ORGANIC LETTERS

XXXX
Vol. XX, No. XX
000–000

Tuning Reactivity of Glycosyl Imidinium Intermediate for 2-Azido-2-deoxyglycosyl Donors in α-Glycosidic Bond Formation

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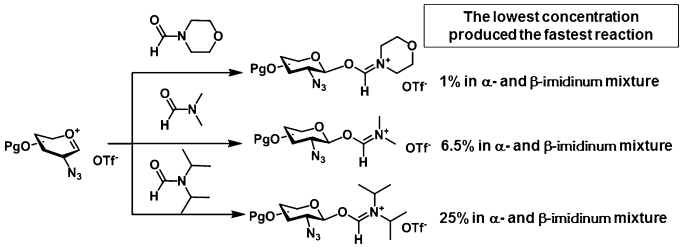
Received September 2, 2013

ABSTRACT



The chemical properties of nucleophile additives were investigated in a modulated glycosylation context. *N*-Formylmorpholine (NFM) was found to be an effective modulator for glycosylation with less reactive 2-azido-2-deoxythioglucosyl and thiogalactosyl donors.

Different glycosylation approaches have been developed to achieve pure and structurally defined oligosaccharide compounds.[1−3] Oligosaccharide synthesis involves regio- and stereospecific assembly of glycosyl building units. Such requirements are usually fulfilled by designing glycosyl building blocks with suitable reactivity profiles and stereodirecting functional groups.[4−7]

The reactivity of glycosyl building blocks is generally modified by protecting functions. For example, a glycosyl donor with acyl-protecting functions is less reactive in glycosylation than a glycosyl donor carrying benzyl ether protection.[8] This electronic effect was elaborated by Frasier-Reid et al. in relation to the seminal armed-disarmed glycosylation concept.[9] Alternatively, external nucleophiles can be used to modulate the reactivity of the glycosyl donor. A bromide nucleophile was exploited as a catalyst in the conversion of α-glycosyl bromide to the more reactive *β*-glycosyl bromide for glycosylation coupling.[10] *β*-Glucosyl onium adducts prepared from substitution of α-glucosyl bromide with dimethyl sulfide (Me_2S), triethylamine (Et_3N), and phosphine (Ph_3P) nucleophiles exhibited different glycosylation reactivity.[11] A difference in reactivity was also observed for glycosyl sulfonium adducts prepared from different thioether nucleophiles.[12,13]

In 2011, we described an α-selective glycosylation method[14] that used dimethylformamide (DMF) to trap glycosyl oxacarbenium ions as glycosyl imidinium adducts. Nucleophilic displacement of the glycosyl imidinium adducts with an acceptor furnishes a glycosylation product with good α-selectivity. We coined the term "DMF-modulated glycosylation" for this method. Later studies found that this method was impractically slow for

(1) Seeberger, P.-H. *Chem. Soc. Rev.* **2008**, *37*, 19–28.
(2) Hsu, C.-H.; Hung, S.-C.; Wu, C.-Y.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2011**, *50*, 11872–11923.
(3) Ranade, S. C.; Demchenko, A. V. *J. Carbohydr. Chem.* **2013**, *32*, 1–43.
(4) Kerns, R.; Wei, P. *ASC Symp. Ser.* **2012**, 235–263.
(5) Enugala, R.; Carvalho, L. C. R.; Pires, M. J. D.; Marques, M. M. B. *Chem.—Asian J.* **2012**, *7*, 2482–2501.
(6) Manabe, S.; Ito, Y. *Curr. Bioactive Compd.* **2008**, *4*, 258–281.
(7) Yang, L.; Qin, Q.; Ye, X.-S. *Asian J. Org. Chem.* **2013**, *2*, 30–49.
(8) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.
(9) (a) Fraser-Reid, B.; Wu, Z.; Udodong, U.; Ottoson, H. *J. Org. Chem.* **1990**, *55*, 6068–6070. (b) Fraser-Reid, B.; Wu, Z.; Andrews, C. W.; Skowrinski, E. *J. Am. Chem. Soc.* **1991**, *113*, 1434–1435.
(10) Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
(11) West, A.; Schuerch, C. *J. Am. Chem. Soc.* **1973**, *95*, 1333–1335.
(12) Nokami, T.; Shibuya, A.; Manabe, S.; Ito, Y.; Yoshida, J. *Chem.—Eur. J.* **2009**, *15*, 2252–2255.
(13) Nokami, T. *Trends Glycosci. Glycotechnol.* **2012**, *24*, 203–214.
(14) Lu, S.-R.; Lai, Y.-H.; Chen, J.-H.; Liu, C.-Y.; Mong, K.-K. T. *Angew. Chem., Int. Ed.* **2011**, *50*, 7315–7320.

