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Lipid-conjugated Smac analogs

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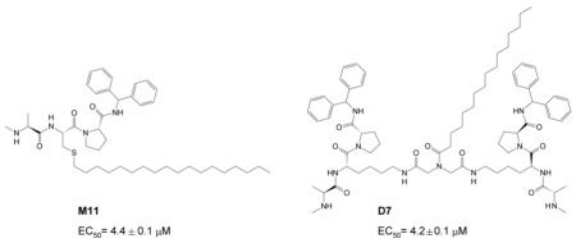
Abstract

A small library of monovalent and bivalent Smac mimics was synthesized based on 2 types of monomers, with general structure NMeAla-Xaa-Pro-BHA (Xaa=Cys or Lys). Position 2 of the compounds was utilized to dimerize both types of monomers employing various bis-reactive linkers, as well as to modify selected compounds with lipids. The resulting library was screened *in vitro* against metastatic human breast cancer cell line MDA-MB-231, and the two most active compounds selected for *in vivo* studies. The most active lipid-conjugated analogue M11, showed *in vivo* activity while administered both subcutaneously and orally. Collectively, our findings suggest that lipidation may be a viable approach in the development of new Smac-based therapeutic leads.

Graphical Abstract



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Keywords

Smac mimics; Anticancer agents; Lipids-conjugated peptides; S-Alkylation of peptides; Apoptosis

Apoptosis, also called programmed cell death (PCD) is an important mechanism controlling a variety of physiological processes including: host defense, development, homeostasis, and suppression of oncogenesis with implications in human pathologies spanning from cancer[1–5] to inflammation[6,7] and neurodegeneration[8,9]. Regulation of apoptosis depends on Inhibitors of Apoptosis Proteins (IAPs)[10–12]. Structurally, IAPs contain one or more of Baculovirus IAP Repeat (BIR) domains[12,13], which are capable of binding to and inhibition of various caspases, enzymes belonging to cysteine–aspartyl proteases family, which are crucial for apoptotic process[14]. To date, eight mammalian IAPs have been identified: neuronal IAP (NIAP), cellular IAP1 (cIAP1), cellular IAP2 (cIAP2), X chromosome-linked IAP (XIAP), survivin, ubiquitin-conjugating BIR domain enzyme apollon, melanoma IAP (ML-IAP) and IAP-like protein 2, with the most potent caspase inhibitor family member being XIAP[15,16], which simultaneously inhibits caspases -3, -7, and -9[17–20]. Except XIAP, only cIAP1, cIAP2, and ML-IAP, were shown to play a direct role in the regulation of apoptosis by inhibiting caspases' activity or their activation[12]. Anti-apoptotic activity of IAPs is in turn regulated by the second mitochondria derived activator of caspases (Smac), also called direct IAP binding protein with low pI (DIABLO)[21,22], which acts as their endogenous pro-apoptotic antagonist promoting programmed cell death[21–25]. Structurally, N-terminal tetrapeptide AVPI (Ala-Val-Pro-Ile), so called binding motif[21,22], is responsible for pro-apoptotic effects of mature Smac. In the case of XIAP, homodimeric form of Smac is capable of binding to both BIR2 and BIR3 domains of the protein abrogating its inhibition of caspases-3, -7, and -9[24,26]. For cIAP1 and cIAP2 only BIR3 domain is targeted by a single AVPI binding motif[27].

Over the past decade Smac mimics have become a promising therapeutic modalities in anti-cancer treatment[28–47] with several compounds advancing into clinical trials[32,41,42,46,48–50]. Among these bivalent Smac analogues containing two AVPI mimics tethered with a linker and capable of binding to both BIR2 and BIR3 XIAP domains became the focus of investigation due to their high potency[35–37,39,43,44,47]. On the other hand, monovalent Smac mimics are also desirable due to their favorable pharmaceutical properties: low molecular weight, favorable pharmacokinetics and potential oral bioavailability[41,46]. Nonetheless, review of available literature revealed that lipid-derivatized Smac mimics were not synthesized to date which prompted our investigation.

Lipidation is extensively used in the drug development, including therapeutic peptides[51–53]. Its application usually leads to new therapeutic entities with significantly changed physicochemical and pharmacological properties. In the case of peptides, such modification(s) may result in increased peptide stability, permeability and intestinal absorption[54–63]. An increased oral availability was also described[64,65]. Mechanistically, observed beneficial effects of lipidation are usually associated with binding of therapeutic entity to circulating albumin[54,66–68], and/or targeted excretion by the liver rather than by the

kidney[54,63,65]. An alternative mechanism employing high-density lipoprotein (HDL), low-density lipoprotein (LDL) and their receptors was also proposed[69].

To ascertain whether lipidation can be a useful approach in the development of Smac mimics, we decided to synthesize and test small library of analogues with structure(s) schematically shown in Figure 1. Available data[39,47] suggest that overall hydrophobicity of the Smac mimics may be important for their biological activity, most likely promoting cell permeability and increasing intracellular concentration of analogues, resulting in more potent therapeutic effects. In this context, lipidation seems to be viable modification approach.

Generally monomeric (**M**) analogues had a sequence NMeAla-Cys/Lys-Pro-BHA which is closely related to various potent analogues developed by *Wang group*[35,37–39,43,44]. Based on literature data[70], position 2, which in our analogues is occupied by either (L)Lys or (L)Cys, was chosen as viable modification/dimerization point. Synthesis of monomers was carried out in solution according to reaction Scheme 1 and 2. Dimerization of monomers was carried out utilizing either bis-amine-reactive linkers (**D1-D8**, Figure 4) or bis-thiol-reactive linkers (**D9-D16**, Figure 5) listed in Figure 1. This report describes synthesis and biological properties of these novel compounds. Detailed experimental methods, analytical data for obtained peptides as well as an example of MS-spectra and corresponding analytical RP-HPLC profile are presented in Supplementary Material.

