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Preparation of tetrasubstituted pyrimido[4,5-*d*]pyrimidine diones

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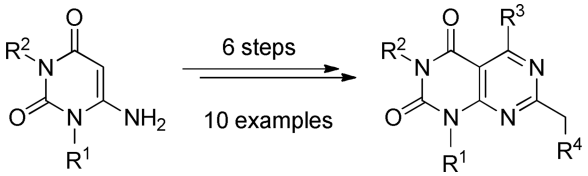
Abstract

A novel synthetic route to 1,3,5,7-tetrasubstituted pyrimido[4,5-d]pyrimidine-2,4-diones, of interest for potential antitumor activity, is reported. The route uses 1,3-disubstituted 6-amino uracils as starting materials. The key step is a hydrazine-induced cyclization reaction to form the fused pyrimidine ring. By choosing different uracils, acylation reagents and alkylation reagents, substituents at N-1, N-3, C-5, and C-7 may be selectively varied to provide a structurally diverse set of compounds for biological evaluation.

Graphical Abstract



Keywords

pyrimidopyrimidines; synthesis; heterocycles; antitumor; inhibitor

Introduction

Pyrimidopyrimidines have attracted considerable attention in medicinal chemistry due to their significant and diverse biological activity,[1] including antitumor,[2] antiviral,[3] antimicrobial,[4] and anti-allergic effects.[5] As part of an ongoing effort to investigate the ability of various scaffolds to act as antitumor agents by inhibiting monocarboxylate transporters (MCTs),[6,7] the investigation of this scaffold (**1**) was of particular interest due to its structural homology with the pteridinone MCT1 inhibitor **2** (Figure 1). [6,6]-fused scaffolds such as compounds **2–5** are potent MCT1 inhibitors with antitumor activity,[7] as are related [6,5]-fused ring compounds such as **6** and **7**.[8]

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Supplementary Data

^{1}H NMR spectra for all new compounds are provided in supporting information.

To our knowledge, general synthetic methods to prepare 1,3,5,7-tetrasubstituted pyrimido[4,5-d]pyrimidines (general structure **1**) have not been extensively investigated. Hamed's method[9] has limitations in scope because it requires that the compounds produced have identical substituents at the C-5 and C-7 positions. Yoneda's method[10] also suffers from limitations of providing only products with an aryl substituent at C-7 and the requirement for relatively inaccessible starting materials. Herein we report our efforts toward developing a general route to prepare compounds in this scaffold.

Results and Discussion

Due to its enamine character, the commercially available 6-amino uracil **8** (Scheme 1) can in principle act as a nucleophile at either a carbon or nitrogen atom. The amine group of **8** is only very weakly basic, however. For example, attempted amide coupling reactions of **8** using carboxylic acids and conventional coupling reagents, such as EDCI and HOBt, were unsuccessful and gave only recovered starting material. Reactions reported in the literature involving the amine group of **8** and its analogues are rare but include some very reactive electrophiles, including imidoyl derivatives,[11] DMF-DMA[12] and succinic anhydride.[13]

Interestingly, even chloroformates are unreactive toward the amine group: only electrophilic attack at C-5 occurs.[14] In our hands the use of ethyl chloroformate in pyridine at 90 °C cleanly gave ester **9** in 70% yield (Scheme 1). The nucleophilicity of the amine group in the ester product **9** should be even lower than that of **8**, given the additional conjugation to the ester group. Thus we opted to use a strong base (LiHMDS), expecting that the anion thus provided might be acylated by an acid chloride, such as the naphthyl-substituted acetyl chloride **10**. Indeed this approach was successful, giving the desired product **11** in 52% yield.[15] Attempted cyclization to the [6,6]-fused pyrimidinone **12**, however, proved to be problematic. Only low yields (<5%) of **12** were obtained using 7N NH_3 in MeOH for 2 weeks at room temperature or under a variety of more harsh microwave reaction conditions. Amidine or amide-promoted ring closure using 2-naphthalen-1-ylacetamidine[16] (neat, 170 °C, 1 h) or 2-(naphthalene-1-yl)-acetamide (DMF, reflux, 14 h, or DMF, microwave 250 °C, 30 min) were similarly unsuccessful.

