Stereoselective Assignment of the Protein–Protein Interaction Inhibitor JBIR-22 by Total Synthesis**

Alan R. Healy, Miho Izumikawa, Alexandra M. Z. Slawin, Kazuo Shin-ya, and Nicholas J. Westwood*

Abstract: Recent reports have highlighted the biological activity associated with a subfamily of the tetramic acid class of natural products. Despite the fact that members of this subfamily act as protein–protein interaction inhibitors that are of relevance to proteasome assembly, no synthetic work has been reported. This may be due to the fact that this subfamily contains an unnatural 4,4-disubstituted glutamic acid, the synthesis of which provides a key challenge. A highly stereoselective route to a masked form of this unnatural amino acid now enabled the synthesis of two of the possible diastereomers of JBIR-22 and allowed the assignment of its relative and absolute stereochemistry.

Natural products that contain the tetramic acid motif have been studied extensively, and their complexity and biological profiles have led to several total syntheses.[1] For example, equisetin, a close structural analogue of the compounds studied here, has been prepared.[1a–c] However, the synthesis of members of a subfamily that contain an unnatural 4,4-disubstituted glutamic acid unit (1–4, Figure 1) is an unmet challenge.[2] The biological activity displayed by members of this subfamily justifies the development of a concise and general approach for their synthesis.

Examples of the important activity shown by this subfamily include the inhibition of the CCR5 receptor by JBIR-22 (2).[2c,d] A number of CCR5 receptor antagonists are in clinical trials or in use as antiretroviral drugs.[3, 4] In addition, JBIR-22 (2) is the first example of a tetramic acid that acts as a protein–protein interaction (PPI) inhibitor.[2c,d] Compound 2 inhibits the homodimerization of the proteasome assembly chaperone 3 (PAC3), an important protein involved in the formation of the proteasomal machinery. The clinical success of bortezomib,[5] a proteasome inhibitor, supports the study of compounds that target the proteasome or its formation. The fact that the stereosechemical assignment of 2[2a] was incomplete when our work began further highlights the need for synthetic studies on this subfamily of tetramic acids.

Although chemical[6] and enzymatic[7] syntheses of 4-hydroxy-4-methylglutamic acid have been developed, a synthesis of 4-hydroxy-4-iso-propylglutamic acid has not yet been reported, which could be a factor in the lack of synthetic work done on this subfamily. Here we report a short, stereoselective synthesis of a 4,4-disubstituted glutamic acid derivative and the application of this methodology to the first total synthesis of 2. Our studies enabled the assignment of the relative and absolute stereochemistry of 2.

Our initial synthetic plan was based on the synthesis of 1,3-amino alcohols (e.g. 5). This methodology involves the diastereoselective addition of a metalloenamine 6 to an aldehyde followed by diastereoselective imine reduction (Scheme 1).[6] We proposed that the reaction of 7 with ethyl dimethylpyruvate could establish the required stereogenic center of the tertiary alcohol. Subsequent diastereoselective reduction of the resulting 3-hydroxy-N-sulfinyl ketimine 8 could give 9, a precursor of a protected form of the unnatural amino acid 10 (Scheme 1). If accessible, 10 could potentially be used in the synthesis of 2 in an analogous manner to that previously demonstrated for other tetramic acids containing...
natural amino acids, such as equisetin\(^{[1a–c,9]}\). It also seemed plausible that the tertiary alcohol in 8 or 9 may cyclize to generate a lactone (e.g. 11 from 9). If this occurred, N-methylation of 11 and removal of the N-sulfinyl group could give the masked 4,4-disubstituted glutamic acid derivative 12.

Conversion of 12 to members of this subfamily was considered achievable.

