唾液酸类似物库的化学-酶法构建及其对血凝素的识别特性

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摘 要 甲型流感病毒血凝素(HA)在演化过程中受体识别特性不断改变,且天然唾液酸与 HA 的亲和力极弱,限制了病毒识别过程的机理研究与干预探索。本研究旨在构建结构多样性的非天然唾液酸类似物库,探究化学修饰对不同演化阶段毒株 HA 蛋白识别模式的影响。采用化学-酶法合成策略,利用一锅多酶法(OPME)精准构建 α-2,6-糖苷键,通过引入 1,3-丙二硫醇/三乙胺体系,实现叠氮基团与苄氧羰基的正交选择性还原,得到系列氨基糖骨架。基于该通用骨架,通过平行化衍生,在唾液酸 C-5 和 C-9 位点引入不同的磺酰基修饰基团,构建了包含 18 种非天然唾液酸类似物的化合物库。糖芯片分析显示,化合物 3a(二糖)和 **5c**(三糖)对 2019-H1N1 HA 蛋白均表现出强结合活性(**3a** 提升约 120 倍,**5c** 峰值约 25000RFU),且 **5c** 对 2009-H1N1 亦有显著结合作用。分子对接结果揭示,相同 C-9 位修饰不同糖链骨架时,HA 识别活性存在明显差异,表明配体识别受末端修饰、骨架长度及 HA 蛋白的协同调控。本研究为针对 HA 的高亲和力配体设计提供了先导结构和实验依据。

关键词 甲型流感病毒 血凝素 化学酶法 唾液酸类似物 糖芯片

Chemo-enzymatic Construction of a Sialic Acid Analog Library and Their Recognition Properties Toward Hemagglutinins

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Abstract The receptor-binding properties of hemagglutinin (HA) from influenza A virus undergo continuous evolution, and the inherent weak affinity between natural sialic acids and HA limits the effective study and intervention of viral recognition processes. This study aims to construct a structurally diverse library of non-natural sialic acid analogs to explore the impact of chemical modifications on the recognition patterns of influenza strains at different evolutionary stages. A chemo-enzymatic synthesis strategy was employed, utilizing a one-pot multienzyme (OPME) system to precisely construct α-2,6-glycosidic linkages. By introducing a 1,3-propanedithiol/triethylamine system, the orthogonal selective reduction of azide groups in the presence of benzyloxycarbonyl (Cbz) protecting groups was achieved, yielding a series of universal amino-sugar scaffolds. Based on these scaffolds, a library comprising 18 non-natural sialic acid analogs was constructed through parallel derivatization, incorporating acyl and sulfonyl groups with varying electronic and hydrophobic properties at the C-5 and C-9 positions. Glycan microarray analysis revealed that compounds **3a** (disaccharide) and **5c** (trisaccharide) exhibited strong binding activity against the 2019-H1N1 HA protein, with **3a** showing an approximately 120-fold increase in fluorescence intensity compared to the natural disaccharide control and **5c** displaying a peak RFU value of approximately 25000. Moreover, **5c** also showed significant binding to the 2009-H1N1 HA protein. Molecular docking further revealed that the same C-9 modification led to markedly different activities on different glycan backbones, indicating that ligand recognition is synergistically regulated by the terminal modification, the glycan chain length, and the specific HA protein. These findings provide lead structures and experimental evidence for the design of high-affinity ligands targeting HA.

Keywords Influenza A virus, Hemagglutinin, Sialic acid analogs, Glycan microarray, One-pot multienzyme synthesis

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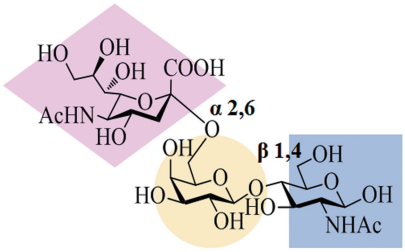
2026-04-14 收稿,2026-05-08 接受

流感病毒侵染宿主细胞的起始环节,依赖于病毒表面血凝素(HA)与宿主细胞受体末端唾液酸糖链的特异性识别[1~5]。在人类呼吸道上皮细胞中,HA 主要识别以 α-2,6 连接的唾液酸残基[6,7]。三糖单元 Neu5Acα2,6Galβ1,4GlcNAc 是流感病毒识别宿主受体的关键最小功能基元(图式 1)[8]。HA 的受体结合位点(RBS)通过其内部高度保守的残基(如 Tyr-98、Trp-153 等)通过氢键和疏水作用锁定唾液酸单元[9~11]。然而,随着病毒持续演化,RBS 周边的环区突变显著改变了不同毒株的识别特性,这给病毒监测和干预带来了挑战[12~15]。天然唾液酸配体与 HA 蛋白之间的单价亲和力通常较为微弱(毫摩尔每升级别)[16,17],为提升结合效能,研究者尝试通过针对 RBS 周边的 Lys133 等残基开发共价抑制剂,展现了受体修饰在提升结合力方面的巨大潜力[18]。此外,在唾液酸外周位点引入非天然基团,可有效利用 RBS 边缘的亚位点。Xu 等[19]报道了一种新型唾液酸五环三萜结合物的合成,实验结果证实 C-5 乙酰胺基和 C-9 羟基是 HA 蛋白识别过程中的关键位点,这两个位点在调控病毒识别性能及扩展亲和力空间方面具有核心价值。



图式 1 人流感病毒受体核心三糖单元的化学结构

Scheme 1 Chemical structure of the core trisaccharide unit of human influenza virus receptors

粉色、黄色及蓝色背景分别代表唾液酸(Neu5Ac)、半乳糖(Gal)及 *N*-乙酰葡糖胺(GlcNAc)残基

在合成策略上,传统的全化学合成法步骤冗长且立体选择性控制难以精准调控。一锅多酶法虽能模拟生物体内糖链合成路径,实现温和、高效的区域选择性构建[20,21],但在引入多样化人工修饰方面存在局限。鉴于此,本研究利用“化学-酶法”模块化合成策略,构建了系列 C-5/C-9 位点差异化修饰的类似物库,并采用聚糖微阵列技术[22]系统评价了其对 1918 年至今多种典型 H1N1 及 H3N2 流感毒株 HA 蛋白的识别差异性。

