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DOI: 10.1002/hlca.202000220

FULL PAPER

HELVETICA chimica acta

Synthesis and Biological Studies of O3-Aryl Galactosides as Galectin Inhibitors

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Dedicated to Prof. *Antonio Togni* at the occasion of his 65th birthday

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β-Galactose derivatives have recently been reported to selectively inhibit galectin-3, and a library of O3-arylated galactosides with varying substitution patterns was designed to study such inhibitions further. The O3-arylated galactosides were synthesized using diaryliodonium salts under mild and transition metal free conditions, providing the target products in moderate to good yields. An O3-trifluoroethylated galactoside was also synthesized using iodonium salt chemistry. Azido-substituted products were subsequently transformed into the corresponding triazoles. After deprotection, a selection of galactoside derivatives were evaluated for inhibitory potencies against galectins-1, 3, 4 N (N-terminal domain), 4 C (C-terminal domain), 7, 8 N, 8 C, 9 N, and 9 C and one compound with promising affinity and selectivity for both the N- and C-terminal domain of galectin-9 was discovered.

Keywords: arylation, carbohydrates, galactosides, galectin inhibitor, hypervalent compounds.

Introduction

Carbohydrates are among the most abundant biomacromolecules and play important roles in living organisms. They serve as energy storage, are found in structural components and facilitate cell signalling.[1] Consequently, carbohydrates are of great interests within medicinal chemistry.[2]

Derivatization of carbohydrates through introduction of functional groups at the oxygens gives rise to a vast array of products with potential biological activity, and methodology development to reach *O*-functionalized carbohydrates is thus of importance. Common methods include the stepwise addition of leaving groups followed by nucleophilic substitution, leading to inversion of carbohydrate carbon stereochemistry,[3] and esterification, which is performed under harsh conditions causing scope limitations.[4] Recently, a Cu-mediated site-selective *O*-arylation of carbohydrates with arylboronic acids was reported.[5]

To increase the synthetic routes to these targets, we recently developed an efficient *O*-arylation of carbohydrates with retention of stereochemistry. The reactions were performed using diaryliodonium salts under mild and transition metal-free conditions and provided a broad substrate scope (*Scheme 1*).[6] The reaction could also be extended to *O*-trifluoroethylation using trifluoroethyl(mesityl)iodonium triflate.[6]

β-D-Galactopyranosides are found terminally or internally in glycoconjugates and have the ability to

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/hlca.202000220

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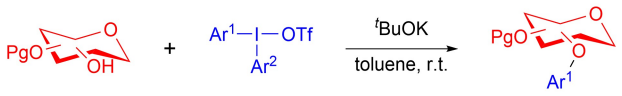
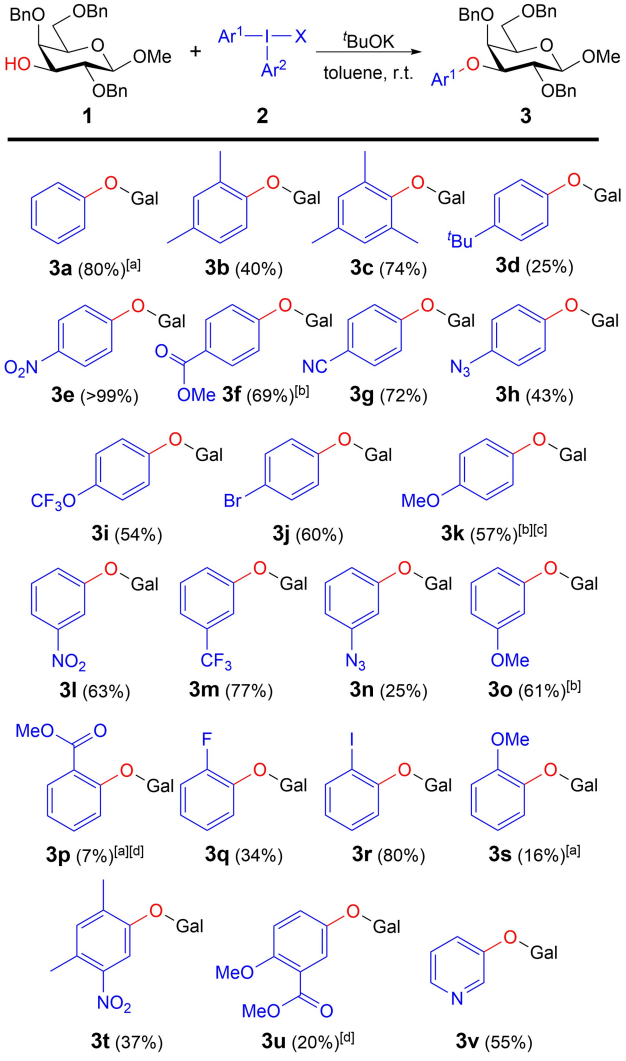


