Published in final edited form as:
J Am Chem Soc. 2009 October 14; 131(40): 14337–14344. doi:10.1021/ja9052625.

A New Model for the Presentation of Tumor-Associated Antigens and the Quest for an Anticancer Vaccine: A Solution to the Synthesis Challenge via RCM

Insik Jeon[1], **Dongjoo Lee**[1], **Isaac J. Krauss**[1], and **Samuel J. Danishefsky**[1,2,*]

[1]Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065

[2]Department of Chemistry, Columbia University, Havemeyer Hall, 3000 Broadway, New York, NY 10027

Abstract

Fully synthetic, carbohydrate-based antitumor vaccine candidates have been synthesized in highly clustered modes. Multiple copies of tumor-associated carbohydrate antigens, Tn and STn, were assembled on a single cyclic peptide scaffold in a highly convergent manner. Ring-closing metathesis-mediated incorporation of an internal cross-linker was also demonstrated. In particular, this rigidified cross-linked construct would enhance a cluster-recognizing antibody response by retaining an appropriate distance between glycans attached to the peptide platform. Details of the design and synthesis of highly clustered antigens are described herein.

Introduction

Our laboratory is seeking the development of novel, fully synthetic carbohydrate-based vaccines for the treatment of cancer. This research program is based on the finding that malignantly transformed cells often exhibit significant alteration in the nature and quantity of carbohydrates presented on their cell surfaces, either as glycosphingolipids or as glycoproteins.[1] Presumably, when properly introduced to the immune system, a tumor-associated carbohydrate-based antigen could set into motion an exploitable immune response, leading to the generation of antibodies which would selectively bind to and hopefully eliminate those tumor cells which over-express the carbohydrates in question. We are certainly not alone in this area. In particular, impressive advances of carbohydrate based anticancer vaccines have been reported by Boons,[2] Kunz,[3] and Schmidt.[4] The viability of this carbohydrate vaccine concept has been confirmed experimentally in our laboratory. Thus, when these tumor-associated antigens are presented to the immune system as glycoconjugates appended to immunogenic carrier molecules (such as KLH carrier protein)[5] and co-administered with an immunological adjuvant (such as QS21),[6] a carbohydrate-specific antibody response may be induced. A number of complex, fully synthetic carbohydrate-based constructs, synthesized in our laboratories, have shown significant promise in preclinical, and even clinical settings.[7] In designing a carbohydrate-based vaccine construct, it may be of considerable value to consider the way in which the antigen is displayed in its natural environment, i.e. on the surface of the transformed cell, to more directly mimic this natural setting in the context of the vaccine.

s-danishefsky@ski.mskcc.org.

Supporting Information. Experimental procedures and characterization data (PDF). This material is available free of charge *via* the Internet at http://pubs.acs.org

Along these lines, we have taken note of the mucin-bound carbohydrate antigens, Tn and STn, which are over-expressed on the surfaces of a variety of epithelial cancers, such as prostate, breast, colon, and ovarian.[8] On the tumor cell surface, Tn and STn are presented in broadly conserved "clusters" of 2–4 carbohydrate units, O-linked to the mucin peptide through serine or threonine residues.

Approaches using monomeric Tn or STn antigen, in which one glycan unit is covalently appended to an immunogenic carrier protein, have proven beneficial.[9] However, it has been shown that clustered vaccines – wherein multiple copies of the carbohydrate are incorporated on a peptide backbone (Figure 1) –induce higher titers against some carbohydrate epitopes.[10a] Indeed, recent studies with the antitumor monoclonal antibody (MAb) B72.3 revealed that it preferentially recognized clustered STn in preference to monovalent STn.[9a,10b–c]

To some extent, the lowered entropic penalty associated with the increase in valency seems to enhance carbohydrate-protein interactions. However, the choice of template for multivalent carbohydrate display may be crucial. Excessively flexible scaffolds will permit attached glycans to remain far apart in most conformations thereby perhaps decreasing effective clustering of the antigen. In considering a template for the presentation of the clustered carbohydrates, we have been attracted to the model, depicted in Figure 2, upon which the clustered glycans would be displayed in a well-defined orientation. Our design, clearly inspired by Dumy[11] and Robinson[12] is amenable to variations in the number and type of carbohydrates displayed, as well as the spacing of the carbohydrate domains. Moreover, the promise of such templates is evidenced by recent studies demonstrating their use for clustered antigen syntheses in our lab and elsewhere.[13,14] In fact, we have recently employed this scaffold in the context of a separate program, directed toward the development of an HIV vaccine.[15]

The essential element of our cyclic peptide design is a pair of β-turn-inducing D-Pro-L-Pro sequences[16] at both ends of the macrocycle. Positions A–F (red, with side chain projecting "above" the plane of the scaffold) may contain handles for glycan attachment, whereas position G is a cysteine residue (blue, with side chain projecting from the "bottom" face of the scaffold), suitable for linkage to a carrier protein or biological marker. This scaffold is tunable in that differential placement of aspartate residues in positions A–F permits variation in the number and spacing of the glycan attachments (**1**, Figure 2).

In an effort to identify an optimal presentation of the carbohydrates on the cyclic peptide scaffold, we chose as our targets the four constructs shown in Figure 3. Structures **4** and **5** incorporate six and four replicate copies of the Tn antigen, respectively, and construct **6** presents four copies of the STn disaccharide. We also sought to prepare a multiantigenic construct, **7**, incorporating both the Tn and STn antigens.

Protected O-linked glycosylamino acids **2** and **3** (Figure 3) were prepared from the L-hydroxynorleucine benzyl ester, according to our previously established protocol.[17] These Tn and STn "cassettes," which we originally employed in earlier approaches to the synthesis of clustered antigens, serve as useful building blocks for glycal assembly. In this system, the N-termini of both the Tn and STn cassettes serve as handles for coupling to the peptide scaffold, and the remaining carboxyl function may provide a handle for further elaboration (i.e., as T-helper or additional B-epitope attachments).

Cyclic peptides **9**, **10**, and **11** (containing 4 or 6 aspartate residues) were prepared in parallel through automated solid-phase synthesis from the prolinated trityl resin **8** (Scheme 1). Cleavage from the resin, macrocyclization,[18] and deprotection of the *tert* butyl esters of the aspartate and tyrosine residues furnished **1**, **12**, and **13** in good overall yields.

With the components of the target structures in hand, we directed our efforts toward the covalent attachment of the carbohydrate antigens to the scaffolds. The Lansbury aspartylation reaction[19] is often employed in glycopeptide synthesis. However, the standard Lansbury aspartylation protocol employs a glycosidic amine as the coupling partner of activated aspartic acid, rather than the primary amino acid nitrogen which would be required by our strategy. In considering the application of this protocol to our own system, there was concern that altered nucleophilicity of the amine might favor in the emergence of nonproductive pathways. For example, the competing, relatively facile cyclization of the peptide itself might lead to the formation of aspartimide.[20] Indeed, this side reaction was found to be a problem in the coupling of peptide **12** with glycosylamino acid **2**. Under standard conditions (HOAt, HATU, DIEA/DMSO), formation of the undesired aspartimide was predominant, with little indication of the requisite hexavalent product, **14**. Upon close investigation, it was found that the HOAt, which is generally used as an activation additive, along with HATU, plays a significant role in promoting undesired aspartimide formation. Fortunately, activation of the aspartic acids with HATU alone in the presence of DIEA in DMSO effectively minimized aspartimide formation, and consequently allowed high yielding conversion to the desired hexavalent product, **14** (Scheme 2).