1 实验部分

1.1 材料、试剂与仪器

CMP-唾液酸合成酶[23](NmCSS,来源于 *Neisseria meningitidis*)、α-2,6-唾液酸转移酶[24](Pd2,6ST,来源于 *Photobacterium damselae*)及唾液酸醛缩酶[25](aldolase,来源于 *Escherichia coli*)均由中国科学院上海药物研究所提供。重组流感病毒 HA 蛋白:A/California/07/2009(H1N1)、A/Guangdong-Maonan/SWL1536/2019(H1N1)、A/Wisconsin/67/2022(H1N1)及 A/Cambodia/e0826360/2020(H3N2),购自北京义翘神州科技股份有限公司;1918 年 H1N1 HA 蛋白由中国科学院上海药物研究所提供。所有重组 HA 蛋白均含有 His 纯化标签,其生物学活性在实验前均已通过聚糖微阵列验证。

Q90 离子交换填料(瑞典 Praesto 公司);石墨化炭黑填料(GCB,纳谱分析技术(苏州)有限公司);Bio-Gel P-2 凝胶(Bio-RAD 公司)。链霉亲和素 Streptavidin-AlexaFluor 647(上海翌圣生物);生物素化的 Anti-Histidine 抗体(北京百普赛斯)。D-半乳糖、唾液酸(上海宝森生物技术有限公司);三磷酸胞苷(CTP)、三氟甲磺酸三甲基硅酯(TMSOTf)、1,3-丙二硫醇、Tween 20、系列取代磺酰氯(上海毕得医药科技股份有限公司)。

Avance 500MHz 核磁共振仪(德国 Bruker);Agilent 6545 Q-TOF 高分辨质谱仪(美国安捷伦);sciFLEXARRAYER S3 糖芯片点样仪(德国 Scienion);InnoScan 710 芯片扫描仪(法国 Innopsys)。

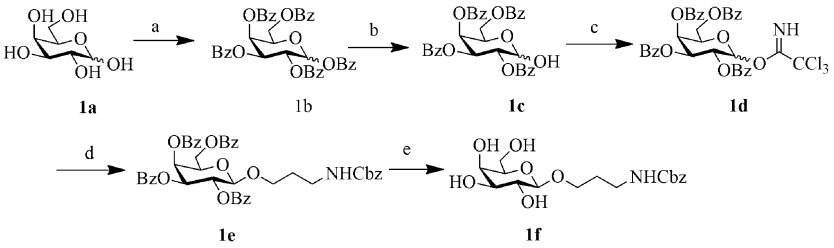
1.2 合成方法

1.2.1 关键中间体的制备

9-叠氮-唾液酸与 5-叠氮-唾液酸:参照文献[26,27]合成,所得核磁共振(NMR)与高分辨质谱(HRMS)数据均与文献报道一致。

受体 **1f** 的合成(图式 2):以 D-半乳糖(25g,0.14mol)为原料,用苯甲酰氯(96mL,0.83mol)全羟基保护,氨甲醇溶液(7mol/L,300mL)选择性脱除异头位苯甲酰基,加入 13mL 三氯乙腈制备三氯乙酰亚胺酯供体;随后与苄氧羰基(Cbz)保护的 3-氨基丙醇(1.8g,8.8mmol)在 TMSOTf(534μL,2.7mmol)催化下进行糖苷化反应,最后经甲醇钠脱保护得到 8g 白色固体。

受体 **1m** 的合成(图式 3):以 *N*-乙酰乳糖

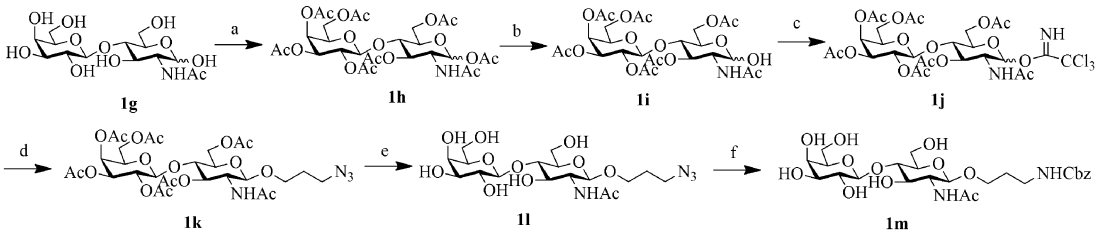


图式 2 受体 1f 的合成路线

Scheme 2 Synthetic route to receptor 1f

(a)苯甲酰氯,吡啶,室温,12h,97%;(b)氨甲醇溶液,四氢呋喃,室温,12h,33%;(c)三氯乙腈,碳酸钾,二氯甲烷,室温,12h;(d)3-(苄氧羰基氨基)-1-丙醇,4Å 分子筛,TMSOTf,二氯甲烷,2h,50%;(e)甲醇钠,甲醇,室温,12h,65%。

胺(LacNAc)为原料(10g,0.026mol),乙酸酐/吡啶(140mL,140mL)全乙酰化保护,用苄胺(2.8mL,0.039mol)选择性脱除异头位乙酰基,加入 13mL 三氯乙腈和 3mL 1,8-二氮杂二环[5.4.0]十一碳-7-烯(DBU)制备三氯乙酰亚胺酯供体。供体与叠氮丙醇(1.28g,0.0127mol)在 TMSOTf 催化下实现具有 β-构型的糖苷化反应。脱除乙酰基后,经 Pd/C 催化氢化还原叠氮为伯胺,再用苯甲氧羰酰琥珀酰亚胺(Cbz-OSu)引入 Cbz 保护基,得 1.2g 白色固体。



图式 3 受体 1m 的合成路线

Scheme 3 Synthetic route to receptor 1m

(a)醋酸酐,吡啶,室温,12h,80%;(b)苄胺,四氢呋喃,室温,12h,55%;(c)三氯乙腈,DBU,二氯甲烷,室温反应 1h,85%;(d)3-叠氮基-1-丙醇,4Å 分子筛,TMSOTf,1,2-二氯乙烷,90℃,5h,50%;(e)甲醇钠,甲醇,室温,12h,89%;(f)i:氢气,钯碳,室温,12h;ii:苯甲氧羰酰琥珀酰亚胺,碳酸氢钠,水和丙酮,两步总收率 30%。

