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Design and Synthesis of Functionalized Glycomers as Non-Peptidic Ligands for SH2 Binding and as Inhibitors of A-431 Human Epidermoid and HT-29 Colon Carcinoma Cell Lines

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Abstract—A set of *O*-substituted aryl β-D-glucopyranosides were prepared and found to have inhibitory activity on the growth of two carcinona cell lines. © 2000 Elsevier Science Ltd. All rights reserved.

Cellular signal transduction is a vital physiological process (for recent reviews, see refs 1–4), through which important recognition events among proteins are regulated. Distinct functional modules composed of independently folded amino acids termed Src homology 2 (SH2) domains (for recent reviews, see refs 5–8), and present in many intracellular proteins, bind to phosphotyrosine units of peptides. A cascade of sequential protein–protein interactions follows leading to a complex set of cellular responses. The aberration of some of these signaling pathways may have important consequences in diverse pathologies such as cancer and immune disorders. Grb-2 is a key transducer containing the SH2 domain, and it is particularly important for EGF (e.g. refs 9–12) and HGF (e.g. refs 13 and 14) receptors which are expressed in A-431 human epidermoid and HT-29 colon carcinoma cell lines. The discovery of antagonist molecules to these domains may have great potential in the development of anticancer and antiinflammatory drugs for example.[1–4,15–18]

Elegant studies on the crystal structures of high affinity phosphotyrosyl peptide ligands such as (Y^{p}VNV) to Grb2-SH2 have been reported (see, for example, refs 19–21) (Fig. 1).

We envisaged the synthesis of a series of aryl β-D-glucopyranosides 'glycomers' as scaffolds onto which we would deploy diverse functionality to probe their binding affinities (Fig. 1, Types A, B, and C). Such glycomers could also be viewed as constrained spacial glycomimetics of the YVNV tetrapeptide.

The synthesis of the intended glycomers proceeded in a straightforward fashion and in good yields for individual steps (>70%). Scheme 1 depicts the synthesis of glycomers of Type A, where an 6-*O*-(*m*-trifluoromethyl benzenesulfonyl) and a 3-*O*-allyl groups were present in each of the 2-ester derivatives (**6**, R_1 = acyl). The choice of a *p*-iodophenyl aglycone was based on the presence of the tyrosine unit in YVNV and on the opportunities offered by cross-coupling reactions in subsequent transformations. To that end, the *p*-iodophenyl, *p*-bromophenyl and *p*-hydroxylphenyl β-D-glucopyranosides were also easily obtained (for glycoside synthesis via stannyl ethers, see refs 22–25). The protected ethyleneglycol glycoside **4** was prepared by the Koenigs–Knorr method using $Hg(CN)_2/HgBr_2$ as activators (for a review, see ref 26).

Utilizing a common precursor **2** (R = *p*-iodophenyl), a series of 6-carbamates, 6-esters and 6-ethers were also synthesized by standard methods (Scheme 2).

Scheme 3 shows the elaboration of glycomers in which a distal carbamate group was introduced at C-3 in

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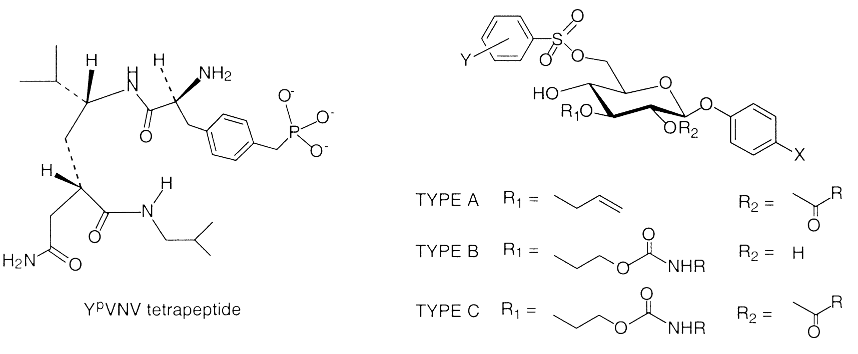
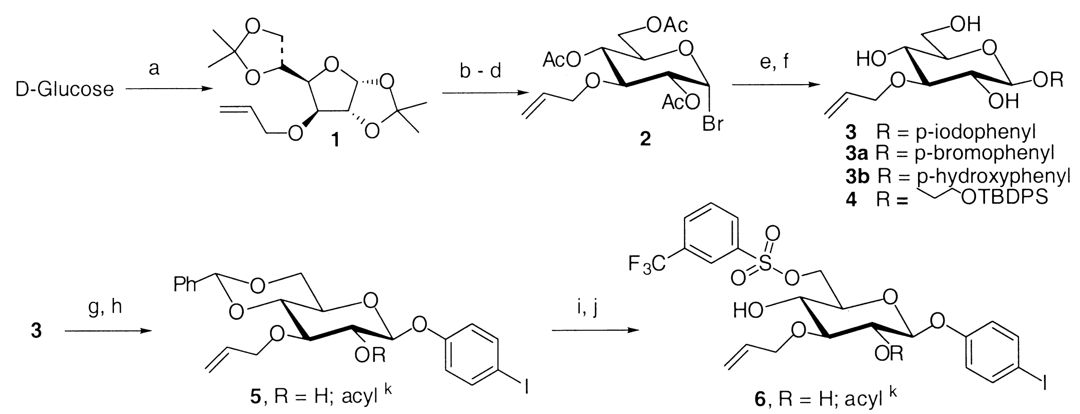
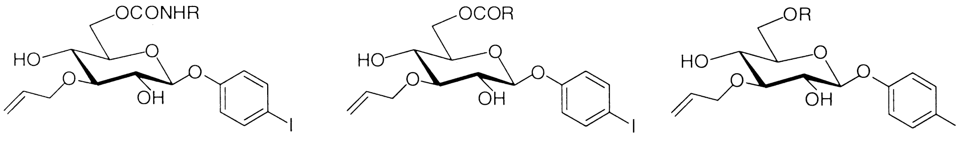


