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Solid-Phase Synthesis of β-Lactams via the Miller Hydroxamate Approach

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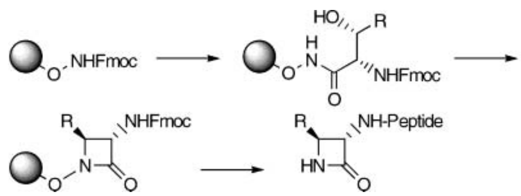
ABSTRACT



β-Lactams were prepared on solid phase starting from serine, threonine, or other β-hydroxyacids derived from naturally occurring amino acids and a resin bound hydroxylamine. The ring closure was carried out under Mitsunobu conditions. The amino group present on the β-lactam was used to assemble a short peptide. After a reductive cleavage with SmI_2, β-lactam-containing peptides were obtained

The β-lactam ring is the key component of commonly used antibiotics such as penicillins, chephalosporins, carbapenems, and monobactams.[1] Moreover, several examples of peptides and peptidomimetics containing the β-lactam ring have been recently described as effective proteases inhibitors and, consequently, as potential drugs for a wide range of diseases implicating proteases.[2]

Despite their importance, the solid-phase synthesis of β-lactams has been barely reported.[3] The favorite synthetic approach to β-lactams on support have been the [2 + 2] cycloaddition[3a−c,j] and the enolate−imine condensation.[3d,g−i]

Following our interest in the synthesis of heterocyclic-containing peptidomimetics,[4] we became interested in the solid-phase synthesis of peptides containing a β-lactam as potential protease inhibitors. In this case the cyclization of a β-hydroxyhydroxamate derived from an amino acid could be a real straightforward approach.[5]

We now report, to the best of our knowledge, the first example of Miller hydroxamate synthesis of β-lactams[6] carried out on solid phase.

The strategy chosen was to link the amino acid derivative to a polystyrene-supported hydroxylamine, then carry out the cylization under Mitsunobu conditions, and finally assemble a short peptide on the NH_2 present on the ring. This approach could be particularly suitable for solid-phase synthesis as the supported β-lactam could be easy separated from the byproducts of the Mitsunobu reaction.

The linker employed was a polystyrene resin carrying a *O*-trityl-hydroxylamine linker prepared as described in the

(1) Mandell, G. L.; Petri, W. A., Jr. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*; Hardman J. G., Limbird, L. E., Molinoff, P. B., Ruddon, R. W., Goodman Gilman, A., Eds.; McGraw-Hill: New York, 1996; p 1073.

(2) Yoakim, C.; Ogilvie, W. W.; Cameron, D. R.; Chabot, C.; Guse, I; Haché, B.; Naud, J.; O'Meara, J. A.; Plante, R.; Déziel, R. *J. Med. Chem*. **1998**, *41*, 2882. Linder, M. R.; Podlech, J. *Org. Lett*. **1999**, *1*, 869. Abell, A. D.; Gardiner, J. *J. Org. Chem*. **1999**, *64*, 9668. Palomo, C.; Aizpurua, J. M.; Benito, A.; Galarza, R.; Khamrai, U. K.; Vasquez, J.; de Pascual-Teresa, B.; Nieto, P. M.; Linden, A. *Angew. Chem., Int. Ed.* **1999**, *38*, 3056.

(3) See: (a) Ruhland, B.; Bhandari, A.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc*. **1996**, *118*, 253. (b) Pei, Y.; Houghten, R. A.; Kiely, J. S. *Tetrahedron Lett*. **1997**, *38*, 3349. (c) Singh, R.; Nuss, J. M. *Tetrahedron Lett*. **1999**, *40*, 1249. (d) Molteni, V.; Annunziata, R.; Cinquini, M.; Cozzi, F.; Benaglia, M. *Tetrahedron Lett*. **1998**, *39*, 1257. (e) Furman, B.; Thurmer, R.; Kaluza, Z.; Lysek, R.; Voelter, W.; Chmielewski, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 1121. (f) Delpiccolo, M. L.; Mata, E. G. *Tetrahedron: Asymmetry* **1999**, *10*, 3893. (g) Benaglia, M.; Cinquini, M.; Cozzi, F. *Tetrahedron Lett*. **1999**, *40*, 2019. (h) Annunziata, R.; Benaglia, M.; Cinquini, M.; Cozzi, F. *Chem. Eur. J.* **2000**, *6*, 133. (i) Schunk, S.; Enders, D. *Org. Lett*. **2000**, *2*, 907 (j) Hafez, A. M.; Taggi, A. E.; Wack, H.; Drury, W. J., III; Lectka, T. *Org. Lett*. **2000**, *2*, 3963.

