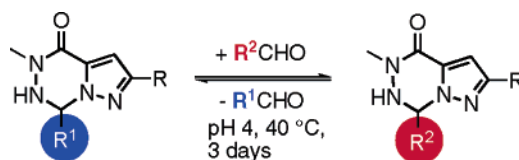# Metathesis Reactions of Pyrazolotriazinones Generate Dynamic Combinatorial Libraries

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ABSTRACT

Reversible metathesis reactions of pyrazolotriazinones and aliphatic aldehydes or ketones proceed in aqueous, phosphate-buffered media at pH 4 and 40–60 °C to generate thermodynamically controlled mixtures of heterocycles.

Dynamic combinatorial chemistry (DCC) is a promising new strategy with potential applications in drug discovery, host–guest chemistry, and catalysis. The principle of DCC is based on the use of reversible reactions to generate compound mixtures that respond to the presence of a template (enzyme, protein, small molecule, or ion) or a change in environment. Ideally, these dynamic combinatorial libraries (DCLs) rapidly evolve toward major products that possess the highest affinity to the template or that adapt best to the reaction environment. The concept of DCL has been widely reviewed, and successful applications have been described.[1]

While the chemical literature offers a wide selection of protocols for essentially irreversible processes, relatively few reversible reactions are available for DCL generation. These include transesterification,[2] disulfide exchange,[3] Schiff base exchange,[4] oxime and hydrazone metathesis,[5,6] transpepti-

dation,[7] Diels–Alder reaction,[8] olefin metathesis,[9] and boronic ester exchange.[10] A further expansion of reactions suitable for DCC is clearly desirable, especially if an aqueous environment can be tolerated.[11]

We have been interested in the development of reversible heterocycle formations suitable for DCL generation and hit identification. The pyrazolotriazine scaffold was particularly attractive in this context since it could be constructed from readily available carbonyl compounds and acylhydrazides. The 6,7-dihydro-5*H*-pyrazolo[1,5-*d*][1,2,4]triazin-4-one was first reported by Ainsworth in 1955,[12] and this scaffold was subsequently shown to have anti-inflammatory properties.[13]

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The parent pyrazolotriazinone heterocycles were synthesized in a four-step sequence as shown in Scheme 1.

Scheme 1. Synthesis of Pyrazolotriazinones

6a; R=CH₃, 90%
6b; R=CH₂CH₂Ph, 89%
6c; R=CH(CH₃)₂, 80%
6d; R=c-(C₆H₁₁), 67%
6e; R=CH₂CH(CH₃)₂, 80%
6f; R= , 53%
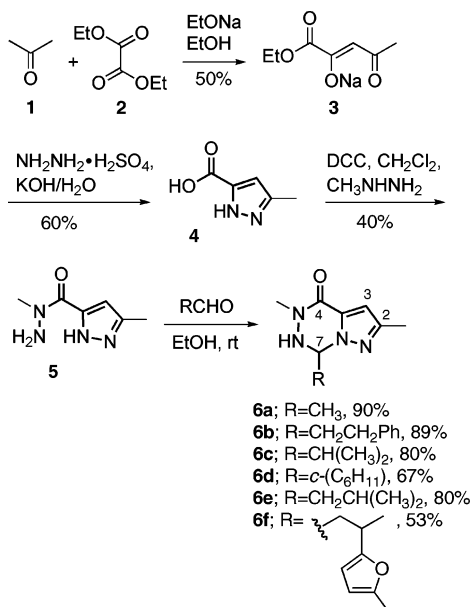
Ketoesters **3** were obtained by aldol condensation of acetone (**1**) and diethyloxalate (**2**) in EtOH/EtONa at room temperature. The cyclocondensation of **3** with hydrazine in basic aqueous media led the pyrazole carboxylic acid **4**,[14] which could be converted into pyrazolecarboxylic hydrazide **5** by acylation of methyl hydrazine using DCC as an activating agent. Ring closure of **5** was achieved with a range of aliphatic aldehydes to give the corresponding pyrazolotriazinone derivatives **6**.[15] However, attempts to synthesize pyrazolotriazinones from aromatic aldehydes or ketones failed.