1.2.2 核心骨架的模块化合成

通用酶促体系(OPME):将唾液酸供体(1.5eq)、受体 **1f** 或 **1m**(1.0eq)及 CTP(2.0eq)溶于 Tris-HCl 缓冲液(1mol/L,pH 7.4)和 $MgCl_2$ 溶液(200mmol/L)中。加入 NmCSS 和 Pd2,6ST 酶液,反应底物浓度为 40mmol/L。37℃反应,全程维持 pH 在 8.0 左右。经薄层色谱(TLC)监测(乙酸乙酯/甲醇/水=10∶3∶2,体积比)反应完全后,加入无水乙醇沉淀蛋白(-80℃)。滤液经浓缩后,依次通过 P-2 凝胶柱(水为洗脱剂)与 Praesto Q90 离子交换柱(0~100 mmol/L NaCl 溶液梯度洗脱)纯化,除盐后冻干。

采用上述通用酶促路线合成化合物 **1**、**2**、**2a**、**2b**、**2e**、**2f**。化合物 **1**(二糖对照):化合物 **1f**(10mg,0.027mmol)、唾液酸(15mg,0.04mmol),最终得 18mg 白色粉末,产率 70%。^{1}H NMR (500MHz,D_2O) δ:7.43 (m,5H),5.13 (s,3H),4.34 (d,J=8.1 Hz,1H),3.98~3.91 (m,3H),3.91~3.84 (m,2H),3.82 (d,J=10.1 Hz,1H),3.77~3.68 (m,4H),3.67~3.57 (m,4H),3.49 (dd,J=9.9、7.9 Hz,1H),3.25 (m,2H),2.74 (dd,J=12.4、4.7 Hz,1H),2.04 (s,3H),1.82 (m,2H),1.70 (t,J=12.1 Hz,1H);^{13}C NMR (126MHz, D_2O) δ: 173.41, 158.39, 128.77, 128.31, 127.62, 102.84, 100.43, 73.40, 72.64, 72.55,71.70,70.66,68.59,68.18,67.79,66.77, 63.37,62.60,51.85,40.18,37.29,28.86,22.00; HRMS(ESI) m/z: $C_{28}H_{42}N_2O_{16}$ [M-H]$^-$ 理论值 661.2462,实测值 661.2462。

化合物 **2**(三糖对照):**1m**(10mg,0.017mmol)、唾液酸(15mg,0.03mmol),最终得 15mg 白色粉末,产率 67%。^{1}H NMR (500MHz, D_2O) δ:7.43 (m,5H),4.48 (dd,J=30.0、8.0 Hz,2H),4.06(m,1H),4.01~3.89(m,4H),3.85

~3.66(m,8H),3.65~3.56(m,3H),3.56~3.50(m,3H),3.39(dd,J=13.1、3.0 Hz,1H),3.17(m,2H),3.00(m,1H),2.68(m,1H),2.04(s,6H),1.75(m,2H);^{13}C NMR(126MHz,D_2O)δ:174.87,174.46,173.48,165.60,158.25,157.02,141.62,128.73,128.29,127.57,103.43,100.84,100.10,96.36,96.12,90.18,89.29,82.93,82.87,80.63,74.40,74.20,73.91,73.61,72.50,72.39,71.66,70.68,69.34,68.47,68.34,68.15,67.58,66.69,63.55,63.29,62.61,60.64,60.28,54.79,51.87,40.02,37.22,28.72,22.20,22.01;HRMS(ESI) m/z:$C_{36}H_{55}N_3O_2$[M-H]$^-$,理论值864.3255,实测值864.3258。

化合物**2a**:**1f**(3g,8.1mmol)、9-叠氮-唾液酸(4g,12.1mmol),最终得3.7g白色粉末,产率66%。^{1}H NMR(500MHz,D_2O)δ:7.44(m,5H),5.12(s,2H),4.38~4.26(m,1H),4.07~3.87(m,4H),3.83(t,J=10.1Hz,1H),3.77~3.55(m,8H),3.53~3.38(m,2H),3.24(s,2H),2.73(dd,J= 12.4、4.7 Hz,1H),2.05(s,3H),1.81(d,J=5.6Hz,2H),1.69(t,J=12.2Hz,1H);^{13}C NMR(126MHz,D_2O)δ:175.03,173.38,158.40,136.62,128.78,128.32,127.62,102.87,100.49,73.42,72.57,72.46,70.66,70.33,68.87,68.56,68.16,67.80,66.78,63.35,53.09,51.85,46.65,40.19,37.30,28.87,22.01;HRMS(ESI) m/z:$C_{28}H_{41}N_5O_{15}$[M-H]$^-$,理论值686.2526,实测值686.2529。

化合物**2b**:**1f**(3g,8.1mmol)、5-叠氮-唾液酸(4g,12.1mmol),最终得4g白色粉末,产率70%。^{1}H NMR(500MHz,D_2O)δ:7.44(m,5H),5.12(s,2H),4.34(d,J=7.9Hz,1H),3.94(m,4H),3.89(m,2H),3.86(d,J=2.5Hz,1H),3.82(dd,J=10.4、1.8Hz,1H),3.75(m,2H),3.70~3.67(m,1H),3.66~3.57(m,4H),3.49(dd,J=10.0、7.8 Hz,1H),3.25(m,2H),2.75(dd,J=12.4、4.7 Hz,1H),1.82m,2H),1.72(t,J=12.2Hz,1H);^{13}C NMR(126MHz,D_2O)δ:173.42,171.21,128.78,128.32,127.63,102.85,100.46,73.42,72.56,72.31,71.77,70.67,68.61,68.14,68.04,67.80,66.79,63.40,62.59,51.93,51.88,40.20,37.31,28.88;HRMS(ESI) m/z:$C_{28}H_{41}N_5O_{16}$[M-H]$^-$,理论值702.2476,实测值702.2479。

化合物**2e**:**1m**(500mg,0.8mmol)、9-叠氮-唾液酸(400mg,1.2mmol),最终得712mg白色粉末,产率62%。^{1}H NMR(500MHz,D_2O)δ:7.44(m,5H),5.11(s,2H),4.50(dd,J=5.7、2.8 Hz,1H),4.44(d,J=7.9Hz,1H),4.03(m,2H),3.99~3.95(m,2H),3.95~3.89(m,3H),3.85~3.78(m,3H),3.77~3.70(m,3H),3.70~3.65(m,3H),3.63~3.54(m,4H),3.52~3.48(m,1H),3.23~3.11(m,2H),2.67(dd,J=12.4、4.7 Hz,1H),2.05(d,J=11.4Hz,6H),1.78~1.72(m,3H);^{13}C NMR(126MHz,D_2O)δ:174.87,174.53,173.51,158.33,128.78,128.34,127.63,103.47,100.87,100.19,80.72,74.45,73.66,72.41,72.35,70.70,70.26,69.04,68.35,68.17,67.62,66.75,63.32,60.34,54.83,53.12,51.89,40.09,22.24,22.04;HRMS(ESI) m/z:$C_{36}H_{54}N_6O_{20}$[M-H]$^-$,理论值889.3320,实测值889.3318。