10.1021/ol402519c

glycosylations of secondary glycosyl acceptors with 2-azido-2-deoxy-glycosyl donors. Because 2-azido-2-deoxy-glycosyl donors are widely used for α-glycoside formation,[4,5,15] a more effective modulated glycosylation method is necessary.

Because glycosyl imidinium adducts are the key in modulated glycosylation, we speculated that their reactivity might be tuned by variation of the nucleophile additive. Accordingly, we designed experiments to evaluate the properties of different nucleophiles in the modulated glycosylation context using model glycosyl substrates **1**, **2**, and **3** (Scheme 1, Table 1). At the outset, thiogalactosyl donor **1** undertaken in the modulated glycosylation procedure was coupled with galactosyl acceptor **3**.[14] In the present study, *N*,*N*-diisopropylformamide (DIPF), *N*-formylpiperidine (NFP), *N*-formylmorpholine (NFM), dimethylacetamide (DMA),[16] tetramethylurea (TMU),[17] triphenylphosphine oxide (TPP),[18] and diphenyl sulfoxide (DPSO)[19] were used as the nucleophiles for evaluation (Table 1, entries 1−9).

In general, formamide compounds offer higher α-selectivity of glycosylation than do nonformamide compounds (entries 1−3 vs 4−6). No glycosylation product was obtained when DPSO was applied. Among the formamide compounds examined, DIPF offered the highest α-selectivity of glycosylation, but the longest reaction time of 6 h was required (entry 4). In contrast, NFM-modulated glycosylation could be completed within 2 h, but the α-selectivity obtained was moderate (entry 6). Next, we increased the amount of DIPF and NFM to 4.0 equiv (entries 7 and 8).

Although the DIFP-modulated glycosylation produced an excellent 36:1 α/β ratio of disaccharide **4**, the reaction yield dropped from 74% to 68%, and the reaction time was prolonged from 6 to > 10 h (entry 4 vs 7). In contrast, the reaction time and yield of the NFM-modulated glycosylations were similar at 2.0 and 4.0 equiv of NFM, and to our delight, the α/β ratio of **4** was improved from 5:1 (at 2 equiv) to 13:1 (at 4 equiv). Along this line, we increased the amount of NFM to 16.0 equiv, and the α/β ratio was further raised to 39:1 (entry 9).[20]

These evaluation studies convinced us to apply NFM as the modulator for glycosylations with 2-azido-2-deoxy-thioglycosyl donors. To validate the reaction procedure, 2-azido-2-deoxythiogalactosyl donor **2** was activated with NIS and TMSOTf in the presence of 2.0, 4.0, 8.0, and 16.0 equiv of NFM, followed by coupling with the galactosyl acceptor **3** (entries 10−13). The glycosylations were complete in ca. 5−6 h with excellent 91% yield despite the stoichiometric amount of NFM, and the highest 13:1 α/β ratio of glycosylation product **5** was obtained with 16.0 equiv of NFM.

After validating the glycosylation procedure, we applied the low-temperature NMR spectroscopy method to probe the glycosyl imidinium adducts. Thus, 2-azido-2-deoxy thiogalactoside **6** in $CDCl_3$ was activated with NIS and TMSOTf promoters in the presence of 2.0 equiv of the DMF, DIPF, or NFM nucleophile, and the preactivation mixture was taken for NMR analysis.