The synthesis of 7 was achieved by condensation of \((R\text{)}_2\) tert-butanesulfinamide with ethyl pyruvate (8). Using the reported conditions\(^{[10]}\), 7 was obtained in only 30\% yield with the major product being lactone 13 (13:7 = 5:3, Scheme 2). The formation of 13 likely occurred in situ through an \(\text{Ti(OEt)}_2\)-catalyzed aldol reaction of 7 with ethyl pyruvate (8) followed by lactonization (Scheme S1). Although 13 was not required for the preparation of 2, it could be used in a future synthesis of 1. Optimization of the synthesis of 7 resulted in its isolation in 60\% yield (Table S1). Reaction of 7 with ethyl dimethylpyruvate gave the related lactone 14 (Scheme 2) with excellent diastereoselectivity and yield. As expected, 14 was confirmed as the \((R\text{)}_2\text{S}\text{)}_2\) diastereomer by X-ray analysis (Scheme 2).\(^{[8,11]}\) N-methylation of 14 proceeded in high yield to provide 15. While an initial screening of reducing agents gave only recovered lactone 15, the use of \(\text{NaBH}_3\text{CN} \text{ with HCl (4N in dioxane)}\) resulted in the diastereoselective (d.r. > 98\%) reduction of 15 with cleavage of the N-sulfinyl group to give 12 (Scheme 2). The stereochemistry of 12 was assigned by NOE analysis (Scheme 2 and Figure S1). Further analysis suggested that this reaction proceeded by acid deprotection of the N-sulfinyl group followed by the reduction with \(\text{NaBH}_3\text{CN}\) (Scheme S2). The observed diastereoselectivity was rationalized based on the preferred approach of the reducing agent from the same side as the ester. This efficient route provided the masked 4-hydroxy-4-isopropyl glutamic acid 12 in just four steps from 8.

With 12 in hand, a synthesis of 2 was attempted because of its unique activity as a PPI inhibitor and the uncertainty associated with its stereochemical assignment. Izumikawa et al. had shown that 2 could be assigned as one of the four stereoisomers shown in Table 1 (diastereomers 2a and 2b and their enantiomers 2c and 2d).\(^{[2c]}\) Given the relatively large distance between the decalin moity and the unnatural amino acid stereogenic center in 2, it is difficult to assign the relative configuration of these two units. A convergent route to access optically enriched samples of diastereomers 2a and 2b was therefore investigated (Scheme 3).

The tetramic acid core in 2a would be formed at a late stage, inspired by the conversion of 3-oxo-homoserine
lactones to simple tetramic acids through a Claisen-like intramolecular reaction (Scheme S3). A Lacey–Dieckmann condensation of fragment 16 would form the tetramic acid core and provide the unnatural 4,4-disubstituted glutamic acid side chain in one step. Fragment 16 could be accessible through the coupling of 12 and the β-ketothioester 17a. A late-stage convergent step such as this could ultimately facilitate the coupling of alternate β-ketothioesters to enable access to the other members of this subfamily (Figure 1) or novel analogues. We envisaged that the decalin β-ketothioester could be assembled through an asymmetric Diels–Alder cycloaddition followed by manipulation to introduce the thioester functionality (Schemes 4 and 5).

**Scheme 4.** Synthesis of 23. Reagents and conditions: a) KHMD, diethyl 2-butenylyphosphonate (19), DME, –78°C – RT, 69%, E: Z = 8:1; b) Aq, HCl, THF, RT, 12 h, 94%; c) (i) 1,3-dioxolan-2-ylmethyltriphenylphosphonium bromide (22), tBuOK, THF, 0°C, 3.5 h. (ii) 10% aq. oxalic acid, RT, 1 h, 89%.

Assembly of 17a-b began with an Schreiber ozonolysis of cyclohexene to give acetal 18. Horner–Wadsworth–Emmons (HWE) olefination of 18 using phosphonate 19 provided 20 (8:1 mixture of inseparable E:E:E,Z isomers, Scheme 4). The acid-mediated deprotection of 20 gave dienal 21, which was reacted with Wittig reagent 22, followed by acetyl hydrolysis to give the trienal 23 (85% E:E:E geometry). Trienal 23 was then subjected to an organocatalytic intramolecular Diels–Alder (IMDA) reaction using MacMillan’s conditions (Scheme 5). Both enantiomers of 24 were accessed with good enantioselectivities (see Scheme 5 and the Supporting Information for chiral GC analysis). The minor E:E:E isomer present in the sample of 23 was inert in this IMDA reaction, thus enabling the purification to give either 24a or 24b, depending on which enantiomer of the organocatalyst was used (Scheme S4).[14] Elaboration of 24a and 24b to give β-ketothioesters 17a and 17b, respectively, was achieved through an aldol reaction using S-tert-butyl thioacetate to give 25a or 25b, respectively, as an inconsequential mixture of diastereomers, followed by oxidation with Dess–Martin periodinane (Scheme 5).[15]

