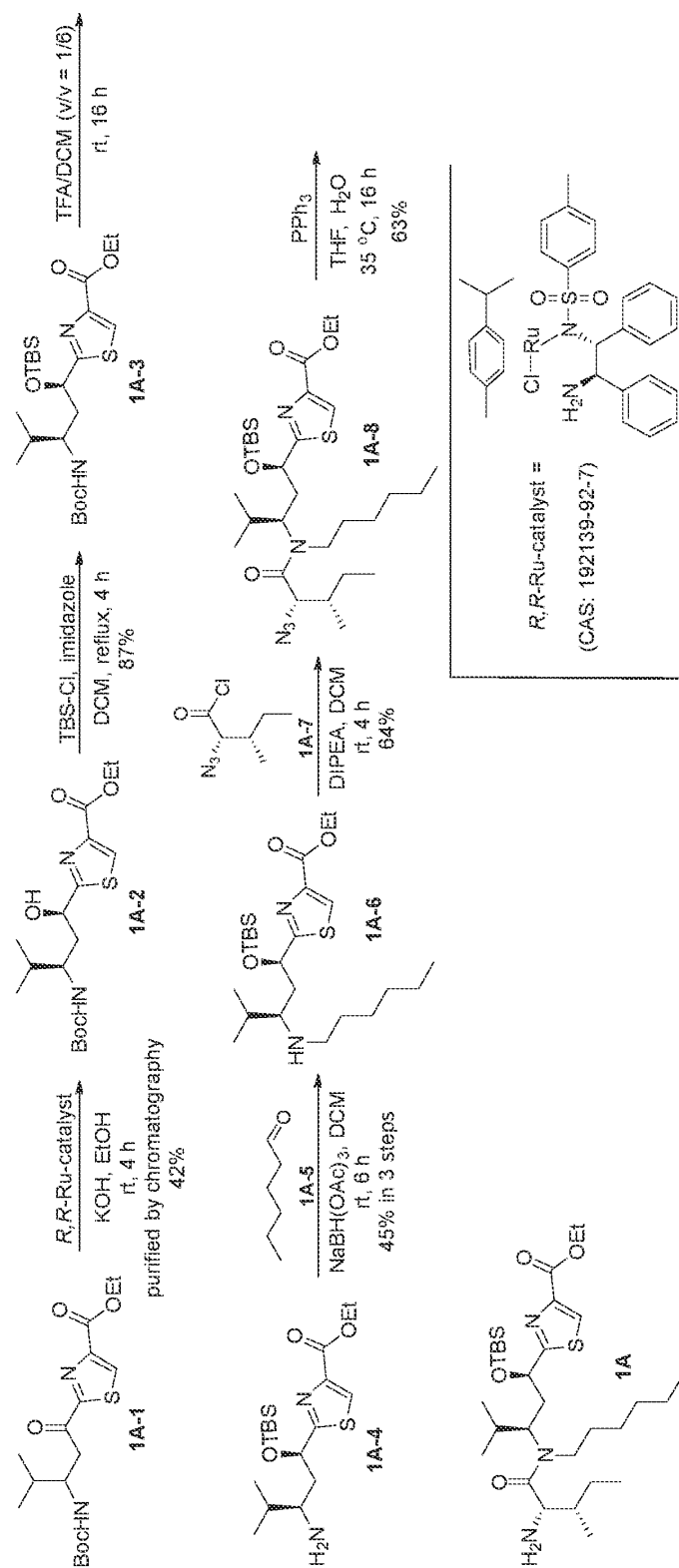


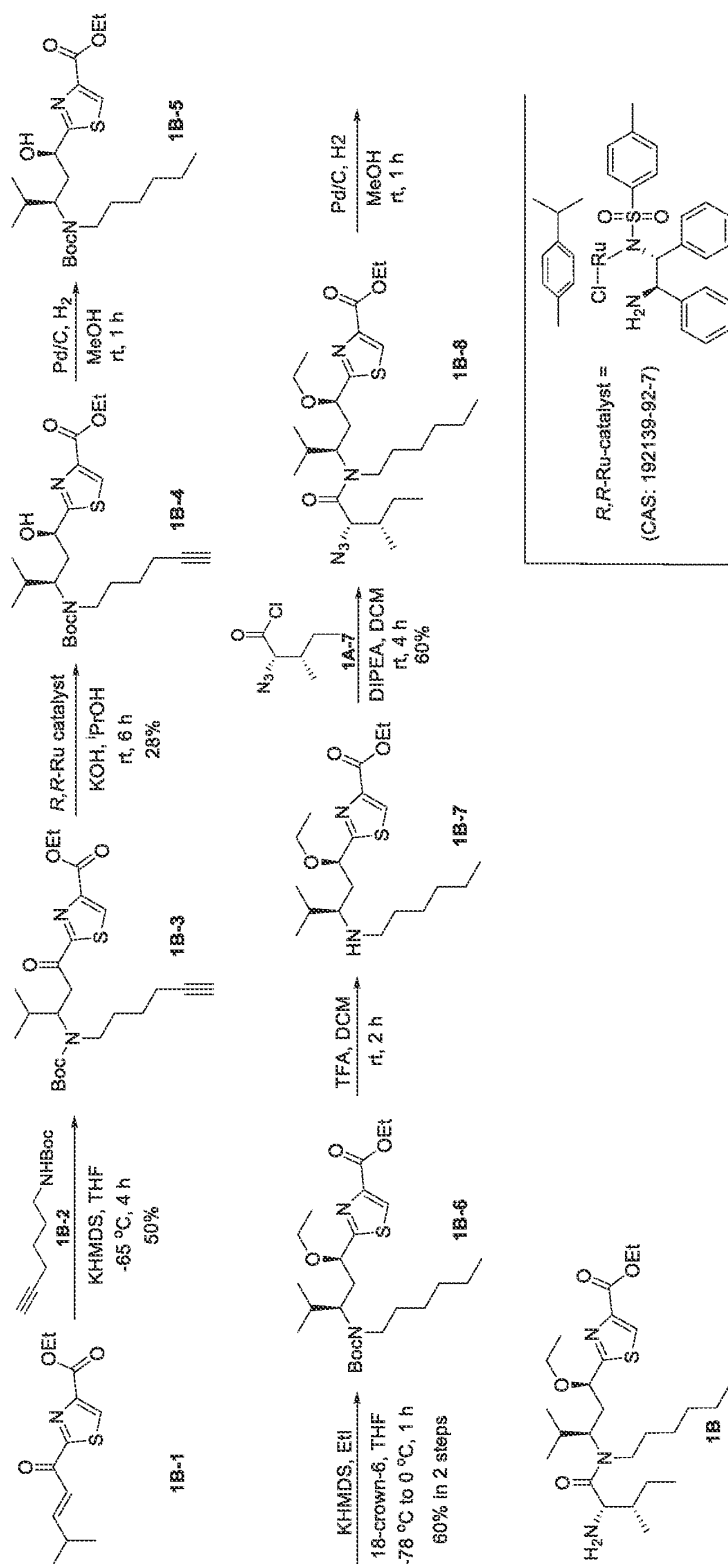
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FIG. 1. Synthesis of Intermediate 1A



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FIG. 2. Synthesis of Intermediate 1B



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FIG. 3. Synthesis of Intermediate 1C