A set of signals at chemical shifts (δ) of 6.18, 6.30, and 6.26 ppm were found in the ^{1}H NMR spectra of the preactivation mixture (Figure 1). Referring to spectroscopic data of known α-imidinium adducts,[14] the signals were assigned to the α-anomeric ^{1}H signals ($H_{1\alpha}$) of the

Scheme 1. Glycosylation with Nucleophile (Nu) Additives

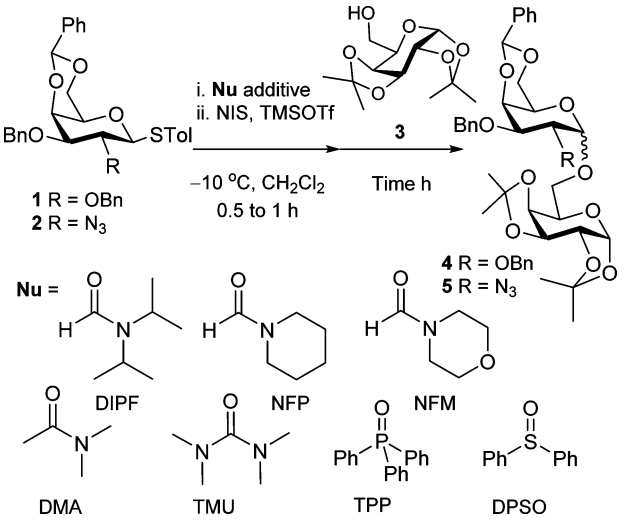


Table 1. Results of Glycosylation of Galactosyl Acceptor **3** with Thiogalactoside **1** or 2-Azido-2-deoxythiogalactoside **2**

entry	donor	nucleophile (equiv)	time (h)	product, yield (%), α/β^a
1	**1**	DMA (2)	5	**4**, 77, 3:1
2	**1**	TMU (2)	6	**4**, 70, 1:1
3	**1**	TPP (2)	2	**4**, 83, 1.2:1
4	**1**	DIPF (2)	6	**4**, 74, 10:1
5	**1**	NFP (2)	3	**4**, 77, 8:1
6	**1**	NFM (2)	2	**4**, 85, 5:1
7	**1**	DIPF (4)	>10[b]	**4**, 68, 36:1
8	**1**	NFM (4)	2	**4**, 92, 13:1
9	**1**	NFM (16)	2	**4**, 92, 39:1
10	**2**	NFM (2)	2	**5**, 90, 4:1
11	**2**	NFM (4)	5	**5**, 92, 8.5:1
12	**2**	NFM (8)	5	**5**, 90, 11:1
13	**2**	NFM (16)	6	**5**, 91, 13:1

[a] α/β of **4** and **5** were determined by HPLC analysis. [b] The reaction was not complete, and ca. 10−20% of acceptor **3** was recovered.

(15) (a) Paulsen, H.; Kalar, C.; Stenzel, W. *Chem. Ber.* **1978**, *111*, 2358–2369. (b) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244–1251.

(16) (a) Koto, S.; Morishima, N.; Owa, M.; Zen, S. *Carbohydr. Res.* **1984**, *130*, 73–83. (b) Koto, S.; Asami, K.; Hirooka, M.; Nagura, K.; Takiazawa, M.; Yamamoto, S.; Okamoto, N.; Sato, M.; Tajima, H.; Yoshida, T.; Nonaka, N.; Sato, T.; Zen, S.; Yago, K.; Tomonaga, F. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 765–777.

(17) (a) Jiaang, W.-T.; Hsiao, K.-F.; Chen, S.-T.; Wang, K.-T. *Synlett* **1999**, 1687–1690. (b) Jiaang, W.-T.; Chang, M.-Y.; Tseng, P.-H.; Chen, S.-T. *Tetrahedron Lett.* **2000**, *41*, 3727–3730.

