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# Concise route to α-acylamino-β-keto amides: application to the synthesis of a simplified azinomycin A analogue

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**Abstract**—Condensation of acid chlorides (alkyl, aryl or heteroaryl) with *N*,*N*′-dialkyl α-acylamino malonamides in the presence of magnesium ethoxide provides a direct route to α-acylamino-β-keto amides in moderate to good yields (46–95%). Using this method, a concise route to an enantiomerically enriched 1-azabicyclo[3.1.0]hexane containing most of the elements of the 'right-hand' domain of azinomycin A has been developed. 

Azinomycins A **1** and B **2** are naturally occurring antibiotics which possess potent in vitro cytotoxic activity, significant in vivo antitumour activity and which appear to act by disruption of cellular DNA replication by interstrand cross-link (ISC) formation (Fig. 1).[1] The epoxide and aziridine are known to be responsible for the cross-linking process which occurs between bases two residues apart on the complementary DNA strands, with specificity for 5′–PuPyPy–3′ sequences.[2] The azinomycins possess a highly unusual molecular architecture which, combined with their interesting biological properties, has stimulated considerable interest from the scientific community.[1] The synthetic problems presented by these natural products are formidable, because in addition to the density of functional groups, the azinomycins are unstable in aqueous solution,[3] and prone to opening by nucleophiles at C-10 and C-21.[4] In 2001, Coleman overcame these synthetic hurdles and reported the first total synthesis of azinomycin A.[5]

As part of our own synthetic efforts, we have developed a novel reductive cyclisation to construct the dehydroamino acid fragment.[6] This chemistry involved homologation of acid chloride **3** to α-amino-β-keto ester **4** and subsequent catalytic hydrogenation in the presence of hydrochloric acid to give pyrrolidine **5** (Scheme 1). Furthermore, we were able to convert **5** into epoxy aziridine **6** and demonstrate that it cross-links DNA in a manner similar to the natural products.[6] To further progress our synthetic efforts towards azinomycin A itself, and to quantify the role of the amide side-chain in DNA binding, we required structures incorporating the azinomycin A side-chain (C–1 to N–5). Unfortunately, all our attempts to selectively hydrolyse the ethyl ester of **5** to facilitate the introduction of this substituent were entirely unsuccessful. To circumvent this problem, we sought a way to introduce

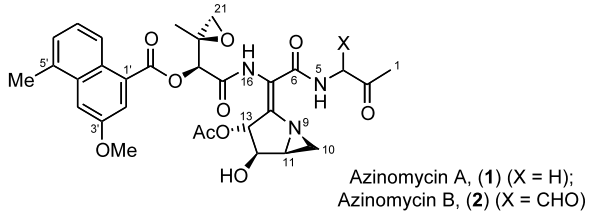
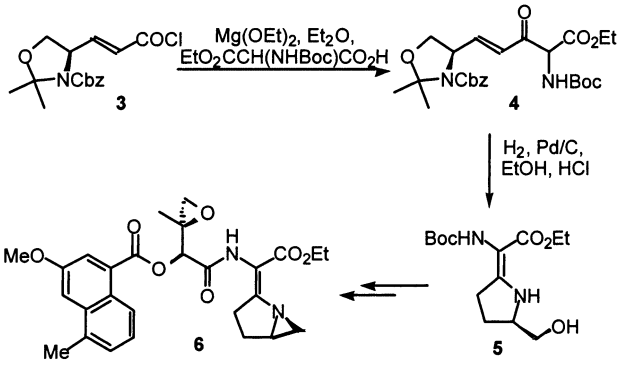



**Figure 1.** Structures of the azinomycins.




**Scheme 1.** Reductive cyclisation approach to azinomycin analogue **6**.

*Keywords*: antitumour compounds; azinomycins; aziridines; carzinophilin; coupling reactions.

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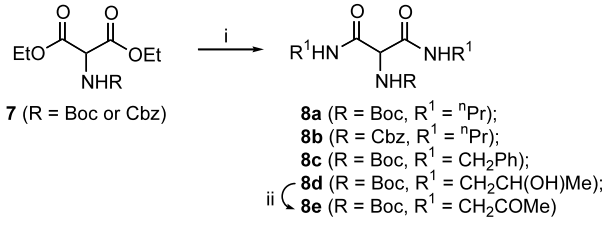
the amide side-chain prior to implementing the reductive cyclisation. In this Letter, we describe a new direct method for the synthesis of α-acylamino-β-keto amides and demonstrate that it can be used to make a 1-azabicyclo[3.1.0]hexane containing the amide side-chain of azinomycin A.

