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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 7007–7009

Synthesis of C^{α} methylated carboxylic acids: isosteres of arginine and lysine for use as N-terminal capping residues in polypeptides

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Received 18 July 2005; revised 9 August 2005; accepted 11 August 2005

Abstract—Replacement of the N-terminal α-amine with the isosteric methyl functionality in bioactive peptides can influence various pharmacokinetic parameters, including hydrophobicity and stability. C^{α} methylated amino acid analogues are thus of great interest to expand the repertoire of nonnatural synthons available as N-terminal 'capping' residues for peptide-based drug design. Several novel arginine and lysine analogues stereoselectively modified in the C^{α} position with a methyl group in place of the α-amine were prepared.

Lack of stability in biological matrices is a key obstacle in developing peptides as therapeutic entities. One approach used to address this issue is derivatization of peptide N-termini, for example by N^{α}-acetylation or N^{α}-methylation.[1,2] Such modification provides resistance to cleavage by aminopeptidases, the predominant route of degradation for peptides containing an unprotected N-terminal amine.[3–5] Previous work in this laboratory has produced a library of side-chain alkylated analogues of the cationic amino acids L-arginine (Arg) and L-lysine (Lys).[6–9] Recently, a subset of these analogues featuring an α-methyl group substituted for the α-amine has been synthesized[10] and incorporated at the N-terminus of peptides.[11] The C^{α} methyl group is virtually isosteric to the N^{α} ammonium ion, and its substitution removes the positive charge, thereby increasing the overall hydrophobicity of the peptide in which the replacement is made. The resulting compounds shared dramatically increased biological stability and, in certain cases, increased central nervous system activity following oral administration.[11,12] Demonstration of the substantial biological effects resulting from stereochemically defined C^{α} methyl incorporation makes production of enantiomerically pure α-methyl acids of great interest. Accordingly, synthesis of the Arg analogue (2*S*)-5-guanidino-2-methylvaleric acid (**1**) and the ornithine (Orn), Lys, and homolysine (HLys) analogues (2*S*)-5-amino-2-methylvaleric acid (**12a**), (2*S*)-6-amino-2-methylhexanoic acid (**12b**), and (2*S*)-7-amino-2-heptanoic acid (**12c**), respectively, are reported herein.

The initial attempt at the synthesis of **1** followed the method of Hadden et al.[10] This procedure utilizes the Evans chiral auxiliary, (*S*)-(−)-4-benzyl-2-oxazolidinone (X_P), to effect stereoselective introduction (>95% enantiomeric purity)[10] of the methyl group at the α-position on the appropriate ω-bromo acid (Scheme 1). However, in contrast to previous reports,[10,13] removal of the chiral auxiliary from **2a** following generation of the stereocenter resulted in unacceptably low yields (10–20%). Upon further investigation, the cyclic lactone side product (**4**) was identified following saponification along with **3a** and the free chiral auxiliary.

It appears that base-induced cyclization to produce **4** is highly favorable and competes with formation of the protonated form of the acid following carboximide hydrolysis, thus the unprotected carboxylate of **3a** may have limited utility as a substrate in this reaction. While ω-bromo acids work well with respect to the preparation of α-azido acids,[13] the α-methyl group employed here seems to destabilize the carboxylate during the ensuing

Keywords: Amino acid analogues; Isostere; Arginine; Lysine; N-terminal capping.
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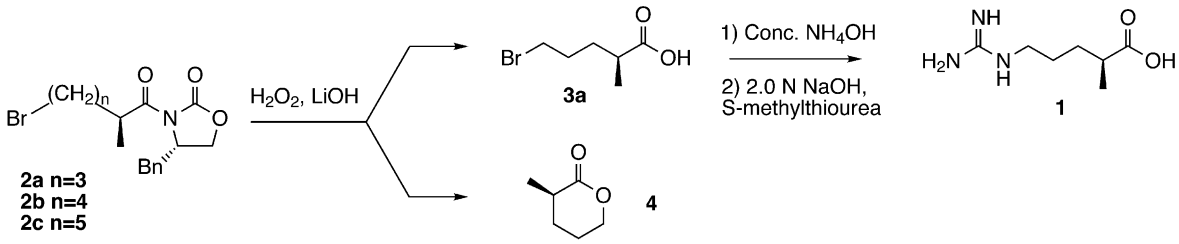
doi:10.1016/j.tetlet.2005.08.056



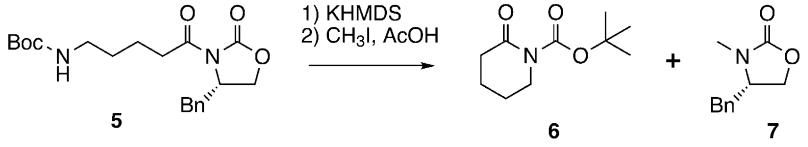
Scheme 1.

removal of the chiral auxiliary. Further, similar problems were observed in the subsequent derivatization of isolated **3a** where a clean product in acceptable yield was not obtained under a variety of conditions.

