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Article

O-Aminoalkyl-*O*-Trimethyl-2,3-Dehydrosilybins: Synthesis and *in vitro* Effects towards Prostate Cancer Cells

Bao Vue [1], Sheng Zhang [1], Andre Vignau [1], Guanglin Chen [1], Xiaojie Zhang [1], William Diaz [1], Qiang Zhang [2], Shilong Zheng [2], Guangdi Wang [2], and Qiao-Hong Chen [1,*]

[1] Department of Chemistry, California State University, Fresno, 2555 E. San Ramon Avenues, M/S SB70, Fresno, CA 93740, USA;

[2] Department of Chemistry and RCMI Cancer Research Center, Xavier University of Louisiana, 1 Drexel Drive, New Orleans, LA 70125, USA;

* Correspondence: qchen@csufresno.edu; Tel.: +01-559-278-2394

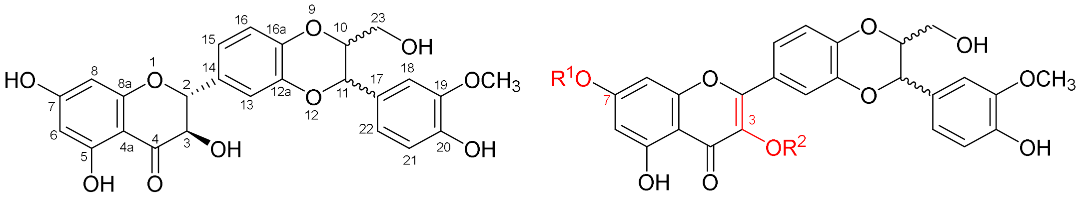
Abstract: As part of our ongoing silybin project, this study aims to introduce a basic nitrogen-containing group to 7-OH of 3,5,20-*O*-trimethyl-2,3-dehydrosilybin or 3-OH of 5,7,20-O-trimethyl-2,3-dehydrosilybin via an appropriate linker for *in vitro* evaluation as potential anti-prostate cancer agents. The synthetic approaches to 7-*O*-substituted-3,5,20-*O*-trimethyl-2,3-dehydrosilybins through a five-step procedure and to 3-*O*-substituted-5,7,20-*O*-trimethyl-2,3-dehydrosilybins via a four-step transformation have been developed. Thirty-two nitrogen-containing derivatives of silybin have been achieved through these synthetic methods for the evaluation of their antiproliferative activities towards both androgen-sensitive (LNCaP) and androgen-insensitive prostate cancer cell lines (PC-3 and DU145) using WST-1 cell proliferation assay. These derivatives exhibited greater *in vitro* antiproliferative potency than silybin. Among them, **11**, **29**, **31**, **37**, and **40** were identified as five optimal derivatives with IC_{50} values in the range of 1.40–3.06 μM, a 17- to 52-fold improvement in potency as compared with silybin. All these five optimal derivatives can arrest the PC-3 cell cycle in the G_0/G_1 phase and promote PC-3 cell apoptosis. Derivatives **11**, **37**, and **40** are more effective than **29** and **31** in activating PC-3 cell apoptosis.

Keywords: silybin; prostate cancer; 2,3-dehydrosilybin; cell proliferation; cell apoptosis.

1. Introduction

Silybin (**1**, Figure 1), also named as silibinin, exists in nature as an approximately equimolar mixture of two diastereomers of silybin A and silybin B with opposite configurations at C-10 and C-11 [1]. They are hard to separate by the conventional purification methods, but can be separated by HPLC method [2]. Silybin A and silybin B are biogenetically originated from a taxifolin moiety (flavonoid) and a coniferyl alcohol unit (lignan), which was presumed to follow a non-stereoselective radical coupling reaction [3]. Silybin (**1**) is the first and well-studied member of flavonolignans [4] and the key chemical and medicinal component of milk thistle (*Silybum marianum* L. Gaertner, Asteraceae) [5]. The earliest record on the medicinal merit of Milk thistle for preventing and treating various hepatotoxicity is Hieronymus Bock's book published in 1539 [6,7]. Milk thistle and silybin are now attractive to scientists not only for its well-known chemotherapeutic use for hepatotoxicity in Europe [8], but also for the potential in treating and preventing cancers [9-12]. Silymarin (crude extract of milk thistle), silybin (diastereomeric mixture), silybin A (optically pure), and silybin B (optically pure) have been demonstrated by various in vitro cell-based and in vivo animal-based assays to possess therapeutic potential in treating prostate cancer [11,13,14]. Additionally, the non-toxic profiles of silybin have been confirmed by its long-term use as dietary supplements and a Phase I clinical trial of silybin-phytosome, a formula of silybin, at a dose of 13 g/day [15]. However, the development of silybin as anti-prostate cancer drug is hindered, at least partly, by its moderate potency with its IC$_{50}$ values of 40-106 μM in prostate cancer cell models [11,13,16,17]. Structural modification of silybin can serve as a viable strategy to enhance its potency. Methylated silybins have been reported to be capable of increasing antiproliferative activities towards prostate cancer cells [18]. Additionally, 2,3-dehydrosilybin has been shown to be a significantly better anticancer agent than silybin [19].

Our previous studies on structure-activity relationships of silybin revealed that the antiproliferative potency of 2,3-dehydrosilybin in three prostate cancer cell models could be further improved through introducing a suitable alkyl group on 7-OH and 3-OH, as exemplified by 7-*O*-ethyl-2,3-dehydrosilybin (**3**) and 3-*O*-propyl-2,3-dehydrosilybin (**4**) (Figure 1) [20,21]. This encouraged us to further investigate the effects of nitrogen-containing groups on 7-OH and 3-OH of 2,3-dehydrosilybin on the biological profiles in prostate cancer cell models. Consequently, this study started with the development of general synthetic approaches to 7-*O*-substituted-3,5,20-*O*-trimethyl-2,3-dehydrosilybins and 3-*O*-substituted-5,7,20-*O*-trimethyl-2,3-dehydrosilybins followed by the synthesis of thirty-two new derivatives of silybin, including six 7-*O*-aminoalkyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybins and twenty-six 3-*O*-aminoalkyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybins. However, it is worth noting that 2,3-dehydrosilybin A is more potent than 2,3-dehydrosilybin B in cell and whole organism models to prolong lifespan and block Aβ aggregation [22]. It is thus imperative to confirm the respective in vitro antiproliferative potency of each enantiomer for the optimal *O*-aminoalkyl-*O*-trimethyl-2,3-dehydrosilybins before moving further for *in vivo* animal studies and various mechanism investigations. Additionally, the phenolic hydroxyl groups in all synthetic derivatives were converted to methoxyl groups. This conversion was expected to overcome, to some degree, pharmacokinetic limitations caused by the phenolic hydroxyl groups and to pave an avenue to selective incorporation of a basic nitrogen-containing group to the phenolic hydroxyl group either at C-7 or at C-3. The in vitro anticancer activities of these derivatives have been evaluated in three prostate cancer cell models. The design, synthesis, antiproliferative activity, and structure-activity relationships of these silybin derivatives were presented in this paper. The cell apoptosis induction and cell cycle regulation by five representative derivatives were also reported.



silybin (**1**) {silybin A: 10*R*, 11*R*
silybin B: 10*S*, 11*S*

2,3-dehydrosilybin (**2**); R^1 = R^2 = H
7-*O*-ethyl-2,3-dehydrosilybin (**3**): R^1 = ethyl; R^2 = H
3-*O*-propyl-2,3-dehydrosilybin (**4**): R^1 = H; R^2 = propyl

Figure 1. Structures of silybin and derivatives.

2. Results and Discussion

2.1. Chemistry

2.1.1. Synthesis of 7-O-aminoalkyl-3,5,20-O-trimethyl-2,3-dehydrosilybins (**9**-**14**)

As illustrated in Scheme 1, synthesis of 7-*O*-substituted-2,3-dehydrosilybins (**9**-**14**) started with selective benzylation (81%) of C-7 phenolic hydroxyl group of silybin according to the procedure reported by Kren *et al.* and us [20,23]. It is worth noting that anaerobic conditions are essential to achieving high yields for the selective benzylation. This is because the simultaneous presence of base and air led to the aerobic oxidation of silybin to 2,3-dehydrosilybin [7,21] and the 3-OH in 2,3-dehydrosilybin is readily benzylated or alkylated [7,21,23] that has been rationalized by the

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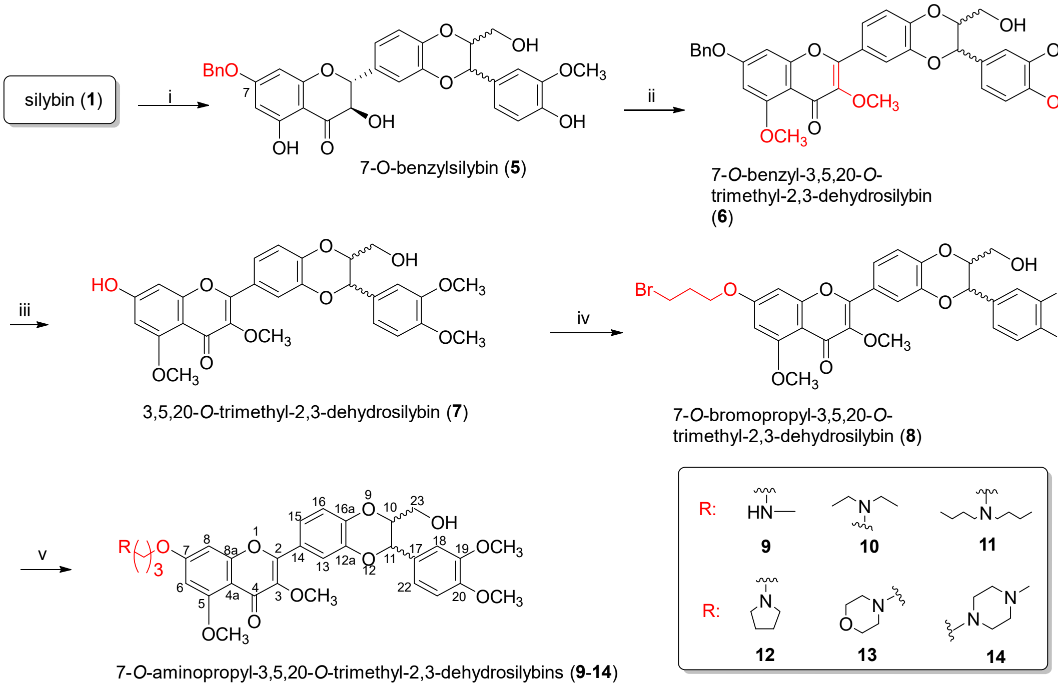
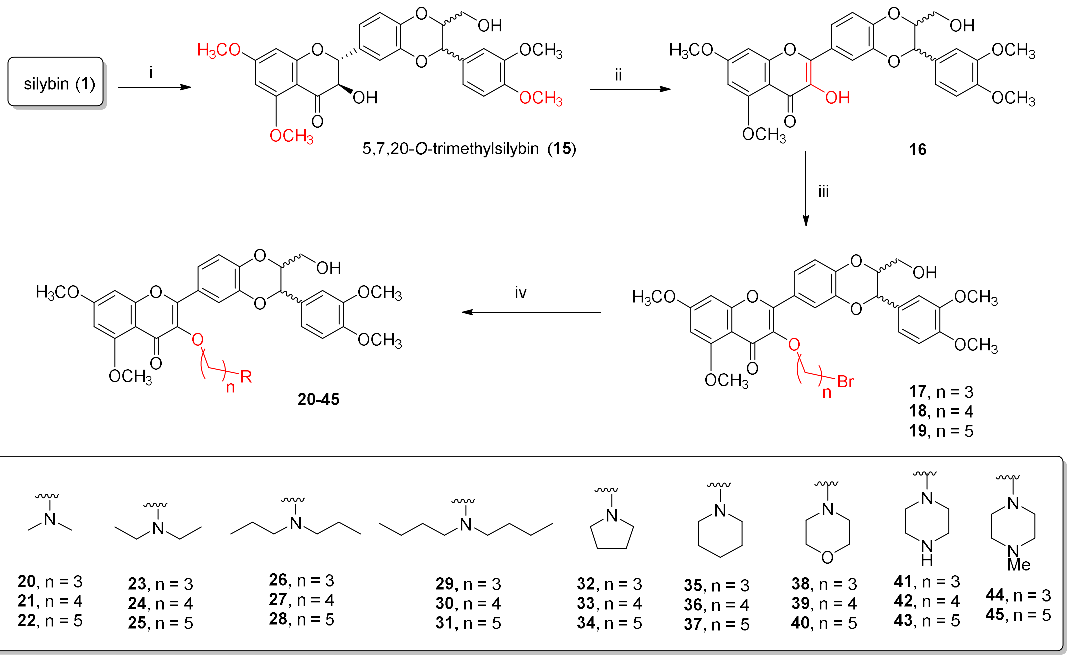
electrochemistry measurements and bond dissociation energy calculations [24]. 7-*O*-Benzyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**6**) was then achieved by the one-pot reaction of base-mediated oxidation of 7-*O*-benzylsilybin (**5**) followed by trimethylation of the corresponding 7-*O*-benzyl-2,3-dehydrosilybin. 3,5,20-*O*-Trimethyl-2,3-dehydrosiliybin (**7**) was obtained by debenzylation of aryl benzyl ether **6** using ammonium formate as the hydrogen source catalyzed by palladium on carbon. 7-*O*-bromopropyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**8**) was prepared by *O*-alkylating **7** with 1,3-dibromopropane mediated by potassium carbonate. 7-*O*-Aminoalkyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybins (**9**-**14**) were achieved by *N*-alkylation of the bromoalkyl side chain of **8** with the appropriate amine.



