Solid-Phase Synthesis of Pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones

Nadège Graveleau, Thierry Masquelin*

Combinatorial & Parallel Chemistry, Basilea Pharmaceutica Ltd, Grenzacherstrasse 487, 4002 Basel, Switzerland
Fax thierry.masquelin@basileapharma.com
Received 7 April 2003

Abstract: We report a novel, and versatile solid-phase synthesis of pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones starting from the polymer-bound pyrimidine 3. The key step is based on the reaction of the support-bound pyrimidine 3 with isocyanates 4, involving formation of a carbamate intermediate, followed by a base-catalysed intramolecular ring closure, to give polymer-bound 3-monosubstituted 1H-pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones 5. At this stage, subsequent treatment with alkyl halides 6 lead to 1,3-disubstituted 2H-pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione intermediates 7, which after oxidation and cleavage with various amines 8 gave 1,3-disubstituted 7-amino-2H-pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione intermediates 9 in moderate yields and high purity.

Key words: solid-phase, pyrimidine, base-catalysed ring closure, intramolecular cyclisation, pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones

Combinatorial chemistry has undergone a rapid development during the last decade and has provided a new paradigm for drug discovery.1–7 The potential of this technology integrating other disciplines such as computational chemistry, laboratory automation, analytical chemistry and high-throughput screening is widely recognised and may provide a number of unique approaches to satisfy the ever-growing need for new chemical entities with pharmaceutical activities. In this context, our interest first started with the development of a new solid-phase approach toward heterocycles to facilitate the automated parallel generation of arrays of compounds. Among them, pyrimidinedione systems represent attractive targets due to their broad biological activity. In particular, pyrimidopyrimidine-2,4-dione derivatives have found application in drug development for the treatment of central nervous system disorders, of bacterial infections, as well as analgesic and antiallergic agents.8–15 To our knowledge, there are only a few reports on the synthesis and the biological activities of pyrimido[4,5-d]-pyrimidine-2,4(1H,3H)-diones. The most promising methods are multi-step syntheses starting from 1,3-disubstituted 5-cyanoaracils,16–18 or from a polymer bound 2-(alkylsulfanyl)-4-aminopyrimidine-5-carbonitrile.19

As an extension of our studies towards combinatorial and parallel synthesis of versatile heterocycles on solid-support, we report herein a novel general solid-phase method for the synthesis of pyrimido[4,5-d]-pyrimidine-
2,4(1\(H\),3\(H\))-diones. Our strategy efficiently combines the reaction of a support-bound pyrimidine 3 with isocyanates to form carbamate intermediates, which by a base-catalysed intramolecular ring closure gives the pyrimidopyrimidine-2,4-dione skeleton, with the known nucleophilic displacement of the 2-alkylsulfanyl group of pyrimidine with nucleophiles.\(^{20-23}\)

This sequence appeared ideally suited for the production of combinatorial heterocyclic on solid-phase (Scheme 1). Thus, when resin-bound pyrimidine 3, prepared by reaction of ethyl 4-amino-2-sulfanylpyrimidine-5-carboxylate 2 with commercially available Merrifield resin 1 (1.8 mmol/g),\(^{23-25}\) was reacted with isocyanates 4 (3.0 equiv) in the presence of 1-tert-butyl-4,4,4-tris(dimethylamino)-2,2-bis[tris(dimethylamino)phosphoranenylideamino]-\(2\)(\(3\),\(4\),\(5\)-catenadi(phosphazene) (phosphazene, 3.2 equiv) in anhydrous DMF at 95 °C for 18 hours, the corresponding polymer 3-monosubstituted 1\(H\)-pyrimido[4,5-\(d\)]pyrimidine-2,4(1\(H\),3\(H\))-diones of type 5 were obtained (Scheme 1). The formation of the resin-bound compounds was followed by ATR/FT-IR (attenuated total reflection method).\(^{26}\)

As a key step in the sequence, we assume that this reaction involves an initial formation of a carbamate intermediate, followed by a direct conversion of the urea to the corresponding pyrimido[4,5-\(d\)]pyrimidine-2,4(1\(H\),3\(H\))-diones 5 via a base-catalysed intramolecular addition of the ureido-nitrogen anion to the carbonyl group of the ester (Scheme 2).\(^{27}\)

