

Supporting Information

Impact of Dipeptide on ADC Physicochemical Properties and Efficacy Identifies Ala-Ala as the Optimal Dipeptide

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| Chemical Synthesis | 5 |
| (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (C1)..... | 5 |
| tert-Butyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate. | 5 |
| (S)-2-Amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide. | 6 |
| (S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (LD1). | 6 |
| (9H-Fluoren-9-yl)methyl ((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate. | 6 |
| (S)-2-Amino-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide. | 7 |
| (9H-Fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-1-oxopropan-2-yl)carbamate. | 7 |
| (S)-2-((S)-2-Aminopropanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide..... | 8 |
| (S)-2-((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H- | |

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| naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide..... | 8 |
| (S)-2-((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-guanidinopentanamide (LD2)..... | 8 |
| <i>tert</i> -Butyl (S)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate. | 9 |
| <i>tert</i> -Butyl (S)-4-amino-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate. | 9 |
| <i>tert</i> -Butyl (S)-4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate. | 10 |
| <i>tert</i> -Butyl (S)-4-((S)-2-aminopropanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate. | 10 |
| <i>tert</i> -Butyl (S)-4-((S)-2-aminopropanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate. | 11 |
| (S)-4-((S)-2-(2-Bromoacetamido)propanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD4)..... | 11 |
| (S)-2-(2-Bromoacetamido)-N-(2-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)propanamide (LD5)..... | 11 |
| <i>tert</i> -butyl 4-((S)-2-amino-3-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-3-oxopropyl)-1H-imidazole-1-carboxylate. | 12 |
| <i>tert</i> -butyl 4-((S)-2-((S)-2-((<i>tert</i> -butoxycarbonyl)amino)propanamido)-3-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-3-oxopropyl)-1H-imidazole-1-carboxylate. | 12 |
| (S)-2-((S)-2-Aminopropanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-(1H-imidazol-4-yl)propanamide..... | 12 |
| (S)-2-((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-(1H-imidazol-4-yl)propanamide (LD6)..... | 13 |
| (9H-Fluoren-9-yl)methyl <i>tert</i> -butyl ((S)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexane-1,5-diyl)dicarbamate..... | 13 |
| <i>tert</i> -Butyl ((S)-5-amino-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate. | 13 |
| (9H-Fluoren-9-yl)methyl ((S)-1-(((S)-6-((<i>tert</i> -butoxycarbonyl)amino)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxohexan-2-yl)amino)-1-oxopropan-2-yl)carbamate..... | 14 |
| <i>tert</i> -Butyl ((S)-5-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo- | |

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| 2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate..... | 14 |
| <i>tert</i> -Butyl ((S)-5-((S)-2-aminopropanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate. | 15 |
| <i>tert</i> -Butyl ((S)-5-((S)-2-(2-bromoacetamido)propanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate. | 15 |
| (S)-6-Amino-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)hexanamide (LD7)..... | 15 |
| (9H-Fluoren-9-yl)methyl ((S)-3-(<i>tert</i> -butoxy)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)carbamate..... | 16 |
| (S)-2-Amino-3-(<i>tert</i> -butoxy)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide. | 16 |
| (9H-Fluoren-9-yl)methyl ((S)-1-(((S)-3-(<i>tert</i> -butoxy)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate. | 16 |
| (S)-2-((S)-2-Aminopropanamido)-3-(<i>tert</i> -butoxy)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide. | 17 |
| (S)-2-((S)-2-(2-Bromoacetamido)propanamido)-3-(<i>tert</i> -butoxy)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide. | 17 |
| (S)-2-((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-hydroxypropanamide (LD8). | 17 |
| (S)-2-((S)-2-(2-Bromoacetamido)propanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)succinamide (LD9). | 18 |
| (S)-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-methylbutanamide (LD10). | 18 |
| (S)-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-phenylpropanamide (LD11). | 18 |
| (S)-2-(2-(2-Bromoacetamido)acetamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide (LD12). | 19 |
| (S)-2-(2-(2-Bromoacetamido)acetamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)pentanediamide (LD13)..... | 19 |
| (S)-4-(2-(2-Bromoacetamido)acetamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD14)..... | 19 |
| 2-Bromo-N-(2-((2-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)amino)-2-oxoethyl)acetamide (LD15). | 20 |

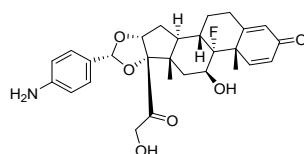
| | |
|--|----|
| (S)-2-(2-(2-Bromoacetamido)acetamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-hydroxypropanamide (LD16). | 20 |
| (S)-6-Amino-2-((S)-2-(2-bromoacetamido)-3-phenylpropanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)hexanamide (LD17). | 20 |
| (S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)-3-hydroxypropanamide (LD18). | 21 |
| (S)-2-((S)-2-(2-Bromoacetamido)-3-hydroxypropanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)succinamide (LD19). | 21 |
| (S)-2-((S)-2-(2-Bromoacetamido)-3-hydroxypropanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)pentanediamide (LD20). | 21 |
| (S)-4-((S)-2-(2-Bromoacetamido)-3-hydroxypropanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD21). | 22 |
| (S)-2-(2-Bromoacetamido)-N-(2-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)-3-hydroxypropanamide (LD22). | 22 |
| (S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (LD23). | 22 |
| (S)-2-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-guanidinopentanamide (LD24). | 23 |
| (S)-2-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-ureidopentanamide (LD25). | 23 |
| (S)-2-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)succinamide (LD26). | 23 |
| (S)-2-((S)-2-(2-bromoacetamido)-3-methylbutanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)pentanediamide (LD27). | 24 |
| (S)-4-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD28). | 24 |
| (S)-2-(2-Bromoacetamido)-N-(2-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)-3-methylbutanamide (LD29). | 24 |
| (S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-3-hydroxy-1-oxopropan-2-yl)-3-methylbutanamide (LD30). | 24 |
| LCMS Conditions | 25 |
| Table S1. | 25 |
| Synthesis of Antibody-Drug Conjugates..... | 25 |

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|---|----|
| DAR4 ADC Preparation..... | 25 |
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Chemical Synthesis

All the reagents and solvents were purchased from TCI China, Accela ChemBio, Bide Pharmatech Ltd and Sigma Aldrich and used without further purification. ¹H NMR spectra were recorded on a Bruker AVANCE III 400MHz spectrometer. The data were processed with MestReNova software, measuring proton shifts in parts per million (ppm) downfield from an internal standard tetramethyl silane. Whenever possible, reactions were monitored by LCMS. Linker payloads were synthesized according to procedures described in the Supporting Information (S2). All purified compounds were ≥95% purity based on analytical HPLC. HPLC conditions are detailed in Table S1 in the Supporting Information. Compound names were generated in ChemDraw 20.1 and properties (cLogP, cLogD, PSA and pI) calculated in ChemDraw 20.1 or in-house proprietary software. The mouse anti-TNF antibody is IgG2a.

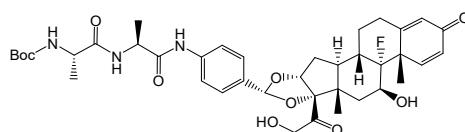
(6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (C1).



To a solution of (8S,9R,10S,11S,13S,14S,16R,17S)-9-fluoro-11,16,17-trihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-3-one (25.4 g, 64.4 mmol) and *tert*-butyl (4-formylphenyl)carbamate (15.0 g, 67.8 mmol) in acetonitrile (500 mL) was added perchloric acid (20.4 mL, 339 mmol) at 20 °C. The resulting mixture was stirred at 20 °C for 2 h. The reaction was quenched with saturated aqueous NaHCO₃ and extracted with DCM (2 × 1 L). The combined organic layer was dried with Na₂SO₄ (20 g), filtered and concentrated under reduced pressure. The residue was purified by prep-HPLC to afford the title compound (7 g, 21% yield) as a yellow solid. LCMS (Method a, Table S1) R_t = 2.10 min; MS m/z: 498.0 (M+H)⁺.

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 7.31 – 7.29 (m, 1H), 7.05 – 7.03 (m, 2H), 6.51 – 6.49 (m, 2H), 5.26 – 5.05 (m, 4H), 4.87 – 4.86 (m, 1H), 4.50 – 4.44 (m, 1H), 4.20 – 4.14 (m, 2H), 2.52 – 2.50 (m, 2H), 2.22 – 2.17 (m, 1H), 2.02 – 1.65 (m, 6H), 1.62 – 1.41 (m, 4H), 0.85 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ ppm 209.54, 185.70, 167.16, 153.08, 150.66, 129.51, 128.34, 124.80, 122.95, 118.54, 113.57, 104.27, 101.96, 101.09, 97.30, 81.30, 70.90, 70.72, 66.58, 48.35, 48.23, 45.42, 43.68, 36.65, 33.15, 30.59, 27.95, 23.36, 17.18. HRMS: found 498.2286 C₂₈H₃₂FNO₆ requires 498.2292.

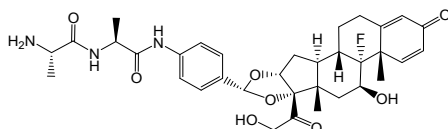
***tert*-Butyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate.**



To a mixture of 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium hexafluorophosphate(V) (115 mg, 0.30 mmol), (S)-2-((S)-2-((*tert*-butoxycarbonyl)amino)propanamido)propanoic acid (63 mg, 0.24 mmol) and (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-6a,6b,7,8,8a,8b,11a,12,12a,12b-decahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4(2H)-one (0.100 g, 0.201 mmol) in DMF (3 mL) was added 2,6-dimethylpyridine (65 mg, 0.60 mmol). The mixture was

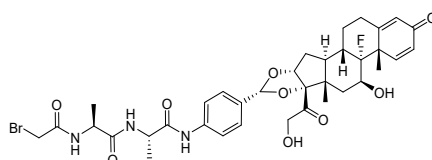
stirred for 2 h at RT and then subjected to purification by prep-HPLC to afford the title compound (60 mg, 40% yield) as a white solid. LCMS (Method b, Table S1) $R_t = 1.43$ min; MS m/z : 740.4 (M+H)⁺.

(S)-2-Amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide.



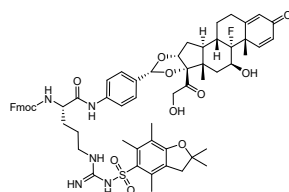
To a solution of *tert*-butyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (400 mg, 0.541 mmol) in EtOAc (10 mL) was added HCl (0.14 mL, 0.54 mmol) at 0 °C. The mixture was stirred for 3 h at RT, evaporated to dryness and subjected to purification by prep-HPLC to afford the title compound (200 mg, 58% yield) as a yellow solid. LCMS (Method c, Table S1) $R_t = 0.70$ min; MS m/z : 640.4 (M+H)⁺.

(S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (LD1).



To a solution of 2-bromoacetic acid (78 mg, 0.56 mmol) in DMF (5 mL) was added 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (174 mg, 0.70 mmol) and (S)-2-amino-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)propanamide (300 mg, 0.47 mmol) at RT. The reaction mixture was stirred at for 2 h at RT and then subjected to purification by prep-HPLC to afford the title compound (33.4 mg, 9% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 2.37$ min; MS m/z : 762.0 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.00 (s, 1H), 8.48 (br d, $J = 7.1$ Hz, 1H), 8.28 (br d, $J = 7.1$ Hz, 1H), 7.60 (br d, $J = 8.5$ Hz, 2H), 7.36 (br d, $J = 8.5$ Hz, 2H), 7.29 (br d, $J = 10.1$ Hz, 1H), 6.23 (br d, $J = 10.0$ Hz, 1H), 6.03 (br s, 1H), 5.43 (br s, 2H), 4.93 (br d, $J = 4.0$ Hz, 1H), 4.52 (br d, $J = 19.5$ Hz, 1H), 4.43 - 4.27 (m, 3H), 4.24 - 4.16 (m, 2H), 3.90 (s, 2H), 2.34 (br d, $J = 13.9$ Hz, 2H), 2.21 - 2.11 (m, 1H), 2.08 - 2.00 (m, 1H), 1.89 - 1.80 (m, 1H), 1.68 (br d, $J = 12.6$ Hz, 3H), 1.50 (s, 3H), 1.30 (br d, $J = 7.0$ Hz, 3H), 1.21 (br d, $J = 7.0$ Hz, 3H), 0.87 (s, 3H).