The desired cyclization was observed when the more nucleophilic reagent hydrazine was used in place of ammonia (Scheme 2).[17] Thus, treatment of **11** with hydrazine gave product **13**, which required cleavage of the N-N bond to give compound **12**. This was achieved under diazotization conditions ($NaNO_2$, AcOH) followed by heating to reflux to give **12** in 38% yield for the two step procedure.[18] While efforts to improve the yield for this conversion were unsuccessful, compound **12** was readily converted to the triflate **14** (triflic anhydride, triethylamine) in 64% yield.[19] Access to triflate **14** permitted the facile preparation of the desired thioethers **15–17**, analogues of pteridinone **2**, in 67–77% yield by treatment with ω-mercaptoalcohols.[20]

We investigated a number of alternative strategies to access the fused pyrimidinone **12**, given the modest yield of the two step conversion from ester **11**. This included the reaction of acyl cyanates[21] with enamine **8**. Such alternative strategies were unfortunately ill-suited for suitable for efficient and scalable preparation of this compound.

Encouraged by the ability of the Scheme 1–2 route to give the desired products, albeit in modest yields, the generality of the approach was then investigated (Scheme 3). Using three different 6-aminouracil starting materials (**8**, **18** and **19**[22]), the same six step sequence was followed. The acid chloride used to install the R^4 substituent was varied, as was the thiol nucleophile used to install the R^3 group in the final step. With these modifications, seven additional analogues of compounds **15–17** were synthesized (Figure 2, compounds **36**–**42**). Because structural analogues of pteridinone **2** were desired, only aryl acetyl chlorides **22** and **23** were used, but a variety of other acid chlorides would likely be well-suited for use in this process. Similarly, triflates **32**–**35** would likely be quite useful substrates for transition metal-mediated conversions. Thus diverse structures in the scaffold are accessible using these methods.

The isolated yields for material obtained in each step during the synthesis of compounds **15–17** and **36–42** was fairly consistent. Step 1 yields ranged from 60–70%, step 2 from 47–52%, steps 3–4 (combined) from 31–54%, step 5 from 52–83%, and step 6 from 45–82%. Thus while the overall yield for the final products made by this six-step process was low (4–7%), the method gave access to densely-substituted heterocycles, compounds that to our knowledge are inaccessible using previously-reported methods.

Conclusion

In this study a six-step sequence was devised to prepare ten 1,3,5,7-tetrasubstituted pyrimido[4,5-d]pyrimidine-2,4-diones. By choosing different starting materials and reagents, substituents were varied at N-1, N-3, C-5, and C-7. Efforts to optimize these synthetic procedures and the evaluation of the biological properties of the final products will be the subject of future reports from our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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15.

Synthesis of ethyl 1-isobutyl-3-methyl-6-(2-(naphthalene-1-yl)acetamido)-2,4-dioxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (**11**): A solution of **9** (757 mg, 2.81 mmol) in THF (42 mL) was cooled to −78 °C under N_2. A solution of LiHMDS in THF (3.23 mL, 3.23 mmol, 1 M in THF) was added dropwise. The reaction mixture was stirred at −78 °C for 10 min, 0 °C for 30 min, and cooled to −78 °C. Acid chloride **10** (748 mg, 3.66 mmol) was added in one portion. The resultant mixture was slowly warmed up to room temperature and stirred overnight. The reaction was quenched with saturated NH_4Cl, extracted with ethyl acetate (EA). The combined organic extracts were washed with brine and dried over Na_2SO_4. The crude product was concentrated and purified by flash chromatography on silica gel (hexanes:ethyl acetate=2:3) to afford 633 mg (52%) of **11** as a yellow solid. R_f= 0.34 (hexanes:ethyl acetate=1:1); LC-MS (ESI): m/z 438 $[M+1]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.14 (d, J=6.8 Hz, 6H), 1.15 (t, J=7.2 Hz, 3H), 1.44 (sep, J=6.8 Hz, 1H), 3.11 (s, 3H), 3.24 (d, J=7.6 Hz, 2H), 3.99 (s, 2H), 4.03 (q, J=7.2 Hz, 2H), 7.08 (s, 1H), 7.33–7.43 (m, 3H), 7.70–7.83 (m, 3H), 9.36 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 14.1, 19.1, 27.4, 28.5, 43.3, 53.3, 61.9, 97.2, 123.2, 125.9, 126.4, 127.3, 128.7, 129.1, 129.2, 129.3, 131.9, 134.1, 150.0, 151.8, 159.1, 166.1, 170.5.