化合物**2f**:**1m**(500mg,0.8mmol)、5-叠氮-唾液酸(400mg,1.2mmol),最终得435mg白色粉末,产率60%。^{1}H NMR(500MHz,D_2O)δ:7.44(m,5H),5.12(s,2H),4.47(m,2H),4.07(d,J=1.4Hz,2H),4.02~3.94(m,3H),3.94~3.86(m,5H),3.77(d,J=4.4Hz,5H),3.75~3.70(m,3H),3.62(m,4H),3.20(m,3H),2.05(s,2H),1.79~1.71(m,3H),1.29(t,J=7.3Hz,2H);^{13}C NMR(126MHz,D_2O)δ:174.61,173.40,171.01,158.33,128.79,128.36,127.63,103.45,100.87,100.10,80.63,74.43,73.65,72.41,72.21,72.05,68.35,66.74,62.48,60.32,54.85,51.98,42.46,40.03,35.80,35.72,28.94,22.28;HRMS(ESI) m/z:$C_{36}H_{54}N_6O_{21}$[M-H]$^-$,理论值905.3269,实测值905.3272。

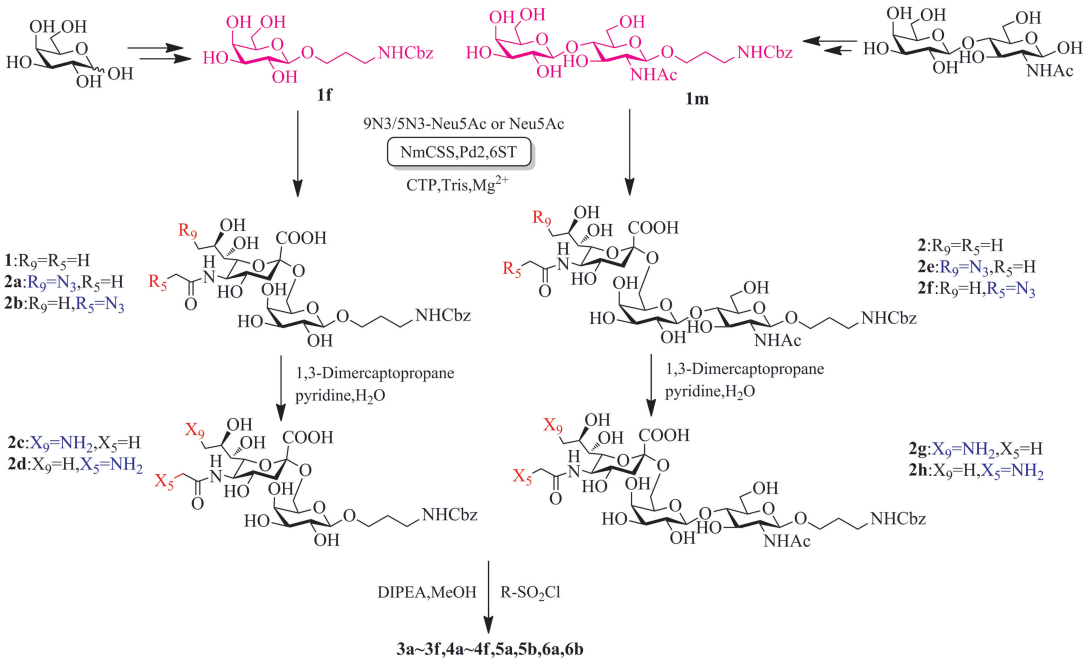
化合物**2d**的合成:将3g(4.4mmol)化合物**2b**溶于吡啶与水的混合溶剂(1∶1,11mL)中,加入18mL三乙胺。在冰浴下缓慢滴加4.4mL(44mmol)1,3-丙二硫醇,室温搅拌反应5h。经TLC监测(异丙醇/氨水/水=7∶3∶2与乙酸乙酯/甲醇/水=10∶3∶2等体积混合液)反应完全后,减压浓缩。粗产物经GCB反相色谱柱纯化,以乙腈/10mmol·L^{-1} NH_4HCO_3水溶液为洗脱剂,冷冻干燥得2.4g白色固体,产率81%。^{1}H NMR(500MHz,D_2O)δ:7.53~7.32(m,5H),5.12(s,2H),4.34(d,J=7.9Hz,1H),3.97~3.92(m,

4H), 3.92 ~ 3.88 (m, 2H), 3.86 (d, J = 2.8Hz, 2H), 3.81 (d, J = 1.7Hz, 1H), 3.76 ~ 3.72 (m, 1H), 3.69 (m, 3H), 3.65 ~ 3.57 (m, 4H), 3.49 (m, 1H), 3.24 (d, J = 6.6Hz, 2H), 2.75 (dd, J = 12.4、4.7 Hz, 1H), 1.81 (t, J = 6.5Hz, 2H), 1.72 (t, J = 12.2Hz, 1H); ^{13}C NMR (126MHz, D_2O) δ: 173.33, 167.58, 158.41, 136.60, 128.78, 127.62, 102.85, 100.40, 73.41, 72.55, 72.37, 71.59, 70.65, 68.63, 68.19, 68.12, 67.81, 66.79, 63.45, 62.50, 51.91, 40.51, 40.20, 37.31, 28.87; HRMS (ESI) m/z: $C_{28}H_{43}N_3O_{16}$ [M-H]$^-$, 理论值 676.2571, 实测值 676.2576。