Scheme 1. Synthesis of 7-*O*-aminopropyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybins (**9**-**14**). Reagents and conditions: i. K_2CO_3 (4 equiv.), BnBr (1 equiv.), acetone (0.1 M), reflux overnight, 80%; ii. (a) K_2CO_3 (3 equiv.), DMF (0.5 M), rt, 3 h; (b) MeI (6 equiv.), rt, overnight, 48%; iii. $HCOONH_4$ (10 equiv.), Pd/C (10%, w/w), MeOH (0.2 M), reflux, overnight, 67%; iv. 1,3-dibromopropane (4 equiv.), K_2CO_3 (4 equiv.), DMF (1 M), 60 °C, overnight; v. amine (3 equiv.), K_2CO_3 (3 equiv.), acetone (0.1 M), reflux, overnight, 40%.

2.1.2. Synthesis of 3-O-aminoalkyl-5,7,20-O-trimethyl-2,3-dehydrosilybins (**20**-**45**)

3-*O*-Substituted-2,3-dehydrosilybins (**20**-**45**) were synthesized following a four-step procedure as shown in Scheme 2. Specifically, 5,7,20-*O*-trimethylsilybin (**15**) was achieved by treating silybin (**1**) with dimethylsulfate in the presence of potassium carbonate under strictly anaerobic conditions. Note that small amount of 3,5,7,20-*O*-tetramethyl-2,3-dehydrosilybin can be formed if anaerobic conditions were not well-controlled, which would complicate the purification process and decrease the yield. Even though 5,7,20-*O*-tribenzylsilybin was much easier to be aerobically oxidized than that in silybin [25], oxidation of 5,7,20-*O*-trimethylsilybin (**15**) under the same conditions led to a mixture of products instead of the desired oxidation product. After several trials with different oxidation conditions, 5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**16**) was eventually obtained by oxidation of **15** with sodium hydroxide and hydrogen peroxide. 10-14 Hours reaction time serves as a critical factor for the optimal yield (40-55%) of this oxidation reaction. We also found this oxidation cannot be quenched with hydrochloric acid because it selectively demethylated 5-OMe of the product. 5,7,20-*O*-Trimethyl-2,3-dehydrosilybin (**16**) was then converted to 3-*O*-bromoalkyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybins (**17**-**19**) via *O*-alkylation with the appropriate dibromoalkanes using potassium carbonate as the base and DMF as the aprotic solvent. The subsequent *N*-alkylation of the 3-*O*-bromoalkyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybins (**17**-**19**) with the corresponding amine furnished the respective 3-*O*-aminoalkyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**20**-**45**).



Scheme 2. Synthesis of 3-*O*-aminoalkyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybins (**20**-**45**). Reagents and conditions: i. dimethyl sulfate (8 equiv.), K_2CO_3 (8 equiv.), acetone, argon, reflux 4 h; ii. H_2O_2 (30%), NaOH (16%), methanol/THF, rt overnight; iii. Dibromoalkane (10 equiv.), K_2CO_3 (6 equiv.), DMF, rt 24-48 h; iv. Amine (16 equiv.), K_2CO_3 (6 equiv.), acetone, rt 48 h.

2.2. Antiproliferative activity towards prostate cancer cell lines and structure-activity relationships.

The in vitro antiproliferative activities of six 7-O-substituted and twenty-six 3-O-substituted silybin derivatives were evaluated using WST-1 cell proliferation assay according to the procedure as described in the Experimental Section in both androgen-sensitive (LNCaP) and androgen-insensitive (PC-3 and DU145) human prostate cancer cell lines. Silybin was used as a positive control for comparison in the parallel experiments and the IC$_{50}$ values calculated from the dose-response curves were summarized in Table 1. Clearly, 7-O-aminoalkyl-3,5,20-O-trimethyl-2,3-dehydrosilybins and 3-O-aminoalkyl-5,7,20-O-trimethyl-2,3-dehydrosilybins are more ogen-insensitive prostate cancer cell proliferation than silybin. This conclusion is supported by the following data: i) the optimal 7-O-substituted derivative (**11**) is 52-, 51-, and 24-fold more potent than silybin toward PC-3, DU145, and LNCaP prostate cancer cell lines; and ii) the optimal 3-O-substituted derivatives (**29**, **31**, **37**, and **40**) are 26-27, 31-37, and 17-22 times more potent than silybin. Additionally, the dibutylamino group in derivatives **11**, **29** and **31**, the morpholino moiety in **37**, and the piperidino unit in **40** are the favorable nitrogen-containing groups for the greater potency. A three-carbon linker in **11** and **29**, and a five-carbon linker in **31**, **37**, and 40 are beneficial to the potency.

Table 1. Anti-Proliferative Activity of dialkylaminoalkyl-2,3-dehydrosilybins.

Compd	IC$_{50}$ (μM)^{a}			IC$_{50}$ (silybin)/IC$_{50}$(derivative)		
	PC-3^{b}	DU145^{c}	LNCaP^{d}	PC-3^{b}	DU145^{c}	LNCaP^{d}
silybin	72.65 ± 3.15	93.34 ± 13.67	43.73 ± 10.90	1	1	1
8	26.09 ± 3.58	11.47 ± 2.38	5.71 ± 2.13	3	8	8
9	42.64 ± 6.61	39.64 ± 9.49	12.08 ± 1.81	2	2	4
10	9.92 ± 0.43	8.62 ± 0.32	7.49 ± 0.16	7	11	6
11	1.40 ± 0.17	1.84 ± 0.14	1.82 ± 0.14	52	51	24
12	25.05 ± 1.00	19.59 ± 0.47	11.00 ± 0.99	3	5	4
13	26.47 ± 1.00	45.10 ± 11.53	12.72 ± 6.28	3	2	3
14	25.65 ± 3.39	19.42 ± 0.88	16.35 ± 3.47	3	5	3
19	5.76 ± 1.36	8.13 ± 0.42	5.19 ± 2.08	13	11	8
20	9.09 ± 1.48	32.71 ± 5.32	20.69 ± 5.34	8	3	2
21	5.15 ± 2.13	9.97 ± 3.34	5.13 ± 0.89	14	9	9
22	3.47 ± 2.23	6.73 ± 0.45	5.07 ± 0.52	21	14	9
23	5.16 ± 0.94	9.21 ± 0.37	3.09 ± 0.15	14	10	14
24	5.76 ± 1.36	8.18 ± 0.42	5.19 ± 2.08	11	8	13
25	6.41 ± 0.40	6.64 ± 0.54	6.33 ± 0.34	11	14	7
26	2.03 ± 0.69	8.39 ± 1.38	3.89 ± 0.54	36	11	11
27	3.77 ± 0.41	5.39 ± 0.53	4.02 ± 1.55	19	17	11
28	3.30 ± 0.86	5.78 ± 1.36	2.70 ± 0.47	22	16	16
29	2.73 ± 0.12	2.51 ± 0.04	2.21 ± 0.17	27	37	20
30	3.77 ± 0.41	3.07 ± 0.51	3.84 ± 0.51	19	30	11
31	2.84 ± 0.10	2.85 ± 0.23	2.51 ± 0.31	26	33	17
32	2.86 ± 0.79	14.12 ± 2.79	5.89 ± 0.30	25	7	7
33	7.49 ± 1.98	19.84 ± 2.38	8.06 ± 1.44	10	5	5
34	2.95 ± 0.76	5.90 ± 1.30	3.92 ± 1.67	25	16	11
35	2.37 ± 0.70	7.26 ± 1.12	2.28 ± 0.42	31	13	19
36	24.09 ± 10.55	66.96 ± 13.65	27.30 ± 5.42	3	1	2
37	2.71 ± 0.23	2.69 ± 0.03	1.99 ± 0.23	27	35	22
38	2.94 ± 0.13	10.60 ± 0.63	1.74 ± 0.97	25	9	25

39	3.49 ± 0.24	6.36 ± 0.37	2.84 ± 0.22	21	15	15
40	2.72 ± 0.08	3.06 ± 0.13	2.23 ± 0.18	27	31	20
41	9.98 ± 4.94	> 50	11.41 ± 5.91	7	< 2	4
42	5.30 ± 0.66	6.85 ± 0.77	4.58 ± 1.77	14	14	10
43	7.87 ± 1.47	48.12 ± 16.27	14.39 ± 7.49	9	2	3
44	8.45 ± 2.89	18.57 ± 7.67	12.80 ± 7.80	9	5	3
45	4.72 ± 0.88	8.59 ± 2.23	5.66 ± 0.44	15	11	8

[a] IC_{50} value is the compound concentration effective in inhibiting 50% of the cell viability measured by WST-1 cell proliferation assay after 3 days exposure. The data were presented as the mean ± SD from n = 3.

[b] Human androgen-insensitive prostate cancer cell line derived from bone metastasis of prostate tumor.

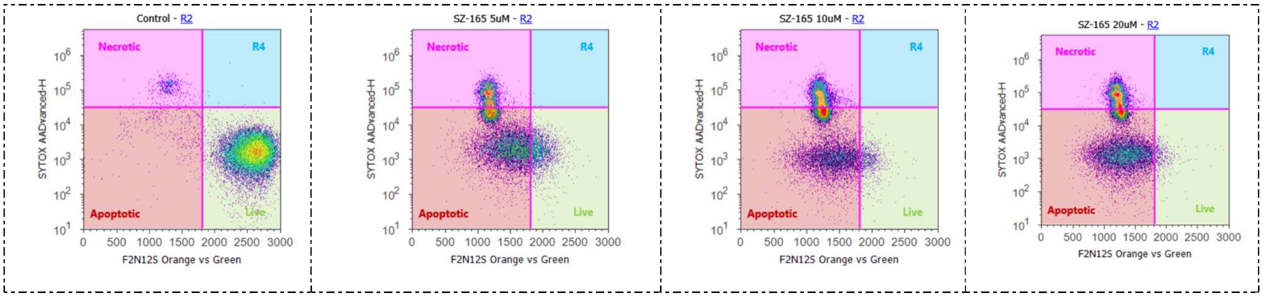
[c] Human androgen-insensitive prostate cancer cell line derived from brain metastasis of prostate tumor.

[d] Human androgen-sensitive prostate cancer cell line derived from lymph node metastasis of prostate tumor.

2.3. Cell Cycle Regulation and Cell Apoptosis.

Silybin can arrest rat (H-7 and I-8) and human prostate cancer cell (LNCaP) cycle at G_1 phase [26,27], and cause G_1 and G_2-M PC-3 prostate cancer cell cycle arrest [28]. Five optimal derivatives, **11**, **29**, **31**, **37**, and **40**, were selected for flow cytometry evaluation of their effect on PC-3 cell cycle regulation because they exhibited optimal cell proliferation inhibition on both androgen-dependent LNCaP and androgen-independent PC-3 prostate cancer cell models with ≤ 3.0 μM IC_{50} values. At 20 μM, all these five derivatives can cause PC-3 cell accumulation in a G_0/G_1 phase by increasing the cell population in this phase at 16 hours from 55.7% (control) to 66.3% (treated with **11**), from 36.2% (control) to 50.3% (treated with **29**), from 33.1% (control) to 35.9% (treated with **31**), from 33.1% (control) to 34.6% (treated with **37**), and from 33.1% (control) to 43.4% (treated with **40**).

