Communication

Enzyme-Catalyzed Transetherification of Alkoxysilanes

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Abstract: We report the first evidence of an enzyme-catalyzed transetherification of model alkoxysilanes. During an extensive enzymatic screening in the search for new biocatalysts for silicon-oxygen bond formation we found that certain enzymes promoted the transetherification of alkoxysilanes when tert-butanol or 1-octanol were used as the reaction solvents.

Keywords: enzyme; biomimetic catalysis; transetherification; alkoxysilane.

1. Introduction

Biotransformations are chemical processes which occur under the influence of biological materials such as peptides and proteins. Amongst the myriad examples of bio-mediated transformations we have focused our attention on enzyme-catalyzed reactions at a silicon centre.

In the literature, there are several examples of organo-silicon biotransformations, such as the selective synthesis of organosilicon esters under mild reaction conditions [1], enzymatic silicone oligomerization catalyzed by a lipid-coated lipase [2], and the hydrolysis of silatranes catalyzed by an esterase obtained from the yeast *Rhodotorula mucilaginosa* [3]. In addition, nature provides the many

1 examples reactions from simple to very complex with Si-substrates, where peptides and proteins are
2 generally considered to be the undisputed arbiters [4, 5]. Examples include silica formation in diatoms
3 and other silica-forming organisms [4, 5].

4   Our group has extensively studied silica precipitation [6] and the enzyme-catalyzed hydrolysis and
5 condensation of alxokysilanes [7, 8, 9] under mild conditions. We have discovered several enzymatic
6 candidates which were able to perform such reactions at room temperature and neutral pH and have
7 investigated the potential involvement of their respective active sites in the biocatalyzed organo-silicon
8 transformations.

9   In this contribution, we report a new enzyme-mediated reaction, namely the transetherification of
10 alkoxysilanes, under mild conditions.

11 **2. Results and Discussion**

12   During our previous work on enzyme-catalyzed organo-silicon transformations, we were interested
13 in siloxane-bond formation in the presence of biocatalysts both in aqueous and aqueous-organic media.
14 Several monophasic and biphasic aqueous-organic systems were investigated as reaction solvents. One
15 of the biphasic-aqueous organic systems employed during the alkoxysilane studies consisted of 1-
16 octanol saturated with tris-buffered water. In addition to the enzyme-catalyzed hydrolysis and
17 condensation of alkoxysilanes [9], the formation of the octylsilyl ethers as a result of
18 transetherification and/or silanol-alcohol condensation/exchange was observed in this solvent whereas
19 no equivalent reaction was observed in the negative control reactions (Scheme 1). The "side-product"
20 octyl-silyl ether was identified as a new peak which appeared in the gas-chromatogram following
21 reaction and work-up when compared to our standard set of peaks which arise from solvents (THF,
22 ethanol and 1-octanol), unreacted starting material, hydrolyzed silanol, and the disiloxane
23 condensation product.

24   The identity of the structure was further investigated by means of GC-MS (not shown) and was
25 confirmed to be the alkoxyoctylsilyl ether.

26   **Scheme 1.** Enzyme-catalyzed transetherification and/or silanol-alcohol condensation.

