Tetrahedron Letters 54 (2013) 1198–1201

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

An efficient synthesis of C3 C-alkylated Neu5Ac2en derivatives

Santosh Rudrawar, Mauro Pascolutti, Beenu Bhatt, Robin J. Thomson, Mark von Itzstein *

Institute for Glycomics, Griffith University, Gold Coast Campus, Queensland 4222, Australia

ARTICLE INFO

Article history:
Received 13 October 2012
Revised 29 November 2012
Accepted 13 December 2012
Available online 25 December 2012

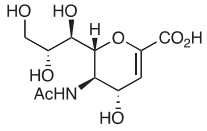
Keywords:
Influenza
Sialidase
Carbohydrate
Neu5Ac2en
Sialic acid

ABSTRACT

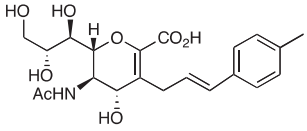
C3-modified Neu5Ac2en derivatives can be used to study the flexible 150-loop region of influenza virus sialidases and also provide a new template for the development of next-generation sialidase inhibitors. This Letter describes an efficient synthetic route towards the large scale synthesis of C3 C-alkylated Neu5Ac2en derivatives. The key intermediate, a 3-*eq*-allyl-Neu5Ac derivative, is formed by stereoselective addition of the allyl group in an equatorial configuration at C3, regardless of the stereochemistry of the bromohydrin precursor.

Influenza is a significant human disease, as evidenced by seasonal epidemics that exact a high toll in morbidity and mortality, and worldwide pandemics, for example the 2009 'swine-origin' influenza A pandemic. Two influenza virus-specific drugs, Relenza® and Tamiflu®, target the viral enzyme sialidase, and are effective against all wild type influenza virus strains.[1] Resistance development[2] to the widely used drug, Tamiflu®, however, has fuelled a drive to better understand fundamental aspects of sialidase inhibitor interactions and to develop new sialidase-inhibitor anti-influenza drugs. Recent X-ray crystal structures of a sub-group of influenza A virus sialidases, that includes the N1 sub-types associated with currently circulating and pandemic influenza A viruses, indicate the flexibility of a protein loop (the '150-loop') adjacent to the enzyme active site.[3] When this loop is in an 'open' conformation, a significant cavity is formed adjacent to the active site.[3]

Development of probes and inhibitors which bind the extended sialidase active site is an area of growing interest.[4–7] Our work in this area has shown that suitable functionality introduced at the C3 position of the general sialidase inhibitor Neu5Ac2en (**1**), can extend into the 150-cavity and lock the flexible 150-loop in an open conformation.[6] C3-Modified Neu5Ac2en derivatives such as **2** (a micromolar inhibitor of a number of N1 sialidases[6]) provide tools to study the 150-loop region of influenza virus sialidases, and also provide a new template for the development of more potent sialidase inhibitors.



1 Neu5Ac2en



2 3-(*p*-tolylallyl)-Neu5Ac2en

The synthesis of C3-substituted Neu5Ac2en derivatives was until recently an unexplored field. For the synthesis of **2**, a reaction pathway was developed[6] in which an allyl group was initially installed at C3 on the saturated template, giving 3-*eq*-allyl *N*-acetylneuraminic acid (Neu5Ac) derivative **6** (Scheme 1). Subsequent manipulation of **6** to introduce a halide at C2 allowed the generation of the corresponding C2–C3 unsaturated derivative by base-promoted beta-elimination of HCl.[6] The key 3-*eq*-allyl-Neu5Ac derivative **6** is prepared via radical-mediated allylation of the *trans*-2,3-diequatorial bromohydrin **4**.[6,8] A low isolated yield (38%) of the required bromohydrin **4**,[6] however, was a bottle-neck in this synthesis.

