·综述·

肿瘤相关糖抗原 GM3 及其衍生物合成方法研究进展

白国静[1,2],边晓杰[2],姚 阔[2],吴俊琪[2],俞世冲[2],孟庆国[1],吴秋业[1,2](1.烟台大学药学院,山东 烟台 264005;2.第二军医大学药学院,上海 200433)

[摘要] GM3 及其衍生物合成中的糖苷化反应条件苛刻,端基碳的立体构型较难控制,能否高效地得到 α 糖苷键是评价反应优劣的重要标志之一。笔者综述了近年来 GM3 及其衍生物的合成方法,涉及糖供体化合物的选择、糖受体的确定、立体选择性的控制及新糖苷化反应催化剂的发展。

[关键词] GM3 及其衍生物;合成;糖供体;糖受体;糖苷化反应;立体选择性
[中图分类号] R914.5 [文献标志码] A [文章编号] 1006-0111(2016)01-0005-04
[DOI] 10.3969/j.issn.1006-0111.2016.01.002

Research progress in the synthesis of tumor-associated carbohydrate antigens (TACAs) GM3 and derivatives

BAI Guojing[1,2], BIAN Xiaojie[2], YAO Kuo[2], WU Junqi[2], YU Shichong[2], MENG Qingguo[1], WU Qiuye[1,2](1.College of Pharmacy, Yantai University, Yantai 264005, China; 2.School of Pharmacy, Second Military Medical University, Shanghai 200433, China)

[Abstract] Glycosylation is the key step of the synthesis of GM3, its reaction conditions are very harsh, the stereoselectivities are usually poor, and the configuration of anomeric carbon is difficult to control. Whether α glycosidic bond can be constructed efficiently in sialylation reactions is an important criteria used to evaluate the reaction quality. Studies of GM3 and derivatives methods generally relates to following areas: the choice of the donor compounds and receptor compounds, the control of stereoselectivity, and the development of some new glycosidic reaction catalyst. In recent years, important progress has been made in this research area. Now, we predominately make a summary and review on the progress of methods for the synthesis of GM3 and derivatives.

[Key words] GM3 and derivatives; synthesis; glycosyl donor; glycosylacceptor; glycosylation; stereoselectivity

1 前言

1952 年,Yamakawa[1]首次从马血红细胞中得到三糖抗原 *N*-乙酰基唾液酸-α-吡喃型半乳糖-β-吡喃型葡萄糖(GM3,结构见图 1),其在生物体内表现出重要生物学作用,如感知流感病毒 A、诱导脑脊髓瘤中白血球变异、提高或抑制蛋白激酶活性等[2],且 GM3 大量表达于消化道肿瘤、白血病和黑色素瘤等肿瘤细胞表面[3],可作为诊断和治疗癌症的新型分子靶标,其研究开发对预防治疗肿瘤具有重要意义。

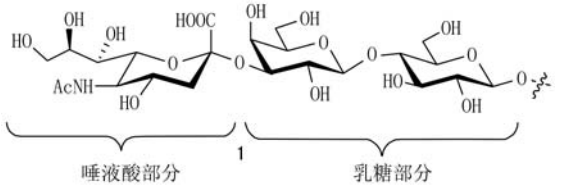

图 1 GM3 的化学结构

2 合成方法

2.1 化学合成法 化学合成法是 GM3 及其衍生物的主要合成方法。文献报道 GM3 及其衍生物的合成方法多以端位取代的唾液酸为糖供体,乳糖为受体,通过糖苷键连接得到含有唾液酸的三糖,该中间体具有良好的异头立体选择性,再与神经酰胺先导物进一步反应,得到 GM3 及其衍生物[4,5]。几种比较常见的 GM3 及其衍生物的合成方法如下。

2.1.1 以唾液酸亚磷酸酯为供体 叶新山[6]于 2012 年合成了 *N*-修饰的 GM3 衍生物。以二乙基

[基金项目] 国家自然科学基金青年项目(21202200),国家科技部重大专项重大新药创制(2012ZX09502001-005),第二军医大学本科生创新基金项目
[作者简介] 白国静,硕士研究生.E-mail:baigj2008@126.com
[通讯作者] 吴秋业,博士,教授,博士生导师.研究方向:抗肿瘤、抗真菌药物研究.Tel:(021)81871228-15;E-mail:wuqy640319@163.com

