JOC Article

Amino Acid Bromides: Their N-Protection and Use in the Synthesis of Peptides with Extremely Difficult Sequences

Alma DalPozzo,*,† Minghong Ni,† Laura Muzi,† Andrea Caporale,† Roberto de Castiglione,† Bernard Kaptein,‡ Quirinus B. Broxterman,‡ and Fernando Formaggio§

G. Ronzoni Institute for Chemical and Biochemical Research, via G. Colombo 81, 20133 Milano, Italy, Department of Fine Chemicals, Advanced Synthesis and Catalysis, DSM Research, P.O. Box 18, 6160 MD Geleen, The Netherlands, and Department of Organic Chemistry, University of Padova, Institute of Biomolecular Chemistry, CNR, 35131 Padova, Italy

dalpozzo@ronzoni.it

Received April 19, 2002

N^{α}-Protected α-amino acid bromides were easily generated in situ with 1-bromo-*N*,*N*-2-trimethyl-1-propenylamine from the corresponding amino acids under very mild conditions. *o*-Nbs and the azido moieties proved to be compatible with these overactivated halides and were successfully applied in difficult peptide bond formations. N-Deprotection methods and the total step-by-step solution synthesis of a peptide containing up to seven consecutive L-(αMe)Valine residues are also reported. The assembly of this homopeptide was achieved in a short time and in very high yields by the azido/bromide system in a single repetitive operation.

Introduction

Serious difficulties may be encountered during the synthesis of peptides containing unusual α-amino acids, the main problems arising from the incorporation of sterically hindered or poorly electro- or nucleophilic residues into the peptide chain. Even strongly activated amino acid chlorides or fluorides fail to give condensation with some extremely hindered C^{α}-tetrasubstituted or N^{α}-substituted amino acids. This is also the case with α-fluoroalkylamino acids, molecules in which researchers have shown much biological interest,[1,2] where, in addition, the nucleophilicity of the α-amino group is dramatically decreased by the electron-withdrawing effect of the α-trifluoromethyl substituent.

Recently, we described a new activating method for the synthesis of particularly difficult peptide bonds,[3] using N^{α}-phthaloyl-amino acid bromides as acylating agents. Bromides were obtained from the corresponding N^{α}-phthaloyl amino acids by the readily available brominating reagent proposed by Ghosez.[4] Amino acid bromides (AA-Br), which had never been used before in chemical practice, proved to be very efficient reagents for the in situ acylation, affording the amide bond formation usually in a few minutes and in all cases without a loss of chirality at the carboxylic site. This method allowed us to introduce, for the first time, *β*-fluorine-substituted α-amino acids at the C-terminus of peptides in excellent yields.

The present work is aimed at (i) exploring alternative N^{α}-protecting groups, compatible with AA-Br, and (ii) testing the performance of the acyl bromide method under the repetitive coupling/deprotection steps normally required for peptide synthesis.

As for the first target, it is worth reminding that, as compared to chlorides and fluorides,[5] AA-Brs undergo very fast cyclization to Leuch's anhydrides when N^{α}-protected as carbamates (e.g., with the Boc, Cbz, or Fmoc group). N^{α}-Protecting groups stable enough to survive to an AA-Br generation are usually difficult to remove and not generally suitable for peptide synthesis.

This is the case with phthaloyl, and also with tosyl- and Pmc-sulfonamide groups.[6] Therefore, to improve the applicability of the acyl bromide method, there is a need for N^{α}-protecting groups that are unable to take part in the cyclization and are removable under mild conditions.

