CHEMISTRY
A EUROPEAN JOURNAL

ChemPubSoc
Europe

DOI: 10.1002/chem.201202276

Multivalent Design of Apoptosis-Inducing Bid-BH3 Peptide–Oligosaccharides Boosts the Intracellular Activity at Identical Overall Peptide Concentrations

Martin Richter,[b, c] Alokta Chakrabarti,[d] Ivo R. Ruttekolk,[d] Burkhard Wiesner,[b] Michael Beyermann,[b] Roland Brock,[d] and Jörg Rademann*[a, b]

Abstract: Multivalent peptide–oligosaccharide conjugates were prepared and used to investigate the multivalency effect concerning the activity of Bid-BH3 peptides in live cells. Dextran oligosaccharides were carboxyethylated selectively in the 2-position of the carbohydrate units and activated for the ligation of N-terminally cysteinylated peptides. Ligation through maleimide coupling was found to be superior to the native chemical ligation protocol. Monomeric Bid-BH3 peptides were virtually inactive, whereas pentameric peptide conjugates induced apoptosis up to 20-fold stronger at identical peptide concentrations. Comparison of lowly multivalent and highly multivalent peptide dextrans proved a multivalency effect in life cells which was specific for the BH3 peptide sequence.

Keywords: apoptosis · cancer · oligosaccharides · peptides · polymers · protein–protein interactions

Introduction

Intracellular protein–protein interactions (PPIs) are essential for the transmission of information within and between living cells.[1] Whereas numerous protein–protein interactions have been predicted by proteome-wide interaction screens such as yeast two-hybrid systems,[2] such methods are prone to produce high numbers of false positives and the functional relevance of many of the postulated protein–protein interactions remains unclear. Therefore, chemical tools to specifically disrupt and modulate protein–protein interactions are in high demand. Whereas in some cases, peptides corresponding to parts of the native interaction domains can effectively modulate PPIs, in other cases, they fail to do so due to a lack of activity. Multivalency is a biologically relevant mechanism for converting low-affinity binding into high-affinity interactions found ubiquitously in nature.[3] Consequently, multivalent arrangements of peptides should be able to potentiate the biological activity by increasing the local ligand concentration and by targeting proteins that present multivalent binding sites, for example, by the incorporation into membranes.[4–6] Therefore, the development of a flexible synthetic access to multivalent and structurally defined peptide-oligosaccharide conjugates ("peptide-dextrans") was investigated.[7] Ideally, such a material was expected to enhance the biological potency of a multivalent peptide conjugate relative to a monovalent peptide at identical peptide concentrations, if the targeted receptor protein indeed was accessible to multivalent binding.

To test this hypothesis, the activity of multivalent Bid-BH3-dextrans was investigated. Bid-BH3 is a pro-apoptotic peptide, derived from the apoptosis-activating Bid protein.[8,9] Expression of the Bid-protein is stimulated by binding of the tetrameric oncoprotein p53 to damaged DNA and leads to the induction of apoptosis through the interaction of the BH3 helix with anti-apoptotic Bcl-proteins.[10,11] Mutations of the p53 gene, which are found in $>95\,\%$ of all tumors, are responsible for the shut-down of apoptosis in cancer cells, constituting a crucial switch towards the survival of cancer cells.[12–14] Therefore, the re-stimulation of the pro-apoptotic pathway downstream of p53 leads to the efficient reversion of cancer cell survival and might have the potential to kill cancer cells with mutated p53 selectively. The Bid-BH3 peptide has been found to interact with the anti-apoptotic Bcl-proteins Bcl-xl, Bcl-2, and Bcl-w, but also the pro-apoptotic Bax protein in mitochondrial membranes,

[a] Prof. Dr. J. Rademann
Pharmaceutical/Medicinal Chemistry
Institute of Pharmacy, University of Leipzig
Brüderstrasse 34, 04103 Leipzig (Germany)
Fax: (+49) 341-97-36709
E-mail: rademann@uni-leipzig.de

[b] Dr. M. Richter,[+] Dr. B. Wiesner, Dr. M. Beyermann, Prof. Dr. J. Rademann
Leibniz Institut für Molekulare Pharmakologie (FMP)
Robert-Rössle-Strasse 10, 13125 Berlin (Germany)

[c] Dr. M. Richter[+]
Freie Universität Berlin
Institute of Chemistry and Biochemistry
Takustrasse 3, 14195 Berlin (Germany)

[d] A. Chakrabarti,[+] Dr. I. R. Ruttekolk, Prof. Dr. R. Brock
Nijmegen Centre for Molecular Life Sciences
Radboud University Nijmegen Medical Centre
PO Box 9101, 6500 HB Nijmegen (The Netherlands)

[+] These authors contributed equally to this work.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201202276.

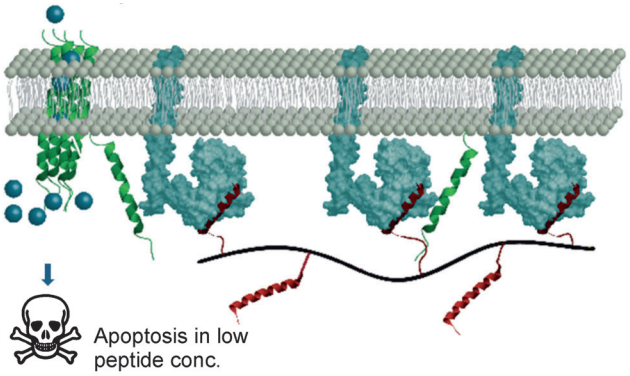


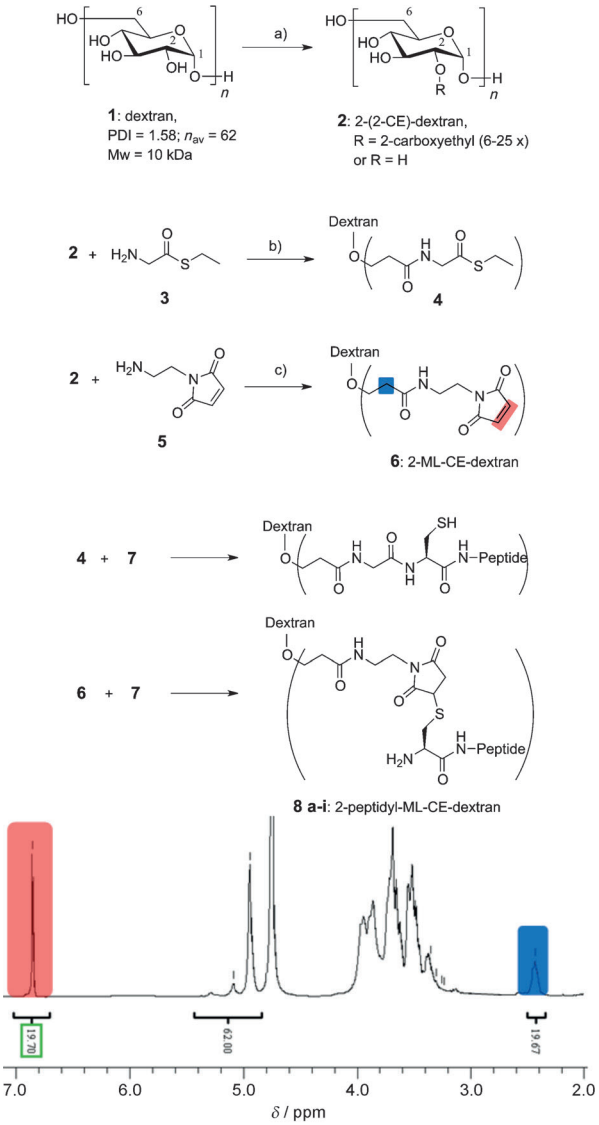
Figure 1. Design principles for bioactive BH3 peptides. Helical BH3 peptides (red) bind to Bcl-xl proteins (blue) in the outer mitochondrial membrane replacing the Bax protein (green). Oligomerization of Bax leads to the formation of membrane pores and the release of cytochrome c into the cytosol triggering the activation of caspases and resulting in apoptosis.

