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Dynamic Combinatorial Thiolester Libraries for Efficient Catalytic Self-Screening of Hydrolase Substrates

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Dynamic combinatorial thiolester libraries were efficiently generated from pools of thiols and acyl functionalities through reversible transthiolesterification in aqueous media at neutral pH. The dynamic features of the library generation were investigated, and the libraries were screened against acetylcholinesterase, clearly demonstrating the catalytic self-screening of its substrates from the constituents. Acetyl- and propionylthiocholine were easily identified as the best substrates for the enzyme, whereas other constituents showed lower efficiency or were inactive. A range of hydrolases was furthermore screened for rapid substrate identification, clearly demonstrating the differences in selectivity. The results show that transthiolesterification is a useful method to generate dynamic libraries, and that the catalytic self-screening concept is highly valuable for substrate identification.

Introduction

Constitutional Dynamic Chemistry (CDC) enables the generation of constitutional diversity from molecular or supramolecular dynamic processes, yielding systems that are responsive to internal and/or external factors and amenable to adaptive activity.[1–3] A specific expression of CDC is Dynamic Combinatorial Chemistry (DCC), which relies on the use of reversible processes to generate potent libraries of chemical compounds based on the continuous interchange of different building blocks within the library. The components are spontaneously assembled to encompass all possible combinations, through the implementation of non-covalent or reversible covalent bonds, resulting in pools of continuously interchanging library constituents. The concept has proven to be a highly useful tool for screening and rapid identification of ligands with high affinity to target molecules such as receptors and enzymes.[4–9]

One major advantage with dynamic combinatorial libraries (DCLs) over their static counterparts is their potential susceptibility to change in response to an external selection pressure. If, for instance, the dynamic library is exposed to a receptor showing affinity for one or more of the constituents formed, the dynamic system will shift in favor to the specific compounds bound. According to Le Châtelier's principle,[10] the library will adapt to the selection pressure and the constituents bound to the receptor will be enriched (amplified) in relation to the unbound compounds.[4–9] Further, if the binding event can be coupled to a secondary process, the selection may be enhanced by allowing the bound species to be removed from the equilibrating pool. This process generates more of the best bound species by re-equilibration of the DCL (Figure 1).

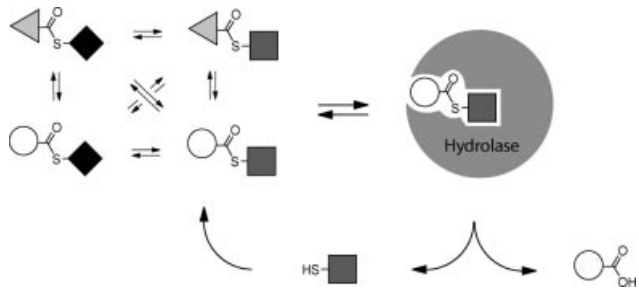

Figure 1. Schematic representation of the dynamic combinatorial catalytic self-screening process with thiolesters.

An important challenge in DCC is the need for new means to generate dynamics in the systems, and in particular reversible reactions that are compatible with biological target entities. Until now, mainly imines,[11–13] acyl hydrazones,[14–16] and disulfides,[17–20] have been used for DCLs in these applications, since these formats have proven to be the most efficient in the systems studied at mild conditions in aqueous media. Another challenge of the method is the development of improved screening and identification protocols. Different approaches of the technique have to date been developed, including the adaptive approach,[11,12,17,21,22] the pre-equilibrated approach,[14,18] and the iterative approach,[23] all of which address different specific challenges. A related technique is also exerted by the interesting pseudo-dynamic or deletion approach.[24]

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A new way to generate dynamics in DCC systems using transthiolesterification has previously been reported by our group.[25] It was shown that this reversible reaction type was rapid and sufficiently stable under mild conditions in aqueous media,[25–27] where DCL generation and screening could be efficiently established. It was further demonstrated that coupled to a biocatalyst, the dynamic process generated more of the best recognized constituent of the library. Following the catalytic action, the products were expelled from the active site, thus rendering the site free to host more of the DCL constituents and forcing the dynamic system to run to completion. Substrates to the biocatalyst could be selectively produced and easily identified using this self-screening dynamic system.