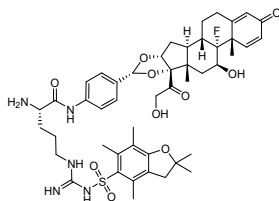
(9H-Fluoren-9-yl)methyl ((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate.



To a solution of N²-(((9H-fluoren-9-yl)methoxy)carbonyl)-N^W-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-L-arginine (1.95 g, 3.0 mmol) in DMF (10 mL) was added 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (0.75 g, 3.0 mmol) and (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (1.0 g, 2.0 mmol) at RT. The mixture was stirred for 12 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford

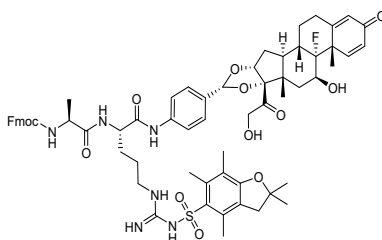
the title compound (10 g, 441% crude yield) as yellow solid that was used directly in the next step without further purification. LCMS (Method c, Table S1) $R_t = 0.85$ min; MS m/z : 1128.7 (M+H)⁺.

(S)-2-Amino-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide.



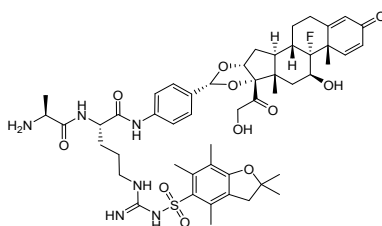
To a solution of (9H-fluoren-9-yl)methyl ((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate (4 g, 3.6 mmol) in DMF (30 mL) was added piperidine (0.30 g, 3.6 mmol) at RT. The mixture was stirred for 12 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (2 g, 31% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 0.67$ min; MS m/z : 906.6 (M+H)⁺.

(9H-Fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-1-oxopropan-2-yl)carbamate.

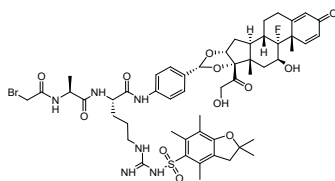


To a solution of (((9H-fluoren-9-yl)methoxy)carbonyl)-L-alanine (172 mg, 0.55 mmol) in DMF (3 mL) was added BEP (227 mg, 0.83 mmol) and DIEA (214 mg, 1.66 mmol), (S)-2-amino-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide (500 mg, 0.552 mmol) at RT. The mixture was stirred for 2 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (600 mg, 37.8% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 0.84$ min; MS m/z : 1199.9 (M+H)⁺.

(S)-2-(((S)-2-Aminopropanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide.

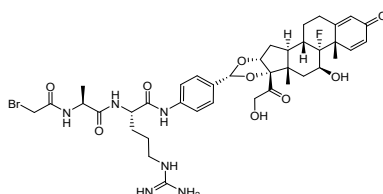


To a solution of (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxo-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-1-oxopropan-2-yl)carbamate (900 mg, 0.75 mmol) in DMF (4 mL) was added piperidine (64 mg, 0.75 mmol) at RT. The mixture was stirred for 1 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (300 mg, 41% yield) as a yellow solid. LCMS (Method c, Table S1) $R_t = 1.20$ min; MS m/z : 977.5 (M+H)⁺.
(S)-2-(((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide.



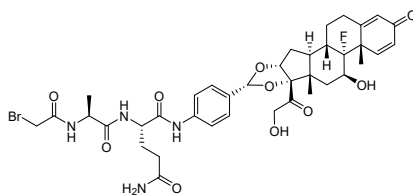
To a solution of 2-bromoacetic acid (26 mg, 0.18 mmol) in DMF (1 mL) was added BEP (30 mg, 0.11 mmol), DIEA (0.03 mL, 0.18 mmol) and (S)-2-(((S)-2-aminopropanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide (60 mg, 0.06 mmol) at RT. The reaction mixture was stirred for 2 h at RT, combined with 3 identical reactions, added dropwise to ice-water (200 mL) and stirred for 30 min. The mixture was filtered to afford the title compound (250 mg, 74% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 0.74$ min; MS m/z : 1099.7 (M+H)⁺.

(S)-2-(((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-guanidinopentanamide (LD2).



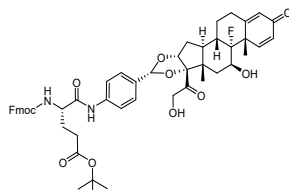
To a solution of (S)-2-(((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-(3-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentanamide (100 mg, 0.09 mmol) in DCM (5 mL) was added TFA (5 mL) at RT. The mixture was stirred for 1 h at RT and then subjected to purification by prep-HPLC to afford the title compound (33.2 mg, 42% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 1.90$ min; MS m/z : 833.3 (M+H)⁺. ¹H NMR: (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.21 (br d, $J=6.88$ Hz, 3 H) 1.43 (br d, $J=14.76$ Hz, 1 H) 1.50 (s, 4 H) 1.57 - 1.77 (m, 5 H) 1.80 - 1.90 (m, 1 H) 2.02 (br d, $J=13.38$ Hz, 1 H) 2.09 - 2.20 (m, 1 H) 2.36 (br s, 1 H) 2.58 - 2.64 (m, 1 H) 3.10 (q, $J=6.25$ Hz, 2 H) 3.86 - 3.94 (m, 2 H) 4.20 (br d, $J=19.39$ Hz, 2 H) 4.30 - 4.44 (m, 2 H) 4.52 (br d, $J=19.51$ Hz, 1 H) 4.94 (br d, $J=3.75$ Hz, 1 H) 4.99 - 5.23 (m, 1 H) 5.44 (s, 2 H) 6.03 (s, 1 H) 6.23 (br d, $J=10.13$ Hz, 1 H) 6.51 - 6.98 (m, 2 H) 7.29 (br d, $J=10.01$ Hz, 1 H) 7.37 (br d, $J=8.38$ Hz, 2 H) 7.44 (br t, $J=5.32$ Hz, 1 H) 7.60 (br d, $J=8.38$ Hz, 2 H) 8.30 (br d, $J=7.75$ Hz, 1 H) 8.52 (d, $J=7.00$ Hz, 1 H) 10.12 (s, 1 H).

(S)-2-(((S)-2-(2-Bromoacetamido)propanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)pentanediamide (LD3).



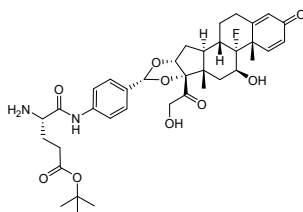
Prepared in a similar manner to compound **LD1** (17 mg, 14% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.02 min; MS m/z : 817.2 (M+H)⁺. ¹H NMR: (400 MHz, DMSO-*d*₆) δ ppm 0.75 - 0.95 (m, 3 H) 1.21 (d, J =7.06 Hz, 3 H) 1.34 - 1.56 (m, 4 H) 1.60 - 1.75 (m, 3 H) 1.77 - 1.89 (m, 2 H) 1.89 - 1.99 (m, 1 H) 2.00 - 2.08 (m, 1 H) 2.09 - 2.24 (m, 3 H) 2.29 - 2.42 (m, 1 H) 2.55 - 2.73 (m, 2 H) 3.87 - 3.95 (m, 2 H) 4.14 - 4.24 (m, 2 H) 4.25 - 4.39 (m, 2 H) 4.52 (br d, J =19.40 Hz, 1 H) 4.93 (d, J =4.63 Hz, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.14, 1.76 Hz, 1 H) 6.70 - 6.82 (m, 1 H) 7.19 - 7.42 (m, 4 H) 7.60 (d, J =8.60 Hz, 2 H) 8.27 (d, J =7.28 Hz, 1 H) 8.49 (d, J =7.06 Hz, 1 H) 9.99 (s, 1 H).

tert-Butyl (S)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate.



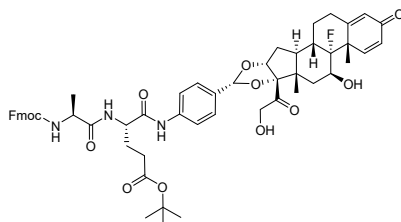
To a solution of (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(*tert*-butoxy)-5-oxopentanoic acid (2.052 g, 4.82 mmol) in DMF (10 mL) was added BEP (1.3 g, 4.8 mmol), DIEA (2.1 mL, 12.1 mmol) and (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-1,2,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-4H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4-one (2.0 g, 4.0 mmol) at RT. The mixture was stirred for 1 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (3 g, 82% yield) as a yellow solid. LCMS (Method c, Table S1) R_t = 1.47 min; MS m/z : 906.1 (M+H)⁺.

tert-Butyl (S)-4-amino-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate.



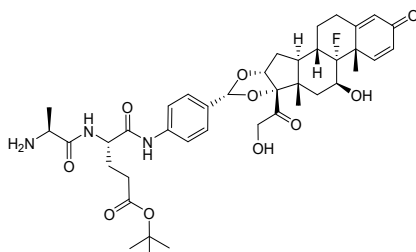
To a solution of *tert*-butyl (S)-4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate (3 g, 3.3 mmol) in DMF (10 mL) was added piperidine (0.28 g, 3.3 mmol) at RT. The mixture was stirred for 1 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (1.5 g, 2.197 mmol, 66.3% yield) as yellow solid. LCMS (Method c, Table S1) R_t = 1.48 min; MS m/z : 683.3 (M+H)⁺.

tert-Butyl (S)-4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate.



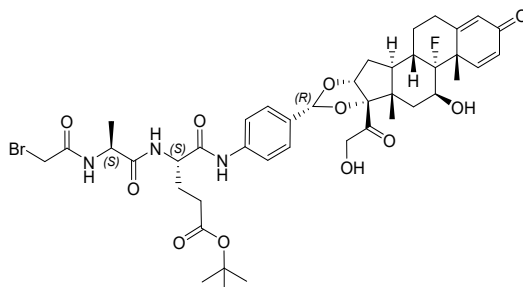
To a solution of (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino propanoic acid (274 mg, 0.9 mmol) in DMF (5 mL) was added BEP (241 mg, 0.9 mmol), DIEA (0.384 mL, 2.2 mmol) and *tert*-butyl (S)-4-amino-5-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate (500 mg, 0.7 mmol) at RT. The mixture was stirred for 1 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (265 mg, 37.1% yield) as a white solid. LCMS (Method c, Table S1) R_t = 1.02 min; MS m/z : 976.6 (M+H)⁺.

***tert*-Butyl (S)-4-((S)-2-aminopropanamido)-5-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate.**



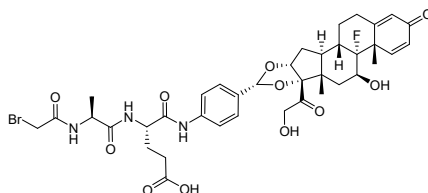
To a solution of *tert*-butyl (S)-4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-5-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate (265 mg, 0.3 mmol) in DMF (5 mL) was added piperidine (23 mg, 0.27 mmol) at RT. The mixture was stirred for 1 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (185 mg, 90% yield) as a white solid. LCMS (Method c, Table S1) R_t = 0.85 min; MS m/z : 754.5 (M+H)⁺.