(4) Filigheddu, S. N.; Taddei, M. *Tetrahedron Lett.* **1998**, *39*, 3853. Filigheddu, S. N.; Masala, S.; Taddei, M. *Tetrahedron Lett*. **1999**, *40*, 6503.

(5) For a comprehensive review on the possible synthetic approaches to azetidinones see: Perrone, E.; Franceschi, G. In *Recent Progress in the Chemical Synthesis of Antibiotics*; Lukacs, G., Ohno, M., Eds.; Springer-Verlag: Berlin, 1990; p 613.

(6) Miller, M. J.; Mattingly, P. G.; Morrison, M. A.; Kerwin, J. F., Jr. *J. Am. Chem. Soc*. **1980**, *102*, 7026.

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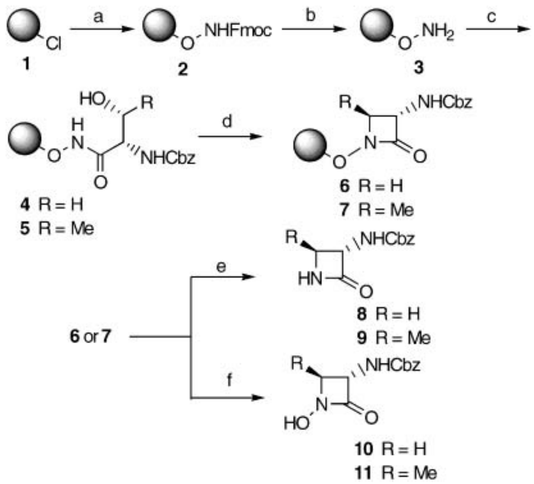
literature.[7] Following Fmoc deprotection with 20% piperidine in DMF, L-Cbz-serine (or L-Cbz-threonine) was coupled using DMTMM[8] in *N*-methylpyrrolidone (NMP) in the presence of *N*,*N*-diisopropylethylamine (DIPEA).

Scheme 1[a]



[a] (a) FmocNHOH, 2 eq. DIPEA, rt, 48 h.b) Piperidine, DMF, rt, 20 min. c) (*L*)-Cbz-Ser-OH or (*L*)-Cbz-Thr-OH, 4 eq. DMTMM, 4 eq. DIPEA, NMP, rt, 12 h. d) 5 eq. DEAD, 10 eq. PPh_3, THF, rt, 24 h. e) SmI_2 0.1 M in THF, rt, 4 h, work up as in ref 12. f) 5% TFA in CH_2Cl_2, rt, 1 h followed by aqueous workup.

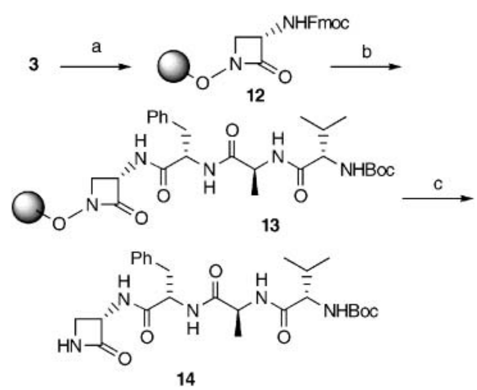
The choice of this coupling agent was due to its low reactivity with alcohol, which allows the use of a free OH group in the serine and threonine components. The coupling was achieved efficiently,[9] and the presence of the free OH was assayed with our TCT-AliR test.[10]

Attempted cyclization of compound **4** following the conditions originally reported by Miller[6] failed to produce appreciable β-lactam products (color test positive, which is indicative of the presence of a free OH). Then the amounts of DEAD and PPh_3 were increased, and the reaction was attempted in different solvents at room temperature or with heating. In each case the TCT-AliR test was always positive. On the other hand, FT-IR of the beads showed the presence of a weak signal around 1770 cm^{-1} showing that the β-lactam was partially formed. Finally we decided to use freshly distilled DEAD,[11] and the cyclization occurred in THF giving **6** with high conversion. The TCT-AliR test was negative (all beads observed at the microscope were gray), and the FT-IR showed a strong band at 1780 cm^{-1} typical of C=O stretching for *N*-alkoxy β-lactams. The same procedure was followed with compound **5** giving product **7**.