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18.

Synthesis of 1-isobutyl-3-methyl-7-(naphthalen-1-ylmethyl) pyrimido[4,5-d]pyrimidine-2,4,5(1H,3H,6H)-trione (**12**): A suspension of **11** (224 mg, 0.512 mmol) in *n*-BuOH (40 mL) was treated with $NH_2NH_2•H_2O$ (0.77 mL) under N_2. The resultant mixture was refluxed for 30 min. The reaction

was concentrated to afford crude 6-amino-1-isobutyl-3-methyl-7-(naphthalen-1-ylmethyl)pyrimido[4,5-d]pyrimidine-2,4,5(1H,3H,6H)-trione (**13**), which was used directly in the next step. LC-MS (ESI): m/z 406 $[M+1]^+$. A solution of crude **13** in acetic acid (30 mL) was treated with $NaNO_2$ (283 mg, 4.10 mmol). The resultant mixture was refluxed for 5 min and cooled to room temperature. Additional $NaNO_2$ (283 mg, 4.10 mmol) was added and the solution was refluxed for 5 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in dichloromethane (DCM), washed with H_2O, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (DCM:MeOH=19:1 to 9:1 gradient) to afford 75 mg (38% for two steps) of **12** as a yellow solid. R_f= 0.07 (DCM:MeOH=19:1); LC-MS (ESI): m/z 391 $[M+1]^+$; ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm) 0.34 (d, J=6.8 Hz, 6H), 1.50 (sep, J=6.8 Hz, 1H), 3.10 (s, 3H), 3.46 (d, J=7.6 Hz, 2H), 4.44 (s, 2H), 7.46–7.54 (m, 4H), 7.86–8.10 (m, 3H) 13.05 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d6) δ (ppm) 19.2, 26.5, 27.5, 37.4, 48.8, 94.2, 124.3, 125.4, 125.6, 126.1, 127.8, 128.2, 128.4, 131.4, 131.9, 133.4, 150.7, 155.1, 158.2, 158.3, 164.6.

19. Synthesis of 8-isobutyl-6-methyl-2-(naphthalen-1-ylmethyl)-5,7-dioxo-5,6,7,8-tetrahydropyrimido[4,5-d]pyrimidin-4-yl trifluoromethanesulfonate (**14**): A solution of **12** (20 mg, 0.051 mmol) in DCM (3 mL) was cooled to 0 °C. Et_3N (15 mg, 0.15 mmol) was added followed by Tf_2O (26 mg, 0.092 mmol). The reaction was stirred at 0 °C for 30 min, quenched with saturated NH_4Cl and extracted with DCM. The combined organic extracts were washed with brine and dried over Na_2SO_4. The crude product was purified by flash chromatography on silica gel (hexanes:ethyl acetate=9:1) to afford 17 mg (64%) of triflate **14** as a white solid. R_f= 0.34 (hexanes:ethyl acetate=4:1); LC-MS (ESI): m/z 523 $[M+1]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.63 (d, J=6.8 Hz, 6H), 1.77 (sep, J=6.8 Hz, 1H), 3.39 (s, 3H), 3.83 (d, J=7.2 Hz, 2H), 4.70 (s, 2H), 7.44–7.51 (m, 4H), 7.79–8.08 (m, 3H); ^{19}F NMR (400 MHz, CDCl3) δ (ppm) −73.3.