Having identified workable coupling conditions, we were able to assemble the tetravalent constructs **15** and **16** in a similar fashion. These less congested glycopeptides exhibit different spatial arrangements, yet they still express the epitopes in highly clustered fashions. Following global deprotection, the highly clustered antigens **4**, **5**, and **6** were in hand.

We next turned to the synthesis of the unimolecular multiantigenic construct, **7**,[21] wherein both the Tn and STn carbohydrate antigens are displayed on the peptidic backbone. This type of multivalent construct is intended to reflect the degree of carbohydrate heterogeneity associated with most cancers.[22] There is significant variation in the types of carbohydrates which are over-expressed on the tumor cell surface, even within a particular cancer type. By combining clusters of both the Tn and STn antigens within a single cyclic peptide scaffold, we would hope to induce a more effective immune response, in which the antibodies raised would target a greater proportion of transformed tumor cells. It is not unlikely that careful strategic considerations in the peptide scaffold design could well influence the effectiveness of a multiantigenic construct.

Initial efforts to reach cyclic peptide **17** were unsuccessful, due to complications arising from aspartimide formation at position 8, leading to the undesired product, **17a** (Scheme 3). Unlike the bulky Asp-β-*tert*-butyl esters, the less hindered Asp-β-allyl ester is susceptible to undesired aspartimide formation, arising from intramolecular nucleophilic attack by the amide nitrogen at the aspartyl C-terminus.[23] However, aspartimide formation of the Asp-β-allyl ester at position 5 was impeded by the presence of proline in position 6. Thus, we initiated a design decision to transpose the -β-allyl and -β-*tert*-butyl protecting groups of positions 8 and 12. Gratifyingly, no evidence of aspartimide formation was found in the synthesis of cyclic peptide **18**.

Following selective *tert*-butyl deprotection of **18** under the conditions described in Scheme 1, intermediate **13** was in hand. We then undertook the sequential attachment of the Tn and STn carbohydrate antigens. In the event, coupling of Tn **2** with peptide **13** proceeded efficiently to produce the Tn glycopeptide construct, as shown in Scheme 4. The liberation of additional reaction sites by palladium-mediated allyl deprotection[24] resulted in a high yield of divalent product **20**. Addition of STn **3** to activated **20** and subsequent global deacetylation completed the synthesis of tetravalent antigen **7**.

An intriguing question concerning the clustered antigen design could be the potential flexibility of linkers which attach the glycans to the cyclic peptide scaffold. Cross-linking elements

between carbohydrates would rigidify the construct and prevent undesired spreading of glycan units. This should better mimic a tightly-clustered antigen and enhance the cluster-recognizing antibody response. Ring closing metathesis (RCM) has proven to be a powerful tool for the formation of peptide macrocycles in numerous settings.[25] In particular, fascinating works for the synthesis of multivalent glycoconjugates by Kiessling[26] have further encouraged us to explore the ruthenium-catalyzed metathesis for the incorporation of cross-linker in our system, which would lead to highly clustered cross-linked constructs. Our initial attempted RCM of di-Tn precursor **23** employing Grubbs catalyst (**24**) proceeded to generate spacer-linked construct **27** in modest yield (Scheme 5). Variation of the reaction conditions, including higher catalyst loading, was unable to induce complete conversion of **23**. Additionally, mass spectra of isolated RCM products indicated the existence of inseparable contaminants in which a methylene group has been deleted,[27(a)] presumably due to the ruthenium complex-mediated isomerization[27] of the terminal olefin of **23** (Figure 4, top) prior to ring closure. Although a significant increase in yield was observed when the Hoveyda-Grubbs II catalyst (**25**) [28] was employed, terminal olefin isomerization could not be suppressed. Gratifyingly, no isomerization of **23** occurred with the use of Hoveyda-Grubbs I catalyst (**26**) [29], which afforded the desired RCM product **27** in good yield (Scheme 5 and Figure 4 (bottom)). The product olefins, a mixture of *E*- and *Z*- isomers, were then hydrogenated to furnish **28**. The protected aspartyl residues of **28** may serve as additional handles for further modification of the construct; i.e. attachment of multiple different antigens, to appropriately reflect the heterogeneity of target cancers.

Conclusion

In summary, we have described the design and synthesis of multivalent anticancer vaccine constructs. The individual carbohydrate-based antigens were prepared in well-defined and highly clustered modes by convergent synthesis. In addition, the successful introduction of a cross-linker provides a wider scope of future modifications in epitope design either by spacer length variations or additional glycan attachments on the scaffolds. Upon conjugation to KLH carrier protein, the anticipated higher molecular ratio of Tn or STn versus KLH could offer an additional benefit in terms of immunogenicity. We expect that these highly clustered anticancer constructs will help in gaining further insight into the way in which carbohydrate clusters are recognized by the immune system. Conjugation to KLH carrier protein and immunological investigations will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by a grant from the National Institutes of Health (CA28824 to SJD). DL is grateful for support from the Terri Brodeur Breast Cancer Foundation.

Reference

1. (a) Danishefsky SJ, Allen JR. Angew. Chem., Int. Ed 2000;39:836–863. (b) Livingston PO, Zhang S. Cancer Immunol. Immunother 1997;45:1–9. [PubMed: 9353421] (c) Livingston PO, Ragupathi G. Cancer Immunol. Immunother 1997;45:10–19. [PubMed: 9353422] (d) Toyokuni T, Singhal AK. Chem. Soc. Rev 1995:231–242. (e) Hakomori SI. Advances in Exp. Med. Biol 2001;491:369–402. [PubMed: 14533809]
2. (a) Ingale S, Wolfert MA, Gaekwad J, Buskas T, Boons G-J. Nat. Chem. Biol 2007;3:663–667. [PubMed: 17767155] (b) Buskas T, Ingale S, Boons G-J. Angew. Chem., Int. Ed 2005;44:5985–5988.

3. (a) Kunz H, Dziadek S, Wittrock S, Becker T. ACS Symp. Ser 2008;989:293–310. (b) Westerlind U, Hobel A, Gaidzik N, Schmitt E, Kunz H. Angew. Chem., Int. Ed 2008;47:7551–7556. (c) Wittrock S, Becker T, Kunz H. Angew. Chem., Int. Ed 2007;46:5226–5230. (d) Dziadek S, Brocke C, Kunz H. Chem. Eur. J 2004;10:4150–4162. (e) Sprengard U, Schudok M, Schmidt W, Kretzschmar G, Kunz H. Angew. Chem., Int. Ed 1996;35:321–324.