The synthesis of monomers proceeded efficiently and due to simplicity of final products, was carried out with minimal purification of the intermediates. Four different lipids were used to modify **M** compounds in position 2: palmitic acid (Pal, C_{16}), lignoceric acid (Lig, C_{24}), cholesterol (Chol, C_{27}) and stearyl chain (Ste, C_{18}) afforded by 1-bromooctadecane, with first two using side chain amine group of Lys for modifications with palmitoyl chloride or lignoceroyl chloride respectively. Cholesterol was introduced in similar manner using cholesteryl chloroformate giving urethane type connectivity (see Scheme 2). Analogue **M11** was synthesized using a previously described 1,1,3,3-tetramethylguanidine (TMG) driven alkylation of thiol(s) in organic solvents[71] that we adapted to peptides[72]. Notably, the same S-alkylation protocol was successfully employed in the synthesis of dimers **D9-D16**. Among all dimers synthesized for this study only three, **D7**, **D8** and **D16** underwent lipidation. **D7** and **D8** were modified with palmityl moiety using either physiologically stable amide bond (**D7**) or cleavable ester type (**D8**) connectivity. **D16** was modified with stearyl chain afforded by mBMPB-3,5-bis(bromomethyl)-1-(methyl-S-palmityl)-benzene however efficiency of the reaction was particularly low (<3%).

An initial evaluation of bioactivity of our Smac mimics was carried out *in vitro* using exclusively growth inhibition assay (PrestoBlue™, Invitrogen, Carlsbad, CA) and MDA-MB-231 metastatic human breast cancer cell line, which in our view provides more reliable data than pure biophysical method(s) e.g. measurement of binding affinity to BIR2/BIR3 XIAP domain as it takes into account many factors like the compound's cell permeability, its binding potency, stability in the cell's microenvironment, etc. Notably, we had previously employed this approach in the synthesis of Smac mimics candidates with positive results[47].

Obtained results are summarized in Figure 1 and an example of cell growth curves is presented in Figure 2A.

Initial screening of monovalent Smac analogues (**M1-M11**) (Figure 1) suggested that indeed optimal hydrophobicity plays an important role in overall bioactivity of position 2 modified compounds and observed activity gain can be significant in both ^{2}Cys (**M2<M3<M11**, EC_{50} (μM): NA<49.9±0.7<4.4±0.1 respectively) and ^{2}Lys series (**M4<M6<M7<M8**, EC_{50} (μM): NA<44.7±15.0<19.0±1.6<6.1±0.1 respectively). Simultaneously exceedingly hydrophobic substituents (Lig, Chol) in position 2 seem to be undesirable as is the presence of hydrophilic/ionizable amine and guanidine moieties (**M4** and **M5** respectively). The similar conclusions could be drawn from dimeric mimics as analogues utilizing hydrophilic linkers have shown low potency (**D1, D6, D9, D10**). Lipidation is also clearly beneficial, as introduction of lipid moiety (Pal) into otherwise inactive **D6** compound leads to highly active derivatives **D7** and **D8**. Effects of dimerization are also apparent with dimeric analogue **D3** being ~13.5× more potent than its monomeric counterpart, **M6** (EC_{50} (μM): 44.7±15.0 *versus* 3.3±0.2 respectively). previously reported results, Similarly to previously reported results[43,44], our most active dimeric analogues contain relatively hydrophobic linker(s) with ~13–14 atoms (Cα↔C′α). Moreover, spatial geometry of linkers(s) is also an important for overall potency as compounds utilizing relatively similar linkers show diversified bioactivity (**D13-D15**). Interestingly, for analogous shorter linkers (**D11&D12**), a specific linker geometry (*para- versus meta*-positions of substituents) seems to have an opposite effect on overall potency. Nonetheless, reported to date *in vitro* results for similar Smac mimics showed markedly better bioactivity in low nanomolar range[35,39,43–45,47] *versus* low micromolar range in the case of our most active analogues (Figure 1).

To confirm that our newly synthesized Smac mimics indeed can promote apoptosis, we measured enzymatic activity of caspases-3/7 and -9 in a metastatic breast cancer cell line, MDA-MB-231 that was treated with selected, most active analogues: **M11, D3, D7** and **D13**. Direct comparison of the effects of the treatment at 10 μM concentration is presented in Figure 2B. Interestingly, only caspase-3/7 seems to be selectively affected by the treatment resulting in ~2.7–6.7 fold increase in enzymatic activity and is approximately 50% less effective than our previously described *tail-to-tail* dimer, **SMAC17-2X**[47]. All tested dimers (**D3, D7, D13**) are also approximately ~2.5× more potent than monomer, **M11** and in the case of lipid-conjugated analogues **M11** and **D7**, observed effects are dose dependent (Figure 2C).

To further characterize most potent lipid-conjugated analogues **M11** and **D7**, we performed pharmacokinetic (PK) studies using 2 different delivery routes: subcutaneous (SC, 10 mg/kg dose) and oral (OR, 10 mg/kg dose). For analogue **M11** observed plasma half-life ($t_{1/2}$) is ~2.2 h for SC and ~5.2 h for OR delivery (Figure 2D&2E). Analogue **D7** was bioavailable only *via* SC route giving value of $t_{1/2}\approx$2.8 h.

