Multiple ligand co-recognition of 3’-sulfogalactosylceramide (SGC) and sulfotyrosine initiated the comparison of SGC and sulfotyrosine and, subsequently, phosphotyrosine (pY) binding. SGC is a receptor for ligands involved in cell adhesion/microbial pathology. pY forms a Src homology domain 2 recognition motif in intracellular signaling. Using hsp70, anti-SGC, and anti-pY antibodies, ligand binding is retained following phosphate/sulfate and tyrosine/galactose substitution in SGC and sulfate/phosphate exchange in pY. Remarkable lipid-dependent binding to phosphatidyethanolamine-conjugated sulfotyrosine suggests “microenvironmental” modulation of sulfotyrosine-containing receptors, similar to glycosphingolipids. Based on an aryl substrate-bound co-crystal of arylsulfatase A, a sulfogalactose and phosphotyrosine esterase, modeling provides a solution basis for co-recognition. c-Src/Src homology domain 2:SGC/phosphogalactosylceramide binding confirms this hypothesis, heralding a carbohydrate-based approach to regulation of phosphotyrosine-mediated phosphorylation.

Sulfogalactolipids (SGLs) play a central role in spermatogenesis and nerve function (1) and are highly expressed in gastrointestinal and renal tissue (2). Our glycolipid receptor studies showed that 3’-sulfogalactosylceramide (sulfatide, SGC) bound hsp70 family members (3–7). Interaction of the N-terminal domain of hsp70 with SGC or its derivatives results in the inhibition of hsp70 ATPase activity (8), suggesting that hsp70/SGC binding might modulate chaperone function. Our finding that cytosolic hsp70s showed a similar SGL binding specificity (9) yet were unlikely co-localized with membrane SGC in cells, questioned the physiological significance of this mechanism of regulation of ATPase activity of such chaperones.

In addition, the similar SGL binding of hsp70s from prokaryotes, which contain no SGLs, questions whether SGL binding is an evolutionarily conserved or fortuitous function. hsp70 binding to the sulfogalactosyl residue is dependent on (6) and modulated by (9, 10) the lipid moity. Lipid modulation of glycosphingolipid ( GSL) receptor function is observed frequently (11).

Three other proteins, which specifically bind SGC, i.e. the human immunodeficiency virus adsatin (glycoprotein 120) (12), the coagulation protein (von Willebrand factor VIII) (13), and the eukaryotic selectin adsatin family (14, 15), also bind tyrosine sulfate (16–18). Therefore, we considered whether tyrosine sulfate and SGC recognition were related and, subsequently, whether phosphotyrosine and sulfogalactose recognition could be related. Peptide mimics of carbohydrate epitopes are well known (19). Therefore, the reverse was also considered possible. Considerable evidence in the literature is consistent with this concept.

MATERIALS AND METHODS

Tyrosine sulfated conjugated to dihexadecyl (DHD) phosphatidyethanolamine (PE) prepared as described previously (18) was kindly supplied by Dr. T. Feizi (Imperial College, Harrow, United Kingdom). Tyrosine and tyrosine sulfate coupled to PE were prepared by the same procedure in our laboratory using both DHD and Escherichia coli diacyl-PE. 4’-SGC, 6’-SGC, and 6’-phosphogalactosylceramide (6’-PGC) were synthesized from GalCer to retain ceramide heterogeneity. The structure of these compounds was verified by mass spectrometry, and the synthetic procedure will be described elsewhere. The binding of mAb anti-SGC (Sulf1), kindly provided by Dr. P. Friedman (University of Goteborg, Goteborg, Sweden), and DnaK by TLC overlay assay was detected immunologically as described previously (9, 20), and bound rabbit polyclonal anti-pY (Upstate Biotechnology Inc., Lake Placid, NY) were detected according to the supplier’s instructions.

Recombinant fusion gene constructs between SH2 domains from human c-Src kinase (residues 440–766) or the tyrosine phosphatase SHP-1 and N-terminal (residues 1–345), C-terminal (residues 328–654), and SHP-1 domains (C+N-terminal SH2 domains) (21) were generated.

