E-19-AAF
#4

Silicon biocatalysis in novel reaction media: results and perspective for next steps
Report to Dow Corning, August 16, 2006

Executive Summary

Coupling siloxane electrophiles (model compounds: siloxane-substituted benzyl chloride, SiBzCl, and methyl dimethiconate) with amino acids, polypeptides, proteins, or polyols, such as ascorbic acid or sucrose, could lead to enormous scientific and economic benefits. In our project in the first year we have pursued two routes to achieve this goal – phase transfer catalysis (PTC) and enzymatic methods. Both routes have now shown very promising initial results, and fruition is clearly in sight, as we have ironed out the initial issues regarding side reactions in both cases. We have used our expertise in synthesis and PTC (Liotta) to synthesize new siloxyl phase transfer catalysts to achieve an example of the desired coupling in both neat and solvent-based systems. This project has met all of the goals outlined in the original proposal and can be considered a complete success. We have carried out extensive investigations of possible solvent media, and we are using our expertise in phase equilibria (Eckert) to open new vistas along these lines. Polyols are not sufficiently soluble in standard organic media. Therefore, to have success in this area, novel solvent systems are required. Further, we are exploring media and reaction conditions for enzymatic coupling of siloxane electrophiles with polyols, including sucrose, ascorbic acid, glycerol and derivatives. The enzymatic work is coupled to the solvent development as such triphasic reaction systems (liquid siloxane, solid polyol, solid enzyme) are very hampered by mass transfer conditions. Our experience in biocatalysis (Bommarius) is leading to the application of standard enzymes in parallel to existing processes for fatty acid couplings, again with the goal of delineating the conditions for commercially successful processes. We can currently couple sucrose octaacetate and glycidol to siloxanes; however, yield is not enough to allow for purification. We have also developed standard procedures for the acetylation and trimethysilylation of compounds to further their solubility in organic media, and perhaps, increase their nucleophilicity for reaction. This report outlines our success over the past 10 to 11 months.

Results

Topic 1: Amino acid-siloxane conjugation

We have shown the use of siloxane-modified phase-transfer catalysts (PTCs) for the purpose of amino acid-siloxane conjugation. PTCs are often used to couple hydrophilic and hydrophobic species residing in orthogonal phases. Here, we have modified quaternary ammonium salts with siloxane functionality to create novel phase-transfer materials.

Work to date has focused on three main areas:

1) Synthesis of siloxane-based PTCs
2) Activity of organic and siloxane-based PTCs on a model nucleophilic displacement reaction onto a siloxane electrophile
3) Coupling of a siloxane electrophile with L-lysine and other amino acids

Area 1: Synthesis of siloxane-based phase-transfer catalysts

Three different siloxane-based PTCs have been synthesized: 1) tri(ethylene pentamethyl disiloxy)**methyl**ammonium chloride; 2) tri(ethylene pentamethyldisiloxy)**benzyl**ammonium chloride; and 3) triethyl(*p*-ethylene pentamethyldisiloxybenzyl)ammonium chloride (Figure 1). These are all novel materials and a patent disclosure has been sent to Dow Corning for review. (1) and (2) were synthesized using pentamethyldisiloxane, triallyl amine and either methyl chloride or benzyl choride. (3) was synthesized using triethyl amine and the SiBzCl provided by Dow Corning. Structures were verified using NMR, ESI-MS and elemental analysis.

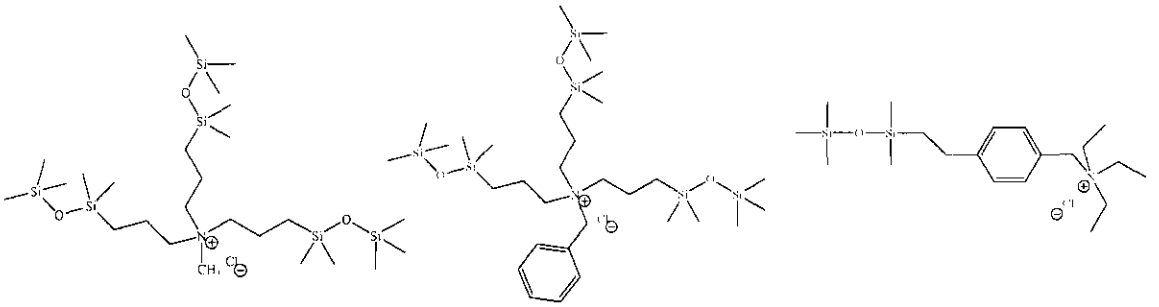

Figure 1. Novel siloxane-based PTCs synthesized for this project. Si-PTC #1 (left) Si-PTC #2 (center) Si-PTC #3 (right).

Area 2: Activity of PTCs with siloxane-based electrophiles

In order to simplify analysis, the siloxane electrophile, 2-pentamethyldisiloxy- *p*-ethyl benzyl chloride (SiBzCl), was coupled with various inorganic and organic nucleophiles for analysis by GC-MS. The nucleophiles included potassium cyanide (KCN), potassium thiocyanate (KSCN), and potassium acetate (KOAc). The activity of this reaction was examined both neat and with 80% toluene or ethyl acetate as a solvent and also with various PTCs. Both commercially available organic-based PTCs (tetrabutylammonium chloride (TBACl), tetrabutylammonium bromide (TBAB), tetraoctylammonium chloride (TOACl) and trioctylmethylammonium chloride (Aliquat 336)) and the three novel siloxane-based PTCs were utilized. Pertinent control reactions were also performed.

