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EDGE ARTICLE

Zinc complex mediated regioselective *O*-acylation of therapeutic agents†

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Several Zn β -diiminato reagents were developed and used to mediate regioselective *O*-acylation reactions of therapeutic agents with anhydrides. Various prodrugs were obtained in excellent yield and high regioselectivity, including derivatives of rapamycin and paclitaxel. Furthermore, the application of regioselective acylation was extended to one-pot reactions between therapeutics and carboxylic acids with various pendant functional groups. This rapid functionalization strategy will find application in either prodrug synthesis or bioconjugation for drug delivery.

Introduction

The hydroxyl group is the most abundant functional group in natural products: it is found in approximately 65% of approximately 78,000 known pharmacophores.¹ Of various strategies being pursued in drug discovery, derivatization of natural products through acylation of their hydroxyl groups (*O*-acylation) is among the quickest and most cost-effective approaches for the synthesis of new therapeutic agents and prodrugs with improved properties, such as increased water solubility, enhanced therapeutic efficacy, and reduced side effects.^{2,3} However, precisely controlled, highly regioselective *O*-acylation is difficult to achieve, especially for compounds with complex structures and various functional groups.⁴ To date, only a few approaches have been reported for the *O*-acylation of natural products; these approaches involve the use of enzymes,^{5–9} amino acid derivatives,^{10,11} or oligopeptides^{12–15} as *O*-acylation catalysts. However, these agents usually have only modest activities and require prolonged reaction times. Therefore, a highly active and highly regioselective catalyst for the *O*-acylation of complex molecules (*e.g.*, polyol therapeutics) is greatly needed.

We recently reported the drug-initiated ring-opening polymerization of lactide mediated by (BDI)ZnN(TMS)₂ (**1**, BDI = β -diiminato, Fig. 1),^{16,17} a group of complexes originally developed by Coates and coworkers.¹⁸ When **1** is mixed with hydroxyl-containing drug molecules, (BDI)Zn-drug alkoxides are formed

quantitatively *in situ*. Activation of the hydroxyl group of the drugs by the Zn complex initiates well-controlled ring-opening polymerization of lactide, resulting in ester-linked polylactide-drug conjugates (see ESI, Scheme S1A†).¹⁷ Moreover, similar Zn alkoxides efficiently induce copolymerization of cyclic anhydrides and epoxides.^{19,20} These findings suggest that this class of Zn complex may be useful for controlled, regioselective *O*-acylation of therapeutics with anhydrides (Fig. 1). In this study, we tested this hypothesis and developed a general strategy for *O*-acylation of polyol therapeutics with remarkable regioselectivity and efficiency (see ESI, Scheme S1B†).

Results and discussion

O-acylation with anhydrides and Zn complex

First, we evaluated whether **1** effectively mediated the *O*-acylation of a model hydroxyl-containing drug, 2-naphthalene-ethanol (Naph), with several commercially available anhydrides (Fig. 1). When mixed with Naph at a 1 : 1 molar ratio, **1** showed remarkable activity for acylation with methacrylic anhydride (MA), resulting in Naph-MA in quantitative yield within 4 h (Table 1, entry 1). In sharp contrast, the same reaction mediated by triethylamine (TEA) under similar conditions afforded Naph-MA in less than 10% yield (entry 2). These experiments demonstrated that the activity of this Zn complex for *O*-acylation was much higher than that of organocatalysts. Various functional groups, including several routinely used for bioconjugation reactions²¹ (*e.g.*, $-\text{COC}(\text{CH}_3)=\text{CH}_2$ in Naph-MA, entry 1; $-\text{Cl}$ in Naph-ClA, entry 3; $-\text{I}$ in Naph-IA, entry 4; and $-\text{COOH}$ in Naph-SA,²² entry 5) or for bioorthogonal reactions (*e.g.*, $-\text{C}\equiv\text{CH}$ in Naph-PA,²³ entry 6, used for “click” chemistry²⁴), were readily incorporated onto Naph in >90% yields after 2–4 h of reaction with the corresponding anhydrides.

