



Title	1,2-Disubstituted 1,2-Dihydro-1,2,4,5-tetrazine-3,6-dione as a Dynamic Covalent Bonding Unit at Room Temperature
Author(s)	Kawai, Kentaro; Ikeda, Kazuki; Sato, Akane; Kabasawa, Akira; Kojima, Masahiro; Kokado, Kenta; Kakugo, Akira; Sada, Kazuki; Yoshino, Tatsuhiko; Matsunaga, Shigeki
Citation	Journal of the American Chemical Society, 144(3), 1370-1379 https://doi.org/10.1021/jacs.1c11665
Issue Date	2022-01-18
Doc URL	http://hdl.handle.net/2115/88127
Rights	This document is the Accepted Manuscript version of a Published Work that appeared in final form in Journal of the American Chemical Society, copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see https://pubs.acs.org/articlesonrequest/AOR-JKCBT3BXPURU3JRJMTVT3 .
Type	article (author version)
File Information	manuscript_rev.pdf



[Instructions for use](#)

1,2-Disubstituted-1,2-dihydro-1,2,4,5-tetrazine-3,6-dione as a Dynamic Covalent Bonding Unit at Room Temperature

Kentaro Kawai,[†] Kazuki Ikeda,[†] Akane Sato,[†] Akira Kabasawa,[‡] Masahiro Kojima,[†] Kenta Kokado,[§] Akira Kakugo,[‡] Kazuki Sada,[‡] Tatsuhiko Yoshino,^{*,†,¶} and Shigeki Matsunaga^{*,†,¶}

[†]Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12 Nishi-6, Kita-ku, Sapporo 060-0812, Japan

[‡]Department of Chemistry, Faculty of Science, Hokkaido University, Kita-10 Nishi-8, Kita-ku, Sapporo 060-0810, Japan

[§]Research Institute for Electronic Science, Hokkaido University, Sapporo, 001-0020, Japan

[¶]Global Station for Biosurfaces and Drug Discovery, Hokkaido University, Kita-12 Nishi-6, Kita-ku, Sapporo 060-0812, Japan

ABSTRACT: Dynamic covalent bonds are useful tools in a wide range of applications. Although various reversible chemical reactions have been studied for this purpose, the requirement for harsh conditions, such as high temperature, and low or high pH to activate generally stable covalent bonds limits their potential applications involving biomolecules or household utilization. Here, we report the design, synthesis, characterization, and dynamic covalent bonding properties of 1,2-disubstituted-1,2-dihydro-1,2,4,5-tetrazine-3,6-diones (TETRAD). Hetero-Diels–Alder reactions of TETRAD with furan derivatives and their retro-reactions proceeded rapidly at room temperature under neutral conditions, enabling a chemically induced sol–gel transition system.

INTRODUCTION

Some covalent bonds in organic molecules can be reversibly formed and cleaved under certain reaction conditions; this phenomenon is known as dynamic covalent bonding. Such dynamic processes are realized by reversible chemical reactions such as imine/acetal formation, transesterification, Diels–Alder reactions, etc.¹ These reversible reactions guide the whole system to the most thermodynamically stable state under the given conditions, and adaptable dynamic changes can be achieved when an external perturbation is applied to the system (Figure 1a). In the past few decades, reactions for dynamic covalent bond formation have been broadly studied not only in the context of fundamental organic chemistry, but also in applied chemical sciences. Compared to non-covalent bonding interactions, which have also been extensively investigated in supramolecular chemistry and related research fields, dynamic covalent bonding reactions can potentially provide functional molecules with both static and dynamic characteristics. These reactions have been widely utilized for various applications, such as macromolecular chemistry,² covalent organic frameworks (COFs),³ drug discovery/delivery,⁴ and construction of well-defined molecular architectures⁵ (Figure 1b).

Although various kinds of reversible chemical reactions have been exploited to develop such functional molecules,^{1–5} the cleavage of covalent bonds under mild reaction conditions remains a challenging and important issue (Figure 1c). Because the formation of stable covalent bonds is usually highly exergonic, the retro-reaction to cleave them exhibits a high activation barrier, and thus requires high/low pH, particular transition metal catalysts, or high temperature. These harsh conditions can limit the applications of dynamic covalent bonding in situations where such drastic conditions are not suitable, e.g. applications involving unstable biomolecules⁴ or household utilization.

Therefore, the development of a novel reversible covalent bonding system that can be activated by a mild external perturbation is of great importance and could significantly expand the applications of reversible covalent bonding.

Here, we report the synthesis of 1,2-disubstituted-1,2-dihydro-1,2,4,5-tetrazine-3,6-diones (**1**; TETRAD), and their dynamic covalent bonding properties. TETRAD **1** undergoes an instantaneous hetero-Diels–Alder (HDA) reaction with furan derivatives, and the HDA adducts also undergo a very fast retro-Diels–Alder reaction under ambient conditions (Figure 1d). We demonstrated the utility of TETRAD **1** through the development of furan-containing polyurethanes gelled by a TETRAD-based cross-linker, which exhibited fast and chemically-controlled sol–gel transition ability at room temperature.

Our design of TETRAD **1** was inspired by 4-substituted-1,2,4-triazoline-3,5-diones (TAD, **2**). TAD is a five-membered heterocyclic compound containing a highly electrophilic *cis*-fixed azo group, which is activated by two carbonyls. TAD **2** participates in HDA reactions, Alder-ene reactions, thermal [2+2] cycloadditions, and electrophilic aromatic substitutions under mild conditions (Figure 2a).⁶ Due to its high reactivity under ambient conditions without any catalysts, TAD **2** has been used as a clicking unit for numerous applications involving polymer synthesis/functionalization⁷ and bioconjugation.⁸ Additionally, some covalent-bond-forming reactions of TAD have been reported to be reversible and have found applications in dynamic covalent bonding systems.^{9–12} For example, a reversible hetero-ene reaction of 4-methyl-TAD (MeTAD) and indole derivatives was used for the protection of indoles in natural product synthesis¹⁰ as well as reversible polymer conjugation/recombination with click reaction characteristics.^{11,12} Although TAD represents the prominent properties as a dynamic covalent binding unit, retro-reactions to regenerate the TAD

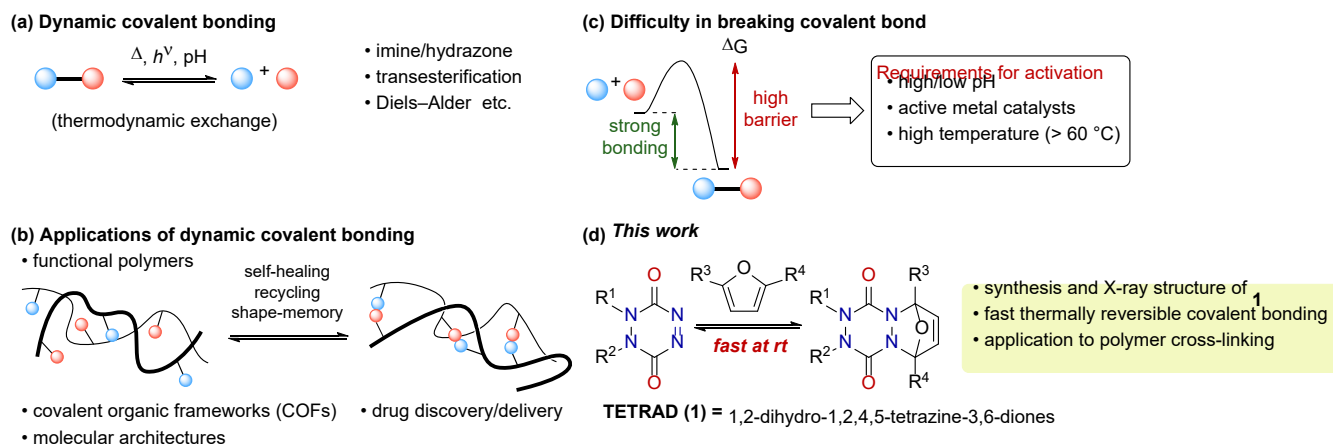


Figure 1. Dynamic covalent bonding and the concept of this work. (a) Concept of dynamic covalent bonding. (b) Application of dynamic covalent bonding. (c) Difficulty in breaking covalent bonds (d) This work: TETRAD as a dynamic covalent bonding unit.

