SUPPORTING INFORMATION

Facile Acetal Dynamic Combinatorial Library

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I. General:

NMR analyses were done in a Bruker Avance DPX200 or in a Bruker Avance DMX500 NMR. Spectra were calibrated on the residual solvent signal. MALDI-TOF analyses were done in a Bruker Reflex-4; the chromatograms were realized using the positive reflectron mode. Dithranol was used as the MALDI matrix. Separations and analysis by preparative HPLC were performed on a Jasco MD-1515 photodiode array detector equipped with a reversed phase Luna C18 (2) 10 μm column (250 × 21.2 mm) (Phenomenex). The elution solvent was isocratic 80% acetonitrile / 20% water (v/v), at a flow rate of 20 ml/min. The injection loop was 1ml and typical injections were 20 mg/ml concentration. All glassware was flame dried before use and reactions were run under a dry nitrogen atmosphere.

II. Acetal DCL of p-nitrobenzaldehyde and triethylene glycol:

Freshly distilled TEG (2.2ml, 16.5mmol); an equimolar concentration of p-nitrobenzaldehyde (2.5g, 16.5mmol) and a catalytic amount of sulfuric acid (0.044ml, 0.825mmol) were refluxed in freshly distilled toluene (200ml) in a 500ml flask equipped with a Dean-Stark water trap for 20h. After the reaction was deemed complete, the mixture was cooled, followed by addition of excess anhydrous K_2CO_3 and filtered.

Analysis of the library members:

O_1: ^{1}H NMR ($CDCl_3$, 200MHz) δ 8.22 (2H, d, *J* = 8.5), 7.70 (2H, d, *J* = 8.5), 5.76 (1H, s), 3.55-3.80 (26H, m). MS (MALDI-TOF): 456.5 ($M+Na^+$).

O_2: ^{1}H NMR ($CDCl_3$, 200MHz) δ 8.20 (4H, d, *J* = 8.5), 7.68 (4H, d, *J* = 8.5), 5.72 (2H, s), 3.55-3.75 (38H, m). MS (MALDI-TOF): 739.5 ($M+Na^+$).

O_3: ^{1}H NMR ($CDCl_3$, 200MHz) δ 8.15-8.23 (6H, m), 7.64-7.72 (6H, m), 5.72 (2H, s), 5.70 (1H, s), 3.55-3.75 (50H, m). MS (MALDI-TOF): 1022.5 ($M+Na^+$).

O_4: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.16-8.21 (8H, m), 7.66-7.72 (8H, m), 5.72 (2H, s), 5.70 (2H, s), 3.58-3.76 (62H, m). MS (MALDI-TOF): 1305.5 ($M+Na^+$).

O_5: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.16-8.24 (10H, m), 7.65-7.72 (10H, m), 5.72 (2H, s), 5.70 (3H, bs), 3.58-3.77 (74H, m). MS (MALDI-TOF): 1588 ($M+Na^+$).

O_6: ^{1}H NMR ($CDCl_3$, 200MHz) δ 8.15-8.22 (12H, m), 7.65-7.72 (12H, m), 5.72 (2H, s), 5.70 (4H, bs), 3.56-3.80 (86H, m). MS (MALDI-TOF): 1871 ($M+Na^+$).

O_7: ^{1}H NMR ($CDCl_3$, 200MHz) δ 8.13-8.23 (14H, m), 7.64-7.72 (14H, m), 5.72 (2H, s), 5.70 (5H, bs), 3.55-3.75 (98H, m). MS (MALDI-TOF): 2154 ($M+Na^+$).

O_8: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.15-8.22 (16H, m), 7.65-7.71 (16H, m), 5.72 (2H, s), 5.70 (6H, bs), 3.55-3.75 (110H, m). MS (MALDI-TOF): 2437 ($M+Na^+$).

