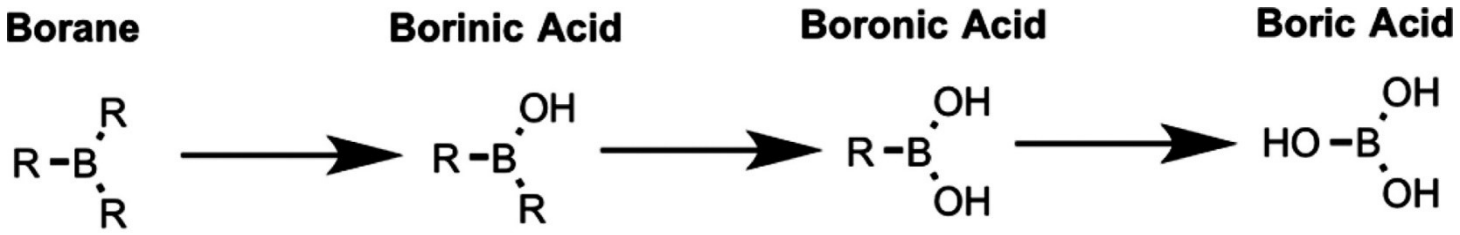
Boronic Acid Assisted Bioorthogonal Chemistry

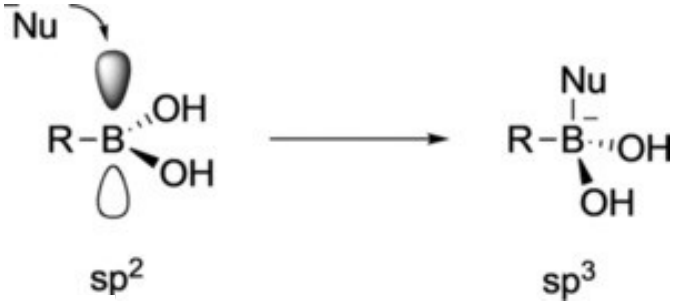
M2 Siqi Xi

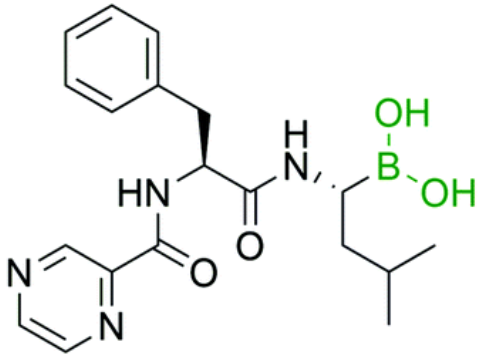
2019.08.22

Contents

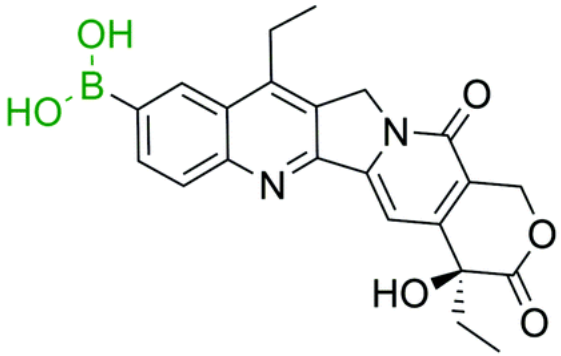
Boronic Acid





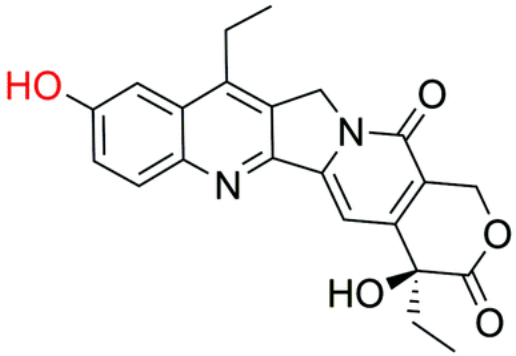


Bortezomib



SN-38 Prodrug

ROS



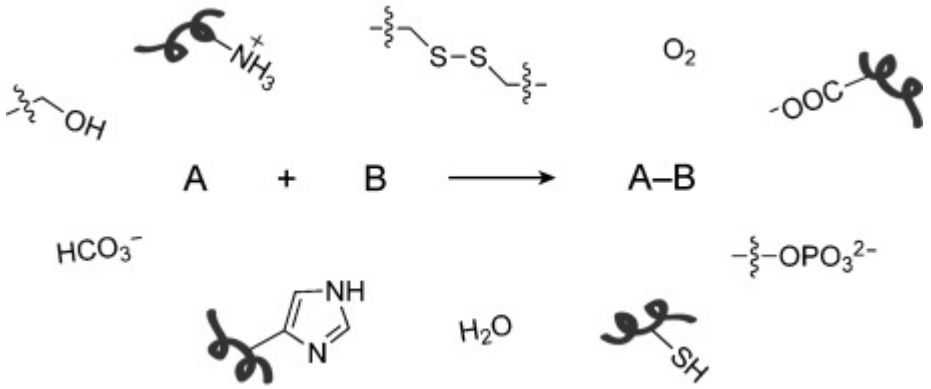
Active SN-38

Sumerlin. B. S *et al. Chem. Rev.* **2015**, *116*, 1375-1397.
Hall. D. G *et al. Angew. Chem. Int. Ed.* **2018**, *57*, 13028-13044.
Gois. P. M. P *et al. Chem. Sco. Rev.* **2019**, *48*, 3513-3536.

Bioorthogonal Chemistry

Requirements of Bioorthogonal Chemistry

- ✓ Selectivity
- ✓ Biological inertness
- ✓ Chemical inertness
- ✓ Kinetics
- ✓ Reaction biocompatibility
- ✓ Accessible engineering

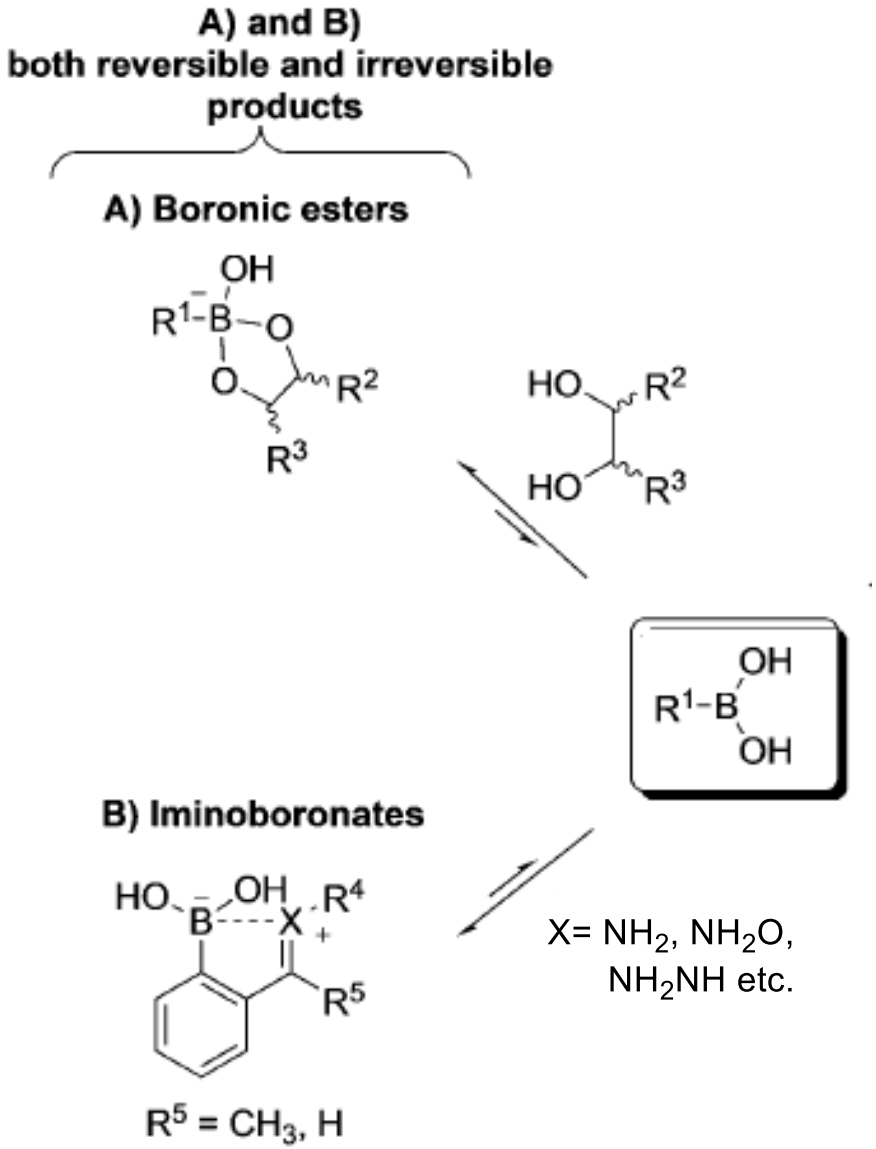


Usual Concerns of Current Biocompatible Chemistry:

- ✓ Off-target labeling
- ✓ Unintended immune
- ✓ Slow kinetics
- ✓ Side reactions with endogenous molecules.

Bertozzi, C. R *et al. Acc. Chem. Res.* **2011**, *44*, 666-676.
Gao, J *et al. Acc. Chem. Res.* **2018**, *51,* 2198-2206.

Boronic Acid for Bioorthogonal Chemistry

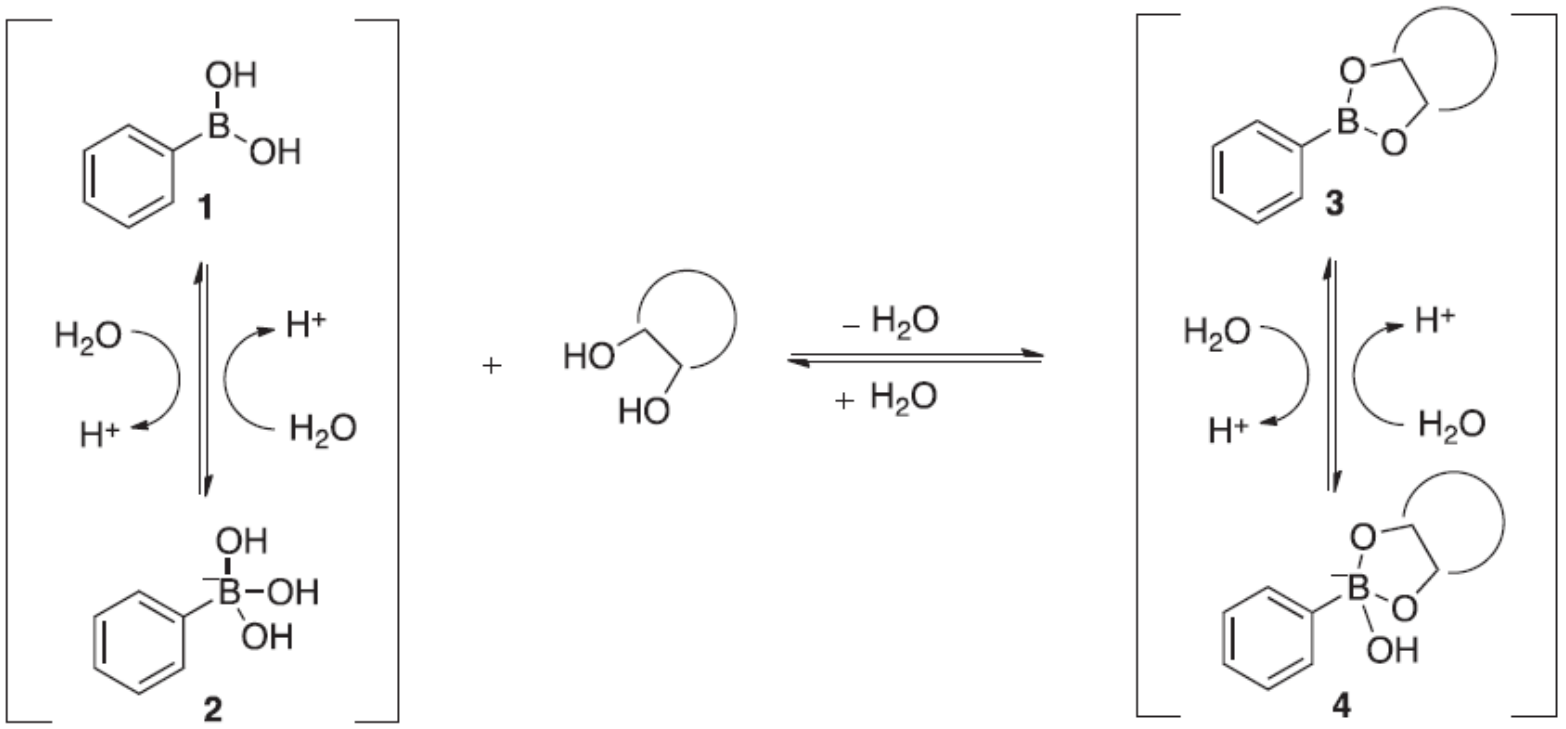


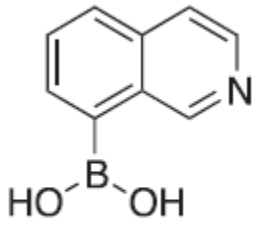
- ✓ High kinetics
- ✓ Reaction biocompatibility

Hall. D. G *et al. Angew. Chem. Int. Ed.* **2018**, *57*, 13028-13044.

Contents

Boronic Ester with Sugars



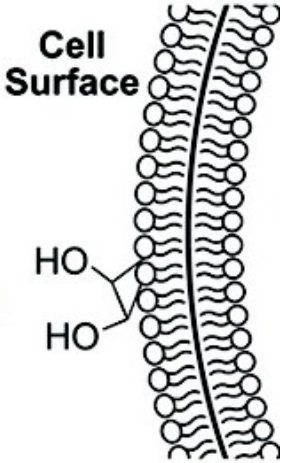


8-isoquinolinylboronic acid (8-IQBA)

D-Sugar	8-IQBA			
	k_{on} ($M^{-1}\,s^{-1}$)	k_{off} (s^{-1})	Calculated K_a (M^{-1})	Literature K_a[a] (M^{-1})
Fructose[a]	287	0.38	755	1493 ± 25
Tagatose[b]	169	0.36	469	1183 ± 367
Mannose[b]	17	0.38	45	84 ± 16
Glucose[a]	0.6	0.13	5	46 ± 12

Wang. B *et al. Bio. Med. Chem.* **2012**, 2957-2961.

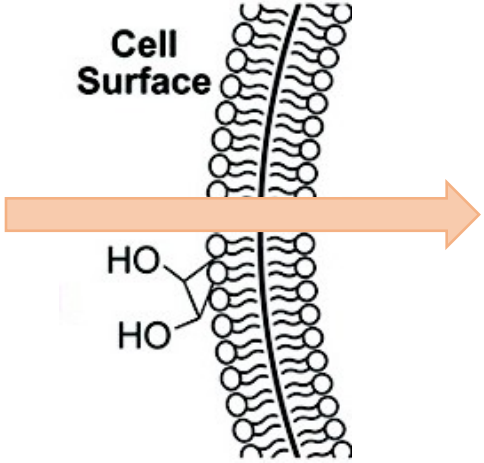
Boronate-Mediated Biologic Delivery



Role of Glycoproteins

- ✓ Cell-cell communication
- ✓ Infection
- ✓ Inflammation
- ✓ Metastasis
- ✓ Reproduction

Protein



Biologic drug delivery helped by recognition parts of Glycoproteins

Raines, R. T *et al. J. Am. Chem. Soc*. **2012**, *8*, 3631-3634.

Boronate-Mediated Biologic Delivery

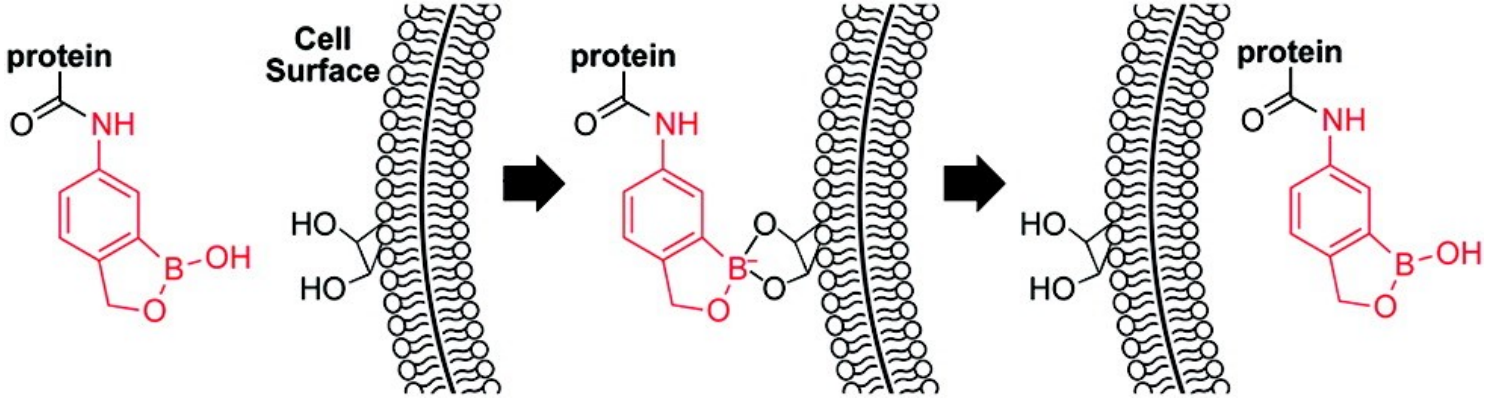
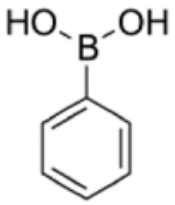
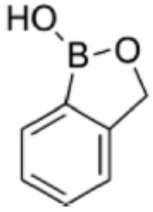


