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Repetitive solid-phase synthesis of polyamines

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Abstract—A repetitive solid-phase method for the synthesis of polyamines is described. Primary amino groups attached to a crosslinked polystyrene resin are monoalkylated by acid labile, benzhydryl-based alkyl chlorides. Reductive alkylation of the resulting secondary amino group by Fmoc-protected aminoaldehydes gives a *N*-benzhydryl polyamine backbone. Treatment of the resin with trifluoroacetic acid cleaves both the benzhydryl protective group and the polyamine derivative from the resin. By using benzhydryl protective groups with different acid stability, unbranched, branched and partly branched polyamines are synthesized.

Polyamines such as spermine and spermidine are a class of natural products that have recently received increased attention. This is mainly a result of the putative role of endogenous polyamines in the regulation and rectification of different ion channels and their role in cell proliferation. Polyamines isolated from spider venoms e.g. Agel-416 and PhTx-433 have also been found to interact with cation selective ion channels and have antagonistic properties when bound to glutamate receptors and Ca^{2+} channels. Partly for these reasons, an increasing number of studies have recently been devoted to the synthesis and pharmacological characterization of different polyamines.[1]

Synthesis of polyamines in homogenous solution is a time-consuming and tedious task requiring extensive elaboration with protecting groups and difficult purification steps due to the basicity and polarity of the substances. For these reasons, strategies for a repetitive solid-phase synthesis of polyamines, analogous to polypeptide or oligonucleotide synthesis have received attention and different approaches for either derivatisation of commercially available polyamines or stepwise construction of a polyamine backbone have been reported.[2–13,24]

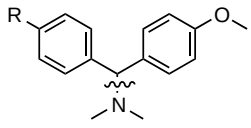

Figure 1. 4-Methoxy dityl, Mmd (R = H-) and 4,4′-dimethoxy dityl, Dod (R = MeO-).

Including this study, four methods for repetitive solid-phase synthesis of polyamines have been presented. Hone et al.[2] presented a repetitive route where Dde was used, as temporary protection, in combination with Mitsunobu alkylation for the synthesis of Agel-416. Uriac et al.[3] presented a methodology where amino alcohol building blocks were used to displace on-resin generated mesyl groups for prolongation of the polyamine. Chhabra et al.[4] used Dde as linker and extended the polyamine by reductive alkylation of primary amines via isolation of the imine on solid-phase. After removal of excess aldehyde by extensive washing, the protonated imine was reduced with $NaCNBH_3$. The success of this approach will largely be dependent on the stability of the imine during washes and reduction. The resulting secondary amine is protected from alkylation by introduction of a Boc group, a step that needs 20 h for completion.

In this communication we present an alternative route for the repetitive synthesis of polyamines employing readily available Fmoc-protected aminoaldehydes and reductive alkylation under mild reaction conditions. By using acid labile *N*-alkyl protective groups, this approach minimizes the risks of incomplete alkylation or dialkylation, associated with the isolation of the imine.

We have previously used this strategy for the selective monomethylation of N-terminal amino groups in peptides.[14] The key step of the method is the selective monoalkylation of primary amino groups by 4-

