Access to the Bicyclic Core of Isatisine, and an Investigation of Its Antibacterial Activity

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Received 15 November 2010

Abstract: A chemoselective Dieckmann ring closure using an oxazolidine derived from serine may be used to generate a tetramic acid, the further manipulation of which by reduction and ring closure leads to the bicyclic core of isatisine; depending on the nature of the ring closing electrophile, different diastereomers are obtained. None of the compounds from this sequence exhibited activity against *S. aureus* but several showed activity against *E. coli*.

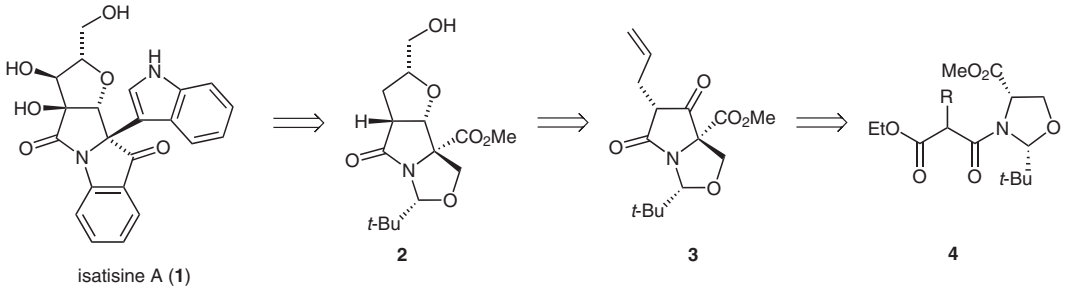
Key words: heterocycles, natural products, cyclisation, antibiotics, lactams

The discovery of new antibiotics has become imperative by reason of the emergence of bacterial strains resistant to current clinically-effective drugs,[1] and the urgency of the task has recently been emphasised.[2] However, since there is a paucity of New Chemical Entities (NCE) entering the antibacterial pipeline,[3] a number of new strategies for more efficient drug development have been elaborated in recent years[4] and re-examination of the function and availability of natural products has been pivotal.[5] In particular, it has recently become apparent that the physicochemical characteristics of antibacterial compounds differ sufficiently from the property space of commonly available compound libraries that re-investigation of natural products to identify novel antibacterials is fully justified[6] and that the structural information thus obtained will be useful for the design of NCE suitable for fragment-based drug design.[7] Libraries based upon such natural product leads have several key benefits which do not apply to combinatorially-derived systems: they will have benefited from the optimisation of bioactivity for a given receptor as a result of natural selection; they will be expected to provide an enhanced rate of positive hits for a given library size; they are more likely to provide novel structural chemotypes not currently in use in existing therapeutic regimes; they would not be immediately susceptible to resistance-conferring genes in the bacterial and DNA pools and they are likely to provide novel new target proteins and receptors.[8] Mindful of the long-standing application of traditional Chinese medicine,[9] and to develop further the compound and bioactivity space encompassed by pyrrolidin(on)es as commonly occurring core structural components of natural products,[10–12] we examined isatisine A as an unusual lead structure. Isatisine A (**1**) is a structurally novel alkaloid shown to have moderate anti-HIV activity (EC_{50} 37.8 μM) and cytotoxicity against C8166 cells (CC_{50} 302 μM).[13] This compound was first isolated from the leaves of the Chinese plant *Isatis indigotica* Fort. (cruciferae) in 2007, and the roots and leaves of *I. indigotica* have been used in traditional Chinese medicine for the treatment of bacterial and viral infections, and immunoregulatory and tumour diseases.[14] The first total synthesis of (+)-isatisine A was completed earlier this year by Karadeolian and Kerr in 14 steps with an overall yield of 5.8%.[15]