Figure 1.



Scheme 1. (a) i. Acetone, H_2SO_4, ii. NaH, DMF, iii. allyl bromide; (b) IRa-1 20 (H^+), H_2O; (c) Ac_2O, pyridine; (d) HBr, AcOH; (e) for **3**: tributylstannyl *p*-iodophenoxide, $SnCl_4$, CH_2Cl_2, 85%; for **3a**: tributyistannyl *p*-bromophenoxide, $SnCl_4$, CH_2Cl_2, 88%; for **3b**: *p*-resorcinol, $CdCO_3$, toluene, 70%; for **4**: β-diphenyl *tert*-butylsilyloxyethanol, $Hg(CN)_2$, $HgBr_2$, benzene, 75%; (f) MeONa/MeOH; (g) benzaldehyde dimethylacetal, DMF, HBF_4; (h) RCOCl, Et_3N, CH_2Cl_2; (i) TFA, H_2O; (j) *m*-trifluorobenzenesulfonyl chloride, pyridine; (k) R_1 = acetyl **6a,** R_1 = propionyl **6b**, R_1 = butyryl **6b**,A_1 = benzoyl **6d**, R_1 = phenylacetyl **6e**, R_1 = phenylpropionyl **6f** (yields 80–87%).

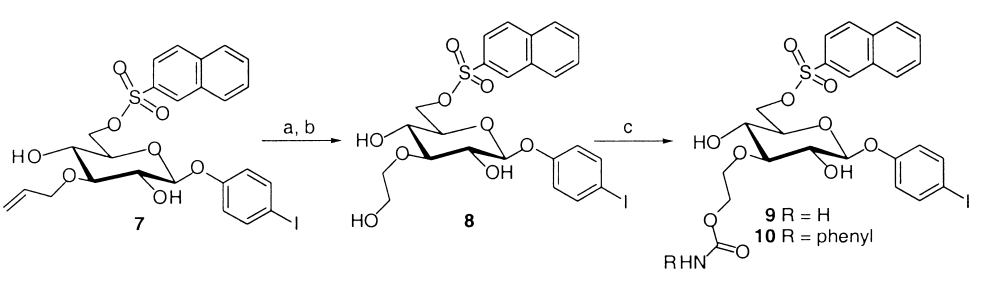


R = Et; Ph; p-toluyl; allyl

R = α-naphthyl, p-chlorobenzyl, p-methoxybenzyl, benzyl

R = p-methoxybenzyl, benzyl

Scheme 2.

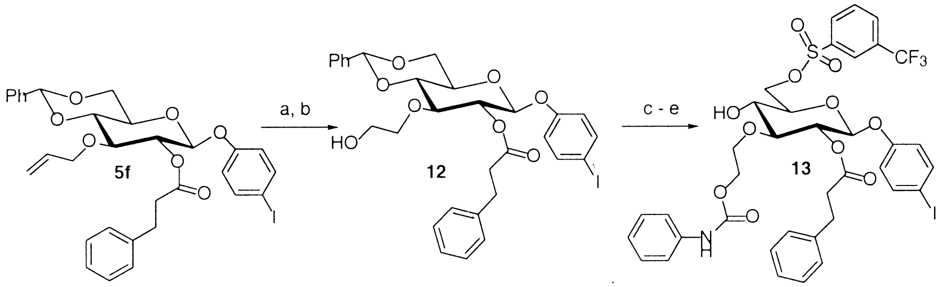


Scheme 3. (a) O_3, MeOH; (b) $NaBH_4$; (c) for **9**: i. *p*-nitrophenyl chloroformate, ii. ammonium formate; for **10,** PhNCO.

conjunction with a 6-*O*-(2-naphthylsulfonyl) ester. Related carbamates in the *m*-trifluoromethyl benzenesulfonate series were also prepared.