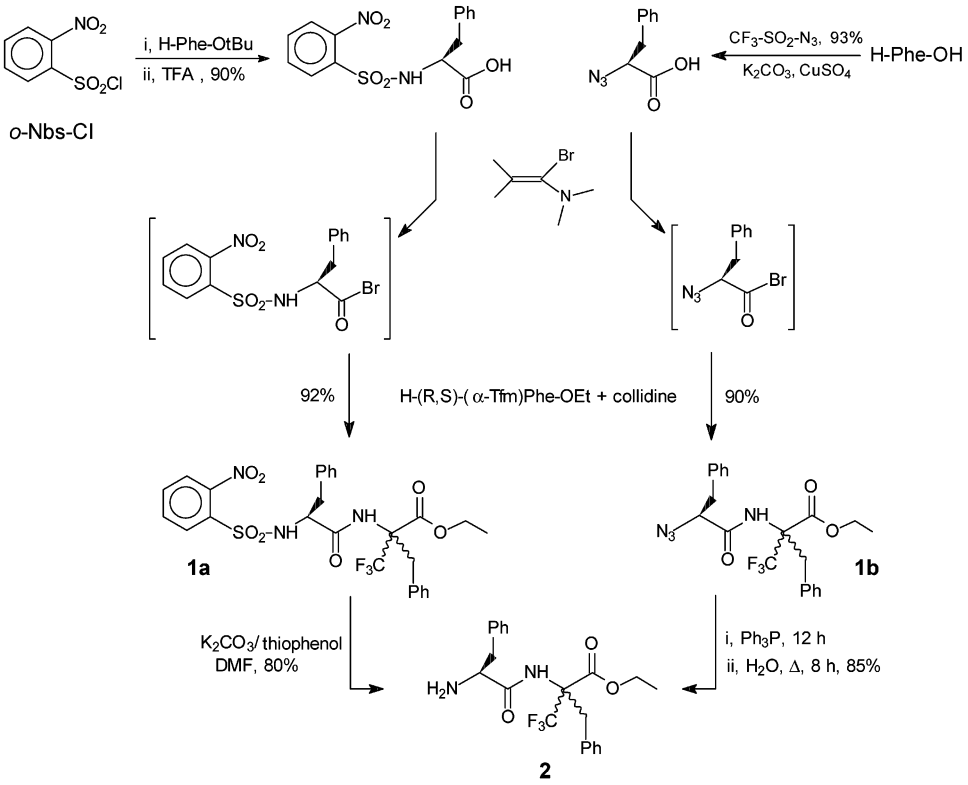
In this paper, we report the successful application of both *o*-Nbs[7] and N_3[8] as N-protecting groups in a difficult peptide bond formation involving the (α-Tfm)Phe[9] residue

* To whom correspondence should be addressed. Phone: +39-02-70600223. Fax: +39-02-70641634.
† Ronzoni Institute.
‡ DSM Research.
§ University of Padova.

(1) Ojima, I. In *Organofluorine Compounds in Medicinal Chemistry and Biochemical Applications;* Filler, R., Ed.; Elsevier: Oxford, 1993; pp 241−273.
(2) Koksch, B.; Sewald, N.; Jakubke, H.-D.; Burger, K. In *Biochemical Frontiers of Fluorine Chemistry*; Ojima, I., McCarthy, J. R., Welch, J. T., Eds.; ACS Symposium Series 639; American Chemical Society: Washington, DC, 1996; pp 42−58.
(3) DalPozzo, A.; Bergonzi, R.; Ni, M.-H. *Tetrahedron Lett.* **2001**, *42*, 3925−3927.
(4) Devos, A.; Remion, J.; Frisque-Hesbain, A.; Colens, A.; Ghosez, L. *J. Chem. Soc., Chem. Commun.* **1979**, 1180−1181.
(5) Carpino, L. A.; Beyrmann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29*, 268−274.
(6) DalPozzo, A.; Bergonzi, R.; Ni, M.-H.; Cossettini, P. In *Peptides: The Wave of the Future*; Lebl, M., Houghten, R. A., Eds.; Kluwer: Dordrecht, The Netherlands, 2002; pp 93−94.
(7) Fukuyama, T.; Jow, C. K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6573−6574.
(8) Zaloom, J.; Roberts, D. C. *J. Org. Chem.* **1981**, *46*, 5173−5176.
(9) Sewald, N.; Burger, K. In *Fluorine-Containing Amino Acids*; Kukhar, V. P., Soloshonok, V. A., Eds.; Wiley: New York, 1995; pp 130−220.

10.1021/jo020280w CCC: $22.00
Published on Web 08/14/2002

SCHEME 1



as a nucleophile. Indeed, the amino function of this amino acid is known to be extremely poorly reactive because of the steric hindrance and the polarizing effect of the trifluoromethyl group.

As far as the second target of this work is concerned, we chose to test the efficacy of the acyl bromide activation method, in combination with the azido N-protecting group, in the step-by-step solution synthesis of an L-(αMe)Val (C^{α}-methylvaline) homopeptide series. Peptides based on C^{α}-tetrasubstituted amino acids are interesting because they show a great tendency to adopt well-defined, rigid three-dimensional structures that can be exploited as spacers or templates in bioorganic chemistry. In particular, L-(αMe)Val homopeptides form very stiff β-turn or 3_{10}-helices depending on the number of residues.[10] However, the synthesis of these peptide molecular rulers is very difficult, as (αMe)Val is one of the most sterically demanding β-branched tetrasubstituted α-amino acids. Here, we compare the results obtained by means of the azido/bromide method with those reported for the same sequence, $-[$L-(αMe)Val$]_n-$ (n = 2−8),[11] prepared by using Cbz-(αMe)Val-F as the stepwise acylating agent.

