Synthesis of an Azide- and Tetrazine-Functionalized [60]Fullerene and Its Controlled Decoration with Biomolecules

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ABSTRACT: Bingel cyclopropanation between Buckminster fullerene and a heteroarmed malonate was utilized to produce a hexakis-functionalized C60 core, with azide and tetrazine units. This orthogonally bifunctional C60 scaffold can be selectively one-pot functionalized by two pericyclic click reactions, that is, inverse electron-demand Diels–Alder and azide–alkyne cycloaddition, which with appropriate ligands (monosaccharides, a peptide and oligonucleotides tested) allows one to control the assembly of heteroantennary bioconjugates.

INTRODUCTION

Since the discovery of Buckminster fullerene ([60]fullerene, by Kroto et al.), scientists have invested significant effort by investigating potential applications of this fascinating carbon allotrope. Its synthetic availability, controlled functionalization techniques, and efficient conjugation chemistries have enabled the preparation of sophisticated C60 conjugates, which have had a significant impact in material and biomedical sciences. [60]Fullerene as such shows interesting physical and biological properties, and the spherical structure that allows radially symmetric and dense functionalization makes it an excellent example to probe multivalent biomolecular interactions. For example, C60-based glycoballs have received marked interest as potential lectin binders. More recently, 12-armed fullerene has been used for the assembly of molecular spherical nucleic acids (SNAs), which is an attractive delivery and formulation option for therapeutic oligonucleotides. In most of the applications above, one type of ligand (e.g., sugars and oligonucleotides) is multiply on an appropriately functionalized C60 hexakis adduct. However, some biomolecular applications would need a combination of different biomolecules (i.e., heteroantennary C60 bioconjugates). For example, a drug delivery vehicle may need a system that allows an orthogonal loading of tissue-specific and cell-penetrating ligands and the drug payloads. Appropriate heterovalency is also needed to introduce reporter groups selectively to C60 conjugates or to integrate them with other functionalities. A controlled mono Bingel cyclopropanation of [60]fullerene, followed by full-decoration with the same reaction and using two different malonates, has enabled the synthesis of 1:5-, 1:1:1-, and even 1:1:1:10-heterosubstituted C60 scaffolds, which have been used for an orthogonal ligation, for example, via alkane–azide and thiol–ene click reactions. Recently, stereodefined C60 [3:3] hexa-adducts were prepared by two subsequent click reactions. For the controlled assembly, a C60 tris-adduct was first regioselectively prepared using a macrocyclic malonate, which was then exposed to a Bingel cyclopropanation with another malonate.

RESULTS AND DISCUSSION

A Bingel cyclopropanation between [60]fullerene and heteroarm malonate S was used for the synthesis of 1 (Scheme 1). For the synthesis of malonate S, one would use Meldrum’s acid and a stepwise reaction with 3 and 4. However, the described one step-approach was chosen, as it gave S in an acceptable yield (21%) with a mixture of readily available alcohols (Scheme S1). A Bingel cyclopropanation between C60 and S under standard conditions, using CBr4 as a bromination agent and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a strong organic base in o-dichlorobenzene for 3 d at rt under argon, gave the hexakis-substituted C60 core 1. The crude product mixture was purified first by silica gel
column chromatography (in 24% yield) and then further by reversed-phase (RP) high-performance liquid chromatography (HPLC) to give homogenized 1 in 5% overall yield. The authenticity of 1 was verified by NMR spectroscopy and mass spectrometry (MS) (electrospray ionization—time-of-flight (ESI-TOF)) (Figures S5–S8). It may be worth of mentioning that, while the cyclopropanated C60 moiety of 1 is a well-organized structure, with pyritohedral symmetry, 1 is obtained as a stereoisomeric mixture (in fact 2^5 = 32 stereoisomers) due to the heteroarm malonate. The corresponding stereodefined structure would increase the biological and supramolecular value of these C60-derivatives. However, prior to a marked synthetic effort, needed to obtain the corresponding stereo-defined C60 derivative, we wanted to evaluate first in this article how the tetrizine-azide combination on 1 works for the catalyst free assembly of heteroarm bioconjugates.

