New Silybin Scaffold for Chemical Diversification: Synthesis of Novel 23-Phosphodiester Silybin Conjugates

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Received: 01.10.2012; Accepted after revision: 02.11.2012

Abstract: Silybin is the major component (ca. 30%) of the silymarin complex extracted from the seeds of Silybum marianum, with multiple biological activities operating at various cell levels. As an ongoing effort toward the exploitation of natural products as scaffolds for chemical diversification at readily accessible positions, we present here an efficient synthetic procedure to obtain new 23-phosphodiester silybin conjugates with different labels. A key point in our approach is the new 3,5,7,20-tetraacetylsilybin-23-phosphoramidite, useful for a variety of derivatizations following a reliable and well-known chemistry. The feasibility of the procedure has been demonstrated by preparing new 23-silybin conjugates, exploiting standard phosphoramidite chemistry.

Key words: natural products, flavonolignans, silybin, phosphorylation, drugs

Flavonoids and flavonolignans are widely distributed among various citrus plants and are frequently found in the human diet. They may act as enzyme inhibitors, free-radical scavengers, antitumor agents, antibacterial agents, anti-inflammatory agents, and antioxidants.1–3

Silybin is the major component (ca. 30%) of the silymarin complex extracted from the seeds of Silybum marianum consisting of two diastereomers A and B in a ratio of approximately 1:1.

Figure 1 Chemical structure of silybin A and silybin B

Silybin is a natural compound with multiple biological activities operating at various cell levels most of them related to its radical-scavenging activity. Along with the beneficial activities resulting from the antioxidant and radical-scavenging properties,5,6 silybin has recently received attention due to its anticancer and chemopreventive actions,5,6 as well as hypcholesterolemic, cardioprotective, and neuroprotective activities.5,7 In vivo applications of silybin are rather hampered by its very low bioavailability. In an attempt to improve its biological properties and facilitate in vivo applications of silybin, only limited structural modifications have been proposed3,8–12 and the available analogues are still unsatisfactory. Therefore new synthetic approaches for selectively modifying silybin are of interest.

As a part of our continuing research effort towards the synthesis of new natural product analogues,13,14 we present here the preliminary results of an efficient synthetic procedure to obtain new 23-phosphodiester silybin conjugates with different labels.

The introduction of a phosphate group may bring pharmaceutical and pharmacokinetic benefits.15 Conjugation is usually considered as an efficient route in drug discovery to improve the biological properties of a large number of drugs and can improve the bioavailability and delivery as well as the biological activity.

We chose to start from the new 23-phosphoramidite building block 3 (Scheme 1) which could be transformed into a series of conjugates using a solution-phase parallel array protocol, exploiting standard and reliable phosphoramidite chemistry.15 We initially converted silybin (1) into its 23-ODMT ether by a reaction with DMT-chloride in pyridine at 50 °C. After exhaustive acetylation with an excess of acetic anhydride in pyridine, subsequent treatment with 5% formic acid in dichloromethane allowed the removal of the DMT protecting group to give 2 in 75% yield and this could be converted into the corresponding phosphoramidite derivatives 3. Thus intermediate 2 was reacted with 2-cyanoethyl-N,N-diisopropylamino-chlorophosphoramidite and DIPEA in anhydrous dichloromethane. In these preliminary studies, the silybin used was a mixture of diastereomers, and the derivatives 3 were obtained as a mixture of inseparable diastereomers, although the 1H NMR and 31P NMR spectra appeared to be of a single compound. After purification the identities of compounds 3, obtained in good yields (65%), were confirmed by NMR (1H, 13C, and 31P) and ESI-HRMS analysis.16

Subsequently we selected a group of model molecules having a free hydroxyl group (A–E, Scheme 1), useful for coupling with the key intermediate 3.17 In particular, we selected molecules known for their ability to act as molecular carriers (steroids, bile acids),18–21 to improve water solubility (polyethers)22 and as radical scavengers (nucleosides).23,24 While A, B, and C are commercially available, D and E were efficiently obtained starting from 2′-deoxyadenosine and 3′,7′a,12′a,24-tetrahydroxycoclaine.
respectively. Exploiting the different reactivity of hydroxyl groups our studies were carried out using TBDMS or DMTCl for the transient protection of the primary OH moiety while all other groups were protected by acetylation. 2′-Deoxyguanosine and 3α,7α,12α,24-tetrahydroxycholane were protected by reaction with TBDMS and DMTCl, respectively. 25,26 The products were acetylated with acetic anhydride to yield fully protected products. Finally, the TBDMS group was cleaved by Et₃N·3HF and DCA to yield N-2-acetyl-2′,3′-O-diacetyl-deoxyguanosine (D) and 3α,7α,12α-O-triacetyl-24-hydroxycholane (E) in overall yields of 80 and 75%, respectively.

The coupling of 3 with A–E (Scheme 1) was carried out by using the classic coupling reagent (0.45 M tetrazole in MeCN), and then treatment with 5.5 M tert-butyl hydroperoxide solution in decane led to phosphotriesters 4a–e. After purification by flash chromatography, the derivatives 4 were treated with concentrated aqueous ammonia and MeOH (1:1, v:v) at room temperature, allowing full deprotection, leading to the desired phosphodiester derivatives 5a–e in good yields (Scheme 1).27 All final derivatives 5a–e were purified by flash chromatography and then characterized by NMR (¹H and ³¹P) and MS analysis. All the intermediates and final derivatives were obtained as mixtures of two diastereomers, that were inseparable by chromatography, although the NMR spectra of many of them appeared to indicate a single compound.