Several conditions were screened to identify a thermodynamically controlled cyclocondensation−carbonyl exchange process in aqueous media (Table 1). The reaction of **6a** with equimolar amounts of hydrocinnamaldehyde and acetaldehyde at room temperature and pH 4 over 2 days proceeded only slowly (entry 1). Furthermore, at pH 5 and 36 °C only starting material could be detected (entry 5).

Thermodynamic equilibration was achieved at pH 4 after 3 d at 40 °C (entries 2 and 3). After 5 d, these compounds were still stable in the aqueous environment, and the distribution was maintained (entry 4). We also probed the reversibility of the system by starting from products **6a** or **6b** (entries 2 and 3, respectively) and obtained an identical product ratio either way. The yields of recovered **6a** and **6b** were quantitative.

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(15) (a) Tihanyi, E.; Gál, M.; Feher, Ö.; Dvortsák, P. *J. Heterocycl. Chem.* **1989**, *26*, 1045. (b) Tihanyi, E.; Gál, M.; Feher, Ö.; Janaky, J. HU 42771, 1987.

Table 1. Optimization of Pyrazolotriazinone Formation

entry	starting material	reaction cond[a]	time (h)	6a/6b (ratio)[b]
1	**6a**	pH 4, rt	48	97:3
2	**6a**	pH 4, 40 °C	72	1:1
3	**6b**	pH 4, 40 °C	72	48:52
4	**6a**	pH 4, 40 °C	120	52:48
5	**6a**	pH 5, 36 °C	48	1:0

[a] The concentration of each component was 1 mM in a buffered phosphate solution. [b] The ratio was determined by GC, using 1,4-dimethoxybenzene as an internal standard, and confirmed by preparative isolation.

Similar results could be observed with either **7a** or **7b** as starting materials using the same equilibrating conditions, as shown in Table 2 (entries 1 and 2).[16]

Table 2. Equilibration Condition for Compounds **7a** and **7b**

entry	starting material	reaction cond[a]	time (h)	7a/7b (ratio)[b]
1	**7a**	pH 4, 40 °C	72	3:7
2	**7b**	pH 4, 40 °C	72	3:7

[a] The concentration of each component was 1 mM in a buffered phosphate solution. [b] The ratio was determined by GC, using 1,4-dimethoxybenzene as an internal standard, and confirmed by preparative isolation (**7a**, 25%; **7b**, 67%).

These results demonstrate that despite the acidic aqueous reaction conditions, heterocycles **6** and **7** were not irreversibly hydrolyzed to the acyl hydrazide precursors. In all cases, pyrazolotriazinones proved stable to the mildly acidic aqueous enviroment, but the yield and rate of heterocycle formation were dependent on pH, concentration, and temperature. As further proof of principle, a small DCL was generated by incubating pyrazolotriazinone **6c** with aldehydes **8**−**11** (Scheme 2). The starting concentrations of aldehydes were kept at 1 mM each, and a 1 mM concentration of **6c** was established at reaction onset. The product distribution was established by GC analysis using independently prepared

(16) Compounds **7a** and **7b** were synthesized using the same four-step sequence as shown in Scheme 1, starting with 3-methyl-2-butanone: (1) EtOH/EtONa, 87%; (2) NH₂NH₂/KOH, 80%; (3) BOP, DIPEA, THF, 45%; (4) **7a**, EtOH, rt, OHCCH(CH₃)₂, 70%; **7b**, EtOH, rt, OHC(CH₂)₂Ph, 90%.