Results and Discussion

The synthesis of N^{α}-protected AA-Br was achieved by simple addition of the corresponding N-protected AA to a solution of the bromoenamine, as described in the Experimental Section.

In Schemes 1 and 2, alternative methods for N-protection of AA-Br and deprotection of the products are illustrated. The *o*-Nbs group is stable to both strongly acidic and basic conditions but easily removed upon treatment with potassium thiophenolate. The alkylazido group is stable under basic conditions, but it can be removed by two different methods: either by treatment with Ph_3P/H_2O (Scheme 1) or by catalytic transfer hydrogenation with $HCOONH_4$ and Pd/C (Scheme 2b). Under the latter conditions, the N_3 function is converted to NH_2 in a few minutes with a 90% average yield, independently of the length of the peptide.

In Scheme 1, the synthesis of the model diastereomeric dipeptide **2**, obtained from two differently N^{α}-protected AA-Brs, is described. Due to the poorly reactive amino function of (α-Tfm)Phe in peptide bond formation, a 5-fold excess of the AA-Br was necessary for complete acylation. To favor the coupling reaction over the competing decomposition of the acyl bromide, we found it more convenient to add the reagent solution in two portions. In this way, we could obtain the protected dipeptides **1a** and **1b** in about 90% yield after purification.

As expected, the coupling involving (αMe)Val residues (Scheme 2) proved to be much easier as compared to that observed for the (α-Tfm)-substituted amino acid: lower amounts of acylating reagent, 2-azido-2,3-dimethylbutyric acid (N_3-DMB) bromide, and shorter reaction times were required. A homopeptide containing seven residues of L-(αMe)Val was prepared, each step giving good yields in times ranging from a few minutes to 1 h (Table 1). The reaction rates and yields tend to decrease when the length of the oligomer increases. The improvement of this synthetic procedure over the acylation via N^{α}-Cbz-amino acid fluoride, previously used for the same peptide,[10,11]

(10) Polese, A.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. *Chem. Eur. J.* **1996**, *2*, 1104−1111.

(11) Rainaldi, M. Chemistry Doctor Degree Thesis, , University of Padova, Padova, Italy, 1999.

SCHEME 2

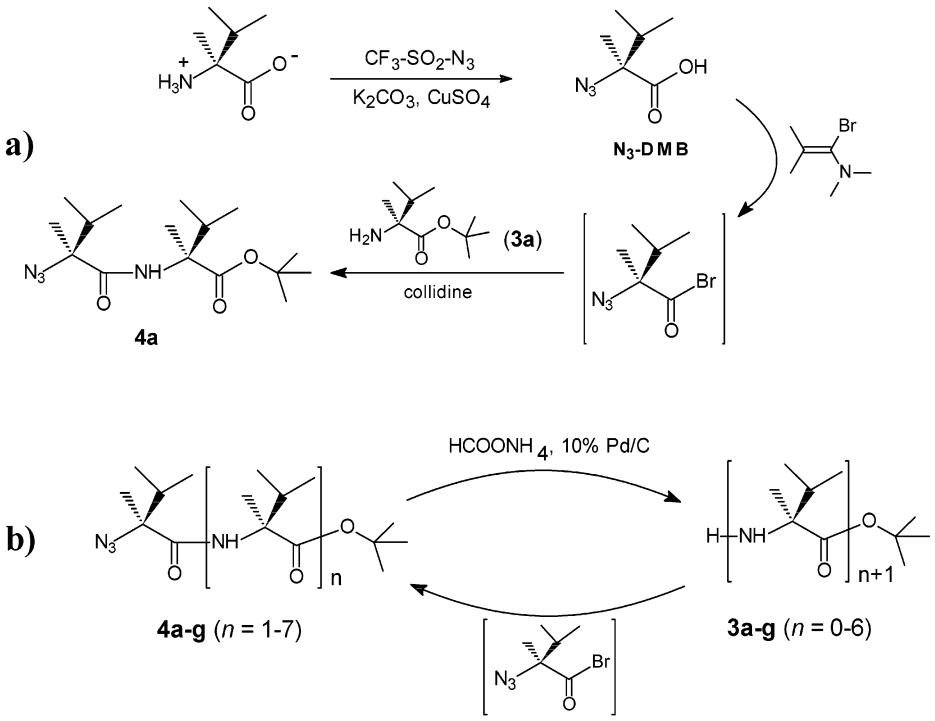


TABLE 1. Reaction Conditions for the Synthesis of N_3-Dmb-[L-(αMe)Val]$_n$-OtBu (n = 1−7 for 4a−g, respectively) Peptides

