Tetrahedron Letters 49 (2008) 4958–4961

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A Fmoc-based submonomeric strategy for the solid phase synthesis of optically pure chiral PNAs

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ARTICLE INFO

Article history:
Received 14 April 2008
Revised 20 May 2008
Accepted 23 May 2008
Available online 17 June 2008

Keywords:
Chiral PNAs
Submonomeric strategy
Optical purity
Fmoc strategy

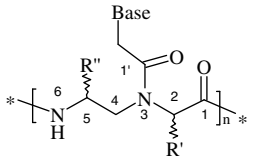
ABSTRACT

A new submonomeric approach for the Fmoc solid phase synthesis of chiral PNAs is reported here. The design and synthesis of a new D-lysine-based Fmoc submonomer obtained by replacing the nucleobase residue with an Alloc group, compatible with Fmoc chemistry, is described starting from D-lysine and Fmoc-aminoacetaldehyde. The desired submonomer was obtained in high yield with no racemization. The conditions for synthesizing a chiral PNA dimer on solid support by a submonomeric approach have been optimized, allowing to obtain the desired PNA with a very high optical purity.

Peptide nucleic acids (PNAs) are well-known DNA mimics introduced by Nielsen and co-workers in 1991.[1] Their structure is based on a neutral and achiral pseudopeptide backbone based on *N*-(2-aminoethyl)glycyl units, with nucleobases linked to the secondary amines by carboxymethylene linkers. PNAs are able to bind complementary DNA/RNA sequences through standard Watson–Crick hydrogen bonds with high sequence specificity and affinity.[2] For their outstanding hybridization properties, together with their high chemical and biological stability, PNAs have found many applications in different areas, including bioorganic chemistry, drug discovery, molecular biology, diagnostics, prebiotic evolution and material science.[3]

Many examples of modified PNAs have been developed by introducing functional groups with different charge, polarity and flexibility.[4,5] Amongst modified PNAs, chiral amino acid-based analogues substituted in position 2, position 5 or both (Fig. 1) have recently gained increasing attention, due to their peculiar DNA binding abilities easily tunable according to the different configurations,[6,7] to the new properties induced by the aminoacidic residues[8,9] and to the possibility to use amino acid side chains in order to link new functionalities.[10,11]

Since the performances of chiral PNAs in terms of binding specificity and selectivity are strongly affected by the configurations of the stereogenic centers, their optical purity is a primary issue, although often neglected. In particular, PNA synthesis of chiral derivatives substituted in position 2 can be strongly affected by racemization, as demonstrated by a GC method developed by our group.[12] In order to maintain a very high optical purity, a Boc-based 'submonomeric strategy' for the solid phase synthesis of these oligomers was developed:[13] according to this strategy, the Boc-protected chiral backbone, carrying a Fmoc group on the secondary amine in place of the nucleobase acetyl moiety, was coupled to the PNA growing chain on the resin, followed by Fmoc deprotection and resin coupling of the nucleobase acetic acid. The assembly of the full chiral PNA residue directly on resin allowed for a fast and almost racemization-free synthesis of chiral PNAs. Although successful, this protocol has the main drawback of using the harsh synthetic conditions of the Boc-chemistry. Since the conjugation of PNAs with fluorophores and other labelling molecules, which are often labile at strong acidic conditions, is becoming of great interest, the use of a milder chemistry, such as in Fmoc-based strategies, should be advisable. Recently, Seitz and co-workers[14] have proposed a submonomeric approach in the synthesis of achiral PNAs as FIT (Force Intercalation of Thiazole orange) probes.



R '= R" = H: standard achiral PNA
R' = amino acid side chain, R"=H: chiral 2-substituted PNA
R' = H, R" = amino acid side chain: chiral 5-substituted PNA
R' = R"= amino acid side chain: chiral 2,5-substituted PNA

Figure 1. Schematic representation of achiral and chiral PNAs.