亚磷酸保护的唾液酸(**2**)为糖苷化供体,3, 2′,3′,4′位具有裸露羟基的乳糖(**3**)为糖苷化受体,在 TMSOTf(三甲硅基三氟甲磺酸酯)催化下,于 −72 ℃的乙腈/二氯甲烷(2∶3)中得到三糖中间体(**4**),并通过对其 *N*-乙酰基的结构修饰,进一步合成了两个系列的 *N*-修饰的 GM3 衍生物。

2.1.2　以唾液酸三氟乙酰亚胺酯为供体　李相鹏等[7]以较高收率制备了 α-构型的 GM3 衍生物。以 6 位 TBDPs(叔丁基二苯基硅基)保护乳糖二醇受体和供体唾液酸 *N*-苯基三氟亚胺酯(**6**),在 TMSOTf 催化下,于 −70 ℃乙腈中反应,制得 α-构型的神经节苷脂 GM3 三糖衍生物(**7**);将 **7** 的保护基 Bn(苄基)转化为 Ac(乙酰基),制得全乙酰化 GM3 三糖对甲氧基苯酚苷,收率 70%。

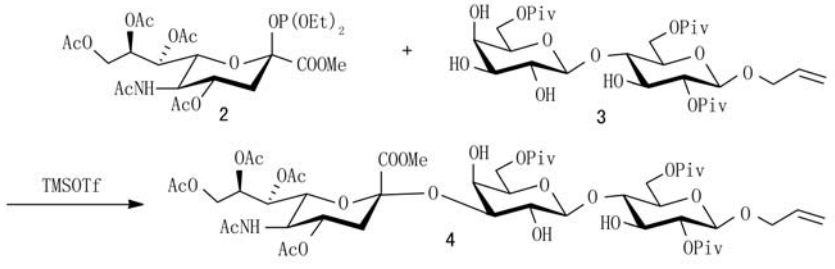

图 2　化合物 4 的合成路线

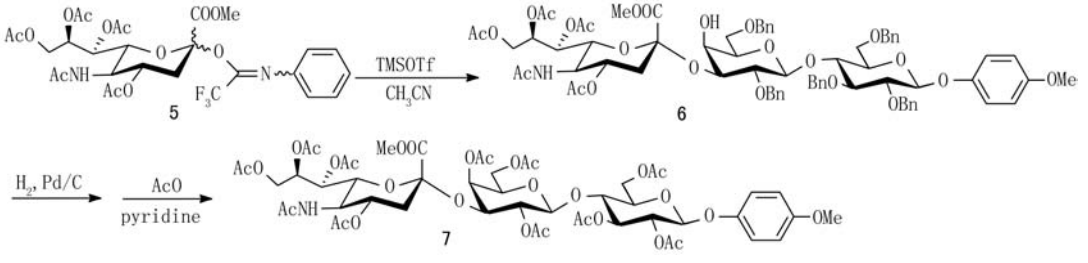

图 3　化合物 7 的合成路线

2.1.3　以唾液酸硫苷为供体　郭忠武[8-12]以唾液酸硫苷为供体,合成了一系列 GM3 衍生物。以唾液酸和乳糖为原料,制备糖苷化供体唾液酸硫苷(**8**)及糖苷化受体 3,4 位具有裸露羟基的乳糖衍生物(**9**),然后在 NIS(*N*-碘代丁二酰亚胺)、TfOH(三氟甲磺酸)催化下,得到乙酰化的 GM3(**10**)。

唾液酸硫苷为具有广泛应用的糖苷化供体,以 NIS/TMSOTf 为催化剂,可有效活化硫苷供体。但是,硫醇具有较强挥发性,实验过程中往往会伴随不良气味产生。

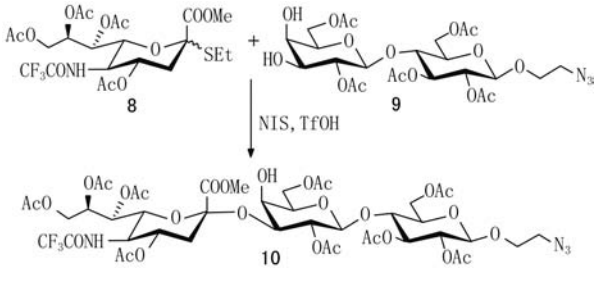

图 4　化合物 10 的合成路线

2.1.4　分子内糖基化　2008 年,Kohki[13]首次利用分子内糖基化合成了一种新型 GM3 衍生物。先用唾液酸与半乳糖反应制备糖苷化供体——唾液酸半乳糖苷(**11**)[14-16],再与糖苷化受体植物神经酰胺葡萄糖苷(**12**)在 TMSOTf 催化下,得到全保护 GM3 衍生物,收率 70%,进一步脱保护得化合物 **13**。