***tert*-Butyl (S)-4-((S)-2-aminopropanamido)-5-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate.**



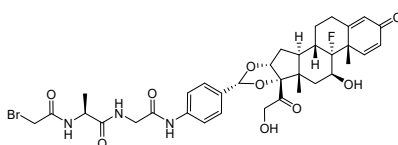
To a solution of 2-bromoacetic acid (60 mg, 0.43 mmol) in DMF (5 mL) was added BEP (109 mg, 0.40 mmol) and DIEA (0.17 mL, 1.0 mmol), *tert*-butyl (S)-4-((S)-2-aminopropanamido)-5-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate (250 mg, 0.33 mmol) at RT. The mixture was stirred for 1 h at RT and then evaporated under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (80 mg, 28% yield) as an off-white solid. LCMS (Method c, Table S1) R_t = 0.86 min; MS m/z : 754.5 (M+H)⁺.

(S)-4-((S)-2-(2-Bromoacetamido)propanamido)-5-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD4).



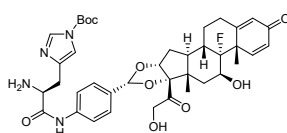
To a solution of *tert*-butyl (S)-4-((S)-2-aminopropanamido)-5-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoate (200 mg, 0.23 mmol) in DCM (6 mL) and TFA (2 mL) at RT. The mixture was stirred for 1 h at RT and then subjected to purification by prep-HPLC to afford the title compound (4.8 mg, 3% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 2.07$ min; MS m/z : 820.3 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.14 - 1.29 (m, 3 H) 1.35 - 1.44 (m, 1 H) 1.50 (s, 3 H) 1.60 - 1.73 (m, 3 H) 1.79 - 1.91 (m, 2 H) 1.92 - 2.08 (m, 2 H) 2.11 - 2.19 (m, 1 H) 2.20 - 2.30 (m, 2 H) 2.36 (br s, 1 H) 2.55 - 2.63 (m, 1 H) 3.91 (d, $J=2.25$ Hz, 1 H) 4.10 (s, 1 H) 4.14 - 4.25 (m, 2 H) 4.26 - 4.41 (m, 2 H) 4.52 (br d, $J=19.51$ Hz, 1 H) 4.93 (br d, $J=4.38$ Hz, 1 H) 5.38 - 5.53 (m, 2 H) 6.03 (s, 1 H) 6.23 (br d, $J=10.13$ Hz, 1 H) 7.29 (br d, $J=10.13$ Hz, 1 H) 7.36 (br d, $J=8.38$ Hz, 2 H) 7.60 (br d, $J=8.50$ Hz, 2 H) 8.21 - 8.31 (m, 1 H) 8.58 (br d, $J=7.88$ Hz, 1 H) 8.38 - 8.54 (m, 1 H) 9.90 - 10.19 (m, 1 H).

(S)-2-(2-Bromoacetamido)-N-(2-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)propanamide (LD5).



Prepared in a similar manner to compound **LD1** (6 mg, 3% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 2.06$ min; MS m/z : 746.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.89 (s, 1H), 8.59 (br d, $J=6.8$ Hz, 1H), 8.44 - 8.35 (m, 1H), 7.62 (br d, $J=8.4$ Hz, 2H), 7.36 (br d, $J=8.4$ Hz, 2H), 7.29 (br d, $J=10.1$ Hz, 1H), 6.27 - 6.19 (m, 1H), 6.03 (s, 1H), 5.43 (s, 2H), 4.93 (br d, $J=4.4$ Hz, 1H), 4.52 (br d, $J=19.7$ Hz, 1H), 4.34 - 4.25 (m, 1H), 4.24 - 4.14 (m, 2H), 3.90 (br dd, $J=5.1, 18.6$ Hz, 4H), 2.21 - 2.11 (m, 3H), 2.04 (br d, $J=13.2$ Hz, 2H), 1.90 - 1.80 (m, 2H), 1.72 - 1.63 (m, 3H), 1.50 (s, 3H), 1.45 - 1.38 (m, 1H), 1.24 (br d, $J=7.0$ Hz, 3H), 0.87 (s, 3H).

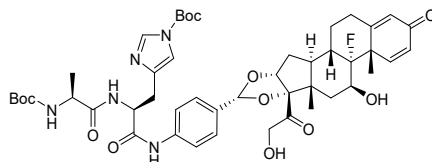
***tert*-butyl 4-((S)-2-amino-3-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-3-oxopropyl)-1H-imidazole-1-carboxylate.**



To a solution of *tert*-butyl 4-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((4-(((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-3-oxopropyl)-1H-imidazole-1-carboxylate (5.0 g, 5.2 mmol) in DMF (50 mL) was added piperidine (2.5 mL, 5.2 mmol) at RT. The reaction mixture was stirred for 10 min at RT, combined with two identical reactions and subjected to purification by prep-HPLC to afford the title compound (2.8 g, 61% yield) as a white solid. LCMS (Method f, Table S1) $R_t = 1.17$ min; MS m/z : 735.4 (M+H)⁺.

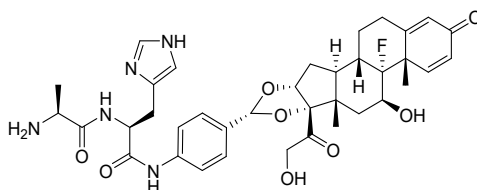
tert-butyl

4-((*S*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)propanamido)-3-((4-((6*aS*,6*bR*,7*S*,8*aS*,8*bS*,10*R*,11*aR*,12*aS*,12*bS*)-6*b*-fluoro-7-hydroxy-8*b*-(2-hydroxyacetyl)-6*a*,8*a*-dimethyl-4-oxo-2,4,6*a*,6*b*,7,8,8*a*,8*b*,11*a*,12,12*a*,12*b*-dodecahydro-1*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-10-yl)phenyl)amino)-3-oxopropyl)-1*H*-imidazole-1-carboxylate.



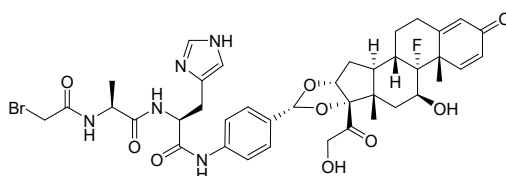
To a solution of (*tert*-butoxycarbonyl)-L-alanine (324 mg, 1.7 mmol) in dimethyl formamide (2 mL) was added BEP (470 mg, 1.7 mmol), DIEA (0.5 mL, 2.9 mmol) and *tert*-butyl 4-((*S*)-2-amino-3-((4-((6*aS*,6*bR*,7*S*,8*aS*,8*bS*,10*R*,11*aR*,12*aS*,12*bS*)-6*b*-fluoro-7-hydroxy-8*b*-(2-hydroxyacetyl)-6*a*,8*a*-dimethyl-4-oxo-2,4,6*a*,6*b*,7,8,8*a*,8*b*,11*a*,12,12*a*,12*b*-dodecahydro-1*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-10-yl)phenyl)amino)-3-oxopropyl)-1*H*-imidazole-1-carboxylate (700 mg, 1.0 mmol) at RT. The reaction mixture was stirred for 2 h at RT, combined with two identical reactions and subjected to purification by prep-HPLC to afford the title compound (350 mg, 38% yield) as a white solid. LCMS (Method f, Table S1) $R_t = 3.05$ min; MS m/z : 906.4 ($M+H$)⁺.

(*S*)-2-((*S*)-2-Aminopropanamido)-*N*-(4-((6*aS*,6*bR*,7*S*,8*aS*,8*bS*,10*R*,11*aR*,12*aS*,12*bS*)-6*b*-fluoro-7-hydroxy-8*b*-(2-hydroxyacetyl)-6*a*,8*a*-dimethyl-4-oxo-2,4,6*a*,6*b*,7,8,8*a*,8*b*,11*a*,12,12*a*,12*b*-dodecahydro-1*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-10-yl)phenyl)-3-(1*H*-imidazol-4-yl)propanamide.



To a solution of *tert*-butyl 4-((*S*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)propanamido)-3-((4-((6*aS*,6*bR*,7*S*,8*aS*,8*bS*,10*R*,11*aR*,12*aS*,12*bS*)-6*b*-fluoro-7-hydroxy-8*b*-(2-hydroxyacetyl)-6*a*,8*a*-dimethyl-4-oxo-2,4,6*a*,6*b*,7,8,8*a*,8*b*,11*a*,12,12*a*,12*b*-dodecahydro-1*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-10-yl)phenyl)amino)-3-oxopropyl)-1*H*-imidazole-1-carboxylate (350 mg, 0.4 mmol) in DCM (10 mL) was added TFA (0.2 mL) at RT. The reaction mixture was stirred for 1 h at RT and then dried by lyophilization to afford the title compound (260 mg, 95% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 1.07$ min; MS m/z : 706.4 ($M+H$)⁺.

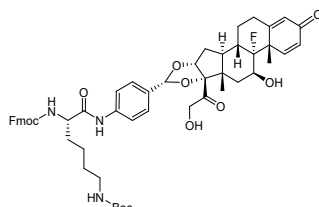
(*S*)-2-((*S*)-2-(2-Bromoacetamido)propanamido)-*N*-(4-((6*aS*,6*bR*,7*S*,8*aS*,8*bS*,10*R*,11*aR*,12*aS*,12*bS*)-6*b*-fluoro-7-hydroxy-8*b*-(2-hydroxyacetyl)-6*a*,8*a*-dimethyl-4-oxo-2,4,6*a*,6*b*,7,8,8*a*,8*b*,11*a*,12,12*a*,12*b*-dodecahydro-1*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-10-yl)phenyl)-3-(1*H*-imidazol-4-yl)propanamide (LD6).



To a solution of 2-bromoacetic acid (25.6 mg, 0.18 mmol) in DMF (2 mL) was added BEP (37.8 mg, 0.138 mmol), DIEA (0.05 mL, 0.28 mmol) and (*S*)-2-((*S*)-2-aminopropanamido)-*N*-(4-((6*aS*,6*bR*,7*S*,8*aS*,8*bS*,10*R*,11*aR*,12*aS*,12*bS*)-6*b*-fluoro-7-hydroxy-8*b*-(2-hydroxyacetyl)-6*a*,8*a*-dimethyl-4-oxo-2,4,6*a*,6*b*,7,8,8*a*,8*b*,11*a*,12,12*a*,12*b*-dodecahydro-1*H*-naphtho[2',1':4,5]indeno[1,2-*d*][1,3]dioxol-10-yl)phenyl)-3-(1*H*-imidazol-4-yl)propanamide (65 mg, 0.09 mmol) at RT. The reaction mixture was stirred for 2 h at RT, combined with three identical reactions and subjected to purification by prep-HPLC to afford the title compound (36 mg, 12% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 1.91$ min; MS m/z : 828.3 ($M+H$)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.89 (s, 3 H) 1.21 (d, $J=7.00$ Hz, 3 H)

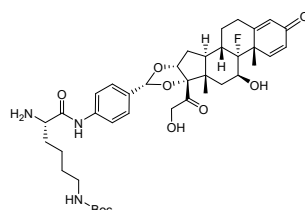
1.34 - 1.55 (m, 4 H) 1.65 - 1.74 (m, 3 H) 1.83 - 1.92 (m, 1 H) 2.04 (br d, $J=13.63$ Hz, 1 H) 2.11 - 2.23 (m, 1 H) 2.37 - 2.48 (m, 1 H) 2.56 - 2.67 (m, 1 H) 3.00 - 3.10 (m, 1 H) 3.18 (br dd, $J=15.20, 5.82$ Hz, 1 H) 3.85 - 3.97 (m, 2 H) 4.16 - 4.33 (m, 3 H) 4.54 (br d, $J=19.39$ Hz, 1 H) 4.67 - 4.76 (m, 1 H) 4.96 (d, $J=4.00$ Hz, 1 H) 5.09 - 5.21 (m, 1 H) 5.44 - 5.51 (m, 2 H) 6.05 (s, 1 H) 6.25 (dd, $J=10.13, 1.63$ Hz, 1 H) 7.31 (d, $J=10.13$ Hz, 1 H) 7.36 - 7.44 (m, 3 H) 7.60 (d, $J=8.50$ Hz, 2 H) 8.50 (d, $J=7.88$ Hz, 1 H) 8.61 (d, $J=6.63$ Hz, 1 H) 8.97 (s, 1 H) 10.07 (s, 1 H) 14.14 (br s, 1 H).

