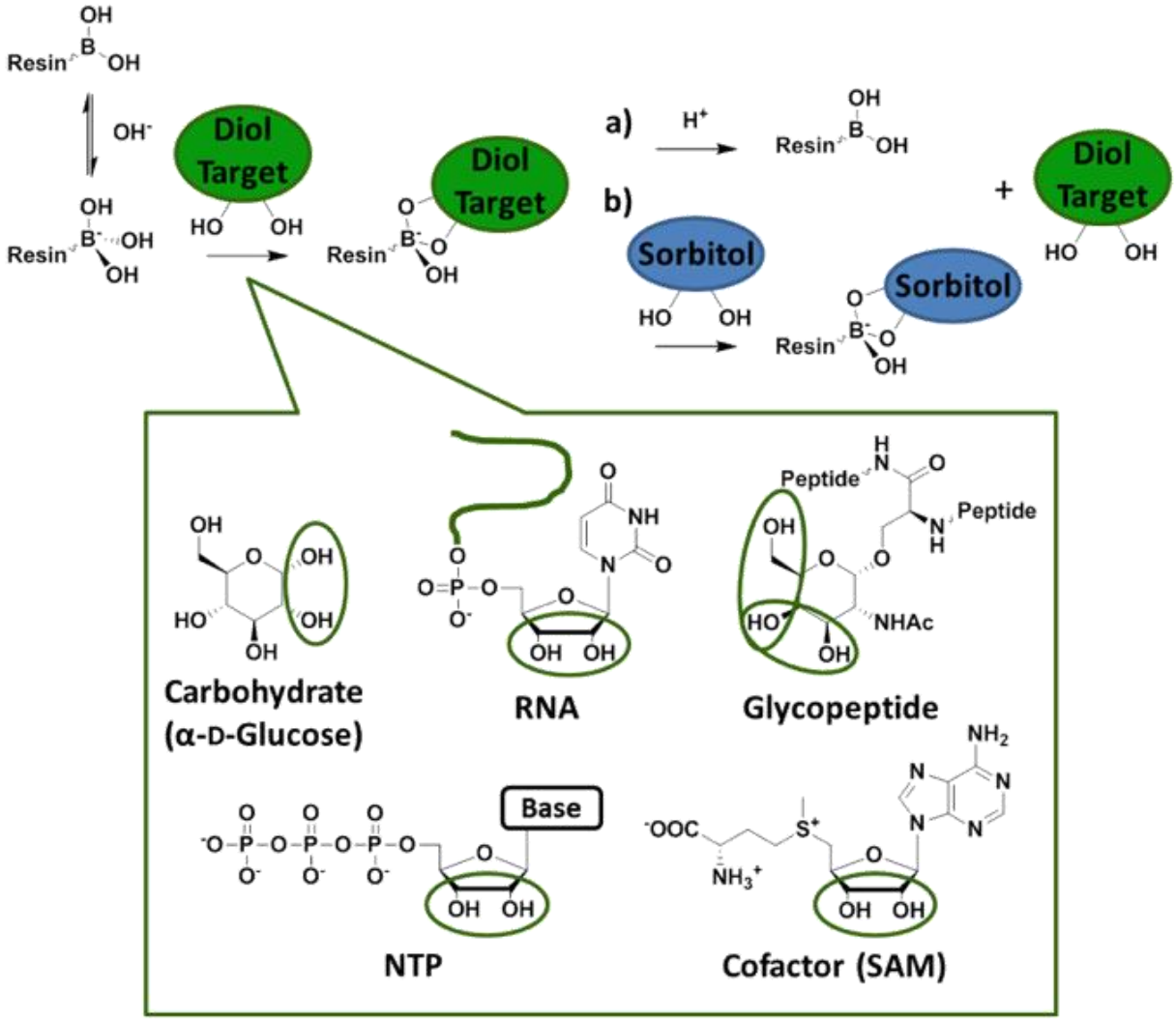
Affinity Chromatography of sugar derivatives

Catch and Release of Diols with Boronic Acid Agarose

Boronic acids bind diol compounds with high selectivity.[1] Since many biomolecules are **diol-decorated sugar derivatives**, **immobilized boronic acids** permit the single step enrichment of

- **Carbohydrates**[2] • **Nucleotides**[3] • **Cofactors**[4] • Entire **Glycoproteins**[5] and **RNAs**[6,7]

To this end, **agarose resins** stand out for their large exclusion limit (1 x 10^4 - 4 x 10^5 Da) and are therefore particularly well suited for the complexation of **large biomolecules**.[8,9] Capitalizing on the **reversibility** of boronate ester formation, captured compounds can be **easily retrieved under mild conditions** for further applications or analysis.



Scheme 1: Depending on the *pH* of the solution, **boronic acids coexist in equilibrium with their corresponding boronates** (pK_a ~ 9). While boronic esters are prone to fast hydrolysis, their tetragonal boronate counterparts are stable and allow for the efficient immobilization of ***cis*-diol** functionalities. Trapped diols are then either released by **a) lowering the *pH*** or by **b)** replacement with **sorbitol**.