20. General procedure for synthesis of thioethers **15**–**17**: A solution of triflate **14** (1.0 eq) in THF was treated with a ω-mercaptoalcohol (1.5 eq.) and Et_3N (2.0 eq.). The reaction was stirred at room temperature for 12–16 hours. The solvent was removed *in vacuo* and the residue was purified by column chromatography.

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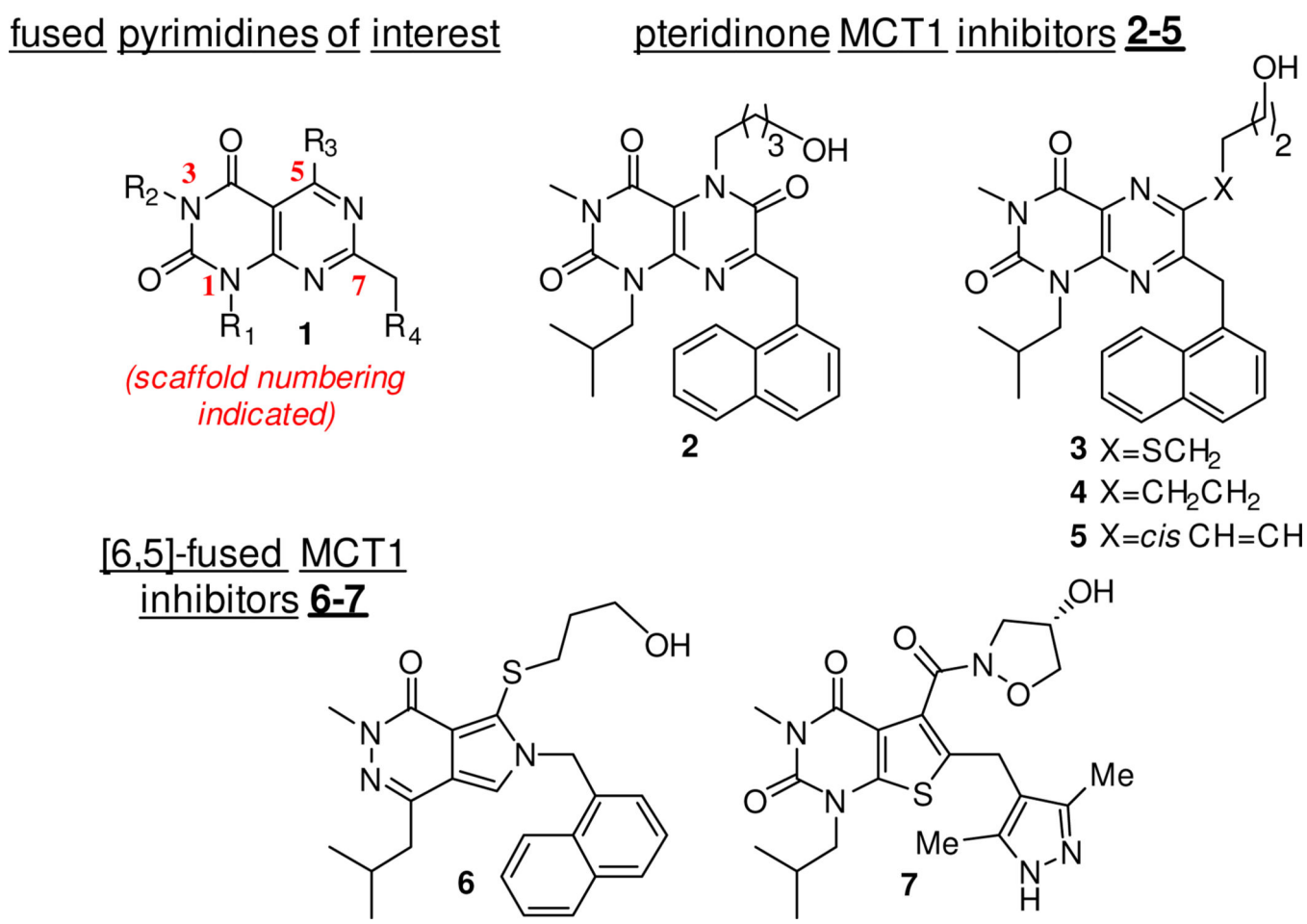