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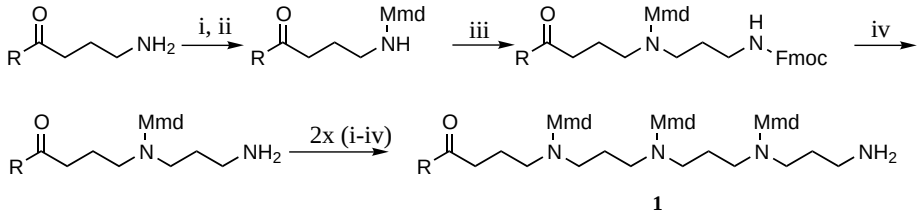
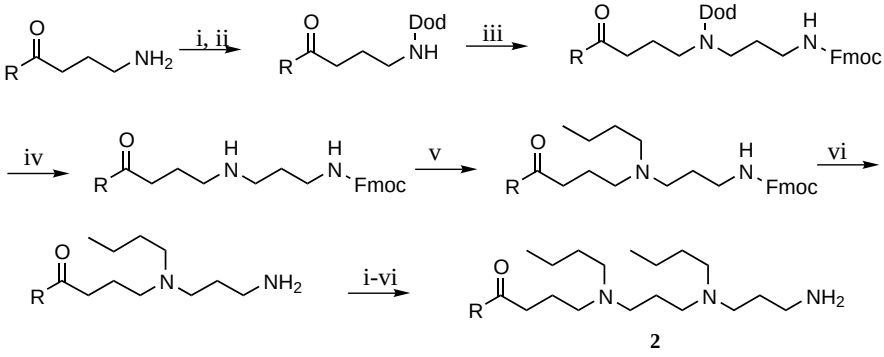
methoxy-dityl-chloride (Mmd-Cl)[15] and/or 4,4′-dimethoxy dityl-chloride (Dod-Cl)[16] (Fig. 1) followed by reductive alkylation. After the reductive alkylation, Dod and Mmd can be cleaved by TFA, but as Mmd is more stable to TFA, the Dod group can with satisfying selectivity be cleaved in the presence of the Mmd group. These differences in acid stability between the Mmd and the Dod group were exploited by using the Mmd group to synthesize unbranched polyamine skeletons, completely branched (tertiary) amines with the Dod group or selectively branched with a combination of Mmd and Dod (Schemes 1–3).

In order to evaluate the method and to facilitate both UV detection during HPLC analysis and characterisation of the product by MALDI-TOF, we performed the syntheses of the polyamine backbones on a model peptide[18] consisting of Merrifield resin-Ala-Phe(p-NO_2) (R in Schemes 1–3).

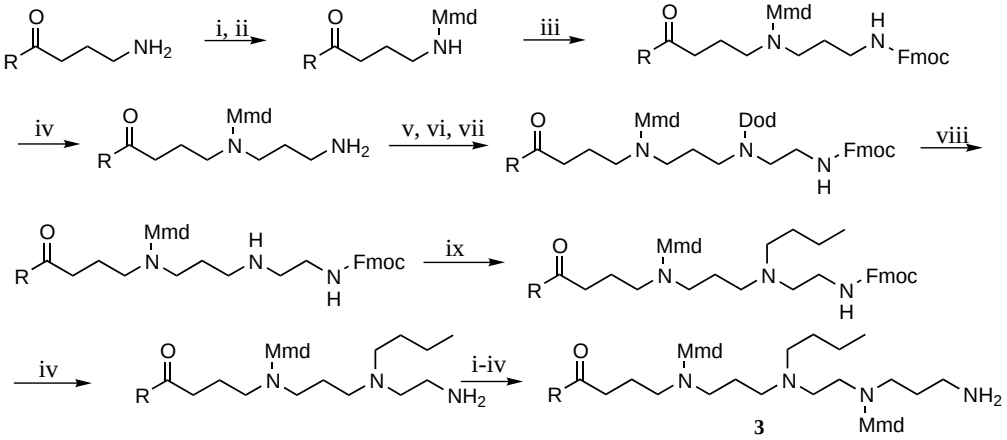
Synthesis of an unbranched polyamine (Scheme 1): In step i, the primary amine is alkylated with excess Mmd-Cl, which results in a mixture of mono- or dialkylated amines. By treating the resin with dilute TFA in step ii, the Mmd group is cleaved from tertiary but not sec-



Scheme 1. (i) 4 Equiv. of Mmd-Cl, 10 equiv. of DIPEA, DCM, 1 h; (ii) 6×10 s with 10% TFA in DCM; (iii) 3 equiv. Fmoc-aminopropanal,[17] 3 equiv. $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (iv) 20% piperidine in DMF, 30 min.



Scheme 2. (i) 4 Equiv. of Dod-Cl, 10 equiv. of DIPEA, DCM, rt, 1 h; (ii) 6×10 s with 5% TFA in DCM; (iii) 3 equiv. Fmoc-aminopropanal,[17] 3 equiv. $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (iv) 5% TFA in DCM, rt, 15 min; (v) 3 equiv. butyraldehyde, 3 equiv. $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (vi) 20% piperidine in DMF, 30 min.



