Synthesis I. Avan Paper

Microwave-Assisted Synthesis of 2,2′-Azopyridine-Labeled Amines, Amino Acids, and Peptides

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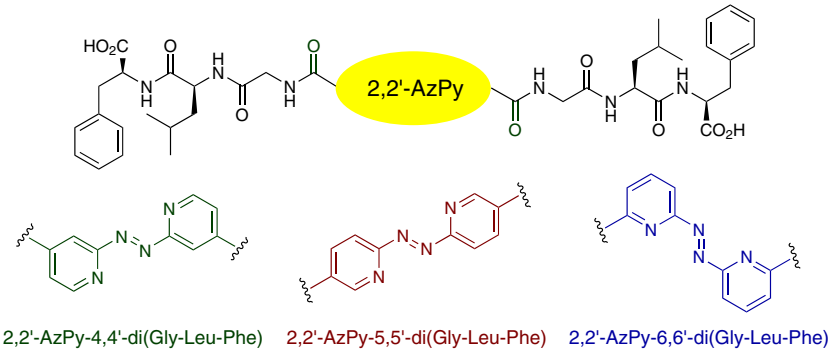
Dedicated to the memory of Alan Roy Katritzky (1928–2014)



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Abstract A microwave-assisted procedure for labeling amines, amino acids, and peptides with 2,2′-azopyridines (2,2′-AzPy) is described using the corresponding 2,2′-azopyridine-diacylbenzotriazoles. The efficiency of the procedure is proven by the generation of three constitutionally isomeric sets of 2,2′-AzPy-X,X′-labeled amino conjugates (where X = 4, 5, 6) including amines, amino acids, and peptides. Microwave-assisted synthesis conditions provide good to excellent yields in less than 15 minutes with retention of original chirality. A *trans*-to-*cis* isomerization of the 2,2′-azopyridine-labeled amino conjugate upon laser irradiation at 325 nm is visualized with UV and NMR spectroscopy.

Key words azopyridine, microwave synthesis, *N*-acylbenzotriazole, acylation, peptides, photoisomerization, *cis*–*trans* isomerization

Peptides and proteins are essential biomolecules that have diverse functions such as hormones, neurotransmitters, and neuromodulators in living systems. Their inherent interaction with biological systems makes them suitable candidates for therapeutic and biological use.[1] Peptides have long been studied and prepared as drugs, immunogens, hormones, vaccines, and peptidic probes.[2] In fact, the secrets of biofunction of peptides are mostly hidden in their constitutions and well-defined three-dimensional structures. Introduction of shape or size labile modules into peptide chains, which response to external stimuli such as light, heat, pH, or physicochemical change, might affect folding pattern, and thus allow a temporal control on peptide's bioactivity.[3] Based on aforementioned approach, many smart-modules and polymeric fragments are devised and utilized for the manipulation of peptide-protein function.[3,4] Among them, azoaromatic compounds (especially azobenzene) are the most prominent moieties due to their effective light-driven *trans*-to-*cis* isomerization.[5] Azobenzene derivatives are used in a variety of light-driven bioprocess including protein and DNA folding,[6] enzymatic,[7] and ion-transfer activity through channels.[8]

Azopyridine (AzPy) is also an azoaromatic compound that is fashioned by two pyridyl group attached to N=N bond and show similar *trans-cis* isomerization. Azopyridines have attracted considerable attention in the field of coordination chemistry due to their high propensity to coordinate metallic fragments through electron pairs of pyridyl and azo nitrogen atoms. Structural identity, gas sorption ability, thermal stability, and magnetic property of AzPy-metal complexes are of current interest.[9,10] The earliest examples concerning photoresponsive AzPy compounds are reported by Shinkai et al. who described the synthesis of 2,2′-AzPy-bridged crown ether, which enables extraction of substantial amounts of heavy metal ions; Cu^{2+}, Ni^{2+}, Co^{2+}, and Hg^{2+}.[11] Since the *trans*-isomer is vertically positioned over the crown ether ring, it builds better coordination with metal ions, and thus, extracts greater amounts of metal ions than the *cis*-isomer does. Later on, the same group reported a thiacrown ether attached 2,2′-Azopyridines.[12] In this case, the *cis*-isomer formed by photoirradiation shows better binding affinity to Cu^{2+} and Hg^{2+} than the *trans*-form.[12] Consequently, when thiacrown ether is used as an ion carrier on a membrane, Cu^{2+} transport rate is enhanced by UV irradiation. Recently, Bardaji et al.[10] reported the synthesis of photosensitive 2,2′- and 4,4′-Azpy gold(I) and silver(I) complexes, which undergo *trans*-to-*cis* isomerization in solution. Azo-metal coordination of an Ag-2,2′-AzPy complex, having a confirmed structure in solid state via X-ray, is simultaneously broken by UV irradiation during photoisomerization in solution.

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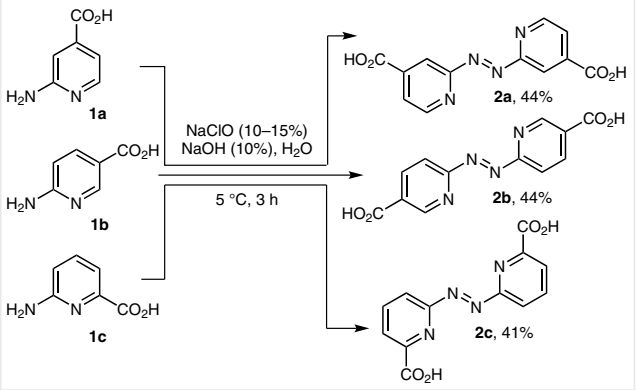
Despite the coordination ability over other azoaromatics and stimuli-responsive behavior of azopyridines, they were not reported as labeling agent for amino acids and peptides. Here, a general microwave assisted procedure is presented for labeling amines, amino acids, and peptides with AzPy in good to excellent yields in less than 15 minutes. The efficiency of the procedure is shown by the synthesis of AzPy-labeled peptides without causing detectable racemization. Preparations of AzPy precursors, microwave-assisted synthesis conditions for AzPy labeling, and preliminary results on light-driven *trans-cis* isomerization are discussed. The methodology presented here expands labeling toolbox for amino acids-peptides, and might be used for future light-driven bioapplications.

Preparation of 2,2′-AzPy-Dicarboxylic Acids 2a–c

Up to date, several methods have been employed for the synthesis of azo compounds including (i) oxidation of aromatic amines and (ii) hydrazo derivatives; reduction of aromatic (iii), nitro, and (iv) azoxy compounds; (v) coupling of primary arylamines with nitroso compounds; and (vi) coupling of aryldiazonium salts.[13] Synthetic methods have been selected based on starting material availability and substituent dependent reaction efficacy. Herein, symmetric 2,2′-AzPy-dicarboxylic acids **2a**–**c** were obtained by the oxidation of 2-aminopyridine carboxylic acids **1a**–**c** as previously applied by Shinkai et al.[11] using aqueous sodium hypochlorite (10–15%), an inexpensive reagent, in moderate yields of 41–44% (Scheme 1, Table 1).



Scheme 1 Synthesis of 2,2′-AzPy-dicarboxylates **2a**–**c**

Preparation of 2,2′-AzPy-Diacylbenzotriazoles 3a–c

In the published methods, azoaromatic carboxylic acids were linked to biomoieties by using coupling reagents such as (i) DCC, EDCI, HBTU, HATU, CDI;[14] (ii) acid chlorides;[11,15] and (iii) other carboxylic acid activators including *N*-hydroxysuccinimide ester,[16] and benzotriazole.[17–19] In the last few decades, Katritzky and co-workers showed synthetic utilities of *N*-acylbenzotriazoles as stable and easy to handle acylation reagents for the preparation of tetra-, penta-, hexa- and cyclic heptapeptides in solution phase,[20,21] 'difficult' sequence peptides on solid phase,[22] and peptidomimetics.[23,24–26] Recently, Katritzky et al. reported the preparation of azobenzene-labeled amino acids, amines, nucleosides, terpenes, sugar, and steroids through activated benzotriazole intermediates under conventional methods.[17–19] Benzotriazole-activated intermediates were prepared under reflux condition for 5 hours in THF from the corresponding azobenzene-carboxylic acids by reacting 1-(methylsulfonyl)-1*H*-benzotriazole. Furthermore, benzotriazole-activated azobenzene carboxylic acids were reacted with free amino acids and amines under reflux conditions or at room temperature for 1–48 hours to give azo-dye-labeled amino acids and amines in 74–100% yields.[17] The treatment of benzotriazole-activated carboxylic acids with terpenes, sugar, and steroids at room temperature for 12–36 hours gave azobenzene-labeled adjuncts in 45–82% yields.[18] Azobenzene-labeled nucleosides were obtained in 30–79% yields after 24-hour reaction of benzotriazole-activated intermediates with corresponding nucleosides at room temperature. Herein, microwave-assisted reaction conditions enable the coupling of 2,2′-AzPy-diacylbenzotriazoles **3a**–**c** with amino conjugates in less than 15 minutes to give 2,2′-AzPy-labeled amines, amino acids, and peptides in good to excellent yields (40–91%) with retention of chirality.

Table 1 2,2′-AzPy-Dicarboxylates **2a**–**c** and 2,2′-AzPy-Diacylbenzotriazoles **3a**–**c**

Entry	Acyl position	**2**, Yield (%)	**2**, Mp (°C)	**3**, Yield (%)	**3**, Mp (°C)
1	4,4′	**2a**, 44	>340	**3a**, 89	255–260
2	5,5′	**2b**, 44	323–326	**3b**, 62	238–242
3	6,6′	**2c**, 41	252–254	**3c**, 62	236–238

Active intermediates, 2,2′-AzPy-diacylbenzotriazoles (2,2′-AzPy-diBt) **3a**–**c**, were prepared from 2,2′-AzPy-dicarboxylic acids **2a**–**c** in 15 minutes by treating with 1-(methylsulfonyl)-1*H*-benzotriazole in anhydrous dimethylformamide under microwave irradiation with 30 W irradiation power at 70 °C in the presence of triethylamine (Scheme 2, Table 1).

