Short and efficient diastereoselective synthesis of pyrrolidinone-containing dipeptide analogues†

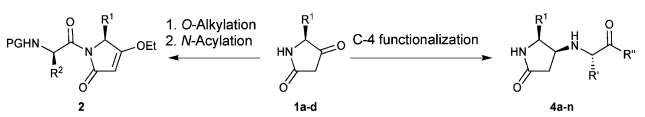
Masood Hosseini,[a,b] Jakob S. Grau,[a] Kasper K. Sørensen,[a] Inger Søtofte,[a] David Tanner,[a] Anthony Murray[b] and Janne E. Tønder*[a,b]

Received 3rd April 2007, Accepted 25th May 2007
First published as an Advance Article on the web 7th June 2007
DOI: 10.1039/b705093c

The pyrrolidine-2,4-diones have been identified as a convenient starting point for the synthesis of peptide analogues. Herein we describe an optimized two-step reductive amination procedure, which provides a small library of pyrrolidinone-containing dipeptide analogues in high yield and excellent diastereoselectivity.

In the last decade, peptide drugs have received renewed interest due to their high activity and specificity.[1] Recently Fuzeon (enfuvirtide, T-20), the first peptide drug produced by large scale solid phase peptide synthesis, has been introduced to the market. However, peptide drugs are known to display only a very low bioavailability when administered orally and their use as pharmaceuticals would be greatly enhanced if their biostability was improved. While many novel bicyclic scaffolds for dipeptide analogues have been developed,[2] only a few amino acid analogues exist; the most prominent being β-amino acids,[3] α-aminoxy acids,[4] and the peptoids.[5] Current research in our group aims at developing new amino acid analogues that can be synthesized readily and incorporated into peptides, thereby increasing the biostability of the latter.

We have focused on 5-substituted 2,4-pyrrolidinediones[6] (**1**), also known as tetramic acids (Scheme 1), which can be synthesized in gram scale from cheap and commercially available *N*-protected amino acids.[7] Furthermore, we can preserve the stereogenic center in the side chain, which is vital for interaction with biological targets. In our work towards incorporating pyrrolidinones into peptides, we have recently published a method for *N*-acylation of *O*-alkylated 3-pyrrolin-2-ones (**2**) with activated amino acids[6] (Scheme 1, left). In the present paper we have turned our attention to the attachment of amino acids at the C-4 carbonyl (Scheme 1, right).[8]



Scheme 1 Functionalization of pyrrolidinediones with amino acids.

[a]*Department of Chemistry, Technical University of Denmark, Building 201, DK-2800 Kgs. Lyngby, Denmark*
[b]*Medicinal Chemistry Research, Novo Nordisk A/S, Novo Nordisk Park, DK-2760, Måløv, Denmark. E-mail: jejr@novozymes.com*
† Electronic supplementary information (ESI) available: Experimental details and spectra. See DOI: 10.1039/b705093c

Based on reports describing the reductive alkylation of amino acids[9] and amino acid derivatives,[10] we investigated different reaction conditions, but found that direct reductive amination was not possible for the pyrrolidinediones. Instead we had to employ a stepwise procedure comprising enamine formation followed by reduction. The amino acid analogues *py*Val,[8] *py*Leu, *py*Phe and *py*Ala were prepared according to our in-house modification[6] of a literature procedure,[11] by which multi gram synthesis can be performed in less than a day. Initial tests of the condensation reaction showed that it was successful for amino acids protected at the carbonyl carbon as esters or amides, but not for unprotected amino acids.

By screening different solvents (THF, dioxane, toluene, DCE, MeCN, i-PrOH, DMF and MeOH) we found that the optimal solvent was i-PrOH with the addition of AcOH. MeCN–AcOH solvent mixtures also furnished the product albeit with a slower reaction rate, while the use of MeOH led to decomposition of the starting material. All other solvents provided at best only trace amounts of the desired product.

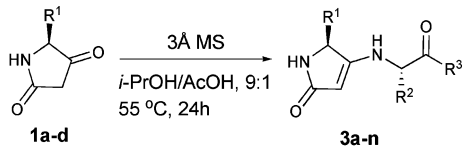
By using i-PrOH–AcOH as solvent at 55 °C for 24 h, we could obtain good to excellent yields of the enamines with no epimerization (Table 1), but we noted that epimerization did occur at higher temperatures. Varying the amount of AcOH co-solvent and the amount of added molecular sieves revealed that 5–10 vol% AcOH provided the highest reaction rate, just as increasing amounts of molecular sieves did. Use of $MgSO_4$ or Na_2SO_4 as dehydrating agents did not furnish any product.

