Pergamon

Tetrahedron Letters 42 (2001) 835–837

TETRAHEDRON LETTERS

Synthesis of nucleopeptides by an enzyme labile urethane protecting group

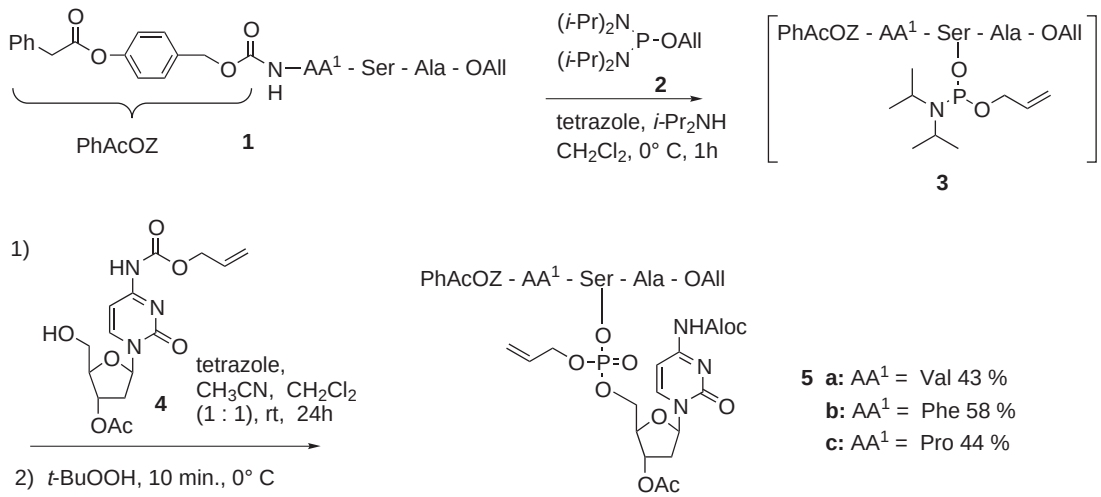
Duraiswamy A. Jeyaraj and Herbert Waldmann*

Max-Planck-Institut für molekulare Physiologie, Abteilung Chemische Biologie, Otto-Hahn-Str. 11, D-44227 Dortmund, Germany and Universität Dortmund, Fachbereich 3, Organische Chemie

Received 24 October 2000; accepted 16 November 2000

Abstract—The synthesis of acid- and base-labile nucleopeptides is accomplished by employing the enzyme labile phenylacetoxy benzyloxycarbonyl (PhAcOZ) urethane protecting group as the key technique.

Nucleoproteins are naturally occurring biopolymers in which the hydroxy group of a serine, a threonine or a tyrosine is linked via a phosphodiester group to the 3′- or 5′-end of a nucleic acid.[1] These protein conjugates play decisive roles in important biological processes like viral replication.[2] For the study of the biological phenomena in which nucleoproteins are involved, nucleopeptides embodying the characteristic linkage between the peptide chain and the oligonucleotide may serve as powerful tools. However, due to the multifunctionality of nucleopeptides their synthesis requires the application of a variety of orthogonally stable blocking groups. In addition, fully protected serine/threonine nucleopeptides are very acid- and base-labile making most of the established blocking function not applicable to nucleopeptide synthesis. Thus, it is not surprising that only a few reports on the successful synthesis of nucleopeptides have appeared.[3–6] In particular, protecting groups allowing for the assembly of sensitive serine/threonine nucleopeptides via sequential selective N-terminal deprotection and chain elongation have not been developed yet. In this paper we report that the enzyme-labile phenylacetoxy benzyloxycarbonyl (PhAcOZ) group[7] (Scheme 1) fulfills the extraordinary

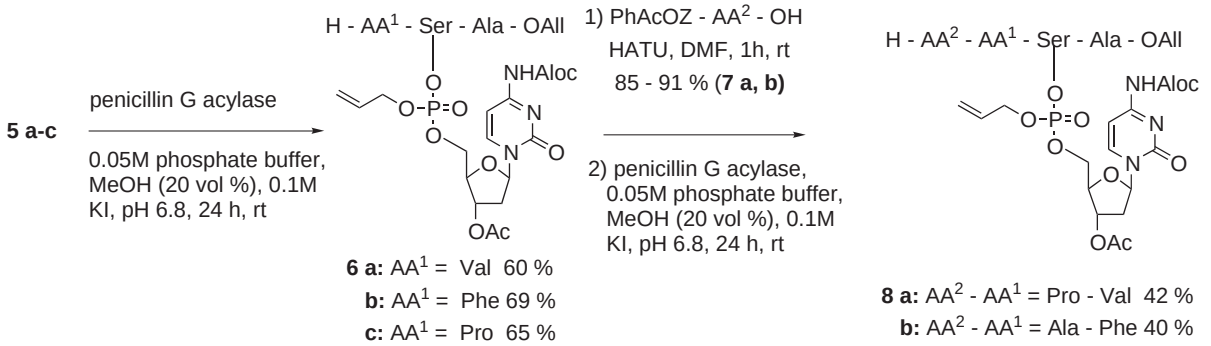


Scheme 1. Synthesis of model nucleopeptides **5**.