Table 1 presents results for the reaction of *p*-SiBzCl with a variety of nucleophilic salts in ethyl acetate (EtOAc) as solvent. Pseudo-first order rate constants were calculated based on an excess of nucleophile. Results were analyzed by GC-FID with decane used as an internal standard. It is apparent from these data that tetrabutylammonium chloride (TBACl) was the most effective phase transfer agent, with the siloxane-based PTCs performing poorest. This result is somewhat expected because of the organic nature of the solvent system, which is less favorable for siloxane-based compounds. Additionally, the nature of the nucleophile played a vital role both in the speed of conversion and also the intensity of the PTC-related effects. The stronger nucleophile KOAc converted 600 times faster than KCN, and TBACl was nearly 100 times more effective than SiBz PTC for this nucleophile. Meanwhile, the rates varied by only a factor of 2.5

for the weaker nucleophile KSCN, which is a weaker nucleophile than KOAc and also less soluble in EtOAc. These results differed greatly from the solventless reactions discussed below.

Table 1. Pseudo-first order rate constants for the reaction of several nucleophiles with *p*-siloxane electrophile and various PTCs at 70 °C and 900 rpm stirring. 5x excess KOAc was used in all conditions and equimolar KCl salt added to ensure constant ionic composition.

	Pseudo-First Order Rate Constant (1/s) [k1]			
PTC (5%)	**KCN**	**KSCN**	**KOAc**	**Lysine**
None	No Rxn	-	No Rxn	No Rxn
TBACl	1.3E-06	4.0E-05	8.0E-04	9.1E-06
A336	-	2.8E-05	4.0E-04	
SiMePTC	-	1.8E-05	3.5E-05	
SiBzPTC	-	1.15E-05	6.9E-06	

Table 2 presents results for the reaction of *o*-SiBzCl, a second isomer found in the starting material, in the same EtOAc reactions as in Table 1. Pseudo-first order rate constants followed similar trends for this isomer also, with rates generally slightly faster than for the *para* isomer.

Table 2. Pseudo-first order rate constants for the reaction of several nucleophiles with *o*-siloxane electrophile and various PTCs at 70 °C and 900 rpm stirring. 5x excess KOAc was used in all conditions and equimolar KCl salt added to ensure constant ionic composition.

	Pseudo-First Order Rate Constant (1/s) [k2]			
PTC (5%)	**KCN**	**KSCN**	**KOAc**	**Lysine**
None	No Rxn	-	No Rxn	No Rxn
TBACl	2.0E-06	4.1E-05	9.2E-04	1.2E-05
A336	-	2.5E-05	4.6E-04	
SiMePTC	-	1.7E-05	4.5E-05	
SiBzPTC	-	1.28E-05	9.9E-06	

Table 3 presents sample results for the displacement of KOAc onto the siloxane electrophile catalyzed by TBACl under solventless conditions. 98% conversion at 70°C was achieved in 105 min using 10% PTC (relative to electrophile) and 5x excess KOAc. The reaction was approximately 2.5-fold faster at 70°C compared to 50°C. We determined an activation energy for the EtOAc-based reactions of 65 kJ/mol. The control reaction showed no conversion in 1100 minutes in the absence of PTC. Figure 2 compares time-dependent behavior for each of these conditions.

Table 3. Reaction of KOAc with siloxane electrophile and various amounts of TBACl PTC at 70 and 50 °C. 5x excess KOAc was used in all conditions.

% PTC (TBACl)	Temperature (°C)	% Conversion (105 min)	Pseudo-first order rate constant (s^{-1})	Second-order rate constant (mL·s/mol)
0	70	0	0	0
1.2	70	17	3.1×10^{-5}	0.011
5.0	70	51	1.3×10^{-4}	0.045
10.1	71	89	3.3×10^{-4}	0.11
5.3	50.5	15	3.0×10^{-5}	0.011

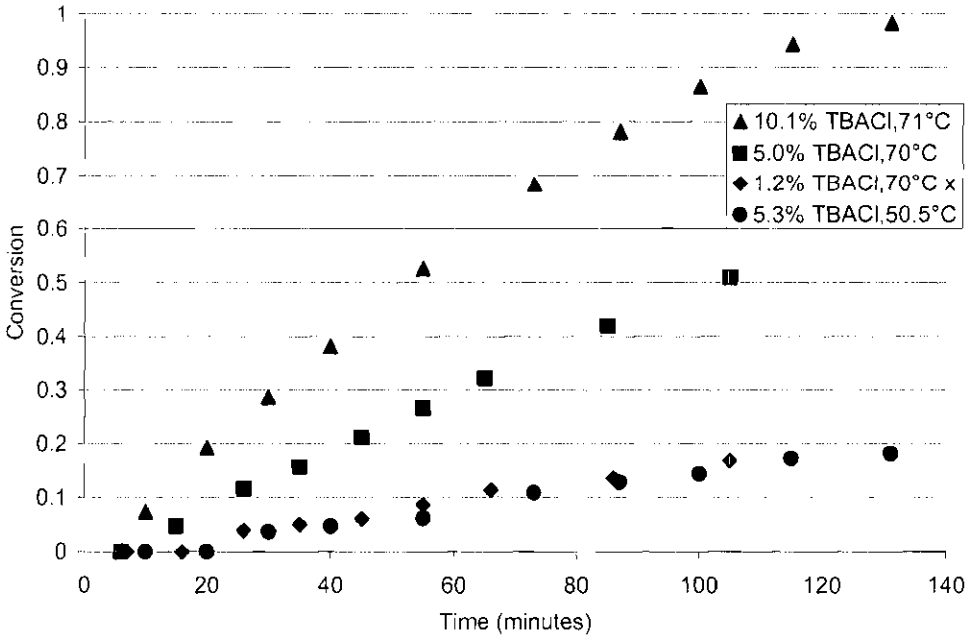

Figure 2. Time-dependent behavior for results shown in Table 1.