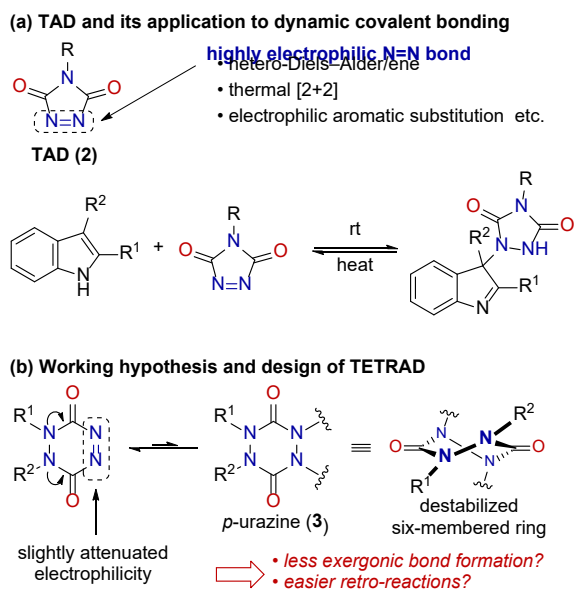


Figure 2. Design of TETRAD inspired by TAD.

unit from the stable adducts still usually require high temperatures. Diels–Alder (DA) reactions with aromatic compounds are noticeable exceptions, but irradiation with light is required for the highly endothermic forward reactions in many cases.^{9b,c,f,12a} The use of anthracene derivatives is essential for the forward reactions under thermal conditions.^{9e,12b} We speculated that the high barriers for the retro-reactions could be related to the high exergonicity of the forward reactions, and thus, the retro-reaction could be facilitated by destabilizing the products. While TAD adducts possess a stable five-membered planar 6π system, TETRAD **1** is expected to form adducts (1,2,4,5-tetrazinane-3,6-diones; *p*-urazines **3**) possessing a distorted twist-boat-like six-membered ring, which might lead to the destabilization of the adducts and decreased exergonicity of the forward reactions. In addition, we envisioned that TETRAD **1** might exhibit attenuated electrophilicity compared with that of TAD **2** because the extra nitrogen atom could be partially electron-donating to the carbonyl groups. As the violent electrophilicity of TAD **2** sometimes causes undesired side reactions and decomposition, attenuation of its electrophilicity would be advantageous for various applications (Figure 2b).

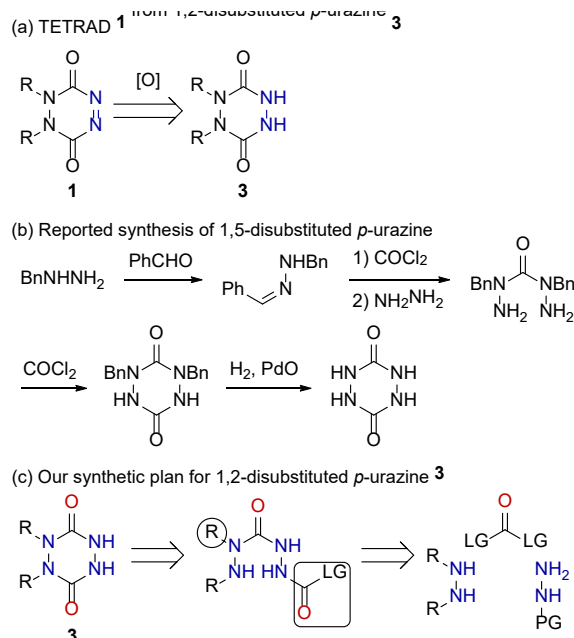
RESULTS AND DISCUSSION

Synthesis of *p*-urazines and TETRAD. Based on the synthesis of TAD **2**,⁶ we speculated that TETRAD **1** would be accessible via the oxidation of 1,2-disubstituted-1,2,4,5-tetrazinane-3,6-dione (1,2-disubstituted-*p*-urazine) **3**, although such seemingly simple compounds were not found in the literature (Scheme 1a).

To the best of our knowledge, only a few successful syntheses of *p*-urazines with other substitution patterns have been reported.¹³ In 1982, Neugebauer and Fischer reported the synthesis of 1,5-dibenzyl- and non-substituted *p*-urazines by intramolecular acylation of a carbonylhydrazone derivative and the further removal of the benzyl groups (Scheme 1b).^{13a,b} They also reported the X-ray crystal structure of 1,2,4,5-tetramethyl-*p*-urazine and revealed its twist-boat-like structure.^{13d} Importantly, they pointed out that most earlier synthetic studies of *p*-urazine derivatives since the 1880s¹⁴ had misassigned the products; the synthesized products would not be *p*-urazines, but instead their five-membered ring isomers or linear bisureas.^{13a} These previous studies suggested that the formation of the distorted six-membered ring of *p*-urazines is unfavorable compared with the competing five-membered ring formation. An appropriate cyclization precursor that cannot undergo the undesired five-membered ring formation should be prepared. Accordingly, we planned to synthesize a 1,2-disubstituted-*p*-urazine **3** via intramolecular cyclization as shown in Scheme 1c.

Initially, we examined the cyclization from an ethyl carbamate-derived intermediate (R = Bn, LG = OEt in Scheme 1c) under basic conditions, but the desired product was not obtained, probably due to the deprotonation of an acidic NH proton of the carbamate. After several attempts, we found that the cyclization reaction under neutral conditions from a more reactive intermediate (LG = imidazole) effectively provides a *p*-urazine derivative. The optimized synthetic route is shown in Scheme 2. The successive one-pot condensation of *tert*-butyl carbamate, CDI (1,1'-carbonyldiimidazole), and *N,N'*-dibenzyl hydrazine provided **4** in 54% after recrystallization. The acidic removal of the Boc-group and neutralization by aqueous K_2CO_3 furnished **5** in 95% yield. Gratifyingly, the desired 1,2-dibenzyl-*p*-urazine **3a** was obtained by sequential intermolecular and intramolecular carbonylation of **5** with CDI. The cyclization step was sluggish, but the yield was improved by removing basic imidazole via

Scheme 1. (a) TETRAD 1 from 1,2-disubstituted *p*-urazine; (b) Reported synthesis of 1,5-disubstituted *p*-urazine; (c) Our synthetic plan for 1,2-disubstituted *p*-urazine.

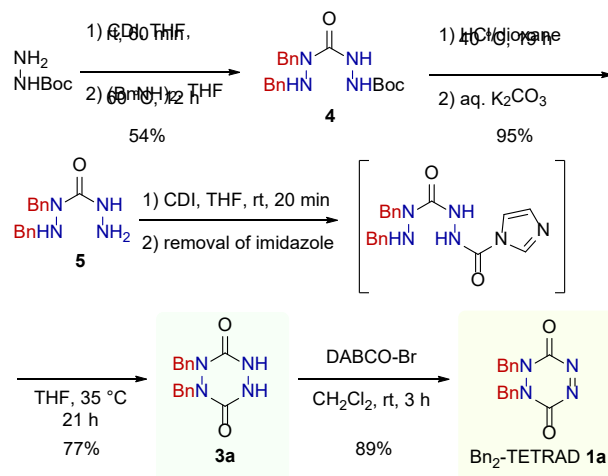


aqueous work-up soon after the first carbonylation. Essentially pure **3a** was obtained after filtration in 77% yield. As expected, the oxidation of **3a** with DABCO-Br¹⁵ proceeded smoothly, affording the desired 1,2-dibenzyl-TETRAD (Bn₂-TETRAD) **1a** as a deep-orange solid. The established synthetic route does not require any chromatographic purification and thus is scalable, which allowed further intensive studies of the reactivities and properties of **1** and **3**.

The steric and electronic effects of substituents can be important factors to control the reactivity and reversibility of TETRAD **1**, prompting us to seek rapid access to various derivatives. As **1a** and **3a** are easy to prepare and contain removable benzyl groups on the nitrogen atoms, we examined the synthesis of other derivatives using **1a** and **3a** as common intermediates (Scheme 3). Dimethylation of **3a** with MeI and subsequent debenzylation under hydrogenolysis conditions^{13a} furnished dimethyl *p*-urazine **3b**, which was then oxidized to Me₂-TETRAD **1b** in almost quantitative yield. The high electrophilicity of **1a** also enabled diversification with nucleophilic reagents. Hetero-Diels–Alder (HDA) reactions of **1a** with butadiene or 1,3-cyclohexadiene rapidly proceeded to give bicyclic *p*-urazines **7** in high yield. Hydrogenolysis of the benzyl groups of **7** along with the reduction of the olefin and subsequent oxidation afforded the TETRAD products with bicyclic backbones (**1c** and **1d**).