Human full-length c-Src cDNA was generated by transposing residues 1–875 of the 5’-cDNA sequence from human c-Src cDNA in the Bluescript plasmid (a kind gift of Dr. D. Fujita, University of Calgary, Calgary, Alberta, Canada) and residues 576–1611 of the 3’-cDNA sequence isolated from human MCF-10A cells using PCR cloning based on the published sequence (22, 23). The two c-Src fragments were recombined at a KpnI site, the construct was cloned using TA-cloning vector, and the entire sequence was verified (ACGT, Toronto, Ontario, Canada). The cDNA was then introduced into pcDNA3.1/His A (Invitrogen) vector and used as a basic plasmid for construction of GST-c-SrcSH2, cDNA for c-SrcSH2 was generated from pcDNA3 using the primer pair 5’-CGCGGCGGCTGAGGAGTTATTGTTG-3’ corresponding to nt 440–461 of c-Src and the 5’-primer 5’-CTCGAGTCTGCGGCTTGGACGTGGGGCA-3’ complementary to nt 742–766 of c-Src.
The full-length SHP-1 from peripheral blood mononuclear cells encoding amino acids 1–595 (bb) were amplified with oligonucleotides SHPA (5′-GATCAGGAATTCATCCATGGTAGGTGTC-3′) and SHPB (5′-TCGCTCAGGACACTGAGGACACGCTGACG-3′). For making SHP-1/SH2N, SHP-1/SH2C, and SHP-1/SH2N-SH2C DNA fragments, a jumping PCR approach was applied from above full-length SHP-1 DNA template using the 5′-primer SHPA, corresponding to nt 1–16 of SHP-1, and SHP-1 SH2C-F (5′-GATCAGGAATTCATCCATGGTAGGTGG-TACCATGGCCACATC-3′), corresponding to nt 9–328 of SHP-1, respectively, and the 3′-primer SHP-1 SH2N-R (5′-CTCGAGGCTCTGACTATGGGATCGGA-3′), complementary to nt 307–327 of SHP-1, respective.

PCRs were performed in 50 μl of reaction mixture using 50 ng of primers and 2.5 units of Taq polymerase (Invitrogen) according to the recommendation of manufacturer. The PCR products contained an EcoRI as well as XhoI restriction sites at the N and C termini. These fragments were ligated into pGEX-4T-2 (Amersham Biosciences) vector at the same sites located downstream of glutathione S-transferase (GST) driven by tac promoter and in-frame with GST. The region that contained the truncation was completely sequenced to assure the effectiveness of the deletion and that no other mutations were introduced during deletion or cloning.

GST-c-Src/SH2, SHP-1/SH2 C, SHP-1/SH2 N, and SHP-1/SH2 C+N proteins were overexpressed in E. coli strain BL21 (DE3) (Novagen) and transformed with the pGEX-4T-2 constructs, and the GST fusion proteins were produced as described previously (24). A 500-ml culture of E. coli cells expressing each pGEX-4T-2/SH2 construct was grown at 37 °C until an optical density of 0.6 at 600 nm was reached. The culture was then induced with 1 mM isopropyl-β-D-thiogalactopyranoside, and the incubation continued for an additional 2 h. The cells were harvested and disrupted by sonication (six cycles of 50 s with a 1-min pause in between on ice). Triton X-100 to final concentration of 1% was added to the disrupted cells, and then cells were centrifuged at 10,000 × g for 5 min at 4 °C. 1 ml of a 50% slurry of glutathione-Sepharose 4B beads was added to the supernatant and mixed gently for 5 min at room temperature. The beads were washed three times by adding 50 ml of ice-cold phosphate-buffered saline and centrifuging for 10 s at 500 × g. The fusion protein was eluted by adding 1 ml of 50 mM Tris-HCl, pH 8.0, and 5 mM reduced glutathione, and the purified GST protein fractions were analyzed by SDS-PAGE for appropriate size.

GST binding of the SH2 domain fusion proteins was determined by TLC overlay, either by dot blot or chromatographically separated species. TLC chips or plates were blocked with bovine serum albumin/phosphate-buffered saline and incubated with fusion proteins (~1 μg/ml) overnight at room temperature. After washing, bound SH2-GST was detected using an anti-GST (9)/peroxidase/chloronaphthol system. GST alone was used as control.

In the molecular modeling studies, the minimum energy conformations of tyrosine phosphate and of 3′-sulfogalactose were obtained from ab initio Restricted Hartree-Fock/31G* geometry optimization using the GAUSSIAN98 program (25). The initial structure of 3′-sulfogalactose was derived from Protein Data Bank structure 1ONQ of a CD1-sulfatide complex (26) followed by initial geometry optimization at the Slater Type Orbital (STO-3G) level. Point charges for the O atoms of the sulfate and phosphate groups were obtained from the CHELPG (charges from electrostatic potentials using a grid) method (27).