化合物 **2c** 的制备方法同 **2d**, 得 3.27g 白色固体, 产率 85%。^{1}H NMR (500MHz, D_2O) δ: 7.49 ~ 7.23 (m, 5H), 5.02 (s, 2H), 4.25 (d, J = 7.9 Hz, 1H), 4.00 (m, 1H), 3.90 ~ 3.79 (m, 3H), 3.74 (t, J = 10.0Hz, 1H), 3.69 ~ 3.56 (m, 4H), 3.55 ~ 3.45 (m, 3H), 3.40 (dd, J = 9.9、7.9 Hz, 1H), 3.34 (dd, J = 13.1、3.1Hz, 1H), 3.15 (m, 2H), 2.94 (dd, J = 13.1、9.5Hz, 1H), 2.66 (dd, J = 12.6、4.8Hz, 1H), 1.96 (s, 3H), 1.72 (m, 2H), 1.62 (t, J = 12.1Hz, 1H); ^{13}C NMR (126MHz, D_2O) δ: 175.13, 173.31, 158.38, 136.59, 128.80, 128.34, 127.64, 102.87, 100.50, 73.41, 72.52, 72.40, 70.66, 70.16, 68.73, 67.96, 67.89, 66.79, 63.60, 51.83, 42.23, 40.15, 37.32, 28.93, 22.05; HRMS (ESI) m/z: $C_{28}H_{43}N_3O_{15}$ [M-H]$^-$, 理论值 660.2626, 实测值 660.2621。

化合物 **2g** 的制备方法同 **2d**, 得 546mg 白色固体, 产率 79%。^{1}H NMR (500MHz, D_2O) δ: 7.43 (m, 5H), 5.11 (s, 2H), 4.53 ~ 4.48 (m, 1H), 4.45 (d, J = 7.9Hz, 1H), 4.06 (m, 1H), 4.01 ~ 3.87 (m, 4H), 3.86 ~ 3.76 (m, 3H), 3.76 ~ 3.71 (m, 3H), 3.70 ~ 3.66 (m, 2H), 3.60 (m, 3H), 3.56 ~ 3.50 (m, 3H), 3.39 (dd, J = 13.1、3.0 Hz, 1H), 3.17 (m, 2H), 3.00 (dd, J = 13.1、9.5 Hz, 1H), 2.68 (dd, J = 12.4、4.6 Hz, 1H), 2.04 (s, 6H), 1.80 ~ 1.71 (m, 3H); ^{13}C NMR (126MHz, D_2O) δ: 174.97, 174.52, 173.45, 158.35, 128.77, 128.34, 128.33, 127.62, 127.61, 103.50, 100.82, 100.25, 80.66, 74.42, 73.54, 72.39, 70.72, 70.30, 68.31, 68.21, 67.98, 67.62, 66.76, 63.21, 60.30, 54.88, 51.82, 42.34, 40.03, 28.75, 22.21, 22.01; HRMS (ESI) m/z: $C_{36}H_{56}N_4O_{20}$ [M-H]$^-$, 理论值 863.3422, 实测值 863.3415。

化合物 **2h** 的制备方法同 **2d**, 得 410mg 白色固体, 产率 83%。^{1}H NMR (500MHz, D_2O) δ: 7.43 (m, 5H), 5.12 (s, 2H), 4.52 ~ 4.48 (m, 1H), 4.45 (d, J = 7.9Hz, 1H), 3.99 (m, 2H), 3.95 ~ 3.88 (m, 4H), 3.88 ~ 3.85 (m, 1H), 3.85 ~ 3.78 (m, 3H),



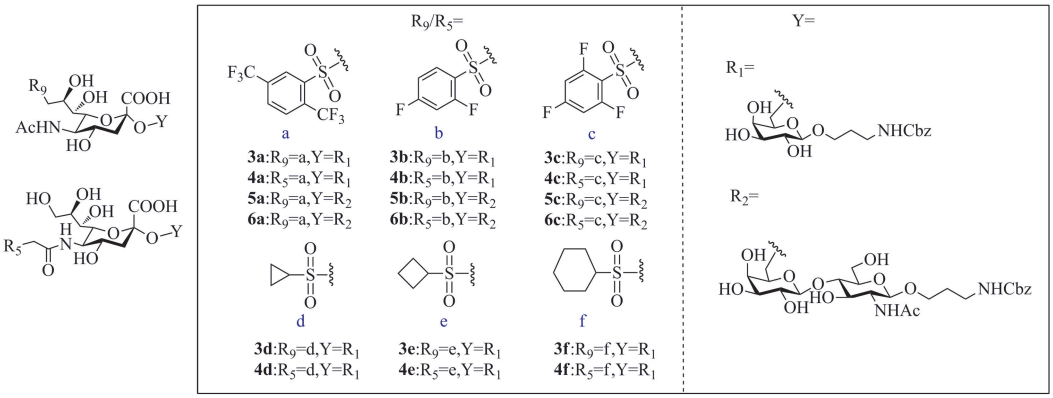
图式 4 化学酶法合成唾液酸类似物

Scheme 4 Chemo-enzymatic synthesis of sialic acid analogs

3.76(t,J=2.3Hz,1H),3.75~3.70(m,2H),3.70~3.61(m,6H),3.61~3.51(m,4H),3.18(m,2H),2.69(dd,J=12.4、4.6 Hz,1H),2.05(s,3H),1.85~1.62(m,3H);^{13}C NMR(126MHz,D_2O) δ:174.53,173.47,171.40,158.35,128.78,128.34,127.60,103.42,100.88,100.17,80.54,74.47,73.66,72.39,71.62,70.71,68.37,68.31,68.17,67.61,66.76,62.55,60.32,54.85,51.86,42.00,40.11,37.24,28.74,22.24; HRMS(ESI) m/z:$C_{36}H_{56}N_4O_{21}[M-H]^-$,理论值 879.3375,实测值 879.3364。

1.2.3 唾液酸类似物的模块化合成

以四种氨基糖为通用骨架,通过其 C-5 或 C-9 位活泼氨基与系列磺酰氯的平行衍生化反应,完成了目标类似物库的模块化构建。通用合成方法:将二糖或三糖骨架(0.01mmol)溶解于无水甲醇(50μL)中,加入二异丙基乙胺(DIPEA)(4.0eq.)和相应的磺酰氯(2.0eq.),室温搅拌反应 3h。经 TLC 监测(乙酸乙酯/甲醇/水=10∶3∶2)反应完全后,制备型高效液相色谱(HPLC)纯化(C18 柱,5%~60%乙腈/10mmol/L NH_4HCO_3,210nm 检测),收集组分,冻干得终产物。由于化合物库成员较多且具有相似的糖链骨架,文中仅列出具有代表性的化合物(如 **3a**、**4b**、**5c** 等)的 NMR 和 HRMS 数据(见表 1)。



