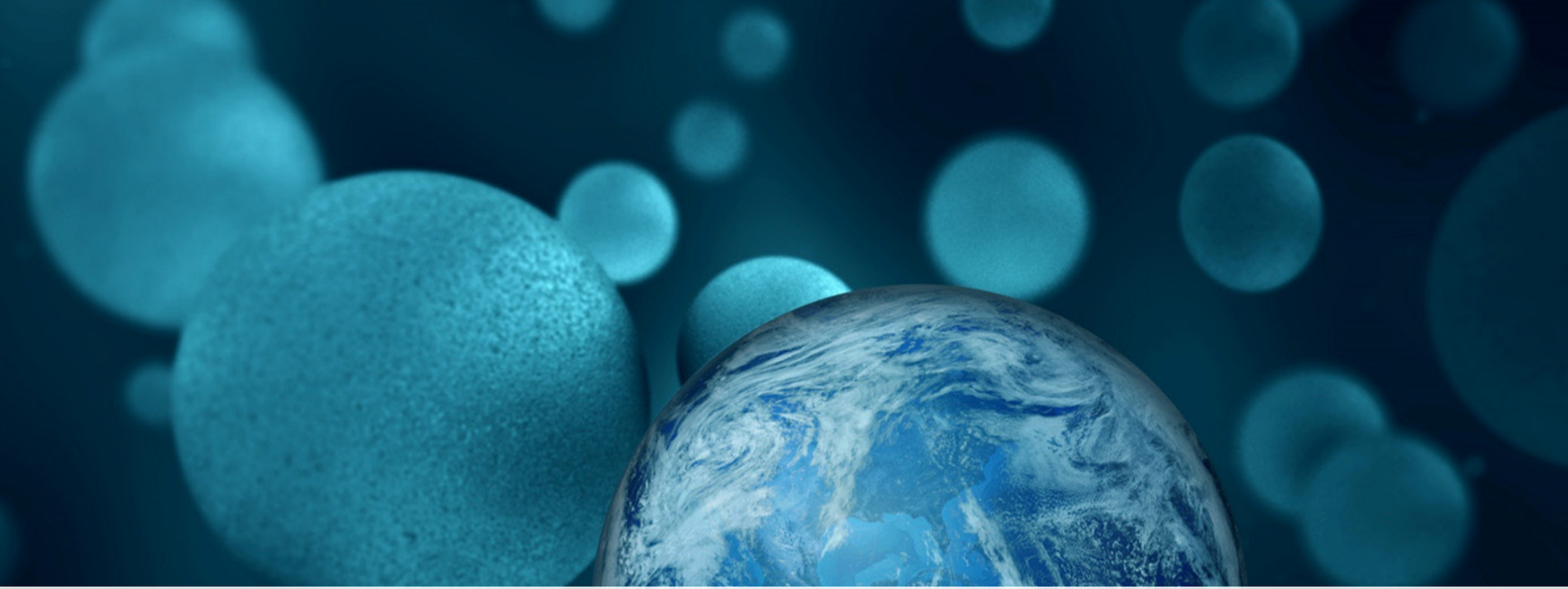
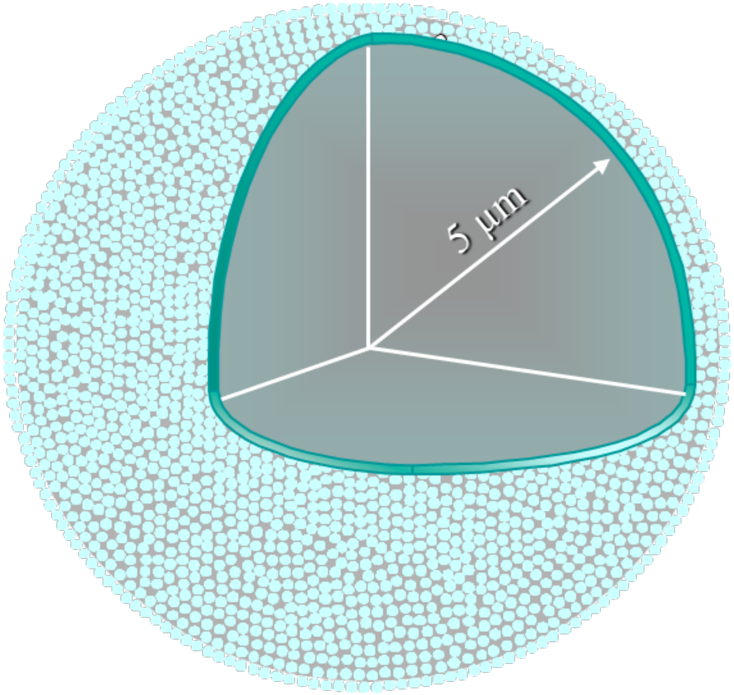


New Hyperbranched Anion Exchange Phases for High pH Separation of Carbohydrates

Chris Pohl
Vice President, Chromatography Chemistry

…of high pH carbohydrate separations



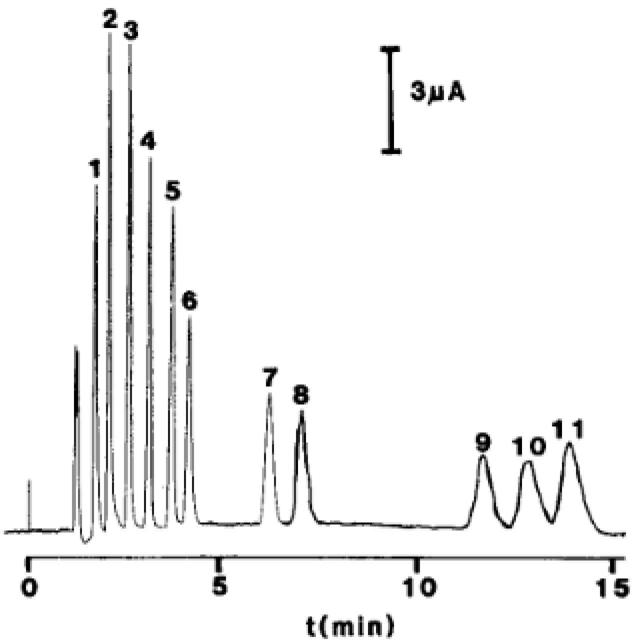


Figure 4. Separation of sugar alcohols and saccharides. Listed in order, they are: 25 ppm xylitol (1); 50 ppm sorbitol (2), rhamnose (3), arabinose (4), glucose (5), fructose (6), lactose (7); 100 ppm sucrose (8), raffinose (9), stachyose (10); 150 ppm maltose (11). 0.15 M NaOH eluent at 36°C.

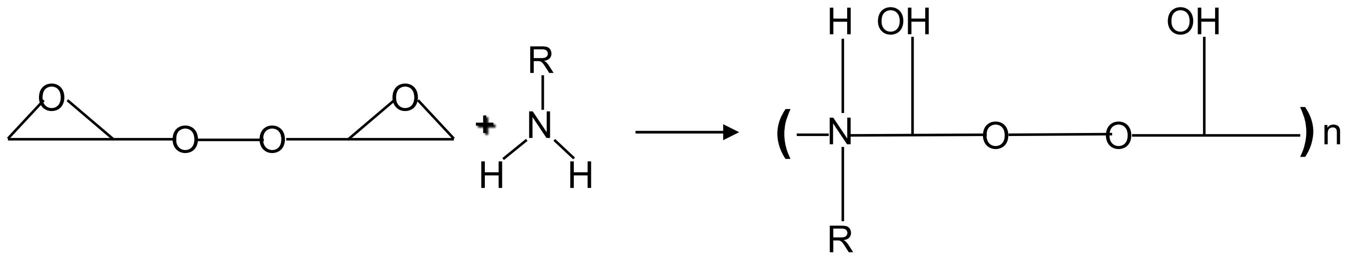
R. Rocklin, C. A. Pohl, J. Liquid Chrom. 6 (1983) 1577.

Synthesis of New Anion-Exchange Phases…

…for the separation of carbohydrates

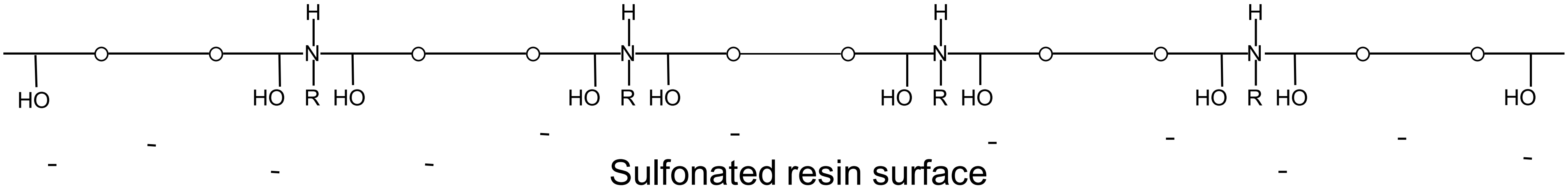
- Latex based anion-exchange phases lack the capacity to provide sufficient retention of sugar alcohols at an eluent pH sufficient for ionization
- The CarboPac MA1 column does have sufficient capacity but has poor mass transport and bed stability characteristics
- An ideal material should have both high stationary phase pH and high capacity to provide both good retention and good selectivity for sugar alcohols
- Hyperbranched structures should be able to achieve both high capacity and high stationary phase pH