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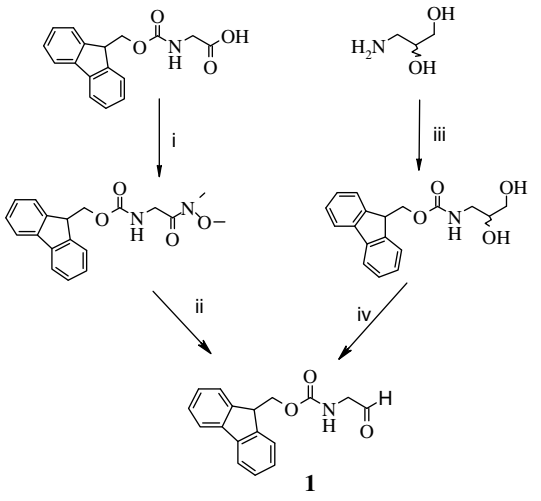
doi:10.1016/j.tetlet.2008.05.114

Hence, in this Letter we propose for the first time a new Fmoc-based submonomeric strategy for the synthesis of optically pure chiral PNAs. We report the design and the synthesis of a new Fmoc-compatible submonomeric unit carrying a lysine side chain at the carbon 2. This new submonomer has been used in the synthesis of a chiral PNA dimer on solid phase support by Fmoc protocol. The preservation of the optical purity all along the synthetic steps was also demonstrated.

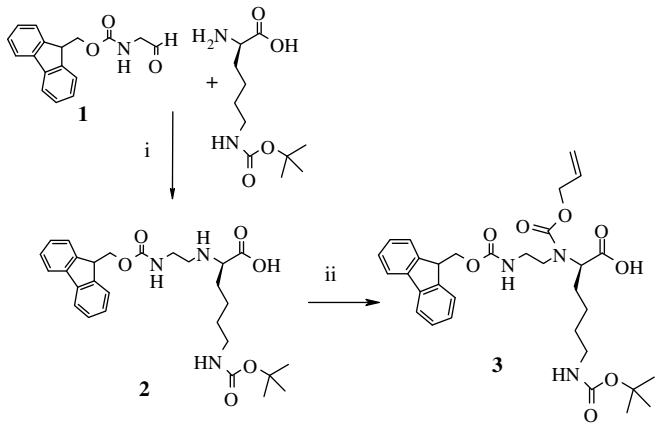
Synthesis of the Fmoc-submonomeric unit based on 2D-Lysine backbone: In order to obtain a chiral PNA submonomer Fmoc protected at the N-terminus, Fmoc-*N*-aminoacetaldehyde **1** was first synhtesized. Its synthesis has been reported in the literature, although with low yields, by reduction with $LiAlH_4$ of the Fmoc glycine Weinreb amide.[15] We tried this approach in comparison with a new one based on the oxidation of the Fmoc-*N*-(±)-3-amino-1,2-propandiol with KIO_4 (Scheme 1). In both strategies the first steps, the synthesis of the Weinreb amide from the Fmoc-glycine in one case and the introduction of the Fmoc group on the (±)-3-amino-1,2-propandiol in the other case, were obtained with quite high yields. In contrast, the reduction with $LiAlH_4$ of the Weinreb amide was not very efficient, whereas the diol oxidation by periodate was almost quantitative, making the second route preferable.

Then, the 2D-lysine backbone **2** was obtained by reductive amination of **1** with H-D-Lys(Boc)-OH. Although this reaction is usually performed with the carboxyl function protected as an ester,[13] in this case the reaction was performed directly on the amino acid residue without protecting the carboxyl function (Scheme 2). Consistent with studies performed by Gilon and co-workers[16] on other amino acid derivatives, this procedure gave good yields and, as a further advantage, the product was easily purified by direct precipitation from the reaction mixture.

