOI: 10.1039/b411770k



Scheme 2 (i) 1 equiv. of **3a**–**c** or **6**, 3 equiv. **7** or **8**, **9** (20 mol%), CH_2Cl_2, reflux, N_2 flow, (**10a**, 69%; **10b**, 45%, **10c**, 64%, **11**, 39%, **12a**, 55%; **12b**, 33%, **12c**, 65%; (ii) **12c** 10% Pd–C, 74 PSI H_2, quant.

at 74 PSI to give the fully deprotected and side chain reduced analogue **13**.

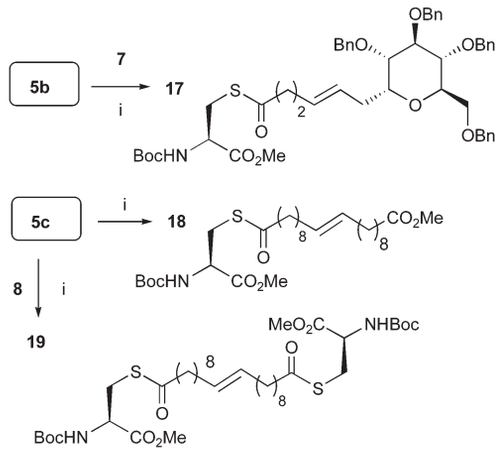
An Fmoc-protected lysine analogue with a pre-attached olefin tether (**16**) was also prepared as a building block for use in peptide synthesis, Scheme 3. The incorporation of **16** into an oligopeptide provides a site for subsequent attachment of a sugar analog (or another olefin), *i.e.* post-translational glycopeptide synthesis.



Scheme 3 (i) EDCI, HOBT, **2c**, 60%; (ii) LiOH, THF, 96%.

The *S*-acyl cysteine analogues **5b** and **5c** also underwent cross metathesis with the sugar-based olefin **7** and the fatty acid analogue **8**, respectively, to give **17** (43%) and **18** (61%), Scheme 4. We also successfully carried out a dimerization of the chain-extended analogue **5c** to give **19** in 36% (Scheme 3) in what is a new and versatile method for the cross linking of cysteine residues.

In summary, we have identified a new and important method that allows conjugation of a suitably functionalised fatty acid or sugar to the side chains of lysine and cysteine. The methodology should also be amenable to a wide range of other biologically important olefins. While reaction yields are not optimized it is apparent that the couplings are favored when either α,β-unsaturated (**3a**) or long chain tether (**3c**) is employed. An ability to couple these amino acids to a sugar is particularly significant since it paves the way for the preparation of important peptide–sugar complexes. The ability to introduce of an olefin linker of variable length is of particular interest for the preparation of immunoconjugates where optimum spacer-linker combinations are varied and ill-defined.[15] It is also worth noting that important olefin coupling partners for use in this sequence are generally available from nature, or *via* standard functional group manipulations.



Scheme 4 (i) **9** (20 mol%), CH_2Cl_2, reflux, N_2 flow.

Experimental

General procedure for preparation of *N*-acyl lysines 3b and 3c

The acids **2b** or **2c** (typically 6.13 mmol) and diisopropylethylamine (1.25 mL, 6.74 mmol) were added to a solution of N_α-Boc-L-Lys-OMe·HCl (2.0 g, 6.74 mmol), EDCI (1.53 g, 7.96 mmol), and HOBt (1.24 g, 9.19 mmol) in dry CH_2Cl_2 (50 mL) under an atmosphere of N_2. The solution was stirred at room temperature overnight, diluted with CH_2Cl_2 and washed twice with 3 M aqueous NaCl. The combined aqueous layers were re-extracted with CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure to give a yellow oil that was purified by silica gel flash chromatography to give **3b** and **3c**, respectively.

General procedure for preparation of *S*-acyl cysteines 3b and 3c

Triethylamine (0.82 mL, 5.83 mmol) was added to a solution of *N*-Boc-L-Cys-OMe (572 mg, 2.43 mmol) in CH_2Cl_2 (15 mL) under an atmosphere of N_2. The mixture was cooled in an ice-bath and after 10 minutes a solution of BOP-Cl (743 mg, 2.92 mmol) and either **2b** or **2c** (typically 2.67 mmol), in CH_2Cl_2 (15 mL), was added dropwise. The reaction was warmed to room temperature and stirred for a further four hours. The solution was concentrated under reduced pressure, diluted with ethyl acetate, and washed successively with water, 1 M aqueous $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4), and concentrated under reduced pressure to give an oil that was purified by silica gel flash chromatography (1 : 4 ethyl acetate : petroleum ether) to give **5b** and **5c**, respectively.