2.2　化学酶合成法　2013 年,SUN[17]为了监测 GM3 在生物体内的表达情况,利用化学酶法合成丹磺酸和生物素二取代荧光性神经节苷酯 GM3。他们先通过化学方法合成唾液酸糖供体和受体[18,19],再利用重组 β-半乳糖-(1″→3′/4′)-*N*-乙酰葡萄糖-α-(2‴-3″)唾液酸转移酶得到化合物(**15**),并证明其为合成 GM3 的中间体。经 13 步反应,总收率 16.2%。

化学酶法合成 GM3 可以完全控制糖苷连接反应的区域选择性和立体构型,从而得到 α 构型 GM3。但由于乳糖苷神经酰胺的唾液酸转移酶活性较低,需要附加步骤来合成更适合该酶的基底,使合成步骤更加烦琐,不适于大量生产。

3　结语

随着 GM3 及其衍生物合成研究的深入,各种

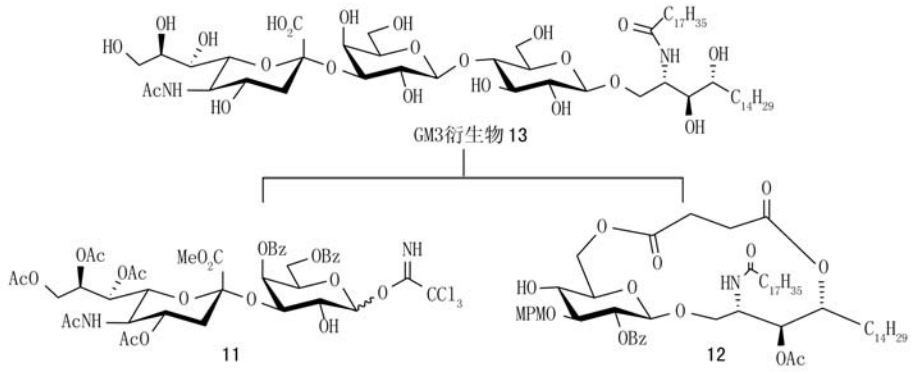

图 5　神经节苷酯 GM3 衍生物 13 的逆合成路线

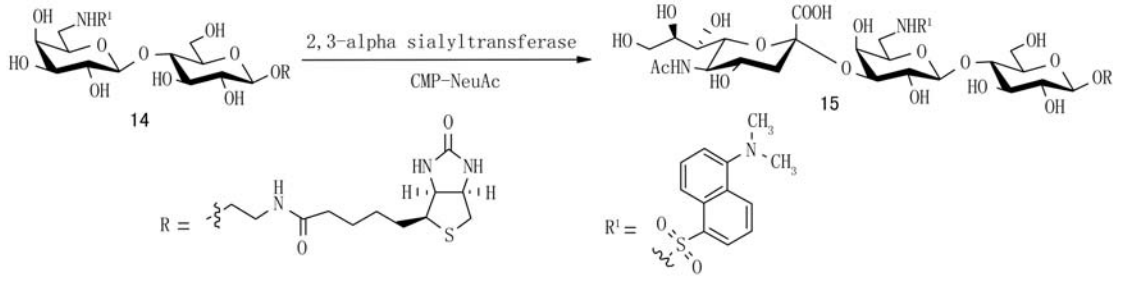

图 6　丹磺酸和生物素二取代荧光性神经节苷酯 GM3 15 的合成路线

不同结构的唾液酸糖苷化供体及糖受体相继被设计与合成出来，以提高糖苷化反应产率和 α 立体选择性。其中，设计唾液酸硫苷、唾液酸亚磷酸酯和唾液酸三氟乙酰亚胺酯等有效的糖苷化供体，通过糖苷键与各种乳糖受体连接来制备 GM3 及其衍生物，仍是 GM3 合成的主要方法。除化学合成法，化学酶法也是 GM3 合成的常见方法，但需先用化学方法合成糖供体与受体，再在酶催化下生成 GM3，可以完全控制糖苷连接反应的区域选择性和立体构型，但是步骤烦琐，需要多种酶的参与。

总之，一种理想的 GM3 合成路线应满足以下条件：①合成路线短，制备过程简便；②反应活性和产率高；③具有较高的立体选择性。在此基础上，相信在不久的将来，一些更加高效的合成方法将被研究出来。随着研究的深入将为其他糖类的合成研究提供依据及方法支持。

【参考文献】

[1] Yamakawa T, Suzuki S. The chemistry of the lipids of posthemolytic residue or stroma of erythrocytes I concerning the ether-insoluble lipids of lyophilized horse Bblood stroma [J]. J Biochem, 1951, 38 (2): 199-212.