A set of TCO- and BCN-modified biomolecule ligands (cf. the tool box in Scheme 2) were used for the decoration of 1. The ligands were prepared by a carbamate coupling from commercially available TCO and BCN precursors, an amino-modified peptide, amino-modified oligonucleotides, and p-nitrophenyl carbonate-modified sugars (cf. Supporting Information, Schemes S2 and S3).

To evaluate the applicability of core 1 in the preparation of heteroarm bioconjugates, we first synthesized C60-glycoconjugate C4. 1 (0.33 μmol) was dissolved in DMSO (100 μL) and exposed to iEDDA with TCO-Gal (9 equiv in 100 μL of DMSO added) to yield intermediate glycoconjugate C1, and then BCN-Glu (9 equiv in 100 μL of DMSO) was added to the same reaction mixture. The completion of the successive click reactions (both overnight reactions) was verified by RP HPLC (Figure 1 and Scheme S4), and the authenticity of the intermediate conjugate C1 and of the end product C4 was verified by MS (ESI-TOF) (Figures S15 and S16). The isolated C4 was characterized also by NMR spectroscopy (1H, COSY, HSQC, HMBC), in which the correct 1:1 ratio of the galactose and glucose units could be clearly seen (Figures S17–S20 and Table S1). Because of the small-scale synthesis, the molar quantity of the obtained C4 was extracted from the 1H NMR spectra by comparing the intensity of 1H signals to an internal standard (a known quantity of acetonitrile used). Accordingly, C4 was obtained in 66% isolated yield. The other heteroarm glycoconjugates C5 and C6 and the peptide-glycoconjugate C7 were next assembled in a similar manner by using 0.1 μmol of 1 and adjusting the excesses of the reagents and solvent volumes accordingly. As seen in the RP HPLC profiles (Figure 1) of the crude product mixtures, the selective assembly on the C60 core (1) worked well in each case to yield the desired heteroarm conjugates C5—C7 in S6, 69, and 61% isolated yields (based on the absorbance at λ = 260 nm of the products compared to that of a known concentration of C4, ε = 120 × 10^3 L mol⁻¹ cm⁻¹). It may be worth mentioning that the idea of these small-scale trials was to further evaluate the proof of concept, in which the applicability of the scaffold 1 for the successive one-pot iEDDA and SPAAC conjugations was validated. Because of the difficulties related to the stereoisomeric mixture (and the small scale), we did not invest in an effort for a complete NMR characterization, but the authenticity of the intermediate conjugates (C2 and C3) and of the end products (C5—C7) was verified by MS (ESI-TOF) (Figures S15, S16, and S21) only.