In the course of our investigation, we first tried to promote the transformation of the resin-bound pyrimidine 3 into pyrimidopyrimidine-2,4-dione 5 with bases such as \(N\)-ethylidisopropylamine (DIPEA), DBU, 2-tert-butyliminono-2-diethylamino-1,3-dimethylperhydro-1,3,2-diaza-phosphorine (BEMP), or DABCO, unfortunately without significant success, even at reflux for prolonged time and with excess of reagents. In all cases, a mixture of pyrimidine, urea and of pyrimidopyrimidine-2,4-dione was isolated in yields ranging from 12 to 54%. During our method development studies, we have used phosphazene successfully as a strong base under similar conditions. We observed complete consumption of starting pyrimidine 3, and good isolated yields of 3-monosubstituted 1\(H\)-pyrimido[4,5-\(d\)]pyrimidine-2,4(1\(H\),3\(H\))-diones 5 (typically 45–90%), generally with high levels of chemical purity [the purity of the crude product as assessed by HPLC-UV-ELSD (Evaporation Light Scattering Detection) peak area]. General observation of the obtained results suggest that the difference in reactivity may be due to steric hindrance, and to the influence of electronic factors (substitution pattern) on the course of the cyclisation.

Additionally, we observed that the use of a polar solvents such as DMF or \(N\),\(N\)-dimethylacetamide (DMA) is beneficial. When less polar solvents (dioxane, dichloromethane) were used, significant amounts of uncharacterised side products and of the corresponding carbamate intermediate were often generated. In all cases, it is very important to exclude traces of water, which caused a dramatic decrease in yield. The use of large excess of reagents led also to erratic results. Under the developed conditions, all results clearly show the crucial action of a strong base such as phosphazene, which acts as an initiator for the formation of a carbamate intermediate, and efficiently promotes the intramolecular cyclisation.

At this stage a second point of diversity was introduced into the resin-bound 3-monosubstituted 1\(H\)-pyrimido[4,5-\(d\)]pyrimidine-2,4(1\(H\),3\(H\))-diones 5 by reacting with alkyl halides 6 under basic conditions to give the corresponding polymer-bound 1,3-disubstituted 2\(H\)-pyrimido[4,5-\(d\)]-py-
dimidine-2,4(1H,3H)-diones. After extensive examination of the reaction conditions, the condensation was conventionally performed in DMF at room temperature, in the presence of DBU as the base. To further extend the scope of the process, we applied the previous conditions to α-bromoketones, which appeared less successful, affording a mixture of the expected 1,3-disubstituted and of the 3-monosubstituted products, easily separable after cleavage from the support. Other conditions were explored to fully convert 5, unfortunately without success.

At this stage of the process, a third vector of diversity was introduced after a selective sulfur oxidation of 7 with 1.3 equivalents of N-(phenylsulfonyl)-3-phenylaziridine in CHCl₃ at room temperature furnishing the alkylsulfinyl intermediate, which was subjected to subsequent cleavage from the support with various amines in dioxane at 85 °C leading to 7-amino-1,3-disubstituted-2H-pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones 9 in good yields and high purity (Table 1). N-(Phenyisulfonyl)-3-phenyl-

oxaziridine was selected as the reagent of choice in terms of selectivity, purity and reaction work-up. The formation of the resin-bound compounds was easily followed by ATR/FT-IR.

Finally, having optimised the chemistry, in order to demonstrate the potential of the described method for a parallel preparation of heterocyclic derivatives, starting from 20 isocyanates, 10 alkyl halides, and 10 amines, a library of 2000 individual pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones 9 was synthesised in good purity [55–99%]: HPLC Purity of the crude material with UV detection and confirmed by ¹H NMR spectroscopy and moderate yields [30–75%]: based on weight of crude material and are relative to the initial loading of polymer-bound pyrimide 3 (1.5 mmol/g). This sequence appeared ideally suited for the parallel synthesis of compound arrays on solid-phase.