The structures of *p*-urazines **3a** (CCDC 2119672) and **3d** (CCDC 2119675) and TETRAD **1a** (CCDC 2119673) and **1d** (CCDC 2119676) were determined via single crystal X-ray crystallography (Figure 3). The solid-state structure of **3** adopts a twist-boat-like conformation,^{13de} probably due to the electronic repulsion between lone pairs of two adjacent nitrogen atoms, while all six atoms constituting the core ring of **1** are coplanar, suggesting stabilized π -conjugation. The lengths of the N–N bonds are summarized in Table 1. The short lengths of the reactive N–N bonds in **1a** (1.260 Å) and **1d** (1.261 Å) clearly show the double-bond character, and these bond lengths are not

Scheme 2. Chromatography-free synthesis of *p*-urazine 3a and TETRAD 1a.



strongly affected by the substituents. In contrast, the difference in the backbone N–N bond lengths of **1d** and **3d** ($\Delta = 0.062$ Å) is somewhat larger than that of **1a** and **3a** ($\Delta = 0.014$ Å). We speculate that this difference could be attributed to the enhanced strain derived from the tricyclic structure of **3d**.

Evaluation of reversibility of hetero-ene reactions of TETRAD with indole. Having synthesized several TETRAD derivatives (**1a–1d**), we next investigated the reversibility of the hetero-ene reactions of these molecules with indole **8**, because these reversible reactions had been studied in depth for TAD **2**.^{9d,10,11} The expected ene products **9** were obtained within 10 min from **1** and **8**, except for **1d**; this transformation can be easily monitored via the color change from deep-red/orange to colorless (Figure 4a). The reactions using TETRAD **1** were slightly slower than those using TAD **2**,^{11a,c} which indicates the attenuated electrophilicity of TETRAD **1**. The resulting adducts **9** were then subjected to a kinetic reversibility test according to the previously reported procedure for TAD **2**,^{11a,c} the adduct **9** in DMSO-*d*₆ was treated with a slight excess of (2*E*,4*E*)-2,4-hexadien-1-ol (HDEO, **10**), which rapidly and irreversibly traps a liberated TETRAD **1** to form a thermodynamically stable HDA adduct **11**. After heating at various temperatures for 15 minutes, the conversion of **9** and **10** to **11** was measured and used as an indicator of the reversibility (Figure 4a). As a control experiment, PhTAD **2a** and MeTAD **2b** were used for the same test in our hands.

The results are summarized in Figure 4b. The conversions (reversibility) are plotted as a function of the reaction temperatures, and thus, the plotted curve moves to the left as the reversibility increases. TETRAD **1** exhibited reversibility similar to that of TAD **2**, but the rate of the retro-reaction depends significantly on the substituents. TETRAD **1a** has the lowest reversibility, and a temperature of over 140 °C was required to achieve 50% conversion. TETRAD **1b** and tricyclic **1d** showed similar results. Whilst the reversibilities of **1a**, **1b**, and **1d** are similar to that of MeTAD **2b** and lower than that of PhTAD **2a**, bicyclic TETRAD **1c** exhibited slightly greater reversibility than **2a**. Although the reversibility using TETRAD **1a–1d** with indole **8** is not significantly different from that for TAD **2**, the large substituent effects observed imply the potentially tunable nature of TETRAD **1**. We speculated that the bicyclic structure of **1c** would further destabilize the adduct **11** by increasing the strain and thus improve the reversibility, whilst the more strained

Scheme 3. Derivatization of *p*-urazines **3 and TETRAD **1**.**

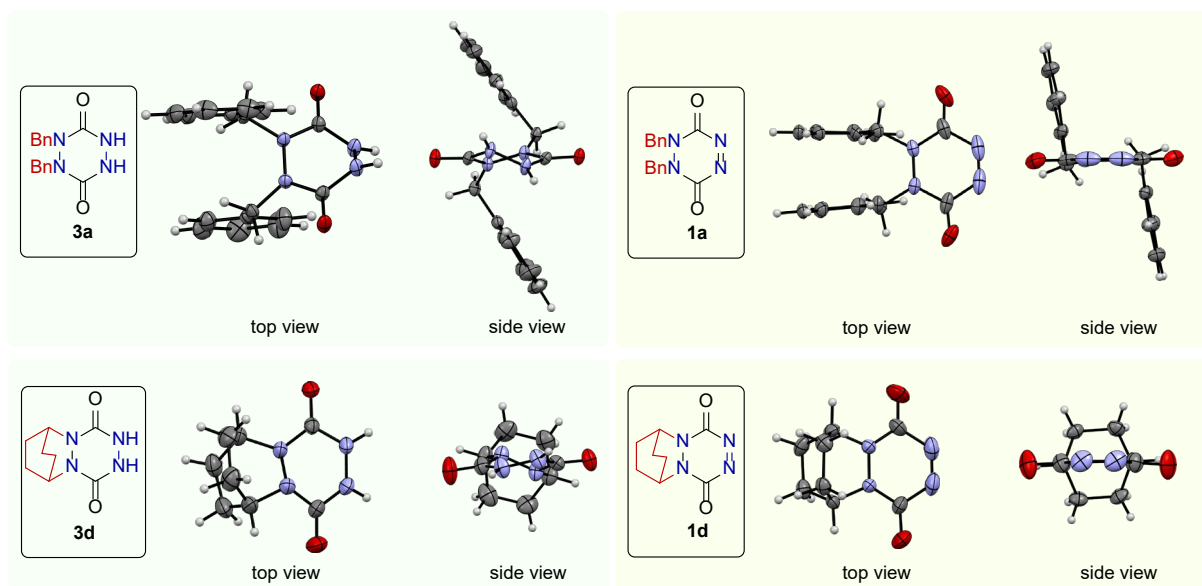
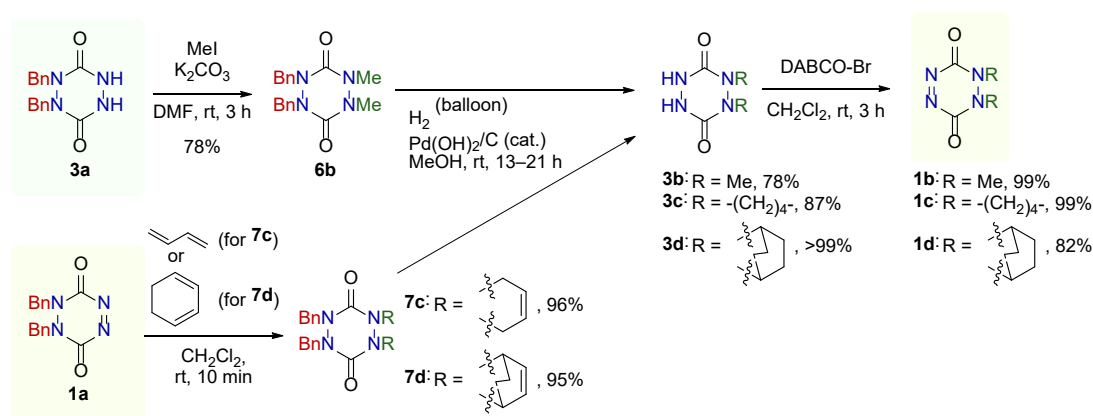
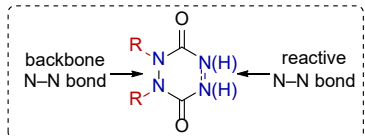


Figure 3. X-ray crystal structures of **3a**, **1a**, **3d**, and **1d** drawn with thermal ellipsoids at 50% probability.

Table 1. N–N bond lengths in X-ray structures of **1 and **3****

	Backbone N–N bond (Å)		Reactive N–N bond (Å)	
	3	1	3	1
a	1.410	1.396	1.426	1.260
d	1.446	1.384	1.396	1.261



tricyclic structure of **1d** would destabilize both the adduct and **1d** itself.