27
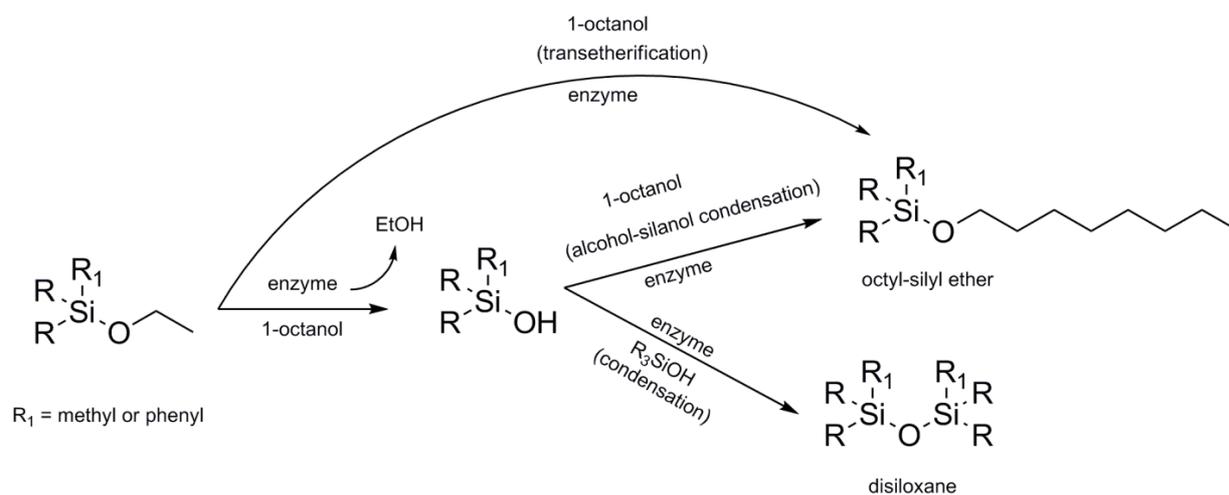
1　　　The reactions were formulated with approximately 5:1 alkoxysilane to enzyme weight ratio in wet
2　　(water-saturated) 1-octanol (5:1 solvent to alkoxysilane weight ratio) and conducted in inert glass
3　　vials. After 24 hours of stirring at room temperature, the reactions were filtered and analyzed by GC-
4　　FID. The gas chromatography analysis was performed with an Agilent 6890 Series injector on an
5　　Agilent 6890 plus gas chromatograph with a flame ionization detector. Dodecane was used as an
6　　internal standard to quantitate the chromatographic analyses. The samples were prepared at ~1% (w/w)
7　　product in a THF solution containing 1% (w/w) dodecane. Based on triplicate measurements, the
8　　response factors for the analytes were calculated, and determined to be linear as a function of
9　　concentration over four orders of magnitude (i.e. 0.01-10% w/w).

10　　　　　　　　　　**Scheme 2:** Chemical synthesis of the octyl-silylethers.

11

R_1 = methyl or phenyl　　　　　　　　octyl-silyl ether
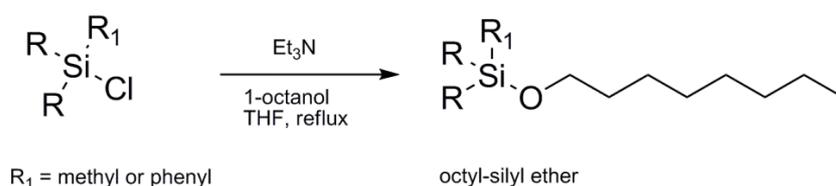
12　　　In　　order　　to　　chromatographically　　quantify　　the　　trimethyloctyloxysilane　　and
13　　phenyldimethyloctyloxysilane products, the two compounds had to be synthesized, as they were not
14　　commercially available. The synthetic procedures are detailed in the Experimental Section. In general,
15　　the appropriate chlorosilane was refluxed with 1-octanol in THF in the presence of triethylamine
16　　(Scheme 2). The products were subsequently purified by vacuum distillation, characterized and used as
17　　standard references in the gas-chromatography analyses.

18　　　As shown in Figure 1 and detailed in Tables 1-2, selected enzymes such as trypsin, *Rhizopus*
19　　*Oryzae* lipase (ROL) and lysozyme were able to catalyze the formation of the octyltrimethyl-silyl ether
20　　(Figure 1, top) and/or the octylphenyldimethylsilyl ether (Figure 1, bottom) after 24 hours at room
21　　temperature. In similar conditions, no condensation was observed in the negative control reactions.

22　　　**Figure　1:**　Enzyme-catalyzed　transetherification　and/or　silanol　alcohol
23　　　　　　　condensation/exchange　study　between　trimethylethoxysilane　(top　figure)　or
24　　　　　　　phenyldimethylethoxysilane (bottom figure) with 1-octanol (blue bars) or *tert*-butanol (red
25　　　　　　　bars), after 24 hours at 25 °C.