Silybin was revealed by Agarwal and co-workers to activate cell apoptosis in PC-3 tumor xenografts [29]. F2N12S and SYTOX AADvanced double staining flow cytometry-based assay was used to discriminate PC-3 cells dying from apoptosis from those dying from necrosis in response to various concentrations of derivatives **11**, **29**, **31**, **37**, and **40**. PC-3 cells were incubated with the test compound for 16 h. As shown in Figure 2, derivatives **11**, **37**, and **40** induced appreciable levels of apoptotic cell death in the androgen-insensitive PC-3 prostate cancer cell line in a dose-responsive manner after a 16-hour treatment. Specifically, 5 μM of derivatives **11**, **37**, and **40** could induce substantial early phase of apoptosis (26-59%) in PC-3 cells as compared with control cells; treatment with 10 μM of these three optimal derivatives led to 56-76% early apoptotic cells and 6-40% late apoptotic/necrotic cells; 20 μM of derivatives **11**, **37**, and **40** activated notable apoptosis as well, with 54-75% early apoptotic cells and 16-44% late apoptotic/necrotic cells. The apoptotic cell population reached maximum when PC-3 cancer cells were exposed to derivative **11**, **37**, and **40** at 5μM, 10 μM, and 30 μM, respectively. In contrast, derivatives **29** and **31** did not induce significant levels of apoptotic cell death (less than 10%) up to 10 μM concentration. Only 50 μM of derivatives **29** and **31** results in the maximum apoptotic cell population (71% and 95%, respectively).



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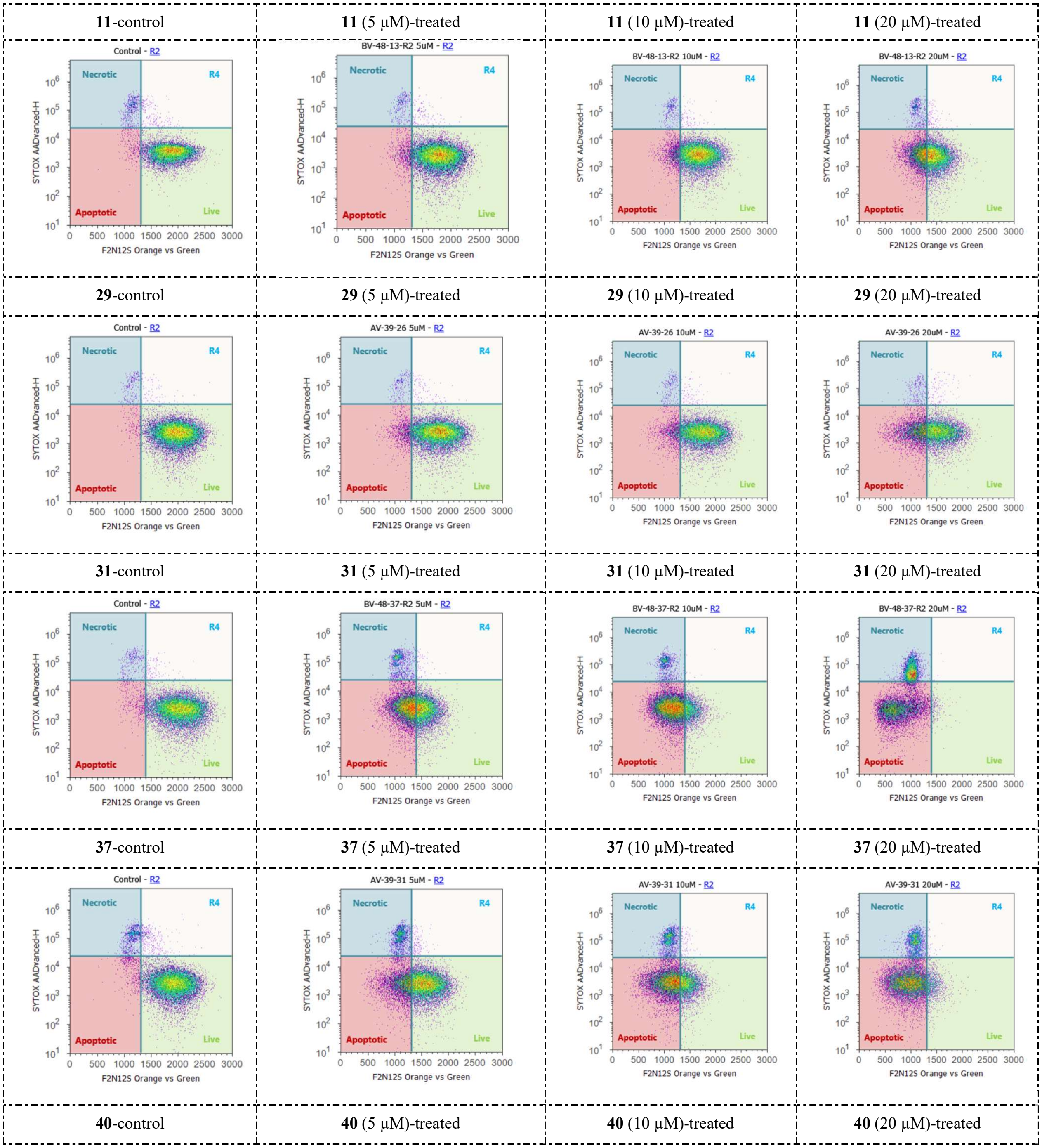

11-control | **11** (5 μM)-treated | **11** (10 μM)-treated | **11** (20 μM)-treated

29-control | **29** (5 μM)-treated | **29** (10 μM)-treated | **29** (20 μM)-treated

31-control | **31** (5 μM)-treated | **31** (10 μM)-treated | **31** (20 μM)-treated

37-control | **37** (5 μM)-treated | **37** (10 μM)-treated | **37** (20 μM)-treated

40-control | **40** (5 μM)-treated | **40** (10 μM)-treated | **40** (20 μM)-treated

Figure 2. Apoptosis in PC-3 cells treated with 11, 29, 31, 37, and 40 at 5 μM, 10 μM, 20 μM (by F2N12S and SYTOX AADvanced double staining).

3. Materials and Methods

3.1. General Procedures.

HRMS were obtained on an Orbitrap mass spectrometer with electrospray ionization (ESI). NMR spectra were obtained on a Bruker Fourier 300 spectrometer in $CDCl_3$, or DMSO-d_6. The chemical shifts are given in ppm referenced to the respective solvent peak, and coupling constants are reported in Hz. Anhydrous THF and dichloromethane were purified by PureSolv MD 7 Solvent Purification System from Innovative Technologies (MB-SPS-800). All other reagents and solvents

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were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using silica gel (32-63 μm). Preparative thin-layer chromatography (PTLC) separations were carried out on thin layer chromatography plates loaded with silica gel 60 GF254 (EMD Millipore Corporation, MA, USA). Silybin (> 98.0%) was purchased from Fisher Scientific (TCI America, Cat # 50-014-46874).

*3.2. Synthesis of 7-O-benzylsilybin (**5**)*

Following the procedure described in the literature [20,23], 7-*O*-benzylsilybin (**5**) was prepared from silybin in 80% yield as a light yellow solid. m.p. 93-95 ^{o}C. IR (film) ν_{max}: 3432, 2937, 1634, 1571, 1507 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (11.24) (s, 1H), 7.43-7.34 (m, 5H), 7.19 (dd, *J* = 4.2, 1.5 Hz, 1H), 7.10-7.01 (m, 2H), 6.97-6.88 (overlapped, 3H), 6.21 (d, *J* = 1.8 Hz, 1H), 6.13 (6.12) (d, *J* = 2.4 Hz, 1H), 5.96 (br.s, 1H), 5.07 (s, 2H), 4.99 (d, *J* = 11.7 Hz, 1H), 4.93 (d, *J* = 8.4 Hz, 1H), 4.52 (dd, *J* = 11.7, 3.3 Hz, 1H), 4.09-3.99 (m, 1H), 3.89 (s, 3H), 3.80 (dd, *J* = 12.3, 2.1 Hz, 1H), 3.55 (dd, *J* = 12.3, 3.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 96.2, 167.8, 163.6, 162.8, 147.0, 146.4, 144.1, 143.9, 135.6, 129.5, 128.8, 128.4, 127.9, 127.5, 121.2 (121.1), 120.8, 117.3 (117.2), 116.6, 114.9, 109.8, 101.1, 96.4, 95.4, 83.0, 78.4, 76.3, 72.4, 70.5, 61.6, 56.1; HRMS-ESI *m*/*z* [M+H]$^{+}$ calcd for $C_{32}H_{29}O_{10}$: 573.1761, found: 573.1769.

*3.3. Synthesis of 7-O-benzyl-3,5,20-O-trimethyl-2,3-dehydrosilybin (**6**)*

Potassium carbonate (3 eq.) was added to a solution of benzylsilybin (1 eq.) in DMF (0.5 M) and the reaction mixture was opened to air with stirring at room temperature (or 60 ^{o}C) for 3 hours. When most of 7-*O*-benzylsilybin was oxidized to 7-*O*-benzyl-2,3-dehydrobenzylsilybin as monitored by TLC, the reaction mixture was cooled down to room temperature. Potassium carbonate (3 eq.) followed by methyl iodide (6 eq.) were added to the reaction mixture and the reaction was allowed to proceed at room temperature overnight prior to being quenched with HCl (1 M). The subsequent mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were rinsed with brine and dried over anhydrous sodium sulfate. After filtration, the volatile components were evaporated under vacuum to give the crude product, which was purified by column chromatography over silica gel or PTLC eluting with 5% methanol in dichloromethane to generate 7-*O*-benzyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**6**): 48% yield, yellow solid, m.p. 118-119 ^{o}C. IR (film) ν_{max}: 3401, 2932, 1601, 1504, 1440 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, *J* = 2.1 Hz, 1H), 7.73 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.48-7.34 (m, 5H), 7.06 (d, *J* = 8.7 Hz,1H), 7.04 (dd, *J* = 8.4, 2.1Hz, 1H), 6.98 (d, *J* = 1.8 Hz, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 6.56 (d, *J* = 2.1 Hz, 1H), 6.41 (d, *J* = 2.4 Hz, 1H), 5.12 (s, 2H), 5.02 (d, *J* = 8.4 Hz,1H), 4.17-4.10 (m,1H), 3.94 (s, 3H), 3.924 (s, 3H), 3.916 (s, 3H), 3.89 (s, 3H), 3.85 (dd, *J* = 12.3, 2.1 Hz, 1H), 3.58 (dd, *J* = 12.6, 3.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 163.1, 161.2, 158.8, 152.1, 150.0, 149.6, 145.3, 143.8, 141.6, 135.8, 128.9, 128.6, 128.4, 127.7, 124.5, 122.4, 120.3, 117.3, 117.2, 111.5, 110.3, 109.8, 96.5, 93.4, 78.8, 76.5, 70.6, 61.8, 60.1, 56.6, 56.2, 56.1; HRMS-ESI *m*/*z* [M+H]$^{+}$ calcd for $C_{35}H_{33}O_{10}$: 613.2074, found: 613.2071.

*3.4. Synthesis of 3,5,20-O-trimethyl-2,3-dehydrosilybin (**7**)*

To the solution of 7-*O*-benzyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**6**, 1 eq) in methanol (0.2 M), Pd-C (50% wet, 10% w/w) and ammonium formate (10 eq) were sequentially added. The reaction mixture was refluxed overnight under argon. After being cooled to room temperature, the reaction mixture was filtered through a silica gel pad eluting with THF. The filtrate was concentrated under vacuum to afford 3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**7**) in 67% yield as a light yellow solid. M.p. 235-236 ^{o}C. IR (film) ν_{max}: 3545, 2955, 2924, 2853, 2177, 2159, 2028, 1992, 1978, 1968, 1728, 1593, 1557, 1508 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.74-7.64 (m, 1H), 7.60-7.55 (m, 2H), 7.13-6.98 (m, 6H), 6.42 (s, 1H), 6.33 (s, 1H), 5.02 (d, *J* = 7.8 Hz, 1H), 4.36-4.28 (m, 1H), 3.78 (s, 12H), 3.76 (s, 3H), 3.57 (dd, *J* = 12.9, 2.1 Hz, 1H), 3.35 (dd, *J* = 12.9, 4.8 Hz, 1H). HRMS-ESI *m*/*z* [M+H]$^{+}$ calcd for $C_{28}H_{27}O_{10}$: 523.1604, found: 523.1598.