Diepoxide and Primary Amine Reactions



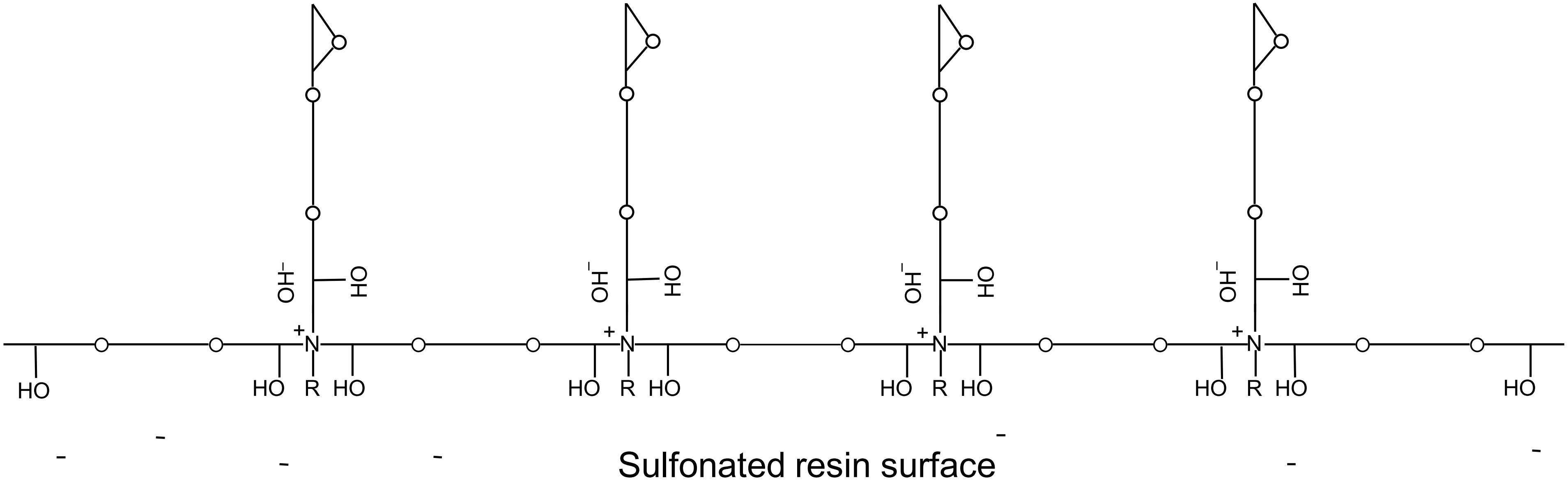
Hypothetical product of 1:1 ratio (diepoxide:amine)

Basement coating [1:1 ratio (diepoxide:amine)]