4. (a) Hermans IF, Silk JD, Gileadi U, Salio M, Mathew B, Ritter G, Schmidt R, Harris Adrian L, Old L, Cerundolo V. J. Immunol 2003;171:5140–5147. [PubMed: 14607913] (b) Schmidt RR, Castro-Palomino JC, Retz O. Pure Appl. Chem 1999;71:729–744.

5. (a) Helling F, Shang A, Calves M, Zhang SL, Ren SL, Yu RK, Oettgen HF, Livingston PO. Cancer Res 1994;54:197–203. [PubMed: 8261439] (b) Helling F, Zhang S, Shang A, Adluri S, Calves M, Koganty R, Longenecker BM, Yao TJ, Oettgen HF, Livingston PO. Cancer Res 1995;55:2783–2788. [PubMed: 7796403]

6. (a) Kensil CR, Patel U, Lennick M, Marciani D. J. Immunol 1991;146:431–437. [PubMed: 1987271] (b) Marciani DJ, Press JB, Reynolds RC, Pathak AK, Pathak VL, Gundy E, Farmer JT, Koratich MS, May RD. Vaccine 2000;18:3141–3151. [PubMed: 10856794] (c) Kim S-K, Ragupathi G, Musselli C, Choi SJ, Park YS, Livingston PO. Vaccine 1999;18:597–603. [PubMed: 10547417] (d) Kim S-K, Ragupathi G, Cappello S, Kagan EP, Livingston O. Vaccine 2000;19:530–537. [PubMed: 11027818]

7. (a) Ragupathi G, Park TK, Zhang SL, Kim IJ, Graber L, Adluri S, Lloyd KO, Danishefsky SJ, Livingston PO. Angew. Chem., Int. Ed 1997;36:125–128. (b) Slovin SF, Ragupathi G, Adluri S, Ungers G, Terry K, Kim S, Spassova M, Bornmann WG, Fazzari M, Dantis L, Olkiewicz K, Lloyd KO, Livingston PO, Danishefsky SJ, Scher HI. Proc. Natl. Acad. Sci. U.S.A 1999;96:5710–5715. [PubMed: 10318949] (c) Gilewski T, Ragupathi G, Bhuta S, Williams LJ, Musselli C, Zhang XF, Bencsath KP, Panageas KS, Chin J, Hudis CA, Norton L, Houghton AN, Livingston PO, Danishefsky S. J. Proc. Natl. Acad. Sci. U.S.A 2001;98:3270–3275. (d) Krug LM, Ragupathi G, Hood C, Kris MG, Miller VA, Allen JR, Keding SJ, Danishefsky SJ, Gomez J, Tyson L, Pizzo B, Baez V, Livingston PO. Clin. Cancer Res 2004;10:6094–6100. [PubMed: 15447995] (e) Sabbatini PJ, Kudryashov V, Ragupathi G, Danishefsky SJ, Livingston PO, Bornmann W, Spassova M, Zatorski A, Spriggs D, Aghajanian C, Soignet S, Peyton M, O'Flaherty C, Curtin J, Lloyd KO. Int. J. Cancer 2000;87:79–85. [PubMed: 10861456]

8. (a) Springer GF. Science 1984;224:1198–1206. [PubMed: 6729450] (b) Itzkowitz SH, Yuan M, Montgomery CK, Kjeldsen T, Takahashi HK, Bigbee WL, Kim YS. Cancer Res 1989;49:197–204. [PubMed: 2908846] (c) Kim YS, Gum J, Brockhausen I. Glyconjugate J 1996;13:693–707. (d) Springer GF. J. Mol. Med 1997;75:594–602. [PubMed: 9297627] (e) Springer GF. Crit. Rev. Oncogenesis 1995;6:57–85. [PubMed: 8573608]

9. (a) Adluri S, Helling F, Ogata S, Zhang S, Itzkowitz SH, Lloyd KO, Livingston PO. Cancer Immunol. Immunother 1995;41:185–192. [PubMed: 7553688] (b) MacLean GD, Reddish M, Koganty RR, Wong T, Gandhi S, Smolenski M, Samuel J, Nabholtz JM, Longenecker BM. Cancer Immunol. Immunother 1993;36:215–222. [PubMed: 8439984] (c) MacLean GD, Reddish MA, Bowen-Yacyshyn MB, Poppema S, Longenecker BM. Cancer Invest 1994;12:46–56. [PubMed: 7506634] (d) MacLean GD, Miles DW, Rubens RD, Reddish MA, Longenecker BM. J. Immunother 1996;19:309–316. (e) MacLean GD, Reddish MA, Koganty RR, Longenecker BM. J. Immunother 1996;19:59–68. (f) O'Boyle KP, Markowitz AL, Khorshidi M, Lalezari P, Longenecker BM, Lloyd KO, Welt S, Wright KE. Hybridoma 1996;15:401–408. [PubMed: 8985750]

10. (a) Kudryashov V, Glunz PW, Williams LJ, Hintermann S, Danishefsky SJ, Lloyd KO. Proc. Natl. Acad. Sci. U.S.A 2001;98:3264–3269. [PubMed: 11248067] (b) Slovin SF, Ragupathi G, Musselli C, Olkiewicz K, Verbel D, Kuduk SD, Schwarz JB, Sames D, Danishefsky SJ, Livingston PO, Scher HI. J. Clin. Oncol 2003;21:4292–4298. [PubMed: 14645418] (c) Zhang S, Walberg LA, Ogata S, Itzkowitz SH, Koganty RR, Reddish M, Gandhi SS, Longenecker BM, Lloyd KO, Livingston PO. Cancer Res 1995;55:3364–3368. [PubMed: 7614472]

11. (a) Dumy P, Eggleston IM, Cervigni S, Sila U, Sun X, Mutter M. Tetrahedron Lett 1995;36:1225–1258. (b) Dumy P, Renaudet O. Org. Lett 2003;5:243–246. [PubMed: 12556162] (c) Singh Y, Dolphin GT, Razkin J, Dumy P. ChemBioChem 2006;7:1298–1314. [PubMed: 16892470]

12. (a)Jiang L, Moehle K, Dhanapal B, Obrecht D, Robinson JA. Helv. Chim. Acta 2000;83:3097–3112.3112 (b)Favre M, Moehle K, Jiang J, Pfeiffer B, Robinson JA. J. Am. Chem. Soc 1999;121:2679–2685.2685 For a review, see (c)Robinson JA. Synlett 2000:429–441.441

