The final authenticated version is available online at: https://doi.org/10.1039/D2OB01834A

COMMUNICATION

Immobilization of a flavin molecule onto poly(methacrylic acid)s and its application in aerobic oxidation catalysis: effect of polymer stereoregularity

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Yukihiro Arakawa,* Yoshiko Sogabe, Keiji Minagawa, Miyuki Oshimura, Tomohiro Hirano, Koichi Ute and Yasushi Imada*

The isoalloxazine ring system, called flavin, was successfully immobilized on poly(methacrylic acid)s, PMAAs, with different tacticity via post-polymerization modification under suitable conditions. The resulting flavin-containing polymers showed catalytic activities for aerobic oxidation reactions, in which the polymer stereoregularity clearly influenced their catalysis.

Flavins are molecules that contain the heterocyclic isoalloxazine ring system found in many biologically important compounds such as flavin cofactors in a flavoenzyme. The concept of mimicking the catalytic function of flavoenzymes with a simple flavin molecule is highly attractive since it allows in principle catalytic aerobic oxidation in an environmentally friendly manner without utilizing scarce resources, such as rare metals, and producing toxic wastes.[1] We have pioneered some flavin-catalyzed aerobic oxidation reactions including sulfoxidation,[2a,2e,2g,2i] amine oxidation,[2a,2e] Baeyer-Villiger reaction,[2b,2g,2h] and hydrazine oxidation-triggered olefin hydrogenation,[2c,2d,2f] and recently extended our attention to the immobilization of flavin catalysts onto synthetic polymers to improve their practicability as well as catalysis. For example, we reported highly reusable poly(styrene-*co*-divinylbenzene)-supported flavin catalysts for the aerobic reduction of olefins, which was the first example of immobilizing a flavin catalyst onto insoluble supports via covalent bond.[3a] We also introduced a flavin-containing mesoporous network polymer, poly(riboflavin tetramethacrylate-*co*-ethylene glycol dimethacrylate), that exhibited high catalytic activities comparable with low-molecular-weight flavin catalysts in aerobic reduction of olefins as well as photooxidation of benzyl alcohols.[3b] In addition, we achieved the first *N*5-unmodified neutral flavin-catalyzed aerobic oxygenations by means of the polystyrene resin-supported flavopeptide catalyst, in which both the peptide moiety theoretically designed and the resin played a crucial role in stabilizing a key active species, 4a-hydroperoxide of the flavin ring system.[2g,2i,3c] It should be noted that, prior to our previous studies, there had been many reports on flavin-containing soluble polymers including those designed for their application as an oxidation catalyst.[4] As a result, such a collaboration with polymer has been demonstrated to be highly potential for enhancing or bringing out the aerobic catalysis of flavin molecules, like a flavoenzyme where flavin cofactor and apoenzyme collaborate with each other in nature.

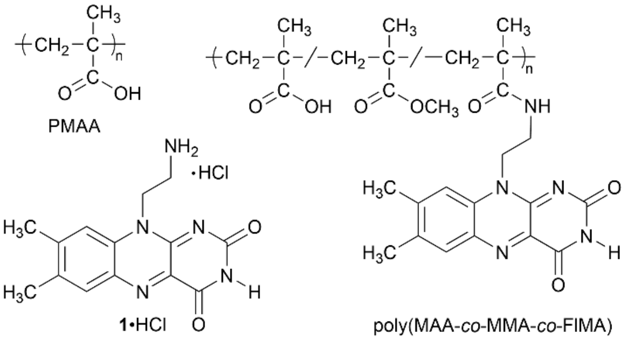


Fig. 1 Structures of PMAA, **1**•HCl, and poly(MAA-*co*-MMA-*co*-FIMA)

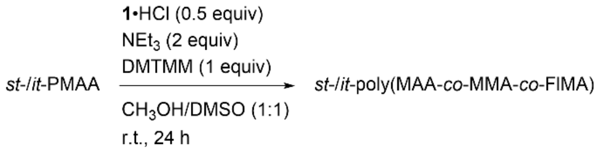
In this article, we present a new type of immobilized flavin catalysts that use poly(methacrylic acid), PMAA (Fig. 1), as a support polymer. Besides the fact that it has not so far been used for developing immobilized flavin catalysts, the reasons we have focused on PMAA include that a) various synthetic methods with control of factors such as tacticity and average molecular weight have been established,[5] b) they can be easily modified with functionalized flavin molecules via an ester/amide bond formation, and c) the immobilized flavin molecules may be activated or stabilized depending on their redox forms by interaction with surrounding carboxy groups. We herein demonstrate that PMAA is a useful material for immobilizing *N*5-unmodified flavin molecules through its post-