Scheme 1. Methodology for O-arylation of carbohydrates. Pg = protecting group.



Scheme 2. Arylation scope with galactoside **1**. Reaction conditions: **1** (0.1 mmol), salt **2** (2 equiv.) and ^{t}BuOK (2 equiv.) were stirred in toluene (2 mL, anhydrous conditions not required) for 1 h. Additional **2** and ^{t}BuOK (1 equiv. each) were then added and the reaction continued another 1–2 h. Unsymmetric salts **2** (dummy group): **2b**, **2m**, **2n**, **2r**, **2s**, **2v** (anisyl), **2e**, **2f**, **2g**, **2l** (Ph), **2h** (TMP), **2o**, **2p** (mesityl).[a] Additional **2** and ^{t}BuOK (2 equiv. each) used.[b] The conditions from reference [6] were used.[c] Reaction at 50 °C for 16 h, regioisomeric mixture **3k**:**3o** 3.7:1.[d] Reaction at 60 °C for 18 h.

bind to the protein family of galectins.[7] Such binding influences the cellular trafficking, localization, and molecular interactions of glycoconjugates and thereby regulate their functions. For example, galectins can interact with such cell surface glycoproteins on T cell receptors responsible for recognizing antigens,[8] and the membrane transport protein CD98.[9] Galectins can also interact with the transforming growth factor-β TGF-β-R, which is involved in paracrine signalling/cell-cell communication,[10] and receptors for vascular endothelial growth (VEGF2-R), which are involved in the formation of the circulatory system and the growth of blood vessels.[11]

In 2016, *Nilsson* and co-workers reported that a C3-derivatized β-galactose derivative could selectively inhibit galectin-3. The derivative proved successful in a bleomycin-induced mouse model of lung fibrosis and thus proved promising for the development of anti-fibrotic drugs.[7]

The *O*-arylated galactosides generated through the methodology in *Scheme 1* were interesting targets for biological studies as galectin inhibitors. The previous scope only included one O3-arylated galactose,[6] and we hypothesized that such compounds have the potential for discovery of novel galectin-inhibitory structural classes as C3-derivatised galactosides are known drug-like inhibitors.[6] Herein, we describe the results from a targeted synthesis of a variety of O3-arylated galactosides and the investigation of their biological properties.

Results and Discussion

Synthesis of O3-Arylated Galactosides

Benzyl-protected galactoside **1** was chosen as the key starting material for diversifying 3-*O*-arylations and the reaction with diphenyliodonium triflate (**2a**) was examined first. Product **3a** was obtained in 65% yield together with recovered starting material under the previously developed conditions.[6] To increase the conversion, additional **2a** and base were added to the reaction after 1 h, resulting in 80% yield of **3a**. This stepwise addition set-up was next applied to a series of functionalized diaryliodonium salts **2**, which were either symmetric or unsymmetric with a phenyl, mesityl, anisyl or trimethoxyphenyl (TMP) dummy group. The choice of iodonium reagent and the observed chemoselectivities are detailed in the *Supporting Information*.[12,13] The reactions were performed without individual optimizations of the reaction conditions, as the main focus was to obtain products **3** for biological investigations (*Scheme 2*).