*3.5. Synthesis of 7-O-(3′-bromo)propyl-3,5,20-O-trimethyl-2,3-dehydrosilybin (**8**)*

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To a solution of 3,5,20-*O*-trimethyl-2,3-dehydrosilybin in DMF (1 M) were added potassium carbonate (4 eq.) followed by 1,3-dibromopropane (4 eq.). The reaction mixture was stirred at 60 °C overnight prior to being quenched by addition of 1 M HCl. The subsequent reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were rinsed with brine, dried over anhydrous sodium sulfate, concentrated under vacuum to yield a crude product, which was subjected to column chromatography or PTLC purification over silica gel eluting with 5% methanol in dichloromethane to afford 7-*O*-(3'-bromo)propyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**8**) as a yellow solid. M.p. 115-116 °C. IR (film) ν_{ma}x: 2932, 1604, 1506, 1463, 1441, 1345, 1264 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.76 (d, J = 2.1 Hz, 1H), 7.73 (dd, J = 8.7, 2.4 Hz, 1H), 7.07-6.97 (m, 3H), 6.92 (d, J = 8.4 Hz, 1H), 6.48 (d, J = 2.1 Hz, 1H), 6.32 (d, J = 2.1 Hz, 1H), 5.02 (d, J = 8.4 Hz, 1H), 4.20-4.11 (m, 1H), 4.17 (t, J = 5.7 Hz, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.85 (dd, J = 12.6, 2.7 Hz, 1H), 3.66-3.56 (m, 1H), 3.61 (t, J = 6.3 Hz, 2H), 2.36 (quin, J = 6.0 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.2, 163.0, 161.2, 158.8, 152.1, 149.9, 149.6, 145.3, 143.8, 141.5, 128.4, 124.4, 122.3, 120.3, 117.3, 117.2, 111.4, 110.2, 109.7, 96.1, 93.0, 78.8, 76.4, 66.0, 61.8, 60.1, 56.5, 56.2, 56.1, 32.1, 29.6; HRMS-ESI *m*/z [M+H]$^+$ calcd for $C_{31}H_{32}BrO_{10}$: 643.1179, 645.1158, found: 643.1173, 645.1151.

3.6. General procedure for the synthesis of 7-O-(N,N-dialkylamino)propyl-3,5,20-O-trimethyl-2,3-dehydrosilybins

Potassium carbonate (3 eq.) and the appropriate amine (3 eq.) were added to a solution of 7-*O*-(3'-bromo)propyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**8**) in dry acetone (0.1 M). The reaction mixture was refluxed overnight before removal of acetone under vacuum. The residue was diluted with water and extracted with ethyl acetate. The combined organic extracts were rinsed with brine, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to generate the crude products, which was subjected to PTLC purification eluting with 10% methanol in dichloromethane to yield the respective 7-*O*-(3'-amino)propyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin.

3.6.1. 7-*O*-(*N*-Methylaminopropyl)-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**9**). 40% yield, light yellow solid, mp. 125-127 °C. IR (film) $\nu_{\max}$: 3401, 2928, 1625, 1606, 1507 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.59 (d, J = 2.1 Hz, 1H), 7.53 (dd, J = 8.7, 2.1 Hz, 1H), 7.04 (dd, J = 8.1, 1.2 Hz, 1H), 6.97 (d, J = 1.5 Hz, 1H), 6.93 (dd, J = 8.7, 2.7 Hz, 2H), 6.22 (d, J = 1.8 Hz, 1H), 6.18 (d, J = 1.8 Hz, 1H), 4.99 (d, J = 8.4 Hz, 1H), 4.17-4.06 (overlapped, 3H), 3.92 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.80 (s, 3H), 3.85-3.80 (overlapped, 1H), 3.57 (dd, J = 12.9, 3.9 Hz, 1H), 3.25 (t, J = 6.3 Hz, 2H), 2.81 (s, 3H), 2.34-2.30 (m, 2H), 2.01 (d, J = 1.2 Hz, 1H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.4, 162.9, 160.4, 158.1, 152.6, 150.0, 149.6, 145.6, 143.7, 140.9, 128.3, 123.5, 122.0, 120.3, 117.2, 117.1, 111.5, 110.4, 108.7, 96.3, 92.9, 78.9, 76.3, 65.2, 61.6, 59.9, 56.8, 56.2, 56.1, 47.5, 34.2, 25.7; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{32}H_{36}NO_{10}$: 594.2339, found: 594.2334.

3.6.2. 7-*O*-(*N*,*N*-Diethylaminopropyl)-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**10**). 29% yield, yellow solid, m.p. 107-109 °C. IR (film) $\nu_{\max}$: 3419, 3182, 2981, 2844, 1624, 1602 cm^{-1}; ^{1}H NMR (300 MHz, CD$_3$OD): δ 7.59-7.48 (overlapped, 1H), 7.51 (s, 1H), 7.03 (s, 1H), 7.00-6.94 (overlapped, 2H), 6.93 (d, J = 8.4 Hz, 1H), 6.36 (s, 1H), 6.23 (s, 1H), 4.89-4.86 (overlapped, 1H), 4.09-3.92 (overlapped, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 3.75 (s, 3H), 3.69 (dd, J = 12.9, 2.4 Hz, 1H), 3.43 (dd, J = 12.3, 3.9 Hz, 1H), 2.74 (t, J = 7.5 Hz, 2H), 2.70 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 2.02-1.86 (m, 2H), 1.10 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, CD$_3$OD): δ 175.5, 165.0, 161.7, 159.7, 153.9, 151.1, 150.6, 147.2, 145.0, 141.9, 130.3, 124.4, 123.0, 121.6, 118.1, 117.9, 112.8, 112.3, 109.6, 97.0, 94.1, 80.3, 77.5, 67.9, 61.9, 60.2, 56.6, 56.5, 56.4, 50.3, 47.9, 26.5, 11.0; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{35}H_{42}NO_{10}$: 636.2808, found: 636.2801.

3.6.3. 7-*O*-(*N*,*N*-Dibutylaminopropyl)-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**11**). 25% yield, yellow solid, mp. 69-71 °C. IR (film) $\nu_{\max}$: 3404, 2930, 2871, 1625, 1602, 1505 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.75 (d, J = 1.8 Hz, 1H), 7.72 (dd, J = 8.4, 2.1 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 7.03 (dd, J = 8.1, 1.8 Hz, 1H), 6.97 (d, J = 1.8 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.46 (d, J = 2.1 Hz, 1H), 6.31 (d, J = 2.1 Hz, 1H), 5.02

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(d, *J* = 8.4 Hz, 1H), 4.13 (dt, *J* = 8.1, 2.7 Hz, 1H), 4.08 (t, *J* = 6.3 Hz, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.91 (s, 3H), 3.88 (s, 3H), 3.84 (dd, *J* = 12.6, 2.7 Hz, 1H), 3.57 (dd, *J* = 12.3, 3.6 Hz, 1H), 2.66 (t, *J* = 6.3 Hz, 2H), 2.49 (t, *J* = 7.2 Hz, 4H), 1.98 (quin, *J* = 6.3 Hz, 2H), 1.50-1.40 (m, 4H), 1.36-1.24 (m, 4H), 0.89 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 163.4, 161.1, 158.8, 152.0, 149.9, 149.6, 145.3, 143.8, 141.5, 128.5, 124.4, 122.3, 120.3, 117.3, 117.2, 111.5, 110.3, 109.5, 96.1, 93.0, 78.8, 76.4, 66.8, 61.7, 60.0, 56.5, 56.2, 56.1, 53.9, 50.5, 28.9, 26.7, 20.8, 14.2; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{39}H_{50}NO_{10}$: 692.3434, found: 692.3431.

3.6.4. 7-*O*-Pyrrolidinopropyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**12**). 29% yield, yellow solid, mp. 111-113 °C. IR (film) ν_{max}: 3415, 2933, 1625, 1605, 1578, 1506 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74 (d, *J* = 1.8 Hz, 1H), 7.71 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.03 (d, *J* = 8.7 Hz, 2H), 6.97 (d, *J* = 1.5 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.46 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.01 (d, *J* = 8.1 Hz, 1H), 4.16-4.12 (m, 1H), 4.09 (t, *J* = 6.3 Hz, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.84 (dd, *J* = 12.9, 2.4 Hz, 1H), 3.56 (dd, *J* = 12.6, 3.9 Hz, 1H), 2.71 (t, *J* = 7.2 Hz, 2H), 2.67-2.57 (m, 4H), 2.07 (quin, *J* = 6.0 Hz, 2H), 1.88-1.79 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 163.3, 161.0, 158.8, 152.1, 149.9, 149.6, 145.3, 143.8, 141.4, 128.5, 124.3, 122.2, 120.3, 117.3, 117.2, 111.4, 110.2, 109.5, 96.1, 93.0, 78.8, 76.4, 66.7, 61.7, 60.0, 56.5, 56.2, 56.1, 54.3, 53.0, 28.0, 23.6; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{35}H_{40}NO_{10}$: 634.2652, found: 634.2650.

3.6.5. 7-*O*-Mopholinopropyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**13**). 19% yield, yellow solid. mp. 132-133 °C. IR (film) ν_{max}: 3406, 2932, 1625, 1606, 1506, 1464, 1443 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (s, 1H), 7.72 (d, *J* = 10.8 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.44 (s, 1H), 6.29 (s, 1H), 5.02 (d, *J* = 8.1 Hz, 1H), 4.18-4.04 (overlapped, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 3.87-3.76 (overlapped, 4H), 3.57 (dd, *J* = 12.3, 3.3 Hz, 1H), 3.06-2.99 (m, 1H), 2.79-2.57 (overlapped, 6H), 2.18-2.05 (overlapped, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 163.2, 161.0, 158.8, 152.1, 149.9, 149.6, 145.3, 143.8, 141.4, 128.4, 124.3, 122.2, 120.3, 117.23, 117.17, 111.4, 110.2, 109.5, 96.1, 92.9, 78.8, 76.4, 66.4, 66.2, 61.7, 60.0, 56.5, 56.2, 56.1, 55.4, 53.4, 42.3; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{35}H_{40}NO_{11}$: 650.2601, found: 650.2598.

3.6.6. 7-*O*-(4-Methylpiperazin-1-yl)propyl-3,5,20-*O*-trimethyl-2,3-dehydrosilybin (**14**). 10% yield, light yellow solid. Mp. 142-143 °C. IR (film) ν_{max}: 3406, 2917, 2850, 1625, 1604, 1505 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77-7.71 (overlapped, 2H), 7.06 (d, *J* = 9.0 Hz, 1H), 7.04 (dd, *J* = 10.8, 1.5 Hz, 1H), 6.97 (d, *J* = 1.5 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 1H), 6.30 (d, *J* = 1.8 Hz, 1H), 5.02 (d, *J* = 8.4 Hz, 1H), 4.17-4.01 (m, 1H), 4.06 (t, *J* = 6.0 Hz, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H), 3.85 (dd, *J* = 12.3, 2.1 Hz, 1H), 3.57 (dd, *J* = 12.6, 3.9 Hz, 1H), 2.95-2.72 (overlapped, 8H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.55 (s, 3H), 2.07-2.02 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.2, 163.2, 161.1, 158.8, 152.0, 150.0, 149.6, 145.3, 143.8, 141.5, 128.4, 124.4, 122.4, 120.3, 117.2, 117.2, 111.5, 110.3, 109.6, 96.2, 92.8, 78.8, 76.5, 66.3, 61.7, 60.1, 56.6, 56.2, 56.1, 54.3, 54.0, 50.9, 44.5, 26.1; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{36}H_{43}N_2O_{10}$: 663.2917, found: 663.2917.

*3.7. Synthesis of 5,7,20-O-trimethylsilybin (**15**).*

A 3-neck round bottom flask was charged with silybin (2.01 g, 4.2 mmol) and potassium carbonate (3.43 gram, 25.1 mmol), which was vacuumed three times under argon prior to the addition of acetone (30.0 mL). The reaction mixture was refluxed for 15 minutes before dimethylsulfate (3.13 mL, 33.1 mmol) was added through a needle. The reaction was continued with refluxing for an additional 4 hours when the reaction was completed as monitored by TLC. After cooling down to room temperature, saturated ammonium chloride was added to quench the reaction, and the subsequent mixture was extracted with ethyl acetate for 3 times. The organic layers were combined, washed with brine twice, and dried over anhydrous sodium sulfate. Purification of the crude product through column chromatography, eluting with ethyl acetate/hexane (50/50 to 70/30, v/v), gave the product (**15**) as a white crystal in 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (dd, *J* = 9.9, 1.8 Hz, 1H), 7.08(dd, *J* = 8.4, 2.1 Hz, 1H), 7.04 (d, *J* = 3.3 Hz, 1H), 7.00 (dd, *J* = 8.7, 2.7 Hz,

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1H), 6.96 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 6.12 (d, *J* = 2.1 Hz, 1H), 6.11 (d, *J* = 1.5 Hz, 1H), 4.98 (d, *J* = 9.0 Hz, 1H), 4.94 (d, *J* = 12.3 Hz, 1H), 4.42 (dd, *J* = 12.3, 5.1 Hz, 1H), 4.06-4.03 (m, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.90 (s, 3H), 3.81 (d, *J* = 1.8 Hz, 3H), 3.81 (dd, *J* = 12.0, 2.4 Hz, 1H), 3.55 (dd, *J* = 12.3, 3.9 Hz).