Figure 3 compares five PTCs (two organics, three siloxanes) for the reaction of KCN with the siloxane electrophile under solventless conditions. At 70°C and 16 h, the more nonpolar PTCs (Aliquat 336 and Si-PTC #3, see Figure 1) performed best, with 8.5 and 9.5% conversion. The low nucleophilicity of cyanide results in very low reaction rates.

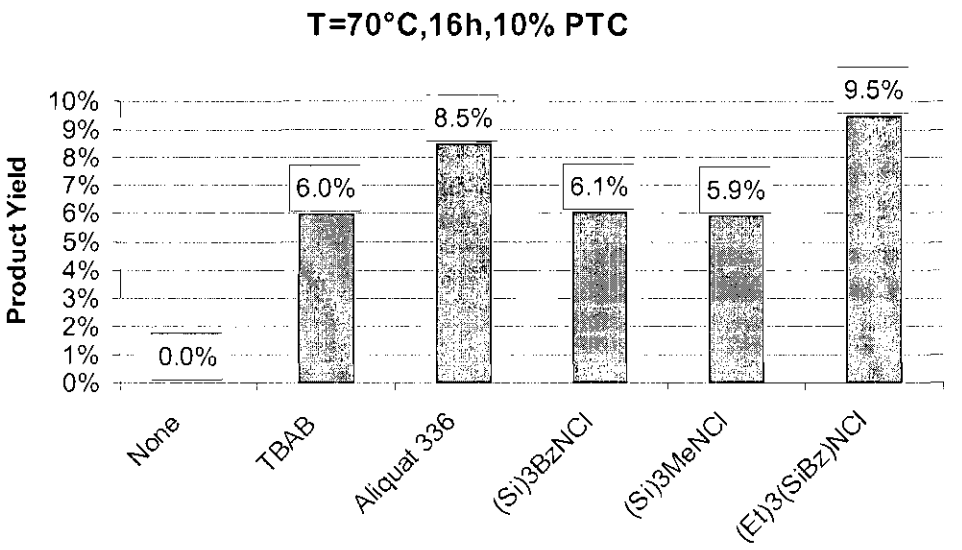

Figure 3. Comparison of PTCs for KCN displacement at 70°C, 10% PTC and 16 hours.

Figure 4 compares two different nucleophiles (KCN and KSCN) with TBAB and Si-PTC #3. Conversions were much higher for KSCN, and the siloxane-based PTC outperformed TBAB, 100% to 49% conversion in 16 h at 67°C. Only the KSCN control showed any conversion (10% in 1100 min). Again, under solventless conditions, the less polar siloxane-based PTCs outperformed the organic PTCs. This trend is the opposite of that found for EtOAc and toluene-based solvent systems. The polarity (or siloxane-character) of the reaction medium clearly dictates which PTC will be most effective. The reaction rates are also 7-10 times higher for solventless systems due to the increase in substrate concentration. Reaction kinetics are more difficult to obtain accurately in the absence of solvent due to limited sample size, leading to more significant errors in the solventless rate measurements.

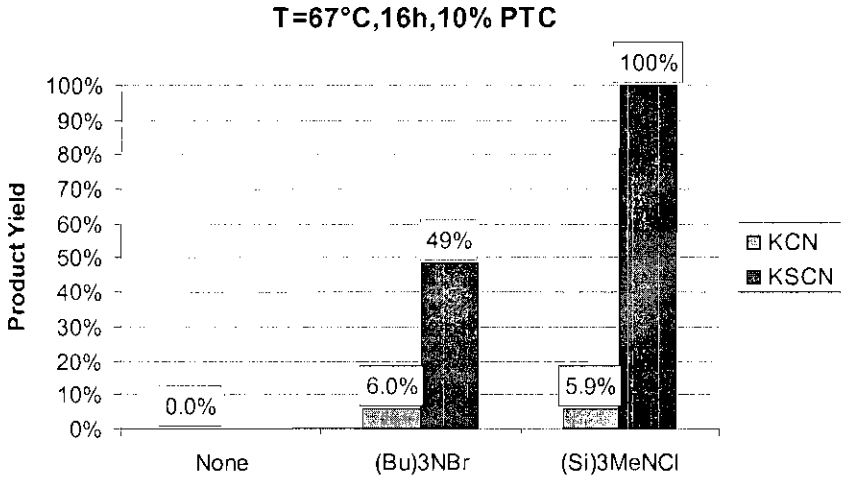

Figure 4. Comparison of KCN vs. KSCN displacement at 67°C, 10% PTC and 16 hours.