For **M11** and **D7** analogues we also assessed their plasma stability. Obtained results (Figure 2F) indicated that both analogues are remarkably stable in experimental conditions (<31 h) and even after prolonged exposure (144h) both compounds were mainly intact (plasma stability: **M11**=60.4±0.3% and **D7**=80.1±0.5%). Notably, under the same experimental

conditions unrelated control peptide, **mHS1**[72] was quickly degraded falling to less than 1% of initial content within 6 h.

To test utility of our lipidated compounds we performed *in vivo* studies using subcutaneous engraftment mouse model and human metastatic breast cancer line, MDA-MB-231. Based on PK studies we decided to administer monovalent analogue **M11** *via* both SC and OR routes. Bivalent lipidated analogue **D7** was administered only SC. The treatment of the experimental, cancer bearing animals with **M11** resulted in dose dependent anticancer effects (Figure 3). Animals treated with 10 doses of the **M11** at the escalating dosage from 2.5 to 15 mg/kg showed progressively longer tumor growth delay values (Table 1) reaching ~11.0 days of delay at the 15 mg/kg SC dose. Oral administration of **M11** at 30 mg/kg dose resulted in ~7.6 days of tumor growth delay which suggested that oral bioavailability of **M11** is ~23%, based on anticancer response. Surprisingly, bivalent **D7** analogue showed low anticancer *in vivo* activity and was ~2.7× less potent than monovalent **M11** (SC route). Comparison of our *in vivo* results with published data is somewhat difficult due to the differences in experimental conditions[35,39,44]. Nonetheless, comparing to previously reported analogue, SMAC17-2X[47], our best lipidated compound **M11** is significantly less active as reported tumor growth delay values for **SMAC17-2X** were: ~10.2 days at 2.5 mg/kg dose and ~23.4 days at 7.5 mg/kg dose. Similarly, *in vivo* results reported for **SM-164**[35], compound 27[39] and **SM-1200**[44] showed markedly improved activity profiles with **SM-1200** promoting complete and durable tumor regression in mouse model. Nevertheless, our data suggest that modification of Smac mimics with lipids may be beneficial as certain lipid-modified analogues showed improved bioactivity comparing to "lipid-less" counterparts. (**M11** *versus* **M2; M8** *versus* **M4; D7/D8** *versus* **D6**). Certainly, optimal overall hydrophobicity is also important and is directly related to solubility of the compound(s) which in turn can influence delivery, distribution and pharmacokinetics of the drug(s), and this arguably might explain inactivity of Lig-containing analogue (**M9**) which is perhaps too hydrophobic. Successful application of lipidation to the development of Smac mimics will certainly require extensive future experimentation. Considering overall size of the Smac mimics, there is obvious that localization of lipid-modification(s) within the prospective analogues is of paramount importance, and we explored only position 2 for that purpose. Judging from various multimerization studies[35,39,41,47] the C-terminus is quite accommodating and perhaps may be better suited for such derivatization.

Despite a small size of our analogues we decided to probe their secondary structure using Fourier transform infrared (FTIR) spectroscopy and summarized our findings in Table 2. Both tested peptides, **M11** and **D7**, showed dominant turn conformations in hydrated self-films and in POPC multilayers (Figure S2A–D and Table 2). In self-films **M11** had broad absorption from 1682 cm^{-1} to 1662 cm^{-1} suggesting the peptide assumed number of turn conformations. There was also present a well-defined β-sheet absorbance centered at 1924 cm^{-1}, indicating the peptide may self-associate forming anti-parallel β-sheets at these experimental conditions. When the **M11** was incorporated into a membrane-like environment of POPC multilayers, the turn absorption was better defined with a peak centered around 1675 cm^{-1} and some loss of β-sheet to disordered conformations occured (Figure S2B). In both, self-films and lipid multilayers **M11** showed very little α-helical

structure. The infrared signature of **D7** amide I conformational band although similar to that of **M11** had better defined turn band than the latter, which was centered at 1664 cm^{-1} indicating typical 310-helix or type III turn conformations (Figure S2C). The deuterium hydrated self-film of **D7** also had a well-defined β-sheet band round 1924 cm^{-1} similar to that observed for the **M11** peptide. **D7** also showed a loss of β-sheet conformation to more disordered structures in the POPC environment similar to that of **M11**. Nonetheless, in POPC multilayers environment, **D7** showed a dominant absorption around 1664 cm^{-1} (Figure S2D) indicating that the dimeric lipo-peptide assumed a more stable type III turn structure than **M11**.

In conclusion, a new group of monomeric and dimeric anticancer Smac peptides, including novel lipid-conjugated Smac mimics, was synthesized, characterized and screened for anticancer activity against human metastatic breast cancer cell line, MDA-MB-231. Selected lipidated analogues, monomeric (**M11**) and dimeric (**D7**), were characterized further showing favorable pharmacokinetics, plasma stability and *in vivo* efficacy in murine model. The most active lipid-conjugated analogue **M11**, showed *in vivo* activity while administered both subcutaneously and orally. Collectively, our findings suggest that modification of Smac mimics with various lipids may be a viable approach in the development of novel anticancer leads.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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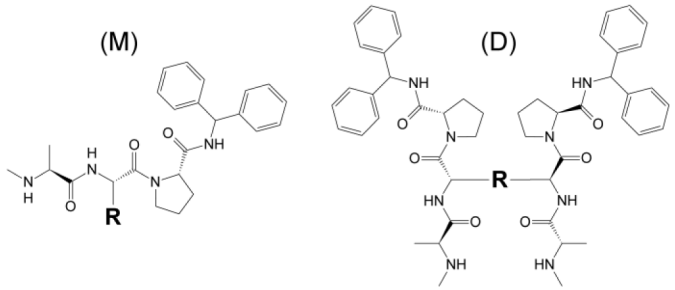