We next explored whether this highly efficient reaction could be extended to complex therapeutic molecules. As a prototype complex molecule, we chose dasatinib (Dasa), a Bcr/Abl tyrosine kinase inhibitor bearing a primary hydroxyl group (Table 1), for

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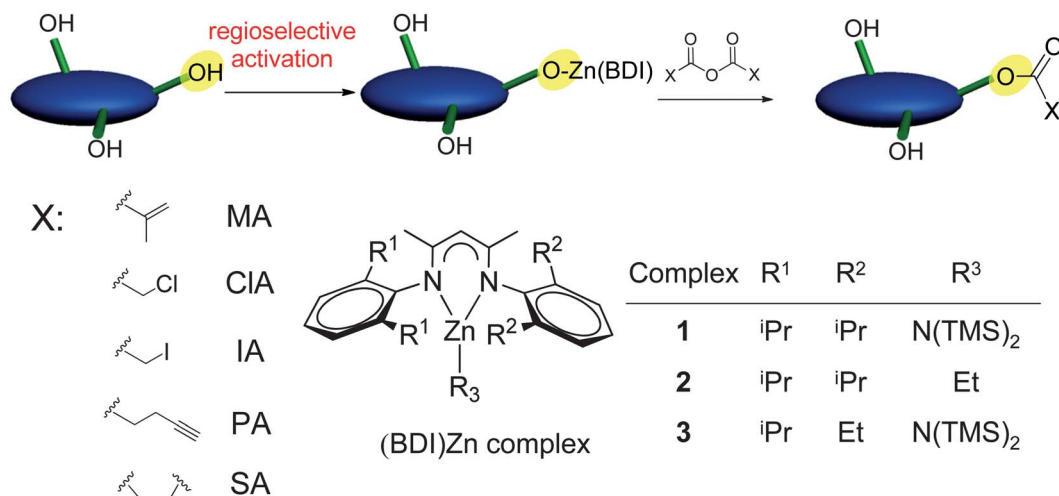


Fig. 1 Regioselective *O*-acylation catalyzed by (BDI)Zn complexes. Polyols (blue ovals) were first regioselectively activated by the (BDI)Zn complexes (yellow) to generate active Zn alkoxides *in situ*. Subsequent reactions with various anhydrides efficiently provided the *O*-acyl products. Three (BDI)Zn complexes (1–3) are described in this paper.

reaction with MA in the presence of **1**. The reaction was rapid: Dasa-MA was obtained in 91% yield within 2 h (entry 7). When **1** was used for acylation of 20(*S*)-camptothecin (Cpt, Table 1), a topoisomerase I inhibitor, substantially reduced reactivity was observed (12 h reaction at 40 °C, 26% yield, entry 8).²⁵ The low reactivity was presumably associated with the tertiary 20-OH group in Cpt: steric hindrance around the 20-OH prevented efficient formation of the (BDI)Zn-Cpt alkoxide required for the subsequent *O*-acylation reaction. Attempts to catalyze the reaction using **3**, which has a less bulky BDI ligand, resulted in a pronounced increase in the yield of Cpt-MA (4 h reaction at 40 °C, 82% yield, entry 9). Conventional acylation reagents (*e.g.*, TEA and 4-dimethylaminopyridine/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) failed to convert the 20-OH of Cpt to the expected *O*-acylated derivative (<15% yield after 12 h at 40 °C; ESI, Fig. S1†).

Table 1 Evaluation of *O*-acylation substrates and reaction conditions^a

Entry	R-OH	Anhydride	Reagent	Temp. (°C)	Time (h)	Yield (%)
1	Naph	MA	1	25	4	>98
2	Naph	MA	TEA	25	4	<10
3	Naph	CIA	1	25	4	>98
4	Naph	IA	1	25	4	>98
5	Naph	SA	1	40	2	>98
6	Naph	PA	2	40	2	92
7	Dasa	MA	1	25	2	91
8	Cpt	MA	1	40	12	26
9	Cpt	MA	3	40	4	82

^a R-OH/anhydride/reagent = 0.01/0.01/0.01 mmol in 800 μL THF. Abbreviations: CIA, chloroacetic anhydride; MA, methacrylic anhydride; PA, pent-4-ynoic anhydride; SA, succinic anhydride. Yields were determined by HPLC.