The use of decane as an internal standard enabled detection of a slow side reaction which consumed siloxane electrophile. The reaction was occurring only in the presence of quaternary ammonium salts, not with neat siloxane, siloxane + L-lysine, siloxane + ionic liquid or siloxane + nucleophilic salt (KSCN, KCN, KOAc). Table 4 compares the side reaction conversion in the absence of nucleophile with several PTCs and other additives. On the time scale of L-lysine coupling (< 14 hrs) the conversion is generally limited to 10% for 5% PTC. The siloxane-based PTCs showed slightly slower rates for the undesired reaction than organic PTCs. The product has MW = 395 and is presently unidentified.

Table 4. Conversion of side reaction of SiBzCl with various PTCs at 70°C. TBAB = tetrabutylammonium bromide; TOACl = tetraoctylammonium chloride; bmim Cl = 1-butyl-3-methylimidazolium chloride (ionic liquid). All reactions were performed in 4:1 toluene:siloxane as solvent unless otherwise indicated. A "-" means not determined.

PTC	14 hr	21 hr	42 hr	4 days
TBAB (5%)	-	37%	-	-
TBAB (5%), diluted	-	11.5%	-	-
TBAB (5%), no solvent	-	28%	-	-
TBAB (100%)	41%	30%	-	-
TBAB (5%), no solvent, anhydrous	8%	52%	56%	71%
TBAB (5%), 45°C	-	-	-	33%
Aliquat 336 (5%)	-	27%	-	-
TBACl (5%)	-	34%	-	-
TOAB (5%)	19%	29%	34%	48%
Si-PTC #1 (5%)	11%	12%	11%	12%
Bmim Cl (5%)	-	0.7%	-	-
L-lysine (5%)	-	0%	-	-
None	-4% (gain)	-	-	-3% (gain)

To determine the cause of this side reaction, we synthesized the siloxane electrophile for comparison with that provided by Dow Corning. Our synthesis coupled *p*-vinyl benzyl chloride with pentamethyldisiloxane using Pt-DVDS as catalyst. The material was purified by column chromatography, resulting in three fractions with isomeric ratios (para:meta) of 2:1, 3:1 and 28:1. The Dow Corning material has an isomeric ratio of 2:1. The side reaction was completely suppressed in the 28:1 and 2:1 fractions. The presence of residual catalyst in the 3:1 fraction prevented its use. Elemental analysis, ESI-MS and NMR confirmed the structure. The Dow Corning material also showed no impurities by elemental analysis, leading us to speculate that residual organometallic catalysts in the starting material might be responsible for the side reaction.

Area 3: Coupling of siloxanes with L-lysine

The model amino acid for coupling with the SiBzCl is L-lysine. The proposed reaction scheme is shown as Figure 5. The reaction has been performed by Dow Corning in 1:1 (v:v) methanol:acetonitrile solvent mixture with equimolar reagents. We duplicated this experiment, observing 70% conversion in 22 hours, 82% after 42 hours and 91% after 66 hours. By using no solvent and TBACl, we were able to achieve 100% conversion in just 16 hours. This experiment used 10% PTC relative to SiBzCl and fivefold excess of L-lysine, similar to the PTC displacements. Product analysis was performed by NMR and ESI-MS and indicates predominantly mono-substituted product, though neither technique has been conclusive. We believe that both α- and ε-substitution may be occurring.

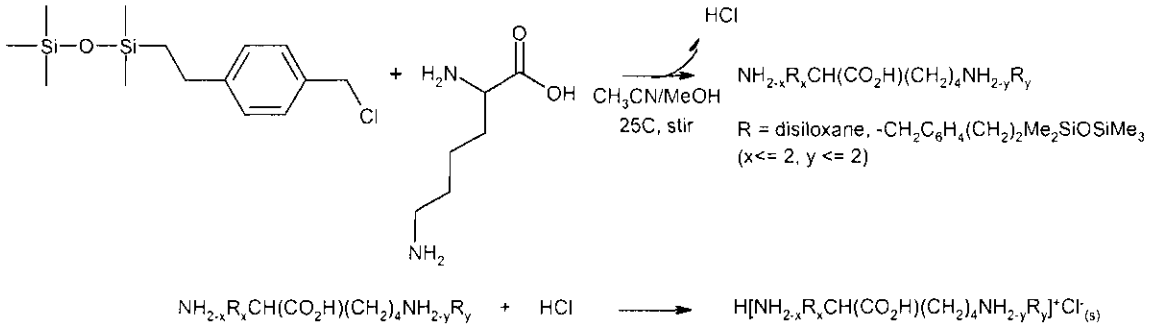

Figure 5. Coupling of SiBzCl with L-lysine.

Using 5% TBACl at 70 °C, multiple runs in EtOAc have yielded a pseudo-first order rate constant of 1.06 * 10^{-5} s^{-1} for this reaction, using 3x excess lysine. This rate is similar to that observed for the displacement of KOAc, the fastest nucleophile we employed. This is an excellent result, as it indicates that lysine is a very active nucleophile for the coupling reaction. In the absence of any PTC, the reaction does not yield any conversion after 24 hrs.

Coupling of SiBzCl with six other amino acids (L-glutamic acid, D-phenylglycine, glycine, L-arginine, L-aspargine, L-glutamine) gave varying degrees of conversion in 16 hours. However, we were unable to verify product structure by NMR. Coupling of SiBzCl with poly(L-lysine) showed 10% disappearance of siloxane by GC-FID in 16 hours and quantitative conversion after 72 hours. Product analysis has yet to be performed to verify coupling occurred.

