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Dipeptide Analogues Containing 4-Ethoxy-3-pyrrolin-2-ones

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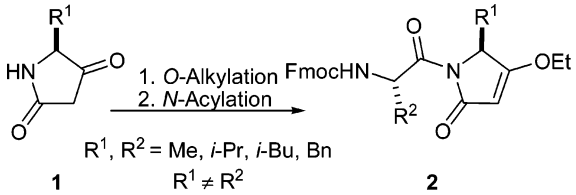
ABSTRACT



Pyrrolidine-2,4-diones (1) are naturally occurring analogues of amino acids. We herein present a facile synthesis of N-acylated, O-alkylated pyrrolin-2-ones (2) in high yield and excellent enantiopurity. Molecular mechanics calculations suggest that the resulting dipeptide analogues adopt a linear, extended conformation.

N-Acylated pyrrolidine-2,4-diones (also known as N-acylated tetramic acids) are present in a range of natural products,[1] many of these displaying interesting biological activities, e.g., antibiotic, antiviral, or cytotoxic activities. The structure of the pyrrolidine-2,4-diones is closely related to that of amino acids, and the biosynthetic pathway has indeed been suggested to occur via an intramolecular condensation of N-acetyl amino acid methyl esters.[2]

In nature, only the O-methylated form of N-acylated pyrrolidine-2,4-diones is seen (Figure 1), hence the pyrrolidinone analogue of glycine methyl ester (in the following named *py*Gly-OMe) is present in the natural products malyngamide A,[3] althiomycin,[4] and the pukeleimides.[5] Likewise, *py*Ala-OMe is present in mirabamide E[6] and dysideapyrrolidone[7], *py*Val-OMe is present in dysidin and mirabimide A−D,[8] *py*Ser-OMe is present in the malyngamides Q and R,[9] *py*Phe-OMe is present in dolastatin 15,[10]

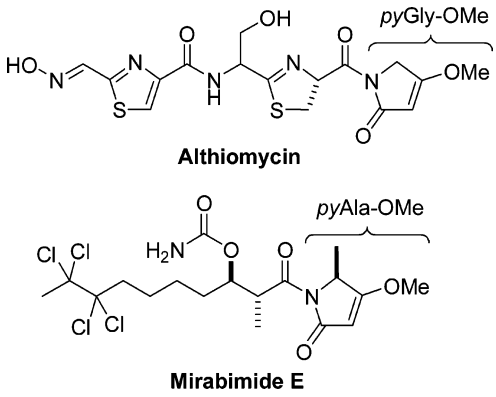


Figure 1. Natural compounds containing N-acylated, O-alkylated pyrrolin-2-ones.

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(1) For a review on tetramic acids, see: Royles, B. J. L. *Chem. Rev.* **1995**, *95*, 1981.
(2) Pettit, G. R.; Kamano, Y.; Dufresne, C.; Cerny, R. L.; Herald, C. L.; Schmidt, J. M. *J. Org. Chem.* **1989**, *54*, 6005.
(3) Cardellina, J. H., II.; Marner, F.-J.; Moore, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 240.
(4) Yamaguchi, H.; Nakayama, Y.; Takeda, K.; Tawara, K. *J. Antibiot. Ser. A* **1957**, *10*, 195.
(5) (a) Simmons, C. J.; Marner, F.-J.; Cardellina, J. H., II.; Moore, R. E.; Seff, K. *Tetrahedron Lett.* **1979**, *20*, 2003. (b) Cardellina, J. H., II.; Moore, R. E. *Tetrahedron Lett.* **1979**, *20*, 2010.

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and the 3-methylated form of *py*Phe-OMe is present in palau'imide.[11]

In our group, we are interested in the possibility of using the pyrrolidine-2,4-diones as amino acid analogues. Because pyrrolidine-2,4-diones are connected to the rest of the molecule via an imide bond, we have focused on the creation of this bond.

N-Acylation of sterically hindered chiral cyclic amides has previously been described; especially the oxazolidinones have attracted much interest due to their use as chiral auxiliaries.[12] Similar pyrrolidinones have been N-acylated using an *n*-BuLi protocol with electrophiles such as acid chlorides[13] and Pfp-esters.[14]

