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One-Pot Sequences of Reactions with Sol-Gel Entrapped Opposing Reagents: An Enzyme and Metal-Complex Catalysts

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Abstract: We extend our sol-gel methodology of one-pot sequences of reactions with opposing reagents to an enzyme/metal-complex pair. Sol-gel entrapped lipase and sol-gel entrapped $RhCl[P(C_6H_5)_3]_3$ or $Rh_2Co_2(CO)_{12}$ were used for one-pot esterification and C−C double bond hydrogenation reactions, leading to saturated esters in good yields. When only the enzyme is entrapped, the homogeneous catalysts quench its activity and poison it. Thus, when 10-undecenoic acid and 1-pentanol were subjected in one pot to the entrapped lipase and to homogeneously dissolved $RhCl[P(C_6H_5)_3]_3$ under hydrogen pressure, only 7% of the saturated 1-pentyl undecanoate was obtained. The yield jumped 6.5-fold when both the enzyme *and* the catalyst were immobilized separately in silica sol-gel matrixes. Similar one-pot esterifications and hydrogenations by sol-gel entrapped lipase and heterogenized rhodium complexes were carried out successfully with the saturated nonoic, undecanoic, and lauric acids together with several saturated and unsaturated alcohols. The use of (*S*)-(−)-2-methylbutanol afforded an optically pure ester. The heterogenized lipase is capable of inducing asymmetry during esterification with a prochiral alcohol. Both the entrapped lipase and the immobilized rhodium catalysts can be recovered simply by filtration and recycled in further runs without loss of catalytic activity.

Background

Heterogenization of reagents and catalysts by their direct or physical entrapment in sol-gel inorganic matrixes has become a widespread method.[1] Many useful families of reactions have been explored in this context, some major ones being reactions for analytical and sensing purposes,[1,2] catalytic reactions,[1,3] electrochemical reactions,[4] and reactions with entrapped proteins[5] and cells.[6] The fast growth of these applications is mainly due to a remarkable property of these functional doped materials, namely that, on one hand, the dopant is well isolated and protected within the porous matrix and yet, on the other hand, it is accessible to substrate molecules which enter the matrix from the environment by a diffusion process through the pore network, reach the entrapped reagent, interact with it, and release a product back to the environment through the same pore network. This property can be rationalized, in the case of silica sol-gel matrixes, by assuming the molecules to be entangled in cyclic cage-like Si_mO_n fragments which are known to be formed during the early stages of the sol−gel process[7] in such a way that, while being tightly held, their active moieties are still open to the pore network of the ceramic matrix.

With this picture in mind, it becomes clear that, when in the neighborhood of a chemical which is entrapped within a sol−gel matrix, there is another sol-gel matrix doped with another reagent, these two chemicals cannot interact with each other when dispersed in a solvent, and these entrapped chemicals are still accessible to react with substrate molecules which are dissolved in that solution. Therefore, in principle, even an acid

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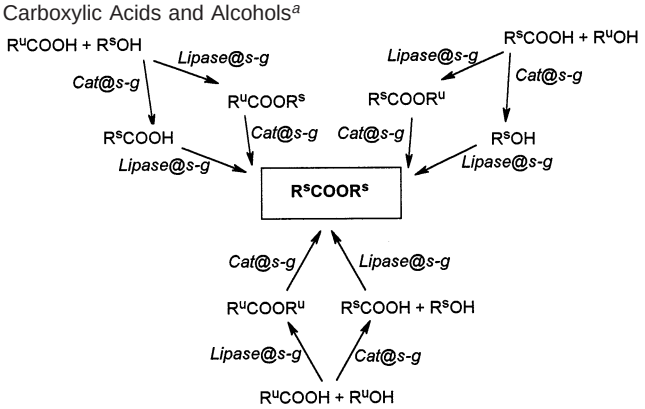
and a base can be put in the same pot without mutual annihilation, if properly protected by the sol−gel matrix. Where can this property be put to use?

Synthetic chemistry is governed traditionally by linear, consecutive sequences of reactions linking starting materials to products through intermediate molecules. Tedious as this route may be, there is not much choice, because each synthetic step is carried out with a different reagent or catalyst, which quite often quenches each other if put in one pot. Efforts to address this one-pot problem by anchoring reactive species to polymeric supports are known,[8] but this approach never gained popularity, apparently because of a host of problems such as the noninertness of the polymeric support, destructive exposure of anchored opposing moieties to each other, and complicated synthetic routes for the preparation of derivatized polymers, all of which make the saving of steps in a one-pot procedure not worthwhile. Experience gained with the porous, high surface area, inert sol-gel matrixes and the ease of their functionalization, simple one-step direct physical or covalent entrapment, have made this approach an attractive alternative as a heterogenizing support for multiple reagent one-pot procedures.