2,2′-AzPy-diacylbenzotriazoles **3a**–**c** are readily stable solid compounds at room temperature with high melting points (>200 °C). However, NMR data are not available, since they are not well soluble in organic solvents. Addition of trifluoroacetic acid (TFA) to NMR tube improves solubility, nevertheless they decompose over time. The structures

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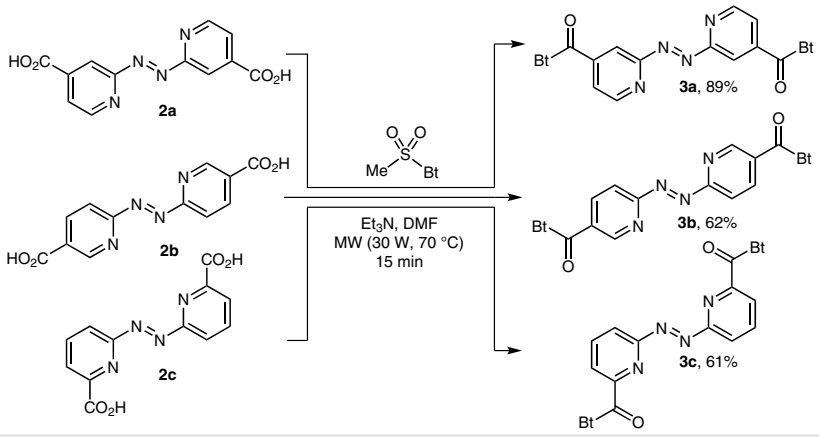


Scheme 2 Preparation of 2,2′-AzPy-diacylbenzotriazoles **3a–c**

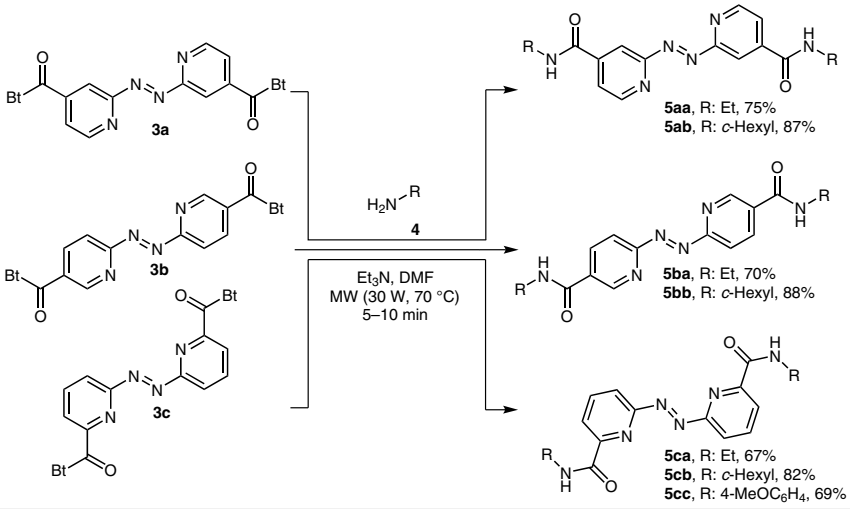
of intermediates **3a–c** are confirmed by elemental analysis and further reactions for the preparation of 2,2′-AzPy-labeled amines, amino acids, and peptides.

Preparation of 2,2′-AzPy-Diacylamines 5aa–cc

Reaction of 2,2′-AzPy-diacylbenzotriazoles **3a–c** with primary amines **4** in anhydrous DMF under microwave exposure with 30 W irradiation power for 5–10 minutes at 70 °C afforded the corresponding amide derivatives **5aa–cc** (67–88%) (Scheme 3 and Table 2). While the reactions of aliphatic amines took 5 minutes, the coupling reaction of aromatic amine for the preparation of **5cc** took 10 minutes because of its lower reactivity.

Table 2 2,2′-AzPy-Diacylamines **5aa–cc**

Entry	Acyl position	Reactant	Amine	**5**, Yield (%)	Mp (°C)
1	4,4′	**3a**	ethylamine	**5aa**, 75	231–232
2	4,4′	**3a**	cyclohexyl-amine	**5bb**, 87	260–261
3	5,5′	**3b**	ethylamine	**5ba**, 70	262–264
4	5,5′	**3b**	cyclohexyl-amine	**5bb**, 88	286–288
5	6,6′	**3c**	ethylamine	**5ca**, 67	208–210
6	6,6′	**3c**	cyclohexyl-amine	**5cb**, 82	188–190
7	6,6′	**3c**	4-methoxy-aniline	**5cc**, 69	234–235



Scheme 3 Preparation of 2,2′-AzPy-diacylamines **5aa–cc**

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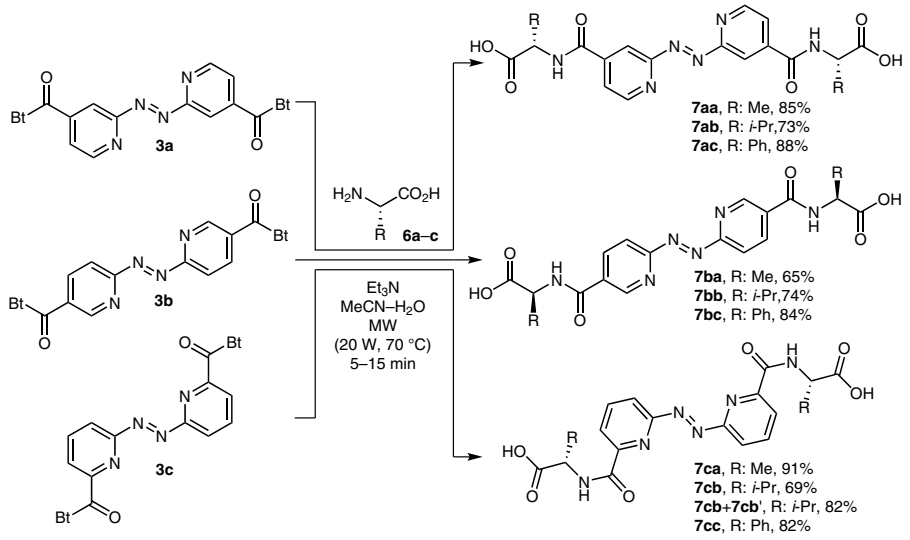
Preparation of 2,2′-AzPy-Labeled Amino Acids 7aa–cc

N-Acylbenzotriazoles are advantageous peptide coupling reagents that allow the use of unprotected amino acids in aqueous media without causing racemization.[21] In initial experiments, the reaction of 2,2′-AzPy-diacylbenzotriazoles **3a** and **3b** with free amino acids **6a**–**c** were not completed after stirring for 24 hours at room temperature, while the reactions of **3c** with amino acids were completed after 6 hours at room temperature. When the reaction was heated up to 50 °C by conventional heating, the reaction of **3a** resulted after 3 hours at 50 °C with the formation of product **7ab** and some starting material **2a** as an inseparable mixture. Therefore, the reaction temperature was elevated to 70 °C. At this temperature, the reactions of **3a** with amino acids were completed after 30 minutes, but yielded the formation of some small amount of starting material **2a**. When the conventional heating was replaced by microwave irradiation in a modified procedure, the reaction time of **3a** was shortened to 15 minutes providing pure products **7ab** without formation of starting material [see Supporting Information (SI), pages S2–S3]. Microwave-assisted coupling reactions of 2,2′-AzPy-diacylbenzotriazoles **3a**–**c** with free amino acids **6a**–**c** in a mixture of water, acetonitrile, and triethylamine with 20 W microwave irradiation power for 5–15 minutes at 70 °C afforded 2,2′-AzPy-labeled amino acids **7aa**–**cc** and (**7cb** + **7cb′**) (65–91%) (Scheme 4, Table 3). While the complete conversion of **3c** took 5 minutes, coupling reaction of **3b** took 10 minutes and reactions with **3a** took 15 minutes because of their lower reactivity. The end of reactions could not be determined by TLC analysis since the active intermediates, 2,2′-AzPy-diacylbenzotriazoles **3**, were not soluble in common organic solvents. The coupling reactions were carried out until the disappearance of all colored solid intermediates **3** in the reaction vessel.

Table 3 2,2′-AzPy-Labeled Amino Acids **7aa**–**cc**

Entry	Acyl position	Reactant	Amino acid	Reaction time (min)	**7**, Yield (%)	Mp (°C)
1	4,4′	**3a**	L-Ala	15	**7aa**, 85	205–206
2	4,4′	**3a**	L-Val	15	**7ab**, 73	199–201
3	4,4′	**3a**	L-Phe	15	**7ac**, 88	210–212
4	5,5′	**3b**	L-Ala	10	**7ba**, 65	224–225
5	5,5′	**3b**	L-Val	10	**7bb**, 74	91–92
6	5,5′	**3b**	L-Phe	10	**7bc**, 84	203–205
7	6,6′	**3c**	L-Ala	5	**7ca**, 91	240–241
8	6,6′	**3c**	L-Val	5	**7cb**, 69	195–197
9	6,6′	**3c**	DL-Val	5	**7cb** + **7cb′**, 82	193–196
10	6,6′	**3c**	L-Phe	5	**7cc**, 82	205–207

Chiral integrity of compounds **7aa**–**cc** was supported by the NMR spectroscopy. Compound **7cb**, valine analogue of 2,2′-AzPy-6,6′-dicarboxylate, showed duplication of signals in the ^{1}H and ^{13}C NMR spectra (Figure 1). For example, methyl protons of isopropyl group provided two separate doublets at 0.98 ppm (J = 5.1 Hz) and 0.96 ppm (J = 5.1 Hz), and they appeared again as two separate doublets at 0.72 ppm (J = 6.8 Hz) and 0.69 ppm (J = 6.8 Hz). For this particular compound, those minor repetitive signals were scrutinized to figure out whether they originate from impurities or possible in situ racemization. Considering racemization of acidic proton on alpha carbon for duplication of signals, the racemic mixture (**7cb** + **7cb′**) of this compound was prepared from DL-valine and **3c**. The racemic mixture (**7cb** + **7cb′**) also provided similar duplicate signals within the same integration ratio (20:1) and appear as chirally pure



Scheme 4 Preparation of 2,2′-AzPy-labeled amino acids **7aa**–**cc**

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compound **7cb** in the ^{1}H NMR spectrum, and each repeating signals form their own racemate splitting patterns (see SI, page S31). For instance, while shifted signals of isopropyl group of **7cb** provided two separate doublets at 0.72 ppm and 0.69 ppm, its racemic mixture (**7cb** + **7cb′**) gave multiple signals in the range of 0.80–0.67 ppm due to further splitting of parent signals. The ^{13}C NMR spectrum of racemic mixture (**7cb** + **7cb′**) was almost identical to the ^{13}C NMR spectrum of chirally pure compound **7cb**, except the mixture of **7cb** and **7cb′** provided two separated signals at 116.4 and 116.3 ppm, whereas **7cb** gave one signal at 116.4 ppm. Attempts for chiral separation of **7cb** and **7cb′** were not successful via analytical HPLC using Chirobiotic T column. Based on those findings, these minor signals on ^{1}H NMR of **7cb** were not formed because of racemization. Apparently, *trans*-to-*cis* isomerization over azo bond (N=N) was another possibility. More stable *trans*-isomer of **7cb** could form the *cis*-isomer via a conformational change by absorbing UV light at wavelength of its maximum absorbance (λ_{max}). UV/Vis spectra of 30 μM of 2,2′-AzPy-valine adjuncts **7ab**, **7bb**, **7cb** in DMSO were measured to determine λ_{max} values (Figure 2, A). In particular, azopyridine compounds showed quite similar characteristic of azobenzene compounds in their UV/Vis spectra having a high-intensity π–π* band in UV region and a low-intensity n–π* band in visible region (Table 4).[27] Those λ_{max} values in the UV region closely fit for irradiation with He-Cd laser at 325 nm. When a 60 μM solution of **7cb** in DMSO was exposed to laser irradiation at 325 nm for 2 minutes, UV/Vis spectra of **7cb** changed by a decrease at π–π* band and an increase at n–π* band upon the *trans*-to-*cis* photoisomerization (Figure 2, B). An NMR tube containing a solution of compound **7cb** in DMSO-d_6 was exposed to laser irradiation at 325 nm for 10 minutes to monitor the possible changes in NMR spectra. Intensities of minor NMR signals contributed by *cis*-isomer were increased from a ratio of 12:1.6 to 12:8 upon laser irradiation, because *trans*-**7cb** was transformed to *cis*-**7cb** isomer (Figure 1, A). The ^{1}H NMR chemical shifts of *cis*-**7cb** were shifted to upfield and coupling constants of methyl protons of **7cb** were amplified from 5.1 Hz to 6.8 Hz.