The enamines obtained were sensitive to degradation on silica gel, however using dry flash chromatography[12] provided a fast and simple gram scale purification of the product.

With this procedure in hand, we synthesized a range of dipeptide enamine analogues comprising a pyrrolidinone and an amino acid protected as methyl and *t*-butyl esters or as amides (Table 1).

These condensations were facile reactions leading to the desired products in good yield and excellent d.e. They were furthermore stable solids with no decomposition being observed even after one year of storage on the shelf. After testing a range of reducing agents in the enamine reduction ($NaBH_3CN$,[13] $NaBH(OAc)_3$,[14] $NaBH_4$,[15] Stryker's reagent,[16] L-selectride,[17] $LiBH_4$, Zn–AcOH,[18] BH_3,[19] $Cu(OAc)_2$–PMHS–Binap,[20] H_2 with Pd/C,[21] Raney Nickel,[21] Rh/Al[22] or PtO_2[15]) we found that only two reagents ($NaBH_3CN$ and H_2/PtO_2) gave the desired product, all others left the starting material unchanged. In reactions with free or polymer supported $NaBH_3CN$,[23] the diastereoselectivity varied substantially (6–99% d.e.) depending on the nature of the side-chains, the main product having the expected[11] *cis*-conformation with yields ranging from 29–65%. These results

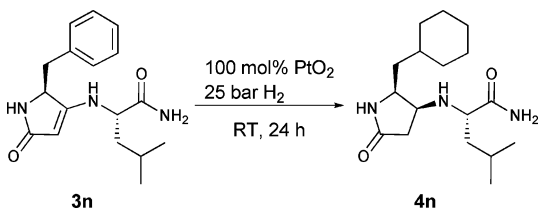
Table 1 Synthesis of enamine dipeptide analogues



Compound	R^1	R^2		Yield (%)[a]
*py*Ala-Ala-NH_2	Me	Me	(**3a**)	95
*py*Ala-Val-NH_2	Me	i-Pr	(**3b**)	88
*py*Ala-Leu-NH_2	Me	i-Bu	(**3c**)	85
*py*Val-Ala-NH_2	i-Pr	Me	(**3d**)	88
*py*Val-Val-NH_2	i-Pr	i-Pr	(**3e**)	82
*py*Val-Leu-NH_2	i-Pr	i-Bu	(**3f**)	91
*py*Val-D-Ala-NH_2	i-Pr	Me	(**3g**)	86
*py*Val-Ala-OMe	i-Pr	Me	(**3h**)	89
*py*Val-Ala-O*t*-Bu	i-Pr	Me	(**3i**)	93
*py*Val-Pro-NH_2	i-Pr	—	(**3j**)	88
*py*Leu-Ala-NH_2	i-Bu	Me	(**3k**)	88
*py*Leu-Val-NH_2	i-Bu	i-Pr	(**3l**)	86
*py*Leu-Leu-NH_2	i-Bu	i-Bu	(**3m**)	87
*py*Phe-Leu-NH_2	Bn	i-Bu	(**3n**)	88

[a] Only one diastereomer was observed by ^{1}H NMR (d.e. >95%).

Table 2 Reduction of *py*Phe-Leu-NH_2 in protic and aprotic solvents



Entry	Solvent	Conversion (%)[a]
1	MeOH	>95[b,c]
2	i-PrOH	>95[b,c]
3	*t*-BuOH	>95[c]
4	MeCN	0[d]
5	THF	0[d]
6	Dioxane	0[d]

[a] Determined by ^{1}H NMR of the crude mixture. [b] Trace of byproducts. [c] Only one diastereomer was observed by ^{1}H NMR (d.e. >95%). [d] Only starting material observed.

were, however, not reproducible regardless of extensive attempts at optimizing the reaction conditions. In contrast, reduction with hydrogen over PtO_2 (Adams catalyst),[15] resulted in a very clean reaction and an excellent degree of diastereoselectivity for the expected *cis*-configuration. Performing a solvent screen[24] (Table 2), we identified alcoholic solvents as being necessary for the reduction to proceed, and the sterically hindered *t*-BuOH proved to be the solvent of choice (no byproducts), while MeOH and i-PrOH afforded trace amounts of unidentifiable byproducts. THF, dioxane and MeCN completely suppressed the reduction.

