Enantiospecific Synthesis of Analogues of the Diketide Intermediate of the Erythromycin Polyketide Synthase (PKS)

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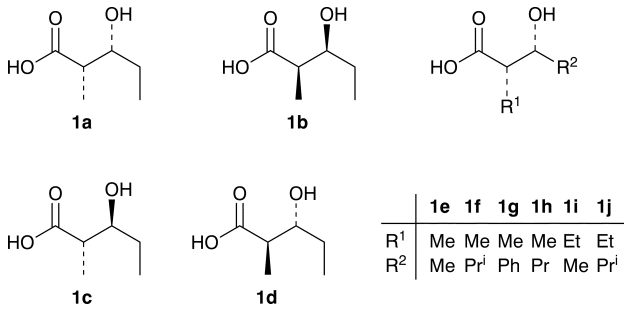
The four stereoisomers of 3-hydroxy-2-methylpentanoic acid (**1a–d**) and the structurally modified acids **1e–j** have been synthesised enantiospecifically and converted into *p*-nitrophenyl ester and thioester derivatives; as the activated derivatives; they are available for investigations into the substrate selectivity of polyketide synthase (PKS) domains.

In recent years, the substrate specificity of modular polyketide synthases, the enzymes responsible for a range of clinically important antibiotics, have been probed through feeding studies with analogues of biosynthetic intermediates.[1] In our studies of the 6-deoxyerythronolide B synthase (DEBS), the erythromycin-producing PKS, we desired a range of diketide analogues. We synthesised all four stereoisomers of 3-hydroxy-2-methylpentanoic acid **1a–d**, as well as analogues with the stereochemistry of **1a** which incorporated further structural modifications, **1e–j** (Fig. 1). All diketides (**1a–j**) were converted into activated *p*-nitrophenyl esters and/or *N*-acetylcysteamine thioesters.[2]

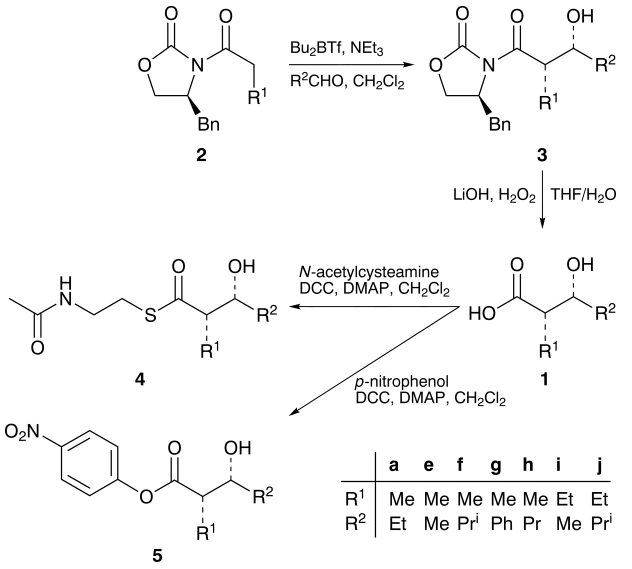


	1e	1f	1g	1h	1i	1j
R^1	Me	Me	Me	Me	Et	Et
R^2	Me	Pr^i	Ph	Pr	Me	Pr^i

Fig. 1 Structures of diketide acids

The synthetic strategy used in all cases centred on Evans aldol methodology.[3–6] The appropriate chiral auxiliary was acylated, C-2, C-3 stereochemistry established through a boron-mediated aldol reaction, and then the resulting acid was cleaved from the auxiliary using lithium hydroperoxide (Scheme 1). We chose to derivatise the acids as their *N*-acetylcysteamine (NAC) thioesters and *p*-nitrophenyl esters; the NAC moiety is a good structural mimic for the 4′-phosphopantetheine group of PKS acyl carrier proteins,[7] while nitrophenyl esters, though less accurate structural analogues, are well-known serine protease substrates whose reactions can be monitored by UV–VIS spectroscopy.[8] Synthesis of the derivatives was achieved by coupling the acids with *N*-acetylcysteamine or *p*-nitrophenol in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP);[9] excess NAC was removed by flash column chromatography with $CuSO_4$-impregnated silica gel (Scheme 1).[7]

The derivatives **4e–i**, incorporating the same stereochemistry as **1a** but with a range of functionalities, were accessed by the same route as for **1a** (Scheme 1); functional variation was introduced through the choices of acid chloride in the acylating step and aldehyde in the aldol reaction. Compound **4j** was generated directly from **3j**, by reacting



	a	e	f	g	h	i	j
R^1	Me	Me	Me	Me	Me	Et	Et
R^2	Et	Me	Pr^i	Ph	Pr	Me	Pr^i

Scheme 1

it with *N*-acetylcysteamine in the presence of lithium bis-(trimethylsilylamide).[7]

Techniques used: ^1H and ^{13}C NMR, column chromatography, mass spectra, polarimetry

References and notes: 10

Schemes: 4

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