In summary, a new and efficient process for the solid-phase synthesis of pyrimido[4,5-d]pyrimidine-

### Table 1 Pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-diones 9 Library

<table>
<thead>
<tr>
<th>R¹NCO (4)</th>
<th>R²CH₂Br (6)</th>
<th>R²R³NH (8)</th>
<th>Yield (%)</th>
<th>Product</th>
<th>Purity (%)</th>
<th>Observed MS⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>d</td>
<td>pyrrolidin-1-yl</td>
<td>68</td>
<td>9a</td>
<td>90</td>
<td>310 [M + H⁺]</td>
</tr>
<tr>
<td>Ph</td>
<td>p-NCC₆H₄</td>
<td>pyrrolidin-1-yl</td>
<td>61</td>
<td>9b</td>
<td>81</td>
<td>425 [M + H⁺]</td>
</tr>
<tr>
<td>Ph</td>
<td>cyclohexyl</td>
<td>pyrrolidin-1-yl</td>
<td>65</td>
<td>9c</td>
<td>87</td>
<td>406 [M + H⁺]</td>
</tr>
<tr>
<td>Ph</td>
<td>cyclopropyl</td>
<td>pyrrolidin-1-yl</td>
<td>48</td>
<td>9d</td>
<td>64</td>
<td>364 [M + H⁺]</td>
</tr>
<tr>
<td>Ph</td>
<td>NCC₆H₄,CH₂</td>
<td>pyrrolidin-1-yl</td>
<td>70</td>
<td>9e</td>
<td>93</td>
<td>377 [M + H⁺]</td>
</tr>
<tr>
<td>o-FC₆H₄</td>
<td>d</td>
<td>pyrrolidin-1-yl</td>
<td>42</td>
<td>9f</td>
<td>60</td>
<td>328 [M + H⁺]</td>
</tr>
<tr>
<td>o-FC₆H₄</td>
<td>p-NCC₆H₄</td>
<td>pyrrolidin-1-yl</td>
<td>72</td>
<td>9g</td>
<td>94</td>
<td>443 [M + H⁺]</td>
</tr>
<tr>
<td>o-FC₆H₄</td>
<td>NCC₆H₄,CH₂</td>
<td>pyrrolidin-1-yl</td>
<td>69</td>
<td>9h</td>
<td>90</td>
<td>395 [M + H⁺]</td>
</tr>
<tr>
<td>o-FC₆H₄</td>
<td>cyclopropyl</td>
<td>3-morpholin-4-yl-propylamino</td>
<td>45</td>
<td>9i</td>
<td>61</td>
<td>455 [M + H⁺]</td>
</tr>
<tr>
<td>C₆H₅</td>
<td>d</td>
<td>pyrrolidin-1-yl</td>
<td>67</td>
<td>9j</td>
<td>90</td>
<td>276 [M + H⁺]</td>
</tr>
<tr>
<td>m-ETO₂CC₆H₄</td>
<td>NCCH₆H₄,CH₂</td>
<td>3-morpholin-4-yl-propylamino</td>
<td>59</td>
<td>9k</td>
<td>78</td>
<td>508 [M + H⁺]</td>
</tr>
<tr>
<td>m-ETO₂CC₆H₄</td>
<td>m-FCC₆H₄</td>
<td>3-morpholin-4-yl-propylamino</td>
<td>52</td>
<td>9l</td>
<td>73</td>
<td>549 [M + H⁺]</td>
</tr>
<tr>
<td>m-ETO₂CC₆H₄</td>
<td>p-NCC₆H₄</td>
<td>3-morpholin-4-yl-propylamino</td>
<td>55</td>
<td>9m</td>
<td>73</td>
<td>556 [M + H⁺]</td>
</tr>
<tr>
<td>Ph-Ala-OMe</td>
<td>p-CNC₆H₄</td>
<td>pyrrolidin-1-yl</td>
<td>59</td>
<td>9n</td>
<td>81</td>
<td>396 [M + H⁺]</td>
</tr>
<tr>
<td>Ph-Ala-OEt</td>
<td>d</td>
<td>3-morpholin-4-yl-propylamino</td>
<td>65</td>
<td>9o</td>
<td>85</td>
<td>483 [M + H⁺]</td>
</tr>
<tr>
<td>Ph-Ala-OMe</td>
<td>p-NCC₆H₄</td>
<td>pyrrolidin-1-yl</td>
<td>75</td>
<td>9p</td>
<td>99</td>
<td>511 [M + H⁺]</td>
</tr>
<tr>
<td>Ph-Ala-OEt</td>
<td>p-NCC₆H₄</td>
<td>3-morpholin-4-yl-propylamino</td>
<td>61</td>
<td>9q</td>
<td>82</td>
<td>584 [M + H⁺]</td>
</tr>
<tr>
<td>Ph-Ala-OEt</td>
<td>d</td>
<td>2-pyridin-2-yl-ethylamino</td>
<td>70</td>
<td>9r</td>
<td>91</td>
<td>461 [M + H⁺]</td>
</tr>
<tr>
<td>Ph-Ala-OEt</td>
<td>ETO₂C₆H₄</td>
<td>3-morpholin-4-yl-propylamino</td>
<td>72</td>
<td>9s</td>
<td>93</td>
<td>569 [M + H⁺]</td>
</tr>
</tbody>
</table>