interactions that have been characterized functionally as well as structurally (Figure 1).[15–17]

Apoptosis is triggered by induction of Bax oligomers in the mitochondrial membrane. Whereas Bid-BH3 peptides bind to Bcl-xl in homogeneous, cell-free in-vitro systems with affinities at high nM to μM concentrations, no stimulation of apoptosis has been found for the free peptide in cells.[26] Only upon peptide lipidation or conformational fixation of the BH3 helix by hydrocarbon staples the induction of apoptosis was observed.[10,19] In addition, small molecules have been developed as BH3 analogues and are in clinical trials to date.[20] Since activation of Bax involves formation of a multimeric complex, we ask here whether multivalency may be useful to increase the activity of Bid-BH3 peptides.

Results and Discussion

For the synthesis of peptide–oligosaccharide conjugates dextran (α-(1→6)-linked oligo-D-glucose) was selected as a well characterized, biocompatible polymer, which we have demonstrated recently as an efficient carrier in intracellular applications.[21] Dextran **1** displayed an average molecular weight of 10 kDa and a polydispersity index of 1.58 (Scheme 1). Thus, an average dextran molecule **1** was constructed of 62 glucose monomers, spanning a distance of 25 nm in its extended conformation, which corresponds to the size of 8 globular proteins of 24 kDa. Dextran **1** was alkylated with acrylamide and the amides were hydrolyzed yielding 2-carboxyethyl dextran (2-CED) **2**, which was isolated by precipitation followed by dialysis.[21,22] Using the reported, optimized reaction conditions, carboxyethylation was found to be entirely selective for the 2-position of glucose as revealed by HMQC-HMBC NMR spectroscopic analysis of **2** (see the Supporting Information, Figures S2 and S3). Loading of 2-CED was varied in a range between 6 and 25 carboxyethyl (CE) groups per dextran molecule and was quantified by ^{1}H NMR spectroscopy (see Figure 2). Results were confirmed by colorimetric titration of polymer **2**. All subsequent experiments were conducted employing 2-CED **2** with a loading of 1.2 mmol g^{-1} polymer (13 CE groups per dextran molecule). For the ligation with unprotected peptides, the carboxy groups of polymers were condensed either with ethyl glycine thioester **3** and *N*-ethyl-*N*′-3-dimethylamino-propyl carbodiimide (EDC) furnishing polymer **4** for native chemical ligation or with EDC and 2-(aminoethyl)-maleimide **5** yielding polymer **6** for maleimide-thiol couplings (Scheme 1).



Scheme 1. Synthesis of peptide dextrans **8a–i**: a) acryl amide, 1 N NaOH, 30–50 °C, 0.1 N HCl; b) *N*-(2-aminoethyl)-maleimide 5, EDC, pH 6.5; c) cysteinyl peptide **7**, TCEP, pH 6.5.

In both cases the complete amidation of the carboxyethyl groups could be confirmed by NMR spectroscopy of the polymer (see the Supporting Information, Figures S4 and S5). Activated dextrans **4** and **6** (each at 1 mM with 13 mM activated carboxylates) were tested using both ligation protocols with N-cysteinylated R9-peptide **7** at concentrations

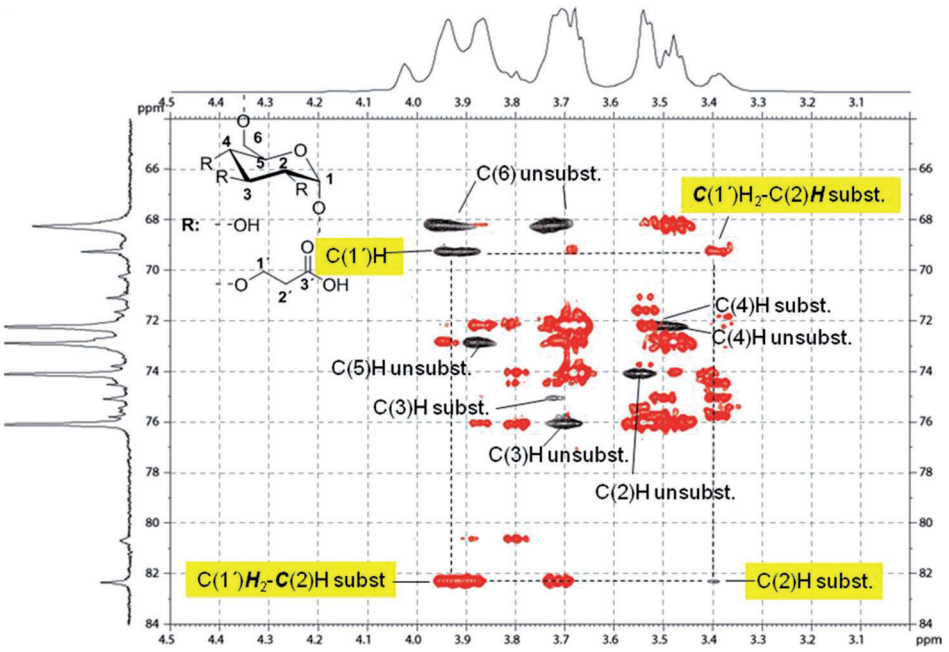


Figure 2. Overlay of HMBC (red) and HMQC (black) spectra showed that signals of CE-methylene groups 1′ correlated only with the signals of the glucose units in position 2, but not in position 3 or 4.

of 5 and 7 mM. The efficiency of the maleimide couplings was found to be superior compared with native chemical ligation (max. yield of 75 vs. 30%, see the Supporting Information, Tables S4 and S5). Thus, maleimide couplings at pH 6.5 were preferred for all subsequent ligation reactions. Polymer **6** was loaded with mixtures of two different N-cysteinyl peptides yielding nine different peptide dextrans **8a–i** in one step (Table 1). Cysteinyl peptides were applied in 1.5–2 fold excess to the desired degree of substitution. The remaining maleimide groups were deactivated by using 2-mercaptoethanol for 16 h. Obtained peptide dextrans **8a–i** were analyzed after precipitation and dialysis by gel permeation chromatography, NMR, and quantitative amino acid analysis. Fluorescence correlation spectroscopy was employed to verify the polymer attachment of the fluorescein dye. No traces of free, unattached fluoresceinyl peptides were detected in the polymer product. Coupling efficiencies correlated directly with peptide concentration and provided oligosaccharides with 3 to 7 peptide copies; relative stoichiometries of peptides on the polymer reflected the stoichiometry in the reaction mixture with no significant effect of peptide sequences and lengths.