Regioselective *O*-acylation of rapamycin

With these results in hand, we next investigated the *O*-acylation of therapeutics with more complex structures, in particular polyols. We selected rapamycin (Rapa, Table 2 and Fig. 2), a macrolide polyol and an important antifungal, immunosuppressive, anti-proliferative and anti-neoplastic agent,^{26–30} for study because of its obvious importance in medicine and its

Table 2 Regioselective *O*-acylation of Rapa and Ptxl^a

Entry	R-OH	Anhydride	Catalyst	Temp. (°C)	Time (h)	Yield (%)	RS (%)
1	Rapa	MA	1	25	6	96	>99
2	Rapa	PA	2	25	4	67	>99
3	Rapa	MA	Novazym 435	40	6	<10	>99
4	Rapa	MA	TEA	40	24	<10	31
5	Rapa	MA	TEA/NMI	25	12	20	43
6	Ptxl	MA	1	25	3	>98	>99
7	Ptxl	MA	TEA	25	4	13	>99
8	Ptxl	CIA	1	25	4	90	>99
9	Ptxl	PA	2	40	2	81	>99

^a R-OH/anhydride/catalyst = 0.01/0.01/0.01 mmol. Abbreviations: CIA, chloroacetic anhydride; MA, methacrylic anhydride; NMI, *N*-methylimidazole; PA, pent-4-ynoic anhydride; RS: regioselectivity (for Ptxl, it is the ratio of Ptxl-2'-derivative *versus* other acylation derivatives; for Rapa, it is the ratio of Rapa-40-derivative *versus* other acylation derivatives). Yields and regioselectivities were determined by HPLC.