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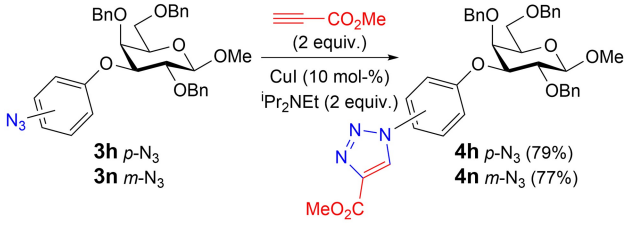
Electron-donating alkyl-substituted aryl groups could be transferred to provide products **3a**–**3d**. Arylated products with a variety of electron-withdrawing functional groups in the *para*-position (**3e**–**3j**) were easily obtained, and the yield of nitro-substituted **3e** increased from 78%[6] to quantitative under these conditions. Even the *p*-methoxy-substituted product **3k** was successfully obtained, although the arylation resulted in a regioisomeric mixture (3.7:1) of **3k**:**3o**, likely formed through an aryne mechanism.[14] The scope with *meta*-substituted aryl groups was subsequently examined and delivered products **3l**–**3o**. The azido-functionalized products **3h** and **3n**, which are very interesting for further derivatization, were formed in moderate yields that could not be improved by changing the reaction temperature. Reactions with the 4-azidophenyl (TMP) salt proved to give better yield than the corresponding anisyl salt, see the *Supporting Information* for further details.

ortho-Substituted products **3p**–**3t** proved more difficult to obtain and the synthesis of *ortho*-ester decorated **3p** required heating to 60 °C and prolonged reaction time. The *ortho*-iodinated product **3r** was the only exception to this trend and could be isolated in 80% yield. Reactions with the unsymmetric 2-methoxyphenyl(4-methoxyphenyl)iodonium triflate (**2s**) delivered a separable product mixture of **3s** and **3k** (3:1) through incomplete chemoselectivity in the ligand coupling, which decreased the yield of isolated **3s**. Products **3t** and **3u**, carrying functional groups with both electron-withdrawing and electron-donating properties, were obtained in modest amounts. The library was completed by the synthesis of pyridyl galactoside **3v** in 55% yield.

Derivatization of the O*-Arylated Galactosides* **3**

The azido-functionalized galactosides **3h** and **3n** were efficiently transformed into triazoles **4h** and **4n** through CuAAC reactions[15,16] (*Scheme 3*).



Scheme 3. CuAAC reactions with azido-functionalized galactosides **3h** and **3n**.

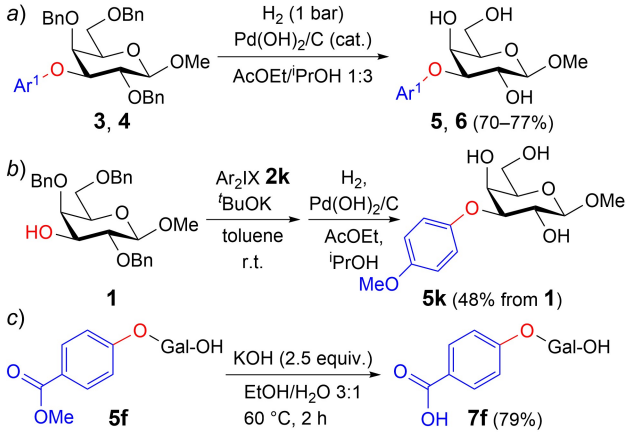
The benzyl protecting groups in O3-aryl galactosides **3** and **4** were subsequently removed through hydrogenation to provide target compounds **5** and **6** (*Scheme 4,a*). Most products were obtained in good yields, but the deprotection of substrates **3e** and **3v** failed. The arylation and hydrogenation could also be combined into a sequential one-pot procedure without purification of compound **3**. In this fashion, product **5k** was obtained in improved overall yield (*Scheme 4,b*). Basic hydrolysis of methyl ester **5f** provided carboxylic acid **7f** (*Scheme 4,c*).

Synthesis of an O3-Trifluoroethylated Galactoside

We have previously demonstrated that trifluoroethyl (mesityl)iodonium triflate (**8**)[17] can be utilized to transfer a trifluoroethyl moiety to carbohydrates.[6] This methodology was applied to synthesize target product **9** (*Scheme 5*), but the trifluoroethylation of substrate **1** did not proceed under our reported conditions. A small optimization revealed that product **9** could be obtained by addition of the reagents at −78 °C followed by reaction at room temperature overnight. Subsequent deprotection by hydrogenation delivered target product **10**.