(9H-Fluoren-9-yl)methyl *tert*-butyl ((S)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexane-1,5-diyl)dicarbamate.



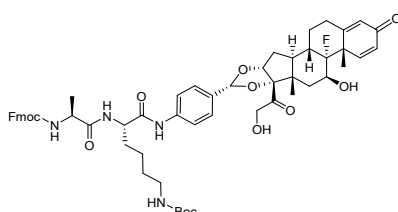
To a solution of (((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(*tert*-butoxycarbonyl)-L-lysine (1.13 g, 2.412 mmol) in DMF (5 mL) was added BEP (0.60 g, 2.2 mmol) and DIEA (1.05 mL, 6.03 mmol) at RT, (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4(2H)-one (1.0 g, 2.01 mmol) was added. The mixture was stirred for 2 h at RT and then subjected to purification by prep-HPLC to afford the title compound (900 mg, 36% yield) as a white solid. LCMS (Method e, Table S1) $R_t = 2.56$ min; MS m/z 948.4 (M+H)⁺.

***tert*-Butyl ((S)-5-amino-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate.**



To a solution of (9H-fluoren-9-yl)methyl *tert*-butyl ((S)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexane-1,5-diyl)dicarbamate (900 mg, 0.95 mmol) in DMF (5 mL) was added piperidine (404 mg, 4.75 mmol) at RT. The mixture was stirred for 2 h at RT and then subjected to purification by prep-HPLC to afford the title compound (545 mg, 48% yield) as a white solid. LCMS (Method e, Table S1) $R_t = 1.95$ min; MS m/z : 725.8 (M+H)⁺.

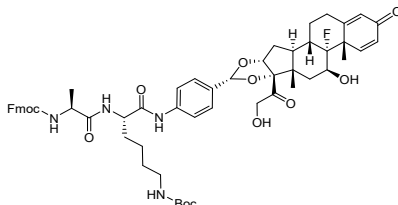
(9H-Fluoren-9-yl)methyl ((S)-1-(((S)-6-((*tert*-butoxycarbonyl)amino)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxohexan-2-yl)amino)-1-oxopropan-2-yl)carbamate.



To a solution of (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (103 mg, 0.33 mmol) in DMF (5 mL) was added BEP (83 mg, 0.30 mmol) and DIEA (0.14 mL, 0.83 mmol) at RT was added *tert*-butyl ((S)-5-amino-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-

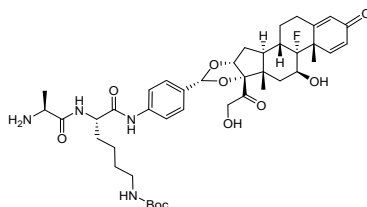
2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate (200 mg, 0.27 mmol). The mixture was stirred for 2 h at RT and then subjected to purification by prep-HPLC to afford the title compound (100 mg, 29% yield) as white solid. LCMS (Method e, Table S1) R_t = 1.29 min; MS m/z : 1020.1 (M+H)⁺.

***tert*-Butyl ((S)-5-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate.**



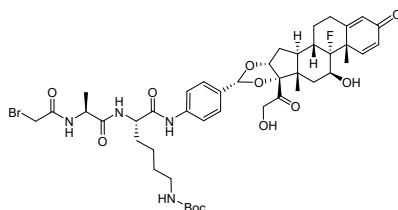
To a solution of (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (103 mg, 0.331 mmol) in DMF (5 mL) was added BEP (83 mg, 0.30 mmol) and DIEA (0.14 mL, 0.83 mmol) was added *tert*-butyl ((S)-5-amino-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate (200 mg, 0.276 mmol) at RT. The mixture was stirred for 2 h at RT and then subjected to purification by prep-HPLC to afford the title compound (100 mg, 29% yield) as a white solid. LCMS (Method e, Table S1) R_t = 1.29 min; MS m/z : 919.4 (M+H-100)⁺.

***tert*-Butyl ((S)-5-((S)-2-aminopropanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate.**



A solution of (9H-fluoren-9-yl)methyl ((S)-1-(((S)-6-((*tert*-butoxycarbonyl)amino)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxohexan-2-yl)amino)-1-oxopropan-2-yl)carbamate (400 mg, 0.39 mmol) in DMF (5 mL) and piperidine (3.3 mg, 0.04 mmol) was stirred at for 2 h at RT. The mixture was concentrated under reduced pressure and subjected to purification by prep-HPLC to afford the title compound (95 mg, 15% yield) as a white solid. LCMS (Method e, Table S1) R_t = 1.39 min; MS m/z : 797.4 (M+H-100)⁺.

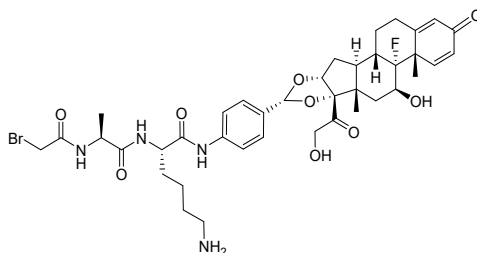
***tert*-Butyl ((S)-5-((S)-2-(2-bromoacetamido)propanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate.**



To a solution of 2-bromoacetic acid (75 mg, 0.54 mmol) in DMF (1 mL) was added BEP (111 mg, 0.41 mmol) and DIEA (0.09 mL, 0.54 mmol) at RT. The mixture was stirred at 20 °C for 1 h. Then *tert*-butyl ((S)-5-((S)-2-

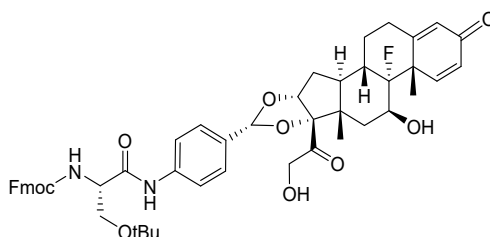
aminopropanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate (215 mg, 0.27 mmol) was added and the mixture was stirred for 2 h at RT. The mixture was concentrated under reduced pressure and subjected to purification by prep-HPLC to afford the title compound (248 mg, 73% yield) as a white solid. LCMS (Method e, Table S1) $R_t = 0.79$ min; MS m/z : 919.3 (M+H)⁺.

(S)-6-Amino-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)hexanamide (LD7).



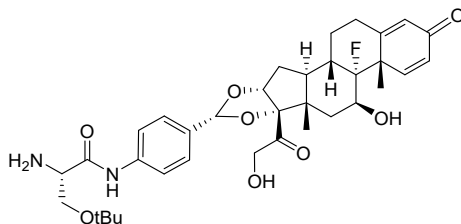
The mixture of *tert*-butyl ((S)-5-((S)-2-(2-bromoacetamido)propanamido)-6-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-6-oxohexyl)carbamate (200 mg, 0.218 mmol) in DCM (0.5 mL) and TFA (0.5 mL) was stirred at 20 °C for 2 h. LCMS showed that the starting material was consumed, and the product was detected. The mixture was concentrated under reduced pressure and subjected to purification by prep-HPLC to afford the title compound (53 mg, 29% yield) as a yellow solid. LCMS (Method d, Table S1) $R_t = 1.91$ min; MS m/z : 819.3 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.19 - 1.26 (m, 3 H) 1.28 - 1.44 (m, 3 H) 1.47 - 1.57 (m, 5 H) 1.62 - 1.74 (m, 4 H) 1.80 - 1.90 (m, 1 H) 2.03 (br d, $J=13.20$ Hz, 1 H) 2.10 - 2.21 (m, 1 H) 2.31 - 2.39 (m, 1 H) 2.61 - 2.70 (m, 1 H) 2.76 (br d, $J=6.36$ Hz, 2 H) 3.86 - 3.95 (m, 2 H) 4.14 - 4.24 (m, 2 H) 4.29 - 4.42 (m, 2 H) 4.52 (br d, $J=19.56$ Hz, 1 H) 4.94 (d, $J=3.91$ Hz, 1 H) 5.03 - 5.21 (m, 1 H) 5.41 - 5.50 (m, 2 H) 6.03 (s, 1 H) 6.23 (m, 1 H) 7.29 (d, $J=10.27$ Hz, 1 H) 7.37 (d, $J=8.80$ Hz, 2 H) 7.55 - 7.72 (m, 5 H) 8.20 - 8.29 (m, 1 H) 8.51 (d, $J=7.34$ Hz, 1 H) 10.08 (s, 1 H).

(9H-Fluoren-9-yl)methyl ((S)-3-(*tert*-butoxy)-1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)carbamate.



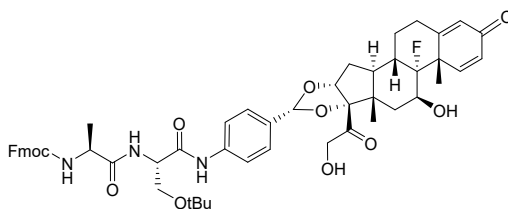
To a solution of N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-(*tert*-butyl)-L-serine (12 mg, 0.03 mmol) and DIEA (10.5 μ L, 0.06 mmol) in DMF (2 mL), was added (6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-10-(4-aminophenyl)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-4(2H)-one (10 mg, 0.02 mmol) at 0 °C. The mixture was stirred for 12 h at RT and then dried under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (17 mg, 10% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 1.13$ min; MS m/z : 863.6 (M+H)⁺.

(S)-2-Amino-3-(*tert*-butoxy)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide.



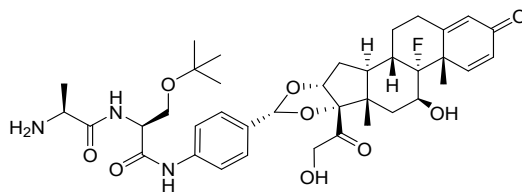
To a solution of (9H-fluoren-9-yl)methyl ((S)-3-(*tert*-butoxy)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)carbamate (6 g, 7.0 mmol) in DMF (1 mL) was added piperidine (0.59 g, 7.0 mmol) at RT. The reaction mixture was stirred for 2 h at RT, combined with three identical reactions and subjected to purification by prep-HPLC to afford the title compound (2.5 g, 56% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 1.429$ min; MS m/z : 641.3 (M+H)⁺.

(9H-Fluoren-9-yl)methyl ((S)-1-(((S)-3-(*tert*-butoxy)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate.



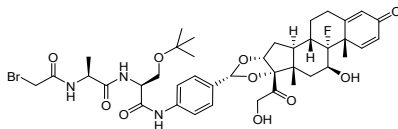
To a solution of (((9H-fluoren-9-yl)methoxy)carbonyl)-L-alanine (233 mg, 0.75 mmol) in DMF (5 mL) was added BEP (205 mg, 0.75 mmol), DIEA (0.33 mL, 1.9 mmol) and (S)-2-amino-3-(*tert*-butoxy)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide (400 mg, 0.62 mmol) at RT. The mixture was stirred for 2 h at RT and then dried under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (615 mg, 105% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 0.83$ min; MS m/z : 934.7 (M+H)⁺.