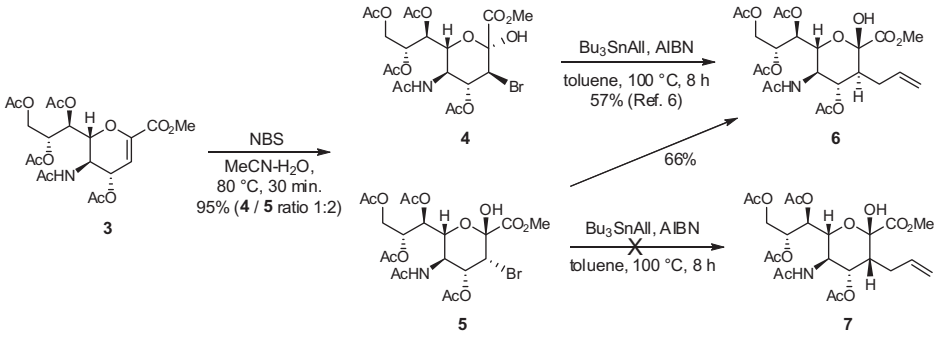
In addition, a moderate yield of the allylation reaction to give **6**,[6] led us to further investigate the synthetic strategy to 3-*eq*-allyl-Neu5Ac derivative **6** that would provide efficient, and scalable access to novel C3 C-alkylated Neu5Ac2en derivatives.

Herein, we report a synthesis of C3-substituted Neu5Ac2en derivatives that is successful on a multi-gram scale.

The high-yielding, regioselective, bromohydroxylation of protected Neu5Ac2en **3**, using *N*-bromosuccinimide (NBS) and water in the presence of a co-solvent, gives a mixture of *trans*-2,3-diequatorial **4** and *trans*-2,3-diaxial **5** bromohydrins with the ratio varying from 1:0.1 to 1:3.[9] The stereoselectivity of the NBS-mediated

* Corresponding author. Tel.: +61 7 5552 7025; fax: +61 7 5552 9040.
E-mail address: m.vonitzstein@griffith.edu.au (M. von Itzstein).

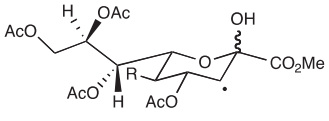
0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.12.064



Scheme 1.

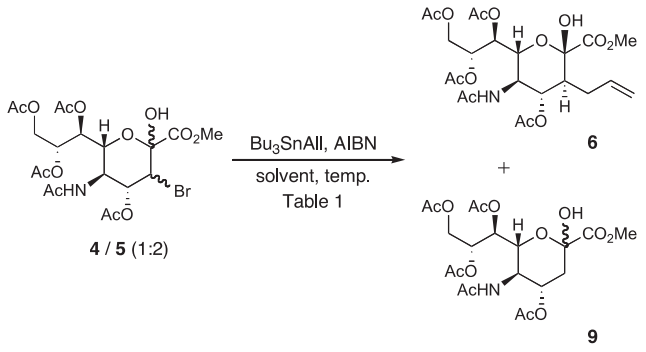
bromohydroxylation is governed by solvent composition, reaction temperature and the stereoelectronic nature of the substituent at C4.[10] The two bromohydrins formed are, however, difficult to separate on silica gel in a number of different solvent combinations, and require several chromatographic purifications to isolate the pure isomers. Given that the *trans*-2,3-diaxial bromohydrin **5** is always formed in the bromohydroxylation of **3**, we examined the reactivity of **5** towards C3 allylation (Scheme 1). Reaction of **5** at 100 °C in toluene with allyltributyltin using AIBN as the free-radical initiator afforded a single C3-allylated product in 66% yield. Interestingly, the main product was assigned as the C3 equatorially-allylated derivative **6**, rather than the C3 axially-substituted product **7**, based on the large $J_{3,4}$ value (10.5 Hz) of the product, and comparison with NMR data for **6** prepared previously from the C3 equatorial bromohydrin.[6,8] The yield of **6** was slightly improved when compared to reaction with *trans*-2,3-diequatorial bromohydrin **4** (57%[6]) under the same reaction conditions, and importantly was reproducible on a large scale (up to 25 g, 44 mmol, of **5**).