13. Grigalevicius S, Chierici S, Renaudet O, Lo-Man R, Deriaud E, Leclerc C, Dumy P. Bioconjugate Chem 2005;16:1149–1159.
14. Wang J, Li H, Zou G, Wang LX. Org. Biomol. Chem 2007;5:1529–1540. [PubMed: 17571181]
15. (a) Krauss IJ, Joyce JG, Finnefrock AC, Song HC, Dudkin VY, Geng XJ, Warren D, Chastain M, Shiver JW, Danishefsky SJ. J. Am. Chem. Soc 2007;129:11042–11044. [PubMed: 17711286] (b) Joyce JG, Krauss IJ, Song HC, Opalka DW, Grimm KM, Nahas DD, Esser MT, Hrin R, Feng M, Dudkin VY, Chastain M, Shiver JW, Danishefsky SJ. Proc. Natl. Acad. Sci. USA 2008;105:15684–15689. [PubMed: 18838688]
16. Bean JW, Kopple KD, Peishoff CE. J. Am. Chem. Soc 1992;114:5328–5334.
17. Keding SJ, Endo A, Danishefsky SJ. Tetrahedron 2003;59:7023–7031.
18. The macrocyclization was essentially instantaneous at room temperature, indicating that cross-strand hydrogen bonding may preorganize the acyclic peptide for cyclization. Moreover, downfield ^{1}H NMR chemical shifts of selected N**H** resonances, slow D_2O exchange times, and the temperature profile of these chemical shifts provided preliminary evidence of the desired β-sheet character of the peptide scaffold.
19. Cohen-Anisfeld ST, Lansbury PT. J. Am. Chem. Soc 1993;115:10531–10537.
20. (a) Bodanszky M, Natarajan S. J. Org. Chem 1975;40:2495–2499. [PubMed: 1165511] (b) Bodanszky M, Kwei JZ. Int. J. Pept. Protein Res 1978;12:69–74. [PubMed: 711372] (c) Tam JP, Riemen MW, Merrifield RB. Pept. Res 1988;1:6–18. [PubMed: 2980781] (d) Mergler M, Dick F, Sax B, Weiler P, Vorherr T. J. Peptide Sci 2003;9:36–46. [PubMed: 12587881] (e) Lauer JL, Fields CG, Fields GB. Lett. Peptide Sci 1994;1:197–205.
21. (a) Ragupathi G, Koide F, Livingston PO, Cho YS, Endo A, Wan Q, Spassova MK, Keding SJ, Allen J, Ouerfelli O, Wilson RM, Danishefsky SJ. J. Am. Chem. Soc 2006;128:2715–2725. [PubMed: 16492059] (b) Allen JR, Harris CR, Danishefsky SJ. J. Am. Chem. Soc 2001;123:1890–1897. [PubMed: 11456809] (c) Ragupathi G, Coltart DM, Williams LJ, Koide F, Kagan E, Allen J, Harris C, Glunz PW, Livingston PO, Danishefsky SJ. Proc. Natl. Acad. Sci. U.S.A 2002;99:13699–13704. [PubMed: 12359877] (b) Keding SJ, Danishefsky SJ. Proc. Natl. Acad. Sci. U.S.A 2004;101:11937–11942. [PubMed: 15280546]
22. (a) Zhang SL, Cordon-Cardo C, Zhang HS, Reuter VE, Adluri S, Hamilton WB, Lloyd KO, Livingston PO. Int. J. Cancer 1997;73:42–49. [PubMed: 9334808] (b) Zhang SL, Zhang HS, Cordon-Cardo C, Reuter VE, Singhal AK, Lloyd KO, Livingston PO. Int. J. Cancer 1997;73:50–56. [PubMed: 9334809]
23. Mergler M, Dick F, Sax B, Weiler P, Vorherr T. Peptide Sci 2003;9:36–46.
24. Kunz H, Waldmann H. Angew. Chem., Int. Ed 1984;23:71–72.
25. (a) Blackwell HE, Sadowsky Jack D, Howard RJ, Sampson JN, Chao JA, Steinmetz WE, O'Leary DJ, Grubbs RH. J. Org. Chem 2001;66:5291–5302. [PubMed: 11485448] (b) Miller SJ, Blackwell HE, Grubbs RH. J. Am. Chem. Soc 1996;118:9606–9614. (c) Blackwell HE, Grubbs RH. Angew. Chem. Int. Ed 1998;37:3281–3284. (d) Schmiedeberg N, Kessler H. Org. Lett 2002;4:59–62. [PubMed: 11772090] (e) Kazmaier U, Maier S. Org. Lett 1999;1:1763–1766. (f) Jarvo ER, Copeland GT, Papaioannou N, Bonitatebus PJ Jr, Miller SJ. J. Am. Chem. Soc 1999;121:11638–11643.
26. (a) Yang Z-Q, Puffer EB, Pontrello JK, Kiessling LL. Carbohydr. Res 2002;337:1605–1613. [PubMed: 12423961] (b) Gestwicki JE, Cairo CW, Strong LE, Oetjen KA, Kiessling LL. J. Am. Chem. Soc 2002;124:14922–14933. [PubMed: 12475334] (c) Strong LE, Kiessling LL. J. Am. Chem. Soc 1999;121:6193–6196. (d) Kanai M, Mortell KH, Kiessling LL. J. Am. Chem. Soc 1997;119:9931–9932. (e) Schuster MC, Mortell KH, Hegeman AD, Kiessling LL. J. Mol. Catal. A: Chem 1997;116:209–216. (f) Mortell KH, Weatherman RV, Kiessling LL. J. Am. Chem. Soc 1996;118:2297–2298. (g) Mortell KH, Gingras M, Kiessling LL. J. Am. Chem. SOC 1994;116:12053–12054.
27. (a) Wan Q, Cho YS, Lambert TH, Danishefsky SJ. J. Carbohydr. Chem 2005;24:425–440. (b) Hu Y-J, Dominique R, Das SK, Roy R. Can. J. Chem 2000;78:838–845. (c) Wipf P, Rector SR, Takahashi H. J. Am. Chem. Soc 2002;124:14848–14849. [PubMed: 12475317] (d) Cadot C, Dalko PI, Cossy J. Tetrahedron Lett 2002;43:1839–1841.
28. Garber SB, Kingsbury JS, Gray BL, Hoveyda AH. J. Am. Chem. Soc 2000;122:8168–8179.
29. Kingsbury JS, Harrity JPA, Bonitatebus PJ Jr, Hoveyda AH. J. Am. Chem. Soc 1999;121:791–799.