Keywords: nucleopeptides; enzyme labile protecting group; PGA; enzymatic cleavage.

* Corresponding author. E-mail: herbert.waldmann@mpi-dortmund.mpg.de

0040-4039/01/$ - see front matter
PII: S0040-4039(00)02121-3



Scheme 2. Selective penicillin G acylase catalyzed removal of the N-terminal PhAcOZ urethane from nucleotri- and tetrapeptides.

demands of nucleopeptide chemistry concerning chemoselectivity and mildness of cleavage conditions. The PhAcOZ group is an enzyme-labile urethane that is cleaved under very mild conditions by penicillin G acylase-mediated hydrolysis of the phenylacetic acid phenyl ester and subsequent fragmentation of the generated phenolate to give a quinone methide (which is trapped by nucleophiles) and the desired unmasked amino acid ester. In order to determine if the conditions for the selective removal of this enzyme-sensitive blocking group are mild enough to allow for an efficient nucleopeptide synthesis model nucleotripeptides were synthesized. To this end, PhAcOZ protected tripeptides **1** were synthesized according to the literature[7] from PhAcOZ-masked amino acids and H-Ser-Ala-OAll by means of established peptide coupling procedures. The central serine of tripeptides **1** was then coupled to selectively masked deoxycytidine **4**[8] via a phosphodiester bond (Scheme 1). Upon treatment of **1** with phosphordiamidite **2** in the presence of tetrazole, hydrolysis-sensitive intermediates **3** were formed. They were converted without isolation into the desired peptide conjugates **5**[9] by reaction with deoxycytidine building block **4** and subsequent oxidation of the formed phosphites to the corresponding phosphates.

Completely masked nucleotripeptides **5** were then subjected to selective enzymatic deprotection with penicillin G acylase. To this end the PhAcOZ protected compounds were treated with the enzyme in a mixture of 0.05 M phosphate buffer and methanol (20 vol%) at pH 6.8 and room temperature and in the presence of 0.1 M KI as a trapping reagent for the quinone methide formed in the enzyme-initiated cleavage of the urethanes. Under these conditions the enzyme smoothly cleaved the phenylacetic acid and the selectively unmasked nucleotripeptides **6** were formed in high yield (Scheme 2). In order to assure that this method is widely applicable, the peptide chain of compounds **6** was elongated with a further PhAcOZ-protected amino acid to yield nucleotetrapeptides **7** in high yields. Once more treatment of these peptide conjugates with penicillin G acylase under the conditions described above resulted in a smooth and clean deprotection of the N-terminus to yield selectively deprotected nucleotetrapeptides **8**.[9]

In all enzyme-catalyzed deprotections undesired side reactions did not occur. Thus, the high selectivity of penicillin G acylase for the phenylacetic acid group guarantees that the peptide bonds, the allyl ester, the allyl urethane, the acetate protecting group and the phosphate remain fully intact. The conditions of the enzymatic transformation are so mild that the glycosidic bond is not affected and that a β-elimination of the phosphate (which occurs at pH>7) is not observed at all. These findings prove that the PhAcOZ group is an efficient protecting function for the selective synthesis of sensitive and multifunctional nucleopeptides.

Acknowledgements

This research was supported by the Fonds der Chemischen Industrie and Dr. D. A. Jeyaraj is grateful to the Alexander von Humboldt foundation (AvH) for a fellowship.

