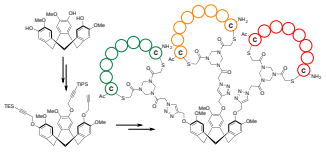
An orthogonally protected CycloTriVeratrylene (CTV) as a highly pre-organized molecular scaffold for subsequent ligation of different cyclic peptides towards protein mimics

Leave this area blank for abstract info.

Ondřej Longin, Helmus van de Langemheen, Rob M.J. Liskamp*

School of Chemistry, Joseph Black Building, University of Glasgow, University Avenue, Glasgow G12 8QQ (UK)



An Orthogonally Protected CycloTriVeratrylene (CTV) as a Highly Pre-organized Molecular Scaffold for Subsequent Ligation of Different Cyclic Peptides towards Protein Mimics

Ondřej Longin[a], Helmus van de Langemheen[a] and Rob M.J. Liskamp*[,a]

[a] *School of Chemistry, Joseph Black Building, University of Glasgow, University Avenue, Glasgow G12 8QQ (UK)*
E-Mail: Robert.liskamp@glasgow.ac.uk

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Protein mimic
Peptide cyclisation
Orthogonal functionalization
Molecular scaffold
Synthetic antibody

ABSTRACT

The synthesis of a (semi)orthogonally protected CycloTriVeratrilene (CTV) scaffold derivative as well as the sequential introduction of three different peptide loops onto this molecular scaffold *via* Cu(I)-catalyzed azide alkyne cycloaddition towards a medium-sized protein mimic is described. This approach for the construction of medium-sized protein mimics is illustrated by the synthesis of a paratope mimic of the monoclonal antibody Infliximab (Remicade®) and provides access to a range of highly pre-organised molecular constructs bearing three different peptide segments. This approach may find wide applications for development of protein-protein interaction disruptors as well as synthetic vaccines.

1. Introduction

Protein mimics form a category of medium sized molecules, which might have a large impact because they could become viable medium-sized molecule alternatives of biologics such as antibodies and vaccines. Thus, they may have advantages closer to those of smaller molecules such as better bio-availability and stability and be less immunogenic. We have provided bases for realization of molecular construction of protein mimics by (1) the development of syntheses of different scaffolds for attachment of (cyclic) peptides,[1-4] (2) synthetic approaches for attachment of different (cyclic) peptides to scaffolds,[5-10] and (3) the generation of collections of the resulting protein mimics.[9,11,12] The latter aspect is especially note worthy since design of an optimal protein mimic is often hardly possible even when a significant amount of structural data is available, so there has to be a versatile approach to prepare collections or libraries of protein mimics, which can be screened to find protein mimic "hits"[9,12] For this purpose a non-orthogonally protected scaffold is used, providing direct access to the required unprotected protein mimics, which can be screened as mixtures or as single compounds, followed by MS-identification of the hit(s).[11,12] However, this/these hit(s) then have to be re-synthesized using the an orthogonally protected scaffold to obtain appreciable quantities of single protein mimic compounds for validation of the hit and subsequent structural and biological activity studies Thus, a scalable (re)synthesis approach for any individual hit is absolutely essential. So far, we have developed semi-orthogonally protected TAC, ATAC and TACO scaffolds,[1,3,4]

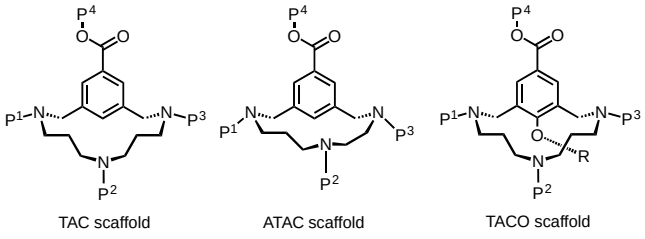


Figure 1. Orthogonally protected TAC[1,3,13,14], ATAC[3], and TACO[4] scaffolds

which can be applied for the convenient synthesis of selected protein mimics (Figure 1). Other important orthogonally protected molecular scaffolds include the RAFT-peptide (Mutter and co-workers[15]), cholic acid derivatives (Savage and co-workers[16]), cyclic β-peptide and penta-erythrityltetramine derivatives (Lönnberg and co-workers[17,18]), a pentaerythritol derivative (De Clerq and co-workers[19]) as well as a cyclicpeptide derivative by Eichler and co-workers.[20]

* Corresponding author. Tel.: +0-000-000-0000; fax: +0-000-000-0000; e-mail: author@university.edu

In the past we have used the highly pre-organized CycloTriVeratrylene (CTV) scaffold successfully for development of effective collagen mimics.[2] More recently, preparation of collections of gp120 protein mimics, using these different scaffolds, showed that CTV-scaffolded gp120 epitope mimics were the most powerful protein mimics (IC_{50}-values of the gp120-CD4 interaction in ELISA of 1.7 and 2.2 μM).[12] Therefore, we felt it was necessary to develop an orthogonally protected CTV derivative, so that the selected protein mimics can be re-synthesized. In this research we describe the synthesis of the highly pre-organized semi-orthogonally protected CTV scaffold as well as an example of sequential introduction of three different cyclic peptides towards individual protein mimics.

2. Results and discussion

Similar to the recently described semi-orthogonal alkyne functionalized TAC-scaffold **1** (Figure 2), we wished to develop a semi-orthogonally alkyne functionalized CTV scaffold **2**.

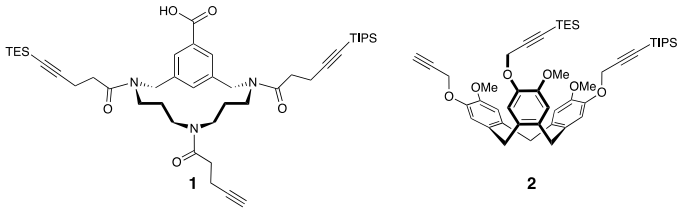
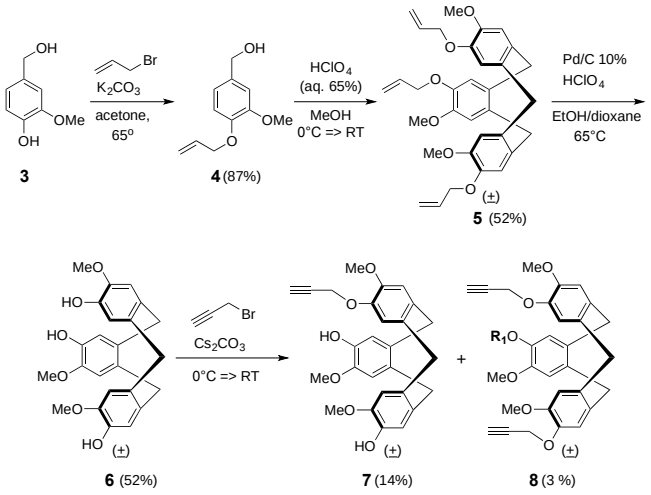


Figure 2. Recently developed orthogonally protected TAC-scaffold[13] **1** and the orthogonally protected (±)-CTV scaffold **2**.

However, in contrast to the TAC-scaffold,[1,3] **1** orthogonality of protection cannot be achieved during synthesis of the CTV scaffold[21] **6** (Scheme 1) itself, but has to be introduced post-synthesis, thus, after completion of its synthesis. First, it was attempted to mono-functionalize the CTV scaffold **6** with propargyl bromide (**14**). This gave only low yields of CTV propargyl ether **7**, together with di-alkylated derivative **8** and starting material, which were hardly separable.

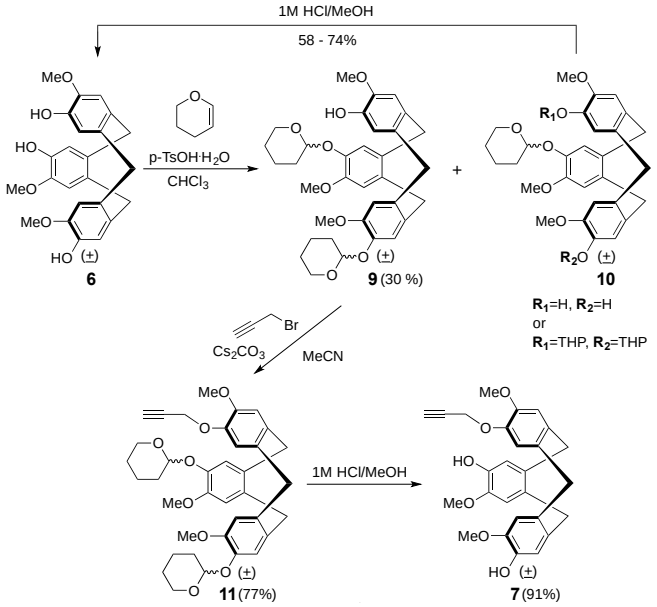


Scheme 1. Synthesis of CTV scaffold **6** and mono-alkylation attempt.