(S)-2-(((S)-2-Aminopropanamido)-3-(*tert*-butoxy)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide.



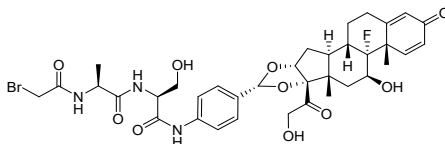
To a solution of (9H-fluoren-9-yl)methyl ((S)-1-(((S)-3-(*tert*-butoxy)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (615 mg, 0.66 mmol) in DMF (1 mL) was added piperidine (56 mg, 0.66 mmol) at RT. The mixture was stirred for 2 h at RT and then dried under reduced pressure. Water (100 mL) was added, and the mixture was filtered to afford the title compound (450 mg, 96% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 0.61$ min; MS m/z : 712.5 (M+H)⁺.

(S)-2-(((S)-2-(2-Bromoacetamido)propanamido)-3-(*tert*-butoxy)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide.



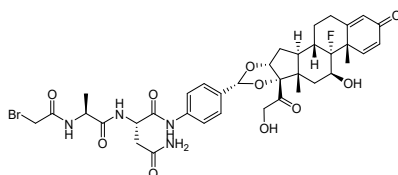
To a solution of 2-bromoacetic acid (105 mg, 0.76 mmol) in DMF (5 mL) was added BEP (208 mg, 0.76 mmol) and DIEA (0.33 mL, 1.9 mmol), (S)-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide (450 mg, 0.63 mmol) at RT. The mixture was stirred for 1 h at RT and then dried under reduced pressure. Water (100 mL) was added, and the mixture filtered to afford the title compound (200 mg, 38.0% yield) as an off-white solid. LCMS (Method c, Table S1) $R_t = 0.72$ min; MS m/z : 834.4 (M+H)⁺.

(S)-2-((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-hydroxypropanamide (LD8).



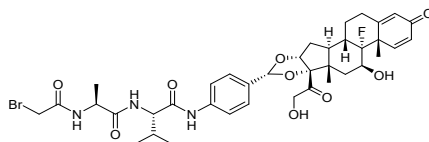
To a solution of (S)-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide (160 mg, 0.19 mmol) in DCM (6 mL) and TFA (3 mL) at RT. The mixture was stirred for 1 h at RT and then subjected to purification by prep-HPLC to afford the title compound (15 mg, 9% yield) as a yellow solid. LCMS (Method d, Table S1) $R_t = 2.02$ min; MS m/z : 778.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.17 - 1.27 (m, 3 H) 1.41 (br dd, $J=12.76, 4.63$ Hz, 1 H) 1.50 (s, 3 H) 1.60 - 1.74 (m, 3 H) 1.80 - 1.94 (m, 1 H) 2.04 (br d, $J=13.51$ Hz, 1 H) 2.16 (td, $J=11.82, 7.25$ Hz, 1 H) 2.36 (s, 1 H) 2.55 - 2.64 (m, 1 H) 3.64 (br d, $J=5.63$ Hz, 2 H) 3.92 (s, 1 H) 4.11 (s, 1 H) 4.20 (br d, $J=19.51$ Hz, 2 H) 4.33 - 4.46 (m, 2 H) 4.52 (d, $J=19.39$ Hz, 1 H) 4.93 (d, $J=4.63$ Hz, 1 H) 5.40 - 5.47 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, $J=10.13, 1.63$ Hz, 1 H) 7.29 (d, $J=10.13$ Hz, 1 H) 7.36 (d, $J=8.63$ Hz, 2 H) 7.63 (d, $J=8.63$ Hz, 2 H) 8.16 (d, $J=7.88$ Hz, 1 H) 8.51 (d, $J=7.25$ Hz, 1 H) 10.00 (s, 1 H).

(S)-2-((S)-2-(2-Bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)succinamide (LD9).



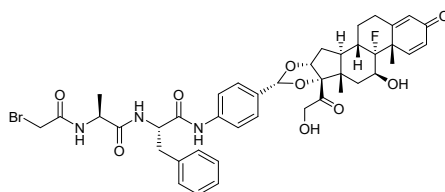
Prepared in a similar manner to compound LD1 (30 mg, 16% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 2.01$ min; MS m/z : 803.0 (M+H)⁺. ¹H NMR: (400 MHz, DMSO-*d*₆) δ ppm 9.91 - 9.84 (m, 1H), 8.60 - 8.52 (m, 1H), 8.37 - 8.28 (m, 1H), 7.62 (br d, $J=6.8$ Hz, 2H), 7.36 (br d, $J=8.6$ Hz, 3H), 7.31 - 7.25 (m, 1H), 6.96 - 6.89 (m, 1H), 6.29 - 6.18 (m, 1H), 6.03 (br s, 1H), 5.44 (br d, $J=1.5$ Hz, 2H), 4.96 - 4.89 (m, 1H), 4.69 - 4.59 (m, 1H), 4.56 - 4.46 (m, 1H), 4.31 - 4.25 (m, 1H), 4.23 - 4.15 (m, 2H), 3.95 - 3.89 (m, 2H), 2.61 (br d, $J=2.6$ Hz, 6H), 2.40 - 2.35 (m, 1H), 2.22 - 2.12 (m, 1H), 2.08 - 2.00 (m, 1H), 1.89 - 1.81 (m, 1H), 1.74 - 1.63 (m, 3H), 1.50 (br s, 3H), 1.44 - 1.36 (m, 1H), 1.25 - 1.15 (m, 3H), 0.87 (br s, 3H).

(S)-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-methylbutanamide (LD10).



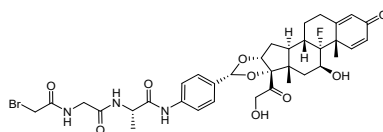
Prepared in a similar manner to compound **LD1** (30 mg, 6% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.28 min; MS m/z : 788.3 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.15 (s, 1H), 8.83 - 8.33 (m, 1H), 8.08 (br d, J =9.0 Hz, 1H), 7.59 (d, J =8.5 Hz, 2H), 7.36 (d, J =8.5 Hz, 2H), 7.29 (d, J =10.1 Hz, 1H), 6.23 (dd, J =1.5, 10.1 Hz, 1H), 6.03 (s, 1H), 4.93 (d, J =4.4 Hz, 1H), 4.52 (br d, J =19.5 Hz, 2H), 4.43 (br dd, J =7.3, 14.4 Hz, 2H), 4.29 (br t, J =7.9 Hz, 2H), 4.23 - 4.17 (m, 2H), 4.10 (s, 1H), 2.69 - 2.59 (m, 1H), 2.56 - 2.51 (m, 1H), 2.48 - 2.43 (m, 1H), 2.40 - 2.33 (m, 1H), 2.16 (dt, J =7.2, 11.7 Hz, 1H), 2.08 - 1.97 (m, 2H), 1.89 - 1.80 (m, 1H), 1.73 - 1.61 (m, 3H), 1.50 (s, 3H), 1.46 - 1.36 (m, 1H), 1.24 - 1.18 (m, 3H), 0.92 - 0.85 (m, 9H).

(S)-2-((S)-2-(2-bromoacetamido)propanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-phenylpropanamide (LD11).



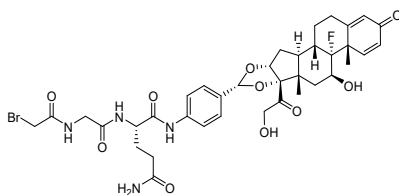
Prepared in a similar manner to compound **LD1** (35 mg, 7% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.39 min; MS m/z : 836.3 (M+H)⁺. ¹H NMR: (400 MHz, DMSO-*d*₆) δ ppm 10.02 (s, 1H), 8.64 - 8.38 (m, 1H), 8.35 - 8.24 (m, 1H), 7.60 - 7.54 (m, 2H), 7.36 (br d, J =8.3 Hz, 2H), 7.29 (d, J =10.3 Hz, 1H), 7.25 (d, J =4.0 Hz, 4H), 7.20 - 7.13 (m, 1H), 6.23 (br d, J =9.9 Hz, 1H), 6.03 (br s, 1H), 5.43 (s, 2H), 5.13 (s, 1H), 4.94 (d, J =4.4 Hz, 1H), 4.64 (br d, J =4.6 Hz, 1H), 4.52 (d, J =19.4 Hz, 1H), 4.40 - 4.26 (m, 1H), 4.24 - 4.16 (m, 2H), 4.14 - 4.04 (m, 1H), 3.90 (br d, J =3.4 Hz, 1H), 3.06 (br dd, J =4.1, 13.3 Hz, 1H), 2.90 (br dd, J =9.2, 13.4 Hz, 1H), 2.71 - 2.53 (m, 2H), 2.35 (br d, J =13.8 Hz, 1H), 2.23 - 2.10 (m, 1H), 2.05 (br d, J =13.5 Hz, 1H), 1.89 - 1.79 (m, 1H), 1.74 - 1.60 (m, 3H), 1.50 (s, 3H), 1.45 - 1.36 (m, 1H), 1.20 - 1.10 (m, 3H), 0.87 (s, 3H).

(S)-2-(2-(2-Bromoacetamido)acetamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)propanamide (LD12).



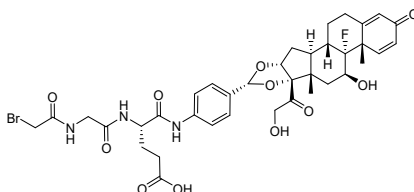
Prepared in a similar manner to compound **LD1** (23 mg, 7% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.07 min; MS m/z : 748.0 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.29 (br d, J =7.13 Hz, 3 H) 1.41 (br dd, J =12.51, 4.38 Hz, 1 H) 1.50 (s, 3 H) 1.61 - 1.73 (m, 3 H) 1.80 - 1.90 (m, 1 H) 2.04 (br d, J =13.13 Hz, 1 H) 2.16 (td, J =11.82, 7.25 Hz, 1 H) 2.37 (br s, 1 H) 2.54 - 2.65 (m, 1 H) 3.79 (br dd, J =5.32, 2.69 Hz, 2 H) 3.93 (s, 2 H) 4.20 (br d, J =19.39 Hz, 2 H) 4.38 - 4.46 (m, 1 H) 4.52 (br d, J =19.51 Hz, 1 H) 4.93 (d, J =4.50 Hz, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.13, 1.50 Hz, 1 H) 7.29 (d, J =10.13 Hz, 1 H) 7.36 (br d, J =8.50 Hz, 2 H) 7.61 (d, J =8.51 Hz, 2 H) 8.33 (br d, J =7.25 Hz, 1 H) 8.52 (br t, J =5.38 Hz, 1 H) 10.02 (s, 1 H).

(S)-2-(2-(2-Bromoacetamido)acetamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)pentanediamide (LD13).



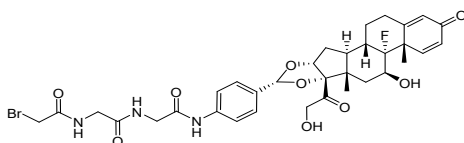
Prepared in a similar manner to compound **LD1** (18 mg, 15% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.00 min; MS m/z : 803.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.33 - 1.45 (m, 1 H) 1.50 (s, 3 H) 1.60 - 1.74 (m, 3 H) 1.75 - 1.99 (m, 3 H) 2.00 - 2.23 (m, 4 H) 2.29 - 2.41 (m, 1 H) 2.54 - 2.73 (m, 2 H) 3.80 (br dd, J =5.07, 2.43 Hz, 3 H) 3.94 (s, 2 H) 4.13 - 4.27 (m, 2 H) 4.32 - 4.43 (m, 1 H) 4.52 (d, J =19.40 Hz, 1 H) 4.93 (d, J =4.63 Hz, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.14, 1.76 Hz, 1 H) 6.78 (br s, 1 H) 7.29 (br d, J =9.92 Hz, 2 H) 7.36 (d, J =8.60 Hz, 2 H) 7.61 (d, J =8.38 Hz, 2 H) 8.33 (d, J =7.50 Hz, 1 H) 8.49 - 8.59 (m, 1 H) 10.07 (s, 1 H).