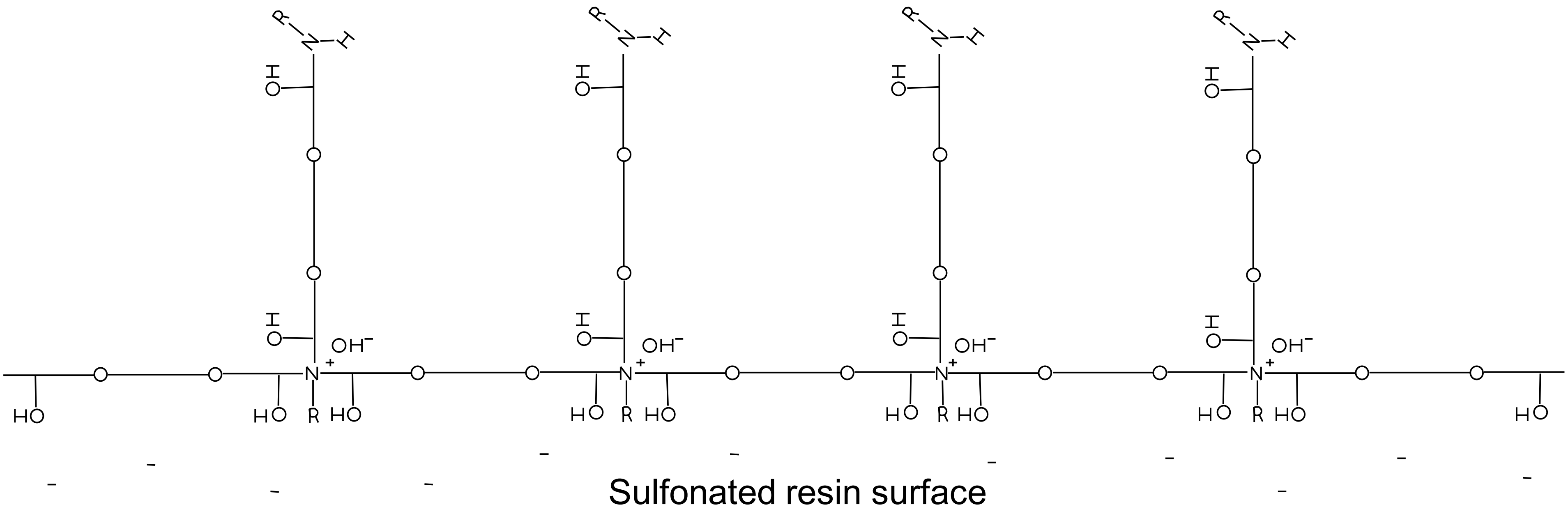


ThermoFisher SCIENTIFIC

Layer 1 after diepoxide treatment

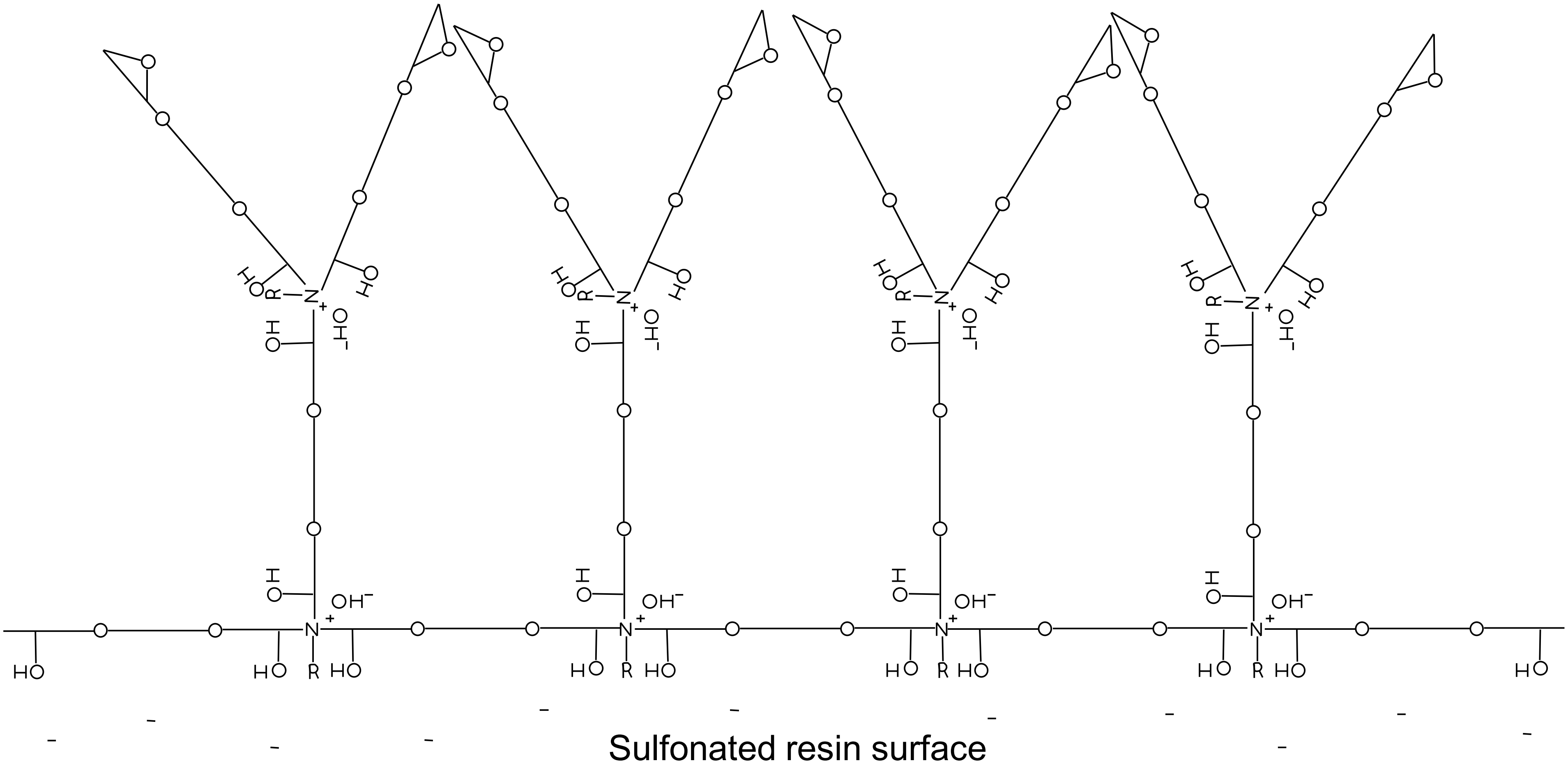


Layer 1 after diepoxide and amine treatment

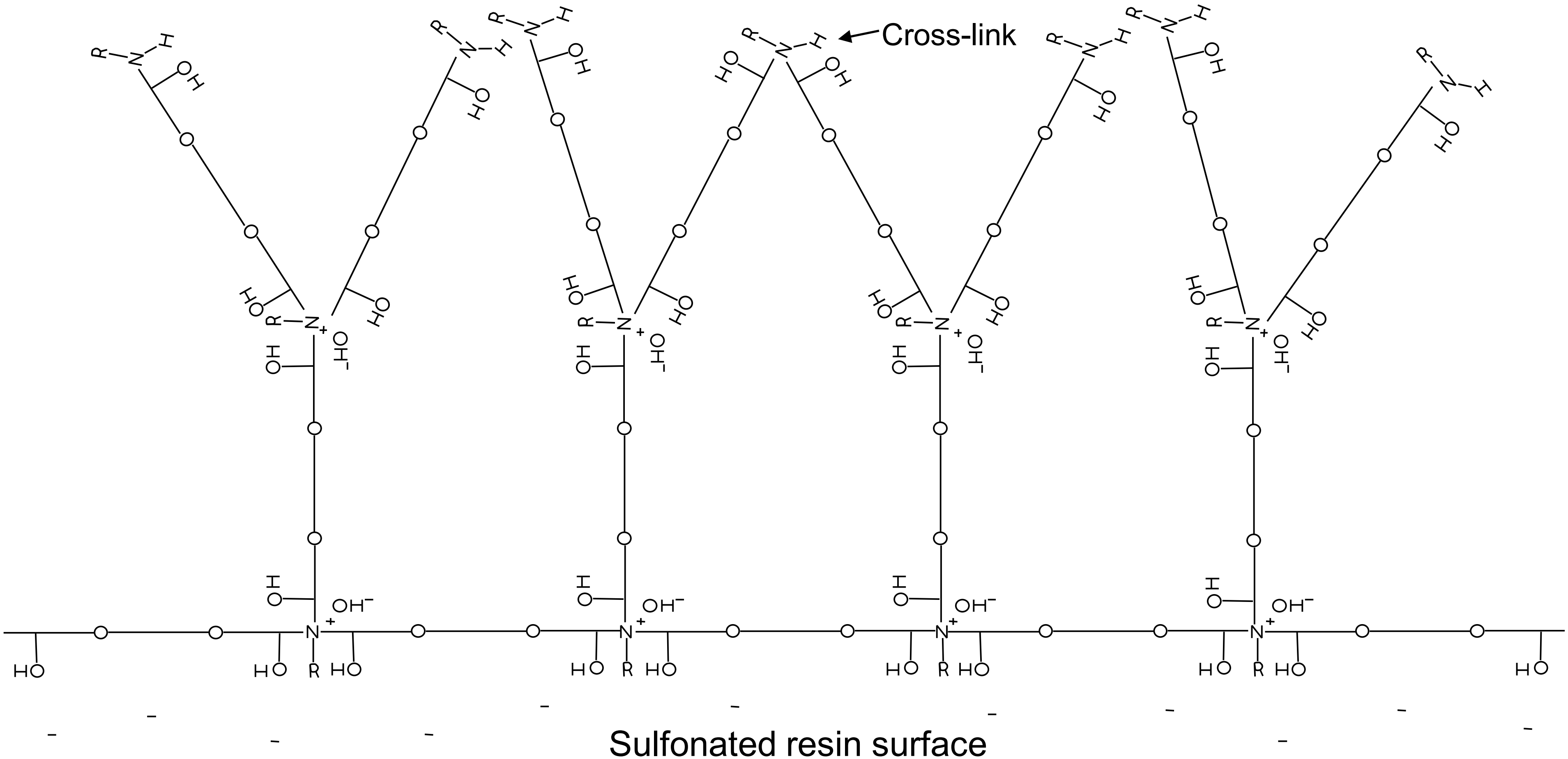


Sulfonated resin surface

Layer 2 after diepoxide treatment



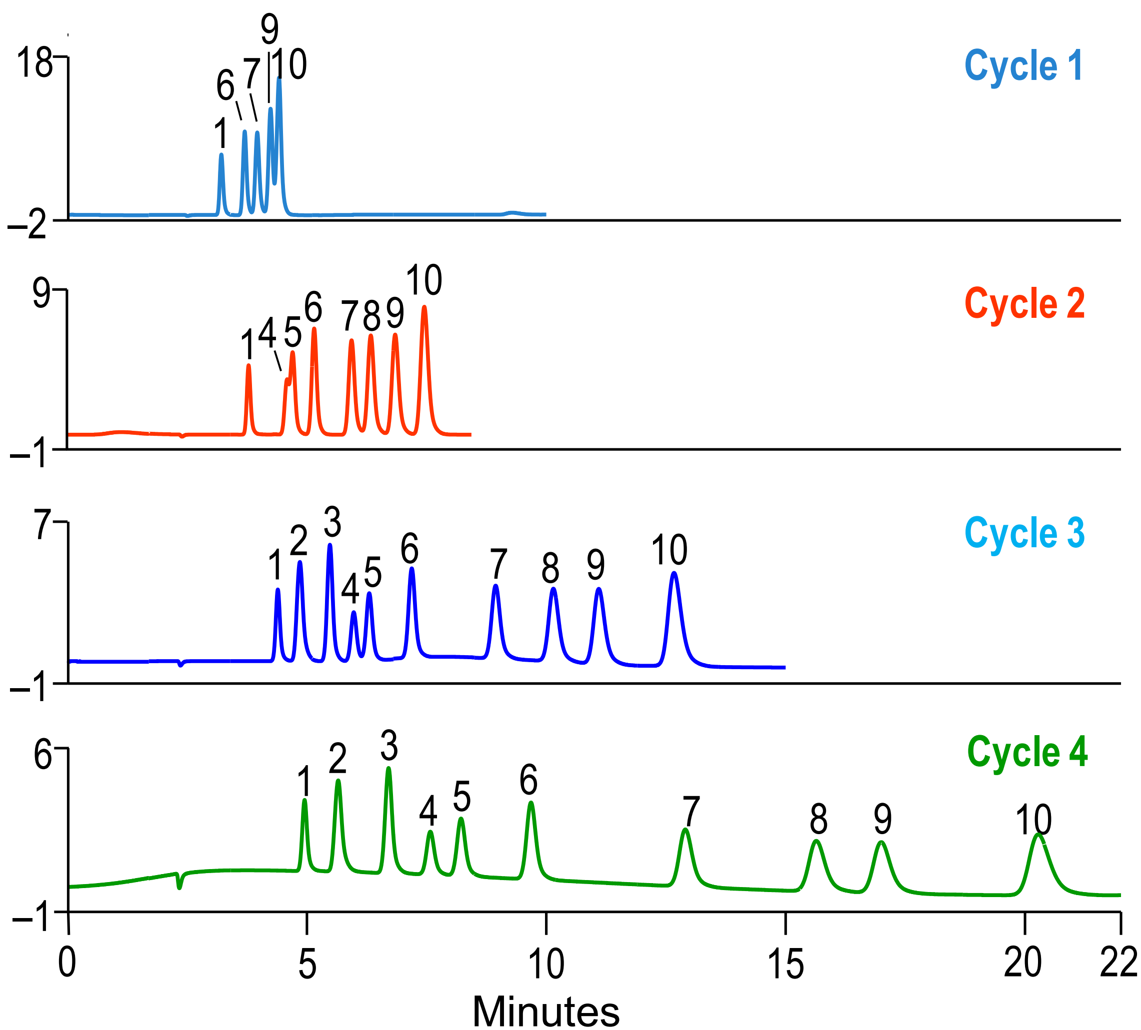
Layer 2 after diepoxide and amine treatment



Application of Layers In-Column

- The basement layer is applied to a packed column:
  - Pass diepoxide-amine solution mixture (1:1 mole ratio) through column for 10 minutes, allow to react at 60 ° C for 60 minutes
- Each layer consists of the following reaction “cycle”:
  - Pass diepoxide solution through column for 10 minutes, allow to react at 60 ° C for 30 minutes
  - Pass amine solution through column for 10 minutes, allow to react 60 ° C for 30 minutes

All In-Column Preparation



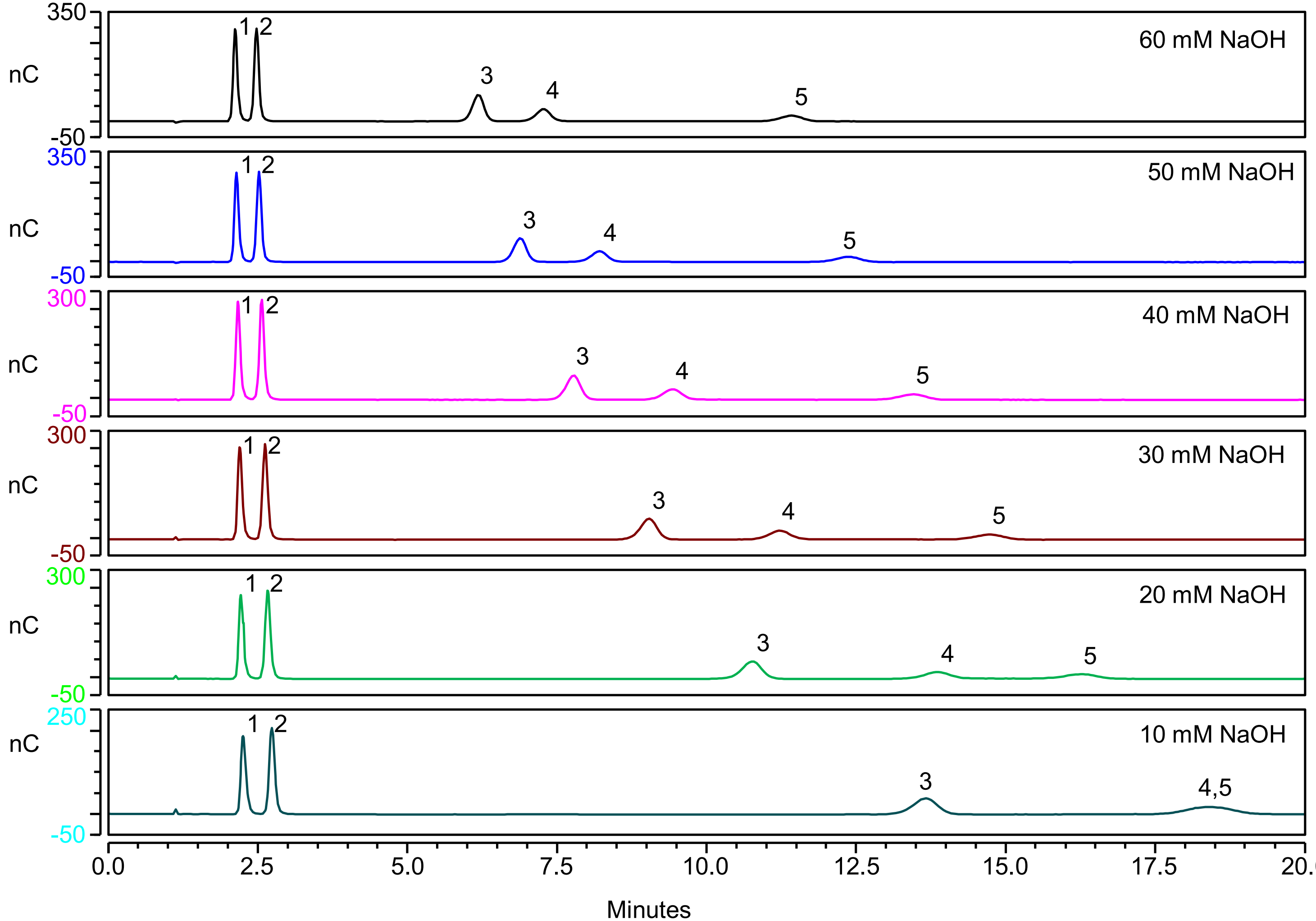
Column:	Prototype Thermo Scientific™ Dionex™ IonPac™ AS19
Eluent:	5 mM KOH
Flow rate:	1 mL/min
Inj. volume:	25 µL