The final stages involved a silver trifluoroacetate mediated coupling of 12 with either enantiomer of fragment 17 to give 26a and 26b, following the protocol developed by the Ley group for the synthesis of equisetin (Scheme 6).[16,17] Finally, cyclization onto the lactone in 26a and 26b and microwave-assisted ester hydrolysis gave separate samples of the optically enriched diastereomers 2a and 2b, which were purified by reverse-phase chromatography. No evidence of epimerization at the Cs’ position was observed.[18]

The assignment of the relative stereochemistry of 2 was completed by comparison of the reported spectroscopic data[19] for 2 with those obtained for our synthetic samples of 2a and 2b. This analysis revealed very similar 1H NMR signals, but clear differences in the 13C NMR spectra, with the signals reported for the isolated sample of 2 all being within ±0.1 ppm of those obtained for diastereomer 2a. In contrast, there were significant differences when the data was compared to that for diastereomer 2b (Figure 2 for selected examples and Table S2). Further evidence for the identical relative stereochemistry in 2 and diastereomer 2a came from the optical purity of a sample of natural 2 (retention time = 3.3 min) with 2a, an increase in the size of the peak at 3.3 min was observed, whereas doping of natural 2...
future synthesis and biological assessment of all members of the convergent nature of this approach should facilitate the development of a short, stereoselective synthesis of occurring protein–protein interaction inhibitor both the relative and absolute configuration of the naturally two of the possible stereoisomers facilitated the assignment of 10.1% and 11.3% overall yield, respectively. The synthesis of sized in ten steps (longest linear route from cyclohexene) in vergent strategy. The diastereomers

![Figure 2](image_url)

**Figure 2.** A) UPLC-TOFMS doping experiment. B) Selected $^{13}$C NMR signals of 2a and 2b with 2a/b (a 1:1 mixture of 2a and 2b synthesized following an alternative route, Scheme S5). C) Selected $^1$H NMR signals of 2a and 2b with 2a/b. D) Selected $^{13}$C NMR chemical shifts of isolated 2a and 2b (see Supporting Information for full table). UPLC-TOFMS-ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry.

[2] Sch210972 (1): a) S. Yang, R. Mierzwa, J. Terracciano, M. Patel, V. Gullo, N. Wagner, B. Baroudy, M. Puar, T. Chan, M. Chu, J. Antibiot. **2007**, 60, 524–528; b) S.-W. Yang, R. Mierzwa, J. Terracciano, M. Patel, V. Gullo, N. Wagner, B. Baroudy, M. Puar, T.-M. Chan, A. T. McPhail, et al., J. Nat. Prod. **2006**, 69, 1025–1028; J-BIR-22 (2) by a concise, convergent strategy. The diastereomers 2a and 2b were synthesized in ten steps (longest linear route from cyclohexene) in 10.1% and 11.3% overall yield, respectively. The synthesis of two of the possible stereoisomers facilitated the assignment of both the relative and absolute configuration of the naturally occurring protein–protein interaction inhibitor 2. The development of a short, stereoselective synthesis of 12 coupled with the convergent nature of this approach should facilitate the future synthesis and biological assessment of all members of this subfamily of natural products as well as novel analogues.

Chen, W. R., Roush, Org. Lett. **2012**, 14, 426–428; T. Yoshinari, K. Ohmori, M. G. Schrems, A. Pflaltz, K. Suzuki, Angew. Chem. Int. Ed. **2010**, 49, 881–885; Angew. Chem. **2010**, 122, 893–897; k) O. Hartmann, M. Kalesse, Angew. Chem. Int. Ed. **2014**, 53, 7335–7338; Angew. Chem. **2014**, 126, 7463–7466.

In summary, a highly stereoselective synthesis of the masked 4,4-disubstituted glutamic acid 12 enabled the first total synthesis of highly enantiomeric samples of two of the possible diastereomers of JBIR-22 (2) by a concise, convergent strategy. The diastereomers 2a and 2b were synthesized in ten steps (longest linear route from cyclohexene) in 10.1% and 11.3% overall yield, respectively. The synthesis of two of the possible stereoisomers facilitated the assignment of both the relative and absolute configuration of the naturally occurring protein–protein interaction inhibitor 2. The development of a short, stereoselective synthesis of 12 coupled with the convergent nature of this approach should facilitate the future synthesis and biological assessment of all members of this subfamily of natural products as well as novel analogues.
Rotshteyn, A. J. Marozsan, et al., *Antimicrob. Agents Chemother.* 2010, 54, 4137 – 4142.