Scheme 2. Reversible Exchange Reactions between Pyrazolotriazinone **6c** and the Aldehydes **8–11**
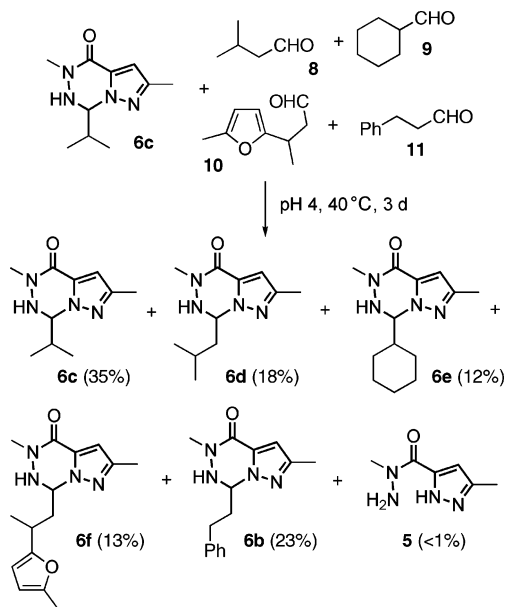

6b–f as reference compounds for the identification of each peak. In addition to the metathesis products, only traces of hydrazide **5** were detected by ^{1}H NMR and GC–MS in the reaction mixture.[17] It is interesting to note that the thermodynamic distribution of these heterocycles is not exactly even. This could be due to differential steric interactions of side chain substituents with the heterocyclic cores or variation in the free energy of aqueous solvation which is expected to change considerably depending on the exact substitution pattern of the pyrazolotriazinones.[18] Heterocycles **6** and **7** are stable in acidic, neutral, and basic aqueous media over several days; however, after several weeks exposure to air, small amounts of the oxidized 5*H*-pyrazolo[1,5-*d*][1,2,4]-triazin-4-ones are detectable by LC–MS.

For the screening of DCLs in biological assays, it is important to minimize the presence of free aldehydes which are capable of covalently modifying active site residues. Therefore, we tested the possibility for direct side-chain metathesis of pyrazolotriazinones. An equimolecular mixture of **6c** and **7a**, which differ by their substitutions at the 2-positions of the heterocycles, was equilibrated during 3 d at pH 4 and 40 °C (Scheme 3). As expected, a mixture of four products (the original starting materials and two crossover derivatives) was formed. The equilibration distribution was identical when **6b** and **7b** were used as starting materials.[19]

(17) **Typical Procedure.** A mixture of compound **6c** (6.4 mg, 0.031 mmol) and aldehydes **8–11** (0.031 mmol each) in a phosphate buffer solution at pH 4 was stirred for 3 d (72 h) at 40 °C. The pH was raised to 7 with NaHCO₃, and the reaction mixture was extracted with AcOEt. The combined organic layers were dried (MgSO₄), filtered, and concentrated in vacuo. The residue was analyzed by GC using dimethoxyphenol as an internal standard, or alternatively, by LC–MS.

(18) See, for example: (a) Jorgensen, W. L.; Tirado-Rives, J. *Persp. Drug Discuss. Design* **1995**, *3*, 123. (b) Perrin, C. L.; Fabian, M. A.; Rivero, I. A. *J. Am. Chem. Soc.* **1998**, *120*, 1044.

Scheme 3. Metathesis of Pyrazolotriazinones[a]
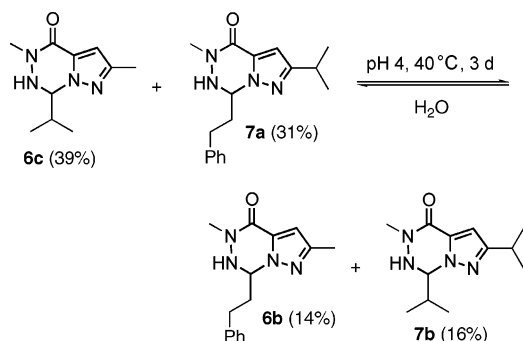
[a] The yields in parentheses reflect the equilibrium distribution.