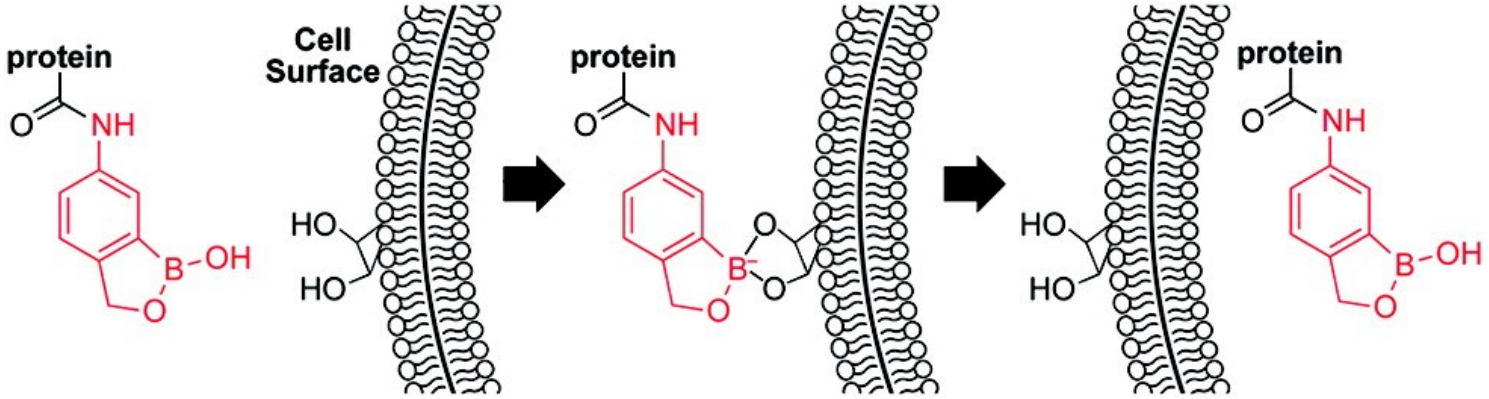
Table 1. Values of K_a (M^{-1}) for Boronic Acids and Saccharides[a]

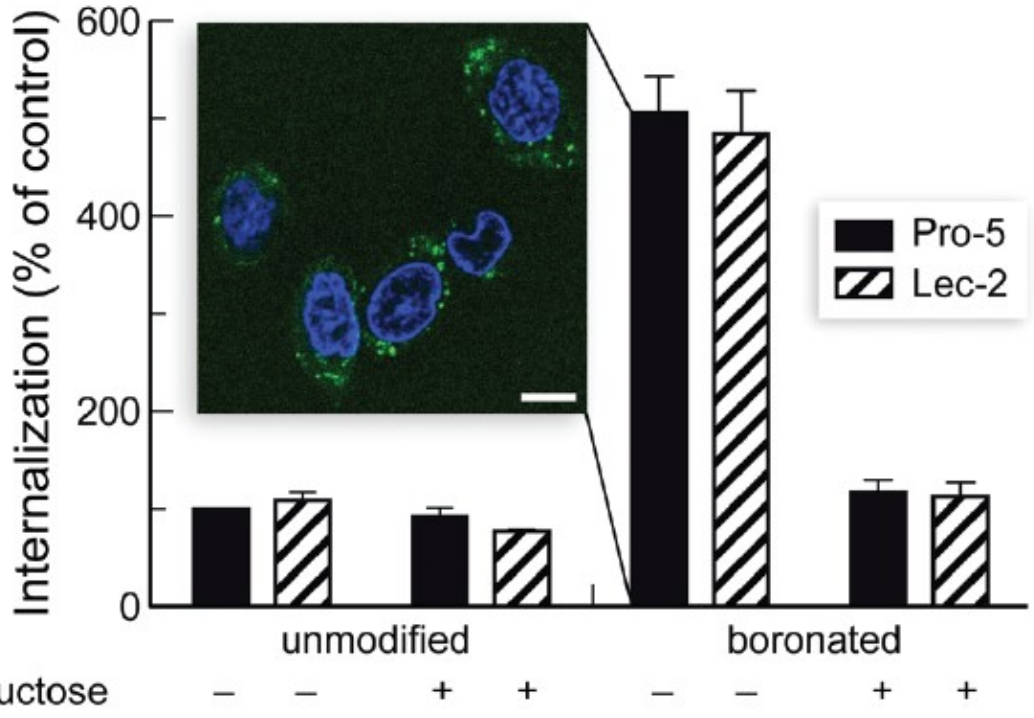
	D-fructose	D-glucose	Neu5Ac
	128 ± 20	5 ± 1	13 ± 1
	336 ± 43	28 ± 4	43 ± 5

- ✓ Benzoxaborole has a greater affinity than phenyl boronic acid for each saccharide.

Raines, R. T *et al. J. Am. Chem. Soc*. **2012**, *8*, 3631-3634.

Boronate-Mediated Biologic Delivery



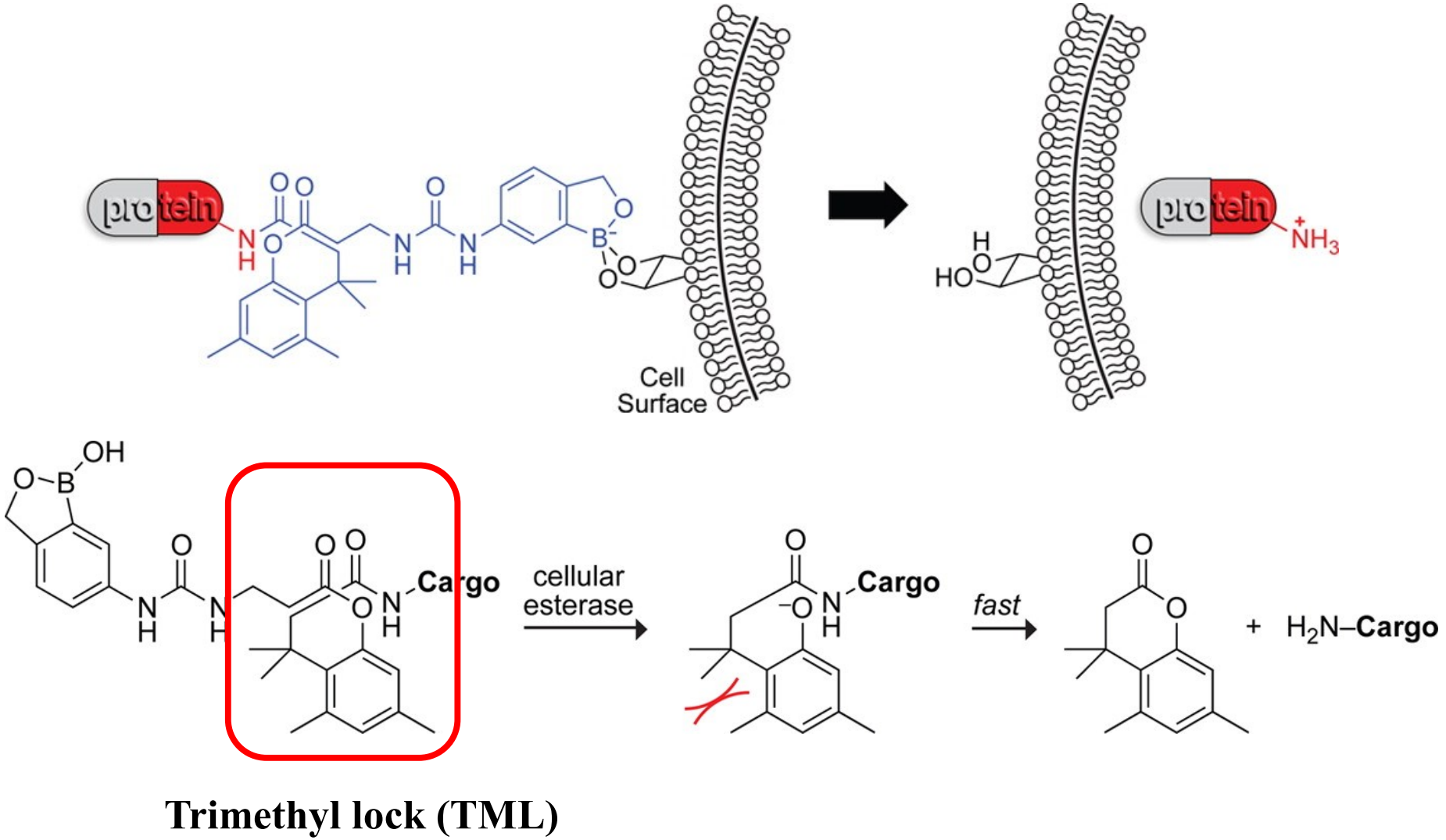


- ✓ Lec-2 have lower levels of sialic acid in their glycoprotein than Pro-5.
- ✓ Fructose decreased the enhancement.
- ✓ Cell-surface sialic acid content did not affect uptake significantly.

Limitation: irreversible of protein.

Raines, R. T *et al. J. Am. Chem. Soc*. **2012**, *8*, 3631-3634.

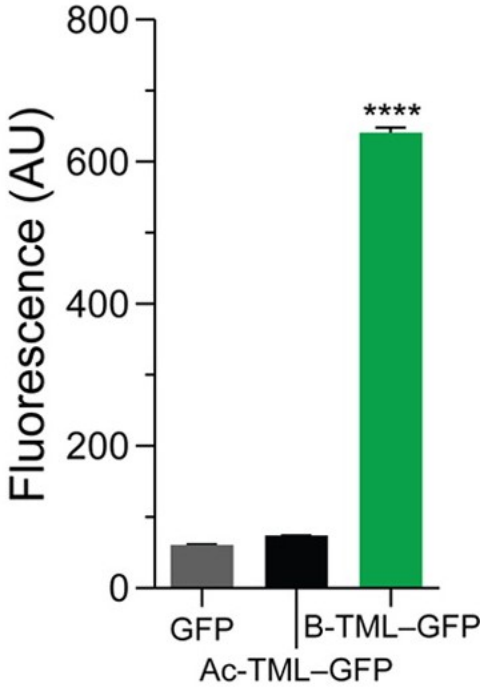
Boronate-Mediated Biologic Delivery

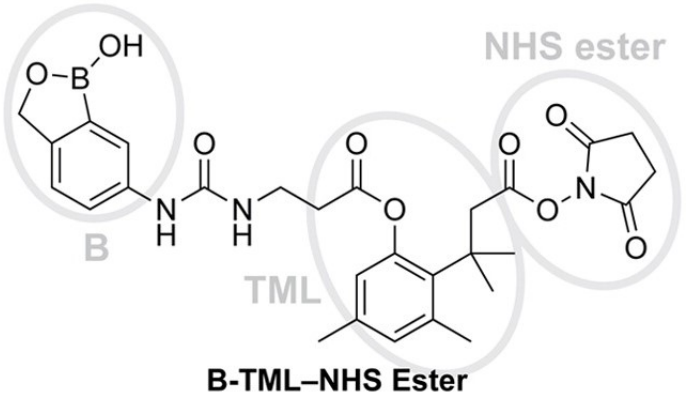


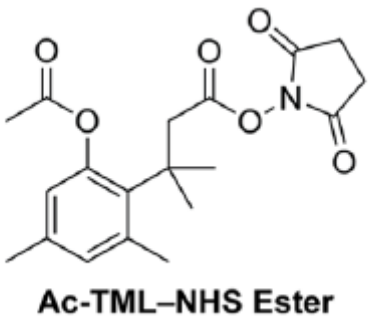
Trimethyl lock (TML)

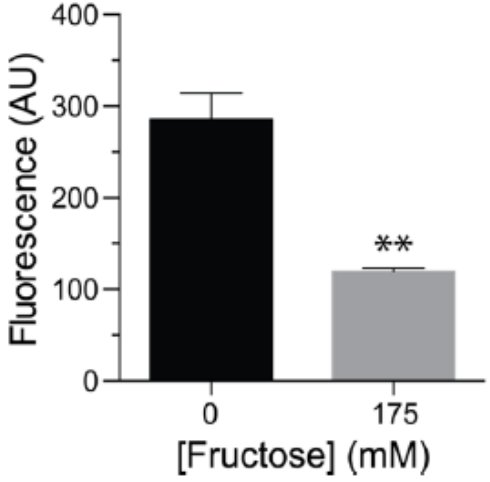
Raines, R. T *et al. ACS Chem. Biol.* **2016**, *11*, 319-323.

Boronate-Mediated Biologic Delivery







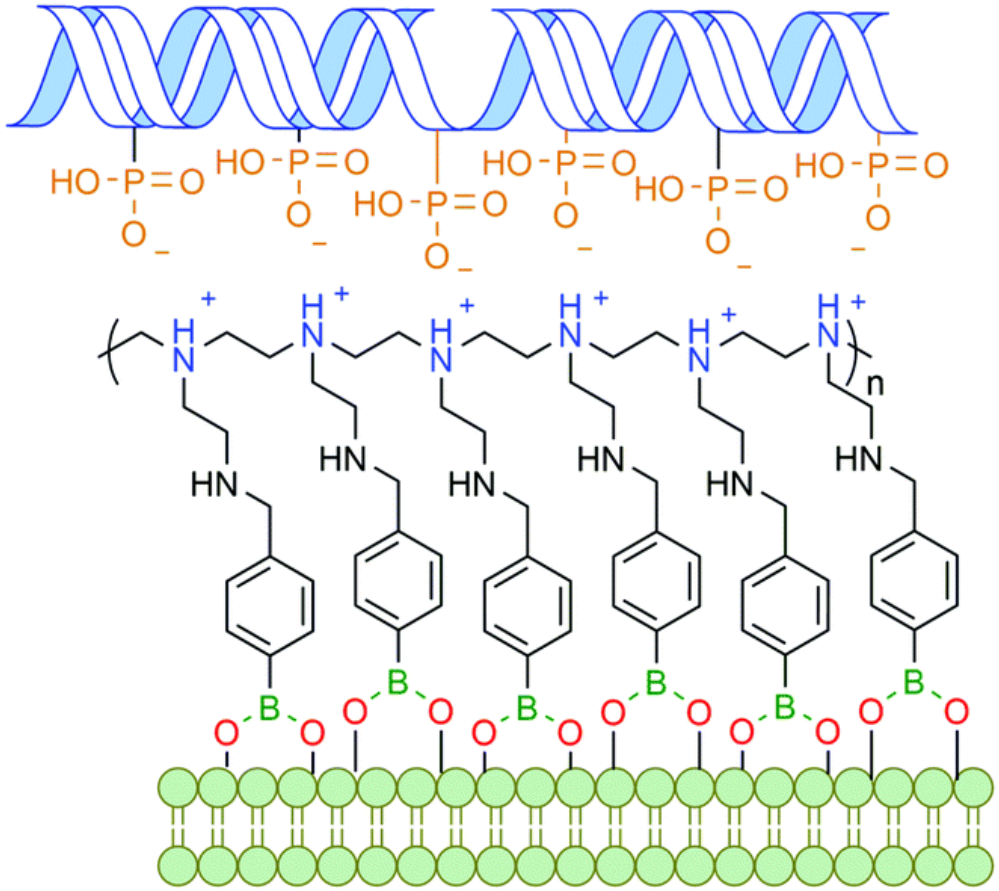


- ✓ B-TML-GFP shows dramatic increase compared with two others.
- ✓ Fructose decreases the cellular uptake.

Advantage: reversible biomodification.

Raines, R. T *et al. Chem. Sci.* **2012**, *3*, 2412-2420.

Boronate Assisted DNA Transfection



Polymer/DNA electrostatic interactions

Improved Gene Transfection

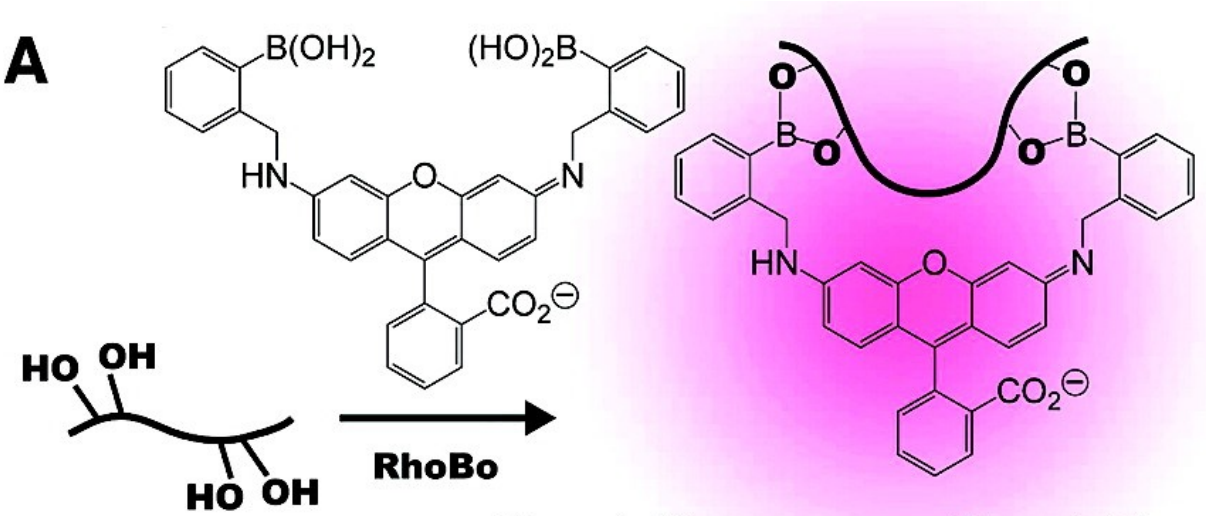
BE formation with sialic acid at cell surface

- ✓ Cations and anions interaction.
- ✓ Boronic ester improves gene cellular uptake (2 to 3 orders of magnitude).

Gois. P. M. P *et al. Chem. Sco. Rev.* **2019**, *48*, 3513-3536.

Rhodamine-derived Bisboronic Acid

A



B

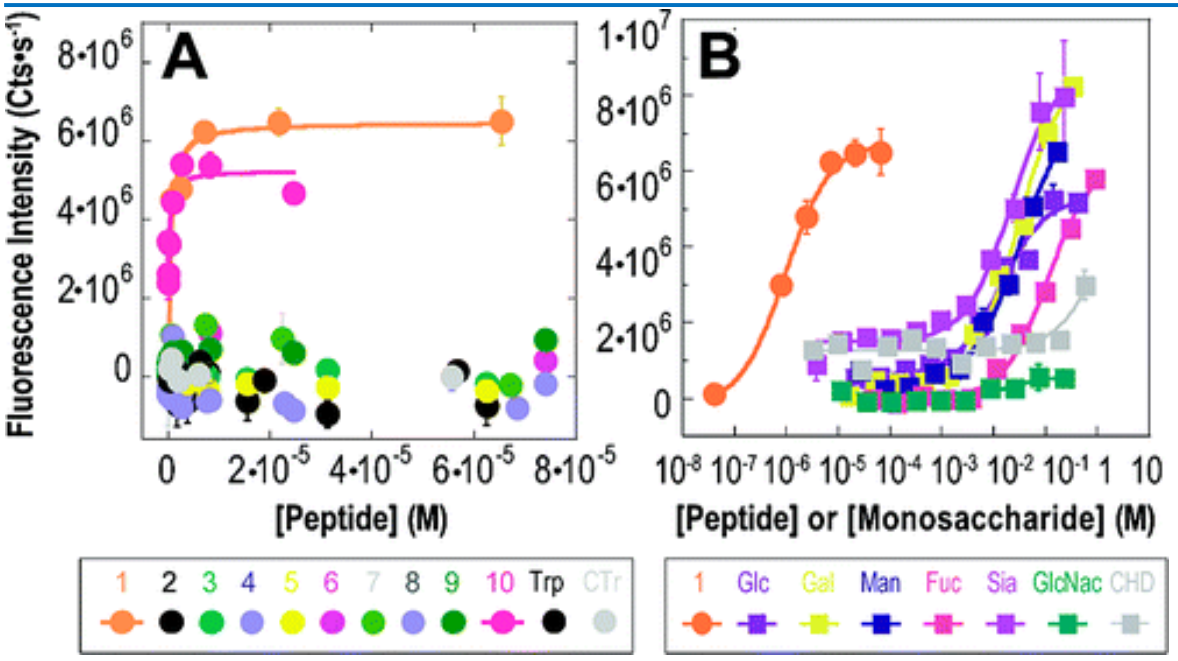
		K_{app} (μM)		K_{app} (μM)
1	Ac-WD**SS**PG**SS**K-NH_2	0.45 ± 0.11	**Gal**	29,000 ± 5,000
2	Ac-WDAAPGG**SS**K-NH_2	No ΔF	**Glc**	13,000 ± 3,000
3	Ac-WD**SS**P**SS**K-NH_2	No ΔF	**Man**	29,000 ± 6,000
4	Ac-WD**SS**K**SS**K-NH_2	No ΔF	**Fuc**	445,000 ± 16,000
5	Ac-WD**SS**PGG**SS**K-NH_2	No ΔF	**Sia**	21,000 ± 4,000
6	Ac-WD**SS**GG**SS**K-NH_2	No ΔF	**GlcNAc**	No ΔF
7	Ac-WD**TT**PG**TT**K-NH_2	No ΔF	**CHD**	No ΔF
8	Ac-WD**YY**PG**YY**K-NH_2	No ΔF	**10**	0.35 ± 0.23
9	Ac-WD**DD**PG**DD**K-NH_2	No ΔF		

- ✓ Cell-permeable
- ✓ Turn-on fluorescent sensor

- ✓ SSPGSS in **1** shows high affinity.