To investigate the intracellular multivalency effect of the 22-mer Bid-BH3 peptide and to substantiate the findings with all necessary control experiments, nine peptide dextrans **8a–i** and four free peptides **9–12** were designed and synthesized (Table 1). Multivalent peptide dextrans **8c**,**d**,**e**,**h**, and **8i** were produced as low valency (divalent) and dextrans **8a**,**b**,**f**, and **8g** as high valency (pentavalent) derivatives to dissect the multivalency effect from the effect of oligosaccharide attachment alone. In addition, two routes of administration were investigated; delivery by an oscillating electric field, denominated as electroporation (or nucleofection), and delivery using a cell-penetrating peptide. Peptide dextrans **8a–d** were prepared without attachment of a cell-penetrating peptide and were delivered by nucleofection, thereby challenging the possibility that cell-penetrating peptide (CPP) delivery was required for multivalent enhancement. Dextrans **8e–i** were synthesized with two copies of the N-cysteinylated CPP nonaarginine **7** to enable delivery through endosomal uptake. For this group of polymers, peptide dextran **8e**, carrying only two copies of the CPP, served as a control to exclude that the CPP alone was sufficient to induce apoptosis.

Peptide dextrans **8a**,**c**,**f**,**h** were obtained from the maleimide-dextran **6** by coupling with the N-cysteinyl derivative of the native 22-mer BH3 peptide (C-BH3), whereas **8b**,**d**,**g**,**i** were ligated with the N-cysteinyl derivative of the mutated, inactive control peptide mBH3 (C-mBH3) to prove that the observed effect was specific to the BH3 sequence. The mBH3 peptide was rendered inactive by three alanine mutations in positions 12, 17, and 19, which have been described as essential for the interaction with Bcl-xl protein.[15] For fluorescencelabeling, either *N*-cysteinyl-lysinyl-5(6)-carboxyfluorescein was used in the coupling mixture, or fluoresceinyl-lysine was added C-terminally to both the BH3 and mBH3 peptides.[23] Finally, non-conjugated, "free" peptides were prepared for comparison, namely the 23-mer

Table 1. Composition of tested peptide dextrans **8a–i** and peptides (**9–12**).

	Structure	Name
7	CRRRRRRRRR-NH_2	CR_9
8a	Dextran 6 with 5 C-BH3[a] and 1 C-Fluo[b]	Dex-5B
8b	Dextran 6 with 5 C-mBH3[c] and 1 C-Fluo	Dex-5mB
8c	Dextran 6 with 2 C-BH3 and 1 C-Fluo	Dex-2B
8d	Dextran 6 with 2 C-mBH3 and 1 C-Fluo	Dex-2mB
8e	Dextran 6 with 2 CR9[d] and 1 C-Fluo	Dex-2R
8f	Dextran 6 with 5 C-BH3-Fluo and 2 CR9	Dex-5B-2R
8g	Dextran 6 with 5 C-mBH3-Fluo and 2 CR9	Dex-5mB-2R
8h	Dextran 6 with 2 C-BH3-Fluo and 2 C-R9	Dex-2B-2R
8i	Dextran 6 with 2 C-mBH3-Fluo and 2 C-R9	Dex-2mB-2R
9	Fluo-BH3	BH3
10	Fluo-mBH3	mBH3
11	Fluo-BH3-R9	BH3-R9
12	Fluo-mBH3-R9	mBH3-R9

[a] BH3: EDIIRNIARHLAQVGDSMDRSI-NH_2. [b] C-Fluo: CK(*N*-fluoresceinyl-carboxy). [c] mBH3: EDIIRNIARHAAQVGASADRSI-NH2. [d] R9: RRRRRRRRR-NH_2.

Fluo-BH3 **9** with an N-terminal lysinyl-*N*-ε-fluoresceinyl-carboxy residue, the mutated derivative Fluo-mBH3 **10**, and the two C-terminal nonaarginine conjugates, **11** and **12**, both being 32 mer peptides.

Preparation of all free peptides (**9**–**12**) and the ligated peptides (C-BH3 and C-mBH3) required the careful optimization of the synthesis protocols to obtain products of sufficient quality and without deletion sequences. Efficient synthesis of the BH3 sequence required the employment of the pseudo-dipeptide building blocks Asp-Ser(ΨMe,MePro) and Ala-Ser(ΨMe,MePro) in position 17 and 18 of the 23 mers C-BH3 C-mBH3, FluoBH3 (**9**), FluomBH3 (**10**), as well as in the 32 mers Fluo-BH3-R9 and Fluo-mBH3-R9 (**11** and **12**, respectively).[27]

The apoptosis-inducing activity of peptide dextrans **8a**–**i** (Table 1) was investigated at Jurkat E6.1 cells, an established lymphoma cell line (Figures 3 and 5). Peptide dextrans **8a**–**d** were imported into Jurkat E6.1 cells by nucleofection, a gentle way of electroporation, in which the plasma membrane is temporarily permeabilized by an externally applied electric field. Peptide dextrans **8e**–**i** were imported into cells by covalently attached nonaarginine, an established cell-penetrating peptide.[24] After loading of cells with peptide dextrans at various concentrations the cellular distribution of peptide dextrans was evaluated by confocal laser scanning microscopy (Figure 3) and uptake was quantified employing flow cytometry. First, apoptotic cells were visualized by addition of annexin-V labeled with the fluorescent dye Alexa Fluor 647 to cells after washing. Annexin-V is a protein that binds specifically to the membrane phospholipid phosphatidylserine (PS), which is externalized to the outer leaflet of the plasma membrane in early apoptosis. Integrity of the plasma membrane was validated by addition of trypan blue (Figure 3) and propidium iodide (Figure 4), dyes, which do

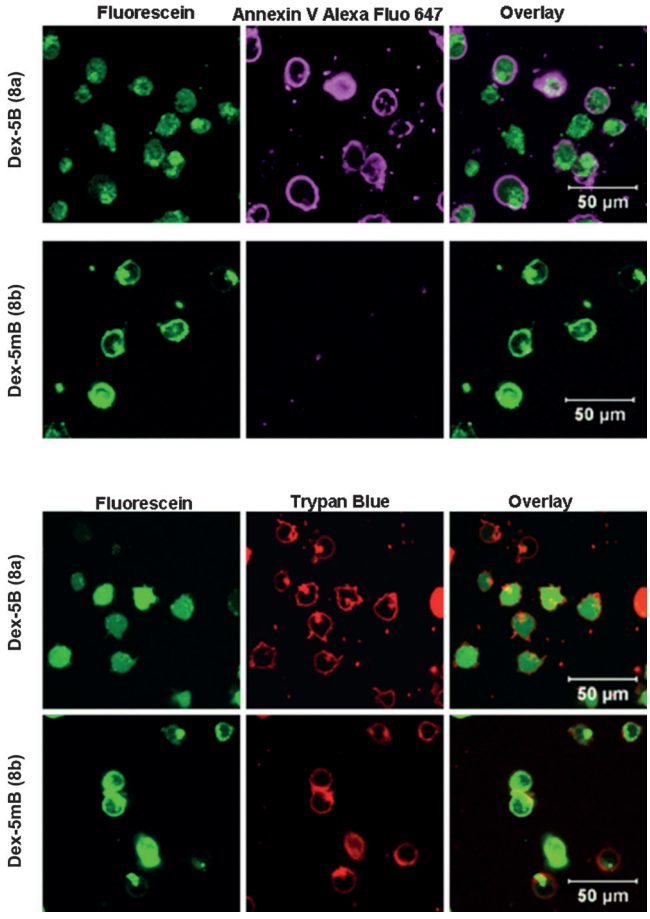


Figure 3. Induction of apoptosis by the pentavalent BH3 peptide dextran **8a** (10 µM total peptide concentration) after 8 h in cells of the human cell line Jurkat E6.1. Compound **8a** was transferred into the cells by nucleofection, a mild form of electroporation. Compounds **8a** and the mutated pentavalent control peptide dextran **8b** were homogeneously distributed in the cells as indicated by staining with the fluorescein label (left column). Only **8a** led to the membrane staining of cells with Annexin-V (purple), characteristic for cells in early apoptosis. At the same time, trypan-blue staining (red) was detectable only outside of living cells for both **8a** and **8b**, indicative for intact, not yet permeabilized outer cell membranes.