O_9: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.14-8.21 (18H, m), 7.64-7.72 (18H, m), 5.72 (2H, s), 5.70 (7H, bs), 3.57-3.75 (122H, m). MS (MALDI-TOF): 2720 ($M+Na^+$).

O_{10}: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.12-8.22 (20H, m), 7.62-7.72 (20H, m), 5.72 (2H, s), 5.69 (8H, bs), 3.55-3.76 (134H, m). MS (MALDI-TOF): 3003 ($M+Na^+$).

M_1: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.21 (2H, d, *J* = 8.5), 7.69 (2H, d, *J* = 8.5), 5.8 (1H, s), 3.55-3.75 (12H, m). MS (MALDI-TOF): 306.3 ($M+Na^+$).

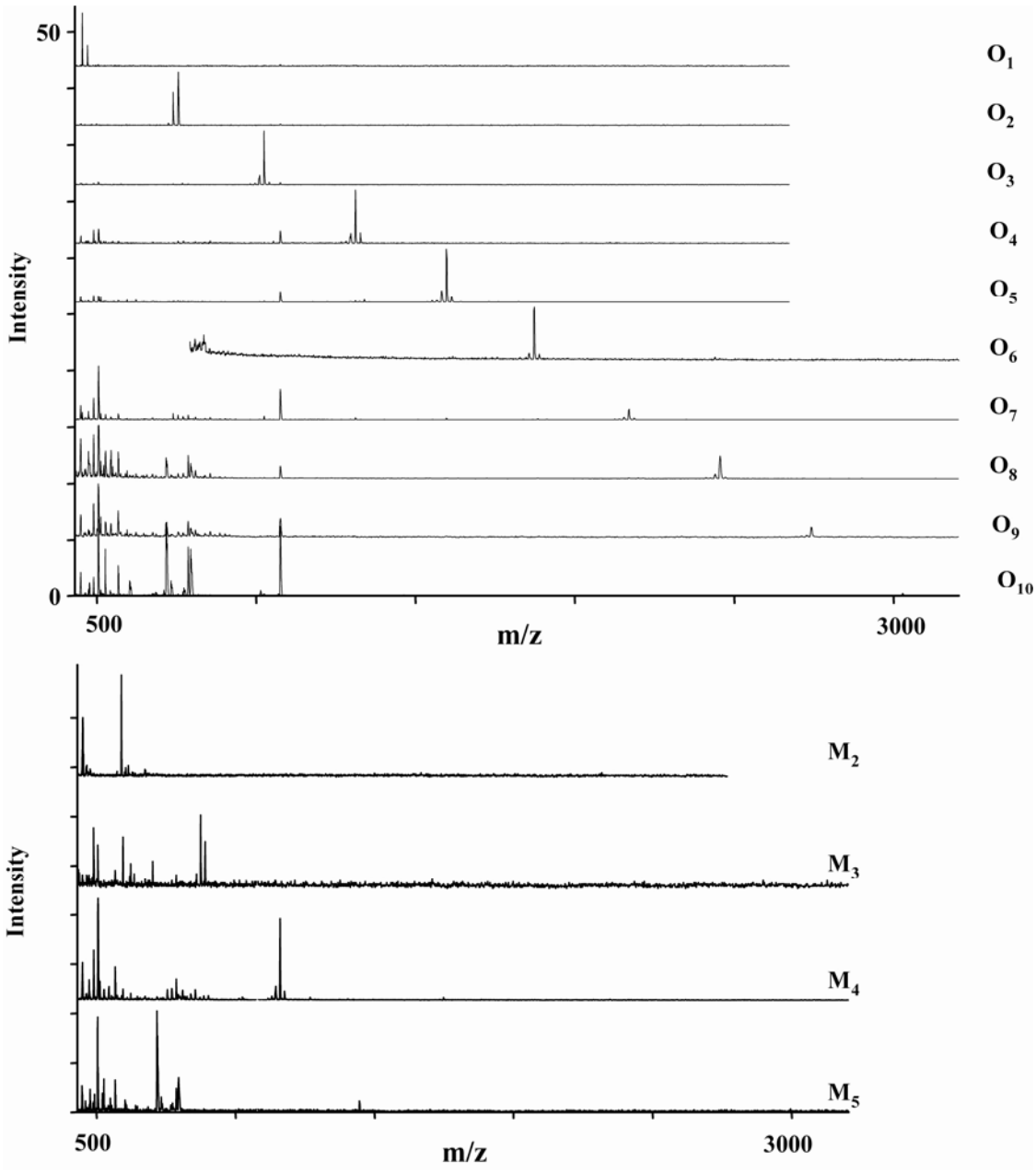
M_2: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.21 (4H, d, *J* = 3.5), 7.69 (4H, d, *J* = 3.5), 5.76 (2H, s), 3.61-3.80 (24H, m). MS (MALDI-TOF): 589.5 ($M+Na^+$).