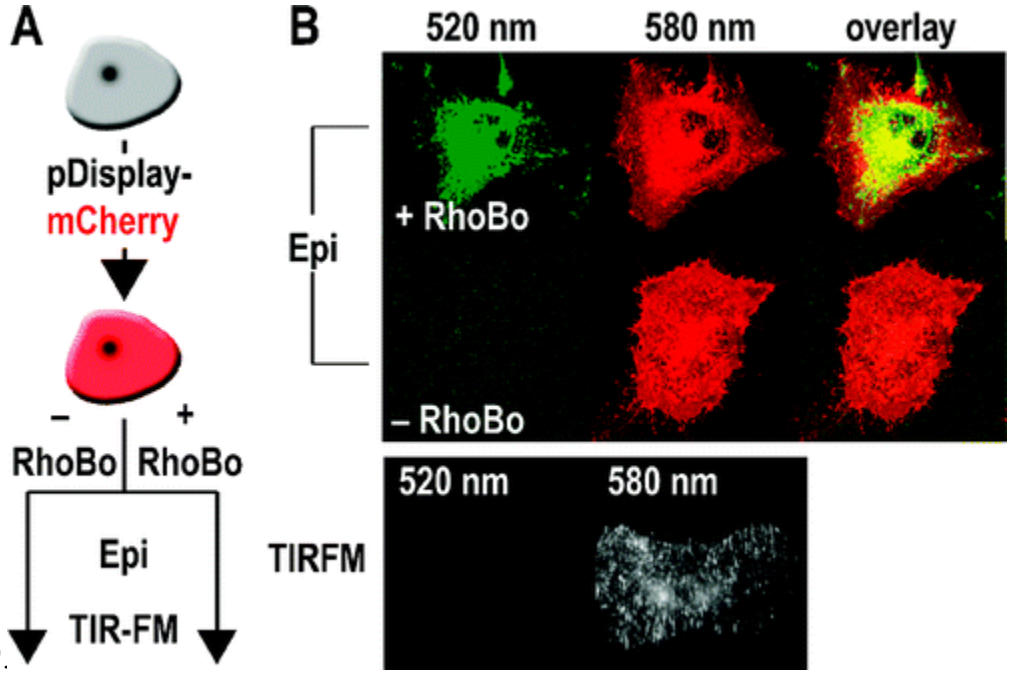
Schepartz. A *et al. J. Am. Chem. Soc.* **2009**, *131*, 438-4339. Rhodamine-derived bisboronic acid (RhoBo)

Rhodamine-derived Bisboronic Acid



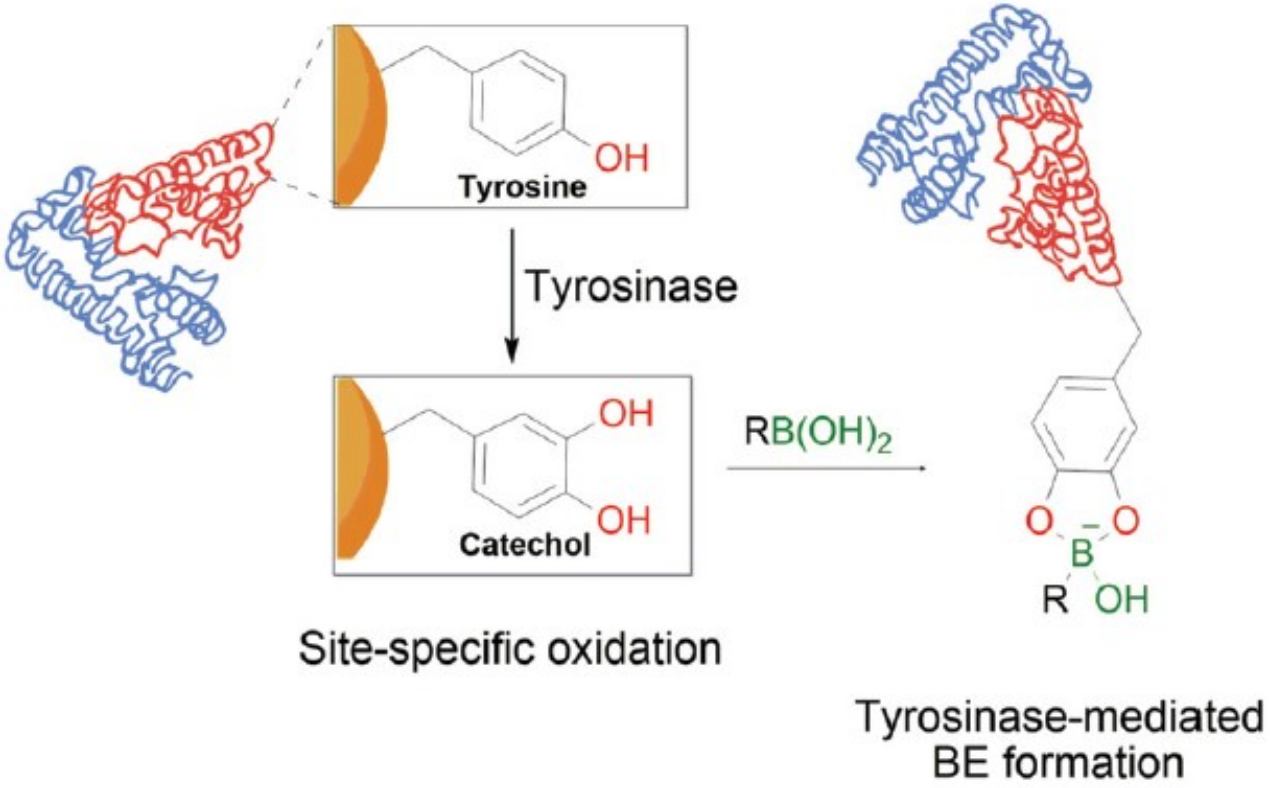
- ✓ **1** and **10** show high fluorescence change.
- ✓ **2-9** show no detectable fluorescence change.

- ✓ High fluorescent intensity in the cytoplasmic region
- ✓ Lower fluorescence intensity in nucleus and outer plasma membrane.



Schepartz. A *et al. J. Am. Chem. Soc.* **2009**, *131*, 438-4339.

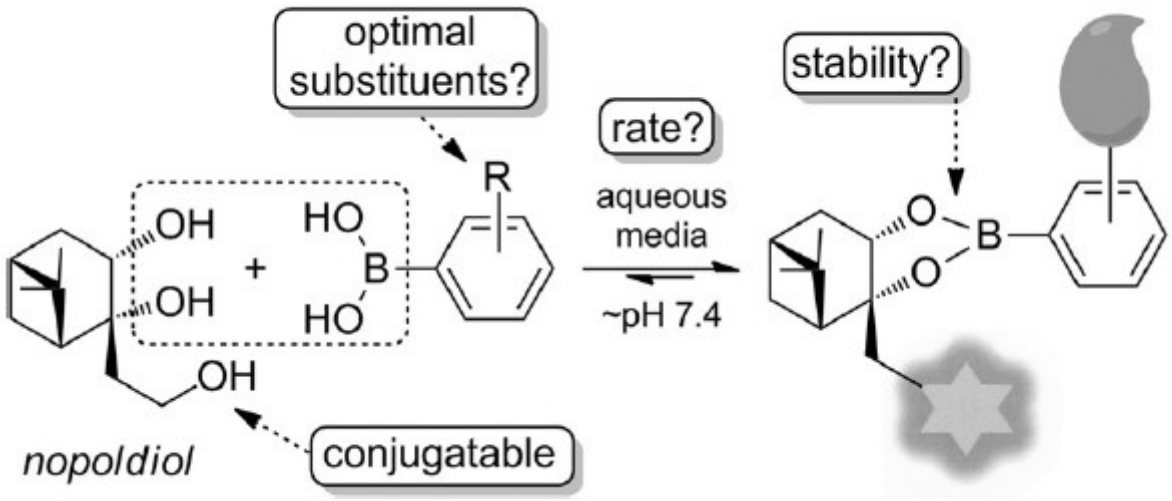
Tyrosinase-Mediated Bioconjugation



- ✓ Applied to different proteins using the hemagglutinin-derived HA-tag that can introduce exposed Tyr groups onto proteins.

Tirelli. N *et al. Bioconjugate Chem.* **2018**, *29*, 2550-2560.

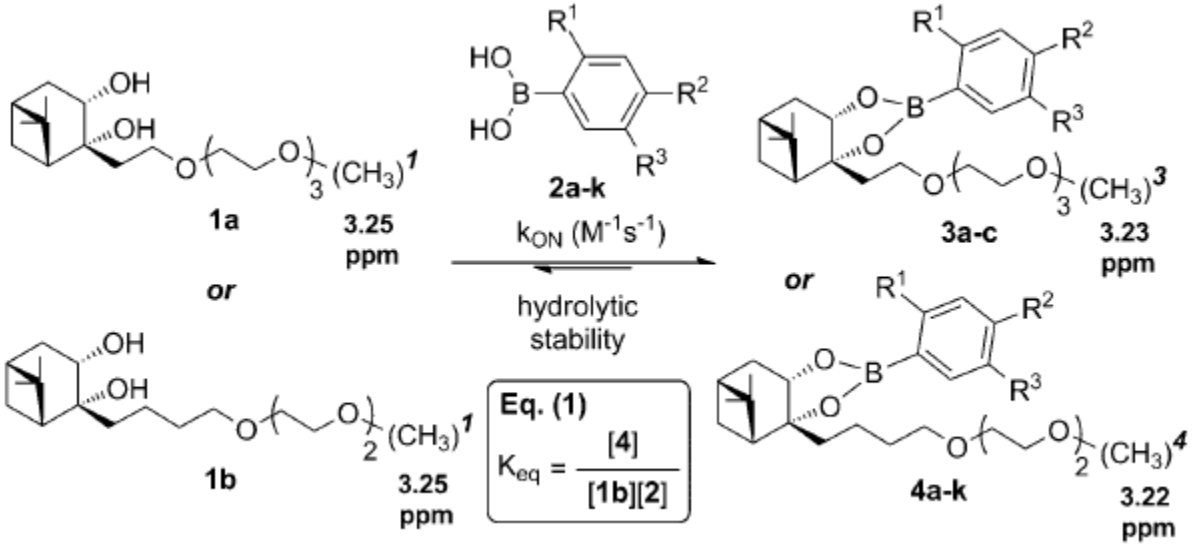
Synergic “Click” Boronate/Thiosemicarbazone System



- ✓ Hindered, pre-organized vicinal diols mitigate the loss of entropy in the diol substrate.
- ✓ Tight-binding.

Hall. D. G *et al. Angew. Chem. Int. Ed.* **2016**, *55*, 3909-3913.

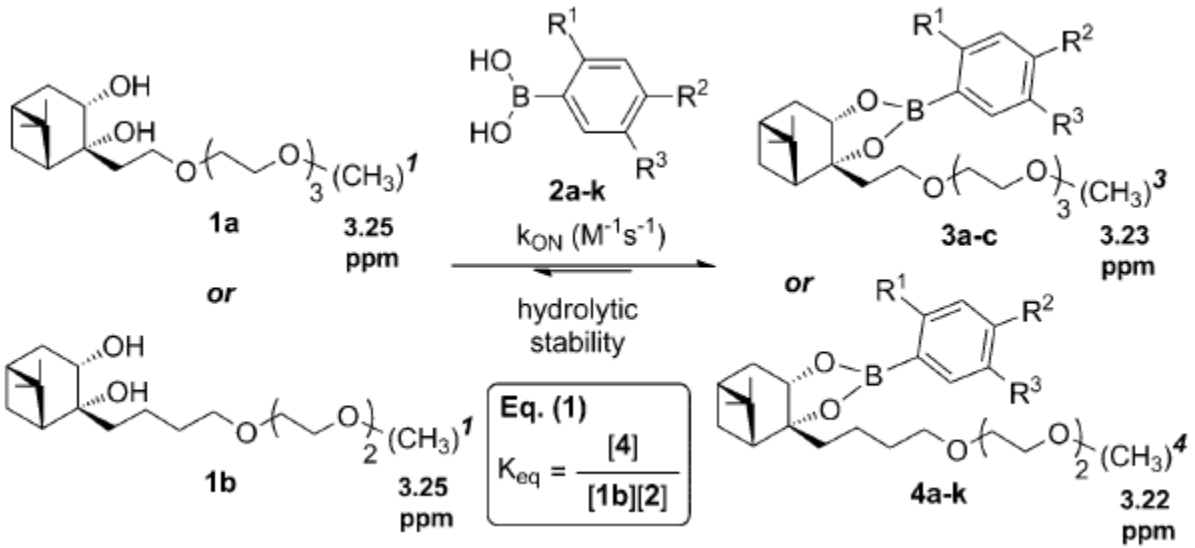
Synergic "Click" Boronate/Thiosemicarbazone System



Entry (1a/b)	2a–k: R^1, R^2, R^3	Hydrolytic stability[b] (3/1a or 4/1b)	K_{eq} $\times 10^3$ [M^{-1}][c]	k_{ON} [$M^{-1}s^{-1}$][d]
1 (1a)	2a: Me, H, H	3a/1a: 87:13	–	1.6 ± 0.1
2 (1a)	2b: F, H, H	3b/1a: 78:22	–	18 ± 4
3 (1a)	2c: CN, H, H	3c/1a: 70:30	–	> 50[e]
4 (1b)	2a: Me, H, H	4a/1b: 93:7	180	2.3 ± 0.2
5 (1b)	2b: F, H, H	4b/1b: 84:16	27	33 ± 2
6 (1b)	2c: CN, H, H	4c/1b: 83:17	25	> 50[f]
7 (1b)	2d: Me, H, CO_2Me	4d/1b: 91:9	120	6.9 ± 0.6
8 (1b)	2e: Me, CO_2Me, H	4e/1b: 90:10	91	7.8 ± 0.7
9 (1b)	2f: Me, H, OMe	4f/1b: 92:8	130	3.3 ± 0.6
10 (1b)	2g: Me, OMe, H	4g/1b: 94:6	330	1.0 ± 0.2
11 (1b)	2h: F, H, $CONMe_2$	4h/1b: 75:25	15	> 50[f]
12 (1b)	2i: F, H, OMe	4i/1b: 82:18	25	> 50[f]
13 (1b)	2j: F, OMe, H	4j/1b: 85:15	40	18 ± 1
14 (1b)	2k: CN, H, CO_2Me	4k/1b: 78:22	12	> 50[f]

Hall. D. G *et al. Angew. Chem. Int. Ed.* **2016**, *55*, 3909-3913.

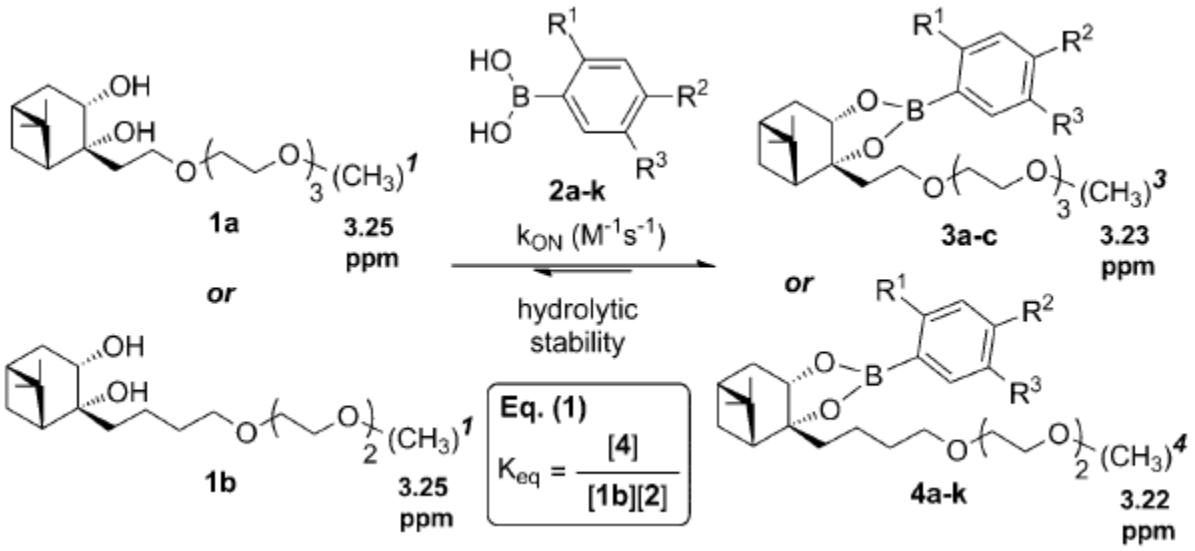
Synergic "Click" Boronate/Thiosemicarbazone System



Entry (1a/b)	2a–k: R^1, R^2, R^3	Hydrolytic stability[b] (3/1a or 4/1b)	K_{eq} $\times 10^3$ [M^{-1}] [c]	k_{ON} [$M^{-1}s^{-1}$] [d]
1 (1a)	2a: Me, H, H	3a/1a: 87:13	–	1.6 ± 0.1
2 (1a)	2b: F, H, H	3b/1a: 78:22	–	18 ± 4
3 (1a)	2c: CN, H, H	3c/1a: 70:30	–	> 50[e]

Hall. D. G *et al. Angew. Chem. Int. Ed.* **2016**, *55*, 3909-3913.