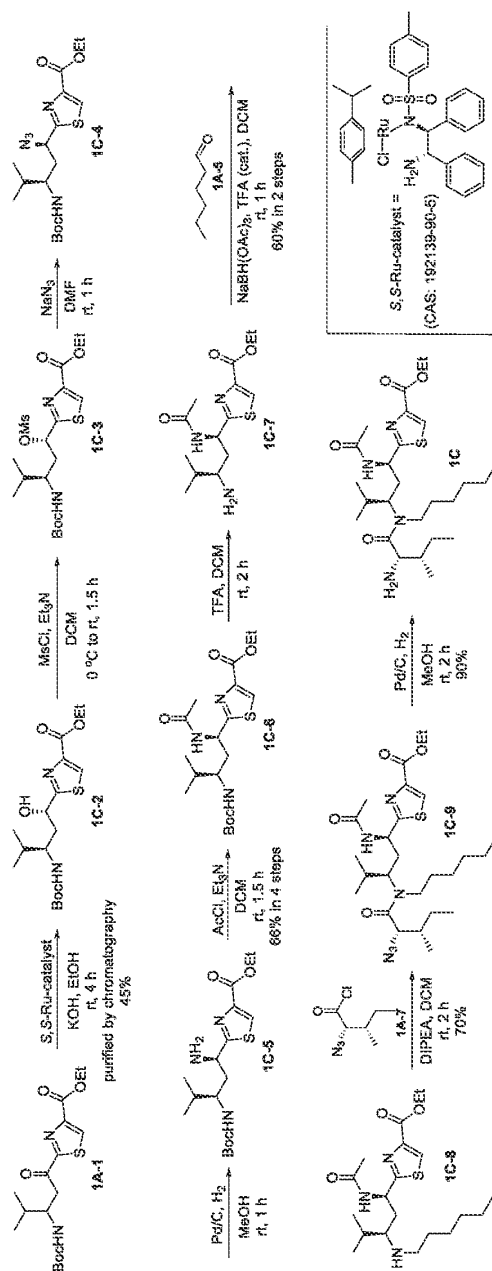


FIG. 4. Synthesis of Intermediate 1G

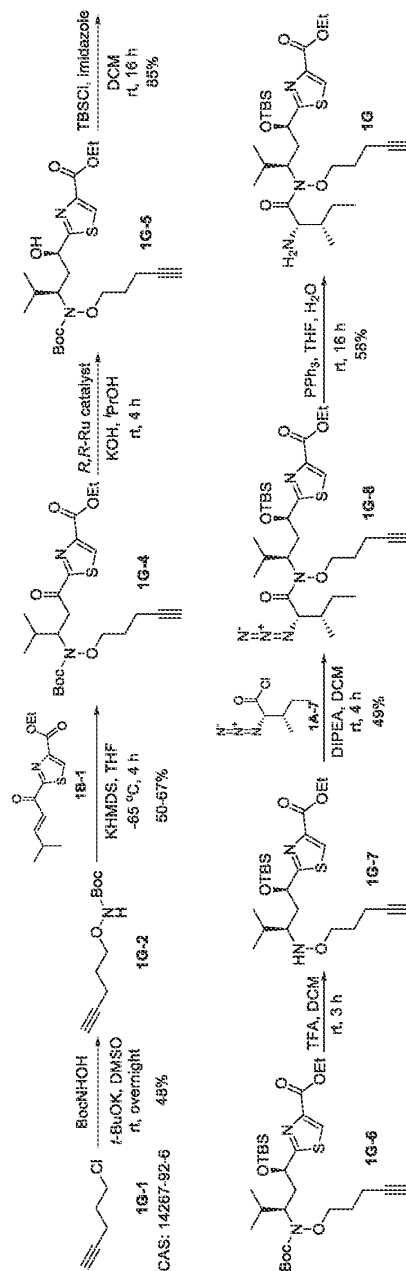