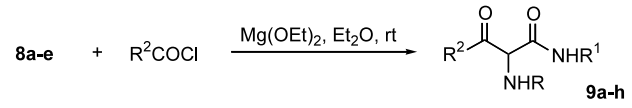
Whilst methods for the formation of α-acylamino-β-keto esters by acylation of $EtO_2CCH(NHCOR)CO_2H$ with acid chlorides are known,[7] we are unaware of any published methods for the preparation of α-acylamino-β-keto amides via the same disconnection. Somewhat serendipitously, we have discovered that this transformation can be smoothly accomplished by reacting *N*,*N*′-dialkyl α-acylamino-malonamides with acid chlorides in the presence of magnesium ethoxide.

Five representative dialkyl malonamides were used. Malonamides **8a–d** were made directly by treating amino malonate **7** (R = Boc or Cbz) with an excess of the amine in refluxing xylene (Scheme 2). Further double oxidation of **8d** under Swern conditions provided **8e** containing the amide side-chain required for azinomycin A.

Treatment of dialkyl malonamides **8a–e** (1.0 equiv.) with a variety of acid chlorides (2.0 equiv.) in diethyl ether using magnesium ethoxide (4.0 equiv.) as base directly provided the corresponding α-acylamino-β-keto amides **9a-h** in moderate to good yields (Table 1).[8,9] The reaction works with alkyl, aromatic and heteroaromatic acid chlorides (Table 1, entries 1–3) and with either Cbz and Boc protection of the α-amino substituent (Table 1, entry 1 cf. entry 4). The reactions were typically performed using a two-fold excess of the malonamide although it seems that reasonable yields can be obtained using just 1.1 equivalents of this coupling partner (Table 1, entry 1 cf. entry 9).



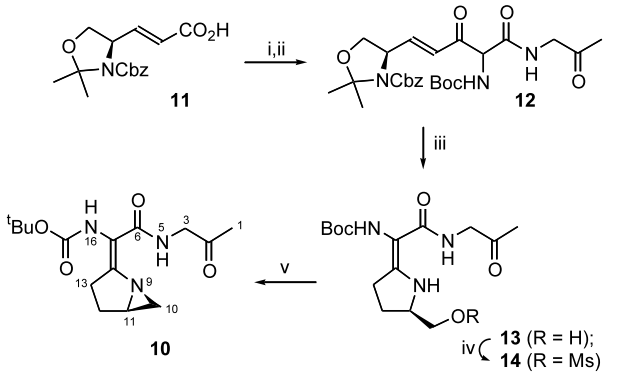

**Scheme 2.** Preparation of dialkyl malonamides **8a–e**. *Reagents and conditions*: (i) $R^1NH_2$ (8 equiv.), xylene, 140°C, 2 h, **8a** (88%), **8b** (92%), **8c** (89%), **8d** (87%); (ii) $(COCl)_2$ (2.2 equiv.), DMSO (4.4 equiv.), $Et_3N$ (10 equiv.), $CH_2Cl_2$, −78°C, 82%.




**Scheme 3.** Synthesis of α-acylamino-β-keto amides **9a–h**.

When benzoyl chloride was reacted with **8a** (2 equiv.) in the presence of excess magnesium ethoxide, in addition to **9a** (92%), $PrHNCO_2Et$ (80%) was isolated along with unreacted **8a** (90% of theoretical assuming 100% conversion). This experiment provides us with a basic working hypothesis as to the reaction pathway. We suggest that malonamide **8** is deprotonated by the base then is *C*-acylated with the acid chloride. Further reaction with ethoxide then provides **9** along with $PrHNCO_2Et$ by subsequent C–C bond fission.