In the present study, the characteristics of the catalytic self-screening approach have been further addressed. The dynamic features of the transthiolesterification reaction have been probed for a range of components of different character, resulting in potent dynamic thiolester libraries. These libraries were further exposed to a series of different hydrolases, where the performance and selectivity of the self-screening process were investigated.

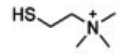
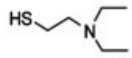
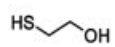
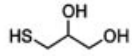
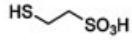
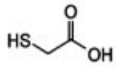
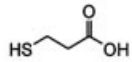
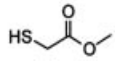
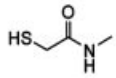
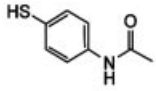
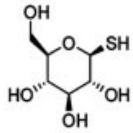
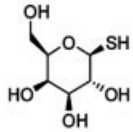
Results and Discussion

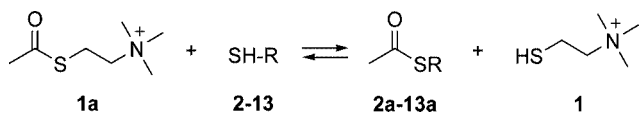
The catalytic self-screening libraries were generated from a series of thiols and thiolesters, respectively. In order to probe the performance of the library generation, a range of thiols were investigated (Table 1), differing with respect to structure and functionality. Thus, thiols possessing an adjacent amine/ammonium group (**1**,**2**), hydroxy group (**3**–**4**, **11**–**12**), sulfonate group (**5**), carboxylate group (**6**–**7**), carboxylic ester group (**8**), carboxylic amide group (**9**–**10**), or trifluoromethyl group (**13**) were tested for their performances. An aromatic thiol (**10**) and two cyclic thiols (**11**–**12**) were furthermore studied. All thiols were chosen so as to be easily soluble in aqueous media at neutral pH. The dynamic features of the thiols were subsequently evaluated in transthiolesterification reactions with acetylthiocholine (**1a**), where the formation/thiolysis of the each thiolester was followed (Scheme 1).

The exchange rate and equilibrium composition of each combination was determined by mixing equivalents of the thiols together with acetylthiocholine in $NaOD/D_3PO_4$ buffer at pD 7.0. The exchange taking place was then measured by ^1H NMR at different time intervals. For the rapidly reacting thiols ($t_{½} < 15$ min) only one measurement was possible.

From the results presented in Table 1, it is evident that the rate of exchange directly correlates to the pK_a of the thiols. The lower the pK_a, the faster the exchange reaction, where thiols having pK_a values lower than 8.5 all reached equilibrium very rapidly ($t_{½} < 15$ min). The results also indicate that the majority of thiols produce equilibrium concentrations that are close to the concentration of acetylthiocholine, thus showing near isoenergetic behavior. Thiolesters from secondary thiols were however considerably

Table 1. Thiol structures, and their respective pK_a values, tested in the transthiolesterification reactions. Thiolester equilibrium composition (ratio), and exchange rate ($t_{½}$) in reactions with acetylthiocholine (**1a**).[31–40]

	Thiol	pK_a	Exchange with acetylthiocholine	
			ratio	$t_{½}$ (min)
1		7.7[31]	–	–
2		7.7[32]	1.0:0.6	<15
3		9.7[33]	1.0:1.2	40
4		9.5[34]	1.0:1.7	55
5		9.1[35]	1.0:0.9	25
6		9.8[34]	1.0:1.0	40
7		10.3[36]	1.0:1.0	40
8		7.8[33]	1.0:1.0	<15
9		8.5[37]	1.0:1.2	<15
10		6.1[38]	1.0:0.1	<15
11		7.7[39]	1.0:0.2	<15
12		7.7[39]	1.0:0.2	<15
13		7.3[40]	1.0:1.0	<15



Scheme 1. Transthiolesterification with acetylthiocholine (**1a**), and different thiols (**2**–**13**).

less stabilized compared to acetylthiocholine. For the aromatic thiol **10**, and the 1-thio-β-D-glycopyranosides **11**–**12**, the ratio was clearly shifted in favor of the reactants, and only about 10–20% of the thiols were present as the corresponding thiolesters at equilibrium. When comparing all thiols, these components showed the largest differences in reactivity.