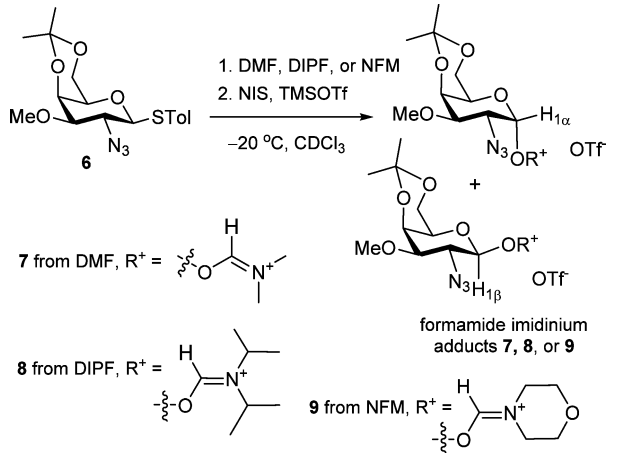
(18) Kobashiy, Y.; Mukaiyama, T. *Chem. Lett.* **2004**, *33*, 874–875.

(19) Crich, D.; Li, W. *Org. Lett.* **2006**, *8*, 959–962.

(20) A further increase in NFM did not improve the α-selectivity of the reaction.

DMF−, DIPF−, and NFM−imidinium adducts **7**, **8**, and **9**, respectively (Scheme 2). From HSQC- and HMBC-NMR analysis, the corresponding α-anomeric ^{13}C signals of **7**, **8**, and **9** were also identified (see the Supporting Information).

Scheme 2. Production and NMR Detection of Formamide Imidinium Adducts **7**−**9**



Closer examination of the ^1H spectrum of the DIPF−imidinium adduct **8** revealed a smaller signal at chemical shifts (δ) of 5.56 ppm (Figure 1b). Driven by curiosity, we magnified the spectra of DMF− and NFM−imidinium adducts **7** and **9**. As such, two tiny but distinctive signals emerged at chemical shifts (δ) of 5.47 and 5.50 ppm (Figure 1a and c). The $^3J_{H/H}$ coupling constants of these signals lie between 7.0 and 8.5 Hz, implicating a β-configuration if they were anomeric protons.[21] In addition, the corresponding ^{13}C signals of these protons were also identified through HSQC- and HMBC-NMR analysis.[22] On the basis of the NMR data and the literature,[23] we assigned these ^1H and ^{13}C signals as the anomeric ^1H and ^{13}C signals of the β-imidinium adducts **7**−**9**. Additional support of the assignment was given by the formyl ^1H signals of α/β-imidinium adducts (at ca. δ = 9 ppm).

From the ratio of the α/β-anomeric ^1H signals, the equilibrium constants (K_{eq}) of 0.07, 0.34, and 0.01 were determined for formamide−imidinium adducts **7**, **8**, and **9**, respectively.[12,13,24] The concentration of β-imidinium adduct **9** was the lowest among the three β-imidinium adducts, implying that the β-imidinium adduct **9** is also the least stable species in modulated glycosylation.

With this new NMR information, we propose the following mechanism of the formamide-modulated glycosylation

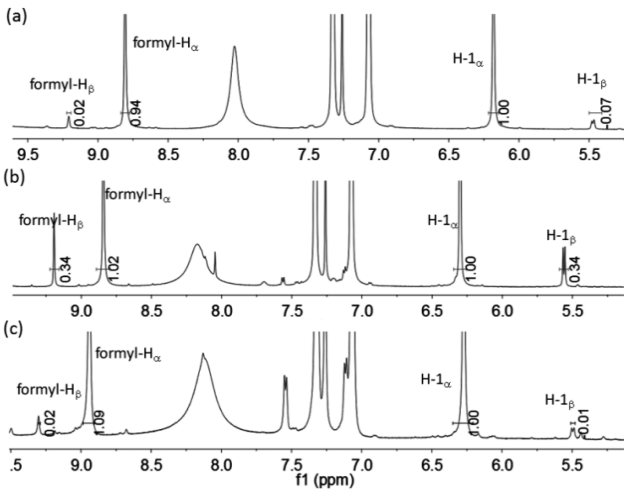


Figure 1. (a) Selected ^1H spectrum of DMF−imidinium adduct **7**. (b) Selected ^1H spectrum of DIPF−imidinium adduct **8**. (c) Selected ^1H spectrum of NFM−imidinium adduct **9**.