In conclusion a facile and efficient protocol for the synthesis of a broad array of new 23-phosphodiester silybin structured conjugates has been achieved. The feasibility of this procedure has been demonstrated by preparing new 23-silybin conjugates, exploiting standard phosphoramidite chemistry. A key point in our strategy is the new silybin building block 3, useful for a variety of derivatizations following established chemistry. In principle, this methodology can be readily extended to other molecules which have a free hydroxyl group.15,28

Acknowledgment
This study was supported by AIPRAS Onlus (Associazione Italiana per la Promozione delle Ricerche sull’Ambiente e la Saluta umana). We also thank Dr. Vincenzo Perino of the CIMCF, Università degli Studi di Napoli ‘Federico II’, for access to NMR facilities.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

References and Notes
Efficient Synthesis of Novel 23-Phosphodiester Silybin Analogues


General Procedure for the Preparation of Phosphoramidite 3

To 3.57,20-tetra-O-acetyl silylibin (2, 240.0 mg, 0.37 mmol) dissolved in anhydrous CH2Cl2 (5 mL), DIEA (390 μL, 2.22 mmol), and 2-cyanoethyl-N,N-diisopropylaminomethane phosphoramidite (107 μL, 0.48 mmol) were mixed under argon. After 10 min the mixture was diluted with EtOAc, and the organic phase was washed twice with brine and then concentrated. Silica gel chromatography of the residue (eluents n-hexane–EtOAc = 3:7, v/v, in the presence of 1% of Et3N), afforded desired compound 3 (205.0 mg, 0.24 mmol) in a 65% yield. δf = 0.8 (n-hexane–EtOAc = 7:3, v/v), ppm.

1H NMR (500 MHz, CDCl3, r.t., mixture of diastereomers): δ = 7.11–6.90 (6 H, overlapped signals, H-13, H-15, H-16–H-18, H-21, H-22), 6.81 (1 H, s, H-6), 6.58 (1 H, s, H-6), 5.66 (1 H, d, J = 11.6 Hz, H-3), 5.37 (1 H, d, J = 11.6 Hz, H-2), 5.03 (1 H, d, J = 7.6 Hz, H-11), 4.13 (1 H, m, H-10), 3.90–3.60 (9 H, complex signals, OCH3–H3, 2671; and references cited therein.

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Synlett 2013, 24, 45–48
CDCl3): δ = 3.1 ppm. HRMS (MALDI-TOF, negative ions): m/z calc for C36H44O18P: 795.2271; found: 795.2271 [M – H].

Compound 5c: 1H NMR (500 MHz, CD3OD, r.t., mixture of diastereomers): δ = 7.79 (1 H, s), 7.11–6.72 (6 H, complex signals), 6.41 (1 H, dd, J = 6.8, 6.8 Hz), 5.91 (1 H, d, J = 1.5 Hz), 5.85 (1 H, m), 4.97–4.74 (2 H, complex signal), 4.65 (1 H, m), 4.41 (1 H, d, J = 11.5 Hz), 4.22–4.10 (4 H, m), 4.00–3.85 (5 H, complex signals), 2.44 (2 H, m), 1.99 (3 H, s) ppm. 31P NMR (161.98 MHz, CDCl3): δ = 3.3 ppm. HRMS (MALDI-TOF, negative ions): m/z calc for C35H34N2O17P: 785.1600; found: 785.1602 [M – H].

Compound 5d: 1H NMR (500 MHz, CD3OD, r.t., mixture of diastereomers): δ = 8.56 (1 H, s), 8.29 (1 H, s), 7.11–6.72 (6 H, complex signals), 6.05 (1 H, d, J = 6.0 Hz), 5.91 (1 H, d, J = 1.5 Hz), 5.85 (1 H, m), 4.90 (2 H, complex signals), 4.63 (1 H, m), 4.54 (1 H, m), 4.41 (1 H, d, J = 11.5 Hz), 4.35 (1 H, m), 4.20 (2 H, m), 4.21–4.05 (3 H, m), 3.88–3.68 (3 H, s) ppm. 31P NMR (161.98 MHz, CDCl3): δ = 3.1 ppm. HRMS (MALDI-TOF, negative ions): m/z calc for C35H33N5O16P: 810.1665; found: 810.1666 [M – H].

Compound 5e: 1H NMR (500 MHz, CD3OD, r.t., mixture of diastereomers): δ = 7.08–6.81 (6 H, complex signals), 5.95–5.89 (2 H, m), 5.07 (1 H, m), 4.97 (1 H, m), 4.52 (2 H, m), 4.24 (1 H, m), 4.06 (1 H, m), 3.95–3.68 (7 H, complex signals), 3.30 (1 H, m), 2.01–0.70 (33 H, complex signals) ppm. 31P NMR (161.98 MHz, CDCl3): δ = 4.3 ppm. ESI-MS (positive ions): m/z calc for C49H63O16P: 938.39; found: 939.48 [MH]+; [MNa]+ = 961.37. HRMS (MALDI-TOF, negative ions): m/z calc for C49H62O16P: 937.3781; found: 937.3782 [M – H].