Solid Phase Synthesis of Fumitremorgin, Verruculogen and Tryprostatin Analogs based on a Cyclization/Cleavage Strategy

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Abstract

A solid phase synthesis towards structural analogs of fumitremorgins, verruculogens and tryprostatins using a cyclization/cleavage strategy was developed. To prove the general applicability of the route, a representative set of 42 single compounds (as diastereomeric mixtures) was prepared by parallel synthesis and analyzed with LC/MS. © 1998 Elsevier Science Ltd. All rights reserved.
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Fumitremorgins, verruculogens and (cyclo)tryprostatins belong to the class of indolyl diketopiperazine alkaloids, first isolated as fungal secondary metabolites from *Aspergillus fumigatus* [1], *Penicillium verruculosum* [2] and the marine fungal strain BM939 [3], respectively.
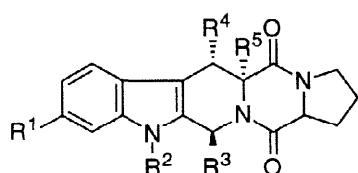
R^1 = OCH$_3$ or H

R^2 = H or -CH$_2$-CH=C(CH$_3$)$_2$

R^3 = -CH=C(CH$_3$)$_2$ or -CH$_2$-C(CH$_3$)$_2$-OH

R^4 = OCH$_3$, OH, H or =O

R^5 = OH or H

Fumitremorgins and verruculogens were found to be tremorgenic mycotoxins, interfering with mechanisms responsible for the release of neurotransmitters in the CNS[1] [4], whereas members of all three families showed inhibitory effects on the mammalian cell cycle [5]. Therefore, it appeared worthwhile to initiate a program on the chemical preparation of a range of indolyl diketopiperazine analogs as potential tools in CNS receptor studies or as candidates for cancer chemotherapy.

Our goal was the development of a generally applicable solid phase synthesis towards the fumitremorgin, verruculogen and tryprostatin class, in order to get access to analogs *via*

multiple parallel synthesis. We chose for a cyclization/cleavage strategy [6], *i.e.* formation of the diketopiperazine ring proceeds with simultaneous cleavage from the resin in the key step.[2] The major advantage of this approach, with the solid support acting as a leaving group during final cyclization of the resin-bound precursor, lies in the optional intrinsic product purification: upon introduction of the functionality required for cyclization with the last building block, cleavage is essentially restricted to the anticipated product, whereas side products (*i.e.* resulting from incomplete conversions) remain attached to the solid support.

We developed a synthesis allowing the application of a diverse set of commercially available building blocks. Hydroxyethyl functionalized polystyrene resin[3] was loaded [7] with L-tryptophan and subjected to Pictet-Spengler condensation with excess aldehydes [8,9,10,11]. In our hands, optimal conditions were found to comprise reaction in dichloromethane in the presence of TFA at room temperature.[4] Use of ketones (acetone, cyclopentanone, cyclohexanone) under identical conditions also led to the desired tetrahydro-ß-carboline systems, but at a lower rate.
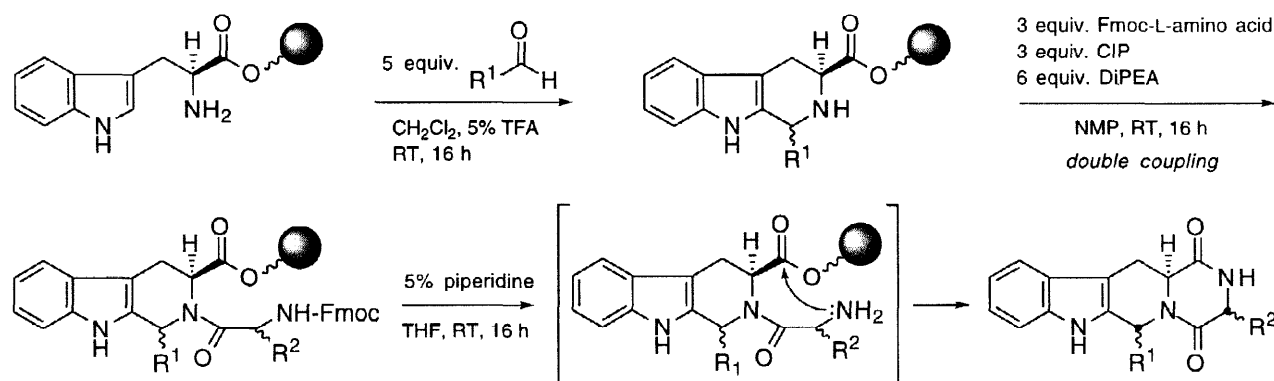
Unfortunately, subsequent coupling of the resulting secondary amine with protected amino acids remained elusive under various conditions, despite the use of PyBroP® [12], PyBroP®/HOAt [13] and HATU [14], particularly effective reagents for the coupling of sterically demanding components. Only a slight improvement was achieved by use of Boc- or Fmoc-protected amino acid fluorides, synthesized prior to coupling (cyanuric fluoride) [15] or generated *in situ* with TFFH [16]. A more successful approach involved reaction with highly reactive Fmoc-amino acid chlorides,[5] conveniently prepared by treatment with $SOCl_2$ followed by isolation [18], or generated *in situ* with TCFH [16] or commercially available CIP [19]. Efficient acylation proceeded in the presence of an excess DiPEA at room temperature in NMP. Extension of either of the *in situ* activation procedures to the