Retrosynthetic analysis (Scheme 1) of isatisine **1** suggested that functional group simplification could give tricycle **2**, which would in turn be readily generated from tetramic acid **3** by reduction and cyclisation; such systems are available from oxazolidines **4** using our previously published approaches by aldol[16] or Dieckman[17] cyclisation. An initial investigation of this strategy (Scheme 2) using oxazolidine **7a** (prepared by acylation of oxazolidine **5** with the malonate half ester **6a**) involved allylation using cinnamyl bromide to give **7b**, confirmation of the relative stereochemistry being readily achieved by single crystal X-ray analysis.[18] Chemoselective Dieckmann cyclisation gave tetramate **8a** in 63% yield. Reduction by a well-established[19] procedure using $NaBH_4$–AcOH gave epimeric alcohols **10a** and **11a** in a ratio of 1:1.5 and modest overall yield (relative stereochemistry assigned on the basis of nOe data; see Supporting Information). This stereochemical outcome resulted from epimerisation at the acidic C(7) position, although fully diastereoselective *endo*-hydride addition at C-6 was observed.[20] Epoxidation (MCPBA) of **10a** and **11a** proceeded without diastereocontrol to give **12** and **13**, both in good yield, and ring closure of **12** under basic conditions gave the desired tricyclic core **14** as a mixture of four inseparable diastereomers, as shown by NMR and MS analysis. As expected, similar treatment of epimeric epoxide **13** returned unreacted starting material. This approach demonstrated that a strategy based upon cycloetherification was possible, but that improvements in yield and diastereoselectivity would be required.

To develop this approach further, allylation of oxazolidine **7a** proved to be possible, but the yield was only 36%, and we found that the synthesis of the required allyl derivative **7c** could be better achieved in 58% yield by acylation of oxazolidine **5** with ethyl allylmalonate **6b** directly (prepared by partial hydrolysis of diethyl allylmalonate) using

SYNLETT 2011, No. 3, pp 0378–0382
Advanced online publication: 19.01.2011
DOI: 10.1055/s-0030-1259330; Art ID: D30410ST



Scheme 1

DCCI–DMAP, conditions which we found to be superior to a similar recently reported coupling using MsCl–Et_3N.[21] Dieckmann ring closure of oxazolidine **7c** to give allyl tetramate **8b** proceeded smoothly and in excellent yield (92%), but column chromatographic purification required the use of alumina, since silica gel led to epimerisation at C(7); the relative stereochemistry was determined by an NOE experiment (see Supporting Information). Moreover, we found that variable quantities of **9** were also obtained from this cyclisation, presumably as a result of adventitious water leading to ester hydrolysis and decarboxylation, and this material could not be separated from the major product **8b**; careful exclusion of water successfully prevented this side reaction, as has been noted previously.[17] Application of the above reduction procedure using excess $NaBH_4$–AcOH led to formation of alcohols **10b** and **11b** (ratio 2:1), along with significant recovery of starting material **8b** (18–41%). Alternative conditions were investigated (2–15 equiv of $NaBH_4$ with addition of $CeCl_3$ or $CaCl_2$; use of $LiBH_4$), but selective formation of **10b** could not be achieved. The relative stereochemistries of **10b** and **11b** were readily established by NOE experiments (see Supporting Information). Attempted conversion of **11b** to **10b** by deprotonation (LDA, –78 °C) and low temperature quench with ammonium chloride gave a 49% recovery of **11b**, along with some of the desired alcohol **10b** (13% isolated yield).

With the desired allylpyroglutamate **10b** in hand, investigation of electrophilic tetrahydrofuran ring closure was investigated (Scheme 2). Treatment with I_2–$NaHCO_3$ resulted in 5-*exo*-*tet* ring closure giving diastereomers **15a** and **16a** in a ratio of 10:3 (61% yield). The two epimers were inseparable by column chromatography, but careful recrystallisation (CH_2Cl_2–*n*-heptane) provided access to the pure major epimer **15a**, whose structure was determined by an NOE experiment and single crystal X-ray analysis (see Supporting Information and Figure 1).[18] Reaction of the **15a/16a** mixture with potassium *p*-nitrobenzoate and a catalytic amount of 18-crown-6 gave inseparable benzoates **15b/16b** in 31% overall yield, and another separable by-product **17** (24%), presumably arising by elimination from **15b/16b** followed by double bond equilibration. Hydrolysis of the mixture of esters **15b/16b** proceeded smoothly under basic conditions and furnished alcohols **15c/16c** in quantitative yield. Unsurprisingly, epimeric alcohol **11b** did not ring close under these conditions, since this would have led to a *trans*-fused bicyclic ring system.