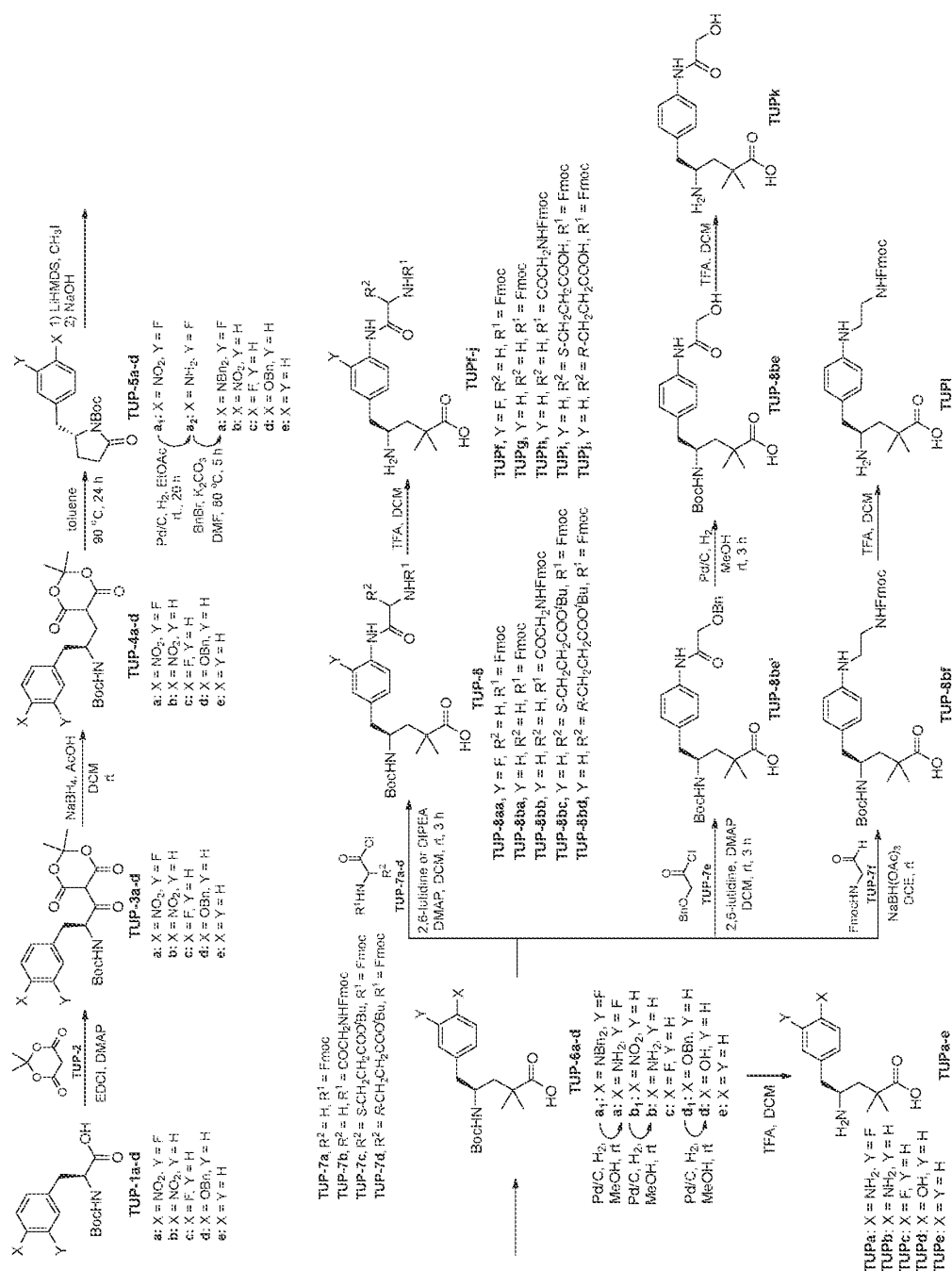
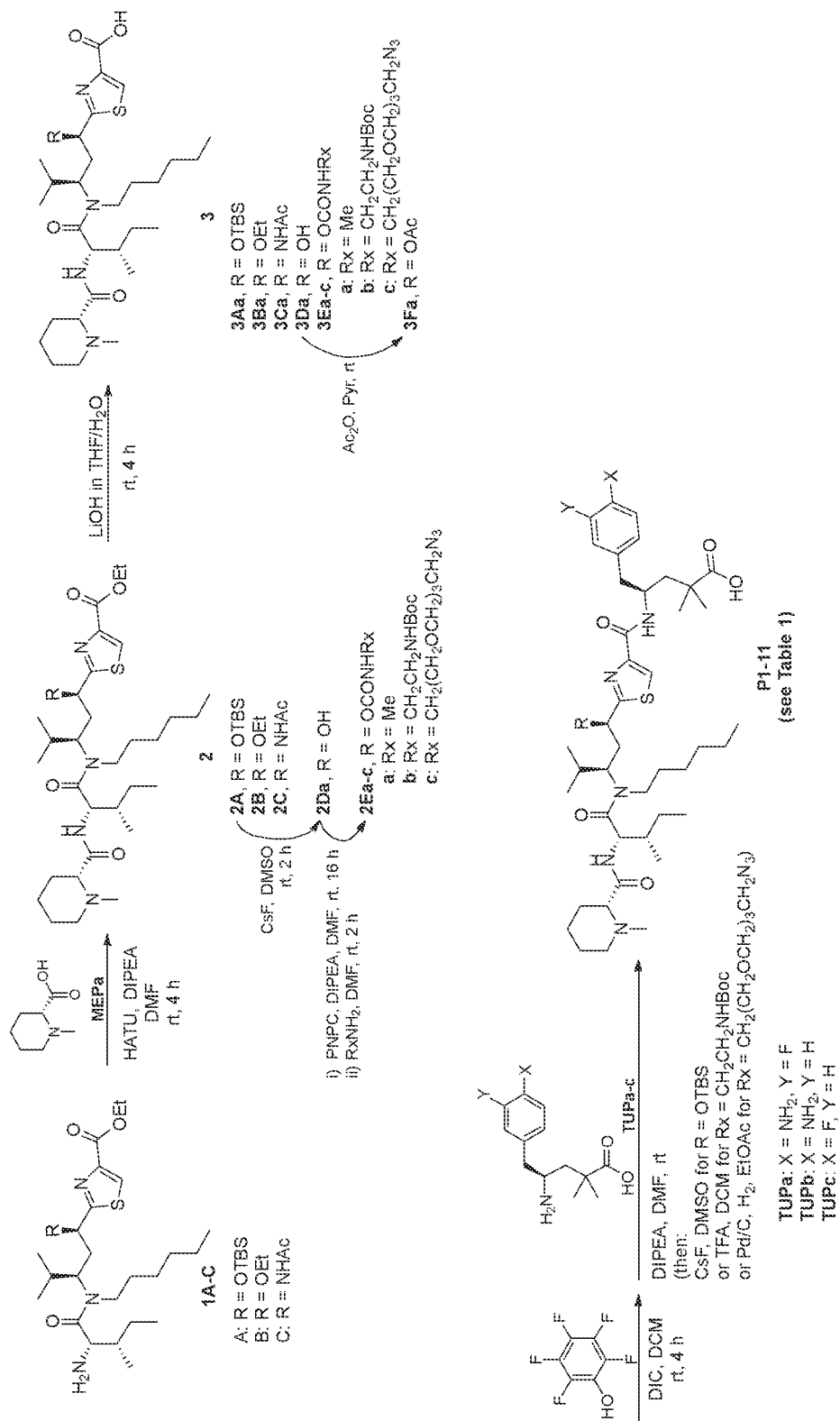