Reversible hetero-Diels-Alder reaction with furan. With the promising properties of TETRAD **1** as dynamic covalent bonding units in hand, we next examined their HDA reactions with furan. DA reactions of furan with maleimides are well-known reversible processes and are widely used in dynamic covalent bonding, especially for polymer materials.^{16,17} Generally, most of the forward reactions proceed within several minutes to hours

under ambient conditions around room temperature to 50 °C, and the retro-reactions proceed on the same time scale when heated to over 90 °C. We envisioned that faster and more reversible HDA reactions of a furan could be achieved using TETRAD **1** as the dienophiles. In addition, the HDA reactions using TETRAD **1** would not afford diastereomers unlike those using maleimides, which simplifies the overall system.

The HDA and retro-HDA reactions of **1** and 2,5-dimethylfuran **12a** were examined using a protocol similar to that for the above-mentioned ene reactions, and the results are summarized in Table S2 and Figure 5. The forward HDA reactions were monitored using ¹H NMR at 10, 60, and 120 min (T1) after mixing **1** and **12a** in C₆D₆. After 80 min (T1), HDEO **10** was added to a portion of the reaction mixture, and the concentration of each component was monitored using ¹H NMR after 15, 30, and 90 min (T2), observing the retro-HDA reaction of **13** to regenerate **12a** with the irreversible formation of **11** (Figure 5b). All the reactions and analyses were performed at ambient temperature (22±2 °C). The initial concentration for **1a** and **1c** was 10 mM, while that for **1c** and **1d** was 2.5 mM due to their limited solubility. In the forward reactions, the ratio of **12a**, **1**, and **13** remains almost unchanged from 10 min to 120 min, indicating

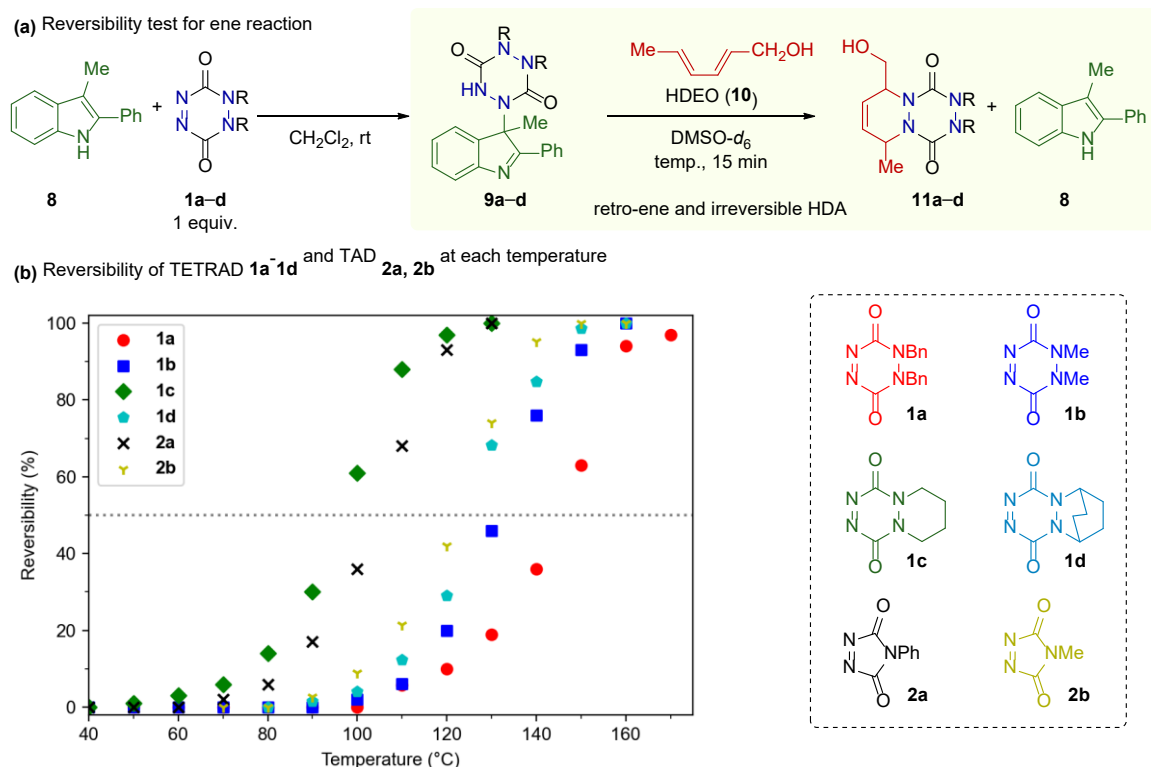


Figure 4. Evaluation of the reversibility of hetero-ene reactions of TETRAD **1** or TAD **2** and indole **8**.^{11a,c}

that the reaction systems reach thermodynamic equilibrium within 10 min in all cases. On the other hand, the equilibrium constant varies significantly depending on the substituents on **1**. The experimental Gibbs free energy changes of the reactions were calculated using the concentrations at T1 = 60 min (T2 = 0 min in Figure 5b) and are shown in Table 2. The benzyl substituents stabilize the adduct (**1a/13aa**), while the tricyclic structure leads to destabilization (**1d/13ad**). To our delight, after the addition of HDEO **10**, the concentration of **13** smoothly decreased with a concomitant increase in those of **11** and **12a** (Figure 5b), confirming the rapid retro-HDA reaction from **13**. It is noteworthy that even when using **1a**, which afforded the most stable adduct (exp. $\Delta G_r^\circ = -6.0$ kcal/mol) among **1a–1d**, most of the adduct **13ad** underwent the retro-reaction and was trapped by **10** within 90 min (T2) at room temperature. We observed some degradation when **1c** was used, leading to a bad mass balance. We also performed DFT calculations for these HDA reactions, which found two possible concerted transition states, i.e., *endo*- and *exo*-TS. The *endo*-TS is lower in energy than *exo*-TS in all cases (Table 2, calc. ΔG^\ddagger). The calculated ΔG_r° and activation energies for the forward and retro-reactions are reasonable compared with experimental observations, although the exergonicity for the forward reactions are slightly overestimated, especially for **1b** and **1c**. The DFT studies also suggest that **13ad** is significantly destabilized compared to other adducts. The calculated backbone N–N bond length of **13ad** (1.420 Å) is greater than those of the other adducts (**13aa** : 1.395 Å; **13ab** : 1.393 Å; **13ac** : 1.394 Å). This difference is similar to that observed in the X-ray structures of **1** and **3**, as discussed above, and we speculate that the enhanced strain derived from the tricyclic structure of **13ad** might lead to its destabilization.

We also experimentally and computationally examined the reactivity of PhTAD **2a**, MeTAD **2b**, and *N*-Me-maleimide with **16** for comparison with that of **1** under the same conditions (Scheme 4). Although **2a** and **2b** provided the corresponding HDA adducts **14** at the initial point, some unidentified byproducts were observed and increased with prolonged reaction time, resulting in a complex mixture after 60 min (**14**, <5%), probably due to the violent electrophilicity of TAD **2** (Scheme 4a).¹⁸ Further addition of HDEO **10** did not form the corresponding adducts **15**, and the concentration of **12a** did not increase, indicating the some irreversible reactions between **2** and **12a**. In particular, the reaction using **2a** gradually formed an insoluble, probably polymeric material. Unexpectedly, the calculated exergonicities for the reactions of **2** and **12a** are moderate and similar to those for **1** and **12a**. Therefore, we concluded that an important feature of **1** in terms of successful reversible HDA reaction was the attenuated electrophilicity. Additionally, *N*-methylmaleimide **16** did not react with **12a** under these mild reaction conditions (Scheme 4b). Our DFT calculations may slightly underestimate the stability of the adducts in this case,¹⁹ but the higher calculated activation barrier for maleimide **16** compared to those for **1** and **2** reflects the experimentally observed lower reactivity.

The same experiments using **1a** and non-substituted furan **12b** or 2-methylfuran **12c** were performed (see Supporting Information for details). Furan **12b** exhibited a reactivity similar to that of **12a**, albeit in slightly lower conversion at the equilibrium point. On the other hand, when using **12c**, several unidentified side reactions competed and the reaction profile was complicated.