In a recent series of publications, we have shown the versatility of this sol-gel approach to one-pot sequences of reactions with several reaction pairs.[9] These include (i) indeed reaction sequences in which one step requires an acid while the other requires a base, an example being the acid-catalyzed pinacol−pinacolone rearrangement followed by a base-promoted condensation of the ketone with malononitrile;[9a] (ii) one-pot oxidation/reduction sequences, with an example being the conversion of 1-(4-nitrophenylethanol) into 4-aminoacetophenone, where the oxidant was SiO_2−sol-gel entrapped pyridinium dichromate and the reductant H_2 activated by entrapped $RhCl[P(C_6H_5)_3]_3$;[9b] and (iii) one-pot sequences requiring metallic catalysts in the presence of catalyst inhibitors, with an example being the base-catalyzed dehydrohalogenation of phenethylbromide by an immobilized diamine, followed by $RhCl[P(C_6H_5)_3]_3$-catalyzed hydrogenation of the resulitng styrene.[9c,d]

Here, we report the further generalization of the one-pot sol-gel methodology to the important, versatile class of enzymatically functionalized sol-gel materials. The past decade has witnessed a very rapid growth in the preparation and utilization of these bioactive materials,[5,10,11] a growth which has been mainly due to the synthetic ease of enzyme entrapment procedures and to the significantly enhanced stability of the entrapped enzyme. As such, this class of reactive sol-gel materials seemed to be suitable for one-pot reaction sequences in the presence of entrapped chemicals which otherwise quench the enzymatic activity. In particular, we report the coexistence of an entrapped enzyme and entrapped metal-complex catalysts in one-pot reaction pairs, despite the fact that the free catalyst *in solution* inhibits the action of the enzyme.

Scheme 1. Reaction Routes of the Saturated and Unsaturated Carboxylic Acids and Alcohols[a]



[a] R^u (acid): $CH_2{=}CH(CH_2)_8$ (**1**). R^s (acid): $CH_3(CH_2)_9$ (**2**), $CH_3(CH_2)_7$ (**3**), $CH_3(CH_2)_{10}$ (**4**). R^u (alcohol): $CH{=}CHCH_2$ (**5**), $CH{=}CHCH(CH_3)CH_2$ (**6**). R^s (alcohol): $CH_3(CH_2)_2$ (**7**), $CH_3(CH_2)_4$ (**8**), (*S*)-(−)-$CH_3CH_2CH(CH_3)CH_2$ (**9**).

Results and Discussion

The system we selected in order to study the feasibility of the one-pot Enzyme@s-g + Catalyst@s-g approach (we use the entrapment notation compound@s-g) consists of lipase and a hydrogenation catalyst. Sol-gel entrapped lipases, particularly those which were immobilized in hydrophobic sol-gel matrixes (as used here),[11] are among the most successful examples of this enzyme-heterogenization methodology.[5] We mention here also that some enzyme/catalyst couples have already been used for chiral syntheses and enantiomeric resolutions, either with soluble catalysts[12] or with Pd,[13] where the catalyst did not interfere with the activity of the enzyme.

The specific reaction pairs represent two simultaneous catalytic reactions: hydrogenation of a C−C double bond catalyzed either by entrapped $RhCl[P(C_6H_5)_3]_3$ (Cat1@s-g)[14] or by the immobilized efficient bimetallic hydrogenation catalyst, $Rh_2Co_2(CO)_{12}$ (Cat2@s-g)[15] and an esterification reaction catalyzed by the entrapped lipase (Lipase@s-g).[16] The resulting saturation of the final ester can be achieved through various possible routes, depending on which of the starting materials carries the unsaturation. All of the three main possible routes were tested in this study, and these are as follows (Scheme 1 and Table 1): (I) interaction between an unsaturated carboxylic acid and a saturated alcohol (two parallel routes may lead to the same saturated ester (Scheme 1, top left) by esterification followed by reduction of the double bond of the resulting unsaturated ester or by saturation of the acid followed by its esterification); (II) reaction of a saturated acid with an unsaturated alcohol (Scheme 1, top right), with two possible routes