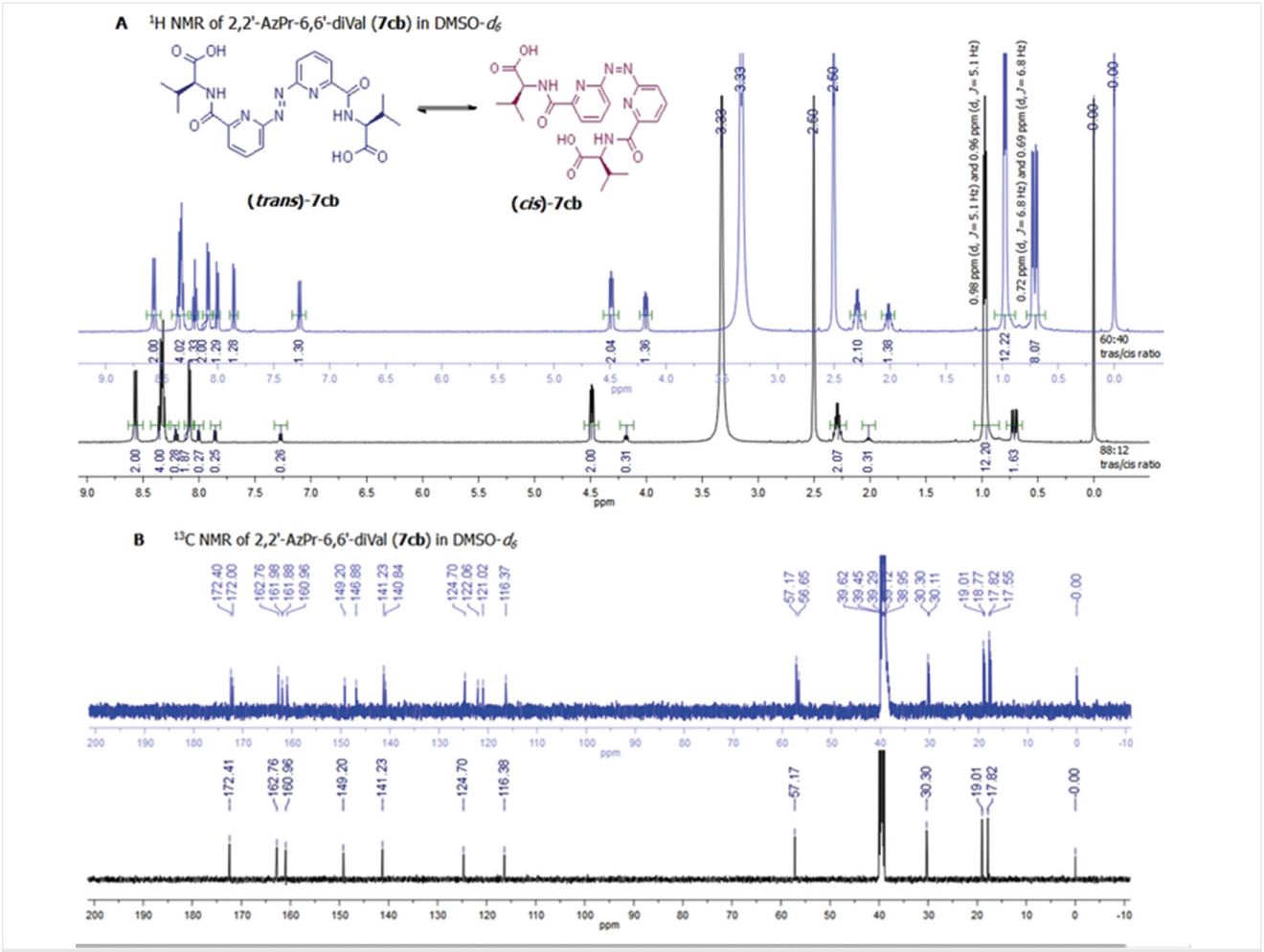

Figure 1 (A) ^{1}H NMR and (B) ^{13}C NMR spectra change upon *trans*-to-*cis* photoisomerization of **7cb** via laser irradiation at 325 nm for 10 minutes (blue spectra were obtained after 10 minutes laser exposure of NMR tube).

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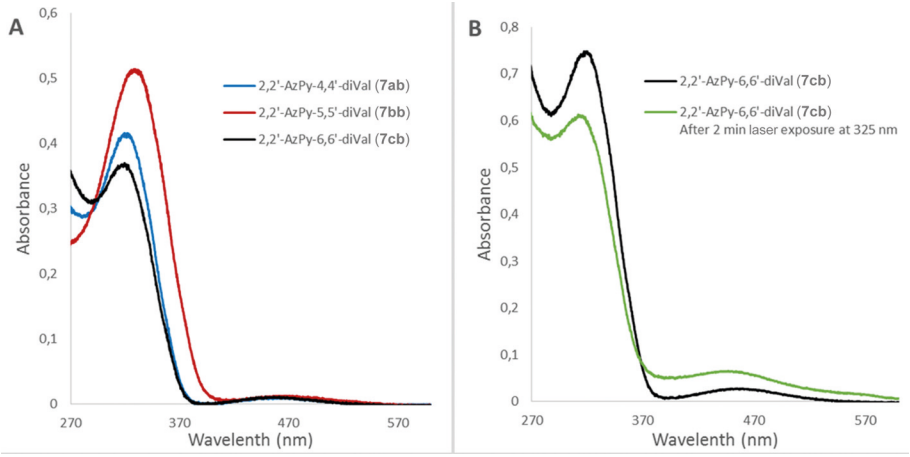

Figure 2 (A) UV/Vis spectra of 30 μM 2,2′-AzPy-valine conjunctions **7ab** (blue), **7bb** (red), and **7cb** (black) in DMSO. (B) UV/Vis spectral changes upon photoisomerization of 60 μM **7cb** in DMSO before (black) and after (green) 2 minutes laser irradiation at 325 nm.

Table 4 UV-Vis Bands of 2,2′-AzPy-Labeled Valine Compounds

2,2′-AzPy-valine compound	Acyl position	UV bands		Vis bands	
		λ_{max} (nm)	ε_{max} (M^{-1}·cm^{-1})	λ_{max} (nm)	ε_{max} (M^{-1} cm^{-1})
7ab	4,4′	322	13 833	460	333
7bb	5,5′	329	17 133	470	433
7cb	6,6′	319	12 667	460	367

These results indicate that the electron density of aforementioned molecule was increased while its molecular volume was decreased, since pyridines were *cis*-oriented over the azo bond, and amino acid arms of *cis*-**7cb** are much closer to themselves than before. The ^{13}C NMR spectrum of **7cb** also presented the duplicate signals due to photoisomerization upon laser irradiation (Figure 1, B). When the NMR tube containing an enriched amount of *cis*-**7cb** isomer was left under sunlight for 30 minutes, the initial ratio levels (12:1.8) of isomers in the mixture was set again by reverse *cis*-to-*trans* isomerization. ^{1}H NMR spectrum containing only *trans*-**7cb** was obtained, when the NMR sample was prepared avoiding sunlight. For this sample, the photodynamic equilibrium between *trans*- and *cis*-isomers of **7cb** was established in 15 minutes under sunlight (see SI, page S30).

Preparation of 2,2′-AzPy-Labeled Di- and Tripeptides

Synthetic utilities of 2,2′-AzPy-diacylbenzotriazoles in coupling reactions were extended by reacting them with small peptides. Free di- and tripeptides were obtained by following previously published method for depsipeptides[25,26] (see SI, page S4). Amino acid types and sequences in a small peptide were selected by considering NMR signals of subgroups to monitor racemization during reactions via NMR spectroscopy (in order to eliminate possible overlaps or interferences to individual NMR signals). Reactions of 2,2′-AzPy-diacylbenzotriazoles **3a**–**c** with free dipeptide-amide **8** in anhydrous DMF under microwave with 20 W irradiation power in 10 minutes at 70 °C afforded 2,2′-AzPy-labeled dipeptide-amides **9a**–**c** (61–76%) (Scheme 5 and Table 5). 2,2′-AzPy-6,6′-diacylbenzotriazole **3c** was treated with free dipeptides **10a**, **10b**, and free tripeptide **11a** in a mixture of H_2O–MeCN–DMF in the presence of triethylamine under microwave irradiation (20 W, 70 °C) for 10 minutes to afford 2,2′-AzPy-6,6′-diacyl-labeled di- and tripeptides **12a**, **12b**, and **13** (72–89%) (Scheme 6 and Table 5). The reactions of 2,2′-AzPy-4,4′-diacylbenzotriazole **3a** and 2,2′-AzPy-5,5′-diacylbenzotriazole **3b** with free dipeptides **10a**, **10b**, and free tripeptide **11a** using various reaction conditions provided a mixture of coupling products and starting materials, 2,2′-AzPy-4,4′-dicarboxylic acid **2a** and 2,2′-AzPy-5,5′-dicarboxylic acid **2b**; however, those mixtures in low quantities could not be separated. Therefore, active intermediates **3a** and **3b** were allowed to react with a free tripeptide ester **11b** under microwave conditions (20 W, 70 °C) for 10 minutes in anhydrous DMF to give 2,2′-AzPy-diacyltripeptide methyl esters **14a** and **15a**, which further underwent deprotection by LiOH in DMF–MeOH–H_2O to give the unprotected 2,2′-AzPy-diacyltripep-