*3.8. Synthesis of 5,7,20-O-trimethyl-2,3-dehydrosilybin (**16**)*

A 10-mL round flask was charged with 5,7,20-*O*-trimethylsiybin (150.0 mg, 0.23 mmol) in methanol (2.0 mL) and tetrahydrofuran (2.0 mL). The solution was stirred for 10 minutes at room temperature prior to being added hydrogen peroxide (0.85 mL, 30%) and sodium hydroxide aqueous solution (0.65 mL, 16%) at 0 °C. The reaction mixture was slowly warmed to room temperature and then stirred overnight before being quenched with saturated ammonium chloride. The subsequent mixture was extracted with dichloromethane for three times, and the combined extracts were dried over sodium sulfate and concentrated under vacuum. The crude product was obtained in 49% yield, which is pure enough for the next step reaction without purification. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.89 (s, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 8.7 Hz, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 6.98 (s, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 6.53 (d, *J* = 1.8 Hz, 1H), 6.35 (d, *J* = 2.1 Hz, 1H), 5.03 (d, *J* = 8.1 Hz, 1H), 4.17 – 4.11 (m, 1H), 3.98 (s, 3H), 3.93 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.85 (d, *J* = 13.8 Hz, 1H), 3.59 (d, *J* = 10.5 Hz, 1H).

*3.9. General procedure for the synthesis of 3-O-bromoalkyl-5,7,20-O-trimethyl-2,3-dehydrosilybins (**17**-**19**)*

A round bottom flask (10 mL) was charged with 5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**16**, 83.2 mg, 0.16 mmol), potassium carbonate (352.0 mg, 2.55 mmol), and DMF (5.0 mL). The mixture was stirred for 10 minutes prior to being added of 1,3-, 1,4-, or 1,5-dibromalkane (2.56 mmol, 16 equiv.). The reaction was continued with stirring at room temperature for 24-48 hours before the reaction was quenched with water. The subsequent mixture was extracted with ethyl acetate for three times, and the combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. The crude products was subjected to PTLC purification eluting with DCM/methanol (100/5, v/v) to yield the respective 3-*O*-bromoalkyl-5,7,10-*O*-trimethyl-2,3-dehydrosilybin.

3.9.1. 3-*O*-(3'-Bromo)propyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**17**). 81% yield, ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (s, 1H), 7.70 (d, *J* = 9.6 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 7.06 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.00 (d, *J* = 1.8 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.49 (d, *J* = 2.1 Hz, 1H), 6.35 (d, *J* = 2.4 Hz, 1H), 5.05 (d, *J* = 8.4 Hz, 1H), 4.14 (t, *J* = 6.0 Hz, 2H), 4.16-4.12 (overlapped, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.86 (dd, *J* = 12.6, 2.7 Hz, 1H), 3.60 (t, *J* = 6.9 Hz, 2H), 3.63-3.58 (overlapped, 1H), 2.31-2.26 (m, 2H).

3.9.2. 3-*O*-(4'-Bromo)butyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**18**). 62% Yield, ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.71 (s, 1H), 7.69 (dd, *J* = 7.2, 1.5 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 1.8 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.32 (d, *J* = 2.4 Hz, 1H), 5.03 (d, *J* = 8.1 Hz, 1H), 4.16-4.09 (m, 1H), 4.03 (t, *J* = 6.0 Hz, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.81 (dd, *J* = 7.2, 2.4 Hz, 1H), 3.57 (dd, *J* = 12.3, 3.6 Hz, 1H), 3.46 (t, *J* = 6.6 Hz, 2H), 2.09-2.00 (m, 2H), 1.90-1.81 (m, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.2, 164.1, 161.1, 158.9, 152.6, 149.9, 149.6, 145.3, 143.8, 140.4, 128.5, 124.4, 122.4, 120.3, 117.3, 117.1, 111.4, 110.3, 109.5, 95.9, 92.5, 78.8, 76.4, 61.7, 56.5, 56.2, 56.1, 55.9, 34.1, 28.8.

3.9.3. 3-*O*-(5'-Bromo)pentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**19**). 77% yield, ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (s, 1H), 7.71 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 2H), 6.97 (d, *J* = 1.5 Hz, 1H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.03 (d, *J* = 8.4 Hz, 1H), 4.14-4.11 (m, 1H), 4.02 (t, *J* = 6.3 Hz, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.85 (s, 3H), 3.85 (d, *J* = 12.6 Hz, 1H), 3.57 (d, *J* = 11.7 Hz, 1H), 3.34 (t, *J* = 6.9 Hz, 2H), 1.85 (quin, *J* = 7.2 Hz, 2H), 1.74 (quin, *J* = 7.2 Hz, 2H), 1.59-1.49 (m, 2H).

3.10. General Procedure for the synthesis of 3-O-(alkylamino)alkyl-5,7,20-O-trimethyl- 2,3-dehydrosilybins.

A round bottom reaction flask (10 mL) was charged with 3-*O*-bromoalkyl-5,7,10-*O*-trimethyl-2,3-dehydrosilybin (1 eq.) and potassium carbonate (10 eq.) in acetone (2.0 mL, 0.029 M). The solution was stirred for 10 minutes prior to being added the appropriate amine (16 eq.). The reaction was allowed to proceed with stirring at room temperature for 24-48 hours before being quenched with water. The subsequent mixture was extracted with ethyl acetate for three times, and the combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was subjected to PTLC purification eluting with DCM/methanol (100:5, v/v). Each desired nitrogen-containing compound was retrieved from PTLC silica gel by washing with dichloromethane/methanol/ammonium hydroxide (100:10:5, v/v/v).

3.10.1. 3-*O*-(*N*,*N*-Dimethylamino)propyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**20**). 97% yield, white solid, white wax. IR (film) ν_{max}: 3364, 2940, 2837, 1625, 1604, 1517, 1505, 1492, 1462 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.64 (d, *J* = 6.2 Hz, 1H), 7.63 (s, 1H), 7.10 (d, *J* = 10.1 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.98 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.47 (s, 1H), 6.33 (s, 1H), 5.05 (d, *J* = 7.7 Hz, 1H), 4.19-4.16 (m, 1H), 4.00-3.97 (overlapped, 2H), 3.94 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.86-3.83 (overlapped, 1H), 3.57 (dd, *J* = 12.2, 2.9 Hz, 1H), 3.52-3.42 (m, 2H), 2.88 (s, 6H), 2.33-2.22 (m. 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.4, 164.5, 160.8, 158.9, 153.4, 149.8, 149.4, 145.8, 143.9, 139.6, 128.2, 123.3, 122.2, 120.2, 117.5, 117.0, 111.4, 110.3, 108.9, 96.2, 92.5, 78.7, 76.3, 69.3, 61.4, 56.5, 56.1, 56.0, 55.9, 43.3, 42.3, 25.4; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{33}H_{38}NO_{10}$: 608.2496, found: 608.2490.

3.10.2. 3-*O*-(*N*,*N*-Dimethylamino)butyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**21**). 86% yield, yellow wax. IR (film) ν_{max}: 3397, 2938, 1625, 1603, 1505, 1460 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (dd, *J* = 8.7 Hz, 2.1 Hz, 1H), 7.72 (d, *J* = 1.8 Hz, 1H), 7.04 (d, *J* = 9.3 Hz, 1H), 7.03 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.97 (d, *J* = 1.5 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.04 (d, *J* = 8.1 Hz, 1H), 4.13 (dt, *J* = 7.8, 3.3 Hz, 1H), 4.05-401 (m, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.85-3.81 (overlapped, 1H), 3.57 (dd, *J* = 12.3, 3.9 Hz, 1H). 2.45 (t, *J* = 7.5 Hz, 2H), 2.29 (s, 6H), 1.77-1.65 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 161.0, 158.8, 152.3, 149.7, 149.4, 145.2, 143.6, 140.4, 128.5, 124.3, 122.4, 120.2, 117.2, 117.0, 111.3, 110.2, 109.4, 95.8, 92.3, 78.7, 76.2, 71.8, 61.5, 59.1, 56.4, 56.1, 56.0, 55.7, 44.8, 27.9, 23.5; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{34}H_{40}NO_{10}$: 622.2652, found: 622.2651.

3.10.3. 3-*O*-(*N*,*N*-Dimethylamino)pentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**22**). 76% yield, yellow solid, mp. 90-91 ºC. IR (film) ν_{max}: 3398, 2919, 1625, 1608, 1509 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.74-7.67 (overlapped, 2H), 7.03 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 1.5 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.06 (d, *J* = 7.8 Hz, 1H), 4.14 (dt, *J* = 7.8, 3.3 Hz, 1H), 4.03-3.98 (m, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.85-3.81 (overlapped, 1H), 3.57 (dd, *J* = 12.6, 3.9 Hz, 1H), 2.42 (t, *J* = 7.5 Hz, 2H), 2.34 (s, 6H), 1.72 (quin, *J* = 6.9 Hz, 2H), 1.61-1.51 (m, 2H), 1.47-1.37 (m, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 161.0, 158.8, 152.3, 149.7, 149.4, 145.1, 143.6, 140.5, 128.5, 124.3, 122.4, 120.2, 117.2, 116.9, 111.3, 110.2, 109.4, 95.8, 92.3, 78.7, 76.1, 72.0, 61.5, 59.1, 56.4, 56.0, 55.9, 55.7, 44.5, 29.9, 26.1, 23.7; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{35}H_{42}NO_{10}$: 636.2809, found: 636.2807.

3.10.4. 3-*O*-(*N*,*N*-Diethylamino)propyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**23**). 90% yield, yellow wax. IR (film) ν_{max}: 3375, 2936, 2837, 1624, 1604, 1579, 1517, 1505, 1492, 1462 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.81-7.56 (overlapped, 1H), 7.68 (s, 1H), 7.06 (d, *J* = 9.0 Hz, 1H), 7.03 (d, *J* = 9.3 Hz, 1H), 6.97 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.47 (s, 1H), 6.33 (s, 1H), 5.03 (d, *J* = 7.9 Hz, 1H), 4.16-4.13 (m, 1H), 4.04-4.01 (m, 2H), 3.94 (s, 3H), 3.91 (s, 6H), 3.86 (s, 3H), 3.88-3.84 (overlapped, 1H), 3.57 (dd, *J* = 12.9, 2.7 Hz, 1H), 3.07 (t, *J* = 6.9 Hz, 2H), 2.90-2.83 (m, 4H), 2.86-2.78 (m, 2H), 1.19 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.2, 164.2, 161.1, 159.0, 152.9, 149.9, 149.6, 145.6, 143.9, 140.2, 128.5, 124.1, 122.4, 120.4, 117.4, 117.3, 111.5, 110.3, 109.4, 96.1, 92.5, 78.9, 76.5, 70.0, 61.7, 56.6, 56.2, 56.1, 55.9, 49.9, 46.9, 26.4, 10.1; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{35}H_{42}NO_{10}$: 636.2809, found: 636.2806.

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3.10.5. 3-*O*-(*N*,*N*-Diethylamino)butyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**24**). 69% yield, yellow solid, mp. 64-65 °C. IR (film) ν_{max}: 3397, 2938, 1625, 1606, 1506, 1463 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (d, J = 6.3 Hz, 1H), 7.71 (s, 1H), 7.03 (d, J = 9.0 Hz, 2H), 6.97 (s, 1H), 6.91 (d, J = 8.1 Hz, 1H), 6.45 (s, 1H), 6.31 (s, 1H), 5.03 (d, J = 7.8 Hz, 1H), 4.15-4.10 (m, 1H), 4.02-4.98 (m, 2H), 3.95 (s, 3H), 3.93 (s, 6H), 3.90 (s, 3H), 3.85-3.81 (overlapped, 1H), 3.56 (dd, J = 12.3, 3.3 Hz, 1H), 2.96 (br.s, 2H), 2.63 (q, J = 7.2 Hz, 4H), 1.80-1.59 (m, 4H), 1.06 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.8, 164.5, 161.6, 159.4, 153.0, 150.3, 150.0, 145.8, 144.2, 141.0, 129.1, 124.9, 123.0, 120.8, 117.7, 117.6, 111.9, 110.8, 110.0, 96.4, 92.9, 79.3, 76.8, 72.5, 62.1, 57.0, 56.6, 56.6, 56.4, 52.7, 47.1, 28.7, 22.9, 11.3; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for C$_{36}$H$_{44}$NO$_{10}$: 650.2966, found 650.2963.