References

1. (a) Juodka, B. A. *Nucleosides & Nucleotides* **1984**, *3*, 445–483; (b) Wang, J. C. *Ann. Rev. Biochem.* **1985**, *54*, 665–697; (c) Vartapetian, A. B.; Bogdanov, A. A. *Prog. Nucleic Acid Res. Mol. Biol.* **1987**, *34*, 209–251; (d) Kelly, T. J.; Wold, M. S.; Li, J. *Adv. Virus Res.* **1988**, *34*, 1–42; (e) M. Salas In *The Viruses*, Vol. 1 *The Bacteriophages*; R. Calender, Ed.
2. (a) Review: Salas, M. *Ann. Rev. Biochem.* **1991**, *160*, 39–71; (b) Review: Salas, M. *Virus Strategies–Molecular Biology and Pathogenesis*, VCH, Weinheim, 1993, pp. 1–23; (c) Blanco, L.; Lázaro, J. M.; de Vega, M.; Bonnin, A.; Salas, M. *Proc. Natl. Acad. Sci.* **1994**, *91*, 12198.
3. (a) Juodka, B. A.; Kirveliene, V.; Povilionis, P. *Nucleosides & Nucleotides* **1982**, *1*, 497–508; (b) Kuyl-Yeheskiely, E.; van der Klein, P. A. M.; Visser, G. M.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pay-Bas* **1986**, *105*, 69–70; (c) Kuyl-Yeheskiely, E.; Tromp, C. M.; Lefeber, A. W. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1988**, *44*, 6515–6523; (d) Kuyl-Yeheskiely, E.; Dreef-Tromp, C. M.; Geluk, A.; van der Marel, G. A.; van

Boom, J. H. *Nucleic Acids Res.* **1989**, *17*, 2897–2905; (e) Dreef-Tromp, C. M.; van der Marel, J. C. M.; van den Elst, H.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1992**, *20*, 4015–4020.

4. (a) Robles, J.; Pedroso, E.; Grandas, A. *Tetrahedron Lett.* **1994**, *35*, 4449–4452; (b) Robles, J.; Pedroso, E.; Grandas, A. *J. Org. Chem.* **1994**, *59*, 2482–2486; (c) Robles, J.; Beltran, M.; Marchan, V.; Perez, Y.; Travesset, I.; Pedroso, E.; Grandas, A. *Tetrahedron* **1999**, *55*, 13252–13264 and references cited therein.
5. Flohr, S.; Jungmann, V.; Waldmann, H. *Chem. Eur. J.* **1999**, *5*, 669–681 and references cited therein.
6. Ueno, Y.; Saito, R.; Hata, T. *Nucleic Acids Res.* **1993**, *21*, 4451–4457.
7. Pohl, T.; Waldmann, H. *J. Am. Chem. Soc.* **1997**, *119*, 6702–6710.
8. Compound **4** was synthesized from 2-deoxycytidine via introduction of the Aloc group into the nucleobase, masking of the 5′-OH group as TBDMS ether, acetylation of the 3′-OH group and selective removal of the TBDMS ether by treatment with TBAF (S. Gabold, Dissertation, Univ. Karlsruhe 1998).
9. Characteristic data of compound **5b**: ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 8.40–8.60 (bs, 1H); 7.66–7.81 (bs, 1H); 7.13–7.43 (m, 13H); 6.99–7.01 (d, $J=7.52$ Hz, 2H); 6.12–6.26 (m, 1H); 5.72–5.99 (m, 4H); 5.17–5.45 (m, 7H); 4.2–5.06 (m, 16H); 3.85 (s, 2H); 3.55–3.70 (m, 1H); 3.35–3.50 (m, 1H); 3.15–3.25 (m, 1H); 2.90–3.02 (m, 1H); 2.64–2.78 (m, 1H); 2.30–2.50 (m, 1H); 2.06 (s, 3H); 1.44 (d, $J=7.28$ Hz, 3H). $[\alpha]_D^{22}=+8.0$ (*c* 0.35, MeOH). ESI-MS: Calc. 1087.04. Found 1087.1 (100%, positive mode). **8b**: ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 8.18–8.20 and 8.17–8.18 (two d, $J=7.52$ Hz, 1H); 7.32–7.34 and 7.31–7.33 (two d, $J=7.52$ Hz, 1H); 7.20–7.27 (m, 5H); 6.18–6.22 (m, 1H); 5.87–6.03 (m, 3H); 5.20–5.43 (m, 7H); 4.56–4.70 (m, 8H); 4.23–4.46 (m, 6H); 3.83–3.87 (m, 1H); 3.20–3.21 and 3.16–3.18 (two d, $J=5.52$ Hz, 1H); 2.92–3.00 (m, 1H); 2.62–2.71 (m, 1H); 2.25–2.37 (m, 1H); 2.11 (s, 3H); 1.47–1.48 (d, $J=7.04$ Hz, 3H); 1.40–1.42 (d, $J=7.28$ Hz, 3H). ESI-MS: Calc. 889.84. Found 890.2 (100%, positive mode).