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General Linker Diversification Approach to Bivalent Ligand Assembly: Generation of an Array of Ligands for the Cation-Independent Mannose 6-Phosphate Receptor

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Abstract

A generalized strategy is presented for the rapid assembly of a set of bivalent ligands with a variety of linking functionalities from a common monomer. Herein, an array of phosphatase-inert mannose-6-phosphonate-presenting ligands for the cation-independent-mannose 6-phosphate receptor (CI-MPR) is constructed. Receptor binding affinity varies with linking functionality—the simple amide and 1,5-triazole(tetrazole) being preferred over the 1,4-triazole. This approach is expected to find application across chemical biology, particularly in glycoscience, wherein multivalency often governs molecular recognition.

Graphical Abstract

There is currently great interest in the chemistry and biology of functionalized phosphonates as mimics of biological phosphates,¹ an area in which we too have longstanding interest,² including in developing unnatural phosphonate-based ligands for the mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R = CI-MPR).³ Evidence suggests that over-expression of the receptor may be associated with prostate cancer⁴ and neurodegenerative disease.⁵ The CI-MPR regulates intracellular trafficking of lysosomal glycoproteins through multiple interactions with M6P-terminated N-glycans, including uPAR, granzyme B, proliferin, and TGF-beta precursor (Figure 1).⁶ This receptor also binds

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ASSOCIATED CONTENT

Supporting Information

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Detailed experimental procedures (synthesis and radio-ligand displacement) and characterization data (¹H, ¹³C, and ³¹P NMR spectra) (PDF)

The authors declare no competing financial interest.

and internalizes the growth factor IGF2.⁷ York et al. have shown that human β -glucuronidase (hGUS) accelerates the CI-MPR-mediated endocytosis of IGF2 by 3- to 4-fold.⁸ This is likely a result of receptor dimerization stabilized by a bivalent contact between hGUS and the receptor.⁹

Indeed, very recent studies by Zavorka et al. demonstrate that artificial glycopeptides bearing pentamannosyl phosphate (PMP) oligosaccharides have a high affinity for the CI-MPR and accelerate receptor-mediated IGF2-uptake and degradation in the lysosome.¹⁰ Further, there are other beautiful examples from Chen,¹¹ Wang,¹² Zhou,¹³ Overkleeft,¹⁴ and Sen Gupta¹⁵ of the construction of well-defined artificial glycoconjugates that bear complex M6P-terminated oligosaccharides to specifically target this receptor. However, as has been pointed out, a major drawback to these approaches lies in the lability of the phosphomonoester bond of M6P to digestive phosphatases.¹⁶ Therefore, it is critical that complementary phosphatase-inert CI-MPR ligands be developed.

Herein, we present a linker diversification strategy for constructing phosphonate-based ligands for the receptor. This approach is generalizable for the assembly of a set of structurally diverse multivalent ligands for lectin-like targets in general, an area of great current interest.¹⁷ Diversification is achieved by exploitation of the azide-terminated carbohydrate monomer. To be sure, copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) has been used in all aspects of chemical biology.¹⁸ However, as Raines has pointed out, this intense focus on 1,4-triazole chemistry is really a missed opportunity.¹⁹ *Herein, we describe the confluence of five chemistries to diversify any modular ligand constructed from an azide-terminated monomer. Of particular note, we highlight the use of a Sharpless-Demko cycloaddition/SNAr sequence²⁰ to provide tetrazole linked ligands, and show that this chemistry is especially conducive to the assembly of a tailored set of PEG-spaced ligands, wherein spacer length is varied.*

Monovalent analogues of M6P have been independently synthesized by the Montero and Berkowitz groups.^{3a,21} Among a handful of surrogates studied, the phosphonate analogue has shown the highest binding affinity. In this case, exploiting our triflate-displacement chemistry, monomer 4 was smoothly assembled (Scheme 1). Pentabutylene glycol (PBG) 5 was selected as a model tether that can be conveniently synthesized and modified (Scheme 2).²²