---

Department of Applied Chemistry, Tokushima University, Minamijosanjima, Tokushima 770-8506, Japan

polymerization modification by condensing with 10-(2-aminoethyl)-7,8-dimethylisoalloxazine hydrochloride (**1**•HCl, Fig. 1) under suitable conditions. In addition, we show that the resulting flavin-containing polymers, poly(MAA-*co*-MMA-*co*-FlMA)s (Fig. 1, MMA = methyl methacrylate unit, FlMA = flavin-containing methacrylamide unit), exhibit aerobic oxidation catalysis depending on the flavin as well as the main chain stereoregularity.

Table 1 Preparation of *st*-/*it*-poly(MAA-*co*-MMA-*co*-FlMA)

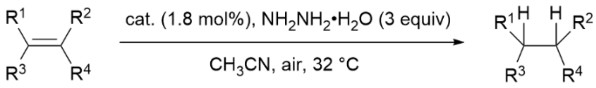


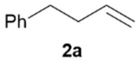
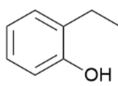
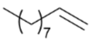
	st-/*it*-PMAA		*st*-/*it*-poly(MAA-*co*-MMA-*co*-FlMA)		
Entry	Triad tacticity[a] (%) *mm*:*rm*:*rr*	M_n[b] (kg mol^{-1})	DFl[a,c] (%)	Fl loading[d] (mmol g^{-1})	Yield[e] (%)
1	5:36:59 (*st*-)	286	14	1.11	79
2	3:31:66 (*st*-)	21.3	13	0.99	83
3	97:2:1 (*it*-)	33.6	16	1.13	55

[a] Determined by ^{1}H NMR. [b] Determined by SEC. [c] The degree of substitution with FlMA. [d] Calculated from nitrogen content determined by elemental analysis. [e] Calculated from the weight of the obtained product and the theoretical weight of the product. DMTMM: 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; Fl: flavin.

We prepared **1**•HCl according to the literature procedure from riboflavin[6] and started our investigation by exploring whether commercially available syndiotactic (*st*-)PMAA with the *rr* triad content of 59%, whose number-average molar mass (M_n) is 286 kg mol^{-1}, can be modified as desired via condensation with **1**•HCl that forms an amide bond. The fact that **1**•HCl is well soluble in methanol while nearly not in aprotic solvents such as THF and DMSO led us to use 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as a condensing agent that is usable in methanol for amide bond formation.[7] On the other hand, PMAA is well soluble in DMSO while nearly not in protic solvents such as methanol. Therefore, a mixed solvent of methanol and DMSO (1:1) was envisioned to be effective, and the reaction of the commercial *st*-PMAA with 0.5 equivalents of **1**•HCl using 1 equivalent of DMTMM and 2 equivalents of triethylamine in the mixed solvent system was carried out under ambient conditions. As expected, the corresponding flavin-containing polymer partially methyl esterificated, *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{LG}$, was obtained as a yellow solid after appropriate pH adjustment, dialysis for purification followed by lyophilization (Table 1, entry 1). The flavin loading of the product was calculated to be 1.11 mmol g^{-1} from its nitrogen content determined by elemental analysis, while the degree of substitution with FlMA was determined to be 14% by ^{1}H NMR. The ratio of MAA and MMA units in the product was estimated to be nearly 1:1 through ^{1}H NMR analysis (see supporting information). The yield was calculated to be 79% from the weight of the obtained *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{LG}$ and the theoretical weight based on the copolymer composition. A similar result was observed with another *st*-PMAA, prepared according to literature procedures,[8b] having slightly higher syndiotacticity while much lower M_n (*rr* triad : 66%, M_n : 21.3 kg mol^{-1}), providing *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{SM}$ (Table 1, entry 2). The post-polymerization modification of isotactic (*it*-)PMAA with *mm* triad content of 97% (M_n : 33.6 kg mol^{-1}), prepared according to literature procedures,[8a] was also explored under identical conditions, which almost equally proceeded as in the case of *st*-PMAAs to afford *it*-poly(MAA-*co*-MMA-*co*-FlMA) with a similar flavin loading and copolymer composition (Table 1, entry 3, 1.13 mmol g^{-1}, MAA:MMA ≈ 1:0.8). Note that the amount of flavin loading could be controlled to some extent by the stoichiometry of **1**•HCl and DMTMM, regardless of the polymer tacticity (see supporting information). In addition, all the products above exhibited an intense fluorescence emission around 520 nm, characteristic of the isoalloxazine ring, in solution state and emitted light apparently even in solid state with irradiation of 365 nm UV light, indicating that the flavin molecules are certainly immobilized and evenly distributed to the polymer chain.[3a,3b]