At this point two alternatives were possible for removal of the products from the resin: cleave the N−O bond to give β-lactams **8** and **9** or cleave the *N*-trityl bond to give 1-hydroxy-β-lactams **10** and **11.** The first approach was efficiently accomplished using a reductive cleavage with SmI_2 recently described by Abell and co-workers.[12] The resin was treated with a commercially available solution of SmI_2 in THF, and the products were recovered from the solution after hydrolytic workup and passage through a short silica gel column. Products **8** and **9** were obtained in acceptable yields (45% and 52% calculated from the loading of the original trityl-hydroxylamine resin **2**). Alternatively, acidic cleavage with 5% TFA in CH_2Cl_2 for 3 h followed by quench with Et_3N and aqueous workup gave compounds **10** and **11** in modest yields (about 35% in both cases, calculated as above).[13]

After determining that Miller's synthesis could be carried out successfully on solid phase, we tried the construction of a simple peptide in position 3 of the β-lactam ring. Thus *N*-Fmoc-Ser-OH was coupled on resin **3** following standard conditions. The ring closure was carried out with DEAD/PPh_3 in THF to give product **12** (IR, 1765 cm^{-1}).

Scheme 2[a]



[a] (a) *N*-Fmoc-Ser-OH, DMTMM, NMM, NMP, rt, 4 h followed by 5 equiv of DEAD, 10 equiv of PPh_3, THF, rt, 24 h. (b) Series of Fmoc deprotections with 25% piperidine in DMF followed by couplings using DMTMM in NMP with *N*-Fmoc-Phe-OH; *N*-Fmoc-Ala-OH, and *N*-Boc-Val-OH. (c) SmI_2 0.1 M in THF, rt, 4 h.

The nitrogen was deprotected following standard conditions, and a tripetide was assembled using DMTMM as the coupling agent. The efficiency of all steps was controlled using the classical Kaiser's test, and the integrity of the β-lactam was verified with FT-IR of the beads. Cleavage was carried out using SmI_2. In this case the workup procedure

(7) Mellor, S. L.; McGuire, C.; Chan, W. C. *Tetrahedron Lett*. **1997**, *38*, 3311. Different linkers, such as, for example, a Wang-type resin carrying an hydroxylamine group, were also tried with unsatisfactory results.

(8) (4,6-Dimethoxy-[1,3,5]-triazin-2-yl)-4-methyl-morpholinium chloride. See: Falchi, A.; Giacomelli, G.; Porcheddu, A.; Taddei, M. *Synlett* **2000**, 277. DMTMM is commercially available from Acros Organics.

(9) The terminal NH_2 of hydroxylamine does not give a fully positive ninhydrine test. Following Kaiser's conditions we osberved, for compound **3**, a pale yellow solution and red beads (microscope, 10X). After the coupling, the test was completely negative (uncolored beads). For Kaiser's test see: Kaiser, E.; Colescott, R. L.: Bossinger, C. D.; Cook, P. I. *Anal. Biochem*. **1970**, *34*, 595

(10) Attardi, M. E.; Falchi, A.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7395.