图式 5 本研究合成的 18 种非天然唾液酸类似物的化学结构

Scheme 5 Chemical structures of the 18 non-natural sialic acid analog library

1.3 聚糖微阵列实验

1.3.1 芯片点样制备

为将化合物固定于芯片表面,需脱除连接子末端的 Cbz 保护基以暴露氨基。将系列类似物(如 **3a~3c**,**4a~4g**)配制成 10mg/mL 的水溶液,取样品溶液 50μL,加入 20μL Pd(OH)$_2$/C 悬浮液(20mg/mL)和 100μL 超纯水。氢气氛围下室温反应,至 TLC 监测原料消失(异丙醇/氨水/水=7∶3∶2,体积比)。过滤,滤液冻干。将脱保护后化合物溶于磷酸钠缓冲液(250mmol/L,pH 8.5)中,浓度为 100μmol/L,使用 sciFLEXARRAYER S3 点样仪点样于 *N*-羟基丁二酰亚胺(NHS)活化的玻璃片上(每组 4 个重复,点样体积约为 400pL)。玻片于 75%相对湿度下孵育 8h,用乙醇胺封闭液(50mmol/L,pH 9.0)于 50℃封闭 90min。经去离子水洗涤,离心干燥后备用。

1.3.2 缓冲液的配制

结合缓冲液(TSMTB):20mmol/L Tris-Cl(pH 7.4),150mmol/L NaCl,2mmol/L $CaCl_2$,2mmol/L $MgCl_2$,0.05% 吐温 20,1% BSA。洗涤缓冲液(TSMT):20mmol/L Tris-Cl(pH 7.4),150mmol/L NaCl,2mmol/L $CaCl_2$,2mmol/L $MgCl_2$,0.05%吐温 20。TSM 缓冲液:20mmol/L Tris-Cl(pH 7.4),150mmol/L NaCl,2mmol/L $CaCl_2$,2mmol/L $MgCl_2$。

1.3.3 孵育检测

将 5 种 HA 蛋白用 TSMTB 稀释至 50μg/mL(经预实验确定的最佳浓度)与芯片室温孵育 1h,依次用 TSMT、TSM 缓冲液及去离子水洗涤。将生物素化的 Anti-His 抗体(10μg/mL)与链霉亲和素-AlexaFluor® 647(5μg/mL)的混合液避光孵育芯片 1h。清洗干燥后,使用 Innopsys Innoscan710 扫描采集荧光信号,通过调整增益(Gain)和光电倍增管(PMT)参数确保信号强度处于线性范围

表 1 代表性唾液酸类似物的^{1}H NMR、^{13}C NMR 谱和高分辨质谱数据

Tab. 1 ^{1}H NMR, ^{13}C NMR and HRMS data of representative sialic acid analogs

化合物	^{1}H NMR(500MHz, D_2O) δ	^{13}C NMR(126MHz, D_2O) δ	HRMS *m/z*
3a(白色固体)	7.68(m,1H),7.57~7.42(m,2H),7.42~7.26(m,5H),5.02(s,2H),4.36(d,*J*=7.9Hz,1H),3.99~3.90(m,2H),3.86(dd,*J*=9.8、6.6Hz,1H),3.83~3.72(m,3H),3.71~3.56(m,5H),3.56~3.44(m,2H),3.34(d,*J*=13.5Hz,1H),3.21(m,2H),3.06(m,1H),2.71(m,1H),2.03(s,3H),1.84~1.76(m,2H),1.69(t,*J*=12.2Hz,1H)	175.12,172.96,158.23,136.45,130.87,128.67,128.20,127.52,120.81,120.62,102.93,100.20,73.19,72.59,70.69,69.99,69.26,68.28,67.91,66.67,62.73,51.85,45.26,40.16,37.26,28.93,22.02	$C_{36}H_{45}F_6N_3O_{17}S$ [M-H]$^-$,理论值 936.2301,实测值 936.2314
3b(白色固体)	7.90(m,1H),7.40(m,5H),7.18(m,2H),5.08(s,2H),4.36(d,*J*=7.9Hz,1H),3.90~3.66(m,8H),3.61(m,2H),3.55~3.44(m,2H),3.36(dd,*J*=14.0、2.7Hz,1H),3.23(m,3H),3.07(dd,*J*=14.0、7.5Hz,1H),2.71(m,1H),2.04(s,3H),1.82(m,2H),1.69(t,*J*=12.2Hz,1H)	175.12,173.02,158.36,131.87,131.79,128.74,128.28,127.55,102.90,100.23,73.30,72.58,70.68,69.93,69.21,68.42,67.93,66.72,63.05,54.34,51.82,45.19,40.12,37.26,21.98	$C_{34}H_{45}F_2N_3O_{17}S$ [M+H]$^+$,理论值 838.2511,实测值 838.2508
3c 白色固体	7.43(m,5H),7.06(t,*J*=9.4Hz,2H),5.12(s,2H),4.34(d,*J*=8.0Hz,1H),3.94(m,3H),3.91~3.86(m,4H),3.86~3.72(m,4H),3.72~3.68(m,1H),3.64(m,3H),3.58~3.53(m,1H),3.49(t,*J*=8.9Hz,1H),3.25(d,*J*=7.2Hz,2H),2.74(dd,*J*=12.4、4.6Hz,1H),1.82(m,2H),1.70(t,*J*=12.1Hz,1H)	173.41,171.51,158.41,128.78,128.31,127.62,102.85,102.35,100.44,73.42,72.56,72.41,71.82,70.67,68.61,68.24,67.93,63.41,62.71,51.99,44.81,40.23,37.30,28.66	$C_{34}H_{44}F_3N_3O_{18}S$ [M+H]$^+$,理论值 872.2365,实测值 872.2362
3d(白色固体)	7.43(m,5H),5.10(s,3H),4.35~4.25(m,1H),4.03~3.94(m,2H),3.94~3.84(m,4H),3.84~3.77(m,1H),3.72(m,3H),3.66(s,1H),3.61(d,*J*=8.8Hz,2H),3.58~3.46(m,2H),3.31~3.18(m,3H),2.80~2.71(m,1H),2.70~2.64(m,1H),2.05(s,3H),1.80(m,2H),1.70(t,*J*=12.1Hz,1H),1.11(d,*J*=6.2Hz,2H)	175.13,173.32,158.40,136.62,128.78,128.31,127.61,102.87,100.47,73.35,72.57,70.67,70.46,69.19,68.47,68.08,67.78,66.78,63.17,56.92,54.34,51.88,45.05,42.53,40.23,37.29,34.64,31.94,31.92,28.87,24.31,22.00,17.69,16.23	$C_{31}H_{47}N_3O_{17}S$ [M+H]$^+$,理论值 766.2699,实测值 766.2698
4b(白色固体)	7.93(m,1H),7.44(m,5H),7.26~7.12(m,2H),5.13(s,2H),4.34(d,*J*=8.0Hz,1H),3.94(t,*J*=7.8Hz,3H),3.91~3.84(m,2H),3.84~3.77(m,3H),3.72(m,4H),3.66~3.59(m,3H),3.56~3.46(m,2H),3.29~3.19(m,2H),2.73(dd,*J*=12.5、4.7Hz,1H),1.82(m,2H),1.69(t,*J*=12.1Hz,1H)	173.40,171.70,158.42,136.62,131.85,128.77,128.30,127.61,112.29,102.84,100.43,73.42,72.56,72.41,71.80,70.66,68.60,68.22,67.91,67.80,66.78,63.40,62.70,54.34,51.96,44.76,40.22,37.29,28.86	$C_{34}H_{45}F_2N_3O_{18}S$ [M+H]$^+$,理论值 854.2460,实测值 854.2454
5c(白色固体)	7.34(m,5H),7.01~6.94(m,2H),5.03(s,2H),4.00(s,1H),3.95~3.87(m,2H),3.86~3.80(m,4H),3.79(s,1H),3.73(m,4H),3.69~3.61(m,3H),3.61~3.56(m,2H),3.56~3.42(m,7H),3.14~3.10(m,1H),3.08(d,*J*=11.2Hz,1H),2.89(s,1H),2.59(m,1H),1.94(d,*J*=6.0Hz,3H),1.66(dd,*J*=13.6、7.7 Hz,2H)	174.98,173.28,158.31,138.94,130.41,128.75,128.32,127.91,127.60,103.44,100.88,100.09,80.76,74.46,73.67,72.42,72.39,70.70,69.88,69.36,68.39,68.00,67.57,66.73,63.44,60.37,54.80,51.87,45.53,40.07,37.20,28.71	$C_{42}H_{57}F_3N_4O_{22}S$ [M-H]$^-$,理论值 1057.3094,实测值 1057.3077