Evaluation of **5**–**7** *and* **10** *as Galectin Inhibitors*

With a variety of diverse 3-*O*-arylated galactoside derivatives at hand we proceeded to evaluate selected compounds (**5a**–**5f**, **5k**, **5m**, **5o**, **5q**, **6h**, **6n**, **7f** and **10**) for their galectin inhibitory properties (*Figure 1*). The compounds were evaluated for binding to galectins-1, 3, 4 N (N-terminal domain), 7, 8 N,



Scheme 4. *a*) Hydrogenation of galactosides **3**. *b*) Sequential one-pot procedure. *c*) Saponification of ester **5f**.

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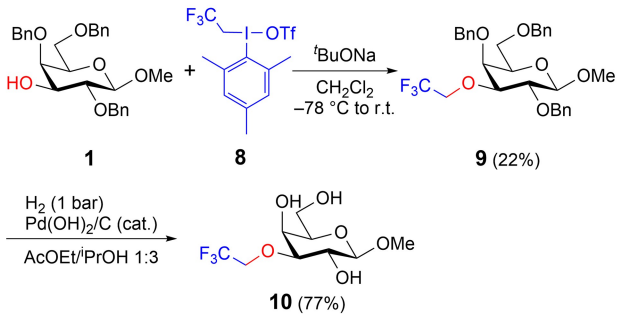

Scheme 5. Synthesis of O3-trifluoroethylated target product **10**.

8 C (C-terminal domain), 9 N, and 9 C in a reported competitive fluorescence anisotropy assay[18,19] with specific experimental conditions as earlier described[20,21] (*Table 1*). Analysis of the affinities for galectin-1 in comparison to the unsubstituted methyl β-D-galactopyranoside (**11**) shows that several 3-*O*-arylated derivatives were significantly better ligands. Affinities were nevertheless mediocre with K_d values in the range of 1–2 mM. A similar trend was observed for galectin-3, 4 N, 4 C, 7, and 8 C, however with fewer compounds binding with K_d 1–3 mM and more non-binding compounds. Interestingly, galectin-9 N and galectin-9 C found the PMP-derivative **5k** as a lower μM inhibitor with a K_d of 260 and 250 μM, respectively, which is significantly better than the unsubstituted reference **11**. Furthermore, the *tert*-butyl derivative **5d** also revealed a reasonably good affinity for galectin-9 N. Hence, among the galectins investigated, galectin-9 galactoside ligand binding is discovered to benefit from 3-*O*-arylation as both carbohydrate-recognizing domains, the N-terminal and the C-terminal, show low μM-affinity for **5k**. Other evaluated galactosides presented less pronounced affinity enhancements upon binding to the galectin-9 N- and C-terminal domains than **5k**, their interactions with the tested compound are highly dependent on the position and chemical nature of the aryl substituent. The *meta*-OMe analog **5o** is 3–4-fold worse inhibitor than the *para*-OMe **5k** and other para substituents are less efficient than methoxy. Intriguingly, this methoxy-substituent effect is reverse to that recently reported for the corresponding 3-*N*-arylated galactosides, for which the *meta*-OMe (K_d 140 μM) was three times more potent inhibitor than the *para*-OMe (K_d 440 μM).[22]

Conclusions

A series of O3-functionalized galactosides was successfully synthesized using iodonium salts under