We recently communicated that multiarm C60-branched units (e.g., 1) may be contaminated by structurally similar and hardly identified derivatives. Therefore, prior to the assembly of C60-based macromolecules, in which plausible errors may remain hidden, the homogeneity and applicability of the cores should be evaluated with small molecular ligands. After a successful synthesis of the glycoconjugates (C4—C7), we validated the applicability of 1 for the assembly of a molecularly defined SNA, in which both strands of the double helices were covalently bound to the C60 core (C9). Relatively short oligonucleotides (TCO-ON1 and BCN-ON2) were used for the assembly. 1 (5 nmol) was exposed to iEDDA with TCO-ON1 (45 nmol, 9 equiv) in DMSO (45 μL). The mixture was stirred overnight at room temperature and purified by RP HPLC (Scheme S4) to give the hexa-arm intermediate conjugate (C8) in 50% isolated yield (according to the UV absorbance at λ = 260 nm). The authenticity of C8 was verified by MS (ESI-TOF) spectroscopy (Figure S22). Because of the complementarity of the ON1 and ON2 strands, we did not try a one-pot assembly here. An aliquot (1.0 nmol) of purified C8 was exposed to SPAAC with BCN-ON2 (9.0 nmol) in H2O (20 μL). The mixture was stirred overnight at room temperature and purified by an RP HPLC (Figure 1), which gave the homogenized SNA C9 in 57% isolated yield (according to the UV absorbance at λ = 260 nm). As usual with SNAs, the MS-spectroscopic characterization of C9 proved complex. An aliquot of C9 was then introduced to Size Exclusion Chromatography equipped with a Multiple Angle Light Scattering detector (SEC-MALS), which showed a well-behaving and pure (95.7% mass fraction of the total sample mass) macromolecule with a molecular weight of 41.4 ± 1.4 kDa (expected: 41.4 kDa) (Figure S23). We also evaluated the homogeneity of C8, C9, and C8 in the presence of complementary strand (6 equiv of ON2, that is, hybridization-mediated SNA C10) by native PolyAcrylamide Gel Electrophoresis (PAGE). As seen in the electrophoreogram (Figure 1, cf. also Figure S25), each of the SNAs showed a distinct band. C9 eluted markedly slower compared to C10. This is a surprising behavior, as the only difference between C9 and C10 is the covalent link of ON2 to the C60 core. The thermal stability of the double helices on the SNAs C9 and...
C10 was next evaluated by a UV-melting profile ($T_m$) analysis. C10 resulted in an 8 °C decrease in the $T_m$ value, when compared to the corresponding free duplex (ON1 + ON2) (Figure 2). This decreased duplex stability is consistent with the previous findings of SNAs,\(^8\)\(^{28−31}\) caused by an electrostatic and steric repulsion between the densely packed oligonucleotides. Interestingly, C9 showed no melting at all in the measured temperature range of 10−90 °C. This indicates very stable double helices on C9. One may wonder whether this is a result of a negligible hyperchromic effect, even if an unwinding of the strands occurs on C9. (Note: a relative hyperchromic effect is considered in the melting profiles, Figure 2). Circular dichroism (CD) measurements with C9 and C10 (Figure 2) were then performed to verify changes of helicity upon the temperature ramp. CD profiles of C10 represent a typical B-type double helix. The characteristic minimum at 250 nm gradually disappears upon heating, which indicates a thermal denaturation of the double helices. Typical B-type CD profiles may be observed also on C9, but what makes these data fascinating is that there is no marked change between the profiles upon heating. In the profile even at 90 °C (the bold red line) a deep minimum at 250 nm can be seen, demonstrating that B-type double helices exist there. Very stable cyclic double helices are known structures,\(^32\) but C9 seems to be an interesting example of dendritic nucleic acids, in which neighboring hairpin-type double helices stabilize each other via steric and electrostatic repulsion, in this manner preventing an unwinding of the strands. Polydisperse gold nanoparticle SNAs with covalently bound RNA double helices have been reported,\(^28\) but no similar stability phenomenom has been studied in detail.

We also analyzed an aliquot of C9 on a polyethylenimine (PEI)-coated mica using atomic force microscopy (AFM). Particles of ca. 10 nm height, representing the monomeric C9
were observed. We mention that the sample contained also larger but rather control-sized particles (ca. 25 nm height) that indicated aggregates of C9 on the PEI-coated mica (Figure S24). The SNAs like C9 may find interesting applications as well-organized constructs in medicinal and supramolecular chemistry. Further studies considering a deeper understanding of the stability requirements (the effect of the sequence and its length, hydrogen-bonding stability, Dicer-mediated cleavage of therapeutically relevant siRNAs on these SNAs, etc.) are currently under way in our laboratory.

**CONCLUSION**

In conclusion, a bifunctional C60 core (1) has been described, which can be selectively functionalized by two subsequent pericyclic click reactions (iEDDA and SPAAC) in catalyst-free conditions. The applicability of the core in a one-pot assembly has been demonstrated for the synthesis of heteroantennary glycoballs (and one glyco-peptide C60-conjugate C4−C7, which were obtained in 56−69% isolated yields. Furthermore, a novel type SNA (C9) with extraordinary stable covalently bound double helices, verified by UV- and CD-melting profile
experiments, has been assembled in 29% overall yield. PAGE, SEC-MALS, and AFM imaging of C9 demonstrated a homogeneous and uniform biomacromolecule.

**EXPERIMENTAL SECTION**

**General Remarks.** All reactions involving air- or moisture-sensitive conditions were routinely performed and under an inert atmosphere. All reagents from commercial suppliers were used without further purification. The NMR spectra were recorded using 500 and 600 MHz instruments. The chemical shifts in the $^1$H and $^{13}$C NMR spectra are given in parts per million (ppm) from the residual signal of the deuterated solvents.