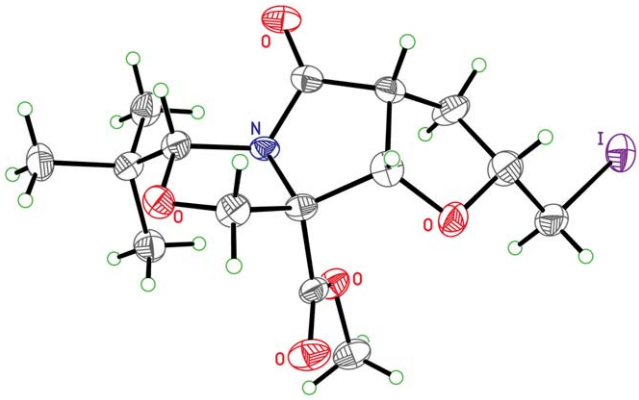
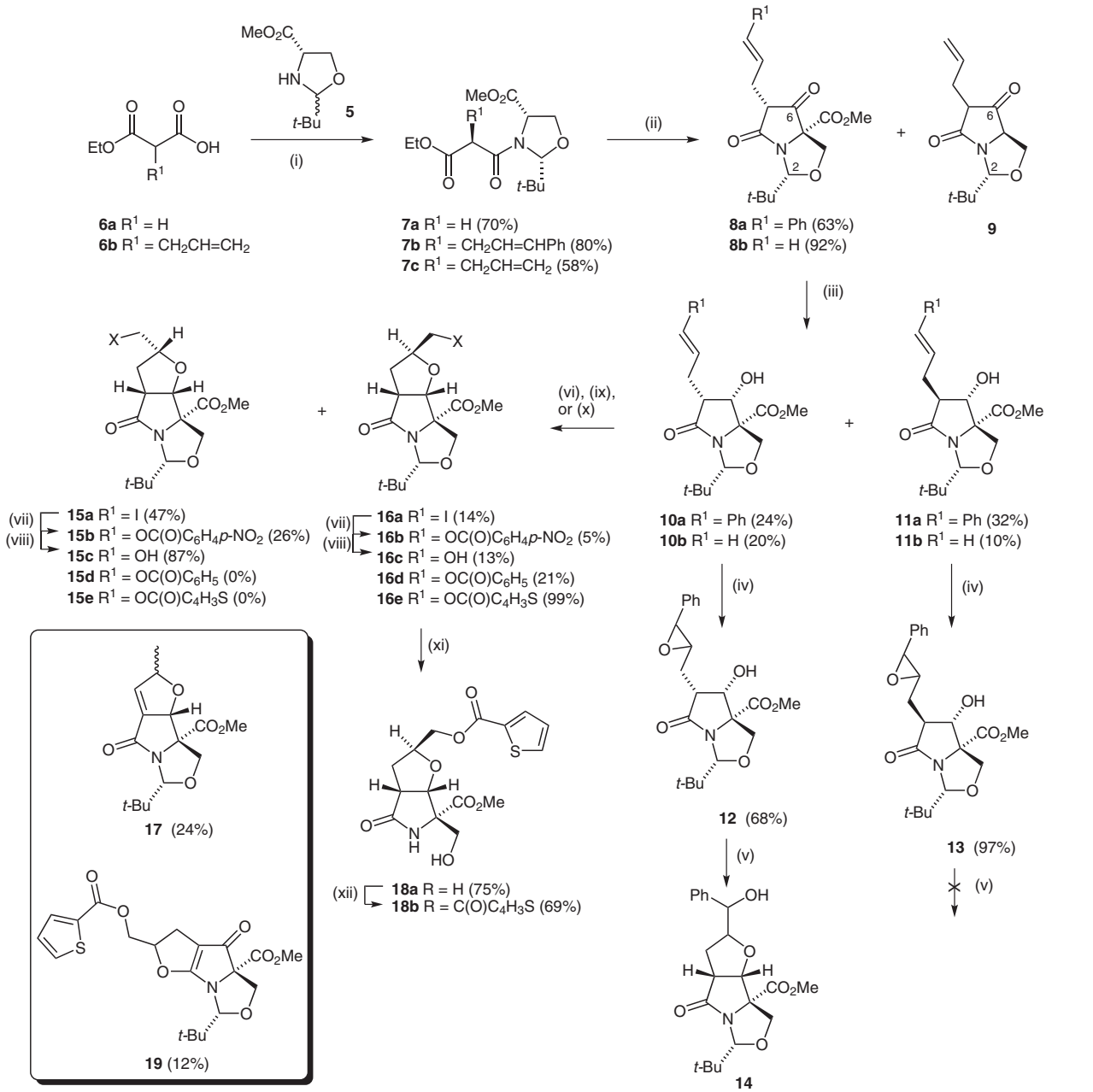