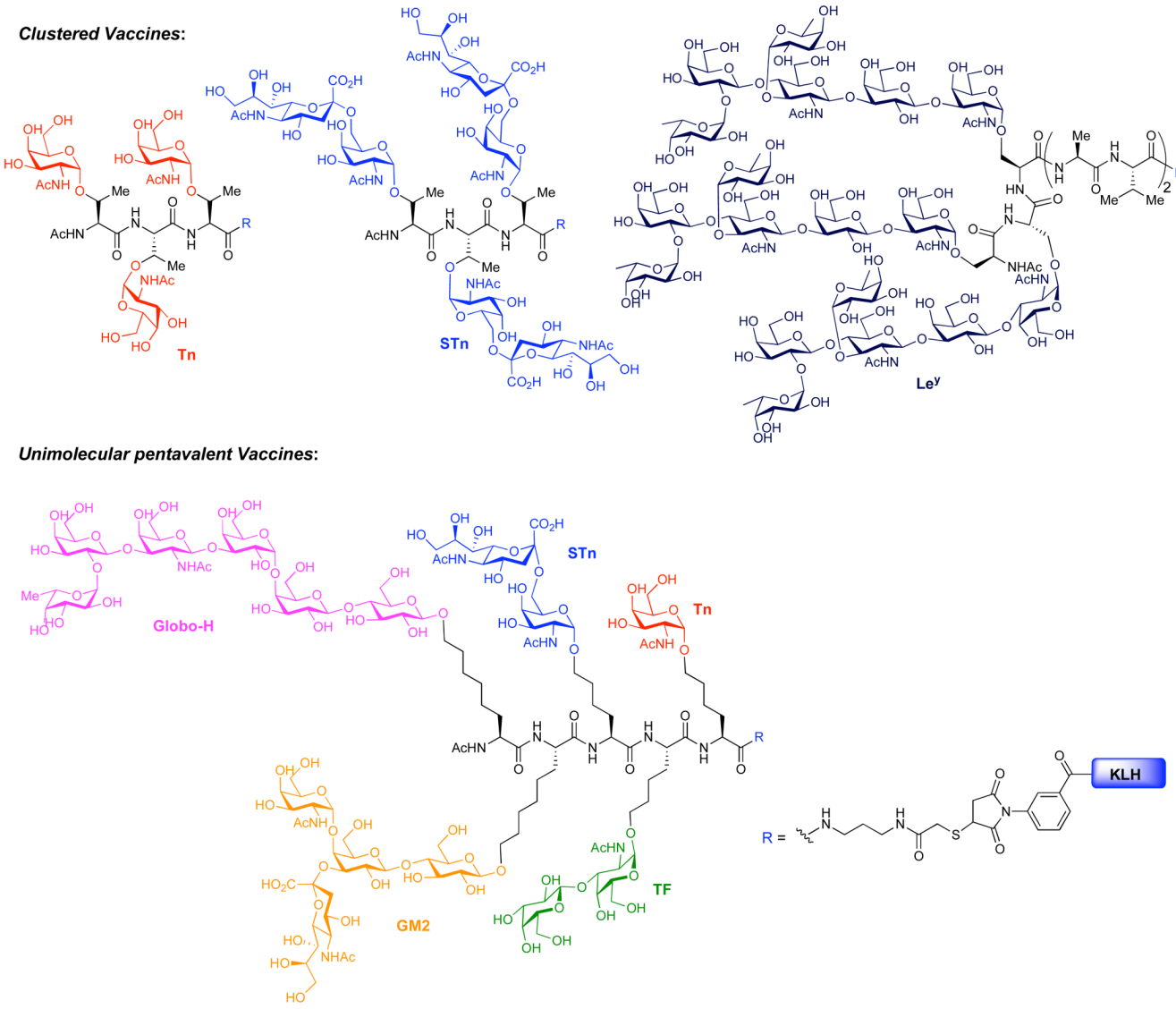


Figure 1.
Representative anticancer vaccine constructs.

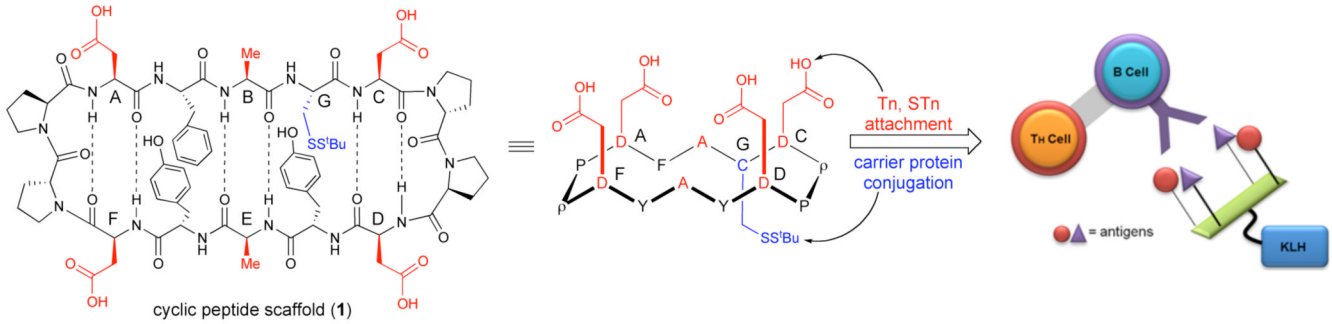


Figure 2.
Cyclic peptide scaffold **1** and antibody response to multivalent-KLH conjugate. TH Cell = T helper cell.