entry	equiv of N_3-DMB	reaction time (min)	yield (%)
4a	1	10	98.6
4b	1	10	90.2
4c	2	20	99.0
4d	2	20	86.9
4e	2	60	76.8
4f	3	120	68.5
4g	3	120	67.0

is dramatic. As a matter of fact, the reaction via Cbz/acyl fluoride was very sluggish, each coupling taking 1 week on the average, with modest yields (total yield for the final peptide) equaling about 5%.

Another great advantage of the acyl bromide method comes from the in situ generation of the acylating species, thus favoring a rapid step-by-step solution-phase peptide assembly. Indeed, in most cases, the isobutyramide formed from the bromoenamine reagent is easily removed by simple filtration through silicagel at the end of the coupling reaction. On the contrary, α-amino acid fluorides need to be isolated before use to achieve satisfactory results.

The N_3 group at the N-terminus, besides preventing the aminoacyl bromide intramolecular cyclization, endows the intermediate and final oligomers with higher solubility as compared to the Cbz-protected analogues. Moreover, this group does not interfere with the tendency of L-(αMe)Val homopeptides to adopt turn/helical conformations, as clearly shown in Figure 1. As the peptide main-chain length increases, the relative intensities of the IR band associated with the hydrogen-bonded amide N−H stretching modes (below 3400 cm^{-1}) grow and, concomitantly, the frequency of the absorption maximum

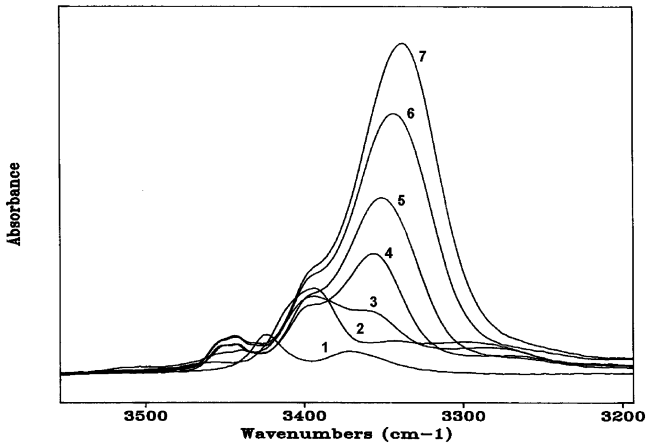


FIGURE 1. FT-IR absorption spectra in the conformationally informative region 3500−3200 cm^{-1} for the N_3-DMB-[L-(αMe)-Val]$_n$-OtBu (n = 1−7 for **4a**−**g**, respectively) homopeptide series in $CDCl_3$ solution. Peptide concentration: 0.1 mM.

decreases. This behavior clearly indicates that the β-turn type of folding, already observed at the level of the tetrapeptide (n = 3 in Figure 1), evolves into a 3_{10}-helical structure in the longest oligomers.[12]

Conclusions

α-Amino acid bromides, when suitably N$^{\alpha}$-protected, have been shown to be superior reagents for the solution synthesis of difficult peptide sequences. Because of the mild conditions involved, the bromides can be generated in situ and directly used for coupling, thus avoiding the disadvantages associated with their isolation and purification.

(12) Toniolo, C.; Bonora, G. M.; Barone, V.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Grimaldi, P.; Lelj, F.; Pavone, V.; Pedone, C. *Macromolecules* **1985**, *18*, 895−902.

The different protecting groups used in combination with amino acid bromides provide enough orthogonality for application to the synthesis of a variety of peptide sequences. In particular, α-azido acids appear to be ideal substrates for conversion to the corresponding acyl bromides and their subsequent utilization in the coupling reactions.

The successful results reported here suggest that this new coupling procedure is not restricted to special situations, but rather should find extensive application in current peptide synthesis. Moreover, it might be applied to other interesting classes of peptidomimetics hitherto practically unavailable.