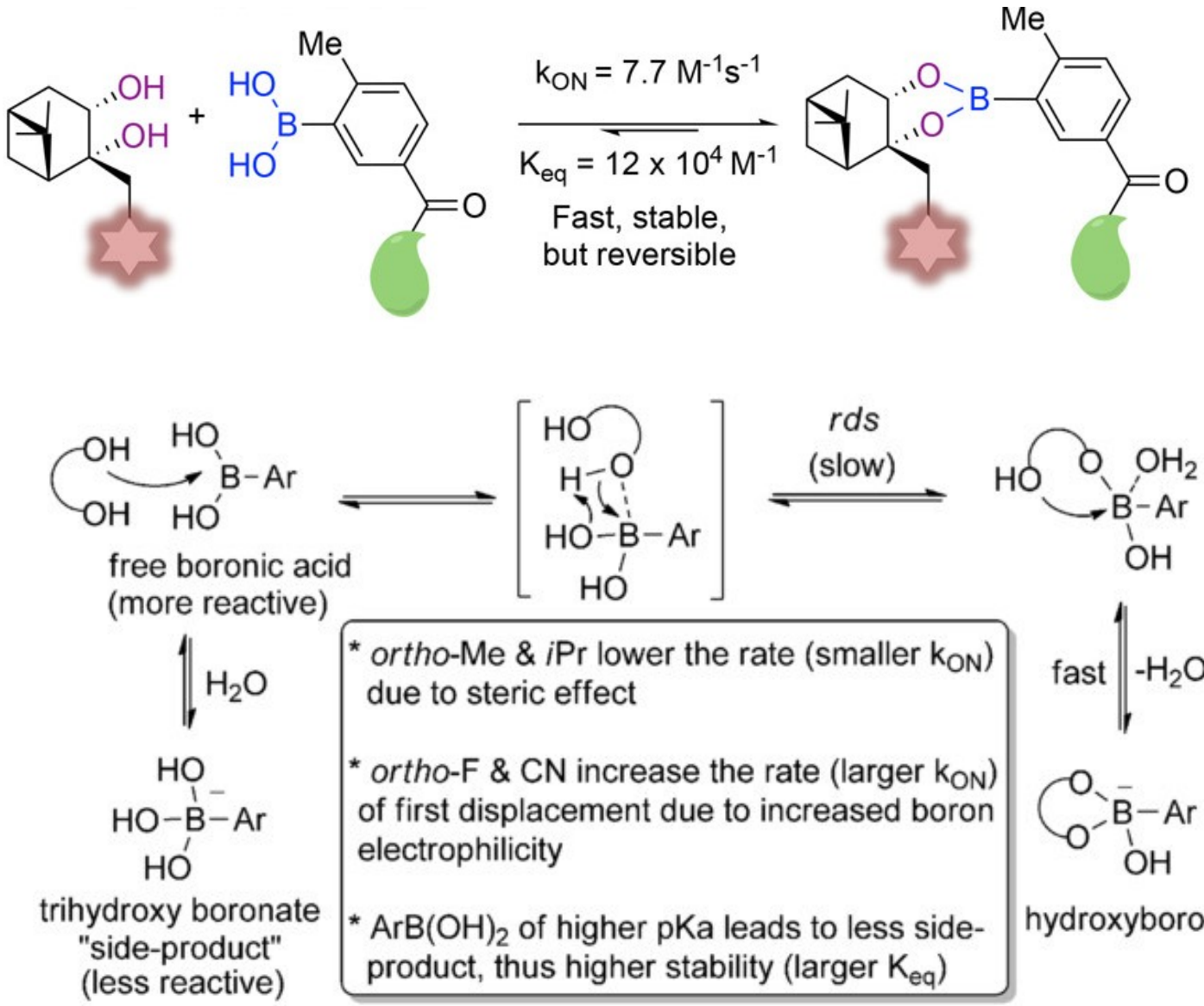
Synergic "Click" Boronate/Thiosemicarbazone System



Entry (1a/b)	2a–k: R^1, R^2, R^3	Hydrolytic stability[b] (3/1a or 4/1b)	K_{eq} $\times 10^3$ [M^{-1}] [c]	k_{ON} [$M^{-1}s^{-1}$] [d]
4 (1b)	2a: Me, H, H	4a/1b: 93:7	180	2.3 ± 0.2
5 (1b)	2b: F, H, H	4b/1b: 84:16	27	33 ± 2
6 (1b)	2c: CN, H, H	4c/1b: 83:17	25	>50[f]
7 (1b)	2d: Me, H, CO_2Me	4d/1b: 91:9	120	6.9 ± 0.6
8 (1b)	2e: Me, CO_2Me, H	4e/1b: 90:10	91	7.8 ± 0.7
9 (1b)	2f: Me, H, OMe	4f/1b: 92:8	130	3.3 ± 0.6
10 (1b)	2g: Me, OMe, H	4g/1b: 94:6	330	1.0 ± 0.2
11 (1b)	2h: F, H, $CONMe_2$	4h/1b: 75:25	15	>50[f]

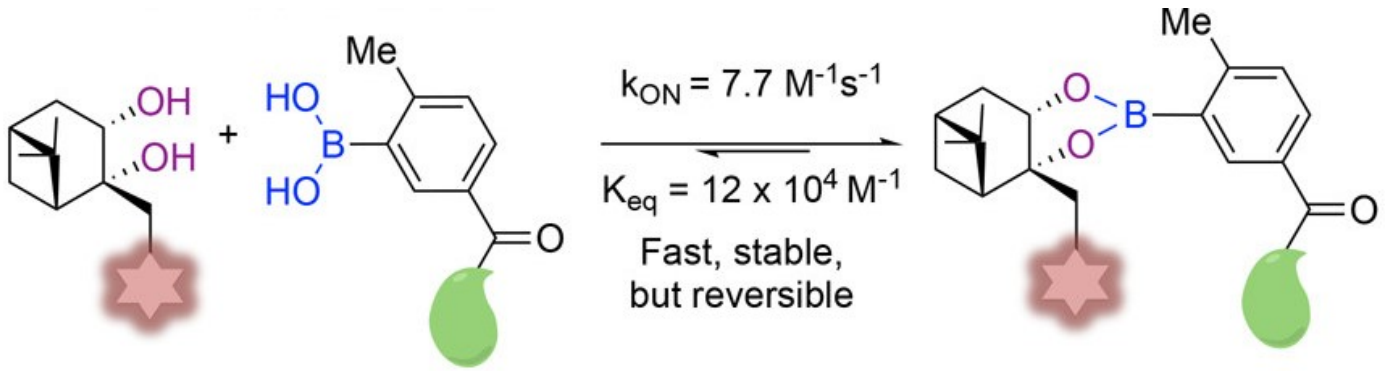
Hall. D. G *et al. Angew. Chem. Int. Ed.* **2016**, *55*, 3909-3913.

Synergic "Click" Boronate/Thiosemicarbazone System

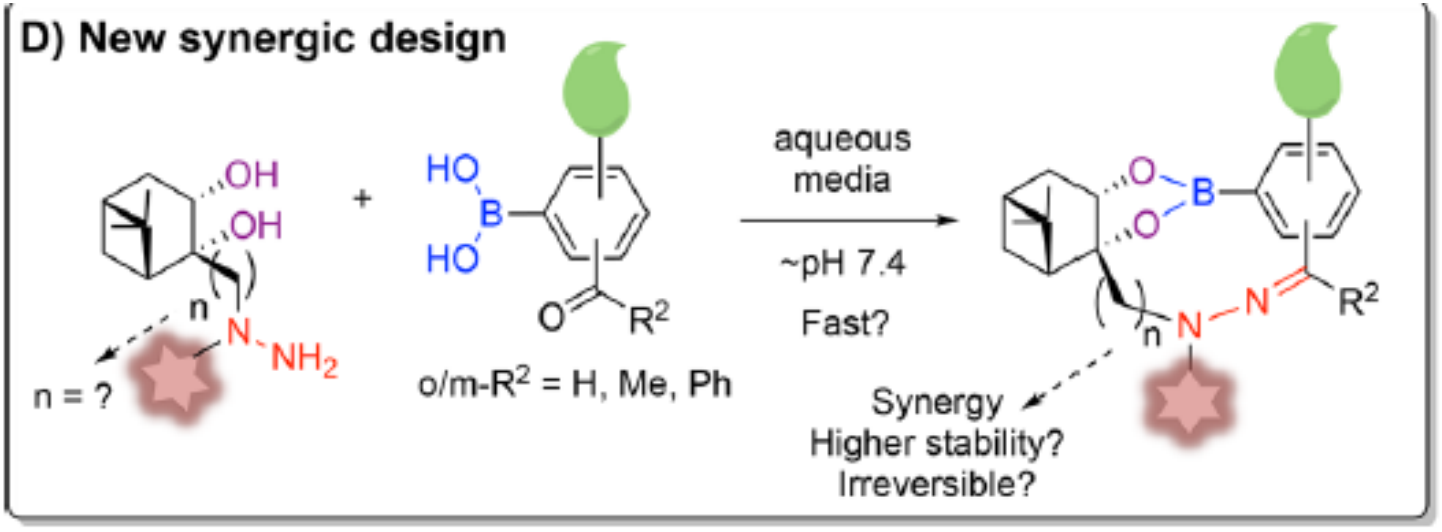


Hall. D. G *et al. Angew. Chem. Int. Ed.* **2016**, *55*, 3909-3913.

Synergic "Click" Boronate/Thiosemicarbazone System (Irreversible)

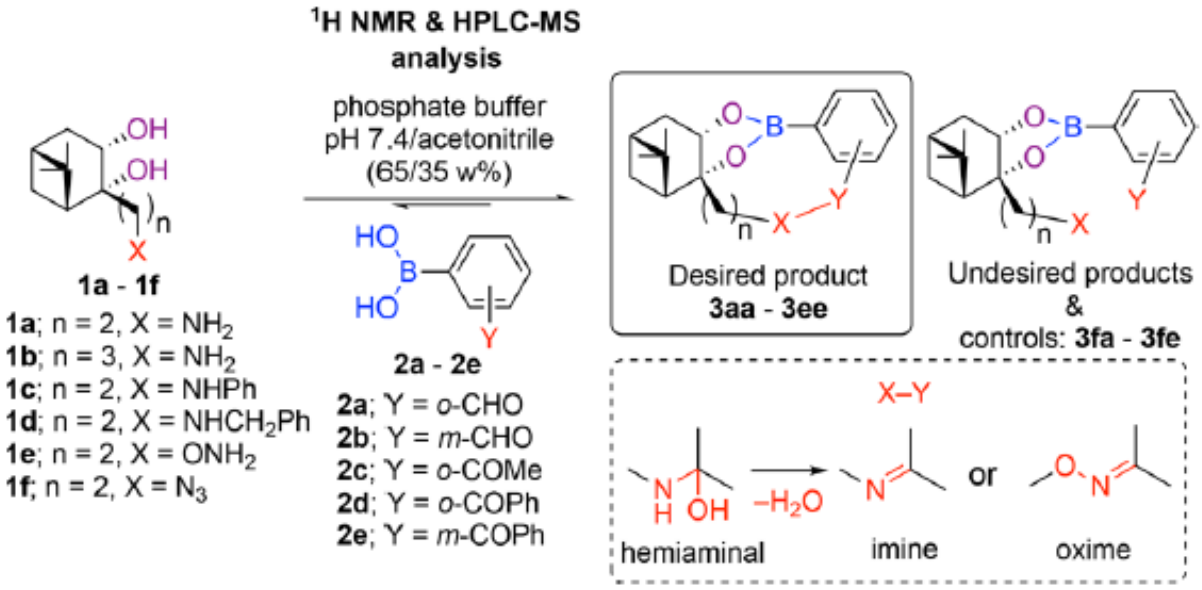


Undesirable cleavage!



Hall. D. G *et al. J. Am. Chem. Soc.* **2017**, *139*, 14285-14291.

Synergic "Click" Boronate/Thiosemicarbazone System (Irreversible)

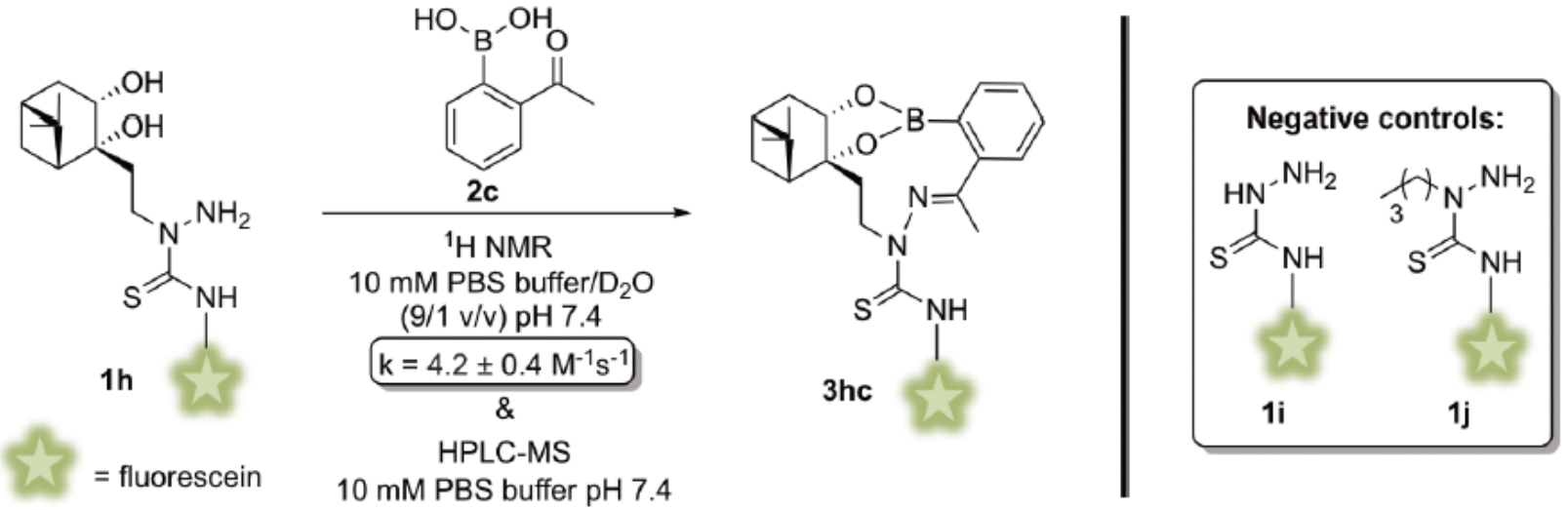


entry (1a–1f)	boronic acid	product (2 h) (1 mM) 3/1[a]	product (24 h) (1 mM) 3/1[a]	imine/oxime formation?
1 (1a)	2a	3aa/3aa•H_2O/1a: 11/67/22	71/21/8	yes[b,c]
2 (1a)	2c	3ac/1a: 43/57	43/57	yes[b]
3 (1b)	2a	3ba/1b: 79/21	79/21	no
4 (1c)	2a	3ca/1c: 68/32[d]	68/32[d]	no
5 (1d)	2a	3da/1d: 86/14[d]	86/14[d]	no
6 (1e)	2a	3ea/1e: 100/0	100/0	yes[b]
7 (1e)	2c	3ec/1e: 100/0	100/0	yes[b]
8 (1e)	2d	3ed/1e: 100/0	100/0	yes[b]
9 (1f)	2a	3fa/1f: 73/27	73/27	no

- ✓ n=2 as ideal length.
- ✓ Second reaction clearly improved the conversion.
- ✓ **2a** side selectivity with *N*-terminal cysteine.
- ✓ **2c** exhibits lower hydrophobicity.

Hall. D. G *et al. J. Am. Chem. Soc.* **2017**, *139*, 14285-14291.

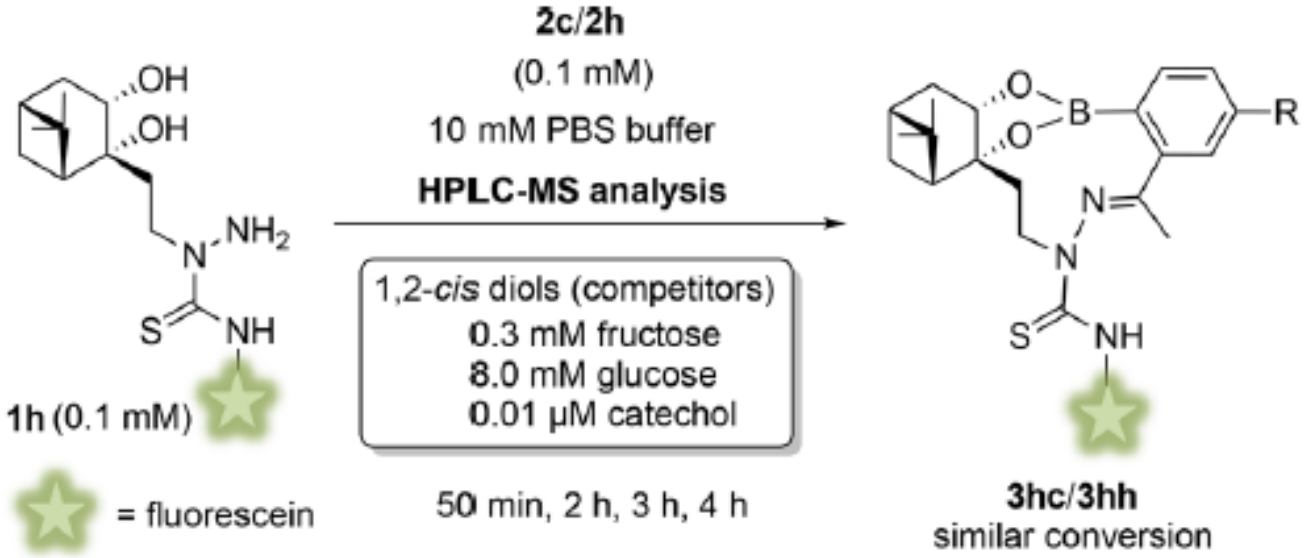
Synergic "Click" Boronate/Thiosemicarbazone System (Irreversible)

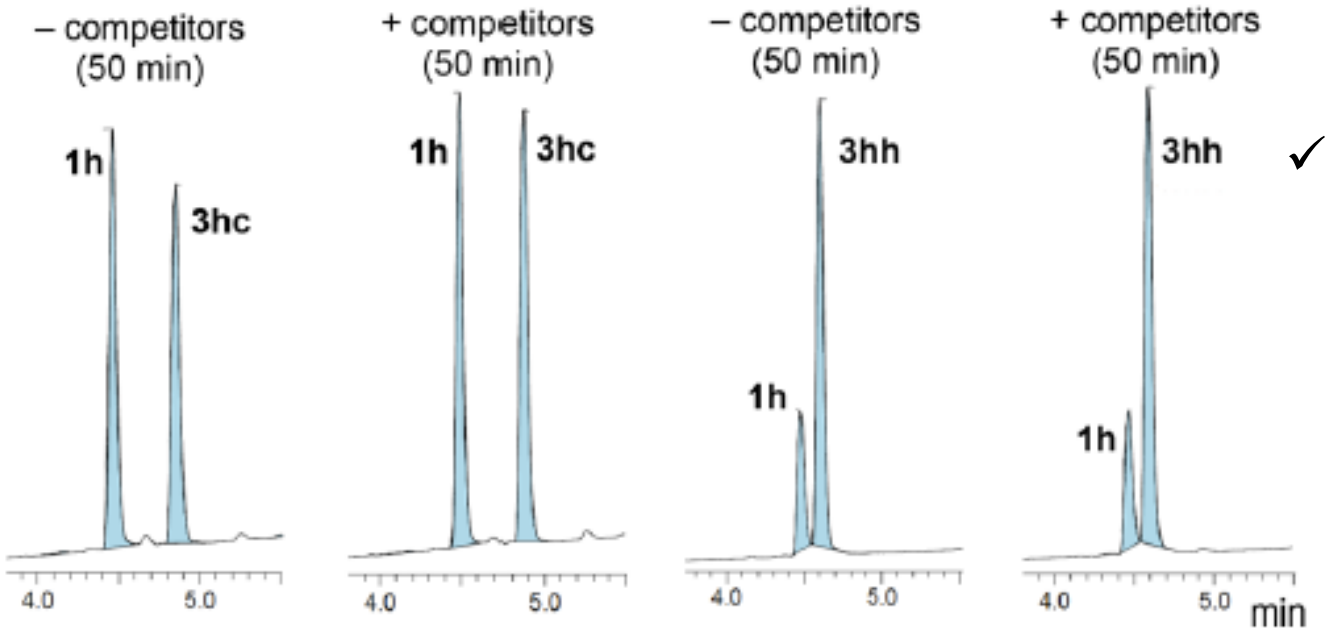


entry	% proportions of 1/3					
	10 min	30 min	60 min	120 min	3.5 h	24 h
1 (1h)	90/10	86/14	64/36	35/65	0/100	0/100
2 (1i)	73/27 (maximal conversion)					
3 (1j)	100/0 (no product)					

Hall. D. G *et al. J. Am. Chem. Soc.* **2017**, *139*, 14285-14291.