26
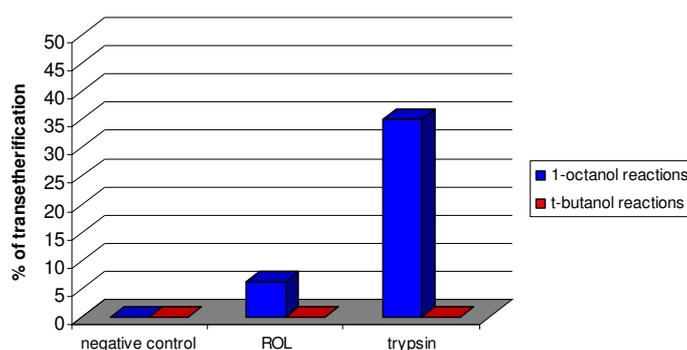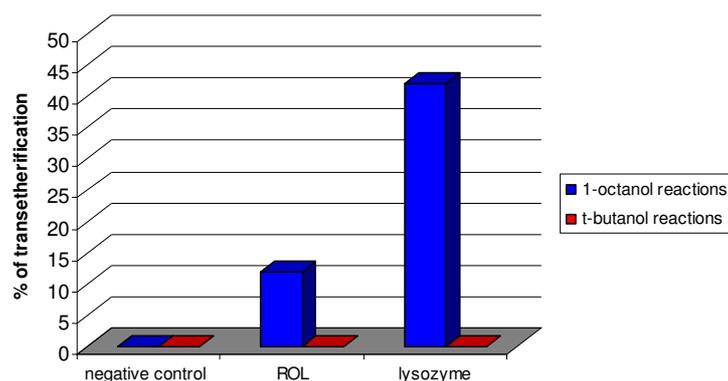
Table 1. Enzyme-catalyzed transetherification reactions between trimethylethoxysilane and 1-octanol or *tert*-butanol after 24 hours at 25 °C.

	% yield (normalized)			
Reaction	**Me₃SiOEt**[1]	**Me₃SiOH**[2]	**HMDS**[3]	**Me₃SiOR**[4]
negative control, 1-octanol	91.3	5.3	3.4	0.0
negative control, 5% water in *tert*-butanol	34.7	64.4	1.0	0.0
Rhizopus oryzae lipase, 1-octanol	81.8	13.0	3.4	1.8
Rhizopus oryzae lipase, 5% water in *tert*-butanol	77.0	23.0	0.0	0.0
bovine pancreatic trypsin, 1-octanol	7.0	52.9	2.3	34.9
bovine pancreatic trypsin, 5% water in *tert*-butanol	3.8	92.1	4.1	0.0

[1] Me_3SiOEt = trimethylethoxysilane
[2] Me_3SiOH = trimethylsilanol
[3] HMDS = hexamethyldisiloxane
[4] Me_3SiOR = trimethyloctyloxysilane and trimethyl *tert*butoxysilane in the 1-octanol and *tert*- butanol reactions, respectively

Figure 1 shows percentage yield of octyl-ether formation based on the quantitative chromatographic data, the mass balance being completed by either unreacted alkoxysilane, silanol formed by simple hydrolysis, or the corresponding disiloxane from the condensation product with another molecule of silanol (see Scheme 1). Notably, in the absence of any biocatalyst (negative control), no octyl-silyl ether was observed, denoting the critical role of the enzyme in the alkoxysilane transetherification transformation.

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Table 2: Enzyme-catalyzed transetherification reactions between phenyldimethyethoxysilane and 1-octanol or *tert*-butanol after 24 hours at 25 °C.

	% yield[1]			
Reaction	**PhMe$_2$SiOEt[2]**	**PhMe$_2$SiOH[3]**	**(PhMe$_2$OSi)$_2$-O[4]**	**PhMe$_2$SiOR[5]**
negative control, 1-octanol	100.0	0.0	0.0	0.0
negative control, 5% water in *tert*-butanol	88.8	11.2	0.0	0.0
Rhizopus oryzae lipase, 1-octanol	66.8	21.5	0.0	11.7
Rhizopus oryzae lipase, 5% water in *tert*-butanol	95.8	4.2	0.0	0.0
chicken egg white lysozyme, 1-octanol	3.2	55.0	0.0	41.8
chicken egg white lysozyme, 5% water in *tert*-butanol	71.5	28.5	0.0	0.0

[1] % Yield = qualitative (i.e. area percent) values
[2] PhMe$_2$SiOEt = phenyldimethylethoxysilane
[3] PhMe$_2$SiOH = phenyldimethylsilanol
[4] (PhMe$_2$OSi)$_2$-O = phenyldisiloxane
[5] PhMe$_2$SiOR = = phenyldimethyloctyloxysilane and phenyldimethyl*tert*butoxy-silane in the 1-octanol and *tert*-butanol reactions, respectively

To our knowledge, this is the first case of an enzyme-mediated transetherification reaction of an organo-silicon substrate under mild conditions. It is apparent that there is advantage in using an enzyme at room temperature over the conventional synthetic procedure the synthesis of the octyl-silyl products by avoiding the use of harsh chemicals and elevated reaction temperature.