Peaks:

1.	Fluoride	1	ppm
2.	Acetate	10	
3.	Formate	5	
4.	Chlorite	5	
5.	Bromate	10	
6.	Chloride	3	
7.	Nitrite	5	
8.	Chlorate	10	
9.	Bromide	10	
10.	Nitrate	10	

Effect of Hydroxide Concentration

on Thermo Scientific™ Dionex™ CarboPac™ PA1selectivity



1. Dulcitol
2. Mannitol
3. Glucose
4. Fructose
5. Sucrose

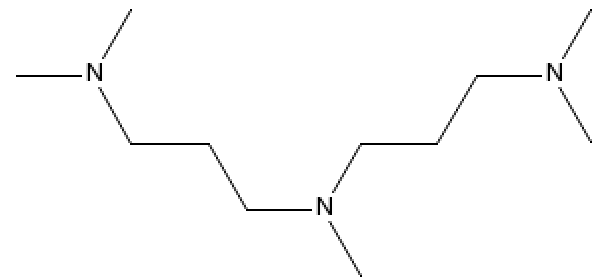
Why Not Use the Amine/Epoxide Approach for Carbohydrates?

- The beta hydroxyl groups distributed throughout the amine/epoxide hyperbranched structure have pKa values similar to carbohydrates
- When amine/epoxide hyperbranched structures are in the hydroxide form, a significant portion of the beta hydroxyl groups are ionized, dropping the stationary phase pH and preventing ionization and thus retention of carbohydrates

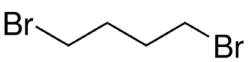
	pKa
Glucose	12.28
Galactose	12.35
Fructose	12.03
Sucrose	12.62
Lactose	11.98
Sorbitol	13.60
Choline	13.9

Alternative Chemistry for Hyperbranched Ion Exchange Architectures

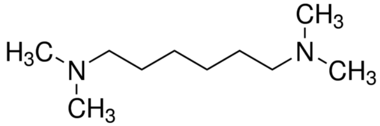
- Preferred reagents:
  - 2,6,10-Trimethyl-2,6,10-triazaundecane (pentamethyldipropyltriamine, PMDPTA)



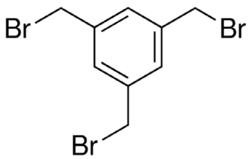
  - 1,4-dibromobutane (DBB)



- Secondary reagents:
  - N,N,N′,N′-Tetramethyl-1,6-hexanediamine (TMHDA)



  - 1,3,5-Tri(bromomethyl)benzene (TBMB)

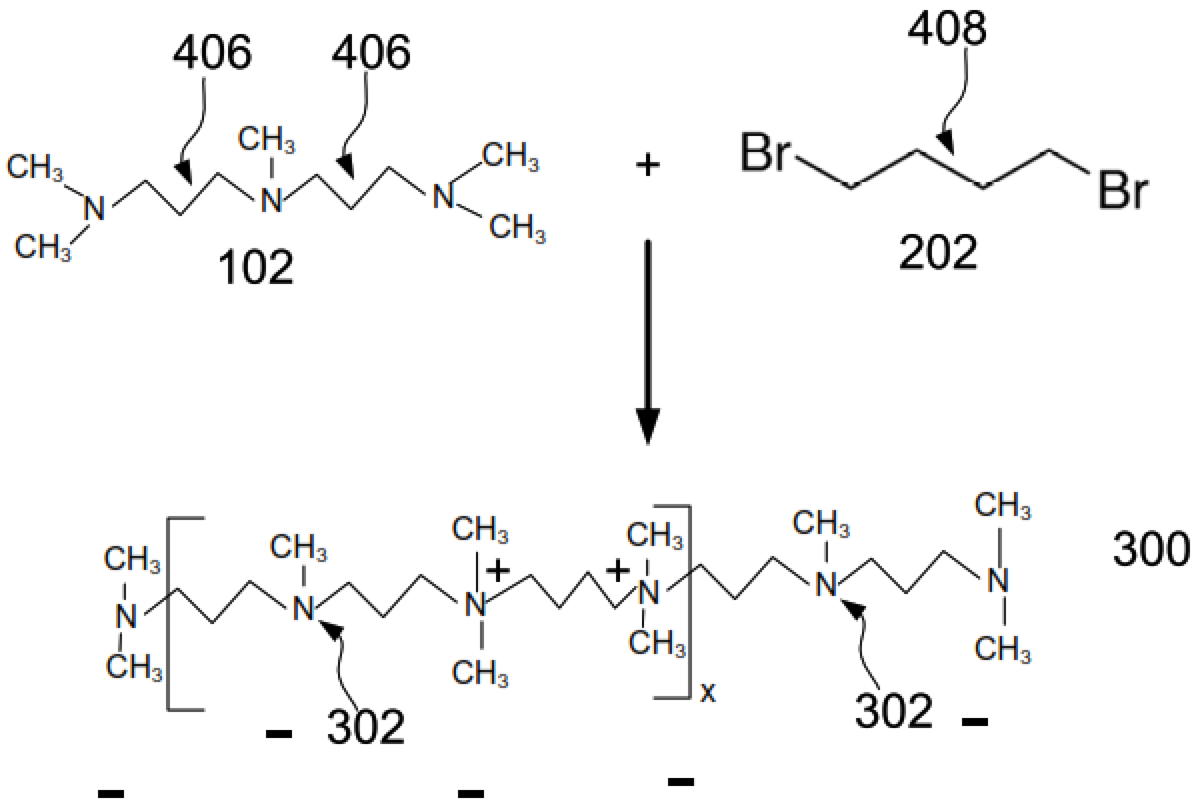


  - 2,9,13-Trimethyl-2,9,13-triazaheptadecane (pentamethyldihexyltriamine, PMDHTA)

Challenges with Amine-Alkylhalide Reactions

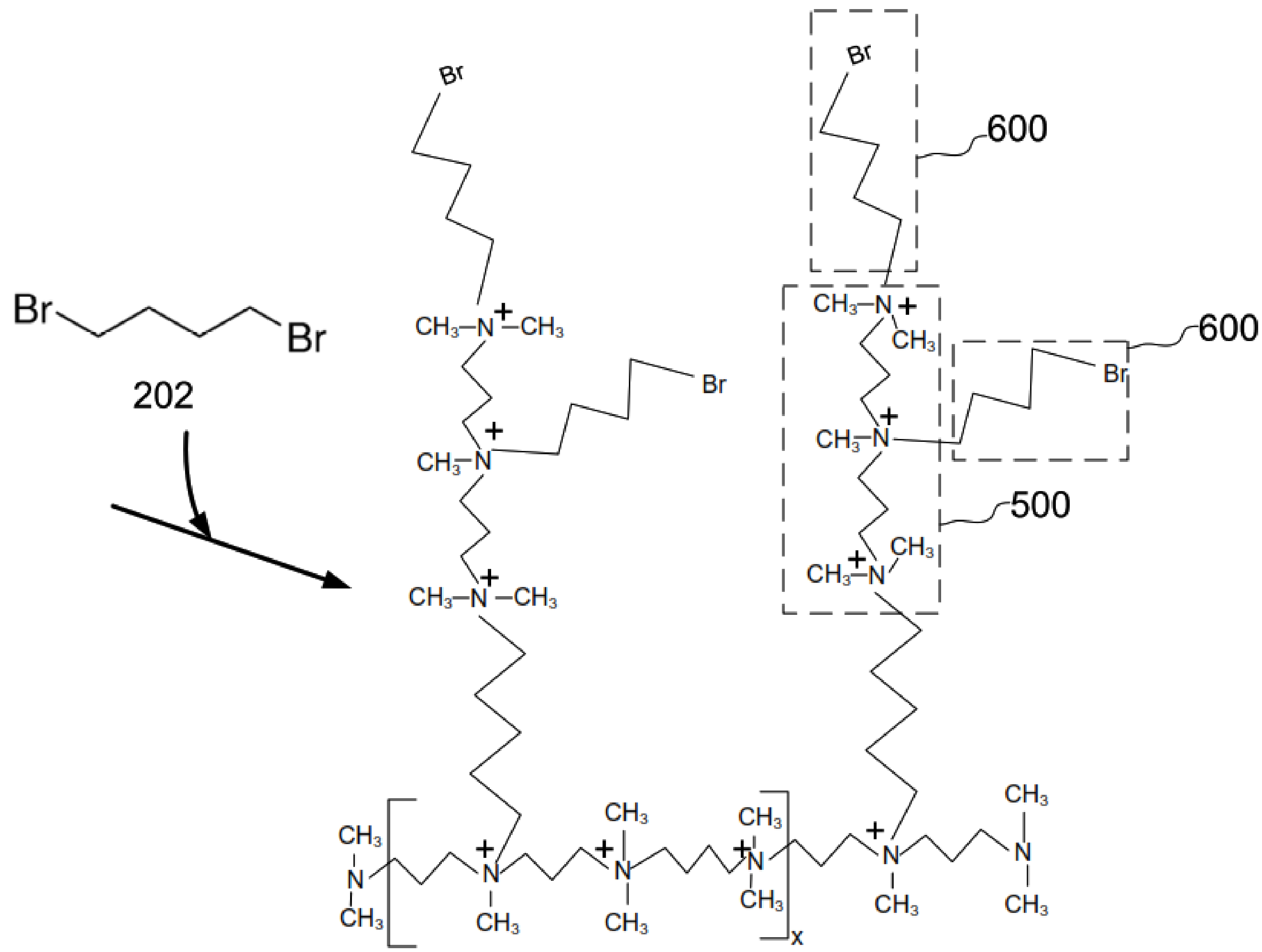
- Alkylhalides:
  - Aren’t soluble in water
  - Are less reactive than epoxides (possible exception: alkyliodides)
  - Form unreactive intermediates with primary and secondary amines
  - Good solvents for the reaction products result in slow reaction kinetics
  - Poor solvents for the reaction products exhibit good reaction kinetics but reaction products precipitate, limiting molecular weight of the polymer
  - Best solvents are IPA or 4 parts acetonitrile to 1 part methanol