内且无饱和现象。使用 Mapix 软件提取荧光强度值,扣除背景信号后,以 4 个重复点的平均值(Mean±SD)表示最终结合信号。

1.4 分子对接实验

HA 蛋白晶体结构从 RCSB Protein Data Bank 数据库获取(PDB ID: 3UBJ,来源:A/California/04/2009 H1N1)。对接前对蛋白进行去水、加氢及电荷平衡处理,小分子配体经能量最低化后进行对接优化。通过分析最低结合自由能及相互作用模式,探讨糖链骨架对识别特性的影响。

2 结果与讨论

本研究建立了一种基于化学-酶法偶联的模块化合成方案。首先,利用 OPME 体系在水相(pH 8.0)中实现非天然叠氮供体与受体 **1f/1m** 的 α-2,6 偶联,产率稳定在 60%~70%。利用 1,3-丙二硫醇/三乙胺体系选择性还原叠氮基团(产率>80%),同时保留了 Cbz 保护基及酸碱敏感的糖苷键。体系副产物较少,产物经 GCB 固相萃取柱脱盐即可获得核心骨架。基于所得骨架末端伯胺的亲核活性,通过平行衍生化策略,构建了包含 18 种非天然唾液酸类似物的化合物库。该模块化方案缩短了合成周期,不仅限于本研究展示的磺酰基修饰,可快速扩充至成百上千种类似物。

利用聚糖微阵列技术横向评价了库中 20 个唾液酸类似物(含对照二糖和三糖)对 5 种典型流感病毒 HA 蛋白的识别特性,热图分析显示(图 1),该化合物库表现出明显的毒株选择性。在二糖类似物系列中,C-9 位芳香磺酰基修饰(**3a**,**3b**)表现出最强的结合倾向,且其识别活性

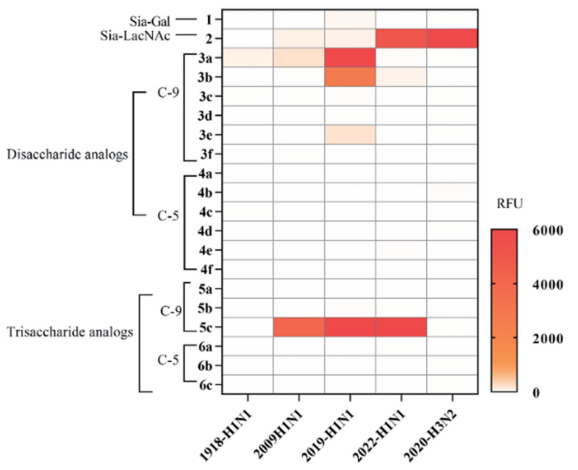


图 1 化合物库对 HA 蛋白的结合活性热图

Fig. 1 Heatmap of binding activity of the compound library against HA proteins

颜色基于 RFU 值映射,最大颜色阈值设为 6000RFU(超过 6000 的值为深红色),具体数值和组间差异参见图 2 的定量分析

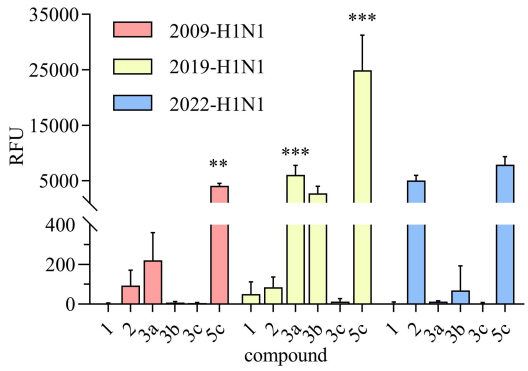


图 2 代表性化合物与不同 H1N1 毒株 HA 蛋白结合强度的定量分析

Fig. 2 Binding intensity analysis of representative compounds to HA proteins from different H1N1 strains

数据以 Mean ± SD 表示($n=4$);采用单因素方差分析(one-way ANOVA)进行统计学评价;二糖类似物以天然二糖 **1** 为对照进行显著性分析,三糖类似物以天然三糖 **2** 为对照进行显著性分析;*** $p < 0.001$, ** $p < 0.01$,未标注的比较均无统计学显著差异(ns)

