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SCIENCE DIRECT
Tetrahedron 61 (2005) 6046–6055
Tetrahedron

Fukuyama–Mitsunobu alkylation in amine synthesis on solid phase revisited: N-alkylation with secondary alcohols and synthesis of curtatoxins

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Received 14 January 2005; revised 29 March 2005; accepted 15 April 2005

Available online 17 May 2005

Abstract—The Fukuyama–Mitsunobu amination strategy has emerged as an efficient means of N-alkylation of peptides and sulfonamides, as well as a method for synthesis of polyamines on solid phase. Here, an array of reagent combinations for solid-phase alkylation with secondary alcohols was examined in various solvents. The classical reagents DEAD–PPh_3 as well as DEAD–PEt_3 proved applicable for a single alkylation step. Sharply dropping yields in successive alkylation steps were identified as the most serious limitation of the use of Fukuyama–Mitsunobu reaction in SPS of polyamines using primary and in particular secondary alcohols.

1. Introduction

Polyamines and their derivatives play important roles in biochemistry and their pharmacological significance continues to increase. Simple polyamines such as spermidine (**1**) and spermine (**2**) are ubiquitous in eukaryotic cells, where they conduct important roles in cellular physiology, for example, in cell proliferation and DNA synthesis.[1] Relatively simple *n*-alkylated analogs of **2** have been shown to possess anticancer activity in various cell lines.[2] Polyamine-based cationic lipids have proved to be efficient gene-delivery agents.[3] Moreover, certain polyamine derivatives have been shown to interact with ion channels in the central nervous system (CNS).[4] Thus, polyamine neurotoxins isolated from venoms of spiders and wasps antagonize various classes of ionotropic receptors such as nicotinic acetylcholine receptors (nAChRs) and ionotropic glutamate receptors (iGluRs).[5] A variety of polyamine neurotoxins with closely related structures have been isolated from venoms of the funnel web spiders *Hololena curta*[6] and *Agelenopsis aperta*.[7] Some of these indole-containing toxins, known as curtatoxins, are shown in Figure 1. Compounds **4** and **6**, for instance, were isolated from both venom mixtures (which explains the two different names assigned to each of these compounds). Only the *H. curta* venom contains long-chain non-hydroxylated toxins such as compound **5**. The structurally similar wasp toxin component, philanthotoxin-433 (**7**),[8] has been the subject of extensive structure–activity relationship (SAR) studies on various receptor types.[9]

In order to obtain pure natural toxins in useful quantities as well as unnatural analogs for biological studies, efficient synthetic strategies are necessary. Since solution-phase methods for polyamine synthesis[10] often require extensive use of protecting groups as well as tedious purification steps, the development of solid-phase synthesis (SPS) methodologies has received considerable attention in recent years. The subsequent attachment of the amino acid and/or acyl residues to the polyamines may be achieved by well-established solid-phase peptide synthesis (SPPS) protocols. The synthetic strategies for construction of polyamines on solid phase may be classified into three major groups: (1) simple S_N2 alkylation reactions;[11] (2) methods based on reduction of intermediary imines[12] or amides;[13] and (3) Fukuyama amination reactions.[14] In the latter approach,

Keywords: Solid-phase synthesis; Fukuyama–Mitsunobu alkylation; Amines; Spider toxins.
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doi:10.1016/j.tet.2005.04.027