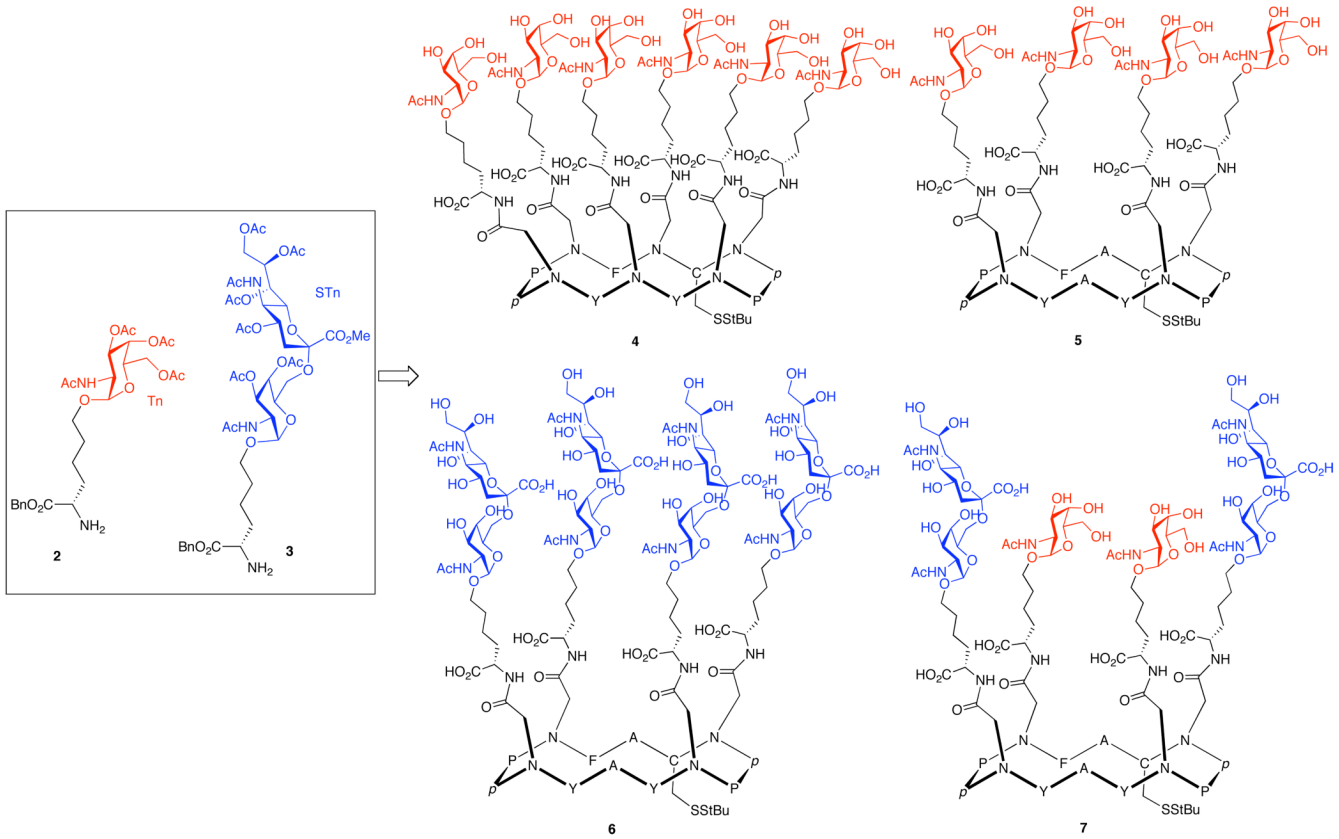


Figure 3.
Protected glycosylamino acids and clustered antigens.

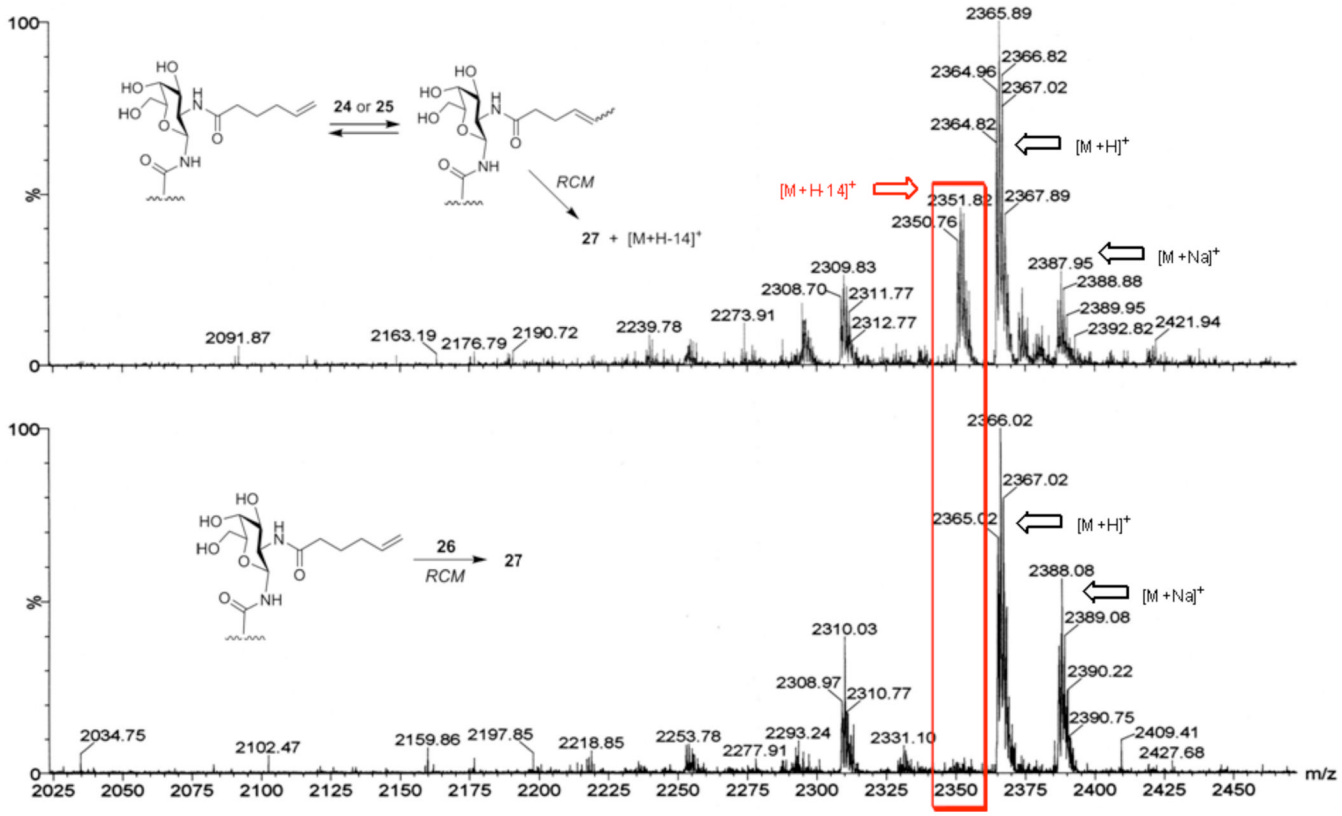
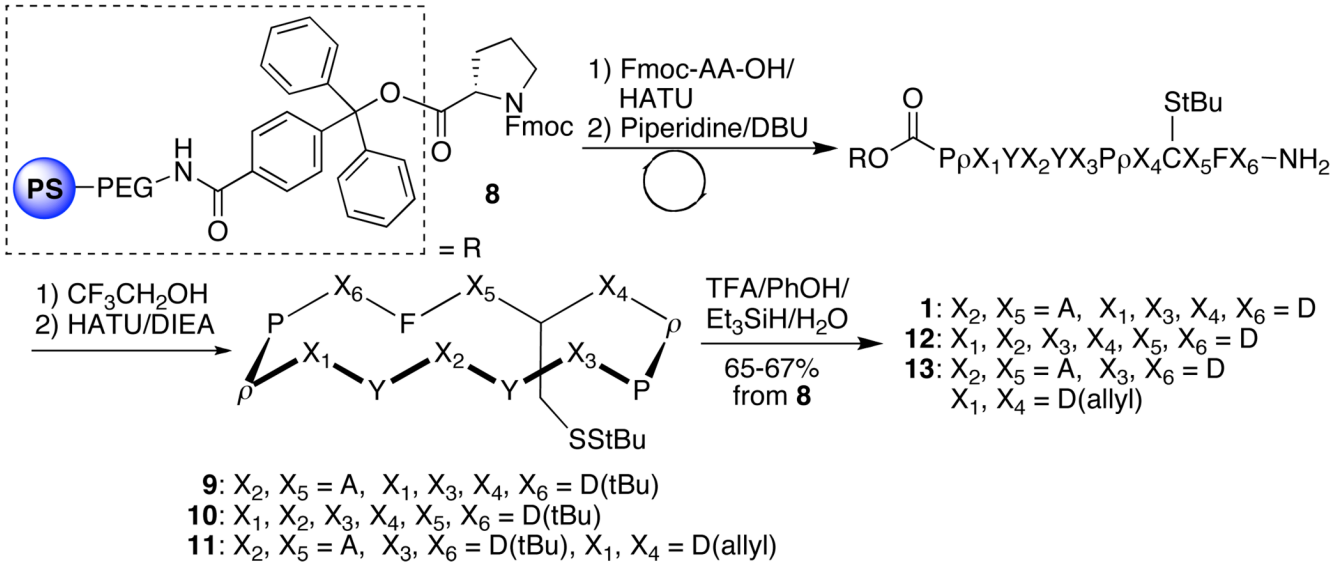
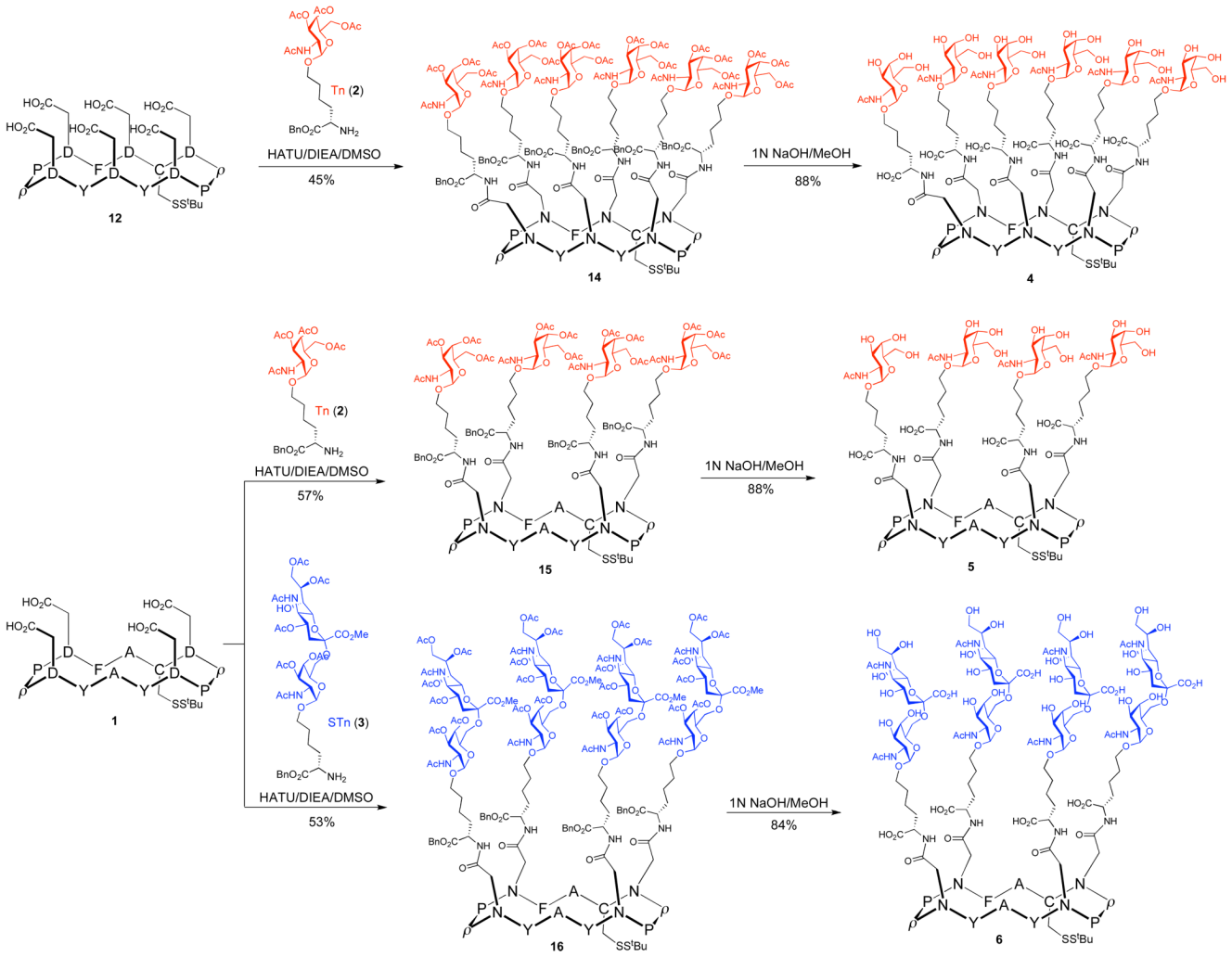