The use of gas-expanded methanol (mixture of CO_2 and methanol) as a solvent for this reaction has been explored briefly. A saturated solution of methanol with L-lysine was added to an immiscible portion (equimolar to L-lysine) of SiBzCl. At approximately 35 bar CO_2 the siloxane dissolved in the mixture, creating a single phase. At 60 bar, the L-lysine began to precipitate from solution. For reaction, a mixture of L-lysine in methanol at 50% of saturation has been coupled with equimolar SiBzCl at 40-60 bar CO_2 pressure. The reaction has been performed several times and conversions are, on average, similar to the acetonitrile/methanol system, with conversions of 40-60% observed in the first 24 hrs and 70-90% after 48 hrs. This system is a promising alternative for the coupling reactions and should be explored further.

Next steps
We plan to extend the current PTCs to polypeptides and proteins. Some of this work is already underway.

Topic 2: Enzymatic coupling of polyols with alkylsiloxane (di)acids

The model reactions chosen to explore methods to couple polyols and siloxane-containing carboxylic acid moieties are the coupling of sucrose, glycerol and ascorbic acid to methyl esters of a siloxane-based mono- and diacid (Figure 6). One main engineering problem to be solved is one of phase behavior since the long-chain siloxane ester is very hydrophobic and the polyols are very hydrophilic. Therefore, the two are not miscible and solvents that dissolve one usually do not dissolve the other.

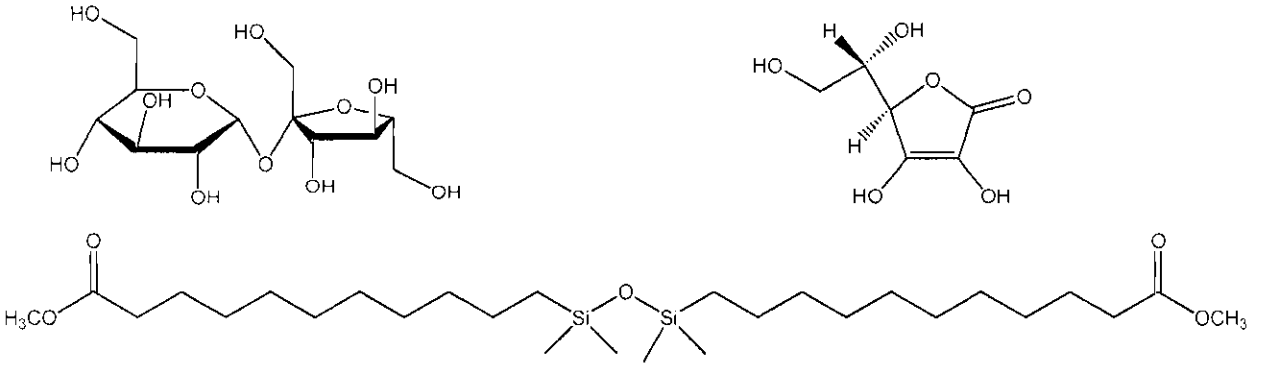

Figure 6. D-Sucrose (top left); L-ascorbic acid (top right); dimethoxy "dimethiconate" (bottom).

Work to date has been focused on three main areas:

1) Traditional organic solvents and mixtures thereof
2) Ionic liquids and deep eutectic solvents
3) Manipulating the solubility and nucleophilicity of the polyol via chemical modifications.

Area 1: Single and Mixed Traditional Organic Solvents

Both sucrose solubility and enzyme activity of the immobilized *Candida antarctica* lipase B (CALB) N435 were tested in a wide range of organic solvents including methanol, ethanol, isopropanol, 1-butanol, 1-hexanol,1-octanol, *t*-butanol, *t*-amyl alcohol, n-heptane, chloroform, acetone, acetonitrile, 1,4-dioxane, dimethyl sulfoxide (DMSO), diethyl ether, toluene, the ionic liquid (bmim)PF_6, pyridine, tetrahydrofuran, dimethyl formamide (DMF), dimethyl acetamide (DMA), N-methyl pyrrolidone (NMP), hexamethylphosphoramide (HMPA), and hexamethylphosphortriamide (HMPTA). Sucrose is only sparingly soluble in most of these, with the exceptions of DMSO (> 300 g/L), methanol (80 g/L), DMF, pyridine, or HMPA (10 g/L), DMA (8 g/L), and NMP or HMPTA (5 g/L). Methanol is not favorable for enzymatic activity. We were unable to detect product in reactions of sucrose and the methyl ester of dimethiconate performed in DMF, DMA, NMP, HMPA, pyridine, or HMPTA, or in reactions

performed in 50:50 DMSO/t-butanol or mixtures of t-butanol and water. Mixtures involving water caused hydrolysis of the methyl ester of the dimethiconate. Although CALB tends not to be active in basic solvents like pyridine or NMP, there is some evidence that subtilisin is stable in these solvents. Thus, immobilized subtilisin was explored as a catalyst in DMF, DMA, NMP, and pyridine. However, only trace amounts of sucrose-ester coupling were detected.

To understand if the lack of reaction is caused by the enzyme not accepting the methyl ester of the siloxane as a substrate, we performed two control reactions. First, the methyl ester of dimethiconate was hydrolyzed to yield the carboxylic acid using N435 in water/*t*-butanol. The reaction proceeded to completion within 24 hours. Second, the methyl ester of dimethiconate was converted to the ethyl ester using ethyl acetate as a solvent/reactant. This reaction also proceeded to completion within 24 hours, whereas a control reaction without enzyme showed no conversion. The conversion achieved was similar to the conversion of ethyl palmitate to its methyl ester using methyl acetate as a solvent.