Interestingly, the enzymatic screening conducted during the hydrolysis and condensation study of monoalkoxysilanes in wet *tert*-butanol (see [9] and Figure1) did not lead to any *tert*-butylsilyl ether product formation. This may be due to the steric hindrance of *tert*-butyl groups, as opposed to the longer but more flexible octyl chains, which may be more accessible to the enzyme cavities. Notably, ROL was observed to catalyze both octyl ether formation. Lipases normally interact with long-chain alcohols and/or carboxylic acids as natural substrates. Our results show that ROL catalyzes the formation of octyl-silyl ethers. Conversely, the lipase was not able to catalyze the formation of *tert*-butyl silyl ethers. This is in agreement with the natural substrate-selectivity of the ROL, and suggests the involvement of the active site during the catalysis. Trypsin was a good biocatalyst for trimethyloctyl silyl ether formation, and this is in line with our previous studies on the alkoxysilane hydrolysis and condensation reactions [7, 9]. Lysozyme, which was already observed to be a good siloxane-bond biocatalyst [8], produced the highest yield of the phenyldimethyloctyl silyl ether in this study. The reason for the unusual selectivity of this glycoside hydrolase is not yet understood and will be the subject of further investigations. The work proves the potential of the use of (bio)-macromolecules as catalytic aids on unusual substrates under facile and mild reaction conditions.

1　**3. Experimental Section**

2　*3.1. Materials*

3　3.1.1. Synthesis of Trimethyloctyloxysilane

4　　1-octanol (31.25g, 0.24mol) and triethylamine (24.28g, 0.24mol) were dissolved in anhydrous THF
5　(300ml) under nitrogen in a 3-necked round-bottomed flask, and a solution of chlorotrimethylsilane
6　(15.21g, 0.14mol) in anhydrous THF (100ml) was added dropwise to the mixture over one hour. After
7　gently refluxing at approximately 70°C, a white solid precipitated (triethylammonium chloride). The
8　solid was filtered off and the filtrate recovered. The solvent and excess triethylamine were removed
9　using a rotary evaporator, and the transparent liquid purified by distillation at 120 °C and
10　approximately 85 mbar. The product was characterized by NMR spectroscopy and GC-MS, and it was
11　used as a GC-standard in order to understand the product distribution during the enzyme-catalyzed
12　transetherification studies. Yield 67%. ^{1}H NMR (CDCl$_3$, 300 MHz): δ 3.45 (*t*, 2H, *J* 7.4, O-C**H$_2$**), 1.41
13　(*m*, 2H, OCH$_2$-**CH$_2$**), 1.16 (*m*, 10H, OCH$_2$CH$_2$-(**CH$_2$**)$_5$), 0.77 (3H, *t*, *J* 7.4, O(CH$_2$)$_7$-**CH$_3$**) and 0.0 (*s*,
14　9H, Si(C**H$_3$**)$_3$) ppm. ^{13}C NMR (CDCl$_3$, 75.45 MHz) δ 63.2 (O-**CH$_2$**), 33.2 (OCH$_2$-**CH$_2$**), 32.3
15　(OCH$_2$CH$_2$-**CH$_2$**), 29.8 (O(CH$_2$)$_3$-**CH$_2$**), 29.7 (O(CH$_2$)$_4$-**CH$_2$**), 26.3 (O(CH$_2$)$_5$-**CH$_2$**), 23.1 (O(CH$_2$)$_6$-
16　**CH$_2$**), 14.5 (O(CH$_2$)$_7$-**CH$_3$**) and 0.0 (Si-(**CH$_3$**)$_3$). ^{29}Si NMR (CDCl$_3$, 79.3 MHz): δ 17.5 ppm. Mass m/z
17　(EI) 202 (M$^+$), 187 (M$^+$-CH$_3$). NMR data were consistent with those reported [10].

