ORGANIC LETTERS

2004
Vol. 6, No. 4
497−500

Synthesis of All Nineteen Appropriately Protected Chiral α-Hydroxy Acid Equivalents of the α-Amino Acids for Boc Solid-Phase Depsi-Peptide Synthesis

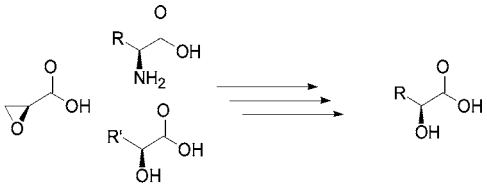
Songpon Deechongkit, Shu-Li You, and Jeffery W. Kelly*

Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California 92037

jkelly@scripps.edu

Received October 28, 2003

ABSTRACT



The R group is appropriately protected in cases where side-chain protection is necessary.
The R' group is an unprotected side-chain in need of protection.

The preparation of depsi-peptides, amide-to-ester-substituted peptides used to probe the role of hydrogen bonding in protein folding energetics, is accomplished by replacing specific L-α-amino acid residues by their α-hydroxy acid counterparts in a solid-phase synthesis employing a *t*-Boc strategy. Herein we describe the efficient stereoselective synthesis of all 19 appropriately protected α-hydroxy acid equivalents of the L-α-amino acids, employing commercially available materials, expanding the number of available α-hydroxy acids from 9 to 19.

Polypeptide backbone modifications enable the role of backbone hydrogen bonding in protein folding, in protein−protein interactions, in protein−small molecule recognition, and in peptide hormone structure activity relationships to be assessed.[1−5] One strategy for modifying the backbone is to replace amide bonds with ester bonds. Proteins and peptides containing an ester bond(s) as part of the backbone are called depsi-peptides ("depsi" comes from the *Greek* word, *depsidi*, which means ester).[6] An amide-to-ester (A-to-E) substitution affords an ester carbonyl oxygen that has a lower partial charge relative to an amide and as such displays reduced hydrogen bond acceptor capacity, the extent depending on the dielectric constant of the local environment. The same substitution also replaces the −NH− with an sp^3-hybridized −O− that cannot serve as hydrogen bond donor.[7] A-to-E substitutions represent the minimal tractable structural perturbation for probing the role of backbone hydrogen bonding.[3,5,8] The conformational preferences (ϕ and ψ space)

(1) Carlson, H. A.; Briggs, J. M.; McCammon, J. A. *J. Med. Chem.* **1999**, *42*, 109−117.
(2) Hong, A. L.; Newlander, K. A.; Foster, J. E.; Bowers, C. Y.; Reynolds, G. A. *Pept.: Struct. Funct., Proc. Am. Pept. Symp., 8th* **1983**, 385−388.
(3) Blankenship, J. W.; Balambika, R.; Dawson, P. E. *Biochemistry* **2002**, *41*, 15676−15684.
(4) Beligere, G. S.; Dawson, P. E. *Biopolymers* **1999**, *51*, 363−369.
(5) (a) Chapman, E.; Thorson, J. S.; Schultz, P. G. *J. Am. Chem. Soc.* **1997**, *119*, 7151−7152. (b) Koh, J. T.; Cornish, V. W.; Schultz, P. G. *Biochemistry* **1997**, *36*, 11314−11322. (c) Wales, T. E.; Fitzgerald, M. C. *J. Am. Chem. Soc.* **2001**, *123*, 7709−7710.
(6) Shemyakin, M. M.; Ovchinnikov, Y. A. *Recent Dev. Chem. Nat. Carbon Compd.* **1967**, *2*, 1−46.
(7) Jaffe, H. H.; Freedman, L. D.; Doak, G. O. *J. Am. Chem. Soc.* **1953**, *75*, 2209−2211.
(8) Shin, I.; Ting, A. Y.; Schultz, P. G. *J. Am. Chem. Soc.* **1997**, *119*, 12667−12668.

10.1021/ol036102m CCC: $27.50
Published on Web 01/16/2004

for a dipeptide (Ala−Ala) and the analogous didepsi-peptide (Ala−Lac) are virtually identical.[9] Furthermore, amides and esters both strongly prefer a trans conformation, are planar, and have similar bond angles and lengths.[9−12] As a result, depsi-peptides are isostructural to proteins with an all amide backbone.