[2] For a preliminary announcement see the 6th Ibn Sina International Conference on Pure and Applied Heterocyclic Chemistry, Cairo, Egypt, December 13-16, 1997, abstract PLIIc-2, page 214.

[3] Polystyrene A OH, particle size 200-400 mesh, crosslinked with 1% divinylbenzene, capacity 1.2 mmol/g, was purchased from Rapp Polymere GmbH, Tübingen, Germany.

[4] At the start of the project, we optimized conditions in solution phase using L-tryptophan methylester. During translation of this reaction to the solid support (using L-tryptophan esterified to hydroxyethyl-functionalized polystyrene resin), Pictet-Spengler reactions performed on solid phase cited in the text appeared in literature.

[5] Unfortunately, Boc-amino acid chlorides are unstable under the conditions applied [17], whereas hydrogenolysis of the Z-group is incompatible with parallel solid-phase chemistry, thus limiting our choice of protective group to Fmoc.

MULTIBLOCK[6] manual multiple/library synthesizer required a 3-fold excess of reagents, and in certain cases a double coupling procedure, as indicated by bromophenolblue functional group tests [20] and single-bead microIR analysis. Most probably due to severe steric restrictions, acylation of the secondary amine of tetrahydro-ß-carbolines obtained from Pictet-Spengler reaction with ketones remained fruitless in all cases.

Fmoc-deprotection and subsequent cyclization/cleavage takes place in THF containing 5% piperidine at room temperature. Reaction times for this step depend on the amino acid residue of the last building block; in the case of proline –giving the pentacyclic skeleton of the fumitremorgins, verruculogens and cyclotryprostatins– the reaction is very fast, probably due to correct pre-orientation of the precursor ("template-effect"). Evaporation of solvents[7] followed by removal of N-(9-fluorenylmethyl)piperidine[8] gave the desired products in moderate to high overall yields and high chemical purity.

A representative library of 42 single compounds was prepared by parallel synthesis in the MULTIBLOCK. A suspension of the resin loaded with L-tryptophan (0.99 mmol/g) was distributed over the matrix to 0.18 mmol per vessel. Six aldehydes and seven Fmoc-L-amino acids (with orthogonally protected side-chain if necessary) were used following the optimized conditions. Crude product fractions were collected and concentrated, followed by work-up. Yields were gravimetrically determined and products were subjected to LC/MS analysis.[9] In all cases, the main peaks (diastereoisomers) corresponded to the expected molecular masses.[10]