Synergic "Click" Boronate/Thiosemicarbazone System (Irreversible)

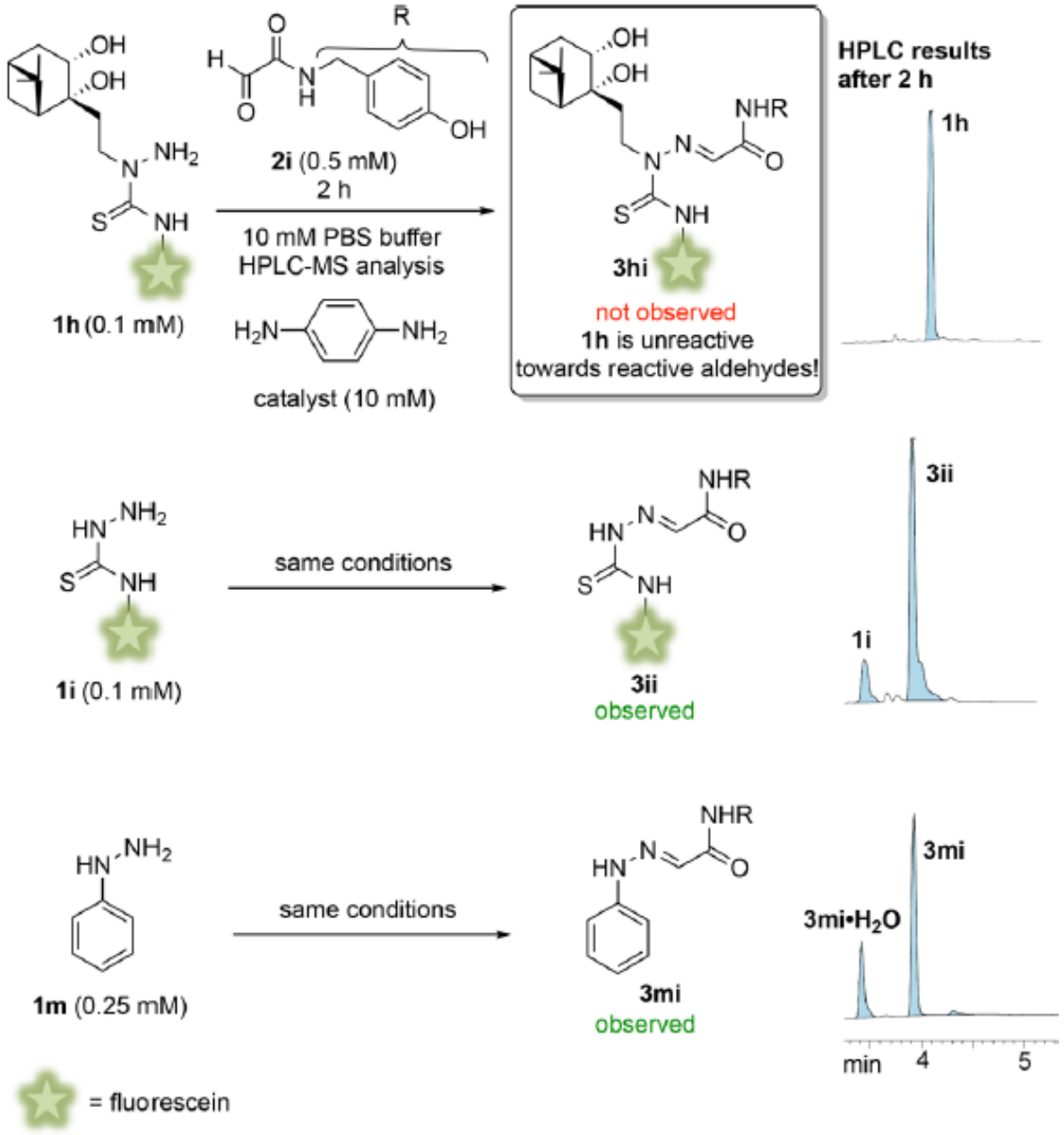




✓ Biological inertness with biological diols.

Hall. D. G *et al. J. Am. Chem. Soc.* **2017**, *139*, 14285-14291.

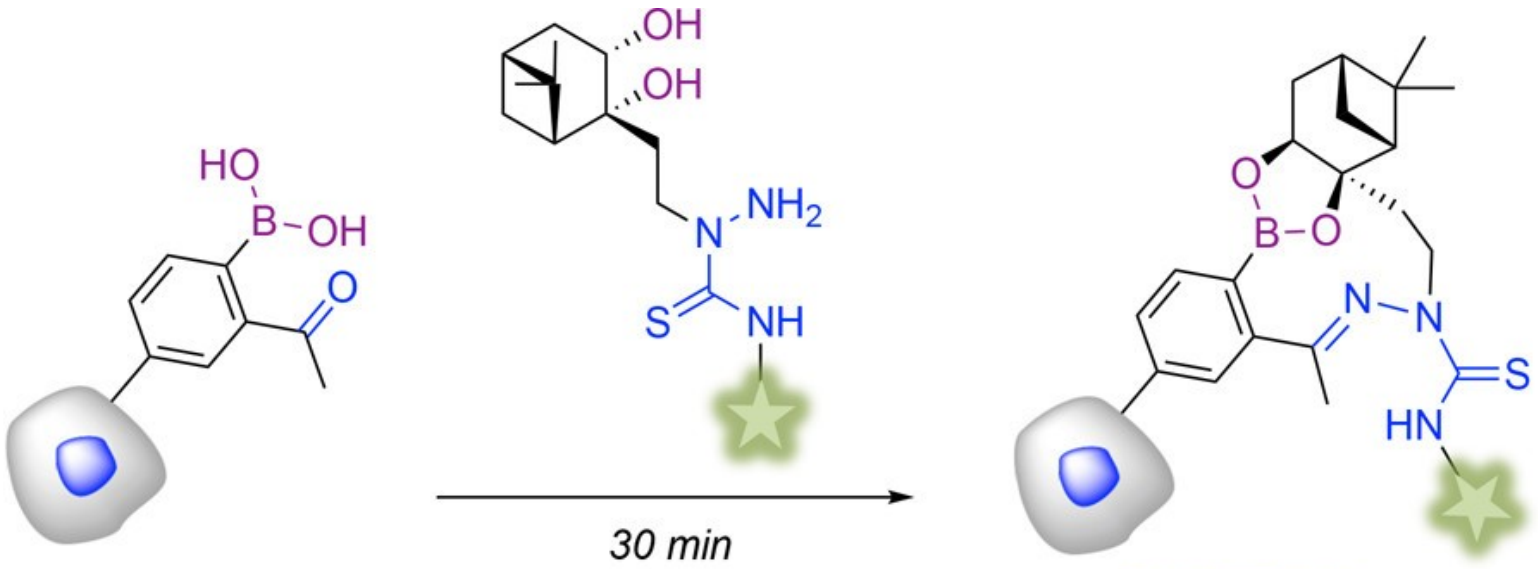
Synergic "Click" Boronate/Thiosemicarbazone System (Irreversible)



- ✓ Inert towards biological electrophiles

Hall. D. G *et al. J. Am. Chem. Soc.* **2017**, *139*, 14285-14291.

Synergic “Click” Boronate/Thiosemicarbazone System (Irreversible)

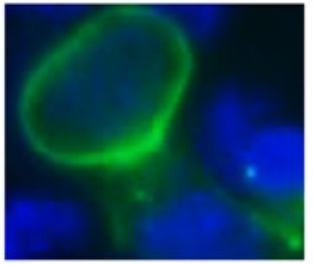


Live HEK293T labeled with boronic acid on cell membrane via SNAPtag approach

***Fast (9 $M^{-1}s^{-1}$), irreversible**

***Benign reagents, compatible with biological polyols**

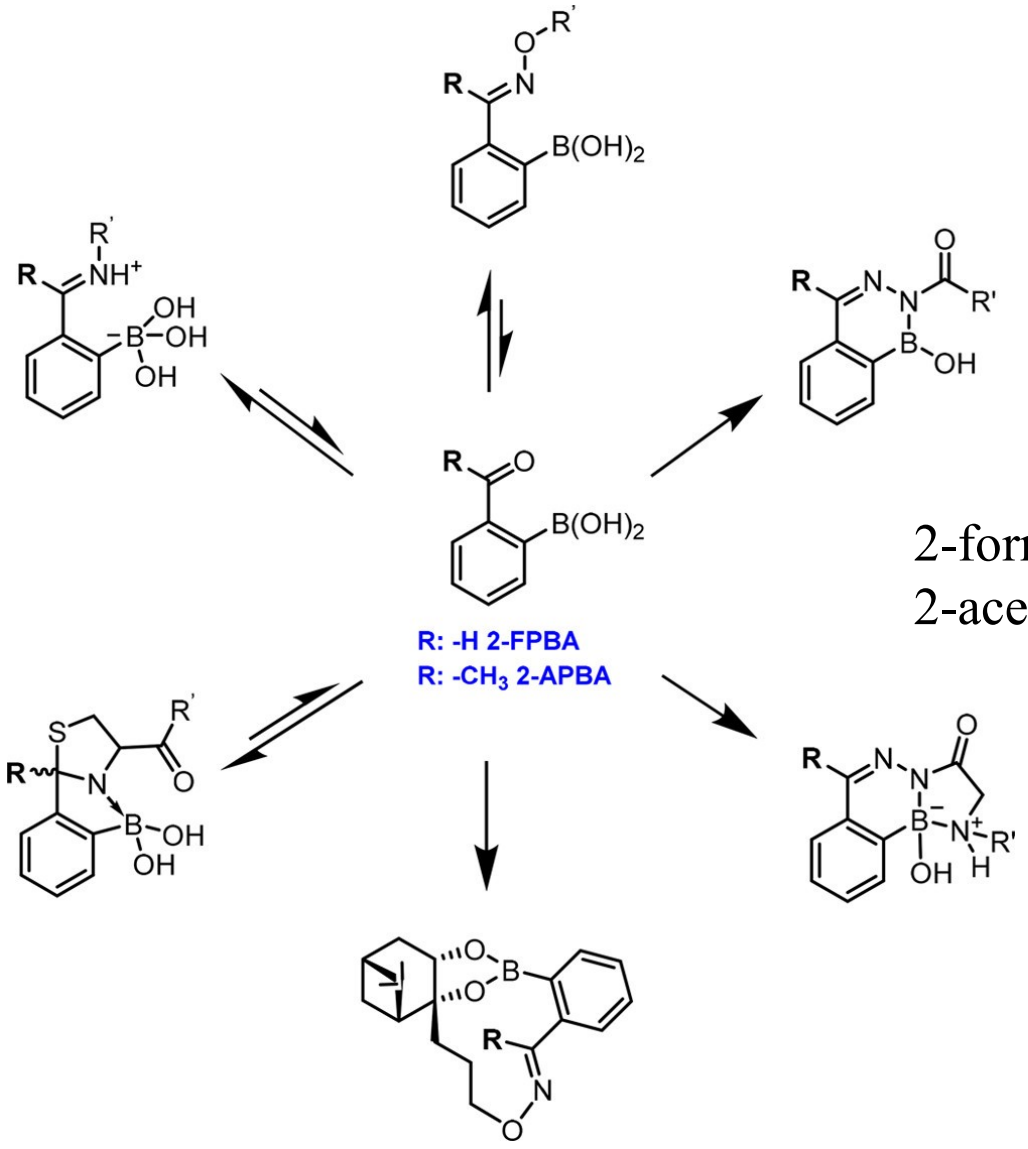
***Inert towards biological electrophiles**



Hall. D. G *et al. J. Am. Chem. Soc.* **2017**, *139*, 14285-14291.

Contents

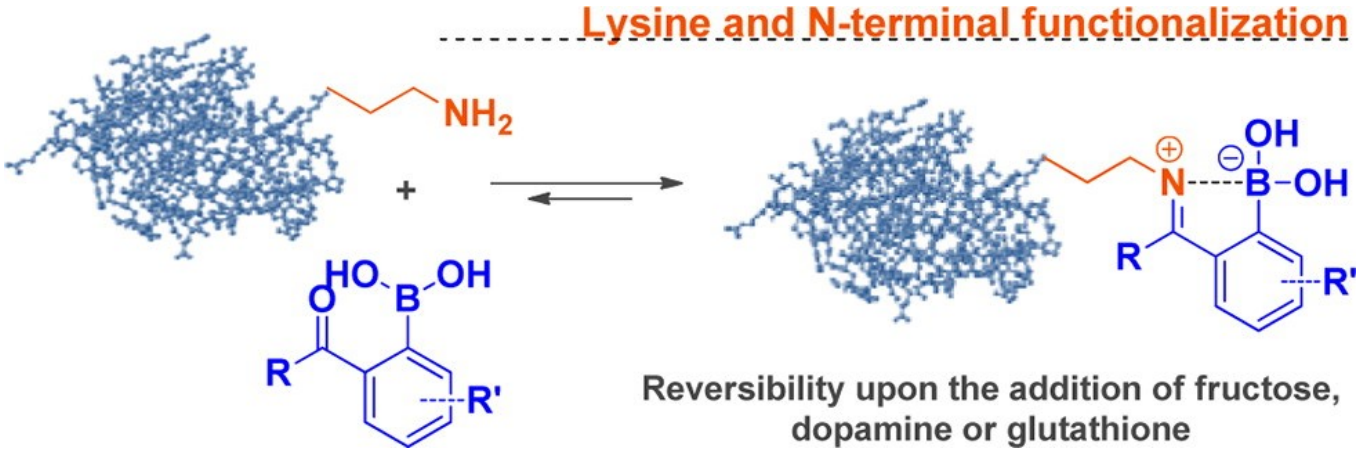
Iminoboronate



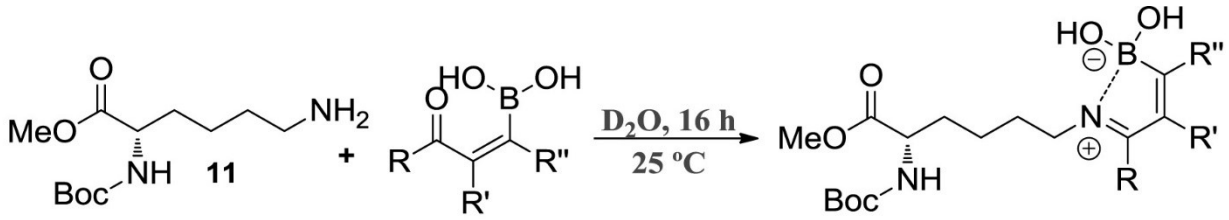
2-formayl phenylboronic acid (2-FPBA)
2-acetyl phenylboronic acid (2-APBA)

Gao. J *et al. Acc. Chem. Res.* **2018**, *51*, 2198-2206.