Therefore, it was attempted to temporarily protect two phenolic hydroxy functionalities and subsequently alkylate the remaining free hydroxy group. Attempts to protect two hydroxy functionalities as bis-esters or bis-silylethers were unsuccessful. Nevertheless, the desired temporary protection was achieved using THP protecting group, which afforded the desired di-THP derivative **9** from CTV **6** under mild conditions in a decent 30% yield (Scheme 2). Moreover, the di-THP derivative **9** was easily separable from mono and tri-THP-ethers **10**, which could then be converted back to CTV **6** in a moderate yield. Subsequently, di-THP ether **9** was alkylated to give **11** followed by removal of the THP-protecting group leading to mono-propargyl ether **7** in a good (70% over 2 steps) yield.

Scheme 2. Synthesis of mono-propargyl CTV **7** via di-THP-CTV ether **9**

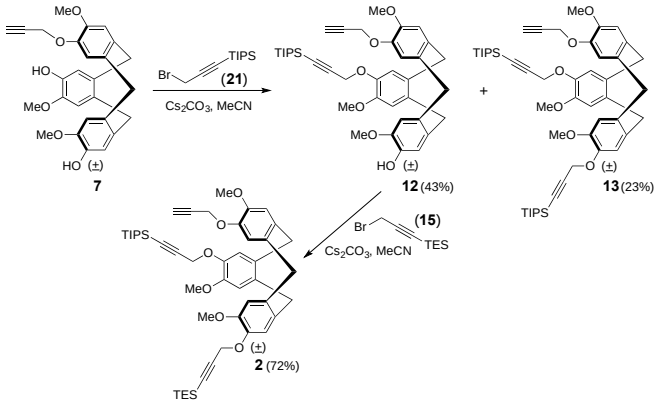
Next, silyl protected alkynes were introduced onto the mono alkylated scaffold **7** (Scheme 3). Since the TIPS-group is more stable, alkyne bromide **21** was introduced first onto the propargyl

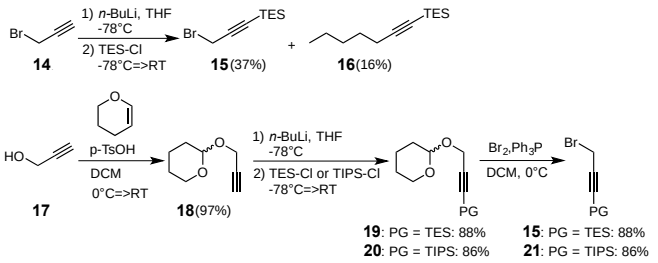


CTV derivative **7**. Along with the desired mono-TIPS derivative **12** the di-TIPS side product **13** was formed. Because of the formation side product **13** and only 1.05 eq. of **21** used in the reaction, some starting material **7** remained unreacted and was recovered (25%). Finally, the mono-TIPS derivative **12** was alkylated with TES-protected alkyne bromide **15** to give the desired semi-orthogonally protected CTV scaffold derivative **2** in a good yield (72%) (Scheme 3).

Scheme 3. Completion of the synthesis of the semi-orthogonally protected CTV scaffold **2**.

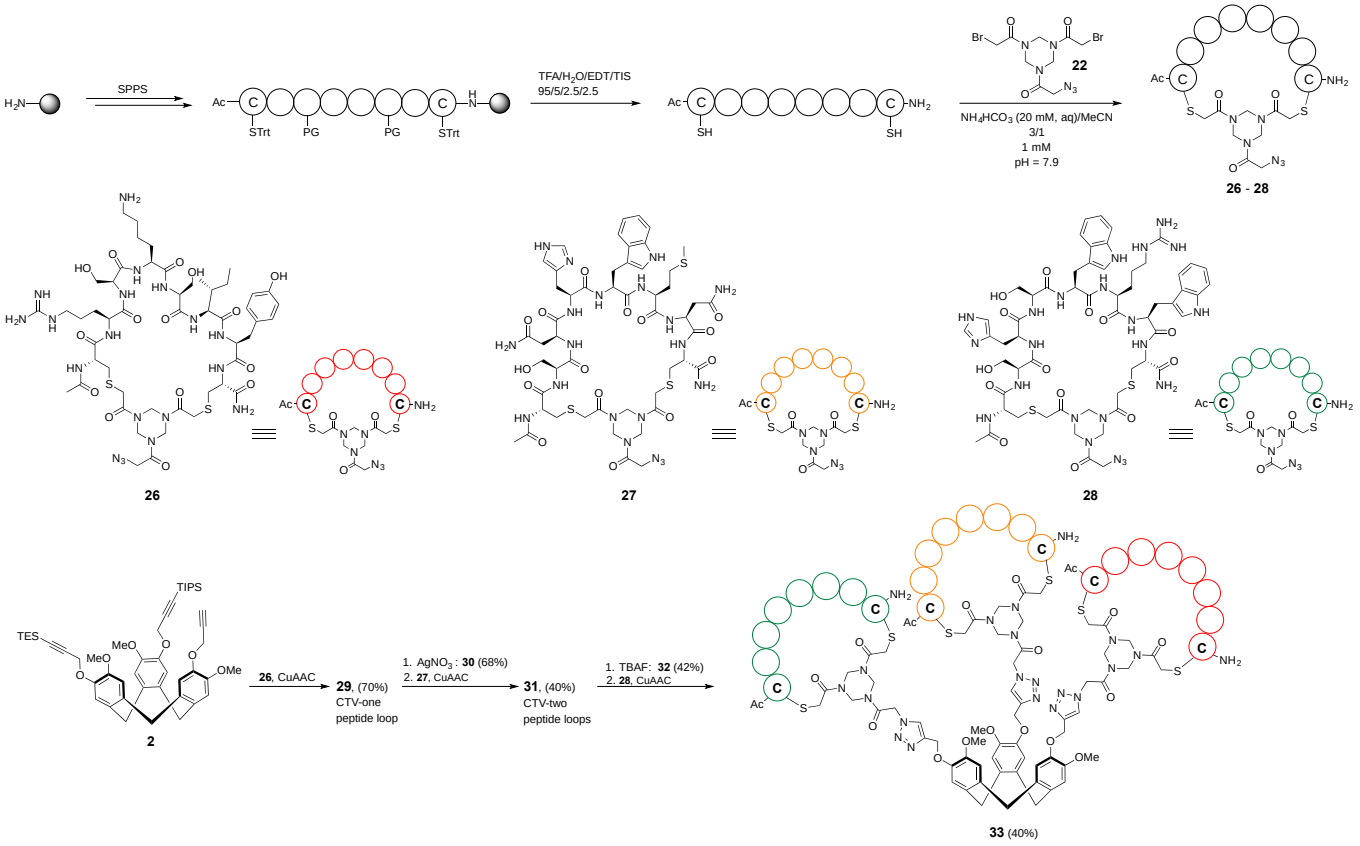
The preparation of the required silyl protected alkyne bromides **15** and **21** is shown in Scheme 4. First, a direct silylation of propargyl bromide **14** with TES-Cl was attempted (Scheme 4).





Scheme 4. Synthesis of silyl protected alkyne bromides **15** and **21.**

However, the target TES-protected alkyne bromide **15** was obtained in a low yield partly due to formation of the substitution by-product **16**. Here too, the THP group offered a remedy. Hence, the desired silyl protected alkyne bromides **15** and **21** were prepared starting from propargyl alcohol **17** which was protected as a THP ether **18**. Following the protection, the free alkyne of the THP ether **18** was silylated with either TES-Cl or TIPS-Cl to give corresponding TES and TIPS protected analogues **19** and **20** respectively. Lastly, bromination and concomitant removal of the THP ethers of the silyl protected alkynes **19** and **20** led to the formation of the desired silyl protected alkyne bromides **15** and **21**.