FIG. 5. Synthesis of Intermediate TUP



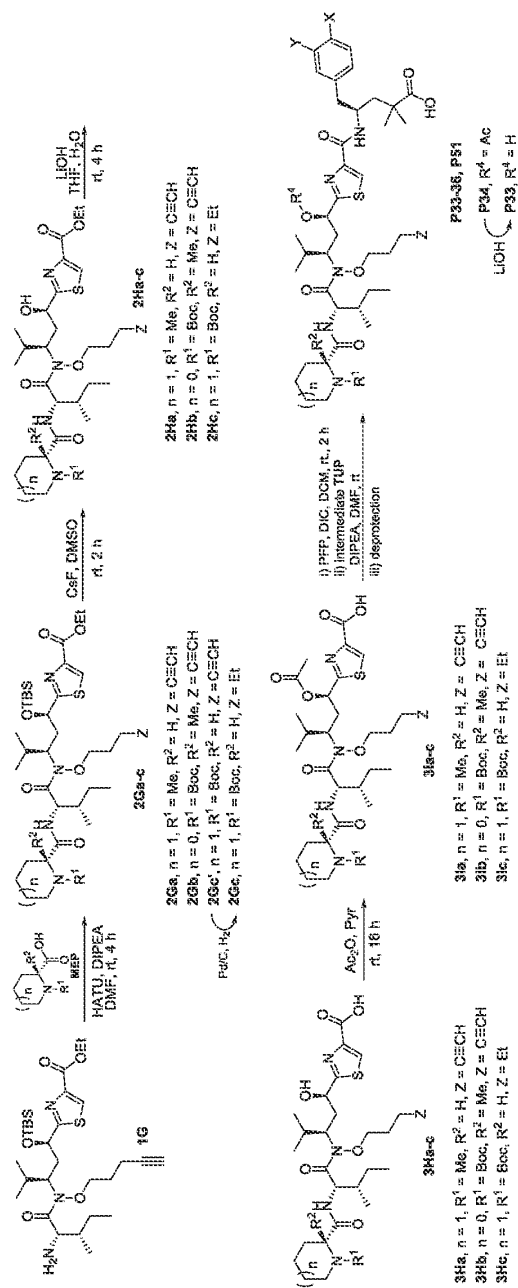
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FIG. 6. Synthesis of Tubulysin Payloads in Table 1



Scheme 1 illustrates the synthesis of various **2Da-e** and **3Ba-f** derivatives. The scheme shows the reaction of **1A** (a substituted thiazole derivative) with **MEP a-e** (various amino acids) under conditions of HATU, DIPEA, and DMF at room temperature for 4 hours. The resulting products are **2Aa-e** (where $R^1 = \text{Me}$, $R^2 = R^3 = \text{H}$; $R^1 = \text{Boc}$, $R^2 = R^3 = \text{H}$; $R^1 = \text{Me}$, $R^2 = \text{H}$, $R^3 = \text{Me}$; $R^1 = \text{Boc}$, $R^2 = \text{H}$, $R^3 = \text{Me}$; $R^1 = \text{Boc}$, $R^2 = \text{Me}$, $R^3 = \text{H}$) and **2Ba-e** (where $R^1 = \text{Me}$, $R^2 = R^3 = \text{H}$; $R^1 = \text{Boc}$, $R^2 = R^3 = \text{H}$; $R^1 = \text{Me}$, $R^2 = \text{H}$, $R^3 = \text{Me}$; $R^1 = \text{Boc}$, $R^2 = \text{H}$, $R^3 = \text{Me}$; $R^1 = \text{Boc}$, $R^2 = \text{Me}$, $R^3 = \text{H}$). The products are then converted to **3Ba-f** (where $R^4 = \text{Et}$; $R^4 = \text{H}$; $R^4 = \text{CONHMe}$; $R^4 = \text{Ac}$) using TFA, DCM, and MeOH/H₂O, followed by Pst/C, H_2 . The final products are **3Ba-f** (where $R^4 = \text{Et}$; $R^4 = \text{H}$; $R^4 = \text{CONHMe}$; $R^4 = \text{Ac}$). The scheme also shows the conversion of **2Da-e** to **3Ba-f** via **3Ba-f** (where $R^4 = \text{Et}$; $R^4 = \text{H}$; $R^4 = \text{CONHMe}$; $R^4 = \text{Ac}$) using TFA, DCM, and MeOH/H₂O, followed by Pst/C, H_2 .