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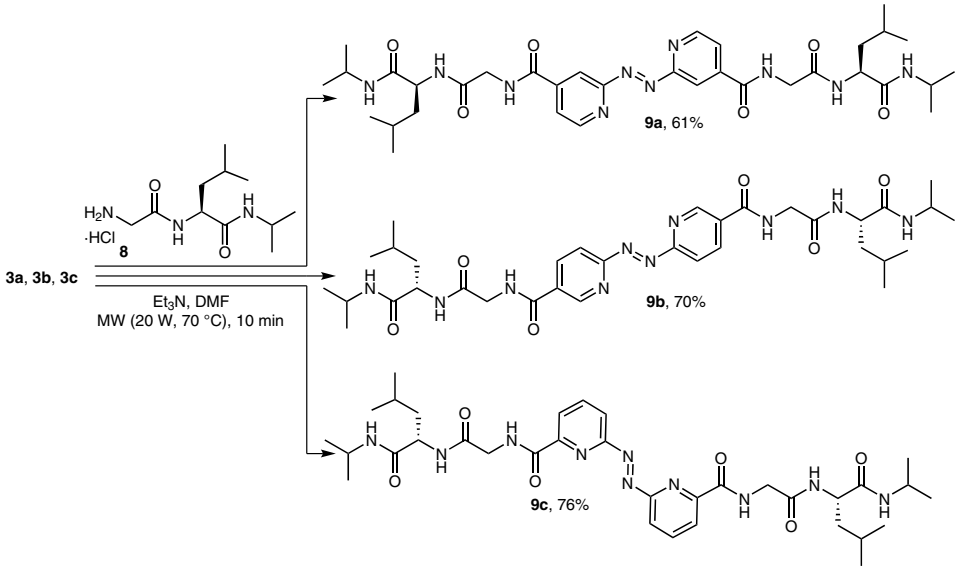
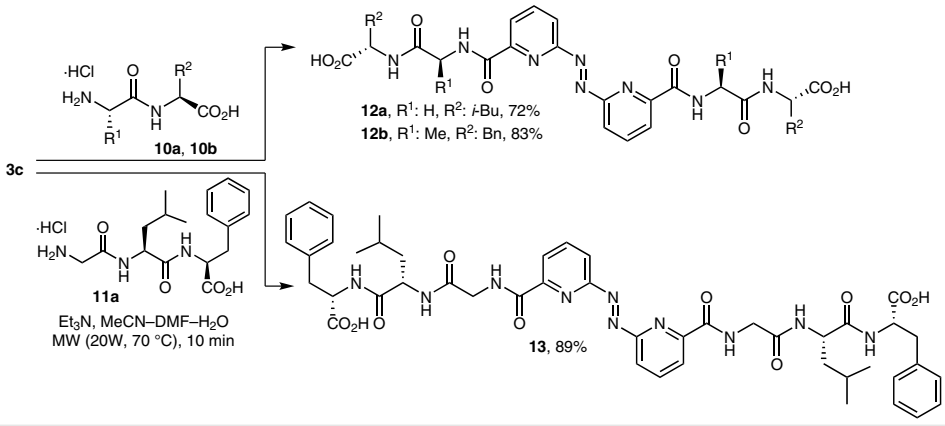
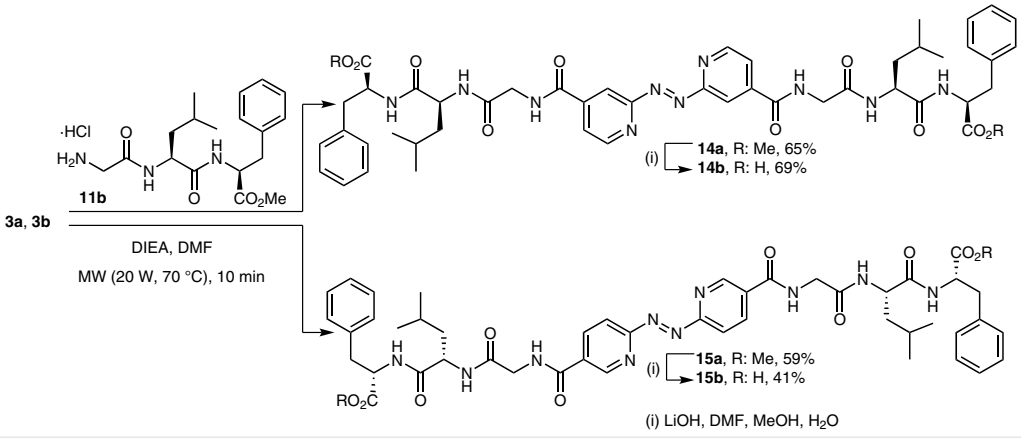


Scheme 5 Preparation of 2,2′-AzPy-labeled dipeptide-amides **9a–c**



Scheme 6 Preparation of 2,2′-AzPy-6,6′-diacyldipeptides **12a**, **12b** and -tripeptide **13**



Scheme 7 Preparation of 2,2′-AzPy-4,4′-diacyltripeptides **14a**, **14b** and 2,2′-AzPy-5,5′-diacyltripeptides **15a**, **15b**

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Table 5 2,2′-AzPy-Labeled Di- and Tripeptides **9a–c**, **12a**, **12b**, **13**, **14a**, **14b**, **15a**, and **15b** Prepared

Entry	Acyl position	Reactant	Product, yield (%)	Peptide part	Mp (°C)
1	4,4′	**3a**	**9a**, 61	Gly-Leu-NH*i*-Pr	242–244
2	5,5′	**3b**	**9b**, 70	Gly-Leu-NH*i*-Pr	212–216
3	6,6′	**3c**	**9c**, 76	Gly-Leu-NH*i*-Pr	244–246
4	6,6′	**3c**	**12a**, 72	Gly-Leu	113–115
5	6,6′	**3c**	**12b**, 83	Ala-Phe	134–138
6	6,6′	**3c**	**13**, 89	Gly-Leu-Phe	212–214
7	4,4′	**3a**	**14a**, 65	Gly-Leu-Phe-OMe	176–180
8	4,4′	**3a**	**14b**,[a] 69	Gly-Leu-Phe	138–142
9	5,5′	**3b**	**15a**, 59	Gly-Leu-Phe-OMe	196–200
10	5,5′	**3b**	**15b**,[a] 41	Gly-Leu-Phe	180–184

[a] Hydrolysis product.

tides **14b** and **15b** (Scheme 7, Table 5). Chiral integrity of 2,2′-AzPy-labeled peptides was confirmed by the NMR spectroscopic data. Notably, there was no duplication of peaks in the ^{1}H and ^{13}C NMR spectra of **9a–9c**, **12a**, **12b**, **13**, **14a**, **14b**, **15a**, and **15b**.

In conclusion, microwave-assisted syntheses of novel 2,2′-AzPy-labeled amines **5aa–cc**, amino acids **7aa–cc**, and peptides **9a–c**, **12a–c**, **13**, **14a**,**b**, and **15a**,**b** were achieved by treating 2,2′-AzPy-diacylbenzotriazoles with primary amines, unprotected amino acids, and peptides. All amino acid and peptide derivatives were prepared in less than 15 minutes under microwave reaction conditions in good to excellent yields and without any detectable racemization. *Trans*-to-*cis* isomerization of 2,2′-AzPy-labeled amino acid conjugate was brought about by laser exposure at 325 nm and monitored using UV and NMR spectroscopy. More importantly, these newly prepared photoactive 2,2′-AzPy derivatives could be useful tools for remotely modulating the folding pattern of a peptide and thus allowing a temporal control of their bioactivity.

Melting points were determined with Mettler Toledo MP90 apparatus and are uncorrected. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Bruker Biospin 500 MHz spectrometer and ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Agilent DD2 400 MHz spectrometer in $CDCl_3$, DMSO-d_6, D_2O, or TFA-d_1 with TMS as internal standard. NMR spectra were analyzed using MestRe Nova 5.2 software. Elemental analyses were performed on a Vario EL III CHNOS elemental analyzer. HRMS analyses were measured on a Waters SYNAPT MS-TOF or Agilent TOF system. Microwave-assisted amino acid and peptide coupling reactions were performed by using a single-mode cavity CEM Discover microwave synthesizer with a continuous irradiation at 2450 MHz and equipped with an infrared temperature control system. DMF was dried and distilled over CaH_2. THF was dried and distilled over metallic Na in the presence of benzophenone. CH_2Cl_2 and EtOAc were dried and distilled over CaO. The *trans*-to-*cis* photoisomerization was carried out by using a Kimmon He-Cd laser system (325 nm, 50 mW). Starting materials, aminopyridine carboxylic acids **1a**, **1b**, and **1c** were prepared by following reported methods.[28] Primary amines **4** and unprotected amino acids **6** were purchased from commercial sources. 1-(Methylsulfonyl)-1*H*-benzotriazole ($BtSO_2Me$), Boc-Gly (**16a**), Boc-Ala (**16b**), Boc-Gly-Bt (**17a**), and Boc-Ala-Bt (**17b**) were prepared by previously reported methods.[24,26,29] Unprotected dipeptides **10a** and **10b** were characterized by ^{1}H and ^{13}C NMR spectroscopy and used for the next coupling step without further purification. The structures of 2,2′-AzPy-diacylbenzotriazoles **3a**, **3b**, and **3c** were confirmed by elemental analysis, IR spectroscopy, and following reactions. NMR data of these compounds are not available because of their insufficient solubility in common NMR solvents.

2,2′-AzPy-Dicarboxylic Acids (2,2′-AzPy-DiCAs) 2a–c; General Procedure

A solution of 6-aminopyridinecarboxylic acid **1a–c** (1 g, 7.25 mmol) in aq 10% NaOH (5 mL) was added dropwise in 15 min to a solution of aq 10–15% NaOCl (15 mL) at 0 °C. After the mixture had been stirred for 1 h at 0 °C, additional aq 10–15% NaOCl (10 mL) was added to the reaction mixture. After further stirring (3 h at 5 °C), the resulting orange precipitate was filtered through a sintered glass filter. The remaining aqueous filtrate was treated with NaOH pellets (~5 g) to form more precipitate, which was filtered later. The collected solid portions were dissolved in aq 1 M NaOH solution (200 mL) to give a red solution. The red solution was heated to 50 °C and treated with active charcoal. The solution was filtered through a pad of Celite (2 cm). The solution was cooled to 10 °C, then acidified with concd HCl to pH 3–4. A pale yellow precipitate, which was formed upon acidification was collected by filtration, washed with cold H_2O, and dried under reduced pressure to give 2,2′-AzPy-dicarboxylic acids **2a–c** as microcrystals (Table 1).

(*E*)-2,2′-(Diazene-1,2-diyl)diisonicotinic Acid (2,2′-AzPy-4,4′-diCA, 2a)

Yield: 0.43 g (44%); orange microcrystals; mp >340 °C.

^{1}H NMR (500 MHz, 0.1 M K_2CO_3 in D_2O): δ = 8.72 (d, *J* = 4.9 Hz, 2 H), 8.20 (s, 2 H), 7.92 (dd, *J* = 4.9, 1.3 Hz, 2 H).

^{13}C NMR (125 MHz, 0.1 M K_2CO_3 in D_2O): δ = 174.7, 168.6, 152.5, 151.1, 128.9, 119.1.

Anal. Calcd for $C_{12}H_8N_4O_4$: C, 52.95; H, 2.96; N, 20.58. Found: C, 52.49; H, 2.96; N, 20.44.