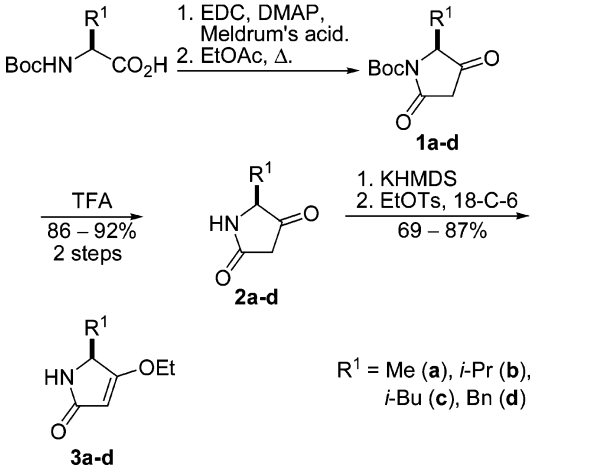
The corresponding N-acylation of 4-alkylated pyrroline-2-ones has been reported previously in the syntheses of althiomycin,[15] pukeleimide A,[16] dysidin,[17] and mirabamide E.[6] However, only one group has reported an acylation in which it was assumed that the stereochemistry at C-5 had remained unchanged.[6]

The pyrrolidine-2,4-diones were synthesized efficiently via a modified literature procedure[18] (Scheme 1), in which a Boc-protected amino acid was activated with EDC, condensed with Meldrum's acid, and finally cyclized to give the Boc-protected pyrrolidine-2,4-diones (**1**). The following N-deprotection was accomplished with TFA treatment giving the parent pyrrolidine-2,4-diones (**2**). This sequence can be run in 1 day, furnishing up to 30 g of the products.

In nature, the pyrrolinones appear in their O-methylated form. However, preliminary experiments showed that the methyl group was more difficult to remove than the ethyl group,[19] which was therefore used in the further work.

Conversion of the pyrrolidine-2,4-diones (**2**) into their O-ethylated derivatives (**3**) was accomplished by deprotonation with KHMDS followed by alkylation with ethyl tosylate in the presence of 18-crown-6.

Scheme 1. Synthesis of 4-Ethoxy-3-pyrrolin-2-ones



In the O-alkylation, it was found that the use of lithium bases (LiHMDS and *n*-BuLi) led to prolonged reaction times due to a precipitation of the anion, which could not be reversed by addition of 15-crown-5. However, among the potassium bases tested, KHMDS afforded yields in the range of 69−87%,[20] with no racemization of the final product. This was superior to both KO^{t}Bu in terms of yield and K_2CO_3 in terms of enantiopurity.

For the acylation step, we had to be aware of two important issues: (1) the poor nucleophilicity of the pyrrolinone anion which has been described in an earlier report[15a] and (2) the retention of enantiopurity in the products.

Because the anions of compounds **3** can obtain the aromatic 4-alkoxy-1*H*-pyrrol-2-olate structure by intermolecular proton transfer, we initially focused on avoiding racemization in the deprotonation step.

Preliminary findings based on ^{1}H NMR detection showed, surprisingly, that no incorporation of deuterium was observable even after quenching the anion of **3d** with CF_3COOD at 20 °C. However, when the product after a water quench were analyzed by HPLC, full racemization had actually taken place at 20 °C (Table 1). Lowering the temperature to −30 °C led to a rise in the enantiomeric excess to 91%, and by lowering the temperature further to below −45 °C, no epimerization was observed.

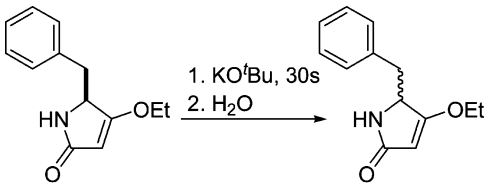
For the acylations, Fmoc-protected amino acids were preferred over Boc-protected acids because the O-ethyl group was to be removed under acidic conditions,[19] and it was therefore considered advantageous with a base labile N-protecting group.

Initial acylation experiments were conducted at −78 °C. These experiments quickly showed that, in contrast to what we observed in the alkylation reactions, lithium bases (LiHMDS, *n*-BuLi) were now superior to the potassium

