A chemoselective ligation for the synthesis of amino acid derivatives of virginiamycin M1

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Abstract—An efficient chemoselective ligation approach using an oxime bond was developed for the synthesis of amino acid derivatives of virginiamycin M1, a highly sensitive streptogramin antibiotic.
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Chemoselective ligation was first developed by protein chemists1,2 to circumvent the difficulties encountered with conventional solid phase peptide synthesis (SPPS) when making large peptides. The principle is to specifically link two completely unprotected peptides, under mild aqueous conditions, via the chemoselective reaction of a pair of mutually and uniquely reactive functional groups introduced on the peptides during SPPS. Thiol and carbonyl chemistries are extensively used for that purpose. Since its initial development in the protein field, this interesting concept has been extended to the synthesis of numerous groups of molecules, otherwise difficult to obtain, such as cyclic peptides,3 lipopeptides,4,5 glycopeptides,4,6,7 carbohydrate–oligonucleotide conjugates,8 and steroid derivatives.9

Virginiamycin, a streptogramin antibiotic produced by Streptomyces virginiae, is composed of two classes of compounds, M and S (for a review see Crooy and De Neys)10 and inhibits protein synthesis in Gram positive bacteria.11 Virginiamycin is used as a growth promoting agent in animal feeds, under the commercial name Statfac®. Its therapeutic uses have been limited.12 Nowadays, the rising number of nosocomial infections has renewed interest in streptogramins. Indeed, dalfopristin, a water-soluble derivative of pristinamycin M1 factor (virginiamycin M1) combined with quinupristin, a semi-synthetic derivative of pristinamycin S, is used for the treatment of infections caused by multiple resistant Gram positive bacterial strains (e.g., Staphylococcus aureus, Streptococcus pneumoniae...).13 Therefore, a demand for new synthetic routes to access streptogramin derivatives is currently arising. In this paper, we introduce the chemoselective ligation strategy to link amino acids or peptides to virginiamycin M1.

The M1 factor (Fig. 1) is the major component of virginiamycin (over 60% by weight); highly purified M1 (HPLC-UV (214 nm) purity >99%) used throughout this study was obtained as previously described.14 In qualitative studies, it has been reported that virginiamycin M factors are subject to degradation reactions in acidic and basic media.15 In the present study, the stability of purified M1 factor was monitored by RP-HPLC as...
a function of time, using buffers covering the 2–9 pH range. The data presented in Figure 2 confirm pH-mediated modification of M₁, which shows optimal stability at pH 5. This clearly precludes standard Boc or Fmoc protections when linking amino acids or peptides to M₁ through an ester bond involving the hydroxyl group of M₁, which would not resist to the severe deprotection conditions required. Therefore, chemoselective ligation was considered as an alternative and oxime bond formation was chosen as it can selectively involve the ketone function of M₁ within the less disfavourable pH 4–6 range.¹⁶

Commercially available (Boc-aminooxy)acetic acid (Boc-AOA) was selected to introduce the aminooxy segment on the amino acid moiety. In a preliminary step, in order to check the capability of AOA as connecting arm, unprotected AOA was linked to M₁ via an oxime bond, (AOA.1/2 HCl, pyridine 1.1 equiv, ethanol, 20 h, room temperature) to deliver the expected oxime conjugate M₁AOA. RP-HPLC analysis of the reaction medium confirmed on the one hand that the oxime M₁AOA was produced in almost quantitative yield and on the other hand that both Z and E stereoisomers have been synthesised in a 55:45 ratio. Z and E oximes were efficiently purified and separated by preparative RP-HPLC.

As in the case of M₁AOA, Z/E oxime couples were obtained and purified by preparative RP-HPLC (same column as Figure 3; isocratic elution with H₂O and CH₃CN + 0.05% TFA in a ratio depending on the amino acid linked, 23 mL/min). In contrast with M₁AOA, repeated re-injections in analytical RP-HPLC did not reveal any isomerisation within 24 h, which enabled easy purification of Z and E isomers. This is of particular interest as the biological activity of the virginiamycin derivatives may depend on their configuration. Figure 4 shows the chromatogram of the reaction medium for the synthesis of the M₁AOA–AA conjugate 12: two major peaks corresponding to the Z/E couple can be observed without any major side product.

The mass spectra of all oximes 9–12 were recorded by ESI-MS on a Micromass QT of II (Micromass, Man-
oxime conjugates were indeed produced. As an illustration, compared NMR data for M1 and the separable stereoisomeric lysine conjugates 8a and 8b are discussed.18,19 The 13C shift of the keto group in M1 is 201.4 ppm: upon oxime formation, C-16 shifts upfield to 152.3 and 153.6 ppm, depending on the configuration. Both α-carbons to oxime also shift upfield: C-17 shifts from 43.7 in M1 to 28.1 and 34.2 in 8a and 8b, respectively, and similarly, C-15 shifts from 49.3 in M1 to 40.9 and 37.3, respectively. For the β-carbons, a slight downfield shift is observed: +1.0 and +1.1 ppm for C-18; +1.3 and +3.3 for C-14. The above observations are in accordance with the data reported by Hawkes et al.20 on a large number of ketones and their corresponding oximes. With the exception of H-11, H-13, H-14, H-15 and H-17 (mainly in isomer 12b), the protons in M1 conjugates are not significantly affected by oxime formation.

The original chemoselective pathway established within the present study is of particular interest as compared to previously reported works.21,22 Anteunis et al.21 made the synthesis of M1AOATrpOMe by linking Trp methyl ester to the preformed M1AOA oxime via a classical peptide synthesis strategy. The major drawback of such a procedure was that the final product had to remain protected as no suitable deprotection method was reported. Similarly, using protecting groups Lin et al.22 linked a tripeptide siderophore to the virginiamycin S1 factor, which is far more stable than M1 in acid media.23 In the present work, irrespective of the side chain of amino acid to be linked to M1, the chemoselective ligation using oxime bond, which is known to be stable both in vitro and in vivo,24 could be applied to the synthesis of derivatives of many other polyfunctional, very sensitive biologically active natural compounds.

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References and notes

18. Selected spectral data for M 1AOALys isomer