Precursor *trans*-2,3-diequatorial **4** and *trans*-2,3-diaxial **5** bromohydrins each give rise to the same C3 equatorially-substituted product **6**, indicating that the stereoselectivity of the free-radical reaction is evidently not influenced by the configuration of the activating group (bromide) on the C3 position. Substituents adjacent to the radical centre in glycopyranosyl radicals influence the direction of attack on that centre:[11] where the radical centre is flanked by equatorial substituents there is a marked preference for an equatorial product; where the substituents are axial, the preference is for axial attack.[11] In the case of the radical species **8** formed from bromohydrins **4**/**5**, the bulkiest substituents β to the radical centre (4-OAc, 2-CO_2Me) are equatorial. The formation of the equatorially allylated product **6** is therefore consistent with the reported[11] trend.



8 R = NHAc

In light of this result, the reactivity of bromohydrin mixture **4**/**5** towards the allylation reaction was examined. Reaction of a mixture of **4**/**5** (ratio 1:2) at 100 °C in toluene with allyltributyltin in the presence of AIBN afforded the single C3 equatorially-allylated product **6** in 68% yield (Scheme 2, Table 1, entry 1). Using the bromohydrin mixture, the overall yield of C3 *C*-allyl derivative **6** from glycal **3** over two steps was 63%, a substantial increase in yield over that previously achieved (22%[6]) via *trans*-2,3-diequatorial bromohydrin **4**.



Scheme 2.

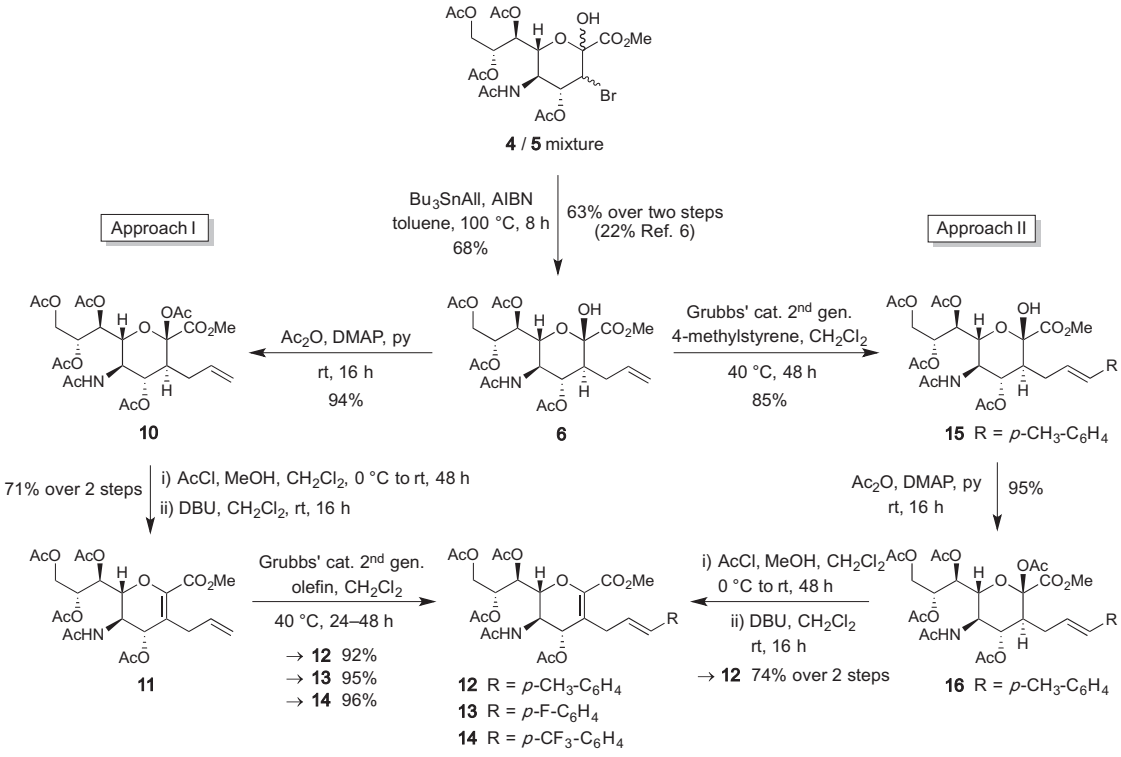
Table 1
Reaction conditions evaluated for C3 allylation of bromohydrins **4**/**5** using Bu_3SnAll in the presence of AIBN[a]