**RP HPLC of C$_{60}$ Conjugates.** For the analysis of the product mixture and purification of C$_{60}$ conjugates 1, C1−C7, and C9 an analytical RP-HPLC column Phenomenex, Aeries 3.6 μm WIDEPORE XB-C8 200 Å, LC Column 150×4.6 mm, at a flow rate of 1.0 mL min$^{-1}$ and detection at 260 nm was used. Linear gradients from 40% to 100% MeCN in H$_2$O over 30 min for 1 and run time of 20 min were used.

**SEC-MALS.** For the SEC-MALS analysis of C9 an Agilent Technologies 1260 Infinity II HPLC system (sampler, pump, and UV/vis detector) equipped with a Wyatt Technologies miniDAWN light-scattering detector and a Wyatt Technologies Optilab refractive index detector was used. An Agilent AdvanceBio SEC 300 Å 2.7 μm 4.6×300 mm column, 150 mM sodium phosphate pH 7.0 as mobile phase eluting at a rate of 0.2 mL min$^{-1}$ and run time of 20 min were used. Four microliters of a sample of C9 (1 mg mL$^{-1}$ in Milli-Q water) was loaded onto the pre-equilibrated column. Detector signals were aligned with a bovine serum albumin (BSA) standard, which was analyzed prior to the C9 sample. The RI and MALS signals were used for the molecular weight (MW) calculations using an average refractive index increment (dn/dc) of 0.1703 mL/g.

**PAGE Analysis of SNAs.** A native 6% Tris base, boric acid, ethylenediaminetetraacetic acid (EDTA), and acrylamide (TBE) gel were used to check SNAs purity. A pre-cast gel into a vertical electrophoresis chamber, and 10% Tris-borate-EDTA running buffer (VWR Life Science) was filled into an electrophoresis chamber. SNA samples (C8−C10, 5 μL of 0.3 μM SNAs mixed with 5 μL of 6X TriTrack DNA Loading Dye) and 5 μL of Gene Ruler Ultra Low Range DNA ladder 10−300 bp (Thermo Scientific) were loaded and electrophoresed at 150 V constant (45 mA) for ~35 min. After the completion of the electrophoresis, the gel was removed from the electrophoresis chamber and stained by SYBRRTM Gold Nucleic Acid Stain (Thermo Fisher Scientific) for 1 h and imaged under G-Box camera (Syngene).

**Running Buffer Preparation.** 100 mL of (10X concentrated solution of 0.9 M Tris, 0.9 M borate, and 0.02 M EDTA, 8.3 pH in distilled, deionized water) was dissolved in 900 mL of distilled, deionized water. Sample preparations: 5 μL of (6× TriTrack DNA Loading Dye) and 5 μL of SNAs in water (0.3 μM based on SNAs concentration). Staining solution preparation: 5 μL of SYBRRTM Gold Nucleic Acid Stain was dissolved in 50 mL of running buffer.

**UV Melting Profile (T$_{m}$) Experiments.** The melting curves (absorbance vs temperature) were measured at 260 nm on a UV−vis spectrometer equipped with a multiple cell holder and a Peltier temperature controller. The temperature was changed at a rate of 0.5 °C min$^{-1}$ between 10 and 80 °C (ON1 + ON2-duplex and C10) and between 10 and 90 °C (C9). The measurements were performed in 10 mmol L$^{-1}$ sodium cacodylate (pH 7.0) with 0.1 mol L$^{-1}$ NaCl and using 1.0 μmol L$^{-1}$ ON1 + ON2 and 2.0 μmol L$^{-1}$ C9 and C10. The T$_{m}$ values were determined as the maximum of the first derivative of the melting curve. The relative increased absorbance (hyperchromicity) of the C9 and C10 samples was compared to that of ON1 + ON2.

**CD Spectroscopic Analysis.** CD spectra of C9 and C10 were measured on an Applied Photophysics Chirascan spectrometer. The same mixtures used for the UV melting profile analysis were used. A quartz cell of diameter 10 mm was used for the measurements. The sample temperatures were changed from 20 to 90 °C at a rate of 1 °C min$^{-1}$.