We also tested the stability of soluble forms of CALB, the large-substrate variant of CALB (ICR), and several variant subtilisins (FNA, GG-36, and P-3000) supplied by Genencor in mixtures of water and acetone (Figure 7). Both CALB and ICR lose activity very quickly with the introduction of acetone, whereas the proteases tend to retain a larger proportion of their activity.

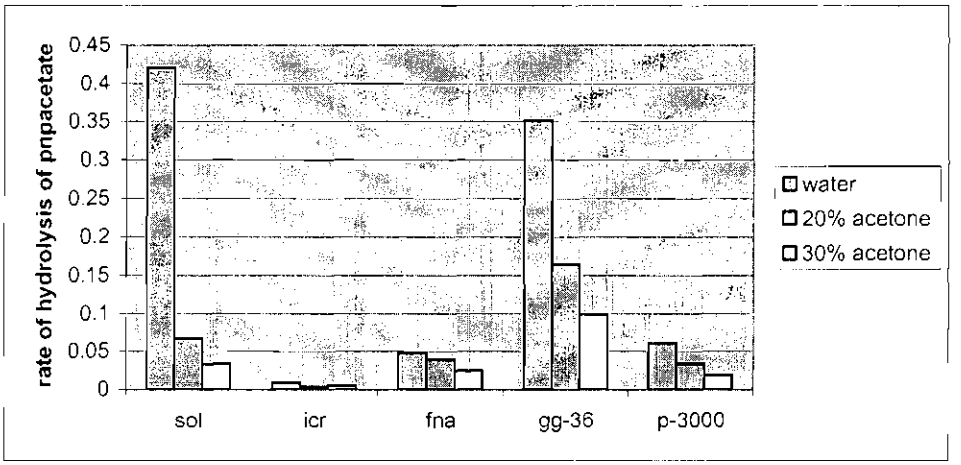

Figure 7. Comparison of the activity of 5 enzymes in water-acetone mixtures.

Since Genencor has dropped out as a partner during the reporting period, we stopped testing its esterase and protease constructs and focused on commercially available and widely known and used N435 for this period. We also obtained a commercially available form of subtilisin and immobilized it and ICR on Eupergit to test activity.

Ascorbic acid ester synthesis was also attempted using unprotected ASA and methyl dimethiconate in t-butanol and water mixtures. Less hydrolysis was detected than with the sucrose case, but no measurable levels of product were present.

Area 2: Ionic Liquids and Deep Eutectic Solvents

Prior work by Sheldon and colleagues [2] tested the solubility of sucrose in a range of ionic liquids. Those with a dicyanamide anion were able to dissolve substantial amounts of sucrose. The highest solubility of 250 g/L was reported in butylmethylimidazolium dicyanamide, which also is miscible with methyl dimethiconate. We combined sucrose at 170 g/L with an equal molar amount of methyl dimethiconate and 10 mg of N435 immobilized catalyst and allowed the reaction to occur at 50°C. After 2 weeks, the reaction mixture was extracted with hexane three times, then with ethyl acetate three times. The hexane and ethyl acetate fractions were evaporated to remove the solvent and the residual powder dissolved for NMR. No product was detected in the NMR samples. We also attempted the reaction of ascorbic acid and dimethiconate in bmim-dca. No product was detected in these NMR samples. We are unable to conclusively prove that the product is not formed and just retained in the ionic liquid, as this would be a likely result.

Because the dicyanamide anion seems to dissolve a large amount of sucrose, we explored the possibility of creating a novel ionic liquid with an electronically similar anion (Figure 8) by reacting butyl imidazole with malononitrile. The result was a brownish liquid that did not dissolve sucrose.

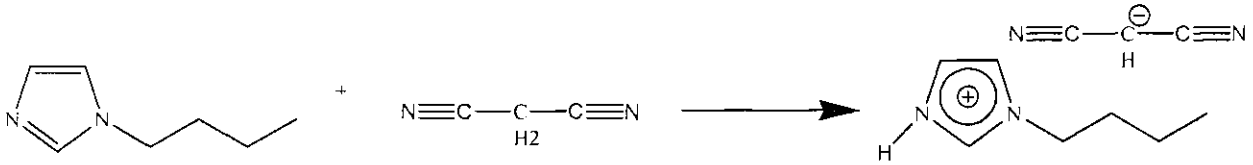

Figure 8. Reaction of butyl imidazole with malononitrile to form a novel ionic liquid.

Finally, we explored solubility of sucrose in mixtures of choline chloride and urea, which form a deep eutectic solvent at room temperature [3]. Sucrose solubility was less than 1 g/L in this mixture. In addition, although choline chloride carboxylic acid mixtures have been known to form deep eutectics [4], the mixture of dimethiconic acid and choline is not a liquid even up to 50°C.

Area 3: Chemical Modification of the Polyols

Addition of trimethylsilyl groups to the hydroxyls of ascorbic acid (Figure 9- TMS-ASA) increases the solubility of the ascorbic acid and promotes reaction with the methyl ester of dimethiconate. The coupling of TMS-ASA and the ester of dimethiconate in *t*-amyl alcohol and *t*-butanol shows evidence of conversion when analyzed by thin layer chromatography.

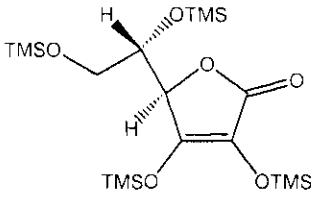

Figure 9. TMS-ASA.