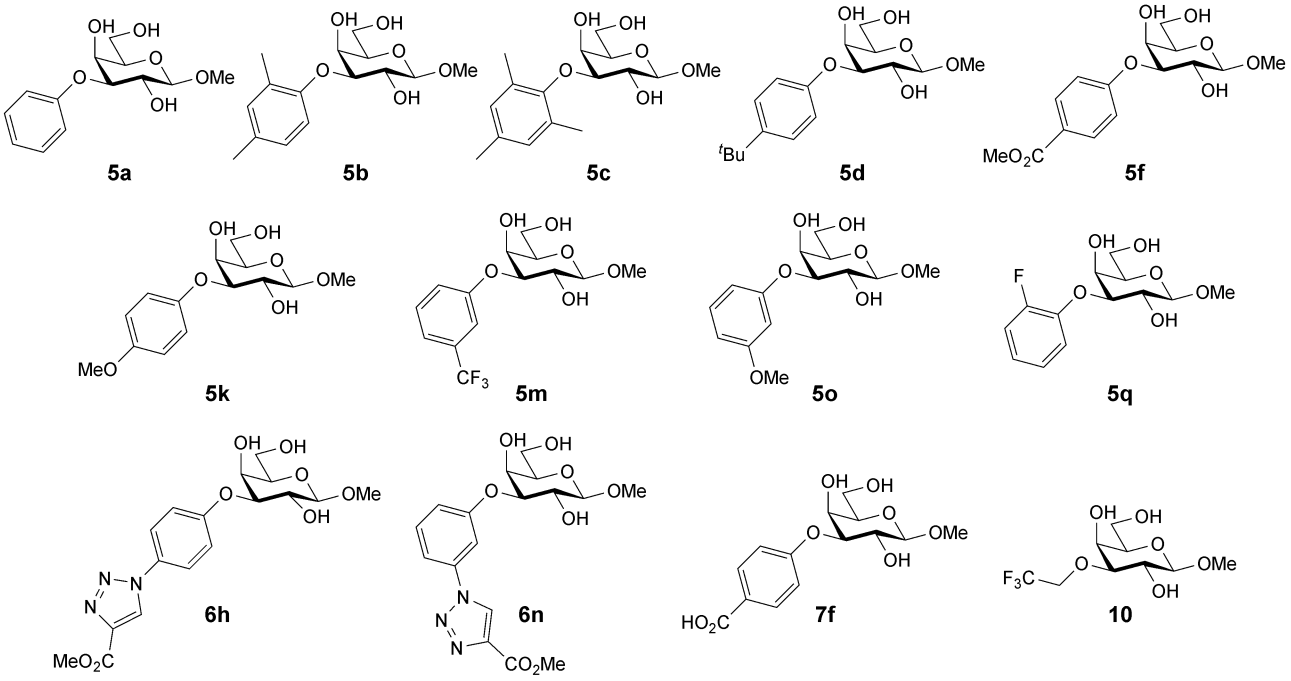

Figure 1. 3-*O*-Arylated and alkylated compounds evaluated for galectin binding affinities.

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Table 1. K_d-Values [μM] of **5a**–**5f**, **5k**, **5m**, **5o**, **5q**, **6h**, **6n**, **7f** and **10** against human galectin-1, 3, 4N, 4C, 7, 8N, 8C, 9N, and 9C as measured by a fluorescence anisotropy assay. Methyl β-D-galactopyranoside **11** is included as a reference compound.

	Galectin 1	3	4N	4C	7	8N	8C	9N	9C
5a	750 ± 37	n.b.	n.b.	n.b.	n.b.	n.b.	2700 ± 210	1500 ± 340	910 ± 10
5b	n.b.[a]	n.b.	1800 ± 120	n.b.	n.b.	n.b.	n.b.	1900 ± 80	1700 ± 120
5c	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
5d	1300 ± 160	n.b.	990 ± 160	2700 ± 50	n.b.	n.b.	n.b.	460 ± 68	2200 ± 290
5f	1600 ± 290	n.b.	n.b.	n.b.	3200 ± 110	n.b.	n.b.	1300 ± 50	1700 ± 140
5k	1100 ± 160	770 ± 170	360 ± 64	1900 ± 450	2800 ± 170	n.b.	2200 ± 250	260 ± 30	250 ± 20
5m	n.b.	n.b.	n.b.	2700 ± 480	n.b.	n.b.	n.b.	620 ± 90	930 ± 90
5o	2400 ± 170	n.b.	1800 ± 550	2500 ± 170	n.b.	n.b.	3000 ± 440	780 ± 55	860 ± 80
5q	730 ± 100	1100 ± 300	1900 ± 210	n.b.	n.b.	n.b.	2400 ± 200	1500 ± 100	1000 ± 30
6h	1800 ± 190	n.b.	n.b.	n.b.	n.t.[b]	n.b.	n.b.	n.b.	n.b.
6n	n.b.	1500 ± 110	1300 ± 270	1200 ± 130	n.b.	n.b.	n.b.	1400 ± 10	2300 ± 80
7f	930 ± 20	n.b.	n.b.	n.b.	2200 ± 610	n.b.	n.b.	1700 ± 190	2200 ± 610
10	1600 ± 70	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
11[22]	> 10000	4100	6600	10000	4800	6300	> 30000	3300	8600