Depsi-peptides can be synthesized recombinantly[5] or chemically.[3,4] The recombinant approach requires charging *t*RNA with the desired α-hydroxy acid, while the chemical synthesis approach necessitates the availability of appropriate protected α-hydroxy acids.[5] Chemical synthesis approaches to depsi-peptides have been amply described in the literature.[3,4] Typically, solid-phase peptide synthesis utilizing appropriately protected *N*-Boc-α-amino acids and α-hydroxy acids is employed. The side-chain protecting groups (when necessary) on both the α-hydroxy acids (Figure 1) and the

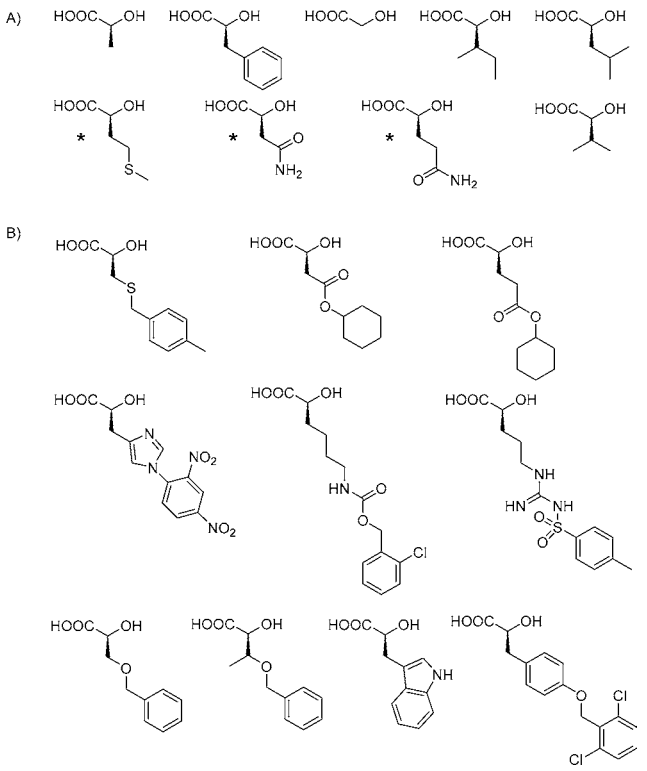


Figure 1. Nineteen α-hydroxy acids suitably protected for solid-phase depsi-peptide synthesis using a *t*-Boc strategy, employing diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole hydrate (HOBt), and *N*-ethylmorpholine (NEM) to activate the carboxyl group: (A) commercially available or previously synthesized (denoted by an asterisk) α-hydroxy acids and (B) α-hydroxy acids synthesized herein.

α-amino acids must be stable to the TFA deprotection step used to liberate the free amine in order to elongate the peptide chain with high fidelity. Only 6 of the 19 required α-hydroxy acids for A-to-E substitutions are commercially available (Figure 1A). Previous A-to-E studies utilized one or a few commercially available α-hyrdroxy acids to replace all the amino acids, often necessitating side chain structural alterations that complicate data interpretation, especially when these changes alter the hydrophobic core and hence thermodynamic stability. It is highly desirable to utilize α-hydroxy acids with the same side-chain as the α-amino acid residue being replaced to eliminate side chain effects in H-bonding studies.

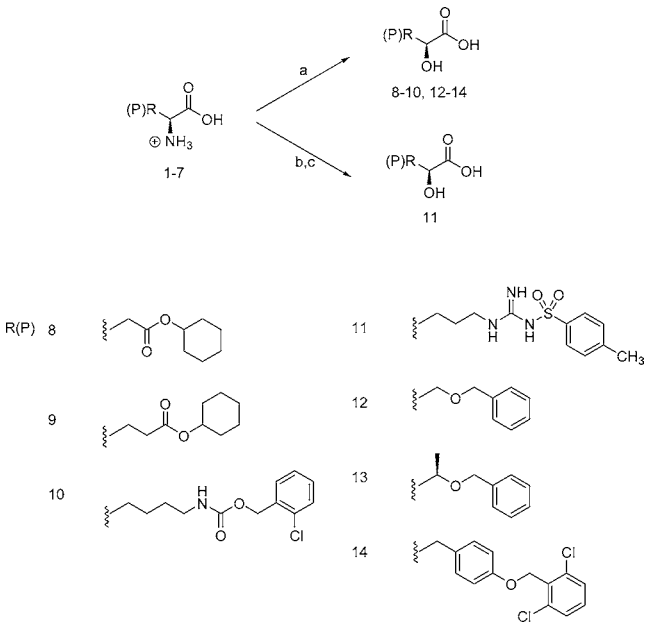
A number of approaches have been reported for the synthesis of chiral α-hydroxy acids.[13−23] Asymmetry can be achieved either by using chiral starting materials or chiral catalysts; however, none of the approaches affords all 19 of the α-hydroxy acid α-amino acid equivalents with complete stereocontrol.[14,21−23] Of the previously reported approaches, diazotization of chiral α-amino acids with sodium nitrite in acid appears to be the most efficient route for the stereospecific synthesis of α-hydroxy acids containing hydrocarbon side-chains.[15] Herein, we attempt to prepare all 19 of the α-hydroxy acids using diazotization chemistry and report the scope and limitations of this approach. Alternative stereocontrolled approaches are reported to deliver the protected α-hydroxy acids that cannot be synthesized efficiently by a diazotization strategy.