Figure 1.
Scaffold **1** and potent MCT1 inhibitors **2–7**

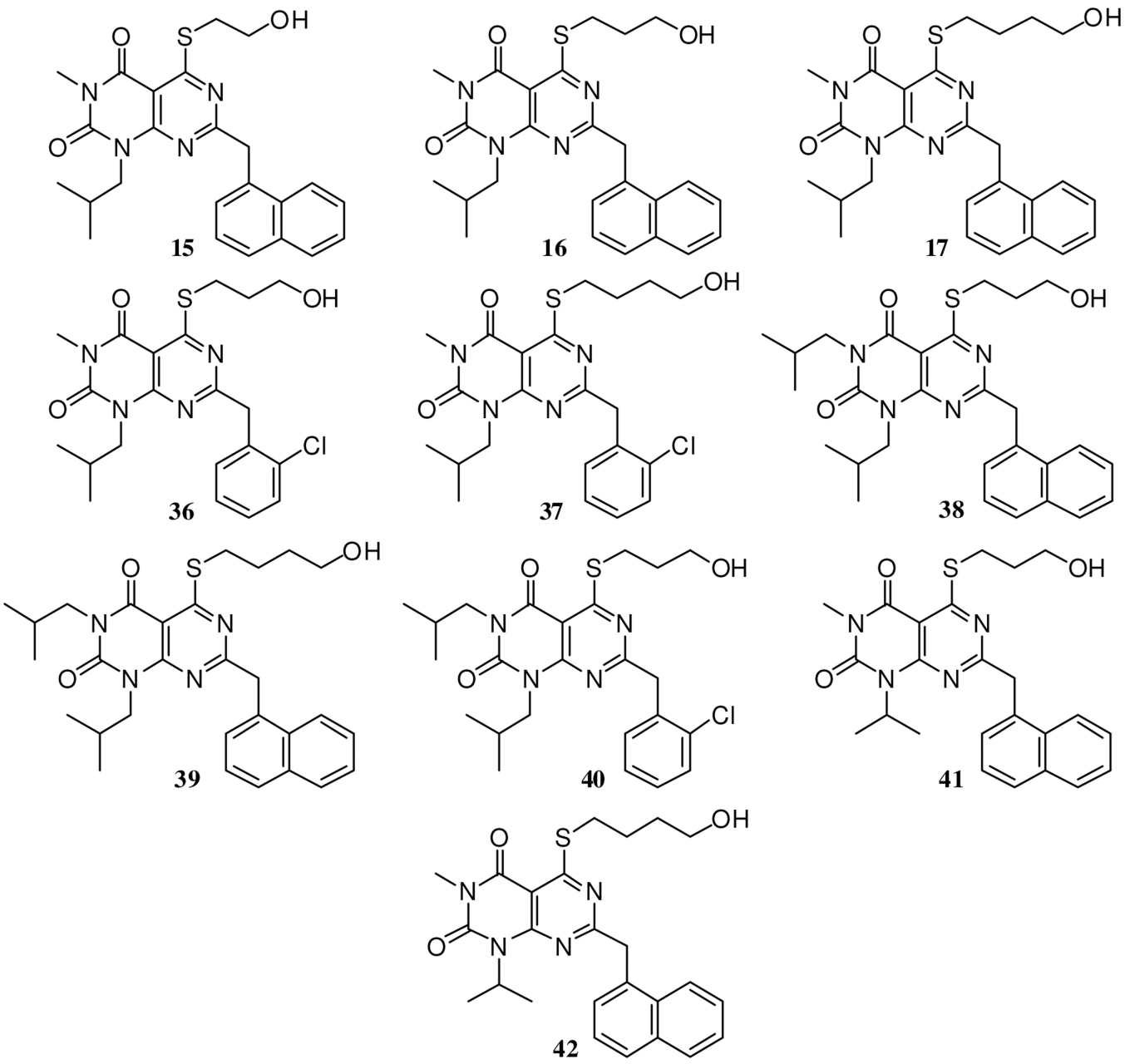
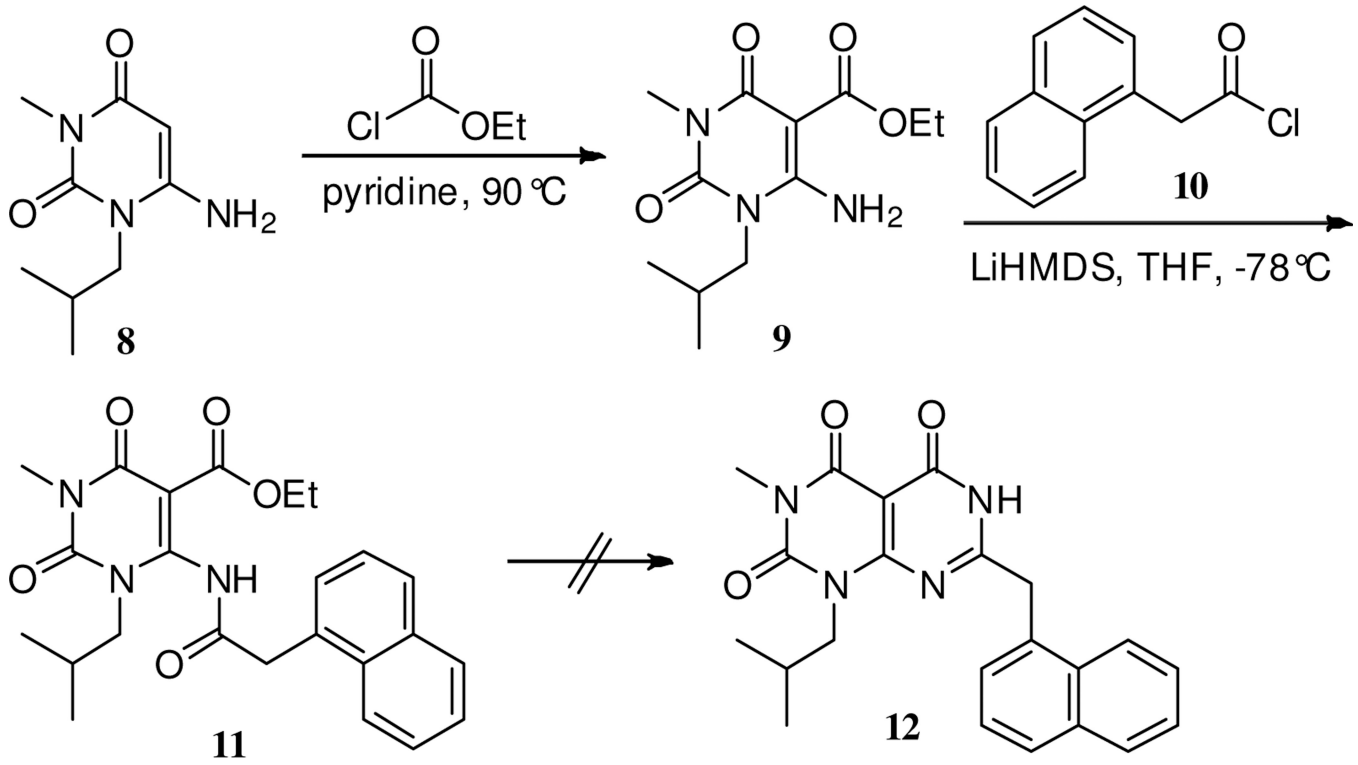