Basement Layer Synthesis



Structure After Additional Reaction Steps

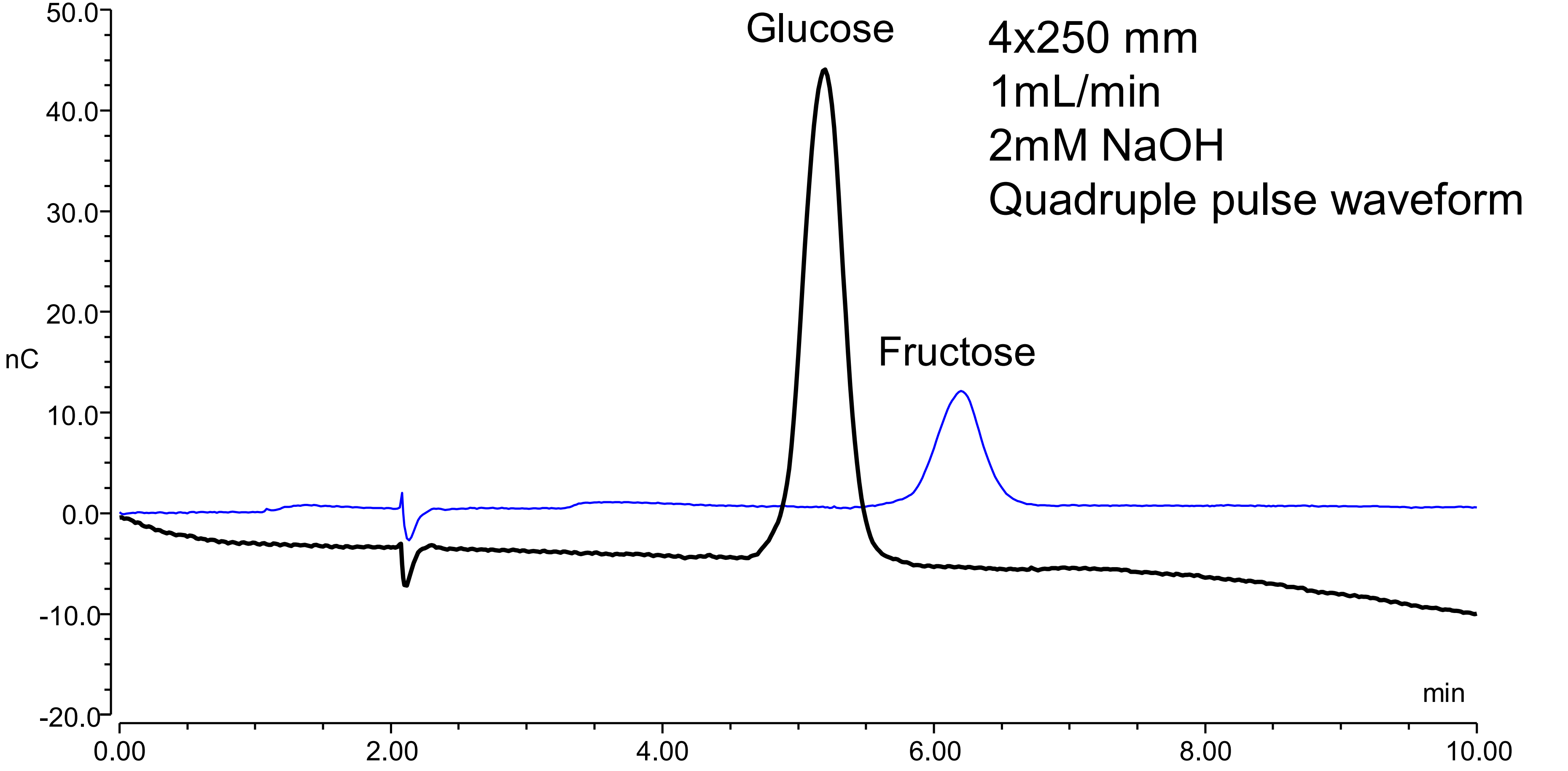
FIG 6



Protocol for In-Column Synthesis with PMDPTA and DBB

- The basement layer is applied to a packed column:
  - Pass PMDPTA-DBB mixture (1:1 mole ratio in 20% MeOH-80% ACN) through column at 70^{o}C for 90 min
- 4 additional layers, each layer consists of the following reaction "cycle":
  - Pass 38% DBB solution in 20% MeOH-80% ACN through column for 60 min at 70^{o}C
  - Pass a 50% PMDPTA solution in 20% MeOH-80% ACN through column for 60 min at 70^{o}C

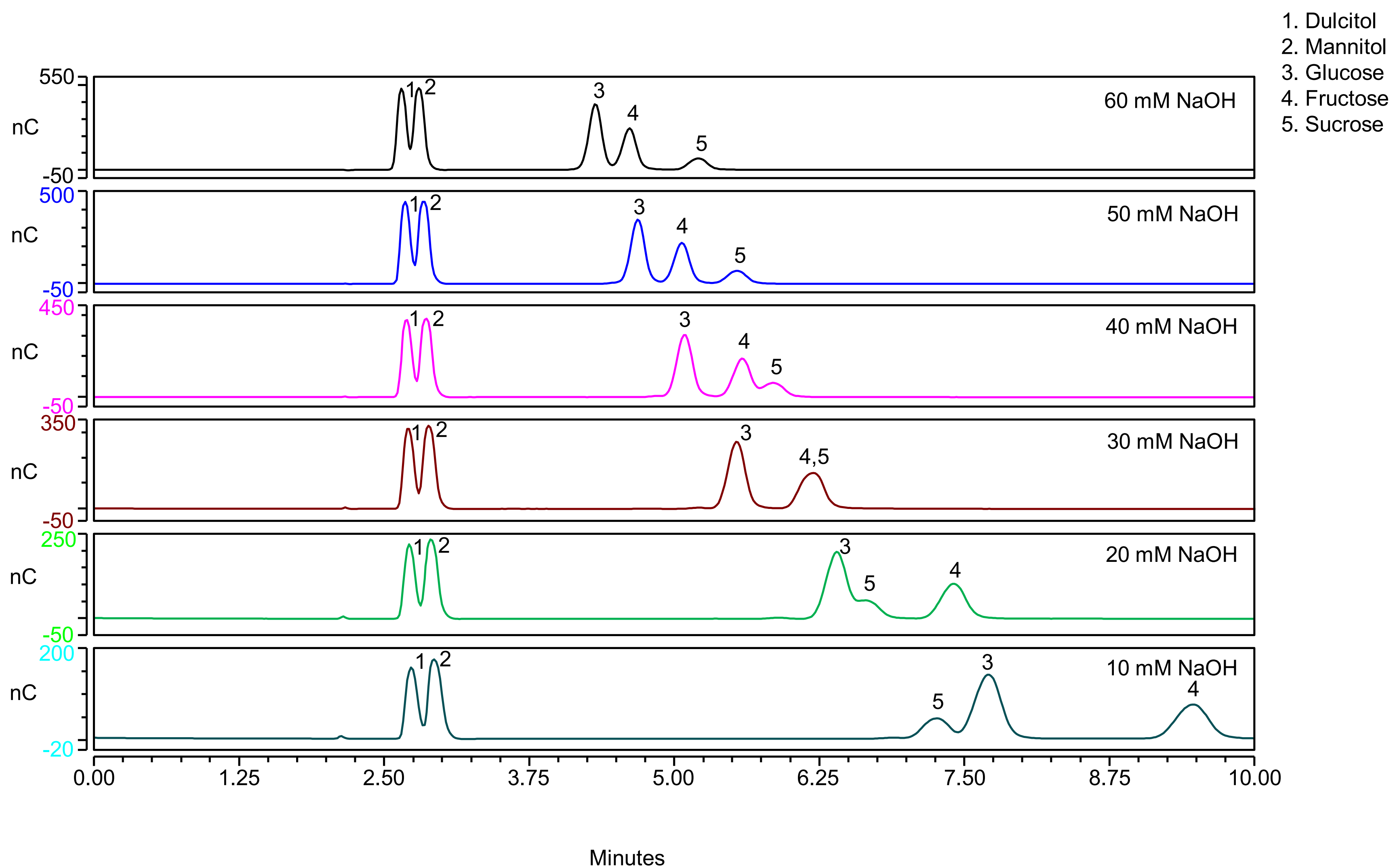
…on a hyperbranched anion-exchange phase