Table 2 Flavin-catalyzed aerobic reduction of olefins[a]

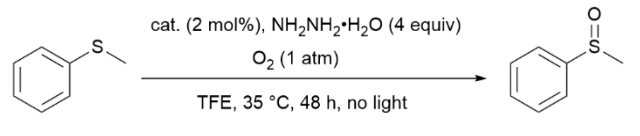


Entry	Substrate	Catalyst	Yield (%)[b]
1	 **2a**	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{SM}$[c]	81
2	**2a**	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{LG}$[d]	75
3	**2a**	*it*-poly(MAA-*co*-MMA-*co*-FlMA)[e]	46
4	**2a**	DMRFl	47
5	 **2b**	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{SM}$[f]	96
6	**2b**	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{LG}$[d]	85
7	**2b**	*it*-poly(MAA-*co*-MMA-*co*-FlMA)[g]	33
8	 **2c**	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{SM}$[f]	92
9	**2c**	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{LG}$[d]	65
10	**2c**	*it*-poly(MAA-*co*-MMA-*co*-FlMA)[g]	51

[a] Reactions were performed using 0.125 mmol of the olefin and 0.375 mmol of hydrazine monohydrate in 1 mL of acetonitrile in the presence of 1.8 mol% of the catalyst under air at 32 °C for either 24 h (entries 1–4), 6 h (entries 5–7), or 12 h (entries 8–10). [b] Determined by GC analysis. [c] 0.99 mmol g^{-1}. [d] 1.11 mmol g^{-1}. [e] 1.13 mmol g^{-1}. [f] 0.65 mmol g^{-1}. [g] 1.04 mmol g^{-1}.

With the flavin-containing polymers with different stereoregularities and similar flavin loadings in hand, we evaluated their catalytic activity in the aerobic hydrogenation of olefins with hydrazine that was previously developed by ourselves as one of the unique flavin-catalyzed reactions.[2c,2d,2f] 4-Phenyl-1-butene (**2a**) was selected as a test substrate, and reactions were carried out with 3 equivalents of hydrazine monohydrate to the substrate in acetonitrile under air at 32 °C in the presence of 1.8 mol% of either *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{LG}}$, *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{SM}}$, or *it*-poly(MAA-*co*-MMA-*co*-FlMA) for 24 h (Table 2, entries 1–3). In all cases, the catalyst was insoluble in the reaction solvent and the reaction system was heterogeneous. Nevertheless, they all showed catalytic activity comparable with or even higher than that of 2′,4′:3′,5′-di-*O*-methyleneriboflavin (DMRFl, Table 2, entry 4),[2d,2f] which is a homogeneous catalyst. This is probably due to the well-dispersed flavin molecules on the support, which suppresses self-quenching of redox-active flavin species that is more likely to occur in solution. It should be noted that *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{SM/LG}}$ showed obviously higher catalytic activity than *it*-poly(MAA-*co*-MMA-*co*-FlMA), although there is no apparent difference in their solubility as well as swelling properties in acetonitrile (see supporting information). The same tendency in the correlation between tacticity and catalytic activity of poly(MAA-*co*-MMA-*co*-FlMA) was observed in the reduction of other olefins including *o*-allylphenol (**2b**, Table 2, entries 5–7) and 1-decene (**2c**, Table 2, entries 8–10), clearly indicating the positive effect of the syndiotacticity. It is also interesting to find that the catalytic activity of *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{SM}}$ was somewhat better than that of *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{LG}}$ in all cases. Also note that the heterogeneity of these catalysts allows for their easy recovery and reuse (see supporting information).