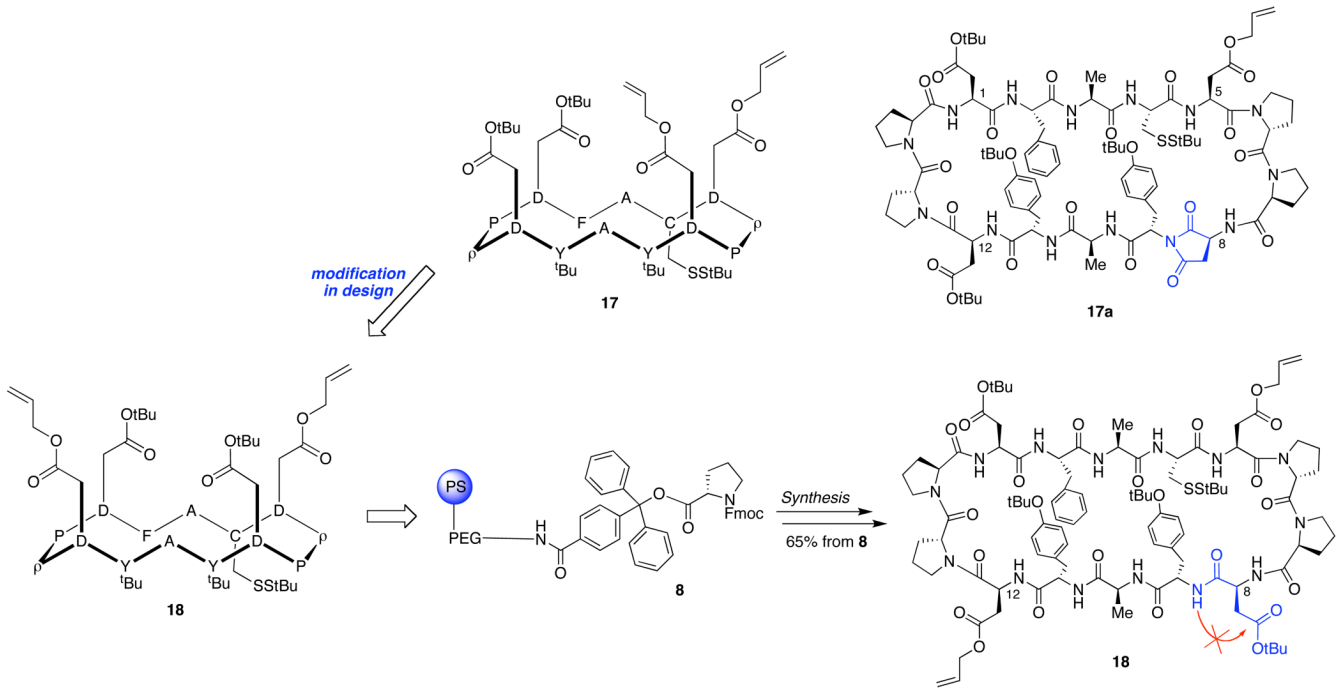
Figure 4.
Low-resolution mass spectrums of RCM products.



