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Chemically Programmed Antibodies As HIV-1 Attachment Inhibitors

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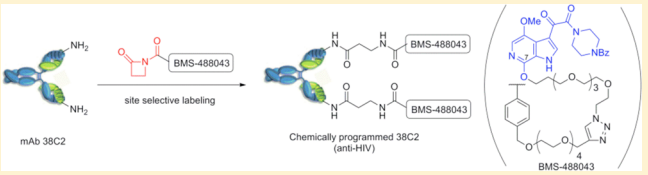
Supporting Information

ABSTRACT: Herein, we describe the design and application of two small-molecule anti-HIV compounds for the creation of chemically programmed antibodies. *N*-Acyl-*β*-lactam derivatives of two previously described molecules BMS-378806 and BMS-488043 that inhibit the interaction between HIV-1 gp120 and T-cells were synthesized and used to program the binding activity of aldolase antibody 38C2. Discovery of a successful linkage site to BMS-488043 allowed for the synthesis of chemically programmed antibodies with affinity for HIV-1 gp120 and potent HIV-1 neutralization activity. Derivation of a successful conjugation strategy for this family of HIV-1 entry inhibitors enables its application in chemically programmed antibodies and vaccines and may facilitate the development of novel bispecific antibodies and topical microbicides.



KEYWORDS: *Bioconjugation, anti-HIV agent, chemically programmed antibody, microbicide, entry inhibitor*

The retrovirus HIV-1, which causes acquired immune deficiency syndrome (AIDS), has infected 34 million people worldwide, and this number is expected to increase by 2.5 million each year into the near future.[1] Although the combination reverse transcriptase inhibitor/protease inhibitor treatment known as HAART has proven successful,[2,3] side effects and viral escape are significant issues, and new treatments are needed. The viral envelope protein gp120, the primary target for antibody mediated viral neutralization, is an emerging target for small molecule treatment of HIV infection.[4,5] This protein is responsible for the entry of HIV into host cells. In the initial step of entry, gp120 binds to the CD4 glycoprotein expressed on the surface of human immune cells. Bristol−Myers Squibb Pharmaceutical Research Institute discovered small molecules BMS-378806 (**1**) and BMS-488043 (**2**) that bind to gp120 (Figure 1) and block its interaction with

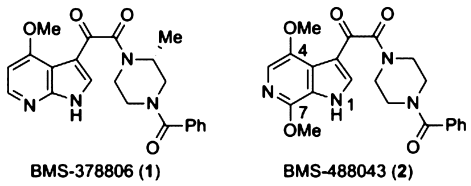


Figure 1. Chemical structures of gp120 inhibitors.

CD4.[6−11] However, the short pharmacokinetic profiles of these small molecule inhibitors (half-lives after intravenous injection are 0.3 and 2.4 h, respectively) may limit their clinical application.

We hypothesize that the pharmacokinetic properties of these small molecule gp120 inhibitors can be improved by conjugation with a monoclonal antibody (mAb) (Scheme 1).[12−21] Furthermore, coupling of the small molecule to the mAb could further enhance their activity in vivo through antibody effector functions such as antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). Recently, we have described the development of chemically programmed antibodies based on the use of mAb 38C2, an aldolase antibody generated by reactive immunization by using a 1,3-diketone hapten.[22−24] This antibody possesses a low pK_a lysine residue in its binding site that is key to its aldolase activity that can be site-selectively labeled with *N*-acyl-*β*-lactams to produce a chemically programmed antibody. Chemically programmed antibodies have duration times after systemic dosing that depend on the properties of the antibody rather than on those of the conjugated small molecule, providing for very significant extensions in the pharmacokinetic profiles of the attached molecule.[18,20] We have demonstrated the utility of this approach by preparing mAb conjugates that show promising activity in a variety of cancer models but also in the area of anti-infectives through the preparation of CCR5 blocking mAbs that inhibit HIV-1 entry and neuraminidase inhibitors that neutralize influenza.[18−20]