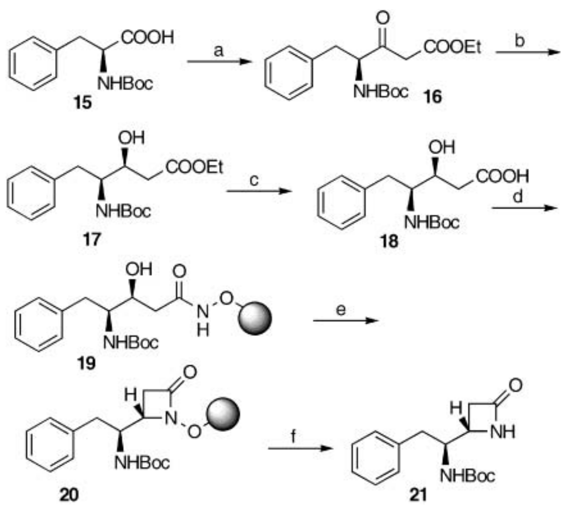
(11) Distillation should be conducted using a temperature-controlled bath because of the danger of explosion on overheating DEAD. See: Pansare, S. V.; Huyer, G.; Arnold, L. D.; Vederas, J. C. *Org. Synth*. **1991**, *70*, 1.

(12) Myers, R. M.; Langston, S. P.; Conway, S. P.; Abell, C. *Org. Lett*. **2000**, *2*, 1349. See also: Yang, H. W.; Romo, D. *J. Org. Chem*. **1999**, *64*, 7657.

was modified regarding to the literature.[12] After cleavage, the resin was washed with CH_2Cl_2 and MeOH. After evaporation, the dark residue was treated several times with water. The residual solid obtained was crude **14**, which required purification by chromatography to obtain a product with a purity higher than 95%. The final yield of **14** (calculated with respect to the loading of resin **2**) was 36%.

To extend the potential of the method we loaded on the resin the γ-amino β-hydroxyacid **18,** which was obtained starting from *N*-Boc-Phe-OH **15**. Claisen-type condensation of **15**, after carbonyl diimidazole (CDI) activation, gave β-keto ester **16** in 85% yield. The carbonyl was selectively reduced under chelation control using $TiCl_4$ and BH_3–pyridine complex at −78 °C.[14] Product **17** was obtained in high diastereomeric excess (approximately 90% from ^{1}H NMR analysis of the crude). After column chromatography, **17** was isolated in 75% yield as a single diastereoisomer. Finally, hydrolysis with LiOH in THF/H_2O gave **18** in 95% yield.[15] Compound **18** was loaded on the resin with DMTMM in NMP and NMM, and the cyclization was carried out under standard conditions.

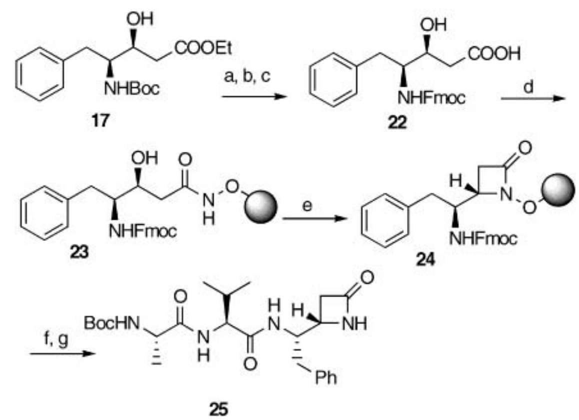
Scheme 3[a]



[a] (a) CDI, THF, rt, 24 h followed by EtAc/LDA in THF, −78 °C, 1 h. (b) $TiCl_4$ in CH_2Cl_2, 30 min, followed by BH_3·Py, −78 °C, 1 h. (c) LiOH, THF/H_2O, 24 h, followed by aqueous citric acid. (d) **3**, NMP, DMTMM, NMM, 2 h. (e) 5 equiv of DEAD, 10 equiv of PPh_3, THF, rt, 24 h. (f) SmI_2 0.1 M in THF, rt, 4 h, workup as in ref 12.

All of the steps were controlled with colorimetric tests on the beads. The ninhydrin test was negative, and TCT-AliR was positive for the loading. Finally, the TCT-AliR test was negative after cyclization. Reductive cleavage with SmI_2 gave product **21** in 52% yield.[16] Unfortunately the Boc protection on **20** could not be removed without cleaving the product from the resin. The only protection compatible with the trityl resin is Fmoc, which is, on the other hand, unstable under the reaction conditions used for carbonyl reduction. Consequently, compound **17** was deprotected with LiOH in THF/H_2O followed by formic acid. The crude material obtained after evaporation of the acid was treated with Fmoc-Cl and Na_2CO_3 in dioxane/water to give the corresponding Fmoc derivative **22** in 75% yield (Scheme 4). Compound **22** was