Boronic Acid with ε-residue Lysine



Reversibility upon the addition of fructose, dopamine or glutathione



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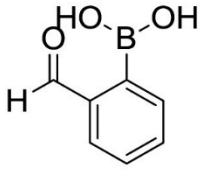
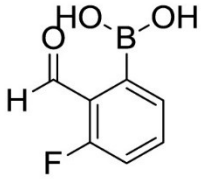
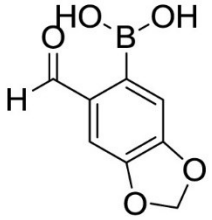
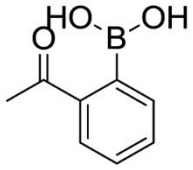
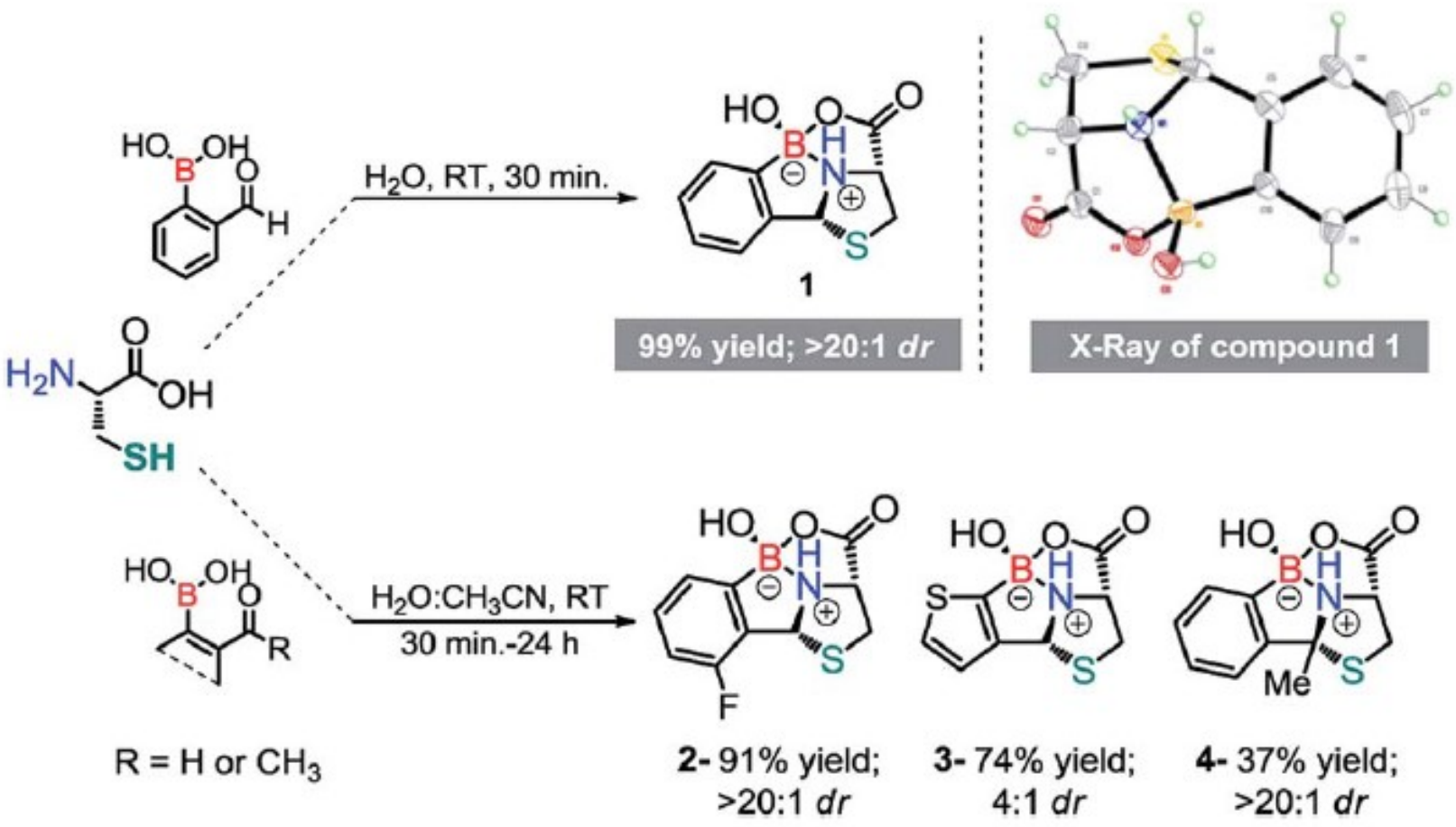
1		61%	11
4		33%	12

	Boronic acid	Conv. %	Product
5		56%	13
9		71%	14

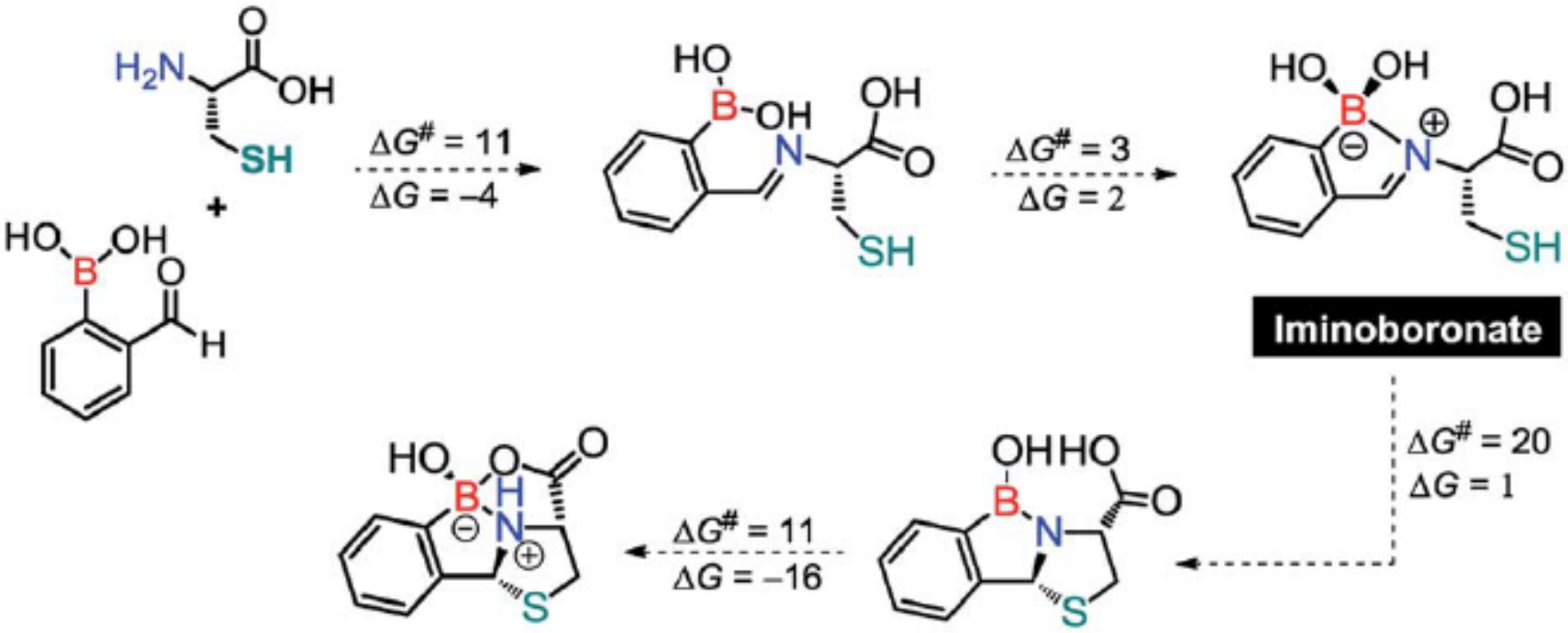
Gois. P. M. P *et al. J. Am. Chem. Soc.* **2012**, *134*, 10299-10305.

Boronic Acid with *N*-terminal Cysteine



Gois. P. M. P *et al. Chem. Sci.* **2016**, *7*, 5052-5058.

Boronic Acid with *N*-terminal Cysteine



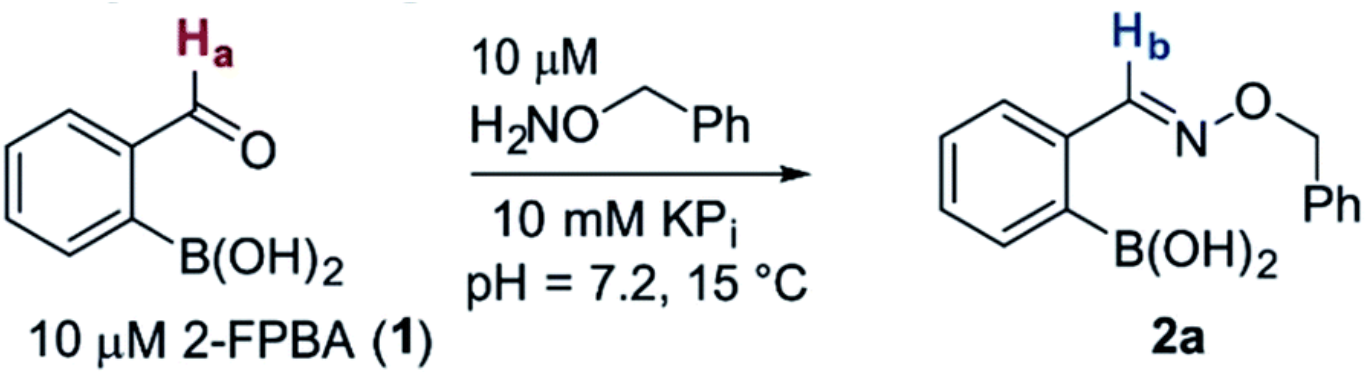
Gois. P. M. P *et al. Chem. Sci.* **2016**, *7*, 5052-5058.

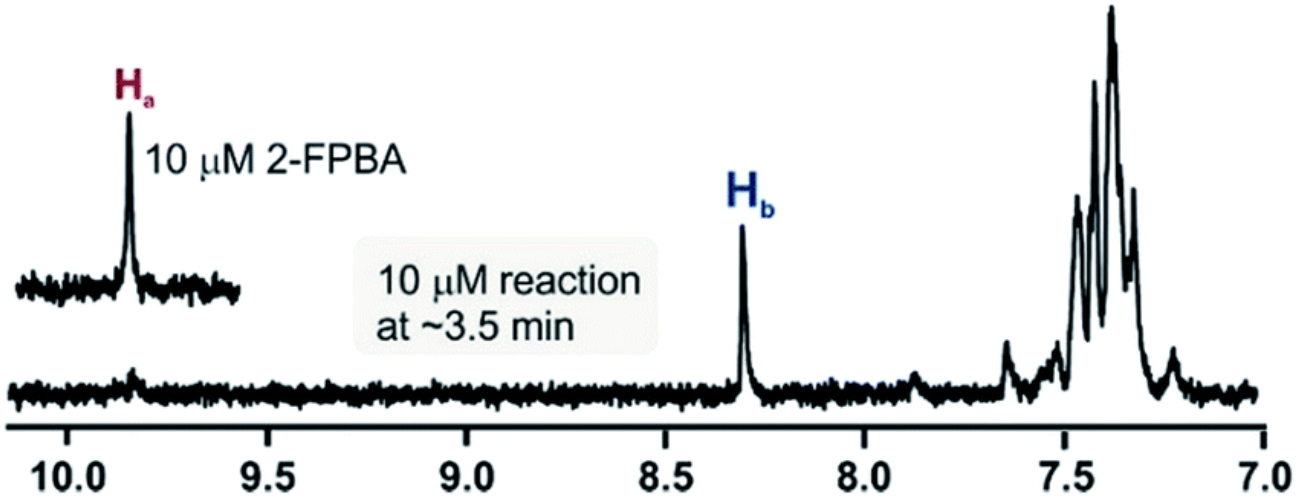
Boronic Acid with *N*-terminal Cysteine

Potential Inhibitor	% Inhibition	Implication
Fructose (5 mM)	0	Boron chelators do not interfere
Serine (5 mM)	0	1, 2-amino alcohols do not interfere
Lysine (15 mM)	0	Biological amines do not interfere
GSH (5 mM)	0	Biological thiols/internal cysteines do not interfere
Cystine (1 mM)	0	Oxidized cysteines do not interfere
Cysteine (1 mM)	50	TzB formation is specific to 1, 2-aminothiols

Gao. J *et al. Chem. Sci.* **2016**, *7*, 4589-4593.

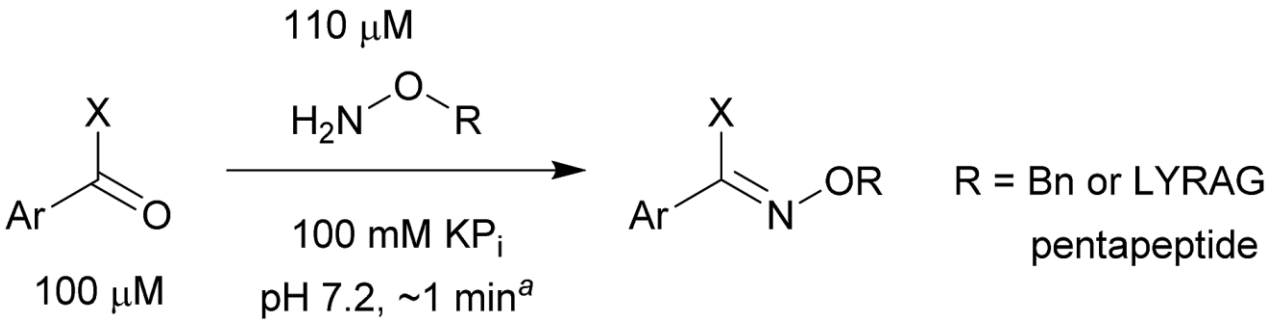
Boronic Acid with Oxyamines

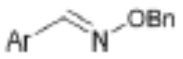
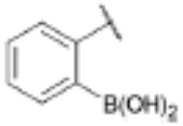
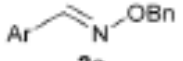
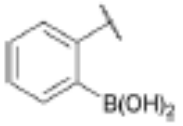
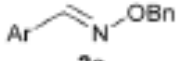
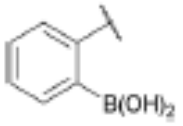
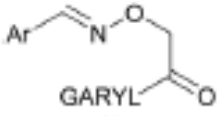
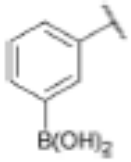
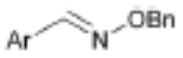
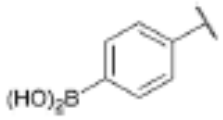
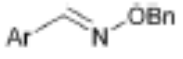




Gillingham. D *et al. Chem. Sci.* **2015**, *6*, 3329-3333.

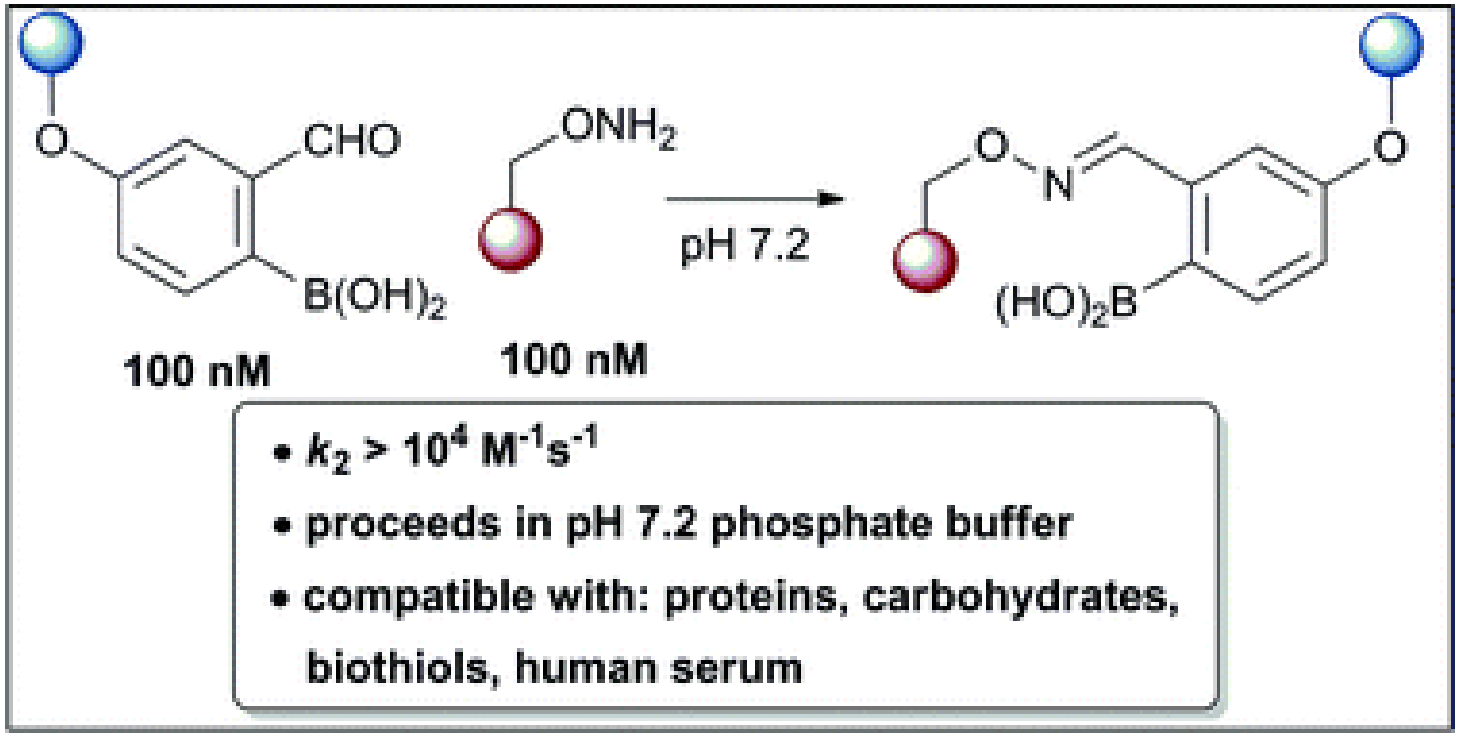
React with Oxyamines



Entry	Ar	X	Product	Conc (μM)	Conv[b] (%)
1[c]	Ph	H		100	<5
2		H	 **2a**	100	>98
3		H	 **2a**	10	>98
4		H	 **2b**	100	>98
5[c]		H	 **2c**	100	<5
6[c]		H	 **2d**	100	<5

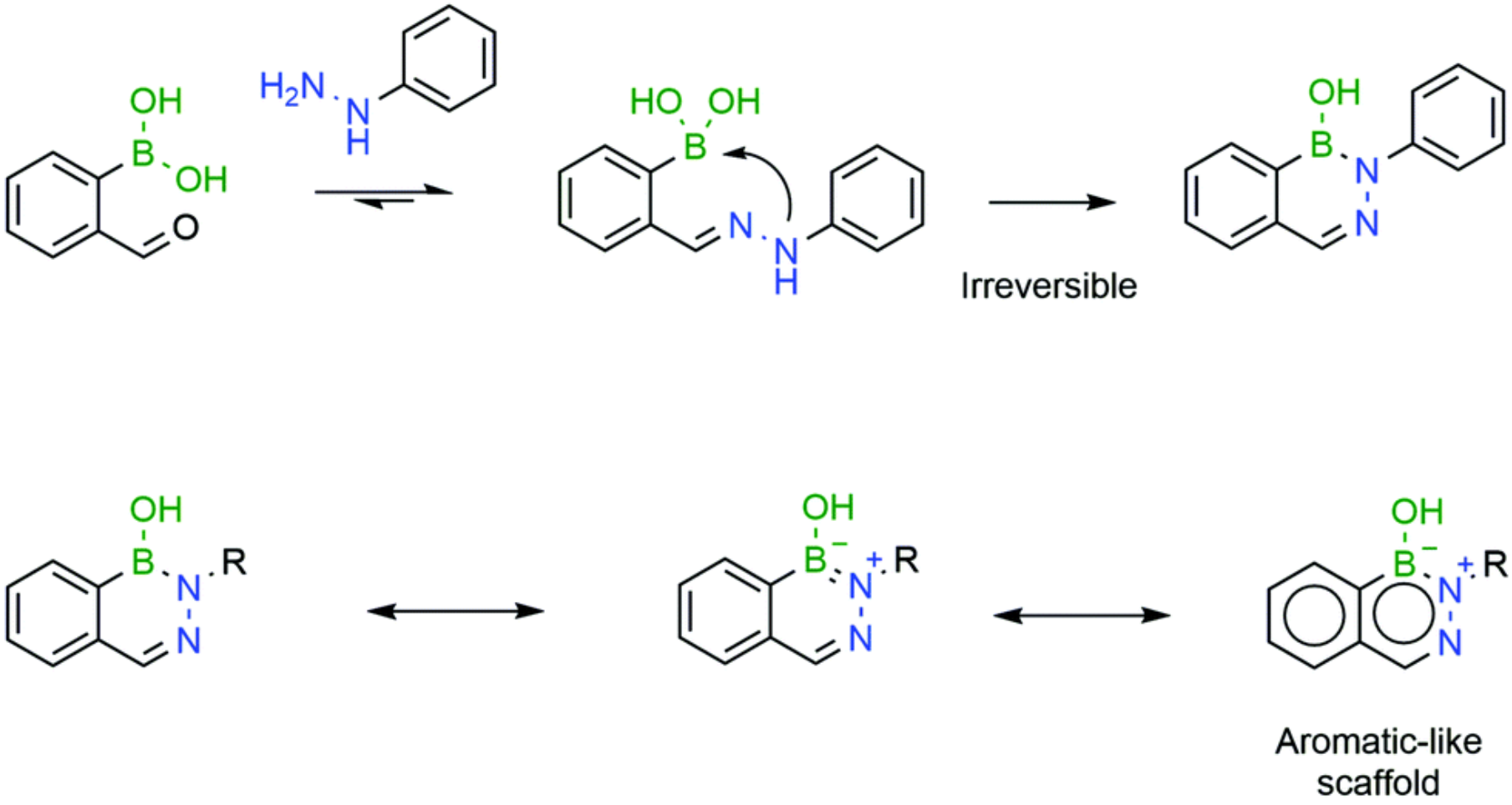
Gillingham. D *et al. Chem. Sci.* **2015**, *6*, 3329-3333.