3.10.6. 3-*O*-(*N*,*N*-Diethylamino)pentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**25**). 33% yield, yellow wax. IR (film) ν_{max}: 3397, 2938, 1624, 1606, 1507, 1459 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.74 (dd, J = 9.3, 2.1 Hz, 1H), 7.74 (d, J = 2.1 Hz, 1H), 7.05 (d, J = 9.3 Hz, 1H), 7.04 (dd, J = 8.4, 2.1 Hz, 1H), 6.98 (d, J = 1.8 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 6.46 (d, J = 2.1 Hz, 1H), 6.32 (d, J = 2.1 Hz, 1H), 5.07 (d, J = 8.1 Hz, 1H), 4.16 (dt, J = 7.2, 2.4 Hz, 1H), 4.05-3.98 (m, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.85 (dd, J = 12.6, 2.7 Hz, 1H), 3.58 (dd, J = 12.6, 3.9 Hz, 1H), 2.75 (q, J = 7.2 Hz, 4H), 2.63 (t, J = 8.4 Hz, 2H), 1.73 (quin, J = 6.9 Hz, 2H), 1.56-1.51 (m, 2H), 1.46-1.41 (m, 2H), 1.13 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 161.0, 158.8, 152.5, 149.7, 149.4, 145.3, 143.6, 140.3, 128.5, 124.2, 122.3, 120.2, 117.2, 117.0, 111.3, 110.2, 109.4, 95.8, 92.3, 78.7, 76.1, 71.7, 61.5, 56.4, 56.1, 56.0, 55.7, 51.1, 46.3 (2 × C), 29.5, 23.7, 9.4; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for C$_{37}$H$_{46}$NO$_{10}$: 663.3122, found: 664.3116.

3.10.7. 3-*O*-(3-(*N*,*N*-Dipropyl)propyl)-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**26**). 78% yield, yellow wax. IR (film) ν_{max}: 3338, 3052, 2958, 2934, 2873, 2837, 1624, 1603, 1578, 1517, 1505, 1456 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.70 (d, J = 7.7 Hz, 1H), 7.68 (s, 1H), 7.05 (t, J = 7.9 Hz, 1H), 7.03 (d, J = 6.8 Hz, 1H), 6.97 (s, 1H), 6.91 (d, J = 8.2 Hz, 1H), 6.46 (s, 1H), 6.32 (s, 1H), 5.03 (d, J = 8.0 Hz, 1H), 4.13 (d, J = 7.8 Hz, 1H), 4.08-4.00 (m, 2H), 3.93 (s, 3H), 3.90 (s, 6H), 3.85 (s, 3H), 3.85-3.82 (overlapped, 1H), 3.57 (dd, J = 12.4, 3.3 Hz, 1H), 2.97-2.85 (m, 2H), 2.59 (t, J = 7.4 Hz, 4H), 2.05-2.00 (m, 2H), 1.56 (sextet, J = 6.8 Hz, 4H), 0.86 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.2, 164.1, 161.1, 158.9, 152.7, 149.9, 149.6, 145.5, 143.8, 140.3, 128.5, 124.2, 122.5, 120.3, 117.3, 117.2, 111.5, 110.3, 109.5, 96.0, 92.5, 78.9, 76.4, 70.3, 61.6, 56.5, 56.2, 56.1, 55.9, 55.4, 51.0, 26.7, 18.7, 11.8; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for C$_{37}$H$_{46}$NO$_{10}$: 664.3122, found: 664.3118.

3.10.8. 3-*O*-(*N*,*N*-dipropylamino)butyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**27**). 59% yield, yellow solid, mp. 80-81 °C. IR (film) ν_{max}: 3415, 2956, 1625, 1607, 1507, 1462, 1433 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.75 (d, J = 10.2 Hz, 1H), 7.73 (s, 1H), 7.04 (d, J = 8.4 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.97 (s, 1H), 6.92 (d, J = 8.1 Hz, 1H), 6.46 (s, 1H), 6.32 (s, 1H), 5.04 (d, J = 8.1 Hz, 1H), 4.15-4.12 (m, 1H), 4.05-3.99 (m, 2H), 3.94 (s, 3H), 3.91 (s, 6H), 3.86 (s, 3H), 3.88-3.82 (overlapped, 1H), 3.57 (dd, J = 12.3, 3.3 Hz, 1H), 2.55 (t, J = 5.7 Hz, 2H), 2.44 (t, J = 7.5 Hz, 4H), 1.80-1.56 (m, 4H), 1.52-1.43 (m, 4H), 0.85 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.8, 164.5, 161.6, 159.4, 152.9, 150.4, 150.0, 145.7, 144.2, 141.1, 129.0, 125.0, 123.1, 120.8, 117.7, 117.6, 111.9, 110.7, 110.1, 96.4, 92.9, 79.3, 76.9, 72.7, 62.2, 57.0, 56.7, 56.6, 56.4, 56.2, 54.1, 28.7, 23.3, 20.1, 12.5; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for C$_{38}$H$_{48}$NO$_{10}$: 678.3279, found: 678.3275.

3.10.9. 3-*O*-(*N*,*N*-Dipropylamino)pentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**28**). 60% yield, white solid, mp. 94.0-94.5 °C. IR (film) ν_{max}: 3388, 2936, 2873, 2838, 1624, 1605, 578, 1490, 1462 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.73 (d, J = 7.2 Hz, 1H), 7.72 (s, 1H), 7.06 (d, J = 9.3 Hz, 2H), 6.99 (s, 1H), 6.93 (d, J = 8.4 Hz, 1H), 6.47 (s, 1H), 6.33 (s, 1H), 5.07 (d, J = 7.8 Hz, 1H), 4.16 (d, J = 7.8 Hz, 1H), 4.03 (t, J = 6.0 Hz, 2H), 3.94 (s, 3H), 3.91 (s, 6H), 3.87 (s, 3H), 3.87-3.83 (overlapped, 1H), 3.67 (dd, J = 12.3, 3.0 Hz, 1H), 2.68-2.51 (overlapped, 6H), 1.75 (quin, J = 6.9 Hz, 2H), 1.60-1.50 (m, 6H), 1.43 (quin, J = 6.9 Hz, 2H), 0.90 (t, J = 7.5 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 161.0, 158.8, 152.3, 149.7, 149.4, 145.1, 143.6, 140.4, 128.4, 124.4, 122.4, 120.1, 117.2, 116.9, 111.3, 110.2, 109.5, 95.8, 92.3, 78.6, 76.2, 72.0, 61.5, 56.4, 56.1, 56.0, 55.7, 55.2, 53.2, 29.7, 23.8, 18.8, 18.7. 11.7; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for C$_{39}$H$_{50}$NO$_{10}$: 692.3435, found: 692.3436.

3.10.10. 3-*O*-(3-(*N*,*N*-Dibutylamino)propyl)-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**29**). 79% yield, yellow wax. IR (film) ν_{max}: 3352, 2957, 2933, 2872, 2838, 1624, 1604, 1578, 1505, 1491, 1457 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.67 (d, *J* = 7.2 Hz, 1H), 7.65 (s, 1H), 7.04 (d, *J* = 8.7 Hz, 1H), 6.98 (d, *J* = 7.2 Hz, 1H), 6.96 (s, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 6.45 (s, 1H), 6.31 (s, 1H), 5.02 (d, *J* = 8.1 Hz, 1H), 4.12 (d, *J* = 7.8 Hz, 1H), 4.05-3.95 (m, 2H), 3.92 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H), 3.84-3.81 (overlapped, 1H), 3.55 (dd, *J* = 12.6, 3.3 Hz, 1H), 3.04 (t, *J* = 6.9 Hz, 2H), 2.72 (t, *J* = 7.8 Hz, 4H), 2.13-2.05 (m, 2H), 1.62-1.51 (m, 4H), 1.30 (quin, *J* = 7.2 Hz, 4H), 0.89 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.2, 164.2, 161.0, 158.9, 152.8, 149.9, 149.5, 145.5, 143.9, 140.2, 128.5, 124.1, 122.5, 120.3, 117.3, 117.2, 111.4, 110.3, 109.4, 96.0, 92.5, 78.9, 76.4, 70.1, 61.6, 56.6, 56.2, 56.1, 55.9, 53.3, 51.1, 27.1, 26.5, 20.6, 14.0; HRMS-ESI *m/z* [M+H]$^+$ calcd for C$_{39}$H$_{50}$NO$_{10}$: 692.3435, found 692.3427.

3.10.11. 3-*O*-(*N*,*N*-dibutylamino)butyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**30**). 74% yield, yellow wax. IR (film) ν_{max}: 3397, 2931, 2870, 1624, 1605, 1579, 1506, 1460 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.75 (dd, *J* = 9.9, 1.8 Hz, 1H), 7.73 (s, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.97 (s, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.46 (d, *J* = 1.8 Hz, 1H), 6.32 (d, *J* = 2.1 Hz, 1H), 5.05 (d, *J* = 8.1 Hz, 1H), 4.16-4.12 (m, 1H), 4.02 (t, *J* = 5.7 Hz, 2H), 3.94 (s 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.86-3.81 (overlapped, 1H), 3.58 (dd, *J* = 12.6, 3.9 Hz, 1H), 2.59 (t, *J* = 7.5 Hz, 2H), 2.51 (t, *J* = 7.5 Hz, 4H), 1.78-1.60 (m, 4H), 1.50-1.40 (m, 4H), 1.33-1.21 (m, 4H), 0.89 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 161.0, 158.8, 152.3, 149.7, 149.4, 145.1, 143.6, 140.4, 128.4, 124.4, 122.4, 120.2, 117.1, 117.0, 111.3, 110.1, 109.4, 96.8, 92.3, 78.6, 76.2, 72.0, 61.5, 56.4, 56.0, 55.9, 55.7, 53.3, 53.2, 28.1, 22.5, 20.6, 14.0; HRMS-ESI *m/z* [M+H]$^+$ calcd for C$_{40}$H$_{52}$NO$_{10}$: 706.3592, found: 706.3591.

3.10.12. 3-*O*-(*N*,*N*-Dibutylamino)pentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**31**). 85% yield, yellow wax. IR (film) ν_{max}: 3398, 2931, 1625, 1606, 1506, 1462 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.71 (s, 1H), 7.04 (d, *J* = 8.1 Hz, 2H), 6.98 (d, *J* = 1.8 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.46 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.06 (d, *J* = 8.1 Hz, 1H), 4.17-4.13 (m, 1H), 4.02 (t, *J* = 6.3 Hz, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.86-3.81 (overlapped, 1H), 3.58 (dd, *J* = 12.3, 3.9 Hz, 1H), 2.54 (t, *J* = 7.5 Hz, 6H), 1.74 (quin, *J* = 7.5 Hz, 2H), 1.51-1.25 (m, 12H), 0.90 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.8, 164.5, 161.6, 159.4, 153.0, 150.3, 150.0, 145.8, 144.2, 141.1, 129.1, 124.9, 123.0, 120.8, 117.8, 117.6, 111.9, 110.8, 110.0, 96.4, 92.9, 79.3, 76.8, 72.7, 62.1, 57.0, 56.7, 56.6, 56.4, 53.9, 53.8, 30.5, 28.3, 25.7, 24.5, 21.2, 14.6; HRMS-ESI *m/z* [M+H]$^+$ calcd for C$_{41}$H$_{54}$NO$_{10}$: 720.3748, found: 720.3741.