Table 3 Flavin-catalyzed aerobic oxidation of thioanisole[a]



Entry	Catalyst	Yield (%)[b]
1	*it*-poly(MAA-*co*-MMA-*co*-FlMA)[c]	99
2	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{LG}}$[d]	41
3	*st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{SM}}$[e]	29
4	3-methyllumiflavin	5

[a] Reactions were performed using 0.1 mmol of thioanisole, 0.4 mmol of hydrazine monohydrate in 0.5 mL of 2,2,2-trifluoroethanol (TFE) in the presence of 2 mol% of the catalyst under 1 atm of O_2 at 35 °C. [b] Determined by GC analysis. [c] 1.13 mmol g^{-1}. [d] 1.11 mmol g^{-1}. [e] 0.96 mmol g^{-1}.

We then turned our attention to investigate sulfoxidation that is one of the most common aerobic oxygenation reactions flavoenzymes efficiently catalyze in nature. The aerobic oxidation of thioanisole was used as a test reaction to evaluate the catalytic activities of the flavin-containing polymer catalysts under the conditions that were previously developed by our group for reactions with artificial flavin catalysts.[2a,2e,2g,2i] *it*-Poly(MAA-*co*-MMA-*co*-FlMA), *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{LG}}$, and *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{\mathrm{SM}}$ all promoted the desired sulfoxidation with a catalytic amount of 2 mol% in the presence of 1 atm of molecular oxygen (O_2) and 4 equivalents of hydrazine monohydrate in 2,2,2-trifluoroethanol (TFE) at 35 °C while protected from light to give methyl phenyl sulfoxide in significantly different yields after 48 h (Table 3, entries 1–3). In contrast to the case of olefin hydrogenation (Table 2), these reaction systems were all homogeneous, and the best result in catalytic activity was obtained with *it*-poly(MAA-*co*-MMA-*co*-FlMA) while the others were rather less active. It should be noted that no significant reactivity was observed when 3-methyllumiflavin was used as a catalyst under otherwise identical conditions (Table 3, entry 4), likely due to the lability of 4a-hydroperoxyflavin that has been recognized as a key active species for the flavoenzyme-catalyzed monooxygenation[9] as well as its artificially simulated systems.[2g–2i,10]

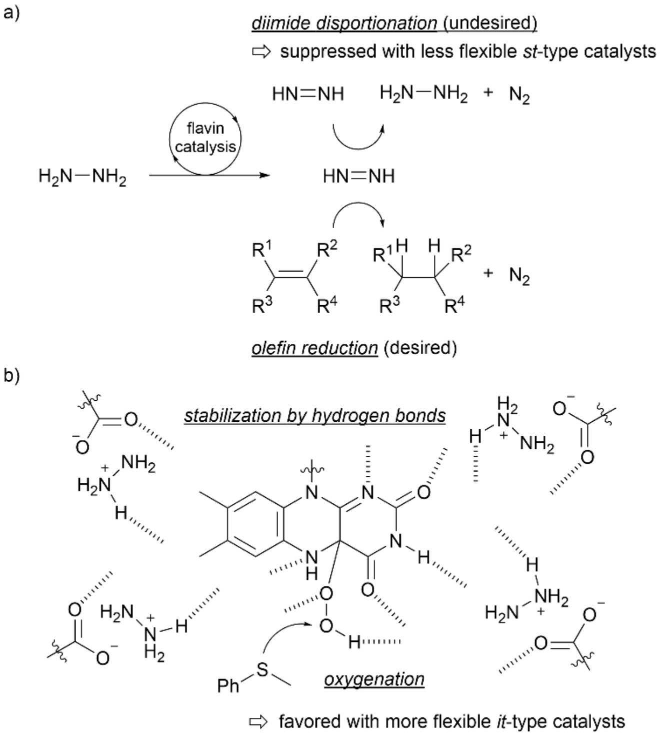


Fig. 2 A plausible explanation for the effect of polymer stereoregularity in the poly(MAA-*co*-MMA-*co*-FlMA)-catalyzed reactions: (a) aerobic reduction of olefins, (b) aerobic oxidation of sulfides