R1 \ R2	CH3	N (pyrrolidine)	benzyl	NH-Boc	O-t-Bu	C(=O)O-t-Bu	indole
	57 (72)	71 (79) *	93 (87)	87 (85)	83 (85)	53 (78)	91 (79)
	78 (80)	71 (58)	91 (62)	87 (61)	85 (91)	75 (89)	99 (74)
	66 (83)	74 (71)	99 (82)	99 (77)	85 (88)	61 (72)	97 (89)
	81 (76)	99 (82)	86 (87)	85 (88)	86 (81)	57 (80)	99 (86)
	73 (81)	96 (81)	99 (79)	89 (90)	80 (81)	56 (88)	99 (88)
	50 (82)	99 (66)	99 (82)	96 (86)	84 (90)	57 (86)	99 (88)

Gravimetrically determined yields (%), followed by -in between brackets- purities (%) according to HPLC (detection at λ = 254 nm; cumulative relative peak areas of all diastereoisomers as confirmed by MS)

[6] The MULTIBLOCK (commercially available from CSPS, San Diego, USA) is a Teflon block holding 42 polypropylene syringes, equipped with a plastic frit, as reactors. The vessels are closed by Teflon plates holding 42 stoppers. An adapter that connects each reactor to a vacuum line is used for work-up. Reactions in the MULTIBLOCK were not performed in an inert atmosphere.

[7] Evaporation after parallel synthesis was achieved by gently heating with continuous nitrogen purge, and further drying in vacuo.

[8] Parallel repetitive trituration was carried out by dissolving the residue in a minimal amount of THF, followed by portionwise addition of PE 60/80 to precipitate the product and vortexing. After centrifugation, the liquid fractions were decanted. Unfortunately, removal of the dibenzofulvene residue by immobilization on a piperidine resin was insufficiently effective.

[9] A Zorbax ODS Reversed Phase column was used with gradient elution of 10 to 90% acetonitrile in water containing 0.1% TFA. For MS, ESI ionization technique was used; generally, both MH^+ and $M(CH_3CN)H^+$ are detected.

[10] NMR data for the compound marked (*) in the matrix (single diastereoisomer after separation; absolute configuration not determined): ^1H NMR (CDCl$_3$, 400 MHz), δ [ppm]: 0.84 (t, $J = 7.4$ Hz, 3H); 1.79-2.12 (m, 4H); 2.27-2.48 (m, 2H); 3.13 (dd, $J = 15.7$, 11.7 Hz, 1H); 3.53 (dd, $J = 15.8$, 5.1 Hz, 1H); 3.63 (m, 2H); 4.04-4.13 (m, 2H); 5.43 (dd, $J = 7.6$, 4.1 Hz, 1H); 7.18 (m, 2H); 7.37 (d, $J = 7.8$ Hz, 1H); 7.58 (d, $J = 7.6$ Hz, 1H); 8.78 (br s, 1H). ^{13}C NMR (CDCl$_3$, 100 MHz, APT), δ [ppm]: 9.32; 21.47; 22.98; 28.44; 29.59; 45.19; 53.44; 56.70; 59.06; 106.56; 110.97; 118.05; 119.69; 121.75; 125.94; 133.50; 135.78; 165.63; 169.77.

Except for the products containing the proline residue, compounds were formed as a mixture of four diastereoisomers[11], indicating that –apart from the two diastereoisomers formed in the Pictet-Spengler condensation– racemization of the stereogenic center of the finally introduced building block must occur during coupling of the activated Fmoc-amino acid. Though the amount of residual N-(9-fluorenylmethyl)piperidine after workup never exceeds 5 mol% (based on peak areas and molar extinction coefficients), the degree of this contamination is proportional to the lipophilicity of the side-chains R^1 and R^2.

In summary, we have developed an efficient solid phase synthesis towards structural analogues of the fumitremorgin, verruculogen and (cyclo)tryprostatin class, suitable for incorporation of a wide variety of commercially available building blocks.[12] Compounds made *via* this method are currently being screened for biological activity.

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[11] Diastereoisomeric ratios, determined by relative HPLC peak areas, were substrate-dependent for both the Pictet-Spengler condensation and the amino acid coupling.

[12] During preparation of this manuscript, a solid phase synthesis following comparable strategy was published [21], employing a PyBOP-mediated coupling of protected glycine and β-alanine to the tetrahydro-β-carboline system. In the light of our findings, it is not excluded that the scope of this approach may be limited to α-unsubstituted amino acids, whereas our approach seems more generally applicable.