To illustrate the potential of the semi-orthogonally protected CTV scaffold **2** for the construction of protein mimics, three cyclized peptides obtained by using our recently described polar hinge[10], were sequentially introduced in a convenient "click and cleavage" approach. The peptide segments represent CDR-loops of the monoclonal antibody Infliximab (Remicade®), which is a powerful tumor necrosis factor α (TNF α) inhibitor. The amino acid sequences of these peptides were derived from the X-ray structure of an Infliximab-TNFα-trimer complex[22] After their solid phase syntheses, the N,C-terminal dicysteine containing peptides were cleaved and deprotected, followed by chemoselective cyclization using polar hinge **22** leading to cyclic peptides **26**-**28** (Scheme 5). The azide functionality in the hinge **22** allowed ligation of the resulting cyclic peptides by Cu(I)-catalyzed azide alkyne cycloaddition (CuAAC) onto the CTV scaffold derivative **2** in a similar manner as was described previously for semi-orthogonally protected TAC scaffold **1**.[13,14] However, no microwave irradiation was necessary and the solvent mixture was changed to *i*-PrOH/DMF/H_2O 4/3/1, v/v/v for complete dissolution of the CTV scaffold derivative **2**. Thus, first cyclic peptide **26** was ligated to **2** *via* CuAAC to give the CTV scaffold containing one peptide loop (**29**), in a

Scheme 5. Sequential ligation of cyclic peptides **26**, **27**, **28** onto the semi-orthogonally protected CTV scaffold **2**.

good yield (70%) (Scheme 5). Next, the TES protecting group was mildly removed using $AgNO_3$ leading to deprotected scaffold derivative **30** (68%) and cyclic peptide **27** was ligated *via* CuAAC to afford the CTV scaffold containing two peptide loops i.e. **31** (40%). The last peptide loop **28** was introduced by CuAAC after removal of the TIPS protecting group (TBAF·3H_2O in DMF, 42%) to complete the molecular construction of the three peptide loops containing protein mimic **33** of Infliximab in 40% yield.

3. Conclusion

We have described the construction of an orthogonally protected molecular scaffold, which is probably the most pre-organized scaffold presently available for the construction of protein mimics. Moreover, owing to the protection group strategy desired different peptide segments can be introduced corresponding to different epitopes, paratopes, protein hot-spots etc., which offers a great potential for applications of this CTV scaffold including antibody mimics and synthetic vaccines.

In this research we have described a versatile construction of a mimic (**33**) of the anti-TNFα monoclonal antibody Infliximab (Remicade®) containing three different cyclic peptides

corresponding to three CDR-loops of this antibody. These cyclic peptides were introduced in "click and cleavage" approach, in which sequentially a peptide loop was ligated, followed by cleavage of the protecting group and repeating this procedure. In establishing this procedure the mono and dipeptide loop constructs were purified and characterized, but we think that this procedure has possibilities for a fast ~~and easy~~ preparation of protein mimics in a "kit-like" manner with only purification after the last stage.

Under present investigation is the biological evaluation of the obtained bio-molecular construct. Although a strong biological activity is of course hoped for, realistically, probably libraries of this antibody mimic have to be prepared first, before finding hits with decent biological activities. However, this will be possible using our earlier described combinatorial approach for obtaining collections of discontinuous epitopes[11], now to be applied to the paratope of Infliximab. Promising hits can then be re-synthesized for validation and on a larger scale using the orthogonally protected CTV scaffold described here.

Finally, it is important to realize that also other (bio) molecular constructs e.g. different carbohydrate constructs, nucleic acid can be selectivity ligated to this semi-orthogonally protected CTV scaffold, which may open up a plethora of applications.

4. Experimental part

4.1. General information

All reagents and solvents were used as received. ^{1}H NMR and the ^{13}C NMR spectra were recorded on Bruker 400 MHz Spectrospin spectrometer (400 MHz, 100 MHz) in $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) relative to trimethylsilane (TMS, 0.00 ppm) or $CDCl_3$ (7.26 ppm). Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). TLC was carried out on silica gel plates (Merck $60F_{254}$) and the visualization was performed by both, the UV detection (254 nm) and staining solutions (cerium molybdenate, potassium permanganate) followed by heating. For column chromatography, silica gel Geduran® Si 60 (40 - 63 μm) was used. All chemicals and solvents were purchased from regular commercial sources in analytical or HPLC grade and were not further purified. Dry solvents (THF, DCM) were dispensed from Pure Solv™ 500 Solvent Purification System and other dry solvents (acetonitrile) were prepared from commercially available HPLC grade solvents by removal of residual water with 4Å molecular sieves overnight. HRMS-ESI was recorded on Bruker microTOFq High Resolution Mass Spectrometer in a positive mode. EI-MS was recorded on Jeol MSTATION JMS-700 in a positive mode.

Fmoc-amino acids were obtained from Activotec (Cambridge, United Kingdom) and *N*,*N*,*N*′,*N*′-Tetramethyl-*O*-(6-chloro-1*H*-benzotriazol-1-yl)uranium hexafluorophosphate (HCTU) was obtained from Matrix Innovation (Quebec, Canada). Tentagel S RAM resin (particle size 90μm, capacity 0.25 mmol.g^{-1}) was obtained from IRIS Biotech (Marktredwitz, Germany). Methyl *tert*-butyl ether (MTBE), *n*-hexane (HPLC grade) and TFA were obtained from Aldrich (Milwaukee, USA). DMF (Peptide grade) was obtained from VWR (Lutterworth, United Kingdom). Piperidine and D*i*PEA were obtained from AGTC Bioproducts (Hessle, United Kingdom), and 1,2-ethanedithiol (EDT) was obtained from Merck (Darmstadt, Germany). HPLC grade CH_2Cl_2 and acetonitrile were obtained from Fischer Scientific (Loughborough, United Kingdom). Solid phase peptide synthesis was performed on a PTI Tribute-UV peptide synthesizer. Lyophilizations were performed on a Christ Alpha 2-4 LD*plus* apparatus. Analytical high pressure liquid chromatography (HPLC) was carried out on a Shimadzu instrument comprising a communication module (CBM-20A), autosampler (SIL-20HT), pump modules (LC-20AT), UV/Vis detector (SPD-20A) and system controller (Labsolutions V5.54 SP), with a Phenomenex Gemini C18 column (110 Å, 5 μm, 250 × 4.60 mm). UV measurements were recorded at 214 and 254 nm, using a standard protocol: 100% buffer A (acetonitrile/H_2O 5:95 with 0.1% TFA) for 2 min followed by a linear gradient of buffer B (acetonitrile/H_2O 95:5 with 0.1% TFA) into buffer A (0-100%) over 30 min at a flow rate of 1.0 mL·min^{-1}. Purification of peptidic compounds was performed on an Agilent Technologies 1260 infinity preparative system using UV detector with a Phenomenex Gemini C18 column (110 Å, 10 μm, 250 × 20 mm). Auto-collection of fractions was based on the UV measurements at 214 nm, using either 100% buffer A or 95% buffer A with 5% buffer B for 5 min followed by and linear gradient of buffer B into buffer A (specified for each compound) over 65 min at a flow rate of 12.5 mL·min^{-1} using the same buffers as described for analytical HPLC. Liquid chromatography mass spectrometry (LCMS) was carried out on a Thermo Scientific LCQ Fleet quadrupole mass spectrometer with a Dionex Ultimate 3000 LC using a Dr. Maisch Reprosil Gold 120 C18 column (110 Å, 3 μm, 150 × 4.0 mm), using a 0-100% linear gradient of buffer B into buffer A and the same flow rate and buffers as described for analytical HPLC.