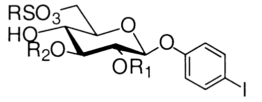
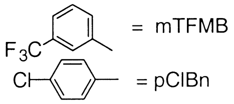
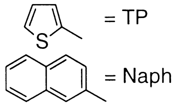
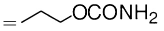
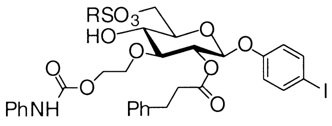
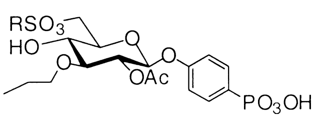
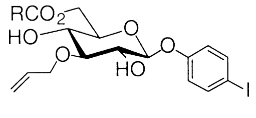
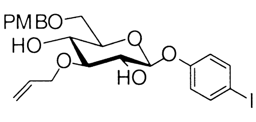
A representative glycomer of the Type C family in which ester and carbamate groups were deployed at C-2 and C-3, respectively, was synthesized from precursor **5f** as shown in Scheme 4.

The results for the inhibition of Grb2-SH2 were disappointing, with at best 25–30% inhibition at 100 μM for compounds in entries 5, 9 and 15 (Table 1). Much



Scheme 4. (a) O_3, MeOH; (b) $NaBH_4$; (c) phenylisocyanate, CH_2Cl_2; (d) TFA, H_2O; (e) *m*-trifluorobenzenesulphonyl chloride, pyridine.

Table 1. Cytotoxicity of glycomers against A-431 and HT-29 cell lines[a]

Entry	Structure			A-431 (IC_{50} μM)	HT-29 (IC_{50} μM)
					
1	R = mTFMB	R_1 = H	R_2 = allyl	36.80	35.45
2	R = mTFMB	R_1 = MeCO	R_2 = allyl	6.10	23.07
3	R = mTFMB	R_1 = EtCO	R_2 = allyl	2.17	4.65
4	R = mTFMB	R_1 = PrCO	R_2 = allyl	2.60	4.00
5	R = mTFMB	R_1 = iPrCO	R_2 = allyl	3.30	4.20
6	R = mTFMB	R_1 = PhCO	R_2 = allyl	7.00	7.50
7	R = mTFMB	R_1 = $PhCH_2CO$	R_2 = allyl	5.15	4.95
8	R = mTFMB	R_1 = $Ph(CH_2)_2CO$	R_2 = allyl	2.00	3.00
9	R = TP	R_1 = MeCO	R_2 = allyl	12.75	—
10	R = pClBn	R_1 = MeCO	R_2 = allyl	9.77	—
11	R = 2-naphthyl	R_1 = H	R_2 = allyl	8.81	—
12	R = 2-naphthyl	R_1 = MeCO	R_2 = allyl	5.20	11.55
13	R = 2-naphthyl	R_1 = H	R_2 = 	9.10	23.10
14	R = 2-naphthyl	R_1 = H	R_2 = 	5.10	3.67
15			R = mTFMB	4.52	7.15
16			R = mTFMB	38.80	35.45
17			R = 2-naphthyl	>50	>50
					
18	R = phenyl			>50	>50
19	R = 4-chlorophenyl			20.5	25.16
20	R = 4-methoxyphenyl			24.6	55.10
21	R = 2-naphthyl			>50	>50
22				10.6	19.72

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[a] Cytotoxicity was determined as described in refs 27 and 28.

more encouraging were the results of inhibitions of the growth of A-431 and HT-29 human cell lines (Table 1). In particular *m*-trifluoromethyl benzenesulfonate esters of Type A bearing a carboxylate moiety at C-2 (entries 4–8), 2-naphthylsulfonate esters of Type B (entry 14), and the *m*-trifluoromethyl benzenesulfonate esters with carboxylate and carbamate esters at C-2 and C-3, respectively (Type C, entry 15), exhibited IC_{50} values ranging from 2.0 to 7.5 μM for both cell lines. Ester and ether groups at C-6 were much less active (Table 1, entries 18–22). In an effort to mimic the phosphotyrosine group, we prepared the glycomers shown in entries 16 and 17, but they were found to be inactive, perhaps because of limited cell penetration due to the charged phosphate.

Although the mechanism of action of these compounds is still unknown, it should be noted that the A-431 cells overexpress the EGF receptor whose signal transduction also involves a Grb2–SH2 domain interaction (e.g. refs 9–12). Perhaps of greater interest is the finding that the active glycomers shown in Table 1 are neutral molecules.

The observed activity against the A-431 and H-29 cell lines is exciting since it implies that such glycomers are capable of penetrating the cells. Thus, the prospects of improving antitumor activity by functional group tuning and modification of the glycomer skeleton may prove to be highly beneficial, particularly for the much dreaded human epidermoid carcinomas.

In conclusion, we have devised a new carbohydrate-based scaffold which is amenable to systematic functionalizations using the hydroxyl groups as anchors to provide a series of glycomers. Exploration of a limited subset of hydrophobic functional groups, and allowing for some latitude for H-bonding or polar interactions at some sites, has led to compounds that inhibit A-431 and A-29 human tumor cell lines at levels as low as 2 μM. These encouraging results should pave the way to the design and synthesis of related non-peptidic neutral compounds,[29] with improved antitumor activities, especially utilizing parallel or combinatorial synthesis protocols.[30]

Acknowledgement

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