Studies of the amount of added PtO_2 revealed that 25 mol% was required for full conversion within reasonable reaction time (Fig. 1). To increase the reaction rate, the reaction mixture was heated gradually to 55 °C, and we observed that the onset of

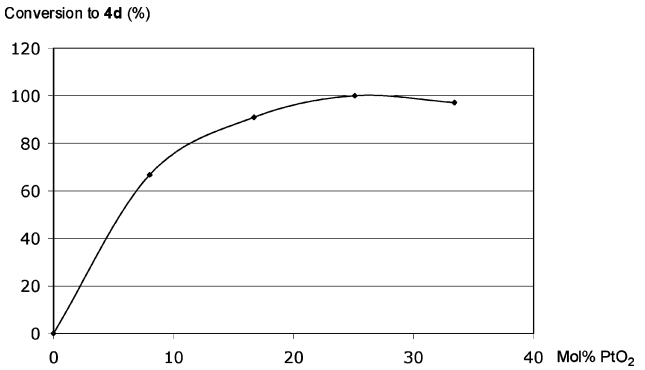


Fig. 1 Reduction of *py*Val-Ala-NH_2 (**3d**), at 24 h and 55 °C as a function of mol% PtO_2.

epimerization depended on the size of the pyrrolidinone side-chain (*py*Ala 25 °C, *py*Leu 35 °C, *py*Val/*py*Phe 55 °C).

To assess the effects of trace amounts of AcOH and amino acid from the condensation reaction, comparative studies were performed with the addition of either AcOH (10 mol%) or H-Val-NH_2 (100 mol%), which did not result in any change in reactivity in the reduction, where we anticipated an increase with AcOH as additive[25] and a decrease with H-Val-NH_2 as a result of coordination to the catalyst.

By using this stepwise reductive amination strategy, we reduced the enamine library compounds (**3a–n**) (Table 3) and synthesized a range of dipeptide analogues (**4a–n**).

In these reactions, the protective group of the amino acid (OMe, O*t*-Bu, NH_2) did not influence the d.e. nor was there any influence when proline was employed as the amino acid group. When *py*Phe was used as the pyrrolidinone part, the phenyl group could not be preserved and was reduced to the corresponding *c*-Hex group. Extensive variations of reaction conditions (solvent, temperature, H_2 pressure, addition of acid) did not lead to a chemoselective reduction. To our satisfaction the reduction of ^{cis}*py*Val-D-Ala-NH_2 still gave the *cis*-product, thereby showing that the diastereoselectivity only depends on the pyrrolidinone configuration. To validate the relative configuration of the products, we analyzed ^{cis}*py*Val-Pro-NH_2 (**4j**) by means of X-ray crystallography (Fig. 2),

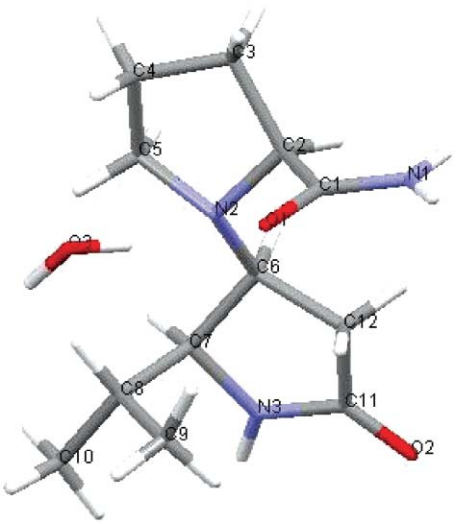
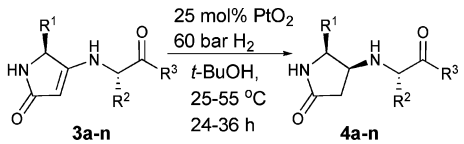


Fig. 2 Crystal structure of ^{cis}*py*Val-Pro-NH_2 (**4j**).