Numerous publications report variable conditions for the diazotization of L-α-amino acids using sodium nitrite in acid.[15−18,20,24−26] Variables include the concentration and identity of the aqueous acid and the sodium nitrite stoichiometry relative to the starting material. All but one of the optimizations herein led to the use of 2 equiv of sodium nitrite in 20% aqueous acetic acid (Method A). Diazotization of Arg(Tos) (Method B) is the exception, requiring glacial acetic acid for an efficient reaction. While the literature consensus suggests that a higher stoichiometry of sodium nitrite should be utilized, we found that 2 equiv relative to α-amino acid was sufficient, resulting in milder reaction conditions and less sodium nitrite to decompose at the end of the reaction. Acetic acid was preferable to other acids used previously, including hydrochloric acid, because chloride ion and related counterions can be nucleophilic enough

(9) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935−5943.
(10) Ingwall, R. T.; Goodman, M. *Macromolecules* **1974**, *7*, 598−605.
(11) Ohyama, T.; Oku, H.; Yoshida, M.; Katakai, R. *Biopolymers* **2001**, *58*, 636−642.
(12) Aravinda, S.; Shamala, N.; Das, C.; Balaram, P. *Biopolymers* **2002**, *64*, 255−267.
(13) Kunz, H.; Lerchen, H. G. *Tetrahedron Lett.* **1987**, *28*, 1873−1876.
(14) Wang, Z.; La, B.; Fortunak, J. M.; Meng, X. J.; Kabalka, G. W. *Tetrahedron Lett.* **1998**, *39*, 5501−5504.
(15) Winitz, M.; Bloch-Frankenthal, L.; Izumiya, N.; Birnbaum, S. M.; Baker, C. G. et al. *J. Am. Chem. Soc.* **1956**, *78*, 2423−2430.
(16) Takahashi, S.; Yanagida, Y. (Kanegafuchi Chemical Industry Co., Ltd., Japan). Eur. Pat. Appl. EP430234, 1991; pp 8 pp.
(17) Shin, I.; Lee, M.-r.; Lee, J.; Jung, M.; Lee, W.; Yoon, J. *J. Org. Chem.* **2000**, *65*, 7667−7675.
(18) Naito, T.; Nakagawa, S. (Bristol-Myers Co., USA). Br. Patent GB 1466001, 1977; pp 5 pp.
(19) Tang, L.; Deng, L. *J. Am. Chem. Soc.* **2002**, *124*, 2870−2871.
(20) Yoneta, T.; Shibahara, S.; Seki, S.; Fukatsu, S. (Meiji Seika Kaisha, Ltd., Japan). U.S. Patent, 1981; pp 6 pp. Cont.-in-part of U.S. Patent 4290972 Ser. No. 805, abandoned.
(21) Corey, E. J.; Link, J. O.; Shao, Y. *Tetrahedron Lett.* **1992**, *33*, 3435−3438.
(22) Ramachandran, P. V.; Brown, H. C.; Pitre, S. *Org. Lett.* **2001**, *3*, 17−18.
(23) Chang, J.-W.; Jang, D.-P.; Uang, B.-J.; Liao, F.-L.; Wang, S.-L. *Org. Lett.* **1999**, *1*, 2061−2063.