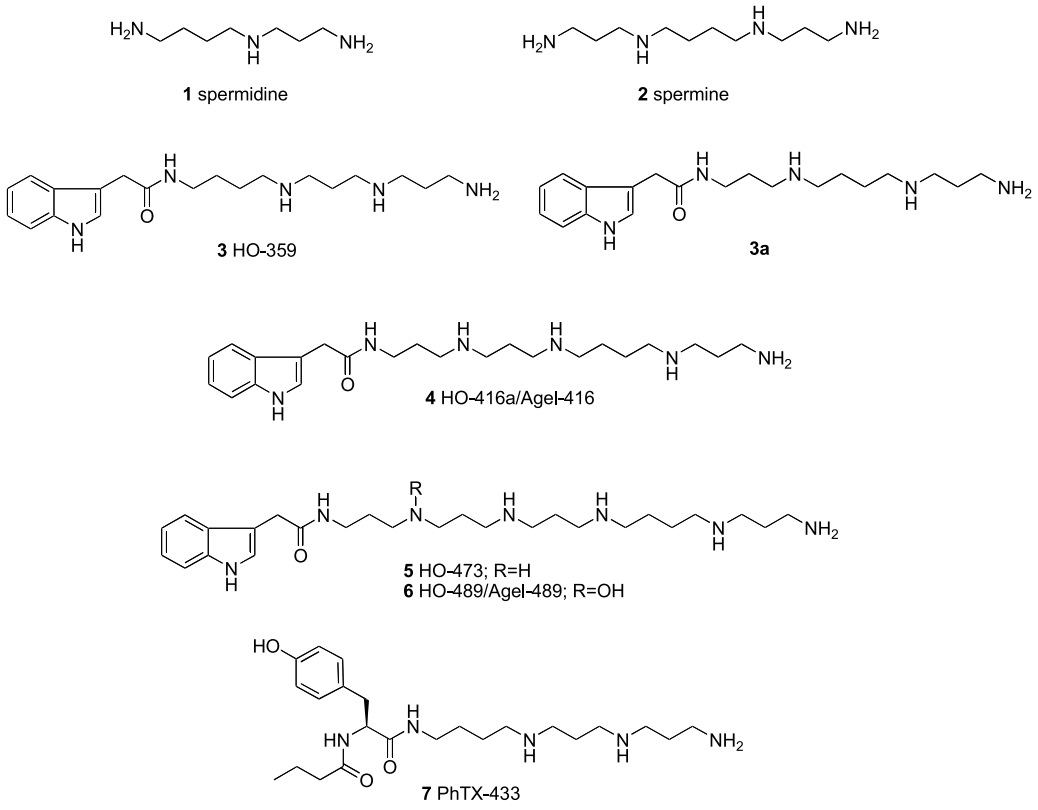

Figure 1. Structures of polyamines and acylpolyamine neurotoxins isolated from venom mixtures of spiders and wasps. The numerals in the names of **3**–**6** denote the molecular masses. In PhTX-433 (**7**) the digits denote the number of methylene groups that separate the amino functionalities, starting from the tyrosine end.

alkylation of secondary sulfonamides is achieved with alkyl halides under mildly basic conditions or with alcohols under Mitsunobu conditions.[15] Fukuyama and co-workers have applied the alkyl bromide approach in mixed solution/solid-phase total syntheses of several spider and wasp toxins. Ns-Protected acylpolyamines (corresponding to **4**[16] and **6**[16b]) were prepared in solution, and the syntheses were completed after anchoring of the terminal amino group to a solid support. Furthermore, two Fukuyama-type approaches based entirely on SPS have been employed in the total synthesis of philanthotoxins,[17] and two SPS Ns-strategies for site-selective N-methylation in peptide synthesis were reported by Miller and Scanlan.[18] The Fukuyama–Mitsunobu conditions have recently emerged as a versatile and general SPS method used, for instance, in N-alkylation of peptides,[19] peptide nucleic acid (PNA) monomer synthesis,[20] preparation of N-alkylated sulfonamides,[21] and in synthesis of polyamine neurotoxins.[22]

Although a relatively large amount of work has already been devoted to SPS of amines using Fukuyama–Mitsunobu alkylation, we addressed two important issues in order to define the scope and limitations of this strategy. The first issue concerns investigation of alkylation conditions suitable for SPS employing sterically hindered alcohols as building blocks (application of secondary alcohols is also challenging in solution-phase Mitsunobu reactions[23]). The second issue concerns the number of consecutive chain elongation steps that are possible to carry out in a satisfactory overall yield, that is, the length limit of polyamines obtainable by this method. The latter question is of interest, since the Fukuyama–Mitsunobu method has been optimized to give >99% yield in a single alkylation step,[22c] yet the overall yields of philanthotoxins obtained after two alkylations only amounted to 23–40%.[22c,e] Accordingly, the present work reports on the use of Fukuyama–Mitsunobu protocols for the SPS of curtatoxins, which contain polyamine moieties of different lengths, and thus constitutes an interesting test case.

2. Results and discussion

2.1. Screening of SPS reagent combinations for N-alkylation with secondary alcohols

Numerous combinations of solvents, bases, azo reagents and phosphines[24] have previously been applied in Mitsunobu reactions, and it was decided to select a range of reagents for a combinatorial screening in order to identify suitable conditions for Fukuyama–Mitsunobu SPS alkylations with secondary alcohols. Bycroft and co-workers[22a] as well as Hone and Payne[22b] have employed the traditional reagent pair diethyl azocarboxylate (DEAD) and PPh_3 in their original reports on polyamine SPS using Fukuyama–Mitsunobu amination, and hence these reagents were included in the present investigation. The two azo reagents used under Tsunoda conditions, that is, 1,1′-(azodicarbonyl)dipiperidide (ADDP) and *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD),[25] were likewise included, since both of these reagents in combination with PMe_3 were previously found to give acceptable to good yields in alkylation of