Table 3 Reduction of enamine dipeptide analogues



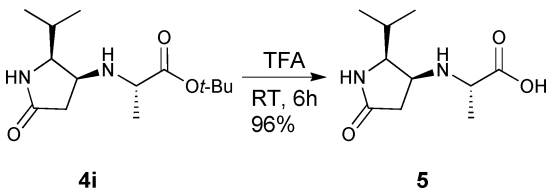
Compound		R^1	R^2	Temp/°C	Yield (%)[a]
^{cis}*py*Ala-Ala-NH_2	(**4a**)	Me	Me	25[b]	89
^{cis}*py*Ala-Val-NH_2	(**4b**)	Me	i-Pr	25[b]	94
^{cis}*py*Ala-Leu-NH_2	(**4c**)	Me	i-Bu	25[b]	89
^{cis}*py*Val-Ala-NH_2	(**4d**)	i-Pr	Me	55[c]	94
^{cis}*py*Val-Val-NH_2	(**4e**)	i-Pr	i-Pr	55[c]	99
^{cis}*py*Val-Leu-NH_2	(**4f**)	i-Pr	i-Bu	55[c]	94
^{cis}*py*Val-D-Ala-NH_2	(**4g**)	i-Pr	Me	55[c]	94
^{cis}*py*Val-Ala-OMe	(**4h**)	i-Pr	Me	55[c]	99
^{cis}*py*Val-Ala-O*t*-Bu	(**4i**)	i-Pr	Me	55[c]	94
^{cis}*py*Val-Pro-NH_2	(**4j**)	i-Pr	—	55[c]	99
^{cis}*py*Leu-Ala-NH_2	(**4k**)	i-Bu	Me	35[b]	89
^{cis}*py*Leu-Val-NH_2	(**4l**)	i-Bu	i-Pr	35[b]	84
^{cis}*py*Leu-Leu-NH_2	(**4m**)	i-Bu	i-Bu	35[b]	99
^{cis}*py*Cha-Leu-NH_2	(**4n**)	CH_2-*c*-Hex[d]	i-Bu	55[c]	99

[a] Only one diastereomer was observed by ^{1}H NMR (d.e. >95%). [b] Reaction time 36 h. [c] Reaction time 24 h. [d] R^1 is reduced from Bn to CH_2-*c*-Hex.

which confirmed the structural framework with the expected *cis*-relationship.‡

We are currently testing the biological stability of these dipeptide analogues and are developing a method for the preservation of the phenyl group when using phenylalanine or *py*Phe. Results will be reported in due course.

As we are interested in further elongation of the peptide chain at both the N- and C-termini we deprotected ^{cis}*py*Val-Ala-O*t*-Bu to ^{cis}*py*Val-Ala-OH by standard treatment with TFA, providing a starting point for further elongation at the C-terminal (Scheme 2).



Scheme 2 Deprotection of *py*Val-Ala-O*t*-Bu by TFA.

Conclusions

In conclusion we have developed a straightforward synthetic strategy for the synthesis of dipeptidomimetics comprising a pyrrolidinone and a protected amino acid. This method provides a facile functionalization of pyrrolidinones at the C-4 position in high yield and excellent d.e. *via* a stepwise reductive amination procedure, and we are currently exploring the incorporation of these new scaffolds into peptides.

Acknowledgements

This work was supported by the Lundbeck Foundation, Corporate Research Affairs at Novo Nordisk A/S, Torkil Holm's Foundation, The Danish Natural Science Research Council, The Augustinus Foundation, and Ib Henriksen's Foundation. We thank Ulrik Jørgensen for NMR at elevated temperatures.

Notes and references

‡ CCDC reference number 642750. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b705093c

1 A. Loffet, *J. Pept. Sci.*, 2002, **8**, 1.
2 (*a*) W. Maison and A. H. G. P. Prenzel, *Synthesis*, 2005, **7**, 1031; (*b*) S. Hanessian, G. McNaughton-Smith, H.-G. Lombart and W. D. Lubell, *Tetrahedron*, 1997, **53**, 12789; (*c*) L. Halab, F. Gosselin and W. D. Lubell, *Biopolymers*, 2000, **55**, 101; (*d*) T. E. Nielsen, S. Le Quement and M. Meldal, *Org. Lett.*, 2005, **7**, 3601; (*e*) J. M. Ndungu, X. Gu, D. E. Gross, J. Ying and V. J. Hruby, *Tetrahedron Lett.*, 2004, **45**, 3245; (*f*) M. Angiolini, S. Araneo, L. Belvisi, E. Cesarotti, A. Checchia, L. Crippa, L. Manzoni and C. Scolastico, *Eur. J. Org. Chem.*, 2000, 2571; (*g*) R. Kaul, S. Surprenant and W. D. Lubell, *J. Org. Chem.*, 2005, **70**, 3838.
3 D. Seebach, M. Overhand, F. N. M. Kühnle, B. Martinoni, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 913.
4 D. Yang, F.-F. Ng, Z.-J. Li, Y. D. Wu, K. W. K. Chan and D.-P. Wang, *J. Am. Chem. Soc.*, 1996, **118**, 9794.
5 R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Tan, A. D. Frankel, D. V. Santi, F. E. Cohen and P. A. Bartlett, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 9367.
6 M. Hosseini, H. Kringelum, A. Murray and J. E. Tønder, *Org. Lett.*, 2006, **8**, 2103.
7 (*a*) P. Jouin, B. Castro and D. Nisato, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1177; (*b*) D. Ma, J. Ma, W. Ding and L. Dai, *Tetrahedron: Asymmetry*, 1996, **7**, 2365; (*c*) J. Courcambeck, F. Bihel, C. De Michelis, G. Quéléver and J. L. Kraus, *J. Chem. Soc., Perkin Trans. 1*, 2001, **12**, 1421.
8 For simplicity and clarity, we have developed a peptide-like nomenclature for the pyrrolidinone amino acid analogues using the prefix *py* before the three letter amino acid code to indicate the pyrrolidinone structure (*e.g. py*Ala) This nomenclature will be used throughout this paper and in future work.
9 (*a*) Y. Ohfune, N. Kurokawa, N. Higuchi, M. Saito, M. Hashimoto and T. Tanaka, *Chem. Lett.*, 1984, 441; (*b*) A. Ando and T. Shioiri, *Tetrahedron*, 1989, **45**, 4969; (*c*) B. Müller, D. Besser, P. Kleinwächter,