(*E*)-6,6′-(Diazene-1,2-diyl)dinicotinic Acid (2,2′-AzPy-5,5′-diCA, 2b)

Yield: 0.43 g (44%); pale red-orange microcrystals; mp 323–326 °C.

^{1}H NMR (500 MHz, 0.1 M K_2CO_3 in D_2O): δ = 8.96 (s, 2 H), 8.40 (d, *J* = 8.2 Hz, 2 H), 7.92 (d, *J* = 8.2 Hz, 2 H).

^{13}C NMR (125 MHz, 0.1 M K_2CO_3 in D_2O): δ = 174.6, 166.4, 152.6, 143.1, 137.7, 120.1.

Anal. Calcd for $C_{12}H_8N_4O_4$: C, 52.95; H, 2.96; N, 20.58. Found: C, 52.46; H, 2.99; N, 20.41.

(*E*)-6,6′-(Diazene-1,2-diyl)dipicolinic Acid (2,2′-AzPy-6,6′-diCA, 2c)

Yield: 0.40 g (41%); orange microcrystals; mp 252–254 °C (Lit.[11] mp 143.3–144.5 °C).

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^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.62 (br s, 2 H), 8.34–8.27 (m, 4 H), 8.04 (dd, J = 6.7 Hz, 2.0 Hz, 2 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 165.5, 162.0, 148.5, 140.6, 127.2, 116.5.

Anal. Calcd for $C_{12}H_8N_4O_4$: C, 52.95; H, 2.96; N, 20.58. Found: C, 52.71; H, 3.00; N, 20.44.

2,2′-AzPy-Diacylbenzotriazoles (2,2′-AzPy-DiacylBt) 3a–c; General Procedure

Et_3N (0.22 g, 2.2 mmol) was added to a suspension of 2,2′-AzPy-dicarboxylic acid **2a–c** (0.275 g, 1 mmol) in anhydrous DMF (4 mL) in a dried 10 mL heavy-walled Pyrex tube containing a long stir bar. $BtSO_2Me$ (0.43 g, 2.2 mmol) was added to this solution and exposed to microwave irradiation (30 W) for 15 min at 70 °C with vigorous stirring and simultaneous air cooling. After completion of the reaction, the mixture was cooled to r.t. and poured onto crushed ice-water mixture (~20 g). The precipitate formed was filtered and washed with aq 10% Na_2CO_3 (3 × 5 mL), H_2O, and Et_2O. The solid was dried to yield 2,2′-AzPy-diacylbenzotriazoles **3a–c** as microcrystals (Table 1).

(*E*)-[Diazene-1,2-diylbis(pyridine-2,4-diyl)]bis[(1*H*-benzo[*d*][1,2,3]triazol-1-yl)methanone] (2,2′-AzPy-4,4′-diBt, 3a)

Yield: 0.42 g (89%); pale red-brown microcrystals; mp 255–260 °C (dec.).

Anal. Calcd for $C_{24}H_{14}N_{10}O_2$: C, 60.76; H, 2.97; N, 29.52. Found: C, 60.38; H, 2.98; N, 29.08.

(*E*)-[Diazene-1,2-diylbis(pyridine-6,3-diyl)]bis[(1*H*-benzo[*d*][1,2,3]triazol-1-yl)methanone] (2,2′-AzPy-5,5′-diBt, 3b)

Yield: 0.29 g (62%); pale red-brown microcrystals; mp 238–242 °C (dec.).

Anal. Calcd for $C_{24}H_{14}N_{10}O_2$: C, 60.76; H, 2.97; N, 29.52. Found: C, 60.17; H, 3.04; N, 29.14.

(*E*)-[Diazene-1,2-diylbis(pyridine-6,2-diyl)]bis[(1*H*-benzo[*d*][1,2,3]triazol-1-yl)methanone] (2,2′-AzPy-6,6′-diBt, 3c)

Yield: 0.29 g (62%); pale red-orange microcrystals; mp 236–238 °C (dec.).

Anal. Calcd for $C_{24}H_{14}N_{10}O_2$: C, 60.76; H, 2.97; N, 29.52. Found: C, 60.40; H, 3.06; N, 29.24.

2,2′-AzPy-Diacylamines 5aa–cc; General Procedure

Et_3N (0.11 g, 1.1 mmol) was added to a mixture of 2,2′-AzPy-diacylbenzotriazole **3a–c** (0.10 g, 0.21 mmol) and primary amine hydrochloride salt **4** (0.84 mmol) in anhydrous DMF (2.5 mL) in a dried 10 mL heavy-walled Pyrex tube containing a long stir bar. The reaction mixture was exposed to microwave irradiation (30 W, 70 °C) for 5 min with vigorous stirring and simultaneous air cooling. After completion of the reaction, the mixture was cooled to r.t. and poured onto crushed ice-water mixture (~20 g). The precipitate formed was filtered and washed with aq 10% Na_2CO_3 (3 × 5 mL), aq 1 N HCl (3 × 5 mL), H_2O, and Et_2O, respectively. The solid was dried under vacuum to yield 2,2′-AzPy-diacylamines **5aa–cc** as microcrystals. Et_3N was not used in the case of cyclohexylamine (1.26 mmol). In the preparation of **5cc**, 4-methoxyaniline (0.104 g, 0.84 mmol) and Et_3N (0.43 g, 0.42 mmol) were used and MW reaction took 10 min (if a precipitate did not form upon acidification, the solution was saturated by adding solid NaCl and cooled for 2–3 h) (Table 2).

(*E*)-2,2′-(Diazene-1,2-diyl)bis(*N*-ethylisonicotinamide) (2,2′-AzPy-4,4′-diEtA, 5aa)

Yield: 51 mg (75%); pale orange microcrystals; mp 231–232 °C.

^{1}H NMR (500 MHz, $CDCl_3$ + TFA): δ = 9.11 (d, J = 5.6 Hz, 2 H), 8.84 (d, J = 1.1 Hz, 2 H), 8.60 (dd, J = 5.6, 1.4 Hz, 2 H), 3.62 (q, J = 7.3 Hz, 4 H), 1.33 (t, J = 7.3 Hz, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$ + TFA): δ = 164.0, 156.5, 150.6, 146.6, 129.6, 115.8, 37.0, 13.5.

HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{16}H_{18}N_6O_2$: 327.1569; found: 327.1571.

Anal. Calcd for $C_{16}H_{18}N_6O_2$: C, 58.88; H, 5.56; N, 25.75. Found: C, 58.75; H, 5.64; N, 26.16.

(*E*)-2,2′-(Diazene-1,2-diyl)bis(*N*-cyclohexylisonicotinamide) (2,2′-AzPy-4,4′-dicHA, 5ab)

Yield: 79 mg (87%); pale orange microcrystals; mp 260–261 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.10 (d, J = 5.6 Hz, 2 H), 8.78 (d, J = 1.1 Hz, 2 H), 8.58 (dd, J = 5.6, 1.4 Hz, 2 H), 4.05–3.95 (m, 2 H), 2.08–2.00 (m, 4 H), 1.90–1.80 (m, 4 H), 1.76–1.68 (m, 2 H), 1.50–1.30 (m, 8 H), 1.30–1.18 (m, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$ + TFA): δ = 163.1, 156.6, 150.5, 146.5, 129.4, 115.6, 51.8, 32.8 (2 C), 25.2, 24.9 (2 C).

Anal. Calcd for $C_{24}H_{30}N_6O_2$: C, 66.34; H, 6.96; N, 19.34. Found: C, 66.31; H, 7.26; N, 19.73.

(*E*)-6,6′-(Diazene-1,2-diyl)bis(*N*-ethylnicotinamide) (2,2′-AzPy-5,5′-diEtA, 5ba)

Yield: 48 mg (70%); pale red microcrystals; mp 262–264 °C.

^{1}H NMR (500 MHz, $CDCl_3$ + TFA): δ = 9.50 (s, 2 H), 9.12 (d, J = 7.8 Hz, 2 H), 8.59 (d, J = 8.0 Hz, 2 H), 3.63 (q, J = 7.3 Hz, 4 H), 1.34 (t, J = 7.3 Hz, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$ + TFA): δ = 164.0, 156.7, 146.8, 144.5, 136.4, 121.6, 37.2, 13.7.

Anal. Calcd for $C_{16}H_{18}N_6O_2$: C, 58.88; H, 5.56; N, 25.75. Found: C, 58.71; H, 5.99; N, 25.37.

(*E*)-6,6′-(Diazene-1,2-diyl)bis(*N*-cyclohexylnicotinamide) (2,2′-AzPy-5,5′-dicHA, 5bb)

Yield: 80 mg (88%); orange microcrystals; mp 286–288 °C.

^{1}H NMR (500 MHz, $CDCl_3$ + TFA): δ = 9.43 (d, J = 18 Hz, 2 H), 9.04 (dd, J = 8.4, 2.0 Hz, 2 H), 8.52 (d, J = 8.4 Hz, 2 H), 4.06–3.96 (m, 2 H), 2.10–2.00 (m, 4 H), 1.90–1.80 (m, 4 H), 1.76–1.68 (m, 2 H), 1.50–1.36 (m, 8 H), 1.30–1.16 (m, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$ + TFA): δ = 163.0, 156.9, 145.9, 144.6, 136.0, 120.6, 51.6, 32.3 (2 C), 25.2, 24.9 (2 C).

HRMS [ESI(+)-TOF]: m/z [M + Na]$^+$ calcd for $C_{16}H_{18}N_6O_2Na$: 457.2322; found: 457.2343.

(*E*)-6,6′-(Diazene-1,2-diyl)bis(*N*-ethylpicolinamide) (2,2′-AzPy-6,6′-diEtA, 5ca)

Yield: 46 mg (67%); pale orange-yellow microcrystals; mp 208–210 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.44 (d, J = 7.5 Hz, 2 H), 8.18–8.10 (m, 4 H), 8.01 (d, J = 7.8 Hz, 2 H), 3.64–3.52 (m, 4 H), 1.32 (t, J = 7.3 Hz, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.2, 161,2, 150.4, 140.0, 124.9, 116.4, 34.5, 14.9.

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HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{16}H_{18}N_6O_2$: 327.1569; found: 327.1570.

(*E*)-6,6′-(Diazene-1,2-diyl)bis(*N*-cyclohexylpicolinamide) (2,2′-AzPy-6,6′-dicHA, 5cb)

Yield: 75 mg (82%); pale orange microcrystals; mp 188–190 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.44 (dd, J = 7.6 Hz, 0.8 Hz, 2 H), 8.11 (t, J = 7.8 Hz, 2 H), 8.04–7.96 (m, 4 H), 4.08–3.96 (m, 2 H), 2.12–2.04 (m, 4 H), 1.86–1.76 (m, 4 H), 1.73–1.64 (m, 2 H), 1.52–1.32 (m, 8 H), 1.28–1.16 (m, 2 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 162.4, 161.3, 150.6, 125.0, 115.9, 48.7, 33.1 (2c), 25.6, 25.1 (2C).

HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{24}H_{30}N_6O_2$: 435.2508; found: 435.2504.