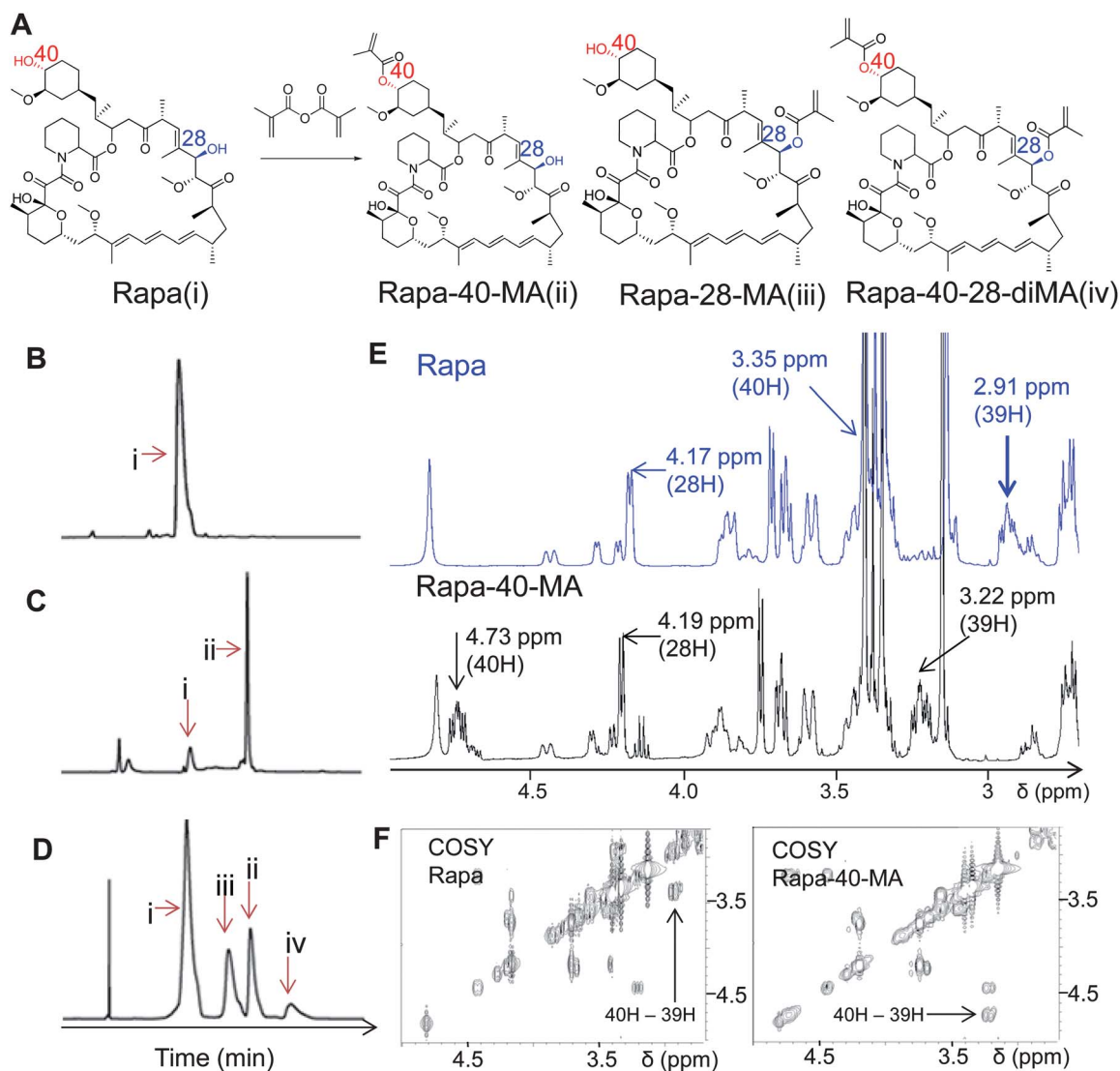


Fig. 2 (A) Reaction of Rapa and MA. (B–D) HPLC traces of Rapa and products of reactions of Rapa, MA, and **1** (Table 2, entry 1) and Rapa, MA, TEA, and *N*-methylimidazole (entry 5), respectively. (E) ¹H-NMR spectra (δ : 3–5 ppm) of Rapa and Rapa-40-MA (Table 2, entry 1). (F) 2D COSY-NMR of Rapa-40-MA. The cross peak of 40H–39H is labeled. The 40H in Rapa is obscured by a broad peak \sim 3.4 ppm; after acylation on 40-OH, both 40H and 39H peaks shifted downfield. Detailed NMR assignments and mass spectroscopy results are described in the ESI, Table S1 and Fig. S4.†

multiple hydroxyl groups. *O*-Acyl derivatives of Rapa have already been extensively investigated. For example, temsirimolimus (CCI-779), a 40-hemiester derivative of Rapa, has been approved by the U.S. Food and Drug Administration as an mTOR inhibitor for the treatment of renal cell carcinoma, and a dozen other 40-*O*-acyl derivatives of Rapa are undergoing clinical trials.³¹ However, the synthesis of 40-*O*-acyl Rapa prodrugs in high yield is difficult,^{32,33} the regioselective derivatization of Rapa at the 40-OH position is difficult to control and requires an auxiliary agent that exhibits kinetic preference. To evaluate (BDI)Zn-mediated *O*-acylation of Rapa, we first tested the stability of Rapa in the presence of **1** because poor Rapa stability under basic conditions has been observed in several acylation studies.^{32,33} No degradation of Rapa was detected after incubation with **1** for 4 h at 40 °C (ESI, Fig. S2†). When MA was used for **1**-mediated Rapa acylation, Rapa-MA was obtained in 96% yield after 6 h at room temperature

(Table 2, entry 1). Analysis of the ¹H NMR spectrum of Rapa-MA confirmed that the *O*-acylation was 100% regioselective for the C-40 position; the chemical shifts of the C28-H were the same before and after *O*-acylation, whereas the chemical shift of C40-H shifted downfield from 3.4 ppm to 4.7 ppm (Fig. 2 and Table S1†).³⁴ In an *in vitro* anti-proliferation study using pluripotent human embryonic carcinoma NTERA-2 cells,²⁹ Rapa-MA showed activity comparable to that of Rapa, suggesting that the regioselective modification of the 40-OH of Rapa did not affect its binding to mTOR and FKBP12 (ESI, Fig. S3†).³⁵

Complex **2** also showed high activity and regioselectivity for acylation of the 40-OH of Rapa with pent-4-ynoic anhydride (PA), affording Rapa-PA in 67% yield in 4 h with >99% regioselectivity (Table 2, entry 2). The reactivity and regioselectivity of both **1** and **2** were drastically different from those of previously reported enzymes or organocatalysts (*e.g.*, *N*-methylimidazole