4.2. Scaffold synthesis and derivatization

*1-O-allyl-vanillyl alcohol (**4**)* Compound **4** was prepared according to a modified literature procedure.[21] To the solution of vanillyl alcohol **3** (55.0 g, 356.8 mmol) in acetone (82 mL), K_2CO_3 (49.8 g, 360.3 mmol) and allyl bromide (34.0 mL, 392.5 mmol) were added. The resulting mixture was stirred under reflux for 4.5 h. Afterwards, acetone was removed under reduced pressure and the residue was partioned between CH_2Cl_2 (500 mL) and water (400 mL). The organic phase was then dried over $MgSO_4$, and filtered. Crude **4** (65.1 g, 94.0%) was obtained upon removal of CH_2Cl_2 under reduced pressure. The crude product was crystallized from MTBE/*n*-hexane affording **4** (60.3 g, 87.0%) as white crystals. R_f = 0.32 (*n*-hexane/EtOAc 6/4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.94 (s, 1H), 3.89 (s, 3H), 4.61 - 4.63 (m, 4H), 5.29 (dd, J = 1.4 Hz, 10.5 Hz, 1H), 5.41 (dd, J = 1.5 Hz, 17.3 Hz, 1H), 6.04 - 6.14 (m, 1H), 6.86 (s, 2H), 6.94 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.9, 65.2, 70.0, 110.9, 113.5, 117.9, 119.3, 133.3, 134.1, 147.5, 149.6. HRMS-ESI: m/z calcd for $C_{11}H_{14}NaO_3$ $[M+Na]^+$, 217.0835; found, 217.0834.This compound has been also previously reported.[21]

*Tris(O-allyl) CTV (**5**)* Compound **5** was prepared according to a literature procedure[21] in the following manner. Compound **4** (57.4 g, 295.5 mmol) was dissolved in MeOH (340 mL) and the solution was cooled to 0°C resulting in the formation of a precipitate. Next, upon the dropwise addition of 60% (aq) $HClO_4$ (171 mL) the precipitate dissolved and the solution became pink. Once $HClO_4$ was added, the reaction mixture was allowed to warm up to RT and stirred for 16.5 h during which white precipitate gradually formed. Afterwards, the reaction mixture was diluted with CH_2Cl_2 (570 mL) and washed with water (7 × 570 mL) till the neutral pH was reached, which can be also recognized by the change of the color of the organic layer from pink to yellow. The organic phase was then dried over $MgSO_4$, filtered, concentrated under reduced pressure to give pale yellow solid which was suspended in Et_2O (100 mL) and stirred for 2 h before being filtered off. The filtrate was washed with Et_2O

(3 × 30 mL) and dried on high vacuum. Compound **5** (27.1 g, 52.0%) was obtained as a white solid. R_f = 0.47 (*n*-hexane/EtOAc 6/4). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.54 (d, J = 13.9 Hz), 3.86 (s, 9H), 4.56 - 4.66 (m, 6H), 4.77 (d, J = 13.7 Hz, 3H), 5.28 (dd, J = 1.4 Hz, 10.5 Hz, 3H), 5.39 (dd, J = 1.6 Hz, 17.3 Hz, 3H), 6.04 - 6.13 (m, 3H), 6.82 (s, 3H), 6.88 (s, 3H). ^{13}C NMR (100 MHz, CDCl3): δ = 36.3, 55.9, 70.0, 113.5, 115.5, 117.3, 131.6, 132.2, 133.6, 146.6, 148.0. HRMS-ESI: m/z calcd for $C_{33}H_{36}NaO_6$ $[M+Na]^+$, 551.2404; found, 551.2391.This compound has been also previously reported.[21]

*CTV-triOH (**6**)* Compound **6** was prepared according to a modified literature procedure.[21] To the solution of **5** (26.0 g, 49.2 mmol) in dioxane/EtOH (130 mL/230 mL), 10% Pd/C (5.4 g), 65% (aq) $HClO_4$ (5.4 mL) were added and the resulting reaction mixture was stirred under nitrogen atmosphere at 65°C for 24 h and then for 48 h at RT. Next, catalyst was filtered off and washed with dioxane (50 mL) and CH_2Cl_2 (200 mL). The filtrate was then washed with water (2 × 700 mL, 2 × 500 mL), aqueous layer was extracted with CH_2Cl_2 (100 mL) and the combined organic layer was dried over $MgSO_4$, filtered, concentrated under reduced pressure to ca 100 mL, and allowed to crystallize. The crystals were filtered off, washed with cold CH_2Cl_2 (3 × 30 mL) and dried on a high vacuum to afford **6** (10.5 g, 52.1%) as white crystals. R_f = 0.18 (*n*-hexane/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.50 (d, J = 13.9 Hz), 3.85 (s, 9H), 4.72 (d, J = 13.7 Hz, 3H), 5.41 (s, 3H), 6.79 (s, 3H), 6.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 36.3, 56.1, 112.3, 115.5, 131.2, 132.5, 144.2, 145.3. HRMS-ESI: m/z calcd for $C_{24}H_{24}NaO_6$ $[M+Na]^+$, 431.1465; found, 431.1464.This compound has been also previously reported.[23]

*Di(O-THP) CTV-OH (**9**), Mono(O-THP) CTV-diOH and Tri(O-THP) CTV (**10**)*

To a suspension of CTV **6** (5.0 g, 12.4 mmol) in $CHCl_3$ (124 mL), DHP (2.3 mL, 25.4 mmol) and p-TsOH monohydrate (0.02 g, 0.1 mmol) were added. After 2 h of stirring, during which the reaction mixture became pink, additional DHP (1.2 mL, 12.3 mmol) was added, which resulted in the formation of a solution which was stirred for an additional 1 h. Afterwards, the reaction mixture was diluted with Et_2O (125 mL) followed by immediate addition of saturated $NaHCO_3$ (50 mL). Direct addition of $CHCl_3$ or saturated $NaHCO_3$ to reaction mixture was attempted too but lead to partial decomposition in certain cases. Next, the aqueous layer was extracted with Et_2O (100 mL) and the combined organic layer was dried over $MgSO_4$, filtered, and solvents were removed under reduced pressure. The crude product was purified by a column chromatography (*n*-hexane/EtOAc 2.5/1 to *n*-hexane/EtOAc 1/1). Compound **6** (2.1 g, 29.9%) was obtained as a yellow foam. R_f = 0.33 (*n*-hexane/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.52 - 1.74 (m, 6H), 1.79 - 1.95 (m, 4H), 1.99 - 2.08 (m, 2H), 3.47 - 3.59 (m, 5H), 3.81 - 3.91 (m, 10H), 3.99- 4.06 (m, 1H), 4.69 - 4.75 (m, 3H), 5.17 - 5.19 (m, 1H), 5.40 - 5.45 (m, 1H), 5.47 (s, 1H), 6.81 (d, J = 3.1 Hz, 1H), 6.84 (d, J = 1.9 Hz, 1H), 6.85 (s, 1H), 6.90 (dd, J = 1.8 Hz, 5.7 Hz, 1H), 7.11- 7.15 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.8, 19.0, 19.1, 25.4, 30.4, 30.6, 36.4, 36.5, 55.9, 56.0, 56.3, 56.4, 56.5, 56.6, 62.0, 62.3, 97.2, 97.3, 98.2, 98.3, 112.3, 113.9, 114.1, 114.3, 114.4, 115.5, 115.6, 115.7, 115.8, 118.7, 119.0, 119.5, 120.0, 120.1, 131.5, 131.6, 131.7, 131.9, 132.0, 132.1, 132.3, 132.4, 133.1, 133.3, 133.9, 134.0, 144.1, 144.2, 144.9, 145.0, 145.1, 145.3, 145.4, 148.6, 148.7, 149.1. HRMS-ESI: m/z calcd for $C_{34}H_{40}NaO_8$ $[M+Na]^+$, 599.2615; found, 599.2592. Along with the product **9**, a mixture of **10** was obtained as a yellow foam. For analytical purposes ^{1}H NMR spectra of pure Mono(O-THP) CTV-diOH and Tri(O-THP) CTV were measured before the compounds were mixed for recovery of compound **6**. Mono(O-THP) CTV-diOH: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.56 - 1.71 (m, 3H), 1.81 - 2.06 (m, 3H), 3.47 - 3.59 (m, 4H), 3.82 - 3.89 (m, 9.5H), 4.00 - 4.05 (m, 0.5H), 4.65 - 4.71 (m, 3H), 5.17 (s, 0.5H), 5.42 (s, 0.5H), 5.56 (s, 2H), 6.78 - 6.91 (m, 5H), 7.12 (s, 1H). R_f = 0.20 (*n*-hexane/EtOAc 1/1). Tri(O-THP) CTV: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.52 - 1.69 (m, 9H), 1.79 - 2.03 (m, 9H), 3.47 - 3.58 (m, 6H), 3.80 - 3.90 (m, 10.5H), 3.98 - 4.04 (m, 1.5H), 4.68 - 4.72 (m, 3H), 5.18 (m, 1.5H), 5.39 (m, 1.5H), 6.84 (s, 3H), 7.11 - 7.13 (m, 3H). R_f = 0.52 (*n*-hexane/EtOAc 1/1).