Treatment as well as prophylaxis of HIV-1 infection requires the development of a cocktail of inhibitors. In order to complement our anti-CCR5 blockade based on this strategy,[18] we envisioned that the conjugate of mAb 38C2 and the small-molecule gp120 inhibitor would bind to gp120 and inhibit CD4-mediated entry of HIV-1 into cells (Scheme 2). In related work, Spiegel and co-workers recently reported that a derivative of HIV-1 inhibitor **1** modified with a 1,3-dinitrophenyl hapten moiety binds to HIV gp120.[25] Their compound was designed to bind noncovalently with polyclonal anti-1,3-dinitrophenyl

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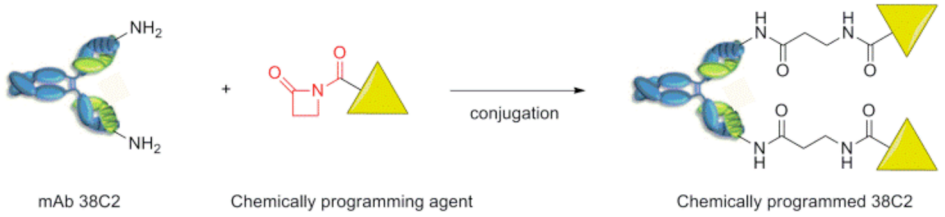
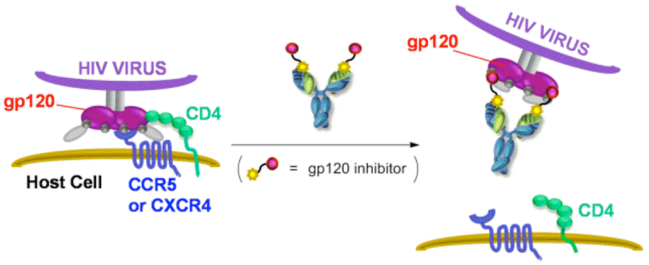
Scheme 1. Chemoselective Modification of Aldolase Antibody 38C2 to Yield a Chemically Programmed Antibody



Scheme 2. Schematic Representation of the Inhibition of the HIV Entry by gp120 Inhibitor-Programmed mAb 38C2



(DNP) antibodies in situ, with the aim of enhancing the activity of **1**. The activity of **1**, however, was severely compromised upon the addition of the DNP linker in their report. Parental **1** has HIV-1 neutralization activity in the nanomolar range, whereas DNP linked **1** demonstrated micromolar activity in binding studies and was not shown to neutralize HIV-1. Our conjugate strategy differs since we use a defined monoclonal antibody covalently linked to **1**. We hypothesized that our strategy might allow us to recover the potent activity of **1** directly if the lack of activity of their DNP derivative of **1** was due to the noncovalent nature of attachment to antibody. Alternatively, modification of the linkage strategy to this family of inhibitors might be key to restoring the activity of the small molecule.

To prepare derivatives of the Bristol–Myers Squibb compounds for conjugation to mAb, we first prepared β-lactam **3** (Figure 2) derived from BMS-378806 (**1**) from the known compound **5** (Scheme 3).[7] Substitution of the nitro group by alcohol **6** followed by the treatment of PCl_3 gave BMS-378806 derivative **7** bearing an azide group. The Huisgen reaction of **7** with β-lactam **8** possessing a terminal alkyne group in the presence of $CuSO_4$, tris(3-hydroxypropyltriazolylmethyl)amine (THPTA), and sodium-(L)-ascorbate proceeded smoothly to yield desired compound **3** with the linker now at the Northern sector of the molecule as suggested by Spiegel et al.[26]

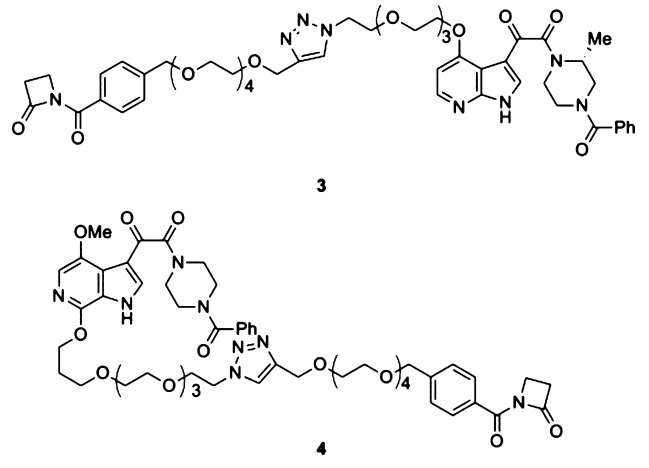


Figure 2. Synthetic targets for this study.