**AFM Images.** 2.5 mg/mL PEI solution was deposited on cleaved mica. After 10 min of adsorption, the mica was rinsed with water and dried with nitrogen. A mixture of 10 nM C9 in water (10 μL) was deposited on the PEI-coated mica. After 10 min of desorption, 50 μL of water was added, and the particles were scanned in water. The AFM images were obtained using a MultiMode 8 atomic force microscope (Bruker) with a NanoScope V controller, working in tapping mode with a ScanAsyst Fluid+ probe (Bruker).

2-(2-(2-(4-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenoxy)-ethoxy)ethoxy)ethoxy)ethanol (3). 4-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenol (1.0 g, 5.3 mmol), 2-(2-(2-(hydroxyethoxy)-ethoxy)-ethoxy)ethyl acetate (6, 1.63 g, 6.9 mmol), and triphenylphosphine (1.81 g, 6.9 mmol) were dissolved in tetrahydrofuran (25 mL) (cf. Scheme S1). The reaction mixture was cooled to 0 °C and diisopropyl azodicarboxylate (1.35 mL, 6.8 mmol) was added dropwise over a period of 40 min. The reaction mixture was stirred for 2 h at 0 °C and for 1 h at rt. The volatiles were evaporated, and the residue was dissolved in ethyl acetate and washed with water. The organic layer was separated, dried over Na$_2$SO$_4$, filtered, and evaporated to dryness. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate (EtOAc)$_2$, 4:6, v/v) to yield 1.59 g (74%) of 2-(2-(2-(2-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenoxy)ethoxy)ethoxy)ethoxy)ethyl acetate (7) as a pink solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.53 (d, 2H, J = 9.0 Hz), 7.10 (d, 2H, J = 9.0 Hz), 4.26−4.22 (m, 2H, 3.91 (t, 2H, J = 4.7 Hz), 3.77−3.75 (m, 2H), 3.72−3.66 (m, 8H), 3.06 (s, 3H), 2.08 (s, 3H), $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 171, 166.6, 163.8, 162.5, 129.7, 124.3, 115.3, 70.9, 70.7, 70.65, 70.6, 69.6, 69.1, 67.7, 63.6, 21.9; high-resolution mass spectrometry (HRMS) (ESI-TOF) m/z: [M + Na]$^+$ requires 429.1745, found 429.1745. Compound 7 (1.59 g) was dissolved in 0.1 mol L$^{-1}$ K$_2$CO$_3$/MeOH solution (100 mL), and the reaction mixture was stirred for 2 h at room temperature. The completion of the acetal removal was verified by thin-layer chromatography (TLC). The reaction mixture was neutralized by an addition of 10% acetic acid (AcOH) in H$_2$O and evaporated to dryness. The residue was dissolved in ethyl acetate and washed with water. The organic layer was separated, dried over Na$_2$SO$_4$, filtered, and evaporated to dryness. The residue was purified by silica gel chromatography (n-hexane/EtOAc$_2$, 1:9, v/v) to yield 1 g (70%) of the product (3) as a pink solid. $^1$H NMR (500 MHz,
CDCl₃ $\delta$ 8.54 (d, 2H, $\ J = 8.9$ Hz), 7.11 (d, 2H, $\ J = 9.0$ Hz), 4.26 (t, 2H, $\ J = 4.9$ Hz), 3.92 (t, 2H, $\ J = 4.7$ Hz), 3.79–3.77 (m, 2H), 3.75–3.70 (m, 8H), 3.63 (t, 2H, $\ J = 4.32$ Hz), 3.08 (s, 3H); $^{13}$C NMR (125 MHz, CDCl₃) $\delta$ 166.6, 163.8, 162.5, 129.7, 124.7, 115.3, 72.5, 70.9, 70.7, 70.6, 70.4, 69.6, 67.7, 67.1, 61.8, 21.1; HRMS (ESI-TOF) $m/z$: [M + Na]$^+$ requires 387.1639, found 387.1639.