Entry	Solvent and reaction conditions[b]	Outcome[c] (yield %)		
		4/**5**	**6**	**9**
1	Toluene, 100 °C, 8 h	—	68	22
2	Benzene, 80 °C, 10 h	—	79	8
3	THF, 60 °C, 10 h	5	8	80
4	1,4-Dioxane, 100 °C, 8 h	10	20	35
5	MeCN, 80 °C, 10 h	15	40	35
6	1,2-Dichloroethane, 80 °C, 10 h	18	26	40

[a] All reactions were carried out on 0.17 mmol (0.1 g) of a mixture of **4**/**5** mixture (ratio = 1:2), except for entries 1 (1.75 mmol, 1 g) and 2 (0.35 mmol, 0.2 g).
[b] Reaction composition: **4**/**5** (0.17 mmol), Bu_3SnAll (0.84 mmol), AIBN (0.017 mmol), anhydrous solvent (8 mL).
[c] Isolated yields.

To investigate if the yield of **6** could be further improved, we studied the effects of solvent on the outcome of the allylation reaction. The use of toluene (chosen[6] to achieve reaction at high temperature) as a solvent for the free-radical allylation of bromohydrin **4** always proceeded with the formation of de-brominated Neu5Ac derivative **9** as a by-product. (e.g., 22% under the optimised reaction conditions; Table 1, entry 1).

Presumably, the formed radical at the C3 position abstracts a hydrogen atom from the solvent (toluene). Bromohydrin mixture **4**/**5** was therefore reacted with allyltributyltin in alternative solvents (benzene, THF, 1,4-dioxane, acetonitrile, 1,2-dichloroethane) (Scheme 2). The results of these reactions are summarised in Table 1. Interestingly, replacing toluene with anhydrous benzene led to an increase in reaction yield (79%, up from 68%), with the de-brominated derivative **9** formed in only ~8% yield (Table 1, entry 2). Reaction in THF, 1,4-dioxane, acetonitrile or 1,2-dichloroethane (DCE), however, resulted in less than satisfactory yields of **6** (Table 1, entries 3–6). While reaction in benzene gave the most



Scheme 3.

satisfactory outcome, its use for large-scale reactions is disfavoured due to its known toxicity.[12]

We next explored two approaches (Scheme 3) for the conversion of **6** into C3 C-alkylated Neu5Ac2en derivatives. Approach I (which was carried-out on the indicated scale) provided the advanced stage key intermediate, 3-allyl-Neu5Ac2en derivative **11**. This can be further elaborated through manipulation of the allyl substituent to prepare a diverse range of compounds to explore structure–activity relationships. Acetylation of the C2 hydroxyl group of **6** (29 mmol; Ac_2O, DMAP, anhydrous pyridine) gave 2-O-acetate derivative **10** in 94% yield (Scheme 3, approach I). Unsaturation was introduced between C2 and C3 using a two step reaction sequence: conversion of **10** (14 mmol) into the 2-β-chloro derivative by reaction with in situ generated HCl gas, followed by treatment with DBU in CH_2Cl_2 affording the desired 3-allyl-Neu5Ac-2en derivative **11**[6] in 71% yield over two steps. In this larger-scale reaction, the yield of **11** from **10** was substantially increased over that previously reported (46%[6]), by extending the reaction time from 8[6] to 16 h.

To illustrate the synthetic potential of **11**, we examined its cross-metathesis reactions with aromatic olefins to give C3-extended Neu5Ac2en derivatives. The cross-metathesis reaction of **11** (7.6 mmol) with 4-methylstyrene using the second-generation Grubbs catalyst (15 mol %) in CH_2Cl_2 at 40 °C provided **12** in 92% yield. For this larger scale reaction, as compared to the original synthesis,[6] addition of the ruthenium catalyst in two batches (10 mol % at the beginning of the reaction and 5 mol % after 24 h) and extension of the reaction time from 24 to 48 h resulted in an improved yield of **12** (92%, compared to 69%[6]). Similarly, cross-metathesis reactions of **11** (0.4 mmol scale) with 4-fluorostyrene and 4-(trifluoromethyl)styrene proceeded smoothly to give coupled products **13** (95%) and **14** (96%), respectively.