Inhibitor **2** presented us with opportunities to explore the southern sector of the molecule for attachment. Structure–activity relationship studies of **2**[9−11] found that bulky substituents at the 4-position of the azaindole unit decreased the inhibition activity of the compound. Thus, a northern sector connection would be ill-advised. Protection at the 1-position also gave diminished biological activities, whereas the piperazine of **2** was already optimized. In contrast, substitution was tolerated at the 7-position of the azaindole. ON the basis of these data, we designed **4** bearing the linker at 7-position of the azaindole (southern sector connection).

Target compound **4** was synthesized as shown in Scheme 4. Commercially available 2-hydroxy pyridine derivative **9** was subjected to bromination to afford **10** in good yield. The hydroxy group of **10** was allylated using Ag_2CO_3. Formation of the core azaindole structure was achieved by treatment of **11** with *N*,*N*-dimethylformamide dimethylacetal followed by reduction of nitro group in the presence of Fe in AcOH. The bromo group of **12** was replaced by a methoxy group, and **13** was treated with borane-dimethylsulfide complex followed by oxidation with hydrogen peroxide to replace the terminal olefin with a primary alcohol. The reactivity of the substituent-free nitrogen atom at the 1-position of the azaindole in **14** was problematic. After analysis of a number of protecting groups, we found that the trimethylsilylethoxymethyl (SEM) group could be utilized.[27] Protection of the reactive azaindole moiety yielded **15**, which was subjected to etherification with **16**[28] to obtain **17**. Removal of the SEM group was performed using tetrabutylammonium fluoride (TBAF). A Friedel–Crafts reaction of **18** and methyl-2-chloro-2-oxoacetate was accomplished in the presence of an excess amount of $AlCl_3$.[29] The resulting compound **19** was hydrolyzed and condensed with 1-benzoylpiperazine **20** mediated by 3-(diethoxy-phosphoryloxy)-3*H*-benzo[*d*][1.2.3]triazine-4-one (DEPBT)[30] to afford the derivative of BMS-488043 **21**. As the final step, a Huisgen reaction was performed under conditions described for synthesis of **3** to obtain the desired compound **4**.

Conjugation of agent **3** with mAb 38C2 to form **22a** was carried out by incubating 38C2 with six equivalents of **3** in 10 mM PBS (pH 7.4) at room temperature for two hours (Scheme 5). We evaluated the conjugation by measuring the catalytic activity of retro-aldol reaction of methodol as per the standard method.[15] Once a conjugate is formed, the antibody cannot catalyze the retro-aldol reaction of methodol. Compound **22a** had undetectable catalytic activity indicating that each of the key catalytic lysine residues had reacted with the lactam (Figure 3A). The MALDI-TOF mass analysis of **22a** supported the effective conjugation of 38C2 with **3** (Figure 3B). The difference in mass between 38C2 and our preparation of **22a**