* Yield are based on weight of crude material and are relative to the initial loading of resin-bound pyrimidine 3 (1.5 mmol/g).
* HPLC Purity of the crude material (confirmed by ¹H NMR), measured on YMC-Pack Pro C18 column (75 × 4.6 mm) with a gradient 12% MeCN–H₂O → 95% MeCN within 5.4 min; flow rate, 2.64 mL/min; UV detection at 200–300 nm.
* Confirmed by mass spectra (ESI).
* No alkyl halide was added.
2,4(1H,3H)-diones 9 has been developed taking advantage of the use of a strong base such as phosphazene in a base-catalysed intramolecular ring closure. Furthermore, we have emphasised the potential of the polymer-bound pyrimidine 3, which appeared to us as a highly versatile building block for the generation of polyfunctional and fused-heterocycles, and demonstrated the potential of a sulfur-based safety-catch linkage strategy for the traceless synthesis of heterocyclic compounds on solid supports. Further applications of this strategy toward different functionalised fused-heterocycles, as well as extension to other heterocycles are in progress.

All chemicals were purchased from Fluka AG and Aldrich. Solvents were purified before use or purchased in anhyd quality. The chloromethylpolystyrene (1.8 mmol/g, 1% crosslinking, 200 µm) was from LCC Engineering & Trading GmbH. ATR/FTIR: Nicolet-860 FT-IR spectrometer with an IR-microscope NICPLAN; resolution 4 cm⁻¹, 200 or 500 co-added scans, MCT detector, characteristic bands in cm⁻¹.

To a suspension of polymer-bound alkyl halide (3) in anhyd DMF (6 mL) under argon at r.t., was added phosphazene (0.67 mL, 0.96 mmol) in anhyd DMF (6 mL) at 250 MHz; in DMSO-d₆ or CDCl₃, NMR Spectra: Bruker-AC-250 spectrometer, at 200 MHz; in DMSO-d₆ or CDCl₃ TMS as internal standard; chemical shift of signal centers and ranges in ppm (δ), J in Hz. EI-MS: Finnigan MS9-AEI or Mat 90; m/z (rel. int., %); ESIMS: PE Sciex API 300; m/z (rel. int., %).

Polymer-Bound Ethyl 4-Amino-2-sulfanylpyrimidinecarboxylic acid (3)

A mixture of Merrifield resin (33.2 g, 6.3 mmol, 1.80 mmol/g), ethyl 4-amino-2-sulfanylpyrimidinecarboxylate (2: 15 g, 7.5 mmol), and N-ethylidiospropylamine (DPEA, 15 mL, 8.7 mmol) in anhyd DMF (500 mL) was shaken at 65 °C for 20 h and then was washed with DMF (1 × 5 min), isopropyl alcohol (2 × 4 min), DMF (6 × 4 min), isopropyl alcohol (3 × 4 min) and hexane (5 × 3 min) at r.t. using an automated washing station. Drying under high vacuum for 20 h afforded polymer-bound 3; 92% of conversion based on elemental analysis.

FT-IR: 3352m, 1690s, 1596s, 1509m, 1520m, 1180m, 969s cm⁻¹. Anal. Found: N, 5.91; O, 4.95; S, 4.23.

Polymer-Bound 3-Monosubstituted 7-Sulfanyl-1H-pyrimido-[4,5-d]pyrimidine-2,4(1H,3H)-diones 5; General Procedure

To a suspension of polymer-bound 3 (200 mg, 0.3 mmol, 1.5 mmol/g), and 1-tert-butyl-4,4,4-tris(dimethylamino)-2,2-bis[tris(dimethylamino)phosphoranylidene]imino-2,4,5,6,7,8-hexahydroaziridine (0.235 g, 0.9 mmol). After 6 h at r.t., the reaction mixture was washed successively with a 1:1 mixture of formic acid-isopropyl alcohol (1 × 5 min) at 65 °C, a (1:1) mixture of dioxane–H₂O (2 × 4 min), isopropyl alcohol (5 × 4 min), and dioxane (3 × 4 min) at r.t. using an automated washing station. To the washed resin (0.3 mmol) was added anhyd dioxane (4 mL) under argon at r.t., followed by amine 8 (0.24 mmol). The reaction mixture was then stirred for 6 h at 85 °C. This eluate and one subsequent wash with dioxane (4 mL) were collected and combined, and the solvent was removed to yield 9 in high purity (Table 1).