We have successfully used this methodology to make 1-azabicyclo[3.1.0]hexane **10** (Scheme 4). Thus, treatment of malonamide **8e** with acid chloride **3**, made from **11**, provided **12** in 55% yield. Hydrogenation of




**Scheme 4.** Synthesis of 1-azabicyclo[3.1.0]hexane **10**. *Reagents and conditions*: (i) $(COCl)_2$, cat. DMF, $CH_2Cl_2$; (ii) **8e**, $Mg(OEt)_2$, $Et_2O$, 55% (from **11**); (iii) $H_2$, Pd/C, HCl, EtOH, 80%; (vi) MsCl, $CH_2Cl_2$, 0°C, 88%; (v) KHMDS, THF, 0°C→rt, 61%.

**Table 1.** α-acylamino-β-keto amides **9a–h** produced via Scheme 3

| Entry | Amide | R | $R^1$ | $R^2$ | Product[a] (yield%) |
|---|---|---|---|---|---|
| 1 | **8a** | Boc | $^n$Pr | Ph | **9a** (91) |
| 2 | **8a** | Boc | $^n$Pr | 2-Thiophenyl | **9b** (85) |
| 3 | **8a** | Boc | $^n$Pr | $^n$Pr | **9c** (46) |
| 4 | **8b** | Cbz | $^n$Pr | Ph | **9d** (90) |
| 5 | **8b** | Cbz | $^n$Pr | 2-Thiophenyl | **9e** (89) |
| 6 | **8c** | Boc | $CH_2Ph$ | Ph | **9f** (95) |
| 7 | **8d** | Boc | $CH_2CH(OH)Me$ | Ph | **9g** (73) |
| 8 | **8e** | Boc | $CH_2COMe$ | 2-Thiophenyl | **9h** (89) |
| 9 | **8a** | Boc | $^n$Pr | Ph | **9a** (68) |

[a] All reactions were performed using 2 equiv. of amide **8** with respect to the acid chloride except entry 9 where 1.1 equiv. was used.

**13** according to our previously developed conditions,[6] facilitated cyclisation to pyrrolidine **13** in 80% yield. This pyrrolidine was produced as essentially a single stereoisomer which was tentatively assigned as possessing the (*E*)-stereochemistry on the basis of NOE experiments.[10] Chiral HPLC analysis indicated that **13** had been produced in ca. 90% ee,[11] revealing that a small amount of racemisation had occurred in the coupling/cyclisation sequence. Comparable levels of racemisation had been noted in our earlier synthesis of ester **5** (Scheme 1).[6] Mesylation of alcohol **13** provided **14** which was ring closed using potassium hexamethyldisilazide to **10**.[12] Aziridine **10** was isolated in a reasonable state of purity ($\geq$90% as judged by $^{13}$C NMR spectroscopy) after rapid aqueous work up and precipitation of the impurities using hexane/$CH_2Cl_2$ (10:1).

Gratifyingly, it possessed the same (*E*)-geometry about the tetrasubstituted double bond as the natural products themselves. This conclusion was reached on the basis of several pieces of spectroscopic data. Firstly, irradiation of the amide hydrogen (H-5) produced small but measurable NOE enhancements of H–3 (6.2%), H–10$_{exo}$ (1.2%) and H–11 (0.9%); whilst simultaneous irradiation of H–13 and H–13′ produced NOE enhancements of H–10$_{endo}$ (3.3%) and H–16 (1.4%). Secondly, a significant downfield shift ($\delta$ 10.3) of the amide hydrogen (H–5) was observed. A similar chemical shift was seen for this hydrogen in azinomycin A itself ($\delta$ 10.09). Yokoi et al. rationalised this observation by invoking a hydrogen bond to the nitrogen atom of the aziridine (N–9) which necessitates the (*E*)-geometry of the double bond.[13] Further evidence in support of this assignment came from the fact that the $^{13}$C NMR spectrum of **10** closely agrees with that of azinomycin A (Table 2).

In summary, we have devised a new simple method for the synthesis of α-acylamino β-keto amides which we have demonstrated is of utility in the synthesis of simplified azinomycin A analogues possessing the correct (*E*)-geometry about the tetrasubstitued double bond. Work to assemble more complex azinomycin analogues and to evaluate their therapeutic potential is ongoing in our laboratory.