Scheme 4[a]



[a] (a) LiOH, THF/H_2O, 24 h. (b) HCOOH (as solvent), rt, 6 h. (c) Fmoc-Cl, Na_2CO_3, dioxane/water, rt, 12 h. (d) **3**, NMP, DMTMM, NMM, 2 h. (e) 5 equiv of DEAD, 10 equiv of PPh_3, THF, rt, 24 h. (f) Series of Fmoc deprotections with 25% piperidine in DMF followed by couplings using DMTMM in NMP with *N*-Fmoc-Val-OH and *N*-Boc-Ala-OH. (g) SmI_2 0.1 M in THF, rt, 4 h.

loaded on resin **3** and cyclized to **24** following standard conditions. The deprotection of the Fmoc (positive Kaiser's test) and the further assembling of amino acids was carried out as described for compound **14**. Final cleavage with SmI_2 gave product **25** in 36% overall yield.[17]

Unfortunately attempts to cleave the *O*-trityl bond of compounds **13** and **24** with TFA 5% in CH_2Cl_2 were unsuccessful.

(13) Products **8**−**11** showed melting points, spectroscopical data, and optical rotations comparable with those reported in the literature. **8**: mp 46−48 °C; $[\alpha]_D$ = −13.8 (*c* 1.4 in MeOH); IR (KBr, cm^{-1}) 1735. See: Cainelli, G.; Giacomini, D.; Galletti, P.; DaCol, M. *Tetrahedron Asymmetry* **1997**, *8*, 3231. **9**: mp 92−94 °C; $[\alpha]_D$ = −17.68 (*c* 4.0 in $CDCl_3$); IR (KBr, cm^{-1}) 1745. See: Andreoli, P.; Billi, L.; Cainelli, G.; Panunzio, M.; Bandini, E. *Tetrahedron* **1991**, *47*, 9061. **10**: mp 130−131 °C; IR (KBr, cm^{-1}) 1765. See: Gordon, E. M.; Ondetti, M. A.; Pluscec, J.; Cimarusti, C. M.; Bonner, D. P.; Sykes, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 6053. **11**: mp 112−117 °C; IR (KBr, cm^{-1}) 1760. See: Woulfe, S. R.; Miller, M. J. *J. Org. Chem.* **1986**, *51*, 3133

(14) Marcantoni, E.; Alessandrini, S.; Malavolta, M.; Bartoli, G.; Bellucci, M. C.; Sambri, L.; Dalpozzo, R. *J. Org. Chem.* **1999**, *64*, 1986.

(15) The *syn* relative stereochemistry of compund **18** was expected on the basis of the chelation control. The assignment was based on the values of melting point observed, 153−154 °C (lit. mp 153.2−153.4 °C): Bänzinger, M.; McGarrity, J. F.; Meul, T. *J. Org. Chem.* **1993**, *58*, 4010. The melting point reported for the *anti* isomer is 187.5 °C: Rich, D.; Sun, D. H.; Edgar, U. *J. Med. Chem.* **1980**, *23*, 27.

(16) Compound **21**: mp 102−104 °C; IR (KBr, cm^{-1}) 3200, 3010, 2950, 1735, 1680, 1600; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20−7.00 (m, 5H), 5.48 (bs, 1H), 5.10 (bs, 1H), 4.60 (m, 1H), 4.12 (m, 1H), 3.36 (A part of an ABX system, 1H), 3.06 (B part of an ABX system, 1H), 2.50−2.30 (m, 2H), 1.25 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.7, 156.4, 141.3, 128.7, 127.4, 125.8, 71.7, 59.8, 48.6, 39.8, 31.7, 24.1.

(17) Compounds **14** and **25** were characterized by ^{1}H and ^{13}C NMR and mass spectrometry (ES/MS).

In conclusion, we have demonstrated that peptides containing a β-lactam ring can be prepared on solid phase using the Miller approach. The reaction can be successfully carried out on cross-linked polystyrene using a trityl linker and the reductive cleavage of the N−O bond with SmI_2 in THF. We are currently employing this approach to prepare small libraries of β-lactam peptidomimetics.

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