With both coupling components in hand, we examined a variety of conjugation reactions (Figure 2). The phosphinoaryl esters 9 and 11 afforded two distinct amide linkages from the same type of reaction with azide 4. The modified Staudinger reactions developed by Saxton and Bertozzi utilize aryl carboxylate esters to trap the aza-ylide intermediates; both such reactions proceeded efficiently to give traceless amide 12 or phosphine oxide appended amide 13.²³ This proved to be a more efficient route to the simple amide than Williams ligation here (20%).²⁴ Global debenzoylation of 12 and 13 then gave the active compounds **BL1** and **BL2**. [Note: **BL2** was also used as a model compound to demonstrate phosphatase resistance; see the SI.] CuAAC has been extensively used in ligand assembly.²⁵ Recently, Ru(II)-mediated azide-alkyne cycloaddition (RuAAC) has emerged as an alternative strategy, offering 1,5-triazole linkage.²⁶ Here, both metal-mediated Huisgen cycloadditions

were performed leading to the 1,4-triazole-linked ligand **BL3** and the 1,5-triazole-linked **BL4**.

Finally, we set out to explore an underexploited ligation strategy, featuring azide-TsCN cycloaddition first introduced by Demko and Sharpless, giving 1,5-tetrazoles.²⁰ Over the past decade, it has been used by few groups in chemical biology.²⁷ We examined possible metal catalysis of the cycloaddition at rt.²⁸ A variety of Cu catalysts and ZnCl₂ gave modest yields and regioselectivity (Table 1, entries 1–4). With Fokin's Ru(II) catalyst, no tetrazole was detected (entry 5). On the other hand, under thermal conditions, the functionally dense azide **4** reacts with TsCN efficiently (72%), yielding only the 1-substituted 5-sulfonyl tetrazole **16** (entry 6). The reaction time can be reduced to 1 h under microwave irradiation (entry 7). The glycosyl tetrazole **16** was then displaced by sodium alkoxides of pentabutyl glycol **5** in excellent yield (79% for bidirectional coupling). Standard deprotection then gave the 1,5-tetrazole-linked bivalent ligand **BL5**.

Relative binding affinities (RBAs) of the ligands were determined by displacement assay using radiolabeled PMP-BSA. The influence of the linkages was examined in terms of five features, namely, (i) number of H-bond donors, (ii) number of H-bond acceptors, (iii) MW, (iv) log *P*, and (v) angular trajectory of the overall linkage (Table 2). The data clearly show that the simple amide (**BL1**) binds significantly better than the phosphine oxide bearing amide (**BL2**) to the CI-MPR. Interestingly, the “bent” 1,5-disubstituted triazole-linked **BL4** has a highest mean RBA, and it is clearly a better binder than the ligand bearing the much more common 1,4-triazole-linkage (**BL3**). Notice also that the tetrazole linkage generated in the Sharpless-Demko chemistry is similar in shape to the favored 1,5-triazole (“acute” trajectories in both **BL4** and **BL5**).

Previously, we reported an alkene cross-metathesis approach to carbon chain-tethered bivalent ligands as the first “measuring sticks” for this receptor.²⁹ Due to the hydrophobic nature of those ligands, we sought to develop a complementary approach here that would allow for a more druglike linking functionality. In fact, a combination of microwave-facilitated azide-TsCN cyclo-addition and S_NAr coupling with polyethylene glycol (PEG) alkoxides allows efficient assembly of PEG-spaced bivalent ligand arrays (see the SI). Preliminary assays of these tetrazole-linked ligands, however, show little difference in binding to the receptor. According to Flory radius estimations, the longest spacer here extends to 15 Å, but this fully extended length likely overestimates PEG-tether length in solution.³⁰ From the aforementioned studies of glycopeptide-based CI-MPR ligand development,^{10,12–15} it would appear that to achieve high binding affinity it may be necessary to incorporate additional oligosaccharide recognition elements while retaining the useful mannose 6-phosphonate termini.