(*E*)-6,6′-(Diazene-1,2-diyl)bis[*N*-(4-methoxyphenyl)picolinamide] (2,2′-AzPy-6,6′-diMeOPhA, 5cc)

Yield: 70 mg (69%); orange microcrystals; mp 234–235 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.92 (s, 2 H), 8.54 (d, J = 7.5 Hz, 2 H), 8.19 (t, J = 7.8 Hz, 2 H), 8.08 (d, J = 7.8 Hz, 2 H), 7.75 (d, J = 8.8 Hz, 4 H), 6.95 (d, J = 8.8 Hz, 4 H), 3.83 (s, 6 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 161.1, 160.9, 150.4, 140.3, 130.7, 125.1, 121.8 (2 C), 116.6, 144.3 (2 C), 55.5.

Anal. Calcd for $C_{26}H_{22}N_6O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.36; H, 4.62; N, 17.43.

2,2′-AzPy-Diacylamino Acids 7aa–cc and 7cb + 7cb′; General Procedure

Et_3N (0.16 g, 1.58 mmol) was added to a suspension of amino acid **6a–c** (1.58 mmol) in H_2O (1.5 mL) in a 10 mL heavy-walled Pyrex tube containing a long stir bar. MeCN (3.5 mL) was added to the amino acid solution and stirred for 5 min at r.t. 2,2′-AzPy-diacylbenzotriazole **3** (0.15 g, 0.32 mmol) was added to this white suspension. The mixture was exposed to microwave irradiation (20 W, 70 °C) for the specified reaction time (15 min for **3a**, 10 min for **3b**, and 5 min for **3a**) with vigorous stirring and simultaneous air cooling. The reactions were carried out until the disappearance of all colored solid starting materials **3** in the reaction vessel. After completion of the reaction, the mixture was cooled to r.t. and poured onto crushed ice-water mixture (~10 g). The mixture was acidified with aq 2 N HCl to pH 3–4. The precipitate formed was filtered and washed with aq 1 N HCl (3 × 5 mL), H_2O, and Et_2O, respectively (if a precipitate did not form upon acidification, the solution was saturated by adding solid NaCl and cooled for 2–3 h). Compound **7aa** and **7ba** were quite water soluble, so the compound in aqueous part (at pH 3–4) was recovered by extracting with EtOAc (3 × 10 mL). The combined organic phases were washed with brine (3 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The solid obtained was washed with Et_2O and dried under vacuum to yield 2,2′-AzPy-diacylamino acids (Table 3).

(2*S*,2′*S*)-2,2′-({2,2′-[(*E*)-Diazene-1,2-diyl]bis(isonicotinoyl)}bis(azanediyl))dipropionic Acid (2,2′-AzPy-4,4′-diAla, 7aa)

Yield: 112 mg (85%); pale red microcrystals; mp 205–206 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.74 (br s, 2 H), 9.28 (d, J = 7.0 Hz, 2 H), 8.96 (d, J = 4.9 Hz, 2 H), 8.27 (s, 2 H), 8.08 (d, J = 4.4 Hz, 2 H), 4.54–4.44 (m, 2 H), 1.44 (d, J = 7.23 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 173.6, 163.7, 163.0, 150.4, 143.8, 124.2, 111.1, 48.3, 16.7.

HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{18}H_{18}N_6O_6$: 415.1361; found: 415.1358.

(2*S*,2′*S*)-2,2′-({2,2′-[(*E*)-Diazene-1,2-diyl]bis(isonicotinoyl)}bis(azanediyl))bis(3-methylbutanoic Acid) (2,2′-AzPy-4,4′-diVal, 7ab)

Yield: 110 mg (73%); pale orange microcrystals; mp 199–201 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.78 (br s, 2 H), 9.08 (d, J = 8.1 Hz, 2 H), 8.94 (d, J = 4.9 Hz, 2 H), 8.24 (s, 2 H), 8.06 (d, J = 4.9 Hz, 2 H), 4.36 (t, J = 7.2 Hz, 2 H), 2.26–2.16 (m, 2 H), 1.00 (d, J = 6.4 Hz, 6 H), 0.98 (d, J = 6.4 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.6, 164.6, 162.8, 150.2, 144.0, 124.4, 111.5, 58.5, 16.7, 29.41, 19.16, 18.63.

HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{22}H_{26}N_6O_6$: 471.1992; found: 471.1992.

(2*S*,2′*S*)-2,2′-[{2,2′-[(*E*)-Diazene-1,2-diyl]bis(isonicotinoyl)}bis(azanediyl)]bis(3-phenylpropanoic Acid) (2,2′-AzPy-4,4′-diPhe, 7ac)

Yield: 160 mg (88%); pale brown microcrystals; mp 210–212 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.93 (br s, 2 H), 9.31 (d, J = 8.2 Hz, 2 H), 8.94 (d, J = 5.0 Hz, 2 H), 8.16 (s, 2 H), 7.96 (dd, J = 5.0, 1.4 Hz, 2 H), 7.35–7.10 (m, 8 H), 7.22–7.14 (m, 2 H), 4.76–4.66 (m, 2 H), 3.22 (dd, J = 13.9, 4.5 Hz, 2 H), 3.08 (dd, J = 13.9, 10.8 Hz, 2 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.5, 163.9, 162.9, 150.4, 143.7, 137.8, 128.9 (2 C), 128.1 (2 C), 126.4, 124.0, 111.1, 54.2, 36.1.

HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{30}H_{26}N_6O_6$: 567.1992; found: 567.2002.

(2*S*,2′*S*)-2,2′-[{6,6′-[(*E*)-Diazene-1,2-diyl)bis(nicotinoyl)}bis(azanediyl)]dipropionic Acid (2,2′-AzPy-5,5′-diAla, 7ba)

Yield: 86 mg (65%); pale red-brown microcrystals; mp 224–225 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.69 (br s, 2 H), 9.22 (d, J = 1.8 Hz, 2 H), 9.12 (d, J = 7.1 Hz, 2 H), 8.54 (dd, J = 8.4, 2.1 Hz, 2 H), 7.93 (d, J = 8.3 Hz, 2 H), 4.54–4.44 (m, 2 H), 1.45 (d, J = 7.3 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 173.7, 163.8, 163.5, 148.8, 138.4, 131.4, 113.5, 48.2, 16.7.

HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{18}H_{18}N_6O_6$: 415.1361; found: 415.1366.

Anal. Calcd for $C_{18}H_{18}N_6O_6$: C, 52.17; H, 4.38; N, 20.28. Found: C, 51.71; H, 4.02; N, 20.11.

(2*S*,2′*S*)-2,2′-({6,6′-[(*E*)-Diazene-1,2-diyl]bis(nicotinoyl)}bis(azanediyl))bis(3-methylbutanoic Acid) (2,2′-AzPy-5,5′-diVal, 7bb)

Yield: 112 mg (74%); pale red-brown microcrystals; mp 91–92 °C.

^{1}H NMR (500 MHZ, DMSO-d_6): δ = 12.79 (br s, 2 H), 9.20 (d, J = 1.6 Hz, 2 H), 8.92 (d, J = 8.1 Hz, 2 H), 8.54 (dd, J = 8.3, 1.9 Hz, 2 H), 7.92 (d, J = 8.3 Hz, 2 H), 4.37 (t, J = 7.4 Hz, 2 H), 2.30–2.18 (m, 2 H), 1.02 (d, J = 6.9 Hz, 6 H), 1.01 (d, J = 6.9 Hz, 6 H).

^{13}C NMR (125 MHZ, DMSO-d_6): δ = 172.6, 164.7, 163.5, 148.9, 138.6, 131.7, 113.4, 58.4, 29.5, 19.2, 18.6.

HRMS [ESI(+)-TOF]: m/z [M + H]$^+$ calcd for $C_{22}H_{26}N_6O_6$: 471.1992; found: 471.1984.

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(2*S*,2′*S*)-2,2′-({6,6′-[(*E*)-Diazene-1,2-diyl]bis(nicotinoyl)}bis(azanediyl))bis(3-phenylpropanoic Acid) (2,2′-AzPy-5,5′-diPhe, 7bc)

Yield: 152 mg (84%); orange microcrystals; mp 203–205 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.90 (br s, 2 H), 9.18 (d, *J* = 8.1 Hz, 2 H), 9.10 (d, *J* = 1.9 Hz, 2 H), 8.42 (dd, *J* = 8.3, 2.1 Hz, 2 H), 7.89 (d, *J* = 8.3 Hz, 2 H), 7.38–7.26 (m, 8 H), 7.24–7.17 (m, 2 H), 4.76–4.66 (m, 2 H), 3.25 (dd, *J* = 13.8, 4.5 Hz, 2 H), 3.08 (dd, *J* = 13.8, 10.8 Hz, 2 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.6, 164.0, 163.5, 148.6, 138.3, 137.8, 131.4, 129.0 (2 C), 128.2 (2 C), 126.4, 113.6, 54.2, 36.3.

Anal. Calcd for $C_{30}H_{26}N_6O_6$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.27; H, 4.69; N, 14.87.

(2*S*,2′*S*)-2,2′-({6,6′-[(*E*)-Diazene-1,2-diyl]bis(picolinoyl)}bis(azanediyl))dipropionic Acid (2,2′-AzPy-6,6′-diAla, 7ca)

Yield: 120 mg (91%); pale orange microcrystals; mp 240–241 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.86 (br s, 2 H), 8.93 (d, *J* = 7.6 Hz, 2 H), 8.38–8.28 (m, 4 H), 8.09 (d, *J* = 7.6 Hz, 2 H), 4.62–4.52 (m, 2 H), 1.48 (d, *J* = 7.2 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 173.6, 162.6, 161.1, 149.6, 141.0, 124.7, 115.9, 47.8, 17.2.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{18}H_{18}N_6O_6$: 415.1361; found: 415.1366.

(2*S*,2′*S*)-2,2′-({6,6′-[(*E*)-Diazene-1,2-diyl]bis(picolinoyl)}bis(azanediyl))bis(3-methylbutanoic Acid) (2,2′-AzPy-6,6′-diVal, 7cb)

Yield: 104 mg (69%); pale orange microcrystals; mp 195–197 °C.

***E*-Isomer**

^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.11 (br s, 2 H), 8.57 (d, *J* = 8.8 Hz, 2 H), 8.38–8.28 (m, 4 H), 8.08 (dd, *J* = 7.4, 0.9 Hz, 2 H), 4.49 (dd, *J* = 8.8, 5.3 Hz, 2 H), 2.34–2.24 (m, 2 H), 0.98 (d, *J* = 5.1 Hz, 6 H), 0.96 (d, *J* = 5.1 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.4, 162.8, 161.0, 149.2, 141.2, 124.7, 116.4, 57.2, 30.3, 19.0, 17.8.