We have developed procedures for the trimethylsilylation of glucose and sucrose based on literature procedures [5]. The sugars are dissolved in anhydrous pyridine, followed by the addition of hexamethydisilazane and trimethylchlorosilane. After vigorous agitation for 30 seconds to 1 minutes, the mixture is allowed to stand at room temperature for 30 minutes. GC-MS analysis confirms that both glucose and sucrose are trimethylsilylated using this procedure.

An additional modification to increase both solubility and reactivity [6] is acetylation (Figure 10). Literature suggests that sucrose octaacetate esters do retain their surface activity [7] and that the acyl groups can be selectively removed by treatment with trifluoroacetic acid [8] and possibly in certain positions enzymatically [9,10]. We have shown that commercially available sucrose octaacetate in neat t-butanol at 50°C can be transesterified with the siloxane. Following reaction, the mixture was evaporated by rotary evaporation and extracted with hexane to remove the product and leftover siloxane starting material. The sample was then subjected to NMR for analysis, which showed evidence of product. Conversion is estimated at 5% (please see section below on side reaction). We have been unable to purify the product by extraction procedures, most likely due to the low level of conversion. An analogous reaction using unacylated sucrose (0.4 g/L) did not show any conversion after one week of reaction. Control reactions without enzyme did not produce any product. Reactions using a different side siloxane side chain (Figure 11) also produced similar conversion.

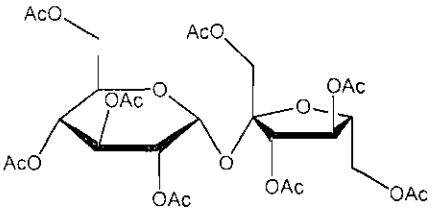

Figure 10. D-(+)-Sucrose octaacetate.

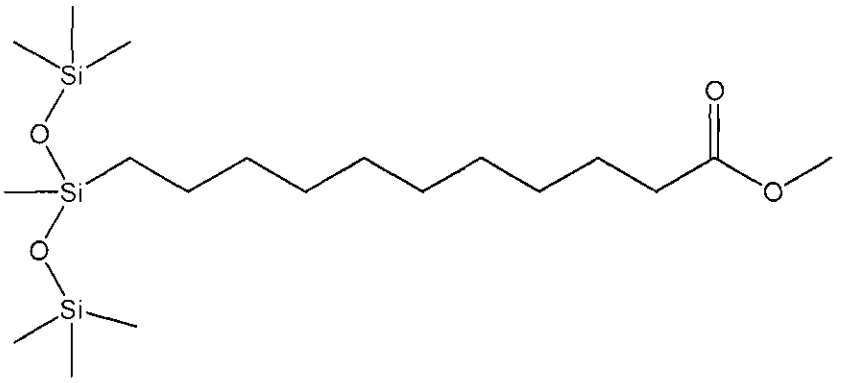

Figure 11. Methoxy monoester of new "t-shaped" siloxane side chain provided by Dow Corning.

In the analogous case of synthesis of fatty acid esters of sucrose very few reports have been able to get significant conversion using alkyl esters due to the low reactivity [11]. The yield on the reaction can be improved via the use of vinyl esters, which are proven to be more reactive than alkyl esters [12]. Additionally, although we use molecular sieves to dry the reaction medium as well as to sequester the methanol produced during the reaction, conversion may be increased by using more stringent procedures for these purposes such as reacting under vacuum or using azeotropic distillation.

Another factor that may be limiting conversion to the desired product is the existence of a rapid and substantial side reaction. This reaction is enzymatically catalyzed and involves only the siloxane side chain. The reaction is favored in t-butanol over t-amyl alcohol and can result in as much as 90% of the starting material being siphoned off. In the case of the dimethiconate, there is a single side product. For the t-shaped siloxane side chain, there are two products. Conversion is much higher in the latter case. We have successfully ruled out hydrolysis of the ester as the cause. The products in question are actually of higher molecular weight than the starting material and have similar volatility in GC-MS analyses. They may represent some sort of condensation product. We are currently attempting to isolate the side product via distillation.

We are currently trying to develop other analytical procedures to aid in the determination of reaction kinetics and to optimize for yield. There are other, more expensive modifications to sucrose, such as the use of trimethylsilyl (TMS) derivatives, t-butoxycarbonyl (boc) derivatives or acetals that could increase the solubility and nucleophilicity of sucrose but be easily removed by chemical modification. Some of these are currently being explored, as well as alternate derivatizations of ascorbic acid and glucose (per-acylation) based on literature procedures (compound plus an excess of acetic anhydride reacted under nitrogen at 85-90°C for 3 hours) [13]. We are able to create both glucose pentaacetate and ascorbyl tetraacetate based on GC-MS analysis.

Glycerol has become an important feedstock because it is inexpensive and easily recovered from a wide variety of natural products. We have examined the reactivity of glycidol (Figure 12), a more reactive derivative of glycerol. Coupling of the siloxane monoester with glycidol in both t-butanol and t-amyl alcohol proceeded using immobilized subtilisin as the enzymatic catalyst. The product(s) were detected by GC-MS, with reaction in t-butanol greatly exceeding that in t-

amyl alcohol. Reactions utilizing N435 did not show any product after 40 hours. Also, reactions with glycerol using subtilisin yielded a large range (4-6) of products in small yield after 65 hrs. Coupling of siloxanes with glycerol also yielded no product when N435 was used as catalyst.