[a] n.b. = Non-binding at the highest concentration tested (1 mM) indicating that the $K_d \geqslant 2$ mM.[b] n.t. = not tested.

mild and transition metal-free conditions. Further derivatization of the obtained products included click reactions, hydrolysis and deprotections, delivering 13 target products. Evaluation of these products revealed compounds with some affinity enhancement over simple methyl β-D-galactopyranoside (**11**) for all galectins except for galectin-8 N. Galectin-9 N and galectin-9 C stood out by having a good μM affinity for the *para*-OMe derivative **5k** and displaying a strong dependence on the aryl substituent structure and position. Hence, the *para*-OMe **5k** constitutes a first promising lead for further development of more potent and selective galectin-9 inhibitors. This is particularly important in light of the key roles of galectin-9 in T reg cell stimulation[23] and influencing check point inhibition via binding to TIM-3.[24]

Experimental Section

Arylation of Galactoside **1**

Galactoside **1** (0.1 mmol) was added to a microwave vial and dissolved in toluene (2 mL) followed by addition of diaryliodonium salt **2** (2 equiv.) and ^{t}BuOK (2 equiv.). The mixture was stirred at r.t. for 1 h, then additional **2** (1–2 equiv.) and ^{t}BuOK (1–2 equiv.) were added. The mixture was stirred at r.t. until it was deemed complete by TLC (1–2 h). The mixture was then concentrated onto *Celite* under reduced pressure and purified by column chromatography to deliver target product **3**.

CuAAC Reaction of Azido-Substituted Galactosides **3h** *and* **3n**

Azide **3h** (21.0 mg, 36 μmol) was dissolved in CH_2Cl_2 (2 mL). Methyl propiolate (6.4 μL, 72 μmol), CuI (1 mg, 10 mol%) and $^i Pr_2NEt$ (13 μL, 72 μmol) were added, and the mixture was stirred at r.t. for 48 h. The solvent was removed under reduced pressure, the residue was dissolved in AcOEt and the solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by column chromatography (hexane/AcOEt 7:1–2:1) to give triazole **4h** as a colorless oil (19.0 mg, 29 μmol, 79%).

Hydrogenation of Galactosides **3** *and* **4**

A solution of *O*-aryl galactoside **3** or **4** (28 μmol) in AcOEt/^{i}PrOH (1:3, 2 mL) was stirred with $Pd(OH)_2$/C

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(10 wt-%, 4 mg) under hydrogen atmosphere at r.t. for 12 h. The mixture was then filtered through *Celite* and washed with MeOH. The filtrate was concentrated under reduced pressure and purified by column chromatography (CH_2Cl_2/MeOH) to give the desired compound **5** or **6**.

Acknowledgements

Olle Engkvist Byggmästare foundation (2014/645) is kindly acknowledged for project funding and *G. L. T.*'s postdoctoral scholarship.

The Swedish Research Council (621-2016-03667), the *Knut and Alice Wallenberg Foundation* (KAW 2013.0022), and *Galecto Biotech AB*, Lund, Sweden are acknowledged for financial support.

We thank Mrs. *Barbro Kahl-Knutsson* for assistance with fluorescence anisotropy experiments.

Author Contribution Statement

G. K., *K. B. P.*, *G. L. T.* and *M. M.* all participated in the synthesis and characterization of the products. *H. L.* designed and supervised the fluorescence anisotropy experiments. *B. O.* and *U. J. N.* designed the study, supervised the project, participated in data interpretation and wrote the article.

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Received November 21, 2020
Accepted December 22, 2020

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