*Recovery of CTV-triOH (**6**)* To the suspension of side products **10** in MeOH (13 mL per 1 g of mixture), 1M HCl (2.6 mL per 1 g of mixture) was added and the reaction mixture was stirred for 2 h during which a white precipitate was formed. Afterwards, MeOH was removed under reduced pressure and the creamy residue was dissolved in EtOAc/MeOH (40/1, v/v, 130 mL per 1 g of mixture) and washed with water/brine (1/1, v/v, 40 mL per 1 g of mixture). The aqueous layer was extracted with EtOAc (25 mL per 1 g of mixture) and the combined organic layer was washed with brine (25 mL per 1 g of mixture), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to a low volume. White precipitate was formed during removal of solvents. The suspension was kept at +4°C overnight before the white precipitate was filtered off and washed with EtOAc to give compound **6** (typically between 58% - 74%).

*Di(O-THP)-O-propargyl CTV (**11**)* To the solution of compound **9** (1.0 g, 1.7 mmol) in acetonitrile (17 mL) Cs_2CO_3 (0.6 g, 1.9 mmol) was added, followed by the addition of propargyl bromide (80% in toluene) (0.23 mL, 2.1 mmol) and the resulting reaction mixture was stirred for 2.5 h. Afterwards, acetonitrile was removed under reduced pressure and the viscous residue was diluted with EtOAc (100 mL) and washed with water (100 mL). Aqueous layer was extracted with EtOAc (50 mL) and combined organic layer was washed with brine (50 mL), dried over $MgSO_4$, filtered, and EtOAc was removed under reduced pressure. The crude product was purified by a column chromatography (*n*-hexane/EtOAc 9/1 to *n*-hexane/EtOAc 7/3). Compound **11** (0.8 g, 76.8%) was obtained as a white foam. R_f = 0.51 (*n*-hexane/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.53 - 1.69 (m, 6H), 1.80 - 2.05 (m, 6H), 2.39 (s, 1H), 3.47 - 3.57 (m, 5H), 3.80 - 3.83 (m, 10H), 3.98 - 4.03 (m, 1H), 4.67 - 4.73 (m, 5H), 5.17 - 5.20 (m, 1H), 5.40 (s, 1H), 6.83 - 6.84 (m, 2H), 6.87 (s, 1H), 7.01 (d, J = 5.8 Hz, 1H), 7.12 - 7.15 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.7, 19.0, 25.3, 30.4, 30.5, 36.4, 36.5, 55.8, 56.0, 56.2, 56.3, 56.4, 56.6, 56.7, 57.0, 61.9, 62.2, 75.8, 79.1, 97.1, 97.2, 98.1, 98.2, 98.3, 113.4, 113.5, 113.8, 114.1, 114.6, 114.7, 114.8, 116.4, 116.5, 116.6, 118.6, 118.9, 119.0, 119.6, 119.7, 119.8, 131.3, 131.4, 131.5, 131.6, 131.7, 131.9, 132.0, 132.1, 132.2, 132.3, 133.1, 133.2, 133.8, 133.9, 134.0, 145.0, 145.1, 145.2, 145.3, 148.4, 148.5, 148.6, 148.9, 149.1. HRMS-ESI: m/z calcd for $C_{37}H_{42}NaO_8$ $[M+Na]^+$, 637.2772; found, 637.2755.

*O-Propargyl CTV-diOH (**7**)* Compound **11** (790 mg, 1.29 mmol) was suspended in MeOH (20 mL) and then 1M HCl (1 mL) was added. The reaction mixture was stirred for 1 h during which the suspension turned into a yellow solution. Afterwards, MeOH was removed under reduced pressure and the viscous residue was diluted with EtOAc (40 mL) and washed with water (30 mL). Next, the aqueous phase was extracted with EtOAc (20 mL) and the combined organic layer was washed with brine (30 mL), dried over $MgSO_4$, filtered, and EtOAc was removed under reduced pressure. The crude product was purified by a column chromatography ($CHCl_3$ to $CHCl_3$/MeOH 50/1).

Compound **7** (524 mg, 91.4%) was obtained as a white foam. R_f = 0.25 (*n*-hexane/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.44 (t, J = 2.4 Hz, 1H), 3.49 - 3.56 (m, 3H), 3.84 (s, 3H), 3.86 (s, 6H), 4.70 - 4.77 (m, 5H), 5.39 (s, 1H), 5.41 (s, 1H), 6.79 (s, 1H), 6.84 (s, 1H), 6.85 (s, 1H), 6.89 (s, 1H), 6.90 (s, 1H), 7.01 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 56.8, 57.9, 76.4, 78.0, 79.9, 113.0, 113.1, 114.4, 116.3, 117.4, 131.9, 132.5, 132.9, 133.3, 134.3, 144.9, 145.9, 146.0, 149.2. HRMS-ESI: m/z calcd for $C_{27}H_{26}NaO_6$ $[M+Na]^+$, 469.1622; found, 469.1603.

*O-Propargyl-O-(TIPS)propargyl CTV-OH (**12**), O-Propargyl bis[O-(TIPS)propargyl] CTV (**13**)* Cs_2CO_3 (181 mg, 0.56 mmol) was added to the solution of compound **7** (226 mg, 0.51 mmol) in dry acetonitrile (20 mL). Next, a solution of compound **21** (146 mg, 0.53 mmol) in dry acetonitrile (3 mL) was added dropwise. The reaction mixture was stirred under nitrogen atmosphere for 20 h. Afterwards, acetonitrile was removed under reduced pressure and the residue was suspended in Et_2O (40 mL) and washed with 1M $KHSO_4$ (40 mL). The aqueous layer was extracted with Et_2O (20 mL) and the combined organic layer washed with brine (40 mL), dried over $MgSO_4$, filtered, and Et_2O was removed under reduced pressure. The crude product was purified by a column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 100/1) to afford compound **13** (93 mg, 23.2%) as a pale yellow solid. R_f = 0.87 (*n*-hexane/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.02 (m, 42H), 2.45 (t, J = 2.4 Hz, 1H), 3.50 - 3.57 (m, 3H), 3.83 - 3.84 (m, 9H), 4.57 - 4.86 (m, 9H), 6.83 (s, 2H), 6.85 (s, 1H), 7.00 (s, 1H), 7.14 (s, 1H), 7.16 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.1, 18.6, 36.5, 56.0, 56.2, 57.0, 58.6, 75.7, 79.1, 89.2, 102.5, 113.5, 113.9, 116.6, 117.6, 117.9, 131.5, 131.7, 133.3, 133.6, 145.3, 145.9, 146.0, 148.5, 148.8. HRMS-ESI: m/z calcd for $C_{51}H_{70}NaO_6Si_2$ $[M+Na]^+$, 857.4603; found, 857.4569. Compound **12** (139 mg, 42.9%) was obtained as a yellow amorphous solid. R_f = 0.62 (*n*-hexane/EtOAc 1/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (m, 21H), 2.44 (m, 1H), 3.47 - 3.54 (m, 3H), 3.83 (s, 9H), 4.58 - 4.84 (m, 7H), 5.44 (d, J = 9.4 Hz, 1H), 6.79 - 6.85 (m, 3H), 6.89 (d, J = 6.8 Hz, 1H), 6.99 (d, J = 6.3 Hz, 1H), 7.13 (d, J = 3.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.1, 18.6, 36.4, 56.0, 56.1, 56.2, 57.0, 57.1, 58.6, 75.6, 75.7, 79.1, 79.2, 89.2, 102.6, 112.1, 112.4, 113.5, 113.7, 113.9, 115.7, 116.6, 116.8, 117.9, 131.1, 131.3, 131.4, 131.6, 131.7, 131.9, 132.4, 133.6, 133.7, 144.2, 145.3, 145.4, 145.9, 148.5, 145.7, 145.8. HRMS-ESI: m/z calcd for $C_{39}H_{48}NaO_6Si$ $[M+Na]^+$, 663.3112; found, 663.3092. Starting material, compound **7**, (57 mg, 25.2%) was recovered.