M_3: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.20 (6H, d, *J* = 3.5), 7.68 (6H, d, *J* = 3.5), 5.72 (3H, s), 3.60-3.73 (36H, m). MS (MALDI-TOF): 872.5 ($M+Na^+$).

M_4: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.18 (8H, d, *J* = 3.5), 7.67 (8H, d, *J* = 3.5), 5.70 (4H, s), 3.61-3.71 (48H, m). MS (MALDI-TOF): 1155.1 ($M+Na^+$).

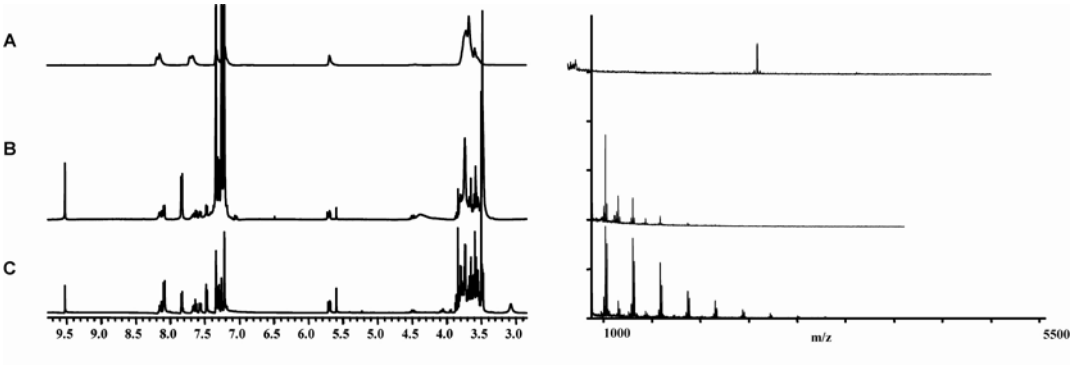
M_5: ^{1}H NMR ($CDCl_3$, 500MHz) δ 8.17 (10H, d, *J* = 3.5), 7.67 (10H, d, *J* = 3.5), 5.70 (5H, s), 3.61-3.71 (48H, m). MS (MALDI-TOF): 1438 ($M+Na^+$).

MALDI-TOF of the library members:



Part III. Library regeneration from one library member:

O_6 (13mg, 7μmol) was dissolved in deuterated toluene (0.51 ml) in a septum sealed Wilmad® screw cap NMR tube 507-TR. To the top of the NMR tube were inserted a few A4 molecular sieves that were large enough not to enter the tube and remain in the wider top part. Sulfuric acid (2.25μl, 42μmol) was added and the mixture was heated under inert nitrogen atmosphere for 48h. The reaction progress was directly observed by ^{1}H-NMR and MALDI-TOF analysis:

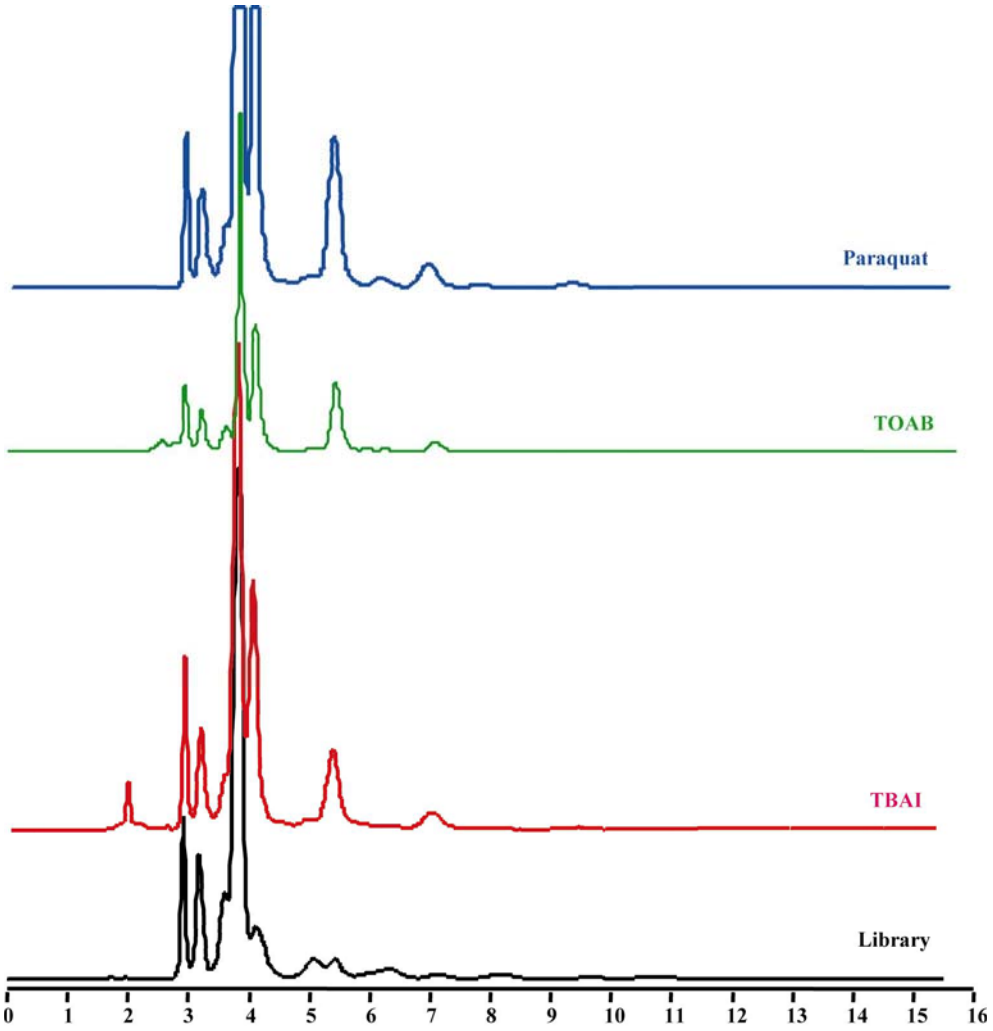


A. Starting material **O_6**. **B.** The equilibrated mixture after 48hrs with acid catalysis. **C.** The original library mixture.

^{1}HNMR shows that after 48h with acid catalysis **O_6** displays the typical acetal peaks observed for the DCL obtained directly from TEG and p-nitrobenzaldehyde. The MALDI spectrum shows that the original library and the library reformed from **O_6** are not identical; this may be to the different starting stoichiometry of starting materials.

Part IV. Amplifications:

HPLC chromatograms for quaternary ammonium ions (not including $DBAPF_6$):



	M_2/O_1	M_3/O_1	M_4/O_1
Original Library	0.14	0.090	0.059
TBAI	0.86	0.39	0.10
TOAB	2.46	0.47	0.12
Paraquat	2.76	0.61	0.15

Ratios measured by HPLC at 262nm.