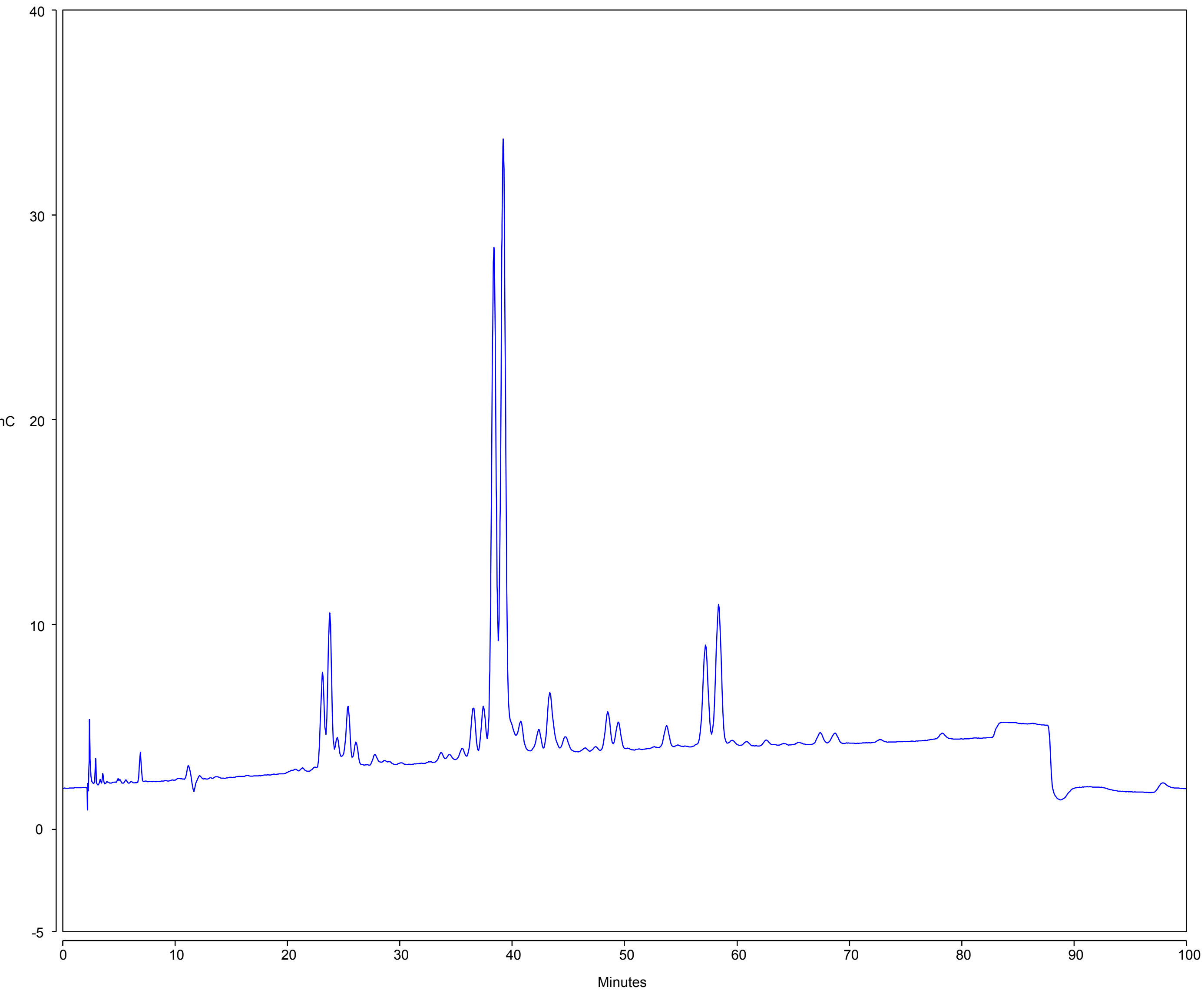
Protocol for Batch Synthesis with PMDHTA and DBB

- The basement layer is applied to bulk resin:
  - Rinse 6.2 g SMP resin with solvent (IPA) to remove water, transfer resin to vial and add 6.0 g IPA, 2.9 g PMDHTA and 2.1 g DBB and allow to react at 70 ^{o}C for 165 minutes. Filter and wash with IPA.
- 3 additional layers, each layer consists of the following reaction “cycle”:
  - Add 6 g of IPA and 2 g of DBB to vial containing resin. Allow to react at 70 ^{o}C for 120 minutes. Filter and wash with IPA.
  - Add 2 g of IPA and 1.5 g of PMDHTA to vial containing resin. Allow to react at 70 ^{o}C for 120 minutes. Filter and wash with IPA.
- Quaternize remaining tertiary amines
  - Add 2 g of IPA and 1.5 g of methyliodide to vial containing resin. Allow to react at 70 ^{o}C for 120 minutes. Filter and wash with IPA, water and 1 M NaOH.