Ordering information:

Immobilized m-AminoPhenyl-Boronic acid **APIEZ0-AC-160, 5ml**

30 µmol *m*-Aminophenylboronic acid/ml gel;
Beads/Particle size: 45 - 165 µm ((for 1 x 104 - 4 x 105); Recommended linear flow rate 30 cm/h ; Maximum pressure: 0.25 bar (3.6 psi)
Stable at pH4-9 (short term) – 7.5 for long term); and to all solutions commonly used in gel filtration including 8 M urea and 6 M guanidine hydrochloride Not stable in organic solvents! ; Not autoclavable!; Store at +4°C (H)

Selected references:

[1] Siegel (2012) Applications of reversible covalent chemistry in analytical sample preparation. Analyst. 137:5457.
[2] Weith et al. (1970) Synthesis of cellulose derivatives containing the dihydroxyboryl group and a study of their capacity to form specific complexes with sugars and nucleic acid components. Biochemistry. 9:4396.
[3] Moore et al. (1974) Separation of ribonucleotides and deoxyribonucleotides on columns of borate covalently linked to cellulose. Application to the assay of ribonucleoside diphosphate reductase. Biochemistry. 13:2904.
[4] Gellekink et al. (2005) Stable-isotope dilution liquid chromatography-electrospray injection tandem mass spectrometry method for fast, selective measurement of S-adenosylmethionine and S-adenosylhomocysteine in plasma. Clin. Chem. 51:1487.
[5] Mallia et. al. (1981) Preparation and use of a boronic acid affinity support for the separation and quantitation of glycosylated hemoglobins. Anal. Lett. 14:649.
[6] Schott et al. (1973) Dihydroxyboryl-substituted methacrylic polymer for the column chromatographic separation of mononucleotides, oligonucleotides, and transfer ribonucleic acid. Biochemistry. 12:932
[7] Nübel et al. (2017) Boronate affinity electrophoresis for the purification and analysis of cofactor-modified RNAs. Methods. 117:14.
[8] Chen et al. (2017) Novel boronate material affords efficient enrichment of glycopeptides by synergized hydrophilic and affinity interactions. Anal. Bioanal. Chem. 409:519.
[9] Grundy et al. (2016) PARP3 is a sensor of nicked nucleosomes and monoribosylates histone H2B(Glu2). Nat. Commun. 7:12404.

Applications using boronate affinity media.

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Application	Application Buffer	Molecules in Vo	Retained Molecules	Elution Buffer	Support Used	References
Adenylate cyclase assay	HEPES pH 7.5 + MgCI2	cAMP	ATP, AMP, and adenosine	0.05 M NaOAc	Bio-Gel P-150 boronate gel	Hageman and Kuehn 1977
Isolation of catecholamines from urine	0.1 M phosphate pH 7.0 + EDTA	Other urine components and DOPA	Norepinephrine, epinephrine	0.025 N HCI	Boric acid gel [0]	Higa et al. 1977
Modified nucleosides in urine	0.25 M NH4OAc, pH 8.8	Thymidine, adenine	Pseudouridine	0.1 M HCOOH	Bio-Gel P-2 gel, 200–400 mesh, boronate	Davis et al. 1977; Uziel et al. 1976
Separation of mono- and oligonucleotides	Triethyl ammonium	Deoxyribo-nucleotides	Ribonucleotides	H2O and others	Dihydroxyboryl methacrylate	Schott et al. 1973
Sugars	0.05 M N-methyl-morpholinium-CI, pH 7.5 + 1 M NaOAc	Erythritol,adonitol, sucrose, and D-glucose	L-arabinitol, xylitol, D-mannitol, dulcitol, sorbitol, and D-fructose*	Isocratic run, elution buffer same as application buffer	Dihydroxyboryl cellulose	Weith et al., 1970
Assay of ribonucleotide reductase	Tris, MgCI2	dUMP, dCMP	CDP	Citrate	Dihydroxyboryl cellulose Dowex 1(AG) resin	Moore et al. 1974

Regeneration and Storage

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Regeneration of the Boronic gel is facilitated by washing with starting buffer if a low-pH elution buffer has been used. If boric acid buffer or diol solutions have been used for elution, the gel must first be washed with 0.1 M acetic acid before reequilibrating with starting buffer

Typical Procedure

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Stepwise elution is typical for separation of a ribonucleotide (such as GMP) from a cyclic nucleotide (cGMP):
1. Prepare a 1 x 4 cm column of Affi-Gel boronate in 0.1 M HEPES, pH 8.5.
2. Load a 200 µl solution containing 1 µmol each cGMP and GMP in the starting buffer onto the column.
3. Wash the column with 5 column volumes of starting buffer at a flow rate of 1 ml/min and collect the cyclic nucleotide.
4. Elute the ribonucleotide with 0.1 M NaPO 4 , pH 6.0.

Related products/documents

• Other affinity resins for the purification of nucleotide-binding, phosphorylated or click-tagged proteins.
Boronic acid on magnetic beads, 1µm, 2.5% (w/v) #7A2610, 2ml
#
• Other Boronic-based conjugates [PH-BB032n], e.g. labeled probes (fluorescent, biotinylated, tagged).

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