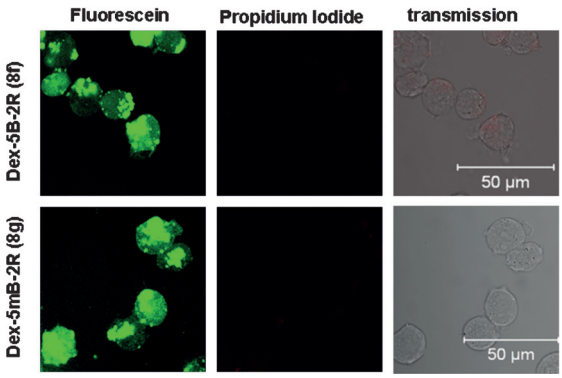


Figure 4. Cellular uptake of peptide dextrans **8f,g** (10 µM total BH3-peptide concentration) after 30 min in Jurkat E6.1 cells. The green fluorescent dextran was homogeneously distributed inside the cells. The absence of propidium iodide staining in the cells showed their good viability.

not enter healthy and early apoptotic cells. After this initial demonstration of the activity of the conjugate, we investigated the impact of multivalency on the induction of apoptosis using conjugates with different numbers of BH3 peptides per polymers. For a reliable quantification of apoptosis induction, the activity of the protease caspase-3 was measured.[25] This specific enzyme was selected as a biomarker, as in every apoptotic process, caspase-3 is the central effector protease, which is activated by a pro-apoptotic signal (such as the presence of BH3 peptides) and then leads to the proteolytic degradation of the cell. Notably, caspase-3 is activated only during apoptosis, but not in other processes leading to cell death such as necrosis. Caspase-3 activation by peptide-dextrans without nonaarginine was determined following nucleofection of cells with peptide dextrans or with free peptides. After washing with buffer, cells were incubated for 6 h at 37 °C and lyzed. The protein content of the cell lysate was determined by the Bradford assay and equal amounts of protein (30 µg) of each sample were incubated with a fluorogenic substrate of caspase-3 (Ac-DEVD-AMC = N-(*N*-acetyl-aspartyl-glutamyl-valinyl-aspartyl)-7-amino-5-methyl

coumarin)[25] in a 96-well plate and analyzed in a fluorescence spectrometer. Fluorescence intensity was recorded after 3 h and the activity of caspase-3 was compared after subtraction of the initial fluorescence (Figure 5 A).

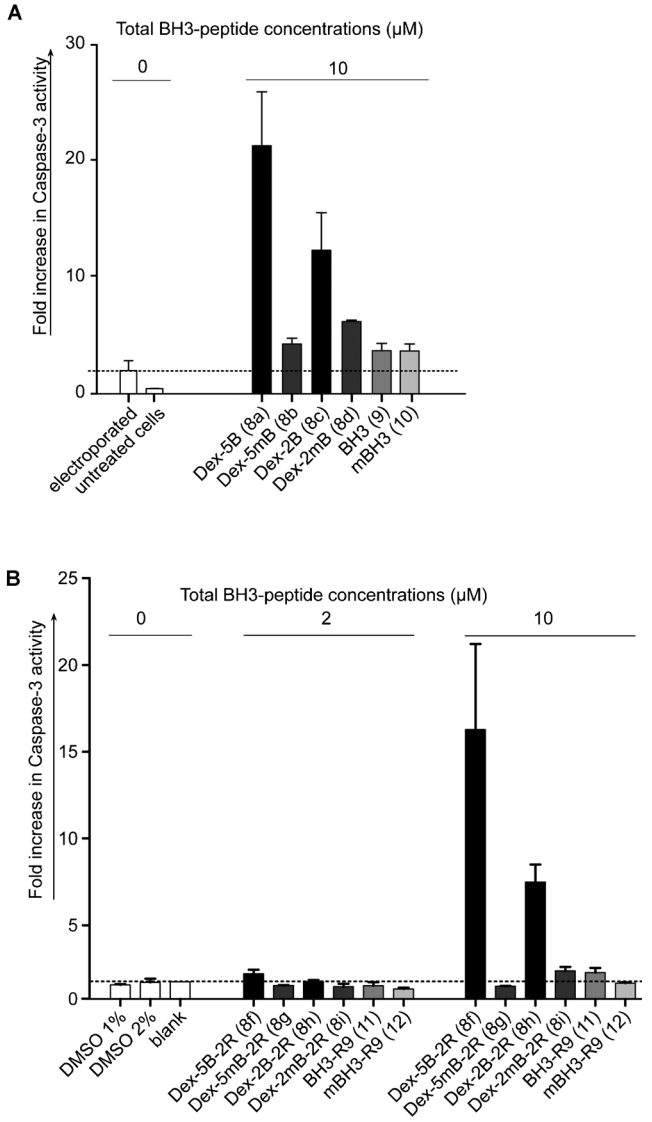


Figure 5. Induction of apoptosis by Bid-BH3-containing peptide dextrans in Jurkat E6.1 cells. A) Activation of caspase-3 in nucleofected cells and B) with CPP-mediated delivery of peptide dextrans. Error bars reflect the standard deviation of values in three independent sets of experiments.

Whereas significant induction of apoptosis was observed neither for the monovalent BH3 peptide **9** nor for the mono-valent mutated BH3 (mBH3) peptide **10**, and the mutated BH3-dextran conjugates **8b**,**d**, a considerable, concentration-dependent induction of apoptosis was recorded for the bi- and pentavalent BH3 peptide dextrans **8a**,**c** at identical overall peptide concentrations of 10 μM. Remarkably, the bivalent Dex-2B **8c** increased the caspase activity by a factor of 12, whereas caspase-3 activity in cells treated with the pentavalent Dex-5B **8a** increased by 21-fold, demonstrating a clear multivalency effect for the pentavalent peptide polymer architecture. In the next step, the same set of experiments was conducted with peptide dextrans **8f–i** delivered by the attachment of two copies of nonaarginine through endosomal uptake without nucleofection. Dextran **8e** labeled with *N*-cysteinyl-nonaarginine amide and *N*-cysteinylfluorescein amide as well as dextrans **8f–i** functionalized with *N*-cysteinyl-nonaarginine amide and fluorescently labeled Cys-BH3(Fluo) showed good uptake into cells with two copies of the cell-penetrating peptide and no signs of toxicity within the tested concentration range. After washing, the integrity of the cell membranes was indicated by the complete exclusion of propidium iodide from the interior of cells. Confocal laser scanning microscopy revealed a homogeneous distribution of fluorescein fluorescence in the cells (the Supporting Information, Figure S3). No induction of apoptosis was observed at up to 10 μM total peptide concentration by the monomeric, R9-conjugated peptides **11** and **12** or by the dextran-conjugated R9 with mutated BH3-dextrans (**8g**,**i**, Figure 3 B).