React with Oxyamines



Gillingham. D *et al. Chem. Sci.* **2015**, *6*, 3329-3333.

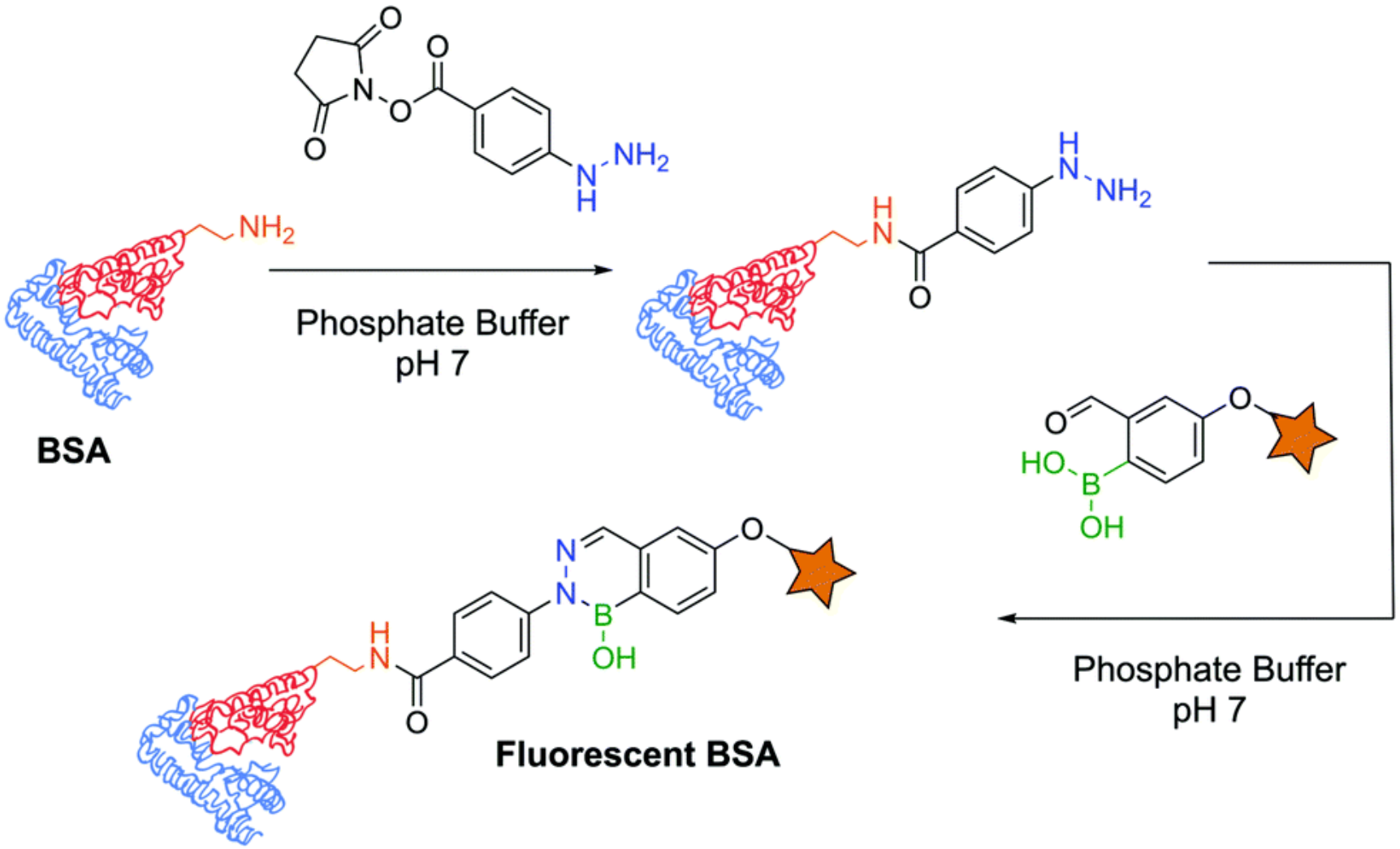
Boronic Acid with Hydrazides



- ✓ Stable aromatic-like structure.

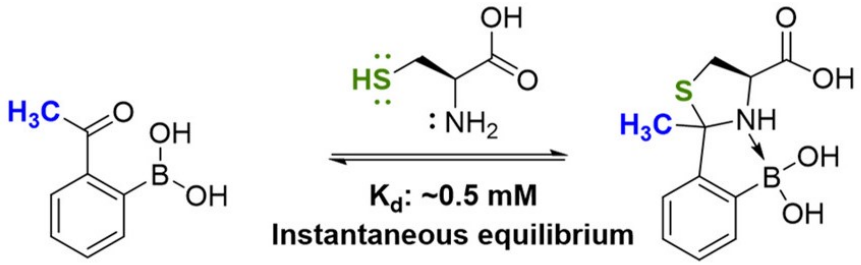
Gois. P. M. P *et al. Chem. Sco. Rev.* **2019**, *48*, 3513-3536.

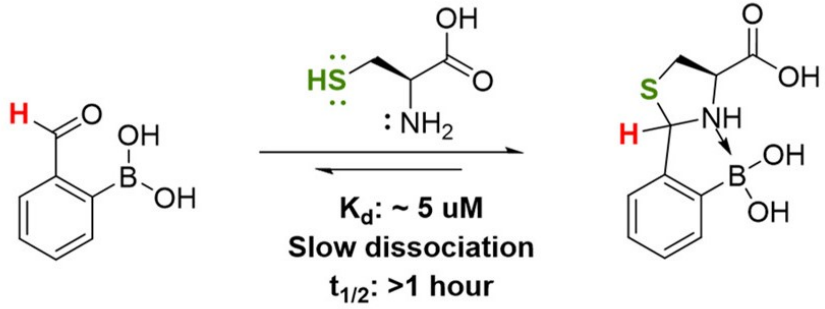
Boronic Acid with Hydrazides

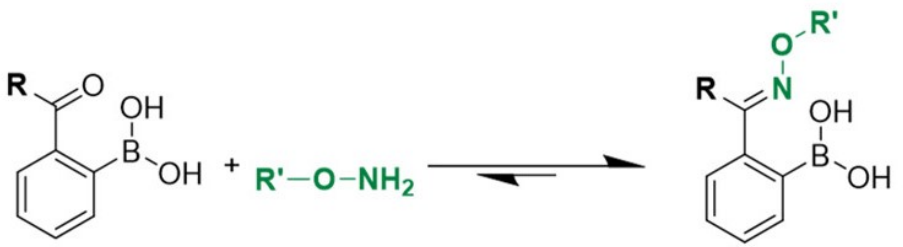


Gois. P. M. P *et al. Chem. Sco. Rev.* **2019**, *48*, 3513-3536.

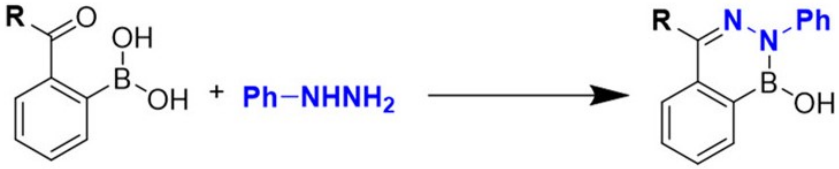
Short Summary







R	K_d µM	Dissociation ($t_{1/2}$)	Exchange ($t_{1/2}$)
H	0.004	ND	Yes (6.6 hr)
CH_3	10	Yes (<10 sec)	NA

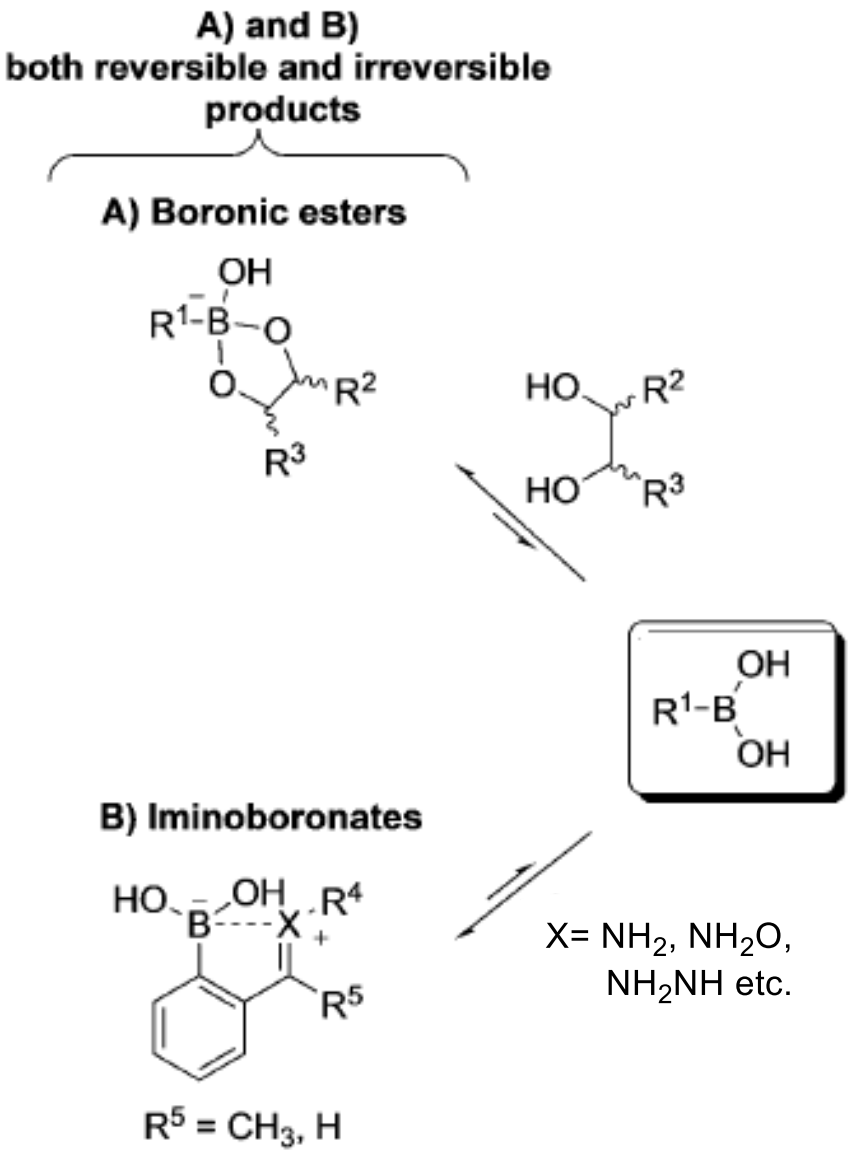


R	K_d µM	Dissociation ($t_{1/2}$)	Exchange ($t_{1/2}$)
H	ND	ND	ND
CH_3	ND	ND	Yes (4-6 hr)

Gois. P. M. P *et al. Chem. Sci.* **2016**, *7*, 5052-5058.

Contents

Summary



Diol Sources:

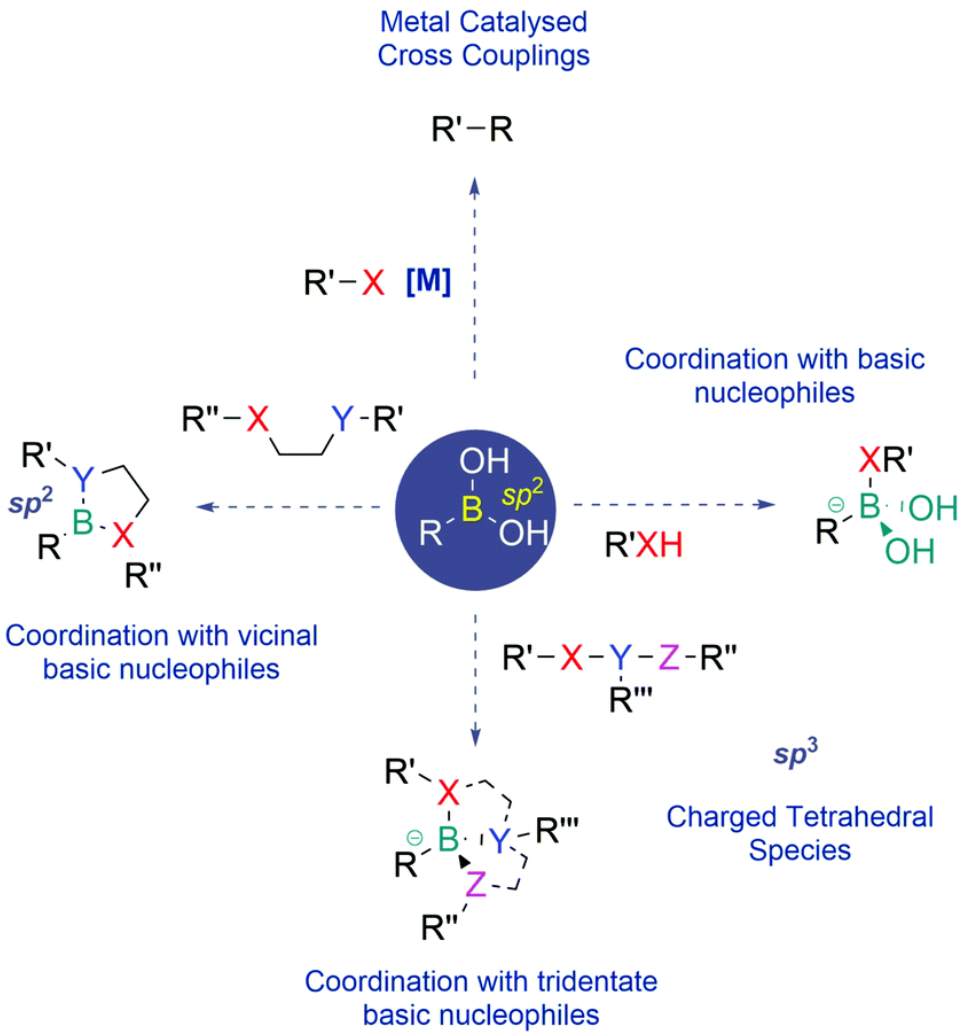
- ✓ Sugars
- ✓ Tetraserines
- ✓ Catachol
- ✓ Nopoldiol

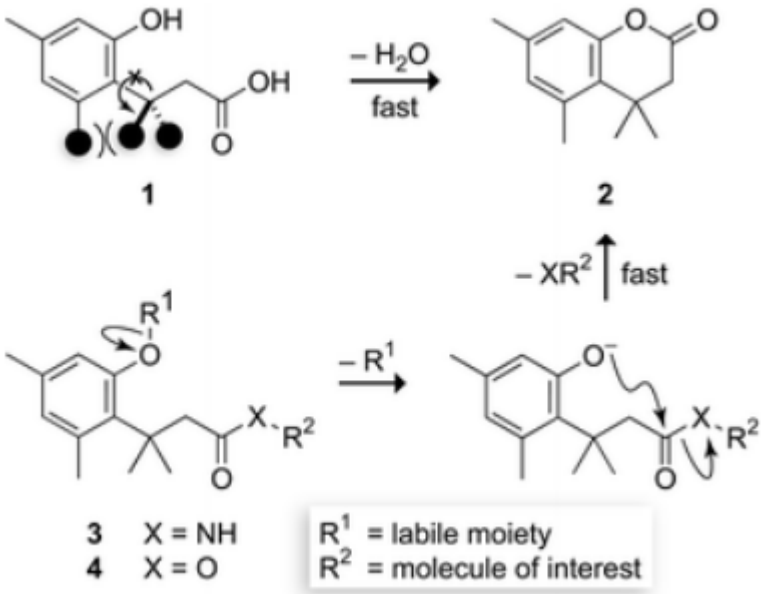
Reversible order:

- ✓ Primary amine, *N*-terminal cysteine, oxyamine, hydrazide (irreversible)

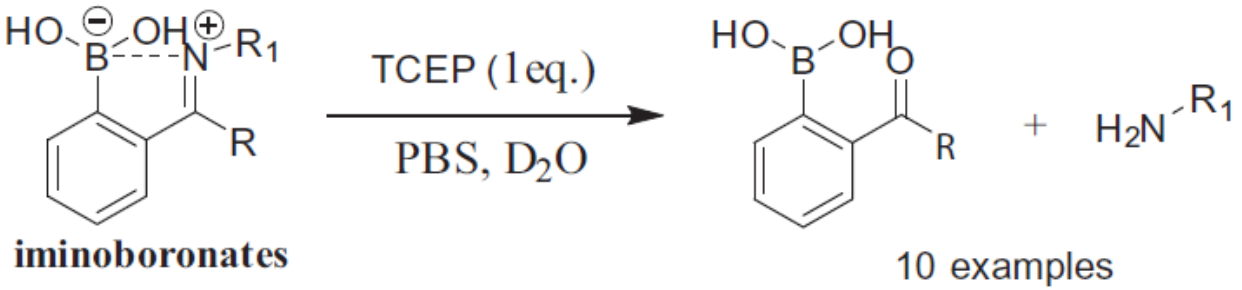
Appendix

Boronic Acid



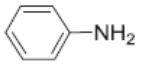
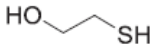
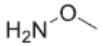
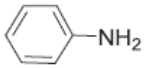
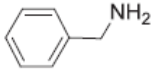
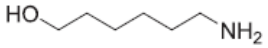
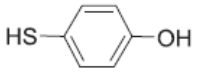
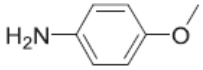
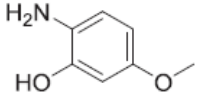
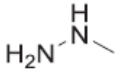