Scheme 3. (i) 4 Equiv. of Mmd-Cl, 10 equiv. of DIPEA, DCM, 1 h; (ii) 6×10 s with 10% TFA in DCM; (iii) 3 equiv. Fmoc-aminopropanal,[17] 3 equiv. $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (iv) 20% piperidine in DMF, 30 min; (v) 4 equiv. of Dod-Cl, 10 equiv. of DIPEA, DCM, 1 h; (vi) 4×10 s with 5% TFA in DCM; (vii) 3 equiv. Fmoc-aminoethanal,[17] 3 equiv. $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h; (viii) 5% TFA in DCM, rt, 15 min; (ix) 3 equiv. butyraldehyde, 3 equiv. $NaCNBH_3$, NMP with 3% AcOH, 40°C, 3×1 h.

ondary amines, leaving the amine mono protected. The cleavage is easily monitored by the strong yellow color from the Mmd carbonium ions, which diminishes with each wash. The reductive alkylation in step iii is a robust reaction and secondary amines are normally alkylated fast and in high yields at room temperature. As a result of the steric hindrance of the Mmd group, reductive alkylation with Fmoc-protected aminoaldehydes had to be performed three times at 40°C for 1 h each, to ensure completion. The Fmoc group is then cleaved with 20% piperidine in DMF for 30 min and the synthetic cycle is repeated.

Product **1**, after final Fmoc deprotection, was incubated in neat TFA for 2 h at 50°C. These conditions removed the tertiary Mmd groups and cleaved the ester linkage to the Merrifield resin. The TFA solution was filtered and triethylsilane was added to decolorize the solution by scavenging of the cations. After evaporation, the products were dissolved in 0.1 M HCl (aq.) and extracted with EtOAc. RP-HPLC[19] (Fig. 2) and MALDI-TOF[20] analyses of the aqueous phase confirmed the correct product in high purity and 30% yield.[21]

Synthesis of a completely branched polyamine (Scheme 2): Steps i–iii are the same as in the syntheses of unbranched polyamines with the exception that Dod-Cl is used instead of Mmd-Cl. The key step to obtain branched polyamines is step iv, where cleavage of the Dod group renders the secondary amine susceptible to reductive alkylation. This cleavage, which is possible only when the amine is tertiary, can be performed with 5% TFA in DCM for 15 min.

At this point, derivatisation is possible with most aliphatic aldehydes and no problems were encountered when branched aldehydes such as isobutyraldehyde were used (data not shown). Branching of the polyamine backbone is possible if amino aldehydes, protected with protecting groups orthogonal to TFA and piperidine, e.g. Alloc, are used.

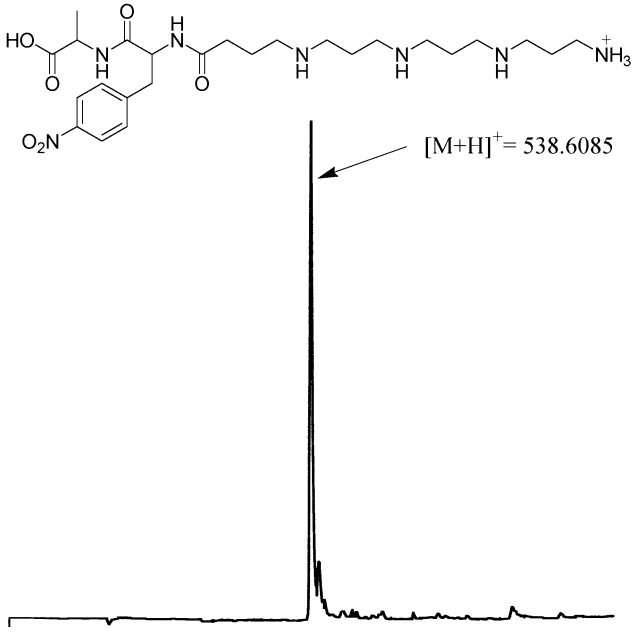


Figure 2. Analytical RP-HPLC chromatogram of cleaved product **1**.[19]

Cleavage of the Fmoc group, step vi, yields a primary amine and steps i–v can be repeated. Cleavage and characterisation of product **2** was carried out as described above. RP-HPLC and MALDI-TOF analyses confirmed the correct product in 39% yield and 76% purity.[21]