3.10.13. 3-*O*-Pyrrolidinopropyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**32**). 84% yield, yellow oil. IR (film) ν_{max}: 3361, 2935, 2879, 2837, 1668, 1624, 1604, 1578, 1517, 1505, 1459 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69 (s, 1H), 7.68 (dd, *J* = 7.8, 2.1 Hz, 1H), 7.06-7.01 (overlapped, 2H), 6.96 (d, *J* = 1.5 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 1H), 6.32 (d, *J* = 2.1 Hz, 1H), 5.03 (d, *J* = 8.4 Hz, 1H), 4.13 (dt, *J* = 7.8, 3.3 Hz, 1H), 4.07-3.96 (m, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.85 (s, 3H), 3.85-3.81 (overlapped, 1H), 3.56 (dd, *J* = 12.3, 3.6 Hz, 1H), 3.05 (t, *J* = 7.8 Hz, 2H), 2.95-2.83 (m, 4H), 2.16-2.00 (m, 2H), 1.92 – 1.83 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.2, 164.2, 161.1, 159.0, 152.8, 149.9, 149.6, 145.5, 143.8, 140.2, 128.5, 124.2, 122.4, 120.4, 117.3, 117.3, 111.5, 110.4, 109.5, 96.0, 92.5, 78.9, 76.4, 70.0, 61.6, 56.6, 56.2, 56.1, 55.9, 53.7, 53.3, 28.2, 23.6; HRMS-ESI *m/z* [M+H]$^+$ calcd for C$_{35}$H$_{40}$NO$_{10}$: 634.2652, found: 634.2648.

3.10.14. 3-*O*-Pyrrolidinobutyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**33**). 69% yield, yellow solid, mp. 109-110 °C. IR (film) ν_{max}: 3398, 2936, 1625, 1606, 1507, 1458, 1433 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.70 (s, 1H), 7.69 (d, *J* = 5.4 Hz, 1H), 7.04 (d, *J* = 9.0 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.97 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.45 (s, 1H), 6.31 (s, 1H), 5.05 (d, *J* = 8.1 Hz, 1H), 4.15-4.12 (m, 1H), 4.00-3.90 (overlapped, 2H), 3.93 (s, 3H), 3.90 (s, 6H), 3.85 (s, 3H), 3.85-3.81 (overlapped, 1H), 3.56 (dd, *J* = 12.6, 3.6 Hz, 1H), 3.35-3.19 (m, 2H), 2.88-2.71 (m, 4H), 1.95-1.76 (m, 8H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.8, 164.6, 161.5, 159.4, 153.2, 150.3, 150.0, 145.9, 144.2, 140.9, 129.1, 124.8, 123.0, 120.8, 117.74, 117.70, 111.9, 110.8, 110.0, 96.4, 93.0, 79.3, 76.8, 72.2, 62.0, 57.0, 56.7, 56.6, 56.4, 56.3, 54.2, 28.4, 24.8, 24.0; HRMS-ESI *m/z* [M+H]$^+$ calcd for C$_{36}$H$_{42}$NO$_{10}$: 648.2809, found: 648.2806.

3.10.15. 3-*O*-Pyrrolidinopentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**34**). 77% yield, reddish wax. IR (film) ν_{max}: 3348, 2935, 2874, 2838, 2802, 1667, 1623, 1604, 1578, 1517, 1505, 1460 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.70 (s, 1H), 7.69 (dd, *J* = 7.3, 2.0 Hz, 1H), 7.03 (d, *J* = 9.3 Hz, 2H), 6.98 (d, *J* = 1.3 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.06 (d, *J* =7.9 Hz, 1H), 4.16-4.13 (m, 1H), 4.03-3.96 (m, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.85-3.81 (overlapped, 1H), 3.57 (dd, *J* = 12.5, 3.8 Hz, 1H), 2.74 (t, *J* = 6.6 Hz, 4H), 2.58 (t, *J* = 7.9 Hz, 2H), 1.90-1.81 (m, 4H), 1.76-1.55 (m, 4H), 1.42 (quin, *J* = 7.8 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 161.0, 158.8, 152.4, 149.7, 149.4, 145.2, 143.5, 140.5, 128.6, 124.3, 122.4, 120.1, 117.2, 116.9, 111.3, 110.2, 109.4, 95.8, 92.3, 78.7, 76.1, 72.1, 61.4, 56.4, 56.0, 55.9, 55.8, 55.7, 53.5, 29.8, 27.1, 23.9, 23.4; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{37}H_{44}NO_{10}$: 662.2965, found: 662.2970.

3.10.16. 3-*O*-Piperidinopropyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**35**). 51% yield, yellow wax. IR (film) ν_{max}: 3380, 2934, 2853, 1702, 1626, 1606, 1578, 1518, 1506, 1460 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.70 (s, 1H), 7.69 (d, *J* = 7.2 Hz, 1H), 7.06 (d, *J* = 9.0 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.97 (s, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 6.46 (d, *J* = 1.6 Hz, 1H), 6.32 (d, *J* = 1.7 Hz, 1H), 5.03 (d, *J* = 8.1 Hz, 1H), 4.14 (dd, *J* = 11.2, 3.2 Hz, 1H), 4.08-3.97 (m, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H), 3.86-3.82 (overlapped, 1H), 3.57 (dd, *J* = 12.5, 3.7 Hz, 1H), 2.94-2.83 (m, 2H), 2.76-2.63 (m, 4H), 2.12-2.08 (m, 2H), 1.79-1.76 (m, 4H), 1.54-1.43 (m, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 164.0, 160.9, 158.8, 152.6, 149.8, 149.4, 145.3, 143.7, 140.1, 128.3, 124.0, 122.3, 120.2, 117.2, 117.2, 111.3, 110.2, 109.3, 95.9, 92.4, 78.7, 76.3, 70.1, 61.5, 56.4, 56.1, 56.0, 55.77, 55.76, 53.9, 26.2, 24.3, 23.4; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{36}H_{42}NO_{10}$: 648.2809, found: 648.2807.

3.10.17. 3-*O*-Piperidinobutyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**36**). 78% yield, yellow solid, mp. 91-92 °C. IR (film) ν_{max}: 3392, 2937, 2837, 1625, 1606, 1506, 1458 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.70-7.65 (overlapped, 2H), 7.02 (d, *J* = 9.0 Hz, 2H), 6.97 (s, 1H), 6.90 (d, *J* = 8.1 Hz, 1H), 6.44 (s, 1H), 6.30 (s, 1H), 5.03 (d, *J* = 7.8 Hz, 1H), 4.15-4.10 (m, 1H), 3.97 (t, *J* = 5.7 Hz, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.84 (s, 3H), 3.84-3.81 (overlapped, 1H), 3.56 (dd, *J* = 12.3, 3.6 Hz, 1H), 2.70-2.42 (overlapped, 6H), 1.74-1.64 (m, 8H), 1.52-1.36 (m, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.8, 164.6, 161.5, 159.4, 153.0, 150.3, 150.0, 145.9, 144.2, 141.0, 129.1, 124.8, 123.0, 120.8, 117.71, 117.65, 111.9, 110.8, 110.0, 96.4, 93.0, 79.3, 76.8, 72.4, 62.0, 59.1, 57.0, 56.7, 56.6, 56.4, 54.5, 28.6, 25.4, 24.3, 22.9; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{37}H_{44}NO_{10}$: 662.2966, found: 662.2959.

3.10.18. 3-*O*-Piperidinopentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**37**). 91% yield, white solid, mp. 89.5-90.0 °C. IR (film) ν_{max}: 3404, 2935, 2858, 1624, 1605, 1578, 1538, 1517, 1490, 1462 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.71 (s, 1H), 7.70 (d, *J* = 6.5 Hz, 1H), 7.03 (d, *J* = 8. 2 Hz, 2H), 6.98 (s, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.45 (s, 1H), 6.31 (s, 1H), 5.06 (d, *J* = 7.9 Hz, 1H), 4.16-4.13 (m, 1H), 4.03-3.97 (m, 2H), 3.93 (s, 3H), 3.90 (s, 6H), 3.85 (s, 3H), 3.85-3.82 (overlapped, 1H), 3.58 (dd, *J* = 11.8, 2.1 Hz, 1H), 2.59-2.45 (m, 4H), 2.41 (t, *J* = 7.1 Hz, 2H), 1.76-1.62 (m, 6H), 1.60-1.52 (m, 2H), 1.51-1.37 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 74.3, 164.0, 161.1, 158.9, 152.5, 149.9, 149.6, 145.3, 143.7, 140.6, 128.7, 124.5, 122.6, 120.3, 117.4, 117.1, 111.5, 110.3, 109.6, 95.9, 92.5, 78.8, 76.3, 72.3, 61.6, 58.9, 56.5, 56.2, 56.1, 55.9, 54.1, 30.0, 25.6, 25.0, 24.2, 23.9; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{38}H_{46}NO_{10}$: 676.3122, found: 676.3117.

3.10.19. 3-*O*-Morpholinopropyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**38**). 54% yield, yellow wax. IR (film) ν_{max}: 3379, 2937, 2838, 1625, 1606, 1518, 1506, 1493, 1462 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.69 (s, 1H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.07 (d, *J* = 8.9 Hz, 1H), 7.04 (d, *J* = 10.8 Hz, 1H), 6.97 (s, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 6.48 (s, 1H), 6.34 (s, 1H), 5.03 (d, *J* = 8.3 Hz, 1H), 4.16-4.14 (m, 1H), 4.02-3.90 (overlapped, 2H), 3.94 (s, 3H), 3.91 (s, 6H), 3.87 (s, 3H), 3.94-3.87 (overlapped, 4H), 3.87-3.83 (overlapped, 1H), 3.58 (dd, *J* = 12.2, 2.9 Hz, 1H), 3.24-3.10 (m, 2H), 3.03-2.83 (m, 4H), 2.29-2.13 (m, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.2, 164.3, 161.1, 159.0, 153.0, 150.0, 149.6, 145.6, 143.9, 140.1, 128.4, 124.0, 122.4, 120.4, 117.4, 117.3, 111.5, 110.4, 109.4, 96.1, 92.6, 78.9, 76.5, 69.6, 65.0, 61.7, 56.6, 56.2, 56.2, 56.1, 56.0, 52.9, 25.6; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{35}H_{40}NO_{11}$: 650.2601, found: 650.2602.

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3.10.20. 3-*O*-Morpholinobutyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**39**). 90% yield, yellow wax. IR (film) ν_{max}: 3415, 2938, 1624, 1605, 1579, 1491 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.73 (d, *J* = 6.9 Hz, 1H), 7.72 (s, 1H), 7.02 (d, *J* = 9.0 Hz, 2H), 6.96 (s, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.45 (d, *J* = 1.5 Hz, 1H), 6.31 (s, 1H), 5.03 (d, *J* = 8.1 Hz, 1H), 4.13-4.10 (m, 1H), 4.07-4.01 (m, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.85 (s, 3H), 3.85-3.82 (overlapped, 1H), 3.68 (t, *J* = 4.5 Hz, 4H), 3.56 (dd, *J* = 12.3, 3.6 Hz), 2.42-2.35 (overlapped, 6H), 1.77-1.62 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.8, 164.5, 161.6, 159.4, 152.8, 150.4, 150.0, 145.8, 144.2, 141.1, 129.0, 125.0, 123.0, 120.8, 117.8, 117.6, 111.9, 110.7, 110.0, 96.4, 92.9, 79.3, 76.9, 72.6, 67.4, 62.2, 59.3, 57.0, 56.7, 56.6, 56.4, 54.1, 28.7, 23.3; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for $C_{36}H_{42}NO_{11}$: 664.2758, found: 664.2757.

3.10.21. 3-*O*-Morpholinopentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**40**). 77% yield, light yellow wax. IR (film) ν_{max}: 3397, 2936, 1625, 1603, 1505, 1456, 1428 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72-7.69 (overlapped, 2H), 7.02 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.01 (d, *J* = 9.3 Hz, 1H), 6.97 (s, 1H), 6.90 (d, *J* = 8.1 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 1H), 6.30 (d, *J* = 2.1 Hz, 1H), 5.03 (d, *J* = 8.1 Hz, 1H), 4.12 (dt, *J* = 8.1, 4.8 Hz, 1H), 4.01-3.98 (m, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.84 (s, 3H), 3.84-3.80 (overlapped, 1H), 3.72 (t, *J* = 4.5 Hz, 4H), 3.56 (dd, *J* = 12.6, 3.9 Hz, 1H), 2.47 (br,s, 4H), 2.35 (t, *J* = 7.2 Hz, 2H), 1.73 (quin, *J* = 6.9 Hz, 2H), 1.53-1.23 (m, 4H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 160.9, 158.7, 152.2, 149.7, 149.4, 145.1, 143.6, 140.5, 128.4, 124.4, 122.4, 120.2, 117.1, 116.9, 111.3, 110.2, 109.4, 95.7, 92.3, 78.7, 76.2, 72.0, 66.5, 61.5, 58.9, 56.3, 56.0, 55.9, 55.7, 53.4, 29.9, 25.7, 23.8; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for $C_{37}H_{44}NO_{11}$: 678.2914, found: 678.2915.