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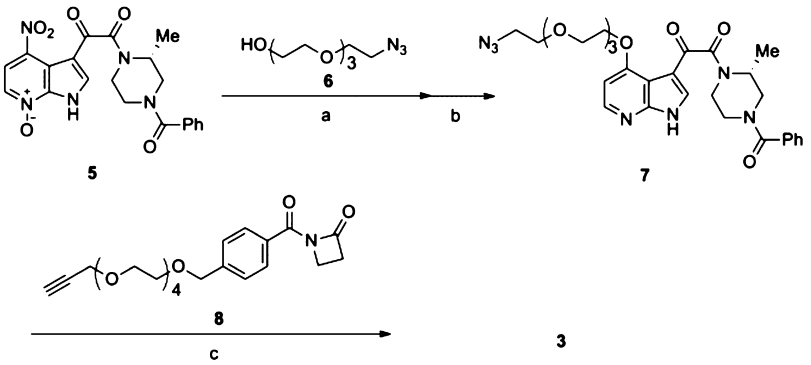
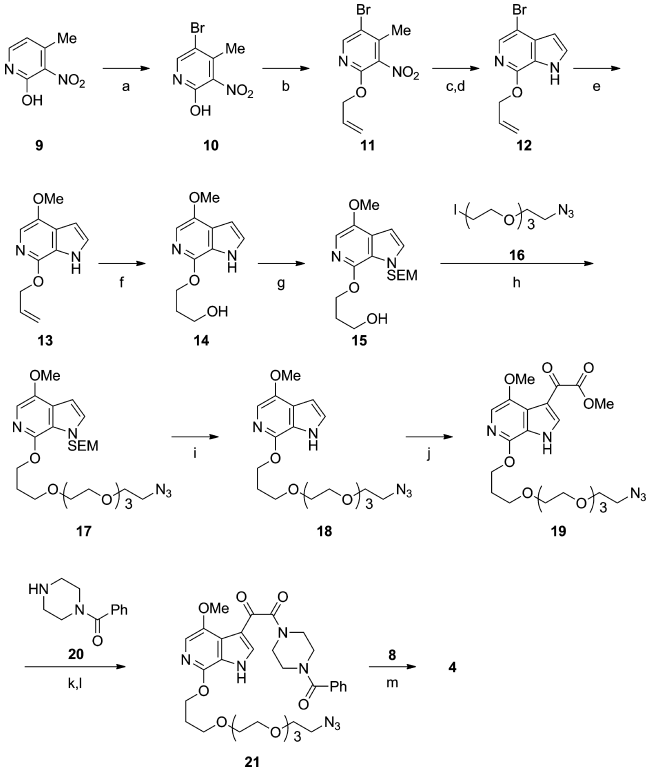
Scheme 3. Synthesis of the BMS-378806 Programming Agent 3[a]



[a]Reagents and conditions: (a) NaH, DME, RT, 2 h then 50 °C, 3 h. (b) PCl_3, EtOAc, RT, 2.5 h (37% in two steps). (c) $CuSO_4{\cdot}5H_2O$, THPTA, Na-(L)-ascorbate, *t*BuOH, H_2O, RT, 30 min (57%).

Scheme 4. Synthesis of the BMS-488043 Programming Agent 21[a]



[a]Reagents and conditions: (a) Br_2, AcOH, AcONa, RT, 1 h (75%). (b) Ag_2CO_3, AllylBr, toluene, RT, 16 h (quant). (c) *N*,*N*-dimethylformamide dimethylacetal, DMF, 130 °C, 2 h. (d) Fe, AcOH, 100 °C, 90 min (40% in two steps). (e) CuI, MeONa, MeOH, DMF, RT to 110 °C, 19 h (87%). (f) BH_3-Me_2S, THF, 0 °C to RT, 4 h then H_2O_2, NaOH, H_2O, 0 °C to RT, 15 h (42%). (g) KOH, SEMCl, THF, RT, 30 min (88%). (h) NaH, DMF, RT, 19 h, (55%). (i) TBAF, ethylenediamine, THF, RT to 70 °C, 21 h (85%). (j) $AlCl_3$, $ClCOCO_2Me$, CH_3NO_2, CH_2Cl_2, RT, 4 h (40%). (k) NaOH, H_2O, MeOH, RT, 1 h. (l) DEPBT, DIPEA, RT, 10 h (38% in two steps). (m) $CuSO_4{\cdot}5H_2O$, THPTA, Na-(L)-ascorbate, *t*BuOH, H_2O, RT, 3 h (69%).

corresponded to two equivalents of the small molecule derivative of **3**. ESI-MS analysis also indicated that both of the two catalytic lysine moieties of 38C2 were modified (see Supporting Information). Conjugate **22b** was similarly prepared from **4** and 38C2 and characterized (Figure 3A,C).