Seven different acyl groups (**a**–**g**), ranging from acetyl to *tert*-butyl (Figure 2), were prepared with 3-sulfanylpropionic acid (**7**) to generate the thiolesters **7a**–**7g** used to probe the acyl components. These acyl groups were primarily chosen to present a homologous series of alkyl chains, including linear and branched structures. The 3-sulfanylpropionic acid was used as thiol counterpart to keep the acyl compounds soluble at neutral pH.

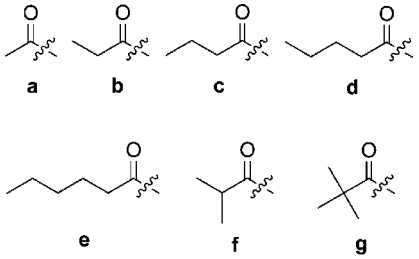


Figure 2. Acyl groups R–C(=O)– used in dynamic thiolester libraries.

The performance of the acyl groups were estimated from two different dynamic libraries. In the first of these DCL-A, five of the thiolesters **7a**–**7e** were treated with thiocholine (**1**), and the exchange monitored by ^{1}H NMR at 25 °C at pD 7.0. The exchange rate was in this case relatively rapid and equilibration of the library was reached with a $t_{½}$ value of 50 min (Figure 3), with the 3-sulfanylpropionic acid derivatives formed in equal to slightly higher amounts than their thiocholine counterparts (1.0:0.6–1.0). The second dynamic library DCL-B was instead composed of thiolesters **7a**–**7c** and **7f**–**7g** and subjected to the same conditions as DCL-A. Library generation was in this case less efficient, and equilibrium from the reaction with thiol **1** was attained considerably slower ($t_{½}$ = 110 min) (Figure 3). As expected, the results from these libraries implied that the branched acyl groups **f** and **g** reduced the exchange rate of the libraries, the carbonyl group being more sterically hindered than for the linear acyl groups.

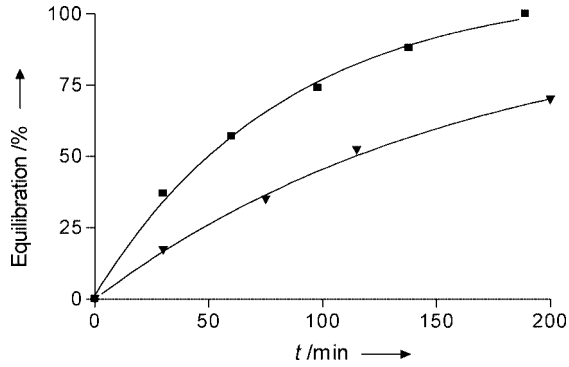
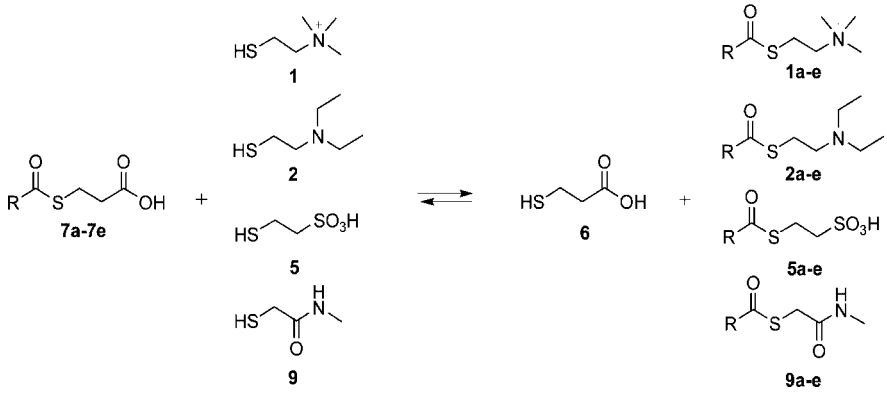


Figure 3. Equilibration of dynamic thiolester libraries: (■) DCL-A from thiol **1** and thiolesters **7a**–**7e**; (▼) DCL-B from thiol **1** and thiolesters **7a**–**7c**, **7f**–**7g**. Both DCLs were made using equimolar amounts of substrates.