Synthesis of a selectively branched polyamine (Scheme 3): This strategy consists of a combination of Schemes 1 and 2 with use of either Mmd or Dod as the protecting group depending on whether further derivatisation of a particular nitrogen is desired. In step viii, where the introduced Dod group is cleaved in the presence of the Mmd group, completely selective cleavage of the Dod is not possible. However, cleavage of the Mmd group can be held at a low level by using 5% TFA in DCM for 15 min. In 5% TFA in DCM, approximately 0.1% of Mmd is cleaved per minute, resulting in branching of the polyamine backbone as a minor side reaction. This problem should be minimized by using a slightly more acid-stable protecting group (compared to the Mmd group). The obtained product **3** was cleaved and characterized as described above. RP-HPLC and MALDI-TOF analyses confirmed the correct product in 40% yield and 88% purity.[21]

In this communication, we have presented novel protocols for the syntheses of branched or unbranched polyamines by repetitive synthesis utilizing benzhydryl-based *N*-alkyl protective groups for semi-permanent and temporary protection. The protocols should be easy to implement in automated syntheses, as the chemistry used is robust and straightforward, and contribute to the collection of methods for syntheses of polyamine compounds. Further studies will be needed in order to evaluate the relative merits of these and other strategies for repetitive solid-phase synthesis of polyamines.

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15. 4-Methoxy dityl chloride (Mmd-Cl): 4-Methoxy benzophenone (10 g, 47.1 mmol) was dissolved in EtOH (250 ml). $NaBH_4$ (0.9 g, 23.6 mmol, 0.5 equiv.) was added slowly and the reduction was left with stirring overnight. TLC analysis (petroleum ether/EtOAc, 9/1) indicated completion of the reduction and the solution was poured into water (250 ml), stirred for 1 h and 4-methoxy benzhydrol filtered off and dried. 4-Methoxy benzhydrol (5 g, 23.3 mmol) was dissolved in anhydrous DCM (50 ml) and oxalyl chloride (2.25 ml, 25.7 mmol, 1.1 equiv.) was added dropwise and the reaction was left stirring for 2 h at room temperature. The solvent was evaporated and the 4-methoxy dityl chloride was crystallized and then recrystallized from warm petroleum ether. Yield: 5.4 g, 99%.
16. 4,4′-Dimethoxy dityl chloride (Dod-Cl): 4,4′-Dimethoxy benzhydrol (6.0 g, 24.6 mmol) was dissolved in anhydrous DCM (60 ml) and oxalyl chloride (2.36 ml, 27 mmol, 1.1 equiv.) was added dropwise and the reaction was left with stirring for 1.5 h at room temperature. The solvent was evaporated and the 4,4′-dimethoxy benzhydryl chloride was crystallized and then recrystallized from warm petroleum ether. Yield: 5.8 g, 90%.
17. Synthesis of Fmoc-amino aldehydes: 3-Amino propanol or 2-aminoethanol (40 mmol) was dissolved in DCM (150 ml) and cooled with an ice bath. Fmoc-Cl (20 mmol) dissolved in DCM (120 ml) was added through a funnel over 30 min. After warming to room temperature, the mixtures were stirred for another 1.5 h and then extracted with 0.5 M HCl (3×100 ml), dried and evaporated. Fmoc-protected amino alcohols were obtained in almost quantitative yield and were converted to the Fmoc-protected amino aldehydes under Swern conditions.[22]
18. The model peptide γ-Abu-Phe(p-NO_2)-Ala-OH was synthesized by standard peptide protocol. Boc-Ala-OH was attached to Merrifield-Cl resin as the cesium salt and deprotected with 25% TFA in DCM. Fmoc-Phe(p-NO_2)-OH was coupled using the TBTU/HOBt method and deprotected with 20% piperidine in DMF. Boc-γ-Abu-OH was coupled using the TBTU/HOBt method and deprotected with 25% TFA in DCM. A quantitative ninhydrin test after final deprotection indicated a loading of 0.5 mmol/g.[23]
19. HPLC analyses were performed on a Machery–Nagel KS 100/4 Nucleosil® 120–3 C_{18} column. The elution gradient was 10–70% of B in 15 min. Solvent A was 0.1% TFA/H_2O and solvent B was 0.1% TFA/acetonitrile.
20. MALDI-TOF analyses were performed on a Voyager-DE STR, Applied Biosystems, USA.
21. Yields were calculated by comparing the obtained amount of product, measured by UV absorbance at 278 nm and the theoretical maximum yield.
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