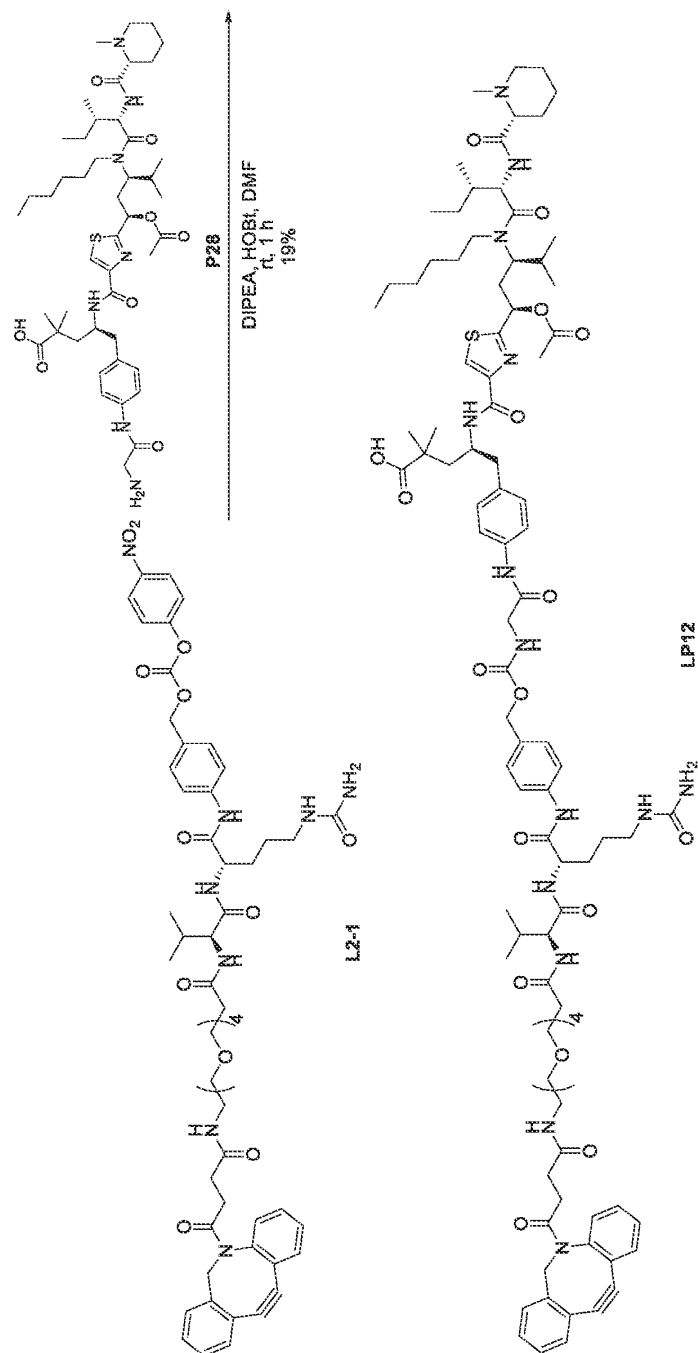
FIG. 9. Synthesis of Tubulysin Payloads in Table 4





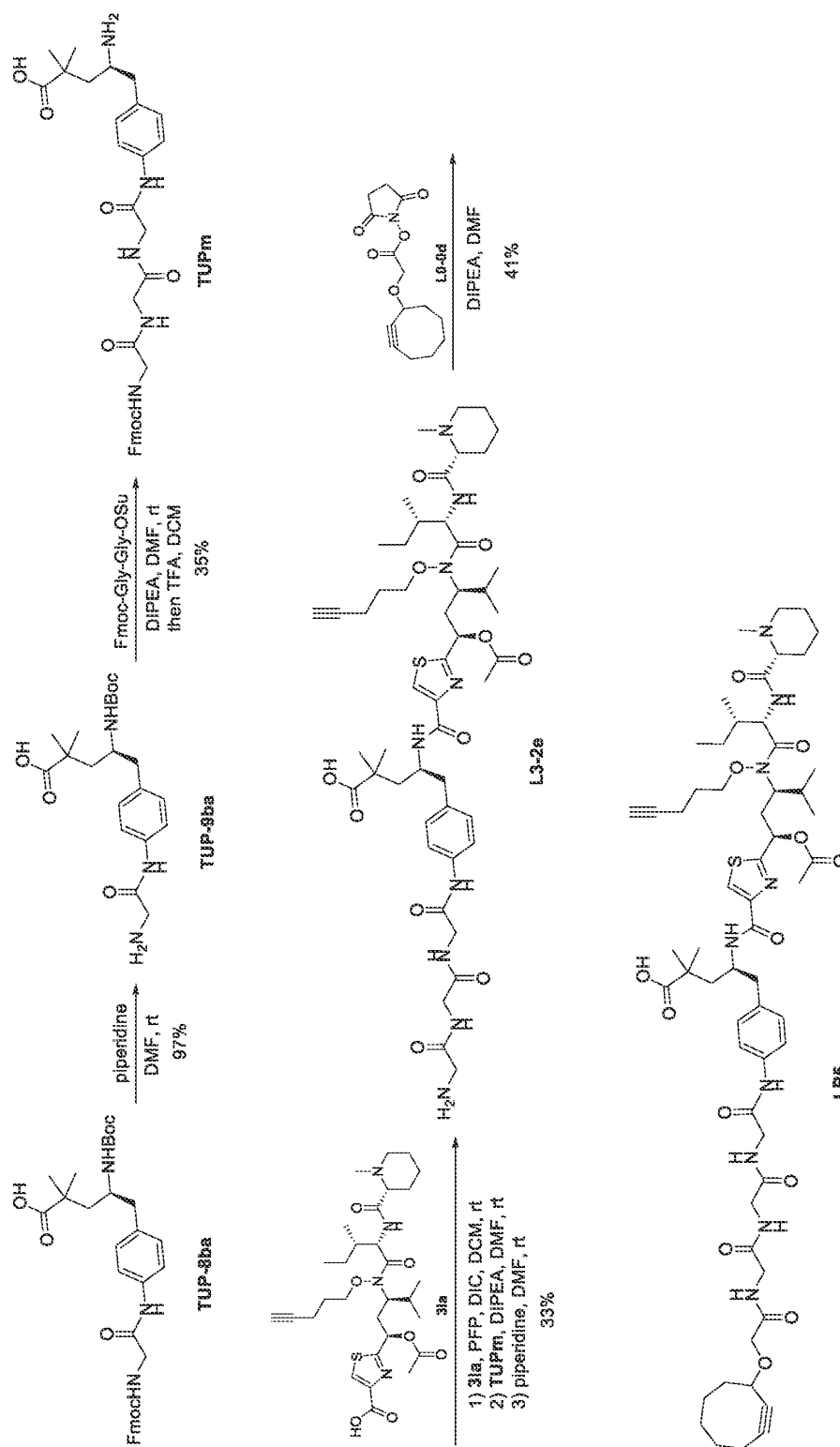
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FIG. 12B. Synthesis of Linker-Payload LP12