*O-Propargyl-O-(TIPS)propargyl-O-(TES)propargyl CTV (**2**)* To the suspension of compound **12** (303 mg, 0.47 mmol) in dry acetonitrile (5 mL) Cs_2CO_3 (162 mg, 0.50 mmol) was added followed by the addition of the solution of compound **15** (121 mg, 0.52 mmol) in dry acetonitrile (0.5 mL). The resulting mixture was stirred for 5.5 h and then acetonitrile was removed under reduced pressure. The viscous residue was suspended in Et_2O (40 mL) and washed with water (30 mL). The aqueous layer was extracted with Et_2O (30 mL) and the combined organic layer washed with brine (20 mL), dried over $MgSO_4$, filtered, and Et_2O was removed under reduced pressure. The crude product was purified by a column chromatography (*n*-hexane/Et_2O 3/1 to *n*-hexane/Et_2O 2/1). Compound **2** (269 mg, 71.7%) was obtained as a white foam. R_f = 0.60 (*n*-hexane/EtOAc 3/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.58 (m, 6H), 0.94 (m, 9H), 1.02 (m, 21H), 2.45 (m, 1H), 3.50 - 3.57 (m, 3H), 3.84 (m, 9H), 4.56 -- 4.85 (m, 9H), 6.83 - 6.85 (m, 3H), 7.00 (s, 1H), 7.09 - 7.15 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 4.2, 7.4, 11.1, 18.6, 36.5, 56.0, 56.1, 57.0, 58.5, 58.6, 58.7, 75.7, 79.1, 89.2, 90.3, 101.8, 102.5, 113.5, 113.6, 114.0, 116.7, 117.9, 131.5, 131.6, 133.4, 133.5, 133.7, 145.4, 145.9, 146.0, 148.5, 148.6, 148.8. HRMS-ESI: m/z calcd for $C_{48}H_{64}NaO_6Si_2$ $[M+Na]^+$, 815.4134; found, 815.4102.

4.3. Preparation of silyl alkyne bromides

*O-THP Propargyl alcohol (**18**)* Compound **18** was prepared according to a literature procedure.[24] To the solution of propargyl alcohol **17** (2.6 mL, 44.6 mmol) and p-TsOH monohydrate (0.09 g, 0.5 mmol) in CH_2Cl_2 (45 mL) DHP (4.3 mL, 46.8 mmol) was added dropwise at 0°C. After stirring for 5 min at 0°C, the reaction mixture was allowed to warm up to RT and was stirred for 1 h. Next, the reaction mixture was washed with saturated $NaHCO_3$ (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (45 mL). The combined organic layer dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give compound **18** (6.1 g, 97.2%) as a yellow oil. R_f = 0.30 (*n*-hexane/Et_2O 95/5). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.50 - 1.67 (m, 4H), 1.70 - 1.89 (m, 2H), 2.41 (t, J = 2.4 Hz, 1H), 3.52 - 3.57 (m, 1H), 3.82 - 3.87 (m, 1H), 4.27 (ddd, J = 2.4 Hz, 11.3 Hz, 15.8 Hz, 2H), 4.83 (t, J = 3.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.0, 25.3, 30.2, 53.9, 61.9, 74.0, 79.7, 96.8. MS-CI: m/z calcd for $C_8H_{12}O_2$ $[M+H]^+$, 141; found, 141. This compound has been also previously reported.[25]

*O-THP (TIPS)Propargyl alcohol(**20**)*Compound **20** was prepared according to a modified literature procedure.[26] A solution of compound **18** (1.0 g, 7.1 mmol) in dry THF (20 mL) was cooled to -78°C. Next, *n*-BuLi (3.0 mL, 7.5 mmol, 2.5M in hexane) was added dropwise and the reaction mixture was stirred under nitrogen atmosphere at -78°C for 1 h before TIPS-Cl (1.7 mL, 7.9 mmol) was added. The reaction mixture was then allowed to warm up to RT and stirred under nitrogen atmosphere for additional 4 h before it was quenched by addition of saturated NH_4Cl (15 mL). The reaction mixture was extracted with Et_2O (2 × 25 mL), dried over $MgSO_4$, filtered, and Et_2O was carefully removed under reduced pressure with water bath kept at RT. The crude product was purified by a column chromatography (*n*-hexane to *n*-hexane/Et_2O 99/1) to afford **20** (1.8 g, 85.9%) as a clear oil. R_f = 0.44 (*n*-hexane/Et_2O 95/5). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.07 (m, 21H), 1.51 - 1.65 (m, 4H), 1.71 - 1.87 (m, 2H), 3.50 - 3.55 (m, 1H), 3.83 - 3.89 (m, 1H), 4.31 (ddd, J = 2.4 Hz, 6.3 Hz, 16.1 Hz, 2H), 4.91 (t, J = 3.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 18.6, 19.2, 25.4, 30.4, 54.7, 62.2, 87.0, 96.3, 103.4. HRMS-ESI: m/z calcd for $C_{17}H_{32}NaO_2Si$ $[M+Na]^+$, 319.2064; found, 319.2049. This compound has been also previously reported.[27]

*(TIPS)Propargyl bromide (**21**)* Compound **21** was prepared according to a modified literature procedure.[26] Br_2 (130 µL, 2.53 mmol) was added dropwise at 0°C to a solution of Ph_3P (697 mg, 2.66 mmol) in dry CH_2Cl_2 (10 mL) and the solution was stirred under nitrogen atmosphere for 30 min during which a white precipitate was formed. Next, compound **20** (750 mg, 2.53 mmol) was added dropwise and the reaction mixture was stirred under nitrogen atmosphere at 0°C for additional 5 h during which the white precipitate dissolved. The reaction mixture was then diluted with water (15 mL) and extracted with *n*-hexane (2 × 20 mL). The combined organic layer was washed with saturated $NaHCO_3$ (10 mL), dried over $MgSO_4$, filtered, and *n*-hexane was carefully removed under reduced pressure with water bath kept at RT. The crude product was purified by a column chromatography using (*n*-hexane) to afford **21** (682 mg, 98.0%) as a clear oil. R_f = 0.69 (*n*-hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.07 (m, 21H), 3.95 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.2, 15.0, 18.5, 89.2, 101.9. This compound has been also previously reported.[27]

*O-THP (TES)Propargyl alcohol (**19**)* Compound **19** was prepared according to a modified literature procedure.[27] A solution of compound **18** (1.0 g, 7.1 mmol) in dry THF (20 mL) was cooled to -78°C. Next, *n*-BuLi (3.0 mL, 7.5 mmol, 2.5M in hexane) was added dropwise and the reaction mixture was stirred under nitrogen atmosphere at -78°C for 1 h before TES-Cl (1.3 mL, 7.9 mmol) was added. The reaction mixture was then allowed to warm up to RT and stirred under nitrogen atmosphere for additional 4 h before it was quenched by addition of saturated NH_4Cl (15 mL). The reaction mixture was extracted with Et_2O (2 × 25 mL), dried over $MgSO_4$, filtered, and Et_2O was carefully removed under reduced pressure with water bath kept at RT. The crude product was purified by a column chromatography using (*n*-hexane to *n*-hexane/Et_2O 99/1) to afford **19** (1.6 g, 87.9%) as a clear oil. R_f = 0.42 (*n*-hexane/Et_2O 95/5). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.57 - 0.64 (m, 6H), 0.95 - 1.02 (m, 9H), 1.50 - 1.67 (m, 4H), 1.70 - 1.87 (m, 2H), 3.50 - 3.55 (m, 1H), 3.82 - 3.88 (m, 1H), 4.29 (s, 2H), 4.86 (t, J = 3.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 4.3, 7.4, 19.1, 25.4, 30.3, 54.7, 62.0, 82.3, 96.5, 102.7. HRMS-ESI: m/z calcd for $C_{14}H_{26}NaO_2Si$ $[M+Na]^+$, 277.1594; found, 277.1584. Although compound **8** has been also previously reported,[28] no spectroscopic data of compound **8** are available.