General procedure for cross metathesis reactions

The amino acid coupling partner (one equiv.) and either **7**[10] or **8** (3 equiv.) were stirred under a pressure of nitrogen in dry, distilled CH_2Cl_2. Grubbs 2nd generation catalyst **9** (0.2 equiv. or 20 mol%) was added and the reaction mixture heated to reflux under a flow of nitrogen. After refluxing for 6 hours in these conditions, DMSO (50 equiv. relative to catalyst) was added and the reaction stirred at room temperature overnight under an air atmosphere. Typically, a brown oily residue was isolated, and this was purified using silica gel column chromatography to give the cross-coupled product.

Acknowledgements

The generous financial support of a Royal Society of New Zealand Marsden grant is gratefully acknowledged. We also thank FiRST for a Top Achievers scholarship (AV)

Notes and references

1 *Glycoproteins and Disease*, J. Montreuil, J. F. G. Vliegenthart and H. Schachter (Eds), Elsevier, NY, 1996; T. Feizi, *Curr. Opin. Struct. Biol.*, 1993, **3**, 701.
2 A. Fersht, *Enzyme Structure and Function*, W. H. Freeman and Co, New York, 1985.

3 H. Zola, *Monoclonal Antibodies: from Background to Bench*, BIOS Scientific, Oxford, 2000.
4 J. A. Gerrard, *Aust. J. Chem.*, 2002, **55**, 2999; P. J. Beisswenger, B. S. Szwergold and K. T. Yeo, *Clin. Lab. Med.*, 2001, **21**, 53.
5 M. Griffin, R. Casadio and C. M. Bergamini, *Biochem. J.*, 2002, **368**, 377.
6 Exceptions do exist, see A. Dondoni and A. Marra, *Chem. Rev.*, 2000, **100**, 4395.
7 In recent times cross metathesis (CM) has emerged as an important, and general synthetic reaction, in which alkyl groups from two different substrates are coupled to give a new olefin. see A. J. Vernall and A. D. Abell, *Aldrichimica Acta*, 2003, **36**, 93; S. J. Connon and S. Blechert, *Angew. Chem., Int. Ed.*, 2003, **42**, 1900; H. E. Blackwell, D. J. O'Leary, A. K. Chatterjee, R. A. Washenfelder, D. A. Bussmann and R. H. Grubbs, *J. Am. Chem. Soc.*, 2000, **122**, 58.
8 G. J. McGarvey, T. E. Benedum and F. W. Schmidtmann, *Org. Lett.*, 2002, **4**, 3591.
9 E. G. Nolen, A. J. Kurish, K. A. Wong and M. D. Orlando, *Tetrahedron Lett.*, 2003, **44**, 2449.
10 T. D. Perrine, C. P. J. Glaudemans, R. K. Ness, J. Kyle and H. G. Fletcher Jr., *J. Org. Chem.*, 1967, **32**, 664; R. P. Spencer, C. L. Cavallaro and J. Schwartz, *J. Org. Chem.*, 1999, **64**, 3987; E. Brenna, C. Fuganti, P. Grasselli, S. Serra and S. Zambotti, *Chem. Eur. J.*, 2002, **8**, 1872.
11 T. M. Trnka and R. H. Grubbs, *Acc. Chem. Res.*, 2001, **34**, 18; M. Scholl, S. Ding, C. W. Lee and R. H. Grubbs, *Org. Lett.*, 1999, **1**, 953.
12 The alkenes were obtained in >90% (*E*) in all cases by ^{1}H NMR.
13 T. Choi, A. K. Chatterjee and R. H. Grubbs, *Angew. Chem., Int. Ed.*, 2001, **40**, 1277.
14 A. J. Phillips and A. D. Abell, *Aldrichimica Acta*, 1999, **32**, 75.
15 J. R. Allen and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1999, **121**, 10875.