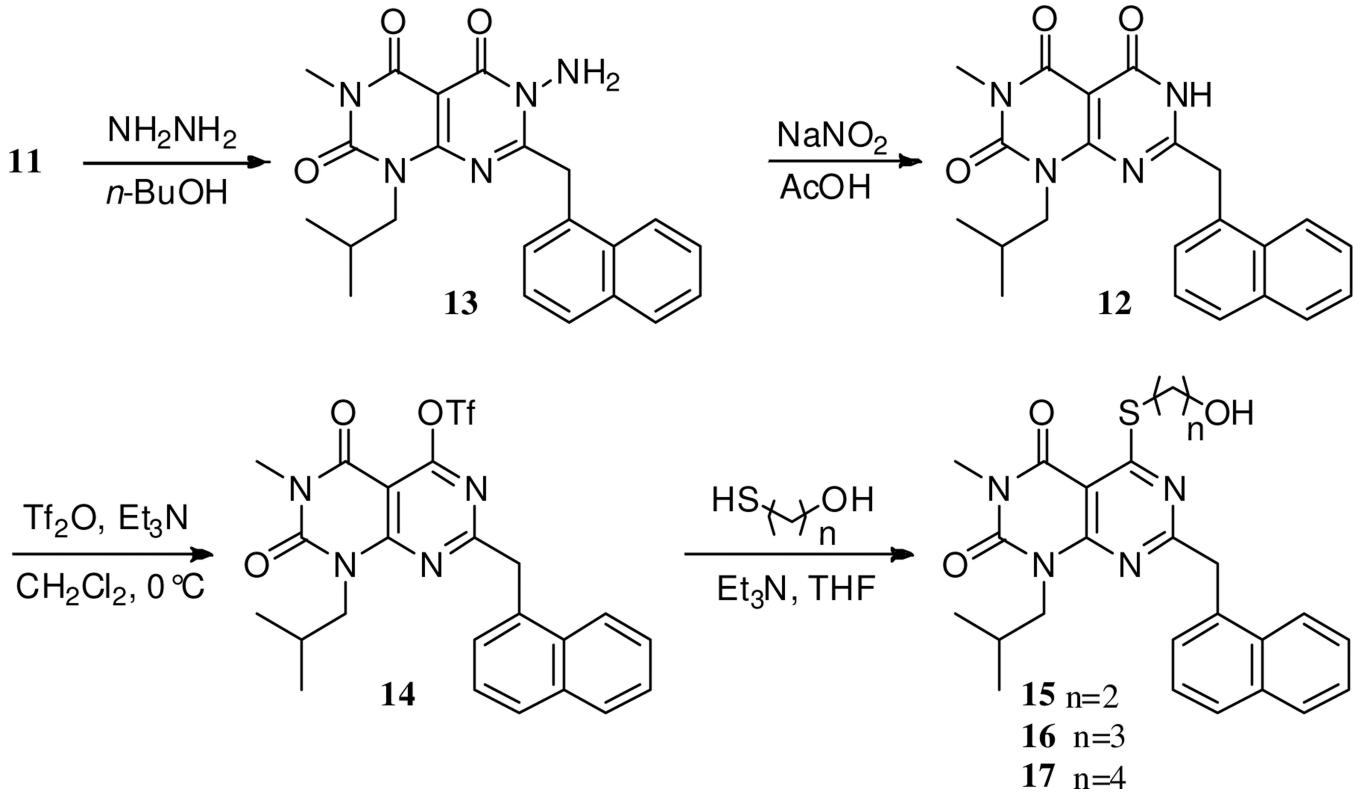