*(TES)Propargyl bromide (**15**)* Compound **15** was prepared according to a modified literature procedure.[26] Br_2 (0.3 mL, 5.9 mmol) was added dropwise at 0°C to a solution of Ph_3P (1.6 g, 6.2 mmol) in dry CH_2Cl_2 (23.6 mL) and the solution was stirred under nitrogen atmosphere for 30 min during which a white precipitate was formed. Next, compound **19** (1.5 g, 5.9 mmol) was added dropwise and the reaction mixture was stirred under nitrogen atmosphere at 0°C for additional 5 h during which the white precipitate dissolved. The reaction mixture was then diluted with water (15 mL) and extracted with *n*-hexane (2 × 20 mL). The combined organic layer was washed with saturated $NaHCO_3$ (10 mL), dried over $MgSO_4$, filtered, and *n*-hexane was carefully removed under reduced pressure with water bath kept at RT. The crude product was purified by a column chromatography using (*n*-hexane) to afford **15** (1.1 g, 82.3%) as a clear oil. R_f = 0.66 (*n*-hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.61 (q, J = 7.9 Hz, 6H), 0.99 (t, J = 7.8 Hz, 9H), 3.95 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 4.2, 7.3, 14.8, 90.1, 101.2. This compound has been also previously reported.[29]

4.4. Peptide synthesis and preparation of synthetic antibody

4.4.1. Linear peptides

General method for automated peptide synthesis: The peptides were synthesized on a PTI Tribute-UV peptide synthesizer. Tentagel S RAM resin (1.0 g, 0.25 mmol, 1.0 equiv or 400 mg, 0.1 mmol, 1.0 equiv) was allowed to swell (3 x 10 min). Deprotection of the Fmoc group was achieved by treatment of the resin with 20% piperidine in DMF using the RV_top_UV_Xtend protocol from the Tribute-UV peptide synthesizer followed by a DMF washing step (5 x 30 sec). The Fmoc-protected amino acids (with the 0.1 mmol scale 5 equiv was used and with the 0.25 mmol scale 4 equiv was used) were coupled using HCTU (with the 0.1 mmol scale 5 equiv was used and with the 0.25 mmol scale 4 equiv was used) and D*i*PEA (with the 0.1 mmol scale 10 equiv was used and with the 0.25 mmol scale 8 equiv was used) in DMF, as a coupling system, with 2 min pre-activation. The coupling time was 10 min when the peptide was synthesized on a 0.1 mmol scale and 20 min when the 0.25 mmol scale was conducted. After every coupling the resin was washed with DMF (6 x 30 sec). After the last amino acid coupling, the Fmoc group was cleaved using the normal deprotection conditions (described above) and the resulting free N-terminus was acetylated by treatment of the resin bound peptide with acetic anhydride (250 µL) and D*i*PEA (10 equiv for the 0.1 mmol scale and 8 equiv for the 0.25 mmol scale) in DMF using the standard coupling times (described above). After the last step the resin was washed with DMF (5 x 30 sec), DCM (5 x 30 sec), dried over a nitrogen flow for 10 min, followed by the cleavage of the resin-bounded peptide. Cleavage and global deprotection was achieved by treatment of the resin with TFA/H_2O/TIS/EDT (10 mL for the 0.25 mmol scale and 5 mL for the 0.1 mmol scale, 90/5/2.5/2.5, v/v/v/v) for 3 hours. Next, the peptide was precipitated by dropwise addition of the TFA mixture to a cold (4 °C) solution of MTBE/*n*-hexane (1/1, 90 mL for the 0.25 mmol scale and 45 mL for the 0.1 mmol scale). After centrifugation (3500 rpm, 5 min) the supernatant was decanted and the pellet was re-suspended in MTBE/*n*-hexane (1/1, v/v) and centrifuged again. Finally, the pellet was washed twice with MTBE/*n*-hexane 50 mL (1/1, v/v), each time collected by centrifugation, dissolved in *t*-BuOH/H_2O (1/1, v/v) and lyophilized to yield the crude linear peptide. Hence following peptides were obtained:

*Peptide 1·2TFA (**23**)*, Ac-CRSKSIYC-NH_2, t_R = 13.6 min. LCMS-ESI : average mass calcd $[M]^+$: 1000.2; found: 1000.5.

*Peptide 2·TFA (**24**)*, Ac-CSNHWMNC-NH_2, t_R = 15.4 min. LCMS-ESI: average mass calcd $[M]^+$: 1035.2; found: 1035.4.

*Peptide 2·2TFA (**25**)*, Ac-CSHSWRWC-NH_2, t_R = 15.7 min. LCMS-ESI: average mass calcd $[M]^+$: 1105.3; found: 1105.5.

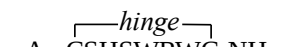
4.4.2. Cyclic peptides

All linear peptides were cyclized at the concentration of 1 mM in the following way. The crude linear peptide and the perhydro triazine hinge **22** were placed into a flask. Then, acetonitrile was added followed by the addition of aqueous solution of NH_4HCO_3 (20 mM, pH = 7.9) to form a 1/3 (acetonitrile/NH_4HCO_3 v/v) mixture. The progress of the cyclization was checked by analytical HPLC after 30 min. In case of all the peptides, 30 min were sufficient for the complete cyclization. Next, acetonitrile was removed under reduced pressure and the remaining aqueous solution/suspension was lyophilized. The crude cyclic peptides were purified using preparative HPLC. The fractions containing product were combined and lyophilized yielding the desired cyclic peptides as a white fluffy solid.

*Cyclic peptide·2TFA (**26**)*, Purified using 5% to 30% of buffer B in buffer A. Average yield 22% (19 steps, 92% per step). t_R = 14.1 min. LCMS-ESI: average mass calcd $[M]^+$: 1250.4; found: 1250.5.

*Cyclic peptide 2·TFA (**27**)*, Purified using 5% to 30% of buffer B in buffer A. Average yield 20% (19 steps, 92% per step). t_R = 15.3 min. LCMS-ESI: average mass calcd $[M]^+$: 1285.4; found: 1285.5.

*Cyclic peptide 2·2TFA (**28**),*. Purified using 5% to 40% of buffer B in buffer A. Average yield 11% (19 steps, 89% per step). t_R = 16.0 min. LCMS-ESI: average mass calcd $[M]^+$: 1355.5; found: 1355.5.



4.4.3. Preparation of a synthetic antibody mimic

*CTV Scaffold derivative (**29**)* Semi-orthogonally protected CTV scaffold **2** (5.2 mg, 6.50 µmol), cyclic peptide **26** (10.6 mg, 7.15 µmol), TBTA (1.0 mg, 1.95 µmol) were placed into a flask and degassed DMF (975 µL) was added and the mixture was

stirred under nitrogen atmosphere until clear solution was obtained. Next, degassed *i*-PrOH (1300 μL), deionized H_2O (290 μL) were added followed by the addition of 0.2M aqueous sodium ascorbate (19.5 μL, 3.90 μmol) and 0.12M aqueous $CuSO_4$ (16.3 μL, 1.95 μmol). The reaction mixture was stirred under nitrogen atmosphere and monitored by analytical HPLC which showed complete conversion after 1 h. Then, the solvents were removed with nitrogen stream giving red liquid residue (ca 50 μL) which was diluted with buffer B (150 μL) and buffer A (1200 μL). The resulting solution was centrifuged (4500 rpm, 5 min) and purified using 5% to 100% of buffer B in buffer A. Scaffold derivative **29**·2TFA (10.5 mg, 71.0%) was obtained as a white fluffy solid after lyophilization. t_R = 29.1 min. LCMS-ESI: average mass calcd $[M+2H]^{2+}$: 1022.8; found: 1022.5.

*CTV Scaffold derivative (**30**)* To a solution of scaffold derivative **29**·2TFA (10.3 mg, 4.53 μmol) in degassed DMF (680 μL), degassed *i*-PrOH (910 μL), and deionized H_2O (225 μL), $AgNO_3$ (3.9 mg, 22.65 μmol) was added and the reaction mixture was stirred under nitrogen atmosphere and monitored by analytical HPLC which showed complete conversion after 1 h. Then, the solvents were removed with nitrogen stream giving clear honey which was diluted with buffer A (1300 μL). The resulting solution was centrifuged (4500 rpm, 5 min) and purified using 5% to 100% of buffer B in buffer A. Scaffold derivative **30**·2TFA (6.3 mg, 64.3%) was obtained as a white fluffy solid after lyophilization. t_R = 24.9 min and 25.1 min (diastereoisomers). LCMS-ESI: average mass calcd $[M+2H]^{2+}$: 965.7; found: 965.5.