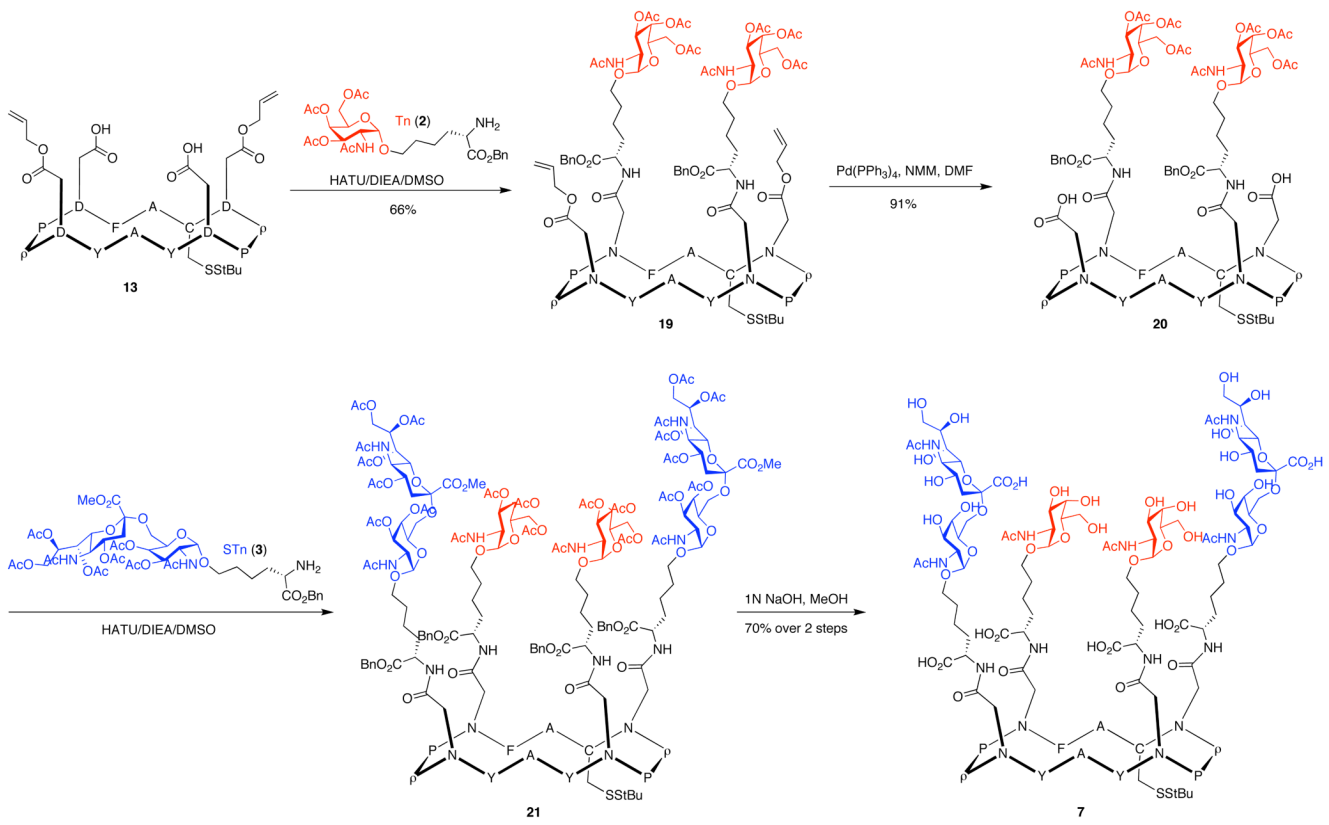
Scheme 1.
Synthesis of cyclic peptide scaffolds.



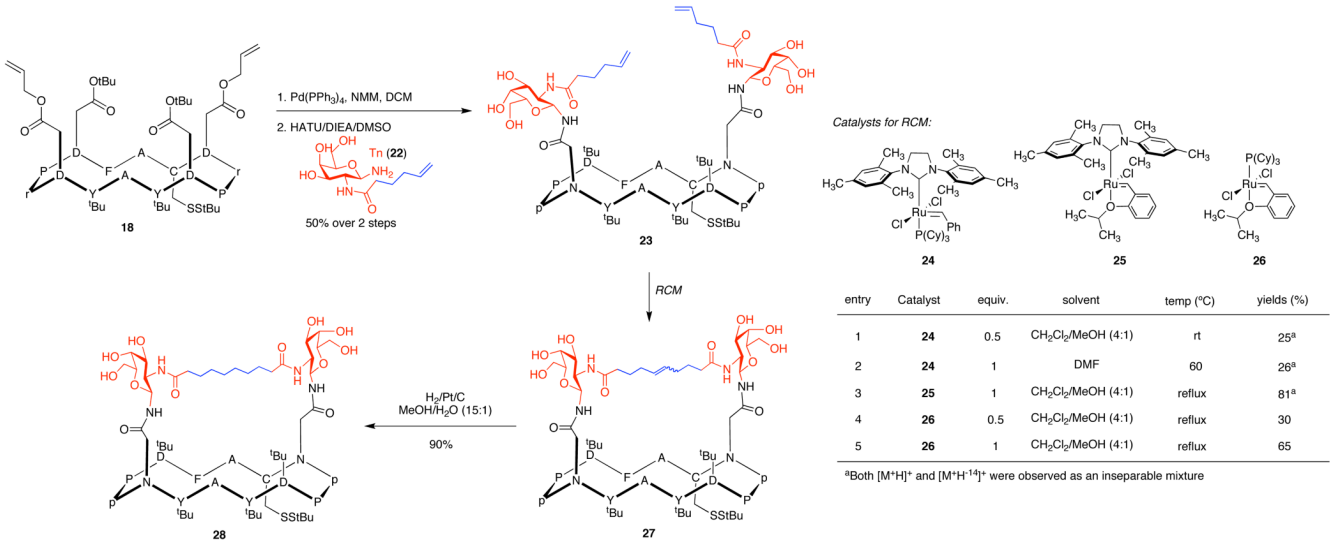
Scheme 2.
Syntheses of clustered glycopeptides.



Scheme 3.
Cyclic peptide scaffold design for multi-antigen attachments.



Scheme 4.
Synthesis of unimolecular multiantigenic glycopeptide **7**.



entry	Catalyst	equiv.	solvent	temp (°C)	yields (%)
1	**24**	0.5	CH_2Cl_2/MeOH (4:1)	rt	25[a]
2	**24**	1	DMF	60	26[a]
3	**25**	1	CH_2Cl_2/MeOH (4:1)	reflux	81[a]
4	**26**	0.5	CH_2Cl_2/MeOH (4:1)	reflux	30
5	**26**	1	CH_2Cl_2/MeOH (4:1)	reflux	65

[a]Both $[M^+H]^+$ and $[M^+H^{-14}]^+$ were observed as an inseparable mixture

Scheme 5.
Synthesis of cross-linked glycopeptide **28**.