In order to avoid creating biased libraries, it is desirable to include components that show comparable reactivities, and for this reason some components were excluded from the subsequent libraries. Due to the slower exchange rate for some of the thiols and the branched acyl components, these were generally excluded. In addition, the secondary thiols were excluded because of their unfavorable equilibria. In a broader perspective, however, the excluded thiol or acyl components tested are not generally disqualified for transthiolesterification libraries, but may well be part of dynamic libraries for other purposes. All of these components are still taking part in the dynamic exchange, albeit showing slower kinetics or unfavorable equilibria. A means to increase the rate of transthiolesterification for the less reactive thiols is also to increase the basicity of the solution, and the dynamic exchange was also efficient at higher pH. However, increasing the basicity not only accelerates thiolyses, but also the competing and unproductive hydrolyses of the thiolesters. In the present study, a neutral pD was generally chosen, because of negligible hydrolysis at the time scale used. Hydrolysis was thus considerably less pronounced at pD 7.0 (3.7%) compared to pD 8.0 (13.5%) and pD 9.0 (17.9%) after three days.



Scheme 2. Generation of dynamic thiolester library DCL-C; for R–C(=O)– see **a**–**e** in Figure 2.

Following the initial screening of thiol and acyl components, a series of dynamic libraries was devised and generated. The reaction between the five thiolesters **7a**–**7e** and the four thiols **1**,**2**,**5** and **9** generated library DCL-C (Scheme 2). For every thiol added, five additional thiolesters are being formed. Thus, during formation of the dynamic libraries these components may form 25 different thiolesters, all of which being in exchange with all others during the whole process. The library generation process was initiated with equimolar amounts of all acyl components and five equivalents of each of the thiols. Since thiol component **7** is connected to the five different acyl functionalities at t_0, this ensured equal quantities of all thiol components in the system. Experiments where the 3-sulfanylpropionic acid (**7**) was left in excess to the other thiols, yielded the same final results albeit showing slightly longer reaction time. In agreement with the results for DCL-B, the resulting concentrations of the formed thiolester constituents were relatively comparable, and the libraries showed close to isoenergetic behavior. The ^{1}H NMR spectrum of a mixture of the five original acyl components and five original thiol components is displayed in Figure 4 (a) and the spectrum after formation of the full library is shown in Figure 4 (b).

The resulting library DCL-C was subsequently exposed to/coupled to hydrolase action in order to initiate the self-screening process. Thus, the library was further analyzed in the presence of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7), a serine hydrolase that catalyzes the hydrolysis of the neurotransmitter acetylcholine to acetate and choline at neuromuscular synapses.[28] Immediately upon addition, the best substrates were recognized by the enzyme and swiftly hydrolyzed. As a result of this thiolester hydrolysis, the library then had to reconstitute, adjusting for the diminished concentrations of selected and hydrolyzed thiolesters, as well as the increased concentrations of selected thiols formed.

Over time, two of the acyl functionalities, the acetyl and propionyl groups, proved to be mainly acted upon by the enzyme, with the acetyl species being more rapidly hydrolyzed than the propionyl counterpart. Figure 4 (c) shows the library in presence of acetylcholinesterase after complete hydrolysis of the acetate and propionate functionalities. The overall rates of formation proved to be: for acetic acid, $t_{½}$ = 260 min and for propionic acid, $t_{½}$ = 310 min (Figure 5). These products were formed at a significantly faster rate than butyric acid ($t_{½} > 1800$ min). The long lag phase of butyric acid formation is likely caused by the higher selectivity for the other substrates, and/or due to inhibitory activities of the present thiolesters.[29]

To further probe the effects of each thiol unit, dynamic libraries were made in the same way as DCL-B. These additional libraries were prepared using one of the thiols **2**,**5** or **9** together with acyl constituents **7a**–**7e**, yielding libraries DCL-D, DCL-E, and DCL-F, respectively (Table 2). Thus, these libraries were composed of 10 different thiolester constituents. The resulting libraries, together with DCL-B, were subsequently subjected to acetylcholinesterase action, and the hydrolysis measured in each case. The half-lives of for-