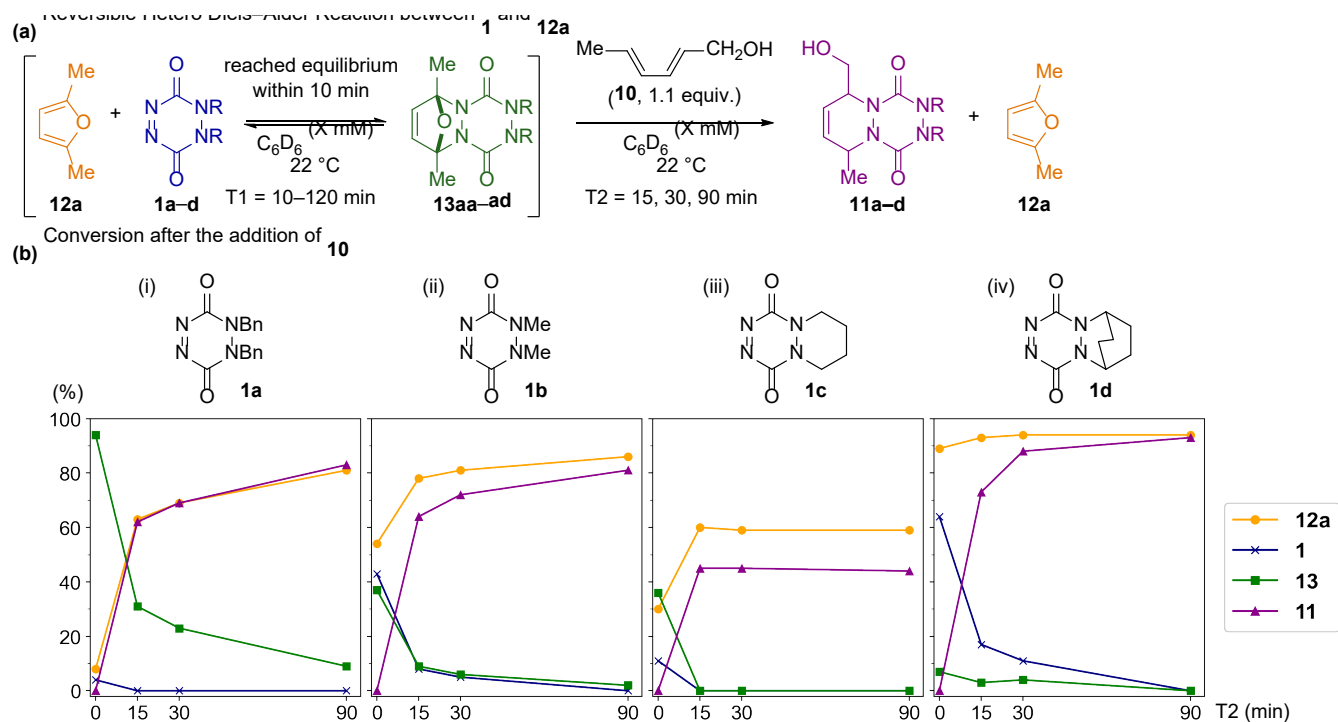


Figure 5. (a) Reversible hetero Diels–Alder (HDA) reactions between TETRAD **1** and 2,5-dimethylfuran **12a**. X = 10 for **1a** and **1c**, 2.5 for **1b** and **1d**. HDEO **10** was added 80 min (T1) after mixing **1** and **12a**. For more details, see Table S2 in the Supporting Information. (b) Yields of each component after the addition of **10**. The values at T2 = 0 min are those observed before the addition of **10** (T1 = 60 min).

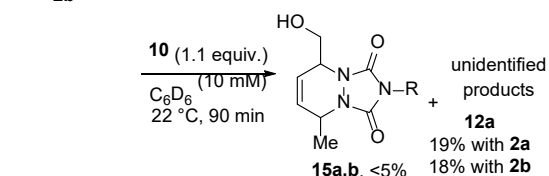
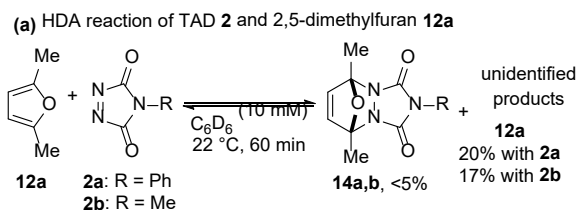
Table 2. Experimental and calculated Gibbs free energies of the of the HDA reactions between **1** and **12a** in Figure 5.

1	exp. $\Delta G_r^{o a}$ (kcal/mol)	calc. $\Delta G_r^{o b,c}$ (kcal/mol)	calc. $\Delta G_{endo}^{\ddagger b}$ (kcal/mol)	calc. $\Delta G_{exo}^{\ddagger b}$ (kcal/mol)
1a	-6.0	-7.2	+16.2	+27.0
1b	-3.8	-6.3	+16.6	+27.8
1c	-4.1	-5.7	+19.7	+27.7
1d	-2.3	-2.7	+18.7	+29.8

^aCalculated using the results at T1 = 60 min in Figure 5 and Table S2 (T = 295.15 K). ^bCalculated at the M06-2X/def2-TZVPP+SMD(benzene)/M06-2X/def2-SVP+SMD(benzene) level of theory (T = 295.15 K). ^cCalculated as the difference between the Gibbs energy of the starting materials and that of the more stable conformer of the adduct among the two conformational isomers that are formed from the *endo* or *exo* transition states.

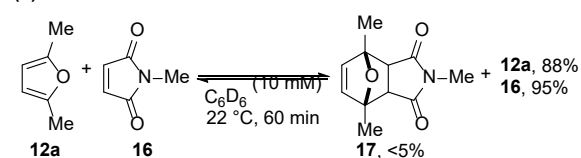
Reversible sol–gel transition of polyurethane at room temperature. Based on the fundamental studies of the reversible HDA reactions of TETRAD **1** with a furan, we designed a new functional polymer that undergoes fast sol–gel transition under neutral conditions at room temperature in response to a chemical stimulus, i.e., the addition of a furan (Figure 6a). Goussé, Gandini, and Hodge reported polystyrene gels cross-linked by a maleimide/furan DA reaction, which underwent de-cross-linking by heating at 130 °C for 3–48 h in the presence of 2-methylfuran via a retro-DA reaction and DA reaction with 2-methylfuran.²⁰ We envisioned that fast cross-linking and de-cross-linking at room temperature with no heating, no light, and no pH change could be realized by using TETRAD **1** as the cross-linking unit, and that the cross-linking and de-cross-linking could be controlled by the addition/removal of a volatile furan derivative that reversibly caps the cross-linking moiety.

Scheme 4. HDA reactions using TAD **2** and maleimide **16**



	calc. ΔG_r^{\ddagger}	calc. $\Delta G_{endo}^{\ddagger}$	calc. $\Delta G_{exo}^{\ddagger}$
2a : R = Ph	-6.2	+12.5	+20.5
2b : R = Me	-6.1	+13.2	+21.3

(b) HDA reaction of *N*-methylmaleimide **16** and 2,5-dimethylfuran **12a**



	calc. ΔG_r^{\ddagger}	calc. ΔG^{\ddagger}
<i>endo</i> adduct	+1.8	+26.3
<i>exo</i> adduct	+1.3	+27.5

For this purpose, we synthesized a polyurethane bearing a 2,5-dialkylfuran unit (**PU-furan 18**, $M_n \sim 5.1 \times 10^4$) and cross-linking reagent **19** (Figure 6a, see the Supporting Information for