Compound	R	EC_{50} [μM] MDA-MB-231
M1	Ser^{OH}	NA
M2	Cys^{SH}	NA
M3	$Cys^{S\text{-}StBu}$	49.9±0.7
M4	Lys	NA
M5	Lys^{Gu}	NA
M6	$Lys^{NH\text{-}DNFB}$	44.7±15.0
M7	$Lys^{NH\text{-}Fmoc}$	19.0±1.6
M8	$Lys^{NH\text{-}Pal}$	6.1±0.1
M9	$Lys^{NH\text{-}Lig}$	NA
M10	$Lys^{NH\text{-}Chol}$	9.7±0.5
M11	$Cys^{S\text{-}Ste}$	4.4±0.1
D1	$Lys^{NH\text{-}Urea}$	NA
D2	$Lys^{NH\text{-}Sub}$	NA
D3	$Lys^{NH\text{-}DFDNB}$	3.3±0.2
D4	$Lys^{NH\text{-}PDI}$	34.8±2.7
D5	$Lys^{NH\text{-}OPI}$	14.4±1.4
D6	$Lys^{NH\text{-}Ida}$	NA
D7	$Lys^{NH\text{-}Ida\text{-}Pal}$	4.2±0.1
D8	$Lys^{NH\text{-}Ida\text{-}N\text{-}EtO\text{-}Pal}$	4.3±0.1
D9	$Cys^{S\text{-}DVS}$	52.4±4.3
D10	$Cys^{S\text{-}CAEDA}$	NA
D11	$Cys^{S\text{-}pBMB}$	9.6±0.6
D12	$Cys^{S\text{-}mBMB}$	7.4±0.4
D13	$Cys^{S\text{-}Bip}$	4.0±0.1
D14	$Cys^{S\text{-}CMPB}$	18.4±1.1
D15	$Cys^{S\text{-}BMBB}$	NA
D16	$Cys^{S\text{-}mBMPB}$	11.3±0.6

Figure 1.

General structure of synthesized monomeric (M) and dimeric (D) Smac derivatives and their respective *in vitro* activity in cell growth inhibition assay, using MDA-MB-231 human metastatic breast cancer cell line.

Abbreviations: Bip–4,4′-Bis(bromomethyl)biphenyl; BMBB–1-(Bromo-methyl)-3-[3-(bromomethyl)benzyl]benzene; CAEDA–N,N′-Bis(2-chloro-acetylo)ethylenediamine; CMPB–1-(Chloromethyl)-4-[4-(chloromethyl)-phenoxy]benzene; Chol–Cholesterol; DFDNB–1,5-difluoro-2,4-dinitroben-zene; DNFB–1-Fluoro-2,4-dinitrobenzene; Fmoc–Fluorenylmethyloxy-carbo-nyl; Gu–guanidine; Ida–Iminodiacetic acid; Lig–Lignoceric acid; mBMB–1,3-Bis(bromomethyl)benzene; mBMPB-3,5-Bis(bromomethyl)-1-(methyl-S-palmityl)-benzene; OPI–4,4′-Oxybis(phenyl isocyanate); Pal–Palmitic acid; pBMB–1,4-Bis(bromomethyl)benzene; PDI–1,4-Phenylene diisocyanate; StBu–S-tertbutylthio; Ste–Stearyl; Sub–Suberic acid; NA–not active. All peptides were synthesized as C–terminal benzhydrylamides. EC_{50} values higher than 100 μM are denoted as NA.

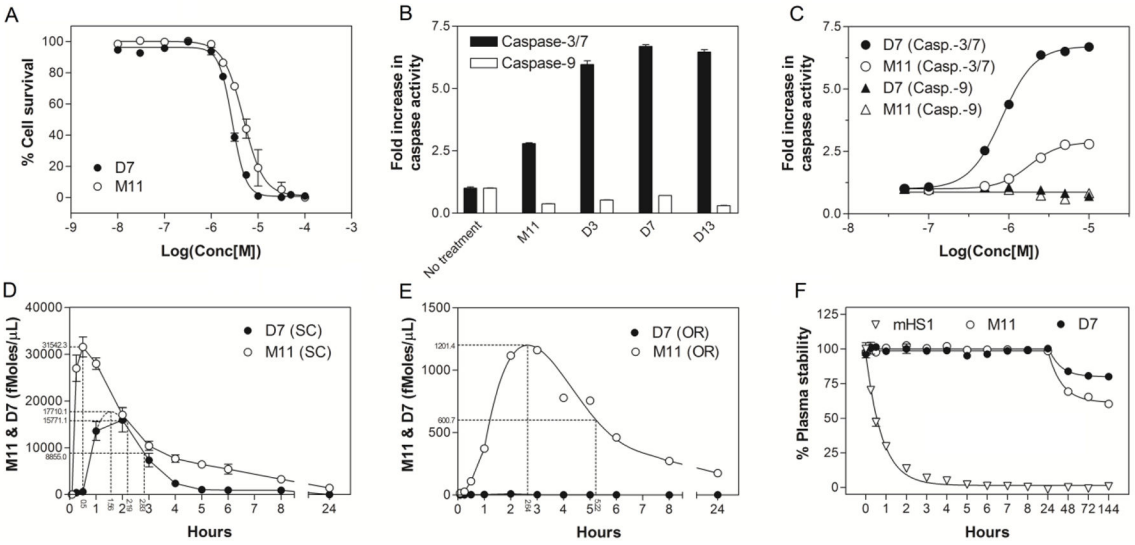


Figure 2.