(6) Paik, S.; Carmeli, S.; Cullingham, J.; Moore, R. E.; Patterson, G. M. L.; Tius, M. A. *J. Am. Chem. Soc.* **1994**, *116*, 8116.
(7) Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 6336.
(8) Carmeli, S.; Moore, R. E.; Patterson, G. M. L. *Tetrahedron* **1991**, *47*, 2087.
(9) Milligan, K. E.; Márquez, B.; Williamson, R. T.; Davies-Coleman, M.; Gerwick, W. H. *J. Nat. Prod.* **2000**, *63*, 965.
(10) Akaji, K.; Hayashi, Y.; Kiso, Y.; Kuriyama, N. *J. Org. Chem.* **1999**, *64*, 405.
(11) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *Tetrahedron* **2002**, *58*, 7959.
(12) (a) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. (b) Ager, D. J.; Prakash, I.; Schaad, D. R. *Aldrichimica Acta* **1997**, *30*, 3. and references therein.
(13) Davies, S. G.; Dixon, D. J.; Doisneau, G. J.-M.; Prodger, J. C.; Sanganee, H. J. *Tetrahedron: Asymmetry* **2002**, *13*, 647.
(14) Andrus, M. B.; Li, W.; Keyes, R. F. *J. Org. Chem.* **1997**, *62*, 5542.
(15) (a) Inami, K.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 352. (b) Toogood, P. L.; Hollenbeck, J. J.; Lam, H. M.; Li, L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1543. (c) Zarantonello, P.; Leslie, C. P.; Ferritto, R.; Kazmierski, W. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 561.
(16) James, G. D.; Mills, S. D.; Pattenden, G. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2581.
(17) Köhler, H.; Gerlach, H. *Helv. Chim. Acta* **1984**, *67*, 1783.
(18) (a) Jouin, P.; Castro, B.; Nisato, D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1177. (b) Ma, D.; Ma, J.; Ding, W.; Dai, L. *Tetrahedron: Asymmetry* **1996**, *7*, 2365. (c) Courcambeck, J.; Bihel, F.; de Michelis, C.; Quéléver, G.; Kraus, J. L. *J. Chem. Soc., Perkin Trans. 1* **2001**, *12*, 1421.
(19) Preliminary removal conditions were 10% HBr in MeCN.

(20) To a solution of tetramic acid (1 g, 1.0 equiv) in THF (30 mL) at 0 °C was added dropwise KHMDS (0.50 M in toluene, 1.05 equiv), and the suspension was stirred for 10 min at 0 °C. To this was added EtOTs (1.1 equiv) and 18-crown-6 (1.1 equiv), and the mixture was slowly heated to room temperature. Upon completion of the reaction (TLC, EtOAc), the mixture was evaporated with 50 mL of silica, and flash chromatography (EtOAc) provided the pure product.

Table 1. Racemization Investigations



(*S*)-**3d** (*R*/*S*)-**3d**

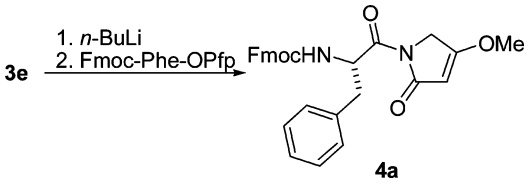
entry	temperature (°C)	ee (%)[a]
1	20	0
2	0	79
3	−30	91
4	−45	>99

[a] Determined by HPLC; Chiralcel AD column, gradient 7 → 5% ^{i}PrOH in hexane, $R_{T(S)-H\text{-}py\text{Phe-OEt}} = 7.6$ min, $R_{T(R)-H\text{-}py\text{Phe-OEt}} = 10.7$ min.

(KO^{t}Bu, KHMDS) and magnesium (^{i}-PrMgCl) bases. This difference may be due to a diminished reactivity of the nucleophile when a larger counterion is present. For the final optimizations, we used the commercially available H-*py*Gly-OMe (**3e**) to minimize steric hindrance.

In these experiments, we found that the electrophile (Fmoc-AA-OPfp) had to be added slowly (over 15 min) to avoid degradation and that using an excess of either electrophile or nucleophile did not lead to an increase in yield (Table 2).

Table 2. Optimizing the N-Acylation Reaction



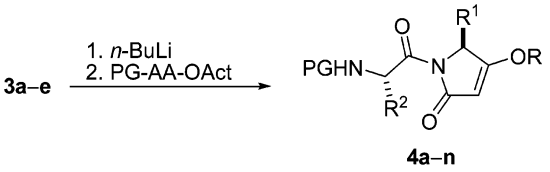
4a

entry	equiv electrophile	temperature (°C)	isolated yield (%)
1	0.5	−78	72
2	1.1	−78	76
3	2.0	−78	77
4	1.1	−55 → −45	91

Satisfyingly, it was observed that raising the temperature from −78 °C to −50 °C led to the increase in nucleophilic reactivity which was necessary for obtaining a synthetically useful reaction.[21] Thus, it is possible to balance the nucleophilicity and possibility of racemization inherent in the anion structure by maintaining the reaction temperature around −50 °C.