***Z*-Isomer**

NMR spectra of samples containing a mixture of both isomers (*E* and *Z*) were recorded after laser irradiation at 325 nm of the NMR tube for 10 min (see SI, p S28–S29).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.11 (br s, 2 H), 8.20 (t, *J* = 7.8 Hz, 2 H), 8.00 (d, *J* = 7.9, 2 H), 7.86 (d, *J* = 8.6 Hz, 2 H), 7.27 (d, *J* = 9.1 Hz, 2 H), 4.18 (dd, *J* = 9.0, 5.4 Hz, 2 H), 2.34–2.24 (m, 2 H), 0.72 (d, *J* = 6.8 Hz, 6 H), 0.69 (d, *J* = 6.8 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.0, 162.0, 161.9, 146.9, 140.8, 122.1, 121.0, 56.6, 30.1, 18.8, 17.6.

Anal. Calcd for $C_{22}H_{26}N_6O_6$: C, 56.16; H, 5.57; N, 17.86. Found: C, 55.79; H, 5.58; N, 17.74.

(*E*)-2,2′-{[6,6′-(Diazene-1,2-diyl)bis(picolinoyl)]bis(azanediyl)}bis(3-methylbutanoic Acid) [2,2′-AzPy-6,6′-di(DL)Val, (7cb + 7cb′)]

Yield: 123 mg (82%); pale orange microcrystals; mp 193–196 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.11 (br s, 2 H), 8.66–8.54 (m, 2 H), 8.38–8.28 (m, 4 H), 8.14–8.06 (m, 2 H), 4.54–4.44 (m, 2 H), 2.36–2.24 (m, 2 H), 1.08–0.90 (m, 12 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.4, 162.8, 161.0, 149.2, 141.2, 124.7, 116.4, 116.3, 57.2, 30.3, 19.0, 17.8.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{22}H_{26}N_6O_6$: 471.1992; found: 471.1997.

(2*S*,2′*S*)-2,2′-({6,6′-[(*E*)-Diazene-1,2-diyl]bis(picolinoyl)}bis(azanediyl))bis(3-phenylpropanoic Acid) (2,2′-AzPy-6,6′-diPhe, 7cc)

Yield: 148 mg (82%); pale orange microcrystals; mp 205–207 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.07 (br s, 2 H), 8.84 (d, *J* = 8.2 Hz, 2 H), 8.34–8.22 (m, 4 H), 7.98 (d, *J* = 7.7 Hz, 2 H), 7.28–7.22 (m, 8 H), 7.22–7.14 (m, 2 H), 4.86–4.76 (m, 2 H), 3.29–3.24 (m, 4 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.4, 162.8, 161.2, 149.4, 141.1, 137.4, 129.1 (2 C), 128.2 (2 C), 126.5, 124.7, 116.1, 53.4, 36.3.

Anal. Calcd for $C_{30}H_{26}N_6O_6$: C, 63.60; H, 4.63; N, 14.83. Found: C, 63.12; H, 4.35; N, 14.82.

2,2′-AzPy-Diacyldipeptide-Amides 9a–c; General Procedure

2,2′-AzPy-diacylbenzotriazole **3** (0.050 g, 0.10 mmol) was added to a mixture of free dipeptide-amide hydrochloride salt **8** (0.135 g, 0.5 mmol) and Et_3N (0.10 g, 1.0 mmol) in anhydrous DMF (2.0 mL) in a dried 10 mL heavy-walled Pyrex tube containing a long stir bar. The reaction mixture was exposed to microwave irradiation (20 W, 70 °C) for 10 min with vigorous stirring and simultaneous air cooling. After completion of the reaction, the mixture was cooled to r.t. and poured onto crushed ice-water mixture (~5 g). The mixture was acidified with aq 1 N HCl (3 mL). The precipitate formed was filtered and washed with aq 10% Na_2CO_3 (3 × 5 mL), aq 1 N HCl (3 × 5 mL), H_2O and Et_2O, respectively. The solid product was dried under vacuum to yield 2,2′-AzPy-diacyldipeptide-amides **9a**–**c** (Table 5).

2,2′-[(*E*)-Diazene-1,2-diyl]bis[*N*-(2-{[(*S*)-1-(isopropylamino)-4-methyl-1-oxopentan-2-yl]amino}-2-oxoethyl)isonicotinamide] [2,2′-AzPy-4,4′-di(Gly-Leu-NHPr-*i*), 9a]

Yield: 42 mg (61%); pale pink-orange microcrystals; mp 242–244 °C.

^{1}H NMR (400 MHz, TFA-d_1): δ = 9.58 (d, *J* = 5.7 Hz, 2 H), 9.46 (d, *J* = 1.4 Hz, 2 H), 9.58 (dd, *J* = 5.7, 1.5 Hz, 2 H), 5.10–5.02 (m, 2 H), 4.96–4.82 (m, 4 H), 4.56–4.44 (m, 2 H), 2.20–1.96 (m, 6 H), 1.60 (d, *J* = 3.7 Hz, 6 H), 1.58 (d, *J* = 3.7 Hz, 6 H), 1.32 (d, *J* = 5.8 Hz, 6 H), 1.28 (d, *J* = 5.8 Hz, 6 H).

^{13}C NMR (100 MHz, TFA-d_1): δ = 176.2, 173.6, 167.0, 157.2, 152.9, 148.2, 131.5, 121.9, 55.7, 46.7, 45.6, 42.4, 26.9, 23.2, 22.4, 22.3.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{50}N_{10}O_6$: 695.3988; found: 695.3986.

6,6′-[(*E*)-Diazene-1,2-diyl]bis[*N*-(2-{[(*S*)-1-(isopropylamino)-4-methyl-1-oxopentan-2-yl]amino}-2-oxoethyl)nicotinamide] [2,2′-AzPy-5,5′-di(Gly-Leu-NHPr-*i*), 9b]

Yield: 49 mg (70%); pale brown microcrystals; mp 212–216 °C.

^{1}H NMR (400 MHz, TFA-d_1): δ = 9.99 (s, 2 H), 9.62 (d, *J* = 8.4 Hz, 2 H), 9.07 (d, *J* = 8.3 Hz, 2 H), 5.10–5.00 (m, 2 H), 4.96–4.80 (m, 4 H), 4.56–4.44 (m, 2 H), 2.18–1.96 (m, 6 H), 1.58 (d, *J* = 3.0 Hz, 6 H), 1.57 (d, *J* = 3.2 Hz, 6 H), 1.32 (d, *J* = 5.6 Hz, 6 H), 1.28 (d, *J* = 5.6 Hz, 6 H).

^{13}C NMR (100 MHz, TFA-d_1): δ = 176.2, 173.8, 166.8, 157.9, 149.0, 147.6, 137.5, 124.4, 55.8, 46.8, 45.6, 42.5, 26.9, 23.2, 22.5, 22.4.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{50}N_{10}O_6$: 695.3988; found: 695.3998.

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6,6′-[(*E*)-Diazene-1,2-diyl]bis[*N*-(2-{[(*S*)-1-(isopropylamino)-4-methyl-1-oxopentan-2-yl]amino}-2-oxoethyl)picolinamide] [2,2′-AzPy-6,6′-di(Gly-Leu-NHPr-*i*), 9c]

Yield: 53 mg (76%); pale orange microcrystals; mp 244–246 °C.

^{1}H NMR (400 MHz, TFA-d_1): δ = 9.05–8.95 (m, 4 H), 8.92–8.84 (m, 2 H), 5.08–5.00 (m, 2 H), 4.94–4.80 (m, 4 H), 4.52–4.42 (m, 2 H), 2.12–1.94 (m, 6 H), 1.56 (d, *J* = 2.6 Hz, 6 H), 1.54 (d, *J* = 2.6 Hz, 6 H), 1.28 (d, *J* = 6.0 Hz, 6 H), 1.24 (d, *J* = 5.8 Hz, 6 H).

^{13}C NMR (100 MHz, TFA-d_1): δ = 176.3, 174.0, 165.9, 159.9, 148.4, 147.6, 130.2, 123.6, 55.7, 46.9, 45.4, 42.4, 26.9, 23.2, 22.4 (2 C).

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{50}N_{10}O_6$: 695.3988; found: 695.4009.

2,2′-AzPy-6,6′-Di(di- and tripeptides) 12a, 12b, and 13; General Procedure

Et_3N (0.10 g, 1.00 mmol) was added to a suspension of the appropriate free peptide hydrogen chloride salt **10a**,**b**, **11a** (0.50 mmol) in H_2O (0.5 mL) in a 10 mL heavy-walled Pyrex tube containing a long stir bar. MeCN (1.5 mL) and DMF (0.2 mL) was added to the peptide solution and stirred for 2 min at r.t. 2,2′-AzPy-6,6′-diacylbenzotriazole **3c** (0.05 g, 0.10 mmol) was added to this solution. The mixture was exposed to microwave irradiation (20 W, 70 °C) for 10 min with vigorous stirring and simultaneous air cooling. After completion of the reaction, the mixture was cooled to r.t. and poured onto crushed ice-water mixture (~10 g). The mixture was acidified with aq 2 N HCl to pH 3–4. The precipitate formed was filtered and washed with aq 1 N HCl (3 × 5 mL), H_2O, and Et_2O, respectively (if a precipitate did not form upon acidification, the solution was saturated by adding solid NaCl and cooled for 2–3 h). The solid product was dried under vacuum to yield 2,2′-AzPy-6,6′-diacylpeptides (Table 5).

(2*S*,2′*S*)-2,2′-[{2,2′-[{6,6′-[(*E*)-Diazene-1,2-diyl]bis(picolinoyl)}bis(azanediyl)]bis(acetyl)}bis(azanediyl)]bis(4-methylpentanoic Acid) [2,2′-AzPy-6,6′-di(Gly-Leu), 12a]

Yield: 44 mg (72%); pale orange microcrystals; mp 113–115 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.61 (br s, 2 H), 8.94 (t, *J* = 5.6 Hz, 2 H), 8.36–8.26 (m, 6 H), 8.02 (d, *J* = 7.4 Hz, 2 H), 4.32–4.26 (m, 2 H), 4.04 (m, 4 H), 1.74–1.60 (m, 2 H), 1.60–1.48 (m, 2 H), 0.90 (d, *J* = 6.5 Hz, 6 H), 0.86 (d, *J* = 6.5 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 173.8, 168.3, 163.1, 161.1, 149.7, 141.0, 124,6 116,0 50.2, 41.9, 40.1, 24.2, 22.7, 21.3.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{28}H_{36}N_8O_8$: 613.2729; found: 613.2728.