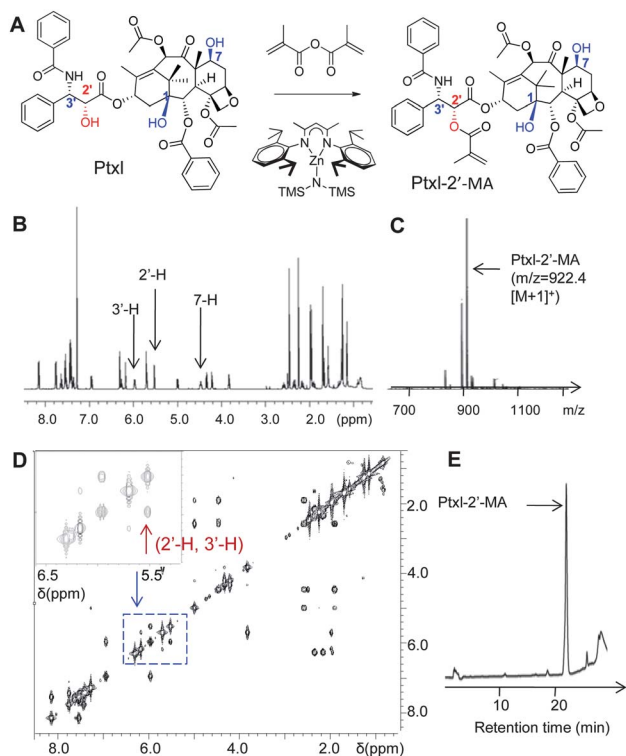


Fig. 3 (A) Scheme of site-specific conjugation of Ptxl with MA to synthesize Ptxl-2'-MA; (B) $^1\text{H-NMR}$ spectrum of pure Ptxl-2'-MA. The arrow in the spectrum indicates the assigned peak; (C) ESI-MS analysis of the reaction mixture. The spectrum indicated that Ptxl-2'-MA ($m/z = 922.4$) was obtained in the reaction. No diacrylate side product (Ptxl-diMA) was detected; (D) COSY-NMR analysis of pure Ptxl-2'-MA. The arrow shows the correlation peak of 2'-H and 3'-H of Ptxl-2'-MA, assisting the assignment of this complex molecule; (E) HPLC analysis of the Ptxl/MA/1 reaction mixture.

(NMI); compare entries 1 and 2 with entries 3–5; also see ESI, Fig. S4†).^{8,14,32}

Regioselective *O*-acylation of paclitaxel

We next tested whether regioselective *O*-acylation chemistry mediated by this class of Zn complexes could be extended to other natural products with multiple hydroxyl groups. Paclitaxel (Ptxl, Table 2), a well-known anticancer agent, has two reactive hydroxyl groups (C2'-OH and C7-OH) that compete in many acylation reactions.^{5,36,37} *O*-Acylation of Ptxl with MA in the presence of **1** afforded Ptxl-MA in 98% yield (entry 6). The one- and two-dimensional ^1H NMR spectra of Ptxl-MA confirmed that *O*-acylation occurred exclusively at the C2'-OH; the chemical shift of the C2'-H of Ptxl-MA noticeably shifted downfield to 5.50 ppm from 4.78 ppm upon acylation of Ptxl, whereas the chemical shift of the C7-H was the same before and after acylation (4.40 ppm; Table S2† and Fig. 3). The high *O*-acylation activity and regioselectivity of **1** contrasted sharply with the low activity of TEA under similar reaction conditions, which yielded Ptxl-2'-MA in only 12.7% (entry 7). Highly regioselective Ptxl acylation was also observed with other *O*-acylation agents, such as chloroacetic anhydride (CIA) and PA (entries 8 and 9), in the presence of **1**. These Ptxl derivatives can be used for conjugation reactions. For instance, the copper(I)-catalyzed azide-alkyne cycloaddition reaction of Ptxl-2'-PA (1 equiv) with 1-azido-1-deoxy- β -D-glucopyranoside tetraacetate (2 equiv) catalyzed by an immobilized CuI catalyst³⁸ readily afforded nearly quantitative yields of 1,4-disubstituted 1,2,3-triazoles, which were purified by means of commercially available copper- and azide-scavenging beads (ESI, Fig. S5†).³⁹

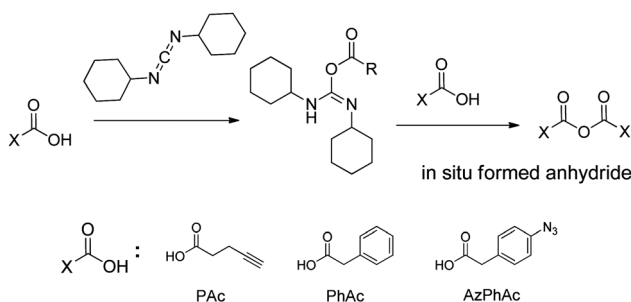
Regioselective *O*-acylation with carboxylic acids