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Table 1. One-Pot Reactions of Saturated and Unsaturated Carboxylic Acids and Alcohols in the Presence of Immobilized Lipase and Homogeneous or Heterogenized Rhodium Catalysts

entry	alcohol	acid	catalyst	H_2 pressure, atm	reaction time, h	products (yield,%)[a]
1	1-pentanol (**8**)	**1**	Cat1@s-g	14	24	**2**−**8** (46), **2** (54)
2	1-pentanol (**8**)	**1**	$RhCl[P(C_6H_5)_3]_3$	14	24	**2**−**8** (7), **2** (93)
3	1-propanol (**7**)	**1**	Cat2@s-g	7	4	**2**−**7** (71), **2** (29)
4	1-propanol (**7**)	**1**	$Rh_2Co_2(CO)_{12}$	7	4	**2**−**7** (19), **1**−**7** (4), **2** (50)
5	1-propanol (**7**)	**1**	Cat2@s-g	7	1	**2**−**7** (30),[b] **2** (70)
6	2-propenol (**5**)	**1**	Cat2@s-g	7	4	**2**−**7** (58), **2**−**5** (5), **2** (37)
7	2-methyl-3-butenol (**6**)	**1**	Cat2@s-g	7	6	**2**−**9** (82, ee = 19%), **2** (18)
8	(*S*)-(−)-2-methyl-butanol (**9**)	**1**	Cat2@s-g	7	6	**2**−**9** (91, optically pure), **2** (9)
9	2-propenol (**5**)	**3**	Cat2@s-g	7	4	**3**−**7** (67)
10	2-propenol (**5**)	**4**	Cat2@s-g	7	4	**4**−**7** (60)[c]

[a] The missing percentage reflects on the unreacted starting acid. [b] Under the same reaction conditions, the yield of **2**−**7** in the following five runs were 29, 30, 29, 28, and 27%, respectively. [c] Both of the catalysts were used after recycling.

(again saturation either prior or after esterification); and (III) interaction of an unsaturated acid with an unsaturated alcohol, where several possible routes can take place, two of which are shown in Scheme 1.

An example for route I (unsaturation in the acid) is provided by the pair of 10-undecenoic acid (CH_2═$CH(CH_2)_8COOH$, **1**) and 1-pentanol, **8**. As the hydrogenation process proved to be faster than the esterification, the reaction pair involved primarily the sequence in which the H_2/Cat1@s-g catalyzed hydrogenation of the double bond of **1** (to yield the saturated 1-undecanoic acid ($CH_3(CH_2)_9COOH$, **2**)) was followed by the Lipase@s-g catalyzed esterification with 1-pentanol, **8**. After 24 h (in benzene at 60°C under 14 atm of H_2), 46% of the reduced ester, 1-pentyl undecanoate ($CH_3(CH_2)_9COO(CH_2)_4CH_3$, **2**−**8** (the ester notation is **acid number**−**alcohol number**)) was obtained. In a blank experiment under the same conditions with entrapped lipase but with *nonentrapped* $RhCl[P(C_6H_5)_3]_3$, all of the 10-undecenoic acid, **1**, was reduced to 1-undecanoic acid, **2**, but only 7% of it was esterified to **2**−**8** by the entrapped enzyme. This experiment indicates that *both* the lipase and the metallic catalyst must be entrapped in sol-gel matrixes, since nonentrapped $RhCl[P(C_6H_5)_3]_3$ acts as a lipase inhibitor, even if the latter is entrapped.

In another route I two-step sequence with acid **1**, the bimetallic Cat2@s-g was used along with 1-propanol, **7**, resulting under the conditions of Table 1 in 71% of the saturated ester 1-propyl undecanoate [$CH_3(CH_2)_9COO(CH_2)_2CH_3$, **2**−**7**] (Scheme 1). The conditions in this process were even milder: 4 h at 50 °C and 7 atm of H_2, in hexane. An important observation made with this system is the good recyclability of the sol-gel materials: the immobilized lipase and the heterogenized bimetallic catalyst could be used for six runs without any significant reduction of their catalytic activities (footnote b of Table 1). In a blank experiment that was performed under the same conditions with Lipase@s-g and nonentrapped $Rh_2Co_2(CO)_{12}$, the yield dropped from 71% to 19%, (along with 4% of the corresponding unsaturated ester, CH_2═$CH(CH_2)_8COO(CH_2)_2CH_3$, **1**−**7**, and 50% of the hydrogenated acid **2**). Although subjecting the enzyme to nonentrapped $Rh_2Co_2(CO)_{12}$ in the *absence* of hydrogen has no synthetic rational, we did perform this blank reaction as well and, interestingly, found that nonentrapped $Rh_2Co_2(CO)_{12}$ in the presence of H_2 is a stronger quencher of the enzymatic activity compared to its absence: when the saturated undecanoic acid, **2**, was taken as a substrate for the enzyme (because it is the main substrate for lipase in the presence of hydrogen), 35% of the saturated ester **2**−**7** was obtained. This suggests that the quenching of the enzymatic activity by $H_2/Rh_2Co_2(CO)_{12}$ involves not only metal poisoning but also reductive destruction of the enzyme.