Initially, the binding of antibody conjugates **22a** and **22b** to gp120 was evaluated using an ELISA with gp120-coated plates (Figure 4). Neither unconjugated mAb or conjugate **22a** bound to gp120 at 200 nM. Signal in these cases was similar to the negative control of buffer alone (PBS). In contrast, the **22b** bound strongly to gp120 at this concentration as did the positive control broadly neutralizing antibody b12.[31] The lack of binding by **22b** is consistent with the results of the structure−activity relationship study of related compounds that the bulky substituent at 4-position of the azaindole **1** diminished the biological activity.[9−11] Loss of binding activity at this concentration is consistent with the reported low binding activity of the DNP conjugate study and indicates that the northern site of the linker attachment is likely responsible for the loss in binding, not the fact that DNP conjugates with antibodies are reversibly formed.

The anti-HIV activities of the conjugates **22a** and **22b** were measured in neutralization assays with a single round of infectious virus (JRFL) as described previously.[32] Conjugate **22a** showed very weak neutralization activity, consistent with the low gp120 binding activity observed. Confirming our hypothesis that the substituent at the northern sector 4-position of **1** disrupted gp120 binding, neither **3** nor **7** were effective in the assay (Figure 5A). The IC_{50} values of **4** and **21** with the linker at southern 7-position were 67.5 and 25.4 nM, respectively. The conjugate **22b** also blocked infection with an IC_{50} of 128 nM (Figure 5B). The unmodified mAb 38C2 had no relevant anti-HIV activity. Evident from these studies is an impact on activity on linker attachment to the southern 7-position; however, significant neutralization activity was preserved following linker addition at this site. We had anticipated that conjugate **22b** might exhibit significantly enhanced activity over **4** and **21** given the bivalent display of the compound on the antibody following conjugation as we have noted with other antibody targeting agents. The lack of enhanced activity following conjugation suggests that **22b** is unable to engage the HIV-1 virion in a bivalent interaction. Monovalent binding of natural antibodies that react with the CD4-binding site on gp120 has been suggested in the literature.[33] As previously reported, the chemically programmed antibody strategy has been shown to significantly extend the half-life of the targeting molecule relative to the unconjugated molecule in studies concerned with small molecule, peptide,

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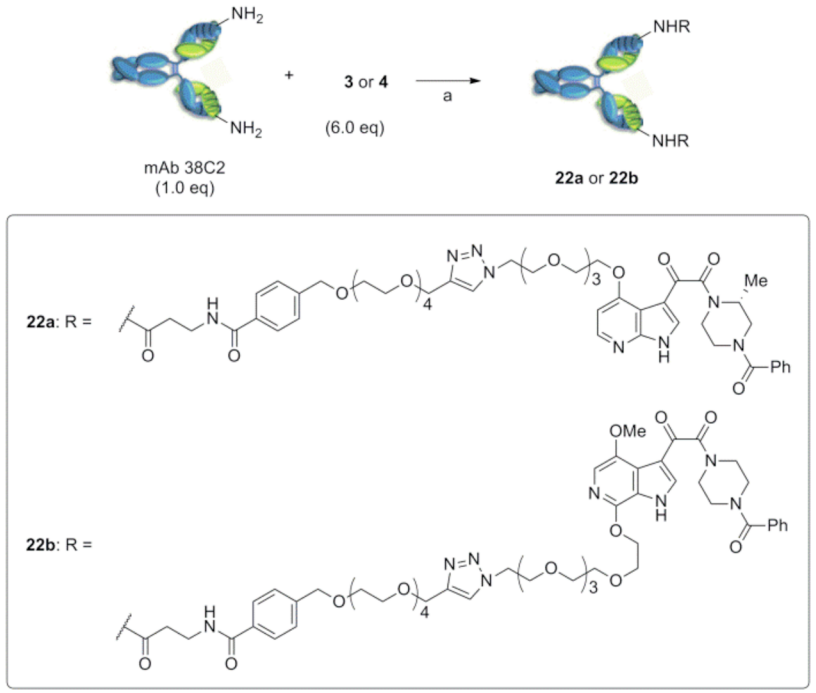
Scheme 5. Preparation of the gp120 Inhibitor Programmed Antibodies 22a and 22b[a]



[a]Reagents and conditions: (a) PBS (pH 7.4), RT, 2 h.