Effect of Hydroxide Concentration on PMDHTA-DBB Phase



Fetuin N-Linked Oligosaccharide Alditols



Column: 4 x 250 mm

Eluents: A: DI H2O
B: 0.1M NaOH
C: 0.1MNaOH, 0.25M NaOAc

Flow: 1.0 mL/min

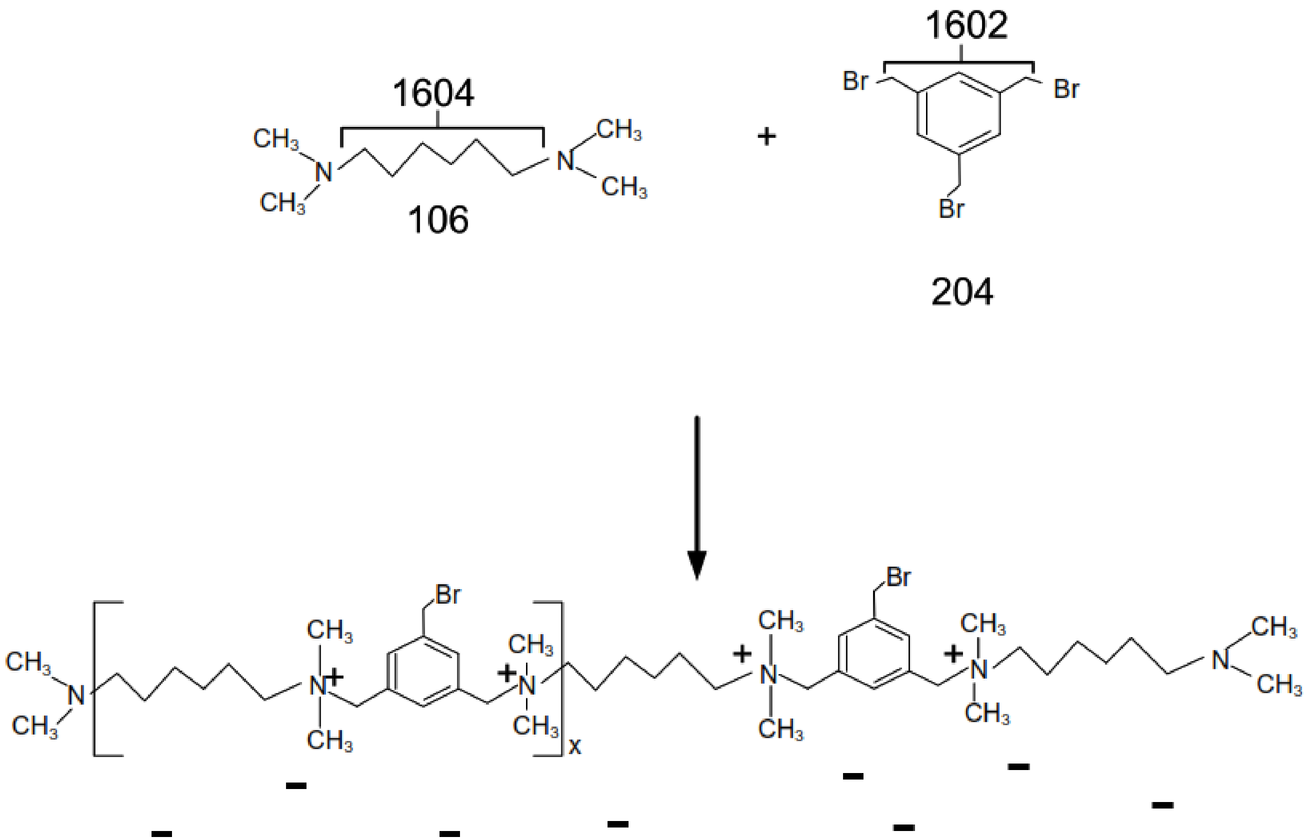
Temp: 30 ºC

Inj: 10 µL 50µM Fetuin Alditols

Det: IntAmp
Standard Quad WF

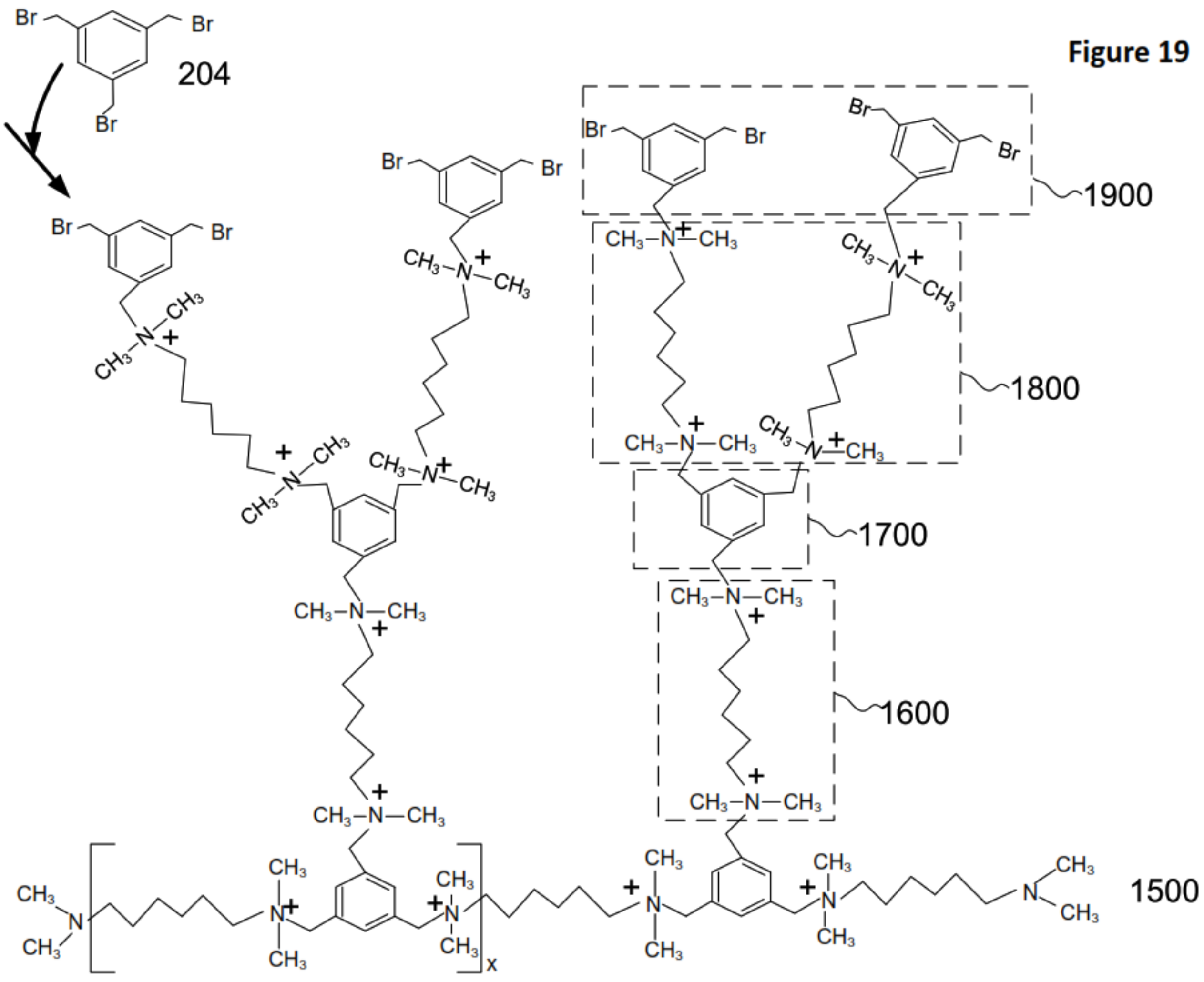
Gradient: 20-225 mM NaOAc
(0.1M NaOH) in 80 min