TCEP for Reversible Improvement

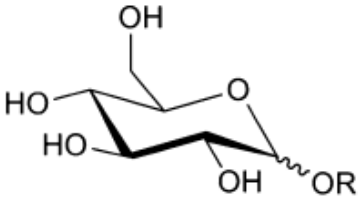


iminoboronates

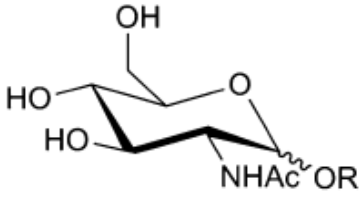
10 examples
98-100% yield
within 5 minutes

Entry	R	R_1-NH_2	Yield %
1	CH_3		100
2			100
3			100
4			24
5			86
6	H		100
7			100
8			100
9			100
10			100
11			99
12			98
13			87

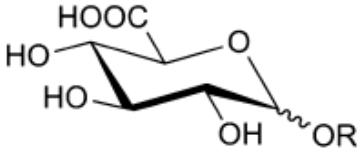
Sugar



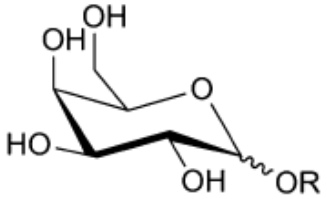
D-glucose (GLc)
2.5 %



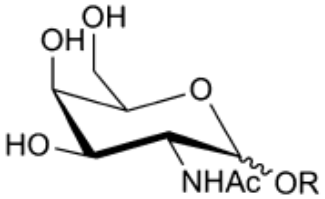
D-N-acetylglucosamine (GLcNAc)
31.8 %



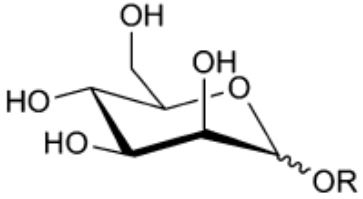
D-glucuronic acid (GlcA)
0.3 %



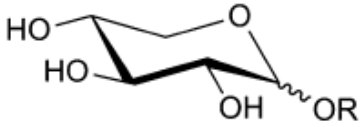
D-galactose (Gal)
24.8%



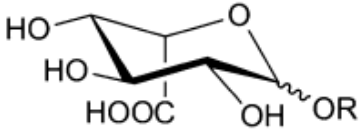
D-N-acetylgalactose (GalNAc)
4.8%



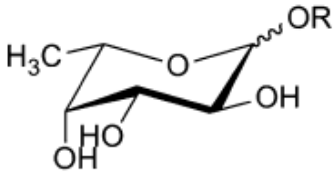
D-mannose (Man)
18.9 %



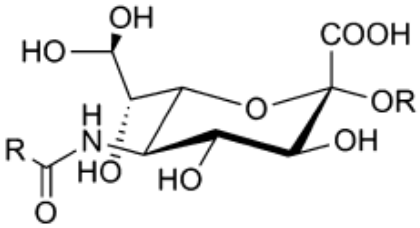
D-xylose (Xyl)
0.1 %



L-iduronic acid (IdoA)
0.1 %



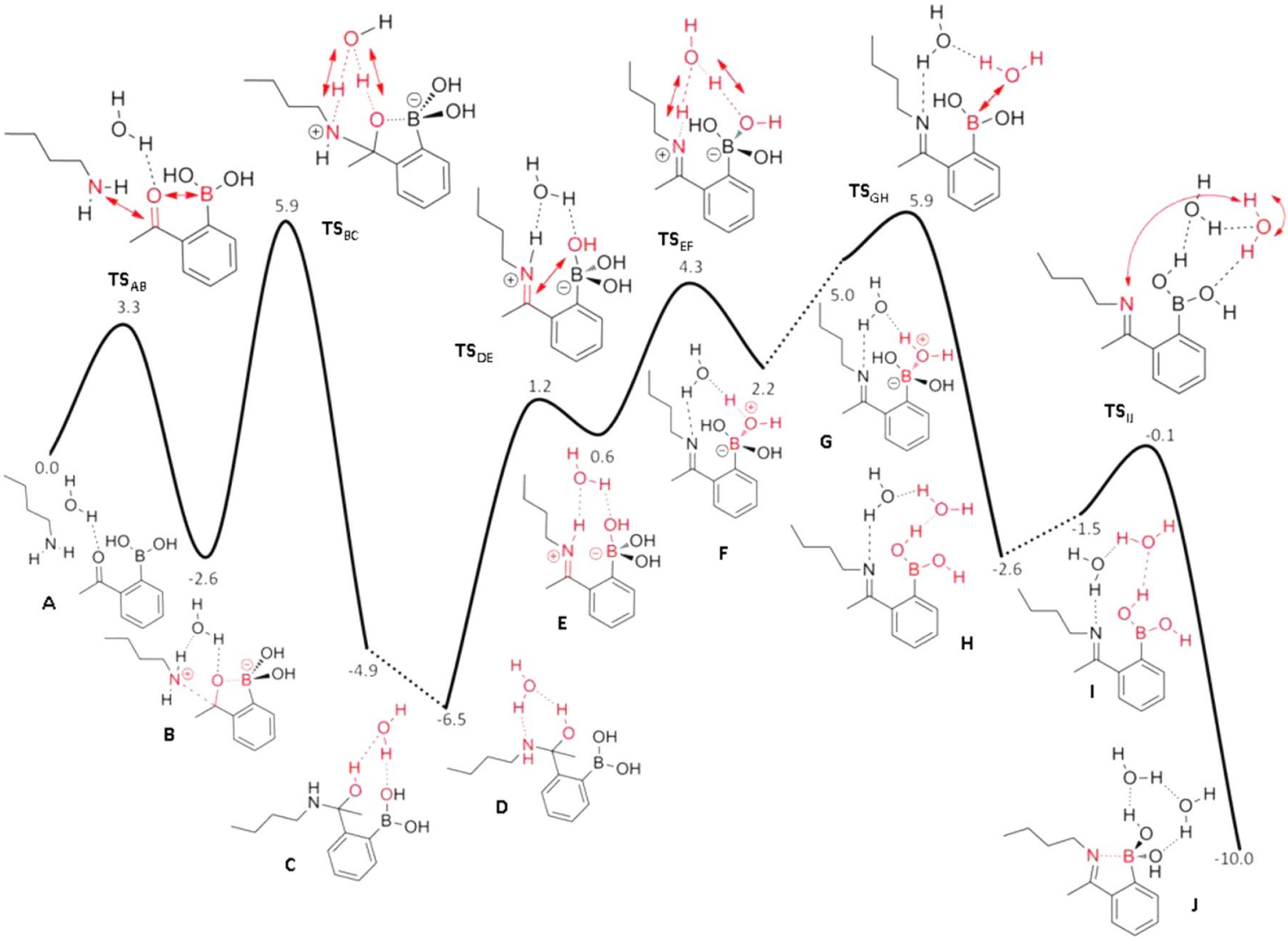
L-fucose (Fuc)
7.2 %



Sialic acid (Sia)
8.3 %

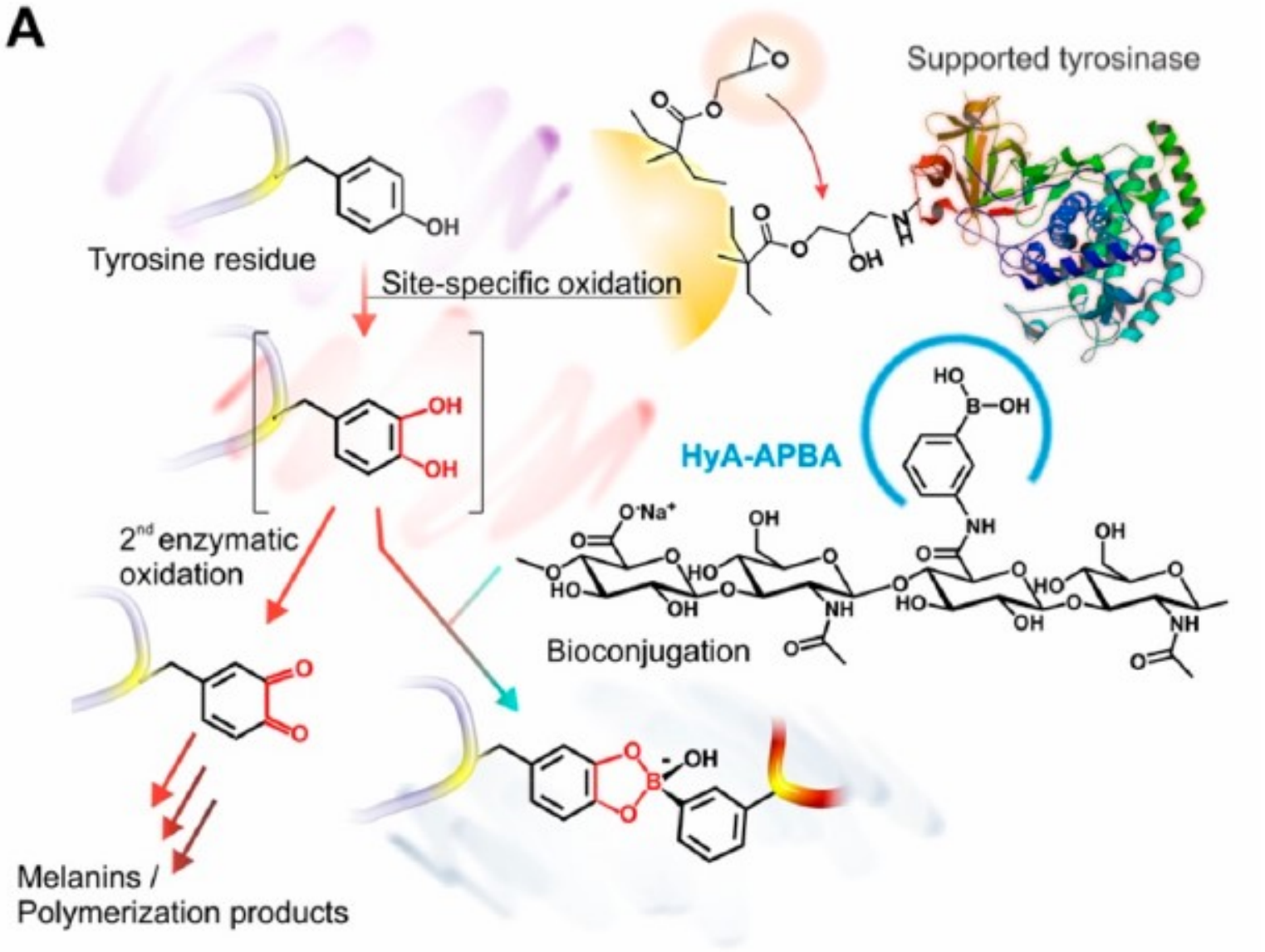
R = CH_3 or CH_2OH

Boronic Acid with *N*-terminal Lysine

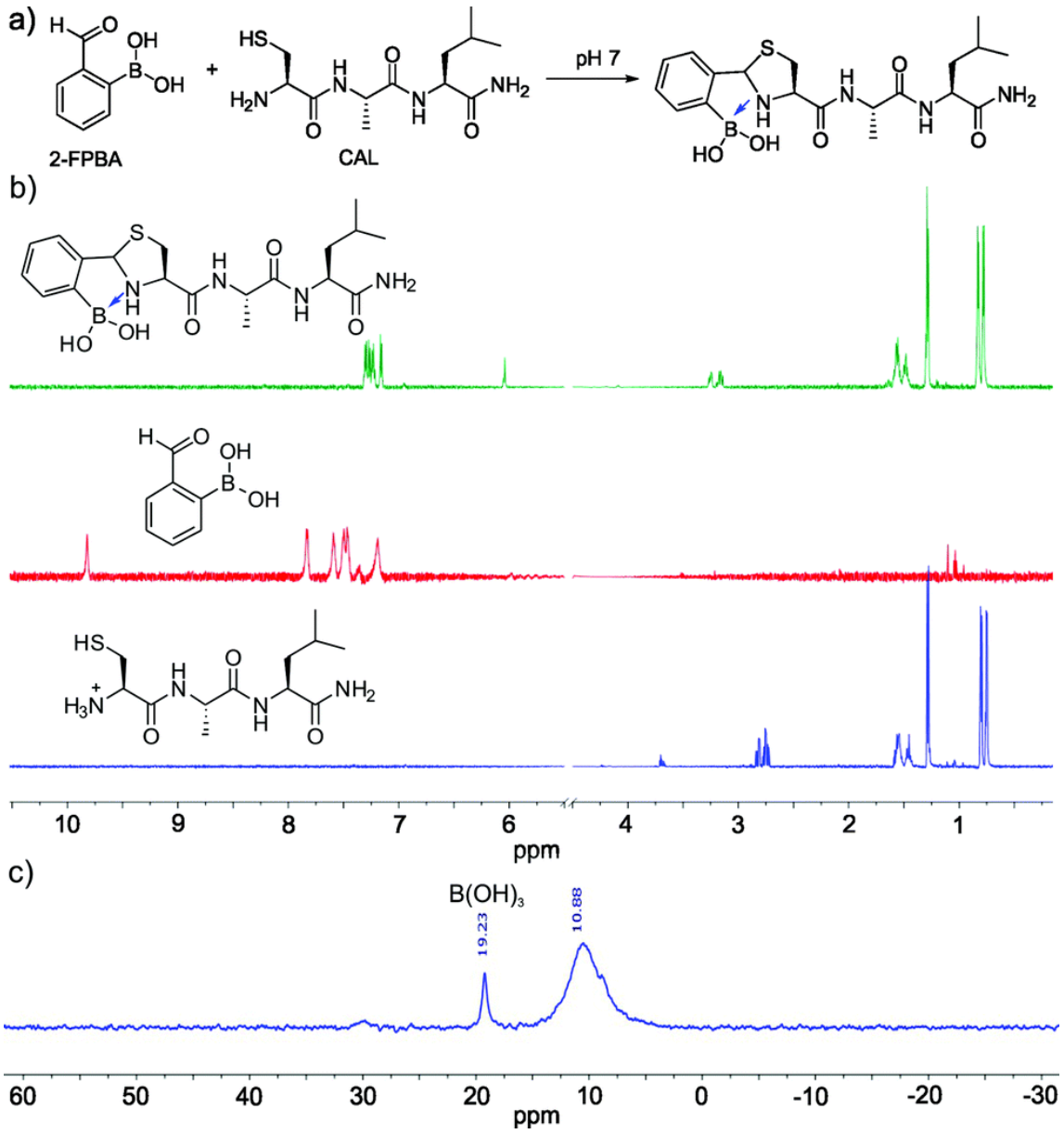


Gois. P. M. P *et al. J. Am. Chem. Soc.* **2012**, *134*, 10299-10305.

Tyr-


A
Supported tyrosinase
Tyrosine residue
Site-specific oxidation
HyA-APBA
2nd enzymatic oxidation
Bioconjugation
Melanins / Polymerization products

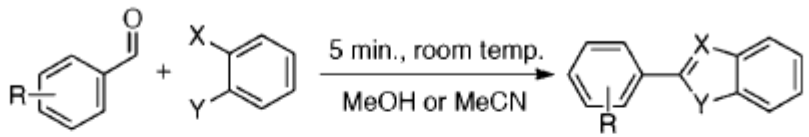
Boronic Acid with *N*-terminal Cysteine



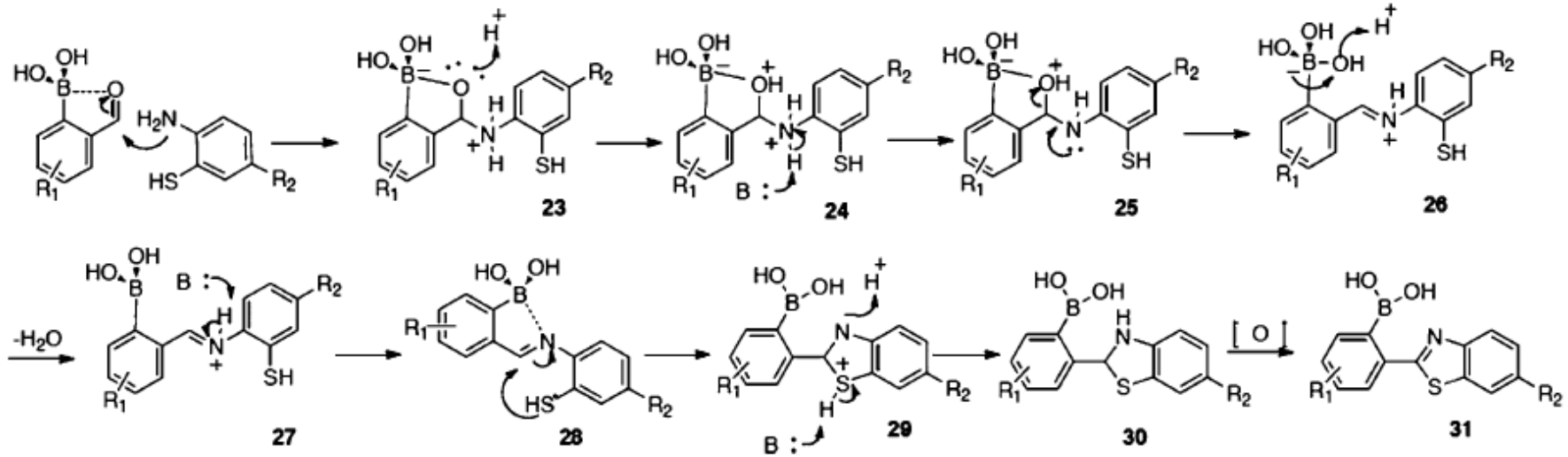
Gao. J *et al. Chem. Sci.* **2016**, *7*, 4589-4593.

Boronic Acid with *N*-terminal Cysteine

Table 1 Design of boronic acid facilitated "click" reaction



Compound	X	Y	R	Isolated yield (%)
1	NH_2	OH	2-$B(OH)_2$	—
2	NH_2	NH_2	2-$B(OH)_2$	—
3	NH_2	SH	2-$B(OH)_2$	85
4	NH_2	SH	3-$B(OH)_2$	—
5	NH_2	SH	4-$B(OH)_2$	—
6	NH_2	SH	H	10



Gao. J *et al. Chem. Sci.* **2016**, *7*, 4589-4593.

Boronic Acid with Hydrazides

Entry	Inhibiting reagent	Yield(%)
1	no inhibitor	94
2	Lysine (2mM)	82
3	Fructose (2mM)	90
4	Glutathione (2mM)	92
5	BSA (2mg/mL)	82

Gao. J *et al. Chem. Eur. J.* **2015**, *21*, 14748-14752.

Combine BE and Iminoboronate