Experimental Section

General. DCM was distilled over P_2O_5 and stored under argon. Optically pure L-(αMe)Val was prepared according to a published chemoenzymatic method[13] by DSM Research. Thin-layer chromatography (TLC) was routinely carried out to monitor reactions, and the products were located with UV light (254 nm), ninhydrin, or $KMnO_4$ according to their chemical structure. Analytical liquid chromatography (RP-HPLC) was carried out with a Ultrasphere ODS (5μ, 10 × 250) Beckmann column. H-(*R*,*S*)-(α-Tfm)Phe-OEt was synthesized following published methods.[14]

o-Nbs-Phe-OH was prepared from H-Phe-O*t*Bu and *o*-nitrobenzene-sulfonyl chloride by the method described by Fukuyama et al.,[7] followed by treatment with TFA. α-Azido acids were synthesized by diazotransfer reaction from the corresponding α-amino acids as described by Alper et al.[15] 1-Bromo-*N*,*N*-2-trimethyl-1-propenylamine was obtained by the method of Ghosez et al.[16] The reagent can be stored in vials containing an approximately 0.5 M solution in DCM, under Ar, at −18 °C, for several months. The title of the solution was determined before use by converting Pht-Phe-OH (Pht, phthaloyl) into its bromide and quenching with dry methanol: disappearance of the acid and appearance of the methyl ester was monitored by TLC, under UV light. N^{α}-protected amino acid bromides: in a typical procedure 1.15 mmol of amino acid was dissolved into 2.4 mL of a 0.5 M solution of bromoenamine in DCM and stirred under Ar for 15 min. The solution of the bromide was used immediately for acylation of the desired α-amino acid ester or peptide.

H-Phe-(*R*,*S*)-(α-Tfm)Phe-OEt (2). A solution of H-(*R*,*S*)-(α-Tfm)Phe-OEt[14] (100 mg, 0.383 mmol) and collidine (50 μL, 0.383 mmol) in 1 mL of DCM at 0 °C was treated with the desired acyl bromide solution (1.15 mmol of the in situ, freshly prepared *o*-Nbs-Phe-Br or 2-azido-3-phenylpropionyl bromide) and, after 10 min, with an additional amount (0.766 mmol) of the bromide solution. After 3 h at room temperature, the substrate disappeared (monitoring by HPLC). The solvent was evaporated and the residue redissolved in 60 mL of a 1:2 mixture of THF−5% $NaHCO_3$ and stirred for 20 min. After removal of THF, the aqueous solution was extracted with DCM (1 × 40 mL, 2 × 20 mL). The combined organic phases were washed with water (10 mL), 1 N HCl (10 mL), and water and then evaporated to dryness. **Deprotection of 1a:** After flash chromatography (75:25 hexanes−EtOAc), *o*-Nbs-Phe-(*R*,*S*)-(α-Tfm)Phe-OEt was obtained as a thick oil (yield 92%). Compound **1a (**100 mg, 0.168 mmol) was dissolved in 1 mL of DMF containing thiophenol (35 μL, 0.336 mmol) and K_2CO_3 (92.8 mg, 0.672 mmol), under Ar. After 10 min, the substrate disappeared (TLC, 1:1 hexanes−EtOAc). The solvent was evaporated and the residue redissolved in 30 mL of EtOAc and washed with water (2 × 20 mL) and brine (20 mL). After evaporation of the solvent, the residue was purified by flash chomatography (99:1 $CHCl_3$−MeOH), affording 54.5 mg of the pure N^{α}-deprotected peptide **2** (yield 80%). **Deprotection of 1b:** After flash chromatography (80:20 hexanes−EtOAc), 2-azido-3-phenylpropionyl-(*R*,*S*)-(α-Tfm)Phe-OEt was obtained as a yellowish syrup (yield 90%). Compound **1b** (100 mg, 0.23 mmol) was dissolved in 1.5 mL of THF; Ph_3P (181 mg, 0.69 mmol) in 1.5 mL of THF was slowly added and the mixture left under stirring at room temperature overnight. Then, 150 μL of water was added and the solution refluxed for 8 h. After evaporation of the solvent, the residue was purified by flash chromatography (60:40 hexanes−EtOAc) giving **2** (yield 85%).

2 (Diastereomeric Mixture): RP-HPLC (50% CH_3CN in water + 0.1% TFA); diastereomer I, R_t 12.89 min; diastereomer II, R_t 13.62 min.; ^{1}H NMR ($CDCl_3$) δ 7.30−6.90 (m), 4.44−4.25 (m), 4.15 and 3.48 (2 dd, J = 12.87 and 11.38), 3.61−3.55 (m), 3.30, 2.68 and 2.40 (dd, J = 9.54, 13.79), 1.34 (2t); ^{19}F NMR ($CDCl_3$, TFA) δ 1.59 and 1.57. Anal. Calcd. for $C_{21}H_{23}F_3O_3 \cdot H_2O$: C, 59.15; H, 5.86; N, 6.57; F; 13.38. Found: C, 60.37; H, 5.89; N, 6.52; F, 13.18.