Given that carboxylic acids are more readily available commercially than anhydrides, we attempted to improve the acylation pathway by developing a method for *in situ* preparation of

Table 3 Regioselective *O*-acylation by *in situ* anhydride formation from carboxylic acids^a

Entry	R-OH	Acid	Complex	Temp. ($^{\circ}\text{C}$)	Time (h)	Yield (%)	RS (%)
1	Ptxl	AzPhAc	1	25	4	>98	>99
2	Ptxl	PhAc	1	25	4	>98	>99
3	Rapa	PAc	2	25	4	82	>99

^a Conditions: R-OH/carboxylic acid/(BDI)Zn/DCC = 0.01/0.02/0.01/0.01 mmol. Abbreviation: RS, regioselectivity (for Ptxl, it is the ratio of Ptxl-2'-derivative versus other acylation derivatives; for Rapa, it is the ratio of Rapa-40-derivative versus other acylation derivatives); Temp., temperature. Yields and regioselectivities were determined by HPLC. Anhydrides were prepared 4 h before the acylation reaction.



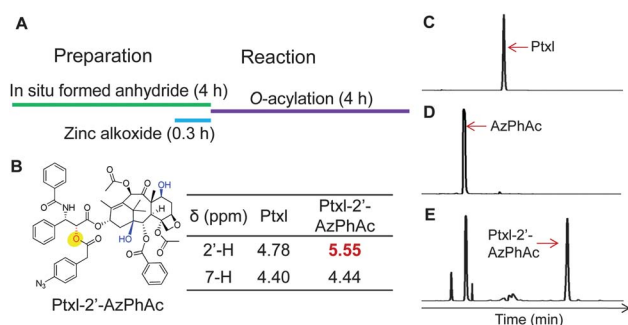


Fig. 4 Regioselective (BDI)Zn-mediated *O*-acylation with *in situ* generated anhydrides. (A) Time frame for the entire process. (B) Chemical structure of Ptxl-2'-AzPhAc and comparison of selected ^1H -NMR chemical shifts with those of Ptxl. (C–E) Overlay of HPLC chromatograms of Ptxl, AzPhAc, and the Ptxl/AzPhAc/DCC/I reaction mixture (Table 3, entry 1).

anhydrides from the corresponding carboxylic acids assisted by *N,N'*-dicyclohexylcarbodiimide (DCC). With 1 equiv of DCC, we obtained almost complete conversion of 2 equiv of three acids to the corresponding anhydrides (Table 3). For example, 2 equiv of 4-azido phenylacetic acid (AzPhAc; Fig. 4) in dichloromethane was mixed with DCC in a glove box at -20°C until conversion to anhydride (as determined by the appearance of the anhydride peak at 1820 cm^{-1} in the FT-IR spectrum) was quantitative (4 h). Prior to acylation, **1** was allowed to react with Ptxl to generate an active Zn-alkoxide species. Mixing of the two solutions induced rapid acylation of Ptxl at room temperature in 3 h, yielding Ptxl-2'-AzPhAc in >99% yield (Fig. 4; Table 3, entry 1); no product of acylation at the 7-position was observed. Similar satisfactory results were also obtained for Ptxl-phenylacetic acid (PhAc) and Rapa-pent-4-ynoic acid (PAC) reactions (Table 3, entries 2–3; ESI, Fig. S6 and Table S3†).

Conclusion

This Zn-mediated regioselective *O*-acylation is analogous to our previously reported site-selective polyester polymerization. We increased the number of potential acyl sources by switching from anhydrides to carboxylic acids. These complexes not only greatly speed up the acylation reactions described herein but also provide a common scaffold that might be effective for various orthogonal functional group transfer reactions (see ESI, Fig. S7 for examples†). This strategy can be expected to provide access to prodrugs with enhanced bioavailability and to allow site-specific bioconjugation for drug delivery, which is related to nanomedicine research being conducted in our laboratory. These reagents may also provide a means to quickly determine the relative reactivities of hydroxyl groups in polyols.^{40–42}

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