The contrasting effects of polymer stereoregularity in the above two flavin-catalyzed reactions may be explained by the flexibility of a polymer chain. In general, *it*-PMMA has a higher flexibility than *st*-PMMA: *it*-PMMA exhibits lower glass transition temperature[11a,11b] and spreads easier in solvents[11c, 11d, 11e] than *st*-PMMA, which is also probably the case for poly(MAA-*co*-MMA-*co*-FlMA). In the aerobic reduction of olefins, as we have previously reported,[2c,2d,2f] diimide generated by the flavin-catalyzed dehydrogenation of hydrazine can reduce olefins, while diimide is so labile that two diimide molecules can rapidly disproportionate to hydrazine and N_2 when they approach each other. From this point of view, *st*-poly(MAA-*co*-MMA-*co*-FlMA) can be advantageous because of its less flexibility that makes the proportion of flavin molecules in close proximity rather lower to suppress the disproportionation of diimides (Fig. 2a). This hypothesis is currently supported by additional experiments showing that the activity of more flexibile *it*-type catalyst is enhanced at lower temperature (see supporting information). On the other hand, the oxygenation catalysis of a flavin molecule immobilized onto poly(MAA-*co*-MMA) can be understood by considering the contribution of surrounding carboxy groups that should be present as hydrazine salts in actual case, which may stabilize the active species, 4a-hydroperoxyflavin, by donating/accepting hydrogen bonds effectively, especially in the case of *it*-poly(MAA-*co*-MMA-*co*-FlMA) that is more flexible (Fig 2b). Provided that the catalysis of poly(MAA-*co*-MMA-*co*-FlMA) is significantly affected by their molar mass, one with larger M_n should be less flexible than the other, due to its higher glass transition temperature, to be more active in the aerobic reduction of olefins while less active in the aerobic oxidation of sulfides. This is, however, in contrast to the present results with *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{SM}$ and *st*-poly(MAA-*co*-MMA-*co*-FlMA)$_{LG}$, which is more likely due to the small difference in their syndiotacticity rather than the large difference in their molar masses.

In summary, we introduced flavin-containing poly(MAA-*co*-MMA)s with different stereoregularity, *st*-poly(MAA-*co*-MMA-*co*-FlMA) and *it*-poly(MAA-*co*-MMA-*co*-FlMA), which were prepared by the post polymerization modification of *st*-/*it*-PMAA with **1**•HCl by means of DMTMM as a condensing agent under mild conditions. These novel polymers showed flavin-dependent aerobic oxidation catalysis with different catalytic activity depending on their stereoregularity. The flavin-catalyzed aerobic reduction of olefins was promoted more efficiently by *st*-poly(MAA-*co*-MMA-*co*-FlMA), while *it*-poly(MAA-*co*-MMA-*co*-FlMA) was more suited for the flavoenzyme-mimetic aerobic oxidation of sulfides that is typically difficult to achieve under apoenzyme-free conditions. These observations can be explained by the flexibility of the polymer catalysts expected from their stereoregularity.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by JSPS KAKENHI (Grant-in-Aid for Scientific Research (C), no. 19K05457) and the Research Clusters program of Tokushima University (no. 1802001).

References

1 (a) H. Iida, Y. Imada, S.-I. Murahashi, *Org. Biomol. Chem.*2015, **13**, 7599–7613; (b) R. Cibulka, *Eur. J. Org. Chem.* 2015, **2015**, 915–932; (c) G. de Gonzalo, M. W. Fraaije, *ChemCatChem* 2013, **5**, 403–415; (d) Y. Imada, T. Naota, *Chem. Rec.* 2007, **7**, 354–361.

2 (a) Y. Imada, H. Iida, S. Ono, S.-I. Murahashi, *J. Am. Chem. Soc.* 2003, **125**, 2868–2869; (b) Y. Imada, H. Iida, S.-I. Murahashi, T. Naota, *Angew. Chem. Int. Ed.* 2005, **44**, 1704–1706; (c) Y. Imada, H. Iida, T. Naota, *J. Am. Soc. Chem.* 2005, **127**, 14544–14545; (d) Y. Imada, T. Kitagawa, T. Ohno, H. Iida, T. Naota, *Org. Lett.* 2010, **12**, 32–35; (e) Y. Imada, H. Iida, S. Ono, Y. Masui, S.-I. Murahashi, *Chem.–Asian J.*, 2006, **1**, 136–147; (f) Y. Imada, H. Iida, T. Kitagawa, T. Naota, *Chem. Eur. J.* 2011, **17**, 5908–5920; (g) Y. Arakawa, K. Yamanomoto, H. Kita, K. Minagawa, M. Tanaka, N. Haraguchi, S. Itsuno, Y. Imada, *Chem. Sci*. 2017, **8**, 5468–5475; (h) K. Yamanomoto, H. Kita, Y. Arakawa, K. Minagawa, Y. Imada, *Chimia* 2018, **72**, 866–869; (i) Y. Arakawa, N. Takechi, K. Yamanomoto, K. Minagawa, Y. Imada, *Tetrahedron Lett.* 2021, **73**, 153107.