(S)-4-(2-(2-Bromoacetamido)acetamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD14).



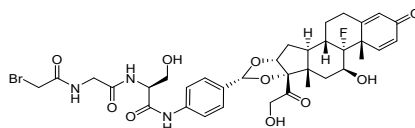
Prepared in a similar manner to compound **LD4** (13 mg, 13% yield) as a yellow solid. LCMS (Method d, Table S1) R_t = 2.02 min; MS m/z : 804.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.37 - 1.45 (m, 1 H) 1.50 (s, 3 H) 1.62 - 1.72 (m, 3 H) 1.77 - 1.89 (m, 2 H) 1.91 - 1.98 (m, 1 H) 2.00 - 2.09 (m, 1 H) 2.12 - 2.19 (m, 1 H) 2.21 - 2.30 (m, 2 H) 2.37 (br d, J =2.38 Hz, 1 H) 2.59 - 2.65 (m, 2 H) 3.77 - 3.85 (m, 2 H) 3.93 (s, 1 H) 4.13 (s, 1 H) 4.17 - 4.23 (m, 2 H) 4.39 - 4.46 (m, 1 H) 4.52 (br d, J =19.64 Hz, 1 H) 4.93 (d, J =4.63 Hz, 1 H) 5.40 - 5.48 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.13, 1.50 Hz, 1 H) 7.29 (d, J =10.13 Hz, 1 H) 7.37 (d, J =8.50 Hz, 2 H) 7.61 (d, J =8.50 Hz, 2 H) 8.28 - 8.33 (m, 1 H) 8.41 - 8.57 (m, 1 H) 10.06 (s, 1 H) 12.07 - 12.22 (m, 1 H).

2-Bromo-N-(2-((2-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)amino)-2-oxoethyl)acetamide (LD15).



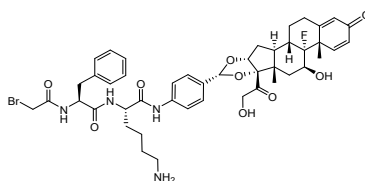
Prepared in a similar manner to compound **LD1** (17 mg, 6% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.03 min; MS m/z : 732.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.95 (s, 1H), 8.59 (br t, J =5.6 Hz, 1H), 8.34 (t, J =5.7 Hz, 1H), 7.60 (d, J =8.6 Hz, 2H), 7.36 (d, J =8.6 Hz, 2H), 7.29 (d, J =10.1 Hz, 1H), 6.23 (dd, J =1.8, 10.1 Hz, 1H), 6.03 (s, 1H), 5.43 (s, 2H), 4.93 (d, J =4.6 Hz, 1H), 4.52 (d, J =19.4 Hz, 1H), 4.25 - 4.15 (m, 2H), 3.95 (s, 2H), 3.90 (br d, J =5.7 Hz, 2H), 3.80 (d, J =5.6 Hz, 2H), 2.64 - 2.59 (m, 1H), 2.37 (br d, J =3.2 Hz, 1H), 2.16 (dt, J =6.8, 12.1 Hz, 2H), 2.05 (br d, J =13.3 Hz, 2H), 1.89 - 1.81 (m, 1H), 1.73 - 1.63 (m, 3H), 1.50 (s, 3H), 1.41 (br dd, J =4.5, 13.1 Hz, 1H), 0.87 (s, 3H).

(S)-2-(2-(2-Bromoacetamido)acetamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-3-hydroxypropanamide (LD16).



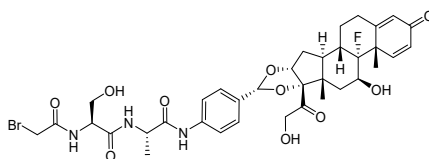
Prepared in a similar manner to compound **LD8** (41 mg, 29% yield) as a white solid. LCMS (Method d, Table S1) R_t = 1.98 min; MS m/z : 764.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.33 - 1.44 (m, 1 H) 1.50 (s, 3 H) 1.59 - 1.73 (m, 3 H) 1.79 - 1.92 (m, 1 H) 1.99 - 2.08 (m, 1 H) 2.16 (td, J =12.07, 6.63 Hz, 1 H) 2.37 (br s, 1 H) 2.57 - 2.65 (m, 1 H) 3.55 - 3.72 (m, 3 H) 3.83 (d, J =5.50 Hz, 2 H) 3.94 (s, 2 H) 4.15 - 4.27 (m, 2 H) 4.43 - 4.57 (m, 2 H) 4.93 (d, J =4.63 Hz, 1 H) 5.39 - 5.49 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.13, 1.75 Hz, 1 H) 7.29 (d, J =10.13 Hz, 1 H) 7.36 (d, J =8.50 Hz, 2 H) 7.63 (d, J =8.50 Hz, 2 H) 8.21 (d, J =7.75 Hz, 1 H) 8.53 (t, J =5.50 Hz, 1 H) 10.01 (s, 1 H).

(S)-6-Amino-2-((S)-2-(2-bromoacetamido)-3-phenylpropanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)hexanamide (LD17).



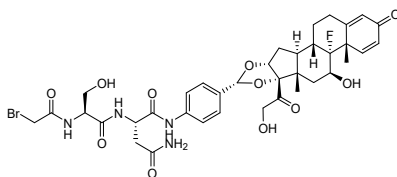
Prepared in a similar manner to compound **LD7** (53 mg, 29% yield) as a yellow solid. LCMS (Method d, Table S1) R_t = 2.05 min; MS m/z : 895.4 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.28 - 1.45 (m, 3 H) 1.48 - 1.58 (m, 5 H) 1.61 - 1.77 (m, 5 H) 1.81 - 1.90 (m, 1 H) 2.00 - 2.08 (m, 1 H) 2.11 - 2.22 (m, 1 H) 2.31 - 2.39 (m, 1 H) 2.62 - 2.70 (m, 1 H) 2.72 - 2.82 (m, 3 H) 3.04 (m, 1 H) 3.85 (s, 2 H) 4.17 - 4.24 (m, 3 H) 4.38 - 4.44 (m, 1 H) 4.50 (s, 1 H) 4.54 - 4.63 (m, 2 H) 4.94 (d, J =3.97 Hz, 1 H) 5.45 (s, 2 H) 6.02 (s, 1 H) 6.23 (d, J =10.14 Hz, 1 H) 7.11 - 7.25 (m, 5 H) 7.29 (d, J =10.14 Hz, 1 H) 7.38 (d, J =8.60 Hz, 2 H) 7.61 (d, J =8.38 Hz, 2 H) 7.69 (br s, 3 H) 8.42 (br d, J =7.72 Hz, 1 H) 8.50 (d, J =7.94 Hz, 1 H) 10.14 (s, 1 H).

(S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)-3-hydroxypropanamide (LD18).



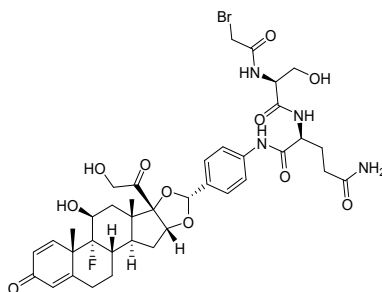
Prepared in a similar manner to compound **LD8** (7 mg, 14% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.06 min; MS m/z : 778.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.86 (s, 3 H) 1.28 - 1.43 (m, 4 H) 1.50 (s, 3 H) 1.60 - 1.73 (m, 3 H) 1.79 - 1.90 (m, 1 H) 1.99 - 2.08 (m, 1 H) 2.16 (td, J =11.76, 7.25 Hz, 1 H) 2.36 (br s, 1 H) 2.54 - 2.63 (m, 1 H) 3.97 (s, 2 H) 4.15 - 4.23 (m, 2 H) 4.34 - 4.46 (m, 2 H) 4.52 (br d, J =19.39 Hz, 1 H) 4.93 (d, J =4.38 Hz, 1 H) 5.39 - 5.51 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.07, 1.56 Hz, 1 H) 7.29 (d, J =10.13 Hz, 1 H) 7.36 (d, J =8.50 Hz, 2 H) 7.59 (d, J =8.63 Hz, 2 H) 8.46 (br t, J =8.25 Hz, 2 H) 9.81 (s, 1 H).

(S)-2-((S)-2-(2-Bromoacetamido)-3-hydroxypropanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)succinamide (LD19).



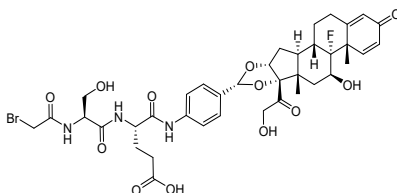
Prepared in a similar manner to compound **LD8** (15 mg, 16% yield) as a white solid. LCMS (Method d, Table S1) R_t = 1.96 min; MS m/z : 821.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.31 - 1.46 (m, 1 H) 1.50 (s, 3 H) 1.58 - 1.75 (m, 3 H) 1.80 - 1.91 (m, 1 H) 2.04 (br d, J =13.45 Hz, 1 H) 2.16 (td, J =11.85, 7.61 Hz, 1 H) 2.29 - 2.41 (m, 1 H) 2.53 - 2.75 (m, 4 H) 3.51 - 3.58 (m, 2 H) 3.66 (br dd, J =10.25, 5.40 Hz, 2 H) 3.98 (s, 1 H) 4.12 - 4.25 (m, 3 H) 4.32 - 4.44 (m, 1 H) 4.52 (d, J =19.62 Hz, 1 H) 4.65 - 4.77 (m, 1 H) 4.93 (d, J =4.63 Hz, 1 H) 5.44 (s, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.14, 1.76 Hz, 1 H) 6.95 (br s, 1 H) 7.29 (d, J =10.14 Hz, 1 H) 7.33 - 7.44 (m, 3 H) 7.60 (d, J =8.60 Hz, 2 H) 8.34 - 8.57 (m, 2 H) 9.67 (d, J =3.09 Hz, 1 H).

(S)-2-((S)-2-(2-Bromoacetamido)-3-hydroxypropanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)pentanediamide (LD20).



Prepared in a similar manner to compound **LD8** (10 mg, 18% yield) as a white solid. LCMS (Method d, Table S1) R_t = 1.96 min; MS m/z : 835.3 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.87 (s, 3 H) 1.33 - 1.44 (m, 1 H) 1.50 (s, 3 H) 1.59 - 1.74 (m, 3 H) 1.76 - 1.91 (m, 2 H) 1.94 - 2.07 (m, 2 H) 2.10 - 2.22 (m, 3 H) 2.37 (br s, 1 H) 2.57 - 2.65 (m, 1 H) 3.66 (br d, J =4.75 Hz, 2 H) 3.93 - 4.03 (m, 2 H) 4.12 - 4.25 (m, 3 H) 4.37 (q, J =6.34 Hz, 2 H) 4.52 (br d, J =19.51 Hz, 1 H) 4.93 (d, J =4.50 Hz, 1 H) 5.44 (s, 2 H) 6.04 (s, 1 H) 6.23 (dd, J =10.01, 1.38 Hz, 1 H) 6.79 (br s, 1 H) 7.23 - 7.32 (m, 2 H) 7.36 (d, J =8.50 Hz, 2 H) 7.60 (d, J =8.50 Hz, 2 H) 8.38 - 8.52 (m, 2 H) 9.82 (s, 1 H).

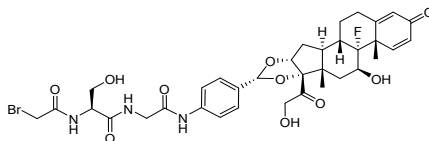
(S)-4-((S)-2-(2-Bromoacetamido)-3-hydroxypropanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD21).



Prepared in a similar manner to compound **LD8** and **LD4** (17 mg, 12% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.03 min; MS m/z : 834.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.86 (s, 3 H) 1.35 - 1.44 (m, 1 H) 1.49 (s, 3 H) 1.62 - 1.73 (m, 3 H) 1.77 - 1.89 (m, 2 H) 1.98 - 2.09 (m, 2 H) 2.16 (td, J =11.85, 7.32 Hz, 1 H) 2.24 - 2.31 (m, 2 H) 2.37 (br d, J =3.38 Hz, 1 H) 2.57 - 2.64 (m, 1 H) 3.55 (br dd, J =10.63, 6.00 Hz, 1 H) 3.65 (br dd, J =10.51, 5.63 Hz, 1 H) 3.97 (s, 2 H) 4.11 - 4.25 (m, 3 H) 4.32 - 4.45 (m, 2 H) 4.52 (d, J =19.39 Hz, 1 H) 4.93 (d, J =4.63 Hz, 1 H) 5.40 - 5.48 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.13, 1.63 Hz, 1 H) 7.29 (d, J =10.13 Hz, 1 H) 7.36 (d, J =8.50 Hz, 2 H) 7.58 (d, J =8.63 Hz, 2 H) 8.32 - 8.40 (m, 1 H) 8.47 (d, J =7.50 Hz, 1 H) 9.80 (s, 1 H).

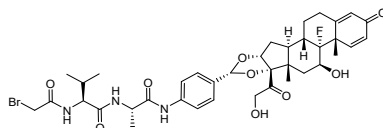
(S)-2-(2-Bromoacetamido)-N-(2-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-

naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)-3-hydroxypropanamide (LD22).



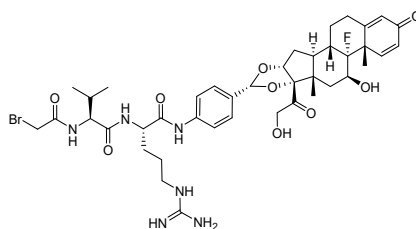
Prepared in a similar manner to compound **LD8** (30 mg, 18% yield) as a yellow solid. LCMS (Method d, Table S1) $R_t = 1.83$ min; MS m/z : 764.2 (M+H)⁺. ¹H NMR: 15024011-2032-p1 (400 MHz, DMSO-*d*₆) δ ppm 0.80 - 0.95 (m, 3 H) 1.41 (br dd, $J=12.59, 4.03$ Hz, 1 H) 1.50 (s, 3 H) 1.61 - 1.74 (m, 3 H) 1.80 - 1.90 (m, 1 H) 1.95 - 2.09 (m, 2 H) 2.11 - 2.22 (m, 1 H) 2.36 (br s, 1 H) 2.55 (br s, 1 H) 2.55 - 2.64 (m, 2 H) 3.87 - 3.95 (m, 3 H) 3.99 - 4.04 (m, 1 H) 4.13 - 4.26 (m, 3 H) 4.52 (br d, $J=19.56$ Hz, 1 H) 4.93 (br d, $J=4.40$ Hz, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.18 - 6.28 (m, 1 H) 7.25 - 7.40 (m, 3 H) 7.60 (br d, $J=8.31$ Hz, 2 H) 8.34 - 8.51 (m, 2 H) 9.97 (s, 1 H)

(S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-1-oxopropan-2-yl)-3-methylbutanamide (LD23).



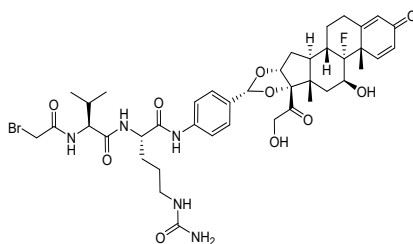
Prepared in a similar manner to compound **LD1** (6 mg, 3% yield) as a white solid. LCMS (Method c, Table S1) $R_t = 1.64$ min; MS m/z : 790.4 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.76 - 0.93 (m, 9 H) 1.29 (d, $J=7.00$ Hz, 3 H) 1.37 - 1.54 (m, 4 H) 1.59 - 1.72 (m, 3 H) 1.79 - 1.90 (m, 1 H) 1.92 - 2.08 (m, 2 H) 2.10 - 2.21 (m, 1 H) 2.36 (br s, 1 H) 2.55 - 2.66 (m, 1 H) 3.84 - 4.03 (m, 2 H) 4.14 - 4.26 (m, 3 H) 4.38 (br t, $J=6.94$ Hz, 1 H) 4.52 (d, $J=19.39$ Hz, 1 H) 4.93 (d, $J=4.38$ Hz, 1 H) 5.39 - 5.49 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, $J=10.13, 1.75$ Hz, 1 H) 7.29 (d, $J=10.13$ Hz, 1 H) 7.35 (d, $J=8.50$ Hz, 2 H) 7.58 (d, $J=8.50$ Hz, 2 H) 8.29 - 8.39 (m, 2 H) 10.04 (s, 1 H).

(S)-2-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-guanidinopentanamide (LD24).



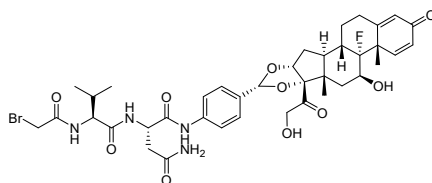
Prepared in a similar manner to compound **LD2** (17 mg, 15% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 2.04$ min; MS m/z : 875.1 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.78 - 0.91 (m, 9 H) 1.32 - 1.56 (m, 6 H) 1.59 - 1.78 (m, 5 H) 1.79 - 1.90 (m, 1 H) 1.91 - 2.07 (m, 2 H) 2.09 - 2.20 (m, 1 H) 2.35 - 2.46 (m, 1 H) 2.55 - 2.65 (m, 1 H) 3.11 (q, $J=6.69$ Hz, 2 H) 3.89 - 4.00 (m, 2 H) 4.13 - 4.27 (m, 3 H) 4.33 - 4.44 (m, 1 H) 4.51 (d, $J=19.40$ Hz, 1 H) 4.94 (d, $J=3.53$ Hz, 1 H) 5.40 - 5.49 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, $J=10.14, 1.76$ Hz, 1 H) 6.63 - 7.14 (m, 2 H) 7.29 (d, $J=10.14$ Hz, 1 H) 7.36 (d, $J=8.60$ Hz, 2 H) 7.47 (br dd, $J=5.07, 1.10$ Hz, 1 H) 7.58 (d, $J=8.60$ Hz, 2 H) 8.32 (t, $J=8.27$ Hz, 2 H) 10.10 (s, 1 H).

(S)-2-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-N-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)-5-ureidopentanamide (LD25).



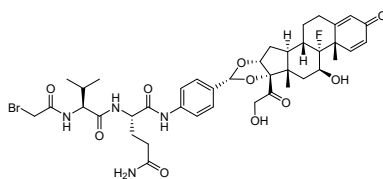
Prepared in a similar manner to compound **LD2** (16 mg, 9% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.14 min; MS m/z : 876.3 (M+H)⁺. ¹H NMR: (400 MHz, DMSO-*d*₆) δ ppm 0.75 - 0.95 (m, 9 H) 1.28 - 1.45 (m, 3 H) 1.50 (s, 3 H) 1.55 - 1.61 (m, 1 H) 1.62 - 1.74 (m, 4 H) 1.79 - 1.90 (m, 1 H) 1.94 - 2.08 (m, 2 H) 2.11 - 2.22 (m, 1 H) 2.37 (br s, 1 H) 2.57 - 2.67 (m, 1 H) 2.84 - 3.14 (m, 1 H) 3.88 - 4.05 (m, 4 H) 4.08 - 4.30 (m, 4 H) 4.34 - 4.43 (m, 1 H) 4.52 (br d, J =19.56 Hz, 1 H) 4.93 (br d, J =4.52 Hz, 1 H) 5.42 (s, 2 H) 5.93 - 6.12 (m, 2 H) 6.23 (br d, J =10.15 Hz, 1 H) 7.29 (d, J =10.03 Hz, 1 H) 7.35 (br d, J =8.44 Hz, 2 H) 7.60 (d, J =8.56 Hz, 2 H) 8.13 - 8.45 (m, 2 H) 10.08 (s, 1 H).

(S)-2-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)succinamide (LD26).



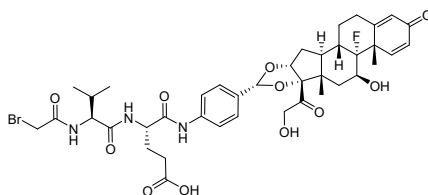
Prepared in a similar manner to compound **LD1** (16 mg, 8% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.11 min; MS m/z : 833.2 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.76 - 0.91 (m, 9 H) 1.34 - 1.45 (m, 1 H) 1.50 (s, 3 H) 1.59 - 1.73 (m, 3 H) 1.79 - 1.89 (m, 1 H) 1.91 - 2.09 (m, 2 H) 2.16 (td, J =12.04, 7.07 Hz, 1 H) 2.45 (br d, J =7.88 Hz, 1 H) 2.59 (br dd, J =15.70, 5.94 Hz, 2 H) 3.89 - 3.94 (m, 1 H) 3.98 - 4.03 (m, 1 H) 4.07 - 4.27 (m, 4 H) 4.52 (d, J =19.51 Hz, 1 H) 4.66 (q, J =7.00 Hz, 1 H) 4.93 (d, J =4.63 Hz, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.23 (dd, J =10.07, 1.81 Hz, 1 H) 6.93 (br s, 1 H) 7.26 - 7.38 (m, 4 H) 7.60 (d, J =8.50 Hz, 2 H) 8.25 (d, J =8.50 Hz, 1 H) 8.32 - 8.42 (m, 1 H) 9.92 (s, 1 H).

(S)-2-((S)-2-(2-bromoacetamido)-3-methylbutanamido)-N1-(4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)pentanediamide (LD27).



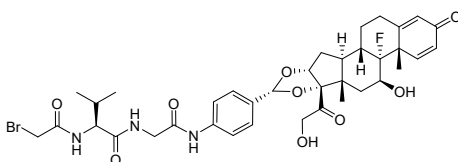
Prepared in a similar manner to compound **LD1** (12 mg, 18% yield) as a white solid. LCMS (Method d, Table S1) R_t = 2.14 min; MS m/z : 847.3 (M+H)⁺. ¹H NMR: (400 MHz, DMSO-*d*₆) δ ppm 0.79 - 0.90 (m, 9 H) 1.37 - 1.45 (m, 1 H) 1.50 (s, 3 H) 1.62 - 1.73 (m, 3 H) 1.79 - 2.22 (m, 9 H) 2.28 - 2.42 (m, 2 H) 2.60 - 2.68 (m, 1 H) 3.84 - 4.07 (m, 2 H) 4.10 - 4.39 (m, 4 H) 4.52 (d, J =19.40 Hz, 1 H) 4.93 (d, J =4.41 Hz, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.16 - 6.31 (m, 1 H) 6.78 (br d, J =1.54 Hz, 1 H) 7.25 - 7.41 (m, 4 H) 7.59 (d, J =8.60 Hz, 2 H) 8.27 - 8.40 (m, 2 H) 10.06.

(S)-4-((S)-2-(2-Bromoacetamido)-3-methylbutanamido)-5-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-5-oxopentanoic acid (LD28).