N_3-DMB-L-(αMe)Val-O*t*Bu (4a). To a solution of HCl·H-L-(αMe)Val-O*t*Bu (**3a**)[10] (295 mg, 1.32 mmol) and collidine (525 μL, 3.96 mmol) in 6 mL of DCM, at 0 °C, was added 2.5 mL of the N_3-DMB bromide solution (1.32 mmol). After 10 min at room temperature, the substrate disappeared (TLC, 1:1 hexanes−EtOAc). After evaporation of the solvent, 25 mL of 5% $NaHCO_3$ and 12 mL of THF were added to the residue and the mixture was stirred for 30 min. After evaporation of THF, the aqueous solution was extracted with DCM and the combined organic phases were washed with water, 1 N HCl, and water to neutrality. The solvent was dried and evaporated, and the crude residue was dissolved in 70:30 hexanes−EtOAc and filtered through silica gel. The title compound **4a** was obtained as a colorless oil (yield 98.6%): $[\alpha]_D^{20}$ −17.0° (*c* 0.3, MeOH); IR (KBr) 3360, 2113, 1713, 1669, 1514 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.13 (s), 2.22 and 2.16 (2 m), 1.54 and 1.51 (2s), 1.47 (s), 1.00 and 0.92 (2 m).

N_3-DMB-[L-(αMe)Val]$_n$-O*t*Bu (*n* = 2−7; 4b−g). To a solution of **4a** (1.3 mmol) in 7 mL of MeOH were added 5.2 mmol of ammonium formate and 250 mg of 10% Pd/C, and the mixture was stirred for 30 min at rt. The reaction mixture was filtered through Celite (2.5 g) and the filtrate evaporated to dryness; the residue was redissolved in 30 mL of DCM and the solution washed with brine. The organic phase was dried and evaporated affording 354.7 mg of the dipeptide H-[L-(αMe)-Val]$_2$-O*t*Bu (**3b**) (97%), which was immediately coupled to N_3-DMB bromide, as described above for **4a**, to give **4b**. Further iterative synthetic steps to build the azido-peptide **4g** were performed using the same procedure (Scheme 2b). When required, the intermediates were purified by flash chromatography. **4g:** mp 239−240 °C; $[\alpha]_D^{20}$ +6.3° (*c* 0.3, MeOH); IR (KBr) 3334, 2106, 1727, 1660, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.70, 7.27, 7.22, 7.15, 6.95 and 6.21 (s), 2.40−1.70 (m), 1.62−1.20 (m), 1.07−0.83 (m); ^{13}C NMR ($CDCl_3$) δ 173.58, 173.17, 173.16, 172.72, 172.39, 172.23, 171.23, 170.23, 79.92, 70.47, 63.60, 63.46, 63.43, 63.38, 62.51, 62.44, 37.10, 36.32, 36.11, 35.91, 35.90, 35.63, 33.84, 27.91, 20.29, 19.27, 19.24, 19.18, 18.17, 17.81, 17.75, 17.77, 17.59, 17.56, 17.54, 17.50, 17.42, 17.37, 17.27, 17.14, 17.12, 17.08, 17.06, 17.04, 16.86; MS calcd for (M + Na^+) 1028, found 1028; MS calcd for (M + K^+) 1044, found 1044.

Acknowledgment. The authors are indebted to Prof. Claudio Toniolo (University of Padova) for helpful suggestions and discussions.

JO020280W

(13) Sonke, T.; Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Kamphuis, J.; Formaggio, F.; Toniolo, C.; Rutjes, F. P. J. T.; Schoemaker, H. E. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Dekker: New York, 2000; pp 23−58.

(14) Burger, K.; Gaa, K. *Chem. Zeit.* **1990**, *114*, 101−104.

(15) Alper, P. B.; Hung, S. C.; Wong, C. H. *Tetrahedron Lett.* **1996**, *37*, 6029−6032

(16) Haveaux, B.; Dekoker, A.; Rens, M.; Sidani, A. R.; Toye, J.; Ghosez, L. *Org. Synth.* **1980**, *59*, 26−34.