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Supplementary material

Glycosylated cell-penetrating peptides and their conjugates to a proapoptotic peptide: preparation by click chemistry and cell viability studies

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Table 1. Conditions for the click reaction on the model peptide: Ac-Arg-Pra-Arg-Ahx-Rink amide-resin.

Cu salt[a] (equivalent)	Compound **2**[c] equivalent	DIEA, equivalent	Time Hours[d]	Conversion[e]
$Cu(OAc)_2$ (40) Pyridine[b]	20	50	15	Complete
$Cu(OAc)_2$ (20) Pyridine[b]	10	25	15	Complete
$Cu(OAc)_2$ (10) Pyridine[b]	5	12.5	15	Complete
$Cu(OAc)_2$ (4) Pyridine[b]	2 2	5 5	15 5	Complete Complete
CuI (2) Lutidine[b]	2	4	5	Complete
CuI (2) Piperidine[b]	2	4	5	Complete

[a] Ascorbic acid in a 1:2 ratio with $Cu(OAc)_2$ and a 1:1 ratio with CuI salt. [b] 30% pyridine, lutidine or piperidine in dimethylformamide. [c] Compound **2**: equivalents calculated from the amino-substitution of the resin used, thus for a total coupling of the propargylglycine residue. [d] Overnight (15h) or 5h coupling at room temperature. [e] The conversion was monitored after cleavage of the peptidyl-resin and RP-HPLC analysis; the 5 hours coupling was considered as optimal conditions for this model peptide and for larger peptides.

Fig 1. Azido-propyl rhodamine **9.**

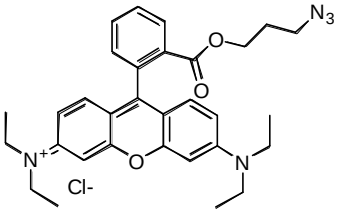

Table 2. Characterization of the different peptides **8** – **24**.

Peptides	Retention time, min[a]	Yield (%)[b]	MALDI-TOF MS[c]
8 - Ac-R-[Pra-Gal(OAc)]-R-Ahx-NH_2	10.0 (0-95%)	n.d.	$[MH]^+$ 953.48 *(953.48)*
10 - (R6/W3)S-S*t*Bu	17.0 (15-55%)	14	$[MH]^+$ 1744.94 *(1744.93)*
11 - (R6/W3)-SH	11.7 (15-55%)	87[d]	$[MH]^+$ 1658.00 *(1657.90)*
12 - (R6/W2)-Pra-S-S*t*Bu	12.5 (15-55%)	n.d.	$[MH]^+$ 1654.82 *(1654.89)*
13 - (R6/W1)-Pra_2-S-S*t*Bu	12.9 (15-55%)	n.d.	$[MH]^+$ 1563.56 *(1563.85)*
14 - (R6/W0)-Pra_3-S-S*t*Bu	9.9 (15-55%)	n.d.	$[MH]^+$ 1472.80 *(1472.81)*
15 - (R6/W2)-[Pra-Gal(OAc)]S-S*t*Bu	14.5 (15-55%)	3	$[MH]^+$ 2027.96 *(2028.00)*
16 - (R6/W2)-[Pra-Gal(OH)]-SH	8.2 (15-55%)	70[d]	$[MH]^+$ 1771.95 *(1771.93)*
17 - (R6/W1)-$[Pra\text{-}Gal(OAc)]_2$S-S*t*Bu	18.0 (10-60%)	4.5	$[MH]^+$ 2309.83 *(2310.07)*
18 - (R6/W0)-$[Pra\text{-}Gal(OAc)]_3$S-S*t*Bu	18.2 (10-60%)	7.5	$[MH]^+$ 2591.87 *(2592.14)*
19 - (R6/W0)-$[Pra\text{-}Gal(OH)]_3$-SH	8.0 (0-55%)	38[d]	$[MH]^+$ 2000.15 *(1999.98)*
20 - (R6/W2)-[Pra-Gal$(CH_2)_3$(OAc)]S-S*t*Bu	14.5 (15-55%)	4.8	$[MH]^+$ 2085.92 *(2086.04)*
21 - (R6/W2)-[Pra-Gal$(CH_2)_3$(OBz)]S-S*t*Bu	24.0 (15-55%)	2.3	$[MH]^+$ 2334.38 *(2334.11)*
22 - (R6/W2)-[Pra-Gal$(CH_2)_3$(OH)]S-S*t*Bu	9.7 (15-55%)	3.4	$[MH]^+$ 1917.59 *(1918.00)*
23 - (R6/W2)-[Pra-Gal$(CH_2)_3$(OH)]-SH	7.0 (15-55%)	46[d]	$[MH]^+$ 1829.71 *(1829.97)*
24 - (R6/W2)-[Pra-Gal$(CH_2)_3$(OAc)]-SH	10.5 (20-60%)	80[d]	$[MH]^+$ 1997.60 *(1998.00)*
[a] Linear gradient (30 min) of acetonitrile in 0.1%TFA. [b] Calculated from MBHA resin substitution: click-chemistry, cleavage, deprotection of galactose, (depending of the sequence), purification. [c] Given as found and *(calculated)*. [d] Yield including deacetylation, if necessary, and disulfide reduction with DTT.			