Detailed amplification by $DBAPF_6$:

	Library	24h templation	3 days templation
O_1	14.71%	2.07%	1.52%
O_2	16.01%	1.76%	1.30%
Aldehyde	27.88%	7.64%	3.26%
M_1	5.58%	51.92%	56.40%
O_5	2.60%	0.69%	0.49%
M_2	2.02%	5.57%	4.35%
O_6	5.40%	1.29%	2.11%
M_3	1.62%	1.05%	0.65%
O_7	2.11%	0.21%	0.36%
M_4	0.80%	0.51%	0.50%
O_8	1.24%	0.28%	0.23%
M_5	0.38%	0.22%	0.30%
O_9	0.14%	0.08%	0.18%
O_3+ O_4	19.52%	26.72%	28.36%

Detailed amplification by TBAI:

	Library	24h templation	3 days templation
O_1	14.71%	9.41%	10.41%
O_2	16.01%	9.99%	10.23%
Aldehyde	27.88%	11.28%	11.83%
M_1	5.58%	37.22%	35.48%
O_5	2.60%	0.07%	0.28%
M_2	2.02%	8.63%	10.88%
O_6	5.40%	1.12%	1.37%
M_3	1.62%	3.60%	4.29%
O_7	2.11%	0.46%	0.52%
M_4	0.80%	0.93%	1.07%
O_8	1.24%	0.24%	0.28%
M_5	0.38%	0.14%	0.24%
O_9	0.14%	0.00%	0.12%
O_3 + O_4	19.52%	16.92%	13.01%

Due to the overlap of some of the signals in the HPLC chromatograms (that include **O_3**, p-nitrobenzaldehyde, **O_4** and **M_1**), and the much stronger absorbance of p-nitrobenzaldehyde relative to the acetal members, HNMR signal integrals of the overlapping fractions were used to calculate the ratio of each component. After this the

percentages for all members were calculated from the HPLC (without the overlapping fractions), taking into account the ratios from the HNMR.

Part V. Binding constant calculations:

$DBAPF_6$ (2mg, 5.83μmol) was titrated with **M_2**. The shift of the benzyl HNMR peak of the salt was used to calculate K. Solubility concerns dictated the use of a solution of deuterated toluene and 17% deuterated acetonitrile, which probably lowers the constant existing in the library itself.

Initial volume (in the NMR tube) was 480μl. It contained 0.0121 M $DBAPF_6$, as mentioned above. **M_2** concentration in the solution used for the titration was 0.0394 M.

The equation used was derived as follows:

$$K = \frac{[C]}{[DBA] * [M]}$$

$$[M]t = [C] + [M] = \frac{x * [M]0}{x + v0}$$

$$[DBA]t = [C] + [DBA] = \frac{v0 * [DBA]0}{x + v0}$$

$$y = dobs = dDBA * \frac{[DBA]}{[DBA]t} + dC * \frac{[C]}{[DBA]t}$$

Where, K is the association constant, [C] is the complex concentration, [DBA] is the free salt concentration, [M] is free **M_2** concentration, t stands for total forms in the solution – free and complexed, [M]0 stands for initial concentration of the stock solution, [DBA]0 stands for initial concentration before any addition of **M_2**, x is the volume of **M_2** solution added by titration, dobs is the benzyl proton chemical shift observed in the NMR upon complexation, dDBA is the benzyl proton chemical shift of pure salt and dC is the benzyl proton chemical shift of the complex.

The equations as entered to OriginPro 7.5 computer program are:

A = 1+k*vo*dbao/(x+vo)+k*mo*x/(x+vo)

B = k*dbao*mo*vo*x/((x+vo)^2)

C = (A-(A^2-4*k*B)^0.5)/(2*k)

y = ddba*((vo*dbao/(x+vo))-C)/(vo*dbao/(x+vo))+dcomp*C/(vo*dbao/(x+vo))

The fit gave a K of about 200, as shown below: