

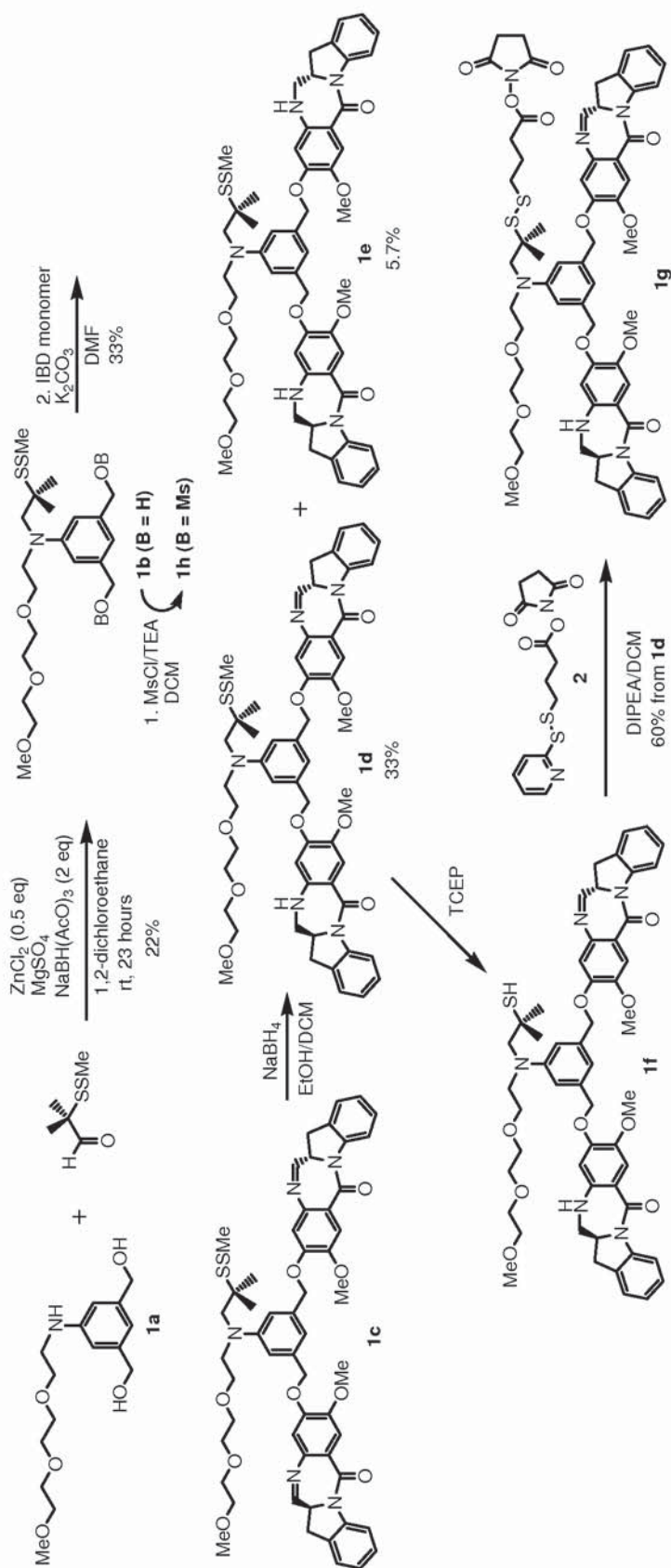
Figure 1. Synthetic Scheme of Linker **1b** and Dimers **1d-g**

Figure 2. Synthetic Scheme of Dimers 2a-b

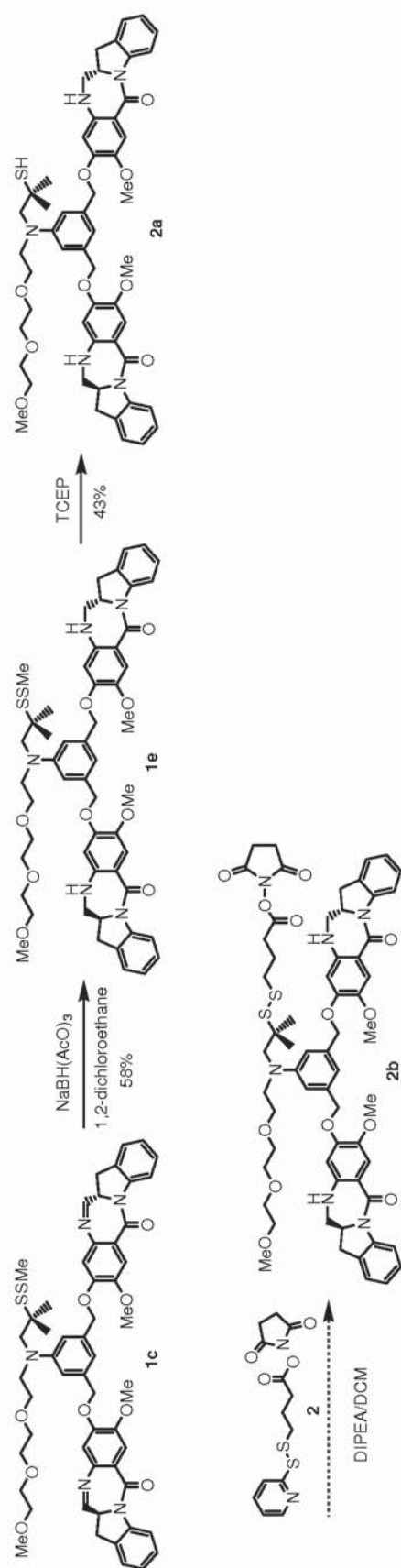


Figure 3. Synthetic Scheme of Dimers 3e-g

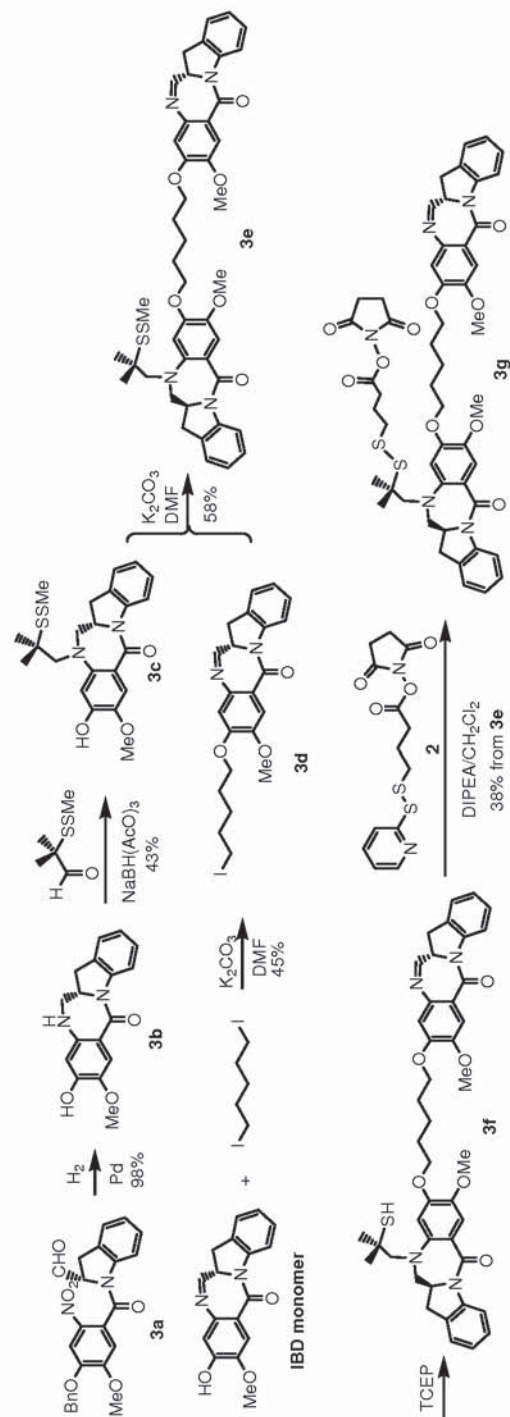


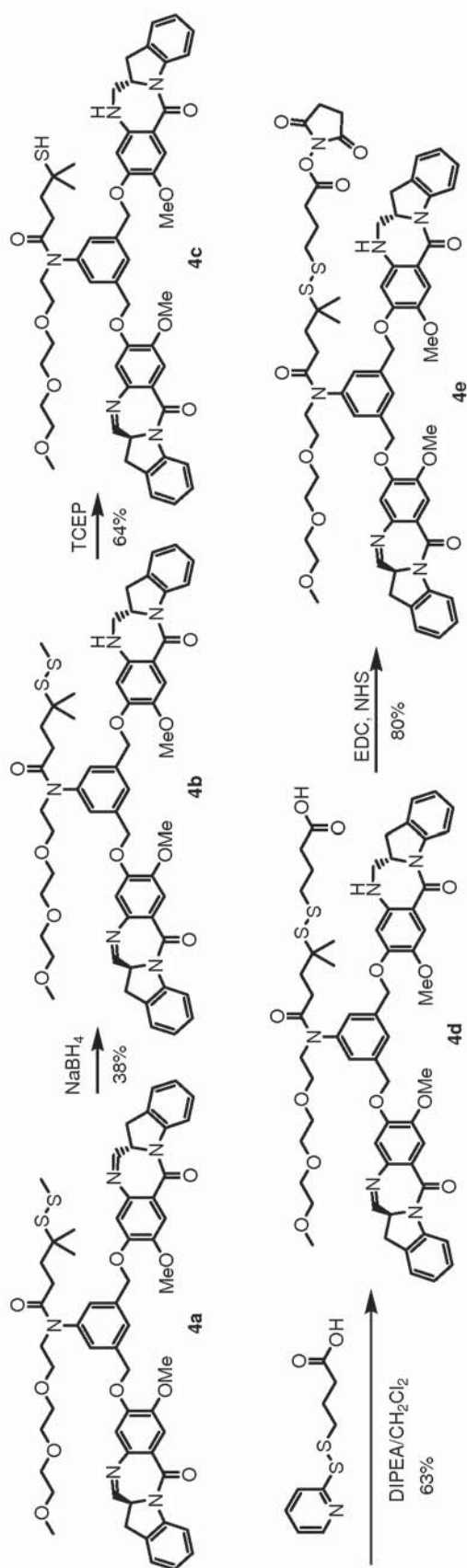
Figure 4. Synthetic Scheme of Dimers **4b-e**

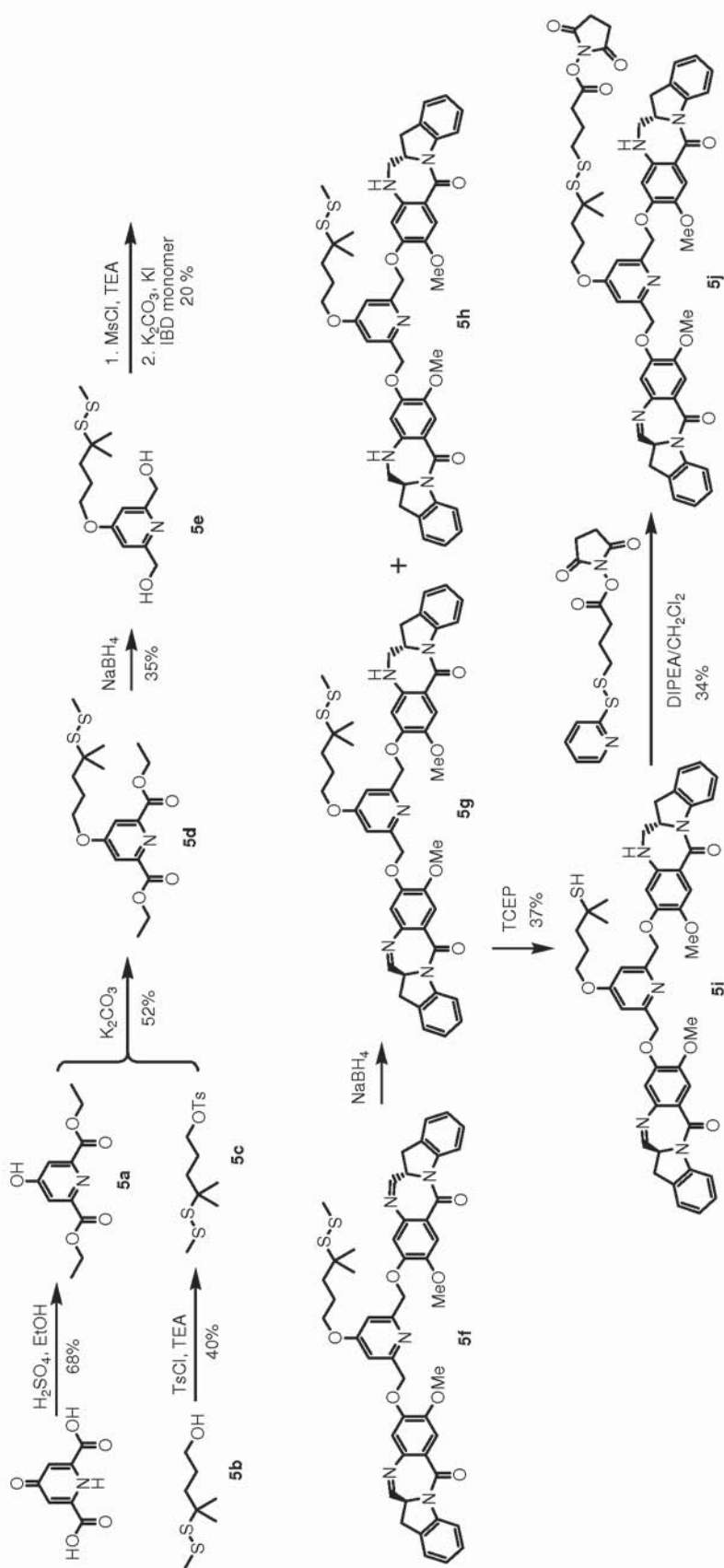
Figure 5. Synthetic Scheme of Linker **5e** and Dimers **5g-j**

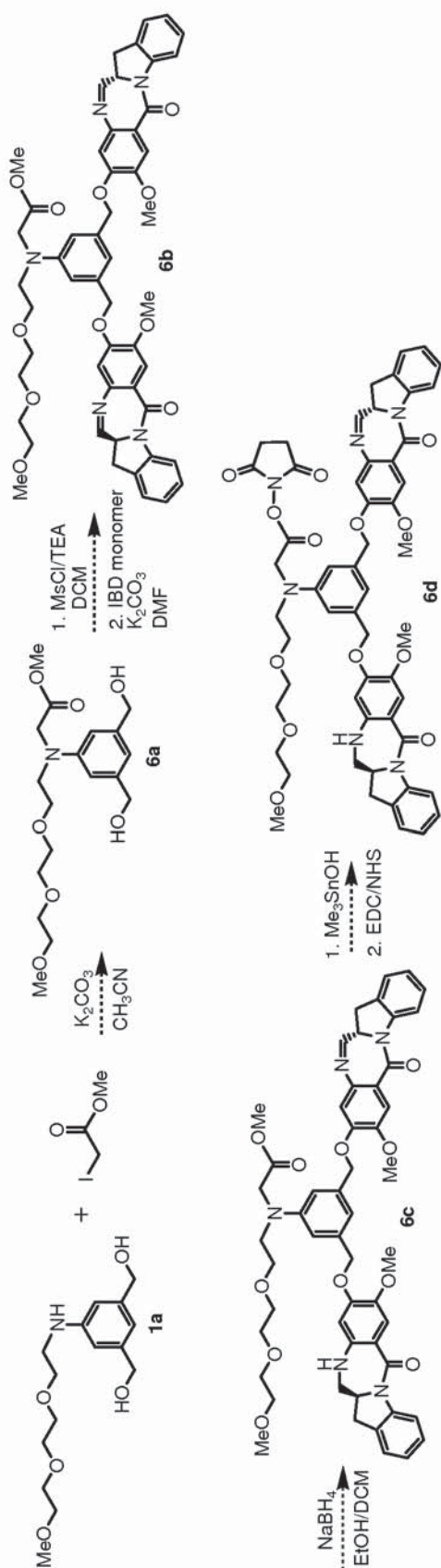
Figure 6. Synthetic Scheme of Linker **6a** and Dimers **6c-d**

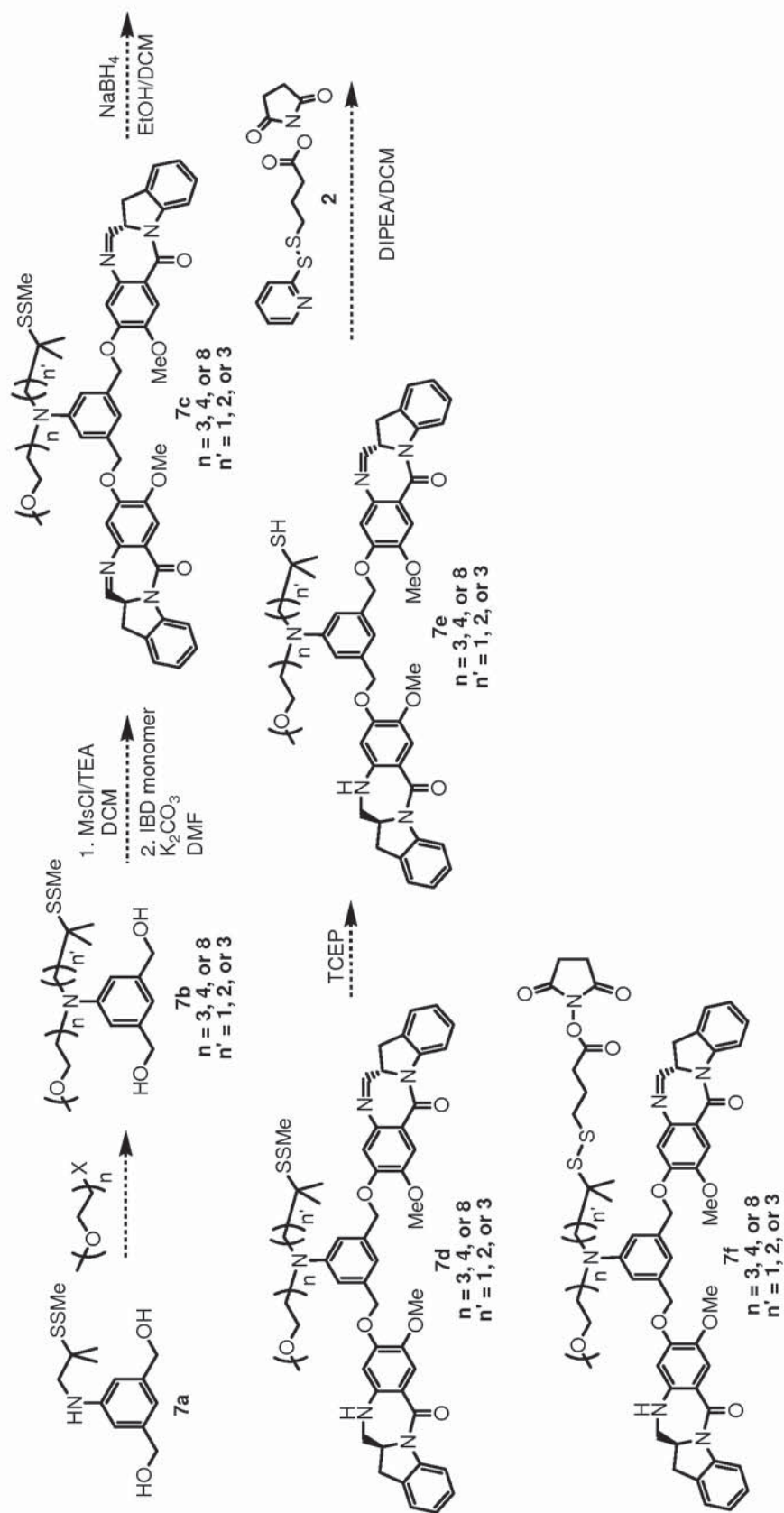
Figure 7. Synthetic Scheme of PEG Modified Linkers **7b** and Dimers **7d-f**

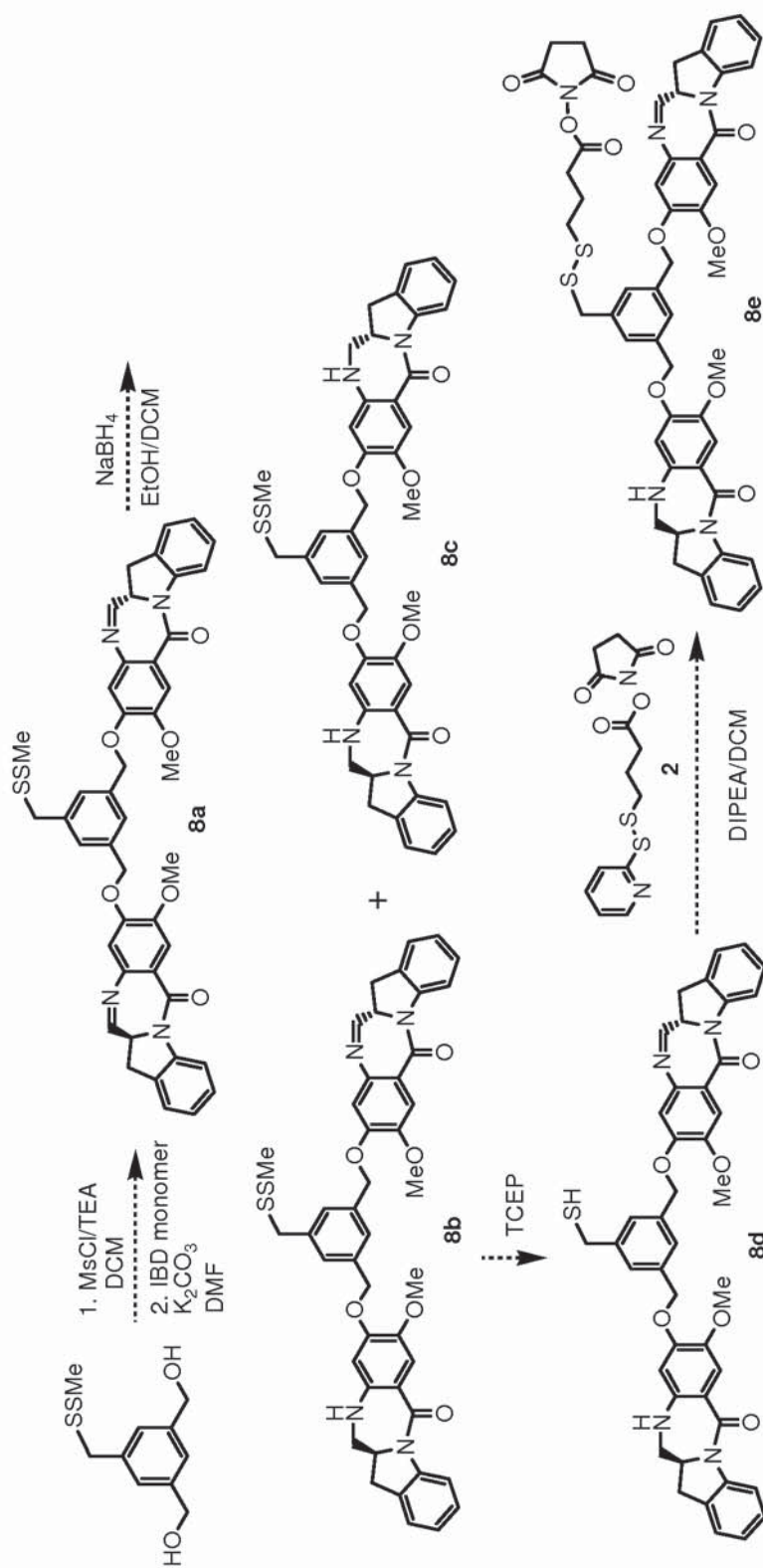
Figure 8. Synthetic Scheme of Thiol-containing Dimers **8b-e**

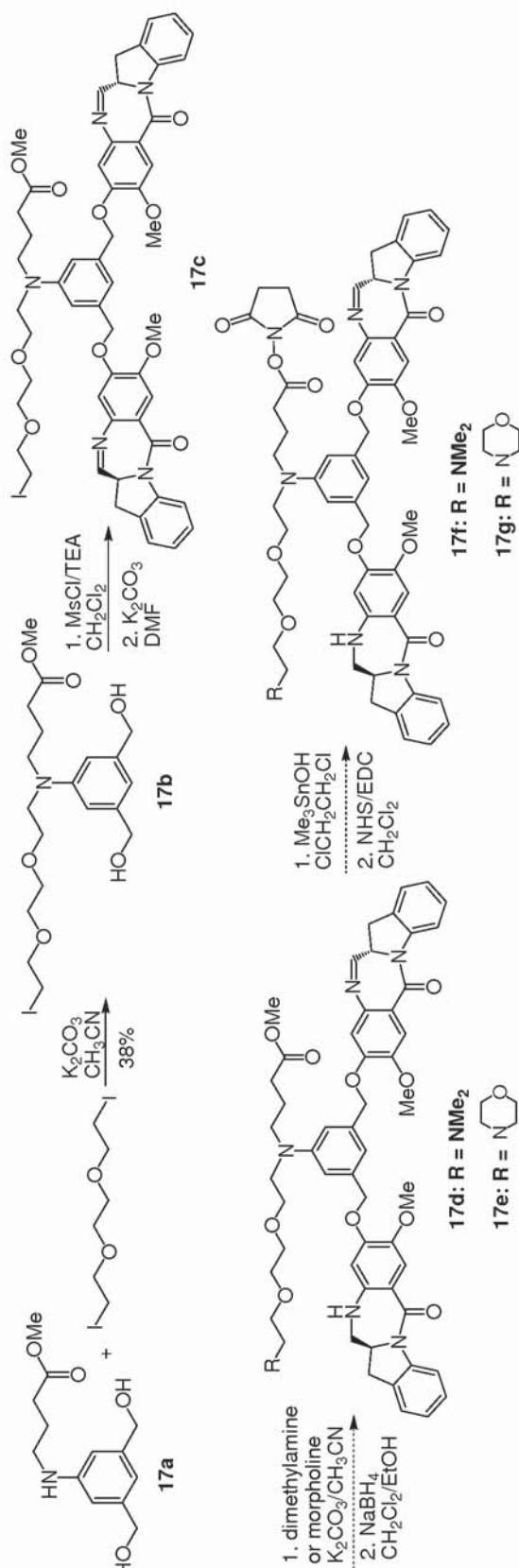
Figure 9. Synthetic Scheme of Dimers **17d-g** Containing a Tertiary Amine

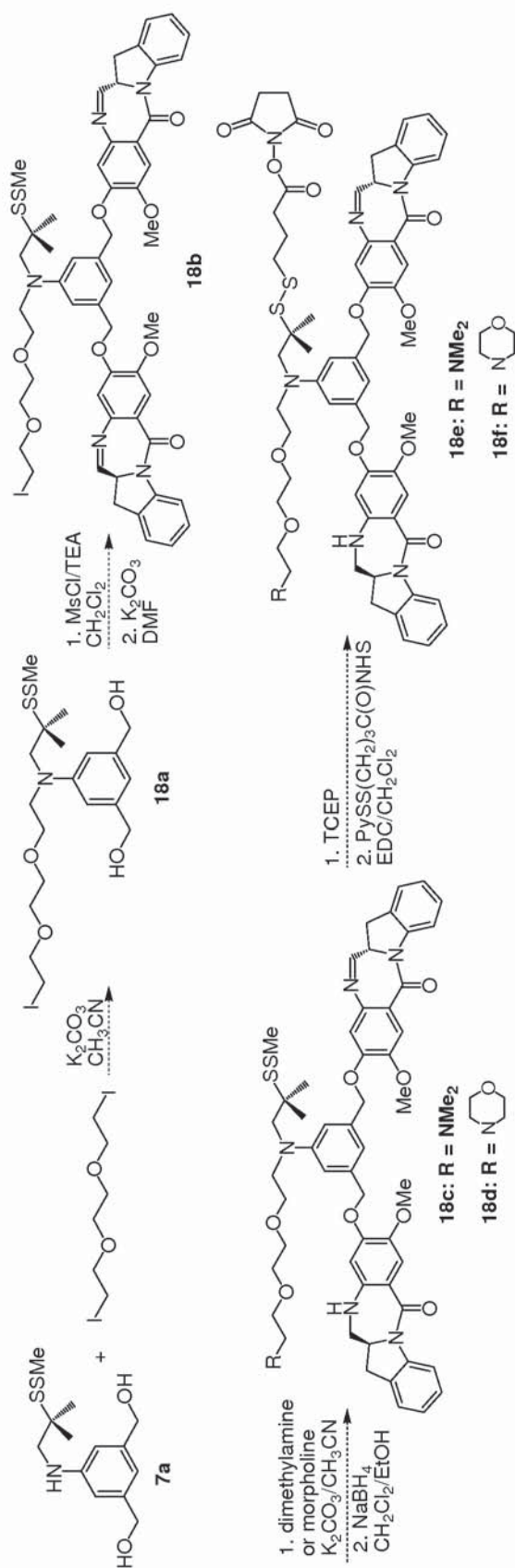
Figure 10. Synthetic Scheme of Dimers **18c-f** Containing a Tertiary Amine

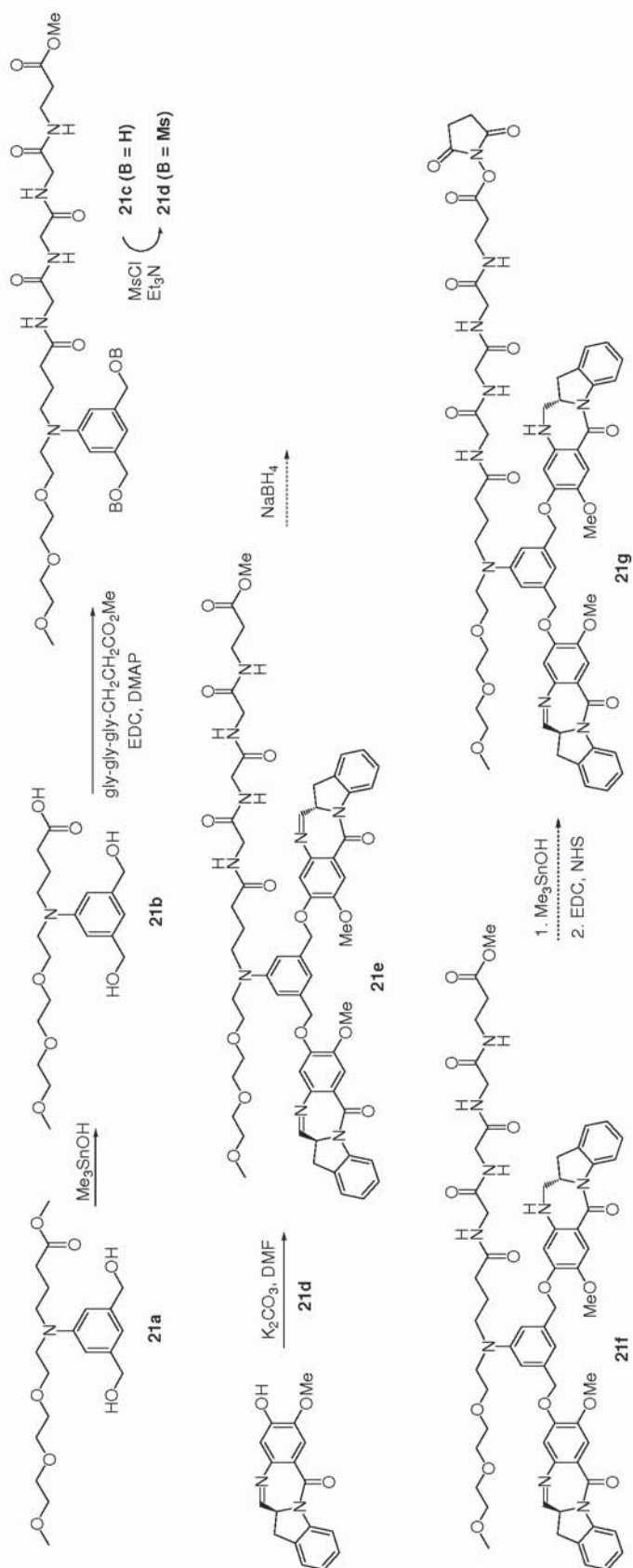
Figure 11. Synthetic Scheme of Dimers **21f-g** Containing a Peptide Linker

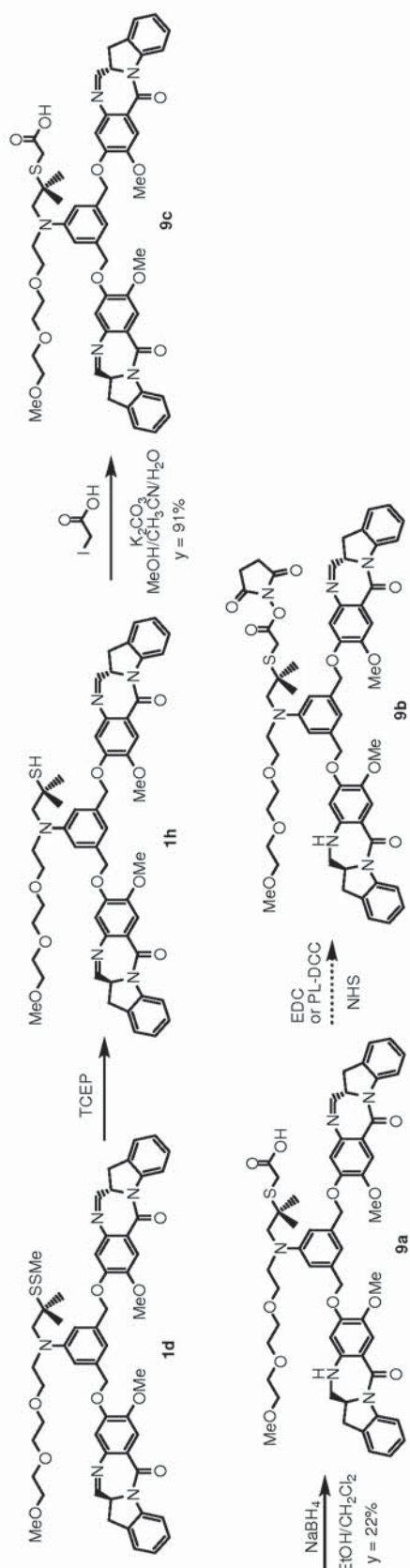
Figure 12A. Synthetic Scheme of the One-step Thioacetyl Linkable Dimers **9a-b**

Figure 12B. Alternative Synthetic Scheme of the One-step Thioacetyl Linkable Dimers **9a-b**

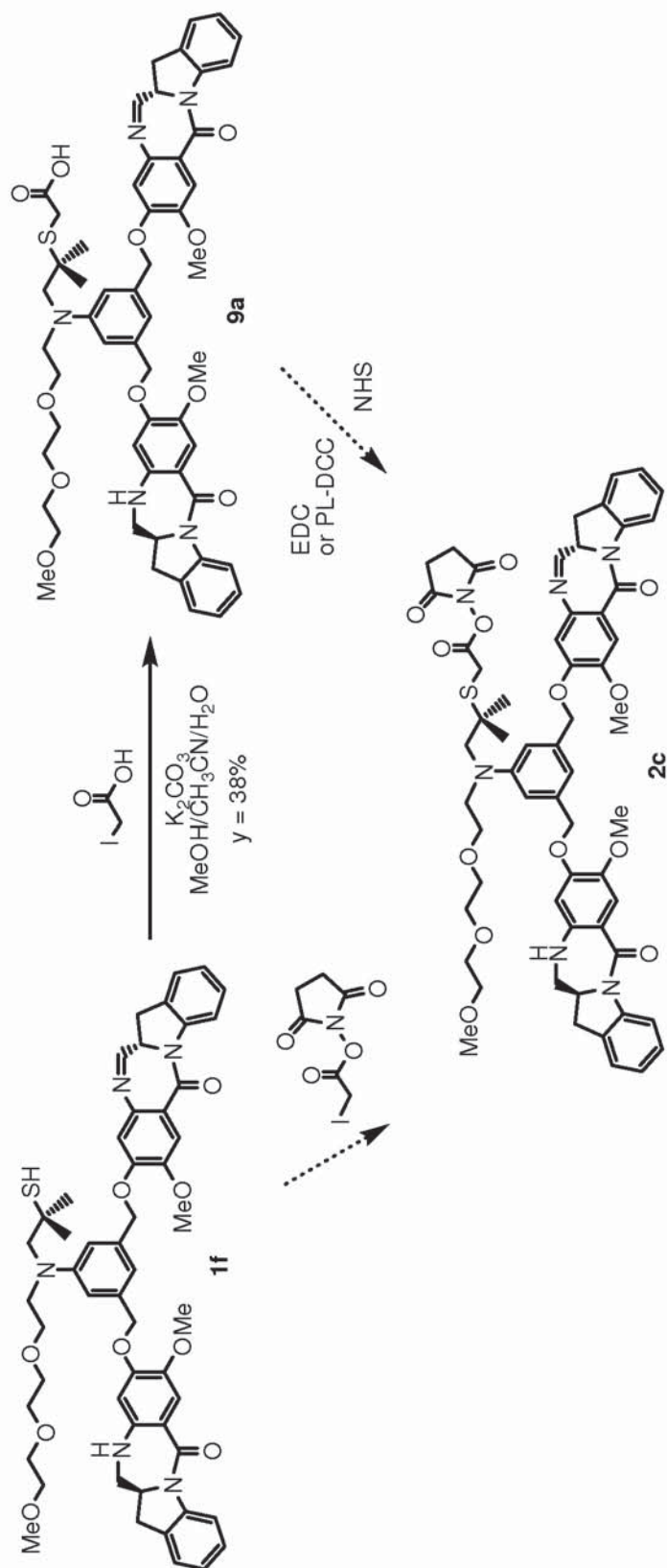


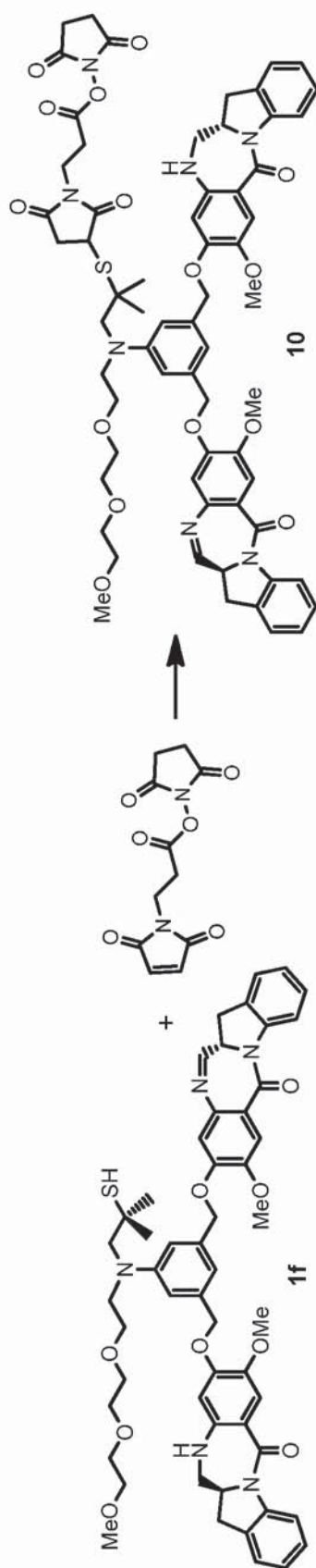
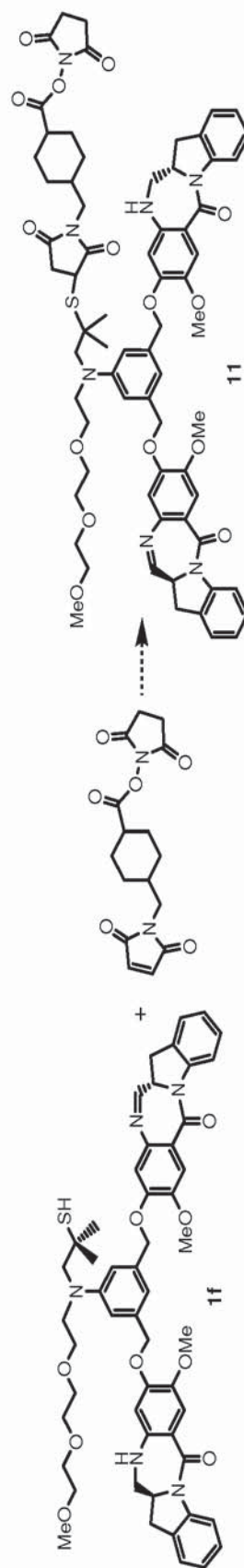
Figure 13. Synthetic Scheme of the One-step BMPS Linkable Dimer **10**Figure 14. Synthetic Scheme of the One-step SMCC Linkable Dimer **11**

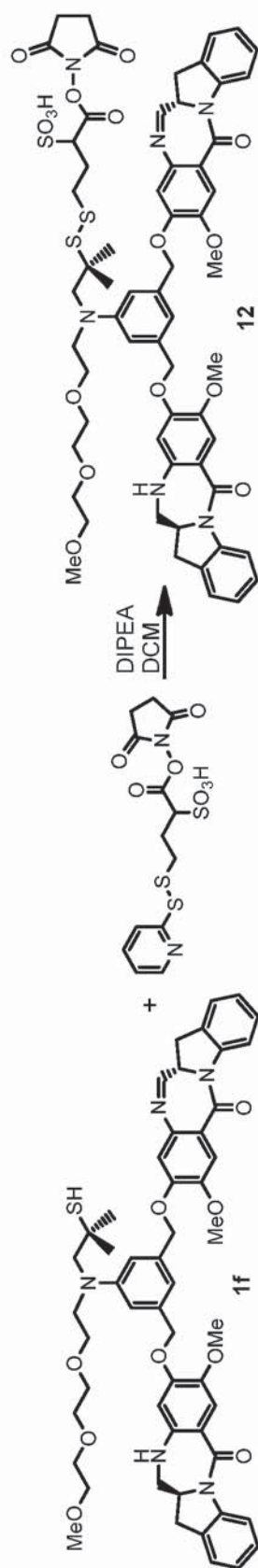
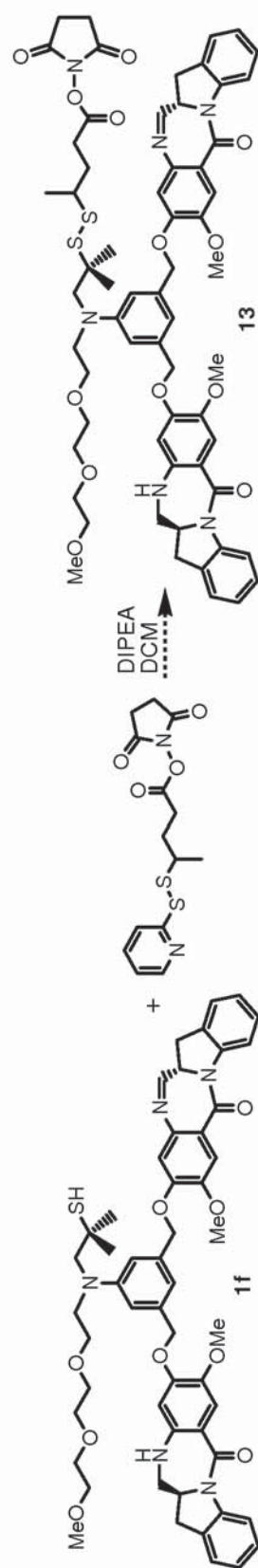
Figure 15. Synthetic Scheme of the One-step Sulfo-SPDB Linkable Dimer **12**Figure 16. Synthetic Scheme of the One-step SPP Linkable Dimer **13**

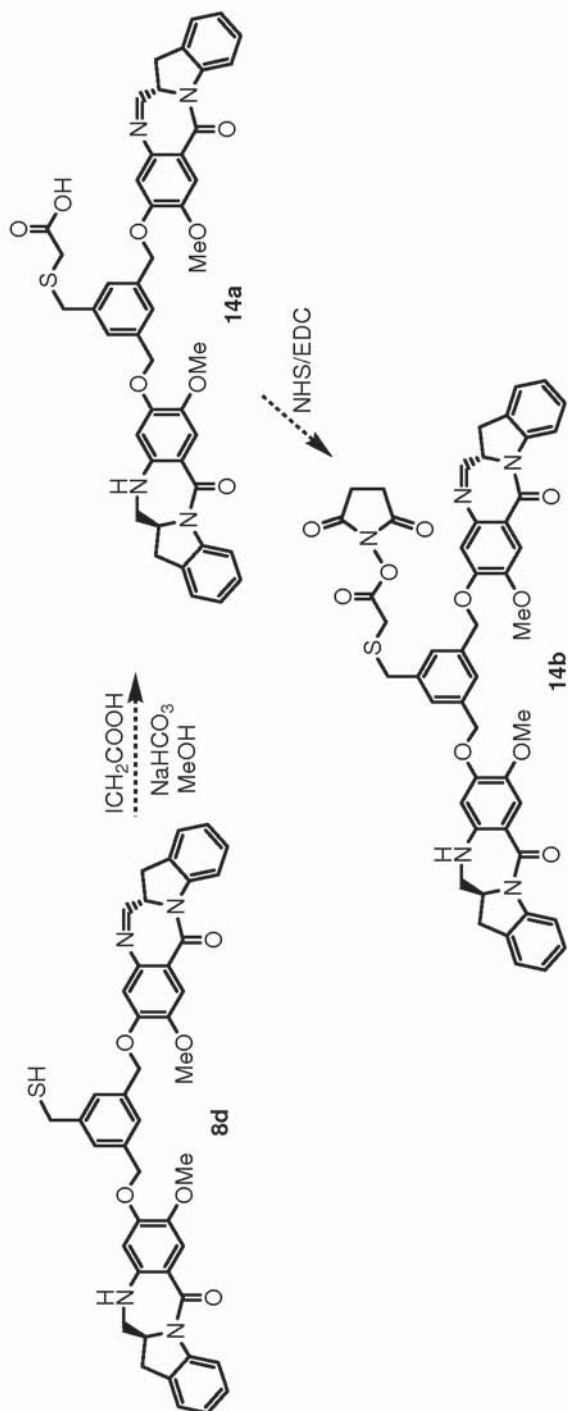
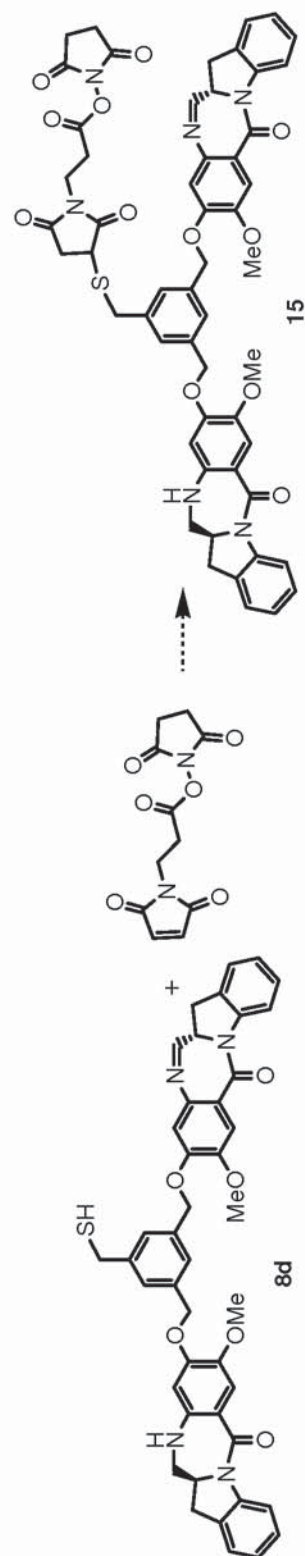
Figure 17. Synthetic Scheme of the One-step Thioacetyl Linkable Dimers **14a-b**Figure 18. Synthetic Scheme of the One-step BMPS Linkable Dimer **15**

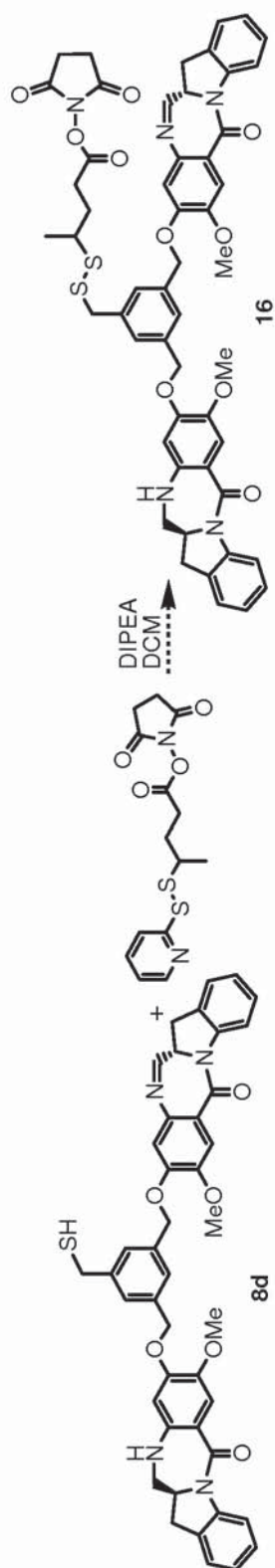
Figure 19. Synthetic Scheme of the One-step SPP Linkable Dimer **16**

Figure 20. Synthetic Scheme for a Two-step Mono-imine Dimer Synthesis

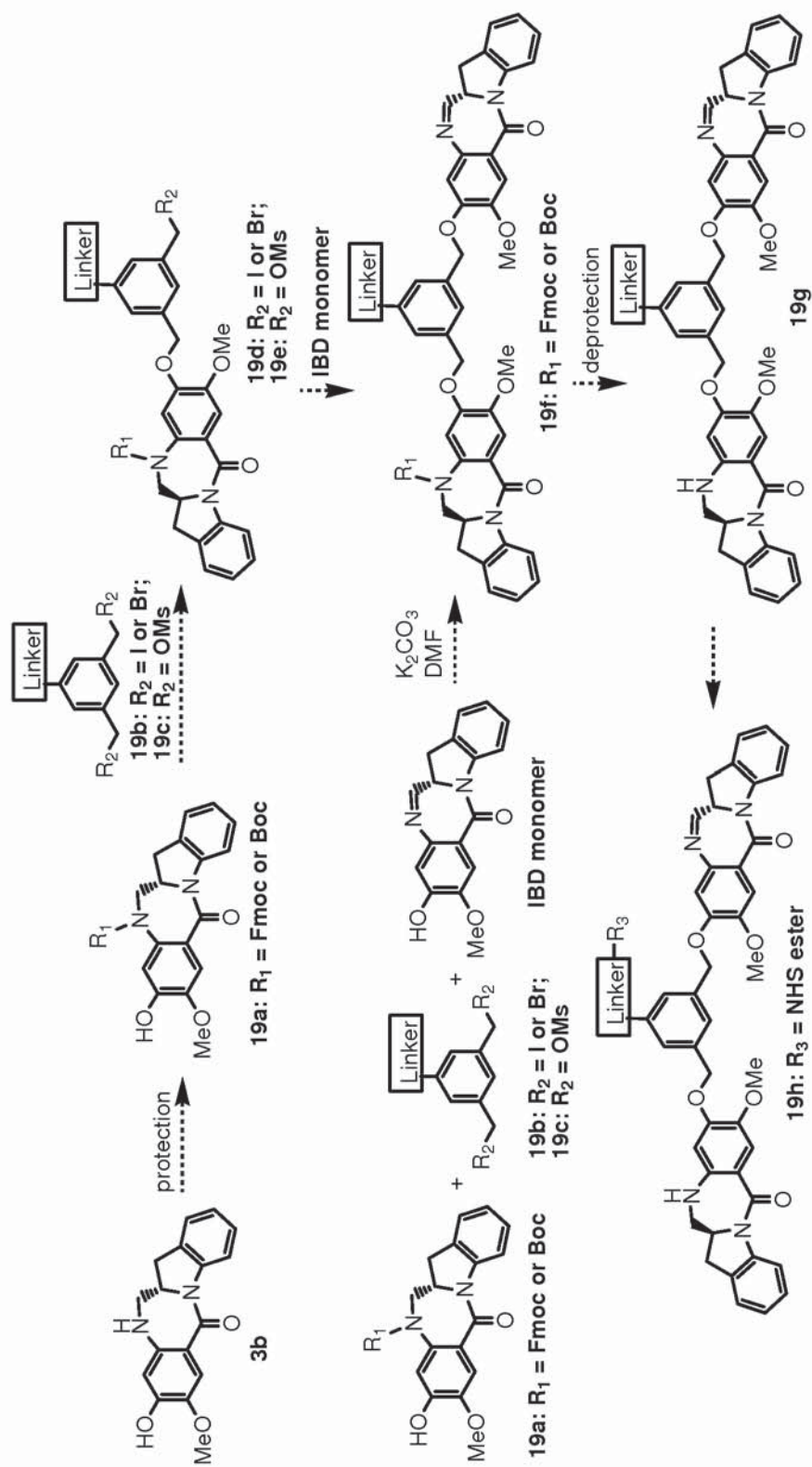


Figure 21. Synthetic Scheme for a Two-step Di-reduced Dimer Synthesis

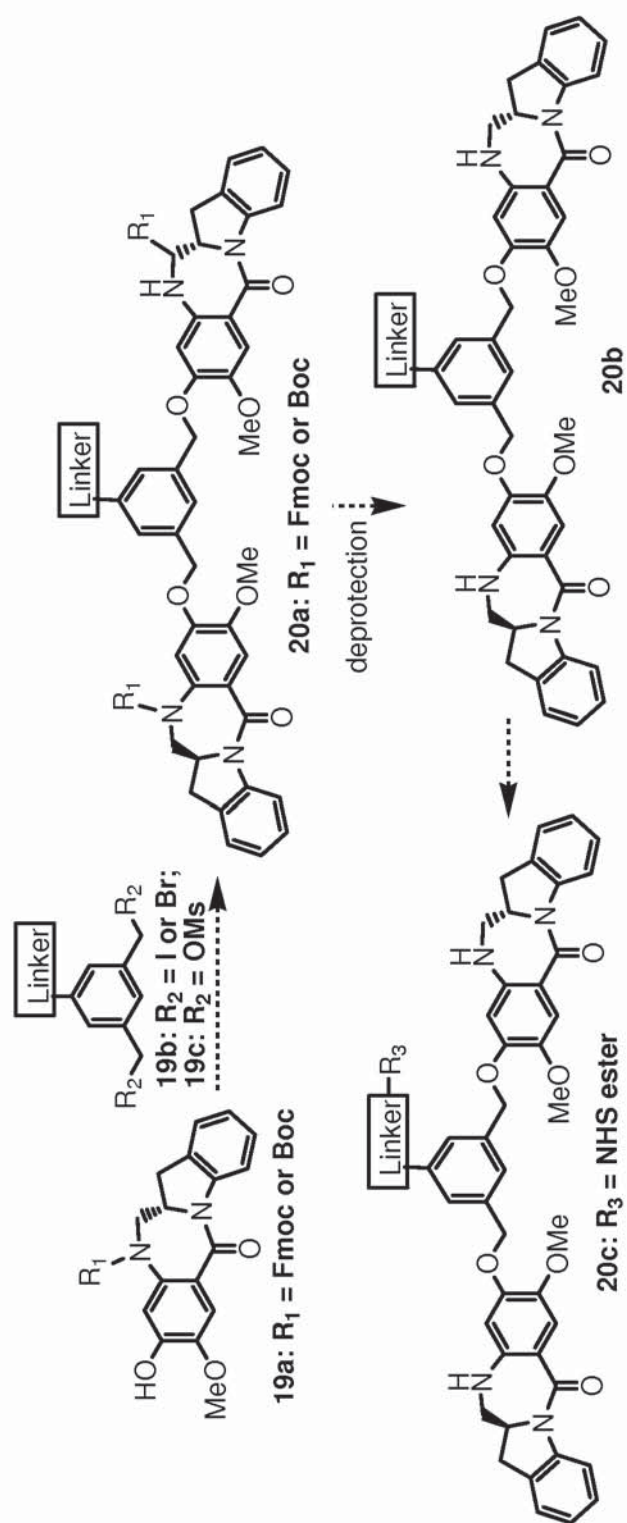


Figure 22. One Step Conjugation Scheme

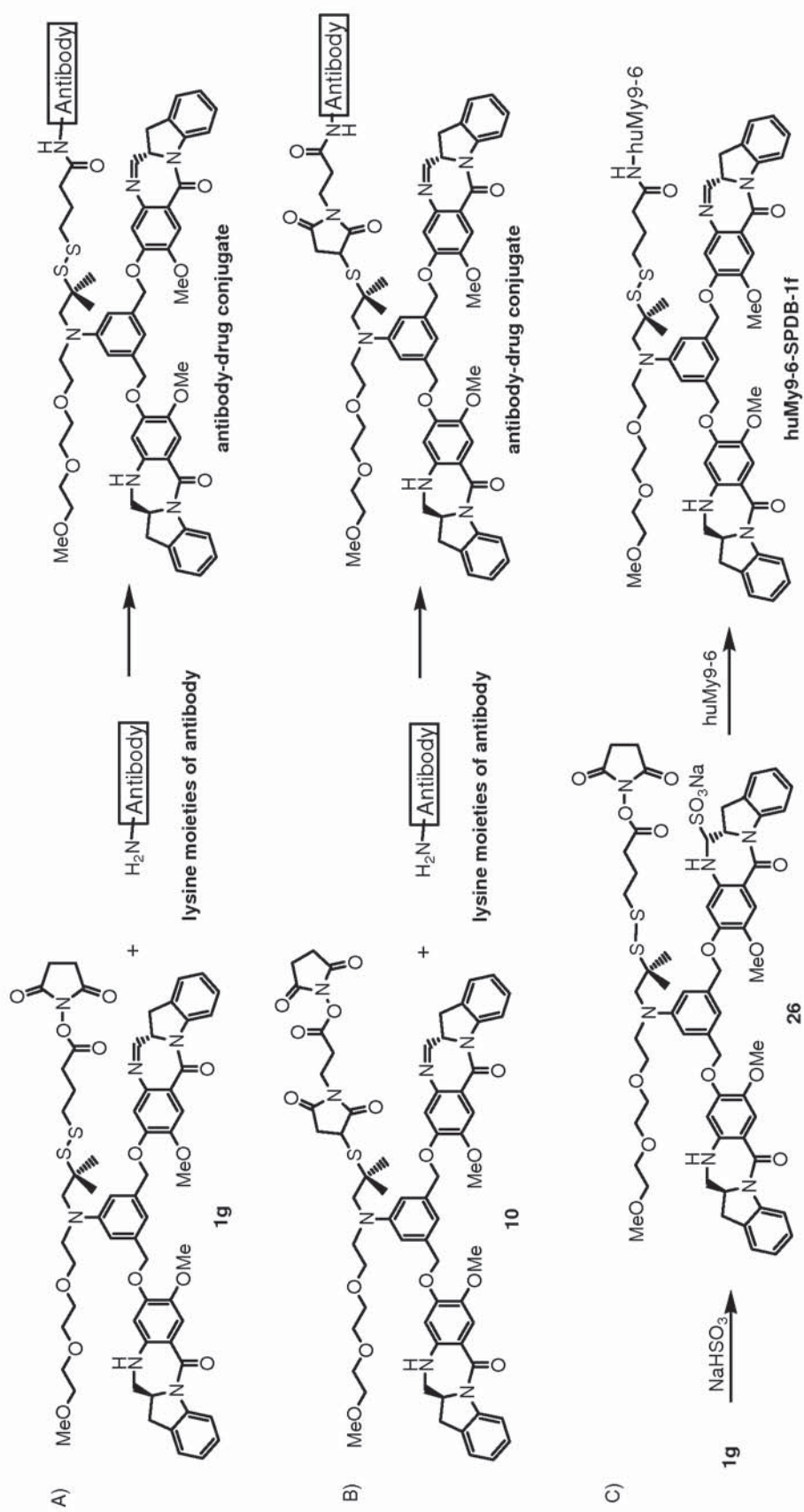


Figure 23. Two-step Conjugation Scheme

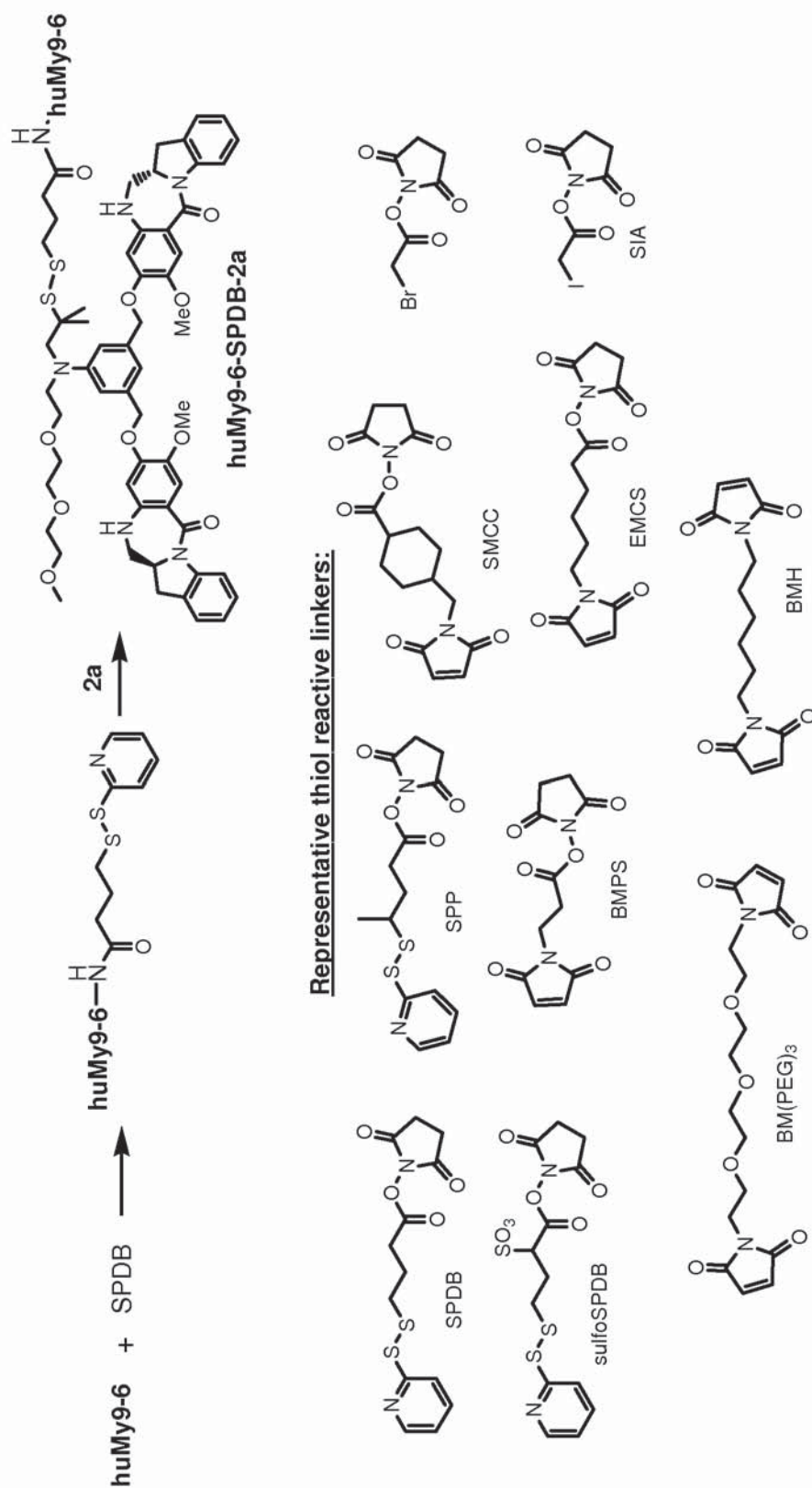


Figure 24. Antiproliferative Activity of Methylthio Dimer **1d** against (A) Namalwa cells, (B) HL60/QC cells, and (C) KB cells

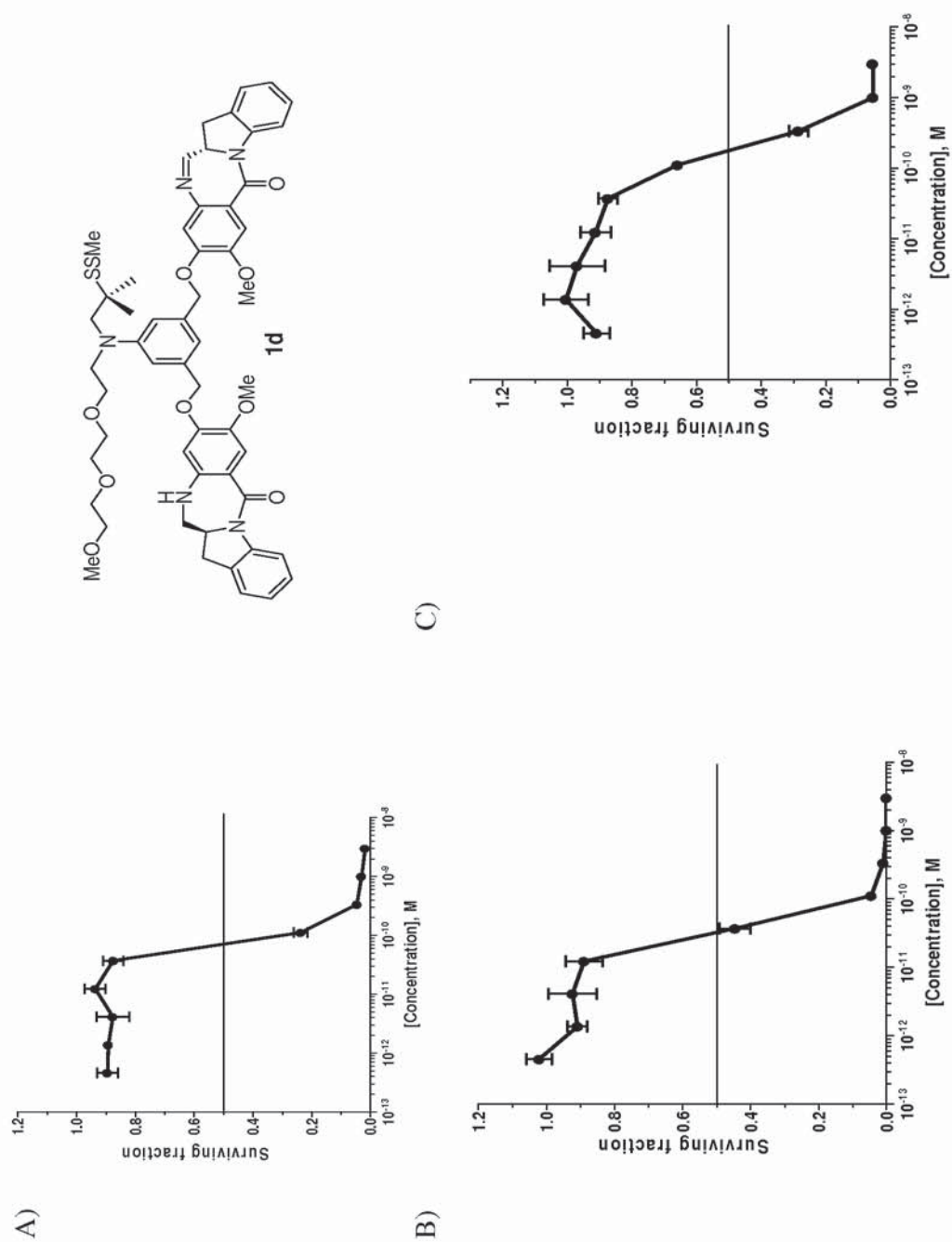


Figure 25. Antiproliferative Activity of huMY9-6-SPDB-1f against (A) HL60/QC (Ag^+) cells with and without blocking of antigen binding sites, (B) HL60/ATCC (Ag^+) cells, and (C) NB-4 (Ag^+) cells

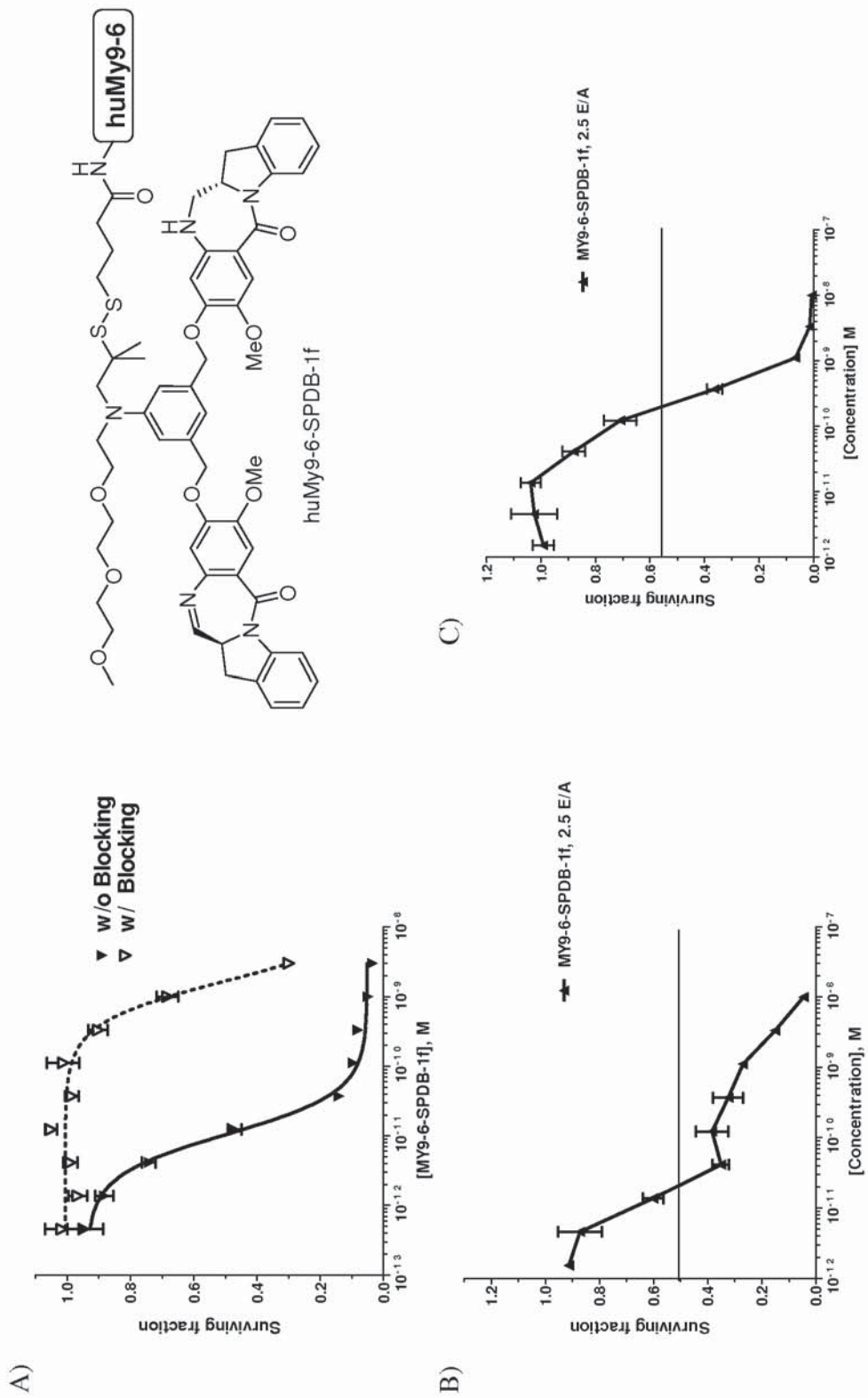


Figure 26. Antiproliferative Activity of huFOLR1-SPDB-1f against KB (Ag^+) cells with and without blocking of antigen binding sites

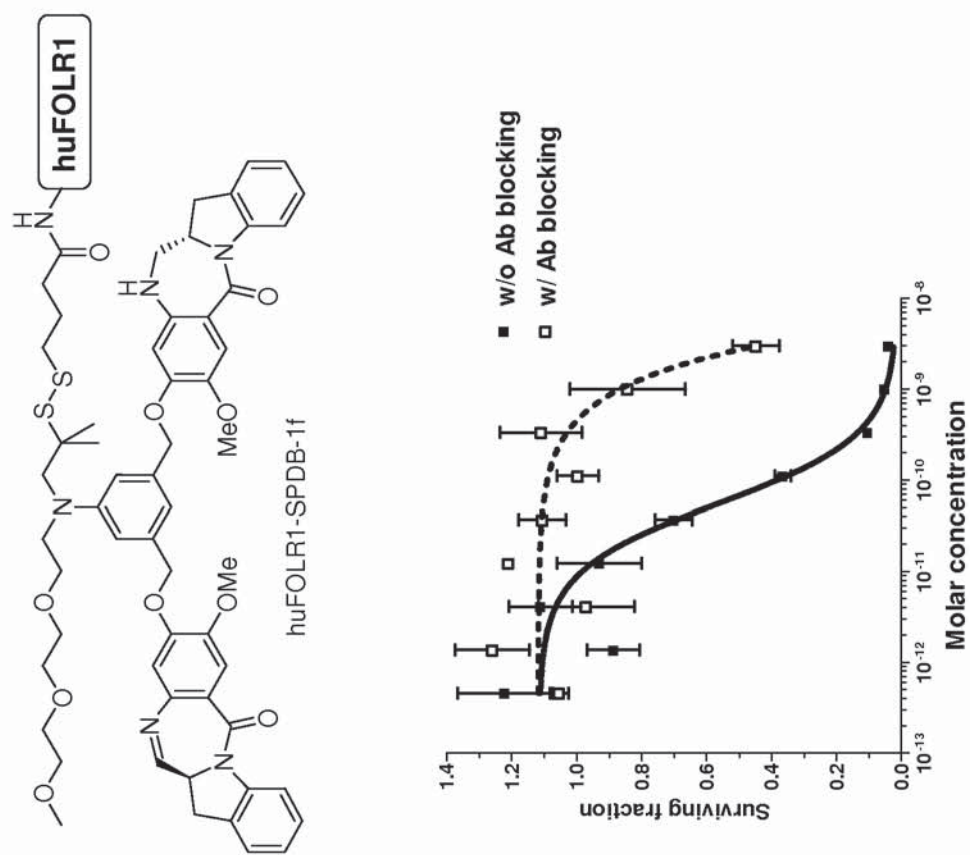


Figure 27. Binding Affinity of huMY9-6-SPDB-1f (FACS analysis)

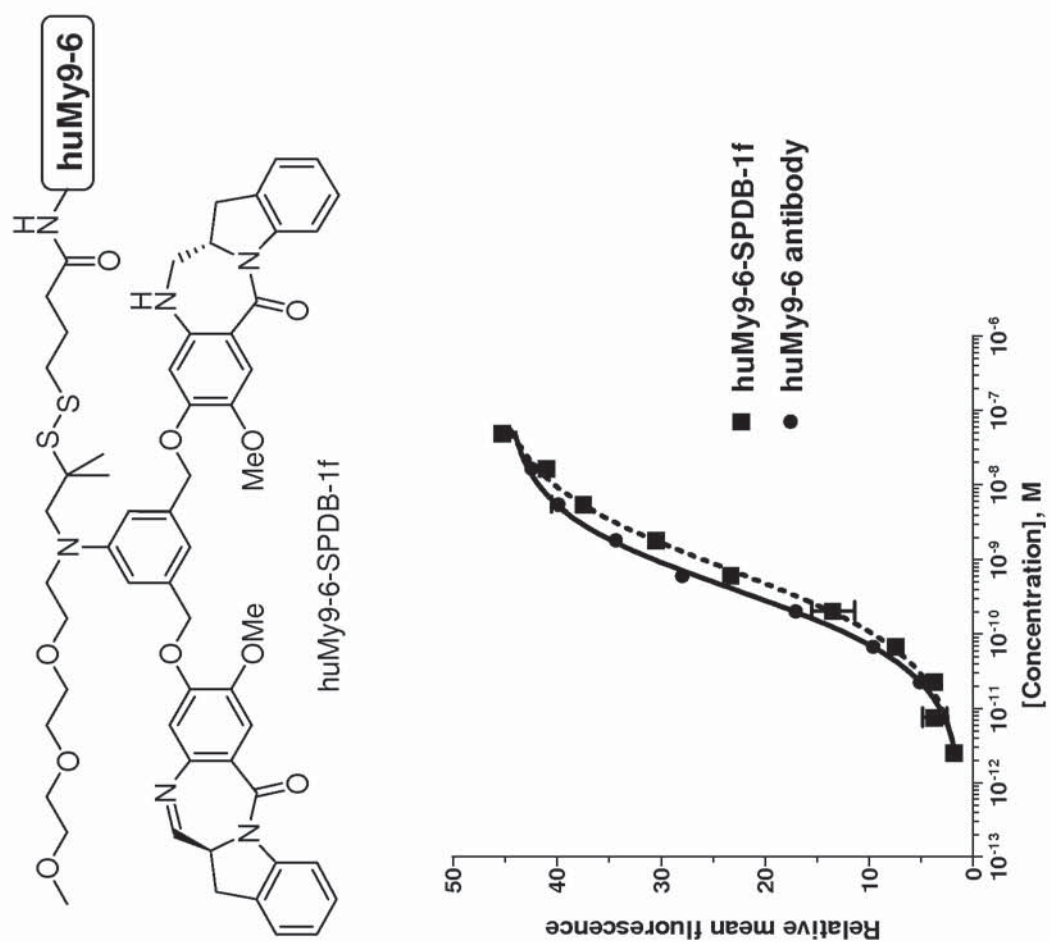


Figure 28. *In vivo* Efficacy of huMy9-6-SPDB-1f in HL60/QC Tumor Bearing Nude Mice

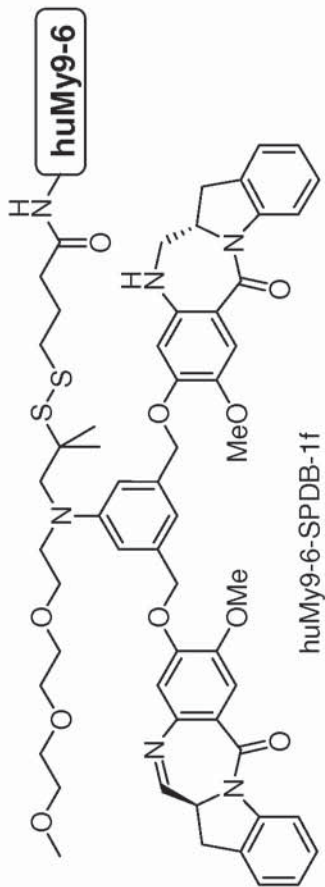
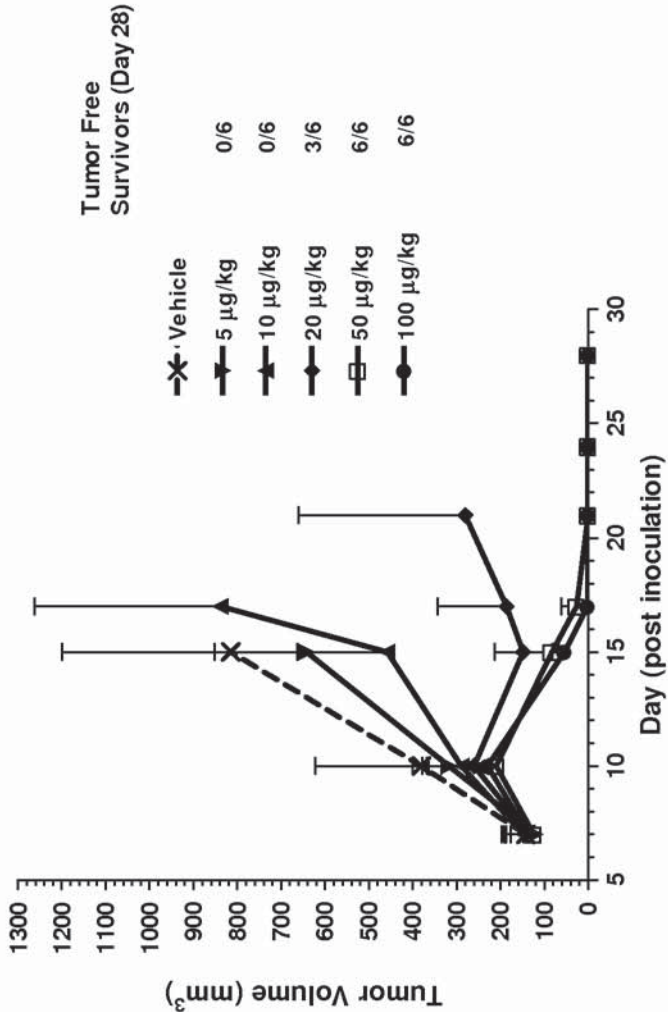


Figure 29. Antiproliferative Activity of huMY9-6-SPDB-1f against Antigen Positive Cell Line (A) HL60/QC cells, (B) HL60/ATCC cells, (C) NB-4 cells, and HEL 92.1.7 cells (D)

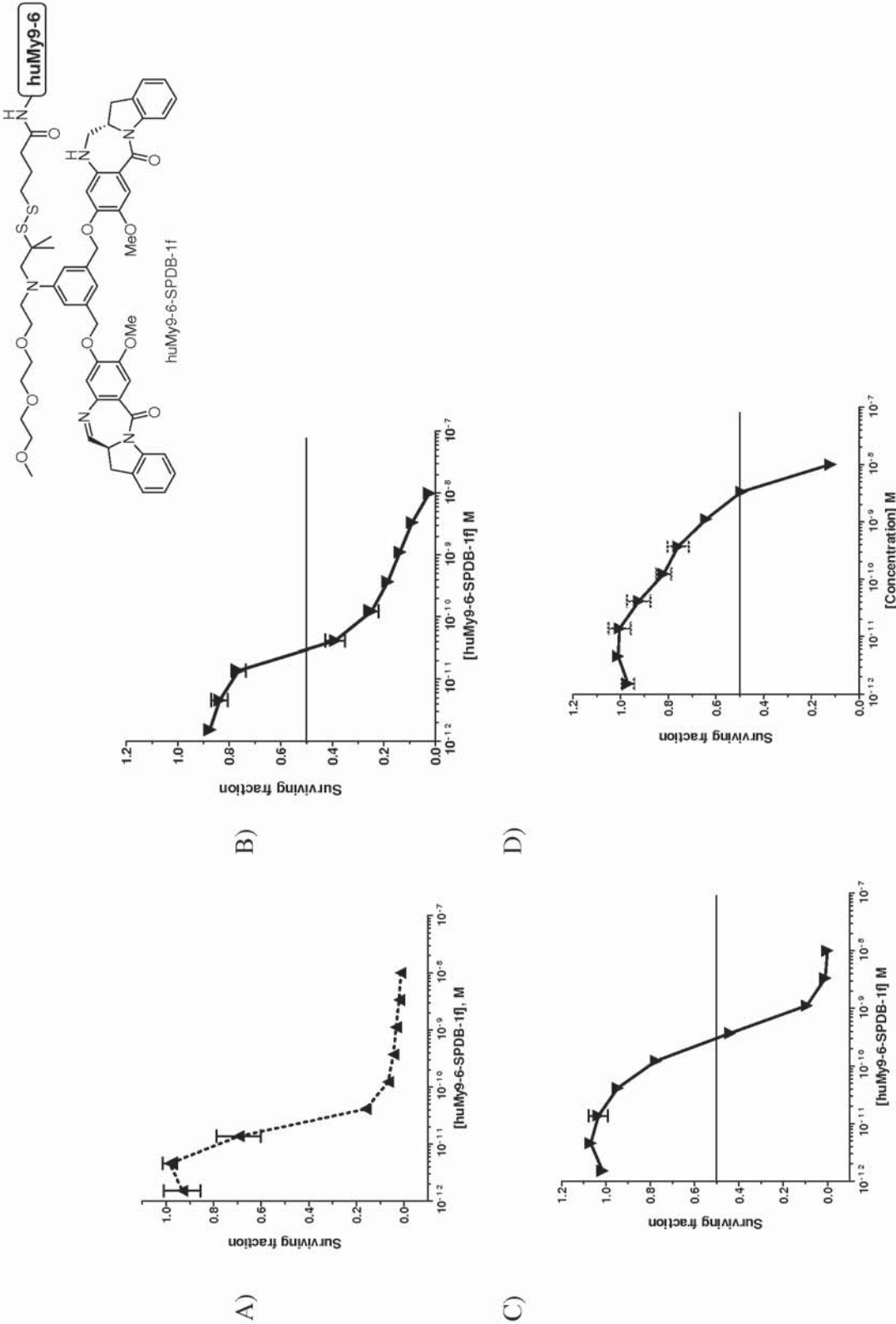


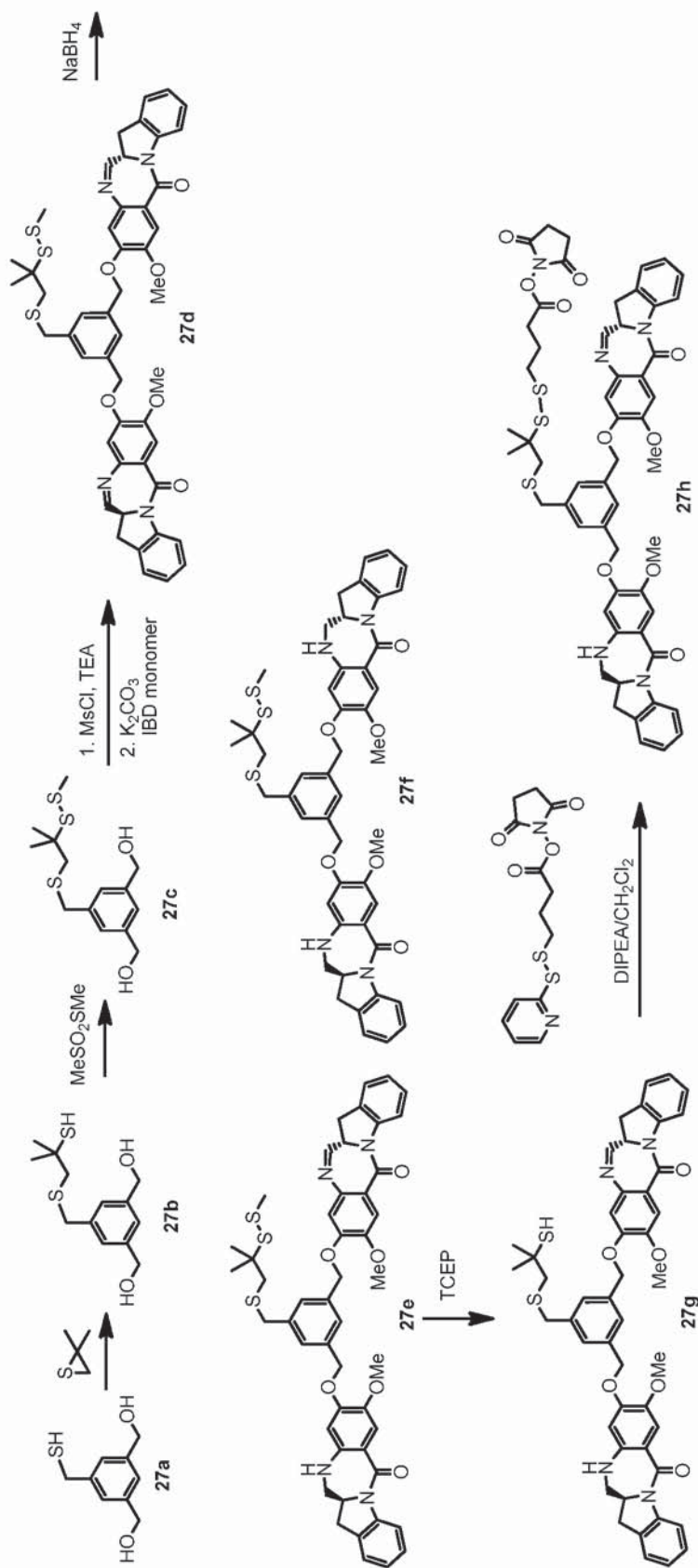
Figure 30. Synthetic Scheme for Thioether-containing Linker Disulfides **27e-h**.

Figure 31. Synthetic Scheme of Dimers 28c-f

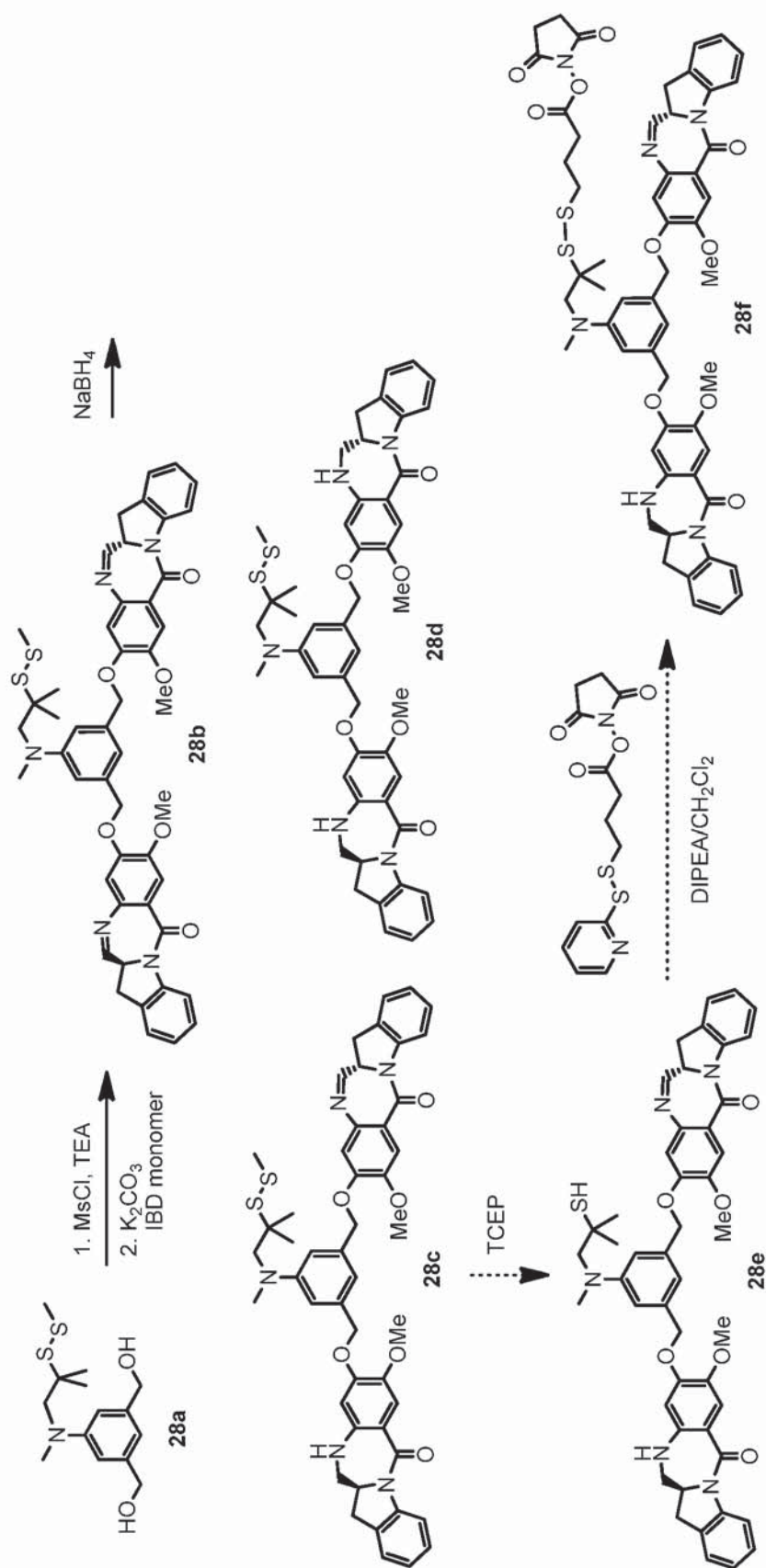


Figure 32. Synthetic Scheme for Phenyl Linked Dimers **29b-c**

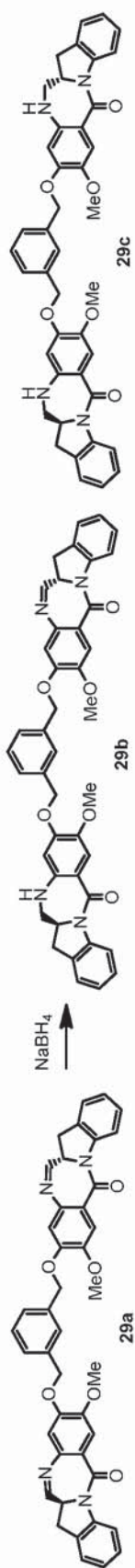


Figure 33. Alternate Two-step Mono-imine Dimer Synthesis

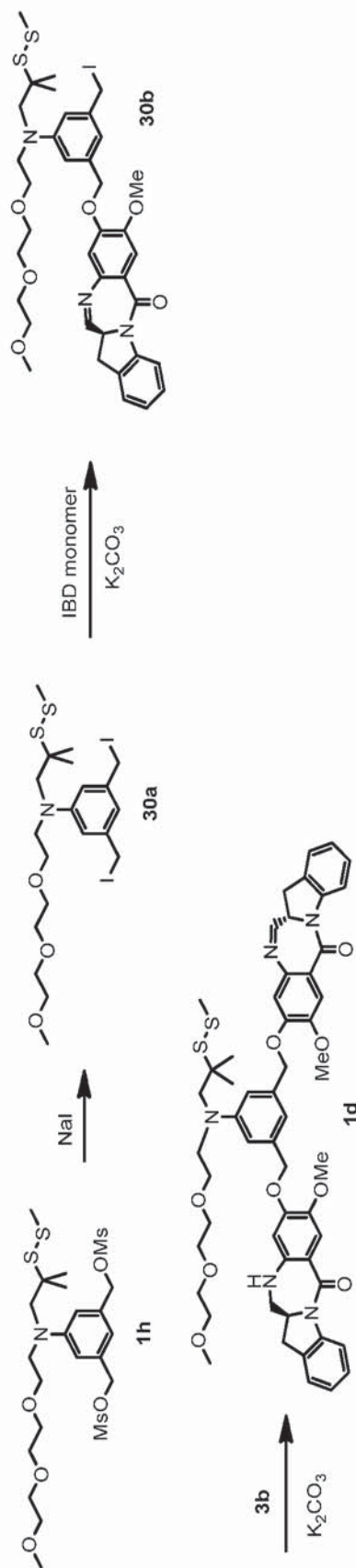


Figure 34. Antiproliferative Activity Comparing (A) huMy9-6-SPDB-1f and (B) huMy9-6-sulfSPDB-1f, and (C) huMy9-6-BMPS-1f against HL60/QC (Ag⁺) Cells with and without Blocking of Antigen Binding Sites

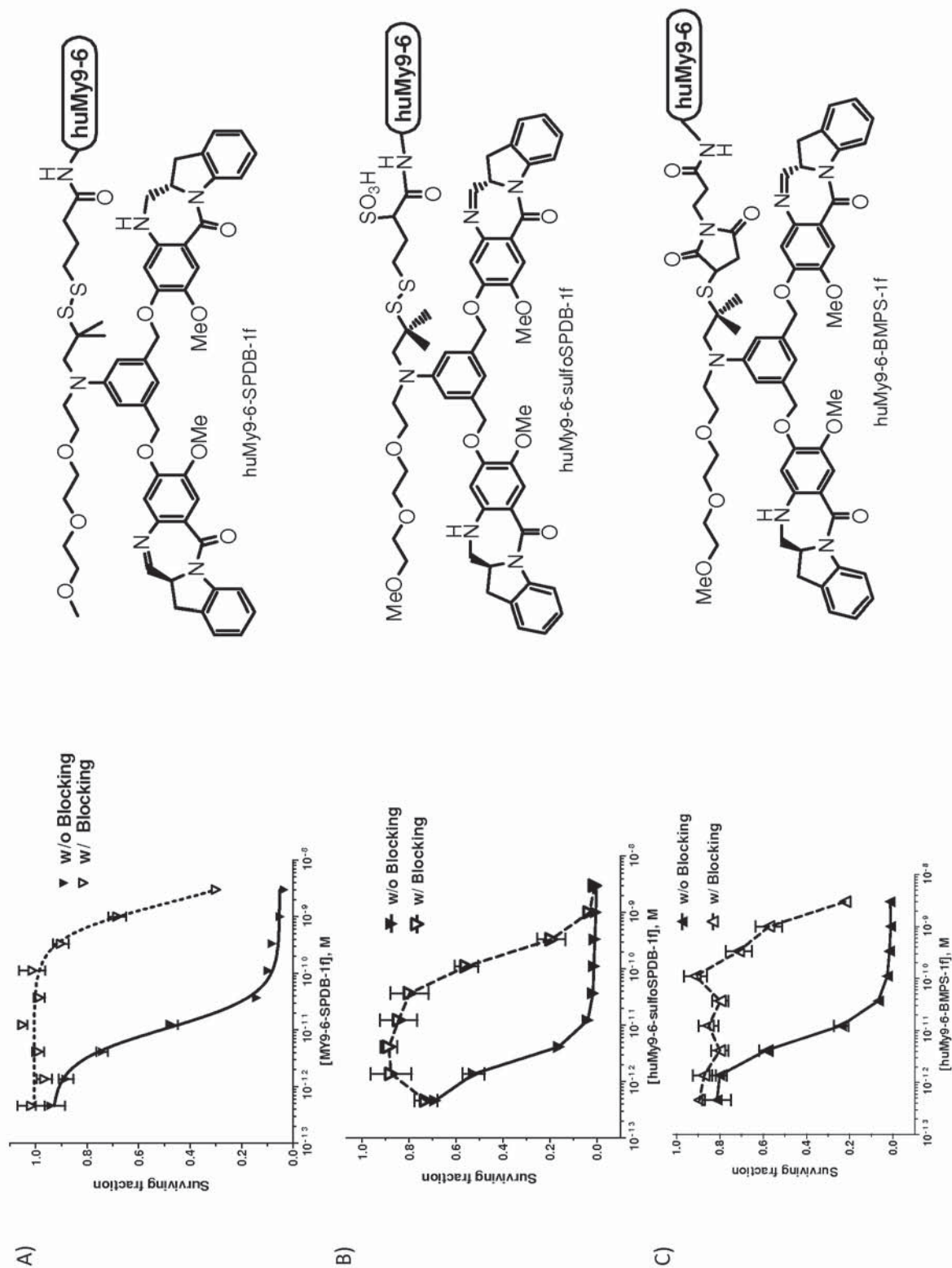


Figure 35. Antiproliferative Activity Comparing (A) chB38.1-SPDB-1f and (B) chB38.1-sulfoSPDB-1f against COLO205 (Ag⁺) cells

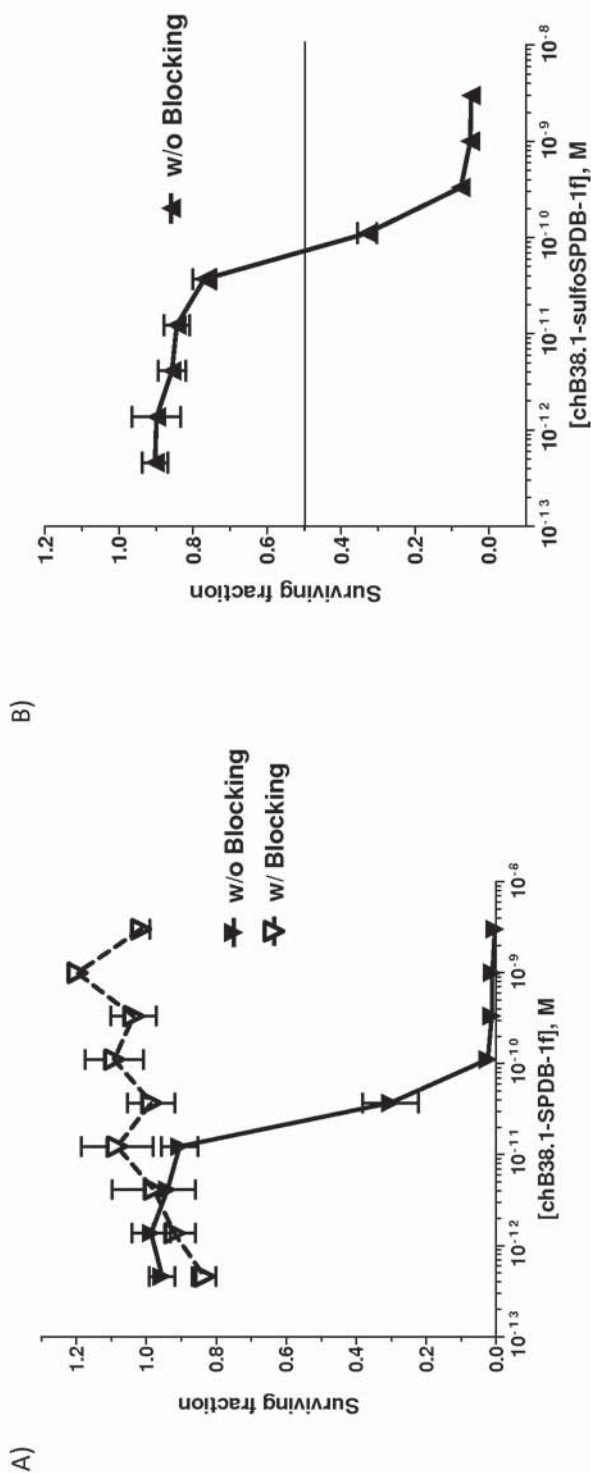


Figure 36. *In vivo* Efficacy of huMy9-6-SPDB-1f in HL60/QC Tumor Bearing Mice

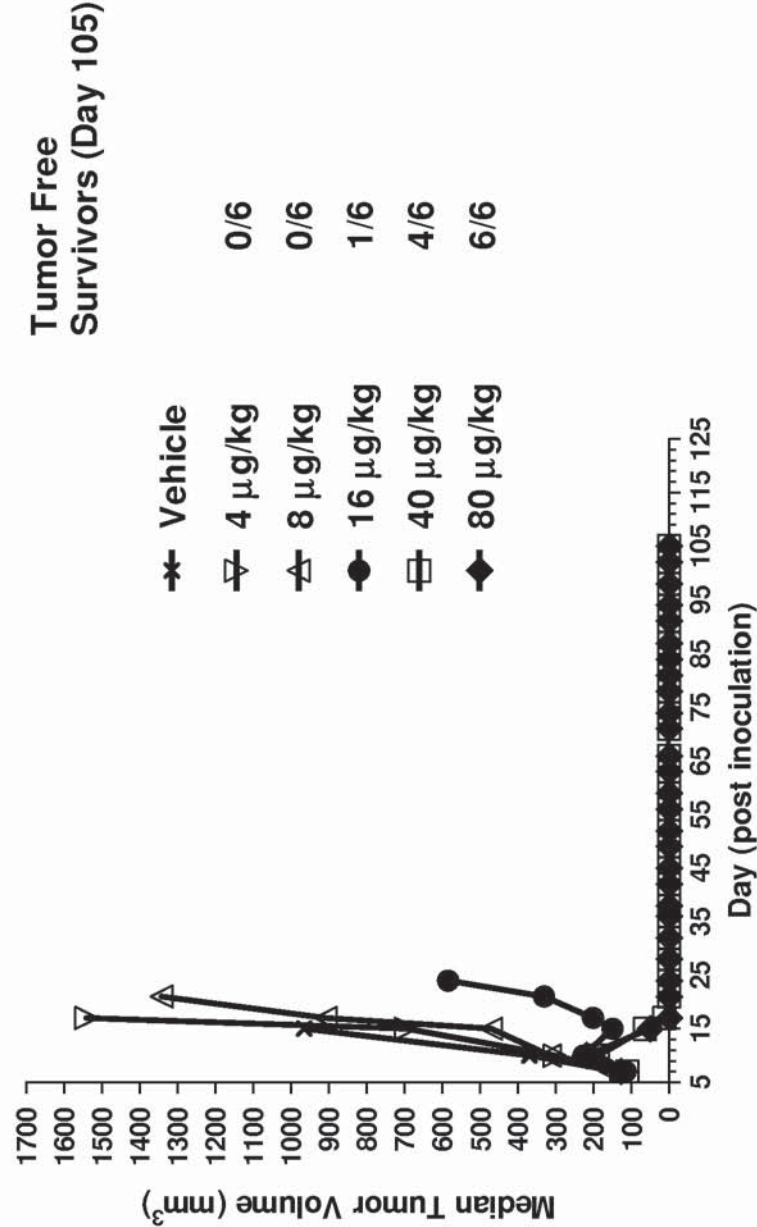


Figure 37. *In vivo* Efficacy of huFOLR1-SPDB-1f in KB Tumor Bearing Mice

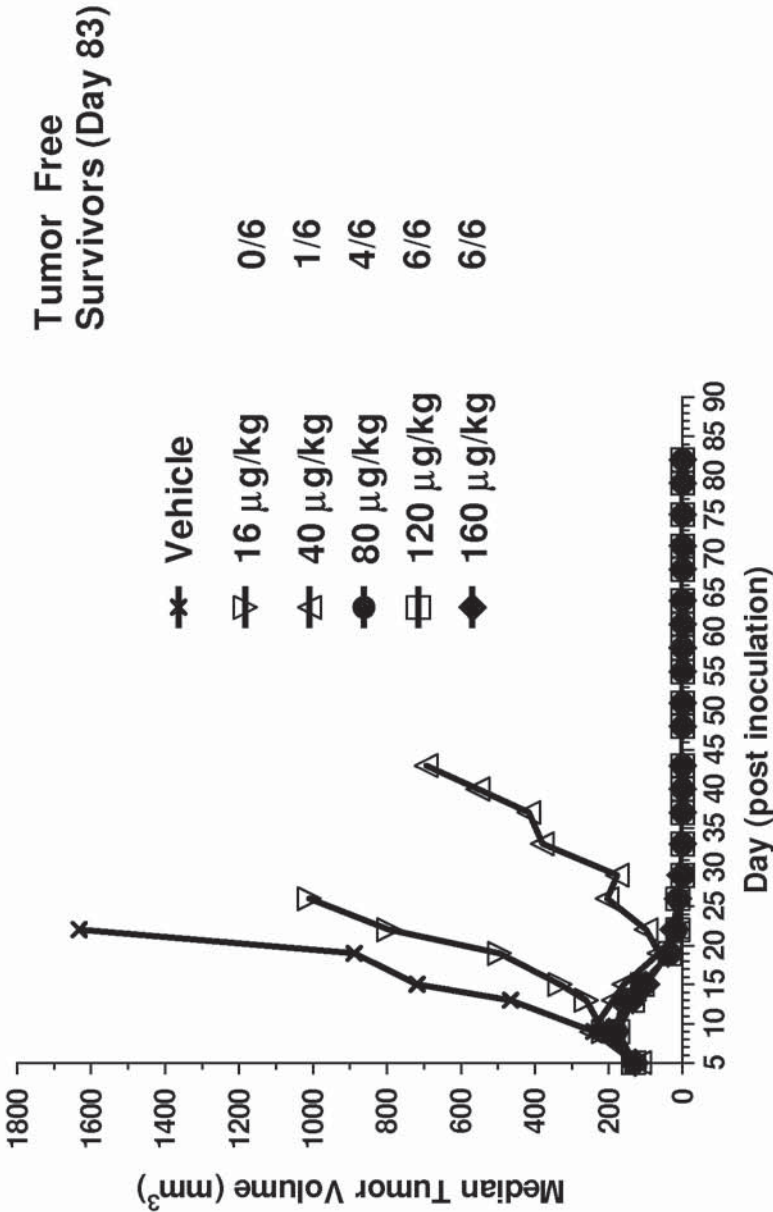


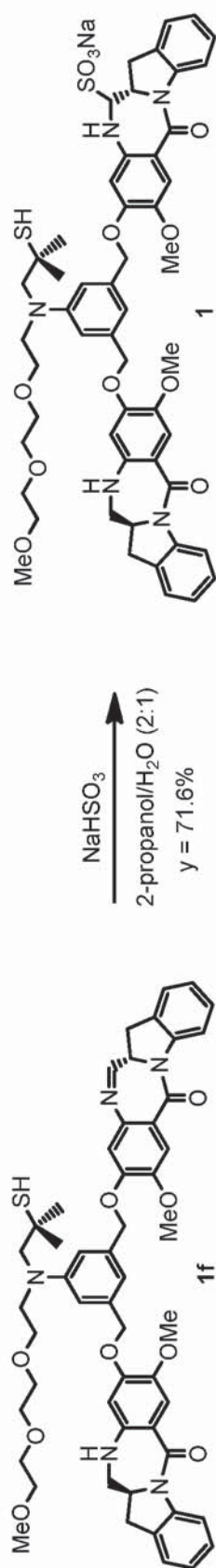
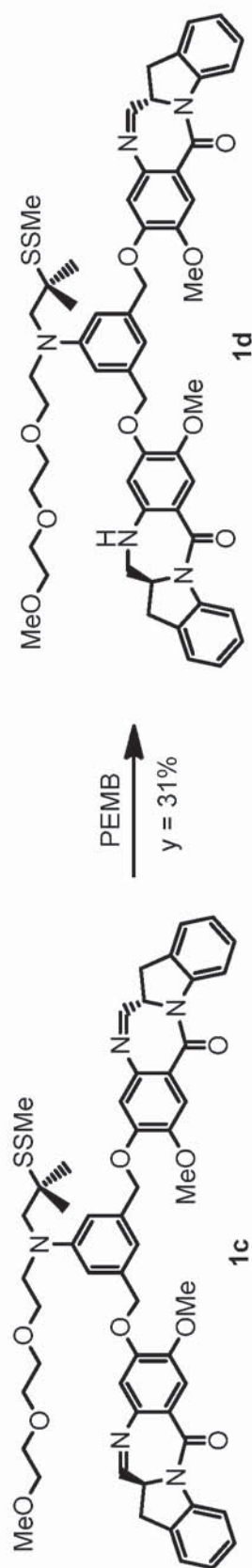
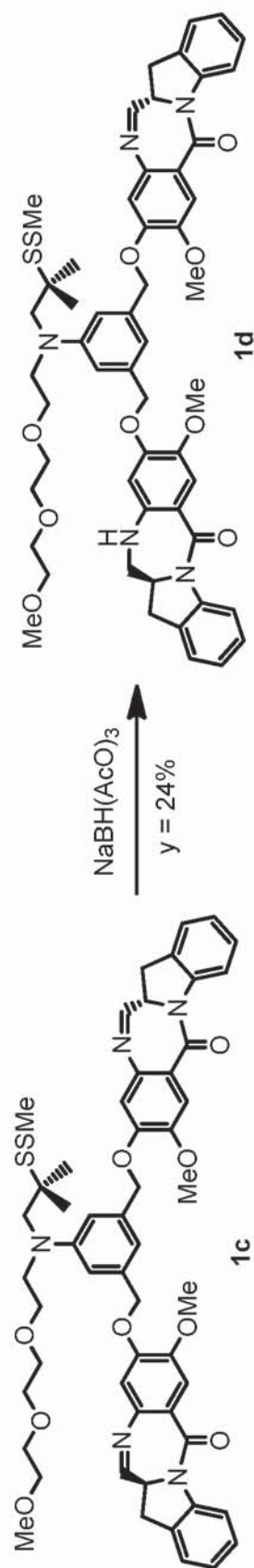
Figure 38. Synthetic Scheme of Compound **1**Figure 39. Synthetic Scheme of Compound **1d** with 5-ethyl-2-methylpyridine borane (PEMB)Figure 40. Synthetic Scheme of Compound **1d** with sodium triacetoxyborohydride (STAB)

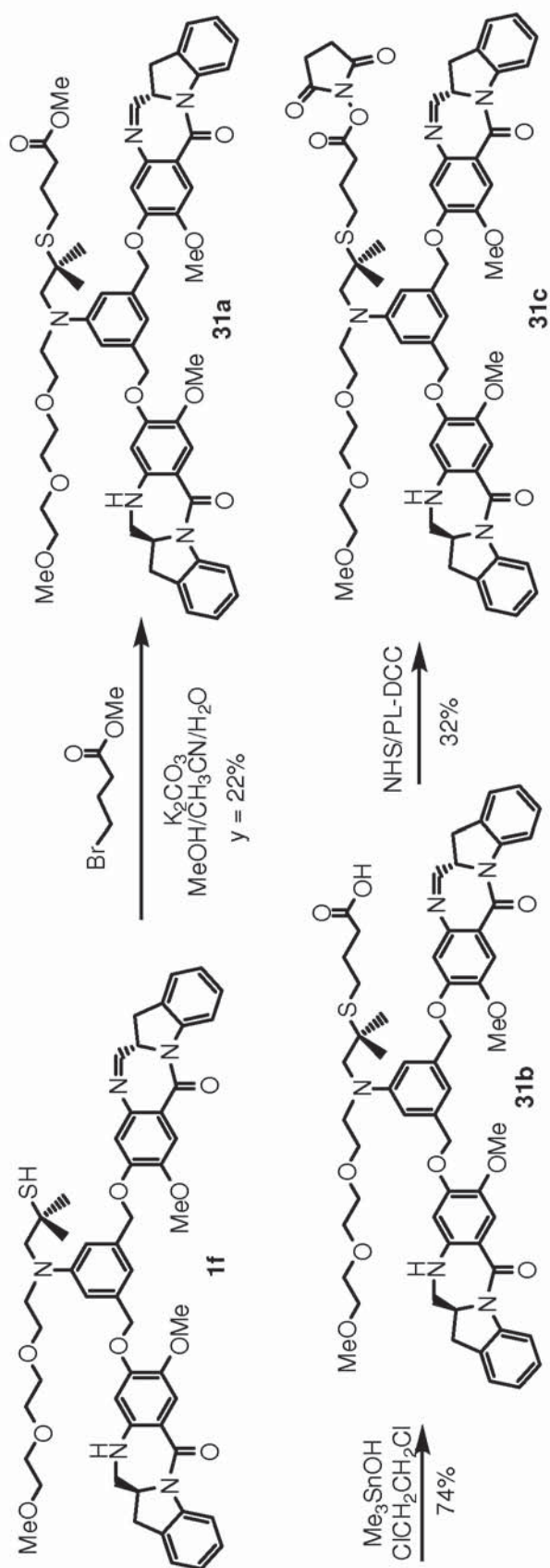
Figure 41. Synthetic Scheme of Compound **31a-c**.

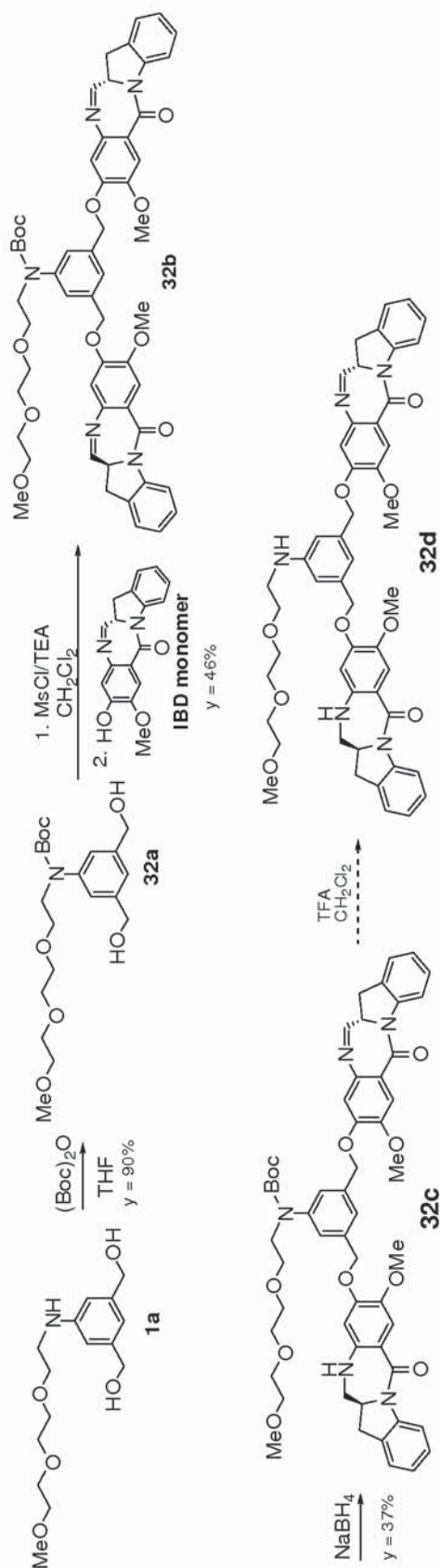
Figure 42. Synthetic Scheme of Compound **32d**

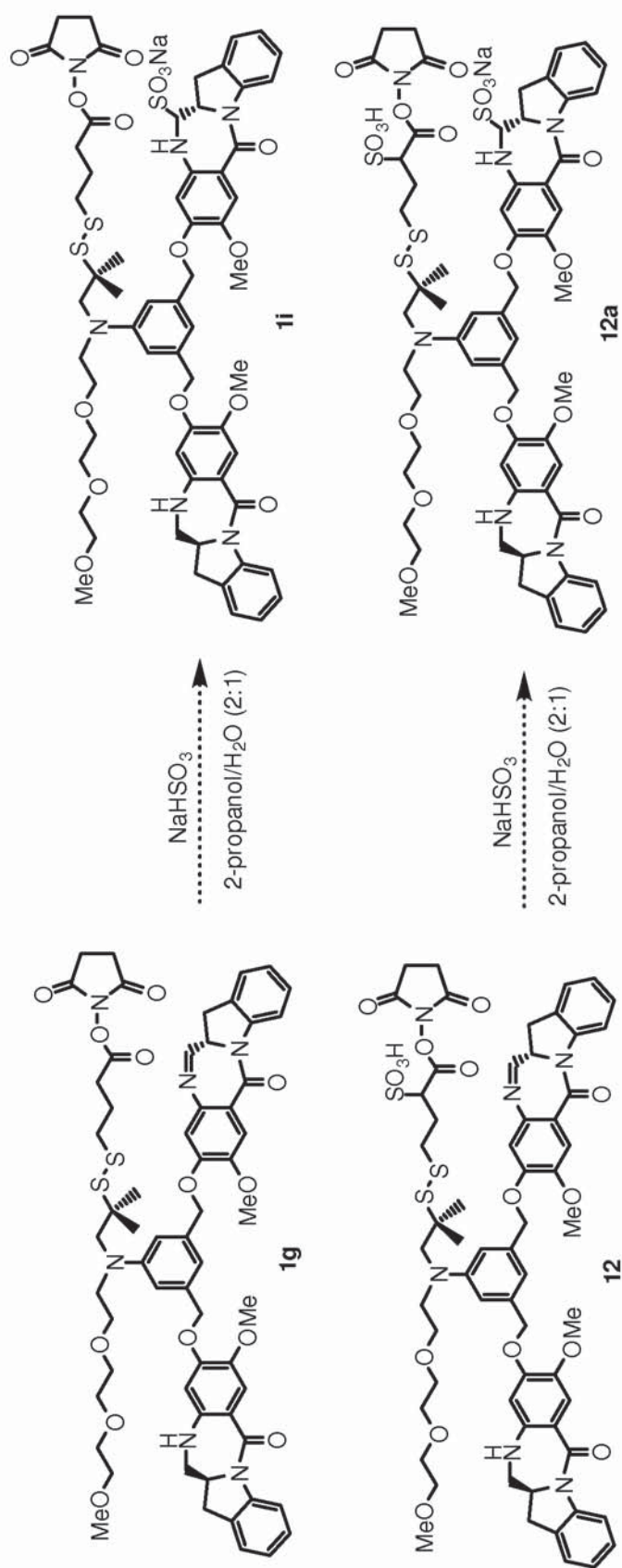
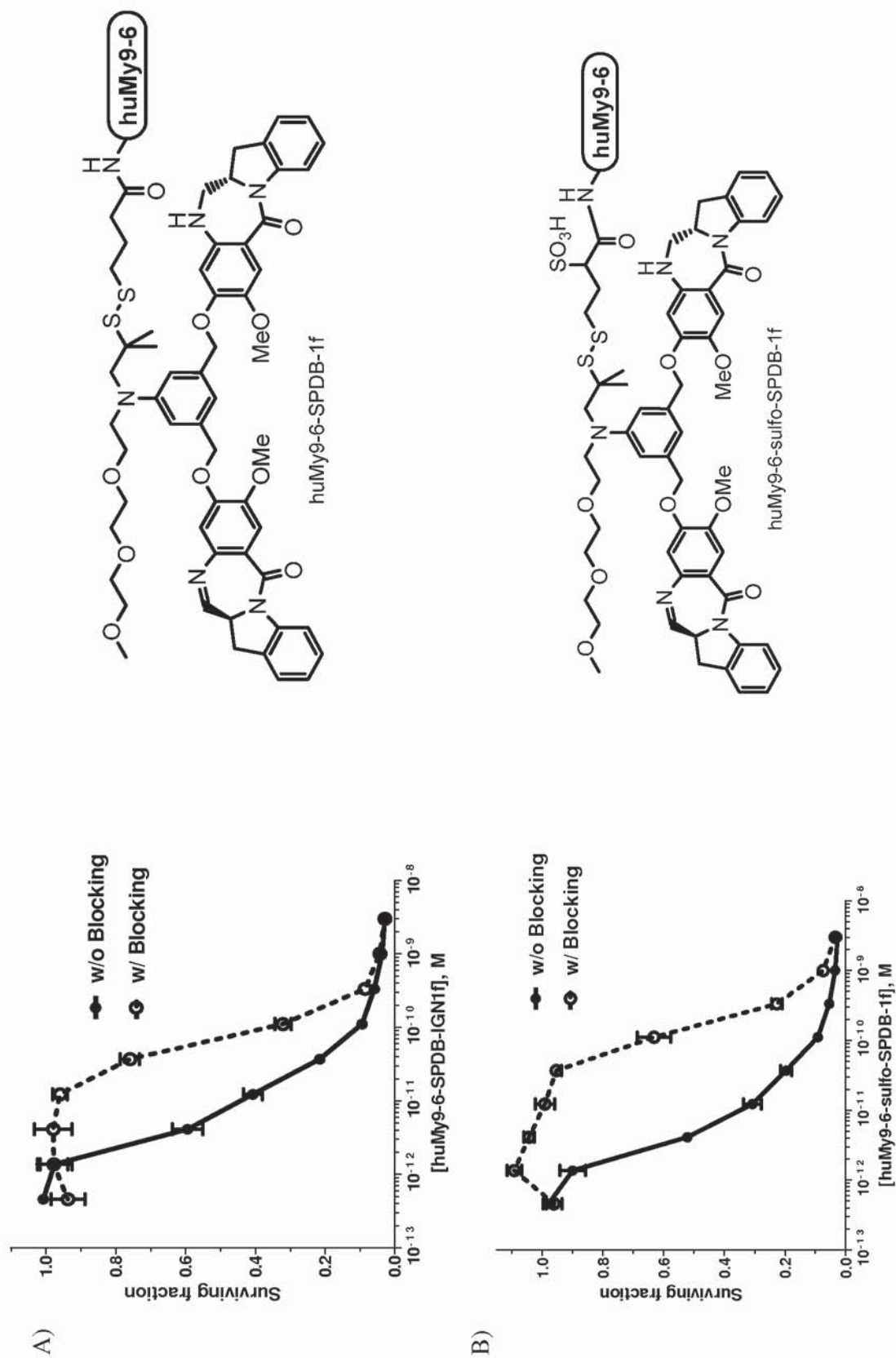
Figure 43. Synthetic Scheme of Compounds **1i** and **12a**

Figure 44. Antiproliferative Activity comparing (A) huMy9-6-SPDB-1f, (B) huMy9-6-sulfSPDB-1f, and, (C) huMy9-6-BMPS-1f



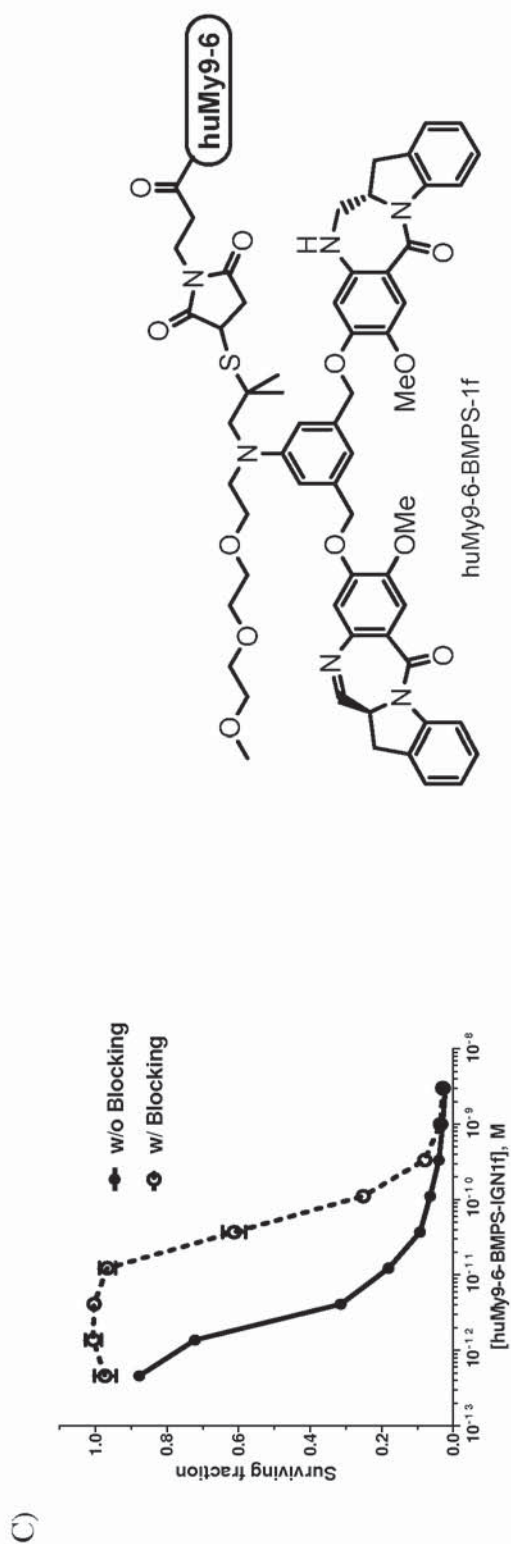


Figure 45. Alternate Synthesis of 4-(benzyloxy)-5-methoxy-2-nitrobenzoic acid used in the Preparation of IBD Monomer



Figure 46. Alternate Synthesis of (5-((2-(2-methoxyethoxy)ethoxy)ethyl)(2-methyl-2-(methyl(disulfanyl)propyl)amino)-1,3-phenylene)dimethanol (**1b**)

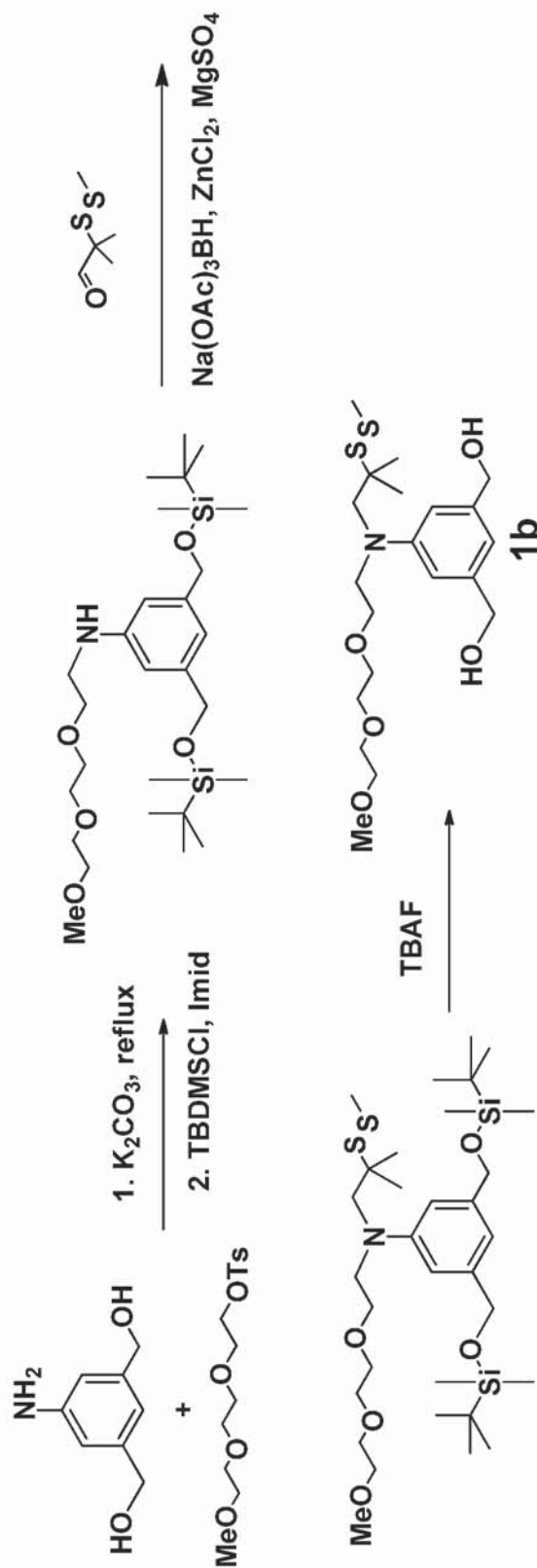


Figure 47. Alternate Synthesis of (5-((2-(2-methoxyethoxy)ethoxy)ethyl)(2-methyl-2-(methyl(disulfanyl)propyl)amino)-1,3-phenylene)dimethanol (**1b**)

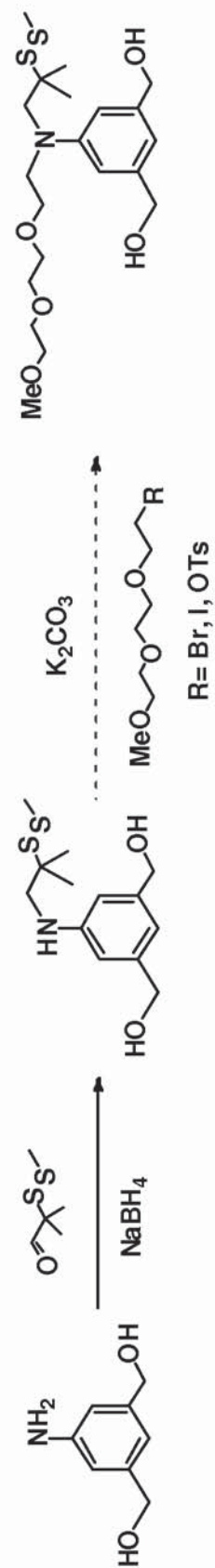
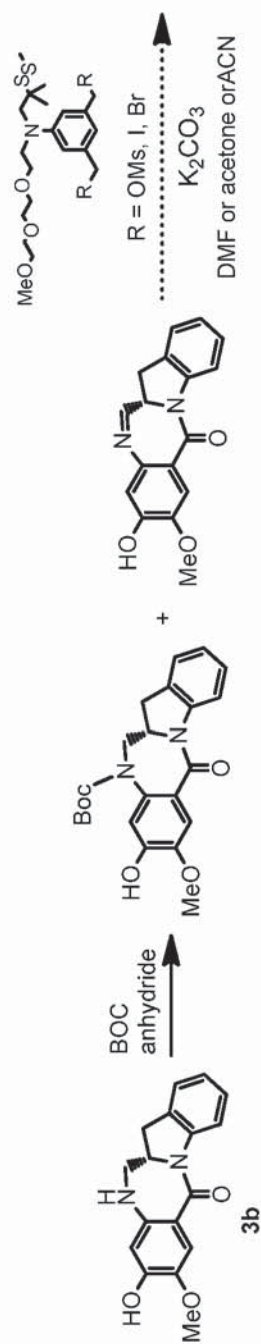
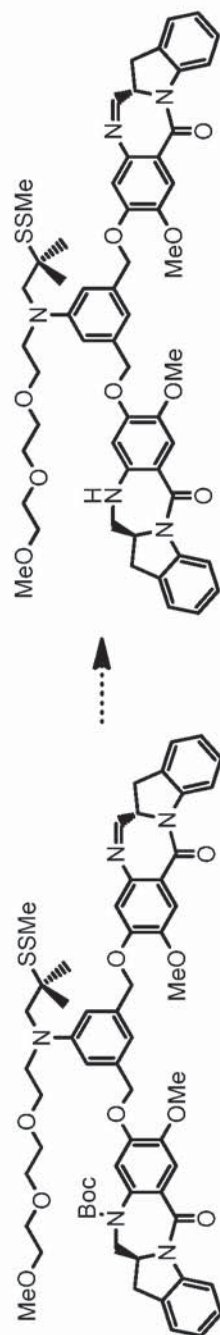


Figure 48. Alternate Synthetic Scheme for a Two-step Mono-imine Dimer Synthesis

Figure 49. Potency of Conjugates against Various Cell Lines Measured by IC₅₀ values (nM)

conjugate	HL60/QC	MOLM-13	NB4	HEL92.1.7	OCI-AML3
huMy9-6-SPDB-1f	0.005	0.003	0.3	0.5	0.01
huMy9-6-sulfo-SPDB-1f	0.006	0.002	0.4	0.05	0.008
huMy9-6-BMPS-1f	0.003	0.001	0.04	0.04	0.003

Figure 50. *In Vivo* Efficacy of huMy9-6-sulfo-SPDB-1f in MOLM-13 Tumor Bearing Mice

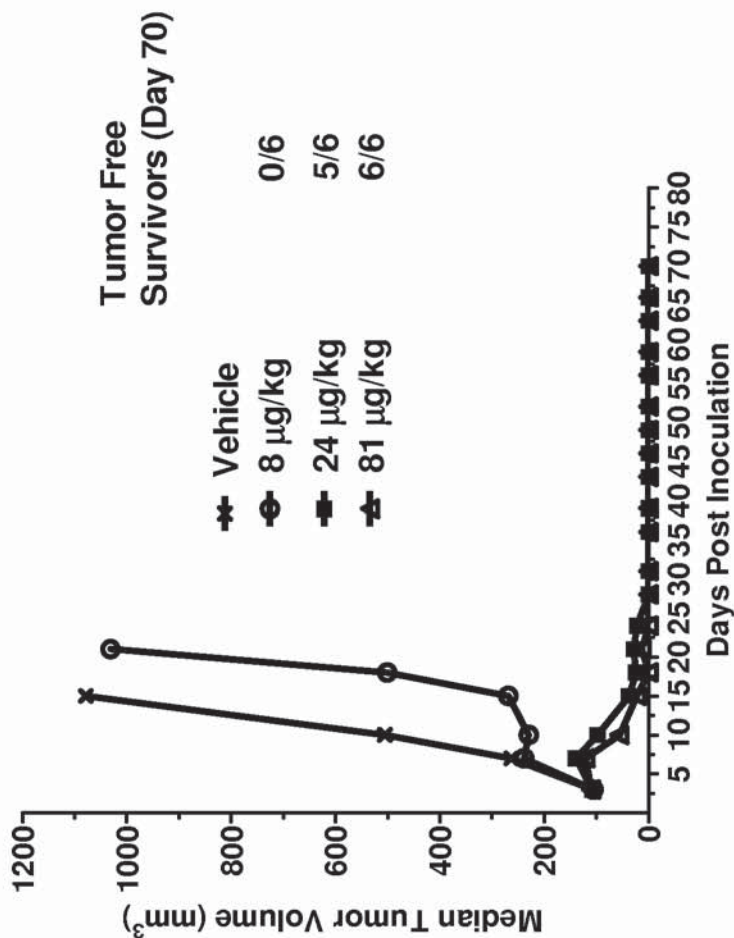


Figure 51. *In Vivo* Efficacy of huMy9-6-sulfo-SPDB-1f in NB4 Tumor Bearing Mice

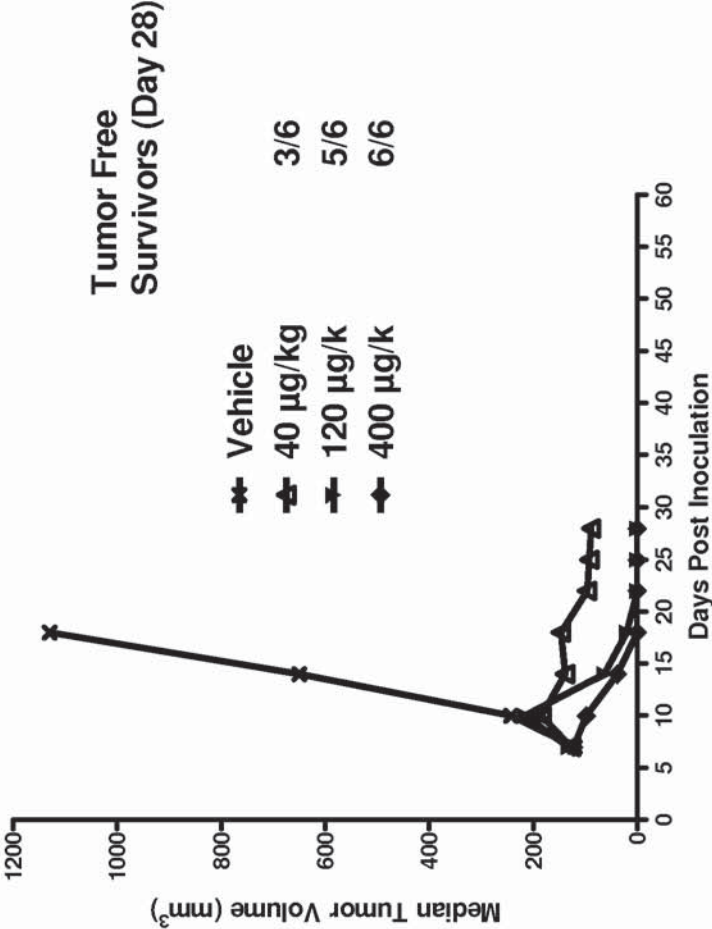


Figure 52. *In Vivo* Efficacy of huMy9-6-BMPS-1f in HL60/QC Tumor Bearing Mice

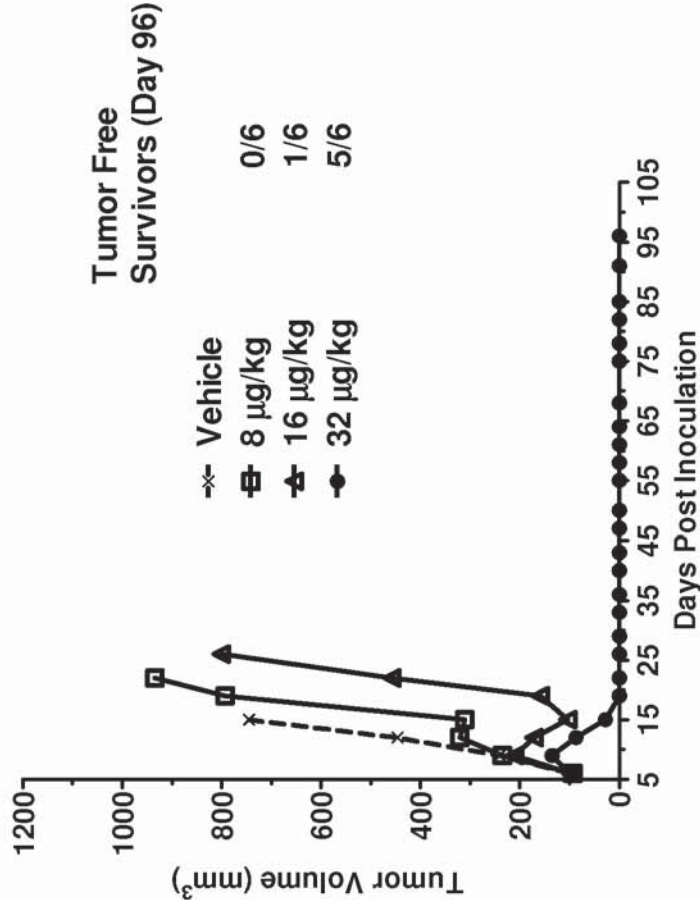


Figure 53. *In vivo* Efficacy of huMy9-6-BMPS-1f in MOLM-13 Tumor Bearing Mice

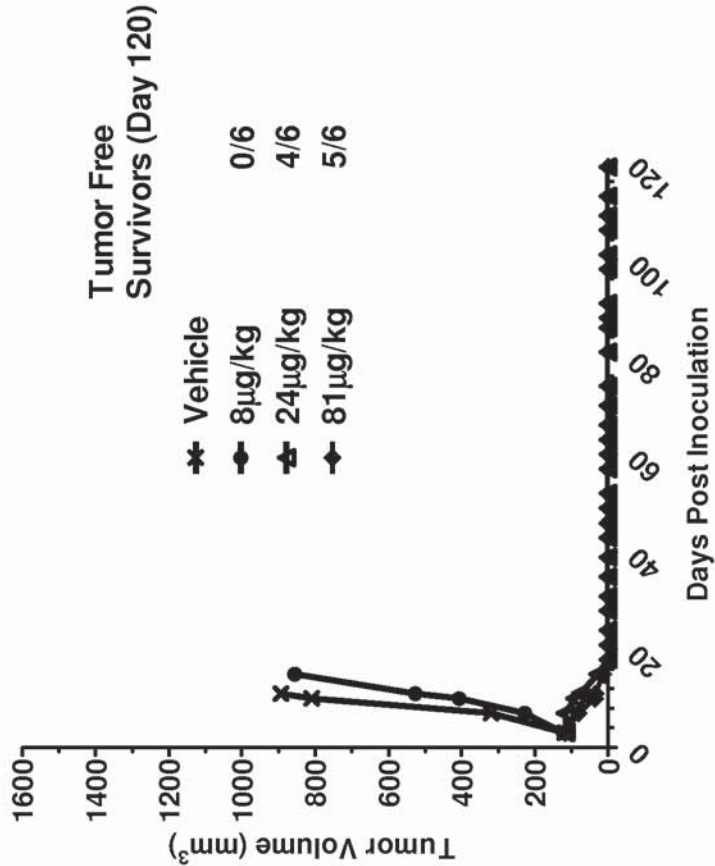


Figure 54. Representative Synthesis Scheme for a Sulfonated Folate / Cytotoxic Compound Conjugate

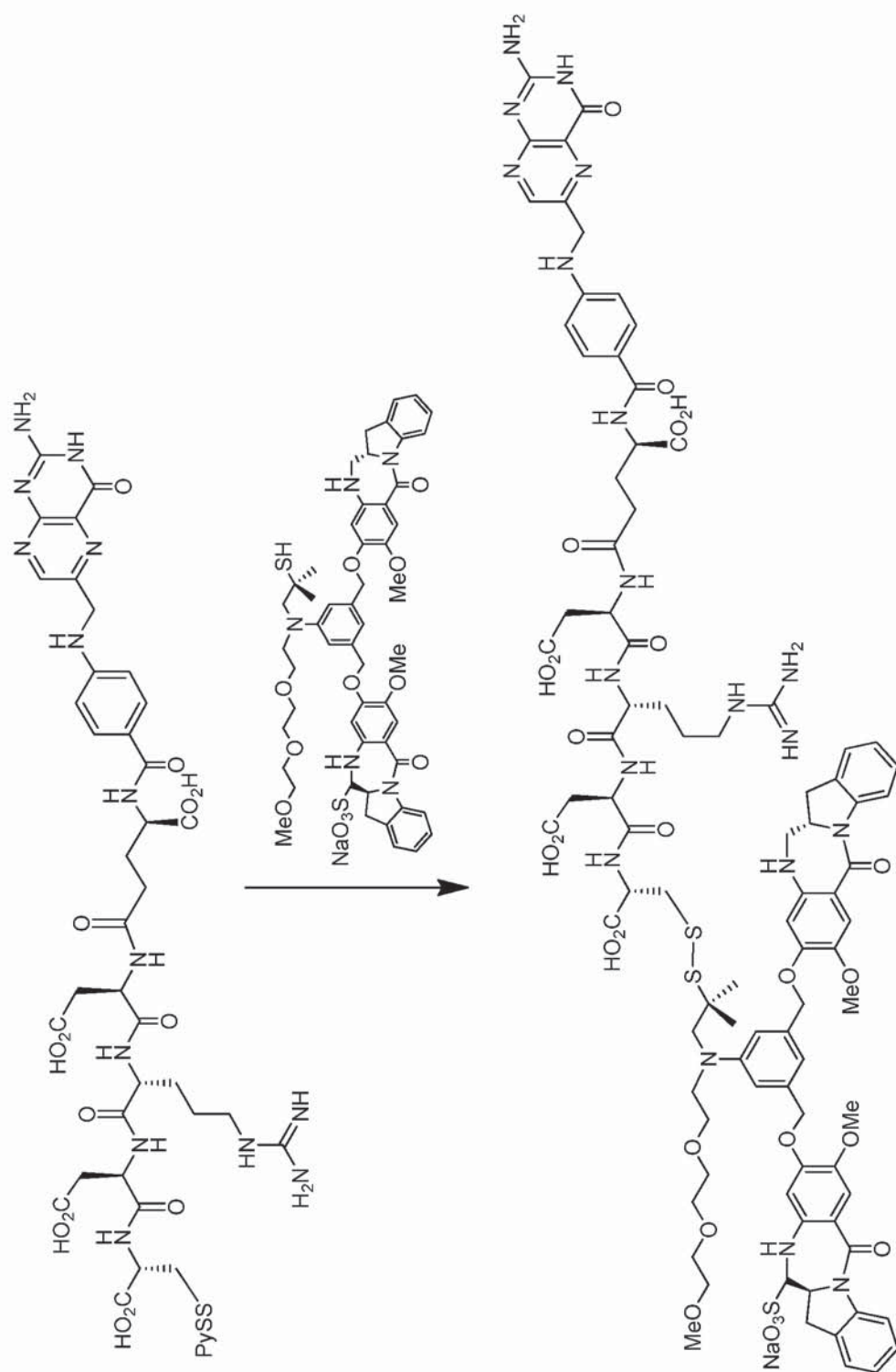


Figure 55. Structures of Several Sulfonated Drug-Antibody (huMy9-6) Conjugates

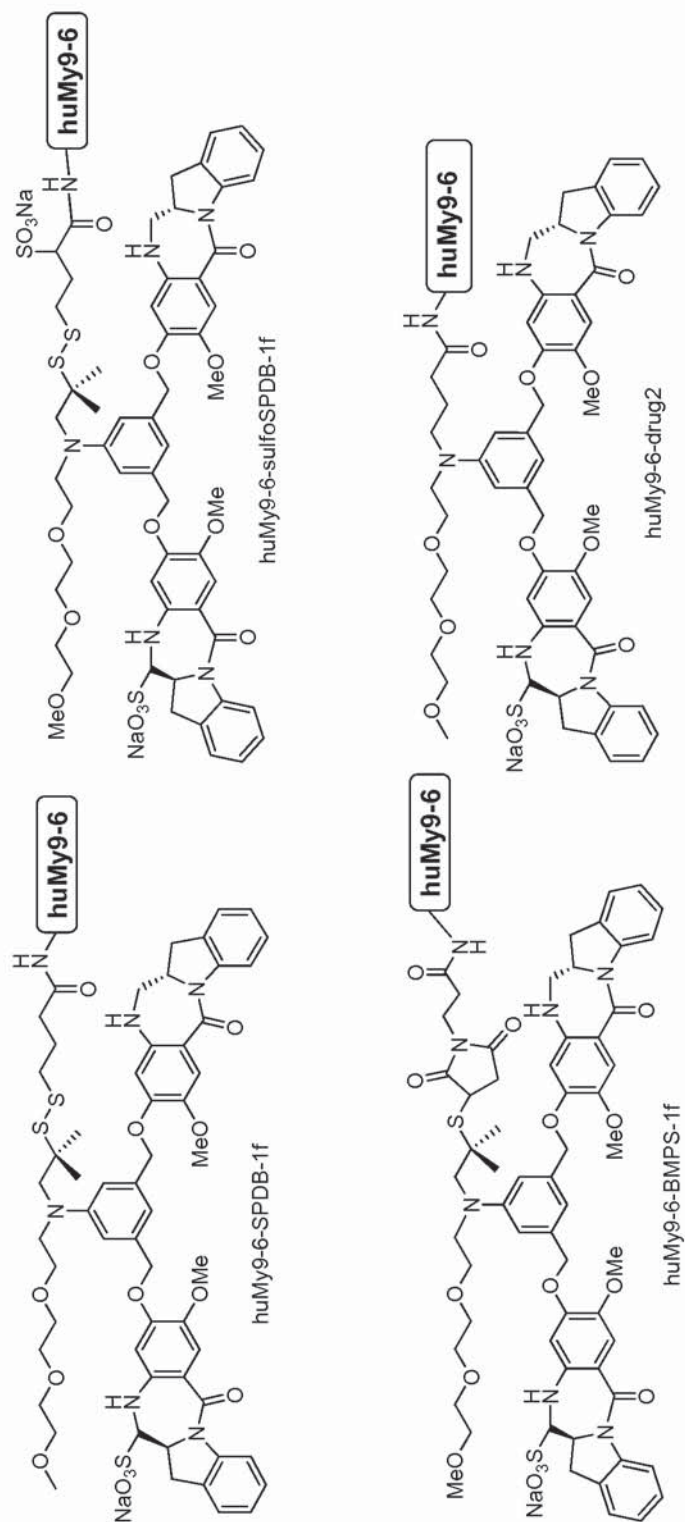
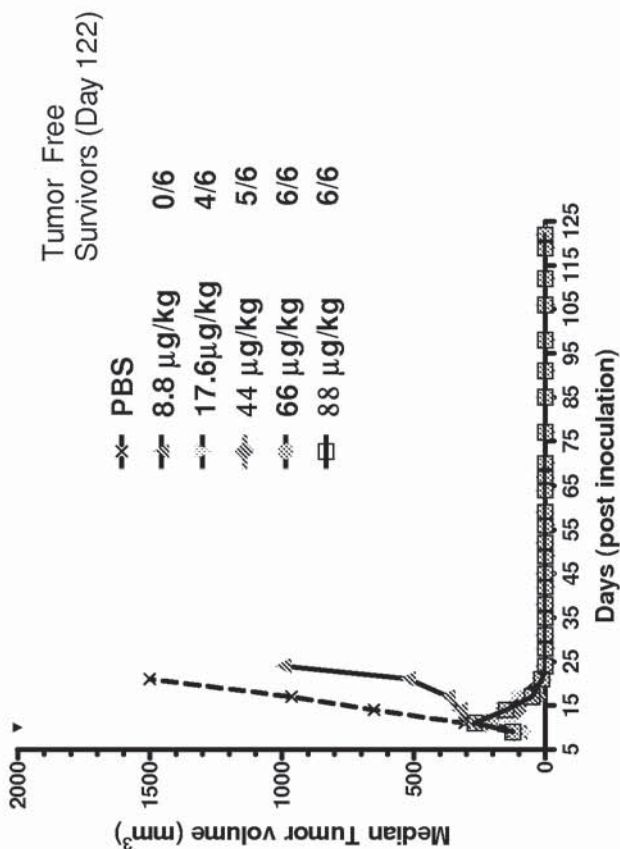
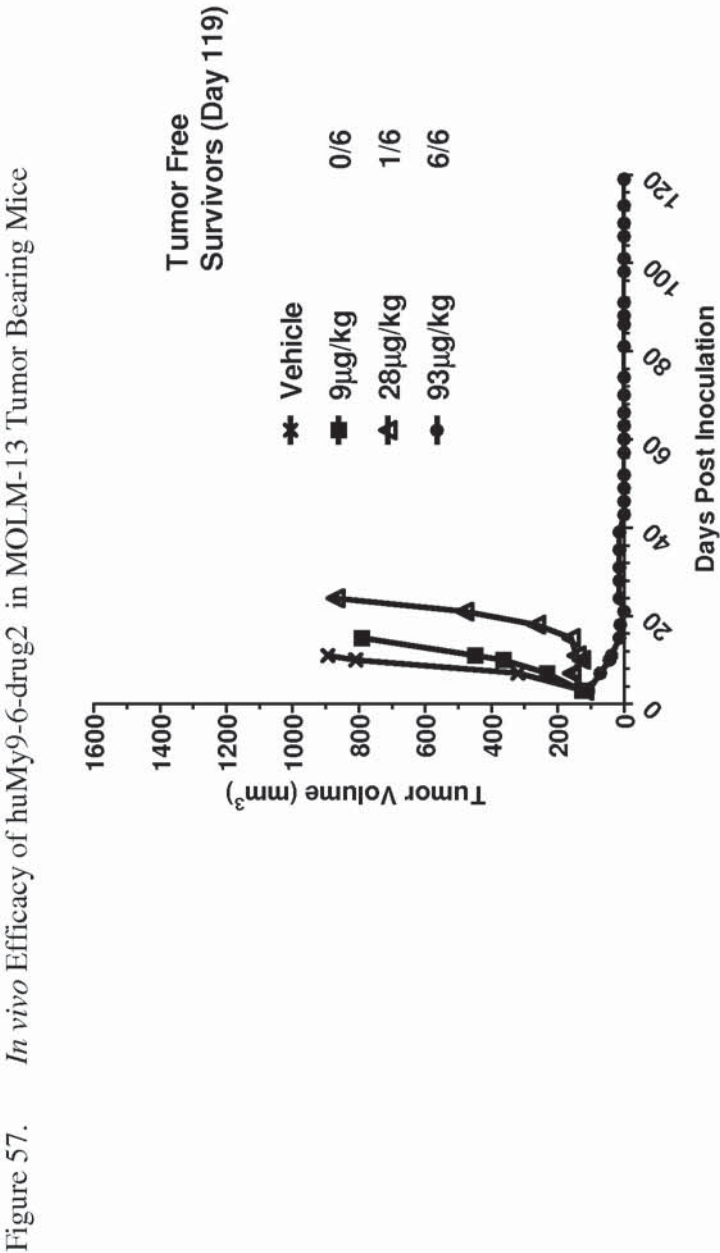


Figure 56. *In vivo* Efficacy of huMy9-6-drug2 in HL60/QC Tumor Bearing Mice





HuMy9-6-2 conjugates prepared without and with sodium bisulfite show similar *in vitro* cytotoxicity toward CD33-antigen expressing HL60 cells

Figure 58.

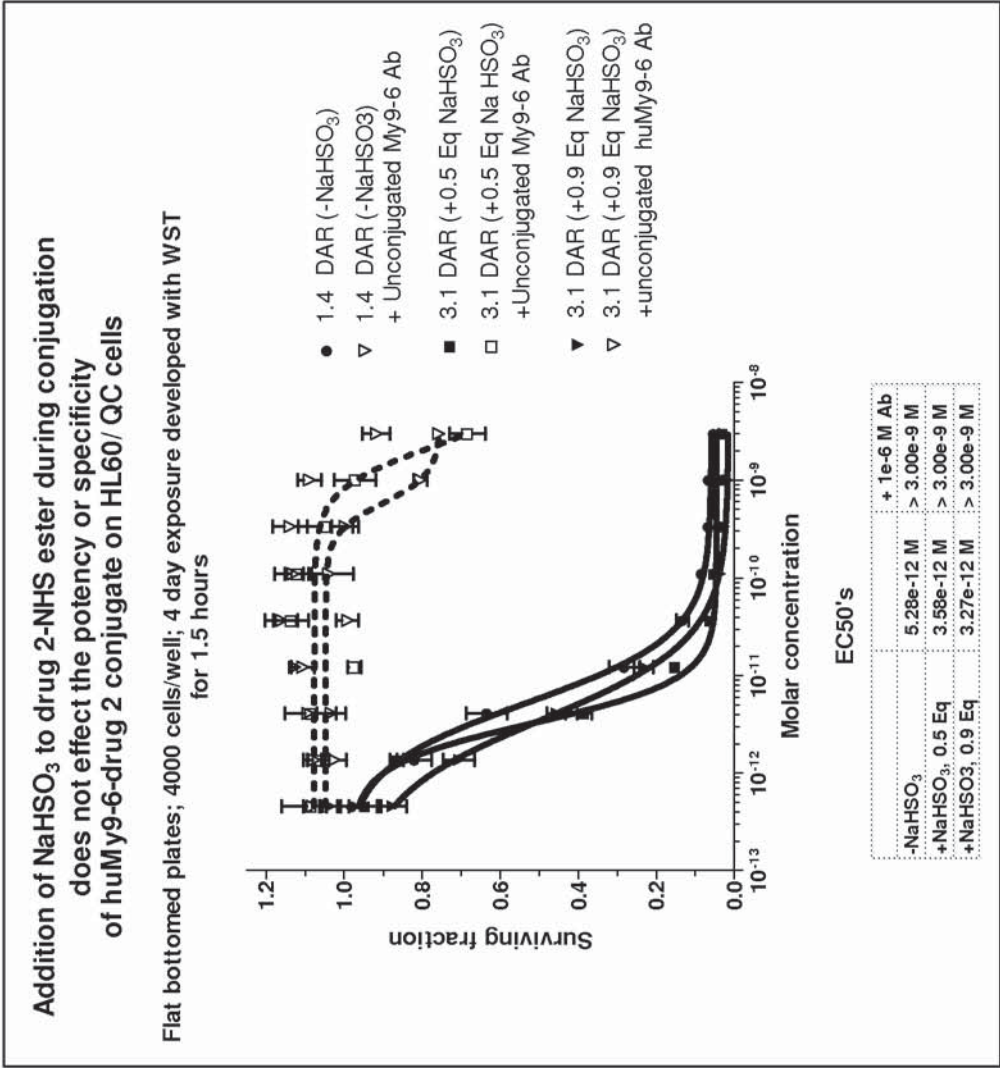


Figure 59.

Anti-CD22 Ab-2 conjugates prepared without and with sodium bisulfite show similar *in vitro* cytotoxicity toward CD22-antigen expressing BJAB cells.

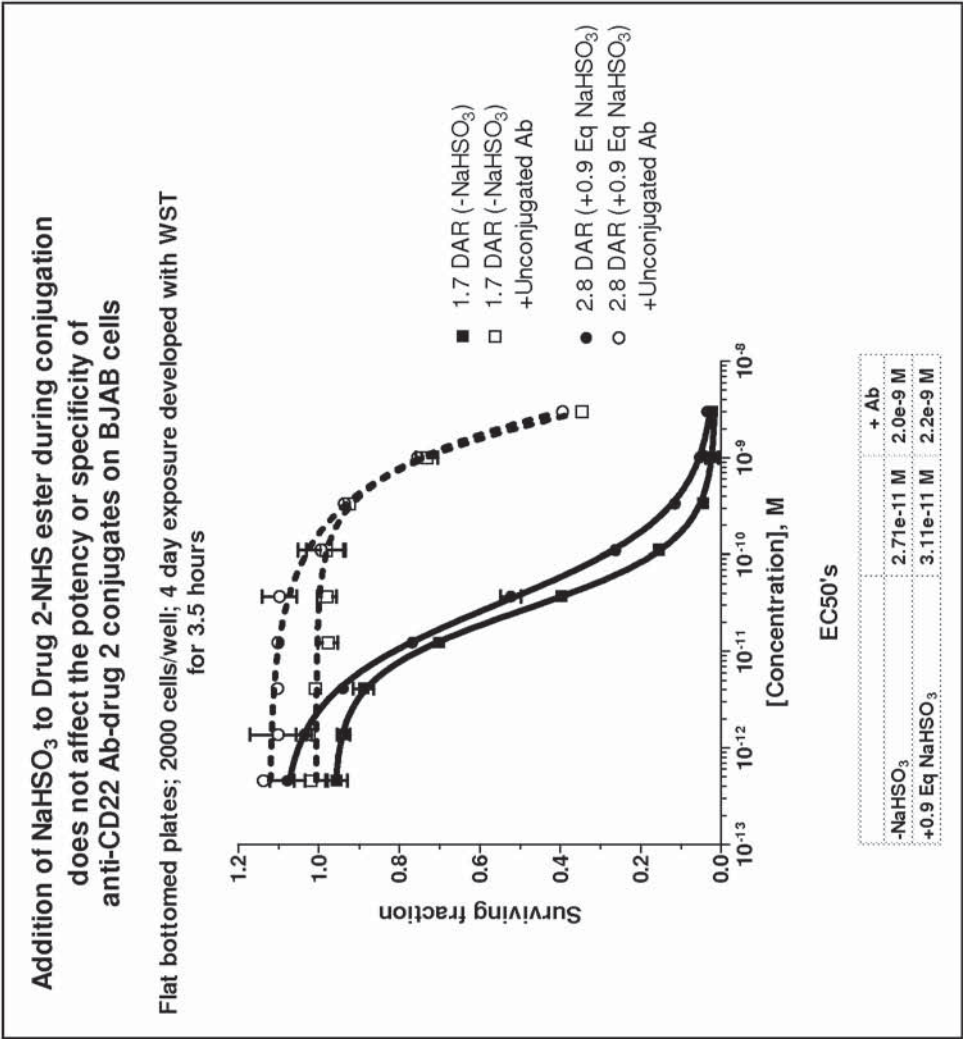
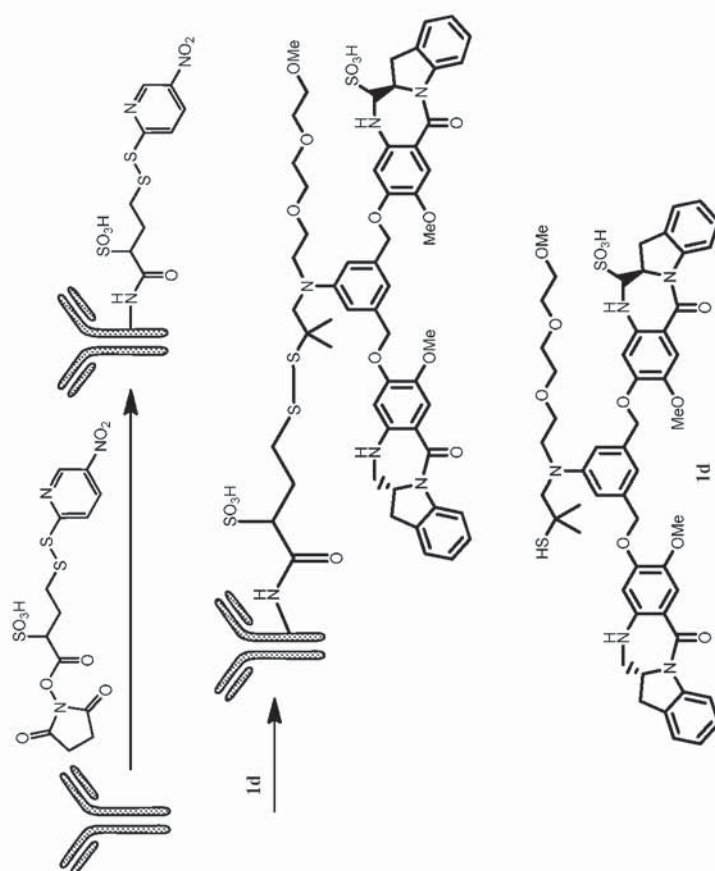


Figure 60.



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Thr Arg Tyr Asp Gly Ser Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr
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Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
 130 135 140
 Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
 145 150 155 160
 Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
 165 170 175
 Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
 180 185 190
 Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
 195 200 205
 Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
 210 215 220
 His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
 225 230 235 240
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
 245 250 255
 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
 260 265 270
 Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 275 280 285
 Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
 290 295 300
 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 305 310 315 320
 Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
 325 330 335
 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
 340 345 350
 Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
 355 360 365
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 370 375 380
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
 385 390 395 400
 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser

405 410 415
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
 420 425 430
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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 Gln Pro Ala Ile Ile Ser Cys Lys Ala Ser Gln Ser Val Ser Phe Ala
 20 25 30
 Gly Thr Ser Leu Met His Trp Tyr His Gln Lys Pro Gly Gln Gln Pro
 35 40 45
 Arg Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ala Gly Val Pro Asp
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Lys Thr Asp Phe Thr Leu Asn Ile Ser
 65 70 75 80
 Pro Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Arg
 85 90 95
 Glu Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 115 120 125
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 130 135 140
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 145 150 155 160
 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 165 170 175
 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
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Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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20 25 30

Gly Thr Ser Leu Met His Trp Tyr His Gln Lys Pro Gly Gln Gln Pro
35 40 45

Arg Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ala Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Lys Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Pro Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Arg
85 90 95

Glu Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
Page 6

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200

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Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 11
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Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
 20 25 30

Phe Met Asn Trp Val Lys Gln Ser Pro Gly Gln Ser Leu Glu Trp Ile
 35 40 45

Gly Arg Ile His Pro Tyr Asp Gly Asp Thr Phe Tyr Asn Gln Lys Phe
 50 55 60

Gln Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala His
 65 70 75 80

Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Phe Ala Val Tyr Tyr Cys
 85 90 95

Thr Arg Tyr Asp Gly Ser Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr
 100 105 110

Thr Val Thr Val Ser Ser
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<210> 12
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<400> 12
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 1 5 10 15

Gln Pro Ala Ile Ile Ser Cys Lys Ala Ser Gln Ser Val Ser Phe Ala
 20 25 30

Gly Thr Ser Leu Met His Trp Tyr His Gln Lys Pro Gly Gln Gln Pro
35 40 45

Arg Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ala Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Lys Thr Asp Phe Thr Leu Asn Ile Ser
65 70 75 80

Pro Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Arg
85 90 95

Glu Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
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1 5 10 15

Gln Pro Ala Ile Ile Ser Cys Lys Ala Ser Gln Ser Val Ser Phe Ala
20 25 30

Gly Thr Ser Leu Met His Trp Tyr His Gln Lys Pro Gly Gln Gln Pro
35 40 45

Arg Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ala Gly Val Pro Asp
50 55 60

Arg Phe Ser Gly Ser Gly Ser Lys Thr Asp Phe Thr Leu Thr Ile Ser
65 70 75 80

Pro Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Arg
85 90 95

Glu Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
100 105 110

<210> 14
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