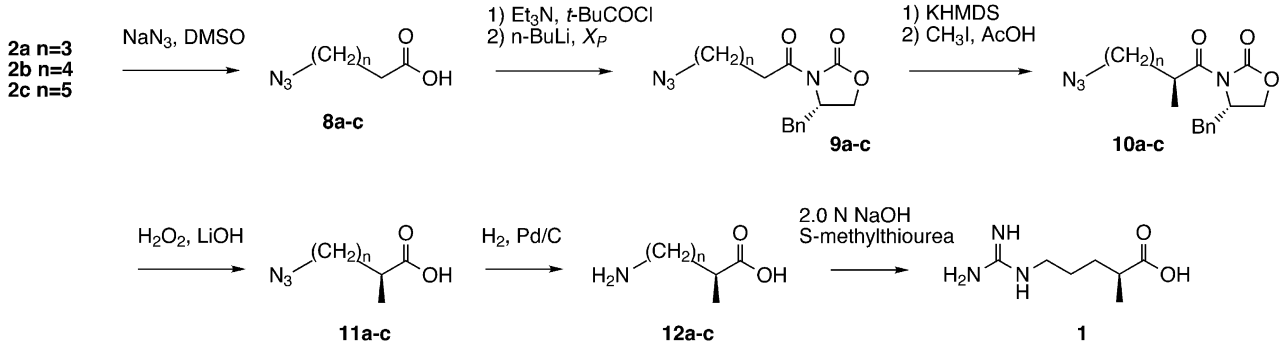
Replacing the ω-bromo moiety from Scheme 1 with a Boc-protected amine was also ineffective (Scheme 2). Instead of the desired enolate formation, the amide proton of **5** is abstracted under the conditions of the methylation reaction, allowing displacement of the chiral auxiliary with concomitant formation of the six-membered lactam, **6**. Methylation then occurs on the free auxiliary upon methyl iodide addition to generate **7** as the major product.

The synthesis strategy was revised further by substituting the bromo moiety of the ω-bromo acids **2a**–**c** with the azide ion as the initial step in the synthesis (Scheme 3). Many procedures for the introduction of azides onto various backbone structures require complex workups, extensive reaction times, elevated temperatures, and result in low yields.[14–16] However, the straightforward method of Alvarez and Alvarez[17] was extended to produce the primary azido acids **8a**–**c**, usually in quantitative yield, at ambient temperature. This approach effectively masks the amine during construction of the stereocenter and subsequent removal of the chiral auxiliary. Substitution of the bromine can be accomplished at any step prior to removal of the chiral auxiliary without loss of efficiency, having been performed on the 5-bromo counterparts of **9a** and **10a** with equal effectiveness (96% and 95% yields, respectively). The use of this azide substitution strategy circumvents the problems of cyclization noted here and observed with similar compounds.[8,10] With the azide in the ω-position, transformation from **10a**–**c** to **11a**–**c** proceeded as expected. Azide reduction then afforded the Orn, Lys, and HLys analogues (**12a**–**c**) in excellent yields from **11a**–**c**, allowing guanylation with *S*-methylthiourea to give **1** from **12a** in 65% overall yield (Scheme 3).

Peptides featuring N-terminal (*S*)-C^{α}-azido homolysine and (*S*)-C^{α}-methyl homolysine residues have been prepared.[12,11] However, in these cases either (2*S*)-2-azido-7-bromoheptanoic acid or (2*S*)-7-bromo-2-methylheptanoic acid was coupled to the peptidyl-resin directly and the bromine was substituted by ammonia in approximately 70% yield following cleavage from the solid phase. It is demonstrated herein that substitution of the bromine with the azide group is both more efficient and widely useful than the previous approach. The present work constitutes the first report of (*S*)-C^{α} methyl amino acid analogues of Arg, Orn, Lys, and HLys as monomers for solid-phase peptide synthesis. The HCl



Scheme 2.



Scheme 3.

salt of **1** and Boc-protected **12a–c** have been coupled to peptidyl-resin by standard methods without difficulty and the resulting peptides and their corresponding properties will be reported elsewhere. Scheme 3 also provides an efficient route to the production of 2(*S*)-methyl versions of a number of other side-chain alkylated Arg analogues via reaction of **12a** with the requisite isothiourea salts.

The novel enantiomerically pure (*S*)-C^{α} methylated analogues of Arg and Lys reported here lack the α-amine and therefore will resist aminopeptidase degradation when incorporated at the N-terminus of peptides.[3,18] These compounds are useful as N-terminal capping residues that simultaneously increase peptide stability and hydrophobicity resulting from the loss of the charged amine. Such a strategy improves peptide biostability without drastically altering the structure of the molecule, leaving the backbone and amino acid side-chain functional groups free to mediate the interactions required for full biological activity of the peptide. This C^{α}-methyl substitution should find widespread use in peptide drug design since it is essentially isosteric with the N^{α}-ammonium ion and thus without the adverse steric effects possible with N^{α}-acetylation and N^{α}-methylation.

Acknowledgements

This research was supported by NIH grant MH-65088. K.S.O. is an American Foundation for Pharmaceutical Education predoctoral scholar. The authors thank Drs. Craig Beeson, Erika Büllesbach, and John Oatis for useful discussions and technical assistance. The MUSC NMR Facility was used in support of this work.

Supplementary data

Supplementary data: 400 MHz ^{1}H NMR for the mixture of **3a** and **4**, ^{1}H NMR. HSQC and HMBC spectra for **7**, **8a–c**, **9a–c**, **10a–c**, **11a–c**, **12a–c**, and **1** as well as experimental procedures. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tetlet.2005.08.056.

References and notes

1. Brown, J. L.; Roberts, W. K. *J. Biol. Chem.* **1976**, *251*, 1009–1014.
2. Machida, R.; Tokumura, T.; Sasaki, A.; Tsuchiya, Y.; Abe, K. *J. Chromatogr. A* **1990**, *534*, 190–195.
3. Delange, R. J. *The Enzymes*, 3rd ed.; Academic Press: New York, 1971; Vol. 3.
4. Lugrin, D.; Vecchini, F.; Doulut, S.; Rodriguez, M.; Martinez, J.; Kitabgi, P. *Eur. J. Pharmacol.* **1991**, *205*, 191–198.
5. Kokko, K. P.; Dix, T. A. *Anal. Biochem.* **2002**, *308*, 34–41.
6. Kennedy, K. J.; Lundquist, I.; Joseph, T.; Simandan, T. L.; Beeson, C. C.; Dix, T. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1937–1940.
7. Kennedy, K. J.; Simandan, T. L.; Dix, T. A. *Synth. Commun.* **1998**, *28*, 741–746.
8. Lundquist, J. T.; Orwig, K. S.; Dix, T. A. *J. Org. Chem.* **1999**, *64*, 9265–9267.
9. Kennedy, K. J.; Lundquist, J. T. I.; Simandan, T. L.; Kokko, K. P.; Beeson, C. C.; Dix, T. A. *J. Pept. Res.* **2000**, *55*, 348–358.
10. Hadden, M.; Kokko, K.; Dix, T. *Synth. Commun.* **2005**, *35*, 1675–1680.
11. Hadden, M. K.; Orwig, K. S.; Mazella, J.; Dix, T. A. *Neuropharmacology*, in press.
12. Kokko, K. P.; Hadden, M. K.; Orwig, K. S.; Mazella, J.; Dix, T. A. *J. Med. Chem.* **2003**, *46*, 4141–4148.
13. Lundquist, J. T. I. V.; Dix, T. A. *Tetrahedron Lett.* **1998**, *39*, 775–778.
14. Hassner, A.; Fibiger, R.; Andisik, D. *J. Org. Chem.* **1984**, *49*, 4237–4244.
15. Dyke, J. M.; Groves, A. P.; Morris, A.; Ogden, J. S.; Dias, A. A.; Oliveira, A. M. S.; Costa, M. L.; Barros, M. T.; Cabral, M. H.; Moutinho, A. M. C. *J. Am. Chem. Soc.* **1997**, *119*, 6883–6887.
16. Tornoe, C. W.; Davis, P.; Porreca, F.; Meldal, M. *J. Pept. Sci.* **2000**, *6*, 594–602.
17. Alvarez, S. G.; Alvarez, M. T. *Synthesis* **1997**, 413–414.
18. Luciani, N.; Marie-Claire, C.; Ruffet, E.; Beaumont, A.; Roques, B. P.; Fournie-Zaluski, M. C. *Biochemistry* **1998**, *37*, 686–692.