高度集中于 2019-H1N1 毒株。相比之下,1918-H1N1 表现出极弱的整体识别活性,而 2022-H1N1 和 2020-H3N2 则表现出对天然二糖受体 2(Sia-LacNAc)的强烈偏好。所有 C-5 位修饰的类似物(**4a~4f**, **6a~6c**)在相同实验条件下均未检测到明显结合,这反映了 HA 结合口袋对 C-5 位空间构型的敏感性。

进一步对三种典型 H1N1 毒株的结合强度进行统计学分析(图 2)。为排除糖链骨架长度对识别强度的基础影响,本研究分别以天然二糖 **1** 和三糖 **2** 作为对应骨架的基准对照,对修饰后的类似物进行了统计学评价。对于 2019-H1N1,化合物 **3a** 和 **5c** 表现出显著的结合。其中二糖类似物 **3a** 的荧光强度较二糖对照 **1** 提升了约 120 倍($p < 0.001$),而三糖类似物 **5c** 的 RFU 峰值接近 25000,显示出极强的结合活性。对于 2009-H1N1,化合物 **5c** 仍能产生显著的结合信号($p = 0.0048$),表明 C-9 位磺酰化修饰在该演化阶段的 HA 中同样具有良好的接纳度。对于 2020-H1N1,该流行株的 HA 蛋白对天然三糖受体本身具有较高的基础识别力,引入非天然修饰后,**5c** 等化合物的荧光值虽维持在 6000 左右,但较天然受体并未产生统计学意义上的显著提升。提示该 HA 蛋白无法依靠非天然修饰基团的引入实现结合强度的大幅提升。

分子对接结果揭示了糖链延伸导致的识别模式的转变(图 3)。二糖衍生物 **3a** 的双三氟甲基苯环深入 Val135、Leu194 和 Trp153 构成的疏水口袋,通过 π-烷基相互作用实现空间填充,且苯环氟原子与 Val135 侧链形成了关键的卤键作用。相比之下,三糖衍生物 **5c** 的末端修饰基团的空间取向发生了显著偏移,转而与 Asp225、Gln226、Lys222 等残基建立了相互作用。尽管两个化合物计算结合自由能接近(分别为 -7.1 和 -7.4 kcal/mol,表 2),但相互作用发生转变。上述结果表明:糖链骨架的延伸并非简单的结构叠加,可能通过调控末端修饰基团与 HA 蛋白识别位点的空间适配度,诱导了识别模式的转变。

表 2 化合物 3a 和 5c 与 HA 蛋白的分子对接结合能

Tab. 2 Docking binding energies of compounds 3a and 5c with HA protein

化合物	Protein	PDB ID	Binding energy /(kcal/mol)
3a	HA	3UBJ	-7.1
5c			-7.4

综合活性筛选数据,C-9 位修饰的类似物均表现出不同程度的结合活性,而所有 C-5 位修饰的类似物在相同条件下均未检测到显著结合信号。这一极端的活性差异反映了 HA 蛋白结合位点对空间构型的严苛限制,提示 C-5 位乙酰胺区域可能处于位阻敏感区,而 C-9 位则具有更好的空间容受性。在 C-9 位修饰的化合物中,芳香磺酰基修饰展现出优于烷基磺酰基(三至六元环)的结合倾向,特别是当苯环引入强电负性基

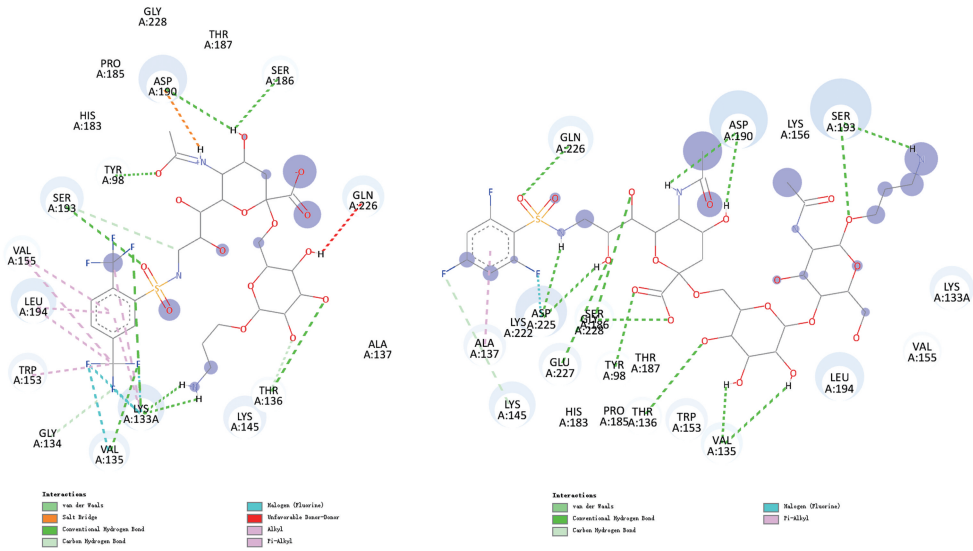


图 3 化合物 3a(左)与 5c(右)与 HA 蛋白对接示意图

Fig. 3 Docking diagrams of compound 3a(left) and 5c(right) with HA protein

团(如氟、三氟甲基)时,结合强度显著提高。此外,相同修饰基团在不同糖链骨架上的活性表现并不具有简单的叠加性:二糖系列中以 **3a**(2,5-双三氟甲基苯磺酰基)结合活性最优,而三糖系列中含氟原子较多且电子云分布更均匀的 **5c**(2,4,6-三氟苯磺酰基)则表现出更强的结合信号。这种协同效应提示,在开发高亲和力糖类似物时,需综合考量骨架长度与末端修饰基团的适配性。

3 结论

本研究通过化学-酶法模块化合成策略,建立了一套适用于非天然唾液酸类似物快速构建的技术路线,为多样化糖类配体库的构建提供了参考。聚糖微阵列分析表明 HA 蛋白的结合活性不仅取决于 C-9 位的修饰类型,还依赖于糖链骨架的长度。特定的 C-9 位功能单元能够与三糖骨架产生共同作用,从而实现跨骨架的亲和力提升,而 C-5 位修饰则普遍导致结合力丧失。化合物 **5c** 展示了对不同演化阶段 H1N1 蛋白的稳定识别特征,提示其具有作为 H1N1 亚型流感病毒 HA 蛋白高亲和力配体的开发潜力。可进一步开展细胞水平的抗病毒活性评价,验证其体外生物活性。

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