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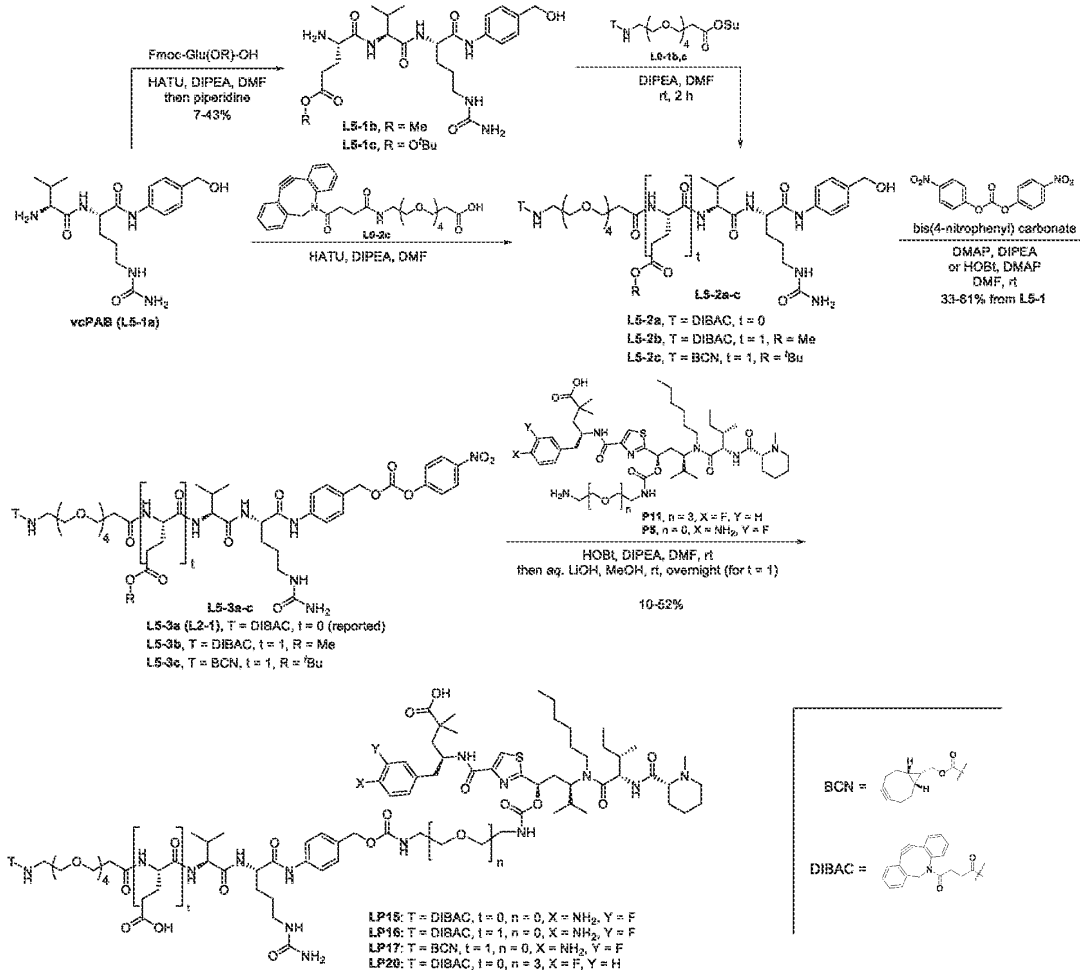
FIG. 13B. Synthesis of Linker-Payload LP5