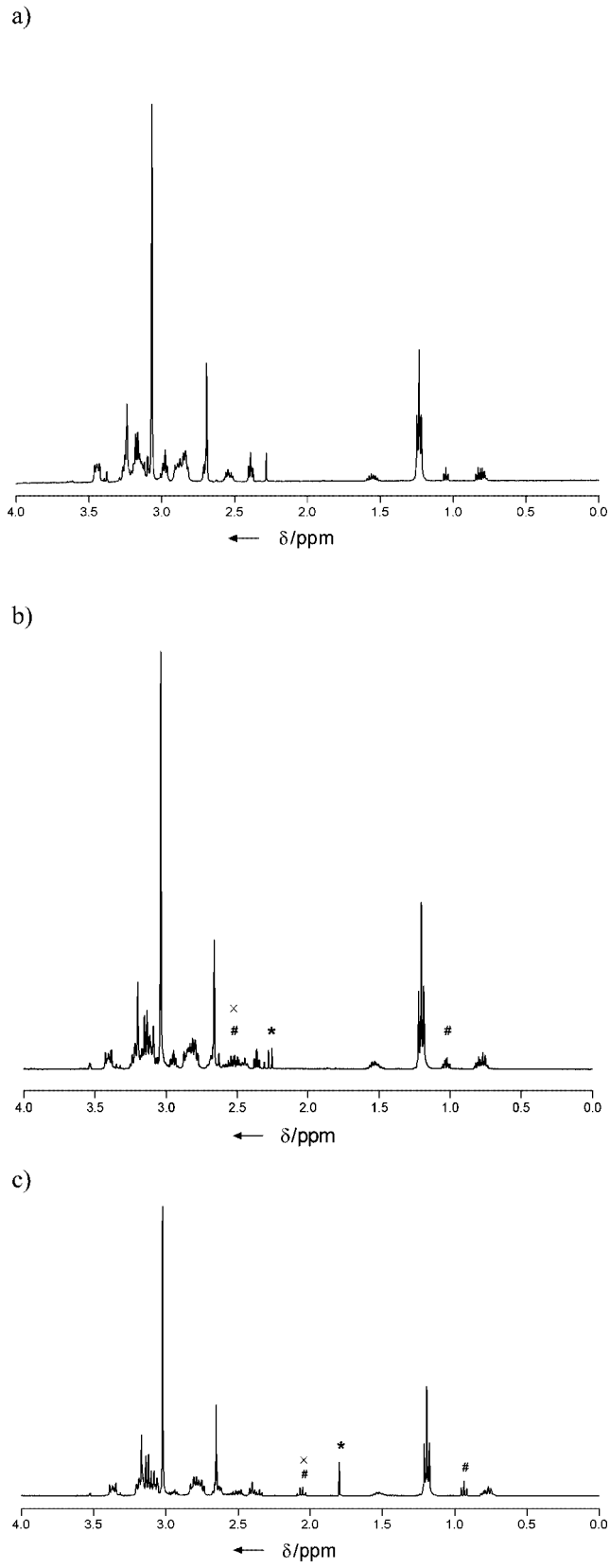


Figure 4. ^{1}H NMR spectra of libraries/components: a) Components for DCL-C before library generation; b) DCL-C in the absence of AChE c) DCL-C in the presence of AChE, *, #, and × indicate the signals for acetate, propionate, and butyrate groups, respectively.