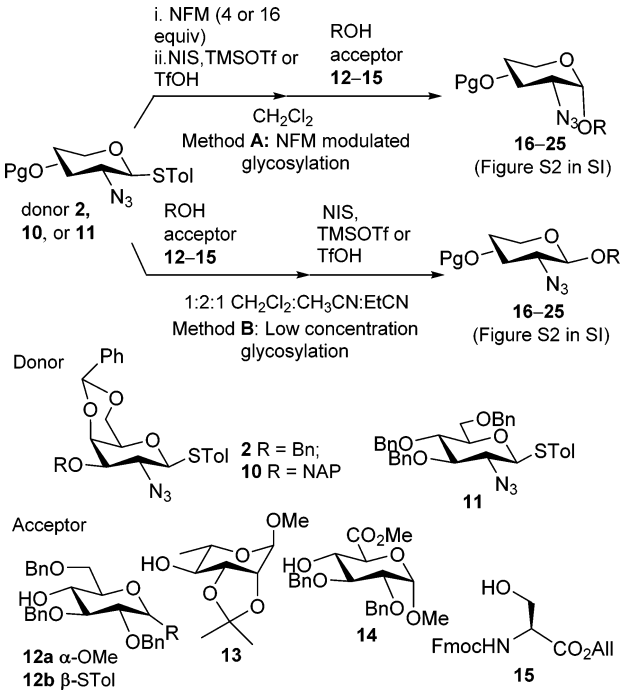
(see Scheme S1 in the Supporting Information). In preactivation, glycosyl donor is activated to form glycosyl oxacarbenium ions, which are trapped by formamide molecules and converted to a mixture of α- and β-glycosyl imidinium adducts.[25] The subsequent reaction of the β-imidinium adduct with the acceptor furnishes the α-glycosylation product. Such a mechanism is consistent with Curtin−Hammett kinetics.[26]

Next, we investigated the scope of the NFM-modulated glycosylation. The 2-azido-2-deoxythioglucoside ($GlcN_3$) **11** and 2-azido-2-deoxythiogalactosides ($GalN_3$) **2** and **10** were coupled with glycosyl acceptors **12**−**14**, and serine acceptor **15** using the NFM-modulated glycosylation procedure (Scheme 3, Table 2). For comparison, the NFM-modulated glycosylation (method A) and the low-concentration glycosylation (LCG) procedures (method B) were performed in parallel.[27] In the LCG method, a 1:2:1 CH_2Cl_2−CH_3CN−EtCN solvent mixture was used to obtain a high β-selectivity in glycosylation.

The glycosylations of the acceptors **12a**−**15** with the $GlcN_3$ donor **11** under the NFM modulation conditions furnished the disaccharides **16**−**19** in yields of 70−80%. The α/β ratios of the glycosylation products spanned from 11:1 to 19:1 (entries 1, 3, 5, and 7). Note that the disaccharide **18** is a repeating unit in heparin sulfate oligomers.[28] With the exception of the acceptor **14**, under the LCG conditions, the glycosylations of the acceptors **12a**, **13**, and **15** with the same $GlcN_3$ donor gave the β-anomers of the disaccharides **16**, **17**, and **19** with high selectivity (entries 2, 4, and 8).

(21) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.
(22) HSQC and/or HMBC correlation NMR spectra of the imidinium adducts **7**−**9** are provided in the Supporting Information.
(23) Lin, Y.-H.; Ghosh, B.; Mong, K.-K. T. *Chem. Commun.* **2012**, *48*, 10910–10912.
(24) Park, J.; Kawatkar, S.; Kim, J. H.; Boons, G. J. *Org. Lett.* **2007**, *9*, 1959–1962.
(25) Bohé, L.; Crich, D. *C. R. Chim.* **2011**, *14*, 3–16.
(26) Seeman, J. I. *Chem. Rev.* **1983**, *83*, 83–134.
(27) Chao, C.-S.; Li, C.-W.; Chen, M.-C.; Chang, S.-S.; Mong, K.-K. T. *Chem.—Eur. J.* **2009**, *15*, 10972–10982.
(28) Bishop, J. R.; Schuksz, M.; Esko, J. D. *Nature* **2007**, *446*, 1030–1037.
(29) When NIS and TMSOTf were used for the glycosylation of **15** with **11**, the reaction was sluggish. Therefore, we applied NIS and TfOH as glycosylation promoters, which gave a cleaner reaction.