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FIG. 15A. Synthesis of Linker-Payloads LP15-LP17 and LP20



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FIG. 15B. Synthesis of Linker-Payloads LP18 and LP19

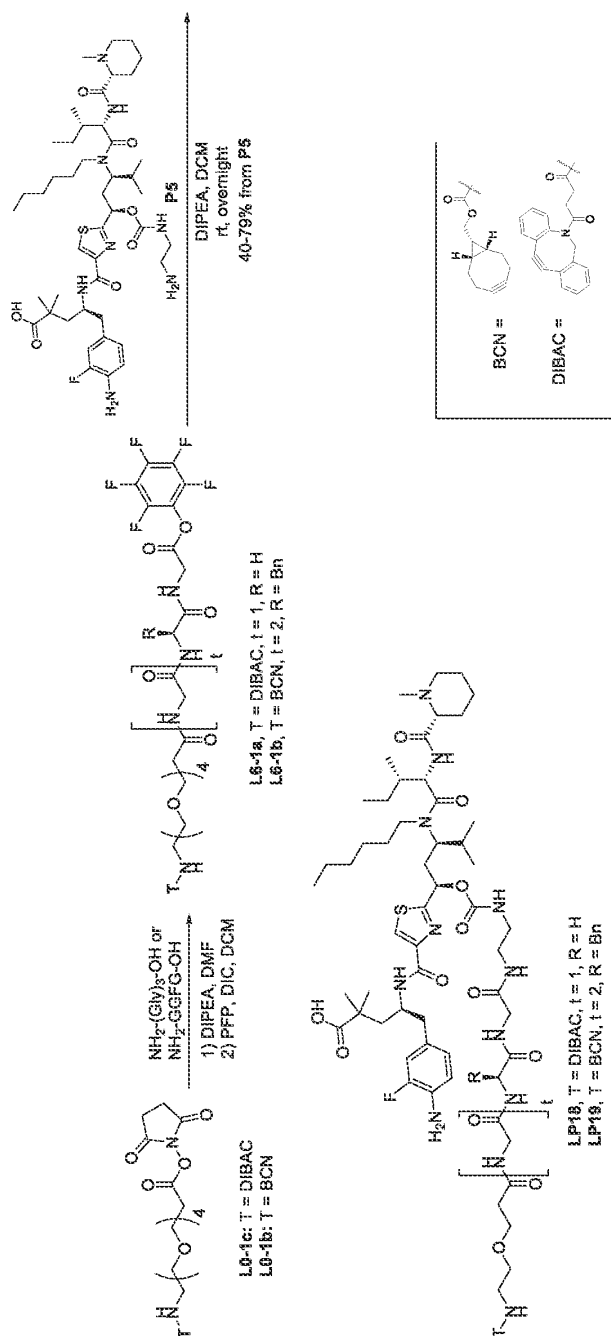
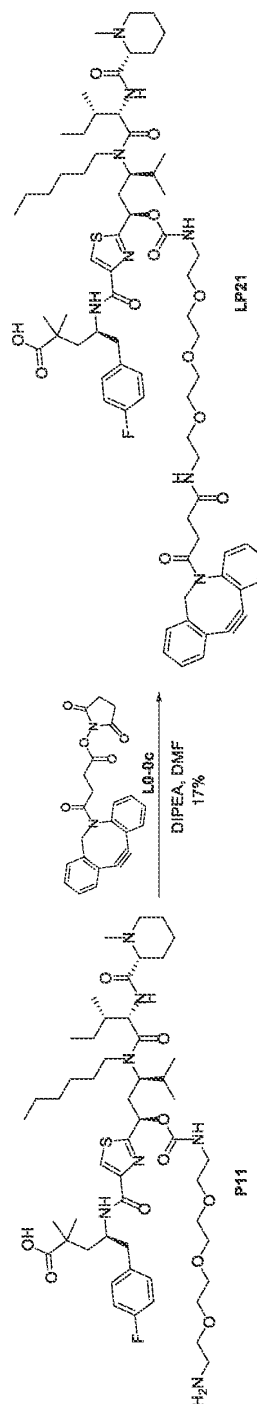


FIG. 15C. Synthesis of Linker-Payload LP21



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FIG. 16. Synthesis of Linker-Payloads LP22-LP25