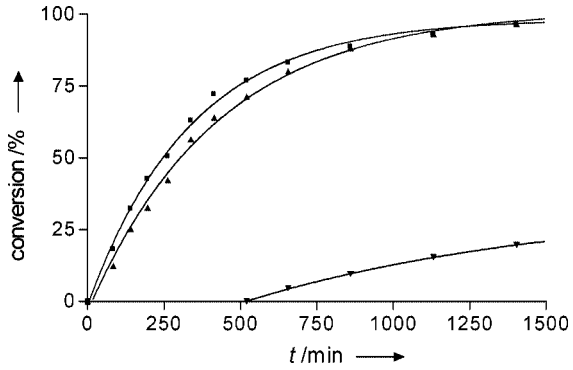


Figure 5. Formation of acetate (■), propionate (▲) and butyrate (▼) hydrolysis products in DCL-C.

mation of the different hydrolysis products, acetate, propionate and butyrate, in the five DCLs, are summarized in Table 2. For DCL-B, using only thiocholine (**1**) together with each thiolester **7a**–**7e**, the results were very similar to DCL-C, and the acetyl and propionyl acyl groups were instantly recognized by the enzyme. The rates of formation were thus comparable to DCL-C and the $t_{½}$ values of acetate, propionate and butyrate were estimated to 210 min, 270 min, and >1500 min, respectively (Table 2).[25] The slightly shorter time required is a consequence of the smaller library size. For every other 10-compound library, the overall rates of formation were considerably less sufficient, well in accordance with the known substrate specificity of acetylcholinesterase. It was anticipated that the strong resemblance between compounds **1** and **2** could lead to the latter substrate(s) also being hydrolyzed by the enzyme (compare DCL-B and DCL-D). The acetate constituent (**2a**) was in this case also the best substrate for the enzyme. However, despite the similarity, the overall formation of acetate was in this case about 12 times slower for **2a** ($t_{½}$ ≈ 2500 min) compared to **1a** ($t_{½}$ = 210 min).

Table 2. Summary of the rates of formation of the different hydrolysis products in five DCLs.

Library	Thiol components	$t_{½}$ (min)		
		Acetate	Propionate	Butyrate
DCL-C	**1**,**2**,**5**,**7**,**9**	260	310	>1800
DCL-B	**1**,**7**	210	270	>1500
DCL-D	**2**,**7**	≈ 2500	>4000	>>4000
DCL-E	**5**,**7**	–	–	–
DCL-F	**7**,**9**	≈ 4000	>4000	–

For propionyl and butyryl, the only other two acyl functionalities being hydrolyzed, even larger differences were recorded. The estimated half-times of formation are well beyond 4000 min for both substrates. Interestingly, the ratio of the hydrolyzed acyl groups appeared in this case shifted. In the libraries containing thiocholine (**1**) (DCL-B, DCL-C), formation of acetate was around 20% faster than propionate, with butyrate forming only after substantial hydrolysis of the two main substrates (≈ 600 min). In the experiments with DCL-D, devoid of thiocholine (**1**), but containing 2-diethylaminoethanethiol (**2**), the difference between propionate and butyrate formation, compared to acetate formation, was around 40% and 55% slower, respectively, at 3000 min.

In contrast to both DCL-B and DCL-D, the DCL in which *N*-(methyl)mercaptoacetamide (**9**) was used together with **7a**–**7e** (DCL-F), did not show any hydrolysis of the butyryl functionality. As with DCL-D, the $t_{½}$ values recorded for the formation of acetate and propionate, respectively, were well beyond that of DCL-B. The library with mercaptoethanesulfonate (**5**) (DCL-F) failed to give any hydrolysis products within the time measured (≈ 4500 min), identical to the effects of thiol **7**.

To further test the selectivity of the self-screening process, six other enzymes belonging to the hydrolase family were tested under the same set of conditions as in DCL-B. These enzymes were: butyrylcholinesterase (BChE, EC 3.1.1.8), horse liver esterase (HLE, EC 3.1.1.1), *Candida cylindracea* lipase (CCL, EC 3.1.1.3), β-galactosidase (β-Gal, EC 3.2.1.23), trypsin (EC, 3.4.21.4), and subtilisin (EC, 3.4.21.62). The dynamic libraries were thus exposed to each of the enzymes and the formation of the hydrolysis products analyzed. The results are summarized in Table 3, recorded as percent product formation after 210 min ($t_{½}$ for acetate in presence of AChE). All hydrolases acting on carboxylic ester bonds (EC, 3.1.1.X) showed some hydrolysis, although the lipase from *Candida cylindracea* (CCL) only very modestly. In contrast to acetylcholinesterase, butyrylcholinesterase (BChE) acted on all acyl groups and hydrolyzed all groups in roughly the same time. This result is well in accordance with the known substrate pattern for this enzyme. The esterase from horse liver (HLE) show a pattern in which the longer acyl chains being slightly faster hydrolyzed than its shorter counterparts. For the two proteases trypsin and subtilisin, only the latter shows some activity under these conditions, also with some selectivity for the longer acyl chains. The hydrolase belonging to the glycosylases, β-galactosidase (β-Gal), did not show any activity. Control experiments with bovine serum albumin (BSA) also failed to give any hydrolysis products, as expected.