Figure 1 Thermal ellipsoid plot of **15a** with the heteroatoms labelled and drawn at 50% probability

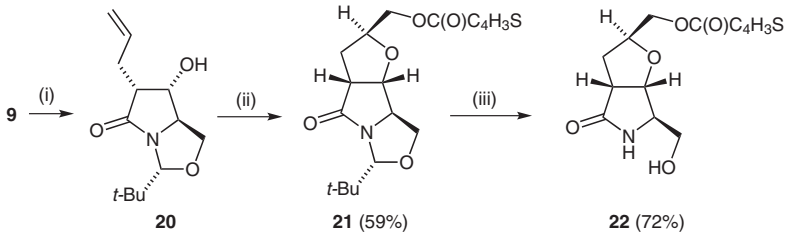
With the expectation that this route might be shortened by one step, it was of interest to apply our recently published protocol for plumbolactonisation,[22] a modification of which was found to be also suitable for plumboetherification;[23] we found that cyclisation of tetramate **10b** using lead tetrabenzoate or lead tetra(thiophene-2-carboxylate) gave the desired products, but only as the unwanted diastereomers **16d** and **16e**, respectively (yields of 21% and 99%) with no trace of the other stereoisomers **15d** and **15e**; relative stereochemistry being again established by NOE experiments (see Supporting Information). We have observed that the thiophene-2-carboxylate system gives better yields and is also much more reactive than other arylcarboxylates in these cyclisations[22–24] and believe that the change in stereochemical outcome in this case arises because the reaction proceeds by initial co-ordination of the C(6) hydroxy function rather than the alkene to the lead(IV) followed by *syn*-oxyplumbylation.[25] Deprotection of **16e** using Corey–Reichard conditions[26] gave the bicyclic product **18a** in excellent yield (75% yield) and this material was readily converted to diester **18b** by DCC–DMAP coupling. Once again, epimeric alcohol **11b** did not ring close under these conditions, but of interest is that tetramate **8b** cyclised to give tricycle **19**, whose structure was established by NMR analysis, although it was formed in only 12% yield.



Scheme 2 *Reagents and conditions*: (i) EDAC or DCC, DMAP, **5**; (ii) NaOMe, MeOH, r.t., 3 d; (iii) $NaBH_4$ (15 equiv), AcOH (10%), CH_2Cl_2, r.t.; (iv) MCPBA, CH_2Cl_2; (v) K_2CO_3, MeOH; (vi) I_2, $NaHCO_3$, MeCN; (vii) $KO_2CC_6H_4NO_2$, 18-C-6, MeCN; (viii) Na_2CO_3, H_2O, EtOH; (ix) $(PhCO_2)_4Pb$, F_3CPh; (x) $(C_4H_3SCO_2)_4Pb$, F_3CPh; (xi) $HS(CH_2)_3SH$, F_3CCH_2OH, r.t.; (xii) DCC, DMAP, $C_4H_3SC(O)Cl$.