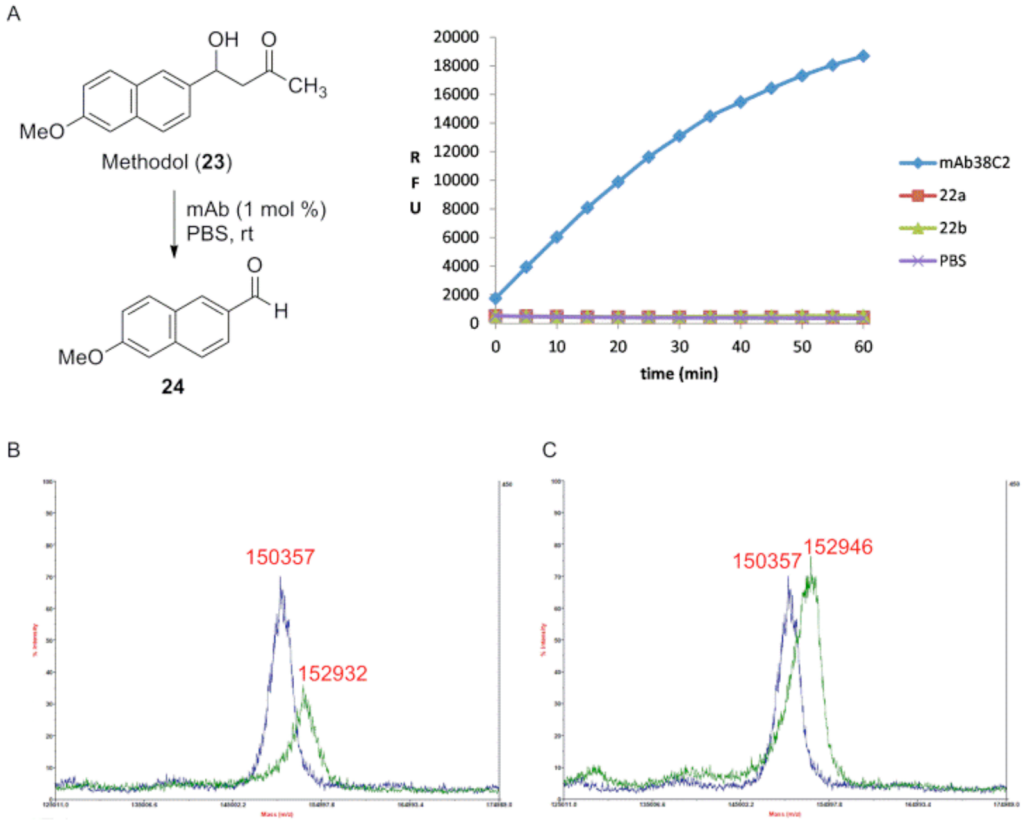


Figure 3. Analysis of **22a** and **22b**. (A) Catalytic activity of **22a**, **22b**, and mAb 38C2 in the retro-aldol reaction of methodol. (B) Overlay of MALDI mass spectra of mAb 38C2 (blue, MW_{av} = 150 357) and **22a** (green, MW_{av} = 152 932). (C) Overlay of MALDI mass spectra of mAb 38C2 (blue, MW_{av} = 150 357) and **22b** (green, MW_{av} = 152 946).

and aptamer targeting molecules.[18−21] Additional biological activities not accessible to the small molecule itself but rather characteristic of the antibody conjugate would be expected to be seen in vivo for **22b** such as ADCC and CDC activity, and these activities may be important to the activities of natural anti-HIV-1 antibodies.[34]