Table 3. Acyl product formation for seven hydrolases and bovine serum albumin (BSA), with DCL-B.

Enzyme	Product yield (%)[a]				
	Acetate	Propionate	Butyrate	Valerate	Caproate
AChE	50	45	–	–	–
BChE	37	42	44	44	43
HLE	16	19	20	23	31
CCL	–	<5	<5	<5	<5
β-Gal	–	–	–	–	–
Trypsin	–	–	–	–	–
Sub[b]	–	<5	<5	9	14
BSA	–	–	–	–	–

[a] t = 210 min ($t_{½}$ for acetate/AChE). [b] Subtilisin Carlsberg.

The results clearly indicate that the catalytic self-screening process is efficient in identifying enzyme substrates. Of all the constituents formed in the DCLs, two of the thiolesters (**1a**, **1b**) were easily recognized by the method as essentially being the best substrates for acetylcholinesterase, well

in accordance to the recorded selectivity of the enzyme. The technique could however also distinguish constituents with considerably lower substrate activities, such as **1c** and **2a**. For acyl functionalities longer than butyryl, all constituents failed to give any hydrolysis products with acetylcholinesterase, and thiolesters having a negative charge at neutral pH were inefficient as substrates in all cases. The selectivity of the method was furthermore clearly demonstrated in self-screening with a range of different hydrolases. Under the same set of conditions, these hydrolases showed selective activities for the library constituents, differing from the performance of acetylcholinesterase. Butyrylcholinesterase and horse liver esterase both showed broader specificities than acetylcholinesterase, and horse liver esterase as well as subtilisin displayed more selectivity towards longer acyl groups.

Conclusion

It has been demonstrated that enzymes can be used as efficient catalysts for targeting and rapid identification of the best substrates formed in a dynamic combinatorial library. Transthiolesterification has proven to be a highly useful means to generate DCLs under mild conditions in aqueous media, and the resulting system can be subjected to self-screening in the presence of enzymes. The outcome of the self-screening system relying on the selectivity of the specific enzyme used. This approach also enables screening of complex DCLs without the necessity of using equimolar amounts of targets. This system is, however, not restrained to enzyme catalysis; it may be extended to any catalytic system, including organic and inorganic catalysts, and may be employed to rapidly screen reactions of catalysts for new substrates.

Experimental Section

General: *S*-Acetylcholine (Lancaster) was hydrolyzed with 6 M HCl to give thiocholine. *N*-(Methyl)-mercaptoacetamide (Sigma–Aldrich) was purified by column chromatography (EtOAc/EtOH, 5:1). 2-diethylaminoethanethiol (Sigma–Aldrich) was reduced by treatment with zinc granules for 15 min.[30] Compounds **7a**–**7e** were prepared as previously reported.[25] Enzymes used were: Acetylcholinesterase (AChE, EC 3.1.1.7, type VI-S, Sigma–Aldrich), butyrylcholinesterase (BChE, EC 3.1.1.8, Sigma–Aldrich), β-galactosidase from *Escherichia coli* (β-Gal, EC 3.2.1.23, Fluka), esterase from horse liver (HLE, EC 3.1.1.1, Fluka), lipase from *Candida cylindracea* (CCL, EC 3.1.1.3, Fluka), subtilisin Carlsberg (EC, 3.4.21.62, type VIII, Sigma–Aldrich), and trypsin (EC, 3.4.21.4, DPCC treated, Fluka). All other reagents used were purchased from commercially available resources and used as received. ^{1}H and ^{13}C NMR spectroscopic data were recorded with a Bruker Avance 400 spectrometer at 400 (100) MHz and/or a Bruker Avance DMX 500 at 500 (125) MHz, respectively. Chemical shifts are reported as δ values (ppm) with $CDCl_3$ (^{1}H NMR: δ = 7.26 ppm, ^{13}C NMR: δ = 77.0 ppm) or D_2O (4.79) as internal standard. *J* values are given in Hertz (Hz). pD Values were measured with a Ecoscan pH meter with a Mettler Toledo inlab 421 pH electrode. Thin-layer chromatography (TLC) was performed with precoated Polygram® SIL G/UV254 silica plates (0.20 mm, Macherey–Nagel) and was visualized by UV detection and/or by spraying with bromothymol blue or an acidified $PdCl_2$ solution. Flash column chromatography was performed on silica gel 60, 0.040–0.063 mm (SDS). Elemental analyses were performed by Analytische Laboratorien GmbH, Lindlar, Germany.