Table 3. Characterization of the glycopeptides conjugated to the KLAK peptides.

DTT was eliminated by rapid HPLC purification to isolate the thiol peptides, which were then immediately mixed to the KLAK peptide **25**. After completion of the coupling the final conjugates were purified by HPLC; their retention times different from those of the corresponding initial peptides attested the disappearance of the starting materials. The MH^+ ion of the glycoconjugates **27** and **28** was not observed on the MALDI-TOF mass spectra, as we already experienced with some dissymmetric disulfide-brigded peptides. Fragments coming from the cleavage of the S-S bond were observed, the isotopic distribution of these peaks and the presence of (±32, ±34 peaks) signed the presence of the expected products [1].

Number - Peptides	Retention time, min[a]	Yield (%)[b]	MALDI-TOF MS[c]
26 - (R6/W3)S-S-KLAK	20.0 (15-35%)	46	$[MH]^+$ [(R6/W3)S-S-KLAKH]$^+$ 3775.28 *(3775.18)*
27 - (R/6W2)-[Pra-Gal(OH)]S-S-KLAK	14.0 (15-55%)	28	$[MH]^+$ [(R6/W2)-[Pra-Gal(OH)]-SHH]$^+$ 1771.91 *(1771.93)* $[MH]^+$ [KLAK-SHNa]$^+$ 2133.85 *(2134.24)*[d]
28 - (R6/W2)-[Pra-Gal$(CH_2)_3$(OH)]S-S-KLAK	14.0 (15-55%)	33	$[MH]^+$ [(R6/W2)-[Pra-Gal$(CH_2)_3$(OH)]-SHH]$^+$ 1829.73 *(1829.96)* $[MNa]^+$ [KLAK-SHNa]$^+$ 2134.03 *(2134.24)*[d]
29 - (R6/W2)-[Pra-Gal$(CH_2)_3$(OAc)]S-S-KLAK	13.5 (20-60%)	22	$[MH]^+$ [(R6/W2)-[Pra-Gal$(CH_2)_3$(OAc)]S-S-KLAKH]$^+$ 4107.11 *(4107.22)*
30 - (R6/W0)-[Pra-Gal(OH)]$_3$S-S-KLAK	19.5 (0-55%)	72	$[MH]^+$ [(R6/W0)-[Pra-Gal(OH)]$_3$S-S-KLAKH]$^+$ 4109.92 *(4109.20)*

[a] Linear acetonitrile (0.1%TFA) gradient (in 30 min) in 0.1%TFA expressed as (% acetonitrile). [b] Coupling with the KLAK cargo. [c] Given as found *(calculated)*, respectively. [d] All these dissymmetric disulfide-bonded peptides are cleaved during the MALDI-TOF MS procedures; the major peak corresponding to the $[MH]^+$ or $[MNa]^+$ of each monomeric peptide, even though these monomeric peptides are not detectable in the HPLC chromatogram.

1. Schnaible V, Wefing S, Resemann A, Suckau D, Bucker A, Wolf-Kummeth S, Hoffmann D (2002) Anal Chem 74:4980-4988.