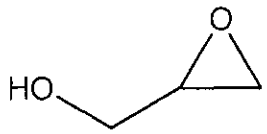

Figure 12. Glycidol, a more reactive derivative of glycerol.

Next steps

During the next phase, we plan to test the reactivity of sucrose, glucose, ascorbic acid, and glycerol, as well as their trimethylsilylated and acetylated derivatives. We also plan to explore the use of sulfur-dioxide expanded organics (liquid sulfur dioxide is one of the best solvents for sucrose- [1]) to dissolve more sucrose in enzymatically favorable media which also dissolve substantial amounts of siloxane. We believe that media which dissolve substantial amounts of sucrose will also be favorable for ascorbic acid solubility.

Summary of Current Results:

Our work on this topic has focused in three main areas. We have successfully synthesized and fully characterized three novel siloxane-based phase-transfer catalysts for use in coupling hydrophilic and hydrophobic compounds, such as siloxanes with amino acids, polypeptides or proteins. We have tested these three PTCs along with several organic PTCs on a model nucleophilic displacement reaction involving a siloxane electrophile and various inorganic and organic ionic nucleophiles, demonstrating that the PTC method is effective and that the siloxane-based PTCs generally outperform traditional organic PTCs under solventless conditions, while the organic PTCs are more effective when organic solvents are employed. We have also successfully coupled several amino acids, such as L-lysine, with the siloxane electrophile using the siloxy PTCs. These reactions proceeded to quantitative conversion of siloxane, indicating significant PTC activity.

Additionally, the coupling reaction proceeds under gas-expanded liquid conditions, with a CO_2/methanol mixture utilized as solvent. The homogeneous reaction yields comparable results to an acetonitrile/methanol mixture, indicating that CO_2 is an acceptable replacement for acetonitrile, with easier product separations and lower solvent usage. It is not yet known if the solvent system has any effect on product distribution.

Currently, the best method of obtaining a sucrose ester of dimethiconate is the reaction of acylated sucrose and the methyl ester of dimethiconate in *t*-butanol using immobilized lipase N435. The acylation of sucrose increases the solubility in *t*-butanol from 0.4 g/L to 25 g/L and also increases the nucleophilicity of the hydroxyl groups. The resulting product should be

surface active and schemes for removal of the acyl groups have been published in the literature. Additionally, synthesis of esters of TMS-ASA and dimethiconate proceeds in *t*-butanol and *t*-amyl alcohol. We believe that solvent systems that are suitable for unprotected sucrose will also translate to the production of esters of unprotected ascorbic acid. Table 5 gives a summary of the enzymatic results to date.

Table 5. Enzymatic results of conversion with various polyols

		siloxane
Polyol	dimethiconate	t-shaped
ascorbic acid	no conversion	no conversion
Sucrose	no conversion	no conversion
acetyl sucrose	5%	5%
Glycerol	no conversion	< 1%
Glycidol	not tested	14%

Not tested yet: acetyl ascorbic acid, TMS-sucrose, glucose, acetyl glucose, TMS-glucose, and glycerol acetate. It is apparent from Table 5 that methyl esters of siloxane-containing esters do not react well and that measures to increase solubility and, most likely, nucleophilicity, are required for high conversions.

We have also recently determined that sucrose has a significant solubility (10-25 wt%) in piperylene sulfone, a novel reversible solvent that has DMSO-like properties but can be easily removed at modest temperatures. [14] The use of this new solvent could provide a method of combining DMSO-like solvent power with easy product purification.

References

1. Dobrzycki J (1984) Chemiczne Podstavy Technologii Cukru Wyd. (Chemical Principles of Sugar Technology). Warsaw Poland.
2. Liu, Q, Janssen MHA, van Rantwijk F and RA Sheldon (2005) Green Chemistry, 7, 39-42
3. Abbott AP, Capper G, Davies DL, Rasheed RK, and V Tembyrajah. (2003) Chemical Communications, 70-71
4. Abbott AP, Boothby D, Capper G, Davies DL and RK Rasheed. (2004) Journal of the American Chemical Society, 126, 9142-9147.
5. Sweeley CC, Bentley R, Makita M, Wells WW. (1963) Journal of the American Chemical Society, 85, 2497-2507.
6. Steverink MC, Kneepkens MFM, de Waard P, Woudenberg-van Oosterom M, Gotlieb KF, Slaghek TM. (1999) Green Chemistry, 1, 153-156.
7. Prey V. Ger. Offen. (1974), 17 pp.
8. Obaje OJ. U.S. Pat. Appl. Publ. (2002), 8 pp.
9. Hennen WJ, Sweers M, Wang Y-F, Wong C-H. (1988) Journal of Organic Chemistry, 53, 4939-4945.
10. Bornemann S, Cassells JM, Dordick JS, Hacking AJ. (1992) Biocatalysis, 7, 1-12.
11. Reyes-Duarte D, Lopez-Cortes N, Ferrer M, Plou FJ, Ballesteros A. (2005) Biocatalysis and Biotransformations, 23, 19-27.
12. Wang Y-F, Lalonde JJ, Momongan M, Bergbreiter DE, Wong C-H. (1988) Journal of the American Chemical Society, 110, 7200-7205.
13. Ranu BC, Dey SS, Hajra A. (2003) Green Chemistry, 5, 44-46.
14. Liotta/Eckert unpublished results.