Galactose 3′-sulfate was docked into the binding site of p-nitrophenyl catalase co-crystallized within the arylsulfatase substrate binding site (Protein Data Bank code 1E2S) (28). The sulfate group of galactose 3′-sulfate was forced to occupy the same position as that of pNCS with harmonic restraints, and the potential energy of the system in vacuo was minimized with a fully flexible ligand and binding pocket using the program CHARMM (Chemistry at Harvard Molecular Mechanics) (29) together with the CHARMM22 force field (30) and carbohydrate parameters developed by Brady and co-workers (31, 67).

RESULTS AND DISCUSSION

Substitution of Phosphate for Sulfate and Tyrosine for Galactose: DnaK and mAb Anti-SGC Sulfogalactolipid Binding—Positional isomers of SGC were made together with the phosphate analogue of 6′-SGC, 6′-PGC. To address the effect of tyrosine substitution for galactose on ligand binding, tyrosine and tyrosine sulfate derivatives of PE, originally generated to demonstrate P and L-selectin binding (18), were constructed. Fig. 1a shows that, in addition to 3′-SGC, both (non-physiological) 4′- and 6′-SGC are effectively bound by DnaK (E. coli hsp70). A comparison of the binding of 6′-SGC with 6′-PGC (Fig. 1a) shows the retention of DnaK binding following phosphate-sulfate substitution at this position. However, the tolerance for phosphate substitution within SGC is ligand-selective. The mAb anti-3′-SGC antibody, SulFI (20), while effectively binding 6′-SGC (although less than 3′-SGC), does not recognize 6′-PGC (Fig. 1c). Thus, in this case, phosphate cannot substitute for sulfate within the galactosphingolipid. Unlike DnaK, SulFI does not bind 4′-SGC (Fig. 1c). The doublet for 6′-SGC on TLC (hydroxyl and non-hydroxylated fatty acids) is bound by...
DnaK, whereas only the upper (non-hydroxylated) species is bound by Sulf1, a clear demonstration of lipid modulation of SGC binding. Both DnaK and Sulf1 tolerate galactose substitution by tyrosine.

Commercially available anti-pY antibodies are routinely used to screen for phosphotyrosine proteins (32) following up-regulation of intracellular signal transduction pathways. These antibodies do not bind phosphoserine or phosphothreonine (33) but may (34) or may not (35, 36) bind sulfotyrosine. The target antigens are specific pY residues within proteins in signal transduction cascades generated by a balance between cytosolic tyrosine kinases (37) and an equivalent family of phosphatases (38). DnaK, Sulf1, and anti-pY antibodies show significant binding to (DHD) PE-sulfotyrosine (Fig. 1, a, c, and d). However, Sulf1 preferentially bound sulfotyrosine- E. coli PE (diacyl, containing a complex mixture of fatty acid (C16:0, C16:1, and C18:1) esters (39)). Remarkably, the binding of both anti-pY and DnaK to sulfotyrosine- E. coli PE was not observed, whereas Sulf 1 showed a significant preference for sulfotyrosine- E. coli PE (Fig. 1). Thus, the character of sulfotyrosine-PE binding is typical of GSL receptors in that the lipid moiety can have a major effect on recognition.

Thus, for DnaK, sulfate can be replaced by phosphate and galactose can be substituted by tyrosine. For mAb anti-SGC, galactose can be replaced by tyrosine but sulfate cannot be replaced by phosphate, at least in the 6’ position. For anti-pY, phosphate can be replaced sulfate (in a select “chemoenvironment”) but galactose cannot replace tyrosine.

Recognition of the phosphotyrosine motifs within signaling proteins by downstream signaling molecules is a highly selective process that involves not only the phosphotyrosine moiety itself but its molecular environment as well (adjacent amino acids) (40). Particularly, tyrosine residues within the cytosolic domains of transmembrane proteins are prevalent adjacent to the membrane domain (41). Thus, the membrane environment may play a role in the receptor function of such residues when phosphorylated, as we have observed for GSLs (42). The recognition of SGLs by their ligands is modulated by their “molecular environment” (the lipid moiety of the SGL) (9, 10). Both sulfotyrosine (Fig. 1) and sulfogalactose (10) ligand binding is similarly modulated by lipid conjugation, supporting a structural relationship between these epitopes.