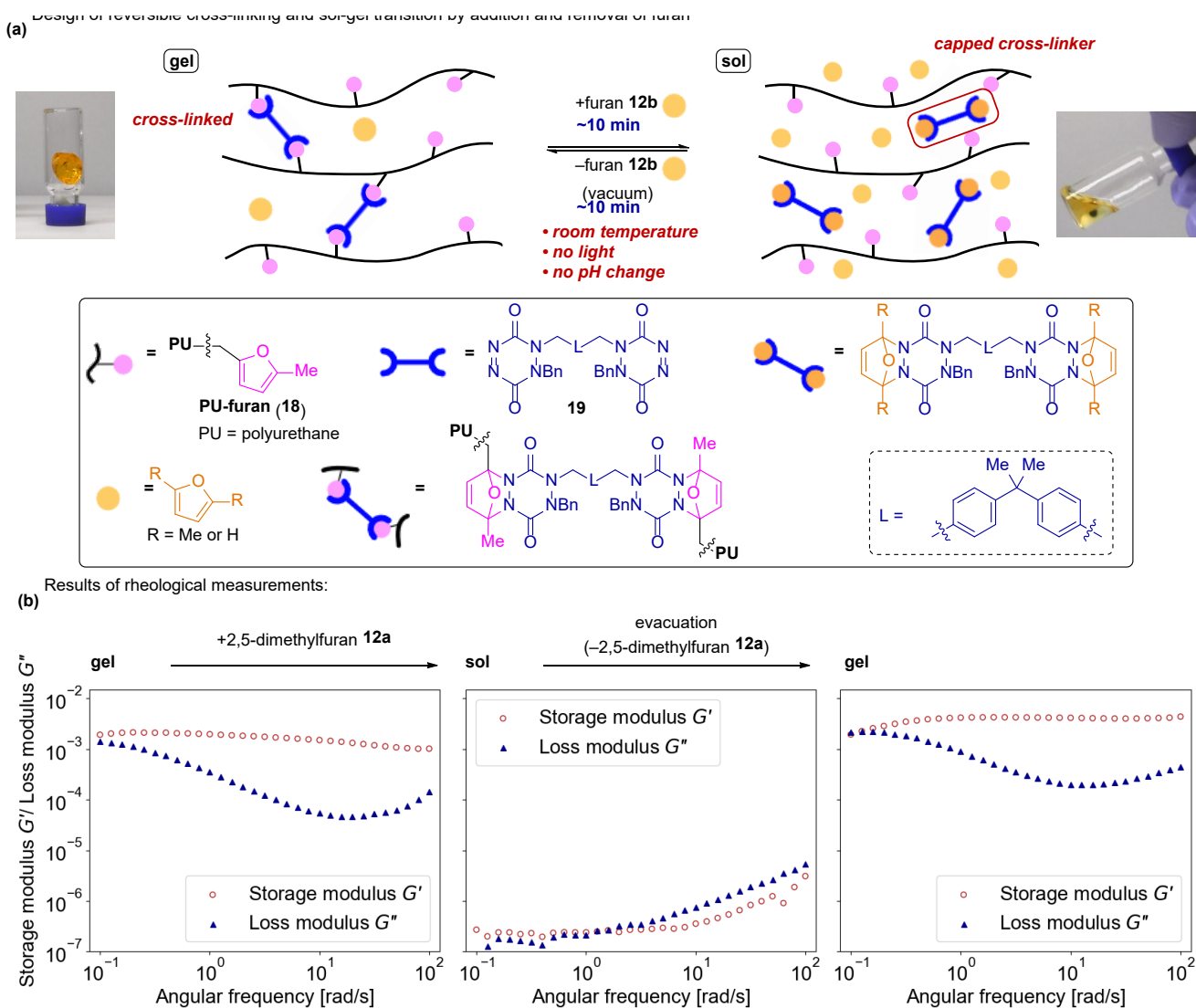


Figure 6. (a) Reversible cross-linking and sol–gel transition based on the HDA reaction of TETRAD and furan. (b) Results of rheological measurements.

details). Before the cross-linking studies, we confirmed that the reversible HDA reaction was viable for the synthesized polymer. Namely, the treatment of PU-furan **18** with Bn₂-TETRAD **1a** led to changes in the chemical shifts of the ¹H NMR signals corresponding to the desired HDA reaction, and the subsequent addition of HDEO caused the formation of **11a** and the original PU-furan **18** (see the Supporting Information for details).

Then, we conducted a reversible sol–gel transition experiment using PU-furan **18** and **19**. Although the direct reaction of **18** and **19** in DMF afforded a gel, an extremely fast cross-linking reaction irreproducibly provided a heterogeneous mixture. Therefore, the cross-linking reagent **19** was first capped by furan **12b** and mixed with **18** to give a solution. When this solution was moderately evacuated at ambient temperature to remove furan **12b**, it gradually transformed into a gel. After the gelation, the addition of an excess of **12b** again afforded a solution. This sol–gel transition triggered by the addition/removal of **12b** proceeded in about 10 min and was successfully repeated (see the Supporting Information).²¹ To confirm the sol–gel transition, the rheological properties (dynamic viscoelasticity) were measured for both the sol and gel states. We used 2,5-dimethylfuran **12a** (b.p. 92–94 °C) instead of non-substituted furan **12b**

as the capping reagent for the rheological measurements to avoid gelation due to the spontaneous volatilization of **12b** (b.p. 31 °C) during the measurements (see the Supporting Information for details). The results are shown in Figure 6b. In the presence of **12a** (sol), the *G'* and *G''* values were similar and exhibited some frequency dependency, indicating their properties as a concentrated solutions. On the other hand, before the addition or after the removal of **12a** (gel), the *G'* value was much higher than the *G''* value and exhibited little dependence on the frequency, which corresponds to gel behavior. In addition, the *G'* and *G''* were several orders of magnitude greater than those in the presence of **12a**. In the low angular-frequency region, the *G''/G'* (tanδ) became large. We speculate that this is due to the fast exchange of the covalent cross-linking bonds via the retro-HDA and HDA reactions, which could follow the given stress and allow the structural change of the material at low frequency.

CONCLUSIONS

In summary, we have synthesized and characterized 1,2-disubstituted-1,2-dihydro-1,2,4,5-tetrazine-3,6-dione derivatives (TETRAD **1**) with different substituents and revealed their

properties as dynamic covalent bonding units. TETRAD **1** exhibited moderately reversible reactivity in hetero-ene reactions with 2-phenyl-3-methylindole **8** similarly to TAD **2**. Furthermore, hetero-Diels–Alder (HDA) reactions of **1** with a furan and the corresponding retro-reactions rapidly proceeded at room temperature to enable efficient dynamic covalent bonding under mild conditions. Based on this reversible reaction, we developed a reversible sol–gel transition system that consists of a polyurethane bearing furan units (PU-furan **18**) and a cross-linking reagent containing two TETRAD moieties (**19**). The sol–gel transition was triggered and controlled by the addition/removal of an external furan additive and proceeded smoothly without heating, reflecting the high reversibility of the TETRAD/furan HDA reactions. This process does not require drastic pH change, high temperature, or irradiation with light, and thus could be potentially useful in applications in which such harsh reaction conditions are not appropriate, e.g. applications involving biomolecules or household utilization. Further studies on the fundamental reactivities of TETRAD **1**, as well as their applications, are now ongoing in our group.²²

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

Experimental details, characterization data, and copies of NMR spectra (PDF)

Crystallographic Information (CIF)

Cartesian coordinates of calculated structures (XYZ)

Movie of a sol-gel transition experiment (3GP)

AUTHOR INFORMATION

Corresponding Authors

Tatsuhiko Yoshino – Faculty of Pharmaceutical Science, Hokkaido University, Sapporo 060-0812, Japan; Global Station for Biosurfaces and Drug Discovery, Hokkaido University, Sapporo 060-0812, Japan; orcid.org/0000-0001-9441-9272; Email: tyoshino@pharm.hokudai.ac.jp

Shigeki Matsunaga – Faculty of Pharmaceutical Science, Hokkaido University, Sapporo 060-0812, Japan; Global Station for Biosurfaces and Drug Discovery, Hokkaido University, Sapporo 060-0812, Japan; orcid.org/0000-0003-4136-3548; Email: smatsuna@pharm.hokudai.ac.jp

Authors

Kentaro Kawai – Faculty of Pharmaceutical Science, Hokkaido University, Sapporo 060-0812, Japan

Akane Sato – Faculty of Pharmaceutical Science, Hokkaido University, Sapporo 060-0812, Japan

Kazuki Ikeda – Faculty of Pharmaceutical Science, Hokkaido University, Sapporo 060-0812, Japan

Akira Kabasawa – Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo 060-0810, Japan; Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan

Masahiro Kojima – Faculty of Pharmaceutical Science, Hokkaido University, Sapporo 060-0812, Japan; orcid.org/0000-0002-4619-2621

Kenta Kokado – Research Institute for Electronic Science, Hokkaido University, Sapporo, 001-0020, Japan; orcid.org/0000-0003-3880-9094

Akira Kakugo – Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo 060-0810, Japan; Faculty

of Science, Hokkaido University, Sapporo 060-0810, Japan; orcid.org/0000-0002-1591-867X

Kazuki Sada – Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo 060-0810, Japan; Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan; orcid.org/0000-0001-7348-0533

ACKNOWLEDGMENT

This work was supported in part by JSPS KAKENHI Grant Number 20H04796 (K.S.) 20H04794 (T.Y.) in Hybrid Catalysis.