O. Arad and S. Reissmann, *J. Pept. Res.*, 1999, **54**, 383; (*d*) M. K. Levadala, S. R. Banerjee, K. P. Maresca, J. W. Babich and J. Zubieta, *Synthesis*, 2004, **11**, 1759.
10 A. F. Abdel-Magid, K. G. Carson, B. D. Harris, C. A. Maryanoff and R. D. Shah, *J. Org. Chem.*, 1996, **61**, 3849.
11 N. Galeotti, J. Poncet, L. Chiche and P. Jouin, *J. Org. Chem.*, 1993, **58**, 5370.
12 D. S. Pedersen and C. Rosenbohm, *Synthesis*, 2001, **16**, 2431.
13 R. F. Borch, M. D. Bernstein and H. D. Durst, *J. Am. Chem. Soc.*, 1971, **93**, 2897.
14 T. Ruckle, B. Dubray, F. Hubler and M. Mutter, *J. Pept. Sci.*, 1999, **5**, 56.
15 (*a*) U. Schmidt, B. Riedl, G. Haas, H. Griesser, A. Vetter and S. Weinbrenner, *Synthesis*, 1993, **2**, 216; S. R. Hussaini and M. G. Moloney, *Org. Biomol. Chem.*, 2006, **4**, 2600; (*b*) S. R. Hussaini and M. G. Moloney, *Org. Biomol. Chem.*, 2003, **1**, 1838; (*c*) N. Ikemoto, D. M. Tellers, S. D. Dreher, J. Liu, A. Huang, N. Rivera, E. Njolita, Y. Hsiao, J. C. McWilliams, J. M. Williams, J. D. Armstrong, III, Y. Sun, D. Mathre, E. J. J. Grabowski and R. D. Tillyer, *J. Am. Chem. Soc.*, 2004, **126**, 3048.
16 A. I. Meyers and T. R. Elworthy, *J. Org. Chem.*, 1992, **57**, 4732.
17 A. S. Fisyuk and Y. G. Bundel, *Chem. Heterocycl. Compd.*, 1999, **35**, 125.
18 S. D. Koulocheri, P. Magiatis and S. A. Haroutounian, *J. Org. Chem.*, 2001, **66**, 7915.
19 J. Froborg, G. Magnusson and S. Thoren, *Tetrahedron Lett.*, 1975, 1621.
20 M. P. Rainka, Y. Aye and S. L. Buchwald, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 5821.
21 For a review on hydrogenation over heterogeneous catalysts see: M. Besson and C. Pinel, *Top. Catal.*, 1998, **5**, 25.
22 H. Niwa, O. Okamoto, Y. Miyachi, Y. Uosaki and K. Yamada, *J. Org. Chem.*, 1987, **52**, 2942.
23 R. O. Hutchins, N. R. Natale and I. M. Taffer, *J. Chem. Soc., Chem. Commun.*, 1978, 1088.
24 We used *py*Phe-Leu-NH_2 as we also wanted to asses the relative reduction of the aromatic system against the enamine system in different solvents.
25 Y. Hsiao, N. R. Rivera, T. Rosner, S. W. Krska, E. Njolito, F. Wang, Y. Sun, J. D. Armstrong, III, E. J. J. Grabowski, R. D. Tillyer, F. Spindler and C. Malan, *J. Am. Chem. Soc.*, 2004, **126**, 9918.