*CTV Scaffold derivative (**31**)* Scaffold derivative **30**·2TFA (6.0 mg, 2.78 μmol), cyclic peptide **27** (4.3 mg, 3.06 μmol), TBTA (0.4 mg, 0.83 μmol) were placed into a flask and degassed DMF (415 μL) was added and the mixture was stirred under nitrogen atmosphere until clear solution was obtained. Next, degassed *i*-PrOH (555 μL), deionized H_2O (125 μL) were added followed by the addition of 0.2M aqueous sodium ascorbate (8.4 μL, 1.67 μmol) and 0.12M aqueous $CuSO_4$ (7.0 μL, 0.83 μmol). The reaction mixture was stirred under nitrogen atmosphere and monitored by analytical HPLC which showed complete conversion after 19 h. Then, the solvents were removed with nitrogen stream giving red thick solid which was diluted with buffer B (50 μL) and buffer A (470 μL). The resulting solution was centrifuged (4500 rpm, 5 min) and purified using 5% to 90% of buffer B in buffer A. Scaffold derivative **31**·3TFA (4.0 mg, 40.4%) was obtained as a white fluffy solid after lyophilization. t_R = 21.7 min. LCMS-ESI: average mass calcd $[M+3H]^{3+}$: 1072.6; found: 1072.4.

*CTV Scaffold derivative (**32**)* To a solution of scaffold derivative **31**·3TFA (3.8 mg, 1.07 μmol) in degassed DMF (430 μL) TBAF·$3H_2O$ (3.4 mg, 10.70 μmol) was added and the reaction mixture was stirred under nitrogen atmosphere and monitored by analytical HPLC which showed complete conversion after 17.5 h. Then, DMF was removed with nitrogen stream giving clear solid which was diluted with buffer A (515 μL). The resulting solution was centrifuged (4500 rpm, 5 min) and purified using 5% to 80% of buffer B in buffer A. Scaffold derivative **32**·3TFA (1.5 mg, 41.7%) was obtained as a white fluffy solid after lyophilization. t_R = 18.1 min. LCMS-ESI: average mass calcd $[M+3H]^{3+}$: 1020.5; found: 1020.3.

*Synthetic antibody mimic (**33**)* CTV Scaffold derivative **32**·3TFA (1.4 mg, 0.41 μmol), cyclic peptide **29** (0.7 mg, 0.45 μmol), TBTA (0.1 mg, 0.25 μmol) were placed into a flask and degassed DMF (130 μL) was added and the mixture was stirred under nitrogen atmosphere until clear solution was obtained. Next, degassed *i*-PrOH (60 μL), deionized H_2O (15 μL) were added followed by the addition of 0.2M aqueous sodium ascorbate (2.5 μL, 0.49 μmol) and 0.12M aqueous $CuSO_4$ (2.1 μL, 0.25 μmol). The reaction mixture was stirred under nitrogen atmosphere and monitored by analytical HPLC which showed complete conversion after 89 h. Then, the solvents were removed with nitrogen stream giving red solid which was diluted with buffer B (50 μL) and buffer A (460 μL). The resulting solution was centrifuged (4500 rpm, 5 min) and purified using 5% to 80% of buffer B in buffer A. Synthetic antibody **33**·5TFA (0.8 mg, 40.0%) was obtained as a white fluffy solid after lyophilization. t_R = 16.9 min. LCMS-ESI: average mass calcd $[M+3H]^{3+}$: 1472.3; found: 1472.1.

References and notes

1. Opatz, T.; Liskamp, R.M.J. *Org. Lett.* **2001**, *3*, 3499-3502.
2. Rump, E.T.; Rijkers, D.T.S.; Hilbers, H.W.; de Groot, P.G.; Liskamp, R.M.J. *Chem. Eur. J.* **2002**, *8*, 4613-4621.
3. Brouwer, A.J.; van de Langemheen, H.; Liskamp, R.M.J. *Tetrahedron* **2014**, *70*, 4002-4007.
4. Brouwer, A.J.; van de Langemheen, H.; Ciaffoni, A.; Schilder, K.E.; Liskamp, R.M.J. *Org. Lett.* **2014**, *16*, 3106-3109.
5. Hijnen, M.; van Zoelen, D.J.; Chamorro, C.; van Gageldonk, P.; Mooi, F.R.; Berbers, G.; Liskamp, R.M.J. *Vaccine* **2007**, *25*, 6807-6817**.**
6. Chamorro, C.; Liskamp, R.M.J. *J. Comb. Chem*. **2003**, *5*, 794-801.
7. Chamorro, C.; Kruijtzer, J.A.W.; Farsaraki, M.; Balzarini, J.; Liskamp, R.M.J. *Chem. Commun*. **2009,** 821-823.
8. Van de Langemheen, H.; van Ufford, H.C.Q.; Kruijtzer, J.A.W.; Liskamp, R.M.J. *Org. Lett*. **2014,** *16*, 2138-2141**.**
9. Van de Langemheen, H.; van Hoeke, M.; van Ufford, H.C.Q.; Kruijtzer, J.A.W.; Liskamp, R.M.J. *Org. Biomol. Chem.* **2014,** *12*, 4471-4478**.**
10. Van de Langemheen, H.; Korotkovs, V.; Bijl, J.; Wilson, C.; Kale, S.S.; Heinis, C.; Liskamp, R.M.J. *ChemBioChem* **2017**, *18*, 387-395.
11. Mulder, G.E.; Kruijtzer, J.A.W.; Liskamp, R.M.J. *Chem. Commun*. **2012**, *48*, 10007-10009.
12. Mulder, G.E.; van Ufford, H.C.Q.; van Ameijde, J.; Brouwer, A.J.; Kruijtzer, J.A.W.; Liskamp, R.M.J. *Org. Biomol. Chem*. **2013**, *11*, 2676-2684.
13. Werkhoven, P.R.; Elwakiel, M.; Meuleman, T.J.; Van Ufford, H.C.Q.; Kruijtzer, J.A.W.; Liskamp, R.M.J. *Org. Biomol. Chem*., **2016**, *14*, 701-706.
14. Werkhoven, P. R.; van de Langemheen, H.; van der Wal, S.; Kruijtzer, J. A. W.; Liskamp, R. M. J. *J. Pept. Sci.* **2014** *20*, 235-239.
15. Peluso, S.; Dumy, P.; Nkubana, C.; Yokokawa, Y.; Mutter, M. *J. Org. Chem.* **1999**, *64*, 7114- 7120.
16. Zhou, X.-T.; Rehman, A.-U.; Li, C.; Savage, P.B. *Org. Lett.* **2000**, *2*, 3015-3018.
17. Virta, P.; Karskela, M. Lönnberg, H. *J. Org. Chem.* **2006**, *71*, 1989-1999.
18. Virta, P.; Leppänen, M. Lönnberg, H. *J. Org. Chem.* **2004**, *69*, 2008-2016.
19. Farcy, N.; De Muynck, H.; Madder, A.; Hosten, N.; De Clerq, P.J. *Org. Lett.* **2001**, *3*, 4299-4301.
20. Franke, R.; Doll, C.; Wray, V.; Eichler, J. *Protein Peptide Lett.* **2003**, *10*, 531-539.
21. Canceill, J.; Collet, A.; Gottarelli, G. *J. Am. Chem. Soc*. **1984**, *106*, 5997-6003.
22. Liang, S.; Dai, J.; Hou, S.; Su, L.; Zhang, D.; Guo, H.; Hu, S.; Wang, H.; Rao, Z.; Guo, Y.; Lou, Z. *J. Biol. Chem.* **2013**, *288*, 13799-13807.
23. Collet, A.; Gabard, J. *J. Org. Chem.* **1980**, *45*, 5400-5401.
24. Allegretti, P. A.; Ferreira, E. M. *Org. Lett.* **2011**, *13*, 5924-5927.
25. Cordova, A.; Lin, S. Z.; Tseggai, A. *Adv. Syn. Cat.* **2012**, *354*, 1363-1372.
26. Bom, D.; Curran, D. P.; Kruszewski, S.; Zimmer, S. G.; Thompson Strode, J.; Kohlhagen, G.; Du, W.; Chavan, A. J.; Fraley, K. A.; Bingcang, A. L.; Latus, L. J.; Pommier, Y.; Burke, T. G. *J. Med. Chem.* **2000**, *43*, 3970-3980.
27. Ogoshi, S.; Nishiguchi, S.; Tsutsumi, K.; Kurosawa, H. *J. Org. Chem.* **1995**, *60*, 4650-4652.
28. Ostwald, R.; Chavant, P. Y.; Stadtmuller, H.; Knochel, P. *J. Org. Chem.* **1994**, *59*, 4143-4153.

29. Watanabe, T.; Imaizumi, T.; Chinen, T.; Nagumo, Y.; Shibuya, M.; Usui, T.; Kanoh, N.; Iwabuchi, Y. *Org. Lett.* **2010**, *12*, 1040-1043.

Supplementary Material

Supplementary material is included