to displace the diazonium ion generated in situ from the α-amino acid, resulting in undesired products.[27,28] Functionally, acetate was less nucleophilic than water, especially under the specified conditions. In the event that the *O*-acetyl α-hydroxy acid side product was formed, it is easily converted to desired α-hydroxy acid by hydrolysis. Excess sodium nitrite remaining after completion of the reaction was quenched by addition of methylamine (2 M solution in tetrahydrofuran). The resulting sodium salts of several of the α-hydroxy acids were acidified and extracted with ethyl acetate. Highly water-soluble α-hydroxy carboxylic acids were not extractable. Instead, the carboxylates were acidified with 1 N HCl and passed through a cation exchange column (Amberlite IR-120(plus) resin). The sodium ions (from the NaCl produced by acidification) were retained on the column, yielding the partially or fully purified, protonated α-hydroxy acids after elution with deionized water. Further purification, when necessary, was achieved by standard silica gel chromatography using 90:9:1 chloroform/methanol/acetic acid as an eluent. Diazotization produced 15 of the 18 desired α-hydroxy acids; however, the His(Dnp), Trp, and Cys(4-Me-Bzl) hydroxy acid equivalents could not be produced in this fashion (Scheme 1).[5]

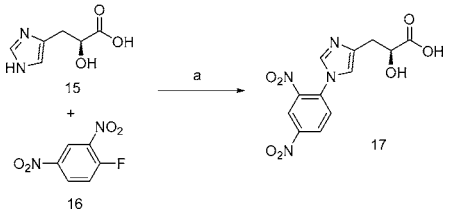
Scheme 1. Synthesis of the α-Hydroxy Acid Equivalents of the Boc-Protected α-Amino Acids by Diazotization (Method A was used for synthesis of **8**−**10** and **12**−**14**; Method B Was Utilized for Synthesis of **11**)[a]



[a] Conditions: (a) $NaNO_2$, 2:8 AcOH/H_2O (v/v), 25 °C, 16 h; (b) $NaNO_2$, AcOH, 25 °C, 16 h; (c) LiOH, 3:1:1 THF/MeOH/H_2O, 25 °C, 16 h.

The preparation of the α-hydroxy acid equivalent of His(Dnp) by diazotization may ultimately be a solvable problem; however, the reaction was not homogeneous. The product mixture was quite polar, precluding the use of standard silica gel chromatography. Alternatives to isolating the desired product were purifying the mixture by reverse-phase HPLC or chromatography on a nonpolar matrix, both considered undesirable for large-scale reactions. We lacked the incentive to optimize purification because L-*β*-imidazolelactic acid (**15**) is commercially available, which can be side-chain protected with the dinitrophenyl group simply by reacting this unprotected α-hydroxy acid with dinitrofluorobenzene (**16**) in the presence of Et_3N, affording the suitably protected α-hydroxy acid variant of His (Method C, Scheme 2).[29,30]

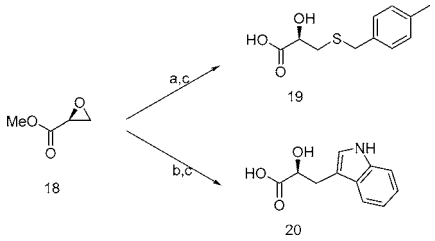
Scheme 2. Synthesis of the α-Hydroxy Acid Counterpart of His, (*S*)-3-[1-(2,4-Dinitro-phenyl)-1*H*-imidazol-4-yl]-2-hydroxy-propionic Acid (**17**) (Method C)[a]



[a] Conditions: (a) Et_3N, acetonitrile 25 °C, flask was protected from light using aluminum foil, 16 h.

Diazotization of Trp and Cys(4-Me-Bzl) did not yield the desired products because the indole ring of Trp was invariably oxidized and the 4-Me-Bzl group on Cys was subject to removal under the diazotization conditions. Since both the Trp and Cys(4-Me-Bzl) α-hydroxy acid equivalents are *β*-substituted α-hydroxy acids, it seemed feasible that they could be synthesized by nucleophilic ring opening of commercially available chiral glycidate ester (Scheme 3).

Scheme 3. Synthesis of the Trp and Cys(4-Me-Bzl) α-Hydroxy Acid Equivalents from Methyl-(2*S*)-glycidate (**18**) Using the *γ* Atom as the Nucleophile to Open the Epoxide Regio- and Stereoselectively (Method D for Synthesis of **19** and Method E for Synthesis of **20**)[a]



[a] Conditions: (a) Et_3N, 4-methylbenzenethiol, MeOH, reflux, 2 h; (b) $SnCl_4$, indole, 0-25 °C, 1 h; (c) LiOH, 3:1:1 THF/MeOH/H_2O, 25 °C, 16 h.