Basement Layer Synthesis



Negatively Charged Polymeric Particle -
Sulfonated Resin Surface

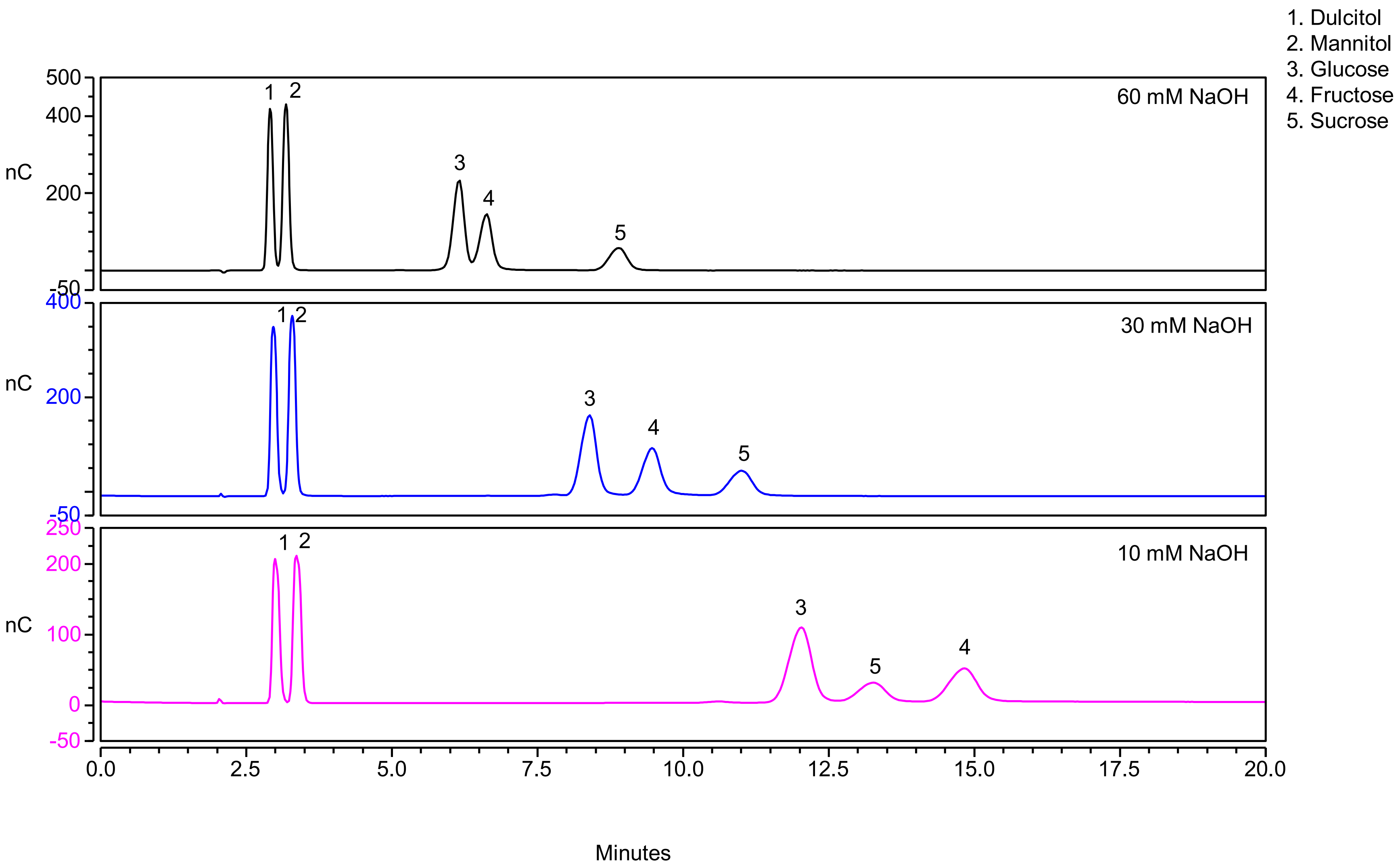
After 2 Reaction Cycles



Protocol for Batch Synthesis with TMHDA and TBMB

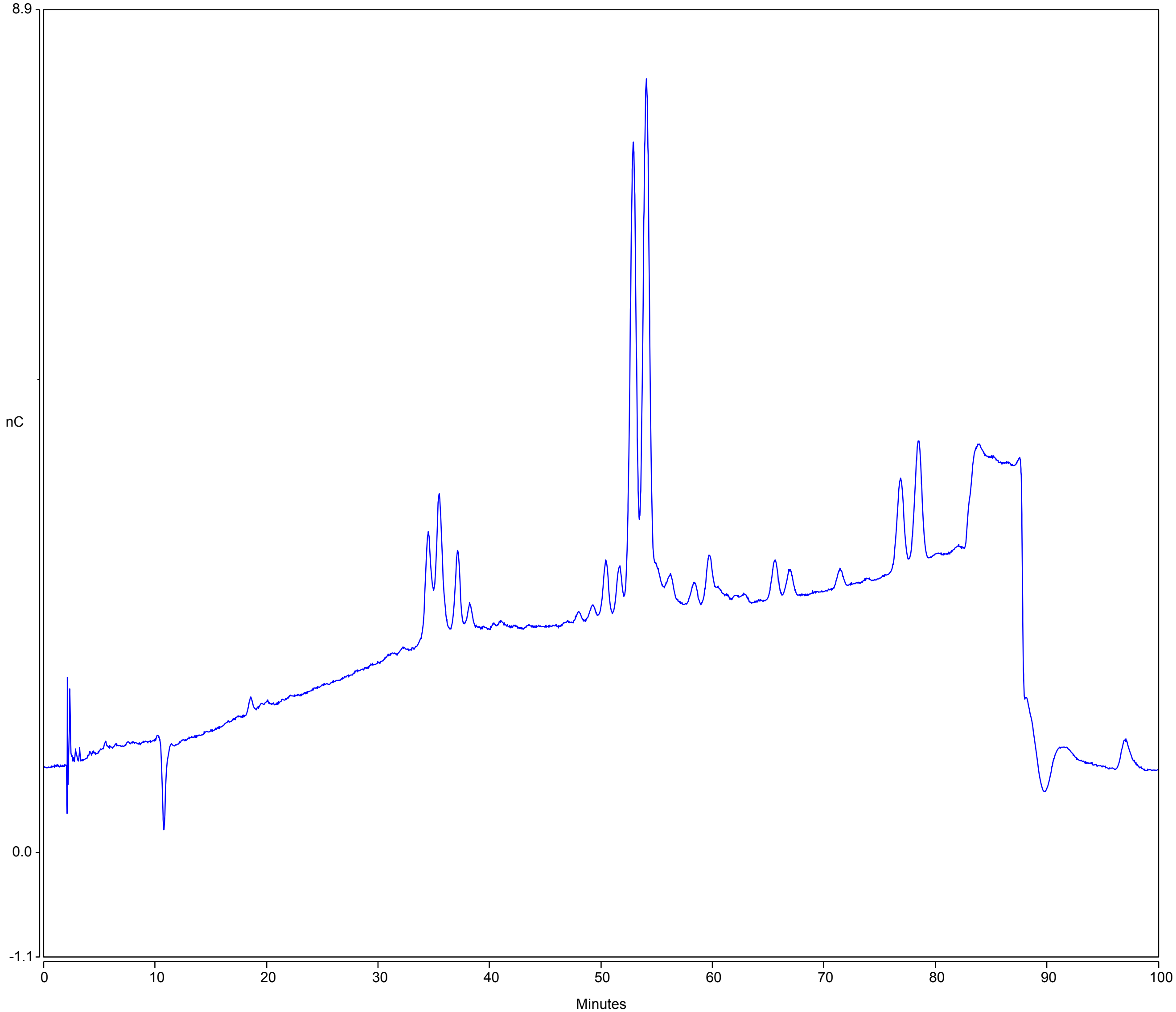
- The basement layer is applied to bulk resin:
  - Rinse 6.4 g SMP resin with solvent mixture (4:1 ACN:MeOH) to remove water, transfer resin to vial and add 6.0 g solvent mixture and 1.35 g TBMB and heat capped vial in 50 ^{o}C oven for 10 minutes to dissolve the TBMB. Mix 0.65 g TMHDA and 1.5 g solvent mixture and add dropwise while stirring. Cap vial and allow to react at 50 ^{o}C for 30 minutes. Filter and wash with solvent mixture.
- 3 additional layers, each layer consists of the following reaction “cycle”:
  - Add 3 g of solvent mixture and 0.9 g of TMHDA to vial containing resin. Allow to react at 50 ^{o}C for 30 minutes. Filter and wash with solvent mixture.
  - Add 3 g of solvent mixture and 0.9 g of TBMB to vial containing resin. Allow to react at 50 ^{o}C for 30 minutes. Filter and wash with solvent mixture.
- React remaining bromomethyl groups
  - Add 2 g of solvent mixture and 1.5 g of 33% trimethylamine in ethanol to vial containing resin. Allow to react at ambient temperature overnight. Filter and wash with solvent mixture, water and 1 M NaOH.

Effect of Hydroxide Concentration on TBMB-TMHDA Phase



Thermo Fisher SCIENTIFIC

Fetuin N-Linked Oligosaccharide Alditols



Column: 4 x 250 mm

Eluents: A: DI H2O
B: 0.1M NaOH
C 0.1MNaOH, 0.25M NaOAc

Flow: 1.0 mL/min

Temp: 30 ºC

Inj: 10 µL 50µM Fetuin Alditols

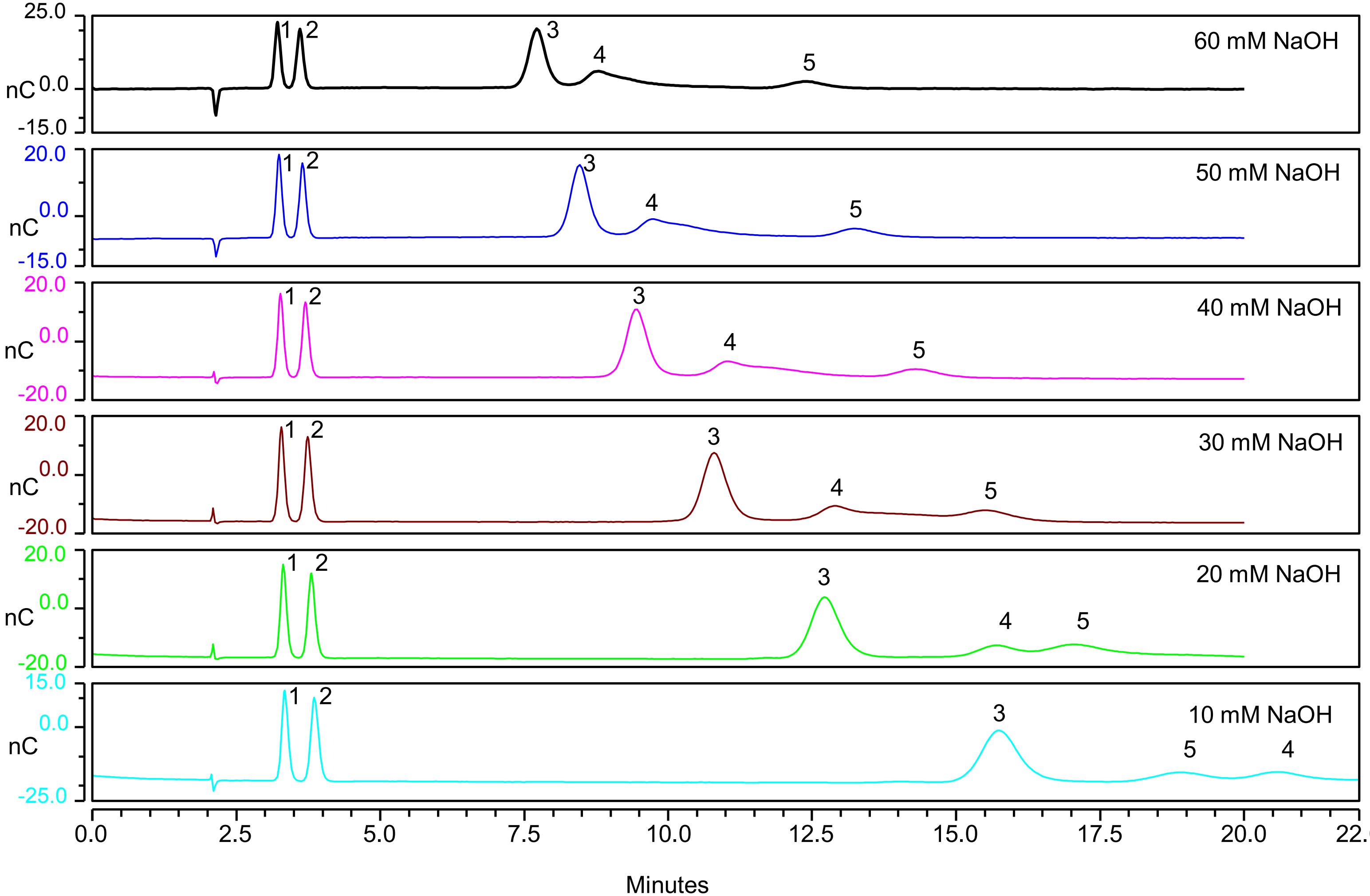
Det: IntAmp
Standard Quad WF

Gradient: 20-225 mM NaOAc
(0.1M NaOH) in 80 min

Protocol for Batch Synthesis with PMDHTA, DBB and TBMB

- The basement layer is applied to bulk resin:
  - Rinse 6.2 g SMP resin with solvent (IPA) to remove water, transfer resin to vial and add 6.0 g IPA, 2.9 g PMDHTA and 2.1 g DBB and allow to react at 80 ^{o}C for 165 minutes. Filter and wash with IPA.
- 1 layer consisting of the following reaction “cycle”:
  - Add 6 g of IPA and 2 g of DBB to vial containing resin. Allow to react at 80 ^{o}C for 120 minutes. Filter and wash with IPA.
  - Add 2 g of IPA and 1.5 g of PMDHTA to vial containing resin. Allow to react at 80 ^{o}C for 120 minutes. Filter and wash with IPA.
- 2 additional layers, each layer consists of the following reaction “cycle”:
  - Add 3 g of IPA and 0.9 g of TBMB to vial containing resin. Allow to react at 50 ^{o}C for 30 minutes. Filter and wash with IPA.
  - Add 3 g of solvent mixture and 1.5 g of PMDHTA to vial containing resin. Allow to react at 50 ^{o}C for 30 minutes. Filter and wash with IPA.
- Quaternize remaining tertiary amines
  - Add 2 g of IPA and 1.5 g of methyliodide to vial containing resin. Allow to react at 80 ^{o}C for 120 minutes. Filter and wash with IPA, water and 1 M NaOH.

Effect of Hydroxide Concentration on PMDHTA-DBB-TBMB Phase



1. Dulcitol
2. Mannitol
3. Glucose
4. Fructose
5. Sucrose

Conclusions

- Hyperbranched structures that contain no beta hydroxyl groups are possible
- Phases based on pentamethyldipropylenetriamine although suitable for preparation of hyperbranched phases is not sufficiently stable
- Tribromomethylbenzene is more reactive but provide lower resolution
- Phases based on pentamethyldihexyltriamine overcomes the stability issue of pentamethyldipropylenetriamine but the stationary phase pH is too low for chemically released O-linked glycans
- Phases combining both pentamethyldihexyltriamine and tribromomethylbenzene show promise but have yet to be tested with chemically released O-linked glycans
- Future work will include extending the number of reaction cycles with phases combining both pentamethyldihexyltriamine and tribromomethylbenzene to further increase capacity