Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 14, pp. 1937-1940, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00335-1

ASYMMETRIC SYNTHESIS OF NON-NATURAL HOMOLOGUES OF LYSINE

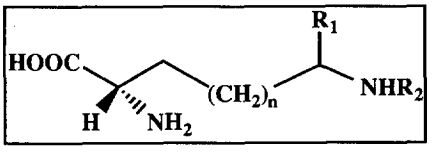
Kevin J. Kennedy,[I] Joseph T. Lundquist, IV,[I] Tiberiu L. Simandan, Craig C. Beeson[Y] and Thomas A. Dix*

Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, South Carolina 29425

[Y]Department of Chemistry, The University of Washington, Seattle, Washington 98195

Abstract: A series of non-natural amino acid homologues of L- and D-lysine have been synthesized and protected for use in solid-phase peptide synthesis. With these residues, electrostatics and hydrophobicity of peptides can be enhanced or altered.
© 1997 Elsevier Science Ltd.

Ion-pair interactions between anions and the naturally occurring cationic amino acids arginine and lysine (**1**, Table 1) contribute significantly to protein structure and stability, and can provide a substantial proportion of the binding energy of peptide ligands. Previous results from this laboratory have demonstrated that alkyl substituents attached or adjacent to a cation can increase its tendency to form an ion pair through destabilization of the aqueous solvation shell of the positive charge.[2] Based on these results, non-natural analogues of arginine have been designed and synthesized.[3] In this paper, the synthesis of a series of non-natural amino acid homologues of lysine (Table 1, **2a–h**) are described. The analogues were designed to manipulate the properties of this cationic side-chain, specifically to enhance desolvation and hence increase the propensity to enter into an ion pair. The series was constructed by adding alkyl substituents adjacent to or directly on the ε-amine of lysine, which serves to increase the hydrophobicity of the side chain with retention of the cation.

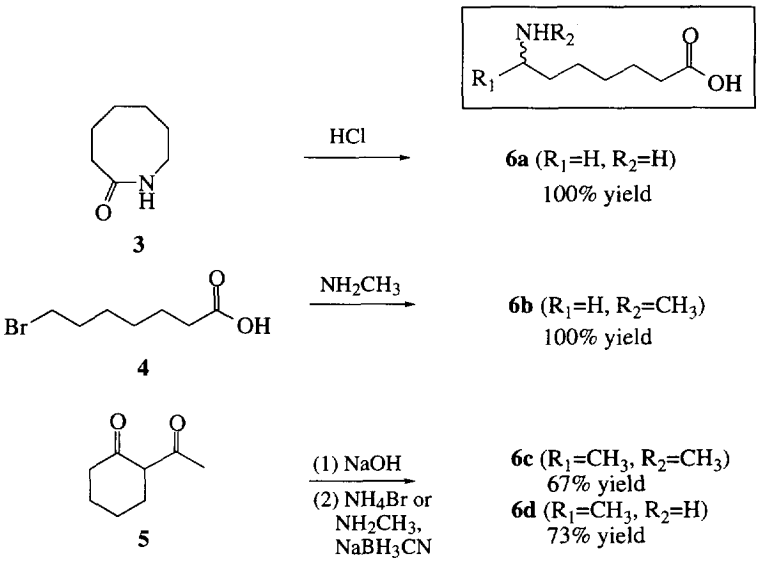


Molecule	n	R_1	R_2
1 (lysine)	2	H	H
2a (homolysine)	3	H	H
2b	3	H	CH_3
2c	3	CH_3	CH_3
2d	3	CH_3	H
2e-h	3	as 2a–d but α–D–epimer	

Table 1. Non-natural Homologues of Lysine

The homologues also have a second potential benefit with regard to substitution in peptides of pharmaceutical interest. All homologues are more hydrophobic than either lysine or arginine,[4] and hence may improve pharmacokinetic and pharmacodynamic properties of peptides apart from their ion-pairing ability when substituted for the naturally occurring cationic amino acids.

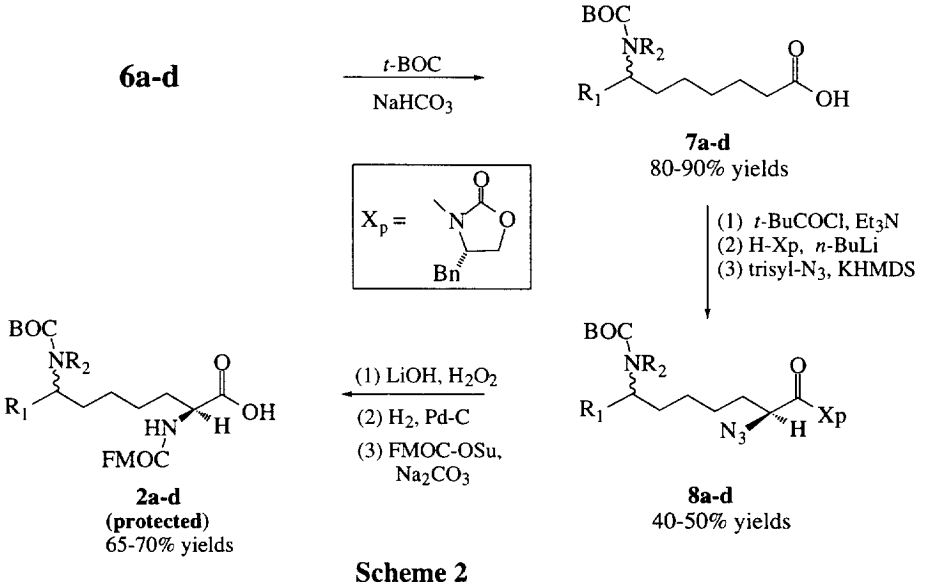
All of the lysine homologues shown above are accessible through related amino-substituted carboxylic acid intermediates (**6a–d**) as depicted in Scheme 1. Intermediate **6a** is obtained quantitatively by acid-catalyzed ring-opening of azacyclooctanone (**3**). It was envisioned that the precursor of *N*-methylated homolysine (**6b**) could be obtained by methylation of **3** prior to ring-opening, but poor yields were obtained from this route. An alternative procedure - reaction of bromoheptanoic acid (**4**) with an excess of methylamine[5] - was both direct and quantitative. Intermediates **6c** and **6d** were obtained in 67 and 73% yields, respectively, by base-catalyzed ring opening of acetylcyclohexanone (**5**) and reductive amination[6] of the resultant ketoacid with a suitable amine.



Scheme 1

The related intermediates were next t-BOC protected[7] (Scheme 2) and the α–amino functionalities introduced stereospecifically using a chiral oxazolidinone auxilary using the methodology of Evans et al. (Scheme 2).[8] All electrophilic azide additions had ees of >95%, as assayed by HPLC. The α-azido carboxylic acids (R or S) thus obtained were reduced by catalytic hydrogenation and FMOC protected[9,10] to give the fully protected non-natural lysine analogues suitable for solid-phase peptide synthesis in 19% yield from **3**, 21% yield from **4** and 20% yield from **5**. The D-amino acids (**2e–g**) also were prepared using this route. L- and D-Homolysine (**2a,e**)

are known compounds while analogues **2b–d** and **2f–h** are new compounds. Typical yields are provided in Scheme 2.



Scheme 2

Compounds **2c** and **2d** have an additional chiral center in the side chains. Extensive efforts were made to separate the diastereomers at different steps of the synthesis, but were unsuccessful. Surprisingly, peptides in which these diastereomeric residues were incorporated initially showed evidence of separation,[4] suggesting that peptides incorporating these residues can be separated if deemed necessary.

Note that while the compounds are chemically more closely related to lysine, they also can be considered isosteres for arginine. Hence, substitution for either cationic amino acid in peptides of interest may be appropriate. This is illustrated by the first set of results in which these and other non-natural analogues were substituted into a biologically relevant peptide. Tri- and tetrapeptides of the general formula D-Phe-Pro-Arg-X are inhibitors of the blood serine protease thrombin, and thus have considerable pharmaceutical interest.[11] Substitution of **2d** for arginine in the tetrapeptide D-Phe-Pro-Arg-Ala resulted in a peptide whose K_i for thrombin inhibition in vitro was 40% lower than the parent peptide.[4] Substitution with other non-natural analogues of arginine[3] gave peptides that were equipotent to the parent peptide,[4] but may possess pharmacokinetic parameters (such as enhanced hydrophobicity) that will make them attractive therapeutic leads.

Analytical NMR data for each of the L-amino acid analogues are provided below.[12]

Acknowledgment: This work was supported by a Faculty Research Award (to T. A. D.) and a Postdoctoral Fellowship (to T. L. S.) from the Medical University of South Carolina. K. J. K. and J. T. L. are American Foundation for Pharmaceutical Education predoctoral scholars.

REFERENCES AND NOTES:

1. These authors contributed equally to this paper.
2. Beeson, C.; Dix, T. A. *J. Am. Chem. Soc.* **1993**, *115*, 10275.
3. Kennedy, K.; Simandan, T.; Dix, T. A., manuscript submitted.
4. Kennedy, K.; Lundquist, J. T., IV; Simandan, T.; Kokko, K.; Dix, T. A., manuscript in preparation.
5. March J. *Advanced Organic Chemistry. Reactions, Mechanism and Structure*; Third Edition, John Wiley & Sons, New York, 1985, pp 364–365.
6. Borch, R. F.; Bernstein, M. D.; Durst, H. D.; *J. Am. Chem. Soc.* **1971**, *93*, 2897.
7. Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 730.
8. Evans, D. A.; Britton, T. C.; Ellman, J. C.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011.
9. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.
10. Fields, C. G.; Fields, G. B.; Noble, R. L.; Cross, T. A. *Int. J. Peptide Protein Res.* **1989**, *33*, 298.
11. Markwardt, F.; Hauptmann, J. *The Design of Synthetic Inhibitors of Thrombin*, Claeson, G.; Scully, M. F.; Kakkar, V. V.; Deadman, J., Eds.; Plenum Press: New York, 1993, pp 143–171.
12. **N-α-FMOC-N-ϕ-BOC-(2S)-2,7-diaminoheptanoic acid (2a):** ^{1}H NMR (300 MHz, $CDCl_3$) 7.8–7.2 (8H, m), 4.5–4.3 (3H, m), 4.18 (1H, t), 3.05 (2H, m), 2.0-1.4 (8H, m), 1.45 (9H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) 175.5, 156.1, 143.8, 143.7, 141.3, 127.7, 127.1, 125.1, 119.9, 79.2, 67.1, 53.7, 47.3, 40.3, 32.3, 29.6, 28.4, 26.2, 24.6. **N-α-FMOC-N-ϕ-BOC-(2S)-7-(N-methylamino)-2-aminoheptanoic acid (2b):** ^{1}H NMR (300 MHz, $CDCl_3$) 7.8-7.2 (8H,m), 4.5-4.3 (3H, m), 4.18 (1H, t), 3.20 (2H, m), 2.82 (3H,s) 2.0–1.3 (8H, m) 1.45(9H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) 175.6, 156.2, 143.9, 143.8, 141.3, 127.7, 127.1, 125.2, 120.0, 79.6, 67.1, 53.7, 48.5, 47.2, 34.2, 32.3, 28.5, 27.4, 26.2, 24.7. **N-α-FMOC-N-ϕ-BOC-(2S)-7-(N-methylamino)-2-aminooctanoic acid (2c):** ^{1}H NMR (300 MHz, $CDCl_3$) 7.8–7.2 (8H, m), 4.5-4.3 (3H, m), 4.21 (1H, t), 4.05 (1H, m), 2.65 (3H, s), 2.0–1.4 (8H, m), 1.45 (9H, s), 1.12 (3H, d); ^{13}C NMR (75.5MHz, $CDCl_3$) 175.9, 156.2, 143.9, 143.8, 141.3, 127.7, 127.1, 125.1, 120.0, 80.1, 67.1, 53.6, 50.4, 47.2, 33.8, 32.3, 28.5, 27.2, 26.0, 24.8, 18.3. **N-α-FMOC-N-ϕ-BOC-(2S)-2,7-diaminooctanoic acid (2d):** ^{1}H NMR (300 MHz, $CDCl_3$) 7.8–7.2 (8H, m), 4.5–4.3 (4H, m), 4.18 (1H, t), 3.60 (1H, m), 2.0–1.4 (8H, m), 1.45 (9H, s), 1.12 (3H, d); ^{13}C NMR (75.5 Hz, $CDCl_3$) 175.8, 156.4, 143.8, 143.7, 141.6, 127.7, 127.1, 125.2, 120.0, 80.1, 67.0, 53.9, 47.2, 46.3, 36.8, 32.1, 28.5, 25.4, 24.9, 21.6.

(*Received in USA* 13 *May* 1997; *accepted* 23 *June* 1997)