3-Phenyl-7-pyrrolidin-1-yl-1H-pyrimido-[4,5-d]pyrimidine-2,4(1H,3H)-dione (9a)

White solid.

IR (KBr): 3185w, 2967w, 1725s, 1674s, 1658s, 1537s, 1482s, 795s, 1111s, 817s, 757s cm⁻¹.

1H NMR (250 MHz, DMSO-d₆ or CDCl₃): δ = 11.90 (s, 1 H, NH), 8.71 (s, 1 H₇), 7.49–7.38 (m, 3 H₇), 7.29–7.27 (m, 2 H₉), 3.61–3.59 (m, 2 H₂), 3.58–3.55 (m, 2 H₂), 2.10–1.95 (m, 5 H₄). Drying under high vacuum for 20 h afforded polymer-bound 3; 92% of conversion based on elemental analysis.

Polymer-Bound 1,3-Diisubstituted 7-Sulfanyl-2H-pyrimido-[4,5-d]pyrimidine-2,4(1H,3H)-diones 7; General Procedure

To a suspension of polymer-bound 5 (0.3 mmol), and DBU (0.14 mL, 0.96 mmol) in anhyd DMF (6 mL) under argon at r.t., was added alkyl halide (6) (0.9 mmol). After 6 h at r.t., the reaction mixture was washed successively with isopropyl alcohol (1 × 5 min), DMF (2 × 4 min), isopropyl alcohol (5 × 4 min), and CH₂Cl₂ (3 × 4 min) at r.t. using an automated washing station. Drying under high vacuum for 20 h afforded polymer-bound 7.

1,3-Diisubstituted 7-Amino-2H-pyrimido-[4,5-d]pyrimidine-2,4(1H,3H)-diones 9

To a suspension of polymer-bound 7 (0.3 mmol) in anhyd CHCl₃ (6 mL) under argon at r.t., was added N-(phenylsulfonyl)-3-phenoxaziridine (0.235 g, 0.9 mmol). After 6 h at r.t., the reaction mixture was washed successively with a 1:1 mixture of formic acid-isopropyl alcohol (1 × 5 min) at 65 °C, a (1:1) mixture of dioxane–H₂O (2 × 4 min), isopropyl alcohol (5 × 4 min), and dioxane (3 × 4 min) at r.t. using an automated washing station. To the washed resin (0.3 mmol) was added anhyd dioxane (4 mL) under argon at r.t., followed by amine 8 (0.24 mmol). The reaction mixture was then stirred for 6 h at 85 °C. This eluate and one subsequent wash with dioxane (4 mL) were collected and combined, and the solvent was removed to yield 9 in high purity (Table 1).
3-Propyl-7-pyridin-1-yl-1H-pyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione (9j)

White solid.

IR (KBr): 3286 m, 2939 w, 1716 s, 1666 s, 1620 s, 1552 s, 1247 s, 692 cm⁻¹.

1H NMR (250 MHz, DMSO-d₆): δ = 11.86 (s, 1 H, NH), 8.73–8.43 (m, 2 H arom), 7.52–7.24 (m, 7 H arom), 5.38–5.35 (m, 2 H αiph), 2.06–1.95 (m, 4 H αiph), 1.57–1.50 (m, 2 H αiph), 0.87–0.83 (t, J = 7.25 Hz, 3 H αiph), 2.10–1.95 (m, 4 H aliph), 1.57–1.50 (m, 2 H αiph), 0.87–0.83 (t, J = 7.25 Hz, 3 H αiph).

13C NMR (400 MHz, DMSO-d₆): δ = 160.8 (s), 158.5 (d), 150.4 (s), 131.8 (d), 130.9 (d), 124.9 (d), 123.1 (s), 116.3 (d), 97.6 (s), 74.3 (2 t), 25.1 (2 t).

ESIMS: m/z = 483 ([M + H]+, 100%).

Acknowledgements

We gratefully thank our colleagues in F. Hoffmann-La Roche AG for analytical support. We wish to thank Dr. Gerard Schmid for his valuable advice and stimulating discussions.

References