In contrast, apoptosis was induced efficiently by multivalent BH3-carrying peptide dextrans **8f**,**h**. At 10 μM overall BH3-peptide concentration the bivalent BH3-peptide dextran Dex-2B-2R **8h** induced caspase-3 to a sevenfold level of activity. For comparison, the pentavalent BH3-peptide dextran Dex-5B-2R **8f** increased the caspase-3 activity 17-fold at the same overall BH3-peptide concentration corresponding to a dextran concentration of 2 μM, demonstrating again that the degree of multivalency had a strong positive impact on activity. Whereas the sevenfold activity enhancement for the divalent peptide polymer, **8h**, can be attributed partially to the protection from proteolytic degration exerted by the polymer,[26] the additional amplification observed for the pentavalent architecture (compound **8f**) can only result from the multivalency of the construct. Therefore, comparison of the biological activities of differentially loaded peptide dextrans **8a**, **8c**, **8f**, and **8h** proved conclusively a strong multivalency effect for the intracellular interactions of BH3 peptides. Furthermore, it could be confirmed from the conducted control experiments that the observed effect can be contributed to specific interactions of the BH3 peptides, because neither the nonaarginine nor the mutated BH3 peptides had an effect on the biological activity.

Conclusion

The presented results demonstrate the efficient and modular synthesis of multivalent and multifunctional peptide dextrans. For this purpose, a protocol for selective carboxyethylation of dextran in the 2-hydroxy position has been developed that was employed for the diverse derivatization of dextran over a broad concentration range between 6 to 25 carboxyethyl groups per 10 kDa dextran. The biocompatible CE-dextran carriers were activated either as maleimides or as thioesters and were ligated with mixtures of N-terminally cysteinylated peptides in a single step. By using this method,

a small collection of differentially loaded, multivalent peptide dextrans was designed and synthesized to investigate the multivalency effect for the BH3 peptide in living cells. BH3 peptide dextrans were delivered to the interior of cells either by nucleofection or through the attachment of two copies of *N*-cysteinyl nonaarginine as cell-penetrating peptides. The obtained multivalent peptide dextrans displayed specific biological activity in the induction of apoptosis. Independent of the mode of cellular import and of the protection against proteolytic degradation exerted by the polymer, this activity was significantly potentiated by multivalent presentation of the peptides. To our knowledge this is the first example of a multivalency effect proven with synthetic peptide constructs for an intracellular protein target. The demonstrated methodology should enable the investigation of homo- and hetero-multivalency effects for other PPIs in cells as well. The flexible access to peptide dextrans should pave the way to further biological applications of these new materials.

Experimental Section

Materials: All chemicals for synthesis were purchased from Sigma Aldrich (Germany) and used without further purification. Annexin-V Assay Kit was obtained from Invitrogen (Karlsruhe, Germany), resins for SPPS from Rapp Polymere (Tübingen, Germany), PD-10 columns from Sigma Aldrich (Germany) and cell culture medium from PAN biotech (Aidenbach, Germany). Automated peptide synthesis was performed at an Activo P11 (Cambridge, England) synthesizer. Cell Line Nucleofector Kit was purchased from AMAXA (Cologne, Germany). ^{1}H and ^{13}C NMR measurements were performed on a Bruker AVANCETM 300 MHz spectrometer. 2D NMR measurements were performed on a Bruker AVANCETM 600 MHz spectrometer. UV spectra were obtained with a JASCO V-550 UV/Vis spectrometer. Amino acid analysis was performed by Genaxxon Bioscience GmbH (Ulm, Germany).

2-Carboxyethyl dextran (2-CED) 2: Dextran (5 g, 0.5 mmol, M_w 10 kDa, PDI = 1.58) was dissolved in a 1 N NaOH solution (50 mL). Acryl amide (0.9 g, 12.5 mmol) was added and stirred at 30 °C. After 24 h the reaction temperature was increased to 50 °C and stirred for additional 24 h. The solution was neutralized the oligosaccharide products were precipitated with methanol, solvents were removed after centrifugation (4000 × rpm, 5 min). After washing of precipitate with methanol the precipitate was dried. The white solid was dissolved in water (25 mL) and dialyzed (benzoylated dialysis tubing, with a pore size (nominal molecular weight cut-off (NMWCO)) of 2000, Sigma Aldrich) twice with 0.1 N HCl (10 L) and water (2 × 10 L) over 24 h. The loading with carboxyethyl groups was quantified by 1H NMR spectroscopy and confirmed by titration against 0.1 N NaOH as described below. Positions of the CE-substitution were determined by using HMBC-HMQC NMR spectroscopy as described below. Substitution was found selectively in the 2-position under both conditions described (see also Figure 2 and the Supporting Information, S2). After lyophilization a white powder was obtained in a yield of 4 g (72 %, 13 carboxyethyl groups per dextran). ^{1}H NMR (D_2O, 300 MHz): δ = 2.65 (t, 2H, CH_2COOH), 3.25–4.1 (m, CH_2CH$_2$COOH, C(2–6)H (glucose units)), 4.93–5.30 ppm (m, 1H, C(1)H); ^{13}C NMR (D_2O, 75 MHz): δ = 34.20 (*C*H$_2$COOH), 65.03 (O*C*H$_2$CH$_2$COOH), 69.03 (C(4) glucose unit), 69.67 (C(2) glucose unit), 70.89 (C(4) glucose unit), 72.89(C(3) glucose unit), 97.18 (C(1) glucose unit, 175.39 ppm (CH$_2$*C*OOH). CE substitution of dextran was varied by using different concentrations of acryl amide in the reaction mixture. Under the reported conditions about 50 % of the employed acrylamide was attached to the polymer (the Supporting Information, Table S1). With 5 g (0.5 mmol) dextran 25, 12.5, and 6.25 mmol of acrylamide yielded a loading of 25, 13, and 6 carboxyethyl groups per polymer, respectively. CE groups per dextran were quantified by titration with 0.1 N NaOH and phenolphthalein as indicator (the Supporting Information, Table S2).

Glycine thioethyl ester hydrochloride 3: Compound **15** (23.1 g, 105.3 mmol) was stirred in 4 N HCl in dioxane (10 mL) for 90 min. The mixture was concentrated in vacuo and the product precipitated in cold Et_2O. The precipitate was filtered, washed with Et_2O and dried to provide the product as white solid (15.34 g, 96 %). ^{1}H NMR (D_2O, 300 MHz): δ = 1.22 (t, 3H, J = 7.4 Hz, SCH$_2$CH_3), 2.98 (q, 2H, J = 7.4 Hz, SCH_2CH$_3$), 4.11 ppm (s, 2H, CH_2COSEt); ^{13}C NMR (D_2O, 75 MHz): δ = 13.3 (SCH$_2$*C*H$_3$), 23.08 (S*C*H$_2$CH$_3$), 46.63 (*C*H$_2$COSEt), 194.92 ppm (*C*OSEt); HRMS (ESI-TOF): m/z: calcd for C_4H_9NOS: 120.0478 $[M+H]^+$; found: 120.0476.

***N*-(2-Aminoethyl)maleimide (5)**: Compound **14** (4.6 g, 19.2 mmol) was dissolved in CH_2Cl_2 (35 mL) at 0 °C. To this solution, TFA (27 mL) was added and stirred for 1 h, during which time the solution was allowed to reach room temperature. The mixture was concentrated and precipitated with cold Et_2O (50 mL). Trituration with cold Et_2O (3 × 50 mL) followed by evaporation of remaining solvent provided a white solid as product (2.5 g, 93 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.18 (t, 2H, J = 5.8 Hz, NH$_3^+$CH_2CH$_2$), 3.79 (t, 2H, J = 5.8 Hz, NH$_3^+$CH$_2$CH_2N), 6.86 ppm (s, 2H, COCH_2CH_2CON); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 34.92 (NH$_3^+$*C*H$_2$CH$_2$), 38.28 (NH$_3^+$CH$_2$*C*H$_2$), 134.6 (CO*C*H$_2$CH$_2$CO), 172.53 ppm (*C*OCH$_2$CH$_2$*C*O); HRMS (ESI-TOF): m/z: calcd for $C_6H_8N_2O_2$: 141.0659 $[M+H]^+$; found: 141.0662.