**Comparison of Galactose 3’-Sulfate and Phosphotyrosine Conformation**—Molecular superimposition of the minimal energy conformers of phosphotyrosine and 3’-sulfogalactose shows an essential overlap of the rings and a significant coincidence of the orientation of the sulfate and phosphate moieties. The energy-minimized conformation and charge distribution for phosphotyrosine were obtained from ab initio calculations (Fig. 2A). The conformation of 3’-sulfogalactose from the structure of the SGC/CD1a co-crystal (28) was used as the starting point for geometry optimization (Fig. 2B). The charge distribution within the phosphate oxygen atoms is not greatly dissimilar from that of the sulfate oxygen atoms, despite the phosphate double and the sulfate single charge. This is explained by the partial delocalization of the phosphate charge within the tyrosine ring.

**Arylsulfatase Provides a Model for Phosphotyrosine-Sulfogalactose Equivalence**—Precedent for the structural convergence of 3’-sulfogalactose and phosphotyrosine with regard to ligand binding can be found from a well studied example in the literature. The enzyme responsible for the degradation of SGC in *vivo* is termed arylsulfatase A. Deficiency in this enzyme activity results in the lysosomal storage disease, metachromatic leukodystrophy, due to intracellular SGC accumulation (43). The name “aryl sulfatase” is used because the enzyme will also desulfate various aryl substrates. Indeed, this activity was demonstrated before the natural substrate (SGC) was identified (44). The several aryl sulfate substrates have a substituted tyrosine-like ring structure. Arylsulfatase has recently been found to have tyrosine phosphatase activity (45). Phosphotyrosine inhibits arylsulfatase-mediated SGC desulfation (46). Phosphotyrosine and methylumbelliferyl phosphate can bind within the aryl sulfate/SGC binding site to inhibit desulfation. Moreover, the phosphorylated enzyme intermediate in phosphotyrosine cleavage has been crystallized. The covalently bound phosphate was localized in the same position as the sulfate in the enzyme p-nitrocatechol sulfate co-crystal (47). The substrate binding site activity (45) is in a cleft lined primarily with polar residues (Fig. 3). Surprisingly, a non-polar domain within the cleft (Leu<sup>68</sup> and Val<sup>91</sup>) is uninvolved in binding the hydrophobic aryl substrates. Since SGC has not been crystallized with the enzyme, we have modeled 3’-sulfogalactose within the substrate binding site as defined by the co-crystal (Fig. 3) (28). The galactose ring occupies a position intermediate between the two conformations of the aryl ring in the co-crystal, and its hydroxyl groups can form several hydrogen bonds with the protein (Fig. 3, Table I). In addition, the 6’-CH<sub>2</sub> makes hydrophobic contact with Val<sup>91</sup> in the hydrophobic domain, providing additional binding energy for
the natural SGL substrate (Fig. 3B). Thus, although the aryl ring provides the more non-polar substrate, it is the galactosyl substrate that utilizes the hydrophobic patch within the arylsulfatase substrate binding site. In the catachol sulfate/arylsulfatase co-crystal, a water molecule is resolved adjacent to the aryl ring, which is H-bonded to the sulfate oxygen to assist in the coordination of the bound sulfate. A water molecule immobilized in the pNCS co-crystal is shown in B, which is virtually coincident with the 4'-OH of the docked galactose sulfate. The mean position of the ring is intermediate between the two alternate orientations of the pNCS ring proposed in the crystallographically determined structure (28).

Table 1
| Atom 1        | Atom 2        | Separation (nm) | Comments        |
|---------------|---------------|-----------------|-----------------|
| Galactose atom (CHARMM) |              |                 |                 |
| C-6           | Val<sup>93</sup>, Cy2 | 0.32            | Hydrophobic contact |
| O-4           | His<sup>139</sup>, Nε2 | 0.37            | Weak HB         |
| O-4           | Ser<sup>131</sup>, Oγ | 0.36            | Weak HB         |
| O-4           | Sulfate OS3   | 0.31            | Intramolecular HB |
| O-1           | Arg<sup>364</sup>, Nγ2 | 0.35            | HB              |
| O₂            | Glu<sup>195</sup>, Oε2 | 0.29            | HB              |
| O₃            | Lys<sup>233</sup>, Nε | 0.27            | HB              |
| O-3 (sulfate) | Lys<sup>233</sup>, Nε | 0.31            | HB              |
| O-3 (sulfate) | His<sup>229</sup>, Nε2 | 0.27            | HB              |
| OS1 (sulfate) | Lys<sup>193</sup>, Nε | 0.27            | HB              |
| OS2 (sulfate) | Lys<sup>204</sup>, Nε | 0.30            | HB              |
| OS2 (sulfate) | Ala<sup>54</sup>, NH (backbone amide) | 0.34            | HB              |