Applying these optimized conditions to a range of substrates (Table 3) furnished the desired dipeptide analogues

Table 3. Acylation of 4-Alkoxy-3-pyrrolin-2-ones



4a–n

compound[a]	isolated yield (%)[b]
Fmoc-Phe-*py*Gly-OMe (**4a**)	91
Fmoc-Val-*py*Ala-OEt (**4b**)	92
Fmoc-Leu-*py*Ala-OEt (**4c**)	88
Fmoc-Phe-*py*Ala-OEt (**4d**)	85
Fmoc-Ala-*py*Val-OEt (**4e**)	89
Fmoc-Leu-*py*Val-OEt (**4f**)	78
Fmoc-Phe-*py*Val-OEt (**4g**)	78
Fmoc-Ala-*py*Leu-OEt (**4h**)	84
Fmoc-Val-*py*Leu-OEt (**4i**)	81
Fmoc-Phe-*py*Leu-OEt (**4j**)	80
Fmoc-Ala-*py*Phe-OEt (**4k**)	84
Fmoc-Val-*py*Phe-OEt (**4l**)	88
Fmoc-Leu-*py*Phe-OEt (**4m**)	85
Boc-Phe-*py*Phe-OEt (**4n**)	92

[a] Fmoc-AA-OPfp and Boc-Phe-ONp were used as electrophiles. [b] Only one diastereomer was observed by ^{1}H NMR (de > 95%).

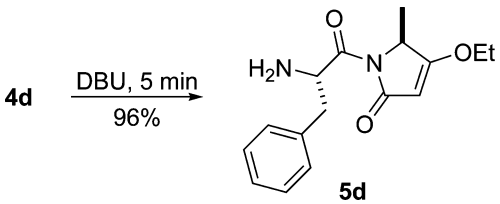
in very good isolated yields and with complete retention of enantiopurity.

In addition to the Fmoc-protected amino acids, Boc-Phe-ONp was employed in the reaction giving **4n** in 92% yield and again with full retention of enantiopurity.

It can be seen that steric hindrance may have some influence on this reaction, as the highest yields are obtained for smaller reactants such as *py*Gly, Ala, *py*Ala, Val, and *py*Val. *py*Phe is ranked almost as good as *py*Ala, reflecting that the benzylic side chain may be able to bend away from the reaction center.

To show that these novel dipeptide analogues can be employed in further synthesis, the Fmoc protecting group in compound **4d** was removed by DBU treatment affording **5d** in excellent yield (Scheme 2).

Scheme 2. N-Deprotection



5d

(21) *n*-BuLi (1.58 M in hexanes, 1.0 equiv) was added dropwise to a solution of H-*py*AA-OEt (**3**) (50 mg, 1.0 equiv) in THF (3 mL) at −55 °C, and stirring was continued for 10 min. To this yellow solution was added Fmoc-AA-OPfp (1.1 equiv) dissolved in THF (2 mL) by a syringe pump over 15 min. The mixture was allowed to stir for an additional 5 min. Over this 30 min period, the temperature had been allowed to reach −45 °C. The mixture was quenched with AcOH (0.1 mL) and evaporated with 5 mL of silica. Column chromatography (30→50% EtOAc in heptane) afforded the pure compounds (**4**) as white solids.

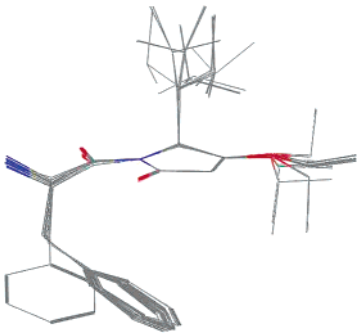


Figure 2. All low-energy conformations for **5j**. (Hydrogens are omitted for clarity.)

To investigate the structure of the desired dipeptide analogues, conformational searches on the N-deprotected compounds (**5b**−**m**) were carried out.[22]

In all cases, the conformational search gives rise to the expected almost planar pyrrolinone ring (Figures 2 and 3) with values for φ and ψ varying between −161.8 to −173.2 and 171.0 to 177.3, respectively.

In the low energy conformations of H-Phe-*py*AA-OEt (**5d**,**g**,**j**), an electrostatic interaction could be observed between the π-systems on the lactam carbonyl on the pyrrolinone ring system and the phenyl from the benzyl group, respectively (Figure 2).

In all low energy conformations of compounds **5a**−**m**, the backbone exists in the extended form, and the different conformations represent rotation of the side chains R^1, R^2, and the OEt group.

(22) See Supporting Information for details and results of the molecular modeling study.

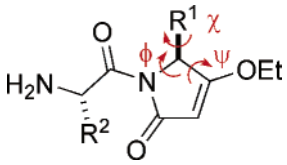


Figure 3. Torsional angles used in the computational study.

In conclusion, we have developed a facile synthesis of dipeptide analogues containing pyrrolin-2-ones. These transformations have been shown to afford a robust and general method, where the products could be achieved in high yield and excellent enantiopurity.

As expected, the computational investigations of these analogues have suggested that they should adopt a linear, extended conformation with some conformational constraints in the pyrrolinone ring.

We are currently investigating the physical and biological properties of these compounds, and results will be reported in due time.

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Supporting Information Available: Experimental procedures, full spectroscopic data for all new compounds, in addition to detailed procedures and results from the molecular modeling study. This material is available free of charge via the Internet at http://pubs.acs.org.

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