REFERENCES

- (1) (a) Jin, Y.; Yu, C.; Denman, R. J.; Zhang, W. Recent advances in dynamic covalent chemistry. *Chem. Soc. Rev.* **2013**, *42*, 6634-6654. (b) Herrmann, A. Dynamic combinatorial/covalent chemistry: A tool to read, generate and modulate the bioactivity of compounds and compound mixtures. *Chem. Soc. Rev.* **2014**, *43*, 1899-1933. (c) Zhang, W.; Jin, Y. *Dynamic Covalent Chemistry: Principles, Reactions, and Applications*, Wiley-VCH, Weinheim, 2017.
- (2) (a) Zhang, Z. P.; Rong, M. Z.; Zhang, M. Q. Polymer engineering based on reversible covalent chemistry: A promising innovative pathway towards new materials and new functionalities. *Prog. Polym. Sci.* **2018**, *80*, 39-93. (b) Sun, H.; Kabb, C. P.; Sims, M. B.; Sumerlin, B. S. Architecture-transformable polymers: Reshaping the future of stimuli-responsive polymers. *Prog. Polym. Sci.* **2019**, *89*, 61-75. (c) Chakma, P.; Konkolewicz, D. Dynamic covalent bonds in polymeric materials. *Angew. Chem., Int. Ed.* **2019**, *58*, 9682-9695. (d) Jin, Y.; Lei, Z.; Taynton, P.; Huang, S.; Zhang, W. Malleable and Recyclable Thermosets: The Next Generation of Plastics. *Matter* **2019**, *1*, 1456-1493.
- (3) (a) Feng, X.; Ding, X.; Jiang, D. Covalent organic frameworks. *Chem. Soc. Rev.* **2012**, *41*, 6010-6022. (b) Diercks, C. S.; Yaghi, O. M. The atom, the molecule, and the covalent organic framework. *Science* **2017**, *355*, eaal1585. (c) Lohse, M. S.; Bein, T. Covalent organic frameworks: Structures, synthesis, and applications. *Adv. Funct. Mater.* **2018**, *28*, 1705553.
- (4) (a) Mondal, M.; Hirsch, A. K. H. Dynamic combinatorial chemistry: A tool to facilitate the identification of inhibitors for protein targets. *Chem. Soc. Rev.* **2015**, *44*, 2455-2488. (b) Bandyopadhyay, A.; Gao, J. Targeting biomolecules with reversible covalent chemistry. *Curr. Opin. Chem. Biol.* **2016**, *34*, 110-116. (c) Ulrich, S. Growing prospects of dynamic covalent chemistry in delivery applications. *Acc. Chem. Res.* **2019**, *52*, 510-519.
- (5) (a) Jin, Y.; Wang, Q.; Taynton, P.; Zhang, W. Dynamic Covalent Chemistry Approaches Toward Macrocycles, Molecular Cages, and Polymers. *Acc. Chem. Res.* **2014**, *47*, 1575-1586. (b) Hasell, T.; Cooper, A. I. Porous organic cages: soluble, modular and molecular pores. *Nat. Rev. Mater.* **2016**, *1*, 16053. (c) Huang, S.; Lei, Z.; Jin, Y.; Zhang, W. By-design molecular architectures via alkyne metathesis. *Chem. Sci.* **2021**, *12*, 9591-9606.
- (6) Cookson, R. C.; Gilani, S. S. H.; Stevens, I. D. R. 4-Phenyl-1,2,4-triazolin-3,5-dione: A powerful dienophile. *Tetrahedron Lett.* **1962**, *3*, 615-618. (b) Korobitsyna, I. K.; Khalikova, A. V.; Rodina, L. L.; Shusherina, N. P. 4-Phenyl-1,2,4-triazoline-3,5-dione in organic synthesis (review). *Chem. Heterocycl. Comp.* **1983**, *19*, 117-136. (c) De Bruycker, K.; Billiet, S.; Houck, H. A.; Chattopadhyay, S.; Winne, J. M.; Du Prez, F. E. Triazolinediones as highly enabling synthetic tools. *Chem. Rev.* **2016**, *116*, 3919-3974.
- (7) For selected recent examples, see: (a) Vandewalle, S.; Billiet, S.; Driessen, F.; Du Prez, F. E. Macromolecular coupling in seconds of triazolinedione end-functionalized polymers prepared by RAFT polymerization. *ACS Macro Lett.* **2016**, *5*, 766-771. (b) Becker, G.; Vlaminck, L.; Velencoso, M. M.; Du Prez, F. E.; Wurm, F. R. Triazolinedione-“clicked” poly(phosphoester): Systematic adjustment of thermal properties. *Polym. Chem.* **2017**, *8*, 4074-4078. (c) Chen, J.; Sun, R.; Liao, X.; Han, H.; Li, Y.; Xie, M. Tandem metathesis polymerization-induced self-assembly to nanostructured block copolymer and the controlled triazolinedione modification for enhancing dielectric properties. *Macromolecules* **2018**, *51*, 10202-10213. For other examples, see ref. 6c.