The thiol of 4-methylbenzenethiol effected the nucleophilic ring opening of methyl-(2*S*)-glycidate (**18**) in the presence

(24) Senkpeil, R. F.; Pantaleone, D. P.; Taylor, P. P. (PCBU Services, Inc., USA). PCT Int. Appl. WO 02/33110, 2002; pp 32 pp.
(25) Naito, T.; Nakagawa, S. (Bristol-Myers Co.). U.S. Patent US3823187, 1974; pp 2 pp.

of base (Et_3N) (Method D) affording (*S*)-2-hydroxy-3-(4-methyl-benzylsulfanyl)-propionic acid (**19**).[28] For the Trp α-hydroxy acid equivalent, indole effected the nucleophilic ring opening of methyl-(2*S*)-glycidate (**18**) in the presence of Lewis acid ($SnCl_4$) (Method E), affording (*S*)-2-hydroxy-3-(1*H*-indol-3-yl)-propionic acid (**20**).[31,32]

In general, diazotization of α-amino acids to form α-hydroxy acids is compatible with most side-chains. However, two limitations can be problematic. First, the conditions for diazotization can be too acidic or too oxidizing for some side-chains, thus resulting in undesired side products. Competing intramolecular attack by nucleophilic atoms attached to the *β*-substituent (SH of Cys) and the (guanidine group of Arg) can also complicate the diazotization reaction. The severity of this problem varies with the chemical nature of the side-chains. Second, diazotization does not always yield only the desired product. Often, the product mixture resulting from diazotization requires further purification. While most reaction mixtures can be purified by flash column chromatography on silica gel, some reaction mixtures (notably His(Dnp)) are too polar to be purified by this standard approach. Herein we have established cation exchange chromatography as a method of choice to isolate several highly water-soluble α-hydroxy acids from inorganic salts.[7]

In summary, chiral α-hydroxy acids can be prepared from their chiral α-amino acid counterparts by diazotization in the case of 16 out of 19 residues (Table 1 and Figure 1A). Stereoselectivity is excellent (>95% ee), and the yields are generally satisfactory (28−100%; 79% average yield). His(Dnp) and especially Trp and Cys(4-Me-Bzl) did not easily yield to diazotization in attempts to prepare the corresponding α-hydroxy acids. Practical alternatives were developed for the preparation of the α-hydroxy acid versions of His(Dnp), Cys(4-Me-Bzl), and Trp as depicted in Schemes 2 and 3. The methods optimized and developed herein may be applicable to the synthesis of unnatural α-hydroxy acids as well. These results make it practical for protein and medicinal chemists to synthesize the α-hydroxy acids required to make amide-to-ester substitutions in polypeptides of interest using solid-phase peptide synthesis.

Table 1. Summary of the Syntheses of α-Hydroxy Acids Prepared by the Methods Indicated

compounds	methods	yield	ee
8	A	100%	>98%
9	A	100%	>98%
10	A	80%	>95%
11	B	28%	>96%
12	A	77%	>98%
13	A	78%	>98%
14	A	90%	>95%
17	C	100%	>99%
19	D	45%	>98%
20	E	42%	>98%

Acknowledgment. We gratefully acknowledge financial support from NIH (GM 51105), The Skaggs Institute of Chemical Biology, The Lita Annenberg Hazen Foundation, and Norton B. Gilula Graduate Fellowship (S.D.).

Supporting Information Available: Experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL036102M

(26) Urban, F. J. (Pfizer Inc., USA). Eur. Pat. Appl. EP338735, 1989; pp 20 pp.
(27) Gouesnard, J. P. *Bull. Soc. Chem. Fr.* **1989**, 88−94.
(28) Roush, W. R.; Brown, B. B. *J. Org. Chem.* **1992**, *57*, 3380−3387.
(29) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B. *Org. Lett.* **2002**, *4*, 3767−3769.
(30) Henkart, P. *J. Biol. Chem.* **1971**, *246*, 2711−2713.
(31) Akita, H.; Kawaguchi, T.; Enoki, Y.; Oishi, T. *Chem. Pharm. Bull.* **1990**, *38*, 323−328.
(32) Lewis, R. T.; Macleod, A. M.; Merchant, K. J.; Kelleher, F.; Sanderson, I. et al. *J. Med. Chem.* **1995**, *38*, 923−933.