Biosynthesis of Tetronasin: Part 3
Preparation of Deuterium Labelled Tri- and Tetraketides as Putative Biosynthetic Precursors of Tetronasin

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\[ \text{Abstract: The preparation of seven deuterium labelled N-acetyl cysteamine thioesters (2a), (2b), (3a), (3b), (4), (5) and (6) as putative biosynthetic precursors of the acyl tetronic acid ionophore tetronasin is described.} \]

In the preceding two Letters we have discussed incorporation experiments, with proposed tri- and tetraketide precursors in the biosynthesis of the ionophore antibiotic tetronasin (1), the results of which are shown in Scheme 1. Here we report the synthetic routes used to prepare the deuterium labelled compounds (2a), (2b), (3a), (3b), (4), (5) and (6) that were required for our studies.

The routes to these N-acetyl cysteamine thioester derivatives were designed to be flexible in order to access the maximum number of compounds. Those compounds labelled at the C-2 methyl group (i.e. all except (3b)) were produced via a linked synthetic route and their preparation shall be dealt with together. The C-8 labelled analogue (3b) was produced via a different route and will be dealt with independently of the others.

**Synthesis of the tri- and tetraketide analogues (2a), (2b), (3a), (4), (5) and (6).**

Scheme 2 shows synthetic routes leading to the triketide analogues. The racemic form of the acyl residue was produced by methylation of the di-anion of (4E)-hexenoic acid. The homochiral forms were prepared using Evans chiral oxazolidinones to direct the addition of the CD3 group followed by cleavage of the auxiliary under standard conditions. All three acids were coupled with N-acetyl cysteamine using DCC-DMAP.
Scheme 2: (i) LDA 2 equiv., THF, 0°C; 5 equiv. CD$_3$I (78%). (ii) N-acetyl cysteamine, DCC, DMAP, CH$_2$Cl$_2$ (70%). (iii) nBuLi, THF; then add to mixed anhydride formed from (4R)-hexenoic acid, Et$_3$N, Me$_2$COCl, THF (91%). (iv) NaN(SiMe$_3$)$_2$, THF, -78°C; 5 equiv CD$_3$I (65%). (v) LiOH, H$_2$O$_2$, THF-H$_2$O 3:1 (95%).

The four diastereoisomers of the tetraketide were synthesised by complementary routes employing an aldol reaction of different propionate derivatives with the common aldehyde (7). This was produced from the intermediate (8), itself prepared by a method analogous to that shown above in Scheme 2.

(i) LiAlH$_4$, Et$_2$O, 0°C (70%). (ii) (COCl)$_2$, DMSO, CH$_2$Cl$_2$, -78°C; then Et$_3$N, -30°C (87%).

The tetraketide precursors (3a) and (4) having an anti relationship between the C-2 and C-3 centres were produced by employing a Heathcock anti aldol condensation between 2',6'-dimethylphenyl-[3-2H$_3$]-propionate (9) and the aldehyde (7) as shown in Scheme 3.

Scheme 3: (i) nBuLi, THF, 0°C; then acetyl chloride (100%). (ii) NaN(SiMe$_3$)$_2$, THF, -78°C; 5 equiv. CD$_3$I (67%). (iii) LDA, -78°C, 45 min; then -118°C and add (7) and warm to -78°C (53% overall). (iv) KOH 5 equiv, dioxane-H$_2$O 30:1. (v) (PhCH$_2$)$_2$NH 1.05 equiv, recrystallise from Et$_2$O/hexanes (87% overall over two steps; 55% of (10), 32% of (11)). (vi) Dowex 50-H$^+$ (99%). (vii) N-acetyl cysteamine, DCC, DMAP, CH$_2$Cl$_2$ (54%).
The reaction produced a mixture of two diastereoisomers that were separated by conversion to the dibenzylammonium salts (via the acids), followed by recrystallisation from ether/hexane to give the pure diastereomeric salts (10) and (11). These salts were reconverted to the acids by treatment with Dowex-50 (H⁺ form) and coupled to N-acetyl cysteamine as before to give (3a) and (4). The two isomers (5) and (6) having a syn relationship between the C-2 and C-3 centres were prepared using Evans' syn aldol methodology as shown in Scheme 4. Thus condensation of the boron enolates of the CD₃-propionate oxazolidinones (12) with the aldehyde (7) under standard conditions gave the diastereomically pure products (13) and (14). Hydrolysis of the auxiliary under standard conditions followed by coupling of the resulting acids to N-acetyl cysteamine gave the derivatives (5) and (6) as shown.