Route I may lead of course to chiral esters if a chiral alcohol is used, and this has been demonstrated by using the pair of acid **1** and (*S*)-(−)-2-methylbutanol, **9**. The saturated chiral ester, (*S*)-(−)-2-methylbutyl undecanoate ($CH_3(CH_2)_9COOCH_2CH(CH_3)CH_2CH_3$, **2**−**9**) was obtained in 91% yield (along with 9% of the saturated acid **2**) by employing Cat2@s-g and Lipase@s-g (hexane**,** 50°C, 7 atm of H_2, 6 h). In fact, this chiral ester could be obtained also through route III, namely where both the acid and the alcohol are unsaturated. Thus, esterification of **1** with the *racemic* 2-methyl-3-butenol, **6**, afforded **2**−**9** in an enantiomerically enriched racemic mixture (ee = 19%), as determined from synthesized (*S*)-**2**−**9** ($[\alpha]_D^{20}$ = 2.4°, heptane, *c* = 0.6 (lit.[17] 1.9°)).

The feasibility of route II (unsaturation in the alcohol) was demonstrated in a reaction between nonoic acid, **3**, and 2-propenol, **5** (*n*-hexane, 50°C, 7 atm of H_2, 4 h) resulting in 67% of 1-propyl nonanoate, **3**−**7**. Here, yet another demonstration of the recyclability was performed, by using the same recycled catalytic system (Cat2@s-g and Lipase@s-g) for a *different* acid/alcohol pair, namely for the reaction with lauric acid, **4**, and 2-propenol, **5**. Under the same reaction conditions 60% of 1-propyl laurate, **4**−**7**, was obtained.

It should be noted that although the hydrogenation of the C−C double bonds of both the acid and the alcohol is, under our reaction conditions, faster than the esterification, the routes of esterification prior to reduction (leading to the same product, Scheme 1) were found, by ^1H NMR monitoring, to take place as well. Thus, esterification of **1** with 2-propen-1-ol, **5**, (route III, in hexane, 50°C, 7 atm of H_2, 4 h) led not only to the fully reduced ester **2**−**7** as a major product but also to 5% of 2-propen-1-yl undecanoate [$CH_3(CH_2)_9COOCH_2CH$═CH_2, **2**−**5**]. Likewise, when the reaction of **1** and saturated 1-propanol, **7**, in the presence of Lipase@s-g and Cat2@s-g was stopped after 1 h, a 5:1 mixture of the saturated 1-propyl undecanoate, **2**−**7**, and the unsaturated 1-propyl 10-undecenoate, **1**−**7**, was obtained.

In conclusion, we believe that the positive results of this feasibility-of-concept study should be applicable to many other enzyme-based one-pot reaction sequences as well.

Experimental Details

Typically, a suspension of 0.1 g of Lipase@s-g[16] (12 U), 0.3 g of Cat2@s-g[15] (containing 0.01 mmol of $Rh_2Co_2(CO)_{12}$), 0.12 g (0.6 mmol) of acid, and 2.6 mmol of the carbinol in 3 mL hexane was placed in

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an autoclave. After the autoclave was purged with H_2, it was charged with 7 atm of H_2 and heated at 50 °C for the desired length of time (for time durations and other pressures, see Table 1). After the autoclave was cooled to room temperature, the sol-gel materials were filtered off and the solvent was evaporated. The resulting products were identified by GC, GC−MS, and NMR. For recycling, the catalysts were sonicated three times in 10 mL of hexane for 10 min. The reaction pair with Cat1@s-g was performed with 0.6 g of ceramic material containing 0.015 mmol of entrapped catalyst and 2.6 mmol carbinol at 60 °C, 14 atm of H_2, in 3 mL of benzene. For the detection of the two parallel sequences in the synthesis of **2**−**7**, 0.6 mmol of **1** and 2.6 mmol of 1-propanol, **7**, were reacted at 50 °C, under 3.5 atm of H_2 in the presence of 0.08 g of Cat2@s-g (containing 0.003 mmol of $Rh_2Co_2(CO)_{12}$) and 0.1 g of Lipase@s-g. The reaction was stopped prematurely after 1 h revealing, along with unreacted starting materials, a mixture of 11% of the fully saturated 1-propyl undecanoate, **2**−**7**, and 2.2% of the unsaturated 1-propyl 10-undecenoate, **1**−**7**.

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