In order to obtain a suitable submonomer to be used in a solid phase Fmoc-PNA synthesis, it appeared necessary to have a protecting group on the secondary amine, in place of the nucleobase acetyl moiety, which could be cleaved without affecting neither the temporary Fmoc group at the N-terminus nor the orthogonal Boc-protecting group used for the lysine side chain. Since the presence of a carbamate as protecting group in this position was found to be essential in order to maintain a high optical purity during coupling,[13] the allyloxycarbonyl (Alloc) group was chosen. This group is removed by nucleophilic cleavage in the presence of palladium, being thus orthogonal both to Fmoc and Boc-groups, and it has been already tested on the achiral PNAs chemistry.[14] The Alloc



Scheme 1. Reagents and conditions: (i) 0.97 equiv *N*-methyl-*N*-mehoxy amine. HCl, HBTU, DIEA, DMF, η: 98%; (ii) 4.6 equiv $LiAlH_4$ (1 M in THF), THF, η: 30%; (iii) FmocOSu, Na_2CO_3, 1,4-dioxane, η: 80%; (iv) KIO_4, H_2O/acetone, η: 99%.



Scheme 2. Reagents and conditions: (i) 1 equiv $NaBH_3CN$, 1.1 equiv CH_3COOH, CH_3OH, η: 53%; (ii) 2 equiv Alloc–Cl, 1.5 equiv DIPEA THF, η: 91%.

group introduction was initially carried out by following the same conditions used for the introduction of Fmoc group on Boc-lysine backbone: Alloc–Cl/DIEA in DCM with bis(trimethylsilyl)acetamide (BTSA). BTSA was added in order to increase the reagent solubility by formation of a transient silyl ester, which was also previously found essential in order to avoid racemization.[13] Unfortunately, the reaction was totally unsuccesful by this strategy. Also the substitution of Alloc–Cl with Alloc anydride did not improve the yields. As a further attempt, BTSA was excluded from the reaction mixture and DCM was replaced with a more polar solvent (THF). In these conditions the reaction was successful and the submonomer **3** was obtained with high yields (90%) (Scheme 2).

In order to check if the lack of the in situ protection of the carboxylic function had caused racemization, a chiral analysis was performed by using the chiral GC method previously developed.[12] For both **2** and **3** an ee of 99% was found, indicating that this synthetic procedure does not affect the optical purity of the lysine backbone.

All the compounds described here were characterized by ^1H, ^{13}C NMR and ESI mass spectrometry. The characterization of compounds **1**, **2** and **3** is reported.[17]

Solid phase synthesis of a chiral PNA dimer based on 2D-lysine by Fmoc submonomeric strategy: The submonomer synthesized above was used for the solid phase synthesis of the chiral PNA dimer **4** by Fmoc-based submonomeric strategy (Fig. 2).

Starting from the resin preloaded with an achiral T PNA monomer (Fmoc-T-Linker-AM Champion resin, 0.17 mmol/g), the submonomer **3** was successfully inserted by manual coupling using a standard HBTU/DIEA protocol.[13]

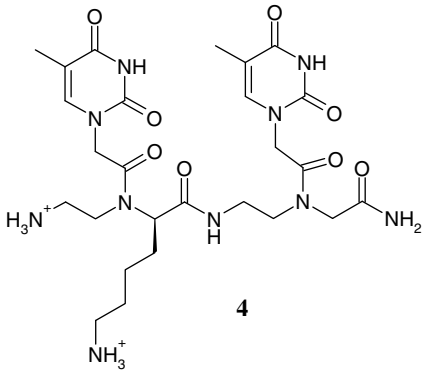
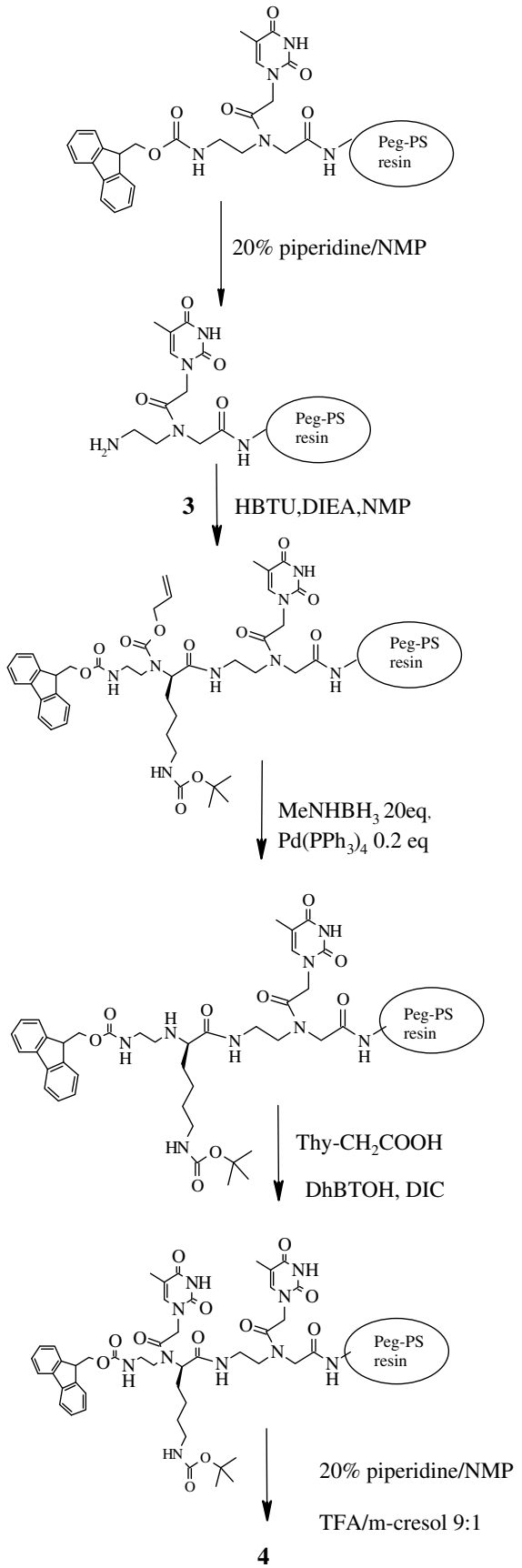


Figure 2. Chiral PNA dimer H–$T_{(D\text{-}Lys)}$T–NH_2 **4**.



Scheme 3. Scheme of the submonomeric cycle for the insertion of a chiral monomer on a PNA chain on solid phase by Fmoc protocol.

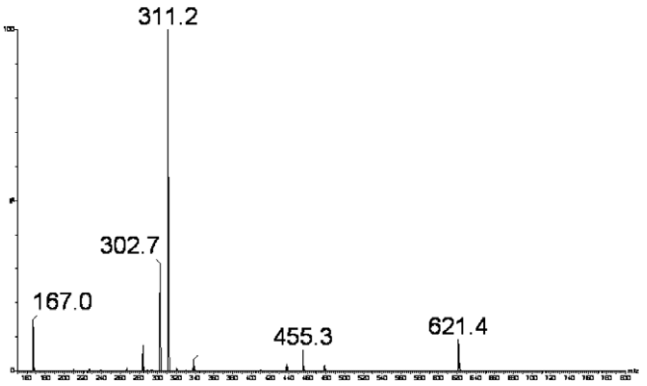


Figure 3. ESI-MS spectrum of the purified chiral PNA dimer **4**. *m/z*: 621.4: $[M+H^+]^+$, 455.3: $[M–ThyCH_2CO^+{+}2H^+]^+$, 311.1: $[M+2H^+]^{2+}$, 302.7: $[M-NH_3{+}2H^+]^{2+}$, 167.0: $ThyCH_2CO^+$.

The next step was the deprotection of the N^{α}-Alloc protecting group. The Alloc removal is usually done through the allyl group transfer *via* nucleophylic substitution, which takes place in the presence of a palladium complex. According to the literature,[18] the dimethyl amino borane complex was used as cleaving reagent, by using the same conditions reported by Seitz and co-workers[14] (0.1 equiv $[Pd(PPh_3)_4]$, 8 equiv Me_2NHBH_3 in DCM at room temperature for 40 min). After cleavage of the product from the resin, the reaction completion was monitored by HPLC/ESI-MS analysis. Unfortunately, these conditions were not sufficient to completely cleave the Alloc, even after long reaction times, very likely for the high steric hindrance of the protected amino acid side chain nearby. Thus, the Alloc deprotection reaction was performed by increasing the amounts of $[Pd(PPh_3)_4]$ and Me_2NHBH_3. Complete removal of the protecting group was finally achieved by using a DCM solution containing 0.2 equiv of $[Pd(PPh_3)_4]$ and 20 equiv of Me_2NHBH_3 and by repeating the reaction two times for 1 h each.

For the introduction of the thymine acetic acid, the same coupling reaction conditions already used for the Boc submonomeric strategy,[13] which are consistent with the Fmoc group, were applied, by using DIC/DhBTOH in NMP. After removal of the terminal Fmoc group by a 20% solution of piperidine in NMP, the chiral PNA dimer **4** was cleaved from the solid support with a TFA/*m*-cresol = 9:1 mixture (Scheme 3).

The crude product was obtained after precipitation with diethyl ether (yield 75% as determined by HPLC/MS). The chiral PNA dimer **4** was purified by semipreparative HPLC and characterized by ESI-MS (Fig. 3). Its optical purity was checked by chiral GC:[12] an outstanding ee of 98% was found.

From these results it is clear that the new Fmoc submonomeric strategy reported here is fully suitable for the obtainment of PNAs incorporating optically pure chiral residues: the enantiomeric excess observed is higher than that obtained by standard PNA solid phase synthesis;[19] the preparation of the submonomer can be made in fewer synthetic steps than the previously reported Boc protocol, and the protocol can be used for conjugating PNAs with fluorophores or other labelling molecules labile in strong acidic conditions.

Acknowledgement

Financial support from Italian Ministry of Instruction, University and Research (MIUR) through Projects of Relevant National Interest (PRIN 2005, Grant No. 200503874) is gratefully acknowledged.

References and notes

1. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497.
2. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566.
3. Lundin, K. E.; Good, L.; Stromberg, R.; Gaslund, A.; Smith, C. I. E. *Adv. Genet.* **2006**, *56*, 1–51.
4. Kumar, V. A.; Ganesh, K. N. *Acc. Chem. Res.* **2005**, *38*, 404–412.
5. Corradini, R.; Sforza, S.; Tedeschi, T.; Totsingan, F.; Marchelli, R. *Curr. Top. Med. Chem.* **2007**, *7*, 681–694.
6. Sforza, S.; Haaima, G.; Marchelli, R.; Nielsen, P. E. *Eur. J. Org. Chem.* **1999**, 197–204.
7. Sforza, S.; Tedeschi, T.; Corradini, R.; Marchelli, R. *Eur. J. Org. Chem.* **2007**, 5879–5885.
8. Dragulescu-Andrasi, A.; Rapireddy, S.; He, G.; Bhattacharya, B.; Hyldig-Nielsen, J.J.; Zon, G.; Ly, D.H.; *J. Am. Chem. Soc.*, **2006**, 128, 50, 16104.
9. Rapireddy, S.; He, G.; Roy, S.; Armitage B.A.; Ly, D.H.; *J. Am. Chem. Soc.*, **2007**, 129, 50, 15596.
10. Englud, E.; Appella, D.H.; *Org. Lett.*, **2005**, 7, 16, 3465.
11. Englund, E. A.; Appella, D. H. *Angew. Chem., Int. Ed.* **2007**.
12. Corradini, R.; Di Silvestro, G.; Sforza, S.; Palla, G.; Dossena, A.; Nielsen, P. E.; Marchelli, R. *Tetrahedron: Asymmetry* **1999**, *10*, 2063.
13. Sforza, S.; Tedeschi, T.; Ciavardelli, D.; Corradini, R.; Dossena, A.; Marchelli, R. *Eur. J. Org. Chem.* **2003**, 1056.
14. Jarikote, D. V.; Kohler, O.; Socher, E.; Seitz, O. *Eur. J. Org. Chem.* **2005**, 3187–3195.
15. Baghavanthy, S. B.; Gallazzi, F.; Jia, F.; Lewis, M. R. *Bioconjugate Chem.* **2006**, *17*, 551–558.
16. Bitan, G.; Muller, D.; Kasher, R.; Gluhov, E. V.; Gilon, C. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1501–1510.
17. Compound **1**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.6 (s, 1H, C–H aldehyde), 7.7 (d, 2H, J = 7.5, C–H aromatic Fmoc), 7.6 (d, 2H, J = 7.1, C–H aromatic Fmoc), 7.4–7.2 (m, 4H, C–H aromatic Fmoc), 5.5 (br s, 1H, N–H carbamate), 4.4 (d, 2H, J = 6.7 CH_2 Fmoc), 4.2 (t, 1H, J = 6.7, C–H Fmoc), 4.1 (d, 2H, J = 4.5 CH_2 glycine). ^{13}C NMR (75 MHz,$CDCl_3$): δ 196.5, 156.2, 143.7, 141.3, 127.7, 127, 124.9, 119.9, 67.1, 51.6, 47. ESI (CH_3OH, positive ions) calcd m/z: 336.1 ($M+CH_3OH+Na^+$), 352.1($M+CH_3OH+K^+$), found: 336.3 ($M+CH_3OH+Na^+$), 352.3($M+CH_3OH+K^+$).
Compound **2**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.9–7.8 (m, 2H, aromatic Fmoc), 7.7–7.6 (m, 2H, aromatic Fmoc), 7.4–7.3(m, 4H, aromatic Fmoc), 6.7 (br s, 1H, N–H carbamate), 6.3 (br s, 1H, N–H carbamate), 4.3–4.2 (m, 3H, CH+CH_2 Fmoc), 3.2–3.1 (m, 3H, C(2)–H + C(5)–H_2), 2.9–2.7 (m, 4H, C(4)–H_2 + CH_2 lysine side chain), 1.5–1.3 (m, 15H, CH_2 lysine side chain + CH_3 Boc). ^{13}C NMR (75 MHz, DMSO-d_6): δ 72.2, 156, 155.3, 143.7, 142.2, 128.7, 127.4, 127.1, 126.9, 124.9, 121.2, 120, 119.8, 77.2, 66, 61.7, 46.5, 40.1, 38.1, 30.7, 29.3, 28.1, 22.3, 21.4. ESI (CH_3OH, positive ions) calcd m/z: 512.3 (MH^+), 534.3 (MNa^+), 549.3 (MK^+), found: 512.5, 534.5, 549.5. Ee (chiral GC–MS according to the method reported in the literature[12]): 99%.
Compound **3**: ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.3: δ 10.3 (br s, 1H, CO_2–H carboxylic acid), 7.8 (d, 2H J = 7.3, aromatic Fmoc), 7.6 (d, 2H, J = 7.3 aromatic Fmoc), 7.4 (t, 2H, J = 7.3, aromatic Fmoc), 7.3 (t, 2H, J = 7.3, aromatic Fmoc), 5.9–5.8 (m, 1H, C–H Alloc group), 5.3–5.2 (m, 2H, CH_2 alloc group), 4.6–4.5 (m, 2H, CH_2 alloc group), 4.3–4.2 (m, 3H, CH + CH_2 Fmoc), 3.9 (br s, C(2)–H), 3.4–3.3 (m, 2H, C(5)–H_2), 3.2–3.1 (m, 2H, C(4)–H_2), 2.9–2.8 (m, 2H CH_2 lysine side chain), 1.4–1.2 (m, 15H, CH_2 lysine side chian + CH_3 Boc). ^{13}C NMR (75 MHz, DMSO-d_6): δ 72.6, 155.9, 155.4, 155.2, 149.6, 143.7, 140.6, 132.9, 127.4, 126.8, 124.9, 119.9, 116.5, 77.1, 65.2, 61.6, 59.6, 46.5, 45.4, 41.4, 39.5, 29.1, 28.3, 28.1, 23.3. ESI (CH_3OH, positive ions) calcd m/z: 618.3(MNa^+), 633.3(MK^+), found: 618.5, 633.5. Ee (chiral GC–MS according to the method reported in the literature[12]): 99%.
18. Gomez-Martinez, P.; Dessolin, M.; Guibè, F.; Albericio, F. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2871–2874.
19. Tedeschi, T.; Corradini, R.; Marchelli, R.; Pushl, A.; Nielsen, P. E. *Tetrahedron: Asymmetry* **2002**, *13*, 1629.