3.10.22. 3-*O*-(Piperazin-1-yl)propyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**41**). 74% yield, white solid, mp. 158-159 °C. IR (film) ν_{max}: 3347, 3207, 2937, 2836, 1623, 1604, 1578, 1517, 1490, 1458 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.67 (s, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 2H), 6.97 (s, 1H), 6.90 (d, *J* = 8.1 Hz, 1H), 6.44 (s, 1H), 6.31 (s, 1H), 5.73 (br.s, 1H), 5.04 (d, *J* = 7.8 Hz, 1H), 4.13 (d, *J* = 7.5 Hz, 1H), 4.04 (t, *J* = 5.4 Hz, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.85-3.82 (overlapped, 1H), 3.55 (dd, *J* = 12.3, 3.0 Hz, 1H), 2.97 (br.s, 4H), 2.49 (br.s., 6H), 1.90-1.79 (m, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 160.9, 158.8, 152.5, 149.7, 149.3, 145.3, 143.5, 140.3, 128.5, 124.1, 122.5, 120.2, 117.3, 116.9, 111.3, 110.2, 109.4, 95.8, 92.3, 78.7, 76.1, 70.4, 61.2, 56.4, 56.0, 55.9, 55.8, 55.3, 51.7, 44.2, 27.2; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for $C_{35}H_{41}N_2O_{10}$: 649.2761, found: 649.2764.

3.10.23. 3-*O*-(Piperazin-1-yl)butyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**42**). 68% yield, yellow wax. IR (film) ν_{max}: 3379, 2934, 1625, 1604, 1505, 1456 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.68 (d, *J* = 7.8 Hz, 1H), 7.66 (s, 1H), 7.03 (d, *J* = 7.8 Hz, 2H), 6.99 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.45 (s, 1H), 6.32 (s, 1H), 5.06 (d, *J* = 7.8 Hz, 1H), 4.17-4.09 (m, 1H), 4.09-3.99 (m, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.85-3.79 (overlapped, 1H), 3.56 (d, *J* = 11.1 Hz, 1H), 3.11-2.99 (m, 2H), 2.61-2.43 (m, 4H), 2.39-2.26 (m, 2H), 1.74-1.47 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.8, 164.5, 161.6, 159.4, 153.2, 150.3, 150.0, 145.8, 144.2, 141.0, 129.1, 124.9, 123.3, 120.8, 117.9, 117.5, 111.9, 110.8, 110.0, 96.4, 92.9, 79.4, 76.7, 72.7, 61.8, 58.6, 57.0, 56.7, 56.6, 56.4, 52.0, 44.9, 28.4, 23.7; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for $C_{36}H_{43}N_2O_{10}$: 663.2918, found: 663.2912.

3.10.24. 3-*O*-(Piperazin-1-yl)pentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**43**). 98% yield, light yellow wax. IR (film) ν_{max}: 3365, 3003, 2937, 2861, 2836, 1667, 1623, 1604, 1578, 1517, 1491, 1459 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (d, *J* = 8.1 Hz, 1H), 7.67 (s, 1H), 7.04 (d, *J* = 8.1 Hz, 2H), 6.98 (s, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.46 (s, 1H), 6.32 (s, 1H), 5.05 (d, *J* = 7.8 Hz, 1H), 4.12 (d, *J* = 7.2 Hz, 1H), 4.02 (t, *J* = 4.5 Hz, 2H), 4.02 (s, 3H), 3.93 (s, 6H), 3.91 (s, 6H), 3.91-3.83 (overlapped, 1H), 3.56 (d, *J* = 12.3 Hz, 1H), 3.28-2.98 (m, 4H), 2.62-2.50 (m, 4H), 2.41-2.25 (m, 2H), 1.78-1.60 (m, 2H), 1.50-1.33 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.3, 164.1, 161.1, 159.0, 152.7, 149.9, 149.6, 145.3, 143.8, 140.6, 128.7, 124.5, 122.8, 120.4, 117.3, 117.0, 111.5, 110.4, 109.6, 95.9, 92.5, 79.0, 76.4, 72.2, 61.3, 58.5, 56.5, 56.2, 56.1, 55.9, 51.0, 43.9, 30.0, 26.2, 24.1; HRMS-ESI *m*/*z* [M+H]$^+$ calcd for $C_{37}H_{45}N_2O_{10}$: 677.3074, found: 677.3064.

3.10.25. 3-*O*-(4-Methylpiperazin-1-yl)propyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**44**). 75% yield, yellow wax. IR (film) ν_{max}: 3398, 3002, 2937, 2879, 2836, 2806, 1625, 1606, 1578, 1517, 1506, 1491, 1460

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cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.70 (s, 1H), 7.68 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 1.5 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.04 (d, *J* = 8.1 Hz, 1H), 4.17-4.12 (m, 1H), 4.10-4.04 (m, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.85-3.82 (overlapped, 1H), 3.58 (dd, *J* = 12.3, 3.9 Hz, 1H), 2.62-2.47 (overlapped, 8H), 2.32 (s, 3H), 1.93 (quin, *J* = 6.6 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.0, 163.9, 161.0, 158.8, 152.3, 149.7, 149.4, 145.2, 143.6, 140.3, 128.5, 124.2, 122.4, 120.2, 117.3, 117.0, 111.3, 110.2, 109.4, 95.8, 92.3, 78.7, 76.2, 70.4, 61.4, 56.4, 56.1, 56.0, 55.7, 55.2, 54.3, 52.3, 45.4, 27.2; HRMS-ESI *m/z* [M+H]$^+$ calcd for $C_{36}H_{43}N_2O_{10}$ 663.2918, found: 663.2914.

3.10.26. 3-*O*-(4-Methylpiperazin-1-yl)pentyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybin (**45**). 93% yield, light yellow wax. IR (film) ν_{max}: 3384, 2941, 2838, 1624, 1606, 1579, 1517, 1506, 1491, 1463 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.72 (dd, *J* = 7.7, 2.1 Hz, 1H), 7.70 (s, 1H), 7.05-7.01 (overlapped, 2H), 6.98 (d, *J* = 1.8 Hz, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 6.31 (d, *J* = 2.2 Hz, 1H), 5.06 (d, *J* = 8.1 Hz, 1H), 4.13 (dt, *J* = 7.9, 3.1 Hz, 1H), 4.02 (t, *J* = 6.4 Hz, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.85 (dd, *J* = 12.5, 2.6 Hz, 1H), 3.56 (dd, *J* = 12.5, 3.8 Hz, 1H), 2.89-2.70 (overlapped, 4H), 2.63-2.45 (m, 6H), 2.32 (s, 3H), 1.71 (quin, *J* = 6.3 Hz, 2H), 1.51-1.35 (m, 4H); ^{13}C NMR (75 MHz, CDCl$_3$): δ 174.1, 163.9, 161.0, 158.8, 152.3, 149.7, 149.4, 145.1, 143.6, 140.5, 128.5, 124.4, 122.5, 120.2, 117.2, 116.9, 111.3, 110.2, 109.5, 95.8, 92.3, 78.7, 76.1, 72.2, 61.4, 58.4, 56.4, 56.1, 56.0, 55.7, 54.5, 52.5, 45.6, 29.9, 26.1, 23.9; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{38}H_{47}N_2O_{10}$: 691.3231, found 691.3233.

3.11. Cell culture

All cell lines were initially purchased from American Type Culture Collection (ATCC™). The PC-3 and LNCaP prostate cancer cell lines were routinely cultured in RPIM-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. The DU145 prostate cancer cells were routinely cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS and 1% penicillin/streptomycin. Cultures were maintained in a high humidity environment supplemented with 5% carbon dioxide at a temperature of 37°C.

3.12. WST-1 cell proliferation assay

PC-3, DU145, or LNCaP cells were plated in 96-well plates at a density of 3,200 each well in 200 μL of culture medium. The cells were then treated with silybin, or synthesized silybin derivatives separately at 5 different doses for 3 days, while equal treatment volumes of DMSO (0.25%) were used as vehicle control. The cells were cultured in a CO_2 incubator at 37 °C for three days. 10 μL of the premixed WST-1 cell proliferation reagent (Clontech) was added to each well. After mixing gently for one minute on an orbital shaker, the cells were incubated for additional 3 hours at 37 °C. To ensure homogeneous distribution of color, it is important to mix gently on an orbital shaker for one minute. The absorbance of each well was measured using a microplate reader (Synergy HT, BioTek) at a wavelength of 430 nm. The IC_{50} value is the concentration of each compound that inhibits cell proliferation by 50% under the experimental conditions and is the average from at least triplicate determinations that reproducible and statistically significant. For calculating the IC_{50} values, a linear proliferative inhibition was made based on at least five dosages for each compound.

3.13. Cell cycle analysis

PC-3 cells were plated in 24-well plates at a density of 200,000 each well in 400 μL of culture medium. After 3 hours of cell attachment, the cells were then treated with compound 30 at 5 μM for 15 hours, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in CO_2 incubator at 37°C for 22 hours and 31 hours, respectively. Both attached and floating cells were collected in a centrifuge tube by centrifugation at rcf value of 450 g for 5 minutes. After discarding the supernatant, the collected cells were re-suspended with 500 μL 80% cold ethanol to fix for 30 minutes in 4°C. The fixed cell could store at -20°C for one week. After fixation, the ethanol was removed after centrifuging and the cells were washed with PBS. The cells were then re-suspend with 100 μL of 100 mg/mL ribonuclease and were cultured at 37°C for 30 minutes to degrade all

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RNA. The cells were stained with 200 μL of 50 μg/mLpropidium iodide stock solution for 30 minutes at -20°C, and then the fluorescence intensity of PI was detected in individual PC-3 cells using an Attune flow cytometer (Life Technologies) within 0.5 to 1 hour after staining.

3.14. F2N12S and SYTOX AADvanced double staining assay

PC-3 cells were plated in 24-well plates at a density of 200,000 each well in 400 μL of culture medium. After 3 hours of cell attachment, the cells were then treated with each test compound at different concentration for 15 hours, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in CO_2 incubator at 37°C for 15 hours. Both attached and floating cells were collected in a centrifuge tube by centrifugation at rcf value of 450 g for 5 to 6 minutes. The collected cells were re-suspended with 500 μL HBSS to remove proteins which may affect flow signal and centrifuged again. After discarding the supernatant, the collected cells were re-suspended with 300 μL HBSS and stained with 0.3 μL of F2N12S for 3-5 minutes followed by 0.3 μL SytoxAAdvanced for an additional 5 minutes. The fluorescence intensity of the two probes was further measured in individual PC-3 cells using an Attune flow cytometer (Life Technologies) 0.5 to 1 hour after staining.

3.15. Statistical analysis:

All data are represented as the mean ± standard deviation (S.D.) for the number of experiments indicated. Other differences between treated and control groups were analyzed using the Student's t-test. A p-value < 0.05 was considered statistically significant.

4. Conclusions

In summary, six 7-*O*-aminoalkyl-3,5-20-*O*-trimethyl-2,3-dehydrosilybins and twenty-six 3-*O*-aminoalkyl-5,7,20-*O*-trimethyl-2,3-dehydrosilybins have been successfully synthesized through a five-step and four-step sequence, respectively. The synthetic methods can be used for the general synthesis of 7-*O*-substituted-3,5,20-*O*-trimethyl-2,3-dehydrosilybins and 3-*O*-substituted-5,7,20-O-trimethyl-2,3-dehydrosilybins. The antiproliferative activities of the thirty-two derivatives against three prostate cancer cell lines have been evaluated using WST-1 cell proliferation assay. All of them showed better prostate cancer cell proliferation inhibition than silybin. Derivatives **11**, **29**, **31**, **37**, and **40** were identified as the optimal derivatives with IC_{50} values in the range of 1.40–3.06 μM toward these three prostate cancer cell lines, a 17- to 52-fold improvement in potency as compared with silybin. All these five optimal derivatives can cause PC-3 cell accumulation in a G_0/G_1 phase by increasing the cell population in this phase at 16 hours. Derivatives **11**, **37**, and **40** show stronger ability than derivatives **29** and **31** in activating PC-3 cell apoptosis by inducing appreciable levels of apoptotic cell death at 5 μM concentration after a 16-hour treatment. In contrast, derivatives **29** and **31** did not induce significant levels of apoptotic cell death (less than 10%) up to 10 μM concentration.

Supplementary Materials: NMR-spectra (^{1}H and ^{13}C) of the new silybin derivatives.

Author Contributions: conceptualization and designed, Q.C., B.V., and G.W.; performed the experiment and analyzed data, B.V., S.Z., A.V., G.C., X.Z., W.D., Q.Z., S.Z., and Q.C.; Drafted and proofread the paper: Q.C., B.V., S.Z., G.W.; All authors finally reviewed and approved the manuscript.

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