18　3.1.2. Synthesis of Phenyldimethyloctyloxysilane

19　　1-octanol (28.65g, 0.22mol) and triethylamine (22.26g, 0.22mol) were dissolved in anhydrous THF
20　(250ml) under nitrogen in a 3-necked round-bottomed flask and a solution of
21　chlorodimethylphenylsilane (20.48g, 0.12mmol) in anhydrous THF (100ml) added dropwise to the
22　mixture over one hour. After gently refluxing at approximately 70°C for 2 hours, the
23　triethylammonium chloride was filtered off and the filtrate collected. After removing the solvent and
24　excess of triethylamine using a rotary evaporator, the product was purified by distillation at 142 °C and
25　approximately 85 mbar. The product was characterized by NMR spectroscopy and GC-MS and it was
26　used as a GC-standard in order to understand the product distribution during the enzyme-catalyzed
27　transetherification studies. Yield 44%. ^{1}H NMR (CDCl$_3$, 300 MHz): δ 7.22-7.02-7.01 (*m*, 5H, aromatic
28　**H**), 3.26 (*t*, 2H, *J* 6.6, O-C**H$_2$**), 1.17 (*m*, 2H, OCH$_2$-**CH$_2$**), 0.90 (*m*, 10H, OCH$_2$CH$_2$-(**CH$_2$**)$_5$), 0.51 (*t*,
29　3H, *J* 3.0, O(CH$_2$)$_7$-C**H$_3$**) and 0.0 (*s*, 6H, Si(C**H$_3$**)$_2$) ppm.　　　^{13}C NMR (CDCl$_3$, 75.45 MHz): δ 138.1-
30　133.4-129.5-127.7 (aromatic **C**), 63.0 (O-**CH$_2$**), 32.6 (OCH$_2$-**CH$_2$**), 31.8 (O(CH$_2$)$_2$-**CH$_2$**), 29.4
31　(O(CH$_2$)$_3$-**CH$_2$**), 29.2 (O(CH$_2$)$_4$-**CH$_2$**), 25.7 (O(CH$_2$)$_5$-**CH$_2$**), 22.6 (O(CH$_2$)$_6$-**CH$_2$**), 14.0 (O(CH$_2$)$_7$-**CH$_3$**)
32　and -1.7 (Si-(**CH$_3$**)$_3$). ^{29}Si NMR (CDCl$_3$, 79.3 MHz): δ 7.5 ppm. Mass m/z (EI) 264 (M$^+$), 249 (M$^+$-
33　CH$_3$). NMR data were consistent with those reported [11].

1   *3.2. Enzyme-Catalyzed Transetherification Reactions*

2   The reactions were formulated with a 5:1 alkoxysilane (100mg) to enzyme (20mg) weight ratio in
3   0.5 g of alcohol (water-equilibrated 1-octanol or tert-butanol containing 5% (v/v) buffered water).
4   Prior to analysis, the reactions were filtered through a Whatman Autovial® 5 0.45-µm Teflon® filter.
5   The closed (screw capped) two-phase reactions were conducted in inert glass vials at 25 °C with
6   magnetic stirring for 24 hours. The reaction products were isolated and analyzed by GC-FID
7   (quantitative) and GC-MS (qualitative).
8   Control reactions are defined as non-enzymatic reactions. Specifically, experiments conducted in
9   the absence of a protein are defined as negative control reactions.

10 *3.3. Gas Chromatography-Flame Ionization Detection*

11   The gas chromatography (GC) analyses were performed with an Agilent 6890 Series injector on an
12 Agilent 6890 plus gas chromatograph (GC) with a flame-ionization detector (FID).

Table 1: GC-FID experimental parameters.

Parameter	Setting
Carrier gas	99.9995% Ultra high purity helium (UHP)
GC inlet, split	250 °C, split ratio=100:1, constant flow (rate = 1.0ml/min.)
Detector	Flame ionization detector at 275 °C, H_2 = 40ml/min, Make up N_2 = 45ml/min.
GC column	HP-5MS crosslinked 5% phenylmethylsiloxane film (30m x 0.25mm, 0.25 µm film)
GC temperature program	50(2) → 250 (8) @ 10 °C/min, 30 min total run time
Internal standard	~1%(w/w) dodecane in THF
Data system	Agilent Technologies ChemStation