- (8) (a) Ban, H.; Gavrilyuk, J.; Barbas III, C. F. Tyrosine bioconjugation through aqueous ene-type reactions: A click-like reaction for tyrosine. *J. Am. Chem. Soc.* **2010**, *132*, 1523-1525. (b) Naik, A.; Alzeer, J.; Triemer, T.; Bujalska, A.; Luedtke, N. W. Chemoselective modification of vinyl DNA by triazolinediones. *Angew. Chem., Int. Ed.* **2017**, *56*, 10850-10853. (c) Wilke, P.; Kunde, T.; Chattopadhyay, S.; ten Brummelhuis, N.; Du Prez, F. E.; Börner, H. G. Easy access to triazolinedione-encapped peptides for chemical ligation. *Chem. Commun.* **2017**, *53*, 593-596. (d) Alvarez-Dorta, D.; Thobie-Gautier, C.; Croyal, M.; Bouzelha, M.; Mével, M.; Deniaud, D.; Boujtita, M.; Gouin, S. G. Electrochemically promoted tyrosine-click-chemistry for protein labeling. *J. Am. Chem. Soc.* **2018**, *140*, 17120-17126. For a related study, see: (e) Sato, S.; Nakamura, K.; Nakamura, H. Tyrosine-Specific Chemical Modification with *in Situ* Hemin-Activated Luminol Derivatives. *ACS Chem. Biol.* **2015**, *10*, 2633-2640.
- (9) (a) Seymour, C. A.; Greene, F. D. Mechanism of triazolinedione-olefin reactions. Ene and cycloaddition. *J. Am. Chem. Soc.* **1980**, *102*, 6384-6385. (b) Kjell, D. P.; Sheridan, R. S. Photochemical cycloaddition of *N*-methyltriazolinedione to naphthalene. *J. Am. Chem. Soc.* **1984**, *106*, 5368-5370. (c) Hamrock, S. J.; Sheridan, R. S. Para photoaddition of *N*-methyltriazolinedione to benzene. Synthesis of energy-rich azo compounds comprising benzene + nitrogen. *J. Am. Chem. Soc.* **1989**, *111*, 9247-9249. (d) Baran, P. S.; Guerrero, C. A.; Corey, E. J. The first method for protection-deprotection of the indole 2,3- π bond. *Org. Lett.* **2003**, *5*, 1999-2001. (e) Roy, N.; Lehn, J.-M. Dynamic covalent chemistry: A facile room-temperature, reversible, Diels-Alder reaction between anthracene derivatives and *N*-phenyltriazolinedione. *Chem. Asian J.* **2011**, *6*, 2419-2425. (f) Southgate, E. H.; Pospech, J.; Fu, J.; Holycross, D. R.; Sarlah, D. Dearomative dihydroxylation with arenophiles. *Nat. Chem.* **2016**, *8*, 922-928. (g) Matsuoka, W.; Ito, H.; Sarlah, D.; Itami, K. Diversity-oriented synthesis of nanographenes enabled by dearomative annulative π -extension. *Nat. Commun.* **2021**, *12*, 3940.
- (10) Baran, P. S.; Guerrero, C. A.; Corey, E. J. Short, enantioselective total synthesis of okaramine N. *J. Am. Chem. Soc.* **2003**, *125*, 5628-5629.
- (11) (a) Billiet, S.; De Bruycker, K.; Driessen, F.; Goossens, H.; Van Speybroeck, V.; Winne, J. M.; Du Prez, F. E. Triazolinediones enable ultrafast and reversible click chemistry for the design of dynamic polymer systems. *Nat. Chem.* **2014**, *6*, 815-821. (b) Roling, O.; De Bruycker, K.; Vonhören, B.; Stricker, L.; Körsgen, M.; Arlinghaus, H. F.; Ravoo, B. J.; Du Prez, F. E. Rewritable polymer brush micropatterns grafted by triazolinedione click chemistry. *Angew. Chem., Int. Ed.* **2015**, *54*, 13126-13129. (c) Houck, H. A.; De Bruycker, K.; Billiet, S.; Dhanis, B.; Goossens, H.; Catak, S.; Van Speybroeck, V.; Winne, J. M.; Du Prez, F. E. Design of a thermally controlled sequence of triazolinedione-based click and transclick reactions. *Chem. Sci.* **2017**, *8*, 3098-3108. (d) Houck, H. A.; De Bruycker, K.; Barner-Kowollik, C.; Winne, J. M.; Du Prez, F. E. Tunable blocking agents for temperature-controlled triazolinedione-based cross-linking reactions. *Macromolecules* **2018**, *51*, 3156-3164. (e) Van Herck, N.; Du Prez, F. E. Fast healing of polyurethane thermosets using reversible triazolinedione chemistry and shape-memory. *Macromolecules* **2018**, *51*, 3405-3414.
- (12) Applications of DA reactions of TAD with aromatic compounds in polymer chemistry: (a) Houck, H. A.; Blasco, E.; Du Prez, F. E.; Barner-Kowollik, C. Light-stabilized dynamic materials. *J. Am. Chem. Soc.* **2019**, *141*, 12329-12337. (b) Mondal, P.; Jana, G.; Behera, P. K.; Chattaraj, P. K.; Singha, N. K. A new healable polymer material based on ultrafast Diels-Alder 'click' chemistry using triazolinedione and fluorescent anthracyl derivatives: A mechanistic approach. *Polym. Chem.* **2019**, *10*, 5070-5079.
- (13) (a) Neugebauer, F. A.; Fischer, H. 1,2,4,5-Tetrahydro-1,2,4,5-tetrazin-3,6-dion (*p*-Urazin) und seine Umwandlung in 1,2,4,5-Tetrazinderivate. *Liebigs Ann. Chem.* **1982**, 387-395. (b) Neugebauer, F. A.; Fischer, H.; Siegel, R.; Krieger, C. 2,4-Dialkylsubstituierte Carbono- und Thiocarbonohydrazide, Reaktionen mit Carbonylverbindungen. *Chem. Ber.* **1983**, *116*, 3461-3481. (c) Neugebauer, F. A.; Fischer, H.; Krieger, C. 1,4-Dimethyl-3,6-dioxo-1,2,4,5-tetrazin-1-ium-5(4H)-id, a new six-membered heterocyclic betaine. *Tetrahedron Lett.* **1984**, *25*, 629-632. (d) Nelsen, S. F.; Kim, Y.; Neugebauer, F. A.; Krieger, C.; Siegel, R.; Kaftory, M. Tetraalkyl-*p*-urazines and their cation radicals. *J. Org. Chem.* **1991**, *56*, 1045-1051. (e) Mills, A. M.; Wu, J.-Z.; Bouwman, E.; Reedijk, J.; Spek, A. L. 1,5-Dimethyl-1,2,4,5-tetrazinane-3,6-dione. *Acta Cryst. E* **2004**, *60*, o2485-o2487.
- (14) For selected examples of earlier synthetic studies on *p*-urazines, see: (a) Pinner, A. Einwirkung von Harnstoff auf Hydrazine. *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 1219-1227. (b) Curtius, T.; Heidenreich, K. Hydrazide und Azide organischer Säuren. XI Abhandlung. 36. Die Hydrazide und Azide der Kohlensäure. *J. Prakt. Chem.* **1895**, *52*, 454-489. (c) Busch, M. Die Constitution der Urazine. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 2311-2320. (d) Stolle, R. 69. Über die Überführung von Hydrazinabkömmlingen in Heterocyclische Verbindungen. *J. Prakt. Chem.* **1907**, *75*, 416-432. (e) Lutz, A. W. "p-Urazine" and "Dithio-p-urazine." *J. Org. Chem.* **1964**, *29*, 1174-1177.
- (15) Zolfigol, M. A.; Chehardoli, G.; Ghaemi, E.; Madrakian, E.; Zare, R.; Azadbakht, T.; Niknam, K.; Mallakpour, S. *N*-Bromo reagent mediated oxidation of urazoles to their corresponding triazolinediones under mild and heterogeneous conditions. *Monatsh. Chem.* **2008**, *139*, 261-265.
- (16) For reviews, see: (a) Tasdelen, M. A. Diels-Alder "click" reactions: recent applications in polymer and material science. *Polym. Chem.* **2011**, *2*, 2133-2145. (b) Gandini, A. The furan/maleimide Diels-Alder reaction: A versatile click-unclick tool in macromolecular synthesis. *Prog. Polym. Sci.* **2013**, *38*, 1-29. See also refs. 1 and 2.
- (17) For selected examples, see: (a) Chen, X.; Dam, M. A.; Ono, K.; Mal, A.; Shen, H.; Nutt, S. R.; Sheran, K.; Wudl, F. A thermally remendable cross-linked polymeric material. *Science* **2002**, *295*, 1698-1702. (b) Asadiriad, A. M.; Boutault, S.; Erno, Z.; Branda, N. R. Controlling a polymer adhesive using light and a molecular switch. *J. Am. Chem. Soc.* **2014**, *136*, 3024-3027. (c) Sun, H.; Kabb, C. P.; Dai, Y.; Hill, M. R.; Ghiviriga, L.; Bapat, A. P.; Sumerlin, B. S. Macromolecular metamorphosis via stimulus-induced transformations of polymer architecture. *Nat. Chem.* **2017**, *9*, 817-823. (d) Kida, J.; Imato, K.; Goseki, R.; Aoki, D.; Morimoto, M.; Otsuka, H. The photoregulation of a mechanochemical polymer scission. *Nat. Commun.* **2018**, *9*, 3504.
- (18) Madder and co-workers reported an irreversible electrophilic aromatic substitution reaction of a peptide with a furan moiety and PhTAD **2a**. Hoogewijs, K.; Buyst, D.; Winne, J. M.; Martins, J. C.; Madder, A. Exploiting furan's versatile reactivity in reversible and irreversible orthogonal peptide labeling. *Chem. Commun.* **2013**, *49*, 2927-2929.
- (19) Boutelle, R. C.; Northrop, B. H. Substituent Effects on the Reversibility of Furan-Maleimide Cycloadditions. *J. Org. Chem.* **2011**, *76*, 7994-8002.
- (20) Goussé, C.; Gandini, A.; Hodge, P. Application of the Diels-Alder reaction to polymers bearing furan moieties. 2. Diels-Alder and retro-Diels-Alder reactions involving furan rings in some styrene copolymers. *Macromolecules* **1998**, *31*, 314-321.
- (21) The sol-gel transition could be repeated for five times, but the sixth removal of furan led to the irreversible gelation, possibly due to some competing side reactions to form non-dynamic covalent bonds.
- (22) TETRAD **1a** underwent ene-reactions with alkenes. Amines reacted with **1a**, resulting in a complex mixture. **1a** was not decomposed when treated with CH₂Cl₂/H₂O at room temperature, while MeTAD **2b** was completely decomposed under the same conditions. For details of these experiments, see the Supporting Information.