***S*-Ethyl-thiocarbonyl-ethyl amidoethyl dextran (thioester dextran) 4**: Compound **2** (0.2 g, 0.02 mmol) and **3** (0.23 g, 1.49 mmol) were dissolved in water (1 mL). After adjusting the pH value of 6.5 with sodium bicarbonate, EDC (0.14 g, 0.74 mmol) was added. After 1 h (0.14 g, 0.74 mmol) EDC was added again and the solution was stirred overnight. The product was precipitated with MeOH and washed several times with MeOH. The precipitate was dissolved in water (2.5 mL) and purified on a PD-10 column. After lyophilization a white solid was obtained as product (0.19 g, 77 %). ^{1}H NMR (D_2O, 300 MHz): δ = 1.17 (t, 3H, J = 7.4, SCH$_2$CH_3), 2.59 (t, 2H, J = 5.4, CH_2COSEt), 2.86 (q, 2H, J = 7.4, SCH_2CH$_3$), 3.3–4.1 (m, C(2–6) H-dextran, OCH_2CH$_2$CO), 4.92 (s, anomeric H (unsubstituted)), 4.9–5.3 ppm (m, anomeric H); ^{13}C NMR (D_2O, 75 MHz): δ = 13.32 (SCH$_2$*C*H$_3$), 22.50 (S*C*H$_2$CH$_3$), 35.49 (*C*H$_2$CONH), 48.70 (NHCH$_2$CO), 59.95 (C(6)), 64.97 (O*C*H$_2$CH$_2$CO), 68.98 (C(5)), 69.64 (C(3)), 70.87 (C(2)), 72.87 (C(4)), 97.16 (C(1)), 174.14 (*C*ONHCH$_2$), 200.97 ppm (COSEt).

Maleimidoethyl amidoethyl dextran (maleimide dextran) 6: Compound **2** (0.2 g, 0.02 mmol) with 13 carboxy groups (0.26 mmol) and **5** (0.69 g, 2.69 mmol) were dissolved in water (1 mL). A pH of 6.5–7 was adjusted and EDC (0.26 g, 1.35 mmol) added. After 1 h EDC (0.26 g, 1.35 mmol) was added again and the solution was allowed to stir for 4 h. The mixture was precipitated with methanol. The precipitate was washed with methanol and DMF. The solid was dissolved in water (2.5 mL) and purified over Sephadex G-25. Lyophilization led to product as a white solid (0.19 g, 82 %). Quantification of maleimide groups per dextran was performed using 1H NMR spectroscopy (the Supporting Information, Figure S4). It could be proven that under the conditions used, all the carboxyl groups on the dextran reacted to the corresponding maleimidoethyl amide groups. ^{1}H NMR (D_2O, 300 MHz): δ = 2.48 (t, 2H, J = 4.7, CH$_2$CH_2CO), 3.3–4.1 (m, CH_2CH$_2$CO, *H* (glucose units)), 4.98, –5.3 (m, anomeric H), 6.89 ppm (s, 2H, COC*HCH*CO); ^{13}C NMR (D_2O, 75 MHz): δ = 36.38 (*C*H$_2$CONH), 37.12 (NHCH$_2$*C*H$_2$NH$_2$), 39.97 (NH*C*H$_2$CH$_2$N), 65.35 (*C*(6)H$_2$), 65.58 (C(O*C*H$_2$CH$_2$CONH), 67.94 (*C*(4) Glucose), 71.41 (*C*(5) Glucose), 72.57 (*C*(3)H Glucose), 74.68 (*C*(2) Glucose), 98.71 (*C*(1) Glucose), 179.29 (*C*ONH), 181.82 ppm (CH$_2$*C*ONH).

Peptide synthesis (7, 9, 10, 11, and 12): Peptides were synthesized manually or by automated Fmoc-SPPS on TentaGel S Ram amide (0.2 mmol g^{-1}) resins. Fluorescein-labeled peptides were synthesized by using preloaded Nα-carboxyfluorescein-lysinyl amide TentaGel R Ram resin. To avoid aspartimide formation, Fmoc-groups were cleaved using 20 % piperidine and 0.1 N acetic acid solution in DMF. To decrease impurities by sequence termination pseudo-prolines were used. For the synthesis of BH3-peptides, Asp17-Ser18 were substituted by Asp-Ser(ΨMe,-

MePro) and Ala17-Ser18 were substituted by Ala-Ser(ΨMe,MePro)[27]. Purification was performed by preparative RP-HPLC. Peptides were identified by LCMS, HRMS and MALDI TOF.

General procedure for synthesis of dextran-peptide conjugates (8a–g): 6 (5 mg, 0.4 μmol) with 13 maleimide groups was dissolved in an MES buffer (1 mL, pH 6.5). After degassing, cysteinyl-BH3(Fluo)-peptide (7 mg, 2 μmol for **8f** and 3 mg, 1 μmol for **8h**) and cysteinyl-nonaarginine amide **7** (2 mg, 1 μmol) were added under a nitrogen atmosphere. The pH value was checked and the mixture stirred for 16 h. The solution was concentrated and the product precipitated with MeOH. After several washing steps with MeOH and DMF the solid was dissolved in water (2.5 mL) and purified twice using PD-10 columns. The desired conjugate was obtained after lyophilization. Purity of the conjugates was checked using fluorescence correlation spectroscopy (FCS). Results of amino acid analysis showed how much peptide per polymer was coupled (see the Supporting Information, Table S3 (**8f**: 7 mg, 60%, 5 BH3(Fluo)- and 2 R9 groups per dextran)). Cellular uptake was shown in Jurkat E6.1 cells. Viability of cells was checked with propidium iodide (see the Supporting Information, Figure S6).

***N*-(*tert*-Butoxycarbonyl)-ethyl-1,2-diamine 13**: A solution of Boc-anhydride (8.7 g, 40 mmol) in $CHCl_3$ (200 mL) was added dropwise to an ice-cold solution of 1,2-diaminoethane (27 mL, 400 mmol) in $CHCl_3$ (400 mL) over 3 h. After complete addition, the mixture was stirred at 0°C for 30 min and afterwards allowed to warm to room temperature overnight. The solvent was removed under reduced pressure and the residue dissolved in 3 N Na_2CO_3 (300 mL) solution. The mixture was extracted with $CHCl_3$ (3×200 mL), the organic phase dried over $MgSO_4$ and evaporated to provide the product as a colorless oil (6.4 g, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.35 (s, 9H, CCH_3), 2.69 (t, 2H, CH_2NHBoc), 3.07 (q, 2H, CH_2CH$_2$NHBoc), 5.11 ppm (brs, 1H, N*H*Boc); ^{13}C NMR (75 MHz, $CDCl_3$): δ=27.87 (*C*CH$_3$), 41.36 (H$_2$N*C*H$_2$CH$_2$), 42.93 (*C*H$_2$NHBoc), 78.52 (*C*CH$_3$), 155.73 ppm (NH*C*OO); HRMS (ESI-TOF): *m*/*z*: calcd for $C_7H_{16}N_2O_2$: 161.1285 [*M*+H]$^+$; found: 161.1285.