Characterization of selected Smac mimics: (A) Examples of cell viability curves obtained for MDA-MB-231 human metastatic breast cancer cell line treated with lipidated compounds M11 and D7, Increase in enzymatic activity of caspases-3/7 and -9 in MDA-MB-231 cells treated with peptides: (B) M11, D3, D7, and D13 at 10 μM concentration, (C) M11, and D7 at various concentrations (dose response), (D) PK experiments of SC and (E) OR administered compounds M11 and D7, (F) Plasma stability studies of M11 and D7 analogues.

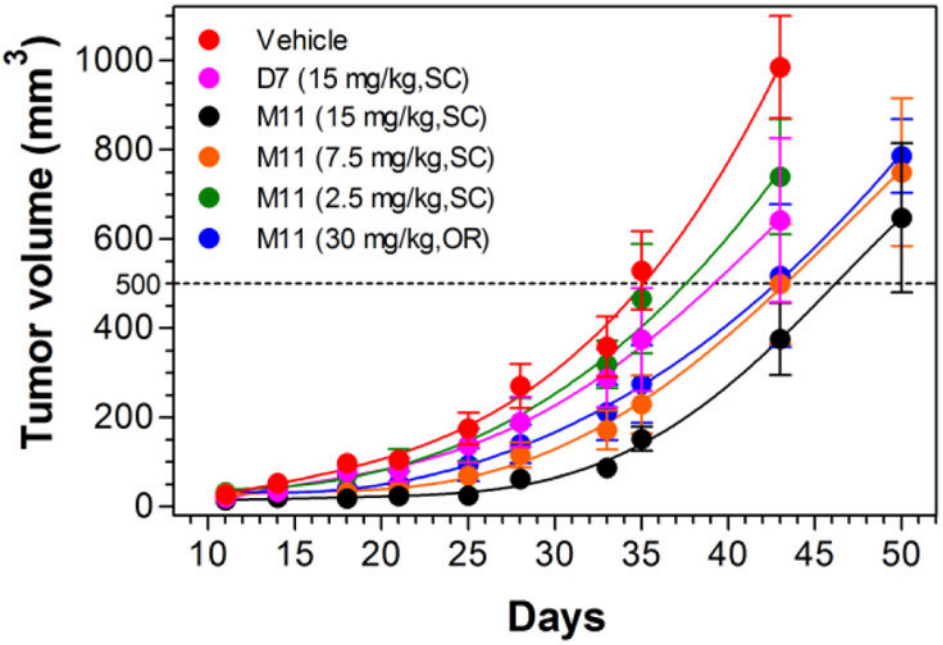


Figure 3.
Anticancer effects of M11 and D7 treatment in xenograft mouse model.

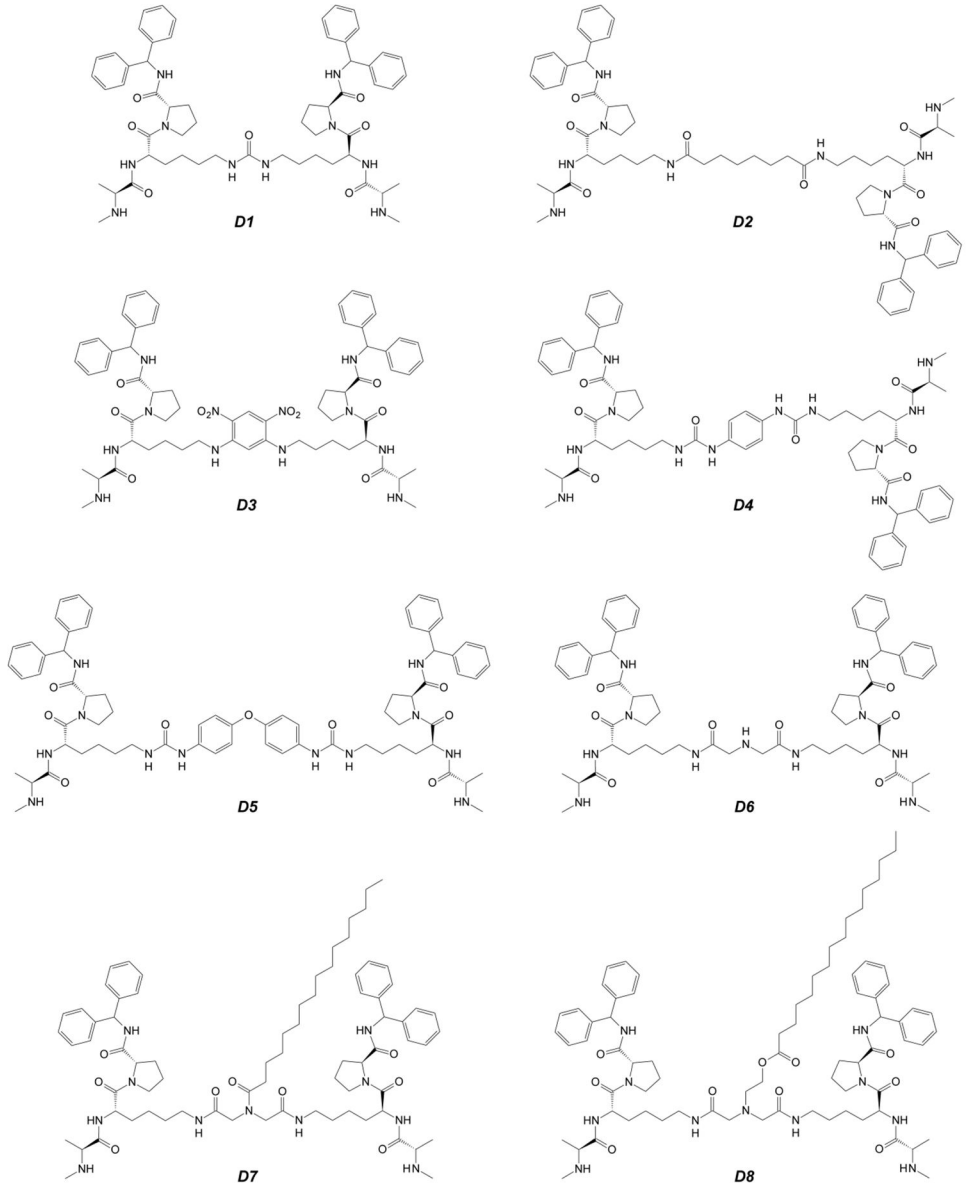


Figure 4.