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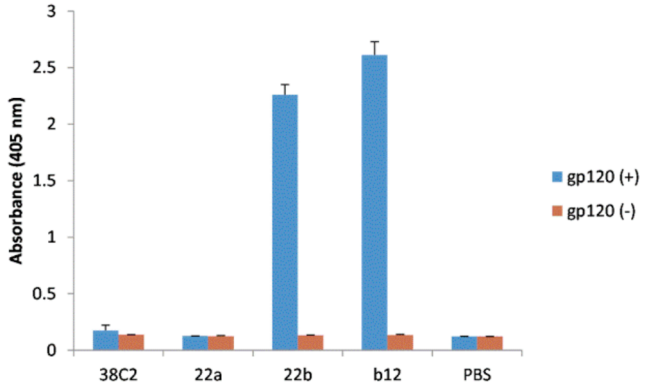

Figure 4. Binding of mAb 38C2 (200 nM), **22a** (200 nM), **22b** (200 nM), and mAb b12 (2 nM) to JRFL gp120 as evaluated by ELISA. PBS indicates the background control.

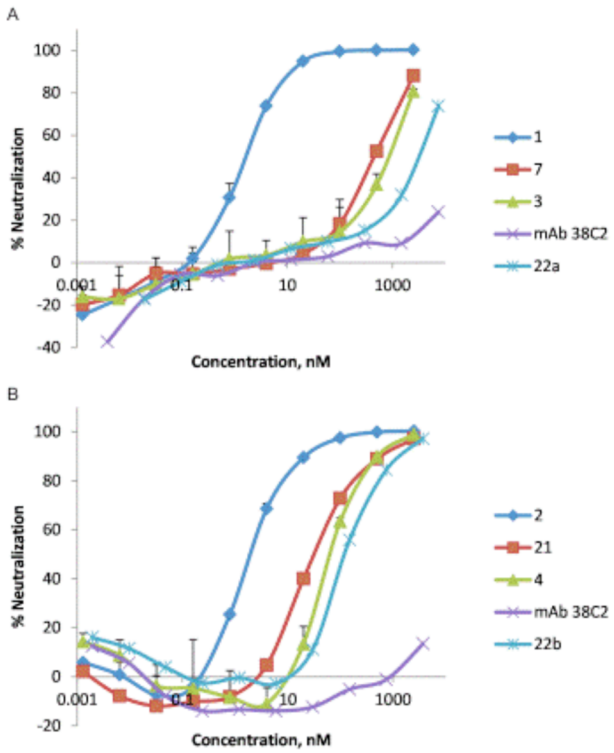

Figure 5. Evaluation of small molecule gp120 inhibitors and mAb conjugates in a single-round neutralization assay using U87.CD4.CCR5 cells and HIV-1 JRFL: (A) **1** (IC_{50} 1.05 nM), **3** (IC_{50} > 200 nM), **7** (IC_{50} > 200 nM), **22a** (IC_{50} > 1000 nM), and mAb 38C2 (IC_{50} > 1000 nM); (B) **2** (IC_{50} 1.98 nM), **4** (IC_{50} 67.50 nM), **21** (IC_{50} 25.41 nM), **22b** (IC_{50} 128.6 nM), and mAb 38C2 (IC_{50} >1000 nM).

In conclusion, synthesis of **3** and **4** allowed for the exploration of two linkage strategies for the BMS series attachment inhibitors **1** and **2** and their conjugation to mAb 38C2 to create chemically programmed antibodies **22a** and **22b**. Compound **4** and its antibody conjugate **22b** possessed good biological activity and effectively neutralized HIV-1, validating a southern site for linkage of this family of attachment inhibitors. The northern linkage site explored in **3** and **22a** destroyed biological activity. We anticipate that conjugation to the antibody should improve the bioactivity and pharmacokinetic properties significantly, and therefore, **22b** warrants further testing in anti-HIV models. While the discovery of a viable site of conjugation for this promising family of attachment inhibitors[35] has allowed us to establish good antiviral activity in the case of a chemically programmed antibody, active conjugation to this family of inhibitors should also facilitate their application in chemically programmed vaccines,[36] chemical approaches to bispecific antibodies,[37] and topical microbicides whose construction is hereby facilitated.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, analytical data, and procedures for ELISA and neutralization assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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