[2] Giussani P, Tringali C, Riboni L, *et al*. Sphingolipids: key regulators of apoptosis and pivotal players in cancer drug resistance [J]. Int J Mol Sci, 2014, 15 (3): 4356-4392.

[3] Tringali C, Silvestri I, Testa F, *et al*. Molecular subtyping of metastatic melanoma based on cell ganglioside metabolism profiles [J]. BMC cancer, 2014, 14 (1): 560.

[4] Duclos JR. The total synthesis of ganglioside GM3 [J]. Carbohydr Res, 2000, 328 (4): 489-507.

[5] Imamura A, Yoshikawa T, Komori T, *et al*. Design and synthesis of versatile ganglioside probes for carbohydrate microarrays [J]. Glycoconj J, 2008, 25 (3): 269-278.

[6] Zheng M, Ye XS. Synthesis of *N*-modified ganglioside GM3 derivatives [J]. Tetrahedron Lett, 2012, 68 (5): 1475-1482.

[7] 李相鹏，王　鹏，李英霞. 全乙酰 GM3 三糖对甲氧基苯酚苷的合成[J].合成化学，2010，18 (5)：538-542.

[8] Chefalo P, Pan Y, Nagy N, *et al*. Harding CV. Efficient metabolic engineering of GM3 on tumor cells by *N*-phenylacetyl-D-mannosamine [J]. J Biochem, 2006, 45 (11): 3733-3739.

[9] Yu S, Wang Q, Zhang J, *et al*. Synthesis and evaluation of protein conjugates of GM3 derivatives carrying modified sialic acids as highly immunogenic cancer vaccine candidates [J]. Medchemcomm, 2011, 2 (6): 524-530.

[10] Wang Q, Zhou Z, Tang S, *et al*. Carbohydrate-monophosphoryl lipid A conjugates are fully synthetic self-adjuvanting cancer vaccines eliciting robust immune responses in the mouse [J]. ACS Chem Biol, 2011, 7 (1): 235-240.

[11] Tang S, Wang Q, Guo Z. Synthesis of a monophosphoryl derivative of escherichia coli lipid A and its efficient coupling

（下转第 15 页）

Flow Metab, 2014, 34(3):369-375.

[2] Heneka MT, Kummer MP, Andrea Stutz, *et al*. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice[J]. Nature, 2013, 493(7434):674-678.

[3] Weber MD, Frank MG, Tracey KJ, *et al*. Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male Sprague Dawley rats: a priming stimulus of microglia and the NLRP3 inflammasome[J]. J Neurosci, 2015, 35(1):316-324.

[4] Ying P, Yang CX, Yu ZQ, *et al*. Microglial NLRP3 inflammasome activation mediates IL-1β-related inflammation in prefrontal cortex of depressive rats[J]. Brain Behav Immun, 2014, 41:90-100.

[5] Cribbs DH, Berchtold NC, Perreau V, *et al*. Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study[J]. J Neuroinflammation, 2012, 9: 179-196.

[6] Shao BZ, Wei W, Ke P, *et al*. Activating cannabinoid receptor 2 alleviates pathogenesis of experimental autoimmune encephalomyelitis via activation of autophagy and Inhibiting NLRP3 inflammasome[J]. CNS Neurosci Ther, 2014, 20(12):1021-1028.

[7] Weber MD, Frank MG, Tracey KJ, *et al*. Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male Sprague Dawley rats: a priming stimulus of microglia and the NLRP3 inflammasome[J]. J Neurosci, 2015, 35:316-324.

[8] Walsh JG, Muruve DA, Paver C. Inflammasomes in the CNS[J]. Nat Rev Neurosci, 2014, 15(2):84-97.

[9] Kumar M, Roe K, Orillo B, *et al*. Inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD (ASC) is critical for the immune response and survival in West Nile virus encephalitis[J]. Virol, 2013, 87:3655-3667.