Structures of dimeric Smac derivatives containing Lys in position 2.

Dimerization conditions: Compound **7** was dimerized in DMF using: **D1**-p-Nitrophenyl chloroformate, **D2**-Suberic acid bis(N-hydroxysuccinimide ester), **D3**-1,5-Difluoro-2,4-dinitrobenzene, **D4**-1,4-Phenylene diisocyanate, **D5**-4,4′-Oxybis(phenyl isocyanate), **D6**-(1) Fmoc-Ida-OH/TCTU, (2) 50% piperidine in DMF/1h, **D7**-(1) Fmoc-Ida-OH/TCTU, (2) 50% piperidine in DMSO/1h, (3) Palmitoyl chloride/1,4-Dioxane/NMM/2h, **D8**- Pal-O-Et-N-Ida-OH/TCTU. Boc groups were removed using 4M HCl in 1,4-dioxane (30 min).

Abbreviations: DMF-N,N-Dimethylformamide, Fmoc-Ida-OH–N-Fluorenylmethyloxycarbonyl-iminodiacetic acid, Pal-O-Et-N-Ida-OH–N,N-bis(carboxymetyl)-O-palmitoyl-ethanolamine, NMM–N-methylmorpholine, TCTU–O-(6-Chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

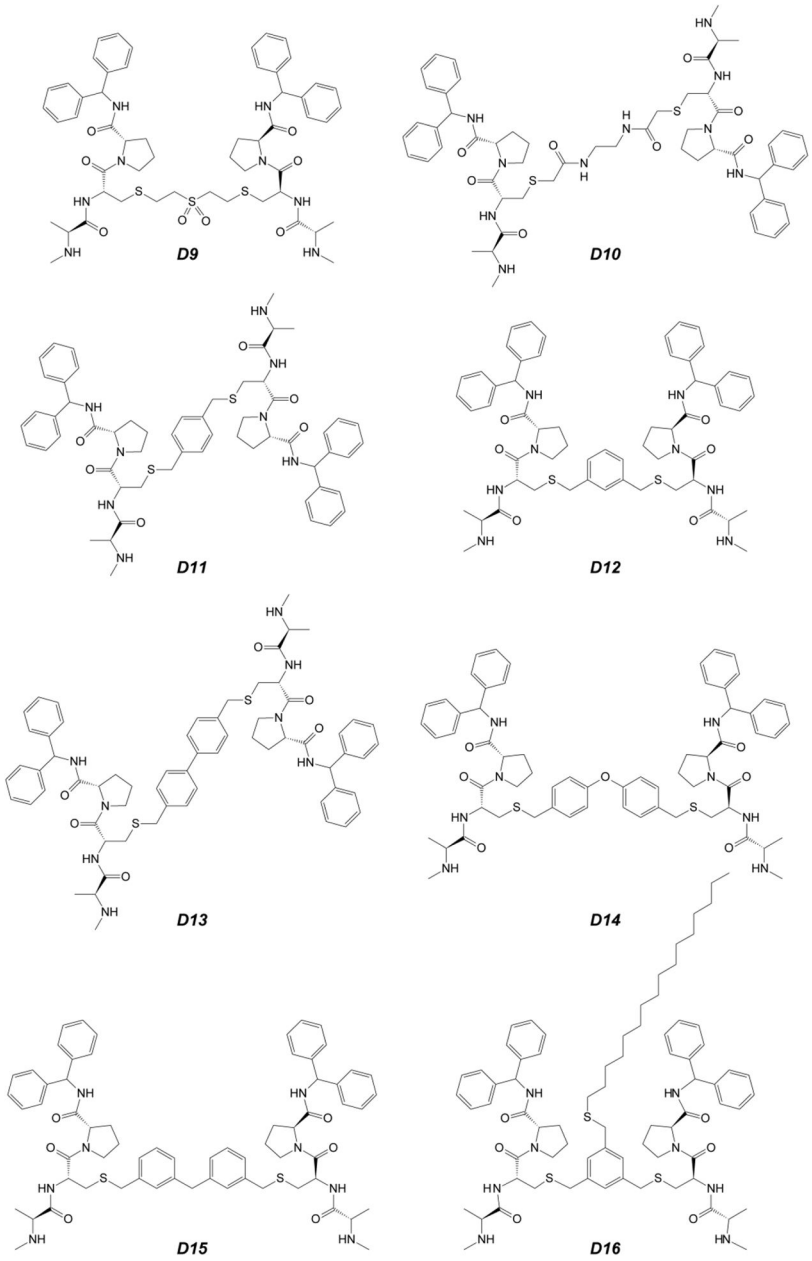
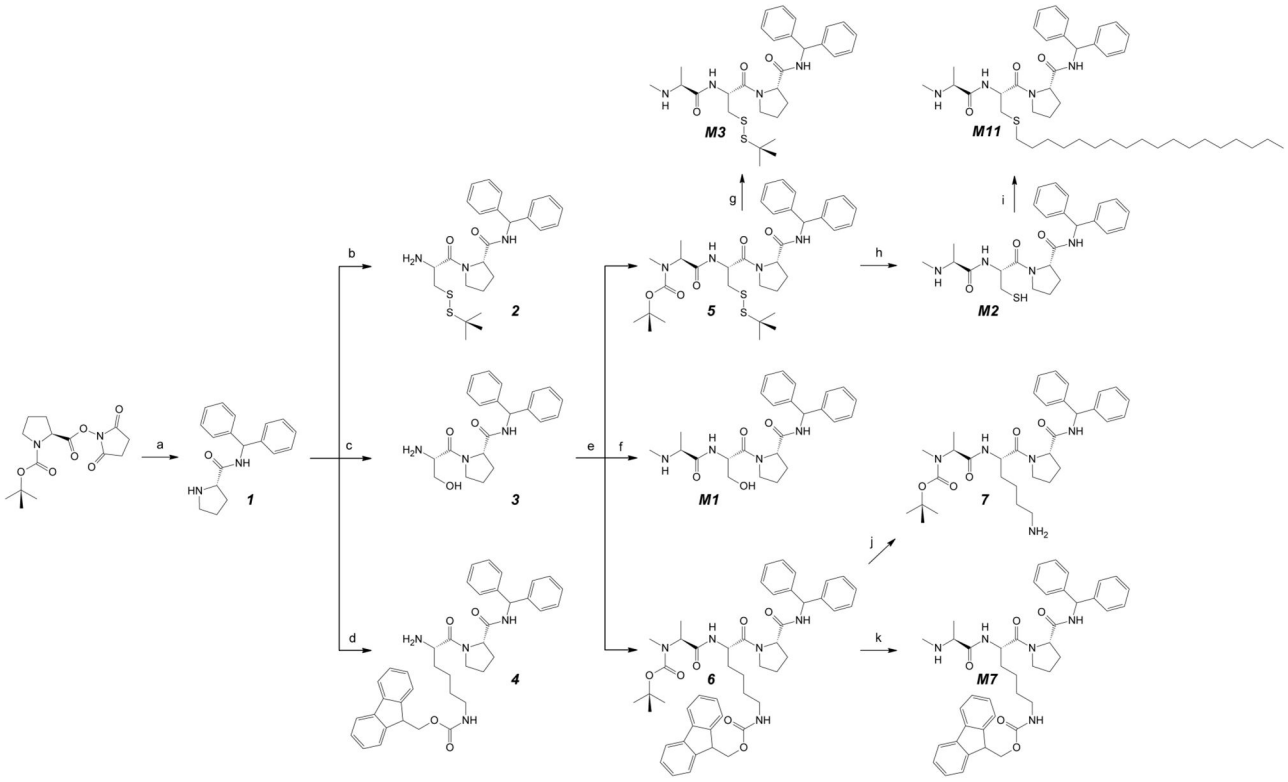


Figure 5.

Structures of dimeric Smac derivatives containing Cys in position 2.

Dimerization conditions: Compound **M2** was dimerized using: **D9**-Divinyl sulfone, **D10**-N,N′-Bis(2-chloroacetylo)ethylenediamine, **D11**-1,4-Bis(bromomethyl)benzene, **D12**-1,3-Bis(bromomethyl)benzene, **D13**-4,4′-Bis(bromomethyl)biphenyl, **D14**-1-(Chloromethyl)-4-[4-(chloromethyl)phenoxy]benzene, **D15**-1-(Bromomethyl)-3-[3-(bromomethyl)benzyl]benzene, **D16**-1-(Palmityl-S-methyl)-3,5-bis(bromomethyl)-benzene.

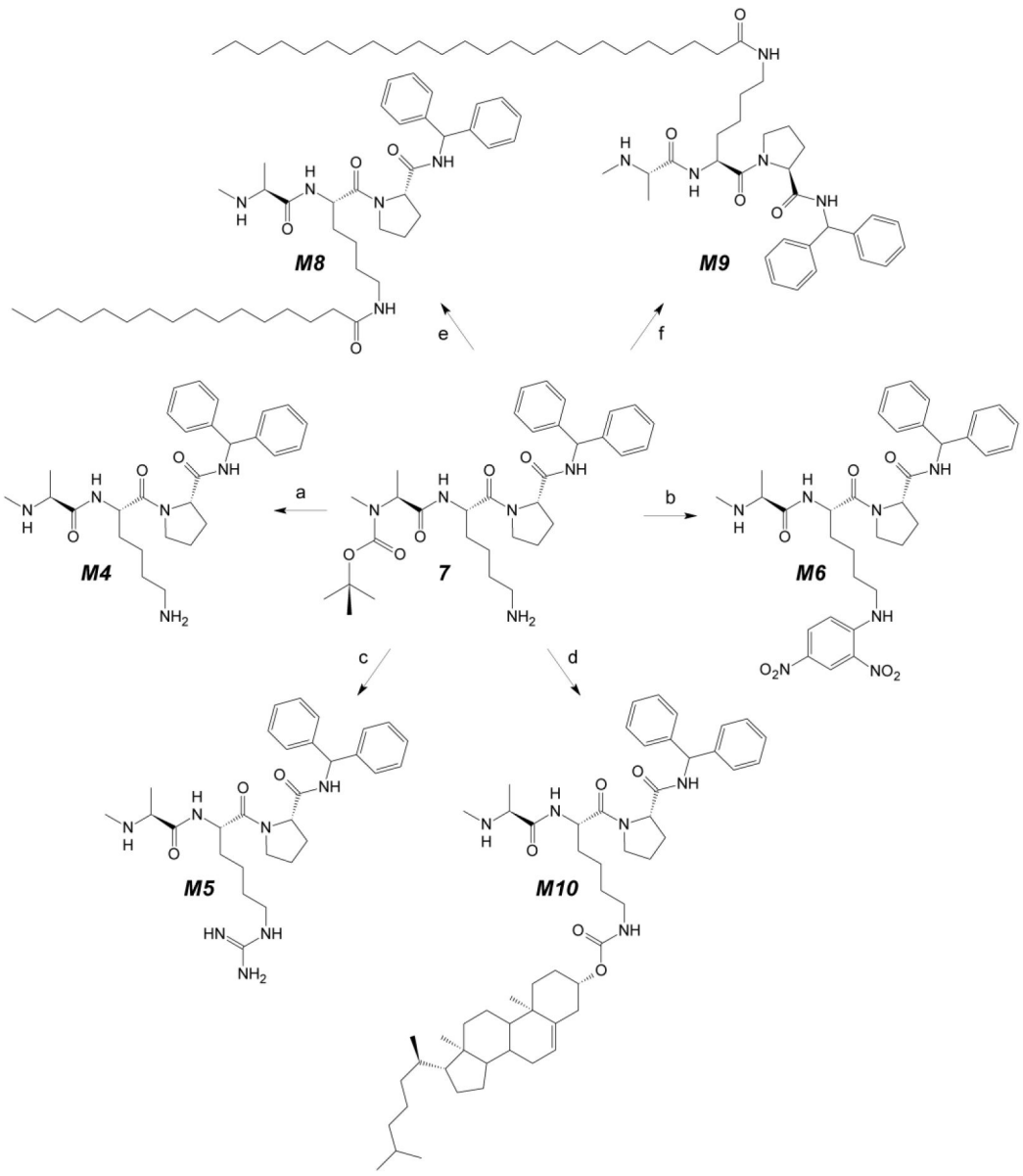


Scheme 1.

Synthesis of monomeric Smac derivatives.

Conditions: a: (1) BHA/1,4-dioxane/2h; (2) 4M HCl in 1,4-dioxane/30 min; **b**: (1) Boc-(L)-Cys(SStBu)-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min **c:** (1) Boc-(L)-Ser(tBu)-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min; **d**: (1) Boc-(L)-Lys(Fmoc)-OH/TCTU/NMM/DMSO/75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min; **e**: Boc-N-Me-(L)-Ala-OH/TCTU/NMM/DMSO/75°C/10 min/MW; **f**: 4M HCl in 1,4-dioxane/30 min; **g**: 4M HCl in 1,4-dioxane/30 min; **h**: TFA/DTT/30 min; **i**: C_{18}-Br/CH_3OH/TMGU/1h/50oC; **j**: 4M NaOH in CH_3OH (1:9)/r.t./30 min; **k**: 4M HCl in 1,4-dioxane/30 min.

Abbreviations: BHA–benzhydrylamine, Boc-(L)-Cys(SStBu)-OH–N-tertbutoxycarbonyl-S-tert-butylthio-L-cysteine, Boc-(L)-Lys(Fmoc)-OH–N^2-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N^6-{[(2-methyl-2-propanyl)oxy]-carbonyl}-L-lysine, Boc-N-Me-(L)-Ala-OH–N-tertbutoxycarbonyl-N-methyl-L-alanine, Boc-(L)-Ser(tBu)-OH–N-tertbutoxycarbonyl-O-tert-butyl-L-serine, C_{18}-Br–1-Bromooctadecane, DMSO–dimethylsulfoxide, DTT–DL_Dithiothreitol, MW–microwave assisted synthesis, NMM–N-methylmorpholine, TCTU–O-(6-Chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TFA–Trifluoroacetic acid, TMGu–1,1,3,3-Tetramethylguanidine.



Scheme 2.

Synthesis of monomeric Smac derivatives containing Lys in position 2.

Conditions: a: 4M HCl in 1,4-dioxane/30 min; **b**: (1) DNFB/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min **c:** (1) N,N-Di-Boc-1H-pyrazole-1-carboxamidine/1,4-Dioxane/NMM//75°C/10 min/MW; (2) 4M HCl in 1,4-dioxane/30 min; **d**: (1) Cholesteryl chloroformate/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min; **e**: (1) Palmitoyl chloride/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min; **f**: (1) Lignoceroyl chloride/1,4-dioxane/NMM/2h; (2) 4M HCl in 1,4-dioxane/30 min.

Abbreviations: DNFB–1-Fluoro-2,4-dinitrobenzene, MW–microwave assisted synthesis, NMM–N-methylmorpholine.

Table 1

Tumor growth delay values obtained for M11 and D7 analogues.

Compound	Dose (mg/kg)	Delivery route	Tumor Growth Delay at 500 mm^3 (days)
M11	2.5	SC	~2.5
M11	7.5	SC	~8.1
M11	15	SC	~11.0
M11	30	OR	~7.6
D7	15	SC	~4.1

Table 2

Proportions of different components of secondary structure for M11 and D7 peptides in hydrated self-films and lipid multilayers based on infrared spectroscopic analysis.

Peptide*	% Conformation			
	α-helix	β-sheet	turns	disordered
M11 self-film	11.90	26.75	50.98	10.37
M11 in POPC	6.71	18.29	35.27	39.73
D7 self-film	4.43	23.17	55.32	17.08
D7 in POPC	6.40	25.09	36.96	32.55

* Deuterium hydrated peptide self-films and POPC - peptide multilayers were studied with a germanium ATR accessory as described in methods. All IR spectra analyzed for secondary conformation based on secondary structural analysis using GRAMS/AI deconvolution-curve-fitting software (Methods). Peak area error for these estimates is ±5%.