3 (a) Y. Arakawa, R. Kawachi, Y. Tezuka, K. Minagawa, Y. Imada, *J. Polym. Sci. Part A: Polym. Chem.* 2017, **55**, 1706–1713; (b) Y. Arakawa, F. Sato, K. Ariki, K. Minagawa, Y. Imada, *Tetrahedron Lett.* 2020, **61**, 151710; (c) Y. Arakawa, K. Minagawa, Y. Imada, *Polym. J.* 2018, **50**, 941–949.

4 For soluble polymer-supported flavins as oxidation catalysts, see: (a) S. Shinkai, S. Yamada, T. Kunitake, *Macromolecules* 1978, **11**, 65–68; (b) S. Shinkai, R. Ando, T. Kunitake, *Biopolymers* 1978, **17**, 2757–2760; (c) W. J. Spetnagel, I. M. Klotz, *Biopolymers* 1978, **17**, 1657–1668; (d) S. Shinkai, K. Mori, Y. Kusano, O. Manabe, *Bull. Chem. Soc. Jpn*. 1979, **52**, 3606–3610; (e) J. P. C. Bootsma, G. Challa, F. Müller, *J. Polym. Sci: Polym. Chem. Ed.* 1984, **22**, 705–719; (f) H. F. M. Schoo, G. Challa, *Polymer* 1990, **31**, 1559–1563; (g) H. F. M. Schoo, G. Challa, *Macromolecules* 1992, **25**, 1633–1638; (h) H. Iida, S. Iwahana, T. Mizoguchi, E. Yashima, *J. Am. Chem. Soc.* 2012, **134**, 15103–15113.

5 (a) K. Hatada, T. Kitayama, K. Ute, *Prog. Polym. Sci*. 1988, **13**, 189–276; (b) T. Nakano and Y. Okamoto, in *Controlled Radical Polymerization*, ed. K. Matyjaszewski, ACS Symposium Series 685, American Chemical Society, Washington, D.C., 1998, pp 451–462; (c) K. Hatada, T. Kitayama, *Polym. Int.* 2000, **49**, 11–47; (d) E. Y.-X. Chen, *Chem. Rev*. 2009, **109**, 5157–5214; (e) K. Satoh, M. Kamigaito, *Chem. Rev*. 2009, **109**, 5120–5156.

6 T. Matsuo, T. Hayashi, Y. Hisaeda, *J. Am. Chem. Soc.* 2002, **124**, 11234–11235.

7 (a) M. Kunishima, C. Kawachi, J. Morita, K. Terao, F. Iwasaki, S. Tani, *Tetrahedron*, 1999, **55**, 13159–13170; (b) J. M. Pelet, D. Putnam, *Bioconjugate Chem.* 2011, **22**, 329–337.

8 (a) H. Yuki, K. Hatada, T. Niinomi, Y. Kikuchi, *Polym. J.* 1970, **1**, 36–45; (b) M. Kamigaito, K. Ishitake, K. Satoh, Y. Okamoto, *Macromolecules*, 2011, **44**, 9108–9117.

9 (a) L. L. Poulsen, D. M. Ziegler, *J. Biol. Chem.* 1979, **254**, 6449–6455; (b) V. Massey, P. Hemmerich, *Biochem. Soc. Trans.* 1980, **8**, 246–257; (c) N. B. Beaty, D. P. Ballou, *J. Biol. Chem.* 1980, **255**, 3817–3819.

10 (a) Y. Chevalier, Y. L. T. Ki, D. le Nouen, J.-P. Mahy, J.-P. Goddard, F. Avenier, *Angew. Chem. Int. Ed.* 2018, **57**, 16412–16415; (b) L. Zhao, J. Cai, Y. Li, J. Wei, C. Duan, *Nat. Commun.* 2020, **11**, 2903.

11 (a) F. E. Karasz and W. J. MacKnight, *Macromolecules* 1968, **1**, 537–540; (b) K. Ute, N. Miyatake, K. Hatada, *Polym. J.* 1995, **36**, 1415-1419; (c) K. Ute, N. Miyatake, Y. Osugi, K. Hatada, *Polym. J*. 1993, **25**, 1153–1160; (d) M. Kamijo, N. Sawatari, T. Konishi, T. Yoshizaki, H. Yamakawa, *Macromolecules* 1994, **27**, 5697–5703; (e) N. Sawatari, T. Konishi, T. Yoshizaki, H. Yamakawa, *Macromolecules* 1995, **28**, 1089-1094.