[10] Hoegen T, Tremel N, , *et al*. The NLRP3 inflammasome contributes to brain injury in pneumococcal meningitis and is activated through ATP-dependent lysosomal cathepsin B release[J]. J Immunol, 2011, 187:5440-5451.

[11] Pradillo JM, Denes A, Greenhalgh AD, *et al*. Delayed administration of interleukin-1 receptor antagonist reduces ischemic brain damage and inflammation in comorbid rats[J]. J Cereb Blood Flow Metab, 2012, 32:1810-1819.

[12] Lupfer C, Kanneganti TD. Unsolved Mysteries in NLR Biology[J]. Front Immunol, 2013, 4:285-294.

[13] Richter K, Kiefer KP, Grzesik BA, *et al*. Hydrostatic pressure activates ATP-sensitive K^+ channels in lung epithelium by ATP release through pannexin and connexin hemichannels[J]. FASEB J, 2014, 28(1):45-55.

[14] Kampen JMV, Baranowski D, Kay DG. Progranulin gene delivery protects dopaminergic neurons in a mouse model of Parkinson's disease[J]. PLoS One, 2014, 9(5):e97032.

[15] Codolo G, Plotegher N, Pozzobon T, *et al*. Triggering of inflammasome by aggregated α-synuclein, an inflammatory response in synucleinopathies[J]. PLoS One, 2013, 8(1): e55375.

[16] Lu M, Sun XL, Qiao C, *et al*. Uncoupling protein 2 deficiency aggravates astrocytic endoplasmic reticulum stress and nod-like receptor protein 3 inflammasome activation[J]. Neurobiology Aging, 2014, 35:421-430.

[17] Guillot-Sestier MV, Town T. Innate immunity in Alzheimer's disease: a complex affair[J]. CNS Neurol Disord Drug Targets, 2013, 12:593-607.

[18] Halle A, Hornung V, Petzold GC, *et al*. The NALP3 inflammassome is involved in the innate immune reponse to amyloid-beta[J]. Nat Immunol, 2008, 9(8):857-865.

[19] Tan Ms, Yu JT, Jiang T, *et al*. The NLRP3 inflammasome in Alzheimer's disease[J]. Mol Neurobiol, 2013, 84(3):875-82.

[收稿日期] 2015-07-09 [修回日期] 2015-10-15
[本文编辑] 陈 静

(上接第 7 页)

to a tumor-associated carbohydrate antigen[J]. Chemistry, 2010, 16(4): 1319-1325.

[12] Pan Y, Chefalo P, Nagy N, *et al*. Synthesis and immunological properties of *N*-modified GM3 antigens as therapeutic cancer vaccines[J]. J Med Chem, 2005, 48(3): 875-883.

[13] Fujikawa K, Imamura A, Ishida H, *et al*. Synthesis of a GM3 ganglioside analogue carrying a phytoceramide moiety by intramolecular glycosylation as a key step[J]. Carbohydr Res, 2008, 343(16): 2729-2734.

[14] Ando H, Koike Y, Ishida H, *et al*. Extending the possibility of an *N*-Troc-protected sialic acid donor toward variant sialoglycoside synthesis[J]. Tetrahedron Lett, 2003, 44(36): 6883-6.

[15] Ando H, Ishida H, Kiso M, *et al*. A synthetic approach to the c-series gangliosides containing sialyl-α(2→8) sialyl-α(2→8) sialic acid: Synthesis of ganglioside GT4, α(2→6) GT4 and GT3[J]. Carbohydr Res, 1997, 300(3):207-217.

[16] Kameyama A, Ishida H, Kiso M, *et al*. Stereoselective synthesis of sialyl-lactotetraosylceramide and sialylneo-lactotetraosylceramide[J]. Carbohydr Res, 1990, 200: 269-285.

[17] Sun B, Jiang H. Total synthesis of dansyl and biotin functionalized ganglioside GM3 by chemoenzymatic method[J]. Sci China Chem, 2013, 56(7): 933-938.

[18] Sun B, Jiang H. An efficient approach for total synthesis of aminopropyl functionalized ganglioside GM1b[J]. Tetrahedron Lett, 2012, 53(42): 5711-5715.

[19] Sun B, Yang B, Huang X. Total synthesis of the aminopropyl functionalized ganglioside GM1[J]. Sci China Chem, 2012, 55(1): 31-35.

[收稿日期] 2014-12-03 [修回日期] 2015-03-27
[本文编辑] 陈 静