1-(*N*-*tert*-Butoxycarbonyl)-2-aminoethyl) maleimide 14: Mono-protected *N*-Boc-ethylenediamine **13** (5.1 g, 31.9 mmol) and Et_3N (6.6 mL, 47.79 mmol) were dissolved in Et_2O (60 mL) at 0°C. Maleic anhydride (3.1 g, 31.9 mmol) in 60 mL Et_2O was added dropwise and the reaction mixture was stirred for 4 h, during which time the solution was allowed to reach room temperature. After concentrating the solution the residue was dissolved in acetone (150 mL). Et_3N (8.8 mL, 63.7 mmol) was added and the mixture was heated to reflux. Acetic anhydride (4.5 mL, 47.8 mmol) was added and the solution was heated to reflux for 20 h. After the solvent was removed under reduced pressure chromatography of the dark brown oily residue over silica gel (EtOAc/hexane 1:1) gave the product as a white solid (3.7 g, 48.1%). ^{1}H NMR (CDCl3, 300 MHz): δ=1.4 (s, 9H, CCH_3), 3.34 (q, 2H, J=4.9 Hz, CH_2CH_2NHBoc), 3.66 (t, 2H, J=5.1 Hz, CH_2CH$_2$NHBoc), 4.74 (brs, 1H, N*H*Boc), 6.71 ppm (s, 2H, C*HCH*CON); ^{13}C NMR ($CDCl_3$, 75 MHz): δ=27.83 (C*C*H$_3$), 37.5 (*C*H$_2$CH$_2$NHBoc), 38.91 (CH$_2$*C*H$_2$NHBoc), 79.07 (*C*CH$_3$), 133.96 (*CH*CHCON), 155.46 (NH*C*OO), 170.34 ppm (*C*ON*C*O); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{11}H_{16}N_2O_4$: 241.1183 [*M*+H]$^+$; found: 241.1188.

***N*-*tert*-Butoxycarbonyl-glycine thioethyl ester 15**: *N*-Boc-glycine (24.7 g, 141.5 mmol) and carbonyldiimidazole (25 g, 154.2 mmol) were stirred in dry THF (125 mL) at 20°C under a nitrogen atmosphere for 30 min. Mercaptoethanol (31.39 mL, 424.3 mmol) was added and the mixture was stirred overnight. After addition of 4 N NaOH (250 mL), the solution was rapidly extracted with CH_2Cl_2 (3×150 mL). The organic phase was dried over $MgSO_4$, evaporated and redissolved in ethyl acetate. The solution was filtered over silica gel and the solvent removed under reduced pressure to provide pure product as yellowish oil (23 g, 75%). ^{1}H NMR (D_2O, 300 MHz): δ=1.23 (t, 3H, J=7.2 Hz, SCH$_2$CH_3), 1.44 (s, 9H, OC(CH_3)$_3$), 2.89 (q, 2H, J=7.2, SCH_2CH$_3$), 4.00 (s, 2H, NHCH_2COSEt), 5.19 ppm (brs N*H*Boc); ^{13}C NMR (D_2O, 75 MHz): 14.03 (SCH$_2$*C*H$_3$), 22.47 (S*C*H$_2$CH$_3$), 27.80 (C(*C*H$_3$)$_3$)), 49.86 (*C*H$_2$NHBoc), 79.76 (*C*(CH$_3$)$_3$), 155.04 (NH*C*OOC), 197.82 ppm (*C*OSEt); HRMS (ESI-TOF): *m*/*z*: calcd for $C_9H_{17}NO_3S$: 220.1002 [*M*+H]$^+$; found: 220.1004.

Synthesis of nonaarginine dextran conjugates using maleimide thiol coupling 16, 17: Compound **6** (10 mg, 1 μmol) loaded with 12 maleimide groups (12 μmol) was dissolved in MES buffer (1 mL, pH 6.5). After degassing, cysteinyl-nonaarginine amide **7** (6 mg, 5 μmol) was added under nitrogen atmosphere. The pH value was checked and the mixture stirred for 16 h. The solution was concentrated and the product precipitated with MeOH. After several washing steps with MeOH and DMF the solid was dissolved in water (2.5 mL) and purified twice using PD-10 columns. After lyophilization the resulting conjugate was obtained as a white powder. Results of amino acid analysis showed how much peptide per polymer was coupled (see the Supporting Information, Table S4: 8 mg, 68%, 3 R9 groups per dextran).

Synthesis of nonaarginine dextran conjugates using native chemical ligation 18, 19: Thioester dextran **4** (10 mg, 1 μmol) loaded with 12 thioester groups (12 μmol) was dissolved in phosphate buffer (1 mL, 100 mM KH_2PO_4/Na_2PO_4 pH 7.4). After degassing, cysteinyl-nonaarginine amide **7** (6 mg, 5 μmol), TCEP (9 mg, 50 equiv) and 4-mercaptophenyl acetic acid (5 mg, 32 equiv) were added under a nitrogen atmosphere. The pH value was checked and the mixture stirred for 16 h. The solution was concentrated and the product precipitated with MeOH. After several washing steps with MeOH and DMF the solid was dissolved in water (2.5 mL) and purified twice using PD-10 columns. The resulting conjugate was obtained after lyophilization. Results of amino acid analysis showed how much peptide per polymer was coupled (see the Supporting Information, Table S5: 8 mg, 73%, 1.2 R9 groups per dextran).

Comparison of both ligation methods: To compare NCL and maleimide thiol coupling, compounds **4** and **6** were reacted with different amounts of cysteinyl-nonaarginine amide. Polymers were worked up as previously described and analyzed by using amino acid analysis. Under the optimized conditions 25% of the available cysteinyl peptides were coupled to (*S*)-ethyl-thiocarbonyl-ethyl dextran using NCL. In contrast to that 75% of the available cysteinyl peptides were coupled on **4** using maleimide thiol coupling. Advantages and disadvantages of both ligation methods are summarized in the Supporting Information, Table S6.

Laser scanning microscopy: HeLa-S cells seeded on cover slips were incubated with peptides or peptide dextrans, dissolved in HEPES buffer, for 30 min at 37°C under a LSM-510 or LSM 710 laser scanning microscope (Carl Zeiss MicroImaging, Jena, Germany) equipped with a 63 water objective. Immediately after adding the substance, pictures were recorded every 60 sec for 30 min. After removing the peptide solution and washing the coverslips with HEPES buffer, trypan blue was added to prove the viability of treated cells and their membrane integrity.

Fluorescence correlation spectroscopy: FCS measurements were performed at room temperature on an LSM 510-ConfoCor 2 system and on an LSM 710-ConfoCor 3 system (Carl Zeiss MicroImaging, Jena). Fluorescent BH3-peptides, BH3-dextrans **8f–i**, respective mixtures of unreactive CED 2, and fluorescent BH3-peptides were dissolved in water at a concentration of 10–8 mol L^{-1}. Each solution on cover slips (50 μL per sample) were fixed in a measurement chamber. An argon ion laser (488 nm) was used for excitation of fluorescein. Calculations of the autocorrelation function as well as fitting of the autocorrelation function were performed using ZEN 2010 software. Correlation functions accounting for free diffusion in three dimensions were fitted for one component and yielded diffusional autocorrelation times of particles through the confocal volume (the Supporting Information, Figure S7). In two component fittings, it could be shown that the investigated peptide polymers (e.g., **8f**) contained no free peptides.

Electroporation: Jurkat E6.1 cells (10^6) were resuspended in Nucleofector Solution V (100 μL). Peptide or peptide polymer was added in the respective concentration and the solution was pipetted into LONZA nucleofection cuvettes. Nucleofection was performed in an AMAXA Nucleofector using program X-001 in accordance with the recommendations of the manufacturer. Following nucleofection the medium (900 μL) was added carefully and the cells incubated for 5 min at RT. Subsequently cells were washed and resuspended in fresh medium.