The superimposition of 3'-sulfogalactose in the substrate binding site of arylsulfatase. Galactose 3'-sulfate was docked into the binding site of pNCS (pale orange) co-crystallized within the arylsulfatase substrate binding site. A, non-polar carbohydrate face. B, polar face view. Hydroxyl groups 2 and 4 can form several hydrogen bonds as highlighted with dashed lines (Table I). In addition, the methylene group at position 6 forms a hydrophobic contact with Val<sup>93</sup>, whereas the hydroxyl groups 2 and 6 are at least partly exposed to solvent. A water molecule immobilized in the pNCS co-crystal is shown in B, which is virtually coincident with the 4'-OH of the docked galactose sulfate. The mean position of the ring is intermediate between the two alternate orientations of the pNCS ring proposed in the crystallographically determined structure (28).
older post-translational modification than sulfogalactosylation. Interaction of prokaryote hsp70s with phosphotyrosine motifs could provide an evolutionary basis of the conserved SGL binding that we have observed (9). Thus, under appropriate circumstances, sulfogalactose-based analogues might modify tyrosine kinase/phosphatase activity and signaling pathways.

**SH2 Domain Sulfogalactolipid Binding**—SH2 domains provide the central recognition mechanism for binding pY motifs mediating protein:phosphoprotein interactions in signal transduction (50). Recombinant fusion proteins of c-Src (panel b), SHP-1 C-terminal domain (panel c), SHP-1 N-terminal domain (panel d), and SHP-1 C + N-terminal SH2 domain (panel e). Left column from top, lipids are cholesterol sulfate, GM3 ganglioside, tyrSO4-DHD PE. Right column, 6'-SGC, 3'-SGC, tyrSO4-E. coli PE. Middle panels, a comparison of DnaK (panel a) and c-SrcSH2 domain (panel b) TLC overlay binding. Lane 1, GalCer, 3'-SGC and GM3. Lane 2, 4'-SGC. Lane 3, 6'-SGC. Lane 4, 6'-PGC. Each lane contains 1 μg of GSL. Lower panels, c-SrcSH2 domain TLC overlay binding to tyrosine-PE derivatives. Panel a, c-SrcSH2 binding. Panel b, molybdemenum blue staining for phosphate. Lane 1, from top, E. coli PE, tyr-E. coli PE, and tyrSO4-E. coli PE. Lane 2, from top, DHD PE, tyr-DHD PE, and tyrSO4-DHD PE. Lane 3, phosphatidylcholine + cholesterol sulfate.

**FIG. 4. Sulfogalactolipid binding by SH2 domains.** Upper panels, dot blot TLC binding comparing Sulfl mAb anti-SGC (panel a) and GST fusion proteins of c-Src (panel b), SHP-1 C-terminal domain (panel c), SHP-1 N-terminal domain (panel d), and SHP-1 C + N-terminal SH2 domain (panel e). Left column from top, lipids are cholesterol sulfate, GM3 ganglioside, tyrSO4-DHD PE. Right column, 6'-SGC, 3'-SGC, tyrSO4-E. coli PE. Middle panels, a comparison of DnaK (panel a) and c-SrcSH2 domain (panel b) TLC overlay binding. Lane 1, GalCer, 3'-SGC and GM3. Lane 2, 4'-SGC. Lane 3, 6'-SGC. Lane 4, 6'-PGC. Each lane contains 1 μg of GSL. Lower panels, c-SrcSH2 domain TLC overlay binding to tyrosine-PE derivatives. Panel a, c-SrcSH2 binding. Panel b, molybdemenum blue staining for phosphate. Lane 1, from top, E. coli PE, tyr-E. coli PE, and tyrSO4-E. coli PE. Lane 2, from top, DHD PE, tyr-DHD PE, and tyrSO4-DHD PE. Lane 3, phosphatidylcholine + cholesterol sulfate.

These results validate the prediction that sulfogalactose can substitute for phosphotyrosine recognition. Demonstration of c-SrcSH2 as a carbohydrate binding motif identifies a potential new avenue for the generation of inhibitors of SH2 domain/phosphoprotein binding. In the c-SrcSH2-activated platelet-derived growth factor receptor co-crystal (51), a water molecule is coordinated in the binding of the (tyrosine) phosphate in a manner analogous to the aromatic sulfate that we highlighted in arylsulfatase. This could explain the selective binding of SGC by this SH2 domain. Solvation of the “receptor ring” may provide a link between GSL and pY recognition.