(2*S*,2′*S*)-2,2′-[{(2*S*,2′*S*)-2,2′-[{6,6′-[(*E*)-Diazene-1,2-diyl]bis(picolinoyl)}bis(azanediyl)]bis(propanoyl)}bis(azanediyl)]bis(3-phenylpropanoic Acid) [2,2′-AzPy-6,6′-di(Ala-Phe), 12b]

Yield: 59 mg (83%); pale orange microcrystals; mp 134–138 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.79 (br s, 2 H), 8.70 (d, *J* = 7.9 Hz, 2 H), 8.48 (d, *J* = 8.0 Hz, 2 H) 8.36–8.26 (m, 4 H), 8.04 (dd, *J* = 7.3, 0.9 Hz, 2 H), 7.28–7.18 (m, 8 H), 7.18–7.12 (m, 2 H), 4.64–4.56 (m, 2 H), 4.52–4.42 (m, 2 H), 3.09 (dd, *J* = 13.8, 5.0 Hz, 2 H), 2.82 (dd, *J* = 13.8, 9.3 Hz, 2 H), 1.32 (d, *J* = 6.9 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.5, 171.5, 162.0, 161.0, 149.4, 141.0, 137.3, 129.0 (2C), 128.3 (2C), 126.3, 124.6, 115.8, 53.4, 48.1, 36.5, 18.7.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{36}H_{36}N_8O_8$: 709.2729; found: 709.2739.

(2*S*,2′*S*)-2,2′-[{(2*S*,2′*S*)-2,2′-[{2,2′-[{6,6′-[(*E*)-Diazene-1,2-diyl]bis(picolinoyl)}bis(azanediyl)]bis(acetyl)}bis(azanediyl)]bis(4-methylpentanoyl)}bis(azanediyl)]bis(3-phenylpropanoic Acid) [2,2′-AzPy-6,6′-di(Gly-Leu-Phe), 13]

Yield: 81 mg (89%); pale orange microcrystals; mp 212–214 °C.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.64 (br s, 2 H), 8.96 (t, *J* = 5.5 Hz, 2 H), 8.36–8.26 (m, 4 H), 8.24 (d, *J* = 7.7 Hz, 2 H), 8.13 (d, *J* = 8.3 Hz, 2 H), 8.02 (d, *J* = 7.4 Hz, 2 H), 7.28–7.18 (m, 8 H), 7.18–7.14 (m, 2 H), 4.44–4.36 (m, 4 H), 4.06–3.96 (m, 4 H), 3.04 (dd, *J* = 13.9, 5.2 Hz, 2 H), 2.92 (dd, *J* = 13.7, 8.9 Hz, 2 H), 1.64–1.54 (m, 2 H), 1.46–1.36 (m, 4 H), 0.86 (d, *J* = 6.4 Hz, 6 H), 0.86 (d, *J* = 6.4 Hz, 6 H).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 172.6, 171.7, 167.9, 163.1, 161.1, 149.7, 140.1, 137.4, 129.0 (2 C), 128.0 (2 C), 126.2, 124.6, 115.9, 53.3, 50.6, 42.2, 40.9, 36.4, 23.9, 23.0, 21.6.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{46}H_{54}N_{10}O_{10}$: 907.4097; found: 907.4124.

2,2′-AzPy-Di(tripeptides) 14a, 14b, 15a, and 15b

2,2′-AzPy-diacyltripeptide-esters **14a** and **15a** were prepared by following the same general procedure as described for 2,2′-AzPy-diacyldipeptide-amides **9a**–**c** (Table 5).

Dimethyl 2,2′-[{(2*S*,2′*S*)-2,2′-[{2,2′-[{2,2′-[(*E*)-Diazene-1,2-diyl]bis(isonicotinoyl)}bis(azanediyl)]bis(acetyl)}bis(azanediyl)]bis(4-methylpentanoyl)}bis(azanediyl)](2*S*,2′*S*)-bis(3-phenylpropanoate) [2,2′-AzPy-4,4′-di(Gly-Leu-Phe-OMe), 14a]

Yield: 61 mg (65%); brown microcrystals; mp 176–180 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.38–9.28 (m, 2 H), 8.96 (d, *J* = 4.4 Hz, 2 H), 8.41 (d, *J* = 7.0 Hz, 2 H), 8.25 (s, 2 H), 8.13 (d, *J* = 7.9 Hz, 2 H), 8.06 (d, *J* = 4.1 Hz, 2 H), 7.36–7.10 (m, 10 H), 4.56–4.34 (m, 4 H), 4.04–3.90 (m, 4 H), 3.55 (s, 6 H), 3.10–2.90 (m, 4 H), 1.70–1.50 (m, 2 H), 1.50–1.34 (m, 4 H), 0.88 (d, *J* = 6.2 Hz, 6 H), 0.85 (d, *J* = 6.2 Hz, 6 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 171.9, 171.6, 167.9, 164.0, 162.9, 150.4, 143.8, 137.0, 129.0 (2 C), 128.1 (2 C), 126.4, 124.0, 111.0, 53.4, 51.6, 50.4, 42.3, 40.8, 36.3, 23.9, 22.9, 21.6.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{48}H_{58}N_{10}O_{10}$: 935.4410; found: 935.4445.

(2*S*,2′*S*)-2,2′-[{(2*S*,2′*S*)-2,2′-[{2,2′-[{2,2′-[(*E*)-Diazene-1,2-diyl]bis(isonicotinoyl)}bis(azanediyl)]bis(acetyl)}bis(azanediyl)]bis(4-methylpentanoyl)}bis(azanediyl)]bis(3-phenylpropanoic Acid) [2,2′-AzPy-4,4′-di(Gly-Leu-Phe), 14b)]

LiOH (8 mg, 0.33 mmol) was added to a suspension of **14a** (30 mg, 0.032 mmol) in DMF–MeOH–H_2O ((3:1:1), 2 mL) in a test tube at 10 °C. After stirring the reaction mixture for 2 h at 10 °C, ice-cold water (5 mL) was added to this mixture and acidified with aq 1 N HCl until pH 3–4. The precipitate formed was collected by suction filtration and washed with cold H_2O and Et_2O. The solid product was dried under vacuum to give **14b** as pale brown microcrystals; yield: 20 mg (69%); mp 138–142 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.68 (br s, 2 H), 9.38–9.28 (m, 2 H), 8.96 (d, *J* = 4.9 Hz, 2 H), 8.32–8.20 (m, 4 H), 8.13 (d, *J* = 8.4 Hz, 2 H), 8.06 (d, *J* = 4.2 Hz, 2 H), 7.32–7.14 (m, 10 H), 4.46–4.34 (m, 4 H), 4.04–3.88 (m, 4 H), 3.05 (dd, *J* = 13.8, 5.2 Hz, 2 H), 2.92 (dd, *J* = 13.8, 9.0 Hz, 2 H), 1.66–1.54 (m, 2 H), 1.50–1.36 (m, 4 H), 0.86 (d, *J* = 6.4 Hz, 6 H), 0.84 (d, *J* = 6.4 Hz, 6 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.6, 171.8, 167.9, 164.0, 162.9, 150.4, 143.9, 137.4, 129.0 (2C), 128.0 (2C), 126.3, 124.1, 111.0, 53.3, 50.6, 42.3, 40.9, 36.4, 23.9, 23.0, 21.6.

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HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{46}H_{54}N_{10}O_{10}$: 907.4097; found: 907.4135.

Dimethyl 2,2′-[{(2*S*,2′*S*)-2,2′-[{2,2′-[{6,6′-[(*E*)-Diazene-1,2-diyl]bis(nicotinoyl)}bis(azanediyl)]bis(acetyl)}bis(azanediyl)]bis(4-methylpentanoyl)}bis(azanediyl)](2*S*,2′*S*)-bis(3-phenylpropanoate) [2,2′-AzPy-5,5′-di(Gly-Leu-Phe-OMe), 15a]

Yield: 55 mg (59%); pale red microcrystals; mp 196–200 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.28–9.16 (m, 4 H), 8.52 (dd, *J* = 8.3, 1.9 Hz, 2 H), 8.42 (d, *J* = 7.4 Hz, 2 H), 8.13 (d, *J* = 8.3 Hz, 2 H), 7.93 (d, *J* = 8.3 Hz, 2 H), 7.32–7.18 (m, 10 H), 4.52–4.36 (m, 4 H), 4.04–3.92 (m, 4 H), 3.56 (s, 6 H), 3.04 (dd, *J* = 13.7, 5.9 Hz, 2 H), 2.97 (dd, *J* = 13.7, 8.7 Hz, 2 H), 1.66–1.54 (m, 2 H), 1.48–1.38 (m, 4 H), 0.88 (d, *J* = 6.5 Hz, 6 H), 0.85 (d, *J* = 6.5 Hz, 6 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.0, 171.6, 168.1, 164.2, 163.4, 148.7, 138.3, 137.0, 131.5, 128.9 (2 C), 128.1 (2 C), 126.4, 113.6, 53.5, 51.6, 50.5, 42.4, 40.8, 36.3, 23.9, 22.9, 21.6.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{48}H_{58}N_{10}O_{10}$: 935.4410; found: 935.4438.

(2*S*,2′*S*)-2,2′-[{(2*S*,2′*S*)-2,2′-[{2,2′-[{6,6′-[(*E*)-Diazene-1,2-diyl]bis(nicotinoyl)}bis(azanediyl)]bis(acetyl)}bis(azanediyl)]bis(4-methylpentanoyl)}bis(azanediyl)]bis(3-phenylpropanoic Acid) [2,2′-AzPy-5,5′-di(Gly-Leu-Phe), 15b]

LiOH (8 mg, 0.33 mmol) was added to a suspension of **15a** (25 mg, 0.027 mmol) in DMF–MeOH–H_2O (3:1:1, 2 mL) in a test tube at 10 °C. After stirring the reaction mixture for 2 h at 10 °C, ice-cold H_2O (5 mL) was added to this mixture and acidified with aq 1 N HCl until pH 3–4. The precipitate formed was collected by suction filtration and washed with cold H_2O and Et_2O. The solid product was dried under vacuum to give **15b** as red-brown microcrystals; yield: 10 mg (41%); mp 180–184 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.68 (br s, 2 H), 9.26–9.16 (m, 4 H), 8.51 (dd, *J* = 8.3, 1.9 Hz, 2 H), 8.24 (d, *J* = 7.7 Hz, 2 H), 8.12 (d, *J* = 8.6 Hz, 2 H), 7.93 (d, *J* = 8.3 Hz, 2 H), 7.32–7.18 (m, 10 H), 4.46–4.36 (m, 4 H), 4.02–3.94 (m, 4 H), 3.06 (dd, *J* = 13.8, 5.4 Hz, 2 H), 2.94 (dd, *J* = 13.8, 8.9 Hz, 2 H), 1.66–1.54 (m, 2 H), 1.50–1.36 (m, 4 H), 0.88 (d, *J* = 6.5 Hz, 6 H), 0.84 (d, *J* = 6.5 Hz, 6 H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 172.6, 171.8, 168.0, 164.1, 163.4, 148.7, 138.3, 137.4, 131.5, 129.0 (2 C), 128.1 (2 C), 126.3, 113.6, 53.5, 50.6, 42.3, 40.9, 36.4, 23.9, 23.0, 21.6.

HRMS [ESI(+)-TOF]: *m*/*z* [M + H]$^+$ calcd for $C_{46}H_{54}N_{10}O_{10}$: 907.4097; found: 907.4132.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1560523.

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