**Synthesis of the Heteroarm Malonate (5).** Tetrazine alcohol 3 (1.0 g, 2.7 mmol) and 2-(2-(2-azidoethoxy)-ethoxy)ethan-1-ol (0.6 g, 2.7 mmol) were dissolved in dry dichloromethane (DCM) (60 mL), and NaHCO₃ (0.92 g, 4.4 equiv) was added. The reaction mixture was cooled to 0 °C, and a mixture of malonyl chloride (0.24 mL, 2.4 mmol) was added dropwise over a period of 40 min via a dropping funnel under N₂. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched by an addition of water, and the mixture was extracted with dichloromethane (40 mL) was added. The mixture was filtered, and evaporated to dryness. The residue was purified by silica gel chromatography (Et₂O/acetone, 95:5, v/v) to yield 0.34 g (21%) of the product 5 as a pink oil. $^{1}$H NMR (600 MHz, CDCl₃) $\delta$ 8.54 (d, 2H, $\ J = 8.9$ Hz), 7.11 (d, 2H, $\ J = 8.9$ Hz), 4.31 (t, 4H, $\ J = 4.8$ Hz), 3.78 (dd, 2H, $\ J = 6.5$ and 4.1 Hz), 3.74–3.67 (m, 20H), 3.46 (s, 2H), 3.40 (t, 2H, $\ J = 5.0$ Hz), 3.08 (s, 3H); $^{13}$C NMR (125 MHz, CDCl₃) $\delta$ 166.6, 163.8, 162.5, 129.7, 124.3, 115.3, 72.5, 70.9, 70.7, 70.6, 70.4, 69.6, 67.7, 67.1, 64.60, 64.58, 50.7, 45.2, 21.1; MS (ESI-TOF): M requires 4618.6, found 4618.6 (calculated from $[(M+2H)/2]^+$). The purity of the product was verified by MS (ESI-TOF) (Figure S16) and by $^1$H NMR (Figure S17), HMQC (Figures S18 and S19), and HMBC (Figure S20) spectroscopy. The assignment of the signals is shown in Table S1. $^1$H NMR was also used to determine the accurate quantity of the product, by using a known amount of acetonitrile as an internal standard and comparing the $^1$H NMR peak areas of 5 to the methyl signal of acetonitrile. 5 was obtained in 66% isolated yield.

**Synthesis of Conjugates C5–C7.** The same procedure described for 5 was used to obtain two other C₆₀-glyco conjugates C5 and C6 and C₆₀-glyco-peptide-conjugate C7. 0.1 µmol of 1 was used for the assembly. The reagent excesses and solvent volumes were adjusted accordingly. The RP HPLC analysis of the individual reaction steps is described in Scheme S4. The authenticity of the intermediate products (C2 and C3, aliquots purified prior MS-characterization) and of the end products (C5–C7) was verified by MS (ESI-TOF) spectroscopy (Figures S15, S16, and S21). The isolated yields were extracted from the UV absorbance of the isolated products at $\lambda = 260$ nm. (The absorbance was compared to that of a known concentration of C4, $\epsilon = 120 \times 10^3$ L mol⁻¹ cm⁻¹; in the case of C7, the absorbance of the peptide moiety was considered.) Accordingly, C5, C6, and C7 were isolated in 56, 69, and 61% yields, respectively.

**Synthesis of C8.** TCO-ON1 (40 nmol in 40 µL of DMSO) was added to a mixture of 1 (5.0 nmol) in DMSO (5 µL) in a microcentrifuge tube. The mixture was mixed overnight at room temperature. Reaction mixture was introduced to an RP-HPLC (Scheme S4), and the product fractions were lyophilized to dryness. C8 was obtained in 50% isolated yield (based on UV absorbance at $\lambda = 260$ nm). The authenticity and homogeneity of C8 was verified by MS (ESI-TOF) (Figure S22) and by PAGE (Figure 1).

**Synthesis of C9.** BCN-ON2 (9.0 nmol in 10 µL of H₂O) was added to a mixture of C8 (1.0 nmol) in H₂O (10 µL) in a microcentrifuge tube. The mixture was mixed overnight at room temperature and introduced to an RP HPLC (Scheme S4), and the product fractions were lyophilized to dryness. C9 was obtained in 57% isolated yield (based on the UV absorbance at $\lambda = 260$ nm). The authenticity and homogeneity of C9 were verified by SEC-MALS (Figure S23) and by PAGE (Figure 1).

## Associated Content

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c05955.

Experimental and characterization details for the synthesis of 1, 3, 5, BCN, and TCO-modified carbohydrates, oligonucleotides, and BCN-modified peptide and C4–C9 (PDF)
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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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