Furthermore, the co-crystal structure of a *Yersinia* tyrosine phosphatase (YopH) complexed with a small molecule inhibitor has been solved (52). This inhibitor is p-nitrocatechol sulfate, the same compound that was co-crystalized in the substrate binding site of arylsulfatase (Fig. 3). YopH is a homologue of SHP-1 and pNCS was found to be a competitive inhibitor of many such SHP-1 homologous tyrosine phosphatases (52). Because pNCS is bound by both arylsulfatase and YopH, we predict SGC binding for YopH. pNCS binds in a solvent-exposed positively charged cleft in YopH where solvation is likely to play a significant role. Indeed, Sun *et al.* (52) identify a bound water coordinating the charge in much the same way as is seen for arylsulfatase (and c-Src) and suggest that hydroxyl groups might be used as an advantage in the generation of more effective inhibitors. The hydrophobic interactions of the aryl ring with YopH occur from the more protein-adjacent face, whereas the polar and solvent-mediated interactions (with the exception of the direct coordination of the sulfate) occur from the other more solvent-exposed face, generating a polarity asymmetry such as might be mimicked by a sugar ring.

The overall relationship that we propose is summarized in Scheme 1. Our data establish a continuum of ligand binding from sulfogalactose to phosphotyrosine, either by tyrosine substitution for galactose or by phosphate substitution for sulfate. In terms of the latter, only one of the carbohydrate positional isomers has been studied thus far, the 6'-sulfo- and 6'-phosphogalactosylceramide. Phosphogalactose is an uncommon moiety in arylsulfatase. This could explain the selective binding of SGC by this SH2 domain. Solvation of the “receptor ring” may provide a link between GSL and pY recognition.

**Scheme 1. Receptor mimicry between sulfogalactose and phosphotyrosine moieties: relationship between “intermediate” structures and binding ligands from the literature and the present work.**

**FIG. 4.** Sulfogalactolipid binding by SH2 domains. Upper panels, dot blot TLC binding comparing Sulfl mAb anti-SGC (panel a) and GST fusion proteins of c-Src (panel b), SHP-1 C-terminal domain (panel c), SHP-1 N-terminal domain (panel d), and SHP-1 C + N-terminal SH2 domain (panel e). Left column from top, lipids are cholesterol sulfate, GM3 ganglioside, tyrSO4-DHD PE. Right column, 6'-SGC, 3'-SGC, tyrSO4-E. coli PE. Middle panels, a comparison of DnaK (panel a) and c-SrcSH2 domain (panel b) TLC overlay binding. Lane 1, GalCer, 3'-SGC and GM3. Lane 2, 4'-SGC. Lane 3, 6'-SGC. Lane 4, 6'-PGC. Each lane contains 1 μg of GSL. Lower panels, c-SrcSH2 domain TLC overlay binding to tyrosine-PE derivatives. Panel a, c-SrcSH2 binding. Panel b, molybdemenum blue staining for phosphate. Lane 1, from top, E. coli PE, tyr-E. coli PE, and tyrSO4-E. coli PE. Lane 2, from top, DHD PE, tyr-DHD PE, and tyrSO4-DHD PE. Lane 3, phosphatidylcholine + cholesterol sulfate.

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**Scheme 1. Receptor mimicry between sulfogalactose and phosphotyrosine moieties: relationship between “intermediate” structures and binding ligands from the literature and the present work.**

![Diagram](image-url)
Sulfogalactose Is a Mimic of Phosphotyrosine

Frotional cancer (54), correlating with the up-regulation of Src in this disease (55). Because SGC can function as a partial structural mimic of phosphotyrosine, the effects microbial pathogens which bind SGC (4, 56), have on host cell tyrosine phosphorylation (57, 58) should be considered. Such a SrcSH2-mediated effect may have already been reported (59). Similarly, the effect of human immunodeficiency virus glycoprotein 120 on host cell tyrosine phosphorylation (60) could relate to its SGL binding.

Tyrosine sulfate is a more common cell surface post-translational modification. As indicated in Scheme 1, there are more examples, both from the literature and our present work, that indicate this may be the more physiologically relevant mimicry with SGLs. The fact that, like SGL biosynthesis (1, 61), tyrosine phosphorylation (60) could relate to its SGL binding.

Tyrosine sulfate is a more common cell surface post-translational mimic of phosphotyrosine, the effects microbial pathogens with SGLs. The fact that, like SGL biosynthesis (1, 61), tyrosine phosphorylation (60) could relate to its SGL binding.

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