13   The system was configured as detailed in Table 1. Dodecane was used as an internal standard to
14 gravimetrically quantitate the chromatographic analyses. The samples were prepared at ~1% (w/w)
15 product in a THF solution containing 1% (w/w) dodecane. Based on triplicate measurements, the
16 response factors for the analytes were calculated (Equation **1**), and found to be linear as a function of
17 concentration over four orders of magnitude (i.e. 0.01-10% (w/w) (Table 3).
18     $RF_{analyte} = ([analyte]/Area_{analyte}) \times (Area_{IS}/[IS]) \times RF_{IS}$ (**1**)

19   where $RF_{analyte}$ = response factor for the analyte, [analyte] = concentration of the analyte, $Area_{analyte}$
20 = peak area of the analyte, $Area_{IS}$ = peak area of the internal standard, [IS] = concentration of the
21 internal standard, RF_{IS} = response factor for the internal standard = 1. Equation **1** was then solved to
22 quantitatively calculate the concentration of an analyte in the presence of an internal standard
23 (Equation **2**).

1

2    $[analyte] = (Rf_{analyte} \times Area_{analyte}) \times ([IS]/Area_{IS})$ (**2**)

3 **4. Gas Chromatography-Mass Spectrometry**

4   The gas chromatography-mass spectrometry (GC-MS) analyses were performed with an Agilent
5 6890 Series injector on an Agilent 6890 plus gas chromatograph with a 5973 MS detector. The MS
6 detector was autotuned with perfluorotributylamine (PFTBA) prior to analysis. The system was
7 configured as detailed in Table 4.

Table 3: GC-FID analyte retention times and response factors.

Analyte[1]	Retention Time (m)	Response Factor (RF)		
		Average	Standard Deviation	RSD[2]
Me_3SiOEt	2.75	2.00	0.007	3.3%
Me_3SiOH	2.63	2.35	0.091	3.9%
HMDS	3.22	2.01	0.052	2.6%
$PhMe_2SiOEt$	10.37	1.35	0.007	0.5%
$PhMe_2SiOH$	9.92	ND	ND	ND
phenyl disiloxane	17.42	ND	ND	ND
$PhMe_2SiO(CH_2)_7CH_3$	17.48	2.02	0.038	0.2%
$Me_3SiO(CH_2)_7CH_3$	10.97	2.15	0.027	1.3%
dodecane	11.11	1.00	---	---

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9
10
11

[1] Me_3SiOEt = trimethylethoxysilane, Me_3SiOH = trimethylsilanol, HMDS = hexamethyldisiloxane, $PhMe_2SiOEt$ = phenyldimethylethoxysilane, $PhMe_2SiOH$ = phenyldimethylsilanol, phenyl disiloxane = diphenyltetramethyldisiloxane, $PhMe_2SiO(CH_2)_7CH_3$ = phenyldimethyloctyloxysilane, $Me_3SiO(CH_2)_7CH_3$ = trimethyloctyloxysilane, dodecane = internal standard.

[2]   RSD = relative standard deviation

Table 4: GC-MS Experimental Parameters.

Parameter	Setting
Carrier gas	99.999% high purity helium
GC inlet, split	250 °C, split ratio= 50:1, constant flow (rate = 1.0 mL/min.)
GC column	HP-5MS crosslinked 5% phenyl methylsiloxane film (30m x 0.25mm, 0.25 um film)
GC temperature program	50(2) → 250 (8) @ 10 °C/min, 30 min total run time
GC-MS transfer line temperature	350 °C
MS ionization	electron impact
MS full scanning mass range	15-500 amu, 1scan/sec

Data system	Agilent Technologies ChemStation

4. Conclusions

The use of wet 1-octanol as a solvent system for the enzyme-catalyzed hydrolysis and condensation of alkoxysilanes led to the observation that some enzymatic candidates promoted the transetherification and/or alcohol exchange of alkoxysilanes. To our knowledge, this is the first example of a biocatalyzed process leading to the synthesis of a new alkoxysilane. The route offers a clear example for further expanding the potential of bio-catalyzed reactions at a silicon centre and would offer several advantages with respect to conventional chemical procedures, such as benign reaction conditions and the use of non-toxic catalysts. Further studies with different alcohols and in the absence of water are planned to explore the full potential of this novel biomimetic transetherification.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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