We further investigated the scope of this new DCC methodology by varying the substituents on the hydrazine segment and using ketones as exchange components. Attempts to equilibrate the system composed of 5,6-disubstituted pyrozolotriazinones **12** and **13** under the standard conditions failed; no exchange product was detected (Figure 1). Heterocycle **12** was further incubated with 1 equiv of **6e**
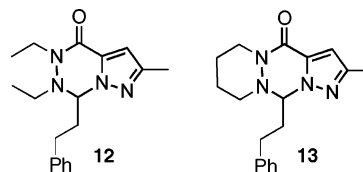
Figure 1. *N*(5),*N*(6)-Disubstituted pyrazolotriazinones are stable under the metathesis reaction conditions.

in the aqueous buffer solution for 4 d, but only starting materials were recovered. Heterocycle **13** was allowed to equilibrate with cylohexanecarboxaldehyde in a 1 mM buffer solution, and exchange product was not detected either. We hypothesized that substitution at *N*(6) of the pyrazolotriazinone scaffold stabilizes the ring system sufficiently to prevent reversible ring opening.

In contrast, the equilibration of ketone-derived *C*(7)-disubstituted pyrazoltriazinones was feasible but required a prolonged equilibration time and slightly harsher conditions (Scheme 4). After a 4 d exposure to pH 4 phosphate buffer at 60 °C, a 1 mM solution of **14a**[20] and ketones **15**, **16**, and **17** was fully equilibrated. LC–MS analyses detected a small amount of an unidentified byproduct; otherwise, the reaction was clean.

In summary, we developed a new exchange reaction between pyrazolotriazinone heterocycles and carbonyl com-

(19) We currently do not know the exact mechanism of the exchange process. Both a direct metathesis as well as an exchange reaction mediated by small amounts of hydrolyzed intermediates are feasible.

(20) Compound **14a** was synthesized from hydrazide **5** and cyclohexanone in EtOH at rt in 70% yield.

Scheme 4. Metathesis of *C*(7)-Disubstituted Pyrazolotriazinones
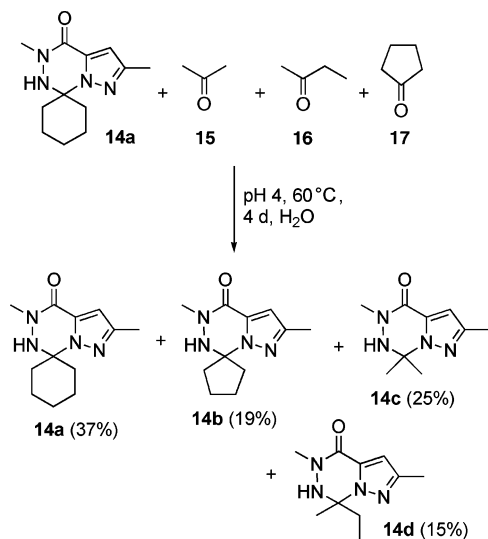
pounds. This dynamic exchange proceeds in a reversible fashion in a mildly acidic aqueous environment and repre-

sents a significant extension of current DCC methodologies. Structural diversity of the core scaffolds can be accomplished by modifications at the 2- and 7-positions of the heterocycles. The pyrazolotriazinones are stable in buffered media over several days, and these conditions are suitable for the generation of DCLs as well as for the direct biological screening of these libraries.[21] Moreover, the exchange reaction can be stopped by raising the pH to 7, thus providing a convenient way to analyze the compound distribution patterns.

Supporting Information Available: Experimental procedures, GC and LC−MS traces, and spectral data for compounds **6b−f**, **7a,b**, and **12−14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) The optimal pH stability of many biological targets ranges from pH 4 to 8; therefore, DCLs of pyrazolotriazinones can be directly exposed to proteins in order to discover new binding agents. Schaefer, M.; Sommer, M.; Karplus, M. *J. Phys. Chem. B* **1997**, *101*, 1663.