Scheme 4: (i) nBuLi, THF, 0°C; then acetyl chloride (99%). (ii) Na[N(SiMe₃)₂]₂, DMPU 10 equiv., THF, -78°C, 5 equiv. CD₃I (74%). (iii) Bu₂BOTf, iPr₂EtN, CH₂Cl₂, 0°C; then -78°C and add (7) (65%). (iv) LiOH, H₂O₂, THF-H₂O 3-1, 0°C (95%). (v) N-acetyl cysteamine, DCC, DMAP, CH₂Cl₂ (57%).

Synthesis of the tetraketide analogues (3b).

The synthesis of (3b), the precursor that was required to complete our biosynthetic studies, was achieved as shown in Scheme 5. The preparation of the initial building block (15) is reported in a subsequent paper in this series, since it is also a precursor for the C19-C26 tetrahydrofuran fragment required for the total synthesis studies. Compound (15) was reacted with triethyl phosphonoacetate, using the Masamune-Roush conditions, to give an intermediate homologated ester which upon reduction with DIBAL-D gave the deuteriated allylic alcohol (16). This was further reduced via conversion to the corresponding chloride, using tosyl chloride in CH₂Cl₂ containing DMAP and treatment with lithium triethyl borodeuteride to give the trideuterio derivative (17) in excellent overall yield. Deprotection of (17) with lithium in ammonia at -78°C, and oxidation with tetra-n-propylammonium perruthenate (TPAP) under catalytic conditions gave the aldehyde (18) also in excellent yield. The last steps toward (3b) were then straightforward. Oxidation of (18) with sodium chlorite, using 2-methyl-2-butene as a chlorine scavenger, gave an acid which was converted to the (3b) on coupling with N-acetyl cysteamine using DCC/DMAP, and subsequent deprotection with HF/pyridine.
Scheme 5 (i) EtO$_2$CH$_2$P(O)(OEt)$_2$, LiCl, DIPEA, CH$_3$CN (93%). (ii) DIBAL-H, THF. -78°C (85%). (iii) TsCl, DMAP, CH$_2$Cl$_2$ (90%). (iv) LiE@D, THF, -78°C (85%). (v) Li$\text{NH}_2$, Et$_2$O, -78°C (97%). (vi) Pr$_4$NReO$_4$, NMO, 4Å powdered sieves, CH$_2$Cl$_2$ (95%). (vii) NaClqL, 2-methyl-2-buten, KH$_2$PO$_4$, Li$_2$SO$_4$. $\text{H}_2$O (99%). (viii) N-acycyleysteamine, DCC, DMAP, CH$_2$Cl$_2$ (80%). (ix) IIF, pyridine, MeCN (80%).

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References and Notes
1. (a) See one of the proceeding papers in this series: Biosynthesis of Tetronasin: Part 1. (b) See preceding paper Biosynthesis of Tetronasin: Part 2 and references therein.
2. All new compounds gave satisfactory analytical data.
8. Formation of aldo products gave 4% of a minor diastereoisomer due to racemisation of (7) and resulting epimerisation at C-4.
10. See following paper in this series: Two New Routes to the C19-C26 Tetrahydrofuran Fragment of the Acyl Tetrionic Acid Ionophore Tetronasin (ICI M139603).
14. Use of sodium in ammonia at -78°C gave the silyl migrated product in 87% yield along with 10% of the desired primary alcohol.

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