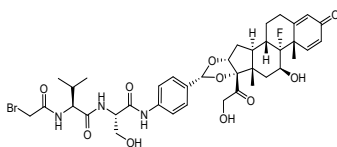
Prepared in a similar manner to compound **LD4** (5 mg, 3% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 2.184$ min; MS m/z : 848.3 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.77 - 0.96 (m, 9 H) 1.43 (br dd, $J=12.32$, 4.19 Hz, 1 H) 1.52 (s, 3 H) 1.62 - 1.75 (m, 3 H) 1.80 - 1.92 (m, 2 H) 1.93 - 2.02 (m, 2 H) 2.03 - 2.12 (m, 1 H) 2.13 - 2.20 (m, 1 H) 2.20 - 2.27 (m, 1 H) 2.30 (br dd, $J=10.57$, 4.06 Hz, 1 H) 2.38 (br s, 1 H) 2.60 - 2.67 (m, 1 H) 3.88 - 3.97 (m, 1 H) 3.98 - 4.07 (m, 1 H) 4.16 - 4.28 (m, 3 H) 4.33 - 4.42 (m, 1 H) 4.54 (br d, $J=19.39$ Hz, 1 H) 4.95 (d, $J=4.50$ Hz, 1 H) 5.39 - 5.53 (m, 2 H) 6.05 (s, 1 H) 6.25 (dd, $J=10.13$, 1.50 Hz, 1 H) 7.25 - 7.42 (m, 3 H) 7.60 (d, $J=8.50$ Hz, 2 H) 8.35 (br dd, $J=11.69$, 8.07 Hz, 2 H) 10.09 (s, 1 H).

(S)-2-(2-Bromoacetamido)-N-(2-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-2-oxoethyl)-3-methylbutanamide (LD29).



Prepared in a similar manner to compound **LD1** (3 mg, 2% yield) as a white solid. LCMS (Method d, Table S1) $R_t = 2.19$ min; MS m/z : 776.3 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.80 - 0.95 (m, 9 H) 1.41 (br dd, $J=12.59$, 4.03 Hz, 1 H) 1.50 (s, 3 H) 1.61 - 1.74 (m, 3 H) 1.80 - 1.90 (m, 1 H) 1.95 - 2.09 (m, 2 H) 2.11 - 2.22 (m, 1 H) 2.36 (br s, 1 H) 2.55 (br s, 1 H) 2.55 - 2.64 (m, 1 H) 3.87 - 3.95 (m, 3 H) 3.99 - 4.04 (m, 1 H) 4.13 - 4.26 (m, 3 H) 4.52 (br d, $J=19.56$ Hz, 1 H) 4.93 (br d, $J=4.40$ Hz, 1 H) 5.43 (s, 2 H) 6.03 (s, 1 H) 6.18 - 6.28 (m, 1 H) 7.25 - 7.40 (m, 3 H) 7.60 (br d, $J=8.31$ Hz, 2 H) 8.34 - 8.51 (m, 2 H) 9.97 (s, 1 H).

(S)-2-(2-Bromoacetamido)-N-((S)-1-((4-((6aS,6bR,7S,8aS,8bS,10R,11aR,12aS,12bS)-6b-fluoro-7-hydroxy-8b-(2-hydroxyacetyl)-6a,8a-dimethyl-4-oxo-2,4,6a,6b,7,8,8a,8b,11a,12,12a,12b-dodecahydro-1H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-10-yl)phenyl)amino)-3-hydroxy-1-oxopropan-2-yl)-3-methylbutanamide (LD30).



Prepared in a similar manner to compound **LD8** (30 mg, 20% yield) as an off-white solid. LCMS (Method d, Table S1) $R_t = 2.16$ min; MS m/z : 806.3 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 0.78 - 0.92 (m, 9 H) 1.37 - 1.45 (m, 1 H) 1.50 (s, 3 H) 1.61 - 1.73 (m, 3 H) 1.80 - 1.89 (m, 1 H) 1.94 - 2.08 (m, 2 H) 2.16 (td, $J=11.94$, 7.00 Hz, 1 H) 2.37 (br d, $J=2.38$ Hz, 1 H) 2.56 - 2.65 (m, 1 H) 3.64 (br dd, $J=5.82$, 1.94 Hz, 2 H) 3.88 - 4.03 (m, 2 H) 4.15 - 4.23 (m, 2 H) 4.28 (dd, $J=8.63$, 6.63 Hz, 1 H) 4.39 - 4.47 (m, 1 H) 4.52 (d, $J=19.51$ Hz, 1 H) 4.93 (d, $J=4.75$ Hz, 1 H) 5.00 - 5.25 (m, 1 H) 5.40 - 5.48 (m, 2 H) 6.03 (s, 1 H) 6.23 (dd, $J=10.07$, 1.69 Hz, 1 H) 7.29 (d, $J=10.13$ Hz, 1 H) 7.35 (d, $J=8.63$ Hz, 2 H) 7.61 (d, $J=8.50$ Hz, 2 H) 8.23 (br d, $J=7.38$ Hz, 1 H) 8.35 (d, $J=8.75$ Hz, 1 H) 10.04 (s, 1 H).

LCMS Conditions

Table S1.

| Method | Description |
|--------|--|
| a | Gradient: 25-100% B in 3.4 min with a hold at 100% B for 0.45 min, 100-25% B in 0.01 min, and then held at 25% B for 0.65 min. Mobile phase A was 0.0375% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column: Kinetex C18 50*2.1mm, 5um particles. Column: 2.1x 30 mm SunFire C18 3.5 μ m. Flow rate: 0.8 mL/min flow rate. |
| b | Gradient: 10-90% B in 1.15 with a hold at 90% B for 0.4 min, 90-10% B in 0.01 min, and then hold at |

| | |
|---|--|
| | 10% B for 0.54 min. Mobile phase A was 0.0375% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column: 2.1 x 30 mm Halo C18, 2.7 μ m particles. Flow rate: 1.0 mL/min. |
| c | Gradient: 5-95% B in 2.00 min. 5% B in 0.01 min, 5-95% B (0.01-1.00 min), 95-100% B (1.00 -1.80 min), 5% B in 1.81 min with a hold at 5% B for 0.19 min. Mobile phase A was 0.037% TFA in water, and mobile phase B was 0.018% TFA in MeCN. Column: Luna-C18 2.0x30mm, 3 μ m particles. Flow rate: 1.0 mL/min (0.00-1.80 min) and 1.2 mL/min (1.81 -2.00 min). |
| d | Gradient: 5%B in 0.40 min and 5-95% B at 0.40-3.00 min, hold on 95% B for 1.00min, and then 95-5%B in 0.01min. Mobile phase A was 0.037% TFA in water, mobile phase B was 0.018% TFA in MeCN. Column: Kinetex C18 50*2.1mm, 5 μ m particles. Flow rate: 1.0 ml/min. |
| e | Gradient: 10-80% B in 2.0 min, 80-80% B in 0.5 min, 80-10% B in 0.01 min, and then hold at 10% B for 0.5 min. Mobile phase A was 10 mM NH ₄ HCO ₃ , mobile phase B was MeCN. Column: 2.1 x 50 mm Xbridge C18 column, 5 μ m particles. Flow rate: 1.0 mL/min. |
| f | Gradient: 10-80% B in 4 min, held at 80% B for 0.9 min, 80-10% B in 0.01 min, and then held at 10% B for 1 min. Mobile phase A was 0.0375% TFA, mobile phase B was 0.018% TFA in MeCN. Column: 2.0 x 50 mm phenomenex Luna-C18 column, 5 μ m particles. Flow rate: 0.8 mL/min. |

Synthesis of Antibody-Drug Conjugates

DAR4 ADC Preparation

Mouse anti-TNF antibody 8C11 in PBS containing 5 mM of EDTA (10.6 mg/mL) was treated with DPhPEA (2.7 equivalents) at 4 °C overnight. To the reduced mAb was added the linker drugs (10 equivalents /antibody) in DMSO followed by addition of 1 M Tris buffer (pH 8.5) to a final concentration of 50 mM. The mixture was gently rocked at RT for 4 h before buffer exchange to 15 mM histidine buffer (pH 6.0) by elution through Sephadex G25 column. The ADC was sterile filtered through a 0.2 μ m filter and stored at -78 °C before analysis and *in vitro* test.

DAR10 ADC Preparation

Mouse anti-TNF antibody 8C11 in PBS containing 5 mM of EDTA (10.6 mg/mL) was treated with TCEP (5.75 equivalents) at 4 °C overnight. To the reduced mAb was added the drug linker (18-60 equivalents /antibody) in DMSO followed by addition of 1 M Tris buffer (pH 8.5) to a final concentration of 50 mM. The mixture was left at 4 °C for conjugation overnight before buffer exchange to 15 mM histidine buffer (pH 6.0) by elution through Sephadex G25 column. The ADC was sterile filtered through a 0.2 μ m filter and stored at -78 °C before analysis and *in vitro* test.

HPLC Analysis of ADCs

Hydrophobic Interaction Chromatography (HIC)

Approximately 25 μ g of the ADC was loaded onto an Ultimate 3000 Dual LC system equipped with a TSK-GEL Butyl-NPR column (4.6 mm \times 3.5 cm, Tosoh Bioscience, cat. 14947). The column was equilibrated with 100% buffer A followed by a 10 min linear gradient to 100% buffer B at a flow rate of 0.8 mL/min, where buffer A was 1.5 M ammonium sulfate, 25 mM potassium phosphate, pH 7.0; and buffer B was 20 mM potassium phosphate with 20% isopropanol, pH 7.0.

Size Exclusion Chromatography (SEC)

Approximately 15 μ g of the ADC was loaded onto an Ultimate 3000 Dual LC system equipped with a TSK-gel 3000 SWXL column (7.8 mm \times 30 cm, Tosoh Bioscience, cat. 08541). The column was eluted with an isocratic gradient of 100 mM sodium phosphate, 100 mM sodium sulfate, pH 6.8 for 17 min at a flow rate of 1.0 mL/min.

DAR LCMS Analysis

ADC samples were reduced with 5 mM TCEP at rt for 30 min. Approximately 50 ng of reduced ADC was injected onto an Agilent 1290 Infinity II UHPLC equipped with an Agilent PLRP-S column (4000 Å, 8 μ m, 2.1 x 50 mm) and coupled to an Agilent 6545XT QTOF MS system. The gradient was 10% B for 0.5 min, 10-90% B in 0.1 min and held at 90% B for 1.1 min, 90-10% B in 0.1 min, and then held at 10% B for 0.2 min. The mobile phases were A: 0.1% formic acid in water and B: 0.1% formic acid in MeCN. The flow rate was 0.6 mL/min, and the column compartment was maintained at 60 °C. Agilent DAR Calculator was used to calculate DAR of the ADC samples.

GRE Luciferase Reporter Assay

The HEK-293 GRE luciferase reporter cell line 293/GR-luc was purchased from Panomics (#RC0018). Cells were cultured in DMEM medium supplemented with 10% FBS, 1% Penicillin-Streptomycin and 100 μ g/mL Hygromycin B at 37 °C and 5% CO₂ according to the manufacturer's instructions. The cells were seeded at 4 x 10⁴ cells/well into a 96-well cell assay plate (Corning, #3842) in complete cell culture medium at 37 °C and 5% CO₂ overnight. After cell culture medium was removed, cells were incubated with various concentrations of the test compound in DMEM medium supplemented with 2% FBS, and 1% Penicillin-Streptomycin for overnight. On the following day, after the supernatants were removed, GRE luciferase reporter activity was measured according to the manufacturer's instructions using a SteadyLitePlus (PerkinElmer, #6066751) prior to being read on an Envision (PerkinElmer, serial

#1040867). The full response for stimulation of HEK293 GRE reporter gene was determined by 10 μ M of prednisolone. The dose response data were fitted to a sigmoidal curve using nonlinear regression, and the EC₅₀ values calculated with the aid of GraphPad 5.0 (GraphPad Software, Inc.).