In those reactions in which tetramate **9** was formed along with **8b** (Scheme 2), reduction of the mixture permitted isolation of alcohol **20** (Scheme 3) and plumboetherification gave tricycle **21** (59% yield). Assignment of the formation of the five-membered ring and the stereochemistry was made by comparison of the chemical shifts with those of product **16e** [C(8)H, the two C(9)H and the two C(12)H have very similar chemical shifts in both compounds] and deprotection to lactam **22** proceeded efficiently (72% yield).

Of interest is that bioassay[27] against *S. aureus* and *E. coli* indicated that no compounds were active against the former, but that several were active against the latter, typically with a potency of about 0.05–0.07% of the cephalosporin standard; this outcome is of interest given that Gram-negative bacteria are intrinsically more resistant to antibacterials as a result of a more impermeable outer cell membrane, and particularly efficient efflux pumps.[6] Bioactivity and chemical informatics[28] analysis is of interest (see Table in Supplementary Information). Although oxazolidines **7c**, tetramates **8a**,**8b**, pyroglutamates **10a**,**b**, **11a**,**b**, **12**, **13** and **18b** are active against *E. coli*, tricycles **15a**, **16a**, **16d** and **16e** (which might be considered most closely to mimic the core ring system of isatisine) are totally inactive against both organisms. For the active mol-



Scheme 3 *Reagents and conditions*: (i) $NaBH_4$, AcOH (10%), CH_2Cl_2, r.t.; (ii) $(C_4H_3SCO_2)_4Pb$, F_3CPh; (iii) $HS(CH_2)_3SH$, F_3CCH_2OH, r.t.

ecules, the van der Waals molecular surface area is in the range 452–587 Å^2,[29] and the CMR value, which is a measure of the van der Waals attractive forces that act in drug-receptor interactions[30] is in the range 74–107; this is to be expected from the common structural skeleton of these compounds. The active molecules have ClogD, %PSA and CMR average values of 2.5±0.76, 14.5±1.3 and 91.6±12.9, respectively. The polar surface area parameter (PSA), which correlates the presence of polar atoms with membrane permeability and therefore gives an indication of drug transport properties,[31] has been reported to have an optimal value of 70 < PSA < 120 Å^2 for a non-CNS orally absorbable drug,[32] and of interest is that the compounds active against *E. coli* all lie at the lower end of this range, with the exception of diester **18b**. The use of this approach, guided by bioactive natural product structures, is likely to be valuable for the characterisation of chemical space[33] and may provide a useful start point for the identification of novel chemotypes for fragment-based drug design, especially where target identity or structure is not known.[34]

We have recently demonstrated the low intrinsic antibacterial activity of simple pyroglutamates[12] and tetramates,[11] but that modest manipulation of the skeletal functionality[10,35] or homologation to longer chain side-units[36] can restore activity. It may be that similar adjustment of the tricyclic skeletons synthesised in this work will be required before antibacterial bioactivity is observed; however, it is noted that no antibacterial activity of isatisine **1** *per se*, as opposed to the plant extracts, has been reported,[13] and it is possible that the reported antibacterial plant extract activity may not result from this compound alone.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(18) The diffraction data for **7b** and **15a** were collected at 150 K[37] using an Enraf-Nonius KCCD diffractometer.[38] Structures were solved using SIR92,[39] and refined using the CRYSTALS software suite[40] as per the supplementary information (CIF file). The Flack *x* parameter [41] for **7b** refined to –0.2 (8), however analysis of the Bijvoet pairs gave a Hooft *y* parameter[42] of 0.0(3) giving a 99.2% probability that the structure is of the correct handedness (assuming full enantiopurity).[43] In the absence of a strong anomalous signal, the Friedel pairs were merged for the final refinement. The Flack *x* parameter for **15a** refined to –0.02 (3). Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre [CCDC 800835 (**7b**), CCDC 800836 (15a)] and copies of these data can be obtained via www.ccdc.cam.ac.uk/data_request/cif.
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