AnnexinV assay: Electroporated Jurkat E6.1 cells were washed with medium, transferred to a 24-well tissue culture plate and incubated for 14 h in RPMI medium. The cells were washed in cold PBS buffer and in-

cubated for 15 min with 5 μL AnnexinV Alexa Fluor 647 in 95 μL Annexin-binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4) on ice. Following the annexin-binding buffer (400 μL) was added to each sample. Analysis was performed by flow cytometry on a FACS Calibur System (Becton Dickinson, Heidelberg).

Caspase-3 assay: Jurkat E6.1 cells (10^6) were incubated with peptide or peptide dextran conjugate at the respective concentration for 6 h. After washing with ice-cold PBS buffer, the cells were spun down at 1900 rpm for 5 min and pellets were resuspended in lysis buffer (20 mM Tris/HCl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 protease inhibitor cocktail tablet (Roche Diagnostics Mannheim, Germany) per 50 mL buffer, pH 7.7). After 30 min cooling on ice, the lysate was separated from the remaining solid by centrifugation and protein content estimated by commercially available Bradford Protein Assay Kit (Bio-Rad Laboratories Munich, Germany). 30 μg protein aliquots of each sample were diluted in caspase activity buffer (20 mM HEPES, 100 mM NaCl, 10 mM dithiothreitol, 10% glycerol, pH 7.5). Finally, fluorogenic caspase-3 substrate (Ac-DEVD-AMC) was added to a concentration of 2 μM. Cleavage efficiency of active caspase-3 in solution was measured immediately after addition of the substrate and after 3 h at 37 °C by measuring fluorescence increase using a luminescence spectrometer LS50B (Perkin–Elmer, Norwalk, CT, USA).

Acknowledgements

The authors gratefully acknowledge the DFG (Ra895–4–6, SFB 765, and FOR 806; BR2443–5) and the Fonds der Chemischen Industrie for continuous support.

[1] M. C. Souroujon, D. Mochly-Rosen, *Nat. Biotechnol.* **1998**, *16*, 919–924.
[2] W. Kelly, M. Stumpf, *Curr. Opin. Biotechnol.* **2008**, *19*, 396–403.
[3] a) M. Mammen, G. Dahmann, G. M. Whitesides, *J. Med. Chem.* **1995**, *38*, 4179–4190; b) M. Mammen, S. K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794.
[4] J. A. Kritzer, O. M. Stephens, D. A. Guarracino, S. K. Reznik, A. Scheppartz, *Bioorg. Med. Chem.* **2005**, *13*, 11–16.
[5] R. Langer, *Nature* **1998**, *392*, 5–10.
[6] R. Haag, F. Kraatz, *Angew. Chem.* **2006**, *118*, 1218–1237; *Angew. Chem. Int. Ed.* **2006**, *45*, 1198–1215.
[7] M. Barth, R. Fischer, R. Brock, J. Rademann, *Angew. Chem.* **2005**, *117*, 1584–1588; *Angew. Chem. Int. Ed.* **2005**, *44*, 1560–1563.
[8] K. Wang, X. M. Yin, D. T. Chao, C. L. Milliman, S. J. Korsmeyer, *Genes Dev.* **1996**, *10*, 2859–2869.
[9] K. J. Oh, S. Barbuto, K. Pitter, J. Morash, L. D. Walensky, S. J. Korsmeyer, *J. Biol. Chem.* **2006**, *281*, 36999–37008.
[10] L. D. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine, S. J. Korsmeyer, *Science* **2004**, *305*, 1466–1470.
[11] E. P. Holinger, T. Chittenden, R. J. Lutz, *J. Biol. Chem.* **1999**, *274*, 13298–13304.
[12] I. R. Ruttekolk, R. Fischer, F. Duchardt, K.-H. Wiesmüller, J. Rademann, R. Brock, *Bioconjugate Chem.* **2008**, *19*, 2081–2087.
[13] R. F. Boynton, P. L. Blount, J. Yin, V. L. Brown, Y. Huang, Y. Tong, T. McDaniel, C. Newkirk, J. H. Resau, W. H. Raskind, R. C. Haggitt, B. J. Reid, S. J. Meltzer, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3385–3388.
[14] F. Chakrani, J. P. Armand, G. Lenoir, L. Y. Ju, J. P. Liang, E. May, P. May, *Int. J. Cancer* **1995**, *6*, 1316–1320.
[15] S. Dickman, *Science* **1997**, *277*, 1605–1606.
[16] A. Letai, M. C. Bassik, L. D. Walensky, M. D. Sorcinelli, S. Weiler, S. J. Korsmeyer, *Cancer Cell* **2002**, *2*, 183–192.
[17] E. F. Lee, J. D. Sadowsky, B. J. Smith, P. E. Czabotar, K. J. Peterson-Kaufman., P. M. Colman, S. H. Gellman, W. D. Fairlie, *Angew. Chem.* **2009**, *121*, 4382–4386; *Angew. Chem. Int. Ed.* **2009**, *48*, 4318–4322.
[18] E. Gavathiotis, M. Suzuki, M. L. Davis, K. Pitter, G. H. Bird, S. G. Katz, H.-C. Tu, H. Kim, E. H.-Y. Cheng, N. Tjandra, L. D. Walensky, *Nature* **2008**, *455*, 1076–1081.
[19] J. Wang, Z. Zhang, S. Choksi, S. Shan, Z. Lu, C. M. Croce, E. S. Alnemri, R. Korngold, Z. Huang, *Cancer Res.* **2000**, *60*, 1498–1502.
[20] T. Oltersdorf, S. W. Elmore, A. R. Shoemaker, R. C. Armstrong, D. J. Augeri, B. A. Belli, M. Bruncko, T. L. Deckwerth, J. Dinges, P. J. Hajduk, M. K. Joseph, S. Kitada, S. J. Korsmeyer, A. R. Kunzer, A. Letai, C. Li, M. J. Mitten, D. G. Nettesheim, S. Ng, P. M. Nimmer, J. M. O'Connor, A. Oleksijew, A. M. Petros, J. C. Reed, W. Shen, S. K. Tahir, C. B. Thompson, K. J. Tomaselli, B. Wang, M. D. Wendt, H. Zhang, S. W. Fesik, S. H. Rosenberg, *Nature* **2005**, *435*, 677–681.
[21] S. Weinert, S. Jabs, C. Supanchart, M. Schweizer, N. Gimber, M. Richter, J. Rademann, T. Stauber, U. Kornak, T. J. Jentsch, *Science* **2010**, *328*, 1401–1403.
[22] A. A. Iozep, N. K. Bessonova, B. V. Passet, *Russ. J. Appl. Chem.* **1998**, *71*, 335–337.
[23] R. Fischer, O. Mader, G. Jung, R. Brock, *Bioconjugate Chem.* **2003**, *14*, 653–660.
[24] P. A. Wender, D. J. Mitchell, K. Pattabiraman, E. T. Pelkey, L. Steinman, J. B. Rothbard, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13003–13008.
[25] M. Schmidt, A. El-Dahshan, S. Keller, J. Rademann, *Angew. Chem.* **2009**, *121*, 6464–6467; *Angew. Chem. Int. Ed.* **2009**, *48*, 6346–6349.
[26] I. R. Ruttekolk, A. Chakrabarti, M. Richter, F. Durchardt, H. Glauner, W. P. R. Verdurmen, J. Rademann, R. Brock, *Mol. Pharmacol.* **2011**, *79*, 692–700.
[27] M. Mutter, S. Vuilleumier, *Angew. Chem.* **1989**, *101*, 551–571; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 535–554.

Received: June 27, 2012
Published online: November 4, 2012