This study demonstrates the utility of the Sharpless-Demko cyclocondensation/S_NAr coupling approach to PEG-spaced biomimetic ligand assembly. We have shown that with azide termini, one can rapidly generate diversity at the linking stage, even with functionally dense sugar-phosphonate ligands. This is particularly useful when building libraries with Lipinski's rules in mind (see Table 2),³¹ with variation in H-bond donor/acceptor patterns, hydrophobicity, and overall trajectory, all from a common monomer. This sequence should

find broad application in chemical biology wherein the widespread usage of azide-tagging can presumably be leveraged more fully through the linker diversification approach described herein.

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Figure 1. Structure and function of M6P/IGF2R: (A) domains 1–3, N-terminal M6P-binding site showing bound M6P monomer at repeat 3; (B) domains 11–13 with IGF-II bound; (C) schematic of the entire CIMP2R illustrating the 15 homologous extracellular repeats and dimeric structure. Color coding: black, uPAR binding site; orange, M6P-binding sites; red, M6P-GlcNAc diester binding site; blue, IGF-II binding site; (D) schematic of the intracellular trafficking for CI-MPR-mediated transport of M6P-decorated glycoproteins to the lysosome for sorting or degradation.

Figure 2.
Divergent synthesis of bivalent M6P-mimicking ligands with diversification in the linking chemistry.

Scheme 1.
Synthesis of Azide-Terminated Mannosyl Phosphonate Monomer

Scheme 2.
Synthesis of Pentabutylene Glycol Tethers

Table 1.

Optimization of the Azide Sulfonyl Cyanide 1,3-Dipolar Cycloaddition

entry	catalyst	solvent	temp (°C)	Time (h)	yield (%)	sel ^a
1	Cu ₂ (OTf) ₂ ·C ₆ H ₆	DCM	23	22	31	75/25
2	Cu ₂ (OTf) ₂ ·C ₇ H ₈	DCM	23	22	48	70/30
3	Cu(OTf) ₂	DCM	23	22	13	71/29
4	ZnCl ₂	DCM	23	22	15	93/7
5	Cp*(COD) RuCl	DCM	23	48	0	NA
6	none	none	95	20	72	>99/1
7	none	none	95 ^b	1	70	>99/1

^a Selectivity of the cycloaddition reaction with ratios of 1,5-tetrazole over 1,4-tetrazole.^b This reaction temperature was achieved by microwave irradiation.

Table 2.

Molecular Features and RBAs of Bivalent Ligands

ligand	BL1	BL2	BL3	BL4	BL5
linkage ^a
functional group	amide	amide/ & triphenyl phosphine oxide	1,4-triazole	1,5-triazole	1,5-tetrazole
RBA ^b	2.1±0.78	0.67±0.24	1.3± 1.0	3.9±2.4	2.4±1.1
<i>P</i> values ^c	0.047	0.008	0.529	0.065	0.251
H-bond donor	1	1	0	0	0
H-bond acceptor	1	2	2	2	3
M.W.	43	319	67	67	68
logP	-0.72	2.38	0.58	0.58	1.62
trajectory	linear	obtuse	obtuse	acute	acute

^aRBA (Relative binding affinity) and *P*values are for the ligands themselves; all other values in the table are for the linking functionalities only.

^bRBA = (IC50(M6P))/(IC50(bivalent ligand)).

^cTests of significance were done by Student's *t* test (**BL2** vs M6P) or by oneway ANOVA with Dunnett's posthoc test (**BL1** and **BL3** vs M6P; **BL4** and **BL5** vs M6P). Differences were considered significant at *P* < 0.05. Thus, **BL1** and **BL2** are significantly different from M6P, while **BL4** and **BL5** are trending in that direction.