[4] a) Q. Tan, Y. Zhu, J. Li, Z. Chen, G. W. Han, I. Kufareva, T. Li, L. Ma, G. Fenalti, J. Li, et al., *Science* 2013, 341, 1387 – 1390; b) B. L. Gillam, D. J. Riedel, R. R. Redfield, *J. Transl. Med.* 2011, 9 Suppl 1, S9.

[5] a) J. Adams, M. Kauffman, *Cancer Invest.* 2004, 22, 304 – 311; b) A. L. Goldberg, *J. Cell Biol.* 2012, 199, 583 – 588.

[6] S. Baldwin, A. Long, *Org. Lett.* 2004, 6, 1653 – 1656.

[7] a) V. Helaine, J. Bolte, *Tetrahedron: Asymmetry* 1998, 9, 3855 – 3861; b) V. Helaine, J. Rossi, T. Gefflaut, S. Alaux, J. Bolte, *Adv. Synth. Catal.* 2001, 343, 692.

[8] a) T. Kochi, T. Tang, J. Ellman, *J. Am. Chem. Soc.* 2002, 124, 6518 – 6519; b) T. Kochi, T. P. Tang, J. Ellman, *J. Am. Chem. Soc.* 2003, 125, 11276 – 11282.

[9] For reviews see: a) B. J. L. Royles, *Chem. Rev.* 1995, 95, 1981 – 2001; b) R. Schobert, A. Schlenk, *Bioorg. Med. Chem.* 2008, 16, 4203 – 4221; c) R. Schobert, *Naturwissenschaften* 2007, 94, 1 – 11.

[10] L. Reddy, A. Gupta, Y. Liu, *J. Org. Chem.* 2011, 76, 3409 – 3415.

[11] CCDC1034467 (14) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[12] a) G. F. Kaufmann, R. Sartorio, S.-H. Lee, C. J. Rogers, M. M. Meijler, J. A. Moss, B. Clapham, A. P. Brogan, T. J. Dickerson, K. D. Janda, *Proc. Natl. Acad. Sci. USA* 2005, 102, 309 – 314; b) C. A. Lowery, J. Park, C. Gloeckner, M. M. Meijler, R. S. Mueller, H. I. Bosshoff, R. L. Ulrich, C. E. Barry, D. H. Bartlett, V. V. Kravechenko, et al., *J. Am. Chem. Soc.* 2009, 131, 14473 – 14479.

[13] a) S. Schreiber, R. Claus, J. Reagan, *Tetrahedron Lett.* 1982, 23, 3867 – 3870; b) R. E. Claus, S. L. Schreiber, *Org. Synth.* 1986, 64, 150.

[14] R. M. Wilson, W. S. Jen, D. W. C. Macmillan, *J. Am. Chem. Soc.* 2005, 127, 11616 – 11617.

[15] D. B. Dess, J. C. Martin, *J. Org. Chem.* 1983, 48, 4155 – 4156.

[16] S. V. Ley, P. R. Woodward, *Tetrahedron Lett.* 1987, 28, 3019 – 3020.

[17] CS epimerization was reported in the total synthesis of equisetin, see Ref. [1b]; for further discussion, see Refs. [9a,c] and a) J. Poncet, P. Jouin, B. Castro, L. Nicolas, M. Boutilier, A. Gaudemer, *J. Chem. Soc. Perkin Trans. 1* 1990, 611; b) U. Marquardt, D. Schmidt, G. Jung, *Synlett* 2000, 1131 – 1132; c) S. V. Ley, C. S. Smith, P. R. Woodward, *Tetrahedron* 1992, 48, 1145 – 1174.

[18] The reported specific rotation of natural 2 in this manuscript ($\alpha_{D}^{	ext{23}} = +62.0^{\circ}, c = 0.1, \text{MeOH}$) is a correction of the originally reported specific rotation reported, see Ref. [2e].

Received: November 17, 2014
Published online: February 4, 2015