In approach II (Scheme 3), the reaction sequence was explored in reverse, beginning with olefin cross-metathesis of 3-allyl-Neu5Ac derivative **6** (6 mmol) with 4-methylstyrene. This afforded product **15** (85%) in a similar high yield to the reactions carried out on unsaturated derivative **11**. Subsequent processing of **15** as for **6**—2-O-acetylation, 2-β-chlorination, and subsequent DBU-catalysed β-elimination of HCl—provided the 2,3-unsaturated derivative **12** (70% yield over 3 steps from **15**). The overall yield of **12** from **6** via either Approach I or II was approximately 60%.

In summary, an efficient synthetic route to the synthesis of C3 C-alkylated Neu5Ac2en derivatives has been developed. The formation of key 3-*eq*-allyl-Neu5Ac derivative **6**, required for the later introduction of the C2–C3 double bond, is facilitated by a highly stereoselective addition of the allyl group in an equatorial configuration at C3, regardless of the stereochemistry of the bromohydrin precursor. This outcome provides a significant improvement over our previously reported synthesis of these derivatives.[6] Subsequent elaboration provides access to C3 C-alkylated Neu5Ac2en derivatives. This pathway is amenable to scale-up, with reaction conditions optimised to prepare the C3 *C*-allyl Neu5Ac2en derivative **6** on a 15 g scale. This work provides access to a new inhibitor template for exploration of influenza virus sialidases and other Neu5Ac2en-recognising proteins.

Acknowledgments

This work was supported by the Honda Foundation (Australia) through a Grant to the Institute for Glycomics.

Supplementary data

Supplementary data (experimental details and data for new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.12.064.

References and notes

1. von Itzstein, M. *Nat. Rev. Drug Disc.* **2007**, *6*, 967–974.
2. Ison, M. G. *Curr. Opin. Virol.* **2011**, *1*, 563–573.

3. (a) Russell, R. J.; Haire, L. F.; Stevens, D. J.; Collins, P. J.; Lin, Y. P.; Blackburn, G. M.; Hay, A. J.; Gamblin, S. J.; Skehel, J. J. *Nature* **2006**, *443*, 45–49; (b) Wang, M.; Qi, J.; Liu, Y.; Vavricka, C. J.; Wu, Y.; Li, Q.; Gao, G. F. J. *Virology* **2011**, *85*, 8431–8435.
4. Wen, W.-H.; Wang, S.-Y.; Tsai, K.-C.; Cheng, Y.-S. E.; Yang, A.-S.; Fang, J.-M.; Wong, C.-H. *Bioorg. Med. Chem.* **2010**, *18*, 4074–4084.
5. Mohan, S.; McAtamney, S.; Haselhorst, T.; von Itzstein, M.; Pinto, B. M. *J. Med. Chem.* **2010**, *53*, 7377–7391.
6. Rudrawar, S.; Dyason, J. C.; Rameix-Welti, M.-A.; Rose, F. J.; Kerry, P. S.; Russell, R. J. M.; van der Werf, S.; Thomson, R. J.; Naffakh, N.; von Itzstein, M. *Nat. Commun.* **2010**, *1*, 113.
7. Ye, D.; Shin, W.-J.; Li, N.; Tang, W.; Feng, E.; Li, J.; He, P.-L.; Zuo, J.-P.; Kim, H.; Nam, K.-Y.; Zhu, W.; Seong, B.-L.; No, K. T.; Jiang, H.; Liu, H. *Eur. J. Med. Chem.* **2012**, *54*, 764–770.
8. Paulsen, H.; Matschulat, P. *Liebigs Ann. Chem.* **1991**, 487–495.
9. Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 631–636.
10. Kok, G. B.; van Phan, T.; von Itzstein, M. J. *Carbohydr. Chem.* **2001**, *20*, 359–374.
11. (a) Giese, B.; Gröninger, K. *Tetrahedron Lett.* **1984**, *25*, 2743–2746; (b) Giese, B. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 969–980.
12. Rana, S. V.; Verma, Y. *J. Environ. Biol.* **2005**, *26*, 157–168.