**Table 2.** Selected $^{13}$C NMR chemical shifts for azinomycin A **1** and **10**

| Carbon[a] | **1**[b] | **10** |
|---|---|---|
| C–1 | 27.2 | 27.2 |
| C–2 | 202.6 | 203.5 |
| C–3 | 50.6 | 50.3 |
| C–6 | 163.2 | 164.5 |
| C–7 | 120.1 | 117.6 |
| C–8 | 149.6 | 153.3 |
| C–10 | 35.8 | 34.9 |
| C–11 | 45.4 | 44.5 |
| C–12 | 76.9 | 23.3 |
| C–13 | 84.0 | 26.6 |
| C–17 | 163.8 | 157.4 |

[a] Spectra recorded in $CDCl_3$ (100 MHz).
[b] Data from Ref. 13.

## Acknowledgements

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4. For example, 4-*O*-methyl-13-desacetyl-12,13-di-*O*-benzyl azinomycin B readily reacts with thiophenol at both the epoxide and the aziridine, see Hashimoto, M.; Terashima, S. *Heterocycles* **1998**, *47*, 59–64.
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7. For example, see: (a) Schmidt, U.; Griesser, H.; Lieberknecht, A.; Schmidt, J.; Gräther, T. *Synthesis*, **1993**, 765–766; (b) Krysan, D. J. *Tetrahedron Lett.* **1996**, *37*, 3303–3306.
8. *Typical procedure:* To magnesium (0.16 g, 6.58 mmol) was added $CCl_4$ (0.15 ml) then ethanol (0.8 ml) dropwise at room temperature. The reaction was stirred for 20 min, then diethyl ether (4 ml) was added and the reaction refluxed for 40 min. On cooling to room temperature, diamide **8a** (1.0 g, 3.32 mmol) was added followed by ethanol (2 ml) and diethyl ether (2 ml). The mixture was stirred for 1 h, then benzoyl chloride (190 μl, 1.64 mmol) added. After stirring for 1 h, saturated sodium hydrogen carbonate (8 ml) was added and the mixture extracted with ethyl acetate (3×15 ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by column chromatography (20% ethyl acetate in light petroleum) gave **9a** as a white solid (0.48 g, 91%); m.p. 91°C; $\nu_{max}$ (thin film) 3292 (NH), 2966 (CH), 1686 (C=O), 1643 (C=O) cm$^{-1}$; $\delta_H$ (300 MHz, $CDCl_3$) 8.07 (2H, d, $J$=7.3 Hz, Ar), 7.60 (1H, t, $J$=7.4 Hz, Ar), 7.48 (2H, t, $J$=7.4 Hz, Ar), 6.63 (1H, bs, NH), 6.21 (1H, bs, NH), 5.66 (1H, bs, CH), 3.18 (2H, m, $CH_2N$), 1.52-1.44 (11H, m, $^t$Bu, $NCH_2CH_2$), 0.86 (3H, t, $J$=7.4 Hz, $CH_3$); $\delta_C$ (75 MHz, $CDCl_3$) 193.5 (s), 166.5 (s), 155.4 (s), 134.5 (s), 134.3 (d), 129.3 (d), 128.6 (d), 80.9

(s), 61.1 (d), 41.4 (t), 28.2 (q), 22.5 (t), 11.2 (q); $m/z$ ($CI^+$) 321 ($MH^+$), 265, 221; Observed ($MH^+$): 321.1821; $C_{17}H_{25}N_2O_4$ requires 321.1814.

9. All new compounds were fully characterised by standard spectroscopic and analytical techniques.
10. Partial NOE data for **13** (azinomycin numbering): Irradiation of H–16 enhanced H–5 (5.8%), H–13 and H–13′ (2.5%); simultaneous irradiation of H–13 and H–13′ enhanced H–16 (1.8%), H–12 (1.1%) and H–12′ (1.2%).
11. HPLC performed using a Daicel chiralpak AD column (0.7 ml $min^{-1}$; 17.5% IPA in hexane; $\lambda$ 254 nm): 23.5 min (major), 25.1 min (minor).
12. For the first application of this methodology, see: Hashimoto, M.; Matsumoto, M.; Yamada K.; Terashima, S. *Tetrahedron Lett*. **1994**, *35*, 2207–2210.
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