Generation of DCLs and Catalytic Self-Screening: Stock solutions of each of the compounds/constituents were prepared in D_2O/D_3PO_4/NaOD buffer. The libraries were subsequently generated by combining aliquots of each solution to give final concentrations in D_3PO_4/NaOD buffer solution (100 mM, pD 7.0); DCL-C: 4 mM of esters **7a**–**7e** and 20 mM each of thiols **1**,**2**,**5** and **9**; DCL-A and DCL-D-F: 4 mM of esters **7a**–**7e** and 20 mM of thiols **1**,**2**,**9** or **5**, respectively. DCL-B: 4 mM of esters **7a**–**7c** and **7f**–**7g**, respectively, and 20 mM of thiol **1**. For the catalytic self-screening was further added the respective enzyme (2.5 U). The formation of hydrolysis products in all cases were followed by ^{1}H NMR at different time intervals.

3-(Isobutyrylsulfanyl)propionic Acid (7f): Isobutyryl chloride (3.5 mL, 33.4 mmol) was added dropwise to a cooled solution of mercaptopropionic acid (1.0 mL, 11.4 mmol) in CH_2Cl_2 (2.0 mL) and acetic acid (3.0 mL). The reaction was maintained at room temperature for 36 h. The excess of the chloride and acetic acid was evaporated in vacuo. The residue was purified by column chromatography (hexane/EtOAc, 8:2) to give the final product (1.54 g, 76.6%) as a white solid. R_f = 0.56 (hexane/EtOAc, 7:3). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.25 [d, *J* = 6.8 Hz, 6 H, CH–(*CH*$_3$)$_2$] 2.74 (t, *J* = 6.9 Hz, 2 H, *CH*$_2$–COOH) 2.79 [m, 1 H, *CH*–(CH$_3$)$_2$] 3.15 (t, 2 H, SCH$_2$). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 19.7, 23.7, 34.7, 43.5, 177.6, 204.2. $C_7H_{12}O_3S$ (176.23): calcd. C 47.71, H 6.86; found C 47.85, H 6.98.

3-(2,2-Dimethylpropionylsulfanyl)propionic Acid (7g): 2,2-Dimethylpropionyl chloride (5.5 mL, 44.7 mmol) was added dropwise to a cooled solution of mercaptopropionic acid (1.0 mL, 11.4 mmol) in CH_2Cl_2 (5.0 mL) and acetic acid (5.0 mL). The reaction was maintained at room temperature for 36 h. The excess of the 2,2-dimethylpropionyl chloride and acetic acid was evaporated in vacuo. The residue was purified by column chromatography (hexane/EtOAc, 9:1) to give the final product (1.81 g, 83.4%) as a white solid. R_f = 0.49 (hexane/EtOAc, 7:3). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ = 1.29 [s, 9 H, C–(*CH*$_3$)$_3$] 2.72 (t, *J* = 6.9 Hz, 2 H, *CH*$_2$–COOH), 3.14 (t, 2 H, SCH$_2$). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K): δ = 23.7, 27.8, 34.6, 46.9, 178.1, 207.3. $C_8H_{14}O_3S$ (190.26): calcd. C 50.50, H 7.42; found C 50.41, H 7.34.

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