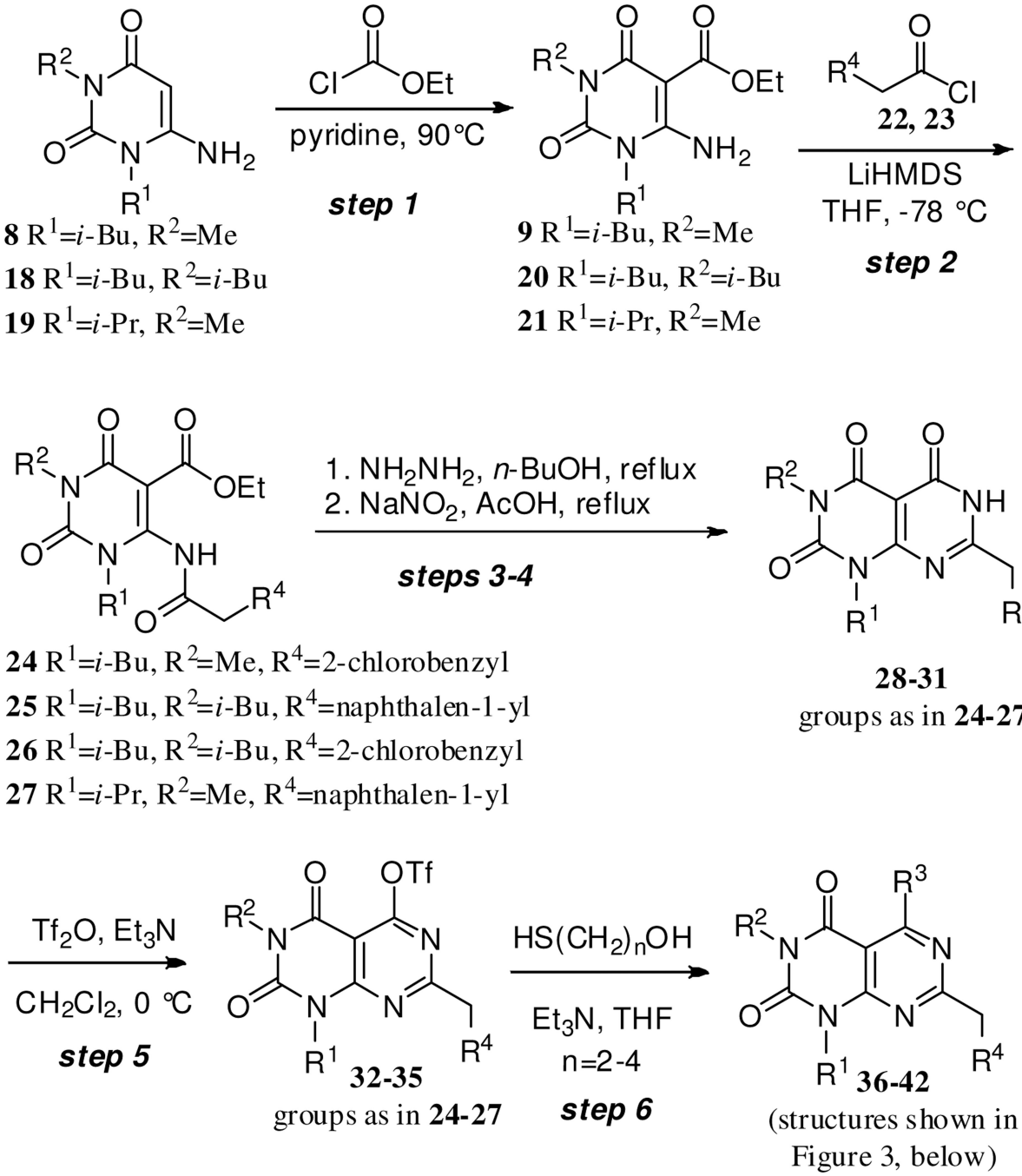
Figure 2.
Test library produced, following methods from Schemes 1–2



Scheme 1.
Attempts to prepare compound **12**



Scheme 2.
Successful cyclization and conversion to test compounds



8 R^1=*i*-Bu, R^2=Me
18 R^1=*i*-Bu, R^2=*i*-Bu
19 R^1=*i*-Pr, R^2=Me

9 R^1=*i*-Bu, R^2=Me
20 R^1=*i*-Bu, R^2=*i*-Bu
21 R^1=*i*-Pr, R^2=Me

24 R^1=*i*-Bu, R^2=Me, R^4=2-chlorobenzyl
25 R^1=*i*-Bu, R^2=*i*-Bu, R^4=naphthalen-1-yl
26 R^1=*i*-Bu, R^2=*i*-Bu, R^4=2-chlorobenzyl
27 R^1=*i*-Pr, R^2=Me, R^4=naphthalen-1-yl

28-31
groups as in **24-27**

32-35
groups as in **24-27**

36-42
(structures shown in Figure 3, below)

Scheme 3.
Generalization of the six step sequence.