Scheme 3. Glycosylation Studies of Donors **2**, **10**, and **11** with Acceptors **12**–**15**



Concerning the glycosylations of the acceptors **12a**–**15** with $GalN_3$ donors **2** and **10**, the disaccharides **20**, **21**, **22**, and **23** were furnished with high α-selectivity under the NFM modulation conditions (entries 9, 11, 13, and 15). The α/β ratios of the disaccharides spanned from 13:1 to α-exclusive. Under LCG conditions, the glycosylations of the same set of glycosyl acceptors with the $GalN_3$ donors produced the disaccharides **20**, **21**, **22**, and **23** in good yields, but the selectivity of the reactions was reversed (entries 10, 12, 14, and 16). We also examined the glycosylation of thioglucosyl acceptor **12b** with 2-azido-2-deoxythioglycosyl donors **10** and **11** (entries 17 and 18). The glycosylation furnished the disaccharide thioglycosides **24** and **25** in acceptable yields with high α-selectivity.

We observed that the α-selectivity of glycosylation is generally higher for the $GalN_3$ donor than for the $GlcN_3$ donor. For example, in modulated glycosylation of **13** with $GalN_3$ **10**, 4.0 equiv of NFM was enough to afford an excellent α/β ratio of 32:1 (entry 11). However the glycosylation of **13** with $GlcN_3$ donor **11** required 16.0 equiv of NFM, although the α/β ratio of the product was slightly lower (entry 3). Thus far, the selectivity of glycosylation of primary glycosyl acceptors with $GlcN_3$ donor is inadequate under the NFM modulation conditions (unpublished data).

Table 2. NFM-Modulated Glycosylation and Low Concentration Glycosylation (LCG) with 2-Azido-2deoxythioglycosyl Donors **2**, **10**, and **11**

entry	donor/acceptor	method[a] (NFM equiv)	time (h), T (°C)	product, yield (%), α:β[b]
1	**11/12a**	A (16)	18, −5	**16**, 70, 19:1
2	**11/12a**	B	2, −60	**16**, 76, 1:16
3	**11/13**	A (16)	12, −10	**17**, 84, 19:1
4	**11/13**	B	1.5, −60	**17**, 60, 1:16
5	**11/14**	A (16)	12, −5	**18**, 75, 16:1
6	**11/14**	B	5, −60	**18**, 74, 1:2
7	**11/15**	A (16)[c]	12, −5	**19**, 81, 11:1
8	**11/15**	B[c]	1, −60	**19**, 74, 1:9
9	**10/12a**	A (16)	17, 0	**20**, 83, 13:1
10	**10/12a**	B	1, −60	**20**, 80, 1:19
11	**10/13**	A (4)	3, −10	**21**, 82, 32:1
12	**10/13**	B	2.5, −60	**21**, 70, 1:19
13	**2/14**	A (16)	24, −10	**22**, 89, α only
14	**2/14**	B	1, −60	**22**, 85, 1:10
15	**2/15**	A (16)	18, −10	**23**, 90, α only
16	**2/15**	B	1.5, −60	**23**, 79, 1:12
17	**11/12b**	A (8)	18, −10	**24**, 60, 15:1
18	**10/12b**	A (16)	7, −5 to 0	**25**, 65, α-only

[a] Method A refers to NFM-modulated glycosylation, and method B refers to LCG. [b] The α/β ratios of **16**–**23** were determined by HPLC analysis except for **24** and **25**. [c] TfOH was used as an acid promoter.[29]

In this study, we evaluated the chemical properties of nucleophiles in a modulated glycosylation context and identified *N*-formyl morpholine (NFM) as a reactive modulator for less reactive 2-azido-2-deoxythioglycosyl donors.

Acknowledgment. We thank the National Science Council of Taiwan (NSC 102-2113-M-009-009) and the Center for Interdisciplinary Science of NCTU for support. Thanks to Dr. C.S. Chao for initial exploration on NFM modulation.

Supporting Information Available. Synthetic procedures, HPLC chromatographs for determination of α- to β-anomer ratio in Tables 1 and 2, and data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.